

**POLY(ETHYLENE GLYCOL) BASED DENDRIMER-LIKE HYPERBRANCHED
MACROMOLECULES, METHODS OF PREPARATION AND USE THEREOF**

TECHNICAL FIELD

[0001] In certain embodiments, the present invention relates to dendrimer-like hyperbranched
5 macromolecules for several uses such as medical or biopharmaceutical applications, or non-
medical or industrial uses, such as antibody purification, cosmetic applications, catalytic
applications, applications in electronics, agriculture, food, filtration and further applications. In
medical applications, the hyperbranched molecules are conjugated with active agents, such as
drugs, peptides or proteins. Furthermore, the present invention relates in certain embodiments to
10 a hyperbranched macromolecule for drug delivery, comprising polyethylene glycol (PEG) units
and at least one active agent conjugated to the hyperbranched macromolecules. In certain further
embodiments, the present invention relates to methods for synthesizing, purifying and
characterizing such dendrimer-like hyperbranched macromolecules. The present invention also
relates in certain embodiments to methods of treatment of a medical condition such as treatment
15 of an ocular disease.

BACKGROUND

[0002] Controlled delivery and stabilization of therapeutic agents is a large area of research in
recent years. A controlled delivery improves therapies, facilitates administration, and leads to
enhanced efficacy, better compliance, less side effects and overall better therapeutic results.

20 [0003] The eye is a unique organ of perfection and complexity and is a microcosm of the body in
many ways. It provides a great opportunity for nanomedicine since it is readily accessible
allowing for direct drug/gene delivery to maximize the therapeutic effect and minimize side
effects. The development of appropriate delivery systems that can sustain and deliver
therapeutics to the target tissues is a key challenge that can be addressed by nanotechnology.

25 Current delivery systems for anterior ocular segment disorders such as punctum plug, micro- and
nano-particle encapsulation, microneedle system, iontophoresis, different types of intravitreal
implants, etc., represent state-of-the-art tools for sustained and controlled drug release in the eye.

[0004] Dendrimers and hyperbranched polymers have attracted the attention of scientists in the area of drug and gene delivery over the last two decades for their versatility, complexity and multi-branching properties. Dendrimers are tree-like, highly symmetric, monodisperse, branched nanostructured polymers that have repeatable building blocks with well-defined size, tailorable structure, and potentially favorable ocular biodistribution. Dendrimers have been widely explored as a new platform for delivery of bioactives owing to unique biological properties such as high drug load, lipid bilayer interactions, targeting potential, blood plasma retention time, filtration, intracellular internalization, biodistribution, transfection, good colloidal and biological stability. A number of dendrimers have been explored for drug delivery including polymer-based dendrimers such as polyamidoamine (PAMAM), poly(propylene imine) (PPI), polyester, polyether, poly-L-lysine, triazine, melamine, poly(glycerol-co-succinic acid), poly(glycerol), and poly[2,2-bis(hydroxymethyl) propionic acid] dendrimers, and other types of dendrimers made of peptide, liquid crystal forming dendrimers, carbosilane, etc. (for an overview, see, e.g. “Dendrimer as nanocarrier for drug delivery” Prashant Kesharwani, Keerti Jain, Narendra Kumar Jain, Progress in Polymer Science 39 (2014) 268– 307; and “Dendrimer-based drug delivery systems: history, challenges, and latest developments” Juan Wang, Boxuan Li, Li Qiu, Xin Qiao and Hu Yang, Journal of Biological Engineering (2022) 16:18).

[0005] Among the applications of dendrimers in drug delivery, those related to treating and managing ocular diseases are of special interest. Ocular drug therapies suffer from some significant disadvantages, including frequent administration, poor penetration and/or rapid elimination. The use of dendrimers as a strategy for overcoming obstacles to the traditional treatment of ocular diseases shows promising progress in this field, and the approach to ocular safety with dendrimers is intended that accounts for the most advanced science to date. Several ocular applications of dendrimers and dendrimeric delivery systems are known, cf. “Dendrimer as nanocarrier for drug delivery” Prashant Kesharwani, Keerti Jain, Narendra Kumar Jain, Progress in Polymer Science 39 (2014) 268– 307. However, most of these applications are still in the early laboratory exploration stage, and only very few commercial products for ocular disease treatment with dendrimer delivery are known so far.

[0006] There is accordingly a need to provide strategies for optimizing drug delivery and site-specific targeting. There is also a need for providing delivery systems for sustained release drug delivery that allow increasing half-life of the active agent, and enhancement of efficacy and

bioavailability of active agents. For delivery of biomolecules such as peptides and proteins there is further a need to improve avidity and to extend half-life of receptor binding biomolecules by providing multivalent delivery possibilities.

5 [0007] Furthermore, dendrimers can have various applications in non-medical fields or industrial uses, such as antibody purification, applications in cosmetics, catalysis, electronics, agriculture, food, filtration, energy storage, construction materials, and further applications.

[0008] All references cited herein are incorporated by reference in their entireties for all purposes.

OBJECTS AND SUMMARY OF INVENTION

10 [0009] It is thus an object of certain embodiments of the present invention to provide a dendrimer platform based on polyethylene glycol building blocks that are highly variable and flexible for being adapted to several uses and applications, and easy to synthesize.

[0010] A further object of certain embodiments of the present invention is to provide systems with optimized drug delivery and site-specific targeting, specifically for treating a condition at
15 the eye.

[0011] It is another object of certain embodiments of the present invention to provide delivery systems for sustained release drug delivery that allow increasing half-life of the active agent, an enhancement of efficacy, avidity and bioavailability of active agents.

[0012] It is another object of certain embodiments of the present invention to provide
20 biodegradable drug delivery systems that can be tuned for their degradation rate and active agent release rate using a wide range of different biodegradable molecular groups, including hydrolyzable groups and linkages that are built into the molecular structure of the drug delivery system. The biodegradable drug delivery systems of certain embodiments should be fully resorbable and degradable to the initial building blocks that can be easily cleared from the local
25 tissues and ultimately the body.

[0013] It is another object of certain embodiments of the present invention to provide biodegradable drug delivery systems leading to enhanced binding affinity and/or avidity of the active agent, e.g., biomolecules such as peptides or proteins to biological targets.

5 [0014] It is a further object of certain embodiments of the invention, and an aspect, to provide methods for treating a disease/medical condition of a patient.

[0015] A further object of certain embodiments of the present invention to provide dendrimers for applications also in non-medical fields or industrial uses, such as antibody purification, cosmetic applications, catalytic applications, applications in electronics, agriculture, food, filtration, energy storage, construction materials, coatings, adhesives, water purification, oil
10 recovery, fragrance release, paper making, environmental sensing and release Systems, membranes, textiles, printing inks, surface chemistry applications, thickeners, detergents, rheology modifiers, scaffolding, or 3D-printing.

[0016] The above objects are solved by the inventions as described in the independent claims. Advantageous modifications are disclosed in the dependent claims.

15 [0017] Some aspects of the present disclosure are directed to a hyperbranched macromolecule comprising a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit at the connectivities c , at least one of the polymeric arms being connected by a hydrolyzable bond to a dendritic constitutional repeating unit (DCRU), the dendritic constitutional repeating unit /DCRU) comprising a branch unit connected to at least two
20 polymeric arms each comprising an end group or being connected to a next dendritic constitutional repeating unit that may again be connected to a further dendritic constitutional repeating unit, the polymeric arms of the outermost dendritic constitutional repeating unit each comprising an end group; wherein the polymeric arms consist of polyethylene glycol (PEG) units; wherein optionally at least one active agent is conjugated to at least one of the outermost
25 polymeric arms. In some embodiments, at least 10%, preferably about 20 to 100 % of the chemical bonds of the connections can be cleaved by hydrolysis. The bonds cleavable by hydrolysis are preferably ester bonds. In certain embodiments, the ester bonds are introduced by using linkers derived from organic diacids.

[0018] In some embodiments, the building blocks or fragments of the hyperbranched macromolecule obtained/obtainable after cleaving all hydrolyzable bonds of the connections have an average molecular weight (M_n) of less than 50,000 Daltons. The active agent may be covalently or non-covalently bound to the hyperbranched macromolecule. In certain
5 embodiments, the active agent is covalently conjugated to the hyperbranched macromolecule.

[0019] In certain aspects, the hyperbranched macromolecule is a generation G_0 dendrimer-like branched macromolecule wherein the surface end groups of the branched macromolecule are the end groups at the polymeric arms connected to the core unit without further connections to DCRU's. In other aspects, the hyperbranched macromolecule is a higher generation G_x
10 dendrimer-like hyperbranched macromolecule, with x being an integer of 1 to 10 defining the number of consecutively connected dendritic constitutional repeating units in the hyperbranched macromolecule, the polymeric arms of the outermost dendritic constitutional repeating unit each comprising an end group, wherein the at least one active agent is conjugated to at least one of the outermost polymeric arms.

[0020] In certain aspects of the present disclosure, the core unit and the branch units of the hyperbranched macromolecule are the same or different and independently of each other have a connectivity c of 3 to 10, or 4 to 8, or 4 to 6, or 4. In certain aspects, the core unit and the branch unit are the same or different and are each derived from a polyol having at least 3 hydroxyl
15 groups. In certain aspects, the polyol is selected from the group consisting of glycerol, pentaerythritol, xylitol, dipentaerythritol, tripentaerythritol, hexaglycerol, isomalt, lactitol,
20 maltitol, mannitol, or sorbitol.

[0021] In certain aspects of the present disclosure, the polymeric arms in the hyperbranched macromolecule comprise PEG units having an average molecular weight (M_w) in the range from about 1,000 to about 80,000 Daltons, or from about 10,000 to about 60,000 Daltons, or from
25 about 15,000 to about 50,000 Daltons. In certain aspects, the average molecular weight of the polymeric arm PEG units attached to the core is the same or different than that of the polymeric arms in the dendritic constitutional repeating units. The average molecular weight of the polymeric arm PEG units attached to the core can be higher or lower than that of the polymeric arms in the dendritic constitutional repeating units. For higher generation G_x hyperbranched
30 macromolecules, with x being an integer of 2 to 10, the average molecular weight of the

polymeric arm PEG units can decrease or increase from the innermost polymeric arms to the outermost polymeric arms.

[0022] In certain aspects of the present disclosure, the end groups attached to the outermost polymeric arms are grafted to the termini of the polymeric arms directly or via a difunctional linker comprising hydrolyzable bonds such as a carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group. In further embodiments, the end groups attached to the outermost polymeric arms are functional groups selected from electrophiles such as activated ester groups such as succinimidyl esters, succinimidyl carbonates; nitrophenyl carbonates, aldehydes, ketones, acrylates, acrylamides, maleimides, vinylsulfones, iodoacetamides, alkenes, alkynes, azides, norbornenes, epoxides, mesylates, tosylates, tresyls, cyanurates, orthopyridyl disulfides, or halogen; nucleophiles such as an amine, such as a primary amine, a hydroxyl, an alcohol, a thiol, an azide anion, and a carboxyl group; functional groups for click chemistry; functional groups for cycloadditions, such as 1,3-dipolar cycloadditions, [3+2] cycloadditions such as alkene-nitrone cycloadditions or alkyne-nitrone cycloadditions, [4+2] cycloadditions; functional groups for thiol-ene reactions; hetero-Diels-Alder cycloadditions; functional groups for nucleophilic ring openings, functional groups for non-aldol type carbonyl reactions; functional groups for addition reactions to carbon-carbon multiple bonds, polymerizable vinyl groups, or combinations thereof.

[0023] In certain aspects of the present disclosure, the end groups attached to the outermost polymeric arms are linker-spaced functional groups selected from succinimidyl succinate (SS), succinimidyl glutarate (SG), succinimidyl adipate (SAP), succinimidyl azelate (SAZ), and succinimidyl glutaramide (SGA).

[0024] In certain aspects of the present disclosure, the end groups attached to the outermost polymeric arms are functional groups selected from an alkyne compound such as a dibenzocyclooctyne (DBCO), or a bicyclo[6.1.0]-nonyne (BCN); or a norbornene, or a trans-cyclooctene (TCO); an azide, a 3,4 dihydroxyphenylacetic acid (DHPA), or a tetrazine (Tz).

[0025] In certain aspects of the present disclosure, the connection between the polymeric arms connected to the core unit and the first dendritic constitutional repeating unit and/or the

connections between consecutive dendritic constitutional repeating units are formed by click chemistry.

5 [0026] In certain aspects of the present disclosure, the connection is formed by click chemistry, wherein the connection is formed by reacting a polymeric arm functionalized with an alkyne, cycloalkyne, or strained or terminal alkene moiety with a polymeric arm functionalized with an azide or a tetrazine moiety in a SPAAC or an IEDDA type click chemistry coupling reaction. In certain aspects, the alkyne moiety is a dibenzocyclooctyne moiety.

10 [0027] In certain aspects of the present disclosure, the connection between the polymeric arms connected to the core unit and the first dendritic constitutional repeating unit and/or the connections between consecutive dendritic constitutional repeating units are formed between the polymeric arms connected to the core unit and polymeric arms connected to the branch unit of the dendritic constitutional repeating units and/or between the polymeric arms of a dendritic constitutional repeating unit and polymeric arms of consecutive dendritic constitutional repeating units.

15 [0028] In certain aspects of the present disclosure, the active agent conjugated to at least one of the end groups located at the surface of the hyperbranched macromolecule is selected from the group consisting of therapeutically or diagnostically active agents.

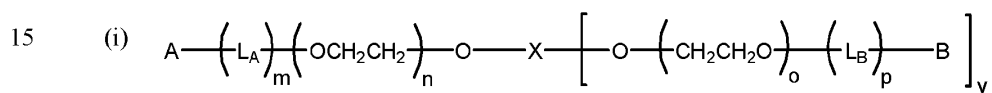
20 [0029] In certain aspects of the present disclosure, the active agent conjugated to at least one of the end groups located at the surface of the dendrimer is selected from steroids; non-steroidal anti-inflammatory drugs (NSAIDS) such as Diclofenac, Ibuprofen, Meclofenamate, Mefenamic A, Salsalate, Sulindac, Tolmetin, Ketoprofen, Diflunisal, Piroxicam, Naproxen, Etodolac, Flurbiprofen, Fenoprofen C, Indomethacin, Celecoxib, Ketorolac, Nepafenac; intraocular pressure lowering drugs; antibiotics such as Ciprofloxacin; pain reliever such as Bupivacaine; calcium channel blockers such as Nifedipine; cell cycle inhibitors such as Simvastatin; proteins such as insulin; small molecule hydrophilic drugs, including carboxylic acid salts and amine salts; small molecule hydrophobic drugs, hydrophilic peptides and protein drugs, such as insulin, single chain antibody fragments, Fab fragments, IgG antibodies, fusion antibodies, etc.; aptamers; particularly Bupivacaine (BPV-HCl or base), Ropivacaine (RPV), Dexamethasone, Travoprost, Axitinib, non-steroidal anti-inflammatory drugs (NSAIDS), steroids, antibiotics, 25 pain relievers, calcium-channel blockers, cell cycle inhibitors, chemotherapeutics, anti-viral 30

drugs, anesthetics, hormones, anticancer drugs, antineoplastic agents, viruses, viruses for gene delivery such as AAV, protein binders such as nanobodies, affibodies, ankyrins, DARPs, etc., or any combinations thereof.

[0030] In certain aspects of the present disclosure, the active agent covalently or non-covalently conjugated to at least one of the end groups located at the surface of the hyperbranched macromolecule is a peptide selected from the group consisting of compstatin, APL-1, and Fc-III-4C, Beovu (Brolucizumab), Zimura (Avacincaptad Pegol), Pegcetacoplan, Abicipar Pegol, Lampalizumab, Fovista, Risuteganib, AXT107, Elamipretide, THR149, ALM201, VGB3, and Largazole.

[0031] In certain aspects of the present disclosure, the active agent is covalently bound to at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or up to 100% of the end groups located at the surface of the hyperbranched macromolecule.

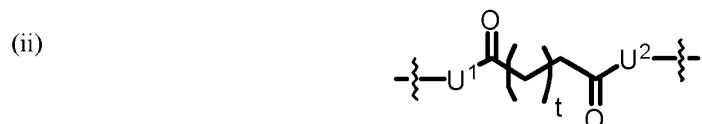
[0032] In certain embodiments of the disclosure, the dendritic constitutional repeating unit is represented by the formula (i):



wherein A is a connection to a polymeric arm that is connected to the core unit, or A is a connection to B of a preceding dendritic constitutional repeating unit represented by Formula (i), L_A is a linker, m is either 0 or 1, n is an integer from 20 to 2000, o is an integer from 20 to 2000, n and o can be different or the same, X is the branch unit such as a polyol derived unit, L_B is a linker, p is either 0 or 1, B comprises an end group located at the surface of the hyperbranched macromolecule or is a connection to A of a consecutive dendritic constitutional repeating unit or a connection to an active agent, L_A and L_B can be different or the same, m and p can be different or the same, and y is an integer from 2 to 9, wherein $y = c - 1$ with c being the connectivity c of the branch unit X; and wherein the dendritic constitutional units in the hyperbranched macromolecules may be the same or different.

[0033] In certain further aspects, A comprises a functional group formed by click chemistry such as a triazole or dihydropyrazine and/or the linker L_A and/or L_B comprise a diacid and/or an acid diamido group, a carboxyl and/or a carboxamide group such as succinate, glutarate, adipate,

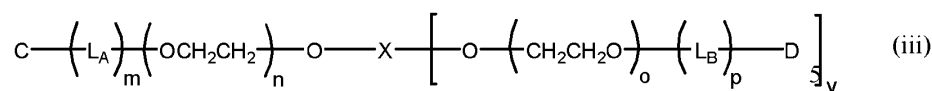
azelate, or glutaramide. In certain embodiments, the linker L_A and/or L_B comprises a structure represented by Formula (ii):



- 5 wherein U^1 and U^2 are independently NH or O and can be the same or different, and wherein t is an integer from 0 to 10. The linker L_A and/or L_B may further comprise a polyethylene glycol unit between the bond to B and the carboxyl group, carboxamide group or structure of Formula (ii).

[0034] In certain embodiments of the disclosure, the present invention provides a method for
 10 manufacturing a hyperbranched macromolecule as described herein by divergent synthesis, comprising the steps of (a) providing a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit having functional groups suitable for click chemistry at the termini of the polymeric arms; (b) providing dendritic constitutional repeating unit
 15 precursors comprising one polymeric arm comprising a functional group suitable for forming a connection by click chemistry with the corresponding functional groups of the polymeric arms connected to the core, (such as an azide, alkyne, alkene or tetrazine), and at least two polymeric arms comprising functional groups not reactive in click chemistry; (c) forming a connection by click chemistry between the polymeric arms connected to the core and the polymeric arms of the
 20 dendritic constitutional repeating unit precursors, (d) optionally converting the functional groups of the at least two polymeric arms comprising functional groups not reactive in click chemistry into functional groups suitable for click chemistry, and (e) conjugating an active agent comprising a functional group to the outermost polymeric arms by reacting with the functional groups of the outermost polymeric arms, thereby forming a hyperbranched macromolecule-active agent conjugate. For a higher generation G_x hyperbranched macromolecule, with x being
 25 an integer of 2 to 10, step (d) can be compulsory and further consecutive dendritic constitutional repeating unit precursors are connected to the functional groups suitable for click chemistry obtained in step (d) by click chemistry to the hyperbranched macromolecule before conjugating the active agent in step (f).

[0035] In certain embodiments of the method, the dendritic constitutional repeating unit precursor in step (c) is represented by the Formula (iii):



wherein C comprises a functional group suitable for click chemistry (such as an alkyne, alkene, azide, or tetrazine), D comprises functional groups not suitable for click chemistry such as succinimidyl, and L_A , m, n, X, o, L_B , p and y are as defined above, and wherein the dendritic constitutional repeating units may be the same or different.

[0036] In a further embodiment of the method, the invention relates to a method for manufacturing a hyperbranched macromolecule as described herein by convergent synthesis, comprising the steps of (I) providing dendritic constitutional repeating unit precursors comprising one polymeric arm comprising a functional group suitable for forming a connection by click chemistry (such as an azide, alkyne, alkene or tetrazine), and at least two polymeric arms comprising functional groups not reactive in click chemistry; (II) conjugating active agents comprising a functional group to at least one of the at least two polymeric arms comprising functional groups not reactive in click chemistry of the dendritic constitutional repeating unit precursors; (III) providing a core unit having at least 3 connectivities c, a plurality of polymeric arms connected to the core unit having functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine) at the termini of the polymeric arms; and (IV) forming a connection by click chemistry between the polymeric arms connected to the core provided in step III) and the polymeric arm comprising a functional group suitable for forming a connection by click chemistry of the active agent conjugated dendritic constitutional repeating unit precursors obtained in step II), thereby forming a hyperbranched macromolecule-active agent conjugate. In embodiments of this method, the dendritic constitutional repeating unit precursor in step I) is represented by the Formula (iii) as described above.

[0037] For a higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, the active agent conjugated dendritic constitutional repeating unit precursors obtained in step II) can be connected by click chemistry to reverse dendritic constitutional repeating unit precursors comprising one polymeric arm comprising a functional group not reactive in click chemistry, and at least two polymeric arms comprising functional groups suitable for click chemistry (such as an

azide, alkyne, alkene or tetrazine), wherein the functional group not reactive in click chemistry of the one polymeric arm is subsequently converted to a functional group suitable for click chemistry before connecting to further reverse dendritic constitutional repeating unit precursors or before forming a connection by click chemistry with the polymeric arms connected to the core in step IV), thereby forming higher generation hyperbranched macromolecules. In an aspect, dendritic constitutional repeating unit precursors having different active agents conjugated to the polymeric arms can be obtained by performing steps I) and II) for each active agent conjugated dendritic constitutional repeating unit precursor, and a mixture of the obtained active agent conjugated dendritic constitutional repeating unit precursors is then used for step IV), thereby forming a hyperbranched macromolecule-active agent conjugate having different active agents at different regions of the surface of the hyperbranched macromolecule.

[0038] In a further embodiment, the invention relates to a hyperbranched macromolecule as described herein, for use as a medicament. In a further embodiment, the invention relates to a method of treatment, wherein the method comprises treating a disease or medical condition in a patient with a hyperbranched macromolecule of embodiments of the invention. In an aspect thereof, the hyperbranched macromolecule is used for an ocular treatment, such as the treatment of an ocular disease such as back-of-the-eye diseases such as any ocular disease of the posterior segment that affects the vasculature and integrity of the retina, macula or choroid leading to visual acuity disturbances, loss of sight or blindness, particularly disease states of the posterior segment resulting from age, trauma, surgical interventions, such as age-related macular degeneration (AMD) cystoid macular edema (CME), diabetic macular edema (DME), posterior uveitis, and diabetic retinopathy.

[0039] In further aspects, the hyperbranched macromolecule is used in the treatment of an ocular disease selected from the group consisting of retinal neovascularization, choroidal neovascularization, Wet AMD, Dry AMD, retinal vein occlusion, diabetic macular edema, retinal degeneration, hyphema, presbyopia, corneal graft rejection, retinoblastoma, melanoma, myosis, mydriasis, glaucoma, conjunctivitis, intraocular infections, choroidal neovascularization (CNV), intraocular tumors, retinal neuroinflammation, inflammation, autoimmune uveitis, uveitis, proliferative vitreoretinopathy, and corneal degeneration, acute and chronic macular neuroretinopathy, central serous chorioretinopathy, macular edema, acute multifocal placoid pigment epitheliopathy, Behcet's disease, birdshot retinochoroidopathy, posterior uveitis,

- posterior scleritis, serpiginous choroiditis, subretinal fibrosis, uveitis syndrome, Vogt-Koyanagi-Harada syndrome, retinal arterial occlusive disease, central retinal vein occlusion, disseminated intravascular coagulopathy, branch retinal vein occlusion, hypertensive fundus changes, ocular ischemic syndrome, retinal arterial microaneurysms, Coat's disease, parafoveal telangiectasis,
- 5 hemi-retinal vein occlusion, papillophlebitis, carotid artery disease (CAD), frosted branch angiitis, sickle cell retinopathy, angioid streaks, familial exudative vitreoretinopathy, Eales disease, proliferative vitreal retinopathy, diabetic retinopathy, retinal disease associated with tumors, congenital hypertrophy of the retinal pigment epithelium (RPE), posterior uveal melanoma, choroidal hemangioma, choroidal osteoma, choroidal metastasis, combined
- 10 hamartoma of the retina and retinal pigmented epithelium, retinoblastoma, vasoproliferative tumors of the ocular fundus, retinal astrocytoma, intraocular lymphoid tumors, myopic retinal degeneration, acute retinal pigment epithelitis, glaucoma, endophthalmitis, cytomegalovirus retinitis, retinal cancers, retinitis pigmentosa, Leber's Congenital Amaurosis, Choroideremia, X-linked retinitis pigmentosa, best vitelliform macular dystrophy, x-linked retinoschisis,
- 15 achromatopsia CNGA3, achromatopsia CNGB3, LHON, Stargardt disease, Usher syndrome, Norrie disease, Bardet-Biedl syndrome, and red-green color blindness.

- [0040] In certain embodiments, the hyperbranched macromolecule is formulated for direct injection at a treatment site of a patient, for example by parenteral administration, intra-tumoral injection, injection into the eye such as intravitreal, intracameral, subconjunctival, retrobulbar,
- 20 sub-tenon, subretinal, or suprachoroidal injections. The hyperbranched macromolecule can be administered by direct injection, by oral application, incorporated in gels, or incorporated in implants.

DEFINITIONS

- [0041] The terms “hyperbranched macromolecule” or “hyperbranched polymer”, or simply
- 25 “branched polymer” or “branched macromolecule” are all interchangeably used herein to designate dendrimer-like branched macromolecules or polymers that have a tree-like structure like dendrimers, and the term “dendrimer” is used herein as a synonym thereof. However, while dendrimers are monodispersed, highly symmetric molecules of exactly defined composition, the hyperbranched macromolecules of the present invention are polydisperse molecules because they
- 30 include polyethylene glycol arms or units that have a certain polydispersity like most synthetic

polymeric structures. Polydispersity of PEG chains and precursor molecules including them may be small, but it infers polydispersity also to dendrimer-like hyperbranched macromolecules as described herein.

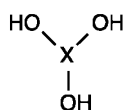
- 5 [0042] Polydispersity is given by the polydispersity index D with $D = M_w/M_n$, where M_w is the weight-average molar mass and M_n is the number-average molar mass, determined by gel permeation chromatography. For most polyethylene glycol (PEG) materials, the polydispersity index is a parameter given in product specifications by the manufacturer, as an indicator of uniformity and quality of the material. Polydispersity of PEG multi-arm precursors can be less than 1.3, less than 1.2, less than 1.1.
- 10 [0043] The term “biodegradable” refers to a material or object (such as the hyperbranched macromolecules according to the present invention) which becomes degraded in vivo, i.e., when placed in the human or animal body or in vitro when immersed in an aqueous solution under physiological conditions such as pH 7.2-7.4 at 37 °C. In the context of the present invention, as disclosed in detail herein below, the hyperbranched macromolecules once administered or
- 15 deposited in the human or animal body slowly biodegrade and are cleared over time. In certain embodiments, biodegradation takes place at least in part via ester hydrolysis in the aqueous environment of the body. Biodegradation may take place by hydrolysis or enzymatic cleavage of the covalent connection or conjugation bonds, in linker groups and/or within the polymer arms. The hyperbranched macromolecules slowly disintegrate, resulting in clearance through
- 20 physiological pathways. In certain embodiments, the hyperbranched macromolecules of the present invention are degradation stable over extended periods of time (e.g., about 1 month, 3 months or 6 months). In certain embodiments, the hyperbranched macromolecules only biodegrade, e.g., until after the active agent or at least a major amount (e.g., at least 50%, at least 75% or at least 90%) thereof has been released therefrom.
- 25 [0044] The terms “precursor” or “component” or “building block” herein refers to those molecules or compounds that are reacted with each other and that are thus connected via covalent bonds to form a hyperbranched macromolecule.

- [0045] The parts of the precursor molecules that are still present in a final dendrimer-like hyperbranched macromolecules are also called “units” or “polymeric arms” herein. The “units”
- 30 or “polymeric arms” thus belong to the main building blocks or constituents of a polymeric

hyperbranched macromolecule. For example, a hyperbranched macromolecule suitable for use in the present invention may contain identical or different polyethylene glycol units or arms, in addition to core units and branch units as further disclosed herein.

[0046] The term “core unit” as used herein refers to a constitutional unit in the center of a hyperbranched macromolecule, from which the polymeric arms or dendritic constitutional repeating units (DCRU) or dendrons emanate. The core unit has at least 3 connectivities c (or valences) to each of which a polymeric arm or a dendritic constitutional repeating unit is connected, i.e., covalently bound. For example, for a multi-arm PEG branched macromolecule of generation $G0$ the core unit may be derived from a polyol compound, which is poly(ethoxylated) on each of its hydroxyl groups.

[0047] An exemplary core unit structure with three connectivities with the connectivities c shown as OH is shown below:



[0048] The terms “branch unit” or “branch point” used herein refers to a constitutional unit within a dendritic constitutional repeating unit with at least 3 connectivities c' (or valences) to each of which a polymeric arm or another dendritic constitutional repeating unit is connected. The branch unit can have the same or different chemical structure as the core unit.

[0049] The term “dendritic constitutional repeating unit” (DCRU), sometimes referred to as “dendron”, as used herein refers to a constitutional repeating unit of connectivity $c' \geq 3$, including a branch point and polymeric arms emanating from it. It may be connected to a total of c polymeric arms emanating from the core unit and/or other DCRU's consecutively to form a dendrimer-like hyperbranched structure.

[0050] The term “end-group” as used herein refers to a constitutional unit, for example a functional group, which is located at an extremity of a polymeric arm or DCRU. In a hyperbranched macromolecule, the end groups at the outermost surface of the hyperbranched macromolecule can be used for conjugating or bonding active agent molecules to the

hyperbranched macromolecule. The end-group may consist of a linker having hydrolyzable groups connected to a terminal functional group.

[0051] The term “generation”, abbreviated “*G*”, refers to the set of dendritic constitutional repeating units separated from the free valence of a dendron by the same number of dendritic constitutional repeating units.

[0052] The term “dendron” as used herein refers to a part of the hyperbranched macromolecule with only one free valence, comprising exclusively DCRU’s and end-groups, and in which each path from the free valence to any end-group comprises the same number of constitutional repeating units.

10 [0053] The term “conjugated” as used herein includes covalent or non-covalent binding of an active agent to the hyperbranched macromolecule. Conjugation comprises non-covalent binding, such as to a hyperbranched macromolecule end group with affinity for the active agent molecule, which may also be a means of linking to an active agent molecule to the hyperbranched macromolecule.

15 [0054] The term “release” (and accordingly the terms “released”, “releasing” etc.) as used herein refers to the chemical separation and provision of active agents from the hyperbranched macromolecules of the present invention to the surrounding environment. The released agents may or may not have molecular fragments of the hyperbranched macromolecule still bound to them. The surrounding environment may be an *in vitro* or *in vivo* environment as described
20 herein. In certain specific embodiments, the surrounding environment is the vitreous humor and/or ocular tissue, such as the retina and the choroid. The linkage of the API to the hyperbranched macromolecule can be a covalent linkage, wherein the API can be detached from the hyperbranched macromolecule by a chemical event such as hydrolysis of a linkage formed by a linker group. Furthermore, multiple hydrolyzable linkage chemistries may be employed to
25 release the API at multiple rates from the same or comingled hyperbranched macromolecules to achieve a desired release profile. In addition, the hyperbranched macromolecule may be functionalized with end groups that bind an API non-covalently, releasing the API according to the binding affinity kinetics of the hyperbranched macromolecule end group-API pair. Multiple non-covalently bound end group-API pairs can be employed to achieve a desired release profile.

[0055] The term “100% release of the active agent” should be construed as from 95% to 100%. The way this controlled release is achieved is by a number of parameters that are characteristics of the drug-delivery system as disclosed herein. Each such characteristic feature of the drug-delivery system alone or in combination with each other can be responsible for the controlled release.

[0056] The term “sustained release” for the purposes of the present invention is meant to characterize products such as biodegradable hyperbranched macromolecules, which are formulated to make an active agent available over an extended period of time, thereby allowing a reduction in dosing frequency compared to an immediate release dosage form, such as for example a solution of an active agent that is topically applied onto the eye (i.e. eye drops). Other terms that may be used herein interchangeably with “sustained release” are “extended release” or “controlled release”. Within the meaning of the invention, the term “sustained release” comprises constant active agent release, tapered active agent release, ascending active agent release as well as any combination thereof such as a constant active agent release followed by a tapered active agent release. Within the meaning of the invention, the term “tapered”, or “tapering” refers to a decrease of active agent release over time. Specifically, the term “sustained release” refers to release of an active agent from the hyperbranched macromolecules or drug-delivery system including them in a predetermined way and is in contrast to an immediate release like a bolus injection. In certain embodiments, the controlled release refers to the amount of the active agent release over the total number of days required for 100% release of the active agent in an aqueous solution under *in-vitro* physiological conditions such as at pH 7.2-7.4 and 37 °C.

[0057] The term “extended period of time” as used herein refers to any period of time that would be considered by those of ordinary skill in the art as being extended with respect to treating a disease, and in particular refers to periods such as at least about 1 week, or at least about 1 month or longer, such as up to about 12 months, or any intermediate periods such as about 1 to about 6 months, about 2 to about 4 months, about 2 to about 3 months or about 3 to about 4 months or as otherwise disclosed herein.

[0058] A “zero order” release or “substantially zero order” release or “near zero order” release is defined as exhibiting a relatively straight line in a graphical representation of percent of the active agent released versus time. In certain embodiments of the present invention, substantially

zero order release is defined as the amount of the active agent released which is proportional within 20% to elapsed time.

- [0059]** The terms “API”, “active (pharmaceutical) ingredient”, “active (pharmaceutical) agent”, “active (pharmaceutical) principle”, “(active) therapeutic agent”, “active”, and “drug” are used interchangeably herein and refer to the substance used in a finished pharmaceutical product (FPP) as well as the substance used in the preparation of such a finished pharmaceutical product, intended to furnish pharmacological activity or to otherwise have direct effect in the diagnosis, cure, mitigation, treatment or prevention of a disease, or to have direct effect in restoring, correcting or modifying physiological functions in a patient.
- [0060]** The active agent used according to the present invention may be an active agent for the treatment and/or prevention of a disease or disorder, or a diagnostic agent such as a marker. In an embodiment of the invention, the active agent is a low water solubility active agent (i.e., having a solubility in water of less than about 1000 µg/mL or less than about 100 µg/mL). In other embodiments of the invention, the active agent is a highly water-soluble active agent (i.e., having a solubility in water of greater than about 1000 µg/mL or even greater than 10 mg/mL). This definition is not dependent on the agent being approved by a governmental agency.

- [0061]** For the purposes of the present invention, an active agent in all its possible forms, including free acid, free base, polymorphs or any pharmaceutically acceptable salts, anhydrides, hydrates, co-crystals, or other solvates or derivatives, such as pro-drugs or conjugates, can be used. For conjugating to the hyperbranched macromolecule, the active agent may need to be functionalized, unless it already comprises a suitable functional group for conjugation. Whenever in this description or in the claims an active agent is referred to without further specification, even if not explicitly stated, it also refers to the active agent in the form of any such polymorphs, pharmaceutically acceptable salts, anhydrides, or solvates (including hydrates) thereof. With respect to the active agent, suitable solid forms include without limitation the pure substance form in any physical form known to the person of ordinary skill in the art.

- [0062]** As used herein, the term “therapeutically effective” refers to the amount of active agent needed to produce a desired therapeutic result after administration. For example, in the context of the present invention, one desired therapeutic result would be the reduction of symptoms associated with dry eye disease (DED), e.g., as measured by *in vivo* tests known to the person of

ordinary skill in the art, such as an increase of a Schirmer's tear test score, a reduction of Staining values as measured by conjunctival lissamine green staining or corneal fluorescein staining, a reduction of the eye dryness severity and/or eye dryness frequency score on a visual analogue scale (VAS), a reduction of the Ocular Surface Disease Index and/or the Standard Patient Evaluation of Eye Dryness score as well as a reduction of the best corrected visual acuity. In one embodiment, "therapeutically effective" refers to an amount of active agent in a sustained release intracanalicular insert capable of achieving a tear fluid concentration which is equivalent in terms of therapeutic effect to a cyclosporine concentration of 0.236 µg/mL (which is considered to be required for immunomodulation, Tang-Liu and Acheampong, Clin. Pharmacokinet. 44(3), pp. 247-261)) over an extended period of time and in particular over substantially the whole remaining wearing period of the insert once said tear fluid concentration is achieved.

[0063] The term "patient" herein includes both human and animal patients. The biodegradable drug-delivery systems according to the present invention are therefore suitable for human or veterinary medicinal applications. Generally, a "subject" is a (human or animal) individual to which a drug-delivery systems according to the present invention is administered. A "patient" is a subject in need of treatment due to a particular physiological or pathological condition. A "patient" does not necessarily have a diagnosis of the particular physiological or pathological condition prior to receiving the drug-delivery system.

[0064] The molecular weight of a hyperbranched macromolecule, polymer precursor, polymer unit, arm or the like as used for the purposes of the present invention and as disclosed herein may be determined by analytical methods known in the art. The molecular weight of polyethylene glycol may for example be determined by any method known in the art, including gel electrophoresis such as SDS-PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis), gel permeation chromatography (GPC), including GPC with static light scattering detectors (SLS) or dynamic light scattering (DLS), liquid chromatography (LC), as well as mass spectrometry such as matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) spectrometry or electrospray ionization (ESI) mass spectrometry. The molecular weight of a polymer, including a polyethylene glycol precursor as disclosed herein, is an average molecular weight (based on the polymer's molecular weight distribution), and may therefore be indicated by means of various average values, including the weight average molecular weight

(Mw) and the number average molecular weight (Mn). In the case of the multi-arm PEG precursors as used in some aspects of the present invention, the molecular weight indicated herein is the number average molecular weight (Mn) determined by gel permeation chromatography using a suitable molecular weight standard, such as a polyethylene glycol or polystyrene standard, according to standard methods known in the art. Typically, the materials, especially the multi-arm precursors are purchased with a specified molecular weight and polydispersity defined by the vendor. Suitable PEG precursors are for example available from a number of suppliers, such as Jenkem Technology, Xiamen SinoPeg Biotech Co. Ltd., and others.

[0065] The term “day 1” as used herein refers to a time point that immediately follows after “day 0”. Thus, whenever “day 1” is used, it refers to an already elapsed time period of one day or about 24 hours after administration of the drug-delivery system.

[0066] As used herein, the term “about” in connection with a measured quantity, refers to the normal variations in that measured quantity, as expected by one of ordinary skill in the art in making the measurement and exercising a level of care commensurate with the objective of measurement and the precision of the measuring equipment.

[0067] The term “at least about” in connection with a measured quantity refers to the normal variations in the measured quantity, as expected by one of ordinary skill in the art in making the measurement and exercising a level of care commensurate with the objective of measurement and precisions of the measuring equipment and any quantities higher than that.

[0068] The term “average” as used herein refers to a central or typical value in a set of data(points), which is calculated by dividing the sum of the data(points) in the set by their number (i.e., the mean value of a set of data).

[0069] As used herein, the singular forms “a,” “an,” and “the” include plural references unless the context clearly indicates otherwise.

[0070] The term “and/or” as used in a phrase such as “A and/or B” herein is intended to include both “A and B” and “A or B”.

[0071] Open terms such as “include,” “including,” “contain,” “containing” and the like mean “comprising.” These open-ended transitional phrases are used to introduce an open-ended list of

elements, method steps, or the like that does not exclude additional, unrecited elements or method steps.

[0072] The term “up to” when used herein together with a certain value or number is meant to include the respective value or number.

- 5 [0073] The terms “from A to B”, “of from A to B”, and “of A to B” are used interchangeably herein and all refer to a range from A to B, including the upper and lower limits A and B.

[0074] Throughout this disclosure, various aspects of this invention are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention.

- 10 Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6, etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This
- 15 applies regardless of the breadth of the range. Numeric ranges recited are inclusive of the numbers defining the range and include each integer within the defined range.

[0075] The abbreviation “PBS” when used herein means phosphate-buffered saline.

[0076] The abbreviation “PEG” when used herein means polyethylene glycol.

BRIEF DESCRIPTION OF THE DRAWINGS

- 20 **Figure 1** schematically illustrates **a)-c)** different synthesis methods of dendrimers or hyperbranched macromolecules.

Figure 2 schematically illustrates the generations of a dendrimer or hyperbranched macromolecule.

- Figure 3** shows **a)** a schematic image of PEG hyperbranched macromolecule formation via
- 25 **DBCO-azide coupling**; and **b)** schematically illustrates a 3-D model of an 8-arm PEG core with eight 4-arm PEG branched repeating units and conjugated with peptides.

Figure 4 illustrates the structure of peptides compstatin, APL-1 (Mod2) and APL-1 (Mod3).

Figure 5 illustrates a purification setup by **a)** dialysis, and **b)** SEC column filtration of Examples 6 and 7.

Figure 6 is a diagram of a UHPLC analysis of hyperbranched macromolecule-compstatin conjugates purified by dialysis of Example 6.

Figure 7 is a diagram of a UHPLC analysis of hyperbranched macromolecule-compstatin conjugates purified by SEC column filtration of Example 7.

Figure 8 is a UHPLC graph of **a)** compstatin sample standard curve (12.5, 25, 50, 100, 200 µg/mL) with calibration plot, and **b)** a 4arm G0 hyperbranched macromolecule-compstatin conjugate of Example 1.

Figure 9 shows a graph of substitution rate comparison of different linker PEGs with compstatin, and compstatin-lysine based on Example 8.

Figure 10 is a graph of optimization conditions of compstatin conjugation from Example 8.

Figure 11 illustrates SPR results of Example 9: C3 binding: **a1-4)** KD of 4 compstatin samples from different vendors; **b)** equilibrium analysis.

Figure 12 illustrates SPR results of Example 9: C3b binding: **a1-4)** KD of 4 compstatin samples from different vendors; **b)** equilibrium analysis.

Figure 13 illustrates SPR results of Example 9: C3 and C3b binding: **a)** APL-1 (amine acetylated), **b)** APL-1 (amine, acetate salt), **c)** APL-1 (lysine end).

Figure 14 illustrates SPR results of IgG binding: **a-c)** Fc-III 4C, and **d)** Fc-III.

Figure 15 illustrates an SPR comparison of free compstatin and multi-valency compstatin.

Figure 16 illustrates SPR results of **a)** 8a-40k-PEG-[(4a-2kPEG-(comp)₃]₈, **b)** 4a-40kPEG-SGA-(comp)₄, **c)** 4a-40kPEG-SS-(comp)₄, and **d)** SS-comp (hydrolysis) compared to free compstatin.

Figure 17 is a plot of the dissociation constant K_D of hyperbranched macromolecule-compstatin conjugates against the corresponding number of peptide substitution.

Figure 18 is an illustration of an alternative pathway (AP) hemolysis assay.

Figure 19 AP hemolysis results of IC_{50} for hyperbranched macromolecule-compstatin
5 conjugates.

Figure 20 is a calibration curve of the hydrodynamic radius R_h versus the half-life $T_{1/2}$ determined in New Zealand White Rabbit Vitreous Humor for predicting sustained release of dendrimer drug conjugates.

Figures 21 a) to c) show the degradation effect of temperature variation from 35 °C to 39°C
10 at constant pH of 7.4.

Figures 22 a) to c) show the degradation effect of pH variation from pH 7.0 to pH 8.5 to at constant temperature of 37°C.

DETAILED DESCRIPTION OF THE INVENTION

15 **[0077]** The present invention is directed, in certain aspects, to hyperbranched macromolecules (dendrimers) comprising polyethylene glycol polymer units and an active agent covalently bound or conjugated to at least one of the outermost arms of the hyperbranched macromolecule. Conjugation comprises covalent binding and non-covalent binding, such as to a peptide hyperbranched macromolecule end group with affinity for the active agent molecule, and this
20 may also be a means of linking an active agent to the hyperbranched macromolecule.

[0078] Dendrimers are monodisperse macromolecules with several reactive end groups at their surface. Dendrimers are often compared with tree-like structures, i.e., a branched molecular architecture providing a large variety of possible terminal groups and extraordinary structural control. Elements are added to a dendrimer structure by a chemical reaction series and build a
25 branching spheroidal structure from a starting atom or core unit. The central core unit has at least two or at least three reactive functional groups, and the repeated branches are organized in a series of “radially concentric layers” called “generations”. Hyperbranched macromolecules can

have the same molecular architecture as dendrimers without being monodisperse, as they can be built by using polydisperse precursors or units.

[0079] The inventors have found that strategies for optimizing drug delivery and site-specific targeting using dendrimer-like hyperbranched macromolecules as described herein provide several advantages in drug delivery and can utilize advantages of dendrimers also for hyperbranched macromolecules that have a similar structure as dendrimers, without being monodisperse molecules. For example, dendrimer-like hyperbranched macromolecules provide various terminal functionalities that can be used to adjust the hydrophobicity/hydrophilicity of the hyperbranched macromolecule used as a carrier for an active agent, or it can be used as conjugation precursor to target molecules to enhance the interaction between API and hyperbranched macromolecule, for example by multivalent binding to receptors and/or improvement of avidity of conjugated biomolecules such as peptides or proteins.

[0080] The multiple number of surface groups on hyperbranched macromolecules can anchor more API with desired bonding methods and achieve controlled release via different degradation conditions or degradation kinetics. Hyperbranched macromolecule-drug conjugates can enhance stability and solubility of the therapeutics to be delivered and reduce systemic effects and increase efficacy at the targeted site compared with free drugs. Further, a hyperbranched macromolecule may have a symmetric structure which provides numerous intramolecular cavities to trap unbound API molecules. Additionally, the large outer hydration radius of specifically PEG based dendrimer structures extends the half-life in vivo, such as in the vitreous body, of the dendrimer drug conjugates, which can be used for controlling and adjusting sustained release of active agents.

[0081] Furthermore, biodegradable synthetic dendrimers offer the advantage of built-in controllably degradable functional groups, such as hydrolyzable or enzymatically cleavable bonds, which upon degradation yield smaller molecular weight fragments with lower radius of hydration and a different half-life which dictates their clearance from the body. The built-in degradable groups can be used to tailor and control the release rate of active agents associated with the dendrimer.

Hyperbranched macromolecules

[0082] In certain aspects of the present invention, a hyperbranched macromolecule is provided that is formed from several building blocks of the dendritic structure (not including the active agent), such as the core unit, polymeric arms, branch unit, linkers and/or extenders, and dendritic constitutional repeating units. In an embodiment, a hyperbranched macromolecule is provided comprising a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit at the connectivities c , each polymeric arm comprising an end group or being connected to a dendritic constitutional repeating unit comprising a branch unit connected to at least two polymeric arms each comprising an end group or being connected to a next dendritic constitutional repeating unit that may again be connected to further dendritic constitutional repeating units, the polymeric arms of the outermost dendritic constitutional repeating unit each comprising an end group; wherein the polymeric arms comprise or consist of linear polyethylene glycol (PEG) units; and wherein at least one active agent is conjugated to at least one of the end groups located at the outermost polymeric arms of the hyperbranched macromolecule. The hyperbranched macromolecule includes chemical bonds that can be cleaved by hydrolysis, rendering the hyperbranched macromolecule biodegradable in aqueous environments.

[0083] In embodiments the hyperbranched molecule is formed from building blocks at least partially connected by hydrolyzable bonds or connections located at positions such that a complete hydrolysis of all hydrolyzable bonds in the macromolecule produces hydrolysis fragments that each have a molecular weight of less than 40 kDa. This may be achieved by selection of suitable building blocks or precursors having a molecular weight of less than 40 kDa, and connecting them via hydrolyzable, typically acid labile, chemical bonds such as esters or amide bonds as further described herein. For example, the inclusion of diacid linkers to connect constitutional repeating units each having a molecular weight of less than 40 kDa allows hydrolytic degradation producing hydrolysis fragments that each meet the desired molecular weight limit. If non hydrolyzable connections are used, for example bonds formed by some click chemistry reactions such as alkyne-azide coupling, they should be located between building blocks that together meet the molecular weight limit for hydrolysis fragments of less than 40 kDa.

[0084] In certain aspects of the invention, the overall molecular size and number of surface groups of the hyperbranched macromolecules gradually increase with the addition of successive

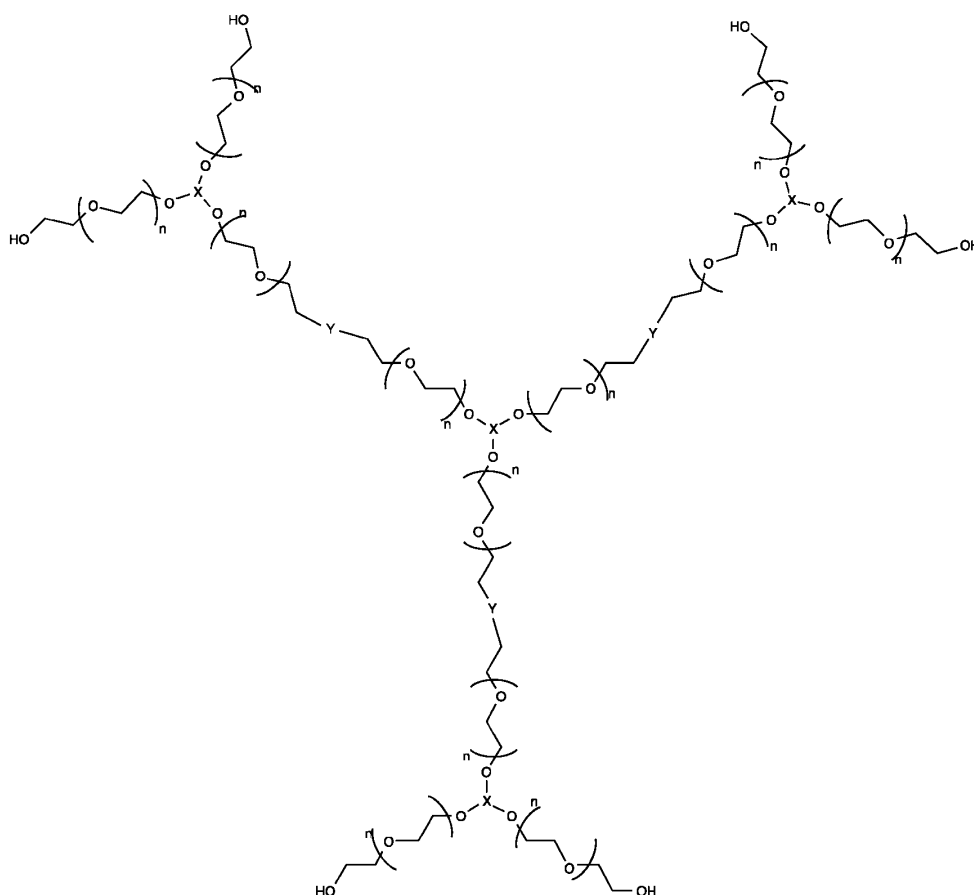
layers of monomers which is called a generation. The biodegradable hyperbranched macromolecules can be synthesized by divergent or convergent synthesis, or a combination of both, see FIG. 1. The divergent method involves addition of monomers or so-called dendritic constitutional repeating units (DCRU) in repeated sequence and starts from a multivalent core to surface molecules with continuous enhancement in the number of branching. The molecular size and number of surface groups gradually increase with the addition of successive layers of monomers which is called generations. While the convergent method involves the synthesis of hyperbranched macromolecules from the surface to core and leads to the formation of conical wedge-shaped units or dendrons, these are joined to a multivalent core at the last step.

- 10 [0085] The hyperbranched macromolecules of certain embodiments of the present invention include as principal building units a core unit and optionally a plurality of branch units that may be derived from polyols, a plurality of polymeric arms comprising polyethylene (PEG) units, optional hydrolyzable linker groups, connection groups between building units, end-groups and conjugated active agents such as, e.g., peptides. All these constitutional elements or building units are further described herein below.

- [0086] Connections formed between different polymeric arms in the hyperbranched macromolecules may include hydrolyzable bonds by introduction of suitable linker groups between the PEG arms and the functional groups for connecting the different units to form the hyperbranched macromolecule. Such linkers forming hydrolyzable bonds facilitate biodegradation in aqueous environments such as the human or animal body *in vivo*. For example, the hydrolyzable chemical bonds may be acid-labile, to facilitate cleavage in more acidic environments that may be found for example within tumors at a cellular level.

- [0087] The hydrolyzable chemical bonds can include bonds or linkages selected from the group consisting of amine, amide, urethane, ester, anhydride, ether, acetal, ketal, nitrile, isonitrile, isothiocyanate, or imine bonds, and combinations thereof. These bonds are typically formed by condensation reactions or click chemistry of suitably functionalized precursors during synthesis of the hyperbranched macromolecule. In certain preferred embodiments, the hydrolyzable bonds are ester bonds, such as ester bonds formed by using diacid linkers such as succinic acid, glutaric acid, adipic acid and higher homologues.

[0088] An exemplary structure of a hyperbranched macromolecule of certain embodiments of the invention is shown below:



[0089] In this Formula of a generation *G1* hyperbranched macromolecule, X designates a core unit, or a branch unit derived from a polyol, such as glycerol, the core or branch unit being each
 5 connected to three polyethylene glycol arms, and the branch units are connected with one of their polyethylene glycol arms via a linker group Y to the polyethylene glycol arms of the core unit in the center. The linker Y comprises hydrolyzable bonds such as ester or amide bonds as further defined herein. n designates the number of polyethylene glycol repeating units in the polymeric arm.

[0090] The building blocks of which the hyperbranched macromolecules are formed comprise a core unit, polymeric arms, such as arms consisting of polyethylene glycol (PEG), bifunctional linking groups or linkers, bifunctional extenders, dendritic constitutional repeating units that comprise a branch unit, functional end groups. In embodiments of the invention, the building blocks have an average molecular weight (M_n) of less than 50,000 Daltons, such as less than 45,000 Daltons, or less than 40,000 Daltons, or less than 35,000 Daltons, or less than 30,000 Daltons.

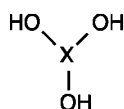
Core unit

[0091] The core unit is the centre of the hyperbranched macromolecule from which the polymeric arms or dendritic constitutional repeating units (DCRU) or dendrons emanate. The core unit has at least 3 connectivities c (or valences) to each of which a polymeric arm or a dendritic constitutional repeating unit is connected, i.e., covalently bound. The polymeric arms may be bonded by a hydrolyzable bond to the core unit, are, preferably by non-hydrolyzable bonds such as ether bonds.

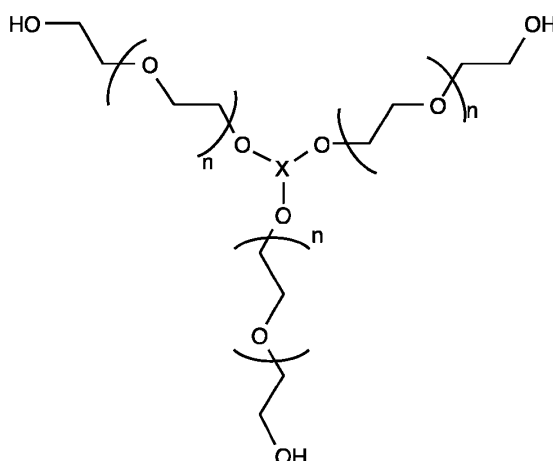
[0092] In certain embodiments, the core unit has a connectivity c of 3 to 10, or 4 to 8, or 4 to 6, or 4. The core unit may be derived from a molecule or a chemical structure having a number of c functional groups to which polymeric arms are bound. For example, in certain embodiments the core unit is derived from a polyol having at least 3 hydroxyl groups, or 4, 5, 6, 7, 8, 9 or 10 hydroxyl groups.

[0093] In such embodiments, the polyol can be selected from glycerol, pentaerythritol, xylitol, dipentaerythritol, tripentaerythritol, hexaglycerol, isomalt, lactitol, maltitol, mannitol, or sorbitol. In certain embodiments, the core unit derived from a polyol is ethoxylated at each of its hydroxyl groups to form a multi-arm precursor with the arms being polymeric PEG arm that are endcapped with an end-group or functional group.

[0094] An exemplary core unit structure with three connectivities may be depicted by the following formula, with the connectivities c shown as OH:



[0095] The core unit of a multi-arm precursor that can be used to form the hyperbranched macromolecule of certain embodiments of the present invention is thus a structure appropriate to provide the desired number of arms of the precursor. For example, for 4-arm precursors, the core unit can be a pentaerythritol or ethylenediamine structure, whereas for 8-arm precursors, the core unit can be a hexaglycerol structure. In certain embodiments of the invention, the core unit is pegylated at its connectivities *c* with polyethylene glycol arms such as in the structure depicted below. The connectivities at the end are again shown as OH groups, where a linker, functional group or another DCRU can be connected.



10

[0096] The connectivities or end groups at the end are again shown as OH groups, where a linker, functional group, extender, or another DCRU can be connected for further hyperbranched macromolecule generations.

[0097] In embodiments of the invention, a building block consisting of a multi-arm PEG precursor as further defined below is used, derived from ethoxylated polyol core units, and these multi-arm PEG precursor building blocks have an average molecular weight (M_n) of less than

15

50,000 Daltons, such as less than 45,000 Daltons, or less than 40,000 Daltons, or less than 35,000 Daltons, or less than 30,000 Daltons.

Generations

[0098] Like in dendrimers, the sequence of the constitutional repeating units in the
5 hyperbranched macromolecules of the invention can be designated by generations. In certain
embodiments, the hyperbranched macromolecule may be a *G0* hyperbranched macromolecule,
or a *G1* to *G10* hyperbranched macromolecule, such as a *G1*, *G2*, *G3*, *G4* or *G5* hyperbranched
macromolecule, in general terms a *Gx* hyperbranched macromolecule, with *x* being an integer of
1 to 10. The abbreviation *G* refers to generation, and the number designates the overall number
10 of dendritic constitutional repeating units that are consecutively bonded to each other in a row.

[0099] As shown in FIG. 2, branching and the number of outermost end groups increases from
generation to generation.

[0100] In an exemplary embodiment, the hyperbranched macromolecule is a *G0* branched
macromolecule wherein the end groups located at the surface of the hyperbranched
15 macromolecule are the end groups of the polymeric arms connected to the core unit. The *G0*
branched macromolecule may also be described as a multi-arm PEG molecule with an active
agent covalently bound to at least one of its arms.

[0101] In another exemplary embodiment, the hyperbranched macromolecule is a higher
generation *Gx* hyperbranched macromolecule, with *x* being an integer of 1 to 10 defining the
20 number of consecutively connected dendritic constitutional repeating units in the hyperbranched
macromolecule, the polymeric arms of the outermost dendritic constitutional repeating unit each
comprising an end group, wherein the at least one active agent is conjugated to at least one of the
outermost polymeric arm.

[0102] Exemplary embodiments of the present invention include *G1* to *G10* hyperbranched
25 macromolecules, such as *G1* to *G8*, *G1* to *G6*, or *G1* to *G4*, such as *G1*, *G2*, *G3* or *G4*
hyperbranched macromolecules.

[0103] The same or different DCRU's can be used for forming different generations in a
hyperbranched macromolecule, such as DCRU's having different molecular weights (due to
different PEG arm lengths) or different number of arms. Further, different generation DCRU

within a hyperbranched macromolecule may be connected to each other using the same linker and functional groups or with different linkers and functional groups, e.g., for controlling the degradation rate at different junctions within the hyperbranched macromolecule.

Branch units

5 [0104] Branch units can be selected from the same chemical entities as core units described above. Like the core unit, a branch unit is a branched chemical structure including a branching point and a plurality of connectivities.

[0105] While the core unit is in the center of the hyperbranched macromolecule and only occurs once in the hyperbranched macromolecule, a branch unit occurs within the dendritic constitutional
10 repeating units (DCRU) of the hyperbranched macromolecule. A G_0 hyperbranched macromolecule includes a core unit but no branch unit. A higher generation hyperbranched macromolecule of generation G_x includes a plurality of branch units. The branch units in a hyperbranched macromolecule may have the same chemical structure as the core unit or may be different. For example, the core unit of a G_1 hyperbranched macromolecule may be derived from
15 pentaerythritol with a connectivity $c = 4$, and the hyperbranched macromolecule may include 4 DCRU with branch units each derived from glycerol with a connectivity of $c' = 3$, so that the G_1 hyperbranched macromolecule overall has 8 terminal (outermost) end groups. If the 4 DCRU's have a branch unit also derived from pentaerythritol, like the core unit, the hyperbranched macromolecule will have a total of 12 outermost end groups.

20 **Polymeric arms**

[0106] In certain embodiments, the polymeric arms of the hyperbranched macromolecules are made of polyethylene glycol (PEG) polymer units. In a generation G_0 branched macromolecule, the polymeric arms are connected to the core unit, for example via ether bonds, and have terminal end groups located at the surface of the branched macromolecule. At least to some of
25 these terminal end groups the active agent is covalently bound. In a higher generation G_x hyperbranched macromolecule, the polymeric arms additionally occur in consecutively connected dendritic constitutional repeating units.

[0107] Thus, in some embodiments, the polymeric arms comprised in the hyperbranched macromolecule are made of or include at least one polyethylene glycol unit. Polyethylene glycol

(PEG, also referred to as polyethylene oxide) refers to a polymer with a repeating group $(\text{CH}_2\text{CH}_2\text{O})_n$, with n being at least 3.

5 [0108] A polymeric arm having a polyethylene glycol thus has at least three of these repeating groups connected to each other in a linear series. The PEG polymeric arm terminates in an end group such as a nucleophile or electrophile, a dibenzocyclooctyne (or other strained alkyne), a strained alkene, a tetrazine or an azide, and can be used for conjugation with an active agent or for connecting with a precursor of a DCRU to build up the next generation hyperbranched macromolecule.

10 [0109] The polymeric arms may comprise PEG units having an average molecular weight (M_n) in the range from about 1,000 to about 100,000 Daltons, or from about 10,000 to about 60,000 Daltons, or from about 15,000 to about 50,000 Daltons.

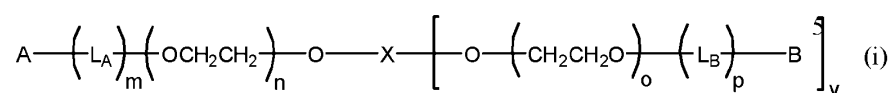
[0110] In certain embodiments, the average molecular weight (M_n) of the polymeric arm PEG units attached to the core can be the same or different than that of the polymeric arms in the dendritic constitutional repeating units. For example, the average molecular weight of the
15 polymeric arm PEG units attached to the core can be higher or lower than that of the polymeric arms in the dendritic constitutional units. In an aspect thereof, for a higher generation G_x biodegradable hyperbranched macromolecule, with x being an integer of 2 to 10, the average molecular weight of the polymeric arm PEG units may decrease or increase from the innermost polymeric arms to the outermost polymeric arms. For example, the polymeric arms connected to
20 the core unit may have a large molecular weight, and that of DCRU's may have a shorter molecular weight, or vice versa. Molecular weight of the polymeric arms may also vary from generation to generation DCRU. As an example, a G_2 hyperbranched macromolecule having 24 outermost conjugation sites may be constructed from a 4arm 40k PEG core attached to four 4arm 20k PEG DCRU's, that may be again connected to twelve 3-arm 30k PEG. K in this context
25 refers to kilo Daltons (kDa), so a 4 arm 40k PEG has 4 polymeric PEG arms and a total molecular weight of 40 kDa.

Dendritic constitutional repeating units

[0111] A dendritic constitutional repeating unit (DCRU) is a partial structure within the hyperbranched macromolecule of higher generations G_x as defined herein, having a connectivity
30 $c' \geq 3$, and including a branch point and polymeric arms emanating from it. It may be connected

to a total of c' polymeric arms emanating from the core unit and/or other DCRU's consecutively to form a tree-like dendrimeric structure.

[0112] In certain embodiments, the dendritic constitutional repeating unit in the dendrimer can be represented by the general Formula (i):



[0113] Within this Formula (i), A is a connection to a polymeric arm that is connected to the core unit, or A is a connection to B of a preceding dendritic constitutional repeating unit represented by Formula (i), L_A is a linker group, m is either 0 or 1 meaning that the linker may be absent or present, n is an integer from 3 to 2000, or 20 to 2000, o is an integer from 3 to 2000, or 20 to 2000, while n and o can be different or the same, X is a branch unit, L_B is a linker group, p is either 0 or 1 meaning that the linker may be absent or present, B comprises an end group located at the surface of the hyperbranched macromolecule or is a connection to A of a consecutive dendritic constitutional repeating unit or a connection to an active agent, L_A and L_B can be different or the same, m and p can be different or the same, and y is an integer from 2 to 9, wherein $y = c' - 1$ with c' being the connectivity c' of the branch unit X. In certain embodiments of the hyperbranched macromolecule including DCRU's of Formula (i), the connection between A and B may comprise a functional group formed by click chemistry, such as a triazole or dihydropyrazine.

[0114] The branch unit may be derived from a polyol, such as glycerol, pentaerythritol, xylitol, dipentaerythritol, tripentaerythritol, hexaglycerol, isomalt, lactitol, maltitol, mannitol, or sorbitol. In Formula (i) above, the branch unit is ethoxylated at all its connectivities c , so it is connected via an ether linkage to one PEG polymeric arm connected to A, optionally via linker group L_A , and y PEG polymeric arms each connected to B, optionally via a linker group L_B . The same or different DCRU's can be used in a hyperbranched macromolecule such as DCRU's having different molecular weights (due to different PEG arm lengths) or different number of arms.

Linker groups

[0115] Inclusion of linker groups that are labile to hydrolysis into the dendritic constitutional unit allow a biodegradation of the hyperbranched macromolecule under physiological conditions.

The high molecular weight hyperbranched macromolecule conjugate can be degraded into small constitutional unit having low molecular weight and can be cleared from the body by usual physiological pathways.

[0116] In certain embodiments of the hyperbranched macromolecule including DCRU's of Formula (i), the linker groups L_A and/or L_B comprise a dicarboxyl and/or a carboxamide moiety or combinations thereof of varying chain length, and these may be derived from diacid groups such as succinate, glutarate, adipate, azelate, or an acid diamido group such as glutaramide. These groups can be connected to the PEG polymeric arms, A, and/or B via ester or amide bonds that are hydrolyzable under physiologic conditions *in vivo* at different rates, depending on the acid chain length. In certain embodiments, the linkers form ester bonds and are derived from diacids.

[0117] In certain embodiments, the linker L_A and/or L_B comprises a structure represented by Formula (ii):



wherein U^1 and U^2 are independently NH or O and can be the same or different, and wherein t is an integer from 0 to 10. For example, in a succinate linker, U^1 and U^2 are both oxygen, and t is 2. For a linker-end group, the linker of Formula (ii) comprises a terminal functional group on one of its ends. For the above example of a succinate linker, reacting with N-hydroxysuccinimidyl results in a succinimidylsuccinate linker-end group that may be used to conjugate amine functionalized active agents to a hyperbranched macromolecule having this linker-end group on its outermost polymeric arms. In certain embodiments, the linker L_A and/or L_B further comprises a polyethylene glycol unit between the bond to B and the carboxyl group, carboxamide group or structure of Formula (ii).

[0118] The linker of Formula (ii) introduces hydrolyzable bonds into the hyperbranched macromolecule that can be used to tune the degradation rate of the hyperbranched macromolecule and/or the release rate of conjugated active agents from the hyperbranched macromolecule. For example, the rate of biodegradation-/hydrolyzation of ester bonds at these linkers decreases from succinate (C4) to azelate (C9). In embodiments of the invention, this can be used to control the degradation rate of the hyperbranched macromolecule and/or the release of

active agents conjugated via these linkers to the hyperbranched macromolecule. For example, succinimidyl succinate groups (SS) can degrade in the order of a few days, while succinimidyl glutarate groups (SG) degrade in the order of weeks.

5 [0119] At the other end of the linker groups L_A and L_B , an end group such as an ester may be connected, for example succinimidyl (NHS) groups formed by esterification of the linker acid group with N-hydroxy succinimide or click chemistry functional groups such as DBCO or an azide, as further described below.

Extenders

10 [0120] In addition to or instead of linkers, bifunctional extender units may be incorporated as additional building blocks to extend polymeric arms in length, for example between arms connected to core and branch units, to provide further flexibility and /or to provide further hydrolytic cleaving points in the dendrimer. Such extenders are typically linear bifunctional polymer chains, such as linear PEG extenders.

15 [0121] In certain embodiments, the hyperbranched macromolecule comprises at least one extender unit comprising or consisting of polyethylene glycol (PEG) units, wherein the extender unit is linear, difunctional and connected to the polymeric arm of a dendritic constitutional repeating unit or the polymeric arm connected to the core unit and to either an end group or a polymeric arm of a next dendritic constitutional repeating unit.

20 [0122] In embodiments thereof, the extender unit comprises at least one linker, wherein the linker can be located at either terminus or both termini of the extender unit and is a difunctional linker comprising hydrolyzable bonds comprising a carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.

25 [0123] Inclusion of extenders can be used to enlarge the hydration radius of the dendrimer and to increase the half-life of the hyperbranched molecules in vivo.

PEG Precursors for G0 branched macromolecules and DCRU precursors

[0124] The core element of the hyperbranched macromolecule of certain embodiments of the present invention may comprise one or more multi-arm PEG precursors having from 2 to 10

arms, or 4 to 8 arms, or 4, 5, 6, 7 or 8 arms. It has to be noted that since multi-arm precursors have a core, a 2-arm PEG precursor, for example, differs from simple linear PEG by the presence of the core structure. The PEG precursors used in a hyperbranched macromolecule may have a different or the same number of arms. In certain embodiments, the PEG precursors used in the hyperbranched macromolecule of the present invention have 3, 4 and/or 8 arms. In certain embodiments, a combination of 4- and 3-arm or a combination of 4- and 8-arm PEG precursors is utilized, and any combinations thereof. For example, an 8-arm core unit may be combined with eight 4-arm DCRU precursors, which may again be connected with 24 precursors having 3-arms, resulting in a hyperbranched macromolecule having 48 conjugation sites at the outermost arms. In another exemplary embodiment, a 4-arm core unit may be combined with four 3-arm precursors, that may again be connected to eight 4 arm precursors, resulting in a hyperbranched macromolecule having 24 conjugation sites at the outermost arms. Multi-arm PEG precursors for G0 branched macromolecules and DCRU's in embodiments of the invention are commercially available, for example from JenKem Technology USA, SinoPEG, or Sigma-Aldrich, optionally including various functional end groups for further derivatization.

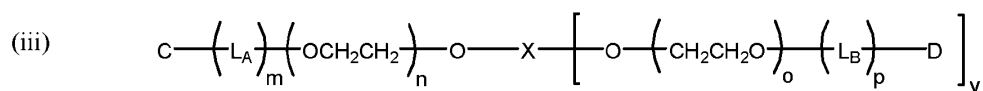
[0125] In certain embodiments of the present invention, polyethylene glycol units used as core building block or as DCRU precursors have an average molecular weight in the range from about 1,000 to about 80,000 Daltons, or in a range from about 10,000 to about 60,000 Daltons, or in a range from about 15,000 to about 50,000 Daltons. In some embodiments, the polyethylene glycol units have an average molecular weight in a range from about 10,000 to about 40,000 Daltons, or of about 20,000 Daltons. PEG precursors of the same average molecular weight may be used, or PEG precursors of different average molecular weight may be combined with each other. The average molecular weight of the PEG precursors used in the present invention is given as the number average molecular weight (M_n), which, in certain embodiments, may be determined by gel permeation chromatography against polystyrene standard according to standardized methods.

[0126] In a 4-arm PEG, each of the arms may have an average arm length (or molecular weight) of the total molecular weight of the PEG divided by 4. A 4a20kPEG precursor, which is one precursor that can be utilized in the present invention thus has 4 arms with an average molecular weight of about 5,000 Daltons (+/- 500) each, attached to a pentaerythritol core unit. An 8a20k PEG precursor, which may be used in addition to the 4a20kPEG precursor in the present

invention, thus has 8 arms each having an average molecular weight of 2,500 (+/-250) Daltons, attached to a tripentaerythritol or hexaglycerol core unit.

[0127] In general, when referring to a polymer precursor having a certain average molecular weight, such as a 15kPEG-precursor, the indicated average molecular weight (i.e., a Mn of 15,000 or 20,000, respectively) refers to the polymer unit part of the precursor, before end groups are added (“20k” here means 20,000 Daltons (+/- 2,000 Da), and “15k” means 15,000 Daltons (+/- 1,500 Da)– the same abbreviation is used herein for other average molecular weights of PEG or other polymer precursors). In certain embodiments, the Mn of the polymer unit part of the precursor is determined by gel permeation chromatography against polystyrene standard according to standardized methods. The degree of substitution with end groups as disclosed herein may be determined by means of ¹H-NMR after end group functionalization.

[0128] In certain embodiments, the precursors suitable for use in forming DCRU's are generally represented by the Formula (iii):



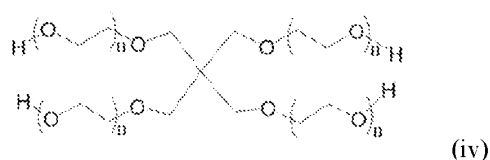
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wherein C comprises a functional group suitable for click chemistry, (such as an alkyne, alkene, azide, or tetrazine), D comprises functional groups not reactive in click chemistry (such as succinimidyl), and L_A is a linker group, m is either 0 or 1 meaning that the linker may be absent or present, n is an integer from 3 to 2000, or 20 to 2000, o is an integer from 3 to 2000, or 20 to 2000, while n and o can be different or the same, X is a branch unit, L_B is a linker group, p is either 0 or 1 meaning that the linker may be absent or present, L_A and L_B can be different or the same, m and p can be different or the same, and y is an integer from 2 to 9, wherein y = c' - 1 with c' being the connectivity c' of the branch unit X.

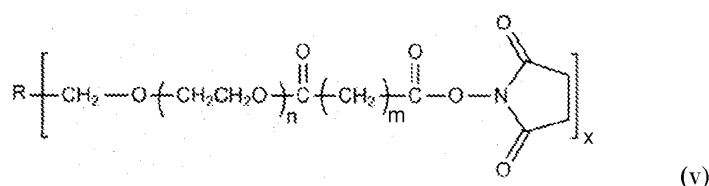
[0129] In certain embodiments, the PEG-precursor useful for forming the DCRU's of the hyperbranched macromolecule is an NHS dicarboxylic acid ester-terminated multi-arm PEG precursor derived from commercially available multi-arm PEG compounds such as Formula (iv), an example of a 4-arm structure derived from pentaerythritol.

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[0130] A PEG-precursor useful for forming a DCRU of certain embodiments can be represented by the following Formula (v):



- 5 wherein n is determined by the molecular weight of the respective PEG-arm, m is an integer from 0 to 10, and specifically is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, and x is the number of arms (and thus can e.g., 2, 4, 8, etc., see above). Where m is 1, each arm is terminated with a succinimidylsuccinate (SS) end group, where m is 2, each arm is terminated with a succinimidylglutarate (SG) group, where m is 3, each arm is terminated with a
- 10 succinimidyladipate (SAP) group, and where m is 6, each arm is terminated with a succinimidylazelaate (SAZ) group. With these specific electrophilic end groups, multi-arm PEG units may be abbreviated in the form of e.g., 4a20kPEG-SAP, referring to a 4-arm PEG with a succinimidyladipate end group and a molecular weight of 20,000 Da. In the above formula, R is a core unit structure appropriate to provide the desired number of arms. For 4-arm PEG units and
- 15 precursors as shown in the formula above, R can be a pentaerythritol structure, whereas for 8-arm PEG units and precursors, R can be a hexaglycerol structure.

[0131] In certain embodiments, the PEG precursor used is 4a20kPEG-SG or 4a20kPEG-SAP.

- [0132] Instead of electrophilic end groups, precursors having nucleophilic end groups may also be used. In certain embodiments, nucleophilic end groups for use as hyperbranched
- 20 macromolecule PEG precursors are amine (denoted as "NH₂") end groups. Thiol (-SH) end groups or other nucleophilic end groups are also possible.

[0133] In certain embodiments, 4-arm PEGs with an average molecular weight of about 20,000 Daltons and 4-arm PEGs with an average molecular weight of about 40,000 Daltons can be used for forming the hyperbranched macromolecules according to the present invention.

Functional groups for connecting hyperbranched macromolecule building blocks

5 [0134] To synthesize the hyperbranched macromolecule, the polymeric arms or precursors have pairs of functional groups that react with each other, i.e., a first functional group on a first polymeric arm or precursor is capable of reacting with a second functional group on a second polymeric arm or precursor on a different DCRU precursor.

10 [0135] In an embodiment, a first multi-arm precursor including the core unit and PEG arms connected to it comprises first functional groups, and a second multi-arm precursor DCRU comprises one second functional group capable of reacting with the first functional groups, whereas all other end groups of that second DCRU precursor do not react with the first functional group, the functional groups being located at the terminus of the arms of the precursor or DCRU. The first and second functional groups may be directly grafted to the arms termini, or
15 via a linker, preferably a hydrolyzable linker as defined herein elsewhere. The functional groups are capable to react with each other and form a covalent bond, for example, in click chemistry reactions or electrophile-nucleophile reactions, or are configured to participate in other chemical crosslinking reactions as described below.

20 [0136] In certain embodiments of the invention, the first functional group and the second functional group are selected from an electrophile and a nucleophile, functional groups for click chemistry, functional groups for cycloadditions, particularly 1,3-dipolar cycloadditions, hetero-Diels-Alder cycloadditions, functional groups for nucleophilic ring openings, functional groups for non-aldol type carbonyl reactions, functional groups for addition reactions to carbon-carbon multiple bonds, polymerizable vinyl groups, or combinations thereof. The skilled person will
25 know that certain pairs of functional groups may be classified in more than one of these groups. For example, in click chemistry, an azide reacting with dibenzocyclooctyne may be also seen as an electrophile-nucleophile reaction pair.

[0137] In certain embodiments of the hyperbranched macromolecules of the present invention, the connections between different parts of the hyperbranched macromolecule such as the
30 polymeric arms connected to the core unit and the DCRU's are formed by click chemistry

reactions such as strain promoted alkyne-azide cycloaddition (SPAAC), also termed as the Cu-free click reaction, or inverse electron demand Diels-Alder ligation (IEDDA) type click chemistry coupling reactions. An overview of such types of reaction is given in H. C. Kolb; M. G. Finn; K. B. Sharpless (2001). "Click Chemistry: Diverse Chemical Function from a Few Good Reactions", *Angewandte Chemie International Edition*, **40** (11): 2004–2021), incorporated herein by reference.

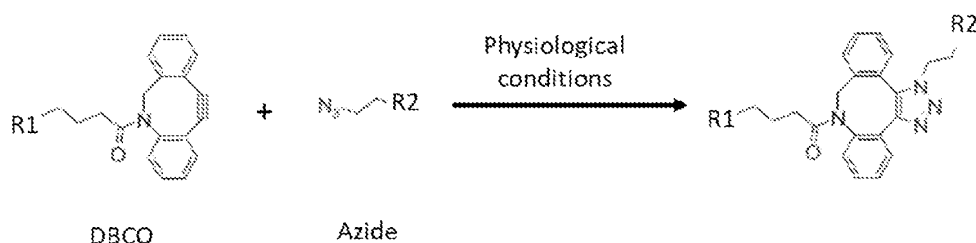
[0138] Other suitable click chemistry reactions for connecting constitutional units of hyperbranched macromolecules of certain embodiments include aldehyde/ketone condensation, cyanobenzothiazole condensation; strain-promoted, oxidation-controlled cyclooctyne-1,2-quinone cycloaddition (SPOCQ); 1,3-dipolar cycloadditions, [3+2] cycloadditions such as alkene-nitrone cycloadditions or alkyne-nitrone cycloadditions, [4+2] cycloadditions; and hetero-Diels-Alder reactions.

[0139] SPAAC requires a ring-structured alkyne such as dibenzylcyclooctyne (DBCO) and Bicyclo[6.1.0] nonyne (BCN) to react with an aliphatic azide. This strained chemistry causes the reaction to happen efficiently without the need of a copper catalyst required in copper(I)-catalyzed azide-alkyne click chemistry reactions (CuAAC). Similarly, IEDDA requires the reaction of norbornene and tetrazine without the need of a catalyst. Thus, the advantage of SPAAC and IEDDA over CuAAC and electrophile-nucleophile reactions such as NHS-NH₂ is that there is no catalyst needed and no byproduct exists after the reaction is completed.

[0140] SPAAC and IEDDA coupling reactions are bioorthogonal reactions with selective and quantitative yields under mild conditions that can occur even inside of living systems without interfering with native biochemical processes. These click chemistry reactions utilize a pair of reagents, for example cyclooctynes and azides, that exclusively and efficiently react with each other while remain inert to naturally occurring functional groups:

[0141] Scheme A:

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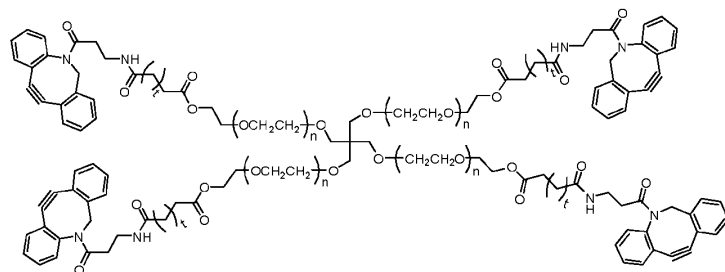
with R1 and R2 being any same or different residues.

- [0142] This reaction is suitable for forming the hyperbranched macromolecules of embodiments of the invention from correspondingly functionalized precursors and DCRU's as described
- 5 herein. Among the considerable number of known cyclooctynes, the dibenzocyclooctynes (DBCO) compounds comprise a class of reagents that possesses reasonably fast kinetics and good stability in aqueous buffers. Within physiological temperature and pH ranges, the DBCO group will not react with amines or hydroxyls that are naturally present in many biomolecules, or present as different functional groups on parts of the hyperbranched macromolecule.
- 10 Additionally, reaction of the DBCO group with the azide group is significantly fast and high yielding.

- [0143] The advantages of DBCO-based SPAAC are, for example, its biocompatibility as there are no cytotoxic copper catalysts required that may remain in undesirable traces in the hyperbranched macromolecules. Another advantage is the use of mild reaction conditions:
- 15 Connecting DCRU's or conjugation of active agents is possible in aqueous buffered media or common organic solvents at physiological conditions. Furthermore, DBCO and azide moieties are long term stable and have a high selectivity and specificity as azide groups react only with DBCO in the presence of amine, hydroxyl, thiol, and acid groups, as well as other protein functional groups. Also, the reactions lead to the formation of a stable triazole in quantitative
- 20 yield with high reaction rate and leave no byproducts. Similar advantages are provided by IEDDA coupling reactions and other types of catalyst free click chemistry reactions mentioned herein before.

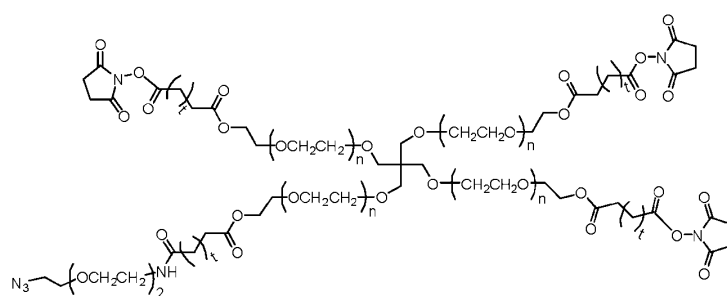
[0144] In exemplary embodiments, hyperbranched macromolecules including diacid derived hydrolyzable linkers can be formed by using the following precursors for click chemistry:

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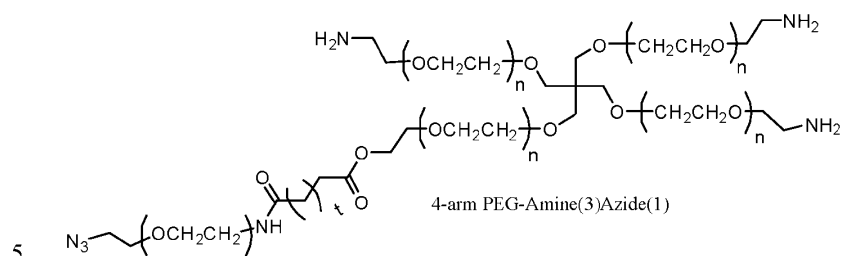
4-arm-PEG-DBCO

and either



4-arm-PEG-(DBCO)3(Azide)1

or

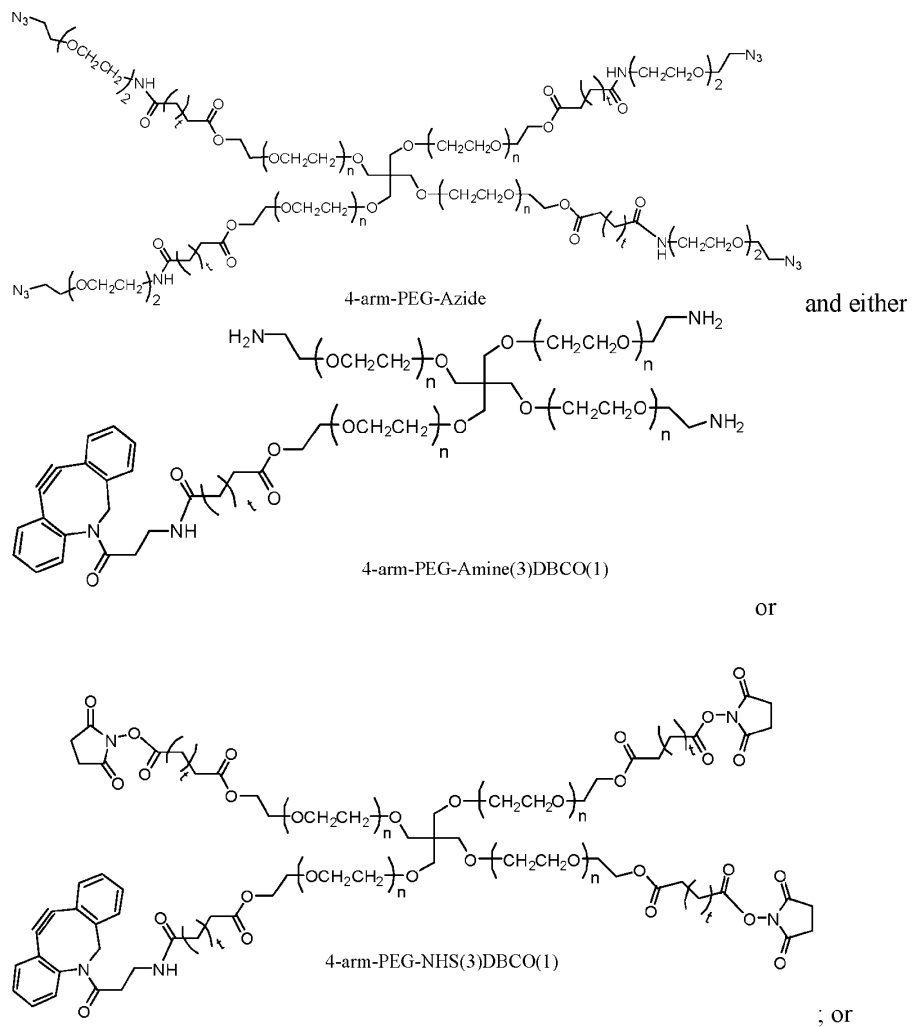


4-arm PEG-Amine(3)Azide(1)

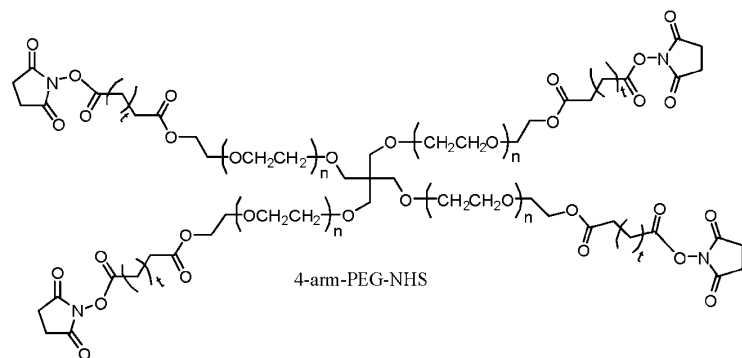
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; or

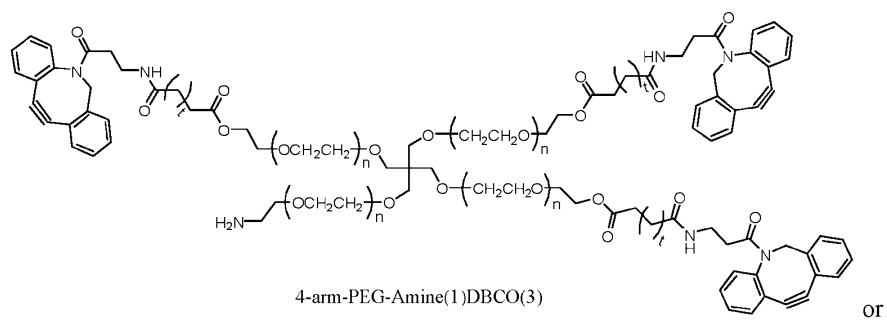
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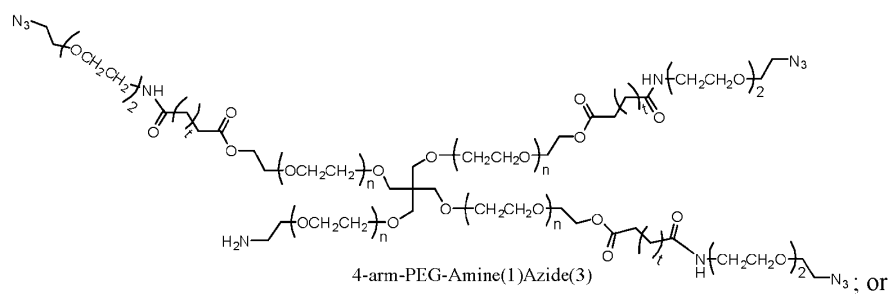
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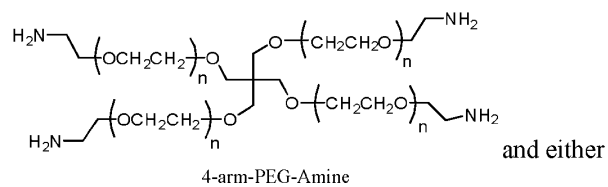
and either



or

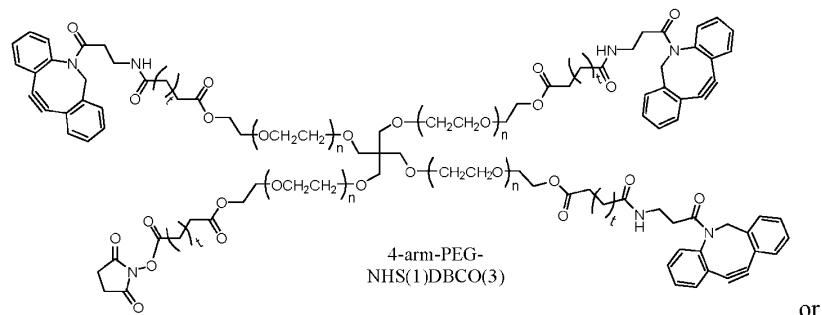


or

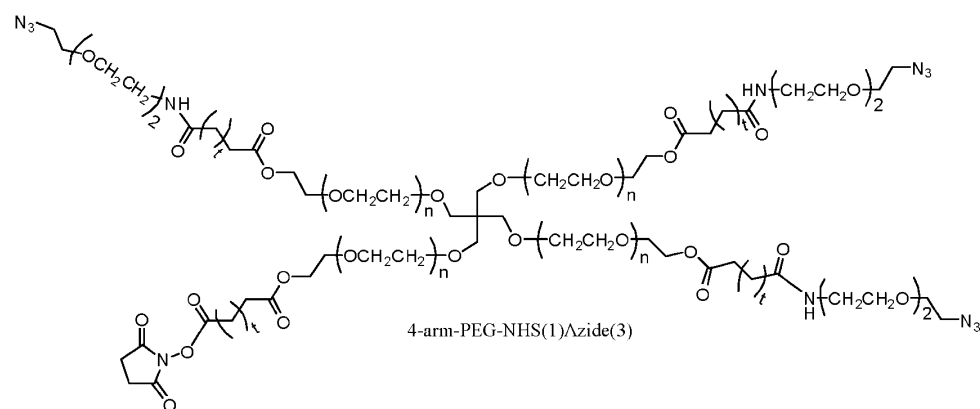


and either

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and



wherein t is m , and n and m are defined as for formula (v) herein before.

- 5 **[0145]** Instead of 4-arm PEG, other core/branch unit connectivities can also be used as described herein. Also, in other embodiments, the above precursors may include hydrolyzable linkages including carboxamide bonds instead of ester bonds, or an ester and amide bonds such as in SGA linker units further described herein above.

- 10 **[0146]** Using click chemistry functional groups, in certain embodiments connections in the hyperbranched macromolecule can be formed selectively using DCRU-precursors such as those above that include one functional group for the click chemistry bond formation, whereas the other terminal functional groups of the DCRU remain unreactive and can be used subsequently for other follow-up reactions such as hyperbranched macromolecule growth or conjugation. In other embodiments, connections in the hyperbranched macromolecule can be formed selectively
- 15 using electrophile-nucleophile-precursors or other functional groups not reactive in click chemistry, whereas the other terminal functional groups of the DCRU include a functional group

for click chemistry bond formation and remain unreactive and can be used subsequently for other follow-up reactions such as hyperbranched macromolecule growth or conjugation with click chemistry reactions.

[0147] The functional group pairs for click chemistry can be selected functional groups for cycloadditions, particularly 1,3-dipolar cycloadditions, [3+2] cycloadditions such as alkene-nitrone cycloadditions or alkyne-nitrone cycloadditions, [4+2] cycloadditions, hetero-Diels-Alder cycloadditions; functional groups for thiol-ene reactions; functional groups for nucleophilic ring openings; functional groups for non-aldol type carbonyl reactions; functional groups for addition reactions to carbon-carbon multiple bonds; functional groups for Michael-type additions.

[0148] For example, the first functional group is an alkyne compound such as a dibenzocyclooctyne (DBCO), or a bicyclo[6.1.0]-nonyne (BCN); or a norbornene, or a trans-cyclooctene (TCO); and the second functional group is an azide, a 3,4 dihydroxyphenylacetic acid (DHPA), or a tetrazine (Tz). In these embodiments, the DBCO, BCN, norbornene, TCO, azide, DHPA and Tz functional groups can be grafted to the termini of the multi-arm precursor via a hydrolyzable linker such as an acid group, a diacid group, an amide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group, or may be directly connected to the PEG.

[0149] In another embodiment, the first and second functional groups are selected for a [3+2] cycloaddition reaction such as alkene-nitrone cycloadditions or alkyne-nitrone cycloadditions. In a further embodiment, the first and second functional group are selected for a [4+2] cycloaddition reaction, particularly a hetero Diels-Alder reaction, wherein the first functional group is an aldehyde or imine compound, and the second functional group is a 1,3-diene compound, an unsaturated carbonyl compound, or a nitroso-alkene compound. In a further embodiment, the first and second functional group are selected for nucleophilic ring openings, wherein the first functional group is selected from an epoxide, thiirane, aziridine, or lactam, and the second functional group is nucleophile as mentioned above. In another embodiment, the first and second functional group are selected for non-aldol type carbonyl reactions, wherein the first functional group is an aldehyde or ketone compound, and the second functional group is a primary amine, a hydrazide, acyl hydrazide or aminooxy compound, to form an imine, amide, isourea, hydrazone, acyl hydrazone or oxime linkage.

Conjugation of active agents

[0150] Active agent bonding or conjugation to the outermost polymeric arms of the hyperbranched macromolecule can be done also by click chemistry as described above for connecting hyperbranched macromolecule building blocks, or by electrophile-nucleophile reactions and other types of coupling reactions as mentioned herein.

[0151] Thus, in one embodiment, the first functional group on the outermost polymeric arms of the hyperbranched macromolecule may be a nucleophile and the second functional group on the active agent may be an electrophile, or *vice versa*, and the reaction between the first functional group and second functional group is an electrophile-nucleophile reaction that forms a covalent bond.

[0152] Nucleophiles may be selected from one of amine such as a primary amine, a hydroxyl, a thiol, a carboxyl, or a hydrazide group. In certain embodiments, one of the functional groups comprises a nucleophile, such as a primary amine.

[0153] Electrophiles that can be used in embodiments of the present invention may be selected from activated ester groups such as succinimidyl esters, succinimidyl carbonates; nitrophenyl carbonates, aldehydes, ketones, acrylates, acrylamides, maleimides, vinylsulfones, iodoacetamides, alkenes, norbornenes, epoxides, mesylates, tosylates, tresyls, cyanurates, orthopyridyl disulfides, or halogen. These electrophiles comprise functional groups that participate in the electrophile-nucleophile reaction, and they preferably additionally include reactive groups forming linkers to the PEG that include hydrolyzable groups or bonds, such as glutarate. For example, in an embodiment of the invention, a succinimidyl ester may comprise a reactive group such as succinimidyl succinate (SS), succinimidyl glutarate (SG), succinimidyl adipate (SAP), succinimidyl azelate (SAZ), or succinimidyl glutaramide. Such electrophile-nucleophile reactions for connecting multi-arm PEG-precursors are for example described in US 2002/0042473A1, which is incorporated by reference.

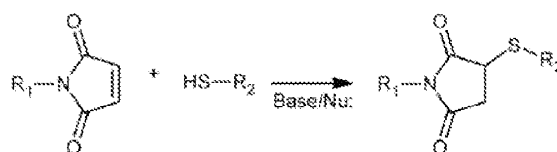
[0154] The active agent may be suitably derivatized with functional groups as mentioned above, unless it already has a suitable functional group for connecting with the hyperbranched macromolecule. For example, peptides having primary amino groups may be conjugated via an electrophile-nucleophile reaction to a hyperbranched macromolecule having an activated ester group at its surface.

[0155] In certain embodiments, the active agent, particularly peptides, may be conjugated via click chemistry reactions to the hyperbranched macromolecule. In embodiments thereof, the active agent or peptide having a terminal primary amino group is first reacted with DBCO-NHS or azide-NHS compounds, to produce an active agent or peptide functionalized with DBCO or an azide group suitable for reacting with its counterpart functional group on the terminal ends of the hyperbranched macromolecule, yielding conjugates with high reproducibility.

[0156] Suitable reactants for click chemistry functionalization of active agents or peptides having a terminal primary amino group are for example the azidoacetic acid *N*-hydroxysuccinimidylester (NHS-azide), azidobutyric acid *N*-hydroxysuccinimidylester or other azidoacid-NHS esters, and dibenzocyclooctyne-*N*-hydroxysuccinimidylester (DBCO-NHS) of varying acid chain length. Both azide-NHS esters and DBCO-NHS esters may be used with different chain length acids (such as discussed as linkers herein before) in order to vary the biodegradation rate and active agent release from the hyperbranched macromolecules. Such reagents for click chemistry are commercially available, e.g., from Sigma-Aldrich or Thermo Fisher Scientific and other vendors.

[0157] In certain embodiments, the active agent or peptide having a thiol group functionality for conjugation, such as a cysteine thiol group, may be conjugated to the hyperbranched macromolecule via maleimide-thiol click chemistry reactions according to the following reaction scheme:

[0158] Scheme B:

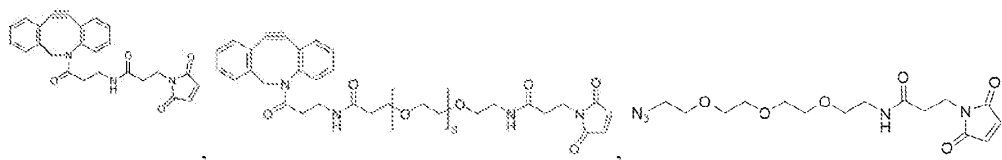


with R1 being the hyperbranched macromolecule terminal end and R2 being a peptide or an active agent. The thiol-maleimide reaction is a thiol Michael-addition type reaction yielding thio-succinimide linkages. The reaction is fast and chemoselective for thiols at a pH of 6.5 to pH 7.5.

[0159] For example, maleimide functionalized terminal ends of the hyperbranched macromolecule can be used to conjugate peptides or active agents via maleimide-thiol reactions.

- In another embodiment, DBCO or azide functionalized terminal ends of the hyperbranched macromolecule can be provided with a maleimide terminal functionalization by reacting with click chemistry linkers having azide or DBCO functionality and a maleimide group at their other end, which is then used for conjugation with thiol groups at a peptide or active agent. Suitable
- 5 DBCO-maleimide or azide-maleimide linkers may optionally be prolonged with PEG parts and are commercially available from Sigma-Aldrich, TCI, Thermo Fisher, etc.

[0160] Examples are compounds such as DBCO-maleimide, DBCO-PEG3-maleimide, DBCO-PEG4-maleimide, azido-PEG3-maleimide, with the following exemplary structures:



- 10 [0161] In certain embodiments, the active agent is bound to at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or up to 100% of the outermost polymeric arms. The average substitution rate of active agent conjugated to surface end groups of the hyperbranched macromolecule may be determined by UHPLC as further described herein.

Active Agents:

- 15 [0162] The active agent in the biodegradable microparticles of embodiments of the invention can be a therapeutically active agent or a diagnostically active agent, or combinations thereof. It may be a single active agent or a plurality of active agents.

- [0163] In some embodiments, the hyperbranched macromolecule comprises two or more different active agents at different dendrons or regions on the surface of the hyperbranched
- 20 macromolecule. Two or more active agents may be attached each with the same or with different hydrolyzable groups to control the release of the active agents at different rates. Further, the active agents may be attached to the dendrimer with or without hydrolyzable links or arms/extendors, or combinations thereof, to control the release of the active agents at different rates.

- [0164] In certain embodiments, the active agent conjugated to at least one of the outermost polymeric arms of the hyperbranched macromolecule is a peptide selected from the group consisting of Compstatin, APL-1, Fc-III-4C, Beovu (Brolucizumab), Zimura (Avacincaptad Pegol), Pegcetacoplan, Abicipar Pegol, Lampalizumab, Fovista, Risuteganib, AXT107, Elamipretide, THR149, ALM201, VGB3, and Largazole.
- [0165] Therapeutically active agents may be steroids; non-steroidal anti-inflammatory drugs (NSAIDs) such as Diclofenac, Ibuprofen, Meclofenamate, Mefenamic A, Salsalate, Sulindac, Tolmetin, Ketoprofen, Diflunisal, Piroxicam, Naproxen, Etodolac, Flurbiprofen, Fenoprofen C, Indomethacin, Celecoxib, Ketorolac, Nepafenac; intraocular pressure lowering drugs; antibiotics such as Ciprofloxacin; pain reliever such as Bupivacaine; calcium channel blockers such as Nifedipine; cell cycle inhibitors such as Simvastatin; proteins such as insulin; small molecule hydrophilic drugs, including carboxylic acid salts and amine salts; small molecule hydrophobic drugs, hydrophilic peptides and protein drugs, such as insulin, single chain antibody fragments, Fab fragments, IgG antibodies, fusion antibodies, etc.; aptamers; particularly Bupivacaine (BPV-HCl or base), Ropivacaine (RPV), Dexamethasone, Travoprost, Axitinib, non-steroidal anti-inflammatory drugs (NSAIDs), steroids, antibiotics, pain relievers, calcium-channel blockers, cell cycle inhibitors, chemotherapeutics, anti-viral drugs, anesthetics, hormones, anticancer drugs, antineoplastic agents, viruses, viruses for gene delivery such as AAV, protein binders such as nanobodies, affibodies, ankyrins, DARPs, etc., or any combinations thereof.
- [0166] In some embodiments, steroids may be corticosteroids that can comprise hydrocortisone, loteprednol, cortisol, cortisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone, aldosterone, or fludrocortisone.
- [0167] In some embodiments, NSAIDs can comprise diclofenac (e.g., diclofenac sodium), flurbiprofen (e.g., flurbiprofen sodium), ketorolac (e.g., ketorolac tromethamine), bromfenac, or nepafenac.
- [0168] In some embodiments, IOP lowering agents and/or glaucoma medications can comprise prostaglandin analogs (e.g., bimatoprost, latanoprost, travoprost, or latanoprostene bunod), rho kinase inhibitor (e.g., netarsudil), adrenergic agonists (epinephrine or dipivefrin), beta-adrenergic antagonists also known as beta blockers (e.g., timolol, levobunolol, metipranolol, carteolol, or betaxolol), alpha2-adrenergic agonists (e.g., apraclonidine, brimonidine, or brimonidine tartrate),

carbonic anhydrase inhibitors (e.g., brinzolamide, dichlorphenamide, methazolamide acetazolamide, acetazolamide, or dorzolamide), pilocarpine, echothiophate, demercarium, physostigmine, and/or isofluorophate.

5 [0169] In some embodiments, anti-infective can comprise antibiotics comprising ciprofloxacin, tobramycin, erythromycin, ofloxacin, gentamicin, fluoroquinolone antibiotics, moxifloxacin, and/or gatifloxacin; antivirals comprising ganciclovir, idoxuridine, vidarabine, and/or trifluridine; and/or antifungals comprising amphotericin B, natamycin, voriconazole, fluconazole, miconazole, clotrimazole, ketoconazole, posaconazole, echinocandin, caspofungin, and/or micafungin.

10 [0170] In some embodiments, antimetabolites can comprise methotrexate, mycophenolate, or azathioprine. In some embodiments, antifibrotic agents can comprise mitomycin C or 5-fluorouracil.

[0171] In some embodiments, angiogenesis inhibitors can comprise anti-VEGF agents (e.g., aflibercept, ranibizumab, bevacizumab), PDGF-B inhibitors (e.g., Fovista[®]), complement 15 antagonists (e.g., eculizumab), tyrosine kinase inhibitors (e.g., sunitinib, axitinib), and/or integrin antagonists (e.g., natalizumab and vedolizumab).

[0172] In some embodiments, nanobodies can be conjugated to the hyperbranched macromolecules. Nanobodies are described, for example, in Yang et al. (2020), Nanobodies: Next Generation of Cancer Diagnostics and Therapeutics, Front. Oncol. 10:1182, which is 20 incorporated herein by reference in its entirety. Nanobodies may be selected from ⁶⁸GaNOTA-Anti-HER2-VHH1, ⁶⁸GaNOTA-Anti-HER2-VHH1, ^{99m}Tc-NM-02, ¹³¹I-SGMIB-Anti-HER2-VHH1, ⁶⁸GaNOTA-Anti-MMR-VHH2, ^{99m}Tc-Anti-PD-L1, L-DOS47 + Doxorubicin, L-DOS47 + Cisplatin/Vinorelbine, KN035 + Trastuzumab/Docetaxel, KN035, KN044, TC-210 T Cells, CD19/CD20 bispecific CAR T cells, BCMA CAR T cells, or TAS266 nanobodies.

25 [0173] In some embodiments, non-immunoglobulin affinity proteins such as affibodies can be conjugated to the hyperbranched macromolecules. Affibody molecules are described, for example, in Ståhl et al., Affibody Molecules in Biotechnological and Medical Applications, Trends in Biotechnology 2017, 35 (8) p.691-712, which is incorporated herein by reference in its entirety.

[0174] In some embodiments, binding proteins such as ankyrins and DARPins can be conjugated to the hyperbranched macromolecules. Ankyrins and DARPins are described, for example, in a review by Caputi et al., *Current Opinion in Pharmacology* 2020, 51:93–101, which is incorporated herein by reference in its entirety. Ankyrins and DARPins may be selected from
5 MP0250, a tri-specific DARPIn drug candidate that can bind VEGF-A and hepatocyte growth factor (HGF) as well as one molecule of MP0250 binding two molecules of human serum albumin (HSA); Abicipar pegol (MP0112 or AGN-150998); Brolucizumab, Ranibizumab, or Aflibercept.

[0175] In some embodiments, cytoprotective agents can comprise ebselen, sulforaphane, oltipraz
10 or dimethyl fumarate. In some embodiments, neuroprotective agents can comprise ursodiol, memantine or acetylcysteine. In some embodiments, anaesthetic agents can comprise lidocaine, proparacaine or bupivacaine.

[0176] In some embodiments, the active agent can be dexamethasone, ketorolac, diclofenac, vancomycin, moxifloxacin, gatifloxacin, besifloxacin, travoprost, 5-fluorouracil, methotrexate,
15 mitomycin C, prednisolone, bevacizumab (Avastin®), ranibizumab (Lucentis®), sunitinib, pegaptanib (Macugen®), timolol, latanoprost, brimonidine, nepafenac, bromfenac, triamcinolone, difluprednate, fluocinolide, aflibercept, or combinations thereof. In some embodiments, the agent may be dexamethasone, ketorolac, diclofenac, moxifloxacin, travoprost, 5-fluorouracil, or methotrexate.

[0177] In alternative embodiments, active agents that can be utilized with the dendrimers and methods of the present invention include but are not limited to immunosuppressants, complement inhibitors (e.g., C5 inhibitors such as eculizumab or avacincaptad pegol), steroids, anti-inflammatories such as steroidal and non-steroidal anti-inflammatories (e.g., COX1 or COX
20 2 inhibitors), antivirals, antibiotics, anti-glaucoma agents, anti-VEGF agents, analgesics, tyrosine kinase inhibitors, integrin inhibitors, IL-6 blockers, reactive aldehyde species (RASP) inhibitors,
25 nitric oxide donating PgAs, antihistamines, mast cell stabilizers, rho kinase inhibitors, plasma kallikrein inhibitors, BCL-2 blockers, semaphorin antagonists, HtRA1 blockers, IGF-1R inhibitors, VEGF combination agents (multi-specific antiangiogenic agents) and combinations thereof.

[0178] Immunosuppressants include but are not limited to cyclosporine, mTOR inhibitors (e.g., rapamycin, tacrolimus, temsirolimus, sirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, or Torin-2), cyclophosphamide, atropine, thiopental, methotrexate, azathioprine, mercaptopurine, interferons, infliximab, etanercept, mycophenolate mofetil, 15-
5 deoxyspergualin, thalidomide, glatiramer, leflunomide, vincristine, cytarabine, pharmaceutically acceptable salts thereof and combinations thereof.

[0179] Non-steroidal anti-inflammatory compounds include inhibitors of the cyclooxygenase (COX) enzyme such as cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isozymes. General classes of non-steroidal anti-inflammatory compounds include salicylates, propionic
10 acid derivatives, acetic acid derivatives, enolic acid derivatives, and anthranilic acid derivatives. Examples of non-steroidal anti-inflammatory compounds include acetylsalicylic acid, diflunisal, salsalate, ibuprofen, dex-ibuprofen, naproxen, fenoprofen, ketoprofen, dex-ketoprofen, flurbiprofen, oxaprozin, loxoprofen, indomethacin, tolmetin, sulindac, etodolac, ketorolac, diclofenac, aceclofenac, nabumetone, piroxicam, tenoxicam, tenoxicam, lornoxicam,
15 phenylbutazone, mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, celecoxib, pharmaceutically acceptable salts thereof and combinations thereof.

[0180] Anti-inflammatory agents that may be utilized with the dendrimers and methods of the present invention may include agents that target inflammatory cytokines such as TNF α , IL-1, IL-4, IL-5, or IL-17, or CD20. Such agents may include etanercept, infliximab, adalimumab,
20 daclizumab, rituximab, tocilizumab, certolizumab pegol, golimumab, pharmaceutically acceptable salts thereof and combinations thereof.

[0181] Analgesics that may be utilized with the dendrimers and methods of the present invention include acetaminophen, acetaminosalol, aminochlorphenoxazin, acetylsalicylic 2-amino-4-
25 picoline acid, acetylsalicylsalicylic acid, anileridine, benoxaprofen, benzylmorphine, 5-bromosalicylic acetate acid, buccetin, buprenorphine, butorphanol, capsaicin, cinchophen, ciramadol, clometacin, clonixin, codeine, desomorphine, dezocine, dihydrocodeine, dihydromorphine, dimepheptanol, dipyracetyl, eptazocine, ethoxazone, ethylmorphine, eugenol, floctafenine, fosfosal, glafenine, hydrocodone, hydromorphone, hydroxypethidine, ibufenac, p-lactophenetide, levorphanol, meptazinol, metazocine, metopon, morphine, nalbuphine,
30 nicomorphine, norlevorphanol, normorphine, oxycodone, oxymorphone, pentazocine, phenazocine, phenocoll, phenoperidine, phenylbutazone, phenylsalicylate, phenylramidol,

salicin, salicylamide, tiorphan, tramadol, diacerein, actarit, pharmaceutically acceptable salts thereof and combinations thereof.

- [0182]** Antibiotic that may be utilized with the dendrimers and methods of the present invention include aminoglycosides, penicillins, cephalosporins, fluoroquinolones, macrolides, and combinations thereof. Aminoglycosides may include tobramycin, kanamycin A, amikacin, dibekacin, gentamicin, sisomicin, netilmicin, neomycin B, neomycin C, neomycin E, streptomycin, paramomycin, pharmaceutically acceptable salts thereof and combinations thereof. Penicillins may include amoxicillin, ampicillin, bacampicillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, nafcillin, oxacillin, penicillin G, penicillin V, piperacillin, pivampicillin, pivmecillinam, ticarcillin, pharmaceutically acceptable salts thereof and combinations thereof. Cephalosporins may include cefacetile, cefadroxil, cefalexin, cefaloglycin, cefalonium, cefaloridine, cefalotin, cefapirin, cefatrizine, cefazaflur, cefazedone, cefazolin, cefradine, cefroxadine, ceftezole, cefaclor, cefamandole, cefmetazole, cefonicid, cefotetan, cefoxitin, cefprozil, cefuroxime, cefuzonam, cefcapene, cefdaloxime, cefdinir, cefditoren, cefetamet, cefixime, cefmenoxime, cefodizime, cefotaxime, cefpimizole, cefpodoxime, cefteram, ceftibuten, ceftiofur, ceftiolene, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime, cefclidine, cefepime, cefluprenam, cefoselis, cefozopran, cefpirome, cefquinome, ceftobiprole, ceftaroline, cefaclomezine, cefaloram, cefaparole, cefcanel, cefedrolor, cefempidone, cefetrixole, cefivitril, cefmatilen, cefmepidium, cefovecin, cefoxazole, cefrotil, cefsumide, cefuracetime, ceftioxide, pharmaceutically acceptable salts thereof and combinations thereof. Fluoroquinolones may include ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, ofloxacin, norfloxacin, pharmaceutically acceptable salts thereof and combinations thereof. Macrolides may include azithromycin, erythromycin, clarithromycin, dirithromycin, oxithromycin, telithromycin, pharmaceutically acceptable salts thereof and combinations thereof.
- [0183]** Antivirals that may be utilized with the dendrimers and methods of the present invention include nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, fusion inhibitors, integrase inhibitors, nucleoside analogs, protease inhibitors, and reverse transcriptase inhibitors. Examples of antiviral agents include, but are not limited to, abacavir, aciclovir, acyclovir, adefovir, amantadine, amprenavir, ampligen, arbidol, atazanavir, boceprevir, cidofovir, darunavir, delavirdine, didanosine, docosanol, edoxudine, efavirenz, emtricitabine, enfuvirtide, entecavir, famciclovir, fomivirsen, fosamprenavir, foscarnet, fosfonet,

ganciclovir, ibacitabine, imunovir, idoxuridine, imiquimod, indinavir, inosine, interferon type III, interferon type II, interferon type I, interferon, lamivudine, lopinavir, loviride, maraviroc, moroxydine, methisazone, nelfinavir, nevirapine, nexavir, oseltamivir, peginterferon alfa-2a, penciclovir, peramivir, pleconaril, podophyllotoxin, raltegravir, ribavirin, rimantadine, ritonavir, 5 pyramiding saquinavir, stavudine, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir, tromantadine, truvada, valaciclovir, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, zidovudine, pharmaceutically acceptable salts thereof and combinations thereof.

[0184] Steroidal anti-inflammatory agents that may be utilized with the dendrimers and methods 10 of the present invention include dexamethasone, budenonide, triamcinolone, hydrocortisone, fluocinolone, loteprednol, prednisolone, mometasone, fluticasone, rimexolone, fluorometholone, beclomethasone, flunisolide, pharmaceutically acceptable salts thereof and combinations thereof.

[0185] Anti-glaucoma agents that may be utilized with the dendrimers and methods of the 15 present invention include beta-blockers such as atenolol propranolol, metipranolol, betaxolol, carteolol, levobetaxolol, levobunolol timolol, pharmaceutically acceptable salts thereof and combinations thereof; adrenergic agonists or sympathomimetic agents such as epinephrine, dipivefrin, clonidine, apraclonidine, brimonidine, pharmaceutically acceptable salts thereof and combinations thereof; parasympathomimetics or cholinergic agonists such as pilocarpine, carbachol, phospholine iodine, physostigmine, pharmaceutically acceptable salts thereof and 20 combinations thereof; carbonic anhydrase inhibitor agents, including topical or systemic agents such as acetazolamide, brinzolamide, dorzolamide; methazolamide, ethoxzolamide, dichlorphenamide, pharmaceutically acceptable salts thereof and combinations thereof; mydriatic-cycloplegic agents such as atropine, cyclopentolate, succinylcholine, homatropine, phenylephrine, scopolamine, tropicamide, pharmaceutically acceptable salts thereof and 25 combinations thereof; prostaglandins such as prostaglandin F2 alpha, antiprostaglandins, prostaglandin precursors, or prostaglandin analog agents such as bimatoprost, latanoprost, travoprost, unoprostone, tafluprost, pharmaceutically acceptable salts thereof and combinations thereof.

[0186] Anti-VEGF agents that may be utilized with the dendrimers and methods of the present 30 invention include bevacizumab, pegaptanib, ranibizumab, brolucizumab, conbercept, aflibercept, pharmaceutically acceptable salts thereof and combinations thereof.

[0187] Tyrosine kinase inhibitors that may be utilized with the dendrimers and methods of the present invention include deucravacitinib, axitinib, avapritinib, capmatinib, pegmatinib, ripretinib, selpercatinib, selumetinib, tucatinib, entrectinib, erdaftinib, fedratinib, pexidartinib, upadacatinib, zanubrutinib, baricitinib, binimetinib, dacomitinib, fostamatinib, gilteritinib, 5 larotrectinib, lorlatinib, acalabrutinib, brigatinib, midostaurin, neratinib, alectinib, cobimetinib, lenvatinib, osimertinib, ceritinib, nintedanib, afatinib, ibrutinib, trametinib, bosutinib, cabozantinib, ponatinib, regorafenib, tofacitinib, crizotinib, ruxolitinib, vandetanib, pazopanib, lapatinib, nilotinib, dasatinib, sunitinib (vorolanib), sorafenib, erlotinib, gefitinib, imatinib, afatinib, bosutinib, cabozantinib, cediranib, ceritinib, crizotinib, dabrafenib, dasatinib, erlotinib, 10 everolimus, gefitinib, imatinib, lestaurtinib, nilotinib, palbociclib, pazopanib, ponatinib, regorafenib, ruxolitinib, semaninib, sirolimus, sorafenib, temsirolimus, tofacitinib, trametinib, vandetanib, and vemurafenib. In another embodiment, the tyrosine kinase inhibitor is a Src family tyrosine kinase inhibitor, such as but not limited to, A419259, AP23451, AP23464, AP23485, AP23588, AZD0424, AZM475271, BMS354825, CGP77675, CU201, ENMD 2076, 15 KB SRC 4, KX2361, KX2-391, MLR 1023, MNS, PCI-32765, PD166285, PD180970, PKC-412, PKI166, PP1, PP2, SRN 004, SU6656, TC-S7003, TG100435, TG100948, TX-1123, VAL 201, WH-4-023, XL 228, altenusin, bosutinib, damnacanthal, dasatinib, herbimycin A, indirubin, neratinib, lavendustin A, pelitinib, piceatannol, saracatinib, Src11, foretinib, motesanib, tivozanib, LY2457546, MGCD-265, MGCD-510, tivantinib, AMG458, JNJ-3887, 20 EMD1214063, BMS794833, PHI1665752, SGX-523, INCB280, pharmaceutically acceptable salts thereof and combinations thereof.

[0188] Complement pathway modulators that may be utilized with the dendrimers and methods of the present invention include those that target, e.g., C1/C1Q, C3, C3 Convertase, C5, C5 convertase, C5a, C5aR, C6, C7, C8, C9, CD59, Factor B, Factor D, Factor H, Factor P, or a 25 combination thereof. Particular agents may include cinryze, berinert, ruconest, sutimlimab, pegcetacoplan (GA), eculizumab, ravulizumab, avacopan, pozelimab, nomacopan, zilucoplan, vilobelimab, crovalimab, avacincapted pegol), cemdisiran, BDB-001, tesidolumab, avdoralimab, MOR210, ALXN1720, danicopan, vemircopan, ACH-5228, ACH-5548, BCX-9330, AMY-101, ANX005, ANX007, narsoplimab, iptacopan, CLG561, GT103, ARGX-117, ALXN1820, 30 NGM621, lampalizumab, NGM621, IONIS-FB-Lrx, GEM103, CLG561, pharmaceutically acceptable salts thereof and combinations thereof

[0189] Integrin inhibitors that may be utilized with the dendrimers and methods of the present invention include lifitegrast, vedolizumab, natalizumab, efalizumab, tirofiban, eptifibatide, abciximab, IDL-2965, PLN-74809, PLN-1474, PN-943, 7HP349, MORF-057, OS2966, OTT166, AXT-107, JSM-6427, Risuteganib, THR-687 (D/ced), pharmaceutically acceptable salts thereof and combinations thereof.

[0190] Antihistamines that may be utilized with the dendrimers and methods of the present invention include loradatine, hydroxyzine, diphenhydramine, chlorpheniramine, brompheniramine, cyproheptadine, terfenadine, clemastine, triprolidine, carbinoxamine, diphenylpyraline, phenindamine, azatadine, tripelennamine, dexchlorpheniramine, dexbrompheniramine, methdilazine, and trimprazine doxylamine, pheniramine, pyrilamine, chlorcyclizine, thonzylamine, pharmaceutically acceptable salts thereof and combinations thereof.

[0191] IL-6 inhibitors that may be utilized with the dendrimers and methods of the present invention include sarilumab, tocilizumab, RG6179, pharmaceutically acceptable salts thereof and combinations thereof.

[0192] HtrA1 inhibitors that may be utilized with the dendrimers and methods of the present invention include IC-500, FHTR2163, RG6147, pharmaceutically acceptable salts thereof and combinations thereof.

[0193] RASP inhibitors that may be utilized with the dendrimers and methods of the present invention include reproxalap and pharmaceutically acceptable salts thereof.

[0194] Rho kinase inhibitors that may be utilized with the dendrimers and methods of the present invention include netardusil, ripasudil, HA-1077, Y-27632, H-1152P, INS-115644, Y-39983, SB772077BS, LX71D1, AR-12286, AMA-0076, AR-13533, pharmaceutically acceptable salts thereof and combinations thereof.

[0195] Plasma kallikrein inhibitors that may be utilized with the dendrimers and methods of the present invention include ecallantide, lanadelumab, berotralstat, ATN-249, KVD900, KVD824, THR-149, pharmaceutically acceptable salts thereof and combinations thereof.

[0196] Nitric Oxide Donating PgAs that may be utilized with the dendrimers and methods of the present invention include Latanoprostene Bunod, NCX470, NCX125, pharmaceutically acceptable salts thereof and combinations thereof

5 [0197] Mast Cell Stabilizers that may be utilized with the dendrimers and methods of the present invention include Iodoxamide, nedocromil, pemirolast, cromolyn (e.g., chromolyn sodium), pharmaceutically acceptable salts thereof and combinations thereof.

[0198] IGF-1R Inhibitors that may be utilized with the dendrimers and methods of the present invention include teprotumumab, VRDN-001, VRDN-002, VRDN-003, ganitumab, figitumumab, MEDI-573, cixutumumab, dalotuzumab, robatumumab, AVE1642, BIIB022, xentuzumab, 10 istiratumab, linsitinib, picropodophyllin, BMS-754807, BMS-536924, BMS-554417, GSK1838705A, GSK1904529A, NVP-AEW541, NVP-ADW742, GTx-134, AG1024, KW-2450, PL-2258, NVP-AEW541, NSM-18, AZD3463, AZD9362, B11885578, B1893923, TT-100, XL-228, A-928605, pharmaceutically acceptable salts thereof and combinations thereof.

[0199] TRPV1 antagonists that may be utilized with the dendrimers and methods of the present 15 invention include asivatrep, V116517, fused azabicyclic, heterocyclic, and amide compounds as described, for example, in U.S. Patent Application No. 2004/0157849, U.S. Patent Application No. 2004/0209884, U.S. Patent Application No. 2005/0113576, International Patent Application No. WO 05/016890, U.S. Patent Application No. 2004/0254188, U.S. Patent Application No. 2005/0043351, International Patent Application No. WO 05/040121, U.S. Patent Application 20 No. 2005/0085512, and Gomtsyan et al., 2005, J. Med. Chem. 48:744-752; fused pyridine derivatives as described, for example, in U.S. Patent Application No. 2004/0138454; pyridyl piperazinyl ureas as described, for example, in Swanson et al., 2005, J. Med. Chem. 48:1857-1872 and U.S. Patent Application No. 2005/0049241, as well as AMG8163 (Bannon et al., 2005, 11.sup.th World Congress on Pain) and BCTC (Sun et al., 2003, Chem. Lett. 13:3611-3616); 2- (piperazine-1-yl)-1H-Benzimidazole; pyridazinylpiperazines; urea derivatives as describe, for 25 example, in U.S. Patent Application No. 2005/0107388, U.S. Patent Application No. 2005/0187291, and U.S. Patent Application No. 2005/0154230, as well as A-425619 (El Kouhen et al., 2005, J. Pharmacol. Exp. Ther. 314:400-409); cinnamides, including SB-366791 (Gunthorpe et al., 2004, Neuropharmacology 46:133-149) and AMG 9810 (Gawa et al., 2005, J. 30 Pharmacol. Exp. Ther. 313:474-484).

[0200] In some embodiments, TRPV1 antagonists useful in the methods and compositions as disclosed herein include, for example, TRPV-1 antagonists include capsazepine, (E)-3-(4-
butylphenyl)-N-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylamide (commercially available for
example as AMG9810 from Tocris Bioscience, Bristol, United Kingdom), and 4-tertiary butyl
5 cyclohexane (commercially available as SYMSITIVE 1609 from Symrise GmbH of
Holzminden, Germany, as well as TRPV1 antagonists as disclosed in U.S. Pat. Nos. 8,815,930,
6,933,311, 7,767,705 and U.S. Pat. App. Pub. Nos. 2010/0249203 and 2011/0104301,
International Application WO/2008/013861.

[0201] In some embodiments, TRPV1 antagonists useful in the methods and compositions and
10 devices as disclosed herein include AMG-517 and AMG-628 (Amgen Inc., Thousand Oaks,
Calif.). TRPV1 antagonists useful in the present application are also described, for example, in
International Patent Application No. WO 2006065484; International Patent Application No. WO
2003070247; U.S. Patent Application No. US 2005080095; and International Patent Application
No. WO 2005007642. Additional TRPV1 antagonists useful in the methods and compositions
15 and devices as disclosed herein include TRPV1 antagonists: ABT-102, AMG8562, AMG9810,
BCTC, SB366791, JNJ17203212, I-TTX, JYL-1421, A-425619, N-[4-[6-
[4-(Trifluoromethyl)phenyl]pyrimidin-4-yloxy]benzothiazol-2-yl]acetamide (also known as AL-
49975 or AMG-517), (R)—N-(4-(6-(4-(1-(4-fluorophenyl)ethyl)piperazin-1-yl)pyrimidin-4-
yloxy)benzo[d]thiazol-2-yl)acetamide (AL-49976, also known as AMG-628), pharmaceutically
20 acceptable salts thereof and combinations thereof.

[0202] Other TRPV1 antagonists useful in the methods and compositions and devices as
disclosed herein are those that have a low inhibitory activity against CYP3A4, such as, e.g., 1-(2-
(3,3-dimethylbutyl)-4-(trifluoromethyl)benzyl)-3-(1-methyl-1H-indazol-4-yl)urea; methyl 2,2-
dimethyl-4-(2-((3-(1-methyl-1H-indazol-4-yl)ureido)methyl)-5-(trifluo-
25 romethyl)phenyl)butanoate; 1-(2-(4-hydroxy-3,3-dimethylbutyl)-4-(trifluoromethyl)benzyl)-3-
(1-methyl-1H-indazol-4-yl)urea; 2,2-dimethyl-4-(2-((3-(1-methyl-1H-indazol-4-
yl)ureido)methyl)-5-trifluoromethyl)phenyl)butanoic acid; 1-[4-Chloro-3-(3,3-
dimethylbutyl)benzyl]-3-(1-methyl-1H-indazol-4-yl)urea; 1-(2-isobutyl-4-
(trifluoromethyl)benzyl)-3-(1-methyl-1H-indazol-4-yl)urea; 1-(2-isopropyl-4-
30 (trifluoromethyl)benzyl)-3-(1-methyl-1H-indazol-4-yl)urea; 1-(4-Chloro-3-isopropylbenzyl)-3-

(1-methyl-1H-indazol-4-yl)urea, pharmaceutically acceptable salts thereof and combinations thereof.

[0203] TrkA antagonists that may be utilized with the dendrimers and methods of the present invention include VM902A, Larotrectinib, Entrectinib, Selitrectinib (LOXO-195, BAY 5 2731954), repotrectinib (TPX-0005), pharmaceutically acceptable salts thereof and combinations thereof.

[0204] For the purposes of the present invention, an active agent includes all its possible forms, including free acid, free base, polymorphs, pharmaceutically acceptable salts, anhydrites, hydrates, other solvates, stereoisomers, crystalline forms, co-crystals, pro-drugs, conjugates (e.g., 10 pegylated compounds), complexes and mixtures thereof.

[0205] Diagnostically active agents may be, e.g., imaging agents, markers, or visualization agents. Generally, diagnostic agents may be substances used to examine the body in order to detect impairment of its normal functions. In some cases, diagnostic agents may be agents with a functional purpose, such as for use in the detection of ocular deformities, ailments, and 15 pathophysiological aspects. For example, the diagnostic agent may be an important and effective diagnostic adjuvant, such as a dye (e.g., fluorescein dye, indocyanine green, trypan blue, a dark quencher such as a cyanine dye, an azo dye, an acridine, a fluorene, an oxazine, a phenanthridine, a naphthalimide, a rhodamine, a benzopyrone, a perylene, a benzanthrone, pra benzoxanthrone), to aid in visualization of ocular tissues. The diagnostic agent may comprise 20 paramagnetic molecules, fluorescent compounds, magnetic molecules, radionuclides, x-ray imaging agents, and/or contrast media. In some embodiments, a diagnostic agent may include radiopharmaceuticals, contrast agents for use in imaging techniques, allergen extracts, activated charcoal, different testing strips (e.g., cholesterol, ethanol, and glucose), pregnancy test, breath test with urea ^{13}C , and various stains/markers. In some embodiments, the labelling moiety is a 25 fluorescent dye or a dark quencher, selected from the group consisting of a coumarin, a cyanine dye, an azo dye, an acridine, a fluorene, an oxazine, a phenanthridine, a naphthalimide, a rhodamine, a benzopyrone, a perylene, a benzanthrone, and a benzoxanthrone. In particular non-limiting embodiments, the fluorescent dye is or is the residue of a compound selected from the group consisting of Coumarin, Fluorescein, Cyanine 3 (Cy3), Cyanine 5 (Cy5), Cyanine 7 (Cy7), 30 Alexa dyes, bodipy derivatives, (E)-2-(4-(phenyldiazenyl)phenoxy)acetic acid, 3-(3',3'-dimethyl-

6-nitrospiro[chromene-2,2'-indolin]-1'-yl)propanoate (Spiropyran), 3,5-dihydroxybenzoate and (E)-2-(4-(phenyldiazenyl)phenoxy)acetic acid, or combinations thereof.

[0206] In certain embodiments of the invention, the active agent may additionally be dispersed, embedded or encapsulated in the voids of the hyperbranched macromolecule. In certain
5 embodiments thereof, the active agent may be in particulate form.

Synthesis

[0207] Several methods for manufacturing hyperbranched macromolecules are known to the skilled artisan, and these methods can be principally applied and suitably adapted in embodiments of the present invention.

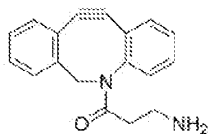
10 [0208] In certain embodiments of the invention, synthetic methods have been developed to produce dendrimers, among them the divergent and convergent synthesis that are the two most common general methods that are used by chemists. These methods can, in principle, also be employed for synthesizing the hyperbranched macromolecules of certain embodiments of the invention. The divergent method involves addition of monomers in repeated sequence and starts
15 from a multivalent core to surface molecules with continuous enhancement in the number of branching. The molecular size and number of surface groups gradually increase with the addition of successive layers of monomers which is called generations (cf. FIG. 2). While the convergent method involves the synthesis of hyperbranched macromolecules from the surface to core and leads to the formation of conical wedge-shaped units or dendrons, these are joined to a
20 multivalent core at the last step. Besides that, combined divergent/convergent method can also be employed in embodiments of the invention. For example, in an embodiment of a combined divergent/convergent synthesis, as shown in FIG. 1, first generation DCRU's are connected to a core unit, and second to higher DCRU's are first connected to each other before being connected to the first generation DCRU's. Any variation of combined divergent and convergent synthesis
25 steps can be used in embodiments of the invention, as desired for the particular hyperbranched macromolecule structure aimed at.

[0209] In an embodiment of the invention, a method for divergently synthesizing the hyperbranched macromolecule is provided that includes the following steps:

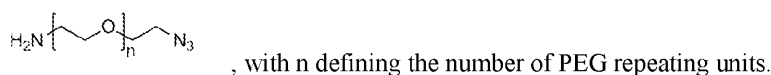
- (a) Providing a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit having functional groups suitable for click chemistry at the termini of the polymeric arms;
- (b) Providing dendritic constitutional repeating unit precursors comprising
- 5 - one polymeric arm comprising a functional group suitable for forming a connection by click chemistry with the corresponding functional groups of the polymeric arms connected to the core, (such as an azide, alkyne, alkene or tetrazine), and
- at least two polymeric arms comprising functional groups not reactive in click chemistry,
- 10 (c) Forming a connection by click chemistry between the polymeric arms connected to the core and the polymeric arms of the dendritic constitutional repeating unit precursors,
- (d) Optionally converting the functional groups of the at least two polymeric arms comprising functional groups not reactive in click chemistry into functional groups suitable for click chemistry, and
- 15 (e) Conjugating an active agent comprising a functional group to the outermost polymeric arms by reacting with the functional groups of the outermost polymeric arms, thereby forming a hyperbranched macromolecule-active agent conjugate.

[0210] In certain embodiments for higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, step (d) is compulsory and further consecutive dendritic constitutional repeating unit precursors are connected to the functional groups suitable for click chemistry obtained in step (d) by click chemistry to the hyperbranched macromolecule before conjugating the active agent in step (f).

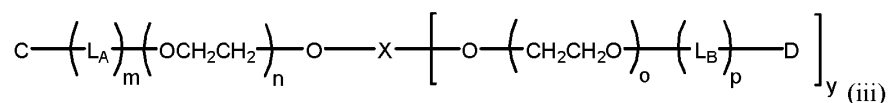
[0211] For example, step d) may be performed by converting PEG arms with SS (Succinimidyl Succinate), SG (Succinimidyl Glutarate), SAP (Succinimidyl Adipate), or SAZ (Succinimidyl Azelate) NHS terminal groups into DS (Dibenzocyclooctyne Amido Succinate), DG (Dibenzocyclooctyne Amido Glutarate), DAP (Dibenzocyclooctyne Amido Adipate), or DAZ (Dibenzocyclooctyne Amido Azelate) groups by reacting the NHS group with a DBCO-Amine click chemistry linker such as:



[0212] Similarly, a conversion of PEG-NHS termini into a PEG arm terminated with azide groups can be done by reacting the NHS group with an azido-amine click chemistry linker such as azido-PEG2-NH2 or the like. Such azido-amine click chemistry linker are commercially available from several vendors and have a structure as shown below:



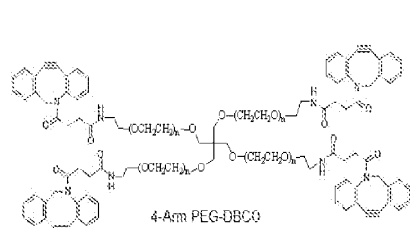
[0213] In the synthesis method, the dendritic constitutional repeating unit precursor in step (c) can be represented by the formula (iii):



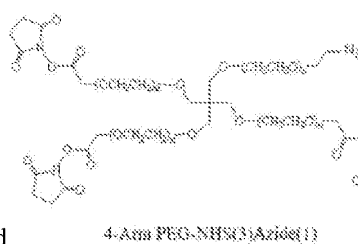
wherein C comprises a functional group suitable for click chemistry such as an alkyne, alkene, azide, or tetrazine, D comprises functional groups not reactive in click chemistry such as succinimidyl or primary amine, L_A is a linker group, m is either 0 or 1 meaning that the linker may be absent or present, n is an integer from 3 to 2000, or 20 to 2000, o is an integer from 3 to 2000, or 20 to 2000, while n and o can be different or the same, X is a branch unit, L_B is a linker group, p is either 0 or 1 meaning that the linker may be absent or present, B comprises an end group located at the surface of the hyperbranched macromolecule or comprises a bond connected to either A of a consecutive dendritic constitutional repeating unit or an active agent, L_A and L_B can be different or the same, m and p can be different or the same, and y is an integer from 2 to 9, wherein $y = c' - 1$ with c' being the connectivity c' of the branch unit X, and wherein the DCRU precursors used for synthesizing a hyperbranched macromolecule may be the same or different.

[0214] Exemplary precursors having 4 arms are 4-aPEG-NHS(3)Azide(1) or 4-arm PEG-NHS(3)DBCO(1) compounds and similar structures, with or without hydrolyzable linker groups

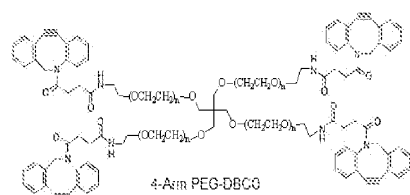
such as those connected via ester bonds, amide bonds or a combination of both, see for example the structures below.



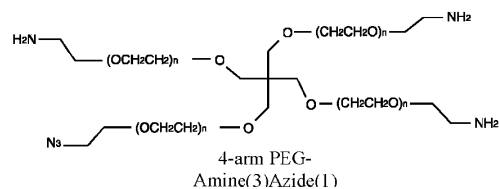
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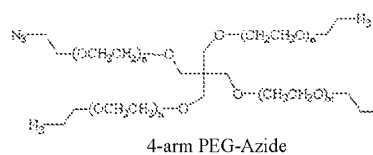
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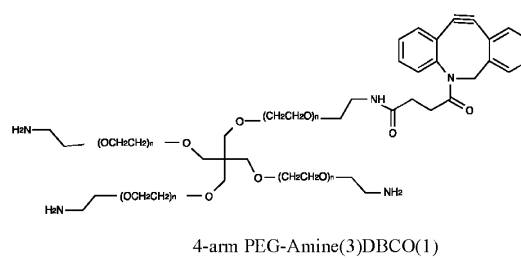
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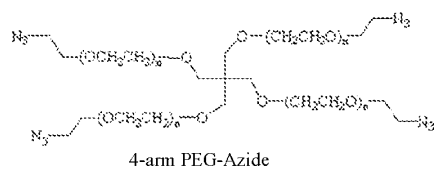
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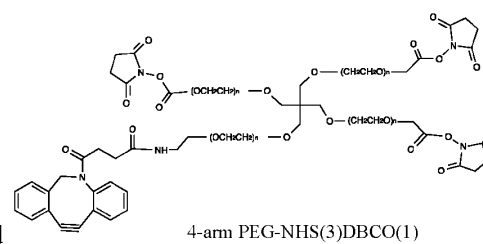
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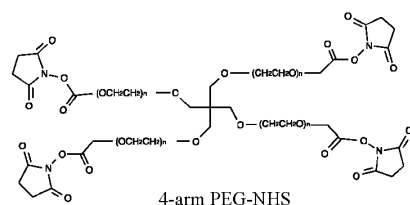
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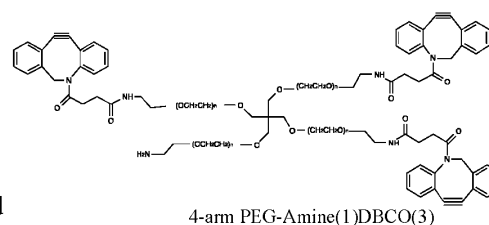
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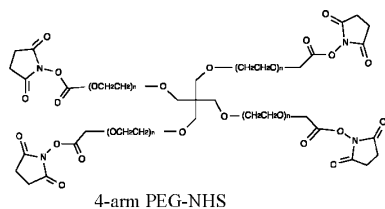


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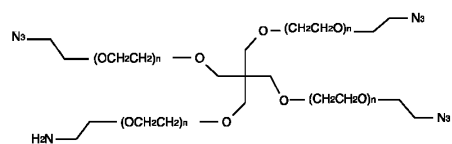


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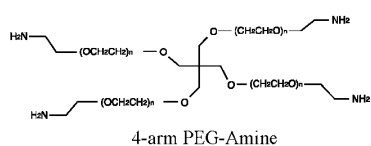
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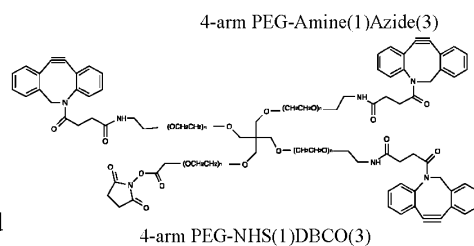
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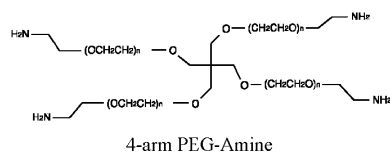
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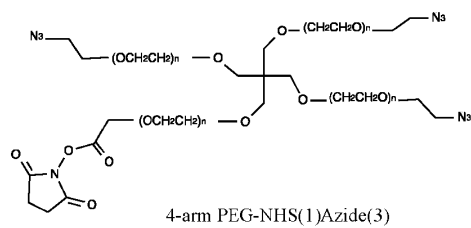
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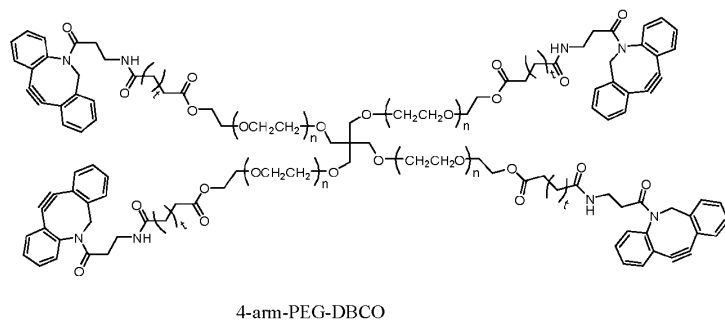


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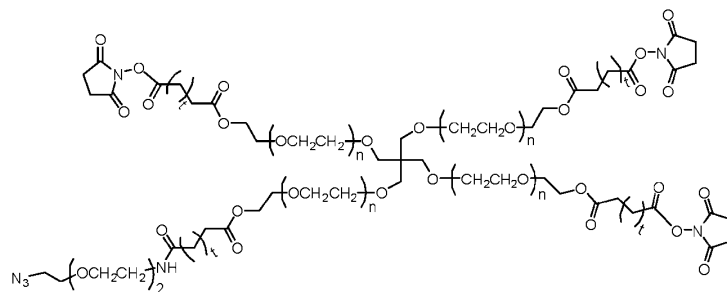


or the following exemplary precursor pairs:

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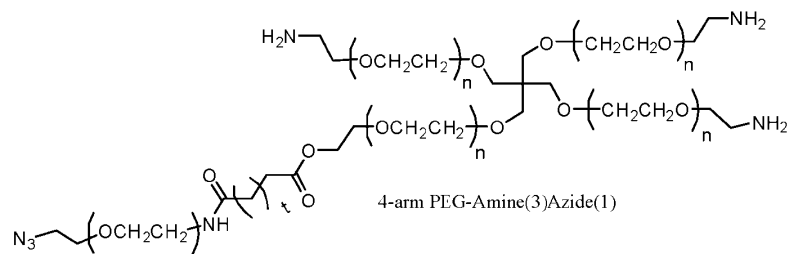
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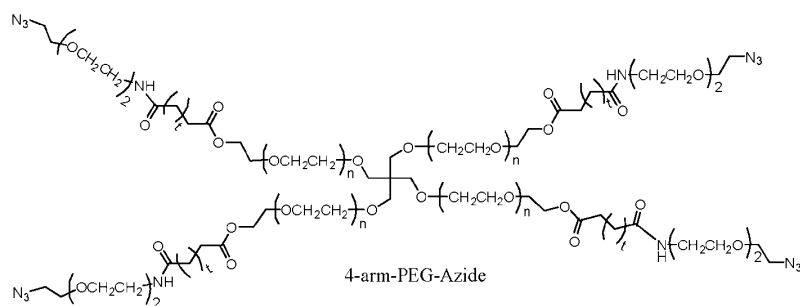
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4-arm-PEG-(DBCO)3(Azide)1

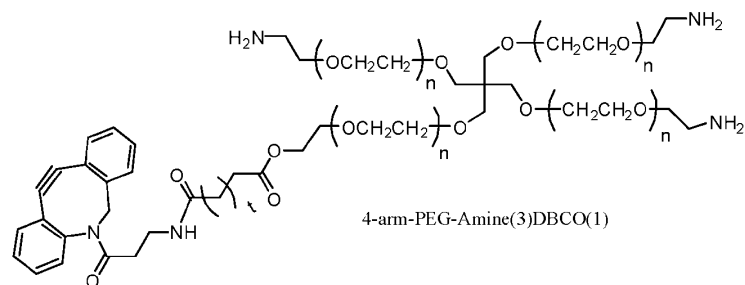
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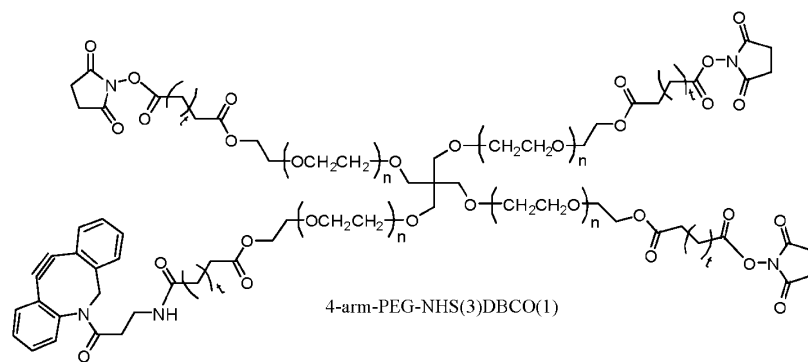
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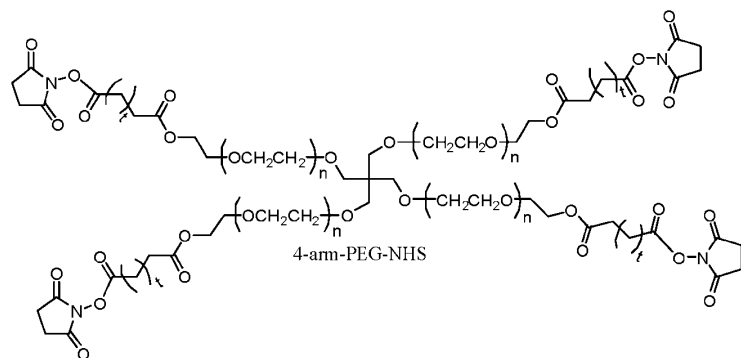


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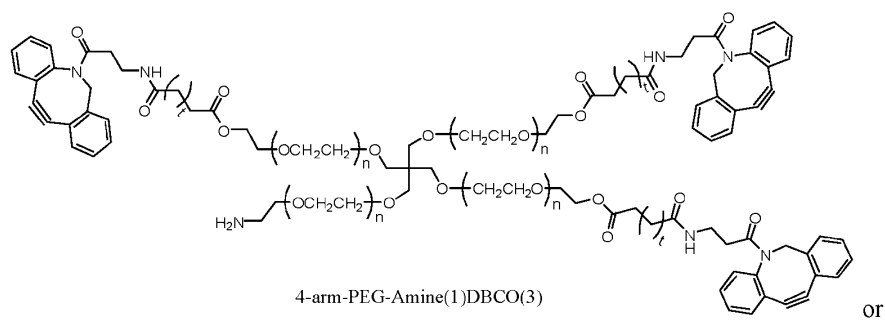


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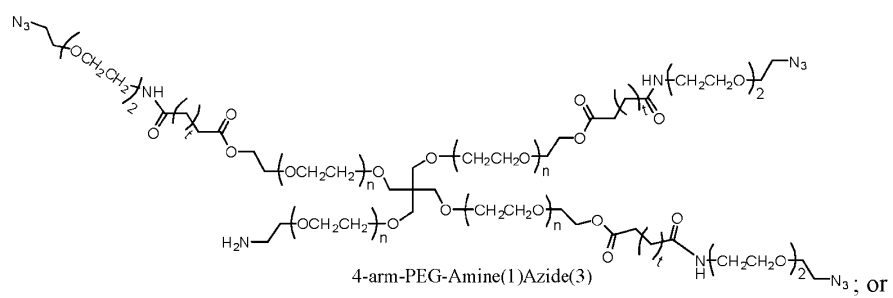
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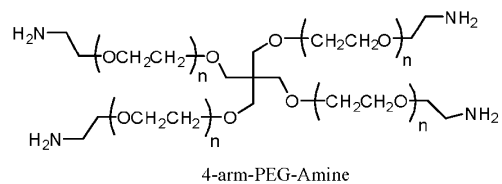
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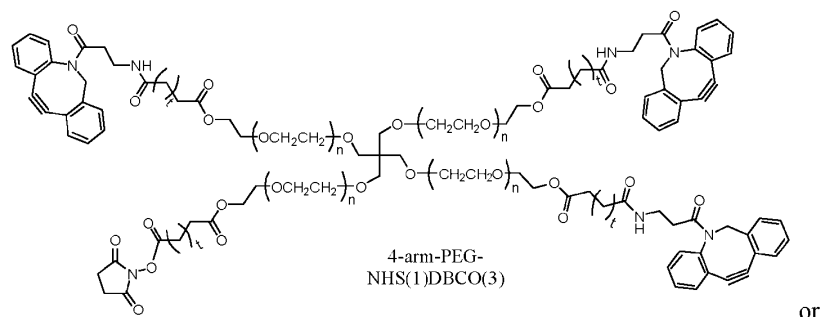


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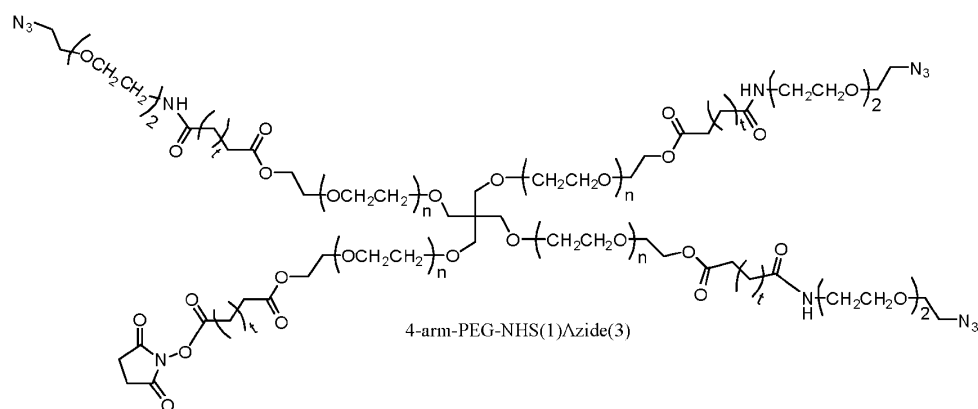


and either

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and



wherein t is m , and n and m are defined as for formula (v) herein before.

- 5 [0215] Referring to FIG. 3, exemplary synthesis schemes for a peptide conjugated $G1$ hyperbranched macromolecule of 4-4arm PEG units (FIG. 3 a)) and an 8-4arm PEG hyperbranched macromolecule according to certain embodiments are shown. In a generic embodiment, a multi-arm PEG with terminal functional groups for click chemistry, such as DBCO or azide, can be used as the core of hyperbranched macromolecule. Next, another
- 10 branched PEG with functional groups will react with the core PEG via click chemistry. The branched PEG will contain two types of functional groups, one group such as azide or DBCO, can couple with the core PEG for hyperbranched macromolecule growth in a click chemistry reaction, while the rest of the branched PEG (i.e. the DCRU) will be inert to the core PEG that can be used for the next generation of hyperbranched macromolecule growth or used as
- 15 precursor for terminal bioconjugation (FIG. 3a). Based on this generic method, multiple generations of hyperbranched macromolecules can be synthesized to achieve different number of

terminal functional groups. FIG. 3b) shows a 3-D structure of a hyperbranched macromolecule starting with an 8arm PEG core and coupled with eight 4arm PEG branches to achieve 24 terminal groups on the surface, and finally conjugated with up to 24 peptides.

5 [0216] As shown in the Examples, two cyclic peptides as C3 binding inhibitor, compstatin and APL-1, and an immunoglobulin G (IgG)-binding peptide ligand, Fc-III-4C, can be exemplarily used as API that are conjugated with PEG hyperbranched macromolecule. The primary amine groups on the peptides can be used as nucleophiles to react with electrophile NHS groups on the outermost polymeric arms of the hyperbranched macromolecule.

10 [0217] Alternatively, by converting the functional groups of the outermost polymeric arms comprising functional groups not reactive in click chemistry into functional groups suitable for click chemistry, and functionalizing the active agent, such as a peptide with a functional group suitable for click chemistry (such as an alkyne, alkene, azide, or tetrazine), conjugation of the active agent can also be done in a click chemistry reaction at the outermost polymeric arms of the hyperbranched macromolecule.

15 [0218] Suitable ester groups on the outermost polymeric PEG arms of the hyperbranched macromolecule of certain embodiments, such as succinic (S-), glutaric (G-), adipic (AP-), and azelaic (AZ-), are hydrolyzable under physiologic conditions and are degraded at controlled pH conditions to release the peptides *in vivo*. The controlled release and binding affinity of the peptide moieties can be characterized by Ultra High Performance Liquid Chromatography
20 (UHPLC), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and surface plasmon resonance (SPR), etc., as further described herein.

[0219] In an alternative embodiment, a convergent synthesis for the hyperbranched macromolecules of the present invention is provided, comprising the following steps:

- 25 I) Providing dendritic constitutional repeating unit precursors comprising
- one polymeric arm comprising a functional group suitable for forming a connection by click chemistry (such as an azide, alkyne, alkene or tetrazine), and
 - at least two polymeric arms comprising functional groups not reactive in click chemistry,

- II) Conjugating active agents comprising a functional group to at least one of the at least two polymeric arms comprising functional groups not reactive in click chemistry of the dendritic constitutional repeating unit precursors,
- 5 III) Providing a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit having functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine) at the termini of the polymeric arms, and
- 10 IV) Forming a connection by click chemistry between the polymeric arms connected to the core provided in step III) and the polymeric arm comprising a functional group suitable for forming a connection by click chemistry of the active agent conjugated dendritic constitutional repeating unit precursors obtained in step II), thereby forming a biodegradable hyperbranched macromolecule-active agent conjugate.

[0220] In this method, the dendritic constitutional repeating unit precursor in step I) is
15 represented by the Formula (iii) as described above.

[0221] In certain embodiments for higher generation G_x biodegradable hyperbranched macromolecules, with x being an integer of 2 to 10, the active agent conjugated dendritic constitutional repeating unit precursors obtained in step II) are connected by click chemistry to reverse dendritic constitutional repeating unit precursors comprising one polymeric arm
20 comprising a functional group not reactive in click chemistry, and at least two polymeric arms comprising functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine), wherein the functional group not reactive in click chemistry of the one polymeric arm is subsequently converted to a functional group suitable for click chemistry before connecting to further reverse dendritic constitutional repeating unit precursors or before forming a connection
25 by click chemistry with the polymeric arms connected to the core in step IV), thereby forming higher generation biodegradable hyperbranched macromolecules.

[0222] In contrast to the divergent synthesis method, the convergent method allows also for the synthesis of hyperbranched macromolecules having two or more different active agents at different dendrons or regions on the surface of the hyperbranched macromolecule. This allows a
30 clustering of more than one active agent on the surface of the hyperbranched macromolecule. In certain embodiments of the convergent method of the invention, dendritic constitutional

repeating unit precursors having different active agents conjugated to the polymeric arms can be obtained by performing steps I) and II) for each active agent conjugated DCRU precursor, and a mixture of the obtained active agent conjugated DCRU precursors is used for step IV), thereby forming a biodegradable hyperbranched macromolecule-active agent conjugate having different
5 active agents at different regions of the surface of the hyperbranched macromolecule. Such clustered hyperbranched macromolecules may for example be used for combination therapies involving the administration of more than one active agent.

[0223] In alternative embodiments of the divergent and convergent synthesis methods discussed above, the methods can also be performed with inversely exchanged functional groups, i.e.,
10 using other reactions and functional groups for forming connections within the hyperbranched macromolecule, and click chemistry functional groups for terminal conjugation. In such “inverse” embodiments of the described synthesis methods, connections in the hyperbranched macromolecule can be formed selectively using electrophile-nucleophile-precursors or other functional groups not reactive with click chemistry functional groups, whereas all other terminal
15 functional groups of the DCRU not participating in the connection to the core or previous DCRU include a functional group for click chemistry bond formation and remain unreactive in the connection formation reaction. These terminal click chemistry functional groups can be used subsequently for other follow-up reactions such as hyperbranched macromolecule growth of conjugation with click chemistry reactions.

20 [0224] Thus, in another embodiment of the invention, a method for divergently synthesizing the hyperbranched macromolecule is provided that includes the following steps:

- (a) Providing a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit having functional groups not reactive in click chemistry at the termini of the polymeric arms;
- 25 (b) Providing dendritic constitutional repeating unit precursors comprising
 - one polymeric arm comprising a functional group suitable for forming a connection in a reaction other than click chemistry with the corresponding functional groups of the polymeric arms connected to the core, (such as an electrophile or nucleophile, e.g., amine, NHS), and

- at least two polymeric arms comprising functional groups suitable for click chemistry,
- (c) Forming a connection between the polymeric arms connected to the core and the polymeric arms of the dendritic constitutional repeating unit precursors by reacting the functional groups not reactive in click chemistry,
- 5 (d) Optionally converting the functional groups of the at least two polymeric arms comprising functional groups suitable for click chemistry into functional groups not reactive in click chemistry, and
- (e) Conjugating an active agent comprising a functional group to the outermost polymeric arms by reacting with the functional groups of the outermost polymeric arms, thereby
- 10 forming a hyperbranched macromolecule-active agent conjugate.

[0225] In certain embodiments for higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, step (d) is compulsory and further consecutive dendritic constitutional repeating unit precursors are connected to the functional groups not reactive in click chemistry obtained in step (d) to the hyperbranched macromolecule before conjugating the

15 active agent in step (f).

[0226] In the synthesis method, the dendritic constitutional repeating unit precursor in step (c) can be represented by the formula (iii) as described above.

[0227] In a further alternative embodiment of the convergent method, a convergent synthesis for the hyperbranched macromolecules of the present invention is provided, comprising the

20 following steps:

- I. Providing dendritic constitutional repeating unit precursors comprising
 - one polymeric arm comprising a functional group not reactive in click chemistry, and
 - at least two polymeric arms comprising functional groups suitable for forming a connection by click chemistry (such as an azide, alkyne, alkene or tetrazine),
- 25 II. Conjugating active agents comprising a functional group to at least one of the at least two polymeric arms comprising functional groups suitable for click chemistry of the dendritic constitutional repeating unit precursors,

- III. Providing a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit having functional groups not reactive in click chemistry at the termini of the polymeric arms, and
- IV. Forming a connection between the polymeric arms connected to the core provided in step III) and the polymeric arm comprising a functional group not reactive in click chemistry of the active agent conjugated dendritic constitutional repeating unit precursors obtained in step II), thereby forming a biodegradable hyperbranched macromolecule-active agent conjugate.

[0228] Exemplary reaction conditions for forming the dendrimers involve reacting the core precursors and DCRU's at relatively mild temperatures such as 10 to 50 °C, such as 30 to 45°C in suitable solvents, such as DMF, for several hours, such as overnight, or up to 24 or even 48 hours.

Purification and Characterization

[0229] Purification of the hyperbranched macromolecule reaction mixtures obtained in the synthesis methods as described herein can be done for example by filtration, dialysis, SEC column filtration, centrifugation, or UHPLC.

[0230] In an exemplary embodiment, the synthesis reaction mixtures of the hyperbranched molecules, optionally conjugated with active agents, such as peptides, are diluted, filtered, e.g. at 0.45µm mesh sizes, then purified by ultra-centrifuge filtration, e.g. using a 100 kDa membrane, and may then be lyophilized after addition of sugar buffers, to render the final product. For administration in therapeutic methods, the lyophilized product may be reconstituted by addition of solvent, optionally including further sugar buffer.

[0231] Sugar buffers may be added as needed to improve solubility and stability of the dendrimer peptide or protein conjugates, e.g., by preventing peptide precipitation, before or after lyophilization. Even with non peptide dendrimer conjugates, the addition of sugar buffers improves stability and solubility, as the PEG based dendrimers of the embodiments of the invention show a behavior similar to synthetic proteins. An exemplary sugar buffer formulation for use with embodiments of the invention may include a solution of sugar, such as trehalose,

mono- and diphosphates in water at a suitable concentration, such as 3 % (or 30mg/mL) and a pH of about 6.4.

[0232] Dialysis is a common purification method based on separating molecules in solution by the difference in their rates of diffusion through a semipermeable membrane, such as a dialysis tubing. For purifying the hyperbranched macromolecule reaction mixtures, which can be a solution containing molecules of different sizes, such as free peptide (MW for example, of about 1.5kDa), free PEG/DCRU precursors (MW for example about 10-40kDa), small hyperbranched macromolecule conjugates such as G0 (MW for example, about 20-50kDa) and large conjugates such as higher generation G_x hyperbranched macromolecule-conjugates (for example, about 50kDa and above), the solution can be loaded into a dialysis tubing with a specific pore size membrane defining the cut-off and soaked into large amount of solvent. Molecules that are smaller than the pore size will elute from the tubing into the solvent, whereas molecules larger than the pore size will keep inside the tubing. Dialysis tubings are commercially available for example from Spectra/Por® Float-A-Lyzer G2 Dialysis Devices, Spectrum® Laboratories, with several different molecular weight cutoffs as desired for the particular separation task. FIG. 5a) shows a corresponding experimental setup for purification by dialysis.

[0233] By selecting proper molecular weight cutoff dialysis tubing and membranes, if necessary, in a series of separation steps with different cut-off dialysis tubings, it is possible to remove most of the impurities, such as excess amount of peptide and not reacted precursors, small size intermediates, from the product.

[0234] Another purification method that can be applied in certain aspects of the present invention is the use of size exclusion chromatography (SEC), such as a SEC column. For example, Zeba™ Spin Desalting columns (from ThermoFisher Scientific) designed for protein purification to remove salts and small size impurities can be used for purification of hyperbranched macromolecule conjugates. Columns with different pore sizes, for example, 7kDa and 40kDa may be used. The purification mechanism is based on size exclusion chromatography, in which small particles will be trapped in the pore on the immobile phase material, and particles with large size such as hyperbranched macromolecule conjugates of certain embodiments of the invention will elute through the column and collected in purified form. FIG. 5b) shows a corresponding experimental setup for purification by SEC column filtration.

[0235] The resulting purified products can be characterized by Ultra-performance liquid chromatography (UHPLC), which is an efficient technique which offers more sensitive analysis with good chromatographic separation and resolution of analytes. It provides benefits including fast analysis, high-resolution separations, reduced solvent and sample usage, enhanced sensitivity and precision, etc. Based on calibration curves with free active agent, free precursor units, and a comparison of the solutions before and after the purification treatment, the amounts of desired product in the purified solution can be determined by peak area integration.

[0236] With purification by dialysis, product solutions comprising greater than 99% of hyperbranched macromolecule-peptide-conjugate can be obtained (see Example 6), as determined by UHPLC based on peak area integration. With purification by SEC column, product solutions comprising greater than 98% of hyperbranched macromolecule-peptide-conjugate can be obtained (see Example 7), as determined by UHPLC based on peak area integration. Both described purification methods show very efficient purification capabilities.

[0237] For determining the molecular weight of hyperbranched macromolecule-conjugates of certain embodiments of the present invention SDS-PAGE can be used. SDS-Page is an analytical technique to separate materials based on their molecular weight. When samples are separated by electrophoresis under an electric potential through a gel matrix, smaller compounds migrate faster due to less resistance from the gel matrix, whereas larger molecules migrate slower. Sodium dodecyl sulfate (SDS) is a surfactant that can exfoliate large molecules such as protein and eliminates the influence of their structure and charge to separate compounds solely based on their molecular size.

[0238] In embodiments of the invention, the hyperbranched molecules are lyophilized to provide a storage stable formulation that can be reconstituted with suitable solvents before therapeutic use.

25 **Multivalent Receptor Binding**

[0239] For biologic efficacy, it is desirable that the biomolecules conjugated to the hyperbranched macromolecules of the invention show the same or similar affinity to a receptor. Additionally, efficacy can be improved if the half-life of receptor binding biomolecules is extended by multivalent binding. For example, in binding of antigens, affinity is defined as the strength required for an interaction between a site of antigen binding at an antibody and an

antigen epitope. Avidity is the total strength required for the interaction between a multivalent antibody and multiple antigenic epitopes. This definition can be applied to other biomolecules binding to specific targets or receptor sites as well. Multivalent binding thus results in an improvement of avidity. The concept of multivalency and the resulting concept of avidity, and a model to quantify avidity has been described by Kitov et al., “On the Nature of the Multivalency Effect: A Thermodynamic Model”, JACS 2003, 125, 16271-16284, which is incorporated herein by reference in its entirety.

[0240] Kitov describes the interaction of Shiga-like toxins with a series of dendrimer-conjugated multivalent oligosaccharide ligands based on PANAM dendrimer structures having varying multivalency. Inter alia, Kitov found that even if extra branches of the multivalent ligand dendrimers do not interact with the receptor in a common sense, they increased the probability of the interaction with the receptors. Further, Kitov concludes that “in a situation when it is necessary to inhibit all binding sites to achieve a desirable effect, the fraction of uninhibited bonding sites can be precisely controlled by choosing the appropriate number of branches for assembly of a multivalent inhibitor”. Thus, for multivalent inhibitor systems as an example, the extra branches with further conjugated inhibitor molecules can secure a higher degree of inhibition, although individual inhibitors are unable to specifically interact with the receptor, resulting in extended half-life, improved efficacy and avidity even by multivalent binding possibilities.

[0241] Khalili et al., “Fab-PEG-Fab as a Potential Antibody Mimetic”, Bioconjugate Chem. 2013, 24, 1870–1882, which is incorporated herein by reference in its entirety, exemplifies a bivalent PEG conjugated to protein binding ligands, exemplifying an avidity improvement from the bivalency.

[0242] Certain embodiments of the invention make use of the described concepts as shown below with reference to Examples 8, 9 and Fig. 18 and 19.

[0243] Embodiments of the invention relate to methods of treatment of a disease with antibodies bound to the dendrimers as described herein. For example, the dendrimers of embodiments of the invention may be used for improving the pharmacokinetics of antibodies delivered as a delivery target bound or conjugated to the hyperbranched molecules as described herein. Suitable delivery targets are, for example, selected from anti-VEGF, aflibercept, faricimab, bevacizumab, anti-

TNF- α , infliximab, etanercept, adalimumab, anti-IL-6R, sarilumab, anti-IL-6, siltuximab, anti-C5, ravulizumab, eculizumab, anti-CD20, ocrelizumab, rituximab, anti-IGF-1R, or teprotumumab. These antibodies bound to and delivered by dendrimers non-covalently bind and chaperone antibody drugs and prolong half-life in blood upon intravenous administration, or
5 upon injection into the vitreous humor (IVT). The high molecular weight of the dendrimer-antibody conjugate prevents clearance of the bound antibody from the blood via the kidneys and slows diffusion from the therapeutic target site such as the vitreous humor.

[0244] The antibodies remain functional while bound to the dendrimers of embodiments of the invention. Gradual release from the dendrimer allows unhindered antibody delivery to target
10 tissue. The dendrimer can be designed to degrade to lower molecular weights, such as fragments having less than 50,000 kDa as described herein, for eventual clearance through the kidney.

[0245] The nanoscale size of dendrimer-antibody conjugates of embodiments of the invention furthermore allows passive targeting of leaky vessels, e.g., tumors or choroidal neovascularization (CNV) through the enhanced permeability and retention (EPR) effect. EPR
15 can enable subcutaneous (SC) or intravenous (IV) administration routes by diminishing off target effects. This could enable SC or IV delivery to CNV areas in the eye.

[0246] Exemplary diseases that can be treated with the dendrimer antibody conjugates of certain embodiments include wet AMD, cancer (e.g., with anti VEGF dendrimer conjugates, IVT or SC); RA, PsA, COPD (e.g., with anti-TNF- α dendrimer conjugates, administration IV or SC);
20 PNH, aHUS, MG, glomerular disease. GA, (e.g., with dendrimer conjugates having anti-C5, ravulizumab or eculizumab, administration IV, IVT, or SC); RA (e.g., with dendrimer conjugates having rifuximab, administration IV); or TED (e.g., with dendrimer conjugates having anti-IGF-1R, administration IV, or SC).

[0247] Further embodiments of the invention relate to methods of treatment of a disease with
25 peptides bound to the dendrimers as described herein. For example, the dendrimers of embodiments of the invention may be used for improving the pharmacokinetics of peptides delivered as a delivery target bound or conjugated to the hyperbranched molecules as described herein. Suitable delivery targets are, for example, selected from anti-C3, C3B, syfovre, GLP-1RA, liraglutide, victosa, saxenda, semaglutide, Ozempic, rybelsus, wegovy, exenatide, hormone
30 therapy, HGH (somatotripin), insulin, esrogen, etc.

[0248] Compared to larger proteins, peptides have the advantage of lower immunogenicity and better stability. However, peptides have the disadvantage of rapid clearance and may have low solubility, which can limit their usefulness in therapy. In embodiments of the invention, conjugation to the dendrimers can be a successful strategy for peptide delivery in therapeutic treatments, e.g., dendrimer Syfovre conjugates, to increase solubility and prolong half-life. Syfovre also benefits from a divalent conjugation, for improved binding avidity. Conjugation to a dendrimer as in embodiments of the invention can go beyond simple PEG conjugation to provide higher molecular weight, more prolonged half-life and higher valency - higher avidity. Gradual dendrimer biodegradability into smaller fragments allows for the clearance of these high molecular weight molecules and prevents accumulation in the body.

[0249] Exemplary diseases that can be treated with the dendrimer peptide conjugates of certain embodiments include GA, PNH (e.g., with anti-C3, C3B dendrimer conjugates, IVT, IV or SC); T2D, obesity (e.g., with GLP-1RA dendrimer conjugates); hormone deficiency syndromes (e.g., with hormone dendrimer conjugates, inhalation, IV or SC).

[0250] Further embodiments of the invention relate to methods of treatment of a disease with aptamers bound to the dendrimers as described herein. For example, the dendrimers of embodiments of the invention may be used for improving the pharmacokinetics of aptamers delivered as a delivery target bound or conjugated to the hyperbranched molecules as described herein. Suitable delivery targets are, for example, selected from anti-C5, Izervay, anti-VEGF165, Macugen, Anti-CXCL12/SDF-1, or NOX-A12.

[0251] Aptamers are similar to peptides with regard to low immunogenicity. However, in vivo stability has been a problem, which can be addressed with conjugation to dendrimers as described herein. Aptamers also have good water solubility. Conjugation to PEG has been a successful strategy for aptamers, e.g., Macugen and Izervay, to prolong half-life. Conjugation to a dendrimer as described herein can go beyond simple PEG conjugation to provide higher molecular weight, more prolonged half-life and higher valency - higher avidity. Gradual dendrimer biodegradability into smaller fragments allows for the clearance of these high molecular weight molecules and prevents accumulation in the body.

[0252] Exemplary diseases that can be treated with the dendrimer aptamer conjugates of certain embodiments include wet AMD (e.g., with anti VEGF165 dendrimer conjugates,); PNH, aHUS,

MG, glomerular disease, GA (e.g., with anti-C5 or izervay dendrimer conjugates); CLL, pancreatic cancer (e.g., with Anti-CXCL12/SDF-1dendrimer conjugates.).

[0253] In order to determine the biologic efficacy of hyperbranched macromolecule-conjugates of certain embodiments of the invention, binding assays tests can be done to analyze binding 5 affinity of peptides conjugated to hyperbranched macromolecules.

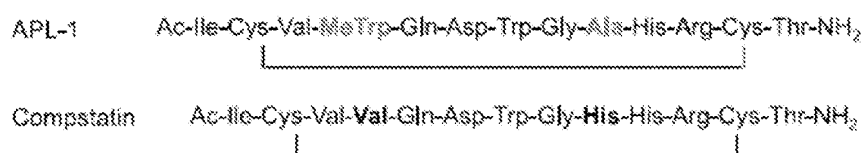
[0254] Complement activation is essential for the development of normal inflammatory responses against foreign pathogens; however, its inappropriate activation has been a cause of tissue injury in many disease states. Complement component C3 is a common denominator in the activation of the classical, alternative, and lectin pathways of complement activation.

10 Uncontrolled complement activation can lead to a wide range of life-threatening or debilitating disorders.

[0255] Compstatin, a 13-mer peptide (le-Cys-Val-Val-Gln-Asp-Trp-Gly-His-His-Arg-Cys-Thr-NH₂) cyclized through a disulfide bridge, is a novel and promising inhibitor of the activation of the complement system and was initially isolated from a phage-displayed random peptide library
15 screened against C3b.

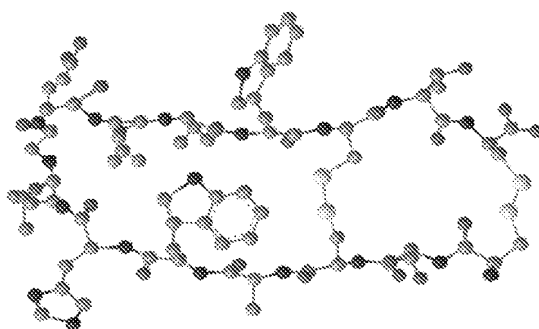
[0256] APL-1 (Ie-Cys-Val-MeTrp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys-Thr-NH₂) has a structure similar to that of compstatin, with 2 different amino acids in the sequence. As reported in the literature, the dissociation constant K_D of APL-1 to C3 is 10nM whereas the K_D of compstatin is 13μM, which is a difference of about one hundreds of times in C3 binding affinity. The structure of compstatin and APL-1 peptide sequences is shown below:

[0257] Scheme C:



[0258] Fc-III-4C is an immunoglobulin G (IgG)-binding peptide ligand, which is composed of 15 residues, where the 4 cysteine residues form 2 disulfide bonds to generate a double cyclic structure. The proposed structure of the Fc-III-4C double cyclic peptide is shown below:

[0259] Scheme D:



5

[0260] The binding affinity of the Fc-III-4C peptide toward human IgG was determined to be 2.45 nM (K_D), which is higher than that of IgG with Protein A/G (Pro-A/G). Importantly, the Fc-III-4C peptide displayed high affinity to various IgGs from different species, so it has also been reported to be used as peptide-based antibody affinity tag.

10 [0261] The three above mentioned peptides can be used to analyze binding affinity of hyperbranched macromolecule-conjugates of these peptides with the use of a surface resonance plasmon setup of Mosaic Biosciences, Inc., USA. Surface plasmon resonance (SPR) binding analysis methodology can be used to study molecular interactions. SPR is an optical technique for detecting the interaction of two different molecules in which one is mobile, and the other is
15 fixed on a thin film. In such an analysis, the C3 target will be fixed on the surface of a thin film or a chip, and a hyperbranched macromolecule-peptide conjugate solution will be passed along it. A difference in signal is monitored when hyperbranched macromolecule-peptide conjugate associate/dissociate on the C3 target.

[0262] These assays demonstrate that the C3 and C3b binding of free compstatin, APL-1 and Fc-
20 III 4C matches well with literature reported values of the dissociation constant K_D , so the chosen assay is a reliable tool.

[0263] The same assay can be applied to test binding affinity of the hyperbranched macromolecule conjugated peptides, in which C3 is immobilized on the assay chip and the hyperbranched macromolecule-peptide conjugate solutions are flown over at a range of concentrations. If multivalent binding of several peptides conjugated to a hyperbranched macromolecule occurs, the hyperbranched macromolecule should dissociate very slowly, while free peptide is expected to dissociate rapidly. This measurement makes it possible to compare dissociation rates of hyperbranched macromolecules of different generations.

[0264] This theoretical prediction has been confirmed by SPR measurement. The comparison of the binding affinity of hyperbranched macromolecule-conjugates of compstatin, APL-1 and Fc-III 4C of certain embodiments of the invention in the same assay show a fast association rate. During the dissociation time, the response from free compstatin drops quickly, the rate is the same as association, as can be expected. On the other hand, hyperbranched macromolecule-peptide conjugates show a much slower dissociation rate. Without wishing to be bound to a theory, it is believed that this is caused by the multiple interaction of several peptides conjugated on the hyperbranched macromolecule, e.g., multivalent binding. The slower dissociation associated phase indicates a cooperative binding of the multivalent conjugated hyperbranched macromolecule-peptides to the C3 surface.

[0265] By similar experiments, it can be shown that the peptide after having been hydrolyzed from the hyperbranched macromolecule during biodegradation has about the same binding affinity as the free peptide. The hydrolyzed peptide contains an acid ester linkage from the degradation (a part of the linker on the hyperbranched macromolecule), and this can be shown not to have any effects to their C3 binding according to the similar SPR signal. It is thus believed that the ester linkage does not change the peptide bioactivity.

[0266] Furthermore, it can be seen in a quantitative K_D analysis that the K_D of hyperbranched macromolecule-conjugates shows a decrease when there is more peptide substituted on the hyperbranched macromolecule.

[0267] With reference to FIG. 18 and 19, an IC_{50} (half maximal inhibitory concentration) has been measured by an alternative pathway (AP) hemolysis assay with four different compstatin conjugated hyperbranched macromolecules of the invention, as described in Example 10. It can be seen that the G1 PEG hyperbranched macromolecule compstatins had improved IC_{50} s

relative to free compstatin suggesting an improvement in potency through avidity. Without wishing to be bound to a theory, it is believed that multiple binding events or multivalent binding being possible with higher generation hyperbranched macromolecule peptide conjugates are contributing to receptor inhibition and increase the efficiency. Further, hyperbranched

5 macromolecule conjugates having longer polymeric arms conjugated to the peptide appear to have improved IC₅₀s, probably due to a higher flexibility for interacting with receptors than shorter polymeric arms that may have steric repulsion problems. These results have been confirmed with a classical pathway (CP) hemolysis assay, showing that higher valency compstatin conjugates of certain embodiments of the invention are more effective at inhibiting

10 CP hemolysis compared to free compstatin alone. Furthermore, as shown in Example 10, dendrimer bound agents, such as APL-1 used in this example, can retain their *in vivo* stability and activity over prolonged periods of time.

Release Kinetics

[0268] In certain embodiments, the hyperbranched macromolecules of the invention can be used

15 for sustained release drug-delivery. In general, conjugation of a therapeutically active agent to a hyperbranched macromolecule can extend the *in vivo* half-life of the agent. The hyperbranched macromolecule structure may be adapted to modify the release of an active agent conjugated at the hyperbranched macromolecule by several measures, to provide a hyperbranched macromolecule-based drug-delivery system. For example, tailoring or suitably selecting the

20 precursor components and DCRU's forming the hyperbranched macromolecule, such as length and molecular weight of polymeric arms, the type of linkers used, and connections formed between the hyperbranched macromolecule parts and used for conjugating etc. have an influence on active agent release.

[0269] Additionally, the release of active agents having multiple binding sites to the dendrimer

25 can be slowed down by multiple binding of the active agent to the dendrimer functional end groups either intramolecularly and/or intermolecularly, i.e. connecting two or more dendrimers via one multiply bonded active agent. For example, multibinding to dendrimers can be used to increase the half-life of active agents, as release of the active agent from the dendrimer requires more than one conjugation bond to be cleaved for fully releasing the active agent.

[0270] Dendrimers for drug delivery can be seen as large support or carrier vehicles. Due to their highly symmetric and regular spheroidal structure, they may be used to extend the hydration or hydrodynamic radius R_h of active agents bound to them. A large hydrodynamic radius of specifically PEG based dendrimer structures can be used to further extend the half-life in vivo, such as in the vitreous body, of the dendrimer drug conjugates, which can be used for controlling and adjusting sustained release of active agents. As can be estimated from the Stokes-Einstein Equation,

$$D = \frac{k_B T}{6\pi\eta R_h}$$

the diffusion rate D of a spherical particle is roughly inverse proportional to the hydrodynamic radius R_h of the particle, given that the temperature T and the viscosity η are relatively constant in physiological fluids in vivo. Thus, the larger the radius of the dendrimer drug conjugate, the slower the diffusion rate, and the longer the half-life $T_{1/2}$ of the active agent in vivo.

[0271] Accordingly, embodiments of the invention make use of the large size of dendrimers to delay the release of active agents in vivo by suitably adjusting the overall size of the dendrimer drug conjugate. Since the hydrodynamic radius R_h can be easily determined, for example by size-exclusion chromatography (SEC), it is possible to predictably adjust the release rate or half-life of an active agent bound to the dendrimer from calibration information of SEC measurements. Example 11 below exemplarily shows how to correlate dendrimer size to release rate.

[0272] Thus, biodegradable synthetic dendrimers of embodiments of the invention offer the advantage of built-in controlled degradable functional groups which upon degradation yield smaller fragments with stepwise lower hydrodynamic radius R_h and different half-lives which dictates their mobility, and/or clearance from the body. As an example, a generation 1 (G1) dendrimer built from a 4 arm 40kDa PEG core and four 4 arm 20kDa dendrons conjugates with 12 peptides or proteins or 1.7kDa each (e.g. 4a40k-PEG(SGA)-[4a20k-PEG(SG)-(Fc-III-4C)₃]₄) has a molecular weight of about 145 kDa. Cleaving off one, two, three or all four of the dendrons will reduce the molecular weight stepwise to produce fragments of about 115 kDa, 90 kDa, 65 kDa, and will finally leave the 40k core and 4 dendrons of each about 25 kDa, with each

fragment having a different hydrodynamic radius and diffusion rate. If in the same macromolecule 20 kDa linear PEG extenders are built in between the core and branch unit arms, the molecular weight cascade includes a dendrimer of about 22 kDa, and degradation fragments of about 175 kDa, 130 kDa, 85 kDa, 45kDa, 40 kDa, 25 and 20 kDa, which dendrimer will have a different, prolonged release rate and a larger distribution of half lives of fragments.

[0273] The multiple half-life aspect of the degradable dendrimers of embodiments of the invention is based on the initial R_h of the dendrimer itself (G1, G2 etc.), followed by another half-life based on the degradable fragments (dendrons or dendron-like or dendron-like with linear PEG extension) formed by cleaving hydrolyzable bonds within the hyperbranched macromolecule structure. These multiple successively degrading species all having different R_h as they degrade and detach from the initial dendrimeric structure, present a range of substructures with different hydrodynamic radius' yielding different clearance rate and half lives. The R_h of these different species is correlated to the different building blocks of different molecular weights, number of arms, linear PEG extensions, and/or linkers. In constructing the hyperbranched dendrimer macromolecules from building blocks of different molecular weight, number of arms, using linear PEG extenders and different length difunctional linkers between the hydrolyzable bonds, the dendrimers of embodiments of the invention can be designed for each individual active agent and/or therapeutic purpose/or administration form to degrade and clear the fragments at multiple rates and half-life.

[0274] Specifically, the built-in degradable linking groups (such as diacid derived esters of linkers based on succinimidyl succinate (SS), succinimidyl glutarate (SG), succinimidyl adipate (SAP), succinimidyl azelate (SAZ), such as succinate diesters (S), glutarate diesters (G), adipate diesters (AP) or azelate diesters (AZ) etc.) can thus be used to tailor and control the release rate of the active agent conjugated or associated with the dendrimer. For example, in one embodiment, dendron building blocks can be made with different degradable linkers (such as SS, SG, SAP, SAZ, etc.) yielding a homogenous dendrimer with all dendrons degrading at the same rate, if the ester linkage is the same.

[0275] In another embodiment, dendron building blocks can be made with different degradable linkages (such as S, G, AP, AZ, etc.) yielding a heterogenous dendrimer with dendrons degrading at different rates if the ester linkages are different. For example, in an embodiment, a

dendrimer can be made with S, G, AP or AZ dendrons or a mixture of such dendrons, clicked on the core structure by click chemistry links.

[0276] In other embodiments, a mixture of several homogenous dendrimers can be blended to tailor a specific release profile and adjust the half-life clearance rate by dry or wet blending.

- 5 [0277] The PEG-based of embodiments of the invention due to their large hydrodynamic radius at low solids content may also be described as nano-droplets. At similar weight or solids content than the agent or peptide itself, the dendrimers have a much higher hydrodynamic radius (see Figure 20 and Example 11), and therefore slower diffusion rate, and longer half-life $T_{1/2}$ of the conjugated active agent in vivo.
- 10 [0278] In certain embodiments, the linker of Formula (ii) used in the hyperbranched macromolecule structure introduces hydrolyzable bonds into the hyperbranched macromolecule that can be used to modify the degradation rate of the hyperbranched macromolecule and/or the release rate of conjugated active agents from the hyperbranched macromolecule. For example, the rate of biodegradation /hydrolyzation of ester bonds at these linkers increases from succinate
- 15 (C4) to azelate (C9). The shorter the diacid linker chain length, the faster the ester bonds it forms are hydrolyzed. Thus, the hydrolysis rate decreases from SS>SG>SAP>SAZ>SGA ester bonds. In embodiments of the invention, this can be used to control the degradation rate of the hyperbranched macromolecule and/or the release of active agents conjugated via these linkers to the hyperbranched macromolecule. For example, esters formed from succinimidyl succinate (SS)
- 20 groups can degrade in the order of a few days, while esters of succinimidyl glutarate (SG) groups degrade in the order of weeks. Different linkers may be used within the hyperbranched macromolecule to control degradation rates among the junctions of different generation DCRU's in the hyperbranched macromolecule, and for conjugating the active agents to control the release of the active agent from the hyperbranched macromolecule.
- 25 [0279] For connections formed by click chemistry reactions, an extension of the spacer structure, e.g., an alkylene chain or a pegylation, between the DBCO/Azide functional group and the functional group with which it binds to a polymeric arm can be used in embodiments of the disclosure to delay hydrolyzation of neighboring ester linkages as well. The larger the distance between the DBCO/Azide functional group and the next hydrolyzable ester group, the slower the
- 30 hydrolysis of the ester occurs. Different linkers within the hyperbranched macromolecule and at

conjugation site of the active agent can be used to enable degradation control. By using short chain linker groups such as succinates for DCRU connections and long chain linkers such as SAZ at the conjugation sites it can be possible during degradation to first break up ester groups within the hyperbranched macromolecule and only later breaking up the DCRU-active agent conjugation. Inversely, first breaking up conjugation sites for active agent release with short chain linkers can be designed on purpose. This can be used to control the half-life of an active agent and to modify the release kinetics.

[0280] Also, depending on chain length of the diacid linkers, the hydrolysis of the ester bonds will depend on pH and/or temperature of the environment. This can be used in certain embodiments to control active agent release for example for site specific release in certain tumor cells having a higher pH than surrounding cells.

[0281] In certain embodiments, the sustained release drug-delivery hyperbranched macromolecule of the present invention is formulated to make an active agent available over an extended period of time, thereby allowing a reduction in dosing frequency compared to an immediate release dosage form, such as for example a solution of an active agent that is topically applied onto the eye (i.e., eye drops). In certain embodiments, the release of the active agent comprises constant active agent release, tapered active agent release as well as any combination thereof such as a constant active agent release followed by a tapered active agent release. The “sustained release” may be measured *in vitro* in an aqueous solution under physiological conditions such as at pH 7.2-7.4 and 37 °C and is considered to be the same or substantially the same when the hyperbranched macromolecule is administered *in vivo* to a subject.

[0282] In various embodiments of the present invention, the active agent release follows zero order release kinetics or substantially zero order release kinetics, preferably without a “burst” of active agent at the beginning of the period.

[0283] Embodiments of the present invention may provide for a release of a therapeutically effective amount of the active agent for a period of time, such as up to 1 year, up to 9 months, up to 6 months, up to 3 months, up to 1 month, or up to about 25 days after administration. Other embodiments of the present invention may provide for a release of a therapeutically effective amount of the active agent of up to about 14 days, or up to about 21 days after administration, or a release of a therapeutically effective amount of the active agent for a period of about 6 hours or

longer after administration, or for a period of about 12 hours, or 24 hours or longer or about 48 or longer, or about 72 hours or longer or about 7 days or longer, or about 10 days or longer after administration. The present invention contemplates all of the above lower and higher time periods in any combination of ranges.

5 [0284] Some aspects of the present disclosure are directed to a pharmaceutically acceptable hyperbranched macromolecule for controlled release of a an active agent conjugated to a hyperbranched macromolecule, wherein the controlled release is characterized by: the amount of the active agent released on day 1 is from 0 to 50% of the total amount of the active agent, the amount of the active agent released per day from day 2 until the last day of release is from 0 to
10 50% of the total amount of the active agent, and/or the number of days required for 100% release of the total amount of the active agent is at least 2 days.

[0285] In one embodiment, the controlled release of the active agent is characterized by the amount of the active agent released on day 1 is from 0 to 50% of the total amount of the active agent, the amount of the active agent released per day from day 2 until the last day of release is
15 from 0 to 50% of the total amount of the active agent, and/or the number of days required for 100% release of the total amount of the active agent is at least 2 days. In another embodiment, the amount of the active agent released on day 1 is from 0 to 25%, 0 to 20% 0 to 10%, 0 to 5%, or about 0% of the total amount of the active agent, the amount of the active agent released per day from day 2 until the last day of release is from 0 to 50% of the total amount of the active
20 agent, and/or the number of days required for 100% release of the total amount of the active agent is at least 3 days but no greater than 30 days, 25 days, or no greater than 16 days.

Sustained release drug delivery system and administration

[0286] The hyperbranched macromolecules of certain embodiments of the invention can be used for drug delivery to a patient, and for example for ophthalmic drug delivery since it offers a
25 number of advantages as a carrier system. The hyperbranched macromolecules can be used for drug delivery, gene delivery, antioxidant delivery, peptide delivery, biomedical imaging, and genetic testing in ophthalmology.

[0287] Hyperbranched macromolecules are able to transport into and out of the cells. Different ocular application routes can be used for drug delivery with the hyperbranched macromolecules,
30 and their tunable properties such as water solubility, permeability, bioavailability, and

biocompatibility can be broadly varied depending on the specific needs of different medical applications.

[0288] In certain embodiments, a sustained release, biodegradable drug-delivery system is provided that comprises the hyperbranched macromolecules as described herein. In certain
5 embodiments of the invention, the hyperbranched macromolecules or the drug-delivery system comprising it may be formulated for direct or indirect administration via diverse routes such as oral, parenteral, or by operative insertion or injection.

[0289] For formulating the drug-delivery system, the hyperbranched macromolecules may be incorporated into a suitable carrier, such as a solvent or solvent mixture, or may be incorporated
10 into a hydrogel, or organogel.

[0290] In certain embodiments, the hyperbranched macromolecules are formulated for direct injection at a treatment site of a patient, for example by parenteral administration, or intra-tumoral injection, injection into the eye such as intravitreal, intracameral, subconjunctival, retrobulbar, sub-tenon, subretinal, and suprachoroidal injections. It may be formulated for
15 injection into the anterior chamber, the vitreous, episcleral, in the posterior subtenon's space (Inferior fornix), subconjunctival, intracameral, peribulbar, retrobulbar, sub-tenon, retinal, subretinal, intracanalicular, intravitreal, intrascleral, choroidal, suprachoroidal, a retina, subretinal, or a lens, a surface of the cornea or the conjunctiva, puncta (canaliculus, upper/lower canaliculus), ocular fornix, upper/lower ocular fornix, subtenon space, choroid, suprachoroid,
20 tenon, cornea, cancer tissue, organ, prostate, breast, joint space, subdural, dental, subcutaneous, carpal tunnel, perivascular, surgically created space or injury, void space, and potential space.

[0291] In embodiments of the invention, the drug-delivery system is used for producing or forming a medical implant, wherein the hyperbranched macromolecules are embedded or dispersed in a hydrogel or organogel matrix.

25 ***Treatment methods***

[0292] According to certain embodiments of the invention, the hyperbranched macromolecules or the biodegradable drug-delivery system comprising the hyperbranched macromolecules are configured for use as a medicament, such as for use in treating a disease or medical condition of a patient.

[0293] In an embodiment, the method for treating a disease or medical condition of a patient comprises administering the hyperbranched macromolecules to the patient in order to release the active agent over an extended period of time.

[0294] A treatment method of an embodiment of the invention comprises an ocular treatment. In such a treatment, the hyperbranched macromolecule is used to release the active agent over an extended period of time in the eye. In an embodiment thereof, the disease or medical condition to be treated is an eye disease, ocular disease such as back-of-the-eye diseases such as any ocular disease of the posterior segment that affects the vasculature and integrity of the retina, macula or choroid leading to visual acuity disturbances, loss of sight or blindness, particularly disease states of the posterior segment resulting from age, trauma, surgical interventions, such as age-related macular degeneration (AMD) cystoid macular edema (CME), diabetic macular edema (DME), posterior uveitis, and diabetic retinopathy.

[0295] The treatment method can also involve treatment of or glaucoma, ocular hypertension, hyphema, presbyopia, cataract, retinal vein occlusion, inflammation, myosis, mydriasis, conjunctivitis, intraocular infections, choroidal neovascularization (CNV), intraocular tumors, and retinal neuroinflammation.

[0296] The ocular disease may further be one of retinal neovascularization, choroidal neovascularization, Wet AMD, Dry AMD, retinal vein occlusion, diabetic macular edema, retinal degeneration, corneal graft rejection, retinoblastoma, melanoma, glaucoma, autoimmune uveitis, uveitis, proliferative vitreoretinopathy, and corneal degeneration, acute and chronic macular neuroretinopathy, central serous chorioretinopathy, macular edema, acute multifocal placoid pigment epitheliopathy, Behcet's disease, birdshot retinochoroidopathy, posterior uveitis, posterior scleritis, serpiginous choroiditis, subretinal fibrosis, uveitis syndrome, Vogt-Koyanagi-Harada syndrome, retinal arterial occlusive disease, central retinal vein occlusion, disseminated intravascular coagulopathy, branch retinal vein occlusion, hypertensive fundus changes, ocular ischemic syndrome, retinal arterial microaneurysms, Coat's disease, parafoveal telangiectasis, hemi-retinal vein occlusion, papillophlebitis, carotid artery disease (CAD), frosted branch angiitis, sickle cell retinopathy, angioid streaks, familial exudative vitreoretinopathy, Eales disease, proliferative vitreal retinopathy, diabetic retinopathy, retinal disease associated with tumors, congenital hypertrophy of the retinal pigment epithelium (RPE), posterior uveal melanoma, choroidal hemangioma, choroidal osteoma, choroidal metastasis, combined

hamartoma of the retina and retinal pigmented epithelium, retinoblastoma, vasoproliferative tumors of the ocular fundus, retinal astrocytoma, intraocular lymphoid tumors, myopic retinal degeneration, acute retinal pigment epithelitis, glaucoma, endophthalmitis, cytomegalovirus retinitis, retinal cancers, retinitis pigmentosa, Leber's Congenital Amaurosis, Choroideremia, X-linked retinitis pigmentosa, best vitelliform macular dystrophy, x-linked retinoschisis, achromatopsia CNGA3, achromatopsia CNGB3, LHON, Stargardt disease, Usher syndrome, Norrie disease, Bardet-Biedl syndrome, and red-green color blindness.

[0297] The methods described in this section can also comprise administration of the hyperbranched macromolecules in combination with another agent, also termed "combination therapy".

[0298] In one embodiment, the combination therapy comprises administering the hyperbranched macromolecules in combination with one or more additional agents either on the same or different day. In one embodiment, the additional agent to be administered in a combination therapy can be a liquid formulation of the agent, or it may be comprised in an oral dosage form. Thus, the additional agent can be any small molecule, large molecule, a protein, a nanoparticle, or any other of the active agents described herein. In another embodiment hyperbranched macromolecules having more than one active agent conjugated to it, such as those available by convergent synthesis described above, may be used for combination therapies involving the administration of more than one active agent. With the hyperbranched macromolecules of certain embodiments it is possible to conjugated different regions on the surface of the hyperbranched macromolecule with different agents.

[0299] The method of treatment comprising administering the hyperbranched macromolecules may comprise any one of intravitreal, intracameral, subconjunctival, retrobulbar, sub-tenon, subretinal, and suprachoroidal injections. The method of administration may also be topical or oral.

[0300] The active agent or the additional agent to be administered in a combination therapy, may also be a diagnostic agent. Diagnostic agents have been described above and may be substances used to examine the body in order to detect impairment of its normal functions. In some cases, diagnostic agents may be agents with a functional purpose, such as for use in the detection of ocular deformities, ailments, and pathophysiological aspects.

[0301] Exemplary embodiments of medical treatments involving drug-conjugated dendrimers of the invention are summarized in Table A below.

[0302] Table A

Treatment	Specific use and advantages of dendrimers
Drug delivery	Dendrimers can encapsulate drugs, improving their solubility, stability, and targeted delivery to specific cells or tissues.
Drug Delivery Systems	The capacity for renal clearance despite the high molecular weight makes these dendritic PEG polymers excellent candidates for the creation of drug delivery systems. They can be engineered to encapsulate therapeutic agents within their structure or attach them to the modifiable end groups, allowing for controlled release. Their biodegradability ensures that they can safely break down within the body after fulfilling their drug delivery function.
Imaging agents	Dendrimers can be functionalized with imaging molecules (e.g., fluorescent dyes or MRI contrast agents) for medical diagnostics and imaging applications.
Imaging and Diagnostics	The unique structure of these dendritic PEGs could enhance contrast agents' delivery for imaging techniques such as MRI or PET scans. Their biocompatibility and renal clearance capability make them safe carriers that can be functionalized with imaging agents.
Nanotechnology	Dendrimers serve as building blocks for nanomaterials and nanodevices, such as sensors, nanoelectronics, and nanocomposites.
Gene therapy	Dendrimers can deliver nucleic acids (e.g., DNA or RNA) for gene therapy applications, enabling efficient transfection and gene expression modulation.
Gene therapy	The ability to introduce different end groups allows these dendrimers to be tailored for gene delivery applications. They can be used to bind and protect nucleic acids, such as DNA or RNA, facilitating their delivery into cells for gene therapy purposes. The biodegradable nature ensures that the carrier degrades after delivering its genetic payload, reducing potential cytotoxicity.
Targeted Cancer Therapy	The adjustable diffusivity and ability to modify surface groups make these polymers ideal for targeted cancer therapies. By conjugating targeting ligands to the surface, these dendritic polymers can be directed towards specific cancer cells, releasing therapeutic agents directly where needed, minimizing side effects on healthy tissue.
Biosensors	Dendrimers can be incorporated into biosensors for detecting various analytes (e.g., biomolecules, pathogens, or pollutants) with high sensitivity and selectivity.

Biomedical materials	Dendrimers can be used in tissue engineering, wound healing, and regenerative medicine due to their biocompatibility and ability to modify surface properties.
Drug Delivery Systems	Dendrimers are utilized in transdermal drug delivery systems, enhancing solubility for drug delivery, and offering targeted drug delivery capabilities
Medical Applications	biomedical studies, magnetic resonance imaging contrast agents, stability enhancement of formulations, and as carriers for nanodrugs
Nanoparticles Synthesis	Dendrimers play a role in synthesizing metallic nanoparticles, such as PAMAM dendrimers used for nanoparticle formation
Therapeutic vectors	Effective amphiphilic dendrimer vectors share two important elements: chemical hydrophilic entities to bind RNA and RNA complex-stabilizing hydrophobicity. These two combined features allow the encapsulation of RNA within a stable complex before its release into the cytosol following endocytosis. This hydrophilic/hydrophobic balance permitted by the structural features of amphiphilic dendrimers plays a determining role in RNA delivery success.
Antimicrobials, antiseptics	
diagnostics	
tissue engineering	Their ability to form hydrogels can be exploited in creating scaffolds for tissue engineering. These scaffolds can support cell growth and tissue regeneration, with the added advantage of controlled release of growth factors or other bioactive molecules to promote healing.
Globular protein mimics	
Therapeutic vaccines	
Tissue Engineering	The ability to form dendrimers through crosslinking makes multi-armed PEG ideal for creating scaffolds that mimic the extracellular matrix, supporting cell growth and tissue regeneration. Wound Healing: These PEG-based hydrogels can be applied as wound dressings that not only provide a moist environment but can also be engineered to release therapeutic agents to promote healing
Tissue Engineering	Dendrimeric polymers can be designed to mimic the extracellular matrix, supporting cell attachment and growth. Their multi-armed structure can be exploited to present multiple signals to cells, promoting tissue formation. The degradation by hydrolysis allows these scaffolds to gradually break down as new tissue forms, making them ideal for regenerative medicine.
Antibacterial and Antifouling Surfaces	By attaching antimicrobial agents to some arms and other functional groups that resist protein adsorption to others, dendrimeric polymers can be used to create surfaces that are both antibacterial and antifouling. This application is particularly

	useful in medical devices and implants, where preventing bacterial colonization is crucial.
Wound healing	Dendrimers can be applied as wound dressings that not only provide a moist environment but can also be engineered to release therapeutic agents to promote healing
targeted therapy	By attaching therapeutic molecules to the modifiable end groups, dendrimers can be designed to release drugs in a controlled manner. This is particularly useful for cancer therapy, where targeted delivery can reduce side effects and improve efficacy
Targeted Drug Delivery	Dendrimeric polymers can be engineered to carry multiple therapeutic agents simultaneously, each attached to different arms or end groups. This allows for the co-delivery of drugs that can synergistically work together, potentially increasing the efficacy of treatments for complex diseases like cancer. The biodegradability of these structures ensures that they can safely break down after their therapeutic payload has been delivered.
Long-term Treatment	For chronic conditions, these polymers can provide a sustained release of medication, reducing the frequency of dosing and improving patient compliance.
Bio adhesives	creation of bio adhesives that can be used in surgical repairs, as sealants to prevent leakage of bodily fluids, or to attach medical devices to tissues.
Smart Delivery Systems	The dendrimeric polymers can be engineered to respond to specific stimuli (pH, temperature, enzymes) to release their load. This can be particularly useful in agriculture for the controlled release of pesticides or fertilizers, minimizing environmental impact and improving efficiency.
Diagnostic Imaging	With different end groups, dendrimeric polymers can be functionalized to carry imaging agents (such as MRI contrast agents or fluorescent molecules) and targeting ligands that seek out specific disease markers. This dual functionality makes them excellent candidates for use in targeted diagnostic imaging, enabling early detection of diseases at the molecular level.
Self-Healing Materials	Incorporating dendritic PEG polymers into composite materials can lead to self-healing materials. The reversible hydrolyzable linkages may allow the material to repair itself when damaged
artificial enzymes	

Industrial and non-medical applications

[0303] In embodiments of the invention, the hyperbranched molecules / dendrimers can be used also for non-medical or industrial applications. In certain embodiments thereof, the dendrimers

do not include hydrolyzable bonds. In other embodiments thereof, the dendrimers may include hydrolyzable bonds as described herein.

[0304] Table B below provides an overview on non-medical and industrial applications and exemplary uses of the dendrimers of embodiments of the present invention.

5 [0305] Table B:

Field of application	Specific use of dendrimers / hyperbranched molecules
Antibody purification	High avidity can reversibly bind antibodies and allow separation from low molecular weight impurities by size-based techniques or by coupling to a solid substrate.
Fragrance release	Can be formulated as an aqueous application that releases fragrance slowly from dendrimer by hydrolysis
Hair products	Bind agents to hair surfaces, dynamic coloring agents (change color over time as different pigments are hydrolyzed away)
Water purification	Can bind specific impurities, allowing separation by size of bound complex. Possibly flocculation and/or filtration. Are recyclable
Coupling agents for high tech adhesives	Structure allows bispecific binding
Sunscreens for skin	Dendrimers can contain titanium dioxide/UV-absorbing moieties as well as other moieties that can associate to the skin (water resistance) but released at high pH (soap)
Solar antennas	
Photocatalysis	
battery	
catalysis	dendrimer can be used to stabilize catalysis agents on surfaces/chips/in solution and still be recovered and separated from final products
catalysis	In catalysis, dendrimers have been proposed to bridge the gap between homogeneous catalysis and surface catalysis, because their sizes enable separation from reaction products by modern membrane techniques
catalysis	Dendrimers with catalytic functionalities can be used in chemical reactions to accelerate or control reactions, improving efficiency and selectivity.
catalysis	The dendrimeric structure, with its large surface area and multiple functional sites, can serve as a support for catalysts. This application could revolutionize various industrial processes, including organic synthesis and the production of renewable energy sources.
magnetic materials	

Electronic devices	Dendrimers can be utilized in electronic devices, such as organic light-emitting diodes (OLEDs) and photovoltaic cells, for improved performance and stability.
Environmental remediation	Dendrimers can be employed for pollutant capture, water purification, and remediation of contaminated sites due to their high surface area and functionalization capabilities
Environmental remediation	The tailored attachment of specific functional groups makes these dendrimers suitable for capturing and removing pollutants from the environment. For example, certain end groups may selectively bind heavy metals or organic pollutants, allowing for their efficient removal from water or soil.
water treatment	Flocculation and Coagulation: Water-soluble polymers are used to aggregate suspended solids in water treatment processes, facilitating their removal. They are used in both municipal and industrial wastewater treatment.
water treatment	Sludge Dewatering: Polymers help in dewatering sludge by binding water molecules, making the sludge easier to filter and dry.
Agriculture	Soil Conditioning: Improve soil structure, promoting water retention and aeration
Agriculture	Water Retention Agents: Used in hydrogels to help soils retain water, reducing the need for frequent irrigation
Coatings and adhesives	Dendrimers can enhance the properties of coatings and adhesives, providing improved adhesion, corrosion resistance, and mechanical strength.
Coatings and adhesives	For agrochemical delivery, these systems can be utilized to release fertilizers, pesticides, or herbicides in a controlled manner, minimizing environmental impact and improving efficiency.
Papermaking	Strength Additives: They are used to enhance the strength of paper.
Retention Aids	dendrimers improve the retention of fillers and fines, reducing the loss of materials and improving the efficiency of the papermaking process.
Light harvesting materials	
Photodynamic therapy	
Transdermal patches/band aids with skin adhesion and localized delivery of antihistamines/antibiotic/sunscreen etc.	
Separating agents	
Enhanced Oil Recovery (EOR)	Water-soluble polymers are injected into oil reservoirs to increase viscosity of the flooding water, improving its effectiveness in pushing oil towards the production wells.

Environmental Sensing and Release Systems	By incorporating sensing mechanisms that respond to environmental stimuli (pH, temperature, enzymes), these dendrimers can be designed to release their cargo only under specific conditions, enhancing the precision of treatments or the protection of sensitive ingredients.
Pollutant Capture and Release	The tunable diffusivity and surface functionality could be exploited in designing systems for the capture and controlled release of pollutants or the delivery of environmental remediation agents. Their biodegradability ensures minimal environmental impact.
Nanofiltration Membranes	The size and structure of these dendritic polymers could make them suitable for creating or enhancing nanofiltration membranes, potentially improving water purification technologies by selectively filtering out pollutants while allowing water molecules to pass through.
textiles	Sizing Agents: Applied to yarns to strengthen and protect them during weaving.
textiles	Finishing Agents: Provide desired properties to textiles, such as softness, crease resistance, and water repellency.
printing inks/nanotubes	
Thickening Agents	Used in cosmetics and personal care products to adjust viscosity and improve texture.
cosmetics	Thickening Agents: Used in cosmetics and personal care products to adjust viscosity and improve texture.
cosmetics	In cosmetic formulations, dendrimers can be used to encapsulate active ingredients, like vitamins or antioxidants, protecting them from degradation and ensuring their controlled release for extended benefits.
detoxification materials	
Food Industry	Thickeners, Gelling Agents, and Stabilizers: Improve texture, consistency, and stability of food products.
Construction	Concrete Additives: Improve the properties of concrete, such as workability, strength, and drying time.
Paint coatings	Paint and Coating Additives: Used to adjust viscosity, improve adhesion, and increase water resistance.
Food Packaging	Incorporating these dendrimers into packaging materials could enable the controlled release of preservatives or antioxidants to extend the shelf life of food products without direct addition to the food.

[0306] Further exemplary application and use embodiments include those of Table C:

[0307] Table C:

Application	Specific use
Surface Chemistry	Tribology, Roughness, contact angle, coatings, lubricating agents

Cosmetics	Color, skin care
detergents	Soaps and dishwashers
membranes	ion specific membranes
fillers	coatings, reinforcements
scaffolds/3D printing	Tissue engineering, bone generation
Phase change materials	diapers, mattresses
Filtration	size/specific separation
wound healing	adhesives
antimicrobials	surgical kits, surfaces
Rheology modifiers	xanthan gum, starches
Energy storage	
Adhesives	Urethanes and epoxy
Food science	flavors, sugars (chewing gum)
Emulsions	polymer synthesis
Stimuli responsive	light sensitive/pH sensitive/pressure sensitive

EXAMPLES

[0308] The following Examples are included to demonstrate certain aspects and embodiments of the invention as described in the claims. It should be appreciated by those of skill in the art, however, that the following description is illustrative only and should not be taken in any way as a restriction of the invention.

[0309] Materials and abbreviations used in the examples:

Degradable di-functional 4arm-PEG-(NHS)3-(azide)1 (10kK, 20k and 40kDa) and 4arm-PEG-DBCO (10k, 20k, 40kDa) were purchased from XIAMEN SINOPEG BIOTECH Co. Ltd. All the NHS terminated PEGs were purchased from JenKem Technology USA.

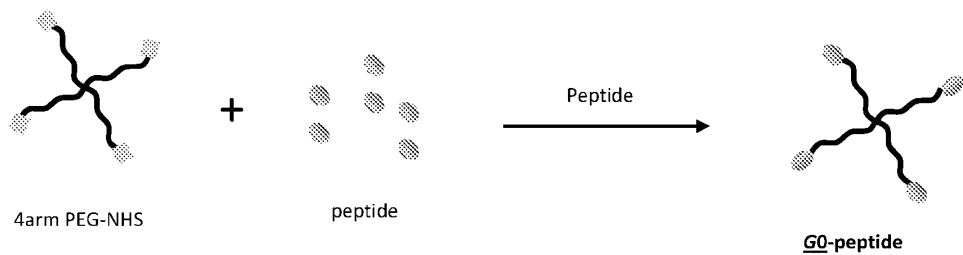
[0310] Compstatin (ICVVQDWGHRCT, Disulfide bridge: Cys2-Cys12, TFA and acetate salt forms) was purchased from MedChemExpress. APL-1 (ICV {L-1-Me-Trp} QDWGAHRCT, Disulfide bridge: Cys2-Cys12, TFA and acetate salt forms), and Fc-III 4C (CDCAWHLGELVWCTC, Disulfide bridge: Cys1-Cys15, Cys3-Cys13, TFA and acetate salt forms) were purchased from Alan Scientific. The structures are shown in FIG. 4.

Solvents and other reagents including methanol, acetonitrile, PBS buffer, triethylamine were purchased from VWR.

EXAMPLE 1

[0311] Divergent synthesis of branched macromolecule G0-peptide conjugates:

[0312] Scheme 1:



[0313] An amount of peptide was weighed and dissolved in anhydrous methanol. 4arm PEG-SS-NHS (MW=40kDa) with molar ratio of 1:6 to peptide was added slowly to the peptide (compstatin) solution with vigorous stirring. A small amount of triethylamine (5-10μL) was dropped into the reaction mixture. The reaction was conducted at room temperature for 1-4 hours. The final product was collected and purified via dialysis against 10kDa molecular weight cutoff tubing in methanol for 24 hours. The purified hyperbranched macromolecule G0-peptide conjugate was collected from the dialysis tubing and the solvent was removed on a rotary evaporator. The dried powder was stored at -20°C for characterization. Formulation is shown in Table 1:

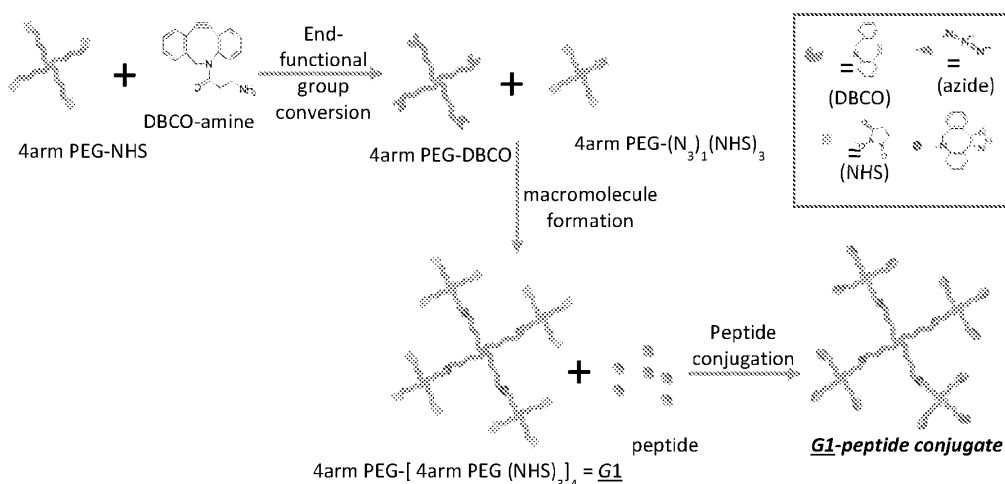
[0314] Table 1:

Synthesis of G0-comp conjugate	Peptide-amine	Ester
	compstatin	4arm 40kPEG(SS)-NHS
End group	NH ₂	NHS
# of functionality/molecule	1	4
MW	1600	40000
theoretical reaction ratio	4	1
Experimental excess reaction ratio	6.25	1
m mole of molecule	0.0125	0.002
m mole of functionality	0.0125	0.008
weight (mg)	20	80
Solvent: anhydrous methanol (mL)	2	2
conc. in reaction mixture (mg/mL)	5	20

EXAMPLE 2

[0315] Divergent synthesis of hyperbranched macromolecule G1-peptide conjugates:

[0316] Scheme 2:



5 End-functional group conversion of 4arm PEG-DBCO:

[0317] An amount of 4arm PEG-NHS (MW=40kDa) was dissolved in anhydrous methanol. DBCO-amine with molar ratio of 1:1 to 4arm PEG-NHS was weighed and dissolved in anhydrous acetonitrile. The DBCO-amine solution was added drop wisely into the 4arm PEG-NHS solution with vigorous stirring. The reaction was conducted at room temperature for 1-4
 10 hours, and the solvent was removed on a rotary evaporator. The dried crude product was used in the next step synthesis. Formulation is shown in Table 2 Step-1.

Hyperbranched macromolecule formation of 4arm PEG-[4arm PEG-(NHS)₃]₄ (G1):

[0318] An amount of 4arm PEG-DBCO was dissolved in anhydrous methanol:acetonitrile=1:1 mixed solvent. 4arm PEG-(N₃)₁(NHS)₃ (MW=20kDa) with molar ratio of 1:1 to 4arm PEG-
 15 DBCO was weighed and dissolved in anhydrous methanol. The 4arm PEG-(N₃)₁(NHS)₃ solution was added slowly into the 4arm PEG-DBCO solution with vigorous stirring. The reaction was conducted at room temperature for 1-4 hours, and the solvent was removed on a rotary

evaporator. The dried crude product can be further purified by dialysis if required. Formulation is shown in Table 2 Step-2.

Peptide conjugation of hyperbranched macromolecule G1-peptide conjugate:

[0319] An amount of peptide was weighed and dissolved in anhydrous methanol. 4arm PEG-
 5 [4arm PEG-(NHS)₃]₄ (G1) with molar ratio of 1:18 to peptide was added slowly to the peptide solution with vigorous stirring. Small amount of triethylamine (5-10μL) was dropped into the reaction mixture. The reaction was conducted at room temperature for 1-4 hours. The final product was collected and purified via dialysis against 40kDa molecular weight cutoff tubing in methanol for 24 hours. The purified hyperbranched macromolecule G1-peptide conjugates was
 10 collected from the dialysis tubing and the solvent was removed on rotary evaporator. The dried powder was stored at -20°C for characterization. Formulation is shown in Table 2 Step-3.

[0320] Table 2

Step 1:	amine	Ester
Synthesis of 4arm 40k PEG-SG-(DBCO) ₄	DBCO-amine	4arm 40kPEG-SG-NHS
End group	NH ₂	NHS
# of functionality/molecule	1	4
MW	276	40000
theoretical reaction ratio	4	1
Experimental excess reaction ratio	6	1
m mole of molecule	0.15	0.025
m mole of functionality	0.15	0.1
weight (mg)	42	1000
Solvent: anhydrous acetonitrile:methanol =1:1 (mL)	2	10
con. In reaction mixture (mg/mL)	3.5	83

Step 2:	azide	DBCO
4arm 40k PEG-SG-[4arm 20k PEG-SG- SG- (NHS) ₃] ₄ = G1	4arm 20k PEG-SG- (N ₃) ₁ (NHS) ₃	4arm 40k PEG-SG- (DBCO) ₄
End group	N ₃	DBCO
# of functionality/molecule	1	4
MW	20000	40000
theoretical reaction ratio	4	1
Experimental excess reaction ratio	4	1
m mole of molecule	0.0125	0.003125

100

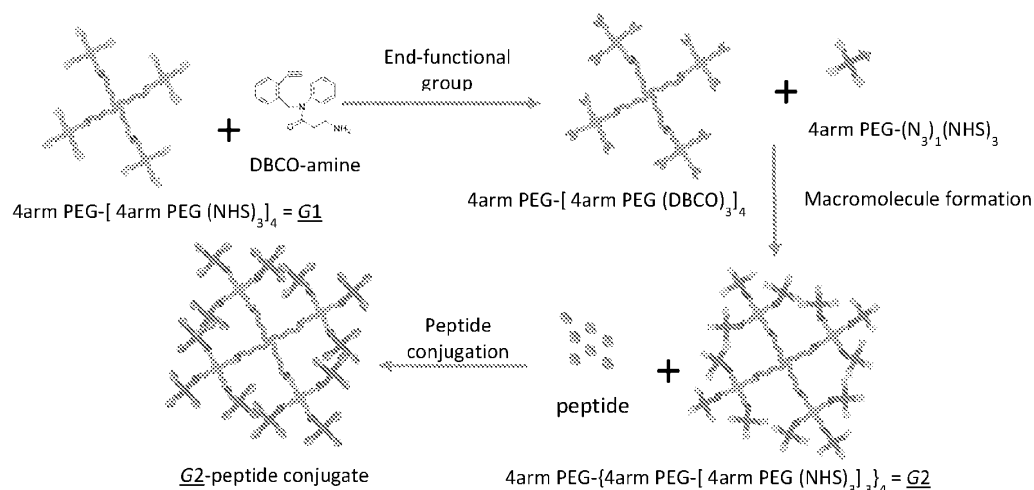
m mole of functionality	0.0125	0.0125
weight (mg)	250	125
Solvent: anhydrous acetonitrile:methanol =1:1 (mL)		1
conc. in reaction mixture (mg/mL)	125	62.5

Step 3:		
Peptide conjugation of 4arm 40k PEG-SG-[4arm 20k PEG-SG-(Comp) ₃] ₄	amine	Ester
	compstatin	4arm 40k PEG-SG-[4arm 20k PEG-SG-(NHS) ₃] ₄ = <u>G1</u>
End group	NH ₂	NHS
# of functionality/molecule	1	3
MW	1550	120000
theoretical reaction ratio	12	1
Experimental excess reaction ratio	24	1
m mole of molecule	0.0129	0.00054
m mole of functionality	0.0129	0.00648
weight (mg)	20	64.5
Solvent: anhydrous acetonitrile:methanol=1:1 (mL)	1	1
conc. in reaction mixture (mg/mL)	10	32

EXAMPLE 3

- 5 [0321] Divergent synthesis of hyperbranched macromolecule G2-peptide conjugates:

[0322] Scheme 3:



End-functional group conversion of 4arm PEG-[4arm PEG (DBCO)₃]₄:

- 5 [0323] An amount of 4arm PEG-[4arm PEG-(NHS)₃]₄ (G1) obtained in step 2 of Example 2 was dissolved in anhydrous methanol. DBCO-amine with molar ratio of 1:1 to 4arm PEG-[4arm PEG-(NHS)₃]₄ (G1) was weighed and dissolved in anhydrous acetonitrile. The DBCO-amine solution was added drop wisely into the 4arm PEG-[4arm PEG-(NHS)₃]₄ (G1) solution with vigorous stirring. The reaction was conducted at room temperature for 1-4 hours, and the solvent
- 10 was removed on a rotary evaporator. The dried crude product was used in the next step synthesis. Formulation is shown in Table 3 Step-1.

Hyperbranched macromolecule formation of 4arm PEG-{4arm PEG-[4arm PEG (NHS)₃]₃]₄

- [0324] An amount of 4arm PEG-[4arm PEG (DBCO)₃]₄ was dissolved in anhydrous methanol:acetonitrile=1:1 mixed solvent. 4arm PEG-(N₃)₁(NHS)₃ (MW=10kDa) with molar
- 15 ratio of 1:1 to 4arm PEG-[4arm PEG (DBCO)₃]₄ was weighed and dissolved in anhydrous methanol. The 4arm PEG-(N₃)₁(NHS)₃ solution was added slowly into the 4arm PEG-[4arm PEG (DBCO)₃]₄ solution with vigorous stirring. The reaction was conducted at room temperature for 1-4 hours, and the solvent was removed on a rotary evaporator. The dried crude product can be further purified by dialysis if required. Formulation is shown in Table 3 Step-2.

- 20 Peptide conjugation of hyperbranched macromolecule G2-peptide conjugate:

- [0325] An amount of peptide was weighed and dissolved in anhydrous methanol. 4arm PEG-
 {4arm PEG-[4arm PEG (NHS)₃]₃}₄ (G2) with molar ratio of 1:18 to peptide was added slowly
 to the peptide solution with vigorous stirring. Small amount of triethylamine (5-10μL) was
 dropped into the reaction mixture. The reaction was conducted at room temperature for 1-4
 5 hours. The final product was collected and purified via dialysis against 40kDa molecular weight
 cutoff tubing in methanol for 24 hours. The purified hyperbranched macromolecule G2-peptide
 conjugates was collected from the dialysis tubing and the solvent was removed on rotary
 evaporator. The dried powder was stored at -20°C for characterization. Formulation is shown in
 Table 3 Step-3.

- 10 [0326] Table 3:

Step 1: Synthesis of 4arm 40k PEG-SG-(DBCO) ₄	amine	Ester
	DBCO-amine	4arm 40k PEG-SG-[4arm 20k PEG-SG-(NHS) ₃] ₄ = <u>G1</u>
End group	NH ₂	NHS
# of functionality/molecule	1	12
MW	276	120000
theoretical reaction ratio	12	1
Experimental excess reaction ratio	24	1
m mole of molecule	0.024	0.001
m mole of functionality	0.024	0.012
weight (mg)	6.6	120
Solvent: anhydrous acetonitrile:methanol=1:1 (mL)	1	9
conc. in reaction mixture (mg/mL)	0.66	12

Step 2: 4arm 40k PEG-SG-{4arm 20k PEG-SG- [4arm 10k PEG-SG-(NHS) ₃] ₃ } ₄ = <u>G2</u>	azide	DBCO
	4a 10k PEG-SG- (N ₃) ₁ (NHS) ₃	4arm 40k PEG-SG-[4arm 20k PEG-SG- (DBCO) ₃] ₄
End group	N ₃	DBCO
# of functionality/molecule	1	12
MW	10000	120000
theoretical reaction ratio	12	1
Experimental excess reaction ratio	12	12
m mole of molecule	0.024	0.001
m mole of functionality	0.024	0.012
weight (mg)	240	120

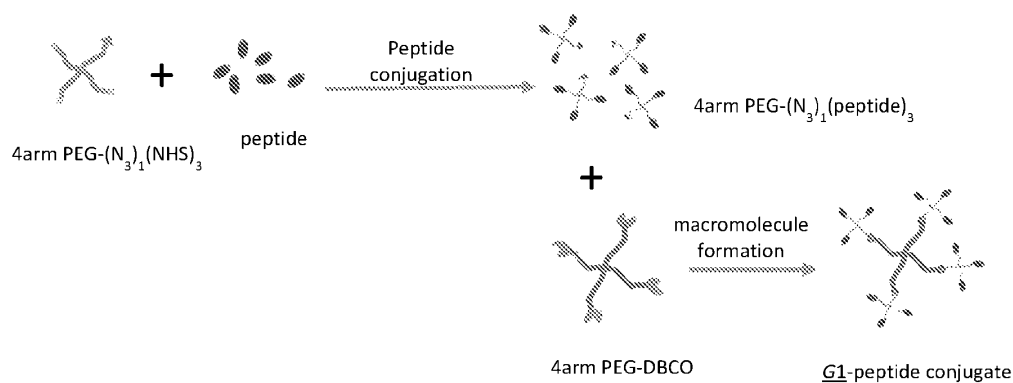
Solvent: anhydrous acetonitrile:methanol=1:1 (mL)	5	5
con. In reaction mixture (mg/mL)	24	12

Step 3: Peptide conjugation of 4arm 40k PEG-SG- {4arm 20k PEG-SG-[4arm 10k PEG-SG- (Comp) ₃] ₃ } ₄	amine compstatin	Ester 4arm 40K PEG-SG-{4arm 20k PEG-SG-[4arm 10k PEG-SG- (NHS) ₃] ₃ } ₄ = <u>G2</u>
End group	NH2	NHS
# of functionality/molecule	1	36
MW	1550	240000
theoretical reaction ratio	36	1
Experimental excess reaction ratio	72	1
m mole of molecule	0.006	0.000083
m mole of functionality	0.006	0.003
weight (mg)	10	20
Solvent: anhydrous acetonitrile:methanol=1:1 (mL)	1	1
conc. in reaction mixture (mg/mL)	5	10

EXAMPLE 4

5 Convergent synthesis hyperbranched macromolecule G1-peptide conjugates:

Scheme 4:



10 Peptide conjugation of 4arm PEG-(N₃)₁(peptide)₃:

[0327] An amount of peptide was weighed and dissolved in anhydrous methanol. 4arm PEG-(N₃)₁(NHS)₃ (MW=20kDa) with molar ratio of 1:4.5 to peptide was added slowly to the peptide solution with vigorous stirring. Small amount of triethylamine (5-10μL) was dropped into the reaction mixture. The reaction was conducted at room temperature for 1-4 hours. The solvent was removed on a rotary evaporator. The dried crude product was used in the next step synthesis. Formulation is shown in Table 4 Step-1.

Hyperbranched macromolecule formation of G1-peptide conjugate:

[0328] An amount of 4arm PEG-(N₃)₁(peptide)₃ was dissolved in anhydrous methanol. 4arm PEG-DBCO (MW=40kDa) with molar ratio of 1:1 to PEG-(N₃)₁(peptide)₃ was weighed and dissolved in anhydrous methanol. The 4arm PEG-(N₃)₁(peptide)₃ solution was added slowly into the 4arm PEG-DBCO solution with vigorous stirring. The reaction was conducted at room temperature for 1-4 hours. The conjugate was collected and purified in a dialysis tubing with molecular weight cutoff of 40kDa in methanol for 24 hours. The solvent was removed on rotary evaporator and the dried powder was stored at -20°C for characterization. Formulation is shown in Table 4 Step-2.

[0329] Table 4:

Step 1:	amine	Ester
Synthesis of 4a 20k PEG-SG-(N ₃) ₁ (Comp) ₃	compstatin	4a 20k PEG-SG-(N ₃) ₁ (NHS) ₃
End group	NH ₂	NHS
# of functionality/molecule	1	3
MW	1550	20000
theoretical reaction ratio	3	1
Experimental excess reaction ratio	6	1
m mole of molecule	0.012	0.002
m mole of functionality	0.012	0.006
weight (mg)	19	40
Solvent: anhydrous methanol (mL)	2	2
conc. in reaction mixture (mg/mL)	5	10

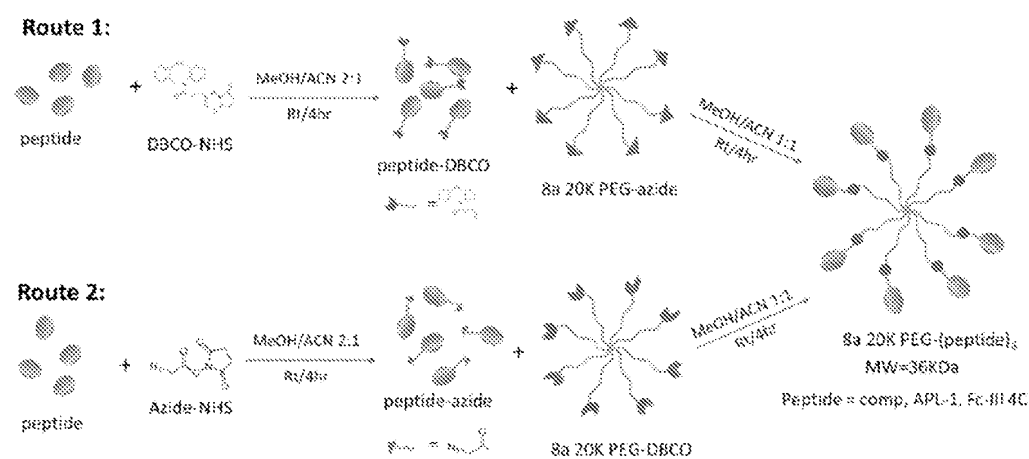
Step 2:	azide	DBCO
4arm 40k PEG-SG-[4arm 20k PEG-SG- (Comp) ₃] ₄	4a 20k PEG-SG-(N ₃) ₁ (Comp) ₃	4arm 40k PEG-SG-(DBCO) ₄
End group	N ₃	DBCO
# of functionality/molecule	1	4

MW	20000	40000
theoretical reaction ratio	4	1
Experimental excess reaction ratio	4	1
m mole of molecule	0.003	0.00075
m mole of functionality	0.003	0.003
weight (mg)	60	30
Solvent: anhydrous methanol (mL)	2	1
conc. in reaction mixture (mg/mL)	20	10

EXAMPLE 5

Peptide conversion and hyperbranched macromolecule G0-conjugation:

Scheme 5:



Route 1:

[0330] *Peptide-DBCO conversion:* Step-1. An amount of peptide (compstatin, MW=1.5kDa) was dissolved in 1mL of mixed solvent (anhydrous methanol:acetonitrile=1:1). DBCO-NHS was weighed and dissolved in 1mL of mixed solvent (anhydrous methanol:acetonitrile=1:1). The peptide solution was added dropwisely into the DBCO-NHS solution with vigorous stirring. The reaction was conducted at room temperature for 1-4 hours. The dried crude product was used in the next step synthesis. Formulation is shown in Table 5 Step-1.

[0331] *Dendrimer formation of 8arm PEG-(peptide)s*: Step-2. An amount of 8arm 20k PEG-Azide was dissolved in 1mL of mixed solvent (anhydrous methanol:acetonitrile=1:1), and then was added slowly into the peptide-DBCO solution with vigorous stirring. The reaction was conducted at room temperature for 1-4 hours, and the solvent was removed on rotary evaporator.

- 5 The dried crude product can be further purified by dialysis if required. Formulation is shown in Table 5 Step-2.

[0332] Table 5:

Step 1: Synthesis of peptide-azide	DBCO-NHS	peptide
MW	402	1550
Weight (mg)	20	37.5
m mole	0.05	0.025
-NHS/amine on molecule	1	1
theoretical reaction ratio	1	1
Experimental excess reaction ratio	2	1
Solvent (acetonitrile: methanol=1:1)	1mL	1mL

Step 2: Synthesis of 8arm PEG-(peptide)s	peptide-DBCO	8arm 20k PEG-Azide
MW	2k	20k
theoretical reaction ratio	8	1
Experimental excess reaction ratio	16	1
Weight (mg)	57.5	36
m mole	0.02875	0.0018
Solvent (acetonitrile: methanol=1:1)	1mL	1mL

10 Route 2:

[0333] *Peptide-Azide conversion*: Step-1. An amount of peptide (compstatin, MW=1.5kDa) was dissolved in 1mL of mixed solvent (anhydrous methanol:acetonitrile=1:1). Azide-NHS was weighed and dissolved in 1mL of mixed solvent (anhydrous methanol:acetonitrile=1:1). The peptide solution was added dropwisely into the Azide-NHS solution with vigorous stirring. The reaction was conducted at room temperature for 1-4 hours. The dried crude product was used in the next step synthesis. Formulation is shown in Table 6 Step-1.

[0334] *Dendrimer formation of 8arm PEG-(peptide)s*: Step-2. An amount of 8arm 20k PEG-DBCO was dissolved in 1mL of mixed solvent (anhydrous methanol:acetonitrile=1:1), and then

was added slowly into the peptide-Azide solution with vigorous stirring. The reaction was conducted at room temperature for 1-4 hours, and the solvent was removed on rotary evaporator. The dried crude product can be further purified by dialysis if required. Formulation is shown in Table 6 Step-2.

5 [0335] Table 6:

Step 1: Synthesis of peptide-azide	azide-NHS	Peptide
MW	198	1550
Weight (mg)	10	37.5
m mole	0.05	0.025
-NHS/amine on molecule	1	1
theoretical reaction ratio	1	1
Experimental excess reaction ratio	2	1
Solvent (acetonitrile: methanol=1:1)	1mL	1mL

Step 2: Synthesis of 8arm PEG-(peptide)s	peptide-azide	8arm 20k PEG-DBCO
MW	2k	20k
theoretical reaction ratio	8	1
Experimental excess reaction ratio	16	1
Weight (mg)	47.5	30
m mole	0.02375	0.0015
Solvent (acetonitrile: methanol=1:1)	1mL	1mL

EXAMPLE 6

Purification of hyperbranched macromolecule and peptide conjugates by dialysis method

10 [0336] Products as obtained in Example 1 to 5 were dissolved in methanol to reach a concentration of 10mg/mL or higher, and the solution (1-5mL) was loaded in a dialysis tubing (Spectra/Por® Float-A-Lyzer G2 Dialysis Devices, Spectrum® Laboratories) with a specific molecular weight cutoff. The tubing was then placed in 500mL of methanol in a beaker at room temperature for 24 hours to separate the lower molecular weight components that diffused out of

15 the tubing. See FIG. 5 a). After treatment, the product was removed from the tubing and collected in a glass vial for characterization.

[0337] Table 7: Dialysis tubing selection

Molecular weight cutoff:	Purpose:
8-10kDa	Remove free peptide
20-50kDa	Remove free peptide, small PEG
50-100kDa	Remove free peptide, small-large PEG

[0338] The products were then characterized by UHPLC. FIG. 6 shows a UHPLC analysis of a G1 4arm 40k PEG-[4arm 20k PEG-SG-(comp)₃]₄ conjugate of Example 2 purified by dialysis in methanol with molecular weight cutoff of 8-10kDa membrane that removes mainly the low molecular weight peptide (MW of about 1.5kDa), in which the blue line is before purification and the green line is after purification. It can be seen that the peak at retention time about 4 minutes, which is attributed to free compstatin, has decreased from 45% to less than 1% in the sample based on peak area integration. On the other hand, a starting material in the mixture, 4arm 40k PEG-SG-(DBCO)₄ (retention time about 9 minutes), MW about 40kDa), which has a molecular weight larger than 8-10kDa, remained in the tubing. Also, the hyperbranched macromolecule conjugate (MW of about 120kDa) remained in the tubing, with a peak shifted slightly to the left from about 8.5 to 9 minutes. Separation of the hyperbranched macromolecule conjugate from the PEG-DBCO precursor can be done by using another dialysis tubing with the higher molecular weight cutoff.

[0339] Table 8: Example 2 product before and after dialysis purification (data based on UHPLC peak area integration).

sample	before purification	after purification
free compstatin	45%	<1%
hyperbranched macromolecule-compstatin conjugate	55%	>99%

20

[0340] These data show that a very efficient purification with greater than 99% purity of hyperbranched macromolecule-peptide-conjugates can be obtained by dialysis.

EXAMPLE 7

Purification of hyperbranched macromolecule and peptide conjugates by SEC column filtration method

[0341] SEC column (Zeba™ Spin Desalting columns) was unpacked, and the column was rinsed with 1mL of methanol for 2 times. Products as obtained in Examples 1 to 5 were dissolved in methanol to reach a concentration of 10mg/mL or higher (1-2mL) and the solution was loaded on the column, the solution flowing through the column by gravity. See FIG. 5b). The eluate was collected in a glass vial for characterization.

[0342] Table 9: SEC column selection

Molecular weight cutoff:	Purpose:
7kDa	Remove free peptide
40kDa	Remove free peptide, small PEG

[0343] FIG. 7 shows a UHPLC analysis of a G0 4arm 40k PEG-SS-compstatin conjugate of Example 1 before (black line) and after purification (blue line). Based on peak area integration, the content of free compstatin decreased from 38.8% to 1.6%, which shows very efficient purification capability.

[0344] Table 10: content change before and after SEC column filtration (data based on UHPLC peak area integration).

sample	before purification	after purification
free compstatin	38.8%	1.6%
PEG-compstatin conjugate	61.2%	98.4%

[0345] These data show that a very efficient purification with greater than 98% purity of hyperbranched macromolecule-peptide-conjugates can be obtained by SEC column filtration.

EXAMPLE 8

Purity and substitution rate by UHPLC

[0346] Ultra-performance liquid chromatography (UHPLC) is an efficient technique which offers more sensitive analysis with good chromatographic separation and resolution of analytes. It provides benefits including fast analysis, high-resolution separations, reduced solvent and sample usage, enhanced sensitivity and precision, etc. A Waters XBridge BEH300 C18 column (3.5 μm, 2.1 x 100 mm, PN1860036080) has been used to characterize hyperbranched macromolecules and hyperbranched macromolecule-peptide conjugates from Examples 1 to 5,

with mobile phase of A (0.1% trifluoroacetic acid in water) and B (0.1% trifluoroacetic acid in acetonitrile).

[0347] For UHPLC analysis, peptide powder was dissolved in PBS : methanol = 9:1 at concentration of 12.5, 25, 50, 100, 200 µg/mL and injected into UHPLC. The peak area of each sample was integrated and used as standard for peptide concentration calculation (cf. FIG. 8a). The inset plot is the standard curve of peptide concentration against peak integration area.

[0348] Dried G0 PEG hyperbranched macromolecule-peptide was dissolved in PBS : methanol = 9:1 at a concentration of 1mg/mL and analyzed by UHPLC. FIG. 8b shows a typical UHPLC graph of a G0 4arm PEG-hyperbranched macromolecule-compstatin conjugate of Example 1, in which the peak with retention time of 22 minutes is from free compstatin, and the peak centered at 42 minutes is from the 4arm PEG-hyperbranched macromolecule-compstatin conjugate. Using the compstatin standard curve, the concentration of each content in the product can be calculated and the peptide substitution can be estimated by the moles of hyperbranched macromolecule-conjugated peptide divided by the moles of PEG as the following equation:

$$peptide\ substitution = \frac{\frac{C\ (conj.\ pep.)}{molecular\ weight\ of\ peptide}}{\frac{C(total\ sample) - [C(conj.\ pep.) + C(free\ pep.)]}{molecular\ weight\ of\ PEG}}$$

[0349] In this equation, C(conj. pep.) is the concentration of conjugated peptide, C(free pep.) is the concentration of free peptide, C(total sample) is the concentration of total solids in the prepared PEG-hyperbranched macromolecule-peptide conjugate sample, i.e. including conjugated peptide, free peptide, and free macromolecule (non-conjugated), the equation resulting in mole of conjugated peptide per mole of macromolecule.

[0350] This method provides a practical way to compare and optimize reaction methods. Compstatin peptide samples from different vendors, Ambeed, Genscript, and MCE, in different salt forms, trifluoro acetate (TFA), acetate, and lysine, were selected to react with the same 4arm 40k PEG-SGA-NHS at the same reaction conditions. Table 11 lists the conjugation results. Overall, the substitution of each peptide on G0 4arm PEG is about 80%, with ±10% variation from each other.

[0351] Table 11:

Vendor	Salt form	# of end groups	Calculated conjugated compstatin per hyperbranched macromolecule	Substitution %
Ambeed	TFA	4	3.7	92.5
Genscript	acetate	4	2.9	72.5
Genscript	Lys	4	3.3	82.5
MCE	Free NH ₂	4	3.2	80.0

- [0352] Compstatin and compstatin-lysine substitution on different PEGs, such as 4arm 40k PEG-SGA and 4arm 40k PEG-SS, have been investigated via the same analysis method. These reactions were run at the same condition with same molar ratio of each compound, only changing the PEGs. Three replicates of each reaction have been applied, and from FIG. 9 these reactions show good reproducibility, with an average substitution of about 3 peptides on 4arm PEGs.
- [0353] Other reaction conditions, such as reaction time, solvent, and use of catalyst, have been investigated and the results are shown in FIG. 10. According to the results, it is clear that reaction in methanol with triethylamine as catalyst is the best condition to achieve highest peptide substitution, whereas reaction time plays a minor role to change the number of substitutions.
- [0354] Table 12 below lists a number of hyperbranched macromolecules, from *G0* to *G2*, that have been synthesized by convergent or divergent method. The highest molecular weight hyperbranched macromolecule is about 240kDa for a *G2* hyperbranched macromolecule, with about 36 end functionalities on the structure. In most of the conjugation results, the peptide substitution was above 50%, which indicated these methods have good reproducibility.
- [0355] Table 12:

Hyperbranched macromolecule structure	Synthesis method	MW of macromolecule	# of end functionality	# of substitution	Substitution (%)
4a 40kPEG-[4a 10k PEG-SG-(comp) ₃] ₄	convergent	80k	12	10.8	90%
4a 40kPEG-[4a 20k PEG-SG-(comp) ₃] ₄	convergent	120k	12	7.0	58%

4a 40k PEG-SS-(comp) ₄	divergent	40k	4	2.9	73%
4a 40k PEG-SG-(comp) ₄	divergent	40k	4	2.6	65%
4a 40kPEG-[4a 10k PEG-SG-(comp) ₃] ₄	convergent	80k	12	6	50%
4a 40kPEG-[4a 10k PEG-SG-(comp) ₃] ₄	convergent	80k	12	6	50%
4a 40kPEG-[4a 20k PEG-SG-(comp) ₃] ₄	convergent	120k	12	5	42%
4a 40kPEG-[4a 20k PEG-SG-(comp) ₃] ₄	convergent	120k	12	8	67%
4a 20kPEG-[4a 10k PEG-SG-(comp) ₃] ₄	divergent	60k	12	3.5	29%
4a 40kPEG-[4a 10k PEG-SG-(comp) ₃] ₄	divergent	80k	12	3.8	32%
4a 20kPEG-[4a 10k PEG-SG-(comp) ₃] ₄	convergent	60k	12	6.1	51%
4a 40kPEG-[4a 10k PEG-SG-(comp) ₃] ₄	convergent	80k	12	8.8	73%
4a 40k PEG-SGA-(comp) ₄	divergent	40k	4	3.7	93%
4a 40k PEG-SGA-(comp) ₄	divergent	40k	4	2.9	73%
4a 40k PEG-SGA-(comp) ₄	divergent	40k	4	3.3	83%
4a 40k PEG-SGA-(comp) ₄	divergent	40k	4	3.2	80%
4a 40kPEG-[4a 20k PEG-SG-(-comp) ₃] ₄	divergent	120k	12	10.8	90%
4a 40kPEG- {[4a 20k PEG-SG-(4a 10k PEG-SG-(comp) ₃] ₃] ₄	divergent	240k	36	29.1	81%

[0356] The same synthesis method has been applied using APL-1 and Fc-III 4C for hyperbranched macromolecule conjugation, and the substitution results are shown in Table 13 and 14. The substitution of APL-1 and Fc-III 4C is lower than that of Compstatin.

[0357] Table 13:

Hyperbranched macromolecule structure	synthesis method	MW of macromolecule	# of end functionality	# of substitution	substitution %
4a 40k PEG-SGA-(APL-1) ₄	divergent	40k	4	1.3	32%

4a 20k PEG-SS-(APL-1) ₄	divergent	20k	4	2.9	72%
4a 40kPEG-[4a 10k PEG-SG-(APL-1) ₃] ₄	convergent	80k	12	5.1	43%
4a 40kPEG-[4a 20k PEG-SG-(APL-1) ₃] ₄	convergent	120k	12	6.4	53%

[0358] Table 14:

Hyperbranched macromolecule structure	synthesis method	MW of macromolecule	# of end functionality	# of substitution	substitution %
4a 20k PEG-[4a 10k PEG-SG-(Fc-III 4C) ₃] ₄	convergent	60k	12	3.3	28%
4a 20k PEG-[4a 20k PEG-SG-(Fc-III 4C) ₃] ₄	convergent	100k	12	3.8	32%
8a 20k PEG-[4a 10k PEG-SG-(Fc-III 4C) ₃] ₈	convergent	100k	24	3.6	15%
8a 20k PEG-[4a 20k PEG-SG-(Fc-III 4C) ₃] ₈	convergent	180k	24	4.5	19%
4a 40k PEG-[4a 10k PEG-SG-(Fc-III 4C) ₃] ₄	convergent	80k	12	1.7	14%
4a 40k PEG-[4a 20k PEG-SG-(Fc-III 4C) ₃] ₄	convergent	120k	12	3.6	30%
4a-40k PEG-SGA-(Fc-III 4C) ₄	divergent	40k	4	1.2	30%
4a-40k PEG-SGA-(Fc-III 4C) ₄	divergent	40k	4	1.1	28%

EXAMPLE 9

Binding assay

[0359] Surface plasmon resonance (SPR) binding analysis methodology obtained from Mosaic Biosciences, Inc. was used to study molecular interactions.

[0360] A Biacore 3000 instrument is used to detect SPR signals. Generally, C3 and C3b were immobilized on the sensor chip surface at a high density (~20 kRU). Aqueous buffered saline solution at pH 7.4 was flowed through the device at a flow rate of 30 μL/min at 25 °C.

Hyperbranched macromolecule-peptide conjugates of embodiments of the invention were injected at concentrations ranging from 1 nM to 300 nM (APL-1 derivatives) or 200 nM to 50 μM (Compstatin derivatives). Association was monitored for 4 minutes, and dissociation for 10 minutes. Equilibrium analysis was performed for compstatin analogs, kinetic analysis with mass transport for APL-1 analogs.

Binding affinity of free peptides

[0361] FIG. 11 and 12 shows C3 and C3b binding of different types of compstatin, and FIG. 13 shows C3 and C3b binding of different types of APL-1. The KD results are summarized in Table 15. From these results it is clear that the free peptides, compstatin and APL-1, show remarkably similar KD value to reported results. This result complies with previous measurements with APL-1 but is lower in affinity than reported by Apellis for APL-2 (200 pM), possibly due to avidity effect for the bivalent APL-2.

[0362] Table 15: KD of C3 and C3b affinity of compstatin and APL-1 and comparison to reference results.

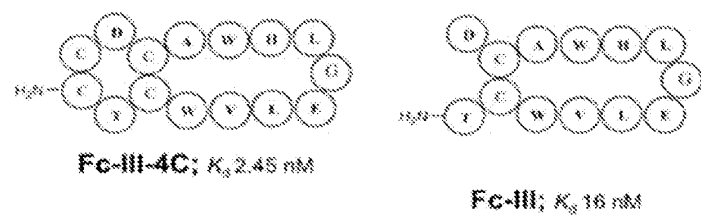
Reagent	Common Derivative	C3 Affinity, K_D	C3b Affinity, K_D	Reported C3 Affinity, K_D
Compstatin-1	Compstatin * (TFA salt)	13.5 μM	8.8 μM	3 – 10 μM
Compstatin-2	Compstatin** (acetate salt)	6.8 μM	4.8 μM	3 – 10 μM
Compstatin-3	Compstatin** (lysine end)	3.3 μM	2.6 μM	3 – 10 μM
Compstatin-4	Compstatin*** (free amine)	10 μM	6.6 μM	3 – 10 μM
APL-1 -1	APL-1** (amine acetylated)	14 nM	15 nM	11 nM (Mosaic)
APL-1 -2	APL-1** (acetate salt)	18 nM	21 nM	11 nM (Mosaic)
APL-1 -3	APL-1** (lysine end)	19 nM	22 nM	11 nM (Mosaic)

*sample purchased from Ambeed, ** sample purchased from Genscript, *** sample purchased from MCE.

[0363] The same experiments have also been conducted for Fc-III 4C. For comparison, another peptide, Fc-III, has also been evaluated under same condition. Fc-III has similar peptide

sequence as Fc-III 4C, but lack of one Cys-Cys bridge. The amino acid sequence structures are as follows:

[0364] Scheme 6:



5 [0365] This structure difference leads to significant antibody binding affinity difference, about 8 times sensitive for Fc-III 4C to antibody (K_D equals 2.45nM vs. 16nM). The SPR results (FIG. 13 and Table 16) also convinced that 3 types of Fc-III 4C shows much smaller binding affinity K_D than that of Fc-III.

[0366] Table 16: K_D of IgG affinity of Fc-III 4C and comparison to reference results.

Reagent	Common Derivative	IgG Affinity, K_D	Reported IgG Affinity, K_D
Fc-III-4c-1	Fc-III-4C* (HCl salt)	7.6 nM	2.5 nM
Fc-III-4c-2	Fc-III-4C* (acetate salt)	11 nM	2.5 nM
Fc-III-4c-3	Fc-III-4C** (acetate salt)	6.7 nM	2.5 nM
Fc-III -4	Fc-III** (acetate salt)	62 nM	16 nM

10 *sample purchased from Genscript, ** sample purchased from Alan Scientific.

Binding affinity of hyperbranched macromolecule conjugated peptides

[0367] The same experiment has been applied to test binding affinity of hyperbranched macromolecule conjugated peptides, in which C3 was immobilized on chip and hyperbranched macromolecule-peptide conjugates were flown over at a range of concentrations.

15 [0368] FIG. 15 shows the comparison of free compstatin and a multi-valency compstatin, 4arm 40k PEG-SGA-(comp)₄. When both of the samples flow over the C3 coated chip, both of them show very fast association rate. After interaction, buffer solution was flown through the chip to

wash away samples associated on it. During this dissociation time, the response from free compstatin drops very quickly, the rate is the same as association. On the other hand, hyperbranched macromolecule-compstatin conjugates show a much slower dissociation rate, which is caused by the multiple interaction of peptides on the hyperbranched macromolecule with the receptors.

FIG. 16 a-c) shows SPR results of the comparison of 3 different hyperbranched macromolecule-comp conjugates to free compstatin. The hyperbranched macromolecule-conjugated compstatin appear to contain both a fast- and slow-dissociating component, in which the slower dissociation associated phase with these constructs indicates cooperative binding of the multivalent hyperbranched macromolecule conjugates to the C3 surface.

[0369] The sample in FIG. 16 d) is a compstatin after hydrolysis from 4a 40k PEG-SS-(comp)₄. This hydrolyzed peptide contains a -succinate- ester linkage from the degradation of the conjugation linker group and it did not show any effects to their C3 binding according to the similar SPR signal. This strongly indicates that the ester linkage did not change the peptide bioactivity.

[0370] The same set of samples with high purity have been detected again to obtain quantitative KD analysis. KD results are listed in Table 17 and plotted against the corresponding number of peptide substitution on each sample. It can be seen that the KD shows a decrease when there is more peptide substituted on hyperbranched macromolecule.

[0371] Table 17: K_D of hyperbranched macromolecule-compstatin conjugates.

Sample	Compstatin substitution	K _D [M]*
-SS-comp (hydrolysis)	1	1.89E-06
4a-40kPEG-SS-(comp) ₄	2.2	1.24E-09
4a-40kPEG-SGA-(comp) ₄	2.4	3.85E-09
4a-40kPEG-SGA-(comp) ₄	2.5	7.46E-09
4a-40k-PEG-[4a-20kPEG-(comp) ₃] ₄	7.1	1.09E-09
4a-40k-PEG-[4a-10kPEG-(comp) ₃] ₄	10.8	1.85E-09

* K_D was calculated using the reported association rate constant for Compstatin ($5e5 \text{ M}^{-1} \text{ s}^{-1}$) and fitting the dissociation rate (k_d) for the multivalent dissociation. The K_D is then calculated as k_d/k_a . K_D for compstatin was measured directly in this experiment.

EXAMPLE 10

5 Alternative Pathway (AP) Hemolysis Assay for IC50 measurement

[0372] The IC50 (half maximal inhibitory concentration) has been measured by an alternative pathway (AP) hemolysis assay. Four compstatin conjugated hyperbranched macromolecules have been used in this assay: Samples No. REA638 (4a 40k-PEG-SGA-(comp)_n TFA salt) and REA639 (4a-40k-PEG-SGA-(comp)_n acetate salt) having 2.6 and 2.5 compstatins per
10 hyperbranched macromolecule, respectively, and samples No. REA640 (4a-40k-PEG-[4a-10kPEG-(comp)_n]₄) and REA641 (4a-40k-PEG-[4a-20k-PEG-(comp)_n]₄) having 10.8 and 7.1 compstatins per hyperbranched macromolecule, respectively (cf. Tables 10 and 15).

[0373] In a 96-well Plate Assay experiment (cf. Fig. 18), Inhibitors (50 μL) are diluted in GVBo (GVBo: 0.1% gelatin, 5 mM barbital, 145 mM NaCl, 0.025% NaN₃, pH 7.3) and incubated with
15 1:2 normal human serum:GVBo at a range of concentration for 30 mins at room temperature. Rabbit RBCs (CompTech) are pelleted at 500 x g for 3 mins and resuspended at 5.0×10^8 cells/mL in MgEGTA (MgEGTA: 0.1 M MgCl₂, 0.1 M EGTA, pH 7.3). Rabbit RBCs (20 μL) was added and incubated at 37°C for 60 mins. Reaction stopped by addition of 200 μL GVBE (GVBE: 0.1% gelatin, 5 mM barbital, 145 mM NaCl, 10 mM EDTA, 0.025% NaN₃, pH 7.3).
20 Cells pelleted at 500 x g for 5 mins and supernatant (150 μL) transferred to new 96-well plate. Calculate % Hemolysis = $(A_{412\text{inhibitor}} / A_{412\text{no inhibitor}}) \times 100$ and fit with 4PL curve fitting to determine IC₅₀. The results are shown in Table 18.

[0374] Table 18: IC50 Results of AP Hemolysis Assay

Sample	IC50 [μM]
Compstatin	6.83
REA638 (4a 40k-PEG-SGA-(comp) _n (TFA salt))	11.7
REA639 (4a-40k-PEG-SGA-(comp) _n acetate salt)	12.3
REA640 (4a-40k-PEG-[4a-10kPEG-(comp) _n] ₄)	3.91
REA641 (4a-40k-PEG-[4a-20k-PEG-(comp) _n] ₄)	1.35

[0375] From the results (FIG. 19 and Table 18), it can be seen that the *G0* 4-armed PEG compstatins (REA638 and REA639 having 2.6 and 2.5 compstatins per hyperbranched macromolecule, respectively) had worse IC₅₀s on a per molecule basis compared to free compstatin. This is not surprising since these molecules have approximately 2 compstatins per molecule and are thus likely unable to achieve avidity from such low substitution. On the other hand, the *G1* PEG hyperbranched macromolecule compstatins (REA640 and REA641 having 10.8 and 7.1 compstatins per hyperbranched macromolecule, respectively) had improved IC₅₀s relative to free compstatin suggesting an improvement in potency through avidity. Additionally, the hill slope of these curves (curve steepness) is lower compared to free compstatin and REA638 and REA639, suggesting multiple binding events contributing to inhibition. Interestingly, REA641, which has 7.1 compstatins, performed better than REA640, which has 10.8 compstatins, despite having a higher valency of the latter. It is believed that the 20k PEG dendrons of REA641 provide a higher flexibility for interacting with C3 than the shorter 10k PEG dendrons of REA640.

15 Classical Pathway (CP) Hemolysis Assay

[0376] The IC₅₀ (half maximal inhibitory concentration) has also been measured by a classical pathway (CP) hemolysis assay, using the same hyperbranched macromolecule conjugates as in the AP hemolysis assay above. This assay is similar in principle to the AP hemolysis assay but uses sheep sensitized red blood cells as the classical pathway initiates with binding of antibodies to cells.

[0377] Serial dilutions of inhibitors (50 μ L) were prepared in gelatin veronal buffer supplemented with Mg²⁺ and Ca²⁺ GVB++ (GVB++: 0.1 % gelatin, 5 mM barbital, 145 mM NaCl, 0.025 % NaN₃, pH 7.3, containing 0.15 mM calcium chloride and 0.5 mM magnesium chloride) in a 96-well plate. C3-depleted human serum supplemented with 12 nM Human C3 was diluted 1:2 in GVB++ (30 μ L) and added to each well and incubated for 30 minutes. Sheep erythrocytes sensitized with anti-sheep pAbs at 5.0 x 10⁸ cells/mL in GVB++ (20 μ L) were added and incubated at 37°C for 30 minutes. The reaction was quenched by the addition of gelatin veronal buffer with EDTA (GVBE, 200 μ L). Cells were pelleted at 500 x g for 5 minutes and supernatant (150 μ L) was transferred to a new 96-well plate. Absorbance at 412 nm was measured on a Molecular Devices SpectraMax M5 plate reader, and the % hemolysis was calculated by % hemolysis = (A_{412inhibitor}/A_{412no inhibitor})*100. IC₅₀s were determined using a

4PL curve fit in GraphPad Prism. The assay was also run in the absence of C3 to determine background hemolysis. The results are shown in Table 19.

[0378] Table 19: IC50 Results of AP Hemolysis Assay

Sample	IC50 [μM]
Compstatin	142
REA638 (4a 40k-PEG-SGA-(comp)n (TFA salt))	56.0
REA639 (4a-40k-PEG-SGA-(comp)n acetate salt)	73.3
REA640 (4a-40k-PEG-[4a-10kPEG-(comp)n]4)	30.8
REA641 (4a-40k-PEG-[4a-20k-PEG-(comp)n]4)	34.6

[0379] Free compstatin had an IC50 = 142 μM. Increasing hyperbranched macromolecule valency to 2.6 and 2.5 for REA638 and REA639 improved the IC50s to 56.0 μM and 73.3 μM respectively. Further increasing the hyperbranched macromolecule valency to 10.8 or 7.1 compstatsins (REA640 and REA641) improved the IC50s to 30.8 and 34.6 μM, respectively. Combined, these data suggest higher valency compstatsins of the invention are more effective at inhibiting CP hemolysis compared to free compstatin alone.

[0380] In the same way as described above, the IC50 (half maximal inhibitory concentration) has also been measured by the classical pathway (CP) hemolysis assay, using samples of a 12 arm G1 hyperbranched molecule 4a40k-PEG(SGA)-[4a20k PEG(SG)-(APL-1)3]4 prepared by convergent synthesis as in Example 4, using APL-1 instead of compstatin. The product has a substitution rate of about 35% of the 12 end groups, which was also used in Examples 11 and 12 below. 32mg x2 (equivalent dendrimer 20 mg x2) of the lyophilized dendrimer was reconstituted in aqueous 90mM sodium phosphate and 360mM NaCl, and 50μL aliquot doses (1.25 mg/eye) thereof where injected into animal eyes (New Zealand white rabbit) for one week. Vitreous humor was harvested from 2 animals/timepoint at 1, 3, 5, and 7 days post dose and analyzed via the classical pathway (CP) hemolysis assay for determining the IC50 over time. The IC50 value of the dendrimer was determined as a control. The results are summarized in Table 20 below.

[0381] Table 20:

Sample	Day 1 IC50 [nM]	Day 3 IC50 [nM]	Day 5 IC50 [nM]	Day 7 IC50 [nM]
Control dendrimer	54.7	-	-	-
Right eye (OD)	43.2	90.2	54.7	39.4
Left eye(OS)	16.4	-	67.2	50.8

[0382] The results show that the dendrimer bound APL-1 retains its in vivo stability and activity over more than 7 days.

5

EXAMPLE 11

Hydrodynamic radius correlation to in vivo half-life

[0383] The hydrodynamic radius R_h of linear pegylated protein (IgG) of different size (IgG 2x40k PEG and 2x20k PEG and their half-life $T_{1/2}$ in New Zealand white rabbit vitreous humor (NZWVH) was determined and compared to the free protein, and several non-conjugated active principles (API's) to obtain a calibration curve allowing the estimation of $T_{1/2}$ of APL-1 conjugated to 12arm120kDa PEG dendrimer based on its R_h determined by SEC. The results are shown in Table 21 below:

[0384] Table 21:

API	Theoretical MW (Mn, kDa)	R _h by SEC (nm)	NZW T1/2 (days)
IgG 2x40k PEG IgG	230	10.39	10.2
2x20k PEG IgG	190	9.27	7.3
IgG	150	4.69	3.3
Bevacizumab	150	4.17	3.7
Aflibercept	115	5.4	4.4
12a120k APL-1**	~120+	9.75	8.7

** 12a120k APL-1 is a generation 1 dendrimer conjugate of the nominal structure 4a40k-

15 PEG(SGA)-[4a20k-PEG(SG)-(APL-1)₃]₄ prepared by convergent synthesis as in Example 4,

using APL-1 instead of compstatin. The product has a substitution rate of about 35% of the 12 end groups.

[0385] As can be seen from Table 20 and Figure 20, the hydrodynamic radius R_h determined by SEC allows for reliable estimates of the half-life of dendrimer drug conjugates of embodiments of the invention depending on dendrimer size, and adjusting sustained release properties thereof.

EXAMPLE 12

Dendrimer degradation effect on in vitro release kinetics

[0386] An APL-1 conjugated generation 1 dendrimer of the nominal structure 4a-40kPEG-[4a-40kPEG-APL-1]₃ prepared by convergent synthesis as in Example 4, using APL-1 instead of compstatin, with a substitution rate of about 35% of the 12 end groups was subjected to in vitro degradation tests under various conditions of temperature and pH to determine degradation / hydrolysis behavior. The tests were done in PBS using HPLC chromatograms to show disappearance of dendrimer and appearance of dendron groups (hydrolysis products) over time.

[0387] Figures 21 a) to c) show the effect of temperature variation from 35 °C to 39°C at constant pH of 7.4. Figure 21 a) shows the decrease of dendrimer concentration over time, figure 21 b) shows the increase of dendron concentration over time, and figure 21 c) shows the impact of temperature on dendrimer % loss rate at pH7.4 on a logarithmic scale, based on first order release kinetics, to determine the rate constant K and the half-life $T_{1/2}$ of active agent release estimated from these degradation rates. The results are in table 22 below:

[0388] Table 22:

Temperature (°C)	$T_{1/2}$	K
35	23.59	0.029
37	21.29	0.033
39	21.37	0.032

[0389] It can be seen that degradation and thus release rate of APL-1 from this generation 1 120kPEG dendrimer is increasing with temperature, with close trends for all three temperatures with the first 26 days. Overall, the temperature effect on half-life of release is low.

[0390] Figures 22 a) to c) show the effect of pH value at constant temperature of 37°C. Figure 22 a) shows the decrease of dendrimer concentration over time, figure 22 b) shows the increase of dendron concentration over time, and figure 22 c) shows the impact of pH on dendrimer % loss rate at pH7.4 on a logarithmic scale, based on first order release kinetics, to determine the rate constant and K and the half-life T1/2 of active agent release estimated from these degradation rates. The results are in table 23 below:

[0391] Table 23:

pH	T1/2	K
7	51.43	0.013
7.4	21.36	0.032
7.8	10.28	0.067
8.5	3.58	0.194

[0392] It can be seen that degradation and thus release rate of APL-1 from this generation 1 120kPEG dendrimer is strongly increasing over time with higher pH. Overall, the pH effect on half-life of release is significant, and follows an exponential model. The higher the pH, the shorter the release half-life.

[0393] These release experiments show that hydrolytic degradation of ester bonds between dendritic building blocks or dendrons can be used to alter or delay the release kinetics of dendrimer conjugated active principles.

Specific sets of embodiments

First set of embodiments

- 1. A hyperbranched macromolecule comprising:
 - a core unit having at least 3 connectivities *c*;
 - a plurality of polymeric arms connected to the core unit at the connectivities *c*,
 - each polymeric arm comprising an end group or being connected to a dendritic constitutional repeating unit,

- the dendritic constitutional repeating unit comprising a branch unit connected to at least two polymeric arms each comprising an end group or being connected to a next dendritic constitutional repeating unit that can again be connected to further dendritic constitutional repeating units, the polymeric arms of the outermost dendritic constitutional repeating unit of the hyperbranched
- 5 macromolecule each comprising an end group;
- wherein the polymeric arms comprise polyethylene glycol (PEG) units;
- wherein at least one active agent is conjugated to at least one of the outermost polymeric arms;
- and
- wherein the hyperbranched macromolecule includes chemical bonds that can be cleaved by
- 10 hydrolysis.
2. The hyperbranched macromolecule according to aspect 1, being a generation G_0 branched macromolecule wherein the end groups of the branched macromolecule are the end groups of the polymeric arms connected to the core unit.
3. The hyperbranched macromolecule according to aspect 1, being a higher generation G_x
- 15 hyperbranched macromolecule, with x being an integer of 1 to 10 defining the number of consecutively connected dendritic constitutional repeating units in the hyperbranched macromolecule.
4. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the core unit and the branch unit are the same or different and independently of each other have a
- 20 connectivity c or c' of 3 to 10, or 4 to 8, or 4 to 6, or 4.
5. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the core unit and the branch unit are the same or different and are each derived from a polyol having at least 3 hydroxyl groups.
6. The hyperbranched macromolecule according to aspect 5, wherein the polyol is selected
- 25 from the group consisting of glycerol, pentaerythritol, xylitol, dipentaerythritol, tripentaerythritol, hexaglycerol, isomalt, lactitol, maltitol, mannitol, or sorbitol.
7. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the polymeric arms comprise polyethylene glycol (PEG) units having an average molecular weight (M_n) in the range from about 1,000 to about 100,000 Daltons, or from about 10,000 to about
- 30 60,000 Daltons, or from about 15,000 to about 50,000 Daltons.

8. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the average molecular weight of the polymeric arm PEG units attached to the core is the same or different than that of the polymeric arms in the dendritic constitutional repeating units.
9. The hyperbranched macromolecule according to any one of the preceding aspects,
5 wherein the average molecular weight of the polymeric arm PEG units attached to the core is higher or lower than that of the polymeric arms in the dendritic constitutional units.
10. The hyperbranched macromolecule according to any one of the preceding aspects, wherein for a higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, the average molecular weight of the polymeric arm PEG units decreases or increases from
10 the innermost polymeric arms to the outermost polymeric arms.
11. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the end groups attached to the outermost polymeric arms are grafted to the termini of the polymeric arms directly or via a suitable difunctional linker comprising hydrolyzable bonds comprising a carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized
15 aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.
12. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the functional groups of the end groups and/or linker-end groups attached to the outermost polymeric arms are functional groups selected from electrophiles such as activated ester groups, such as succinimidyl esters, succinimidyl carbonates; nitrophenyl carbonates, aldehydes, ketones,
20 acrylates, acrylamides, maleimides, vinylsulfones, iodoacetamides, alkenes, alkynes, azides, norbornenes, epoxides, mesylates, tosylates, tresyls, cyanurates, orthopyridyl disulfides, or halogen; nucleophiles such as an amine, such as a primary amine, a hydroxyl, an alcohol, a thiol, an azide, and a carboxyl group; functional groups for click chemistry; functional groups for cycloadditions, such as 1,3-dipolar cycloadditions, [3+2] cycloadditions such as alkene-nitrone
25 cycloadditions or alkyne-nitrone cycloadditions, [4+2] cycloadditions; functional groups for thiol-ene reactions; hetero-Diels-Alder cycloadditions; functional groups for nucleophilic ring openings, functional groups for non-aldol type carbonyl reactions; functional groups for addition reactions to carbon-carbon multiple bonds, polymerizable vinyl groups, or combinations thereof.
13. The hyperbranched macromolecule according to any one of the preceding aspects, wherein
30 the linker-end groups attached to the outermost polymeric arms are functional groups selected from succinimidyl succinate (SS), succinimidyl glutarate (SG), succinimidyl adipate (SAP), succinimidyl azelate (SAZ), and succinimidyl glutaramide (SGA).

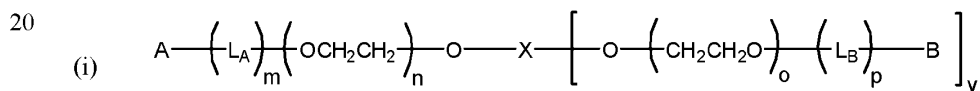
14. The hyperbranched macromolecule according to any one of aspects 1 to 10, wherein the end groups attached to the outermost polymeric arms are functional groups selected from an alkyne compound such as a dibenzocyclooctyne (DBCO), or a bicyclo[6.1.0]-nonyne (BCN); or a norbornene, or a trans-cyclooctene (TCO); an azide, a 3,4 dihydroxyphenylacetic acid (DHPA),
5 or a tetrazine (Tz).
15. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the connection between the polymeric arms connected to the core unit and the first dendritic constitutional repeating unit and/or the connections between consecutive dendritic constitutional repeating units are formed by click chemistry.
- 10 16. The hyperbranched macromolecule according to aspect 15, wherein the connection is formed by reacting a polymeric arm functionalized with an alkyne, cycloalkyne, or strained or terminal alkene moiety with a polymeric arm functionalized with an azide or a tetrazine moiety in a SPAAC or an IEDDA type click chemistry coupling reaction.
- 15 17. The hyperbranched macromolecule according to aspect 16, wherein the alkyne moiety is a dibenzocyclooctyne moiety.
18. The hyperbranched macromolecule according to any one of aspects 15 to 17, wherein the connection between the polymeric arms connected to the core unit and the first dendritic constitutional repeating unit and/or the connections between consecutive dendritic constitutional repeating units are formed between the polymeric arms connected to the core unit and polymeric
20 arms connected to the branch unit of the dendritic constitutional repeating units and/or between the polymeric arms of a dendritic constitutional repeating unit and polymeric arms of consecutive dendritic constitutional repeating units.
19. The hyperbranched macromolecule according to any of the preceding aspects, wherein the active agent conjugated to at least one of the outermost polymeric arms is selected from the group
25 consisting of therapeutically or diagnostically active agents.
20. The hyperbranched macromolecule according to any of the preceding aspects, wherein the active agent conjugated to at least one of the outermost polymeric arms is selected from steroids; non-steroidal anti-inflammatory drugs (NSAIDS) such as Diclofenac, Ibuprofen, Meclofenamate, Mefanamic A, Salsalate, Sulindac, Tolmetin, Ketoprofen, Diflunisal, Piroxicam, Naproxen,
30 Etodolac, Flurbiprofen, Fenoprofen C, Indomethacin, Celecoxib, Ketorolac, Nepafenac; intraocular pressure lowering drugs; antibiotics such as Ciprofloxacin; pain reliever such as Bupivacaine; calcium channel blockers such as Nifedipine; cell cycle inhibitors such as

Simvastatin; proteins such as insulin; small molecule hydrophilic drugs, including carboxylic acid salts and amine salts; small molecule hydrophobic drugs, hydrophilic peptides and protein drugs, such as insulin, single chain antibody fragments, Fab fragments, IgG antibodies, fusion antibodies, etc.; aptamers; particularly Bupivacaine (BPV-HCl or base), Ropivacaine (RPV), Dexamethasone, 5 Travoprost, Axitinib, non-steroidal anti-inflammatory drugs (NSAIDS), steroids, antibiotics, pain relievers, calcium-channel blockers, cell cycle inhibitors, chemotherapeutics, anti-viral drugs, anesthetics, hormones, anticancer drugs, antineoplastic agents, viruses, viruses for gene delivery such as AAV, protein binders such as nanobodies, affibodies, ankyrins, DARPs, etc., or any combinations thereof.

10 21. The hyperbranched macromolecule according to any of the preceding aspects, wherein the active agent conjugated to at least one of the outermost polymeric arms is a peptide selected from the group consisting of Compstatin, APL-1, and Fc-III-4C, Beovu (Brolucizumab), Zimura (Avacincaptad Pegol), Pegcetacoplan, Abicipar Pegol, Lampalizumab, Fovista, Risuteganib, AXT107, Elamipretide, THR149, ALM201, VGB3, and Largazole.

15 22. The hyperbranched macromolecule according to any of the preceding aspects, wherein the active agent is bound to at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or up to 100% of the outermost polymeric arms.

23. The hyperbranched macromolecule according to any of the preceding aspects, wherein a dendritic constitutional repeating unit is represented by Formula (i):



wherein A is a connection to a polymeric arm that is connected to the core unit, or A is a connection to B of a preceding dendritic constitutional repeating unit represented by Formula (i),

25 L_A is a linker,

m is either 0 or 1,

n is an integer from 20 to 2000, o is an integer from 20 to 2000,

n and o can be different or the same,

X is a branch unit having a connectivity c ,

30 L_B is a linker, p is either 0 or 1,

B comprises an end group located at the surface of the hyperbranched macromolecule or is a connection to A of a consecutive dendritic constitutional repeating unit or an active agent,

L_A and L_B can be different or the same,

m and p can be different or the same, and

y is an integer from 2 to 9, wherein $y = c' - 1$ with c' being the connectivity c' of the branch unit X ; and wherein the dendritic constitutional units in the hyperbranched macromolecules may be

5 the same or different.

24. The hyperbranched macromolecule according to aspect 23, wherein the connection between A and B comprises a functional group formed by click chemistry, such as a triazole or dihydropyrazine.

25. The hyperbranched macromolecule according to aspects 23 or 24, wherein the linker L_A and/or L_B comprise a diacid and/or an acid diamido group such as succinate, glutarate, adipate, azelate, or glutaramide.

26. The hyperbranched macromolecule according to any of aspects 23 to 25, wherein the linker L_A and/or L_B comprises a structure represented by Formula (ii):



wherein U^1 and U^2 are independently NH or O and can be the same or different, and wherein t is an integer from 0 to 10.

27. The hyperbranched macromolecule according to aspects 25 or 26, wherein the linker L_A and/or L_B further comprises a polyethylene glycol unit between the bond to B and the carboxyl group, carboxamide group or structure of Formula (ii).

28. A method for manufacturing a hyperbranched macromolecule according to any one of aspects 1 to 27 by divergent synthesis, comprising the following steps:

(a) Providing a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit having functional groups suitable for click chemistry at the termini of the polymeric arms;

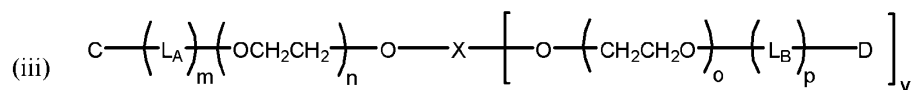
(b) Providing dendritic constitutional repeating unit precursors comprising

- one polymeric arm comprising a functional group suitable for forming a connection by click chemistry with the corresponding functional groups of the polymeric arms connected to the core (such as an azide, alkyne, alkene or tetrazine), and
- at least two polymeric arms comprising functional groups not reactive in click chemistry,

- (c) Forming a connection by click chemistry between the polymeric arms connected to the core and the polymeric arms of the dendritic constitutional repeating unit precursors,
- (d) Optionally converting the functional groups of the at least two polymeric arms comprising functional groups not reactive in click chemistry into functional groups suitable for click chemistry, and
- (e) Conjugating an active agent comprising a functional group to the outermost polymeric arms by reacting with the functional groups of the outermost polymeric arms, thereby forming a hyperbranched macromolecule-active agent conjugate.

29. The method according to aspect 28, wherein for higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, step (d) is compulsory and further consecutive dendritic constitutional repeating unit precursors are connected to the functional groups suitable for click chemistry obtained in step (d) by click chemistry to the hyperbranched macromolecule before conjugating the active agent in step (e).

30. The method according to aspects 28 or 29, wherein the dendritic constitutional repeating unit precursor in step (c) is represented by the Formula (iii):



20

wherein C comprises a functional group suitable for click chemistry, (such as an alkyne, alkene, azide, or tetrazine),

D comprises functional groups not reactive in click chemistry (such as succinimidyl), and L_A , m , n , X , o , L_B , p and y are as defined in aspects 23 to 27; and wherein the dendritic constitutional units may be the same or different.

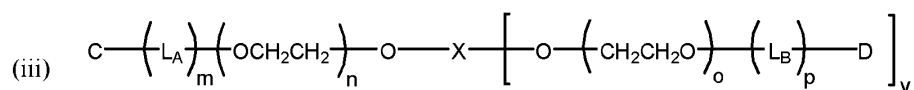
31. The method according to aspects 28 to 30, wherein after a penultimate step (d) of converting the functional groups of the at least two polymeric arms comprising functional groups not reactive in click chemistry into functional groups suitable for click chemistry, the active agent in step (e) is first functionalized with a functional group suitable for click chemistry (such as an alkyne, alkene, azide, or tetrazine) and then conjugated in a click chemistry reaction to the outermost polymeric arms of the hyperbranched macromolecule.

32. The method of aspect 31, wherein the active agent functionalized with a functional group suitable for click chemistry is a peptide.

33. A method for manufacturing a hyperbranched macromolecule according to any one of aspects 1 to 27 by convergent synthesis, comprising the following steps:

- 5 I) Providing dendritic constitutional repeating unit precursors comprising
 - one polymeric arm comprising a functional group suitable for forming a connection by click chemistry (such as an azide, alkyne, alkene or tetrazine), and
 - at least two polymeric arms comprising functional groups not reactive in click chemistry,
- 10 II) Conjugating active agents comprising a functional group to at least one of the at least two polymeric arms comprising functional groups not reactive in click chemistry of the dendritic constitutional repeating unit precursors,
- III) Providing a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit having functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine) at the termini of the polymeric arms, and
- 15 IV) Forming a connection by click chemistry between the polymeric arms connected to the core provided in step III) and the polymeric arm comprising a functional group suitable for forming a connection by click chemistry of the active agent conjugated dendritic constitutional repeating unit precursors obtained in step II), thereby forming a hyperbranched macromolecule-active agent conjugate.

- 20 34. The method according to aspect 33, wherein a dendritic constitutional repeating unit precursor in step I) is represented by the Formula (iii):



- 25 wherein C comprises a functional group suitable for click chemistry (such as an alkyne, alkene, azide, or tetrazine),

D comprises functional groups not reactive in click chemistry (such as succinimidyl), and L_A , L_B , m , n , X , o , p , and y are as defined in aspects 23-27, and wherein the dendritic constitutional units may be the same or different.

- 30 35. The method according to aspects 33 or 34, wherein for higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, the active agent conjugated

dendritic constitutional repeating unit precursors obtained in step II) are connected by click chemistry to reverse dendritic constitutional repeating unit precursors comprising:

- one polymeric arm comprising a functional group not reactive in click chemistry, and
- at least two polymeric arms comprising functional groups suitable for click chemistry

5 (such as an azide, alkyne, alkene or tetrazine),

wherein the functional group not reactive in click chemistry of the one polymeric arm is subsequently converted to a functional group suitable for click chemistry before connecting to further reverse dendritic constitutional repeating unit precursors or before forming a connection by click chemistry with the polymeric arms connected to the core in step IV), thereby forming
10 higher generation hyperbranched macromolecules.

36. The method according to aspects 33 or 34, wherein dendritic constitutional repeating unit precursors having different active agents conjugated to the polymeric arms are obtained by performing steps I) and II) for each active agent conjugated dendritic constitutional repeating unit precursor, and a mixture of the obtained active agent conjugated dendritic constitutional
15 repeating unit precursors is used for step IV), thereby forming a hyperbranched macromolecule-active agent conjugate having different active agents at different regions of the surface of the hyperbranched macromolecule.

37. The method according to any one of aspects 28 to 36, wherein the outermost polymeric arms of the hyperbranched macromolecule have terminal maleimide functional groups, and
20 peptides or active agents are conjugated thereto via maleimide-thiol reaction.

38. The method of aspect 37, wherein the terminal maleimide functional groups are provided by reacting DBCO or azide functionalized terminal functional group of the hyperbranched macromolecule with click chemistry linkers having an azide or DBCO functionality connected to a maleimide group, such as DBCO-maleimide, DBCO-PEG3-maleimide, DBCO-PEG4-
25 maleimide, or azido-PEG3-maleimide.

39. A hyperbranched macromolecule according to any of aspects 1 to 27, for use as a medicament.

40. A method of treatment, wherein the method comprises treating a disease or medical condition in a patient with a hyperbranched macromolecule according to any of aspects 1 to 27.

30 41. The hyperbranched macromolecule for use or the method of treatment according to aspects 39 or 40, wherein the hyperbranched macromolecule is used for an ocular treatment.

42. The hyperbranched macromolecule for use or the method of treatment according to aspects 39 to 41, wherein the hyperbranched macromolecule is used in the treatment of an ocular disease such as back-of-the-eye diseases such as any ocular disease of the posterior segment that affects the vasculature and integrity of the retina, macula or choroid leading to visual acuity
- 5 disturbances, loss of sight or blindness, particularly disease states of the posterior segment resulting from age, trauma, surgical interventions, such as age-related macular degeneration (AMD) cystoid macular edema (CME), diabetic macular edema (DME), posterior uveitis, and diabetic retinopathy.
43. The hyperbranched macromolecule for use or the method of treatment according to
- 10 aspects 39 to 42, wherein the hyperbranched macromolecule is used in the treatment of an ocular disease selected from the group consisting of retinal neovascularisation, choroidal neovascularisation, Wet AMD, Dry AMD, retinal vein occlusion, diabetic macular edema, retinal degeneration, hyphema, presbyopia, corneal graft rejection, retinoblastoma, melanoma, myosis, mydriasis, glaucoma, conjunctivitis, intraocular infections, choroidal neovascularization
- 15 (CNV), intraocular tumors, retinal neuroinflammation, inflammation, autoimmune uveitis, uveitis, proliferative vitreoretinopathy, and corneal degeneration, acute and chronic macular neuroretinopathy, central serous chorioretinopathy, macular edema, acute multifocal placoid pigment epitheliopathy, Behcet's disease, birdshot retinochoroidopathy, posterior uveitis, posterior scleritis, serpiginous choroiditis, subretinal fibrosis, uveitis syndrome, Vogt-Koyanagi-
- 20 Harada syndrome, retinal arterial occlusive disease, central retinal vein occlusion, disseminated intravascular coagulopathy, branch retinal vein occlusion, hypertensive fundus changes, ocular ischemic syndrome, retinal arterial microaneurysms, Coat's disease, parafoveal telangiectasis, hemi-retinal vein occlusion, papillophlebitis, carotid artery disease (CAD), frosted branch angiitis, sickle cell retinopathy, angioid streaks, familial exudative vitreoretinopathy, Eales
- 25 disease, proliferative vitreal retinopathy, diabetic retinopathy, retinal disease associated with tumors, congenital hypertrophy of the retinal pigment epithelium (RPE), posterior uveal melanoma, choroidal hemangioma, choroidal osteoma, choroidal metastasis, combined hamartoma of the retina and retinal pigmented epithelium, retinoblastoma, vasoproliferative tumors of the ocular fundus, retinal astrocytoma, intraocular lymphoid tumors, myopic retinal
- 30 degeneration, acute retinal pigment epithelitis, glaucoma, endophthalmitis, cytomegalovirus retinitis, retinal cancers, retinitis pigmentosa, Leber's Congenital Amaurosis, Choroideremia, X-linked retinitis pigmentosa, best vitelliform macular dystrophy, x-linked retinoschisis,

achromatopsia CNGA3, achromatopsia CNGB3, LHON, Stargardt disease, Usher syndrome, Norrie disease, Bardet-Biedl syndrome, and red-green color blindness.

44. The hyperbranched macromolecule for use or the method of treatment according to any of aspects 39 to 43, wherein the hyperbranched macromolecule is formulated for direct injection at a treatment site of a patient, for example by parenteral administration, intra-tumoral injection, injection into the eye such as intravitreal, intracameral, subconjunctival, retrobulbar, sub-tenon, subretinal, or suprachoroidal injections.

45. The hyperbranched macromolecule for use or the method of treatment according to any of aspects 39 to 44, wherein the hyperbranched macromolecule is administered by direct injection, by oral application, incorporated in gels, or incorporated in implants.

46. The hyperbranched macromolecule for use or the method of treatment according to any of aspects 39 to 46, wherein the hyperbranched macromolecule comprises two or more different active agents at different dendrons or regions on the surface of the hyperbranched macromolecule.

47. The hyperbranched macromolecule for use or the method of treatment according to aspects 46, for use in a combination therapy involving the administration of more than one active agent.

Second set of embodiments

1. A hyperbranched macromolecule comprising building blocks which comprise:
 - a core unit having at least 3 connectivities c ;
 - a plurality of polymeric arms connected to the core unit at the connectivities c ,
 - at least one of the polymeric arms being connected by to a dendritic constitutional repeating unit,
 - the dendritic constitutional repeating unit comprising a branch unit connected to at least two polymeric arms each comprising an end group or being connected by a hydrolyzable bond to a next dendritic constitutional repeating unit that can again be connected by a chemical bond to further dendritic constitutional repeating units,
 - the polymeric arms of the outermost dendritic constitutional repeating unit of the hyperbranched macromolecule each comprising an end group;
- wherein the polymeric arms consist of polyethylene glycol (PEG) units.

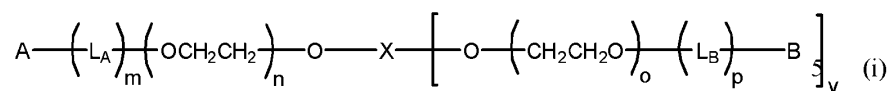
2. The hyperbranched macromolecule according to aspect 1, wherein at least 10%, preferably about 20 to 100 % of the connections in the macromolecule can be cleaved by hydrolysis.
3. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the connections are not hydrolyzable.
- 5 4. The hyperbranched macromolecule according to any one of the previous aspects, being a higher generation G_x hyperbranched macromolecule, with x being an integer of 1 to 10 defining the number of consecutively connected dendritic constitutional repeating units in the hyperbranched macromolecule.
- 10 5. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the core unit and the branch unit are the same or different and independently of each other have a connectivity c or c' of 3 to 10, or 4 to 8, or 4 to 6, or 4.
6. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the core unit and the branch unit are the same or different, and are derived from a polyol having at least 3 hydroxyl groups.
- 15 7. The hyperbranched macromolecule according to aspect 6, wherein the polyol is selected from the group consisting of glycerol, pentaerythritol, xylitol, dipentaerythritol, tripentaerythritol, hexaglycerol, isomalt, lactitol, maltitol, mannitol, or sorbitol.
8. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the polyethylene glycol (PEG) units of the polymeric arms have an average molecular weight (M_n) in the range from about 1,000 to about 100,000 Daltons, or from about 10,000 to about 60,000 Daltons, or from about 15,000 to about 50,000 Daltons, or from about 10,000 to about 40,000 Daltons.
- 20 9. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the average molecular weight of the polymeric arm PEG units attached to the core is the same or different than that of the polymeric arms in the dendritic constitutional repeating units.
- 25 10. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the average molecular weight of the polymeric arm PEG units attached to the core is higher than that of the polymeric arms in the dendritic constitutional units.
- 30 11. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the average molecular weight of the polymeric arm PEG units attached to the core is lower than that of the polymeric arms in the dendritic constitutional units.

12. The hyperbranched macromolecule according to any one of the preceding aspects, wherein for a higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, the average molecular weight of the polymeric arm PEG units
5 decreases from the innermost polymeric arms to the outermost polymeric arms; or wherein the average molecular weight of the polymeric arm PEG units increases from the innermost polymeric arms to the outermost polymeric arms.
13. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the at least one arm connected to the core unit or branch unit is connected to the dendritic constitutional unit via a difunctional linker forming hydrolyzable bonds comprising a
10 carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.
14. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the end groups attached to the outermost polymeric arms are grafted to the termini of the polymeric arms directly, or via a difunctional linker comprising or forming hydrolyzable
15 bonds comprising a carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.
15. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the functional groups of the end groups and/or linker-end groups attached to the outermost polymeric arms are functional groups selected from electrophiles such as activated ester
20 groups, such as succinimidyl esters, succinimidyl carbonates; nitrophenyl carbonates, aldehydes, ketones, acrylates, acrylamides, maleimides, vinylsulfones, iodoacetamides, alkenes, alkynes, azides, norbornenes, epoxides, mesylates, tosylates, tresyls, cyanurates, orthopyridyl disulfides, or halogen; nucleophiles such as an amine, such as a primary
25 amine, a hydroxyl, an alcohol, a thiol, an azide, and a carboxyl group; functional groups for click chemistry; functional groups for cycloadditions, such as 1,3-dipolar cycloadditions, [3+2] cycloadditions such as alkene-nitrone cycloadditions or alkyne-nitrone cycloadditions, [4+2] cycloadditions; functional groups for thiol-ene reactions;
30 hetero-Diels-Alder cycloadditions; functional groups for nucleophilic ring openings, functional groups for non-aldol type carbonyl reactions; functional groups for addition

reactions to carbon-carbon multiple bonds, polymerizable vinyl groups, or combinations thereof.

- 5 16. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the (linker) end groups attached to the outermost polymeric arms are functional groups selected from succinimidyl succinate (SS), succinimidyl glutarate (SG), succinimidyl adipate (SAP), succinimidyl azelate (SAZ), and succinimidyl glutaramide (SGA).
- 10 17. The hyperbranched macromolecule according to any one of aspects 1 to 14, wherein the end groups attached to the outermost polymeric arms are functional groups selected from an alkyne compound such as a dibenzocyclooctyne (DBCO), or a bicyclo[6.1.0]-nonyne (BCN); or a norbornene, or a trans-cyclooctene (TCO); an azide, a 3,4 dihydroxyphenylacetic acid (DHPA), or a tetrazine (Tz).
- 15 18. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the connection between the polymeric arms connected to the core unit and the first dendritic constitutional repeating unit and/or the connections between consecutive dendritic constitutional repeating units are formed by click chemistry, optionally with click chemistry functionalized linkers that include a hydrolyzable bond.
- 20 19. The hyperbranched macromolecule according to aspect 18, wherein the connection is formed by reacting a polymeric arm functionalized, optionally via a linker, with an alkyne, cycloalkyne, or strained or terminal alkene moiety with a polymeric arm functionalized with an azide or a tetrazine moiety in a SPAAC or an IEDDA type click chemistry coupling reaction.
- 25 20. The hyperbranched macromolecule according to aspect 19, wherein the alkyne moiety is a dibenzocyclooctyne moiety.
- 30 21. The hyperbranched macromolecule according to any one of aspects 18 or 20, wherein the connection between the polymeric arms connected to the core unit and the first dendritic constitutional repeating unit and/or the connections between consecutive dendritic constitutional repeating units are formed between the polymeric arms connected to the core unit and polymeric arms connected to the branch unit of the dendritic constitutional repeating units and/or between the polymeric arms of a dendritic constitutional repeating unit and polymeric arms of consecutive dendritic constitutional repeating units, optionally via a difunctional linker forming at least one hydrolyzable bond.

22. A dendritic constitutional repeating unit represented by Formula (i), or the hyperbranched macromolecule according to any of the preceding aspects including the dendritic constitutional repeating unit represented by Formula (i):



wherein A is a connection to a polymeric arm that is connected to the core unit, or A is a connection to B of a preceding dendritic constitutional repeating unit represented by Formula (i),

- 10 L_A is a linker,
 m is either 0 or 1,
 n is an integer from 20 to 2000, o is an integer from 20 to 2000,
 n and o can be different or the same,
 X is a branch unit having a connectivity c' ,
- 15 L_B is a linker, p is either 0 or 1,
 B comprises an end group located at the surface of the hyperbranched macromolecule or is a connection to A of a consecutive dendritic constitutional repeating unit or an active agent,
 L_A and L_B can be different or the same,
- 20 m and p can be different or the same, and
 y is an integer from 2 to 9, wherein $y = c' - 1$ with c' being the connectivity c' of the branch unit X; and wherein the dendritic constitutional units in the hyperbranched macromolecules may be the same or different.
23. The hyperbranched macromolecule according to aspect 22, wherein the connection between A and B comprises a functional group formed by click chemistry, such as a triazole or dihydropyrazine.
24. The hyperbranched macromolecule according to aspects 22 or 23, wherein the linker L_A and/or L_B comprise a diacid and/or an acid diamido group such as succinate, glutarate, adipate, azelate, or glutaramide.
- 30 25. The hyperbranched macromolecule according to any of aspects 22 to 24, wherein the linker L_A and/or L_B comprises a structure represented by Formula (ii):

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wherein U^1 and U^2 are independently NH or O and can be the same or different, and
 wherein t is an integer from 0 to 10.

26. The hyperbranched macromolecule according to aspects 24 or 25, wherein the linker L_A and/or L_B further comprises a polyethylene glycol unit between the bond to B and the carboxyl group, carboxamide group or structure of Formula (ii).

27. The hyperbranched macromolecule according to any of the preceding aspects, wherein the hyperbranched macromolecule further comprises at least one extender unit comprising polyethylene glycol (PEG) units, wherein the extender unit is linear, difunctional and connected to the polymeric arm of a dendritic constitutional repeating unit or the polymeric arm connected to the core unit and to either an end group or a polymeric arm of a next dendritic constitutional repeating unit.

28. The hyperbranched macromolecule according to aspect 27, wherein the extender unit comprises at least one linker, wherein the linker can be located at either terminus or both termini of the extender unit and is a difunctional linker comprising hydrolyzable bonds comprising a carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.

29. The hyperbranched molecule according to any one of the preceding aspects, wherein at least one, or all, preferably all, building blocks selected from core unit, core unit including polymeric arms at the connectivities c , dendritic constitutional repeating unit, linkers and extenders, between hydrolyzable bonds, have a molecular weight less than 50,000 Daltons, such as less than 45,000 Daltons, or less than 40,000 Daltons, or less than 35,000 Daltons, or less than 30,000 Daltons.

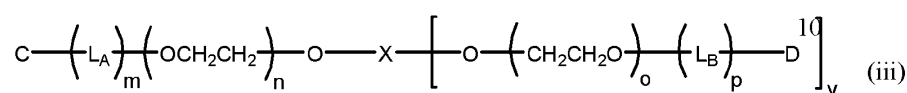
30. The hyperbranched molecule according to any one of the preceding aspects, wherein upon complete hydrolysis of the hydrolyzable bonds all fragments formed of the molecule have a molecular weight less than 50,000 Daltons, such as less than 45,000 Daltons, or less than 40,000 Daltons, or less than 35,000 Daltons, or less than 30,000 Daltons.

31. A dendritic constitutional repeating unit precursor comprising:

- one polymeric arm comprising a functional group suitable for forming a connection by click chemistry (such as an azide, alkyne, alkene or tetrazine), and
- at least two polymeric arms comprising functional groups not reactive in click chemistry,

wherein the polymeric arms are connected to a branch unit having a connectivity c' ,
 wherein the polymeric parts of the polymeric arms consist of polyethylene glycol (PEG) units.

32. The precursor of aspect 31, wherein the compound is represented by the Formula (iii):



wherein C comprises a functional group suitable for click chemistry, (such as an alkyne, alkene, azide, or tetrazine),

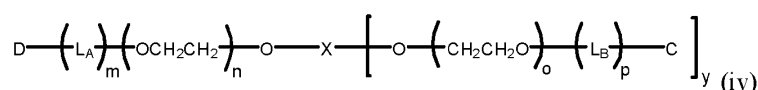
D comprises functional groups not reactive in click chemistry (such as succinimidy), and
 L_A , m, n, X, o, L_B , p and y are as defined in the previous aspects.

33. A reverse dendritic constitutional repeating unit precursor comprising:

- one polymeric arm comprising a functional group not reactive in click chemistry, and
- at least two polymeric arms comprising functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine), and
- a branch unit having a connectivity c' ;

wherein the polymeric parts of the polymeric arms consist of polyethylene glycol (PEG) units.

34. The precursor of aspect 33, wherein the precursor is represented by the Formula (iv):

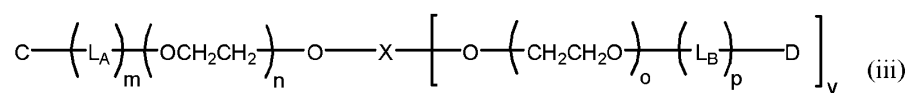


wherein C comprises a functional group suitable for click chemistry, (such as an alkyne, alkene, azide, or tetrazine),

D comprises functional groups not reactive in click chemistry (such as succinimidy), and
 L_A , m, n, X, o, L_B , p and y are as defined in the previous aspects.

35. A method for manufacturing a hyperbranched macromolecule according to any one of aspects 1 to 34 by divergent synthesis, comprising the following steps:

- (a) Providing a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit having functional groups suitable for click chemistry at the termini of the polymeric arms;
- (b) Providing dendritic constitutional repeating unit precursors comprising
- one polymeric arm comprising a functional group suitable for forming a connection by click chemistry with the corresponding functional groups of the polymeric arms connected to the core (such as an azide, alkyne, alkene or tetrazine), and
 - at least two polymeric arms comprising functional groups not reactive in click chemistry,
- (c) Forming a connection by click chemistry between the polymeric arms connected to the core and the polymeric arms of the dendritic constitutional repeating unit precursors,
- (d) Optionally converting the functional groups of the at least two polymeric arms comprising functional groups not reactive in click chemistry into functional groups suitable for click chemistry, and
- (e) Conjugating an active agent comprising a functional group to the outermost polymeric arms by reacting with the functional groups of the outermost polymeric arms, thereby forming a hyperbranched macromolecule-active agent conjugate.
36. The method according to aspect 35, wherein for higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, step (d) is compulsory, and further consecutive dendritic constitutional repeating unit precursors are connected to the functional groups suitable for click chemistry obtained in step (d) by click chemistry to the hyperbranched macromolecule before conjugating the active agent in step (e).
- The method according to aspects 35 or 36, wherein the dendritic constitutional repeating unit precursor in step (c) is represented by the Formula (iii):



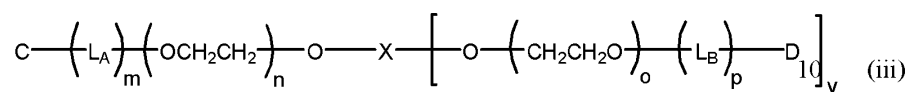
wherein C comprises a functional group suitable for click chemistry, (such as an alkyne, alkene, azide, or tetrazine),

D comprises functional groups not reactive in click chemistry (such as succinimidyl), and L_A , m , n , X , o , L_B , p and y are as defined in the previous aspects; and wherein the dendritic constitutional units may be the same or different.

37. The method according to aspects 35 to 37, wherein after a penultimate step (d) of
5 converting the functional groups of the at least two polymeric arms comprising functional groups not reactive in click chemistry into functional groups suitable for click chemistry, the active agent in step (e) is first functionalized with a functional group suitable for click chemistry (such as an alkyne, alkene, azide, or tetrazine) and then conjugated in a click chemistry reaction to the outermost polymeric arms of the hyperbranched macromolecule.
- 10 38. The method of aspect 38, wherein the active agent functionalized with a functional group suitable for click chemistry is a peptide, such as one of Compstatin, APL-1, Fc-III-4C, Beovu (Brolucizumab), Zimura (Avacincaptad Pegol), Pegcetacoplan, Abicipar Pegol, Lampalizumab, Fovista, Risuteganib, AXT107, Elamipretide, THR149, ALM201, VGB3, and Largazole, or an angiogenesis inhibitors such as anti-VEGF agents (e.g.,
15 aflibercept, ranibizumab, bevacizumab), PDGF-B inhibitors (e.g., Fovista®), complement antagonists (e.g., eculizumab), tyrosine kinase inhibitors (e.g., sunitinib, axitinib), and/or integrin antagonists (e.g., natalizumab and vedolizumab).
39. A method for manufacturing a hyperbranched macromolecule according to any one of aspects 1 to 34 by convergent synthesis, comprising the following steps:
- 20 I) Providing dendritic constitutional repeating unit precursors comprising
- one polymeric arm comprising a functional group suitable for forming a connection by click chemistry (such as an azide, alkyne, alkene or tetrazine), and
 - at least two polymeric arms comprising functional groups not reactive in
25 click chemistry,
- II) Conjugating active agents comprising a functional group to at least one of the at least two polymeric arms comprising functional groups not reactive in click chemistry of the dendritic constitutional repeating unit precursors,
- III) Providing a core unit having at least 3 connectivities c , a plurality of
30 polymeric arms connected to the core unit having functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine) at the termini of the polymeric arms, and

IV) Forming a connection by click chemistry between the polymeric arms connected to the core provided in step III) and the polymeric arm comprising a functional group suitable for forming a connection by click chemistry of the active agent conjugated dendritic constitutional repeating unit precursors obtained in step II), thereby forming a hyperbranched macromolecule-active agent conjugate.

40. The method according to aspect 40, wherein a dendritic constitutional repeating unit precursor in step I) is represented by the Formula (iii):



wherein C comprises a functional group suitable for click chemistry (such as an alkyne, alkene, azide, or tetrazine),

D comprises functional groups not reactive in click chemistry (such as succinimidyl), and

L_A , L_B , m, n, X, o, p, and y are as defined in the previous aspects, and wherein the dendritic constitutional units may be the same or different.

41. The method according to aspects 40 or 41, wherein for higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, the active agent conjugated dendritic constitutional repeating unit precursors obtained in step II) are connected by click chemistry to reverse dendritic constitutional repeating unit precursors comprising:

- one polymeric arm comprising a functional group not reactive in click chemistry, and
- at least two polymeric arms comprising functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine),

wherein the functional group not reactive in click chemistry of the one polymeric arm is subsequently converted to a functional group suitable for click chemistry before connecting to further reverse dendritic constitutional repeating unit precursors or before forming a connection by click chemistry with the polymeric arms connected to the core in step IV), thereby forming higher generation hyperbranched macromolecules.

42. The method according to aspects 40 to 42, wherein dendritic constitutional repeating unit precursors having different active agents conjugated to the polymeric arms are obtained

by performing steps I) and II) for each active agent conjugated dendritic constitutional repeating unit precursor, and a mixture of the obtained active agent conjugated dendritic constitutional repeating unit precursors is used for step IV), thereby forming a hyperbranched macromolecule-active agent conjugate having different active agents at different regions of the surface of the hyperbranched macromolecule.

43. The method according to any one of aspects 40 to 43, wherein the outermost polymeric arms of the hyperbranched macromolecule have terminal maleimide functional groups, and peptides or active agents are conjugated thereto via maleimide-thiol reaction.

44. The method of aspect 44, wherein the terminal maleimide functional groups are provided by reacting DBCO or azide functionalized terminal functional group of the hyperbranched macromolecule with click chemistry linkers having an azide or DBCO functionality connected to a maleimide group, such as DBCO-maleimide, DBCO-PEG3-maleimide, DBCO-PEG4-maleimide, or azido-PEG3-maleimide.

45. A use of a hyperbranched macromolecule according to any of aspects 1 to 34, in at least one application selected from non-medical fields or industrial uses, such as antibody purification, cosmetic applications, catalytic applications, applications in electronics, agriculture, food, filtration, energy storage, construction materials, coatings, adhesives, water purification, oil recovery, fragrance release, paper making, environmental sensing and release Systems, membranes, textiles, printing inks, surface chemistry applications, thickeners, detergents, rheology modifiers, scaffolding, or 3D-printing.

Third set of embodiments

1. A hyperbranched macromolecule comprising building blocks which comprise:

a core unit having at least 3 connectivities c ;

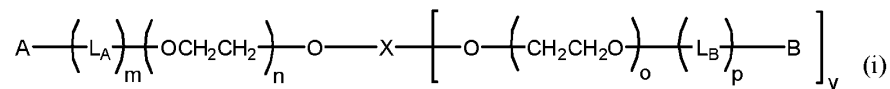
a plurality of polymeric arms connected to the core unit at the connectivities c ,

at least one of the polymeric arms being connected by a hydrolyzable bond to a dendritic constitutional repeating unit,

the dendritic constitutional repeating unit comprising a branch unit connected to at least two polymeric arms each comprising an end group or being connected by a hydrolyzable bond to a next dendritic constitutional repeating unit that can again be connected by a chemical bond to further dendritic constitutional repeating units,

wherein the dendritic constitutional repeating unit is represented by Formula (i):

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wherein A is a connection to a polymeric arm that is connected to the core unit, a connection to an extender unit, or A is a connection to B of a preceding dendritic constitutional repeating unit represented by Formula (i),

L_A is a linker,

m is either 0 or 1,

n is an integer from 20 to 2000, o is an integer from 20 to 2000,

n and o can be different or the same,

X is a branch unit having a connectivity c' ,

L_B is a linker, p is either 0 or 1,

B comprises an end group located at the surface of the hyperbranched macromolecule or is a connection to A of a consecutive dendritic constitutional repeating unit or an active agent,

L_A and L_B can be different or the same,

m and p can be different or the same, and

y is an integer from 2 to 9, wherein $y = c' - 1$ with c' being the connectivity c' of branch unit X; and wherein the dendritic constitutional units in the hyperbranched macromolecules may be the same or different;

wherein the polymeric arms of the outermost dendritic constitutional repeating unit of the hyperbranched macromolecule each comprising an end group; and

wherein at least one active agent is conjugated to at least one of the outermost polymeric arms.

2. The hyperbranched macromolecule according to aspect 1, wherein at least 10%, preferably about 20 to 100 % of the connections in the macromolecule can be cleaved by hydrolysis.
3. The hyperbranched macromolecule according to any one of the preceding aspects, wherein each of the building blocks (fragments) of the hyperbranched macromolecule obtained after cleaving all hydrolyzable bonds of the connections in the macromolecule has an average molecular weight (Mn) of less than 50,000 Daltons, such as less than 45,000 Daltons, or less than 40,000 Daltons, or less than 35,000 Daltons, or less than 30,000 Daltons.

4. The hyperbranched macromolecule according to any one of the preceding aspects, wherein at least one of the building blocks comprises a core unit, or a branch unit, having a plurality of polymeric arms connected by non-hydrolyzable bonds to the core unit or branch unit.
5. The hyperbranched macromolecule according to any one of the previous aspects, being a higher generation G_x hyperbranched macromolecule, with x being an integer of 1 to 10 defining the number of consecutively connected dendritic constitutional repeating units in the hyperbranched macromolecule.
6. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the core unit and the branch unit are the same or different and independently of each other have a connectivity c or c' of 3 to 10, or 4 to 8, or 4 to 6, or 4.
7. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the core unit and the branch unit are the same or different, and are derived from a polyol having at least 3 hydroxyl groups.
8. The hyperbranched macromolecule according to aspect 7, wherein the polyol is selected from the group consisting of glycerol, pentaerythritol, xylitol, dipentaerythritol, tripentaerythritol, hexaglycerol, isomalt, lactitol, maltitol, mannitol, or sorbitol.
9. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the polyethylene glycol (PEG) units of the polymeric arms have an average molecular weight (M_n) in the range from about 1,000 to about 100,000 Daltons, or from about 10,000 to about 60,000 Daltons, or from about 15,000 to about 50,000 Daltons, or from about 10,000 to about 40,000 Daltons.
10. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the average molecular weight of the polymeric arm PEG units attached to the core is the same or different than that of the polymeric arms in the dendritic constitutional repeating units.
11. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the average molecular weight of the polymeric arm PEG units attached to the core is higher than that of the polymeric arms in the dendritic constitutional units.
12. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the average molecular weight of the polymeric arm PEG units attached to the core is lower than that of the polymeric arms in the dendritic constitutional units.

13. The hyperbranched macromolecule according to any one of the preceding aspects, wherein for a higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, the average molecular weight of the polymeric arm PEG units decreases from the innermost polymeric arms to the outermost polymeric arms; or
5 wherein the average molecular weight of the polymeric arm PEG units increases from the innermost polymeric arms to the outermost polymeric arms.
14. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the at least one arm connected to the core unit or branch unit is connected to the dendritic constitutional unit via a difunctional linker forming hydrolyzable bonds comprising a
10 carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.
15. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the end groups attached to the outermost polymeric arms are grafted to the termini of the polymeric arms directly, or via a difunctional linker comprising or forming hydrolyzable
15 bonds comprising a carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.
16. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the functional groups of the end groups and/or linker-end groups attached to the outermost
20 polymeric arms are functional groups selected from electrophiles such as activated ester groups, such as succinimidyl esters, succinimidyl carbonates; nitrophenyl carbonates, aldehydes, ketones, acrylates, acrylamides, maleimides, vinylsulfones, iodoacetamides, alkenes, alkynes, azides, norbornenes, epoxides, mesylates, tosylates, tresyls, cyanurates, orthopyridyl disulfides, or halogen; nucleophiles such as an amine, such as a primary
25 amine, a hydroxyl, an alcohol, a thiol, an azide, and a carboxyl group; functional groups for click chemistry; functional groups for cycloadditions, such as 1,3-dipolar cycloadditions, [3+2] cycloadditions such as alkene-nitrone cycloadditions or alkyne-nitrone cycloadditions, [4+2] cycloadditions; functional groups for thiol-ene reactions; hetero-Diels-Alder cycloadditions; functional groups for nucleophilic ring openings,
30 functional groups for non-aldol type carbonyl reactions; functional groups for addition reactions to carbon-carbon multiple bonds, polymerizable vinyl groups, or combinations thereof.

17. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the (linker) end groups attached to the outermost polymeric arms are functional groups selected from succinimidyl succinate (SS), succinimidyl glutarate (SG), succinimidyl adipate (SAP), succinimidyl azelate (SAZ), and succinimidyl glutaramide (SGA).
- 5 18. The hyperbranched macromolecule according to any one of aspects 1 to 13, wherein the end groups attached to the outermost polymeric arms are functional groups selected from an alkyne compound such as a dibenzocyclooctyne (DBCO), or a bicyclo[6.1.0]-nonyne (BCN); or a norbornene, or a trans-cyclooctene (TCO); an azide, a 3,4 dihydroxyphenylacetic acid (DHPA), or a tetrazine (Tz).
- 10 19. The hyperbranched macromolecule according to any one of the preceding aspects, wherein the connection between the polymeric arms connected to the core unit and the first dendritic constitutional repeating unit and/or the connections between consecutive dendritic constitutional repeating units are formed by click chemistry, optionally with click chemistry functionalized linkers that include a hydrolyzable bond.
- 15 20. The hyperbranched macromolecule according to aspect 19, wherein the connection is formed by reacting a polymeric arm functionalized, optionally via a linker, with an alkyne, cycloalkyne, or strained or terminal alkene moiety with a polymeric arm functionalized with an azide or a tetrazine moiety in a SPAAC or an IEDDA type click chemistry coupling reaction.
- 20 21. The hyperbranched macromolecule according to aspect 20, wherein the alkyne moiety is a dibenzocyclooctyne moiety.
22. The hyperbranched macromolecule according to any one of aspects 19 to 21, wherein the connection between the polymeric arms connected to the core unit and the first dendritic constitutional repeating unit and/or the connections between consecutive dendritic constitutional repeating units are formed between the polymeric arms connected to the core unit and polymeric arms connected to the branch unit of the dendritic constitutional repeating units and/or between the polymeric arms of a dendritic constitutional repeating unit and polymeric arms of consecutive dendritic constitutional repeating units, optionally via a difunctional linker forming at least one hydrolyzable bond.
- 25 23. The hyperbranched macromolecule according to any of the preceding aspects, wherein the active agent conjugated to at least one of the outermost polymeric arms is selected from the group consisting of therapeutically or diagnostically active agents.
- 30

24. The hyperbranched macromolecule according to any of the preceding aspects, wherein the active agent conjugated to at least one of the outermost polymeric arms is selected from steroids; non-steroidal anti-inflammatory drugs (NSAIDS) such as Diclofenac, Ibuprofen, Meclofenamate, Mefenamic A, Salsalate, Sulindac, Tolmetin, Ketoprofen, Diflunisal, Piroxicam, Naproxen, Etodolac, Flurbiprofen, Fenoprofen C, Indomethacin, Celecoxib, Ketorolac, Nepafenac; intraocular pressure lowering drugs; antibiotics such as Ciprofloxacin; pain reliever such as Bupivacaine; calcium channel blockers such as Nifedipine; cell cycle inhibitors such as Simvastatin; proteins such as insulin; small molecule hydrophilic drugs, including carboxylic acid salts and amine salts; small molecule hydrophobic drugs, hydrophilic peptides and protein drugs, such as insulin, single chain antibody fragments, Fab fragments, IgG antibodies, fusion antibodies, etc.; aptamers; particularly Bupivacaine (BPV-HCl or base), Ropivacaine (RPV), Dexamethasone, Travoprost, Axitinib, non-steroidal anti-inflammatory drugs (NSAIDS), steroids, antibiotics, pain relievers, calcium-channel blockers, cell cycle inhibitors, chemotherapeutics, anti-viral drugs, anesthetics, hormones, anticancer drugs, antineoplastic agents, viruses, viruses for gene delivery such as AAV, protein binders such as nanobodies, affibodies, ankyrins, DARPs, etc., or any combinations thereof.
25. The hyperbranched macromolecule according to any of the preceding aspects, wherein the active agent conjugated to at least one of the outermost polymeric arms is a peptide selected from the group consisting of Compstatin, APL-1, and Fc-III-4C, Beovu (Brolucizumab), Zimura (Avacincaptad Pegol), Pegcetacoplan, Abicipar Pegol, Lampalizumab, Fovista, Risuteganib, AXT107, Elamipretide, THR149, ALM201, VGB3, and Largazole.
26. The hyperbranched macromolecule according to any of the preceding aspects, wherein the active agent is bound to at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or up to 100% of the outermost polymeric arms.
27. The hyperbranched macromolecule according to any one of the previous aspects, wherein the connection between A and B in formula (i) comprises a functional group formed by click chemistry, such as a triazole or dihydropyrazine.
28. The hyperbranched macromolecule according to aspect 27, wherein the linker L_A and/or L_B comprise a diacid and/or an acid diamido group such as succinate, glutarate, adipate, azelate, or glutaramide.

29. The hyperbranched macromolecule according to any of aspects 27 or 28, wherein the linker L_A and/or L_B comprises a structure represented by Formula (ii):



wherein U^1 and U^2 are independently NH or O and can be the same or different, and wherein t is an integer from 0 to 10.

30. The hyperbranched macromolecule according to aspects 28 or 29, wherein the linker L_A and/or L_B further comprises a polyethylene glycol unit between the bond to B and the carboxyl group, carboxamide group or structure of Formula (ii).
31. The hyperbranched macromolecule according to any of the preceding aspects, wherein the hyperbranched macromolecule further comprises at least one extender unit comprising polyethylene glycol (PEG) units, wherein the extender unit is linear, difunctional and connected to the polymeric arm of a dendritic constitutional repeating unit or the polymeric arm connected to the core unit and to either an end group or a polymeric arm of a next dendritic constitutional repeating unit.
32. The hyperbranched macromolecule according to aspect 31, wherein the extender unit further comprises at least one linker, wherein the linker can be located at either terminus or both termini of the extender unit and is a difunctional linker comprising hydrolyzable bonds comprising a carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.
33. The hyperbranched molecule according to any one of the preceding aspects, wherein at least one, or all, preferably all, building blocks selected from core unit, core unit including polymeric arms at the connectivities c , dendritic constitutional repeating unit, linkers and extenders, between hydrolyzable bonds, have a molecular weight less than 50,000 Daltons, such as less than 45,000 Daltons, or less than 40,000 Daltons, or less than 35,000 Daltons, or less than 30,000 Daltons.
34. The hyperbranched molecule according to any one of the preceding aspects, wherein upon complete hydrolysis of the hydrolyzable bonds all fragments formed of the molecule have a molecular weight less than 50,000 Daltons, such as less than 45,000

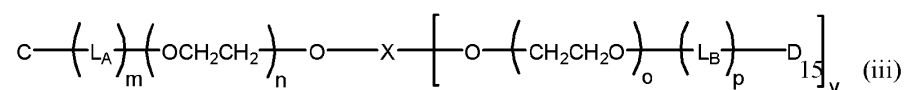
Daltons, or less than 40,000 Daltons, or less than 35,000 Daltons, or less than 30,000 Daltons.

35. A dendritic constitutional repeating unit precursor comprising:

- 5
- one polymeric arm comprising a functional group suitable for forming a connection by click chemistry (such as an azide, alkyne, alkene or tetrazine), and
 - at least two polymeric arms comprising functional groups not reactive in click chemistry,

10 wherein the polymeric arms are connected to a branch unit having a connectivity c' , wherein the polymeric parts of the polymeric arms consist of polyethylene glycol (PEG) units.

36. The precursor of aspect 35, wherein the compound is represented by the Formula (iii):



wherein C comprises a functional group suitable for click chemistry, (such as an alkyne, alkene, azide, or tetrazine),

D comprises functional groups not reactive in click chemistry (such as succinimidyl), and

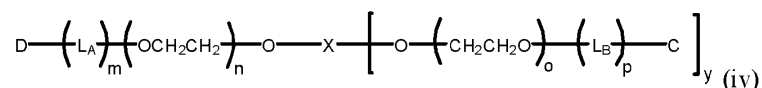
20 L_A , m , n , X , o , L_B , p and y are as defined in the previous aspects.

37. A reverse dendritic constitutional repeating unit precursor comprising:

- 25
- one polymeric arm comprising a functional group not reactive in click chemistry, and
 - at least two polymeric arms comprising functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine), and
 - a branch unit having a connectivity c' ;

wherein the polymeric parts of the polymeric arms consist of polyethylene glycol (PEG) units.

38. The precursor of aspect 37, wherein the precursor is represented by the Formula (iv):



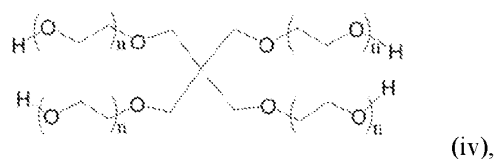
30 wherein C comprises a functional group suitable for click chemistry, (such as an alkyne, alkene, azide, or tetrazine),

150

D comprises functional groups not reactive in click chemistry (such as succinimidyl), and L_A , m, n, X, o, L_B , p and y are as defined in the previous aspects.

39. The hyperbranched macromolecule of any one of aspects 1 to 34, wherein the core unit is a pegylated pentaerythritol compound of formula (iv):

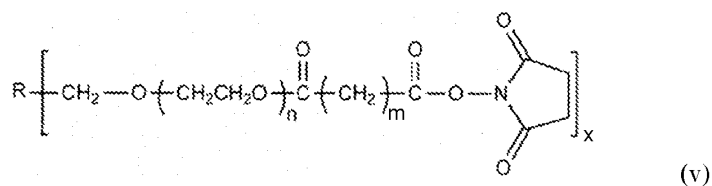
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with n being an integer of 3 to 2,000.

40. The hyperbranched macromolecule of any one of aspects 1 to 34, wherein the core unit is a compound of formula (v):

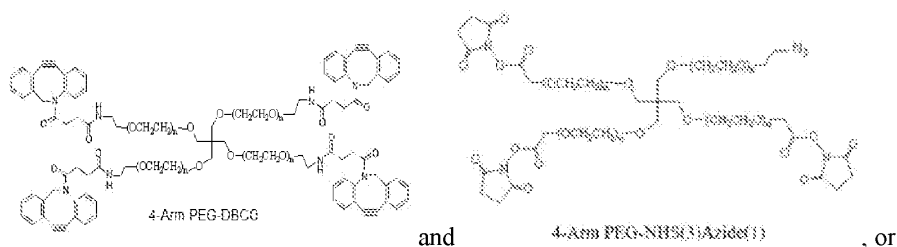
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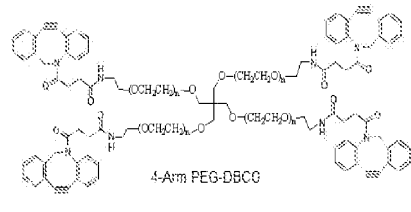
wherein R is the core unit having x connectivities c, n is determined by the molecular weight of the respective PEG-arm and is from 3 to 2,000, or 20 to 2,000, m is an integer from 0 to 10, and x is the number of arms and is an integer from 1 to 10.

15

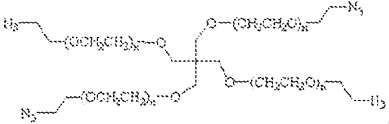
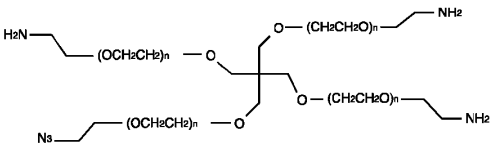
41. The hyperbranched macromolecule of any one of aspects 1 to 34 or 39 to 40, formed from one of the following precursors/dendrons:



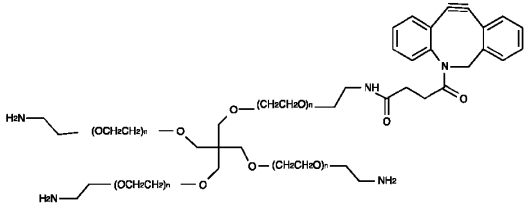
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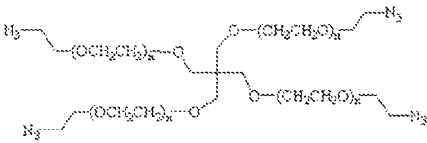
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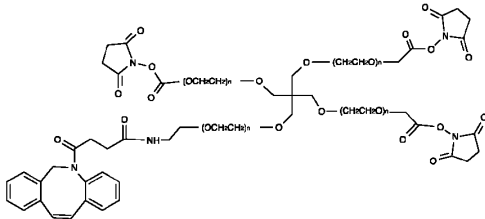
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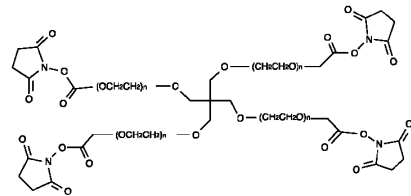
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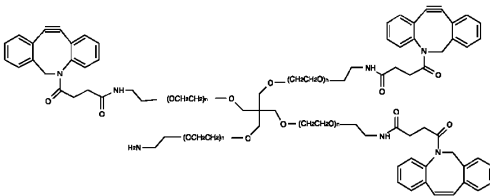
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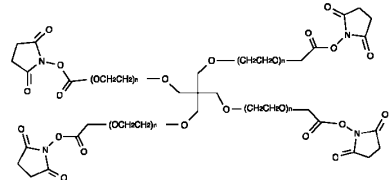
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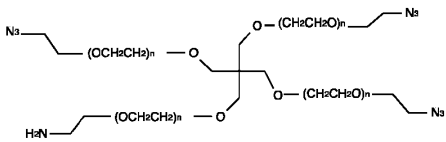
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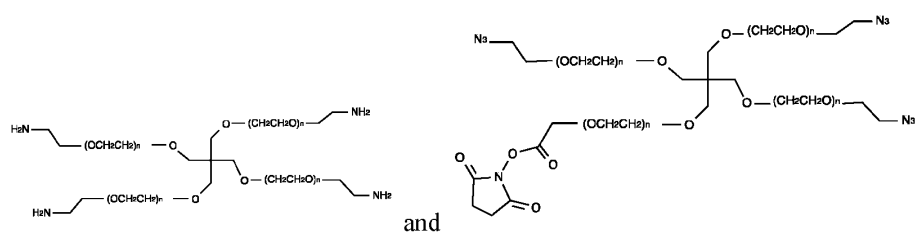
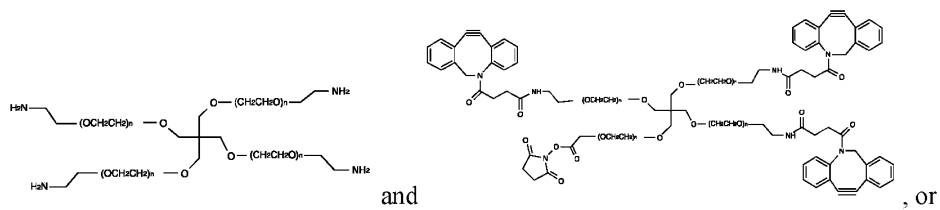


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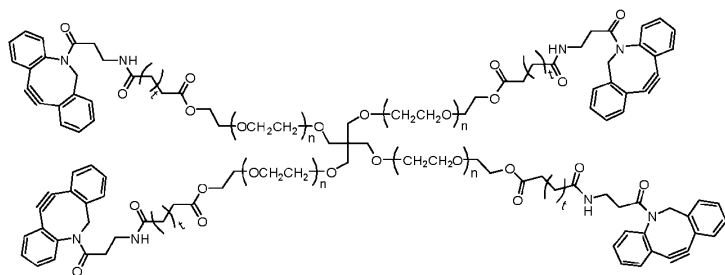


, or

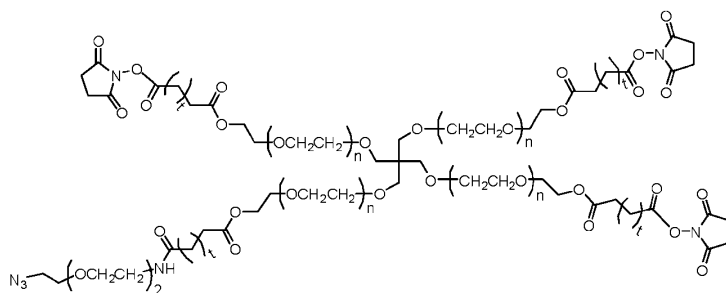
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or the following exemplary precursor pairs:

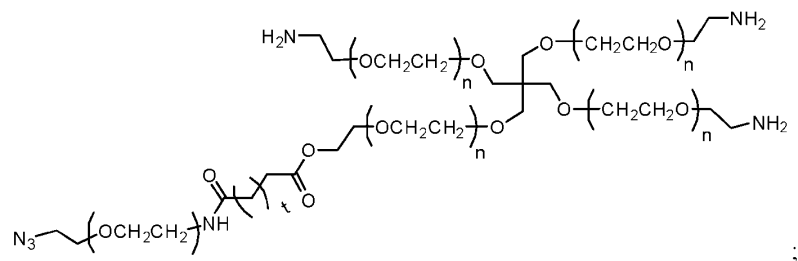


5 and either

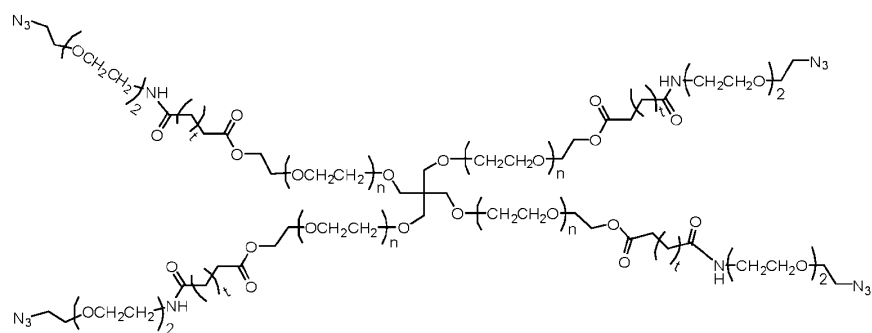


or

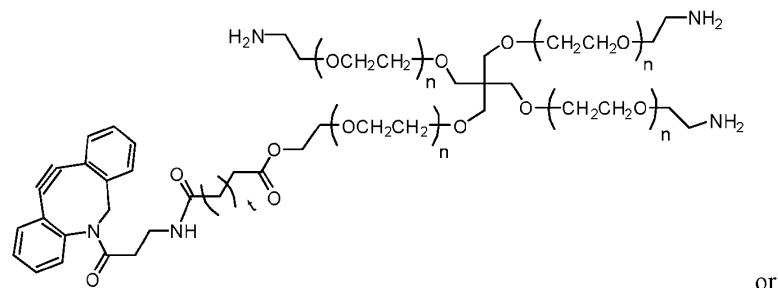
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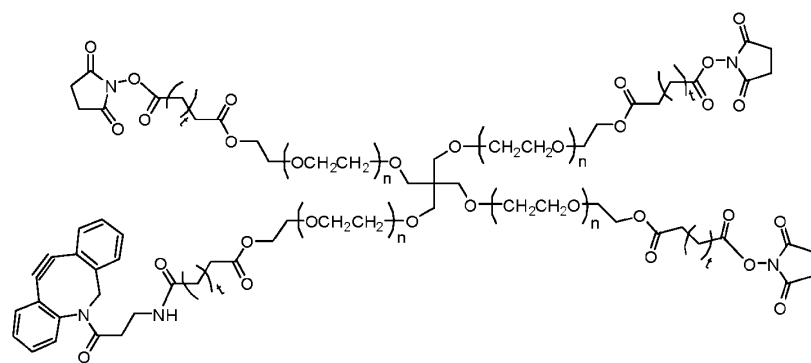
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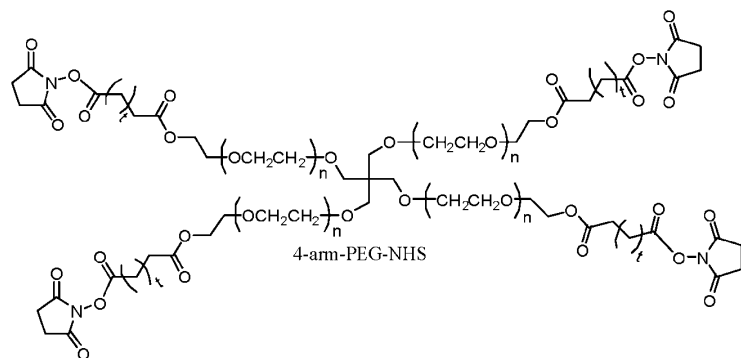


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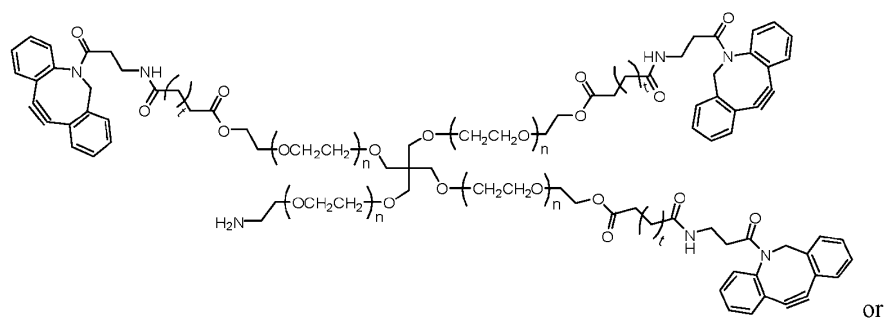


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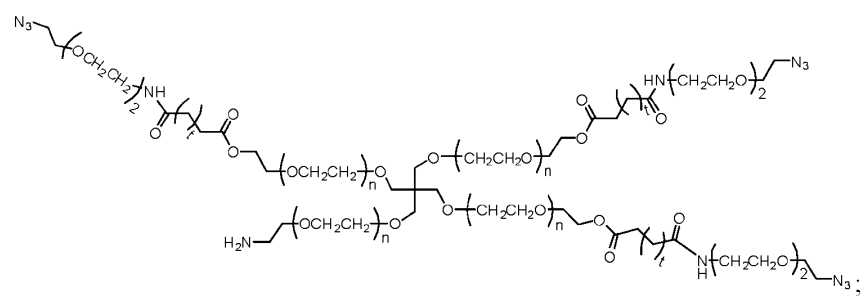
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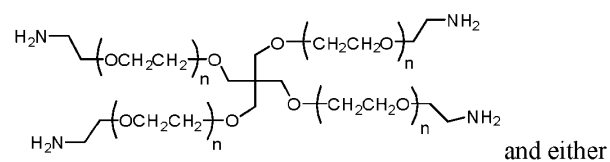
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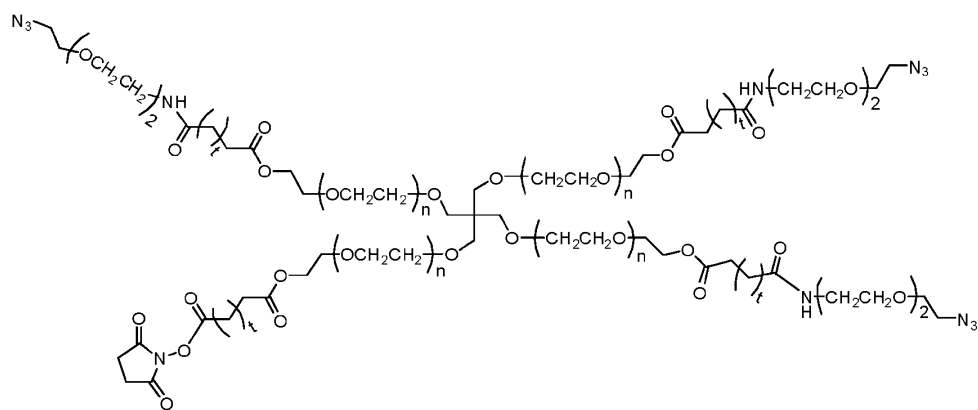
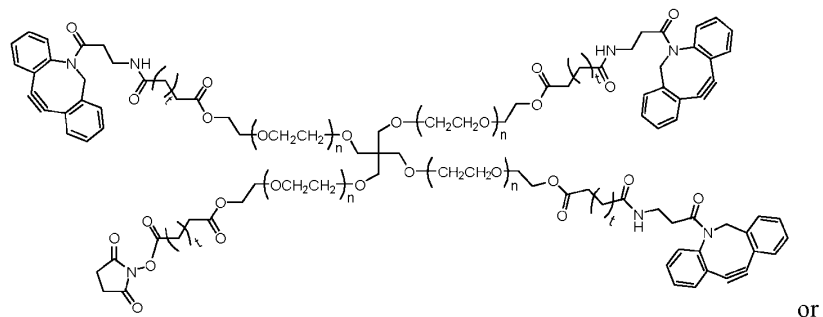


5 or



and either

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wherein t is m, and n and m are defined as for formula (v) in aspect 40.

CLAIMS

1. A hyperbranched macromolecule comprising building blocks which comprise:
 - a core unit having at least 3 connectivities c ;
 - a plurality of polymeric arms connected to the core unit at the connectivities c ,
 - 5 at least one of the polymeric arms being connected by a hydrolyzable bond to a dendritic constitutional repeating unit,
 - the dendritic constitutional repeating unit comprising a branch unit connected to at least two polymeric arms each comprising an end group or being connected by a hydrolyzable bond to a next dendritic constitutional repeating unit that can again
 - 10 be connected by a chemical bond to further dendritic constitutional repeating units,
 - the polymeric arms of the outermost dendritic constitutional repeating unit of the hyperbranched macromolecule each comprising an end group;
 - wherein the polymeric arms consist of polyethylene glycol (PEG) units;
 - wherein at least one active agent is conjugated to at least one of the outermost polymeric
 - 15 arms.
2. The hyperbranched macromolecule according to claim 1, wherein at least 10%, preferably about 20 to 100 % of the connections in the macromolecule can be cleaved by hydrolysis.
- 20 3. The hyperbranched macromolecule according to any one of the preceding claims, wherein each of the building blocks (fragments) of the hyperbranched macromolecule obtained after cleaving all hydrolyzable bonds of the connections in the macromolecule has an average molecular weight (M_n) of less than 50,000 Daltons, such as less than 45,000 Daltons, or less than 40,000 Daltons, or less than 35,000 Daltons, or less than 30,000
- 25 Daltons.
4. The hyperbranched macromolecule according to any one of the preceding claims, wherein at least one of the building blocks comprises a core unit, or a branch unit, having a plurality of polymeric arms connected by non-hydrolyzable bonds to the core unit or branch unit.
- 30 5. The hyperbranched macromolecule according to any one of the previous claims, being a higher generation G_x hyperbranched macromolecule, with x being an integer of 1 to 10

defining the number of consecutively connected dendritic constitutional repeating units in the hyperbranched macromolecule.

- 5 6. The hyperbranched macromolecule according to any one of the preceding claims, wherein the core unit and the branch unit are the same or different and independently of each other have a connectivity *c* or *c'* of 3 to 10, or 4 to 8, or 4 to 6, or 4.
- 10 7. The hyperbranched macromolecule according to any one of the preceding claims, wherein the core unit and the branch unit are the same or different, and are derived from a polyol having at least 3 hydroxyl groups.
- 15 8. The hyperbranched macromolecule according to claim 7, wherein the polyol is selected from the group consisting of glycerol, pentaerythritol, xylitol, dipentaerythritol, tripentaerythritol, hexaglycerol, isomalt, lactitol, maltitol, mannitol, or sorbitol.
- 20 9. The hyperbranched macromolecule according to any one of the preceding claims, wherein the polyethylene glycol (PEG) units of the polymeric arms have an average molecular weight (Mn) in the range from about 1,000 to about 100,000 Daltons, or from about 10,000 to about 60,000 Daltons, or from about 15,000 to about 50,000 Daltons, or from about 10,000 to about 40,000 Daltons.
- 25 10. The hyperbranched macromolecule according to any one of the preceding claims, wherein the average molecular weight of the polymeric arm PEG units attached to the core is the same or different than that of the polymeric arms in the dendritic constitutional repeating units.
- 30 11. The hyperbranched macromolecule according to any one of the preceding claims, wherein the average molecular weight of the polymeric arm PEG units attached to the core is higher than that of the polymeric arms in the dendritic constitutional units.

12. The hyperbranched macromolecule according to any one of the preceding claims, wherein the average molecular weight of the polymeric arm PEG units attached to the core is lower than that of the polymeric arms in the dendritic constitutional units.
- 5 13. The hyperbranched macromolecule according to any one of the preceding claims, wherein for a higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, the average molecular weight of the polymeric arm PEG units decreases from the innermost polymeric arms to the outermost polymeric arms; or
10 wherein the average molecular weight of the polymeric arm PEG units increases from the innermost polymeric arms to the outermost polymeric arms.
14. The hyperbranched macromolecule according to any one of the preceding claims, wherein the at least one arm connected to the core unit or branch unit is connected to the dendritic constitutional unit via a difunctional linker forming hydrolyzable bonds comprising a
15 carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.
15. The hyperbranched macromolecule according to any one of the preceding claims, wherein the end groups attached to the outermost polymeric arms are grafted to the termini of the
20 polymeric arms directly, or via a difunctional linker comprising or forming hydrolyzable bonds comprising a carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.
- 25 16. The hyperbranched macromolecule according to any one of the preceding claims, wherein the functional groups of the end groups and/or linker-end groups attached to the outermost polymeric arms are functional groups selected from electrophiles such as activated ester groups, such as succinimidyl esters, succinimidyl carbonates; nitrophenyl carbonates, aldehydes, ketones, acrylates, acrylamides, maleimides, vinylsulfones, iodoacetamides,
30 alkenes, alkynes, azides, norbornenes, epoxides, mesylates, tosylates, tresyls, cyanurates, orthopyridyl disulfides, or halogen; nucleophiles such as an amine, such as a primary amine, a hydroxyl, an alcohol, a thiol, an azide, and a carboxyl group; functional groups

- for click chemistry; functional groups for cycloadditions, such as 1,3-dipolar cycloadditions, [3+2] cycloadditions such as alkene-nitrone cycloadditions or alkyne-nitrone cycloadditions, [4+2] cycloadditions; functional groups for thiol-ene reactions; hetero-Diels-Alder cycloadditions; functional groups for nucleophilic ring openings, functional groups for non-aldol type carbonyl reactions; functional groups for addition reactions to carbon-carbon multiple bonds, polymerizable vinyl groups, or combinations thereof.
- 5
17. The hyperbranched macromolecule according to any one of the preceding claims, wherein the (linker) end groups attached to the outermost polymeric arms are functional groups selected from succinimidyl succinate (SS), succinimidyl glutarate (SG), succinimidyl adipate (SAP), succinimidyl azelate (SAZ), and succinimidyl glutaramide (SGA).
- 10
18. The hyperbranched macromolecule according to any one of claims 1 to 13, wherein the end groups attached to the outermost polymeric arms are functional groups selected from an alkyne compound such as a dibenzocyclooctyne (DBCO), or a bicyclo[6.1.0]-nonyne (BCN); or a norbornene, or a trans-cyclooctene (TCO); an azide, a 3,4 dihydroxyphenylacetic acid (DHPA), or a tetrazine (Tz).
- 15
19. The hyperbranched macromolecule according to any one of the preceding claims, wherein the connection between the polymeric arms connected to the core unit and the first dendritic constitutional repeating unit and/or the connections between consecutive dendritic constitutional repeating units are formed by click chemistry, optionally with click chemistry functionalized linkers that include a hydrolyzable bond.
- 20
20. The hyperbranched macromolecule according to claim 19, wherein the connection is formed by reacting a polymeric arm functionalized, optionally via a linker, with an alkyne, cycloalkyne, or strained or terminal alkene moiety with a polymeric arm functionalized with an azide or a tetrazine moiety in a SPAAC or an IEDDA type click chemistry coupling reaction.
- 25
- 30

21. The hyperbranched macromolecule according to claim 20, wherein the alkyne moiety is a dibenzocyclooctyne moiety.
22. The hyperbranched macromolecule according to any one of claims 19 to 21, wherein the
5 connection between the polymeric arms connected to the core unit and the first dendritic constitutional repeating unit and/or the connections between consecutive dendritic constitutional repeating units are formed between the polymeric arms connected to the core unit and polymeric arms connected to the branch unit of the dendritic constitutional repeating units and/or between the polymeric arms of a dendritic constitutional repeating
10 unit and polymeric arms of consecutive dendritic constitutional repeating units, optionally via a difunctional linker forming at least one hydrolyzable bond.
23. The hyperbranched macromolecule according to any of the preceding claims, wherein the
15 active agent conjugated to at least one of the outermost polymeric arms is selected from the group consisting of therapeutically or diagnostically active agents.
24. The hyperbranched macromolecule according to any of the preceding claims, wherein the
active agent conjugated to at least one of the outermost polymeric arms is selected from
steroids; non-steroidal anti-inflammatory drugs (NSAIDS) such as Diclofenac, Ibuprofen,
20 Meclofenamate, Mefenamic A, Salsalate, Sulindac, Tolmetin, Ketoprofen, Diflunisal, Piroxicam, Naproxen, Etodolac, Flurbiprofen, Fenoprofen C, Indomethacin, Celecoxib, Ketorolac, Nepafenac; intraocular pressure lowering drugs; antibiotics such as Ciprofloxacin; pain reliever such as Bupivacaine; calcium channel blockers such as Nifedipine; cell cycle inhibitors such as Simvastatin; proteins such as insulin; small
25 molecule hydrophilic drugs, including carboxylic acid salts and amine salts; small molecule hydrophobic drugs, hydrophilic peptides and protein drugs, such as insulin, single chain antibody fragments, Fab fragments, IgG antibodies, fusion antibodies, etc.; aptamers; particularly Bupivacaine (BPV-HCl or base), Ropivacaine (RPV), Dexamethasone, Travoprost, Axitinib, non-steroidal anti-inflammatory drugs (NSAIDS), steroids,
30 antibiotics, pain relievers, calcium-channel blockers, cell cycle inhibitors, chemotherapeutics, anti-viral drugs, anesthetics, hormones, anticancer drugs,

antineoplastic agents, viruses, viruses for gene delivery such as AAV, protein binders such as nanobodies, affibodies, ankyrins, DARPins, etc., or any combinations thereof.

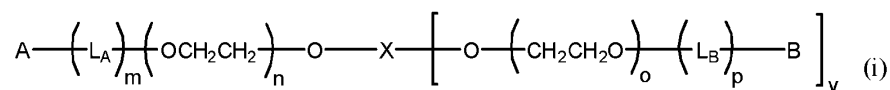
25. The hyperbranched macromolecule according to any of the preceding claims, wherein the active agent conjugated to at least one of the outermost polymeric arms is a peptide selected from the group consisting of Compstatin, APL-1, and Fc-III-4C, Beovu (Brolucizumab), Zimura (Avacincaptad Pegol), Pegcetacoplan, Abicipar Pegol, Lampalizumab, Fovista, Risuteganib, AXT107, Elamipretide, THR149, ALM201, VGB3, and Largazole.

10

26. The hyperbranched macromolecule according to any of the preceding claims, wherein the active agent is bound to at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or up to 100% of the outermost polymeric arms.

15

27. The hyperbranched macromolecule according to any of the preceding claims, wherein a dendritic constitutional repeating unit is represented by Formula (i):



20

wherein A is a connection to a polymeric arm that is connected to the core unit, or A is a connection to B of a preceding dendritic constitutional repeating unit represented by Formula (i),

L_A is a linker,

m is either 0 or 1,

25

n is an integer from 20 to 2000, o is an integer from 20 to 2000,

n and o can be different or the same,

X is a branch unit having a connectivity c^* ,

L_B is a linker, p is either 0 or 1,

30

B comprises an end group located at the surface of the hyperbranched macromolecule or is a connection to A of a consecutive dendritic constitutional repeating unit or an active agent,

L_A and L_B can be different or the same,

m and p can be different or the same, and
 y is an integer from 2 to 9, wherein $y = c' - 1$ with c' being the connectivity c' of the
 branch unit X; and wherein the dendritic constitutional units in the hyperbranched
 macromolecules may be the same or different.

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28. The hyperbranched macromolecule according to claim 27, wherein the connection
 between A and B comprises a functional group formed by click chemistry, such as a
 triazole or dihydropyrazine.

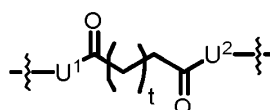
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29. The hyperbranched macromolecule according to claims 27 or 28, wherein the linker L_A
 and/or L_B comprise a diacid and/or an acid diamido group such as succinate, glutarate,
 adipate, azelate, or glutaramide.

15

30. The hyperbranched macromolecule according to any of claims 27 to 29, wherein the
 linker L_A and/or L_B comprises a structure represented by Formula (ii):

(ii)



wherein U^1 and U^2 are independently NH or O and can be the same or different, and
 wherein t is an integer from 0 to 10.

20

31. The hyperbranched macromolecule according to claims 29 or 30, wherein the linker L_A
 and/or L_B further comprises a polyethylene glycol unit between the bond to B and the
 carboxyl group, carboxamide group or structure of Formula (ii).

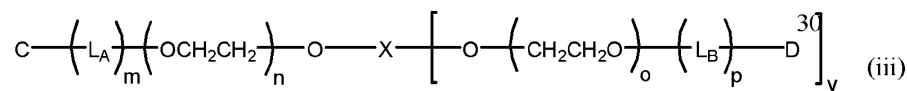
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32. The hyperbranched macromolecule according to any of the preceding claims, wherein the
 hyperbranched macromolecule further comprises at least one extender unit comprising
 polyethylene glycol (PEG) units, wherein the extender unit is linear, difunctional and
 connected to the polymeric arm of a dendritic constitutional repeating unit or the
 polymeric arm connected to the core unit and to either an end group or a polymeric arm
 of a next dendritic constitutional repeating unit.

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33. The hyperbranched macromolecule according to claim 32, wherein the extender unit comprises at least one linker, wherein the linker can be located at either terminus or both termini of the extender unit and is a difunctional linker comprising hydrolyzable bonds comprising a carboxyl group, a dicarboxyl group, carboxamide group, dicarboxamide group, a functionalized aliphatic, heteroaliphatic, or aromatic or heteroaromatic group.
34. The hyperbranched molecule according to any one of the preceding claims, wherein at least one, or all, preferably all, building blocks selected from core unit, core unit including polymeric arms at the connectivities c , dendritic constitutional repeating unit, linkers and extenders, between hydrolyzable bonds, have a molecular weight less than 50,000 Daltons, such as less than 45,000 Daltons, or less than 40,000 Daltons, or less than 35,000 Daltons, or less than 30,000 Daltons.
35. The hyperbranched molecule according to any one of the preceding claims, wherein upon complete hydrolysis of the hydrolyzable bonds all fragments formed of the molecule have a molecular weight less than 50,000 Daltons, such as less than 45,000 Daltons, or less than 40,000 Daltons, or less than 35,000 Daltons, or less than 30,000 Daltons.
36. A dendritic constitutional repeating unit precursor comprising:
- one polymeric arm comprising a functional group suitable for forming a connection by click chemistry (such as an azide, alkyne, alkene or tetrazine), and
 - at least two polymeric arms comprising functional groups not reactive in click chemistry,
- wherein the polymeric arms are connected to a branch unit having a connectivity c' , wherein the polymeric parts of the polymeric arms consist of polyethylene glycol (PEG) units.

37. The precursor of claim 36, wherein the compound is represented by the Formula (iii):



wherein C comprises a functional group suitable for click chemistry, (such as an alkyne, alkene, azide, or tetrazine),

D comprises functional groups not reactive in click chemistry (such as succinimidy), and

L_A , m, n, X, o, L_B , p and y are as defined in claims 27 to 31.

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38. A reverse dendritic constitutional repeating unit precursor comprising:

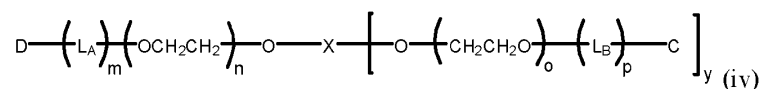
- one polymeric arm comprising a functional group not reactive in click chemistry, and
- at least two polymeric arms comprising functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine), and
- a branch unit having a connectivity c' ;

10

wherein the polymeric parts of the polymeric arms consist of polyethylene glycol (PEG) units.

39. The precursor of claim 34, wherein the precursor is represented by the Formula (iv):

15



wherein C comprises a functional group suitable for click chemistry, (such as an alkyne, alkene, azide, or tetrazine),

D comprises functional groups not reactive in click chemistry (such as succinimidy), and

L_A , m, n, X, o, L_B , p and y are as defined in the previous claims.

20

40. A method for manufacturing a hyperbranched macromolecule according to any one of claims 1 to 39 by divergent synthesis, comprising the following steps:

- (a) Providing a core unit having at least 3 connectivities c , a plurality of polymeric arms connected to the core unit having functional groups suitable for click chemistry at the termini of the polymeric arms;
- (b) Providing dendritic constitutional repeating unit precursors comprising
 - one polymeric arm comprising a functional group suitable for forming a connection by click chemistry with the corresponding functional groups of the polymeric arms connected to the core (such as an azide, alkyne, alkene or tetrazine), and

25

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- at least two polymeric arms comprising functional groups not reactive in click chemistry,
- (c) Forming a connection by click chemistry between the polymeric arms connected to the core and the polymeric arms of the dendritic constitutional repeating unit precursors,
- (d) Optionally converting the functional groups of the at least two polymeric arms comprising functional groups not reactive in click chemistry into functional groups suitable for click chemistry, and
- (e) Conjugating an active agent comprising a functional group to the outermost polymeric arms by reacting with the functional groups of the outermost polymeric arms, thereby forming a hyperbranched macromolecule-active agent conjugate.
41. The method according to claim 40, wherein for higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, step (d) is compulsory, and further consecutive dendritic constitutional repeating unit precursors are connected to the functional groups suitable for click chemistry obtained in step (d) by click chemistry to the hyperbranched macromolecule before conjugating the active agent in step (e).
42. The method according to claims 40 or 41, wherein the dendritic constitutional repeating unit precursor in step (c) is represented by the Formula (iii):
- $$C-\left(L_A\right)_m\left(OCH_2CH_2\right)_n-O-X-\left[O-\left(CH_2CH_2O\right)_o\left(L_B\right)_p-D\right]_y \quad (iii)$$
- wherein C comprises a functional group suitable for click chemistry, (such as an alkyne, alkene, azide, or tetrazine),
- D comprises functional groups not reactive in click chemistry (such as succinimidyl), and L_A , m , n , X , o , L_B , p and y are as defined in the previous claims; and wherein the dendritic constitutional units may be the same or different.
43. The method according to claims 40 to 42, wherein after a penultimate step (d) of converting the functional groups of the at least two polymeric arms comprising functional groups not

reactive in click chemistry into functional groups suitable for click chemistry, the active agent in step (e) is first functionalized with a functional group suitable for click chemistry (such as an alkyne, alkene, azide, or tetrazine) and then conjugated in a click chemistry reaction to the outermost polymeric arms of the hyperbranched macromolecule.

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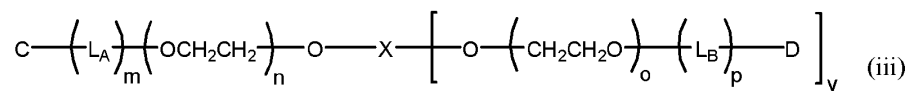
44. The method of claim 43, wherein the active agent functionalized with a functional group suitable for click chemistry is a peptide.

45. A method for manufacturing a hyperbranched macromolecule according to any one of claims 1 to 39 by convergent synthesis, comprising the following steps:
- I) Providing dendritic constitutional repeating unit precursors comprising
 - one polymeric arm comprising a functional group suitable for forming a connection by click chemistry (such as an azide, alkyne, alkene or tetrazine), and
 - 15 - at least two polymeric arms comprising functional groups not reactive in click chemistry,
 - II) Conjugating active agents comprising a functional group to at least one of the at least two polymeric arms comprising functional groups not reactive in click chemistry of the dendritic constitutional repeating unit precursors,
 - 20 III) Providing a core unit having at least 3 connectivities *c*, a plurality of polymeric arms connected to the core unit having functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine) at the termini of the polymeric arms, and
 - IV) Forming a connection by click chemistry between the polymeric arms
25 connected to the core provided in step III) and the polymeric arm comprising a functional group suitable for forming a connection by click chemistry of the active agent conjugated dendritic constitutional repeating unit precursors obtained in step II), thereby forming a hyperbranched macromolecule-active agent conjugate.

30

46. The method according to claim 45, wherein a dendritic constitutional repeating unit precursor in step I) is represented by the Formula (iii):

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wherein C comprises a functional group suitable for click chemistry (such as an alkyne, alkene, azide, or tetrazine),

D comprises functional groups not reactive in click chemistry (such as succinimidyl), and L_A , L_B , m, n, X, o, p, and y are as defined in the previous claims, and wherein the dendritic constitutional units may be the same or different.

47. The method according to claims 45 or 46, wherein for higher generation G_x hyperbranched macromolecule, with x being an integer of 2 to 10, the active agent conjugated dendritic constitutional repeating unit precursors obtained in step II) are connected by click chemistry to reverse dendritic constitutional repeating unit precursors comprising:
- one polymeric arm comprising a functional group not reactive in click chemistry, and
 - at least two polymeric arms comprising functional groups suitable for click chemistry (such as an azide, alkyne, alkene or tetrazine),
- wherein the functional group not reactive in click chemistry of the one polymeric arm is subsequently converted to a functional group suitable for click chemistry before connecting to further reverse dendritic constitutional repeating unit precursors or before forming a connection by click chemistry with the polymeric arms connected to the core in step IV), thereby forming higher generation hyperbranched macromolecules.
48. The method according to claims 45 to 47, wherein dendritic constitutional repeating unit precursors having different active agents conjugated to the polymeric arms are obtained by performing steps I) and II) for each active agent conjugated dendritic constitutional repeating unit precursor, and a mixture of the obtained active agent conjugated dendritic constitutional repeating unit precursors is used for step IV), thereby forming a hyperbranched macromolecule-active agent conjugate having different active agents at different regions of the surface of the hyperbranched macromolecule.

49. The method according to any one of claims 40 to 48, wherein the outermost polymeric arms of the hyperbranched macromolecule have terminal maleimide functional groups, and peptides or active agents are conjugated thereto via maleimide-thiol reaction.
- 5 50. The method of claim 49, wherein the terminal maleimide functional groups are provided by reacting DBCO or azide functionalized terminal functional group of the hyperbranched macromolecule with click chemistry linkers having an azide or DBCO functionality connected to a maleimide group, such as DBCO-maleimide, DBCO-PEG3-maleimide, DBCO-PEG4-maleimide, or azido-PEG3-maleimide.
- 10 51. A hyperbranched macromolecule according to any of claims 1 to 39, for use as a medicament.
52. A method of treatment for treating a disease or medical condition in a patient, with a
15 hyperbranched macromolecule according to any of claims 1 to 39.
53. The hyperbranched macromolecule for use, or the method of treatment according to claims 51 or 52, wherein the hyperbranched macromolecule is used for an ocular treatment.
- 20 54. The hyperbranched macromolecule for use, or the method of treatment according to claims 51 to 53, wherein the hyperbranched macromolecule is used in the treatment of an ocular disease such as back-of-the-eye diseases such as any ocular disease of the posterior segment that affects the vasculature and integrity of the retina, macula or choroid leading to visual acuity disturbances, loss of sight or blindness, particularly
25 disease states of the posterior segment resulting from age, trauma, surgical interventions, such as age-related macular degeneration (AMD) cystoid macular edema (CME), diabetic macular edema (DME), posterior uveitis, and diabetic retinopathy.
- 30 55. The hyperbranched macromolecule for use, or the method of treatment according to claims 51 to 54, wherein the hyperbranched macromolecule is used in the treatment of an ocular disease selected from the group consisting of retinal neovascularisation, choroidal neovascularisation, Wet AMD, Dry AMD, retinal vein occlusion, diabetic macular edema, retinal degeneration, hyphema, presbyopia, corneal graft rejection,

- retinoblastoma, melanoma, myosis, mydriasis, glaucoma, conjunctivitis, intraocular infections, choroidal neovascularization (CNV), intraocular tumors, retinal neuroinflammation, inflammation, autoimmune uveitis, uveitis, proliferative vitreoretinopathy, and corneal degeneration, acute and chronic macular neuroretinopathy, central serous chorioretinopathy, macular edema, acute multifocal placoid pigment epitheliopathy, Behcet's disease, birdshot retinochoroidopathy, posterior uveitis, posterior scleritis, serpiginous choroiditis, subretinal fibrosis, uveitis syndrome, Vogt-Koyanagi-Harada syndrome, retinal arterial occlusive disease, central retinal vein occlusion, disseminated intravascular coagulopathy, branch retinal vein occlusion, hypertensive fundus changes, ocular ischemic syndrome, retinal arterial microaneurysms, Coat's disease, parafoveal telangiectasis, hemi-retinal vein occlusion, papillophlebitis, carotid artery disease (CAD), frosted branch angiitis, sickle cell retinopathy, angioid streaks, familial exudative vitreoretinopathy, Eales disease, proliferative vitreal retinopathy, diabetic retinopathy, retinal disease associated with tumors, congenital hypertrophy of the retinal pigment epithelium (RPE), posterior uveal melanoma, choroidal hemangioma, choroidal osteoma, choroidal metastasis, combined hamartoma of the retina and retinal pigmented epithelium, retinoblastoma, vasoproliferative tumors of the ocular fundus, retinal astrocytoma, intraocular lymphoid tumors, myopic retinal degeneration, acute retinal pigment epithelitis, glaucoma, endophthalmitis, cytomegalovirus retinitis, retinal cancers, retinitis pigmentosa, Leber's Congenital Amaurosis, Choroideremia, X-linked retinitis pigmentosa, best vitelliform macular dystrophy, x-linked retinoschisis, achromatopsia CNGA3, achromatopsia CNGB3, LHON, Stargardt disease, Usher syndrome, Norrie disease, Bardet-Biedl syndrome, and red-green color blindness.
56. The hyperbranched macromolecule for use, or the method of treatment according to any of claims 51 to 55, wherein the hyperbranched macromolecule is formulated for direct injection at a treatment site of a patient, for example by parenteral administration, intra-tumoral injection, injection into the eye such as intravitreal, intracameral, subconjunctival, retrobulbar, sub-tenon, subretinal, or suprachoroidal injections.

57. The hyperbranched macromolecule for use, or the method of treatment according to any of claims 51 to 56, wherein the hyperbranched macromolecule is administered by direct injection, by oral application, incorporated in gels, or incorporated in implants.
58. The hyperbranched macromolecule for use, or the method of treatment according to any
5 of claims 51 to 57, wherein the hyperbranched macromolecule comprises two or more different active agents at different dendrons or regions on the surface of the hyperbranched macromolecule.
59. The hyperbranched macromolecule for use or the method of treatment according to
10 claims 54, for use in a combination therapy involving the administration of more than one active agent.
60. The hyperbranched macromolecule of any one of claims 1 to 39, the hyperbranched
molecule for use, or the method of treatment according to any of 51 to 59, wherein the
hyperbranched macromolecule comprises one active agent at different positions or
regions on the surface of the hyperbranched macromolecule with different hydrolyzable
15 groups for varying the release of the active agent at different rates.
61. The hyperbranched macromolecule of any one of claims 1 to 39, the hyperbranched
molecule for use, or the method of treatment according to any of claims 51 to 59, wherein
the hyperbranched macromolecule comprises two or more different active agents at
different positions or regions on the surface of the hyperbranched macromolecule with
20 different hydrolyzable groups for varying the release of the same or different active
agents at different rates.
62. The hyperbranched macromolecule of any one of claims 1 to 39, the hyperbranched
molecule for use, or the method of treatment according to any of claims 51 to 61, wherein
the active agent(s) are attached to the macromolecule with hydrolyzable or
25 nonhydrolyzable links or connections, optionally via an extender, or combinations
thereof.