Claims

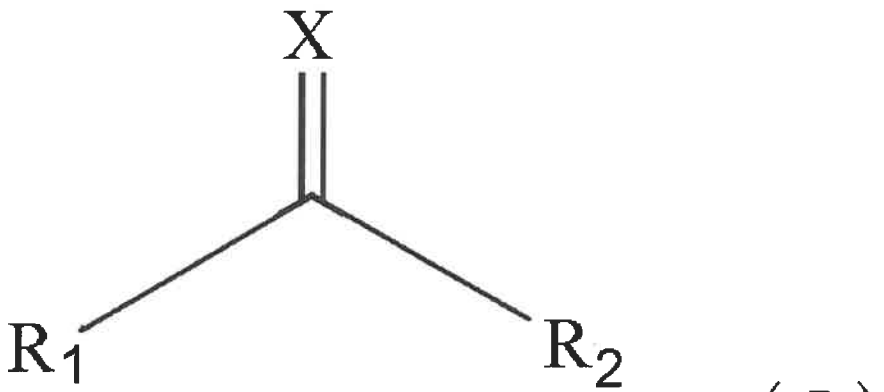
1. A method for efficiently manufacturing a first polypeptide fragment having cysteine at the N-terminal and a second polypeptide fragment having the C-terminal modified which are suitable for the NCL method, comprising:

(1) A step of reacting a polypeptide having the following structure:
(N-terminal) second polypeptide fragment-Cys-W-(His)n-Z-Met-first polypeptide fragment (C-terminal)
[wherein n means an integer from 0 - 10, Cys is cysteine, W means any 1, 2, or 3 amino acids, Z means any 0, 1, or 2 amino acids, His means histidine, Met means methionine, and the N-terminal of the first polypeptide fragment is cysteine]
with CNBr to obtain the following polypeptide fragments:

(A) a first polypeptide fragment having cysteine at the N-terminal and

(B) a third polypeptide fragment having the following structure:
(N-terminal) second polypeptide fragment-Cys-W-(His)n-Z-Met' (C-terminal)
[wherein Met' means a Met derivative];

(2) A step of reacting said third polypeptide fragment with a compound represented by the following formula (I):
[Chemical Formula 1]

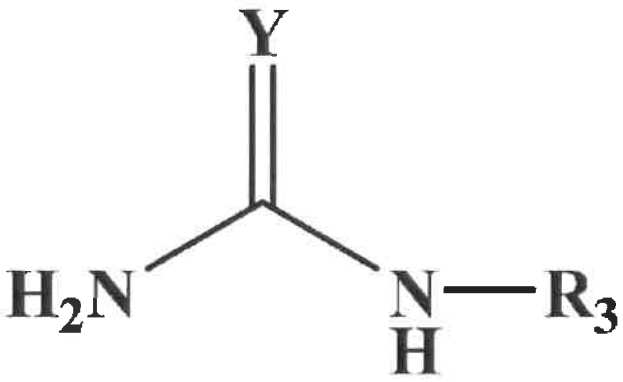



( I )

[wherein

X is a sulfur atom or an oxygen atom, and

$R_1$ and $R_2$ are leaving groups]
and subsequently, in an organic solvent, reacting with a compound represented by the following formula (II):
[Chemical Formula 2]



( I I )

[wherein

Y is an oxygen atom, a sulfur atom, or =NH, and

$R_3$ is a hydrogen atom, an acyl group, or an alkoxycarbonyl group]

to obtain a second polypeptide fragment having the C-terminal modified having the following structure:

(N-terminal) second polypeptide fragment-C(=O)-NH-C(=Y)$NHR_3$ (C-terminal).

2. A manufacturing method according to claim 1, further comprising:

a step of reacting the second polypeptide fragment having the C-terminal modified obtained in said step (2) with further a thiol represented by the following formula

$R_4$-SH

[wherein $R_4$ is any one group selected from the group consisting of a substituted or unsubstituted benzyl group, a substituted or unsubstituted aryl group, and a substituted or unsubstituted alkyl group]

and exchanging the -NH-C(=Y)$NHR_3$ group at the C-terminal with the thiol group to obtain a second polypeptide fragment having the C-terminal modified having the following structure:

(N-terminal) second polypeptide fragment-C(=O)-$SR_4$ (C-terminal).

3. A manufacturing method according to claim 1 or 2, characterized in that X in the compound represented by said formula (I) is a sulfur atom.

4. A manufacturing method according to any one of claims 1 to 3, characterized in that $R_1$ in the compound represented by said formula (I) is an -O-$C_6$ aryl group.

5. A manufacturing method according to any one of claims 1 to 4, characterized in that $R_2$ in the compound represented by said formula (I) is a halogen atom or a substituted or unsubstituted -S-$C_{6\text{-}10}$ aryl group.

6. A manufacturing method according to any one of claims 1 to 5, characterized in that Y in the compound represented by said formula (II) is =NH.

7. A manufacturing method according to any one of claims 1 to 6, characterized in that $R_3$ in the compound represented by said formula (II) is an acetyl group.

8. A manufacturing method according to claims 1 to 7, characterized in that said polypeptide having the following structure:
(N-terminal) second polypeptide fragment-Cys-W-(His)n-Z-Met-first polypeptide fragment (C-terminal)
is a recombinant polypeptide fragment expressed by a cell.

9. A manufacturing method according to claim 8, characterized in that said cell is *E. coli*.

10. A manufacturing method according to any one of claims 1 to 9, characterized in that W is one amino acid, and is any one amino acid selected from the group consisting of Val, Ile, Leu, and Trp.

11. A manufacturing method according to any one of claims 1 to 10, characterized in that n is an integer from 6 - 10.

12. A method for manufacturing a glycosylated polypeptide, comprising:

(1) A step of classifying and designing the peptide sequence of the desired glycosylated polypeptide to be manufactured into at least:

· a sugar chain-containing polypeptide fragment consisting of a polypeptide comprising a glycosylated amino acid,

· a second polypeptide fragment present on the N-terminal side of the sugar chain-containing polypeptide fragment and consisting of a polypeptide comprising the N-terminal side of the desired glycosylated peptide,

· a first polypeptide fragment present on the C-terminal side of the sugar chain-containing polypeptide fragment and consisting of a polypeptide comprising the C-terminal side of the desired glycosylated peptide,

· when it may be present, a polypeptide fragment between the sugar chain-containing polypeptide fragment and the second polypeptide fragment, and

· when it may be present, a polypeptide fragment between the sugar chain-containing polypeptide fragment and the first polypeptide fragment,

wherein the N-terminal of the first polypeptide fragment is designed to be a cysteine;

(2) A step of obtaining a polypeptide having said structure by employing an expression vector comprising a nucleotide sequence encoding a polypeptide having the following structure:
(N-terminal) second polypeptide fragment-Cys-W-(His)n-Z-Met-first polypeptide fragment (C-terminal)
[wherein n means an integer from 0 - 10, Cys is cysteine, W means any 1, 2, or 3 amino acids, Z means any 0, 1, or 2 amino acids, His means histidine, and Met means methionine, and the N-terminal of the first polypeptide fragment is cysteine]
and allowing expression from *E. coli*;

(3) A step of reacting the polypeptide obtained in step (2) with CNBr to obtain the following polypeptide fragments:

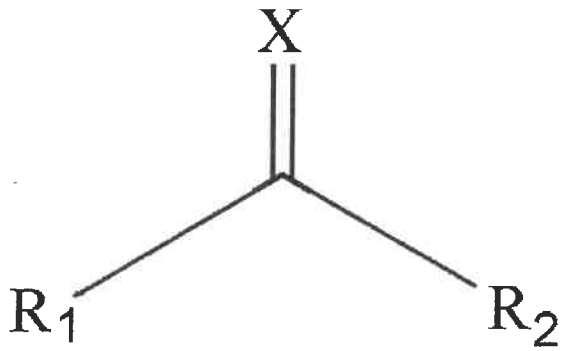
(A) a first polypeptide fragment having cysteine at the N-terminal and

(B) a third polypeptide fragment having the following structure:

(N-terminal) second polypeptide fragment-Cys-W-(His)n-Z-Met' (C-terminal) (wherein Met' means a Met derivative);

(4) A step of reacting said third polypeptide fragment with a compound represented by the following formula (I):

[Chemical Formula 1]

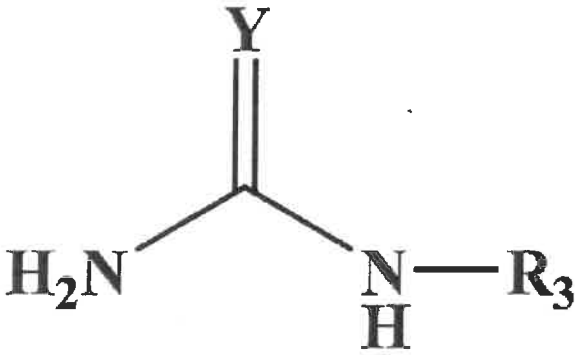


( I )

[wherein X is a sulfur atom or an oxygen atom, and $R_1$ and $R_2$ are leaving groups]

and subsequently, in an organic solvent, reacting with a compound represented by the following formula (II):

[Chemical Formula 2]



( I I )

[wherein

Y is an oxygen atom, a sulfur atom, or a NH group, and

$R_3$ is a hydrogen atom, an acyl group, or an alkoxycarbonyl group]

to obtain a second polypeptide fragment having the C-terminal modified having the following structure:

(N-terminal) second polypeptide fragment-C(=O)-NH-C(=Y)NH$R_3$ (C-terminal);

(5) Arbitrarily, a step of reacting the second polypeptide fragment having the C-terminal modified obtained in said step (4) with further a thiol represented by the following formula

$R_4$-SH

[wherein $R_4$ is any one group selected from the group consisting of a substituted or unsubstituted benzyl group, a substituted or unsubstituted aryl group, and a substituted or unsubstituted alkyl group]

and exchanging the -NH-C(=Y)NHR$_3$ group at the C-terminal with the thiol group to obtain a second polypeptide fragment having the C-terminal modified having the following structure:
(N-terminal) second polypeptide fragment-C(=O)-SR$_4$ (C-terminal); and

(6) A step of binding:

· said sugar chain-containing polypeptide fragment,

· when it may be present, said polypeptide fragment between the sugar chain-containing polypeptide fragment and the second polypeptide fragment, and

· when it may be present, said polypeptide fragment between the sugar chain-containing polypeptide fragment and the first polypeptide fragment

separately prepared by chemical synthesis with:

the first polypeptide fragment having cysteine at the N-terminal obtained in step (3) and

the second polypeptide fragment having the C-terminal modified obtained in step (4) or (5)

in an order that will give the desired glycosylated polypeptide by ligation.

13. A manufacturing method according to claim 12, characterized in that X in the compound represented by said formula (I) is a sulfur atom.

14. A manufacturing method according to claim 12 or 13, characterized in that R$_1$ in the compound represented by said formula (I) is an -O-C$_6$ aryl group.

15. A manufacturing method according to any one of claims 12 to 14, characterized in that R$_2$ in the compound represented by said formula (I) is a halogen atom or a substituted or unsubstituted -S-C$_{6\text{-}10}$ aryl group.

16. A manufacturing method according to any one of claims 12 to 15, characterized in that Y in the compound represented by said formula (II) is =NH.

17. A manufacturing method according to any one of claims 12 to 16, characterized in that R$_3$ in the compound represented by said formula (II) is an acetyl group.

18. A manufacturing method according to any one of claims 12 to 17, characterized in that W is one amino acid, and is any one amino acid selected from the group consisting of Val, Ile, Leu, and Trp.

19. A manufacturing method according to any one of claims 12 to 18, characterized in that n is an integer from 6 - 10.