SPECIFICATION

PREPARATION AND PURIFICATION PROCESS OF MONOMETHYL AURISTAIN E COMPOUND

FIELD

[1] The invention relates to the technical field of compound synthesis, in particular to a preparation and purification process of a monomethyl auristatin E compound (ie. MMAE).

BACKGROUND

[2] MMAE (Monomethyl Auristantin E, also known as methyl auristatin E), a fully synthetic derivative of auristatin, can effectively inhibit mitosis by inhibiting tubulin polymerization, and has been widely used as a cytotoxic component (ie, the drug moiety) to synthesize antibody-drug conjugates to treat cancer.

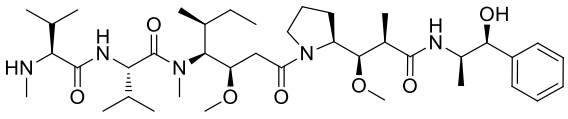
[3] Antibody drug conjugate (ADC) is a class of antitumor drugs, and includes three components: Antibody, Linker and Drug. Its principle is that the selective targeting ability of the antibody is combined with the cytotoxic efficacy of the drug moiety, then an antigen on the surface of a tumor cell is specifically recognized by means of the targeting specificity of the antibody, entry of the cell is achieved through endocytosis of the cell, the drug moiety is released by protease in the cell, and thus, the purposes of killing the tumor cell while avoiding killing of non-target tissues are achieved.

[3a] Currently, a large number of natural and chemically synthesized cytotoxins are known to exist, but only a very small part of the drug structure can be applied to ADCs. This is mainly because toxins that can be used as ADC loads must have complex properties such as high cytotoxic potency and small molecular weight. Therefore, auristatin compounds (such as MMAE) are highly sought after in the ADC field. Currently, the linker-toxin structure on antibodies of many ADC drugs on the market is Mc-Val-Cit-PAB-MMAE. However, the current market price of MMAE is very high. The main reason is that the current synthesis and purification process is still immature, the synthesis process of many drugs is complex, and the purification process is

immature, which result in low yield, low purity and high content of impurities (especially the single impurity) of the final product. While for the safety of clinical medication, the drug used for clinical use must have extremely high purity and extremely low impurities, but most of the current processes cannot meet the standard requirements of clinical medication, which is also one of the main reasons for the high price of the commercially available MMAE.

SUMMARY

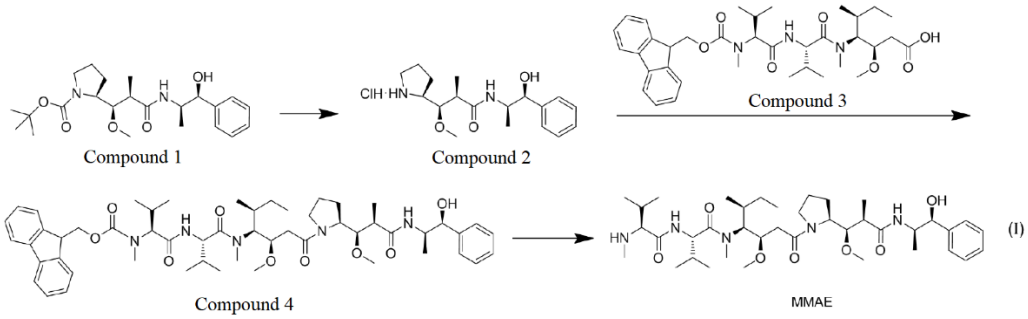
[3b] In an aspect of the present disclosure, there is provided a preparation and purification method of a compound shown in formula (I):



(I)

MMAE

the preparation route of the method being as follows:



Wherein the method comprises the following steps:

(1) dissolving a compound 1 in an appropriate amount of a first organic solvent to form a solution A;

(2) adding a sufficient amount of HCl-1,4-dioxane solution to the solution A for insulation reaction, removing a Boc protecting group;

(3) after the reaction finishes, pouring the reaction solution of step (2) into a sufficient amount of first low-polarity solvent, discarding the filtrate after stirring, and solid residues being compound 2 after drying;

(4) dissolving the obtained compound 2 and an appropriate amount of compound 3 in a second organic solvent to form a solution B;

(5) dissolving a first polypeptide condensing agent in an appropriate amount of third organic solvent to form a solution C, wherein the mole number of the first polypeptide condensing agent is larger than the mole number of the compound 3 of step （4）;

(6) adding the solution C to the solution B to form a solution D;

(7) adding an appropriate amount of first organic base to the solution D for insulation reaction;

(8) after the reaction of step (7) finishes, adding a sufficient amount of second low polarity solvent and purified water to the reaction system of step (7) for extraction, and collecting an organic phase;

(9) washing the organic phase collected in step (8) with an appropriate amount of hydrochloric acid solution, purified water and sodium chloride solution successively, drying by anhydrous sodium sulfate, concentrating under reduced pressure, and drying to obtain a compound 4;

(10) dissolving the compound 4 in a fourth organic solvent to form a solution E;

(11) adding a sufficient amount of diethylamine to the solution E for insulation reaction, removing a Fmoc protecting group;

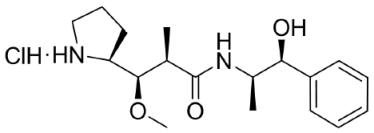
(12) after the reaction finishes, adding an appropriate amount of fifth organic solvent and purified water to the reaction system of step (11) for extraction, collecting an organic phase, drying by anhydrous sodium sulfate, and concentrating under reduced pressure;

(13) carrying out chromatographic purification on the concentrate under reduced pressure obtained in step (12) by an elution system of toluene: methanol, and concentrating the collected eluent under reduced pressure;

(14) after dissolving the concentrated product under reduced pressure obtained in step (13) with a sixth organic solvent, filtering, and concentrating the filtrate under reduced pressure; and

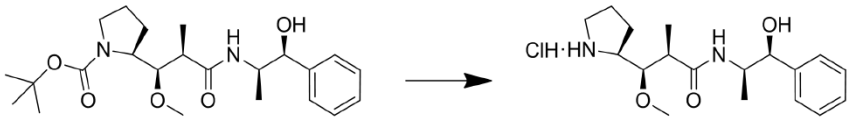
(15) vacuum-drying the concentrated product under reduced pressure obtained in step (14) to obtain the MMAE.

[3c] In another aspect of the present disclosure, there is provided a preparation and purification method of a compound shown in the following formula:



Compound 2

the preparation route of the method being as follows:



Compound 1 Compound 2

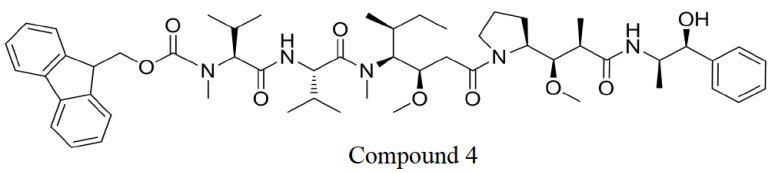
wherein the method comprises the following steps:

(1-1). dissolving a compound 1 in an appropriate amount of seventh organic solvent to form a solution F;

(1-2). adding a sufficient amount of HCl-1,4-dioxane solution to the solution F for insulation reaction, removing a Boc protecting group; and

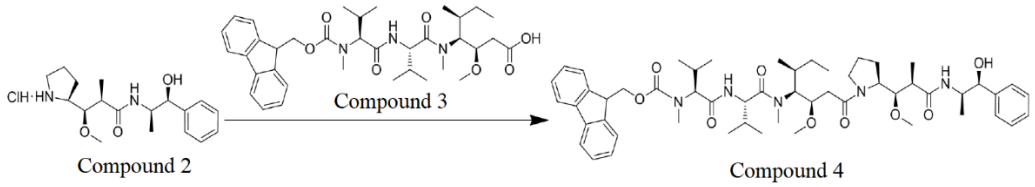
(1-3). after the reaction finishes, pouring the reaction solution of step (1-2) into a sufficient amount of third low-polarity solvent, discarding a filtrate after stirring, and solid residues being a compound 2 after drying.

[3d] In a further aspect of the present disclosure, there is provided a preparation and purification method of a compound shown in the following formula:



Compound 4

the preparation route of the method being as follows:



wherein the method comprises the following steps:

(2-1). dissolving a compound 2 and an appropriate amount of compound 3 in an eighth organic solvent to form a solution G;

(2-2). dissolving a second polypeptide condensing agent in an appropriate amount of ninth organic solvent to form a solution H, wherein the mole number of the second polypeptide condensing agent is larger than the mole number of the compound 3 in step（2-1）;

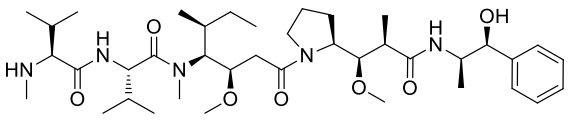
(2-3). adding the solution H to the solution G to form a solution I;

(2-4). adding an appropriate amount of second organic base to the solution I for insulation reaction;

(2-5). after the reaction finishes, adding a sufficient amount of fourth low polarity solvent and purified water to the reaction system of step (2-4) for extraction, and collecting an organic phase; and

(2-6). washing the organic phase collected in step (2-5) with an appropriate amount of hydrochloric acid solution, purified water and sodium chloride solution successively, drying by anhydrous sodium sulfate, concentrating under reduced pressure, and drying to obtain a compound 4.

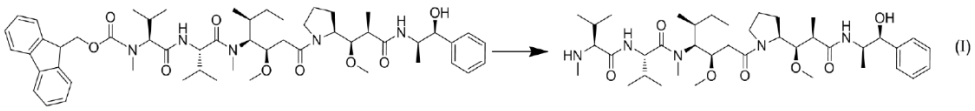
[3e] In another aspect of the present disclosure, there is provided a preparation and purification method of a compound shown in formula (I):



(I)

MMAE

the preparation route of the method being as follows:



Compound 4 MMAE (I)

wherein the method comprises the following steps:

(3-1). dissolving a compound 4 in a tenth organic solvent to form a solution J;

(3-2). adding a sufficient amount of diethylamine to the solution J for insulation reaction, removing a Fmoc protecting group;

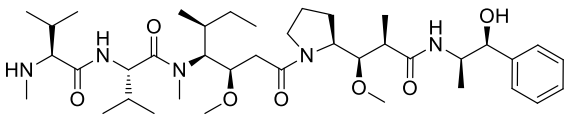
(3-3). after the reaction finishes, adding an appropriate amount of eleventh organic solvent and purified water to the reaction system of step (3-2) for extraction, collecting an organic phase, drying by anhydrous sodium sulfate, and concentrating under reduced pressure;

(3-4). carrying out chromatographic purification on the concentrate under reduced pressure obtained in step (3-3) by an elution system of toluene: methanol, and concentrating the collected eluent under reduced pressure;

(3-5). after dissolving the concentrated product under reduced pressure obtained in step (3-4) with a twelfth organic solvent, filtering, and concentrating the filtrate under reduced pressure; and

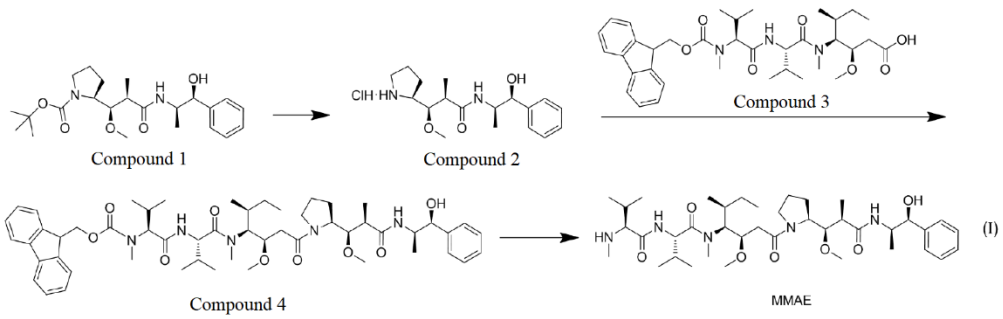
(3-6). vacuum-drying the concentrated product under reduced pressure obtained in step (3-5) to obtain the MMAE.

[4] The disclosure provides a preparation and purification process capable of obtaining extremely high-purity MMAE (structural formula as shown in formula I), which can well meet the quality requirements of clinical drugs.



MMAE (I)

[5] The preparation route of the method is as follows:



[6] The method includes the following steps:

(1) dissolving a compound 1 in an appropriate amount of a first organic solvent to form a solution A;

(2) adding a sufficient amount of HCl-1,4-dioxane solution to the solution A for insulation reaction, and removing a Boc protecting group;

(3) after the reaction finishes, pouring the reaction solution of step (2) into a sufficient amount of first low-polarity solvent, discarding the filtrate after stirring, and solid residues being compound 2 after drying;

20:1; preferably, the elution system is firstly toluene: methanol in a volume ratio (V/V) of about 20:1. When TLC detects that only the product is visible (for example, only product spots), the elution system is changed to toluene: methanol in a volume ratio (V/V) of about 10:1. Of course, the elution system may not be replaced, and the purpose of replacing the elution system here is to make the product eluted more quickly and to save time and cost in the production process.

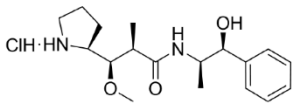
[38] Further, the developing agent of the TLC detection is toluene: methanol in a volume ratio (V/V) of about 5:1.

[39] Further, the sixth organic solvent in step（14） is selected from methanol, toluene and acetonitrile; and preferably, the sixth organic solvent in step（14） is methanol.

[40] Further, the weight-to-volume ratio (g/mL) of the compound 4 in step（10） to the sixth organic solvent in step（14） is about 1:3-10.

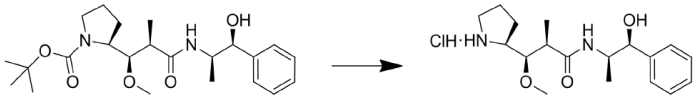
[41] Further, the process in step（14） may be repeated 1-5 times.

[42] The disclosure also provides a preparation and purification method of a compound shown in the following formula:



Compound 2

[43] The preparation route of the method is as follows:



Compound 1 Compound 2

[44] The method includes the following steps:

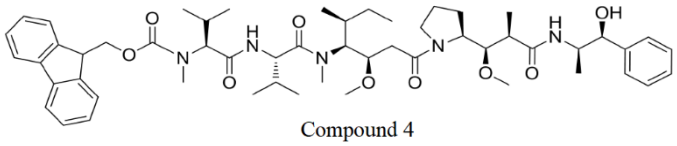
(1-1). dissolving a compound 1 in an appropriate amount of seventh organic solvent to form a solution F;

[50] Further, the temperature of the insulation reaction in step (1-2) is 10-15°C.

[51] Further, the third low-polarity solvent in step (1-3) is selected from n-hexane, petroleum ether, and n-heptane; and preferably, the third low-polarity solvent in step (1-3) is selected from n-hexane.

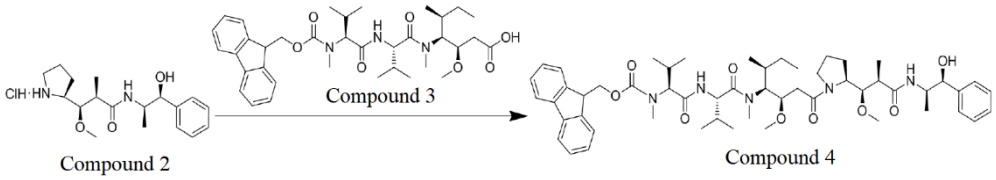
[52] Further, the compound 1 in step (1-1) to the third low-polarity solvent in step (1-3) is about 1:16; preferably, the weight-to-volume ratio (g/mL) of the compound 1 in step (1-1) to the third low-polarity solvent in step (1-3) is 1:10-25; more preferably, the weight-to-volume ratio (g/mL) of the compound 1 in step (1-1) to the third low-polarity solvent in step (1-3) is 1:12-20; and more preferably, the weight-to-volume ratio (g/mL) of the compound 1 in step (1-1) to the third low-polarity solvent in step (1-3) is 1:15-17.

[53] The disclosure also provides a preparation and purification method of a compound shown in the following formula:



Compound 4

[54] the preparation route of the method is as follows:



[55] the method includes the following steps:

(2-1). dissolving a compound 2 and an appropriate amount of compound 3 in an eighth organic solvent to form a solution G;

(2-2). dissolving a second polypeptide condensing agent in an appropriate amount of ninth organic solvent to form a solution H, where the mole number of the second polypeptide condensing agent is larger than the mole number of the compound 3 in step（2-1）;

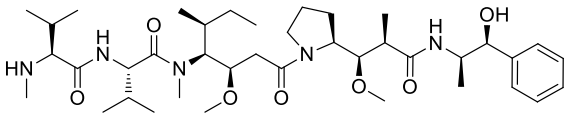
(2-3). adding the solution H to the solution G to form a solution I;

and purified water in step (2-5) is 1:15-25:15-25; more preferably, the weight-to-volume ratio (g/mL) of the compound 3 in step (2-1) to the fourth low-polarity solvent and purified water in step (2-5) is 1:20-24:20-24; and more preferably, the weight-to-volume ratio (g/mL) of the compound 3 in step (2-1) to the fourth low-polarity solvent and purified water in step (2-5) is 1:20-21:20-21.

[68] Further, the volumes of the fourth low-polarity solvent and the purified water in step (2-5) are the same.

[69] Further, the concentration of the hydrochloric acid solution in step (2-6) is about 0.05 mol/L; preferably, the concentration of the hydrochloric acid solution in step (2-6) is 0.02-0.08 mol/L; more preferably, the concentration of the hydrochloric acid solution in step (2-6) is 0.04-0.06 mol/L; and more preferably, the concentration of the hydrochloric acid solution in step (2-6) is 0.05 mol/L.

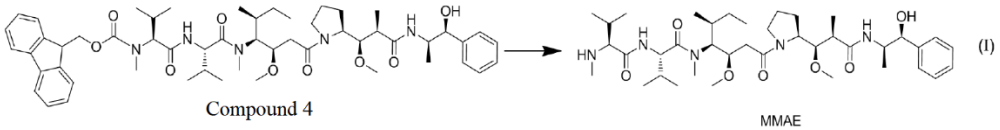
[70] The disclosure also provides a preparation and purification method of a compound shown in formula (I):



(I)

MMAE

[71] The preparation route of the method is as follows:



Compound 4

MMAE

(I)

[72] The method includes the following steps:

(3-1). dissolving a compound 4 in a tenth organic solvent to form a solution J;

(3-2). adding a sufficient amount of diethylamine to the solution J for insulation reaction, removing a Fmoc protecting group;

(3-3). after the reaction finishes, adding an appropriate amount of eleventh organic solvent and purified water to the reaction system of step (3-2) for extraction, collecting an organic phase, drying by anhydrous sodium sulfate, and concentrating under reduced pressure;

volume ratio (g/mL) of the compound 4 in step (3-1) to the eleventh organic solvent and purified water in step (3-3) is 1:5-10:5-15; and more preferably, the weight-to-volume ratio (g/mL) of the compound 4 in step (3-1) to the eleventh organic solvent and purified water in step (3-3) is 1:6-8:9-12.

[79] Further, the silica gel used in the chromatographic purification in step (3-4) is 200-300 mesh silica gel; the elution system is toluene: methanol in a volume ratio (V/V) of 10-20:1; preferably, the elution system is firstly toluene: methanol in a volume ratio (V/V) of about 20:1; and when TLC detects that only the product is visible, the elution system is changed to toluene: methanol in a volume ratio (V/V) of about 10:1.

[80] Further, the developing agent of the TLC detection is toluene: methanol in a volume ratio (V/V) of about 5:1.

[81] Further, the twelfth organic solvent in step (3-5) is selected from methanol, toluene and acetonitrile; and preferably, the twelfth organic solvent in step (3-5) is methanol.

[82] Further, the weight-to-volume ratio (g/mL) of the compound 4 in step (3-1) to the twelfth organic solvent in step (3-5) is about 1:3-10.

[83] Further, the process in step (3-5) may be repeated 1-5 times.

[84] The preparation and purification process of MMAE provided by the present disclosure has mild synthesis and purification conditions, can effectively prevent the change of product chirality caused by excessively high temperature, greatly reduces the generation of degradation impurities, improves the purity of the product, and increases the yield of the product. In addition, the preparation and purification process provided by the present disclosure has good stability and is more suitable for scale-up production. The MMAE prepared by the preparation and purification process provided by the present disclosure has purity of higher than 99%, and can perfectly meet clinical drug requirements.

BRIEF DESCRIPTION OF FIGURES