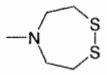
CLAIMS

1. A method of assembling peptide fragments for manufacturing a polypeptide comprising n peptide fragments and at least n-1 amino acids bearing a thiol function, represented by the formula:

$$A_1\text{-}C_1\text{-}A_2\text{-}C_2\text{-}A_3\text{- } \ldots \text{ -}C_{i-1}\text{-}A_i\text{-}\ldots\text{ -}C_{n-1}\text{-}A_n \qquad (I)$$

in which A_1, A_2, A_3, ... A_i ..., A_n are peptide fragments,
C_1, C_2, C_3 ... C_{i-1} ... C_{n-1} are amino acid residues bearing a thiol functional group,
n is comprised between 3 and 50,
and
i is any integer between 2 and n, said method involving:

(a1) at least one step of preparing a fragment Y-A_1-SEAoff (II_1) in which A_1 represents a peptide fragment the C-terminal of which bears a cyclic bis(2-sulphanylethyl)amino group



called SEAoff, and

Y is a fragment capable of reacting with a functional group of a solid support so as to form a bond between A_1 and a solid support,

(b) at least one step of reaction of Y-A_1-SEAoff (II_1) with a solid support designated □-Y', □ representing the solid support itself and Y' representing a reactive functional group capable of reacting with Y to form a group Z according to the diagram:

□-Y' + Y-A_1-SEAoff ⟶ □-Z-A_1-SEAoff

(II_1) (III_1)

(a2) at least one step of preparing a fragment H-C_1(PG_1)-A_2-SEAoff (II_2) in which C_1, A_2 and SEAoff are as defined above and (PG_1) represents H or a protective group of the thiol of the amino acid C_1,

(c1) at least one step of preparing a thioester peptide of formula (III'_1) starting from bis(2-sulphanylethyl)amino peptide □-Z-A_1-SEAoff (III_1) according to the diagram:

□-Z-A_1-SEAoff ⟶ □-Z-A_1-SR

(III_1) (III'_1)

by the action of a thiol R-SH, in the presence of a reducing agent of the cyclic disulphides, where R is an alkyl or aryl radical,

(d1) at least one step of condensation of (III'_1) with the peptide fragment (II_2) in the presence of an aromatic thiol ArSH under conditions in which PG_1 is eliminated when it is different from H:

$$\square\text{-Z-}A_1\text{-SR} + \text{H-}C_1(PG_1)\text{-}A_2\text{-SEAoff} \longrightarrow \square\text{-Z-}A_1\text{-}C_1\text{-}A_2\text{-SEAoff}$$

(III'_1) (II_2) (III_2)

(an-1) at least one step of preparing a fragment $C_{n-1}(PG_{n-1})\text{-}A_n$ where (PG_{n-1}) represents H or a protective group of the thiol of the amino acid C_{n-1},

(dn-1) at least one step of condensation of $C_{n-1}(PG_{n-1})\text{-}A_n$ with

$\square$-Z-A_1- C_1-A_2-...$C_{i-1}A_i$-...$C_{n-2}A_{n-1}$SEAoff (III_{n-1})

in the presence of an aromatic thiol ArSH under conditions in which PG_{n-1} is eliminated when it is different from H in order to give:

$\square$-Z-A_1- C_1-A_2-...$C_{i-1}A_i$-...$C_{n-1}A_n$ (IV_n)

cleaving of the bond between the solid support and the peptide (I) under conditions allowing the peptide (I) to be released

said method further comprising, for every i = 2,...n-2,

(ci) at least one step of conversion of

$\square$-Z-A_1-C_1- ...$C_{i-1}A_i$SEAoff (III_i)

to

$\square$-Z-A_1- C_1- ...C_{i-1}-A_iSR (III'_i)

by the action of a thiol R-SH, optionally in the presence of a reducing agent of cyclic disulphides,

(di) at least one step of condensation of $C_i(PG_i)A_{i+1}$SEAoff where (PG_i) represents H or a protective group of the thiol of the amino acid C_i, in the presence of an aromatic thiol ArSH under conditions in which PG_i, when it is different from H, is eliminated, with

$\square$-Z-A_1- C_1- ...$C_{i-1}A_i$-SR (III'_i)

in order to give

$\square$-Z-A_1-C_1- ...C_iA_{i+1}SEAoff (III_{i+1}), wherein steps (ci) and (di) are carried out before step dn-1).

~~2. The method according to claim 1, which further comprises:~~

~~(e) a step of detaching the peptide A_1-C_1-A_2-...$C_{i-1}A_i$-...$C_{n-1}A_n$ (I) from the solid support.~~

32. The method according to claim 1 ~~or claim 2~~, in which the solid support □ is selected from resins based on polystyrene, polyacrylamide, polyethylene glycol, cellulose, polyethylene, polyester, latex, polyamide, polydimethylacrylamide, synthetic or natural hydrophilic polymers, glass beads, silica gels.

43. The method according to ~~any one of~~ claims 1 ~~to 3~~or 2, in which C_1,...C_i...C_n are cysteines.

54. The method according to any one of claims 1 to 43, in which PG_1,... PG_i... PG_n are tert-butyl sulphenyl groups.

65. The method according to any one of claims 1 to 54, in which:

Y' comprises a function selected from an azide and Y is selected from the groups comprising an alkyne functional group, or

Y' comprises an alkyne functional group and Y is selected from the groups comprising an azide functional group or

Y' comprises an aldehyde functional group, Y is H and the N-terminal amino acid of A_1 is selected from a cysteine, a serine or a threonine, or

Y' comprises an aldehyde functional group, Y comprises an NH_2 group capable of forming a Schiff base.

76. The method according to any one of claims 1 to 65, in which R is selected from an alkyl radical comprising 1 to 12 carbon atoms, linear or branched, or C6-C12 aryl.

~~8. The method according to any one of claims 1 to 7, in which, for every i = 2,...n-2, the method comprises~~

~~(ci) at least one step of conversion of~~

~~□-Z-A_1-C_1-...$C_{i-1}A_i$SEAoff (III$_i$)~~

~~to~~

~~□-Z-A_1-C_1-...C_{i-1}-A_iSR (III'$_i$)~~

~~by the action of a thiol R-SH, optionally in the presence of a reducing agent of cyclic disulphides,~~

~~(di) at least one step of condensation of C_i(PG_i)A_{i+1}SEAoff where (PG_i) represents H or a protective group of the thiol of the amino acid C_i, with~~

~~□-Z-A_1-C_1-...$C_{i-1}A_i$-SR (III'$_i$)~~

~~in order to give~~

~~⊟-Z-A_1-C_1-....C_iA_{i+1}SEAoff (III_{i+1}), wherein steps (ci) and (di) are carried out before step dn-1).~~

~~97~~97. The method according to claim ~~81~~1, in which, for every i = 2,...n-2, the steps di) of the method are carried out under conditions in which PG_i is removed selectively in situ, without affecting SEAoff and the solvent of the reaction is an aqueous buffer, with pH between 4 and 9, containing an aromatic thiol.

~~108~~8. Method of manufacturing a medicinal product comprising at least:

- manufacturing at least one polypeptide by the method according to any one of claims 1 to ~~97~~7, and

- combining it with a pharmaceutically acceptable support.