

INTERMEDIATE FOR PREPARING GLUCAGON AND GLP-1 DUAL AGONIST AND PREPARATION METHOD THEREFOR

5 CROSS REFERENCE TO RELATED APPLICATION

The application claims priority to Chinese application No. 202311494392.7, filed November 10, 2023, which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

10 The present invention relates to an intermediate or a pharmaceutically acceptable salt thereof for preparing a compound that agonizes both a glucagon (Gcg) receptor and a glucagon-like peptide-1 (GLP-1) receptor, and a preparation method therefor, and specifically relates to an intermediate or a pharmaceutically acceptable salt thereof for preparing an oxyntomodulin/glucagon analog mazdutide, and a preparation method therefor.

BACKGROUND

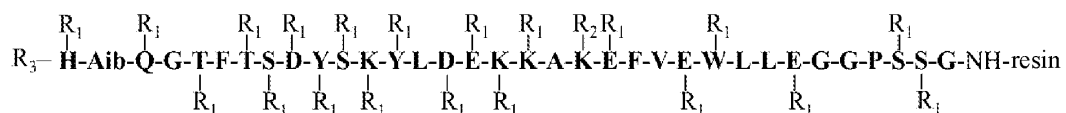
15 Mazdutide is a Gcg and GLP-1 dual agonist peptide that can be used for treating T2D and obesity, and is an oxyntomodulin/glucagon analog, which consists of a main peptide chain containing 34 amino acids and a side chain, wherein 2-aminoisobutyric acid (Aib) at position 2 is a non-natural amino acid, and the C-terminus is an amide; the side chain consists of two 2-(2-(2-aminoethoxy)ethoxy)acetic acid (AEEA) residues, one γ -Glu residue, one eicosanedioic acid residue; the side chain is connected to the Lys20 site of the main peptide chain via two 2-(2-(2-aminoethoxy)ethoxy)acetic acid (AEEA) residues. Methods for preparing such Gcg and GLP-1 dual agonist
20 peptides are described in the prior art.

However, there is still a need for improved methods for preparing Gcg and GLP-1 dual agonist peptides that are more cost-effective, allowing for the generation of peptides with a variety of advantages including commercially desirable purity. At the same time, there is a need for faster and more stable methods, including stable compounds, to provide Gcg and GLP-1 dual agonist peptides with fewer or simpler preparation and/or purification steps. It is well known that there are a number of technical challenges in the preparation of such Gcg and GLP-1 dual agonist peptides that may affect overall yield and purity.

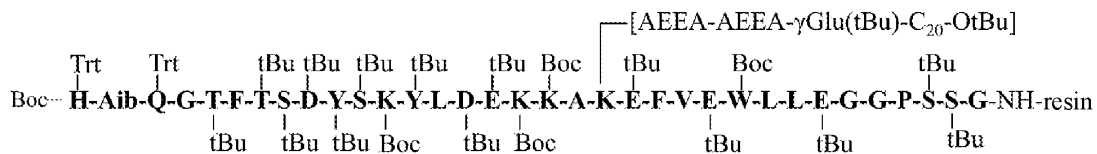
SUMMARY

In the present invention, a coupling of Fmoc-Gly-Gly-OH is used instead of a one-by-one coupling of Fmoc-Gly-OH in solid phase synthesis of peptides, and an OSU activated ester of a side chain synthesized by DIC/HOSu is directly coupled with a peptide chain, such that the reaction period is shortened, and possible racemization and impurities are reduced, resulting in fewer steps, while maintaining and improving quality and purity.

In one aspect, the present invention provides a method for preparing a compound of the following formula (SEQ ID NO: 1):

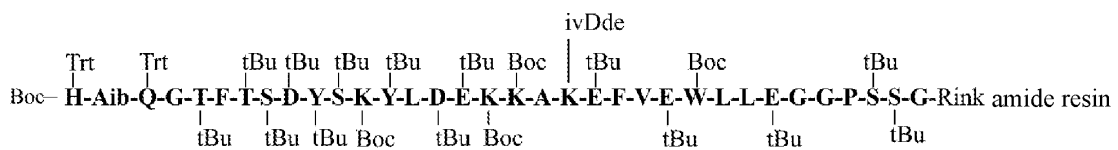


35 the method comprising solid phase synthesis of the compound,
wherein, Fmoc-Gly-Gly-OH is used for the solid phase synthesis.

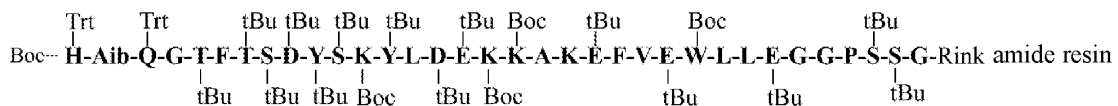


formula (III).

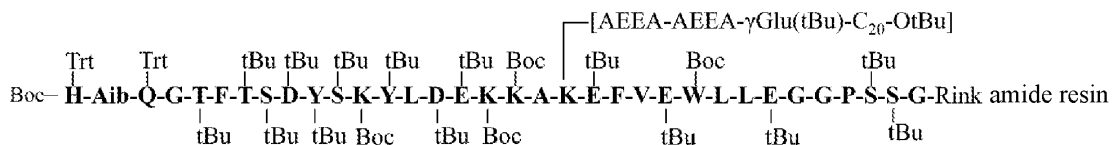
In some embodiments, the compound of formula (I) or the pharmaceutically acceptable salt thereof, the compound of formula (II) or the pharmaceutically acceptable salt thereof, or the compound of formula (III) or the pharmaceutically acceptable salt thereof is selected from the following compounds or pharmaceutically acceptable salts thereof:



(compound 2) (SEQ ID NO: 6),

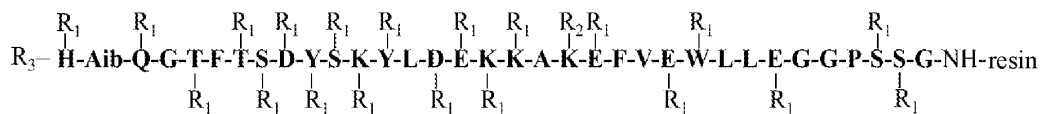


10 (compound 3) (SEQ ID NO: 7),



(compound 4) (SEQ ID NO: 8).

In another aspect, the present application provides a compound of the following formula (SEQ ID NO: 1):



15 made by a method comprising solid phase synthesis of the compound,

wherein, Fmoc-Gly-Gly-OH is used for the solid phase synthesis,

R₁ and R₃ are base-stable side-chain protecting groups, and

R₂ is an ivDde side-chain protecting group.

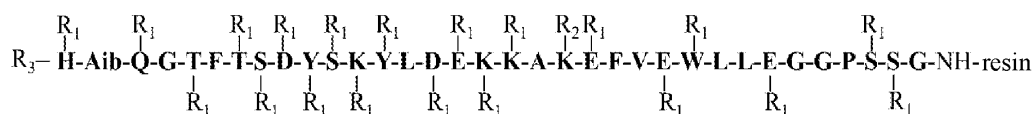
In another aspect, the present application provides a compound of the following formula (SEQ ID NO: 2):

20 **H-Aib-O-G-T-F-T-S-D-Y-S-K-Y-L-D-E-K-K-A-K-E-F-V-E-W-L-L-E-G-G-P-S-S-G-NH₂**

wherein, Lys at position 20 is chemically modified by conjugating an ϵ -amino group of a side chain of Lys at position 20 to ([2-(2-aminoethoxy)-ethoxy]-acetyl)-2-(γ -Glu)-CO-(CH₂)₁₈CO₂H,

made by a method comprising:

(i) solid phase synthesis of a compound of the following formula (SEQ ID NO: 1):



wherein, Fmoc-Gly-Gly-OH is used for the solid phase synthesis,

5 R₁ and R₃ are base-stable side-chain protecting groups, and

R₂ is an ivDde side-chain protecting group;

(ii) selective deprotection of Lys at position 20 and direct coupling of tBuO-C₂₀-γGlu(tBu)-AEEA-AEEA-OSu activated ester obtained via DIC/HOSu to an ε-amino group of a side chain of Lys at position 20;

(iii) cleavage of the compound from a solid support and removal of the base-stable side-chain protecting groups;

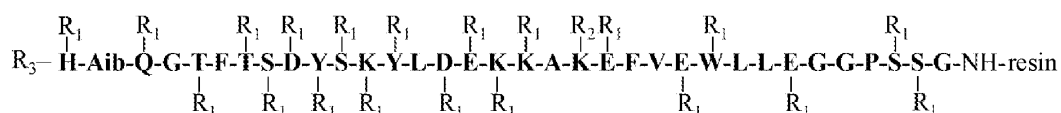
10 and

(iv) purification of the compound.

DETAILED DESCRIPTION

The specific embodiments of the present invention are described in detail below. It should be understood that the specific embodiments described here are merely intended to illustrate and explain the present invention rather than limit the present invention. The endpoints of the ranges and any values disclosed herein are not limited to the precise ranges or values, and these ranges or values should be understood to encompass values close to these ranges or values. For numerical ranges, the endpoint values of each range, the endpoint values of each range and single point values, as well as single point values, can be combined with each other to obtain one or more new numerical ranges, which should be construed as being specifically disclosed herein.

20 In the present invention, provided is a method for preparing a compound of the following formula (SEQ ID NO: 1):



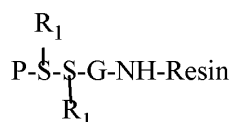
the method comprising solid phase synthesis of the compound,

wherein, Fmoc-Gly-Gly-OH is used for the solid phase synthesis,

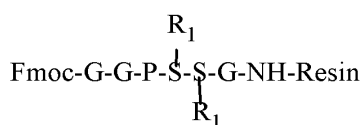
25 R₁ and R₃ are base-stable side-chain protecting groups, and

R₂ is an ivDde side-chain protecting group.

The method comprises a step of coupling Fmoc-Gly-Gly-OH to a solid-phase bound peptide sequence of formula



(A), R_1 . The step of coupling results in a solid-phase bound peptide sequence of formula



(B).

In some embodiments, the solid phase synthesis is performed on an Fmoc amide resin solid support.

In a preferred embodiment, the Fmoc amide resin is a Rink amide resin, which reduces costs.

A variety of base-stable protecting groups are known in the art and can be used in the method of the present invention. In one embodiment of the present invention, the base-stable side-chain protecting group R₁ used for the synthesis of the compound is: (a) *tert*-butoxycarbonyl (Boc) for Trp and Lys; (b) *tert*-butyl ester (OtBu) for Asp and Glu; (c) *tert*-butyl (tBu) for Ser, Thr, and Tyr; (d) triphenylmethyl (trityl, Trt) for Gln; and (e) Trt for His.

In some embodiments, for His, the R₃ protecting group is connected to an amino group of His, and R₃ is Boc.

In a preferred embodiment for the method of the present invention, R₁ is Boc for Trp and Lys; OtBu for Asp and Glu; tBu for Ser, Thr, and Tyr; and Trt for Gln and His.

The solid phase synthesis of the compound is performed on the Fmoc amide resin solid support, wherein the first step is Fmoc deprotection of the amide resin, followed by sequential coupling of Fmoc amino acids of the peptide. In the present invention, a coupling of Fmoc-Gly-Gly-OH is used instead of a one-by-one coupling of Fmoc-Gly-OH for coupling of Gly-Gly at positions 29–30 in a peptide chain, thereby reducing the risk of generating Gly impurities. In addition, a coupling of a glycine-threonine pseudoproline dipeptide is replaced with one-by-one couplings of Gly and Thr at positions 4 and 5, such that the material cost is reduced, and no peptides with corresponding amino acid deletions are found in the crude peptides obtained by the process of the present invention.

Specifically, the solid phase synthesis is performed on the Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following: Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH·H₂O, Fmoc-Gly-Gly-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Lys(ivDde)-OH, Fmoc-Ala-OH·H₂O, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Aib-OH, and Boc-His(Trt)-OH.

In some embodiments, the solid phase synthesis is performed on the Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following Fmoc amino acids: Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH·H₂O, before the step of coupling Fmoc-Gly-Gly-OH. After coupling Fmoc-Gly-Gly-OH, the solid phase synthesis further comprises sequential coupling of the following: Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Lys(ivDde)-OH, Fmoc-Ala-OH·H₂O, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Aib-OH, and Boc-His(Trt)-OH.

In some embodiments, the solid phase synthesis is performed on the Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following: Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH·H₂O, Fmoc-Gly-Gly-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Lys(ivDde)-OH, Fmoc-Ala-OH·H₂O, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Aib-OH, and Boc-His(Trt)-OH.

In some embodiments, the solid phase synthesis is performed on the Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following Fmoc amino acids: Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH·H₂O, before the step of coupling Fmoc-Gly-Gly-OH. After coupling Fmoc-Gly-Gly-OH, the solid phase synthesis further comprises sequential coupling of the following: Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Lys(ivDde)-OH, Fmoc-Ala-OH·H₂O, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Aib-OH, and Boc-His(Trt)-OH.

In a preferred embodiment, naturally occurring amino acids are preferably used in the present application. SPPS contains several basic steps that are repeated as additional amino acids are added to the growing peptide chain to extend the peptide chain. “Solid phase” refers to resin particles (e.g., Rink amide resin) to which the original amino acid — and then the growing peptide chain — is connected. Since the chains are connected to the particles, they can be treated as a set of solid particles (in particular for washing and separation — e.g., filtration — steps).

The repeated steps of SPPS include deprotection, activation, and coupling:

- (1) Deprotection: the last acid on the peptide chain remains “protected” before the beginning of each cycle. As used herein, the term “protected” means that a protecting group is connected to a specified position, that is, an “amino” end of the peptide chain is connected to a functional group that protects the acid from undesired reactions. A variety of protecting groups are well known, and alternative protecting groups may be suitable for a particular method. When the next amino acid is to be added, the “protecting group” is removed (“deprotection” step).
- (2) Activation: a compound (“activating agent”) is added to the reaction to produce an intermediate amino acid species that is more likely to couple with the acid after the deprotection on the peptide chain.
- (3) Coupling: the species after the activation is connected to the existing peptide chain.

The reagent for the deprotection may be one commonly used in the art. In the present invention, a Pip/DMF/NMP solution or a DBU deprotection solution is preferably used, preferably, wherein Pip may be 15 wt%–20 wt%, and DMF and NMP are in a mass ratio of 1:(0.9–1.1). For example, preferably, DMF, Pip, and NMP are in a mass ratio of 2.2:1:2.4.

In a preferred embodiment, the deprotection reaction in the solid phase synthesis of the present invention comprises: performing the deprotection two times in first ten solid phase synthesis cycles, each for 13–17 min

(e.g., 14–16 min, or 15 min); performing the deprotection two times in last 13–33 solid phase synthesis cycles, each for 25–35 min (e.g., 29–31 min, or 30 min). The reduction of the time and frequency of deprotection can shorten the production period and reduce material costs.

In some embodiments, the deprotection can be performed 3 times (each for 15 min) for the nucleotide Val; in addition, the deprotection can be performed 3 times (each for 30 min) for the amino acid Phe at position 22 after Val. In some embodiments, the deprotection can be performed 4 times (each for 30 min) for the amino acids Thr at positions 5 and 7.

One of the most commonly used and studied activation methods for peptide synthesis lies in carbodiimide-based applications. The carbodiimide contains two weakly basic nitrogen atoms that will react with the carboxylic acid of the amino acid derivative to form a highly reactive O-acylisourea compound. In a preferred embodiment, 1-hydroxybenzotriazole (HOBt) or 1-hydroxy-7-azabenzotriazole (HOAt) is incorporated as an additive in the carbodiimide activation method, which significantly improves the carbodiimide activation method. Other additives, e.g., 2-cyano-2-(hydroxyimino)ethyl acetate (Oxyma) or 1-hydroxy-2,5-pyrrolidinedione (NHS), can be used instead of HOBt or HOAt.

In addition, onium salt condensation reagents can be used for the activation, which are highly reactive and fast. For example, 1*H*-benzotriazole-1-yl-oxymethylpyrrolidinyl hexafluorophosphate (PyBOP) or the like can be used. The addition of organic bases such as diisopropylethylamine (DIEA) and *N*-methyldmorpholine (NMM) is required in the use of the reagent, such that the amino acids can be activated. 1-Hydroxybenzotriazole (HOBt) can also be incorporated as an additive in the onium salt condensation reaction.

The activation can also be performed using a phosphate organophosphorus condensation agent, e.g., 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-one (DEPBT). DEPBT is an HOBt-derived phosphate organophosphorus condensation agent, and has the advantages of good solubility, mild reaction conditions, no need of protection of partial amino acid side chains, low racemization rate, and the like. An organic base, e.g., DIEA, can be added to the reaction of the reagent.

For the method of the present invention, a preferred activation system is DIC/HOBt/HOAt/Oxyma in DMF or DMF/NMP; or PyBOP/HOBt in DMF or DMF/NMP; or DEPBT in DMF or DMF/NMP.

In an embodiment of the present invention, the molar amount of the activating agent may be 3 to 7 times greater than that of the resin. Further, the ratio of the molar amount of the activating agent to that of the additive may be about 1. Where the addition of an organic base is required, the amount of the organic base used is preferably the sum of the molar amounts of the activating agent and an optional additive.

In a preferred embodiment, the coupling of the first 11 amino acids (i.e., the first ten solid phase synthesis cycles) is performed using low equivalents (e.g., 2–4 equivalents, preferably, 2, 3, or 4 equivalents) of the Fmoc amino acids relative to the resin; the coupling of the remaining amino acids is performed using high equivalents (e.g., 4–6 equivalents, e.g., 4, 5, or 6 equivalents) of the Fmoc amino acids relative to the resin. In some embodiments, the coupling of His at position 1 and Lys at position 20 can be performed using 3 equivalents of the Fmoc amino acids. In some embodiments, the coupling can be performed at a temperature of 20–30 °C for about 3 h, e.g., 2 h 50 min–3 h 10 min. The reaction time can be adjusted according to the degree of completion of the reaction, but the total reaction time should be ≤ 12 h.

More generally, with respect to the method of the present invention, SPPS construction is preferably accomplished

by standard Fmoc peptide chemistry techniques, using sequential coupling by an automated peptide synthesizer. The preferred resin is a Rink amide resin. DMF/NMP is the preferred solvent system, and DMF is used for the swelling of the resin. A Pip/DMF/NMP solution is preferably used for the deprotection of the resin. A Pip/DMF/NMP solution or a DBU deprotection solution is preferably used for subsequent Fmoc deprotection treatment, in which treatment for 2×15 min is used for the first ten solid phase synthesis cycles, treatment for $2/3 \times 30$ min is preferably used for subsequent couplings of amino acids, and treatment for $2/3 \times 30$ min can also be used for a difficult coupling. After the deprotection, the resin is preferably washed with 7–9 (preferably 8) times the amount of DMF for 8×1 –3 min. The amino acid activation is preferably performed at room temperature (e.g., 20–30 °C) or 0–10 °C for 30 min using an activating agent/DMF/NMP solution, which optionally contains an additive or an inorganic base. The activation is performed for 1–6 min under stirring. For each individual amino acid, a coupling of the activated amino acid to the resin-bound peptide is performed for a specified time. After each coupling, 7–9 (preferably 8) times the amount of DMF washing solvent is preferably used for 8×1 –3 min. For the addition of Val, other washing agents, e.g., DCM and NMP, can also be used, and washing can be performed as many times as necessary.

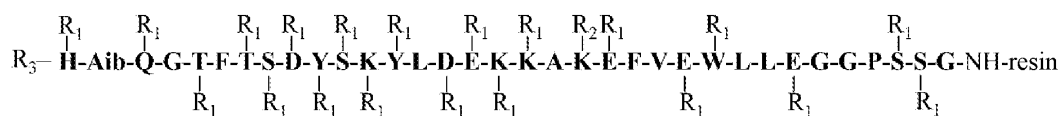
The difficult coupling can be also performed by refeeding, for example, by repeating the steps of activation, coupling, and washing after washing, or by using different activating agents in the refeeding. The solid phase synthesis further optionally comprises an end capping step to block any unreacted amino groups or to acetylate the N-terminus of the intact peptide after the coupling reactions.

In the present invention, provided is a method for preparing a compound of the following formula (SEQ ID NO: 2):

H-Aib-O-G-T-F-T-S-D-Y-S-K-Y-L-D-E-K-K-A-K-E-F-V-E-W-L-L-E-G-G-P-S-S-G-NH₂

wherein, Lys at position 20 is chemically modified by conjugating an ϵ -amino group of a side chain of Lys at position 20 to ([2-(2-aminoethoxy)-ethoxy]-acetyl)-2-(γ -Glu)-CO-(CH₂)₁₈CO₂H,
the method comprising:

(i) solid phase synthesis of a compound of the following formula (SEQ ID NO: 1)



wherein, Fmoc-Gly-Gly-OH is used for the solid phase synthesis,

R₁ and R₃ are base-stable side-chain protecting groups, and

R₂ is an ivDde side-chain protecting group:

(ii) selective deprotection of Lys at position 20 and direct coupling of tBuO-C₂₀-γGlu(tBu)-AEEA-AEEA-OSu activated ester obtained via DIC/HOSu to an ε-amino group of a side chain of Lys at position 20;

(iii) cleavage of the compound from a solid support and removal of the base-stable side-chain protecting groups;
and

(iv) purification of the compound.

In some embodiments, the solid phase synthesis is performed on an Fmoc amide resin solid support.

In a preferred embodiment, the Fmoc amide resin is a Rink amide resin, which reduces costs.

A variety of base-stable protecting groups are known in the art and can be used in the method of the present invention. In one embodiment of the present invention, the base-stable side-chain protecting group R_1 used for the synthesis of the compound is: (a) *tert*-butoxycarbonyl (Boc) for Trp and Lys; (b) *tert*-butyl ester (OtBu) for Asp and Glu; (c) *tert*-butyl (tBu) for Ser, Thr, and Tyr; (d) triphenylmethyl (trityl, Trt) for Gln; and (e) Trt for His.

- 5 In some embodiments, for His, the R_3 protecting group is connected to an amino group of His, and R_3 is Boc. In a preferred embodiment for the method of the present invention, R_1 is Boc for Trp and Lys; OtBu for Asp and Glu; tBu for Ser, Thr, and Tyr; and Trt for Gln and His.

The solid phase synthesis of the compound is performed on the Fmoc amide resin solid support, wherein the first step is Fmoc deprotection of the amide resin, followed by sequential coupling of Fmoc amino acids of the peptide.

- 10 In the present invention, a coupling of Fmoc-Gly-Gly-OH is used instead of a one-by-one coupling of Fmoc-Gly-OH for coupling of Gly-Gly at positions 29–30 in a peptide chain, thereby reducing the risk of generating Gly impurities. In addition, a coupling of a glycine-threonine pseudoproline dipeptide is replaced with one-by-one couplings of Gly and Thr at positions 4 and 5, such that the material cost is reduced, and no peptides with corresponding amino acid deletions are found in the crude peptides obtained by the process of the present invention.

- 15 Specifically, the solid phase synthesis of the compound in step (i) is performed on the Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following: Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH·H₂O, Fmoc-Gly-Gly-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Lys(ivDde)-OH, Fmoc-Ala-OH·H₂O, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Aib-OH, and Boc-His(Trt)-OH.

- 25 In some embodiments, the solid phase synthesis is performed on the Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following Fmoc amino acids: Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH·H₂O, before the step of coupling Fmoc-Gly-Gly-OH. After coupling Fmoc-Gly-Gly-OH, the solid phase synthesis further comprises sequential coupling of the following: Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Lys(ivDde)-OH, Fmoc-Ala-OH·H₂O, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Aib-OH, and Boc-His(Trt)-OH.

- 35 In some embodiments, the solid phase synthesis is performed on the Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following: Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH·H₂O, Fmoc-Gly-Gly-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Lys(ivDde)-OH, Fmoc-Ala-OH·H₂O, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-

L-Glu(OtBu)-OH·H₂O, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Aib-OH, and Boc-His(Trt)-OH.

In some embodiments, the solid phase synthesis is performed on the Fmoc amide resin solid support and comprises

- 5 Fmoc deprotection of the amide resin and sequential coupling of the following Fmoc amino acids: Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH·H₂O, before the step of coupling Fmoc-Gly-Gly-OH. After coupling Fmoc-Gly-Gly-OH, the solid phase synthesis further comprises sequential coupling of the following: Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Lys(ivDde)-OH,
- 10 Fmoc-Ala-OH·H₂O, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Aib-OH, and Boc-His(Trt)-OH.

In a preferred embodiment, naturally occurring amino acids are preferably used in the present application.

- 15 SPPS contains several basic steps that are repeated as additional amino acids are added to the growing peptide chain to extend the peptide chain. “Solid phase” refers to resin particles (e.g., Rink amide resin) to which the original amino acid — and then the growing peptide chain — is connected. Since the chains are connected to the particles, they can be treated as a set of solid particles (in particular for washing and separation — e.g., filtration — steps).

- 20 The repeated steps of SPPS include deprotection, activation, and coupling:

(1) Deprotection: the last acid on the peptide chain remains “protected” before the beginning of each cycle. As used herein, the term “protected” means that a protecting group is connected to a specified position, that is, an “amino” end of the peptide chain is connected to a functional group that protects the acid from undesired reactions.

- 25 A variety of protecting groups are well known, and alternative protecting groups may be suitable for a particular method. When the next amino acid is to be added, the “protecting group” is removed (“deprotection” step).

(2) Activation: a compound (“activating agent”) is added to the reaction to produce an intermediate amino acid species that is more likely to couple with the acid after the deprotection on the peptide chain.

(3) Coupling: the species after the activation is connected to the existing peptide chain.

- 30 The reagent for the deprotection may be one commonly used in the art. In the present invention, a Pip/DMF/NMP solution or a DBU deprotection solution is preferably used, preferably, wherein Pip may be 15 wt%–20 wt%, and DMF and NMP are in a mass ratio of 1:(0.9–1.1). For example, preferably, DMF, Pip, and NMP are in a mass ratio of 2.2:1:2.4.

- In a preferred embodiment, the deprotection reaction in the solid phase synthesis of the present invention comprises: performing the deprotection two times in first ten solid phase synthesis cycles, each for 13–17 min (e.g., 14–16 min, or 15 min); performing the deprotection two times in last 13–33 solid phase synthesis cycles, each for 25–35 min (e.g., 29–31 min, or 30 min). The reduction of the time and frequency of deprotection can shorten the production period and reduce material costs.
- 35

In some embodiments, the deprotection can be performed 3 times (each for 15 min) for the nucleotide Val; in addition, the deprotection can be performed 3 times (each for 30 min) for the amino acid Phe at position 22 after

Val. In some embodiments, the deprotection can be performed 4 times (each for 30 min) for the amino acids Thr at positions 5 and 7.

One of the most commonly used and studied activation methods for peptide synthesis lies in carbodiimide-based applications. The carbodiimide contains two weakly basic nitrogen atoms that will react with the carboxylic acid of the amino acid derivative to form a highly reactive O-acylisourea compound. In a preferred embodiment, 1-hydroxybenzotriazole (HOBt) or 1-hydroxy-7-azabenzotriazole (HOAt) is incorporated as an additive in the carbodiimide activation method, which significantly improves the carbodiimide activation method. Other additives, e.g., 2-cyano-2-(hydroxyimino)ethyl acetate (Oxyma) or 1-hydroxy-2,5-pyrrolidinedione (NHS), can be used instead of HOBt or HOAt.

10 In addition, onium salt condensation reagents can be used for the activation, which are highly reactive and fast. For example, 1*H*-benzotriazole-1-yloxytripyrrolidiny1 hexafluorophosphate (PyBOP) or the like can be used. The addition of organic bases such as diisopropylethylamine (DIEA) and *N*-methylmorpholine (NMM) is required in the use of the reagent, such that the amino acids can be activated. 1-Hydroxybenzotriazole (HOBt) can also be incorporated as an additive in the onium salt condensation reaction.

15 The activation can also be performed using a phosphate organophosphorus condensation agent, e.g., 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-one (DEPBT). DEPBT is an HOBt-derived phosphate organophosphorus condensation agent, and has the advantages of good solubility, mild reaction conditions, no need of protection of partial amino acid side chains, low racemization rate, and the like. An organic base, e.g., DIEA, can be added to the reaction of the reagent.

20 For the method of the present invention, a preferred activation system is DIC/HOBt/HOAt/Oxyma in DMF or DMF/NMP; or PyBOP/HOBt in DMF or DMF/NMP; or DEPBT in DMF or DMF/NMP.

In an embodiment of the present invention, the molar amount of the activating agent may be 3 to 7 times greater than that of the resin. Further, the ratio of the molar amount of the activating agent to that of the additive may be about 1. Where the addition of an organic base is required, the amount of the organic base used is preferably the sum of the molar amounts of the activating agent and an optional additive.

25 In a preferred embodiment, the coupling of the first 11 amino acids (i.e., the first ten solid phase synthesis cycles) is performed using low equivalents (e.g., 2–4 equivalents, preferably, 2, 3, or 4 equivalents) of the Fmoc amino acids relative to the resin; the coupling of the remaining amino acids is performed using high equivalents (e.g., 4–6 equivalents, e.g., 4, 5, or 6 equivalents) of the Fmoc amino acids relative to the resin. In some embodiments, the coupling of His and Lys at position 20 can be performed using 3 equivalents of the Fmoc amino acids.

30 In some embodiments, the coupling can be performed at a temperature of 20–30 °C for about 3 h, e.g., 2 h 50 min–3 h 10 min. The reaction time can be adjusted according to the degree of completion of the reaction, but the total reaction time should be ≤ 12 h.

More generally, with respect to the method of the present invention, SPPS construction is preferably accomplished by standard Fmoc peptide chemistry techniques, using sequential coupling by an automated peptide synthesizer. The preferred resin is a Rink amide resin. DMF/NMP is the preferred solvent system, and DMF is used for the swelling of the resin. A Pip/DMF/NMP solution is preferably used for the deprotection of the resin. A Pip/DMF/NMP solution or a DBU deprotection solution is preferably used for subsequent Fmoc deprotection treatment, in which treatment for 2×15 min is used for the first ten solid phase synthesis cycles, treatment for 2/3

- × 30 min is preferably used for subsequent couplings of amino acids, and treatment for $2/3 \times 30$ min can also be used for a difficult coupling. After the deprotection, the resin is preferably washed with 7–9 (preferably 8) times the amount of DMF for $8 \times 1-3$ min. The amino acid activation is preferably performed at room temperature (e.g., 20–30 °C) or 0–10 °C for 30 min using an activating agent/DMF/NMP solution, which optionally contains an additive or an inorganic base. The activation is performed for 1–6 min under stirring. For each individual amino acid, a coupling of the activated amino acid to the resin-bound peptide is performed for a specified time. After each coupling, 7–9 (preferably 8) times the amount of DMF washing solvent is preferably used for $8 \times 1-3$ min. For the addition of Val, other washing agents, e.g., DCM and NMP, can also be used, and washing can be performed as many times as necessary.
- 10 The difficult coupling can be also performed by refeeding, for example, by repeating the steps of activation, coupling, and washing after washing, or by using different activating agents in the refeeding. The solid phase synthesis further optionally comprises an end capping step to block any unreacted amino groups or to acetylate the N-terminus of the intact peptide after the coupling reactions.
- Preferably, when R_2 is ivDde, Lys at position 20 is selectively deprotected by contacting the compound with a solution containing hydrazine hydrate. Further preferably, the solution contains 1% w/w-15% w/w hydrazine hydrate in DMF, NMP, NBP, or DMSO.
- 15 Still further preferably, the solution contains 8% w/w-9% w/w, for example, about 8.6% w/w hydrazine hydrate in DMF.
- In step (ii), $t\text{BuO-C}_{20}\text{-}\gamma\text{Glu(tBu)-AEEA-AEEA-OSu}$ activated ester obtained via DIC/HOSu is directly coupled to an ϵ -amino group of a side chain of Lys at position 20 of the resin peptide obtained in step (1). Through DIC/HOSu, the side chain is directly bound to the polypeptide, such that the reaction period is shortened, and possible racemization and deletion impurities are reduced. In step (ii), the molar ratio of DIC/HOSu/side chain may be about 1:1:1. HOSu may be slightly more. In step (ii), after the reaction of the side chain with DIC/HOSu, the system is filtered, concentrated, dissolved with DCM, and filtered, then DIEA and DMF are added, and a coupling is performed at room temperature for about 12 h. After the coupling, the system is washed 6 times with 8- to 10-fold amount of NMP; then 8- to 10-fold (e.g., 8-fold or 9-fold) amount of MTBE is added, and the system is stirred for 1–3 min and filtered (this operation is performed 2 times); 8- to 10-fold (e.g., 8-fold or 9-fold) amount of DCM is added, and the system is stirred for 1–3 min and filtered (this operation is performed 2 times); 8- to 10-fold (e.g., 8-fold or 9-fold) amount of MTBE is added, and the system is stirred for 1–3 min and filtered (this operation is performed 2 times); then drying is performed.
- 25 30 For the next step, after ivDde is removed, the system is filtered and washed sequentially with 8-fold amounts of DMF (10 times) and NMP (2 times), each for 1–3 min, and the obtained product is soaked in NMP.
- In step (iii), it is preferred that the mixture with the resin is used directly in the next step after cleaving the compound from the solid support, due to the poor stability of the crude peptide in the cleavage system. That is, after the reaction is completed, the system is directly added to MTBE for crystallization without filtering the resin, and the resin is dissolved and filtered out in subsequent treatment.
- 35 Specifically, in step (iii), the peptide is cleaved from the resin with an acidic mixture preferably consisting of trifluoroacetic acid, triisopropylsilane, dithiothreitol, and water. For example, the cleavage system can be $\text{TFA:H}_2\text{O:TIPS:DTT} = 92.5:4.4:3.1:3$ (V/V; DTT w/v).

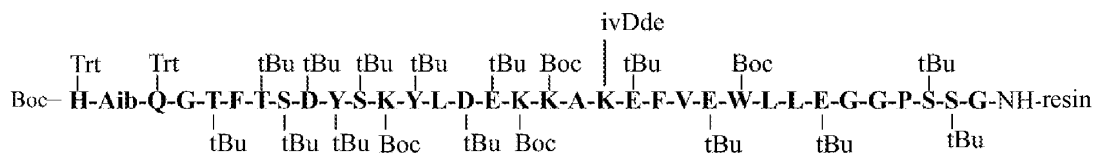
After step (iii), the system after the cleavage is directly added to MTBE for crystallization, filtered, dried and ground, slurried with MTBE, and then dried.

In an embodiment of the method of the present invention, the purification of the compound comprises subjecting a crude solution of the compound of step (iii) to chromatographic purification.

- 5 Preferably, the chromatographic purification is performed by HPLC or reverse-phase HPLC.

Still further preferably, the purification further comprises the following steps: (a) obtaining a product by elution with a large excess of ammonium acetate/acetonitrile (ACN)/water mobile phase, performing salt transfer, and removing trifluoroacetic acid (TFA); (b) performing concentration and removing a solvent; and (c) performing lyophilization.

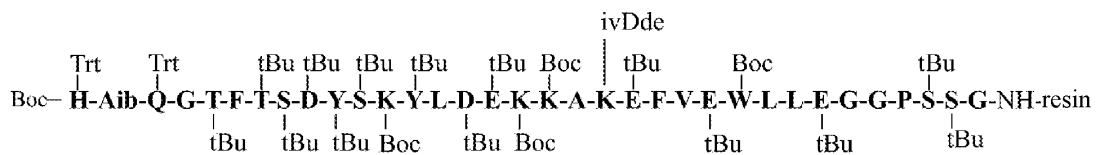
- 10 The present invention provides a method for preparing a compound of the following formula (I) (SEQ ID NO: 3) or a pharmaceutically acceptable salt thereof,



formula (I)

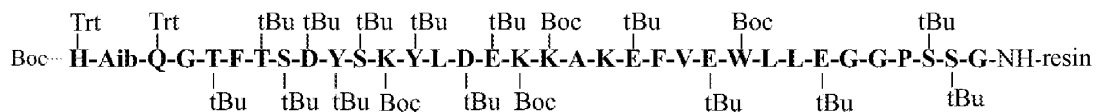
the method comprising:

- 15 (i) solid phase synthesis of a compound of the following formula:



wherein, Fmoc-Gly-Gly-OH is used for the solid phase synthesis.

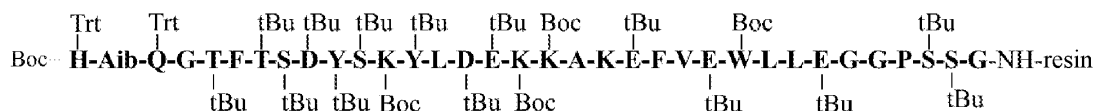
The present invention provides a method for preparing a compound of the following formula (II) (SEQ ID NO: 4) or a pharmaceutically acceptable salt thereof,



formula (II)

the method comprising:

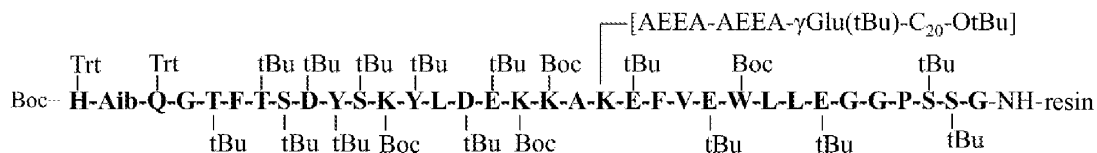
- (i) solid phase synthesis of a compound of the following formula:



- 25 wherein, Fmoc-Gly-Gly-OH is used for the solid phase synthesis; and

(ii) selective deprotection of Lys at position 20.

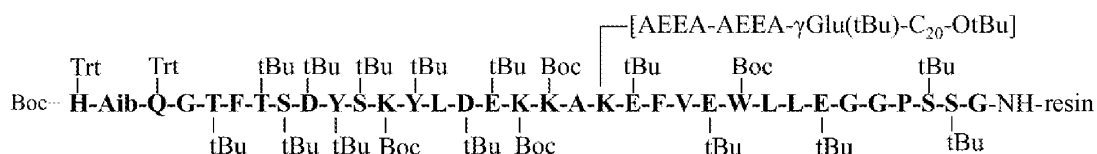
The present invention provides a method for preparing a compound of the following formula (III) (SEQ ID NO: 5) or a pharmaceutically acceptable salt thereof.



5 formula (III)

the method comprising:

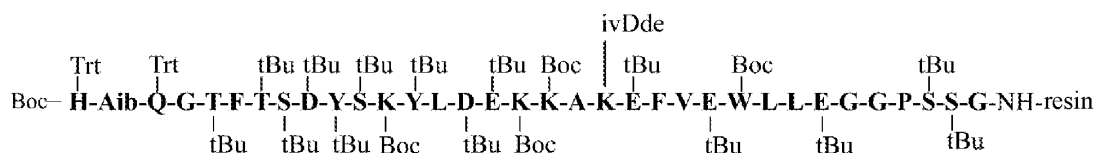
(i) solid phase synthesis of a compound of the following formula:



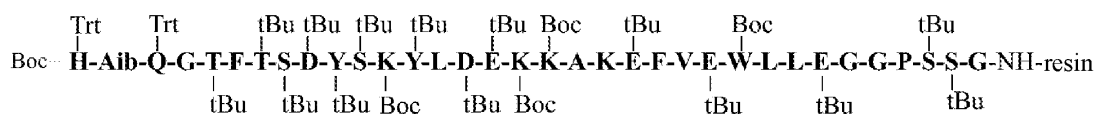
wherein, Fmoc-Gly-Gly-OH is used for the solid phase synthesis; and

10 (ii) selective deprotection of Lys at position 20 and direct coupling of tBuO-C₂₀-γGlu(tBu)-AEEA-AEEA-OSu activated ester obtained via DIC/HOSu to an ε-amino group of a side chain of Lys at position 20.

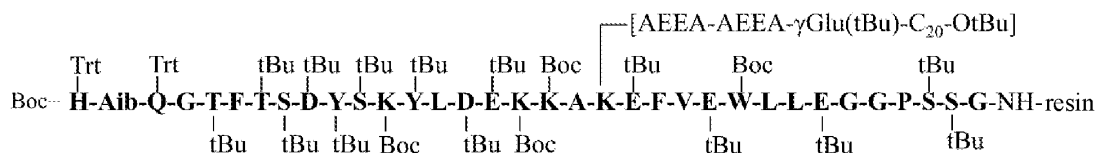
In another aspect, the present application provides a compound of the following formula (I) (SEQ ID NO: 3) or a pharmaceutically acceptable salt thereof, a compound of the following formula (II) (SEQ ID NO: 4) or a pharmaceutically acceptable salt thereof, or a compound of the following formula (III) (SEQ ID NO: 5) or a pharmaceutically acceptable salt thereof:



formula (I)

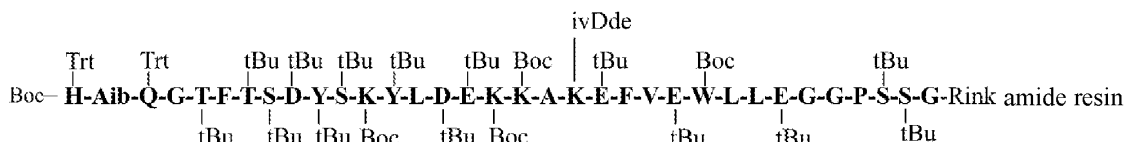


formula (II)

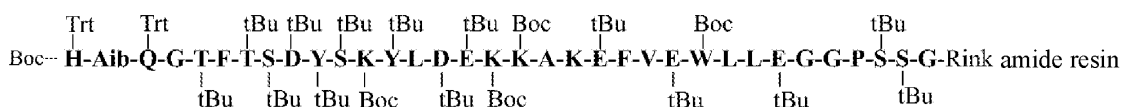


formula (III).

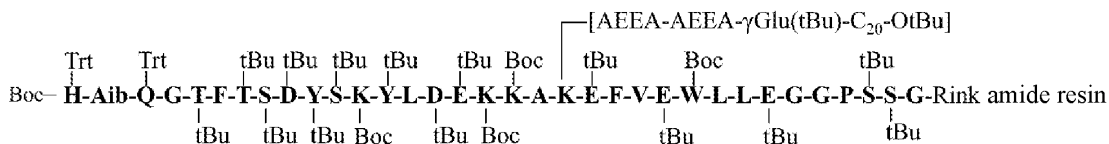
In some embodiments, the compound of formula (I) or the pharmaceutically acceptable salt thereof, the compound of formula (II) or the pharmaceutically acceptable salt thereof, or the compound of formula (III) or the pharmaceutically acceptable salt thereof is selected from the following compounds or pharmaceutically acceptable salts thereof:



(compound 2) (SEQ ID NO: 6),



(compound 3) (SEQ ID NO: 7),



10

(compound 4) (SEQ ID NO: 8).

Definitions

The following abbreviations used herein have the meanings described herein: “SPPS” stands for solid phase peptide synthesis, “Fmoc” stands for fluorenylmethoxycarbonyl chloride, “Boc” stands for *tert*-butoxycarbonyl, “OtBu” stands for *tert*-butyl ester, “tBu” stands for *tert*-butyl, “Trt” stands for triphenylmethyl or trityl, NMP stands for *N*-methylpyrrolidone, “ivDde” stands for 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl, “Pip” stands for piperidine, “DIC” stands for *N,N*-diisopropylcarbodiimide, “Oxyma” stands for ethyl 2-oxime cyanoacetate, “DCM” stands for dichloromethane, DEPBT stands for 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-one, DMF stands for *N,N*-dimethylformamide, “TFA” stands for trifluoroacetic acid, “TIPS” stands for triisopropylsilane, “DTT” stands for dithiothreitol, “AEEA” stands for 17-amino-10-oxo-3,6,12,15-tetraoxa-9-azaheptadecanoic acid, “HOBt” stands for 1-hydroxybenzotriazole, and “PyBOP” stands for 1*H*-benzotriazol-1-yloxytripyrrolidiny hexafluorophosphate. “tBuO-C₂₀-γ-Glu(tBu)-AEEA-AEEA-OH” stands for (*S*)-22-(*tert*-butoxycarbonyl)-45,45-dimethyl-10,19,24,43-tetraoxo-3,6,12,15,44-pentaoxa-9,18,23-triaza-tetracetic acid, “AEEA” stands for (8-amino-3,6-dioxaoctanoic acid), HOAt stands for 1-hydroxy-7-azabenzotriazole, and DIEA stands for *N,N*-diisopropylethylamine.

As shown herein, one-letter amino acid abbreviations are shown in bold text, while atoms are shown in non-bold text, and are typically smaller in font to distinguish them from one-letter amino acid abbreviations. As used herein, an amino acid when abbreviated is accompanied by a number above the amino acid that refers to the corresponding

position of the amino acid in the final mazdutide product. These numbers are provided for convenience, and the presence or absence of these numbers in the sequence does not affect the amino acid sequence or the peptide indicated in such a sequence. As used herein, the term “protected” means that a protecting group is attached at the indicated position. Those skilled in the art will recognize that a variety of protecting groups are well known, and alternative protecting groups may be suitable for a particular method.

The amino acid sequences of the present invention comprise the standard one-letter or three-letter codes for the 20 naturally occurring amino acids. Further, “Aib” is α -aminoisobutyric acid.

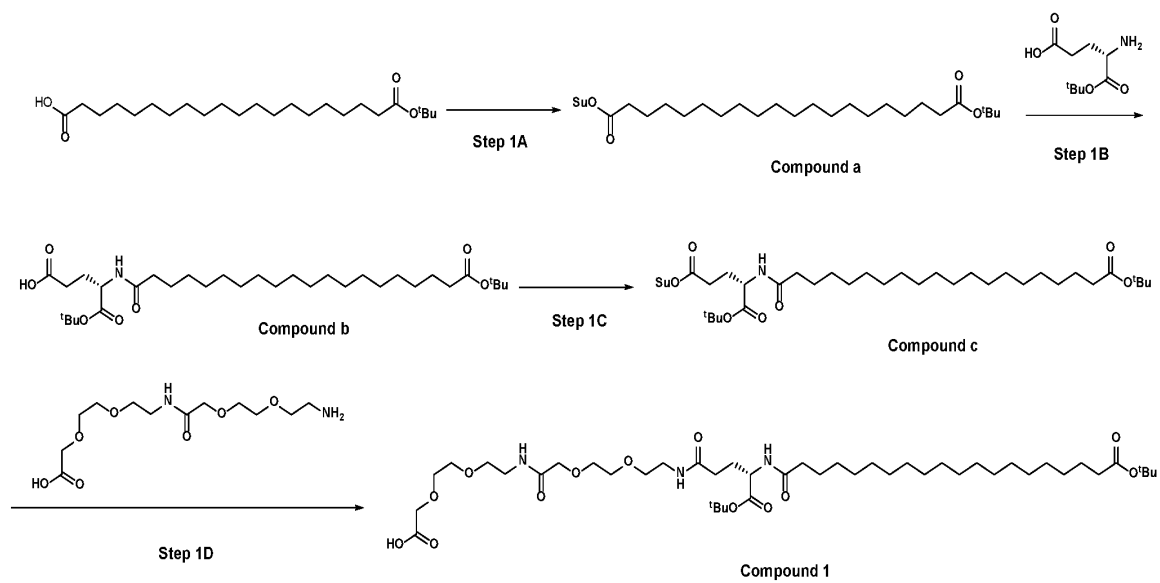
The present invention relates to novel compounds and methods that can be used for the synthesis of the compounds or the pharmaceutically acceptable salts (particularly acetates) thereof disclosed herein. The novel methods and compounds are exemplified in the examples below. Reagents and starting materials are readily available to those of ordinary skill in the art. It will be understood that these examples are not intended to limit the scope of the present invention in any way.

Examples

All reagents used in the present application are commercially available and can be used without further purification.

Example 1. Synthesis of Compound

Synthetic route for side chain



Step 1A: Synthesis of compound a

($8.10 \times W_{1A}$) kg of acetonitrile was added to a 200 L enamel reaction kettle, and the temperature of the system was adjusted to 15–30 °C. W_{1A} kg of mono(1,1-dimethylethyl) docosanediate and ($0.03 \times W_{1A}$) kg of 4-dimethylaminopyridine were added under stirring, and ($0.77 \times W_{1A}$) kg of *N,N*-disuccinimidyl carbonate was added in three batches at intervals of 5–10 min. The funnel was rinsed with ($(0.20-0.40) \times W_{1A}$) kg of acetonitrile. The system in the reaction kettle was heated to 35–45 °C (target temperature: 40 °C), and reacted for at least 3 h with the temperature maintained.

The system in the reaction kettle was cooled to -5–5 °C, stirred for 2–3 h, and then filtered. The reaction kettle was rinsed sequentially with ($1.54 \times W_{1A}$) kg of acetonitrile, ($0.80 \times W_{1A}$) kg of acetonitrile/($0.26 \times W_{1A}$) kg of purified water, and ($1.00 \times W_{1A}$) kg of acetonitrile, cooled to -5–5 °C, and pre-cooled for at least 1 h. The filter cake was soaked in and washed with the rinsing solution for 5–10 min, and then filtered with the temperature controlled to be ≤ 5 °C. The filter cake was dried at 15–30 °C to provide compound a, which was then sealed and stored at 10–30 °C.

W_{1A} was 3.39 kg.

Step 1B: Synthesis of compound b

($7.86 \times W_{1B}$) kg of acetonitrile was added to a 100 L enamel reaction kettle, and the temperature of the system was adjusted to 15–30 °C. W_{1B} kg of compound a and ($0.48 \times W_{1B}$) kg of H-Glu-OtBu were added. The reaction kettle was rinsed with ($(1.00-2.00) \times W_{1B}$) kg of acetonitrile. ($0.31 \times W_{1B}$) kg of *N,N*-diisopropylethylamine was added to the reaction kettle with the temperature controlled to be 15–30 °C. The system in the reaction kettle system was heated to 35–45 °C (target temperature: 40 °C), and reacted for at least 8 h with the temperature maintained.

($0.150 \times W_{1B}$) of acetic acid and ($4.00 \times W_{1B}$) kg of purified water were added to the reaction kettle with the temperature controlled to be 35–45 °C. The system in the reaction kettle was cooled to 10–15 °C with the temperature maintained for 1–2 h, and then heated to 20–25 °C with the temperature maintained for 1–2 h. The system was cooled to -5–5 °C with the temperature maintained for 1–3 h, and then filtered with the temperature controlled to be -5–5 °C. The reaction kettle was rinsed sequentially with ($1.65 \times W_{1B}$) kg of acetonitrile/($0.90 \times W_{1B}$) kg of purified water and ($1.57 \times W_{1B}$) kg of acetonitrile, cooled to -5–5 °C, and pre-cooled for at least 0.5 h. The filter cake was soaked in and washed with the rinsing solution for 5–10 min, and then filtered (the temperature was controlled to be ≤ 5 °C). The filter cake was dried at 15–30 °C to provide compound b, which was then sealed and stored at 10–30 °C.

W_{1B} was 3.85 kg.

Step 1C: Synthesis of compound c

($8.86 \times W_{1C}$) kg of acetonitrile was added to a 100 L enamel reaction kettle, the system was stirred, and the temperature of the system was adjusted to 15–30 °C. W_{1C} kg of compound b and ($0.0200 \times W_{1C}$) kg of 4-dimethylamino pyridine were added under stirring, and ($0.53 \times W_{1C}$) kg of *N,N*-disuccinimidyl carbonate was added in three batches at intervals of 5–10 min. The funnel and the kettle wall were rinsed with ($(1.02-2.04) \times W_{1C}$) kg of acetonitrile. The system in the reaction kettle was heated to 15–25 °C (target temperature: 20 °C), and reacted for at least 3 h with the temperature maintained.

The system in the reaction kettle was cooled to -5–5 °C, and stirred for at least 2 h. The system was filtered with the temperature controlled to be -5–5 °C. The reaction kettle was rinsed with ($4.40 \times W_{1C}$) kg of acetonitrile, cooled

to -5–5 °C, and pre-cooled for at least 0.5 h. The filter cake was soaked in and washed with the rinsing solution four times, each for 5–10 min, and then filtered (the temperature was controlled to be ≤ 5 °C). The filter cake was dried at 15–30 °C to obtain compound c, which was then sealed and stored at 10–30 °C.

W_{IC} was 4.23 kg.

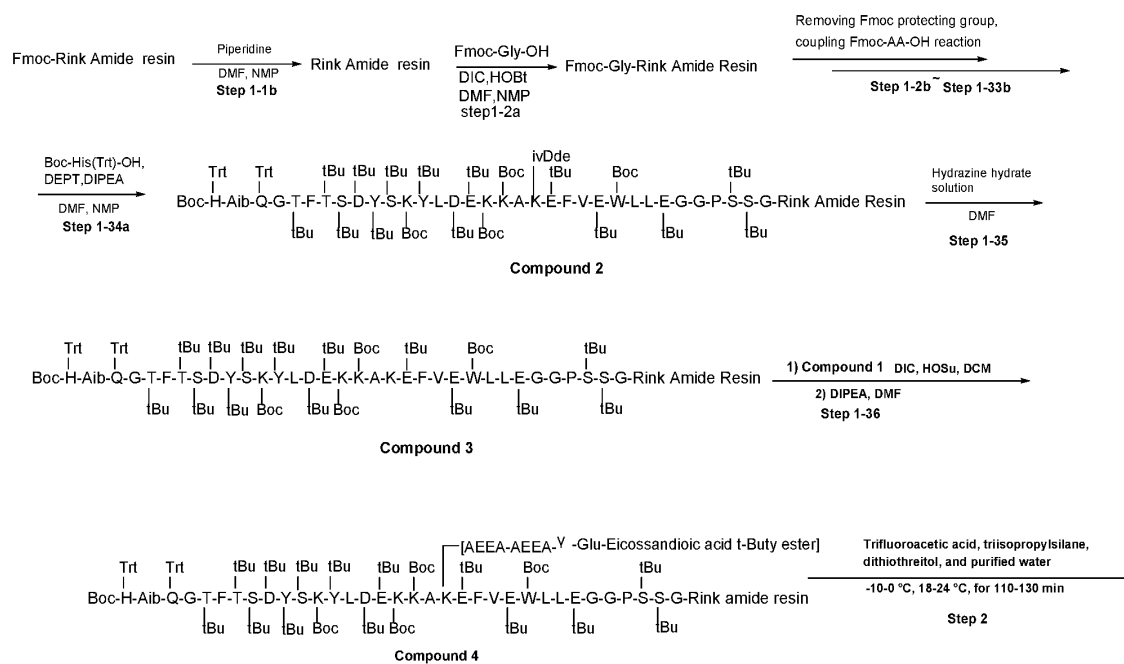
5 Step 1D: Synthesis of compound 1

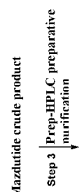
Solution preparation: A solution was prepared with $(13.50 \times W_{ID})$ kg of purified water and $(1.50 \times W_{ID})$ kg of sodium chloride and denoted as N1. A solution was prepared with $(17.60 \times W_{ID})$ kg of purified water, $(1.86 \times W_{ID})$ kg of potassium bisulfate, and $(2.00 \times W_{ID})$ kg of sodium chloride solution, the preparation was repeated two times, and the solution was denoted as N2. A solution was prepared with $(16.00 \times W_{ID})$ kg of purified water, 10 $(1.60 \times W_{ID})$ kg of potassium bisulfate, and $(1.60 \times W_{ID})$ kg of sodium chloride solution and denoted as N3. $(40.00 \times W_{ID})$ kg of dichloromethane was added to a 500 L enamel reaction kettle 2, and the system was stirred. $(0.60 \times W_{ID})$ kg of *N,N*-diisopropylethylamine and $(0.50 \times W_{ID})$ kg of 17-amino-10-oxo-3,6,12,15-tetraoxa-9-azaheptadecanoic acid (purchased from Chengdu Pukang Biotechnology Technology Co., Ltd.) were added. The temperature of the system was adjusted to 15–25 °C, and the system was stirred for 2–4 h. W_{ID} kg of compound c 15 was added to kettle 2 with the temperature controlled, and the funnel and the kettle wall were rinsed with $(3.00 \times W_{ID})$ kg of dichloromethane. The system was reacted at 15–25 °C for 8 h with the temperature maintained. The system in kettle 2 was concentrated at the temperature T of ≤ 30 °C and the reference P of ≤ -0.06 MPa until the residual volume was 5–10 vol. $(10.00 \times W_{ID})$ kg of ethyl acetate was added to kettle 2, and the system was still concentrated until the residual volume was 5–10 vol. (this operation was repeated 3 times). 20 $(10.00 \times W_{ID})$ kg of ethyl acetate was added to kettle 2, and $(9.50 \times W_{ID})$ kg of N3 solution and $(5.00 \times W_{ID})$ kg of N2 solution were added to kettle 2 at the temperature $T \leq 30$ °C. The system was stirred for 15–20 min, and then left to stand for liquid separation. With the temperature controlled to be 15–30 °C, the N2 solution was added to the organic phase for washing 5 times, each time with $(5.00 \times W_{ID})$ kg, and stirring was performed for 15–20 min during each washing. Then the system was left to stand for liquid separation. With the temperature controlled 25 to be 15–30 °C, the N1 solution was added to kettle 2 for washing 2 times, each time with $(5.00 \times W_{ID})$ kg, and stirring was performed for 5–10 min during each washing. Then the system was left to stand for liquid separation. The organic phase was transferred to a 3000 L enamel reaction kettle 1, kettle 2 was rinsed with $((1.00-2.00) \times W_{ID})$ kg of preparative grade acetonitrile, and the rinsing solutions were combined and added to kettle 1. The system in kettle 1 was concentrated until the residual volume was 2–3 vol. at the jacket temperature T of ≤ 30 °C 30 and the reference P of ≤ -0.08 MPa. $(3.50 \times W_{ID})$ kg of preparative grade acetonitrile was added to kettle 1, and the system was still concentrated until the residual volume was 2–3 vol. (this operation was repeated 3 times). $(6.00-8.00 \times W_{ID})$ kg of preparative grade acetonitrile was added to kettle 1, and the stirring was started. The system was cooled to -20 to -10 °C (target: -13 °C), and stirred for 3–5 h with the temperature maintained. The system in kettle 1 was filtered with the temperature controlled to be -20 to -10 °C, and subjected to pressure 35 filtration with precooled nitrogen. Kettle 1 was rinsed with preparative grade acetonitrile $((1.00-3.00) \times W_{ID})$, with the jacket controlled to be cooled to -5–5 °C, and pre-cooled for at least 0.5 h. The filter cake was soaked in and washed with the rinsing solution two times, each for 5–10 min, and then filtered. The system was dried at -15 °C to -5 °C to obtain compound 1, which was then sealed and stored at -25 to -15 °C. W_{ID} was 4.65 kg.

Example 2. Preparation of Mazdutide

Main synthetic route

5





2.1 Preparation of compound 2

Solution preparation

DMF/NMP mixed solution: *N,N*-dimethylformamide and *N*-methylpyrrolidone were mixed at a weight ratio of 1:1.09 and stirred for 30–60 min to prepare a mixed solution.

Pip/DMF/NMP solution: *N,N*-dimethylformamide, piperidine, and *N*-methylpyrrolidone (at a weight ratio of 2.2:1:2.4) were added in batches, and pumped and circulated for 2–4 h by a magnetic pump to prepare a mixed solution.

DBU deprotection solution: Piperidine, 1,8-diazabicycloundec-7-ene, *N,N*-dimethylformamide, and *N*-methylpyrrolidone were added at a weight ratio of 1:1.2:26.3:28.6, and the mixture was stirred for 30–60 min to prepare a mixed solution. The solutions can be prepared in batches.

Step 1-1b

Resin pretreatment: A Rink amide resin (6.82 kg W_1 , 2.99 mol N) was added to a 500 L synthesis reaction kettle, and *N,N*-dimethylformamide was added for washing the resin two times (the amount of *N,N*-dimethylformamide used was 8 W_1 each time; the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min). After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out.

N,N-dimethylformamide was added to the 500 L synthesis reaction kettle for the swelling of the resin (the amount of *N,N*-dimethylformamide used was 8 W_1), and the system was stirred while being purged with nitrogen, with the temperature controlled to be 25 ± 5 °C. After the swelling of the resin was performed for 0.5–1 h, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out.

Step 1-2a and step 1-2b to step 1-34a

Compound 2 was obtained via the following cycle 1-cycle 33.

Fmoc-Gly-OH connection (cycle 1)

1) Deprotection: $8.0 W_1 \pm 1.0 W_1$ L of Pip/DMF/NMP solution was added to a 500 L synthesis reaction kettle through a buffer tank, and the system was stirred after being purged with nitrogen for 3 min, with the temperature controlled to be 20–30 °C. After stirring for 14–16 min, the stirring was stopped, the nitrogen was shut off, and the system was filtered to remove the solution until no droplets dripped out. This operation was performed 2 times.

2) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle for washing the resin, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 8 times.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($3 N \times 0.297$) kg of Fmoc-Gly-OH, and ($3.3 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, ($3.3 N \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled to be 20–30 °C, and the system was stirred for 4–6 min.

4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was $\leq 1.5 N$ L). After being reacted for 1 h 50 min–2 h 10 min, the system was sampled for ninhydrin detection, the reaction was continued to

2 h 50 min–3 h 10 min if the resin was colorless, and the system was filtered.

5) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was reacted for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

Fmoc-Ser(tBu)-OH connection (cycle 2)

1) Deprotection: This operation was performed in the same manner as in cycle 1.

2) Washing: This operation was performed in the same manner as in cycle 1.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($3 N \times 0.383$) kg of Fmoc-Ser(tBu)-OH, and ($3.3 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($3.3 N \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 1.

5) Washing: This operation was performed in the same manner as in cycle 1.

Fmoc-Ser(tBu)-OH connection (cycle 3)

1) Deprotection: This operation was performed in the same manner as in cycle 1.

2) Washing: This operation was performed in the same manner as in cycle 1.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($3 N \times 0.383$) kg of Fmoc-Ser(tBu)-OH, and ($3.3 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($3.3 N \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 1.

5) Washing: This operation was performed in the same manner as in cycle 1.

6) End capping: a) Solution preparation: Under stirring, $4 W_1 \times 0.986$ Kg of mixed solution of *N,N*-dimethylformamide and *N*-methylpyrrolidone (1:1) was added to the 80 L glass reaction kettle, and $3 N \times 0.102$ Kg of acetic anhydride was added, followed by $3 N \times 0.129$ Kg of *N,N'*-diisopropylethylamine (DIPEA). After the dropwise addition was completed, the system was directly transferred into the 500 L synthesis reaction kettle. b) End capping reaction: The system was stirred while being purged with nitrogen, with the temperature controlled to be 20–30 °C, during which the wall can be flushed with *N,N*-dimethylformamide to ensure that resin particles do not adhere to the wall (the total amount for flushing the wall was $\leq 1.5 N$ L). After being reacted for 55–65 min, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. Then the next step was performed. c) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

Fmoc-Pro-OH·H₂O (cycle 4)

1) Deprotection: This operation was performed in the same manner as in cycle 1.

2) Washing: This operation was performed in the same manner as in cycle 1.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($3 N \times 0.353$) kg of Fmoc-Pro-OH·H₂O, and ($3.3 N \times$

0.135) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, *N,N'*-diisopropylcarbodiimide ($3.3 \text{ N} \times 0.126$) was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 1.

5) Washing: This operation was performed in the same manner as in cycle 1.

Fmoc-Gly-Gly-OH connection (cycle 5)

1) Deprotection: This operation was performed in the same manner as in cycle 1.

2) Washing: This operation was performed in the same manner as in cycle 1.

3) Activation: ($4 \text{ W}_1 \times 0.986$) kg of DMF/NMP solution, ($3 \text{ N} \times 0.354$) kg of Fmoc-Gly-Gly-OH, and ($3.3 \text{ N} \times 0.142$) kg of ethyl 2-oxime cyanoacetate were added to an 80 L glass reaction kettle, ($3.3 \text{ N} \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled to be 20–30 °C, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 1.

5) Washing: This operation was performed in the same manner as in cycle 1.

6) End capping: This operation was performed in the same manner as in cycle 3.

Fmoc-L-Glu(OtBu)-OH·H₂O connection (cycle 6)

1) Deprotection: This operation was performed in the same manner as in cycle 1.

2) Washing: This operation was performed in the same manner as in cycle 1.

3) Activation: ($4 \text{ W}_1 \times 0.986$) kg of DMF/NMP solution, ($3 \text{ N} \times 0.443$) kg of Fmoc-L-Glu (OtBu)-OH·H₂O, and ($3.3 \text{ N} \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($3.3 \text{ N} \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 1.

5) Washing: This operation was performed in the same manner as in cycle 1.

Fmoc-Leu-OH connection (cycle 7)

1) Deprotection: This operation was performed in the same manner as in cycle 1.

2) Washing: This operation was performed in the same manner as in cycle 1.

3) Activation: ($4 \text{ W}_1 \times 0.986$) kg of DMF/NMP solution, ($3 \text{ N} \times 0.353$) kg of Fmoc-Leu-OH, and ($3.3 \text{ N} \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($3.3 \text{ N} \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 1.

5) Washing: This operation was performed in the same manner as in cycle 1.

Fmoc-Leu-OH connection (cycle 8)

1) Deprotection: This operation was performed in the same manner as in cycle 1.

2) Washing: This operation was performed in the same manner as in cycle 1.

3) Activation: ($4 \text{ W}_1 \times 0.986$) kg of DMF/NMP solution, ($3 \text{ N} \times 0.353$) kg of Fmoc-Leu-OH, and ($3.3 \text{ N} \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($3.3 \text{ N} \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the

system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 1.

5) Washing: This operation was performed in the same manner as in cycle 1.

Fmoc-Trp(Boc)-OH connection (cycle 9)

1) Deprotection: This operation was performed in the same manner as in cycle 1.

2) Washing: This operation was performed in the same manner as in cycle 1.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($3 N \times 0.527$) kg of Fmoc-Trp(Boc)-OH, and ($3.3 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($3.3 N \times 0.126$) kg of *N,N*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 1.

5) Washing: This operation was performed in the same manner as in cycle 1.

Fmoc-L-Glu(OtBu)-OH·H₂O connection (cycle 10)

1) Deprotection: This operation was performed in the same manner as in cycle 1.

2) Washing: This operation was performed in the same manner as in cycle 1.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($3 N \times 0.443$) kg of Fmoc-L-Glu(OtBu)-OH·H₂O, and ($3.3 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($3.3 N \times 0.126$) kg of *N,N*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 1.

5) Washing: This operation was performed in the same manner as in cycle 1.

Fmoc-Val-OH connection (cycle 11)

1) Deprotection: ($8.0 W_1 \pm 1.0 W_1$) × 0.984 Kg of DBU deprotection solution was added to a 500 L synthesis reaction kettle, and the system was stirred after being purged with nitrogen for 3 min, with the temperature controlled to be 20–30 °C. After stirring for 14–16 min, the stirring was stopped, the nitrogen was shut off, and the system was filtered to remove the solution until no droplets dripped out. This operation was performed 3 times.

2) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle for washing the resin, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 2 times. The resin was washed with $8.0 W_1 \pm 1.0 W_1$ L of dichloromethane (DCM) in the same manner, which was performed 6 times. The resin was washed with $(8.0 W_1 \pm 1.0 W_1) \times 1.028$ Kg of *N*-methylpyrrolidone in the same manner, which was performed 4 times.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.339$) kg of Fmoc-Val-OH, and ($5.5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($5.5 N \times 0.126$) kg of *N,N*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was $\leq 1.5 N$ L). The

reaction was performed for 2 h 50 min–3 h 10 min, and then filtered.

5) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 4 times.

6) Fmoc-Val-OH refeeding and connection: a) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.339$) kg of Fmoc-Val-OH, and ($5.5 N \times 0.135$) kg 1-hydroxybenzotriazole were added to the 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($5.5 N \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min. b) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was $\leq 1.5 N$ L). After being reacted for 1 h 50 min–2 h 10 min, the system was sampled for ninhydrin detection, the reaction was continued to 2 h 50 min–3 h 10 min if the resin was colorless, and the system was filtered. c) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

Fmoc-Phe-OH connection (cycle 12)

1) Deprotection: $8.0 W_1 \pm 1.0 W_1$ L of Pip/DMF/NMP solution was added to a 500 L synthesis reaction kettle through a buffer tank, and the system was stirred after being purged with nitrogen for 3 min, with the temperature controlled to be 20–30 °C. After stirring for 29–31 min, the stirring was stopped, the nitrogen was shut off, and the system was filtered to remove the solution until no droplets dripped out. This operation was performed 3 times.

2) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle for washing the resin, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 8 times.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.387$) kg of Fmoc-Phe-OH, and ($5.5 N \times 0.135$) kg 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($5.5 N \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was $\leq 1.5 N$ L). After being reacted for 1 h 50 min–2 h 10 min, the system was sampled for ninhydrin detection, the reaction was continued to 2 h 50 min–3 h 10 min if the resin was colorless, and the system was filtered.

5) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

Fmoc-L-Glu(OtBu)-OH·H₂O connection (cycle 13)

- 1) Deprotection: $8.0 W_1 \pm 1.0 W_1$ L of Pip/DMF/NMP solution was added to a 500 L synthesis reaction kettle through a buffer tank, and the system was stirred after being purged with nitrogen for 3 min, with the temperature controlled to be 20–30 °C. After stirring for 29–31 min, the stirring was stopped, the nitrogen was shut off, and the system was filtered to remove the solution until no droplets dripped out. This operation was performed 2 times.
- 2) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle for washing the resin, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 8 times.
- 3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.443$) kg of Fmoc-L-Glu(OtBu)-OH·H₂O, and ($5.5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($5.5 N \times 0.126$) kg of *N,N*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.
- 4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was $\leq 1.5 N$ L). After being reacted for 1 h 50 min–2 h 10 min, the system was sampled for ninhydrin detection, the reaction was continued to 2 h 50 min–3 h 10 min if the resin was colorless, and the system was filtered.
- 5) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

Fmoc-Lys(ivDde)-OH coupling reaction (cycle 14)

- 1) Deprotection: This operation was performed in the same manner as in cycle 13.
- 2) Washing: This operation was performed in the same manner as in cycle 13.
- 3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($3 N \times 0.575$) kg of Fmoc-Lys(ivDde)-OH, and ($3.3 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($3.3 N \times 0.126$) kg of *N,N*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.
- 4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was $\leq 1.5 N$ L). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, the reaction was continued to 3 h 50 min–4 h 10 min if the resin was colorless, and the system was filtered.
- 5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Ala-OH·H₂O connection (cycle 15)

- 1) Deprotection: This operation was performed in the same manner as in cycle 13.
- 2) Washing: This operation was performed in the same manner as in cycle 13.
- 3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.329$) kg of Fmoc-Ala-OH·H₂O, and ($5.5 N \times$

0.135) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, (5.5 N × 0.126) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

6) End capping: This operation was performed in the same manner as in cycle 3.

Fmoc-Lys(Boc)-OH connection (cycle 16)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (5 N × 0.469) kg of Fmoc-Lys(Boc)-OH, and (5.5 N × 0.135) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, (5.5 N × 0.126) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was ≤ 1.5 N L). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, and then filtered.

5) Washing: 8.0 $W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 4 times.

6) Fmoc-Lys(Boc)-OH refeeding: a) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (5 N × 0.469) kg of Fmoc-Lys(Boc)-OH, (5 N × 0.520) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and (5 N × 0.135) kg of 1-hydroxybenzotriazole were added to the 80 L glass reaction kettle, the system was cooled to 0–10 °C, (10 N × 0.129) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–15 °C, and the system was stirred for 1–3 min. b) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was ≤ 1.5 N L). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, and then filtered if the resin was colorless or nearly colorless. c) Washing: 8.0 $W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

Fmoc-Lys(Boc)-OH connection (cycle 17)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (5 N × 0.469) kg of Fmoc-Lys(Boc)-OH, (5 N × 0.520) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and (5.5 N × 0.135) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, (10 N ×

0.129) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–10 °C, and the system was stirred for 1–3 min.

4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was ≤ 1.5 N L). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, and then filtered.

5) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 4 times.

6) Fmoc-Lys(Boc)-OH refeeding: a) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.469$) kg of Fmoc-Lys(Boc)-OH, ($5 N \times 0.520$) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and ($5.5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to the 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($10 N \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–10 °C, and the system was stirred for 4–6 min. b) Coupling: After the 80 L glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was ≤ 1.5 N L). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, and then filtered if the resin was colorless or nearly colorless. c) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

Fmoc-L-Glu(OtBu)-OH·H₂O connection (cycle 18)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.443$) kg of Fmoc-L-Glu(OtBu)-OH·H₂O, ($5 N \times 0.520$) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and ($5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($10 N \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–15 °C, and the system was stirred for 1–3 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Asp(OtBu)-OH connection (cycle 19)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.411$) kg of Fmoc-Asp(OtBu)-OH, ($5 N \times 0.520$) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and ($5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($10 N \times$

0.129) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–15 °C, and the system was stirred for 1–3 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Leu-OH connection (cycle 20)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (5 N $\times 0.353$) kg of Fmoc-Leu-OH, and (5.5 N $\times 0.135$) kg 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, (5.5 N $\times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Tyr(tBu)-OH connection (cycle 21)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (5 N $\times 0.460$) kg of Fmoc-Tyr(tBu)-OH, and (5.5 N $\times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, (5.5 N $\times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Lys(Boc)-OH connection (cycle 22)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (5 N $\times 0.469$) kg of Fmoc-Lys(Boc)-OH, (5 N $\times 0.520$) kg of 1*H*-benzotriazole-1-yl-*oxy*tripyrrolidinyll hexafluorophosphate, and (5 N $\times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, (10 N $\times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–15 °C, and the system was stirred for 1–3 min.

4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was ≤ 1.5 N L). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, and then filtered.

5) Washing: 8.0 $W_1 \pm 1.0$ W_1 L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 4 times.

6) Fmoc-Lys(Boc)-OH refeeding: a) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (5 N $\times 0.469$) kg of

Fmoc-Lys(Boc)-OH, ($5\text{ N} \times 0.520$) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and ($5\text{ N} \times 0.135$) kg of 1-hydroxybenzotriazole were added to the 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($10\text{ N} \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–10 °C, and the system was stirred for 4–6 min. b) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was $\leq 1.5\text{ N L}$). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, and then filtered. c) Washing: $8.0\text{ W}_1 \pm 1.0\text{ W}_1\text{ L}$ of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

7) End capping: This operation was performed in the same manner as in cycle 3.

Fmoc-Ser(tBu)-OH connection (cycle 23)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: ($4\text{ W}_1 \times 0.986$) kg of DMF/NMP solution, ($5\text{ N} \times 0.383$) kg of Fmoc-Ser(tBu)-OH, and ($5.5\text{ N} \times 0.136$) kg of 1-hydroxy-7-azabenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($5.5\text{ N} \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Tyr(tBu)-OH connection (cycle 24)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: ($4\text{ W}_1 \times 0.986$) kg of DMF/NMP solution, ($5\text{ N} \times 0.460$) kg of Fmoc-Tyr(tBu)-OH, and ($5.5\text{ N} \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($5.5\text{ N} \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Asp(OtBu)-OH connection (cycle 25)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: ($4\text{ W}_1 \times 0.986$) kg of DMF/NMP solution, ($5\text{ N} \times 0.411$) kg of Fmoc-Asp(OtBu)-OH, ($5\text{ N} \times 0.520$) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and ($5\text{ N} \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($10\text{ N} \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–15 °C, and the system was stirred for 1–3 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Ser(tBu)-OH connection (cycle 26)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (5 N $\times 0.383$) kg of Fmoc-Ser(tBu)-OH, and (5.5 N $\times 0.136$) kg of 1-hydroxy-7-azabenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, (5.5 N $\times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Thr(tBu)-OH connection (cycle 27)

1) Deprotection: 8.0 $W_1 \pm 1.0 W_1$ L of Pip/DMF/NMP solution was added to a 500 L synthesis reaction kettle through a buffer tank, and the system was stirred after being purged with nitrogen for 3 min, with the temperature controlled to be 20–30 °C. After stirring for 29–31 min, the stirring was stopped, the nitrogen was shut off, and the system was filtered to remove the solution until no droplets dripped out. This operation was performed 4 times.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (5 N $\times 0.398$) kg of Fmoc-Thr(tBu)-OH, (5 N $\times 0.520$) kg of 1*H*-benzotriazole-1-ylxytripyrrolidiny1 hexafluorophosphate, and (5 N $\times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, (10 N $\times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–15 °C, and the system was stirred for 1–3 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

6) End capping: This operation was performed in the same manner as in cycle 3.

Fmoc-Phe-OH connection (cycle 28)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (5 N $\times 0.387$) kg of Fmoc-Phe-OH, and (5.5 N $\times 0.136$) kg of 1-hydroxy-7-azabenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, (5.5 N $\times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Thr(tBu)-OH connection (cycle 29)

1) Deprotection: 8.0 $W_1 \pm 1.0 W_1$ L of Pip/DMF/NMP solution was added to a 500 L synthesis reaction kettle through a buffer tank, and the system was stirred after being purged with nitrogen for 3 min, with the temperature controlled to be 20–30 °C. After stirring for 29–31 min, the stirring was stopped, the nitrogen was shut off, and the system was filtered to remove the solution until no droplets dripped out. This operation was performed 4 times.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.398$) kg of Fmoc-Thr(tBu)-OH, ($5 N \times 0.520$) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and ($5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($10 N \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–15 °C, and the system was stirred for 1–3 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

6) End capping: This operation was performed in the same manner as in cycle 3.

Fmoc-Gly-OH reaction (cycle 30)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.297$) kg of Fmoc-Gly-OH, and ($5.5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, ($5.5 N \times 0.126$) kg of *N,N'*-diisopropylcarbodiimide was added dropwise with the temperature controlled to be 20–30 °C, and the system was stirred for 4–6 min.

4) Coupling: This operation was performed in the same manner as in cycle 13.

5) Washing: This operation was performed in the same manner as in cycle 13.

Fmoc-Gln(Trt)-OH connection (cycle 31)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.611$) kg of Fmoc-Gln(Trt)-OH, ($5 N \times 0.520$) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and ($5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($10 N \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–15 °C, and the system was stirred for 1–3 min.

4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was $\leq 1.5 N L$). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, and then filtered.

5) Washing: $8.0 W_1 \pm 1.0 W_1 L$ of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 4 times.

6) Fmoc-Gln(Trt)-OH refueling: a) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.611$) kg of Fmoc-Gln(Trt)-OH, ($5 N \times 0.520$) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and ($5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to the 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($10 N \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–10 °C, and the system was stirred for 4–6 min. b) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled

to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was ≤ 1.5 N L). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, and then filtered. c) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

7) End capping: This operation was performed in the same manner as in cycle 3.

Fmoc-Aib-OH connection (cycle 32)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.325$) kg of Fmoc-Aib-OH, ($5 N \times 0.520$) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and ($5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, ($10 N \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 0–15 °C, and the system was stirred for 1–3 min.

4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was ≤ 1.5 N L). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, and then filtered.

5) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 4 times.

6) Fmoc-Aib-OH refeeding: a) Activation: ($4 W_1 \times 0.986$) kg of DMF/NMP solution, ($5 N \times 0.325$) kg of Fmoc-Aib-OH, ($5 N \times 0.520$) kg of 1*H*-benzotriazole-1-yloxytripyrrolidinyl hexafluorophosphate, and ($5 N \times 0.135$) kg of 1-hydroxybenzotriazole were added to the 80 L glass reaction kettle, ($10 N \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled to be 20–30 °C, and the system was stirred for 4–6 min. b) Coupling: After the 80 L glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 20–30 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was ≤ 1.5 N L). After being reacted for 2 h 50 min–3 h 10 min, the system was sampled for ninhydrin detection, and then filtered if the resin was colorless. c) Washing: $8.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

7) End capping: This operation was performed in the same manner as in cycle 3.

Boc-His(Trt)-OH coupling reaction (cycle 33)

1) Deprotection: This operation was performed in the same manner as in cycle 13.

2) Washing: This operation was performed in the same manner as in cycle 13.

3) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (3 $N \times 0.498$) kg of Boc-L-His(Trt)-OH, and (6 $N \times 0.299$) kg of 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-one were added to an 80 L glass reaction kettle, the system was cooled to 0–10 °C, (6 $N \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled, and the system was stirred for 1–3 min.

4) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 22–28 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was ≤ 1.5 N L). The reaction was performed for 2 h 50 min–3 h 10 min, and then filtered.

5) Washing: 8.0 $W_1 \pm 1.0$ W_1 L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 4 times.

6) Boc-L-His(Trt)-OH refeeding: a) Activation: (4 $W_1 \times 0.986$) kg of DMF/NMP solution, (3 $N \times 0.498$) kg of Boc-L-His(Trt)-OH, and (6 $N \times 0.299$) kg of 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-one were added to the 80 L glass reaction kettle, the system was cooled to 0–10 °C, (6 $N \times 0.129$) kg of *N,N*-diisopropylethylamine was added dropwise with the temperature controlled, and the system was stirred for 1–3 min. b) Coupling: After the glass reaction kettle was activated, the system was added to the 500 L synthesis reaction kettle, and the system was reacted with the temperature controlled to be 22–28 °C, during which the wall of the synthesis reaction kettle can be flushed with *N,N*-dimethylformamide (the total amount was ≤ 1.5 N L). The reaction was performed for 2 h 50 min–3 h 10 min, and then filtered. c) Washing: 8.0 $W_1 \pm 1.0$ W_1 L of *N,N*-dimethylformamide was added to the 500 L synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min. After the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out. This operation was performed 6 times.

7) End capping: This operation was performed in the same manner as in cycle 3.

Polypeptide analysis method:

Agilent 1290 UPLC equipped with a UV detector and a temperature-controlled autosampler or equivalent; chromatographic column: ACQUITY UPLC® Peptide CSH™, 1.7 μ m 2.1 $\times$ 150 mm; and analytical balance with a precision of 0.01 mg.

The analysis was performed according to the following chromatographic conditions:

Chromatographic column	ACQUITY UPLC® Peptide CSH™, 1.7 μ m 2.1 $\times$ 300 mm							
Column temperature	60 °C							
Flow rate	0.25 ml/min							
Injection volume	2 μ L							
Elution gradient	T/min	0	10	60	60.1	65	65.1	70
	B%	15	40	65	95	90	10	5
Mobile phase	Mobile phase A: 0.05% aqueous trifluoroacetic acid solution							

	Mobile phase B: 0.01% trifluoroacetic acid (acetonitrile:water = 90:10) solution
Run time	90 min
Autosampler temperature	5 °C
Ultraviolet detector	210 nm
Diluent	Diluent 1: 0.01 mol/L TRIS buffer (pH = 8.0) Diluent 2: acetonitrile:water = 55:45
Needle washing	Acetonitrile:water = 55:45

The polypeptides obtained from the cycles were assayed based on the polypeptide analysis described above. It was noted that a coupling of Fmoc-Gly-Gly-OH (cycle 5) is used instead of a one-by-one coupling of Fmoc-Gly-OH for coupling of Gly-Gly at positions 29–30 in a peptide chain, thereby reducing the risk of generating Gly impurities. In addition, a coupling of a glycine-threonine pseudoproline dipeptide was replaced with one-by-one couplings of Gly and Thr (corresponding to cycles 29 and 30) at positions 4 and 5, no peptides with corresponding amino acid deletions were found, and the cost was reduced.

The polypeptide cycles described above were also used for the determination of the polypeptides in the following steps.

2.2 Preparation of compound 3

Step 1-35: The iVDde protecting group of Lys at position 20 was removed from compound 2 in the following manner to obtain compound 3.

($16.2 \times W_1 \times 0.944$) kg of *N,N*-dimethylformamide and ($1.8 \times W_1 \times 1.026$) kg of 80% hydrazine hydrate solution were added to an 80L glass reaction kettle, and the system was stirred for 15–20 min to prepare a mixed solution; the mixed solution was added to a synthesis reaction kettle, and the system was stirred for 11 h 50 min–12 h 10 min while being purged with nitrogen, with the temperature controlled to be 22–32 °C, and then filtered to remove *N,N*-dimethylformamide until no droplets dripped out (this operation was performed 2 times). $9.0 W_1 \pm 1.0 W_1$ L of *N,N*-dimethylformamide was added to the synthesis reaction kettle, and the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min; after the washing was completed, the system was filtered to remove *N,N*-dimethylformamide until no droplets dripped out; this operation was performed 10 times. The system was washed with (9 ± 1.0) $W_1 \times 1.028$ Kg of *N*-methylpyrrolidone in the same manner; this operation was performed 2 times. As such, compound 3 was obtained.

N-methylpyrrolidone was added to the synthesis reaction kettle to soak the resin until the next step.

2.3 Preparation of compound 4

Step 1-36: Compound 1 was coupled with compound 3 in the following manner to obtain compound 4.

1) Activation: ($7.5 W_1 \times 1.325$) kg of dichloromethane was added to an 80 L glass reaction kettle, and ($1.8 N \times 0.874$) kg of compound 1, ($1.89 N \times 0.115$) kg of *N*-hydroxysuccinimide (HOSu), and ($1.8 N \times 0.126$) kg of *N,N*-diisopropylcarbodiimide (DIC) were added with the temperature controlled to be 20–30 °C. The system was reacted for 5 h with the temperature maintained at 20–30 °C, and then sampled once every 2–4 h until the peak

area percentage of compound 1 was $\leq 10\%$.

2) Post-treatment: The system was filtered, then transferred to a glass reaction kettle, and concentrated with the temperature T of an external bath controlled to be $\leq 35\text{ }^{\circ}\text{C}$ and the reference pressure P of $\leq -0.06\text{ MPa}$ until the residual volume was $(1-2 \times W_1)\text{ L}$. $(7.5 W_1 \times 1.325)\text{ kg}$ of dichloromethane was added to kettle 2, and the system was stirred until there was no viscous liquid agglomeration visually, and then filtered. The filtered system was transferred to the glass reaction kettle (the glass was washed with DCM after the filtration), and cooled to $5-15\text{ }^{\circ}\text{C}$, and $(3.6 N \times 0.129)\text{ kg}$ of N,N -diisopropylethylamine and $(3 W_1 \times 0.944)\text{ kg}$ of N,N -dimethylformamide were added with the temperature controlled to obtain an active ester solution of compound 1.

3) Coupling: The system after the activation and post-treatment was transferred to a synthesis reaction kettle. The system was reacted with the temperature controlled to be $20-30\text{ }^{\circ}\text{C}$, during which the wall of the synthesis reaction kettle can be flushed with N,N -dimethylformamide (the total amount was $\leq 1.5\text{ N L}$). After being reacted for 11 h 50 min–12 h 10 min, the system was sampled for Kaiser detection, and the reaction liquid was drained if the resin was colorless.

4) Washing, shrinking, and drying: 1) Washing: The system was washed 6 times with $9W_1 \pm 1 W_1\text{ L}$ of N,N -dimethylformamide. 2) Shrinking: $(9W_1 \pm 1 W_1) \times 0.740\text{ Kg}$ of methyl *tert*-butyl ether was added to the synthesis reaction kettle at $25 \pm 5\text{ }^{\circ}\text{C}$, the system was stirred for 1–3 min/time after being purged with nitrogen for 3 min; the stirring was stopped, the nitrogen was shut off, and the system was filtered to remove the methyl *tert*-butyl ether until no droplets dropped put (this operation was repeated 2 times). The system was treated with $9 W_1 \pm 1 W_1\text{ L}$ of dichloromethane in the same manner; this operation was repeated 2 times. The system was treated with $(9 W_1 \pm 1 W_1) \times 0.740\text{ Kg}$ of methyl *tert*-butyl ether in the same manner; this operation was repeated 2 times. 3) Drying: The system was purged with nitrogen for 4–8 h and then transferred to a vacuum drying oven for drying to obtain compound 4.

2.4 Preparation of mazdutide crude product

Step 2: The resin in compound 4 was cleaved and deprotected in the following manner to obtain a crude peptide of mazdutide. The weight of compound 4 used was 24.76 kg and was denoted as W_2 .

1. Crystallization solvent pre-cooling: $(30 \times W_2 \times 0.740)\text{ kg}$ of methyl *tert*-butyl ether was added to a glass reaction kettle, and the system was cooled to $-15-0\text{ }^{\circ}\text{C}$.

2. Peptide resin cleavage: $(10 \times W_2 \times 0.925 \times 1.489)\text{ kg}$ of trifluoroacetic acid, $(10 \times W_2 \times 0.773 \times 0.031)\text{ kg}$ of triisopropylsilane, $(0.3 \times W_2)\text{ kg}$ of dithiothreitol, and $(9 \times W_2 \times 0.044)\text{ kg}$ of purified water were added to the synthesis reaction kettle, and the system was stirred until the solids were completely dissolved. The system was cooled to $-10-0\text{ }^{\circ}\text{C}$, and $W_2\text{ kg}$ of compound 4 was added. The system in the synthesis reaction kettle was heated to $18-24\text{ }^{\circ}\text{C}$, and reacted for 110–130 min (starting timing when the system was heated to $18\text{ }^{\circ}\text{C}$) with the temperature maintained.

3. Crystallization: The temperature of the glass reaction kettle was controlled to be $-15-20\text{ }^{\circ}\text{C}$, and the system in the synthesis reaction kettle was transferred to the glass reaction kettle; the temperature of the system in the glass reaction kettle was adjusted to $0-20\text{ }^{\circ}\text{C}$, the system was stirred for 2–4 h and then filtered; the glass reaction kettle was rinsed with $(5 \times W_2 \times 0.740)\text{ kg}$ of methyl *tert*-butyl ether, and the filter cake soaked in and washed with the rinsing solution for 20–40 min (10 times).

4. Grinding, slurring, and drying: The material was dried at $20-30\text{ }^{\circ}\text{C}$ to be in a loose state and then collected,

and solids were ground; $(10 \times W_2 \times 0.740)$ kg of methyl *tert*-butyl ether was adjusted to the temperature of 10 °C and added to the solids, and the system was stirred for 4–8 h and then filtered; the system was rinsed with $(5 \times W_2 \times 0.740)$ kg of methyl *tert*-butyl ether, and the filter cake was soaked in and washed with the rinsing solution for 20–40 min, and then filtered (3 times); the material was dried at the temperature of 20–30 °C until the LOD was $\leq 5.0\%$ to obtain a mazdutide crude product (purity: 69%–74%).

2.5 Isolation and purification of mazdutide

Step 3: The mazdutide crude product prepared in step 2.4 was purified by chromatography and by a salt transfer method, and then lyophilized.

Chromatography

3.23 kg of mazdutide crude product was dissolved in acetonitrile/water (at a ratio of 1/1.3), and the system was stirred for 1 h–1 h 30 min and determined for the pH value (pH = 2–4, the system can be adjusted with 3% diluted ammonia water or 1% trifluoroacetic acid solution), and then subjected to preparation. The system was then purified by reverse-phase chromatography (mobile phase A1: 0.1% trifluoroacetic acid solution; mobile phase B: preparative grade acetonitrile; chromatographic column inner diameter: 600 mm; chromatographic column height: 23.0 cm; detection wavelength (monitoring wavelength): 210 nm (254 nm)) to obtain a solution containing mazdutide (purity $\geq 85.0\%$). Then the solution was adjusted to pH 7.0–7.5 with 3% diluted ammonia water at 10–20 °C, concentrated until no fraction was left with the system temperature T of ≤ 20 °C and the reference P of ≤ -0.08 MPa, sampled for detection until the content of acetonitrile was 10%, stirred for 1–2 h for uniform mixing, and then subjected to preparation. The system was then purified by reverse-phase chromatography (mobile phase A2: 50 mM aqueous ammonium bicarbonate solution; mobile phase B: preparative grade acetonitrile; chromatographic column inner diameter: 600 mm; chromatographic column height: 23.0 cm; detection wavelength (monitoring wavelength): 210 nm (254 nm)) to obtain a solution containing mazdutide (purity $\geq 97.0\%$).

Salt transfer

The sample component after purification by chromatography was diluted with two-fold mass of purified water, and the system was stirred for 1–2 h. Then the system was subjected to salt transfer preparation using mobile phase A3 (preparative grade acetonitrile:water = 5:95 (v:v)), mobile phase A4 (preparative grade acetonitrile:32 g/L aqueous ammonium acetate solution = 5:95 (v:v)), mobile phase B (preparative grade acetonitrile), and a chromatographic column (chromatographic column inner diameter: 600 mm; chromatographic column height: 23.0 cm) according to the following table. The detection wavelength (monitoring wavelength) was 210 nm (254 nm).

Stage	Time (min)	A3 (%)	B (%)	Flow rate (mL/min)
Equilibration	15	100	0	8800
Injection	The sample was injected by an injection pump at a reference flow rate of 5000–8000 mL/min.			
Stage	Time (min)	A4 (%)	B (%)	Flow rate (mL/min)
Flushing	30	100	0	8800
Stage	Time (min)	A3 (%)	B (%)	Flow rate (mL/min)
Elution	0	100	0	8800

30	100	0	8800
35	50	50	8800
125	50	50	8800

Concentration and lyophilization

Concentration: The component after purification by chromatography or salt transfer in an enamel reaction kettle was cooled to 10–20 °C, and the system was adjusted to pH 7.0–7.5 with prepared 3% diluted ammonia water; the system was concentrated until no fraction was left with the internal temperature T controlled to be ≤ 20 °C and the reference pressure P controlled to be ≤ -0.08 MPa, and sampled for detection until the content of acetonitrile was $\leq 1.0\%$; the system was purged with nitrogen under the liquid surface with the temperature T controlled to be ≤ 20 °C, and sampled every 4–8 h until the residual acetonitrile was ≤ 3000 ppm; the concentrated system was sampled for detection of the pH value to make the pH value of the solution be 7.0–7.5 (the pH value of the solution can be adjusted with 3% diluted ammonia water).

Lyophilization: The solution after the concentration was pumped into lyophilization trays at 1.8–2.4 kg/tray; after the filling was completed, the first lyophilization was performed according to the following parameters.

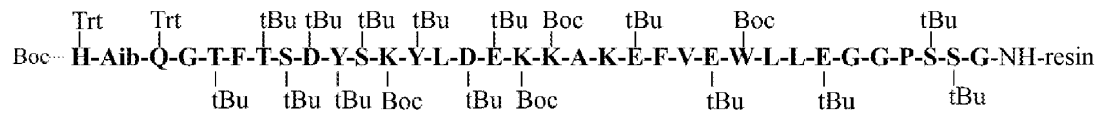
Procedure		Set temperature (°C)	Set vacuum (mbar)	Set time (min)	Duration (min)
Pre-freezing		-40	--	120	180
Primary drying	First stage	-20	0.10	120	480
	Second stage	0	0.10	240	1800
Analytical drying	First stage	30	0.10	180	300

Concentration: The solid after lyophilization was dissolved in 30-fold mass of purified water (the solution was adjusted to pH 7.0–7.5 with the prepared 3% diluted ammonia water solution) in an enamel reaction kettle, and the system was stirred until the solid was completely dissolved with the pH maintained at 7.0–7.5, and detected for the content of acetonitrile until the acetonitrile content was ≤ 200 ppm (if the acetonitrile residue was > 200 ppm, the system can be purged with nitrogen under the liquid surface).

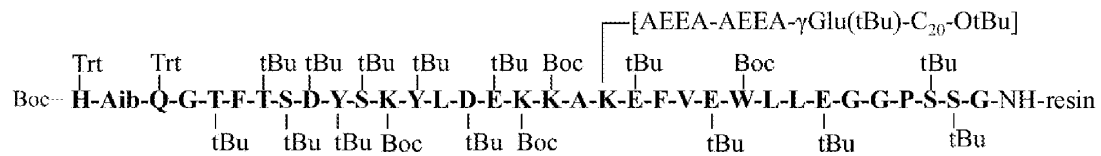
Lyophilization: the solution after the concentration was pumped into lyophilization trays at 1.8–2.4 kg/tray; after the filling was completed, the second lyophilization was performed according to the following parameters.

Procedure		Set temperature (°C)	Set vacuum (mbar)	Set time (min)	Duration (min)
Pre-freezing		-40	--	120	180
Primary drying	First stage	-20	0.10	120	480
	Second stage	-5	0.10	240	960
Analytical drying	First stage	0	0.10	120	1440
	Second stage	30	0.25	180	1080

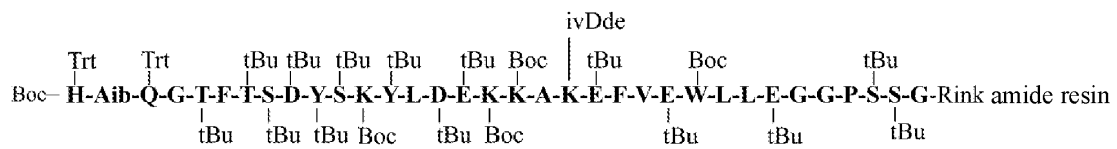
Dissolution: The solid after lyophilization was dissolved in 30-fold mass of purified water (the solution was adjusted to pH 7.0–7.5 with the prepared 3% diluted ammonia water solution) in an enamel reaction kettle, and



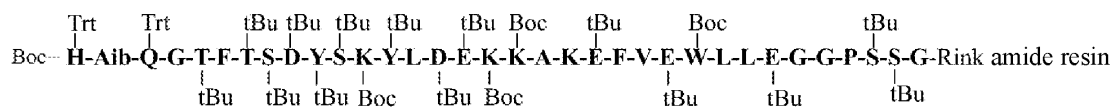
SEQ ID NO: 5



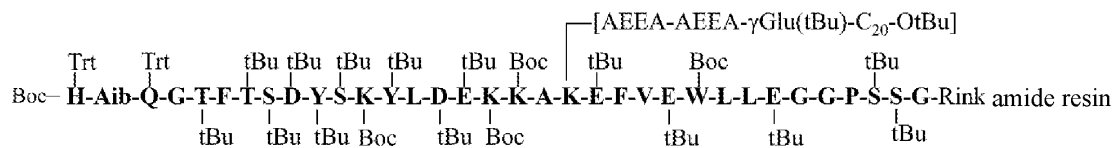
SEQ ID NO: 6



SEQ ID NO: 7

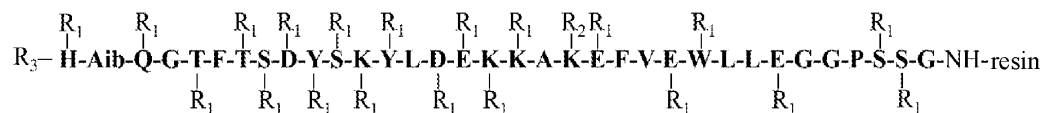


SEQ ID NO: 8



CLAIMS

1. A method for preparing a compound of the following formula (SEQ ID NO: 1):



5 the method comprising solid phase synthesis of the compound,
wherein, Fmoc-Gly-Gly-OH is used for the solid phase synthesis,
R₁ and R₃ are base-stable side-chain protecting groups, and
R₂ is an ivDde side-chain protecting group.

10 2. The method according to claim 1, wherein, the solid phase synthesis is performed on an Fmoc amide resin solid support, and the Fmoc amide resin is a Rink amide resin.

3. The method according to claim 1 or 2, wherein,
R₁ is: (a) Boc for Trp and Lys; (b) OtBu for Asp and Glu; (c) tBu for Ser, Thr, and Tyr; (d) Trt for Gln; and (e) Trt
15 for His;
R₃ is Boc and is connected to an amino group of His.

4. The method according to any one of claims 1–3, wherein, the solid phase synthesis is performed on the Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following: Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH·H₂O, Fmoc-Gly-Gly-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Lys(ivDde)-OH, Fmoc-Ala-OH·H₂O, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Aib-OH, and Boc-His(Trt)-OH.

5. The method according to any one of claims 1–4, wherein, the solid phase synthesis comprises deprotection, activation, and coupling.

6. The method according to claim 5, wherein, the deprotection is performed using a Pip/DMF/NMP solution or a DBU deprotection solution.

7. The method according to claim 5 or 6, wherein, the deprotection reaction in the solid phase synthesis comprises:
35 performing the deprotection two times in first ten solid phase synthesis cycles, each for 14–16 min; and performing the deprotection two times in last 13–33 solid phase synthesis cycles, each for 29–31 min.

8. The method according to claim 5 or 6, wherein, an activation system used in the activation is DIC/HOBt/HOAt/Oxyma in DMF or DMF/NMP; or PyBOP/HOBt in DMF or DMF/NMP; or DEPBT in DMF or DMF/NMP.

9. The method according to claim 5 or 6, wherein, in the first ten solid phase synthesis cycles, the coupling is performed using 2–4 equivalents of the Fmoc amino acids relative to the resin; in the remaining solid phase synthesis cycles, the coupling is performed using 4–6 equivalents of the Fmoc amino acids relative to the resin; or the coupling of His at position 1 and Lys at position 20 is performed using 3 equivalents of the Fmoc amino acids.

10. The method according to claim 5 or 6, wherein, the coupling is performed at a temperature of 20–30 °C for a total reaction time of ≤ 12 h.

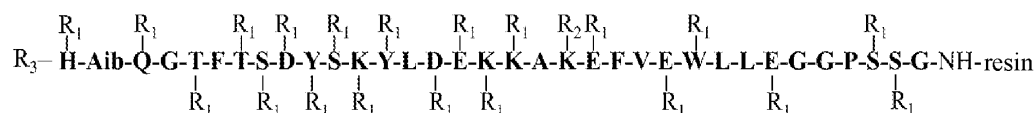
11. The method according to claim 5 or 6, wherein, the solid phase synthesis further optionally comprises an end capping step.

12. A method for preparing a compound of the following formula (SEQ ID NO: 2):

H-Aib-Q-G-T-F-T-S-D-Y-S-K-Y-L-D-E-K-K-A-K-E-F-V-E-W-L-L-E-G-G-P-S-S-G-NH₂

wherein, Lys at position 20 is chemically modified by conjugating an ϵ -amino group of a side chain of Lys at position 20 to ([2-(2-aminoethoxy)-ethoxy]-acetyl)-2-(γ -Glu)-CO-(CH₂)₁₈CO₂H, the method comprising:

(i) solid phase synthesis of a compound of the following formula (SEQ ID NO: 1):



wherein, Fmoc-Gly-Gly-OH is used for the solid phase synthesis,

R₁ and R₃ are base-stable side-chain protecting groups, and

R₂ is an ivDde side-chain protecting group;

(ii) selective deprotection of Lys at position 20 and direct coupling of tBuO-C₂₀- γ Glu(tBu)-AEEA-AEEA-OSu activated ester obtained via DIC/HOSu to an ϵ -amino group of a side chain of Lys at position 20;

(iii) cleavage of the compound from a solid support and removal of the base-stable side-chain protecting groups; and

(iv) purification of the compound.

13. The method according to claim 12, wherein, the solid phase synthesis is performed on an Fmoc amide resin solid support, and the Fmoc amide resin is a Rink amide resin.

14. The method according to claim 12 or 13, wherein,

R₁ is: (a) Boc for Trp and Lys; (b) OtBu for Asp and Glu; (c) tBu for Ser, Thr, and Tyr; (d) Trt for Gln; and (e) Trt for His;

R₃ is Boc and is connected to an amino group of His.

- 5 15. The method according to any one of claims 12–14, wherein, the solid phase synthesis of step (i) is performed on the Fmoc amide resin solid support and comprises Fmoc deprotection of the amide resin and sequential coupling of the following: Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH·H₂O, Fmoc-Gly-Gly-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Glu(OtBu)-OH·H₂O, Fmoc-Lys(ivDde)-OH, Fmoc-Ala-OH·H₂O,
- 10 Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-L-Glu(OtBu)-OH·H₂O, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Aib-OH, and Boc-His(Trt)-OH.
- 15 16. The method according to any one of claims 12–15, wherein, the solid phase synthesis comprises deprotection, activation, and coupling.
17. The method according to claim 16, wherein, the deprotection is performed using a Pip/DMF/NMP solution or a DBU deprotection solution.
- 20 18. The method according to claim 16 or 17, wherein, the deprotection reaction in the solid phase synthesis comprises: performing the deprotection two times in first ten solid phase synthesis cycles, each for 14–16 min; and performing the deprotection two times in last 13–33 solid phase synthesis cycles, each for 29–31 min.
- 25 19. The method according to claim 16 or 17, wherein, an activation system used in the activation is DIC/HOBt/HOAt/Oxyma in DMF or DMF/NMP; or PyBOP/HOBt in DMF or DMF/NMP; or DEPBT in DMF or DMF/NMP.
20. The method according to claim 16 or 17, wherein, in the first ten solid phase synthesis cycles, the coupling is
- 30 performed using 2–4 equivalents of the Fmoc amino acids relative to the resin; in the remaining solid phase synthesis cycles, the coupling is performed using 4–6 equivalents of the Fmoc amino acids relative to the resin; or the coupling of His at position 1 and Lys at position 20 is performed using 3 equivalents of the Fmoc amino acids.
21. The method according to claim 16 or 17, wherein, the coupling is performed at a temperature of 20–30 °C for
- 35 a total reaction time of ≤ 12 h.
22. The method according to claim 16 or 17, wherein, the solid phase synthesis further optionally comprises an end capping step.

23. The method according to any one of claims 12–22, wherein Lys at position 20 is selectively deprotected by contacting the compound (SEQ ID NO: 1) with a solution containing hydrazine hydrate.

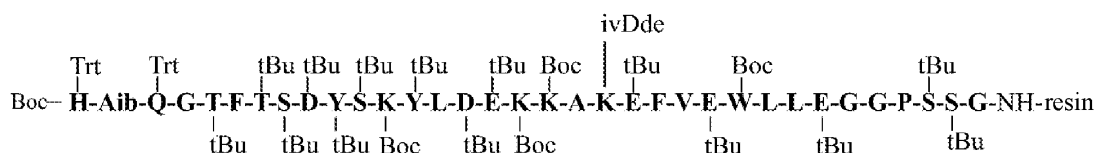
24. The method according to any one of claims 12–23, wherein, in step (iii), a peptide is cleaved from the resin with an acidic mixture consisting of trifluoroacetic acid, triisopropylsilane, dithiothreitol, and water, and then the system after the cleavage is directly subjected to crystallization in MTBE.

25. The method according to any one of claims 12–24, wherein, step (iv) comprises subjecting a crude solution of the compound of step (iii) to chromatographic purification.

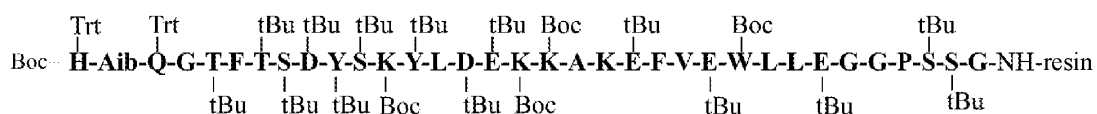
26. The method according to claim 25, wherein, the chromatographic purification is performed by HPLC or reverse-phase HPLC.

27. The method according to claim 25, wherein the purification further comprises the following steps: (a) obtaining a product by elution with an ammonium acetate/acetonitrile (ACN)/water mobile phase, performing salt transfer, and removing trifluoroacetic acid (TFA); (b) performing concentration and removing a solvent; and (c) performing lyophilization.

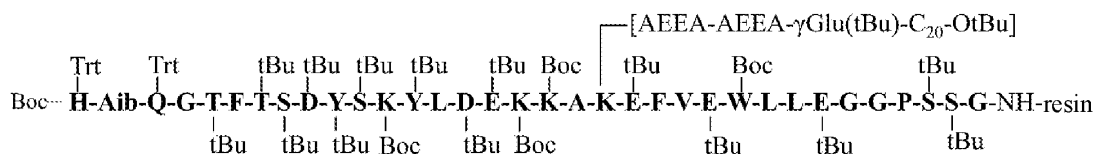
28. A compound of the following formula (I) (SEQ ID NO: 3) or a pharmaceutically acceptable salt thereof, a compound of the following formula (II) (SEQ ID NO: 4) or a pharmaceutically acceptable salt thereof, or a compound of the following formula (III) (SEQ ID NO: 5) or a pharmaceutically acceptable salt thereof:



formula (I)

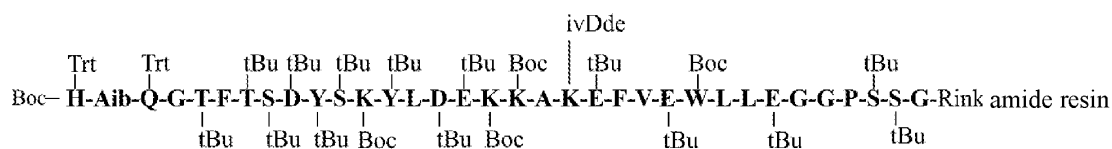


formula (II)



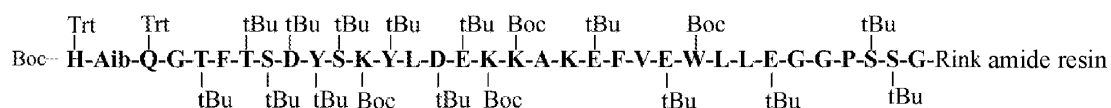
formula (III).

29. The compound or the pharmaceutically acceptable salt thereof according to claim 28, wherein, the compound of formula (I) or the pharmaceutically acceptable salt thereof, the compound of formula (II) or the pharmaceutically acceptable salt thereof, or the compound of formula (III) or the pharmaceutically acceptable salt thereof is selected from the following compounds or pharmaceutically acceptable salts thereof:

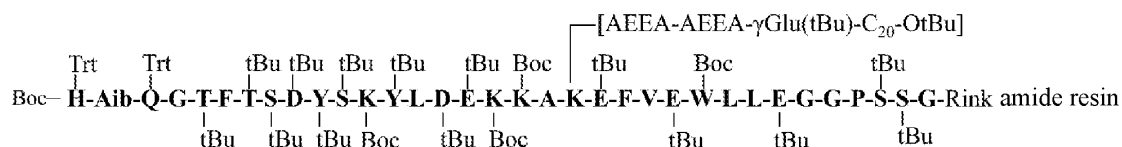


5

(compound 2) (SEQ ID NO: 6):

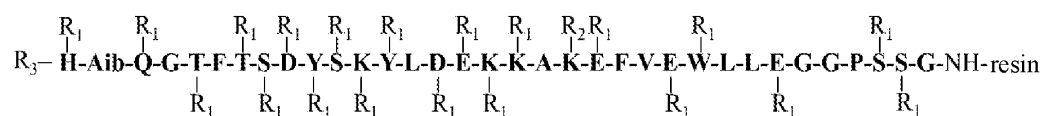


(compound 3) (SEQ ID NO: 7):



10 (compound 4) (SEQ ID NO: 8).

30. A compound of the following formula (SEQ ID NO: 1):



made by the method according to any one of claims 1-11.

15 31. A compound of the following formula (SEQ ID NO: 2):



wherein, Lys at position 20 is chemically modified by conjugating an ϵ -amino group of a side chain of Lys at position 20 to ([2-(2-aminoethoxy)-ethoxy]-acetyl)-2-(γ -Glu)-CO-(CH₂)₁₈CO₂H, made by the method according to any one of claims 12-27.