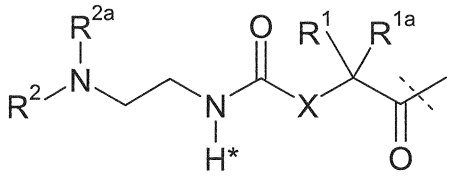
Claims

1. A prodrug or a pharmaceutically acceptable salt thereof comprising an insulin linker conjugate D-L, wherein

D represents the insulin moiety; and

-L is a non-biologically active linker moiety -L^1 represented by formula (I),



(I)

,

wherein the dashed line indicates the attachment to one of the amino groups of the insulin by forming an amide bond;

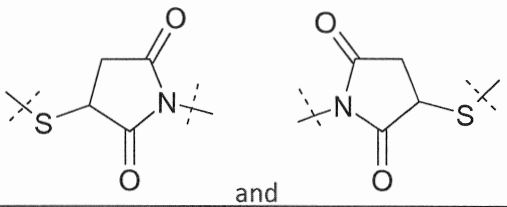
X is $C(R^3R^{3a})$; or $N(R^3)$;

R^{1a}, R^{3a} are independently selected from the group consisting H, $NH(R^{2b})$, $N(R^{2b})C(O)R^4$ and C_{1-4} alkyl;

R^1, R^2 R^{2a}, R^{2b}, R^3, R^4 are independently selected from the group consisting of H and C_{1-4} alkyl,

wherein L^1 is substituted with one L^2-Z ~~and optionally further substituted~~, provided that the hydrogen marked with the asterisk in formula (I) is not replaced by a substituent and 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 Z via a terminal group selected from the group consisting of



and ;

Z is a hydrogel, wherein the hydrogel Z is a biodegradable poly(ethylene glycol) (PEG) based water-insoluble hydrogel;

with a backbone moiety of formula C-(A-Hyp)$_4$, wherein:

- each A is independently selected from the formula -$(CH_2)_{n1}(OCH_2CH_2)_nX'$-, wherein:
  - n1 is 1 or 2;
  - n is an integer ranging from 5 to 50; and
  - X' is an amide linkage linking A and Hyp;
- each Hyp is independently selected from hyperbranched polypeptides comprising lysine;
- the backbone is crosslinked by poly(ethylene glycol)-based crosslinker moieties, comprising m ethylene glycol units, wherein m is an integer ranging from 10-70, and terminated by at least two hydrolytically degradable bonds;

.

2. Prodrug of claim 1, wherein in formula (I) R^2 is replaced by L^2-Z.

3. Prodrug of claim 1, wherein in formula (I) R^1 is replaced by L^2-Z.

4. Prodrug of any of claims 1 to 3, wherein in formula (I) X is $N(R^3)$.

5. Prodrug of any of claims 1 to 3, wherein in formula (I) X is $C(R^3R^{3a})$ and R^{3a} is $N(R^{2b})C(O)R^4$.

6. Prodrug of claim 1, wherein X is $C(R^3R^{3a})$ and R^{3a} is replaced by L^2-Z.

7. Prodrug of claim 1, wherein X is $C(R^3R^{3a})$, R^{3a} is $N(R^{2b})$-L^2-Z.

8. Prodrug of any of claims 1 to 7, wherein L^1 is not further substituted.

9. Prodrug of any of claims 1 to 8, wherein the insulin moiety is attached to L^1 through the nitrogen $N^{\alpha A1}$.

10. Prodrug of any of claims 1 to 9, wherein insulin is recombinant human insulin.

11. Prodrug of claim 10, wherein the recombinant human insulin is attached to L^1 through the nitrogen of a lysine side chain of the insulin moiety.

~~12. Prodrug of any of claims 1 to 11, wherein the hydrogel Z is a biodegradable poly(ethylene glycol) (PEG) based water-insoluble hydrogel.~~

~~13. Prodrug of any of claims 1 to 12, wherein the hydrogel is composed of backbone moieties interconnected by hydrolytically degradable bonds.~~

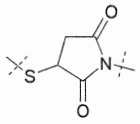
~~14~~12. Prodrug of claim ~~13~~, wherein the backbone moieties have a molecular weight in the range of from 1 kDa to 20 kDa.

~~15. Prodrug of claim 13 or 14, wherein backbone moieties are linked together through crosslinker moieties, each crosslinker moiety being terminated by at least two of the hydrolytically degradable bonds.~~

~~16~~13. Prodrug of claim ~~15~~, wherein the crosslinker moieties have a molecular weight in the range of from 0.5 kDa to 5 kDa.

~~17~~14. Prodrug of claim ~~13 to 16~~, wherein L^2 is connected to a backbone moiety.

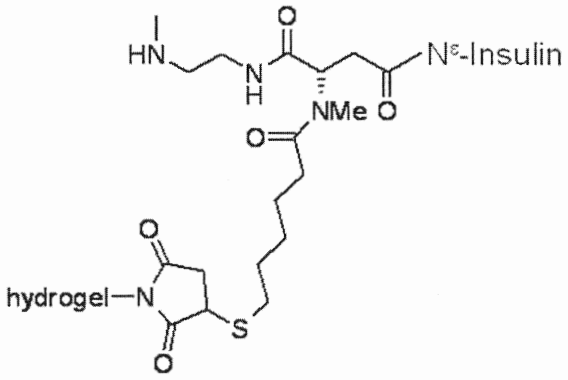
~~18~~15. The prodrug of any of claims 1 to ~~17~~14, wherein L^2 is attached to Z via a terminal group having the following structure



,

wherein the dashed lines indicate the attachment to L^2 and Z, respectively.

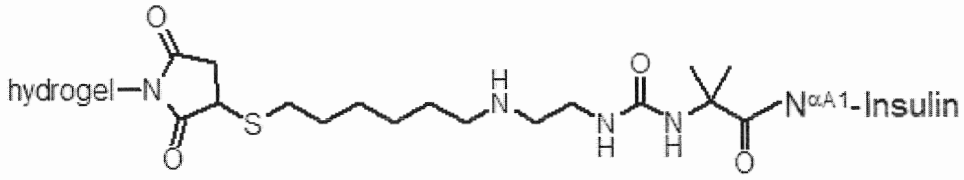
~~19~~16. Prodrug of claim 1, of formula (IIa)



(IIa)

wherein N^{ε}-Insulin refers to insulin connected via one lysine side chain.

~~20~~17. Prodrug of of claim 1 or 2, of formula (IIb)

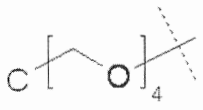


(IIb).

~~21. The prodrug of claim 19 or 20, wherein the hydrogel is a biodegradable polyethylene glycol (PEG) based water-insoluble hydrogel.~~

~~22. The prodrug of any of claims 19 to 21, wherein the hydrogel is composed of backbone moieties interconnected by hydrolytically degradable bonds.~~

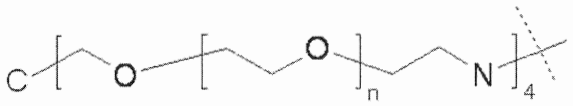
~~23~~18. The prodrug of any of claims ~~13~~ 1 to ~~18, 22,~~17 wherein the backbone moieties comprise a branching core of the following formula:



,

wherein the dashed line indicates attachment to the remainder of the backbone moiety.

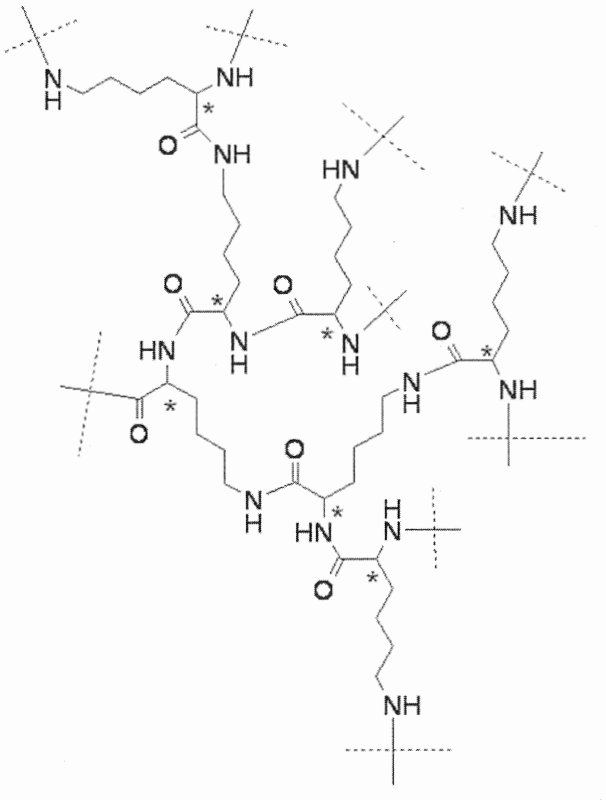
~~24~~19. The prodrug of any of claims 1~~3~~ to 18~~, 22, 23~~ wherein the backbone moieties comprise a structure of the following formula:



wherein n is an integer of from 5 to 50 and the dashed line indicates attachment to the rest of the molecule.

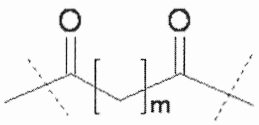
~~25. The prodrug of any of claims 13 to 24, wherein the backbone moiety comprises a hyperbranched moiety Hyp.~~

~~26~~20. The prodrug of claim ~~25~~1, wherein the backbone moiety comprises a hyperbranched moiety Hyp of the following formula:



wherein the dashed lines indicate attachment to the rest of the molecule and carbon atoms marked with asterisks indicate S-configuration.

~~27~~21. The prodrug of any of claims ~~22~~ 1 to ~~26~~20, wherein the backbone moieties are attached to at least one spacer of the following formula:

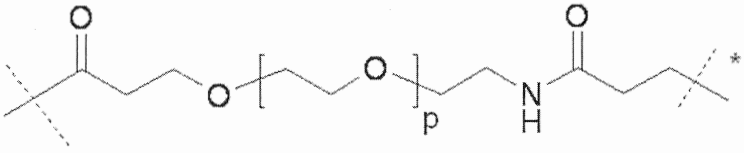


,

wherein one of the dashed lines indicates attachment to the hyperbranched moiety Hyp and the second dashed line indicates attachment to the rest of the molecule; and

wherein m is an integer of from 2 to 4.

~~28~~22. The prodrug of any of claims ~~22 or 27~~1 to 21, wherein the backbone moieties are attached to at least one spacer of the following formula:



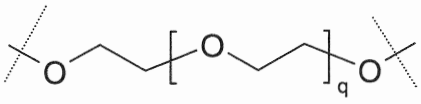
,

wherein the dashed line marked with the asterisk indicates the bond between the hydrogel and the N of the thiosuccinimide group of claim 15~~8~~,

wherein the other dashed line indicates attachment to Hyp, and

wherein p is an integer of from 0 to 10.

~~29~~23. The prodrug of any of claims ~~15~~ 1 to ~~28~~22, wherein the backbone moieties are linked together through crosslinker moieties comprising the following structure



wherein

q is an integer from 3 to 100.

~~30~~24. The prodrug of any of claims 1 to ~~29~~ 23 in the form of microparticles.

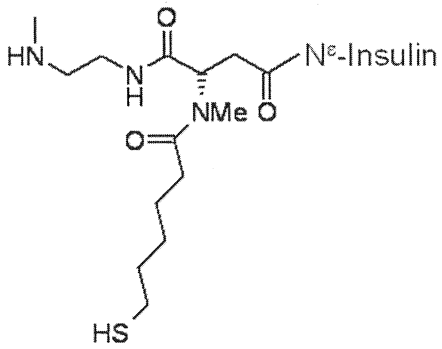
~~31~~25. The prodrug of claim ~~30~~24, wherein the microparticles have a diameter of between 20 and 100 micrometer.

~~32~~26. The prodrug of claim ~~30~~24, wherein the microparticles can be administered by injection through a needle smaller than 0.6 mm inner diameter.

~~33~~27. The prodrug of claims ~~30~~24, wherein the microparticles can be administered by injection through a needle smaller than 0.3 mm inner diameter.

~~34~~28. The prodrug of claim ~~30~~24, wherein the microparticles can be administered by injection through a needle smaller than 0.2 mm inner diameter.

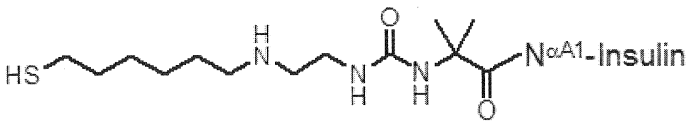
~~35~~29. An insulin-linker conjugate of formula (IIIa)



(IIIa),

wherein N^{ε}-Insulin refers to insulin connected via one lysine side chain.

~~36~~30. An insulin-linker conjugate of formula (IIIb)



(IIIb).

~~37~~31. A pharmaceutical composition comprising a prodrug of any of claims 1 to ~~34~~28 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable excipient.

~~38~~32. A pharmaceutical composition according to claim ~~37~~31, wherein the pharmaceutical composition is dry.

~~39~~33. A pharmaceutical composition according to claim ~~38~~32, wherein the pharmaceutical composition is dried by lyophilization.

~~40.~~ 34. A composition according to any of claims ~~37~~ 31 to ~~39~~33, wherein the insulin hydrogel prodrug is sufficiently dosed in the composition to provide a therapeutically effective amount of insulin for at least three days in one application.

~~41.~~ 35. A composition according to any of claims ~~37~~ 31 to ~~40~~34, wherein it is a single dose composition.

~~42.~~ 36. A composition according to any of claims ~~37~~ 31 to ~~40~~34, wherein it is a multiple dose composition.

~~43.~~ 37. A composition according to any of claims ~~37~~ 31 to ~~42~~36, wherein it contains one or more additional biologically active agents, either in their free form; as prodrugs, especially as hydrogel prodrugs; and wherein the one or more additional biologically active agents are selected from the group consisting of sulfonylureas, such as, for example, chlorpropamide, tolazamide, tolbutamide, glyburide, glipizide, glimepiride, and the like; meglitinides, such as, for example, repaglinide; glucagon-like peptide-1(GLP-1) and its mimetics, glucose-insulinotropic peptide (GIP) and it's mimetics, exendin and its mimetics, and dipeptyl protease inhibitors (DPPIV); biguanides , such as, for example, metformin; thiazolidinediones, such as, for example, rosiglitazone, pioglitazone, troglitazone, isaglitazone (known as MCC-555), 2-[2-[(2R)-4-hexyl-3,4-dihydro-3-oxo-2H-1,4-benzoxazin-2-yl]ethoxy]-benzene acetic acid, and the like; GW2570, and the like; retinoid-X receptor (RXR) modulators, such as, for example, targretin, 9-cis-retinoic acid, and the like; other insulin sensitizing agents , such as, for example, INS-1, PTP-1B inhibitors, GSK3 inhibitors, glycogen phosphorylase a inhibitors, fructose-1,6-bisphosphatase inhibitors, and the like; insulins, including regular or short-acting, intermediate-acting, and long-acting insulins, inhaled insulin and insulin analogues, such as insulin molecules with minor differences in the natural amino acid sequence; small molecule mimics of insulin, including, but not limited to L-783281, TE-17411, and the like; Na-glucose co-transporter inhibitors, such as T-1095, T-1095A, phlorizen, and the like; amylin agonists which include, but are not limited to pramlintide, and the like; glucagon antagonists such as AY-279955, and the like; anti-obesity agents such as orlistat, a pancreatic lipase inhibitor; sibutramine; norepinephrine and dopamine; growth hormone, IGF-1, growth hormone releasing factor; oxyntomodulin and ghrelin modulators; appetite-suppressants such as benzphetamine, phenmetrazine, phentermine, diethylpropion, mazindol, sibutramine, phenylpropanolamine or, ephedrine; appetite-suppressants such as quipazine, fluoxetine, sertraline, fenfluramine, or dexfenfluramine; appetite-suppressant agents such as apomorphine; appetite-suppressant such as histamine mimetics, H3 receptor modulators; enhancers of energy expenditure such as beta-3 adrenergic agonists and stimulators of

uncoupling protein function; leptin and leptin mimetics (eg, metreleptin); neuropeptide Y antagonists; melanocortin-1, 3 and 4 receptor modulators; cholecystokinin agonists; glucagon-like peptide-1 mimetics and analogues like for example exendin; androgens such as dehydroepiandrosterone and derivatives such as etiocholandione; testosterone; anabolic steroids such as oxandrolone, and steroidal hormones; galanin receptor antagonists; cytokine agents such as ciliary neurotrophic factor; amylase inhibitors.

~~44~~38. A container comprising the pharmaceutical composition according to claims ~~37~~ 31 to ~~43~~37.

~~45~~39. A container according to claim ~~44~~38, wherein the container is a dual-chamber syringe.

~~46~~40. A suspension comprising the pharmaceutical composition according to claims ~~37~~ 31 and ~~40~~ 34 to ~~43~~37.

~~47~~41. A method of preparing a suspension according to claim ~~46~~40, comprising the steps of reconstituting the dry pharmaceutical composition according to claims ~~38~~ 32 or ~~39~~ 33 by adding reconstitution solution.

~~48~~42. A kit of parts comprising a needle and a container containing reconstitution solution and the dry composition according to claim ~~38~~ 32 or ~~39~~ 33 for use with the needle.

~~49~~43. A kit of parts according to claim ~~48~~42, wherein the container is a dual-chamber syringe and wherein one of the two-chambers of the dual-chamber syringe contains the dry pharmaceutical composition and the second chamber of said dual-chamber syringe contains the reconstitution solution.

~~50~~44. A prodrug of any of claims 1 to ~~34~~ 28 or a pharmaceutical composition of any of claims ~~37~~ 31 to ~~43~~ 37 for use as a medicament.

~~51~~45. A prodrug of any of claims 1 to ~~34~~ 28 or a pharmaceutical composition of any of claims ~~37~~ 31 to ~~43~~ 37 for use in a method of treating or preventing diseases or disorders which can be treated by insulin.

~~52. A process for acylating the ε-amino group of insulin or an insulin analog, having one or more free α-amino groups and the free ε-amino group with an acylating agent containing one or~~

~~more protected functional groups, which comprises reacting the insulin or insulin analog with a soluble acylating agent containing one or more protected functional groups at a pH of 8.0 to below 9.0 in a polar solvent.~~

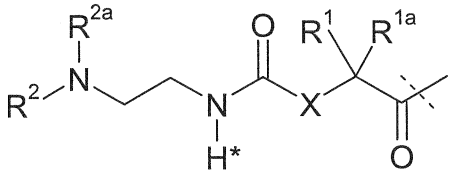
~~53. The process of claim 52, wherein the pH is from 8.0 to 8.9.~~

~~54. An insulin compound characterized by having an acyl group linked to the ε-nitrogen of insulin or an insulin analog and wherein such acyl group has one or more protected functional groups.~~

~~55~~46. An insulin-linker reagent D-L*, wherein

D represents an insulin moiety; and

L* is a non-biologically active linker reagent represented by formula (IV),



(IV),

wherein the dashed line indicates the attachment to one of the amino groups of the insulin by forming an amide bond;

X is $C(R^3R^{3a})$; or $N(R^3)$;

R^{1a}, R^{3a} are independently selected from the group consisting H, $NH(R^{2b})$, $N(R^{2b})C(O)R^4$ and C_{1-4} alkyl;

R^1, R^2 R^{2a}, R^{2b}, R^3, R^4 are independently selected from the group consisting of H and C_{1-4} alkyl,

wherein L* is substituted with one L^{2*}~~ and optionally further substituted~~, provided that the hydrogen marked with the asterisk in formula (IV) is not replaced by a substituent and wherein

L^2* 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; ~~spacer~~ and connected to L* and comprising a chemical functional group intended for conjugation to a reactive biodegradable hydrogel.~~;~~

~~56~~47. The insulin-linker reagent of claim ~~55~~46, wherein in formula (IV) R^2 is replaced by L^2*.

~~57~~48. The insulin-linker reagent of claim ~~56~~47, wherein in formula (IV) R^1 is replaced by L^2*.

~~58~~49. The insulin-linker reagent of any of claims ~~55~~ 46 to ~~57~~48, wherein in formula (IV) X is N(R^3).

~~59~~50. The insulin-linker reagent of any of claims ~~55~~ 46 to ~~57~~48, wherein in formula (IV) X is C(R^3R^{3a}) and R^{3a} is N(R^{2b})C(O)R^4.

~~60~~51. The insulin-linker reagent of claim ~~55~~46, wherein X is C(R^3R^{3a}) and R^{3a} is replaced by L^2*.

~~61~~52. The insulin-linker reagent of claim ~~55~~46, wherein X is C(R^3R^{3a}), R^{3a} is N(R^{2b})-L^2*.

~~62~~53. The insulin-linker reagent of any of claims ~~55~~ 46 to ~~61~~52, wherein L* is not further substituted.

~~63~~54. The insulin-linker reagent of any of claims ~~55~~ 46 to ~~62~~53, wherein L^2* comprises a thiol group.

~~64~~55. The insulin-linker reagent of any of claims ~~55~~ 46 to ~~62~~53, wherein L^2* comprises a maleimide group.

~~65~~56. A process for the preparation of a prodrug of any of claims 1 to ~~34~~ 28 and ~~55~~46, ~~51~~ 45 comprising the steps of

(a) contacting an aqueous suspension comprising maleimide-functionalized hydrogel microparticles with a solution comprising the insulin-linker reagent of claim 63 at temperatures between room temperature and 4°C in a buffered aqueous solution of pH 2-5, resulting in an insulin-linker-hydrogel conjugate;

(b) optionally, treating the insulin-linker-hydrogel conjugate from step (a) with a thiol-containing compound of 34 Da to 500 Da at temperatures between room temperature and 4°C in a buffered aqueous solution of pH 2-5.

~~66~~57. A process for the preparation of a prodrug of any of claims 1 to ~~34~~ 28 and ~~55~~46, ~~51~~ 45 comprising the steps of

(a) contacting an aqueous suspension comprising thiol-functionalized hydrogel microparticles with a solution comprising the insulin-linker reagent of 64 at temperatures between room temperature and 4°C in a buffered aqueous solution of pH 2-5, resulting in an insulin-linker-hydrogel conjugate;

(b) optionally, treating the insulin-linker-hydrogel conjugate from step (a) with a maleimide-containing compound of 100 to 300 Da at temperatures between room temperature and 4°C in a buffered aqueous solution of pH 2-5.

~~67. A process for preparing a needle injectable prodrug comprising the step of~~

~~(a) preparing a prodrug of claim 30 in the form of microparticles;~~

~~(b) sieving the microparticles~~

~~(c) selecting a fraction with a prodrug bead diameter of between 25 and 80 µm.~~

~~(d) suspending the bead fraction of step (c) in an aqueous buffer solution suitable for injection.~~

~~68. Needle injectable prodrug obtainable from the process of claim 67.~~

~~69. Prodrug of claim claim 68, injectable through a needle with an inner diameter of less than 300 µm.~~

~~70. Prodrug of claim 68, injectable through a needle with an inner diameter of less than 225 µm.~~

~~71. Prodrug of claim 68, injectable through a needle with an inner diameter of less than 175 µm.~~