

USE OF LONG-ACTING GROWTH HORMONE FOR TREATING INFLAMMATION-INDUCED DISEASES

The present invention relates to a long-acting growth hormone (GH) for use in the treatment of an inflammation-induced disease.

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Non-alcoholic fatty liver disease (NAFLD) is a disorder affecting as many as 1 in 3 - 5 adults and 1 in 10 children in the United States, and refers to conditions where there is accumulation of excess fat in the liver of people who drink little or no alcohol. Some people with NAFLD may develop a more serious condition called non-alcoholic steatohepatitis (NASH): about 2 - 5% of adult Americans and up to 20% of those who are obese may suffer from NASH. In NASH, fat accumulation in the liver is associated with inflammation and different degrees of scarring/fibrosis. NASH is a potentially serious condition that carries a substantial risk of progression to end-stage liver disease, cirrhosis and hepatocellular carcinoma. Some patients who develop cirrhosis are at risk of liver failure and may eventually require a liver transplant.

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The liver has an abundance of macrophages that upon activation accelerate the development of NASH by means of extensive inflammatory pathways. The majority of macrophages present in liver tissue are the self-renewing, resident phagocytic Kupffer cells which may be split into M1 (proinflammatory) and M2 (immunoregulatory) phenotypes. In a healthy liver, M1 and M2 function is well balanced to control inflammation. In NASH, an imbalance towards M1 has been implicated in causing excess inflammation.

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Historically, a number of pharmacological interventions have been tried in NAFLD/NASH but with overall limited benefit. Antioxidant agents may arrest lipid peroxidation and cytoprotective agents may stabilize phospholipid membranes, but agents tried so far including ursodeoxycholic acid, vitamins E (α-tocopherol) and C, and pentoxifylline demonstrated no or only modest benefit. Most weight-loss studies in NAFLD/NASH have been pilot studies of short duration and limited success, reporting no or only a modest improvement in necroinflammation or fibrosis. A randomized, double-blind, placebo-controlled 6-month trial of weight loss alone against pioglitazone, a thiazolidinedione peroxisome proliferator-activated receptor (PPAR)-γ agonist and insulin sensitizer, failed to demonstrate any improvement for weight loss alone, but treatment with pioglitazone improved glycemic control, insulin sensitivity, indicators of systemic inflammation (including high-sensitivity

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C-reactive protein, tumor necrosis factor- α , and transforming growth factor- β), and liver histology in patients with NASH and impaired glucose tolerance or type 2 diabetes mellitus. Unfortunately, pioglitazone is also associated with a significantly increased risk of weight gain, edema, congestive heart failure, and osteoporotic fractures in both women and men. At the time of writing, Phase III trials for NASH are ongoing for the Thyroid Hormone Receptor- β (THR- β) agonist resmetirom, the C-C Chemokine Receptor Type 2/5 (CCR2/CCR5) inhibitor cenicriviroc, the Stearoyl-CoA desaturase-1 (SCD1) modulator aramchol, the Galectin 3 inhibitor belaepectin and the Selective Sodium Glucose Co Transporter-2 (SGLT-2) inhibitor dapagliflozin. The Farnesoid X Receptor (FXR) agonist obeticholic acid completed a Phase III trial for NASH by achieving one of the two FDA suggested primary endpoints: “ ≥ 1 -stage improvement in liver fibrosis using the NASH Clinical Research Network (CRN) fibrosis score and no worsening of NASH” or “Resolution of NASH and no worsening of liver fibrosis using the NASH CRN fibrosis score”. However, results were not sufficient to obtain regulatory approval, indicating that the predicted benefit based on a surrogate histopathologic endpoint remains uncertain and does not sufficiently outweigh the potential risks for the treatment of patients with liver fibrosis due to NASH. There have been three Phase III trials that failed to achieve one of the two requisite primary endpoints for NASH. The Apoptosis signal-regulating kinase 1 ASK1 inhibitor Selonsertib failed to meet the primary endpoint of ≥ 1 -stage improvement in fibrosis without worsening of NASH in the STELLAR 3 and STELLAR 4 trials. The PPAR α/δ agonist Elafibranor also failed to meet the primary endpoint of NASH resolution without the worsening of fibrosis.

There are other drugs currently in earlier clinical development stage showing potential to treat NAFLD/NASH. These include among others the Fibroblast Growth Factor (FGF)-21 agonists Efruxifermin and Pegbelfermin, the FGF-19 agonist Aldafermin, the Fibroblast Growth Factor Receptor 1- β Klotho (FGFR1-KLB) antibodies BFKB8488A and NGM313, the Glucagon Like Peptide 1 (GLP-1) receptor agonist Semaglutide, the dual receptor agonists with GLP-1 and Glucagon activity Cotadutide and Efinopegdutide, the dual receptor agonists with Gastric Inhibitory Polypeptide (GIP) and GLP-1 Tirzepatide, and the PPAR $\alpha/\delta/\gamma$ agonist Lanifibranor.

In summary there is a need for a more effective treatment of inflammation-induced diseases, in particular of NAFLD/NASH. It is an object of the present invention to at least partially overcome the limitations of current treatment options.

In a first aspect the present invention relates to a long-acting growth hormone (GH) for use in the treatment of an inflammation-induced disease. In certain embodiments the inflammation-induced disease is an inflammation-induced disease of the liver. In certain embodiments the inflammation-induced disease is NAFLD. In certain embodiments the inflammation-induced disease is NASH.

It was surprisingly found that a stable level of growth hormone, such as that obtained from administering a long-acting growth hormone to a patient, triggered the re-balancing of macrophage phenotypes between M1 and M2. Such rebalancing of macrophage phenotypes is in certain embodiments achieved through an M1 reduction. In certain embodiments such rebalancing of macrophage phenotypes is achieved through an M2 induction.

Use of a long-acting growth hormone reduces the administration frequency, which increases patients' compliance and consequently may improve treatment outcomes.

Within the present invention the terms are used having the meaning as follows.

As used herein, the term "growth hormone" or "GH" refers to all growth hormone protein sequences, preferably from mammalian species, more preferably from human and mammalian species, more preferably from human and murine species, and includes in certain embodiments also their variants, analogs, orthologs, homologs, and derivatives and fragments thereof. Growth hormone is characterized by promoting growth in the growing phase and maintains normal body composition, anabolism, and lipid metabolism. In certain embodiments the term "human growth hormone" or "hGH" refers to the hGH polypeptide of SEQ ID NO:1 and includes its variants, homologs and derivatives exhibiting essentially the same biological activity, i.e. promoting growth in the growing phase and maintaining normal body composition, anabolism, and lipid metabolism. In certain embodiments the term "hGH" refers to the sequence of SEQ ID NO:1.

SEQ ID NO:1 has the following sequence:

FPTIPLSRLFDNAMLRAHRLHQLAFDITYQEFEEAYIPKEQKYSFLQNPQTSLCFSES IPT

PSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVVFANSLVYGASDSNVYDLLKDLEEGIQ
TLMGRLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQC
RSVEGSCGF

5 As used herein, the term “GH variant” refers to a GH protein from the same species that differs from a reference GH protein, such as from the hGH of SEQ ID NO:1. In certain embodiments, such GH variants are at least 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference GH, such as the hGH of SEQ ID NO:1. By a protein having an amino acid sequence at least, for example, 95% “identical” to a query amino acid sequence, it is intended that the amino acid sequence of the subject
10 protein is identical to the query sequence except that the subject protein sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. These alterations of the reference sequence may occur at the amino (N-terminal) or carboxy terminal (C-terminal) positions of the reference amino acid sequence or anywhere between those terminal positions or in any combination thereof. These alterations of the reference sequence may either be
15 interspersed among residues in the reference sequence or may be in one or more contiguous groups within the reference sequence. Such GH variants may be naturally occurring variants, such as naturally occurring allelic variants encoded by one of several alternate forms of a GH occupying a given locus on a chromosome or an organism, or isoforms encoded by naturally occurring splice variants originating from a single primary transcript. Alternatively, a GH variant may be a variant
20 that is not known to occur naturally and that can be made mutagenesis techniques known in the art.

As used herein, the term “GH analog” refers to GH of different and unrelated organisms which perform the same functions in each organism, but which did not originate from an ancestral structure that the organisms’ ancestors had in common. Instead, analogous GHs arose separately and then later
25 evolved to perform the same or similar functions. In other words, analogous GH proteins are proteins with quite different amino acid sequences but that perform the same biological activity, namely promoting growth in the growing phase and maintaining normal body composition, anabolism, and lipid metabolism.

30 As used herein the term “GH ortholog” refers to GH within two different species which sequences are related to each other via a common homologous GH in an ancestral species, but which have evolved to become different from each other.

As used herein, the term “GH homolog” refers to GH of different organisms which perform the same functions in each organism, and which originate from an ancestral structure that the organisms’ ancestors had in common. In other words, homologous GH proteins are proteins with quite similar amino acid sequences that perform the same biological activity, namely promoting growth in the growing phase and maintaining normal body composition, anabolism, and lipid metabolism. In certain embodiments such GH homologs may be defined as proteins exhibiting at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identity to a reference GH sequence, such as to the hGH of SEQ ID NO:1.

Thus, a GH according to the invention may be, for example: (i) one in which at least one of the amino acids residues is substituted with a conserved or non-conserved amino acid residue, in certain embodiments a conserved amino acid residue, and such substituted amino acid residue may or may not be one encoded by the genetic code; and/or (ii) one in which at least one of the amino acid residues includes a substituent group; and/or (iii) one in which the GH is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol); and/or (iv) one in which additional amino acids are fused to the hGH polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the above form of the protein or a pre-protein sequence.

The GH protein may be a monomer or multimer. Multimers may be dimers, trimers, tetramers or multimers comprising at least five monomeric polypeptide units. Multimers may also be homodimers or heterodimers. Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent association and/or may be indirectly linked, by for example, liposome formation. In certain embodiments the GH is a monomer, in particular an hGH monomer, such as an hGH monomer of SEQ ID NO:1.

As used herein, the term “GH fragment” refers to any peptide or protein comprising a contiguous span of a part of the amino acid sequence of a GH protein, such as the hGH of SEQ ID NO:1. More specifically, a GH fragment comprises at least 6, preferably at least 8 or 10, more preferably at least 12, 15, 20, 25, 30, 35, 40, 50, 60, 75, 100, 125, 150, 175, 191 consecutive amino acids of GH, such as the hGH of SEQ ID NO:1.

As used herein the term “long-acting growth hormone” refers to a compound which comprises GH either in crystallized form or wherein the GH is embedded, fused or covalently conjugated to at least one other chemical compound or moiety, such as for example a polymer, fatty acid or fatty acid variant moiety, and has an increased clearance half-life in a patient’s body compared to unmodified GH, such as a clearance half-life that is at least 10-fold, at least 20-fold, at least 30-fold, at least 50-fold, at least 100-fold or at least 200-fold higher than the clearance half-life of the corresponding unmodified GH. In certain embodiments the GH is hGH, such as the hGH of SEQ ID NO:1. As used herein the term “clearance half-life” refers to the time until half of all molecules administered to a patient are cleared from the body.

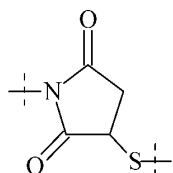
As used herein, the terms “reversible”, “reversibly”, “degradable” or “degradably” with regard to the attachment of a first moiety to a second moiety mean that the linkage that connects said first and second moiety is cleavable under physiological conditions, which are aqueous buffer at pH 7.4, 37°C, with a half-life ranging from one hour to three months, such as from 12 hours to two months, from one day to one month. Cleavage may be enzymatically or non-enzymatically and is in certain embodiments non-enzymatically. Accordingly, the term “stable” or “permanent” with regard to the attachment of a first moiety to a second moiety means that the linkage that connects said first and second moiety is cleavable with a half-life of more than three months under physiological conditions.

As used herein, the term “reagent” means a chemical compound, which comprises at least one functional group for reaction with the functional group of another chemical compound or drug. It is understood that a drug comprising a functional group (such as a primary or secondary amine or hydroxyl functional group) is also a reagent.

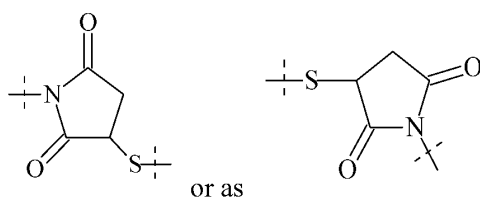
As used herein, the term “moiety” means a part of a molecule, which lacks one or more atom(s) compared to the corresponding reagent. If, for example, a reagent of the formula “H-X-H” reacts with another reagent and becomes part of the reaction product, the corresponding moiety of the reaction product has the structure “H-X-” or “-X-”, whereas each “-” indicates attachment to another moiety. Accordingly, a drug moiety is released from a reversible linkage as a drug.

It is understood that if the sequence or chemical structure of a group of atoms is provided which group of atoms is attached to two moieties or is interrupting a moiety, said sequence or chemical structure

can be attached to the two moieties in either orientation, unless explicitly stated otherwise. For example, a moiety “-C(O)N(R¹)-” can be attached to two moieties or interrupting a moiety either as “-C(O)N(R¹)-” or as “-N(R¹)C(O)-”. Similarly, a moiety



5 can be attached to two moieties or can interrupt a moiety either as



The term “substituted” as used herein means that one or more -H atoms of a molecule or moiety are replaced by a different atom or a group of atoms, which are referred to as “substituent”.

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As used herein, the term “substituent” refers in certain embodiments to a moiety selected from the group consisting of halogen, -CN, -COOR^{x1}, -OR^{x1}, -C(O)R^{x1}, -C(O)N(R^{x1}R^{x1a}), -S(O)₂N(R^{x1}R^{x1a}), -S(O)N(R^{x1}R^{x1a}), -S(O)₂R^{x1}, -S(O)R^{x1}, -N(R^{x1})S(O)₂N(R^{x1a}R^{x1b}), -SR^{x1}, -N(R^{x1}R^{x1a}), -NO₂, -OC(O)R^{x1}, -N(R^{x1})C(O)R^{x1a}, -N(R^{x1})S(O)₂R^{x1a}, -N(R^{x1})S(O)R^{x1a}, -N(R^{x1})C(O)OR^{x1a},
 15 -N(R^{x1})C(O)N(R^{x1a}R^{x1b}), -OC(O)N(R^{x1}R^{x1a}), -T⁰, C₁₋₅₀ alkyl, C₂₋₅₀ alkenyl, and C₂₋₅₀ alkynyl; wherein -T⁰, C₁₋₅₀ alkyl, C₂₋₅₀ alkenyl, and C₂₋₅₀ alkynyl are optionally substituted with one or more -R^{x2}, which are the same or different and wherein C₁₋₅₀ alkyl, C₂₋₅₀ alkenyl, and C₂₋₅₀ alkynyl are optionally interrupted by one or more groups selected from the group consisting of -T⁰, -C(O)O-, -O-, -C(O)-, -C(O)N(R^{x3})-, -S(O)₂N(R^{x3})-, -S(O)N(R^{x3})-, -S(O)₂-,
 20 -S(O)-, -N(R^{x3})S(O)₂N(R^{x3a})-, -S-, -N(R^{x3})-, -OC(OR^{x3})(R^{x3a})-, -N(R^{x3})C(O)N(R^{x3a})-, and -OC(O)N(R^{x3})-;

-R^{x1}, -R^{x1a}, -R^{x1b} are independently of each other selected from the group consisting of -H, -T⁰, C₁₋₅₀ alkyl, C₂₋₅₀ alkenyl, and C₂₋₅₀ alkynyl; wherein -T⁰, C₁₋₅₀ alkyl, C₂₋₅₀ alkenyl, and C₂₋₅₀ alkynyl are
 25 optionally substituted with one or more -R^{x2}, which are the same or different and wherein C₁₋₅₀ alkyl,

C₂₋₅₀ alkenyl, and C₂₋₅₀ alkynyl are optionally interrupted by one or more groups selected from the group consisting of -T⁰-, -C(O)O-, -O-, -C(O)-, -C(O)N(R^{x3})-, -S(O)₂N(R^{x3})-, -S(O)N(R^{x3})-, -S(O)₂-, -S(O)-, -N(R^{x3})S(O)₂N(R^{x3a})-, -S-, -N(R^{x3})-, -OC(OR^{x3})(R^{x3a})-, -N(R^{x3})C(O)N(R^{x3a})-, and -OC(O)N(R^{x3})-;

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each T⁰ is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C₃₋₁₀ cycloalkyl, 3- to 10-membered heterocyclyl, and 8- to 11-membered heterobicycyl; wherein each T⁰ is independently optionally substituted with one or more -R^{x2}, which are the same or different;

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each -R^{x2} is independently selected from the group consisting of halogen, -CN, oxo (=O), -COOR^{x4}, -OR^{x4}, -C(O)R^{x4}, -C(O)N(R^{x4}R^{x4a})-, -S(O)₂N(R^{x4}R^{x4a})-, -S(O)N(R^{x4}R^{x4a})-, -S(O)₂R^{x4}, -S(O)R^{x4}, -N(R^{x4})S(O)₂N(R^{x4a}R^{x4b})-, -SR^{x4}, -N(R^{x4}R^{x4a})-, -NO₂, -OC(O)R^{x4}, -N(R^{x4})C(O)R^{x4a}, -N(R^{x4})S(O)₂R^{x4a}, -N(R^{x4})S(O)R^{x4a}, -N(R^{x4})C(O)OR^{x4a}, -N(R^{x4})C(O)N(R^{x4a}R^{x4b})-, -OC(O)N(R^{x4}R^{x4a})-, and C₁₋₆ alkyl; wherein C₁₋₆ alkyl is optionally substituted with one or more halogen, which are the same or different;

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each -R^{x3}, -R^{x3a}, -R^{x4}, -R^{x4a}, -R^{x4b} is independently selected from the group consisting of -H and C₁₋₆ alkyl; wherein C₁₋₆ alkyl is optionally substituted with one or more halogen, which are the same or different.

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In certain embodiments a maximum of 6 -H atoms of an optionally substituted molecule are independently replaced by a substituent, e.g. 5 -H atoms are independently replaced by a substituent, 4 -H atoms are independently replaced by a substituent, 3 -H atoms are independently replaced by a substituent, 2 -H atoms are independently replaced by a substituent, or 1 -H atom is replaced by a substituent.

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As used herein, the term “fatty acid” refers to a saturated or unsaturated monocarboxylic acid having an aliphatic tail, which may include from 4 to 28 carbon atoms. The fatty acid may be saturated or unsaturated, linear or branched. The term “fatty acid variant” refers to a modified fatty acid in which certain carbon atoms may be replaced by other atoms or groups of atoms and which may be substituted.

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The term “peptide” as used herein refers to a chain of at least 2 and up to and including 50 amino acid monomer moieties linked by peptide (amide) linkages. The term “peptide” also includes peptidomimetics, such as D-peptides, peptoids or beta-peptides, and covers such peptidomimetic chains with up to and including 50 monomer moieties. Also included are cyclic peptides, such as lasso peptides.

As used herein, the term “protein” refers to a chain of more than 50 amino acid monomer moieties, which may also be referred to as “amino acid residues”, linked by peptide linkages, in which in certain embodiments no more than 12000 amino acid monomers are linked by peptide linkages, such as no more than 10000 amino acid monomer moieties, no more than 8000 amino acid monomer moieties, no more than 5000 amino acid monomer moieties or no more than 2000 amino acid monomer moieties.

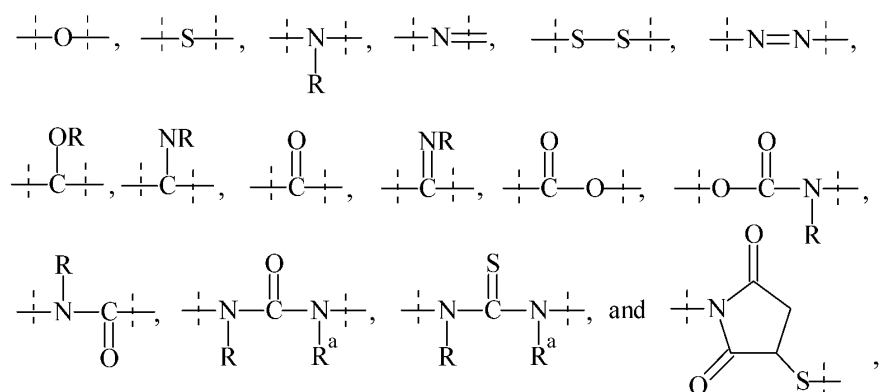
As used herein the term “about” in combination with a numerical value is used to indicate a range ranging from and including the numerical value plus and minus no more than 25% of said numerical value, in certain embodiments plus and minus no more than 20% of said numerical value and in certain embodiments plus and minus no more than 10% of said numerical value. For example, the phrase “about 200” is used to mean a range ranging from and including 200 +/- 25%, i.e. ranging from and including 150 to 250; in certain embodiments 200 +/- 20%, i.e. ranging from and including 160 to 240; and in certain embodiments from and including 200 +/-10%, i.e. ranging from and including 180 to 220. It is understood that a percentage given as “about 50%” does not mean “50% +/- 25%”, i.e. ranging from and including 25 to 75%, but “about 50%” means ranging from and including 37.5 to 62.5%, i.e. plus and minus 25% of the numerical value which is 50.

As used herein, the term “polymer” means a molecule comprising repeating structural units, i.e. the monomers, connected by chemical bonds in a linear, circular, branched, crosslinked or dendrimeric way or a combination thereof, which may be of synthetic or biological origin or a combination of both. It is understood that a polymer may also comprise one or more other chemical groups and/or moieties, such as, for example, one or more functional groups. Likewise, it is understood that also a peptide or protein is a polymer, even though the side chains of individual amino acid residues may be different. In certain embodiments a soluble polymer has a molecular weight of at least 0.5 kDa,

e.g. a molecular weight of at least 1 kDa, a molecular weight of at least 2 kDa, a molecular weight of at least 3 kDa or a molecular weight of at least 5 kDa. If the polymer is soluble, it in certain embodiments has a molecular weight of at most 1000 kDa, such as at most 750 kDa, such as at most 500 kDa, such as at most 300 kDa, such as at most 200 kDa, such as at most 100 kDa. It is understood that for insoluble polymers, such as hydrogels, no meaningful molecular weight ranges can be provided.

As used herein, the term “polymeric” means a reagent or a moiety comprising one or more polymer(s) or polymer moiety/moieties. A polymeric reagent or moiety may optionally also comprise one or more other moiety/moieties, which are in certain embodiments selected from the group consisting of:

- C₁₋₅₀ alkyl, C₂₋₅₀ alkenyl, C₂₋₅₀ alkynyl, C₃₋₁₀ cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, phenyl, naphthyl, indenyl, indanyl, and tetralinyl; and
- linkages selected from the group comprising



wherein

dashed lines indicate attachment to the remainder of the moiety or reagent, and

-R and -R^a are independently of each other selected from the group consisting of -H, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl and 3,3-dimethylpropyl.

The person skilled in the art understands that the polymerization products obtained from a polymerization reaction do not all have the same molecular weight, but rather exhibit a molecular weight distribution. Consequently, the molecular weight ranges, molecular weights, ranges of numbers of monomers in a polymer and numbers of monomers in a polymer as used herein, refer to

the number average molecular weight and number average of monomers, i.e. to the arithmetic mean of the molecular weight of the polymer or polymeric moiety and the arithmetic mean of the number of monomers of the polymer or polymeric moiety.

- 5 Accordingly, in a polymeric moiety comprising “x” monomer units any integer given for “x” therefore corresponds to the arithmetic mean number of monomers. Any range of integers given for “x” provides the range of integers in which the arithmetic mean numbers of monomers lie. An integer for “x” given as “about x” means that the arithmetic mean numbers of monomers lie in a range of integers of $x \pm 25\%$, preferably $x \pm 20\%$ and more preferably $x \pm 10\%$.

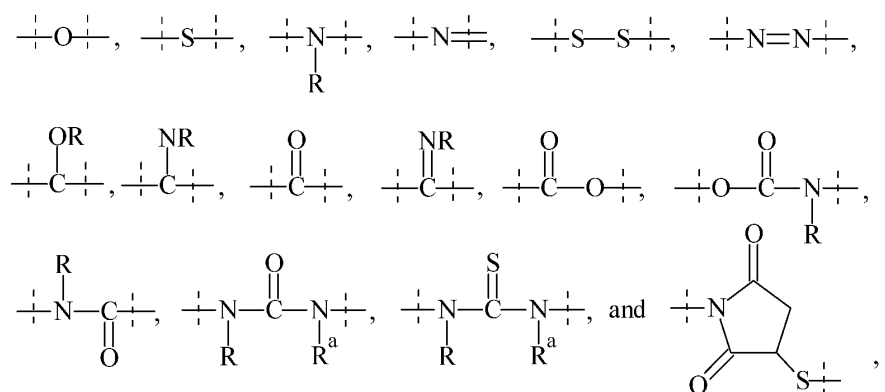
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As used herein, the term “number average molecular weight” means the ordinary arithmetic mean of the molecular weights of the individual polymers.

- As used herein, the term “PEG-based” in relation to a moiety or reagent means that said moiety or reagent comprises PEG. In certain embodiments a PEG-based moiety or reagent comprises at least 10% (w/w) PEG, such as at least 20% (w/w) PEG, such as at least 30% (w/w) PEG, such as at least 40% (w/w) PEG, such as at least 50% (w/w), such as at least 60 (w/w) PEG, such as at least 70% (w/w) PEG, such as at least 80% (w/w) PEG, such as at least 90% (w/w) PEG, such as at least 95%. The remaining weight percentage of the PEG-based moiety or reagent are other moieties that in certain embodiments are selected from the following moieties and linkages:

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- C_{1-50} alkyl, C_{2-50} alkenyl, C_{2-50} alkynyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, phenyl, naphthyl, indenyl, indanyl, and tetralinyl; and
- linkages selected from the group comprising



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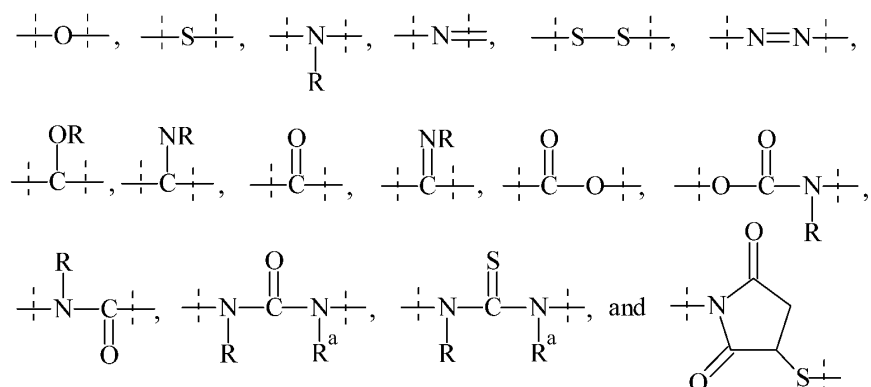
wherein

dashed lines indicate attachment to the remainder of the moiety or reagent, and
 -R and -R^a are independently of each other selected from the group consisting of -H, methyl,
 ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl,
 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-
 5 dimethylbutyl and 3,3-dimethylpropyl.

The term “hyaluronic acid-based” is used accordingly.

As used herein, the term “PEG-based comprising at least X% PEG” in relation to a moiety or reagent
 10 means that said moiety or reagent comprises at least X% (w/w) ethylene glycol units (-CH₂CH₂O-),
 wherein the ethylene glycol units may be arranged blockwise, alternating or may be randomly
 distributed within the moiety or reagent and in certain embodiments all ethylene glycol units of said
 moiety or reagent are present in one block; the remaining weight percentage of the PEG-based moiety
 or reagent are other moieties that in certain embodiments are selected from the following moieties
 15 and linkages:

- C₁₋₅₀ alkyl, C₂₋₅₀ alkenyl, C₂₋₅₀ alkynyl, C₃₋₁₀ cycloalkyl, 3- to 10-membered heterocyclyl, 8-
 to 11-membered heterobicyclyl, phenyl, naphthyl, indenyl, indanyl, and tetralinyl; and
- linkages selected from the group comprising



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wherein

dashed lines indicate attachment to the remainder of the moiety or reagent, and
 -R and -R^a are independently of each other selected from the group consisting of -H, methyl,
 ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl,
 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-
 25 dimethylbutyl and 3,3-dimethylpropyl.

The term “hyaluronic acid-based comprising at least X% hyaluronic acid” is used accordingly.

As used herein, the term “hydrogel” means a hydrophilic or amphiphilic polymeric network composed of homopolymers or copolymers, which is insoluble due to the presence of hydrophobic interactions, hydrogen bonds, ionic interactions, covalent chemical crosslinks or any combination thereof. In certain embodiments a hydrogel is insoluble due to the presence of covalent chemical crosslinks. In general, the crosslinks provide the network structure and physical integrity.

The term “interrupted” means that a moiety is inserted between two carbon atoms or – if the insertion is at one of the moiety’s ends – between a carbon or heteroatom and a hydrogen atom.

As used herein, the term “C₁₋₄ alkyl” alone or in combination means a straight-chain or branched alkyl moiety having 1 to 4 carbon atoms. If present at the end of a molecule, examples of straight-chain or branched C₁₋₄ alkyl are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl. When two moieties of a molecule are linked by the C₁₋₄ alkyl, then examples for such C₁₋₄ alkyl groups are -CH₂-, -CH₂-CH₂-, -CH(CH₃)-, -CH₂-CH₂-CH₂-, -CH(C₂H₅)-, -C(CH₃)₂-. Each hydrogen of a C₁₋₄ alkyl carbon may optionally be replaced by a substituent as defined above. Optionally, a C₁₋₄ alkyl may be interrupted by one or more moieties as defined below.

As used herein, the term “C₁₋₆ alkyl” alone or in combination means a straight-chain or branched alkyl moiety having 1 to 6 carbon atoms. If present at the end of a molecule, examples of straight-chain and branched C₁₋₆ alkyl groups are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl and 3,3-dimethylpropyl. When two moieties of a molecule are linked by the C₁₋₆ alkyl group, then examples for such C₁₋₆ alkyl groups are -CH₂-, -CH₂-CH₂-, -CH(CH₃)-, -CH₂-CH₂-CH₂-, -CH(C₂H₅)- and -C(CH₃)₂-. Each hydrogen atom of a C₁₋₆ carbon may optionally be replaced by a substituent as defined above. Optionally, a C₁₋₆ alkyl may be interrupted by one or more moieties as defined below.

Accordingly, “C₁₋₁₀ alkyl”, “C₁₋₂₀ alkyl” or “C₁₋₅₀ alkyl” means an alkyl chain having 1 to 10, 1 to 20 or 1 to 50 carbon atoms, respectively, wherein each hydrogen atom of the C₁₋₁₀, C₁₋₂₀ or C₁₋₅₀ carbon may optionally be replaced by a substituent as defined above. Optionally, a C₁₋₁₀ or C₁₋₅₀ alkyl may be interrupted by one or more moieties as defined below.

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As used herein, the term “C₂₋₆ alkenyl” alone or in combination means a straight-chain or branched hydrocarbon moiety comprising at least one carbon-carbon double bond having 2 to 6 carbon atoms. If present at the end of a molecule, examples are -CH=CH₂, -CH=CH-CH₃, -CH₂-CH=CH₂, -CH=CHCH₂-CH₃ and -CH=CH-CH=CH₂. When two moieties of a molecule are linked by the C₂₋₆ alkenyl group, then an example for such C₂₋₆ alkenyl is -CH=CH-. Each hydrogen atom of a C₂₋₆ alkenyl moiety may optionally be replaced by a substituent as defined above. Optionally, a C₂₋₆ alkenyl may be interrupted by one or more moieties as defined below.

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Accordingly, the term “C₂₋₁₀ alkenyl”, “C₂₋₂₀ alkenyl” or “C₂₋₅₀ alkenyl” alone or in combination means a straight-chain or branched hydrocarbon moiety comprising at least one carbon-carbon double bond having 2 to 10, 2 to 20 or 2 to 50 carbon atoms. Each hydrogen atom of a C₂₋₁₀ alkenyl, C₂₋₂₀ alkenyl or C₂₋₅₀ alkenyl group may optionally be replaced by a substituent as defined above. Optionally, a C₂₋₁₀ alkenyl, C₂₋₂₀ alkenyl or C₂₋₅₀ alkenyl may be interrupted by one or more moieties as defined below.

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As used herein, the term “C₂₋₆ alkynyl” alone or in combination means a straight-chain or branched hydrocarbon moiety comprising at least one carbon-carbon triple bond having 2 to 6 carbon atoms. If present at the end of a molecule, examples are -C≡CH, -CH₂-C≡CH, -CH₂-CH₂-C≡CH and CH₂-C≡C-CH₃. When two moieties of a molecule are linked by the alkynyl group, then an example is -C≡C-. Each hydrogen atom of a C₂₋₆ alkynyl group may optionally be replaced by a substituent as defined above. Optionally, one or more double bond(s) may occur. Optionally, a C₂₋₆ alkynyl may be interrupted by one or more moieties as defined below.

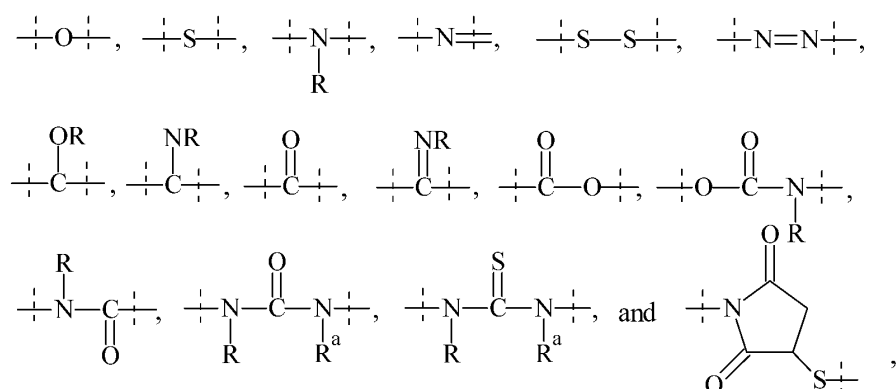
25

Accordingly, as used herein, the term “C₂₋₁₀ alkynyl”, “C₂₋₂₀ alkynyl” and “C₂₋₅₀ alkynyl” alone or in combination means a straight-chain or branched hydrocarbon moiety comprising at least one carbon-carbon triple bond having 2 to 10, 2 to 20 or 2 to 50 carbon atoms, respectively. Each hydrogen atom of a C₂₋₁₀ alkynyl, C₂₋₂₀ alkynyl or C₂₋₅₀ alkynyl group may optionally be replaced by a substituent as

30

defined above. Optionally, one or more double bond(s) may occur. Optionally, a C₂₋₁₀ alkynyl, C₂₋₂₀ alkynyl or C₂₋₅₀ alkynyl may be interrupted by one or more moieties as defined below.

As mentioned above, a C₁₋₄ alkyl, C₁₋₆ alkyl, C₁₋₁₀ alkyl, C₁₋₂₀ alkyl, C₁₋₅₀ alkyl, C₂₋₆ alkenyl, C₂₋₁₀ alkenyl, C₂₋₂₀ alkenyl, C₂₋₅₀ alkenyl, C₂₋₆ alkynyl, C₂₋₁₀ alkynyl, C₂₋₂₀ alkynyl or C₂₋₅₀ alkynyl may optionally be interrupted by one or more moieties which are preferably selected from the group consisting of



wherein

dashed lines indicate attachment to the remainder of the moiety or reagent; and
 -R and -R^a are independently of each other selected from the group consisting of -H, and methyl, ethyl, propyl, butyl, pentyl and hexyl.

As used herein, the term "C₃₋₁₀ cycloalkyl" means a cyclic alkyl chain having 3 to 10 carbon atoms, which may be saturated or unsaturated, e.g. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, cycloheptyl, cyclooctyl, cyclononyl or cyclodecyl. Each hydrogen atom of a C₃₋₁₀ cycloalkyl carbon may be replaced by a substituent as defined above. The term "C₃₋₁₀ cycloalkyl" also includes bridged bicycles like norbornane or norbornene.

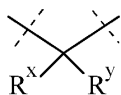
The term "8- to 30-membered carbopolycyclyl" or "8- to 30-membered carbopolycycle" means a cyclic moiety of two or more rings with 8 to 30 ring atoms, where two neighboring rings share at least one ring atom and that may contain up to the maximum number of double bonds (aromatic or non-aromatic ring which is fully, partially or un-saturated). In certain embodiments an 8- to 30-membered carbopolycyclyl means a cyclic moiety of two, three, four or five rings, more preferably of two, three or four rings.

As used herein, the term "3- to 10-membered heterocyclyl" or "3- to 10-membered heterocycle" means a ring with 3, 4, 5, 6, 7, 8, 9 or 10 ring atoms that may contain up to the maximum number of double bonds (aromatic or non-aromatic ring which is fully, partially or un-saturated) wherein at least one ring atom up to 4 ring atoms are replaced by a heteroatom selected from the group consisting of sulfur (including -S(O)-, -S(O)₂-), oxygen and nitrogen (including =N(O)-) and wherein the ring is linked to the rest of the molecule via a carbon or nitrogen atom. Examples for 3- to 10-membered heterocycles include but are not limited to aziridine, oxirane, thiirane, azirine, oxirene, thiirene, azetidine, oxetane, thietane, furan, thiophene, pyrrole, pyrroline, imidazole, imidazoline, pyrazole, pyrazoline, oxazole, oxazoline, isoxazole, isoxazoline, thiazole, thiazoline, isothiazole, isothiazoline, thiadiazole, thiadiazoline, tetrahydrofuran, tetrahydrothiophene, pyrrolidine, imidazolidine, pyrazolidine, oxazolidine, isoxazolidine, thiazolidine, isothiazolidine, thiadiazolidine, sulfolane, pyran, dihydropyran, tetrahydropyran, imidazolidine, pyridine, pyridazine, pyrazine, pyrimidine, piperazine, piperidine, morpholine, tetrazole, triazole, triazolidine, tetrazolidine, diazepane, azepine and homopiperazine. Each hydrogen atom of a 3- to 10-membered heterocyclyl or 3- to 10-membered heterocyclic group may be replaced by a substituent as defined below.

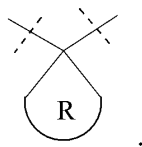
As used herein, the term "8- to 11-membered heterobicyclyl" or "8- to 11-membered heterobicycle" means a heterocyclic moiety of two rings with 8 to 11 ring atoms, where at least one ring atom is shared by both rings and that may contain up to the maximum number of double bonds (aromatic or non-aromatic ring which is fully, partially or un-saturated) wherein at least one ring atom up to 6 ring atoms are replaced by a heteroatom selected from the group consisting of sulfur (including -S(O)-, -S(O)₂-), oxygen and nitrogen (including =N(O)-) and wherein the ring is linked to the rest of the molecule via a carbon or nitrogen atom. Examples for an 8- to 11-membered heterobicycle are indole, indoline, benzofuran, benzothiophene, benzoxazole, benzisoxazole, benzothiazole, benzisothiazole, benzimidazole, benzimidazoline, quinoline, quinazoline, dihydroquinazoline, quinoline, dihydroquinoline, tetrahydroquinoline, decahydroquinoline, isoquinoline, decahydroisoquinoline, tetrahydroisoquinoline, dihydroisoquinoline, benzazepine, purine and pteridine. The term 8- to 11-membered heterobicycle also includes spiro structures of two rings like 1,4-dioxo-8-azaspiro[4.5]decane or bridged heterocycles like 8-aza-bicyclo[3.2.1]octane. Each hydrogen atom of an 8- to 11-membered heterobicyclyl or 8- to 11-membered heterobicycle carbon may be replaced by a substituent as defined below.

Similarly, the term “8- to 30-membered heteropolycyclyl” or “8- to 30-membered heteropolycycle” means a heterocyclic moiety of more than two rings with 8 to 30 ring atoms, preferably of three, four or five rings, where two neighboring rings share at least one ring atom and that may contain up to the maximum number of double bonds (aromatic or non-aromatic ring which is fully, partially or unsaturated), wherein at least one ring atom up to 10 ring atoms are replaced by a heteroatom selected from the group of sulfur (including $-S(O)-$, $-S(O)_2-$), oxygen and nitrogen (including $=N(O)-$) and wherein the ring is linked to the rest of a molecule via a carbon or nitrogen atom.

- 10 It is understood that the phrase “the pair R^x/R^y is joined together with the atom to which they are attached to form a C_{3-10} cycloalkyl or a 3- to 10-membered heterocyclyl” in relation with a moiety of the structure



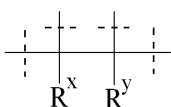
means that R^x and R^y form the following structure:



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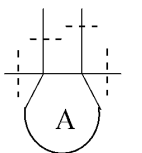
wherein R is C_{3-10} cycloalkyl or 3- to 10-membered heterocyclyl.

It is also understood that the phrase “the pair R^x/R^y is joint together with the atoms to which they are attached to form a ring A” in relation with a moiety of the structure



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means that R^x and R^y form the following structure:



As used herein, "halogen" means fluoro, chloro, bromo or iodo. It is generally preferred that halogen is fluoro or chloro.

As used herein, the term "functional group" means a group of atoms which can react with other groups of atoms. Exemplary functional groups are, for example, carboxylic acid $-(C=O)OH$, primary or secondary amine $(-NH_2, -NH-)$, maleimide, thiol $(-SH)$, sulfonic acid $-(O=S=O)OH$, carbonate, carbamate $(-O(C=O)N<)$, hydroxyl $(-OH)$, aldehyde $-(C=O)H$, ketone $-(C=O)-$, hydrazine $(>N-N<)$, isocyanate, isothiocyanate, phosphoric acid $(-O(P=O)OHOH)$, phosphonic acid $(-O(P=O)OHH)$, haloacetyl, alkyl halide, acryloyl, aryl fluoride, hydroxylamine, disulfide, sulfonamides, sulfuric acid, vinyl sulfone, vinyl ketone, diazoalkane, oxirane, and aziridine.

In case the long-acting GH comprises one or more acidic or basic groups, the invention also comprises its corresponding pharmaceutically or toxicologically acceptable salts, in particular their pharmaceutically utilizable salts. Thus, the long-acting GH comprising acidic groups may be used according to the invention, for example, as alkali metal salts, alkaline earth metal salts or as ammonium salts. More precise examples of such salts include sodium salts, potassium salts, calcium salts, magnesium salts or salts with ammonia or organic amines such as, for example, ethylamine, ethanolamine, triethanolamine or amino acids. Long-acting GH comprising one or more basic groups, i.e. groups which can be protonated, can be present and can be used according to the invention in the form of their addition salts with inorganic or organic acids. Examples for suitable acids include hydrogen chloride, hydrogen bromide, phosphoric acid, sulfuric acid, nitric acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acids, oxalic acid, acetic acid, tartaric acid, lactic acid, salicylic acid, benzoic acid, formic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, malic acid, sulfaminic acid, phenylpropionic acid, gluconic acid, ascorbic acid, isonicotinic acid, citric acid, adipic acid, and other acids known to the person skilled in the art. For the person skilled in the art further methods are known for converting the basic group into a cation, like the alkylation of an amine group resulting in a positively-charge ammonium group and an appropriate counterion of the salt. If long-acting GH simultaneously comprises acidic and basic groups, the invention also includes, in addition to the salt forms mentioned, inner salts or betaines (zwitterions). The respective salts can be obtained by customary methods, which are known to the person skilled in the art like, for example by contacting these compounds with an organic or inorganic acid or base in a solvent or dispersant, or by anion

exchange or cation exchange with other salts. The present invention also includes all salts of the long-acting GH which, owing to low physiological compatibility, are not directly suitable for use in pharmaceuticals but which can be used, for example, as intermediates for chemical reactions or for the preparation of pharmaceutically acceptable salts.

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The term "pharmaceutically acceptable" means a substance that does not cause harm when administered to a patient and preferably means approved by a regulatory agency, such as the EMA (Europe) and/or the FDA (US) and/or any other national regulatory agency for use in animals, such as for use in humans.

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As used herein, the term "excipient" refers to a diluent, adjuvant, or vehicle with which the therapeutic, such as a drug or prodrug, is administered. Such pharmaceutical excipient can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, including but not limited to peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred excipient when the pharmaceutical composition is administered orally. Saline and aqueous dextrose are preferred excipients when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions are preferably employed as liquid excipients for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, mannitol, trehalose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The pharmaceutical composition, if desired, can also contain minor amounts of wetting or emulsifying agents, pH buffering agents, like, for example, acetate, succinate, tris, carbonate, phosphate, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), MES (2-(N-morpholino)ethanesulfonic acid), or can contain detergents, like Tween, poloxamers, poloxamines, CHAPS, Igepal, or amino acids like, for example, glycine, lysine, or histidine. These pharmaceutical compositions can take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders, sustained-release formulations and the like. The pharmaceutical composition can be formulated as a suppository, with traditional binders and excipients such as triglycerides. Oral formulation can include standard excipients such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such compositions will contain a therapeutically effective amount of the drug or biologically active moiety,

together with a suitable amount of excipient so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In general, the terms “comprise” or “comprising” also encompass “consist of” or “consisting of”.

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In certain embodiments the long-acting growth hormone inhibits recruitment of inflammatory monocytes to the site of inflammation. Such site of inflammation is the liver, if the inflammation-induced disease is NAFLD, in particular NASH. In certain embodiments administration of the long-acting growth hormone leads to an increase in HLA-G in the liver, if the inflammation-induced

10 disease is NAFLD, in particular NASH. In certain embodiments administration of the long-acting growth hormone results in an increase in IGF-1 levels. It is understood that in case animal studies are performed that the corresponding genes from such animal species are used, for example the murine ortholog of HLA-G is H2/Bl.

15 In certain embodiments administration of the long-acting growth hormone leads to a change in one or more markers of hepatic inflammation selected from the group consisting of cytokines, chemokines and other transcriptional and histological markers. In certain embodiments the long-acting growth hormone for use in the treatment of an inflammation-induced disease, such as NAFLD and in particular NASH, leads to an improvement of transcriptional or histological markers of fibrosis.

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In certain embodiments administration of the long-acting growth hormone leads to a reduction in steatosis. In certain embodiments the long-acting growth hormone leads to a regeneration of the liver if the inflammation-induced disease is an inflammation-induced disease of the liver.

25 In certain embodiments the long-acting growth hormone is administered to the patient once a week. In certain embodiments the long-acting growth hormone is administered to the patient once every two weeks. In certain embodiments the long-acting growth hormone is administered to the patient once every four weeks. In certain embodiments the long-acting growth hormone is administered to the patient once a month.

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In another aspect the present invention relates to a long-acting GH for use in the treatment of an inflammation-induced disease, wherein the treatment comprises the steps of

- (a) administering at least a first dose of the long-acting GH to a patient having an inflammation-induced disease;
- (b) measuring Insulin-like Growth Factor-1 (IGF-1) levels; and
- (c) reducing the dose of the long-acting GH by at least 5% if IGF-1 levels are above a standard deviation score of +3 and increasing the dose of the long-acting GH by at least 5% if IGF-1 levels are below a standard deviation score of 0.

It is understood that no dose adjustments are required in step (c) if the IGF-1 levels fall into a standard deviation score ranging from 0 to +3. In certain embodiments steps (a) to (c) are repeated until IGF-1 levels are stable in a range from 0 to +3 standard deviation scores.

In certain embodiments the dose of the long-acting GH is reduced in step (c) by at least 5% if IGF-1 levels are above a standard deviation score of +2 and increased by at least 5% if IGF-1 levels are below a standard deviation score of +0.5. Accordingly, in such embodiments no dose adjustments are required in step (c) if the IGF-1 levels fall into a standard deviation score ranging from +0.5 to +2. In certain embodiments steps (a) to (c) are repeated until IGF-1 levels are stable in a range from +0.5 to +2 standard deviation scores.

Embodiments for the inflammation-induced disease, administration frequencies and the long-acting growth hormone are as described elsewhere herein.

In certain embodiments a dose reduction in step (c) is by 5%. In certain embodiments a dose reduction in step (c) is by 6%. In certain embodiments a dose reduction in step (c) is by 7%. In certain embodiments a dose reduction in step (c) is by 8%. In certain embodiments a dose reduction in step (c) is by 9%. In certain embodiments a dose reduction in step (c) is by 10%. In certain embodiments a dose reduction in step (c) is by 11%. In certain embodiments a dose reduction in step (c) is by 12%. In certain embodiments a dose reduction in step (c) is by 13%. In certain embodiments a dose reduction in step (c) is by 14%. In certain embodiments a dose reduction in step (c) is by 15%. In certain embodiments a dose reduction in step (c) is by 16%. In certain embodiments a dose reduction in step (c) is by 17%. In certain embodiments a dose reduction in step (c) is by 18%. In certain embodiments a dose reduction in step (c) is by 19%. In certain embodiments a dose reduction in step (c) is by 20%. In certain embodiments a dose increase in step (c) is by 5%. In certain embodiments a

dose increase in step (c) is by 6%. In certain embodiments a dose increase in step (c) is by 7%. In certain embodiments a dose increase in step (c) is by 8%. In certain embodiments a dose increase in step (c) is by 9%. In certain embodiments a dose increase in step (c) is by 10%. In certain embodiments a dose increase in step (c) is by 11%. In certain embodiments a dose increase in step (c) is by 12%. In certain embodiments a dose increase in step (c) is by 13%. In certain embodiments a dose increase in step (c) is by 14%. In certain embodiments a dose increase in step (c) is by 15%. In certain embodiments a dose increase in step (c) is by 16%. In certain embodiments a dose increase in step (c) is by 17%. In certain embodiments a dose increase in step (c) is by 18%. In certain embodiments a dose increase in step (c) is by 19%. In certain embodiments a dose increase in step (c) is by 20%.

In another aspect the present invention relates to a long-acting GH for use in the treatment of an inflammation-induced disease, wherein the treatment comprises the steps of

- (a) administering at least a first dose of the long-acting GH to a patient having an inflammation-induced disease;
- (b) measuring biomarkers indicative for M1 and M2 macrophages;
- (c) adjusting the dose of the long-acting GH based on the macrophage phenotype change by M1 reduction or M2 induction indicated by said biomarkers.

Embodiments for the inflammation-induced disease, administration frequencies and the long-acting growth hormone are as described elsewhere herein.

In certain embodiments biomarkers indicative of M1 and M2 macrophages are measured prior to initiation of the treatment with the long-acting GH.

In certain embodiments the biomarkers indicative of M1 macrophages are selected from the group consisting of interleukin (IL)-1 β , IL-6, IL-12, IL-23, IL-27, tumor necrosis factor α (TNF- α), interferon γ (IFN- γ), monocyte chemoattractant protein (MCP)-1, CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, CXCL11, CXCL16, CCR2, CCR7, IL1R1, TLR2, TLR4, MARCO, CD11c, CD38 and iNOS. In certain embodiments the biomarkers indicative of M1 macrophages are selected from the group consisting of IL-6, TNF- α , CD11c and iNOS.

In certain embodiments the biomarkers indicative of M2 macrophages are selected from the group consisting of IL-2, IL-4, IL-10, IL-13, CCL17, CCL18, CCL22, CCL24, CCL13, CCL16, CXCR1, CXCR2, CD14, CD23, CD36, CD163, mannose receptor (CD206), scavenger receptor A, Chi3l3/Ym1, Retnla/Fizz-1 and arginase-1. In certain embodiments the biomarkers indicative of M2
5 macrophages are selected from the group consisting of IL-10, CD36, CD206, Retnla/Fizz-1 and arginase-1.

In certain embodiments in step (c) the dose of the long-acting growth hormone is increased, if the M1 to M2 macrophage ratio is unbalanced, such as when the biomarkers indicative of M1 type of
10 macrophage are still increasing or dominant compared to biomarkers indicative of M2 macrophage. In certain embodiments such dose increase is an increase of 5%. In certain embodiments such dose increase is an increase of 6%. In certain embodiments such dose increase is an increase of 7%. In certain embodiments such dose increase is an increase of 8%. In certain embodiments such dose increase is an increase of 9%. In certain embodiments such dose increase is an increase of 10%. In
15 certain embodiments such dose increase is an increase of 11%. In certain embodiments such dose increase is an increase of 12%. In certain embodiments such dose increase is an increase of 13%. In certain embodiments such dose increase is an increase of 14%. In certain embodiments such dose increase is an increase of 15%. In certain embodiments such dose increase is an increase of 16%. In certain embodiments such dose increase is an increase of 17%. In certain embodiments such dose
20 increase is an increase of 18%. In certain embodiments such dose increase is an increase of 19%. In certain embodiments such dose increase is an increase of 20%. If in step (c) the M1 to M2 macrophage ratio is balanced, such as when the biomarkers indicative of M1 and M2 are reaching a steady state, the dose is in certain embodiments not adjusted, but stays constant.

25 In certain embodiments dose adjustments in step (c) are accompanied by measuring IGF-1 levels and adjustments of the dose of the long-acting GH are such that IGF-1 levels are in a range from 0 to +3 standard deviation scores. In certain embodiments dose adjustments in step (c) are accompanied by measuring IGF-1 levels and adjustments of the dose of the long-acting GH are such that IGF-1 levels are in a range from +0.5 to +2 standard deviation scores.

30

In certain embodiments steps (b) and (c) are repeated until macrophage rebalancing is achieved.

In another aspect the present invention relates to a method of treating an inflammation-induced disease, wherein the method comprises the step of administering a pharmaceutically effective amount of a long-acting growth hormone. Embodiments of the long-acting growth hormone and the inflammation-induced disease are described elsewhere herein.

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In another aspect the present invention relates to a method comprising the steps of

- (a) administering at least a first dose of the long-acting GH to a patient having an inflammation-induced disease;
- (b) measuring Insulin-like Growth Factor-1 (IGF-1) levels; and
- 10 (c) reducing the dose of the long-acting GH by at least 5% if IGF-1 levels are above a standard deviation score of +3 and increasing the dose of the long-acting GH by at least 5% if IGF-1 levels are below a standard deviation score of 0.

It is understood that no dose adjustments are required in step (c) if the IGF-1 levels fall into a standard deviation score ranging from 0 to +3. In certain embodiments steps (a) to (c) are repeated until IGF-1 levels are stable in a range from 0 to +3 standard deviation scores.

15

In certain embodiments the dose of the long-acting GH is reduced in step (c) by at least 5% if IGF-1 levels are above a standard deviation score of +2 and increased by at least 5% if IGF-1 levels are below a standard deviation score of +0.5. Accordingly, in such embodiments no dose adjustments are required in step (c) if the IGF-1 levels fall into a standard deviation score ranging from +0.5 to +2. In certain embodiments steps (a) to (c) are repeated until IGF-1 levels are stable in a range from +0.5 to +2 standard deviation scores.

20

25 Embodiments for the dose adjustments of step (c) are as described elsewhere herein.

In another aspect the present invention relates to a method comprising the steps of

- (a) administering at least a first dose of the long-acting GH to a patient having an inflammation-induced disease;
- 30 (b) measuring biomarkers indicative for M1 and M2 macrophages;
- (c) adjusting the dose of the long-acting GH based on the macrophage phenotype change by M1 reduction or M2 induction indicated by said biomarkers.

In certain embodiments steps (b) and (c) are repeated until macrophage rebalancing is achieved.

Embodiments for the biomarkers of step (b) and the dose adjustments of step (c) are described
5 elsewhere herein.

In certain embodiments the long-acting growth hormone comprises at least one human growth hormone (hGH). In certain embodiments the hGH has the sequence of SEQ ID NO:1. In certain
10 embodiments the hGH has a sequence with at least 90% identity to the sequence of SEQ ID NO:1. In certain embodiments the hGH has a sequence with at least 92% identity to the sequence of SEQ ID NO:1. In certain embodiments the hGH has a sequence with at least 94% identity to the sequence of
SEQ ID NO:1. In certain embodiments the hGH has a sequence with at least 95% identity to the
sequence of SEQ ID NO:1. In certain embodiments the hGH has a sequence with at least 96% identity
to the sequence of SEQ ID NO:1. In certain embodiments the hGH has a sequence with at least 97%
15 identity to the sequence of SEQ ID NO:1. In certain embodiments the hGH has a sequence with at
least 98% identity to the sequence of SEQ ID NO:1. In certain embodiments the hGH has a sequence
with at least 99% identity to the sequence of SEQ ID NO:1.

In one embodiment the long-acting GH comprises growth hormone non-covalently embedded or
20 encapsulated in a polymer or lipid-comprising matrix. In certain embodiments the long-acting GH
comprises growth hormone non-covalently embedded or encapsulated in a polymer. A preferred
polymer matrix comprises a polymer selected from the group consisting of 2-methacryloyl-oxyethyl
phosphoyl cholins, poly(acrylic acids), poly(acrylates), poly(acrylamides), poly(alkyloxy) polymers,
poly(amides), poly(amidoamines), poly(amino acids), poly(anhydrides), poly(aspartamides),
25 poly(butyric acids), poly(glycolic acids), poly(butylene terephthalates), poly(caprolactones),
poly(carbonates), poly(cyanoacrylates), poly(dimethylacrylamides), poly(esters), poly(ethylenes),
poly(ethyleneglycols), poly(ethylene oxides), poly(ethyl phosphates), poly(ethyloxazolines),
poly(glycolic acids), poly(hydroxyethyl acrylates), poly(hydroxyethyl-oxazolines),
poly(hydroxymethacrylates), poly(hydroxypropylmethacrylamides), poly(hydroxypropyl
30 methacrylates), poly(hydroxypropyloxazolines), poly(iminocarbonates), poly(lactic acids),
poly(lactic-co-glycolic acids), poly(methacrylamides), poly(methacrylates), poly(methyloxazolines),
poly(organophosphazenes), poly(ortho esters), poly(oxazolines), poly(propylene glycols),

poly(siloxanes), poly(urethanes), poly(vinyl alcohols), poly(vinyl amines), poly(vinylmethylethers), poly(vinylpyrrolidones), silicones, celluloses, carbomethyl celluloses, hydroxypropyl methylcelluloses, chitins, chitosans, dextrans, dextrans, gelatins, hyaluronic acids and derivatives, functionalized hyaluronic acids, mannans, pectins, rhamnogalacturonans, starches, hydroxyalkyl
 5 starches, hydroxyethyl starches and other carbohydrate-based polymers, xylans, and copolymers thereof.

In certain embodiments the polymer is selected from the group consisting of PEG, polylactid-co-glycolid (PLGA) and hyaluronic acid. In certain embodiments the polymer is PEG. In certain
 10 embodiments the polymer is PLGA. In certain embodiments the polymer is hyaluronic acid.

In certain embodiments the polymer matrix is a hydrogel comprising a polymer selected from the group consisting of 2-methacryloyl-oxyethyl phosphoyl cholins, poly(acrylic acids), poly(acrylates), poly(acrylamides), poly(alkyloxy) polymers, poly(amides), poly(amidoamines), poly(amino acids),
 15 poly(anhydrides), poly(aspartamides), poly(butyric acids), poly(glycolic acids), polybutylene terephthalates, poly(caprolactones), poly(carbonates), poly(cyanoacrylates), poly(dimethylacrylamides), poly(esters), poly(ethylenes), poly(ethyleneglycols), poly(ethylene oxides), poly(ethyl phosphates), poly(ethyloxazolines), poly(glycolic acids), poly(hydroxyethyl acrylates), poly(hydroxyethyl-oxazolines), poly(hydroxymethacrylates),
 20 poly(hydroxypropylmethacrylamides), poly(hydroxypropyl methacrylates), poly(hydroxypropyloxazolines), poly(iminocarbonates), poly(lactic acids), poly(lactic-co-glycolic acids), poly(methacrylamides), poly(methacrylates), poly(methyloxazolines), poly(organophosphazenes), poly(ortho esters), poly(oxazolines), poly(propylene glycols), poly(siloxanes), poly(urethanes), poly(vinyl alcohols), poly(vinyl amines), poly(vinylmethylethers),
 25 poly(vinylpyrrolidones), silicones, celluloses, carbomethyl celluloses, hydroxypropyl methylcelluloses, chitins, chitosans, dextrans, dextrans, gelatins, hyaluronic acids and derivatives, functionalized hyaluronic acids, mannans, pectins, rhamnogalacturonans, starches, hydroxyalkyl starches, hydroxyethyl starches and other carbohydrate-based polymers, xylans, and copolymers thereof. In certain embodiments the hydrogel comprises a polymer selected from the group consisting
 30 of PEG, polylactid-co-glycolid (PLGA) and hyaluronic acid. In certain embodiments the hydrogel is a PEG-based hydrogel. In certain embodiments the hydrogel comprises PLGA. In certain embodiments the hydrogel comprises hyaluronic acid.

In certain embodiments the long-acting growth hormone is crystalline growth hormone.

In certain embodiments the long-acting growth hormone comprises a growth hormone moiety fused to at least one natural or unnatural amino acid sequence. It is understood that such amino acid sequence comprises one or more amino acid residues. In certain embodiments such growth hormone fusion protein comprises a recognition sequence for enzymatic cleavage between a growth hormone moiety and a natural or unnatural amino acid sequence. In certain embodiments the growth hormone fusion protein comprises a chemical cleavage site between a growth hormone moiety and a natural or unnatural amino acid sequence. In certain embodiments the amino acid sequence is selected from the group consisting of carboxyl-terminal peptide of the chorionic gonadotropin as described in US 2012/0035101; albumin; XTEN sequences as described in WO2011/123813A2; proline/alanine random coil sequences as described in WO2011/144756A1; proline/alanine/serine random coil sequences as described in WO2008/155134; and Fc fusion proteins. In certain embodiments the long-acting growth hormone comprises a hGH-CTP fusion protein. In certain embodiments the long-acting growth hormone comprises a hGH-XTEN fusion protein. In certain embodiments the long-acting growth hormone comprises a hGH-HSA fusion protein. In certain embodiments the long-acting growth hormone comprises a hGH-Fc fusion protein.

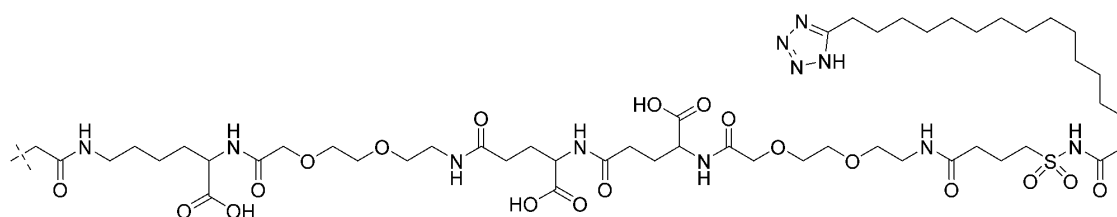
In certain embodiments the long-acting growth hormone comprises a growth hormone moiety covalently conjugated to one or more chemical moiety. In certain embodiments the chemical moiety is a polymeric moiety, such as a polymeric moiety that comprises a polymer selected from the group consisting of 2-methacryloyl-oxyethyl phosphoyl cholins, poly(acrylic acids), poly(acrylates), poly(acrylamides), poly(alkyloxy) polymers, poly(amides), poly(amidoamines), poly(amino acids), poly(anhydrides), poly(aspartamides), poly(butyric acids), poly(glycolic acids), polybutylene terephthalates, poly(caprolactones), poly(carbonates), poly(cyanoacrylates), poly(dimethylacrylamides), poly(esters), poly(ethylenes), poly(ethyleneglycols), poly(ethylene oxides), poly(ethyl phosphates), poly(ethyloxazolines), poly(glycolic acids), poly(hydroxyethyl acrylates), poly(hydroxyethyl-oxazolines), poly(hydroxymethacrylates), poly(hydroxypropylmethacrylamides), poly(hydroxypropyl methacrylates), poly(hydroxypropyloxazolines), poly(iminocarbonates), poly(lactic acids), poly(lactic-co-glycolic acids), poly(methacrylamides), poly(methacrylates), poly(methyloxazolines),

poly(organophosphazenes), poly(ortho esters), poly(oxazolines), poly(propylene glycols), poly(siloxanes), poly(urethanes), poly(vinyl alcohols), poly(vinyl amines), poly(vinylmethylethers), poly(vinylpyrrolidones), silicones, celluloses, carbomethyl celluloses, hydroxypropyl methylcelluloses, chitins, chitosans, dextrans, dextrins, gelatins, hyaluronic acids and derivatives, functionalized hyaluronic acids, mannans, pectins, rhamnogalacturonans, starches, hydroxyalkyl starches, hydroxyethyl starches and other carbohydrate-based polymers, xylans, and copolymers thereof. In certain embodiments the polymeric moiety comprises a PEG-based polymer. In certain embodiments the polymeric moiety comprises a hyaluronic acid-based polymer.

- 10 In certain embodiments the polymeric moiety comprises a linear, branched, dendrimeric or cyclic polymer or any combination thereof. In certain embodiments the polymeric moiety comprises a linear polymer. In certain embodiments the polymeric moiety comprises a branched polymer, such as a polymer with one, two, three, four or five branching points, which branching points may in certain embodiments be selected from the group consisting of $-N<$, $-CR^{b1}<$ and $>C<$, wherein $-R^{b1}$ is selected from the group consisting of $-H$, C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl; wherein C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl are optionally substituted with one or more $-R^{b2}$, which are the same or different, and wherein C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl are optionally interrupted with $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{b3})-$, $-S(O)_2N(R^{b3})-$, $-S(O)N(R^{b3})-$, $-S(O)_2-$, $-S(O)-$, $-N(R^{b3})S(O)_2N(R^{b3a})-$, $-S-$, $-N(R^{b3})-$, $-OC(OR^{b3})(R^{b3a})-$, $-N(R^{b3})C(O)N(R^{b3a})-$, and $-OC(O)N(R^{b3})-$; wherein $-R^{b2}$, $-R^{b3}$ and $-R^{b3a}$ are selected from $-H$, C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl. In certain embodiments the polymeric moiety comprises a dendrimeric polymer. In certain embodiments the polymeric moiety comprises a cyclic polymer.

- 25 In certain embodiments the chemical moiety is a fatty acid or fatty acid variant moiety, which may optionally be substituted. In certain embodiments such fatty acid or fatty acid variant moiety has a structure as disclosed in WO2005/027978A2 and WO2014/060512A1. In certain embodiments the long-acting growth hormone is a growth hormone-fatty acid conjugate. In certain embodiments the long-acting growth hormone is a growth hormone-fatty acid variant conjugate.

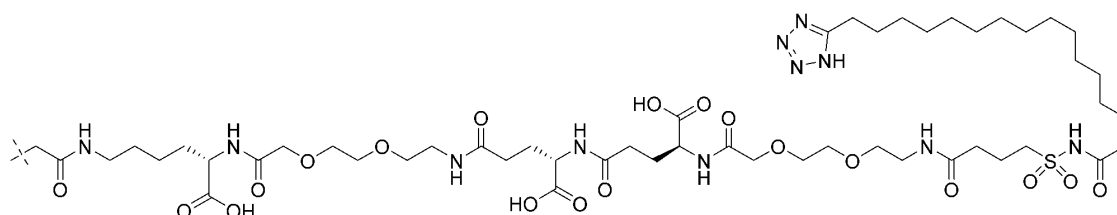
- 30 In certain embodiments the long-acting growth hormone comprises a moiety of formula (F):



(F), wherein the dashed line indicates attachment to the remainder of the long-acting growth hormone.

In certain embodiments the dashed line in formula (F) indicates attachment to a growth hormone moiety. In certain embodiments the dashed line in formula (F) indicates attachment to the sulfur of a thiol of a cysteine side chain of a growth hormone moiety. In certain embodiments the growth hormone moiety is of SEQ ID NO:1, in which the leucine at position 101 is mutated to cysteine and wherein the dashed line of formula (F) indicates attachment to this cysteine at position 101. It is understood that “attachment to this cysteine” means attachment to the sulfur of the thiol of the cysteine.

In certain embodiments the fatty acid or fatty acid variant moiety has the structure of formula (F-i):



(F-i), wherein the dashed line indicates attachment to the remainder of the long-acting growth hormone.

In certain embodiments the dashed line in formula (F-i) indicates attachment to a growth hormone moiety. In certain embodiments the dashed line in formula (F-i) indicates attachment to the sulfur of a thiol of a cysteine side chain of a growth hormone moiety. In certain embodiments the growth hormone moiety is of SEQ ID NO:1, in which the leucine at position 101 is mutated to cysteine and wherein the dashed line of formula (F-i) indicates attachment to this cysteine at position 101. It is understood that “attachment to this cysteine” means attachment to the sulfur of the thiol of the cysteine.

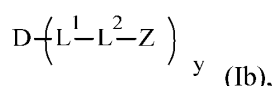
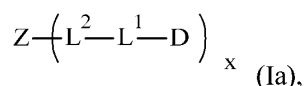
If the long-acting GH is of formula (F-i), with the growth hormone moiety having the sequence of SEQ ID NO:1, in which the leucine at position 101 is replaced by a cysteine and to which cysteine the dashed line indicates attachment via the sulfur of its thiol, the long-acting GH is somapacitan, which is marketed as Sogroya[®].

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In certain embodiments the bond between the growth hormone moiety and the chemical moiety is a stable covalent bond. In certain embodiments the bond between the growth hormone moiety and the chemical moiety is a reversible covalent bond. If the growth hormone moiety is reversibly conjugated to one or more chemical moiety such conjugate may also be referred to as prodrug.

10

In certain embodiments the long-acting growth hormone is a growth hormone conjugate or a pharmaceutically acceptable salt thereof of formula (Ia) or (Ib)



15

wherein

each -D is independently a growth hormone moiety;

each -L¹- is independently a linker moiety covalently and reversibly attached to -D;

each -L²- is independently a chemical bond or is a spacer moiety;

each -Z is independently a polymeric moiety or a fatty acid moiety, which is optionally substituted;

20

x is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16; and

y is an integer selected from the group consisting of 2, 3, 4 and 5.

25

In certain embodiments the growth hormone conjugate is of formula (Ia). In certain embodiments x is 1. In certain embodiments the growth hormone conjugate is of formula (Ib). In certain embodiments y is 2. In certain embodiments y is 3. In certain embodiments y is 4.

In certain embodiments all moieties -D of the conjugate are identical. In certain embodiments the conjugate of the present invention comprises more than one type of -D, such as two, three, four or five different types of moiety -D, provided there is more than one moiety -D present in the conjugate.

- 5 In certain embodiments -D of formula (Ia) and (Ib) has the sequence of SEQ ID NO:1. In certain embodiments -D of formula (Ia) and (Ib) has a sequence with at least 90% identity to the sequence of SEQ ID NO:1. In certain embodiments -D of formula (Ia) and (Ib) has a sequence with at least 92% identity to the sequence of SEQ ID NO:1. In certain embodiments -D of formula (Ia) and (Ib) has a sequence with at least 94% identity to the sequence of SEQ ID NO:1. In certain embodiments -D of formula (Ia) and (Ib) has a sequence with at least 95% identity to the sequence of SEQ ID NO:1. In certain embodiments -D of formula (Ia) and (Ib) has a sequence with at least 96% identity to the sequence of SEQ ID NO:1. In certain embodiments -D of formula (Ia) and (Ib) has a sequence with at least 97% identity to the sequence of SEQ ID NO:1. In certain embodiments -D of formula (Ia) and (Ib) has a sequence with at least 98% identity to the sequence of SEQ ID NO:1. In certain
10
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embodiments -D of formula (Ia) and (Ib) has a sequence with at least 99% identity to the sequence of SEQ ID NO:1.

- In certain embodiments all moieties -L¹- of the conjugate are identical, provided there is more than one moiety -L¹- present in the conjugate. In certain embodiments the conjugate of the present
20 invention comprises more than one type of -L¹-, such as two, three, four or five different moieties -L¹-.

- The moiety -L¹- may be any moiety that allows for the reversible attachment of -D. In certain embodiments -L¹- is a traceless linker, i.e. a linker from the drug D-H is released in its unmodified form, which may also be referred to as free form.

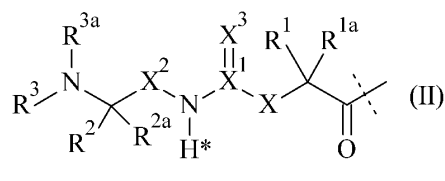
- 25 The moiety -L¹- may be attached to a proteinogenic or non-proteinogenic amino acid residue of -D. In certain embodiments -L¹- is attached to a non-proteinogenic amino acid residue. In certain embodiments attachment of -L¹- is to a proteinogenic amino acid residue. If attachment occurs at a proteinogenic amino acid residue, said proteinogenic amino acid residue is in certain embodiments
30 selected from the group consisting of cysteine, methionine, histidine, lysine, tryptophan, serine, threonine, tyrosine, aspartic acid, glutamic acid, glutamine and arginine. In certain embodiments such proteinogenic amino acid residue is selected from the group consisting of cysteine, histidine, lysine,

tryptophan, serine, threonine, tyrosine, aspartic acid, glutamic acid and arginine. In certain embodiments such proteinogenic amino acid is cysteine. In certain embodiments such proteinogenic amino acid is histidine. In certain embodiments such proteinogenic amino acid is lysine. In certain embodiments such proteinogenic amino acid is tryptophan. In certain embodiments such proteinogenic amino acid is serine. In certain embodiments such proteinogenic amino acid is threonine. In certain embodiments such proteinogenic amino acid is aspartic acid. In certain embodiments such proteinogenic amino acid is glutamic acid. In certain embodiments such proteinogenic amino acid is arginine.

- 10 The moiety $-L^1$ may be connected to $-D$ through any type of linkage, provided that it is reversible, and it is understood that the functional group of $-D$ chosen for attachment influences the linkage between $-L^1$ and $-D$. In certain embodiments $-L^1$ is connected to $-D$ through a linkage selected from the group consisting of amide, ester, carbamate, acetal, aminal, imine, oxime, hydrazone, disulfide and acylguanidine. In certain embodiments $-L^1$ is connected to $-D$ through a linkage selected from the group consisting of amide, ester, carbamate and acylguanidine. It is understood that these linkages may not be reversible per se, but that reversibility may be an effect of certain groups of atoms or moieties present in $-L^1$. In certain embodiments $-L^1$ is connected to $-D$ through an ester linkage. In certain embodiments $-L^1$ is connected to $-D$ through a carbamate linkage. In certain embodiments $-L^1$ is connected to $-D$ through an acylguanidine. In certain embodiments $-L^1$ is connected to $-D$ through an amide linkage.

- 25 In certain embodiments $-D$ is conjugated to $-L^1$ through the nitrogen of an amine functional group of $-D$. Such amine functional group may be the N-terminal amine functional group of $-D$ or may be the amine functional group from the side chain of a lysine residue. In certain embodiments $-D$ is conjugated to $-L^1$ via the nitrogen of the N-terminal amine functional group. In certain embodiments $-D$ is conjugated to $-L^1$ through the nitrogen of an amine functional group from the side chain of a lysine residue. In certain embodiments $-L^1$ is connected to $-D$ via the nitrogen of an amine functional group of a side chain of a lysine residue of $-D$ and the linkage formed between $-D$ and $-L^1$ is a carbamate. In certain embodiments $-L^1$ is connected to $-D$ via the nitrogen of an amine functional group of a side chain of a lysine residue of $-D$ and the linkage formed between $-D$ and $-L^1$ is an amide.

In certain embodiments -L¹- has a structure as disclosed in WO 2009/095479 A2. Accordingly, in certain embodiments the moiety -L¹- is of formula (II):



wherein the dashed line indicates attachment to a nitrogen of -D by forming an amide bond;

-X- is selected from the group consisting of -C(R⁴R^{4a})-; -N(R⁴)-; -O-; -C(R⁴R^{4a})-C(R⁵R^{5a})-; -C(R⁵R^{5a})-C(R⁴R^{4a})-; -C(R⁴R^{4a})-N(R⁶)-; -N(R⁶)-C(R⁴R^{4a})-; -C(R⁴R^{4a})-O-; -O-C(R⁴R^{4a})-; and -C(R⁷R^{7a})-;

X¹ is selected from the group consisting of C and S(O);

-X²- is selected from the group consisting of -C(R⁸R^{8a})- and -C(R⁸R^{8a})-C(R⁹R^{9a})-;

=X³ is selected from the group consisting of =O; =S and =N-CN;

-R¹, -R^{1a}, -R², -R^{2a}, -R⁴, -R^{4a}, -R⁵, -R^{5a}, -R⁶, -R⁸, -R^{8a}, -R⁹, -R^{9a} are independently selected from the group consisting of -H; and C₁₋₆ alkyl;

-R³, -R^{3a} are independently selected from the group consisting of -H; and C₁₋₆ alkyl, provided that in case one of -R³, -R^{3a} or both are other than -H they are connected to N to which they are attached through an SP³-hybridized carbon atom;

-R⁷ is selected from the group consisting of -N(R¹⁰R^{10a}) and -NR¹⁰-(C=O)-R¹¹;

-R^{7a}, -R¹⁰, -R^{10a}, -R¹¹ are independently of each other selected from the group consisting of -H and C₁₋₆ alkyl;

optionally, one or more of the pairs -R^{1a}/R^{4a}, -R^{1a}/R^{5a}, -R^{1a}/R^{7a}, -R^{4a}/R^{5a}, -R^{8a}/R^{9a} form a chemical bond;

optionally, one or more of the pairs -R¹/R^{1a}, -R²/R^{2a}, -R⁴/R^{4a}, -R⁵/R^{5a}, -R⁸/R^{8a},

-R⁹/R^{9a} are joined together with the atom to which they are attached to form a C₃₋₁₀ cycloalkyl or 3- to 10-membered heterocyclyl;

optionally, one or more of the pairs -R¹/R⁴, -R¹/R⁵, -R¹/R⁶, -R¹/R^{7a}, -R⁴/R⁵, -R⁴/R⁶, -R⁸/R⁹, -R²/R³ are joined together with the atoms to which they are attached to form a ring A;

optionally, R³/R^{3a} are joined together with the nitrogen atom to which they are attached to form a 3- to 10-membered heterocycle;

A is selected from the group consisting of phenyl; naphthyl; indenyl; indanyl; tetralinyl; C₃₋₁₀ cycloalkyl; 3- to 10-membered heterocyclyl; and 8- to 11-membered heterobicyclyl; and wherein -L¹- is substituted with at least one -L²-Z and wherein -L¹- is optionally further substituted, provided that the hydrogen marked with the asterisk in formula (II) is not replaced by -L²-Z or a substituent.

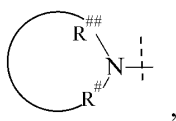
In certain embodiments -L¹- of formula (II) is substituted with one moiety -L²-Z.

In certain embodiments -L¹- of formula (II) is not further substituted.

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It is understood that if -R³/-R^{3a} of formula (II) are joined together with the nitrogen atom to which they are attached to form a 3- to 10-membered heterocycle, only such 3- to 10-membered heterocycles may be formed in which the atoms directly attached to the nitrogen are sp³-hybridized carbon atoms. In other words, such 3- to 10-membered heterocycle formed by -R³/-R^{3a} together with the nitrogen atom to which they are attached has the following structure:

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wherein

the dashed line indicates attachment to the rest of -L¹-;

the ring comprises 3 to 10 atoms comprising at least one nitrogen; and

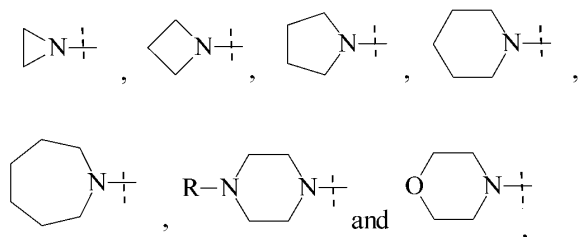
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R[#] and R^{##} represent an sp³-hybridized carbon atom.

It is also understood that the 3- to 10-membered heterocycle may be further substituted.

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Exemplary embodiments of suitable 3- to 10-membered heterocycles formed by -R³/-R^{3a} of formula (II) together with the nitrogen atom to which they are attached are the following:



wherein

dashed lines indicate attachment to the rest of the molecule; and

-R is selected from the group consisting of -H and C₁₋₆ alkyl.

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The moiety -L¹- of formula (II) may optionally be further substituted. In general, any substituent may be used as far as the cleavage principle is not affected, i.e. the hydrogen marked with the asterisk in formula (II) is not replaced and the nitrogen of the moiety

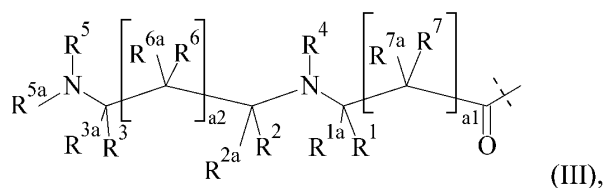


- 10 of formula (II) remains part of a primary, secondary or tertiary amine, i.e. -R³ and -R^{3a} are independently of each other -H or are connected to -N< through an sp³-hybridized carbon atom.

- In one embodiment -R¹ or -R^{1a} of formula (II) is substituted with -L²-Z or -L²-Z'. In another embodiment -R² or -R^{2a} of formula (II) is substituted with -L²-Z or -L²-Z'. In another embodiment -R³ or -R^{3a} of formula (II) is substituted with -L²-Z or -L²-Z'. In another embodiment -R⁴ of formula (II) is substituted with -L²-Z or -L²-Z'. In another embodiment -R⁵ or -R^{5a} of formula (II) is substituted with -L²-Z or -L²-Z'. In another embodiment -R⁶ of formula (II) is substituted with -L²-Z or -L²-Z'. In another embodiment -R⁷ or -R^{7a} of formula (II) is substituted with -L²-Z or -L²-Z'. In another embodiment -R⁸ or -R^{8a} of formula (II) is substituted with -L²-Z or -L²-Z'. In another embodiment -R⁹ or -R^{9a} of formula (II) is substituted with -L²-Z or -L²-Z'.

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In certain embodiments -L¹- has a structure as disclosed in WO2016/020373A1. Accordingly, in certain embodiments the moiety -L¹- is of formula (III):



wherein

the dashed line indicates attachment to a primary or secondary amine or hydroxyl of -D by forming an amide or ester linkage, respectively;

$-R^1$, $-R^{1a}$, $-R^2$, $-R^{2a}$, $-R^3$ and $-R^{3a}$ are independently of each other selected from the group consisting of $-H$, $-C(R^8R^{8a}R^{8b})$, $-C(=O)R^8$, $-C\equiv N$, $-C(=NR^8)R^{8a}$, $-CR^8(=CR^{8a}R^{8b})$, $-C\equiv CR^8$ and $-T$;

$-R^4$, $-R^5$ and $-R^{5a}$ are independently of each other selected from the group consisting of $-H$, $-C(R^9R^{9a}R^{9b})$ and $-T$;

a_1 and a_2 are independently of each other 0 or 1;

each $-R^6$, $-R^{6a}$, $-R^7$, $-R^{7a}$, $-R^8$, $-R^{8a}$, $-R^{8b}$, $-R^9$, $-R^{9a}$, $-R^{9b}$ are independently of each other selected from the group consisting of $-H$, halogen, $-CN$, $-COOR^{10}$, $-OR^{10}$, $-C(O)R^{10}$, $-C(O)N(R^{10}R^{10a})$, $-S(O)_2N(R^{10}R^{10a})$, $-S(O)N(R^{10}R^{10a})$, $-S(O)_2R^{10}$, $-S(O)R^{10}$, $-N(R^{10})S(O)_2N(R^{10a}R^{10b})$, $-SR^{10}$, $-N(R^{10}R^{10a})$, $-NO_2$, $-OC(O)R^{10}$, $-N(R^{10})C(O)R^{10a}$, $-N(R^{10})S(O)_2R^{10a}$, $-N(R^{10})S(O)R^{10a}$, $-N(R^{10})C(O)OR^{10a}$, $-N(R^{10})C(O)N(R^{10a}R^{10b})$, $-OC(O)N(R^{10}R^{10a})$, $-T$, C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl; wherein $-T$, C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are optionally substituted with one or more $-R^{11}$, which are the same or different and wherein C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are optionally interrupted by one or more groups selected from the group consisting of $-T$ -, $-C(O)O$ -, $-O$ -, $-C(O)$ -, $-C(O)N(R^{12})$ -, $-S(O)_2N(R^{12})$ -, $-S(O)N(R^{12})$ -, $-S(O)_2$ -, $-S(O)$ -, $-N(R^{12})S(O)_2N(R^{12a})$ -, $-S$ -, $-N(R^{12})$ -, $-OC(OR^{12})(R^{12a})$ -, $-N(R^{12})C(O)N(R^{12a})$ -, and $-OC(O)N(R^{12})$ -;

each $-R^{10}$, $-R^{10a}$, $-R^{10b}$ is independently selected from the group consisting of $-H$, $-T$, C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl; wherein $-T$, C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are optionally substituted with one or more $-R^{11}$, which are the same or different and wherein C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are optionally interrupted by one or more groups selected from the group consisting of $-T$ -, $-C(O)O$ -, $-O$ -, $-C(O)$ -, $-C(O)N(R^{12})$ -,

-S(O)₂N(R¹²)-, -S(O)N(R¹²)-, -S(O)₂-, -S(O)-, -N(R¹²)S(O)₂N(R^{12a})-, -S-,
-N(R¹²)-, -OC(OR¹²)(R^{12a})-, -N(R¹²)C(O)N(R^{12a})-, and -OC(O)N(R¹²)-;

each T is independently of each other selected from the group consisting of phenyl, naphthyl,
indanyl, indanyl, tetralinyl, C₃₋₁₀ cycloalkyl, 3- to 10-membered heterocyclyl, and 8- to 11-
5 membered heterobicycyl; wherein each T is independently optionally substituted with one or
more -R¹¹, which are the same or different;

each -R¹¹ is independently of each other selected from the group consisting of halogen, -CN,
oxo (=O), -COOR¹³, -OR¹³, -C(O)R¹³, -C(O)N(R¹³R^{13a}), -S(O)₂N(R¹³R^{13a}),
-S(O)N(R¹³R^{13a}), -S(O)₂R¹³, -S(O)R¹³, -N(R¹³)S(O)₂N(R^{13a}R^{13b}), -SR¹³,
10 -N(R¹³R^{13a}), -NO₂, -OC(O)R¹³, -N(R¹³)C(O)R^{13a}, -N(R¹³)S(O)₂R^{13a},
-N(R¹³)S(O)R^{13a}, -N(R¹³)C(O)OR^{13a}, -N(R¹³)C(O)N(R^{13a}R^{13b}),
-OC(O)N(R¹³R^{13a}), and C₁₋₆ alkyl; wherein C₁₋₆ alkyl is optionally substituted with one or
more halogen, which are the same or different;

each -R¹², -R^{12a}, -R¹³, -R^{13a}, -R^{13b} is independently selected from the group consisting of -H,
15 and C₁₋₆ alkyl; wherein C₁₋₆ alkyl is optionally substituted with one or more halogen, which
are the same or different;

optionally, one or more of the pairs -R¹/-R^{1a}, -R²/-R^{2a}, -R³/-R^{3a}, -R⁶/-R^{6a}, -R⁷/-R^{7a} are joined
together with the atom to which they are attached to form a C₃₋₁₀ cycloalkyl or a 3- to 10-
membered heterocyclyl;

20 optionally, one or more of the pairs -R¹/-R², -R¹/-R³, -R¹/-R⁴, -R¹/-R⁵,
-R¹/-R⁶, -R¹/-R⁷, -R²/-R³, -R²/-R⁴, -R²/-R⁵, -R²/-R⁶, -R²/-R⁷, -R³/-R⁴, -R³/-R⁵,
-R³/-R⁶, -R³/-R⁷, -R⁴/-R⁵, -R⁴/-R⁶, -R⁴/-R⁷, -R⁵/-R⁶, -R⁵/-R⁷, -R⁶/-R⁷ are joint together with the
atoms to which they are attached to form a ring A;

A is selected from the group consisting of phenyl; naphthyl; indenyl; indanyl; tetralinyl; C₃₋₁₀
25 cycloalkyl; 3- to 10-membered heterocyclyl; and 8- to 11-membered heterobicycyl;
wherein -L¹- is substituted with at least one -L²-Z and wherein -L¹- is optionally further
substituted.

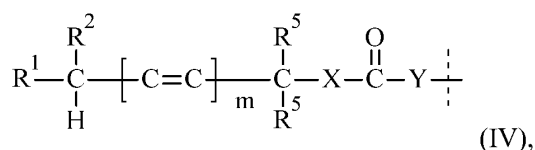
The optional further substituents of -L¹- of formula (III) are preferably as described above.

30 Preferably -L¹- of formula (III) is substituted with one moiety -L²-Z.

In one embodiment -L¹- of formula (III) is not further substituted.

In another embodiment -L¹- has a structure as disclosed in EP1536334B1, WO2009/009712A1, WO2008/034122A1, WO2009/143412A2, WO2011/082368A2, and US8618124B2, which are
5 herewith incorporated by reference.

In certain embodiments -L¹- has a structure as disclosed in US8946405B2 and US8754190B2. Accordingly, in certain embodiments -L¹- is of formula (IV):



10 wherein
the dashed line indicates attachment to -D through a functional group of -D selected from the group consisting of -OH, -SH and -NH₂;
m is 0 or 1;
at least one or both of -R¹ and -R² is/are independently of each other selected from the
15 group consisting of -CN, -NO₂, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkenyl, optionally substituted alkynyl, -C(O)R³, -S(O)R³, -S(O)₂R³, and -SR⁴,
one and only one of -R¹ and -R² is selected from the group consisting of -H, optionally substituted alkyl, optionally substituted arylalkyl, and optionally substituted
20 heteroarylalkyl;
-R³ is selected from the group consisting of -H, optionally substituted alkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, -OR⁹ and -N(R⁹)₂;
-R⁴ is selected from the group consisting of optionally substituted alkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, and
25 optionally substituted heteroarylalkyl;
each -R⁵ is independently selected from the group consisting of -H, optionally substituted alkyl, optionally substituted alkenylalkyl, optionally substituted alkynylalkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl and
30 optionally substituted heteroarylalkyl;

-R⁹ is selected from the group consisting of -H and optionally substituted alkyl;

-Y- is absent and -X- is selected from the group consisting of -O- and -S-; or

-Y- is -N(Q)CH₂- and -X- is -O-;

Q is selected from the group consisting of optionally substituted alkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl and optionally substituted heteroarylalkyl;

optionally, -R¹ and -R² may be joined to form a 3 to 8-membered ring; and

optionally, both -R⁹ together with the nitrogen to which they are attached form a heterocyclic ring;

wherein -L¹- is substituted with -L²-Z and wherein -L¹- is optionally further substituted.

Only in the context of formula (IV) the terms used have the following meaning:

The term “alkyl” as used herein includes linear, branched or cyclic saturated hydrocarbon groups of 1 to 8 carbons, or in some embodiments 1 to 6 or 1 to 4 carbon atoms.

The term “alkoxy” includes alkyl groups bonded to oxygen, including methoxy, ethoxy, isopropoxy, cyclopropoxy, cyclobutoxy, and similar.

The term “alkenyl” includes non-aromatic unsaturated hydrocarbons with carbon-carbon double bonds.

The term “alkynyl” includes non-aromatic unsaturated hydrocarbons with carbon-carbon triple bonds.

The term “aryl” includes aromatic hydrocarbon groups of 6 to 18 carbons, preferably 6 to 10 carbons, including groups such as phenyl, naphthyl, and anthracenyl. The term “heteroaryl” includes aromatic rings comprising 3 to 15 carbons containing at least one N, O or S atom, preferably 3 to 7 carbons containing at least one N, O or S atom, including groups such as pyrrolyl, pyridyl, pyrimidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, quinolyl, indolyl, indenyl, and similar.

In some instance, alkenyl, alkynyl, aryl or heteroaryl moieties may be coupled to the remainder of the molecule through an alkylene linkage. Under those circumstances, the substituent will be referred to as alkenylalkyl, alkynylalkyl, arylalkyl or heteroarylalkyl, indicating that an alkylene moiety is between the alkenyl, alkynyl, aryl or heteroaryl moiety and the molecule to which the alkenyl, alkynyl, aryl or heteroaryl is coupled.

The term “halogen” includes bromo, fluoro, chloro and iodo.

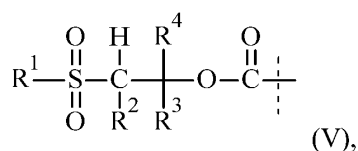
The term “heterocyclic ring” refers to a 4 to 8 membered aromatic or non-aromatic ring comprising 3 to 7 carbon atoms and at least one N, O, or S atom. Examples are piperidinyl, piperazinyl, tetrahydropyranyl, pyrrolidine, and tetrahydrofuranlyl, as well as the exemplary groups provided for the term “heteroaryl” above.

When a ring system is optionally substituted, suitable substituents are selected from the group consisting of alkyl, alkenyl, alkynyl, or an additional ring, each optionally further substituted. Optional substituents on any group, including the above, include halo, nitro, cyano, -OR, -SR, -NR₂, -OCOR, -NRCOR, -COOR, -CONR₂, -SOR, -SO₂R, -SONR₂, -SO₂NR₂, wherein each R is independently alkyl, alkenyl, alkynyl, aryl or heteroaryl, or two R groups taken together with the atoms to which they are attached form a ring.

In certain embodiments -L¹- of formula (IV) is substituted with one moiety -L²-Z.

In certain embodiments -L¹- of formula (IV) is not further substituted.

In certain embodiments -L¹- has a structure as disclosed in WO2013/036857A1. Accordingly, in certain embodiments -L¹- is of formula (V):



wherein

the dashed line indicates attachment to -D through an amine functional group of -D;

-R¹ is selected from the group consisting of optionally substituted C₁-C₆ linear, branched, or cyclic alkyl; optionally substituted aryl; optionally substituted heteroaryl; alkoxy; and -NR⁵₂;

-R² is selected from the group consisting of -H; optionally substituted C₁-C₆ alkyl; optionally substituted aryl; and optionally substituted heteroaryl;

5 -R³ is selected from the group consisting of -H; optionally substituted C₁-C₆ alkyl; optionally substituted aryl; and optionally substituted heteroaryl;

-R⁴ is selected from the group consisting of -H; optionally substituted C₁-C₆ alkyl; optionally substituted aryl; and optionally substituted heteroaryl;

10 each -R⁵ is independently of each other selected from the group consisting of -H; optionally substituted C₁-C₆ alkyl; optionally substituted aryl; and optionally substituted heteroaryl; or when taken together two -R⁵ can be cycloalkyl or cycloheteroalkyl;

wherein -L¹- is substituted with -L²-Z and wherein -L¹- is optionally further substituted.

Only in the context of formula (V) the terms used have the following meaning:

15

“Alkyl”, “alkenyl”, and “alkynyl” include linear, branched or cyclic hydrocarbon groups of 1-8 carbons or 1-6 carbons or 1-4 carbons wherein alkyl is a saturated hydrocarbon, alkenyl includes one or more carbon-carbon double bonds and alkynyl includes one or more carbon-carbon triple bonds. Unless otherwise specified these contain 1-6 C.

20

“Aryl” includes aromatic hydrocarbon groups of 6-18 carbons, preferably 6-10 carbons, including groups such as phenyl, naphthyl, and anthracene “Heteroaryl” includes aromatic rings comprising 3-15 carbons containing at least one N, O or S atom, preferably 3-7 carbons containing at least one N, O or S atom, including groups such as pyrrolyl, pyridyl, pyrimidinyl, imidazolyl, oxazolyl, isoxazolyl, 25 thiazolyl, isothiazolyl, quinolyl, indolyl, indenyl, and similar.

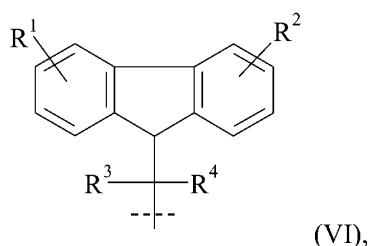
The term “substituted” means an alkyl, alkenyl, alkynyl, aryl, or heteroaryl group comprising one or more substituent groups in place of one or more hydrogen atoms. Substituents may generally be selected from halogen including F, Cl, Br, and I; lower alkyl including linear, branched, and cyclic; 30 lower haloalkyl including fluoroalkyl, chloroalkyl, bromoalkyl, and iodoalkyl; OH; lower alkoxy including linear, branched, and cyclic; SH; lower alkylthio including linear, branched and cyclic; amino, alkylamino, dialkylamino, silyl including alkylsilyl, alkoxysilyl, and arylsilyl; nitro; cyano;

carbonyl; carboxylic acid, carboxylic ester, carboxylic amide, aminocarbonyl; aminoacyl; carbamate; urea; thiocarbamate; thiourea; ketone; sulfone; sulfonamide; aryl including phenyl, naphthyl, and anthracenyl; heteroaryl including 5-member heteroaryls including as pyrrole, imidazole, furan, thiophene, oxazole, thiazole, isoxazole, isothiazole, thiadiazole, triazole, oxadiazole, and tetrazole, 6-member heteroaryls including pyridine, pyrimidine, pyrazine, and fused heteroaryls including benzofuran, benzothiophene, benzoxazole, benzimidazole, indole, benzothiazole, benzisoxazole, and benzisothiazole.

In certain embodiments $-L^1-$ of formula (V) is substituted with one moiety $-L^2-Z$.

In certain embodiments $-L^1-$ of formula (V) is not further substituted.

In certain embodiments $-L^1-$ has a structure as disclosed in US7585837B2. Accordingly, in certain embodiments $-L^1-$ is of formula (VI):



wherein

the dashed line indicates attachment to $-D$ through an amine functional group of $-D$;

R^1 and R^2 are independently selected from the group consisting of hydrogen, alkyl, alkoxy, alkoxyalkyl, aryl, alkaryl, aralkyl, halogen, nitro, $-SO_3H$, $-SO_2NHR^5$, amino, ammonium, carboxyl, PO_3H_2 , and OPO_3H_2 ;

R^3 , R^4 , and R^5 are independently selected from the group consisting of hydrogen, alkyl, and aryl;

wherein $-L^1-$ is substituted with $-L^2-Z$ and wherein $-L^1-$ is optionally further substituted.

Suitable substituents for formula (VI) are alkyl (such as C_{1-6} alkyl), alkenyl (such as C_{2-6} alkenyl), alkynyl (such as C_{2-6} alkynyl), aryl (such as phenyl), heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl (such as aromatic 4 to 7 membered heterocycle) or halogen moieties.

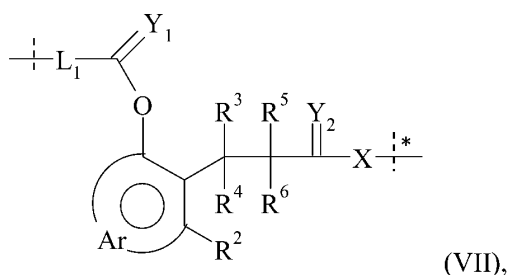
Only in the context of formula (VI) the terms used have the following meaning:

The terms “alkyl”, “alkoxy”, “alkoxyalkyl”, “aryl”, “alkaryl” and “aralkyl” mean alkyl radicals of 1-8, preferably 1-4 carbon atoms, e.g. methyl, ethyl, propyl, isopropyl and butyl, and aryl radicals of 6-10 carbon atoms, e.g. phenyl and naphthyl. The term “halogen” includes bromo, fluoro, chloro and iodo.

In certain embodiments $-L^1-$ of formula (VI) is substituted with one moiety $-L^2-Z$.

10 In certain embodiments $-L^1-$ of formula (VI) is not further substituted.

In certain embodiments $-L^1-$ has a structure as disclosed in WO2002/089789A1. Accordingly, in certain embodiments $-L^1-$ is of formula (VII):



15 wherein

the dashed line indicates attachment to $-D$ through an amine functional group of $-D$;

Y_1 and Y_2 are independently O, S or NR^7 ;

R^2 , R^3 , R^4 , R^5 , R^6 and R^7 are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy, and C_{1-6} heteroalkoxy;

Ar is a moiety which when included in formula (VII) forms a multisubstituted aromatic hydrocarbon or a multi-substituted heterocyclic group;

X is a chemical bond or a moiety that is actively transported into a target cell, a hydrophobic moiety, or a combination thereof,

y is 0 or 1;

wherein $-L^1-$ is substituted with $-L^2-Z$ and wherein $-L^1-$ is optionally further substituted.

Only in the context of formula (VII) the terms used have the following meaning:

The term “alkyl” shall be understood to include, e.g. straight, branched, substituted C₁₋₁₂ alkyls,
5 including alkoxy, C₃₋₈ cycloalkyls or substituted cycloalkyls, etc.

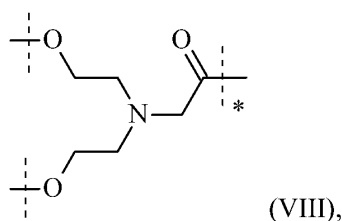
The term “substituted” shall be understood to include adding or replacing one or more atoms contained within a functional group or compounds with one or more different atoms.

10 Substituted alkyls include carboxyalkyls, aminoalkyls, dialkylaminos, hydroxyalkyls and mercaptoalkyls; substituted cycloalkyls include moieties such as 4-chlorocyclohexyl; aryls include moieties such as naphthyl; substituted aryls include moieties such as 3-bromo-phenyl; aralkyls include moieties such as toluy; heteroalkyls include moieties such as ethylthiophene; substituted heteroalkyls include moieties such as 3-methoxythiophene; alkoxy includes moieties such as methoxy; and
15 phenoxy includes moieties such as 3-nitrophenoxy. Halo- shall be understood to include fluoro, chloro, iodo and bromo.

In certain embodiments -L¹- of formula (VII) is substituted with one moiety -L²-Z.

20 In certain embodiments -L¹- of formula (VII) is not further substituted.

In certain embodiments -L¹- comprises a substructure of formula (VIII)



wherein

25 the dashed line marked with the asterisk indicates attachment to a nitrogen of -D by forming an amide bond;

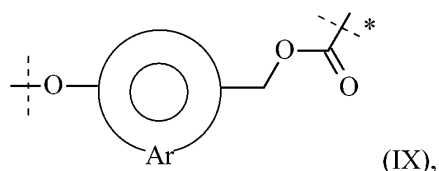
the unmarked dashed lines indicate attachment to the remainder of -L¹-; and

wherein -L¹- is substituted with -L²-Z and wherein -L¹- is optionally further substituted.

In certain embodiments $-L^1-$ of formula (VIII) is substituted with one moiety $-L^2-Z$.

In certain embodiments $-L^1-$ of formula (VIII) is not further substituted.

5 In certain embodiments $-L^1-$ comprises a substructure of formula (IX)



wherein

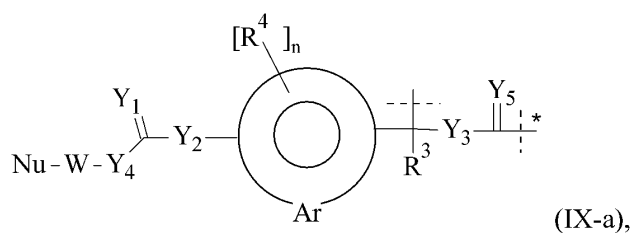
the dashed line marked with the asterisk indicates attachment to a nitrogen of $-D$ by forming a carbamate bond;

10 the unmarked dashed lines indicate attachment to the remainder of $-L^1-$; and
wherein $-L^1-$ is substituted with $-L^2-Z$ and wherein $-L^1-$ is optionally further substituted.

In certain embodiments $-L^1-$ of formula (IX) is substituted with one moiety $-L^2-Z$.

15 In certain embodiments $-L^1-$ of formula (IX) is not further substituted.

In certain embodiments $-L^1-$ is of formula (IX-a):



wherein

20 the dashed line marked with the asterisk indicates attachment to a nitrogen of $-D$ and the
unmarked dashed line indicates attachment to $-L^2-Z$;

n is 0, 1, 2, 3 or 4;

$=Y_1$ and $=Y_5$ are independently selected from the group consisting of $=O$ and $=S$;

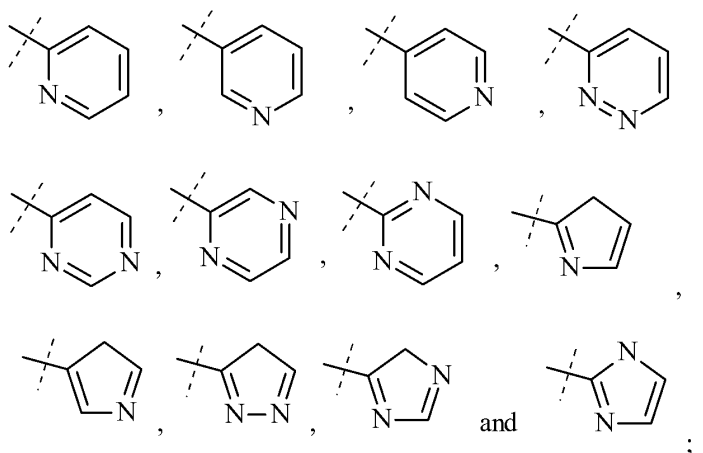
$-Y_2-$ and $-Y_3-$ are independently selected from the group consisting of $-O-$ and $-S-$;

25 $-Y_4-$ is selected from the group consisting of $-O-$, $-NR^5-$ and $-C(R^6R^{6a})-$;

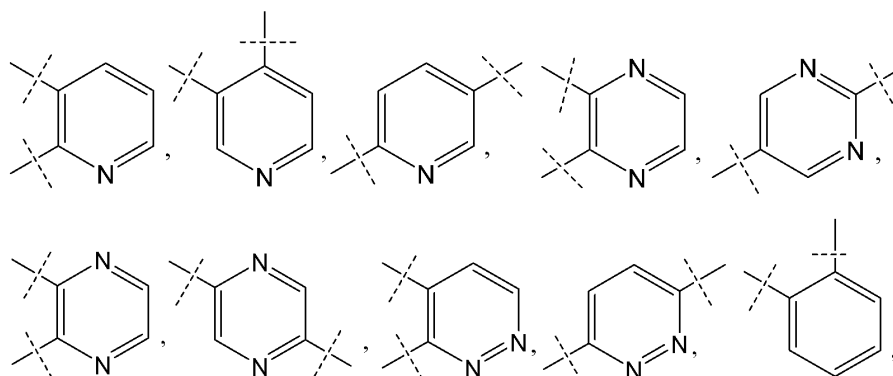
$-R^3$, $-R^5$, $-R^6$ and $-R^{6a}$ are independently of each other selected from the group consisting of -H, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl and 3,3-dimethylpropyl;

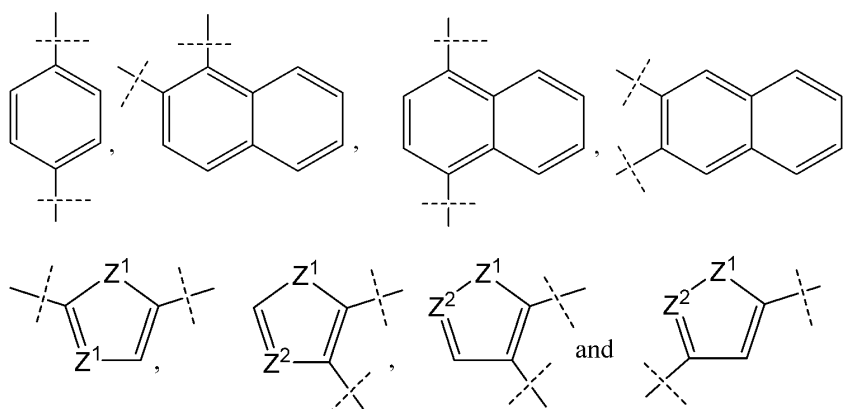
5 $-R^4$ is selected from the group consisting of methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl and 3,3-dimethylpropyl;

10 $-W-$ is selected from the group consisting of C_{1-20} alkyl optionally interrupted by one or more groups selected from the group consisting of C_{3-10} cycloalkyl, 8- to 30-membered carbopolycyclyl, 3- to 10-membered heterocyclyl, $-C(O)-$, $-C(O)N(R^7)-$, $-O-$, $-S-$ and $-N(R^7)-$; $-Nu$ is a nucleophile selected from the group consisting of $-N(R^7R^{7a})$, $-N(R^7OH)$, $-N(R^7)-N(R^{7a}R^{7b})$, $-S(R^7)$, $-COOH$,



15 $-Ar-$ is selected from the group consisting of





wherein

dashed lines indicate attachment to the remainder of $-L^1$,

5 $-Z^1$ is selected from the group consisting of $-O-$, $-S-$ and $-N(R^7)-$, and

$-Z^2$ is $-N(R^7)-$;

$-R^7$, $-R^{7a}$ and $-R^{7b}$ are independently selected from the group consisting of $-H$, C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl; and

wherein $-L^1$ is optionally further substituted.

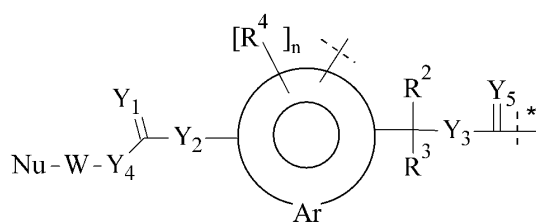
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In certain embodiments $-D$ is attached to $-L^1$ of formula (IX-a) through the nitrogen of an amine functional group of $-D$, such as to the nitrogen of the amine functional group of a lysine side chain of $-D$.

15 In certain embodiments $-L^1$ of formula (IX-a) is substituted with one moiety $-L^2-Z$.

In certain embodiments $-L^1$ of formula (IX-a) is not further substituted.

In certain embodiments $-L^1$ is of formula (IX-b):



(IX-b),

20

wherein

the dashed line marked with the asterisk indicates attachment to a nitrogen of -D and the unmarked dashed line indicates attachment to -L²-Z;

n is 0, 1, 2, 3, or 4;

=Y₁ and =Y₅ are independently selected from the group consisting of =O and =S;

5 -Y₂- and -Y₃- are independently selected from the group consisting of -O- and -S-;

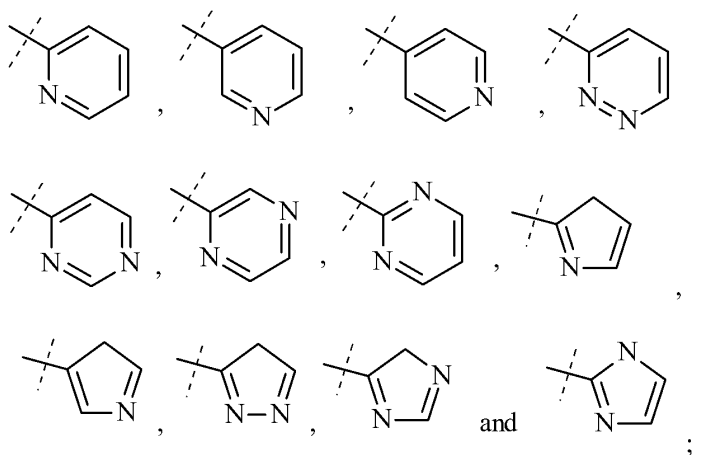
-Y₄- is selected from the group consisting of -O-, -NR⁵- and -C(R⁶R^{6a})-;

-R², -R³, -R⁵, -R⁶, -R^{6a} are independently of each other selected from the group consisting of -H, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl and 3,3-dimethylpropyl;

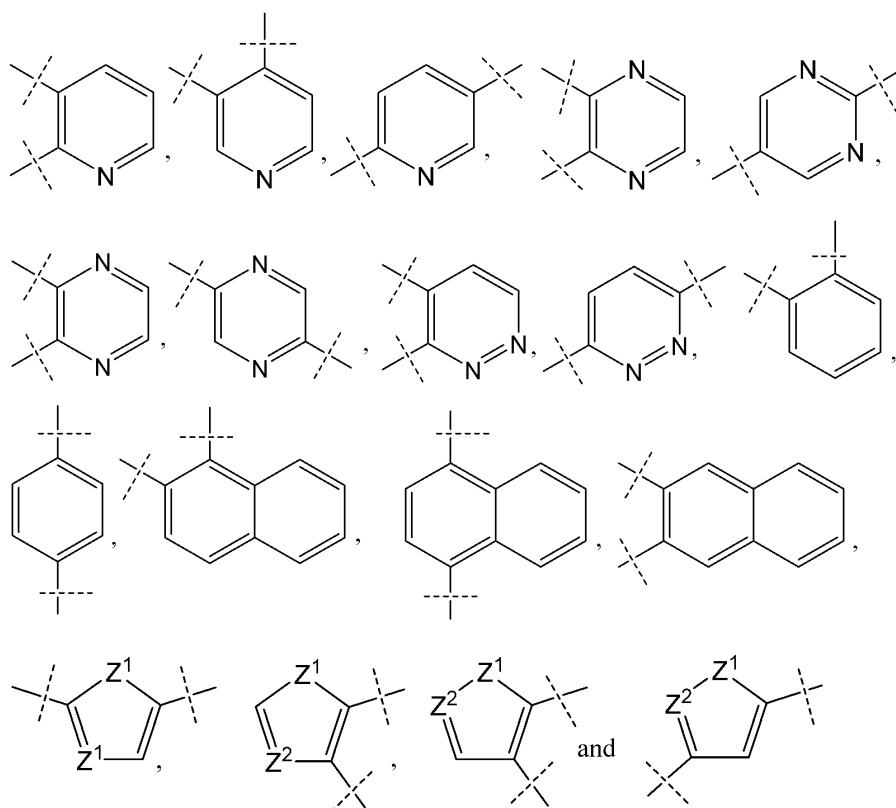
-R⁴ is selected from the group consisting of methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl and 3,3-dimethylpropyl;

-W- is selected from the group consisting of C₁₋₂₀ alkyl optionally interrupted by one or more groups selected from the group consisting of C₃₋₁₀ cycloalkyl, 8- to 30-membered carbopolycyclyl, 3- to 10-membered heterocyclyl, -C(O)-, -C(O)N(R⁷)-, -O-, -S- and -N(R⁷)-;

-Nu is a nucleophile selected from the group consisting of -N(R^{7a}R^{7b}), -N(R⁷OH), -N(R⁷)-N(R^{7a}R^{7b}), -S(R⁷), -COOH,



-Ar- is selected from the group consisting of



5 wherein
dashed lines indicate attachment to the remainder of -L¹-,
-Z¹- is selected from the group consisting of -O-, -S- and -N(R⁷)-, and
-Z²- is -N(R⁷)-;
-R⁷, -R^{7a}, -R^{7b} are independently of each other selected from the group consisting of -H, C₁₋₆
10 alkyl, C₂₋₆ alkenyl and C₂₋₆ alkynyl; and
wherein -L¹- is optionally further substituted.

In certain embodiments -D is attached to -L¹- of formula (IX-b) through the nitrogen of an amine functional group of -D, such as to the nitrogen of the amine functional group of a lysine side chain of -D.

In certain embodiments -L¹- of formula (IX-b) is substituted with one moiety -L²-Z.

In certain embodiments -L¹- of formula (IX-b) is not further substituted.

In certain embodiments $=Y^1$ of formula (IX-a) and (IX-b) is $=O$. In certain embodiments $-Y^2-$ of formula (IX-a) and (IX-b) is $-O-$. In certain embodiments $-Y^3-$ of formula (IX-a) and (IX-b) is $-O-$. In certain embodiments $-Y^4-$ of formula (IX-a) and (IX-b) is $-NR^5-$. In certain embodiments $=Y^5$ of formula (IX-a) and (IX-b) is $=O$.

In certain embodiments n of formula (IX-a) and (IX-b) is 0 or 1. In certain embodiments n of formula (IX-a) and (IX-b) is 0. In certain embodiments n of formula (IX-a) and (IX-b) is 1.

10 In certain embodiments $-R^2$ of formula (IX-b) is selected from the group consisting of $-H$, methyl, ethyl, n -propyl, isopropyl, n -butyl, isobutyl, sec-butyl and tert-butyl. In certain embodiments $-R^2$ of formula (IX-b) is selected from the group consisting of $-H$, methyl, ethyl, n -propyl and isopropyl. In certain embodiments $-R^2$ of formula (IX-b) is selected from $-H$, methyl and ethyl. In certain embodiments $-R^2$ of formula (IX-b) is $-H$.

15

In certain embodiments $-R^3$ of formula (IX-a) and (IX-b) is selected from the group consisting of $-H$, methyl, ethyl, n -propyl, isopropyl, n -butyl, isobutyl, sec-butyl and tert-butyl. In certain embodiments $-R^3$ of formula (IX-a) and (IX-b) is selected from the group consisting of $-H$, methyl, ethyl, n -propyl and isopropyl. In certain embodiments $-R^3$ of formula (IX-a) and (IX-b) is selected from $-H$, methyl and ethyl. In certain embodiments $-R^3$ of formula (IX-a) and (IX-b) is $-H$.

20

In certain embodiments each $-R^4$ of formula (IX-a) and (IX-b) is independently selected from methyl, ethyl, n -propyl, isopropyl, n -butyl, isobutyl, sec-butyl and tert-butyl. In certain embodiments $-R^4$ of formula (IX-a) and (IX-b) is selected from the group consisting of methyl, ethyl, n -propyl and isopropyl. In certain embodiments $-R^4$ of formula (IX-a) and (IX-b) is selected from methyl and ethyl.

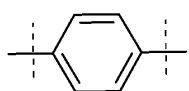
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In certain embodiments $-R^5$ of formula (IX-a) and (IX-b) is selected from the group consisting of $-H$, methyl, ethyl, n -propyl, isopropyl, n -butyl, isobutyl, sec-butyl and tert-butyl. In certain embodiments $-R^5$ of formula (IX-a) and (IX-b) is selected from the group consisting of $-H$, methyl, ethyl, n -propyl and isopropyl. In certain embodiments $-R^5$ of formula (IX-a) and (IX-b) is selected from methyl and ethyl. In certain embodiments $-R^5$ of formula (IX-a) and (IX-b) is methyl.

30

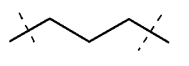
In certain embodiments $-R^6$ and $-R^{6a}$ of formula (IX-a) and (IX-b) are independently selected from the group consisting of -H, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl. In certain embodiments $-R^6$ and $-R^{6a}$ of formula (IX-a) and (IX-b) are independently selected from the group consisting of -H, methyl, ethyl, n-propyl and isopropyl. In certain embodiments $-R^6$ and $-R^{6a}$ of formula (IX-a) and (IX-b) are independently selected from -H, methyl and ethyl. In certain embodiments $-R^6$ and $-R^{6a}$ of formula (IX-a) and (IX-b) are both -H.

In certain embodiments Ar of formula (IX-a) and (IX-b) is phenyl. In certain embodiments Ar of formula (IX-a) and (IX-b) is



wherein the dashed lines indicate attachment to the remainder of the moiety of formula (IX-a) and (IX-b).

In certain embodiments W of formula (IX-a) and (IX-b) is C_{1-20} alkyl, optionally interrupted with C_{3-10} cycloalkyl, $-C(O)-$, $-C(O)N(R^7)-$, $-O-$, $-S-$ and $-N(R^7)-$. In certain embodiments W of formula (IX-a) and (IX-b) is C_{1-10} alkyl, optionally interrupted with C_{3-10} cycloalkyl, $-C(O)-$, $-C(O)N(R^7)-$, $-O-$, $-S-$ and $-N(R^7)-$. In certain embodiments W of formula (IX-a) and (IX-b) is C_{1-6} alkyl, optionally interrupted with C_{3-10} cycloalkyl, $-C(O)-$, $-C(O)N(R^7)-$, $-O-$, $-S-$ and $-N(R^7)-$. In certain embodiments W of formula (IX-a) and (IX-b) is



wherein

the dashed lines indicate attachment to the remainder of the moiety of formula (IX-a) or (IX-b), respectively.

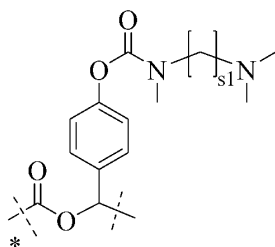
In certain embodiments -Nu of formula (IX-a) and (IX-b) is $-N(R^7R^{7a})$.

In certain embodiments $-R^7$, $-R^{7a}$ and $-R^{7b}$ of formula (IX-a) and (IX-b) are independently of each other selected from the group consisting of -H, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl. In certain embodiments $-R^7$, $-R^{7a}$ and $-R^{7b}$ of formula (IX-a) and (IX-b) are

independently of each other selected from -H, methyl, ethyl, n-propyl and isopropyl. In certain embodiments $-R^7$, $-R^{7a}$ and $-R^{7b}$ of formula (IX-a) and (IX-b) are independently of each other selected from methyl or ethyl. In certain embodiments $-R^7$, $-R^{7a}$ and $-R^{7b}$ of formula (IX-a) and (IX-b) are both methyl.

5

In certain embodiments $-L^1$ is of formula (IX-c)



(IX-c),

wherein

the dashed line marked with the asterisk indicates attachment to a nitrogen of -D;

10

the unmarked dashed line indicates attachment to $-L^2-Z$; and

s_1 is an integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

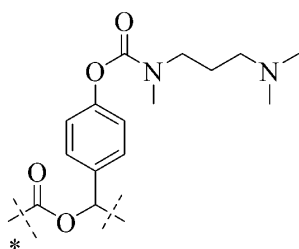
In certain embodiments -D is attached to $-L^1$ of formula (IX-c) through the nitrogen of an amine functional group of -D, such as to the nitrogen of the amine functional group of a lysine side chain of -D.

15

In certain embodiments s_1 of formula (IX-c) is an integer selected from the group consisting of 1, 2, 3, 4 and 5. In certain embodiments s_1 of formula (IX-c) is 1. In certain embodiments s_1 of formula (IX-c) is 2. In certain embodiments s_1 of formula (IX-c) is 3. In certain embodiments s_1 of formula (IX-c) is 4. In certain embodiments s_1 of formula (IX-c) is 5.

20

In certain embodiments $-L^1$ is of formula (IX-d)



(IX-d),

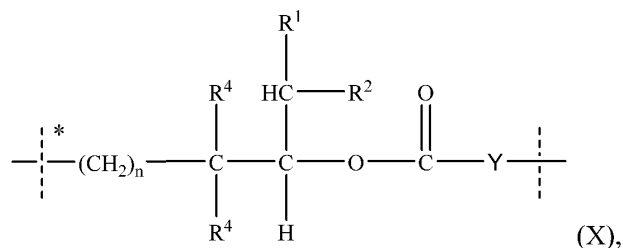
wherein

the dashed line marked with the asterisk indicates attachment to a nitrogen of -D; and
the unmarked dashed line indicates attachment to -L²-Z.

5

In certain embodiments -D is attached to -L¹- of formula (IX-d) through the nitrogen of an amine functional group of -D, such as to the nitrogen of the amine functional group of a lysine side chain of -D.

- 10 In certain embodiments -L¹- has a structure as disclosed in WO2020/206358 A1. Accordingly, in certain embodiments the moiety -L¹- is of formula (X):



(X),

wherein

the unmarked dashed line indicates attachment to -D;

- 15 the dashed line marked with the asterisk indicates attachment to -L²-Z or -L²-Z';

n is an integer selected from the group consisting of 0, 1, 2, 3, 4, 5 and 6;

-R¹ and -R² are independently an electron-withdrawing group, alkyl, or -H, and wherein at least one of -R¹ or -R² is an electron-withdrawing group;

each -R⁴ is independently C₁-C₃ alkyl or the two -R⁴ are taken together with the carbon atom to which they are attached to form a 3- to 6-membered ring; and

20

-Y- is absent when -D is a drug moiety connected through an amine,
or -Y- is -N(R⁶)CH₂- when -D is a drug moiety connected through a phenol, alcohol, thiol,

thiophenol, imidazole, or non-basic amine; wherein $-R^6$ is optionally substituted C_1 - C_6 alkyl, optionally substituted aryl, or optionally substituted heteroaryl.

In certain embodiments $-L^1$ of formula (X) is substituted with one moiety $-L^2-Z$.

5

In certain embodiments $-L^1$ of formula (X) is not further substituted.

In certain embodiments n of formula (X) is an integer selected from 1, 2, 3, 4, 5 and 6. In certain embodiments n of formula (X) is an integer selected from 1, 2 and 3. In certain embodiments n of formula (X) is an integer from 0, 1, 2 and 3. In certain embodiments n of formula (X) is 1. In certain
10 embodiments n of formula (X) is 2. In certain embodiments n of formula (X) is 3.

In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (X) is selected from the group consisting of $-CN$; $-NO_2$; optionally substituted aryl; optionally substituted heteroaryl;
15 optionally substituted alkenyl; optionally substituted alkynyl; $-COR^3$, $-SOR^3$, or $-SO_2R^3$, wherein $-R^3$ is $-H$, optionally substituted alkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, $-OR^8$ or $-NR^8_2$, wherein each $-R^8$ is independently $-H$ or optionally substituted alkyl, or both $-R^8$ groups are taken together with the nitrogen to which they are attached to form a heterocyclic ring; or $-SR^9$, wherein $-R^9$ is
20 optionally substituted alkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, or optionally substituted heteroarylalkyl.

In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (X) is $-CN$. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (X) is $-NO_2$. In certain
25 embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (X) is optionally substituted aryl comprising 6 to 10 carbons. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (X) is optionally substituted phenyl, naphthyl, or anthracenyl. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (X) is optionally substituted heteroaryl comprising 3 to 7 carbons and comprising at least one N, O, or S atom. In certain
30 embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (X) is optionally substituted pyrrolyl, pyridyl, pyrimidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, quinolyl, indolyl, or indenyl. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula

(X) is optionally substituted alkenyl containing 2 to 20 carbon atoms. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (X) is optionally substituted alkynyl comprising 2 to 20 carbon atoms. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (X) is $-\text{COR}^3$, $-\text{SOR}^3$, or $-\text{SO}_2\text{R}^3$, wherein $-\text{R}^3$ is -H, optionally substituted alkyl comprising 1 to 20 carbon atoms, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, $-\text{OR}^8$ or $-\text{NR}^8_2$, wherein each $-\text{R}^8$ is independently -H or optionally substituted alkyl comprising 1 to 20 carbon atoms, or both $-\text{R}^8$ groups are taken together with the nitrogen to which they are attached to form a heterocyclic ring. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (X) is $-\text{SR}^9$, wherein $-\text{R}^9$ is optionally substituted alkyl comprising 1 to 20 carbon atoms, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, or optionally substituted heteroarylalkyl.

In certain embodiments at least one of $-R^1$ or $-R^2$ of formula (X) is $-\text{CN}$, $-\text{SOR}^3$ or $-\text{SO}_2\text{R}^3$. In certain embodiments at least one of $-R^1$ and $-R^2$ of formula (X) is $-\text{CN}$ or $-\text{SO}_2\text{R}^3$. In certain embodiments at least one of $-R^1$ and $-R^2$ of formula (X) is $-\text{CN}$ or $-\text{SO}_2\text{R}^3$, wherein $-\text{R}^3$ is optionally substituted alkyl, optionally substituted aryl, or $-\text{NR}^8_2$. In certain embodiments at least one of $-R^1$ and $-R^2$ of formula (X) is $-\text{CN}$, $-\text{SO}_2\text{N}(\text{CH}_3)_2$, $-\text{SO}_2\text{CH}_3$, phenyl substituted with $-\text{SO}_2$, phenyl substituted with $-\text{SO}_2$ and $-\text{Cl}$, $-\text{SO}_2\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$, $-\text{SO}_2\text{CH}(\text{CH}_3)_2$, $-\text{SO}_2\text{N}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$, or $-\text{SO}_2\text{N}(\text{CH}_2\text{CH}_2\text{OCH}_3)_2$.

In certain embodiments each $-\text{R}^4$ of formula (X) is independently C_1 - C_3 alkyl. In certain embodiments both $-\text{R}^4$ are methyl.

In certain embodiments $-\text{Y}-$ of formula (X) is absent. In certain embodiments $-\text{Y}-$ of formula (X) is $-\text{N}(\text{R}^6)\text{CH}_2-$.

In certain embodiments $-\text{L}^1-$ is of formula (X), wherein n is 1, $-\text{R}^1$ is $-\text{CN}$, $-\text{R}^2$ is $-\text{H}$, and $-\text{R}^4$ is $-\text{CH}_3$. In certain embodiments $-\text{L}^1-$ is of formula (X), wherein n is 1, $-\text{R}^1$ is $-\text{SO}_2\text{N}(\text{CH}_3)_2$, $-\text{R}^2$ is $-\text{H}$, and $-\text{R}^4$ is $-\text{CH}_3$. In certain embodiments $-\text{L}^1-$ is of formula (X), wherein n is 1, $-\text{R}^1$ is SO_2CH_3 , $-\text{R}^2$ is $-\text{H}$, and $-\text{R}^4$ is $-\text{CH}_3$. In certain embodiments $-\text{L}^1-$ is of formula (X), wherein n is 1, $-\text{R}^1$ is $-\text{SO}_2\text{N}(\text{CH}_2\text{CH}_2)_2\text{CHCH}_3$, $-\text{R}^2$ is $-\text{H}$, and $-\text{R}^4$ is $-\text{CH}_3$. In certain embodiments $-\text{L}^1-$ is of formula (X), wherein n is 1, $-\text{R}^1$ is phenyl substituted with $-\text{SO}_2$, $-\text{R}^2$ is $-\text{H}$, and $-\text{R}^4$ is $-\text{CH}_3$. In certain

embodiments -L¹- is of formula (X), wherein n is 1, -R¹ is phenyl substituted with -SO₂ and -Cl, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 1, -R¹ is -SO₂N(CH₂CH₂)₂O, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 1, -R¹ is -SO₂CH(CH₃)₂, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 1, -R¹ is -SO₂N(CH₃)(CH₂CH₃), -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 1, -R¹ is -SO₂N(CH₂CH₂OCH₃)₂, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 1, -R¹ is phenyl substituted with -SO₂ and -CH₃, -R² is -H, and -R⁴ is -CH₃.

10 In certain embodiments -L¹- is of formula (X), wherein n is 2, -R¹ is -CN, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 2, -R¹ is -SO₂N(CH₃)₂, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 2, -R¹ is SO₂CH₃, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 2, -R¹ is -SO₂N(CH₂CH₂)₂CHCH₃, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 2, -R¹ is phenyl substituted with -SO₂, -R² is -H, and -R⁴ is -CH₃. In certain
15 embodiments -L¹- is of formula (X), wherein n is 2, -R¹ is phenyl substituted with -SO₂ and -Cl, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 2, -R¹ is -SO₂N(CH₂CH₂)₂O, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 2, -R¹ is -SO₂CH(CH₃)₂, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of
20 formula (X), wherein n is 2, -R¹ is -SO₂N(CH₃)(CH₂CH₃), -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 2, -R¹ is -SO₂N(CH₂CH₂OCH₃)₂, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 2, -R¹ is phenyl substituted with -SO₂ and -CH₃, -R² is -H, and -R⁴ is -CH₃.

25 In certain embodiments -L¹- is of formula (X), wherein n is 3, -R¹ is -CN, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 3, -R¹ is -SO₂N(CH₃)₂, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 3, -R¹ is SO₂CH₃, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 3, -R¹ is -SO₂N(CH₂CH₂)₂CHCH₃, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula
30 (X), wherein n is 3, -R¹ is phenyl substituted with -SO₂, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 3, -R¹ is phenyl substituted with -SO₂ and -Cl, -R² is -H, and -R⁴ is -CH₃. In certain embodiments -L¹- is of formula (X), wherein n is 3, -R¹

is $-\text{SO}_2\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$, $-\text{R}^2$ is $-\text{H}$, and $-\text{R}^4$ is $-\text{CH}_3$. In certain embodiments $-\text{L}^1$ is of formula (X), wherein n is 3, $-\text{R}^1$ is $-\text{SO}_2\text{CH}(\text{CH}_3)_2$, $-\text{R}^2$ is $-\text{H}$, and $-\text{R}^4$ is $-\text{CH}_3$. In certain embodiments $-\text{L}^1$ is of formula (X), wherein n is 3, $-\text{R}^1$ is $-\text{SO}_2\text{N}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$, $-\text{R}^2$ is $-\text{H}$, and $-\text{R}^4$ is $-\text{CH}_3$. In certain embodiments $-\text{L}^1$ is of formula (X), wherein n is 3, $-\text{R}^1$ is $-\text{SO}_2\text{N}(\text{CH}_2\text{CH}_2\text{OCH}_3)_2$, $-\text{R}^2$ is $-\text{H}$, and $-\text{R}^4$ is $-\text{CH}_3$. In certain embodiments $-\text{L}^1$ is of formula (X), wherein n is 3, $-\text{R}^1$ is phenyl substituted with $-\text{SO}_2$ and $-\text{CH}_3$, $-\text{R}^2$ is $-\text{H}$, and $-\text{R}^4$ is $-\text{CH}_3$.

Only in the context of formula (X) the terms used have the following meaning:

10 The term "alkyl" refers to linear, branched, or cyclic saturated hydrocarbon groups of 1 to 20, 1 to 12, 1 to 8, 1 to 6, or 1 to 4 carbon atoms. In certain embodiments an alkyl is linear or branched. Examples of linear or branched alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, and n-decyl. In certain embodiments an alkyl is cyclic. Examples of cyclic alkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclopentadienyl, and cyclohexyl.

The term "alkoxy" refers to alkyl groups bonded to oxygen, including methoxy, ethoxy, isopropoxy, cyclopropoxy, and cyclobutoxy.

20 The term "alkenyl" refers to non-aromatic unsaturated hydrocarbons with carbon-carbon double bonds and 2 to 20, 2 to 12, 2 to 8, 2 to 6, or 2 to 4 carbon atoms.

The term "alkynyl" refers to non-aromatic unsaturated hydrocarbons with carbon-carbon triple bonds and 2 to 20, 2 to 12, 2 to 8, 2 to 6, or 2 to 4 carbon atoms.

25 The term "aryl" refers to aromatic hydrocarbon groups of 6 to 18 carbons, preferably 6 to 10 carbons, including groups such as phenyl, naphthyl, and anthracenyl. The term "heteroaryl" refers to aromatic rings comprising 3 to 15 carbons comprising at least one N, O or S atom, preferably 3 to 7 carbons comprising at least one N, O or S atom, including groups such as pyrrolyl, pyridyl, pyrimidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, quinolyl, indolyl, and indenyl.

In certain embodiments alkenyl, alkynyl, aryl or heteroaryl moieties may be coupled to the remainder of the molecule through an alkyl linkage. Under those circumstances, the substituent will be referred to as alkenylalkyl, alkynylalkyl, arylalkyl or heteroarylalkyl, indicating that an alkylene moiety is between the alkenyl, alkynyl, aryl or heteroaryl moiety and the molecule to which the alkenyl, alkynyl, aryl or heteroaryl is coupled.

The term "halogen" or "halo" refers to bromo, fluoro, chloro and iodo.

The term "heterocyclic ring" or "heterocyclyl" refers to a 3- to 15-membered aromatic or non-aromatic ring comprising at least one N, O, or S atom. Examples include piperidinyl, piperazinyl, tetrahydropyranyl, pyrrolidine, and tetrahydrofuranyl, as well as the exemplary groups provided for the term "heteroaryl" above. In certain embodiments a heterocyclic ring or heterocyclyl is non-aromatic. In certain embodiments a heterocyclic ring or heterocyclyl is aromatic.

The term "optionally substituted" refers to a group may be unsubstituted or substituted by one or more (e.g., 1, 2, 3, 4 or 5) of the substituents which may be the same or different. Examples of substituents include alkyl, alkenyl, alkynyl, halogen, -CN, -OR^{aa}, -SR^{aa}, -NR^{aa}R^{bb}, -NO₂, -C=NH(OR^{aa}), -C(O)R^{aa}, -OC(O)R^{aa}, -C(O)OR^{aa}, -C(O)NR^{aa}R^{bb}, -OC(O)NR^{aa}R^{bb}, -NR^{aa}C(O)R^{bb}, -NR^{aa}C(O)OR^{bb}, -S(O)R^{aa}, -S(O)₂R^{aa}, -NR^{aa}S(O)R^{bb}, -C(O)NR^{aa}S(O)R^{bb}, -NR^{aa}S(O)₂R^{bb}, -C(O)NR^{aa}S(O)₂R^{bb}, -S(O)NR^{aa}R^{bb}, -S(O)₂NR^{aa}R^{bb}, -P(O)(OR^{aa})(OR^{bb}), heterocyclyl, heteroaryl, or aryl, wherein the alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heteroaryl, and aryl are each independently optionally substituted by -R^{cc}, wherein -R^{aa} and -R^{bb} are each independently -H, alkyl, alkenyl, alkynyl, heterocyclyl, heteroaryl, or aryl, or -R^{aa} and -R^{bb} are taken together with the nitrogen atom to which they attach to form a heterocyclyl, which is optionally substituted by alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkoxy, or -CN, and wherein: each -R^{cc} is independently alkyl, alkenyl, alkynyl, halogen, heterocyclyl, heteroaryl, aryl, -CN, or -NO₂.

In certain embodiments all moieties -L²- of the conjugate are identical, provided there is more than one moiety -L²- present in the conjugate. In certain embodiments the conjugate of the present invention comprises more than one type of -L²-, such as two, three, four or five different moieties -L²-. Such more than one type of -L²- may be connected to only one type of -L¹- or may be connected to more than one type of -L¹-.

In certain embodiments $-L^2-$ is a chemical bond. In certain embodiments $-L^2-$ is a spacer moiety.

In certain embodiments $-L^2-$ is selected from the group consisting of $-T'-$, $-C(O)O-$, $-O-$,
 5 $-C(O)-$, $-C(O)N(R^{y1})-$, $-S(O)_2N(R^{y1})-$, $-S(O)N(R^{y1})-$, $-S(O)_2-$, $-S(O)-$, $-N(R^{y1})S(O)_2N(R^{y1a})-$,
 $-S-$, $-N(R^{y1})-$, $-OC(OR^{y1})(R^{y1a})-$, $-N(R^{y1})C(O)N(R^{y1a})-$, $-OC(O)N(R^{y1})-$, C_{1-50} alkyl, C_{2-50} alkenyl and
 C_{2-50} alkynyl; wherein $-T'-$, C_{1-50} alkyl, C_{2-50} alkenyl and C_{2-50} alkynyl are optionally substituted with
 one or more $-R^{y2}$, which are the same or different and wherein C_{1-50} alkyl, C_{2-50} alkenyl and C_{2-50}
 alkynyl are optionally interrupted by one or more groups selected from the group consisting
 10 of $-T'-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{y3})-$, $-S(O)_2N(R^{y3})-$, $-S(O)N(R^{y3})-$, $-S(O)_2-$,
 $-S(O)-$, $-N(R^{y3})S(O)_2N(R^{y3a})-$, $-S-$, $-N(R^{y3})-$, $-OC(OR^{y3})(R^{y3a})-$, $-N(R^{y3})C(O)N(R^{y3a})-$
 and $-OC(O)N(R^{y3})-$;

$-R^{y1}$ and $-R^{y1a}$ are independently selected from the group consisting of $-H$, $-T'$, C_{1-50} alkyl, C_{2-50}
 15 alkenyl and C_{2-50} alkynyl; wherein $-T'$, C_{1-50} alkyl, C_{2-50} alkenyl and C_{2-50} alkynyl are optionally
 substituted with one or more $-R^{y2}$, which are the same or different, and wherein C_{1-50} alkyl, C_{2-50}
 alkenyl and C_{2-50} alkynyl are optionally interrupted by one or more groups selected from the group
 consisting of $-T'-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{y4})-$, $-S(O)_2N(R^{y4})-$, $-S(O)N(R^{y4})-$, $-S(O)_2-$,
 $-S(O)-$, $-N(R^{y4})S(O)_2N(R^{y4a})-$, $-S-$, $-N(R^{y4})-$, $-OC(OR^{y4})(R^{y4a})-$, $-N(R^{y4})C(O)N(R^{y4a})-$,
 20 and $-OC(O)N(R^{y4})-$;

each T' is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl,
 tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicycyl, 8- to
 30-membered carbopolycyclyl and 8- to 30-membered heteropolycyclyl; wherein each T' is
 25 independently optionally substituted with one or more $-R^{y2}$, which are the same or different;

each $-R^{y2}$ is independently selected from the group consisting of halogen, $-CN$, oxo
 ($=O$), $-C(O)OR^{y5}$, $-OR^{y5}$, $-C(O)R^{y5}$, $-C(O)N(R^{y5})(R^{y5a})$, $-S(O)_2N(R^{y5})(R^{y5a})$, $-S(O)N(R^{y5})(R^{y5a})$,
 $-S(O)_2R^{y5}$, $-S(O)R^{y5}$, $-N(R^{y5})S(O)_2N(R^{y5})(R^{y5a})$, $-SR^{y5}$, $-N(R^{y5})(R^{y5a})$, $-NO_2$, $-OC(O)R^{y5}$,
 30 $-N(R^{y5})C(O)R^{y5a}$, $-N(R^{y5})S(O)_2R^{y5a}$, $-N(R^{y5})S(O)R^{y5a}$, $-N(R^{y5})C(O)OR^{y5a}$,
 $-N(R^{y5})C(O)N(R^{y5})(R^{y5a})$, $-OC(O)N(R^{y5})(R^{y5a})$, and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally
 substituted with one or more halogen, which are the same or different; and

each $-R^{y3}$, $-R^{y3a}$, $-R^{y4}$, $-R^{y4a}$, $-R^{y5}$, $-R^{y5a}$ and $-R^{y5b}$ is independently selected from the group consisting of -H and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different.

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In certain embodiments $-L^2-$ is selected from the group consisting of $-T'-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{y1})-$, $-S(O)_2N(R^{y1})-$, $-S(O)N(R^{y1})-$, $-S(O)_2-$, $-S(O)-$, $-N(R^{y1})S(O)_2N(R^{y1a})-$, $-S-$, $-N(R^{y1})-$, $-OC(OR^{y1})(R^{y1a})-$, $-N(R^{y1})C(O)N(R^{y1a})-$, $-OC(O)N(R^{y1})-$, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl; wherein $-T'-$, C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are optionally substituted with one or more $-R^{y2}$, which are the same or different and wherein C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are optionally interrupted by one or more groups selected from the group consisting of $-T'-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{y3})-$, $-S(O)_2N(R^{y3})-$, $-S(O)N(R^{y3})-$, $-S(O)_2-$, $-S(O)-$, $-N(R^{y3})S(O)_2N(R^{y3a})-$, $-S-$, $-N(R^{y3})-$, $-OC(OR^{y3})(R^{y3a})-$, $-N(R^{y3})C(O)N(R^{y3a})-$, and $-OC(O)N(R^{y3})-$;

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$-R^{y1}$ and $-R^{y1a}$ are independently selected from the group consisting of -H, $-T'$, C_{1-10} alkyl, C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein $-T'$, C_{1-10} alkyl, C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more $-R^{y2}$, which are the same or different, and wherein C_{1-10} alkyl, C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally interrupted by one or more groups selected from the group consisting of $-T'-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{y4})-$, $-S(O)_2N(R^{y4})-$, $-S(O)N(R^{y4})-$, $-S(O)_2-$, $-S(O)-$, $-N(R^{y4})S(O)_2N(R^{y4a})-$, $-S-$, $-N(R^{y4})-$, $-OC(OR^{y4})(R^{y4a})-$, $-N(R^{y4})C(O)N(R^{y4a})-$, and $-OC(O)N(R^{y4})-$;

20

each T' is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, 8- to 30-membered carbopolycyclyl, and 8- to 30-membered heteropolycyclyl; wherein each T' is independently optionally substituted with one or more $-R^{y2}$, which are the same or different;

25

$-R^{y2}$ is selected from the group consisting of halogen, $-CN$, oxo ($=O$), $-C(O)OR^{y5}$, $-OR^{y5}$, $-C(O)R^{y5}$, $-C(O)N(R^{y5})(R^{y5a})$, $-S(O)_2N(R^{y5})(R^{y5a})$, $-S(O)N(R^{y5})(R^{y5a})$, $-S(O)_2R^{y5}$, $-S(O)R^{y5}$, $-N(R^{y5})S(O)_2N(R^{y5a})(R^{y5b})$, $-SR^{y5}$, $-N(R^{y5})(R^{y5a})$, $-NO_2$, $-OC(O)R^{y5}$, $-N(R^{y5})C(O)R^{y5a}$, $-N(R^{y5})S(O)_2R^{y5a}$, $-N(R^{y5})S(O)R^{y5a}$, $-N(R^{y5})C(O)OR^{y5a}$,

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$-N(R^{y5})C(O)N(R^{y5a})(R^{y5b})$, $-OC(O)N(R^{y5})(R^{y5a})$ and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different; and

each $-R^{y3}$, $-R^{y3a}$, $-R^{y4}$, $-R^{y4a}$, $-R^{y5}$, $-R^{y5a}$ and $-R^{y5b}$ is independently selected from the group consisting of -H and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different.

In certain embodiments $-L^2-$ is selected from the group consisting of $-T'-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{y1})-$, $-S(O)_2N(R^{y1})-$, $-S(O)N(R^{y1})-$, $-S(O)_2-$, $-S(O)-$, $-N(R^{y1})S(O)_2N(R^{y1a})-$, $-S-$, $-N(R^{y1})-$, $-OC(OR^{y1})(R^{y1a})-$, $-N(R^{y1})C(O)N(R^{y1a})-$, $-OC(O)N(R^{y1})-$, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl; wherein $-T'-$, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more $-R^{y2}$, which are the same or different and wherein C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally interrupted by one or more groups selected from the group consisting of $-T'-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{y3})-$, $-S(O)_2N(R^{y3})-$, $-S(O)N(R^{y3})-$, $-S(O)_2-$, $-S(O)-$, $-N(R^{y3})S(O)_2N(R^{y3a})-$, $-S-$, $-N(R^{y3})-$, $-OC(OR^{y3})(R^{y3a})-$, $-N(R^{y3})C(O)N(R^{y3a})-$ and $-OC(O)N(R^{y3})-$;

$-R^{y1}$ and $-R^{y1a}$ are independently selected from the group consisting of -H, $-T'$, C_{1-10} alkyl, C_{2-10} alkenyl and C_{2-10} alkynyl;

each T' is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicycyl, 8- to 30-membered carbopolycyclyl and 8- to 30-membered heteropolycyclyl;

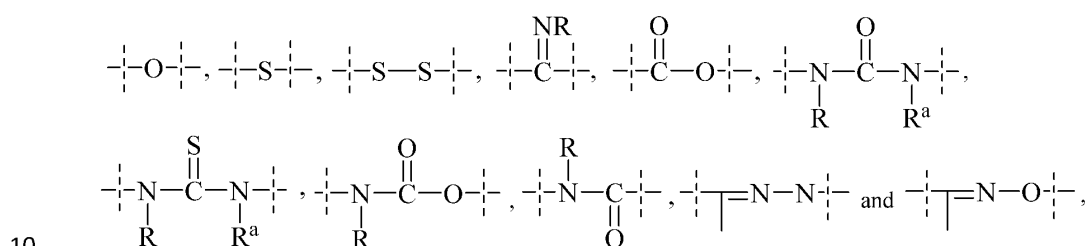
each $-R^{y2}$ is independently selected from the group consisting of halogen, and C_{1-6} alkyl; and each $-R^{y3}$, $-R^{y3a}$, $-R^{y4}$, $-R^{y4a}$, $-R^{y5}$, $-R^{y5a}$ and $-R^{y5b}$ is independently selected from the group consisting of -H and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different.

In certain embodiments $-L^2-$ is a C_{1-20} alkyl chain, which is optionally interrupted by one or more groups independently selected from the group consisting of $-O-$, $-T'-$ and $-C(O)N(R^{y1})-$; and which C_{1-20} alkyl chain is optionally substituted with one or more groups independently selected from the

group consisting of -OH, -T' and -C(O)N(R^{y6}R^{y6a}); wherein -R^{y1}, -R^{y6}, -R^{y6a} are independently selected from the group consisting of H and C₁₋₄ alkyl and wherein T' is selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C₃₋₁₀ cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, 8- to 30-membered carbopolycyclyl and 8- to 30-membered heteropolycyclyl.

In certain embodiments -L²- has a molecular weight in the range of from 14 g/mol to 750 g/mol.

In certain embodiments -L²- comprises a moiety selected from the group consisting of:



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wherein dashed lines indicate attachment to -L¹-, the remainder of -L²- or Z, respectively; and -R and -R^a are independently selected from the group consisting of -H, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, 2-methylbutyl, 2,2-dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl and 3,3-dimethylpropyl.

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In certain embodiments all moieties Z of the conjugate are identical, provided there is more than one moiety Z present in the conjugate. In certain embodiments the conjugate of the present invention comprises more than one type of Z, such as two, three, four or five different moieties Z. Such more than one type of Z may be connected to only one type of -L²- or may be connected to more than one type of -L²-.

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In certain embodiments Z is a fatty acid moiety, which is optionally substituted. In certain embodiments Z is an optionally substituted fatty acid moiety as disclosed in WO 2005/027978 A2 and WO 2014/060512 A1.

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In certain embodiments Z is a polymeric moiety i.e. a moiety that comprises at least one polymer, such as a polymer selected from the group consisting of 2-methacryloyl-oxyethyl phosphocholins,

poly(acrylic acids), poly(acrylates), poly(acrylamides), poly(alkyloxy) polymers, poly(amides), poly(amidoamines), poly(amino acids), poly(anhydrides), poly(aspartamides), poly(butyric acids), poly(glycolic acids), poly(butylene terephthalates), poly(caprolactones), poly(carbonates), poly(cyanoacrylates), poly(dimethylacrylamides), poly(esters), poly(ethylenes),
 5 poly(ethyleneglycols), poly(ethylene oxides), poly(ethyl phosphates), poly(ethyloxazolines), poly(glycolic acids), poly(hydroxyethyl acrylates), poly(hydroxyethyl-oxazolines), poly(hydroxymethacrylates), poly(hydroxypropylmethacrylamides), poly(hydroxypropyl methacrylates), poly(hydroxypropyloxazolines), poly(iminocarbonates), poly(lactic acids), poly(lactic-co-glycolic acids), poly(methacrylamides), poly(methacrylates), poly(methyloxazolines),
 10 poly(organophosphazenes), poly(ortho esters), poly(oxazolines), poly(propylene glycols), poly(siloxanes), poly(urethanes), poly(vinyl alcohols), poly(vinyl amines), poly(vinylmethylethers), poly(vinylpyrrolidones), silicones, celluloses, carbomethyl celluloses, hydroxypropyl methylcelluloses, chitins, chitosans, dextrans, dextrans, gelatins, hyaluronic acids and derivatives, functionalized hyaluronic acids, mannans, pectins, rhamnogalacturonans, starches, hydroxyalkyl
 15 starches, hydroxyethyl starches and other carbohydrate-based polymers, xylans, and copolymers thereof. In certain embodiments the polymeric moiety comprises a PEG-based polymer. In certain embodiments the polymeric moiety comprises a hyaluronic acid-based polymer.

In certain embodiments Z is water-insoluble.

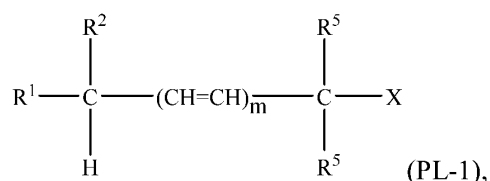
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In certain embodiments Z comprises a hydrogel. Such hydrogel may be degradable or non-degradable, i.e. stable. In certain embodiments such hydrogel is degradable. In certain embodiments such hydrogel is non-degradable. In certain embodiments such hydrogel comprises one or more polymers selected from the group consisting of 2-methacryloyl-oxyethyl phosphoyl cholins,
 25 poly(acrylic acids), poly(acrylates), poly(acrylamides), poly(alkyloxy) polymers, poly(amides), poly(amidoamines), poly(amino acids), poly(anhydrides), poly(aspartamides), poly(butyric acids), poly(glycolic acids), poly(butylene terephthalates), poly(caprolactones), poly(carbonates), poly(cyanoacrylates), poly(dimethylacrylamides), poly(esters), poly(ethylenes), poly(ethyleneglycols), poly(ethylene oxides), poly(ethyl phosphates), poly(ethyloxazolines),
 30 poly(glycolic acids), poly(hydroxyethyl acrylates), poly(hydroxyethyl-oxazolines), poly(hydroxymethacrylates), poly(hydroxypropylmethacrylamides), poly(hydroxypropyl methacrylates), poly(hydroxypropyloxazolines), poly(iminocarbonates), poly(lactic acids),

poly(lactic-co-glycolic acids), poly(methacrylamides), poly(methacrylates), poly(methyloxazolines), poly(organophosphazenes), poly(ortho esters), poly(oxazolines), poly(propylene glycols), poly(siloxanes), poly(urethanes), poly(vinyl alcohols), poly(vinyl amines), poly(vinylmethylethers), poly(vinylpyrrolidones), silicones, celluloses, carbomethyl celluloses, hydroxypropyl methylcelluloses, chitins, chitosans, dextrans, dextrans, gelatins, hyaluronic acids and derivatives, functionalized hyaluronic acids, mannans, pectins, rhamnogalacturonans, starches, hydroxyalkyl starches, hydroxyethyl starches and other carbohydrate-based polymers, xylans, and copolymers thereof. In certain embodiment the hydrogel comprises a PEG-based or hyaluronic acid-based hydrogel. In certain embodiments Z comprises a PEG-based hydrogel. In certain embodiments Z comprises a hyaluronic acid-based hydrogel.

In certain embodiments Z is a hydrogel as disclosed in as disclosed in WO2006/003014, WO2011/012715A1, WO2014/056926A1 or WO2020/064846A1.

In certain embodiments Z is a hydrogel as disclosed in WO2013/036847 A1. In particular, in certain embodiments Z is a hydrogel produced by a method comprising the step of reacting at least a first reactive polymer with a cleavable crosslinker compound, wherein said cleavable crosslinker compound comprises a first functional group $-Y^1$ that reacts with the first reactive polymer and further comprises a moiety that is cleaved by elimination under physiological conditions wherein said moiety comprises a second functional group $-Y^2$ that reacts with a second reactive polymer. In certain embodiments the cleavable crosslinker compound is of formula (PL-1)



wherein

m is 0 or 1;

-X comprises a functional group capable of connecting to a reactive polymer that is amenable to elimination under physiological conditions and said second functional group $-Y^2$;

at least one of $-\text{R}^1$, $-\text{R}^2$ and $-\text{R}^5$ comprises said first functional group $-Y^1$ capable of connecting to a polymer;

one and only one of -R¹ and -R² is selected from the group consisting of -H, alkyl, arylalkyl, and heteroarylalkyl;

optionally, -R¹ and -R² may be joined to form a 3- to 8-membered ring;

at least one or both of -R¹ and -R² is independently selected from the group consisting of -CN, -NO₂, aryl, heteroaryl, alkenyl, alkynyl, -COR³, -SOR³, -SO₂R³ and -SR⁴;

-R³ is selected from the group consisting of -H, alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, -OR⁹ and -NR⁹₂;

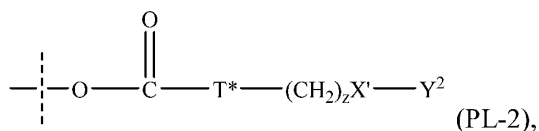
-R⁴ is selected from the group consisting of alkyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl;

each -R⁵ is independently selected from the group consisting of -H, alkyl, alkenylalkyl, alkynylalkyl, (OCH₂CH₂)_pO-alkyl with p being an integer ranging from 1 to 1000, aryl, arylalkyl, heteroaryl and heteroarylalkyl;

each -R⁹ is independently selected from the group consisting of -H and alkyl or both -R⁹ together with the nitrogen to which they are attached form a heterocyclic ring;

and wherein the moiety of formula (PL-1) is optionally further substituted.

In certain embodiments -X of formula (PL-1) is selected from the group consisting of succinimidyl carbonate, sulfosuccinimidyl carbonate halides, thioethers, esters, nitrophenyl carbonate, chloroformate, fluoroformate, optionally substituted phenols and formula (PL-2)



wherein

the dashed line indicates attachment to the remainder of formula (PL-1);

-T* is selected from the group consisting of -O-, -S- and -NR⁶-;

z is an integer selected from the group consisting of 1, 2, 3, 4, 5 and 6;

-X' is absent or is selected from the group consisting of -OR⁷- and -SR⁷-;

-Y² is a functional group capable of connecting with a reactive polymer;

-R⁶ is selected from the group consisting of -H, alkyl, aryl, heteroaryl, arylalkyl, and heteroarylalkyl; and

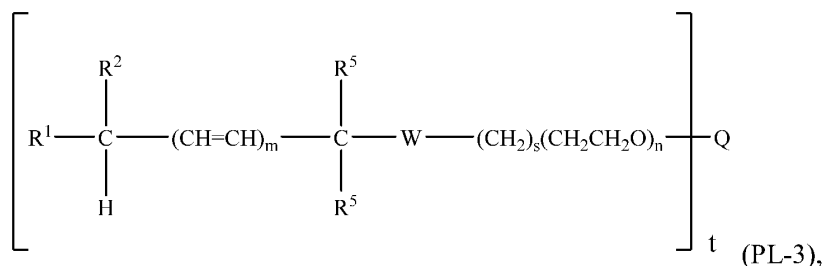
-R⁷ is selected from the group consisting of alkylene, phenylene and (OCH₂CH₂)_p, with p being an integer ranging from 1 to 1000.

In certain embodiments -X of formula (PL-1) comprises an activated carbonate such as succinimidyl carbonate, sulfosuccinimidyl carbonate, or nitrophenyl carbonate. In certain embodiments -X of formula (PL-1) comprises a carbonyl halide such as O(C=O)Cl or O(C=O)F. In certain
 5 embodiments -X of formula (PL-1) has the formula (PL-2). In certain embodiments -X of formula (PL-1) is OR⁷ or SR⁷, wherein R⁷ is optionally substituted alkylene, optionally substituted phenylene or (OCH₂CH₂)_p, wherein p is 1 to 1000.

In certain embodiments p of formula (PL-2) is an integer ranging from 1 to 100. In certain
 10 embodiments p of formula (PL-2) is an integer ranging from 1 to 10.

In certain embodiments -Y¹ of formula (PL-1) and -Y² of formula (PL-2) independently comprise N₃, NH₂, NH-CO^tBu, SH, S^tBu, maleimide, CO₂H, CO₂^tBu, 1,3-diene, cyclopentadiene, furan, alkyne, cyclooctyne, acrylate or acrylamide, wherein ^tBu is tert-butyl, and wherein when one of -Y¹ or -Y²
 15 comprises N₃ the other does not comprise alkyne or cyclooctyne; when one of -Y¹ or -Y² comprises SH the other does not comprise maleimide, acrylate or acrylamide; when one of -Y¹ or -Y² comprises NH₂ the other does not comprise CO₂H; when one of -Y¹ or -Y² comprises 1,3-diene or cyclopentadiene the other does not comprise furan.

20 In certain embodiments the cleavable crosslinker compound is of formula (PL-3)



wherein

m is 0 or 1;

n is an integer selected from 1 to 1000;

25 s is 0, 1 or 2;

t is selected from the group consisting of 2, 4, 8, 16 and 32;

-W- is selected from the group consisting of -O(C=O)O-, -O(C=O)NH-, -O(C=O)S-, -O(C=O)NR⁶CH₂O- and -O(C=O)NR⁶S-;

-Q is a core group having a valency= t ; which connects the multiple arms of the cleavable crosslinking compound,

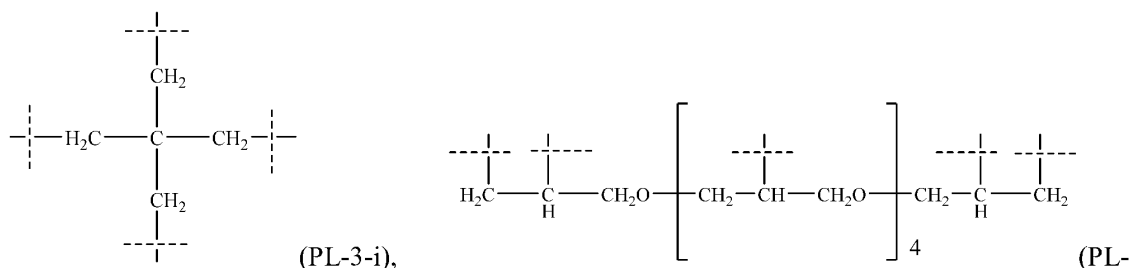
wherein t is an integer selected from 2, 4, 8, 16 and 32, and

wherein $-R^1$, $-R^2$ and $-R^5$ are defined as in formula (PL-1).

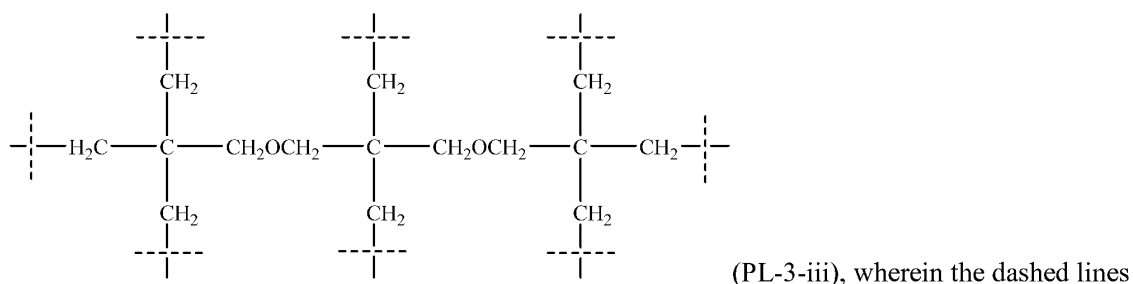
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In certain embodiments t of formula (PL-3) is 2. In certain embodiments t of formula (PL-3) is 4. In certain embodiments t of formula (PL-3) is 8. In certain embodiments t of formula (PL-3) is 16. In certain embodiments t of formula (PL-3) is 32.

10 In certain embodiments -Q of formula (PL-3) has a structure selected from the group consisting of



3-ii) and



indicate attachment to the remainder of the cleavable crosslinker compound.

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In certain embodiments -Q of formula (PL-3) has the structure of (PL-3-i). In certain embodiments -Q of formula (PL-3) has the structure of (PL-3-ii). In certain embodiments -Q of formula (PL-3) has the structure of (PL-3-iii).

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In certain embodiments the cleavable crosslinker compound is of formula (PL-3), wherein m is 0, n is approximately 100, s is 0, t is 4, $-W-$ is $-\text{O}(\text{C}=\text{O})\text{NH}-$, $-Q$ has the structure of (PL-3i), $-R^2$ is H,

one $-R^5$ is $-H$ and the other $-R^5$ is $(CH_2)_5N_3$, and $-R^1$ is (4-chlorophenyl)SO₂, phenyl substituted with $-SO_2$, morpholino-SO₂, or $-CN$.

In certain embodiments $-Y^1$ of formula (PL-3) comprises N_3 , NH_2 , $NH-CO_2^tBu$, SH , S^tBu , maleimide, CO₂H, CO₂^tBu, 1,3-diene, cyclopentadiene, furan, alkyne, cyclooctyne, acrylate or acrylamide, wherein ^tBu is tert-butyl.

In certain embodiments each $-Y^1$ of formula (PL-1) or (PL-3) and $-Y^2$ of formula (PL-2) independently comprises N_3 , NH_2 , $NH-CO_2^tBu$, SH , S^tBu , maleimide, CO₂H, CO₂^tBu, 1,3-diene, cyclopentadiene, furan, alkyne, cyclooctyne, acrylate or acrylamide.

In certain embodiments one of $-Y^1$ and $-Y^2$ is azide and the other is a reactive functional group selected from the group consisting of acetylene, cyclooctyne, and maleimide. In certain embodiments one of $-Y^1$ and $-Y^2$ is thiol and the other is a reactive functional group selected from the group consisting of maleimide, acrylate, acrylamide, vinylsulfone, vinylsulfonamide, and halocarbonyl. In certain embodiments one of $-Y^1$ and $-Y^2$ is amine and the other is a selective reactive functional group selected from carboxylic acid and activated carboxylic acid. In certain embodiments one of $-Y^1$ and $-Y^2$ is maleimide and the other is a selective reactive functional group selected from the group consisting of 1,3-diene, cyclopentadiene, and furan.

20

In certain embodiments the first and any second polymer is selected from the group consisting of homopolymeric or copolymeric polyethylene glycols, polypropylene glycols, poly(N-vinylpyrrolidone), polymethacrylates, polyphosphazenes, polylactides, polyacrylamides, polyglycolates, polyethylene imines, agaroses, dextrans, gelatins, collagens, polylysines, chitosans, alginates, hyaluronans, pectins and carrageenans that either comprise suitable reactive functionalities or is of formula $[Y^3-(CH_2)_s(CH_2CH_2O)_n]_tQ$, wherein $-Y^3$ is a reactive functional group, s is 0, 1 or 2, n is an integer selected from the group ranging from 10 to 1000, $-Q$ is a core group having valency t , and t is an integer selected from the group consisting of 2, 4, 8, 16 and 32.

In certain embodiments the first polymer comprises a multi-arm polymer. In certain embodiments the first polymer comprises at least three arms. In certain embodiments the first polymer comprises at least four arms. In certain embodiments the first polymer comprises at least five arms. In certain

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embodiments the first polymer comprises at least six arms. In certain embodiments the first polymer comprises at least seven arms. In certain embodiments the first polymer comprises at least eight arms.

5 In certain embodiments the second polymer comprises a multi-arm polymer. In certain embodiments the second polymer comprises at least three arms. In certain embodiments the second polymer comprises at least four arms. In certain embodiments the second polymer comprises at least five arms. In certain embodiments the second polymer comprises at least six arms. In certain embodiments the second polymer comprises at least seven arms. In certain embodiments the second polymer comprises at least eight arms.

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In certain embodiments the first polymer comprises a 2-arm polyethylene glycol polymer. In certain embodiments the first polymer comprises a 4-arm polyethylene glycol polymer. In certain embodiments the first polymer comprises an 8-arm polyethylene glycol polymer. In certain embodiments the first polymer comprises a 16-arm polyethylene glycol polymer. In certain
15 embodiments the first polymer comprises a 32-arm polyethylene glycol polymer.

In certain embodiments the second polymer comprises a 2-arm polyethylene glycol polymer. In certain embodiments the second polymer comprises a 4-arm polyethylene glycol polymer. In certain embodiments the second polymer comprises an 8-arm polyethylene glycol polymer. In certain
20 embodiments the second polymer comprises a 16-arm polyethylene glycol polymer. In certain embodiments the second polymer comprises a 32-arm polyethylene glycol polymer.

In certain embodiments the first and a second reactive polymer are reacted with said cleavable crosslinker compound, either sequentially or simultaneously.

25

In certain embodiments the first and second functional groups are the same.

Only in the context of formulas (PL-1), (PL-2) and (PL-3) the terms used have the following meaning:

30 The term "a moiety capable of being cleaved by elimination under physiological conditions" refers to a structure comprising a group $\text{H-C}-(\text{CH}=\text{CH})_m\text{-C-X'}$ wherein m is 0 or 1 and X' is a leaving group, wherein an elimination reaction as described above to remove the elements of HX' can occur at a rate

such that the half-life of the reaction is between 1 and 10,000 hours under physiological conditions of pH and temperature. Preferably, the half-life of the reaction is between 1 and 5,000 hours, and more preferably between 1 and 1,000 hours, under physiological conditions of pH and temperature. By physiological conditions of pH and temperature is meant a pH of between 7 and 8 and a
5 temperature between 30 and 40 degrees centigrade

The term “reactive polymer and reactive oligomer” refers to a polymer or oligomer comprising functional groups that are reactive towards other functional groups, most preferably under mild conditions compatible with the stability requirements of peptides, proteins, and other biomolecules.
10 Suitable functional groups found in reactive polymers include maleimides, thiols or protected thiols, alcohols, acrylates, acrylamides, amines or protected amines, carboxylic acids or protected carboxylic acids, azides, alkynes including cycloalkynes, 1,3-dienes including cyclopentadienes and furans, alpha-halocarbonyls, and N-hydroxysuccinimidyl, N-hydroxysulfosuccinimidyl, or nitrophenyl esters or carbonates.

15 The term “functional group capable of connecting to a reactive polymer” refers to a functional group that reacts to a corresponding functional group of a reactive polymer to form a covalent bond to the polymer. Suitable functional groups capable of connecting to a reactive polymer include maleimides, thiols or protected thiols, acrylates, acrylamides, amines or protected amines, carboxylic acids or
20 protected carboxylic acids, azides, alkynes including cycloalkynes, 1,3-dienes including cyclopentadienes and furans, alpha-halocarbonyls, and N-hydroxysuccinimidyl, N-hydroxysulfosuccinimidyl, or nitrophenyl esters or carbonates.

The term “substituted” refers to an alkyl, alkenyl, alkynyl, aryl, or heteroaryl group comprising one
25 or more substituent groups in place of one or more hydrogen atoms. Substituent groups may generally be selected from halogen including F, Cl, Br, and I; lower alkyl including linear, branched, and cyclic; lower haloalkyl including fluoroalkyl, chloroalkyl, bromoalkyl, and iodoalkyl; OH; lower alkoxy including linear, branched, and cyclic; SH; lower alkylthio including linear, branched, and cyclic; amino, alkylamino, dialkylamino, silyl including alkylsilyl, alkoxy-silyl, and arylsilyl; nitro; cyano;
30 carbonyl; carboxylic acid, carboxylic ester, carboxylic amide; aminocarbonyl; aminoacyl; carbamate; urea; thiocarbamate; thiourea; ketone; sulfone; sulfonamide; aryl including phenyl, naphthyl, and anthracenyl; heteroaryl including 5- member heteroaryls including as pyrrole, imidazole, furan,

thiophene, oxazole, thiazole, isoxazole, isothiazole, thiadiazole, triazole, oxadiazole, and tetrazole, 6-member heteroaryls including pyridine, pyrimidine, pyrazine, and fused heteroaryls including benzofuran, benzothiophene, benzoxazole, benzimidazole, indole, benzothiazole, benzisoxazole, and benzisothiazole.

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The properties of R^1 and R^2 may be modulated by the optional addition of electron-donating or electron-withdrawing substituents. By the term "electron-donating group" is meant a substituent resulting in a decrease in the acidity of the R^1R^2CH ; electron-donating groups are typically associated with negative Hammett σ or Taft σ^* constants and are well-known in the art of physical organic chemistry. (Hammett constants refer to aryl/heteroaryl substituents, Taft constants refer to substituents on non-aromatic moieties.) Examples of suitable electron-donating substituents include lower alkyl, lower alkoxy, lower alkylthio, amino, alkylamino, dialkylamino, and silyl.

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The term "electron-withdrawing group" refers to a substituent resulting in an increase in the acidity of the R^1R^2CH group; electron-withdrawing groups are typically associated with positive Hammett σ or Taft σ^* constants and are well-known in the art of physical organic chemistry. Examples of suitable electron-withdrawing substituents include halogen, difluoromethyl, trifluoromethyl, nitro, cyano, $C(=O)-R^x$, wherein $-R^x$ is H, lower alkyl, lower alkoxy, or amino, or $S(O)_mR^y$, wherein m is 1 or 2 and $-R^y$ is lower alkyl, aryl, or heteroaryl. As is well-known in the art, the electronic influence of a substituent group may depend upon the position of the substituent. For example, an alkoxy substituent on the ortho- or para-position of an aryl ring is electron-donating, and is characterized by a negative Hammett σ constant, while an alkoxy substituent on the meta-position of an aryl ring is electron-withdrawing and is characterized by a positive Hammett σ constant.

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The terms "alkyl", "alkenyl", and "alkynyl" include linear, branched or cyclic hydrocarbon groups of 1 to 8 carbons or 1 to 6 carbons or 1 to 4 carbons wherein alkyl is a saturated hydrocarbon, alkenyl includes one or more carbon-carbon double bonds and alkynyl includes one or more carbon-carbon triple bonds. Unless otherwise specified these contain 1 to 6 carbons.

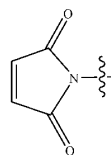
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The term "aryl" includes aromatic hydrocarbon groups of 6 to 18 carbons, preferably 6 to 10 carbons, including groups such as phenyl, naphthyl, and anthracenyl. "Heteroaryl" includes aromatic rings comprising 3 to 15 carbons containing at least one N, O or S atom, preferably 3 to 7 carbons

containing at least one N, O or S atom, including groups such as pyrrolyl, pyridyl, pyrimidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, quinolyl, indolyl, indenyl, and similar.

The term "halogen" includes fluoro, chloro, bromo and iodo.

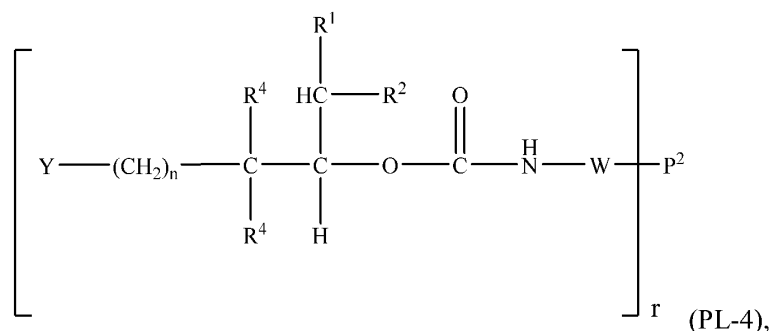
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The term "maleimido" is a group of the formula .

In certain embodiments Z is a hydrogel as disclosed in WO2020/206358 A1. In particular, in certain embodiments Z is a hydrogel produced by a method comprising the steps of

- 10 (a) providing a first prepolymer comprising a multi-arm polymer -P², wherein said first prepolymer is of formula (PL-4)



wherein

n is an integer selected from 0, 1, 2, 3, 4, 5 and 6;

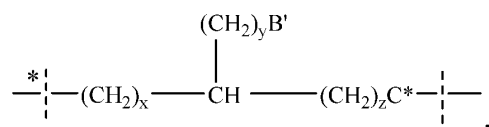
15 r is an integer higher than 2;

-Y is a reactive functional group for connecting said first prepolymer to a second prepolymer;

-R¹ and -R² are independently an electron-withdrawing group, alkyl, or -H, and wherein at least one of -R¹ and -R² is an electron-withdrawing group;

20 each -R⁴ is independently C₁-C₃ alkyl or the two -R⁴ form together with the carbon atom to which they are attached a 3- to 6-membered ring;

-W- is absent or is



wherein the dashed line marked with the asterisk indicates the attachment to -NH- and the unmarked dashed line indicates the attachment to -P²;

each of x, y, and z is independently an integer selected from 0, 1, 2, 3, 4, 5 and 6;

5 -B' is -NH₂, -ONH₂, ketone, aldehyde, -SH, -OH, -CO₂H, carboxamide group, or a group comprising a cyclooctyne or bicyclononyne; and

-C* is carboxamide, thioether, thiosuccinimidyl, triazole, or oxime;

(b) providing the second prepolymer comprising a multi-arm polymer -P¹ wherein each arm is terminated by a reactive functional group -Y'' that reacts with -Y of step (a);

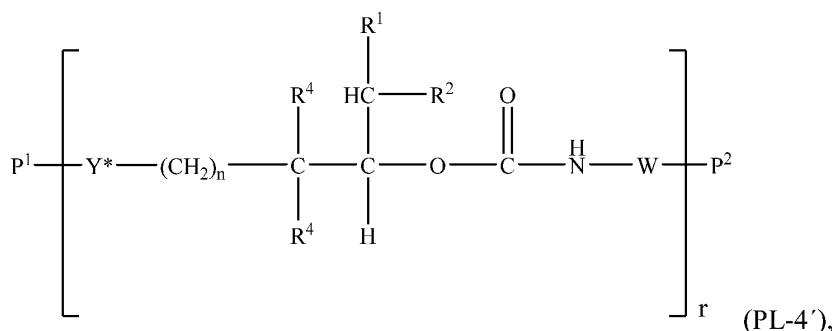
10 (c) mixing the two prepolymers of steps (a) and (b) under conditions wherein -Y and -Y'' react to form a linkage -Y*-; and optionally

(d) isolating the resulting hydrogel.

Accordingly, Z is a hydrogel obtainable from the method described above. In certain embodiments

15 the hydrogel produced by the preceding method is degradable.

In certain embodiments -Y and -Y'' react under step (c) to form an insoluble hydrogel matrix comprising crosslinks of formula (PL-4'):



20 wherein n, r, -P¹, -Y*- , -R⁴, -R¹, -R², -W- and -P² are as defined above.

In certain embodiments n of formula (PL-4) or (PL-4') is an integer selected from 1, 2, 3, 4, 5 and 6.

In certain embodiments n of formula (PL-4) or (PL-4') is an integer selected from 1, 2 and 3. In

certain embodiments n of formula (PL-4) or (PL-4') is an integer selected from 0, 1, 2 and 3. In certain

embodiments n of formula (PL-4) or (PL-4') is 1. In certain embodiments n of formula (PL-4) is 2. In certain embodiments n of formula (PL-4) or (PL-4') is 3.

In certain embodiments the multi-arm -P² of formula (PL-4) or (PL-4') is an r-armed polymer, wherein r is an integer selected from 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. In certain embodiments r of formula (PL-4) or (PL-4') is an integer selected from 2, 3, 4, 5, 6, 7 and 8. In certain embodiments r of formula (PL-4) or (PL-4') is an integer selected from 2, 4, 6 and 8. In certain embodiments r of formula (PL-4) or (PL-4') is 2. In certain embodiments r of formula (PL-4) or (PL-4') is 4. In certain embodiments r of formula (PL-4) or (PL-4') is 6. In certain embodiments r of formula (PL-4) or (PL-4') is 8.

In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of at least 1 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of 1 to 100 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of 1 to 80 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of 1 to 60 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of 1 to 40 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of 1 to 20 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of 1 to 10 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of 1 to 5 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of about 20 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of about 40 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of about 60 kDa. In certain embodiments -P² of formula (PL-4) or (PL-4') has a molecular weight of about 80 kDa.

In certain embodiments the multi-arm polymer -P¹ of step (b) is an r-armed polymer, wherein r is an integer selected from 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. In certain embodiments the multi-arm -P¹ of step (b) is an r-armed polymer, wherein r is an integer selected from 2, 3, 4, 5, 6, 7 and 8. In certain embodiments the multi-arm -P¹ of step (b) is an r-armed polymer, wherein r is an integer selected from 2, 4, 6 and 8. In certain embodiments the multi-arm -P¹ of step (b) is an r-armed polymer, wherein r is 2. In certain embodiments the multi-arm -P¹ of step (b) is an r-armed polymer, wherein r is 4. In certain embodiments the multi-arm -P¹ of step (b) is an r-armed polymer, wherein r is 6. In certain embodiments the multi-arm -P¹ of step (b) is an r-armed polymer, wherein r is 8.

- In certain embodiments $-P^1$ of step (b) has a molecular weight of at least 1 kDa. In certain embodiments the multi-arm polymer $-P^1$ of step (b) has a molecular weight of 1 to 100 kDa. In certain embodiments the multi-arm polymer $-P^1$ of step (b) has a molecular weight of 1 to 80 kDa. In certain
5 embodiments the multi-arm polymer $-P^1$ of step (b) has a molecular weight of 1 to 60 kDa. In certain embodiments the multi-arm polymer $-P^1$ of step (b) has a molecular weight of 1 to 40 kDa. In certain embodiments the multi-arm polymer $-P^1$ of step (b) has a molecular weight of 1 to 20 kDa. In certain embodiments the multi-arm polymer $-P^1$ of step (b) has a molecular weight of 1 to 10 kDa. In certain
10 embodiments the multi-arm polymer $-P^1$ of step (b) has a molecular weight of about 20 kDa. In certain embodiments the multi-arm polymer $-P^1$ of step (b) has a molecular weight of about 40 kDa. In certain embodiments the multi-arm polymer $-P^1$ of step (b) has a molecular weight of about 60 kDa. In certain embodiments the multi-arm polymer $-P^1$ of step (b) has a molecular weight of about 80 kDa.
- 15 In certain embodiments $-P^1$ of step (b) and $-P^2$ of formula (PL-4) or (PL-4') comprise poly(ethylene glycol) (PEG), poly(ethylene oxide) (PEO), poly(ethylene imine) (PEI), dextrans, hyaluronic acids, or co-polymers thereof. In certain embodiments $-P^1$ of step (b) and P^2 of formula (PL-4) or (PL-4') are PEG-based polymers. In certain embodiments $-P^1$ of step (b) and $-P^2$ of formula (PL-4) or (PL-4') are hyaluronic acid-based polymers.
- 20 In certain embodiments $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') are independently electron-withdrawing groups, alkyl, or -H, and wherein at least one of $-R^1$ and $-R^2$ is an electron-withdrawing group.
- 25 In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is -CN, $-\text{NO}_2$, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted alkenyl, optionally substituted alkynyl, $-\text{COR}^3$, $-\text{SOR}^3$, or $-\text{SO}_2\text{R}^3$, wherein $-\text{R}^3$ is -H, optionally substituted alkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, $-\text{OR}^8$ or $-\text{NR}^8_2$, wherein each $-\text{R}^8$ is
30 independently -H or optionally substituted alkyl, or both $-\text{R}^8$ groups are taken together with the nitrogen to which they are attached to form a heterocyclic ring; or $-\text{SR}^9$, wherein $-\text{R}^9$ is optionally

substituted alkyl, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, or optionally substituted heteroarylalkyl.

In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is $-\text{CN}$. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is $-\text{NO}_2$. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is optionally substituted aryl containing 6 to 10 carbons. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is optionally substituted phenyl, naphthyl, or anthracenyl. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is optionally substituted heteroaryl comprising 3 to 7 carbons and containing at least one N, O, or S atom. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is optionally substituted pyrrolyl, pyridyl, pyrimidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, quinolyl, indolyl, or indenyl. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is optionally substituted alkenyl containing 2 to 20 carbon atoms. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is optionally substituted alkynyl containing 2 to 20 carbon atoms. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is $-\text{COR}^3$, $-\text{SOR}^3$, or $-\text{SO}_2\text{R}^3$, wherein R^3 is $-\text{H}$, optionally substituted alkyl containing 1 to 20 carbon atoms, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, $-\text{OR}^8$ or $-\text{NR}^8_2$, wherein each $-\text{R}^8$ is independently $-\text{H}$ or optionally substituted alkyl containing 1 to 20 carbon atoms, or both $-\text{R}^8$ groups are taken together with the nitrogen to which they are attached to form a heterocyclic ring. In certain embodiments the electron-withdrawing group of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is $-\text{SR}^9$, wherein $-\text{R}^9$ is optionally substituted alkyl containing 1 to 20 carbon atoms, optionally substituted aryl, optionally substituted arylalkyl, optionally substituted heteroaryl, or optionally substituted heteroarylalkyl. In certain embodiments at least one of $-R^1$ and $-R^2$ is $-\text{CN}$ or $-\text{SO}_2\text{R}^3$.

In certain embodiments at least one of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is $-\text{CN}$, $-\text{SOR}^3$ or $-\text{SO}_2\text{R}^3$. In certain embodiments at least one of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is $-\text{CN}$ or $-\text{SO}_2\text{R}^3$. In certain embodiments at least one of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is $-\text{CN}$ or $-\text{SO}_2\text{R}^3$, wherein $-\text{R}^3$ is optionally substituted alkyl, optionally substituted aryl, or $-\text{NR}^8_2$. In certain

embodiments at least one of $-R^1$ and $-R^2$ of formula (PL-4) or (PL-4') is $-\text{CN}$, $-\text{SO}_2\text{N}(\text{CH}_3)_2$, $-\text{SO}_2\text{CH}_3$, phenyl substituted with $-\text{SO}_2$, phenyl substituted with $-\text{SO}_2$ and $-\text{Cl}$, $-\text{SO}_2\text{N}(\text{CH}_2\text{CH}_2)_2\text{O}$, $-\text{SO}_2\text{CH}(\text{CH}_3)_2$, $-\text{SO}_2\text{N}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$, or $-\text{SO}_2\text{N}(\text{CH}_2\text{CH}_2\text{OCH}_3)_2$.

- 5 In certain embodiments each $-R^4$ of formula (PL-4) or (PL-4') is independently $\text{C}_1\text{-C}_3$ alkyl or taken together may form a 3- to 6-membered ring. In certain embodiments each $-R^4$ of formula (PL-4) or (PL-4') is independently $\text{C}_1\text{-C}_3$ alkyl. In certain embodiments both $-R^4$ of formula (PL-4) or (PL-4') are methyl.
- 10 In certain embodiments $-Y$ and $-Y''$ are independently selected from the group consisting of amine, aminoxy, ketone, aldehyde, maleimidyl, thiol, alcohol, azide, 1,2,4,6-tetrazinyl, trans-cyclooctenyl, bicyclononyl, cyclooctynyl, and protected variants thereof.

In certain embodiments Y and Y'' may react with each other such as in a selective way. For example, when $-Y$ is amine, $-Y''$ is carboxylic acid, active ester, or active carbonate to yield a residual connecting functional group $-Y^*$ - that is amide or carbamate. As another example, when $-Y$ is azide, $-Y''$ is alkynyl, bicyclononyl, or cyclooctynyl to yield a residual connecting functional group $-Y^*$ - that is 1,2,3-triazole. As another example, when $-Y$ is NH_2O , $-Y''$ is ketone or aldehyde to yield a residual connecting functional group $-Y^*$ - that is oxime. As another example, when $-Y$ is SH, $-Y''$ is maleimide or halocarbonyl to yield a residual connecting functional group $-Y^*$ - that is thiosuccinimidyl or thioether. Similarly, these roles of $-Y$ and $-Y''$ can be reversed to yield $-Y^*$ - of opposing orientation.

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In certain embodiments $-Y^*$ - comprises an amide, oxime, 1,2,3-triazole, thioether, thiosuccinimide, or ether. In certain embodiments $-Y^*$ - is $-\text{L}^2$ -.

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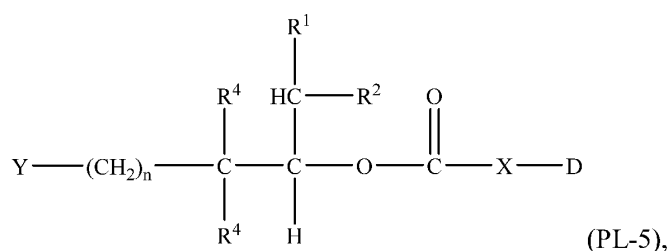
These conjugation reactions may be performed under conditions known in the art, for example when $-Y$ is azide and $-Y''$ is cyclooctyne the conjugation occurs in any solvent wherein both components show adequate solubility, although it is known that aqueous solutions show more favorable reaction rates. When mixed in an appropriate solvent, typically an aqueous buffer at a pH of 2 to 7 when $-Y$ and $-Y''$ are azide/cyclooctyne, or at a pH of 6 to 9 when $-Y$ and $-Y''$ are an activated ester and an amine, the $-Y$ and $-Y''$ groups react to form an insoluble hydrogel matrix comprising

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crosslinks of formula (PL-4'). This process may be carried out in bulk phase, or under conditions of emulsification in a mixed organic/aqueous system so as to form microparticle suspensions such as microspheres that are suitable for injection.

- 5 In certain embodiments a conjugate comprising a hydrogel Z is produced by a method comprising the steps of

- (a) providing a first prepolymer of formula (PL-4)
 (b) reacting the prepolymer of formula (PL-4) with a linker-drug of formula (PL-5)



10 wherein

n , -R^1 , -R^2 , -R^4 and -Y are as defined in formula (PL-4);

-D is a drug moiety;

-X- is absent when -D is a drug moiety connected through an amine,
 or -X- is $\text{-N(R}^6\text{)CH}_2\text{-}$ when -D is a drug moiety connected through a phenol, alcohol, thiol,
 15 thiophenol, imidazole, or non-basic amine; wherein -R^6 is optionally substituted $\text{C}_1\text{-C}_6$ alkyl,
 optionally substituted aryl, or optionally substituted heteroaryl;

so that -Y of formula (PL-5) reacts with -B' of formula (PL-4);

- (c) providing the second prepolymer comprising a multi-arm polymer -P^1 wherein each arm is
 terminated by a reactive functional group -Y'' that reacts with -Y of step (a) and wherein
 20 embodiments for -P^1 are described above;

- (d) mixing the two prepolymers of steps (a) and (b) under conditions wherein -Y and -Y'' react to
 form a residual connecting functional group -Y* ; and optionally
 (e) isolating the resulting hydrogel.

- 25 In certain embodiments a conjugate is obtained by a method comprising the step of reacting a
 hydrogel Z with the linker-drug of formula (PL-5), wherein -B' on the hydrogel Z reacts with -Y of
 formula (PL-5).

Only in the context of formulas (PL-4), (PL-4') and (PL-5) the terms used have the following meaning:

The term "alkyl" refers to linear, branched, or cyclic saturated hydrocarbon groups of 1 to 20, 1 to 12, 1 to 8, 1 to 6, or 1 to 4 carbon atoms. In certain embodiments an alkyl is linear or branched. Examples of linear or branched alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, and n-decyl. In certain embodiments an alkyl is cyclic. Examples of cyclic alkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclopentadienyl, and cyclohexyl.

The term "alkoxy" refers to alkyl groups bonded to oxygen, including methoxy, ethoxy, isopropoxy, cyclopropoxy, and cyclobutoxy.

The term "alkenyl" refers to non-aromatic unsaturated hydrocarbons with carbon-carbon double bonds and 2 to 20, 2 to 12, 2 to 8, 2 to 6, or 2 to 4 carbon atoms.

The term "alkynyl" refers to non-aromatic unsaturated hydrocarbons with carbon-carbon triple bonds and 2 to 20, 2 to 12, 2 to 8, 2 to 6, or 2 to 4 carbon atoms.

The term "aryl" refers to aromatic hydrocarbon groups of 6 to 18 carbons, preferably 6 to 10 carbons, including groups such as phenyl, naphthyl, and anthracenyl. The term "heteroaryl" refers to aromatic rings comprising 3 to 15 carbons comprising at least one N, O or S atom, preferably 3 to 7 carbons comprising at least one N, O or S atom, including groups such as pyrrolyl, pyridyl, pyrimidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, quinolyl, indolyl, and indenyl.

In certain embodiments alkenyl, alkynyl, aryl or heteroaryl moieties may be coupled to the remainder of the molecule through an alkyl linkage. Under those circumstances, the substituent will be referred to as alkenylalkyl, alkynylalkyl, arylalkyl or heteroarylalkyl, indicating that an alkylene moiety is between the alkenyl, alkynyl, aryl or heteroaryl moiety and the molecule to which the alkenyl, alkynyl, aryl or heteroaryl is coupled.

The term "halogen" or "halo" refers to bromo, fluoro, chloro and iodo.

The term "heterocyclic ring" or "heterocyclyl" refers to a 3- to 15-membered aromatic or non-aromatic ring comprising at least one N, O, or S atom. Examples include piperidinyl, piperazinyl, tetrahydropyranyl, pyrrolidine, and tetrahydrofuranyl, as well as the exemplary groups provided for the term "heteroaryl" above. In certain embodiments a heterocyclic ring or heterocyclyl is non-aromatic. In certain embodiments a heterocyclic ring or heterocyclyl is aromatic.

The term "optionally substituted" refers to a group may be unsubstituted or substituted by one or more (e.g., 1, 2, 3, 4 or 5) of the substituents which may be the same or different. Examples of substituents include alkyl, alkenyl, alkynyl, halogen, -CN, -OR^{aa}, -SR^{aa}, -NR^{aa}R^{bb}, -NO₂, -C=NH(OR^{aa}), -C(O)R^{aa}, -OC(O)R^{aa}, -C(O)OR^{aa}, -C(O)NR^{aa}R^{bb}, -OC(O)NR^{aa}R^{bb}, -NR^{aa}C(O)R^{bb}, -NR^{aa}C(O)OR^{bb}, -S(O)R^{aa}, -S(O)₂R^{aa}, -NR^{aa}S(O)R^{bb}, -C(O)NR^{aa}S(O)R^{bb}, -NR^{aa}S(O)₂R^{bb}, -C(O)NR^{aa}S(O)₂R^{bb}, -S(O)NR^{aa}R^{bb}, -S(O)₂NR^{aa}R^{bb}, -P(O)(OR^{aa})(OR^{bb}), heterocyclyl, heteroaryl, or aryl, wherein the alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heteroaryl, and aryl are each independently optionally substituted by -R^{cc}, wherein -R^{aa} and -R^{bb} are each independently -H, alkyl, alkenyl, alkynyl, heterocyclyl, heteroaryl, or aryl, or -R^{aa} and -R^{bb} are taken together with the nitrogen atom to which they attach to form a heterocyclyl, which is optionally substituted by alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkoxy, or -CN, and wherein: each -R^{cc} is independently alkyl, alkenyl, alkynyl, halogen, heterocyclyl, heteroaryl, aryl, -CN, or -NO₂.

In certain embodiments Z is a hyaluronic acid-based hydrogel as disclosed in WO2018/175788A1. In certain embodiments Z is a hyaluronic acid-based hydrogel as disclosed in WO2020/064847A1.

In certain embodiments Z is a water-soluble polymeric moiety.

In certain embodiments Z comprises a polymer selected from the group consisting of linear, branched, dendritic, multi-arm or circular polymers or combination thereof. In certain embodiments Z comprises a linear polymer. In certain embodiments Z is a branched PEG-based polymer. In certain embodiments Z is a branched PEG-based polymer having one, two, three, four, five or six branching points. In certain embodiments Z is a branched PEG-based polymer having one, two or three branching points. In certain embodiments Z is a branched PEG-based polymer having one branching

point. In certain embodiments Z is a branched PEG-based polymer having two branching points. In certain embodiments Z is a branched PEG-based polymer having three branching points. In certain embodiments Z is a branched PEG-based polymer having four branching points. In certain embodiments a branching point is selected from the group consisting of -N<, -CH< and >C<. In certain embodiments Z comprises a dendritic polymer. In certain embodiments Z comprises a multi-arm polymer, such as a polymer comprising 3, 4, 5, 6, 7, or 8 arms. In certain embodiments Z comprises a multi-arm polymer comprising 3 arms. In certain embodiments Z comprises a multi-arm polymer comprising 4 arms. In certain embodiments Z comprises a multi-arm polymer comprising 5 arms. In certain embodiments Z comprises a multi-arm polymer comprising 6 arms. In certain embodiments Z comprises a multi-arm polymer comprising 7 arms. In certain embodiments Z comprises a multi-arm polymer comprising 8 arms. In certain embodiments Z comprises a circular polymer. In certain embodiments Z comprises a polymer comprising any combination of a polymer selected from the group consisting of linear, branched, dendritic, multi-arm or circular polymers.

If Z is a water-soluble polymeric moiety, such polymeric moiety may have a molecular weight ranging from and including 1 kDa to 1000 kDa. In certain embodiments Z has a molecular weight ranging from and including 5 kDa to 1000 kDa. In certain embodiments Z has a molecular weight ranging from and including 6 kDa to 500 kDa. In certain embodiments Z has a molecular weight ranging from and including 7 kDa to 250 kDa. In certain embodiments Z has a molecular weight ranging from and including 10 kDa to 150 kDa. In certain embodiments Z has a molecular weight ranging from and including 12 kDa to 100 kDa. In certain embodiments Z has a molecular weight ranging from and including 15 kDa to 80 kDa. In certain embodiments Z has a molecular weight ranging from and including 10 kDa to 80 kDa. In certain embodiments Z has a molecular weight of about 80 kDa. In certain embodiments Z has a molecular weight of about 70 kDa. In certain embodiments Z has a molecular weight of about 60 kDa. In certain embodiments Z has a molecular weight of about 50 kDa. In certain embodiments Z has a molecular weight of about 40 kDa. In certain embodiments Z has a molecular weight of about 30 kDa. In certain embodiments Z has a molecular weight of about 20 kDa. In certain embodiments Z has a molecular weight of about 10 kDa. In certain embodiments Z has a molecular weight of about 5 kDa.

In certain embodiments Z is a water-soluble polymeric moiety comprising a polymer selected from the group consisting of 2-methacryloyl-oxyethyl phosphoyl cholins, poly(acrylic acids),

poly(acrylates), poly(acrylamides), poly(alkyloxy) polymers, poly(amides), poly(amidoamines), poly(amino acids), poly(anhydrides), poly(aspartamides), poly(butyric acids), poly(glycolic acids), poly(butylene terephthalates), poly(caprolactones), poly(carbonates), poly(cyanoacrylates), poly(dimethylacrylamides), poly(esters), poly(ethylenes), poly(ethyleneglycols), poly(ethylene oxides), poly(ethyl phosphates), poly(ethyloxazolines), poly(glycolic acids), poly(hydroxyethyl acrylates), poly(hydroxyethyl-oxazolines), poly(hydroxymethacrylates), poly(hydroxypropylmethacrylamides), poly(hydroxypropyl methacrylates), poly(hydroxypropyloxazolines), poly(iminocarbonates), poly(lactic acids), poly(lactic-co-glycolic acids), poly(methacrylamides), poly(methacrylates), poly(methyloxazolines), poly(organophosphazenes), poly(ortho esters), poly(oxazolines), poly(propylene glycols), poly(siloxanes), poly(urethanes), poly(vinyl alcohols), poly(vinyl amines), poly(vinylmethylethers), poly(vinylpyrrolidones), silicones, celluloses, carbomethyl celluloses, hydroxypropyl methylcelluloses, chitins, chitosans, dextrans, dextrins, gelatins, hyaluronic acids and derivatives, functionalized hyaluronic acids, mannans, pectins, rhamnogalacturonans, starches, hydroxyalkyl starches, hydroxyethyl starches and other carbohydrate-based polymers, xylans, and copolymers thereof.

In certain embodiments Z is a water-soluble polymeric moiety comprising a protein, such as a protein selected from the group consisting of carboxyl-terminal peptide of the chorionic gonadotropin as described in US 2012/0035101 A1; albumin; XTEN sequences as described in WO 2011/123813 A2; proline/alanine random coil sequences as described in WO 2011/144756 A1; proline/alanine/serine random coil sequences as described in WO 2008/155134 A1 and WO 2013/024049 A1; and Fc-fusion proteins.

In certain embodiments Z comprises a random coil protein moiety of which at least 80%, in certain embodiments at least 85%, in certain embodiments at least 90%, in certain embodiments at least 95%, in certain embodiments at least 98% and in certain embodiments at least 99% of the total number of amino acids forming said random coil protein moiety are selected from alanine and proline. In certain embodiments at least 10%, but less than 75%, in certain embodiments less than 65% of the total number of amino acid residues of such random coil protein moiety are proline residues. In certain embodiments such random coil protein moiety is as described in WO 2011/144756 A1. In certain embodiments Z comprises at least one moiety selected from the group consisting of SEQ ID NO:1,

SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:51 and SEQ ID NO:61 as disclosed in WO2011/144756 which are hereby incorporated by reference. A moiety comprising such
5 random coil protein comprising alanine and proline will be referred to as “PA” or “PA moiety”. Accordingly, in certain embodiments Z comprises a PA moiety.

In certain embodiments Z comprises a random coil protein moiety of which at least 80%, in certain embodiments at least 85%, in certain embodiments at least 90%, in certain embodiments at least 95%,
10 in certain embodiments at least 98% and in certain embodiments at least 99% of the total number of amino acids forming said random coil protein moiety are selected from alanine, serine and proline. In certain embodiments at least 4%, but less than 40% of the total number of amino acid residues of such random coil protein moiety are proline residues. In certain embodiments such random coil protein moiety is as described in WO 2008/155134 A1. In certain embodiments Z comprises at least
15 one moiety selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54 and SEQ ID NO:56 as disclosed in WO
20 2008/155134 A1, which are hereby incorporated by reference. A moiety comprising such random coil protein moiety comprising alanine, serine and proline will be referred to as “PAS” or “PAS moiety”. Accordingly, in certain embodiments Z comprises a PAS moiety.

In certain embodiments Z comprises a random coil protein moiety of which at least 80%, in certain
25 embodiments at least 85%, in certain embodiments at least 90%, in certain embodiments at least 95%, in certain embodiments at least 98% and in certain embodiments 99% of the total number of amino acids forming said random coil protein moiety are selected from alanine, glycine, serine, threonine, glutamate and proline. In certain embodiments such random coil protein moiety is as described in WO 2010/091122 A1. In certain embodiments Z comprises at least one moiety selected from the
30 group consisting of SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184; SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID

NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:208, SEQ ID NO:209, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:216, SEQ ID NO:217, SEQ ID NO:218, SEQ ID NO:219, SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:759, SEQ ID NO:760, SEQ ID NO:761, SEQ ID NO:762, SEQ ID NO:763, SEQ ID NO:764, SEQ ID NO:765, SEQ ID NO:766, SEQ ID NO:767, SEQ ID NO:768, SEQ ID NO:769, SEQ ID NO:770, SEQ ID NO:771, SEQ ID NO:772, SEQ ID NO:773, SEQ ID NO:774, SEQ ID NO:775, SEQ ID NO:776, SEQ ID NO:777, SEQ ID NO:778, SEQ ID NO:779, SEQ ID NO:1715, SEQ ID NO:1716, SEQ ID NO:1718, SEQ ID NO:1719, SEQ ID NO:1720, SEQ ID NO:1721 and SEQ ID NO:1722 as disclosed in WO2010/091122A1, which are hereby incorporated by reference. A moiety comprising such random coil protein moiety comprising alanine, glycine, serine, threonine, glutamate and proline will be referred to as "XTEN" or "XTEN moiety" in line with its designation in WO 2010/091122 A1. Accordingly, in certain embodiments Z comprises an XTEN moiety.

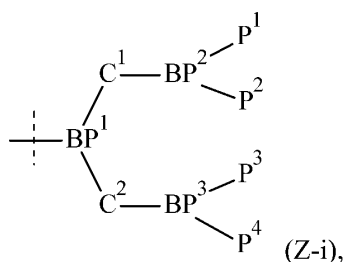
In certain embodiments Z comprises at least 60, such as at least 90 or at least 120, elastin-like peptide structural units selected from any one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:13 of WO2021/030196. In certain embodiments Z comprises the protein of SEQ ID NO:34 of WO2021/030196. In certain embodiments Z comprises the protein of SEQ ID NO:35 of WO2021/030196.

In certain embodiments Z comprises a hyaluronic acid-based polymer.

In certain embodiments Z comprises a polymeric moiety as disclosed in WO 2013/024047 A1. In certain embodiments Z comprises a polymeric moiety as disclosed in WO 2013/024048 A1.

In certain embodiments Z comprises a multi-arm PEG-based polymer. In certain embodiments Z comprises a multi-arm PEG-based polymer having at least 2 PEG-based arms, such as 2, 3, 4, 5, 6, 7 or 8 PEG-based arms.

In certain embodiments Z comprises a branched moiety of formula (Z-i)



wherein

-BP¹<, -BP²<, -BP³< are independently of each other selected from the group consisting of -N< and -C(R⁸)<;

5 R⁸ is selected from the group consisting of H, C₁-₆ alkyl, C₂-₆ alkenyl and C₂-₆ alkynyl;

-P¹, -P², -P³, -P⁴ are independently of each other a PEG-based chain comprising at least 40% PEG and having a molecular weight ranging from 2 to 45 kDa;

-C¹-, -C²- are independently of each other selected from the group consisting of C₁-₅₀ alkyl, C₂-₅₀ alkenyl, and C₂-₅₀ alkynyl; wherein C₁-₅₀ alkyl, C₂-₅₀ alkenyl, and C₂-₅₀ alkynyl are optionally substituted with one or more R⁹, which are the same or different and wherein C₁-₅₀ alkyl, C₂-₅₀ alkenyl, and C₂-₅₀ alkynyl are optionally interrupted by one or more groups selected from the group consisting of -T-, -C(O)O-, -O-, -C(O)-, -C(O)N(R¹⁰)-, -S(O)₂N(R¹⁰)-, -S(O)N(R¹⁰)-, -S(O)₂-, -S(O)-, -N(R¹⁰)S(O)₂N(R¹⁰ᵃ)-, -S-, -N(R¹⁰)-, -OC(OR¹⁰)(R¹⁰ᵃ)-, -N(R¹⁰)C(O)N(R¹⁰ᵃ)-, and -OC(O)N(R¹⁰)-;

15 each T is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C₃-₁₀ cycloalkyl, 3- to 10-membered heterocyclyl, 8- to 11-membered heterobicyclyl, 8- to 30-membered carbopolycyclyl, and 8- to 30-membered heteropolycyclyl; wherein each T is independently optionally substituted with one or more R⁹, which are the same or different;

20 each R⁹ is independently selected from the group consisting of halogen, -CN, oxo (=O), -COOR¹¹, -OR¹¹, -C(O)R¹¹, -C(O)N(R¹¹R¹¹ᵃ), -S(O)₂N(R¹¹R¹¹ᵃ), -S(O)N(R¹¹R¹¹ᵃ), -S(O)₂R¹¹, -S(O)R¹¹, -N(R¹¹)S(O)₂N(R¹¹ᵃR¹¹ᵇ), -SR¹¹, -N(R¹¹R¹¹ᵃ), -NO₂, -OC(O)R¹¹, -N(R¹¹)C(O)R¹¹ᵃ, -N(R¹¹)S(O)₂R¹¹ᵃ, -N(R¹¹)S(O)R¹¹ᵃ, -N(R¹¹)C(O)OR¹¹ᵃ, -N(R¹¹)C(O)N(R¹¹ᵃR¹¹ᵇ), -OC(O)N(R¹¹R¹¹ᵃ), and C₁-₆ alkyl; wherein C₁-₆ alkyl is optionally substituted with one or more halogen, which are the same or different; and

25

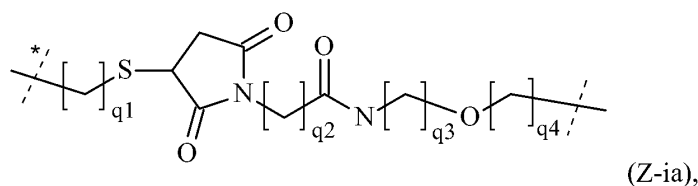
each R^{10} , R^{10a} , R^{11} , R^{11a} and R^{11b} is independently selected from the group consisting of -H, and C_{1-6} alkyl, wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different.

- 5 In certain embodiments BP^1 of formula (Z-i) is -N<. In certain embodiments BP^2 and BP^2 of formula (Z-i) are both -CH<. It is advantageous if the first branching point BP^1 and the attachment site of Z to -L¹- are separated by no more than a certain number of atoms. Accordingly, in certain embodiments the critical distance is less than 60 atoms, such as less than 50 atoms, such as less than 40 atoms, such as less than 30 atoms, such as less than 20 atoms or less than 10 atoms. The term "critical distance"
- 10 refers to the shortest distance measured as the number of atoms between the first branching point BP^1 and attachment site of Z to -L¹-.

- In certain embodiments C^1 and C^2 of formula (Z-i) are C_{1-50} alkyl interrupted by one or more of the groups selected from the group consisting of -O-, -C(O)N(R^{10})- and 3- to 10 membered heterocyclyl;
- 15 wherein the 3- to 10 membered heterocyclyl is substituted with at least one oxo (=O).

- In certain embodiments P^1 , P^2 , P^3 and P^4 have each independently of the other a molecular weight ranging from about 5 to about 40 kDa. In certain embodiments P^1 , P^2 , P^3 and P^4 have each independently of the other a molecular weight ranging from about 8 to about 25 kDa. In certain
- 20 embodiments P^1 , P^2 , P^3 and P^4 have each independently of the other a molecular weight ranging from about 9 to about 22 kDa. In certain embodiments P^1 , P^2 , P^3 and P^4 have each independently of the other a molecular weight ranging from about 9 to about 15 kDa. In certain embodiments P^1 , P^2 , P^3 and P^4 have each independently of the other a molecular weight ranging from 10 to 12 kDa. In certain
- 25 embodiments P^1 , P^2 , P^3 and P^4 have each independently of the other a molecular weight of about 10 kDa. In certain embodiments P^1 , P^2 , P^3 and P^4 have each independently of the other a molecular weight ranging from 15 to 22 kDa. In certain embodiments P^1 , P^2 , P^3 and P^4 have each independently of the other a molecular weight ranging from 18 to 21 kDa. In certain embodiments P^1 , P^2 , P^3 and P^4 have each independently of the other a molecular weight of about 20 kDa.

- 30 In certain embodiments C^1 and C^2 of formula (Z-i) are of formula (Z-ia)



wherein

the dashed line marked with the asterisk indicates attachment to BP¹;

the unmarked dashed line indicates attachment to BP² or BP³, respectively;

5 q_1 is 1, 2, 3, 4, 5, 6, 7 or 8;

q_2 is 1, 2, 3, 4, or 5;

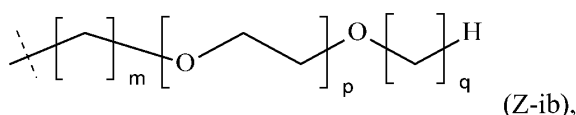
q_3 is 1, 2, 3, 4, 5, 6, 7 or 8;

q_4 is 1, 2 or 3.

10 In certain embodiments q_1 of formula (Z-ia) is 4, 5, 6, 7, or 8. In certain embodiments q_1 of formula (Z-ia) is 5, 6 or 7. In certain embodiments q_1 of formula (Z-ia) is 6. In certain embodiments q_2 of formula (Z-ia) is 1, 2 or 3. In certain embodiments q_3 of formula (Z-ia) is 2, 3, 4 or 5. In certain embodiments q_3 is 2, 3 or 4. In certain embodiments q_3 of formula (Z-ia) is 3. In certain embodiments q_4 of formula (Z-ia) is 1.

15

In certain embodiments P¹, P², P³ and P⁴ of formula (Z-i) are independently of each other of formula (Z-ib)



wherein

20 the dashed line indicates attachment the remainder of R¹, i.e. to BP² or BP³, respectively;

m is 0 or 1;

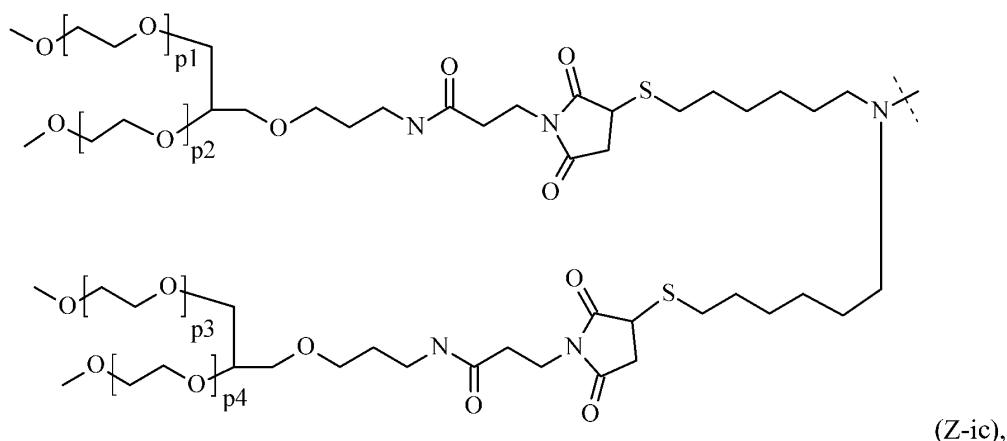
p is an integer ranging from 45 to 1000; and

q is selected from the group consisting of 1, 2, 3, 4, 5, and 6.

25 In certain embodiments p of formula (Z-ib) ranges from 115 to 900. In certain embodiments p of formula (Z-ib) ranges from 180 to 580. In certain embodiments p of formula (Z-ib) ranges from 200 to 340. In certain embodiments p of formula (Z-ib) ranges from 220 to 270. In certain embodiments

p of formula (Z-ib) ranges from 220 to 240. In certain embodiments p of formula (Z-ib) is about 225. In certain embodiments p of formula (Z-ib) ranges from 340 to 500. In certain embodiments p of formula (Z-ib) ranges from 410 to 475. In certain embodiments p of formula (Z-ib) is about 450.

- 5 In certain embodiments Z comprises a moiety of formula (Z-ic):

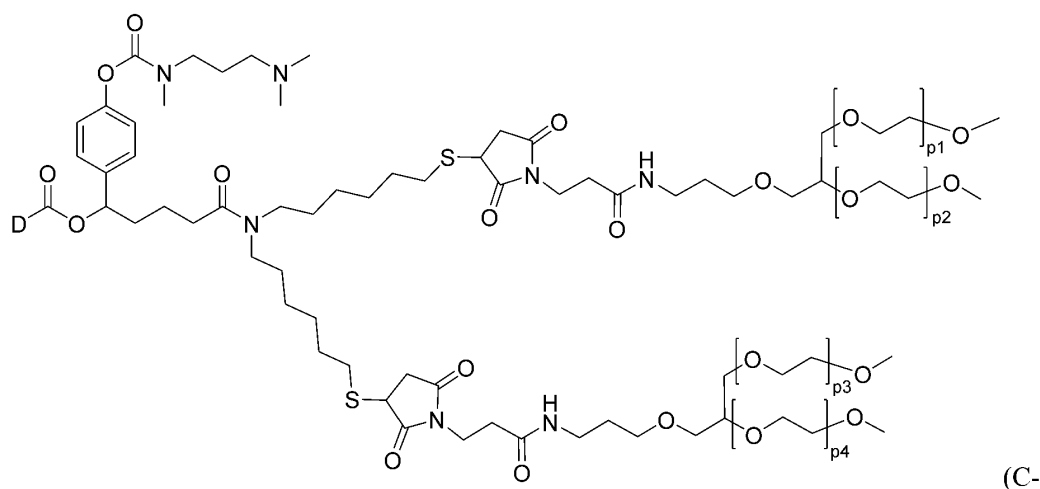


wherein

p1, p2, p3 and p4 are independently an integer ranging from 45 to 1000.

- 10 In certain embodiments p1, p2, p3 and p4 are independently an integer ranging from 115 to 900. In certain embodiments p1, p2, p3 and p4 are independently an integer ranging from 180 to 580. In certain embodiments p1, p2, p3 and p4 are independently an integer ranging from 200 to 340. In certain embodiments p1, p2, p3 and p4 are independently an integer ranging from 220 to 270. In certain embodiments p1, p2, p3 and p4 are independently an integer ranging from 220 to 240.
- 15 In certain embodiments p1, p2, p3 and p4 are independently an integer ranging from 210 to 240. In certain embodiments p1, p2, p3 and p4 are independently about 225. In certain embodiments p1, p2, p3 and p4 are independently an integer ranging from 340 to 500. In certain embodiments p1, p2, p3 and p4 are independently an integer ranging from 410 to 475. In certain embodiments p1, p2, p3 and p4 are independently an integer ranging from 420 to 480. In certain embodiments p1, p2, p3 and p4 are independently about 450.
- 20

In certain embodiments the growth hormone conjugate is a conjugate of formula (C-i), as disclosed for example in WO05099768 A2



i),

wherein

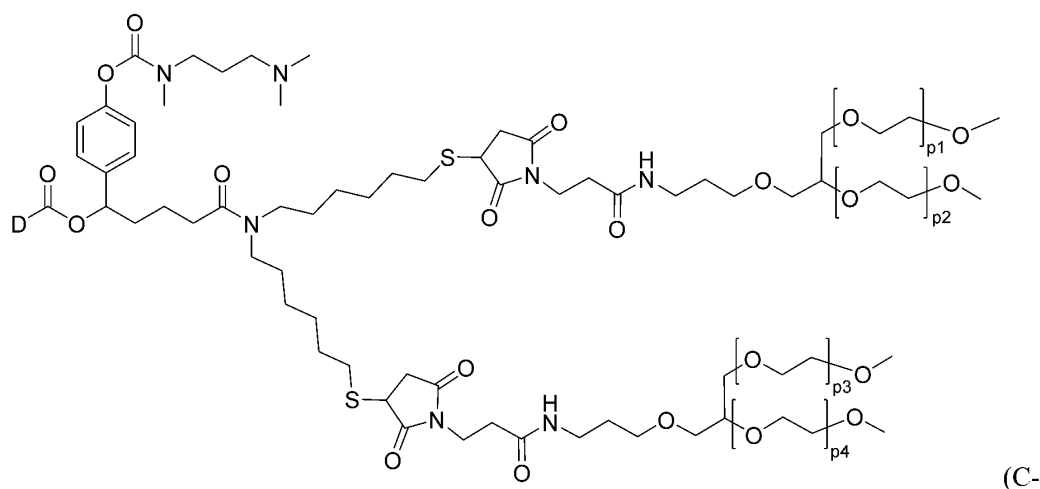
-D is a hGH moiety connected to the rest of the molecule through the nitrogen of an amine functional group of -D; and

p1, p2, p3 and p4 are independently an integer ranging from 400 to 500.

In certain embodiments -D of formula (C-i) is a hGH moiety of SEQ ID NO:1. In certain embodiments -D of formula (C-i) is connected to the rest of the molecule through a nitrogen of an amine functional group provided by a lysine side chain of -D.

In certain embodiments p1, p2, p3 and p4 of formula (C-i) are independently an integer ranging from 420 to 480. In certain embodiments p1, p2, p3 and p4 of formula (C-i) are independently an integer ranging from 420 to 470. In certain embodiments p1, p2, p3 and p4 of formula (C-i) are independently an integer ranging from 440 to 460. In certain embodiments p1, p2, p3 and p4 of formula (C-i) are about 450.

In certain embodiments the growth hormone conjugate is a conjugate of formula (C-ii), as disclosed for example in WO2016/079114A1



ii),

wherein

-D is a hGH moiety connected to the rest of the molecule through the nitrogen of an amine functional group of -D; and

p1, p2, p3 and p4 are independently an integer ranging from 200 to 250.

In certain embodiments -D of formula (C-ii) is a hGH moiety of SEQ ID NO:1. In certain embodiments -D of formula (C-ii) is connected to the rest of the molecule through a nitrogen of an amine functional group provided by a lysine side chain of -D.

In certain embodiments p1, p2, p3 and p4 of formula (C-ii) are independently an integer ranging from 210 to 240. In certain embodiments p1, p2, p3 and p4 of formula (C-ii) are independently an integer ranging from 220 to 240. In certain embodiments p1, p2, p3 and p4 of formula (C-ii) are about 225.

If the long-acting GH is of formula (C-ii) with -D being of SEQ ID NO:1, -D being connected to the rest of the molecule through a nitrogen of an amine functional group provided by a lysine side chain of -D and p1, p2, p3 and p4 independently being an integer ranging from 220 to 240, the long-acting GH is lonapegsomatropin, which is marketed in the US as Skytrofa®.

In certain embodiments the long-acting growth hormone is selected from the group consisting of Somatrogen, Albutropin, ARX201, ALTU-238, PHA-794428, hGH-OctoDex, Norditropin, Nutropin Depot, LB03002, Somatropin Biopartners, Efpegsomatropin, Somapacitan, Jintrolong,

Eftansomatropin and TV-1106. In certain embodiments the long-acting growth hormone is selected from the group consisting of Somatrogen, Albutropin, Norditropin, Nutropin Depot, Somatropin Biopartners, Efpegsomatropin, Somapacitan, Eftansomatropin and Jintrolong. In certain embodiments the long-acting growth hormone is Somatrogen. In certain embodiments the long-acting growth hormone is Albutropin. In certain embodiments the long-acting growth hormone is ARX201. In certain embodiments the long-acting growth hormone is ALTU-238. In certain embodiments the long-acting growth hormone is PHA-794428. In certain embodiments the long-acting growth hormone is hGH-OctoDex. In certain embodiments the long-acting growth hormone is Norditropin. In certain embodiments the long-acting growth hormone is Nutropin Depot. In certain embodiments the long-acting growth hormone is LB03002. In certain embodiments the long-acting growth hormone is Somatropin Biopartners. In certain embodiments the long-acting growth hormone is Efpegsomatropin. In certain embodiments the long-acting growth hormone is Somapacitan. Somapacitan is marketed as Sogroya[®]. In certain embodiments the long-acting growth hormone is TV-1106. In certain embodiments the long-acting growth hormone is Jintrolong. In certain embodiments the long-acting growth hormone is Eftansomatropin.

In another aspect the present invention relates to a pharmaceutical composition comprising at least one long-acting growth hormone and at least one excipient for use in the treatment of an inflammation-induced disease, wherein the at least one long-acting growth hormone and the treatment are as described elsewhere herein. In certain embodiments such pharmaceutical composition has a pH ranging from and including pH 3 to pH 8. In certain embodiments such pharmaceutical composition is a suspension formulation. In certain embodiments such pharmaceutical composition is a liquid formulation. In certain embodiments such pharmaceutical composition is a dry formulation.

Such liquid, suspension or dry pharmaceutical composition comprises at least one excipient. Excipients used in parenteral formulations may be categorized as, for example, buffering agents, isotonicity modifiers, preservatives, stabilizers, anti-adsorption agents, oxidation protection agents, viscosifiers/viscosity enhancing agents, or other auxiliary agents. However, in some cases, one excipient may have dual or triple functions. In certain embodiments the at least one excipient of the pharmaceutical composition of the present invention is selected from the group consisting of

- (i) Buffering agents: physiologically tolerated buffers to maintain pH in a desired range, such as sodium phosphate, bicarbonate, succinate, histidine, citrate and acetate, sulphate, nitrate, chloride, pyruvate; antacids such as $\text{Mg}(\text{OH})_2$ or ZnCO_3 may be also used;
- 5 (ii) Isotonicity modifiers: to minimize pain that can result from cell damage due to osmotic pressure differences at the injection depot; glycerin and sodium chloride are examples; effective concentrations can be determined by osmometry using an assumed osmolality of 285-315 mOsmol/kg for serum;
- 10 (iii) Preservatives and/or antimicrobials: multidose parenteral formulations require the addition of preservatives at a sufficient concentration to minimize risk of patients becoming infected upon injection and corresponding regulatory requirements have been established; typical preservatives include m-cresol, phenol, methylparaben, ethylparaben, propylparaben, butylparaben, chlorobutanol, benzyl alcohol, phenylmercuric nitrate, thimerosal, sorbic acid, potassium sorbate, benzoic acid, chlorocresol, and benzalkonium chloride;
- 15 (iv) Stabilizers: Stabilisation is achieved by strengthening of the protein-stabilising forces, by destabilisation of the denatured state, or by direct binding of excipients to the protein; stabilizers may be amino acids such as alanine, arginine, aspartic acid, glycine, histidine, lysine, proline, sugars such as glucose, sucrose, trehalose, polyols such as glycerol, mannitol, sorbitol, salts such as potassium phosphate, sodium sulphate, chelating agents
- 20 such as EDTA, hexaphosphate, ligands such as divalent metal ions (zinc, calcium, etc.), other salts or organic molecules such as phenolic derivatives; in addition, oligomers or polymers such as cyclodextrins, dextran, dendrimers, PEG or PVP or protamine or HSA may be used;
- 25 (v) Anti-adsorption agents: Mainly ionic or non-ionic surfactants or other proteins or soluble polymers are used to coat or adsorb competitively to the inner surface of the formulation's container; e.g., poloxamer (Pluronic F-68), PEG dodecyl ether (Brij 35), polysorbate 20 and 80, dextran, polyethylene glycol, PEG-polyhistidine, BSA and HSA and gelatins; chosen concentration and type of excipient depends on the effect to be avoided but typically a monolayer of surfactant is formed at the interface just above the CMC value;
- 30 (vi) Oxidation protection agents: antioxidants such as ascorbic acid, ectoine, methionine, glutathione, monothioglycerol, morin, polyethylenimine (PEI), propyl gallate, and

vitamin E; chelating agents such as citric acid, EDTA, hexaphosphate, and thioglycolic acid may also be used;

- (vii) Viscosifiers or viscosity enhancers: retard settling of the particles in the vial and syringe and are used in order to facilitate mixing and resuspension of the particles and to make the suspension easier to inject (i.e., low force on the syringe plunger); suitable viscosifiers or viscosity enhancers are, for example, carbomer viscosifiers like Carbopol 940, Carbopol Ultrez 10, cellulose derivatives like hydroxypropylmethylcellulose (hypromellose, HPMC) or diethylaminoethyl cellulose (DEAE or DEAE-C), colloidal magnesium silicate (Veegum) or sodium silicate, hydroxyapatite gel, tricalcium phosphate gel, xanthans, carrageenans like Satia gum UTC 30, aliphatic poly(hydroxy acids), such as poly(D,L- or L-lactic acid) (PLA) and poly(glycolic acid) (PGA) and their copolymers (PLGA), terpolymers of D,L-lactide, glycolide and caprolactone, poloxamers, hydrophilic poly(oxyethylene) blocks and hydrophobic poly(oxypropylene) blocks to make up a triblock of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) (e.g. Pluronic®), polyetherester copolymer, such as a polyethylene glycol terephthalate/polybutylene terephthalate copolymer, sucrose acetate isobutyrate (SAIB), dextran or derivatives thereof, combinations of dextrans and PEG, polydimethylsiloxane, collagen, chitosan, polyvinyl alcohol (PVA) and derivatives, polyalkylimides, poly (acrylamide-co-diallyldimethyl ammonium (DADMA)), polyvinylpyrrolidone (PVP), glycosaminoglycans (GAGs) such as dermatan sulfate, chondroitin sulfate, keratan sulfate, heparin, heparan sulfate, hyaluronan, ABA triblock or AB block copolymers composed of hydrophobic A-blocks, such as polylactide (PLA) or poly(lactide-co-glycolide) (PLGA), and hydrophilic B-blocks, such as polyethylene glycol (PEG) or polyvinyl pyrrolidone; such block copolymers as well as the abovementioned poloxamers may exhibit reverse thermal gelation behavior (fluid state at room temperature to facilitate administration and gel state above sol-gel transition temperature at body temperature after injection);
- (viii) Spreading or diffusing agent: modifies the permeability of connective tissue through the hydrolysis of components of the extracellular matrix in the interstitial space such as but not limited to hyaluronic acid, a polysaccharide found in the intercellular space of connective tissue; a spreading agent such as but not limited to hyaluronidase temporarily

decreases the viscosity of the extracellular matrix and promotes diffusion of injected drugs;
and

- (ix) Other auxiliary agents: such as wetting agents, viscosity modifiers, antibiotics, hyaluronidase; acids and bases such as hydrochloric acid and sodium hydroxide are
5 auxiliary agents necessary for pH adjustment during manufacture.

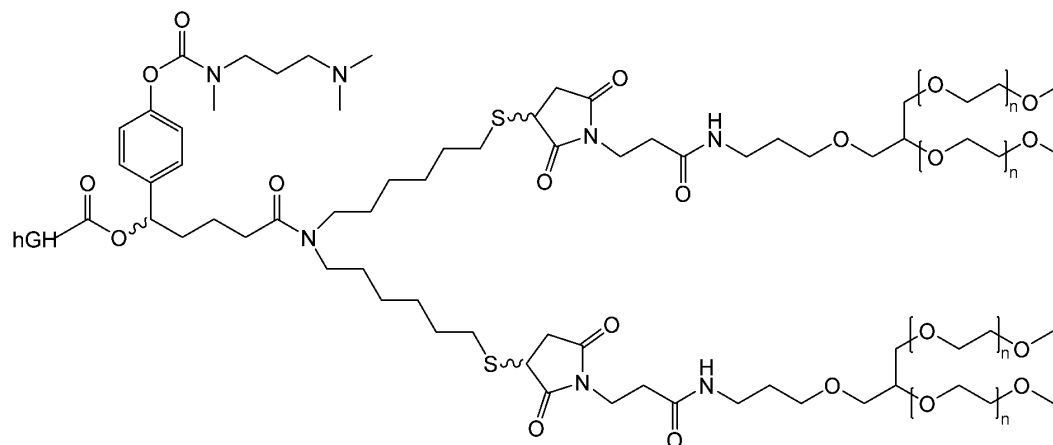
In another aspect the present invention relates to a long-acting growth hormone for use in the treatment of an inflammation-induced disease, wherein administration of the long-acting growth hormone inhibits recruitment of inflammatory monocytes to the site of inflammation. In certain
10 embodiments the inflammation-induced disease is NAFLD, such as NASH. The site of inflammation is the liver, if the inflammation-induced disease is NAFLD, in particular NASH. In certain embodiments administration of the long-acting growth hormone leads to an increase in HLA-G in the liver, if the inflammation-induced disease is NAFLD, in particular NASH. In certain embodiments the long-acting growth hormone for use in the treatment of an inflammation-induced disease results
15 in an increase in IGF-1 levels.

In certain embodiments the long-acting growth hormone for use in the treatment of an inflammation-induced disease, such as NAFLD and in particular NASH, leads to a change in one or more markers of hepatic inflammation selected from the group consisting of cytokines, chemokines and other
20 transcriptional and histological markers. In certain embodiments the long-acting growth hormone for use in the treatment of an inflammation-induced disease, such as NAFLD and in particular NASH, leads to an improvement of transcriptional or histological markers of fibrosis.

In certain embodiments the long-acting growth hormone for use in the treatment of an inflammation-induced disease, such as NAFLD and in particular of NASH, leads to a reduction in steatosis. In certain embodiments the long-acting growth hormone for use in the treatment of an inflammation-induced disease of the liver, such as NAFLD and in particular of NASH, leads to a regeneration of the liver.

30 The long-acting growth hormone and, administration frequencies are as described elsewhere herein.

Examples

Example 1**Synthesis of compound 1**

5 n = 200 - 250

Compound 1 may be synthesized as described in WO2016/079114A1 for compound 2 (example 2) and corresponds to lonapegsomatropin.

10 **Example 2**

Effect of 2 weeks of treatment with compound 1 on metabolic parameters, hepatic pathology, and transcriptomic profile in male DIO-NASH mice

The plasma pharmacokinetics profile was determined in a pre-study after a single injection (14.4 mg/kg) of compound 1 to C57BL/6JRj mice and blood samples were collected over 96 hours. The plasma level of compound 1 was determined using a sandwich ELISA. All concentrations of compound 1 are in protein (hGH) equivalents.

C57BL/6JRj mice (Janvier (France, 5 weeks old)), were fed a non-alcoholic steatohepatitis (NASH) inducing diet consisting with 40% fat, 22% fructose and 2% cholesterol (D09100310, SSNIF, Germany) for a total of 32 weeks.

A liver biopsy was surgically collected from anesthetized animals and fixed in 10% neutral buffered formalin for histology. Based on the evaluation of the picrosirius red and hematoxylin and eosin (HE)

stained liver tissue, animal livers that had a steatosis score of 3, inflammation score of at least 2 and fibrosis stage of at least 1 according to the Kleiner based scoring system (Kleiner, 2005 Hepatology. 2005 Jun;41(6):1313-21. doi: 10.1002/hep.20701. PMID: 15915461) were selected for the study. Animals (n=12 per group) were treated by subcutaneous injection every 48 hours with either vehicle
5 or compound 1 dosed at 14.4 mg/kg or 28.8 mg/kg for 14 days. Blood samples were collected at 24 and 48 hours after first dose and again at day 14, 24 hours after last dose and derived heparinized plasma supernatants were transferred to new tubes and immediately frozen on dry ice and stored at -70°C.

10 After treatment for 14 days the liver was collected from the anesthetized animals before euthanasia. Liver samples were collected for histology assessments, RNA Sequencing (RNAseq), triglycerides, and total cholesterol analyses.

Paraffin embedded sections were de-paraffinated in xylene and rehydrated in series of graded ethanol
15 before HE stain (HE; Dako), CD68 (clone ED1, Abcam, Cat. Ab31630) and CD11b (AbCam, Cat. 133357). IHC was performed using standard procedures. IHC-positive staining was quantified by image analysis using the VIS software (Visiopharm, Denmark). The number of hepatocytes with lipid droplets and inflammatory foci were determined by deep learning apps developed by Gubra using the VIS software.

20 Plasma alanine transaminase (ALT) and aspartate transaminase (AST), and liver homogenate triglycerides (TG) and total cholesterol (TC) was measured using commercial kits (Roche Diagnostics) on the cobas c 501 autoanalyzer.

25 RNA was isolated using the NucleoSpin® kit (MACHEREY-NAGEL) using a total of 10 ng-1 µg purified RNA from each bulk liver sample. Aa cDNA library was generated using the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina (New England Biolabs) and then sequenced on a NextSeq 500 using NextSeq 500/550 High Output Kit V2 (Illumina).

30 The sequencing data was aligned to the mouse genome (Ensembl database) using the Spliced Transcripts Alignment to a Reference (STAR) software. For the bioinformatic analysis, the quality of the data was evaluated using the standard RNA-sequencing quality control parameters, the inter-

and intra-group variability was evaluated using principal component analysis and hierarchical clustering and the differentially expressed genes were identified using the R-package DESeq2.

Results

- 5 Pharmacokinetic evaluation of the pre-study in normal C57BL/6JRj mice resulted in maximum mean plasma concentrations of 80 µg/mL at 24 hours. Exposure of compound 1 was detected up to 96 hours and the DIO-NASH mice were dosed accordingly every 48 hours in the subsequent study.

- 10 Treatment of diet induced (DIO)-NASH mice with compound 1 (14.4 mg/kg and 28.8 mg/kg) led to a significant decrease (73 and 83 %, respectively) in plasma markers of liver damage ALT and AST compared to vehicle. Liver triglycerides were decreased by both doses of compound 1 by 56-57 % compared to vehicle. Liver total cholesterol was decreased 30 % by the high dose treatment. The decrease in steatosis was supported by histological assessment and both the lipid fractional area as well as the number of hepatocytes containing lipid droplets were decreased by both low and high dose
15 compound 1 treatment by 50-61% compared to vehicle. Table 1 summarizes these results.

	Vehicle	Compound 1 14.4 mg/kg	Compound 1 28.8 mg/kg
Plasma ALT U/L	263.0 ± 15.4	67.4 ± 6.9***	44.6 ± 4.2***
Plasma AST U/L	267 ± 17.4	102.0 ± 8.9***	73.1 ± 14.3***
Liver TG (mg/g)	119 ± 4.8	52.6 ± 4.2***	51.5 ± 12.2***
Liver TC (mg/g)	22.3 ± 1.6	19.7 ± 1.4	15.7 ± 4.5**
Liver lipids (% fractional area)	25.4 ± 0.9	11.7 ± 0.7***	10.0 ± 1.2***
Hepatocytes with lipid droplets (%)	85.2 ± 2.1	43.0 ± 3.6***	37.3 ± 8.3***

- 20 *Table 1: Plasma alanine transaminase (ALT) and aspartate transaminase, liver triglycerides (TG), liver total cholesterol (TC), liver lipids and percentage of hepatocytes with lipid droplets (quantified on HE stained liver sections). Values expressed as mean of n = 12 + SEM. Dunnett's test one-factor linear model. **: P < 0.01, ***: P < 0.001 compared to Vehicle.*

The inflammatory state of the liver was examined via histological analysis and gene expression analysis. The immunohistochemistry staining for the inflammation marker cluster of differentiation

11b (CD11b) (expressed by monocytes and macrophages) showed a trend towards a decrease (40 % reduced, $p=0.051$) in CD11b after treatment with compound 1 (28.8 mg/kg). In support, transcription of ITGAM encoding CD11b was significantly downregulated (47 %) by compound 1 (28.8 mg/kg). These results are summarized in Table 2.

5

	Vehicle	Compound 1 14.4 mg/kg	Compound 1 28.8 mg/kg
Itgam (CD11b)	0.49 ± 0.07	0.39 ± 0.05	$0.26 \pm 0.08^*$
CD14	8.0 ± 0.4	7.06 ± 0.42	$5.58 \pm 0.33^{***}$
Galectin-3	28.9 ± 1.56	22.6 ± 2.55	$15.9 \pm 1.17^{***}$
CCR1	0.54 ± 0.077	$0.31 \pm 0.057^*$	$0.3 \pm 0.039^*$
MCP-1 (Ccl2)	4.33 ± 0.28	$2.53 \pm 0.26^{***}$	$2.18 \pm 0.15^{***}$
CCR2	2.07 ± 0.22	1.51 ± 0.12	$1.39 \pm 0.17^*$
CCR7	1.16 ± 0.15	$0.68 \pm 0.054^*$	$0.47 \pm 0.084^{***}$
CD11c	3.93 ± 0.41	3.31 ± 0.23	$2.46 \pm 0.20^{***}$

Table 2: Gene expression of inflammatory markers in the liver. Values expressed as mean of RPKM, $n = 8-9 + SEM$. False discovery rate adjusted p -value (DEseq2 analysis). *: $P < 0.05$, ***: $P < 0.001$, compared to Vehicle

- 10 Likewise, expression of inflammatory markers cluster of differentiation 14 (CD14), Monocyte chemoattractant protein-1 (MCP-1), C-C Motif Chemokine Receptor 1 (CCR1) and C-C Motif Chemokine Receptor 2 (CCR2) associated with monocyte recruitment and macrophage marker Galectin-3 were downregulated by compound 1 (28.8 mg/kg) treatment.
- 15 Finally, downregulated expression of MCP-1, C-X-C Motif Chemokine Ligand 1 (CXCL1), CCR2, C-C Motif Chemokine Receptor 7 (CCR7), cluster of differentiation 11c (CD11c) all correlated to M1 macrophage polarization, was observed (table 2).

- 20 Expression of the gene encoding alpha-smooth muscle actin (α -SMA), a key marker for hepatic stellate cell activation was significantly reduced after both low and high dose treatment. There was furthermore a significant reduction in the gene expression of the fibrotic marker, tissue inhibitor of metalloproteinases 1 (TIMP-1). Results are shown in Table 3.

	Vehicle	Compound 1 14.4 mg/kg	Compound 1 28.8 mg/kg
α -SMA	2.8 ± 0.2	$1.7 \pm 0.1^{**}$	$1.5 \pm 0.3^{***}$
TIMP-1	7.7 ± 0.8	$3.6 \pm 0.5^{***}$	$2.6 \pm 0.4^{***}$

Table 3: Gene expression of markers related to liver fibrosis. Values expressed as mean of RPMK $n = 8-9 + SEM$. False discovery rate adjusted p -value (DEseq2 analysis). **: $P < 0.01$, ***: $P < 0.001$ compared to Vehicle

5 Conclusion

Treatment with compound 1 for 14 days led to a marked improvement of liver steatosis and reduced plasma level of liver enzymes ALT/AST. Moreover, an improvement in liver inflammation as evidenced by reduction in recruitment and accumulation of macrophages and monocytes, downregulation of selected genes related to M1 macrophage polarization and reduced gene expression of key markers of fibrosis was found in a mouse model of NASH.

Abbreviations

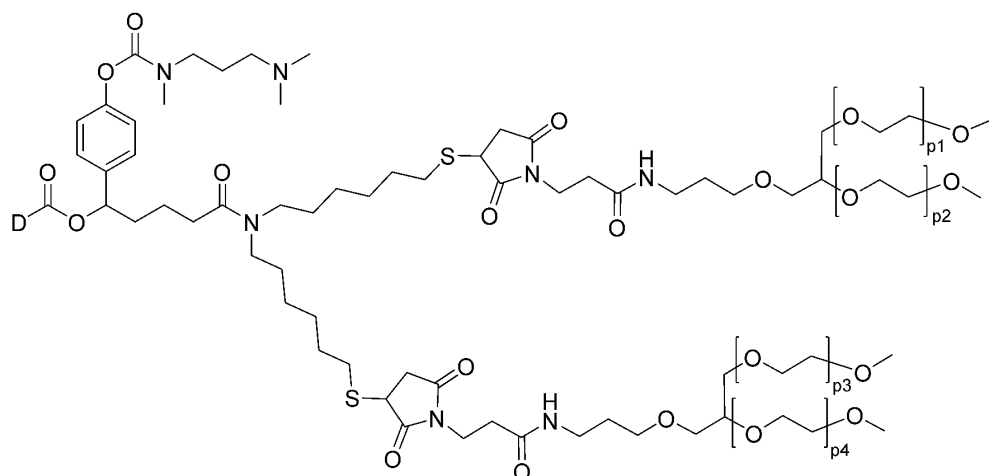
ALT	alanine transaminase
15 AST	aspartate transaminase
CCL2 C-C	Motif Chemokine Ligand 2
CCR1 C-C	Motif Chemokine Receptor 1
CCR2 C-C	Motif Chemokine Receptor 2
CCR7 C-C	Motif Chemokine Receptor 7
20 CD11b	cluster of differentiation 11b
CD14	cluster of differentiation 14
CD68	cluster of differentiation 68
CXCL1	C-X-C Motif Chemokine Ligand 1
DIO-NASH	diet induced non-alcoholic steatohepatitis
25 GH	growth hormone
HE	hematoxylin and eosin
NASH	non-alcoholic steatohepatitis
NBF	neutral buffered formalin
MCP-1	Monocyte chemoattractant protein-1
30 PSR	picrosirius red
RNAseq	RNA Sequencing

TC	total cholesterol
TG	triglycerides
TIMP-1	tissue inhibitor of metalloproteinases 1

Claims

1. A long-acting growth hormone (GH) for use in the treatment of an inflammation-induced disease.
2. The long-acting GH for use of claim 1, wherein the inflammation-induced disease is NAFLD.
3. The long-acting GH for use of claim 1 or 2, wherein the inflammation-induced disease is NASH.
4. The long-acting GH for use of any one of claims 1 to 3, wherein the long-acting GH comprises a growth hormone moiety covalently conjugated to one or more chemical moiety.
5. The long-acting GH for use of claim 4, wherein the chemical moiety is a polymeric moiety.
6. The long-acting GH for use of claim 4 or 5, wherein the chemical moiety is a PEG-based moiety.
7. The long-acting GH for use of any one of claims 4 to 6, wherein the bond between the growth hormone moiety and the chemical moiety is a stable covalent bond.
8. The long-acting GH for use of any one of claims 4 to 6, wherein the bond between the growth hormone moiety and the chemical moiety is a reversible covalent bond.
9. The long-acting GH for use of any one of claims 1 to 3, wherein the long-acting GH comprises growth hormone is non-covalently embedded or encapsulated in a polymer or lipid-comprising matrix.
10. The long-acting GH for use of any one of claims 1 to 3, wherein the long-acting growth hormone comprises a growth hormone moiety fused to at least one natural or unnatural amino acid sequence.
11. The long-acting GH for use of any one of claims 1 to 10, wherein administration of the long-acting GH triggers the re-balancing of macrophage phenotypes between M1 and M2.

12. The long-acting GH for use of any one of claims 1 to 11, wherein administration of the long-acting growth hormone leads to a change in one or more markers of hepatic inflammation selected from the group consisting of cytokines, chemokines and other transcriptional and histological markers.
13. The long-acting GH for use of any one of claims 1 to 12, wherein the long-acting GH inhibits recruitment of inflammatory monocytes to the site of inflammation.
14. The long-acting GH for use of any one of claims 1 to 13, wherein administration of the long-acting growth hormone leads to a reduction in steatosis.
15. The long-acting GH for use of any one of claims 1 to 14, wherein the long-acting GH is administered to the patient once a week.
16. The long-acting GH for use of any one of claims 1 to 6, 8 or 11 to 15, wherein the long-acting GH is of formula (C-ii)



(C-ii),

wherein

-D is a hGH moiety connected to the rest of the molecule through the nitrogen of an amine functional group of -D; and

p1, p2, p3 and p4 are independently an integer ranging from 200 to 250.

17. The long-acting GH for use of claim 16, wherein p1, p2, p3 and p4 of formula (C-ii) are independently an integer ranging from 210 to 240.
18. The long-acting GH for use of claim 16 or 17, wherein p1, p2, p3 and p4 of formula (C-ii) are independently an integer ranging from 220 to 240.
19. The long-acting GH for use of any one of claims 16 to 18, wherein -D is a hGH moiety of SEQ ID NO:1.
20. The long-acting GH for use of any one of claims 16 to 19, wherein -D is connected to the rest of the molecule through a nitrogen of an amine functional group provided by a lysine side chain of -D.
21. The long-acting GH for use of any one of claims 1 to 4, 7 or 11 to 15, wherein the long-acting GH is somapacitan.
22. The long-acting GH for use of any one of claims 1 to 21, wherein the treatment comprises the steps of
- (a) administering at least a first dose of the long-acting GH to a patient having an inflammation-induced disease;
 - (b) measuring Insulin-like Growth Factor-1 (IGF-1) levels; and
 - (c) reducing the dose of the long-acting GH by at least 5% if IGF-1 levels are above a standard deviation score of +3 and increasing the dose of the long-acting GH by at least 5% if IGF-1 levels are below a standard deviation score of 0.
23. The long-acting GH for use of any one of claims 1 to 21, wherein the treatment comprises the steps of
- (a) administering at least a first dose of the long-acting GH to a patient having an inflammation-induced disease;
 - (b) measuring biomarkers indicative for M1 and M2 macrophages;
 - (c) adjusting the dose of the long-acting GH based on the macrophage phenotype change by M1 reduction or M2 induction indicated by said biomarkers.

24. The long-acting GH for use of claim 23, wherein the biomarkers indicative of M2 macrophages are selected from the group consisting of IL-2, IL-4, IL-10, IL-13, CCL17, CCL18, CCL22, CCL24, CCL13, CCL16, CXCR1, CXCR2, CD14, CD23, CD36, CD163, mannose receptor (CD206), scavenger receptor A, Chi3l3/Ym1, Retnla/Fizz-1 and arginase-1.
25. The long-acting GH for use of claim 23 or 24, wherein dose adjustments in step (c) are accompanied by measuring IGF-1 levels and adjustments of the dose of the long-acting GH are such that IGF-1 levels are in a range from 0 to +3 standard deviation scores.
26. The long-acting GH for use of any one of claims 23 to 25, wherein steps (b) and (c) are repeated until macrophage rebalancing is achieved.