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(54) Benævnelse: **Nye polymere høje prodrugs**

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WO-A2-2006/102659
WO-A2-2009/133137

Fortsættes ...

DESCRIPTION

[0001] The present invention relates to a polymeric human growth hormone prodrug. It furthermore relates to them for use as medicaments for the treatment of diseases which can be treated with growth hormone. It also relates to methods of application of such polymeric human growth hormone prodrug or pharmaceutical formulation.

[0002] Human growth hormone (hGH) is a hormone that stimulates growth and cell reproduction in humans and other animals. It is a 191-amino acid, single chain polypeptide hormone which is synthesized, stored, and secreted by the somatotroph cells within the lateral wings of the anterior pituitary gland.

[0003] Growth hormone has a variety of functions in the body, the most noticeable of which is the increase of height throughout childhood, and there are several diseases which can be treated through the therapeutic use of hGH, such as for example pediatric and adult growth hormone deficiency (GHD), idiopathic short stature (ISS), short stature homeobox (SHOX) gene mutations, Turner syndrome (TS), Noonan syndrome (NS), Prader-Willi syndrome (PWS), children born small for gestational age (SGA), chronic renal insufficiency (CRI), wasting due to HIV or AIDS or other malignancies, short bowel syndrome (SBS), sarcopenia, and frailty. Standard treatment of hGH-related diseases is via frequent, usually daily, subcutaneous injections. This is especially inconvenient for the predominantly pediatric patient population. Therefore, various approaches to provide sustained release depots requiring less frequent hGH administrations are under development, such as those described in WO2009/133137 A2.

[0004] It is also desirable to keep the injection volume low to ensure administration of the drug in a manner convenient for the patient. Injection site pain increases significantly when the injection volume is increased from 0.5 to 1.0 mL and injection volumes exceeding 1.0 mL should be avoided. As the majority of patients requiring hGH therapy are children, injection volumes should be maintained at a minimum to ensure proper compliance facilitating desired treatment outcome. The amount of hGH per given volume, however, is restricted and is lowered if certain excipients, covalently and non-covalently bound carriers, such as polymers, are used. In such cases either the administered volume per injection has to increase or more than one injection is needed. If this is not an option, certain diseases requiring higher doses of hGH, such as ISS, Turner Syndrome, Noonan Syndrome, Chronic Kidney Disease, Prader-Willi-Syndrome and pubertal GHD patients, cannot be treated with a given pharmaceutical formulation. Furthermore, pediatric patients requiring growth hormone therapy grow and gain weight and consequently require increasing amounts of hGH to ensure exposure to constant relative hGH concentrations.

[0005] It is therefore desirable to provide sustained release formulations of hGH that can be administered with a high concentration and injection volumes below 1.0 mL across different indications requiring hGH therapy.

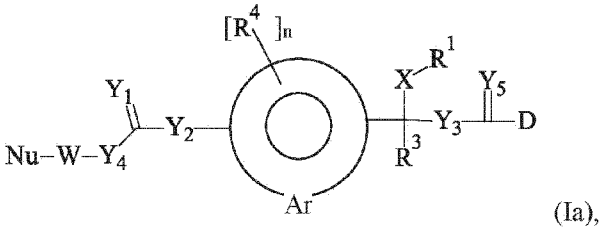
[0006] The viscosity of a pharmaceutical formulation furthermore determines the ability to inject the pharmaceutical formulation through fine gauge needles. With increasing viscosity larger diameter needles are required to ensure that the pharmaceutical formulation can be injected within an acceptable timeframe.

[0007] As the size of the needle required for injection of said hGH formulation influences patient acceptance, it is desirable to provide sustained release formulations of hGH with a viscosity that facilitates administration with a small needle diameter and an acceptable injection time.

[0008] If a pharmaceutical formulation comprising hGH is stored in its dry form, it is desirable that the reconstitution proceeds fast and with as little foam/bubble formation as possible in order to minimize the efforts prior to administration and to ensure proper dosing of the drug.

[0009] It is therefore an object of the present invention to at least partially overcome the above-described shortcomings.

[0010] This object is achieved with a polymeric human growth hormone (hGH) prodrug or a pharmaceutically acceptable salt thereof of formula (Ia):



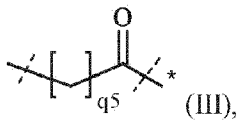
(Ia),

wherein

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

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

-X-
: is of formula (III)



(III),

wherein

the dashed line marked with the asterisk indicates attachment to the R^1;

the unmarked dashed line indicates attachment to remainder of the prodrug;

q5 is 1, 2, 3, 4, 5, 6, 7 or 8;

$=Y_1$

is selected from the group consisting of =O and =S;

$-Y_2-$

is selected from the group consisting of -O- and -S-;

$-Y_3-$

is selected from the group consisting of -O- and -S-;

$-Y_4-$

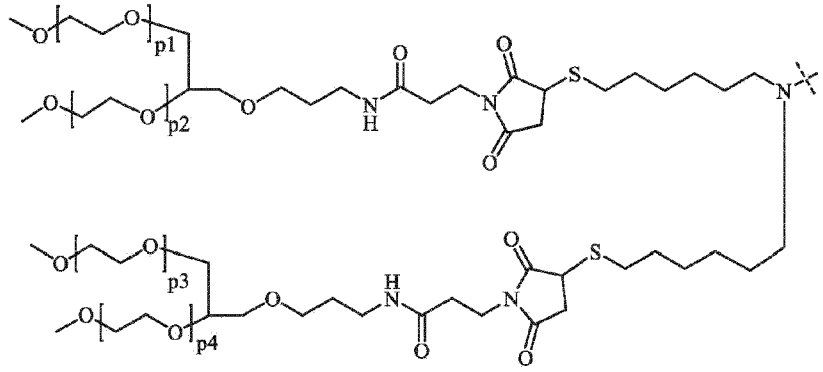
is selected from the group consisting of -O-, $-NR^5-$ and $-C(R^6R^{6a})-$;

$=Y_5$

is selected from the group consisting of =O and =S;

$-R^1$

is of formula (IIc):



(IIc),

wherein

p1, p2, p3, p4 are independently an integer ranging from 220 to 240;

$-R^3$, $-R^5$, $-R^6$, $-R^{6a}$

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;

$-R^4$

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;

-W-

is

wherein

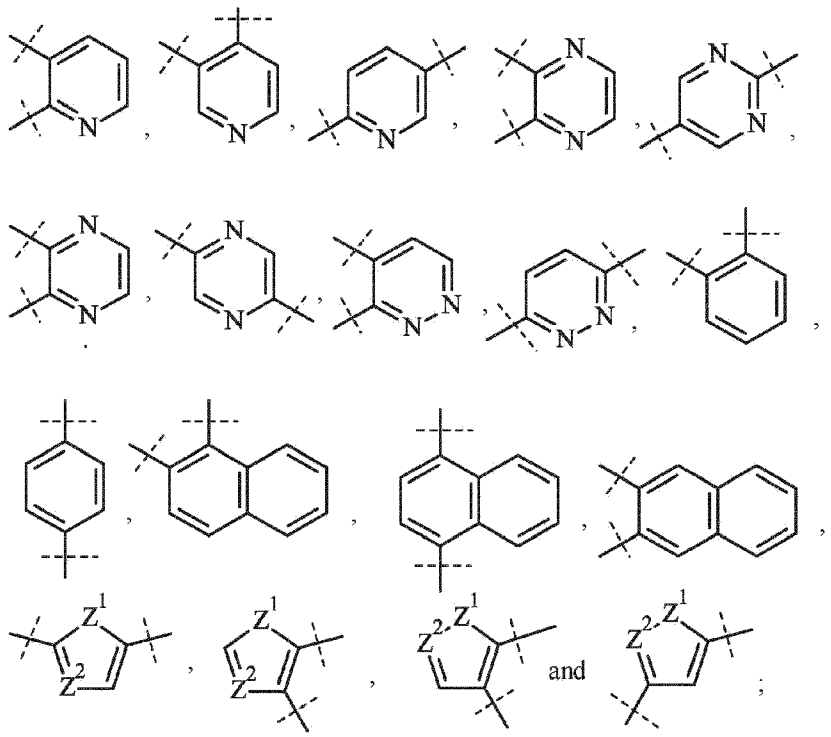
the dashed lines indicate attachment to the rest of the molecule;

-Nu

is $-N(R^7R^{7a})$;

-Ar-

is selected from the group consisting of



wherein

dashed lines indicate attachment to the rest of the prodrug,

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

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

$-R^7$, $-R^{7a}$

are independently of each other selected from the group consisting of -H, C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl.

[0011] It was now surprisingly found that the polymeric hGH prodrug of the present invention exhibits various unexpected properties.

[0012] It is expected that reducing the amount of PEG per hGH moiety increases the amount of hGH equivalents that can be solved in a pharmaceutical formulation with a given viscosity. However, compared to, for example, compound 36 of WO2009/133137 A2 the prodrugs of the present invention allow an increase in the relative hGH concentration that is more than proportional to the reduction of the PEG size. In other words, a pharmaceutical formulation comprising polymeric hGH prodrug with a given viscosity can comprise relatively more hGH if the polymeric hGH prodrug is of the present invention compared to, for example, compound 36 of WO2009/133137 A2.

[0013] This is advantageous, because in order to restrict the pain associated with injectable drugs limited volumes can be administered to a patient. Therefore, being able to administer more hGH per given injection volume opens up new patient populations, namely those patients suffering from diseases requiring higher hGH doses per injection and those patients suffering

from diseases that may require only moderate doses per weight unit, but where the patients are heavy and thus require more hGH equivalents.

[0014] It was also surprisingly found that the polymeric hGH prodrug of the present also has surprising advantages with regard to its manufacturing process. Purification of the polymeric hGH prodrug of the present invention can be done with a loading that is at least threefold higher than for compound 36 of WO2009/133137 A2, for example, without impairing the separation efficiency and product quality. This significantly reduces the number of purifications runs needed.

[0015] Furthermore, if the prodrug of the present invention is comprised in a dry pharmaceutical formulation, said dry pharmaceutical formulation can be reconstituted faster and with the formation of less foam compared to, for example, compound 36 of WO2009/133137 A2. Therefore, reconstituting a dry pharmaceutical formulation of the present invention saves time and ensures administration of the proper dosage.

[0016] Within the present invention the terms are used with the meaning as follows:
As used herein, the term "human growth hormone (hGH)" refers to growth hormone from human that is characterized by promoting growth in the growing phase and in maintaining normal body composition, anabolism, and lipid metabolism. Preferably, the term "hGH" refers to the hGH polypeptide of SEQ ID NO: 1 exhibiting essentially the same biological activity, i.e. promoting growth in the growing phase and in maintaining normal body composition, anabolism, and lipid metabolism. More preferably, the term "hGH" refers to the polypeptide of SEQ ID NO:1.

[0017] SEQ ID NO:1 has the following sequence:
FPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQTSLCFSESIPT
PSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEE
GIQTLMGRLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETF
LRIVQCRSVEGSCGF

[0018] It is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus of a bioactive peptide or protein without substantial loss of biological function.

[0019] It is also recognized by one of ordinary skill in the art that some amino acid sequences of hGH polypeptides can be varied without significant effect of the structure or function of the protein. Such mutants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al. (1990), Science 247:1306-1310, wherein the authors indicate that there are two main approaches for studying the tolerance of the amino acid sequence to change.

[0020] The hGH polypeptide 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. Preferably, the hGH polypeptide is a monomer.

[0021] As used herein, the term "hGH polypeptide fragment" refers to any peptide or polypeptide comprising a contiguous span of a part of the amino acid sequence of a hGH polypeptide, preferably the polypeptide of SEQ ID NO: 1.

[0022] More specifically, a hGH polypeptide 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 a hGH polypeptide, more preferably of the polypeptide of SEQ ID NO:1. A hGH polypeptide fragment may additionally be described as sub-genuses of hGH polypeptides comprising at least 6 amino acids, wherein "at least 6" is defined as any integer between 6 and the integer representing the C-terminal amino acid of a hGH polypeptide, preferably of the polypeptide of SEQ ID No: 1. Further included are species of hGH polypeptide fragments at least 6 amino acids in length, as described above, that are further specified in terms of their N-terminal and C-terminal positions. Also encompassed by the term "hGH polypeptide fragment" as individual species are all hGH polypeptide fragments, at least 6 amino acids in length, as described above, that may be particularly specified by a N-terminal and C-terminal position, i.e. every combination of a N-terminal and C-terminal position that a fragment at least 6 contiguous amino acid residues in length could occupy, on any given amino acid sequence of a hGH polypeptide, preferably the hGH polypeptide of SEQ ID NO: 1.

[0023] It is noted that the above species of polypeptide fragments may alternatively be described by the formula "a to b"; where "a" equals the N-terminal most amino acid position and "b" equals the C-terminal most amino acid position in the polynucleotide; and further where "a" equals an integer between 1 and the number of amino acids of a hGH polypeptide sequence minus 6, and where "b" equals an integer between 7 and the number of amino acids of the hGH polypeptide sequence; and where "a" is an integer smaller then "b" by at least 6, preferably of the hGH polypeptide sequence of SEQ ID NO: 1.

[0024] The term "drug" as used herein refers to a substance used in the treatment, cure, prevention, or diagnosis of a disease or used to otherwise enhance physical or mental well-being. If a drug is conjugated to another moiety, the part of the resulting product that originated from the drug is referred to as "biologically active moiety".

[0025] As used herein the term "prodrug" refers to a biologically active moiety reversibly and covalently connected to a specialized protective group through a reversible prodrug linker moiety comprising a reversible linkage with the biologically active moiety to alter or to eliminate undesirable properties in the parent molecule. This also includes the enhancement of desirable properties in the drug and the suppression of undesirable properties. The specialized non-toxic protective group is referred to as "carrier". A prodrug releases the reversibly and covalently

bound biologically active moiety in the form of its corresponding drug.

[0026] As used herein, the term "free form" of a drug means the drug in its unmodified, pharmacologically active form.

[0027] As used herein the term "liquid formulation" means a formulation comprising the polymeric hGH prodrug of the present invention and at least one solvent. A preferred solvent is water.

[0028] As used herein the term "dry formulation" means that the formulation comprising the polymeric hGH prodrug of the present invention is provided in dry form. Suitable methods for drying are spray-drying and lyophilization which is also referred to as freeze-drying. Such dry formulation comprising polymeric hGH prodrug has a residual water content of a maximum of 10 %, preferably less than 5% and more preferably less than 2% which residual water content is determined according to Karl Fischer. The preferred method of drying is lyophilization. "Lyophilized formulation" means that a formulation comprising the polymeric hGH prodrug of the present invention was first frozen and subsequently subjected to water reduction by means of reduced pressure. This terminology does not exclude additional drying steps which may occur in the manufacturing process prior to filling the formulation into the final container.

[0029] As used herein the term "reconstituted formulation" means the result of adding a solvent which is also referred to as "reconstitution solution" to a dry formulation. Preferably, the amount of solvent is such that the dry formulation is completely dissolved in the resulting reconstituted formulation.

[0030] As used herein, the term "excipient" refers to a diluent, adjuvant, or vehicle with which the therapeutic is administered.

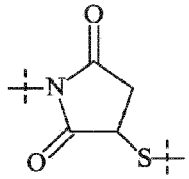
[0031] The term "water soluble" as in a "water-soluble moiety" is a moiety that is soluble in water at room temperature. Typically, a solution of a water-soluble moiety will transmit at least about 75%, more preferably at least about 95% of light, transmitted by the same solution after filtering. On a weight basis, a water-soluble moiety or parts thereof will preferably be at least about 35% (by weight) soluble in water, more preferably at least about 50% (by weight) soluble in water, still more preferably about 70% (by weight) soluble in water, and still more preferably about 85% (by weight) soluble in water. It is most preferred, however, that the water-soluble moiety or parts thereof is about 95% (by weight) soluble in water or completely soluble in water.

[0032] 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 covalent chemical crosslinks. The crosslinks provide the network structure and physical integrity. Hydrogels exhibit a thermodynamic compatibility with water which allows them to swell in aqueous media.

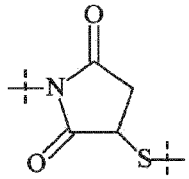
[0033] As used herein, the term "functional group" means a group of atoms which can react with other functional groups. Functional groups include but are not limited to the following groups: carboxylic acid (-(C=O)OH), primary or secondary amine (-NH_2, -NH-), maleimide, thiol (-SH), sulfonic acid (-(O=S=O)OH), carbonate, carbamate (-O(C=O)N<), hydroxy (-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, vinyl sulfone, vinyl ketone, diazoalkane, oxirane, and aziridine.

[0034] As used herein, the term "moiety" means a part of a molecule, which lacks at least one atom 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 biologically active moiety is released from a prodrug as a drug.

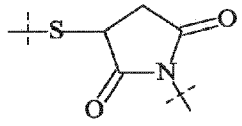
[0035] It is understood that if the sequence or chemical structure of a group of atoms is provided which group of atoms is attached to two moieties or is interrupting a moiety, said sequence or chemical structure can be attached to the two moieties in either orientation, unless explicitly stated otherwise. For example, a moiety "-C(O)N(R)-" can be attached to two moieties or interrupting a moiety either as "-C(O)N(R)-" or as "-N(R)C(O)-". Similarly, a moiety



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



or as



[0036] In case the compounds according to formula (Ia) comprise one or more acidic or basic groups, the invention also comprises their corresponding pharmaceutically or toxicologically acceptable salts, in particular their pharmaceutically utilizable salts. Thus, the compounds of formula (Ia) which comprise acidic groups can 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. Compounds of the formula (Ia) which comprise 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 the compounds of the formula (Ia) simultaneously comprise acidic and basic groups in the molecule, the invention also includes, in addition to the salt forms mentioned, inner salts or betaines (zwitterions). The respective salts according to the formula (Ia) can be obtained by customary methods which are known to the person skilled in the art like, for example by contacting these 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 compounds of the formula (Ia) 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.

[0037] The term "pharmaceutically acceptable" 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, preferably in humans.

[0038] 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 group(s) and/or moiety/moieties, such as, for example, one or more functional group(s). Preferably, 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 preferable 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 crosslinked hydrogels, no meaningful molecular weight ranges can be provided.

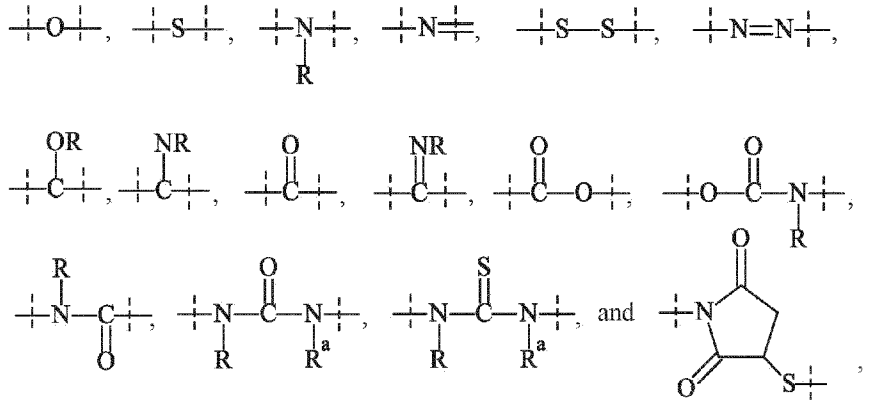
[0039] As used herein, the term "polymeric" means a reagent or a moiety comprising one or more polymer(s).

[0040] 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. As used herein, the term "number average molecular weight" means the ordinary arithmetic means of the molecular weights of the individual polymers.

[0041] As used herein, the term "PEG-based comprising at least X% PEG" in relation to a moiety or reagent means that said moiety or reagent comprises at least X% (w/w) ethylene glycol units (-CH_2CH_2O-), wherein the ethylene glycol units may be arranged blockwise, alternating or may be randomly distributed within the moiety or reagent and preferably 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 preferably selected from the following moieties and linkages:

- 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



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, propyl, butyl, pentyl and hexyl.

[0042] The term "substituted" as used herein means that one or more -H atom(s) of a molecule or moiety are replaced by a different atom or a group of atoms, which are referred to as "substituent" .

[0043] Preferably, the one or more further optional substituents are independently of each other selected from the group consisting of halogen, -CN, -$COOR^{x1}$, -OR^{x1}, -$C(O)R^{x1}$, -$C(O)N(R^{x1}R^{x1a})$, -$S(O)_2N(R^{x1}R^{x1a})$, -$S(O)N(R^{x1}R^{x1a})$, -$S(O)_2R^{x1}$, -$S(O)R^{x1}$, -$N(R^{x1})S(O)_2N(R^{x1a}R^{x1b})$, -$SR^{x1}$, -$N(R^{x1}R^{x1a})$, -$NO_2$, -$OC(O)R^{x1}$, -$N(R^{x1})C(O)R^{x1a}$, -

$N(R^{x1})S(O)_2R^{x1a}$, $-N(R^{x1})S(O)R^{x1a}$, $-N(R^{x1})C(O)OR^{x1a}$, $-N(R^{x1})C(O)N(R^{x1a}R^{x1b})$, $-OC(O)N(R^{x1}R^{x1a})$, $-T^0$, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl; wherein $-T^0$, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more R^{x2}, 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 $-T^0-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{x3})-$, $-S(O)_2N(R^{x3})-$, $-S(O)N(R^{x3})-$, $-S(O)_2-$, $-S(O)-$, $-N(R^{x3})S(O)_2N(R^{x3a})-$, $-S-$, $-N(R^{x3})-$, $-OC(OR^{x3})(R^{x3a})-$, $-N(R^{x3})C(O)N(R^{x3a})-$, and $-OC(O)N(R^{x3})-$;

R^{x1}, R^{x1a}, R^{x1b} are independently of each other selected from the group consisting of -H, $-T^0$, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl; wherein $-T^0$, C_{1-50} alkyl, C_{2-50} alkenyl, and C_{2-50} alkynyl are optionally substituted with one or more R^{x2}, 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 $-T^0-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{x3})-$, $-S(O)_2N(R^{x3})-$, $-S(O)N(R^{x3})-$; $-S(O)_2-$, $-S(O)-$, $-N(R^{x3})S(O)_2N(R^{x3a})-$, $-S-$, $-N(R^{x3})-$, $-OC(OR^{x3})(R^{x3a})-$, $-N(R^{x3})C(O)N(R^{x3a})-$, and $-OC(O)N(R^{x3})-$;

each T^0 is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, and 8- to 11-membered heterobicyclyl; wherein each T^0 is independently optionally substituted with one or more R^{x2}, which are the same or different;

each R^{x2} is independently selected from the group consisting of halogen, -CN, oxo (=O), $-COOR^{x4}$, $-OR^{x4}$, $-C(O)R^{x4}$, $-C(O)N(R^{x4}R^{x4a})$, $-S(O)_2N(R^{x4}R^{x4a})$, $-S(O)N(R^{x4}R^{x4a})$, $-S(O)_2R^{x4}$, $-S(O)R^{x4}$, $-N(R^{x4})S(O)_2N(R^{x4a}R^{x4b})$, $-SR^{x4}$, $-N(R^{x4}R^{x4a})$, $-NO_2$, $-OC(O)R^{x4}$, $-N(R^{x4})C(O)R^{x4a}$, $-N(R^{x4})S(O)_2R^{x4a}$, $-N(R^{x4})S(O)R^{x4a}$, $-N(R^{x4})C(O)OR^{x4a}$, $-N(R^{x4})C(O)N(R^{x4a}R^{x4b})$, $-OC(O)N(R^{x4}R^{x4a})$, and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different;

each R^{x3}, R^{x3a}, R^{x4}, R^{x4a}, R^{x4b} 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.

[0044] More preferably, the one or more further optional substituents are independently of each other selected from the group consisting of halogen, -CN, $-COOR^{x1}$, $-OR^{x1}$, $-C(O)R^{x1}$, $-C(O)N(R^{x1}R^{xla})$, $-S(O)2N(R^{x1}R^{x1a})$, $-S(O)N(R^{x1}R^{x1a})$, $-S(O)_2R^{x1}$, $-S(O)R^{x1}$, $-N(R^{x1})S(O)_2N(R^{x1a}R^{x1b})$, $-SR^{x1}$, $-N(R^{x1}R^{x1a})$, _NOz, $-OC(O)R^{x1}$, $-N(R^{x1})C(O)R^{x1a}$, -

$N(R^{x1})S(O)_2R^{x1a}$, $-N(R^{x1})S(O)R^{x1a}$, $-N(R^{x1})C(O)OR^{x1a}$, $-N(R^{x1})C(O)N(R^{x1a}R^{x1b})$, $-OC(O)N(R^{x1}R^{x1a})$, $-T^0$, C_{1-10} alkyl, C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein $-T^0$, C_{1-10} alkyl, C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more R^{x2}, 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 $-T^0-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{x3})-$, $-S(O)_2N(R^{x3})-$, $-S(O)N(R^{x3})-$, $-S(O)_2-$, $-S(O)-$, $-N(R^{x3})S(O)_2N(R^{x3a})-$, $-S-$, $-N(R^{x3})-$, $-OC(OR^{x3})(R^{x3a})-$, $-N(R^{x3})C(O)N(R^{x3a})-$, and $-OC(O)N(R^{x3})-$;

each R^{x1}, R^{x1a}, R^{x1b}, R^{x3}, R^{x3a} is independently selected from the group consisting of -H, halogen, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl;

each T^0 is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, and 8- to 11-membered heterobicyclyl; wherein each T^0 is independently optionally substituted with one or more R^{x2}, which are the same or different;

each R^{x2} is independently selected from the group consisting of halogen, -CN, oxo (=O), $-COOR^{x4}$, $-OR^{x4}$, $-C(O)R^{x4}$, $-C(O)N(R^{x4}R^{x4a})$, $-S(O)2N(R^{x4}R^{x4a})$, $-S(O)N(R^{x4}R^{x4a})$, $-S(O)_2R^{x4}$, $-S(O)R^{x4}$, $-N(R^{x4})S(O)_2N(R^{x4a}R^{x4b})$, $-SR^{x4}$, $-N(R^{x4}R^{x4a})$, -NOz, $-OC(O)R^{x4}$, $-N(R^{x4})C(O)R^{x4a}$, $-N(R^{x4})S(O)_2R^{x4a}$, $-N(R^{x4})S(O)R^{x4a}$, $-N(R^{x4})C(O)OR^{x4a}$, $-N(R^{x4})C(O)N(R^{x4a}R^{x4b})$, $-OC(O)N(R^{x4}R^{x4a})$, and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different;

each R^{x4}, R^{x4a}, R^{x4b} is independently selected from the group consisting of -H, halogen, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl;

Even more preferably, the one or more further optional substituents are independently of each other selected from the group consisting of halogen, -CN, $-COOR^{x1}$, $-OR^{x1}$, $-C(O)R^{x1}$, $-C(O)N(R^{x1}R^{xla})$, $-S(O)_2N(R^{x1}R^{x1a})$, $-S(O)N(R^{x1}R^{x1a})$, $-S(O)_2R^{x1}$, $-S(O)R^{x1}$, $-N(R^{x1})S(O)_2N(R^{x1a}R^{x1b})$, $-SR^{x1}$, $-N(R^{x1}R^{x1a})$, $-NO_2$, $-OC(O)R^{x1}$, $-N(R^{x1})C(O)R^{x1a}$, $-N(R^{x1})S(O)_2R^{x1a}$, $-N(R^{x1})S(O)R^{x1a}$, $-N(R^{x1})C(O)OR^{x1a}$, $-N(R^{x1})C(O)N(R^{x1a}R^{x1b})$, $-OC(O)N(R^{x1}R^{x1a})$, $-T^0$, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl; wherein $-T^0$, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl are optionally substituted with one or more R^{x2}, which are the same or different and wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl are optionally interrupted by one or more groups selected from the group consisting of $-T^0-$, $-C(O)O-$, $-O-$, $-C(O)-$, $-C(O)N(R^{x3})-$, $-S(O)_2N(R^{x3})-$, $-S(O)N(R^{x3})-$, $-S(O)_2-$, $-S(O)-$, $-N(R^{x3})S(O)_2N(R^{x3a})-$, $-S-$, $-N(R^{x3})-$, $-OC(OR^{x3})(R^{x3a})-$, $-N(R^{x3})C(O)N(R^{x3a})-$, and $-OC(O)N(R^{x3})-$;

each R^{x1}, R^{x1a}, R^{x1b}, R^{x2}, R^{x3}, R^{x3a} is independently selected from the group consisting of -H, halogen, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl;

each T^0 is independently selected from the group consisting of phenyl, naphthyl, indenyl, indanyl, tetralinyl, C_{3-10} cycloalkyl, 3- to 10-membered heterocyclyl, and 8- to 11-membered heterobicyclyl; wherein each T^0 is independently optionally substituted with one or more R^{x2}, which are the same or different.

[0045] Preferably, a maximum of 6 -H atoms of an optionally substituted molecule or moiety 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.

[0046] The term "spacer" as used herein refers preferably to a moiety selected from the group consisting of -T-, -C(O)O-, -O-, -C(O)-, $-C(O)N(R^{z1})-$, $-S(O)_2N(R^{z1})-$, $-S(O)N(R^{z1})-$, $-S(O)_2-$, -S(O)-, $-N(R^{z1})S(O)_2N(R^{z1a})-$, -S-, $-N(R^{z1})-$, $-OC(OR^{z1})(R^{z1a})-$, $-N(R^{z1})C(O)N(R^{z1a})-$, $-OC(O)N(R^{z1})-$, 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^{z2}, 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)-, $-C(O)N(R^{z3})-$, $-S(O)_2N(R^{z3})-$, $-S(O)N(R^{z3})-$, $-S(O)_2-$, -S(O)-, $-N(R^{z3})S(O)_2N(R^{z3a})-$, -S-, $-N(R^{z3})-$, $-OC(OR^{z3})(R^{z3a})-$, $-N(R^{z3})C(O)N(R^{z3a})-$, and $-OC(O)N(R^{z3})-$;

R^{z1} and R^{z1a} are independently of each other selected from the group consisting of -H, -T, 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^{z2}, 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)-, $-C(O)N(R^{z4})-$, $-S(O)_2N(R^{z4})-$, $-S(O)N(R^{z4})-$, $-S(O)_2-$, -S(O)-, $-N(R^{z4})S(O)_2N(R^{z4a})-$, -S-, $-N(R^{z4})-$, $-OC(OR^{z4})(R^{z4a})-$, $-N(R^{z4})C(O)N(R^{z4a})-$, and $-OC(O)N(R^{z4})-$;

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^{z2}, which are the same or different;

each R^{z2} is independently selected from the group consisting of halogen, -CN, oxo (=O), -$COOR^{z5}$, -OR^{z5}, -$C(Q)R^{z5}$ -$C(O)N(R^{z5}R^{z5a})$, -$S(O)_2N(R^{z5}R^{z5a})$, -$S(O)N(R^{z5}R^{z5a})$, -$S(O)_2R^{z5}$, -$S(O)R^{z5}$, -$N(R^{z5})S(O)_2N(R^{z5a}R^{z5b})$, -$SR^{z5}$, -$N(R^{z5}R^{z5a})$, -$NO_2$, -$OC(O)R^{z5}$, -$N(R^{z5})C(O)R^{z5a}$, -$N(R^{z5})S(O)_2R^{z5a}$, -$N(R^{z5})S(O)R^{z5a}$, -$N(R^{z5})C(O)OR^{z5a}$, -$N(R^{z5})C(O)N(R^{z5a}R^{z5b})$, -$OC(O)N(R^{z5}R^{z5a})$, and C_{1-6} alkyl; wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different;

each R^{z3}, R^{z3a}, R^{z4}, R^{z4a}, R^{z5}, R^{z5a} and R^{z5b} 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.

[0047] More preferably, the term "spacer" refers to a moiety selected from the group consisting of -T-, -C(O)O-, -O-, -C(O)-, -$C(O)N(R^{z1})$-, -$S(O)_2N(R^{z1})$-, -$S(O)N(R^{z1})$-, -$S(O)_2$-, -S(O)-, -$N(R^{z1})S(O)2N(R^{z1a})$-, -S-, -$N(R^{z1})$-, -$OC(OR^{z1})(R^{z1a})$-, -$N(R^{z1})C(O)N(R^{z1a})$-, -$OC(O)N(R^{z1})$-, 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^{z2}, 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)-, -$C(O)N(R^{z3})$-, -$S(O)_2N(R^{z3})$-, -$S(O)N(R^{z3})$-, -$S(O)_2$-, -S(O)-, -$N(R^{z3})S(O)_2N(R^{z3a})$-, -S-, -$N(R^{z3})$-, -$OC(OR^{z3})(R^{z3a})$-, -$N(R^{z3})C(O)N(R^{z3a})$-, and -$OC(O)N(R^{z3})$;

R^{z1} and R^{z1a} are independently of each other selected from the group consisting of -H, -T, 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^{z2}, 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)-, -$C(O)N(R^{z4})$-, -$S(O)_2N(R^{z4})$-, -$S(O)N(R^{z4})$-, -$S(O)_2$-, -S(O)-, -$N(R^{z4})S(O)_2N(R^{z4a})$-, -S-, -$N(R^{z4})$-, -$OC(OR^{z4})(R^{z4a})$-, -$N(R^{z4})C(O)N(R^{z4a})$-, and -$OC(O)N(R^{z4})$-;

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^{z2}, which are the same or different;

each R^{z2} is independently selected from the group consisting of halogen, -CN, oxo (=O), -$COOR^{z5}$, -OR^{z5}, -$C(O)R^{z5}$, -$C(O)N(R^{z5}R^{z5a})$, -$S(O)_2N(R^{z5}R^{z5a})$, -$S(O)N(R^{z5}R^{z5a})$, -$S(O)_2R^{z5}$, -

$S(O)R^{z5}$, $-N(R^{z5})S(O)_2N(R^{z5a}R^{z5b})$, $-SR^{z5}$, $-N(R^{z5}R^{z5a})$, -NOz, $-OC(O)R^{z5}$, $-N(R^{z5})C(O)R^{z5a}$, $-N(R^{z5})S(O)_2R^{z5a}$, $-N(R^{z5})S(O)R^{z5a}$, $-N(R^{z5})C(O)OR^{z5a}$, $-N(R^{z5})C(O)N(R^{z5a}R^{z5b})$, $-OC(O)N(R^{z5}R^{z5a})$, 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^{z3}, R^{z3a}, R^{z4}, R^{z4a}, R^{z5}, R^{z5a} and R^{z5b} 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.

[0048] Even more preferably, the term "spacer" refers to a moiety selected from the group consisting of -T-, -C(O)O-, -O-, -C(O)-, $-C(O)N(R^{z1})-$, $-S(O)_2N(R^{z1})-$, $-S(O)N(R^{z1})-$, $-S(O)_2-$, -S(O)-, $-N(R^{z1})S(O)_2N(R^{z1a})-$, -S-, $-N(R^{z1})-$, $-OC(OR^{z1})(R^{z1a})-$, $-N(R^{z1})C(O)N(R^{z1a})-$, $-OC(O)N(R^{z1})-$, 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^{z2}, 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)-, $-C(O)N(R^{z3})-$, $-S(O)_2N(R^{z3})-$, $-S(O)N(R^{z3})-$, $-S(O)_2-$, -S(O)-, $-N(R^{z3})S(O)_2N(R^{z3a})-$, -S-, $-N(R^{z3})-$, $-OC(OR^{z3})(R^{z3a})-$, $-N(R^{z3})C(O)N(R^{z3a})-$, and $-OC(O)N(R^{z3})-$;

R^{z1} and R^{z1a} 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 heterobicyclyl, 8-to 30-membered carbopolycyclyl, and 8- to 30-membered heteropolycyclyl;

each R^{z2} is independently selected from the group consisting of halogen, and C_{1-6} alkyl; and

each R^{z3}, R^{z3a}, R^{z4} , R^{z4a}, R^{z5}, R^{z5a} and R^{z5b} is independently of each other 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.

[0049] The term "interrupted" means that a group of atoms is inserted into a moiety between two carbon atoms or - if the insertion is at one of the moiety's ends - between a carbon and a hydrogen atom. It is understood that if a moiety is interrupted by a group of atoms at one of its ends and if the moiety that is interrupted is connected to a second moiety, the interrupting group of atoms may also be so positioned that it is located between the last atom of said

moiety and the first atom of the second moiety.

[0050] As used herein, the term "C_{1-4} 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 C_{1-4} 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 C_{1-4} alkyl, then examples for such C_{1-4} alkyl groups are $-CH_2-$, $-CH_2-CH_2-$, $-CH(CH_3)-$, $-CH_2-CH_2-CH_2-$, $-CH(C_2H_5)-$, $-C(CH_3)_2-$. Each hydrogen of a C_{1-4} alkyl carbon may optionally be replaced by a substituent as defined above. Optionally, a C_{1-4} alkyl may be interrupted by one or more moieties as defined below.

[0051] As used herein, the term "C_{1-6} 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 C_{1-6} 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 C_{1-6} alkyl group, then examples for such C_{1-6} alkyl groups are $-CH_2-$, $-CH_2-CH_2-$, $-CH(CH_3)-$, $-CH_2-CH_2-CH_2-$, $-CH(C_2H_5)-$ and $-C(CH_3)_2-$. Each hydrogen atom of a C_{1-6} carbon may optionally be replaced by a substituent as defined above. Optionally, a C_{1-6} alkyl may be interrupted by one or more moieties as defined below.

[0052] Accordingly, "C_{1-10} alkyl", "C_{1-20} alkyl" or "C_{1-50} 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 C_{1-10}, C_{1-20} or C_{1-50} carbon may optionally be replaced by a substituent as defined above. Optionally, a C_{1-10} or C_{1-50} alkyl may be interrupted by one or more moieties as defined below.

[0053] As used herein, the term "C_{2-6} 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 $-CH=CH_2$, $-CH=CH-CH_3$, $-CH_2-CH=CH_2$, $-CH=CHCH_2-CH_3$ and $-CH=CH-CH=CH_2$. When two moieties of a molecule are linked by the C_{2-6} alkenyl group, then an example for such C_{2-6} alkenyl is -CH=CH-. Each hydrogen atom of a C_{2-6} alkenyl moiety may optionally be replaced by a substituent as defined above. Optionally, a C_{2-6} alkenyl may be interrupted by one or more moieties as defined below.

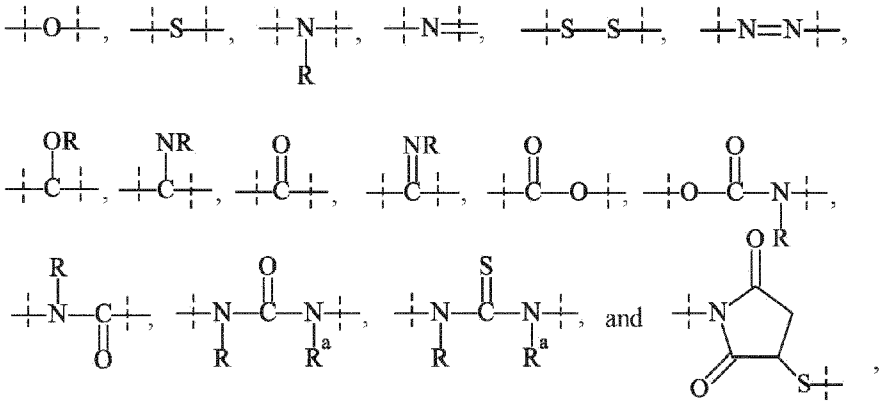
[0054] Accordingly, the term "C_{2-10} alkenyl", "C_{2-20} alkenyl" or "C_{2-50} alkenyl" alone or in combination means a straight-chain or branched hydrocarbon moiety comprising at least one carbon-carbon double bond having 2 to 10, 2 to 20 or 2 to 50 carbon atoms. Each hydrogen atom of a C_{2-10} alkenyl, C_{2-20} alkenyl or C_{2-50} alkenyl group may optionally be replaced by a substituent as defined above. Optionally, a C_{2-10} alkenyl, C_{2-20} alkenyl or C_{2-50} alkenyl may be

interrupted by one or more moieties as defined below.

[0055] As used herein, the term "C_{2-6} alkynyl" alone or in combination means 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, -CH_2-C≡CH, CH_2-CH_2-C≡CH and CH_2-C≡C-CH_3. When two moieties of a molecule are linked by the alkynyl group, then an example is -C≡C-. Each hydrogen atom of a C_{2-6} alkynyl group may optionally be replaced by a substituent as defined above. Optionally, one or more double bond(s) may occur. Optionally, a C_{2-6} alkynyl may be interrupted by one or more moieties as defined below.

[0056] Accordingly, as used herein, the term "C_{2-10} alkynyl", "C_{2-20} alkynyl" and "C_{2-50} 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 C_{2-10} alkynyl, C_{2-20} alkynyl or C_{2-50} alkynyl group may optionally be replaced by a substituent as defined above. Optionally, one or more double bond(s) may occur. Optionally, a C_{2-10} alkynyl, C_{2-20} alkynyl or C_{2-50} alkynyl may be interrupted by one or more moieties as defined below.

[0057] As mentioned above, a C_{1-4} alkyl, C_{1-6} alkyl, C_{1-10} alkyl, C_{1-20} alkyl, C_{1-50} alkyl, C_{2-6} alkenyl, C_{2-10} alkenyl, C_{2-20} alkenyl, C_{2-50} alkenyl, C_{2-6} alkynyl, C_{2-10} alkynyl, C_{2-20} alkenyl or C_{2-50} alkynyl may optionally be interrupted by one or more of the following moieties:



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, propyl, butyl, pentyl and hexyl.

[0058] As used herein, the term "C_{3-10} 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 C_{3-10} cycloalkyl carbon may be replaced by a substituent as defined above. The term "C_{3-10} cycloalkyl" also includes bridged bicycles like norbornane or norbornene.

[0059] 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 comprise up to the maximum number of double bonds (aromatic or non-aromatic ring which is fully, partially or un-saturated). Preferably a 8- to 30-membered carbopolycyclyl means a cyclic moiety of two, three, four or five rings, more preferably of two, three or four rings.

[0060] 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 comprise 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.

[0061] 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 comprise 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-dioxa-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.

[0062] Similary, 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 comprise 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.

[0063] As used herein, "halogen" means fluoro, chloro, bromo or iodo. It is generally preferred that halogen is fluoro or chloro.

[0064] In general, the term "comprise" or "comprising" also encompasses "consist of" or "consisting of".

[0065] In a preferred embodiment $=Y_1$ of formula (Ia) is =O.

[0066] In a preferred embodiment $-Y^2-$ of formula (Ia) is -O-.

[0067] In a preferred embodiment $-Y^3-$ of formula (Ia) is -O-.

[0068] In a preferred embodiment $-Y^4-$ of formula (Ia) is $-NR^5-$.

[0069] In a preferred embodiment $=Y^5$ of formula (Ia) is =O.

[0070] In a preferred embodiment n of formula (Ia) is 0 or 1. Most preferably, n of formula (Ia) is 0.

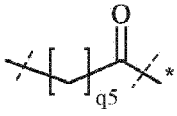
[0071] In a preferred embodiment $-R^3$ of formula (Ia) is selected from the group consisting of -H, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl. More preferably, $-R^3$ of formula (Ia) is selected from the group consisting of -H, methyl, ethyl, n-propyl and isopropyl. Even more preferably $-R^3$ of formula (Ia) is selected from -H, methyl and ethyl. Most preferably, $-R^3$ of formula (Ia) is -H.

[0072] In a preferred embodiment, each $-R^4$ of formula (Ia) is independently selected from methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl. More preferably, $-R^4$ of formula (Ia) is selected from the group consisting of methyl, ethyl, n-propyl and isopropyl. Even more preferably $-R^4$ of formula (Ia) is selected from methyl and ethyl.

[0073] In a preferred embodiment -R^5 of formula (Ia) is selected from the group consisting of -H, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl. More preferably, -R^5 of formula (Ia) is selected from the group consisting of -H, methyl, ethyl, n-propyl and isopropyl. Even more preferably -R^5 of formula (Ia) is selected from methyl and ethyl. Most preferably, -R^5 of formula (Ia) is methyl.

[0074] In a preferred embodiment -R^6 and -R^{6a} of formula (Ia) are independently selected from the group consisting of -H, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl. More preferably, -R^6 and -R^{6a} of formula (Ia) are independently selected from the group consisting of -H, methyl, ethyl, n-propyl and isopropyl. Even more preferably -R^6 and -R^{6a} of formula (Ia) are independently selected from -H, methyl and ethyl. Most preferably, -R^6 and -R^{6a} of formula (Ia) are both -H.

[0075] X of formula (Ia) is of formula (III)



(III),

wherein

the dashed line marked with the asterisk indicates attachment to the R^1;

the unmarked dashed line indicates attachment to remainder of the prodrug;

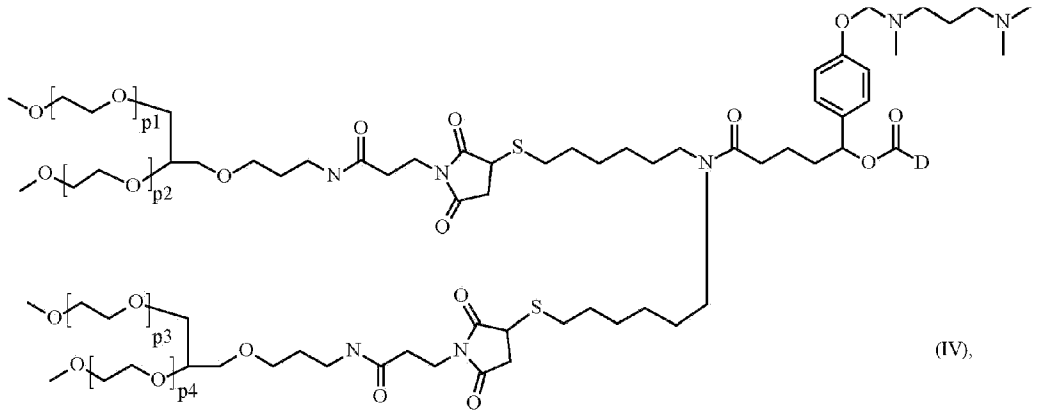
q5 is 1, 2, 3, 4, 5, 6, 7 or 8; preferably q5 is 1, 2, 3, 4, or 5; more preferably q5 is 2, 3 or 4; most preferably q5 is 3;

[0076] Preferably, Ar of formula (Ia) is phenyl. Most preferably, Ar of formula (Ia) is

wherein the dashed lines indicate attachment to the remainder of the prodrug of formula (Ia).

[0077] Preferably, -R^7 and -R^{7a} of formula (Ia) 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. More preferably, -R^7 and -R^{7a} of formula (Ia) are independently of each other selected from -H, methyl, ethyl, n-propyl and isopropyl. Even more preferably, -R^7 and -R^{7a} of formula (Ia) are independently of each other selected from methyl or ethyl. Most preferably, -R^7 and -R^{7a} of formula (Ia) are both methyl.

[0078] Most preferably, the polymeric hGH prodrug of the present invention is of formula (IV)



(IV),

wherein

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

p1, p2, p3, p4 are independently an integer ranging from 220 to 240.

[0079] Most preferably, D of formula (IV) is connected to the rest of the molecule through an amine provided by a lysine side chain.

[0080] Another aspect of the present invention is the polymeric hGH prodrug of the present invention, preferably of formula (IV), for use as a medicament.

[0081] Another aspect of the present invention is the polymeric hGH prodrug of the present invention, preferably the polymeric hGH prodrug of formula (IV), for use in a method of treatment of a disease which can be treated with hGH, wherein the disease is selected from the group consisting of growth hormone deficiency (GHD) in children, idiopathic short stature (ISS), short stature homeobox (SHOX) gene mutations, Turner syndrome (TS), Noonan syndrome (NS), Prader-Willi syndrome (PWS), children born small for gestational age (SGA), chronic renal insufficiency (CRI), growth hormone deficiency (GHD) in adults, wasting due to HIV or AIDS or other malignancies, short bowel syndrome (SBS), sarcopenia, and frailty.

[0082] In one embodiment the disease which can be treated with hGH is GHD in children.

[0083] In another embodiment the disease which can be treated with hGH is GHD in adults.

[0084] In another embodiment the disease which can be treated with hGH is ISS.

[0085] In another embodiment the disease which can be treated with hGH are SHOX gene mutations.

[0086] In another embodiment the disease which can be treated with hGH is TS.

[0087] In another embodiment the disease which can be treated with hGH is NS.

[0088] In another embodiment the disease which can be treated with hGH is PWS.

[0089] In another embodiment the disease which can be treated with hGH is SGA.

[0090] In another embodiment the disease which can be treated with hGH is CRI.

[0091] In another embodiment the disease which can be treated with hGH is wasting due to HIV or AIDS or other malignancies.

[0092] In another embodiment the disease which can be treated with hGH is SBS.

[0093] In another embodiment the disease which can be treated with hGH is sarcopenia.

[0094] In another embodiment the disease which can be treated with hGH is frailty.

[0095] Another aspect of the present invention is the polymeric hGH prodrug or the pharmaceutically acceptable salt thereof for use as described above wherein the prodrug is administered via topical, enteral or parenteral administration or by methods of external application, injection or infusion, including intraarticular, periarticular, intradermal, subcutaneous, intramuscular, intravenous, intraosseous, intraperitoneal, intrathecal, intracapsular, intraorbital, intravitreal, intratympanic, intravesical, intracardiac, transtracheal, subcuticular, subcapsular, subarachnoid, intraspinal, intraventricular, intrasternal injection or infusion, direct delivery to the brain via implanted device allowing delivery to brain tissue or brain fluids, direct intracerebroventricular injection or infusion, injection or infusion into brain or brain associated regions, injection into the subchoroidal space, retro-orbital injection and ocular instillation.

[0096] Preferably, the administration is via subcutaneous injection.

[0097] In a preferred embodiment, the present invention relates to a polymeric hGH prodrug of the present invention, preferably the polymeric hGH prodrug of formula (IV) for use in the treatment of GHD in children via subcutaneous injection.

EXAMPLES

Methods

Cation exchange chromatography

[0098] The purification of conjugates by cation exchange chromatography was performed using an ÄKTA Pure system (GE Healthcare) equipped with a Macrocap SP column with a column volume of 279 mL. The respective reaction mixture was applied to the column which was pre-equilibrated in 20 mM sodium acetate, 10 mM L-methionine buffer, pH 4.0 (buffer A). After loading, the column was washed with three column volumes of buffer A to remove any unreacted PEG reagent. Mono-Conjugates were eluted using a gradient of 0-30% buffer B (20 mM sodium acetate, 1 M sodium chloride, pH 4.5) over 15 column volumes. A gradient of 30-80% B over three column volumes was used to elute unreacted growth hormone. The column was cleaned with 3 column volumes of 100% buffer B. The flow rate was 20 mL/min for loading and 25 mL/min during the elution. The elution was monitored by detection at 280 nm.

SDS-PAGE analysis

[0099] The mPEG-hGH conjugates were analysed by SDS-PAGE using NuPAGE® Novex 4-12% Bis-Tris gels (1.0 mm thick, 12 lanes), NuPAGE MOPS SDS-Running Buffer, HiMark™ Pre-stained High Molecular Weight Protein Standard and Coomassie Colloidal Blue™ Staining Kit (Invitrogen). In each lane 1 µg hGH eq. of the conjugate were applied and the electrophoresis and subsequent staining performed according to the supplier's protocol. Images of the gels were generated using a Digi Image System (Kisker Biotech) and a Power Shot G10 camera (Canon).

Dia-/Ultrafiltration

[0100] Dia- and Ultrafiltration steps were performed using a labscale TFF system (Millipore) equipped with Pellicon XL Biomax membranes with a membrane are of 50 cm^2 and a molecular weight cut-off of 5 or 10 kDa for hGH only, 10 kDa for 4x 10 kDa mPEG-linker-hGH monoconjugate 2 and 50 kDa for 4x 20 kDa mPEG-linker-hGH monoconjugate 1.

RP-HPLC

[0101] The following RP-HPLC parameters were used:
Mobile phase A was composed of 0.05 % aqueous TFA and mobile phase B was composed of 0.04 % TFA in acetonitrile. A Waters UPLC C18 BEH 300Å 1.7µm 2.1x50mm column was used. Flow rate was set to 0.2-0.4 mL/min, detection was at a wavelength of 215 nm, the column running temperature was 30 °C (± 5 °C). The autosampler temperature was set at 4°C and the sample injection load was 20 µL. For peak separation the gradient shown in Table 1 was used.
Table 1: RP-HPLC gradient

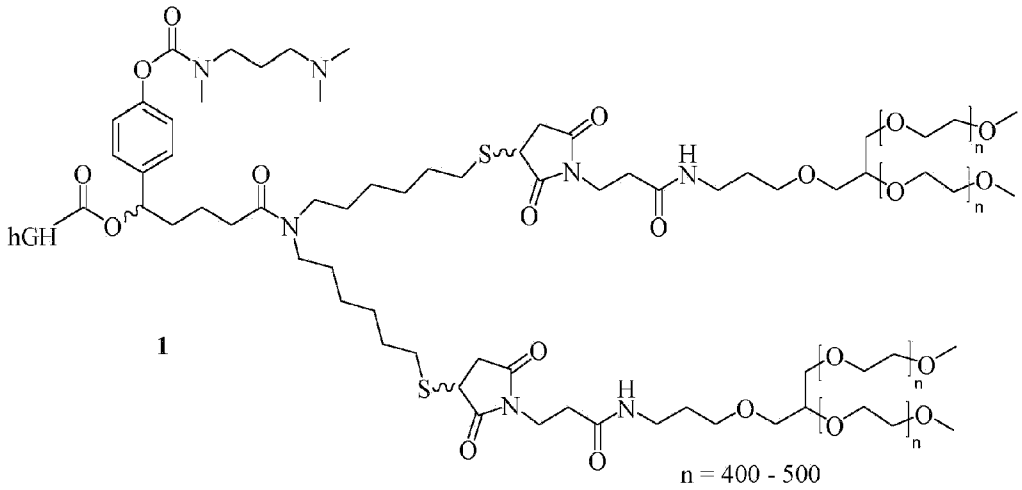
Time [min]	% B
0	25
1	25
8	40
30	60
30.1	90
30.5	90
30.6	25
35	25

Buffer exchange

[0102] Buffer exchange was performed using an ÄKTA explorer system (GE Healthcare) equipped with a HiPrep 26/10 Desalting column or a HiTrap Desalting column.

Example 1: Synthesis of transient 4x 20 kDa mPEG-linker-hGH monoconjugate 1 (reference substance; not according to the invention)

[0103]



1

[0104] 4x 20 kDa mPEG-linker-hGH monoconjugate **1** was synthesized according to a similar procedure as described in WO2009/133137 A2. The formulations of 4x 20 kDa mPEG-linker-hGH monoconjugate **1** as shown in Table 2 were prepared.

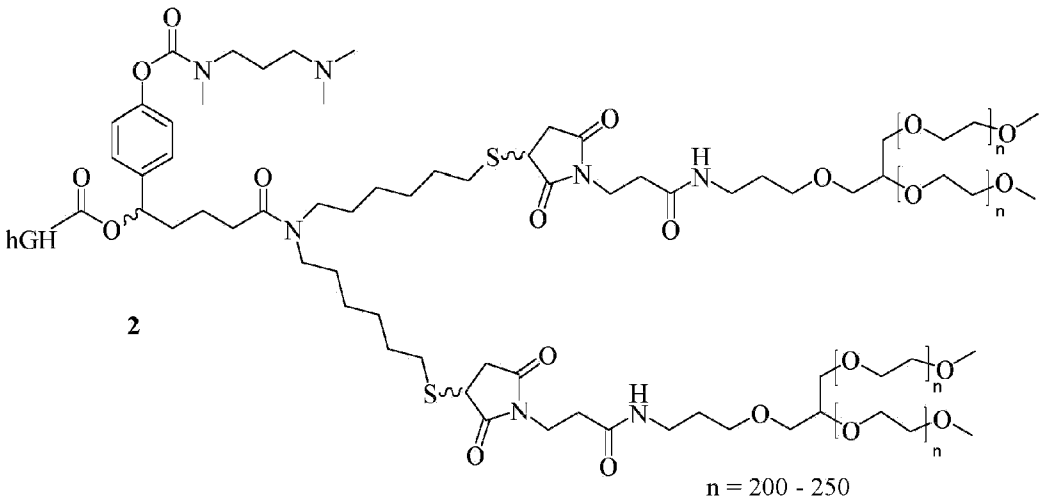
Table 2: Formulations of 4x 20kDa mPEG-linker-hGH monoconjugate **1**

Formulation name:	Concentration of 4x 20 kDa mPEG-linker-hGH monoconjugate **1** formulation [mg conjugate /mL]	Concentration of hGH eq. [mg hGH eq./mL]
1A	30	6
1B	45	9
1C	75	15

Example 2: Synthesis of high strength transient 4x 10 kDa mPEG-linker-hGH monoconjugate

[0105]

2



2

4x 10 kDa mPEG-linker-hGH monoconjugate **2** was synthesized according to a similar procedure as described in WO2009/133137 A2; in detail the manufacturing process was conducted as follows:

hGH was buffer exchanged to 100 mM sodium borate pH 9 and the concentration of hGH was adjusted to 10 mg/mL. A molar excess of 4-arm branched 40kDa mPEG-pentafluorophenyl carbonate derivative relative to the amount of hGH was dissolved in water to form a 6% (w/w) reagent solution. The reagent solution was added to the hGH solution in a 1-to-1 ratio (based on weight) and mixed. The reaction mixture was incubated under stirring for 105 min at 12-16°C and subsequently quenched by adding 4 volumes of a solution comprising 27 mM acetic acid and 12.5 mM L-methionine to 1 volume of the reaction mixture to lower the pH of the solution to 4-4.5. After sterile filtration, the reaction mixture was incubated at room temperature for 16±4 h. 4x 10kDa mPEG-linker-hGH monoconjugate **2** was purified by cation exchange chromatography.

[0106] Buffer exchange and adjustment to the desired concentration of 4x 10kDa mPEG-linker-hGH monoconjugate **2** was achieved using a tangential-flow filtration system. Herewith the eluate from the cation exchange chromatography was ultra-filtrated and dia-filtrated to

formulation buffer (10 mM succinic acid, 85 g/L trehalose dihydrate, pH 5.0 with 1M Tris-solution). Using the same system the trehalose concentration was lowered to 65 g/L and the concentration of this stock solution adjusted to 105±3 mg/mL of 4x 10kDa mPEG-linker-hGH monoconjugate **2** (corresponding to 35 ± 1 mg hGH eq./mL). The formulations as shown in Table 3 were prepared based on this stock-solution of compound **2** by diluting the stock solution with high strength formulation buffer (10 mM succinic acid, 89 g/L trehalose dihydrate, adjusted to pH 5.0 with 1M Tris-base).

Table 3: Formulations of 4x 10kDa mPEG-linker-hGH monoconjugate **2**

Formulation name:	Concentration of 4x 10kDa mPEG-linker-hGH monoconjugate **2** formulation [mg/mL]	Concentration of hGH eq. [mg hGH eq./mL]
2A	103.8	34.6
2B	95.1	31.7
2C	81.9	27.3
2D	65.1	21.7
2E	47.4	15.8

[0107] Individual batches were analyzed by RP-HPLC, SE-HPLC, peptide mapping and SDS-PAGE. SDS-PAGE showed that all formulation have comparable product qualities which are similar to the reference. During method development it was discovered that the load of the cation exchange chromatography column which is used to purify the 4x 10 kDa mPEG-linker-hGH monoconjugate ***2*** could be significantly increased compared to the purification procedure of 4x 20 kDa mPEG-linker-hGH monoconjugate **1.**

Conclusion:

[0108] 4x 10kDa mPEG-linker-hGH monoconjugate **2** could by synthesized by implementing only minor changes to the manufacturing process compared to the manufacturing process described in EP-A 2113256 and showed improved handling and product properties. Loading of the CIEX column for purification could be at least tripled without impairing the separation efficacy and product quality. Additionally, the content of the final product could be increased to above 100 mg/mL of the 4x10kDa mPEG-linker-hGH-conjugate **2** which corresponds to approx. 35 mg hGH eq./mL.

Example 3: Syringability of high strength formulations of 4x 10kDa mPEG-linker-hGH monoconjugate 2 compared to 4x 20kDa mPEG-linker-hGH monoconjugate 1

[0109] Individual formulations from example 1 & 2 were investigated for their ability of being injected through injection needles with various inner diameters. Tests were performed on a

Mecmesin Multitest 1-d stand, equipped with measuring device BFG 200N and using the Emperor Lite software (Vers. no. 1.16-015). Tested injection needles comprised a 27G needle 0.4×13mm 27Gx1/2" from BD (Ref 300635, Lot 101009), a 29G needle, 0.33×13mm from Transcoject, and a 30G needle 0.30×12mm, 30G×1/2", from Sterican (Lot 2G13258811). The measuring device was setup to measure the force for pushing the plunger down for a given constant plunger speed. The applied plunger speeds which correspond to the applied injection speeds were as follows:

Injection speed	688 mm/min	5 sec/mL	12 mL/min
	344 mm/min	10 sec/mL	6 mL/min
	229 mm/min	15 sec/mL	4 mL/min
	172 mm/min	20 sec/mL	3 mL/min
	138 mm/min	25 sec/mL	2.4 mL/min
	115 mm/min	30 sec/mL	2 mL/min

[0110] Testing was performed using the following steps:

1. 1. Charging of a 1ml Luer-lok Syringe, (BD, Ref 309628) with sample (using a 20G needle, 0.90×40mm , 20Gx11/2" from Sterican)
2. 2. Removal of air bubbles
3. 3. Attachment of test needle (starting with the largest inner diameter) onto the syringe
4. 4. Clamping the syringe into the holder
5. 5. Selection of appropriate measuring settings
6. 6. Start measurement and collect the sample in a glass vial (placed underneath the syringe)
7. 7. Removal of syringe from holder
8. 8. Re-charging of the syringe with test material and measuring of subsequent setting -> these steps were repeated for all needles (with descending needle diameter) and for every test sample.

[0111] Formulation buffer without mPEG-linker-hGH monoconjugate **1** or **2** was used as reference solution.

[0112] For all different injection needles and for all injection speeds the injection forces were determined for 4x 10kDa mPEG-linker-hGH monoconjugate **2** and compared with the results for 4x 20kDa mPEG-linker-hGH monoconjugate **1.** Table 4 shows the comparison of injection forces between 4x 10kDa mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1** for the 27G needle 0.4×13mm 27G×1/2" from BD (Ref 300635, Lot 101009).

Table 4: Injection forces of 4x 10kDa mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1** for a 27G needle (0.4×13mm 27G×1/2" from BD)

		Injection Force [N]							
		Formulation of 4x 10kDa mPEG-linker-hGH monoconjugate **2**					Formulation of 4x 20kDa mPEG-linker-hGH monoconjugate 1		
Injection speed [sec/mL]	**Injection speed [mL/min]**	**2E**	**2D**	**2C**	**2B**	**2A**	**1A**	**1B**	**1C**
5	12	5.35	7.35	9.65	22.0	30.0	6.6	12..1	20.3
10	6	2.90	4.00	4.90	11.35	16.0	3.6	6.5	10.7
15	4	2.05	2.95	3.75	7.95	10.8	2.7	4.6	7.5
20	3	1.60	2.40	3.15	6.15	8.85	2.2	3.8	5.7
25	2.4	1.45	2.05	2.65	5.05	7.35	1.8	3.2	4.5
30	2	1.30	1.70	2.25	4.45	6.40	n.d.	n.d.	n.d.

[0113] Table 5 shows the comparison of injection forces between 4x 10kDa mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1** for the 29G needle, 0.33x13mm from Transcoject.

Table 5: Injection forces of 4x 10kDa mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1** for a 29G needle (0.33x13mm from Transcoject)

		Injection Force [N]							
		Formulation of 4x 10kDa mPEG-linker-hGH monoconjugate **2**					Formulation of 4x 20kDa mPEG-linker-hGH monoconjugate 1		
Injection speed [sec/mL]	**injection speed [mL/min]**	**2E**	**2D**	**2C**	**2B**	**2A**	**1A**	**1B**	**1C**
5	12	12.70	20.95	26.70	32.70	n.d.	n.d.	27.3	n.d.
10	6	6.40	10.05	13.25	16.90	25.40	12.0	14.9	28.6
15	4	4.40	6.90	9.20	11.50	19.20	8.0	10.6	20.2
20	3	3.70	5.30	6.75	8.95	13.95	6.3	7.9	15.2
25	2.4	2.80	4.40	5.70	7.50	11.50	5.0	6.5	12.3
30	2	2.50	3.70	4.65	6.05	10.05	n.d.	n.d.	n.d.

[0114] Table 6 shows the comparison of injection forces between 4x 10kDa mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1** for the 30G needle 0.30x12mm, 30Gx1/2", from Sterican (Lot 2G13258811).

Table 6: Injection forces of 4x 10kDa mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1** for a 30G needle (0.30×12mm, 30G×1/2", from Sterican)

		Injection Force [N]							
		Formulation of 4x 10kDa mPEG-linker-hGH monoconjugate 2					Formulation of 4x 20kDa mPEG-linker-hGH monoconjugate **1**		
Injection speed [sec/mL]	**injection speed [mL/min]**	**2E**	**2D**	**2C**	**2B**	**2A**	**1A**	**1B**	**1C**
5	12	26.6	28.50	50.90	n.d.	n.d.	n.d.	45.2	*
10	6	12.95	19.60	26.90	36.50	n.d.	15.0	25.5	51.0
15	4	8.40	13.70	18.90	25.20	34.7	10.3	17.7	37.6
20	3	7.00	10.50	13.90	19.50	28.2	8.2	13.1	28.9
25	2.4	5.50	8.05	11.20	15.70	20.6	7.0	10.5	23.4
30	2	4.75	7.50	9.50	13.15	17.5	n.d.	n.d.	n.d.

Conclusion:

[0115] The injectability of 4x 10 kDa mPEG-linker-hGH monoconjugate **2** was highly improved and the injection force could be reduced 3.5-fold to 4-5 fold compared to 4x 20kDa mPEG linker-hGH monoconjugate **1.**

Example 4: Viscosity measurements of 4x 10kDa mPEG-linker-hGH monoconjugate 2 compared to 4x 20kDa mPEG-linker-hGH monoconjugate 1

[0116] The dynamic viscosity of test samples was determined at Infraserv Knapsack (now synlab Pharma Institute) using a method according to EP method 2.2.10. All measurements were performed with approx. 1-5 mL of test sample at 23.0 ± 0.1°C using a cone/plate measuring system (CP50/1). The shearing rate was in the range of 100 s^{-1} - 10 s^{-1}.

[0117] All tested formulations of 4x 10kDa mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1** were adjusted to an equal osmolality of approx. 290 mOsmol/kg by increasing or decreasing the amount of trehalose in the formulation. The dynamic viscosity values measured for all test samples are summarized in Table 7.

Table 7: Dynamic viscosity values for different formulations of 4x 10kDa mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1** which were adjusted to similar osmolalities.

	Formulation:	Conc. [mg/mL hGH eq.]	Content trehalose in formulation buffer [g/L]	Osmolality	Viscosity [mPa * s]
4x 10kDa mPEG-linker-hGH monoconjugate **2**	2A	34.6	65	286	25.6
	2B	31.7	68	290	18.9
	2C	27.3	71	286	14.9
	2D	21.7	75	283	9.9
	2E	15.8	78	284	6.0
4x 20kDa mPEG-linker-hGH monoconjugate **1**	1A	6	85	291	7.4
	1B	9	80	293	12.8
	1C	15	70	285	31

Conclusion:

[0118] The dynamic viscosity of 4x 10 kDa mPEG-linker-hGH monoconjugate **2** could be significantly reduced about a factor of 4- to 5-fold compared to 4x 20kDa mPEG linker-hGH monoconjugate **1.**

Example 5: Reconstitution time of lyophilisates of 4x 10kDa mPEG-linker-hGH monoconjugate 2

[0119] 1 mL of 4x 10kDa mPEG-linker-hGH monoconjugate **2** was lyophilized in a Din2R vial and after lyophilization the lyo cake was dissolved with 1 mL water for injection. The reconstitution time was compared to the dissolution time of a lyophilisate of 4x 20kDa mPEG-linker-hGH monoconjugate **1.** During reconstitution more gas bubbles were detected for 4x 20kDa mPEG-linker-hGH monoconjugate **1.** While the dissolution of the lyo cake itself was quite fast, the time until a clear solution was obtained with only a minimal amount of gas bubbles remaining, was significantly shorter for 4x 10kDa mPEG-linker-hGH monoconjugate **2.** The results of this reconstitution procedure are summarized in Table 8.

Table 8: Reconstitution times of 4x 10kDa mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1**

	4x 10kDa mPEG-linker-hGH monoconjugate **2**	4x 20kDa mPEG-linker-hGH monoconjugate **1**
Time for dissolution	<1min	<1min
Time until a clear solution is obtained	<5min	>15min
Time for disappearance	<5min	>15min

	4x 10kDa mPEG-linker-hGH monoconjugate **2**	4x 20kDa mPEG-linker-hGH monoconjugate **1**
of most air bubbles		

Conclusion:

[0120] The time of reconstitution until a clear and virtually bubble free solution is achieved is significantly shorter for 4x 10kDa mPEG-linker-hGH monoconjugate **2** compared to 4x 20kDa mPEG linker-hGH monoconjugate **1.**

Example 6: In vitro hydrolysis of 4x 10kDa mPEG-linker-hGH monoconjugate 2

[0121] For the determination of *in vitro* linker cleavage rates of 4x 10kDa mPEG-linker-hGH monoconjugate **2** or 4x 20kDa mPEG-linker-hGH monoconjugate **1,** the compounds were buffer exchanged to PBST buffer at pH 7.4 and the eluted solutions were filtered through a 0.22 µm filter and incubated at 37°C for 1 week. Samples were taken at certain time intervals and analyzed by RP-HPLC. All peaks were integrated and allocated and the relevant peak areas were plotted against incubation time. Curve fitting software was applied to determine first-order cleavage rates. Table 9 shows *in vitro* hydrolysis rates of 4x 10kda mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1** at pH 7.4 and 37°C.

Table 9: *In vitro* hydrolysis rates of 4x 10kDa mPEG-linker-hGH monoconjugate **2** or 4x 20kDa mPEG-linker-hGH monoconjugate **1** at pH 7.4 and 37°C

	Half life time [h]	**95% confidence interval [h]**
4x 10kDa mPEG-linker-hGH monoconjugate **2**	104.7	90.70 - 123.8
4x 20kDa mPEG-linker-hGH monoconjugate **1**	107.2	91.89 - 128.6

Conclusion:

[0122] The *in vitro* hydrolysis rates of conjugates **1** and **2** at pH 7.4 and 37°C were in the range of 105 ± 5h. Both half life times were highly comparable and lay within the 95% confidence interval.

Example 7: Quantification of conjugates 1 and 2 in serum samples from animal studies

[0123] An ELISA based method was used to quantify conjugates **1** and **2** in serum samples from animal studies. The same sandwich ELISA format was used for both conjugates **1** and **2,** which utilized a sheep anti-hGH polyclonal antibody (Abcam, Cat. No. ab64499) as capture antibody and a biotinylated rabbit anti-PEG antibody (Epitomics, Cat. No. 2137-1) as detection antibody. Read-out was done with streptavidin-HRP (Jackson ImmunoResearch, Cat. No. 016-030-084) and a commercial TMB liquid substrate system (Sigma, Cat. No. T0440). Serum standards and samples were diluted 1:50 with a pH 7.0 buffer (50 mM HEPES, 1 mM $CaCl_2$, 0.05 % Tween-20 and 1 % BSA) prior to measurement. Sample incubation on the ELISA plate was performed under shaking for 2 h at 37°C.

Example 8: Quantification of total mPEG40 and 80 in serum samples from animal studies

[0124] An ELISA based method was used to quantify mPEG40 and mPEG80 in serum samples from animal studies. The same sandwich ELISA format was used for both analytes mPEG40 and mPEG80, which utilized an anti-PEG (methoxy group) rabbit monoclonal antibody, (Epitomics, Cat. No. 2061-1) as capture antibody and a biotinylated anti-PEG mouse monoclonal IgM antibody (ANP Tech, Cat. No. 90-1052) as detection antibody. Read-out was done with streptavidin-HRP (Jackson ImmunoResearch, Cat. No. 016-030-084) and a commercial TMB liquid substrate system (Sigma, Cat. No. T0440). Serum standards and samples were diluted 1:50 with a pH 7.0 buffer (50 mM HEPES, 1 mM $CaCl_2$, 0.05 % Tween-20 and 1 % BSA) prior to measurement. Sample incubation on the ELISA plate was performed under shaking for 2 h at 37°C.

Example 9: Comparative pharmacokinetic study in cynomolgus monkeys treated with conjugates 1 and 2

[0125] Two groups of five healthy male non-naive cynomolgus monkeys each received a single subcutaneous administration of conjugate **1** or a single subcutaneous administration of conjugate **2** at a target dose level of 1 mg hGH equivalents per kg (corresponding to 3 mg conjugate **2**/kg and 5 mg conjugate **1**/kg, respectively). For PK-determinations blood samples were collected up to 336 hours post dose and serum generated thereof (for mPEG quantification serum samples were collected up to 56 days). Pharmacokinetic analysis according to Example 7 indicated that both compounds effected a comparable maximal conjugate level (9,200 ng hGH equivalents/mL for conjugate **1** and 7,400 ng hGH equivalents/mL for conjugate **2**) which was reached around 36 hours post dosing. mPEG concentration levels were determined according to Example 8. Both mPEG PK-profiles had their maximum concentration levels at 48 hours post dosing. Clearance of mPEG40 was faster than for mPEG80 as indicated in the terminal elimination half lifes (300 h for mPEG80 and 260 h for mPEG40). This resulted in an overall significant lower mPEG exposure for conjugate **2**

over conjugate **1** in this comparative PK-study.

Abbreviations:

[0126]

AIDS
: acquired immunodeficiency syndrome

CRI
: chronic renal insufficiency

DF
: Diafiltration

ELISA
: Enzyme linked immunosorbent assay

EP
: European Pharmacopoeia

eq
: stoichiometric equivalent

G
: gauge

GHD
: growth hormone deficiency

HIV
: human immunodeficiency virus

ISS
: idiopathic short stature

MW
: molecular weight

NS
: Noonan syndrome

PEG
: polyethylene glycol

PWS
: Prader-Willi syndrome

PK
: Pharmacokinetic

RP-HPLC
: reversed-phase high performance liquid chromatography

rt
: room temperature

SBS
: short bowel syndrome

SDS-PAGE

sodium dodecyl sulfate polyacrylamid gel electrophoresis

SEC
: size exclusion chromatography

SHOX
: short stature hoeobox

SGA
: small for gestational age

TFF
: Tangential flow filtration

Tris
: tris(hydroxymethyl)aminomethane

TS
: Turner syndrome

UF
: Ultrafiltration

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patent documents cited in the description

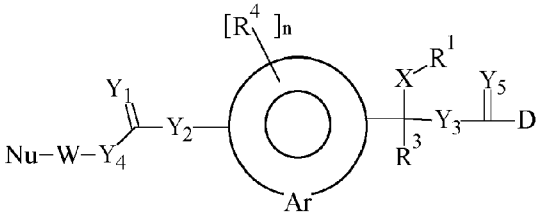
- WO2009133137A2 [0003] [0012] [0012] [0014] [0015] [0104] [0105]
- EP2113256A [0108]

Non-patent literature cited in the description

- **BOWIE et al.**Science, 1990, vol. 247, 1306-1310 [0019]

Patentkrav

1. Polymert humant væksthormon (hGH) prodrug eller et farmaceutisk acceptabelt salt deraf med formlen (Ia)



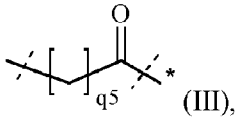
(Ia),

hvor

-D er en hGH-del forbundet med resten af molekylet gennem en aminfunktionel gruppe;

n er 0, 1, 2, 3 eller 4;

-X- har formlen (III)



(III),

hvor

den stiplede linje, der er markeret med asterisken, angiver vedhæftning til -R^1;

den umarkerede stiplede linje angiver binding til resten af prodruget;

q5 er 1, 2, 3, 4, 5, 6, 7 eller 8;

=Y_1 er valgt fra gruppen bestående af =O og =S;

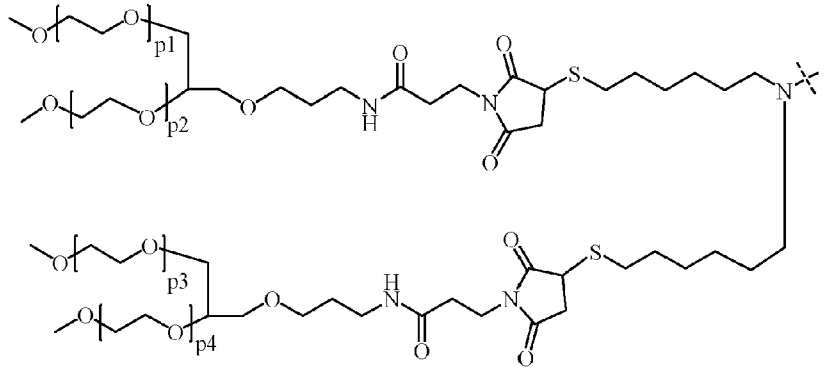
-Y_2- er valgt fra gruppen bestående af -O- og -S-;

-Y_3- er valgt fra gruppen bestående af -O- og -S-;

-Y_4- er valgt fra gruppen bestående af -O-, -NR^5- og -$C(R^6R^{6a})$-;

=Y_5 er valgt fra gruppen bestående af =O og =S;

-R^1 har formlen (IIc):



(IIc),

hvor

p1, p2, p3, p4 uafhængigt er et helt tal i området fra 220 til 240;

-R^3, -R^5, -R^6, -R^{6a} er uafhængigt af hinanden valgt fra gruppen bestående af -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 og 3,3-dimethylpropyl;

-R^4 er valgt fra gruppen bestående af 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 og 3,3-dimethylpropyl;

-W- er

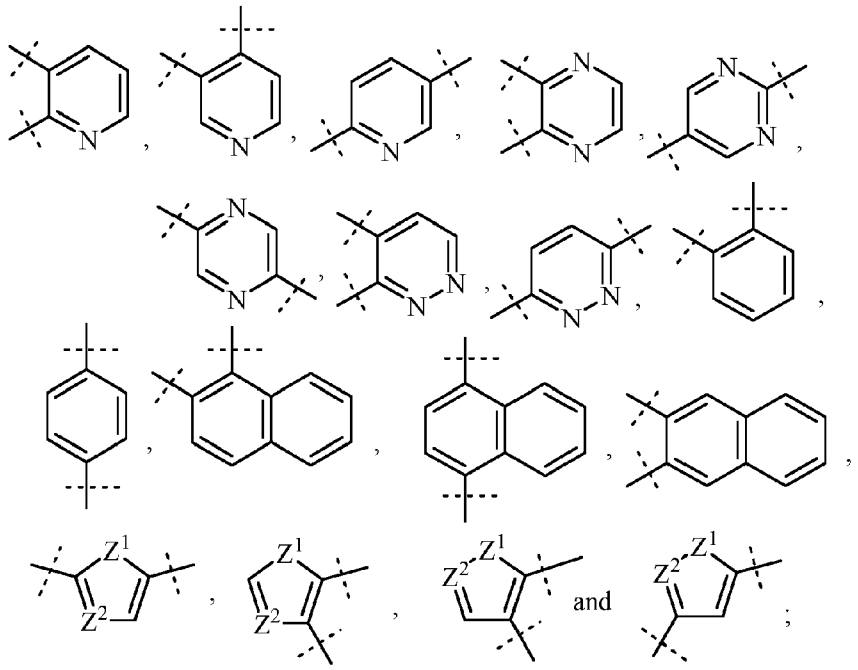
,

hvor

de stiplede linjer indikerer binding til resten af molekylet;

-Nu er -N(R^7R^{7a});

-Ar- er valgt fra gruppen bestående af



hvor

stiplede linjer indikerer binding til resten af prodruget,

-Z^1- er valgt fra gruppen bestående af -O-, -S- og -N(R^7)-, og

-Z^2- er -N(R^7)-; og

-R^7, -R^{7a} er uafhængigt af hinanden valgt fra gruppen bestående af -H, C_{1-6}-alkyl, C_{2-6}-alkenyl og C_{2-6}-alkynyl.

2. Prodrug eller farmaceutisk acceptabelt salt deraf ifølge krav 1, hvor -R^7 og -R^{7a} uafhængigt er valgt fra gruppen bestående af -H, methyl, ethyl, n-propyl og isopropyl.

3. Prodrug eller farmaceutisk acceptabelt salt deraf ifølge krav 1 eller 2, hvor q5 er 2, 3 eller 4.

4. Prodrug eller farmaceutisk acceptabelt salt deraf ifølge et hvilket som helst af kravene 1 til 3, hvor q5 er 3.

5. Prodrug eller farmaceutisk acceptabelt salt deraf ifølge et hvilket som helst af kravene 1 til 4 til anvendelse som et medikament.

6. Prodrug eller farmaceutisk acceptabelt salt deraf ifølge et hvilket som helst af kravene 1 til 4 til anvendelse i en fremgangsmåde til behandling af en sygdom, som kan behandles med hGH, hvor sygdommen er valgt fra gruppen bestående af vækshormonmangel hos børn, idiopatisk kortvækst, homøoboksgenmutationer for kortvoksenhed, Turners syndrom, Noonans syndrom, Prader-Willis syndrom, børn, der er født små i forhold til gestationsalderen, kronisk nyreinsufficiens, vækshormonmangel hos voksne, svind som følge af HIV eller AIDS eller andre maligniteter, korttarmsyndrom, sarkopeni og skrøbelighed.

7. Prodrug eller farmaceutisk acceptabelt salt deraf til anvendelse ifølge krav 6, hvor sygdommen, som kan behandles med hGH, er vækshormonmangel hos børn.

8. Prodrug eller farmaceutisk acceptabelt salt deraf til anvendelse ifølge krav 6, hvor sygdommen, som kan behandles med hGH, er vækshormonmangel hos voksne.

9. Prodrug eller farmaceutisk acceptabelt salt deraf til anvendelse ifølge et hvilket som helst af kravene 5 til 8, hvor prodruget administreres via topisk, enteral eller parenteral administration eller ved metoder til ekstern påføring, injektion eller infusion, herunder intraartikulær, periartikulær, intradermal, subkutan, intramuskulær, intravenøs, intraossøs, intraperitoneal, intrathecal, intrakapsulær, intraorbital, intravitreal, intratympanisk, intravesikal, intrakardiel, transtracheal, subkutikulær, subkapsulær, subaraknoidal, intraspinal, intraventrikulær, intrasternal injektion eller infusion, direkte tilførsel til hjernen via en implanteret anordning, der muliggør tilførsel til hjernevæv eller hjernevæsker, direkte int-

racerebroventrikulær injektion eller infusion, injektion eller infusion i hjerne- eller hjerneassocierede områder, injektion i det subchoroidale rum, retro-orbital injektion og okulær instillation.

10. Prodrug eller farmaceutisk acceptabelt salt deraf til anvendelse ifølge krav 9, hvor prodruget administreres via subkutan injektion.

SEKVENSLISTE

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