

MB-102 RELATED COMPOUND, AND PREPARATION METHOD THEREFOR AND USE THEREOF

FIELD OF INVENTION

The present invention relates to pharmaceutical compounds, particularly to compounds related to 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine (MB-102), and preparation methods therefore and uses thereof.

BACKGROUND

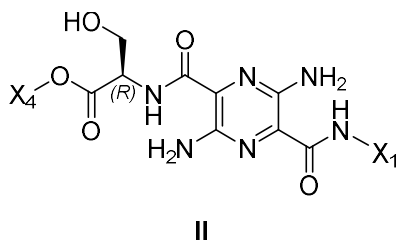
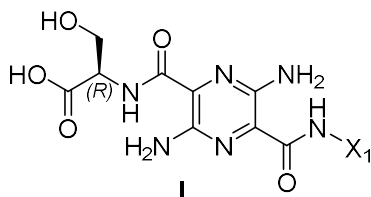
Glomerular Filtration Rate (GFR) is the best indicator for evaluating renal function. 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine (MB-102) is a bis-serine aminopyrazine compound developed by MediBeacon Inc. (USA) for use in the MediBeacon™ Transdermal GFR Measurement System, and has potential application prospects as a fluorescent tracer for evaluating renal function.

There is a need to perform quality control on the active pharmaceutical ingredient of MB-102, formulations thereof and synthesis intermediates therefor during production and storage.

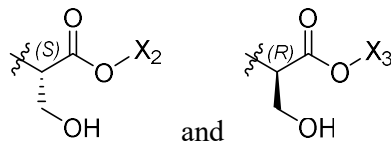
SUMMARY OF THE INVENTION

The inventors have found that compounds of formula I, formula VI, formula X, and formula XI as described in the present disclosure are potentially useful for quality control of the active pharmaceutical ingredient of MB-102 and a formulation thereof, for example, as standards or controls for quality control.

In a first aspect, the present disclosure provides a compound of formula I, and a compound of formula II as a synthesis intermediate for the compound of formula I:



wherein:



X₁ is selected from the group consisting of

X₂ is selected from the group consisting of H, substituted or unsubstituted C₁₋₁₀ alkyl, and -(substituted or unsubstituted C₁₋₁₀ alkyl)-R¹;

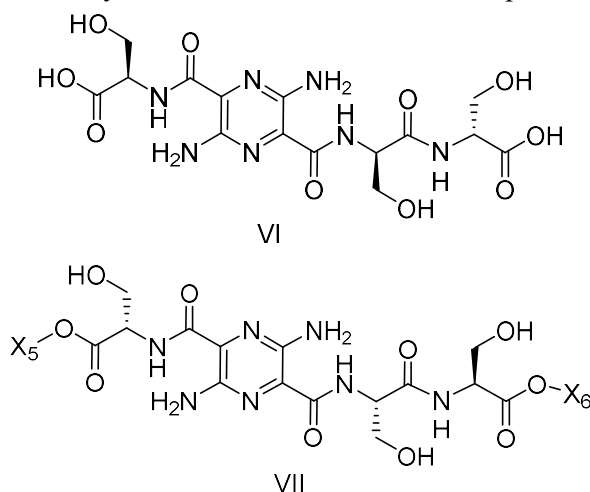
X₃ and X₄ are each independently selected from the group consisting of substituted or unsubstituted C₁₋₁₀ alkyl, and -(substituted or unsubstituted C₁₋₁₀ alkyl)-R¹;

R¹ is selected from the group consisting of C₃₋₁₀ cyclohydrocarbyl, 4-10 membered heterocyclyl, C₆₋₁₀ aryl and 5-10 membered heteroaryl.

In a second aspect, the present disclosure provides a method for preparing the compound of formula I, characterized in that the method comprises the following step:

a) subjecting the compound of formula II to a hydrogenation reaction with hydrogen or a hydrogen-borrowing reagent in a solvent in the presence of a suitable metal catalyst to provide the compound of formula I.

In a third aspect, the present disclosure provides a compound of formula VI, and a compound of formula VII as a synthesis intermediate for the compound of formula VI:



wherein:

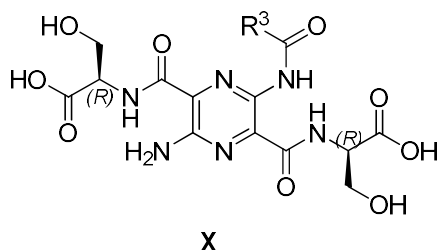
X₅ and X₆ are each independently -(substituted or unsubstituted C₁₋₁₀ alkyl)-R²; and

R² is selected from the group consisting of C₃₋₁₀ cyclohydrocarbyl, 4-10 membered heterocyclyl, C₆₋₁₀ aryl and 5-10 membered heteroaryl.

In a fourth aspect, the present disclosure provides a method for preparing the compound of formula VI, characterized in that the method comprises the following step:

i) subjecting the compound of formula VII to a hydrogenation reaction in a solvent in the presence of a suitable metal catalyst to provide the compound of formula VI.

In a fifth aspect, the present disclosure provides a compound of formula X:

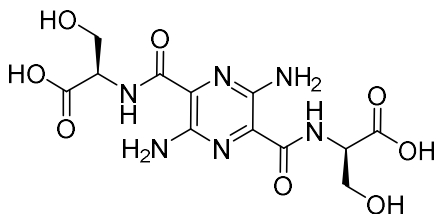


wherein R³ is H, or substituted or unsubstituted C₁₋₄ alkyl, preferably H or methyl.

In some preferred embodiments, R³ is H.

In a sixth aspect, the present disclosure provides a method for preparing the compound of formula X, characterized in that the method comprises the following steps:

x-i) providing a solution of a compound of formula A in a solvent;



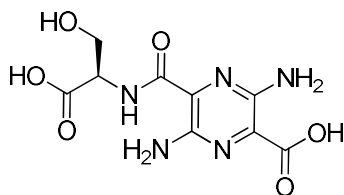
A

x-ii) acylating the compound of formula A with an acid R^3 -COOH at an elevated temperature to provide the compound of formula X; and

x-iii) optionally, isolating and purifying the compound of formula X;

wherein R^3 is H, or substituted or unsubstituted C_{1-4} alkyl, preferably H or methyl, more preferably H.

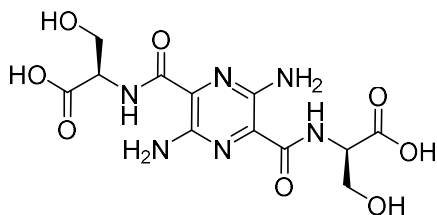
In a seventh aspect, the present disclosure provides a compound of formula XI:



XI

In an eighth aspect, the present disclosure provides a method for preparing the compound of formula XI, characterized in that the method comprises the following steps:

xi-i) hydrolyzing a compound of formula A in water in the presence of a base at an elevated temperature to provide the compound of formula XI or a salt thereof;



A

, and

xi-ii) optionally, isolating and purifying the compound of formula XI or salt thereof.

In a ninth aspect, the present disclosure provides a method for quality control of an active pharmaceutical ingredient of MB-102 or a formulation thereof, comprising determining the content of the compound of formula I, preferably a compound of formula I-1, a compound of formula I-2, or a compound of formula I-3, the compound of formula VI, the compound of formula X, and/or the compound of formula XI in the active pharmaceutical ingredient or the formulation, and optionally comparing with a specified content for each of the said compounds.

In a tenth aspect, the present disclosure provides use of the compound of formula I, preferably a compound of formula I-1, a compound of formula I-2, or a compound of formula I-3, the compound of formula VI, the compound of formula X, and/or the compound of formula XI as a standard or control for quality control of an active pharmaceutical ingredient of MB-102 or a formulation thereof.

DETAILED DESCRIPTION

Definitions

Unless otherwise defined herein, all technical and scientific terms used herein are intended to have the same meanings as commonly understood by one of ordinary skill in the art. Reference to techniques used herein is meant to refer to techniques as commonly understood in the art, including those variations of the techniques or substitutions of equivalent techniques that are obvious to one skilled in the art. While the following terms are believed to be well understood by one skilled in the art, the following definitions are set forth to better explain the present invention.

As used herein, the terms "comprise", "include", "have", "contain", or "involve" and other variants thereof herein are inclusive or open-ended, and do not exclude other unrecited elements or method steps (i.e., these terms also encompass the terms "consist essentially of" and "consist of").

As used herein, the term "alkyl" is a straight or branched saturated monovalent hydrocarbyl. Preferably, the alkyl has 1 to 10 carbon atoms (C₁₋₁₀ alkyl), for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. The alkyl includes C₁₋₈ alkyl, C₁₋₆ alkyl, C₁₋₄ alkyl, C₁₋₃ alkyl, and C₁₋₂ alkyl. Examples of the alkyl include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, and n-decyl. The alkyl may be substituted or unsubstituted.

As used herein, the term "alkenyl" refers to a straight or branched monovalent hydrocarbyl containing one or more double bonds (C=C). Preferably, the alkenyl has 2 to 6 carbon atoms (C₂₋₆ alkenyl), for example, 2, 3, 4, 5, or 6 carbon atoms, and has 1, 2, or 3 double bonds. Examples of the alkenyl include, but are not limited to, vinyl, propenyl, but-1-enyl, but-2-enyl, pent-1-enyl, pent-2-enyl, hex-1-enyl, hex-2-enyl, hex-3-enyl. The alkenyl may be substituted or unsubstituted.

As used herein, the term "alkynyl" refers to a straight or branched monovalent alkynyl group containing one or more triple bonds (C≡C). Preferably, the alkynyl has 2 to 6 carbon atoms (C₂₋₆ alkynyl), for example, 2, 3, 4, 5, or 6 carbon atoms, and has 1, 2, or 3 triple bonds. Examples of the alkynyl include, but are not limited to, ethynyl, propynyl, but-1-ynyl, but-2-ynyl, pent-1-ynyl, pent-2-ynyl, hex-1-ynyl, hex-2-ynyl, hex-3-ynyl. The alkynyl may be substituted or unsubstituted.

As used herein, the term "cyclohydrocarbyl" refers to a saturated (i.e., "cycloalkyl") or partially unsaturated (i.e., having one or more double bonds (cycloalkenyl) and/or triple bonds (cycloalkynyl) in the ring) monocyclic or polycyclic hydrocarbon ring having, for example, 3-10 (suitably 3-8, e.g., 5-7; more suitably 3-6, e.g., 5-6) ring carbon atoms, including but not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclohexenyl, etc. The cyclohydrocarbyl may be substituted or unsubstituted.

As used herein, the term "cycloalkyl" refers to a saturated monocyclic or polycyclic (such as bicyclic) hydrocarbon ring (e.g., monocyclic, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, or bicyclic, including spiro, fused, or bridged systems, such as bicyclo[1.1.1]pentyl, bicyclo[2.2.1]heptyl, bicyclo[3.2.1]octyl or bicyclo[5.2.0]nonyl, decahydronaphthyl, etc.). The cycloalkyl has, for

selected from the group consisting of deuterium (D), halogen, -OH, mercapto, cyano, -CD₃, C₁-C₆ alkyl (preferably C₁-C₃ alkyl), C₂-C₆ alkenyl, C₂-C₆ alkynyl, cycloalkyl (preferably C₃-C₈ cycloalkyl), aryl, heterocyclyl (preferably 3-8 membered heterocyclyl), heteroaryl, aryl-C₁-C₆ alkyl-, heteroaryl-C₁-C₆ alkyl, C₁-C₆ haloalkyl, -OC₁-C₆ alkyl (preferably -OC₁-C₃ alkyl), -OC₂-C₆ alkenyl, OC₁-C₆ alkylphenyl, C₁-C₆ alkyl-OH (preferably C₁-C₄ alkyl-OH), C₁-C₆ alkyl-SH, C₁-C₆ alkyl-O-C₁-C₆ alkyl, OC₁-C₆ haloalkyl, NH₂, C₁-C₆ alkyl-NH₂ (preferably C₁-C₃ alkyl-NH₂), -N(C₁-C₆ alkyl)₂ (preferably -N(C₁-C₃ alkyl)₂), -NH(C₁-C₆ alkyl) (preferably -NH(C₁-C₃ alkyl)), -N(C₁-C₆ alkyl)(C₁-C₆ alkylphenyl), -NH(C₁-C₆ alkylphenyl), nitro, -C(O)-OH, -C(O)OC₁-C₆ alkyl (preferably -C(O)OC₁-C₃ alkyl), -CONRiRii (wherein Ri and Rii are H, D, and C₁-C₆ alkyl, preferably C₁-C₃ alkyl), -NHC(O)(C₁-C₆ alkyl), -NHC(O)(phenyl), -N(C₁-C₆ alkyl)C(O)(C₁-C₆ alkyl), -N(C₁-C₆ alkyl)C(O)(phenyl), -C(O)C₁-C₆ alkyl, -C(O)heteroaryl (preferably -C(O)-5-7 membered heteroaryl), -C(O)C₁-C₆ alkylphenyl, -C(O)C₁-C₆ haloalkyl, -OC(O)C₁-C₆ alkyl (preferably -OC(O)C₁-C₃ alkyl), -S(O)₂-C₁-C₆ alkyl, -S(O)-C₁-C₆ alkyl, -S(O)₂-phenyl, -S(O)₂-C₁-C₆ haloalkyl, -S(O)₂NH₂, -S(O)₂NH(C₁-C₆ alkyl), -S(O)₂NH(phenyl), -NHS(O)₂(C₁-C₆ alkyl), -NHS(O)₂(phenyl) and -NHS(O)₂(C₁-C₆ haloalkyl). When an atom or a group is substituted by multiple substituents, said multiple substituents may be the same or different.

Numerical ranges recited herein are to be understood to encompass any and all subranges contained therein. For example, a range of "1 to 10" should be understood to include not only the explicitly recited values of 1 and 10, but also any individual values (e.g., 2, 3, 4, 5, 6, 7, 8, and 9) and subranges (e.g., 1 to 2, 1.5 to 2.5, 1 to 3, 1.5 to 3.5, 2.5 to 4, 3 to 4.5, etc.) within the range of 1 to 10. This principle also applies to ranges expressed with only one numerical value as the minimum or maximum value.

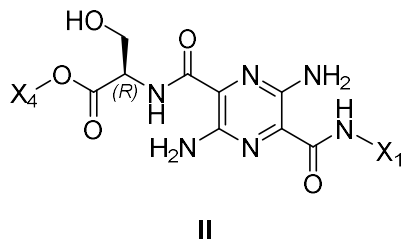
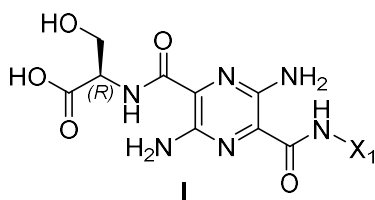
As used herein, the term "room temperature" refers to about 20 to 25°C.

As used herein, the term "elevated temperature" refers to a temperature greater than about 25°C. In some embodiments, "elevated temperature" is a temperature not lower than about 30°C, in some embodiments not lower than about 35°C, in some embodiments not lower than about 40°C, in some embodiments not lower than about 45°C, in some embodiments not lower than about 50°C, in some embodiments not lower than about 55°C, in some embodiments not lower than about 60°C, in some embodiments not lower than about 70°C, in some embodiments not lower than about 80°C, in some embodiments not lower than about 90°C, or in some embodiments not lower than about 100°C, or higher. In some embodiments, "elevated temperature" is a temperature not higher than about 100°C, in some embodiments not higher than about 90°C, in some embodiments not higher than about 80°C, in some embodiments not higher than about 70°C, in some embodiments not higher than about 60°C, in some embodiments not higher than about 55°C, in some embodiments not higher than about 50°C, in some embodiments not higher than about 45°C, or in some embodiments not higher than about 40°C.

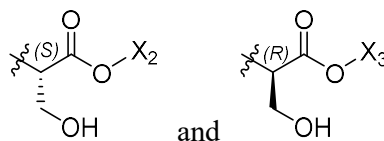
As used herein, the term "about" means within ±10%, preferably within ±5%, more preferably within ±2% of the stated numerical value.

Compounds and Preparation Methods Thereof

In a first aspect, the present disclosure provides a compound of formula I, and a compound of formula II as a synthesis intermediate for the compound of formula I:



wherein:



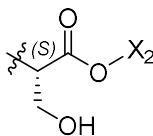
X₁ is selected from the group consisting of

X₂ is selected from the group consisting of H, substituted or unsubstituted C₁₋₁₀ alkyl, and -(substituted or unsubstituted C₁₋₁₀ alkyl)-R¹;

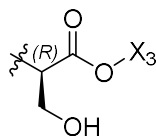
X₃ and X₄ are each independently selected from the group consisting of substituted or unsubstituted C₁₋₁₀ alkyl, and -(substituted or unsubstituted C₁₋₁₀ alkyl)-R¹; and

R¹ is selected from the group consisting of C₃₋₁₀ cyclohydrocarbyl, 4-10 membered heterocyclyl, C₆₋₁₀ aryl and 5-10 membered heteroaryl.

In some embodiments, R¹ is selected from the group consisting of C₃₋₇ cycloalkyl, preferably C₅₋₆ cycloalkyl; 4-7 membered heterocyclyl, preferably 5-6 membered heterocyclyl; phenyl and 5-6 membered heteroaryl. Preferably, R¹ is phenyl.



In some embodiments, X₁ is . In some such embodiments, X₂ is selected from the group consisting of H, substituted or unsubstituted C₁₋₈ alkyl, and -(substituted or unsubstituted C₁₋₈ alkyl)-R¹. In some embodiments, X₂ is selected from the group consisting of H, substituted or unsubstituted C₁₋₆ alkyl, and -(substituted or unsubstituted C₁₋₆ alkyl)-R¹. In some embodiments, X₂ is selected from the group consisting of H, substituted or unsubstituted C₁₋₄ alkyl, and -(substituted or unsubstituted C₁₋₄ alkyl)-R¹. In some embodiments, X₂ is selected from the group consisting of H, unsubstituted C₁₋₂ alkyl, and -(unsubstituted C₁₋₂ alkyl)-R¹. In some preferred embodiments, X₂ is selected from the group consisting of H, ethyl and -CH₂-R¹. More preferably, X₂ is selected from the group consisting of H, ethyl and -CH₂-phenyl.

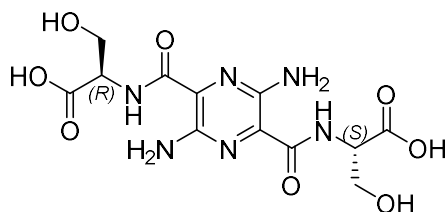


In some other embodiments, X₁ is . In some such embodiments, X₃ is selected from the group consisting of substituted or unsubstituted C₁₋₈ alkyl, and -(substituted or unsubstituted C₁₋₈ alkyl)-R¹. In some embodiments, X₃ is preferably selected from the

group consisting of substituted or unsubstituted C₁₋₆ alkyl, and -(substituted or unsubstituted C₁₋₆ alkyl)-R¹. In some embodiments, X₃ is more preferably selected from the group consisting of substituted or unsubstituted C₁₋₄ alkyl, and -(substituted or unsubstituted C₁₋₄ alkyl)-R¹. In some embodiments, X₃ is more preferably selected from the group consisting of unsubstituted C₁₋₂ alkyl, and -(unsubstituted C₁₋₂ alkyl)-R¹. In some embodiments, X₃ is more preferably selected from the group consisting of ethyl and -CH₂-R¹. More preferably, X₃ is selected from the group consisting of ethyl and -CH₂-phenyl.

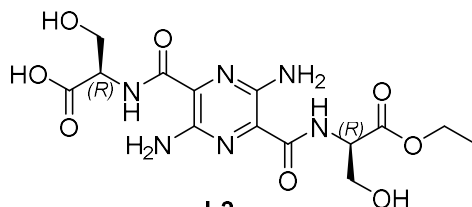
In some embodiments, X₄ is selected from the group consisting of substituted or unsubstituted C₁₋₈ alkyl, and -(substituted or unsubstituted C₁₋₈ alkyl)-R¹. In some embodiments, X₄ is preferably selected from the group consisting of substituted or unsubstituted C₁₋₆ alkyl, and -(substituted or unsubstituted C₁₋₆ alkyl)-R¹. In some embodiments, X₄ is more preferably selected from the group consisting of substituted or unsubstituted C₁₋₄ alkyl, and -(substituted or unsubstituted C₁₋₄ alkyl)-R¹. In some embodiments, X₄ is more preferably selected from the group consisting of unsubstituted C₁₋₂ alkyl, and -(unsubstituted C₁₋₂ alkyl)-R¹. In some embodiments, X₄ is more preferably selected from the group consisting of ethyl and -CH₂-R¹. More preferably, X₄ is selected from the group consisting of ethyl and -CH₂-phenyl. Even more preferably, X₄ is -CH₂-phenyl.

In some embodiments, the compound of formula I is a compound of formula I-1:



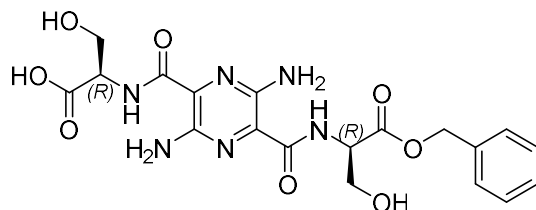
I-1

In some embodiments, the compound of formula I is a compound of formula I-2:



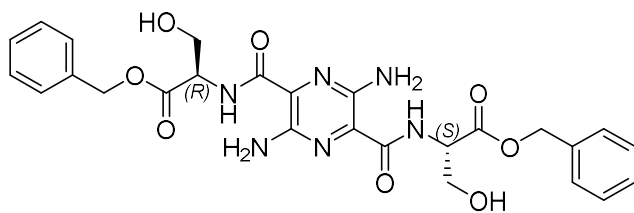
I-2

In some embodiments, the compound of formula I is a compound of formula I-3:



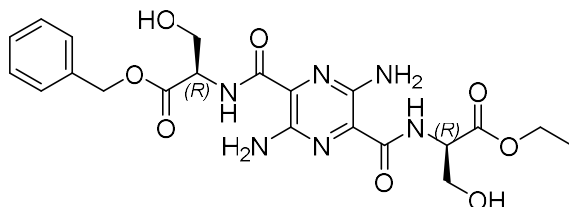
I-3

In some embodiments, the compound of formula II is a compound of formula II-1:



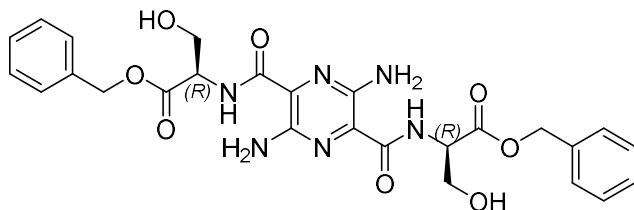
II-1

In some embodiments, the compound of formula II is a compound of formula II-2:



II-2

In some embodiments, the compound of formula II is a compound of formula II-3:



II-3

In a second aspect, the present disclosure provides a method for preparing the compound of formula I as described above, characterized in that the method comprises the following step:

a) subjecting the compound of formula II as described above to a hydrogenation reaction with hydrogen or a hydrogen-borrowing reagent in a solvent in the presence of a suitable metal catalyst to provide the compound of formula I.

In some embodiments, in the step a), the compound of formula II is subjected to the hydrogenation reaction with the hydrogen-borrowing reagent. In some such embodiments, the molar ratio of the compound of formula II to the hydrogen-borrowing reagent is from about 1:2 to 1:20, preferably from about 1:2 to 1:10, for example, about 1:2.5, about 1:4, about 1:5, about 1:6, or about 1:8. In some embodiments, said hydrogen-borrowing reagent is selected from the group consisting of a combination of formic acid and an alkali metal formate, cyclohexadiene, and isopropanol. In some preferred embodiments, the hydrogen-borrowing reagent is a combination of formic acid and an alkali metal formate. Preferably, the molar ratio of the formic acid to the alkali metal formate is from about 1:6 to 6:1, preferably from about 1:1 to 5:1. In some embodiments, the alkali metal formate is sodium formate or potassium formate, preferably potassium formate. In some embodiments, the reaction of the compound of formula II with the hydrogen-borrowing reagent is conducted at a temperature from about 20°C to 100°C, more preferably from about 20°C to 80°C, more preferably room temperature or from about 50°C to 70°C, more preferably about 60°C. In some other embodiments, in the step a), the compound of formula II is subjected to the

hydrogenation reaction with the hydrogen. In some such embodiments, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa. In some embodiments, the reaction of the compound of formula II with the hydrogen is conducted at a temperature from about 15 to 25°C (e.g., from about 20 to 25°C).

The reaction using the hydrogen-borrowing reagent has many advantages, for example, it avoids the use of flammable and explosive hydrogen, greatly reducing safety hazards in industrial production; the reaction time is short; the use of an alkali metal formate instead of ammonium formate avoids the problem of pipeline blockage caused by sublimation when using ammonium formate.

In some embodiments, in the step a), the volume ratio of the solvent to the compound of formula II is from about 10:1 to 80:1, preferably from about 20:1 to 50:1. In some embodiments, the solvent is an aqueous alcohol solution. In some embodiments, the volume ratio of alcohol to water in the aqueous alcohol solution is from about 1:1 to about 8:1 (e.g., about 4:1 or about 6:1). In some embodiments, the alcohol in the aqueous alcohol solution is selected from the group consisting of methanol (MeOH), ethanol (EtOH), isopropanol, and any mixture thereof. Preferably, the alcohol is ethanol. In some other embodiments, the solvent is a mixed solvent of an alcohol and an ester. In some embodiments, the volume ratio of the ester to the alcohol in the mixed solvent of the alcohol and the ester is from about 1:1 to about 1:5, more preferably from about 1:1 to about 1:2. In some embodiments, the alcohol in the mixed solvent of the alcohol and the ester is selected from the group consisting of methanol (MeOH), ethanol (EtOH), isopropanol, and any mixture thereof. Preferably, the alcohol is ethanol. In some embodiments, the ester in the mixed solvent of the alcohol and the ester is selected from the group consisting of ethyl acetate (EA), isopropyl acetate, or a mixture thereof. Preferably, the ester is ethyl acetate (EA). In some embodiments, the alcohol in the mixed solvent of the alcohol and the ester is ethanol, and/or the ester is ethyl acetate (EA).

In some preferred embodiments, the hydrogenation reaction of the compound of formula II with the hydrogen-borrowing reagent is conducted in the aqueous alcohol solution as described above. In some preferred embodiments, the hydrogenation reaction of the compound of formula II with the hydrogen is conducted in the aqueous alcohol solution as described above, or in the mixed solvent of the alcohol and the ester as described above.

In some embodiments, the mass ratio of the compound of formula II to the metal catalyst is from about 1:0.01 to 1:1, preferably about 1:0.2. In some embodiments, the metal catalyst is palladium on carbon (Pd/C). Preferably, the mass fraction of palladium in the Pd/C is from about 5-20%, more preferably about 10% (e.g., the Pd/C catalyst contains about 50 w/w% water).

In some embodiments, the hydrogenation reaction is conducted for a period of time from about 1 to 72 hours (h), preferably from about 1 to 48 h, more preferably from about 6 to 12 h.

In some embodiments, the present disclosure provides the method as described above, the method comprising the following step:

a) subjecting the compound of formula II to a hydrogenation reaction with the hydrogen or with the hydrogen-borrowing reagent in an aqueous ethanol solution or a mixed solvent of ethanol and ethyl acetate under the catalysis of Pd/C for about 1 to 72 h, e.g., about 1 to

48 h, about 1 to 12 h, about 1 to 10 h, about 2 to 5 h, or about 6 to 12 h, to provide the compound of formula I,

wherein:

(1) in some embodiments, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa, and

the reaction of the compound of formula II with the hydrogen is conducted at a temperature from about 20 to 25°C;

or

in some other embodiments, the molar ratio of the compound of formula II to the hydrogen-borrowing reagent is from about 1:2 to 1:10, e.g., about 1:2.5, about 1:4, about 1:5, about 1:6, or about 1:8,

the reaction of the compound of formula II with the hydrogen-borrowing reagent is conducted at a temperature from about 20°C to 100°C, preferably from about 20°C to 80°C, more preferably room temperature or from about 50°C to 70°C, more preferably about 60°C, and

the hydrogen-borrowing reagent is a combination of formic acid and potassium formate, wherein the molar ratio of the formic acid to the potassium formate is from about 1:1 to 5:1;

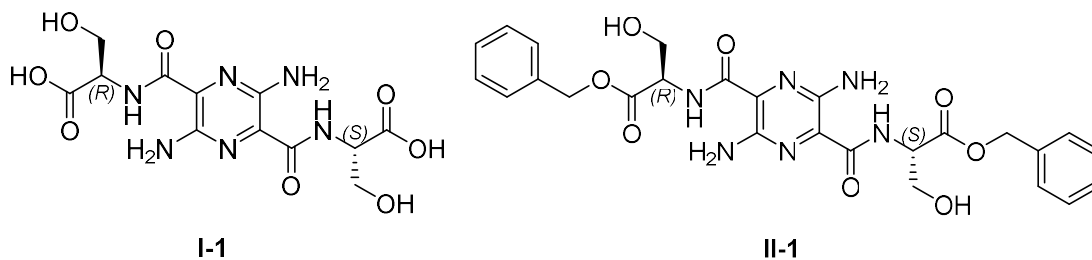
(2) the mass ratio of the compound of formula II to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2;

(3) the volume ratio of the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate to the compound of formula II is from about 10:1 to 80:1, preferably from about 20:1 to 50:1; and

(4) the volume ratio of ethanol to water in the aqueous ethanol solution is from about 1:1 to about 8:1, e.g., about 4:1 or about 6:1; and

the volume ratio of ethyl acetate to ethanol in the mixed solvent of ethanol and ethyl acetate is from about 1:1 to about 1:5, preferably about 1:1 to about 1:2.

In some embodiments of the method according to the present disclosure as described above, the compound of formula I is the compound of formula I-1, and the compound of formula II is the compound of formula II-1:



In some such embodiments, the method comprises the following step:

a) subjecting the compound of formula II-1 to a hydrogenation reaction with the hydrogen-borrowing reagent in the aqueous ethanol solution under the catalysis of the Pd/C for about 1 to 12 h to provide the compound of formula I-1.

In some preferred embodiments, the molar ratio of the compound of formula II-1 to the hydrogen-borrowing reagent is from about 1:2 to 1:10, e.g., about 1:2.5, about 1:4, about 1:5, about 1:6, or about 1:8. In some embodiments, the reaction of the compound of formula II-1 with the hydrogen-borrowing reagent is conducted at a temperature from about 20°C to

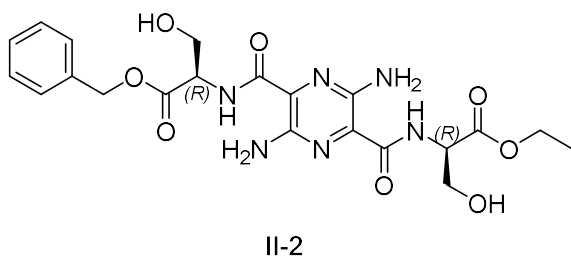
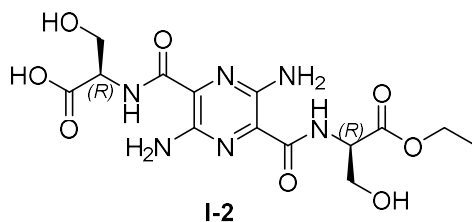
100°C, preferably from about 20°C to 80°C, more preferably room temperature or from about 50°C to 70°C, more preferably about 60°C. In some embodiments, the hydrogen-borrowing reagent is a combination of formic acid and potassium formate, wherein the molar ratio of the formic acid to the potassium formate is from about 1:1 to 5:1.

In some other such embodiments, the method comprises the following step:

a) subjecting the compound of formula II-1 to a hydrogenation reaction with the hydrogen in the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate under the catalysis of the Pd/C for about 6 to 12 h to provide the compound of formula I-1.

In some preferred embodiments, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa. In some embodiments, the reaction of the compound of formula II-1 with the hydrogen is conducted at a temperature from about 20 to 25°C. In some embodiments, the mass ratio of the compound of formula II-1 to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2. In some embodiments, the volume ratio of the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate to the compound of formula II-1 is from about 10:1 to 80:1, preferably from about 20:1 to 50:1. In some embodiments, the volume ratio of ethanol to water in the aqueous ethanol solution is from about 1:1 to about 8:1 (e.g., about 4:1 or about 6:1). In some other embodiments, the volume ratio of ethyl acetate to ethanol in the mixed solvent of ethanol and ethyl acetate is from about 1:1 to about 1:5, preferably from about 1:1 to about 1:2.

In some other embodiments of the method according to the present disclosure as described above, the compound of formula I is the compound of formula I-2, and the compound of formula II is the compound of formula II-2:



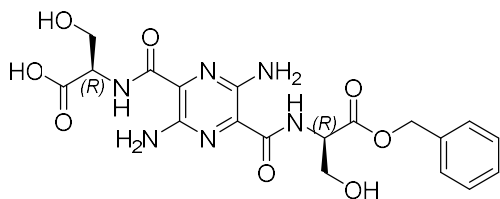
In some such embodiments, the method comprises the following step:

a) subjecting the compound of formula II-2 to a hydrogenation reaction with the hydrogen in the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate under the catalysis of the Pd/C for about 6 to 12 h to provide the compound of formula I-2.

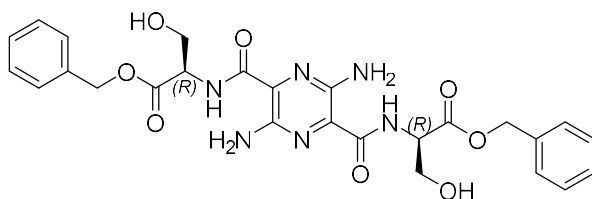
In some preferred embodiments, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa. In some embodiments, the reaction of the compound of formula II-2 with the hydrogen is conducted at a temperature from about 20 to 25°C. In some embodiments, the mass ratio of the compound of formula II-2 to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2. In some embodiments,

the volume ratio of the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate to the compound of formula II-2 is from about 10:1 to 80:1, preferably from about 20:1 to 50:1. In some embodiments, the volume ratio of ethanol to water in the aqueous ethanol solution is from about 1:1 to about 8:1 (e.g., about 4:1 or about 6:1). In some other embodiments, the volume ratio of ethyl acetate to ethanol in the mixed solvent of ethanol and ethyl acetate is from about 1:1 to about 1:5, preferably from about 1:1 to about 1:2.

In some other embodiments of the method according to the present disclosure as described above, the compound of formula I is the compound of formula I-3, and the compound of formula II is the compound of formula II-3:



I-3



II-3

In some such embodiments, the method comprises the following step:

a) subjecting the compound of formula II-3 to a hydrogenation reaction with the hydrogen in the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate under the catalysis of Pd/C for about 1 to 12 h, e.g., about 1 to 10 h, about 2 to 5 h, or about 6 to 12 h, to provide the compound of formula I-3.

In some preferred embodiments, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa. In some embodiments, the reaction of the compound of formula II-3 with the hydrogen is conducted at a temperature from about 20 to 25°C. In some embodiments, the mass ratio of the compound of formula II-3 to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2. In some embodiments, the volume ratio of the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate to the compound of formula II-3 is from about 10:1 to 80:1, preferably from about 20:1 to 50:1. In some embodiments, the volume ratio of ethanol to water in the aqueous ethanol solution is from about 1:1 to about 8:1 (e.g., about 4:1 or about 6:1). In some other embodiments, the volume ratio of ethyl acetate to ethanol in the mixed solvent of ethanol and ethyl acetate is from about 1:1 to about 1:5, preferably from about 1:1 to about 1:2.

In some embodiments of the method according to the present disclosure as described above, the method further comprises the following steps:

b) removing the metal catalyst from the reaction mixture of the step a), then drying the reaction mixture to obtain the compound of formula I; and

c) optionally, further purifying the compound of formula I obtained from the step b).

In the step b), the metal catalyst can be removed by filtration through diatomite; the

reaction mixture after removal of the metal catalyst can be concentrated under reduced pressure to dryness at an elevated temperature (e.g., about 40-50°C, preferably about 45°C).

In the step c), the purifying can be performed by:

slurrying the compound of formula I obtained from the step b) with acetonitrile (e.g., for about 1 to 4 h), further slurrying with water (for about 1 to 4 h), then filtering, and drying the filter cake.

For example, the drying can be performed by concentration under reduced pressure at an elevated temperature (e.g., about 40-50°C, preferably about 45°C).

In the step c), the purifying can also be performed by:

subjecting the compound of formula I obtained from the step b) to passage through a chromatographic column or a high-pressure preparative chromatograph.

There is no particular limitation on the high-pressure preparative chromatograph. Applicable high-pressure preparative chromatographs include, but are not limited to, LAB-ZB-05, the medium or high pressure equipments from Beijing Aonuo Technology Co., Ltd., etc.

In some embodiments of the method according to the present disclosure as described above, wherein the compound of formula I is the compound of formula I-1 and the compound of formula II is the compound of formula II-1, the method comprises performing step c). In some such embodiments, in step c), the compound of formula I-1 obtained from step b) is further purified by slurrying the compound of formula I-1 obtained from step b) with acetonitrile (e.g., for about 1 to 4 h), further slurrying with water (for about 1 to 4 h), then filtering, and drying the filter cake. The drying can be performed by concentration under reduced pressure at a higher temperature (e.g., about 40-50°C, preferably about 45°C).

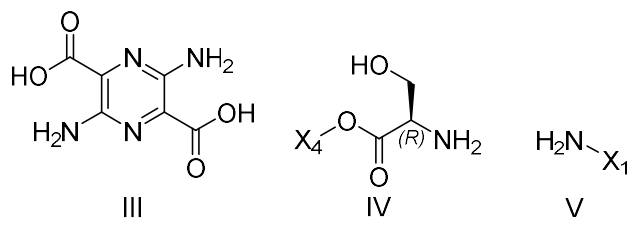
In some other embodiments, wherein the compound of formula I is the compound of formula I-2 and the compound of formula II is the compound of formula II-2, the method comprises performing step c). In some such embodiments, in step c), the compound of formula I-2 obtained from step b) is further purified by slurrying the compound of formula I-2 obtained from step b) with acetonitrile (e.g., for about 1 to 4 h), further slurrying with water (for about 1 to 4 h), then filtering, and drying the filter cake; or subjecting the compound of formula I-2 obtained from step b) to passage through a chromatographic column or a high-pressure preparative chromatograph. The drying can be performed by concentration under reduced pressure at an elevated temperature (e.g., about 40-50°C, preferably about 45°C).

In still other embodiments, wherein the compound of formula I is the compound of formula I-3 and the compound of formula II is the compound of formula II-3, the method comprises performing step c). In some such embodiments, in step c), the compound of formula I-3 obtained from step b) is further purified by subjecting the compound of formula I-3 obtained from step b) to passage through a chromatographic column or a high-pressure preparative chromatograph.

In some embodiments of the method according to the present disclosure as described above, the method further comprises the following step:

a') subjecting a compound of formula III, 3,6-diaminopyrazine-2,5-dicarboxylic acid to an amide condensation reaction with a compound of formula IV or a salt thereof, and a compound of formula V or a salt thereof in a suitable organic solvent in the presence of a

suitable organic base, a suitable coupling agent and a suitable condensing agent, to provide the compound of formula II,

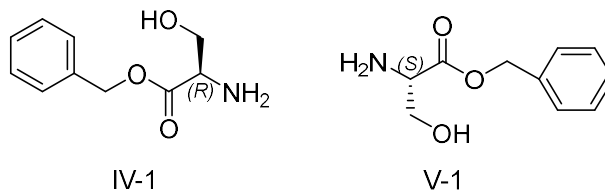


wherein X₁ and X₄ are as defined above.

The salt of the compound of formula IV includes, but is not limited to, a hydrochloride and a hydrobromide, preferably a hydrochloride; the salt of the compound of formula V includes, but is not limited to, a hydrochloride and a hydrobromide, preferably a hydrochloride.

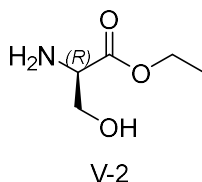
In some embodiments, wherein the compound of formula II is the compound of formula II-1, the method comprises:

reacting the compound of formula III with the hydrochloride of the compound of formula IV having a formula IV-1 and the hydrochloride of the compound of formula V having formula V-1 to provide the compound of formula II-1:



In some other embodiments, wherein the compound of formula II is the compound of formula II-2, the method comprises:

reacting the compound of formula III with the hydrochloride of the compound of formula IV having the formula IV-1 and the hydrochloride of the compound of formula V having a formula V-2 to provide the compound of formula II-2:



In still other embodiments, wherein the compound of formula II is the compound of formula II-3, the method comprises:

reacting the compound of formula III with the hydrochloride of the compound having the formula IV-1 to provide the compound of formula II-3.

In some embodiments of the method according to the present disclosure as described above, the molar ratio of the compound of formula IV to the compound of formula III is from about 0.5:1 to 8:1, preferably from about 1:1 to 4:1.

In some embodiments, the molar ratio of the compound of formula V to the compound of formula III is about (0.5 to 8.0):1.0, preferably from about 1:1 to 4:1.

In some embodiments, the volume ratio of the organic solvent to the compound of formula III is from about 10:1 to 80:1, preferably from about 20:1 to 50:1.

In some embodiments, the molar ratio of the organic base to the compound of formula III is from about 0.5:1 to 9:1, preferably from about 1:1 to 5:1.

In some embodiments, the molar ratio of the coupling agent to the compound of formula III is from about 0.5:1 to 9:1, preferably from about 1:1 to 5:1.

In some embodiments, the molar ratio of the condensing agent to the compound of formula III is from about 0.5:1 to 9:1, preferably from about 1:1 to 5:1.

In some embodiments, the amide condensation reaction is first conducted at a suitable temperature, preferably from about -5 to 20°C, more preferably from about -5 to 10°C, more preferably from about 0 to 10°C for a suitable period of time, e.g., about 10-60 minutes (min), then at room temperature for a suitable period of time, preferably for about 10-72 h, more preferably about 18-36 h.

In some embodiments, the organic solvent is N,N-dimethylformamide (DMF), DCM, DMSO, tetrahydrofuran and/or 1,4-dioxane.

In some embodiments, the organic base is diisopropylethylamine (DIPEA), triethylamine, pyridine and/or DMAP.

In some embodiments, the coupling agent is ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma), 1-hydroxybenzotriazole (HOBt), HATU, HBTU, HCTU, TBTU, TSTU, TNTU, O-(7-azabenzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HAPyU) and/or O-(benzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HBPYU).

In some embodiments, the condensing agent is selected from the group consisting of 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), HOBt, HATU, HBTU, HCTU, TBTU, TSTU, TNTU, (7-azabenzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HAPyU), O-(benzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HBPYU), and any combination thereof.

In some embodiments of the method according to the present disclosure as described above, the method comprises the following step:

a') reacting the compound of formula III with the compound of formula IV and the compound of formula V in DMF at about -5 to 20°C, preferably about -5 to 10°C, more preferably about 0 to 10°C, in the presence of DIPEA, ethyl 2-cyano-2-(hydroxyimino)acetate and EDC·HCl for about 10-60 min, then reacting at room temperature for about 10-72 h, more preferably about 18-36 h.

In some embodiments, the method further comprises the following steps:

b') optionally, adding water to the reaction mixture of the step a') to precipitate the compound of formula II; and

c') optionally, further purifying the compound of formula II obtained from step b').

Preferably, in step b'), the volume ratio of the added water to the reaction mixture is from about 20:1 to 150:1, more preferably from about 35:1 to 75:1.

Preferably, in step c'), the purifying is performed by subjecting the compound of formula II obtained from step b') to passage through a chromatographic column or a high-pressure preparative chromatograph.

In some embodiments of the method according to the present disclosure as described above, wherein the compound of formula II is the compound of formula II-1, the method comprises performing the step c'). In some such embodiments, in the step c'), the compound of formula II-1 is further purified by subjecting the compound of formula II-1 obtained from

the step b') to passage through a chromatographic column.

In some other embodiments, wherein the compound of formula II is the compound of formula II-2, the method comprises performing the step c'). In some such embodiments, in the step c'), the compound of formula II-2 is further purified by subjecting the compound of formula II-2 obtained from the step b') to passage through a chromatographic column or a high-pressure preparative chromatograph.

In still some other embodiments, wherein the compound of formula II is the compound of formula II-3, the method comprises performing the step c'). In some such embodiments, in the step c'), the compound of formula II-3 is further purified by subjecting the compound of formula II-3 obtained from the step b') to passage through a chromatographic column or a high-pressure preparative chromatograph.

In a first specific embodiment, a method according to the present disclosure comprises the following steps:

(1) adding 3,6-diaminopyrazine-2,5-dicarboxylic acid (the compound of formula III) and D-serine benzyl ester hydrochloride as well as L-serine benzyl ester hydrochloride into a vessel, adding DMF and stirring uniformly, wherein preferably, the molar ratio of D-serine benzyl ester hydrochloride to the compound of formula III and the molar ratio of L-serine benzyl ester hydrochloride to the compound of formula III are each from about 0.5:1 to 8:1, preferably from about 1:1 to 4:1; and the volume ratio of DMF to the compound of formula III is from about 10:1 to 80:1, preferably from about 20:1 to 50:1;

(2) after cooling to about -5°C to 20°C (preferably about -5 to 10°C, more preferably about 0 to 10°C), adding DIPEA, adding ethyl 2-cyano-2-(hydroxyimino)acetate after about 30 min, reacting for about 10 min to 60 min, then adding EDC·HCl, reacting for another about 10 min to 60 min, then increasing to room temperature and reacting for about 10 h to 72 h (preferably about 18 to 36 h), wherein preferably, the molar ratio of DIPEA to the compound of formula III is from about 0.5:1 to 9:1, preferably from about 1:1 to 5:1; and the molar ratios of ethyl 2-cyano-2-(hydroxyimino)acetate to the compound of formula III and the molar ratios of EDC·HCl to the compound of formula III are each from about 0.5:1 to 9:1, preferably from about 1:1 to 5:1;

(3) adding purified water at a volume ratio from about 20:1 to 150:1 (preferably from about 35:1 to 75:1) relative to the compound of formula III, filtering after a solid precipitates out, to obtain a crude product of the compound of formula II-1;

(4) subjecting the crude product from step (3) to passage through a chromatographic column to obtain a purified compound of formula II-1;

(5) subjecting the purified compound of formula II-1 to reaction with a hydrogen at about 0.05 MPa to about 0.8 MPa (preferably about 0.1 MPa to about 0.4 MPa) in an aqueous alcohol solution under the catalysis of Pd/C for about 1 to 72 h (preferably about 1 to 48 h, more preferably about 6 to 12 h), removing the Pd/C after completion of the reaction (e.g., by filtration through diatomite), drying the residue (e.g., by concentrating the filtrate under reduced pressure to dryness at about 45°C), to obtain a crude product of the compound of formula I-1, wherein preferably, the mass ratio of the compound of formula II-1 to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2; the mass fraction of palladium in the Pd/C is about 5-20%, more preferably about 10% (e.g., this Pd/C catalyst contains about 50 w/w% water); the volume ratio of alcohol to water in the aqueous alcohol solution is from

about 1:1 to about 8:1 (e.g., about 4:1 or about 6:1); and the alcohol in the aqueous alcohol solution is selected from the group consisting of methanol, ethanol, isopropanol, and any mixture thereof, preferably ethanol;

(6) optionally, slurrying the crude product from step (5) first with acetonitrile for about 1 to 4 h, then with water for about 1 to 4 h, then filtering, drying the filter cake under reduced pressure at an elevated temperature (e.g., about 45°C), to obtain the compound of formula I-1 as the final product.

In a second specific embodiment, the present disclosure provides a method as described in the first specific embodiment, except that the L-serine benzyl ester hydrochloride in the step (1) is replaced with D-serine ethyl ester hydrochloride; the compound of formula II-1 in the steps (3), (4) and (5) is replaced with the compound of formula II-2; and the compound of formula I-1 in the steps (5) and (6) is replaced with the compound of formula I-2.

In a third specific embodiment, the present disclosure provides a method as described in the first specific embodiment, except that the L-serine benzyl ester hydrochloride in the step (1) is replaced with D-serine benzyl ester hydrochloride; the compound of formula II-1 in the steps (3), (4) and (5) is replaced with the compound of formula II-3; and the compound of formula I-1 in the steps (5) and (6) is replaced with the compound of formula I-3.

In a fourth specific embodiment, the present disclosure provides a method as described in the first specific embodiment, except that the step (5) is replaced with the following step (5'):

(5') subjecting the compound of formula II-1 to reaction in an aqueous alcohol solution in the presence of formic acid and potassium formate under the catalysis of Pd/C at about 20°C to 100°C (preferably about 20°C to 80°C, more preferably room temperature or about 50°C to 70°C, more preferably about 60°C) for about 1 to 12 h in a vessel, removing the Pd/C after completion of the reaction (e.g., by filtration through diatomite), drying the residue (e.g., by concentrating the filtrate under reduced pressure to dryness at about 40°C), to obtain crude product of the compound of formula I-1, wherein preferably, the molar ratio of the compound of formula II-1 to formate (HCOO^-) is from about 1:2 to 1:10 (e.g., about 1:2.5, about 1:4, about 1:5, about 1:6, or about 1:8); the mass ratio of the compound of formula II-1 to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2; the mass fraction of palladium in the Pd/C is about 5-20%, more preferably about 10% (e.g., this Pd/C catalyst contains about 50 w/w% water); the volume ratio of alcohol to water in the aqueous alcohol solution is from about 4:1 to about 8:1; and the alcohol in the aqueous alcohol solution is selected from the group consisting of methanol, ethanol, isopropanol, and any mixture thereof, preferably ethanol.

In a fifth specific embodiment, the present disclosure provides a method as described in the fourth specific embodiment, except that the L-serine benzyl ester hydrochloride in the step (1) is replaced with D-serine ethyl ester hydrochloride; the compound of formula II-1 in the steps (3), (4) and (5) is replaced with the compound of formula II-2; and the compound of formula I-1 in the steps (5) and (6) is replaced with the compound of formula I-2.

In a sixth specific embodiment, the present disclosure provides a method as described in the fourth specific embodiment, except that the L-serine benzyl ester hydrochloride in the step (1) is replaced with D-serine benzyl ester hydrochloride; the compound of formula II-1 in the steps (3), (4) and (5) is replaced with the compound of formula II-3; and the compound

of formula I-1 in the steps (5) and (6) is replaced with the compound of formula I-3.

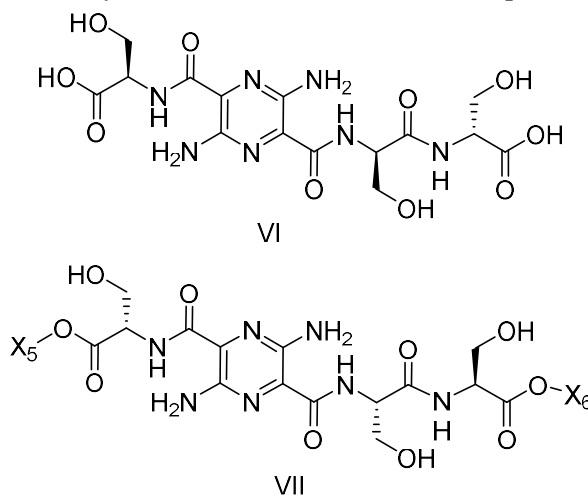
In a seventh specific embodiment, a method according to the present disclosure comprises the following steps:

- (i) performing step (1) of the method according to any one of the first, second, third, fourth, fifth, or sixth specific embodiment as described above;
- (ii) correspondingly performing step (2) of the method according to any one of the above first, second, third, fourth, fifth, or sixth specific embodiment as described above;
- (iii) correspondingly performing step (3) of the method according to any one of the above first, second, third, fourth, fifth, or sixth specific embodiment as described above;
- (iv) correspondingly performing step (5) of the method according to any one of the above first, second, third, fourth, fifth, or sixth specific embodiment as described above;
- (v) subjecting the product from step (iv) to passage through a chromatographic column or a high-pressure preparative chromatograph; and
- (vi) lyophilizing the product from step (v).

In an eighth specific embodiment, a method according to the present disclosure comprises the following steps:

- (i) performing step (5) of the method according to the third specific embodiment as described above;
- (ii) purifying the product from step (ii) by passage through a chromatographic column or by a high-pressure preparative chromatograph;
- (iii) lyophilizing the product from step (ii) to obtain the compound of formula I-3.

In a third aspect, the present disclosure provides a compound of formula VI, and a compound of formula VII as a synthesis intermediate for the compound of formula VI:



wherein:

X₅ and X₆ are each independently -(substituted or unsubstituted C₁₋₁₀ alkyl)-R²; and

R² is selected from the group consisting of C₃₋₁₀ cyclohydrocarbyl, 4-10 membered heterocyclyl, C₆₋₁₀ aryl and 5-10 membered heteroaryl.

In some embodiments, R² is selected from the group consisting of C₃₋₇ cycloalkyl, preferably C₅₋₆ cycloalkyl; 4-7 membered heterocyclyl, preferably 5-6 membered heterocyclyl; phenyl and 5-6 membered heteroaryl. Preferably, R² is phenyl.

In some embodiments, X_5 and X_6 are each independently -(substituted or unsubstituted C_{1-8} alkyl)- R^2 , preferably -(substituted or unsubstituted C_{1-6} alkyl)- R^2 , more preferably -(substituted or unsubstituted C_{1-4} alkyl)- R^2 , more preferably -(unsubstituted C_{1-2} alkyl)- R^2 , more preferably $-CH_2-R^2$. Preferably, X_5 and X_6 are each independently $-CH_2$ -phenyl.

In a fourth aspect, the present disclosure provides a method for preparing a compound of formula VI as described above, characterized in that the method comprises the following step:

i) subjecting the compound of formula VII to a hydrogenation reaction in a solvent in the presence of a suitable metal catalyst to provide the compound of formula VI.

In some embodiments, in the step i), the hydrogenation reaction is conducted under a hydrogen atmosphere. In some such embodiments, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa. In some embodiments, the reaction of the compound of formula VII with the hydrogen is conducted at a temperature from about 20 to 30°C (e.g., from about 20 to 25°C).

In some embodiments, the mass ratio of the compound of formula VII to the metal catalyst is from about 1:0.01 to 1:1, preferably about 1:0.2.

In some embodiments, the metal catalyst is palladium on carbon (Pd/C), wherein preferably, the mass fraction of palladium in the Pd/C is about 5-20%, more preferably about 10% (e.g., the Pd/C catalyst contains about 50 w/w% water).

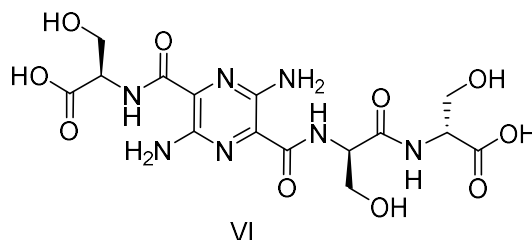
In some embodiments, the volume ratio of the solvent to the compound of formula VII is from about 10:1 to 50:1, preferably from about 10:1 to 30:1.

In some embodiments, the solvent is N,N-dimethylformamide (DMF), dichloromethane (DCM), dimethyl sulfoxide (DMSO), tetrahydrofuran and/or 1,4-dioxane, preferably N,N-dimethylformamide.

In some embodiments, the hydrogenation reaction is conducted for a period of time from about 5 to 72 h, for example, from about 10 to 48 h, or from about 10 to 30 h.

In some embodiments, the present disclosure provides a method for preparing the compound of formula VI or a salt thereof as described above, the method comprising the following step:

i) subjecting a compound of formula VII-1 to a hydrogenation reaction in N,N-dimethylformamide in the presence of about 10% Pd/C under a hydrogen atmosphere of 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa, at a temperature from about 20 to 25°C for a period of time from about 5 to 72 h, preferably from about 10 to 30 h, to provide the compound of formula VI,



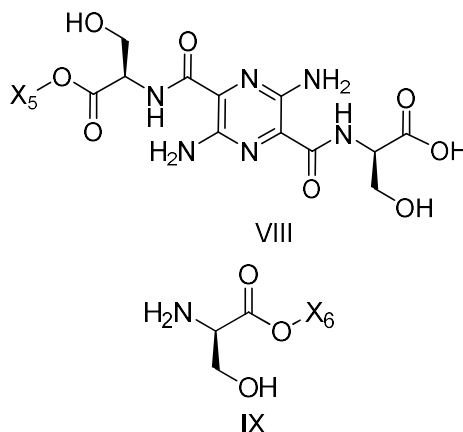
wherein:

the mass ratio of the compound of formula VII-1 to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2; and

the volume ratio of the N,N-dimethylformamide to the compound of formula VII-1 is from about 10:1 to 50:1, preferably from about 10:1 to 30:1.

In some embodiments, the method for preparing the compound of formula VI or a salt thereof as described above further comprises the following steps:

i') subjecting a compound of formula VIII and a compound of formula IX or a salt thereof (preferably a hydrochloride) to an amide condensation reaction in a suitable organic solvent in the presence of a suitable organic base, a suitable coupling agent and a suitable condensing agent under an inert atmosphere, to provide the compound of formula VII,



wherein X₅ and X₆ are each as defined above; and

ii') optionally, filtering the reaction mixture of the step i'), adding water dropwise to the filtrate to crystallize and precipitate the compound of formula VII; and

iii') optionally, adding ethyl acetate to the isolated compound of formula VII for slurrying, then filtering, and vacuum drying at an elevated temperature (e.g., about 50±5°C) for 18 hours or more.

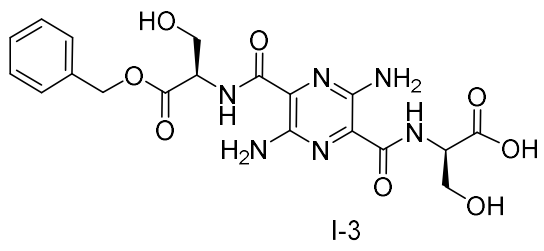
In some embodiments, in the step i'), the inert atmosphere is nitrogen protection. In some embodiments, the volume ratio of the organic solvent to the compound of formula IX or salt thereof is from about 10:1 to 80:1, preferably from about 20:1 to 50:1. In some embodiments, the molar ratio of the organic base to the compound of formula VIII is from about 0.5:1 to 9:1, preferably from about 1:1 to about 5:1. In some embodiments, the molar ratio of the condensing agent to the compound of formula VIII is from about 0.5:1 to 9:1, preferably from about 1:1 to about 5:1. In some embodiments, the molar ratio of the coupling agent to the compound of formula VIII is from about 0.5:1 to 9:1, preferably from about 1:1 to about 5:1. In some embodiments, the amide condensation reaction is conducted at a temperature from about 10 to 30°C, preferably from about 20 to 30°C. In some embodiments, the amide condensation reaction is conducted for a period of time from about 10 to 72 h, preferably from about 18 to 36 h. In some embodiments, the organic solvent is N,N-dimethylformamide (DMF), dichloromethane (DCM), dimethyl sulfoxide (DMSO), tetrahydrofuran and/or 1,4-dioxane. In some embodiments, the organic base is diisopropylethylamine (DIPEA), triethylamine, pyridine and/or 4-dimethylaminopyridine (DMAP). In some embodiments, the coupling agent is ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma), HOBt, HATU, HBTU, HCTU, TBTU, TSTU, TNTU, O-(7-azabenzotriazol-1-yl)-di(pyrrolidiny)carbenium hexafluorophosphate (HAPyU) and/or O-(benzotriazol-1-yl)-di(pyrrolidiny)carbenium hexafluorophosphate (HBPYU). In some

embodiments, the condensing agent is selected from: 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), HOBt, HATU, HBTU, HCTU, TBTU, TSTU, TNTU, O-(7-azabenzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HAPyU), O-(benzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HBPYU), and any combination thereof.

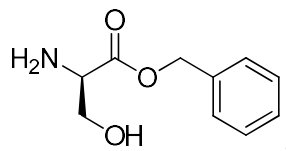
In some embodiments, in the step ii'), the volume ratio of the added water to the filtrate is from about 20:1 to 150:1, preferably from 35:1 to 75:1.

In some embodiments, in the step iii'), the volume ratio of the ethyl acetate to the compound of formula VII is from about 5:1 to 30:1, preferably from about 5:1 to 10:1.

In some other embodiments, the compound of formula VIII is a compound of formula I-3:



the compound of formula IX is a compound of the formula:



the organic solvent is DMF;

the organic base is diisopropylethylamine (DIPEA);

the coupling agent is HOBt; and

the condensing agent is EDC·HCl.

In some embodiments, the method for preparing the compound of formula VI or a salt thereof as described above further comprises the following steps:

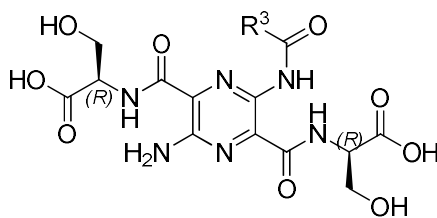
ii) optionally, filtering the reaction mixture of the step i), adding ethyl acetate dropwise to the filtrate to crystallize and precipitate the compound of formula VI; and

iii) optionally, adding ethyl acetate to the isolated compound of formula VI for slurring, then filtering, and vacuum drying at an elevated temperature (e.g., about 35±5°C) for 12 hours or more.

In some such embodiments, in the step ii), the volume ratio of the ethyl acetate to the filtrate is from about 30:1 to 90:1, preferably from about 30:1 to 50:1.

In some such embodiments, in the step iii), the volume ratio of the ethyl acetate to the compound of formula VI is from about 1:1 to 10:1, preferably from about 2:1 to 5:1.

In a fifth aspect, the present disclosure provides a compound of formula X:

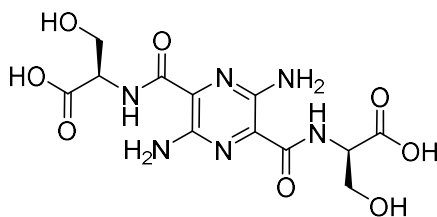


X

wherein R^3 is H, or substituted or unsubstituted C_{1-4} alkyl, preferably H or methyl.
In some preferred embodiments, R^3 is H.

In a sixth aspect, the present disclosure provides a method for preparing the compound of formula X, characterized in that the method comprises the following steps:

x-i) providing a solution of a compound of formula A in a solvent;



A

x-ii) acylating the compound of formula A with an acid R^3 -COOH at an elevated temperature to provide the compound of formula X; and

x-iii) optionally, isolating and purifying the compound of formula X;

wherein R^3 is as defined above.

In some preferred embodiments, R^3 is H.

In some embodiments, in step x-i), the volume ratio of the solvent to the compound of formula A is from about 5:1 to 30:1, preferably from about 5:1 to 10:1. In some embodiments, the solvent in step x-i) is N,N-dimethylformamide (DMF), dichloromethane (DCM), dimethyl sulfoxide (DMSO), tetrahydrofuran and/or 1,4-dioxane, preferably DMF. In some embodiments, the elevated temperature in step x-ii) is from about 55 to 75°C, preferably about 70°C. In some embodiments, in step x-ii), the volume ratio of the acid R^3 -COOH to the compound of formula A is from about 0.5:1 to 10:1, preferably from about 1:1 to 5:1. In some embodiments, in step x-ii), the reaction time is from about 1 to 20 h, preferably from about 1 to 5 h.

In some embodiments, the step x-iii) comprises:

1) concentrating the reaction mixture of the step x-i) until no distillate, then adding ethyl acetate dropwise to the residue to crystallize and precipitate the compound of formula X; and

2) dissolving the isolated compound of formula X in the solvent used in step x-i), followed by adding ethyl acetate dropwise to crystallize and precipitate the compound of formula X, then filtering, and vacuum drying at an elevated temperature (e.g., about 30±5°C);

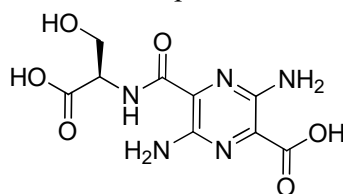
3) repeating the recrystallization of the steps 1) and 2); and

4) slurrying the dried compound of formula X obtained from the step 3) with ethyl acetate, then filtering, and vacuum drying at an elevated temperature (e.g., about 30±5°C)

for 16 hours or more.

In some such embodiments, in the step 1), the volume ratio of the ethyl acetate to the compound of formula A is from about 15:1 to 90:1, preferably from about 15:1 to 30:1. In some embodiments, in the step 2), the volume ratio of the solvent to the compound of formula A is from about 0.5:1 to 5:1, preferably from about 1:1 to 2:1. In some embodiments, in the step 2), the volume ratio of the ethyl acetate to the compound of formula A is from about 1:1 to 10:1, preferably from about 2:1 to 4:1. In some embodiments, in the step 4), the volume ratio of the ethyl acetate to the compound of formula A is from about 1:1 to 10:1, preferably from about 2:1 to 5:1.

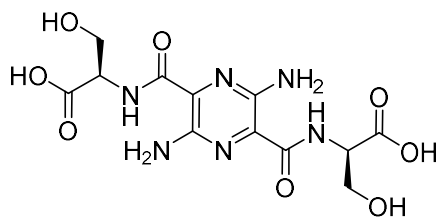
In a seventh aspect, the present disclosure provides a compound of formula XI:



XI

In an eighth aspect, the present disclosure provides a method for preparing the compound of formula XI, characterized in that the method comprises the following steps:

xi-i) hydrolyzing a compound of formula A in water in the presence of a base at an elevated temperature to provide the compound of formula XI or a salt thereof;



A

, and

xi-ii) optionally, isolating and purifying the compound of formula XI or salt thereof.

In some embodiments, in step xi-i), the volume ratio of the water to the compound of formula A is from about 5:1 to 30:1, preferably from about 5:1 to 10:1. In some embodiments, in step xi-i), the molar ratio of the base to the compound of formula A is from about 1:1 to 5:1, preferably from about 2:1 to 5:1. In some embodiments, the base is an inorganic base, preferably potassium hydroxide, sodium hydroxide, or mixtures thereof, more preferably potassium hydroxide. In some embodiments, in step xi-i), the elevated temperature is from about 50 to 100°C, preferably from about 80 to 90°C. In some embodiments, in step xi-i), the hydrolysis reaction is conducted for about 5 to 48 h, preferably about 10 to 20 h.

In some embodiments, the step xi-ii) comprises:

1) adding silica gel to the reaction mixture of the step xi-ii) to concentrate the reaction mixture into a dry silica gel powder, subjecting the dry silica gel powder to silica gel column chromatography to obtain a solid crude product of the compound of formula XI or a salt thereof;

2) slurring the solid crude product from the step xii-1) with methanol, filtering, then

vacuum drying at an elevated temperature (e.g., about 50±5°C) for 16 hours or more to obtain the purified compound of formula XI or a salt thereof.

In some such embodiments, in the step 1), the mass ratio of the added silica gel to the compound of formula A is from about 0.5:1 to 2:1, preferably about 2:1. In some embodiments, in the step 1), the silica gel chromatography column is eluted gradiently using EA/MeOH (1% trifluoroacetic acid (TFA)) as an eluent, wherein the volume ratio of EA : MeOH (1% TFA) is from about 50:1 to 1:1, preferably from about 30:1 to 10:1. In some embodiments, in the step 2), the volume ratio of the methanol to the compound of formula A is from about 1:1 to 10:1, preferably from about 2:1 to 5:1.

Use

In a ninth aspect, the present disclosure provides a method for quality control of an active pharmaceutical ingredient of MB-102 or a formulation thereof, comprising determining the content of the compound of formula I (including the compound of formula I-1, the compound of formula I-2, the compound of formula I-3, and any combination thereof), the compound of formula VI, the compound of formula X, and/or the compound of formula XI in the active pharmaceutical ingredient or the formulation, and optionally comparing with a specified content for each of the said compounds.

In some embodiments, for the compound of formula I, the specified content is not more than about 0.9%, preferably not more than about 0.6%, more preferably not more than about 0.45%, based on the total weight of the active pharmaceutical ingredient.

In some embodiments, for the compound of formula I-1, the specified content is not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15%, based on the total weight of the active pharmaceutical ingredient; or, in some embodiments, is not more than about 0.40%, preferably not more than about 0.3%, more preferably not more than about 0.20%, based on the total weight of the formulation.

In some embodiments, for the compound of formula I-2, the specified content is not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15%, based on the total weight of the active pharmaceutical ingredient.

In some embodiments, for the compound of formula I-3, the specified content is not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15%, based on the total weight of the active pharmaceutical ingredient.

In some embodiments, for the compound of formula VI, the specified content is not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15%, based on the total weight of the active pharmaceutical ingredient.

In some embodiments, for the compound of formula X, the specified content is not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15%, based on the total weight of the active pharmaceutical ingredient.

In some embodiments, for the compound of formula XI, the specified content is not more than about 0.2%, preferably not more than about 0.15%, more preferably not more than about 0.10%, based on the total weight of the active pharmaceutical ingredient; or, in some embodiments, is not more than about 0.40%, preferably not more than about 0.3%, more preferably not more than about 0.20%, based on the total weight of the formulation.

Methods for performing the content determination are known in the art, for example

HPLC processes.

In a tenth aspect, the present disclosure provides a use of the compound of formula I (including the compound of formula I-1, the compound of formula I-2, the compound of formula I-3, and any combination thereof), the compound of formula VI, the compound of formula X, and/or the compound of formula XI as a standard or control for quality control of an active pharmaceutical ingredient of MB-102 or a formulation thereof.

In yet another aspect, the present disclosure provides an active pharmaceutical ingredient of 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine having a low content of related compounds, characterized in that the related compounds are the compound of formula I, preferably the compound of formula I-1, the compound of formula I-2 or the compound of formula I-3, the compound of formula VI, the compound of formula X, and/or the compound of formula XI; and

the active pharmaceutical ingredient has one or more of the following contents:

not more than about 0.9%, preferably not more than about 0.6%, more preferably not more than about 0.45% of the compound of formula I, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15% of the compound of formula I-1, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15% of the compound of formula I-2, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15% of the compound of formula I-3, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15% of the compound of formula VI, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15% of the compound of formula X, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.2%, preferably not more than about 0.15%, more preferably not more than about 0.10% of the compound of formula XI, based on the total weight of the active pharmaceutical ingredient.

In yet another aspect, the present disclosure provides a formulation of 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine having a low content of related compounds, characterized in that the related compounds are selected from the group consisting of the compound of formula I-1 and the compound of formula XI; and

the formulation has one or both of the following contents:

not more than about 0.40%, preferably not more than about 0.3%, more preferably not more than about 0.20% of the compound of formula I-1, based on the total weight of the formulation;

not more than about 0.40%, preferably not more than about 0.3%, more preferably not more than about 0.20% of the compound of formula XI, based on the total weight of the

formulation.

In some embodiments, the formulation as described above is an injectable solution or an injectable lyophilized powder, preferably an injectable solution.

In an eleventh aspect, the present disclosure provides a use of the compound of formula II as described above as a synthesis intermediate for the compound of formula I as described above.

In some embodiments, the present disclosure provides a use of the compound of formula II-1 as described above as a synthesis intermediate for the compound of formula I-1 as described above.

In some embodiments, the present disclosure provides a use of the compound of formula II-2 as described above as a synthesis intermediate for the compound of formula I-2 as described above.

In some embodiments, the present disclosure provides a use of the compound of formula II-3 as described above as a synthesis intermediate for the compound of formula I-3 as described above.

In a twelfth aspect, the present disclosure provides a use of the compound of formula VII as described above as a synthesis intermediate for the compound of formula VI as described above.

In a thirteenth aspect, the present disclosure provides a use of the compound of formula A as described above as a synthesis intermediate for the compound of formula X and the compound of formula XI as described above.

Beneficial Effects

The inventors have provided the compound of formula I as described above, particularly the compound of formula I-1, the compound of formula I-2, and the compound of formula I-3, and the compound of formula VI, the compound of formula X and the compound of formula XI, as well as methods for the preparation thereof, the methods having advantages of simple operation, high purity and high yield. The inventors have for the first time proposed the use of these compounds as standards or controls for quality control of an active pharmaceutical ingredient of MB-102 or a formulation thereof and provided a corresponding quality control method, which is of great significance for quality control during the synthesis and storage of MB-102.

Examples

The present invention is further described in conjunction with examples below, while these examples are not provided for limiting the scope of the present invention.

In the examples of the present invention, experimental methods without indicating specific conditions are usually under conventional conditions, or under the conditions suggested by the raw materials or commodity manufacturers; reagents without indicating sources are usually conventional reagents commercially available or can be prepared from known reagents by conventional methods.

Example 1-1: Preparation of the compound of formula I-1

The compound of formula I-1 was prepared by a method comprising the following steps:

(1) To a three-necked jacketed flask were added 3,6-diaminopyrazine-2,5-dicarboxylic acid (compound III) (10 g, 1 eq.), D-serine benzyl ester hydrochloride (12.86 g, 1.1 eq.) and L-serine benzyl ester hydrochloride (12.86 g, 1.1 eq.), DMF (35 times the volume of compound III) was added and stirred uniformly.

(2) After cooling to 0°C, DIPEA (14.34 g, 2.2 eq.) was added, ethyl 2-cyano-2-(hydroxyimino)acetate (15.77 g, 2.2 eq.) was added after 30 min, EDC·HCl (21.28 g, 2.2 eq.) was added after 20 min, the reaction was carried out for another 40 min, followed by increasing to room temperature to react for 18 h.

(3) Purified water (70 times the volume of compound III) was added, and filtered after a solid precipitated out.

(4) Passage through a chromatographic column (Synthware C184806C chromatographic column with sand plate, φ80 mm) and concentration of the eluate under reduced pressure at 33°C gave the pure compound of formula II-1 (4.8 g).

(5) To a reaction flask was added the compound of formula II-1 (2 g, 1 eq.), followed by sequential addition of ethanol (32 ml, 16 times volume), aqueous formic acid-potassium hydroxide solution (HCOOH: 1.0 g, 6 eq.; KOH: 0.4 g, 6 eq.; purified water: 8 ml, 4 times volume) and Pd/C (0.2 g, 0.1 times volume) to react at 60°C for 2 h. After the TLC detection indicated completion of the reaction, Pd/C was removed by hot filtration through diatomite, the filter cake was washed with aqueous ethanol solution (36% H₂O, v/v) until the filtrate was light yellow, the filtrates were combined. To the filtrate was added 1 mol/L hydrochloric acid (7 ml), stirred for 15 min, then concentrated under reduced pressure to dryness.

(6) The resulting solid was slurried with 20 ml acetonitrile for 2 h, filtered, then slurried with water for 2 h, filtered, and the filter cake was then dried under reduced pressure at 40°C for 12 h to obtain the compound of formula I-1 in solid form (0.79 g): an overall molar yield of 10% (based on the compound of formula III), and a purity of 98.39%.

After analysis by proton nuclear magnetic resonance spectroscopy (Bruker AVANCE III HD), it was confirmed that the chemical structure of the obtained product was as shown by the formula I-1. The obtained proton nuclear magnetic resonance spectrum is shown in Table 1:

Table 1

Chemical Shift (ppm)	Multiplicity (J value, Hz)	Number of Protons	Assignment H
12.97	br. s	2	OH(a)
8.47	d(8.2)	2	NH(c)
6.79	s	4	NH(d)
5.26	br. s	2	OH(b)
4.49	m	2	3
3.90	dd(11.0,10.8)	2	2a
3.77	dd(10.9,10.8)	2	2b

Example 1-2

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (1), the molar ratio of D-serine benzyl ester hydrochloride to the compound of formula III was 0.5:1.0; the molar ratio of L-serine benzyl

ester hydrochloride to the compound of formula III was 0.5:1.0; and the volume ratio of DMF to the compound of formula III was 10:1.

Example 1-3

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (1), the molar ratio of D-serine benzyl ester hydrochloride to the compound of formula III was 1.0:1.0; the molar ratio of L-serine benzyl ester hydrochloride to the compound of formula III was 1.0:1.0; and the volume ratio of DMF to the compound of formula III was 20:1.

Example 1-4

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (1), the molar ratio of D-serine benzyl ester hydrochloride to the compound of formula III was 4.0:1.0; the molar ratio of L-serine benzyl ester hydrochloride to the compound of formula III was 4.0:1.0; and the volume ratio of DMF to the compound of formula III was 50:1.

Example 1-5

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (1), the molar ratio of D-serine benzyl ester hydrochloride to the compound of formula III was 8.0:1.0; the molar ratio of L-serine benzyl ester hydrochloride to the compound of formula III was 8.0:1.0; and the volume ratio of DMF to the compound of formula III was 80:1.

Example 1-6

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (2), the molar ratio of DIPEA to the compound of formula III was 0.5:1.0; the molar ratio of EDC·HCl to the compound of formula III was 0.5:1.0; the temperature was cooled to -5°C; the reaction was carried out for 10 min after adding ethyl 2-cyano-2-(hydroxyimino)acetate; the reaction was carried out for another 10 min after adding EDC·HCl; and the reaction time at room temperature was 10 h.

Example 1-7

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (2), the molar ratio of DIPEA to the compound of formula III was 1.0:1.0; the molar ratio of EDC·HCl to the compound of formula III was 1.0:1.0; the temperature was cooled to 10 °C; the reaction was carried out for 60 min after adding ethyl 2-cyano-2-(hydroxyimino)acetate; the reaction was carried out for another 60 min after adding EDC·HCl; and the reaction time at room temperature was 36 h.

Example 1-8

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (2), the molar ratio of DIPEA to the compound of formula III was 5.0:1.0; the molar ratio of EDC·HCl to the compound of formula III was 5.0:1.0; the temperature was cooled to 15 °C; and the reaction time at room temperature was 72 h.

Example 1-9

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (2), the molar ratio of DIPEA to the

compound of formula III was 9.0:1.0; the molar ratio of EDC·HCl to the compound of formula III was 9.0:1.0; and the temperature was cooled to 20 °C.

Example 1-10

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (3), the volume ratio of the purified water to the compound of formula III was 20:1.

Example 1-11

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (3), the volume ratio of the purified water to the compound of formula III was 35:1.

Example 1-12

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (3), the volume ratio of the purified water to the compound of formula III was 75:1.

Example 1-13

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (3), the volume ratio of the purified water to the compound of formula III was 150:1.

Example 1-14

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (5), the alcohol in the aqueous alcohol solution was selected from methanol with a volume ratio to water of 1:1; the mass ratio of the compound of formula II-1 to Pd/C was 1:0.01; the mass fraction of palladium in Pd/C was 5%; and the reaction time was 1 h.

Example 1-15

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (5), the alcohol in the aqueous alcohol solution was selected from ethanol with a volume ratio to water of 3:1; the mass ratio of the compound of formula II-1 to Pd/C was 1:0.2; the mass fraction of palladium in Pd/C (containing 50% water) was 10%; and the reaction time was 6 h.

Example 1-16

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (5), the alcohol in the aqueous alcohol solution was selected from isopropanol with a volume ratio to water of 5:1; the mass ratio of the compound of formula II-1 to Pd/C was 1:1; the mass fraction of palladium in Pd/C was 20%; and the reaction time was 12 h.

Example 1-17

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (5), the alcohol in the aqueous alcohol solution was selected from methanol with a volume ratio to water of 8:1; the mass ratio of the compound of formula II-1 to Pd/C was 1:1; the mass fraction of palladium in Pd/C was 20%; and the reaction time was 48 h.

Example 1-18

This example provides a method for preparing the compound of formula I-1, differing

from the method of Example 1-1 in that: in step (6), the crude product was slurried with acetonitrile for 1 h and with water for 1 h respectively, and then filtered, and the filter cake was dried under reduced pressure at 45°C to obtain the compound of formula I-1.

Example 1-19

This example provides a method for preparing the compound of formula I-1, differing from the method of Example 1-1 in that: in step (6), the crude product was first slurried with acetonitrile for 4 h, then slurried with water for 4 h, and then filtered, and the filter cake was dried under reduced pressure at 45°C to obtain the compound of formula I-1.

All products of Examples 1-2 to 1-19 were determined to have an overall molar yield of about 10% (based on the compound of formula III), and a purity of about 98%. The results indicate that the compound of formula I-1 prepared under the reaction conditions as described had a high purity and a high yield.

Example 2-A: Preparation of the compound of formula I-2

The compound of formula I-2 was prepared by a method comprising the following steps:

(1) To a three-necked jacketed flask were added compound III (10 g, 1 eq.), D-serine benzyl ester hydrochloride (12.86 g, 1.1 eq.) and D-serine ethyl ester hydrochloride (9.38 g, 1.1 eq.), DMF (35 times the volume of compound III) was added and stirred uniformly.

(2) After cooling to 0°C, DIPEA (14.34 g, 2.2 eq.) was added, ethyl 2-cyano-2-(hydroxyimino)acetate (15.77 g, 2.2 eq.) was added after 30 min, EDC·HCl (21.28 g, 2.2 eq.) was added after 20 min, reaction was carried out for another 40 min, followed by increasing to room temperature to react for 18 h.

(3) Purified water (70 times the volume of compound III) was added, and filtered after a solid precipitated out, to obtain a crude product of the compound of formula II-2 (10 g).

(4) To a hydrogenation autoclave was added the compound of formula II-2 (5 g), and dissolved in 175 ml aqueous ethanol solution (36% H₂O, v/v). Pd/C (0.5 g, 0.1 times volume) was then added. After 3 times of nitrogen replacement, hydrogen replacement was performed 3 times (0.2 MPa) to react for 4 to 12 h. After completion of the reaction, Pd/C was removed by filtration through diatomite, and the filtrate was rotary-evaporated to dryness or concentrated under reduced pressure to dryness.

(5) The obtained solid was subjected to preparative chromatography: column: self-packed spring column (Ø30·250 mm, packing: C18 particles from Huapu); flow rate: 30 ml/min; column temperature: 30°C; gradient was as follows:

Time (min) 0-12-29-29.1-33

A (%) 20-85-30-90-90

B (%) 80-15-70-10-10

Mobile phase A: 0.1% TFA·water

Mobile phase B: acetonitrile (MeCN)

The eluate from 19 min to 22 min was collected.

(6) Rotary evaporation was performed to remove MeCN from the eluate, followed by freeze drying.

1 g of the compound of formula I-2 was obtained, with a purity of 98.5% and an overall molar yield of 11% (based on the compound of formula III).

After analysis by proton nuclear magnetic resonance spectroscopy (Bruker AVANCE III HD), it was confirmed that the chemical structure of the obtained product was as shown by the formula I-2. The obtained proton nuclear magnetic resonance spectrum is shown in Table 2:

Table 2

Chemical Shift (ppm)	Multiplicity (J value, Hz)	Number of Protons	Assignment H
12.98	br. s	1	OH(a)
8.51	d(8.2)	1	NH(e)
8.47	d(8.2)	1	NH(c)
5.43	br. s	6	NH(d), NH(g), OH(f), OH(b)
4.55	m	1	10
4.49	m	1	3
4.16	q(7.1)	2	13
3.90	overlapped	1	11a
3.90	overlapped	1	2a
3.77	overlapped	1	11b
3.77	overlapped	1	2b
1.22	t(7.1)	3	14

Example 2-B: Preparation of the compound of formula I-2

The compound of formula I-2 was prepared by a method comprising the following steps:

(1) To a three-necked jacketed flask were added compound III (10 g, 1 eq.), D-serine benzyl ester hydrochloride (12.86 g, 1.1 eq.) and D-serine ethyl ester hydrochloride (9.38 g, 1.1 eq.), DMF (35 times the volume of compound III) was added and stirred uniformly.

(2) After cooling to 0°C, DIPEA (14.34 g, 2.2 eq.) was added, ethyl 2-cyano-2-(hydroxyimino)acetate (15.77 g, 2.2 eq.) was added after 30 min, EDC·HCl (21.28 g, 2.2 eq.) was added after 20 min, the reaction was carried out for another 40 min, followed by increasing to room temperature to react for 18 h.

(3) Purified water (70 times the volume of compound III) was added, and filtered after a solid precipitated out, to obtain a crude product of the compound of formula II-2 (10 g).

Passage through a chromatographic column (Synthware C184806C chromatographic column with sand plate, φ80 mm) or a high-pressure preparative chromatograph (LAB-ZB-05, domestic medium-high pressure equipment from Aonuo).

(4) To a hydrogenation autoclave was added the compound of formula II-2 (5 g), dissolved in 175 ml aqueous ethanol solution (36% H₂O, v/v). Pd/C (0.5 g, 0.1 times volume) was then added. After 3 times of nitrogen replacement, hydrogen replacement was performed 3 times (0.2 MPa) to react for 4 to 12 h. After completion of the reaction, Pd/C was removed by filtration through diatomite, and the filtrate was rotary-evaporated to dryness or concentrated under reduced pressure to dryness.

(5) The obtained crude product was first slurried with acetonitrile for 1 h, then slurried with water for 1 h, and then filtered, and the filter cake was dried under reduced pressure at 45°C to obtain the compound of formula I-2.

Example 2-1

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (1), the molar ratio of D-serine benzyl ester hydrochloride to compound III was 0.5:1.0; the molar ratio of D-serine ethyl ester hydrochloride to compound III was 0.5:1.0; and the volume ratio of DMF to compound III was 10:1.

Example 2-2

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (1), the molar ratio of D-serine benzyl ester hydrochloride to compound III was 1.0:1.0; the molar ratio of D-serine ethyl ester hydrochloride to compound III was 1.0:1.0; and the volume ratio of DMF to compound III was 20:1.

Example 2-3

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (1), the molar ratio of D-serine benzyl ester hydrochloride to compound III was 4.0:1.0; the molar ratio of D-serine ethyl ester hydrochloride to compound III was 4.0:1.0; and the volume ratio of DMF to compound III was 50:1.

Example 2-4

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (1), the molar ratio of D-serine benzyl ester hydrochloride to compound III was 8.0:1.0; the molar ratio of D-serine ethyl ester hydrochloride to compound III was 8.0:1.0; and the volume ratio of DMF to compound III was 80:1.

Example 2-5

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (2), the molar ratio of DIPEA to compound III was 0.5:1.0; the molar ratio of EDC·HCl to compound III was 0.5:1.0; the temperature was cooled to -5°C; the reaction was carried out for 10 min after adding ethyl 2-cyano-2-(hydroxyimino)acetate; the reaction was carried out for another 10 min after adding EDC·HCl; and the reaction time at room temperature was 10 h.

Example 2-6

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (2), the molar ratio of DIPEA to compound III was 1.0:1.0; the molar ratio of EDC·HCl to compound III was 1.0:1.0; the temperature was cooled to 10 °C; the reaction was carried out for 60 min after adding ethyl 2-cyano-2-(hydroxyimino)acetate; the reaction was carried out for another 60 min after adding EDC·HCl; and the reaction time at room temperature was 36 h.

Example 2-7

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (2), the molar ratio of DIPEA to compound III was 5.0:1.0; the molar ratio of EDC·HCl to compound III was 5.0:1.0; the temperature was cooled to 15 °C; and the reaction time at room temperature was 72 h.

Example 2-8

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (2), the molar ratio of DIPEA to compound III was 9.0:1.0; the molar ratio of EDC·HCl to compound III was 9.0:1.0; and the temperature was cooled to 20 °C.

Example 2-9

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (3), the volume ratio of the purified water to the compound of formula III was 20:1.

Example 2-10

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (3), the volume ratio of the purified water to compound III was 35:1.

Example 2-11

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (3), the volume ratio of the purified water to compound III was 75:1.

Example 2-12

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (3), the volume ratio of the purified water to compound III was 150:1.

Example 2-13

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (4), the alcohol in the aqueous alcohol solution was selected from methanol with a volume ratio to water of 1:1; the mass ratio of the compound of formula II-2 to Pd/C was 1:0.01; the mass fraction of palladium in Pd/C was 5%; the hydrogen pressure was 0.05 MPa; and the reaction time was 1 h.

Example 2-14

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (4), the alcohol in the aqueous alcohol solution was selected from ethanol with a volume ratio to water of 3:1; the mass ratio of the compound of formula II-2 to Pd/C was 1:0.2; the mass fraction of palladium in Pd/C (containing 50% water) was 10%, and the hydrogen pressure was 0.1 MPa; and the reaction time was 6 h.

Example 2-15

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (4), the alcohol in the aqueous alcohol solution was selected from isopropanol with a volume ratio to water of 5:1; the mass ratio of the compound of formula II-2 to Pd/C was 1:1; the mass fraction of palladium in Pd/C was 20%; the hydrogen pressure was 0.4 MPa; and the reaction time was 12 h.

Example 2-16

This example provides a method for preparing the compound of formula I-2, differing from Examples 2-A and 2-B in that: in step (4), the alcohol in the aqueous alcohol solution was selected from methanol with a volume ratio to water of 8:1; the mass ratio of the compound of formula II-2 to Pd/C was 1:1; the mass fraction of palladium in Pd/C was 20%;

the hydrogen pressure was 0.8 MPa; and the reaction time was 48 h.

Example 2-17

This example provides a method for preparing the compound of formula I-2, differing from Example 2-B in that: in step (5), the crude product was first slurried with acetonitrile for 4 h, then slurried with water for 4 h, and then filtered, and the filter cake was dried under reduced pressure at 45°C to obtain the compound of formula I-2.

The products of Examples 2-B and 2-1 to 2-17 were determined to have an overall molar yield of about 11% (based on the compound of formula III), and a purity of about 98%.

The results indicate that the compound of formula I-2 prepared under the reaction conditions as described had a high purity and a high yield.

Example 3-1: Preparation of the compound of formula I-3

The compound of formula I-3 was prepared by a method comprising the following steps:

(1) To a hydrogenation autoclave was added the compound of formula II-3 (5 g), dissolved in 175 ml aqueous ethanol solution (36% H₂O, v/v). Pd/C (0.5 g, 0.1 times volume) was then added. After 3 times of nitrogen replacement, hydrogen replacement was performed 3 times (0.2 MPa) to reacted for 4 to 12 h. After completion of the reaction, Pd/C was removed by filtration through diatomite, and the filtrate was rotary-evaporated to dryness.

(2) The obtained solid was subjected to preparative chromatography: column: self-packed spring column (Ø30*250 mm, packing: C18 particles from Huapu); flow rate: 30 ml/min; column temperature: 30°C; gradient was as follows:

Time (min) 0-20-21-25-26-28

A (%) 70-70-10-10-70-70

B (%) 30-30-90-90-30-30

Mobile phase A: 0.1% TFA·water

Mobile phase B: acetonitrile

The eluate from 13 min to 19 min was collected.

(3) Rotary evaporation was performed to remove MeCN from the eluate, followed by freeze drying.

1 g of the compound of formula I-3 was obtained, with a purity of 98.7% and an overall molar yield of 29% (based on the compound of formula II-3).

After analysis by proton nuclear magnetic resonance spectroscopy (Bruker AVANCE III HD), it was confirmed that the chemical structure of the obtained product was as shown by formula I-3. The obtained proton nuclear magnetic resonance spectrum is shown in Table 3:

Table 3

Chemical Shift (ppm)	Multiplicity (J value, HZ)	Number of Protons	Assignment H
12.97	br. s	1	OH(a)
8.57	d(8.2)	1	NH(e)
8.48	d(8.2)	1	NH(c)
7.36	overlapped	5	15,16,17
6.78	s	4	NH(d)

5.38	br. s	1	OH(f)
5.27	br. s	1	OH(b)
5.20	s	2	13
4.65	m	1	10
4.49	m	1	3
3.96	m	1	11a
3.90	dd(11.0,10.8)	1	2a
3.80	m	1	11b
3.76	dd(11.0,10.8)	1	2b

Example 3-2

This example provides a method for preparing the compound of formula I-3, differing from Example 3-1 in that: in step (1), the mass ratio of the compound of formula II-3 to Pd/C was 1:0.01, and the mass fraction of palladium in Pd/C was 5%; the alcohol in the aqueous alcohol solution was selected from methanol with a volume ratio to water of 1:1; the hydrogen pressure was 0.05 MPa; and the reaction time was 1 h.

Example 3-3

This example provides a method for preparing the compound of formula I-3, differing from Example 3-1 in that: in step (1), the mass ratio of the compound of formula II-3 to Pd/C was 1:1, and the mass fraction of palladium in Pd/C (containing 50% water) was 10%; the alcohol in the aqueous alcohol solution was selected from isopropanol with a volume ratio to water of 3:1; the hydrogen pressure was 0.1 MPa; and the reaction time was 6 h.

Example 3-4

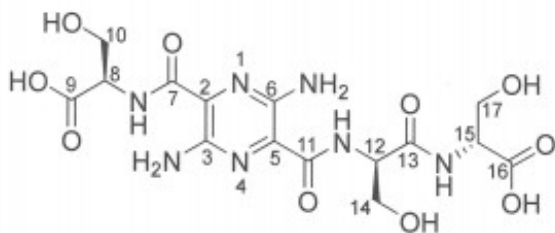
This example provides a method for preparing the compound of formula I-3, differing from Example 3-1 in that: in step (1), the mass ratio of the compound of formula II-3 to Pd/C was 1:0.1, and the mass fraction of palladium in Pd/C was 20%; the alcohol in the aqueous alcohol solution was selected from methanol with a volume ratio to water of 5:1; the hydrogen pressure was 0.4 MPa; and the reaction time was 12 h.

Example 3-5

This example provides a method for preparing the compound of formula I-3, differing from Example 3-1 in that: in step (1), the mass ratio of the compound of formula II-3 to Pd/C was 1:1, and the mass fraction of palladium in Pd/C (containing 50% water) was 10%; the alcohol in the aqueous alcohol solution was selected from methanol with a volume ratio to water of 8:1; the hydrogen pressure was 0.8 MPa; and the reaction time was 48 h.

The products of Examples 3-2 to 3-5 were determined to have an overall molar yield of about 29% (based on the compound of formula II-3) and a purity of about 98%. The results indicate that the compound of formula I-3 prepared under the reaction conditions as described had a high purity and a high yield.

Example 4: Preparation of the compound of formula VI



VI

To a three-necked jacketed flask were added the purified compound of formula II-3 (40.0 g, 1.0 eq.) and ethyl acetate/ethanol (952 mL/952 mL, 23.8 vol/23.8 vol), stirred to dissolve, then 10% Pd/C (4.0 g, 10 wt%) was added. The reactor was sealed, and the interior of the reactor was purged with nitrogen. After purging, hydrogen purging was performed three times, and the temperature of the reaction solution in the reactor was maintained at $25\pm 5^{\circ}\text{C}$ and the hydrogen pressure was maintained at 0.1 to 0.3 MPa, for reaction at a constant temperature for 2 hours. After Filtering, the filter cake was washed with ethanol. The obtained filtrates were combined and concentrated until no obvious distillate, then silica gel (40 g) was added to form a dry silica gel powder. The solid crude dry powder was subjected to column chromatography separation to obtain the compound of formula I-3 in solid form (4.6 g) (elution was performed by sequentially using EA, EA/MeOH (1% TFA) (50/1), and EA/MeOH (1% TFA) (30/1) as eluents).

Under nitrogen protection, DMF (147.2 mL, 32.0 vol) was added into a three-necked flask, D-serine benzyl ester hydrochloride (2.3 g, 1.0 eq.) was added under stirring, and stirred for more than 15 minutes, while controlling the internal temperature at 0 to 10°C . N,N-Diisopropylethylamine (1.29 g, 1.0 eq.) was added dropwise, controlling the internal temperature below 10°C during addition. After the addition was complete, the temperature was maintained at 0 to 10°C for stirring for 5 to 15 minutes. Subsequently, the internal temperature was raised to 20 to 30°C , and the compound of formula I-3 (4.6 g, 1.0 eq.), 1-hydroxybenzotriazole monohydrate (1.52 g, 1.0 eq.), and 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.91 g, 1.0 eq.) were added sequentially. After the addition was complete, the reaction was carried out with stirring at 20 to 30°C for 4 hours. After filtering, purified water (294.4 g, 64.0 vol.) was added dropwise to the filtrate at 10 to 20°C for crystallization. After filtering, the filter cake was rinsed with water, then the filter cake was added to ethyl acetate (46 mL, 10.0 vol.) for slurring. After filtering, the filter cake was vacuum dried at 50°C for 22 hours, to obtain 2.6 g of a crude product of (3,6-diamino-5-(((S)-1-(((S)-1-(benzyloxy)-3-hydroxy-1-oxopropan-2-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamoyl)pyrazine-2-carbonyl)-L-serine benzyl ester.

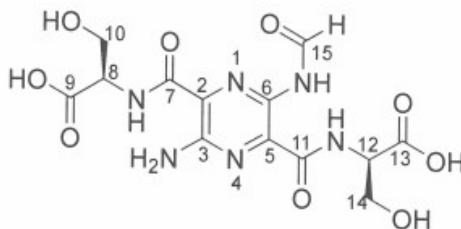
To a hydrogenation reactor were added the crude product of (3,6-diamino-5-(((S)-1-(((S)-1-(benzyloxy)-3-hydroxy-1-oxopropan-2-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamoyl)pyrazine-2-carbonyl)-L-serine benzyl ester (4.5 g, 1.0 eq.), 10% Pd/C (0.9 g, 20 wt%) and DMF (45 mL, 10.0 vol.). The reactor was sealed, and the interior of the reactor was purged with nitrogen. After purging, hydrogen purging was performed three times, the temperature of the reaction solution in the reactor was maintained at $25\pm 5^{\circ}\text{C}$ and the hydrogen pressure was maintained at 0.1 to 0.3 MPa to react for 2 hours. The reaction mixture was filtered, and the filter cake was rinsed with DMF. Ethyl acetate (135 mL, 30.0

vol) was added dropwise to the filtrate for crystallization. After filtering, the filter cake was added to ethyl acetate (22.5 mL, 5.0 vol.) for slurring, then filtered, and the filter cake was vacuum dried at 35°C for 24 hours to obtain the compound of formula VI in solid form (2.1 g). The overall molar yield was 3.7% (based on the compound of formula II-3), and the purity was 92.7%.

Table 4. Proton Nuclear Magnetic Resonance Spectrum of the compound of formula VI

Chemical Shift (ppm)	Multiplicity (J value, HZ)	Number of Protons	Assignment H
8.447	overlapped	1	NH(7)
		1	NH(11)
8.287	d(8.0)	1	NH(13)
6.748	overlapped	4	NH ₂ (3), NH ₂ (6)
4.605	dt(8.0, 5.0)	1	H(12)
4.470	dt(8.0, 4.0)	1	H(8)
4.325	dt(8.0, 4.5)	1	H(15)
3.879	dd(11.0, 4.0)	1	H(10a)
3.757-3.668	overlapped	1	H(10b)
		2	H(14)
		1	H(17a)
3.654	dd(11.0, 4.0)	1	H(17b)

Example 5: Preparation of (2R,2'R)-2,2'-((3-amino-6-formamidopyrazine-2,5-dicarbonyl)bis(azanediyl))bis(3-hydroxypropanoic acid)

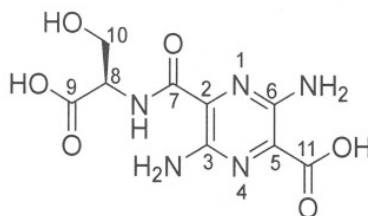


To a three-necked jacketed flask were added MB-102 (15 g, 1.0 eq.) and DMF (150 mL, 10.0 vol.), stirred to clearness, and then raised to 70°C, formic acid (37.5 mL, 2.5 vol) was added to react at a constant temperature of 70°C for 1 hour. Most of DMF was removed by concentration. Ethyl acetate (300 mL, 20.0 vol) was added dropwise for crystallization, and filtered to obtain a crude product of the compound of formula X. This crude product was added to DMF (15 mL, 1.0 vol.), dissolved at room temperature. Then, ethyl acetate (30 mL, 2.0 vol.) was added slowly dropwise for crystallization, slurried, then filtered, vacuum dried at 30°C. The recrystallization operation process was repeated. After recrystallization, filtering was performed, and then the filter cake was added to ethyl acetate (30 mL, 2.0 vol.) for slurring, filtered again, and vacuum dried at 35°C for 16 hours to obtain (2R,2'R)-2,2'-((3-amino-6-formamidopyrazine-2,5-dicarbonyl)bis(azanediyl))bis(3-hydroxypropanoic acid) in solid form (3.0 g). The overall molar yield was 18.6% (based on MB-102), and the purity was 83.4%.

Table 5. Proton Nuclear Magnetic Resonance Spectrum of (2R,2'R)-2,2'-((3-amino-6-formamidopyrazine-2,5-dicarbonyl)bis(azanediyl))bis(3-hydroxypropanoic acid)

Chemical Shift (ppm)	Multiplicity (J value, HZ)	Number of Protons	Assignment H
10.903	br. s	1	NH(6)
9.387	s	1	H(15)
8.772	m	1	NH(11)
8.725	m	1	NH(7)
7.511	br. s	2	NH ₂ (3)
4.234	m	1	H(8)
1.186	m	1	H(12)
3.799-3.672	overlapped	4	H(10); H(14)

Example 6: Preparation of the compound of formula XI



XI

To a three-necked jacketed flask were added purified water (300 mL, 10.0 vol.), potassium hydroxide (11.3 g, 2.5 eq.) and MB-102 (30 g, 1.0 eq.), the reaction solution was raised to 80°C to react with stirring at a constant temperature for 16 hours, then cooled to 25°C. Silica gel (60 g) was added to the reaction solution, concentrated to form a dry silica gel powder, then purified by silica gel column chromatography separation to obtain a solution of the crude compound of formula XI. This solution was concentrated to precipitate a solid which was added to methanol (150 mL, 5.0 vol.) for slurring at 50°C, then cooled and filtered. The wet product was vacuum dried at 35°C to obtain the compound of formula XI in solid form (1.3 g). The overall molar yield was 11.3% (based on MB-102), and the purity was 99.3%.

Table 6. Proton Nuclear Magnetic Resonance Spectrum of the compound of formula XI

Chemical Shift (ppm)	Multiplicity (J value, HZ)	Number of Protons	Assignment H
12.969	br. s	1	OH(9)
8.475	d(8.0)	1	NH(7)
6.682	br. s	2	NH ₂ (3)
5.249	br. s	1	OH(10)
4.478	dt(8.5, 3.5)	1	H(8)
3.880	dd(11.0, 3.5)	1	H(10a)
3.741	dt(11.0, 3.5)	1	H(10b)

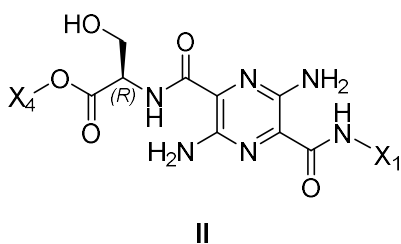
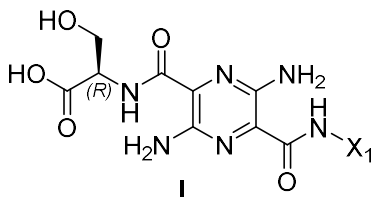
In addition to the embodiments described herein, various modifications of the present invention will be apparent to those skilled in the art based on the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Each reference cited herein (including all patents, patent applications, journal articles, books, and any other publications) is incorporated herein by reference in its entirety.

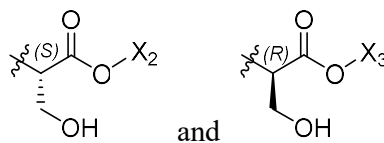
WHAT IS CLAIMED IS:

1. A method for preparing a compound of formula I or a salt thereof, characterized in that the method comprises the following step:

a) subjecting a compound of formula II to a hydrogenation reaction with hydrogen or a hydrogen-borrowing reagent in a solvent in the presence of a suitable metal catalyst to provide the compound of formula I,



wherein:



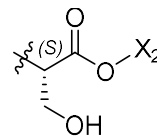
X₁ is selected from the group consisting of

X₂ is selected from the group consisting of H, substituted or unsubstituted C₁₋₁₀ alkyl, and -(substituted or unsubstituted C₁₋₁₀ alkyl)-R¹;

X₃ and X₄ are each independently selected from the group consisting of substituted or unsubstituted C₁₋₁₀ alkyl, and -(substituted or unsubstituted C₁₋₁₀ alkyl)-R¹; and

R¹ is selected from the group consisting of C₃₋₁₀ cyclohydrocarbyl, 4-10 membered heterocyclyl, C₆₋₁₀ aryl and 5-10 membered heteroaryl.

2. The method according to claim 1, characterized in that R¹ is selected from the group consisting of C₃₋₇ cycloalkyl, preferably C₅₋₆ cycloalkyl; 4-7 membered heterocyclyl, preferably 5-6 membered heterocyclyl; phenyl and 5-6 membered heteroaryl; preferably phenyl.



3. The method according to claim 1 or 2, characterized in that X₁ is

4. The method according to any one of claims 1 to 3, characterized in that X₂ is selected from the group consisting of

H, substituted or unsubstituted C₁₋₈ alkyl, and -(substituted or unsubstituted C₁₋₈ alkyl)-

R¹;

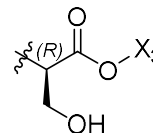
preferably, H, substituted or unsubstituted C₁₋₆ alkyl, and -(substituted or unsubstituted C₁₋₆ alkyl)-R¹;

more preferably, H, substituted or unsubstituted C₁₋₄ alkyl, and -(substituted or unsubstituted C₁₋₄ alkyl)-R¹;

more preferably, H, unsubstituted C₁₋₂ alkyl, and -(unsubstituted C₁₋₂ alkyl)-R¹;

more preferably, H, ethyl and -CH₂-R¹;

more preferably, H, ethyl and -CH₂-phenyl.



5. The method according to claim 1 or 2, characterized in that X₁ is

6. The method according to claim 1, 2 or 5, characterized in that X₃ is selected from the group consisting of

substituted or unsubstituted C₁₋₈ alkyl, and -(substituted or unsubstituted C₁₋₈ alkyl)-R¹;

preferably, substituted or unsubstituted C₁₋₆ alkyl, and -(substituted or unsubstituted C₁₋₆ alkyl)-R¹;

more preferably, substituted or unsubstituted C₁₋₄ alkyl, and -(substituted or unsubstituted C₁₋₄ alkyl)-R¹;

more preferably, unsubstituted C₁₋₂ alkyl, and -(unsubstituted C₁₋₂ alkyl)-R¹;

more preferably, ethyl and -CH₂-R¹;

more preferably, ethyl and -CH₂-phenyl.

7. The method according to any one of claims 1 to 6, characterized in that X₄ is selected from the group consisting of

substituted or unsubstituted C₁₋₈ alkyl, and -(substituted or unsubstituted C₁₋₈ alkyl)-R¹;

preferably, substituted or unsubstituted C₁₋₆ alkyl, and -(substituted or unsubstituted C₁₋₆ alkyl)-R¹;

more preferably, substituted or unsubstituted C₁₋₄ alkyl, and -(substituted or unsubstituted C₁₋₄ alkyl)-R¹;

more preferably, unsubstituted C₁₋₂ alkyl, and -(unsubstituted C₁₋₂ alkyl)-R¹;

more preferably, ethyl and -CH₂-R¹;

more preferably, ethyl and -CH₂-phenyl;

more preferably, -CH₂-phenyl.

8. The method according to any one of claims 1 to 7, characterized in that,

(1) in the step a), the compound of formula II is subjected to the hydrogenation reaction with the hydrogen-borrowing reagent,

wherein preferably, the molar ratio of the compound of formula II to the hydrogen-borrowing reagent is from about 1:2 to 1:20, preferably from about 1:2 to 1:10, for example, about 1:2.5, about 1:4, about 1:5, about 1:6, or about 1:8;

and/or

wherein preferably, the hydrogen-borrowing reagent is selected from the group consisting of a combination of formic acid and an alkali metal formate, cyclohexadiene, and isopropanol, more preferably the combination of formic acid and an alkali metal formate, wherein preferably, the molar ratio of the formic acid to the alkali metal formate is from about 1:6 to 6:1, more preferably from about 1:1 to 5:1;

and/or

wherein preferably, the alkali metal formate is sodium formate or potassium formate, preferably potassium formate;

and/or

wherein preferably, the reaction of the compound of formula II with the hydrogen-borrowing reagent is conducted at a temperature from about 20°C to 100°C, more preferably from about 20°C to 80°C, more preferably room temperature or from about 50°C to 70°C, more preferably about 60°C;

or

in the step a), the compound of formula II is subjected to the hydrogenation reaction with the hydrogen,

wherein preferably, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, more preferably from about 0.1 MPa to about 0.4 MPa;

and/or

wherein preferably, the reaction of the compound of formula II with the hydrogen is conducted at a temperature from about 15 to 25°C;

and/or

(2) the volume ratio of the solvent to the compound of formula II is from about 10:1 to 80:1, preferably from about 20:1 to 50:1;

and/or

(3) the solvent is an aqueous alcohol solution,

wherein preferably, the volume ratio of alcohol to water in the aqueous alcohol solution is from about 1:1 to about 8:1 (e.g., about 4:1 or about 6:1); and/or

wherein preferably, the alcohol in the aqueous alcohol solution is selected from the group consisting of methanol, ethanol, isopropanol, and any mixture thereof, more preferably ethanol;

or

the solvent is a mixed solvent of an alcohol and an ester,

wherein preferably, the volume ratio of the ester to the alcohol in the mixed solvent of the alcohol and the ester is from about 1:1 to about 1:5, more preferably from about 1:1 to about 1:2; and/or

wherein preferably, the alcohol in the mixed solvent of the alcohol and the ester is methanol, ethanol, isopropanol, or any mixture thereof, and/or the ester is ethyl acetate (EA), isopropyl acetate, or a mixture thereof;

and/or

(4) the hydrogenation reaction of the compound of formula II with the hydrogen-borrowing reagent is conducted in the aqueous alcohol solution;

or

the hydrogenation reaction of the compound of formula II with the hydrogen is conducted in the aqueous alcohol solution, or in the mixed solvent of the alcohol and the ester.

and/or

(5) the mass ratio of the compound of formula II to the metal catalyst is from about 1:0.01 to 1:1, preferably about 1:0.2;

and/or

(6) the metal catalyst is palladium on carbon (Pd/C), wherein preferably, the mass fraction of palladium in the Pd/C is from about 5-20%, more preferably about 10% (e.g., the Pd/C catalyst contains about 50 w/w% water);

and/or

(7) the hydrogenation reaction is conducted for a period of time from about 1 to 72 h, e.g., from about 1 to 48 h, from about 1 to 12 h, from about 1 to 10 h, from about 2 to 5 h, or from about 6 to 12 h.

9. The method according to any one of claims 1 to 8, characterized in that the method comprises the following step:

a) subjecting the compound of formula II to a hydrogenation reaction with the hydrogen or with the hydrogen-borrowing reagent in an aqueous ethanol solution or a mixed solvent of ethanol and ethyl acetate under the catalysis of Pd/C for about 1 to 72 h, e.g., about 1 to 48 h, about 1 to 12 h, about 1 to 10 h, about 2 to 5 h, or about 6 to 12 h, to provide the compound of formula I,

wherein:

(1) the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa, and

the reaction of the compound of formula II with the hydrogen is conducted at a temperature from about 20 to 25°C;

or

the molar ratio of the compound of formula II to the hydrogen-borrowing reagent is from about 1:2 to 1:10, e.g., about 1:2.5, about 1:4, about 1:5, about 1:6, or about 1:8,

the reaction of the compound of formula II with the hydrogen-borrowing reagent is conducted at a temperature from about 20°C to 100°C, preferably from about 20°C to 80°C, more preferably room temperature or from about 50°C to 70°C, more preferably about 60°C, and

the hydrogen-borrowing reagent is a combination of formic acid and potassium formate, wherein the molar ratio of the formic acid to the potassium formate is from about 1:1 to 5:1;

(2) the mass ratio of the compound of formula II to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2;

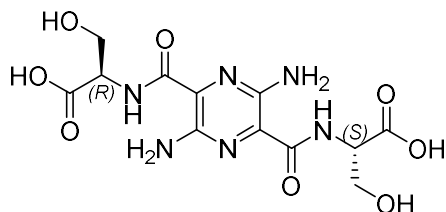
(3) the volume ratio of the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate to the compound of formula II is from about 10:1 to 80:1, preferably from about 20:1 to 50:1; and

(4) the volume ratio of ethanol to water in the aqueous ethanol solution is from about 1:1 to about 8:1 (e.g., about 4:1 or about 6:1); and

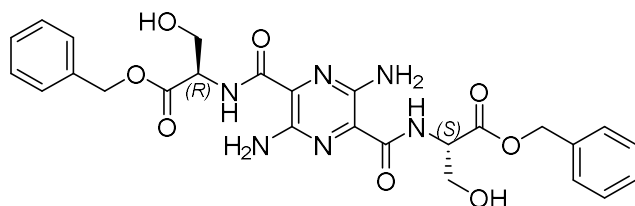
the volume ratio of ethyl acetate to ethanol in the mixed solvent of ethanol and ethyl

acetate is from about 1:1 to about 1:5, preferably about 1:1 to about 1:2.

10. The method according to any one of claims 1 to 4 and 7 to 9, characterized in that the compound of formula I is a compound of formula I-1, and the compound of formula II is a compound of formula II-1:



I-1



II-1

;

wherein preferably, the method comprises the following step:

a) subjecting the compound of formula II-1 to a hydrogenation reaction with the hydrogen-borrowing reagent in the aqueous ethanol solution under the catalysis of the Pd/C for about 1 to 12 h to provide the compound of formula I-1,

wherein preferably, the molar ratio of the compound of formula II-1 to the hydrogen-borrowing reagent is from about 1:2 to 1:10, e.g., about 1:2.5, about 1:4, about 1:5, about 1:6, or about 1:8,

and/or

the reaction of the compound of formula II-1 with the hydrogen-borrowing reagent is conducted at a temperature from about 20°C to 100°C, preferably from about 20°C to 80°C, more preferably room temperature or from about 50°C to 70°C, more preferably about 60°C,

and/or

the hydrogen-borrowing reagent is a combination of formic acid and potassium formate, wherein the molar ratio of the formic acid to the potassium formate is from about 1:1 to 5:1;

or

a) subjecting the compound of formula II-1 to a hydrogenation reaction with the hydrogen in the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate under the catalysis of the Pd/C for about 6 to 12 h to provide the compound of formula I-1,

wherein preferably, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa;

and/or

wherein preferably, the reaction of the compound of formula II-1 with the hydrogen is conducted at a temperature from about 20 to 25°C;

wherein preferably, the mass ratio of the compound of formula II-1 to the Pd/C is from 1:0.01 to 1:1, preferably about 1:0.2;

and/or

wherein preferably, the volume ratio of the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate to the compound of formula II-1 is from about 10:1 to 80:1, preferably from about 20:1 to 50:1;

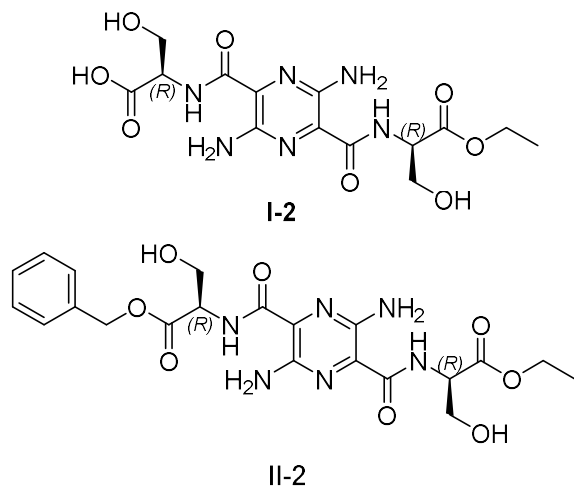
and/or

wherein preferably, the volume ratio of ethanol to water in the aqueous ethanol solution is from about 1:1 to about 8:1 (e.g., about 4:1 or about 6:1);

and/or

wherein preferably, the volume ratio of ethyl acetate to ethanol in the mixed solvent of ethanol and ethyl acetate is from about 1:1 to about 1:5, preferably from about 1:1 to about 1:2.

11. The method according to any one of claims 1 to 2 and 5 to 9, characterized in that the compound of formula I is a compound of formula I-2, and the compound of formula II is a compound of formula II-2:



wherein preferably, the method comprises the following step:

a) subjecting the compound of formula II-2 to a hydrogenation reaction with the hydrogen in the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate under the catalysis of the Pd/C for about 6 to 12 h to provide the compound of formula I-2;

wherein preferably, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa;

and/or

wherein preferably, the reaction of the compound of formula II-2 with the hydrogen is conducted at a temperature from about 20 to 25°C;

and/or

wherein preferably, the mass ratio of the compound of formula II-2 to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2;

and/or

wherein preferably, the volume ratio of the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate to the compound of formula II-2 is from about 10:1 to 80:1, preferably from about 20:1 to 50:1;

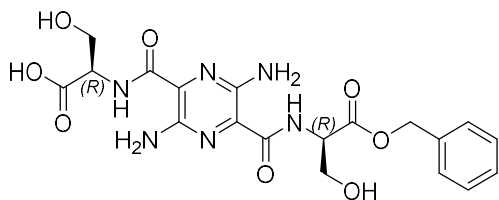
and/or

wherein preferably, the volume ratio of ethanol to water in the aqueous ethanol solution is from about 1:1 to about 8:1 (e.g., about 4:1 or about 6:1);

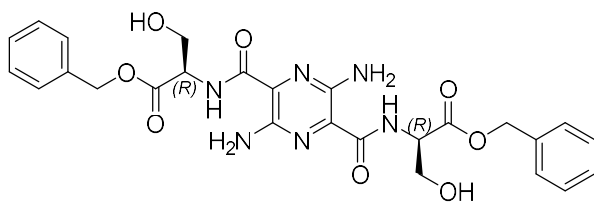
and/or

wherein preferably, the volume ratio of ethyl acetate to ethanol in the mixed solvent of ethanol and ethyl acetate is from about 1:1 to about 1:5, preferably from about 1:1 to about 1:2.

12. The method according to any one of claims 1 to 2 and 5 to 9, characterized in that the compound of formula I is a compound of formula I-3, and the compound of formula II is a compound of formula II-3:



I-3



II-3

;

wherein preferably, the method comprises the following step:

a) subjecting the compound of formula II-3 to a hydrogenation reaction with the hydrogen in the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate under the catalysis of the Pd/C for about 1 to 12 h, e.g., about 1 to 10 h, about 2 to 5 h, or about 6 to 12 h, to provide the compound of formula I-3;

wherein preferably, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa;

and/or

wherein preferably, the reaction of the compound of formula II-3 with the hydrogen is conducted at a temperature from about 20 to 25°C;

and/or

wherein preferably, the mass ratio of the compound of formula II-3 to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2;

and/or

wherein preferably, the volume ratio of the aqueous ethanol solution or the mixed solvent of ethanol and ethyl acetate to the compound of formula II-3 is from about 10:1 to 80:1, preferably from about 20:1 to 50:1;

and/or

wherein preferably, the volume ratio of ethanol to water in the aqueous ethanol solution is from about 1:1 to about 8:1 (e.g., about 4:1 or about 6:1);

and/or

wherein preferably, the volume ratio of ethyl acetate to ethanol in the mixed solvent of ethanol and ethyl acetate is from about 1:1 to about 1:5, preferably from about 1:1 to about 1:2.

13. The method according to any one of claims 1 to 12, characterized in that the method further comprises the following steps:

b) removing the metal catalyst from the reaction mixture of the step a), then drying the reaction mixture to obtain the compound of formula I; and

c) optionally, further purifying the compound of formula I obtained from the step b), preferably by:

slurrying the compound of formula I obtained from the step b) with acetonitrile (e.g., for about 1 to 4 h), further slurrying with water (for about 1 to 4 h), then filtering, and drying the filter cake; or

subjecting the compound of formula I obtained from the step b) to passage through a chromatographic column or a high-pressure preparative chromatograph.

14. The method according to any one of claims 1 to 13, characterized in that,

when the compound of formula I is the compound of formula I-1 and the compound of formula II is the compound of formula II-1, the method comprises performing step c), preferably further purifying the compound of formula I-1 obtained from step b) by slurrying the compound of formula I-1 obtained from step b) with acetonitrile (e.g., for about 1 to 4 h), further slurrying with water (for about 1 to 4 h), then filtering, and drying the filter cake;

or

when the compound of formula I is the compound of formula I-2 and the compound of formula II is the compound of formula II-2, the method comprises performing step c), preferably further purifying the compound of formula I-2 obtained from step b) by slurrying the compound of formula I-2 obtained from step b) with acetonitrile (e.g., for about 1 to 4 h), further slurrying with water (for about 1 to 4 h), then filtering, and drying the filter cake; or subjecting the compound of formula I-2 obtained from step b) to passage through a chromatographic column or a high-pressure preparative chromatograph;

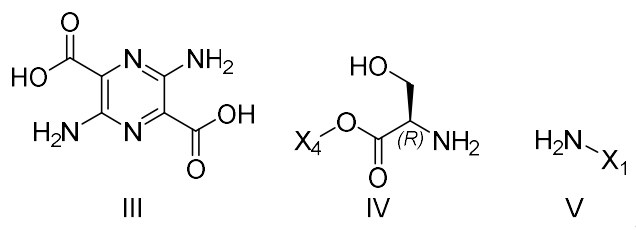
or

when the compound of formula I is the compound of formula I-3 and the compound of formula II is the compound of formula II-3, the method comprises performing step c), preferably further purifying the compound of formula I-3 obtained from step b) by subjecting the compound of formula I-3 obtained from step b) to passage through a chromatographic column or a high-pressure preparative chromatograph.

15. The method according to any one of claims 1 to 14, characterized in that the method further comprises the following step:

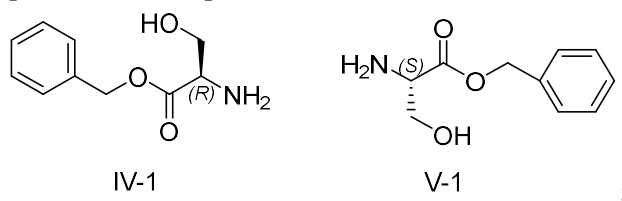
a') subjecting a compound of formula III, 3,6-diaminopyrazine-2,5-dicarboxylic acid to

an amide condensation reaction with a compound of formula IV or a salt thereof (preferably a hydrochloride) and a compound of formula V or a salt thereof (preferably a hydrochloride) in a suitable organic solvent in the presence of a suitable organic base, a suitable coupling agent and a suitable condensing agent, to provide the compound of formula II,



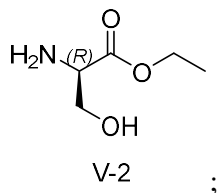
wherein X_1 and X_4 are as defined in any of claims 1 to 14.

16. The method according to claim 15, characterized in that, the compound of formula II is the compound of formula II-1, and the method comprises: reacting the compound of formula III with the hydrochloride of the compound of formula IV having a formula IV-1 and the hydrochloride of the compound of formula V having formula V-1 to provide the compound of formula II-1:



or

the compound of formula II is the compound of formula II-2, and the method comprises: reacting the compound of formula III with the hydrochloride of the compound of formula IV having the formula IV-1 and the hydrochloride of the compound of formula V having a formula V-2 to provide the compound of formula II-2:



or

the compound of formula II is the compound of formula II-3, and the method comprises: reacting the compound of formula III with the hydrochloride of the compound having the formula IV-1 to provide the compound of formula II-3.

17. The method according to claim 15 or 16, characterized in that, the molar ratio of the compound of formula IV to the compound of formula III is from about 0.5:1 to 8:1, preferably from about 1:1 to 4:1; and/or the molar ratio of the compound of formula V to the compound of formula III is about (0.5 to 8.0):1.0, preferably from about 1:1 to 4:1; and/or the volume ratio of the organic solvent to the compound of formula III is from about 10:1 to 80:1, preferably from about 20:1 to 50:1; and/or

the molar ratio of the organic base to the compound of formula III is from about 0.5:1 to 9:1, preferably from about 1:1 to 5:1; and/or

the molar ratio of the coupling agent to the compound of formula III is from about 0.5:1 to 9:1, preferably from about 1:1 to 5:1; and/or

the molar ratio of the condensing agent to the compound of formula III is from about 0.5:1 to 9:1, preferably from about 1:1 to 5:1; and/or

the amide condensation reaction is first conducted at a suitable temperature, preferably from about -5 to 20°C, more preferably from about -5 to 10°C, more preferably from about 0 to 10°C for a suitable period of time, e.g., about 10-60 minutes (min), then at room temperature for a suitable period of time, preferably for about 10-72 h, more preferably about 18-36 h; and/or

the organic solvent is N,N-dimethylformamide (DMF), dichloromethane (DCM), dimethyl sulfoxide (DMSO), tetrahydrofuran and/or 1,4-dioxane; and/or

the organic base is diisopropylethylamine (DIPEA), triethylamine, pyridine and/or 4-dimethylaminopyridine (DMAP); and/or

the coupling agent is ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma), 1-hydroxybenzotriazole (HOBt), HATU, HBTU, HCTU, TBTU, TSTU, TNTU, O-(7-azabenzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HAPyU) and/or O-(benzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HBPYU); and/or

the condensing agent is selected from the group consisting of 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), HOBt, HATU, HBTU, HCTU, TBTU, TSTU, TNTU, (7-azabenzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HAPyU), O-(benzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HBPYU), and any combination thereof.

18. The method according to any one of claims 15 to 17, characterized in that the method comprises the following step:

a') reacting the compound of formula III with the compound of formula IV and the compound of formula V in DMF at about -5 to 20°C, preferably about -5 to 10°C, more preferably about 0 to 10°C, in the presence of DIPEA, ethyl 2-cyano-2-(hydroxyimino)acetate and EDC·HCl for about 10-60 min, then reacting at room temperature for about 10-72 h, more preferably about 18-36 h.

19. The method according to any one of claims 15 to 18, characterized in that the method further comprises the following steps:

b') optionally, adding water to the reaction mixture of the step a') to precipitate the compound of formula II, wherein preferably, the volume ratio of the added water to the compound of formula III is from about 20:1 to 150:1, more preferably from about 35:1 to 75:1; and

c') optionally, further purifying the compound of formula II obtained from the step b'), preferably by subjecting the compound of formula II obtained from step b') to passage through a chromatographic column or a high-pressure preparative chromatograph.

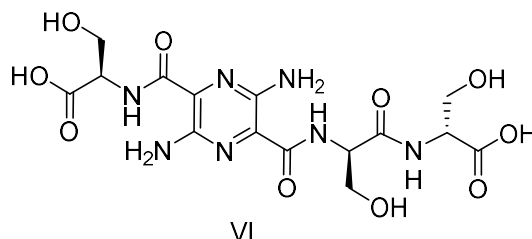
20. The method according to any one of claims 15 to 19, characterized in that,

when the compound of formula II is the compound of formula II-1, the method comprises performing the step c'), wherein the compound of formula II-1 is further purified by subjecting the compound of formula II-1 obtained from the step b') to passage through a chromatographic column; or

when the compound of formula II is the compound of formula II-2, the method comprises performing the step c'), wherein the compound of formula II-2 is further purified by subjecting the compound of formula II-2 obtained from the step b') to passage through a chromatographic column or a high-pressure preparative chromatograph; or

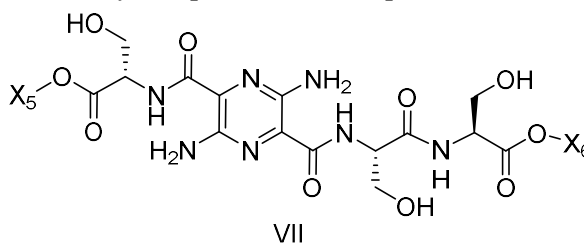
when the compound of formula II is the compound of formula II-3, the method comprises performing the step c'), wherein the compound of formula II-3 is further purified by subjecting the compound of formula II-3 obtained from the step b') to passage through a chromatographic column or a high-pressure preparative chromatograph.

21. A method for preparing a compound of formula VI or a salt thereof,



characterized in that the method comprises the following step:

i) subjecting a compound of formula VII to a hydrogenation reaction in a solvent in the presence of a suitable metal catalyst to provide the compound of formula VI,



wherein:

X₅ and X₆ are each independently -(substituted or unsubstituted C₁₋₁₀ alkyl)-R²; and

R² is selected from the group consisting of C₃₋₁₀ cyclohydrocarbyl, 4-10 membered heterocyclyl, C₆₋₁₀ aryl and 5-10 membered heteroaryl.

22. The method according to claim 21, characterized in that R² is selected from the group consisting of C₃₋₇ cycloalkyl, preferably C₅₋₆ cycloalkyl; 4-7 membered heterocyclyl, preferably 5-6 membered heterocyclyl; phenyl and 5-6 membered heteroaryl; preferably phenyl.

23. The method according to claim 21 or 22, characterized in that X₅ and X₆ are each independently -(substituted or unsubstituted C₁₋₈ alkyl)-R², preferably -(substituted or unsubstituted C₁₋₆ alkyl)-R², more preferably -(substituted or unsubstituted C₁₋₄ alkyl)-R², more preferably -(unsubstituted C₁₋₂ alkyl)-R², more preferably -CH₂-R²; preferably -CH₂-

phenyl.

24. The method according to any one of claims 21 to 23, characterized in that,

(1) in the step i), the hydrogenation reaction is conducted under a hydrogen atmosphere;

wherein preferably, the hydrogen has a pressure from about 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa;

and/or

wherein preferably, the reaction of the compound of formula VII with the hydrogen is conducted at a temperature from about 20 to 30°C;

and/or

(2) the mass ratio of the compound of formula VII to the metal catalyst is from about 1:0.01 to 1:1, preferably about 1:0.2;

and/or

(3) the metal catalyst is palladium on carbon (Pd/C), wherein preferably, the mass fraction of palladium in the Pd/C is about 5-20%, more preferably about 10% (e.g., the Pd/C catalyst contains about 50 w/w% water);

and/or

(4) the volume ratio of the solvent to the compound of formula VII is from about 10:1 to 50:1, preferably from about 10:1 to 30:1;

and/or

(5) the solvent is N,N-dimethylformamide (DMF), dichloromethane (DCM), dimethyl sulfoxide (DMSO), tetrahydrofuran and/or 1,4-dioxane, preferably N,N-dimethylformamide;

and/or

(6) the hydrogenation reaction is conducted for a period of time from about 5 to 72 h, for example, from about 10 to 48 h, or from about 10 to 30 h.

25. The method according to any one of claims 21 to 24, characterized in that the method comprises the following step:

i) subjecting a compound of formula VII-1 to a hydrogenation reaction in N,N-dimethylformamide in the presence of about 10% Pd/C under a hydrogen atmosphere of 0.05 MPa to about 0.8 MPa, preferably from about 0.1 MPa to about 0.4 MPa, at a temperature from about 20 to 25°C for a period of time from about 5 to 72 h, preferably from about 10 to 30 h, to provide the compound of formula VI,

wherein:

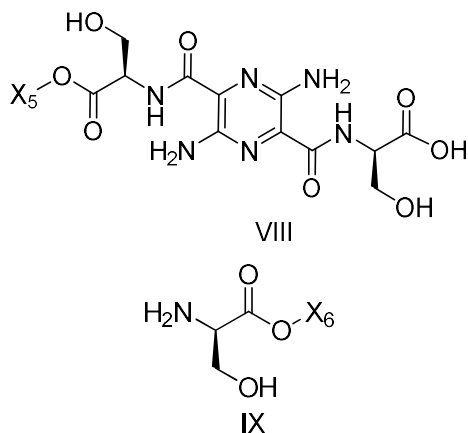
the mass ratio of the compound of formula VII-1 to the Pd/C is from about 1:0.01 to 1:1, preferably about 1:0.2; and

the volume ratio of the N,N-dimethylformamide to the compound of formula VII-1 is from about 10:1 to 50:1, preferably from about 10:1 to 30:1.

26. The method according to any one of claims 1 to 25, characterized in that the method further comprises the following steps:

i') subjecting a compound of formula VIII and a compound of formula IX or a salt thereof (preferably a hydrochloride) to an amide condensation reaction in a suitable organic solvent in the presence of a suitable organic base, a suitable coupling agent and a suitable

condensing agent under an inert atmosphere, to provide the compound of formula VII,



wherein X_5 and X_6 are each as defined in any of claims 21 to 25; and

ii') optionally, filtering the reaction mixture of the step i'), adding water dropwise to the filtrate to crystallize and precipitate the compound of formula VII, wherein preferably, the volume ratio of the added water to the filtrate is from about 20:1 to 150:1, preferably from 35:1 to 75:1; and

iii') optionally, adding ethyl acetate to the isolated compound of formula VII for slurring, then filtering, and vacuum drying at an elevated temperature for 18 hours or more, wherein preferably the volume ratio of the ethyl acetate to the compound of formula VII is from about 5:1 to 30:1, preferably from about 5:1 to 10:1.

27. The method according to claim 26, characterized in that,
the inert atmosphere is nitrogen protection; and/or

the volume ratio of the organic solvent to the compound of formula IX or salt thereof is from about 10:1 to 80:1, preferably from about 20:1 to 50:1; and/or

the molar ratio of the organic base to the compound of formula VIII is from about 0.5:1 to 9:1, preferably from about 1:1 to about 5:1; and/or

the molar ratio of the condensing agent to the compound of formula VIII is from about 0.5:1 to 9:1, preferably from about 1:1 to about 5:1; and/or

the molar ratio of the coupling agent to the compound of formula VIII is from about 0.5:1 to 9:1, preferably from about 1:1 to about 5:1; and/or

the amide condensation reaction is conducted at a temperature from about 10 to 30°C, preferably from about 20 to 30°C; and/or

the amide condensation reaction is conducted for a period of time from about 10 to 72 h, preferably from about 18 to 36 h; and/or

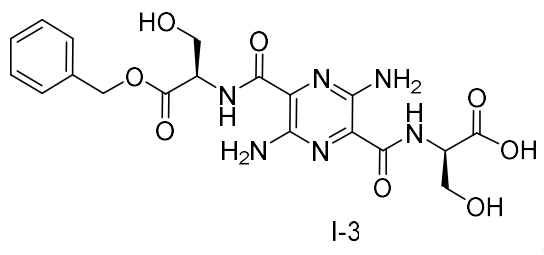
the organic solvent is N,N-dimethylformamide (DMF), dichloromethane (DCM), dimethyl sulfoxide (DMSO), tetrahydrofuran and/or 1,4-dioxane; and/or

the organic base is diisopropylethylamine (DIPEA), triethylamine, pyridine and/or 4-dimethylaminopyridine (DMAP); and/or

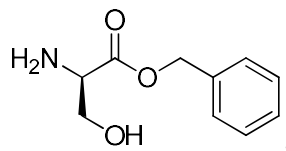
the coupling agent is ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma), HOBt, HATU, HBTU, HCTU, TBTU, TSTU, TNTU, O-(7-azabenzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HAPyU) and/or O-(benzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HBPYU); and/or

the condensing agent is selected from: 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), HOBt, HATU, HBTU, HCTU, TBTU, TSTU, TNTU, O-(7-azabenzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HAPyU), O-(benzotriazol-1-yl)-di(pyrrolidinyl)carbenium hexafluorophosphate (HBPYU), and any combination thereof.

28. The method according to claim 26 or 27, characterized in that, the compound of formula VIII is a compound of formula I-3:



the compound of formula IX is a compound of the formula:



the organic solvent is DMF;

the organic base is diisopropylethylamine (DIPEA);

the coupling agent is HOBt; and

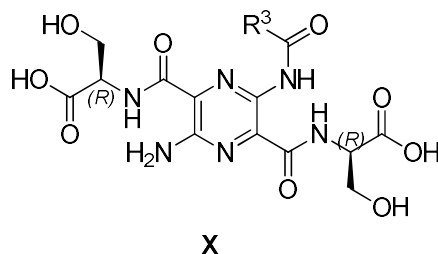
the condensing agent is EDC·HCl.

29. The method according to any one of claims 21 to 28, characterized in that the method further comprises the following steps:

ii) optionally, filtering the reaction mixture of the step i), adding ethyl acetate dropwise to the filtrate to crystallize and precipitate the compound of formula VI, wherein preferably the volume ratio of the ethyl acetate to the filtrate is from about 30:1 to 90:1, preferably from about 30:1 to 50:1; and

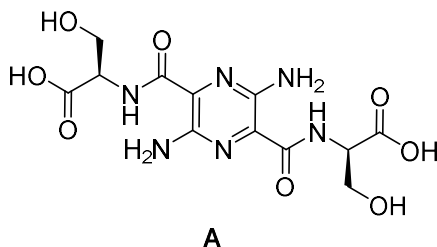
iii) optionally, adding ethyl acetate to the isolated compound of formula VI for slurring, then filtering, and vacuum drying at an elevated temperature for 12 hours or more, wherein preferably the volume ratio of the ethyl acetate to the compound of formula VI is from about 1:1 to 10:1, preferably from about 2:1 to 5:1.

30. A method for preparing a compound of formula X or a salt thereof,



characterized in that the method comprises the following steps:

x-i) providing a solution of a compound of formula A in a solvent;



x-ii) acylating the compound of formula A with an acid R^3 -COOH at an elevated temperature to provide the compound of formula X; and

x-iii) optionally, isolating and purifying the compound of formula X;

wherein R^3 is H, or substituted or unsubstituted C_{1-4} alkyl, preferably H or methyl, more preferably H.

31. The method according to claim 30, characterized in that,

R^3 is H; and/or

in step x-i), the volume ratio of the solvent to the compound of formula A is from about 5:1 to 30:1, preferably from about 5:1 to 10:1; and/or

the solvent in step x-i) is N,N-dimethylformamide (DMF), dichloromethane (DCM), dimethyl sulfoxide (DMSO), tetrahydrofuran and/or 1,4-dioxane, preferably DMF; and/or

the elevated temperature in step x-ii) is from about 55 to 75°C, preferably about 70°C; and/or

in step x-ii), the volume ratio of the acid R^3 -COOH to the compound of formula A is from about 0.5:1 to 10:1, preferably from about 1:1 to 5:1; and/or

in step x-ii), the reaction time is from about 1 to 20 h, preferably from about 1 to 5 h; and/or

the step x-iii) comprises:

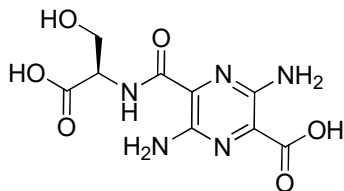
1) concentrating the reaction mixture of the step x-i) until no distillate, then adding ethyl acetate dropwise to the residue to crystallize and precipitate the compound of formula X, wherein preferably, the volume ratio of the ethyl acetate to the compound of formula A is from about 15:1 to 90:1, preferably from about 15:1 to 30:1; and

2) dissolving the isolated compound of formula X in the solvent used in step x-i), followed by adding ethyl acetate dropwise to crystallize and precipitate the compound of formula X, then filtering, and vacuum drying at an elevated temperature, wherein preferably, the volume ratio of the solvent to the compound of formula A is from about 0.5:1 to 5:1, preferably from about 1:1 to 2:1; and/or the volume ratio of the ethyl acetate to the compound of formula A is from about 1:1 to 10:1, preferably from about 2:1 to 4:1;

3) repeating the recrystallization of the steps 1) and 2); and

4) slurring the dried compound of formula X obtained from the step 3) with ethyl acetate, then filtering, and vacuum drying at an elevated temperature for 16 hours or more, wherein preferably, the volume ratio of the ethyl acetate to the compound of formula A is from about 1:1 to 10:1, preferably from about 2:1 to 5:1.

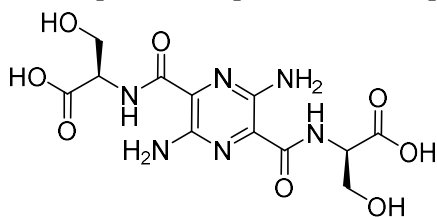
32. A method for preparing a compound of formula XI or a salt thereof,



XI

characterized in that the method comprises the following steps:

xi-i) hydrolyzing a compound of formula A in water in the presence of a base at an elevated temperature to provide the compound of formula XI or a salt thereof;



A

, and

xi-ii) optionally, isolating and purifying the compound of formula XI or salt thereof.

33. The method according to claim 32, characterized in that,

in step xi-i), the volume ratio of the water to the compound of formula A is from about 5:1 to 30:1, preferably from about 5:1 to 10:1; and/or

in step xi-i), the molar ratio of the base to the compound of formula A is from about 1:1 to 5:1, preferably from about 2:1 to 5:1; and/or

the base is an inorganic base, preferably potassium hydroxide, sodium hydroxide, or mixtures thereof, more preferably potassium hydroxide; and/or

in step xi-i), the elevated temperature is from about 50 to 100°C, preferably from about 80 to 90°C; and/or

in step xi-i), the hydrolysis reaction is conducted for about 5 to 48 h, preferably about 10 to 20 h; and/or

the step xi-ii) comprises:

1) adding silica gel to the reaction mixture of the step xi-ii) to concentrate the reaction mixture into a dry silica gel powder, subjecting the dry silica gel powder to silica gel column chromatography to obtain a solid crude product of the compound of formula XI or a salt thereof,

wherein preferably, the mass ratio of the added silica gel to the compound of formula A is from about 0.5:1 to 2:1, preferably about 2:1; and/or the silica gel chromatography column is eluted gradiently using EA/MeOH (1% TFA) as an eluent, wherein the volume ratio of EA : MeOH (1% TFA) is from about 50:1 to 1:1, preferably from about 30:1 to 10:1;

2) slurring the solid crude product from the step xii-1) with methanol, filtering, then vacuum drying at an elevated temperature for 16 hours or more to obtain the purified compound of formula XI or a salt thereof,

wherein preferably, the volume ratio of the methanol to the compound of formula A is from about 1:1 to 10:1, preferably from about 2:1 to 5:1.

34. A method for quality control of an active pharmaceutical ingredient of 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine or a formulation thereof, comprising determining the content of the compound of formula I as defined in any one of claims 1 to 7, preferably the compound of formula I-1 as defined in claim 10, the compound of formula I-2 as defined in claim 11, the compound of formula I-3 as defined in claim 12, the compound of formula VI as defined in claim 21, the compound of formula X as defined in claim 30, and/or the compound of formula XI as defined in claim 32 in the active pharmaceutical ingredient or the formulation, and optionally comparing with a specified content for each of the said compounds;

wherein preferably, for the compound of formula I, the specified content is not more than about 0.9%, preferably not more than about 0.6%, more preferably not more than about 0.45%, based on the total weight of the active pharmaceutical ingredient;

for the compound of formula I-1, the specified content is not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15%, based on the total weight of the active pharmaceutical ingredient; or is not more than about 0.40%, preferably not more than about 0.3%, more preferably not more than about 0.20%, based on the total weight of the formulation;

for the compound of formula I-2, the specified content is not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15%, based on the total weight of the active pharmaceutical ingredient;

for the compound of formula I-3, the specified content is not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15%, based on the total weight of the active pharmaceutical ingredient;

for the compound of formula VI, the specified content is not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15%, based on the total weight of the active pharmaceutical ingredient;

for the compound of formula X, the specified content is not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15%, based on the total weight of the active pharmaceutical ingredient; and/or

for the compound of formula XI, the specified content is not more than about 0.2%, preferably not more than about 0.15%, more preferably not more than about 0.10%, based on the total weight of the active pharmaceutical ingredient; or is not more than about 0.40%, preferably not more than about 0.3%, more preferably not more than about 0.20%, based on the total weight of the formulation.

35. Use of the compound of formula I as defined in any one of claims 1 to 7, preferably the compound of formula I-1 as defined in claim 10, the compound of formula I-2 as defined in claim 11, the compound of formula I-3 as defined in claim 12, the compound of formula VI as defined in claim 21, the compound of formula X as defined in claim 30, and/or the compound of formula XI as defined in claim 32 as a standard or control for quality control of an active pharmaceutical ingredient of 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine or a formulation thereof.

36. An active pharmaceutical ingredient of 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine having a low content of related compounds, characterized in that the related compounds are the compound of formula I as defined in any one of claims 1 to 7, preferably the compound of formula I-1 as defined in claim 10, the compound of formula I-2 as defined in claim 11, the compound of formula I-3 as defined in claim 12, the compound of formula VI as defined in claim 21, the compound of formula X as defined in claim 30, and/or the compound of formula XI as defined in claim 32; and

the active pharmaceutical ingredient has one or more of the following contents:

not more than about 0.9%, preferably not more than about 0.6%, more preferably not more than about 0.45% of the compound of formula I, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15% of the compound of formula I-1, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15% of the compound of formula I-2, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15% of the compound of formula I-3, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15% of the compound of formula VI, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.3%, preferably not more than about 0.25%, more preferably not more than about 0.15% of the compound of formula X, based on the total weight of the active pharmaceutical ingredient,

not more than about 0.2%, preferably not more than about 0.15%, more preferably not more than about 0.10% of the compound of formula XI, based on the total weight of the active pharmaceutical ingredient.

37. A formulation of 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine having a low content of related compounds, characterized in that the related compounds are selected from the group consisting of the compound of formula I-1 as defined in claim 10 and the compound of formula XI as defined in claim 32; and

the formulation has one or both of the following contents:

not more than about 0.40%, preferably not more than about 0.3%, more preferably not more than about 0.20% of the compound of formula I-1, based on the total weight of the formulation;

not more than about 0.40%, preferably not more than about 0.3%, more preferably not more than about 0.20% of the compound of formula XI, based on the total weight of the formulation.

38. The method according to claim 34, the use according to claim 35, the active

pharmaceutical ingredient according to claim 36, or the formulation according to claim 37, wherein the formulation is an injectable solution or an injectable lyophilized powder, preferably an injectable solution.