

Method for preparing pyrazine carboxylic acid derivative as fluorescent tracer

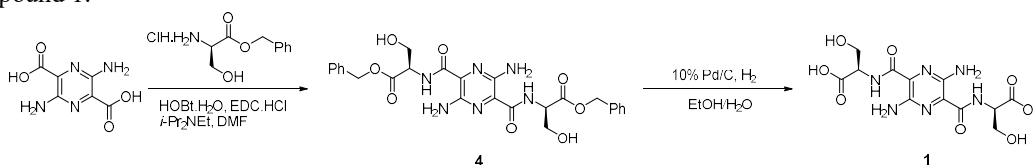
Field of the Invention

The invention relates to a method for preparing 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl}carbamoyl}pyrazine, an intermediate compound for use in the preparation method, and a method for preparing the intermediate compound.

Background of the Invention

Pyrazine derivatives are potentially useful as fluorescent tracers in evaluating renal function. 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl}carbamoyl}pyrazine (compound 1) as a highly clinically valuable fluorescent tracer has attracted widespread attention, but its synthetic route has been rarely reported.

In 2011, Raghavan Rajagopalan *et al.* optimized the synthesis of compound 1 by reducing the reaction steps (J. Med. Chem. 2011, 54, 5048–5058). In this synthesis method, 3,6-diaminopyrazine-2,5-dicarboxylic acid as a raw material is subjected to amidation, and palladium catalyzed hydrogenation steps to obtain the compound 1:



In the method, benzyl D-serinate hydrochloride is used as a reaction substrate in the amidation reaction to obtain intermediate 4 which is then subjected to palladium/carbon catalyzed hydrogenation reaction to generate compound 1. Due to the high price of commercial benzyl D-serinate hydrochloride and palladium/carbon, the production cost of the product is high. In addition, not only the hydrogenation reaction has higher requirements on production equipment and sites, but also hydrogen is a flammable and explosive gas, which is very dangerous to operate and poses great potential safety hazards. Due to the use of palladium/carbon in the hydrogenation reaction, improper treatment will lead to noble metal residue, thus posing a drug safety risk.

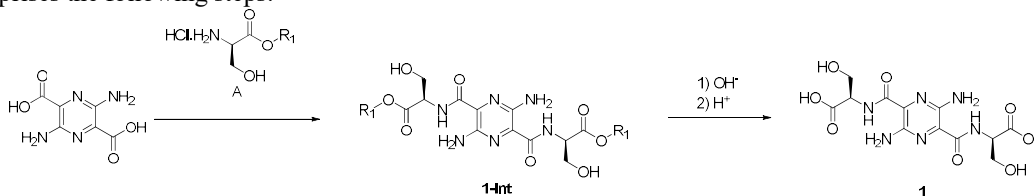
Therefore, there is still a need to develop methods suitable for the industrial production of 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl}carbamoyl}pyrazine with high safety and cost-effectiveness.

Summary of the Invention

The object of the invention is to overcome the problems existing in the prior art and to provide a novel method for preparing 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl}carbamoyl}pyrazine.

The present inventors prepared the intermediate compound of Formula 1-Int for the first time by changing the substrate for the amidation reaction, particularly using low-cost D-serinate hydrochloride of Formula A as a substrate, and optimizing the reaction conditions. The intermediate compound can be subjected to ester hydrolysis reaction, and acidification to give the compound 1. Therefore, the preparation method of the invention does not require palladium catalyzed hydrogenation reaction, thereby greatly improving the safety of industrial production and avoiding the risk of noble metal residue caused by palladium/carbon catalyst. The present inventors have also found that chiral impurities can be generated in the ester hydrolysis reaction. Unexpectedly, the present inventors have found that the chiral purity of compound 1 can be improved by adjusting the conditions of the ester hydrolysis reaction, particularly by selecting a suitable solvent. The preparation method of the invention has the advantages of lower cost, good safety, low noble metal residue risk and high chiral purity of the product.

In a first aspect, the invention provides a method for preparing 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl}carbamoyl}pyrazine of Formula 1, characterized in that the process comprises the following steps:

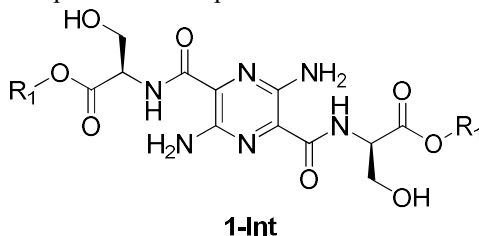


wherein R₁, at each occurrence, is independently selected from the group consisting of C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, and C₃₋₈ cycloalkyl;

Step S1. subjecting a D-serinate hydrochloride of Formula A, 3,6-diaminopyrazine-2,5-dicarboxylic acid, and an amide condensation agent in a first organic solvent to amidation reaction to obtain an intermediate of Formula 1-Int; and

Step S2. hydrolyzing the intermediate of Formula 1-Int in a solvent under a basic condition, and then acidifying the reaction mixture to obtain 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl] carbamoyl}pyrazine of Formula 1, wherein the solvent is water, or a mixture of water and a second organic solvent.

In a second aspect, the invention provides a compound of Formula 1-Int:



wherein each R₁ may be the same or different, and is independently selected from the group consisting of C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, and C₃₋₈ cycloalkyl. The compound of Formula 1-Int may be used as an intermediate for the preparation of compound 1.

In a third aspect, the invention provides a method for preparing the compound of Formula 1-Int.

Detailed Description of the Invention

Definitions

Unless otherwise defined below, all technical and scientific terms used herein are intended to have the same meaning as commonly understood by those skilled in the art. References to technology as used herein are intended to mean technology as commonly understood in the art, including those variations or substitutions of equivalent technology that will be apparent to those skilled in the art. While it is believed that the following terms are well understood by those skilled in the art, the following definitions are set forth to better explain the invention.

As use herein, that terms "include", "comprise", "have", "contain", or "involve", as well as other variants thereof, are either inclusive or open-ended, and do not exclude other non-recited elements or method steps, although other non-recited elements or method steps are not necessarily present (i.e., these terms also encompass the term "consisting essentially of ..." and "consisting of ...").

As use herein, that term "alkyl" refers to a linear or branched saturated aliphatic hydrocarbon radical. The term "C₁₋₈ alkyl" refers to a straight or branched alkyl group having 1 to 8 carbon atoms. For example, as used herein, the term "C₁₋₆ alkyl" refers to a linear or branched alkyl group having 1 to 6 carbon atoms (such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, or n-hexyl). The term "C₁₋₃ alkyl" refers to a straight or branched alkyl group having 1 to 3 carbon atoms, including methyl, ethyl, n-propyl, and isopropyl.

As used herein, the term "C₂₋₈ alkenyl" refers to a straight or branched monovalent hydrocarbon radical containing one or more double bonds, having 2 to 8 carbon atoms (such as "C₂₋₆ alkenyl" or "C₂₋₃ alkenyl"). Examples of alkenyl groups that can be mentioned include, but are not limited to, for example, vinyl, 1-propenyl, 2-propenyl, 2-butenyl, 3-butenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl, 2-methyl-2-propenyl, 4-methyl-3-pentenyl.

As use herein, that term "C₂₋₈ alkynyl" refers to a linear or branched monovalent hydrocarbon radical containing one or more triple bonds, having 2 to 8 carbon atoms (such as "C₂₋₆ alkynyl" or "C₂₋₃ alkynyl"). Examples of alkynyl groups that can be mentioned include, but are not limited to, ethynyl, 1-propynyl, 2-propynyl, 2-butyne, 3-butyne, 2-pentyne, 3-pentyne, 4-pentyne, 2-hexynyl, 3-hexynyl, and the like.

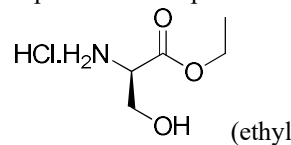
As use herein, that term "C₃₋₈ cycloalkyl" refers to a saturated hydrocarbon ring having 3 to 8 ring carbon atoms. The cycloalkyl group suitably has 3 to 7 carbon atoms, such as 3 to 6 ("C₃₋₆ cycloalkyl"), 4 to 6 ("C₄₋₆ cycloalkyl"), or 5 to 6 ("C₅₋₆ cycloalkyl") carbon atoms. Examples of cycloalkyl groups that may be mentioned include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl (ring).

If the substituents are described as being "independently selected from" a group of functional groups, each substituent is selected independently of the other. Thus, each substituent can be the same as or different from another (other) substituent.

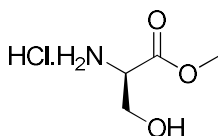
The term "about" means within the range of $\pm 10\%$, preferably $\pm 5\%$, more preferably $\pm 2\%$ of the stated value.

Chemical reaction scheme showing the synthesis of compound **1** from 2,4,6-triaminopyrimidine-5-carboxylic acid and an amino acid **A** (H₂N-CH(R₁)-COOH) in the presence of HCl. The reaction proceeds via intermediate **1-Int** (a dipeptide) to yield compound **1** (a cyclic dipeptide) through a two-step process: 1) OH⁻ and 2) H⁺.

In some embodiments, in the step S1, the D-serinate hydrochloride of Formula A is in the form of a single compound represented by the Formula A. In some other embodiments, in the step S1, the D-serinate hydrochloride of Formula A is in the form of a mixture of two or more different compounds each represented



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D-serinate hydrochloride) and/or (methyl D-serinate hydrochloride).

The present inventors prepared the intermediate compound of Formula 1-Int for the first time by using the D-serinate hydrochloride of Formula A as the substrate for the amidation reaction and optimizing the reaction conditions. The present inventors have found that the operation of adding dropwise the reactants and the amide condensation agent to the first organic solvent under a low temperature condition facilitates the formation of the intermediate and a high yield. The favorable low temperature condition is a temperature from about 0°C to 5°C, and a temperature from about 0°C to 2°C helps to achieve higher yield. The present inventors have also found that there may be a solvent effect in the amidation reaction, the first organic solvent can affect the formation and the yield of the intermediate compound of Formula 1-Int. Preferably, the first organic solvent is DMF.

The prepared intermediate compound of Formula 1-Int can be separated through post-treatment. In some embodiments, the post-treatment includes: removing the first organic solvent by rotary evaporation, and then extracting the residue multiple times with water and a suitable solvent, such as ethyl acetate; washing the combined organic phases with saturated aqueous NaHCO₃ and aqueous KHSO₄, concentrating the organic phases, and then separating through column chromatography.

In some embodiments, in the step S2, the intermediate of Formula 1-Int is hydrolyzed in the solvent under a basic condition and at a suitable temperature, followed by acidification of the reaction mixture to obtain 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine, wherein the solvent is water, or a mixture of water and a second organic solvent.

Preferably, the second organic solvent is a polar organic solvent, for example, selected from the group consisting of tetrahydrofuran, methanol, acetonitrile, ethanol, 1,4-dioxane, and any combination thereof, and more preferably is tetrahydrofuran, acetonitrile, or 1,4-dioxane.

The present inventors have found that chiral impurities can be generated in the ester hydrolysis reaction of step S2. The present inventors have unexpectedly found that the ester hydrolysis reaction in some specific solvents can provide more favorable chiral purity and yield of compound 1. The chiral purity can be represented by a dr value (i.e., a diastereomeric ratio). In step S2 of the method of the invention, the 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine of Formula 1 can have a chiral purity represented by a dr value of about 80:20 to about 94:6, preferably about 95:5 to about 99:1, such as about 96:4, about 97:3, or about 98:2.

In some embodiments, in the step S2, the solvent is water. In some of such embodiments, the hydrolysis is performed at a suitable temperature, such as a temperature of not more than about 20°C, such as about 1 to 15°C, and particularly about 5 to 10°C.

In some other embodiments, in the step S2, the solvent is a mixture of water and the second organic solvent. In some of such embodiments, in the step S2, the hydrolysis is performed at a temperature of not more than 20°C, such as about -10°C to 15°C, in particular about -5°C to 10°C, such as -5°C to 0°C or about 0°C to 5°C.

In other such embodiments, the solvent is a mixture of water and acetonitrile.

In other such embodiments, the solvent is a mixture of water and one selected from the group consisting of tetrahydrofuran, methanol, ethanol, and 1,4-dioxane. In some preferred embodiments, the solvent is a mixture of water and tetrahydrofuran or 1,4-dioxane. Preferably, the hydrolysis is performed at a temperature of about -10°C to 10°C, preferably about -5°C to 5°C, and more preferably about -5°C to 0°C.

In some of the embodiments described above, in the step S2, the solvent comprises an alkali metal hydroxide, such as one, two or more selected from the group consisting of LiOH, NaOH and KOH, and preferably LiOH.

The hydrolysis may be performed, for example, under mixing such as stirring (e.g., for about 1 to 8 hours).

In some embodiments, in the step S2, the acidification is performed by adjusting the pH of the reaction mixture with an acid after the hydrolysis is complete. The completion of the hydrolysis can be indicated, for example, by monitoring the progress of the reaction by TLC until the substrate of the reaction has disappeared.

In some embodiments, the pH is about 2 to about 4, and preferably about 3.

In some embodiments, in the step S2, after the acidification, the reaction mixture is mixed (e.g., by stirring) for a suitable period of time, e.g., about 1 to 4 hours, and preferably about 2 hours.

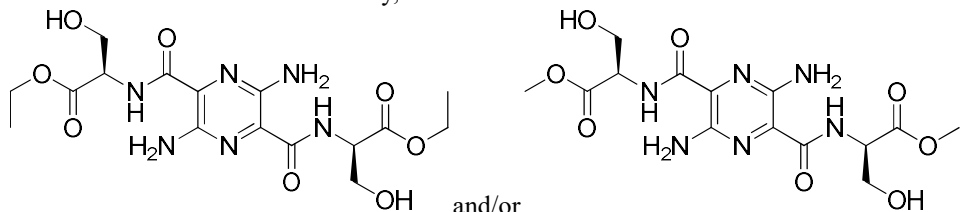
The invention provides a method as described above, wherein in the step S2, the intermediate of Formula 1-Int is hydrolyzed in water or a mixture of water and acetonitrile in the presence of an alkali metal

hydroxide at a temperature not more than about 20°C, and the reaction mixture is adjusted to a pH of about 3 with an acid after the hydrolysis is completed, and subjected to further stirring, to obtain 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine.

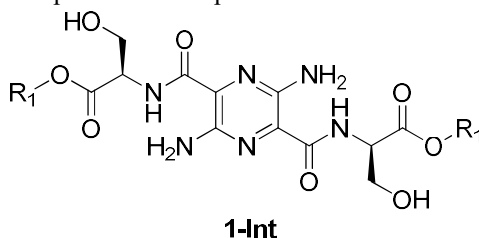
The present invention also provides a method as described above, wherein in the step S2, the intermediate of Formula 1-Int is hydrolyzed in a mixture of water and tetrahydrofuran or 1,4-dioxane in the presence of an alkali metal hydroxide at a temperature of about -10°C to 10°C, such as about -5°C to 5°C, especially about -5°C or 0°C, and the reaction mixture is adjusted to a pH of about 3 with an acid after the hydrolysis is completed and subjected to further stirring for about 2 hours, to provide 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine.

The acid can be one, two or more selected from the group consisting of acetic acid, trifluoroacetic acid, sulfuric acid, citric acid, and hydrochloric acid.

In some embodiments, in the step S2, the intermediate of Formula 1-Int is in the form of a single compound represented by the Formula 1-Int. In some other embodiments, in the step S2, the intermediate of Formula 1-Int is in the form of a mixture of two or more different compounds each represented by the Formula 1-Int. Preferably, the intermediate of Formula 1-Int is



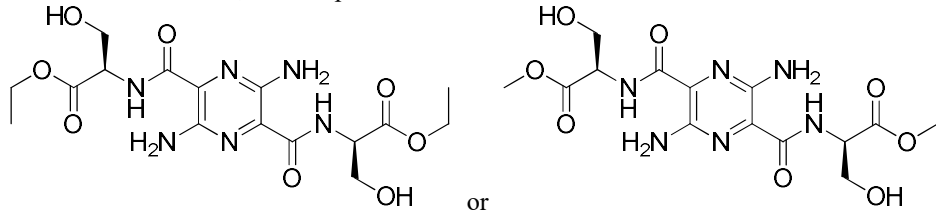
In a second aspect, the invention provides a compound of Formula 1-Int:



wherein each R₁ can be the same or different, and is independently selected from the group consisting of C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, and C₁₋₈ cycloalkyl.

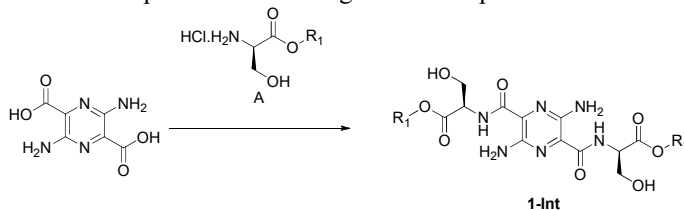
In some preferred embodiments, each R₁ can be the same or different, and is independently selected from C₁₋₆ alkyl, preferably C₁₋₃ alkyl, and more preferably methyl or ethyl.

In some embodiments, the compound of Formula 1-Int is:



The present inventor prepared the intermediate compound of Formula 1-Int for the first time. Compound 1 can be obtained through ester hydrolysis reaction of the intermediate compound and subsequent acidification, thereby avoiding the palladium-catalyzed hydrogenation reaction used in the existing method, greatly improving the safety of industrial production, and avoiding the risk of noble metal residue caused by the palladium/carbon catalyst.

In a third aspect, the invention provides a method for preparing the compound of Formula 1-Int, characterized in that the method comprises the following reaction step:



wherein R₁, at each occurrence, is independently selected from the group consisting of C₁₋₈ alkyl, C₂₋₈

alkenyl, C₂₋₈ alkynyl, and C₁₋₈ cycloalkyl, preferably C₁₋₆ alkyl, more preferably C₁₋₃ alkyl, and more preferably methyl or ethyl;

Step S1. subjecting a D-serinate hydrochloride of Formula A, 3,6-diaminopyrazine-2,5-dicarboxylic acid, and an amide condensation agent in a first organic solvent to amidation reaction to obtain the compound of Formula 1-Int.

In some preferred embodiments, R₁, at each occurrence, is independently C₁₋₆ alkyl, preferably C₁₋₃ alkyl, and more preferably methyl or ethyl.

In some embodiments, in the step S1, the amidation reaction is performed by adding dropwise the D-serinate hydrochloride of Formula A, the 3,6-diaminopyrazine-2,5-dicarboxylic acid, and the amide condensation agent to the first organic solvent under a low temperature condition, and then mixing, to obtain the intermediate of Formula 1-Int.

In some embodiments, in the step S1, the low temperature condition is a temperature of about -10°C to 10°C, preferably about -5°C to 5°C, more preferably about 0°C to 5°C, and particularly preferably about 0°C to 2°C

In some embodiments, in the step S1, the mixing is performed at room temperature. In some embodiments, in the step S1, the mixing is stirring at room temperature for about 8 hours or more, and preferably about 24 hours or more.

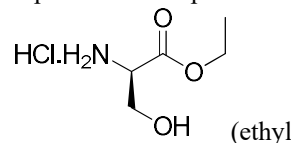
In some embodiments, in the step S1, the first organic solvent is a polar organic solvent, such as one, two or more selected from the group consisting of dimethylformamide (DMF), acetonitrile, dichloromethane, tetrahydrofuran and ethanol, and preferably DMF.

There is no particular limitation on the amide condensation agent used in the step S1, which can be a common amide condensation agent. In some preferred embodiments, in the step S1, the amide condensation agent is a combination of triethylamine, 1-hydroxybenzotriazole (HOBt), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl).

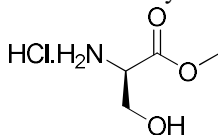
In some embodiments, in the step S1, the D-serinate hydrochloride of Formula A, the 3,6-diaminopyrazine-2,5-dicarboxylic acid, triethylamine, HOBt, and EDC.HCl are added dropwise to DMF at about 0°C to 5°C, preferably about 0°C to 2°C, and then stirred at room temperature for about 24 hours, to obtain the intermediate of Formula 1-Int.

In some embodiments, in the step S1, the D-serinate hydrochloride of Formula A is in the form of a single compound represented by the Formula A. In some other embodiments, in the step S1, the D-serinate hydrochloride of Formula A is in the form of a mixture of two or more different compounds each represented

by the Formula A. Preferably, the D-serinate hydrochloride of Formula A is



D-serinate hydrochloride) and/or



The prepared intermediate compound of Formula 1-Int may be separated through post-treatment. In some embodiments, the post-treatment includes: removing the first organic solvent by rotary evaporation, and then extracting the residue multiple times with water and a suitable solvent, such as ethyl acetate; washing the combined organic phases with saturated aqueous NaHCO₃ and aqueous KHSO₄, concentrating the organic phases, and then separating through column chromatography.

Beneficial effects

The present inventors prepared the intermediate compound of Formula 1-Int for the first time, which can be used to prepare the target compound 1 through ester hydrolysis reaction and acidification, avoiding the palladium catalyzed hydrogenation reaction used in existing technical methods. The preparation method of the invention has the advantages of lower cost, high safety, low risk of noble metal residue, and high chiral purity of the product.

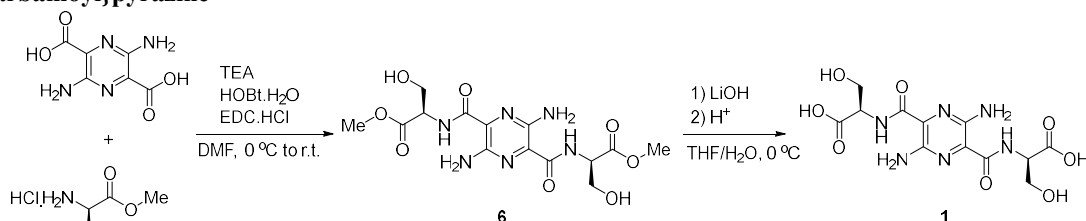
Examples

The invention is further described below in conjunction with examples, but these examples are not intended to limit the scope of the invention.

The experimental methods in the Examples of the invention that do not specify specific conditions are generally under conventional condition or under condition recommended by the raw material or commodity

manufacturer, and reagents of unspecified origin are usually commercially available conventional reagents or can be prepared from known reagents using conventional methods.

Example 1: Preparation of 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl}carbamoyl}pyrazine



(1) Amidation reaction

Methyl D-serinate hydrochloride (18 g), triethylamine (12 g), 3,6-diaminopyrazine-2,5-dicarboxylic acid (20 g), HOBT (14 g), and EDC.HCl (19 g) were each added dropwise to DMF (1000 mL) at 0°C, and then the reaction mixture was stirred at room temperature (r.t.) for 24 hours. After completion of the reaction, DMF was removed by rotary evaporation, and the residue was extracted multiple times with water and ethyl acetate. The combined organic phases were washed with saturated aqueous NaHCO₃ and aqueous KHSO₄, and the organic phases were concentrated and separated through column chromatography to give Intermediate 6 (28 g, yield: 70%).

LCMS: r.t. = 6.9 min, [M+H]⁺ = 401.1.

¹H NMR (500 MHz, DMSO-d₆) δ = 8.51 (d, J = 8.3 Hz, 2H), 6.76 (s, 4H), 5.32 (t, J = 5.5 Hz, 2H), 4.58 (dt, J = 7.9, 3.7 Hz, 2H), 3.90 (ddd, J = 11.0, 5.6, 3.9 Hz, 2H), 3.75 (ddd, J = 11.1, 5.4, 3.7 Hz, 2H), 3.69 (s, 6H).

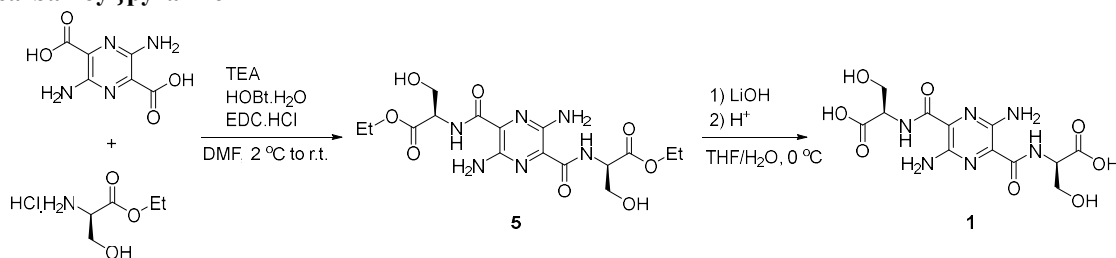
(2) Hydrolysis reaction

A solution of Intermediate 6 (20 g) in tetrahydrofuran was mixed with aqueous LiOH (5.0 g), and stirred at 0°C for 2 hours. The progress of reaction was monitored by TLC. When the reaction substrate disappeared, the reaction solution was acidified with aqueous trifluoroacetic acid (pH adjusted to about 3) and stirred for 2 hours to obtain a red turbid liquid. The red liquid was filtered, and the filter cake was washed and dried to give the target compound 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine (17.7 g, yield: 95%, dr = 98:2).

LCMS: r.t. = 2.9 min, [M+H]⁺ = 373.1.

¹H NMR (500 MHz, DMSO-d₆) δ = 8.47 (d, J = 8.2 Hz, 2H), 6.78 (s, 4H), 4.45 (dt, J = 7.8, 3.7 Hz, 2H), 3.88 (dd, J = 10.9, 3.9 Hz, 2H), 3.74 (dd, J = 11.0, 3.7 Hz, 2H).

Example 2: Preparation of 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl}carbamoyl}pyrazine



(1) Amidation reaction

Ethyl D-Serinate hydrochloride (19 g), triethylamine (12 g), 3,6-diaminopyrazine-2,5-dicarboxylic acid (20 g), HOBT (14 g), and EDC.HCl (19 g) were each added dropwise to DMF (800 mL) at 2°C, and then the reaction mixture was stirred at room temperature for 24 hours. After completion of the reaction, DMF was removed by rotary evaporation, and the residue was extracted multiple times with water and ethyl acetate. The combined organic phases were washed with saturated aqueous NaHCO₃ and aqueous KHSO₄, and the combined organic phases were concentrated and separated through column chromatography to give Intermediate 5 (32.5 g, yield: 75%).

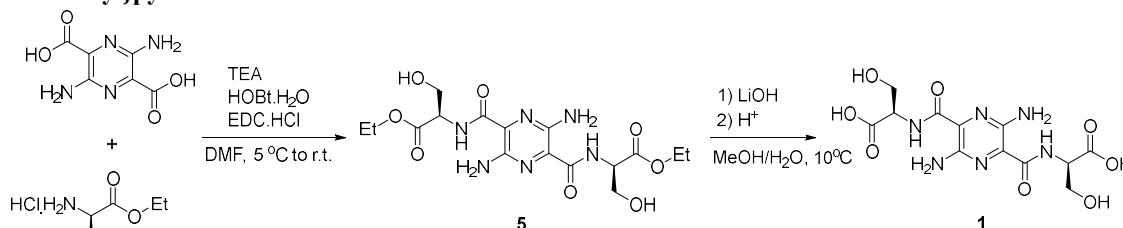
LCMS: r.t. = 6.6 min, [M+H]⁺ = 429.2.

¹H NMR (500 MHz, DMSO-d₆) δ = 8.50 (d, J = 8.2 Hz, 2H), 6.76 (s, 4H), 5.31 (t, J = 5.4 Hz, 2H), 4.55 (dt, J = 7.9, 3.8 Hz, 2H), 4.15 (q, J = 7.1 Hz, 4H), 3.89 (ddd, J = 11.1, 5.7, 4.0 Hz, 2H), 3.75 (ddd, J = 11.0, 5.3, 3.6 Hz, 2H), 1.21 (t, J = 7.1 Hz, 6H).

(2) Hydrolysis reaction

A solution of Intermediate 5 (21 g) in tetrahydrofuran was mixed with aqueous LiOH (5.0 g), and stirred at 0°C for 2 hours. The progress of reaction was monitored by TLC. When the reactants disappeared, the reaction solution was acidified with aqueous acetic acid (pH adjusted to about 3) and stirred for 2 hours to obtain a red turbid liquid. The red liquid was filtered, and the filter cake was washed and dried to give the target compound 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine (18 g, yield: 96%, dr = 98:2). The characterization data are as described in step (2) of Example 1.

Example 3: Preparation of 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine



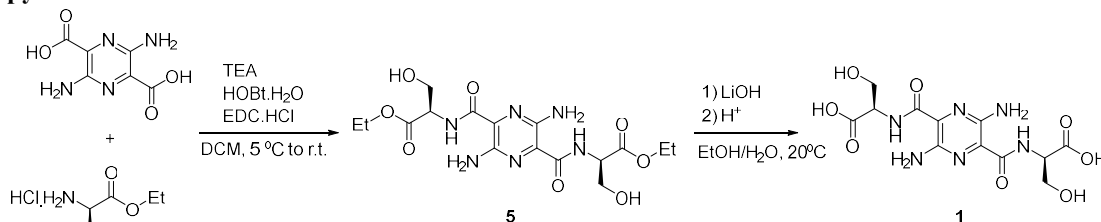
(1) Amidation reaction

Ethyl D-Serinate hydrochloride (19 g), triethylamine (12 g), 3,6-diaminopyrazine-2,5-dicarboxylic acid (20 g), HOBT (14 g), and EDC.HCl (19 g) were each added dropwise to DMF (800 mL) at 5°C, and then the reaction mixture was stirred at room temperature for 24 hours. After completion of the reaction, DMF was removed by rotary evaporation, and the residue was extracted multiple times with water and ethyl acetate. The combined organic phases were washed with saturated aqueous NaHCO₃ and aqueous KHSO₄, and the organic phases were concentrated and separated through column chromatography to give Intermediate 5 (28 g, yield: 65%). characterization data are the same as described in step (1) of Example 2.

(2) Hydrolysis reaction

A solution of Intermediate 5 (21 g) in methanol was mixed with LiOH (5 g), and stirred at 10°C for 2 hours. The progress of the reaction was monitored by TLC. When the reactants disappeared, the reaction solution was acidified with aqueous trifluoroacetic acid (pH was adjusted to about 3), and stirred for 2 hours to obtain a red turbid liquid. The red liquid was filtered, and the filter cake was washed and dried to give the target compound 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine (11 g, yield: 60%, dr = 80:20). The characterization data are as described in step (2) of Example 1.

Example 4: Preparation of 3,6-diamino-2,5-bis { N- (1R) -1-carboxy-2-hydroxyethyl} carbamoyl } pyrazine



(1) Amidation reaction

Ethyl D-Serinate hydrochloride (19 g), triethylamine (12 g), 3,6-diaminopyrazine-2,5-dicarboxylic acid (20 g), HOBT (14 g), and EDC.HCl (19 g) were each added dropwise to dichloromethane (1200 mL) at 5°C, and then the reaction mixture was stirred at room temperature for 24 hours. After completion of the reaction, dichloromethane was removed by rotary evaporation, and the residue was extracted multiple times with water and ethyl acetate. The combined organic phases were washed with saturated aqueous NaHCO₃ and aqueous KHSO₄, and the organic phases were concentrated and separated through column chromatography to give Intermediate 5 (5.2 g, yield: 12%). The characterization data are as described in step (1) of Example 2.

(2) Hydrolysis reaction

A solution of Intermediate 5 (43 g) in ethanol was mixed with LiOH (12 g), and stirred at 20°C for 2 hours. The progress of the reaction was monitored by TLC. When the reactants disappeared, the reaction solution was acidified with aqueous trifluoroacetic acid (pH adjusted to about 3), and stirred for 2 hours to obtain a red turbid liquid. The red liquid was filtered, and the filter cake was washed and dried to give the target compound 3,6-diamino-2,5-bis {N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine (14 g, yield: 38%, dr = 82:18). The characterization data are as described in step (2) of Example 1.

Example 5: Preparation of 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl} carbamoyl}pyrazine

A solution of Intermediate 6 (20 g), prepared according to step (1) of Example 1, in acetonitrile was mixed with an aqueous solution of NaOH (6.0 g), and stirred at 20°C for 2 hours. The progress of the reaction was monitored by TLC. When the reaction substrate disappeared, the reaction solution was acidified with aqueous acetic acid (pH adjusted to about 3), and stirred for 2 hours to obtain a red turbid liquid. The red liquid was filtered, and the filter cake was washed and dried to give the target compound 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl] carbamoyl}pyrazine (16.7 g, yield: 90%, dr = 92:8). The characterization data are as described in step (2) of Example 1.

Example 6: Preparation of 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl} carbamoyl}pyrazine

A solution of Intermediate 5 (20 g), prepared according to step (1) of Example 2, in 1,4-dioxane was mixed with an aqueous solution of KOH (14 g), and stirred at -5°C for 8 hours. The progress of the reaction was monitored by TLC. When the reaction substrate disappeared, the reaction solution was acidified with hydrochloric acid (pH adjusted to about 3), and stirred for 2 hours to obtain a red turbid liquid. The red liquid was filtered, and the filter cake was washed and dried to give the target compound 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine (15.3 g, yield: 88%, dr = 95:5). The characterization data are as described in step (2) of Example 1.

Example 7: Preparation of 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl} carbamoyl}pyrazine

A solution of Intermediate 5 (21 g), prepared according to step (1) of Example 3, in methanol was mixed with LiOH (5 g), and stirred at 0°C for 2 hours. The progress of the reaction was monitored by TLC. When the reactants disappeared, the reaction solution was acidified with aqueous trifluoroacetic acid (pH adjusted to about 3), and stirred for 2 hours to obtain a red turbid liquid. The red liquid was filtered, and the filter cake was washed and dried to give the target compound 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine (11 g, yield: 60%, dr = 86:14). The characterization data are as described in step (2) of Example 1.

Example 8: Preparation of 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl} carbamoyl}pyrazine

An aqueous solution of Intermediate 5 (20 g), prepared according to step (1) of Example 2, was mixed with an aqueous solution of KOH (14 g), and stirred at 10°C for 3 hours. The progress of the reaction was monitored by TLC. When the reaction substrate disappeared, the reaction solution was acidified with aqueous trifluoroacetic acid (pH adjusted to about 3), and stirred for 2 hours to obtain a red turbid liquid. The red liquid was filtered, and the filter cake was washed and dried to give the target compound 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine (16 g, yield: 92%, dr = 98:2). The characterization data are as described in step (2) of Example 1.

Example 9: Preparation of 3,6-diamino-2,5-bis{N-(1R)-1-carboxy-2-hydroxyethyl} carbamoyl}pyrazine

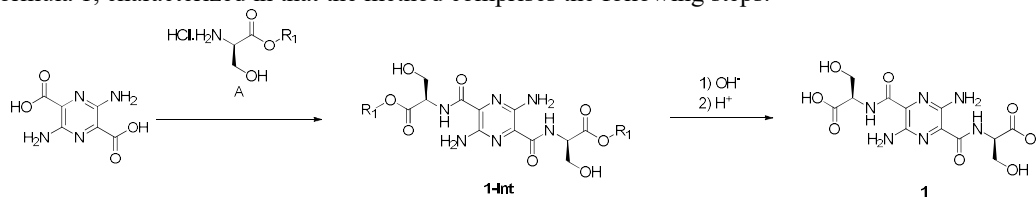
An aqueous solution of Intermediate 5 (20 g), prepared according to step (1) of Example 2, was mixed with an aqueous solution of KOH (14 g), and stirred at 20°C for 3 hours. The progress of the reaction was monitored by TLC. When the reaction substrate disappeared, the reaction solution was acidified with aqueous trifluoroacetic acid (pH adjusted to about 3), and stirred for 2 hours to obtain a red turbid liquid. The red liquid was filtered, and the filter cake was washed and dried to give the target compound 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine (16 g, yield: 92%, dr = 95:5). The characterization data are as described in step (2) of Example 1.

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

Each reference cited in this application, 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 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine of Formula 1, characterized in that the method comprises the following steps:



wherein R₁, at each occurrence, is independently selected from the group consisting of C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, and C₃₋₈ cycloalkyl, preferably C₁₋₆ alkyl, more preferably C₁₋₃ alkyl, and more preferably methyl or ethyl;

Step S1. subjecting a D-serinate hydrochloride of Formula A, 3,6-diaminopyrazine-2,5-dicarboxylic acid, and an amide condensation agent in a first organic solvent to amidation reaction to obtain an intermediate of Formula 1-Int; and

Step S2. hydrolyzing the intermediate of Formula 1-Int in a solvent under a basic condition, and then acidifying the reaction mixture to obtain the 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine of Formula 1, wherein the solvent is water, or a mixture of water and a second organic solvent.

2. The method according to claim 1, characterized in that in the step S1, the amidation reaction is performed by adding dropwise the D-serinate hydrochloride of Formula A, the 3,6-diaminopyrazine-2,5-dicarboxylic acid, and the amide condensation agent to the first organic solvent under a low temperature condition, and then mixing, to obtain the intermediate of Formula 1-Int.

3. The method according to claim 2, characterized in that in the step S1, the low temperature condition is a temperature of about -10°C to 10°C, preferably about -5°C to 5°C, more preferably about 0°C to 5°C, and particularly preferably about 0°C to 2°C.

4. The method according to claim 2 or 3, characterized in that in the step S1, the mixing is performed at room temperature.

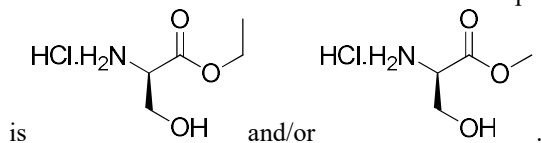
5. The method according to any one of claims 2 to 4, characterized in that in the step S1, the mixing is performed by stirring at room temperature for about 8 hours or more, and preferably about 24 hours or more.

6. The method according to any one of claims 1 to 5, characterized in that in the step S1, the first organic solvent is a polar organic solvent, such as one, two or more selected from the group consisting of dimethylformamide (DMF), acetonitrile, dichloromethane, tetrahydrofuran, and ethanol, and preferably DMF.

7. The method according to any one of claims 1 to 6, characterized in that in the step S1, the amide condensation agent is a combination of triethylamine, 1-hydroxybenzotriazole (HOBt), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl).

8. The method according to any one of claims 1 to 7, characterized in that in the step S1, the D-serinate hydrochloride of Formula A, the 3,6-diaminopyrazine-2,5-dicarboxylic acid, triethylamine, HOBt, and EDC.HCl are added dropwise to DMF at about 0°C to 5°C, preferably about 0°C to 2°C, and then stirred at room temperature, to obtain the intermediate of Formula 1-Int.

9. The method according to any one of claims 1 to 8, characterized in that in the step S1, the D-serinate hydrochloride of Formula A is in the form of a single compound represented by the Formula A or in the form of a mixture of two or more different compounds each represented by the Formula A, preferably



10. The method according to any one of claims 1 to 9, characterized in that in the step S2, the intermediate of Formula 1-Int is hydrolyzed in the solvent under a basic condition and at a suitable temperature, then the reaction mixture is acidified, to obtain 3,6-diamino-2,5-bis{N-[(1R)-1-carboxy-2-hydroxyethyl]carbamoyl}pyrazine.

11. The method according to any one of claims 1 to 10, characterized in that the second organic solvent is a polar organic solvent, in particular selected from the group consisting of tetrahydrofuran, methanol, acetonitrile, ethanol, 1,4-dioxane, and any combination thereof, preferably is tetrahydrofuran, acetonitrile, or 1,4-dioxane.

12. The method according to any one of claims 1 to 11, characterized in that in the step S2, the solvent is water.

13. The method according to any one of claims 1 to 11, characterized in that in the step S2, the solvent is a mixture of water and the second organic solvent.

14. The method according to any one of claims 1 to 13, characterized in that in the step S2, the solvent comprises an alkali metal hydroxide, such as one, two or more selected from the group consisting of LiOH, NaOH, and KOH, and preferably LiOH.

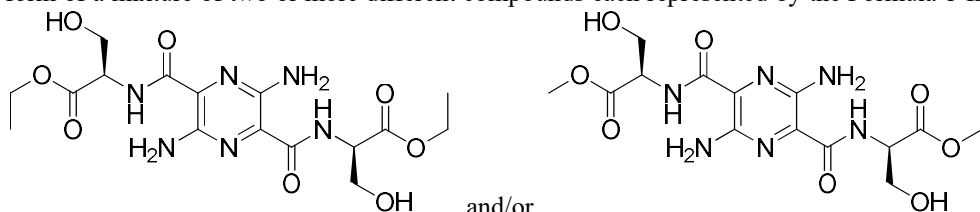
15. The method according to any one of claims 1 to 14, characterized in that the acidification in the step S2 is performed by adjusting the pH of the reaction mixture with an acid after the hydrolysis is complete.

16. The method according to claim 15, characterized in that the pH is about 2 to 4, preferably about 3.

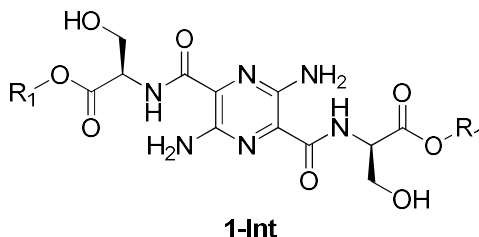
17. The method according to any one of claims 1 to 16, characterized in that in the step S2, after the acidification, the reaction mixture is further mixed for about 1 to 4 hours, preferably about 2 hours.

18. The method according to any one of claims 15 to 17, characterized in that the acid is one, two or more selected from the group consisting of acetic acid, trifluoroacetic acid, sulfuric acid, citric acid, and hydrochloric acid.

19. The method according to any one of claims 1 to 18, characterized in that in the step S2, the intermediate of Formula 1-Int is in the form of a single compound represented by the Formula 1-Int or in the form of a mixture of two or more different compounds each represented by the Formula 1-Int, preferably is

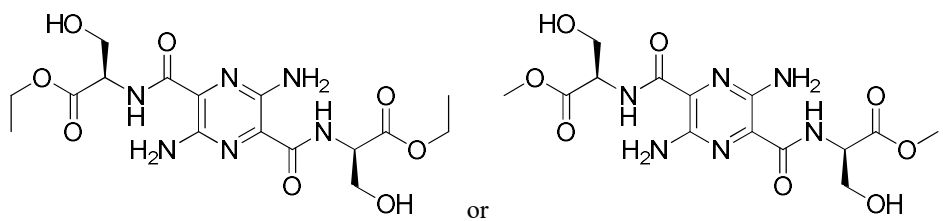


20. A compound of Formula 1-Int:

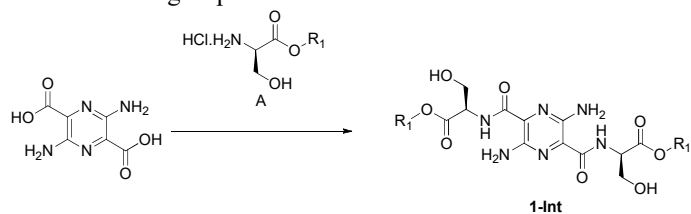


wherein each R₁ can be the same or different, and is independently selected from the group consisting of C₁₋₈ alkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, and C₃₋₈ cycloalkyl, preferably C₁₋₆ alkyl, more preferably C₁₋₃ alkyl, and more preferably methyl or ethyl.

21. The compound according to claim 20, which is:



22. A method for preparing the compound of Formula 1-Int according to claim 20 or 21, characterized in that the method comprises the following step:



Step S1. subjecting a D-serinate hydrochloride of Formula A, 3,6-diaminopyrazine-2,5-dicarboxylic acid, and an amide condensation agent in a first organic solvent to amidation reaction to obtain the compound of Formula 1-Int;

preferably, wherein the step S1 is as defined in any one of claims 2 to 9.