Claims:

1. A process for the preparation of an antibody conjugate comprising the step of reacting an engineered antibody having a single inter-heavy chain disulfide bond with a conjugating reagent that forms a bridge between the two cysteine residues derived from the disulfide bond.

2. A process as claimed in claim 1, wherein the antibody is an IgG1 or IgG4 molecule.

3. A process as claimed in either claim 1 or claim 2, wherein the antibody is prepared by recombinant expression or chemical synthesis.

4. A process as claimed in claim 3, which comprises

a) mutating a nucleic acid sequence encoding a parent antibody, wherein said mutation results in deletion or substitution of one or more inter-heavy chain cysteine residues with an amino acid other than cysteine;
b) expressing the nucleic acid in an expression system; and
c) isolating the engineered antibody.

5. A process as claimed in claim 4, wherein site directed mutagenesis is carried out to incorporate nucleotide changes into the coding sequence of the parent antibody.

6. A process as claimed in any one of the preceding claims, wherein said single inter-heavy chain disulfide bond is at position 226 or 229 of the antibody according to the EU-index numbering system.

7. A process as claimed in any one of the preceding claims, wherein the antibody has a cysteine at position 226 and an amino acid other than cysteine at position 229 according to the EU-index numbering system, or an amino acid other than cysteine at position 226 and a cysteine at position 229 according to the EU-index numbering system.

8. A process as claimed in claim 7, wherein the amino acid other than cysteine does not include a thiol moiety, for example serine, threonine, valine, alanine, glycine, leucine or isoleucine, other polar amino acid, other naturally occurring amino acid, or non-naturally occurring amino acid.

9. A process as claimed in any one of the preceding claims, wherein the antibody has a cysteine at position 226 and a serine at position 229 according to the EU-index numbering system, or a serine at position 226 and a cysteine at position 229 according to the EU-index numbering system.

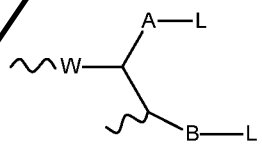
10. A process as claimed in any one of the preceding claims, wherein the antibody is an IgG1 molecule and comprises a sequence of Cys-Pro-Pro-Ser or Ser-Pro-Pro-Cys at positions 226-229 according to the EU-index numbering system; or is an IgG4 molecule and comprises a sequence of Cys-Pro-Ser-Ser or Ser-Pro-Ser-Cys at positions 226-229 according to the EU-index numbering system.

11. A process as claimed in any one of claims 1 to 5, in which the single inter-heavy chain disulfide bond is in the hinge region of the antibody at a location different from a disulfide bond in the parent antibody.

12. A process as claimed in any one of the preceding claims, wherein the reagent includes a diagnostic, therapeutic or labelling agent, or a binding agent capable of binding a diagnostic, therapeutic or labelling agent.

13. A process as claimed in any one of the preceding claims, wherein the reagent includes a polymer.

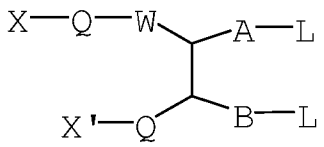
14. A process as claimed in any one of the preceding claims, in which the reagent contains the functional group:



(I)

in which W represents an electron-withdrawing group; A represents a C_{1-5} alkylene or alkenylene chain; B represents a bond or a C_{1-4} alkylene or alkenylene chain; and each L independently represents a leaving group.

15. A process as claimed in claim 14, in which the reagent is of the formula II, III or IV:



(II)

in which one of X and X' represents a polymer and the other represents a hydrogen atom;

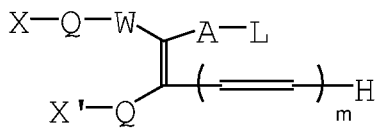
Q represents a linking group;

W represents an electron-withdrawing group; or, if X' represents a polymer, X-Q-W together may represent an electron withdrawing group;

A represents a C_{1-5} alkylene or alkenylene chain;

B represents a bond or a C_{1-4} alkylene or alkenylene chain; and

each L independently represents a leaving group;



(III)

in which X, X', Q, W, A and L have the meanings given for the general formula II, and in addition if X represents a polymer, X' and electron-withdrawing group W together with the interjacent atoms may form a ring, and m represents an integer 1 to 4; or

$$X\text{-}Q\text{-}W\text{-}CR^1R^{1'}\text{-}CR^2.L.L' \qquad (IV)$$

in which X, Q and W have the meanings given for the general formula II, and either

R^1 represents a hydrogen atom or a C_{1-4}alkyl group, $R^{1'}$ represents a hydrogen atom, and each of L and L' independently represents a leaving group; or

R^1 represents a hydrogen atom or a C_{1-4}alkyl group, L represents a leaving group, and $R^{1'}$ and L' together represent a bond; or

R^1 and L together represent a bond and $R^{1'}$ and L' together represent a bond; and

R^2 represents a hydrogen atom or a C_{1-4} alkyl group.

16. A process as claimed in either claim 14 or claim 15, which comprises the additional step of reducing the electron withdrawing group W in the resulting conjugate.

17. A process as claimed in any one of the preceding claims, in which in addition to one molecule of conjugating reagent forming a bridge between the two cysteine residues derived

from said inter-heavy chain disulfide bond, two additional molecules of conjugating reagent form bridges between the cysteine residues derived from the interchain disulfide bonds located between the C_L domain of the light chain and the C_H1 domain of the heavy chain of the antibody.

18. An antibody conjugate in which a conjugating reagent is bound to an antibody via two sulfur atoms derived from a disulfide bridge in the antibody; characterised in that said disulfide bridge is the only inter-heavy chain disulfide bond present in the antibody.

19. An antibody conjugate as claimed in claim 18, which additionally comprises two conjugating reagents bound via sulfur atoms derived the interchain disulfide bonds located between the C_L domain of the light chain and the C_H1 domain of the heavy chain of the antibody.

20. An antibody conjugate as claimed in either claim 18 or claim 19, preparable by a process as claimed in any one of claims 1 to 17.

21. An antibody conjugate as claimed in any one of claims 18 to 20, for use in therapy.

22. An antibody conjugate as claimed in any one of claims 18 to 20, for use in the treatment of cancer.

23. The use of an antibody conjugate as claimed in any one of claims 18 to 20, for the manufacture of a medicament for the treatment of cancer.

24. A method of treating cancer which comprises the administration to a patient of an antibody conjugate as claimed in any one of claims 18 to 20.

25. A pharmaceutical composition comprising a conjugate as claimed in any one of claims 18 to 20, together with a pharmaceutically acceptable carrier; optionally together with an additional therapeutic agent.

CLAIMS

1. A process for the preparation of an antibody conjugate comprising the step of reacting an engineered antibody having a single inter-heavy chain disulfide bond with a conjugating reagent that forms a bridge between the two cysteine residues derived from the disulfide bond.

2. A process according to Claim 1, in which the antibody is an IgG1 or IgG4 molecule.

3. A process according to Claim 1 or 2, in which the antibody is prepared by recombinant expression or chemical synthesis.

4. A process according to Claim 3, which comprises

(a) mutating a nucleic acid sequence encoding a parent antibody, in which the mutation results in deletion or substitution of one or more inter-heavy chain cysteine residues with an amino acid other than cysteine;

(b) expressing the nucleic acid in an expression system; and

(c) isolating the engineered antibody.

5. A process according to Claim 4, in which site directed mutagenesis is carried out to incorporate nucleotide changes into the coding sequence of the parent antibody.

6. A process according to any preceding claim, in which the single inter-heavy chain disulfide bond is at position 226 or 229 of the antibody according to the EU-index numbering system.

7. A process according to any preceding claim, in which the antibody has a cysteine at position 226 and an amino acid other than cysteine at position 229 according to the EU-index numbering system, or an amino acid other than cysteine at position 226 and a cysteine at position 229 according to the EU-index numbering system.

8. A process according to Claim 7, in which the amino acid other than cysteine does not include a thiol moiety, for example serine, threonine, valine, alanine, glycine, leucine or isoleucine, other polar amino acid, other naturally occurring amino acid, or non-naturally occurring amino acid.

9. A process according to any preceding claim, in which the antibody has a cysteine at position 226 and a serine at position 229 according to the EU-index numbering system, or a

serine at position 226 and a cysteine at position 229 according to the EU-index numbering system.

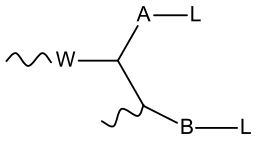
10. A process according to any preceding claim, in which the antibody is an IgG1 molecule and comprises a sequence of Cys-Pro-Pro-Ser or Ser-Pro-Pro-Cys at positions 226-229 according to the EU-index numbering system; or is an IgG4 molecule and comprises a sequence of Cys-Pro-Ser-Ser or Ser-Pro-Ser-Cys at positions 226-229 according to the EU-index numbering system.

11. A process according to any of Claims 1 to 5, in which the single inter-heavy chain disulfide bond is in the hinge region of the antibody at a location different from a disulfide bond in the parent antibody.

12. A process according to any preceding claim, in which the reagent includes a diagnostic, therapeutic or labelling agent, or a binding agent capable of binding a diagnostic, therapeutic or labelling agent.

13. A process according to any preceding claim, in which the reagent includes a polymer.

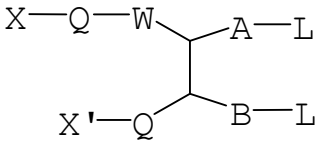
14. A process according to any preceding claim, in which the reagent contains the functional group:



(I)

in which W represents an electron-withdrawing group; A represents a C_{1-5} alkylene or alkenylene chain; B represents a bond or a C_{1-4} alkylene or alkenylene chain; and each L independently represents a leaving group.

15. A process according to Claim 14, in which the reagent is of the formula II, III or IV:



(II)

in which one of X and X' represents a polymer and the other represents a hydrogen atom;

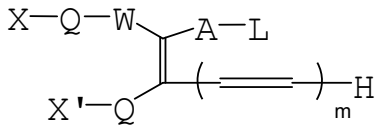
Q represents a linking group;

W represents an electron-withdrawing group; or, if X' represents a polymer, X-Q-W together may represent an electron withdrawing group;

A represents a C_{1-5} alkylene or alkenylene chain;

B represents a bond or a C_{1-4} alkylene or alkenylene chain; and

each L independently represents a leaving group;



(III)

in which X, X', Q, W, A and L have the meanings given for the general formula II, and in addition if X represents a polymer, X' and electron-withdrawing group W together with the interjacent atoms may form a ring, and m represents an integer 1 to 4; or

$$X\text{-}Q\text{-}W\text{-}CR^{1}R^{1'}\text{-}CR^{2}.L.L' \qquad (IV)$$

in which X, Q and W have the meanings given for the general formula II, and either

R^1 represents a hydrogen atom or a C_{1-4}alkyl group, R1' represents a hydrogen atom, and each of L and L' independently represents a leaving group; or

R^1 represents a hydrogen atom or a C_{1-4}alkyl group, L represents a leaving group, and $R^{1'}$ and L' together represent a bond; or

R^1 and L together represent a bond and $R^{1'}$ and L' together represent a bond; and

R^2 represents a hydrogen atom or a C_{1-4} alkyl group.

16. A process according to Claim 14 or 15, which comprises the additional step of reducing the electron withdrawing group W in the resulting conjugate.

17. A process as claimed in any one of the preceding claims, in which in addition to one molecule of conjugating reagent forming a bridge between the two cysteine residues derived from the inter-heavy chain disulfide bond, two additional molecules of conjugating reagent form bridges between the cysteine residues derived from the interchain disulfide bonds located between the C_L domain of the light chain and the C_H1 domain of the heavy chain of the antibody.

18. An antibody conjugate in which a conjugating reagent is bound to an antibody via two sulfur atoms derived from a disulfide bridge in the antibody; characterised in that the disulfide bridge is the only inter-heavy chain disulfide bond present in the antibody.

19. An antibody conjugate according to Claim 18, which additionally comprises two conjugating reagents bound via sulfur atoms derived from the interchain disulfide bonds located between the C_L domain of the light chain and the C_H1 domain of the heavy chain of the antibody.

20. An antibody conjugate according to Claim 18 or 19, preparable by a process as claimed in any one of claims 1 to 17.

21. An antibody conjugate according to Claim 18, 19 or 20, for use in therapy.

22. An antibody conjugate according to Claim 18, 19 or 20, for use in the treatment of cancer.

23. The use of an antibody conjugate according to Claim 18, 19 or 20, for the manufacture of a medicament for the treatment of cancer.

24. A pharmaceutical composition comprising a conjugate according to Claim 18, 19 or 20, together with a pharmaceutically acceptable carrier; optionally together with an additional therapeutic agent.