Prodrugs comprising an GLP-1/Glucagon dual agonist linker hyaluronic acid conjugate

The present invention relates to GLP-1/Glucagon dual agonist prodrugs, pharmaceutical compositions comprising said prodrugs as well as their use as a medicament for treating or preventing diseases or disorders which can be treated by a GLP-1/Glucagon dual agonist, for example in the treatment of disorders of the metabolic syndrome, including diabetes and obesity, as well as for reduction of excess food intake.

BACKGROUND OF THE INVENTION

GLP-1 agonists

Exendin-4 is a 39-amino acid peptide, isolated from the salivary secretions of the venomous Gila monster (Heloderma suspectum). It has some sequence similarity to several members of the glucagon-like peptide family, with the highest homology of 53% being to glucagon-like peptide-1 [7-36]-amide (GLP-1). Exendin-4 acts as a agonist on the GLP-1 receptor and bears GLP-1-like insulin sectretagogue action in isolated rat islets. Exendin-4 is a high potency agonist and truncated GLP-1 agonist-(9-39)-amide is an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. (see e.g. J. Biol. Chem. 268(26):19650-19655). Exendin-4 ("exenatide") was approved recently in the US and EU for improving glycemic control in patients with type 2 diabetes taking metformin and/or a sulfonylurea but have not achieved adequate glycemic control.

The amino acid sequence of exendin-4 is shown as SEQ ID NO: 1

HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2

The amino acid sequence of GLP-1(7-36)-amide is shown as SEQ ID NO 2

HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-NH_2

Glucagon is a 29-amino acid peptide which is released into the bloodstream when circulating glucose is low. Glucagon's amino acid sequence is shown in SEQ ID NO 3.

HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH

During hypoglycemia, when blood glucose levels drop below normal, glucagon signals the liver to break down glycogen and release glucose, causing an increase of blood glucose levels to reach a normal level. Hypoglycemia is a common side effect of insulin treated patients with hyperglycemia (elevated blood glucose levels) due to diabetes. Thus, glucagon's most predominant role in glucose regulation is to counteract insulin action and maintain blood glucose levels.

Holst (Holst, J. J. Physiol. Rev. 2007, 87, 1409) and Meier (Meier, J. J. Nat. Rev. Endocrinol. 2012, 8, 728) describe that GLP-1 receptor agonists, such as GLP-1, liraglutide and exendin-4, have 3 major pharmacological activities to improve glycemic control in patients with T2DM by reducing fasting and postprandial glucose (FPG and PPG): (i) increased glucose-dependent insulin secretion (improved first- and second-phase), (ii) glucagon suppressing activity under hyperglycemic conditions, (iii) delay of gastric emptying rate resulting in retarded absorption of meal-derived glucose.

GLP-1/Glucagon (Glc) agonists

Pocai et al (Obesity. 2012;20:1566–1571; Diabetes 2009, 58, 2258) and Day et al. (Nat Chem Biol 2009;5:749) describe that dual activation of the GLP-1 and glucagon receptors, e.g., by combining the actions of GLP-1 and glucagon in one molecule leads to a therapeutic principle with anti-diabetic action and a pronounced weight lowering effect.

Peptides which bind and activate both the glucagon and the GLP-1 receptor (Hjort et al. Journal of Biological Chemistry, 269, 30121-30124,1994; Day JW et al, Nature Chem Biol, 5: 749-757, 2009) and suppress body weight gain and reduce food intake are described in patent applications WO 2008/071972, WO 2008/101017, WO 2009/155258, WO 2010/096052, WO 2010/096142, WO 2011/075393, WO 2008/152403, WO 2010/070251, WO 2010/070252, WO 2010/070253, WO 2010/070255, WO 2011/160630, WO 2011/006497, WO 2011/152181, WO

2011/152182, WO2011/117415, WO2011/117416, and WO 2006/134340, the contents of which are herein incorporated by reference.

In addition, triple co-agonist peptides which not only activate the GLP-1 and the glucagon receptor, but also the GIP receptor (Gastric inhibitory polypeptide) are described in WO 2012/088116 and by VA Gault et al (Biochem Pharmacol, 85, 16655-16662, 2013; Diabetologia, 56, 1417-1424, 2013).

Bloom et al. (WO 2006/134340) disclose that peptides which bind and activate both the glucagon and the GLP-1 receptor can be constructed as hybrid molecules from glucagon and exendin-4, where the N-terminal part (e.g. residues 1-14 or 1-24) originate from glucagon and the C-terminal part (e.g. residues 15-39 or 25-39) originate from exendin-4.

Otzen et al (Biochemistry, 45, 14503-14512, 2006) disclose that N- and C-terminal hydrophobic patches are involved in fibrillation of glucagon, due to the hydrophobicity and/or high β-sheet propensity of the underlying residues.

WO2014/056872 discloses peptides which bind and activate both the glucagon and the GLP-1 receptor that are derived from exendin-4 wherein at least the aminoacid at position 14 bear a side chain for a prolonged halflife.

Peptides used in this invention are exendin-4 peptide analogues comprising leucine in position 10 and glutamine in position 13 and are described in WO2015/086731, WO2015/086732, WO2015/086733.

Peptides also used in this invention are exendin-4 peptide analogues comprising beta-alanine in position 28 and are described in EP15305899.5.

Further a peptide is used in this invention which is a exendin-4 peptide analogue comprising glutamine at position 3, beta-alanine in position 28 and D-alanines in position 29 and 34.

Peptides used in this invention preferably are soluble not only at neutral pH, but also at pH 4.5. Also the chemical stability at pH values of 4.5 to 5 is an important criterion for the long acting prodrug product. The prodrug is preferably formulated in this pH range in order to obtain a shelflife from at least 6 month at 4°C.

Longacting GLP-1/Glucagon agonists

Ideally, the peptide is formulated in a fashion that provides for a sustained plasma level in human for at least one week after application to a human body resulting in a once-weekly or longer injection frequency.

Current therapy with a long acting GLP-1 agonists is Bydureon® which is exendin-4 in a depot suspension for a once weekly injection based on poly(glycol-co lactic acid) using a 23 gauge needle.

WO2012/173422 describes a GLP-1/Glucagon agonist conjugated to the Fc region of an immunoglobulin for weekly administration wherein the peptide is derived from oxyntomodulin.

Carrier linked prodrugs

To enhance physicochemical or pharmacokinetic properties of a drug in vivo, such as its half-live, such drug can be conjugated with a carrier. If the drug is transiently bound to a carrier and/or a linker, such systems are commonly assigned as carrier-linked prodrugs. According to the definitions provided by IUPAC (as given under http://www.chem.qmul.ac.uk/iupac.medchem, accessed on July 22, 2009), a carrier-linked prodrug is a prodrug that contains a temporary linkage of a given active substance with a transient carrier group that produces improved physicochemical or pharmacokinetic properties and that can be easily removed *in vivo*, usually by a hydrolytic cleavage.

The linkers employed in such carrier-linked prodrugs may be transient, meaning that they are non-enzymatically hydrolytically degradable (cleavable) under physiological conditions (aqueous buffer at pH 7.4, 37°C) with half-lives ranging from, for example, one hour to three months. Suitable carriers are polymers and can either be directly conjugated to the linker or via a non-cleavable spacer.

Transient polymer conjugation through traceless prodrug linkers combines the advantages of prolonged residence time due to polymer attachment and the recovery of the original pharmacology of the native peptide after release from the polymer conjugate.

Using polymer-linker peptide conjugates, native unchanged peptide is slowly released after application to a patient, governed only by release kinetics of the linker and pharmacokinetics of the polymer carrier. Ideally, release kinetics would be independent from the presence of enzymes like proteases or esterases in body fluids to guarantee a consistent and homogenous release pattern.

WO2008/148839, WO2009/095479 and WO2012/035139 refer to prodrugs comprising drug linker conjugates, where the linker is covalently attached via a cleavable bond to a biologically active moiety, such as the GLP 1- agonist exendin-4. The biologically active moiety is released from the prodrug upon cyclization-activation by cyclic imide formation.The release kinetic is dependent on the pH value and is minimum for storage of the prodrug at pH values from 4.5 to 5 and reach its intended release rate at physiological pH of around 7.4 to 7.5. An GLP-1 agonist-prodrug is described in which the linker is based on L-alanine and the polymeric carrier is a PEG-lysine based hydrogel. Not described are dual GLP-1/Glucagon agonist-prodrugs.

Hyaluronic acid (HA)

Dhal et al (Journal of Biomedical Nanotechnology, vol 9, 2013, 1 – 13) report hyaluronic acid as a suitable carrier for drug conjugates. Kong et al. (Biomaterials 31 (2010), 4121-4128) report an exendin-4-hyaluronic acid conjugate which showed an glucose lowering effect over 3 days in mice. The used HA was a linear polymer with a drug load ranging from about 2.4 to 12.%.

In the present invention hydrogels of crosslinked hyaluronic acid are chosen due to their longer residence time as a local depot at the application site than soluble HA. Important criteria for the use of hyaluronic acid (HA) as a carrier polymer is the achievable drug load in the final drug product which is determined by the drug load on the polymer itself and the concentration of the final solution/suspension. Giving the fact that the injection volume for subcutaneous drug depots is practically limited to equal/less than 1 mL, preferably equal/less than 0.6 mL.

The more concentrated the polymer solutions/suspensions of HA is, the more viscous is the formulation which has a negative impact on the syringability of the prodrug formulation. Viscous solutions need injection needles of a larger diameter to limit the

force on the plunger of which the syringe is pressed. Also the time for injection is longer.

It is an object of the present invention to provide a GLP-1/Glucagon agonist prodrug for administering as a subcutaneous depot which releases a GLP-1/Glucagon agonist in an active form over the time period of at least 6 days between administrations and which can be injected through 26 gauge needles or even needles of smaller inner diameter for good patient compliance.

An object of the invention is a prodrug or a pharmaceutically acceptable salt thereof comprising a drug linker conjugate of formula (I)

$$Z - L^1 - L^2 - L - Y - R^{20} \qquad (I)$$

wherein Y is a peptide moiety having the formula (II)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-Asp-Glu-Gln-X18-Ala-X20-X21-Phe-Ile-Glu-Trp-Leu-Ile-X28-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (II)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Arg and Lys
X20 represents an amino acid residue selected from Lys, Gln and His,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val,

or wherein Y is a peptide moiety having the formula (III)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-Asp-Glu-Gln-X18-Ala-X20-X21-Phe-Ile-Glu-Trp-Leu-Ile-X28-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (III)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Leu and His
X20 represents an amino acid residue selected from His, Arg, Lys, and Gln,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Lys, Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val,

or

wherein Y is a peptide moiety having the formula (IV)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Leu-Leu-Asp-Glu-Gln-X18-Ala-Lys-Asp-Phe-Ile-Glu-Trp-Leu-Ile-Ala-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (IV)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Arg and Leu,
X32 represents an amino acid residue selected from Ser and Val,

or

wherein Y is a peptide moiety having the formula (IVa)

H_2N-His-Aib-His-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-X15-Glu-Gln-Leu-Ala-Arg-Asp-Phe-Ile-Glu-Trp-Leu-Ile-Bal-X29-Gly-X31-X32-Ser-X34-X35-Pro-Pro-Pro-X39-R^{20} (IVa)

X15 represents an amino acid residue selected from Asp and Glu, (pref. Asp)
X29 represents an amino acid residue selected from Gly, D-Ala and Pro, (pref) Gly, D-Ala
X31 represents an amino acid residue selected from Pro, His and Trp, (pref. Pro)
X32 represents an amino acid residue selected from Ser, His, Pro and Arg, (pref. Ser, His, Pro),
X34 represents an amino acid residue selected from Gly and D-Ala,

X35 represents an amino acid residue selected from Ala, Pro and Lys, (pref. Ala, Pro)

X39 represents Ser or Pro-Pro-Pro,

or

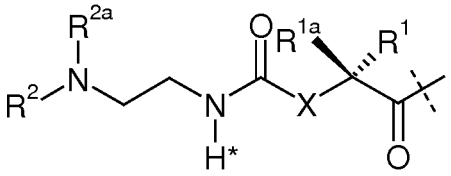
wherein Y is a peptide moiety having the formula (IVb)

H_2N-His-Aib-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Leu-Leu-Glu-Glu-Gln-Arg-Ala-Arg-Glu-Phe-Ile-Glu-Trp-Leu-Ile-Bal-D-Ala-Gly-Pro-Pro-Ser-D-Ala -Ala-Pro-Pro-Pro-Ser-R^{20};

or a salt or solvate thereof;

R^{20} is OH or NH_2;

L is a linker of formula (Ia),



Ia

wherein the dashed line indicates the attachment to the N-Terminus of Y by forming an amide bond;

X is $C(R^4R^{4a})$; $N(R^4)$;

R^1, R^{1a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

R^2, R^{2a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

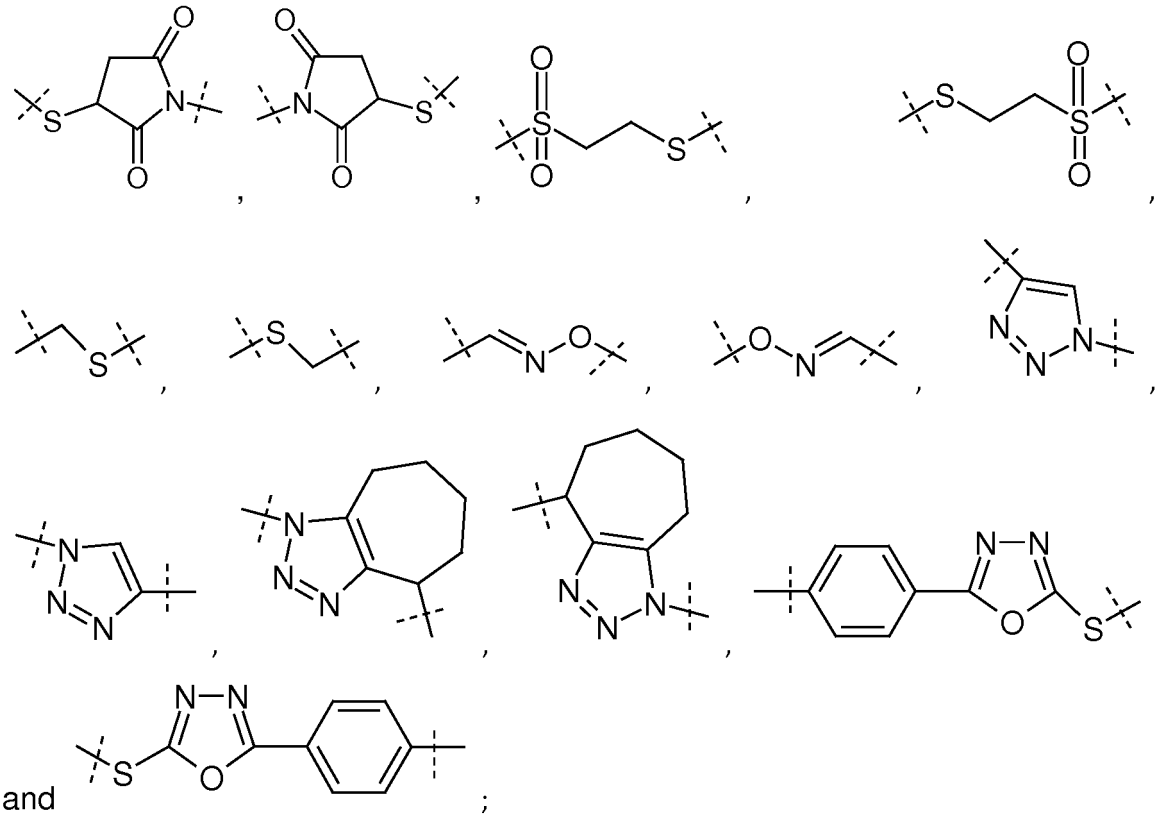
R^4, R^{4a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

wherein R^2 , R^{2a}, R^4 or R^{4a} is substituted with one group L^2-L^1-Z; wherein

L^2 is a single chemical bond or is a C_{1-20} alkyl chain, which is optionally interrupted by one or more groups independently selected from -O- and $C(O)N(R^{3aa})$ and is optionally

substituted with one or more groups independently selected from OH and C(O)N($R^{3aa}R^{3aaa}$), wherein R^{3aa} and R^{3aaa} are independently selected from the group consisting of H and C_{1-4} alkyl; and

L^2 is attached to L^1 via a terminal group selected from the group consisting of



and ;

wherein L^2 is attached to the one position indicated with the dashed line and and L^1 is attached to the position indicated with the other dashed line; and

L^1 is a C_{1-20} alkyl chain, which is optionally interrupted by one or more groups independently selected from -O- and C(O)N(R^{5aa}) and is optionally substituted with one or more groups independently selected from OH and C(O)N($R^{5aa}R^{5aaa}$), wherein R^{5aa} and R^{5aaa} are independently selected from the group consisting of H and C_{1-4} alkyl; and

L^1 is attached to Z via a terminal amino group forming an amide bond with the carboxy group of the beta-1,3-D-glucuronic acid of the hyaluronic acid of Z;

Z is a crosslinked hyaluronic acid hydrogel, in which

0.05 to 20 % of the monomeric disaccharide units are crosslinked by a crosslinker; and 0.2 to 8.5 % of the monomeric disaccharide units bear L^1-L^2 -L-Y-R^{20}.groups.

The present invention relates to a prodrug which provides a GLP-1/Glucagon agonist release from a subcutaneous depot in an active form over the time period of at least 6 days between administrations

This helps patients to reduce the frequency of injections, while being able to maintain optimal control the plasma levels of GLP-1/Glucagon agonist and consequently blood glucose.

Further advantages of the crosslinked hyaluronic acid carrier of the invention are the good injectability through a 26 gauge needle or even a needle of a smaller inner diameter.

LEGENDS TO THE FIGURES

Figure 1a, Figure 1b, Figure 1c show various crosslinking chemistries to synthesize hyaluronic acid hydrogels.

Figure 2a. Magnetic Resonance (MR) images of the HA hydrogel at the injection site, taken at different time points.

Figure 2b. Magnetic Resonance (MR) images of the polymer suspension containing 1:1 (w/w) HA hydrogel- 800 kDa soluble HA at the injection site, taken at different time points.

Figure 3. In vitro release kinetics of Exendin-4 Seq ID No. 26 dual agonist with weekly linker from the HA hydrogel (example 18). The half-life is 5 days.

Figure 4 In vivo Effect of HA Hydrogel – Dual Agonist (Seq ID No. 26) Conjugate on Non-fasting Blood Glucose in db/db Mice: shown is the the difference level of blood glucose in mmol/L versus the treatment time of the animals for different doses .

Figure 4b In vivo Effect of HA Hydrogel – Dual Agonist (Seq ID No. 26) Conjugate on Non-fasting Blood Glucose in db/db Mice: shown is the the difference level of blood

glucose in mmol/L versus the treatment time of the animals for different hydrogel constructs: soluble and crosslinked hyaluronic acid.

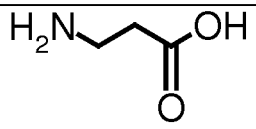
Figure 4c In vivo Effect of HA Hydrogel – Dual agonists (Seq ID No. 45, 46 and 48) Conjugates on Non-fasting Blood Glucose in db/db Mice: shown is the level of blood glucose in mmol/L versus the treatment time of the animals.

Figure 4d In vivo Effect of HA Hydrogel – Dual Agonist (Seq ID No. 49) Conjugate on Non-fasting Blood Glucose in db/db Mice: shown is the level of blood glucose in mmol/L versus the treatment time of the animals.

Figure 5 Injectability study: Extrusion Force on syringe plunger for HA hydrogels of different peptide loading.

Detailed Description

The amino acid sequences of the present invention contain the conventional one letter and three letter codes for naturally occurring amino acids, as well as generally accepted three letter codes for other amino acids, such as Aib (α-aminoisobutyric acid). Furthermore, the following code was used for the amino acid shown in the table below.

Name	Structure	code
beta alanine		Bal
D-alanine		D-Ala

GLP-1/Glucagon agonist bound to linker is referred to as “GLP-1/Glucagon agonist moiety”.

“Protective groups” refers to a moiety which temporarily protects a chemical functional group of a molecule during synthesis to obtain chemoselectivity in subsequent chemical reactions. Protective groups for alcohols are, for example, benzyl and trityl, protective groups for amines are, for example, tert-butyloxycarbonyl, 9-fluorenylmethyloxycarbonyl

and benzyl and for thiols examples of protective groups are 2,4,6-trimethoxybenzyl, phenylthiomethyl, acetamidomethyl, p-methoxybenzyloxycarbonyl, tert-butylthio, triphenylmethyl, 3-nitro-2-pyridylthio, 4-methyltrityl.

"Protected functional groups" means a chemical functional group protected by a protective group.

"Acylating agent" means a moiety of the structure R-(C=O)-, providing the acyl group in an acylation reaction, optionally connected to a leaving group, such as acid chloride, N-hydroxy succinimide, pentafluorphenol and para-nitrophenol.

"Alkyl" means a straight-chain or branched carbon chain. Each hydrogen of an alkyl carbon may be replaced by a substituent.

"Aryl" refers to any substituent derived from a monocyclic or polycyclic or fused aromatic ring, including heterocyclic rings, e.g. phenyl, thiophene, indolyl, napthyl, pyridyl, which may optionally be further substituted.

"Acyl" means a chemical functional group of the structure R-(C=O)-, wherein R is an alkyl or aryl.

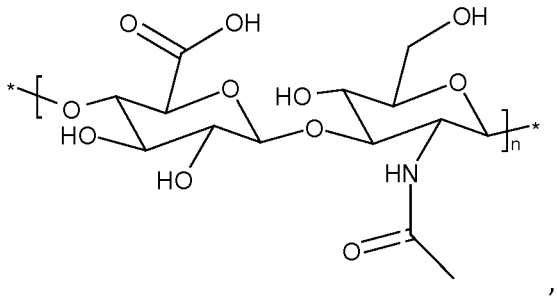
"C_{1-4} alkyl" means an alkyl chain having 1 - 4 carbon atoms, e.g. if present at the end of a molecule: methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl tert-butyl, or e.g. $-CH_2-$, $-CH_2-CH_2-$, $-CH(CH_3)-$, $-CH_2-CH_2-CH_2-$, $-CH(C_2H_5)-$, $-C(CH_3)_2-$, when two moieties of a molecule are linked by the alkyl group. Each hydrogen of a C_{1-4} alkyl carbon may be replaced by a substituent.

"C_{1-6} alkyl" means an alkyl chain having 1 - 6 carbon atoms, e.g. if present at the end of a molecule: C_{1-4} alkyl, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl; tert-butyl, n-pentyl, n-hexyl, or e.g. $-CH_2-$, $-CH_2-CH_2-$, $-CH(CH_3)-$, $-CH_2-CH_2-CH_2-$, $-CH(C_2H_5)-$, $-C(CH_3)_2-$, when two moieties of a molecule are linked by the alkyl group. Each hydrogen of a C_{1-6} alkyl carbon may be replaced by a substituent.

Accordingly, "C_{1-18} alkyl" means an alkyl chain having 1 to 18 carbon atoms and "C_{8-18} alkyl" means an alkyl chain having 8 to 18 carbon atoms. Accordingly, "C_{1-50} alkyl" means an alkyl chain having 1 to 50 carbon atoms.

"Halogen" means fluoro, chloro, bromo or iodo. It is generally preferred that halogen is fluoro or chloro.

"Hyaluronic acid" means a polymer of a disaccharide composed of of beta-1,3-D-glucuronic acid and beta-1,4-N-acetyl-D-glucosamine and their respective sodium salts. These polymers are linear.



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"Disaccharide unit" means the disaccharide composed of beta-1,3-D-glucuronic acid and beta-1,4-N-acetyl-D-glucosamine and their respective sodium salts and is the monomeric building block for HA.

"Crosslinked hyaluronic acid" means a polymer of hyaluronic acid" wherein different chains of HA are covalently connected by a crosslinker, forming a 3-dimensional polymer network. The degree of crosslinking refers the molar ratio of disaccharide units to crosslinker units in the polymer network.

Crosslinked hyaluronic acid" may be derived by different methods. Reaction of HA with the crosslinker, reaction of modified (activated) HA with the crosslinker, reaction of two different modified HA with the crosslinker. Examples may be described in Oh et al, Journal of Controlled Release 141 (2010), 2-12. Example 11 decribes the crosslinking of unmodified HA with divinylsulfone. Further methods for preparing crosslinked HA are also depicted in Figure 1a, Figure 1b and Figure 1c: aldehyde (diol oxidation) and subsequent amine reductive amination, hydroxyl mediated alkylation, amide formation

reaction, Michael Addition Crosslinking (Thiol – maleimide), diol – epoxide chemistry and others.

"Crosslinker" may be a linear or branched molecule or chemical group, preferably is a linear molecule with at least chemical functional groups on each distal ends

"functionalized hyaluronic acid" means a polymer of hyaluronic acid" wherein HA is chemically modified with a group L^1 which bears a chemical functional chemical group at its distal end. The degree of functionalization refers the molar ratio of disaccharide units to L^1 units in the polymer.

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The term "chemical functional group" refers to but not limited to carboxylic acid and activated derivatives, amino, maleimide, thiol and derivatives, sulfonic acid and derivatives, carbonate and derivatives, carbamate and derivatives, hydroxyl, aldehyde, ketone, hydrazine, isocyanate, isothiocyanate, phosphoric acid and derivatives, phosphonic acid and derivatives, haloacetyl, alkyl halides, acryloyl and other alpha-beta unsaturated michael acceptors, arylating agents like aryl fluorides, hydroxylamine, disulfides like pyridyl disulfide, vinyl sulfone, vinyl ketone, diazoalkanes, diazoacetyl compounds, oxirane, and aziridine.

If a chemical functional group is coupled to another chemical functional group, the resulting chemical structure is referred to as "linkage". For example, the reaction of an amine group with a carboxyl group results in an amide linkage.

"Reactive functional groups" are chemical functional groups of the backbone moiety, which are connected to the hyperbranched moiety.

"Functional group" is the collective term used for "reactive functional group", "degradable interconnected functional group", or "conjugate functional group".

The terms "blocking group" or "capping group" are used synonymously and refer to moieties which are irreversibly connected to reactive functional groups to render them incapable of reacting with for example chemical functional groups.

The terms “protecting group” or “protective group” refers to a moiety which is reversibly connected to reactive functional groups to render them incapable of reacting with for example other chemical functional groups under specific conditions.

The term “derivatives” refers to chemical functional groups suitably substituted with protecting and/or activation groups or to activated forms of a corresponding chemical functional group which are known to the person skilled in the art. For example, activated forms of carboxyl groups include but are not limited to active esters, such as succinimidyl ester, benzotriazyl ester, nitrophenyl ester, pentafluorophenyl ester, azabenzotriazyl ester, acyl halogenides, mixed or symmetrical anhydrides, acyl imidazole.

The term “non-enzymatically cleavable linker” refers to linkers that are hydrolytically degradable under physiological conditions without enzymatic activity.

The terms “spacer”, “spacer group”, “spacer molecule”, and “spacer moiety” are used interchangeably and if used to describe a moiety present in the hydrogel carrier of the invention, refer to any moiety suitable for connecting two moieties, such as C_{1-50} alkyl, which fragment is optionally interrupted by one or more groups selected from -NH-, -N(C_{1-4} alkyl)-, -O-, -S-, -C(O)-, -C(O)NH-, -C(O)N(C_{1-4} alkyl)-, -O-C(O)-, -S(O)-, -$S(O)_2$-.

The terms “terminal”, “terminus” or “distal end” refer to the position of a functional group or linkage within a molecule or moiety, whereby such functional group may be a chemical functional group and the linkage may be a degradable or permanent linkage, characterized by being located adjacent to or within a linkage between two moieties or at the end of an oligomeric or polymeric chain.

The phrases “in bound form” or “moiety” refer to sub-structures which are part of a larger molecule. The phrase “in bound form” is used to simplify reference to moieties by naming or listing reagents, starting materials or hypothetical starting materials well known in the art, and whereby “in bound form” means that for example one or more hydrogen radicals (–H), or one or more activating or protecting groups present in the reagents or starting materials are not present in the moiety.

It is understood that all reagents and moieties comprising polymeric moieties refer to macromolecular entities known to exhibit variabilities with respect to molecular weight, chain lengths or degree of polymerization, or the number of functional groups. Structures shown for crosslinking reagents, and crosslinked moieties are thus only representative examples.

A reagent or moiety may be linear or branched. If the reagent or moiety has two terminal groups, it is referred to as a linear reagent or moiety. If the reagent or moiety has more than two terminal groups, it is considered to be a branched or multi-functional reagent or moiety.

The linkers employed in such carrier-linked prodrugs are transient, meaning that they are non-enzymatically hydrolytically degradable (cleavable) under physiological conditions (aqueous buffer at pH 7.4, 37°C) with half-lives ranging from, for example, one hour to three months.

The term “GLP-1/Glucagon agonist hydrogel prodrug” refers to carrier-linked prodrugs of GLP-1/Glucagon agonist, wherein the carrier is a hydrogel. The terms “hydrogel prodrug” and “hydrogel-linked prodrug” refer to prodrugs of biologically active agents transiently linked to a hydrogel and are used synonymously.

A “hydrogel” may be defined as a three-dimensional, hydrophilic or amphiphilic polymeric network capable of taking up large quantities of water. The networks are composed of homopolymers or copolymers, are insoluble due to the presence of covalent chemical or physical (ionic, hydrophobic interactions, entanglements) 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. The chains of the network are connected in such a fashion that pores exist and that a substantial fraction of these pores are of dimensions between 1 nm and 1000 nm.

“Free form” of a drug refers to a drug, specifically to GLP-1/Glucagon agonist, in its unmodified, pharmacologically active form, such as after being released from a polymer conjugate.

The terms "drug", "biologically active molecule", "biologically active moiety", "biologically active agent", "active agent", are used synonymously and refer to GLP-1/Glucagon agonist, either in its bound or free form.

A "therapeutically effective amount" of GLP-1/Glucagon agonist as used herein means an amount sufficient to cure, alleviate or partially arrest the clinical manifestations of a given disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective amount". Effective amounts for each purpose will depend on the severity of the disease or injury as well as the weight and general state of the subject. It will be understood that determining an appropriate dosage may be achieved using routine experimentation, by constructing a matrix of values and testing different points in the matrix, which are all within the ordinary skills of a trained physician.

“Stable” and “stability” means that within the indicated storage time the hydrogel conjugates remain conjugated and do not hydrolyze to a substantial extent and exhibit an acceptable impurity profile relating to GLP-1/Glucagon agonist. To be considered stable, the composition contains less than 5% of the drug in its free form.

The term “pharmaceutically acceptable” means approved by a regulatory agency such as the EMEA (Europe) and/or the FDA (US) and/or any other national regulatory agenciesy for use in animals, preferably in humans.

"Pharmaceutical composition" or “composition” means one or more active ingredients, and one or more inert ingredients, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of the present invention and a pharmaceutically acceptable excipient (pharmaceutically acceptable carrier).

“Dry composition” means that the GLP-1/Glucagon agonist hydrogel prodrug composition is provided in a dry form in a container. Suitable methods for drying are for

example spray-drying and lyophilization (freeze-drying). Such dry composition of GLP-1/Glucagon agonist hydrogel prodrug has a residual water content of a maximum of 10 %, preferably less than 5% and more preferably less than 2% (determined according to Karl Fischer method). The preferred method of drying is lyophilization. "Lyophilized composition" means that the GLP-1/Glucagon agonist hydrogel polymer prodrug composition was first frozen and subsequently subjected to water reduction by means of reduced pressure. This terminology does not exclude additional drying steps which occur in the manufacturing process prior to filling the composition into the final container.

"Lyophilization" (freeze-drying) is a dehydration process, characterized by freezing a composition and then reducing the surrounding pressure and, optionally, adding heat to allow the frozen water in the composition to sublime directly from the solid phase to gas. Typically, the sublimed water is collected by desublimation.

"Reconstitution" means the addition of a liquid to a dry composition to bring it into the form of a liquid or suspension composition. It is understood that the term "reconstitution" is not limited to the addition of water, but refers to the addition of any liquid, including for example buffers or other aqueous solutions.

"Reconstitution solution" refers to the liquid used to reconstitute the dry composition of an GLP-1/Glucagon agonist hydrogel prodrug prior to administration to a patient in need thereof.

"Container" means any container in which the GLP-1/Glucagon agonist hydrogel prodrug composition is comprised and can be stored until reconstitution.

"Buffer" or "buffering agent" refers to chemical compounds that maintain the pH in a desired range. Physiologically tolerated buffers are, for example, sodium phosphate, succinate, histidine, bicarbonate, citrate and acetate, pyruvate. Antacids such as $Mg(OH)_2$ or $ZnCO_3$ may be also used. Buffering capacity may be adjusted to match the conditions most sensitive to pH stability.

“Excipients” refers to compounds administered together with the therapeutic agent, for example, buffering agents, isotonicity modifiers, preservatives, stabilizers, anti-adsorption agents, oxidation protection agents, or other auxiliary agents. However, in some cases, one excipient may have dual or triple functions.

A "lyoprotectant" is a molecule which, when combined with a protein of interest, significantly prevents or reduces chemical and/or physical instability of the protein upon drying in general and especially during lyophilization and subsequent storage. Exemplary lyoprotectants include sugars, such as sucrose or trehalose; amino acids such as arginine, glycine, glutamate or histidine; methylamines such as betaine; lyotropic salts such as magnesium sulfate; polyols such as trihydric or higher sugar alcohols, e.g. glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; ethylene glycol; propylene glycol; polyethylene glycol; pluronics; hydroxyalkyl starches, e.g. hydroxyethyl starch (HES), and combinations thereof.

“Surfactant” refers to wetting agents that lower the surface tension of a liquid.

“Isotonicity modifiers” refer to compounds which minimize pain that can result from cell damage due to osmotic pressure differences at the injection depot.

The term “stabilizers” refers to compouds used to stabilize the polymer prodrug. 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.

“Anti-adsorption agents” refers to mainly ionic or non-ionic surfactants or other proteins or soluble polymers used to coat or adsorb competitively to the inner surface of the composition´s container. 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.

“Oxidation protection agents” refers to antioxidants such as ascorbic acid, ectoine, glutathione, methionine, monothioglycerol, morin, polyethylenimine (PEI), propyl gallate, vitamin E, chelating agents such aus citric acid, EDTA, hexaphosphate, thioglycolic acid.

"Antimicrobial" refers to a chemical substance that kills or inhibits the growth of microorganisms, such as bacteria, fungi, yeasts, protozoans and/or destroys viruses.

"Sealing a container" means that the container is closed in such way that it is airtight, allowing no gas exchange between the outside and the inside and keeping the content sterile.

The term "reagent" or "precursor" refers to an intermediate or starting material used in the assembly process leading to a prodrug of the present invention.

An object of the invention is a prodrug or a pharmaceutically acceptable salt thereof comprising a drug linker conjugate of formula (I)

$$Z - L^1 - L^2 - L - Y - R^{20} \qquad (I)$$

wherein Y is a peptide moiety having the formula (II)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-Asp-Glu-Gln-X18-Ala-X20-X21-Phe-Ile-Glu-Trp-Leu-Ile-X28-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (II)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Arg and Lys
X20 represents an amino acid residue selected from Lys, Gln and His,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val,

or wherein Y is a peptide moiety having the formula (III)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-Asp-Glu-Gln-X18-Ala-X20-X21-Phe-Ile-Glu-Trp-Leu-Ile-X28-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (III)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Leu and His
X20 represents an amino acid residue selected from His, Arg, Lys, and Gln,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Lys, Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val,

or

wherein Y is a peptide moiety having the formula (IV)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Leu-Leu-Asp-Glu-Gln-X18-Ala-Lys-Asp-Phe-Ile-Glu-Trp-Leu-Ile-Ala-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (IV)

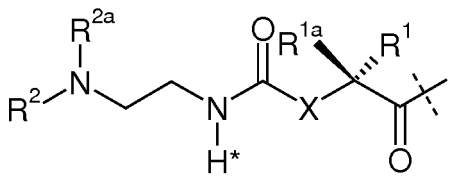
X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Arg and Leu,
X32 represents an amino acid residue selected from Ser and Val,
or a salt or solvate thereof;

or a salt or solvate thereof;

R^{20} is OH or NH_2;

L is a linker of formula (Ia),



Ia

wherein the dashed line indicates the attachment to the N-Terminus of Y by forming an amide bond;

X is $C(R^4R^{4a})$; $N(R^4)$;

R^1, R^{1a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

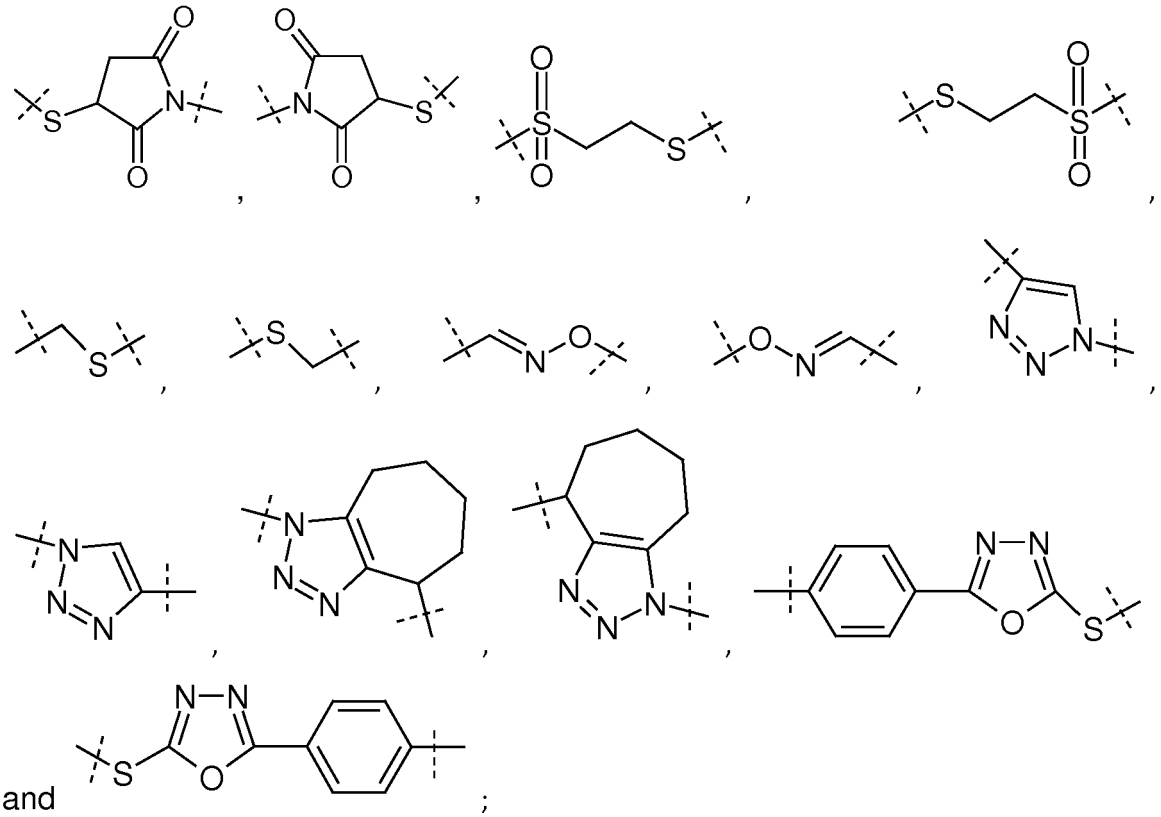
R^2, R^{2a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

R^4, R^{4a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

wherein R^2 , R^{2a}, R^4 or R^{4a} is substituted with one group L^2-L^1-Z; wherein

L^2 is a single chemical bond or is a C_{1-20} alkyl chain, which is optionally interrupted by one or more groups independently selected from -O- and $C(O)N(R^{3aa})$ and is optionally substituted with one or more groups independently selected from OH and $C(O)N(R^{3aa}R^{3aaa})$, wherein R^{3aa} and R^{3aaa} are independently selected from the group consisting of H and C_{1-4} alkyl; and

L^2 is attached to L^1 via a terminal group selected from the group consisting of



and

;

wherein L^2 is attached to the one position indicated with the dashed line and and L^1 is attached to the position indicated with the other dashed line; and

L^1 is a C_{1-20} alkyl chain, which is optionally interrupted by one or more groups independently selected from -O- and $C(O)N(R^{5aa})$ and is optionally substituted with one or more groups independently selected from OH and $C(O)N(R^{5aa}R^{5aaa})$, wherein R^{5aa} and R^{5aaa} are independently selected from the group consisting of H and C_{1-4} alkyl; and

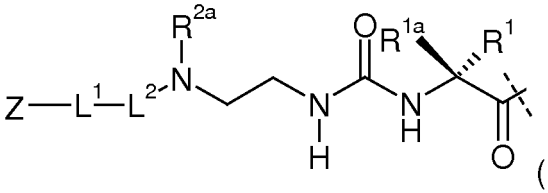
L^1 is attached to Z via a terminal amino group forming an amide bond with the carboxy group of the beta-1,3-D-glucuronic acid of the hyaluronic acid of Z;

Z is a crosslinked hyaluronic acid hydrogel, in which

0.05 to 20 % of the monomeric disaccharide units are crosslinked by a crosslinker; and
0.2 to 8.5 % of the monomeric disaccharide units bear L^1-L^2 -L-Y-R^{20}.groups.

Further embodiments of L, L^1, L^2, Z and Y.

In another embodiment
L is a linker moiety of formula (Ib),



(Ib),

wherein the dashed line indicates attachment to Y by forming an amide bond;
R^1, R^{1a}, R^{2a} are selected independently from the group consisting of H and C_{1-4} alkyl;
L^2-L^1-Z is defined as described above.

In another embodiment
L is a linker moiety of formula (Ib), wherein
R^1 is CH_3;
R^{1a} is H;
R^{2a} is H; and

L^2-L^1-Z is defined as described above.

In another embodiment

L is a linker moiety of formula (Ib), wherein

R^1 is H;

R^{1a} is CH_3;

R^{2a} is H; and

L^2-L^1-Z is defined as described above.

In another embodiment

L is a linker moiety of formula (Ib), wherein

R^1 is CH_3;

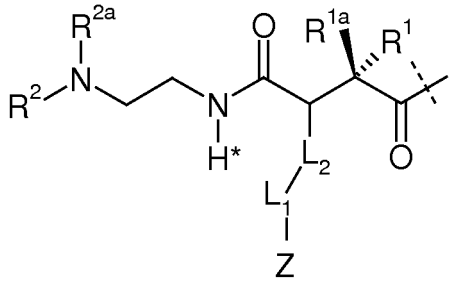
R^{1a} is CH_3;

R^{2a} is H; and

L^2-L^1-Z is defined as described above.

In another embodiment

L is a linker moiety -L of formula (Ic),



(Ic)

wherein the dashed line indicates attachment to Y by forming an amide bond;

R^1 is selected from H or C_{1-4} alkyl, preferably H;

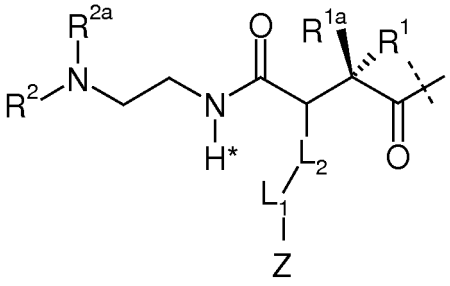
R^{1a} is selected from H or C_{1-4} alkyl, preferably H;

R^2, R^{2a} are independently selected from the group consisting of H and C_{1-4} alkyl;

wherein L^2-L^1-Z is defined as described above.

In another embodiment

L is a linker moiety -L of formula (Ic),



(Ic)

wherein the dashed line indicates attachment to Y by forming an amide bond;

R^1 and R^{1a} are H;

R^2, R^{2a} are independently selected from the group consisting of H and CH_3;

wherein L^2-L^1-Z is defined as described above.

In another embodiment

L is a linker moiety -L of formula (Ic), wherein

R^1 and R^{1a} are H;

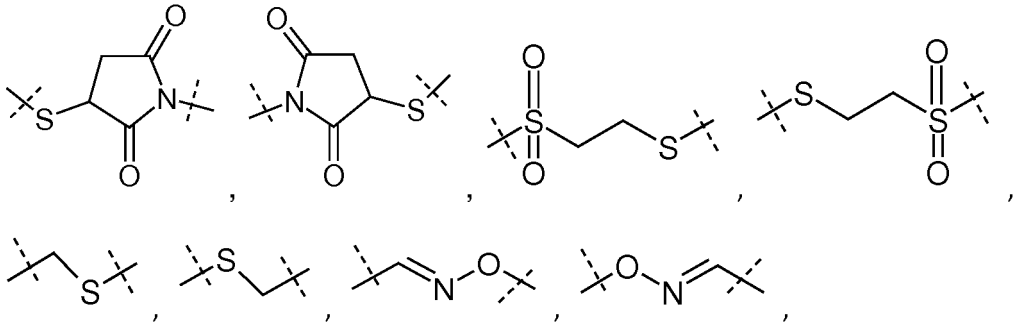
R^2 is H and R^{2a} is CH_3;

wherein L^2-L^1-Z is defined as described above.

In another embodiment

L^2 is a C_{1-10} alkyl chain, which is optionally interrupted by one or two groups independently selected from -O- and C(O)N(R^{3aa}) and, wherein R^{3aa} is independently selected from the group consisting of H and C_{1-4} alkyl; and

L^2 is attached to L^1 via a terminal group selected from the group consisting of

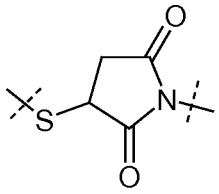
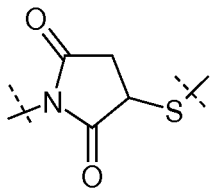


wherein L^2 is attached to the one position indicated with the dashed line and and L^1 is attached to the position indicated with the other dashed line; and

In another embodiment

L^2 is a C_{1-6} alkyl chain, which is optionally interrupted by one group selected from -O- and C(O)N(R^{3aa}) and, wherein R^{3aa} is independently selected from the group consisting of H and C_{1-4} alkyl; and

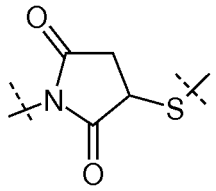
L^2 is attached to L^1 via a terminal group selected from the group consisting of


and

;

wherein L^2 is attached to the one position indicated with the dashed line and and L^1 is attached to the position indicated with the other dashed line.

In another embodiment

L^2 is -CH_2-CH_2-CH_2-CH_2-CH_2-C(O)NH- or -CH_2-CH_2-CH_2-CH_2-CH_2-CH_2- and is attached to L^1 via the terminal group


;

wherein wherein L^2 is attached to the Sulfur atom indicated with the dashed line and and L^1 is attached to nitrogen atom indicated with the dashed line.

In another embodiment

L^1 is a C_{1-10} alkyl chain, with an amino group on one distal end, which is optionally interrupted by one or two groups independently selected from -O- and C(O)N(R^{5aa}) and, wherein R^{5aa} is independently selected from the group consisting of H and C_{1-4} alkyl.

A further embodiment relates to prodrugs, wherein

Z is a crosslinked hyaluronic acid hydrogel, in which

0.05 to 15 % of the monomeric disaccharide units are crosslinked by a crosslinker.

A further embodiment relates to prodrugs, wherein
Z is a crosslinked hyaluronic acid hydrogel, in which
1 to 10 % of the monomeric disaccharide units are crosslinked by a crosslinker.

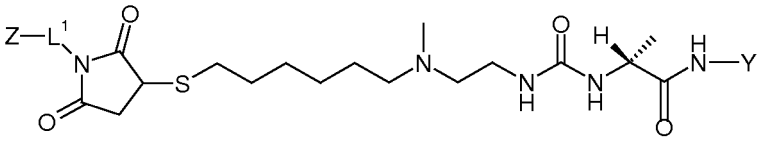
A further embodiment relates to prodrugs, wherein
Z is a crosslinked hyaluronic acid hydrogel, in which
0.2 to 8.5 % of the monomeric disaccharide units bear L^1-L^2 -L-Y-R^{20}.groups.

A further embodiment relates to prodrugs, wherein
Z is a crosslinked hyaluronic acid hydrogel, in which
0.2 to 6 % of the monomeric disaccharide units bear L^1-L^2 -L-Y-R^{20}.groups.

A further embodiment relates to prodrugs, wherein
Z is a crosslinked hyaluronic acid hydrogel, in which
0.2 to 5 % of the monomeric disaccharide units bear L^1-L^2 -L-Y-R^{20}.groups.

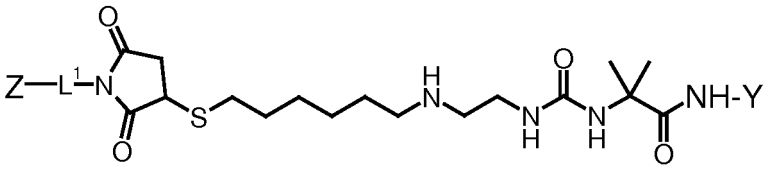
A further embodiment relates to prodrugs, wherein
Z is a crosslinked hyaluronic acid hydrogel, in which
0.4 to 4 % of the monomeric disaccharide units bear L^1-L^2 -L-Y-R^{20}.groups.

In another embodiment the GLP-1/Glucagon agonist prodrug has a structure as represented by formula (V)



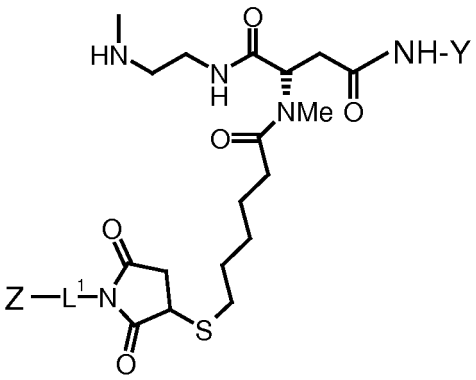
(V).

In another embodiment the GLP-1/Glucagon agonist prodrug has a structure as represented by formula (VI) (Aib-linker)



(VI).

In another embodiment the GLP-1/Glucagon agonist prodrug has a structure as represented by formula (VII)



(VII).

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (II), wherein

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,

X18 represents Arg

X20 represents an amino acid residue selected from Lys, Gln and His,

X21 represents an amino acid residue selected from Asp and Glu,

X28 represents an amino acid residue selected from Ser and Ala,

X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (II), wherein

X2 represents an D-Ser

X3 represents His,

X18 represents Arg
X20 represents Lys,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (II), wherein
X2 represents D-Ser,
X3 represents an amino acid residue selected from Gln and His,
X18 represents Arg,
X20 represents Lys,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (II), wherein
X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents His,
X18 represents Arg,
X20 represents Lys,
X21 represents Asp,
X28 represents Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (II), wherein
X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Arg and Lys,

X20 represents Lys,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (II), wherein
X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid selected from Arg and Lys,
X20 represents an amino acid residue selected from Lys, Gln and His,
X21 represents Asp,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (II), wherein
X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid selected from Arg and Lys,
X20 represents an amino acid residue selected from Lys, Gln and His,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (II), wherein
X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents Arg
X20 represents Lys,
X21 represents an amino acid residue selected from Asp and Glu,

X28 represents Ala,

X32 represents Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (III), wherein

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,

X18 represents Leu

X20 represents an amino acid residue selected from His, Arg, Lys and Gln,

X21 represents an amino acid residue selected from Asp and Glu,

X28 represents an amino acid residue selected from Lys, Ser and Ala,

X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (III), wherein

X2 represents Aib,

X3 represents an amino acid residue selected from Gln and His,

X18 represents an amino acid residue selected from His and Leu;

X20 represents an amino acid residue selected from His, Arg, Lys and Gln,

X21 represents an amino acid residue selected from Asp and Glu,

X28 represents an amino acid residue selected from Lys, Ser and Ala,

X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (III), wherein

X2 represents Aib,

X3 represents His,

X18 represents Leu,

X20 represents Lys,

X21 represents an amino acid residue selected from Asp and Glu,

X28 represents an amino acid residue selected from Ser and Ala,

X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (III), wherein

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents His,
X18 represents an amino acid residue selected from His and Leu,
X20 represents an amino acid residue selected from His, Arg, Lys and Gln,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Lys, Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (III), wherein

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents Gln,
X18 represents Leu,
X20 represents Lys,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents Ala,
X32 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (III), wherein

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from His and Leu,
X20 represents Lys,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Lys, Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (III), wherein

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from His and Leu,
X20 represents an amino acid residue selected from His, Arg, Lys and Gln,
X21 represents Asp,
X28 represents an amino acid residue selected from Lys, Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (III), wherein

X2 represents Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents Leu,
X20 represents an amino acid residue selected from Lys and Gln,
X21 represents Glu,
X28 represents Ala,
X32 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (III), wherein

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from His and Leu,
X20 represents an amino acid residue selected from His, Arg, Lys and Gln,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (III), wherein

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from His and Leu,
X20 represents an amino acid residue selected from His, Arg, Lys and Gln,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Lys, Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety Y of formula (II) or (III), wherein
X2 represents an amino acid residue selected from Aib and D-Ser,
X3 represents His,
X18 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Leu and Arg,
X20 represents Lys,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IV), wherein
X2 represents D-Ser,
X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Arg and Leu,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IV), wherein
X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents His,
X18 represents an amino acid residue selected from Arg and Leu, particularly Leu,
X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IV), wherein

X2 represents D-Ser,

X3 represents Gln,

X18 represents Arg,

X32 represents an amino acid residue selected from Ser and Val.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IV), wherein

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,

X18 represents an amino acid residue selected from Arg and Leu,

X32 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa)

H_2N-His-Aib-His-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-X15-Glu-Gln-Leu-Ala-Arg-Asp-Phe-Ile-Glu-Trp-Leu-Ile-Bal-X29-Gly-X31-X32-Ser-X34-X35-Pro-Pro-Pro-X39-R^{20} (IVa)

wherein

X15 represents an amino acid residue selected from Asp and Glu, (pref. Asp)

X29 represents an amino acid residue selected from Gly, D-Ala and Pro, (pref) Gly, D-Ala

X31 represents an amino acid residue selected from Pro, His and Trp, (pref. Pro)

X32 represents an amino acid residue selected from Ser, His, Pro and Arg, (pref. Ser, His, Pro),

X34 represents an amino acid residue selected from Gly and D-Ala,

X35 represents an amino acid residue selected from Ala, Pro and Lys, (pref. Ala, Pro)

X39 represents Ser or Pro-Pro-Pro,

or a salt or solvate thereof.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein

X15 represents Asp,

X29 represents an amino acid residue selected from Gly, D-Ala and Pro,

X31 represents an amino acid residue selected from Pro, His and Trp,

X32 represents an amino acid residue selected from Ser, His, Pro and Arg,

X34 represents an amino acid residue selected from Gly and D-Ala,

X35 represents an amino acid residue selected from Ala, Pro and Lys,

X39 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein

X15 represents an amino acid residue selected from Asp and Glu,

X29 represents Gly,

X31 represents an amino acid residue selected from Pro, His and Trp,

X32 represents an amino acid residue selected from Ser, His, Pro and Arg,

X34 represents an amino acid residue selected from Gly and D-Ala,

X35 represents an amino acid residue selected from Ala, Pro and Lys,

X39 represents Ser or Pro-Pro-Pro.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein

X15 represents an amino acid residue selected from Asp and Glu,

X29 represents Gly,

X31 represents Pro,

X32 represents an amino acid residue selected from Ser, His and Pro,

X34 represents Gly,

X35 represents Ala,

X39 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein

X15 represents Asp,
X29 represents D-Ala,
X31 represents Pro,
X32 represents Pro,
X34 represents D-Ala,
X35 represents an amino acid residue selected from Ala and Pro,
X39 represents Ser or Pro-Pro-Pro.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein

X15 represents an amino acid residue selected from Asp and Glu,
X29 represents an amino acid residue selected from Gly, D-Ala and Pro,
X31 represents Pro,
X32 represents an amino acid residue selected from Ser, His, Pro and Arg,
X34 represents an amino acid residue selected from Gly and D-Ala,
X35 represents an amino acid residue selected from Ala, Pro and Lys,
X39 represents Ser or Pro-Pro-Pro.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein

X15 represents Asp,
X29 represents Gly,
X31 represents His,
X32 represents Pro,
X34 represents Gly,
X35 represents an amino acid residue selected from Ala and Lys,
X39 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein

X15 represents Asp,
X29 represents an amino acid residue selected from Gly and Pro,
X31 represents Pro,
X32 represents Ser,
X34 represents Gly,
X35 represents Ala,
X39 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein
X15 represents an amino acid residue selected from Asp and Glu,
X29 represents an amino acid residue selected from Gly, D-Ala and Pro,
X31 represents an amino acid residue selected from Pro, His and Trp,
X32 represents Pro,
X34 represents an amino acid residue selected from Gly and D-Ala,
X35 represents an amino acid residue selected from Ala, Pro and Lys,
X39 represents Ser or Pro-Pro-Pro.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein
X15 represents Asp,
X29 represents an amino acid residue selected from Gly and Pro,
X31 represents Pro,
X32 represents His, ,
X34 represents Gly,
X35 represents Ala,
X39 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein
X15 represents an amino acid residue selected from Asp and Glu,
X29 represents an amino acid residue selected from Gly, D-Ala and Pro,

X31 represents an amino acid residue selected from Pro, His and Trp,
X32 represents an amino acid residue selected from Ser, His, Pro and Arg,
X34 represents Gly,
X35 represents an amino acid residue selected from Ala, Pro and Lys,
X39 represents Ser or Pro-Pro-Pro.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein
X15 represents Asp,
X29 represents D-Ala,
X31 represents Pro,
X32 represents an amino acid residue selected from Ser and Pro,
X34 represents D-Ala,
X35 represents an amino acid residue selected from Ala and Pro,
X39 represents Ser or Pro-Pro-Pro.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein
X15 represents an amino acid residue selected from Asp and Glu,
X29 represents an amino acid residue selected from Gly, D-Ala and Pro,
X31 represents an amino acid residue selected from Pro, His and Trp,
X32 represents an amino acid residue selected from Ser, His, Pro and Arg,
X34 represents an amino acid residue selected from Gly and D-Ala,
X35 represents Ala,
X39 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein
X15 represents Asp,
X29 represents Gly,
X31 represents an amino acid residue selected from Pro and His,
X32 represents Pro,

X34 represents Gly,
X35 represents Lys,
X39 represents Ser.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein
X15 represents Asp,
X29 represents an amino acid residue selected from Gly and D-Ala,
X31 represents Pro,
X32 represents Pro,
X34 represents an amino acid residue selected from Gly and D-Ala,
X35 represents Pro,
X39 represents Pro-Pro-Pro.

A further embodiment relates to a group of prodrugs having a peptide moiety of formula (IVa), wherein
X15 represents an amino acid residue selected from Asp and Glu,
X29 represents an amino acid residue selected from Gly, D-Ala and Pro,
X31 represents an amino acid residue selected from Pro, His and Trp,
X32 represents an amino acid residue selected from Ser, His, Pro and Arg,
X34 represents an amino acid residue selected from Gly and D-Ala,
X35 represents an amino acid residue selected from Ala, Pro and Lys,
X39 represents Ser.

In one embodiment Y refers to an GLP-1/Glucagon agonist of Seq. ID No 60.

In one embodiment Y refers to an GLP-1/Glucagon agonist selected from sequences Seq. ID No 4 to 60.

In one embodiment Y refers to an GLP-1/Glucagon agonist selected from sequences Seq. ID No 4 to 44.

In one embodiment Y refers to an GLP-1/Glucagon agonist selected from sequences Seq. ID No 4 to 22.

In one embodiment Y refers to an GLP-1/Glucagon agonist selected from sequences Seq. ID No 23 to 39.

In one embodiment Y refers to an GLP-1/Glucagon agonist selected from sequences Seq. ID No 40 to 44.

In one embodiment Y refers to an GLP-1/Glucagon agonist selected from sequences Seq. ID No 45 to 59.

In one embodiment Y refers to an GLP-1/Glucagon agonist selected from sequences Seq. ID No 18, 21 and 26.

In one embodiment Y refers to an GLP-1/Glucagon agonist selected from sequences Seq. ID No 18, 21, 26, 45, 48, 49 and 60.

Table 1

SEQ ID	sequence
1	H-G-E-G-T-F-T-S-D-L-S-K-Q-M-E-E-E-A-V-R-L-F-I-E-W-L-K-N-G-G-P-S-S-G-A-P-P-P-S-NH2
2	H-A-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-K-E-F-I-A-W-L-V-K-G-R-NH2
3	H-S-Q-G-T-F-T-S-D-Y-S-K-Y-L-D-S-R-R-A-Q-D-F-V-Q-W-L-M-N-T
4	H-S-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-E-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
5	H-S-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-E-F-I-E-W-L-I-A-G-G-P-V-S-G-A-P-P-P-S-NH2

6	H-dSer-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
7	H-dSer-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-V-S-G-A-P-P-P-S-NH2
8	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
9	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-Q-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
10	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-S-G-G-P-S-S-G-A-P-P-P-S-NH2
11	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
12	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-H-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
13	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-E-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
14	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-V-S-G-A-P-P-P-S-NH2
15	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-Q-D-F-I-E-W-L-I-S-G-G-P-S-S-G-A-P-P-P-S-NH2
16	H-S-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-V-S-G-A-P-P-P-S-NH2
17	H-dSer-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-E-F-I-E-W-L-I-A-G-G-P-V-S-G-A-P-P-P-S-NH2
18	H-dSer-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-V-S-G-A-P-P-P-S-NH2
19	H-S-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-V-

	S-G-A-P-P-P-S-NH2
20	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-E-F-I-E-W-L-I-S-G-G-P-S-S-G-A-P-P-P-S-NH2
21	H-dSer-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
22	H-dSer-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-K-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-
23	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
24	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-H-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
25	H-dSer-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
26	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
27	H-S-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
28	H-S-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
29	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-E-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
30	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-S-G-G-P-S-S-G-A-P-P-P-S-NH2
31	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-K-G-G-P-S-S-G-A-P-P-P-S-NH2
32	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-V-S-G-A-P-P-P-S-NH2

33	H-dSer-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
34	H-S-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
35	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-E-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
36	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
37	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-H-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
38	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-Q-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
39	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-Q-E-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
40	H-dSer-Q-G-T-F-T-S-D-L-S-K-L-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
41	H-Aib-H-G-T-F-T-S-D-L-S-K-L-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
42	H-dSer-Q-G-T-F-T-S-D-L-S-K-L-L-D-E-Q-R-A-K-D-F-I-E-W-L-I-A-G-G-P-V-S-G-A-P-P-P-S-NH2
43	H-dSer-H-G-T-F-T-S-D-L-S-K-L-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
44	H-S-H-G-T-F-T-S-D-L-S-K-L-L-D-E-Q-L-A-K-D-F-I-E-W-L-I-A-G-G-P-S-S-G-A-P-P-P-S-NH2
45	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-G-G-P-S-S-G-A-P-P-P-S-NH2
46	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-G-G-P-P-S-G-A-P-P-P-S-NH2
47	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-G-G-P-P-S-G-P-P-P-P-P-P-P-NH2
48	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-dAla-G-

	P-P-S-dAla-P-P-P-P-P-P-P-NH2
49	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-G-G-P-H-S-G-A-P-P-P-S-NH2
50	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-P-G-P-S-S-G-A-P-P-P-S-NH2
51	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-P-G-P-P-S-G-A-P-P-P-S-NH2
52	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-P-G-P-H-S-G-A-P-P-P-S-NH2
53	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-G-G-P-P-S-G-K-P-P-P-S-NH2
54	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-G-G-H-P-S-G-A-P-P-P-S-NH2
55	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-G-G-W-P-S-G-A-P-P-P-S-NH2
56	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-G-G-H-P-S-G-K-P-P-P-S-NH2
57	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-E-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-G-G-P-P-S-G-A-P-P-P-S-NH2
58	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-G-G-P-R-S-G-A-P-P-P-S-NH2
59	H-Aib-H-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-L-A-R-D-F-I-E-W-L-I-Bal-dAla-G-P-P-S-dAla-A-P-P-P-S-NH2
60	H-Aib-Q-G-T-F-T-S-D-L-S-K-L-L-E-E-Q-R-A-R-E-F-I-E-W-L-I-Bal-dAla-G-P-P-S-dAla-A-P-P-P-S-NH2

Another embodiment is the peptide of Seq. ID No. 60 and its use as pharmaceutical.

In case the GLP-1/Glucagon agonist prodrugs comprising the compounds according to formula (I) contain 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 GLP-1/Glucagon agonist prodrugs comprising the compounds of the formula (I) which contain 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. GLP-1/Glucagon agonist prodrugs comprising the compounds of the formula (I) which contain 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. If the GLP-1/Glucagon agonist prodrugs comprising the compounds of the formula (I) simultaneously contain 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 GLP-1/Glucagon agonist prodrugs comprising the formula (I) 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 GLP-1/Glucagon agonist prodrugs comprising the compounds of the formula (I) 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.

Process of making

The peptides Y may be prepared by convenient methods known in the art.

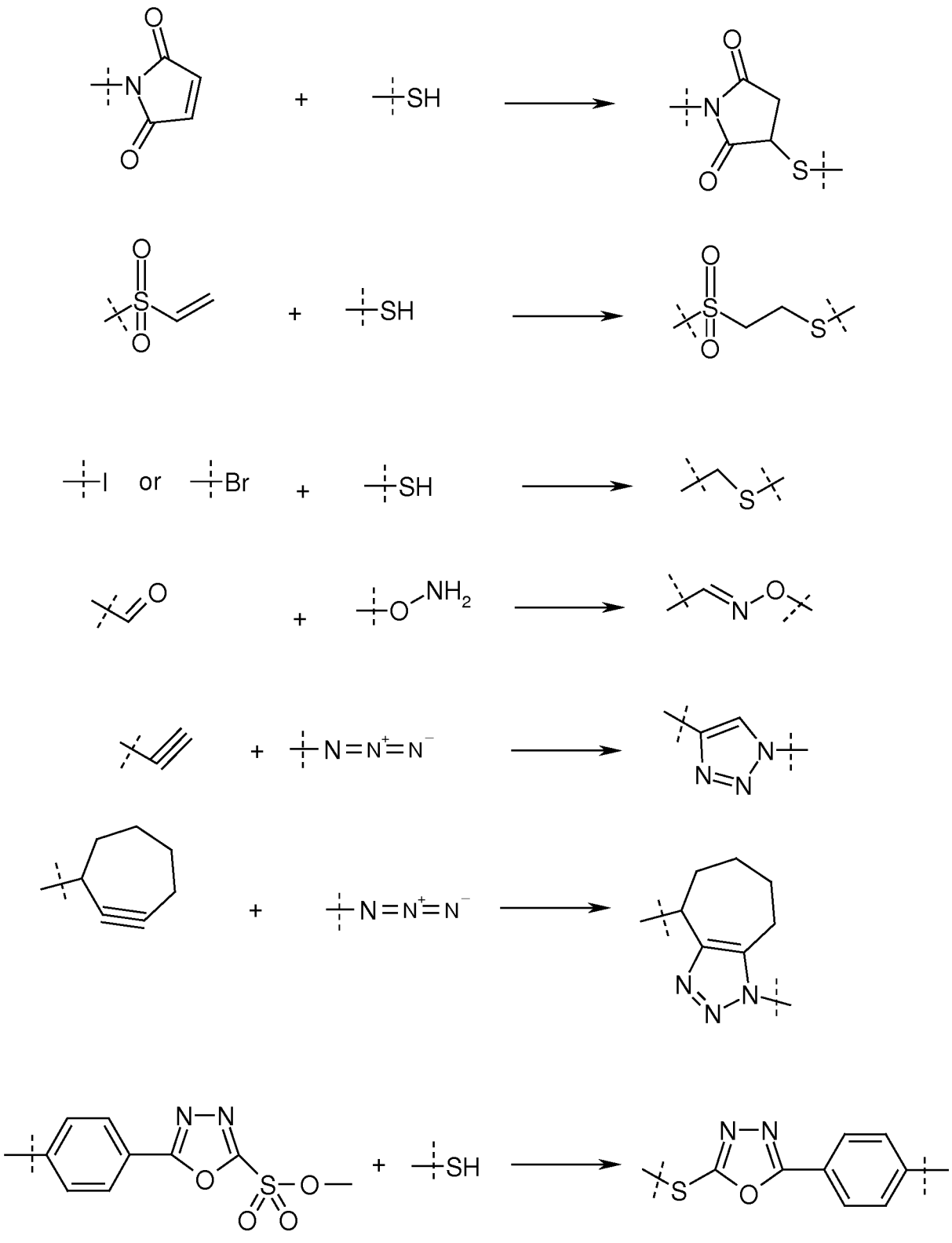
The linkers L are prepared by methods as described in the examples and as disclosed in WO2009/095479, WO2011/012718 and WO2012/035139.
The hydrogel-linked GLP-1/Glucagon agonist prodrug of the present invention can be prepared by synthesizing the building blocks activated hyaluronic acid hydrogel Z-L^{1*} and activated peptide linker conjugate L^{2*}- L -Y.

Activated groups L^{1*} and L^{2*} are used to conjugate peptide to the polymers.
Scheme 1 shows different types of linking chemistries which can be used to conjugate the peptide with self-immolative linkers to the polymer. Thus, besides thiol-maleimide chemistry, other biorthogonal chemistries can be used. In scheme 1 the dashed lines indicates the positions where L^1 and L^2 are attached.

After loading the GLP-1/Glucagon agonist-linker conjugate to the functionalized hyaluronic acid hydrogel, all remaining functional groups are optionally capped with a suitable blocking reagent to prevent undesired side-reactions.

In the case of a functionalized maleimido group-containing HA-hydrogel, a thiol containing compound such as mercaptoethanol is a suitable blocking agent.

Scheme 1



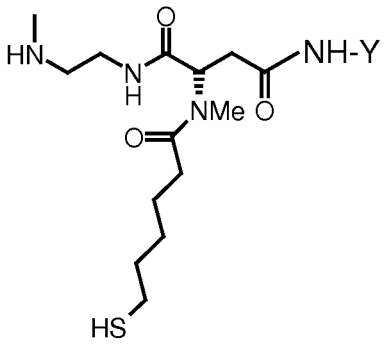
Another aspect of the present invention are functionalized intermediates comprising a GLP-1/Glucagon agonist-linker conjugate L^{2*}- L -Y.

One embodiment of a GLP-1/Glucagon agonist-linker conjugate L^{2*}-L-Y comprises a thiol functionalization, resulting in the formula

HS-L^{2}- L -Y

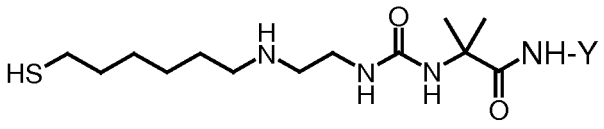
wherein L^{2}, L and Y have the meanings as described above.

One embodiment of thiol functionalized a GLP-1/Glucagon agonist-linker conjugate L^{2*}-L-Y is a GLP-1/Glucagon agonist-linker conjugate of formula (VIII)



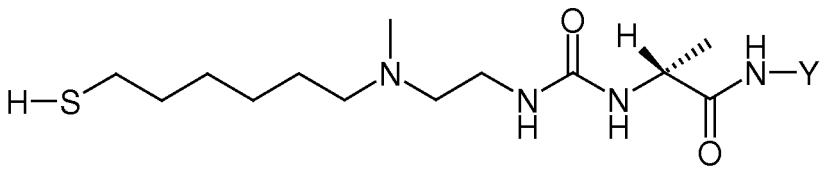
(VIII).

One embodiment of thiol functionalized GLP-1/Glucagon agonist-linker conjugate L^{2*}-L-Y is a GLP-1/Glucagon agonist-linker conjugate of formula (IX)



(IX).

One embodiment of thiol functionalized a GLP-1/Glucagon agonist-linker conjugate L^{2*}-L-Y is a GLP-1/Glucagon agonist-linker conjugate of formula (X)



(X).

Pharmaceutical composition

Another aspect of the present invention is a pharmaceutical composition comprising a prodrug of the present invention or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable excipient. The pharmaceutical composition is further described in the following paragraphs.

The composition of GLP-1/Glucagon agonist-hydrogel prodrug may be provided as a suspension composition or as a dry composition. In one embodiment the pharmaceutical composition of GLP-1/Glucagon agonist-hydrogel prodrug is a dry composition. Suitable methods of drying are, for example, spray-drying and lyophilization (freeze-drying). Preferably, the pharmaceutical composition of GLP-1/Glucagon agonist-hydrogel prodrug is dried by lyophilization.

In another embodiment the pharmaceutical composition of GLP-1/Glucagon agonist-hydrogel prodrug is a ready to use suspension.

In another embodiment the pharmaceutical composition of GLP-1/Glucagon agonist-hydrogel prodrug is a ready to use suspension wherein the prodrug is swollen in water/buffer to a concentration of 0.5 to 8 % (w/v).

In another embodiment the pharmaceutical composition of GLP-1/Glucagon agonist-hydrogel prodrug is a ready to use suspension wherein the prodrug is swollen in water/buffer to a concentration of 1 to 4 % (w/v).

In another embodiment the pharmaceutical composition of GLP-1/Glucagon agonist-hydrogel prodrug is a ready to use suspension wherein the prodrug is swollen in water/buffer to a concentration of 1.5 to 3 % (w/v).

Preferably, the GLP-1/Glucagon agonist hydrogel prodrug is sufficiently dosed in the composition to provide therapeutically effective amount of GLP-1/Glucagon agonist for at least three days in one application. More preferably, one application of the GLP-1/Glucagon agonist hydrogel prodrug is sufficient for one week.

The pharmaceutical composition of GLP-1/Glucagon agonist-hydrogel prodrug according to the present invention contains one or more excipients.

Excipients used in parenteral compositions may be categorized as buffering agents, isotonicity modifiers, preservatives, stabilizers, anti-adsorption agents, oxidation protection agents, viscosifiers/viscosity enhancing agents, or other auxiliary agents. In some cases, these ingredients may have dual or triple functions. The compositions of GLP-1/Glucagon agonist-hydrogel prodrugs according to the present invention contain one or more than one excipient, selected from the groups consisting of:

(i) Buffering agents: physiologically tolerated buffers to maintain pH in a desired range, such as sodium phosphate, bicarbonate, succinate, histidine, citrate and acetate, sulphate, nitrate, chloride, pyruvate. Antacids such as $Mg(OH)_2$ or $ZnCO_3$ may be also used. Buffering capacity may be adjusted to match the conditions most sensitive to pH stability

(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 preparations 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 stater, 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 inon-ionic surfactants or other proteins or soluble polymers are used to coat or adsorb competitively to the inner surface of the composition´s or composition´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) Lyo- and/or cryoprotectants: During freeze- or spray drying, excipients may counteract the destabilising effects caused by hydrogen bond breaking and water removal. For this purpose sugars and polyols may be used but corresponding positive effects have also been observed for surfactants, amino acids, non-aqueous solvents, and other peptides. Trehalose is particulary efficient at reducing moisture-induced aggregation and also improves thermal stability potentially caused by exposure of protein hydrophobic groups to water. Mannitol and sucrose may also be used, either as sole lyo/cryoprotectant or in combination with each other where higher ratios of mannitol:sucrose are known to enhance physical stability of a lyophilized cake. Mannitol may also be combined with trehalose. Trehalose may also be combined with sorbitol or sorbitol used as the sole protectant. Starch or starch derivatives may also be used

(vii) Oxidation protection agents: antioxidants such as ascorbic acid, ectoine, methionine, glutathione, monothioglycerol, morin, polyethylenimine (PEI), propyl gallate, vitamin E, chelating agents such as citric acid, EDTA, hexaphosphate, thioglycolic acid

(viii) Viscosifiers or viscosity enhancers: retard settling of the particles in the vial and syringe and are used in order to facilitate mixing and resuspension of the particles and to make the suspension easier to inject (i.e., low force on the syringe plunger). Suitable viscosifiers or viscosity enhancers are, for example,

carbomer viscosifiers like Carbopol 940, Carbopol Ultrez 10, cellulose derivatives like hydroxypropylmethylcellulose (hypromellose, HPMC) or diethylaminoethyl cellulose (DEAE or DEAE-C), colloidal magnesium silicate (Veegum) or sodium silicate, hydroxyapatite gel, tricalcium phosphate gel, xanthans, carrageenans like Satia gum UTC 30, aliphatic poly(hydroxy acids), such as poly(D,L- or L-lactic acid) (PLA) and poly(glycolic acid) (PGA) and their copolymers (PLGA), terpolymers of D,L-lactide, glycolide and caprolactone, poloxamers, hydrophilic poly(oxyethylene) blocks and hydrophobic poly(oxypropylene) blocks to make up a triblock of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) (e.g. Pluronic®), polyetherester copolymer, such as a polyethylene glycol terephthalate/polybutylene terephthalate copolymer, sucrose acetate isobutyrate (SAIB), dextran or derivatives thereof, combinations of dextrans and PEG, polydimethylsiloxane, collagen, chitosan, polyvinyl alcohol (PVA) and derivatives, polyalkylimides, poly (acrylamide-co-diallyldimethyl ammonium (DADMA)), polyvinylpyrrolidone (PVP), glycosaminoglycans (GAGs) such as dermatan sulfate, chondroitin sulfate, keratan sulfate, heparin, heparan sulfate, hyaluronan, ABA triblock or AB block copolymers composed of hydrophobic A-blocks, such as polylactide (PLA) or poly(lactide-co-glycolide) (PLGA), and hydrophilic B-blocks, such as polyethylene glycol (PEG) or polyvinyl pyrrolidone. Such block copolymers as well as the abovementioned poloxamers may exhibit reverse thermal gelation behavior (fluid state at room temperature to facilitate administration and gel state above sol-gel transition temperature at body temperature after injection).

(ix) 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 but not limited to hyaluronic acid, a polysaccharide found in the intercellular space of connective tissue. A spreading agent such as but not limited to hyaluronidase temporarily decreases the viscosity of the extracellular matrix and promotes diffusion of injected drugs.

(x) 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

In one embodiment the composition of GLP-1/Glucagon agonist-hydrogel prodrug contains one or more than one viscosifier and/or viscosity modifying agent.
In another embodiment the composition of GLP-1/Glucagon agonist-hydrogel prodrug contains hyaluronic acid as viscosifier and/or viscosity modifying agent.

In another embodiment the composition of GLP-1/Glucagon agonist-hydrogel prodrug comprises hyaluronic acid as viscosifier and/or viscosity modifying agent in a concentration of 5 to 30 wt%.

In another embodiment the composition of GLP-1/Glucagon agonist-hydrogel prodrug comprises hyaluronic acid as viscosifier and/or viscosity modifying agent of a molecular weight of 200 kDa to 6 million kDa.
In another embodiment the composition of GLP-1/Glucagon agonist-hydrogel prodrug comprises hyaluronic acid as viscosifier and/or viscosity modifying agent of a molecular weight of 500 kDa to 3 million kDa.

The term "excipient" preferably refers to a diluent, adjuvant, or vehicle with which the therapeutic is administered. Such pharmaceutical excipient can be sterile liquids. Water is a preferred excipient when the pharmaceutical composition is administered orally. Saline and aqueous dextrose are preferred excipients when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions are preferably employed as liquid excipients for injectable solutions.

Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders, sustained-release formulations and the like. Such compositions will contain a therapeutically effective amount of the therapeutic, preferably in purified form, together with a suitable amount of excipient so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a general embodiment a pharmaceutical composition of the present invention whether in dry form or as a suspension or in another form may be provided as single or multiple dose composition.

In one embodiment of the present invention, the dry composition of GLP-1/Glucagon agonist-hydrogel prodrug is provided as a single dose, meaning that the container in which it is supplied contains one pharmaceutical dose.

Thus, in another aspect of the present invention the composition is provided as a single dose composition.

In another aspect of the present invention the composition is comprised in a container. In one embodiment the container is a dual-chamber syringe. Especially the dry composition according to the present invention is provided in a first chamber of the dual-chamber syringe and reconstitution solution is provided in a second chamber of the dual-chamber syringe.

Prior to applying the dry composition of GLP-1/Glucagon agonist-hydrogel prodrug to a patient in need thereof, the dry composition is reconstituted. Reconstitution can take place in the container in which the dry composition of GLP-1/Glucagon agonist-hydrogel prodrug is provided, such as in a vial, syringe, dual-chamber syringe, ampoule, and cartridge. Reconstitution is done by adding a predefined amount of reconstitution solution to the dry composition. Reconstitution solutions are sterile liquids, such as water or buffer, which may contain further additives, such as preservatives and/or antimicrobials. If the GLP-1/Glucagon agonist-hydrogel prodrug composition is provided as single dose, the reconstituion solution may contain one or more preservative and/or antimicrobial. Preferably, the reconstitution solution is sterile water.

An additional aspect of the present invention relates to the method of administration of a reconstituted GLP-1/Glucagon agonist hydrogel prodrug composition. The GLP-1/Glucagon agonist hydrogel prodrug composition can be administered by methods of

injection or infusion, including intradermal, subcutaneous, intramuscular, intravenous, intraosseous, and intraperitoneal.

A further aspect is a method of preparing a reconstituted composition comprising a therapeutically effective amount of an GLP-1/Glucagon agonist hydrogel prodrug, and optionally one or more pharmaceutically acceptable excipients, wherein the GLP-1/Glucagon agonist is transiently linked to a hydrogel, the method comprising the step of

- contacting the composition of the present invention with a reconstitution solution.

Another aspect is a reconstituted composition comprising a therapeutically effective amount of a GLP-1/Glucagon agonist hydrogel prodrug, and optionally one or more pharmaceutically acceptable excipients, wherein the GLP-1/Glucagon agonist is transiently linked to a hydrogel obtainable by the method above.

Another aspect of the present invention is the method of manufacturing a dry composition of GLP-1/Glucagon agonist-hydrogel prodrug. In one embodiment, such suspension composition is made by

(i) admixing the GLP-1/Glucagon agonist-hydrogel prodrug with one or more excipients,
(ii) transfering amounts equivalent to single or multiple doses into a suitable container,
(iii) drying the composition in said container, and
(iv) sealing the container.

Suitable containers are vials, syringes, dual-chamber syringes, ampoules, and cartridges.

Another aspect is a kit of parts. When the administration device is simply a hypodermic syringe then the kit may comprise the syringe, a needle and a container comprising the dry GLP-1/Glucagon agonist-hydrogel prodrug composition for use with the syringe and

a second container comprising the reconstitution solution. In more preferred embodiments, the injection device is other than a simple hypodermic syringe and so the separate container with reconstituted GLP-1/Glucagon agonist-hydrogel prodrug is adapted to engage with the injection device such that in use the liquid composition in the container is in fluid connection with the outlet of the injection device. Examples of administration devices include but are not limited to hypodermic syringes and pen injector devices. Particularly preferred injection devices are the pen injectors in which case the container is a cartridge, preferably a disposable cartridge.

A preferred kit of parts comprises a needle and a container containing the composition according to the present invention and optionally further containing a reconstitution solution, the container being adapted for use with the needle. Preferably, the container is a dual-chamber syringe.

In another aspect, the invention provides a cartridge containing a composition of GLP-1/Glucagon agonist-hydrogel prodrug as hereinbefore described for use with a pen injector device. The cartridge may contain a single dose or multiplicity of doses of GLP-1/Glucagon agonist.

In one embodiment of the present invention the suspension composition of GLP-1/Glucagon agonist-hydrogel prodrug does not only comprise an GLP-1/Glucagon agonist-hydrogel prodrug and one or more than one excipients, but also other biologically active agents, either in their free form or as prodrugs. Preferably, such additional one or more biologically active agent is a prodrug, more preferably a hydrogel prodrug. Such biologically active agents include, but are not limited to, compounds of the following classes:

Injectability

Preferably, the formulation can be administered by injection through a needle smaller than 0.26 mm inner diameter (26 Gauge), even more preferably through a needle smaller than 0.18 mm inner diameter (28 Gauge), and most preferably through a needle small than 0.16 mm inner diameter (30 Gauge).

It is understood that the terms “can be administered by injection”, “injectable” or “injectability”refer to a combination of factors such as a certain force applied to a plunger of a syringe containing the biodegradable HA hydrogel according to the invention swollen in a liquid at a certain concentration (w/v) and at a certain temperature, a needle of a given inner diameter connected to the outlet of such syringe, and the time required to extrude a certain volume of the biodegradable hydrogel according to the invention from the syringe through the needle.

In order to provide for injectability, a volume of 1 mL of the GLP-1/glucagon agonist prodrugs according to the invention swollen in water and contained in a syringe (holding a plunger of a diameter of 4.7 mm) can be extruded at room temperature within 10 seconds by applying a force of equal/less than 20 Newton through a needle of 26 gauge.

A preferred injectability is a volume of 1 mL of the GLP-1/glucagon agonist prodrugs according to the invention swollen in water and contained in a syringe (holding a plunger of a diameter of 4.7 mm) which can be extruded at room temperature within 10 seconds by applying a force of equal/less than 20 Newton through a needle of 30 gauge.

Suprisingly it was found that the HA carrier of the invention needs lesser force for injection the higher the peptide loading on the polymer is (Fig. 5).

In order to provide for injectability, a volume of 1 mL of the GLP-1/glucagon agonist prodrugs according to the invention swollen in water/buffer to a concentration of at least 1.5% (w/v) and contained in a syringe holding a plunger of a diameter of 4.7 mm can be extruded at room temperature within 10 seconds by applying a force of less than 30 Newton through a needle of 30 gauge.

More preferably injectability is achieved for a GLP-1/glucagon agonist prodrug according to the invention swollen in water/buffer to a concentration of at least 2% (w/v) by applying a force of less than 30 Newton through a needle of 30 gauge.

Most preferably injectability is achieved for a GLP-1/glucagon agonist prodrug according to the invention swollen in water/buffer to a concentration of at least 2% (w/v) by applying a force of less than 20 Newton through a needle of 30 gauge.

An important characteristic of the prodrug is the forming of a stable depot which stays its application site. The degradation of the polymer should start after release of the drug.

Combination therapy

The prodrugs of the present invention, dual agonists for the GLP-1 and glucagon receptors, can be widely combined with other pharmacologically active compounds, such as all drugs mentioned in the Rote Liste 2015, e.g. with all weight-reducing agents or appetite suppressants mentioned in the Rote Liste 2015, chapter 1, all lipid-lowering agents mentioned in the Rote Liste 2015, chapter 58, all antihypertensives and nephroprotectives, mentioned in the Rote Liste 2015, or all diuretics mentioned in the Rote Liste 2015, chapter 36.

The active ingredient combinations can be used especially for a synergistic improvement in action. They can be applied either by separate administration of the active ingredients to the patient or in the form of combination products in which a plurality of active ingredients are present in one pharmaceutical preparation. When the active ingredients are administered by separate administration of the active ingredients, this can be done simultaneously or successively.

Most of the active ingredients mentioned hereinafter are disclosed in the USP Dictionary of USAN and International Drug Names, US Pharmacopeia, Rockville 2011.

Other active substances which are suitable for such combinations include in particular those which for example potentiate the therapeutic effect of one or more active

substances with respect to one of the indications mentioned and/or which allow the dosage of one or more active substances to be reduced.

Therapeutic agents which are suitable for combinations include, for example, antidiabetic agents such as:

Insulin and Insulin derivatives, for example: Glargine / Lantus®, 270 - 330U/mL of insulin glargine (EP 2387989 A), 300U/mL of insulin glargine (EP 2387989 A), Glulisin / Apidra®, Detemir / Levemir®, Lispro / Humalog® / Liprolog®, Degludec / DegludecPlus, Aspart, basal insulin and analogues (e.g.LY-2605541, LY2963016, NN1436), PEGylated insulin Lispro, Humulin®, Linjeta, SuliXen®, NN1045, Insulin plus Symlin, PE0139, fast-acting and short-acting insulins (e.g. Linjeta, PH20, NN1218, HinsBet), (APC-002)hydrogel, oral, inhalable, transdermal and sublingual insulins (e.g. Exubera®, Nasulin®, Afrezza, Tregopil, TPM 02, Capsulin, Oral-lyn®, Cobalamin® oral insulin, ORMD-0801, NN1953, NN1954, NN1956, VIAtab, Oshadi oral insulin). Additionally included are also those insulin derivatives which are bonded to albumin or another protein by a bifunctional linker.

GLP-1, GLP-1 analogues and GLP-1 receptor agonists, for example: Lixisenatide / AVE0010 / ZP10 / Lyxumia, Exenatide / Exendin-4 / Byetta / Bydureon / ITCA 650 / AC-2993, Liraglutide / Victoza, Semaglutide, Taspoglutide, Syncria / Albiglutide, Dulaglutide, rExendin-4, CJC-1134-PC, PB-1023, TTP-054, Langlenatide / HM-11260C, CM-3, GLP-1 Eligen, ORMD-0901, NN-9924, NN-9926, NN-9927, Nodexen, Viador-GLP-1, CVX-096, ZYOG-1, ZYD-1, GSK-2374697, DA-3091, MAR-701, MAR709, ZP-2929, ZP-3022, TT-401, BHM-034. MOD-6030, CAM-2036, DA-15864, ARI-2651, ARI-2255, Exenatide-XTEN and Glucagon-Xten.

DPP-4 inhibitors, for example: Alogliptin / Nesina, Trajenta / Linagliptin / BI-1356 / Ondero / Trajenta / Tradjenta / Trayenta / Tradzenta, Saxagliptin / Onglyza, Sitagliptin / Januvia / Xelevia / Tesave / Janumet / Velmetia, Galvus / Vildagliptin, Anagliptin,

Gemigliptin, Teneligliptin, Melogliptin, Trelagliptin, DA-1229, Omarigliptin / MK-3102, KM-223, Evogliptin, ARI-2243, PBL-1427, Pinoxacin.

SGLT2 inhibitors, for example: Invokana / Canaglifozin, Forxiga / Dapagliflozin, Remoglifozin, Sergliflozin, Empagliflozin, Ipragliflozin, Tofogliflozin, Luseogliflozin, LX-4211, Ertuglifozin / PF-04971729, RO-4998452, EGT-0001442, KGA-3235 / DSP-3235, LIK066, SBM-TFC-039,

Biguanides (e.g. Metformin, Buformin, Phenformin), Thiazolidinediones (e.g. Pioglitazone, Rivoglitazone, Rosiglitazone, Troglitazone), dual PPAR agonists (e.g. Aleglitazar, Muraglitazar, Tesaglitazar), Sulfonylureas (e.g. Tolbutamide, Glibenclamide, Glimepiride/Amaryl, Glipizide), Meglitinides (e.g. Nateglinide, Repaglinide, Mitiglinide), Alpha-glucosidase inhibitors (e.g. Acarbose, Miglitol, Voglibose), Amylin and Amylin analogues (e.g. Pramlintide, Symlin).

GPR119 agonists (e.g. GSK-263A, PSN-821, MBX-2982, APD-597, ZYG-19, DS-8500), GPR40 agonists (e.g. Fasiglifam / TAK-875, TUG-424, P-1736, JTT-851, GW9508).

Other suitable combination partners are: Cycloset, inhibitors of 11-beta-HSD (e.g. LY2523199, BMS770767, RG-4929, BMS816336, AZD-8329, HSD-016, BI-135585), activators of glucokinase (e.g. TTP-399, AMG-151, TAK-329, GKM-001), inhibitors of DGAT (e.g. LCQ-908), inhibitors of protein tyrosinephosphatase 1 (e.g. Trodusquemine), inhibitors of glucose-6-phosphatase, inhibitors of fructose-1,6-bisphosphatase, inhibitors of glycogen phosphorylase, inhibitors of phosphoenol pyruvate carboxykinase, inhibitors of glycogen synthase kinase, inhibitors of pyruvate dehydrokinase, alpha2-antagonists, CCR-2 antagonists, SGLT-1 inhibitors (e.g. LX-2761).

One or more lipid lowering agents are also suitable as combination partners, such as for example: HMG-CoA-reductase inhibitors (e.g. Simvastatin, Atorvastatin), fibrates (e.g. Bezafibrate, Fenofibrate), nicotinic acid and the derivatives thereof (e.g. Niacin),

PPAR-(alpha, gamma or alpha/gamma) agonists or modulators (e.g. Aleglitazar),

PPAR-delta agonists, ACAT inhibitors (e.g. Avasimibe), cholesterol absorption inhibitors (e.g. Ezetimibe), Bile acid-binding substances (e.g. cholestyramine, colesevelam), ileal bile acid transport inhibitors, MTP inhibitors, or modulators of PCSK9.

HDL-raising compounds such as: CETP inhibitors (e.g. Torcetrapib, Anacetrapid, Dalcetrapid, Evacetrapid, JTT-302, DRL-17822, TA-8995) or ABC1 regulators.

Other suitable combination partners are one or more active substances for the treatment of obesity, such as for example: Sibutramine, Tesofensine, Orlistat, antagonists of the cannabinoid-1 receptor, MCH-1 receptor antagonists, MC4 receptor agonists, NPY5 or NPY2 antagonists (e.g. Velneperit), beta-3-agonists, leptin or leptin mimetics, agonists of the 5HT2c receptor (e.g. Lorcaserin), or the combinations of bupropione/naltrexone, bupropione/zonisamide, bupropione/phentermine or pramlintide/metreleptin.

Other suitable combination partners are:

Further gastrointestinal peptides such as Peptide YY 3-36 (PYY3-36) or analogues thereof, pancreatic polypeptide (PP) or analogues thereof.

Glucagon receptor agonists or antagonists, GIP receptor agonists or antagonists, ghrelin antagonists or inverse agonists, Xenin and analogues thereof.

Moreover, combinations with drugs for influencing high blood pressure, chronic heart failure or atherosclerosis, such as e.g.: Angiotensin II receptor antagonists (e.g. telmisartan, candesartan, valsartan, losartan, eprosartan, irbesartan, olmesartan, tasosartan, azilsartan), ACE inhibitors, ECE inhibitors, diuretics, beta-blockers, calcium

antagonists, centrally acting hypertensives, antagonists of the alpha-2-adrenergic receptor, inhibitors of neutral endopeptidase, thrombocyte aggregation inhibitors and others or combinations thereof are suitable.

Use

In another aspect, this invention relates to the use of a prodrug according to the invention or a physiologically acceptable salt thereof combined with at least one of the active substances described above as a combination partner, for preparing a medicament which is suitable for the treatment or prevention of diseases or conditions which can be affected by binding to the receptors for GLP-1 and glucagon and by modulating their activity.

Said compositions are for use in a method of treating or preventing diseases or disorders known for GLP-1/Glucagon agonist and GLP-1/Glucagon agonist agonists, for example, for treatment and prevention of hyperglycemia and for treatment and prevention of diabetes mellitus of any type, e.g. insulin-dependent diabetes mellitus, non insulin dependent diabetes mellitus, prediabetes or gestational diabetes mellitus, for prevention and treatment of metabolic syndrome and/or obesity and/or eating disorders, insulin resistance syndrome, lowering plasma lipid level, reducing the cardiac risk, reducing the appetite, reducing the body weight, etc.
The compounds of the invention are useful in the treatment or prevention of hepatosteatosis, preferably non-alcoholic liver-disease (NAFLD) and non-alcoholic steatohepatitis (NASH).

The use of the prodrugs according to the invention, or a physiologically acceptable salt thereof, in combination with one or more active substances may take place simultaneously, separately or sequentially.

The use of the prodrug according to the invention, or a physiologically acceptable salt thereof, in combination with another active substance may take place simultaneously or at staggered times, but particularly within a short space of time. If they are administered simultaneously, the two active substances are given to the patient together; if they are

used at staggered times, the two active substances are given to the patient within a period of less than or equal to 12 hours, but particularly less than or equal to 6 hours.

Consequently, in another aspect, this invention relates to a medicament which comprises a prodrug according to the invention or a physiologically acceptable salt of such a compound and at least one of the active substances described above as combination partners, optionally together with one or more inert carriers and/or diluents.

The compound according to the invention, or physiologically acceptable salt or solvate thereof, and the additional active substance to be combined therewith may both be present together in one formulation, for example a suspension, or separately in two identical or different formulations, for example as so-called kit-of-parts.

Yet another aspect of the present invention is a method of treating, controlling, delaying or preventing in a mammalian patient, preferably in a human, in need of the treatment of one or more conditions comprising administering to said patient a therapeutically effective amount of a prodrug of the present invention or a pharmaceutical composition of the present invention or a pharmaceutically acceptable salt thereof.

EXAMPLES

Materials and Methods

Abbreviations employed are as follows:

AA	amino acid
AcOH	acetic acid
AcOEt	ethyl acetate
cAMP	cyclic adenosine monophosphate

Bn	benzyl
Boc	tert-butyloxycarbonyl
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
BSA	bovine serum albumin
tBu	tertiary butyl
DBU	1,3-diazabicyclo[5.4.0]undecene
DCC	*N,N*-dicyclohexylcarbodiimid
DCM	dichloromethane
Dde	1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-ethyl
ivDde	1-(4,4-dimethyl-2,6-dioxocyclohexylidene)3-methyl-butyl
DIC	N,N'-diisopropylcarbodiimide
DIPEA	N,N-diisopropylethylamine
DMAP	dimethylamino-pyridine
DMEM	Dulbecco's modified Eagle's medium
DMF	dimethyl formamide
DMSO	dimethylsulfoxide
DTT	DL dithiotreitol
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimid
EDT	ethanedithiol
EDTA	ethylenediaminetetraacetic acid
eq	stoichiometric equivalent
EtOH	ethanol
FBS	fetal bovine serum
Fmoc	fluorenylmethyloxycarbonyl
HATU	*O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HBSS	Hanks' Balanced Salt Solution

HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
HOBt	1-hydroxybenzotriazole
HOSu	N-hydroxysuccinimide
HPLC	High Performance Liquid Chromatography
HTRF	Homogenous Time Resolved Fluorescence
IBMX	3-isobutyl-1-methylxanthine
LC/MS	Liquid Chromatography/Mass Spectrometry
Mal	3-maleimido propyl
Mal-PEG6-NHS	N-(3-maleimidopropyl)-21-amino-4,7,10,13,16,19-hexaoxa-heneicosanoic acid NHS ester
Me	methyl
MeOH	methanol
Mmt	4-methoxytrityl
MS	mass spectrum / mass spectrometry
MTBE	methyl *tert.*-butyl ether
MW	molecular mass
NHS	N-hydroxy succinimide
Palm	palmitoyl
iPrOH	2-propanol
PBS	phosphate buffered saline
PEG	polyethylene glycole
PK	pharmacokinetic
PyBOP	benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
Phth	phthalimido
RP-HPLC	reversed-phase high performance liquid chromatography
rpm	rounds per minute
RT	room temperature

SEC	size exclusion chromatography
TCEP	tris(2-carboxyethyl)phosphine hydrochloride
TES	triethylsilane
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TMEDA	N,N,N´N´-tetramethylethylene diamine
Tris	tris(hydroxymethyl)aminomethane
Trt	trityl
UPLC	Ultra Performance Liquid Chromatography
UV	ultraviolet
V	volume

Example 1

General synthesis of peptidic compounds

Materials:

Different Rink-Amide resins (4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucylaminomethyl resin, Merck Biosciences; 4-[(2,4-Dimethoxyphenyl)(Fmoc-amino)methyl]phenoxy acetamido methyl resin, Agilent Technologies) were used for the synthesis of peptide amides with loadings in the range of 0.3-0.4 mmol/g.

Fmoc protected natural amino acids were purchased from Protein Technologies Inc., Senn Chemicals, Merck Biosciences, Novabiochem, Iris Biotech, Nagase or Bachem. The following standard amino acids were used throughout the syntheses: Fmoc-L-Ala-OH, Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Asn(Trt)-OH, Fmoc-L-Asp(OtBu)-OH, Fmoc-L-Cys(Trt)-OH, Fmoc-L-Gln(Trt)-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Ile-OH, Fmoc-L-Leu-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Met-OH,

Fmoc-L-Phe-OH, Fmoc-L-Pro-OH, Fmoc-L-Ser(tBu)-OH, Fmoc-L-Thr(tBu)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Tyr(tBu)-OH, Fmoc-L-Val-OH.

In addition, the following special amino acids were purchased from the same suppliers as above: Fmoc-L-Lys(ivDde)-OH, Fmoc-Aib-OH, Fmoc-D-Ser(tBu)-OH, Fmoc-D-Ala-OH, Boc-L-His(Boc)-OH (available as toluene solvate) and Boc-L-His(Trt)-OH, Fmoc-L-Nle-OH, Fmoc-L-Met(O)-OH, Fmoc-L-Met(O2)-OH, Fmoc-(S)MeLys(Boc)-OH, Fmoc-(R)MeLys(Boc)-OH, Fmoc-(S)MeOrn(Boc)-OH and Boc-L-Tyr(tBu)-OH.

The solid phase peptide syntheses were performed for example on a Prelude Peptide Synthesizer (Protein Technologies Inc) or similar automated synthesizer using standard Fmoc chemistry and HBTU/DIPEA activation. DMF was used as the solvent. Deprotection : 20% piperidine/DMF for 2 x 2.5 min. Washes: 7 x DMF. Coupling 2:5:10 200 mM AA / 500 mM HBTU / 2M DIPEA in DMF 2 x for 20 min. Washes: 5 x DMF.

All the peptides that had been synthesized were cleaved from the resin with King's cleavage cocktail consisting of 82.5% TFA, 5% phenol, 5% water, 5% thioanisole, 2.5% EDT. The crude peptides were then precipitated in diethyl or diisopropyl ether, centrifuged, and lyophilized. Peptides were analyzed by analytical HPLC and checked by ESI mass spectrometry. Crude peptides were purified by a conventional preparative HPLC purification procedure.

Analytical HPLC / UPLC

Method A: detection at 215 nm

column: Aeris Peptide, 3.6 µm, XB-C18 (250 x 4.6 mm) at 60 °C

solvent: H_2O+0.1%TFA : ACN+0.1%TFA (flow 1.5 ml/min)

gradient: 90:10 (0 min) to 90:10 (3 min) to 10:90 (43 min) to 10:90 (48 min) to 90:10 (49 min) to 90:10 (50 min)

Method B: detection at 220 nm

column: Zorbax, 5 µm, C18 (250 x 4.6 mm) at 25 °C

solvent: H_2O+0.1%TFA : 90% ACN + 10% H_2O +0.1%TFA (flow 1.0 ml/min)

gradient: 100:0 (0 min) to 98:2 (2 min) to 30:70 (15 min) to 5:95 (20 min) to 0:100 (25 min) to 0:100 (30 min) to 98:2 (32 min) to 98:2 (35 min)

Method C1: detection at 210 - 225 nm, optionally coupled to a mass analyser Waters LCT Premier, electrospray positive ion mode

column: Waters ACQUITY UPLC® BEH™ C18 1.7 µm (150 x 2.1 mm) at 50 °C

solvent: H_2O+1%FA : ACN+1%FA (flow 0.5 ml/min)

gradient: 95:5 (0 min) to 95:5 (1.80 min) to 80:20 (1.85 min) to 80:20 (3 min) to 60:40 (23 min) to 25:75 (23.1 min) to 25:75 (25 min) to 95:5 (25.1 min) to 95:5 (30min)

Method C2: detection at 210 - 225 nm, optionally coupled to a mass analyser Waters LCT Premier, electrospray positive ion mode

column: Waters ACQUITY UPLC® BEH™ C18 1.7 µm (150 x 2.1 mm) at 50 °C

solvent: H_2O+1%FA : ACN+1%FA (flow 0.6 ml/min)

gradient: 95:5 (0 min) to 95:5 (1 min) to 65:35 (2 min) to 65:35 (3 min) to 45:55 (23 min) to 25:75 (23.1 min) to 25:75 (25 min) to 95:5 (25.1 min) to 95:5 (30 min)

Method C3: detection at 210 - 225 nm, optionally coupled to a mass analyser Waters LCT Premier, electrospray positive ion mode

column: Waters ACQUITY UPLC® BEH™ C18 1.7 µm (150 x 2.1 mm) at 50 °C

solvent: *H_2O+1%FA : ACN+1%FA* (flow 1 ml/min)

gradient: 95:5 (0 min) to 95:5 (1 min) to 65:35 (2 min) to 65:35 (3 min) to 45:55 (20 min) to 2:98 (20.1 min) to 2:98 (25 min) to 95:5 (25.1 min) to 95:5 (30 min)

Method C4:

detection at 210 - 225 nm, optionally coupled to a mass analyser Waters LCT Premier, electrospray positive ion mode

column: Waters ACQUITY UPLC® BEH™ C18 1.7 µm (150 x 2.1 mm) at 50 °C

solvent: H_2O+1%FA : ACN+1%FA (flow 1 ml/min)

gradient: 95:5 (0 min) to 95:5 (1.80 min) to 80:20 (1.85 min) to 80:20 (3 min) to 60:40 (23 min) to 2:98 (23.1 min) to 2:98 (25 min) to 95:5 (25.1 min) to 95:5 (30 min)

Method D: detection at 214 nm

column: Waters X-Bridge C18 3.5 µm 2.1 x 150 mm

solvent: H_2O+0.5%TFA : ACN (flow 0.55 ml/min)

gradient: 90:10 (0 min) to 40:60 (5 min) to 1:99 (15 min)

Method E: detection at 210 - 225 nm, optionally coupled to a mass analyser Waters LCT Premier, electrospray positive ion mode

column: Waters ACQUITY UPLC® BEH™ C18 1.7 µm (150 x 2.1 mm) at 50 °C

solvent: H_2O+1%FA : ACN+1%FA (flow 0.9 ml/min)

gradient: 95:5 (0 min) to 95:5 (2min) to 35:65 (3 min) to 65:35 (23.5 min) to 5:95 (24 min) to 95:5 (26min) to 95:5 (30min)

General Preparative HPLC Purification Procedure:

The crude peptides were purified either on an Äkta Purifier System or on a Jasco semiprep HPLC System. Preparative RP-C18-HPLC columns of different sizes and with different flow rates were used depending on the amount of crude peptide to be purified. Acetonitrile + 0.05 to 0.1% TFA (B) and water + 0.05 to 0.1% TFA (A) were employed as eluents. Alternatively, a buffer system consisting of acetonitrile and water with minor amounts of acetic acid was used. Product-containing fractions were collected and lyophilized to obtain the purified product, typically as TFA or acetate salt.

Solubility and Stability-Testing of exendin-4 derivatives

Prior to the testing of solubility and stability of a peptide batch, its content was determined. Therefore, two parameters were investigated, its purity (HPLC-UV) and the amount of salt load of the batch (ion chromatography).

Example 2

For solubility testing, the target concentration was 1.0 mg/mL pure compound. Therefore, solutions from solid samples were prepared in different buffer systems with a concentration of 1.0 mg/mL compound based on the previously determined content. HPLC-UV was performed after 2 h of gentle agitation from the supernatant, which was obtained by 20 min of centrifugation at 4000 rpm.

The solubility was then determined by comparison with the UV peak areas obtained with a stock solution of the peptide at a concentration of 2 mg/mL in pure water or a variable amount of acetonitrile (optical control that all of the compound was dissolved). This analysis also served as starting point (t0) for the stability testing.

Example 3

For stability testing, an aliquot of the supernatant obtained for solubility was stored for 7 days at 25°C or 40 °C. After that time course, the sample was centrifuged for 20 min at 4000 rpm and the supernatant was analysed with HPLC-UV.

For determination of the amount of the remaining peptide, the peak areas of the target compound at t0 and t7 were compared, resulting in "% remaining peptide", following the equation

% remaining peptide = [(peak area peptide t7) x 100]/peak area peptide t0.

The amount of soluble degradation products was calculated from the comparison of the sum of the peak areas from all observed impurities reduced by the sum of peak areas observed at t0 (i.e. to determine the amount of newly formed peptide-related species). This value was given in percentual relation to the initial amount of peptide at t0, following the equation:

% soluble degradation products = {[(peak area sum of impurities t7) - (peak area sum of impurities t0)] x 100}/peak area peptide t0

The potential difference from the sum of "% remaining peptide" and "% soluble degradation products" to 100% reflects the amount of peptide which did not remain soluble upon stress conditions following the equation

% precipitate = 100-([% remaining peptide] + [% soluble degradation products])

This precipitate includes non-soluble degradation products, polymers and/or fibrils, which have been removed from analysis by centrifugation.

The chemical stability is expressed as “% remaining peptide”.

Anion Chromatography

Instrument: Dionex ICS-2000, pre/column: Ion Pac AG-18 2 x 50 mm (Dionex)/AS18 2 x 250 mm (Dionex), eluent: aqueous sodium hydroxide, flow: 0.38 mL/min, gradient: 0-6 min: 22 mM KOH, 6-12 min: 22-28 mM KOH, 12-15 min: 28-50 mM KOH, 15-20min: 22mM KOH, suppressor: ASRS 300 2 mm, detection: conductivity.

As HPLC/UPLC method, method D or E has been used.

Example 4: In vitro data on GLP-1 and glucagon receptor

Potencies of peptidic compounds at the GLP-1 and glucagon receptors were determined by exposing cells expressing human glucagon receptor (hGlucagon R) or human GLP-1 receptor (hGLP-1 R) to the listed compounds at increasing concentrations and measuring the formed cAMP as described in example 27.

The results are shown in Table 2:

Table 2. EC50 values of exendin-4 derivatives at GLP-1 and Glucagon receptors (indicated in pM)

SEQ ID NO	EC50 hGLP-1R	EC50 hGlucagon-R	SEQ ID NO	EC50 hGLP-1R	EC50 hGlucagon-R
4	1.7	6.2	24	1.2	11.1
5	2.7	5.0	25	4.6	259.0
6	4.9	24.3	26	0.9	33.8

7	8.5	20.6	27	1.0	44.8
8	1.2	5.3	28	1.1	57.7
9	1.5	35.6	29	0.4	21.3
10	1.6	47.5	30	0.5	10.9
11	0.9	68.0	31	0.6	24.6
12	1.0	35.6	32	1.2	29.8
13	1.4	44.9	33	2.5	94.1
14	1.6	77.5	34	1.1	41.5
15	1.0	18.3	35	1.0	555.0
16	3.7	8.2	36	1.0	581.0
17	6.0	10.1	37	1.4	58.1
18	2.6	7.9	38	0.8	11.5
19	1.7	4.7	39	1.0	401.0
20	0.8	26.7	40	3.5	12.1
21	1.9	8.9	41	2.2	25.7
22	4.4	26.1	43	2.0	53.9
23	1.2	3.9	44	1.8	20.0
45	2.1	7.9	46	2.4	8.0
47	5.3	13.4	48	2.1	10.0
49	1.8	6.5	50	1.8	4.2
51	2.3	3.7	52	2.1	4.2

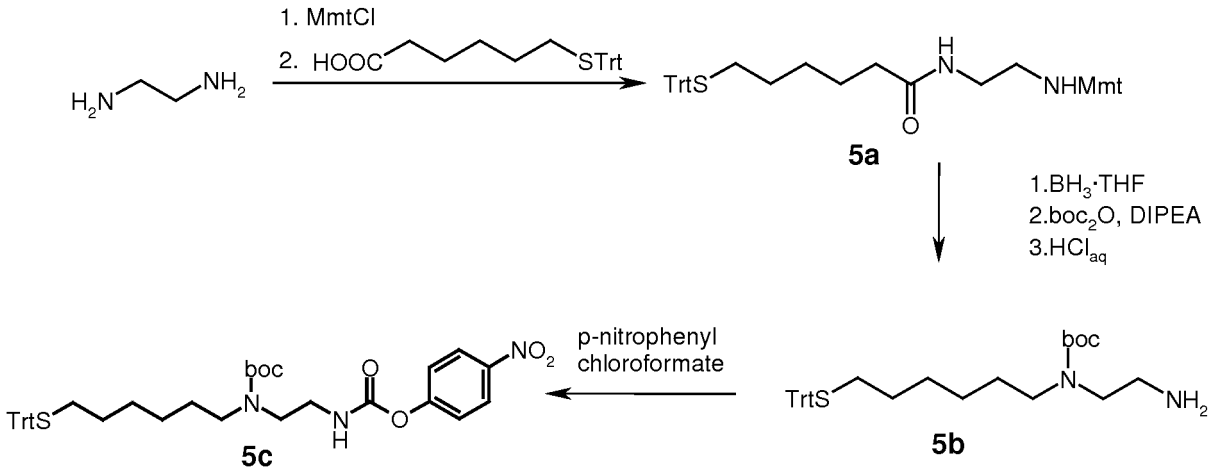
53	2.4	10.5	54	1.4	7.7
55	2.4	10.5	56	1.9	9.4
57	1.4	4.7	58	1.4	11.5
59	n.a.	n.a.	60	1.8	6.5

Linker synthesis

Example 5

Synthesis of linker reagent 5c

Linker reagent 5c was synthesized according to the following scheme:



Synthesis of linker reagent intermediate 5a:

m-Methoxytrityl chloride (3 g, 9.71 mmol) was dissolved in DCM (20 mL) and added dropwise to a solution of ethylenediamine (6.5 mL, 97.1 mmol) in DCM (20 mL). After two hours the solution was poured into diethyl ether (300 mL) and washed three times with 30/1 (v/v) brine/0.1 M NaOH solution (50 ml each) and once with brine (50 mL). The organic phase was dried over Na_2SO_4 and volatiles were removed under reduced pressure . Mmt-protected intermediate (3.18 g, 9.56 mmol) was used in the next step without further purification.

The Mmt-protected intermediate (3.18 g, 9.56 mmol) was dissolved in anhydrous DCM (30 mL). 6-(S-Tritylmercapto)hexanoic acid (4.48 g, 11.47 mmol), PyBOP (5.67 g, 11.47 mmol) and DIPEA (5.0 mL, 28.68 mmol) were added and the mixture was agitated for 30 min at RT. The solution was diluted with diethyl ether (250 mL) and washed three times with 30/1 (v/v) brine/0.1 M NaOH solution (50 mL each) and once with brine (50 mL). The organic phase was dried over Na_2SO_4 and volatiles were removed under reduced pressure. 5a was purified by flash chromatography.
Yield: 5.69 g (8.09 mmol).
MS: m/z 705.4 = $[M+H]^+$ (MW calculated = 705.0).

Synthesis of linker reagent intermediate 5b:

To a solution of 5a (3.19 g, 4.53 mmol) in anhydrous THF (50 mL) was added BH_3·THF (1 M solution, 8.5 mL, 8.5 mmol) and the solution was stirred for 16 h at RT. Further BH_3·THF (1 M solution, 14 mL, 14 mmol) was added and stirred for 16 h at RT. The reaction was quenched by addition of methanol (8.5 mL). *N*,*N*-dimethyl-ethylenediamine (3 mL, 27.2 mmol) was added and the solution was heated to reflux and stirred for three h. Reaction mixture was allowed to cool down to RT and was then diluted with ethyl acetate (300 mL), washed with saturated, aqueous Na_2CO_3 solution (2 x 100 mL) and saturated, aqueous $NaHCO_3$ solution (2 x 100 mL). The organic phase was dried over Na_2SO_4 and volatiles were removed under reduced pressure to obtain crude amine intermediate (3.22 g).

The amine intermediate (3.22 g) was dissolved in DCM (5 mL). Boc_2O (2.97 g, 13.69 mmol) dissolved in DCM (5 mL) and DIPEA (3.95 mL, 22.65 mmol) were added and the mixture was agitated at RT for 30 min. The mitxture was purified by flash chromatography to obtain the crude Boc- and Mmt-protected intermediate (3.00 g).
MS: m/z 791.4 = $[M+H]^+$, 519.3 = $[M\text{-}Mmt+H]^+$ (MW calculated = 791.1).

0.4 M aqueous HCl (48 mL) was added to a solution of the Boc- and Mmt-protected intermediate in acetonitrile (45 mL). The mixture was diluted with acetonitrile (10 mL) and stirred for 1 h at RT. Subsequently, the pH value of the reaction mixture was adjusted to 5.5 by addition of 5 M NaOH solution. Acetonitrile was removed under reduced pressure and the aqueous solution was extracted with DCM (4 x 100 mL). The

combined organic phases were dried over Na_2SO_4 and volatiles were removed under reduced pressure. Crude 5b was used in the next step without further purification.
Yield: 2.52 g (3.19 mmol).
MS: m/z 519.3 = $[M+H]^+$ (MW calculated = 519.8 g/mol).

Synthesis of linker reagent 5c:

Intermediate 5b (985 mg, 1.9 mmol) and *p*-nitrophenyl chloroformate (330 mg, 2.5 mmol) were dissolved in anhydrous THF (10 mL). DIPEA (0.653 mL, 3.7 mmol) was added and the mixture was stirred for 2 h at RT. The solution was acidified by addition of acetic acid (1 mL). 5c was purified by RP-HPLC.
Yield: 776 mg, (1.13 mmol).
MS m/z 706.3 = $[M+Na]^+$ (MW calculated = 706.3).

Synthesis of peptide linker reagent

a) Peptide synthesis

The solid phase peptide syntheses were performed for example on a Prelude Peptide Synthesizer (Protein Technologies Inc) or similar automated synthesizer using standard Fmoc chemistry and HBTU/DIPEA activation. DMF was used as the solvent. Deprotection : 20% piperidine/DMF for 2 x 2.5 min. Washes: 7 x DMF. Coupling 2:5:10 200 mM AA / 500 mM HBTU / 2M DIPEA in DMF 2 x for 20 min. Washes: 5 x DMF.

b) N-terminal elongation with D-Ala

0.9 mmol resin bound peptide (equivalent to 4 g resin) synthesized as described in step a with a free amino group at the N-terminus was split into five equal portions. Each portion was suspended in 15 ml DMF and subsequently 2.5 eq. Fmoc-D-Ala-OH, 2.5 eq. HATU, 2.5 eq. HOAt and 2.5 eq. DIPEA were added. The mixture was agitated for 16 h at ambient temperature. Then the reaction mixture was removed by filtration and the resin was washed 3 times with 18 ml DMF, 18 ml DCM, 18 ml iso-propanol, 18 ml diethyl ether. The remaining solvents were removed in vacuo.

c) Fmoc deprotection, linker attachment and cleavage

0.37 mmol resin bound peptide (equivalent to 1.6 g resin) synthesized as described in step b with a free amino group at the N-terminus was split into two equal portions. Each portion was suspended in 12 ml of a 20 % solution of piperidine in DMF and agitated for 5 min. The solvent was removed and the procedure was repeated twice.

The resin bound peptide was washed 5 times with 12 ml DMF.

Then the resin was suspended in 10 ml DMF and 2.5 eq. of tert-butyl (2-(((4-nitrophenoxy)carbonyl)amino)ethyl)(6-(tritylthio)hexyl)carbamate 5c and 2.5 eq. DIPEA were added. The mixture was agitated for 16 h at ambient temperature. Then the reaction mixture was removed by filtration and the resin was washed 3 times with 15 ml DMF, 15 ml DCM, 15 ml iso-propanol, 15 ml diethyl ether. The remaining solvents were removed in vacuo. The reaction was tested for completion by a Kaisertest

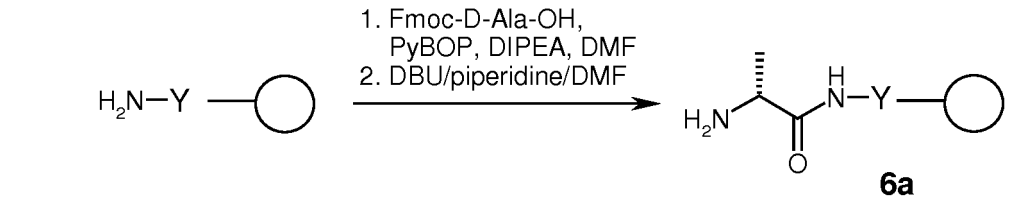
Then a mixture of TFA/DTT/TIS/H_2O/thioanisole/Bu_4NBr (100/3/2/3/1/0.05) was added and the mixture was agitated for 3.5 h. The mixture was filtrated and the resin was washed with 1 ml TFA. The combined filtrates were added to 100 ml cooled diethyl ether. The precipitate was isolated by centrifugation and it was washed 2 times with 100 ml diethyl ether.

The crude product was purified via preparative HPLC on a Waters column (XBridge, BEH130, Prep C18 5µM) using an acetonitrile/water gradient (both buffers with 0,1% TFA). The purified intermediate was imidiately lyophilized and stored in an Ar atmosphere or used directly for the next step.

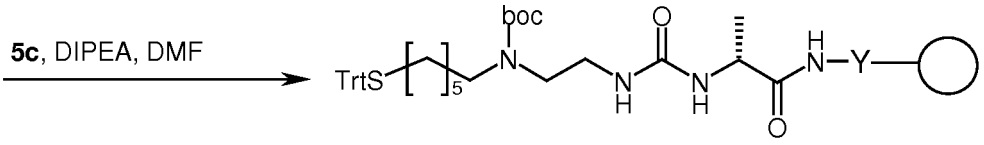
Example 6

Synthesis of GLP-1/Glucagon agonist linker reagent 6d (Ala linker)

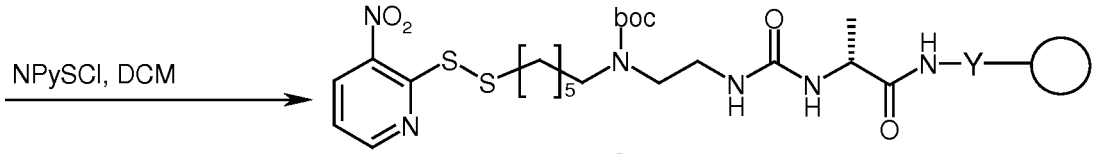
GLP-1/Glucagon agonist linker reagent 6d was synthesized according to the following scheme:



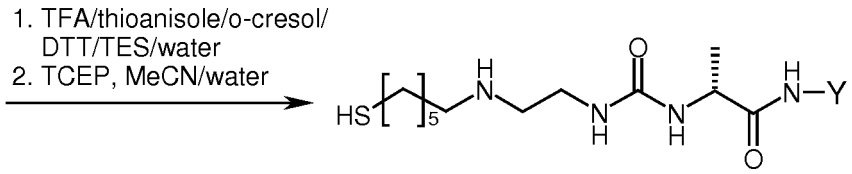
6a



6b



6c



6d

Synthesis of GLP-1/Glucagon agonist linker reagent intermediate 6a:

Fully side chain protected GLP-1/Glucagon agonist with free N-terminus on resin (2.00 g, 0.2 mmol, loading approximately 0.1 mmol/g) was transferred into a 20 mL syringe equipped with a filter frit. 8 mL of anhydrous DMF was drawn into the syringe and the syringe was shaken (600 rpm) for 15 min in order to pre-swell the resin. The solvent was discarded, and a solution of Fmoc-D-alanine-OH (187 mg, 0.6 mol), PyBOP (312 mg, 0.6 mmol), and DIPEA (174 µL, 1.0 mmol) in anhydrous DMF (4 mL) was drawn into the syringe. The syringe was shaken at RT and 600 rpm for 60 min. The solution was discharged, and the resin was washed ten times with DMF.

Fmoc-deprotection was performed as described above.

Synthesis of GLP-1/Glucagon agonist linker reagent intermediate 6b:

A solution of 5c (137 mg, 0,4 mmol) in anhydrous DMF (3 mL) was added to the resin 6a (0.2 mmol), followed by a solution of DIPEA (80 µL, 0.46 mmol) in anhydrous DMF (4.5 mL), and the reaction mixture was shaken (600 rpm) at 22°C for 15 hours.

The resin was washed ten times with DMF and ten times with DCM and dried *in vacuo*.

Synthesis of GLP-1/Glucagon agonist linker reagent intermediate 6c:

3-Nitro-2-pyridine-sulfenyl chloride (48 mg, 0.25 mmol) was given into a syringe containing 6b (0.05 mmol, 0.5 g). Anhydrous DCM (4 mL) was drawn into the syringe and the mixture was shaken (600 rpm) at RT. After 2 h the solution was discarded and the resin was washed 14 times with DCM and dried *in vacuo*.

Synthesis of GLP-1/Glucagon agonist linker reagent intermediate 6d:

In a round bottom flask *o*-cresol (1.5 mL), thioanisole (1.5 mL), DTT (1.125 g), TES (1.125 mL), and water (1.5 mL) were dissolved in TFA (37.5 mL). 6c (0.15 mmol, 1.5 g) was added to the stirred (250-350 rpm) solution at RT in order to obtain a homogeneous suspension. Stirring was continued for 45 min. The solution was separated from the resin beads by filtration, the beads were washed with TFA twice (2 mL each) and the washing solutions were combined with the filtrate. TFA was removed from the combined solutions in a stream of nitrogen.

Crude 6d was precipitated from the concentrated solution (approx. 10 mL) by addition of diethyl ether (30 mL) and vigorous shaking. After centrifugation (2 min, 5000 rpm) the supernatant was discarded and the precipitate was washed with diethyl ether twice (20 mL each).

Dried precipitate was dissolved in a solution of TCEP (114 mg, 0.39 mmol) in 30 ml 1/19 (v/v) acetonitrile/water containing 0.01 % TFA (v/v). Mixture was incubated for 15 hours at RT. 6d was purified by RP-HPLC as described in Materials and Methods using a 150 x 30 mm Waters XBridge™ BEH300 C18 10 µm column and a flow of 40 ml/min.

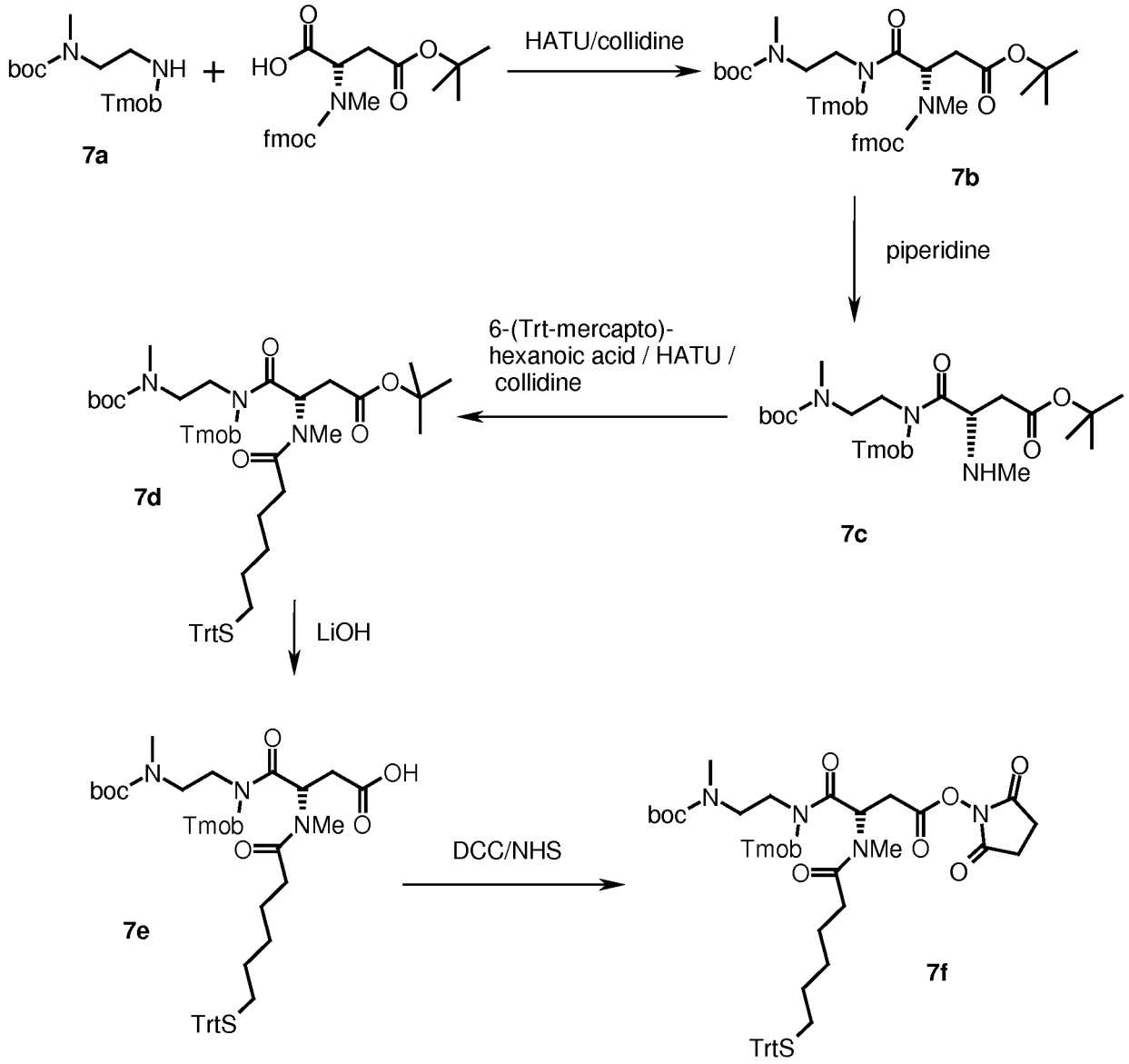
Up to 12 mL of the mixture were loaded on the column. The elution was performed using a linear gradient from 5% to 30% solvent B (5 min) followed by a linear gradient from 30% to 35% solvent B (40 min). Fractions containing product 6d were pooled and lyophilized. Purity: 86 % (215 nm)

Yield: 85.2 mg (19.2 µmol, starting from 2.00 g resin).
MS m/z 1486.7 = $[M+3H]^{3+}$, (MW calculated = 4460.0 g/mol).

Example 7 (Asn linker)

Synthesis of linker reagent 7f

Linker reagent 7f was synthesized according to the following scheme:



To a cooled (0 °C) solution of *N*-Methyl-*N*-boc-ethylendiamine (0.5 mL, 2.79 mmol) and $NaCNBH_3$ (140 mg, 2.23 mmol) in MeOH (10 mL) and acetic acid (0.5 mL) was added a solution of 2,4,6-trimethoxybenzaldehyde (0.547 mg, 2.79 mmol) in EtOH (10 mL). The mixture was stirred at RT for 2 h, acidified with 2 M HCl (1 mL) and neutralized with saturated aqueous Na_2CO_3 (50 mL). Evaporation of all volatiles, DCM extraction of the resulting aqueous slurry and concentration of the organic fractions yielded *N*-Methyl-*N*-boc-*N'*-tmob-ethylendiamine (7a) as a crude oil which was purified by RP-HPLC.

Yield: 593 mg (1.52 mmol)

MS: m/z 377.35 = $[M+Na]^+$, (calculated = 377.14).

N-Fmoc-*N*-Me-Asp(O*t*Bu)-OH (225 mg, 0.529 mmol) was dissolved in DMF (3 mL) and 7a (300 mg, 0.847 mmol), HATU (201 mg, 0.529 mmol), and collidine (0.48 mL, 3.70 mmol) were added. The mixture was stirred at RT for 2 h to yield 7b. For fmoc deprotection, piperidine (0.22 mL, 2.16 mmol) was added and stirring was continued for 1 h. Acetic acid (1 mL) was added, and 7c was purified by RP-HLPC.

Yield: 285 mg (0.436 mmol as TFA salt)

MS: m/z 562.54 = $[M+Na]^+$, (calculated = 562.67).

6-Tritylmercaptohexanoic acid (0.847 g, 2.17 mmol) was dissolved in anhydrous DMF (7 mL). HATU (0.825 g, 2.17 mmol), and collidine (0.8 mL, 6.1 mmol) and 7c (0.78 g, 1.44 mmol) were added. The reaction mixture was stirred for 60 min at RT, acidified with AcOH (1 mL) and purified by RP-HPLC. Product fractions were neutralized with saturated aqueous $NaHCO_3$ and concentrated. The remaining aqueous phase was extracted with DCM and 7d was isolated upon evaporation of the solvent.

Yield: 1.4 g (94%)

MS: m/z 934.7 = $[M+Na]^+$, (calculated = 934.5).

To a solution of 7d (1.40 mg, 1.53 mmol) in MeOH (12 mL) and H_2O (2 mL) was added LiOH (250 mg, 10.4 mmol) and the reaction mixture was stirred for 14 h at 70 °C. The mixture was acidified with AcOH (0.8 mL) and 7e was purified by RP-HPLC. Product fractions were neutralized with saturated aqueous $NaHCO_3$ and concentrated. The aqueous phase was extracted with DCM and 7e was isolated upon evaporation of the solvent.

Yield: 780 mg (60 %)

MS: m/z 878.8 = $[M+Na]^+$, (calculated = 878.40).

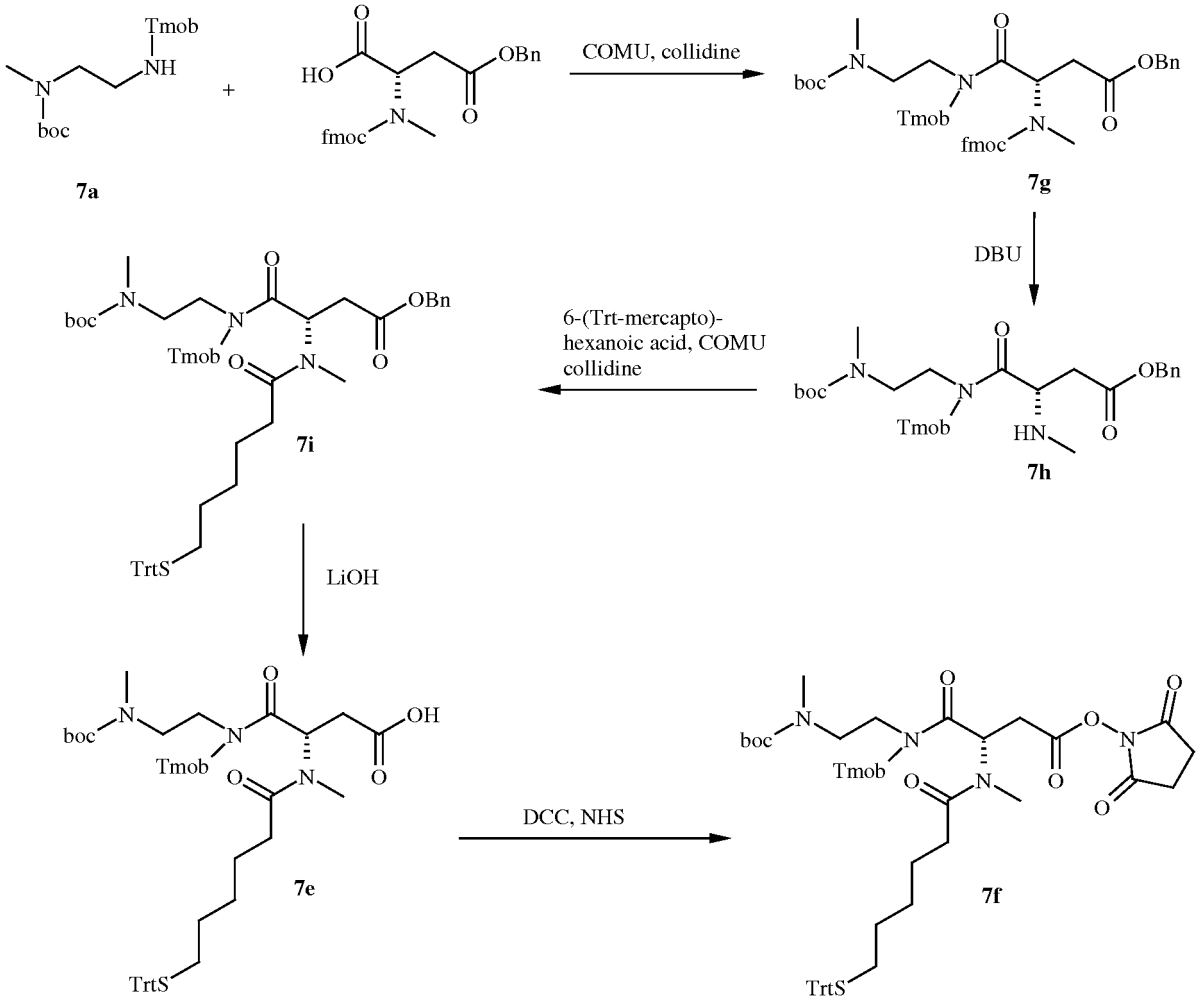
To a solution of 7e (170 mg, 0.198 mmol) in anhydrous DCM (4 mL) were added DCC (123 mg, 0.59 mmol) and *N*-hydroxy-succinimide (114 mg, 0.99 mmol), and the reaction mixture was stirred at RT for 1 h. The mixture was filtered, and the filtrate was acidified with 0.5 mL AcOH and 7f purified by RP-HPLC. Product fractions were neutralized with saturated aqueous $NaHCO_3$ and concentrated. The remaining aqueous phase was extracted with DCM and 7f was isolated upon evaporation of the solvent.

Yield: 154 mg (0.161 mmol)

MS: m/z 953.4 = $[M+H]^+$, (calculated = 953.43).

Alternatively, linker reagent 7f was synthesized according to the following procedure:

Alternative reaction scheme:



To a solution of *N*-Methyl-*N*-boc-ethylenediamine (2 g, 11.48 mmol) and $NaCNBH_3$ (819 mg, 12.63 mmol) in MeOH (20 mL) was added 2,4,6-trimethoxybenzaldehyde (2.08mg, 10.61 mmol) portion wise. The mixture was stirred at RT for 90 min, acidified with 3 M HCl (4 mL) and stirred further 15 min. The reaction mixture was added to saturated $NaHCO_3$ solution (200 mL) and extracted 5 x with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and the solvents were evaporated in vacuo. The resulting *N*-Methyl-*N*-boc-*N'*-tmob-ethylenediamine (7a) was completely dried in high vacuum and used in the next reaction step without further purification.

Yield: 3.76 g (11.48 mmol, 89 % purity, 7a : double Tmob protected product = 8 :1)

MS: m/z 355.22 = $[M+H]^+$, (calculated = 354.21).

To a solution of 7a (2 g, 5.65 mmol) in CH_2Cl_2 (24 ml) COMU (4.84 g, 11.3 mmol), *N*-Fmoc-*N*-Me-Asp(OBn)-OH (2.08 g, 4.52 mmol) and collidine (2.65 mL, 20.34 mmol) were added. The reaction mixture was stirred for 3 h at RT, diluted with CH_2Cl_2 (250 mL) and washed 3 x with 0.1 M H_2SO_4 (100 ml) and 3 x with brine (100 ml). The aqueous phases were re extracted with CH_2Cl_2 (100 ml). The combined organic phases were dried over Na_2SO_4, filtrated and the residue concentrated to a volume of 24 mL. 7g was purified using flash chromatography.

Yield: 5.31 g (148 %, 6.66 mmol)

MS: m/z 796.38 = $[M+H]^+$, (calculated = 795.37).

To a solution of 7g [5.31 g, max. 4.51 mmol ref. to *N*-Fmoc-*N*-Me-Asp(OBn)-OH] in THF (60 mL) DBU (1.8 mL, 3 % v/v) was added. The solution was stirred for 12 min at RT, diluted with CH_2Cl_2 (400 ml) and washed 3 x with 0.1 M H_2SO_4 (150 ml) and 3 x with brine (150 ml). The aqueous phases were re extracted with CH_2Cl_2 (100 ml). The combined organic phases were dried over Na_2SO_4 and filtrated. 7h was isolated upon evaporation of the solvent and used in the next reaction without further purification.

MS: m/z 574.31 = $[M+H]^+$, (calculated = 573.30).

7h (5.31 g, 4.51 mmol, crude) was dissolved in acetonitrile (26 mL) and COMU (3.87 g, 9.04 mmol), 6-Tritylmercaptohexanoic acid (2.12 g, 5.42 mmol) and collidine (2.35 mL, 18.08 mmol) were added. The reaction mixture was stirred for 4 h at RT, diluted with CH_2Cl_2 (400 ml) and washed 3 x with 0.1 M H_2SO_4 (100 ml) and 3 x with brine (100 ml). The aqueous phases were re extracted with CH_2Cl_2 (100 ml). The combined organic phases were dried over Na_2SO_4, filtrated and 7i was isolated upon evaporation of the solvent. Product 7i was purified using flash chromatography.

Yield: 2.63 g (62 %, 94 % purity)

MS: m/z 856.41 = $[M+H]^+$, (calculated = 855.41).

To a solution of 7i (2.63 g, 2.78 mmol) in *i*-PrOH (33 mL) and H_2O (11 mL) was added LiOH (267 mg, 11.12 mmol) and the reaction mixture was stirred for 70 min at RT. The mixture was diluted with CH_2Cl_2 (200 ml) and washed 3 x with 0.1 M H_2SO_4 (50 ml) and 3 x with brine (50 ml). The aqueous phases were re-extracted with CH_2Cl_2 (100 ml). The

combined organic phases were dried over Na_2SO_4, filtrated and 7e was isolated upon evaporation of the solvent. 7j was purified using flash chromatography.

Yield: 2.1 g (88 %)

MS: m/z 878.4 = $[M+Na]^+$, (calculated = 878.40).

To a solution of 7e (170 mg, 0.198 mmol) in anhydrous DCM (4 mL) were added DCC (123 mg, 0.59 mmol), and a catalytic amount of DMAP. After 5 min *N*-hydroxy-succinimide (114 mg, 0.99 mmol) was added and the reaction mixture was stirred at RT for 1 h. The reaction mixture was filtered, the solvent was removed *in vacuo* and the residue was taken up in 90 % acetonitrile plus 0.1 % TFA (3.4 ml). The crude mixture was purified by RP-HPLC. Product fractions were neutralized with 0.5 M pH 7.4 phosphate buffer and concentrated. The remaining aqueous phase was extracted with DCM and 7f was isolated upon evaporation of the solvent.

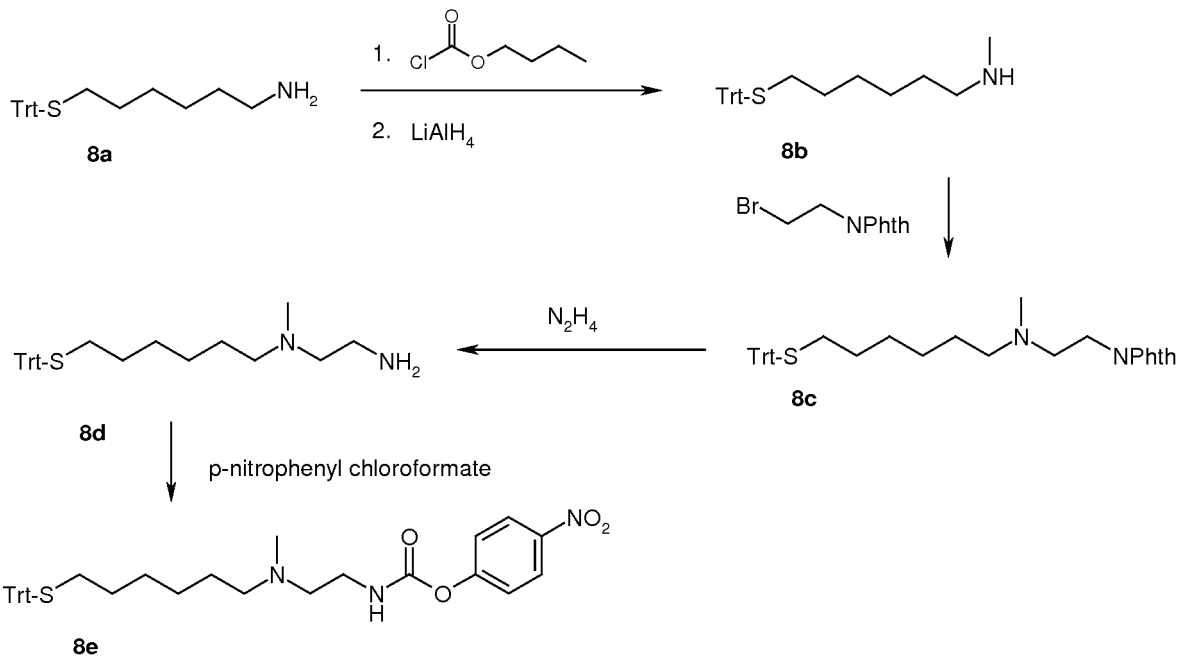
Yield: 154 mg (81%)

MS: m/z 953.4 = $[M+H]^+$, (calculated = 953.43).

Example 8

Synthesis of linker reagent 8e

Linker reagent 8e was synthesized according to the following scheme:



Synthesis of linker reagent intermediate 8b was performed under nitrogen atmosphere. A solution of amine 8a (1.69 g, 4.5 mmol, for preparation see WO-A 2009/133137) in 30 mL THF (dry, mol. sieve) was cooled to 0 °C. Butyl chloroformate (630 µl, 4.95 mmol) in 3 mL THF (dry, mol. sieve) and DIPEA (980 µl, 5.63 mmol) were added. Mixture was stirred for 10 min at 0 °C, cooling was removed and mixture stirred for further 20 min at RT. 1 M $LiAlH_4$ in THF (9 mL, 9 mmol) was added and mixture was refluxed for 1.5 h. Reaction was quenched by slowly adding methanol (11 mL) and 100 mL sat. Na/K tartrate solution. Mixture was extracted with ethyl acetate, organic layer was dried over Na_2SO_4 and solvent was evaporated under reduced pressure. Crude product 8b (1.97 g) was used in the next step without further purification.

MS: m/z 390.2 = $[M+H]^+$ (MW calculated = 389.6).

A solution of crude product 8b (1.97 g), N-(bromoethyl)-phthalimide (1.43 g, 5.63 mmol) and K_2CO_3 (1.24 g, 9.0 mmol) in 120 mL acetonitrile was refluxed for 6 h. 60 mL of a sat. $NaHCO_3$ solution was added and mixture was extracted 3 x with ethyl acetate. Combined organics were dried (Na_2SO_4) and solvent was removed under reduced pressure. Phthalimide 8c was purified on silica by using heptane (containing 0.02 % NEt_3) and an ascending amount of ethyl acetate (containing 0.02 % NEt_3) as eluents.

Yield: 0.82 g (1.46 mmol)

MS: m/z 563.3 = $[M+H]^+$ (MW calculated = 562.8).

Phthalimide 8c (819 mg 1.46 mmol) was dissolved in 35 mL ethanol and hydrazine hydrate (176 µl, 3,64 mmol) was added. Mixture was refluxed for 3 h. Precipitate was filtered off. Solvent was removed under reduced pressure and residue was treated with 15 mL dichloromethane. Precipitate was filtered off and dichloromethane was removed under reduced pressure. Residue was purified by RP HPLC. Pooled HPLC fractions were adjusted to pH 7 by adding $NaHCO_3$ and extracted several times with dichloromethane. Combined organics were dried (Na_2SO_4) and solvent was removed under reduced pressure to yield amine 8d.

Yield: 579 mg (1.34 mmol)

MS: m/z 433.3 = $[M+H]^+$ (MW calculated = 432.7).

Para-nitrophenyl chloroformate (483 mg, 2.40 mmol) was dissolved in 10 mL dichloromethane (dry, mol. sieve). A solution of amine 8d (1.00 g, 2.31 mmol) in 5 mL dichloromethane (dry, mol. sieve) and 1.8 mL of sym-collidine were added and mixture was stirred at room temperature for 40 min. Dichloromethane was removed under reduced pressure, residue was acidified with acetic acid and purified by RP-HPLC to yield para-nitrophenyl carbamate 8e.

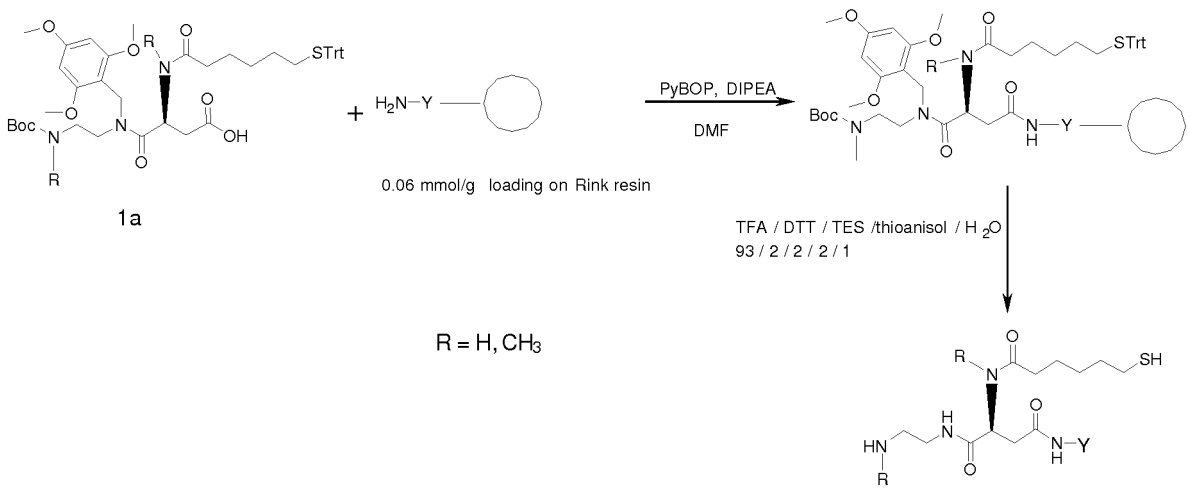
Yield: 339 mg (0.57 mmol)

MS: m/z 598.3 = $[M+H]^+$ (MW calculated = 597.8).

Synthesis of peptide-linker –polymer conjugates

Example 9

Synthesis of GLP/Glucagon agonist thiol linker 1 was synthesized according to the following scheme:



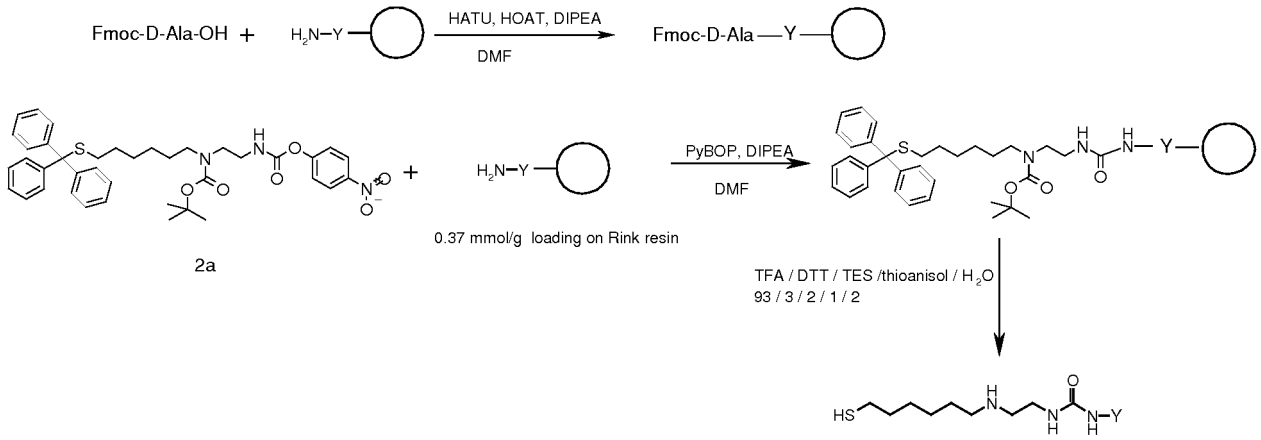
To 400 mg of the resin bound Fmoc-protected Exendin-4 Seq. ID. 26 on rink resin in a peptide synthesis vessel was added 4 ml of 20% piperidine in DMF and the reaction mixture was stirred for 5 minutes. The process was repeated three times. The resin bound peptide was subsequently washed with 4 ml of DMF three times.

To 80 mg (2,5 eqv.) of the linker 1a and 60 mg (3.0 eqv.) of PyBOP was added 5 mL of anhydrous DMF. To this solution was added 16 µl (3.0 eqv.) of diisoproyldiethyamine (DIPEA). The resulting solution was added to above processed resin bound Exendin-4 Seq ID. 3126 -resin. The mixture was allowed to proceed for 18 hr. at 25°C. At the end of this time, the resin filtered and washed with anhydrous DMF (5 ml x 5), dichloromethane (5 ml x 5), isopropanol (5 ml x 5) and diethyl ether (5 ml x 5).

The above resin bound peptide-linker conjugate was treated with 10 ml of a solution containing trifluoroacetic acid (TFA) /triethylsilane(TES) / dithiothreitol (DTT) / Thioanisole / water (100/2/3/1/2). The reaction mixture was shaken at 25°C for 3 hr. At the end of reaction, the solvent was filtered off and the resin was washed with dichloromethane (5 x 10 ml). The filtrate was concentrated under reduced pressure. The residue was poured into 50 ml ice-cold ether. White solid precipitated out. The mixture was centrifuged and decanted. The solid was washed with diethyl ether (2 X 20 ml). The peptide-linker was purified by reverse phase HPLC (30 x 100 mm C18 column, 25-40% AcCN / H_2O w/ 0.1% TFA in 20 min.). The appropriate fractions were collected and evaporated to dryness yielding 16 mg of the desired product as a white solid. The product was identified my mass spectrometry (molecular ion peak, m/z 1469, z=3).

Example 10

GLP/Glucagon thiol linker 2 was synthesized according to the following scheme:



To 1.8 g of the resin bound Fmoc-protected Exendin-4 Seq ID. 26 on rink resin in a peptide synthesis vessel was added 20 ml of 20% piperidine in DMF and the reaction mixture was stirred for 5 minutes. The process was repeated three times. The resin bound peptide was subsequently washed with 20 ml of DMF three times.

To 163 mg (2,5 eqv.) Fmoc-D-Ala-OH, 199 mg (2,5 eqv.) of HATU and 71 mg (2.5 eqv.) of HOAt was added 15 ml of anhydrous DMF. To this solution was added 92 µl (2.5 eqv.) of diisoproyldiethyamine (DIPEA). The resulting solution was added to above processed resin bound Exendin-4 Seq ID. 26 -resin. The mixture was allowed to proceed for 16 hr. at 25°C. At the end of this time, the resin filtered and washed with anhydrous DMF (20 ml x 5), dichloromethane (20 ml x 5), isopropanol (20 ml x 5) and diethyl ether (20 ml x 5).

To the above resin bound Fmoc-protected D-Ala-Exendin-4-Seq ID. 26 on rink resin in a peptide synthesis vessel was added 15 ml of 20% piperidine in DMF and the reaction mixture was stirred for 5 minutes. The process was repeated three times. The resin bound peptide was subsequently washed with 15 ml of DMF three times.

To 352 mg (2,5 eqv.) of the linker 2a was added 15 mL of anhydrous DMF. To this solution was added 92 µl (2.5 eqv.) of diisoproyldiethyamine (DIPEA). The resulting solution was added to above processed resin bound D-Ala-Exendin-4 Seq ID. 26 -resin. The mixture was allowed to proceed for 16 hr. at 25°C. At the end of this time,

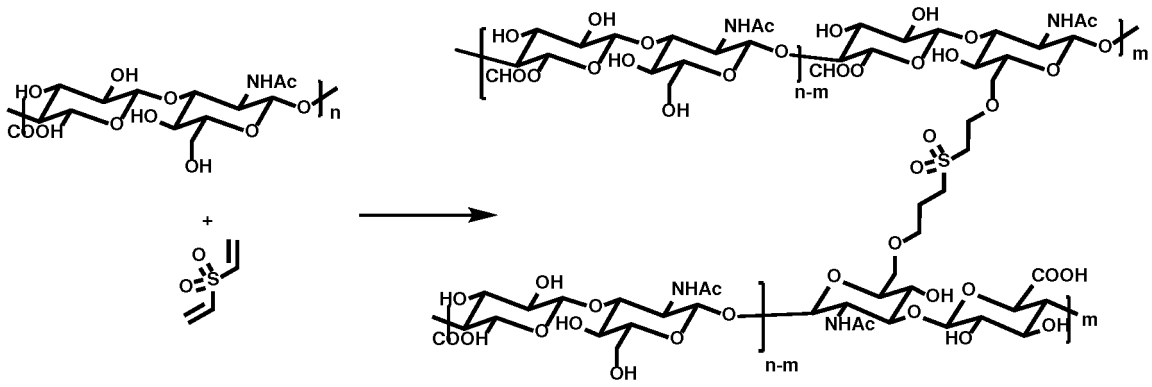
the resin filtered and washed with anhydrous DMF (15 ml x 5), dichloromethane (15 ml x 5), isopropanol (15 ml x 5) and diethyl ether (15 ml x 5).

The above resin bound peptide-linker conjugate was treated with 40 ml of a solution containing trifluoroacetic acid (TFA) /triethylsilane(TES) / dithiothreitol (DTT) / Thioanisole / water (100/2/3/1/2). The reaction mixture was shaken at 25°C for 1 hr. At the end of reaction, the solvent was filtered. The filtrate was poured into 400 ml ice-cold ether. White solid precipitated out. The mixture was centrifuged and decanted. The solid was washed with diethyl ether (2 X 200 ml). The peptide-linker was purified by reverse phase HPLC (30 x 100 mm C18 column, 25-40% AcCN / H_2O w/ 0.1% TFA in 20 min.). The appropriate fractions were collected and evaporated to dryness yielding 104 mg of the desired product as a white solid. The product was identified my mass spectrometry (molecular ion peak, m/z 1465.3, z=3 and m/z 1099.2, z=4).

Synthesis of Hyaloronic acid hydrogels

Example 11.

Divinyl sulfone crosslinked hyaluronic acid was synthesized according to the following scheme:



Example 11a

To 0.2M sodium hydroxide (168.9g) was added sodium chloride (23.4g) with stirring until dissolved. To the solution under rapid mechanical stirring was added sodium hyaluronate (25.4g, 400 – 500KDa) which continued for 2h. The resulting polymer solution has a concentration of ~ 12%w/w. A solution of divinylsulfone (0.41mL, 0.48g)

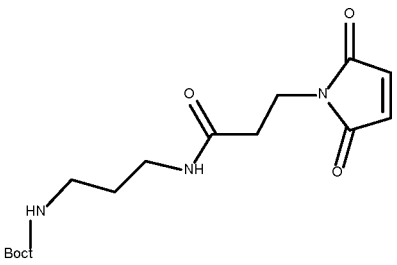
in isopropanol (1.6mL) was prepared and added (5 x 0.4mL) over ~ 30sec. The mixture was stirred for an additional 2min and poured into a 23x28x6.5cm glass tray and sealed with a plastic cover. After standing at RT for 4h the gel was transferred as a single piece to a solution of 1M hydrochloric acid (100.1g) in 0.9% saline (3kg). It was agitated gently at RT. After 24h the pH of the solution was 2.28. The solution discarded leaving a gel (416.2g). To the gel was then added 0.9% saline (3kg) and it was agitated gently at RT for 18h. To the mixture was added 1M sodium hydroxide (9.7mL) at 0,2,4,6 and 8h. The gel was gently agitated for a further 24h at RT at which time the pH of the gel was 6.65. The gel was stored at 2 - 8°C for 120h and then 10mM sodium phosphate solution pH 7.4 (2L) was added. The gel was agitated for an additional 21h and the wash discarded leaving a gel (1036.2g) with a final polymer concentration of 2.4%.

Example 11b. alternative synthesis of Divinylsulphone crosslinked hyaluronic acid

To 35 g of sodium hyaluronate was added 946 mL of sterile water. The reaction mixture was kept at 2-8°C for 7 days, during which time a clear solution has formed. To this solution was added 1M 111mL of 1.0 M sodium hydroxide solution and the resulting reaction mixture was stirred vigorously for 5min. The reaction mixture was kept at 2-8°C for 90min. Subsequently a suspension of 6.7 mL of divinylsulphone in 10 mL of sterile water was to the polymer solution and the resulting reaction mixture was stirred vigorously for 5minutes. Subsequently, reaction mixture was stored at 2-8°C for 150 minutes followed by for 90 minutes at 25°C. The polymer gel thus formed was washed with 0.9% sterile saline for four days. The pH of the suspension was adjusted to 7.0 with either 1.)M NaOH or 1.0 M HCl. The final concentration of the gel suspension was 0.58%.

Example 12

Synthesis of 1-(tert-butoxycarbonyl) amino 3-(3-maleimidopropyl) aminopropane.



In 250 ml round bottomed flask were taken 3.0 g of 1-(tert-Butoxycarbonyl) amino 3-aminopropane) and 100 mL of anhydrous chloroform. The reaction mixture was stirred at 25°C until a clear solution was formed. To this solution was added *N*-succinimidyl 3-maleimidopropionate (5.05g) with stirring until dissolved followed 3.42mL of diisoproylethylamine. The resulting reaction mixture was stirred at 25°C for 18h. The solution was washed with 1M hydrochloric acid (50mL), 10% brine (50mL), saturated sodium bicarbonate (50mL), semi-saturated brine (50mL). The organic phase was isolated and was dried over anhydrous sodium sulfate. After removing the sodium sulfate by filtration, the solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography using ethyl acetate: hexanes gradient as the mobile phase. Removal of the solvent under reduced pressure followed by vacuum drying offered the desired product as an off white solid (4.03 g).

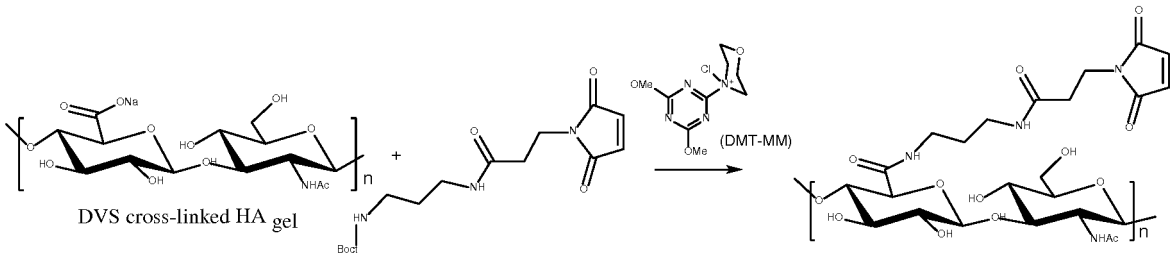
Example 13.

Synthesis of 1-(tert-Butyloxycarbonyl)amino 8-(3-maleimidopropyl) amino-3,6-dioxaoctane.

In 100 ml round bottomed flask were taken 1-(tert-butyloxycarbonyl) amino 8-amino-3,6-dioxaoctane (1.007g) and 25 mL of anhydrous acetonitrile (25mL). The reaction mixture was stirred under nitrogen until dissolved. To this solution was added *N*-succinimidyl 3-maleimidopropionate (1.302g) and the reaction mixture was stirred under nitrogen at 25oC for 6h. At the end of this time, the solvent was removed under reduced pressure. The residue was purified silica gel column chromatography using dichloromethane containing 2 – 6% of methanol. After removing the solvent under reduced pressure and drying the residue under vacuum yielded 1.08 g of the desired product as an off white solid.

Example 14.

Synthesis of 3-(3-maleimido-propyl) aminopropane functionalized hyaluronic acid.



To 15 g of divinyl sulfone crosslinked suspension (Example 1) was added 45 ml of deionized (DI) water and the resulting suspension was stirred at 25oC for 10 minutes. After adding 45 ml of ethanol to the suspension, it was stirred for additional 60 min. It was followed by addition of 60 mL of ethanol and 10 minute of stirring. To this suspension was added 0.24 g of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM) dissolved in 5 mL of ethanol. The resulting reaction mixture was stirred at 25o for 60mins. To 70 mg of 1-(tert-Butoxycarbonyl) amino 3-(3-maleimidopropyl) aminopropane (example 2) dissolved in 2 mL of anhydrous dichloromethane was added 2 mL of trifluoroacetic acid. The resulting reaction was stirred at room temperature for 2h. The solution was concentrated under reduced pressure. The resulting residue was treated with 3 mL of methanol and evaporated to dryness. After repeating this process one more time the residue was dissolved in ethanol (5mL) and the pH adjusted to 6 - 6.5 with 10% N-methylmorpholine in ethanol. The resulting solution was added to the above hyaluronic acid suspension. The vial was rinsed with ethanol (5mL) and added to the slurry. After stirring for 18h at 25oC, saturated brine (2mL) was added to the reaction mixture. The suspension was treated with ethanol (3 x 30mL, 2 x 15mL) to precipitate the polymer gel. The reaction mixture was centrifuged at 4000 rpm and the supernatant decanted. The residue was hydrated in DI water (20mL) for ~ 20 min and was precipitated from ethanol (4 x 10mL). Glucosamine assay method suggests the degree of substitution to be 19 mole%.

Example 15.

Synthesis of 3-(3-maleimido-propyl) aminopropane functionalized hyaluronic acid.

To 20 g of divinyl sulfone crosslinked suspension (Example 1) was added 60 ml of deionized (DI) water and the resulting suspension was stirred at 25oC for 10 minutes. After adding 60 ml of ethanol to the suspension, it was stirred for additional 60 min. It was followed by addition of 60 mL of ethanol and 10 minute of stirring. To this suspension was added 0.325 g of DMT-MM dissolved in 10 mL of ethanol. The resulting reaction mixture was stirred at 25o for 60mins. To 0.38 g of 1-(tert-Butoxycarbonyl) amino 3-(3-maleimidopropyl) aminopropane (example 2) dissolved in 2.5 mL of anhydrous dichloromethane was added 2.5 mL of trifluoroacetic acid. The resulting reaction was stirred at room temperature for 2h. The solution was concentrated under reduced pressure. The resulting residue was treated with 3 mL of methanol and evaporated to dryness. After repeating this process one more time, the residue was dissolved in ethanol (5mL) and the pH adjusted to 6 - 6.5 with 10% N-methylmorpholine in ethanol. The resulting solution was added to the above hyaluronic acid suspension. The vial was rinsed with ethanol (5mL) and added to the slurry. After stirring for 18h at 25oC, saturated brine (2mL) was added to the reaction mixture. The suspension was treated with ethanol (3 x 30mL, 2 x 15mL) to precipitate the polymer gel. The reaction mixture was centrifuged at 4000 rpm and the supernatant decanted. The residue was hydrated in DI water (20mL) for ~ 20 min and was precipitated from ethanol (4 x 10mL). Glucosamine assay method suggests the degree of substitution to be 24 mole%.

Example 16a

General Method for the synthesis of 3-(3-maleimido-propyl) aminopropane functionalized HA hydrogel

To appropriate amount divinylsulphone crosslinked HA suspension (Example 11b) was added sterile saline to obtain a gel concentration of ~1% w/v. The resulting suspension was stirred at 25°C for 15 - 30 minutes. A water miscible organic solvent (preferably ethanol) was added to the suspension and the resulting suspension was stirred for additional 30 - 60 min. To this suspension was added appropriate amount of an ethanolic solution of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM). The container containing DMT-MM solution was rinsed twice with ethanol and the washings were added to the above suspension. The resulting reaction mixture was stirred at 25°C for 90minutes. Appropriate amount of 1-(tert-butoxycarbonyl) amino

3-(3-maleimidopropyl) aminopropane was dissolved in dichloromethane. To this solution was added trifluoroacetic acid to give a 1:1(v/v) solution. After stirring at room temperature for 60 - 90 minutes, the reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in ethanol and was added to the above suspension. The container containing maleimide derivative was rinsed twice with ethanol and the washings were added to the suspension. The pH of the suspension was adjusted to pH 6.4 - 6.6 using an organic or inorganic base (for example10% N-methylmopholine in ethanol). After stirring for 16 - 20h at 25°C, the suspension was treated with ethanol to a volume of 60 - 65% v/v. The solvent was removed from the reaction mixture either by centrifugation at 120G followed by decanting the supernatant or by applying a slight overpressure of N_2 gas to the system and filtering through a glass frit or filter membrane. The residue was subsequently treated with sterile 20 mM succinate saline (0.9%) at pH 3.8 for ~ 15 - 20 min and was precipitated by adding ethanol to a volume of 60 - 65% v/v. The solvent is removed from the reaction mixture by following the above procedure. This procedure was repeated one more time.

Example 16b

Synthesis of 3-(3-maleimido-propyl) aminopropane functionalized HA hydrogels at a degree of substitution of 20 mole%_.

To 5.65 g of the HA hydrogel suspension (Example 11b) was added 7.5 mL of sterile saline and the resulting suspension was gently stirred for 15minutes. To this suspension was added 3 mL of ethanol and the resulting reaction mixture was gently stirred for 60 min. To this suspension was added 94 mg of DMT-MM dissolved in 3 mL of ethanol. The vial containing the DMT-MM was washed with ethanol (2 x 1mL) and the washings were added to the suspension. The reaction mixture was allowed to stir gently at ambient temperature for at 90 minutes. To 21.7 mg of 1-(tert-Butoxycarbonyl) amino 3-(3-maleimidopropyl)aminopropane was added 0.25 mL of dichloromethane and the reaction was gently mixed until a clear solution was formed. To this solution was added 0.25 mL of trifluoroacetic acid and was gently mixed at ambient temperature for 75min. Subsequently, the solution was evaporated to dryness. The residue was dissolved in 3 mL of ethanol and the resulting solution added to the above hydrogel suspension. The vial containing the maleimide reagent was rinsed with ethanol (2 x

1mL) and the washings were added to the reaction mixture. The pH of the reaction mixture was then adjusted to 6.41 using 10% v/v N-methylmorpholine in ethanol and the resulting reaction mixture was shaken gently for 18 hours at ambient temperature. At the end of this time, the pH of the reaction mixture adjusted to pH 3.89 by treating with 1.0 M HCl. The gel was precipitated by adding ethanol (4 x 3.5mL). The suspension was centrifuged at 120G for 2min at 20°C and the supernatant was carefully removed using a pipette. The residue was rehydrated in 12 mL of 20.0 mM SBS buffer (pH 3.8) by gentle shaking/mixing for 15minutes. The suspension was subjected to additional ethanol treatment (4 x 5ml, 1 x 4mL). After each ethanol treatment, the suspension was centrifuged at 120G for 2min at 20°C followed by careful removal of the supernatant. Finally, the vial was inverted, allowed to drain for 15minutes and the supernatant was decanted yielding 0.8876 g of the wet gel.

Example 16c

Synthesis of 3-(3-maleimido-propyl) aminopropane functionalized hyaluronic acid.

To 10 g of divinyl sulfone crosslinked suspension (Example 11a) was added 40 ml of deionized (DI) water and the resulting suspension was stirred at 25°C for 10 minutes. After adding 30 ml of ethanol to the suspension, it was stirred for additional 60 minutes. It was followed by addition of 60 mL of ethanol and 10 minute of stirring. To this suspension was added 0.162 g of DMT-MM dissolved in 7.5 mL of ethanol. The resulting reaction mixture was stirred at 25°C for 60mins. To 29 mg of 1-(tert-Butoxycarbonyl) amino 3-(3-maleimidopropyl) aminopropane (example 2) dissolved in 0.5 mL of anhydrous dichloromethane was added 0.5 mL of trifluoroacetic acid. The resulting reaction was stirred at room temperature for 2h. The solution was concentrated under reduced pressure. The resulting residue was treated with 2 mL of methanol and evaporated to dryness. After repeating this process one more time, the residue was dissolved in ethanol (7.5mL) and 9.5µL of N-methylmorpholine. The solution was added the above hyaluronic acid suspension and pH adjusted to 7.1 with using N-methylmorpholine. The vial was rinsed with ethanol (5mL) and added to the slurry. After stirring for 21h at 25oC, saturated brine (2mL) was added and the gel was precipitated by adding ethanol (4 x 15mL). The slurry was allowed to settle for 15min and the supernatant was decanted. The hydrogel was hydrated in 20mM PBS pH5

(35mL) and was precipitated by adding ethanol (5 x 15mL). The process was repeated, the sample centrifuged at 2500 rpm for 1.5 min, and the supernatant decanted. Glucosamine assay method suggests the degree of substitution to be 24 mole%.

Example 17

Synthesis of 8-(3-maleimidopropyl) amino-3,6-dioxaoctane functionalized hyaluronic acid.

In a 250 mL round bottomed flask were taken 0.5g of lyophilized HA (example 11a) and 50 mL of 0.9% saline solution. The reaction mixture was allowed to stir gently at 25oC for one hr. To this suspension was added 40 mL of ethanol and the suspension was stirred for 5 min. In a 10 mL vial were taken 0.35 g of DMT-MM and 5 mL of ethanol. The solution was added to the HA suspension. The vial was rinsed with 5 mL of ethanol and added to the suspension. The resulting reaction mixture was stirred at 25oC for 1 hr. Subsequently, 86 mg of 1-(tert-Butyloxycarbonyl) amino 8-(3-maleimidopropyl) amino-3,6-dioxaoctane dissolved in 0.5 mL of anhydrous dichloromethane was treated with 0.5 mL of trifluoroacetic acid was added to the. The solution was stirred at room temperature from 1h. The solution was concentrated under reduced pressure, dissolved in 2 mL of ethanol and evaporated to dryness under reduced pressure. The ethanol treatment was repeated twice. The residue was dissolved in 5 mL of ethanol/water (1:1) and the pH of the solution was adjusted to 6.5 using N-methylmorpholine (60µL). The resulting solution was added to the above suspension. After stirring for 4h at 25°C, pH of suspension was adjusted to 3.75 with 0.1M HCl and the gel was precipitated by adding ethanol (7 x 25mL). The slurry was allowed to settle for 30 min and 80% of the supernatant removed decantation. The remaining suspension was centrifuged at 1500rpm for 5 min. After removing the solvent, the residue was treated with 0.9% saline and precipitated from ethanol (7 x 15mL). The residue was dried by lyophilization. The degree substitution, as determined by glucosamine assay was found to be 7.3mol%.

Example 18

Synthesis of 2-pyridyldithiol group containing hyaluronic acid.

To a rapidly stirring solution of 200 mg sodium hyaluronate (molecular weight = 500 kDa) dissolved in 24mL of deionized water was added 16mL acetonitrile in dropwise manner. After addition of acetonitrile was complete, 17.6 mg of chlorodimethoxytriazine in 2 mL of water/acetonitrile (1:1) to the reaction mixture followed by 20μL of *N*-methylmorpholine. The reaction was stirred at 25°C for 1 hr. Subsequently, 26.8 mg of 2-((3-nitropyridin-2-yl)thio)ethanamine hydrochloride dissolved in 1 mL of deionized water was added. The reaction was allowed to stir for 18 hr. The pH of the reaction was adjusted to 6.0 by adding 1M HCl. To the resulting reaction mixture was added 15 mL of pre-washed Amberlite® CG-120 (Na^+ form) and the reaction mixture was stirred for 20 minutes. The resin was filtered off and was washed with deionized water (2 x 5 mL). The Amberlite® CG-120 (Na^+ form) treatment process was repeated twice. The solution was diluted with water to form a solution containing 20 vol% of acetonitrile. The solution was spin-filtered using Macrosep® centrifugal devices (30K molecular weight cutoff). The retentate was washed with deionized water (5 x 200ml). The retentates were combined and lyophilized yielding 120 mg of the product as an off white solid. The degree of modification was 10%. Another 2-pyridyldithiol group containing hyaluronic acid was synthesized using sodium hyaluronate of molecular weight 70 kDa following the similar procedure.

Example 19.

Synthesis of soluble maleimide functionalized HA

In a 100 mL flask were taken 200 mg of hyaluronic acid, sodium salt (mol. wt. = 500 kDa) and 24ml of DI water. It was stirred until a clear solution was obtained. To the rapidly stirred HA solution was added 16mL of ethanol was added in a . 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (55mg, 0.2mmol) in water/ethanol was added along with *N*-methylmorpholine (20μl, 0.2mmol) and the reaction was aged for one hour. An aqueous solution of 1-(2-(2-aminoethoxy)ethyl)-1H-pyrrole-2,5-dione•trifluoroacetate salt (60mg, 0.2mmol) was added. The reaction was left over night. The pH is adjusted to be slightly acidic and pre-washed Amberlite® CG-120, Na^+ form (≥15ml) was added to the reaction mixture and stirred for 20 minutes. The resin was filtered off and washed with water. To the filtrate was again added pre-washed Amberlite® CG-120, Na^+ form (≥15ml) and the same procedure as above was

followed. The whole cycle was repeated once more. The solution was diluted with water to form an aqueous solution containing <20% ethanol. The solution was spin-filtered using four Macrosep® centrifugal devices from PALL, 30K molecular weight cutoff (5000rpm, 15 minutes spin time. The membrane is cleared each time by gently stroking a spatula over it and vigorously shaking the sealed devise). The retentate was washed several times with deionized water (>200ml). The resulting modified HA concentrates were combined and lyophilized. Recovery is between 50-75%. Degree of modification ~ 20% according to NMR.

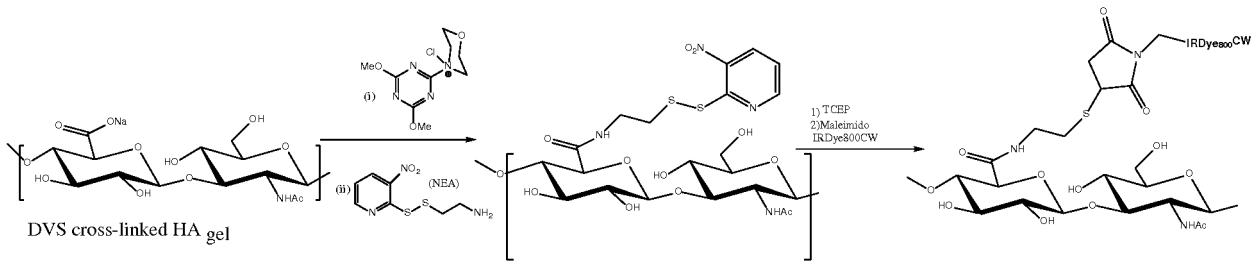
Example 20

Estimation of maleimide content in hyaluronic acid hydrogels.

Estimation of maleimide groups incorporated to HA hydrogels was performed by a colorimetric analysis method. 5-Thio 2-nitrobenzoic acid was prepared by the reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) with *Tris*-(2-carboxyethyl) phosphine hydrochloride (TCEP) in PBS buffer at pH 7.5. A 20mol% excess of 5,5'-dithiobis-(2-nitrobenzoic acid) was used to prevent side reactions with TCEP. A predetermined amount of maleimide functionalized hydrogel suspended in 20mM Succinate buffered saline (SBS) at pH 3.5. Above 5-Thio 2-nitrobenzoic acid solution was added to the hydrogel suspension and the reaction mixture was vortex mixed (2 x 10seconds) and was subsequently stirred gently at 25°C for 45min. The suspension was subsequently centrifuged at 25°C for 10 min and an aliquot of the supernatant taken. The absorbance of the supernatant was measured at 412nm. The concentration 5-thio 2-nitrobenzoic acid in the solution was estimated using a calibration curve. Maleimide concentration in the hydrogels is equivalent to the moles of thiol reacted, which is calculated from the difference between the amount 5-thio 2-nitrobenzoic acid added and that present in the supernatant.

Example 21.

Synthesis of Near Infrared Dye (IRDye800CW) Conjugated HA Hydrogel.



Example 21 a. Synthesis 3-Nitro-2-(2'-amino-ethyldisulfanyl)pyridine hydrochloride (NEA) conjugated divinyl sulfone cross-linked hyaluronic acid.

A suspension containing 5.1g (23 mg/g of water) of divinylsulfone cross-linked hyaluronic acid hydrogel (example 5) and 20 mL of sterile water was gentle stirred at 25°C for 15 min. To this suspension was added ethanol (20mL) and the resulting reaction mixture was stirred 25°C for one hr. To this suspension was added 0.081g of DMT-MM dissolved in 5mL of ethanol. The vial containing DMT-MM solution was rinsed with 2.5mL of ethanol and the washing was added to the suspension. The reaction mixture was stirred at 25°C for 1 hr. followed by addition of 0.017g of NEA. After stirring the reaction mixture for 22h at 25°C, 2mL of brine was added the gel. The solvent was removed by centrifugation was the residue was washed by multiple ethanol treatment (4 x 10mL, 1 x 5mL). The suspension was centrifuged at 2500 rpm for 5min followed by at 5000 rpm for 5min (the temperature of the centrifuge was maintained at 5°C). The supernatant was decanted and residue was allowed equilibrate for 20 min in sterile water (20mL). Subsequently, it was subjected to two ethanol treatment (4 x 10mL). The slurry was allowed to settle for 30 min and the supernatant decanted. The ethanol treatment process was repeated one more time and the wet gel was stored at 2°C until next step.

Example 21b. Synthesis of NIR dye conjugated HA hydrogel.

Under aseptic condition, 0.224 g NEA modified hyaluronic acid (example 15a) and 13mL of sterile water were taken in a 50 mL sterile reaction vial and the mixture was allowed to shake gently for 15 min at 25°C. To this suspension was added 20 mL of ethanol and the suspension was stirred for 1 hr. at 25°C. Subsequently, 14 mg of TCEP was added to the suspension and the reaction mixture was stirred for 16hr. at 25°C. At the end of the reaction, 2 mL of brine was added to the reaction mixture. Subsequently, the gel was subjected to 5 cycles of ethanol treatment (5 mL) and centrifugation (5000 rpm, 5 min., 5°C). After removal of the supernatant, the residue was treated with 10 mL

of in 0.9% sterile saline followed 5 round of ethanol (5 mL) treatment and centrifugation as mentioned above. The residue was suspended in 10mL of sterile 0.9% saline. To this suspension was added 1.8mg of maleimide functionalized IRDye800CW dissolved in sterile 0.9% saline (3mL) using a sterile dehydrogenating filter. The filter was rinsed with 5 mL of ethanol and ethanol/saline 1:1 (2 x 5mL) and the washing were added to the reaction mixture. The reaction vessel was agitated gently in dark for 2hr. at 25°C and was kept at 2°C for 66h. Subsequently 11.5 mg of N-methylmaleimide dissolved in 3 mL of ethanol was added to the reaction mixture and the suspension it was shaken gently for additional 3hr at 25°C. At the end of the reaction, the suspension was centrifuged (5000rpm, 5°C, 2 x 15min) and 20mL of supernatant was removed. The remaining suspension was precipitated from ethanol (5 x 5mL) and further centrifuged (5000rpm, 5°C, 5min) and the supernatant was decanted. 10 mL of 0.9% sterile saline was added to the residue, followed by 5 mL of ethanol. The suspension was centrifuged (5000rpm, 5min, 5°C) and supernatant was decanted. The ethanol treatment and centrifugation process was repeated four more time. The residue was treated with 10 mL of sterile 0.9% saline and stored at 2°C under dark by wrapping the vial with an aluminum foil.

Conjugation of Linker Thio Peptides to Maleimide Functionalized HA Hydrogel

Example 22

General procedure for the thiol terminated trace-linker bearing peptides to maleimide functionalized HA hydrogels.

In a sterile and depyrogenated reactor with medium porosity frit or filter was taken appropriate amount of the maleimide modified HA hydrogel (example 3). Subsequently, appropriate amount of sterile filtered 20mMol SB buffer (containing15% v/v propylene glycol and 0.01% w/v Tween 20, pH 3.8) was added to the reaction such that the concentration of the resulting suspension is~ 1%w/w. The suspension was allowed to mix for 30 - 90minutes with gentle shaking. At the end of this time, appropriate amount of thiol terminated trace-linker bearing peptide dissolved in sterile filtered 20mMol SB buffer (containing15% v/v propylene glycol and 0.01% w/v Tween 20, pH 3.8) was added to the reactor and the resulting reaction mixture was allowed to shake gently at ambient temperature for 1.5 – 24 hours. At the end of the reaction, the supernatant was

removed by filtration using a slight excess pressure of nitrogen or by centrifugation of the suspension. The residue was treated with sterile filtered 20 mM SBS buffer (containing15% v/v propylene glycol and 0.01% w/v Tween 20, pH 3.0) to prepare a suspension 0.7w/v%, shaken for 3 minutes, centrifuged and the supernatant was removed by decantation. This process was repeated five times. The residue was treated with 10mM solution of 1-Hydroxy-2-mercaptoethane dissolved in sterile filtered 20 mM SBS buffer (containing15% v/v propylene glycol and 0.01% w/v Tween 20, pH 3.0) to prepare a ~1wt% suspension and was allowed to stir gently for 30 minutes with gentle shaking/mixing. The solvent was removed by centrifugation followed by decantation as mentioned above. This process of 1-Hydroxy-2-mercaptoethane treatment was repeated four times. The residue was suspended in sterile filtered 20 mM SBS buffer (containing15% v/v propylene glycol and 0.01% w/v Tween 20, pH 3.0) to prepare a suspension of ~0.5wt% concentration and mixed for 3 minutes followed by removal of the by centrifugation and decantation. The resulting residue was suspended in 20 mM SBS buffer (containing15% v/v propylene glycol and 0.01% w/v Tween 20, pH 6.5) to prepare a 0.7wt% suspension and stirred for 20 minutes and filtered. After repeating this process one more time, the residue was in 20 mM SBS buffer (containing15% v/v propylene glycol and 0.01% w/v Tween 20, pH 4.5) to prepare a 0.5 wt.% suspension, stirred for 15 minutes, and filtered. This process was repeated once. The residue was suspended in sterile water (pH 4.5), stirred for 5 minutes, and filtered. The process was repeated five times and. The residue was aseptically filtered using a sterile membrane filter and lyophilized to dryness.

Table 3 summarizes results on the syntheses of various HA hydrogel conjugates of dual agonist peptides obtained by varying reaction conditions and nature of traceless linker.

Amount of maleimide modified HA hydrogel (mg)	Maleimide content in hydrogel (mol%)	Linker type	Amount of Peptide linker used (mg)	Yield of hydrogel-peptide conjugate (mg)	Peptide loading (wt.%)	Peptide loading (mol%)
36	3.4	Asn	2.1	20	4	0.41
35	4.9	Asn	2.4	31	2.4	0.24

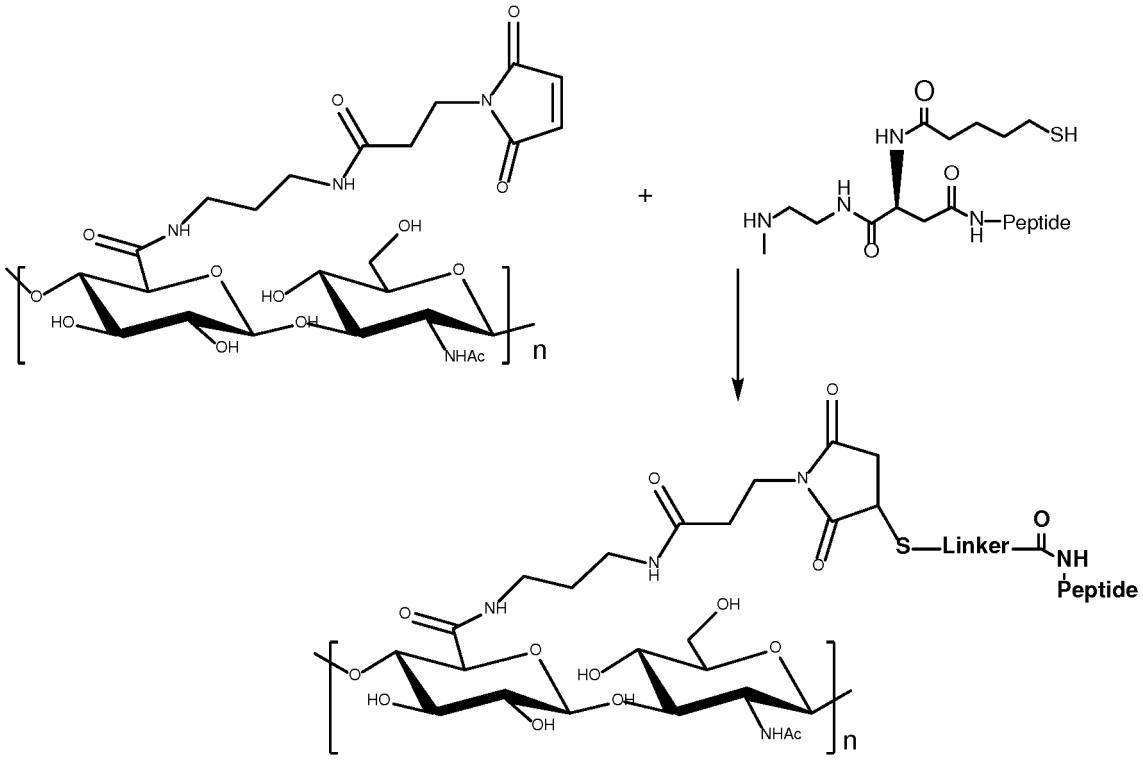
Amount of maleimide modified HA hydrogel (mg)	Maleimide content in hydrogel (mol%)	Linker type	Amount of Peptide linker used (mg)	Yield of hydrogel-peptide conjugate (mg)	Peptide loading (wt.%)	Peptide loading (mol%)
297	10.8	Asn	30.6	228	4	0.41
57.5	3.8	Asn	15	66.3	11.1	1.2
54	3.8	Asn	23	77.8	15.9	2.18
48	5.7	Asn	30	60.8	21.0	2.6
73.2	5.2	Asn	15.8	51.6	11	1.2
137	N.D.	Asn	62.5	150	21.7	2.69
90	9.8	Aib	13.3	78	6.5	0.69
50	8.8	D-Ala	3.7	49.3	N.D.	N.D.
41	6.2	D-Ala	9.2	52	N.D.	N.D.

Table 3b: The following conjugates were prepared as described in examples 14 to 22

Amount of crosslinked HA hydrogel (g)	HA content in gel (%)	Maleimide loading in hydrogel after activation (mol%)	Linker type	Amount of Peptide linker used (mg)	Yield of lyophilized hydrogel-peptide conjugate (g)	Peptide content by NMR (mol%)
200	2.3	12.1	Aib	508	6.4	N.D.
40	2.3	14.8	Aib	102	1.2	N.D.
40	2.3	9.6	Aib	108	0.84	N.D.
40	2.3	13.6	Aib	203	0.89	6.5
40	2.3	11.0	Aib	102	0.82	3.6
40	2.3	12.5	Aib	104	0.71	5.9
40	2.3	16.8	Aib	108	0.49	9.0
40	2.3	8.0	Aib	102	1.2	N.D.

Amount of crosslinked HA hydrogel (g)	HA content in gel (%)	Maleimide loading in hydrogel after activation (mol%)	Linker type	Amount of Peptide linker used (mg)	Yield of lyophilized hydrogel-peptide conjugate (g)	Peptide content by NMR (mol%)
40	2.3	13.5	Aib	102	1.1	4.4
30	2.6	22	Aib	127	0.67	5.3
30	2.6	18.2	Aib	201	0.45	6.5

Conjugation of linker 7 containing thiol functionalized Exendin-4 Seq ID No. 26 with maleimide functionalized hyaluronic acid.



To 53 mg of 3-(3-maleimidopropyl) aminopropane modified DVS-HA hydrogel (example 15) was added 7 mL of the 20mM SBS containing, 0.01wt% Tween® 20, propylene glycol (15% v/v). The pH of buffer is 3.8. The suspension was gently agitated at 25°C for 90min. To this suspension was added 2.1 mg of thiol functionalized Exendin-4 Seq ID 26 with the linker of example 7 dissolved in 1 ml of the above buffer. The peptide vial

was rinsed with buffer (2 x 0.5mL) and the washing was added to suspension. The reaction mixture was gently agitated at 25°C for 18h. The suspension was subsequently centrifuged at 1750 rpm for 2min and the supernatant was carefully decanted. The hydrogel was suspended in 10 mL of 20mM SBS buffer containing 0.01wt% Tween® 20, propylene glycol 15% v/v, pH 3, stirred for 2min, centrifuged at 1750rpm for 2min, and the solvent decanted. This process was repeated four more times. The residue was treated with 2 mL of 10mM 1-Hydroxy-2-mercaptoethane dissolved in 20mM SBS pH 3 and the reaction was allowed to proceed for 30min. The reaction mixture was centrifuged at 2000rpm for 2min and the solvent decanted. This process was repeated three more times. The residue was suspended in 10 mL of 20mM SBS containing 0.01wt% Tween® 20, pH 3, stirred for 2min, centrifuged at 2000rpm for 2min and the solvent decanted. The residue was subsequently suspended in 10 mL of acidified sterile water (pH 3.5)containing 0.01wt% Tween® 20, stirred for 10min, centrifuged at 5000rpm for 5min and the solvent was decanted. This process was repeated with 8, 7 and 6mL of the above acidified sterile water. The sample was lyophilized yielding 78 mg of the HA hydrogel conjugated peptide as an off white solid with 4 wt.% loading of peptide in the hydrogel.

Example 23

Conjugation of linker containing thiol functionalized Exendin-4 Seq ID 26 with maleimide functionalized hyaluronic acid.

Reaction and work up were performed using de-oxygenated buffers and under nitrogen atmosphere. To 36 mg of 3-(3-maleimidopropyl) aminopropane modified DVS-HA hydrogel (example 15) was added 6 mL of 20mM SBS, (containing 0.01wt% Tween® 20, 10% v/v propylene glycol). The pH of the medium was adjusted 6. The suspension was gently shaken at 25°C for 30min. To this suspension was added 2.4 mg of thiol functionalized Exendin-4 Seq ID 26 with linker (example 7) dissolved in 1 ml of the above buffer (pH 6). The peptide vial was rinsed with buffer (2 x 0.5mL) and the washing was added to suspension. The reaction mixture was gently shaken at 25°C for 90min. The suspension was subsequently centrifuged at 4000rpm for 2min and the supernatant was carefully decanted. The hydrogel was suspended in 10 ml of 20mM SBS at pH 3 (containing 0.01wt% Tween® 20, and 10 v/v% propylene glycol), mixed

for 2min, centrifuged at 3005G for 2 min, and the solvent decanted. This process was repeated four more times. The residue was treated with 2 mL of 10mM 1-hydroxy-2-mercaptoethane in 20mM SBS pH3 for 30min, centrifuged at 4000rpm for 2min and the solvent decanted. This process was repeated three more times. The residue was suspended in 10 mL of 20mM SBS containing 0.01wt% Tween® 20 (pH 3), mixed for 2min, centrifuged at 4000rpm for 2min, and the solvent was decanted. This process was repeated four more times. The residue was suspended in acidified (pH 3.5) sterile water containing 0.01wt% Tween® 20, mixed for 10min, centrifuged at 5000rpm for 5min, and the solvent decanted. This process was repeated using 8, 7 and 6mL of the above sterile water. The residue was lyophilized to yield 31mg HA conjugated peptide as an off white solid with 2.4wt% peptide loading in the hydrogel.

Example 24.

Procedure for Estimation of Peptide Loading in the hydrogel-peptide Conjugates.

A predetermined amount HA hydrogel-peptide conjugate was suspended in CHES buffer (pH9.5) and the suspension was allowed to gently stir at 70°C. The suspension was centrifuged and the aliquot was analyzed for peptide content by HPLC method. The HPLC method comprises of using q C-18 Kinetics column (inner diameter = 4.6 mm and length = 100 mm, particle size 2.6 µm, Phenomenex) using Agilent 1100 LC. The composition of mobile phase A is 90% water / 10 % Acetonitrile / 0.1 % Trifluoroacetic acid (TFA) and the mobile phase B is 10% Acetonitrile / 90% water / 0.09 % TFA. The gradient is from mobile phase 25% B to 55% B in 8 minutes. The flow rate was kept at 1 mL / min. Pure peptides were used as standards to quantify the released peptide from the hydrogel.

Example 25

Determination of In Vivo Residence Time of the HA hydrogel.

The residence time of hydrogels in vivo in the subcutaneous space were investigated by magnetic resonance (MR) imaging. The intensity of water proton contrast inside the

hydrogel was used to assess the hydrogels residence time in vivo. For this purpose, : CAnN.Cg-Foxn1nu/Crl mice were used as the animals. The hydrogel was injected using a 31 G needle by following all approved animal care protocols. The MR image of the injection site was taken regularly over a period of time. In one group, the hydrogel was injected and in the other group, a suspension of containing 1:1 (w/w) mixture hydrogel and 800,000 Da soluble HA was used. In the case of pure hydrogel, the gel was evident for 3 weeks with slow loss in intensity (Figure 2). On the other hand, for the mixture containing soluble HA, the MR signal, which was intense on day 1, has significantly reduced on day 4 (2). This suggests that to improve the residence time of the polymer carrier, it needs to be crosslinked to achieve very high molecular weight.

Figure 2a. MR of Image of the HA hydrogel at the injection site as a function of time.

Figure 2b. MR of Image of the polymer suspension containing 1:1 (w/w) HA hydrogel-800 kDa soluble HA at the injection site as a function of time.

Example 26

Release kinetics in vitro

An aliquot of GLP-1/Glucagon agonist linker hydrogel 8 (0.5 mg GLP-1/Glucagon agonist) was transferred into a syringe equipped with a filter frit and washed 5 times with pH 7.4 phosphate buffer (60 mM, 3 mM EDTA, 0.01% Tween-20). The hydrogel was suspended in the same buffer and incubated at 37 °C. At defined time points (after 1 - 7 days incubation time each) the supernatant was exchanged and liberated GLP-1/Glucagon agonist was quantified by RP-HPLC at 215 nm. UV-signals correlating to liberated GLP-1/Glucagon agonist were integrated and plotted against incubation time. Curve-fitting software was applied to estimate the corresponding halftime of release.

Figure 3. In vitro release kinetics of Exendin-4 Seq ID 26 dual agonist with linker from the HA hydrogel (example 23). The half-life is ~ 5 days.

Example 27

In vitro cellular assays for GLP-1 receptor, glucagon receptor and GIP receptor efficacy

Agonism of peptides for the receptors was determined by functional assays measuring cAMP response of HEK-293 cell lines stably expressing human GIP, GLP-1 or glucagon receptor.

cAMP content of cells was determined using a kit from Cisbio Corp. (cat. no. 62AM4PEC) based on HTRF (Homogenous Time Resolved Fluorescence). For preparation, cells were split into T175 culture flasks and grown overnight to near confluency in medium (DMEM / 10% FBS). Medium was then removed and cells washed with PBS lacking calcium and magnesium, followed by proteinase treatment with accutase (Sigma-Aldrich cat. no. A6964). Detached cells were washed and resuspended in assay buffer (1 x HBSS; 20 mM HEPES, 0.1% BSA, 2 mM IBMX) and cellular density determined. They were then diluted to 400000 cells/ml and 25 µl-aliquots dispensed into the wells of 96-well plates. For measurement, 25 µl of test compound in assay buffer was added to the wells, followed by incubation for 30 minutes at room temperature. After addition of HTRF reagents diluted in lysis buffer (kit components), the plates were incubated for 1 hr, followed by measurement of the fluorescence ratio at 665 / 620 nm. In vitro potency of agonists was quantified by determining the concentrations that caused 50% activation of maximal response (EC50).

Example 28

Glucose lowering in female diabetic dbdb-mice

Female diabetic dbdb-mice (BKS.CG-m +/+ Lepr(db)/J) 24-27 weeks of age at study start were used. Mice arrived in the age of 10-13 weeks were habituated to feeding and housing conditions for at least 1 week, then used in a first study and after a washout period of at least 2 weeks finally reused for the present study. 19 days prior to study start, individual HbA1c values were determined to stratify and thereafter allocate the animals into 4 groups with an N=8 per group to provide groups with as equally as

possible mean HbA1c values. Animals had access to food and water ad libitum throughout the entire study period. On the first day of the study blood glucose from tail tip incision was determined just before and 4 hours after single subcutaneous treatment (08:00 – 09:00 am) with either vehicle (sterile succinate buffer) or the HA-conjugate of GLP-1/Glucagon agonist Seq. ID 26 of example 23 in the doses 50, 100 and 200 nmol/kg diluted in vehicle. Thereafter daily blood glucose measurements were performed for the next 16 days all at a similar day time (08:00 – 09:00 am) with the exception of days 11 and 12 (weekend). In addition food intake and water consumption was monitored on a daily basis. Glucose data were analyzed by two-way-ANOVA on repeated measurements, followed by Dunnett's post-hoc test with a significance level of $p< 0.05$. Glucose AUC analysis was done using a one-way-ANOVA followed by Dunnett's post-hoc test with a significance level of $p< 0.05$.

Figure 4 shows the blood glucose concentration relative to the baseline versus time after one injection at various doses of HA-GLP-1/Glucagon agonist conjugate with Seq. ID 26.

Example 28bGlucose lowering effects in female diabetic dbdb-mice

Female, obese diabetic db/db-mice (BKS.CG-m +/+ Lepr(db)/J and healthy, lean controls (BKS.Cg-m +/+ Lepr(db)/J) arrived at the age of 10-11 weeks and were habituated to feeding and vivarium conditions. At the age of 12-13 weeks individual HbA1c values were determined to stratify and allocate animals into different groups with an N=8 per group. Goal wes to provide groups with as equally as possible mean HbA1c values. At the age of 13-14 weeks 2 groups of db/db mice received a s.c. 100 nmol/kg body mass single dose of a conjugate with Seq. ID 26 of either soluble or crosslinked HA. At the same time and a second time on day 7 a third group of db/db mice and the healthy lean references received a s.c. injection of succinate buffer and soluble HA in a 1:1 ratio . In all groups a total volume of 5 ml/kg body mass was injected. Morning-fed blood glucose concentrations were determined just before (between 08:00 – 09:00 am) and four hours after treatment and thereafter daily between 08:00 – 09:00 am. Blood was collected from tail tip incisions and concentrations determined *via* a handheld glucometer (Accu Check). Throughout the entire study period animals had access to food and water ad libitum.

Figure 4b shows the blood glucose concentration relative to the baseline versus time after one injection of cross-linked and soluble HA-GLP-1/Glucagon agonist conjugate with Seq. ID 26.

Example 28c

Glucose lowering effects in female diabetic db/db-mice

After arrival female, obese diabetic db/db-mice (BKS.CG-m +/+ Lepr(db)/J) were habituated to feeding and vivarium conditions and were 12 weeks old at study start. Individual HbA1c values were determined on day 10 of the predose phase to stratify and allocate animals into different groups with an N=8 per group. Goal was to provide groups with as equally as possible mean HbA1c values. On day 1 of the dosing phase db/db mice received a s.c., single dose of 12.75 mg/kg body mass of either conjugate with Seq ID 45, 46, 48 and 49. Db/db animals of the Vehicle group received a s.c. PBS injection. In all groups a total volume of 10 ml/kg body mass was injected. Morning-fed blood glucose concentrations were determined just before (between 08:00 – 09:00 am) and four hours after treatment on day 1 of the dosing phase and thereafter daily between 08:00 – 09:00 am. Blood was collected from tail tip incisions and concentrations determined *via* a handheld glucometer (Accu Check). Throughout the entire study period animals had access to food and water ad libitum.

Figure 4c shows the blood glucose concentration relative to the baseline versus time after one injection at various doses of HA-GLP-1/Glucagon agonist conjugate with Seq. ID 45 (triangles), 46 (squares), 48 (circles).

Figure 4d shows the blood glucose concentration relative to the baseline versus time after one injection at various doses of HA-GLP-1/Glucagon agonist conjugate with Seq. ID 49 (circles).

Example 29

Injectability study

A suspension of 3.75% (mg/mL) HA-peptide conjugate was prepared by dispersing appropriate amount of the peptide conjugate in 20mM succinate buffered saline (SBS)

at pH 4.5. In another vial, a 2 % (mg/mL) solution of native HA polymer was prepared by dissolving lyophilized HA in 20 mM SBS at pH 4.5. Both the samples were allowed to hydrate for 24 to 48 hours at 2-8°C. The vials were brought to room temperature. After equilibration at room temperature, 1 mL of the peptide- HA hydrogel conjugate suspension and HA was taken in a 3 mL syringe. To this suspension was added 0.25 mL of the soluble HA solution. The resulting suspension was subjected to 20 times of back and forth mixing between two 3 mL syringes. Approximately 220µl of this suspension was to a 1ml syringe. To this syringe was attached a 30g, ½ inch needle. It should be ensured that the needle is not primed with test material. The syringe containing the suspension was loaded into the syringe fixture of the Instron equipment and the crosshead of the Instron was aligned with the plunger of the syringe. The speed of the crosshead was adjusted to achieve target injection rate. The testing was initiated. For each sample, three measurements were performed and result presented is an average of these measurements. The results on injectability force for different peptide-HA hydrogel conjugates are shown in Table 4.

Table 4. Effect of HA Modification on Average Injectability Force*

Compound type	Peptide content in the conjugate (wt.%)	(mol%)	Average Injectability Force (N)
HA Conjugated Seq. ID No. 26, Asn linker	11.0	1.2	19.1 ± 0.6
HA Conjugated Seq. ID No. 26, Asn linker	15.9	2.18	16.8 ± 1.8
HA Conjugated Seq. ID No. 26, Asn linker	21.7	2.69	13.7 ± 1.2

*n=3 per group; injection rate = 12 µl/s

Figure 5 shows the average extrusion force by pressing the liquid through a 2.5 cm long 30G needle attached to an 1mL syringe. It is clearly seen that a higher peptide loading on the HA hydrogel leads to lower extrusion forces and therefore enhances injectability.

Example 29b

Injectability study of HA-conjugate with Seq. ID No. 45, Aib-linker

A 1 mL Luer-Lock syringe (BD syringe, inner diameter 4 mm) was filled with approximately 500 µL (460 µL on the syringe scale) of the sample solution and a 29G x 12.7 mm needle was attached. The measurement was carried out at the LF Plus dynamometer from Lloyed Instruments. The plunger rod was pushed until a small droplet appeared at the tip of the 29G needle. The syringe was placed in the syringe holder and the dynamometer was moved so that it touches the plunger rod.
The measurement setting was: an abortion force of 50 N and an injection speed of 5.8 mm/s (which equals 100 µL/s in this case).
The measurement was started and was automatically aborted when the plunger rod reached the bottom of the syringe (syringe is empty) or a force higher than 50 N was reached during the measurement.

Table 5 shows the maximum injection forces for different conjugate concentrations with different mixing ratios of soluble HA (sHA).

Table 5

		600 kD sHA: conjugate mixing ratio/sHA 20mg/mL				
Conjugate [mg/mL]	No sHA	50:50	40:60	30:70	20:80	10:90
20	10.5 N	10 N	11.7 N	11.9 N	11.2 N	10.3 N
25	13.6 N	-	-	-	-	-
30	16.6N	12.3 N	12.1 N	12.4 N	12.6 N	12.6 N

		2600 kD sHA: conjugate mixing ratio/sHA 20mg/mL				
30		15.5 N	14.5 N	16.1 N	14.2 N	18.5 N

The injection forces of pure 20 mg/mL 600 kDa sHA solution was 13.3 N and of 2600 kDa sHA: 18.8 N.

Claims

1. A prodrug or a pharmaceutically acceptable salt thereof comprising a drug linker conjugate of formula (I)

$Z - L^1 - L^2 - L - Y - R^{20}$ (I)

wherein Y is a peptide moiety having the formula (II)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-Asp-Glu-Gln-X18-Ala-X20-X21-Phe-Ile-Glu-Trp-Leu-Ile-X28-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (II)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Arg and Lys
X20 represents an amino acid residue selected from Lys, Gln and His,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val,

or wherein Y is a peptide moiety having the formula (III)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-Asp-Glu-Gln-X18-Ala-X20-X21-Phe-Ile-Glu-Trp-Leu-Ile-X28-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (III)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Leu and His
X20 represents an amino acid residue selected from His, Arg, Lys, and Gln,

X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Lys, Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val,

or

wherein Y is a peptide moiety having the formula (IV)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Leu-Leu-Asp-Glu-Gln-X18-Ala-Lys-Asp-Phe-Ile-Glu-Trp-Leu-Ile-Ala-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (IV)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Arg and Leu,
X32 represents an amino acid residue selected from Ser and Val,

or

wherein Y is a peptide moiety having the formula (IVa)

H_2N-His-Aib-His-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-X15-Glu-Gln-Leu-Ala-Arg-Asp-Phe-Ile-Glu-Trp-Leu-Ile-Bal-X29-Gly-X31-X32-Ser-X34-X35-Pro-Pro-Pro-X39-R^{20} (V)

X15 represents an amino acid residue selected from Asp and Glu, (pref. Asp)
X29 represents an amino acid residue selected from Gly, D-Ala and Pro, (pref) Gly, D-Ala
X31 represents an amino acid residue selected from Pro, His and Trp, (pref. Pro)
X32 represents an amino acid residue selected from Ser, His, Pro and Arg, (pref. Ser, His, Pro),
X34 represents an amino acid residue selected from Gly and D-Ala,
X35 represents an amino acid residue selected from Ala, Pro and Lys, (pref. Ala, Pro)
X39 represents Ser or Pro-Pro-Pro,

or

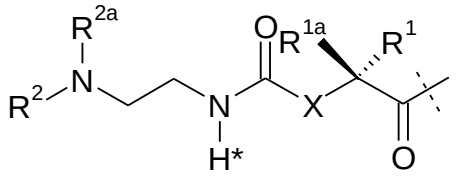
wherein Y is a peptide moiety having the formula (IVb)

H_2N-His-Aib-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Leu-Leu-Glu-Glu-Gln-Arg-Ala-Arg-Glu-Phe-Ile-Glu-Trp-Leu-Ile-Bal-D-Ala-Gly-Pro-Pro-Ser-D-Ala -Ala-Pro-Pro-Pro-Ser-R^{20};

or a salt or solvate thereof;

R^{20} is OH or NH_2;

L is a linker of formula (Ia),



Ia

wherein the dashed line indicates the attachment to the N-Terminus of Y by forming an amide bond;

X is $C(R^4R^{4a})$; $N(R^4)$;

R^1, R^{1a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

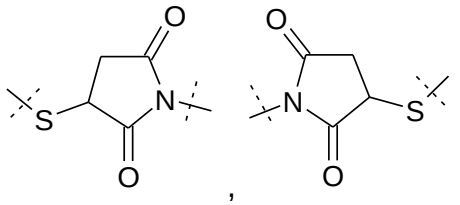
R^2, R^{2a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

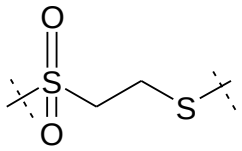
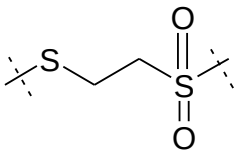
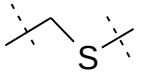
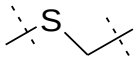
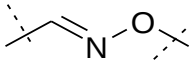
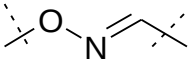
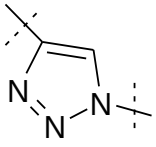
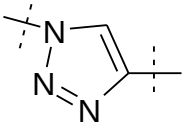
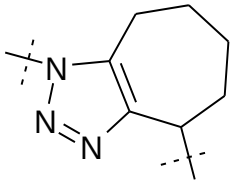
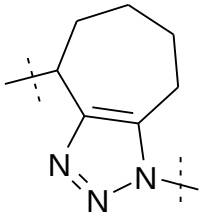
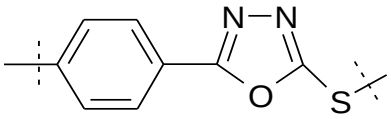
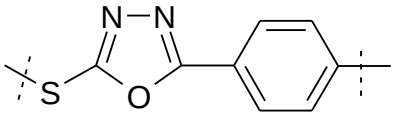
R^4, R^{4a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

wherein R^2 , R^{2a}, R^4 or R^{4a} is substituted with one group L^2-L^1-Z; wherein

L^2 is a single chemical bond or is a C_{1-20} alkyl chain, which is optionally interrupted by one or more groups independently selected from -O- and $C(O)N(R^{3aa})$ and is optionally substituted with one or more groups independently selected from OH and $C(O)N(R^{3aa}R^{3aaa})$, wherein R^{3aa} and R^{3aaa} are independently selected from the group consisting of H and C_{1-4} alkyl; and

L^2 is attached to L^1 via a terminal group selected from the group consisting of


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and

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wherein L^2 is attached to the one position indicated with the dashed line and and L^1 is attached to the position indicated with the other dashed line; and

L^1 is a $C_{1\text{-}20}$ alkyl chain, which is optionally interrupted by one or more groups independently selected from -O- and C(O)N(R^{5aa}) and is optionally substituted with one or more groups independently selected from OH and C(O)N($R^{5aa}R^{5aaa}$), wherein R^{5aa} and R^{5aaa} are independently selected from the group consisting of H and $C_{1\text{-}4}$ alkyl; and

L^1 is attached to Z via a terminal amino group forming an amide bond with the carboxy group of the beta-1,3-D-glucuronic acid of the hyaluronic acid of Z;

Z is a crosslinked hyaluronic acid hydrogel, in which

0.05 to 20 % of the monomeric disaccharide units are crosslinked by a crosslinker; and
0.2 to 8.5 % of the monomeric disaccharide units bear L^1-L^2 -L-Y-R^{20}.groups.

2. A prodrug or a pharmaceutically acceptable salt thereof comprising a drug linker conjugate of formula (I) as claimed in claim 1

$Z - L^1 - L^2 - L - Y - R^{20}$ (I)

wherein Y is a peptide moiety having the formula (II)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-Asp-Glu-Gln-X18-Ala-X20-X21-Phe-Ile-Glu-Trp-Leu-Ile-X28-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (II)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Arg and Lys
X20 represents an amino acid residue selected from Lys, Gln and His,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val,

or wherein Y is a peptide moiety having the formula (III)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-Asp-Glu-Gln-X18-Ala-X20-X21-Phe-Ile-Glu-Trp-Leu-Ile-X28-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (III)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,
X3 represents an amino acid residue selected from Gln and His,
X18 represents an amino acid residue selected from Leu and His
X20 represents an amino acid residue selected from His, Arg, Lys, and Gln,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Lys, Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val,

or

wherein Y is a peptide moiety having the formula (IV)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Leu-Leu-Asp-Glu-Gln-X18-Ala-Lys-Asp-Phe-Ile-Glu-Trp-Leu-Ile-Ala-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (IV)

X2 represents an amino acid residue selected from Ser, D-Ser and Aib,

X3 represents an amino acid residue selected from Gln and His,

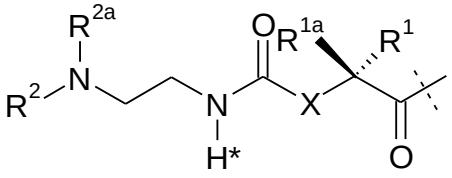
X18 represents an amino acid residue selected from Arg and Leu,

X32 represents an amino acid residue selected from Ser and Val,

or a salt or solvate thereof;

R^{20} is OH or NH_2;

L is a linker of formula (Ia),



Ia

wherein the dashed line indicates the attachment to the N-Terminus of Y by forming an amide bond;

X is $C(R^4R^{4a})$; $N(R^4)$;

R^1, R^{1a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

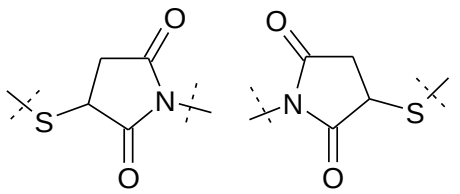
R^2, R^{2a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

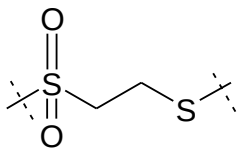
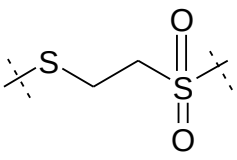
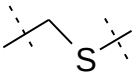
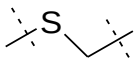
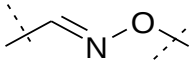
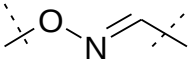
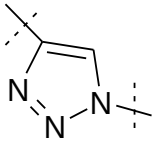
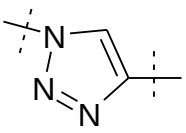
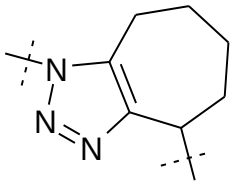
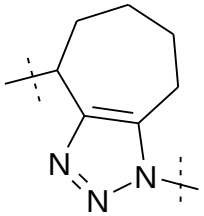
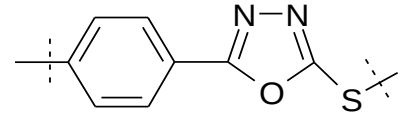
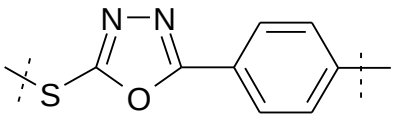
R^4, R^{4a}, are independently selected from the group consisting of H; and C_{1-4} alkyl;

wherein R^2 , R^{2a}, R^4 or R^{4a} is substituted with one group L^2-L^1-Z; wherein

L^2 is a single chemical bond or is a C_{1-20} alkyl chain, which is optionally interrupted by one or more groups independently selected from -O- and C(O)N(R^{3aa}) and is optionally substituted with one or more groups independently selected from OH and C(O)N($R^{3aa}R^{3aaa}$), wherein R^{3aa} and R^{3aaa} are independently selected from the group consisting of H and C_{1-4} alkyl; and

L^2 is attached to L^1 via a terminal group selected from the group consisting of














and ;

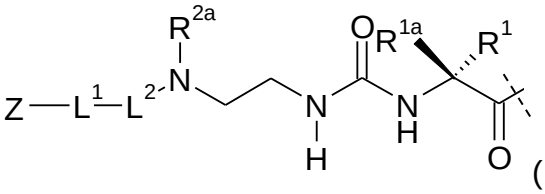
wherein L^2 is attached to the one position indicated with the dashed line and and L^1 is attached to the position indicated with the other dashed line; and

L^1 is a C_{1-20} alkyl chain, which is optionally interrupted by one or more groups independently selected from -O- and C(O)N(R^{5aa}) and is optionally substituted with one or more groups independently selected from OH and C(O)N($R^{5aa}R^{5aaa}$), wherein R^{5aa} and R^{5aaa} are independently selected from the group consisting of H and C_{1-4} alkyl; and

L^1 is attached to Z via a terminal amino group forming an amide bond with the carboxy group of the beta-1,3-D-glucuronic acid of the hyaluronic acid of Z;

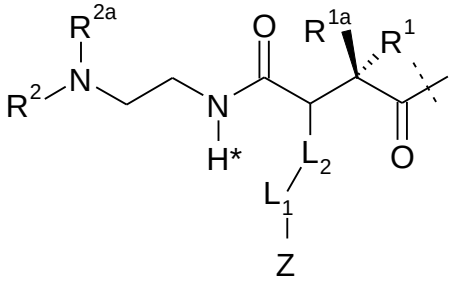
Z is a crosslinked hyaluronic acid hydrogel, in which
0.05 to 20 % of the monomeric disaccharide units are crosslinked by a crosslinker; and
0.2 to 8.5 % of the monomeric disaccharide units bear L^1-L^2 -L-Y-R^{20}.groups.

3. The prodrug of claim 1, wherein
L is a linker moiety of formula (Ib),


(Ib),

wherein the dashed line indicates attachment to Y by forming an amide bond;
R^1, R^{1a}, R^{2a} are selected independently from the group consisting of H and C_{1-4} alkyl;
L^2-L^1-Z is defined as in claim 1.

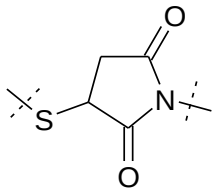
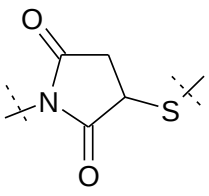
4. The prodrug of claim 1, wherein
L is a linker moiety -L of formula (Ic),


(Ic)

wherein the dashed line indicates attachment to Y by forming an amide bond;
R^1 and R^{1a} are H;
R^2, R^{2a} are independently selected from the group consisting of H and CH_3;
wherein L^2-L^1-Z is defined as in claim 1.

5. The prodrug of any of claims 1 to 4, wherein
L^2 is a C_{1-6} alkyl chain, which is optionally interrupted by one group selected from -O- and C(O)N(R^{3aa}) and, wherein R^{3aa} is independently selected from the group consisting of H and C_{1-4} alkyl; and

L^2 is attached to L^1 via a terminal group selected from the group consisting of


and

;

wherein L^2 is attached to the one position indicated with the dashed line and and L^1 is attached to the position indicated with the other dashed line.

6. The prodrug of any of claims 1 to 5, wherein

wherein Y is a peptide moiety having the formula (II)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-Asp-Glu-Gln-X18-Ala-X20-X21-Phe-Ile-Glu-Trp-Leu-Ile-X28-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (II)

wherein

X2 represents an D-Ser
X3 represents His,
X18 represents Arg
X20 represents Lys,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val;

or wherein Y is a peptide moiety having the formula (III)

His-X2-X3-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-Asp-Glu-Gln-X18-Ala-X20-X21-Phe-Ile-Glu-Trp-Leu-Ile-X28-Gly-Gly-Pro-X32-Ser-Gly-Ala-Pro-Pro-Pro-Ser (III)

wherein

X2 represents Aib,
X3 represents His,
X18 represents Leu,
X20 represents Lys,
X21 represents an amino acid residue selected from Asp and Glu,
X28 represents an amino acid residue selected from Ser and Ala,
X32 represents an amino acid residue selected from Ser and Val.

7. The prodrug of any of claims 1 to 5, wherein Y is a peptide moiety having the formula (IVa)

H_2N-His-Aib-His-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Leu-X15-Glu-Gln-Leu-Ala-Arg-Asp-Phe-Ile-Glu-Trp-Leu-Ile-Bal-X29-Gly-X31-X32-Ser-X34-X35-Pro-Pro-Pro-X39-R^{20} (IVa)

X15 represents an amino acid residue selected from Asp and Glu, (pref. Asp)
X29 represents an amino acid residue selected from Gly, D-Ala and Pro, (pref) Gly, D-Ala
X31 represents an amino acid residue selected from Pro, His and Trp, (pref. Pro)
X32 represents an amino acid residue selected from Ser, His, Pro and Arg, (pref. Ser, His, Pro),
X34 represents an amino acid residue selected from Gly and D-Ala,
X35 represents an amino acid residue selected from Ala, Pro and Lys, (pref. Ala, Pro)
X39 represents Ser or Pro-Pro-Pro.

8. The prodrug of any of claims 1 to 5, wherein Y is a peptide moiety having the formula (IVb)

H_2N-His-Aib-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Leu-Leu-Glu-Glu-Gln-Arg-Ala-Arg-Glu-Phe-Ile-Glu-Trp-Leu-Ile-Bal-D-Ala-Gly-Pro-Pro-Ser-D-Ala -Ala-Pro-Pro-Pro-Ser-R^{20}.

9. The prodrug of any of claims 1 to 8, wherein

Y is GLP-1/Glucagon agonist selected from sequences Seq. ID No 4 to 60.

10. A pharmaceutical composition comprising a prodrug of any of claims 1 to 9 or a pharmaceutical salt thereof together with at least one pharmaceutically acceptable excipient.

11. A pharmaceutical composition comprising a prodrug of any of claims 1 to 9 or a pharmaceutical salt thereof together with at least one pharmaceutically acceptable excipient and a viscosity modifier.

12. A pharmaceutical composition as claimed in claim 11, wherein the viscosity modifier is hyaluronic acid.

13. A pharmaceutical composition as claimed in claims 10 to 12 in form of an injectable formulation.

14. A pharmaceutical composition as claimed in claims 10 to 13 in form of a suspension.

15. A pharmaceutical composition as claimed in claims 10 to 14 in form of a suspension, wherein a prodrug of any of claims 1 to 6 has a concentration of 0.5 to 8 weight/volume percent.

16. A pharmaceutical composition as claimed in claims 10 to 14 in form of a suspension, wherein a prodrug of any of claims 1 to 6 has a concentration of 1.5 to 3 weight/volume percent.

17. A composition according to any of claims 10 to 16, wherein the prodrug is sufficiently dosed in the composition to provide a therapeutically effective amount of GLP1/Glucagon agonist for at least 6 days in one application.

18. A composition according to any of claims 10 to 17, wherein it is a single dose composition.

19. The prodrug of any of claims 1 to 9 or the pharmaceutical composition of claims 10 to 18 for use as a medicament.

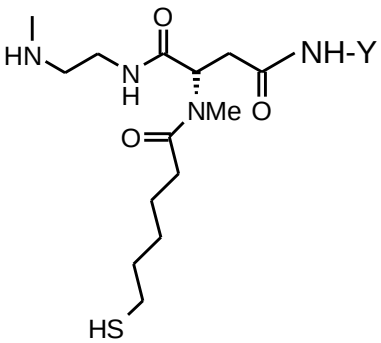
20. The prodrug of any of claims 1 to 9 or the pharmaceutical composition of claims 10 to 18 for use in a method of treating or preventing diabetes.

21. The prodrug of any of claims 1 to 7 or the pharmaceutical composition of claims 10 to 18 for use in a method of treating or preventing dyslipemia.

22. The prodrug of any of claims 1 to 9 or the pharmaceutical composition of claims 10 to 16 for use in a method of treating or preventing metabolic syndrom.

23. The prodrug of any of claims 1 to 9 or the pharmaceutical composition of claims 10 to 18 for use in a method of treating or preventing of hepatosteatosis, preferably non-alcoholic liver-disease (NAFLD) and non-alcoholic steatohepatitis (NASH).

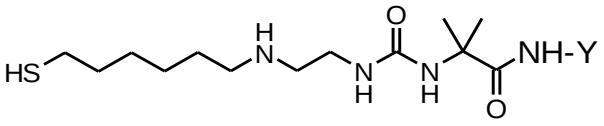
24. A GLP-1/Glucagon agonist-linker conjugate intermediate L^{2*}-L-Y of formula (VIII)



(VIII),

wherein Y is a peptide of Seq ID 4 to 60.

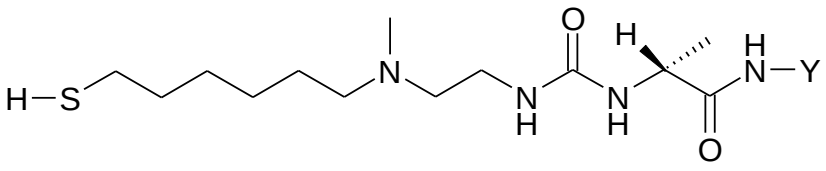
25. GLP-1/Glucagon agonist-linker conjugate intermediate L^{2*}-L-Y of formula (IX)



(IX)

wherein Y is a peptide of Seq ID 4 to 60.

26. An GLP-1/Glucagon agonist-linker conjugate intermediate L^{2*}-L-Y of formula (X)



(X),

wherein Y is a peptide of Seq ID 4 to 60.

27. The composition of claim 18, wherein the prodrug suspension can be administered by injection through a needle smaller than 0.26 mm inner diameter.