Claims:

1. A method of conjugating a water soluble polymer to an oxidized carbohydrate moiety of (1) a glycoprotein other than a blood coagulation protein, (2) a ganglioside, or (3) a drug delivery system comprising a carbohydrate group, comprising contacting the oxidized carbohydrate moiety with the water soluble polymer under conditions that allow conjugation,

wherein said water soluble polymer contains an aminooxy group and an oxime linkage is formed between the oxidized carbohydrate moiety and the aminooxy group on the water soluble polymer, or

wherein said water soluble polymer contains a hydrazide group and a hydrazone linkage is formed between the oxidized carbohydrate moiety and the hydrazide group on the water soluble polymer.

2. The method according to claim 1 wherein the water soluble polymer is polyethylene glycol (PEG) hydroxyethyl cellulose (HEC), dextrin or polyoxazoline.

3. The method according to claim 1 wherein the water soluble polymer is polysialic acid (PSA) or a modified PSA (mPSA).

4. The method according to claim 3 wherein the PSA or mPSA is colominic acid or modified colominic acid.

5. The method according to claim 3 or 4 wherein the PSA or mPSA comprises 2 – 500 sialic acid units.

6. The method according to any previous claim wherein the ganglioside comprises a glycosphingolipid (ceramide and oligosaccharide) with one or more sialic acids linked on the sugar chain.

7. The method according to any previous claim wherein the ganglioside comprises a ceramide linked to glucose, which is linked to a first galactose, which is linked to N-acetylgalactosamine, which is linked to a second galactose.

8. The method according to any one of claims 1-5 wherein the drug delivery system comprises a macromolecular drug delivery system, for instance an antibody

or a neoglycoprotein, or a synthetic polymers, for instance poly(hydroxypropylmethacrylamide), polylysine or polymerised alkyl cyanoacrylates.

9. The method according to any one of claims 1-5 wherein the drug delivery system comprises a particulate drug delivery system, for instance (i) a nanosphere or a microspheres, which comprises biodegradable materials such as albumin or semibiodegradable materials such as dextran and alkylcyanoacrylate polymers, (ii) a vesicle formed of nonionic surfactants, or (iii) a liposome.

10. The method according to any one of claims 1-5 wherein the glycoprotein is selected from cytokines such as interleukins, alpha-, beta-, and gamma-interferons, colony stimulating factors including granulocyte colony stimulating factors, fibroblast growth factors, platelet derived growth factors, phospholipase-activating protein (PUP), insulin, plant proteins such as lectins and ricins, tumor necrosis factors and related alleles, soluble forms of tumor necrosis factor receptors, interleukin receptors and soluble forms of interleukin receptors, growth factors, tissue growth factors, transforming growth factors such as TGFαs or TGFβs and epidermal growth factors, hormones, somatomedins, pigmentary hormones, hypothalamic releasing factors, antidiuretic hormones, prolactin, chorionic gonadotropin, follicle-stimulating hormone, thyroid-stimulating hormone, tissue plasminogen activator, immunoglobulins such as IgG, IgE, IgM, IgA, and IgD, monoclonal antibodies, erythropoietin (EPO), blood factors other than blood coagulation proteins, galactosidases, α-galactosidases, β-galactosidases, DNAses, fetuin, fragments thereof and fusion proteins comprising any of the above mentioned proteins or fragments thereof.

11. The method according to any previous claim, comprising oxidizing the carbohydrate moiety by incubating (1), (2) or (3) with sodium periodate ($NaIO_4$).

12. The method according to any previous claim, comprising oxidizing the water soluble polymer to form an aldehyde group on a terminal unit of the water soluble polymer, and reacting the oxidized water soluble polymer with an aminooxy linker or a hydrazide linker.

13. The method according to claim 12 comprising oxidizing the water soluble polymer using $NaIO_4$.

14. The method according to any previous claim comprising contacting the oxidized carbohydrate moiety with water soluble polymer in a buffer comprising a nucleophilic catalyst selected from aniline and aniline derivatives.

15. The method according to any previous claim, wherein said water soluble polymer contains an aminooxy group and an oxime linkage is formed between the oxidized carbohydrate moiety and the aminooxy group on the water soluble polymer.

16. The method according to claim 15 wherein the aminooxy group is formed by reacting oxidized water soluble polymer with an aminooxy linker, and the aminooxy linker is 3-oxa-pentane-1,5-dioxyamine or 3,6,9-trioxa-undecane-1,11-dioxyamine.

17. The method according to any one of claims 1-14, wherein said water soluble polymer contains a hydrazide group and a hydrazone linkage is formed between the oxidized carbohydrate moiety and the hydrazide group on the water soluble polymer.

18. The method according to claim 17 wherein the hydrazide group is formed by reacting oxidized water soluble polymer with a hydrazide linker.

19. The method according to any previous claim further comprising reducing an oxime linkage or a hydrazone linkage in the conjugated (1), (2) or (3) by incubation in the presence of a reducing compound.

20. The method according to claim 19 wherein the reducing compound is sodium cyanoborohydride ($NaCNBH_3$) or ascorbic acid (vitamin C).

21. A conjugated glycoprotein, ganglioside or drug delivery system obtainable by the method according to any previous claim.

22. A conjugated glycoprotein other than a blood coagulation protein, a ganglioside or a drug delivery system comprising:

(a) the glycoprotein, ganglioside or drug delivery system; and

(b) at least one aminooxy-water soluble polymer bound to the glycoprotein, ganglioside or drug delivery system of (a), wherein said aminooxy-water soluble polymer is attached to the glycoprotein, ganglioside or drug delivery system via one or more carbohydrate moieties.

23. A conjugated glycoprotein according to claim 22, wherein the water soluble polymer is PSA or mPSA.

24. A conjugated glycoprotein other than a blood coagulation protein, a ganglioside or a drug delivery system comprising:

(a) the glycoprotein, ganglioside or drug delivery system; and

(b) at least one hydrazide-water soluble polymer bound to the glycoprotein, ganglioside or drug delivery system of (a), wherein said hydrazide-water soluble polymer is attached to the glycoprotein ganglioside or drug delivery system via one or more carbohydrate moieties.

25. A conjugated glycoprotein according to claim 24, wherein the water soluble polymer is PSA or mPSA.

Claims

1. A method of conjugating polysialic acid (PSA) or a modified PSA (mPSA) to an oxidized carbohydrate moiety of (1) a glycoprotein other than a blood coagulation protein, (2) a ganglioside, or (3) a drug delivery system comprising a carbohydrate group, comprising contacting the oxidized carbohydrate moiety with the PSA or mPSA under conditions that allow conjugation,

wherein said PSA or mPSA contains an aminooxy group and an oxime linkage is formed between the oxidized carbohydrate moiety and the aminooxy group on the PSA or mPSA.

2. The method according to claim 1 wherein the PSA or mPSA is colominic acid or modified colominic acid.

3. The method according to claim 1 or 2 wherein the PSA or mPSA comprises 2 – 500 sialic acid units.

4. The method according to any previous claim wherein the ganglioside comprises a glycosphingolipid (ceramide and oligosaccharide) with one or more sialic acids linked on the sugar chain.

5. The method according to any previous claim wherein the ganglioside comprises a ceramide linked to glucose, which is linked to a first galactose, which is linked to N-acetylgalactosamine, which is linked to a second galactose.

6. The method according to any one of claims 1-3 wherein the drug delivery system comprises a macromolecular drug delivery system, for instance an antibody or a neoglycoprotein, or a synthetic polymers, for instance poly(hydroxypropylmethacrylamide), polylysine or polymerised alkyl cyanoacrylates.

7. The method according to any one of claims 1-3 wherein the drug delivery system comprises a particulate drug delivery system, for instance (i) a nanosphere or a microspheres, which comprises biodegradable materials such as albumin or semibiodegradable materials such as dextran and alkylcyanoacrylate polymers, (ii) a vesicle formed of nonionic surfactants, or (iii) a liposome.

8. The method according to any one of claims 1-3 wherein the glycoprotein is selected from cytokines such as interleukins, alpha-, beta-, and gamma-interferons, colony

stimulating factors including granulocyte colony stimulating factors, fibroblast growth factors, platelet derived growth factors, phospholipase-activating protein (PUP), insulin, plant proteins such as lectins and ricins, tumor necrosis factors and related alleles, soluble forms of tumor necrosis factor receptors, interleukin receptors and soluble forms of interleukin receptors, growth factors, tissue growth factors, transforming growth factors such as TGFαs or TGFβs and epidermal growth factors, hormones, somatomedins, pigmentary hormones, hypothalamic releasing factors, antidiuretic hormones, prolactin, chorionic gonadotropin, follicle-stimulating hormone, thyroid-stimulating hormone, tissue plasminogen activator, immunoglobulins such as IgG, IgE, IgM, IgA, and IgD, monoclonal antibodies, erythropoietin (EPO), blood factors other than blood coagulation proteins, galactosidases, α-galactosidases, β-galactosidases, DNAses, fetuin, fragments thereof and fusion proteins comprising any of the above mentioned proteins or fragments thereof.

9. The method according to any previous claim, comprising oxidizing the carbohydrate moiety by incubating (1), (2) or (3) with sodium periodate ($NaIO_4$).

10. The method according to any previous claim, comprising oxidizing the PSA or mPSA to form an aldehyde group on a terminal unit of the PSA or mPSA, and reacting the oxidized PSA or mPSA with an aminooxy linker.

11. The method according to claim 10 comprising oxidizing the PSA or mPSA using $NaIO_4$.

12. The method according to any previous claim comprising contacting the oxidized carbohydrate moiety with PSA or mPSA in a buffer comprising a nucleophilic catalyst selected from aniline and aniline derivatives.

13. The method according to any previous claim, wherein said PSA or mPSA contains an aminooxy group and an oxime linkage is formed between the oxidized carbohydrate moiety and the aminooxy group on the PSA or mPSA.

14. The method according to claim 13 wherein the aminooxy group is formed by reacting oxidized PSA or mPSAwith an aminooxy linker, and the aminooxy linker is 3-oxa-pentane-1,5-dioxyamine or 3,6,9-trioxa-undecane-1,11-dioxyamine.

15. The method according to any previous claim further comprising reducing an oxime linkage in the conjugated (1), (2) or (3) by incubation in the presence of a reducing compound.

16. The method according to claim 15 wherein the reducing compound is sodium cyanoborohydride ($NaCNBH_3$) or ascorbic acid (vitamin C).

17. A conjugated glycoprotein, ganglioside or drug delivery system obtainable by the method according to any previous claim.

18. A conjugated glycoprotein other than a blood coagulation protein, a ganglioside or a drug delivery system comprising:

(a) the glycoprotein, ganglioside or drug delivery system; and

(b) at least one aminooxy-PSA or aminooxy-mPSA bound to the glycoprotein, ganglioside or drug delivery system of (a), wherein said aminooxy-PSA or aminooxy-mPSA is attached to the glycoprotein, ganglioside or drug delivery system via one or more carbohydrate moieties.