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(54) ONE-POT PREPARATION PROCESS FOR ANTIBODY DRUG CONJUGATE INTERMEDIATE

EINTOPFVERFAHREN ZUR HERSTELLUNG EINES ZWISCHENPRODUKTES EINES ANTIKÖRPER-WIRKSTOFF-KONJUGATS

PROCÉDÉ DE PRÉPARATION MONOTOPE D'UN INTERMÉDIAIRE CONJUGUÉ DE MÉDICAMENT- ANTICORPS

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EP 3 760 233 B1

Description

FIELD

[0001] The present invention relates to the field of antibody-drug conjugates, in particular to a one-pot process for preparing intermediate of antibody-drug conjugate (i.e., linker portion-drug portion conjugate).

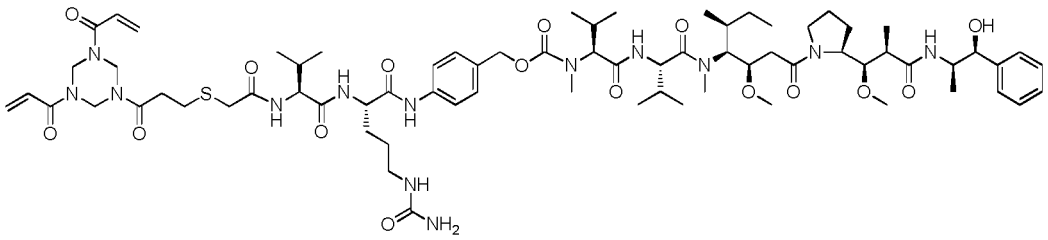
BACKGROUND

[0002] Antibody-drug conjugate (ADC) is a class of anti-tumor drugs comprising three portions: an antibody portion, a linker portion and a drug portion, in which the antibody portion and the drug portion are connected by the linker portion. The mechanism of action is to deliver the drug by virtue of the targeting ability of the antibody to the target cells (such as tumor cells) and then release the drug to kill the tumor cells.

[0003] At present, the most common method for synthesizing antibody-drug conjugates is to covalently link the linker portion and the drug portion in the liquid phase to form a linker-drug conjugate, and then perform thiol or amino coupling with the antibody to form the antibody-drug conjugate. Chinese Patent Publication No. CN107427591A details a general method for synthesis of linker-drug conjugates (see page 34 and pages 47-48 of the specification) (as shown in FIG. 1). The above general synthesis method comprises: in the first step, dissolving the linker containing free benzyl alcohol group in an appropriate solvent, adding bis(4-nitrophenyl)carbonate and diisopropylethylamine to the reaction system, and after several hours of reaction, extracting and purifying the intermediate product; and in the second step, dissolving the above intermediate product and the drug portion containing free amino groups in an appropriate solvent, adding 1-hydroxybenzotriazole and pyridine, and after several hours of reaction, removing the solvent under reduced pressure to obtain the linker-drug conjugate. In addition, CN109200291 discloses a method for synthesis of linker-drug conjugates comprising in a first step reacting the linker containing free benzyl alcohol group with bis(4-nitrophenyl)carbonate and diisopropylethylamine and extracting and purifying the intermediate product, and in the second step reacting the intermediate product with a drug containing free amino groups in the presence of 1-hydroxybenzotriazole and pyridine to obtain the linker-drug conjugate.

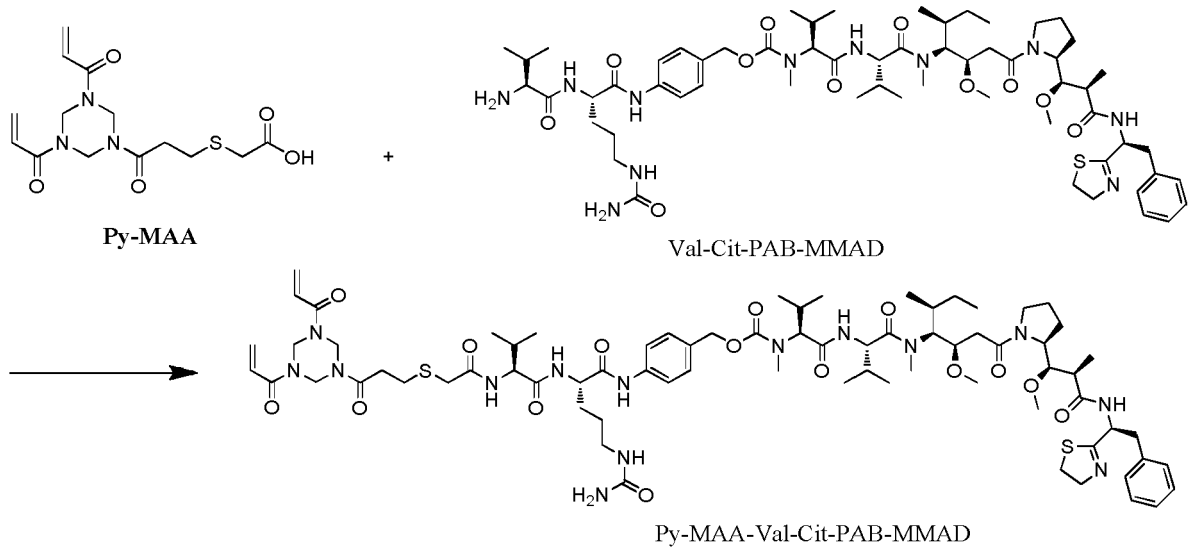
[0004] In the above two-step reaction system, the intermediates need to be extracted and purified, which will affect the final reaction yield. In addition, the above-mentioned preparation process has the inevitable defect of multi-system reaction during production, that is, the need for the concentration, washing and filtration of the reaction liquid in multiple steps, the disposal of the organic waste liquid, and the packaging and storage of the intermediates in the first step, which not only increases the production cost for consumables, labor, equipment, venues, etc., but also generates more waste liquid, increasing the overall production cost and production time.

[0005] Chinese Patent Application Publication No. CN107921030A also discloses a variety of linkers capable of covalently connecting to the antibody in a bridging coupling manner. It discloses an intermediate of antibody-drug conjugate (Py-MAA-Ual-Cit-PAB-MMAE)(wherein, Py is 1,3,5-triacryloylhexahydro-1,3,5-triazine, CAS 959-52-4, available from Bailingwei Technology Co., Ltd. and Nanjing Kangmanlin Chemical Industry Co., Ltd.).



Py-MAA-Val-Cit-PAB-MMAE

[0006] In addition, the patent application also discloses a process for preparing an intermediate of antibody-drug conjugate (Py-MAA-Val-Cit-PAB-MMAD), in which the drug portion is MMAD (Demethyldolastatin 10):

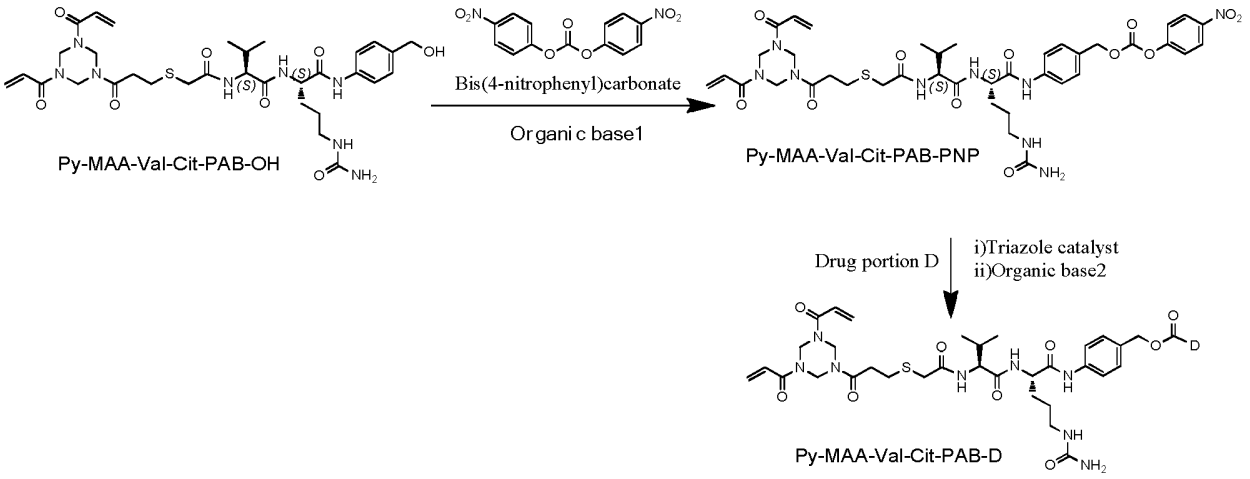


[0007] In this process, Val-Cit-PAB and the drug portion (MMAD) are first coupled together to form Val-Cit-PAB-MMAD conjugate, and then the product obtained after purification is reacted with Py-MAA to generate the intermediate of antibody-drug conjugate Py-MAA-Val-Cit-PAB-MMAD. This process also adopts multi-system synthesis. In addition, since the drug portion (such as MMAD/MMAE or MMAF, etc.) connected in the antibody-drug conjugate participates in the connection reaction in the early stage of the reaction (MMAD is added to the reaction system as a reactant when forming Val-Cit-PAB-MMAD), rather than the last step, the consumption of the drug portion (such as MMAD/MMAE or MMAF, etc.) used in the above process is huge, and since the drug portions (such as MMAD/MMAE or MMAF, etc.) connected in the antibody-drug conjugate are usually relatively expensive, the production cost is greatly increased.

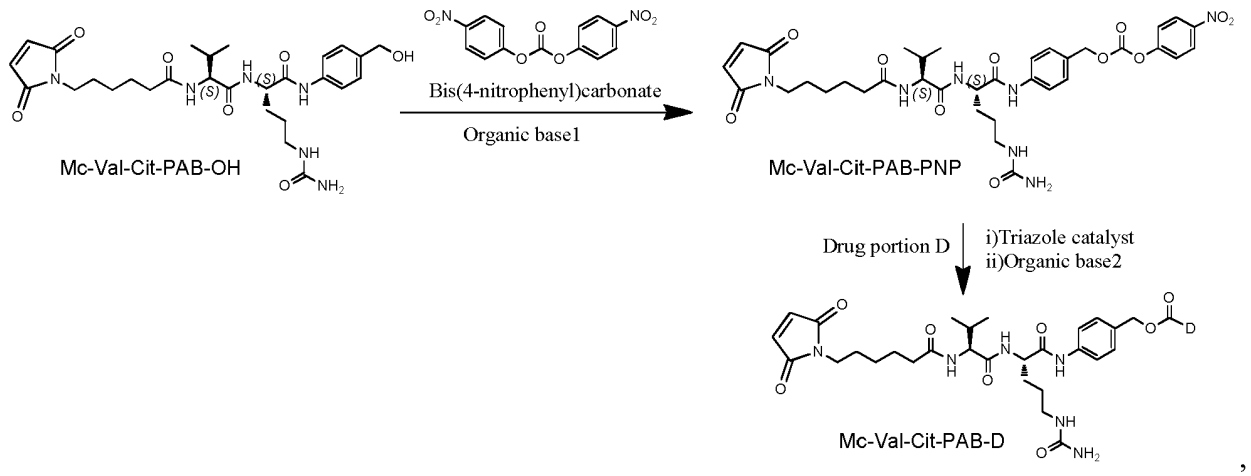
SUMMARY

[0008] In order to solve the above problems, the present invention provides a "one-pot process" for preparing an intermediate of an antibody-drug conjugate (i.e., linker-drug conjugate). Accordingly, the present invention provides subject-matter as set forth in any one and all of the appended claims 1 to 7.

[0009] Specifically, the present invention provides a process for preparing an intermediate of antibody-drug conjugate comprising a linker portion and a drug portion, wherein the intermediate of antibody-drug conjugate is Py-MAA-Val-Cit-PAB-D or MC-Val-Cit-PAB-D, wherein Py-MAA-Val-Cit-PAB or MC-Val-Cit-PAB in the intermediate is the linker portion, and D in the intermediate represents the linked drug portion which is auristatin cytotoxic agent, anthramycin cytotoxic agent, anthracycline cytotoxic agent, puromycin cytotoxic agent, or DX8951 (Exatecan), wherein the process comprises the following reaction route:



or



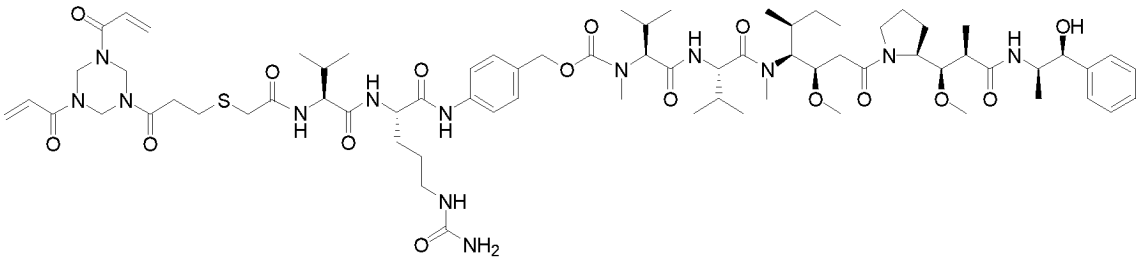
,

wherein the preparation process is a one-pot process in which two steps are carried out in one system.

[0010] Further, the process comprising reacting Py-MAA-Ual-Cit-PAB-OH or MC-Val-Cit-PAB-OH with bis(4-nitrophenyl)carbonate (NPC) in the presence of an organic base, and after the completion of the reaction, further adding organic base, and then 1-hydroxybenzotriazole and the drug portion D into the same reaction system directly for further reaction.

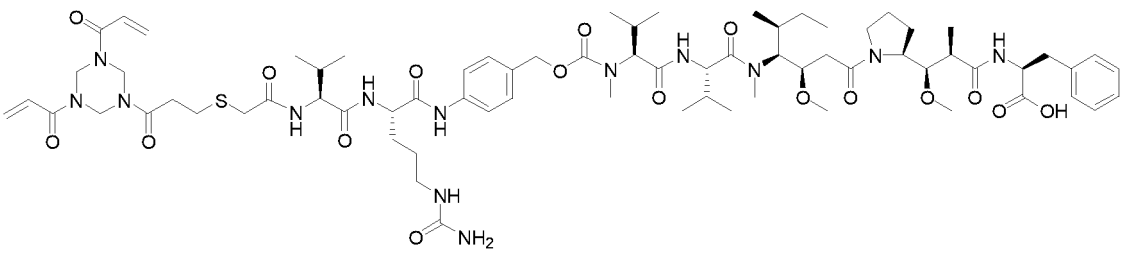
[0011] Further, in embodiments, the auristatin cytotoxic agent is MMAE, MMAF, or MMAD; the anthramycin cytotoxic agent is anthramycin; the anthracycline cytotoxic agent is daunorubicin, doxorubicin, epirubicin, idarubicin, or mitoxantrone; the puromycin cytotoxic agent is puromycin.

[0012] Further, in embodiments, Py-MAA-Val-Cit-PAB-D or MC-Val-Cit-PAB-D has a structure as shown in the following formulas (1-22):



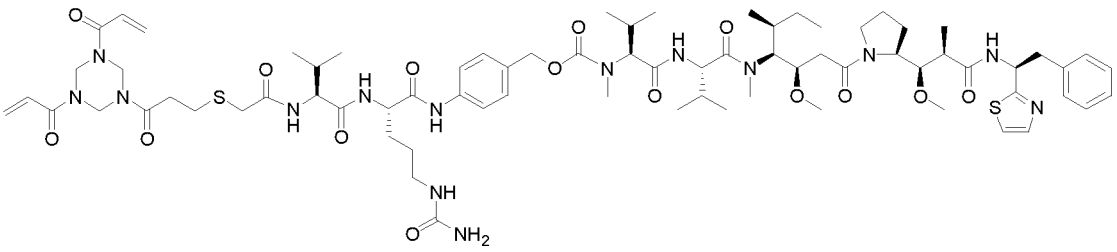
(1)

Py-MAA-Val-Cit-PAB-MMAE



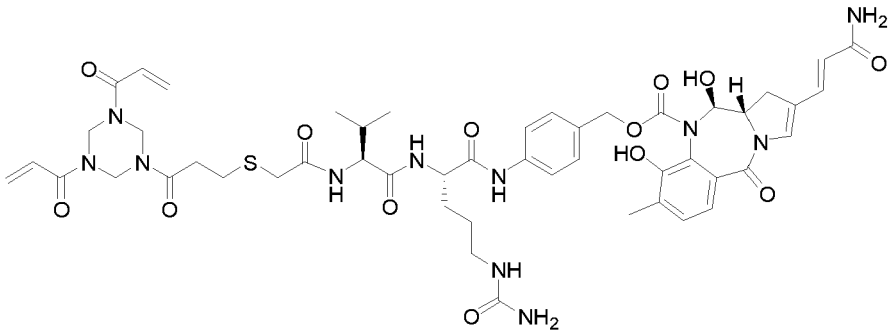
(2)

Py-MAA-Val-Cit-PAB-MMAF



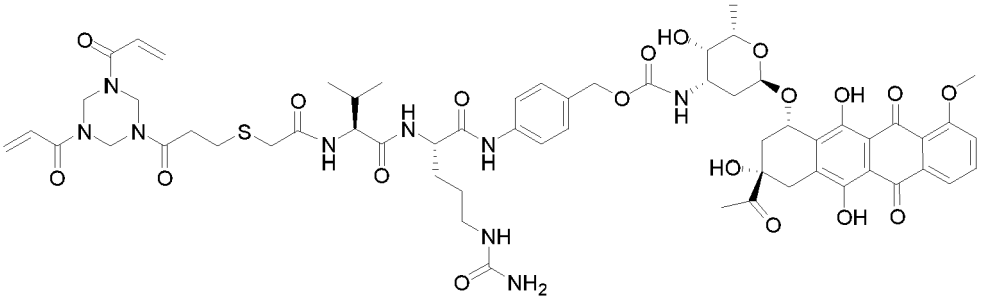
(3)

Py-MAA-Val-Cit-PAB-MMAD



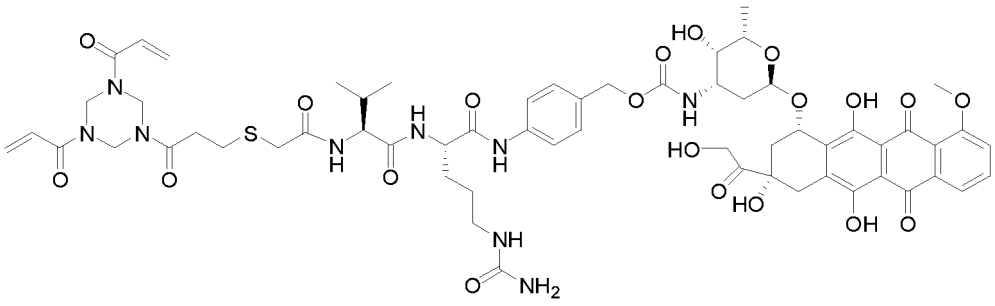
(4)

Py-MAA-Val-Cit-PAB-anthramycin



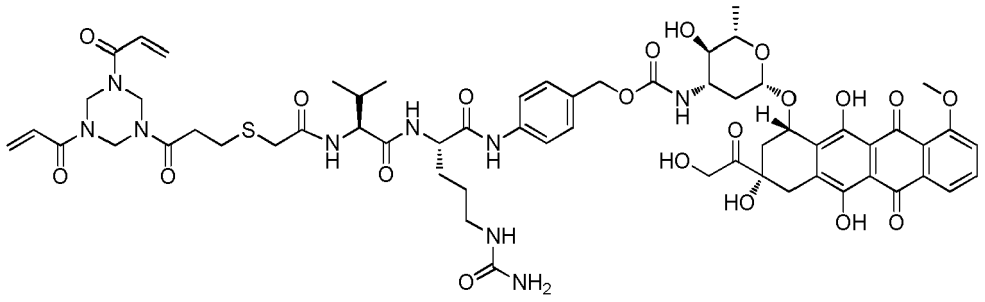
(5)

Py-MAA-Val-Cit-PAB-daunorubicin



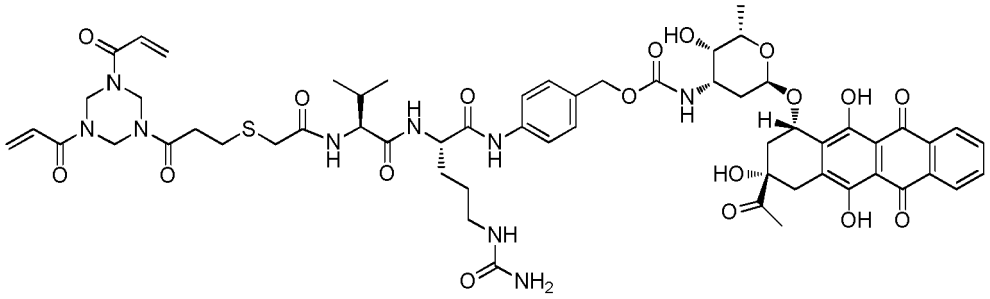
(6)

Py-MAA-Val-Cit-PAB-doxorubicin



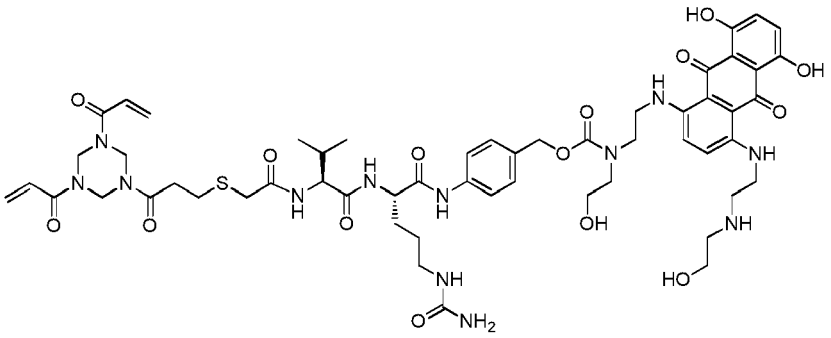
(7)

Py-MAA-Val-Cit-PAB-epirubicin



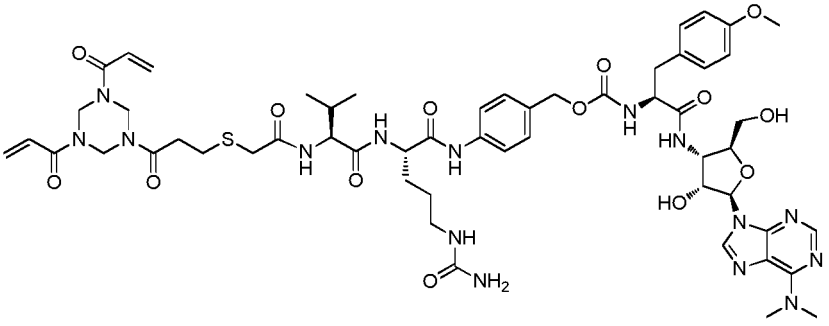
(8)

Py-MAA-Val-Cit-PAB-idarubicin



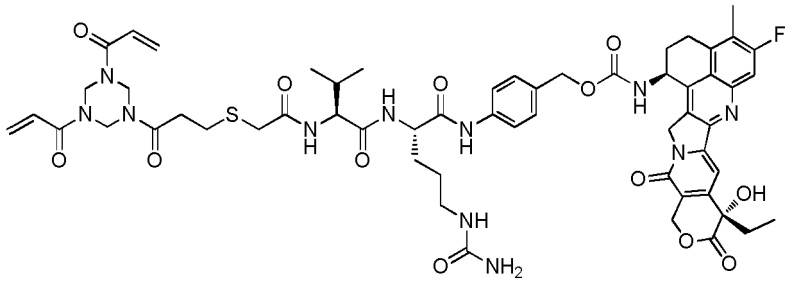
(9)

Py-MAA-Val-Cit-PAB-mitoxantrone



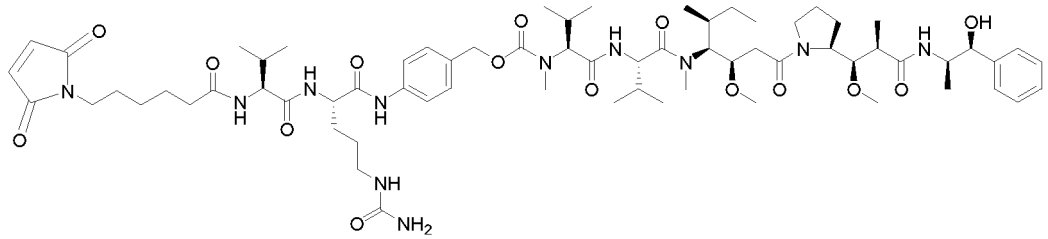
(10)

Py-MAA-Val-Cit-PAB-puromycin



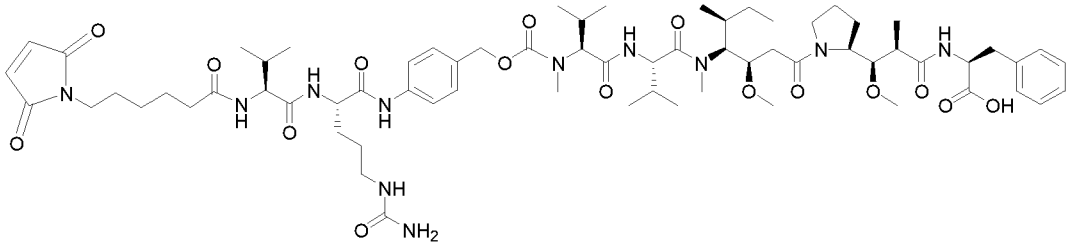
(11)

Py-MAA-Val-Cit-PAB-DX8951



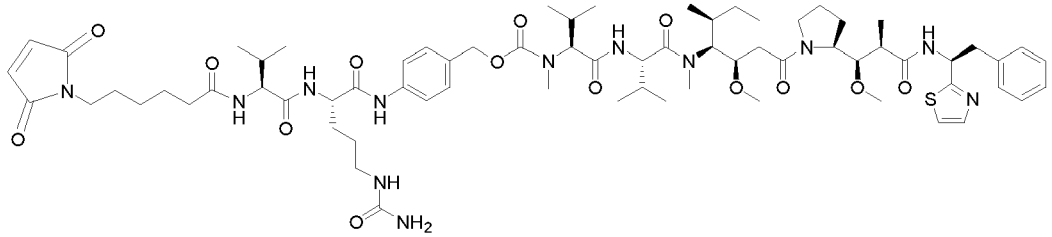
(12)

MC-Val-Cit-PAB-MMAE



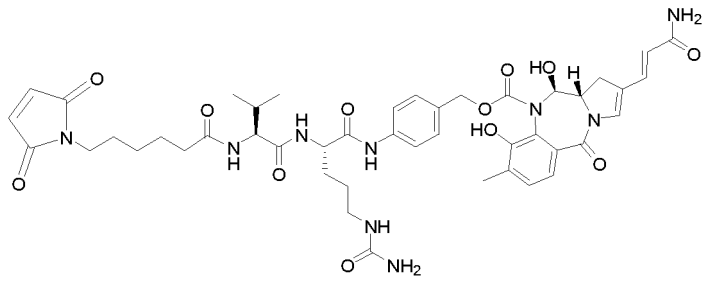
(13)

MC-Val-Cit-PAB-MMAF



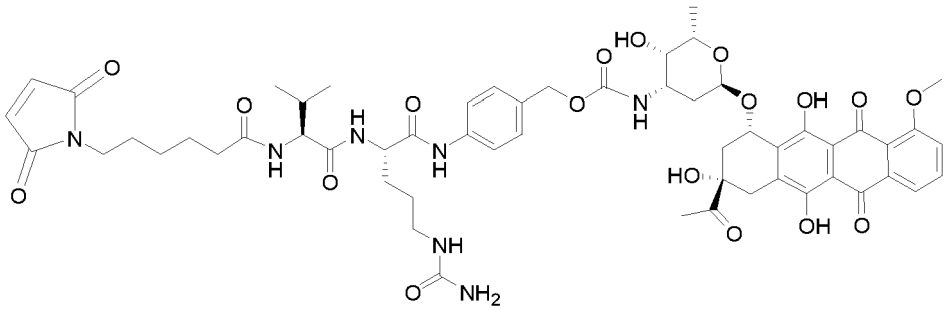
(14)

MC-Val-Cit-PAB-MMAD



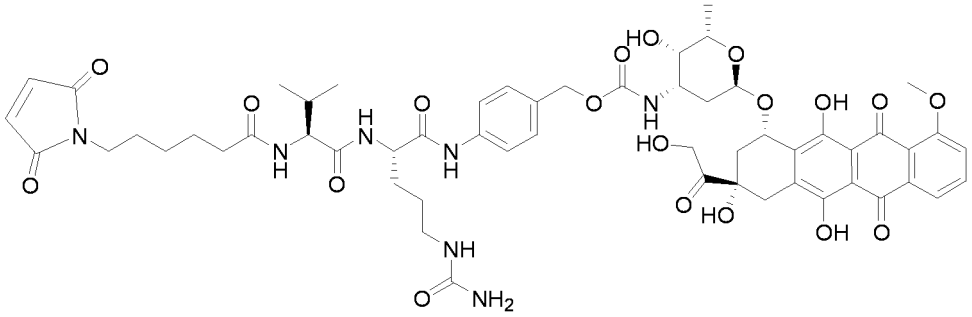
(15)

MC-Val-Cit-PAB-anthramycin



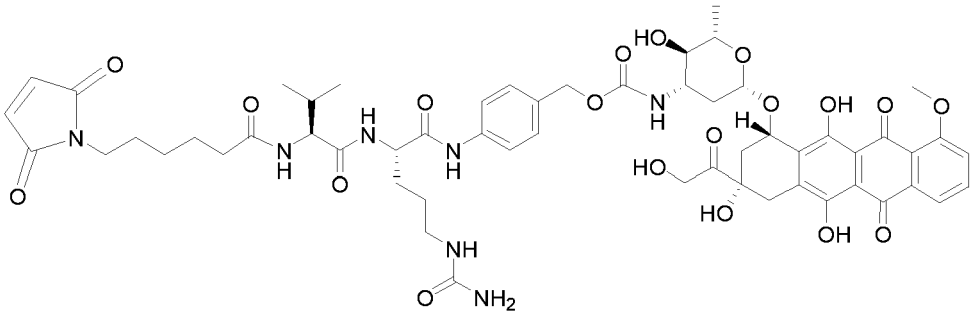
(16)

MC-Val-Cit-PAB-daunorubicin



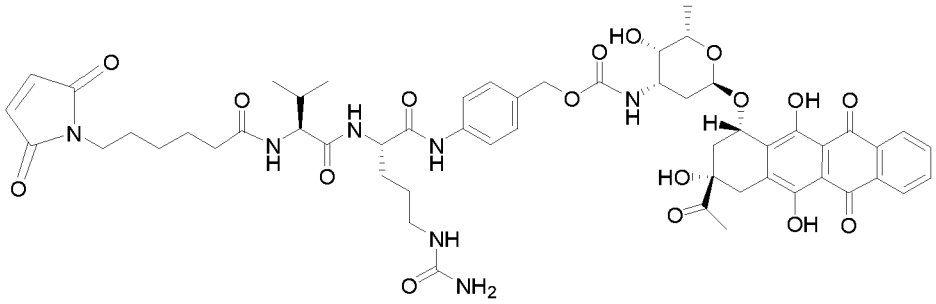
(17)

MC-Val-Cit-PAB-doxorubicin



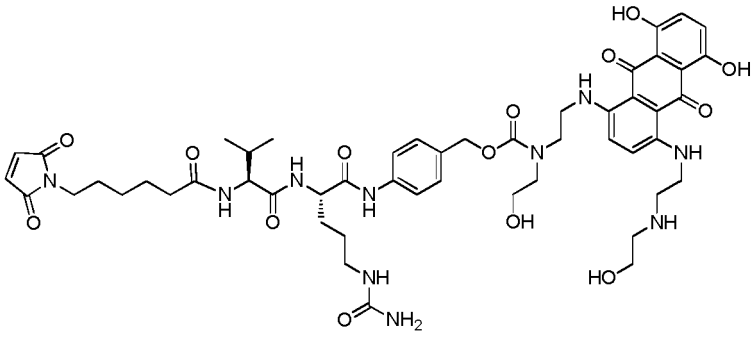
(18)

MC-Val-Cit-PAB-epirubicin



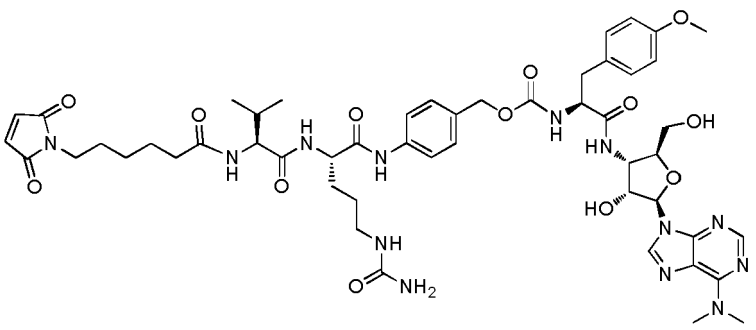
(19)

MC-Val-Cit-PAB-idarubicin



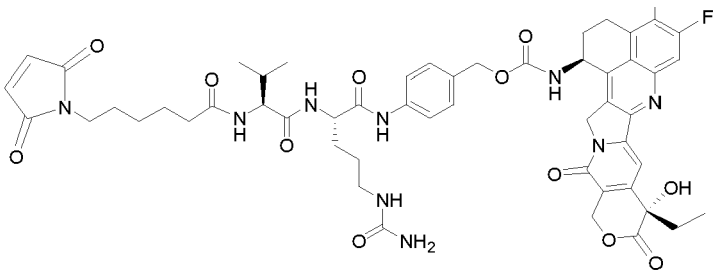
(20)

MC-Val-Cit-PAB-mitoxantrone



(21)

MC-Val-Cit-PAB-puromycin



(22)

MC-Val-Cit-PAB-DX8951

[0013] Further, in embodiments, the organic base 1 and the organic base 2 are each independently one or more of N,N-diisopropylethylamine, triethylamine and pyridine; preferably, the organic base 1 and the organic base 2 are each independently one or two of N,N-diisopropylethylamine and pyridine.

[0014] Further, in embodiments, the triazole catalyst is one or more of 1-hydroxybenzotriazole, 1-hydroxy-7-azobenzotriazole, 1-hydroxy-1H-1,2,3-triazole-4-carboxylic acid ethyl ester, and preferably 1-hydroxybenzotriazole.

[0015] Further, in embodiments, the reaction temperature of the above reaction is about 15-35°C, and further, in embodiments, the reaction temperature may be 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35°C.

[0016] The use of the process described in any one of the above in the preparation of an anti-tumor medicament.

[0017] The use of the process described in any one of the above in the preparation of an antibody-drug conjugate.

[0018] Compared with the traditional two-step reaction system, the "one-pot process" for preparing intermediate of antibody-drug conjugate provided by the present invention is simple in operation, and needs no such steps as the concentration, washing and filtration of the intermediate reaction liquid, the disposal of the organic waste liquid, and the packaging and storage of the intermediates. After the first step of the reaction is completed, the next reaction operation is carried out directly in the same system and the entire reaction system comprises only one separation and purification process, not only saving costs for consumables, labor, equipment, venues, raw materials, etc., but also greatly reducing the production of waste liquid, reducing the production cost, and improving the production efficiency. In addition, during

the reaction process, the linked drug portion is added in the final reaction step, which effectively reduces the consumption of the drug portion (such as MMAD, MMAE or MMAF, DX8951, etc.). Therefore, the "one-pot process" for preparing intermediate of antibody-drug conjugate provided by the present invention is more suitable for scale-up production.

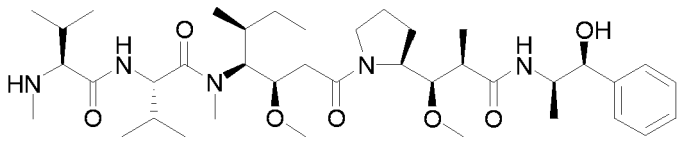
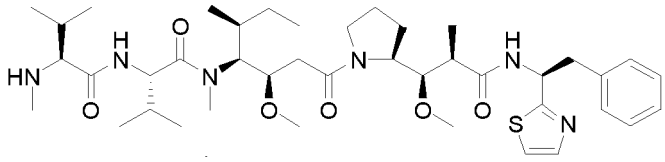
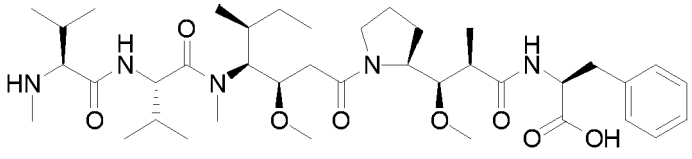
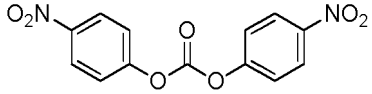
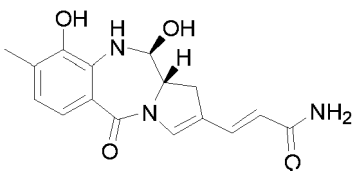
BRIEF DESCRIPTION OF DRAWINGS

[0019] FIG. 1 shows a method for preparing a linker-drug conjugate disclosed on page 34 and pages 47-48 of the specification of Chinese Patent Publication No. CN107427591A.

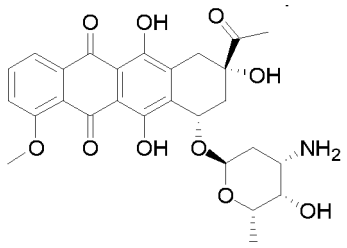
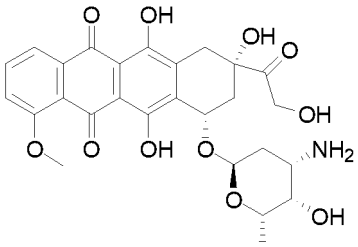
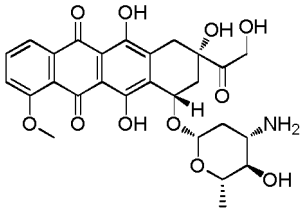
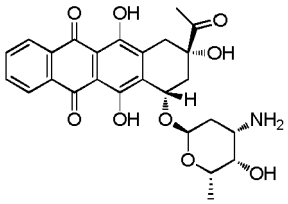
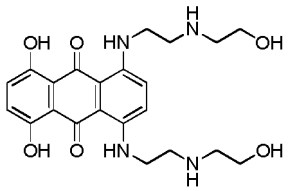
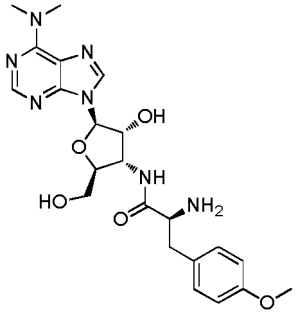
DETAILED DESCRIPTION

ABBREVIATION

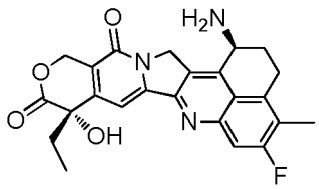
[0020] Unless otherwise stated, all abbreviations used in the present invention have the same meaning as understood by those of ordinary skill in the art. As used in the present invention, commonly used abbreviations and their definitions are as follows:

ABBREVIATION	DEFINE
MC	Maleimidocaproyl
Py	1,3,5-Triacryloylhexahydro-1,3,5-triazineyl
MC-Val-Cit-PAB	Maleimidocaproyl-valine-citrulline-p-amino-benzyloxycarbonyl
Py-MAA-Val-Cit-PAB	1,3,5-Triacryloylhexahydro-1,3,5-triazineyl-mercaptoacetic acid-valine-citrulline-p-amino-benzyloxycarbonyl
NPC	bis(4-nitrophenyl)carbonate
DIPEA	N,N-diisopropylethylamine
HoBt	1-hydroxybenzotriazole
DMF	N,N-dimethylformamide
MMAE	
MMAD	
MMAF	
NPC (bis(4-nitrophenyl) carbonate)	 bis(4-nitrophenyl) carbonate
Anthramycin	

(continued)

ABBREVIATION	DEFINE
Daunorubicin	
Doxorubicin	
Epirubicin	
Idarubicin	
Mitoxantrone	
Puromycin	

(continued)

ABBREVIATION	DEFINE
DX8951 (Exatecan)	

DEFINITION

[0021] Unless otherwise defined, all technical terms used herein have the same meaning as understood by those of ordinary skill in the art.

[0022] The term "antibody-drug conjugate" as used herein refers to a compound in which an antibody/antibody functional fragment, a linker, and a drug portion are linked together through a chemical reaction, and its structure is usually composed of three portions: an antibody or antibody ligand, a drug portion, and a linker that links the antibody or antibody ligand and the drug. At present, the preparation of antibody-drug conjugates usually comprises two steps: in the first step, the linker and the drug portion are chemically reacted to form a "linker-drug" conjugate, and in the second step, the linker portion of the "linker-drug" conjugate is coupled covalently with the antibody/antibody functional fragment via a sulfhydryl group or an amino group. The term "intermediate of antibody-drug conjugate" used herein refers to the above-mentioned "linker-drug" conjugate. Further, the "intermediate of antibody-drug conjugate" mentioned in the present invention generally refers to those "linker-drug" conjugates that are coupled together by a "-CO-NH-" bond formed by amine ester exchange between the linker and the drug.

[0023] The terms "linker" and "linker portion" used herein refer to a portion in an antibody-drug conjugate that connects the antibody to the drug, and may be cleavable or uncleavable. A cleavable linker (i.e., a breakable linker or a biodegradable linker) can be broken in or on the target cell, thereby releasing the drug. In some embodiments, the linker of the invention is selected from cleavable linkers, such as disulfide-based linkers (which are selectively cleaved in tumor cells with higher sulfhydryl groups), peptide linkers (which is cleaved by enzymes in tumor cells), and hydrazone linker. In other embodiments, the linkers of the present invention are selected from uncleavable linkers (i.e., unbreakable linkers), such as thioether linkers. In still other embodiments, the linkers of the present invention are a combination of cleavable linkers and unbreakable linker.

[0024] The terms "drug" and "drug portion" used herein generally refer to any compound having a desired biological activity and having a reactive functional group in order to prepare the conjugate of the present invention. Desired biological activity includes diagnosis, cure, alleviation, treatment, prevention of diseases in humans or other animals. Specifically, the drugs include but are not limited to cytotoxic drugs, cell differentiation factors, stem cell nutrition factors, steroid drugs, drugs for treating autoimmune diseases, anti-inflammatory drugs or drugs for infectious diseases. More specifically, the drugs include but are not limited to tubulin inhibitors or DNA and RNA damaging agents.

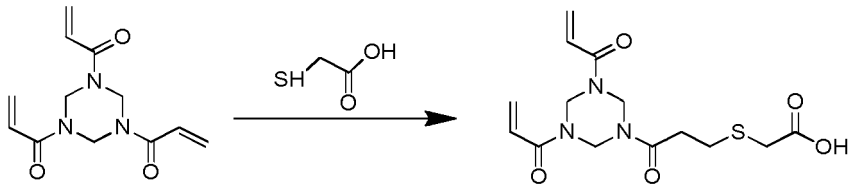
Examples

[0025] The technical solutions of the present invention will be further described in detail in conjunction with specific embodiments below. It should be pointed out that the following examples are only to illustrate the technical concept and features of the present invention, and the purpose thereof is to enable those skilled in the art to understand the content of the present invention and implement it accordingly, but not to limit the protection scope of the present invention.

[0026] General preparation method: Preparation of Py-MAA-Ual-Cit PAB-OH:

(1) Preparation of Py-MAA

[0027]



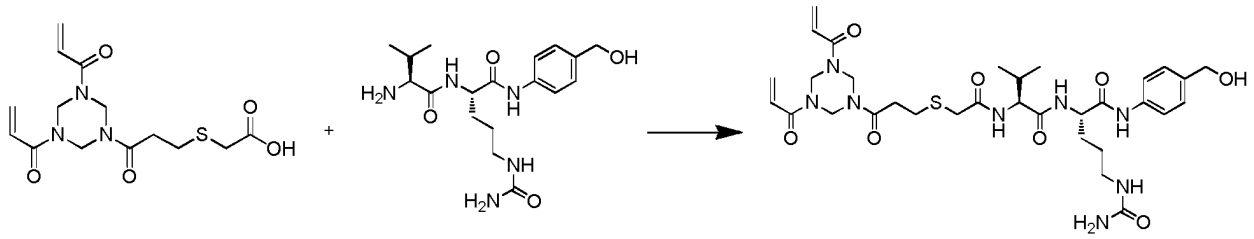
Py

Py-MAA

[0028] Compound Py (1.87 g, 7.51 mmol) and Et_3N (104 μL, 0.75 mmol) were dissolved in anhydrous CH_2Cl_2 (40 mL), and a solution of thioglycolic acid (103.9 μL, 1.50 mmol) in CH_2Cl_2 (40 mL) was added dropwise. After the addition, the system was raised to room temperature and stirred overnight. After the reaction was completed, the solvent was removed under vacuum, and the crude product was purified by column chromatography to obtain white solid Py-MAA (1.87 g).

(2) Preparation of Py-MAA-Val-Cit-PAB-OH

[0029]



Py-MAA

Val-Cit-PAB-OH

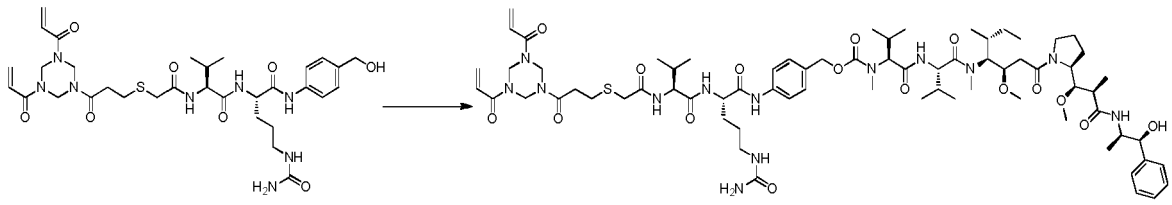
Py-MAA-Val-Cit-PAB-OH

[0030] Compound Py-MAA (10.00 g, 29.33 mmol) was placed in tetrahydrofuran (200 mL), and N'N-carbonyldiimidazole (7.13 g, 44.00 mmol) and Val-Cit-PAB-OH (13.34 g, 35.20 mmol) were added, and then the mixture was stirred at room temperature for 24 hours. Petroleum ether (200 mL) was added, and the mixture was stirred for 0.5 hours, and then filtered to obtain a white solid. The white solid was purified by preparative high-performance liquid chromatography, and the preparation liquid was rotary evaporated under reduced pressure to obtain Py-MAA-Val-Cit-PAB-OH (6.67 g, white solid powder).

Example 1 Preparation of Py-MAA-Val-Cit-PAB-MMAE

(1) Preparation by "one-pot process"

[0031]



Py-MAA-Val-Cit-PAB-OH

Py-MAA-Val-Cit-PAB-MMAE

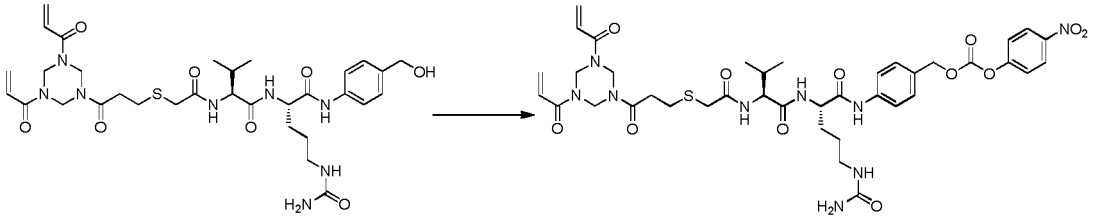
[0032] Py-MAA-Val-Cit-PAB-OH (1.8 g, 1.0 eq) and DMF (40 mL) were added to a three-necked round-bottom flask in sequence, and after stirring to dissolve, NPC (882 mg, 1.1 eq) and DIPEA (336 mg, 1.0 eq) were added, and the mixture was stirred at 24±2°C for 24 hours. Then DIPEA (672 mg, 2.0 eq), pyridine (2.3 mL), HoBt (351 mg, 1.0 eq) and MMAE (1.7 g, 0.9 eq) were added to the reaction solution in sequence, and the reaction continued at 24±2°C for 48 hours. Product Py-MAA-Val-Cit-PAB-MMAE (1.9 g) was obtained after preparative liquid chromatography purification, with a purity of 99.84%, and yield of 51.3% [calculation formula: yield = Py-MAA-Val-Cit-PAB-MMAE amount produced

÷ (Py-MAA-Val-Cit-PAB-OH amount used ÷702.8×1446.8)×100%].

(2) Preparation by "two-step process"

Step 1: Preparation of Py-MAA-Val-Cit-PAB-(4-nitrophenyl)carbonate

[0033]



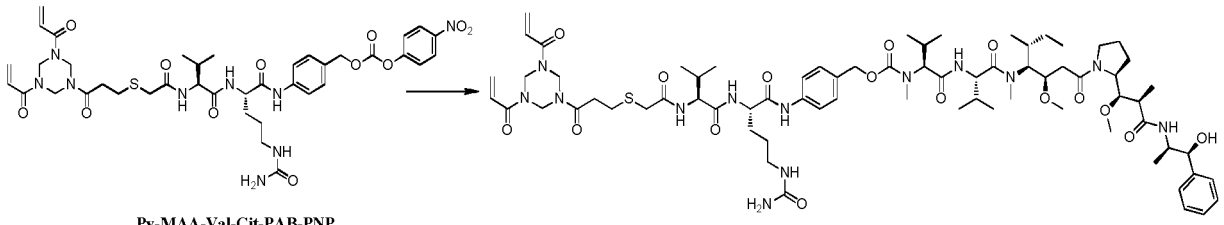
Py-MAA-Val-Cit-PAB-OH

Py-MAA-Val-Cit-PAB-PNP

[0034] DMF (40 mL) and Py-MAA-Val-Cit-PAB-OH (1.8 g, 1.0 eq.) were added to a reaction flask, and after stirring to dissolve, bis(4-nitrophenyl)carbonate (NPC, 882 mg, 1.1 eq.) and DIPEA (336 mg, 1.0 eq.) were added and the reaction was performed at 24±2°C for 24 hours. To the reaction solution, ethyl acetate (mL) was added and petroleum ether (mL) was added dropwise over 20 mins. After the dropwise addition, the mixture was continuously stirred for 10 min and filtered, then washed three times with ethyl acetate and petroleum ether respectively; and spin-dried, to obtain Py-MAA-Val-Cit-PAB-(4-nitrophenyl) carbonate (1.6 g), yield 72.1%, purity: 86%.

Step 2: Preparation of Py-MAA-Val-Cit-PAB-MMAE

[0035]



Py-MAA-Val-Cit-PAB-PNP

Py-MAA-Val-Cit-PAB-MMAE

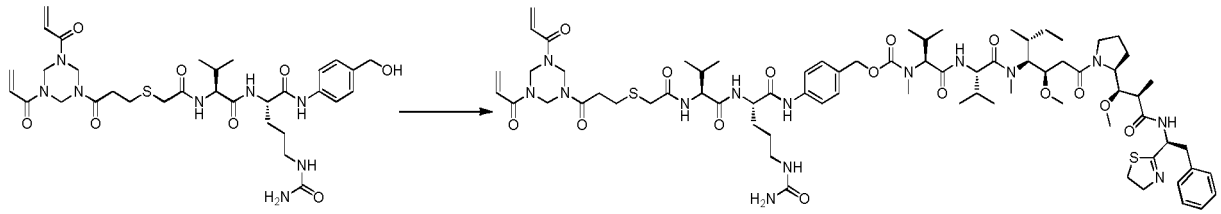
[0036] Py-MAA-Val-Cit-PAB-(4-nitrophenyl)carbonate (1.5 g), 1-hydroxybenzotriazole (HoBt, 234 mg, 1.0 eq.), DMF (30 mL), MMAE (1.1 g, 0.9 eq.), pyridine (1.5 mL) andDIPEA(447 mg, 2.0 eq) were added to a reaction flask, and the reaction was performed at 24±2°C for 48 hours and then spin-dried. Then preparative HPLC was performed to obtain Py-MAA-Val-Cit-PAB-MMAE (1.3 g), yield 52%, purity: 99%.

[0037] It can be known that the final yield of Py-MAA-Val-Cit-PAB-MMAE prepared by the one-pot process is 51.3%, and the final yield of Py-MAA-Val-Cit-PAB-MMAE prepared by the two-step process is 37.49%, with the same amount of main raw materials. After comparison, the final yield of Py-MAA-Val-Cit-PAB-MMAE prepared by the one-pot process is much greater than that of Py-MAA-Val-Cit-PAB-MMAE prepared by the two-step process.

Example 2 Preparation of Py-MAA-Val-Cit-PAB-MMAD

(1) Preparation by "one-pot process"

[0038]



Py-MAA-Val-Cit-PAB-OH

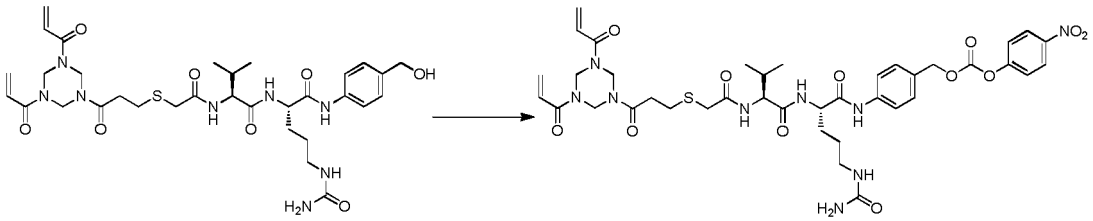
Py-MAA-Val-Cit-PAB-MMAD

[0039] DMF (4 mL) and Py-MAA-Val-Cit-PAB-OH (200 mg, 1.0 eq.) were added to a reaction flask, and after stirring to dissolve, bis(4-nitrophenyl)carbonate (NPC, 95 mg, 1.1 eq.) and DIPEA (36 mg, 1.0 eq.) were added and the reaction was performed at 24±2°C for 24 hours. 1-hydroxybenzotriazole (HoBt, 38 mg, 1.0 eq.), MMAD (197 mg, 0.9 eq.), pyridine (248 μL) and DIPEA (73 mg, 2.0 eq) were added to the reaction flask, and the reaction was performed at 24±2°C for 48hours. The mixture was spin-dried and preparative HPLC was performed to obtain Py-MAA-Val-Cit-PAB- MMAD (208 mg), yield: 48.7%, purity: 99%.

(2) Preparation by "two-step process"

Step 1: Preparation of Py-MAA-Val-Cit-PAB-(4-nitrophenyl)carbonate

[0040]



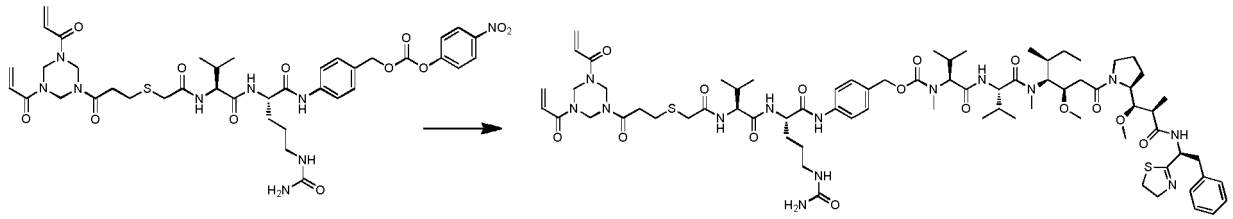
Py-MAA-Val-Cit-PAB-OH

Py-MAA-Val-Cit-PAB-PNP

[0041] DMF (4 mL) and Py-MAA-Val-Cit-PAB-OH (200 mg, 1.0 eq.) were added to a reaction flask, and after stirring to dissolve, bis(4-nitrophenyl)carbonate (NPC, 95 mg, 1.1 eq.) and DIPEA (36 mg, 1.0 eq.) were added and the reaction was performed at 24±2°C for 24 hours. To the reaction solution, ethyl acetate (6 mL) was added and petroleum ether (12 mL) was added dropwise over 20 mins. After the dropwise addition, the mixture was continuously stirred for 10 min and filtered, then washed three times with ethyl acetate and petroleum ether respectively; and spin-dried, to obtain Py-MAA-Ual-Cit-PAB-(4-nitrophenyl) carbonate (170 mg), yield: 68.8%, purity: 86%.

Step 2: Preparation of Py-MAA-Val-Cit-PAB-MMAD

[0042]



Py-MAA-Val-Cit-PAB-PNP

Py-MAA-Val-Cit-PAB-MMAD

[0043] Py-MAA-Val-Cit-PAB-(4-nitrophenyl)carbonate (170 mg), 1-hydroxybenzotriazole (HoBt, 27 mg, 1.0 eq.), DMF (4 mL), MMAD (139 mg, 0.9 eq.), pyridine (174 μL) and DIPEA (52 mg, 2.0 eq) were added to a reaction flask, and the reaction was performed at 24±2°C for 48 hours and then spin-dried. Preparative HPLC was performed to obtain Py-MAA-Val-Cit-PAB-MMAD (139 mg), yield: 47.3%, purity: 99%.

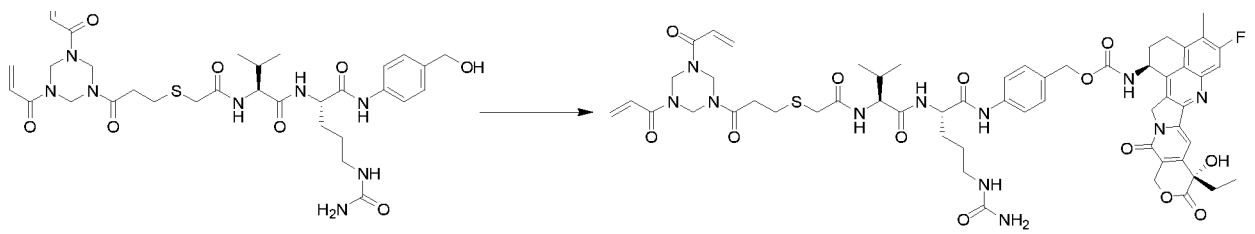
[0044] It can be known that the final yield of Py-MAA-Val-Cit-PAB-MMAD prepared by the one-pot process is 48.7%, and the final yield of Py-MAA-Val-Cit-PAB-MMAD prepared by the two-step process is 32.54%, with the same amount

of main raw materials. After comparison, the final yield of Py-MAA-Val-Cit-PAB-MMAD prepared by the one-pot process is much greater than that of Py-MAA-Val-Cit-PAB-MMAD prepared by the two-step process.

Example 3 Preparation of Py-MAA-Val-Cit-PAB-DX8951

(1) Preparation by "one-pot process"

[0045]



Py-MAA-Val-Cit-PAB-OH

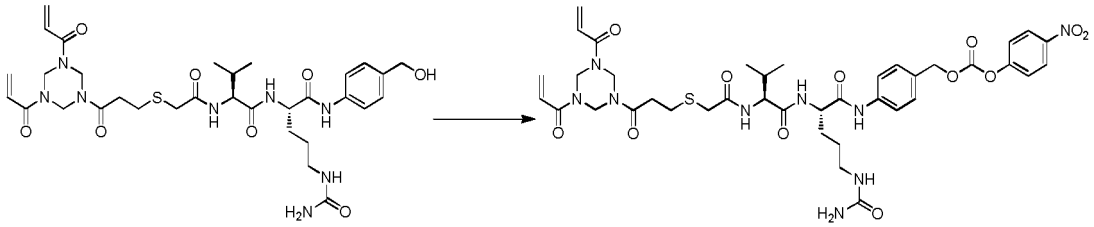
Py-MAA-Val-Cit-PAB-DX8951

[0046] DMF (4 mL) and Py-MAA-Val-Cit-PAB-OH (200 mg, 1.0 eq.) were added to a reaction flask, and after stirring to dissolve, bis(4-nitrophenyl)carbonate (NPC, 95 mg, 1.1 eq.) and DIPEA (36 mg, 1.0 eq.) were added and the reaction was performed at 24±2°C for 24 hours. 1-hydroxybenzotriazole (HoBt, 38 mg, 1.0 eq.), DX8951 (136 mg, 0.9 eq.), pyridine (248 μL) and DIPEA (110 mg, 3.0 eq.) were added, and the reaction continued at 24±2°C for 48hours. The mixture was spin-dried and preparative HPLC was performed to obtain Py-MAA-Val-Cit-PAB-DX8951 (123 mg), yield: 37.1%, purity: 97%.

(2) Preparation by "two-step process"

Step 1: Preparation of Py-MAA-Val-Cit-PAB-(4-nitrophenyl) carbonate

[0047]



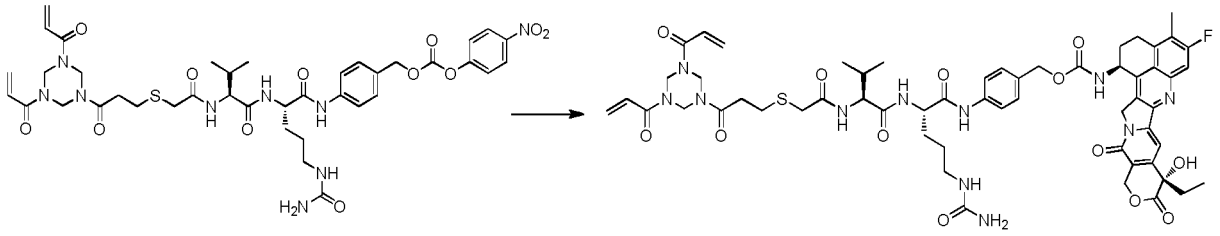
Py-MAA-Val-Cit-PAB-OH

Py-MAA-Val-Cit-PAB-PNP

[0048] DMF (4 mL) and Py-MAA-Val-Cit-PAB-OH (200 mg, 1.0 eq.) were added to a reaction flask, and after stirring to dissolve, bis(4-nitrophenyl)carbonate (NPC, 95 mg, 1.1 eq.) and DIPEA (36 mg, 1.0 eq.) were added and the reaction was performed at 24±2°C for 24 hours. To the reaction solution, ethyl acetate (6 mL) was added and petroleum ether (12 mL) was added dropwise over 20 mins. After the dropwise addition, the mixture was continuously stirred for 10 min and filtered, then washed three times with ethyl acetate and petroleum ether respectively; and spin-dried, to obtain Py-MAA-Val-Cit-PAB-(4-nitrophenyl) carbonate (176 mg), yield: 71.3%, purity: 86%.

Step 2: Preparation of Py-MAA-Val-Cit-PAB-DX8951

[0049]



Py-MAA-Val-Cit-PAB-PNP

Py-MAA-Val-Cit-PAB-DX8951

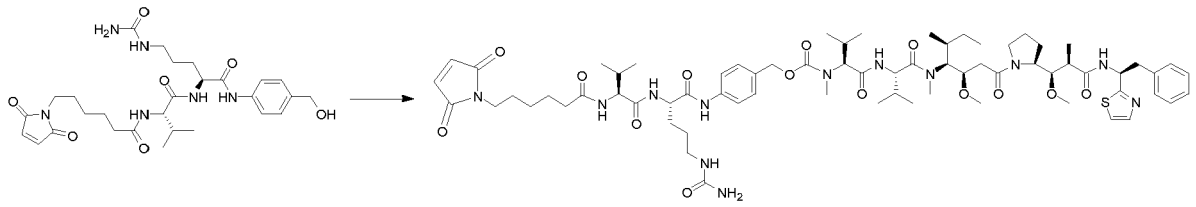
[0050] Py-MAA-Ual-Cit-PAB-(4-nitrophenyl)carbonate (170 mg), 1-hydroxybenzotriazole (HoBt, 27 mg, 1.0 eq.), DMF (4 mL), DX8951 (96 mg, 0.9 eq.), pyridine (174 μL) and DIPEA (78 mg, 3.0 eq) were added to a reaction flask, and the reaction was performed at 24±2°C for 48 hours and then spin-dried. Preparative HPLC was performed to obtain Py-MAA-Val-Cit-PAB-DX8951 (76 mg), yield: 33.3%, purity: 97%.

[0051] It can be known that the final yield of Py-MAA-Val-Cit-PAB-DX8951 prepared by the one-pot process is 37.1%, and the final yield of Py-MAA-Val-Cit-PAB-DX8951 prepared by the two-step process is 23.74%, with the same amount of main raw materials. After comparison, the final yield of Py-MAA-Val-Cit-PAB-DX8951 prepared by the one-pot process is much greater than that of Py-MAA-Val-Cit-PAB-DX8951 prepared by the two-step process.

Example 4 Preparation of Mc-Val-Cit-PAB-MMAD

(1) Preparation by "one-pot process"

[0052]



MC-Val-Cit-PAB-OH

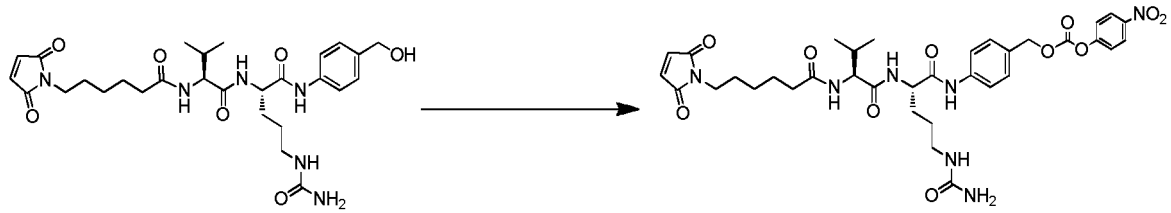
MC-Val-Cit-PAB-MMAD

[0053] DMF (4 mL) and MC-Val-Cit-PAB-OH (200 mg, 1.0 eq.) were added to a reaction flask, and after stirring to dissolve, bis(4-nitrophenyl)carbonate (NPC, 116 mg, 1.1 eq.) and DIPEA (45 mg, 1.0 eq.) were added and the reaction was performed at 24±2°C for 18 hours. 1-hydroxybenzotriazole (HoBt, 47 mg, 1.0 eq.), MMAD (242 mg, 0.9 eq.), pyridine (304 μL) and DIPEA (90 mg, 2.0 eq) were added, and the reaction continued at 24±2°C for 48 hours. The mixture was spin-dried and preparative HPLC was performed to obtain MC-Val-Cit-PAB-MMAD (228 mg), yield: 47.7%, purity: 99%.

(2) Preparation by "two-step process"

Step 1: Preparation of MC-Val-Cit-PAB-(4-nitrophenyl)carbonate

[0054]



Mc-Val-Cit-PAB-OH

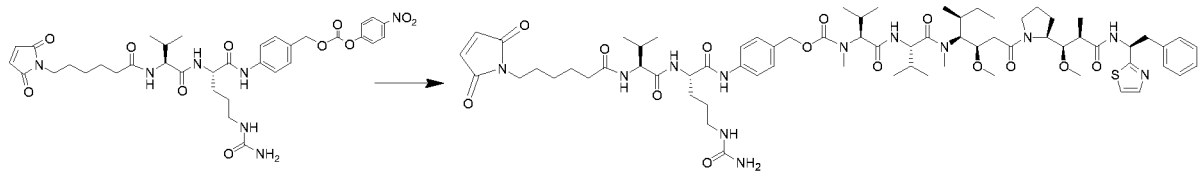
Mc-Val-Cit-PAB-PNP

[0055] DMF (4 mL) and MC-Val-Cit-PAB-OH (200 mg, 1.0 eq.) were added to a reaction flask, and after stirring to dissolve, bis(4-nitrophenyl)carbonate (NPC, 116 mg, 1.1 eq.) and DIPEA (45 mg, 1.0 eq.) were added. The reaction

was performed at 24±2°C for 18 hours. Ethyl acetate (6 mL) was added and petroleum ether (12 mL) was added dropwise over 20 mins. After the dropwise addition, the mixture was continuously stirred for 10 min and filtered, then washed three times with ethyl acetate and petroleum ether respectively; and spin-dried, to obtain MC-Val-Cit-PAB-(4-nitrophenyl) carbonate (177 mg), yield: 68.6%, purity: 90%.

Step 2: Preparation of MC-Val-Cit-PAB-MMAD

[0056]



Mc-Val-Cit-PAB-PNP MC-Val-Cit-PAB-MMAD

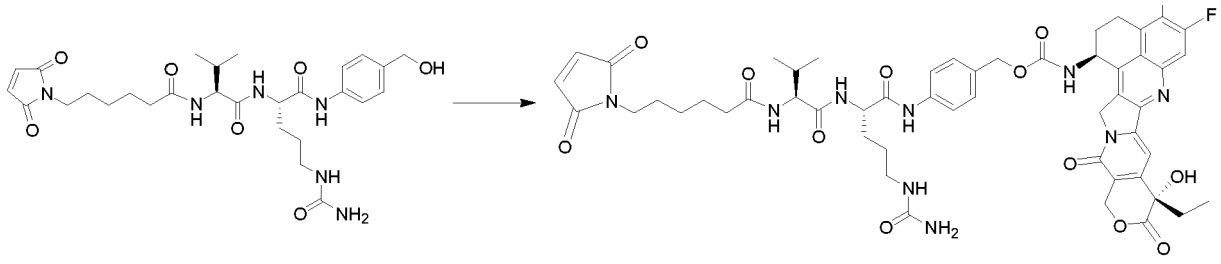
[0057] MC-Val-Cit-PAB-(4-nitrophenyl) carbonate (170 mg) obtained in the first step, 1-hydroxybenzotriazole (HoBt, 31 mg, 1.0 eq), DMF (4 mL), MMAD (160 mg, 0.9 eq.), pyridine (200 μL) and DIPEA (59 mg, 2.0 eq) were added to a reaction flask, and the reaction was performed at 24±2°C for 48 hours and then spin-dried. Preparative HPLC was performed to obtain MC-Val-Cit-PAB-MMAD (156 mg), yield: 49.4%, purity: 99%.

[0058] It can be known that the final yield of MC-Val-Cit-PAB-MMAD prepared by the one-pot process is 47.7%, and the final yield of MC-Ual-Cit-PAB-MMAD prepared by the two-step process is 33.88%, with the same amount of main raw materials. After comparison, the final yield of MC-Val-Cit-PAB-MMAD prepared by the one-pot process is much greater than that of MC-Val-Cit-PAB-MMAD prepared by the two-step process.

Example 5 Preparation of Mc-Val-Cit-PAB-DX8951

(1) Preparation by "one-pot process"

[0059]



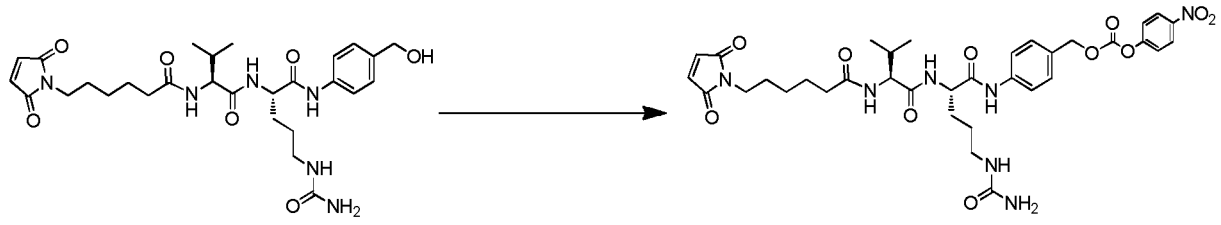
Mc-Val-Cit-PAB-OH Mc-Val-Cit-PAB-DX8951

[0060] DMF (4 mL) and MC-Val-Cit-PAB-OH (200 mg, 1.0 eq.) were added to a reaction flask, and after stirring to dissolve, bis(4-nitrophenyl)carbonate (NPC, 116 mg, 1.1 eq.) and DIPEA (45 mg, 1.0 eq.) were added and the reaction was performed at 24±2°C for 18 hours. 1-hydroxybenzotriazole (HoBt, 47 mg, 1.0 eq.), DMF (4 mL), DX8951 (167 mg, 0.9 eq.), pyridine (304 μL) and DIPEA (135 mg, 3.0 eq) were added, and the reaction was performed at 24±2°C for 48hours. The mixture was spin-dried and preparative HPLC was performed to obtain MC-Val-Cit-PAB-DX8951 (139 mg), yield: 38.4%, purity: 97%.

(2) Preparation by "two-step process"

Step 1: Preparation of MC-Val-Cit-PAB-(4-nitrophenyl) carbonate

[0061]



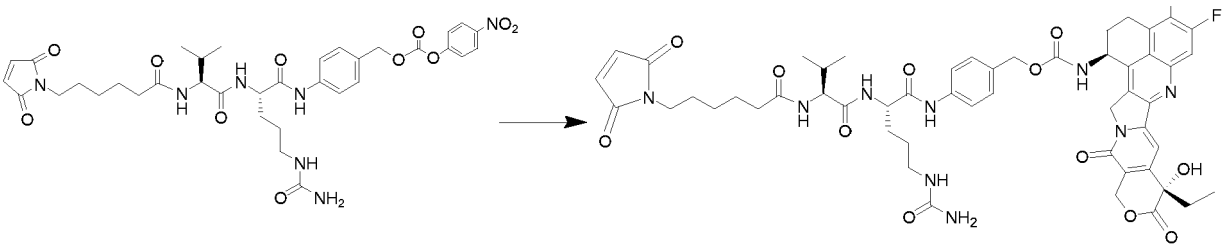
Mc-Val-Cit-PAB-OH

Mc-Val-Cit-PAB-PNP

[0062] DMF (4 mL) and MC-Val-Cit-PAB-OH (200 mg, 1.0 eq.) were added to a reaction flask, and after stirring to dissolve, bis(4-nitrophenyl)carbonate (NPC, 116 mg, 1.1 eq.) and DIPEA (45 mg, 1.0 eq.) were added. The reaction was performed at 24±2°C for 18 hours. Ethyl acetate (6 mL) was added and petroleum ether (12 mL) was added dropwise over 20 mins. After the dropwise addition, the mixture was continuously stirred for 10 min and filtered, then washed three times with ethyl acetate and petroleum ether respectively; and spin-dried, to obtain MC-Val-Cit-PAB-(4-nitrophenyl) carbonate (174 mg), yield: 67.4%, purity: 90%.

Step 2: Preparation of MC-Ual-Cit-PAB-DX8951

[0063]



Mc-Val-Cit-PAB-PNP

Mc-Val-Cit-PAB-DX8951

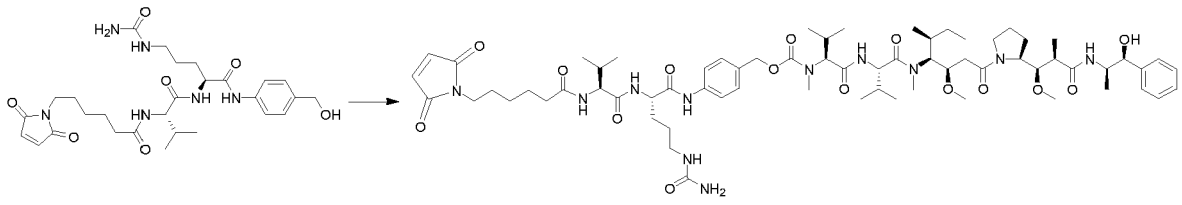
[0064] MC-Ual-Cit-PAB-(4-nitrophenyl) carbonate (170 mg), 1-hydroxybenzotriazole (HoBt, 31 mg, 1.0 eq.), DMF (4 mL), DX8951 (110 mg, 0.9 eq.), pyridine (200 μL) and DIPEA (89 mg, 3.0 eq) were added to a reaction flask, and the reaction was performed at 24±2°C for 48 hours and then spin-dried. Preparative HPLC was performed to obtain MC-Val-Cit-PAB-DX8951 (85 mg), yield: 35.6%, purity: 97%.

[0065] It can be known that the final yield of MC-Val-Cit-PAB-DX8951 prepared by the one-pot process is 38.4%, and the final yield of MC-Val-Cit-PAB-DX8951 prepared by the two-step process is 23.99%, with the same amount of main raw materials. After comparison, the final yield of MC-Val-Cit-PAB-DX8951 prepared by the one-pot process is much greater than that of MC-Val-Cit-PAB-DX8951 prepared by the two-step process.

Example 6 Preparation of Mc-Val-Cit-PAB-MMAE

(1) Preparation by "one-pot process"

[0066]



MC-Val-Cit-PAB-OH

MC-Val-Cit-PAB-MMAE

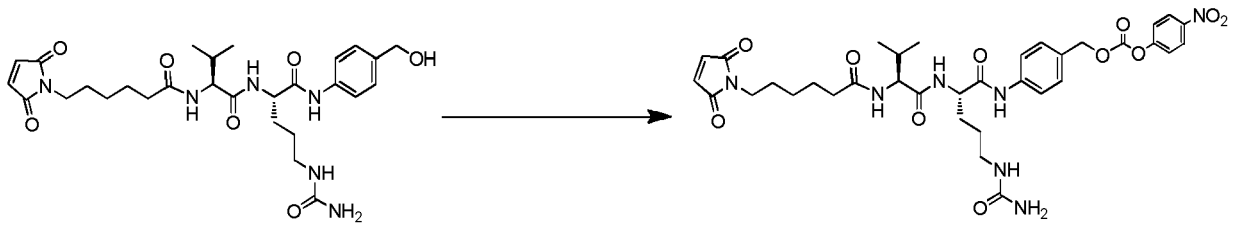
[0067] MC-Val-Cit-PAB-OH (CAS No.: 159857-80-4) (200 mg, 1.0 eq) and DMF (4 mL) were added to a three-necked round-bottom flask in sequence, and after stirring at room temperature to dissolve, NPC (117 mg, 1.1 eq) and DIPEA

(45 mg, 1.0 eq) were added, and the mixture was stirred at 24±2°C for 18 hours. Then DIPEA (90 mg, 2.0 eq), pyridine (0.3 mL, V pyridine/VDIPEA=2.5), HoBt (47 mg, 1.0 eq) and MMAE (251 mg, 1.0 eq) were added to the above reaction solution in sequence, and the reaction continued at 24±2°C for 48 hours. Product MC-Val-Cit-PAB-MMAE (214 mg) was obtained after preparative liquid chromatography purification, with a purity of 99.45%, and yield of 46.5% [calculation formula: yield =MC-Val-Cit-PAB-MMAE amount produced ÷ (MC-Val-Cit-PAB-OH amount used ÷572.7×1316.6)×100%].

(2) Preparation by "two-step process"

Step 1: Preparation of MC-Val-Cit-PAB-(4-nitrophenyl)carbonate

[0068]



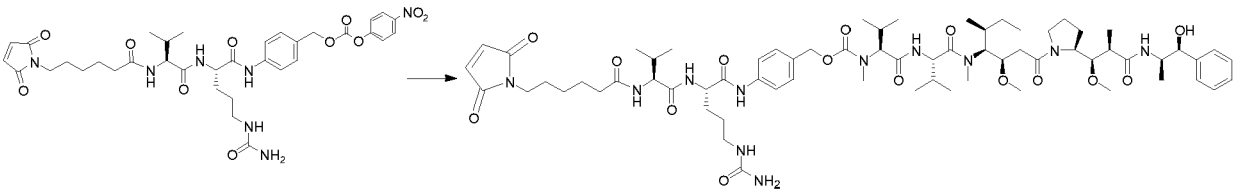
Mc-Val-Cit-PAB-OH

Mc-Val-Cit-PAB-PNP

[0069] MC-Val-Cit-PAB-OH (200 mg, 1.0 eq) and DMF (4 mL) were added to a three-necked round-bottom flask in sequence, and after stirring at room temperature to dissolve, NPC (117 mg, 1.1 eq) and DIPEA (45 mg, 1.0 eq) were added and the reaction was performed at 24±2°C for 18 hours. Ethyl acetate (12 mL) and petroleum ether (18 mL) were added. The mixture was stirred, filtered and spin-dried to obtain a crude product. The crude product was added to acetic acid (2 mL) and methanol (0.3 mL), and after stirring to dissolve, purified water (6 mL) was added dropwise, and the mixture was filtered. The obtained solid was spin-dried on a rotary evaporator to obtain MC-Val-Cit-PAB-(4-nitrophenyl) carbonate (185 mg), yield: 71.8%, purity: 97%.

Step 2: Preparation of MC-Val-Cit-PAB-MMAE

[0070]



MC-Val-Cit-PAB-PNP

MC-Val-Cit-PAB-MMAE

[0071] MC-Val-Cit-PAB-(4-nitrophenyl)carbonate (185 mg), DIPEA (64 mg, 2.0 eq), pyridine (0.2 mL, V pyridine/VDIPEA = 2.5), HoBt (34 mg, 1.0 eq) and MMAE (180 mg, 1.0 eq) were added to a reaction flask in sequence, and the reaction was performed at 24±2°C for 48 hours. Preparative HPLC was performed to obtain the product MC-Val-Cit-PAB-MMAE (115 mg), yield 34.8%, purity 99%.

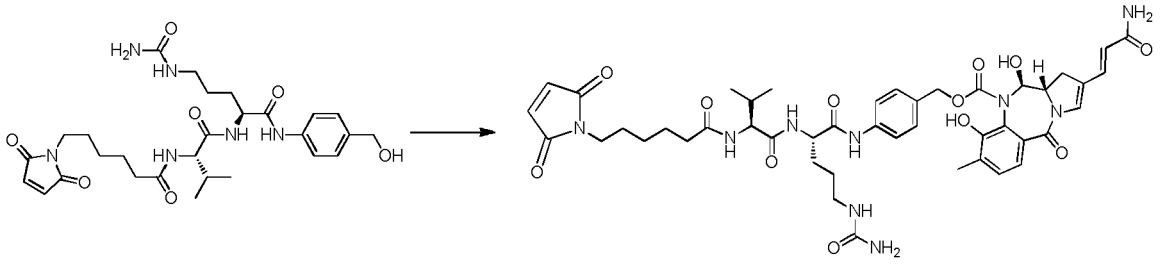
[0072] It can be known that the final yield of MC-Val-Cit-PAB-MMAE prepared by the one-pot process is 46.5%, and the final yield of MC-Ual-Cit-PAB-MMAE prepared by the two-step process is 24.99%, with the same amount of main raw materials. After comparison, the final yield of MC-Val-Cit-PAB-MMAE prepared by the one-pot process is much greater than that of MC-Val-Cit-PAB-MMAE prepared by the two-step process.

Table 1 Comparison of final yields of "one-pot process" and "two-step process"

product	Total yields of "one-pot process"	Total yields of "two-step process"	Absolute increase of the yield	Relative increase of the yield
Py-MAA-Val-Cit-PAB-MMAE	51.30%	37.49%	13.81%	36.84%
Py-MAA-Val-Cit-PAB-MMAD	48.70%	32.54%	16.16%	49.66%
Py-MAA-Val-Cit-PAB-DX8951	37.10%	23.74%	13.36%	56.28%
Mc-Val-Cit-PAB-MMAD	47.70%	33.88%	13.82%	40.79%
Mc-Val-Cit-PAB-DX8951	38.40%	23.99%	14.41%	60.07%
Mc-Val-Cit-PAB-MMAE	46.50%	24.99%	21.51%	86.07%
Note: Absolute increase of the yield = Total yields of "one-pot process" - Total yields of "two-step process" Relative increase of the yield = (Total yields of "one-pot process" - Total yields of "two-step process) / Total yields of "two-step process",				

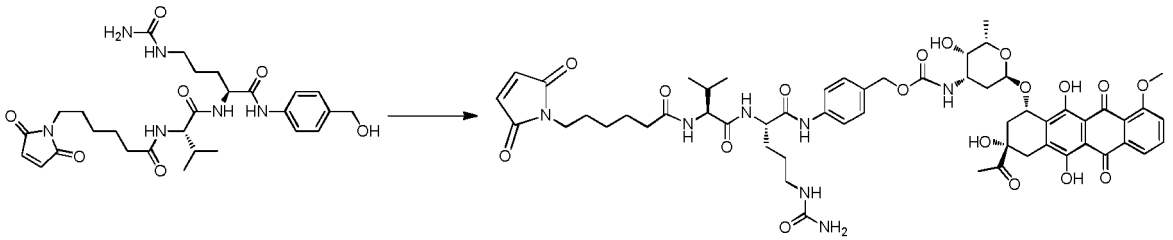
[0073] By comparison of the total yields of the "one-pot process" and the total yields of the "two-step process" for Py-MAA-Val-Cit-PAB-MMAE, Py-MAA-Val-Cit-PAB-MMAD, Py-MAA-Val-Cit-PAB-DX8951, Mc- Val-Cit- PAB- MMAD, Mc-Val-Cit-PAB-DX8951, and Mc-Val-Cit-PAB-MMAE, it can be found that the final yield of "one-pot process" provided by the present invention is greatly improved relative to that of the existing "two-step process". Since drugs (such as MMAE, DX8951) and similar materials are very expensive, the method of the present invention will greatly reduce the production cost of drugs. Therefore, compared with the prior art "two-step process", the "one pot method" of the present invention has significant progress and unexpected technical effects.

[0074] In addition, the one-step preparation process provided by the present invention only needs reactions in the same reaction system, while the traditional preparation process needs reactions in two reaction systems (that is, Py-MAA-Val-Cit -PAB-PNP is extracted and then put into another reaction), which requires the use of organic reagents (ethyl acetate and petroleum ether, etc.) and equipment (for concentration) and a new reaction vessel (for the next reaction), undoubtedly bringing a lot of work to the synthesis process. The use of additional organic reagents and recycling operations not only requires more manpower, but also has a certain impact on the environment. Therefore, the one-step preparation process provided by the present invention is more suitable for large-scale production.



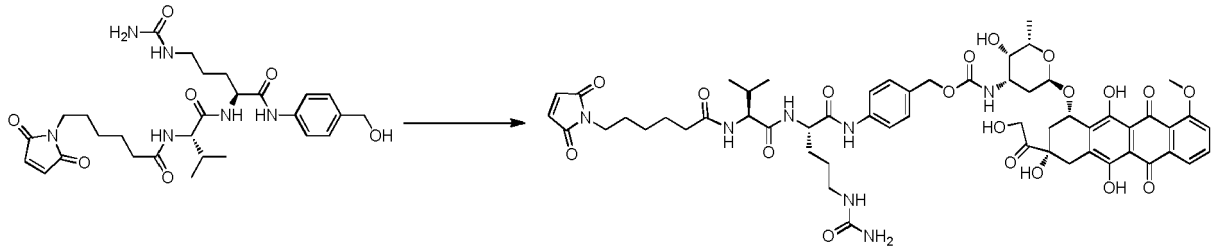
MC-Val-Cit-PAB-OH

MC-Val-Cit-PAB-anthramycin



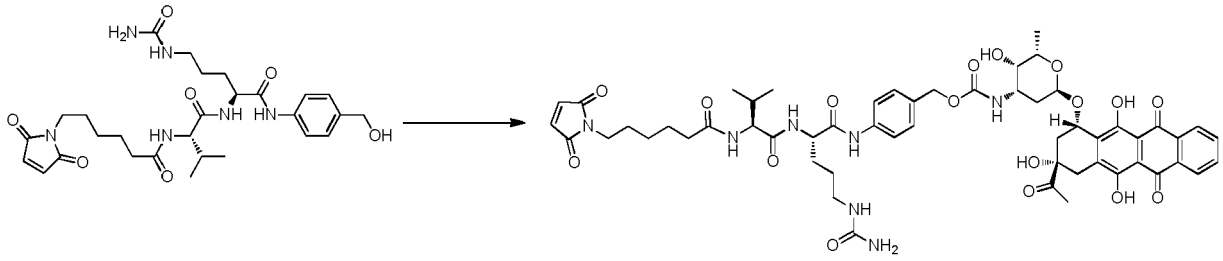
MC-Val-Cit-PAB-OH

MC-Val-Cit-PAB-daunorubicin



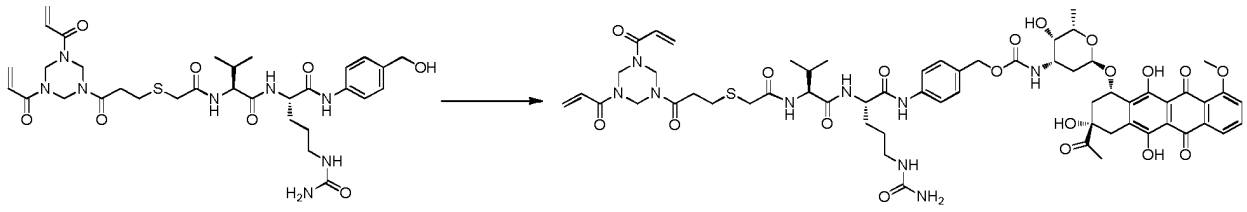
MC-Val-Cit-PAB-OH

MC-Val-Cit-PAB-doxorubicin



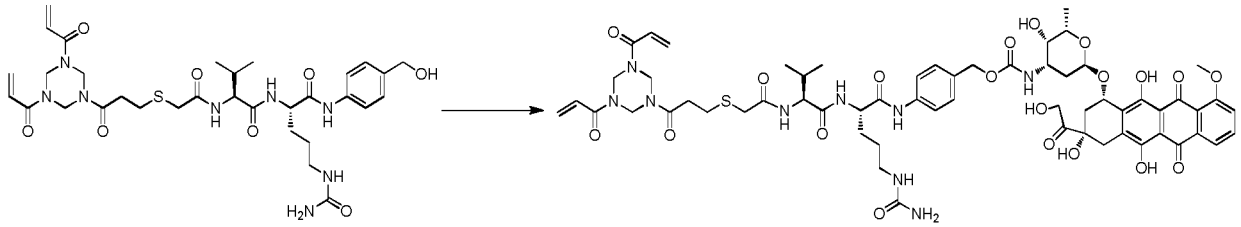
MC-Val-Cit-PAB-OH

MC-Val-Cit-PAB-idarubicin



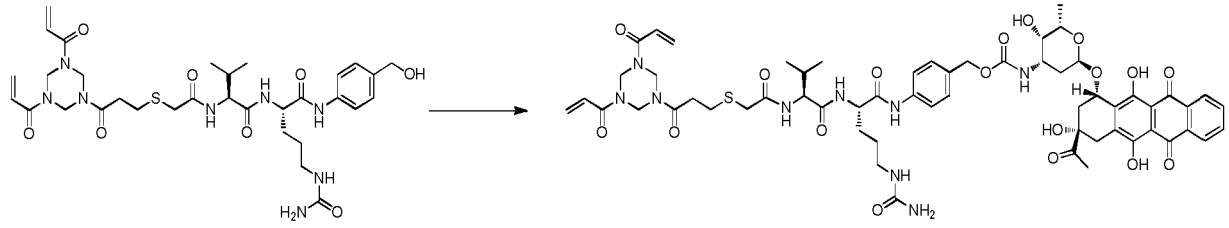
Py-MAA-Val-Cit-PAB-OH

Py-MAA-Val-Cit-PAB-daunorubicin



Py-MAA-Val-Cit-PAB-OH

Py-MAA-Val-Cit-PAB-doxorubicin

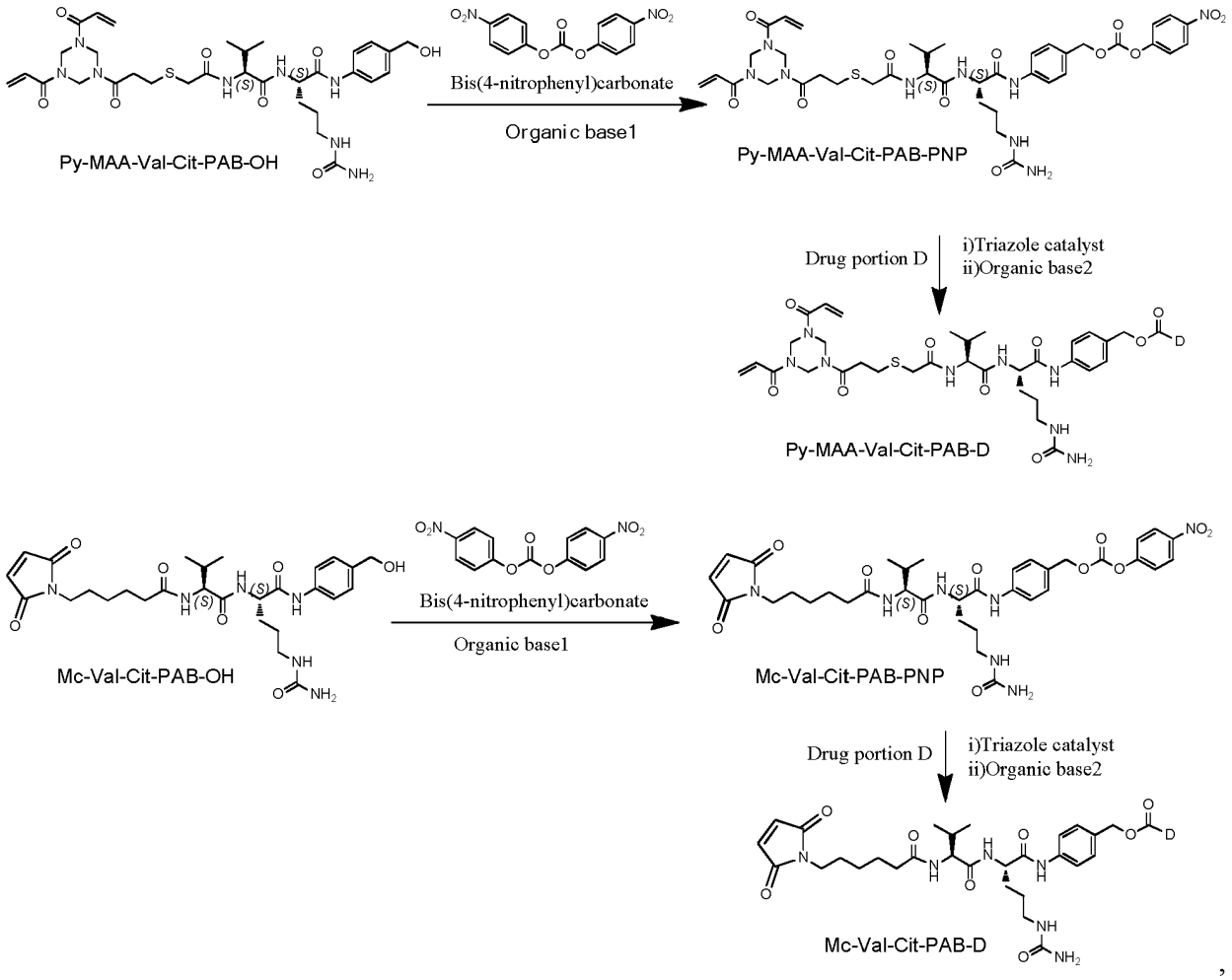


Py-MAA-Val-Cit-PAB-OH

Py-MAA-Val-Cit-PAB-idarubicin

Claims

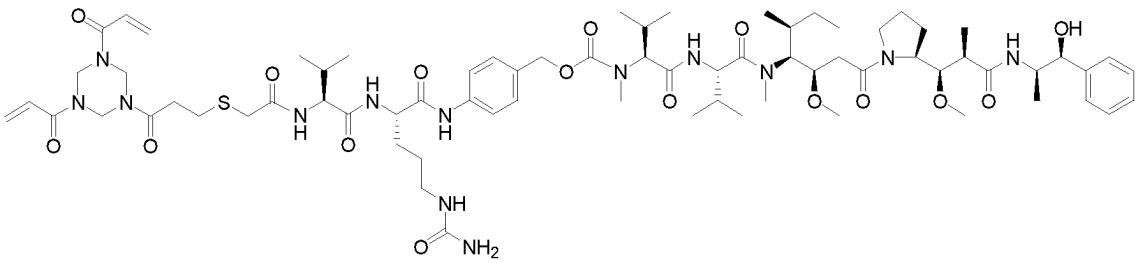
1. A process for preparing an intermediate of antibody-drug conjugate comprising a linker portion and a drug portion, wherein the intermediate of antibody-drug conjugate is Py-MAA-Val-Cit-PAB-D or MC-Val-Cit-PAB-D, wherein Py-MAA-Val-Cit-PAB or MC-Val-Cit-PAB in the intermediate is the linker portion, and D in the intermediate represents drug portion which is auristatin cytotoxic agent, anthramycin cytotoxic agent, anthracycline cytotoxic agent, puromycin cytotoxic agent, or DX8951, wherein the process comprises the following reaction route:



wherein the preparation process is a one-pot process in which two steps are carried out in one system.

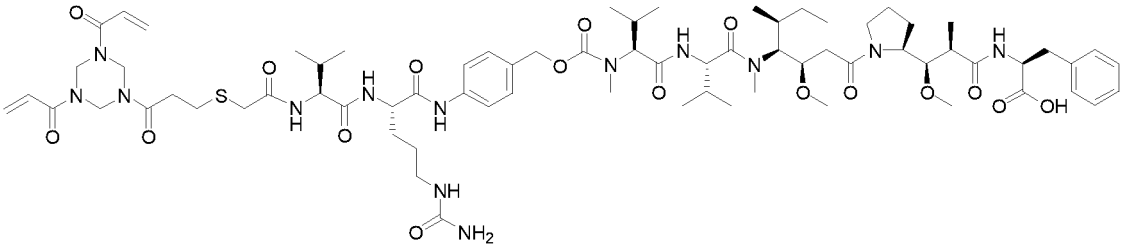
2. The process according to claim 1, wherein the auristatin cytotoxic agent is MMAE, MMAF, or MMAD; the anthramycin cytotoxic agent is anthramycin; the anthracycline cytotoxic agent is daunorubicin, doxorubicin, epirubicin, idarubicin, or mitoxantrone; and the puromycin cytotoxic agent is puromycin.

3. The process according to claim 2, wherein Py-MAA-Val-Cit-PAB-D or MC-Val-Cit-PAB-D has a structure as shown in the following formulas (1-22):



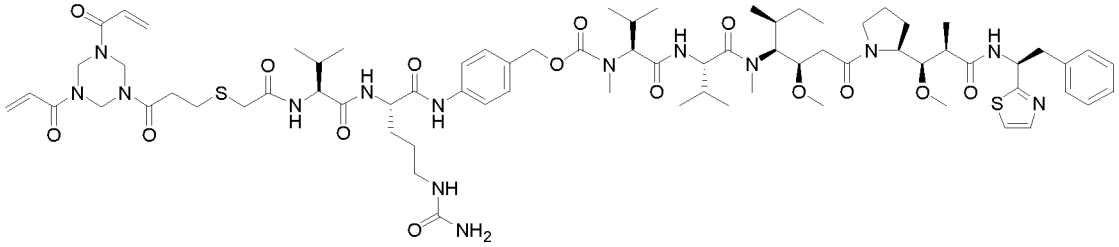
(1)

Py-MAA-Val-Cit-PAB-MMAE



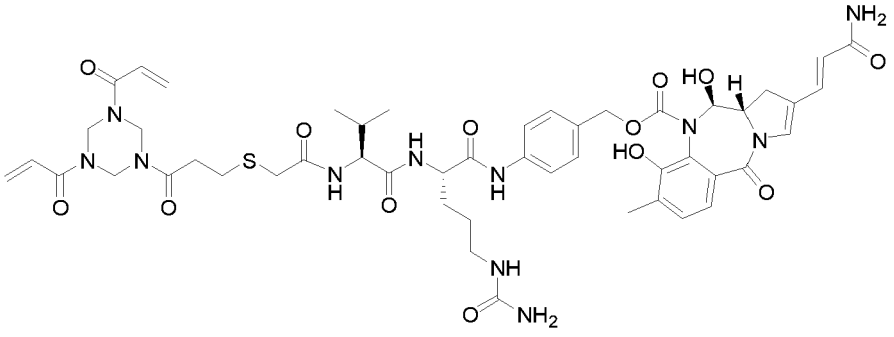
(2)

Py-MAA-Val-Cit-PAB-MMAF



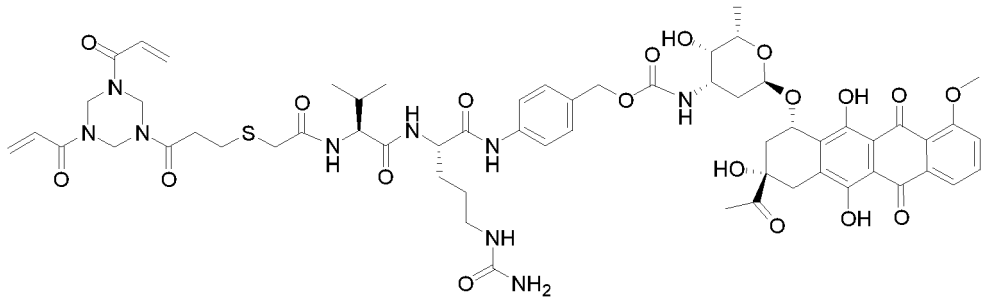
(3)

Py-MAA-Val-Cit-PAB-MMAD



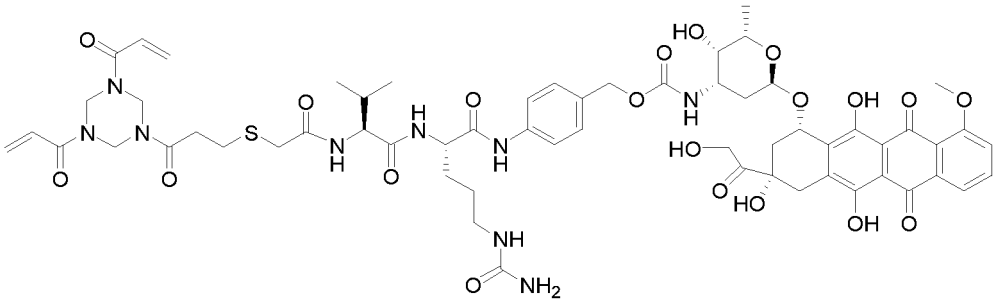
(4)

Py-MAA-Val-Cit-PAB-anthramycin



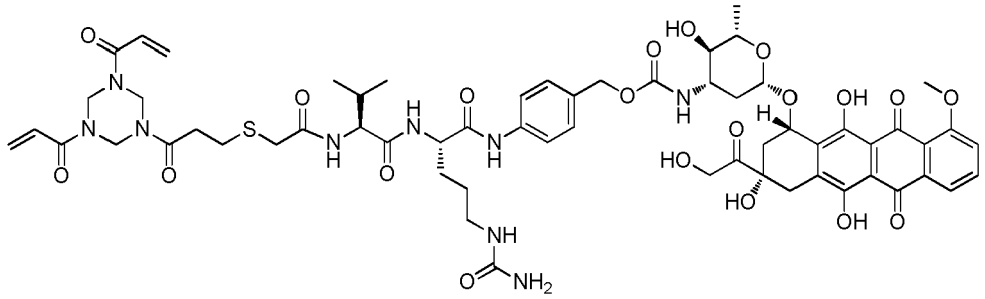
(5)

Py-MAA-Val-Cit-PAB-daunorubicin



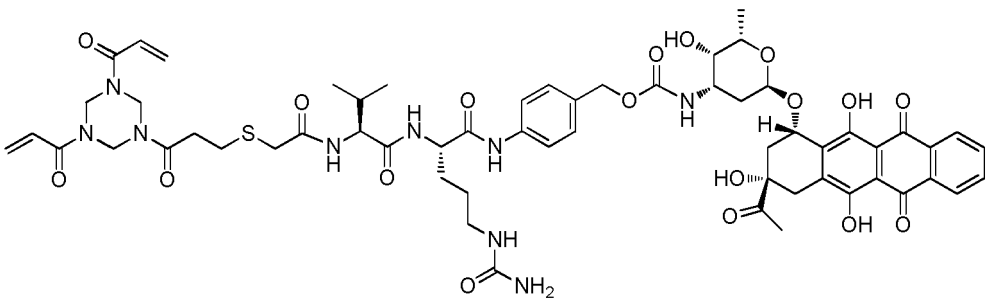
(6)

Py-MAA-Val-Cit-PAB-doxorubicin



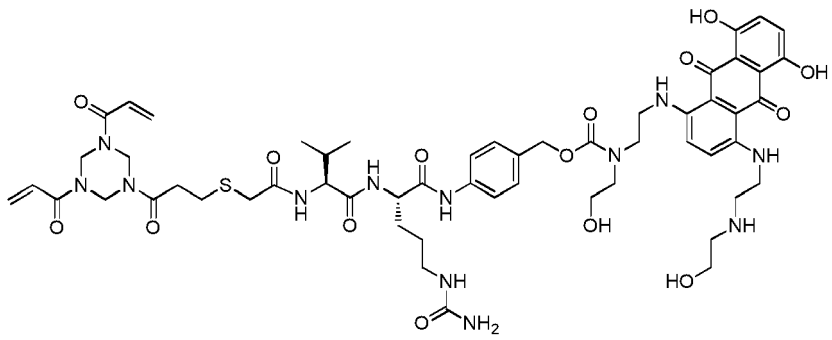
(7)

Py-MAA-Val-Cit-PAB-epirubicin



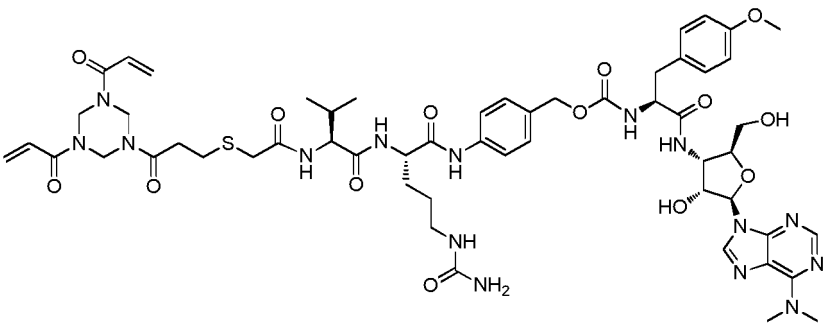
(8)

Py-MAA-Val-Cit-PAB-idarubicin



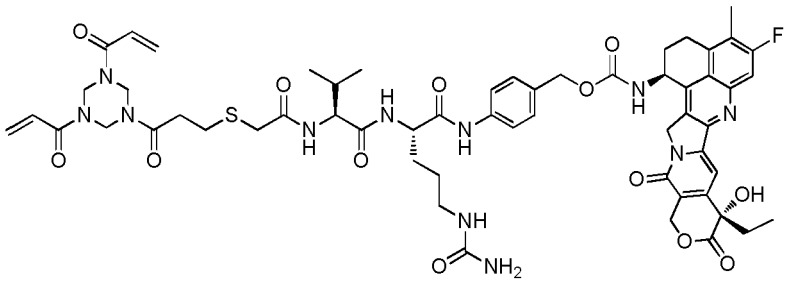
(9)

Py-MAA-Val-Cit-PAB-mitoxantrone



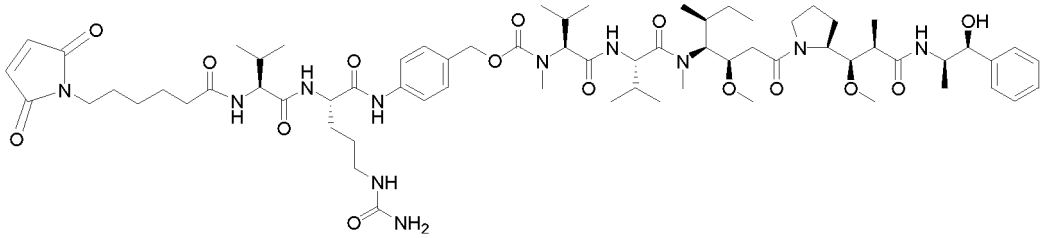
(10)

Py-MAA-Val-Cit-PAB-puromycin



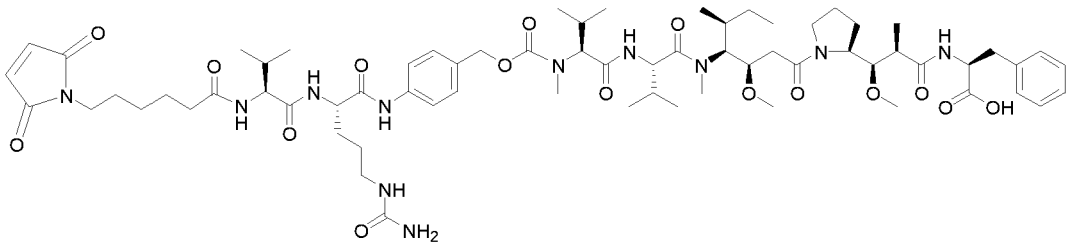
(11)

Py-MAA-Val-Cit-PAB-DX8951



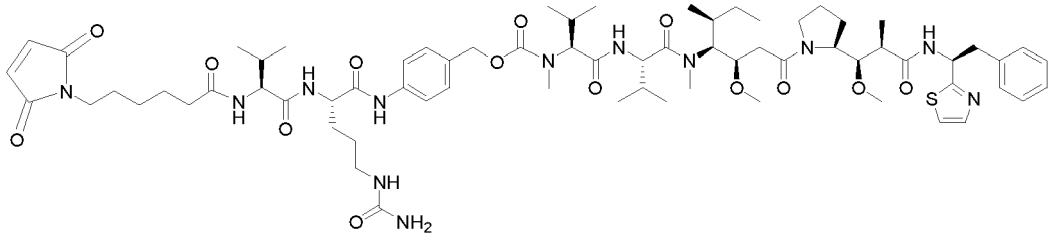
(12)

MC-Val-Cit-PAB-MMAE



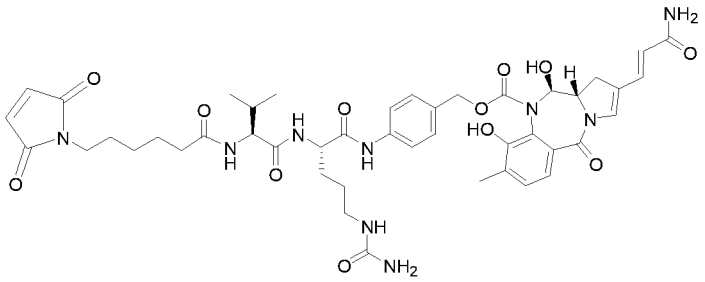
(13)

MC-Val-Cit-PAB-MMAF



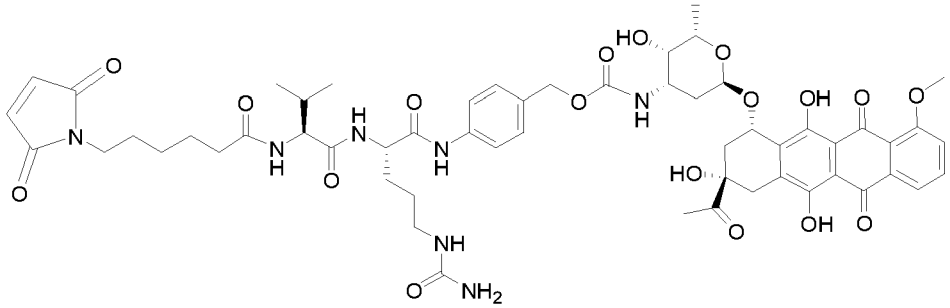
(14)

MC-Val-Cit-PAB-MMAD



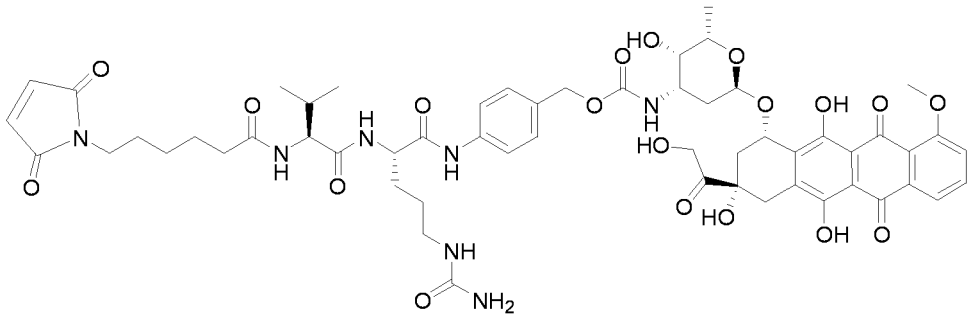
(15)

MC-Val-Cit-PAB-anthramycin



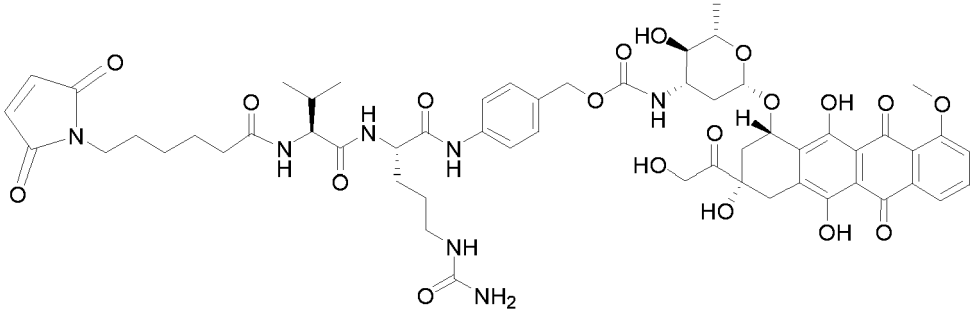
(16)

MC-Val-Cit-PAB-daunorubicin



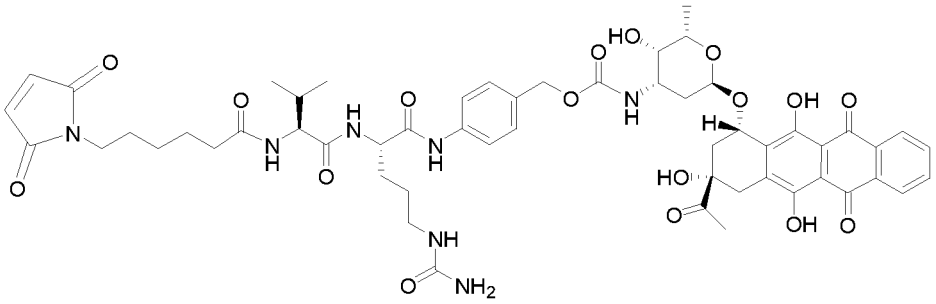
(17)

MC-Val-Cit-PAB-doxorubicin



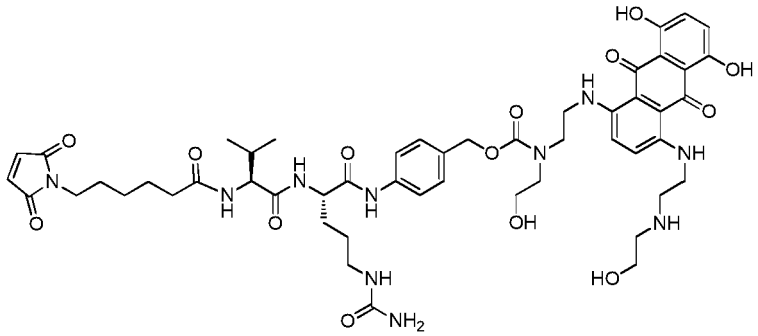
(18)

MC-Val-Cit-PAB-epirubicin



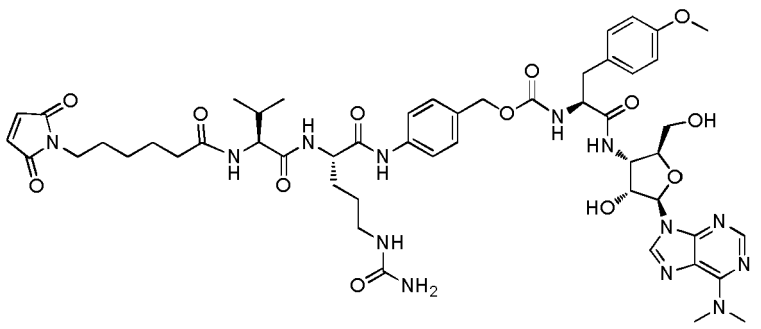
(19)

MC-Val-Cit-PAB-idarubicin



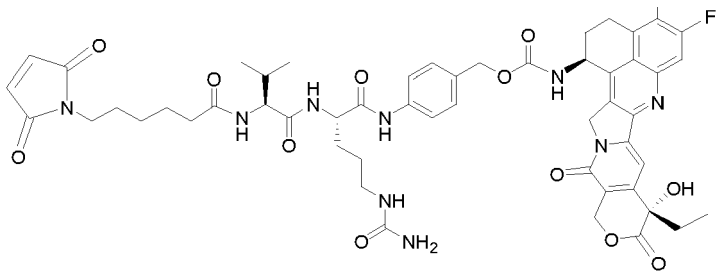
(20)

MC-Val-Cit-PAB-mitoxantrone



(21)

MC-Val-Cit-PAB-puromycin



(22)

MC-Val-Cit-PAB-DX8951

4. The process according to any one of the preceding claims, wherein the organic base 1 and the organic base 2 are each independently one or more of N,N-diisopropylethylamine, triethylamine and pyridine; preferably, the organic base 1 and the organic base 2 are each independently one or two of N,N-diisopropylethylamine and pyridine.

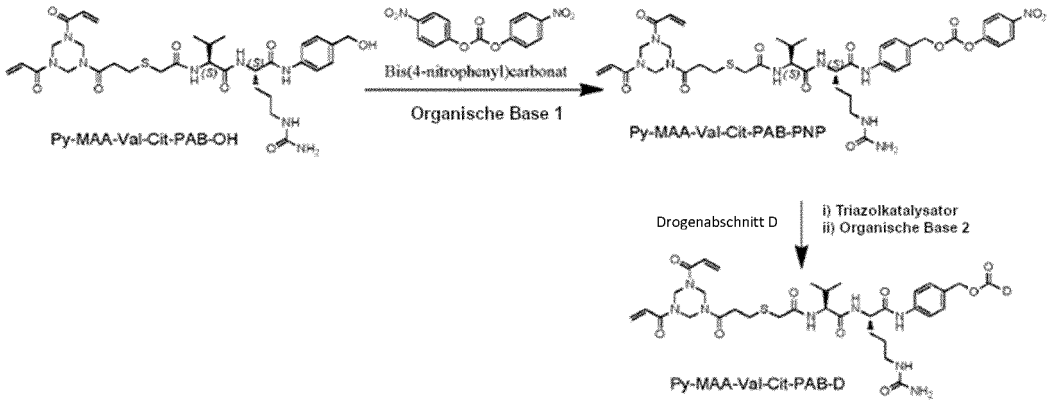
5. The process according to any one of the preceding claims, wherein the triazole catalyst is one or more of 1-hydroxybenzotriazole, 1-hydroxy-7-azobenzotriazole, 1-hydroxy-1H-1,2,3-triazole-4-carboxylic acid ethyl ester, and preferably 1-hydroxybenzotriazole.

6. Use of the process according to any one of claims 1 to 5 in the preparation of an anti-tumor medicament.

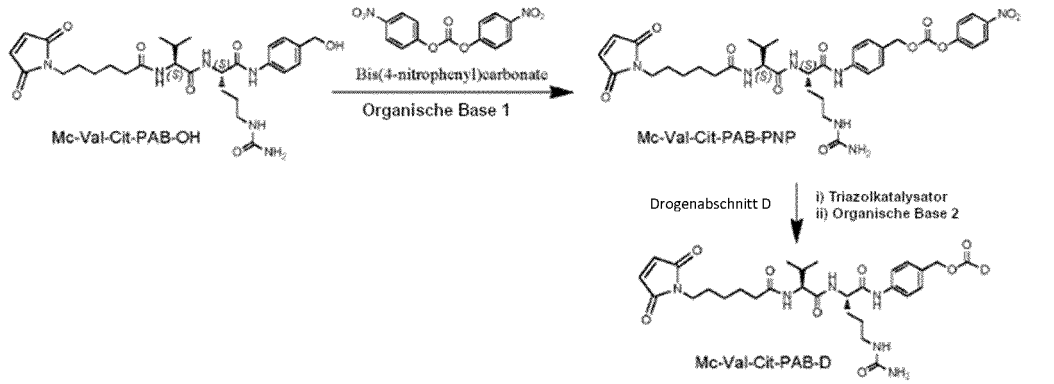
7. Use of the process according to any one of claims 1 to 5 in the preparation of an antibody-drug conjugate.

Patentansprüche

1. Verfahren zum Herstellen eines Zwischenprodukts eines Antikörper-Drogen-Konjugats, umfassend einen Linkerabschnitt und einen Drogenabschnitt, wobei das Zwischenprodukt des Antikörper-Drogen-Konjugats Py-MAA-Val-Cit-PAB-D oder MC-Val-Cit-PAB-D ist, wobei Py-MAA-Val-Cit-PAB oder MC-Val-Cit-PAB im Zwischenprodukt der Linkerabschnitt ist und D im Zwischenprodukt einen Drogenabschnitt darstellt, der ein zytotoxisches Auristatinmittel, ein zytotoxisches Anthramycinmittel, ein zytotoxisches Anthracyclinmittel, ein zytotoxisches Puromycinmittel oder DX8951 ist, wobei das Verfahren den folgenden Reaktionsweg umfasst:



oder



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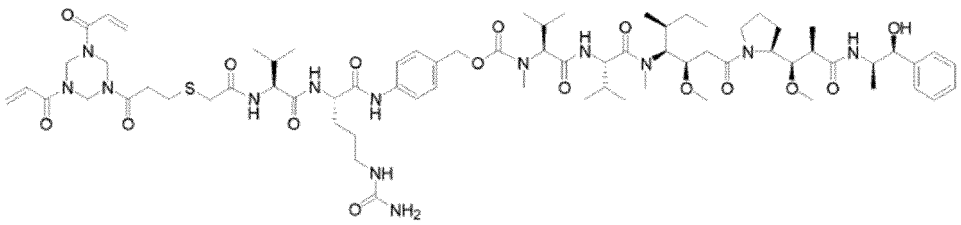
wobei das Herstellungsverfahren ein Eintopfverfahren ist, bei dem zwei Schritte in einem System durchgeführt werden.

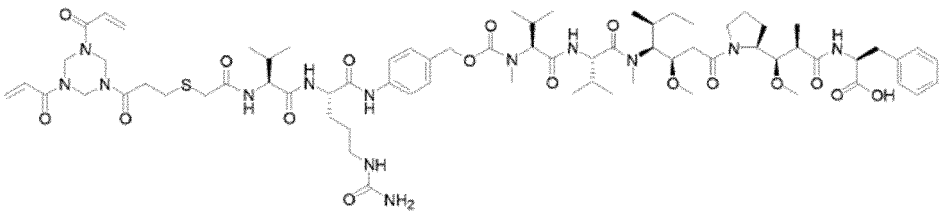
2. Verfahren nach Anspruch 1, wobei das zytotoxische Auristatinmittel MMAE, MMAF oder MMAD ist; das zytotoxische Anthramycinmittel Anthramycin ist; das zytotoxische Anthracyclinmittel Daunorubicin, Doxorubicin, Epirubicin, Idarubicin oder Mitoxantron ist; und das zytotoxische Puromycinmittel Puromycin ist.

3. Verfahren nach Anspruch 2, wobei Py-MAA-Val-Cit-PAB-D oder MC-Val-Cit-PAB-D eine Struktur aufweist wie in den folgenden Formeln (1-22) gezeigt:



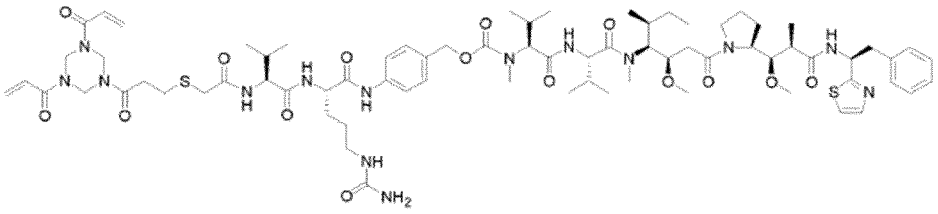
(1)

Py-MAA-Val-Cit-PAB-MMAE



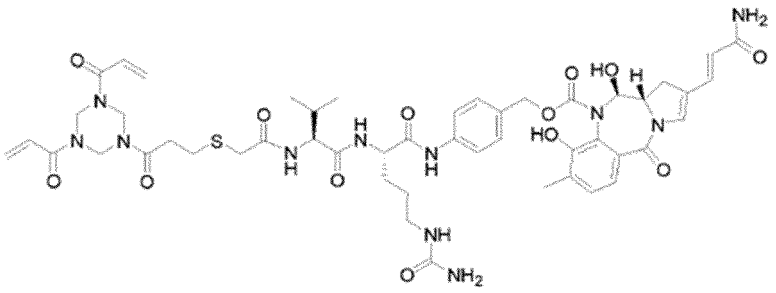
(2)

Py-MAA-Val-Cit-PAB-MMAF



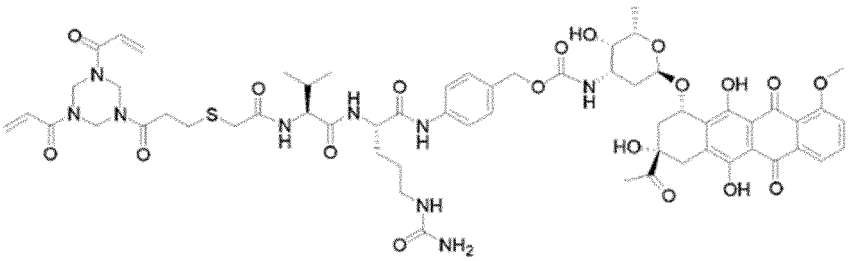
(3)

Py-MAA-Val-Cit-PAB-MMAD



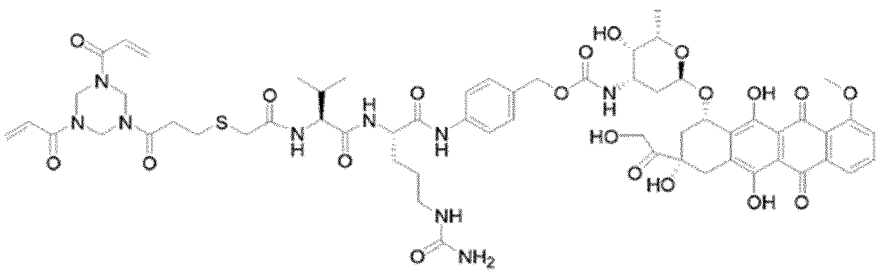
(4)

Py-MAA-Val-Cit-PAB-Anthramycin



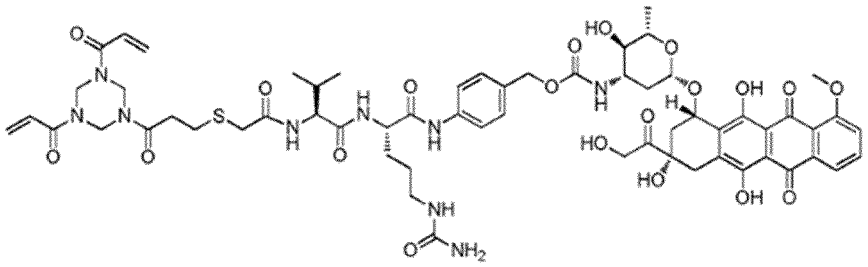
(5)

Py-MAA-Val-Cit-PAB-Daunorubicin



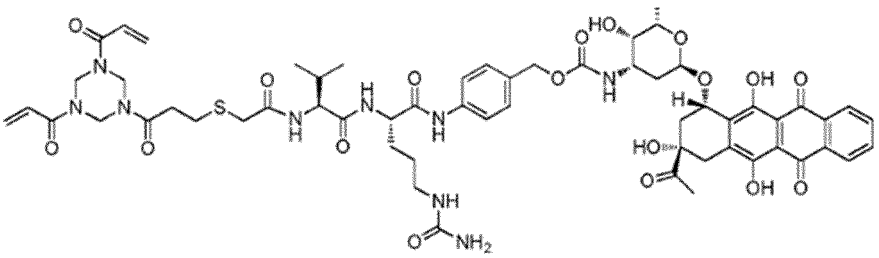
(6)

Py-MAA-Val-Cit-PAB-Doxorubicin



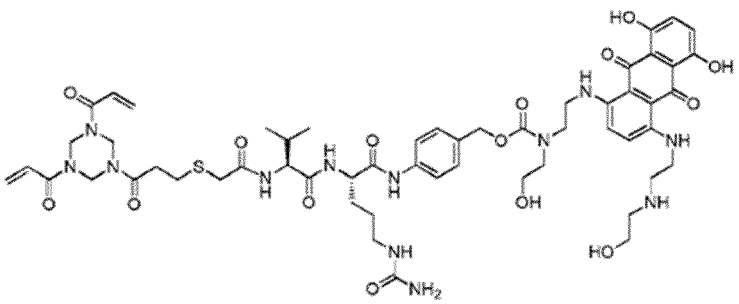
(7)

Py-MAA-Val-Cit-PAB-Epirubicin



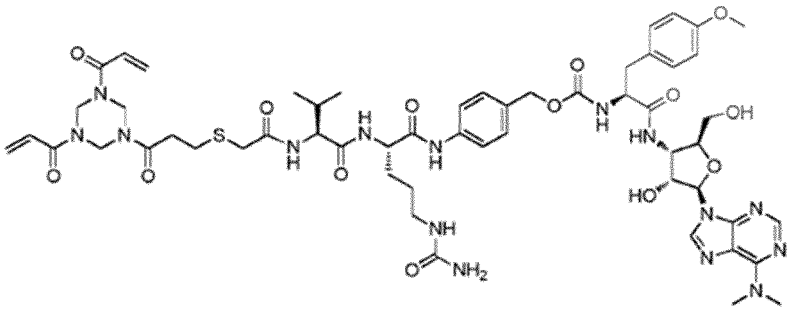
(8)

Py-MAA-Val-Cit-PAB-Idarubicin



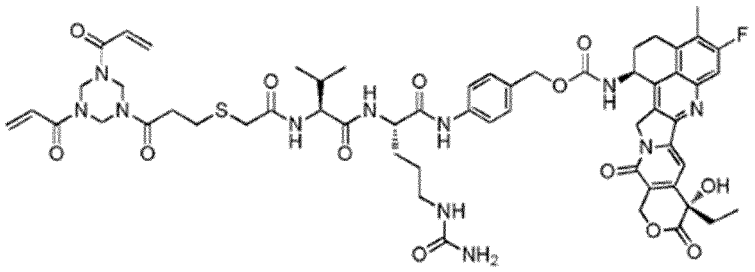
(9)

Py-MAA-Val-Cit-PAB-Mitoxantron



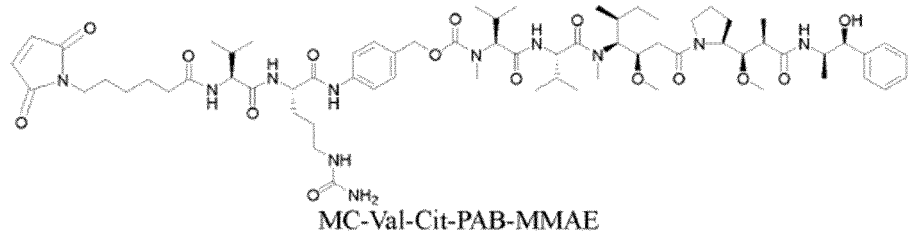
(10)

Py-MAA-Val-Cit-PAB-Puromycin



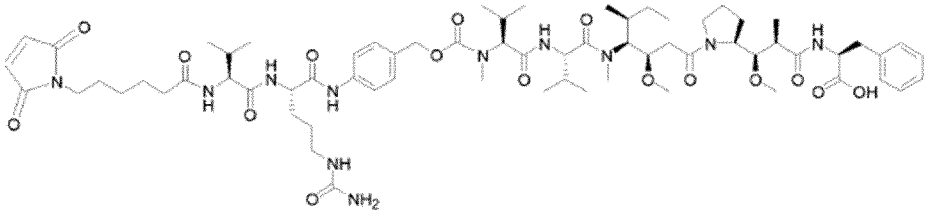
(11)

Py-MAA-Val-Cit-PAB-DX8951



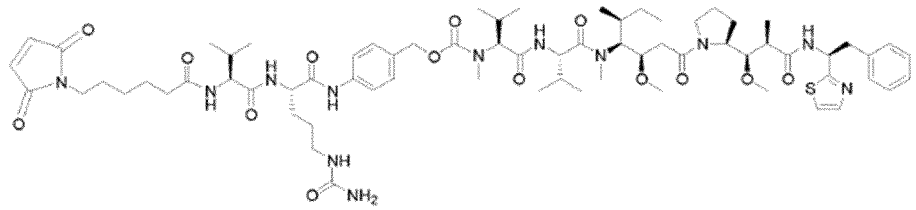
(12)

MC-Val-Cit-PAB-MMAE



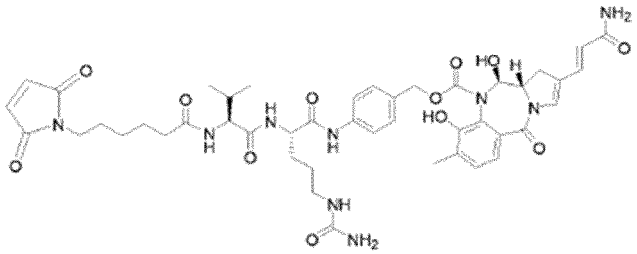
(13)

MC-Val-Cit-PAB-MMAF



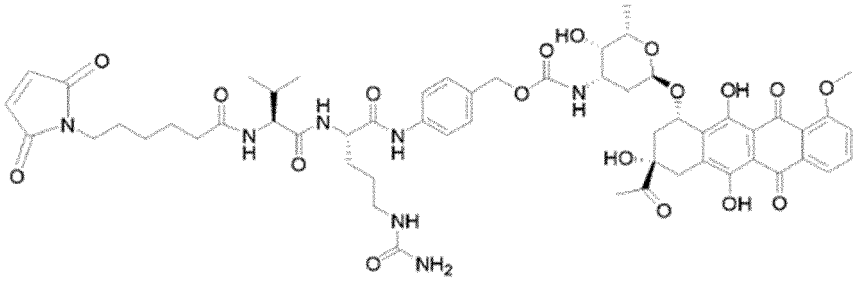
(14)

MC-Val-Cit-PAB-MMAD



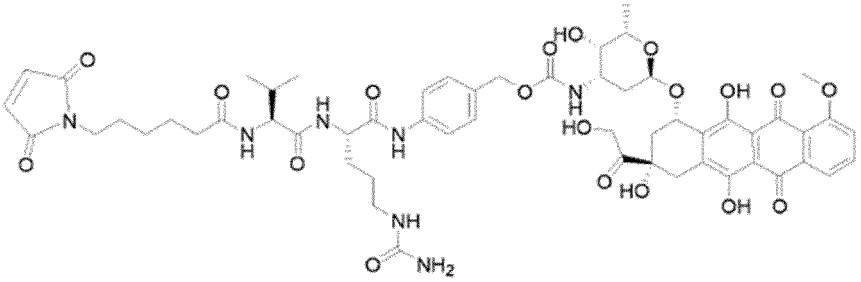
(15)

MC-Val-Cit-PAB-Anthramycin



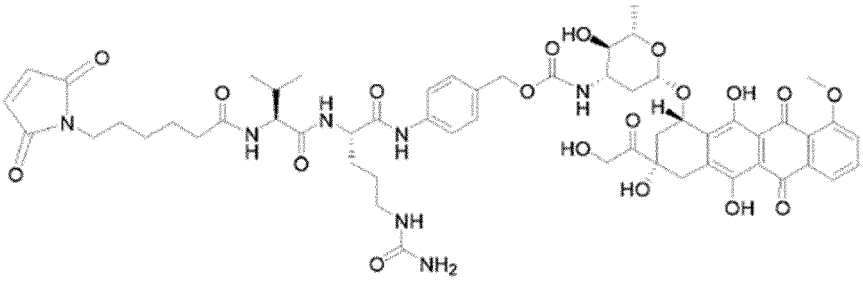
(16)

MC-Val-Cit-PAB-Daunorubicin



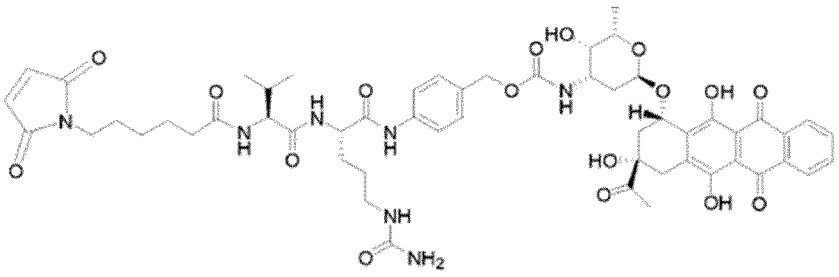
(17)

MC-Val-Cit-PAB-Doxorubicin



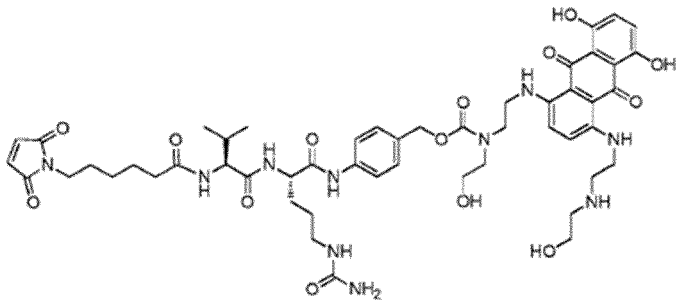
(18)

MC-Val-Cit-PAB-Epirubicin



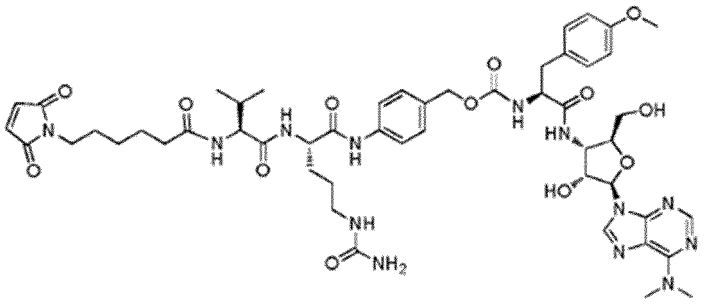
(19)

MC-Val-Cit-PAB-Idarubicin



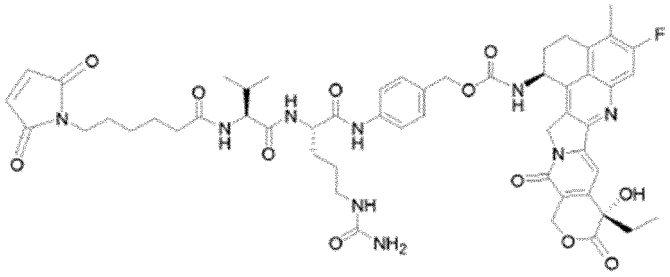
(20)

MC-Val-Cit-PAB-Mitoxantron



(21)

MC-Val-Cit-PAB-Puromycin



(22)

MC-Val-Cit-PAB-DX8951

4. Verfahren nach einem der vorhergehenden Ansprüche, wobei die organische Base 1 und die organische Base 2 jeweils unabhängig eines oder mehrere von N,N-Diisopropylethylamin, Triethylamin und Pyridin sind; vorzugsweise wobei die organische Base 1 und die organische Base 2 jeweils unabhängig eines oder zwei von N,N-Diisopropylethylamin und Pyridin sind.

5. Verfahren nach einem der vorhergehenden Ansprüche, wobei der Triazolkatalysator eines oder mehrere von 1-Hydroxybenzotriazol, 1-Hydroxy-7-azobenzotriazol, 1-Hydroxy-1H-1,2,3-triazol-4-carbonsäureethylester und vorzugsweise 1-Hydroxybenzotriazol ist.

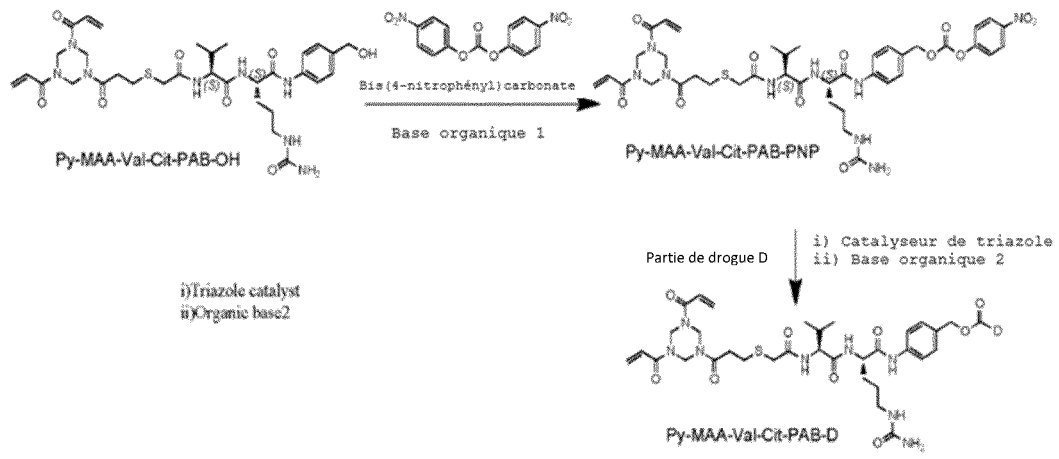
6. Verwendung des Verfahrens nach einem der Ansprüche 1 bis 5 bei der Herstellung eines Antitumor-Medikaments.

7. Verwendung des Verfahrens nach einem der Ansprüche 1 bis 5 bei der Herstellung eines Antikörper-Drogen-Konjugats.

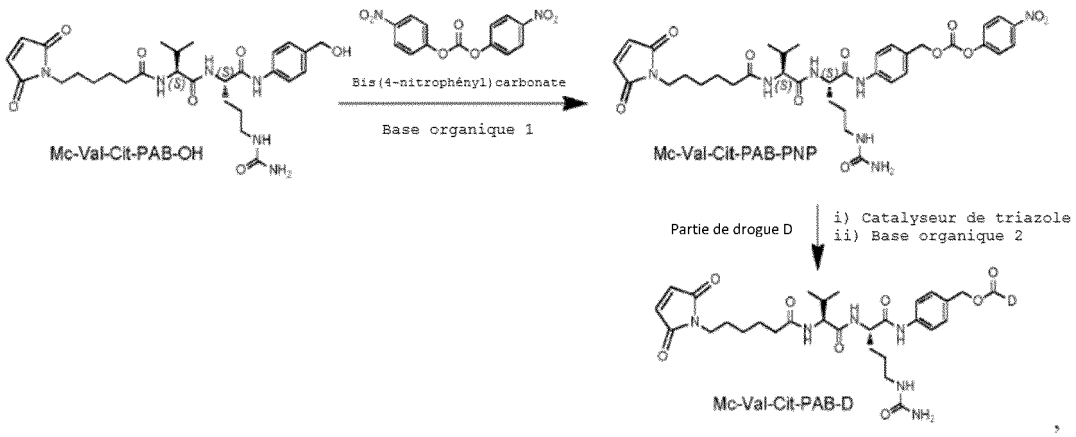
Revendications

1. Procédé pour la préparation d'un intermédiaire de conjugué anticorps-drogue comprenant une partie lieur et une partie drogue, l'intermédiaire de conjugué anticorps-drogue étant Py-MAA-Val-Cit-PAB-D ou MC-Val-Cit-PAB-D,

Py-MAA-Val-Cit-PAB ou MC-Val-Cit-PAB dans l'intermédiaire étant la partie lieur, et D dans l'intermédiaire représentant une partie drogue qui est un agent cytotoxique de type auristatine, un agent cytotoxique de type anthramycine, un agent cytotoxique de type anthracycline, un agent cytotoxique de type puromycine, ou DX8951, le procédé comprenant le mécanisme réactionnel suivant :



ou



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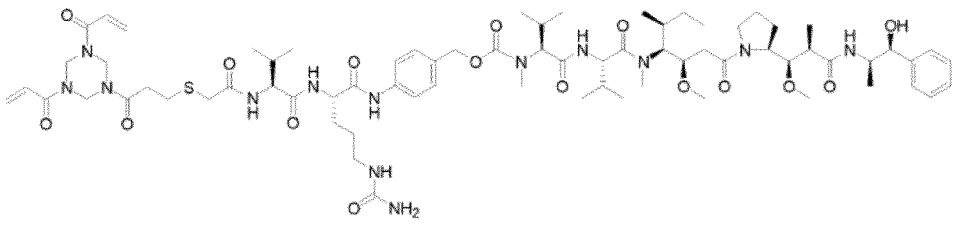
le procédé de préparation étant un procédé en un pot dans lequel deux étapes sont réalisées dans un système.

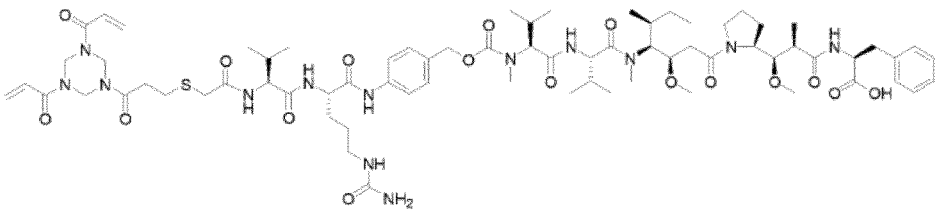
2. Procédé selon la revendication 1, l'agent cytotoxique de type auristatine étant MMAE, MMAF, ou MMAD ; l'agent cytotoxique de type anthramycine étant l'anthramycine ; l'agent cytotoxique de type anthracycline étant la daunorubicine, la doxorubicine, l'épirubicine, l'idarubicine, ou la mitoxantrone ; et l'agent cytotoxique de type puromycine étant la puromycine.

3. Procédé selon la revendication 2, Py-MAA-Val-Cit-PAB-D ou MC-Val-Cit-PAB-D ayant une structure comme présenté dans les formules suivantes (1 à 22) :



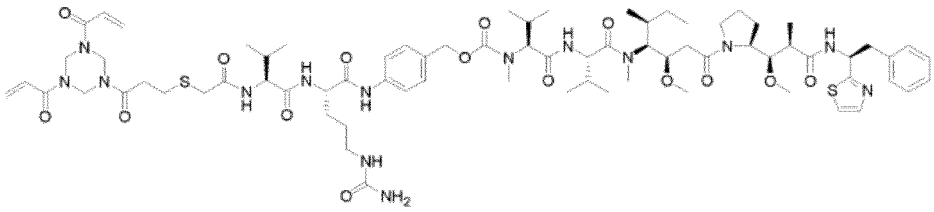
(I)

Py-MAA-Val-Cit-PAB-MMAE



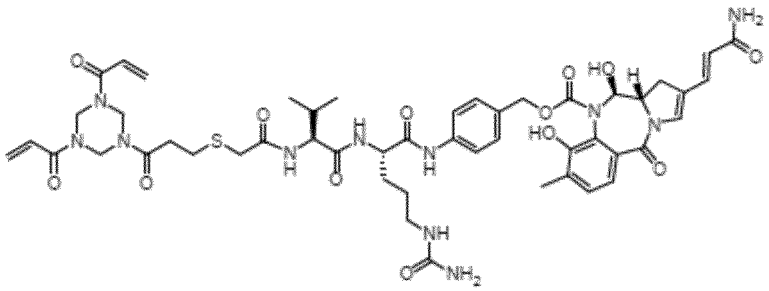
(2)

Py-MAA-Val-Cit-PAB-MMAF



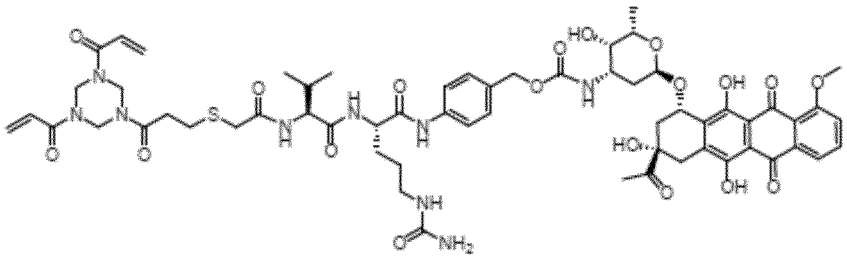
(3)

Py-MAA-Val-Cit-PAB-MMAD



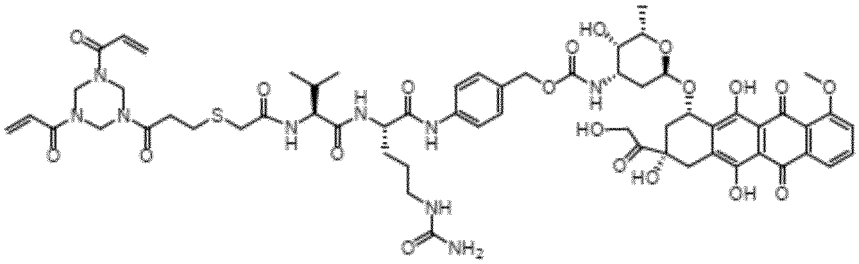
(4)

Py-MAA-Val-Cit-PAB-anthramycine



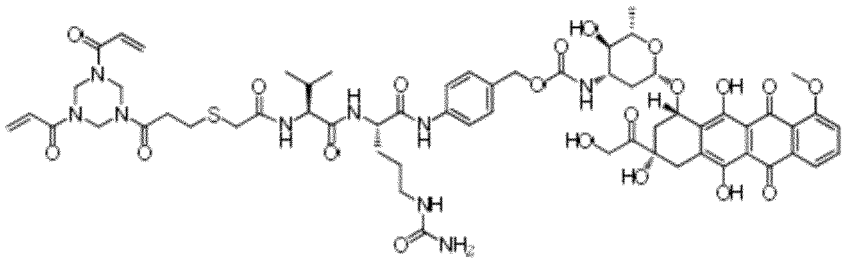
(5)

Py-MAA-Val-Cit-PAB-daunorubicine



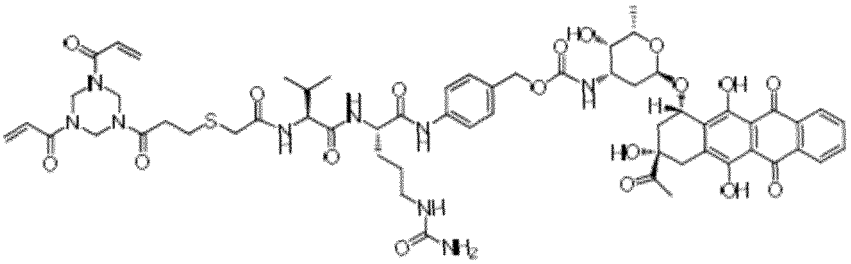
(6)

Py-MAA-Val-Cit-PAB-doxorubicine



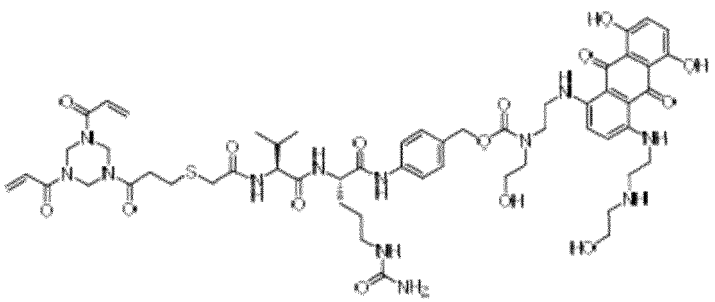
(7)

Py-MAA-Val-Cit-PAB-épirubicine



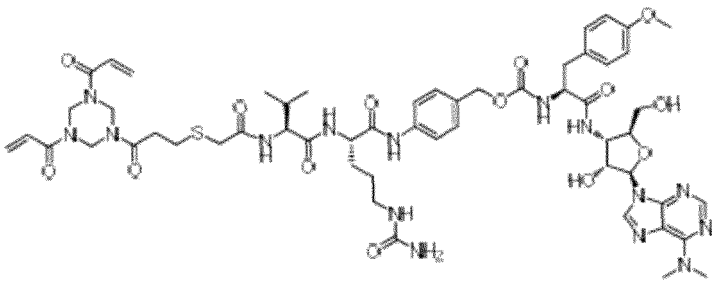
(8)

Py-MAA-Val-Cit-PAB-idarubicine



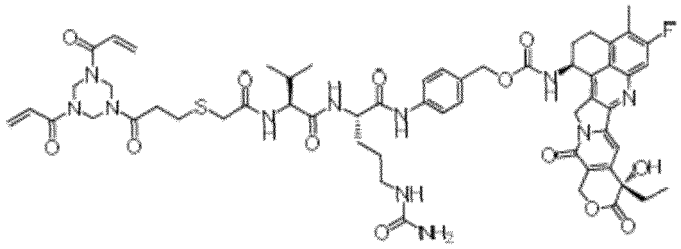
(9)

Py-MAA-Val-Cit-PAB-mitoxantrone



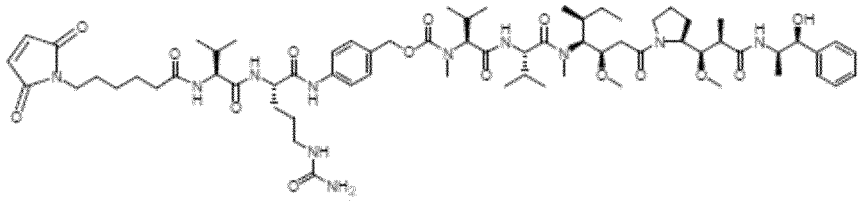
(10)

Py-MAA-Val-Cit-PAB-puromycine



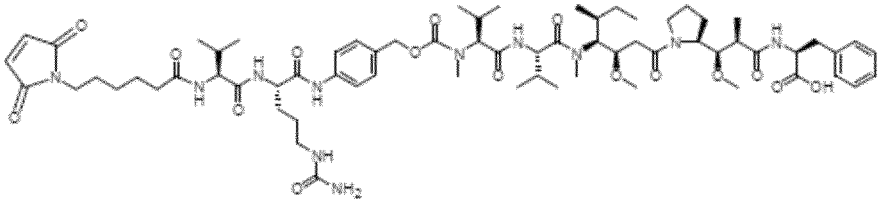
(11)

Py-MAA-Val-Cit-PAB-DX8951



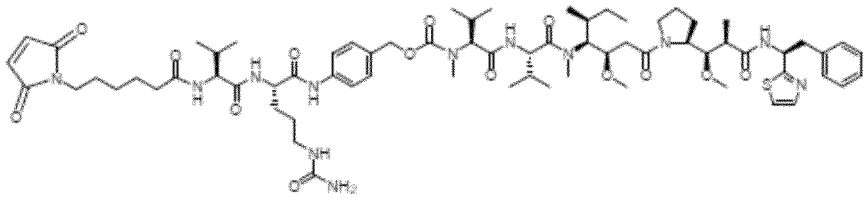
(12)

MC-Val-Cit-PAB-MMAE



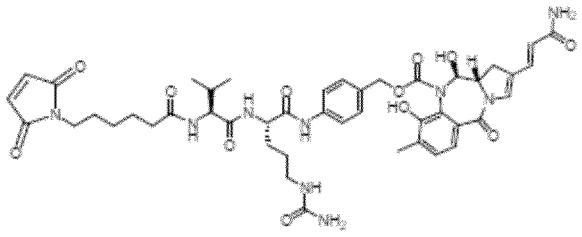
(13)

MC-Val-Cit-PAB-MMAF



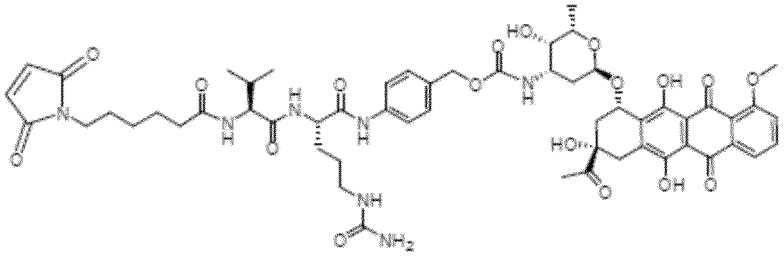
(14)

MC-Val-Cit-PAB-MMAD



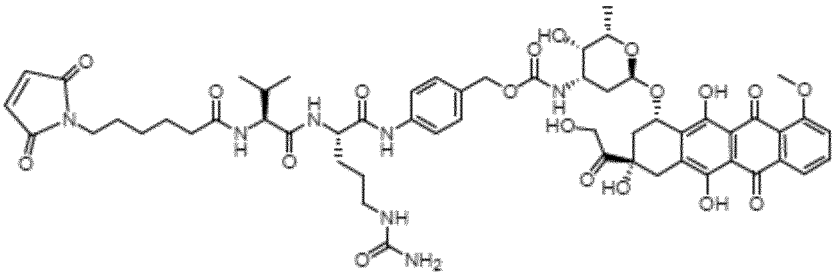
(15)

MC-Val-Cit-PAB-anthramycine



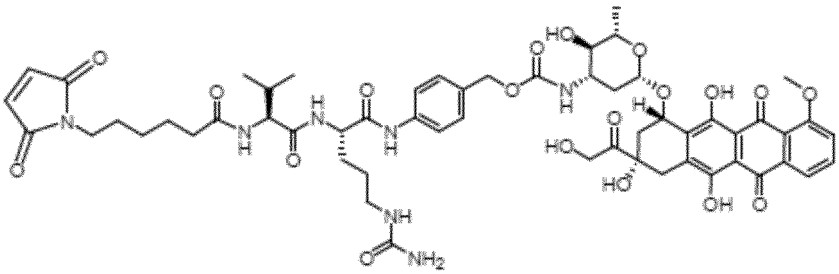
(16)

MC-Val-Cit-PAB-daunorubicine



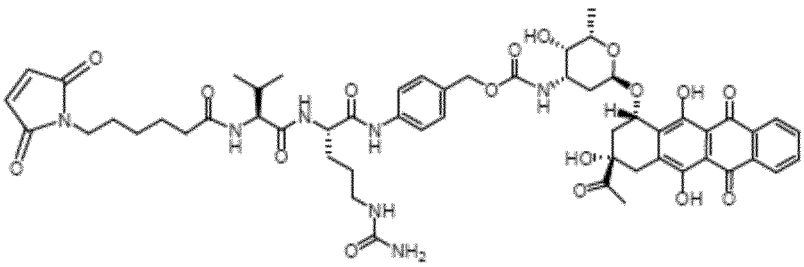
(17)

MC-Val-Cit-PAB-doxorubicine



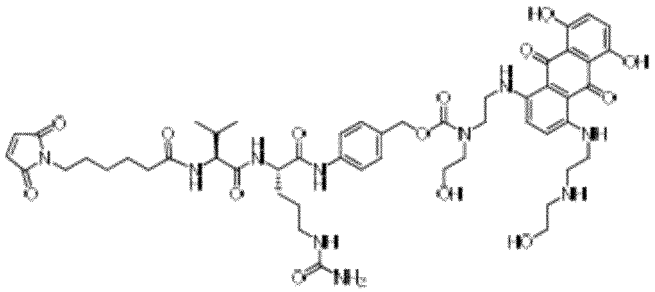
(18)

MC-Val-Cit-PAB-épirubicine



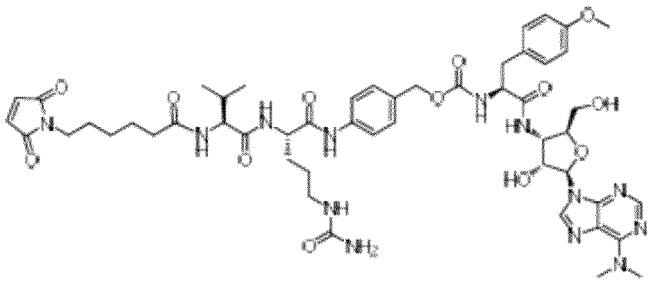
(19)

MC-Val-Cit-PAB-idarubicine



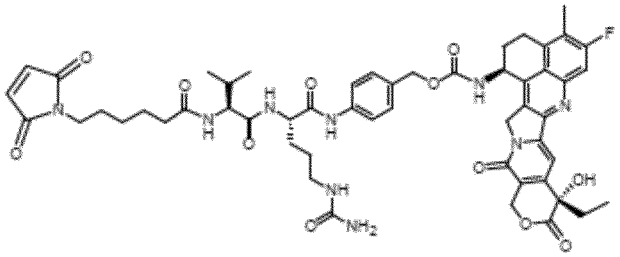
(20)

MC-Val-Cit-PAB-mitoxantrone



(21)

MC-Val-Cit-PAB-puromycine



(22)

MC-Val-Cit-PAB-DX8951

4. Procédé selon l'une quelconque des revendications précédentes, la base organique 1 et la base organique 2 étant chacune indépendamment l'une ou plusieurs parmi la N,N-diisopropyléthylamine, la triéthylamine et la pyridine ; préférablement, la base organique 1 et la base organique 2 étant chacune indépendamment une ou deux parmi la N,N-diisopropyléthylamine et la pyridine.

5. Procédé selon l'une quelconque des revendications précédentes, le catalyseur de triazole étant l'un ou plusieurs parmi le 1-hydroxybenzotriazole, le 1-hydroxy-7-azobenzotriazole, l'ester éthylique de l'acide 1-hydroxy-1H-1,2,3-triazole-4-carboxylique, et préférablement le 1-hydroxybenzotriazole.

6. Utilisation du procédé selon l'une quelconque des revendications 1 à 5 dans la préparation d'un médicament anti-tumoral.

7. Utilisation du procédé selon l'une quelconque des revendications 1 à 5 dans la préparation d'un conjugué anticorps-drogue.

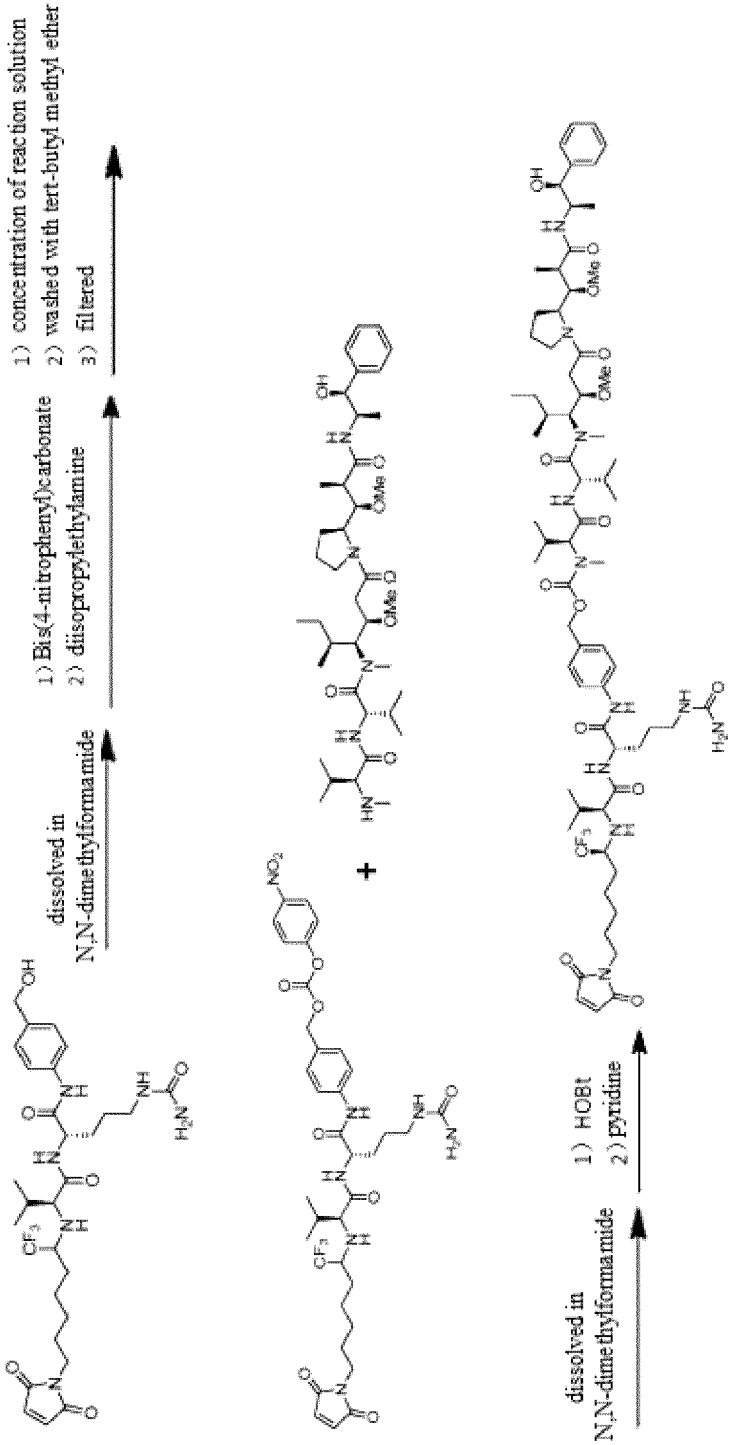

dissolved in
N,N-dimethylformamide
1) Bis(4-nitrophenyl)carbonate
2) diisopropylethylamine
1) concentration of reaction solution
2) washed with tert-butyl methyl ether
3) filtered
dissolved in
N,N-dimethylformamide
1) HOBt
2) pyridine

Fig. 1

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- CN 107427591 A **[0003] [0019]**
- CN 109200291 **[0003]**
- CN 107921030 A **[0005]**

Non-patent literature cited in the description

- *CHEMICAL ABSTRACTS,* 959-52-4 **[0005]**
- *CHEMICAL ABSTRACTS,* 159857-80-4 **[0067]**