

LACTAM-MODIFIED POLYPEPTIDE COMPOUNDS

[0001] The present application claims the right of the following priorities:

CN202011402979.7, the application date is December 02, 2020;

CN202011409947.X, the application date is December 02, 2020;

CN202110432060.0, the application date is April 21, 2021;

CN202110587056.1, the application date is May 27, 2021.

TECHNICAL FIELD

[0002] The present disclosure relates to a class of polypeptide compounds containing lactam modification, and the use thereof in the manufacture of a medicament for treating related diseases.

BACKGROUND

[0003] It is estimated that there are 110 million diabetics in China, accounting for 24% of diabetics in the world. With economic development and lifestyle changes, the prevalence of diabetes in China has increased to 12.8% (as high as 19.9% in some provinces and cities), which is accompanied by obesity or cardiovascular disease (CVD). Recent studies have found that a glucose-dependent insulintropic peptide (GIP)/glucagon-like peptide-1 (GLP-1) dual agonist can be used for treating diabetes.

[0004] GLP-1 plays a role in protecting islet β cells in islets and effectively controls postprandial blood glucose by stimulating the islet β cells to release insulin in a glucose-dependent manner. Due to GLP-1's unique mechanism of action, the risk of hypoglycemia is greatly reduced. Although GLP-1R agonists have demonstrated excellent glucose-lowering effect in clinical practice, there are still many type 2 diabetics who have not achieved their glycemic and weight goals. Therefore, combining GLP-1R with other glucose-lowering targets such as GIP for treating type 2 diabetes is an urgent and promising need. GIP is a polypeptide secreted by neuroendocrine K cells of small intestine, and GIP's physiological actions are mediated by GIPR including non-glucose-dependent insulintropic effects, stimulation of glucagon secretion, and enhancement of lipid metabolism, etc. Although the

beneficial effects of GIPR agonists appear to be attenuated in hyperglycemic symptoms in type 2 diabetics, studies have shown that the diminished insulintropic effect of GIP can be fully restored after a period of normalization of plasma glucose levels. This indicates that co-agonism of GLP-1R/GIPR can exert a synergistic glucose-lowering effect. A GIP and GLP-1 dual agonist has a synergistic effect on the regulation of glucose/lipid metabolism and has better therapeutic effects on lowering blood glucose, reducing body weight, and alleviating liver fat.

CONTENT OF THE PRESENT INVENTION

[0005] The present disclosure provides a compound represented by the following formula or a pharmaceutically acceptable salt thereof,

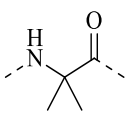
SEQ ID NO1: YAibEGT FTSDY SIAibLD KK¹AZ₀K₀ Z₁FZ₂E₀W LZ₃AGG PSSGA PPPS₀

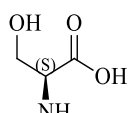
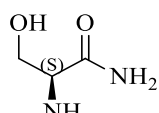
SEQ ID NO2: YAibEGT FTSDY SIAibLD KE₀AQK₀ AFVK¹W LIAGG PSSGA PPPS₀

SEQ ID NO3: YAibEGT FTSDY SIE₀LD KK₀AQK¹ AFVQW LIAGG PSSGA PPPS₀

SEQ ID NO4: YAibEGT FTSDY SIE₀LD K₀IAQK¹ AFVQW LIAGG PSSGA PPPS₀

[0006] wherein

[0007] Aib has a structure of  ;

[0008] S₀ is selected from  and  ;

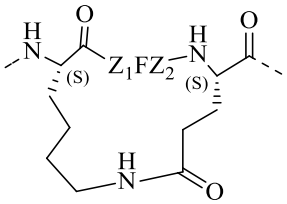
[0009] Z₀ is selected from glutamine (Q) and asparagine (N);

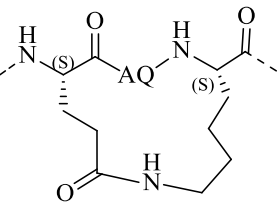
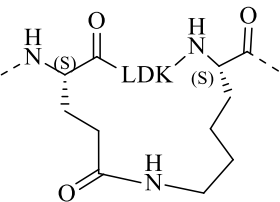
[0010] Z₁ is selected from alanine (A) and glutamic acid (E);

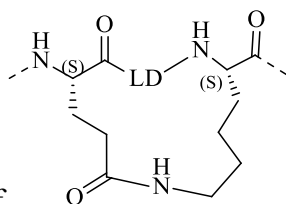
[0011] Z₂ is selected from valine (V) and isoleucine (I);

[0012] Z₃ is selected from isoleucine (I) and leucine (L);

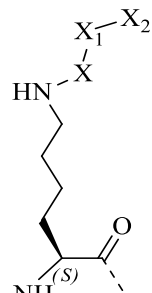
[0013] E₀ and K₀ indicate that the carboxyl group on the side chain of the glutamic acid and the amino group on the side chain of the lysine jointly form a lactam structure, and in this case,

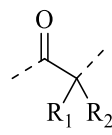
$K_0Z_1FZ_2E_0$ has a structure of , E_0AQK_0 has a structure of

, E_0LDKK_0 has a structure of , and E_0LDK_0 has

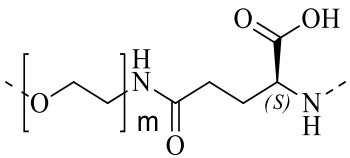
a structure of ;

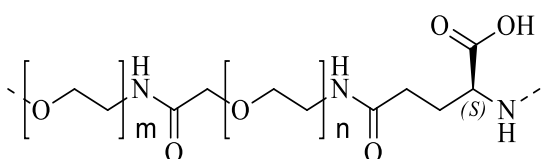
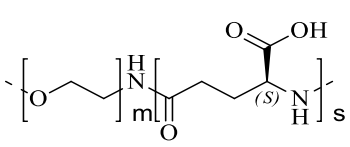
[0014] K^1 indicates that the amino group on the side chain of the lysine is connected to -X-

X_1-X_2 , and has a structure of ;

[0015] X is selected from ;

[0016] R_1 and R_2 are each independently selected from H and CH_3 ;

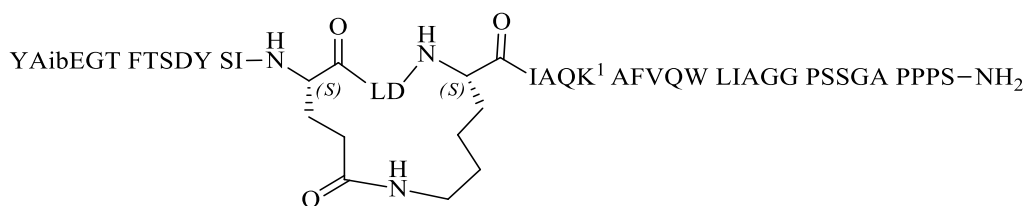
[0017] X_1 is selected from ,

, ,



[0022] In some embodiments of the present disclosure, the compound or the pharmaceutically acceptable salt thereof is selected from:





(P-4)

[0023] wherein

[0024] Aib, Z₀, Z₁, Z₂, Z₃ and K¹ are as defined in the present disclosure.

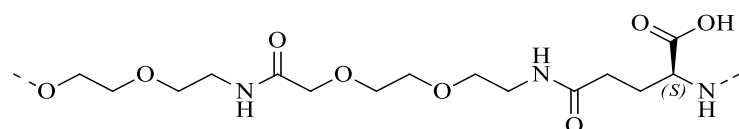
[0025] In some embodiments of the present disclosure, the X is selected from -C(=O)-CH₂-, and other variables are as defined in the present disclosure.

[0026] In some embodiments of the present disclosure, the m, n and p are each independently selected from 2, and other variables are as defined in the present disclosure.

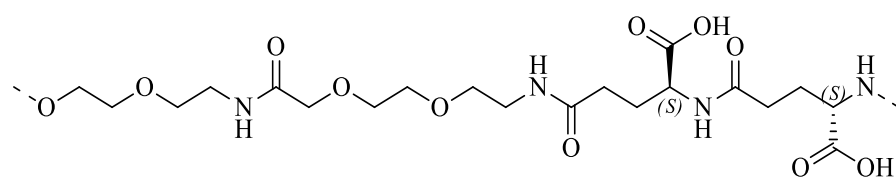
[0027] In some embodiments of the present disclosure, the s is selected from 2, and other variables are as defined in the present disclosure.

[0028] In some embodiments of the present disclosure, the q is selected from 15 and 17, and other variables are as defined in the present disclosure.

[0029] In some embodiments of the present disclosure, the X₁ is selected from

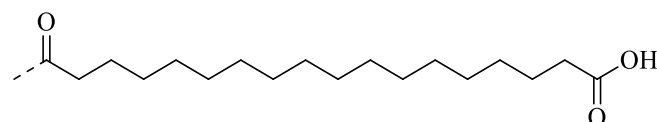


and

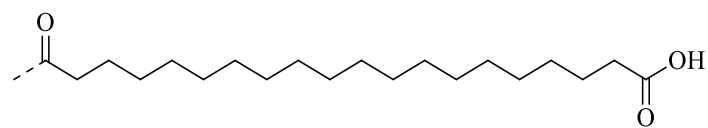


as defined in the present disclosure.

[0030] In some embodiments of the present disclosure, the X₂ is selected from



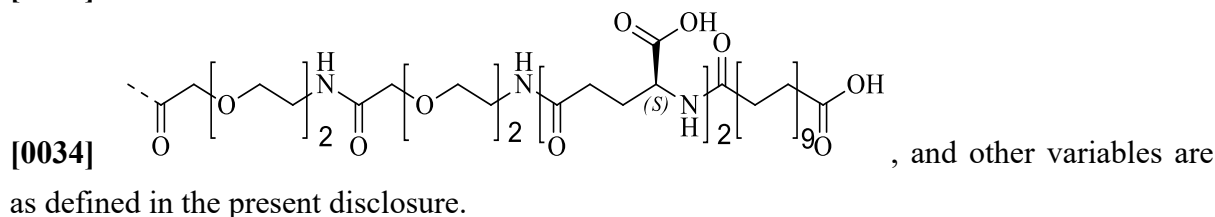
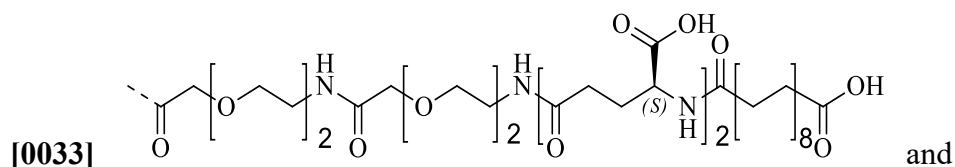
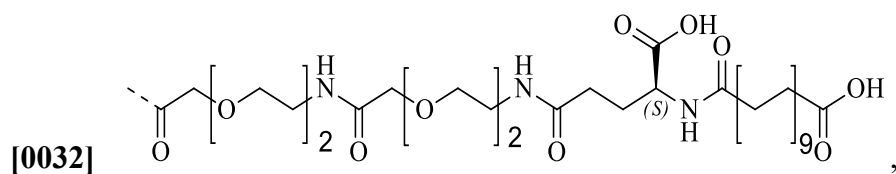
and



, and other variables are as defined in

the present disclosure.

[0031] In some embodiments of the present disclosure, the -X-X₁-X₂ is selected from



[0035] The present disclosure provides a compound represented by the following formula or a pharmaceutically acceptable salt thereof,

YAibEGT FTSDY SIAibLD KK¹AQK₀ AFVE₀W LIAGG PSSGA PPPS₀

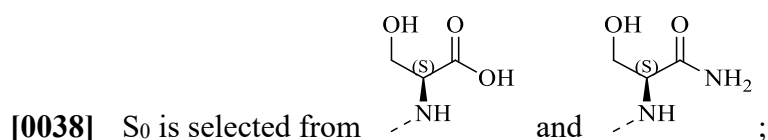
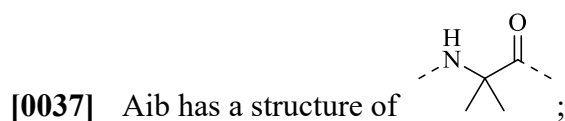
YAibEGT FTSDY SIAibLD KK¹ANK₀ AFVE₀W LIAGG PSSGA PPPS₀

YAibEGT FTSDY SIAibLD KK¹AQK₀ EFVE₀W LIAGG PSSGA PPPS₀

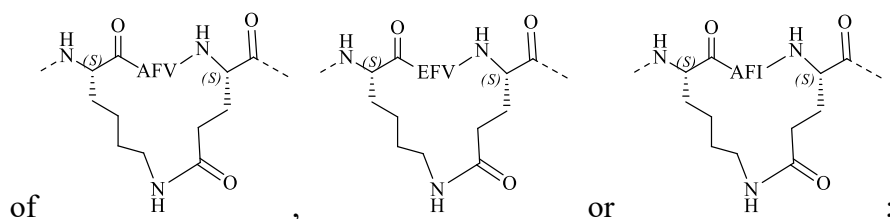
YAibEGT FTSDY SIAibLD KK¹AQK₀ AFIE₀W LIAGG PSSGA PPPS₀

YAibEGT FTSDY SIAibLD KK¹AQK₀ AFVE₀W LLAGG PSSGA PPPS₀

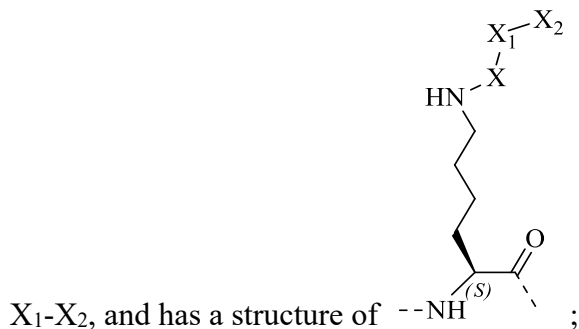
[0036] wherein

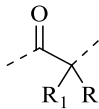


[0039] K₀ and E₀ indicate that the amino group on the side chain of the lysine and the carboxyl group on the side chain of the glutamic acid jointly form a lactam structure, and have a structure

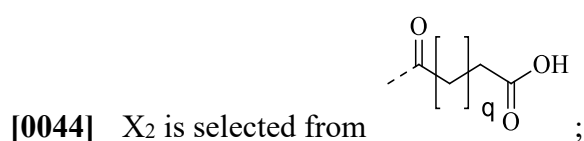
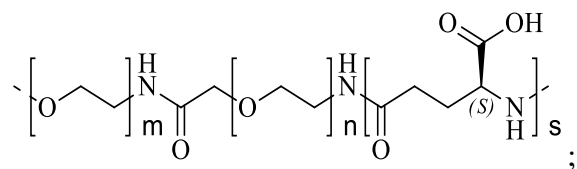
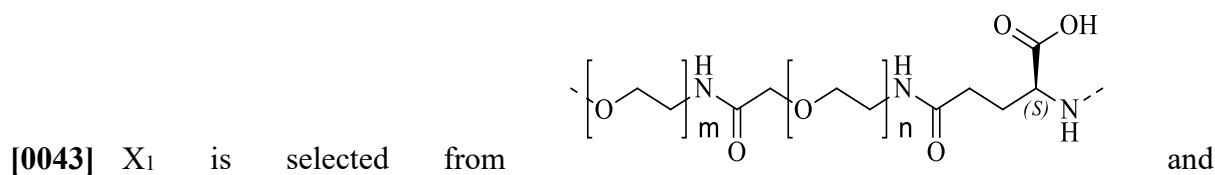


[0040] K¹ indicates that the amino group on the side chain of the lysine is connected to -X-



[0041] X is selected from  ;

[0042] R₁ and R₂ are each independently selected from H;



[0045] m and n are each independently selected from 2 and 3;

[0046] s is selected from 2 and 3;

[0047] q is selected from 15, 16, 17, 18 and 19.

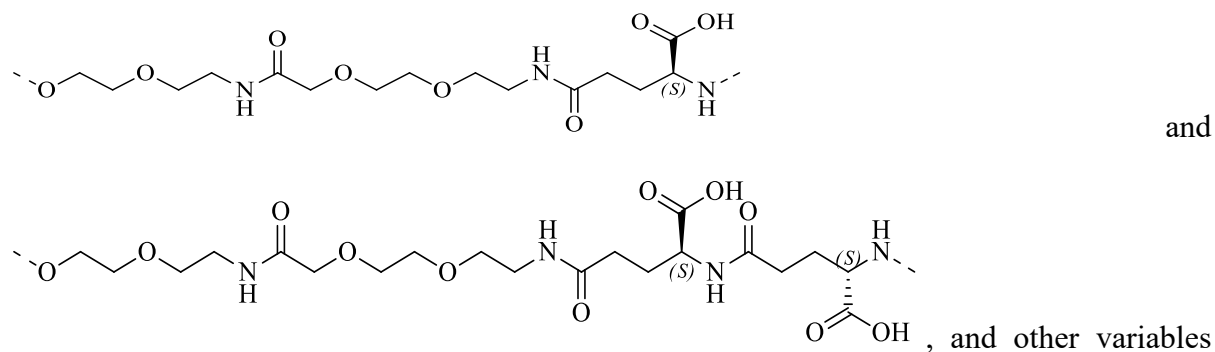
[0048] In some embodiments of the present disclosure, the X is selected from -C(=O)-CH₂-, and other variables are as defined in the present disclosure.

[0049] In some embodiments of the present disclosure, the m and n are each independently selected from 2, and other variables are as defined in the present disclosure.

[0050] In some embodiments of the present disclosure, the *s* is selected from 2, and other variables are as defined in the present disclosure.

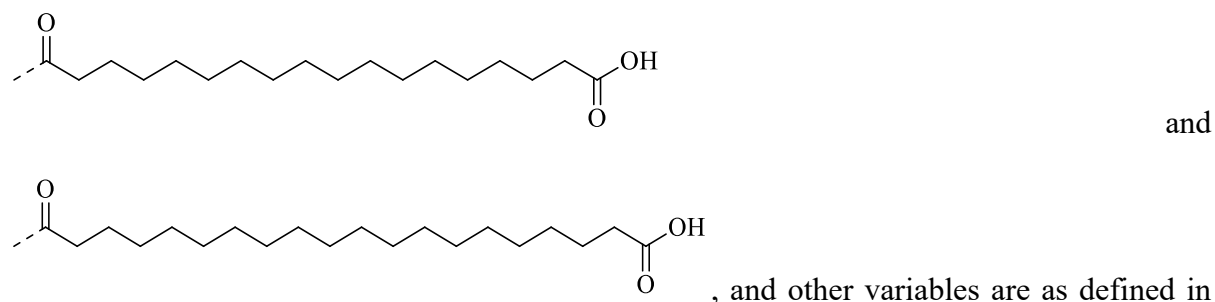
[0051] In some embodiments of the present disclosure, the *q* is selected from 15 and 17, and other variables are as defined in the present disclosure.

[0052] In some embodiments of the present disclosure, the *X*₁ is selected from



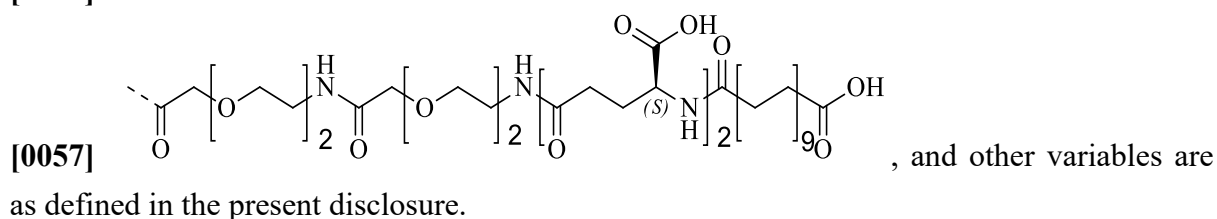
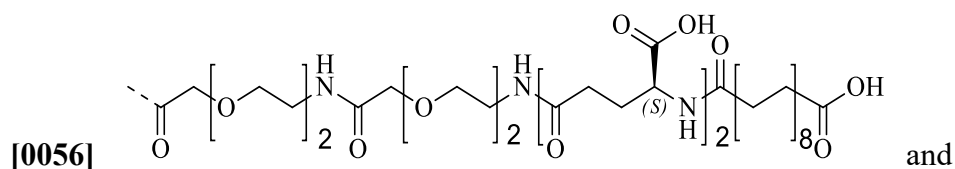
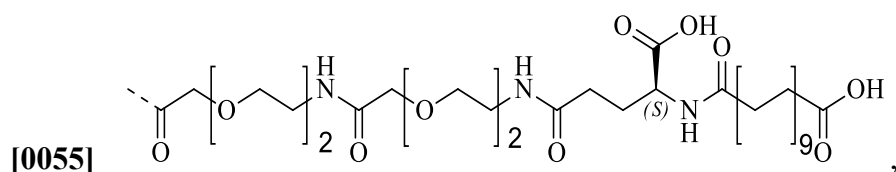
are as defined in the present disclosure.

[0053] In some embodiments of the present disclosure, the *X*₂ is selected from



the present disclosure.

[0054] In some embodiments of the present disclosure, the -*X*-*X*₁-*X*₂ is selected from



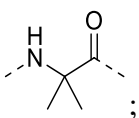
[0058] The present disclosure provides a compound represented by the following formula or a pharmaceutically acceptable salt thereof,

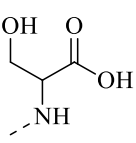
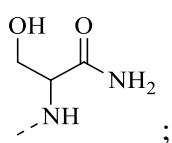
YAibEGT FTSDY SIAibLD KK¹AZ₀K₀ Z₁FZ₂E₀W LZ₃AGG PSSGA PPPS₀

YAibEGT FTSDY SIAibLD KE₀AQK₀ AFVK¹W LIAGG PSSGA PPPS₀

YAibEGT FTSDY SIE₀LD KK₀AQK¹ AFVQW LIAGG PSSGA PPPS₀

[0059] wherein

[0060] Aib has a structure of  ;

[0061] S₀ is selected from  and  ;

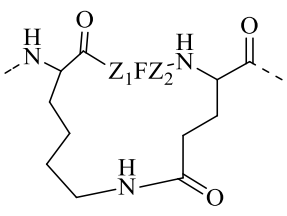
[0062] Z₀ is selected from glutamine (Q) and asparagine (N);

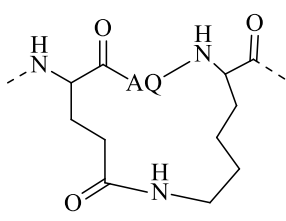
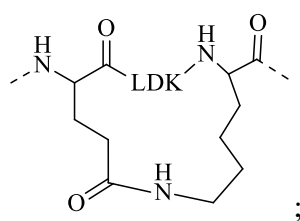
[0063] Z₁ is selected from alanine (A) and glutamic acid (E);

[0064] Z₂ is selected from valine (V) and isoleucine (I);

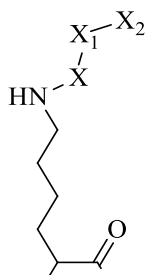
[0065] Z₃ is selected from isoleucine (I) and leucine (L);

[0066] E₀ and K₀ indicate that the carboxyl group on the side chain of the glutamic acid and the amino group on the side chain of the lysine jointly form a lactam structure, and in this case,

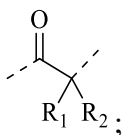
K₀Z₁FZ₂E₀ has a structure of  , E₀AQK₀ has a structure of

 , E₀LDKK₀ has a structure of  ;

[0067] K¹ indicates that the amino group on the side chain of the lysine is connected to -X-



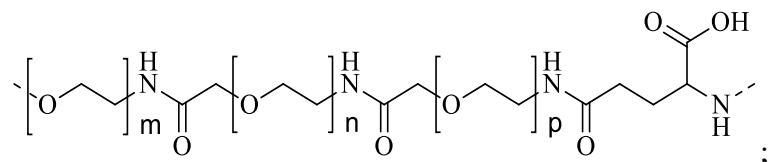
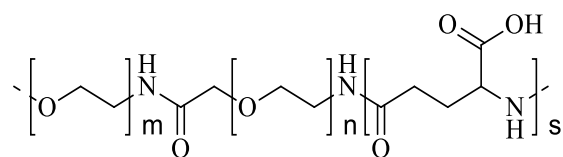
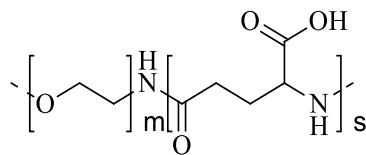
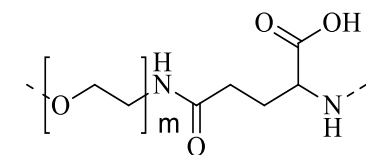
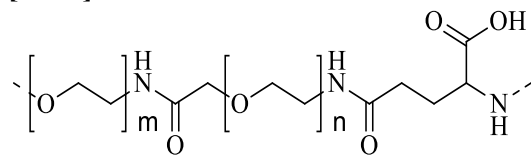
X₁-X₂, and has a structure of --NH-- ;



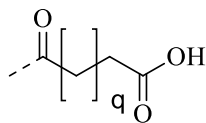
[0068] X is selected from

[0069] R₁ and R₂ are each independently selected from H;

[0070] X₁ is selected from



and



[0071] X₂ is selected from

[0072] m, n and p are each independently selected from 2 and 3;

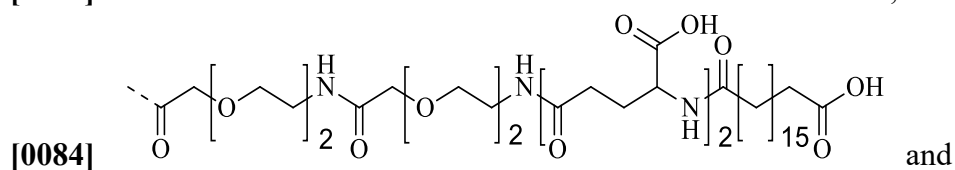
[0073] s is independently selected from 2 and 3;

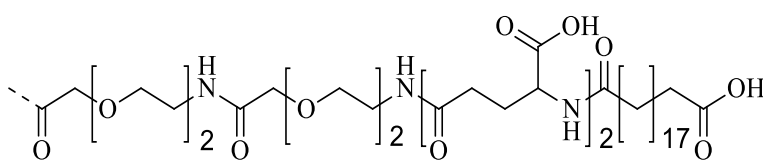
[0074] q is independently selected from 15, 16, 17, 18 and 19.

[0075] In some embodiments of the present disclosure, the X is selected from -C(=O)-CH₂-, and other variables are as defined in the present disclosure.

[0076] In some embodiments of the present disclosure, the m, n and p are each independently selected from 2, and other variables are as defined in the present disclosure.

[0080] In some embodiments of the present disclosure, the X₁ is selected from

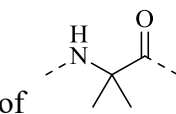


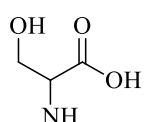
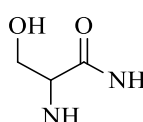
[0085] , and other variables are as defined in the present disclosure.

[0086] The present disclosure provides a compound represented by the following formula or a pharmaceutically acceptable salt thereof,

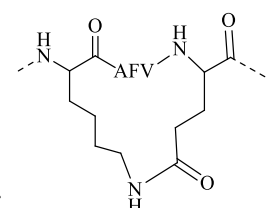


[0087] wherein

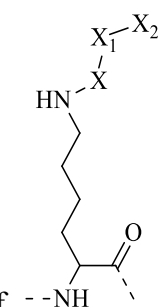
[0088] Aib has a structure of ;

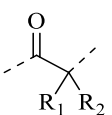
[0089] S_0 is selected from  and ;

[0090] K_0 and E_0 indicate that the amino group on the side chain of the lysine and the carboxyl group on the side chain of the glutamic acid jointly form a lactam structure, and have a structure

of ;

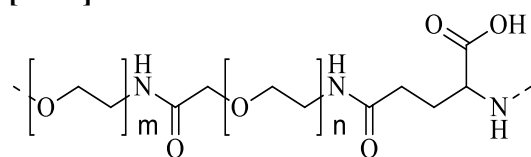
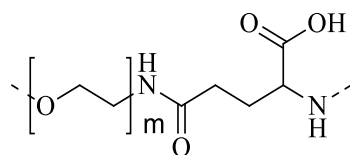
[0091] K^1 indicates that the amino group on the side chain of the lysine is connected to -X-

X_1 - X_2 , and has a structure of ;

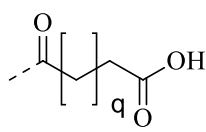
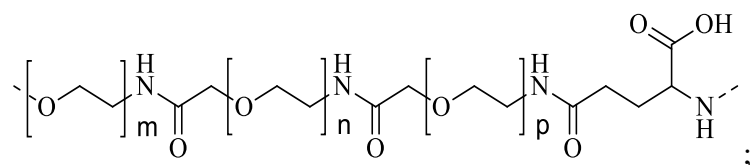
[0092] X is selected from ;

[0093] R_1 and R_2 are each independently selected from H;

[0094] X₁ is selected from



and



[0095] X₂ is selected from

[0096] m, n and p are each independently selected from 2 and 3;

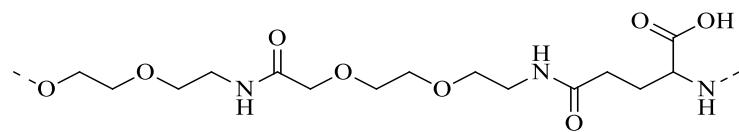
[0097] q is independently selected from 15, 16, 17, 18 and 19.

[0098] In some embodiments of the present disclosure, the X is selected from -C(=O)-CH₂-, and other variables are as defined in the present disclosure.

[0099] In some embodiments of the present disclosure, the m, n and p are each independently selected from 2, and other variables are as defined in the present disclosure.

[0100] In some embodiments of the present disclosure, the q is independently selected from 17, and other variables are as defined in the present disclosure.

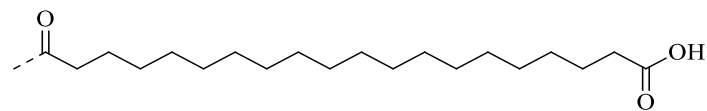
[0101] In some embodiments of the present disclosure, the X₁ is selected from



, and other variables are as defined in

the present disclosure.

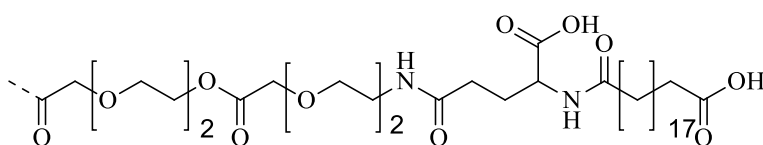
[0102] In some embodiments of the present disclosure, the X₂ is selected from



, and other variables are as defined in

the present disclosure.

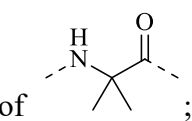
[0103] In some embodiments of the present disclosure, the -X-X₁-X₂ is selected from

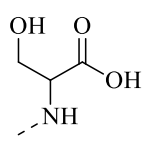
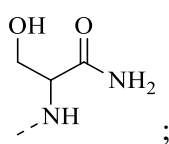
[0104]  , and other variables are as defined in the present disclosure.

[0105] The present disclosure provides a compound represented by the following formula or a pharmaceutically acceptable salt thereof,

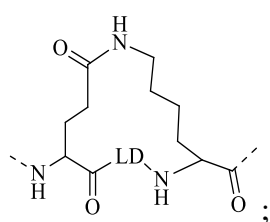


[0106] wherein

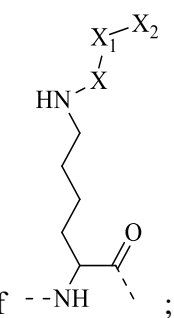
[0107] Aib has a structure of  ;

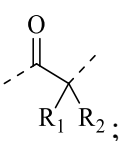
[0108] S_0 is selected from  and  ;

[0109] E_0 and K_0 indicate that the carboxyl group on the side chain of the glutamic acid and the amino group on the side chain of the lysine jointly form a lactam structure, and have a

structure of  ;

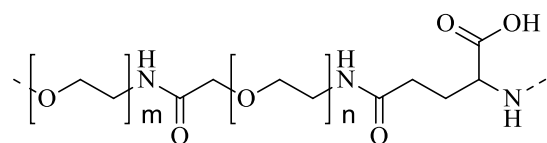
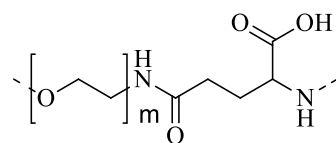
[0110] K^1 indicates that the amino group on the side chain of the lysine is connected to -X-

X_1 - X_2 , and has a structure of  ;

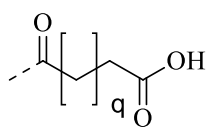
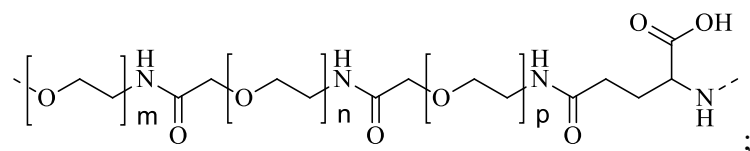
[0111] X is selected from  ;

[0112] R_1 and R_2 are each independently selected from H;

[0113] X₁ is selected from



and



[0114] X₂ is selected from

[0115] m, n and p are each independently selected from 2 and 3;

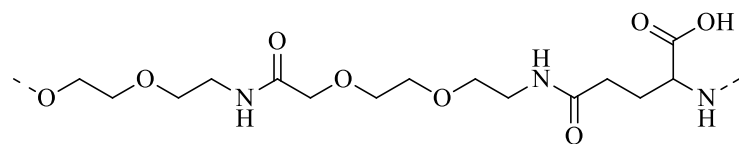
[0116] q is independently selected from 15, 16, 17, 18 and 19.

[0117] In some embodiments of the present disclosure, the X is selected from -C(=O)-CH₂-, and other variables are as defined in the present disclosure.

[0118] In some embodiments of the present disclosure, the m, n and p are each independently selected from 2, and other variables are as defined in the present disclosure.

[0119] In some embodiments of the present disclosure, the q is independently selected from 17, and other variables are as defined in the present disclosure.

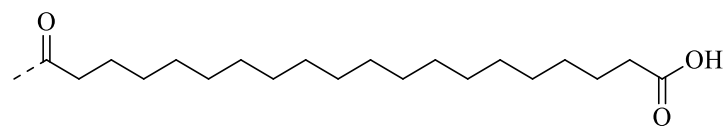
[0120] In some embodiments of the present disclosure, the X₁ is selected from



, and other variables are as defined in

the present disclosure.

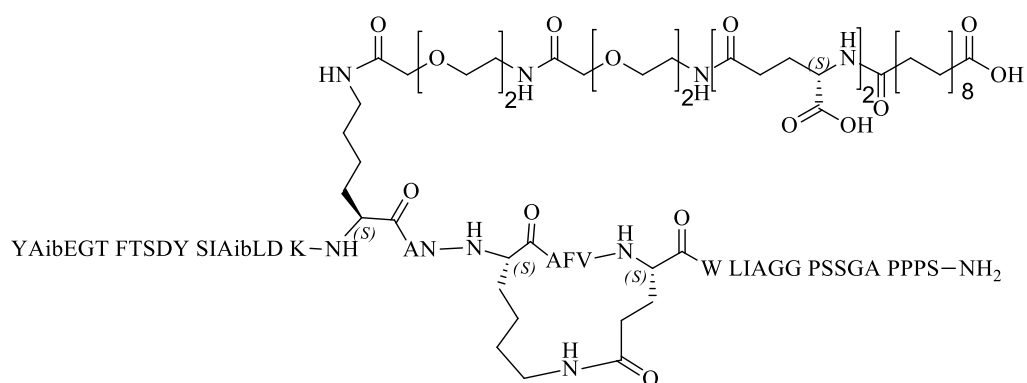
[0121] In some embodiments of the present disclosure, the X₂ is selected from



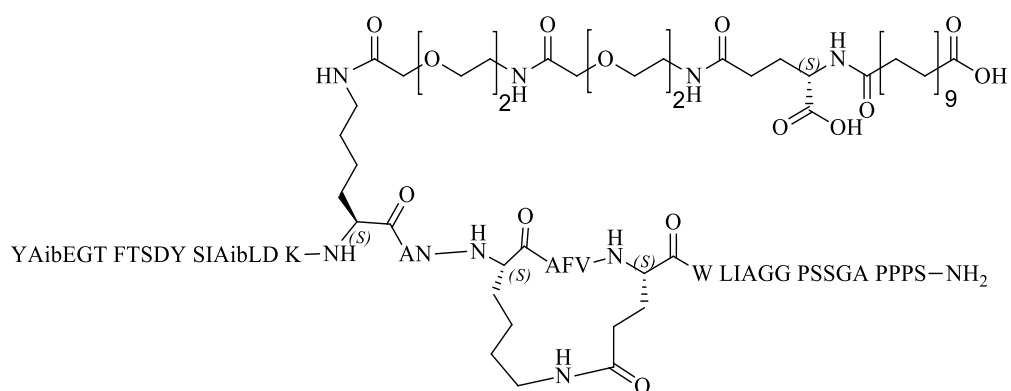
, and other variables are as defined in

the present disclosure.

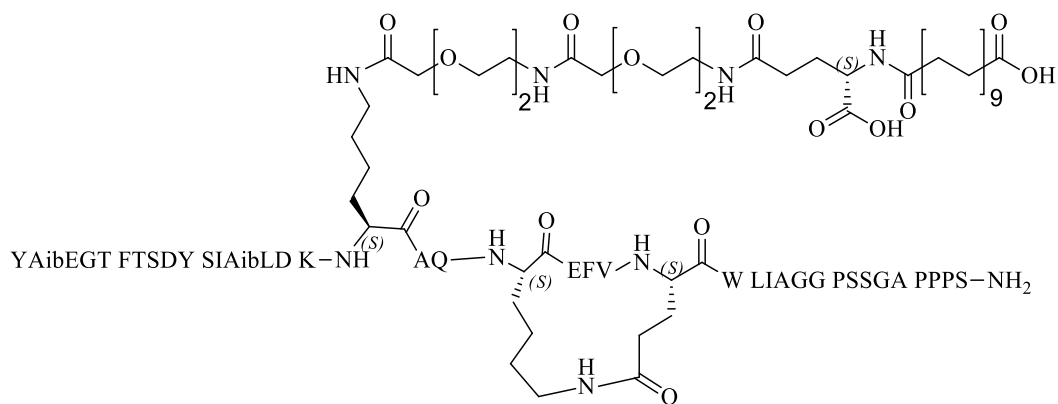
[0122] In some embodiments of the present disclosure, the -X-X₁-X₂ is selected from



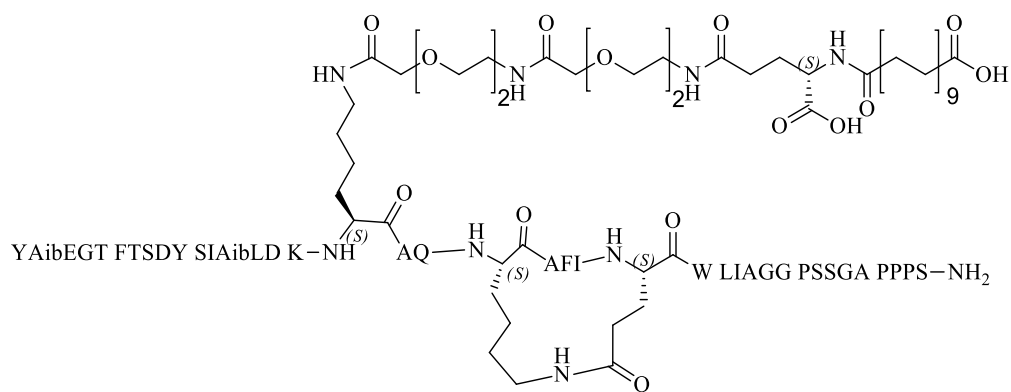
WX-003



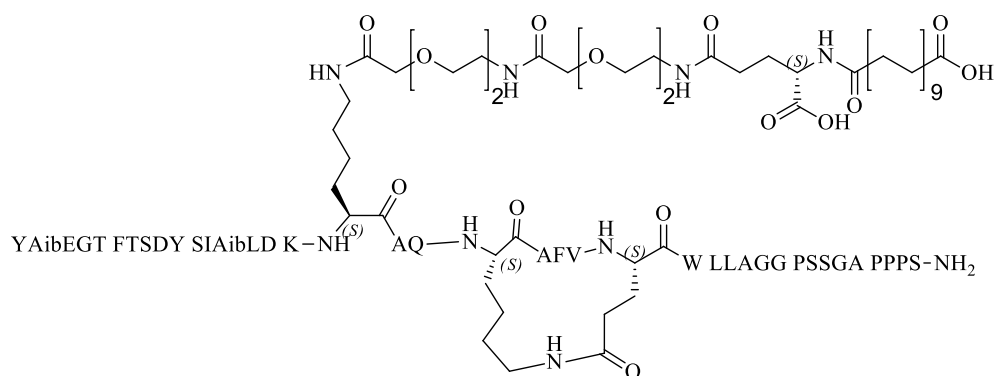
WX-004



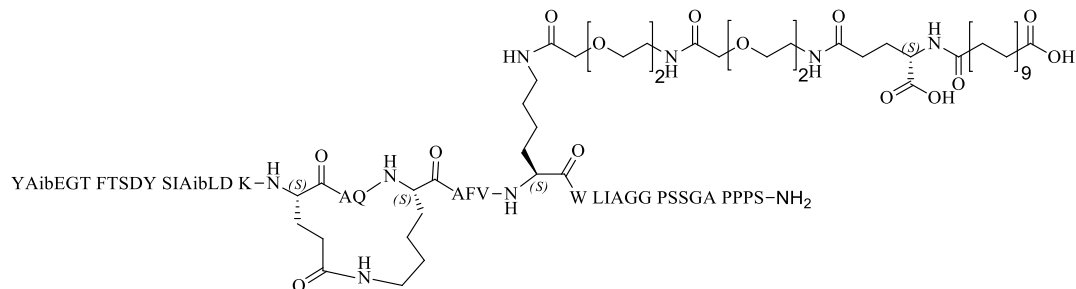
WX-005



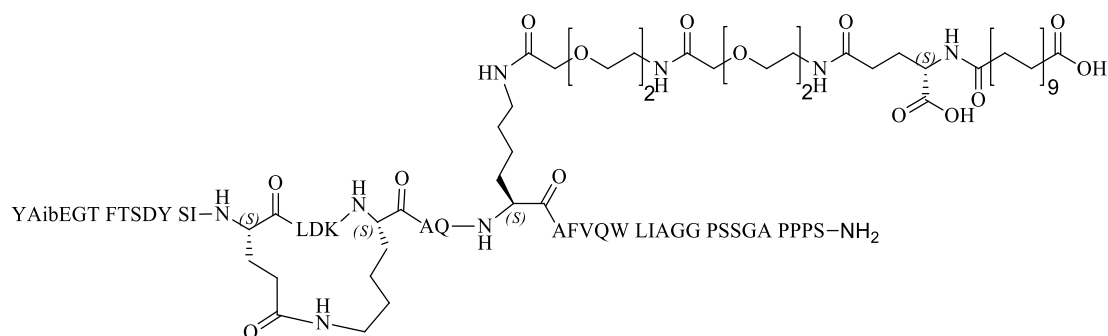
WX-006



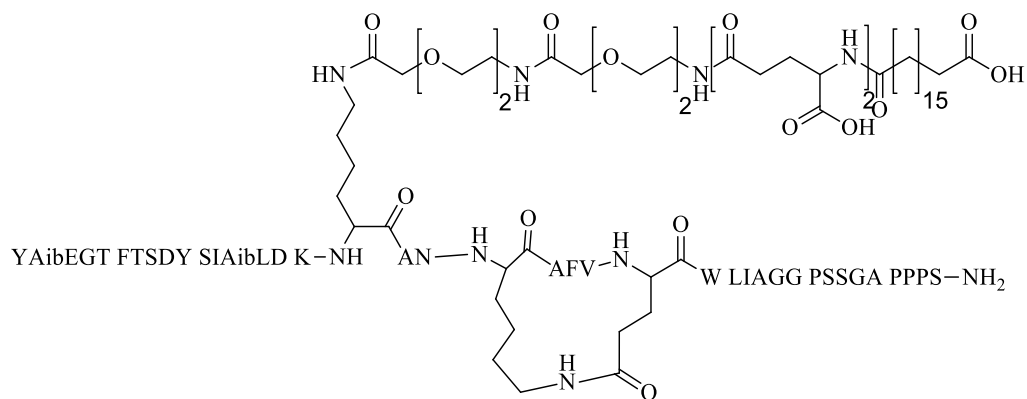
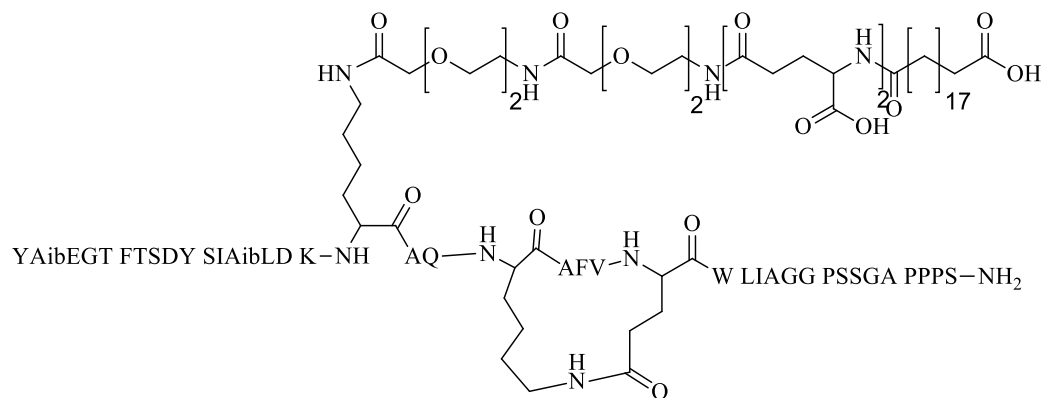
WX-007

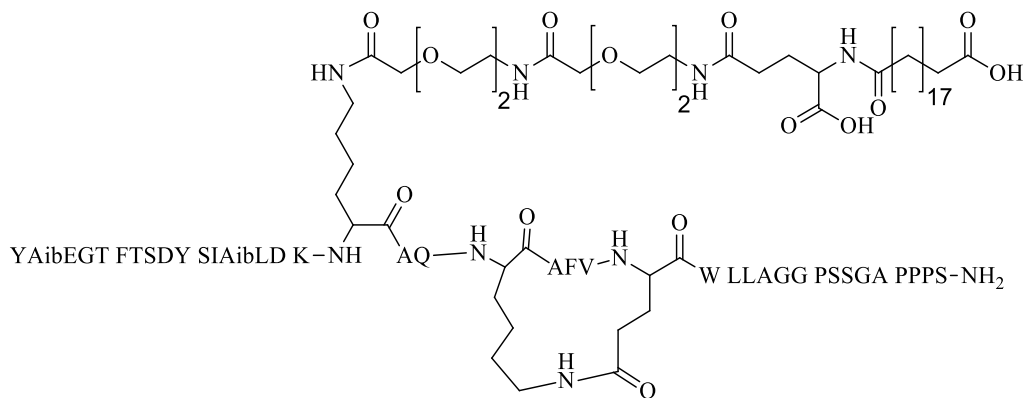
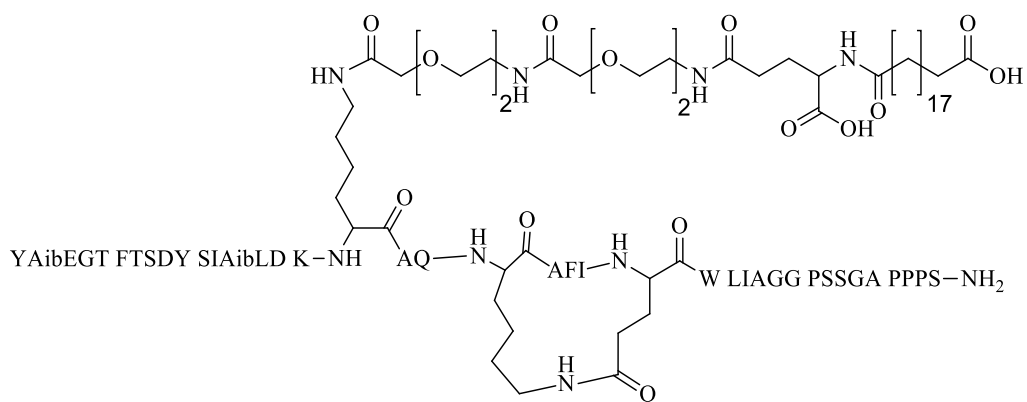
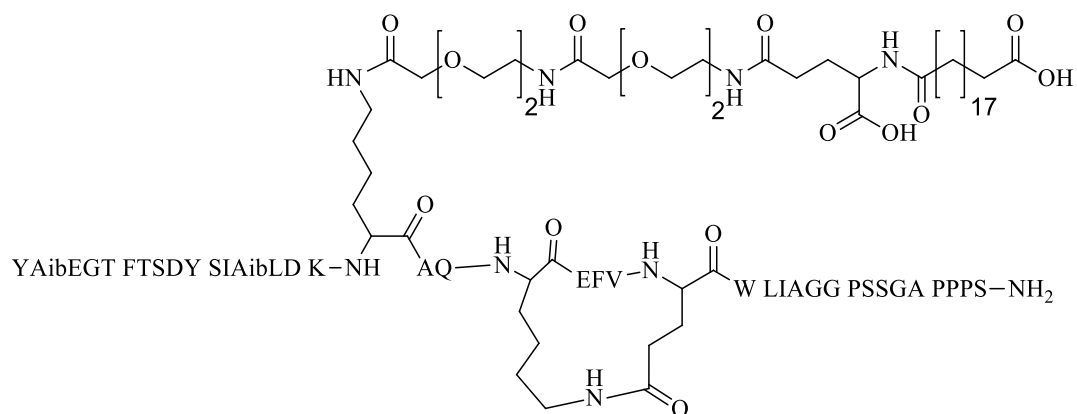
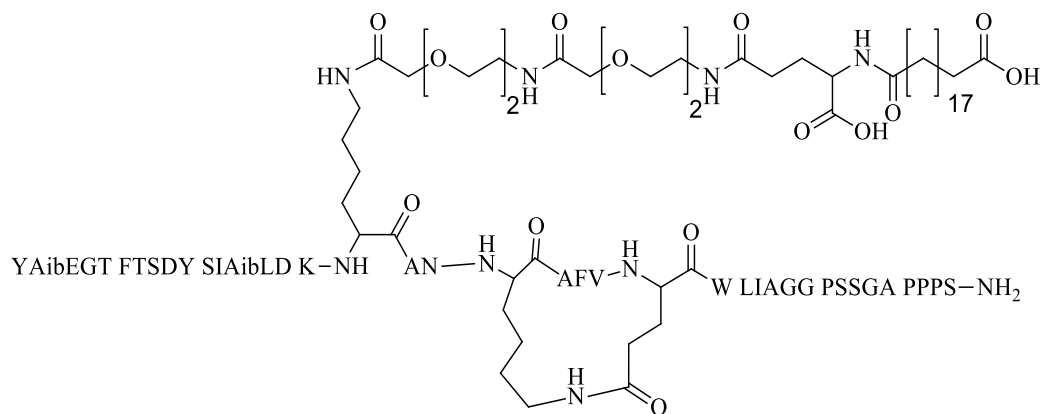


WX-008



WX-009





100 µg/mL G418 (Invitrogen) and 100 µg/mL Hygromycin B (Invitrogen). On the day of the test, the cell culture medium was aspirated, rinsed once with extracellular fluid, and then 0.25% Trypsin-EDTA (Invitrogen) solution was added, followed by digestion at room temperature for 3 to 5 minutes. After the digestion solution was aspirated, the cells were resuspended with extracellular fluid, and transferred to a laboratory dish for electrophysiological recording for further use.

[0135] b) Preparation of Intracellular and Extracellular Fluid

[0136] Extracellular fluid needs to be prepared once a month. Intracellular fluid needs to be subpackaged and frozen at -20°C.

[0137] Extracellular fluid (mM): 145 NaCl, 4 KCl, 2 CaCl₂, 1 MgCl₂, 10 Glucose and 10 HEPES, adjusted to pH = 7.4 with NaOH, with an osmotic pressure of 295 mOsm.

[0138] Intracellular fluid (mM): 120 KCl, 31.25 KOH, 5.374 CaCl₂, 1.75 MgCl₂, 4 Na₂ATP, 10 HEPES and 10 EGTA, adjusted to pH = 7.2 with KOH, with an osmotic pressure of 285 mOsm.

[0139] c) Preparation of Compounds

[0140] The compound is dissolved in DMSO into a 20 mM stock solution. On the day of the test, the compound stock solution is serially 3-fold diluted with DMSO, i.e., 10 µL of the compound stock solution is added to 20 µL of DMSO to obtain 6 intermediate concentrations of the compound serially diluted with DMSO, respectively. The intermediate concentrations are 20, 6.66, 2.22, 0.74, 0.24 and 0.082 mM, respectively. Then 10 µL of the intermediate concentration of the compound is taken and added to 4990 µL of extracellular fluid, and 500-fold diluted to obtain the final concentration to be tested. The highest test concentration is 40 µM, which are 40, 13.3, 4.44, 1.48, 0.49 and 0.16 µM, respectively. The content of DMSO in the final concentration to be tested does not exceed 0.2%, and this concentration of DMSO has no effect on hERG potassium channels.

[0141] d) Electrophysiological Recording Process

[0142] In CHO (Chinese Hamster Ovary) cells stably expressing hERG potassium channels, hERG potassium channel current is recorded by whole cell patch clamp technique at room temperature. The glass microelectrode is made of a glass electrode filament (BF150-86-10, Sutter) pulled by a glass microelectrode puller. The tip resistance after pouring the electrode

internal solution into the electrode is about 2-5 MΩ. The glass microelectrode is inserted into the amplifier probe to connect to an Axopatch 200B (Molecular Devices) patch clamp amplifier. The clamping potential and data record are controlled and recorded by the computer with pClamp 10 software, and the sampling frequency is 10 kHz, and the wave filtering frequency is 2 kHz. After whole-cell recordings are obtained, the cells are clamped at -80 mV, and the step voltage of hERG potassium current (I_{hERG}) is evoked from -80 mV to +20 mV with a depolarizing voltage for 2 s, then repolarized to -50 mV, and returned to -80 mV after 1 s. This voltage stimulation is given every 10 s, and the administration process is started after confirming that the hERG potassium current is stable (1 min). Serial administration is started from a low test concentration of the compounds, with each test concentration administered for at least 1 minute. At least 3 cells ($n \geq 3$) are tested for each concentration of the compounds, and at least 2 cells ($n \geq 2$) are tested for each concentration of the positive compounds.

[0143] e) Data Analysis

[0144] In each complete current recording, the inhibition percentage for each compound effect concentration can be calculated based on the percentage of peak current in the negative control. The dose-effect relationship curve is obtained by fitting the standard Hill equation, and the specific equation is as follows:

$$\text{[0145]} \quad I_{(C)} = I_b + (I_{fr} - I_b) * c^n / (IC_{50}^n + c^n)$$

[0146] c is the compound test concentration, n is the slope

[0147] Both curve fitting and inhibition rate calculation are analyzed and completed by Qpatch analysis software. If the inhibition rate at the lowest concentration exceeds the half inhibition rate or the inhibition rate at the highest concentration does not reach the half inhibition rate, the corresponding IC_{50} of the compound is lower than the lowest concentration or the IC_{50} value is greater than the highest concentration.

[0148] C. Test Results and Conclusions

[0149] The compounds in the present disclosure have no hERG-related risks.

[0150] Experimental Example: Inhibition for Cytochrome P450 Isoenzyme

[0151] A. Experimental Purpose

[0152] To determine the inhibitory effect of the test compounds on the activity of human liver

microsomal cytochrome P450 isozymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4).

[0153] B. Experimental Operation

[0154] First, the test compound (10 mM) is gradient diluted to prepare a working solution (100×final concentration). Concentrations of the working solution are 5 mM, 1.5 mM, 0.5 mM, 0.15 mM, 0.05 mM, 0.015 mM and 0.005 mM, respectively. At the same time, positive inhibitors of the P450 isozymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) and the working solution of the specific substrate mixture thereof are prepared; human liver microsomes frozen in a -80°C refrigerator are thawed on ice. Once the human liver microsomes are completely dissolved, the solution is diluted with PBS (phosphate buffer solution) to prepare a working solution with a certain concentration (0.253mg/mL); 20 µL of substrate mixture solution is added to a reaction plate (20 µL of PB is added to the Blank well). At the same time, 158 µL of human liver microsome working solution is added to the reaction plate, and the reaction plate is placed on ice for use; at this time, 2 µL of the test compound (N=1) and positive inhibitors (N=2) of each concentration are added to the corresponding well, and the group without inhibitors (the test compound or positive inhibitor) is added with the corresponding organic solvent, as the sample of the control group; after pre-incubating in a 37°C water bath for 10 minutes, 20 µL of NADPH solution is added to the reaction plate, and incubated in a 37°C water bath for 10 minutes; 400 µL of cold acetonitrile solution is added to terminate the reaction; the reaction plate is placed on a shaker and shaken for 10 minutes; the mixture is centrifuged at 4,000 rpm for 20 minutes; 200 µL of the supernatant is taken and added to 100 µL of water to dilute the sample; finally the reaction plate is sealed and shaken up for LC/MS/MS detection.

[0155] C. Experimental Results and Conclusions

[0156] The compounds in the present disclosure have no CYP inhibition-related risks.

[0157] Technical Effect

[0158] The compounds in the present disclosure have strong agonist activity on GLP-1R/GIPR; the compounds in the present disclosure have excellent pharmacokinetic properties; the compounds in the present disclosure have excellent plasma stability; the compounds in the

present disclosure have very high plasma-protein binding; the compounds in the present disclosure have excellent *in vivo* efficacy.

[0159] Definition and Description

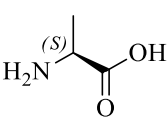
[0160] Unless otherwise specified, the following terms and phrases used herein have the following meanings. A specific term or phrase should not be considered indefinite or unclear in the absence of a particular definition, but should be understood according to the common meaning. When a trade name appears herein, it is intended to refer to its corresponding commercial product or active ingredient thereof.

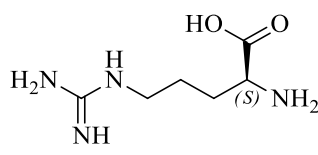
[0161] The term "pharmaceutically acceptable" is used herein in terms of those compounds, materials, compositions, and/or dosage forms, which are suitable for use in contact with human and animal tissues within the scope of reliable medical judgment, without excessive toxicity, irritation, anaphylactic reaction or other problems or complications, commensurate with a reasonable benefit/risk ratio.

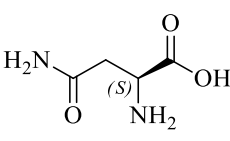
[0162] The term "pharmaceutically acceptable salt" refers to a salt of the compound of the present disclosure that is prepared by reacting the compound having a specific substituent of the present disclosure with a relatively non-toxic acid or base. When the compound of the present disclosure contains a relatively acidic functional group, a base addition salt can be obtained by contacting the compound with a sufficient amount of a base in a pure solution or a suitable inert solvent. The pharmaceutically acceptable base addition salt includes a salt of sodium, potassium, calcium, ammonium, organic amine or magnesium, or similar salts. When the compound of the present disclosure contains a relatively basic functional group, an acid addition salt can be obtained by contacting the compound with a sufficient amount of acid in a pure solution or a suitable inert solvent. Examples of the pharmaceutically acceptable acid addition salt include an inorganic acid salt, wherein the inorganic acid includes, for example, hydrochloric acid, hydrobromic acid, nitric acid, carbonic acid, bicarbonate, phosphoric acid, monohydrogen phosphate, dihydrogen phosphate, sulfuric acid, hydrogen sulfate, hydroiodic acid, phosphorous acid, and the like; and an organic acid salt, wherein the organic acid includes, for example, acetic acid, propionic acid, isobutyric acid, maleic acid, malonic acid, benzoic acid, succinic acid, suberic acid, fumaric acid, lactic acid, mandelic acid, phthalic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, citric acid, tartaric acid, and

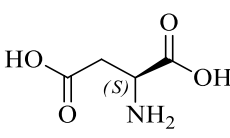
methanesulfonic acid, and the like; and salts of amino acid (such as arginine and the like), and a salt of an organic acid such as glucuronic acid and the like. Certain specific compounds of the present disclosure contain both basic and acidic functional groups, thus can be converted to any base or acid addition salt.

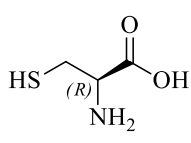
[0163] The pharmaceutically acceptable salt of the present disclosure can be prepared from the parent compound that contains an acidic or basic moiety by conventional chemical methods. Generally, such salt can be prepared by reacting the free acid or base form of the compound with a stoichiometric amount of an appropriate base or acid in water or an organic solvent or a mixture thereof.

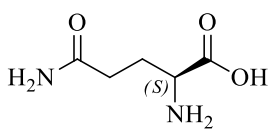
[0164] As used herein, A or Ala represents alanine, and has a structure of  ; R

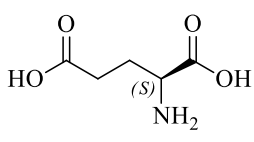
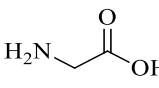
or Arg represents arginine, and has a structure of  ; N or Asn

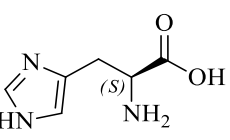
represents asparagine, and has a structure of  ; D or Asp represents aspartic

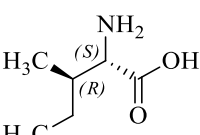
acid, and has a structure of  ; C or Cys represents cysteine, and has a

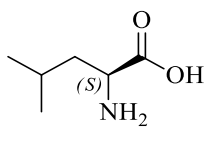
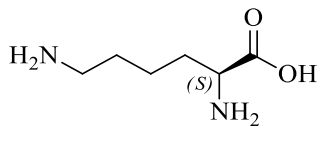
structure of  ; Q or Gln represents glutamine, and has a structure of

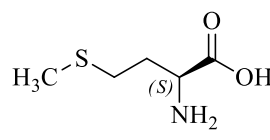
 ; E or Glu represents glutamic acid, and has a structure of

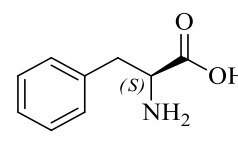
 ; G or Gly represents glycine, and has a structure of  ; H or

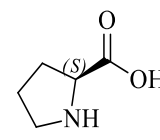
His represents histidine, and has a structure of  ; I or Ile represents isoleucine,

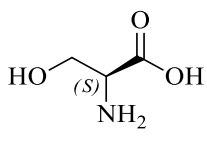
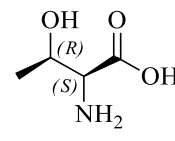
and has a structure of  ; L or Leu represents leucine, and has a structure of

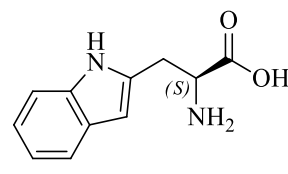
 ; K or Lys represents lysine, and has a structure of  ;

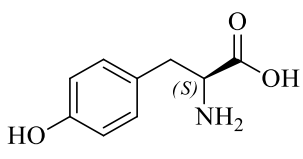
M or Met represents methionine, and has a structure of  ; F or Phe

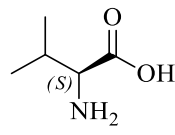
represents phenylalanine, and has a structure of  ; P or Pro represents proline,

and has a structure of  ; S or Ser represents serine, and has a structure of

 ; T or Thr represents threonine, and has a structure of  ; W or Trp

represents tryptophan, and has a structure of  ; Y or Tyr represents

tyrosine, and has a structure of  ; V or Val represents valine, and has a

structure of  .

[0165] The term "treating" includes inhibiting, slowing, stopping or reversing the progression or severity of an existing symptom or disease.

[0166] Unless otherwise specified, the term "isomer" is intended to include a geometric isomers, a *cis-trans* isomer, a stereoisomer, an enantiomer, an optical isomer, diastereoisomers and a tautomeric isomer.

[0167] The compounds of the present disclosure may exist in specific geometric or

stereoisomeric forms. The present disclosure contemplates all such compounds, including *cis* and *trans* isomers, (-)-and (+)-enantiomers, (*R*)-and (*S*)-enantiomers, diastereoisomers, (*D*)-isomers, (*L*)-isomers, and racemic and other mixtures thereof, such as enantiomers or diastereomer enriched mixtures, all of which are within the scope of the present disclosure. Additional asymmetric carbon atoms may be present in substituents such as alkyl. All these isomers and their mixtures are encompassed within the scope of the present disclosure.

[0168] Unless otherwise specified, the term "enantiomer" or "optical isomer" refers to stereoisomers that are mirror images of each other.

[0169] Unless otherwise specified, the term "*cis-trans* isomer" or "geometric isomer" is caused by the inability to rotate freely of double bonds or single bonds of ring-forming carbon atoms.

[0170] Unless otherwise specified, the term "diastereomer" refers to a stereoisomer in which a molecule has two or more chiral centers and the relationship between the molecules is not mirror images.

[0171] Unless otherwise specified, "(+)" refers to dextrorotation, "(-)" refers to levorotation, and or "(±)" refers to racemic.

[0172] Unless otherwise specified, the absolute configuration of a stereogenic center is represented by a wedged solid bond () and a wedged dashed bond (), and the relative configuration of a stereogenic center is represented by a straight solid bond () and a straight dashed bond (), a wave line () is used to represent a wedged solid bond () or a wedged dashed bond (), or the wave line () is used to represent a straight solid bond () and a straight dashed bond ().

[0173] Unless otherwise specified, the terms "enriched in one isomer", "enriched in isomers", "enriched in one enantiomer" or "enriched in enantiomers" refer to the content of one of the isomers or enantiomers is less than 100%, and the content of such isomer or enantiomer is greater than or equal to 60%, or greater than or equal to 70%, or greater than or equal to 80%, or greater than or equal to 90%, or greater than or equal to 95%, or greater than or equal to 96%, or greater than or equal to 97%, or greater than or equal to 98%, or greater than or equal to 99%, or greater than or equal to 99.5%, or greater than or equal to 99.6%, or greater than or

equal to 99.7%, or greater than or equal to 99.8%, or greater than or equal to 99.9%.

[0174] Unless otherwise specified, the term "isomer excess" or "enantiomeric excess" refers to the difference between the relative percentages of two isomers or two enantiomers. For example, if the content of one isomer or enantiomer is 90%, and the content of the other isomer or enantiomer is 10%, the isomer or enantiomer excess (ee value) is 80%.

[0175] Optically active (*R*)- and (*S*)-isomers, as well as *D* and *L* isomers, can be prepared by chiral synthesis or chiral reagents or other conventional techniques. If one enantiomer of a compound of the present disclosure is desired, the pure desired enantiomer can be prepared by asymmetric synthesis or derivatization of chiral auxiliary followed by separating the resulting diastereomeric mixture and cleaving the auxiliary group. Alternatively, when the molecule contains a basic functional group (such as an amino) or an acidic functional group (such as a carboxyl), the compound reacts with an appropriate optically active acid or base to form a salt of the diastereomeric isomer which is then subjected to diastereomeric resolution through the conventional method in the art to give the pure enantiomer. In addition, the enantiomer and the diastereoisomer are generally isolated through chromatography which uses a chiral stationary phase and optionally combines with a chemical derivative method (such as carbamate generated from amine).

[0176] The compound of the present disclosure may contain an unnatural proportion of atomic isotope at one or more atom(s) that constitute the compound. For example, the compound can be labeled with a radioactive isotope, such as tritium (^3H), iodine-125 (^{125}I) or C-14 (^{14}C). For another example, deuterated drugs can be formed by replacing hydrogen with heavy hydrogen, the bond formed by deuterium and carbon is stronger than that by ordinary hydrogen and carbon, compared with non-deuterated drugs, deuterated drugs have the advantages of reduced toxic and side effects, increased drug stability, enhanced efficacy, extended biological half-life of drugs, etc. All isotopic variations of the compound of the present disclosure, whether radioactive or not, are encompassed within the scope of the present disclosure.

[0177] Unless otherwise specified, the term " C_{1-3} alkyl" refers to a linear or branched saturated hydrocarbon group consisting of 1 to 3 carbon atoms. The C_{1-3} alkyl includes C_{1-2} and C_{2-3} alkyl, etc.; it can be monovalent (such as methyl), divalent (such as methylene) or

multivalent (such as methine). Examples of C₁₋₃ alkyl include but are not limited to methyl (Me), ethyl (Et), propyl (including *n*-propyl and isopropyl), etc.

[0178] Unless otherwise specified, "S-NH₂" in the compounds of the present disclosure is used to indicate the replacement of the carboxyl on serine with an amide.

[0179] The structure of the compounds of the present disclosure can be confirmed by conventional methods known to those skilled in the art, and if the disclosure involves an absolute configuration of a compound, then the absolute configuration can be confirmed by means of conventional techniques in the art. For example, in the case of single crystal X-ray diffraction (SXRD), the absolute configuration can be confirmed by collecting diffraction intensity data from the cultured single crystal using a Bruker D8 venture diffractometer with CuK α radiation as the light source and scanning mode: ϕ/ω scan, and after collecting the relevant data, the crystal structure can be further analyzed by direct method (Shelxs97).

[0180] The compounds of the present disclosure can be prepared by a variety of synthetic methods known to those skilled in the art, including the specific embodiments listed below, the embodiments formed by their combination with other chemical synthesis methods, and equivalent alternatives known to those skilled in the art, preferred implementations include but are not limited to the examples of the present disclosure.

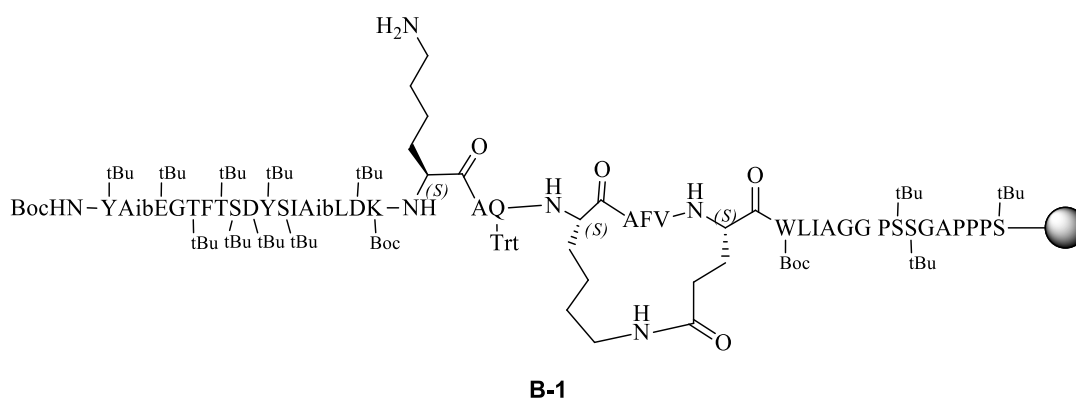
[0181] The solvents used in the present disclosure are commercially available.

[0182] The present disclosure uses the following abbreviations: "aq" represents water; "eq" represents equivalent; "DCM" represents dichloromethane; "PE" represents petroleum ether; "DMSO" represents dimethyl sulfoxide; "MeOH" represents methanol; "BOC" represents *tert*-butoxycarbonyl, which is an amine protecting group; "r.t." represents room temperature; "O/N" represents overnight; "THF" represents tetrahydrofuran; "Boc₂O" represents di-*tert*-butyl dicarbonate; "TFA" represents trifluoroacetic acid; "DIEA" represents diisopropylethylamine; "DMF" represents *N,N*-dimethylformamide; "HBTU" represents O-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate; "HOBT" represents 1-hydroxybenzotriazole; "HOAT" represents 1-hydroxy-7-azabenzotriazole; "DIC" represents *N,N'*-diisopropylcarbodiimide; "DBU" represents 1,8-diazabicyclo[5.4.0]undec-7-ene; "PhSiH" represents phenylsilane; "Pd(PPh₃)₄" represents tetrakis(triphenylphosphine)palladium.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0183] The present disclosure is described in detail by the examples below, but it does not mean that there are any adverse restrictions on the present disclosure. The present disclosure has been described in detail herein, and specific embodiments thereof have also been disclosed; for those skilled in the art, it is obvious to make various modifications and improvements to the embodiments of the present disclosure without departing from the spirit and scope of the present disclosure.

[0184] Intermediate B-1



[0185] Step 1: 1.1 g of 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamidomethylbenzhydryl amine resin (loading value, i.e., Sub =0.28 mmol/g) was weighed and added into a reaction column, and then DMF (50 mL) was added to the reaction column, bubbled with nitrogen for 2 hours. The waste was drained until no liquid flowed out, and the resin was washed with DMF (50 mL) 5 times (1 minute each time), and the waste was drained until no liquid flowed out.

[0186] Step 2: 20% of piperidine/DMF (50 mL) was added to the reaction column, bubbled with nitrogen for 20 minutes, and then the waste was drained until no liquid flowed out. The resin was washed with DMF (50 mL) 5 times (1 minute each time), and the waste was drained until no liquid flowed out. The resin was tested with ninhydrin and showed blue.

[0187] Step 3: Coupling of Amino Acids

[0188] 3.1 Coupling of Fmoc-Ser(tBu)-OH

[0189] 1. Fmoc-Ser(tBu)-OH (3.0 eq) was weighed and added to the above resin, and DIEA (6.00 eq) plus 10 mL of DMF were added to the reaction column, bubbled with nitrogen, and

HBTU (2.85 eq) was added after the amino acid was dissolved. The nitrogen was adjusted to make the resin bulge evenly.

[0190] 2. The reaction was carried out for 0.5 hours at 25°C. The resin was tested with ninhydrin and then showed colorless and transparent.

[0191] 3. The reaction solution was removed, and the resin was washed with DMF 5 times (50 mL each time, 1 min each time). The waste was drained until no liquid flowed out.

[0192] 3.2 Coupling of Fmoc-Pro-OH

[0193] 1. 20% of piperidine/DMF (50 mL) was added to the reaction column, bubbled with nitrogen for 20 minutes, and then the waste was drained until no liquid flowed out. The resin was washed with DMF (50 mL) 5 times (1 minute each time), and the waste was drained until no liquid flowed out. The resin was tested with ninhydrin and showed blue.

[0194] 2. Fmoc-Pro-OH (3.0 eq) was weighed and added to the above resin, and DIEA (6.00 eq) plus 10 mL of DMF were added to the reaction column, bubbled with nitrogen, and HBTU (2.85 eq) was added after the amino acid was dissolved. The nitrogen was adjusted to make the resin bulge evenly.

[0195] 3. The reaction was carried out for 0.5 hours at 25°C. The resin was tested with ninhydrin and then showed colorless and transparent.

[0196] 4. The reaction solution was removed, and the resin was washed with DMF 5 times (50 mL each time, 1 min each time). The waste was drained until no liquid flowed out.

[0197] Step 3.2 was repeated to complete the coupling of the following amino acids

NO.	Raw Material	Coupling Reagent
3.3	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.4	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.5	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.6	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.7	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.8	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.9	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.10	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

3.11	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.12	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.13	Fmoc-Ile-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.14	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.15	Fmoc-Trp(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.16	Fmoc-Glu(OAll)-OH (2.00 eq)	HBTU (1.90 eq) and DIEA (4.00 eq)
3.17	Fmoc-Val-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.18	Fmoc-Phe-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.19	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.20	Fmoc-Lys(Alloc)-OH (2.00 eq)	HOBT (2.00 eq) and DIC (2.00 eq)
3.21	Fmoc-Gln(Trt)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.22	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.23	Fmoc-Lys(Dde)-OH(3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.24	Fmoc-Lys(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3.25	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.26	Fmoc-Leu-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.27	Fmoc-Aib-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.28	Fmoc-Ile-OH (6.00 eq)	HOAT (6.00 eq) and DIC (6.00 eq)
3.29	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.30	Fmoc-Tyr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.31	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.32	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.33	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.34	Fmoc-Phe-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.35	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.36	Fmoc-Gly-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
3.37	Fmoc-Glu(tBu)-OH (6.00 eq)	HOBT (6.00 eq) and DIC (6.00 eq)

3.38	Fmoc-Aib-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
3.39	Boc-Tyr(tBu)-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

[0198] 3.40 Deprotection of Alloc and OAll

[0199] 1. PhSiH₃ (20.0 eq) and DCM (10 mL) were added to the reaction column, after bubbling with nitrogen, Pd(PPh₃)₄ (0.2 eq) was added, and bubbled with nitrogen for 20 min. The reaction was carried out twice, and the waste was drained until no liquid flowed out.

[0200] 2. The resin was washed with DMF 5 times (50 mL each time, 1 min each time). The waste was drained until no liquid flowed out.

[0201] 3.41 Ring Closure of Amide

[0202] 1. DIEA (3.00 eq) plus 10 mL of DMF were added to the reaction column, bubbled with nitrogen, and HATU (1.5 eq) was added after the amino acid was dissolved. The nitrogen was adjusted to make the resin bulge evenly.

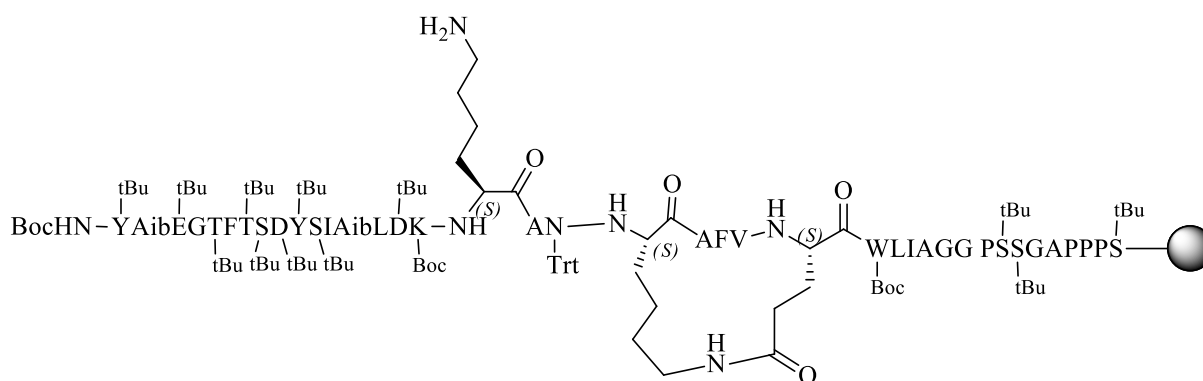
[0203] 2. The reaction was carried out for 0.5 hours at 25°C. The resin was tested with ninhydrin and then showed colorless and transparent.

[0204] 3. The resin was washed with DMF 5 times (50 mL each time, 1 min each time). The waste was drained until no liquid flowed out.

[0205] 3.42 Deprotection of Dde

[0206] 1. 3% of hydrazine hydrate/DMF (50 mL) was added to the reaction column, bubbled with nitrogen for 15 minutes, and then the waste was drained until no liquid flowed out. The resin was washed with DMF 5 times (50 mL each time, 1 min each time) and the waste was drained until no liquid flowed out to obtain intermediate **B-1**. The resin was tested with ninhydrin and showed blue.

[0207] Intermediate B-2



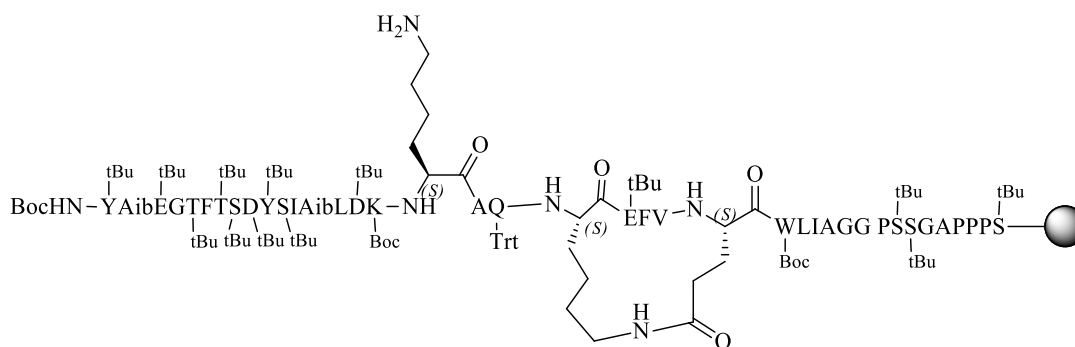
B-2

[0208] Referring to the synthesis method of **B-1**, **B-2** was obtained after the following operations such as coupling of amino acids, deprotection of Alloc and OAll, ring closure of amide, and deprotection of Dde.

NO.	Raw Material	Coupling Reagent
1	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
5	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
6	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
7	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
8	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
9	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
10	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
11	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
12	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
13	Fmoc-Ile-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
14	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
15	Fmoc-Trp(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
16	Fmoc-Glu(OAll)-OH (2.00 eq)	HBTU (1.90 eq) and DIEA (4.00 eq)

17	Fmoc-Val-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
18	Fmoc-Phe-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
19	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
20	Fmoc-Lys(Alloc)-OH (2.00 eq)	HOBT (2.00 eq) and DIC (2.00 eq)
21	Fmoc-Asn(Trt)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
22	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
23	Fmoc-Lys(Dde)-OH(3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
24	Fmoc-Lys(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
25	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
26	Fmoc-Leu-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
27	Fmoc-Aib-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
28	Fmoc-Ile-OH (6.00 eq)	HOAT (6.00 eq) and DIC (6.00 eq)
29	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
30	Fmoc-Tyr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
31	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
32	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
33	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
34	Fmoc-Phe-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
35	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
36	Fmoc-Gly-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
37	Fmoc-Glu(tBu)-OH (6.00 eq)	HOBT (6.00 eq) and DIC (6.00 eq)
38	Fmoc-Aib-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
39	Boc-Tyr(tBu)-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

[0209] Intermediate B-3



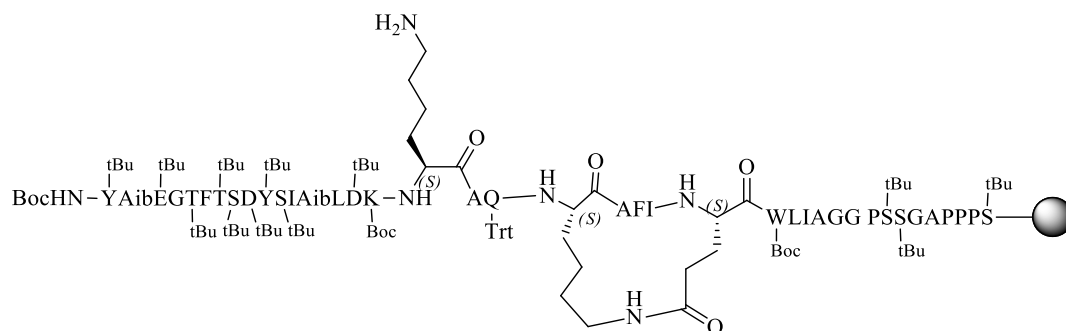
B-3

[0210] Referring to the synthesis method of **B-1**, **B-3** was obtained after the following operations such as coupling of amino acids, deprotection of Alloc and Oall, ring closure of amide, and deprotection of Dde.

NO.	Raw Material	Coupling Reagent
1	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
5	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
6	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
7	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
8	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
9	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
10	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
11	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
12	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
13	Fmoc-Ile-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
14	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
15	Fmoc-Trp(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
16	Fmoc-Glu(OAll)-OH (2.00 eq)	HBTU (1.90 eq) and DIEA (4.00 eq)
17	Fmoc-Val-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

18	Fmoc-Phe-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
19	Fmoc-Glu(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
20	Fmoc-Lys(Alloc)-OH (2.00 eq)	HOBT (2.00 eq) and DIC (2.00 eq)
21	Fmoc-Gln(Trt)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
22	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
23	Fmoc-Lys(Dde)-OH(3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
24	Fmoc-Lys(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
25	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
26	Fmoc-Leu-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
27	Fmoc-Aib-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
28	Fmoc-Ile-OH (6.00 eq)	HOAT (6.00 eq) and DIC (6.00 eq)
29	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
30	Fmoc-Tyr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
31	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
32	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
33	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
34	Fmoc-Phe-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
35	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
36	Fmoc-Gly-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
37	Fmoc-Glu(tBu)-OH (6.00 eq)	HOBT (6.00 eq) and DIC (6.00 eq)
38	Fmoc-Aib-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
39	Boc-Tyr(tBu)-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

[0211] Intermediate B-4



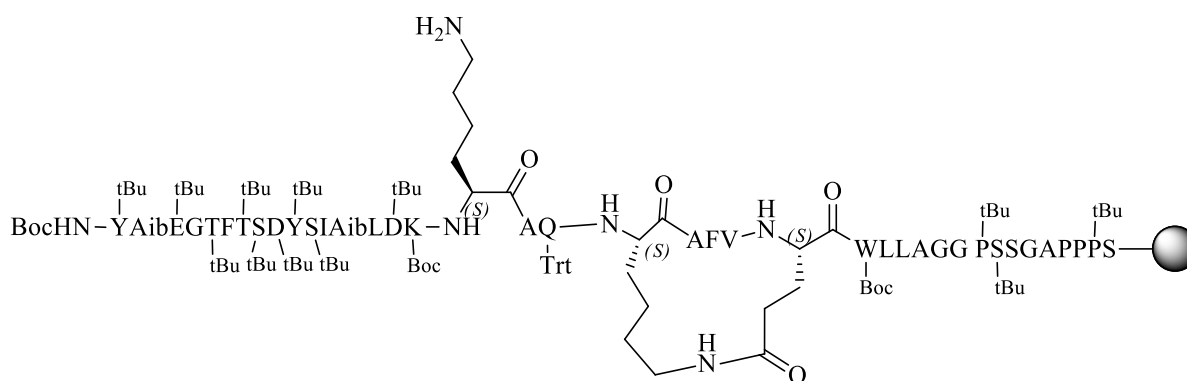
B-4

[0212] Referring to the synthesis method of **B-1**, **B-4** was obtained after the following operations such as coupling of amino acids, deprotection of Alloc and Oall, ring closure of amide, and deprotection of Dde.

NO.	Raw Material	Coupling Reagent
1	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
5	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
6	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
7	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
8	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
9	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
10	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
11	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
12	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
13	Fmoc-Ile-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
14	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
15	Fmoc-Trp(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
16	Fmoc-Glu(Oall)-OH (2.00 eq)	HBTU (1.90 eq) and DIEA (4.00 eq)
17	Fmoc-Ile-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

18	Fmoc-Phe-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
19	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
20	Fmoc-Lys(Alloc)-OH (2.00 eq)	HOBT (2.00 eq) and DIC (2.00 eq)
21	Fmoc-Gln(Trt)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
22	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
23	Fmoc-Lys(Dde)-OH(3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
24	Fmoc-Lys(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
25	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
26	Fmoc-Leu-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
27	Fmoc-Aib-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
28	Fmoc-Ile-OH (6.00 eq)	HOAT (6.00 eq) and DIC (6.00 eq)
29	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
30	Fmoc-Tyr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
31	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
32	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
33	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
34	Fmoc-Phe-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
35	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
36	Fmoc-Gly-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
37	Fmoc-Glu(tBu)-OH (6.00 eq)	HOBT (6.00 eq) and DIC (6.00 eq)
38	Fmoc-Aib-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
39	Boc-Tyr(tBu)-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

[0213] Intermediate B-5



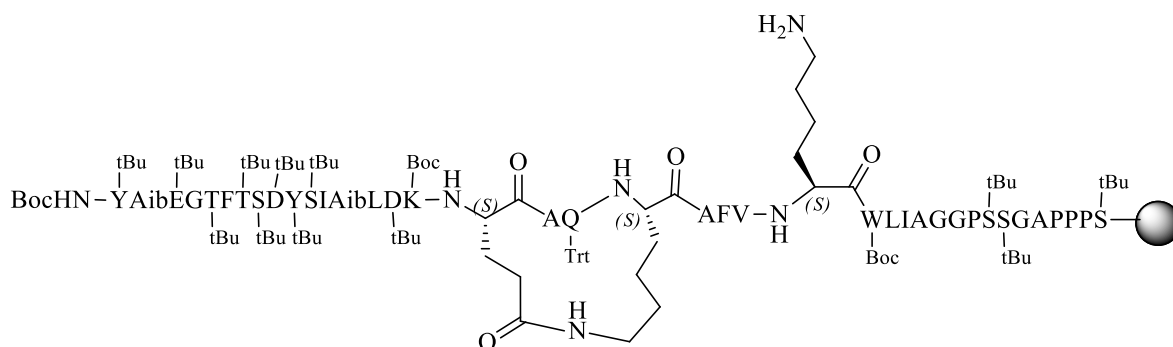
B-5

[0214] Referring to the synthesis method of **B-1**, **B-5** was obtained after the following operations such as coupling of amino acids, deprotection of Alloc and OAll, ring closure of amide, and deprotection of Dde.

NO.	Raw Material	Coupling Reagent
1	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
5	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
6	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
7	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
8	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
9	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
10	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
11	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
12	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
13	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
14	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
15	Fmoc-Trp(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
16	Fmoc-Glu(OAll)-OH (2.00 eq)	HBTU (1.90 eq) and DIEA (4.00 eq)
17	Fmoc-Val-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

18	Fmoc-Phe-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
19	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
20	Fmoc-Lys(Alloc)-OH (2.00 eq)	HOBT (2.00 eq) and DIC (2.00 eq)
21	Fmoc-Gln(Trt)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
22	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
23	Fmoc-Lys(Dde)-OH(3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
24	Fmoc-Lys(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
25	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
26	Fmoc-Leu-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
27	Fmoc-Aib-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
28	Fmoc-Ile-OH (6.00 eq)	HOAT (6.00 eq) and DIC (6.00 eq)
29	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
30	Fmoc-Tyr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
31	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
32	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
33	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
34	Fmoc-Phe-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
35	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
36	Fmoc-Gly-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
37	Fmoc-Glu(tBu)-OH (6.00 eq)	HOBT (6.00 eq) and DIC (6.00 eq)
38	Fmoc-Aib-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
39	Boc-Tyr(tBu)-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

[0215] Intermediate B-6



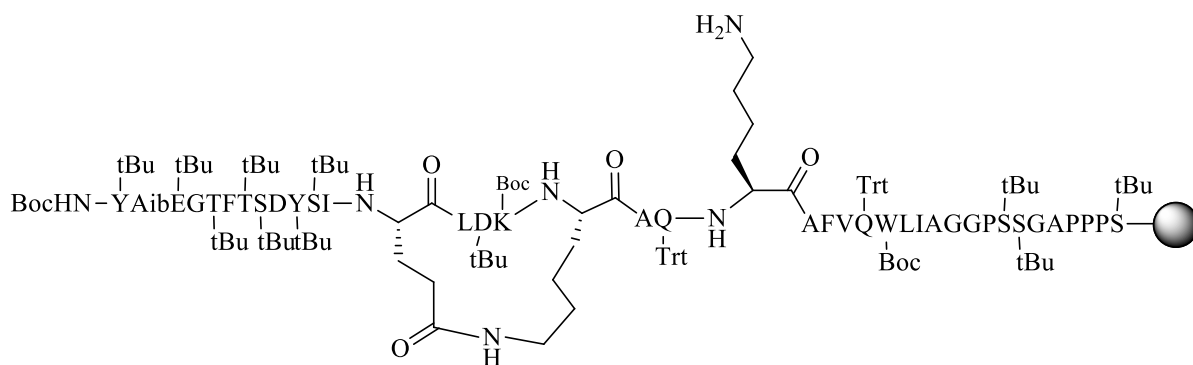
B-6

[0216] Referring to the synthesis method of **B-1**, **B-6** was obtained after the following operations such as coupling of amino acids, deprotection of Alloc and OAll, ring closure of amide, and deprotection of Dde.

NO.	Raw Material	Coupling Reagent
1	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
5	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
6	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
7	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
8	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
9	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
10	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
11	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
12	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
13	Fmoc-Ile-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
14	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
15	Fmoc-Trp(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
16	Fmoc-Lys(Dde)-OH(3.00 eq)	HBTU (1.90 eq) and DIEA (4.00 eq)
17	Fmoc-Val-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

18	Fmoc-Phe-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
19	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
20	Fmoc-Lys(Alloc)-OH (2.00 eq)	HOBT (2.00 eq) and DIC (2.00 eq)
21	Fmoc-Gln(Trt)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
22	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
23	Fmoc-Glu(OAll)-OH (2.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
24	Fmoc-Lys(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
25	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
26	Fmoc-Leu-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
27	Fmoc-Aib-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
28	Fmoc-Ile-OH (6.00 eq)	HOAT (6.00 eq) and DIC (6.00 eq)
29	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
30	Fmoc-Tyr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
31	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
32	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
33	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
34	Fmoc-Phe-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
35	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
36	Fmoc-Gly-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
37	Fmoc-Glu(tBu)-OH (6.00 eq)	HOBT (6.00 eq) and DIC (6.00 eq)
38	Fmoc-Aib-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
39	Boc-Tyr(tBu)-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

[0217] Intermediate B-7



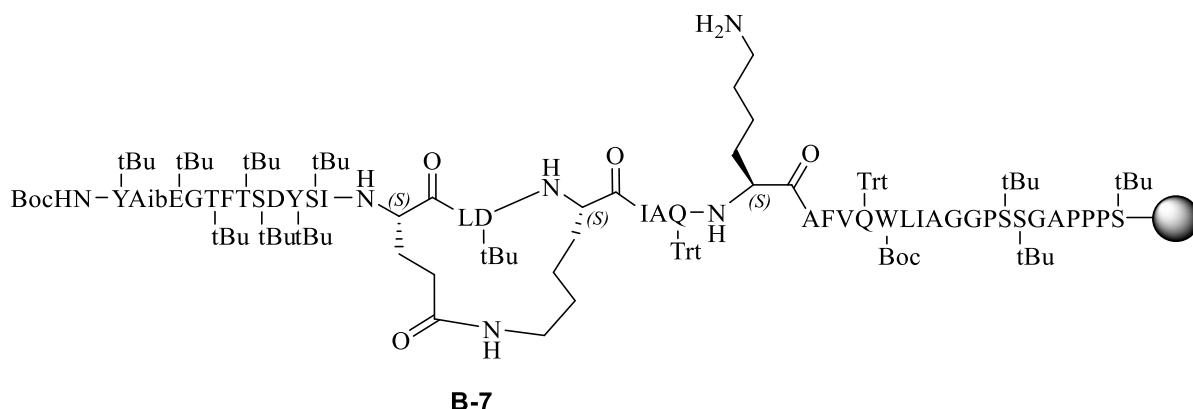
B-7

[0218] Referring to the synthesis method of **B-1**, **B-7** was obtained after the following operations such as coupling of amino acids, deprotection of Alloc and OAll, ring closure of amide, and deprotection of Dde.

NO.	Raw Material	Coupling Reagent
1	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
5	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
6	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
7	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
8	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
9	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
10	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
11	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
12	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
13	Fmoc-Ile-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
14	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
15	Fmoc-Trp(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
16	Fmoc-Gln(Trt)-OH (2.00 eq)	HBTU (1.90 eq) and DIEA (4.00 eq)
17	Fmoc-Val-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

18	Fmoc-Phe-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
19	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
20	Fmoc-Lys(Dde)-OH(3.00 eq)	HOBT (2.00 eq) and DIC (2.00 eq)
21	Fmoc-Gln(Trt)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
22	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
23	Fmoc-Lys(Alloc)-OH (2.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
24	Fmoc-Lys(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
25	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
26	Fmoc-Leu-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
27	Fmoc-Glu(OAll)-OH (2.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
28	Fmoc-Ile-OH (6.00 eq)	HOAT (6.00 eq) and DIC (6.00 eq)
29	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
30	Fmoc-Tyr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
31	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
32	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
33	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
34	Fmoc-Phe-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
35	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
36	Fmoc-Gly-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
37	Fmoc-Glu(tBu)-OH (6.00 eq)	HOBT (6.00 eq) and DIC (6.00 eq)
38	Fmoc-Aib-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
39	Boc-Tyr(tBu)-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

[0219] Intermediate B-8

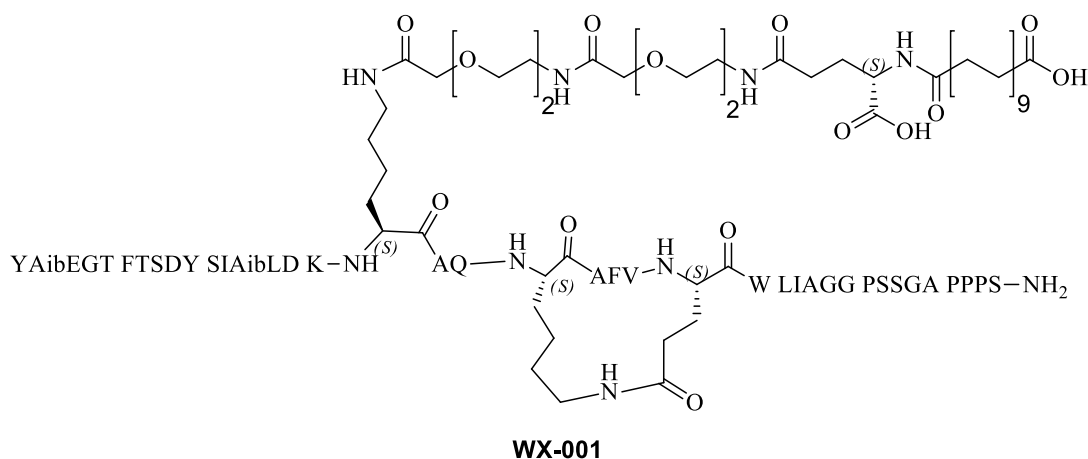


[0220] Referring to the synthesis method of **B-1**, **B-8** was obtained after the following operations such as coupling of amino acids, deprotection of Alloc and OAll, ring closure of amide, and deprotection of Dde.

NO.	Raw Material	Coupling Reagent
1	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
5	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
6	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
7	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
8	Fmoc-Ser(tBu)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
9	Fmoc-Pro-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
10	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
11	Fmoc-Gly-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
12	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
13	Fmoc-Ile-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
14	Fmoc-Leu-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
15	Fmoc-Trp(Boc)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
16	Fmoc-Gln(Trt)-OH (2.00 eq)	HBTU (1.90 eq) and DIEA (4.00 eq)
17	Fmoc-Val-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)

18	Fmoc-Phe-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
19	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
20	Fmoc-Lys(Dde)-OH(3.00 eq)	HOBT (2.00 eq) and DIC (2.00 eq)
21	Fmoc-Gln(Trt)-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
22	Fmoc-Ala-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
23	Fmoc-Ile-OH (3.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
24	Fmoc-Lys(Alloc)-OH (2.00 eq)	HBTU (2.85 eq) and DIEA (6.00 eq)
25	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
26	Fmoc-Leu-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
27	Fmoc-Glu(OAll)-OH (2.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
28	Fmoc-Ile-OH (6.00 eq)	HOAT (6.00 eq) and DIC (6.00 eq)
29	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
30	Fmoc-Tyr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
31	Fmoc-Asp(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
32	Fmoc-Ser(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
33	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
34	Fmoc-Phe-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
35	Fmoc-Thr(tBu)-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
36	Fmoc-Gly-OH (6.00 eq)	HATU (5.70 eq) and DIEA (12.0 eq)
37	Fmoc-Glu(tBu)-OH (6.00 eq)	HOBT (6.00 eq) and DIC (6.00 eq)
38	Fmoc-Aib-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
39	Boc-Tyr(tBu)-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

[0221] Example 1



[0222] **1. Coupling of long-chain fatty acids:** Referring to synthesis step 3.2 of **B-1** to complete the coupling of the following fragments:

NO.	Raw Material	Coupling Reagent
1	Fmoc-AEEA-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-AEEA-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Glu-OtBu (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
4	20-(tBu)-eicosanedioic acid (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

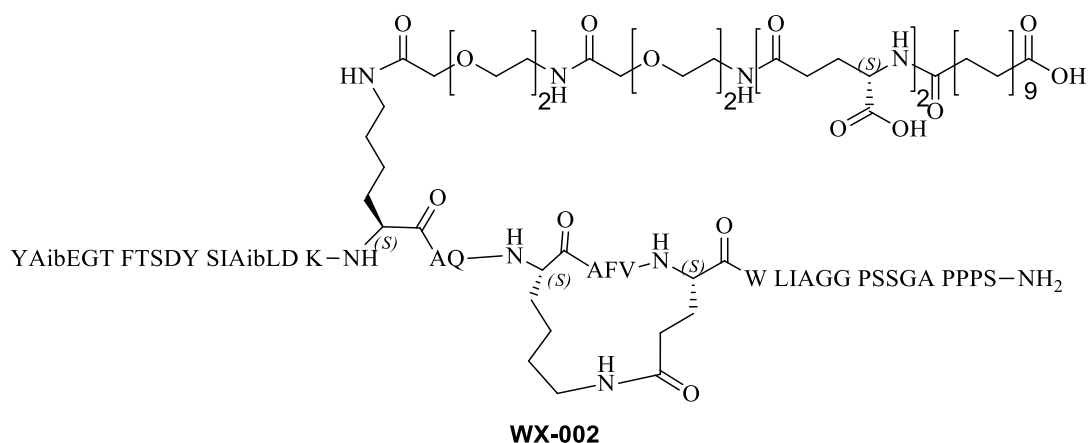
[0223] **2. Cleavage and Drying of Crude Peptide**

[0224] 2.1. The cleavage solution was configured according to the following volume

Reagent	Ratio
Trifluoroacetic acid	91
Triisopropylsilane	3
H ₂ O	3
3-Mercaptopropionic acid	3

[0225] The dried peptide resin was added to the prepared cleavage solution. The mixture was shaken on a shaker for 2.5 hours, and filtered, and the filtrate was added to ice isopropyl ether with 10 times the volume of the filtrate, centrifuged, and washed 3 times with isopropyl ether. After drying in vacuum for 2 hours, the crude peptide was obtained, and the polypeptide compound **WX-001** was obtained after purification. The molecular weight of the polypeptide was confirmed by ESI-MS, calculated for 4811.4, found 4812.0.

[0226] **Example 2**



[0227] **1. Coupling of long-chain fatty acids:** Referring to synthesis step 3.2 of **B-1** to complete the coupling of the following fragments:

NO.	Raw Material	Coupling Reagent
1	Fmoc-AEEA-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-AEEA-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Glu-OtBu (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Glu-OtBu (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
5	20-(tBu)-eicosanedioic acid (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

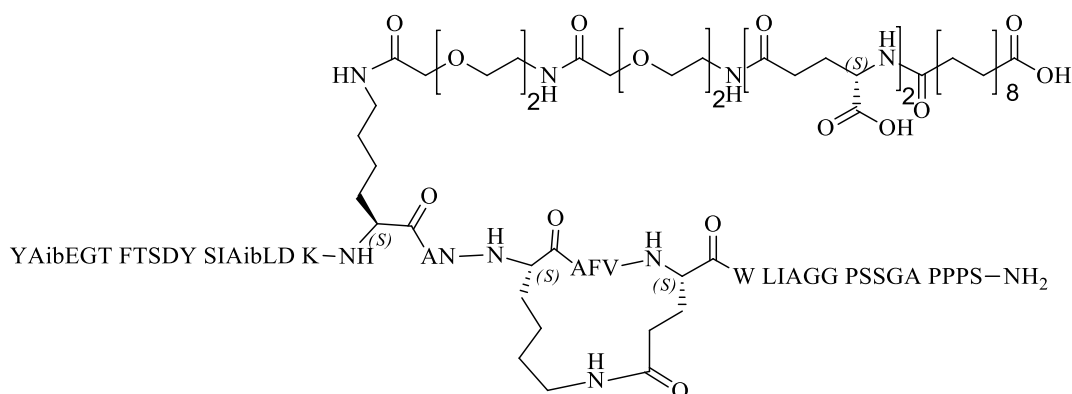
[0228] **2. Cleavage and Drying of Crude Peptide**

[0229] 2.1. The cleavage solution was prepared according to the following volume

Reagent	Ratio
Trifluoroacetic acid	91
Triisopropylsilane	3
H ₂ O	3
3-Mercaptopropionic acid	3

[0230] The dried peptide resin was added to the prepared cleavage solution. The mixture was shaken on a shaker for 2.5 hours, and filtered, and the filtrate was added to ice isopropyl ether with 10 times the volume of the filtrate, centrifuged, and washed 3 times with isopropyl ether. After drying in vacuum for 2 hours, the crude peptide was obtained, and the polypeptide compound **WX-002** was obtained after purification. The molecular weight of the polypeptide was confirmed by ESI-MS, calculated for 4940.5, found 4940.6.

[0231] Example 3



WX-003

[0232] 1. Coupling of long-chain fatty acids: Referring to synthesis step 3.2 of B-1 to complete the coupling of the following fragments:

NO.	Raw Material	Coupling Reagent
1	Fmoc-AEEA-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
2	Fmoc-AEEA-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Glu-OtBu (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-Glu-OtBu (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
5	20-(tBu)-octadecanedioic acid (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)

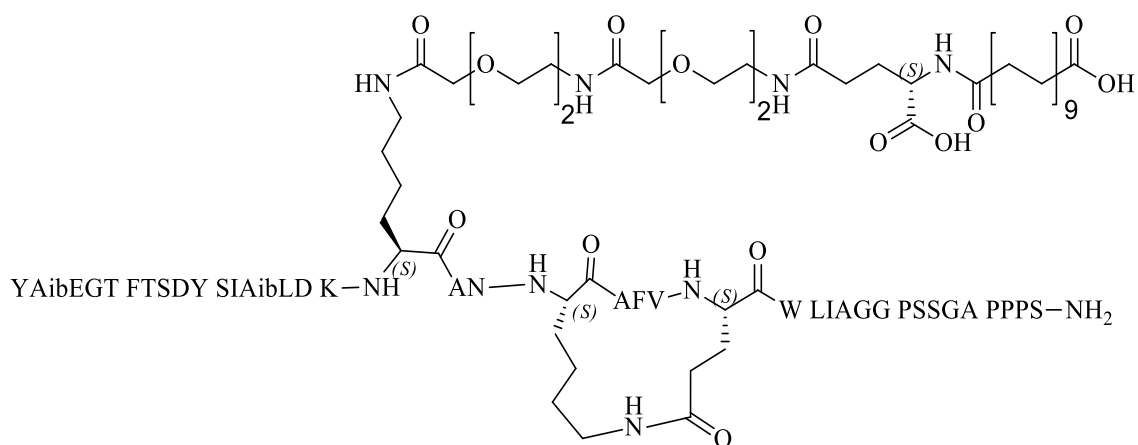
[0233] 2. Cleavage and Drying of Crude Peptide

[0234] 2.1. The cleavage solution was configured according to the following volume

Reagent	Ratio
Trifluoroacetic acid	91
Triisopropylsilane	3
H ₂ O	3
3-Mercaptopropionic acid	3

[0235] The dried peptide resin was added to the prepared cleavage solution. The mixture was shaken on a shaker for 2.5 hours, and filtered, and the filtrate was added to ice isopropyl ether with 10 times the volume of the filtrate, centrifuged, and washed 3 times with isopropyl ether. After drying in vacuum for 2 hours, the crude peptide was obtained, and the polypeptide compound WX-003 was obtained after purification. The molecular weight of the polypeptide was confirmed by ESI-MS, calculated for 4912.5, found 4913.1.

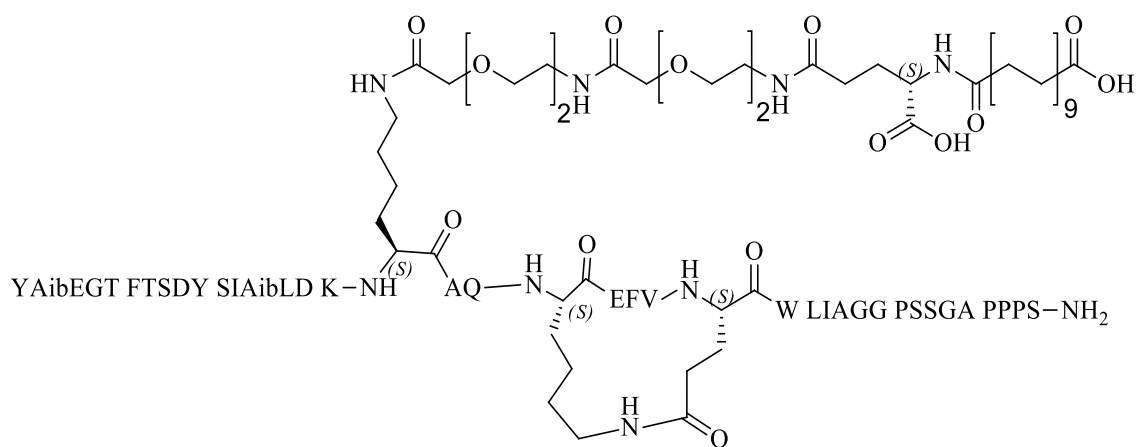
[0236] Example 4



WX-004

[0237] Referring to the synthesis of **WX-001**, **WX-004** was obtained through intermediate **B-2**. The molecular weight of the polypeptide was confirmed by ESI-MS, calculated for 4797.4, found 4797.3.

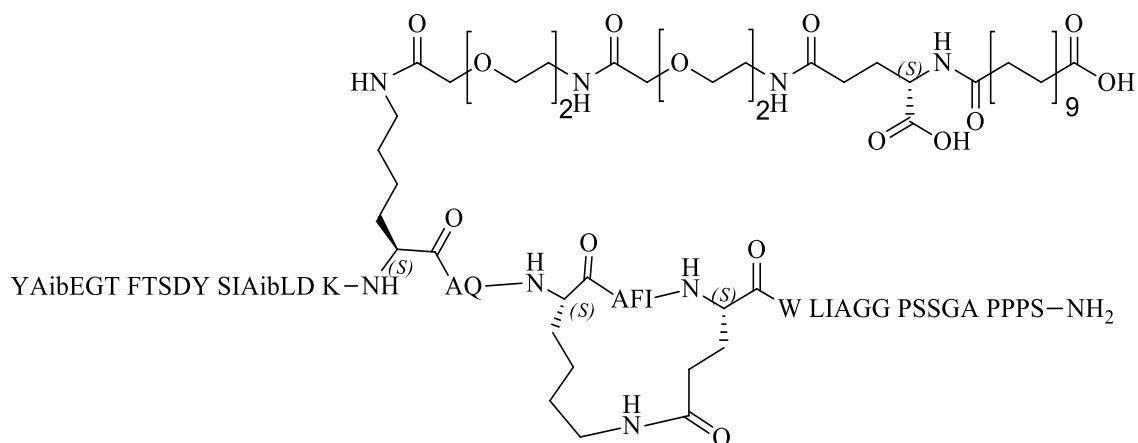
[0238] Example 5



WX-005

[0239] Referring to the synthesis of **WX-001**, **WX-005** was obtained through intermediate **B-3**. The molecular weight of the polypeptide was confirmed by ESI-MS, calculated for 4869.5, found 4869.3.

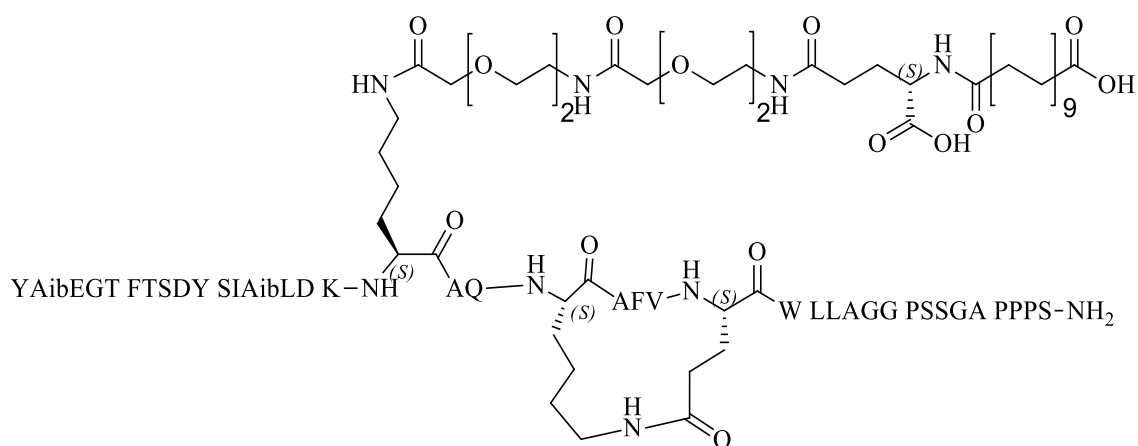
[0240] Example 6



WX-006

[0241] Referring to the synthesis of **WX-001**, **WX-006** was obtained through intermediate **B-4**. The molecular weight of the polypeptide was confirmed by ESI-MS, calculated for 4825.5, found 4825.5.

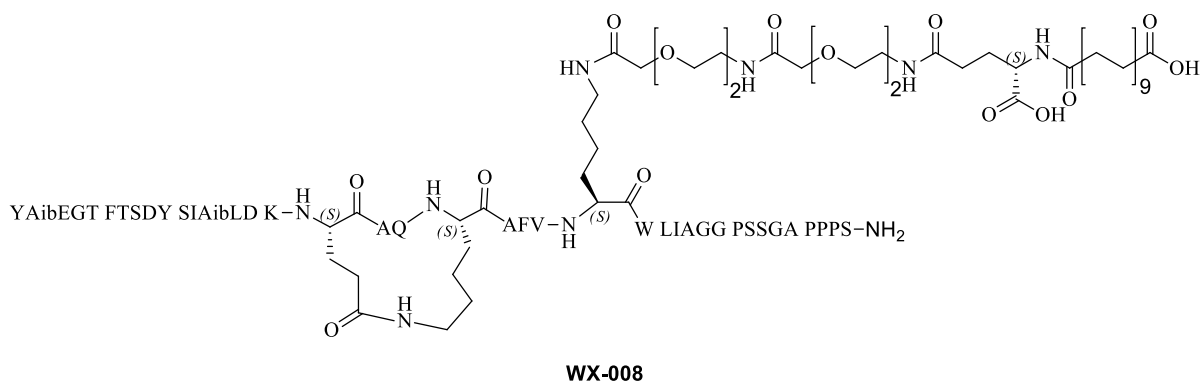
[0242] Example 7



WX-007

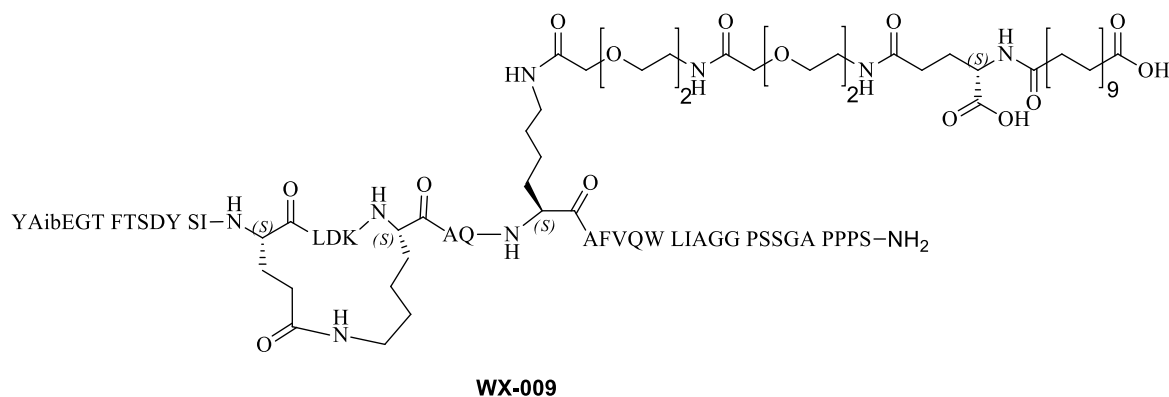
[0243] Referring to the synthesis of **WX-001**, **WX-007** was obtained through intermediate **B-5**. The molecular weight of the polypeptide was confirmed by ESI-MS, calculated for 4811.4, found 4811.1.

[0244] Example 8



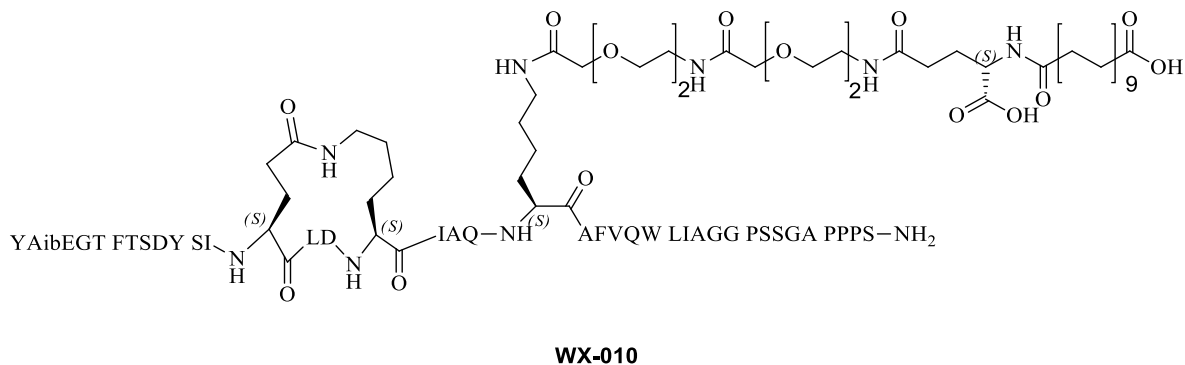
[0245] Referring to the synthesis of **WX-001**, **WX-008** was obtained through intermediate **B-6**. The molecular weight of the polypeptide was confirmed by ESI-MS, calculated for 4811.4, found 4811.4.

[0246] Example 9



[0247] Referring to the synthesis of **WX-001**, **WX-009** was obtained through intermediate **B-7**. The molecular weight of the polypeptide was confirmed by ESI-MS, calculated for 4854.5, found 4854.9.

[0248] Example 10



[0249] Referring to the synthesis of **WX-001**, **WX-010** was obtained through intermediate **B-8**. The molecular weight of the polypeptide was confirmed by ESI-MS, calculated for 4839.4, found 4839.4.

found 4839.6.

[0250] Biological Test Data

[0251] Experimental Example 1: GLP-1R/GIPR Agonist Activity Test *in vitro*

[0252] A. Main Material:

[0253] 1) Cell Strain

[0254] The cell strains were constructed by Shanghai WuXi AppTec. See the table below for details.

Target	Host Cell	Clone
GLP-1R	HEK293	N/A
GIPR	CHO	N/A

[0255] 2) Reagents and Consumables

Name	Batch.	Item NO.	Manufacturer
cAMP assay kit	29F	62AM4PEJ	Cisbio
1M HEPES	2120919	15630-106	Invitrogen
Hanks balanced salt solution (HBSS)	2185775	14025	Invitrogen
Human serum albumin (HSA)	SLCF7301	A1653-10G	Sigma
Casein	SLCC9458	C4765-10mL	Sigma
3-Isobutyl-1-methylxanthine (IBMX)	STBF6061V	I5879-5G	Sigma
ECHO qualified 384-well plate	0006433672	PP-0200	Labcyte
OptiPlate-384	8210-19481	6007299	PerkinElmer

[0256] 3) Instruments

Name	Model	Manufacturer
EnVision	envision2014	PerkinElmer
Vi-cell counter	Vi-CELL™ XR Cell Viability Analyzer	Beckman
Bravo	Bravo V11	Agilent

ECHO	ECHO 555	Labcyte
Centrifuge	Allegra™ 25R Centrifuge	Beckman

[0257] B. Method

[0258] 1) Experimental Materials

[0259] Experimental Buffer

<u>Reagent</u>	<u>Storage Concentration</u>	<u>Volume</u>	<u>Final Concentration</u>
Hanks balanced salt solution	1x	48.7 mL/ 44.7 mL	About 1x
HEPES buffer	1 mol/L	250 µL	5 mmol/L
5% casein solution (HEPES)/ 10% human serum albumin solution (HSA)	5%/ 10%	1000 µL/ 5000 µL	0.10%/ 1%
3-Isobutyl-1-methylxanthine (IBMX)	500 mmol/L	50 µL	0.5 mmol/L

[0260] Detection Reagent Preparation

<u>Reagent</u>	<u>Storage Concentration</u>	<u>Volume</u>	<u>Final Concentration</u>
Cell lysate	1x	9.5 mL	About 1x
D2-cAMP solution	40x	250 µL	1x
cAMP-antibody solution	40x	250 µL	1x

[0261] 2) Experimental Method

[0262] a) Preparation of the Compound Plate:

[0263] The test compound was serially diluted 4-fold with 10 points. The initial concentration was 30 µM, and the dilution was completed with Bravo.

[0264] b) Compound Transference:

[0265] 1) 100 nL of the compound was transferred to OptiPlate-384 plate using Echo.

[0266] 2) The OptiPlate-384 plate was centrifuged at 1000 rpm for 5 seconds.

[0267] c) Preparation of Cell Suspension:

[0268] 1) A GLP-1R/GIPR cell cryopreservation tube was quickly thawed in warm water at 37°C.

[0269] 2) The cell suspension was transferred to a Transfer15 mL centrifuge tube and gently rinsed with 10 mL of HBSS.

[0270] 3) The centrifuge tube was centrifuged at 1000 rpm for 1 minute at room temperature.

[0271] 4) The supernatant was discarded.

[0272] 5) The bottom cells were gently dispersed and gently rinsed with 10 mL of HBSS. The cells were centrifuged to settle, and finally resuspended with the experimental buffer.

[0273] 6) Vi-cell was used to measure cell density and activity.

[0274] 7) The GLP-1R/GIPR cell concentration was diluted to 2.0×10^5 /mL with the experimental buffer.

[0275] 8) 100 nL of the diluted cell suspension was transferred into the OptiPlate-384 plate.

[0276] 9) The diluted cell suspension was incubated at room temperature for 30 minutes.

[0277] d) Addition of Detection Reagent:

[0278] 1) 10 µL of 800 nM gradient-diluted cAMP standard was added to the empty well of the OptiPlate-384 plate.

[0279] 2) 10 µL of cAMP detection reagent was added.

[0280] 3) The OptiPlate-384 plate was covered with TopSeal-A film and incubated at room temperature for 60 minutes.

[0281] TopSeal-A was removed and read in EnVision.

[0282] C. Experimental Results

[0283] The experimental results are shown in Table 1.

[0284] Table 1 GLP-1R/GIPR Agonist Activity Test Results *in vitro*

Polypeptide Drugs	GLP-1R Agonist Activity			GIPR Agonist Activity		
	EC ₅₀ (nM)		Ratio	EC ₅₀ (nM)		Ratio
	(-) 1% HSA	(+) 1% HSA		(-) 1% HSA	(+) 1% HSA	
WX-001	0.014	3.664	265	0.44	23.04	53
WX-002	0.052	NA	NA	0.29	NA	NA

WX-003	0.090	NA	NA	0.30	NA	NA
WX-005	0.042	NA	NA	0.22	NA	NA
WX-006	0.051	NA	NA	0.45	NA	NA
WX-007	0.14	NA	NA	0.13	NA	NA
WX-008	0.08	NA	NA	0.32	NA	NA
WX-010	0.03448	33.96	984.9	11.86	443	37.4

[0285] **Conclusion:** The compounds of the present disclosure have strong agonist activity on GLP-1R/GIPR.

[0286] Experimental Example 2: Pharmacokinetic Evaluation of Compounds in Rats

[0287] A. Experimental Purpose

[0288] To test the pharmacokinetics of compounds in SD rats *in vivo*

[0289] B. Experimental Operation

[0290] The pharmacokinetic characteristics of the compounds in rodents after subcutaneous injection were tested according to the standard protocol. In the experiment, the candidate compounds were formulated into clear solutions, and a single subcutaneous injection (SC, 0.048 mpk) was given to the rats. The injection vehicle was citrate buffer (20 mM, pH=7). Whole blood was collected and prepared to obtain plasma. Drug concentration was analyzed by LC-MS/MS method, and pharmacokinetic parameters were calculated by Phoenix WinNonlin software.

[0291] C. Experimental Results

[0292] The experimental results are shown in Table 2.

[0293] Table 2 Pharmacokinetic Test Results

Compound Number	Maximum Plasma Concentration (nM)	Half-life T_{1/2} (h)	SC Concentration Integral AUC_{0-72 h} (nM.hr)
WX001	22	12	728
WX002	14	17	1316
WX005	14	17	1329

WX006	16	10	846
--------------	----	----	-----

[0294] Conclusion: The compounds of the present disclosure have excellent pharmacokinetic properties in rats.

[0295] Experimental Example 3: Pharmacokinetic Evaluation of Compounds in Rats

[0296] A. Experimental Purpose

[0297] To test the pharmacokinetics of compounds in C57BL/6 mice *in vivo*

[0298] B. Experimental Operation

[0299] The pharmacokinetic characteristics of the compounds in rodents after intravenous injection and subcutaneous injection were tested according to the standard protocol. In the experiment, the candidate compounds were formulated into clear solutions, and a single intravenous injection (IV, 0.048 mpk) and subcutaneous injection (SC, 0.048 mpk) were given to the mice. The vehicle for intravenous injection was PBS buffer (pH=7), and the vehicle for subcutaneous injection was citrate buffer (20 mM, pH=7). Whole blood was collected and prepared to obtain plasma. Drug concentration was analyzed by LC-MS/MS method, and pharmacokinetic parameters were calculated by Phoenix WinNonlin software.

[0300] C. Experimental Results

[0301] The experimental results are shown in Table 3.

[0302] Table 3 Pharmacokinetic Test Results

Compound Number	Clearance (mL/min/kg)	Half-life $T_{1/2}$ (h)	SC	Bioavailability F (%)
			Concentration Integral AUC_{0-72h} (nM.hr)	
WX001	0.088	15.5	1554	85.5%

[0303] Conclusion: The compounds of the present disclosure have excellent pharmacokinetic properties in mice.

[0304] Experimental Example 4: Pharmacokinetic Evaluation of Compounds in Cynomolgus Monkeys

[0305] A. Experimental Purpose

[0306] To test the pharmacokinetics of compounds in cynomolgus monkeys *in vivo*

[0307] B. Experimental Operation

[0308] The pharmacokinetic characteristics of the compounds in mammals after intravenous injection and subcutaneous injection were tested according to the standard protocol. In the experiment, the candidate compounds were formulated into clear solutions, and a single subcutaneous injection (SC, 0.02 mpk) was given to the cynomolgus monkeys. The vehicle for subcutaneous injection was citrate buffer (20 mM, pH=7). Whole blood was collected and prepared to obtain plasma. Drug concentration was analyzed by LC-MS/MS method, and pharmacokinetic parameters were calculated by Phoenix WinNonlin software.

[0309] C. Experimental results

[0310] The experimental results are shown in Table 4.

[0311] Table 4 Pharmacokinetic Test Results

Compound Number	C_{max} (nM)	T_{max} (h)	Half-life T_{1/2} (h)	SC Concentration Integral AUC_{0-240h} (nM.hr)
WX001	30	21	80	3973
WX002	33	29	81	4582
WX005	31	40	98	4553
WX006	30	29	72.4	3640

[0312] Conclusion: The compounds of the present disclosure have excellent pharmacokinetic properties in monkeys.

[0313] Experimental Example 5: Plasma Stability (PLS) Test

[0314] A. Experimental Purpose

[0315] To study the stability of test compounds in the plasma of normal mice.

[0316] B. Experimental Operation

[0317] 1. Before the experiment, the coagulated frozen plasma was thawed in a water bath at 37°C. Plasma was centrifuged at 4000 rpm for 5 min. If there was blood clot, the blood clot was removed and the pH value was adjusted to 7.4±0.1.

[0318] 2. Preparation of the test compound solution: 100 μ M of the solution was prepared by dilution with DMSO.

[0319] 3. 98 μ L of blank control plasma was added to 2 μ L of the test compound solution (100 μ M), so that the final concentration of the mixed solution of the two reached 2 μ M, and the mixed solution was placed in a 37°C water bath for incubation.

[0320] 4. At each time point (0, 10, 30, 60, and 120 min), 100 μ L of H₃PO₄ solution and 800 μ L of stop solution (200 ng/mL of tolbutamide and 200 ng/mL of labetalol in 100% methanol solution) were added to precipitate protein, and mixed thoroughly.

[0321] 5. The samples were centrifuged at 4000 rpm for 20 min, and 100 μ L of supernatant was taken from each well for LC-MS/MS analysis.

[0322] C. Experimental Results

[0323] The experimental results are shown in Table 5.

[0324] Table 5 PLS Test Results

Compound Number	WX001	WX002	WX005	WX006
PLS (H/M) T _{1/2} (min)	> 289/> 289	> 289/> 289	> 289/> 289	> 289/> 289

[0325] Conclusion: The compounds of the present disclosure have excellent plasma stability.

[0326] Experimental Example 6: Plasma Protein Binding (PPB) Test

[0327] A. Experimental Purpose

[0328] To study the binding of the test compounds to human/mouse plasma albumin.

[0329] B. Experimental Operation

[0330] 1. Matrix preparation: On the day of the experiment, the plasma was thawed in cold water and centrifuged at 3220 rpm for 5 min to remove all blood clots. The pH of the obtained plasma was measured and adjusted to 7.4 $\pm$ 0.1 using 1% phosphoric acid or 1N sodium hydroxide as needed.

[0331] 2. Dilution steps of the test compounds: The test compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions at concentrations of 10 mM and 2 mM, respectively. A 40 μ M working solution was prepared by diluting 2 μ L of the stock solution (2 mM) with 98 μ L of DMSO. A 400 μ M working solution of the control compound was

prepared by diluting 10 μL of the stock solution with 240 μL of DMSO. The working solution of the compound (5 μL) was mixed with the blank matrix (995 μL) at a ratio of 1:200 to prepare the loading matrix.

[0332] 3. Analysis Steps

[0333] 3.1 An equal amount of 30 μL of the loading matrix ($n=2$) was transferred to the sample collection plate to prepare samples of time 0 (T_0) for residue determination. The samples were immediately matched to the corresponding blank buffer in a final volume of 60 μL with a 1:1 volume ratio of plasma to buffer in each well. Then, 60 μL of 4% H_3PO_4 in H_2O and 480 μL of stop solution containing an internal standard were then added to the T_0 samples of the test compounds. They were then stored at $2-8^\circ\text{C}$ with other samples pending for further processing.

[0334] 3.2 The remaining plasma samples were pre-incubated in a carbon dioxide incubator at $37\pm 1^\circ\text{C}$ for 30 min. The prepared protein-free samples (F samples) and loading matrix samples (230 μL) were transferred to polycarbonate tubes ($n = 2$) and ultracentrifuged at 37°C and $155000 \times g$ (35000 rpm) for 4 hours.

[0335] 3.3 To prepare T samples (test samples), an additional matrix-containing sample was transferred to a separate 96-well plate (sample incubation plate) and incubated at 37°C for 4 hours.

[0336] 3.4 After centrifugation, 30 μL of protein-free sample and 30 μL of T sample were transferred from the second layer of supernatant (below the upper layer) to a new sample collection plate. Each sample was mixed with the corresponding blank buffer or matrix in a final volume of 60 μL with a matrix:buffer volume ratio of 1:1. 60 μL of 4% H_3PO_4 aqueous solution and 480 μL of stop solution (containing internal standard) were added to all samples. The mixture was centrifuged at 4000 rpm for 20 min, and 100 μL of the supernatant of each sample was taken for LC-MS/MS analysis.

[0337] C. Experimental Results

[0338] The experimental results are shown in Table 6. Note: NA means the plasma-protein binding is too high, and no free drug can be detected under a normal concentration of plasma protein.

[0339] Table 6 PPB Test Results

Compound Number	WX001	WX002	WX005	WX006
PPB% unbound (H/M)	NA/NA	NA/0.21%	NA/0.22%	NA/0.35%

[0340] Conclusion: The compounds of the present disclosure have extremely high plasma-protein binding.

[0341] Experimental Example 7: Kidney Homogenate Stability (BHS) Test

[0342] A. Experimental Purpose

[0343] To study the stability of the test compounds in normal mouse kidney homogenate.

[0344] B. Experimental Operation

[0345] 1. Before the experiment, the frozen kidney homogenate was thawed in a 37°C water bath.

[0346] 2. Test compounds: 10 mM stock solution was diluted with DMSO to prepare 1 mM intermediate solution;

[0347] 3. test compounds: 1 mM intermediate solution was diluted with DMSO to prepare 50 µM dosing solution;

[0348] 4. at each time point (0, 10, 30, 60, 120, 180, 240 min), 100 µL of 4% H₃PO₄ and 800 µL of stop solution were added to precipitate the protein, and mixed thoroughly.

[0349] 5. The samples were centrifuged at 4000 rpm for 10 min, and 100 µL of supernatant was taken from each well to the plate. The samples were shaken at 800 rpm for approximately 10 min before submitting for LC-MS/MS test.

[0350] C. Experimental Results

[0351] The experimental results are shown in Table 7.

[0352] Table 7 BHS Test Results

Compound Number	WX001	WX002	WX005	WX006
BHS (T _{1/2} (min))	14	20	21	234

[0353] Experimental Example 8: Intraperitoneal Glucose Tolerance Test (ipGTT) - *in vivo* Pharmacodynamic Evaluation of Compounds in Mice

[0354] A. Experimental Purpose

[0355] To study the improvement effect of the test compounds on the glucose tolerance of normal mice

[0356] B. Experimental Operation

[0357] 1. After the mice were grouped according to body weight and blood glucose, each group of animals was injected with the test compound (0.3 nmol/kg) and vehicle (20 mM citrate buffer). The mice were fasted overnight, and intraperitoneally injected with glucose solution (2 g/kg, 10 mL/kg) after 18 hours;

[0358] 2. a glucometer was used to measure blood glucose concentrations at -60, 0, 15, 30, 60 and 120 minutes after glucose administration.

[0359] C. Experimental Results

[0360] The experimental results are shown in Table 8.

[0361] Table 8 ipGTT Test Results

Compound Number	Vehicle	WX001	WX002	WX005	WX006
Blood glucose AUC _{0-120min} (mmol.min)	1916	1277	1150	1143	1164

[0362] Conclusion: The compounds of the present disclosure have excellent effects on improving glucose tolerance.

[0363] Experimental Example 9: Pharmacodynamic study – *in vivo* Pharmacodynamic Evaluation in db/db Mice

[0364] A. Experimental Purpose

[0365] To study the glycemic control effect of the test compounds on type II diabetic db/db mice

[0366] B. Experimental Operation

[0367] 1. After the db/db mice arrived at the facility, they were kept in an animal breeding room with strictly controlled environmental conditions. The temperature in the breeding room was maintained at 20-24°C and the humidity was maintained at 30-70%. The temperature and humidity in the breeding room were monitored in real-time by a

hygrothermograph, and the temperature and humidity were recorded twice a day (one time in the morning and one time in the afternoon). The daylighting in the animal breeding room was controlled by an electronic timing lighting system, and the lights were turned on for 12 hours and turned off for 12 hours every day (turned on at 7:00 in the morning and turned off at 19:00 in the afternoon). During the experiment, the animals were kept in single cages, and toys were provided for each cage. During the experiment, the animals had free access to food (feed for growth/breeding of rats and mice) and drinking water.

[0368] 2. The vehicle and the test compound (15 nmol/kg) were subcutaneously injected into each group of animals. The administration time: 9:30-11:00 in the morning, and the administration frequency was once a day, and the administration was continuous for 4 weeks.

[0369] C. Experimental Results

[0370] The experimental results are shown in Table 9.

[0371] Table 9 Glucose-lowering Effect of the Test Compounds in db/db Mice

Compound Number	Vehicle	WX001	WX002	WX005	WX006
Δ HbA1c% (compared with Day 1 after four weeks of continuous administration)	15%	-28%	-21%	-21%	-23%

[0372] Conclusion: The compounds of the present disclosure exhibit excellent glucose-lowering efficacy in db/db mice.

[0373] Experimental Example 10: Pharmacodynamic study – *in vivo* Pharmacodynamic Evaluation in DIO Mice

[0374] A. Experimental Purpose

[0375] To study the weight loss effect of the test compounds in DIO mice

[0376] B. Experimental Operation

[0377] 1. After the DIO mice arrived at the WuXi AppTec facility, they were kept in an animal breeding room with strictly controlled environmental conditions. The temperature in the breeding room was maintained at 20-24°C, and the humidity was maintained at 30-70%. The temperature and humidity in the breeding room were monitored in real-time by a

hygrothermograph, and the temperature and humidity were recorded twice a day (one time in the morning and one time in the afternoon). The daylighting in the animal breeding room was controlled by an electronic timing lighting system, and the lights were turned on for 12 hours and turned off for 12 hours every day (turned on at 7:00 in the morning and turned off at 19:00 in the afternoon). During the experiment, the animals were kept in single cages, and toys were provided for each cage. During the experiment, the animals had free access to food (feed for the growth/breeding of rats and mice) and drinking water.

[0378] 2. The vehicle and the test compound (10 nmol/kg) were subcutaneously injected into each group of animals, respectively. The administration time: 9:30 in the morning, and the administration frequency was once every three days, and the administration cycle was 22 days.

[0379] C. Experimental Results

[0380] The experimental results are shown in Table 10.

[0381] Table 10 Pharmacodynamics of the Test Compounds in DIO Mice

Compound Number	Vehicle	WX001	WX002	WX005	WX006
Δ Body weight % (after 22 days compared with day 1)	0.2%	-29%	-26%	-24%	-32%
Δ Adipose tissue % (after 22 days compared to day 1)	2%	-54%	-48%	-47%	-62%
Δ Non-adipose tissue % (after 22 days compared with day 1)	-3%	-11%	-10%	-8%	-18%

[0382] Conclusion: The compounds of the present disclosure exhibit excellent weight loss efficacy in DIO mice.

What is claimed is:

1. A compound represented by the following formula or a pharmaceutically acceptable salt thereof,

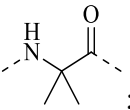
SEQ ID NO1: YAibEGT FTSDY SIAibLD KK¹AZ₀K₀ Z₁FZ₂E₀W LZ₃AGG PSSGA PPPS₀

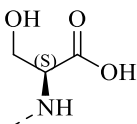
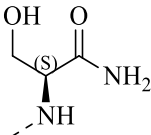
SEQ ID NO2: YAibEGT FTSDY SIAibLD KE₀AQK₀ AFVK¹W LIAGG PSSGA PPPS₀

SEQ ID NO3: YAibEGT FTSDY SIE₀LD KK₀AQK¹ AFVQW LIAGG PSSGA PPPS₀

SEQ ID NO4: YAibEGT FTSDY SIE₀LD K₀IAQK¹ AFVQW LIAGG PSSGA PPPS₀

wherein

Aib has a structure of  ;

S₀ is selected from  and  ;

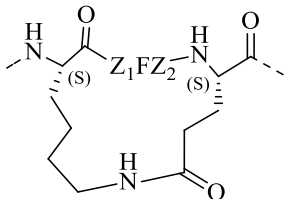
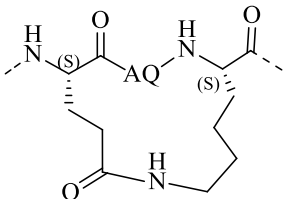
Z₀ is selected from glutamine (Q) and asparagine (N);

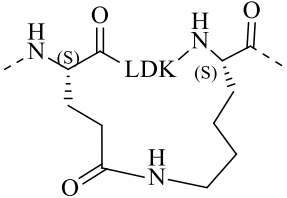
Z₁ is selected from alanine (A) and glutamic acid (E);

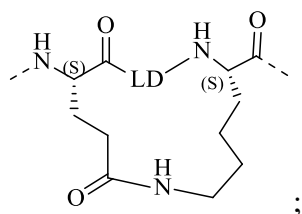
Z₂ is selected from valine (V) and isoleucine (I);

Z₃ is selected from isoleucine (I) and leucine (L);

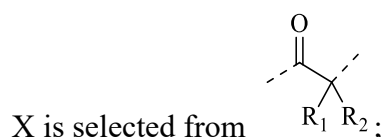
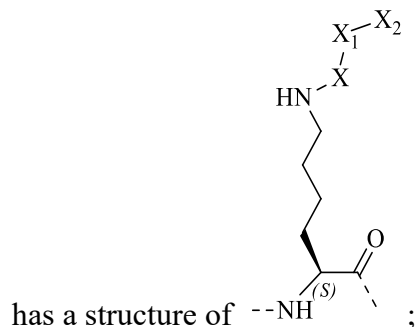
E₀ and K₀ indicate that the carboxyl group on the side chain of the glutamic acid and the amino group on the side chain of the lysine jointly form a lactam structure, and in this case, K₀Z₁FZ₂E₀

has a structure of  , E₀AQK₀ has a structure of  ,

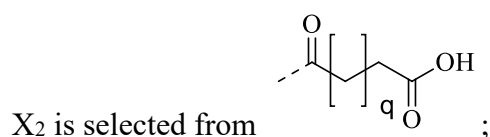
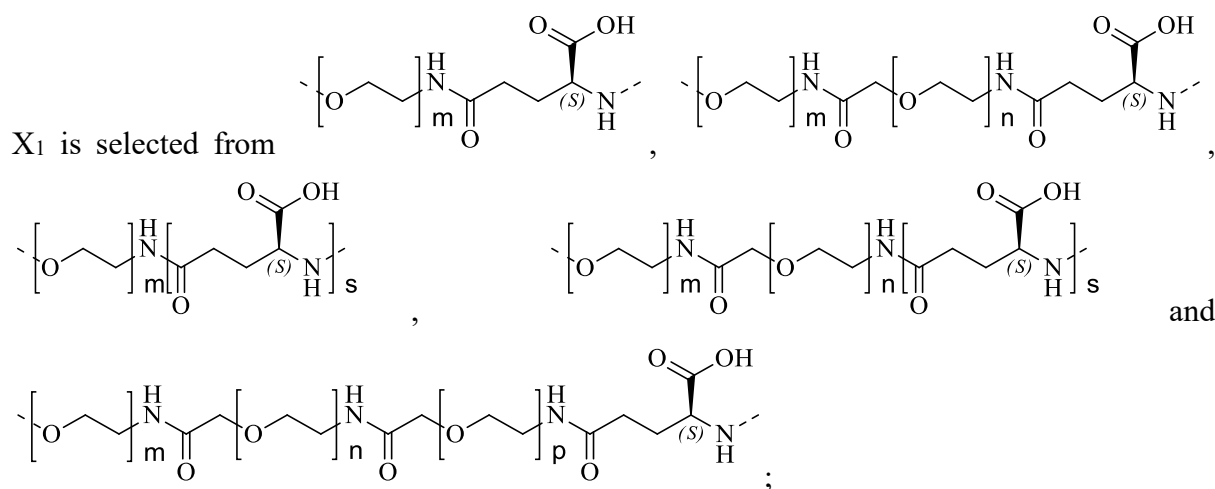
E₀LDKK₀ has a structure of  , and E₀LDK₀ has a structure of



K¹ indicates that the amino group on the side chain of the lysine is connected to -X-X₁-X₂, and



R₁ and R₂ are each independently selected from H and CH₃;

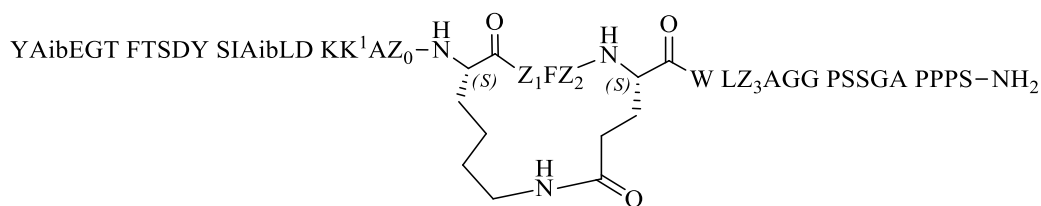


m, n and p are each independently selected from 2 and 3;

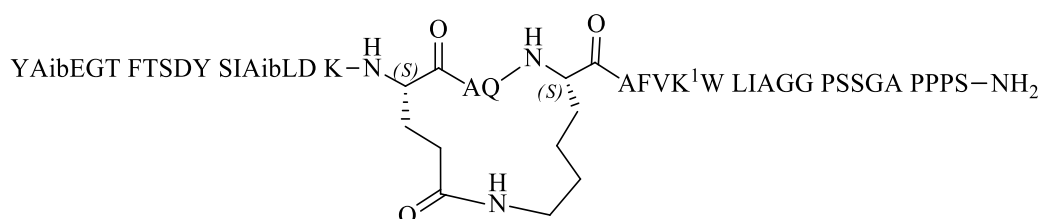
s is selected from 2 and 3;

q is selected from 15, 16, 17, 18 and 19.

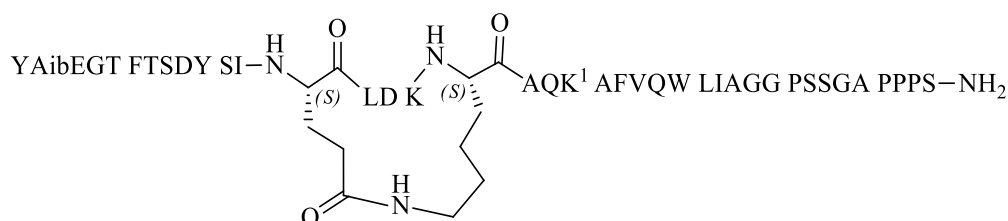
2. The compound or the pharmaceutically acceptable salt thereof according to claim 1, which is selected from:



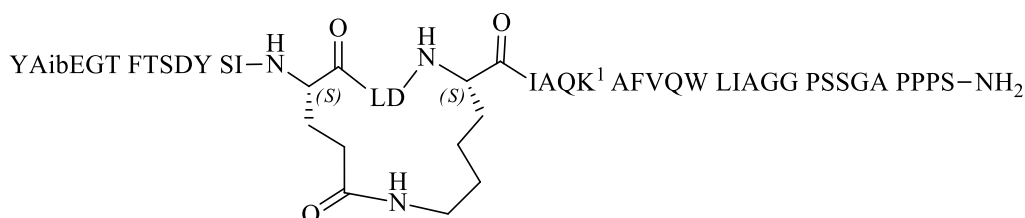
(P-1)



(P-2)



(P-3)



(P-4)

wherein

Aib, Z₀, Z₁, Z₂, Z₃ and K¹ are as defined in claim 1.

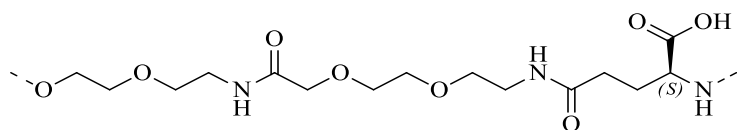
3. The compound or the pharmaceutically acceptable salt thereof according to claim 1 or 2, wherein X is selected from -C(=O)-CH₂-.

4. The compound or the pharmaceutically acceptable salt thereof according to claim 1 or 2, wherein m, n and p are each independently selected from 2.

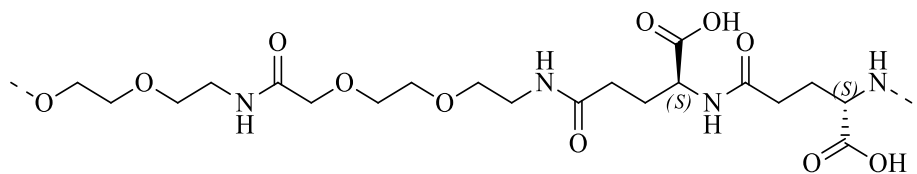
5. The compound or the pharmaceutically acceptable salt thereof according to claim 1 or 2, wherein s is independently selected from 2.

6. The compound or the pharmaceutically acceptable salt thereof according to claim 1 or 2, wherein q is independently selected from 15 and 17.

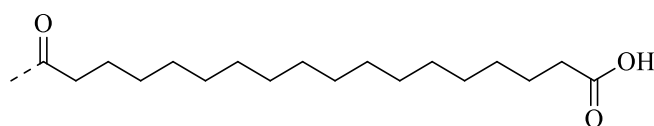
7. The compound or the pharmaceutically acceptable salt thereof according to claim 1 or 2, wherein X₁ is selected from



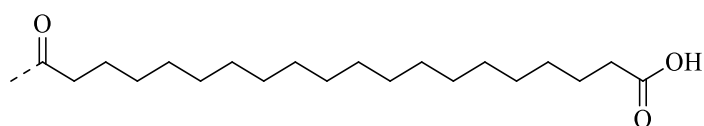
and



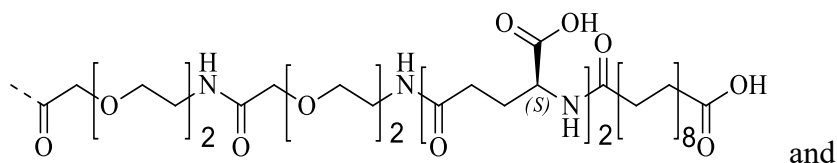
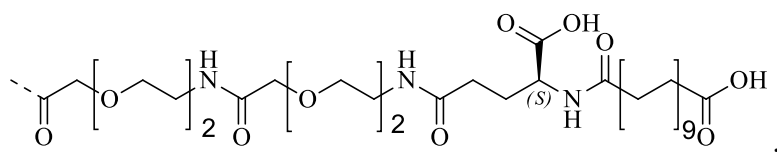
8. The compound or the pharmaceutically acceptable salt thereof according to claim 1 or 2, wherein X₂ is selected from



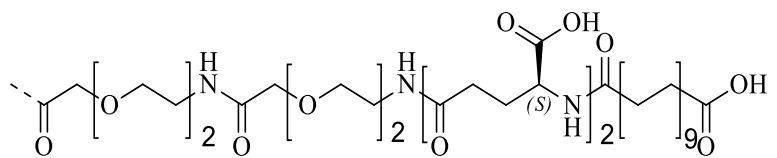
and



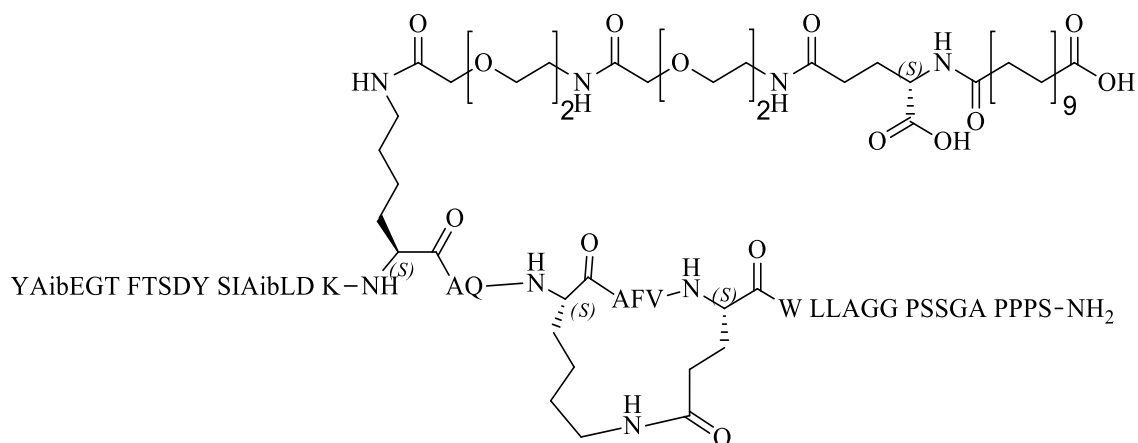
9. The compound or the pharmaceutically acceptable salt thereof according to claim 1 or 2, wherein -X-X₁-X₂ is selected from



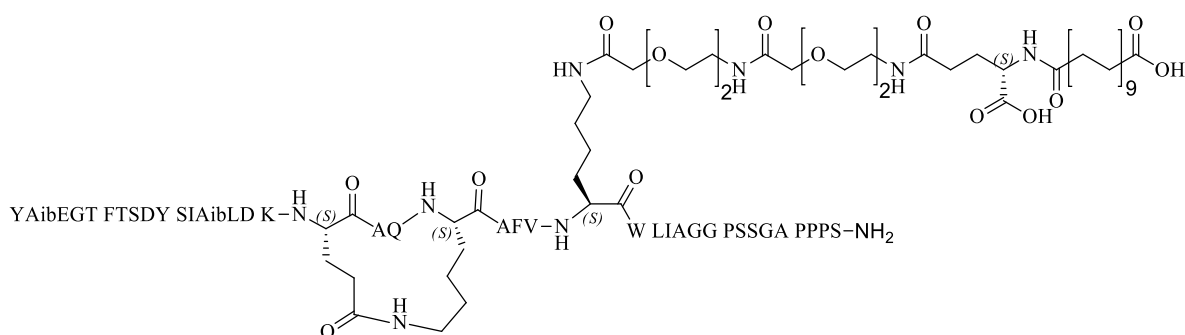
and



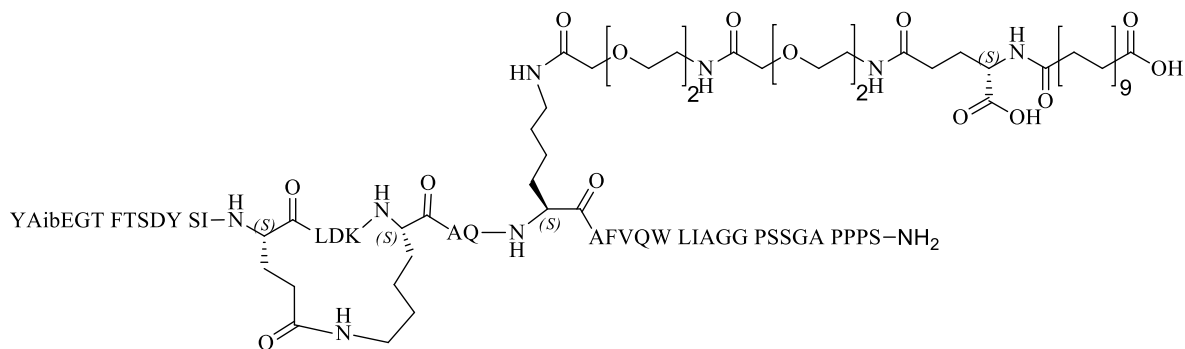
10. A compound represented by the following formula or a pharmaceutically acceptable salt thereof,



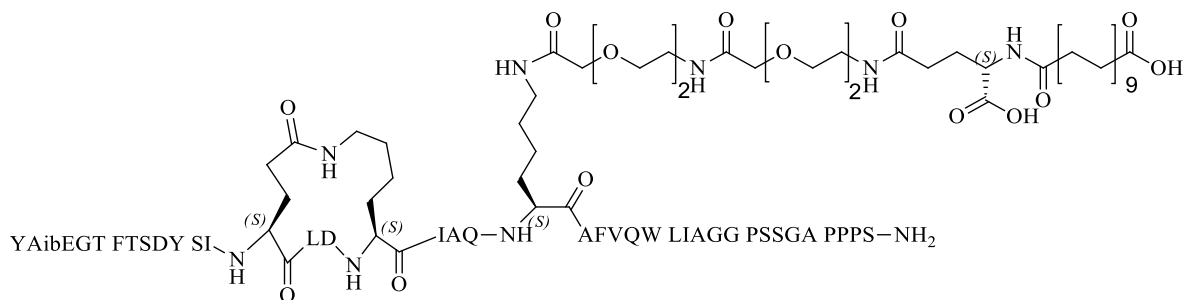
WX-007



WX-008



WX-009



WX-010

11. A use of the compound or the pharmaceutically acceptable salt thereof according to any

one of claims 1 to 10 in the manufacture of a medicament for treating obesity and diabetes.