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(54) **NOVEL POLYMERIC HGH PRODRUGS**

NEUARTIGE POLYMERE HGH-PRODRUGS

NOUVEAUX PROMÉDICAMENTS HGH POLYMÉRIQUES

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Description

[0001] The present invention relates to a polymeric human growth hormone compounds and dry, liquid and reconstituted pharmaceutical formulations comprising said compund. It furthermore relates to their use as medicaments for the treatment of diseases which can be treated with growth hormone

[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.

[0004] 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 and EP 2133256 A1. Soluble polymer conjugates of hGH, in particular PEG conjugates, are also disclosed, for example, in WO 2006/102659 A2 and WO 2005/099768 A2 and related applications EP 1579873 A1 and EP 1625855 A1.

[0005] 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.

[0006] 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.

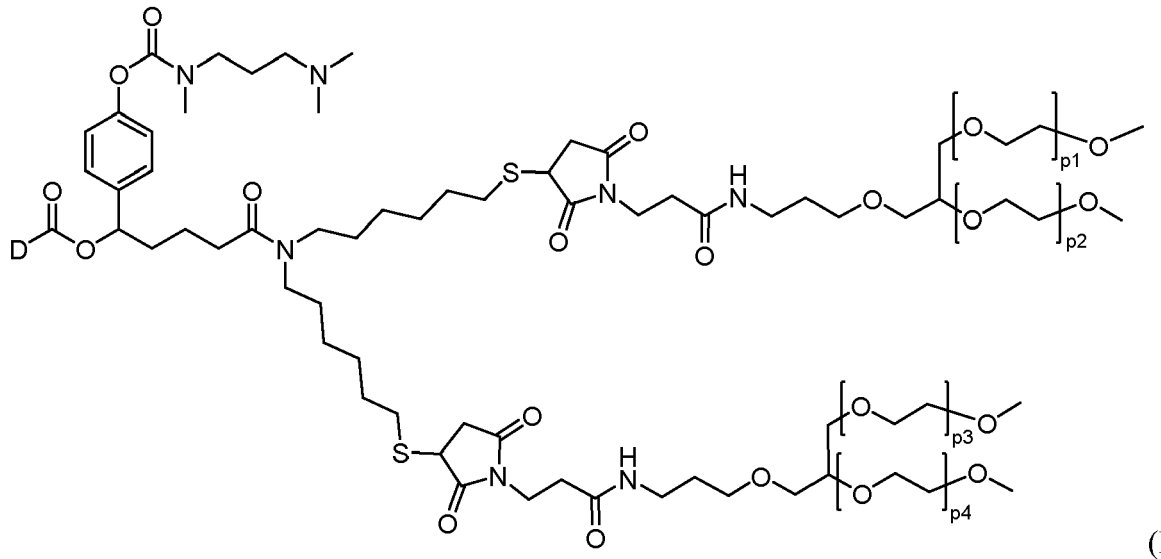
[0007] The viscosity of a pharmaceutical formulation furthermore determines the ability to inject the pharmaceutical formulation through fine gauge needles. With increasing viscosity larger diameter needles are required to ensure that the pharmaceutical formulation can be injected within an acceptable timeframe.

[0008] 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.

[0009] 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.

[0010] It is therefore an object of the present invention to at least partially overcome the above-described shortcomings.

[0011] This object is achieved with a compound of formula (IV)



(IV),

wherein

-D is a hGH polypeptide of SEQ ID NO:1 connected to the rest of the molecule through an amine functional group provided by a lysine side chain; and
each p1, p2, p3, p4 is independently an integer ranging from 210 to 240.

[0012] It was now surprisingly found that the compound of the present invention exhibits various unexpected properties.

[0013] 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 compounds 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 compound of the present invention compared to, for example, compound 36 of WO2009/133137 A2.

[0014] 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.

[0015] It was also surprisingly found that the compound of the present also has surprising advantages with regard to its manufacturing process. Purification of the compound 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.

[0016] Furthermore, if the compound 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.

[0017] Within the present invention the terms are used with the meaning as follows:
As used herein, the term "human growth hormone (hGH)" refers to the polypeptide of SEQ ID NO:1.

[0018] SEQ ID NO:1 has the following sequence:

FPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQTSLCFSESIPT
PSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEE
GIQTLMGRLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETF
LRIVQCRSVEGSCGF

[0019] 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.

[0020] 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".

[0021] 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.

[0022] As used herein, the term "free form" of a drug means the drug in its unmodified, pharmacologically active form.

[0023] As used herein the term "liquid formulation" means a formulation comprising the compound of the present invention and at least one solvent. A preferred solvent is water.

[0024] As used herein the term "dry formulation" means that the formulation comprising the compound 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 the compound 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 compound 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.

[0025] 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.

[0026] As used herein, the term "excipient" refers to a diluent, adjuvant, or vehicle with which the therapeutic is administered.

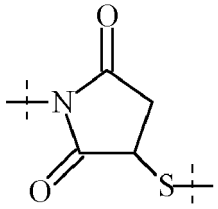
[0027] The term "water soluble" as in a "water-soluble moiety" is a moiety that is soluble in water at room temperature. Typically, a solution of a water-soluble moiety will transmit at least about 75%, more preferably at least about 95% of light, transmitted by the same solution after filtering. On 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.

[0028] 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.

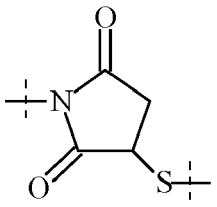
[0029] As used herein, the term "functional group" means a group of atoms which can react with other functional groups. Functional groups include the following groups: carboxylic acid (-(C=O)OH), primary or secondary amine ($-NH_2$, -NH-), maleimide, thiol (-SH), sulfonic acid (-(O=S=O)OH), carbonate, 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.

[0030] 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.

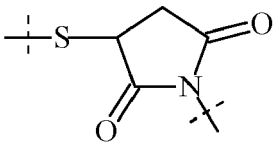
[0031] It is understood that if the sequence or chemical structure of a group of atoms is provided which group of atoms is attached to two moieties or is interrupting a moiety, said sequence or chemical structure can be attached to the two moieties in either orientation, unless explicitly stated otherwise. 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



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[0032] In case the compounds according to formula (IV) comprises 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 (IV) 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 (IV) 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 (IV) 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 (IV) 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 (IV) 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.

[0033] 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.

[0034] 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.

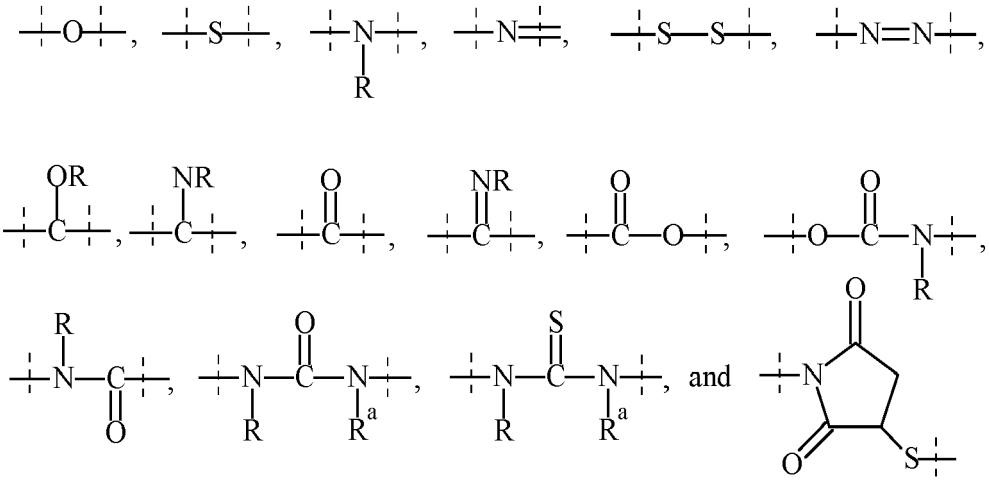
[0035] As used herein, the term "polymeric" means a reagent or a moiety comprising one or more polymer(s).

[0036] 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.

[0037] 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.

[0038] 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".

[0039] 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.

[0040] More preferably, the one or more further optional substituents are independently of each other selected from the group consisting of halogen, $-CN$, $-COOR^{x1}$, $-OR^{x1}$, $-C(O)R^{x1}$, $-C(O)N(R^{x1}R^{x1a})$, $-S(O)_2N(R^{x1}R^{x1a})$, $-S(O)N(R^{x1}R^{x1a})$, $-S(O)_2R^{x1}$, $-S(O)R^{x1}$, $-N(R^{x1})S(O)_2N(R^{x1a}R^{x1b})$, $-SR^{x1}$, $-N(R^{x1}R^{x1a})$, $-NO_2$, $-OC(O)R^{x1}$, $-N(R^{x1})C(O)R^{x1a}$, $-N(R^{x1})S(O)_2R^{x1a}$, $-N(R^{x1})S(O)R^{x1a}$, $-N(R^{x1})C(O)OR^{x1a}$, $-N(R^{x1})C(O)N(R^{x1a}R^{x1b})$, $-OC(O)N(R^{x1}R^{x1a})$, $-T^0$, C_{1-10} 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), $-COOR^{x4}$, $-OR^{x4}$, $-C(O)R^{x4}$, $-C(O)N(R^{x4}R^{x4a})$, $-S(O)_2N(R^{x4}R^{x4a})$, $-S(O)N(R^{x4}R^{x4a})$, $-S(O)_2R^{x4}$, $-S(O)R^{x4}$, $-N(R^{x4})S(O)_2N(R^{x4a}R^{x4b})$, $-SR^{x4}$, $-N(R^{x4}R^{x4a})$, $-NO_2$, $-OC(O)R^{x4}$, $-N(R^{x4})C(O)R^{x4a}$, $-N(R^{x4})S(O)_2R^{x4a}$, $-N(R^{x4})S(O)R^{x4a}$, $-N(R^{X4})C(O)OR^{x4a}$, $-N(R^{x4})C(O)N(R^{x4a}R^{x4b})$, $-OC(O)N(R^{x4}R^{x4a})$, 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^{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;

[0041] Even more preferably, the one or more further optional substituents are independently of each other selected from the group consisting of halogen, $-CN$, $-COOR^{x1}$, $-OR^{x1}$, $-C(O)R^{x1}$, $-C(O)N(R^{x1}R^{x1a})$, $-S(O)_2N(R^{x1}R^{x1a})$, $-S(O)N(R^{x1}R^{x1a})$, $-S(O)_2R^{x1}$, $-S(O)R^{x1}$, $-N(R^{x1})S(O)_2N(R^{x1a}R^{x1b})$, $-SR^{x1}$, $-N(R^{x1}R^{x1a})$, $-NO_2$, $-OC(O)R^{x1}$, $-N(R^{x1})C(O)R^{x1a}$, $-N(R^{x1})S(O)_2R^{x1a}$, $-N(R^{x1})S(O)R^{x1a}$, $-N(R^{x1})C(O)OR^{x1a}$, $-N(R^{x1})C(O)N(R^{x1a}R^{x1b})$, $-OC(O)N(R^{x1}R^{x1a})$, $-T^0$, C_{1-6} 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.

[0042] 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.

[0043] 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)$_2$N(R^{z3})-, -S(O)N(R^{z3})-, -S(O)$_2$-, -S(O)-, -N(R^{z3})S(O)$_2$N(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)$_2$N(R^{z4})-, -S(O)N(R^{z4})-, -S(O)$_2$-, -S(O)-, -N(R^{z4})S(O)$_2$N(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)$_2$N($R^{z5}R^{z5a}$), -S(O)N($R^{z5}R^{z5a}$), -S(O)$_2R^{z5}$, -S(O)R^{z5}, -N(R^{z5})S(O)$_2$N($R^{z5a}R^{z5b}$), -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.

[0044] 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)$_2$N(R^{z1})-, -S(O)N(R^{z1})-, -S(O)$_2$-, -S(O)-, -N(R^{z1})S(O)$_2$N(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)$_2$N(R^{z3})-, -S(O)N(R^{z3})-, -S(O)$_2$-, -S(O)-, -N(R^{z3})S(O)$_2$N(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)$_2$N(R^{z4})-, -S(O)N(R^{z4})-, -S(O)$_2$-, -S(O)-, -N(R^{z4})S(O)$_2$N(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)$_2$N($R^{z5}R^{z5a}$), -S(O)N($R^{z5}R^{z5a}$), -S(O)$_2R^{z5}$, -S(O)R^{z5}, -N(R^{z5})S(O)$_2$N($R^{z5a}R^{z5b}$), -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; 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.

[0045] 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)$_2$N(R^{z1})-, -S(O)N(R^{z1})-, -S(O)$_2$-, -S(O)-, -N(R^{z1})S(O)$_2$N(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)$_2$N(R^{z3})-, -S(O)N(R^{z3})-, -S(O)$_2$-, -S(O)-, -N(R^{z3})S(O)$_2$N(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.

[0046] 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.

[0047] 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.

[0048] 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.

[0049] 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.

[0050] 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 -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 C_{2-6} alkenyl group, then an example for such C_{2-6} alkenyl is -C≡C-. 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.

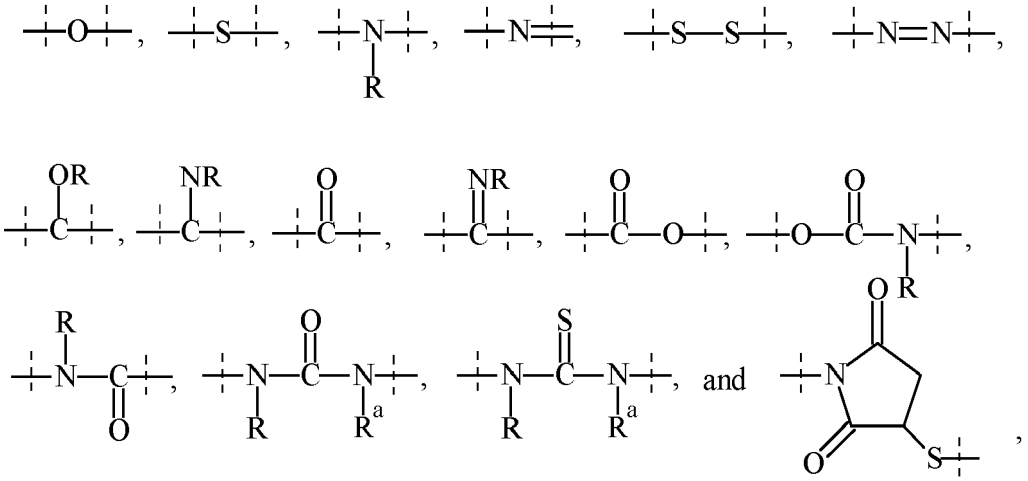
[0051] Accordingly, the term "C_{2-10} alkenyl", "C_{2-20} alkenyl" or "C_{2-50} alkenyl" alone or in combination means a straight-chain or branched hydrocarbon moiety comprising at least one carbon-carbon double bond having 2 to 10, 2 to 20 or 2 to 50 carbon atoms. Each hydrogen atom of a C_{2-10} alkenyl, 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.

[0052] 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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 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.

[0058] 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-aza-spiro[4.5]decane or bridged heterocycles like 8-aza-bicyclo[3.2.1]octane. Each hydrogen atom of an 8- to 11-membered

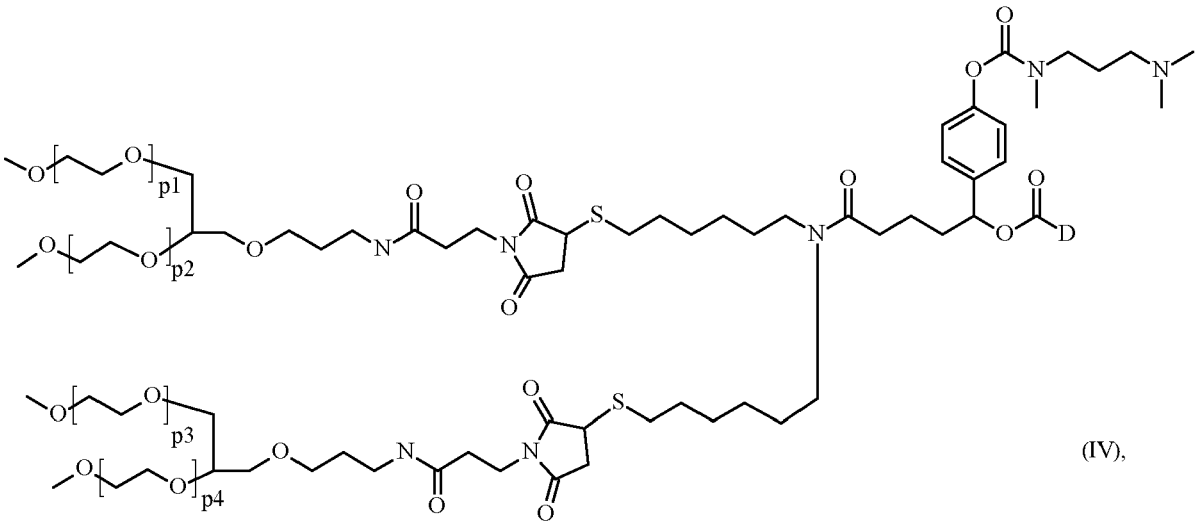
heterobicyclyl or 8- to 11-membered heterobicycle carbon may be replaced by a substituent as defined below.

[0059] 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.

[0060] As used herein, "halogen" means fluoro, chloro, bromo or iodo. It is generally preferred that halogen is fluoro or chloro.

[0061] In general, the term "comprise" or "comprising" also encompasses "consist of' or "consisting of'.

[0062] The compound 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 210 to 240, preferably from 220 to 240.

[0063] - D of formula (IV) is connected to the rest of the molecule through an amine provided by a lysine side chain.

[0064] Another aspect of the present invention is a pharmaceutical formulation comprising at least one compound of formula (IV) and at least one excipient.

[0065] In one embodiment the pharmaceutical formulation is a liquid formulation comprising at least one compound of formula (IV) and at least one excipient.

[0066] Preferably, such liquid formulation comprises from 3 to 300 mg/mL of the compound of formula (IVI (corresponding to 1 to 100 mg hGH equivalents/mL). More preferably the liquid formulation comprises from 9 to 150 mg/mL of the compound of formula (IV) (corresponding to 3 to 50 mg hGH equivalents/mL). Even more preferably the liquid formulation comprises from 15 to 120 mg/mL of the compound of formula (IV) (corresponding to 5 to 40 mg hGH equivalents/mL). Even more preferably the liquid formulation comprises from 30 to 45 mg/mL of the compound of formula (IV) (corresponding to 10 to 15 mg hGH equivalents/mL) or equally preferably the liquid formulation comprises from 75 to 105 mg/mL of the compound of the formula (IV) (corresponding to 25 to 30 mg hGH equivalents/mL). In a particularly preferred embodiment thereof, the liquid formulation comprises 42 or 84 mg/mL of the compound of formula (IV) (corresponding to 14 or 28 mg hGH equivalents/mL).

[0067] The liquid formulation of the compound according to the present invention may comprise one or more excipients. 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. The liquid formulation may comprise one or more than one of the following excipients:

(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 $Mg(OH)_2$ or $ZnCO_3$ may be also used.

(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.

(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, thimerosol, sorbic acid, potassium sorbate, benzoic acid, chlorocresol, and benzalkonium chloride.

(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 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.

(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 gelatines. 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.

(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) Spreading or diffusing agent: modifies the permeability of connective tissue through the hydrolysis of components of the extracellular matrix in the intrastitial space such as hyaluronic acid, a polysaccharide found in the intercellular space of connective tissue. A spreading agent such as hyaluronidase temporarily decreases the viscosity of the extracellular matrix and promotes diffusion of injected drugs.

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

[0068] The liquid formulation of the compound according to the present invention comprises one or more buffering agents. Preferred are such buffering agents which have a pharmaceutically sufficient buffer capacity in the desired pH range. In a preferred embodiment thereof the buffering agent is selected from the group consisting of sodium phosphate, bicarbonate, succinate, histidine, citrate and acetate. Most preferably the buffering agent is succinate. Usually the pH is adjusted by using succinic acid in a concentration of 5-50 mM, more preferably in a concentration of 10 mM and titrating the solution with Tris-base, more preferably with a 1 molar Tris-base solution to the desired pH.

[0069] In a preferred embodiment the pH of a liquid formulation of the present invention ranges from pH 1 to pH 10, more preferably ranges from pH 3 to pH 7, even more preferably ranges from pH 4 to pH 6, even more preferably ranges from pH 4.5 to 5.5 and most preferably has a pH of 5.0. Preferably a buffer concentration and pH is chosen to minimize hGH release during storage, as well as to minimize deamidation, aggregation and precipitation of hGH.

[0070] Preferably, the liquid formulation of the compound of the present invention comprises one or more oxidation protection agent such as antioxidants or chelating agents. A preferred antioxidant is methionine.

[0071] In one embodiment the liquid formulation of the present invention comprises trehalose.

[0072] In one embodiment the liquid formulation of the present invention comprises one or more preservative and/or antimicrobial, such as, for example benzylalcohol and/or cresol.

[0073] In one embodiment the liquid formulation of the present invention comprises the compound of the present invention, an oxidation protection agent and a buffering agent; even more preferably the compound of the present invention, an oxidation protection agent, a stabilizer and a buffering agent.

[0074] Preferably, the liquid formulation of the present invention comprises the compound of the present invention, methionine and succinate; even more preferably the compound of the present invention, methionine, succinate and trehalose, optionally as dihydrate.

[0075] Optionally, the liquid formulation of the present invention also comprises benzylalcohol and/or cresol.

[0076] Preferably, the liquid formulation of the present invention comprises

the compound of formula (IV)	3-300 mg/ml
succinic acid	5-50 mM
optionally trehalose dihydrate	25-150 mg/ml
optionally methionine	1-50 mM

and has a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.The amount of the compound of formula (IV) corresponds to 1-100 mg hGH equivalents/ml.

[0077] More preferably, the liquid formulation of the present invention comprises

the compound of formula (IV)	3-300 mg/ml
succinic acid	5-50 mM
optionally trehalose dihydrate	50-90 mg/ml
optionally methionine	1-50 mM

and has a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.The amount of of the compound of formula (IV) corresponds to 1-100 mg hGH equivalents/ml.

[0078] More preferably, the liquid formulation of the present invention comprises

the compound of formula (IV)	9-150 mg/ml
succinic acid	5-50 mM
optionally trehalose dihydrate	50-90 mg/ml
optionally methionine	1-50 mM

and has a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.The amount of of the compound of formula (IV) corresponds to 1-100 mg hGH equivalents/ml.

[0079] Even more preferably, the liquid formulation of the present invention comprises

the compound of formula (IV)	15-120 mg/ml
succinic acid	5-40 mM
optionally trehalose dihydrate	60-86 mg/ml
optionally methionine	5-40 mM

and has a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution. The amount of of the compound of formula (IV) corresponds to 5-40 mg hGH equivalents/ml.

[0080] Even more preferably, the liquid formulation of the present invention comprises

the compound of formula (IV)	30-45 mg/ml
succinic acid	5-20 mM
optionally trehalose dihydrate	75-86 mg/ml
optionally methionine	5-20 mM

and has a pH ranging from pH 4.5 to pH 5.5 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.The amount of of the compound of formula (IV) corresponds to 10-15 mg hGH equivalents/ml.

[0081] In an equally preferred embodiment, the liquid formulation of the present invention comprises

the compound of formula (IV)	75-105 mg/ml
succinic acid	5-20 mM
optionally trehalose dihydrate	60-81 mg/ml
optionally methionine	5-20 mM

and has a pH ranging from pH 4.5 to pH 5.5 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.The amount of of the compound of formula (IV) corresponds to 25-35 mg hGH equivalents/ml.

[0082] In a preferred embodiment, the liquid formulation of the present invention comprises

the compound of formula (IV)	42 mg/ml
succinic acid	10 mM
optionally trehalose dihydrate	79-86 mg/ml
optionally methionine	10 mM

and has a pH ranging from pH 4.5 to pH 5.5 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution; wherein the compound is the compound of formula (IV). The amount of the compound of formula (IV) corresponds to 14 mg hGH equivalents/ml.

[0083] In another preferred embodiment the liquid formulation of the present invention comprises

the compound of formula (IV)	84 mg/ml
succinic acid	10 mM
optionally trehalose dihydrate	70-80 mg/ml
optionally methionine	10 mM

and has a pH ranging from pH 4.5 to pH 5.5 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.The amount of the compound of formula (IV) corresponds to 28 mg hGH equivalents/ml.

[0084] In one embodiment the liquid formulation of the present invention comprises at least one further biologically active agent, either in its free form or as a prodrug, and wherein the at least one further biologically active agents is preferably selected from the group consisting of IGF-1, ghrelin and ghrelin-like compounds, gonadotropin releasing hormone agonists, gonadotropin releasing hormone analogs, growth hormone releasing factor, growth hormone releasing factor analogs, gonadal steroids, antiandrogens, non-steroidal aromatase inhibitors, HIV combination therapy, free fatty acid regulators, anabolic steroids, estrogen agonists and antagonists, propranolol, appetite suppressants, osteroporosis drugs (including bisphosphonates, bone formation agents, estrogens, parathyroid hormones, selective receptor modulators, and/or anti-diabetic drugs such as insulin, thiazolidinediones, sulfonyl ureas, incretin memetics, meglitinides, biguanides, alpha-glucosidase inhibitors and amylin analogues). Preferably, the at least one additional biological active agent is in its free form.

[0085] In another embodiment the pharmaceutical formulation of the present invention is a dry formulation.

[0086] Preferably, such dry pharmaceutical formulation comprises from 1 to 99.9% (w/w), more preferably from 1.9 to 89% (w/), even more preferably from 3 to 83% (w/w), even more preferably from 9.0 to 71% (w/w), even more preferably from 15 to 63% (w/w), even more preferably from 26 to 36% (w/w) or from 48 to 62% (w/w) and most preferably from 32 to 34% (w/w) or 50 to 54% (w/w) of the compound of formula (IV).

[0087] Preferably, the dry pharmaceutical formulation of the present invention comprises at least one lyoprotectant. The at least one lyoprotectant is preferably selected from the group consisting of amino acids, methylamines, lyotropic salts, polyols, propylene glycol, polyethylene glycol, pluronics, hydroxyalkyl starches, and combinations thereof.

[0088] If the lyoprotectant is an amino acid it is preferably selected from the group consisting of monosodium glutamate and histidine.

[0089] If the lyoprotectant is a polyol, it is preferably selected from the group consisting of sucrose, trehalose, glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol and mannitol.

[0090] If the lyoprotectant is a methylamine, it is preferably betaine.

[0091] If the lyoprotectant is a lyotropic salt, it is preferably magnesium sulfate.

[0092] If the lyoprotectant is a hydroxyalkyl starch, it is preferably hydroxyethyl starch.

[0093] In a preferred embodiment, the lyoprotectant is a non-reducing sugar. Even more preferably, the lyoprotectant

is trehalose or sucrose. Most preferably the lyoprotectant is trehalose.

[0094] Preferably, the dry pharmaceutical formulation of the present invention comprises from 8 to 97% (w/w), more preferably from 14 to 96% (w/w), even more preferably from 24 to 90% (w/w), even more preferably from 32 to 84% (w/w), even more preferably from 60 to 73% (w/w) or from 35 to 52% (w/w) and most preferably 64-66% (w/w) or 45-48% (w/w) of the at least one lyoprotectant, preferably trehalose dihydrate.

[0095] Preferably, the dry formulation of the present invention comprises at least one buffering agent. Preferably the buffering agent is selected from the group consisting of sodium phosphate, bicarbonate, succinate, histidine, citrate and acetate. Most preferably the buffering agent is succinate. Preferably the pH is adjusted by using succinic acid in a concentration of 5-50 mM, more preferably in a concentration of 10 mM and titrating the solution with Tris-base, more preferably with a 1 molar Tris-base solution to the desired pH.

[0096] Preferably, the dry formulation is obtained by a process comprising the steps of

(a) Providing a liquid formulation comprising

the compound of formula (IV)	3-300 mg/ml
succinic acid	5-50 mM
optionally trehalose dihydrate	25-150 mg/ml

and having a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution; and

(b) Drying the liquid formulation of step (a).

[0097] More preferably, the liquid formulation of step (a) comprises

the compound of formula (IV)	3-300 mg/ml
succinic acid	5-50 mM
optionally trehalose dihydrate	50-90 mg/ml

and has a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.

[0098] More preferably, the liquid formulation of step (a) comprises

the compound of formula (IV)	9-150 mg/ml
succinic acid	5-50 mM
optionally trehalose dihydrate	50-90 mg/ml

and has a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.

[0099] Even more preferably, the liquid formulation of step (a) comprises

the compound of formula (IV)	15-120 mg/ml
succinic acid	5-40 mM
optionally trehalose dihydrate	60-86 mg/ml

and has a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.

[0100] Even more preferably, the liquid formulation of step (a) comprises

the compound of formula (IV)	30-45 mg/ml
succinic acid	5-20 mM
optionally trehalose dihydrate	75-86 mg/ml

and has a pH ranging from pH 4.5 to pH 5.5 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.

[0101] Even more preferably, the liquid formulation of step (a) comprises

the compound of formula (IV)	75-105 mg/ml
succinic acid	5-20 mM
optionally trehalose dihydrate	60-81 mg/ml

and has a pH ranging from pH 4.5 to pH 5.5 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.

[0102] Most preferably, the liquid formulation of step (a) comprises

the compound of formula (IV)	42 mg/ml
succinic acid	10 mM
optionally trehalose dihydrate	79-86 mg/ml

and has a pH ranging from pH 4.5 to pH 5.5 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.

[0103] In an equally preferred embodiment, the liquid formulation of step (a) comprises

the compound of formula (IV)	84 mg/ml
succinic acid	10 mM
optionally trehalose dihydrate	70-80 mg/ml,

and has a pH ranging from pH 4.5 to pH 5.5 which is titrated using a suitable buffer, preferably using Tris-base, more preferably using a 1 molar Tris-base solution.

[0104] Preferably, in step (b) the liquid formulation is dried by lyophilization.

[0105] In one embodiment the formulation of step (a) comprises at least one further biologically active agent, either in its free form or as a prodrug, and wherein the at least one further biologically active agents is selected from the group consisting of IGF-1, ghrelin and ghrelin-like compounds, gonadotropin releasing hormone agonists, gonadotropin releasing hormone analogs, growth hormone releasing factor, growth hormone releasing factor analogs, gonadal steroids, antiandrogens, non-steroidal aromatase inhibitors, HIV combination therapy, free fatty acid regulators, anabolic steroids, estrogen agonists and antagonists, propranolol, appetite suppressants, osteroporosis drugs (including bisphosphonates, bone formation agents, estrogens, parathyroid hormones, selective receptor modulators, and/or anti-diabetic drugs such as insulin, thiazolidinediones, sulfonyl ureas, incretin memetics, meglitinides, biguanides, alpha-glucosidase inhibitors and amylin analogues). Preferably, the at least one additional biological active agent is in its free form.

[0106] Another aspect of the present invention is a dry formulation comprising based on the total weight of the formulation:

the compound of formula (IV)	2-89% (w/w)
succinic acid	0.4-1.8% (w/w)
trehalose dihydrate	7-97% (w/w)
Tris	0.4-2% (w/w).

[0107] In a preferred embodiment the dry formulation of the present invention comprises based on the total weight of the formulation:

the compound of formula (IV)	3-83% (w/w)
succinic acid	0.6-1.6% (w/w)
trehalose dihydrate	14-96% (w/w)
Tris	0.6-1.7% (w/w)

[0108] In an even more preferred embodiment the dry formulation of the present invention comprises based on the total weight of the formulation:

the compound of formula (IV)	9.0-71% (w/w)
succinic acid	0.6-2.8% (w/w)
trehalose dihydrate	24-90% (w/w)
Tris	0.6-2.9% (w/w)

[0109] In an even more preferred embodiment the dry formulation of the present invention comprises based on the total weight of the formulation:

the compound of formula (IV)	15-63% (w/w)
succinic acid	0.6-2.5% (w/w)
trehalose dihydrate	32-84% (w/w)
Tris	0.6-2.6% (w/w)

[0110] In an even more preferred embodiment the dry formulation of the present invention comprises based on the total weight of the formulation:

the compound of formula (IV)	26-36% (w/w)
succinic acid	0.5-1.9% (w/w)
trehalose dihydrate	60-73% (w/w)
Tris	0.5-1.9% (w/w)

[0111] In an equally preferred embodiment the dry formulation of the present invention comprises based on the total weight of the formulation:

the compound of formula (IV)	48-62% (w/w)
succinic acid	0.4-1.4% (w/w)
trehalose dihydrate	35-52% (w/w)
Tris	0.4-1.4% (w/w)

[0112] Most preferably the dry formulation of the present invention comprises based on the total weight of the formulation:

the compound of formula (IV)	32-34% (w/w)
succinic acid	0.9-1.0% (w/w)
trehalose dihydrate	64-66% (w/w)
Tris	0.5-1.4% (w/w)

[0113] In an equally preferred embodiment the dry formulation of the present invention comprises based on the total weight of the formulation:

the compound of formula (IV)	50-54 (w/w)
succinic acid	0.7-0.8% (w/w)
trehalose dihydrate	45-48% (w/w)
Tris	0.4-1.1% (w/w)

[0114] In one embodiment the dry formulations of the present invention comprise at least one further biologically active agent, either in its free form or as a prodrug, and wherein the at least one further biologically active agents is selected from the group consisting of IGF-1, ghrelin and ghrelin-like compounds, gonadotropin releasing hormone agonists, gonadotropin releasing hormone analogs, growth hormone releasing factor, growth hormone releasing factor analogs, gonadal steroids, antiandrogens, non-steroidal aromatase inhibitors, HIV combination therapy, free fatty acid regulators, anabolic steroids, estrogen agonists and antagonists, propranolol, appetite suppressants, osteroporosis drugs (including bisphosphonates, bone formation agents, estrogens, parathyroid hormones, selective receptor modulators, and/or anti-

diabetic drugs such as insulin, thiazolidinediones, sulfonyl ureas, incretin memetics, meglitinides, biguanides, alpha-glucosidase inhibitors and amylin analogues). Preferably, the at least one additional biological active agent is in its free form.

[0115] Preferably, the dry formulation of the present invention is obtained from lyophilization.

[0116] Preferably, the dry formulation of the present invention is lyophilized in a vial, syringe, dual-chamber syringe, ampoule, cartridge or dual-chamber cartridge.

[0117] A preferred vial is a glass vial.

[0118] In one embodiment the dry formulation of the present invention is lyophilized in a cartridge for use in a pen injector.

[0119] In another embodiment, the dry formulation is lyophilized in a first chamber of a dual-chamber cartridge, of which second chamber is filled with reconstitution solution.

[0120] Prior to administering the dry formulation of the present invention to a patient in need thereof, the dry formulation is reconstituted. Reconstitution can take place in the container in which the dry formulation of compound of formula (IV) is provided, such as in a vial, syringe, dual-chamber syringe, ampoule, cartridge and dual-chamber cartridge, or the dry formulation of the present invention is transferred to a different container and is then reconstituted.

[0121] Reconstitution is done by adding a predefined amount of reconstitution solution to the dry formulation. The reconstitution solution is a sterile liquid, such as water or buffer, which may comprise further additives, such as preservatives and/or antimicrobials.

[0122] In one embodiment the reconstitution solution is sterile water comprising 0.7-1.1% benzyl alcohol, more preferably comprising 0.9% benzyl alcohol. In another embodiment, the reconstitution solution is sterile water comprising 0.2-0.4% cresol, more preferably comprising 0.3 % cresol. Preferably, the reconstitution solution is sterile water.

[0123] Preferably, the pH of the reconstituted formulation of the present invention ranges from pH 1 to pH 10, more preferably ranges from pH 3 to pH 7, even more preferably ranges from pH 4 to pH 6, even more preferably ranges from pH 4.5 to 5.5 and most preferably has a pH of 5.0.

[0124] Another aspect of the present invention is a method of preparing a reconstituted formulation comprising the compound of formula (IV), wherein the method comprises the step of

- contacting the dry pharmaceutical formulation of the present invention with a reconstitution solution.

[0125] Another aspect of the present invention is a reconstituted formulation obtainable from the method of preparing a reconstituted formulation of the present invention.

[0126] Preferably, the reconstituted formulation of the present invention comprises

the compound of formula (IV)	3-300 mg/ml
succinic acid	5-50 mM
trehalose dihydrate	25-150 mg/ml
Tris	1-50 mM

and has a pH ranging from pH 4.0 to pH 6.0.The amount of compound of formula (IV) corresponds to 1-100 mg hGH equivalents/ml.

[0127] Even more preferably, the reconstituted formulation of the present invention comprises

the compound of formula (IV)	3-300 mg/ml
succinic acid	5-50 mM
trehalose dihydrate	50-90 mg/ml
Tris	5-50 mM

and has a pH ranging from pH 4.0 to pH 6.0.The amount of compound of formula (IV) corresponds to 1-100 mg hGH equivalents/ml.

[0128] In an even more preferred embodiment the reconstituted formulation of the present invention comprises

the compound of formula (IV)	9-150 mg/ml
succinic acid	5-50 mM
trehalose dihydrate	50-90 mg/ml
Tris	5-50 mM

and has a pH ranging from pH 4.0 to pH 6.0.formula (IV). The amount of compound of formula (IV) corresponds to 3-50 mg hGH equivalents/ml.

[0129] In an even more preferred embodiment the reconstituted formulation of the present invention comprises

the compound of formula (IV)	15-120 mg/ml
succinic acid	5-40 mM
trehalose dihydrate	60-86 mg/ml
Tris	5-40 mM

and has a pH ranging from pH 4.0 to pH 6.0.The amount of compound of formula (IV) corresponds to 5-40 mg hGH equivalents/ml.

[0130] Even more preferably, the reconstituted formulation of the present invention comprises

the compound of formula (IV)	30-45 mg/ml
succinic acid	5-20 mM
trehalose dihydrate	75-86 mg/ml
Tris	5-20 mM

and has a pH ranging from pH 4.5 to pH 5.5.The amount of the compound of formula (IV) corresponds to 10-15 mg hGH equivalents/ml.

[0131] In an equally preferred embodiment, the reconstituted formulation of the present invention comprises

the compound of formula (IV)	75-105 mg/ml
succinic acid	5-20 mM
trehalose dihydrate	60-81 mg/ml
Tris	5-20 mM

and has a pH ranging from pH 4.5 to pH 5.5.The amount of the compund of formula (IV) corresponds to 25-35 mg hGH equivalents/ml.

[0132] Most preferably the reconstituted formulation of the present invention comprises

the compound of formula (IV)	42 mg/ml
succinic acid	10 mM
trehalose dihydrate	79-86 mg/ml,
Tris	5-15 mM

and has a pH ranging from pH 4.5 to pH 5.5.The amount of the compound of formula (IV) corresponds to 14 mg hGH equivalents/ml.

[0133] In an equally preferred embodiment the reconstituted formulation of the present invention comprises

the compound of formula (IV)	84 mg/ml
succinic acid	10 mM
trehalose dihydrate	70-80 mg/ml
Tris	5-15 mM

and has a pH ranging from pH 4.5 to pH 5.5.The amount of the compound of formula (IV) corresponds to 28 mg hGH equivalents/ml.

[0134] Optionally, the reconstituted formulation comprises one or more preservative and/or antimicrobial. Preferably, the one or more preservative and/or antimicrobial is benzyl alcohol in a concentration of 0.7-1.1% (w/v), more preferably in a concentration of 0.9% (w/v). In another embodiment, the one or more preservative and/or antimicrobial is cresol in a concentration of 0.2-0.4% (w/v), more preferably in a concentration of 0.3 % (w/v).

[0135] The person skilled in the art is well aware that whenever a dry, liquid or reconstituted formulation of the present invention comprises trehalose dihydrate, the dihydrate form could also be exchanged by other hydration forms of trehalose, including anhydrous trehalose. The skilled artisan would have no difficulty in calculating the corresponding

amounts of trehalose in these other hydration forms including anhydrous trehalose comprised in the corresponding dry, liquid or reconstituted formulation. Therefore, it is understood that a dry, liquid or reconstituted formulation comprising trehalose in hydration forms other than dihydrate are also within the scope of the present invention.

[0136] Another aspect of the present invention is the compound of formula (IV), or the liquid, dry or reconstituted pharmaceutical formulation comprising at least one compound of formula (IV), for use as a medicament.

[0137] Another aspect of the present invention is the use of the compound of formula (IV), or the liquid, dry or reconstituted pharmaceutical formulation comprising at least one compound of formula (IV), in a method of treatment of a disease which can be treated with hGH.

[0138] Preferably, said disease which can be treated with hGH 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.

[0139] In one embodiment the disease which can be treated with hGH is GHD in children.

[0140] In another embodiment the disease which can be treated with hGH is GHD in adults.

[0141] In another embodiment the disease which can be treated with hGH is ISS.

[0142] In another embodiment the disease which can be treated with hGH are SHOX gene mutations.

[0143] In another embodiment the disease which can be treated with hGH is TS.

[0144] In another embodiment the disease which can be treated with hGH is NS.

[0145] In another embodiment the disease which can be treated with hGH is PWS.

[0146] In another embodiment the disease which can be treated with hGH is SGA.

[0147] In another embodiment the disease which can be treated with hGH is CRI.

[0148] In another embodiment the disease which can be treated with hGH is wasting due to HIV or AIDS or other malignancies.

[0149] In another embodiment the disease which can be treated with hGH is SBS.

[0150] In another embodiment the disease which can be treated with hGH is sarcopenia.

[0151] In another embodiment the disease which can be treated with hGH is frailty.

[0152] The compound of formula (IV) or the liquid or reconstituted formulation of the present inventionmay be 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 of the invention to brain tissue or brain fluids (e.g., Ommaya Reservoir), 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.

[0153] Preferably, administering the compound of formula (IV) or the liquid or reconstituted formulation of the present invention is via injection, more preferably via subcutaneous injection.

[0154] In a preferred embodiment, the present invention relates to a compound of formula (IV), or the liquid or reconstituted formulation comprising at least one compound of formula (IV), for use in the treatment of GHD in children via subcutaneous injection.

[0155] Also disclosed is a container comprising the compound of formula (IV) or the liquid or reconstituted formulation of the present invention.

[0156] Preferred containers are syringes, dual-chamber syringes, vials, vials with stopper and seal, ampoules, cartridges, and dual-chamber cartridges.

EXAMPLES

Methods

Cation exchange chromatography

[0157] 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

[0158] 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

[0159] 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

[0160] 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

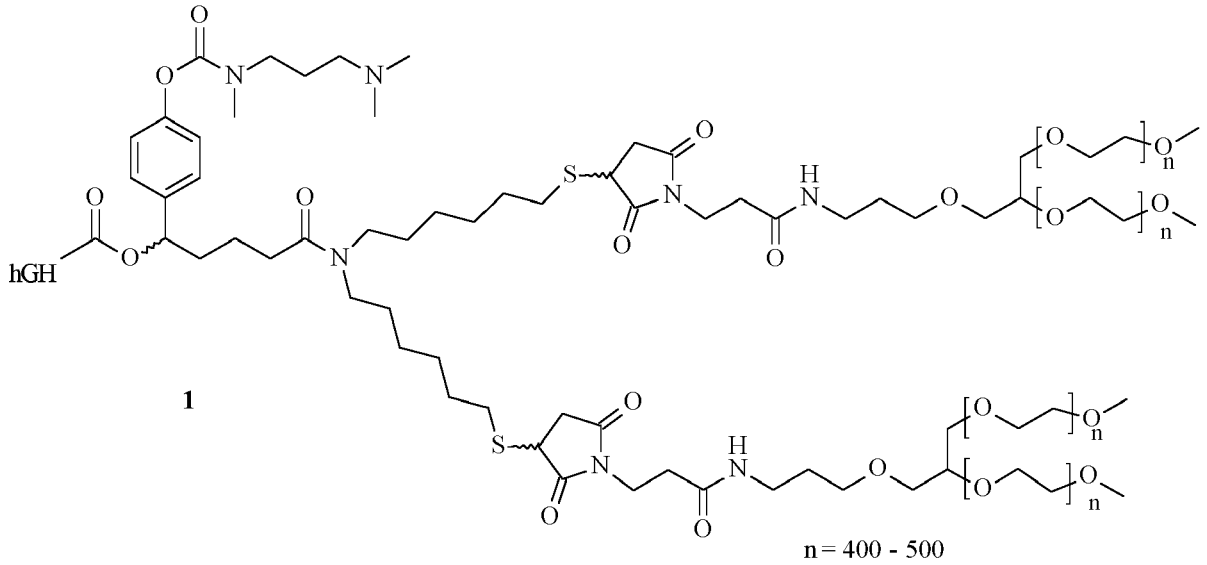
Time [min]	% B
0	25
1	25
8	40
30	60
30.1	90
30.5	90
30.6	25
35	25

Buffer exchange

[0161] 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)

[0162]



1

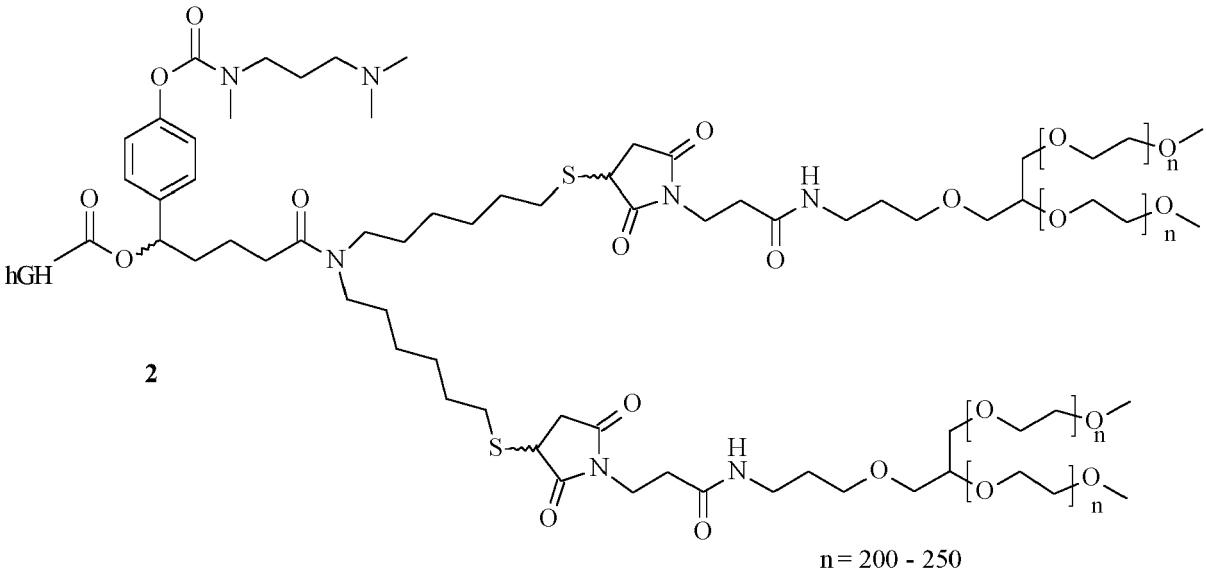
4x 20 kDa mPEG-linker-hGH monoconjugate **1** was synthesized according to a similar procedure as described in WO2009/133137 A2. The formulations of 4× 20 kDa mPEG-linker-hGH monoconjugate **1** as shown in Table 2 were prepared.

Table 2: Formulations of 4× 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 **2**

[0163]



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-pentafluorophenylcarbonate 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 lOkDa mPEG-linker-hGH monoconjugate **2** was purified by cation exchange chromatography.

[0164] Buffer exchange and adjustment to the desired concentration of 4× lOkDa 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 4× lOkDa 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 4× lOkDa mPEG-linker-hGH monoconjugate **2**

Formulation name:	Concentration of 4× lOkDa 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

[0165] 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 4× 20 kDa mPEG-linker-hGH monoconjugate **1.**

[0166] Conclusion:
4x lOkDa 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 4×10kDa mPEG-linker-hGH-conjugate **2** which corresponds to approx. 35 mg hGH eq./mL.

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

[0167] 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 27G×1/2" from BD (Ref 300635, Lot 101009), a 29G needle, 0.33x13mm 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

[0168] Testing was performed using the following steps:

1. Charging of a 1ml Luer-lok Syringe, (BD, Ref 309628) with sample (using a 20G needle, 0.90x40mm , 20G×11/2" from Sterican)
2. Removal of air bubbles
3. Attachment of test needle (starting with the largest inner diameter) onto the syringe
4. Clamping the syringe into the holder
5. Selection of appropriate measuring settings
6. Start measurement and collect the sample in a glass vial (placed underneath the syringe)
7. Removal of syringe from holder
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.

[0169] Formulation buffer without mPEG-linker-hGH monoconjugate **1** or **2** was used as reference solution.

[0170] For all different injection needles and for all injection speeds the injection forces were determined for 4x lOkDa 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 lOkDa 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 lOkDa 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 4× 10kDa mPEG-linker-hGH monoconjugate **2**					Formulation of 4× 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.

[0171] Table 5 shows the comparison of injection forces between 4x lOkDa 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 lOkDa 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 4× 10kDa mPEG-linker-hGH monoconjugate **2**					Formulation of 4× 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.

[0172] Table 6 shows the comparison of injection forces between 4× 10kDa mPEG-linker-hGH monoconjugate **2** and 4x 20kDa mPEG-linker-hGH monoconjugate **1** for the 30G needle 0.30×12mm, 30G×1/2", from Sterican (Lot 2G13258811).

Table 6: Injection forces of 4x lOkDa 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 4× 10kDa mPEG-linker-hGH monoconjugate **2**					Formulation of 4× 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:

[0173] The injectability of 4× 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 lOkDa mPEG-linker-hGH monoconjugate **2** compared to 4x 20kDa mPEG-linker-hGH monoconjugate **1**

[0174] 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}.

[0175] All tested formulations of 4x lOkDa 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 4× 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 lOkDa 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:

[0176] The dynamic viscosity of 4× 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 lOkDa mPEG-linker-hGH monoconjugate **2**

[0177] 1 mL of 4x lOkDa 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 lOkDa mPEG-linker-hGH monoconjugate **2.** The results of this reconstitution procedure are summarized in Table 8.

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

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

Conclusion:

[0178] The time of reconstitution until a clear and virtually bubble free solution is achieved is significantly shorter for 4x lOkDa mPEG-linker-hGH monoconjugate **2** compared to 4x 20kDa mPEG linker-hGH monoconjugate **1.**

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

[0179] For the determination of *in vitro* linker cleavage rates of 4x lOkDa 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 lOkDa 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 lOkDa 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 lOkDa 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:

[0180] The *in vitro* hydrolysis rates of conjugates **1** and **2** at pH 7.4 and 37°C were in the range of 105 $\pm$ 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

[0181] 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.

[0182] **Example 8:** Quantification of total mPEG40 and 80 in serum samples from animal studies 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**

[0183] 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:

[0184]

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

SEQUENCE LISTING

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            20                  25                  30

Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro
        35                  40                  45

Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg
    50                  55                  60

Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu
65                  70                  75                  80

Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val
                85                  90                  95

Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp
            100                 105                 110

Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu
        115                 120                 125

Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser
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Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr
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Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe
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Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe
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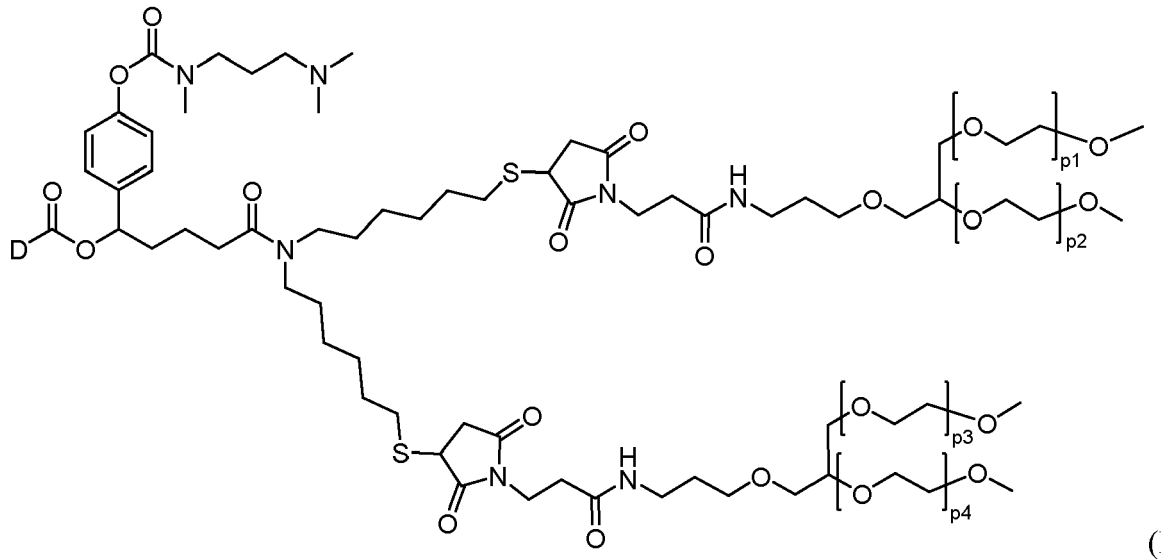
Claims

1. A compound of formula (IV)



(IV),

wherein

D is a hGH polypeptide of SEQ ID NO:1 connected to the rest of the molecule through an amine functional group provided by a lysine side chain; and
each p1, p2, p3, p4 is independently an integer ranging from 210 to 240.

2. The compound according to claim 1, wherein each p1, p2, p3, p4 is independently an integer ranging from 220 to 240.

3. A pharmaceutical formulation comprising the compound of claim 1 or 2 and at least one excipient.

4. The pharmaceutical formulation of claim 3, wherein the pharmaceutical formulation is a liquid formulation comprising

the compound of formula (IV)	15-120 mg/ml
succinic acid	5-40 mM
optionally trehalose dihydrate	60-86 mg/ml
optionally methionine	5-40 mM

having a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer.

5. The pharmaceutical formulation of claim 3 or 4, wherein the pharmaceutical formulation is a liquid formulation comprising

the compound of formula (IV)	30-45 mg/ml
succinic acid	5-20 mM
optionally trehalose dihydrate	75-86 mg/ml
optionally methionine	5-20 mM

having a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer.

6. The pharmaceutical formulation of claim 3 or 4, wherein the pharmaceutical formulation is a liquid formulation comprising

the compound of formula (IV)	75-105 mg/ml
succinic acid	5-20 mM
optionally trehalose dihydrate	60-81 mg/ml
optionally methionine	5-20 mM

having a pH ranging from pH 4.0 to pH 6.0 which is titrated using a suitable buffer.

7. The pharmaceutical formulation of claim 3, wherein the pharmaceutical formulation is a dry formulation comprising

the compound of formula (IV)	15-63% (w/w)
succinic acid	0.6-2.5% (w/w)
trehalose dihydrate	32-84% (w/w)
Tris	0.6-2.6% (w/w)

8. The pharmaceutical formulation of claim 3 or 7, wherein the pharmaceutical formulation is a dry formulation comprising

the compound of formula (IV)	26-36% (w/w)
succinic acid	0.5-1.9% (w/w)
trehalose dihydrate	60-73% (w/w)
Tris	0.5-1.9% (w/w)

9. The pharmaceutical formulation of claim 3 or 7, wherein the pharmaceutical formulation is a dry formulation comprising

the compound of formula (IV)	48-62% (w/w)
succinic acid	0.4-1.4% (w/w)
trehalose dihydrate	35-52% (w/w)
Tris	0.4-1.4% (w/w)

10. The compound of claim 1 or 2 or the pharmaceutical formulation of any one of claims 3 to 9 for use as a medicament.

11. The compound of claim 1 or 2 or the pharmaceutical formulation of any one of claims 3 to 9 for use in a method of treatment of a disease selected from the group consisting of growth hormone deficiency in children, idiopathic short stature, short stature homeobox gene mutations, Turner syndrome, Noonan syndrome, Prader-Willi syndrome, children born small for gestational age, chronic renal insufficiency, growth hormone deficiency in adults, wasting due to HIV or AIDS or other malignancies, short bowel syndrome, sarcopenia, and frailty.

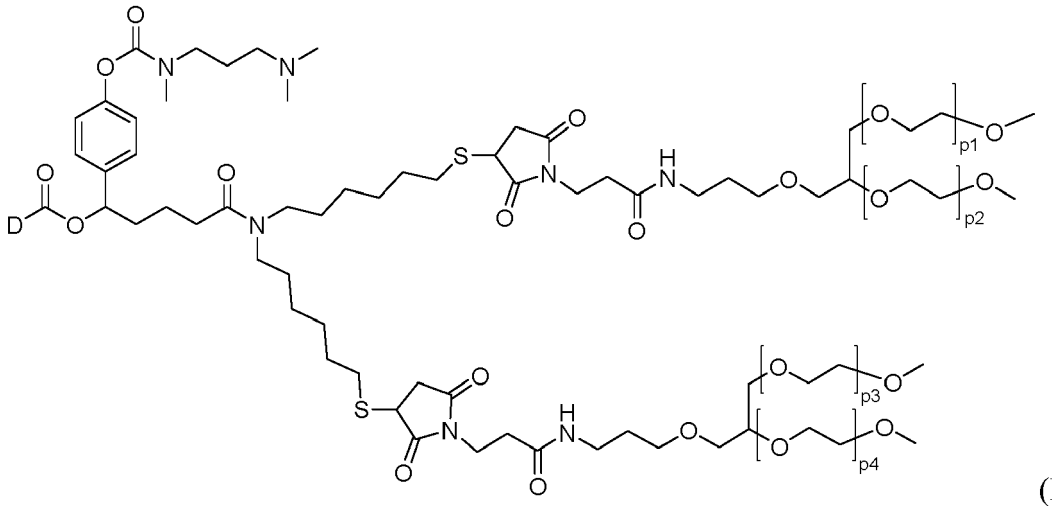
12. The compound or the pharmaceutical formulation for use of claim 11, wherein the disease is growth hormone deficiency in children.

13. The compound or the pharmaceutical formulation for use of claim 11, wherein the disease is growth hormone deficiency in adults.

14. The compound or the pharmaceutical formulation for use of claim 11, wherein the disease is children born small for gestational age.

Patentansprüche

1. Eine Verbindung der Formel (IV)



(IV),

wobei

D ein hGH-Polypeptid der SEQ ID NO:1 ist, das verbunden ist mit dem Rest des Moleküls durch eine funktionelle Amingruppe, die von einer Lysin-Seitenkette bereitgestellt wird; und
jedes p1, p2, p3, p4 unabhängig eine ganze Zahl im Bereich von 210 bis 240 ist.

2. Die Verbindung nach Anspruch 1, wobei jedes p1, p2, p3, p4 unabhängig eine ganze Zahl im Bereich von 220 bis 240 ist.

3. Eine pharmazeutische Formulierung enthaltend die Verbindung nach Anspruch 1 oder 2 und mindestens einen Exzipienten.

4. Die pharmazeutische Formulierung nach Anspruch 3, wobei die pharmazeutische Formulierung eine flüssige Formulierung ist enthaltend

die Verbindung der Formel (IV)	15-120 mg/ml
Bernsteinsäure	5-40 mM
optional Trehalose-Dihydrat	60-86 mg/ml
optional Methionin	5-40 mM

mit einem pH im Bereich von pH 4.0 bis pH 6.0, welcher mit einem geeigneten Puffer titriert wird.

5. Die pharmazeutische Formulierung nach Anspruch 3 oder 4, wobei die pharmazeutische Formulierung eine flüssige Formulierung ist enthaltend

die Verbindung der Formel (IV)	30-45 mg/ml
Bernsteinsäure	5-20 mM
optional Trehalose-Dihydrat	75-86 mg/ml
optional Methionin	5-20 mM

mit einem pH im Bereich von pH 4.0 bis pH 6.0, welcher mit einem geeigneten Puffer titriert wird.

6. Die pharmazeutische Formulierung nach Anspruch 3 oder 4, wobei die pharmazeutische Formulierung eine flüssige Formulierung ist enthaltend

die Verbindung der Formel (IV)	75-105 mg/ml
Bernsteinsäure	5-20 mM
optional Trehalose-Dihydrat	60-81 mg/ml

(fortgesetzt)

optional Methionin	5-20 mM

mit einem pH im Bereich von pH 4.0 bis pH 6.0, welcher mit einem geeigneten Puffer titriert wird.

7. Die pharmazeutische Formulierung nach Anspruch 3, wobei die pharmazeutische Formulierung eine trockene Formulierung ist enthaltend

die Verbindung der Formel (IV)	15-63% (w/w)
Bernsteinsäure	0.6-2.5% (w/w)
Trehalose-Dihydrat	32-84% (w/w)
Tris	0.6-2.6% (w/w)

8. Die pharmazeutische Formulierung nach Anspruch 3 oder 7, wobei die pharmazeutische Formulierung eine trockene Formulierung ist enthaltend

die Verbindung der Formel (IV)	26-36% (w/w)
Bernsteinsäure	0.5-1.9% (w/w)
Trehalose-Dihydrat	60-73% (w/w)
Tris	0.5-1.9% (w/w)

9. Die pharmazeutische Formulierung nach Anspruch 3 oder 7, wobei die pharmazeutische Formulierung eine trockene Formulierung ist enthaltend

die Verbindung der Formel (IV)	48-62% (w/w)
Bernsteinsäure	0.4-1.4% (w/w)
Trehalose-Dihydrat	35-52% (w/w)
Tris	0.4-1.4% (w/w)

10. Die Verbindung nach Anspruch 1 oder 2 oder die pharmazeutische Formulierung nach einem der Ansprüche 3 bis 9 zur Verwendung als Medikament.

11. Die Verbindung nach Anspruch 1 oder 2 oder die pharmazeutische Formulierung nach einem der Ansprüche 3 bis 9 zur Verwendung in einem Verfahren zur Behandlung einer Krankheit ausgewählt aus der Gruppe bestehend aus Wachstumshormonmangel bei Kindern, idiopathischer Kleinwuchs, Kleinwuchs-Homöobox-Genmutationen, Turner-Syndrom, Noonan-Syndrom, Prader-Willi-Syndrom, zu klein für das Gestationsalter geborene Kinder (Small for Gestational Age), chronische Niereninsuffizienz, Wachstumshormonmangel bei Erwachsenen, Auszehrung durch HIV oder AIDS oder andere Malignitäten, Kurzdarmsyndrom, Sarkopenie und Gebrechlichkeit.

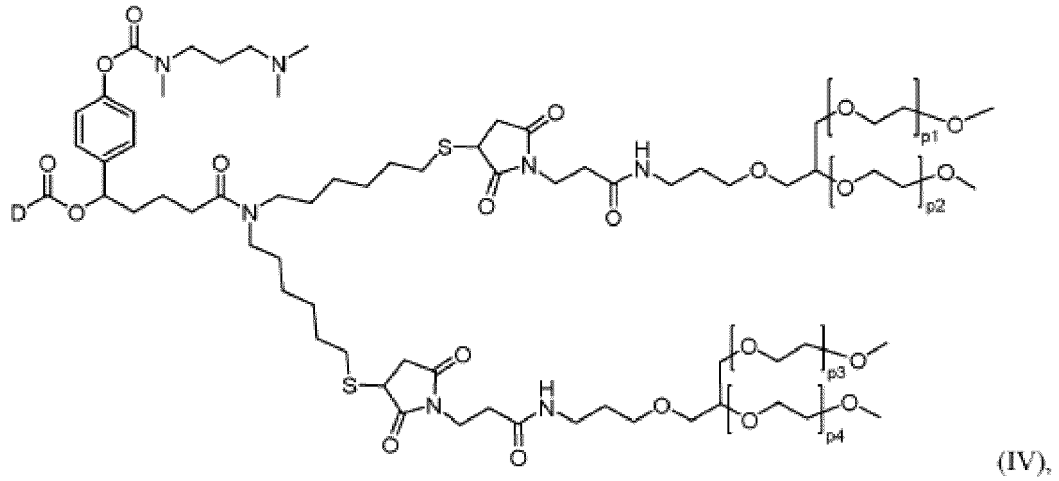
12. Die Verbindung oder die pharmazeutische Formulierung zur Verwendung nach Anspruch 11, wobei die Krankheit Wachstumshormonmangel bei Kindern ist.

13. Die Verbindung oder die pharmazeutische Formulierung zur Verwendung nach Anspruch 11, wobei die Krankheit Wachstumshormonmangel bei Erwachsenen ist.

14. Die Verbindung oder die pharmazeutische Formulierung zur Verwendung nach Anspruch 11, wobei die Krankheit zu klein für das Gestationsalter geborene Kinder (Small for Gestational Age) ist.

Revendications

1. Composé de formule (IV)



(IV),

D étant un polypeptide hGH de SEQ ID N°:1 relié au reste de la molécule par l'intermédiaire d'un groupe fonctionnel de type amine fourni par une chaîne latérale de lysine ; et
chaque p1, p2, p3, p4 étant indépendamment un entier dans la plage de 210 à 240.

2. Composé selon la revendication 1, chaque p1, p2, p3, p4 étant indépendamment un entier dans la plage de 220 à 240.

3. Formulation pharmaceutique comprenant le composé selon la revendication 1 ou 2 et au moins un excipient.

4. Formulation pharmaceutique selon la revendication 3, la formulation pharmaceutique étant une formulation liquide comprenant

le composé de formule (IV)	15 à 120 mg/ml
acide succinique	5 à 40 mM
éventuellement dihydrate de tréhalose	60 à 86 mg/ml
éventuellement méthionine	5 à 40 mM

possédant un pH dans la plage de pH 4,0 à pH 6,0 qui est titré à l'aide d'un tampon approprié.

5. Formulation pharmaceutique selon la revendication 3 ou 4, la formulation pharmaceutique étant une formulation liquide comprenant

le composé de formule (IV)	30 à 45 mg/ml
acide succinique	5 à 20 mM
éventuellement dihydrate de tréhalose	75 à 86 mg/ml
éventuellement méthionine	5 à 20 mM

possédant un pH dans la plage de pH 4,0 à pH 6,0 qui est titré à l'aide d'un tampon approprié.

6. Formulation pharmaceutique selon la revendication 3 ou 4, la formulation pharmaceutique étant une formulation liquide comprenant

le composé de formule (IV)	75 à 105 mg/ml
acide succinique	5 à 20 mM
éventuellement dihydrate de tréhalose	60 à 81 mg/ml
éventuellement méthionine	5 à 20 mM

possédant un pH dans la plage de pH 4,0 à pH 6,0 qui est titré à l'aide d'un tampon approprié.

7. Formulation pharmaceutique selon la revendication 3, la formulation pharmaceutique étant une formulation sèche comprenant

le composé de formule (IV)	15 à 63 % (p/p)
acide succinique	0,6 à 2,5 % (p/p)
dihydrate de tréhalose	32 à 84 % (p/p)
Tris	0,6 à 2,6 % (p/p).

8. Formulation pharmaceutique selon la revendication 3 ou 7, la formulation pharmaceutique étant une formulation sèche comprenant

le composé de formule (IV)	26 à 36 % (p/p)
acide succinique	0,5 à 1,9 % (p/p)
dihydrate de tréhalose	60 à 73 % (p/p)
Tris	0,5 à 1,9 %(p/p).

9. Formulation pharmaceutique selon la revendication 3 ou 7, la formulation pharmaceutique étant une formulation sèche comprenant

le composé de formule (IV)	48 à 62 % (p/p)
acide succinique	0,4 à 1,4 % (p/p)
dihydrate de tréhalose	35 à 52 % (p/p)
Tris	0,4 à 1,4 %(p/p).

10. Composé selon la revendication 1 ou 2 ou formulation pharmaceutique selon l'une quelconque des revendications 3 à 9 pour une utilisation en tant que médicament.

11. Composé selon la revendication 1 ou 2 ou formulation pharmaceutique selon l'une quelconque des revendications 3 à 9 pour une utilisation dans un procédé de traitement d'une maladie choisie dans le groupe constitué par une déficience en hormone de croissance chez des enfants, une petite taille idiopathique, des mutations du gène de l'homéoboîte de la petite taille, le syndrome de Turner, le syndrome de Noonan, le syndrome de Prader-Willi, des enfants nés petits pour leur âge gestationnel, une insuffisance rénale chronique, une déficience d'hormone de croissance chez des adultes, une émaciation due au VIH ou au SIDA ou d'autres affections malignes, le syndrome de l'intestin court, la sarcopénie, et la fragilité.

12. Composé ou formulation pharmaceutique pour une utilisation selon la revendication 11, la maladie étant une déficience d'hormone de croissance chez des enfants.

13. Composé ou formulation pharmaceutique pour une utilisation selon la revendication 11, la maladie étant une déficience d'hormone de croissance chez des adultes.

14. Composé ou formulation pharmaceutique pour une utilisation selon la revendication 11, la maladie étant des enfants nés petits pour leur âge gestationnel.

REFERENCES CITED IN THE DESCRIPTION

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