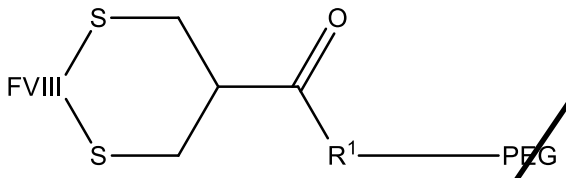
CLAIMS

1. A Factor VIII-polyethylene glycol conjugate, in which one or more polyethylene glycol groups are conjugated to FVIII by a linker group bridging the sulphur atoms of two cysteine residues that formed a disulphide bond in FVIII, in which the conjugate has the structure:



in which R1 is a substituent which is a direct bond, an alkylene group (preferably a C_{1-10} alkylene group), or an optionally-substituted aryl or heteroaryl group; in which the aryl groups include phenyl, benzoyl and naphthyl groups; in which suitable heteroaryl groups include pyridine, pyrrole, furan, pyran, imidazole, pyrazole, oxazole, pyridazine, pyrimidine and purine; in which linkage to the polymer is by a hydrolytically labile bond, or by a nonlabile bond.

2. A Factor VIII-polyethylene glycol conjugate according to Claim 1, in which the polyethylene glycol has a molecular weight of about 5-100kDa.

3. A pharmaceutical composition comprising a Factor VIII-polyethylene glycol conjugate as claimed in any preceding claim.

4. A pharmaceutical composition according to Claim 3 in which the composition comprises a pharmaceutically acceptable diluent, adjuvant or carrier.

5. A pharmaceutical composition according to Claims 3 or 4, further comprising another pharmaceutically active agent.

6. A pharmaceutical composition according to any of Claims 3 to 5, which is suitable for parenteral administration.

7. A pharmaceutical composition according to any of Claims 3 to 6, which is suitable for intradermal, subcutaneous, and intramuscular injections, and intravenous or intraosseous infusions.

8. A pharmaceutical composition according to any of Claims 3 to 7 in the form of a solution, suspension or emulsion.

9. A pharmaceutical composition according to any of Claims 3 to 8, in which the FVIII conjugate has a longer half-life as compared to unmodified FVIII.

10. A pharmaceutical composition according to any of Claims 3 to 9, in which the FVIII conjugate has a higher AUC as compared to unmodified FVIII.

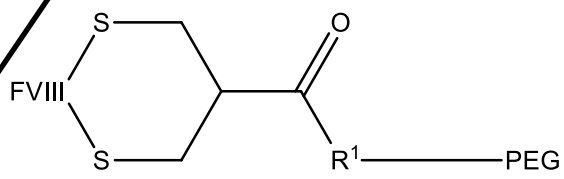
11. A pharmaceutical composition according to any of Claims 3 to 10, in which the FVIII conjugate has a higher bioavailability as compared to unmodified FVIII.

12. A pharmaceutical composition according to any of Claims 3 to 10, in which the FVIII conjugate has a lower immunogenicity as compared to unmodified FVIII.

13. A Factor VIII-polyethylene glycol conjugate according to Claim 1 or Claim 2 for use in the treatment of a blood clotting disease characterised by a loss of function of FVIII, or for use in the treatment of trauma.

14. Use of a Factor VIII -polyethylene glycol conjugate according to Claim 1 or Claim 2 in the preparation of a medicament for the treatment of a blood clotting disease characterised by a loss of function of FVIII, or in the preparation of a medicament for the treatment of trauma.

15. A process for preparing the following Factor VIII-polyethylene glycol conjugate,



in which R1 is a substituent which is a direct bond, an alkylene group (preferably a C1-10 alkylene group), or an optionally-substituted aryl or heteroaryl group; in which the aryl groups include phenyl, benzoyl and naphthyl groups; in which suitable heteroaryl groups include pyridine, pyrrole, furan, pyran, imidazole, pyrazole, oxazole, pyridazine, pyrimidine and purine; in which linkage to the polymer is by a hydrolytically labile bond, or by a nonlabile bond, and in which the process comprises:

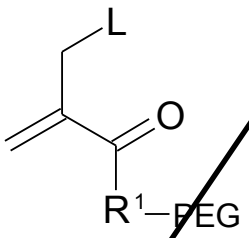
(a) reduction of a native disulphide bond between two cysteine residues in FVIII, to generate two free thiol groups;

(b) a first thiolate addition reaction between a conjugation-reagent comprising a conjugated double bond and a leaving group;

(c) elimination of the leaving group, generating a conjugated double bond; and

(d) a second thiolate addition reaction, forming a 3-carbon bridge between the two sulphur atoms.

16. A process according to Claim 15, in which the conjugation reagent has the formula

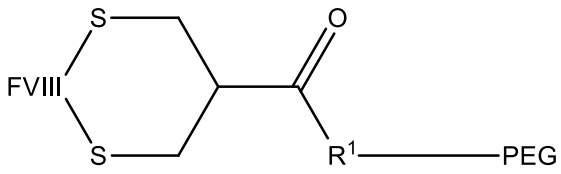


where R1 is a substituent which is a direct bond, an alkylene group (preferably a C1-10 alkylene group), or an optionally-substituted aryl or heteroaryl group; in which the aryl groups include phenyl, benzoyl and naphthyl groups; in which suitable heteroaryl groups include pyridine, pyrrole, furan, pyran, imidazole, pyrazole, oxazole, pyridazine, pyrimidine and purine; in which linkage to the polymer is by a hydrolytically labile bond, or by a nonlabile bond, and L is a leaving group.

17. A Factor VIII-polyethylene glycol conjugate, pharmaceutical composition, use or process substantially as hereinbefore described with reference to and as shown in Figures 1 to 5 of the accompanying drawings.

CLAIMS

1. A Factor VIII-polyethylene glycol (PEG) conjugate, in which one or more polyethylene glycol groups are conjugated to FVIII by a linker group bridging the sulphur atoms of two cysteine residues that formed a disulphide bond in FVIII, in which the conjugate has the structure:



in which R1 is a substituent which is a direct bond, an alkylene group (preferably a C_{1-10} alkylene group), or an optionally-substituted aryl or heteroaryl group; in which the aryl groups include phenyl, benzoyl and naphthyl groups; in which suitable heteroaryl groups include pyridine, pyrrole, furan, pyran, imidazole, pyrazole, oxazole, pyridazine, pyrimidine and purine; in which linkage to the PEG is by a hydrolytically labile bond, or by a nonlabile bond.

2. A Factor VIII-polyethylene glycol conjugate according to Claim 1, in which the polyethylene glycol has a molecular weight of about 5-100kDa.

3. A pharmaceutical composition comprising a Factor VIII-polyethylene glycol conjugate according to Claim 1 or Claim 2.

4. A pharmaceutical composition according to Claim 3 in which the composition comprises a pharmaceutically acceptable diluent, adjuvant or carrier.

5. A pharmaceutical composition according to Claim 3 or 4, further comprising another pharmaceutically active agent.

6. A pharmaceutical composition according to Claim 3, 4 or 5, which is suitable for parenteral administration.

7. A pharmaceutical composition according to any of Claims 3 to 6, which is suitable for intradermal, subcutaneous, and intramuscular injections, and intravenous or intraosseous infusions.

8. A pharmaceutical composition according to any of Claims 3 to 7 in the form of a solution, suspension or emulsion.

9. A Factor VIII-polyethylene glycol conjugate according to Claim 1 or Claim 2 for use in the treatment of a blood clotting disease characterised by a loss of function of FVIII, or for use in the treatment of trauma.

10. A pharmaceutical composition according to any of Claims 3 to 8 for use in treating a blood clotting disease or trauma.

11. A Factor VIII-polyethylene glycol conjugate for use according to Claim 9 or 10, in which the blood clotting disease is haemophilia A.

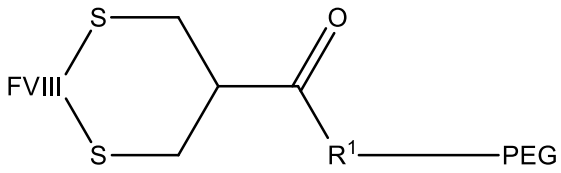
12. A pharmaceutical composition comprising a FVIII conjugate according to any of Claims 3 to 8 for use in reducing the risk of hemarthrosis, hemorrhage, gastrointestinal bleeding and menorrhagia in mammals with haemophilia A or trauma.

13. A pharmaceutical composition for use according to Claim 12, in which the composition is for subcutaneous administration.

14. A pharmaceutical composition for use according to Claim 12, in which the composition is for intravenous administration.

15. A pharmaceutical composition for use according to Claim 12, in which the composition is for administration once every one to fourteen days.

16. A process for preparing the following Factor VIII-polyethylene glycol conjugate,



in which R1 is a substituent which is a direct bond, an alkylene group (preferably a C1-10 alkylene group), or an optionally-substituted aryl or heteroaryl group; in which the aryl groups include phenyl, benzoyl and naphthyl groups; in which suitable heteroaryl groups include pyridine, pyrrole, furan, pyran, imidazole, pyrazole, oxazole, pyridazine, pyrimidine and

purine; in which linkage to the polymer is by a hydrolytically labile bond, or by a nonlabile bond, and in which the process comprises:

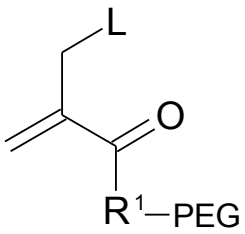
(a) reduction of a native disulphide bond between two cysteine residues in FVIII, to generate two free thiol groups;

(b) a first thiolate addition reaction between a conjugation-reagent comprising a conjugated double bond and a leaving group;

(c) elimination of the leaving group, generating a conjugated double bond; and

(d) a second thiolate addition reaction, forming a 3-carbon bridge between the two sulphur atoms.

17. A process according to Claim 16, in which the conjugation reagent has the formula



where R1 is a substituent which is a direct bond, an alkylene group (preferably a C1-10 alkylene group), or an optionally-substituted aryl or heteroaryl group; in which the aryl groups include phenyl, benzoyl and naphthyl groups; in which suitable heteroaryl groups include pyridine, pyrrole, furan, pyran, imidazole, pyrazole, oxazole, pyridazine, pyrimidine and purine; in which linkage to the polymer is by a hydrolytically labile bond, or by a nonlabile bond, and L is a leaving group.

18. A Factor VIII-polyethylene glycol conjugate substantially as hereinbefore described with reference to and as shown in Figures 1 to 5 of the accompanying drawings.

19. A pharmaceutical composition comprising a Factor VIII-polyethylene glycol conjugate substantially as hereinbefore described with reference to and as shown in Figures 1 to 5 of the accompanying drawings.

20. A Factor VIII-polyethylene glycol conjugate for use in the treatment of a blood clotting disease characterised by a loss of function of FVIII, or for use in the treatment of

trauma substantially as hereinbefore described with reference to and as shown in Figures 1 to 5 of the accompanying drawings.

21. A pharmaceutical composition comprising a Factor VIII-polyethylene glycol conjugate for use in reducing the risk of hemarthrosis, hemorrhage, gastrointestinal bleeding and menorrhagia in mammals with haemophilia A, or trauma substantially as hereinbefore described with reference to and as shown in Figures 1 to 5 of the accompanying drawings.

22. A process for preparing a Factor VIII-polyethylene glycol conjugate substantially as hereinbefore described with reference to and as shown in Figures 1 to 5 of the accompanying drawings.