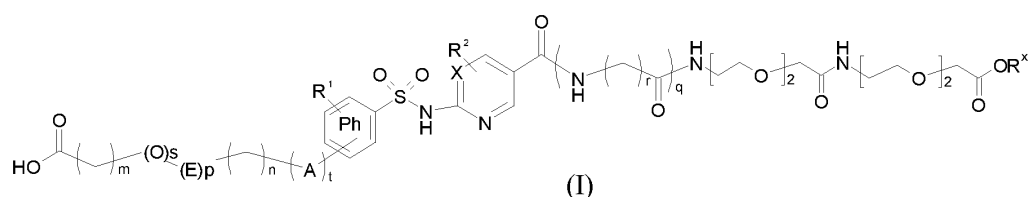


CLAIMS

- 5 1. A method of forming a conjugate of a sulfonamide and a polypeptide, the method comprising:
- a) Providing an activated sulfonamide, wherein the activated sulfonamide corresponds to Formula (I):



10 wherein

A is selected from the group consisting of oxygen atom, -CH₂CH₂- group, -OCH₂- group and -CH₂O- group;

E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;

X represents a nitrogen atom or a -CH- group;

m is an integer in the range from 5 to 17;

n is zero or an integer in the range from 1 to 3;

p is zero or 1;

q is zero or 1:

r is an integer in the range from 1 to 6;

s is zero or 1;

t is zero or 1;

R¹ represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

R² represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

R^x represents an activation group;

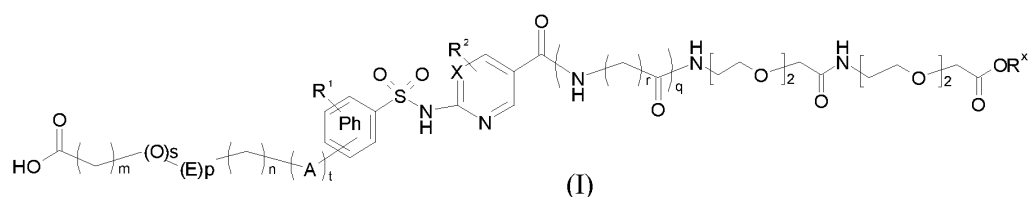
wherein the combination of s being 1, p being zero, n being zero, A being an oxygen atom and t being 1 is excluded for Formula (I);

- b) Providing an aqueous solution of a polypeptide having a free amino group, wherein the aqueous solution optionally comprises an alcohol;
- c) Contacting the aqueous solution of b) with the activated sulfonamide of a); and

- d) Reacting the activated sulfonamide with the polypeptide having a free amino group, obtaining a solution comprising the conjugate of a sulfonamide and a polypeptide, wherein the sulfonamide is covalently bonded to the polypeptide.
- 5 2. The method of claim 1, being a method of forming a conjugate of a sulfonamide and an insulin polypeptide, optionally wherein the activated sulfonamide is an activated albumin binder.
- 10 3. The method of claim 1 or 2, wherein contacting the aqueous solution of b) with the activated sulfonamide of a) according to step c) is done in that the activated sulfonamide of a) is added as a solution of the activated sulfonamide to the aqueous solution of b).
- 15 4. The method of any one of claims 1 to 3, wherein contacting the aqueous solution of b) with the activated sulfonamide of a) according to step c) is done in that the activated sulfonamide of a) is added in solid form to the aqueous solution of b), or at least partially in crystalline form, or at least 90 weight-% in crystalline form; preferably wherein step d) comprises:
- 20 d.1) Reacting the activated sulfonamide with a precursor of the polypeptide having a free amino group at a pH in the range from 9 to 12, or in the range from 9.5 to 11.5 or in the range from 10 to 11, obtaining a pre-conjugate comprising the sulfonamide and the precursor of the polypeptide, wherein the sulfonamide is covalently bonded to the precursor of the polypeptide by an amide bond C(=O)-NH- formed between the -C(=O)-O(R) of the (activated) sulfonamide of Formula (I) and the amino group of the precursor of the polypeptide;
- 25 d.2) Enzymatic digestion optionally at a pH in the range below 9, or at a pH in the range of 7 to 9, of the precursor of the polypeptide of the pre-conjugate obtained according to d.1), obtaining a solution comprising the conjugate of the sulfonamide and the polypeptide.
- 30 5. A conjugate obtained or obtainable from the method of any one of claims 1 to 4.
6. An N-terminally extended insulin A-chain comprising from N- to C-terminus:
- 35 (a) a linker peptide, and
(b) an insulin A-chain,

wherein said N-terminally extended insulin A-chain comprises a cleavage site for trypsin between the last amino acid of the linker peptide and the first amino acid of the A-chain.

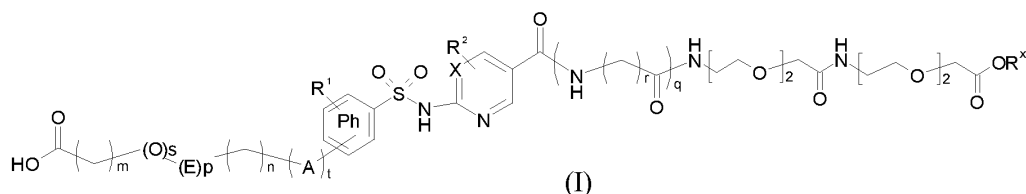
- 5 7. An insulin precursor comprising the N-terminally extended insulin A-chain of claim 6 and an insulin B-chain.
8. A procedure for crystallizing an activated sulfonamide corresponding to Formula (I)



- 10 wherein A, E, X, m, n, p, q, r, s, t, R¹, R² and R^x have the meaning as defined in claim 1, comprising

- A) Providing a solution comprising the activated sulfonamide and an organic solvent;
- B) Removing the organic solvent at least partially, obtaining a phase of the activated sulfonamide having a reduced amount of the organic solvent compared to the solution provided in A);
- 15 C) Adding organic solvent to the phase obtained in B) obtaining a solution of the activated sulfonamide; and
- D) Repeating step B) with the solution obtained in C) obtaining a phase of the activated sulfonamide having a reduced amount of the organic solvent compared to the solution obtained in C);
- 20 E) Optionally repeating steps C) and D) at least one further time.

9. A solid, optionally crystalline, form of the activated sulfonamide corresponding to Formula (I)
- 25



wherein A, E, X, m, n, p, q, r, s, t, R¹, R² and R^x have the meaning as defined in claim 1.

10. A process for generating a conjugate of an albumin binder and a mature insulin, said process comprising
- 30

- 5
- a) Providing a proinsulin comprising from N- to C-terminus an insulin B-chain, a linker peptide and an insulin A-chain,
- b) Cleaving the proinsulin provided in step a) with a first protease between the last amino acid of the insulin B-chain and the first amino acid of the linker peptide, thereby generating an insulin precursor, said insulin precursor comprising the insulin B-chain and an N-terminally extended A-chain comprising the linker peptide and the A-chain,
- 10
- c) contacting said insulin precursor with an albumin binder, wherein the albumin binder comprises a functional group capable of binding to albumin, thereby generating a conjugate of an albumin binder and the insulin precursor,
- d) Cleaving the N-terminally extended A-chain of said insulin precursor comprised by the conjugate with a second protease between the last amino acid of the linker peptide and the first amino acid of the A-chain, thereby generating a conjugate of a sulfonamide and a mature insulin.
- 15
11. The process of claim 10, wherein the last amino acid of the insulin B-chain is a lysine residue.
- 20
12. The process of claim 10 or 11, wherein the linker peptide has a length of at least two amino acid residues, for example wherein the linker peptide has length of 2 to 30 amino acid residues, such as a length of 4 to 9 amino acid residues.
- 25
13. The process of any one of claims 10 to 12, wherein the first amino acid of the linker peptide is a threonine residue, phenylalanine residue, a glutamine residue, a glutamic acid residue, an asparagine residue or an aspartic acid residue and/or wherein the last amino acid of the linker peptide is an arginine residue.
- 30
14. A proinsulin comprising from N- to C-terminus:
- a) an insulin B-chain,
- b) a linker peptide, and
- c) an insulin A-chain,
- wherein said proinsulin comprises a cleavage site for endoproteinase Lys-C between the last amino acid of the insulin B-chain and the first amino acid of the linker peptide and a cleavage site for trypsin between the last amino acid of the linker peptide and the first amino acid of the insulin A-chain.
- 35

15. The process of any one of claims 10 to 13, or the proinsulin of claim 14, wherein the linker peptide comprises the following sequence

Xaa1-Xaa2-Xaa3-Xaa4-Xaa5-Xaa6-Xaa7-Xaa8-Arg (SEQ ID NO: 106)

5

wherein

Xaa1 is any naturally occurring amino acid residue,

Xaa2 is any naturally occurring amino acid residue, or wherein Xaa2 is absent,

10 Xaa3 is any naturally occurring amino acid residue or wherein Xaa3 is absent,

Xaa4 is any naturally occurring amino acid residue, or wherein Xaa4 is absent,

Xaa5 is any naturally occurring amino acid residue, or wherein Xaa5 is absent,

Xaa6 is any naturally occurring amino acid residue, or wherein Xaa6 is absent,

Xaa7 is any naturally occurring amino acid residue, or wherein Xaa7 is absent, and

15 Xaa8 is any naturally occurring amino acid residue, or wherein Xaa8 is absent.

16. The process of any one of claims 10 to 13 and 15, or the proinsulin of claim 14, wherein the linker peptide has the sequence TEGR (SEQ ID NO: 112).