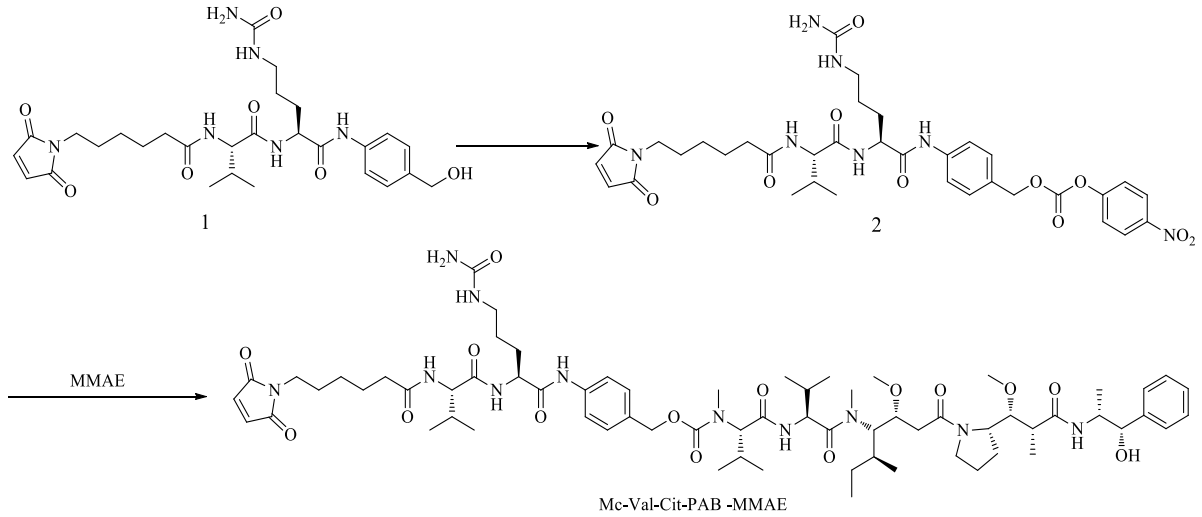
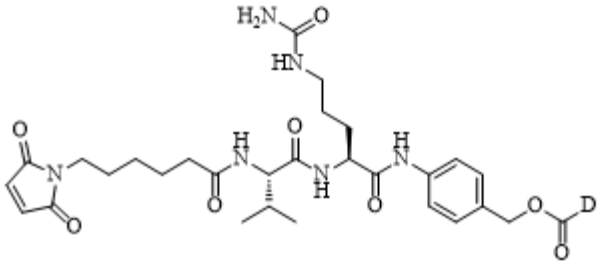


The method is mainly divided into two steps. The first step is to prepare Mc-VC-PAB-PNP with the compound Mc-VC-PABA, and the second step is to prepare Mc-Val-Cit-PAB-MMAE with Mc-VC-PAB-PNP and MMAE. The product Mc-VC-PAB-PNP in the first step is crystallized with petroleum ether and ethyl acetate, and the product is used in the next step without purification, which undoubtedly brings more impurities to the subsequent reaction; and the product purification process in the second step only involves the preparation and purification by HPLC, so the prepared compound often has high impurity content. The Chinese patents with Publication No. CN106999605A and CN108743968A also disclose a preparation method for Mc-Val-Cit-PAB -MMAE on Page 47 of the Description and Page 4 of the Description, but the subsequent product purification methods are the preparation and purification by HPLC, which cannot effectively remove impurities from the product.

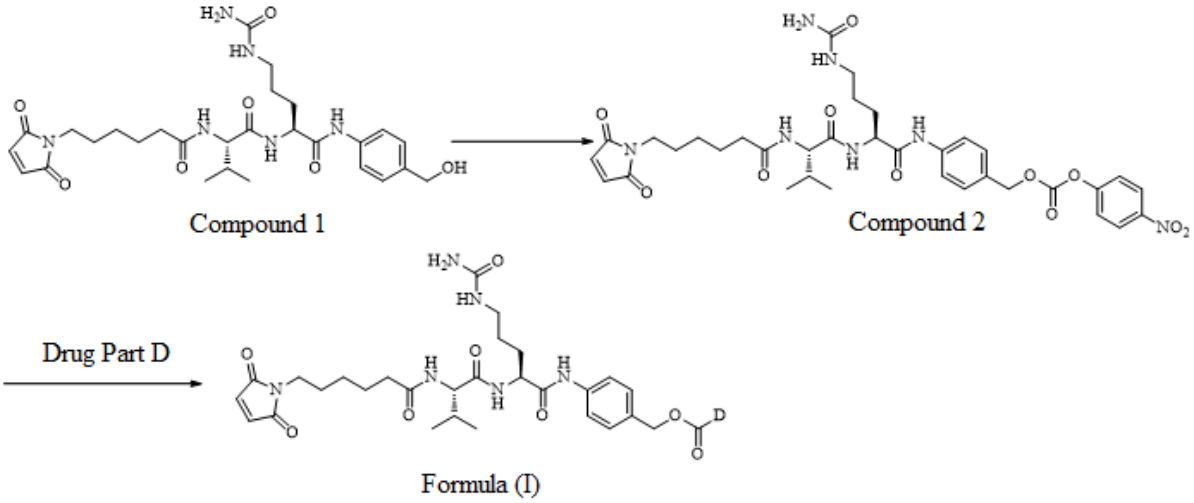
SUMMARY

In an aspect of the present disclosure, there is provided a preparation and purification method for an antibody drug conjugate intermediate, the antibody drug conjugate intermediate being a compound, an enantiomer, a racemate or a pharmaceutically acceptable salt thereof as shown in Formula (I), wherein D represents the linked toxin part:



Formula (I)

the synthetic route of the method is as follows:



the toxin part D is an auristatin cytotoxic agent, an Anthramycin cytotoxic agent, an anthracycline cytotoxic agent or a puromycin cytotoxic agent, wherein the auristatin cytotoxic agent comprises MMAE, MMAF, MMAD or derivatives thereof; the Anthramycin cytotoxic agent comprises anthramycin or derivatives thereof; the anthracycline cytotoxic agent comprises daunorubicin, adriamycin, epirubicin, idarubicin, valrubicin, mitoxantrone or derivatives thereof; the puromycin cytotoxic agent comprises puromycin or derivatives thereof;

the method specifically comprises the following steps:

A. dissolving Compound 1 in an appropriate amount of Solvent 1, and successively adding bis(4-nitrobenzene) carbonate and organic base, wherein the number of moles of the added bis(4-nitrobenzene) carbonate and the number of moles of the added organic base are greater than that of Compound 1;

B. obtaining the filtrate by suction filtration after appropriate reaction time;

C. successively adding a sufficient amount of ethyl acetate and n-hexane to the filtrate

obtained in Step B, stirring for an appropriate time after n-hexane has been dropwise added, and obtaining the filter cake by suction filtration;

D. successively washing the filter cake obtained in Step C with an appropriate amount of ethyl acetate and n-hexane, and obtaining the filter cake by suction filtration;

E. dissolving the filter cake obtained in Step D in a mixed solution of acetic acid and methanol, adding an appropriate amount of purified water, stirring for an appropriate time after the purified water is added, and obtaining the filter cake by suction filtration;

F. successively washing the filter cake obtained in Step E with an appropriate amount of purified water, methanol, ethyl acetate and n-hexane, and obtaining Compound 2 (MC-Val-Cit-PAB-PNP) after suction filtration and drying;

G. dissolving Compound 2 and a triazole-based compound in an appropriate amount of Solvent 2 to form Solution X, dissolving the conjugated toxin part D in Solvent 3 to form Solution Y, adding Solution Y into Solution X, and mixing evenly to form Solution Z;

H. adding an appropriate amount of organic base to Solution Z to adjust the pH of the system and catalyze the reaction;

I. obtaining the filtrate by suction filtration after appropriate reaction time;

J. successively adding an appropriate amount of ethyl acetate and n-hexane to the filtrate in Step I, stirring for an appropriate time, and obtaining the filter cake by suction filtration;

K. successively washing the filter cake obtained in Step J with ethyl acetate and n-hexane, and obtaining the filter cake by suction filtration;

L. dissolving the filter cake obtained in Step K in an appropriate amount of methanol solution, preparing and purifying by preparative liquid chromatography, and collecting a preparation solution;

M. concentrating the preparation solution obtained in Step L under reduced pressure;

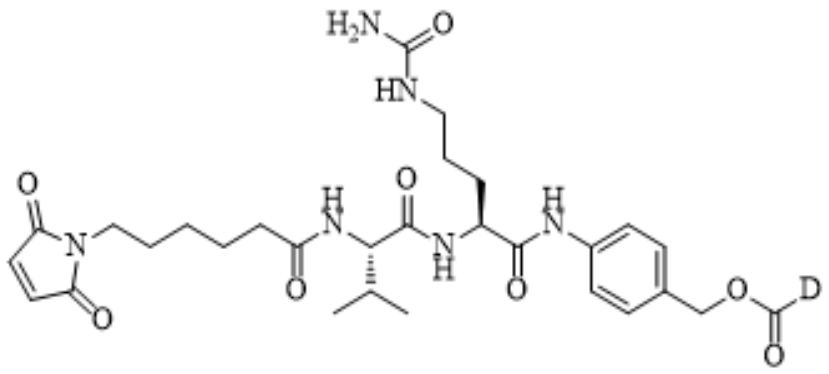
N. dissolving the concentrate obtained under reduced pressure in Step M with an appropriate amount of methanol, and then concentrating under reduced pressure again;

O. vacuum drying the concentrate obtained under reduced pressure in Step N to obtain the purified compound as shown in Formula (I);

wherein:

Solvent 1 in Step A, Solvent 2 and Solvent 3 in Step G are polar solvents; preferably, Solvent 1, Solvent 2 and Solvent 3 are each independently selected from one or more of DMF, DMA and NMP; and more preferably, Solvent 1, Solvent 2 and Solvent 3 are DMF.

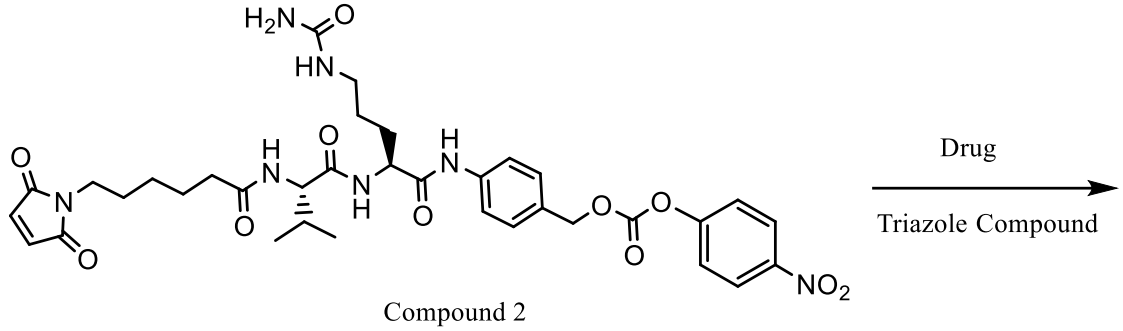
In order to solve the above-identified problems, the disclosure provides new methods of generating purified linker-toxin conjugates that can be used as intermediates to synthesize ADCs. The disclosure also provides purified Mc-Val-Cit-PAB-D of the compound of Formula (I), or a salt thereof, where D represents the linked toxin part.



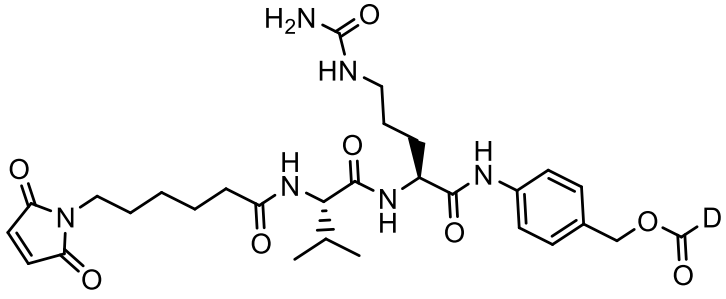
Formula (I)

It will be understood that the methods disclosed herein can also be used to make racemic versions, diastereomers or enantiomers of the compound of Formula (I), or salts thereof.

It has been surprisingly discovered that highly pure compound of Formula (I), or a salt thereof, can be generated reacting Compound 2 and a toxin (D) (e.g., MMAE) in the presence of a triazole-based compound, as depicted below:



Compound 2

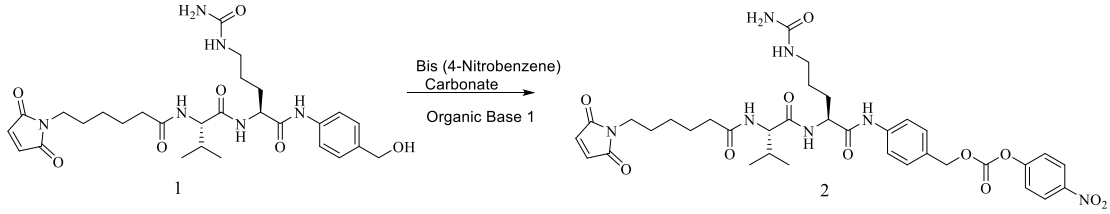


Formula (I)

In some examples, the triazole-based compound is 1-hydroxybenzotriazole, 1-hydroxy-7-azobenzotriazole, ethyl 1-hydroxyl-1H-1,2,3-triazole-4-carboxylate, or combinations thereof. In particular examples, the triazole-based compound is 1-hydroxybenzotriazole.

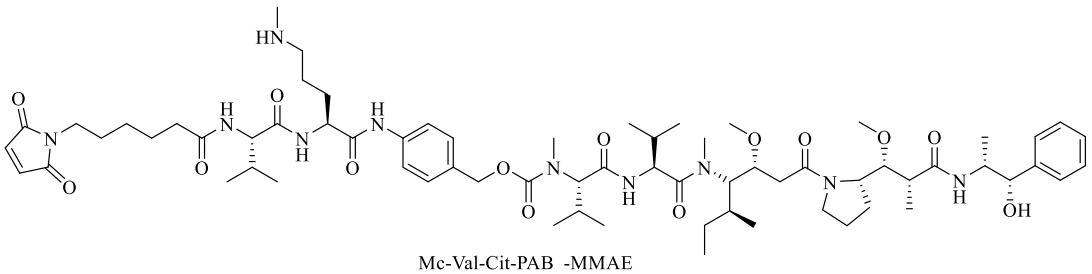
In some examples, the reaction depicted above is run in the presence of one or more bases. For instance, the reactions can be run in the presence of one or more organic bases. In particular examples, the reaction is one in the presence of two organic bases of different alkalinity. It has been found that using two different organic bases with differing alkalinity further reduces the amount of impurities generated in the process. In one example, at least one of the organic bases is N,N-Diisopropylethylamine. In another example, at least one of the organic bases is pyridine. In another example, the two organic bases are N,N-Diisopropylethylamine and pyridine.

The disclosure further provides methods of synthesizing Compound 2. In one example, Compound 2 is prepared by reacting Compound 1 with bis-(4-nitrobenzene) in the presence of an organic base, referred to herein as Organic Base 1. The reaction is depicted below:



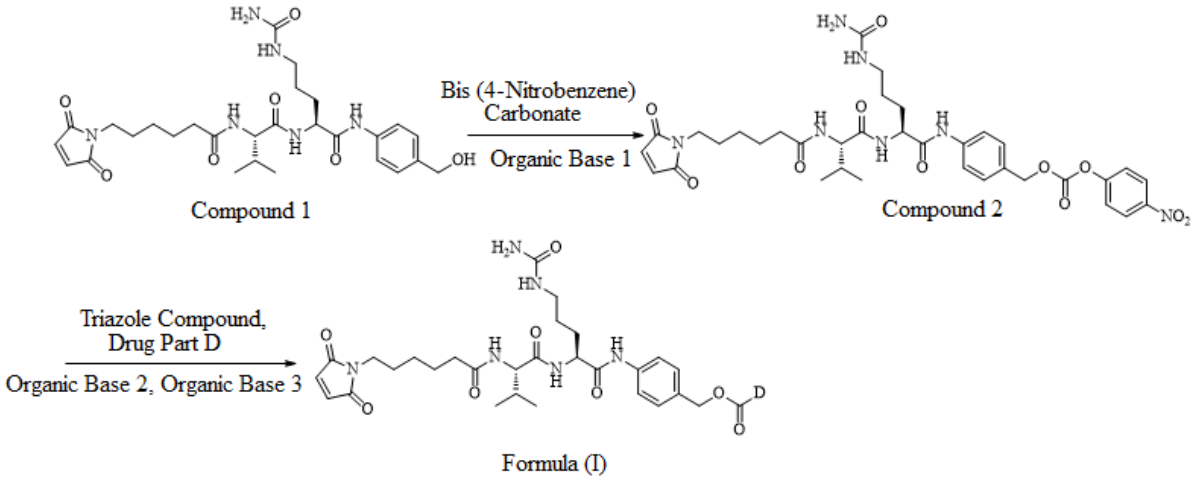
In some examples, the toxin part D is an auristatin cytotoxic agent, an Anthramycin cytotoxic agent, an anthracycline cytotoxic agent or a puromycin cytotoxic agent, wherein the auristatin cytotoxic agent includes MMAE, MMAF, MMAD or derivatives thereof; the Anthramycin cytotoxic agent includes anthramycin or derivatives thereof; the anthracycline cytotoxic agent includes daunorubicin, adriamycin, epirubicin, idarubicin, valrubicin, mitoxantrone or derivatives thereof; the puromycin cytotoxic agent includes puromycin or derivatives thereof;

In particular examples, the toxin (D) is MMAE. In such examples, the purified linker-toxin conjugates produced in accordance with the disclosure is Mc-Val-Cit-PAB-MMAE, which has the following chemical structure:



Mc-Val-Cit-PAB -MMAE

In some examples, Mc-Val-Cit-PAB-D is produced using the following synthetic route:



In particular examples, D is MMAE.

In some examples, the Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of greater than 95%. In some examples, the Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of greater than 96%. In some examples, the Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of greater than 97%. In some examples, the Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of greater than 98%. In some examples, the Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of greater than 99%. In some examples, the Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of greater than 99.5%. In some examples, the Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of greater than 99.8%. %. In other examples, the

Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of from about 95% to about 99.5%. In other examples, the Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of from about 97% to about 99.5%. In other examples, the Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of from about 98% to about 99.8%. In other examples, the Mc-Val-Cit-PAB-D (e.g., Mc-Val-Cit-PAB-MMAE) produced by methods described herein can have a purity of from about 95% to about 98%.

The disclosure also provides highly pure ADCs produced using purified linker-toxin conjugates as prepared herein. In some examples, the ADCs produced in accordance with the disclosure can have a purity of greater than 95%. In other examples, the ADCs produced in accordance with the disclosure can have a purity of greater than 96%. In other examples, the ADCs produced in accordance with the disclosure can have a purity of greater than 97%. In other examples, the ADCs produced in accordance with the disclosure can have a purity of greater than 98%. In other examples, the ADCs produced in accordance with the disclosure can have a purity of greater than 99%. In other examples, the ADCs produced in accordance with the disclosure can have a purity of greater than 99.5%. In other examples, the ADCs produced in accordance with the disclosure can have a purity of greater than 99.8%. In other examples, the ADCs produced in accordance with the disclosure can have a purity of from about 95% to about 99.5%.

In one example, the disclosed method includes the following steps:

A. dissolving Compound 1 in an appropriate amount of Solvent 1, and successively adding bis(4-nitrobenzene) carbonate and organic base, wherein the number of moles of the added bis(4-nitrobenzene) carbonate and the number of moles of the added organic base are greater than that of Compound 1;

B. obtaining the filtrate by suction filtration after appropriate reaction time;

C. successively adding a sufficient amount of ethyl acetate and n-hexane to the filtrate obtained

purified compound as shown in Formula (I);

wherein:

In some examples, solvent 1 in Step A, Solvent 2 and Solvent 3 in Step G are polar solvents; preferably, Solvent 1, Solvent 2 and Solvent 3 are each independently selected from one or more of DMF, DMA and NMP; and more preferably, Solvent 1, Solvent 2 and Solvent 3 are DMF;

The organic base in Step A and the organic base in Step H are selected from one or more of N,N-Diisopropylethylamine, triethylamine and pyridine; preferably, the organic bases are each independently one or two of N,N-Diisopropylethylamine and pyridine. Preferably, the organic base in Step A is N,N-Diisopropylethylamine, and there are two types of organic bases, N,N-Diisopropylethylamine and pyridine, in Step H.

Further, in some examples, the molar ratio of Compound 1 in Step A to bis(4-nitrobenzene) carbonate is about 1:1.8, and the molar ratio of Compound 1 to organic base 1 is about 1:1.2.

Further, in some examples, the molar ratio of Compound 1 in Step A to bis(4-nitrobenzene) carbonate is 1:1.5-2, and the molar ratio of Compound 1 to organic base is 1:1-1.5. Preferably, the molar ratio of Compound 1 in Step A to bis(4-nitrobenzene) carbonate is 1:1.6-1.9 or 1:1.7-1.8; and the molar ratio of Compound 1 to organic base is 1:1.1-1.4 or 1:1.2-1.3. In some specific examples, the molar ratio of Compound 1 in Step A to bis(4-nitrobenzene) carbonate is 1:1.8, and the molar ratio of Compound 1 to organic base is 1:1.2.

Further, in some examples, the weight volume ratio (g/ml) of Compound 1 to ethyl acetate in Step C is about 1:30.0, and the weight volume ratio (g/ml) of Compound 1 to n-hexane in Step C is about 1:60.0.

Further, in some examples, the weight volume ratio (g/ml) of Compound 1 to ethyl acetate in Step C is 1:25-35, 1:27-33, 1:28-32, or 1:29-31; and the weight volume ratio (g/ml) of Compound 1 to n-hexane in Step C is 1:55-65, 1:57-63, 1:58-62, or 1:59-61. In some specific examples, the weight volume ratio (g/ml) of Compound 1 to ethyl acetate in Step C is 1:30, and the weight volume ratio (g/ml) of Compound 1 to n-hexane in Step C is 1:60.

Further, in some examples, the weight volume ratio (g/ml) of Compound 1 to acetic acid in Step E is about 1:7.0, the weight volume ratio (g/ml) of Compound 1 to methanol in Step E is about 1:1.0, and the weight volume ratio (g/ml) of Compound 1 to purified water in Step E is about 1:20.0.

Further, in some examples, the weight volume ratio (g/ml) of Compound 1 to acetic acid in Step E is about 1:6-8, the weight volume ratio (g/ml) of Compound 1 to methanol in Step E is 1:0.5-1.5, and the weight volume ratio (g/ml) of Compound 1 to purified water in Step E is 1:15-25. Preferably, the weight volume ratio (g/ml) of Compound 1 to acetic acid in Step E is 1:6.5-7.5 or 1:6.8-7.3; the weight volume ratio (g/ml) of Compound 1 to methanol in Step E is 1:0.7-1.3 or 1:0.9-1.1; and the weight volume ratio (g/ml) of Compound 1 to purified water in Step E is 1:17-23 or 1:19-21. In some specific examples, the weight volume ratio (g/ml) of Compound 1 to acetic acid in Step E is 1:7.0, the weight volume ratio (g/ml) of Compound 1 to methanol in Step E is 1:1.0; and the weight volume ratio (g/ml) of Compound 1 to purified water in Step E is 1:20.0.

Further, in some examples, the molar ratio of Compound 2 to triazole-based compound in Step G is about 1:1, and the molar ratio of Compound 2 to toxin part D is about 1:1.

Further, in some examples, the molar ratio of Compound 2 to triazole-based compound in Step G is 1:0.8-1.2, and the molar ratio of Compound 2 to toxin part D is 1:0.8-1.2. In some examples, the molar ratio of Compound 2 to triazole-based compound in Step G is 1:0.9-1.1, and the molar ratio of Compound 2 to toxin part D is 1:0.9-1.1. Preferably, the molar ratio of Compound 2 to triazole-based compound in Step G is 1:0.85-1.05, and the molar ratio of Compound 2 to toxin part D is 1:0.95-1.05. In some specific examples, the molar ratio of Compound 2 to triazole-based compound in Step G is 1:1, and the molar ratio of Compound 2 to toxin part D is 1:1.

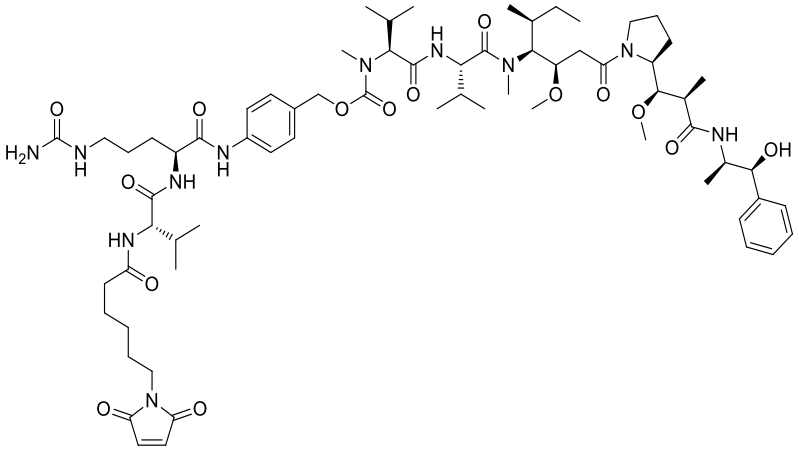
Further, as set forth above, in some examples there are two types of organic bases, N,N-Diisopropylethylamine and pyridine, in Step H. In some examples, the molar ratio of Compound 2 in Step G to the organic base N,N-Diisopropylethylamine added in Step H is about

1:1, and the molar ratio of Compound 2 in Step G to the organic base pyridine added in Step H is about 1:20.5. In other examples, the molar ratio of Compound 2 in Step G to the organic base N,N-Diisopropylethylamine added in Step H is 1:0.8-1.2, and more preferably, the molar ratio is 1:0.9-1.1 or 1:0.95-1.05; the molar ratio of Compound 2 in Step G to the organic base pyridine added in Step H is 1:19-25, and more preferably, the molar ratio is 1:19.5-23, 1:19.5-21.5 or 1:20-21. In some specific examples, the molar ratio of Compound 2 in Step G to organic base 2 is 1:1, and the molar ratio of Compound 2 to organic base 3 is 1:20.5.

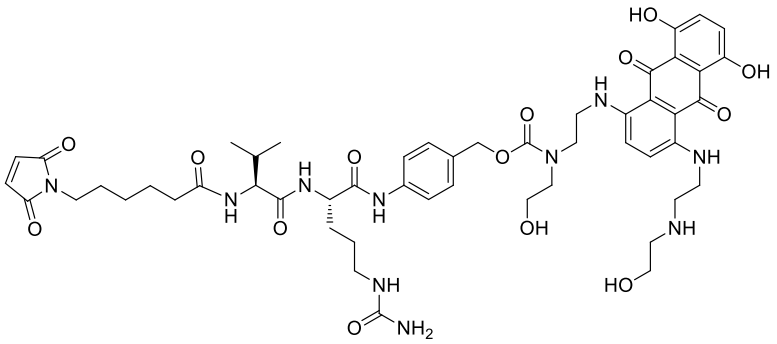
Further, in some examples, the volume of the ethyl acetate added in Step J is 3.5-4.5 times the volume of the filtrate, and the volume of the n-hexane added is 7-9 times the volume of the filtrate. Preferably, the volume of the ethyl acetate added in Step J is 3.7-4.3 times the volume of the filtrate, and the volume of the n-hexane added is 7.5-8.5 times the volume of the filtrate. In some specific examples, the volume of the ethyl acetate added in Step J is 4 times the volume of the filtrate, and the volume of the n-hexane added is 8 times the volume of the filtrate.

Further, in some examples, the preparation conditions of high performance liquid chromatography in Step L are as follows: mobile phase A is an acetic acid aqueous solution with pH=4.0-5.0, mobile phase B is acetonitrile, mobile phase A:B=60:40(V/V), isogradient is used for preparation and purification.

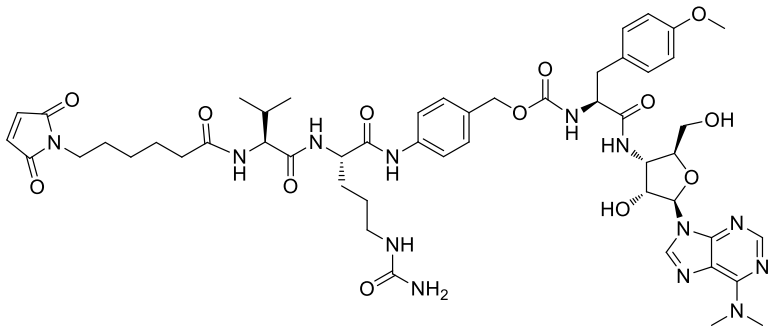
Further, in particular examples, the structure of the antibody drug conjugate intermediate is shown in Formula (1-11):



(1)



(10)



(11)

.

Further, in some examples, the temperature in Step A is controlled within the range of -5-5°C.

Further, in some examples, the temperature in Step B is controlled within the range of 25-30°C

Further, in some examples, Step D can be repeated 1-5 times.

Further, in some examples, the washing times in Step F are 1-5 times.

Further, in some examples, the drying temperature in Step F is 25-30°C.

Further, in some examples, the triazole-based compound in Step G are one or more of 1-hydroxybenzotriazole, 1-hydroxy-7-azobenzotriazole and ethyl 1-hydroxyl-1H-1,2,3-triazole-4-carboxylate, preferably 1-hydroxybenzotriazole.

Further, in some examples, the temperature in Step G is controlled within the range of -5-5°C.

Further, in some examples, the temperature in Step H is controlled within the range of -5-5°C.

Further, in some examples, the reaction temperature in Step I is 25-30°C.

Further, in some examples, the washing times in Step K are 1-5 times.

Further, in some examples, the temperature for concentration under reduced pressure in Step M is 25-35°C.

Further, in some examples, Step M is to concentrate the preparation solution obtained in Step L under reduced pressure to a foamed solid state.

Further, in some examples, the temperature for concentration under reduced pressure in Step N is 25-35°C.

Further, in some examples, Step N is to dissolve the concentrate under reduced pressure in Step M with an appropriate amount of methanol, and then concentrate under reduced pressure again to a foamed solid state.

Further, in some examples, Step N can be repeated 1-5 times.

Further, in some examples, Step A, Step B, Step G, Step H and Step I are all carried out under the protection of nitrogen.

The preparation and purification method for an antibody drug conjugate intermediate provided by the present disclosure can effectively remove the impurities from the target product and by-products in the reaction process, making the purity of the final target product obtained extremely high (e.g., 99% or above), and can realize stable mass production, and well meet the quality standard requirements of clinical drugs, so as to provide a tremendous guarantee for the stable mass production of ADC drugs.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a chromatogram of purified MC-Val-Cit-PAB-PNP.

Figure 2 is a chromatogram of purified MC-VC-PAB-MMAE.

DETAILED DESCRIPTION

Definitions

Unless otherwise defined, all technical terms used in the present invention have the same