CLAIMS

1. A method for conjugating a moiety of interest to an Fc-containing polypeptide (e.g. an antibody), which method comprises:

(i) providing an Fc-containing polypeptide with N-linked glycosylation at a site present in the Fc region of the polypeptide, adjacent a site comprising a glutamine residue;

(ii) treating said polypeptide with an enzyme to trim back the N-linked glycans at the site of N-linked glycosylation to an optionally fucosylated, core N-acetylglucosamine (GlcNAc) moiety; and

(iii)(1) reacting, in the presence of a transglutaminase (TGase), the polypeptide obtained in step (ii) with a moiety of interest comprising an acceptor moiety which is a substrate for the TGase, so that the moiety of interest is attached to the polypeptide via the glutamine residue,

wherein the acceptor moiety is an alpha-effect nucleophile.

2. A method for conjugating a moiety of interest to an Fc-containing polypeptide (e.g. an antibody), which method comprises:

(i) providing an Fc-containing polypeptide with N-linked glycosylation at a site present in the Fc region of the polypeptide, adjacent a site comprising a glutamine residue;

(ii) treating said polypeptide with an enzyme to trim back the N-linked glycans at the site of N-linked glycosylation to an optionally fucosylated, core N-acetylglucosamine (GlcNAc) moiety; and

(iii)(2) reacting, in the presence of a transglutaminase (TGase), the polypeptide obtained in step (ii) with an acceptor moiety which is a substrate for the TGase and which acceptor moiety is an alpha-effect nucleophile and further comprises a reactive group (R) which enables the subsequent attachment of the moiety of interest via a reaction between R and a complementary reactive group (R') attached to the moiety of interest, such that the acceptor moiety is attached to the polypeptide via the glutamine residue; and

(iv) reacting the polypeptide conjugate formed in step (iii)(2) with the moiety of interest attached to R' under conditions such that R reacts with R' to form R-R'.

3. A method for conjugating a first moiety of interest comprising a first drug and a second moiety of interest comprising a second drug to an Fc-containing polypeptide (e.g., an antibody), which method comprises:

(i) providing an Fc-containing polypeptide with N-linked glycosylation at a site present in the Fc region of the polypeptide, adjacent a site comprising a glutamine residue;

(ii) treating said polypeptide with an enzyme to trim back the N-linked glycans at the site of N-linked glycosylation to an optionally fucosylated, core N-acetylglucosamine (GlcNAc) moiety;

(iii)(1) reacting, in the presence of a transglutaminase (TGase), the polypeptide obtained in step (ii) with a first moiety of interest comprising an acceptor moiety which is a substrate for the TGase, so that the moiety of interest is attached to the polypeptide via the glutamine residue; and

(v) reacting, in the presence of a galactosyl transferase (GalT), the polypeptide obtained in step (iii)(1) with a sugar derivative attached to the second moiety of interest, so that the sugar derivative is attached to the polypeptide via the core N-acetylglucosamine (GlcNAc) moiety.

9. A method for conjugating a first moiety of interest comprising a first drug and a second moiety of interest comprising a second drug to an Fc-containing polypeptide (e.g., an antibody), which method comprises:

(i) providing an Fc-containing polypeptide with N-linked glycosylation at a site present in the Fc region of the polypeptide, adjacent a site comprising a glutamine residue;

(ii) treating said polypeptide with an enzyme to trim back the N-linked glycans at the site of N-linked glycosylation to an optionally fucosylated, core N-acetylglucosamine (GlcNAc) moiety;

(iii)(2) reacting, in the presence of a transglutaminase (TGase), the polypeptide obtained in step (ii) with an acceptor moiety which is a substrate for the TGase and which acceptor moiety further comprises a reactive group (R) which enables the subsequent attachment of the first moiety of interest via a reaction between R and a complementary reactive group (R') attached to the first moiety of interest, such that the acceptor moiety is attached to the polypeptide via the glutamine residue; and

(iv) reacting the polypeptide conjugate formed in step (iii)(2) with the first moiety of interest attached to R' under conditions such that R reacts with R' to form R-R';

(v) reacting, in the presence of a galactosyl transferase (GalT), the polypeptide obtained in step (iii)(2) or step (iv) with a sugar derivative attached to a reactive group (R) which enables the subsequent attachment of the second moiety of interest via a reaction between R and a complementary reactive group (R') attached to the second moiety of interest, so that the sugar derivative is attached to the polypeptide via the core N-acetylglucosamine (GlcNAc) moiety; and

(vi) reacting the polypeptide conjugate formed in step (v) with the second moiety of interest attached to R' under conditions such that R reacts with R' to form R-R'.

5. A method according to any one of the preceding claims wherein the acceptor moiety is selected from an aminooxy, a hydrazido, a hydrazino and aryl derivatives thereof.

6. A method according to claim 2 or claim 4 wherein the acceptor moiety is of formula Ac-Sp-R where Ac is selected from an aminooxy, a hydrazido, a hydrazino and aryl derivatives thereof, Sp is absent or a spacer moiety; and R is a reactive group, e.g. an azide.

7. A method according to any one of the preceding claims wherein the enzyme is EndoS.

8. A method according to any one of the preceding claims wherein the N-linked glycosylation site is at position 297 according to the Kabat numbering system, or an equivalent position and/or the site comprising a glutamine residue is at position 295 according to the Kabat numbering system, or an equivalent position.

9. A method according to any one of the preceding claims wherein the moiety or moieties of interest comprise(s) a drug-linker.

10. A method according to any one of claims 3 to 9 wherein the first drug and second drug have different modes of action.

11. An Fc-containing polypeptide conjugate, which comprises:

(i) an asparagine residue present in the Fc region of the polypeptide with an optionally fucosylated, GlcNAc N-glycan core lacking any additional endogenous glycan residues; and

(ii) (Q)-NH-NH-C(O)-L-D; (Q)-NH-NH-C(O)-Ar-L-D; (Q)-NH-NH-L-D; (Q)-NH-NH-Ar-L-D; Q)-NH-O-L-D; (Q)-NH-O-Ar-L-D, or a pharmaceutically acceptable salt or solvate thereof; wherein Q is a glutamine residue present in the Fc region of the polypeptide; L is a linker, which optionally comprises a conditionally-cleavable moiety; and D is a drug moiety;

wherein the asparagine residue is adjacent the glutamine residue.

12. An Fc-containing polypeptide conjugate, which comprises:

(i) (Q)-NH-LA-D1, or a pharmaceutically acceptable salt or solvate thereof; wherein Q is a glutamine residue present in the Fc region of the polypeptide; LA is a linker; and D1 is a first drug moiety;

(ii) (N)-G-S-R_2-R_2'-LB-D2, or a pharmaceutically acceptable salt or solvate thereof; where N is an asparagine residue present in the Fc region of the polypeptide; G is an optionally fucosylated, GlcNAc N-glycan core; S is a sugar derivative; R_2-R_2' is formed by the reaction of a reactive group R_2 and a complementary reactive group R_2'; LB is a linker, which optionally comprises a conditionally-cleavable moiety; and D2 is a second drug moiety; and

wherein the asparagine residue is adjacent the glutamine residue.

13. An Fc-containing polypeptide conjugate according to claim 12, wherein LA comprises LA_1-R_1-R_1'-LA_2;

wherein LA_1 is absent or a spacer moiety, R_1-R_1' is formed by the reaction of a reactive group R_1 and a complementary reactive group R_1'; and LA_2 is a linker or spacer moiety to which D1 is attached and which optionally comprise a conditionally-cleavable moiety.

14. An Fc-containing polypeptide conjugate according to any one of claims 11 to 13 wherein D, D1 and D2 is a cytotoxin or immunostimulatory molecule.

15. An Fc-containing polypeptide conjugate according to any one of claims 12 to 15 wherein the first drug and second drug have different modes of action.

16. An Fc-containing polypeptide conjugate according to any one of claims 11 to 15 wherein the asparagine residue is at position 297 according to the Kabat numbering system, or an equivalent position and/or the glutamine residue is at position 295 according to the Kabat numbering system, or an equivalent position.