LINKER, ANTIBODY-DRUG CONJUGATE AND USE THEREOF

Technical Field

The present invention belongs to the field of biomedicine and relates to a linker, an antibody-drug conjugate, a preparation method therefor and the use thereof in the prevention and/or treatment of diseases related to abnormal cell activity, including but not limited to the prevention and/or treatment of tumor diseases.

Background Art

CD38 is a transmembrane glycoprotein that exerts its biological functions as a cell surface receptor and extracellular enzyme. As a cell surface receptor, CD38 is involved in leukocyte adhesion and migration, and promotes lymphocyte growth, survival, and activation. The enzymatic activity of CD38 is manifested as catalyzing the conversion of the NAD^+ substrate into products such as ADPR and cADPR. As an important second messenger in cells, ADPR/cADPR can affect functions such as cell growth or cell activation by regulating cellular calcium ions. The products ADPR/cADPR produced by the catalysis can be further acted upon by other enzymes to generate adenosine, and the adenosine exerts anti-tumor immunosuppressive functions by recruiting MDSC and Treg cells.*(Malavasi F, Deaglio S, Funaro A, et al. Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiol Rev. 2008;88 (3):841-886. Wo YJ, Gan ASP, Lim X, et al. The Roles of CD38 and CD157 in the Solid Tumor Microenvironment and Cancer Immunotherapy. Cells. 2019;9 (1):26. Published 2019 Dec 20*). CD38 is expressed in multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL), including diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL) and Burkitt's lymphoma *(Calabretta E, Carlo-Stella C. The Many Facets of CD38 in Lymphoma: From Tumor-Microenvironment Cell Interactions to Acquired Resistance to Immunotherapy. Cells. 2020;9 (4):802).*

Daratumumab is a monoclonal antibody targeting CD38 and has been approved by the National Medical Products Administration (NMPA) for the treatment of adult patients with relapsed and refractory MM. However, most MM patients have primary drug resistance to Daratumumab monotherapy, and a small number of MM patients who

respond will eventually acquire resistance *(Saltarella I, Desantis V, Melaccio A, et al. Mechanisms of Resistance to Anti-CD38 Daratumumab in Multiple Myeloma. Cells. 2020;9(1):167).* Clinical trials have shown that Daratumumab monotherapy demonstrates a lower objective response rate (ORR) in MM patients with medium or low CD38 expression compared with MM patients with high CD38 expression *(Nijhof IS, Casneuf T, van Velzen J, et al. CD38 expression and complement inhibitors affect response and resistance to daratumumab therapy in myeloma. Blood. 2016;128(7):959-970).* In addition, Daratumumab monotherapy demonstrates an ORR of only about 8.3% in patients with CD38-expressing NHL, including diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and mantle cell lymphoma (MCL) (*Salles G, Gopal AK, Minnema MC, et al. Phase 2 Study of Daratumumab in Relapsed/Refractory Mantle-Cell Lymphoma, Diffuse Large B-Cell Lymphoma, and Follicular Lymphoma. Clin Lymphoma Myeloma Leuk. 2019;19(5):275-284).* Therefore, there is an urgent need in the art for CD38-targeting antibody drugs with higher biological activity, especially antibody-drug conjugates (ADCs) that are effective against primary resistance or acquired resistance to Daratumumab, so as to further improve efficacy and reduce side effects.

An antibody-drug conjugate (ADC) is composed of an antibody, a payload, and a linker. The bioactive molecular payload (e.g., monomethyl auristatin E (MMAE)) is covalently conjugated to the antibody via a linker. Antibodies (such as monoclonal antibodies) can specifically recognize specific targets on the surface of tumor cells, thereby guiding ADCs to the surface of cancer cells and enabling them to enter into cancer cells through endocytosis. The linker is then broken under the action of low pH values, lysosomal enzymatic reactions, etc. in the cells, and the payload is released, thereby achieving the effect of specifically killing cancer cells without damaging normal tissue cells. The ideal linker should ensure that the ADC drug maintains sufficient stability and integrity in the blood circulation, and upon reaching therapeutic targets (tumor cells), can efficiently release the payload. *(Chau CH, Steeg PS, Figg WD. Antibody-drug conjugates for cancer. Lancet. 2019;394 (10200):793-804. Drago JZ, Modi S, Chandarlapaty S. Unlocking the potential of antibody-drug conjugates for cancer therapy. Nat Rev Clin Oncol. 2021;18 (6):327-344).*

Compared with the Daratumumab antibody drug, the CD38-targeting ADC of the present invention links the CD38-targeting antibody to a potent payload via a novel

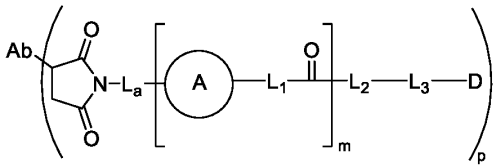
cleavable linker, specifically kills CD38-expressing cancer cells without damaging normal tissue cells, and thus has stronger anti-tumor activity. Currently, no CD38-targeting ADCs with MMAE as a payload have entered clinical research. Only one ADC, i.e., STI-6129, with an MMAF analog as a payload has entered phase I clinical research. However, the MMAF payload is prone to causing clinical ocular toxicity *(Mahalingaiah PK, Ciurlionis R, Durbin KR, et al. Potential mechanisms of target-independent uptake and toxicity of antibody-drug conjugates. Pharmacol Ther. 2019;200:110-125.)*, which obviously does not meet medical needs.

The present invention is dedicated to developing a novel CD38-targeting antibody drug, a novel linker, and an antibody-drug conjugate comprising the linker to meet clinical needs.

Summary of the Invention

The present invention provides a novel linker, an antibody-drug conjugate comprising the linker, and the preparation and use thereof. The present invention also provides a novel anti-CD38 antibody or an antigen-binding fragment thereof that binds to CD38 molecules, an antibody-drug conjugate comprising same, and the preparation and use thereof.

In a first aspect, the present invention provides a drug conjugate represented by formula (III),



(III)

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof,

wherein Ab is a targeting moiety such as an antibody or an antigen-binding fragment thereof;

p is an integer selected from 1-16, for example, an integer selected from 1-10, 1-9, 2-8, 4-10, 6-8, 3-7, 4-8 or 2-6, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 12;

D is a drug active molecule;

L_a is selected from -C_{1-12} alkylene-, -C_{1-12} alkylene-C(O)-, -C_{1-12} alkylene-CONH-$(CH_2CH_2O)_n$-CH_2-C(O)- or -$(CH_2CH_2O)_n$-CH_2-C(O)-; n is an integer selected from 1-18, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

"ring A" is an optionally substituted 3- to 8-membered heterocycloalkylene or 6- to 10-membered heteroarylene;

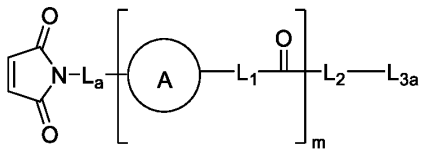
L_1 is selected from -CH_2-CHR_1- or -O-CH_2-, or is absent; R_1 is -OH, -NH_2, -C_{1-4} alkylene-OH or -C_{1-4} alkylene-NH_2;

L_2 is a linking unit selected from short peptide residues consisting of 2-10 amino acids, wherein the amino acids are natural amino acids or non-natural amino acids;

L_3 is a spacer unit; and

m is 1, 2, 3 or 4.

In a second aspect, the present invention provides a compound represented by formula (I):



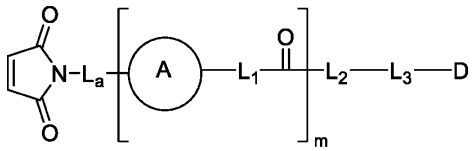
(I) ,

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein

L_a, ring A, L_1, L_2 and m are as defined herein;

L_{3a} is a spacer unit.

In a third aspect, the present invention provides a compound represented by formula (II),



(II)

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein each variable is as defined herein.

In a fourth aspect, the present invention provides a novel anti-CD38 antibody or an antigen-binding fragment thereof that binds to CD38 molecules, which is an isolated anti-

CD38 antibody or an antigen-binding fragment thereof comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the heavy chain variable region comprises three heavy chain complementarity determining regions (HCDRs) in SEQ ID NO: 7 or 9, and the light chain variable region comprises three light chain complementarity determining regions (LCDRs) in SEQ ID NO: 8 or 10.

In another aspect, the present invention provides an isolated nucleic acid encoding any anti-CD38 antibody or antigen-binding fragment thereof provided by the present invention.

In another aspect, the present invention provides a recombinant vector or expression vector comprising one or more nucleic acids provided by the present invention, wherein the vector is suitable for recombinant production of any anti-CD38 antibody or antigen-binding fragment thereof provided by the present invention.

In another aspect, the present invention provides a host cell comprising one or more nucleic acids, recombinant vectors or expression vectors provided by the present invention.

In another aspect, the present invention provides an immunoconjugate or immune fusion comprising the anti-CD38 antibody or the antigen-binding fragment thereof provided by the present invention.

In another aspect, the present invention provides a drug conjugate comprising the anti-CD38 antibody or the antigen-binding fragment thereof provided by the present invention.

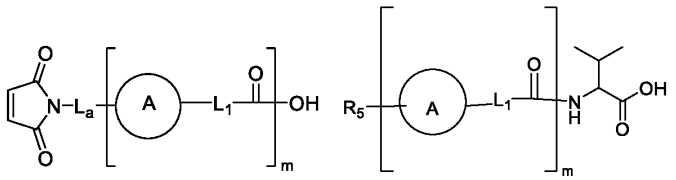
In another aspect, the present invention provides a pharmaceutical composition comprising the compound of the present invention, such as the anti-CD38 antibody or the antigen-binding fragment thereof, the nucleic acid, the recombinant vector or the expression vector, the host cell, the immunoconjugate or the immune fusion, and the drug conjugate provided by the present invention, and optionally at least one pharmaceutically acceptable adjuvant material (such as a pharmaceutical carrier or a pharmaceutical excipient).

In another aspect, the present invention provides a pharmaceutical combination comprising the compound of the present invention, such as the anti-CD38 antibody or the antigen-binding fragment thereof, the nucleic acid, the recombinant vector or the expression vector, the host cell, the immunoconjugate or the immune fusion, and the drug

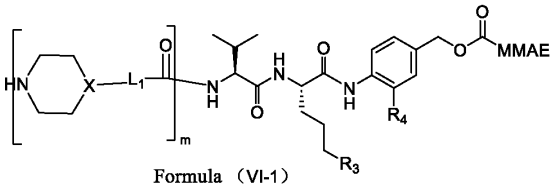
conjugate provided by the present invention, and one or more additional therapeutic agents.

In another aspect, the present invention also provides the use of the anti-CD38 antibody or the antigen-binding fragment thereof, the nucleic acid, the recombinant vector or the expression vector, the host cell, the immunoconjugate or the immune fusion, and the drug conjugate of the present invention in the preparation of a drug for treating cancer.

In a fifth aspect, the present invention provides intermediates represented by formula (IV-1), formula (V-1) and formula (VI-1):



Formula (IV-1) , Formula (V-1) ,



Formula (VI-1) ,

wherein R_5 is an amino protecting group; and each variable, such as L_a, ring A, L_1, X, m, R_3 or R_4, is as defined herein.

Detailed Description of the Invention

Definitions

In the present invention, unless otherwise specified, the scientific and technical terms used herein have the meanings commonly understood by a person skilled in the art, and the laboratory operation steps for cell culture, molecular genetics, nucleic acid chemistry and immunology used herein are all conventional steps widely used in the corresponding fields. Moreover, in order to better understand the present invention, definitions and explanations of relevant terms are provided below.

The term "amino acid residue" refers to an amino acid unit in a peptide chain, that is, the remaining part of an amino acid after dehydration and linked by a peptide bond. The abbreviation for an amino acid residue is a standard three-letter and/or one-letter code used in the field to refer to one of the commonly used amino acids. For example, the

amino acids forming amino acid residues can be selected from phenylalanine (Phe), tyrosine (Tyr), leucine (Leu), glycine (Gly), alanine (Ala), valine (Val), lysine (Lys), citrulline (Cit), serine (Ser), glutamic acid (Glu), aspartic acid (Asp), asparagine (Asn), isoleucine (Ile), arginine (Arg), proline (Pro), glutamine (Gln), etc. The amino acids may be L- or D-isomers, preferably L-isomers.

Unless explicitly stated otherwise, singular forms used in the present application (including the claims) include the corresponding plural forms thereof.

When used to connect two or more optional items, the term "and/or" should be understood to mean any one of the optional items or any two or more of the optional items.

As used in the present invention, the term "comprising" or "including" means to include the mentioned elements, integers or steps, but does not exclude any other elements, integers, or steps. As used herein, when the term "comprising" or "including" is used, unless otherwise stated, examples consisting of the mentioned elements, integers or steps are also encompassed. For example, when referring to an antibody variable region "comprising" a specific sequence, it is also intended to encompass an antibody variable region composed of the specific sequence.

In the present invention, the term "subject" includes human or non-human animals. Exemplary human subjects include human suffering from a disease (e.g., a disease described herein) (referred to as a patient) or a normal subject. The term "non-human animals" in the present invention includes all vertebrates, such as non-mammals (e.g., birds, amphibians, and reptiles) and mammals, such as non-human primates, livestock and/or domesticated animals (e.g., sheep, dogs, cats, cows, and pigs). The term "subject" or "patient" does not denote a particular age or sex. In some embodiments, the subject or patient is a human.

In the present invention, the term "effective amount" refers to an amount of a compound that, after administration, will alleviate to some extent the conditions being treated or one or more symptoms thereof.

The term "alkyl" as used herein refers to a fully saturated straight or branched chain hydrocarbon radical. The alkyl preferably contains 1 to 16 carbon atoms (C_{1-16}), such as 1-12 carbon atoms (C_{1-12}), 1-10 carbon atoms (C_{1-10}), 1-6 carbon atoms (C_{1-6}) or 1-4 carbon atoms (C_{1-4}). Examples of alkyl include, but are not limited to, methyl, ethyl, n-

propyl, isopropyl, n-butyl, s-butyl, i-butyl, t-butyl, n-pentyl, i-pentyl, n-pentyl, n-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl and n-decyl.

The term "alkylene" refers to an alkyl radical as defined above, but which is divalent, i.e., has two single bonds connecting two other radicals. Examples of alkylene include, but are not limited to -CH_2-, -CH_2CH_2-, - $CH_2CH_2CH_2$-, - $CH_2CH_2CH_2CH_2$-, - $CH_2CH_2CH_2CH_2CH_2$-, - $CH_2CH_2CH_2CH_2CH_2CH_2$-, - $CH_2CH_2CH_2CH_2CH_2CH_2CH_2$-, - $CH(-CH_2CH_3)$- or - $CH_2CH(-CH_3)$-.

The term "heterocyclyl" or "heterocycle" can be used interchangeably and each refers to saturated or partially unsaturated non-aromatic cyclic radicals which has, for example, 3-8 ring atoms, such as 4-7 ring atoms (4- to 7-membered heterocyclyl), 5-6 ring atoms (5- to 6-membered heterocyclyl), 5 ring atoms (5-membered heterocyclyl) or 6 ring atoms (6-membered heterocyclyl), wherein the ring atoms include one or more (such as 1, 2 or 3, preferably 1 or 2) heteroatoms independently selected from N, O and S, and the remaining ring atoms are carbon atoms, and which may have 1 or 2 rings, preferably 1 ring, wherein N and S may be optionally oxidized to various oxidation states. The point of attachment of the heterocyclyl can be at a heteroatom or a carbon atom. "Heterocyclyl" includes 5- or 6-membered saturated heterocyclyl containing 1 or 2 nitrogen atoms. Exemplary heterocyclyl includes, but is not limited to, piperidinyl, piperazinyl, pyrrolidinyl and dihydropyridyl.

The term "heterocycloalkylene" refers to a heterocyclyl radical as defined above, but which is divalent, i.e., has two single bonds connecting two other radicals. Examples of heterocycloalkylene include, but are not limited to, piperidinylene, piperazinylene, pyrrolidinylene or dihydropyridylene such as 1,2-dihydropyridylene.

The term "heteroaryl" refers to a monocyclic, bicyclic, or tricyclic aromatic ring system having 5-14 ring atoms (5- to 14-membered heteroaryl), wherein the ring atoms contain one or more, such as 1, 2, 3 or 4 heteroatoms independently selected from N, O and S, with the remaining ring atoms being carbon; wherein at least one of the rings is an aromatic ring, wherein N and S may be optionally oxidized to various oxidation states. When the total number of S and O atoms in the heteroaryl exceeds 1, the S and O heteroatoms are not adjacent to one another. Preferably, the heteroaryl is 6- to 10-

membered heteroaryl, more preferably 6-membered heteroaryl. Examples of heteroaryl include, but are not limited to, imidazolyl, pyridyl, pyrazinyl or pyrimidyl.

The term "heteroarylene" refers to a heteroaryl radical as defined above, but which is divalent, that is, has two single bonds connecting two other radicals. Examples of heteroarylene include, but are not limited to, imidazolylene, pyridylene, pyrazinylene or pyrimidylene.

The term "optional" or "optionally" as used herein means that the subsequently described event or circumstance may or may not occur, and the description includes instances wherein the event or circumstance occur and instances in which it does not occur. For example, when a radical or structure is "optionally substituted", the radical or structure may be substituted or unsubstituted.

The term "substituted", "substituting" or "substituted with" as used herein, means that one or more (such as, 1, 2, 3 or 4) hydrogen on the designated atom or radical are replaced with one or more (such as 1, 2, 3 or 4) substituents, preferably the substituents selected from the indicated group of substituents or radicals, provided that the designated atom's normal valence is not exceeded, wherein the substituents may be the same or different from each other. The term "substituted with one or more groups selected from" or "substituted with one or more" as used herein means that one or more hydrogen on the designated atom or radical are independently replaced with one or more radicals selected from the indicated radical of substituents or radicals, wherein the radicals may be the same or different from each other. An optional substituent can be any radicals, provided that combinations of substituents and/or variables result in a chemically correct and stable compound. A chemically correct and stable compound is meant to imply a compound that is sufficiently robust to survive sufficient isolation from a reaction mixture to be able to identify the chemical structure of the compound. Preferably, substituents are those exemplified in the compounds of the examples of the present application.

Unless otherwise specified, substituents are named into the core structure. For example, it is to be understood that when (cycloalkyl)alkyl is listed as a possible substituent, the point of attachment of this substituent to the core structure is in the alkyl portion.

The "amino protecting group" refers to a group that can protect amino from undesirable reactions during a reaction and can be easily removed after the reaction. Examples of amino protecting groups include Fmoc, Boc, Cbz, etc.

The "carboxyl protecting group" refers to a group that can protect carboxyl from undesirable reactions during a reaction and can be easily removed after the reaction. Examples of carboxyl protecting groups include benzyl, methyl, ethyl, tert-butyl, etc.

In the present invention, the term "conjugate" refers to a substance obtained by linking a payload or active molecule to a targeting moiety. In some embodiments of the present invention, the payload or active molecule is linked to the targeting moiety via a linker. Preferably, the linker can be cleaved in a specific environment (e.g., a low pH environment in a cell) or under a specific action (e.g., the action of a lysosomal protease), thereby releasing the active molecule from the targeting moiety. In some embodiments of the present invention, the linker comprises a cleavable unit, such as a peptide or a disulfide bond.

In some embodiments, the payload or active molecule is a drug active molecule. In the present invention, the term "drug active molecule" refers to a substance that can regulate biological processes, especially change or prevent pathological processes, particularly refers to a substance that inhibits or prevents cell functions and/or causes cell death or destruction. In some embodiments of the present invention, the bioactive substance or drug active molecule in the conjugate is a molecule with anti-tumor biological activity. The "drug active molecules" include "small molecule drugs".

In the present invention, the term "small molecule drug" refers to a drug active molecule that has a low molecular weight and can regulate biological processes, especially change or prevent pathological processes. A "small molecule" is defined as a molecule having a molecular weight of less than 10 kD, typically less than 2 kD and preferably less than 1 kD, more preferably less than 800 D, less than 600 D, less than 500 D or less than 300 D. The small molecule drugs include but are not limited to organic molecules, organic molecules containing inorganic components, molecules containing radioactive atoms, synthetic molecules, peptide mimics and antibody mimics. As therapeutic agents, small molecules can penetrate cells more effectively, are less susceptible to degradation, and are less prone to triggering an immune response than large molecules.

Non-limiting examples of "small molecule drugs" and/or "drug active molecules" include metal platinum complexes (e.g., oxaliplatin) and metal gold complexes; glycopeptide antibiotics, such as bleomycin or pingyangmycin; DNA topoisomerase inhibitors, such as topoisomerase I inhibitors (e.g., Dxd, camptothecin, hydroxycamptothecin, 9-aminocamptothecin, SN-38, irinotecan, topotecan, belotecan or rubitecan), topoisomerase II inhibitors (e.g., actinomycin D, doxorubicin, duocarmycin, daunorubicin, mitoxantrone, podophyllotoxin or etoposide); auristatin compounds such as monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF); drugs that interfere with DNA synthesis, such as methotrexate, 5-fluorouracil, cytarabine, gemcitabine, mercaptopurine, pentostatin, fludarabine, cladribine or nelarabine; drugs that act on structural proteins, such as tubulin inhibitors, vinca alkaloids, vincristine, vinblastine, paclitaxel, docetaxel or cabazitaxel; maytansine derivatives; calicheamicin derivatives; pyrrolobenzodiazepine dimer (PBD) derivatives; melphalan; mitomycin C; chlorambucil; and other active substances that inhibit tumor cell growth and promote tumor cell apoptosis or necrosis.

In the present invention, the term "linker" refers to a fragment that links a drug active molecule to a targeting moiety. It should be understood that a linker, prior to linking a targeting moiety, has a functional group that can form a bond with a functional group of the targeting moiety. Non-limiting examples of linkers include those involved in the embodiments and examples of the present invention.

The "spacer unit" described herein refers to a moiety that helps separate a part of the linker from other moieties of the payload compound described in the present invention. The spacer unit can lead to more effective conjugation and/or more effective cleavage of the antibody with the payload compound described in the present invention. In some embodiments, the spacer unit could self-immolate after effective cleavage/release; in some embodiments, the spacer unit can also stabilize the antibody-drug conjugate and provide improved overall antibody-drug conjugate properties.

The term "targeting moiety" refers to a moiety of a conjugate that can specifically bind to a target (or a portion of the target) on the surface of a cell. Through the interaction of the targeting moiety with the target, the conjugate can be delivered to a specific cell population. Non-limiting examples of cell surface targets include ACTA2, ADGRE1, AGS-16, AIF1, AKR1C1, AKR1C2, ANGPTL4, ApoE, ASLG659, B7-H3, BAFF-R,

BCMA, BMPR1B, BNIP3, Brevican, C1QA, C1QB, CA6, CADM1, CCL5, CCR5, CD11b, CD11c, CD123, CD138, CD19, CD19, CD20, CD21, CD22, CD223, CD30, CD33, CD37, CD38, CD40, CD45(PTPRC), CD49D(ITGA4), CD56, CD66e, CD70, CD72, CD74, CD79a, CD79b, CD79b, CD80, CDCP1, CDH11, CEACAM5, CEACAM5/6, CEACAM6, CLDN18.2, c-Met, COL6A3, COL7A1, CRIPTO, CSF1R, CTGF, CTSD, CTSS, CXCL10, CXCL11, CXCR5, DDIT4, DLL3, DLL4, DR5, E16, EFNA4, EGFR, EGFRvIII, EGLN, EGLN3, EMR2, Endothelin receptor, ENPP3, EpCAM, EphA5, EphB2R, ETBR, FcRH1, FcRH2, FGF2, FGFR2, Fibronectin extra domain B, Folate receptor1, FR alpha, GEDA, GPNMB, Guanylyl cyclase C, GZMB, HER2, Her3, HLA-DOB, HMOX1, IFI6, IFNG, IGF-1R, IGFBP3, IL10RA1, IL20Rα, IL-6, IRTA2, KISS1R, KRT33A, LIV1, LOX, LRRC15, LUM, LY64, LY6E, Ly86, LYPD3, MCPT8, MDP, Mesothelin, MFI2, MMP10, MMP14, MMP16, MPF, MS4A7, MSG783, Mucin-1, Mucin16, NaPi2b, Napi3b, NCA, Nectin-4, NOG, P2X5, pCAD, P-cadherin, PDGFRA, PDK1, PD-L1, PFKFB3, PGF, PGK1, PIK3AP1, PIK3CD, PLOD2, PSCA, PSCA hlg, PSMA, RNF43, ROR1, Sema5b, SERPINEl, SLC39A6, SLITRK6, SLTRK6, Sodiumphosphate cotransporter 2B, STAT1, STC2, STEAP1, STEAP2, TACSTD2, TCF4, TENB2, TGF, TGFB1, TGFB2, TGFBR1, Tissue factor, TNFRSF21, TNFSF9, Trf1, Trop-2, Trophoblast glycoprotein, TrpM4, Tyro7, UPK1B, VEGFA, WNT5A, integrins α5β6, α4β7, 0772P and 5T4.

In the present invention, when a targeting moiety in a conjugate is an antibody or an antigen-binding fragment thereof, the conjugate is referred to as an "immunoconjugate".

In the present invention, when an active molecule in an immunoconjugate is a drug active molecule, the immunoconjugate may be referred to as an "antibody-drug conjugate (ADC)". In the present invention, the "drug conjugate" and "antibody-drug conjugate" can be used interchangeably.

In the present invention, when an antibody or an antigen-binding fragment thereof is linked to one or more other peptides or polypeptides via a covalent bond, it is referred to as an "immune fusion".

The term "linker-payload" refers to a compound formed by linking a payload, such as a drug active molecule (e.g., a small molecule drug), to a linker.

The term "DAR" refers to the ratio of a drug moiety (D) to a targeting moiety (e.g., an Ab moiety, preferably an antibody) described herein, wherein the drug moiety is

conjugated to the targeting moiety (e.g., Ab moiety, preferably an antibody). In some embodiments described herein, the DAR can be determined by p in formula (III), for example, the DAR can be 1 to 16, such as 2-16, 4-16, 5-12, 6-10, 2-8, 3-8, 2-6, 4-6 or 6-10, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. DAR can also be calculated as an average DAR of a molecule population in a product, i.e., the overall ratio of a drug moiety (D) to an Ab moiety described herein in a product as measured by a detection method (e.g., by conventional methods such as mass spectrometry and/or HIC-HPLC), wherein the drug moiety is conjugated to the Ab moiety; and the DAR is referred to herein as an average DAR or a measured DAR. In some embodiments, the conjugate of the present invention has an average DAR value of 1-16, such as 2-16, 4-16, 5-12, 6-10, 2-8, 3-8, 2-6, 4-6 or 6-10, such as 1.0-8.0 or 2.0-6.0, such as 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9 or 10.0, and any range with any two of these values as endpoints. It should be understood that when referring to an average DAR value, the ADC of the present invention refers to an ADC molecule population or an ADC molecule mixture, comprising ADC molecules of the present invention having the same and/or different DARs.

The term "solvate" refers to an association formed by one or more solvent molecules with the antibody-drug conjugate (ADC) of the present invention. The solvents that form solvates include, but are not limited to, water, methanol, ethanol, isopropanol, ethyl acetate, tetrahydrofuran, N, N-dimethylformamide and dimethyl sulfoxide.

As used herein, the "pharmaceutically acceptable" and "pharmaceutical" can be used interchangeably unless there is any contradiction in the context.

As used herein, the term "cancer" refers to a cellular disorder characterized by uncontrolled or dysregulated cell proliferation, decreased cellular differentiation, inappropriate ability to invade surrounding tissue, and/or ability to establish new growth at ectopic sites. The term "cancer" includes, but is not limited to, solid tumors and hematologic malignancies, such as leukemia, lymphoma, or myeloma. The term "cancer" encompasses those of skin, tissues, organs, bone, cartilage, blood, and vessels. The term

"cancer" further encompasses primary cancer, and metastatic cancer, recurrent cancer, and refractory cancer. Examples of cancer include those involved in this document (including the embodiments and examples).

The term "pharmaceutical composition" refers to such a composition that exists in a form that allows the biological activity of the active ingredient contained therein to be effective and does not contain additional ingredients that have unacceptable toxicity to the subject to whom the composition is administrated. In addition to the active ingredient, e.g., ADC, it generally contains ingredients such as pharmaceutically acceptable carriers, stabilizers, diluents, dispersants, suspending agents, thickeners and/or excipients. The pharmaceutical compositions provided herein are in a form that permits administration and subsequently provides the desired biological activity of active ingredients and/or achieves therapeutic effects.

The terms "pharmaceutically acceptable carrier" and "physiologically acceptable carrier" refer to carriers or diluents that do not cause significant irritation to a subject and do not abrogate the biological activity and properties of the administered active ingredients such as ADC compounds or compositions and/or any additional therapeutic agents in the compositions. The pharmaceutically acceptable carrier can enhance or stabilize the composition, or can be used to facilitate the preparation of the composition. Pharmaceutically acceptable carriers may include solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents and antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drug stabilizers, binders, excipients, disintegrants, lubricants, sweeteners, flavoring agents, dyes, etc., and combinations thereof, as known to a person skilled in the art (see, e.g., Remington's Pharmaceutical Sciences, 18th edition, Mack Printing Company, 1990, pp. 1289-1329). Unless incompatible with active ingredients, any of the conventional carriers is considered for use in therapeutic or pharmaceutical compositions. The carrier may be selected to minimize adverse side effects to a subject and/or to minimize degradation of active ingredients. An adjuvant may also be included in any of these formulations.

The term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Formulations for parenteral administration may, for example, contain excipients such as sterile water or physiological saline; and polyalkylene glycols (such as polyethylene glycol), vegetable

oils, or hydrogenated naphthalenes. Other exemplary excipients include, but are not limited to, calcium bicarbonate, calcium phosphate, various sugars and various types of starch, cellulose derivatives, gelatin, ethylene-vinyl acetate copolymer particles, and surfactants, including, for example, polysorbate 20.

The term "pharmaceutically acceptable salt" refers to salts that do not abrogate the biological activity and properties of the compounds of the present invention and do not cause significant irritation to a subject to which they are administered. Examples of such salts include, but are not limited to: (a) acid addition salts formed from inorganic acids, such as hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid and nitric acid; and salts formed from organic acids, such as acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalene disulfonic acid and polygalacturonic acid; and (b) salts formed from basic anions such as chloride ions, bromide ions and iodide ions.

The terms "pharmaceutical combination", "combination product", "drug combination" or "combined product" refer to a non-fixed combination product or a fixed combination product, including but not limited to a kit and a pharmaceutical composition. The term "non-fixed combination" means that active ingredients (e.g., (i) an antibody molecule or ADC molecule of the present invention, and (ii) additional therapeutic agent) are administered as separate entities to a patient simultaneously without specific time limits, or sequentially at equal or different time intervals, wherein such administration provides prophylactically or therapeutically effective levels of two or more active agents in the patient. In some embodiments, the antibody molecule or ADC molecule of the present invention and the additional therapeutic agent used in the pharmaceutical combination are administered at levels not exceeding those when they are used alone. The term "fixed combination" means that two or more active agents are administered as single entity to a patient simultaneously. Preferably, the dosages and/or time intervals of the two or more active agents are selected, so that the combined use of the components produces a greater effect in terms of treating diseases or conditions than that achievable by using any single component alone. Each component may be in a separate formulation form, and the formulation forms may be the same or different.

The term "combination therapy" or "combined therapy" refers to the administration of two or more therapeutic agents or treatment modalities (e.g., radiation therapy or surgery) to treat the diseases described herein. Such administration includes co-administration of the therapeutic agents in a substantially simultaneous manner, for example in a single capsule having a fixed ratio of active ingredients. Alternatively, such administration includes co-administration of the individual active ingredients in multiple or separate containers (e.g., tablets, capsules, powders and liquids). The powder and/or liquid may be reconstituted or diluted to the desired dosages prior to administration. Additionally, such administration also encompasses using each type of therapeutic agent at approximately the same time or at different times in a sequential manner. In either case, the treatment regimen will provide beneficial effects of the pharmaceutical combination in the treatment of the conditions or disorders described herein.

As used herein, "treatment" means to slow, interrupt, arrest, relieve, stop, reduce or reverse the progression or severity of existing symptoms, conditions, disorders or diseases.

As used herein, "prevention" includes the inhibition of the onset or development of diseases or conditions, or symptoms of a particular disease or condition. In some embodiments, a subject with a family history of cancer is a candidate for a prophylactic regimen. Generally, in the context of cancer, the term "prevention" refers to administration of drugs to a subject prior to the onset of conditions or symptoms of cancer, in particular, in a subject at risk of cancer.

The term "antibody" refers to an antibody in any form having a desirable bioactivity. Therefore, it is used in the broadest sense, including but not limited to a monoclonal antibody (including a full-length monoclonal antibody), a polyclonal antibody, a multispecific antibody (such as a bispecific antibody), a humanized antibody, a human antibody, a chimeric antibody, a CrossMab antibody, or a camelized single-domain antibody.

The term "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the various antibodies constituting the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The modifier "monoclonal" indicates the feature of the

antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be constructed as requiring any particular method to produce the antibody.

The term "isolated antibody or antigen-binding fragment" refers to the purified state of the antibody or antigen-binding fragment. For example, “isolated” may mean that the molecule is substantially free of other biomolecules, such as nucleic acids, proteins, lipids, sugars or other substances, such as cell debris and growth medium. However, as is known to a person skilled in the art, the term “isolated” does not mean the complete absence of such substances unless they are present in an amount that significantly interferes with the experimental or therapeutic application of the antibodies described herein. In some embodiments, the isolated antibody or antigen-binding fragment can has a purity of greater than 95%, greater than 96%, greater than 97%, greater than 98% or greater than 99%, which purity is determined by, for example, electrophoresis (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatography (e.g., ion exchange or reverse phase HPLC). For a review of methods of evaluating antibody purity, see, for example, Flatman, S. et al., J. Chrom.B 848 (2007) 79-87.

The terms “full-length antibody” and “intact antibody” can be used interchangeably herein and refer to an antibody that has a structure substantially similar to a native antibody structure. “Native antibodies” refer to naturally occurring immunoglobulin molecules with varied structures. For example, native IgG class antibodies are heterotetrameric glycoproteins of approximately 150,000 daltons, composed of two light chains and two heavy chains interconnected by disulfide bonds. Each of the heavy chains is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region consists of 3 domains (CH1, CH2 and CH3). Each of the light chains is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region consists of the domain CL. The VH and VL regions can be further divided into hypervariable regions (complementarity determining regions or CDRs) interspersed with regions that are more conserved (framework regions or FRs). Each VH or VL consists of three CDRs and four FRs, arranged in the following order from N-terminus to C-terminus: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The constant region is not directly involved in the binding of an antibody to an antigen, but shows multiple effector functions. The heavy chain of an antibody can be classified into one of five types, i.e., α

(IgA), δ (IgD), ε (IgE), γ (IgG) or μ (IgM), some of which can be further divided into subtypes, such as γ1 (IgGl), γ2 (IgG2), γ3 (IgG3), γ4 (IgG4), α1 (IgAl) and α2 (IgA2). The light chains of the antibody can be classified into one of two types, called κ (kappa) and λ (lambda) based on the amino acid sequences of the constant regions thereof.

A "complementary determining region" or "CDR region" or "CDR" is a region in an antibody variable region, which is hypervariable in sequence and forms a structurally established loop ("hypervariable loop") and/or contains an antigen contact residue ("antigen contact point"). CDR is mainly responsible for binding to epitopes. CDRs of heavy chain and light chain are generally called CDR1, CDR2 and CDR3, which are numbered sequentially from the N-terminus. The CDRs located in the antibody heavy chain variable region are called HCDR1, HCDR2 and HCDR3, respectively, while the CDRs located in the antibody light chain variable region are called LCDR1, LCDR2 and LCDR3, respectively. Each VH or VL consists of three CDRs and 4 FRs, which are arranged in the following order from the amino terminus to the carboxyl terminus: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. In a designated VH or VL amino acid sequence, the exact amino acid sequence boundary of each CDR can be determined by using any one of or a combination of various well-known schemes, including, for example: Chothia scheme (Chothia et al., Canonical structures for the hypervariable regions of immunoglobulins", Journal of Molecular Biology, 196, 901-917 (1987)); Kabat scheme (Kabat et al., Sequences of Proteins of Immunological Interest, 4th edition, U.S. Department of Health and Human Services, National Institutes of Health (1987)), AbM (University of Bath) and Contact (University College London); North scheme (North et al., "A New Clustering of Antibody CDR Loop Conformations", Journal of Molecular Biology, 406, 228-256 (2011)). The boundary of the CDR of the antibody in the present invention can be determined according to any schemes or a combination thereof in the art and personal evaluation. In some embodiments, the CDR of the antibody of the present invention can be determined according to the Kabat scheme.

The term "antibody fragment" refers to a molecule that is different from a full antibody and comprises a portion of the full antibody, wherein the portion binds to the antigen that the full antibody binds to. Examples of antibody fragments include, but are not limited to, Fv, such as a disulfide-stabilized Fv fragment (dsFv), $(dsFv)_2$, bispecific dsFv (dsFv-dsFv'), Fab, Fab', Fab'-SH and $F(ab')_2$; a diabody such as a disulfide-

stabilized diabody (ds diabody), a triabody, a tetrabody, and a crossFab fragment; a linear antibody; a single-chain antibody molecule (e.g., scFv) and an scFv dimer (bivalent diabody); and a single-domain antibody. With regard to a review of certain antibody fragments, reference is made to Hudson et al., Nat Med 9, 129-134 (2003). Antibody fragments can be prepared by a variety of techniques including, but not limited to, proteolytic digestion of intact antibodies and production by recombinant host cells (e.g., Escherichia coli or phages).

The term "antigen-binding fragment" includes a fragment of an antibody that binds to the antigen. Generally, the antigen-binding fragment includes at least one fragment (such as one or more CDRs) of the antigen-binding region or variable region of the antibody, and maintains at least some of the binding properties of the antibody. Examples of antigen-binding fragments include, but are not limited to, Fv, such as a disulfide-stabilized Fv fragment (dsFv), $(dsFv)_2$, bispecific dsFv (dsFv-dsFv'), Fab, Fab', Fab'-SH and F $(ab')_2$; a diabody such as a disulfide-stabilized diabody (dS diabody), a triabody, a tetrabody and a crossFab fragment; a linear antibody; a single-chain antibody molecule (e.g., scFv) and an scFv dimer (bivalent diabody); a single-domain antibody; a nanobody and a multispecific antibody. When the antigen binding activity is expressed on a molar concentration basis, the antigen binding fragments generally maintain at least 10% of the antigen binding activity of the antibody from which they are derived. Preferably, the antigen-binding fragments maintain at least 20%, 50%, 70%, 80%, 90%, 95% or 100% or more of the antigen binding activity of the antibody from which they are derived.

The term "chimeric antibody" refers to an antibody having a variable region of a first antibody and a constant domain of a second antibody, wherein the first antibody and the second antibody are from different species. Generally, the variable region is obtained from the antibody of experimental animals such as rodents, while the constant domain sequence is obtained from a human antibody, so that the obtained chimeric antibody is less likely to induce adverse immune response in human subjects than the antibody from the experimental animals.

The term "humanized antibody" refers to an antibody form containing sequences from human and non-human (e.g., mouse and rat) antibodies. In general, the humanized antibody comprises at least one, and generally two, variable regions, in which all or substantially all of the hypervariable loops correspond to those of non-human

immunoglobulins, and all or substantially all of the framework (FR) regions correspond to those of human immunoglobulin. The humanized antibody can optionally comprise at least a portion of the human immunoglobulin constant region (Fc). In some cases, as is known to a person skilled in the art, amino acid mutations can be introduced into humanized antibodies (e.g., variable regions, framework regions, and/or constant regions (if present)), for example, to improve certain properties of the antibodies; such antibody forms still fall within the scope of the "humanized antibody" of the present invention.

As is known to a person skilled in the art, the antibody may have a sugar chain of the cell for producing the antibody. For example, when produced in mice, in mouse cells, or in hybridomas derived from mouse cells, the antibody may contain a mouse sugar chain. Alternatively, when produced in rats, in rat cells, or in hybridomas derived from rat cells, the antibody may contain a rat sugar chain.

The term "Fc region" is used to define the C-terminal region comprising at least a portion of the constant region of an immunoglobulin heavy chain. The term includes native sequence Fc regions and Fc region variants. The Fc region of native sequence covers a variety of naturally occurring immunoglobulin Fc sequences, such as various Ig subtypes and allogeneic Fc regions thereof (Gestur Vidarsson et al., IgG subclasses and allotypes: from structure to effector functions, 20 October 2014, doi:10.3389/fimmu.2014.00520.). In one embodiment, the Fc region of the human IgG heavy chain extends from Cys226 or from Pro230 to the carboxyl terminus of the heavy chain. However, the lysine at the C-terminus (Lys447) of Fc region may or may not be present. Unless otherwise specified herein, amino acid residues in an Fc region or a constant region is numbered in accordance with the EU numbering system, also referred to EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th edition, Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

The terms "Fc region variant" and "variant Fc region" can be used interchangeably herein, and mean that a modified Fc region is comprised relative to a native sequence Fc region. Variants obtained by mutating the Fc region for specific properties are known in the art. The Fc region variants of the present invention are defined according to modifications to the amino acids that constitute the variants. In one embodiment, the Fc region is modified with respect to the properties of the effector function of the Fc region

(such as the complement activation function of the Fc region). In one embodiment, the effector function has been reduced or eliminated relative to a wild-type Fc region. In one embodiment, the effector function is reduced or eliminated by a method selected from: the use of an Fc isotype that naturally has a reduced or eliminated effector function, and the modification of an Fc region. In a preferred embodiment, the Fc region has a reduced effector function mediated by the Fc region, such as a reduced or eliminated ADCC or ADCP or CDC effector function, for example comprising a mutation that achieves the above function.

The "sequence identity" refers to the degree of sequence identity based on one by one nucleotide or amino acid comparing in the comparison window. The "(percent) sequence identity" can be calculated as follows: comparing the two optimally aligned sequences in the comparison window, determining the number of positions having the same nucleic acid base (e.g., A, T, C, G and I) or the same amino acid residue (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) in the two sequences to obtain the number of matching positions, dividing the number of matching positions by the total number of positions in the comparison window (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment for purposes of determining the percentage of sequence identity can be achieved in various ways known in the art, for example, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN (DNASTAR) software. A person skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences or the target sequence region being compared. In the present invention, for antibody sequences, in one embodiment, the percentage of identity of two amino acid sequences is determined by optimally aligning the candidate antibody sequence with the reference antibody sequence, and then performing an optimal alignment in accordance with a numbering rule.

The term "conservative substitution" or "conservative amino acid substitution" refers to the substitution of one or more amino acids with one or more chemically or functionally similar amino acids. Conservative substitution tables providing similar amino acids are well known in the art. Polypeptide sequences having such substitutions are referred to as "conservatively modified variants." Such conservatively modified

variants are in addition to and do not exclude polymorphic variants, interspecies homologs and alleles. Table A illustrates conservative substitutions of one another.

Table A

Original residue	Exemplary substitution	Preferred conservative amino acid substitution
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp; Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; norleucine	Leu
Leu (L)	norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; norleucine	Leu

The term “host cell” refers to a cell into which an exogenous polynucleotide has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived

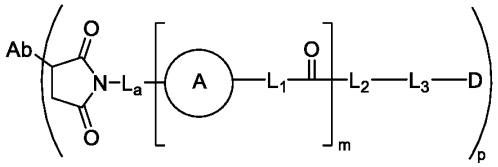
therefrom. In one embodiment, the host cells are eukaryotic. In another embodiment, the host cells are selected from yeast cells or mammalian cells (e.g., CHO cells or 293 cells, such as HEK 293 or 293F cells, 293FT cells or Expi293 cells). In another embodiment, the host cells are prokaryotic. Suitable host cells include prokaryotic microorganisms, such as Escherichia coli, eukaryotic microorganisms such as filamentous fungi or yeast, or various eukaryotic cells, such as Chinese hamster ovary cells (CHO) and insect cells. Mammalian cell lines suitable for suspension culture can be used. Examples of useful mammalian host cell lines include SV40 transformed monkey kidney CV1 line (COS-7); human embryonic kidney line (HEK 293 or 293F cells, 293FT cells or Expi293 cells), baby hamster kidney cells (BHK), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76), human cervical cancer cells (HELA), canine kidney cells (MDCK), Buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), CHO cells, NSO cells, myeloma cell lines such as YO, NS0, P3X63 and Sp2/0.

As used herein, the term "vector" refers to a nucleic acid molecule capable of copying another nucleic acid to which it has been linked. The term includes the vector as a self-replicating nucleic acid structure and the vector integrated into the genome of a host cell into which it has been introduced. Some vectors can direct the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In one embodiment, the vector is an expression vector, such as a prokaryotic expression vector or a eukaryotic expression vector. The vectors include, but are not limited to, viruses, plasmids, cosmids, λ phages or yeast artificial chromosomes (YACs). In a preferred embodiment, the expression vector is pCDNA3.4.

Specific embodiments

Embodiment I

Embodiment I-1. A drug conjugate represented by formula (III),



(III)

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof,

wherein Ab is a targeting moiety such as an antibody or an antigen-binding fragment thereof;

p is an integer selected from 1-16, for example, an integer selected from 1-10, 1-9, 2-8, 4-10, 6-8, 3-7, 4-8 or 2-6, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 12;

D is a drug active molecule;

L_a is selected from -C_{1-12} alkylene-, -C_{1-12} alkylene-C(O)-, -C_{1-12} alkylene-CONH-$(CH_2CH_2O)_n$-CH_2-C(O)- or -$(CH_2CH_2O)_n$-CH_2-C(O)-; n is an integer selected from 1-18, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

"ring A" is an optionally substituted 3- to 8-membered heterocycloalkylene or 6- to 10-membered heteroarylene;

L_1 is selected from -CH_2-CHR_1- or -O-CH_2-, or is absent; R_1 is -OH, -NH_2, -C_{1-4} alkylene-OH or -C_{1-4} alkylene-NH_2;

L_2 is a linking unit selected from short peptide residues consisting of 2-10 amino acids, wherein the amino acids are natural amino acids or non-natural amino acids;

L_3 is a spacer unit; and

m is 1, 2, 3 or 4.

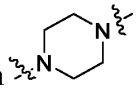
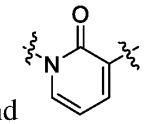
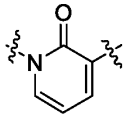
It should be understood that by reducing disulfide bonds to the thiol which undergoes a reaction with a maleimide moiety, Ab is covalently linked to the maleimide moiety. That is, Ab is covalently linked to the maleimide moiety via a sulfur atom.

Embodiment I-2. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment I-1, wherein

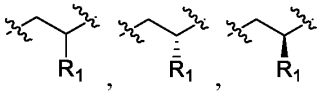
L_a is selected from -C_{1-6} alkylene-, -C_{1-6} alkylene-C(O)-, -C_{1-6} alkylene-CONH-$(CH_2CH_2O)_n$-CH_2-C(O)- or -$(CH_2CH_2O)_n$-CH_2-C(O)-, n is an integer selected from 1-12, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

"ring A" is 5- or 6-membered nitrogen-containing saturated heterocycloalkylene, or 5- or 6-membered nitrogen-containing unsaturated heterocycloalkylene substituted with oxo (=O); and when ring A is 5- or 6-membered nitrogen-containing unsaturated heterocycloalkylene substituted with oxo, L_1 is absent.

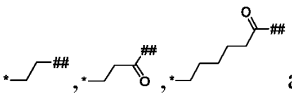
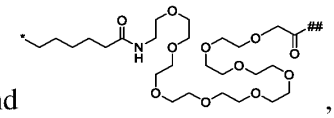
Embodiment I-3. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to either embodiment I-1 or embodiment I-2, wherein

ring A is selected from  and , and when ring A is , L_1 is absent.

Embodiment I-4. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-3, wherein

L_1 is selected from  and-O-CH_2-; R_1 is -OH, -NH_2, -C_{1-4} alkylene-OH or -C_{1-4} alkylene-NH_2.

Embodiment I-5. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-4, wherein

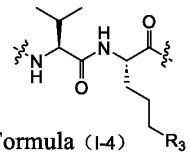
L_a is selected from  and , wherein * represents a position connected to a maleimide group, and ## represents a position connected to ring A.

Embodiment I-6. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-5, wherein

L_2 is a linking unit selected from short peptide residues consisting of 2-10 amino acid residues, wherein the amino acid residues can be independently selected from L- or D-isomers of the following amino acids: alanine (including β-alanine), arginine, aspartic acid, asparagine, cysteine, histidine, glycine, glutamic acid, valine, glutamine, phenylalanine, lysine, leucine, isoleucine, methionine, serine, tyrosine, threonine, tryptophan, proline, ornithine, penicillamine, aminoacetylenic acid, aminoalkanedioic acid, heterocyclyl-carboxylic acid, citrulline, selenocysteine [1-2], pyrrolysine,

diaminoalkanoic acid, and a derivative thereof; preferably L_2 is selected from -Phe-Lys-, -Val-Lys-, -Phe-Phe-Lys-, -D-Phe-Phe-Lys-, -Gly-Phe-Lys-, -Ala-Lys-, -Val-Cit-, -Phe-Cit-, -Leu-Cit-, -Iie-Cit-, -Trp-Cit-, -Phe-Ala-, -Gly-Phe-Leu-Gly-, -Ala-Leu-Ala-Leu-, -Val-Ala-, -Gly-Gly-Phe-Gly, -Gly-Cit-, -Cit-Val-, -Cit-Ala-, -Lys-Val-, -Val-Lys(Ac)-, -Phe-Lys(Ac)-, -Ala-Ala-, -Ala-Ala-Ala-, -Ala-Ala-Asn-, -Ala-Ala-Asp-, -Lys-Ala-Ala-Asn-, -Lys-Ala-Ala-Asp-, -D-Val-Leu-Lys-, -Gly-Gly-Arg-, -Gly-Gly-Phe-, -Val-Lys-Gly-, -Val-Lys-Gly-Gly- and -Lys-Ala-Asn- or a derivative thereof; more preferably, L_2 is -Val-Cit- or a derivative thereof.

Embodiment I-7. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-6, wherein L_2 has a structure represented by formula (I-4):



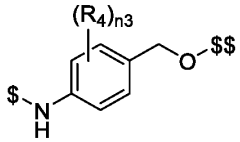
Formula (I-4)

wherein R_3 is selected from $-NHCONH_2$, $-NHCOCH_2(OCH_2CH_2)_{n1}OCH_3$, $-(CH_2)_{n2}SO_2CH_3$ or $-(CH_2)_{n2}P(O)(CH_3)_2$, wherein n_1 is an integer selected from 1-18, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12; n_2 is 0, 1, 2, 3, 4, 5, 6, 7 or 8;

preferably, R_3 is selected from $-NHCONH_2$, $-NHCOCH_2(OCH_2CH_2)_{n1}OCH_3$, $-SO_2CH_3$ or $-P(O)(CH_3)_2$, wherein n_1 is an integer selected from 6-12.

Embodiment I-8. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-7, wherein

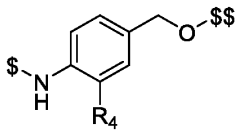
L_3 has a structure represented by formula (II-5):



Formula (II-5)

,

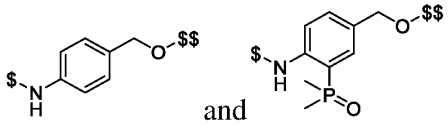
wherein R_4 is selected from $-(CH_2)_{n2}SO_2CH_3$, $-(CH_2)_{n2}P(O)(CH_3)_2$ or hydrogen, wherein n_2 is 0, 1, 2, 3, 4, 5, 6, 7 or 8; n_3 is 1 or 2; $ is an active site connected to L_2, and $$ is a site connected to the drug active molecule;



Formula (II-6)

preferably, L_3 has a structure represented by formula (II-6), wherein each symbol is as defined in formula (II-5).

Embodiment I-9. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment I-8, wherein L_3 has a structure selected from:

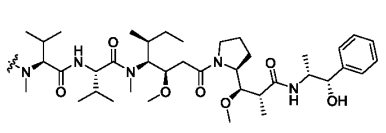


and

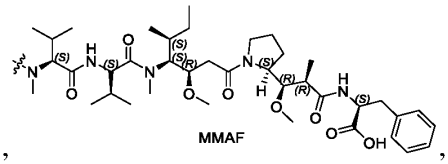
wherein $ represents a connection site to L_2, and $$ represents a connection site to the drug active molecule.

Embodiment I-10. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-9,

wherein D is Dolastatin 10 or a Dolastatin 10 derivative, preferably selected from:



MMAE,



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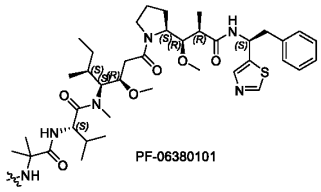
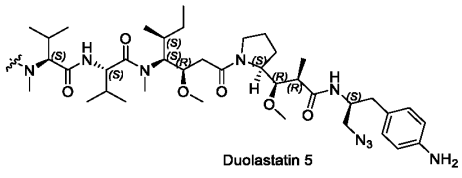
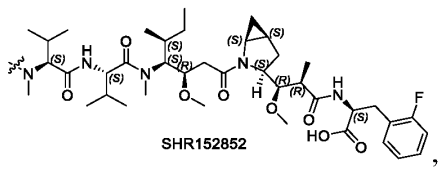


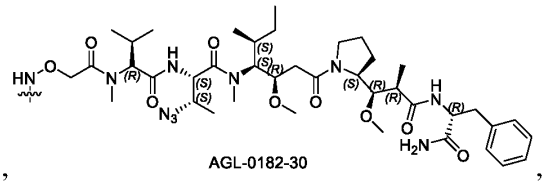
PF-06380101,



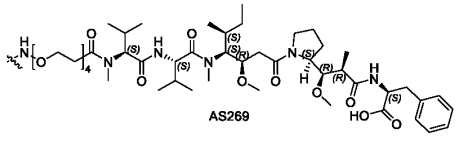
Duolastatin 5,



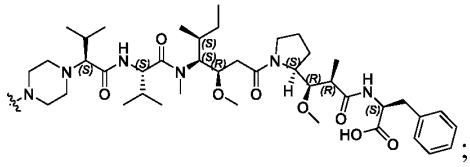
SHR152852,



AGL-0182-30,

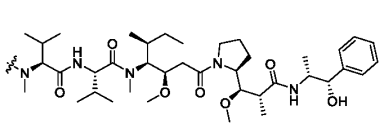


AS269 and



;

preferably, D is a Dolastatin 10 derivative selected from:



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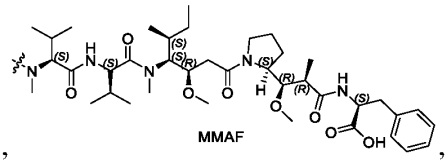

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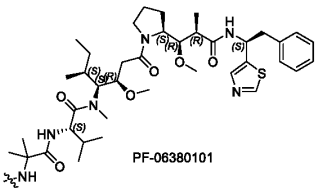
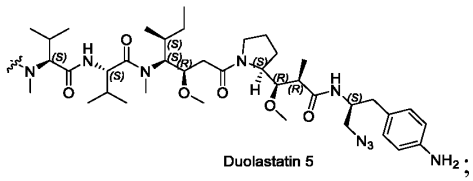
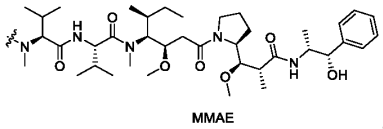


and



;

more preferably, D is selected from:



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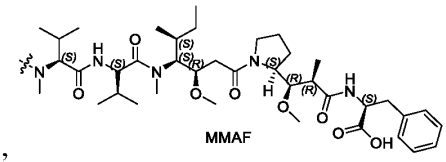


,

wherein the wavy line represents a connection site to L_3.

Embodiment I-11. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-10, wherein p is selected from 2, 3, 4, 5, 6, 7 and 8.

Embodiment I-12. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-11, wherein Ab is an anti-CD38 antibody or an antigen-binding fragment thereof, an anti-CD20 antibody or an antigen-binding fragment thereof, an anti-CD70 antibody or an antigen-binding fragment thereof, or a PD-L1 antibody or an antigen-binding fragment thereof.

In some embodiments, the anti-CD20 antibody comprises the HCDR1, HCDR2, HCDR3 and LCDR1, LCDR2, LCDR3 of Rituximab, Ibritumomab, Ofatumumab, Ocrelizumab, Obinutuzumab, Ripertamab, Ublituximab or Zuberitamab.In some embodiments, the anti-CD20 antibody comprises the variable heavy chain and variable light chain of Rituximab, Ibritumomab, Ofatumumab, Ocrelizumab, Obinutuzumab, Ripertamab, Ublituximab or Zuberitamab. In some embodiments, the anti-CD20 antibody comprises the heavy chain and light chain of Rituximab, Ibritumomab, Ofatumumab, Ocrelizumab, Obinutuzumab, Ripertamab, Ublituximab or Zuberitamab. In some embodiments, the anti-CD20 antibody comprises two heavy chains and two light chains of Rituximab, Ibritumomab, Ofatumumab, Ocrelizumab,

Obinutuzumab, Ripertamab, Ublituximab or Zuberitamab.In some embodiments, the anti-CD70 antibody comprises the HCDR1, HCDR2, HCDR3 and LCDR1, LCDR2, LCDR3 of Vorsetuzumab. In some embodiments, the anti-CD70 antibody comprises the variable heavy chain and variable light chain of Vorsetuzumab. In some embodiments, the anti-CD70 antibody comprises the heavy chain and light chain of Vorsetuzumab. In some embodiments, the anti-CD70 antibody comprises two heavy chains and two light chains of Vorsetuzumab.

In some embodiments, the anti-PD-L1 antibody comprises the HCDR1, HCDR2, HCDR3 and LCDR1, LCDR2, LCDR3 of Adebrelimab, Sugemalimab, Envafolimab, Durvalumab, Avelumab, or Atezolizumab. In some embodiments, the anti-PD-L1 antibody comprises the variable heavy chain and variable light chain of Adebrelimab, Sugemalimab, Envafolimab, Durvalumab, Avelumab, or Atezolizumab. In some embodiments, the anti-PD-L1 antibody comprises the heavy chain and light chain of Adebrelimab, Sugemalimab, Envafolimab, Durvalumab, Avelumab, or Atezolizumab. In some embodiments, the anti-PD-L1 antibody comprises two heavy chains and two light chains of Adebrelimab, Sugemalimab, Envafolimab, Durvalumab, Avelumab, or Atezolizumab.

Embodiment I-13. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-12, wherein the anti-CD38 antibody is selected from the anti-CD38 antibody or the antigen-binding fragment thereof as defined herein, such as the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14, or embodiment I-15; the anti-CD20 antibody is selected from Rituximab, Ibritumomab, Tositumomab, Ofatumumab, Ocrelizumab, Obinutuzumab, Ripertamab, Ublituximab or Zuberitamab; the anti-CD70 antibody is Vorsetuzumab; the PD-L1 antibody is selected from Adebrelimab, Sugemalimab, Envafolimab, Durvalumab, Avelumab or Atezolizumab.

Embodiment I-14. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment I-13, wherein the Ab is an anti-CD38 antibody or an antigen-binding fragment thereof.

Embodiment I-15. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment I-13, wherein the Ab is an anti-CD38 antibody or an antigen-binding fragment thereof comprising a heavy chain variable

region (VH) and a light chain variable region (VL); the VH comprises HCDR1, HCDR2 and HCDR3, and the VL comprises LCDR1, LCDR2 and LCDR3, wherein

the HCDR1 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 17, the HCDR2 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 18, and the HCDR3 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 19; the LCDR1 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 20, the LCDR2 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 21, and the LCDR3 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 22;

optionally, the VH comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 15, and/or the VL comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 16;

optionally, the VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 15, and the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 16; or the VH comprises an amino acid sequence as set forth in SEQ ID NO: 15, and the VL comprises an amino acid sequence as set forth in SEQ ID NO: 16; or the VH consists of an amino acid sequence as set forth in SEQ ID NO: 15, and the VL consists of an amino acid sequence as set forth in SEQ ID NO: 16;

optionally, the anti-CD38 antibody or the antigen-binding fragment thereof is of IgG subtype, such as IgG1, IgG2, IgG3 or IgG4 subtype; for example, the anti-CD38 antibody or the antigen-binding fragment thereof further comprises a heavy chain constant region, such as a heavy chain constant region of IgG1, IgG2, IgG3 or IgG4, for example, a heavy chain constant region of human IgG1, IgG2, IgG3 or IgG4; for example, the heavy chain constant region comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 13 or SEQ ID NO: 23;

optionally, the anti-CD38 antibody or the antigen-binding fragment thereof further comprises a light chain constant region, such as a Kappa light chain constant region or a Lambda light chain constant region, such as a human Kappa light chain constant region

or a human Lambda light chain constant region; for example, the light chain constant region comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 14;

optionally, the anti-CD38 antibody or the antigen-binding fragment thereof further comprises a heavy chain constant region and a light chain constant region, wherein the heavy chain constant region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 13 or SEQ ID NO: 23, and the light chain constant region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 14;

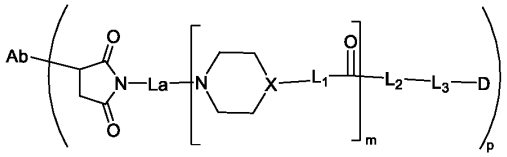
optionally, the anti-CD38 antibody or the antigen-binding fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain comprises or consists of a heavy chain variable region and a heavy chain constant region; and/or the light chain comprises or consists of a light chain variable region and a light chain constant region; for example, the anti-CD38 antibody comprises two heavy chains and two light chains, such as two identical heavy chains and two identical light chains, optionally, wherein the heavy chain comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 11, and/or the light chain comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 12; for example, the heavy chain comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 11, and the light chain comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 12;

optionally, the anti-CD38 antibody is a full-length antibody;

optionally, the anti-CD38 antibody is Daratumumab.

Embodiment I-16. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment I-14, wherein the Ab is the anti-CD38 antibody or the antigen-binding fragment thereof as defined herein, such as the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14, such as Ab1 or huAb1.

Embodiment I-17. A drug conjugate represented by formula (III-2),

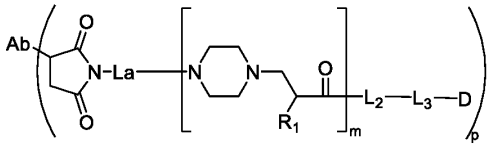


Formula (III-2)

,

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein X is N or CH, and the remaining variables (Ab, L_a, L_1, L_2, L_3, D, m and p) are as defined in formula (III).

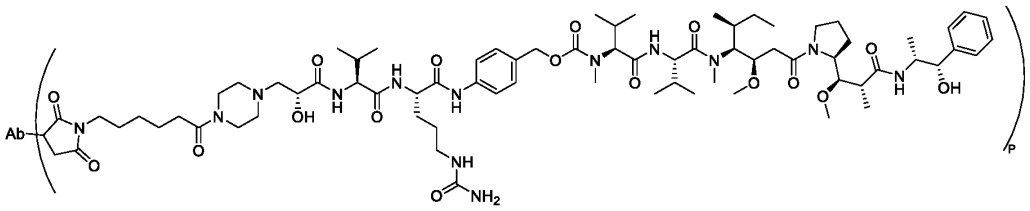
Embodiment I-18. A drug conjugate represented by formula (III-3),



(III-3)

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein each variable (Ab, L_a, R_1, L_2, L_3, D, m and p) is as defined in formula (III).

Embodiment I-19. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-18, wherein the drug conjugate is selected from:



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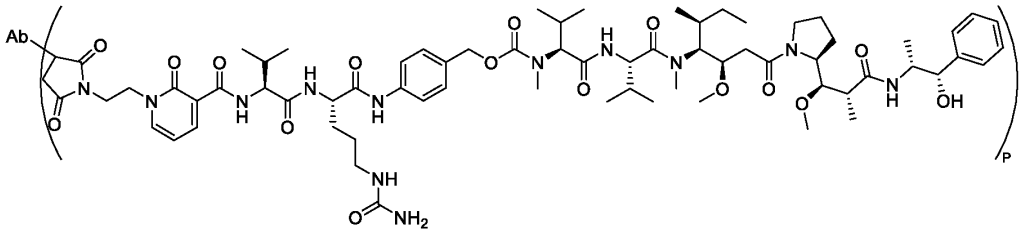

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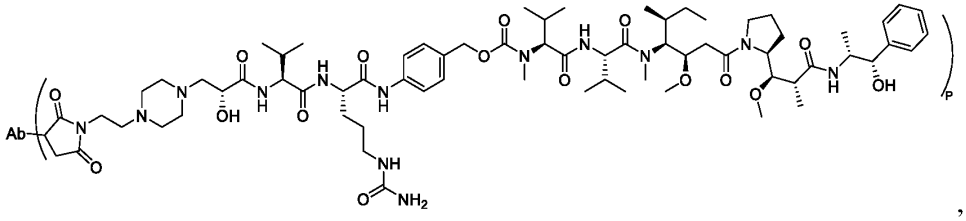

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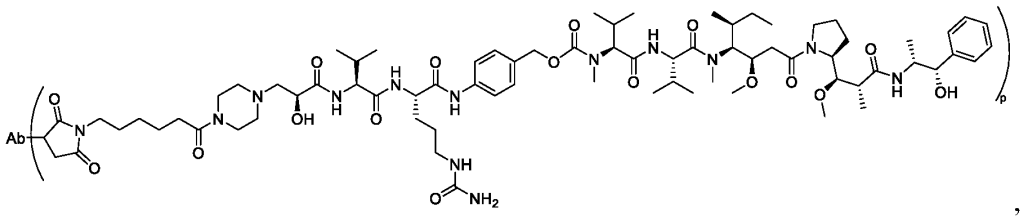

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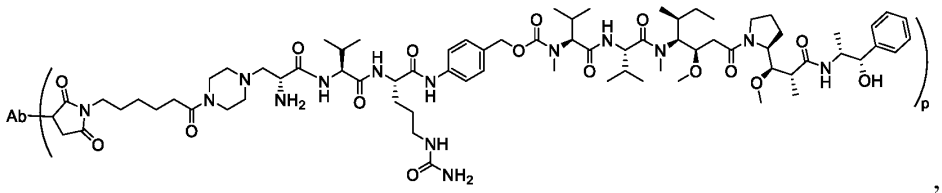

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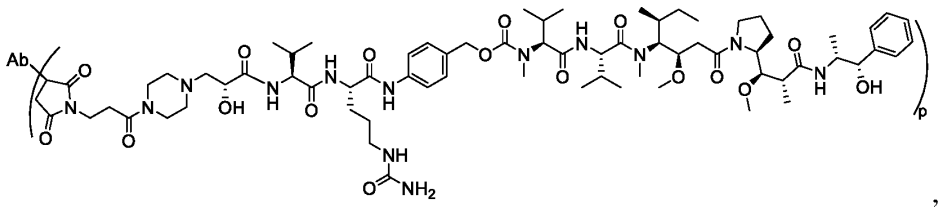

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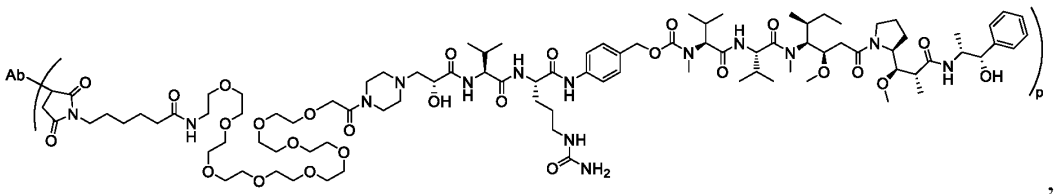

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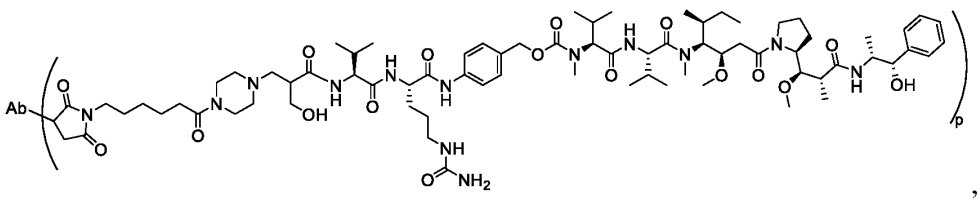

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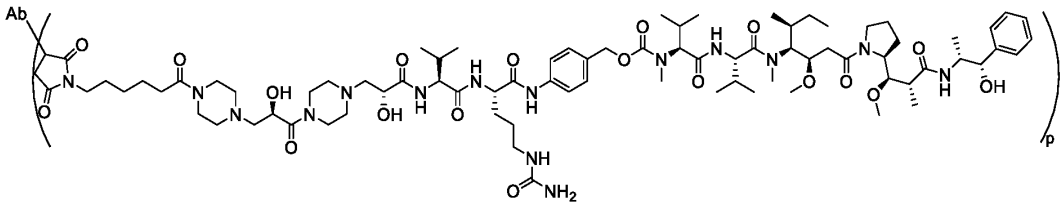

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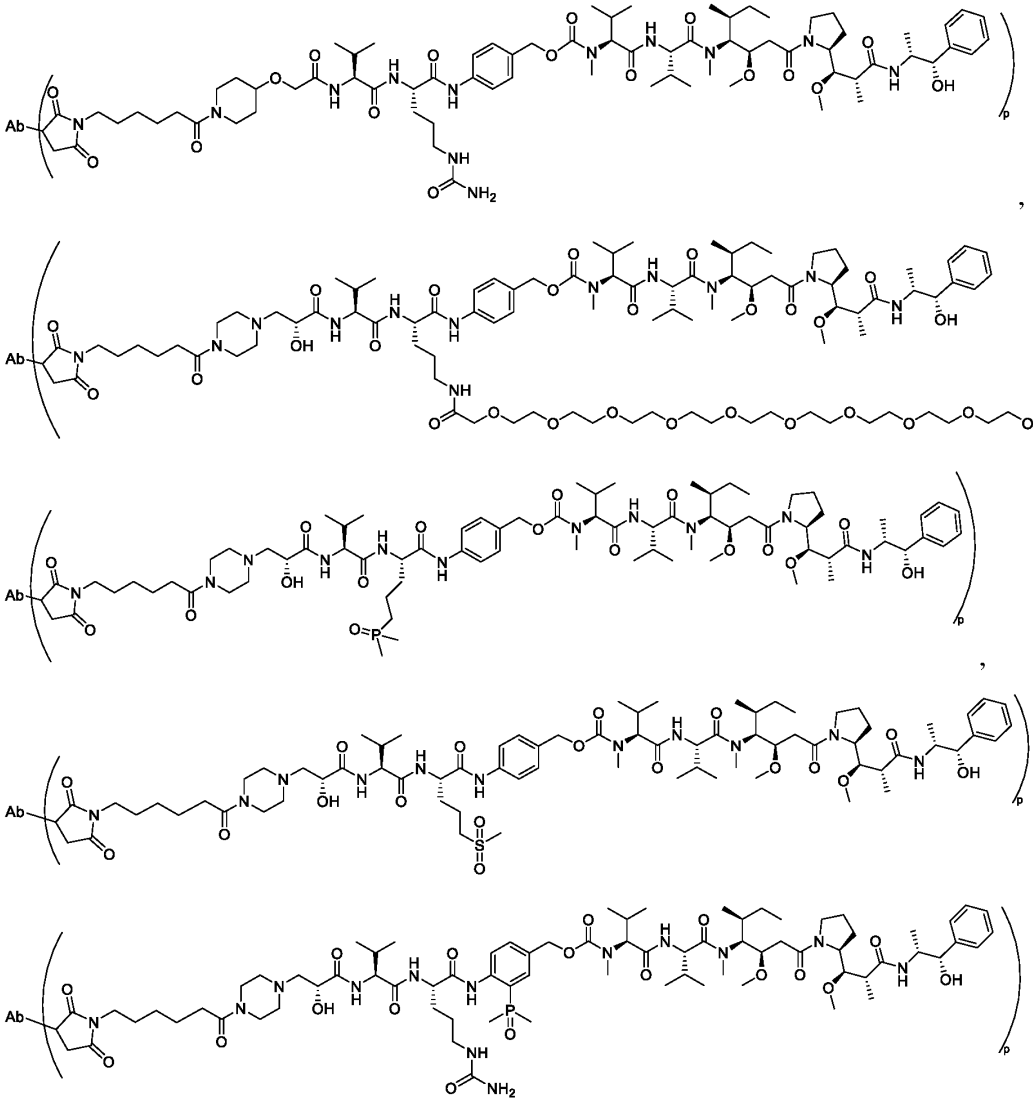


wherein Ab and p are as defined above.

Embodiment I-20. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment I-19, wherein the Ab is Daratumumab, and p is 2, 3, 4, 5, 6, 7 or 8.

Embodiment I-21. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment I-19, wherein the Ab is the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14, such as Ab1 or huAb1; p is 2, 3, 4, 5, 6, 7 or 8.

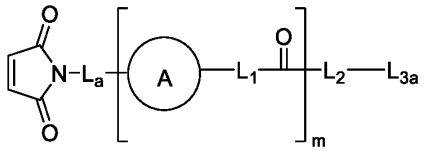
Embodiment I-22. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-21, having an average DAR of 2-8, such as an average DAR of 3-4, 3.8-4.2, 3-5, 4-5.5, 5-7, 6.5-8 or 6-8.

Embodiment I-23. A pharmaceutical composition comprising the drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-22, and optionally a pharmaceutically acceptable carrier

Embodiment I-24. Use of the drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments I-1 to I-22 in the preparation of a drug for treating cancer.

Embodiment II

Embodiment II-1. A compound represented by formula (I):



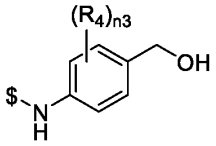
(I) ,

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein

L_a, ring A, L_1, L_2 and m are as defined in embodiment I;

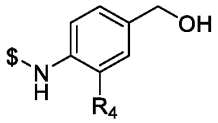
L_{3a} is a spacer unit.

Embodiment II-2. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment **II-1**, wherein L_{3a} has a structure represented by formula (I-5):



Formula (I-5) ,

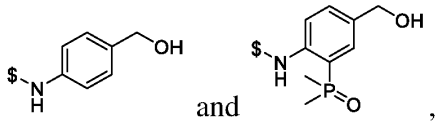
wherein R_4 is selected from $-(CH_2)_{n2}SO_2CH_3$, $-(CH_2)_{n2}P(O)(CH_3)_2$ or hydrogen, wherein n2 is 0, 1, 2, 3, 4, 5, 6, 7 or 8; n3 is 1 or 2; $ is an active site connected to L_2;



Formula (I-6)

preferably, L_{3a} has a structure represented by formula (I-6) , wherein each symbol is as defined in formula (I-5).

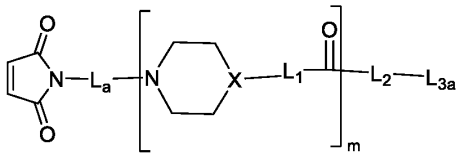
Embodiment II-3. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment **II-2**, wherein L_{3a} has a structure selected from:



and ,

wherein $ represents a connection site to L_2.

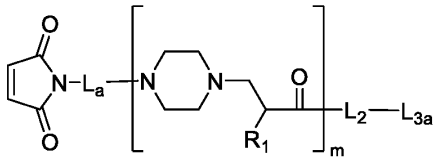
Embodiment II-4. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments **II-1** to **II-3**, wherein the compound is a compound represented by formula (I-2):



Formula (I-2)

wherein X is N or CH; the remaining variables (L_a, L_1, L_2, L_{3a}, and m) are as defined in one of embodiments II-1 to II-3.

Embodiment II-5. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments **II-1** to **II-4**, wherein the compound is a compound represented by formula (I-3):



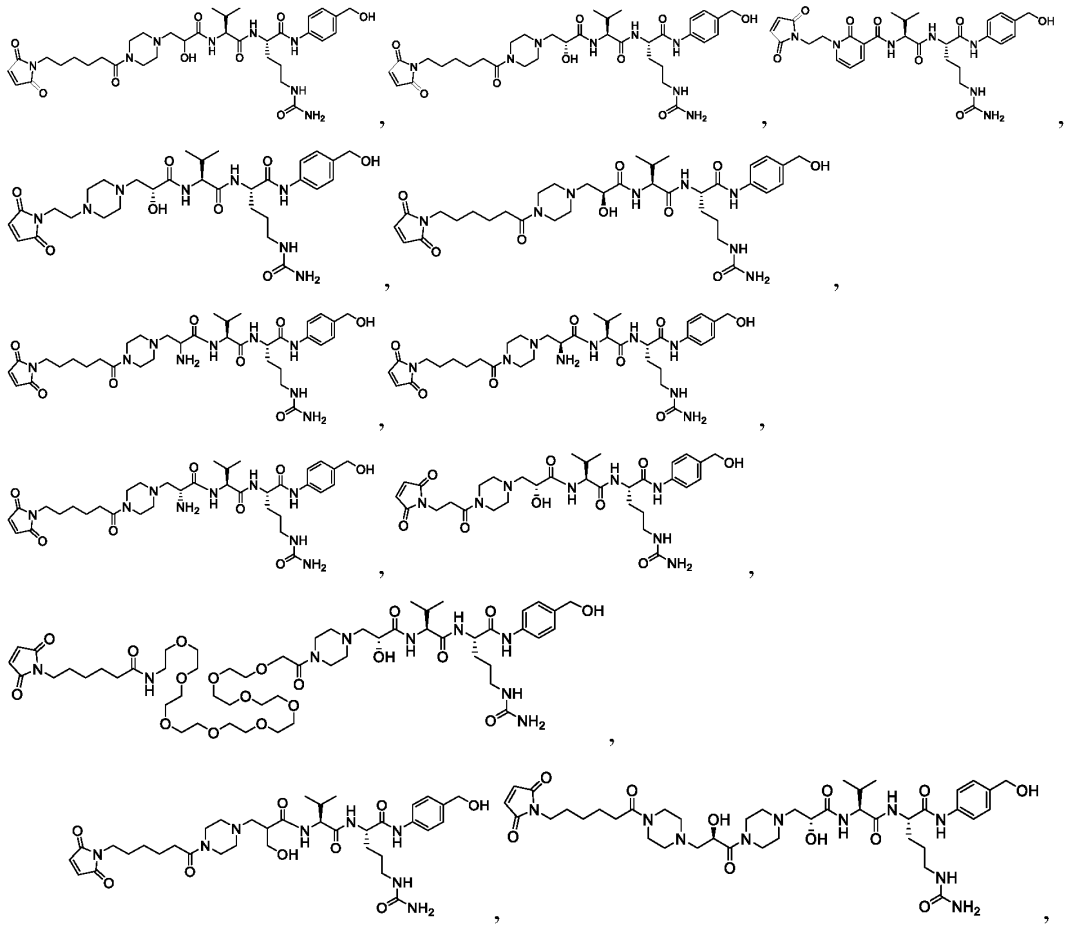
(I-3)

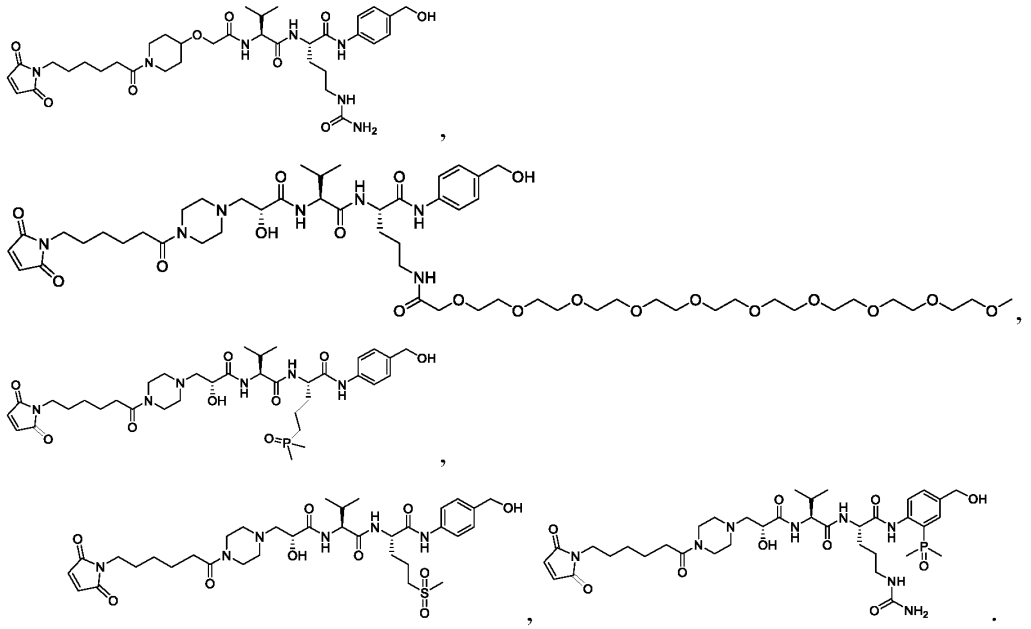
wherein R_1 is as defined in embodiment I-4, and the remaining variables are as defined in one of embodiments **II-1** to **II-4**.

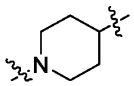
Embodiment II-6. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment **II-5**, wherein

R_1 is -OH, -NH_2, -CH_2-OH or -CH_2-NH_2.

Embodiment II-7. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment **II-1**, wherein the compound is selected from:

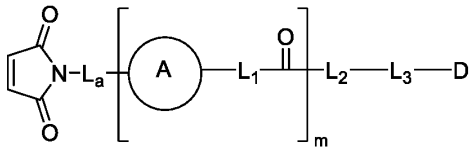




It should be understood that, unless otherwise specified and not contradictory according to the context, the left bond of a divalent group as shown herein is connected to a group on the left of the divalent group in a structure consisting of same, and the right bond of the divalent group is connected to a group on the right of the divalent group in the structure. For example, when ring A is , the bond on the nitrogen atom is connected to L_a, and the bond on the right carbon atom is connected to L_1. This also applies to other embodiments of compounds including ADC.

Embodiment III

Embodiment III-1. A compound represented by formula (II):

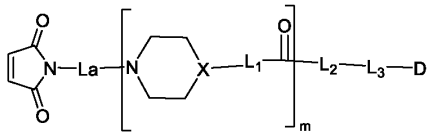


(II)

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein D, L_a, ring A, L_1, L_2, L_3 and m are as defined in embodiment I (e.g., one of embodiments I-1 to I-19) of the present invention.

Embodiment III-2. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer

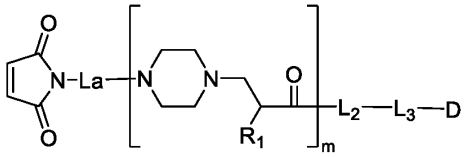
thereof according to embodiment III-1, wherein the compound is a compound represented by formula (II-2):



Formula (II-2)

wherein each variable is as defined in embodiment I, in particular embodiment I-17, of the present invention.

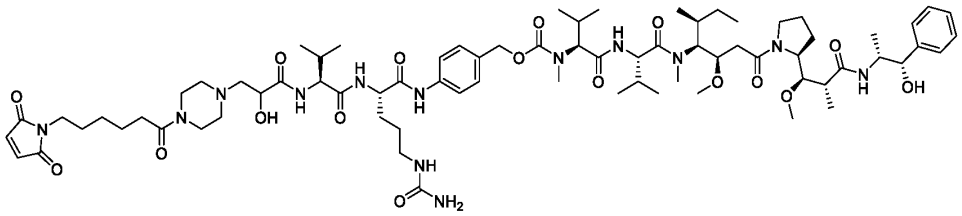
Embodiment III-3. A compound represented by formula (II-3):



(II-3)

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein each variable is as defined in embodiment I, in particular embodiment I-18, of the present invention.

Embodiment III-4. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment III-1, wherein the compound is selected from:



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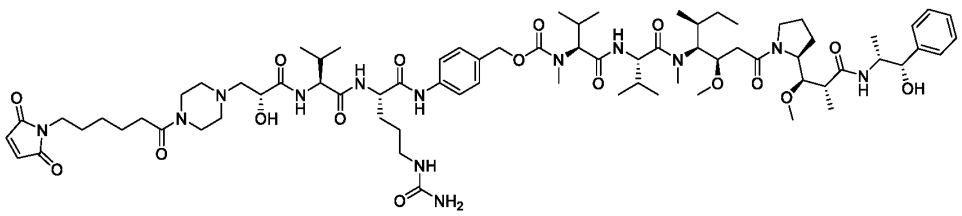

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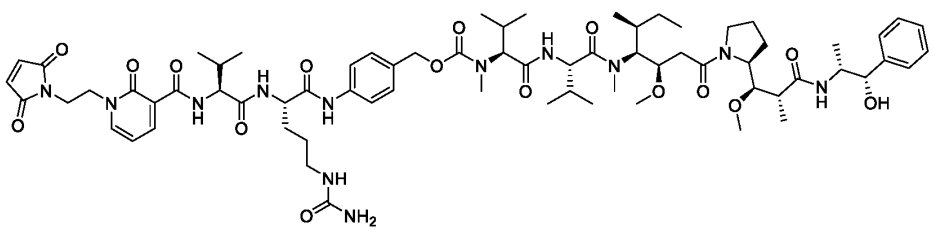

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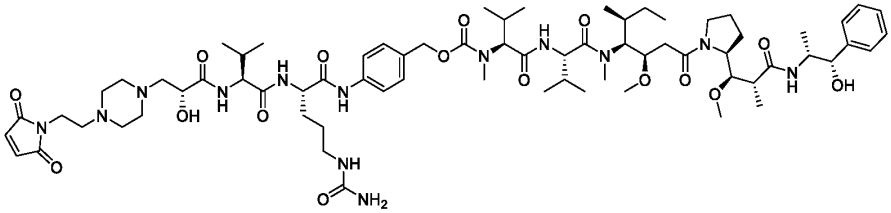
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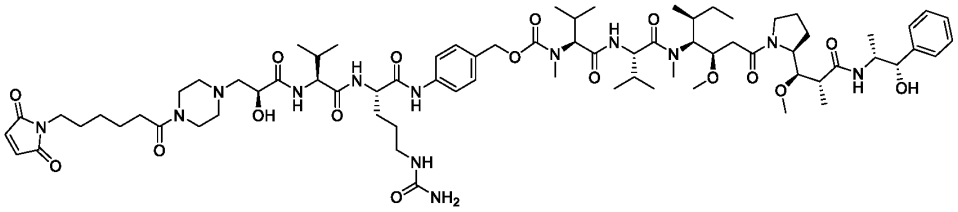
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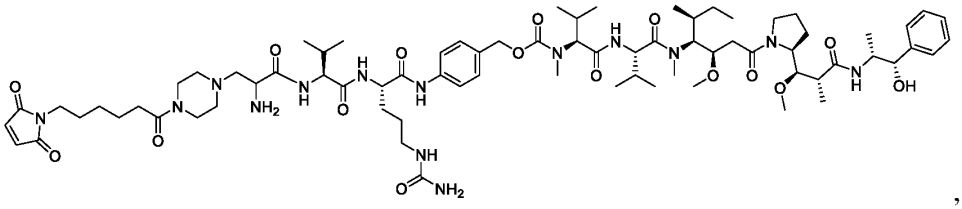
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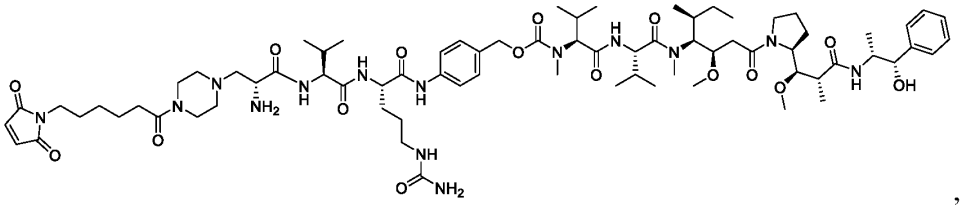
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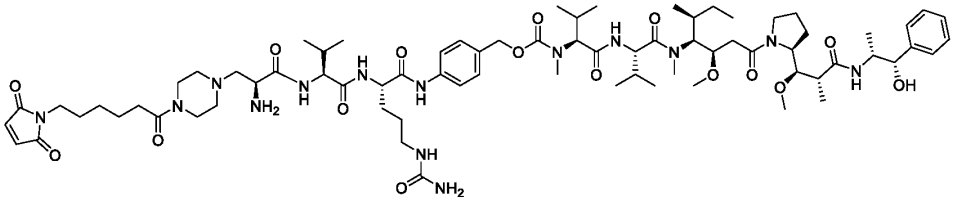
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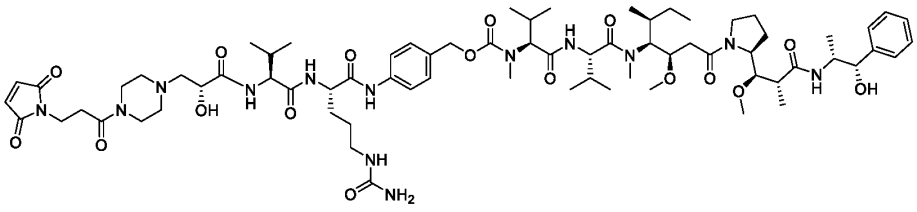
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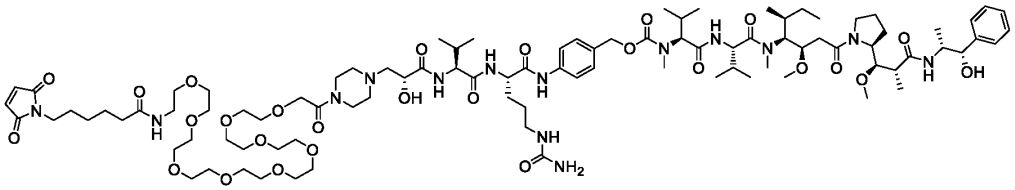
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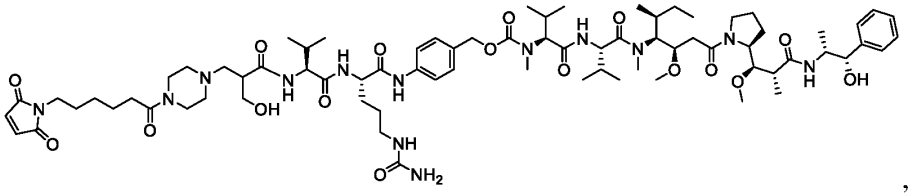
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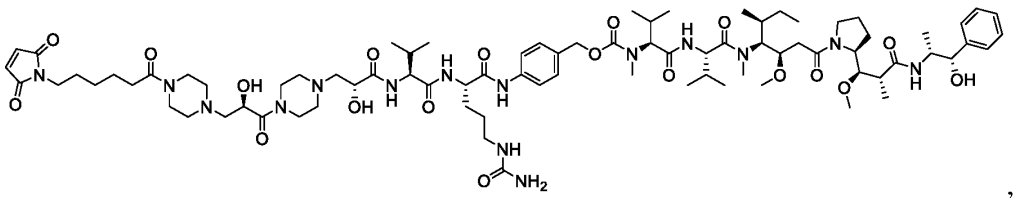
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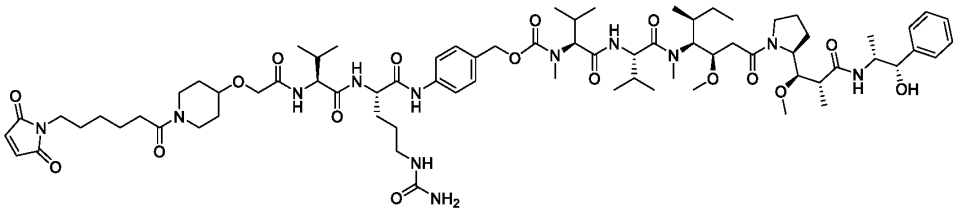
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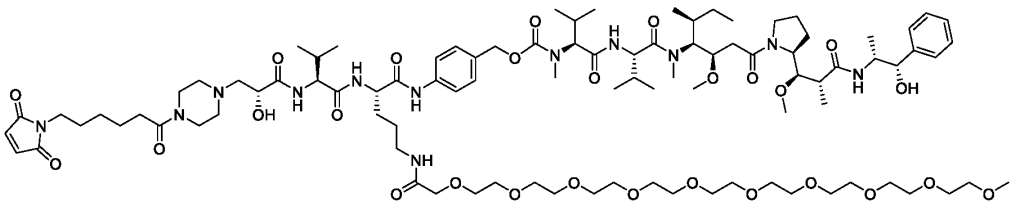
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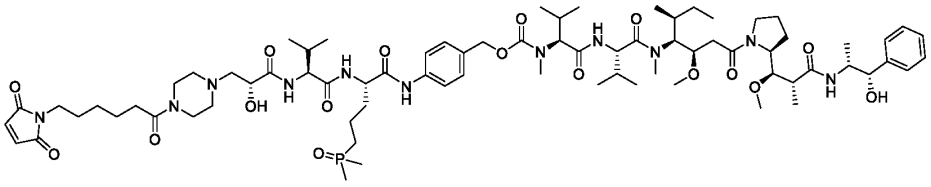
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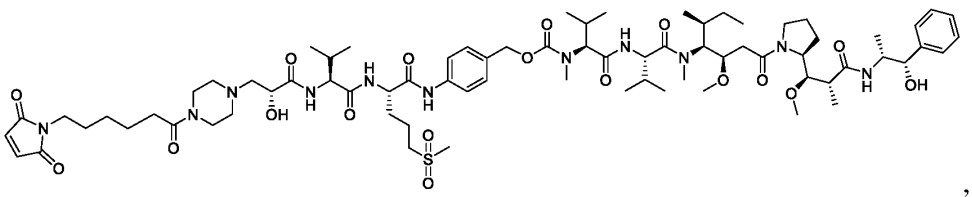
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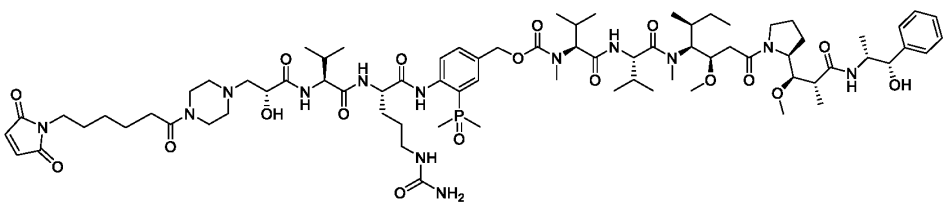
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Embodiment IV

Embodiment IV-1. An isolated anti-CD38 antibody or an antigen-binding fragment thereof, comprising a heavy chain variable region (VH) and a light chain variable region

(VL), wherein the heavy chain variable region comprises three heavy chain complementarity determining regions (HCDRs) in SEQ ID NO: 7 or 9, and the light chain variable region comprises three light chain complementarity determining regions (LCDRs) in SEQ ID NO: 8 or 10;

optionally, the HCDR and LCDR can be determined by any CDR definition scheme known in the art, such as a Kabat scheme, a Chothia scheme, an AbM scheme or a Contact scheme or a combination thereof; preferably, the HCDR and LCDR are determined by the Kabat scheme.

Embodiment IV-2. An isolated anti-CD38 antibody or an antigen-binding fragment thereof, comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises HCDR1, HCDR2 and HCDR3, and the VL comprises LCDR1, LCDR2 and LCDR3, wherein

the HCDR1 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 2, and the HCDR3 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 3; the LCDR1 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 6;

optionally, the HCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 2, and the HCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 3; the LCDR1 comprises an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 comprises an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 6;

optionally, the HCDR1 consists of an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 consists of an amino acid sequence as set forth in SEQ ID NO: 2, and the HCDR3 consists of an amino acid sequence as set forth in SEQ ID NO: 3; the LCDR1 consists of an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 consists of an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 consists of an amino acid sequence as set forth in SEQ ID NO: 6.

Embodiment IV-3. The anti-CD38 antibody or the antigen-binding fragment thereof according to embodiment IV-1 or IV-2, wherein the VH comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 7 or 9, and/or the VL comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 8 or 10.

Embodiment IV-4. The anti-CD38 antibody or the antigen-binding fragment thereof according to embodiment IV-3, wherein the VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 7, and/or the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 8;

optionally, the VH comprises an amino acid sequence as set forth in SEQ ID NO: 7, and the VL comprises an amino acid sequence as set forth in SEQ ID NO: 8;

optionally, the VH consists of an amino acid sequence as set forth in SEQ ID NO: 7, and the VL consists of an amino acid sequence as set forth in SEQ ID NO: 8.

Embodiment IV-5. The anti-CD38 antibody or the antigen-binding fragment thereof according to embodiment IV-3, wherein the VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 9, and/or the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 10;

optionally, the VH comprises an amino acid sequence as set forth in SEQ ID NO: 9, and the VL comprises an amino acid sequence as set forth in SEQ ID NO: 10;

optionally, the VH consists of an amino acid sequence as set forth in SEQ ID NO: 9, and the VL consists of an amino acid sequence as set forth in SEQ ID NO: 10.

Embodiment IV-6. The anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-5, wherein the anti-CD38 antibody or the antigen-binding fragment thereof is of IgG subtype, such as IgG1, IgG2, IgG3 or IgG4 subtype.

Embodiment IV-7. The anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-6, wherein the anti-CD38 antibody or the antigen-binding fragment thereof further comprises a heavy chain constant region, such as a heavy chain constant region of IgG1, IgG2, IgG3 or IgG4, for example, a heavy chain constant region of human IgG1, IgG2, IgG3 or IgG4.

Embodiment IV-8. The anti-CD38 antibody or the antigen-binding fragment thereof according to embodiment IV-7, wherein the heavy chain constant region comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 13 or SEQ ID NO: 23.

Embodiment IV-9. The anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-8, further comprising a light chain constant region, such as a Kappa light chain constant region or a Lambda light chain constant region, such as a human Kappa light chain constant region or a human Lambda light chain constant region.

Embodiment IV-10. The anti-CD38 antibody or the antigen-binding fragment thereof according to embodiment IV-9, wherein the light chain constant region comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 14.

Embodiment IV-11. The anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-10, further comprising a heavy chain constant region and a light chain constant region, wherein the heavy chain constant region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 13 or SEQ ID NO: 23, and the light chain constant region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 14.

Embodiment IV-12. The anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-11, comprising a heavy chain and a light chain, wherein the heavy chain comprises or consists of a heavy chain variable region and a heavy chain constant region; and/or the light chain comprises or consists of a light chain variable region and a light chain constant region;

optionally, the anti-CD38 antibody or the antigen-binding fragment thereof comprises two heavy chains and two light chains, such as two identical heavy chains and two identical light chains.

Embodiment IV-13. The anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-12, wherein the anti-CD38

antibody or the antigen-binding fragment thereof is a murine antibody, a chimeric antibody or a humanized antibody.

Embodiment IV-14. The anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-13, wherein the anti-CD38 antibody or the antigen-binding fragment thereof is a full-length antibody, a disulfide-stabilized Fv fragment (dSFv), a single-domain antibody such as VHH, a Fab antibody (such as a Fab' antibody and Fab'-SH), a $(Fab')_2$ antibody, a single-chain antibody such as scFv, Fv and dAb (domain antibody) or a multispecific antibody such as a bispecific antibody.

Embodiment IV-15 An isolated nucleic acid, wherein the nucleic acid encodes the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14; further, the nucleic acid encodes a heavy chain and/or a light chain of the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14; further, the nucleic acid encodes a heavy chain variable region and/or a light chain variable region of the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14.

Embodiment IV-16. A recombinant vector or expression vector, wherein the recombinant vector or expression vector comprises one or more nucleic acids according to embodiment IV-15; further, the vector is suitable for recombinant production of the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-13, and for example, the vector is a pcDNA vector, such as pcDNA3.1 and pCDNA3.4.

Embodiment IV-17. A host cell comprising one or more nucleic acids according to embodiment IV-15 or the recombinant vector or expression vector according to embodiment IV-16, wherein the host cell is, for example, a CHO cell or a 293 cell, such as Expi293.

Embodiment IV-18. A method of producing the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14, wherein the method comprises

culturing a host cell containing the encoding for a polypeptide chain of the antibody under the condition suitable for expression of the polypeptide chain, and optionally, the

method further comprises assembling the polypeptide chain to produce a molecule under the condition suitable for assembly of the polypeptide chain into the molecule; or

culturing a host cell comprising the nucleic acid according to embodiment IV-15 or the recombinant vector or expression vector according to embodiment IV-16 or culturing the host cell according to embodiment IV-17 under the condition suitable for expression of a chain of the antibody, and optionally recovering the antibody from the host cell (or a culture medium for the host cell).

Embodiment IV-19. An immunoconjugate or immune fusion, wherein the immunoconjugate or immune fusion comprises the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14; further, the immunoconjugate or immune fusion comprises other active molecules, such as the drug active molecules defined in the present invention (such as the Dolastatin 10 and Dolastatin 10 derivative as defined in embodiment I-10). In some embodiments, the antibody or the antigen-binding fragment thereof is conjugated to the drug active molecule via a linker described herein. In some embodiments, the immunoconjugate is an antibody-drug conjugate (ADC).

Embodiment IV-20. A pharmaceutical composition comprising the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14, the nucleic acid according to embodiment IV-15, the recombinant vector or expression vector according to embodiment IV-16, the host cell according to embodiment IV-17, the immunoconjugate or immune fusion according to embodiment IV-19, and optionally at least one pharmaceutically acceptable adjuvant material (such as a pharmaceutical carrier or a pharmaceutical excipient).

Embodiment IV-21. Use of the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14, the nucleic acid according to embodiment IV-15, the recombinant vector or expression vector according to embodiment IV-16, the host cell according to embodiment IV-17, the immunoconjugate or immune fusion according to embodiment IV-19 or the pharmaceutical composition according to embodiment IV-20 in the preparation of a drug for treating cancer.

Embodiment IV-22. The anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14, the nucleic acid according to

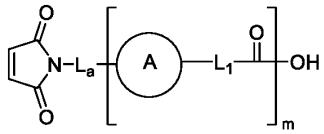
embodiment IV-15, the recombinant vector or expression vector according to embodiment IV-16, the host cell according to embodiment IV-17, the immunoconjugate or immune fusion according to embodiment IV-19 or the pharmaceutical composition according to embodiment IV-20, for use in therapy, for example, for use in the treatment of cancer.

Embodiment IV-23. A method of treating cancer, comprising administering to a subject an effective amount of the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14, the nucleic acid according to embodiment IV-15, the recombinant vector or expression vector according to embodiment IV-16, the host cell according to embodiment IV-17, the immunoconjugate or immune fusion according to embodiment IV-19 or the pharmaceutical composition according to embodiment IV-20.

Embodiment IV-24. Use of the anti-CD38 antibody or the antigen-binding fragment thereof according to any one of embodiments IV-1 to IV-14, the nucleic acid according to embodiment IV-15, the recombinant vector or expression vector according to embodiment IV-16, the host cell according to embodiment IV-17, the immunoconjugate or immune fusion according to embodiment IV-19 or the pharmaceutical composition according to embodiment IV-20 in the treatment of cancer.

Embodiment V

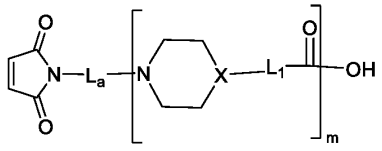
Embodiment V-1. A compound of formula (IV-1), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof:



Formula (IV-1) ,

wherein L_a, ring A, L_1 and m are as defined in embodiment I or II.

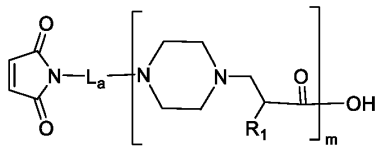
Embodiment V-2. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment V-1, wherein the compound is a compound represented by formula (IV-2):



Formula (IV-2)

wherein L_a, X, L_1 and m are as defined in embodiment I or II.

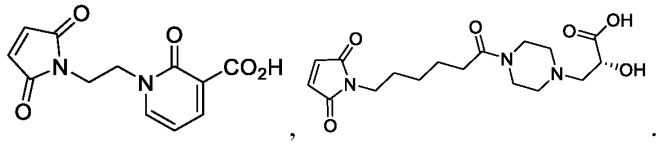
Embodiment V-3. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiment V-1, wherein the compound is a compound represented by formula (IV-3):



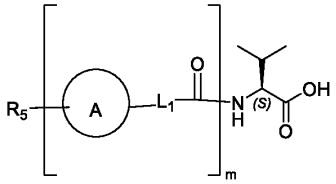
Formula (IV-3)

wherein L_a, R_1, L_1 and m are as defined in embodiment I or II.

Embodiment V-4. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of embodiments V-1 to V-3, wherein the compound is selected from:



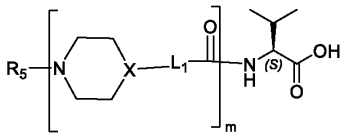
Embodiment V-5. A compound represented by formula (V-1), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof:



Formula (V-1)

wherein R_5 is an amino protecting group; ring A, L_1 and m are as defined in embodiment I or II.

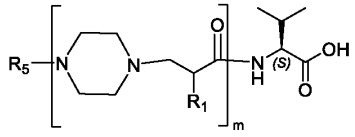
Embodiment V-6. A compound represented by formula (V-2), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof,



Formula (V-2)

wherein R_5 is an amino protecting group; X, L_1 and m are as defined in embodiment I or II.

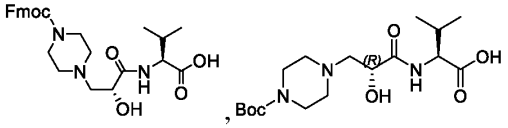
Embodiment V-7. A compound represented by formula (V-3), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof,



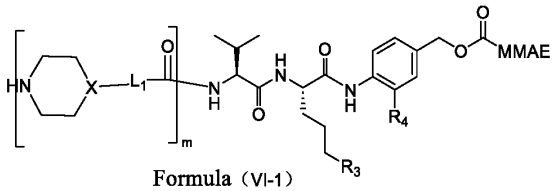
Formula (V-3)

wherein R_5 is an amino protecting group; the remaining variables are as defined in embodiment I or II.

Embodiment V-8. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to embodiments V-5 to V-7, wherein the compound is selected from:



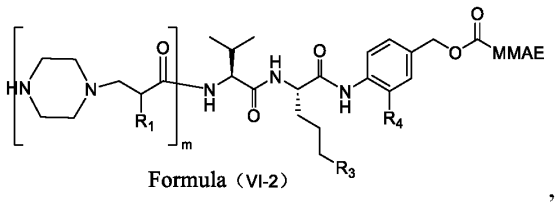
Embodiment V-9. A compound represented by formula (VI-1), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof,



Formula (VI-1) ,

wherein X, L_1, R_3, R_4 and m are as defined in embodiment I or II.

Embodiment V-10. A compound represented by formula (VI-2), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof:

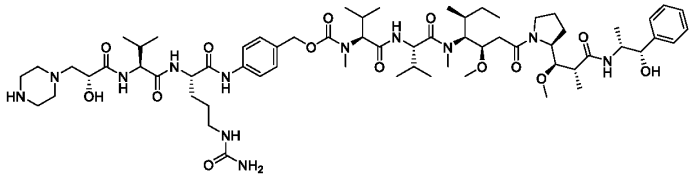


Formula (VI-2)

,

wherein R_1, R_3, R_4 and m are as defined in embodiment I or II.

Embodiment V-11. The compound according to embodiment V-9 or embodiment V-10, selected from:



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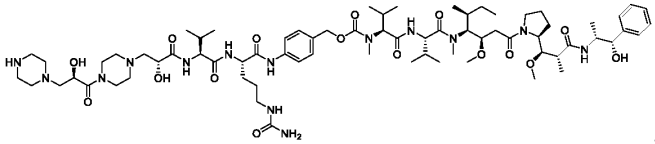

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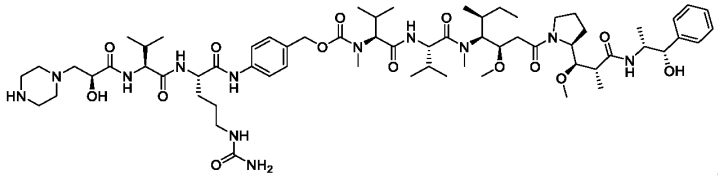

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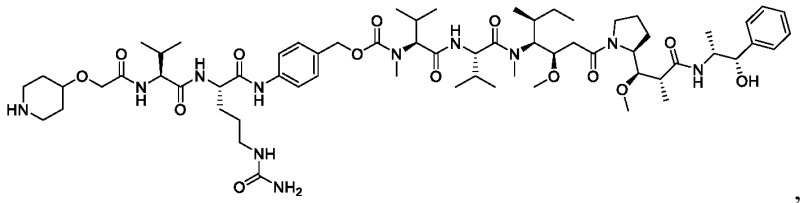

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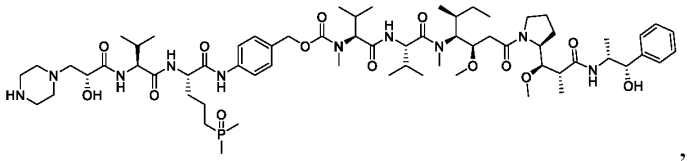

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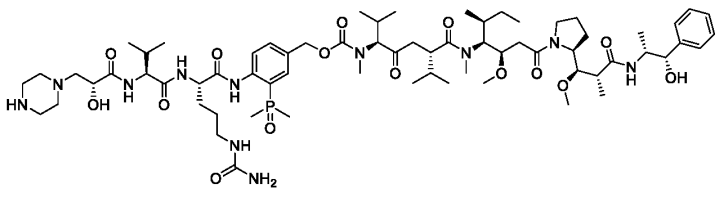

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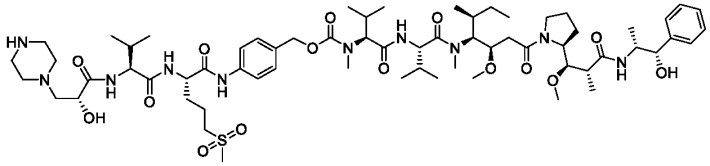


or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof.

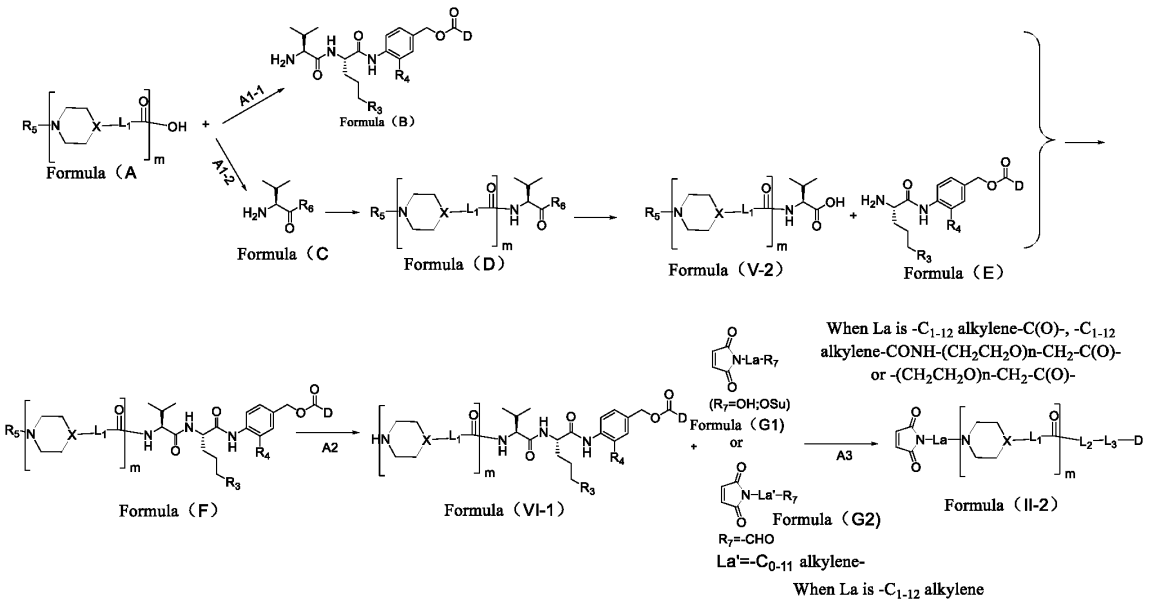
The various embodiments of the present invention (including the following examples) and the features of the various embodiments should be interpreted as being arbitrarily combined with each other, and the various solutions obtained from these mutual combinations are all included in the scope of the present invention, just like the solutions obtained from the mutual combinations specifically and individually set forth herein, unless clearly stated otherwise in the context.

General synthetic methods

The compounds of formula (I), formula (II) and formula (III) and/or the pharmaceutically acceptable salts thereof described in the present invention can be synthesized using commercially available starting materials, by methods known in the art, or methods disclosed in the present patent application. The general synthetic methods of the compounds of the present invention are illustrated below.

In one aspect, the compound represented by formula (II) provided by the present invention can be synthesized by the following general synthetic methods:

Method A:



wherein L_a, X, L_1, L_2, R_3, R_4, R_5, D and m are as defined herein.

Step A1-1: as shown in method A, a compound of formula (A) is reacted with a compound of formula (B) under an alkaline condition (such as but not limited to DIPEA) and in the presence of a condensing agent (such as but not limited to EDCI, HOBT or HATU) to obtain a compound of formula (F);

alternatively, step A1-2: a compound of formula (A) is reacted with a compound of formula (C) under an alkaline condition (such as but not limited to DIPEA) and in the presence of a condensing agent (such as but not limited to EDCI, HOBT or HATU) to obtain a compound of formula (D);

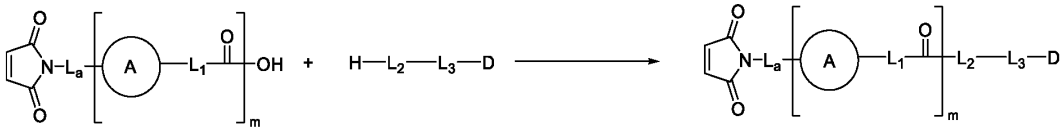
R_6 is a carboxyl protecting group, and based on the specific carboxyl protecting group of R_6, the compound of formula (D) is reacted under a corresponding condition (for example, but not limited to, the compound of formula (D) is reacted under an alkaline condition (such as but not limited to NaOH) or a catalyst condition (such as but not limited to Pd/C) or under an acidic condition (such as but not limited to TFA)) to obtain a compound of formula (V-2);

the compound of formula (V-2) is reacted with a compound of formula (E) under an alkaline condition (such as but not limited to DIPEA) and in the presence of a condensing agent (such as but not limited to EDCI, HOBT or HATU) to obtain a compound of formula (F);

step A2: the compound of formula (F) is reacted under an acidic condition (such as but not limited to TFA) or an alkaline condition (such as but not limited to Et_2NH) to obtain a compound of formula (VI-1);

step A3: when L_a is selected from -C_{1-12} alkylene-C(O)-, -C_{1-12} alkylene-CONH-$(CH_2CH_2O)_n$-CH_2-C(O)- or -$(CH_2CH_2O)_n$-CH_2-C(O)- and R_7 is OH, a compound of formula (G1) is reacted with the compound of formula (VI-1) under an alkaline condition (such as but not limited to DIPEA) and in the presence of a condensing agent (such as but not limited to EDCI, HOBT or HATU) to obtain a compound of formula (II-2); when L_a is selected from -C_{1-12} alkylene, a compound of formula (G2) is reacted with a compound of formula (VI-1) in the presence of a reducing agent (such as but not limited to $NaBH(OAc)_3$) to obtain a compound of formula (II-2); when L_a is selected from -C_{1-12} alkylene-C(O)-, -C_{1-12} alkylene-CONH-$(CH_2CH_2O)_n$-CH_2-C(O)- or -$(CH_2CH_2O)_n$-CH_2-C(O)- and R_7 is Osu, a compound of formula (G1) is reacted with a compound of formula (VI-1) under an alkaline condition (such as but not limited to Et_3N or DIPEA) to obtain a compound of formula (II-2).

Method B:



Formula (IV-1) Formula (II)

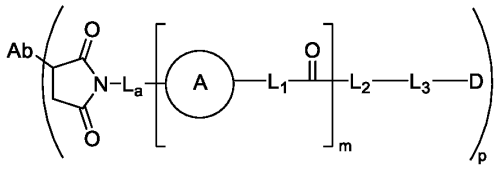
wherein L_a, ring A, L_1, L_2, L_3, D and m are as defined herein.

As shown in method B, a compound of formula (IV-1) is reacted with an H-L_2-L_3-D compound under an alkaline condition (such as but not limited to DIPEA) and in the presence of a condensing agent (such as but not limited to HATU or HBTU) to obtain a compound of formula (II).

The substituents of the compounds thus obtained can be further modified to provide other desired compounds. Synthetic chemistry transformations are described, for example, in R. Larock, Comprehensive Organic Transformations, VCH Publishers (1989); L.Fieser and M.Fieser, Fieser and Fieser's Reagents for Organic Synthesis, John Wiley and Sons (1994); and L. Paquette, eds., Encyclopedia of Reagents for Organic Synthesis, John Wiley and Sons (1995) and subsequent editions.

Before use, the compounds of the present invention can be purified by column chromatography, high performance liquid chromatography, crystallization or other suitable methods.

In another aspect, the present invention provides a method of preparing a drug conjugate represented by formula (III),



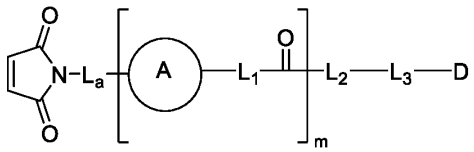
(III)

wherein each variable is as defined herein;

the method comprises the following steps:

(a) adding an Ab (a targeting moiety is preferably an antibody or an antigen-binding fragment thereof) to a buffer solution, adding a reducing agent, and then incubation;

(b) adding a compound of formula (II) or a pharmaceutically acceptable salt thereof, or a solvate thereof to the reaction solution in step (a) for conjugation, so as to obtain a crude product,



(II)

wherein each variable is as defined herein;

and

(c) optionally purifying the crude product to obtain the drug conjugate represented by formula (III).

In some embodiments, the drug conjugate represented by formula (III) can be obtained by conjugating the linker moiety of the compound of formula (II) with the active group of the antibody moiety (such as Daratumumab, Ab1 or HuAb1) in water and/or an organic solvent. Suitable organic solvents may be selected from N,N-dimethylformamide, dimethyl sulfoxide, N-methylpyrrolidone, nitriles (such as acetonitrile), alcohols (such as methanol or ethanol) and any combination thereof. The conjugation method may involve opening a disulfide bond of the antibody by using a reducing agent (such as TCEP) to yield sulfhydryl, then reacting with the linker moiety of

the compound of formula (II) to form a C-S bond, and then performing conjugation. The resulting conjugate product can be purified by a chromatography method (such as one or more of ion exchange chromatography, hydrophobic chromatography, reverse phase chromatography or affinity chromatography).

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

step 1: adding a TCEP solution (10 mmol/L, 2.5-3.0 eq) to a daratumumab solution with a concentration of 4-8 mg/mL prepared with a reaction buffer, and subjecting the resulting solution to a reaction at 0°C-22°C for 1-24 hours, wherein the reaction buffer is one of PBS-D (10 mmol/L, pH 7.0), a histidine-acetate buffer (20 mmol/L, pH 5.5) or a histidine buffer (10 mmol/L, pH 7.0), and TCEP is dissolved in water or a histidine buffer (10 mmol/L, pH 7.0);

step 2: adding a solution of a compound of formula (II) or a pharmaceutically acceptable salt thereof, or a solvate thereof in DMSO (75%)/H_2O (5 mmol/L, 10-15 eq) to the above system, and further performing a reaction at 4°C-25°C for 60-180 min; adding 20 equivalents of L-cystine, quenching at 4°C for 1 hour, transferring the reaction solution to a Slide-A-Lyzer® Dislysis Cassette or directly transferring the reaction solution to an ultrafiltration centrifuge tube (Millipore Amicon®Ultra), and adding a buffer solution for solution exchange to obtain a product, wherein the buffer is one of 10 mmol/L PBS (pH 7.0), 10 mmol/L citric acid buffer (pH 5.5), or 10 mmol/L His/His·HCl (pH 6.2).

In some embodiments, the steps are performed under the specific reaction conditions disclosed in the examples.

It should be noted that the embodiments obtained by floating the ranges or specific values of specific reaction conditions disclosed in the examples by 100%, 80%, 60%, 40%, 20% or 10% are also contemplated by the present invention.

Pharmaceutical composition

The compound of the present invention (e.g., a compound of any of the examples as described herein) can be formulated, alone or in combination with one or more additional therapeutic agents, into a pharmaceutical composition. The pharmaceutical composition comprises: (a) the compound of the present invention; (b) a pharmaceutically acceptable excipient (e.g., one or more pharmaceutically acceptable carriers); and optionally (c) at least one additional therapeutic agent. It should be understood that the active ingredient

(the compound of the present invention) in the pharmaceutical composition is present in an effective amount. In some embodiments, the compounds of the present invention include the compounds as defined in the above embodiments, such as the compounds represented by formula (II), the drug conjugates represented by formula (III), or the pharmaceutically acceptable salts thereof, or the solvates, the racemic mixtures, the enantiomers, the diastereomers or the tautomers thereof; and the antibodies or the antigen-binding fragments thereof, the nucleic acids, the recombinant vectors or the expression vectors, the host cells, the immunoconjugates or the immune fusions as defined in embodiment IV; and the like.

In some embodiments, the additional therapeutic agent is selected from, for example, an anti-tumor active agent, an anti-inflammatory agent or an immunomodulator, wherein the anti-tumor active agent includes a chemotherapeutic agent, an immune checkpoint inhibitor or agonist, and a targeted therapeutic agent. The term "anti-tumor active agent" as used herein refers to any agent that is administered to a subject suffering from cancer for the purposes of treating the cancer, including but not limited to a chemotherapeutic agent, an immune checkpoint inhibitor or agonist, and a targeted therapeutic agent.

The compositions of the present invention may be in a variety of forms. These forms include, for example, liquid, semisolid and solid dosage forms, such as liquid solutions (e.g., injectable solutions and infusible solutions), powders or suspensions, liposomes and suppositories. The preferred form depends on the intended administration mode and therapeutic use.

Pharmaceutical combination and kit

In some embodiments, the present invention also provides a pharmaceutical combination or a pharmaceutical combination product, comprising the compound of the present invention, and one or more additional therapeutic agents, wherein the therapeutic agents are as defined above.

Another object of the present invention is to provide a kit of parts, comprising the pharmaceutical combination of the present invention, wherein the kit is preferably in the form of pharmaceutical dosage units. Dosage units may thus be provided according to a dosage regimen or intervals for drug administration.

In one embodiment, the kit of parts of the present invention comprises in the same package:

- a first container containing a pharmaceutical composition comprising the compound of the present invention;

- a second container containing a pharmaceutical composition comprising an additional therapeutic agent.

The additional therapeutic agent and the compound of the present invention may be administered separately, or may be included in the same pharmaceutical composition, such as a fixed-dose combination drug, according to the present disclosure. The combination may serve to increase efficacy (e.g., by including in the combination a compound potentiating the potency or effectiveness of the compound of the present invention), decrease one or more side effects, or decrease the required dose of the compound of the present invention.

Use and method

The compound of the present invention can be shown to have sufficient pre-clinical practical utility to merit clinical trials hoped to demonstrate a beneficial therapeutic or prophylactic effect, for example, in subjects with cancer.

The compounds of the present invention include the compounds as defined in the above embodiments, such as the compounds represented by formula (II), the drug conjugates represented by formula (III), or the pharmaceutically acceptable salts thereof, or the solvates, the racemic mixtures, the enantiomers, the diastereomers or the tautomers thereof; and the antibodies or the antigen-binding fragments thereof, the nucleic acids, the recombinant vectors or the expression vectors, the host cells, the immunoconjugates or the immune fusions as defined in embodiment IV; and the like.

In one aspect, the present invention provides a method of preventing or treating a disease in a subject, comprising administering to the subject an effective amount of the compound, the pharmaceutical composition, the pharmaceutical combination or the kit of the present invention.

In some embodiments, the disease is tumor, including solid tumors and non-solid tumors, such as hematologic malignancies. In some embodiments, the tumor is cancer. In some embodiments, the cancer includes solid tumors and non-solid tumors, such as

hematologic malignancies. Non-limiting examples of hematologic malignancies include hematopoietic and lymphoid tissue tumors, myelomas, leukemias, etc.

In some embodiments, the hematopoietic and lymphoid tissue tumors include, but are not limited to, non-Hodgkin's lymphoma. In some embodiments, the non-Hodgkin's lymphoma is B-cell lymphoma (such as, but not limited to, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma and Burkitt lymphoma). In some embodiments, the myeloma is, for example, but not limited to, multiple myeloma (MM). In some embodiments, the leukemia is, for example but not limited to, chronic myeloid leukemia, chronic lymphocytic leukemia or acute lymphocytic leukemia. In one embodiment, the disease is a CD38, CD20, CD70 or PD-L1-related disease. In some embodiments, the disease is related to aberrant expression or aberrant activity of CD38, CD20, CD70 or PD-L1.

In some embodiments, the tumor, e.g., cancer, is CD38, CD20, CD70 or PD-L1-positive, and for example, comprises tumor cells expressing CD38, CD20, CD70 or PD-L1. In some embodiments, the tumor, e.g., cancer, is CD38, CD20, CD70 or PD-L1-negative, and for example, comprises tumor cells not expressing or lowly expressing CD38, CD20, CD70 or PD-L1. In some embodiments, the tumor of the patient comprises tumor cells expressing CD38, CD20, CD70 or PD-L1. In some embodiments, the tumor cells of the patient express CD38, CD20, CD70 or PD-L1, such as moderately expressing CD38, CD20, CD70 or PD-L1, and preferably highly expressing CD38, CD20, CD70 or PD-L1. In some embodiments, the tumor tissues of the tumor (e.g., cancer) patient have, for example, an elevated level (such as in terms of nucleic acid or protein levels or activity) of CD38, CD20, CD70 or PD-L1, such as compared to the levels of CD38, CD20, CD70 or PD-L1 in the same tissue of a healthy subject or in a healthy tissue adjacent to the patient's tumor tissue. In some embodiments, the tumor cells of the patient have, for example, an elevated level (such as in terms of nucleic acid or protein levels or activity) of CD38, CD20, CD70 or PD-L1, such as compared to the levels of CD38, CD20, CD70 or PD-L1 in the same cell of a healthy subject or in a healthy cell adjacent to the patient's tumor cell.

The subject may be a mammal, e.g., a primate, preferably a higher primate, e.g., a human (e.g., a subject suffering from a disease described herein or having a risk of suffering from a disease described herein). In one embodiment, the subject suffers from

or has a risk of suffering from a disease described herein (e.g., a cancer). In some embodiments, the subject receives or has received other treatments, such as chemotherapy and/or radiation therapy. In some embodiments, the subject has previously received or is currently receiving immunotherapy.

In other aspects, the present invention provides the use of the above-mentioned compound or pharmaceutical composition or pharmaceutical combination or kit of the present invention in the production or preparation of a medicament for the purposes described herein, such as for preventing or treating the relevant diseases or conditions mentioned herein.

In other aspects, the present invention provides the above-mentioned compound or pharmaceutical composition or pharmaceutical combination or kit of the present invention, for use in therapy, such as for the purposes described herein, such as for preventing or treating the relevant diseases or conditions mentioned herein.

In some embodiments, the compound or pharmaceutical composition or pharmaceutical combination or kit of the present invention can delay the onset of conditions and/or symptoms associated with the conditions.

In some embodiments, the compound or pharmaceutical composition of the present invention can also be administered in combination with one or more additional therapies, such as treatment modalities and/or additional therapeutic agents, for the purposes described herein, such as for preventing and/or treating the relevant diseases or conditions mentioned herein.

In some embodiments, the therapeutic agent is as defined above.

Combination therapies of the present invention encompass combined administration (e.g., two or more therapeutic agents contained in the same formulation or in separate formulations), and separate administration, in which case the compound of the present invention can be administrated prior to, concurrently with, and/or after other agents and/or therapeutic agents.

The pharmaceutical composition is administrated via known methods, for example, orally, by intravenous injection, intraperitoneally, intracerebrally (intraparenchymally), intracerebroventricularly, intramuscularly, intraocularly, intraarterially, intraportally or intralesionally; by a sustained release system or by an implantable device. In certain

embodiments, the composition may be administered by bolus injection, by continuous infusion or by an implantable device.

The composition may also be administered topically via an implanted membrane, a sponge, or another appropriate material into which the desired molecule is absorbed or encapsulated. In certain embodiments, when an implantable device is used, the device can be implanted into any suitable tissue or organ, and the desired molecule can be delivered via diffusion, timed-release bolus, or continuous administration.

The compound of the present invention can be used to achieve a beneficial therapeutic or prophylactic effect, for example, in a subject suffering from cancer.

The drug conjugate of the present invention has the following advantages:

(1) specifically binding to target cells, and having comparable affinity to naked antibodies;

(2) having excellent in vitro cell proliferation inhibitory activity and significant in vivo activity;

(3) having the ability to enter cells through endocytosis and kill target cells;

(4) having high endocytosis efficiency;

(5) having a certain bystander killing effect;

(6) having a better product uniformity;

(7) having excellent physicochemical stability, including plasma stability; and/or

(8) having good drug ability.

Brief Description of the Drawings

Figure 1 shows the binding activity of anti-CD38 antibodies to human CD38 protein.

Figure 2 shows the binding activity of anti-CD38 antibodies to monkey CD38 protein.

Figure 3 shows the binding activity of anti-CD38 antibodies to CD38 on the surface of Daudi cells.

Figure 4 shows the binding activity of anti-CD38 antibodies to CD38 on the surface of HCI-H929 cells.

Figure 5 shows that anti-CD38 antibodies promote phagocytosis of Daudi and HCI-H929 tumor cells by human macrophages.

Figure 6 shows that anti-CD38 antibodies mediate complement killing of Daudi cells.

Figure 7 shows the binding activity of anti-CD38 antibodies to NK cells.

Figure 8 shows internalization signal values of ADC products of the present invention.

Figure 9 shows the anti-tumor activity of Daratumumab and ADC-10 in Daudi subcutaneous transplanted tumors.

Figure 10 shows the anti-tumor activity of Daratumumab and ADC-10 in KMS-11 subcutaneous transplanted tumors.

Detailed Description of Embodiments

The examples below are intended to be purely exemplary and should not be considered to be limiting in any way. Efforts have been made to ensure the accuracy with respect to numbers used (such as amounts and temperature), but a person skilled in the art should understand that some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric. All MS data were determined by Agilent 6120 and Agilent 1100. All NMR data were generated using a Varian 400 MR machine. All reagents and starting materials, except synthesized intermediates, used in the present invention are commercially available. All compound names except the reagents are generated by Chemdraw 20.1.

If there is any atom with empty valence(s) in any one of the structures disclosed herein, the empty balance(s) is (are) the hydrogen atom(s) which is (are) omitted for convenience purpose.

In the present application, in the case of inconsistency of the name and structure of a compound, when the two of which are both given for the compound, it is subject to the structure of the compound, unless the context shows that the structure of the compound is incorrect, and the name is correct.

List of abbreviations used in the following examples:

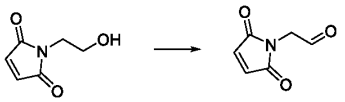
Cbz-Cl	Benzyl chloroformate
Fmoc-OSu	9-Fluorenylmethyl-N-succinimidyl carbonate
DIPEA	N,N-diisopropylethylamine

DMA	N,N-dimethylacetylamide
MsCl	Methanesulfonyl chloride
OXONE	Potassium peroxymonosulfate
TBFA	Tetrabutylammonium fluoride
KI	Potassium iodide
Ag_2O	Silver oxide
Pd/C	Palladium/carbon
HBTU	Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate
HOBT	1-Hydroxybenzotriazole
EDCI	1-Ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride
TFA	Trifluoroacetic acid
MS	Mass spectrometry
PE	Petroleum ether
EA	Ethyl acetate
CH_3CN	Acetonitrile
MMAE	Monomethyl auristatin E
DCM	Dichloromethane
H_2O	Water
MeOH	Methanol
THF	Tetrahydrofuran
Et_2N	Diethylamine
DMF	N,N-dimethylformamide
HATU	2-(7-Azabenzotriazole)-N,N,N',N'-tetramethylurea hexafluorophosphate
ISCO	Fast preparative liquid chromatography
PBS-D	Phosphate buffered saline-Diethylenetriaminepentaacetic acid
HIC-HPLC	Hydrophobic interaction chromatography
His	Histidine

TCEP	Tris(2-carboxyethyl)phosphine
DTPA	Diethylenetriaminepentaacetic acid
DMSO	Dimethyl sulfoxide
ADC	Antibody-drug conjugate
DPPA	Diphenyl azidophosphate
DBU	1,8-Diazabicyclo-undec-7-ene

Example 1 Preparation of intermediates

Preparation of intermediate-1

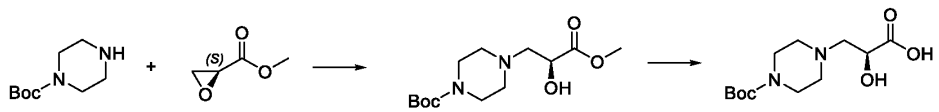


A Intermediate-1

A solution of compound A (1.41 g, 10 mmol) in dichloromethane (100 mL) was cooled to 0°C, and Dess-Martin periodinane (CAS No.: 87413-09-0) (6.36 g, 15 mmol) was added in batches. The resulting reaction solution was transferred to room temperature and stirred for 3 hours, and then filtered to remove the solid. The filtrate was concentrated to obtain intermediate-1 as a yellow solid (1.11 g, yield: 80%).

MS(m/z):140.1$[M+H]^+$

Preparation of intermediate-2



A B Intermediate-2

1) Synthesis of compound B

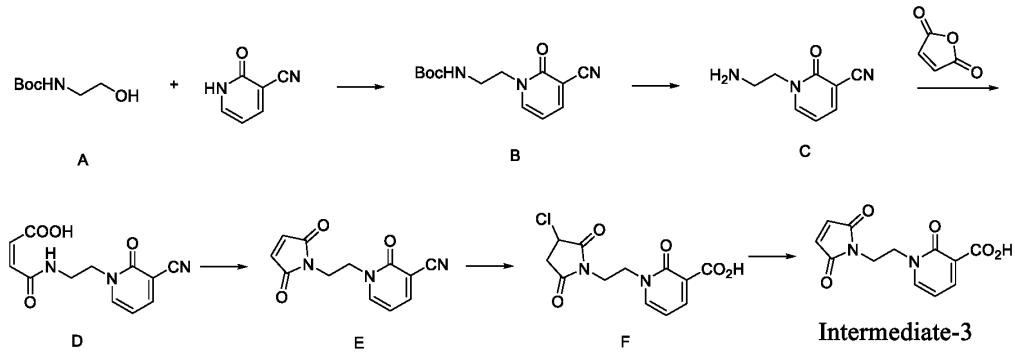
Compound A (4 g, 21.5 mmol), methyl (S)-oxirane-2-carboxylate (2.63 g, 25.8 mmol) and DIPEA (11.2 mL, 64.5 mmol) were dissolved in DMF (30 mL), and the reaction solution was stirred at 80°C for 18 hours. After the reaction was cooled to room temperature, water (40 mL) was added. The resulting mixed solution was extracted with ethyl acetate (25 mL×3). The organic phases were combined, washed once with water (40 mL), dried over anhydrous sodium sulfate and filtered. The resulting filtrate was concentrated to obtain compound B as a light-yellow solid (5.26 g, yield: 85%).

MS(m/z):289.2$[M+H]^+$

2) Synthesis of intermediate-2

Compound B (2.88 g, 10 mmol) and lithium hydroxide monohydrate (2 g, 50 mmol) were dissolved in a mixed solution of methanol/water (15 mL/6 mL), and the reaction solution was heated to 40°C and stirred for 18 hours. The reaction solution was cooled to room temperature, further cooled in an ice-water bath and then adjusted to pH 7.0 with 5 N hydrochloric acid in the ice-water bath. The resulting mixture was concentrated under reduced pressure, and the residue was separated by ISCO (mobile phase: H_2O (0.1% HCOOH)/CH_3CN = 100 : 0–0 : 100) to obtain intermediate-2 as a light-yellow solid (2.41 g, yield: 88%). MS(m/z):275.2[M+H]$^+$

Preparation of intermediate-3



1) Synthesis of compound B

Compound A (19.2 g, 120 mmol), 2-oxo-1,2-dihydropyridine-3-carbonitrile (7.2 g, 60 mmol) and triphenylphosphine (23.6 g, 90 mmol) were dissolved in tetrahydrofuran (200 mL). The reaction solution was cooled to 0°C, and di-tert-butyl azodicarboxylate (20.6 g, 90 mmol) was added in batches. The mixture was stirred at room temperature for 1 hour and then concentrated under reduced pressure. The residue was separated by ISCO (mobile phase: ethyl acetate/petroleum ether = 0 : 100–100 : 0) to obtain compound B as a white solid (6.8 g, yield: 43%). MS(m/z):286.1[M+Na]$^+$

2) Synthesis of compound C

Compound B (6.8 g, 25.8 mmol) was dissolved in dichloromethane (150 mL), and methanesulfonic acid (16.8 mL, 258 mmol) was added. The reaction solution was stirred at room temperature for 30 minutes, neutralized with ammonium hydroxide to pH = 8 and extracted with dichloromethane. The organic phase was concentrated to dryness under reduced pressure to obtain compound C as a yellow oil (4.2 g, yield: 99%). MS(m/z):164.1[M+H]$^+$

3) Synthesis of compound D

Maleic anhydride (2.8 g, 28.4 mmol) was dissolved in ether (100 mL), and then compound C (4.2 g, 25.8 mmol) was added. The mixture was stirred at room temperature for 30 minutes and then filtrated to collect a white precipitate, which was dried to obtain compound D as a white solid (6.5 g, yield: 97%). MS(m/z): 262.1[M+H]$^+$

4) Synthesis of compound E

Compound D (3.6 g, 13.8 mmol) was dissolved in acetic anhydride (50 mL), and sodium acetate (1.6 g, 19.6 mmol) was added. The reaction solution was stirred at 80°C for 1 hour, and then water (50 mL) was added. The mixture was extracted with ether. The organic phase was concentrated under reduced pressure. The residue was separated by ISCO (mobile phase: acetonitrile/water (0.1% trifluoroacetic acid) = 0:100-100:0) to obtain compound E as a white solid (2.1 g, yield: 62%). MS(m/z):244.2[M+H]$^+$

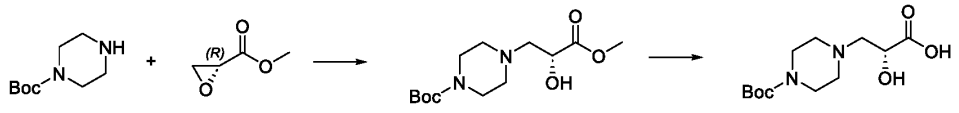
5) Synthesis of compound F

Compound E (1 g, 4.1 mmol) was dissolved in concentrated hydrochloric acid and heated to reflux for 30 minutes. The reaction solution was concentrated under reduced pressure, and the residue was separated by ISCO (mobile phase: acetonitrile/water (0.1% trifluoroacetic acid) = 0 : 100–100 : 0) to obtain compound F as a yellow solid (180 mg, yield: 15%). MS(m/z): 299.1[M+H]$^+$

6) Synthesis of intermediate-3

Compound F (180 mg, 0.6 mmol) and triethylamine (121 mg, 1.2 mmol) were dissolved in dichloromethane (5 mL), and the mixture was stirred at room temperature for 30 minutes and concentrated under reduced pressure. The residue was separated by ISCO (mobile phase: acetonitrile/water (0.1% trifluoroacetic acid) = 0:100–100:0) to obtain intermediate-3 as a yellow solid (60 mg, yield: 38%). MS(m/z): 263.1[M+H]$^+$

Preparation of intermediate-4



A B Intermediate-4

1) Synthesis of compound B

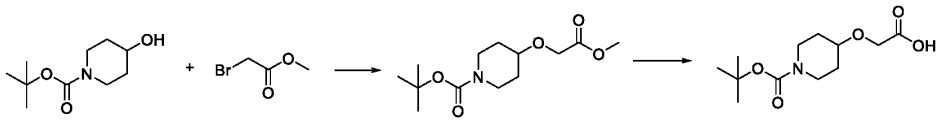
Compound A (4 g, 21.5 mmol), methyl (R)-oxirane-2-carboxylate (2.63 g, 25.8 mmol) and DIPEA (11.2 mL, 64.5 mmol) were dissolved in DMF (30 mL), and the mixture was stirred at 80°C for 18 hours. After the reaction was cooled to room temperature, water (40 mL) was added, and the mixture was extracted with ethyl acetate

(25 mL×3). The organic phases were combined, washed once with water (40 mL) and dried over anhydrous sodium sulfate, and the filtrate was filtered and concentrated to obtain compound B as a light-yellow solid (5.6 g, yield: 90%). MS(m/z): 289.2[M+H]$^+$

2) Synthesis of intermediate-4

Compound B (2.88 g, 10 mmol) and lithium hydroxide monohydrate (2 g, 50 mmol) were dissolved in a mixed solution of methanol/water (15 mL/6 mL), and the mixture was heated to 40°C, stirred for 18 hours, cooled to room temperature and then adjusted to pH 7.0 with 5 N hydrochloric acid in an ice-water bath. The resulting mixture was concentrated under reduced pressure, and the resulting residue was separated by ISCO (mobile phase: H_2O (0.1% formic acid)/CH_3CN =100:0–0:100) to obtain intermediate-4 as a light-yellow solid (2.2 g, yield: 80%). MS(m/z):275.2 [M+H]$^+$

Preparation of intermediate-5



1) Synthesis of compound A

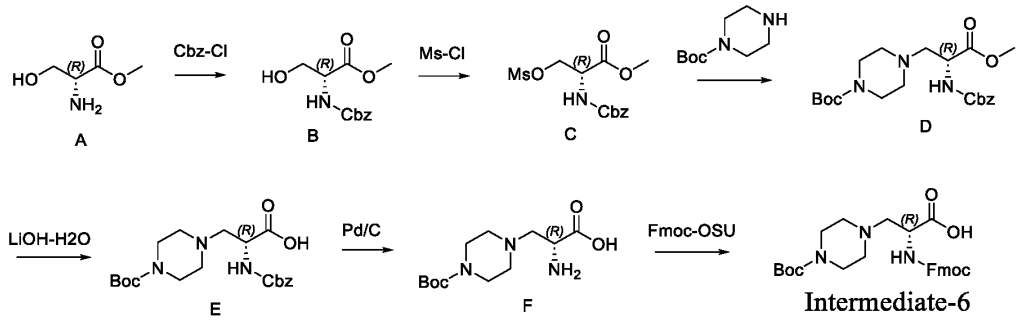
At 0°C, sodium hydride (239 mg, 5.96 mmol) was added to a solution of tert-butyl 4-hydroxypiperidine-1-carboxylate (1 g, 4.96 mmol) in anhydrous THF (20 mL). After stirring for 30 minutes, methyl 2-bromoacetate (912 mg, 5.96 mmol) was added, and the mixture was stirred at room temperature overnight. The reaction was quenched by adding saturated NH_4Cl (100 mL) to the reaction solution, and then extracted with ethyl acetate (50 mL×3). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure to remove the solvent. The residue was purified by ISCO (methanol: water (0.1% formic acid) = 0 : 100-100 : 0) to obtain compound A as a light-yellow oil (500 mg, yield: 37%). MS(m/z) = 218.1[M+H]$^+$

2) Synthesis of intermediate-5

Sodium hydroxide (10 mol/L, 3.7 mL) was added to a solution of compound A (500 mg, 1.83 mmol) in MeOH/THF/H_2O (2 mL/1 mL/1 mL), and the mixture was stirred at room temperature for 4 h. The reaction solution was adjusted to pH 7.0 with 3 mol/L hydrochloric acid, extracted with ethyl acetate (20 mL×3), and concentrated under reduced pressure to remove the solvent. The residue was purified by ISCO (methanol:

water (0.1% formic acid) = 0:100–100:0) to obtain intermediate-5 as a white solid (300 mg, yield: 63%). MS(m/z) = 204.1[M+H]$^+$

Preparation of intermediate-6



1) Synthesis of compound B

Compound A (3.0 g, 25.0 mmol) and DIPEA (7.0 g, 55.0 mmol) were dissolved in dichloromethane (100 mL). Cbz-Cl (4.7 g, 27.5 mmol) was added in an ice bath, and the mixture was stirred at room temperature overnight under nitrogen protection. After the reaction was completed, the reaction mixture was washed with water, and the aqueous phase was extracted with dichloromethane. The resulting organic phases were combined, dried, and then concentrated. The resulting residue was purified by ISCO (water (0.1% TFA)/acetonitrile = 100:0-0:100) to obtain compound B as a white solid (3.5 g, yield: 54.7%). MS(m/z): 254.2[M+H]$^+$

2) Synthesis of compound C

Compound B (3.5 g, 13.8 mmol) and Et_3N (1.5 g, 15.2 mmol) were dissolved in dichloromethane (100 mL). MsCl (1.7 g, 15.2 mmol) was added in an ice bath, and the mixture was stirred at room temperature overnight under nitrogen protection. After the reaction was completed, the reaction mixture was washed with water, and the aqueous phase was extracted with dichloromethane. The resulting organic phases were combined, dried, and then concentrated to obtain the crude compound C as a white solid (3.5 g, yield: 77.8%), and the crude was directly used in the next synthesis. MS(m/z): 354.2[M+Na]$^+$

3) Synthesis of compound D

Compound C (3.5 g, 10.6 mmol) and Et_3N (1.3 g, 12.7 mmol) were dissolved in dichloromethane (100 mL). Tert-butyl piperazine-1-carboxylate (2.4 g, 12.7 mmol) was added in an ice bath, and the mixture was stirred at room temperature overnight under nitrogen protection. After the reaction was completed, the reaction mixture was washed

with water, and the aqueous phase was extracted with dichloromethane. The resulting organic phases were combined, dried, and then concentrated. The resulting residue was purified by ISCO (PE/EA = 100 : 0-0 : 100) to obtain compound D as a white solid (2.0 g, yield: 45.5%). MS(m/z): 422.6[M+H]$^+$

4) Synthesis of compound E

Compound D (2.0 g, 4.8 mmol) and LiOH·H_2O (1.0 g, 24.0 mmol) were added to methanol/water (3 : 1, 50 mL), and the mixture was subjected to nitrogen replacement and then stirred at 40°C for 3 hours. After the reaction was completed, the reaction mixture was concentrated under reduced pressure to remove the solvent. The resulting residue was purified by ISCO (water (0.1% TFA)/acetonitrile = 100:0-0:100) to obtain compound E as a white solid (1.0 g, yield: 52.6%). MS(m/z): 408.6[M+H]$^+$

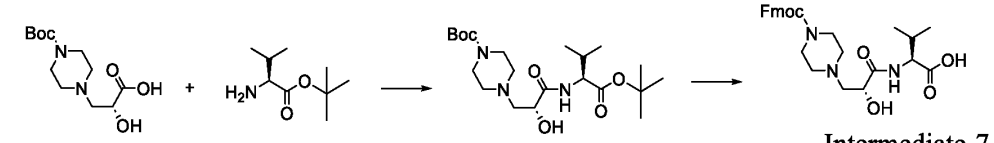
5) Synthesis of compound F

Compound E (1.0 g, 2.5 mmol) and Pd/C (100 mg) were added to methanol (20 mL). The mixture was subjected to hydrogen replacement at normal pressure and then stirred at room temperature for 3 hours. After the reaction was completed, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to remove the solvent to obtain compound F as a light-colored solid (0.65 g, yield: 97.0%). MS(m/z): 274.2[M+H]$^+$

6) Synthesis of intermediate-6

Compound F (0.65 g, 2.4 mmol), Fmoc-OSu (0.88 g, 2.6 mmol) and sodium bicarbonate (0.61 g, 7.2 mmol) were added to dioxane/H_2O (1:1, 20 mL), and the mixture was stirred at room temperature for 5 hours under nitrogen protection. After the reaction was completed, the reaction mixture was concentrated under reduced pressure to remove the solvent. The resulting residue was purified by ISCO (water (0.1% TFA):acetonitrile = 100:0-0:100, gradient elution) to obtain intermediate-6 as a white solid (0.7 g, yield: 59.8%). MS(m/z): 496.4[M+H]$^+$

Preparation of intermediate-7



Intermediate-4 A B Intermediate-7

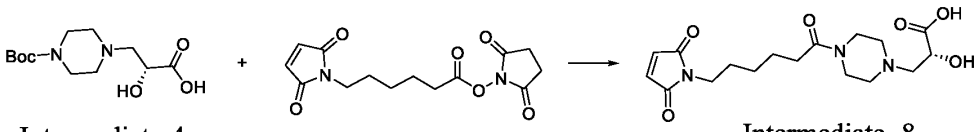
1) Synthesis of compound B

In an ice-water bath and under nitrogen protection, compound A (305 mg, 1.75 mmol), DIPEA (285 mg, 2.19 mmol) and HATU (832 mg, 2.19 mmol) were added to a solution of intermediate-4 (400 mg, 1.46 mmol) in DMF. After the addition was completed, the mixture was stirred at room temperature for 3 hours. After the reaction was completed, the reaction solution was poured into water and extracted with ethyl acetate. The organic phases were combined, dried and concentrated, and the residue was purified by ISCO (mobile phase: CH_3CN/H_2O=0/100-100/0) to obtain compound B as a white solid (470 mg, yield: 75%). MS(m/z): 430.2$[M+H]^+$

2) Synthesis of intermediate-7

In an ice-water bath, compound B (400 mg, 1.46 mmol) was added to a mixed solvent of TFA/DCM (1:1, 4 mL). After the addition was completed, the mixture was stirred at room temperature for 1 hour. After the reaction was completed, the reaction solution was concentrated. The resulting product was dissolved in a mixed solution of dioxane/H_2O (3:2), and then sodium bicarbonate (368 mg, 4.38 mmol) and Fmoc-OSu (492 mg, 1.46 mmol) were added. After the addition was completed, the mixture was stirred at room temperature for 2 h. The resulting reaction solution was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: $CH_3CN:H_2O$=0:100-100:0) to obtain intermediate-7 as a white solid (420 mg, yield: 78%). MS(m/z): 496.4$[M+H]^+$

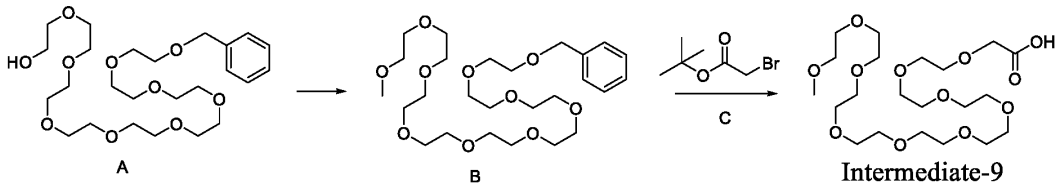
Preparation of intermediate-8



Intermediate-4 A Intermediate -8

Intermediate-4 (500 mg, 1.82 mmol) was added to a solution of TFA/DCM (1:1, 3 mL), and the mixture was stirred at room temperature for 1 hour. After the reaction was completed, the reaction solution was concentrated under reduced pressure. The residue was dissolved in THF, and then DIPEA (712 mg, 5.47 mmol) and compound A (674 mg, 2.19 mmol) were added. After the addition was completed, the mixture was stirred at room temperature for 24 hours. After the reaction was completed, the reaction solution was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: CH_3CN : H_2O = 0 : 100-100 : 0) to obtain intermediate-8 as a light grey solid (260 mg, yield: 38%). MS(m/z): 367.4$[M+H]^+$

Preparation of intermediate-9



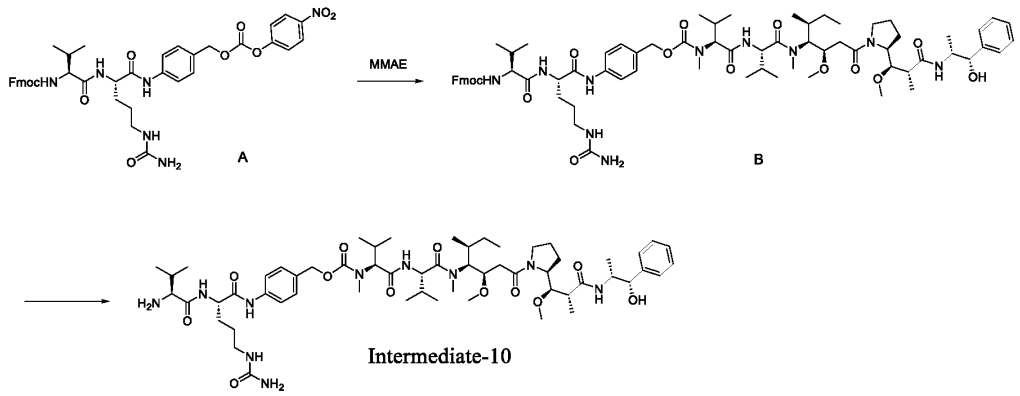
1) Synthesis of compound B

In an ice-water bath and under nitrogen protection, sodium hydride (155 mg, 3.87 mmol) was added in one batch to a solution of compound A (1.3 g, 2.58 mmol) in anhydrous DMF. After the addition was completed, the reaction solution was stirred in the ice-water bath for 15 min. Iodomethane (440 mg, 3.10 mmol) was added dropwise to the reaction solution in one batch. After the addition was completed, the mixture was stirred and reacted at room temperature overnight. After the reaction was completed, the reaction solution was poured into water and extracted with ethyl acetate. The extract was washed with saturated potassium hydrogen sulfate solution and then washed with saturated sodium chloride aqueous solution. The mixture was dried over sodium sulfate and concentrated to obtain compound B as a crude oil, which was directly used in the next step without purification. MS(m/z): 519.2$[M+H]^+$

2) Synthesis of intermediate-9

Pd/C was added to a solution of compound B in THF, and the resulting reaction solution was stirred at 60°C for 3 hours under hydrogen protection. After the reaction was completed, the reaction mixture was filtered, and the filtrate was concentrated. The resulting residue was dissolved in dichloromethane (30 mL), and then silver oxide (155 mg, 3.87 mmol), compound C (155 mg, 3.87 mmol) and potassium iodide (155 mg, 3.87 mmol) were added. The reaction solution was stirred at room temperature for 3 hours. After the reaction was completed, the reaction mixture was filtered, and the filtrate was concentrated. The resulting residue was dissolved in TFA/DCM (3 mL, 1 : 1) and stirred at room temperature for 1 hour. The reaction solution was concentrated, and the resulting residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain intermediate-9 as a colorless oil (770 mg, yield: 61%). MS(m/z): 487.3$[M+H]^+$

Preparation of intermediate-10



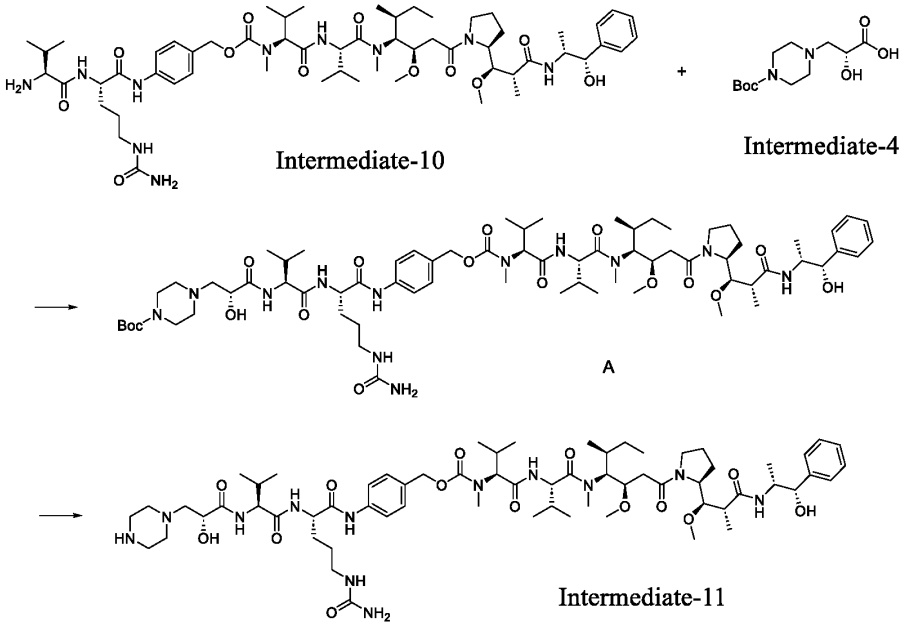
1) Synthesis of compound B

MMAE (1 g, 1.4 mmol), compound A (1.34 g, 1.75 mmol), HOBT (117 mg, 0.87 mmol) and DIPEA (0.61 mL, 3.5 mmol) were dissolved in DMF (12 mL). The reaction solution was stirred at room temperature for 18 hours and then concentrated under reduced pressure. The residue was purified by ISCO (mobile phase: water (0.1% TFA):acetonitrile = 100 : 0–0 : 100) to obtain compound B as a yellow solid (1.12 g, yield: 60%). MS(m/z):1346.8[M+H]$^+$

2) Synthesis of intermediate-10

Compound B (1.12 g, 0.83 mmol) and triethylamine (2.3 mL, 16.6 mmol) were dissolved in DMF (10 mL), and the mixture was stirred at room temperature for 18 hours. The reaction solution was concentrated under reduced pressure and then purified by ISCO (mobile phase: water (0.1% TFA) : acetonitrile = 100 : 0–0 : 100) to obtain intermediate-10 as a yellow solid (700 mg, yield: 75%). MS(m/z):1123.6[M+H]$^+$

Preparation of intermediate-11



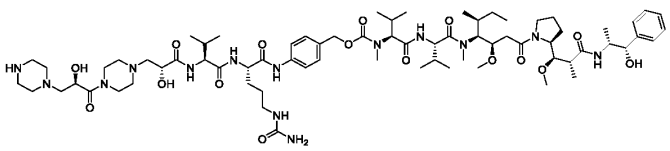
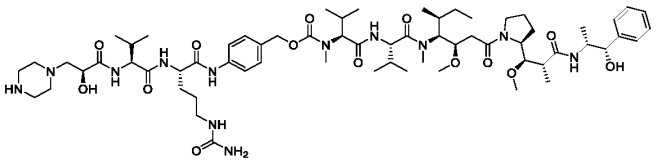
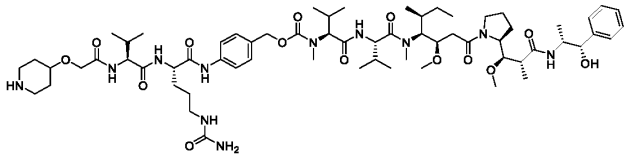
1) Synthesis of compound A

Intermediate-10 (500 mg, 0.45 mmol), intermediate-4 (367 mg, 1.33 mmol), EDCI (171 mg, 0.89 mmol), HOBT (120 mg, 0.89 mmol) and DIPEA (0.78 ml, 4.45 mmol) were dissolved in DMF (10 mL). The resulting reaction solution was stirred at room temperature for 18 hours and then concentrated under reduced pressure. The residue was purified by ISCO (mobile phase: water (0.1%TFA): acetonitrile = 100 : 0–0 : 100) to obtain compound A as a yellow solid (420 mg, yield: 68%). MS(m/z):1379.6[M+H]$^+$

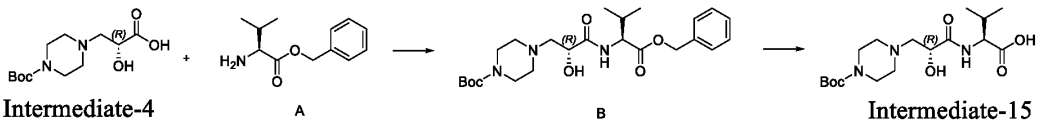
2) Synthesis of intermediate-11

Compound A (420 mg, 0.304 mmol) was dissolved in a mixed solution of dichloromethane and trifluoroacetic acid (3 mL/3 mL), and the mixture was stirred at room temperature for 1 hour. The resulting reaction solution was concentrated under reduced pressure and then purified by ISCO (mobile phase: water (0.1% TFA)/acetonitrile = 100:0–0:100) to obtain intermediate-11 as a yellow solid (390 mg, yield: 98%). MS(m/z):1279.6[M+H]$^+$

The following intermediates were prepared according to the preparation procedure of intermediate-11 using corresponding starting materials and reagents under appropriate conditions that will be recognized by a person skilled in the art.

Intermediate	Structural formula	MS (M+H)$^+$
Intermediate-12		1435.8 [M+H]$^+$
Intermediate-13		1279.6 [M+H]$^+$
Intermediate-14		1265.6 [M+H]$^+$

Preparation of intermediate-15



1) Synthesis of compound B

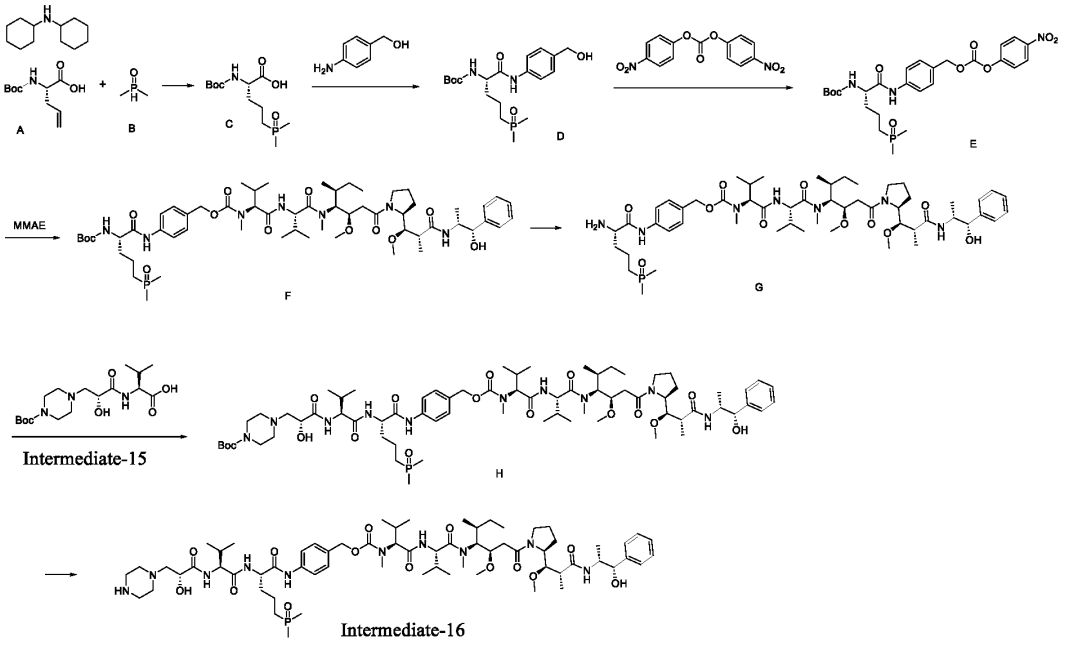
Intermediate-4 (274 mg, 1.0 mmol), compound A (249 mg, 1.5 mmol) and HATU (570 mg, 1.5 mmol) were added to DMA (5 mL), and DIPEA (695 μL, 4.0 mmol) was added dropwise at room temperature. After the addition was completed, the mixture was further reacted at room temperature for 2 hours. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by reverse phase silica gel flash column chromatography (methanol:water (0.1% FA) = 0:100-100:0) to obtain compound B as a yellow oil (284 mg, yield: 61.3%). MS(m/z) = 464.3 [M+H]$^+$

2) Synthesis of intermediate-15

Compound B (284 mg, 0.6 mmol) and 10% Pd/C (30 mg) were added to methanol (10 mL), and the mixture was reacted at room temperature for 4 hours under hydrogen protection. After the reaction was completed, the reaction mixture was filtered through

celite, and the filtrate was concentrated under reduced pressure to obtain intermediate-15 as a white solid (211 mg, yield: 92.2%). MS(m/z) = 374.2[M+H]$^+$

Preparation of intermediate-16



1) Synthesis of compound C

Compound A (350 mg, 0.88 mmol) and compound B (344 mg, 4.41 mmol) were added to n-butanol (10 mL), and under nitrogen protection, the mixture was heated to 120°C and reacted for 4 hours. After the reaction was completed, the reaction mixture was concentrated under reduced pressure to remove the solvent, and the residue was purified by reverse phase silica gel flash column chromatography (methanol: water (0.1% TFA) = 0 : 100-100 : 0, gradient elution) to obtain compound C as a white solid (175 mg, yield: 67.6%). MS(m/z) = 294.4[M+H]$^+$

2) Synthesis of compound D

Compound C (175 mg, 0.60 mmol), (4-aminophenyl)methanol (96 mg, 0.78 mmol) and HATU (342 mg, 0.90 mmol) were added to DMA (5 mL), and DIPEA (313 μL, 1.80 mmol) was added dropwise at room temperature. After the addition was completed, the mixture was further reacted overnight at room temperature. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (methanol:water (0.1% FA) = 0 : 100-100 : 0) to obtain compound D as a yellow solid (120 mg, yield: 50.2%). MS(m/z)=399.2 [M+H]$^+$

3) Synthesis of compound E

Compound D (120 mg, 0.30 mmol) and bis(4-nitrophenyl)carbonate (183 mg, 0.60 mmol) were added to DMA (2 mL), and DIPEA (43 μL, 0.45 mmol) was added dropwise at room temperature. After the addition was completed, the mixture was further reacted overnight at room temperature. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (methanol:dichloromethane = 0:100-100:0) to obtain compound E as a yellow solid (157 mg, yield: 92.9%). MS (m/z) = 564.6 $[M+H]^+$

4) Synthesis of compound F

Compound E (157 mg, 0.28 mmol), MMAE (300 mg, 0.42 mmol) and HOBT (38 mg, 0.28 mmol) were added to a mixed solution of DMF (1.5 mL) and pyridine (0.5 mL), and DIPEA (127 μL, 0.73 mmol) was added dropwise at room temperature. After the addition was completed, the mixture was further reacted at room temperature for 24 hours. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (acetonitrile:water (0.1% TFA) = 0 : 100-100 : 0) to obtain compound F as a white solid (248 mg, yield: 77.5%). MS(m/z)=1142.2 $[M+H]^+$

5) Synthesis of compound G

Compound F (248 mg, 0.22 mmol) was added to dichloromethane (2 mL), and the mixture was cooled to 0°C in an ice bath. Trifluoroacetic acid (1 mL) was added dropwise. After the addition was completed, the mixture was heated to room temperature and reacted for 15 minutes. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (acetonitrile:water (0.1% TFA) = 0 : 100-100 : 0, gradient elution) to obtain compound G as a white solid (228 mg, yield: 100.8%). MS(m/z)=1042.4$[M+H]^+$

6) Synthesis of compound H

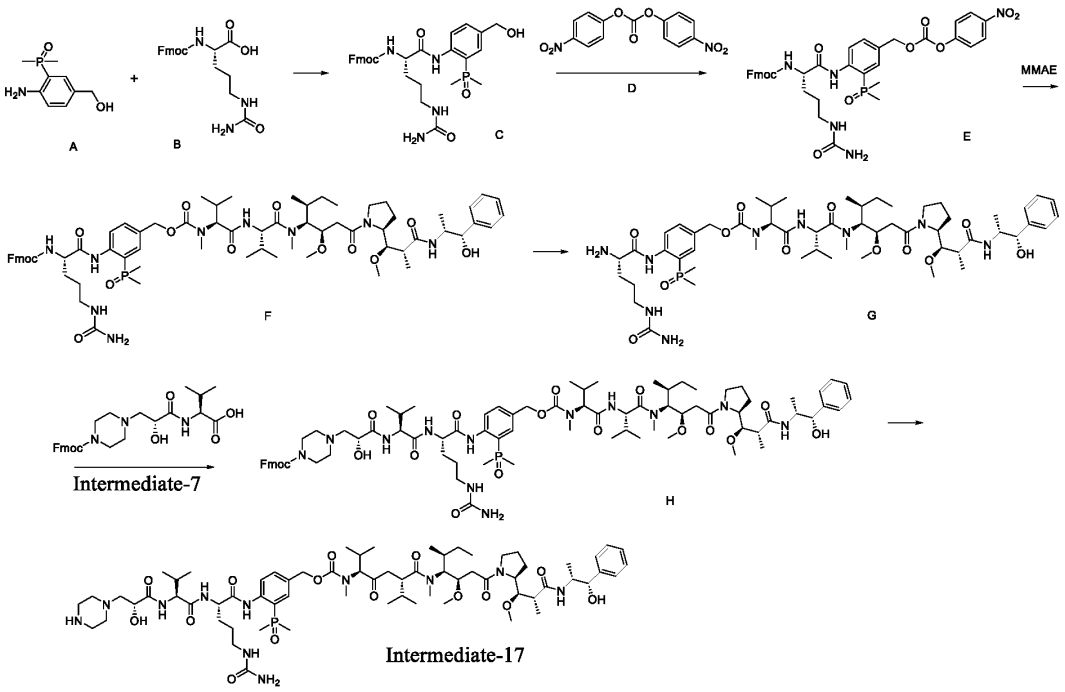
Compound G (115 mg, 0.11 mmol), intermediate-15 (54 mg, 0.14 mmol), EDCI (63 mg, 0.33 mmol) and HOBT (22 mg, 0.17 mmol) were added to DMA (2 mL), and DIPEA (77 μL, 0.44 mmol) was added dropwise at room temperature. After the addition was completed, the mixture was further reacted overnight at room temperature. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (acetonitrile:water (0.1% TFA) = 0 : 100-100 : 0,

gradient elution) to obtain compound H as a white solid (110 mg, yield: 71.5%). MS(m/z)=1398.4 $[M+H]^+$

7) Synthesis of intermediate-16

Compound H (110 mg, 0.08 mmol) was added to dichloromethane (2 mL), and the mixture was cooled to 0°C in an ice bath. Trifluoroacetic acid (1 mL) was added dropwise. After the addition was completed, the mixture was heated to room temperature and reacted for 15 minutes. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (acetonitrile:water (0.1% TFA) = 0 : 100-100 : 0) to obtain intermediate-16 as a white solid (73.8 mg, yield: 72.3%). MS(m/z)=1297.2 $[M+H]^+$

Preparation of intermediate-17



1) Synthesis of compound C

In an ice-water bath and under nitrogen protection, pyridine (1 mL) and HATU (1260 mg, 3.32 mmol) were added to a solution of compound A (330 mg, 1.66 mmol) and compound B (790 mg, 1.99 mmol) in DMF. The reaction solution was stirred at room temperature for 16 hours. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: $MeOH/H_2O$=0/100-100/0) to obtain compound C as a light-yellow solid (360 mg, yield: 37%). MS(m/z)=579.2 $[M+H]^+$

2) Synthesis of compound E

In an ice-water bath and under nitrogen protection, pyridine (0.5 mL) and compound D (379 mg, 1.25 mmol) were respectively added to a solution of compound C (360 mg, 0.62 mmol) in THF. The reaction was stirred at room temperature for 16 hours. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: DCM : MeOH=100 : 0-50 : 50) to obtain compound E as a white solid (420 mg, yield: 90%). MS(m/z)=744.2 $[M+H]^+$

3) Synthesis of compound F

In an ice-water bath and under nitrogen protection, pyridine (0.5 mL), HOBT (84 mg, 0.62 mmol) and MMAE (460 mg, 0.62 mmol) were respectively added to a solution of compound E (420 mg, 0.57 mmol) in DMF. The reaction was stirred at room temperature for 24 hours. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain compound F as a white solid (380 mg, yield: 50%). MS(m/z)=1322.2 $[M+H]^+$

4) Synthesis of compound G

In an ice-water bath, Et_2N (1 mL) was added to a solution of compound F (380 mg) in THF. The reaction was stirred at room temperature for 1 hour. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain compound G as a white solid (290 mg, yield: 90%). MS(m/z)=1100.2 $[M+H]^+$

5) Synthesis of compound H

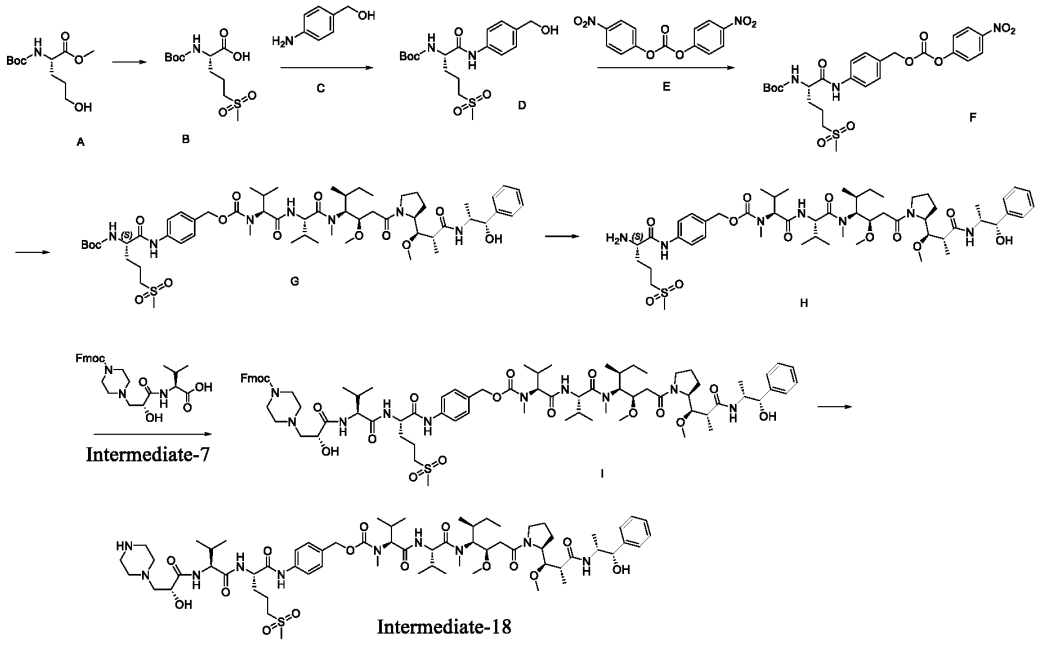
In an ice-water bath and under nitrogen protection, pyridine (35 mg, 0.44 mmol) and HATU (111 mg, 0.29 mmol) were added to a solution of compound G (160 mg, 0.15 mmol) and intermediate-7 (70 mg, 0.15 mmol) in DMF. After the addition was completed, the reaction was stirred at room temperature for 3 hours. After the reaction was completed, the reaction mixture was poured into ice water and filtrated to collect the solid. The filter cake was dissolved in ethyl acetate, dried and then concentrated again to obtain compound H as a white solid (165 mg, yield: 70%). MS(m/z) = 1577.6 $[M+H]^+$

6) Synthesis of intermediate-17

In an ice-water bath, Et_2N (1 mL) was added to a solution of compound H (165 mg) in THF. After the addition was completed, the mixture was stirred at room temperature

for 2 hours. After the reaction was completed, the reaction mixture was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: $MeOH/H_2O$ = 0 : 100-100 : 0) to obtain intermediate-17 as a white solid (128 mg, yield: 90%). MS (m/z)=1355.6 $[M+H]^+$

Preparation of intermediate-18



1) Synthesis of compound B

In an ice-water bath and under nitrogen protection, MsCl (522 mg, 4.25 mmol) and DIPEA (552 mg, 4.25 mmol) were respectively added to a solution of compound A (700 mg, 2.83 mmol) in anhydrous dichloromethane. After the addition was completed, the reaction was stirred at room temperature for 3 hours. After the reaction was completed, the reaction solution was poured into water and extracted with dichloromethane. The extract was dried, concentrated and then dissolved in CH_3SNa/THF (10 mL). The mixture was stirred at room temperature for 3 hours. The reaction system was heated to reflux and stirred for 1 hour. The reaction solution was extracted with ethyl acetate, and the extract was dried and concentrated. $MeOH/H_2O$ and OXONE (2.6 g, 4.25 mmol) were added to the resulting residue. The resulting reaction solution was stirred at room temperature for 1 hour. After the reaction was completed, the reaction mixture was concentrated to remove the solvent, and the residue was purified by ISCO (mobile phase: CH_3CN : H_2O = 0 : 100-100 : 0) to obtain compound B as a colorless oil (530 mg, yield: 63%). MS(m/z)=240.0 $[M-56]^+$

2) Synthesis of compound D

In an ice-water bath and under nitrogen protection, pyridine (0.5 mL) and HATU (820 mg, 2.15 mmol) were added to a solution of compound B (530 mg, 1.79 mmol) and compound C (265 mg, 2.15 mmol) in DMF. After the addition was completed, the reaction solution was stirred at room temperature for 4 hours. The reaction solution was then poured into ice water and filtrated to collect the solid, which was dried to obtain compound D as a white solid (500 mg, yield: 70%). MS(m/z) = 401.2 $[M+H]^+$

3) Synthesis of compound F

In an ice-water bath and under nitrogen protection, DIPEA and compound E (1.1 mg, 3.59 mmol) were respectively added to a solution of compound D (500 mg, 1.25 mmol) in DMF. The reaction solution was stirred at room temperature for 24 hours. After the reaction was completed, the reaction solution was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: EA : PE = 0 : 100-100 : 0, DCM : CH_3CN=1 : 1) to obtain compound F as a white solid (360 mg, yield: 49%). MS(m/z)=588.2 $[M+Na]^+$

4) Synthesis of compound G

In an ice-water bath and under nitrogen protection, pyridine (151 mg, 1.91 mmol), HOBT (87 mg, 0.64 mmol) and MMAE (456 mg, 0.64 mmol) were respectively added to a solution of compound F (360 mg, 0.64 mmol) in DMF. The reaction was stirred at room temperature for 16 h. After the reaction was completed, the reaction solution was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: MeOH : H_2O=0 : 100-100 : 0) to obtain compound G as a white solid (390 mg, yield: 53%). MS(m/z)=1144.4 $[M+H]^+$

5) Synthesis of compound H

In an ice-water bath and under nitrogen protection, compound G (390 mg) was added to a mixed solution of TFA/DCM (1 : 2, 3 mL), and the mixture was stirred for 20 min. After the reaction was completed, the reaction solution was concentrated and the residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain compound H as a white solid (260 mg, yield: 58%). MS(m/z) = 1044.6 $[M+H]^+$

6) Synthesis of compound I

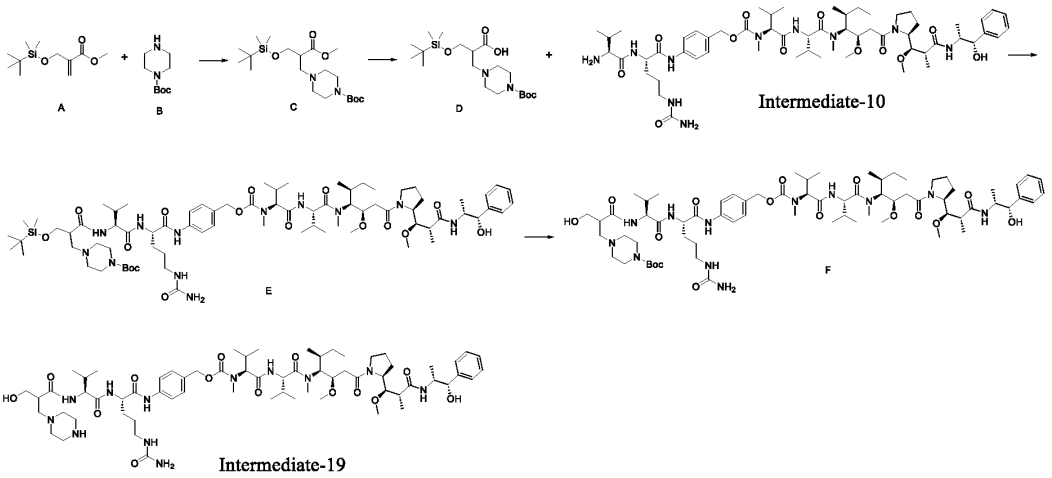
In an ice-water bath and under nitrogen protection, pyridine (47 mg, 0.59 mmol) and HATU (168 mg, 0.44 mmol) were added to a solution of compound H (260 mg, 0.29

mmol) and intermediate-7 (124 mg, 0.29 mmol) in DMF. After the addition was completed, the mixture was stirred at room temperature for 4 h. After the reaction was completed, the reaction solution was concentrated and the residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain compound I as a white solid (296 mg, yield: 66%). MS(m/z) = 1521.6$[M+H]^+$

7) Synthesis of intermediate-18

In an ice-water bath, Et_2N (1 mL) was added to a solution of compound I (296 mg) in THF. The reaction solution was stirred at room temperature for 2 h. After the reaction solution was concentrated, the resulting residue was washed with petroleum ether to obtain intermediate-18 as a white solid (240 mg, yield: 90%). MS(m/z) = 1299.6 $[M+H]^+$

Preparation of intermediate-19



1) Synthesis of compound C

Compound A (1.95 g, 8.5 mmol) and compound B (1.73 g, 9.4 mmol) were dissolved in anhydrous ethanol (30 mL), and the mixture was heated to reflux for 12 hours. After the reaction was completed, the reaction mixture was concentrated under reduced pressure to remove the solvent, and the residue was purified by ISCO (mobile phase: PE : EA = 100 : 0-0 : 100) to obtain compound C as a white solid (200 mg, yield: 5.7%). MS(m/z) = 417.4$(M+1)^+$.

2) Synthesis of compound D

Compound C (200 mg, 0.5 mmol) and $LiOH·H_2O$ (100 mg, 2.5 mmol) were added to methanol/water (3 : 1, 100 mL), and the mixture was stirred at 40°C for 3 h under nitrogen protection. After the reaction was completed, the reaction mixture was concentrated under reduced pressure to remove the solvent, and the residue was purified

by ISCO (mobile phase: water (0.1% TFA)/acetonitrile = 100 : 0–0 : 100) to obtain compound D as a white solid (73 mg, yield: 37.8%). MS(m/z) = 403.4[M+H]$^+$

3) Synthesis of compound E

Compound D (73 mg, 0.18 mmol), intermediate-10 (168 mg, 0.15 mmol), DIPEA (93 mg, 0.72 mmol), EDCI (68 mg, 0.6 mmol) and HOBT (49 mg, 0.36 mmol) were added to DMF (10 mL), and the mixture was stirred at room temperature for 5 hours under nitrogen protection. After the reaction was completed, the reaction mixture was concentrated under reduced pressure to remove the solvent, and the residue was purified by ISCO (mobile phase: water (0.1% TFA)/acetonitrile = 100 : 0–0 : 100) to obtain compound E as a white solid (80 mg, yield: 29.3%). MS(m/z) = 1508.0[M+H]$^+$

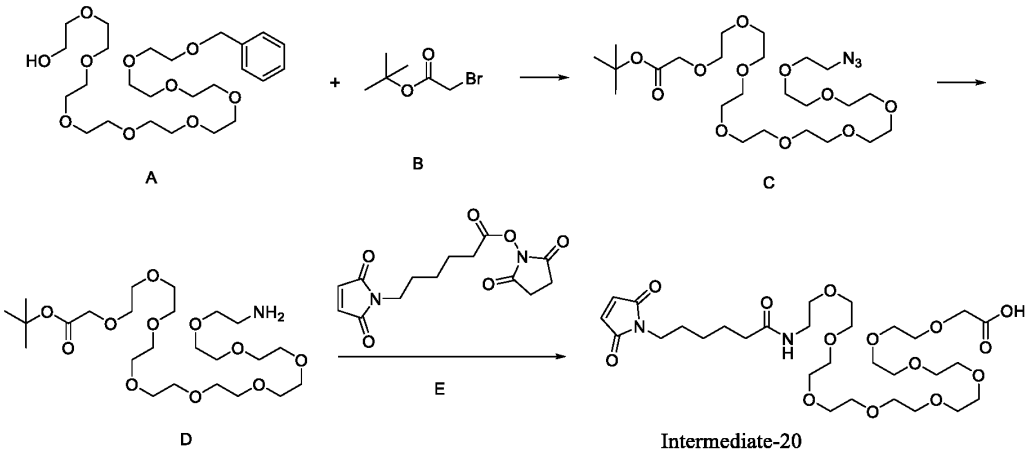
4) Synthesis of compound F

In an ice bath, compound E (80 mg, 0.05 mmol) was added to THF (1 mL), and then TBFA (0.2 mL, 1 M in THF) was added. The mixture was stirred at room temperature for 15 min under nitrogen protection. After the reaction was completed, the reaction mixture was concentrated under reduced pressure to remove the solvent, and the residue was purified by ISCO (mobile phase: water (0.1% TFA)/acetonitrile = 100 : 0–0 : 100) to obtain compound F as an oil (70 mg, yield: 94.6%). MS(m/z) = 697.6[M/2+H]$^+$

5) Synthesis of intermediate-19

Compound F (70 mg, 0.05 mmol) was added to a mixed solution of trifluoroacetic acid/dichloromethane (TFA : DCM = 1 : 1, 10 mL), and the mixture was stirred at room temperature for 0.5 h under nitrogen protection. After the reaction was completed, the reaction mixture was concentrated under reduced pressure to remove the solvent, and the residue was purified by ISCO (mobile phase: water (0.1% TFA)/acetonitrile = 100 : 0–0 : 100) to obtain compound G as an oil (50 mg, yield: 76.9%). MS(m/z) = 1293.8[M+H]$^+$

Preparation of intermediate-20



1) Synthesis of compound C

In an ice-water bath and under nitrogen protection, Ag_2O (4.8 g, 20.83 mmol), KI (1.4 g, 8.33 mmol) and compound B (3.22 g, 16.67 mmol) were added to a solution of compound A (7.0 g, 13.88 mmol) in dichloromethane, and the mixture was stirred at room temperature for 3 hours. After the reaction was completed, the reaction solution was filtered, and the filtrate was washed with a saturated aqueous sodium hydrogen sulfate solution. The organic phase was collected, dried and concentrated. The resulting residue was dissolved in THF, and Pd/C was added. The resulting reaction solution was stirred at 60°C for 3 hours under hydrogen protection. After the reaction was completed, the reaction solution was filtered and concentrated. The resulting residue was dissolved in toluene (60 mL), and DPPA (5.7 g, 20.83 mmol) and DBU (4.2 g, 27.78 mmol) were added. After the addition was completed, the reaction was stirred at 105°C for 20 hours. The mixture was cooled to room temperature, and then poured into water. The mixture was extracted with ethyl acetate. The extract was washed with saturated sodium bicarbonate solution, dried and concentrated. The residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain compound C as a yellow oil (4.1 g, yield: 53%). MS(m/z) = 554.2 $[M+H]^+$

2) Synthesis of compound D

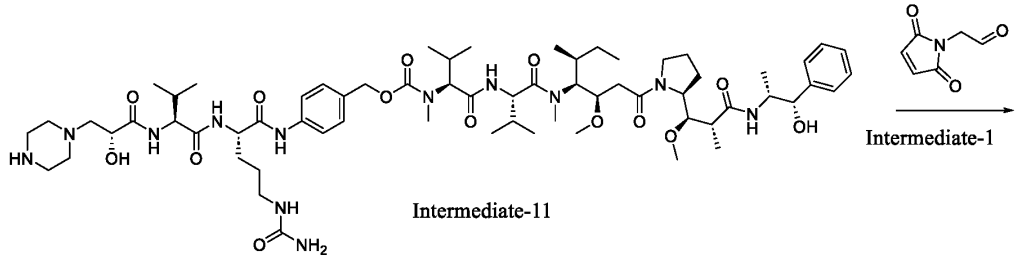
Pd/C was added to a solution of compound C (4.1 g) in THF/H_2O (60 mL), and the mixture was stirred at room temperature for 3 hours under hydrogen protection. The reaction solution was filtered and concentrated, and the residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain compound D as a yellow oil (3.6 g, yield: 90%). MS(m/z) = 528.2 $[M+H]^+$

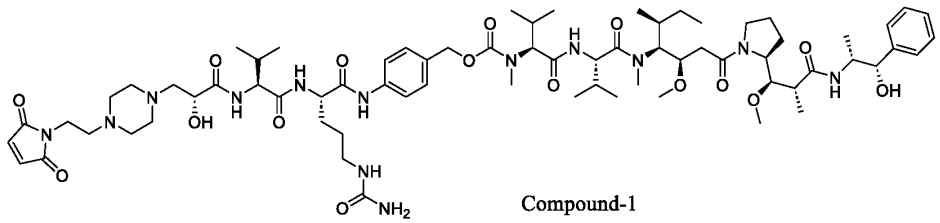
3) Synthesis of intermediate-20

In an ice-water bath and under nitrogen protection, DIPEA (185 mg, 1.42) was added to a solution of compound D (500 mg, 0.95 mmol) and compound E (350 mg, 1.13 mmol) in THF. After the addition was completed, the reaction was stirred at room temperature overnight. The reaction solution was then filtered. The filtrate was concentrated and then dissolved in a mixed solvent of TFA/DCM (volume ratio: 1 : 1) (2 mL). The mixture was stirred at room temperature for 2 hours. The reaction solution was concentrated to remove the solvent, and the residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain intermediate-20 as a colorless oil (560 mg, yield: 88%). MS(m/z) = 665.4 $[M+H]^+$

Example 2 Preparation of compounds 1-14 (linker-payload)

Preparation of compound-1



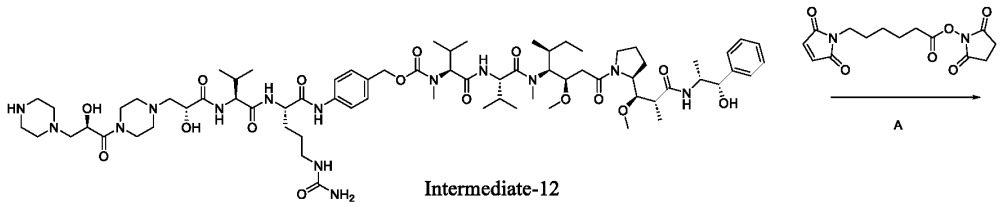


Intermediate-11 (50 mg, 0.039 mmol), $NaBH(OAc)_3$(82 mg, 0.39 mmol) and intermediate-1 (27 mg, 0.195 mmol) were dissolved in methanol (4 mL), and the mixture was stirred at room temperature overnight. The reaction solution was then concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: acetonitrile: water (0.1% TFA) = 0 : 100-100 : 0) to obtain compound-1 as a yellow solid (24 mg, yield: 44%). MS(m/z) = 1402.6$[M+H]^+$

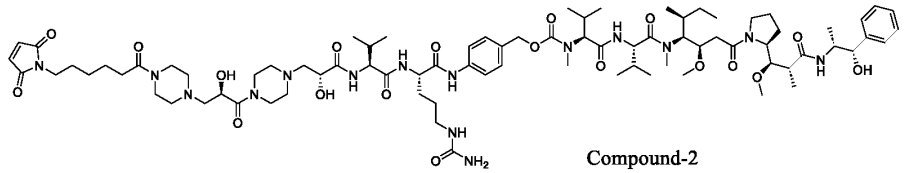
^1H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1H), 8.20 (s, 2H), 7.80-7.70 (m, 1H), 7.56-7.51 (m, 2H), 7.37 – 7.26 (m, 8H), 7.23-7.20 (m, 1H), 6.68 (s, 2H), 6.45-6.35 (m, 1H), 5.80-5.50 (m, 1H), 5.22-4.99 (m, 3H), 4.94-4.80 (m, 2H), 4.69-4.63 (m, 2H), 4.44-4.37 (m, 1H), 4.32-4.17 (m, 4H), 4.16-3.97 (m, 5H), 3.86-3.82 (m, 1H), 3.63-3.60 (m, 3H),

3.37 (s, 3H), 3.35-3.33(s, 1H), 3.32-3.28 (m, 5H), 3.15-3.13 (m, 1H), 3.00 (s, 3H), 2.90-2.85 (m, 6H), 2.71-2.66 (m, 3H), 2.63-2.54 (m, 3H), 2.45 – 2.35 (m, 3H), 2.27 – 2.14 (m, 3H), 2.07-2.01 (m, 2H), 1.95-1.88 (m, 2H), 1.85-1.79 (m, 3H), 1.74 – 1.66 (m, 2H), 1.57-1.51 (m, 2H), 1.23-1.20 (m, 3H), 1.02-1.00 (m, 3H), 0.97-0.94 (m, 8H), 0.88-0.84 (m, 9H), 0.80-0.77 (m, 3H), 0.75-0.71 (m, 3H).

Preparation of compound-2



Intermediate-12

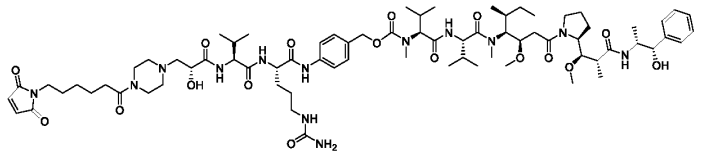
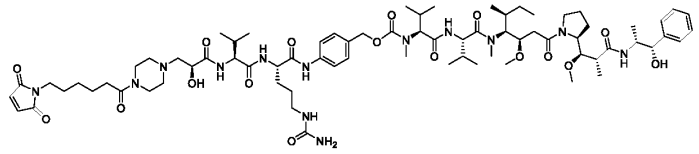
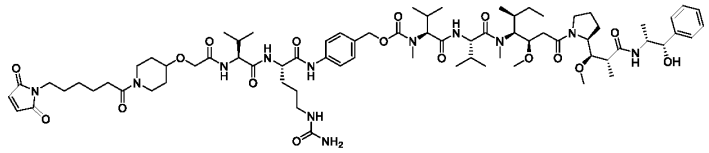
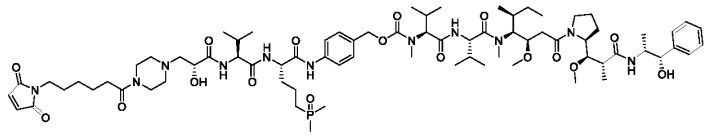
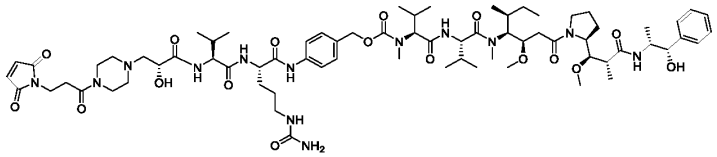
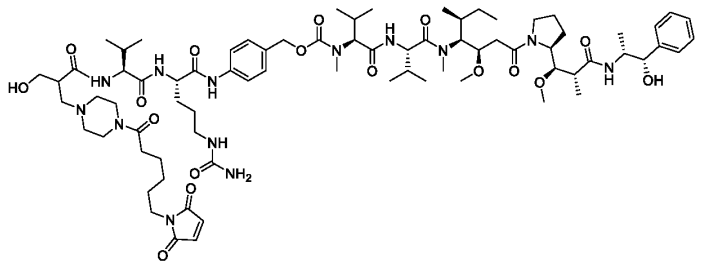


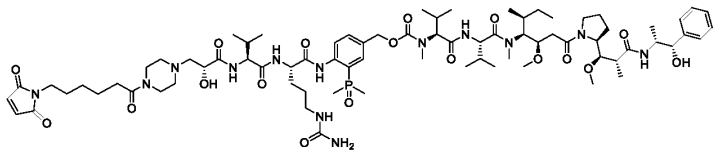
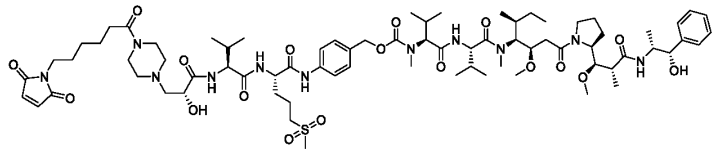
Compound-2

Intermediate-12 (56 mg, 0.039 mmol), compound A (16 mg, 0.051 mmol) and triethylamine (22 μl, 0.156 mmol) were dissolved in DMF (5 mL), and the mixture was stirred at room temperature for 18 hours. The reaction solution was concentrated under reduced pressure, and the residue was separated by ISCO (mobile phase: water (0.1% formic acid) : acetonitrile = 100 : 0–0 : 100) to obtain compound-2 as a yellow solid (44 mg, yield 28%). MS(m/z) = 1628.8$[M+H]^+$

^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (s, 1H), 7.99-7.88 (m, 1H), 7.86-7.72 (m, 1H), 7.61-7.54 (m, 2H), 7.41-7.35 (m, 3H), 7.34 – 7.27 (m, 3H), 7.23-7.17 (m, 1H), 6.79 (s, 2H), 5.36-5.30 (m, 1H), 5.23 – 5.11 (m, 2H), 5.10-5.03 (m, 1H), 4.74-4.58 (m, 3H), 4.54-4.46 (m, 2H), 4.30 – 4.26 (m, 2H), 4.24 – 4.17 (m, 2H), 3.73-3.59 (m, 9H), 3.50-3.46 (m, 2H), 3.35 – 3.33 (m, 5H), 3.27-3.25 (m, 1H), 3.22-3.14 (m, 2H), 3.12-3.07 (m, 2H), 2.96-2.90 (m, 3H), 2.82-2.77 (m, 5H), 2.75-2.67 (m, 5H), 2.64-2.58 (m, 1H), 2.52 – 2.43 (m, 2H), 2.40-2.34 (m, 2H), 2.22 – 2.15 (m, 2H), 2.13 – 2.09 (m, 1H), 2.05 – 1.99 (m, 2H), 1.91 – 1.82 (m, 3H), 1.78 – 1.70 (m, 2H), 1.65-1.50 (m, 10H), 1.20-1.09 (m, 8H), 1.02 – 0.93 (m, 13H), 0.93 – 0.82 (m, 18H).

The following compounds were prepared according to the preparation procedure of compound-2 using corresponding intermediates and reagents under appropriate conditions that will be recognized by a person skilled in the art.

Compound	**Structural formula**	**MS $(M+H)^+$**
Compound-3		1472.6 $[M+H]^+$
Compound-4		1472.8 $[M+H]^+$
Compound-6		1457.6 $[M+H]^+$
Compound-7		1491.6 $[M+H]^+$
Compound-9		716.0 $[M/2+H]^+$
Compound-10		744.1 $[M/2+H]^+$

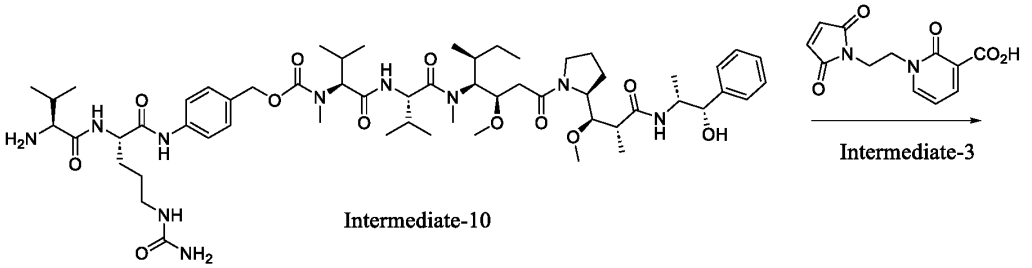
Compound	Structural formula	MS $(M+H)^+$
Compound-11		1548.6 $[M+H]^+$
Compound-12		1492.6 $[M+H]^+$

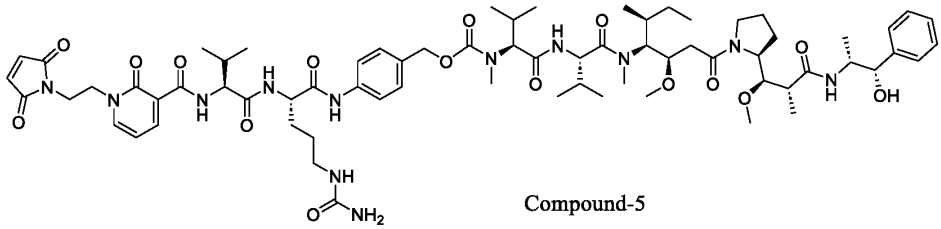
Compound	^{1}H NMR data
Compound-3	^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.82-7.72 (m, 1H), 7.62-7.50 (m, 2H), 7.45-7.26 (m, 8H), 7.25-7.20 (m, 1H), 6.66 (s, 2H), 6.64-6.47 (m, 1H), 6.46- 6.21 (m, 1H), 5.88-5.55 (m, 1H), 5.47-5.26 (m, 1H), 5.24-5.01 (m, 3H), 5.00-4.81 (m, 2H), 4.76 – 4.65 (m, 2H), 4.61 – 4.46 (m, 1H), 4.38-4.22 (m, 4H), 4.18-3.92 (m, 5H), 3.89-3.83 (m, 1H), 3.80-3.71 (m, 1H), 3.63-3.56 (m, 2H), 3.53-3.47 (m, 3H), 3.41-3.37 (m, 3H), 3.34-3.28 (m, 4H), 3.20-3.11 (m, 1H), 3.03-2.97 (m, 4H), 2.91-2.84 (m, 3H), 2.80-2.71 (m, 3H), 2.52 – 2.32 (m, 3H), 2.32-2.23 (m, 3H), 2.22-2.12 (m, 2H), 2.06-1.99 (m, 2H), 1.97-1.88 (m, 2H), 1.85-1.78 (m, 2H), 1.67 – 1.57 (m, 5H), 1.36-1.26 (m, 8H), 1.24-1.20(m, 3H), 1.05-1.01 (m, 3H), 0.99-0.95 (m, 8H), 0.90 – 0.84 (m, 9H), 0.83-0.79 (m, 3H), 0.76-0.71 (m, 3H).
Compound-4	^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96-7.81 (m, 1H), 7.68 – 7.50 (m, 3H), 7.38 – 7.28 (m, 6H), 7.28-7.26 (m, 1H), 7.25 – 7.21 (m, 2H), 6.68 (s, 2H),6.51-6.15 (m, 1H), 5.95-5.45 (m, 1H), 5.28-4.95 (m, 3H), 4.94-4.89 (m, 1H), 4.78 – 4.57 (m, 3H), 4.36-4.29 (m, 1H), 4.28-4.19 (m, 2H), 4.17-4.07 (m, 2H), 4.02-3.95 (m, 1H), 3.87-3.80 (m, 1H), 3.70-3.57 (m, 2H), 3.53 – 3.47 (m, 6H), 3.40-3.35 (m, 4H), 3.32-3.26 (m, 4H), 3.20-3.06 (m, 2H), 3.04-2.94 (s, 3H), 2.92 – 2.84 (m, 4H), 2.67-2.55 (m, 8H), 2.45-2.34 (m, 4H), 2.32 – 2.26 (m, 3H), 2.24-2.14 (m, 3H), 2.10-1.95 (m, 3H), 1.93-1.80 (m, 4H), 1.65-1.58 (m, 5H), 1.54-1.45 (m, 2H), 1.44-1.40 (m, 2H), 1.34-1.29 (m, 3H), 1.25-1.20 (m, 5H), 1.03-1.00 (m, 3H), 0.98-0.94 (m, 8H), 0.88 – 0.85 (m, 9H), 0.81-0.78 (m, 3H), 0.69-0.62 (m, 3H).
Compound-6	^{1}H NMR (400 MHz, DMSO) δ 10.05 (s, 1H), 8.35 (d, J = 7.4 Hz, 1H), 8.16 – 7.82 (m, 1H), 7.59 (dd, J = 25.3, 8.0 Hz, 2H), 7.41 (d, J = 6.4 Hz, 1H), 7.36 – 7.19 (m, 6H), 7.15 (t, J = 6.9 Hz, 1H), 6.98 (s, 2H), 5.98 (s, 1H), 4.99 (dd, J = 37.6, 13.4 Hz, 2H), 4.67 (d, J = 42.1 Hz, 1H), 4.49 – 4.17 (m, 6H), 3.95 (dd, J = 19.5, 15.3 Hz, 9H), 3.58 (d, J = 19.0 Hz, 4H), 3.40 – 3.25 (m,

	3H), 3.24 – 3.07 (m, 10H), 3.04 – 2.92 (m, 4H), 2.84 (dd, J = 15.2, 5.6 Hz, 3H), 2.39 (d, J = 16.1 Hz, 1H), 2.25 (dd, J = 20.8, 13.6 Hz, 3H), 2.03 (ddd, J = 19.6, 15.0, 8.3 Hz, 4H), 1.71 (d, J = 28.8 Hz, 6H), 1.57 – 1.39 (m, 8H), 1.32 (d, J = 8.6 Hz, 3H), 1.18 (dd, J = 14.6, 7.4 Hz, 3H), 1.04 – 0.94 (m, 6H), 0.87 – 0.70 (m, 24H).
Compound-7	^{1}H NMR (400 MHz, DMSO) δ 10.21-10.11 (m, 1H), 9.74 (s, 1H), 8.55-8.37 (m, 1H), 8.34-8.06 (m, 1H), 7.94-7.87 (m, 2H), 7.87-7.80 (m, 1H), 7.67-7.50 (m, 2H), 7.33-7.20 (m, 6H), 7.16-7.09 (m, 1H), 6.99 (s, 2H), 5.09-4.92 (m, 2H), 4.74-4.56 (m, 1H), 4.50-4.36 (m, 5H), 4.28-4.22 (m, 2H), 3.56-3.48 (m, 2H), 3.47-3.40 (m, 2H), 3.37-3.32 (m, 3H), 3.21-3.18 (m, 3H), 3.17-3.15 (m, 2H), 3.14-3.12 (m, 1H), 3.11-3.06 (m, 2H), 3.03-2.99 (m, 1H), 2.96-2.91 (m, 2H), 2.85 (s, 6H), 2.83-2.78 (m, 2H), 2.69 (s, 4H), 2.41-2.33 (m, 1H), 2.30-2.23 (s, 2H), 2.22-2.16 (m, 1H), 2.13-2.03 (m, 2H), 2.02-1.92 (m, 2H), 1.80-1.74 (m, 2H), 1.70-1.63 (m, 4H), 1.49-1.40 (m, 6H), 1.34 (s, 3H), 1.31 (s, 3H), 1.21-1.13 (d, *J* = 7.7 Hz, 3H), 1.03-0.92 (m, 7H), 0.85-0.68 (m, 26H).
Compound-9	^{1}H NMR (400 MHz, CD_3OD) δ 8.53 (s, 1H), 8.00 – 7.88 (m, 1H), 7.78 (s, 1H), 7.56 (s,2H), 7.45 – 7.13 (m, 8H), 6.82 (s, 2H), 5.24 – 5.03 (m, 3H), 4.71 – 4.45 (m, 5H), 4.32 – 4.03 (m, 6H), 3.92 – 3.64 (m, 6H), 3.56 – 3.46 (m, 4H), 3.24 – 3.03 (m, 6H), 3.00 – 2.86 (m, 4H), 2.82 – 2.62 (m, 2H), 2.54 – 2.41 (m, 2H), 2.29 – 2.14 (m, 3H), 2.10 – 1.73 (m, 10H), 1.67 – 1.50 (m, 4H), 1.47 – 1.34 (m,2H), 1.31 – 1.08 (m, 9H), 1.05 – 0.92 (m, 16H), 0.89 – 0.75 (m, 13H).
Compound-10	^{1}H NMR (400 MHz, CD_3OD) δ 9.97 – 9.74 (m,1H), 8.32 (s, 1H), 8.00 – 7.74 (m, 2H), 7.63 – 7.52 (m, 2H), 7.42 – 7.26 (m, 7H), 7.23 – 7.16 (m, 1H), 6.83 – 6.77 (m,2H), 5.21 – 5.04 (m, 3H), 4.71 – 4.46 (m, 4H), 4.30 – 4.01 (m, 6H), 3.89 – 3.76 (m, 4H), 3.73 – 3.60 (m, 4H), 3.56 – 3.45 (m,4H), 3.24 – 3.05 (m, 7H), 3.01 – 2.87 (m, 4H), 2.53 – 2.36 (m, 4H), 2.32 – 2.15 (m, 4H), 2.07 – 1.72 (m, 8H), 1.67 – 1.51 (m, 8H), 1.43 – 1.36 (m, 2H), 1.20 – 1.10 (m, 7H), 1.07 – 0.91 (m, 16H), 0.89 – 0.77 (m, 13H).
Compound-11	^{1}H NMR (400 MHz, DMSO) δ 11.67 (s, 1H), 9.65 (s, 1H), 8.55 (d, *J* = 6.8 Hz, 1H), 8.35 – 8.27 (m, 1H), 8.13 (d, *J* = 8.2 Hz, 0.5H), 7.90 (d, *J* = 8.1 Hz, 0.5H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.66 – 7.46 (m, 2H), 7.30 – 7.21 (m, 4H), 7.14 (d, *J* = 7.1 Hz, 1H), 7.00 (s, 2H), 6.66 (s, 1H), 5.96 (s, 1H), 5.37 (s, 2H), 5.11 – 4.94 (m, 2H), 4.74 – 4.58 (m, 1H), 4.53 – 4.31 (m, 5H), 4.27-4.17 (m, 1H), 4.08-3.90 (m, 5H), 3.42 – 3.26 (m, 10H), 3.23 – 3.16 (m, 7H), 3.14 (s, 2H), 3.09 (s, 2H), 3.03 – 2.80 (m, 9H), 2.43 – 2.34 (m, 1H), 2.33 – 2.20 (m, 3H), 2.13-2.04 (m, 2H), 2.00 – 1.87 (m, 1H), 1.81 – 1.68 (m, 9H), 1.53 – 1.39 (m, 7H), 1.27 – 1.15 (m, 4H), 1.04 – 0.90 (m, 7H), 0.87 – 0.70 (m, 25H).
Compound-12	^{1}H NMR (400 MHz, DMSO) δ 10.11 (s, 1H), 8.45 (d, *J* = 7.3 Hz, 1H), 8.07 (d, *J* = 7.7 Hz, 0.5H), 7.88 (d, *J* = 8.6 Hz, 1H), 7.81 (d, *J* = 7.7 Hz, 1H), 7.62 (d, *J* = 8.5 Hz, 0.5H), 7.53 (d, *J* = 7.4 Hz, 2H), 7.31 – 7.21 (m, 6H), 7.17-7.11 (m, 1H), 6.99 (s,

	2H), 6.65 (s, 1H), 5.07-4.92 (m, 2H), 4.74 – 4.60 (m, 1H), 4.54 – 4.20 (m, 8H), 4.03 – 3.90 (m, 3H), 3.75 (d, *J* = 9.4 Hz, 1H), 3.37 – 3.33 (m, 4H), 3.27 (d, *J* = 10.3 Hz, 1H), 3.23 – 3.15 (m, 8H), 3.14 – 3.08 (m, 5H), 3.05 – 2.98 (m, 2H), 2.95-2.89 (m, 5H), 2.88-2.78 (m, 3H), 2.32-2.16 (m, 3H), 2.11 – 1.94 (m, 4H), 1.82 – 1.65 (m, 7H), 1.51 – 1.40 (m, 6H), 1.30 – 1.15 (m, 5H), 1.02 – 0.93 (m, 7H), 0.86-0.71 (m, 25H).

Preparation of compound-5

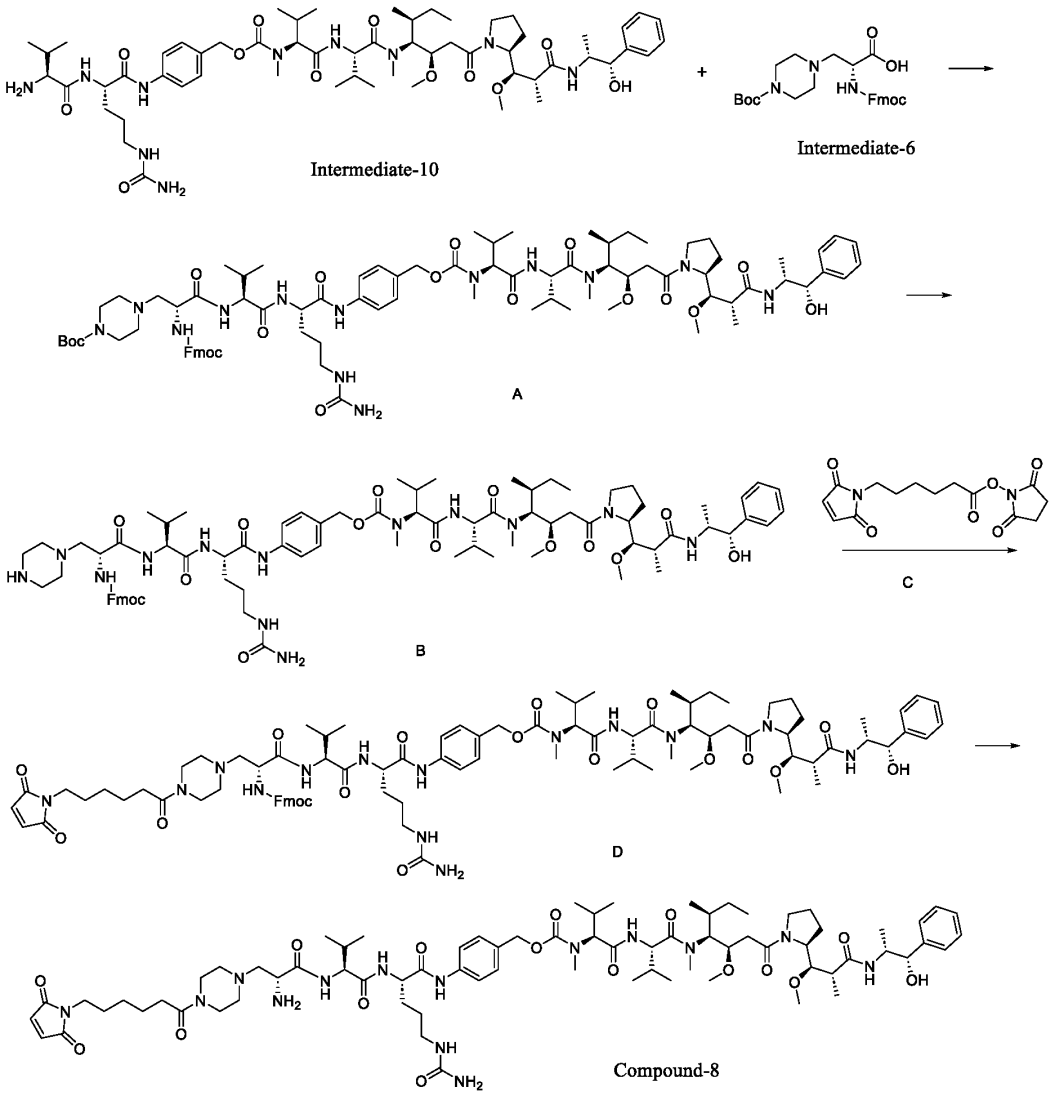




Intermediate-10 (31 mg, 0.12 mmol), intermediate-3 (67 mg, 0.06 mmol), HBTU (46 mg, 0.12 mmol) and DIPEA (77 mg, 0.6 mmol) were dissolved in DMF (4 mL), and the mixture was stirred at room temperature for 3 hours. The reaction solution was concentrated under reduced pressure, and the residue was separated and purified by ISCO (mobile phase: acetonitrile/water (0.1% TFA) = 0 : 100-100 : 0) to obtain compound-5 as a yellow solid (70 mg, yield: 85%). MS(m/z) = 1368.6[M+H]$^+$

^{1}H NMR (400 MHz,CD_3OD) δ 8.53 – 8.48 (m, 1H), 8.44 – 8.38 (m, 1H), 8.35 – 8.32 (m, 1H), 8.29 – 8.26 (m,1H), 8.00 – 7.86 (m, 1H), 7.81 – 7.71 (m, 1H), 7.66 – 7.60 (m, 2H), 7.44 – 7.39 (m, 3H), 7.38 – 7.32 (m, 3H), 7.26 – 7.21 (m, 1H), 7.16 – 7.11 (m, 1H), 6.78 – 6.73 (m, 2H), 5.27 – 5.16 (m, 2H), 5.15 – 5.06 (m, 1H), 4.83 – 4.74 (m, 2H), 4.73 – 4.67 (m, 1H), 4.67 – 4.61 (m, 1H), 4.62 – 4.47 (m, 4H), 4.29 – 4.17 (m, 3H), 4.14 – 4.05 (m, 1H), 4.04 – 3.85 (m, 3H), 3.80 – 3.66 (m, 1H), 3.61 – 3.53 (m, 1H), 3.48 – 3.42 (m, 1H), 3.30 (s, 2H), 3.27 – 3.17 (m, 2H), 3.17 – 3.11 (m, 2H), 3.01 – 2.94 (m, 3H), 2.56 – 2.46 (m, 2H), 2.34 – 2.17 (m, 3H), 2.11 – 2.01 (m, 1H), 1.99 – 1.76 (m, 6H), 1.68 – 1.52 (m, 4H), 1.50 – 1.30 (m, 3H), 1.23 – 1.13 (m, 7H), 1.09 – 0.99 (m, 12H), 0.98 – 0.77 (m, 18H).

Preparation of compound-8



1) Synthesis of compound A

Intermediate-6 (150 mg, 0.3 mmol), intermediate-10 (270 mg, 0.24 mmol), DIPEA (150 mg, 1.2 mmol), EDCI (115 mg, 0.6 mmol) and HOBT (81 mg, 0.6 mmol) were added to DMF (10 mL), and the mixture was stirred at room temperature for 5 h under nitrogen protection. After the reaction was completed, the reaction solution was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: water (0.1% TFA)/acetonitrile = 100 : 0–0 : 100) to obtain compound A as a white solid (200 mg, yield: 41.2%). MS (m/z) = 801.6[M/2+H]$^+$

2) Synthesis of compound B

Compound A (200 mg, 0.13 mmol) was added to a mixed solution of trifluoroacetic acid and dichloromethane (1 : 1, 10 mL), and the mixture was stirred at room temperature

for 0.5 h under nitrogen protection. After the reaction was completed, the reaction solution was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: water (0.1% TFA)/acetonitrile = 100 : 0–0 : 100) to obtain compound B as an oil (90 mg, yield: 48.1%). MS (m/z) = 760.1[M/2+H]$^+$

3) Synthesis of compound D

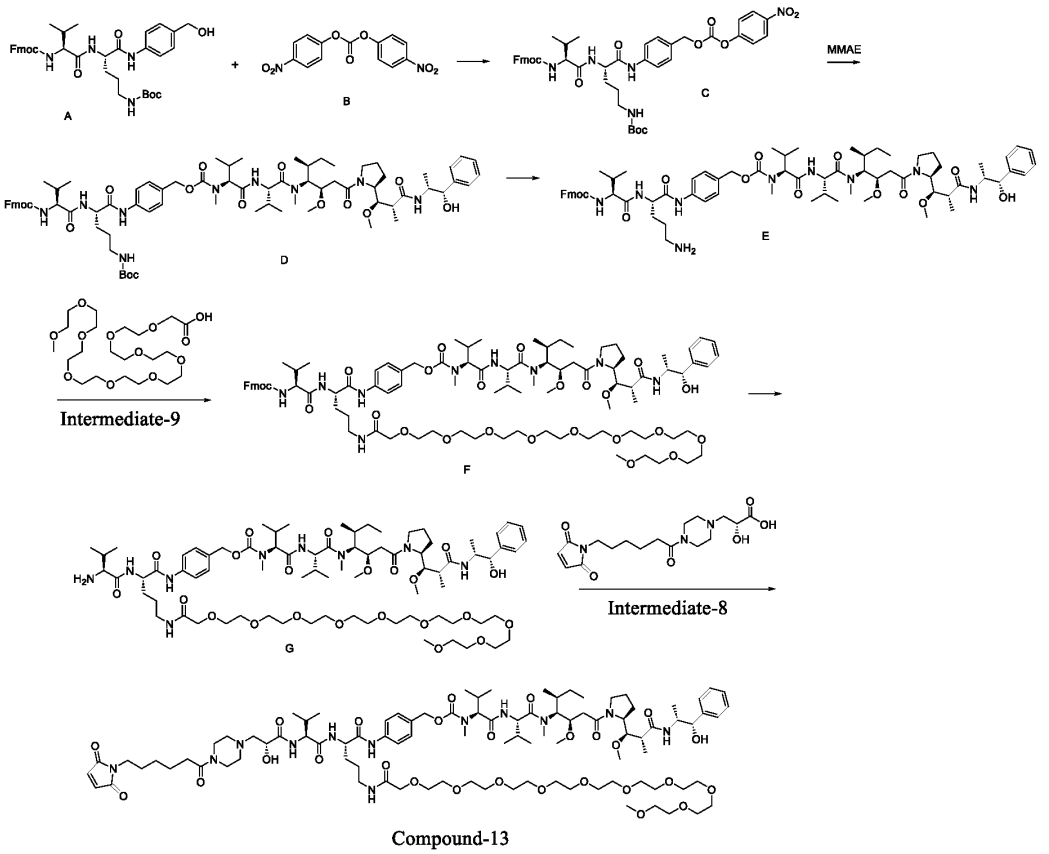
Compound B (90 mg, 0.06 mmol), compound C (28 mg, 0.09 mmol) and DIPEA (31 mg, 0.24 mmol) were added to DMF (3 mL), and the mixture was stirred at room temperature for 5 h under nitrogen protection. After the reaction was completed, the reaction solution was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: water (0.1% TFA) : acetonitrile = 100 : 0–0 : 100) to obtain compound D as a white solid (60 mg, yield: 59.4%). MS(m/z) = 847.8[M/2+H]$^+$

4) Synthesis of compound-8

Compound D (60 mg, 0.03 mmol) and TEA (0.3 mL) were dissolved in DMF (2 mL), and the mixture was stirred at room temperature for 2 hours. The reaction solution was then concentrated under reduced pressure, and the residue was purified and separated by ISCO (mobile phase: water (0.1% formic acid) : acetonitrile = 100 : 0–0 : 100) to obtain compound-8 as a yellow solid (17 mg, yield: 32.7%). MS(m/z) = 1471.8[M+H]$^+$

^{1}H NMR (400 MHz, CD_3OD) δ 9.91 (s, 1H), 8.46 – 8.34 (m, 1H), 8.00 – 7.88 (m, 1H), 7.81 – 7.70 (m, 1H), 7.54 (s, 2H), 7.43 – 7.25 (m, 7H), 7.19 (s, 1H), 6.78 (s, 2H), 5.19 – 5.07 (m, 1H), 4.92 (s, 4H), 4.82 (s, 2H), 4.67 – 4.45 (m, 1H), 4.32 – 4.07 (m, 2H), 3.74 – 3.54 (m, 5H), 3.52 – 3.43 (m, 3H), 3.39 – 3.32 (m, 11H), 3.21 – 3.02 (m, 3H), 2.98 – 2.88 (m, 3H), 2.74 – 2.42 (m, 4H), 2.40 – 2.32 (m, 1H), 2.27 – 1.99 (m, 1H), 1.95 – 1.67 (m, 3H), 1.66 – 1.52 (m, 8H), 1.37 – 1.25 (m, 4H), 1.20 – 1.07 (m, 7H), 1.05 – 0.90 (m, 19H), 0.89 – 0.76 (m, 13H).

Preparation of compound-13



1) Synthesis of compound C

In an ice-water bath and under nitrogen protection, DIPEA (180 mg, 1.39 mmol) and compound B (425 mg, 1.39 mmol) were added to a solution of compound A (460 mg, 0.69 mmol) in DMF. The mixture was stirred at room temperature for 16 h, and then the reaction solution was concentrated. The residue was purified by ISCO (mobile phase: EA : PE = 0 : 100-100 : 0, DCM : CH_3CN = 1 : 1) to obtain compound C as a white solid (560 mg, yield: 90%). MS(m/z) = 845.8 $[M+Na]^+$

2) Synthesis of compound D

In an ice-water bath and under nitrogen protection, pyridine (0.5 mL), HOBT (95 mg, 0.69 mmol) and MMAE (501 mg, 0.69 mmol) were added to a solution of compound C (560 mg, 0.68 mmol) in DMF. The reaction solution was stirred at room temperature for 16 h. After the reaction was completed, the reaction solution was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain compound D as a white solid (370 mg, yield: 37%). MS(m/z) = 1402.4 $[M+H]^+$

3) Synthesis of compound E

In an ice-water bath and under nitrogen protection, compound D (370 mg) was added to a mixed solution of CF_3COOH/DCM (1 : 2, 3 mL), and the mixture was stirred for 20 min. After the reaction was completed, the reaction solution was concentrated, and the residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain compound E as a white solid (170 mg, yield: 50%). MS(m/z) = 1303.4 $[M+H]^+$

4) Synthesis of compound F

In an ice-water bath and under nitrogen protection, DIPEA (105 mg, 0.80 mmol) and HATU (122 mg, 0.32 mmol) were added to a solution of compound E (170 mg, 0.13 mmol) and intermediate-9 (130 mg, 0.26 mmol) in DMF, and the mixture was stirred at room temperature for 2 h. After the reaction was completed, the reaction solution was concentrated, and the residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain compound F as a white solid (57 mg, yield: 25%). MS(m/z) = 1771.6 $[M+H]^+$

5) Synthesis of compound G

In an ice-water bath, Et_2NH (59 mg, 0.80 mmol) was added to a solution of compound F (57 mg) in THF. After the addition was completed, the mixture was stirred at room temperature for 2 h. After the reaction was completed, the reaction solution was concentrated, and the residue was washed with petroleum ether and dried to obtain compound G as a white solid (46 mg, yield: 90%). MS(m/z) = 1549.6 $[M+H]^+$

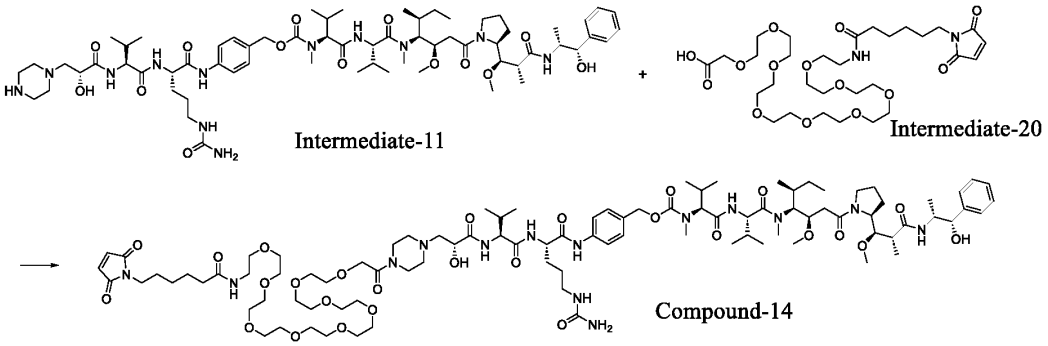
6) Synthesis of compound-13

In an ice-water bath and under nitrogen protection, DIPEA (5 mg, 0.035 mmol) and HATU (14 mg, 0.035 mmol) were added to a solution of compound G (46 mg, 0.029 mmol) and intermediate-8 (14 mg, 0.035 mmol) in DMF. After the addition was completed, the mixture was stirred at room temperature for 2 h. After the reaction was completed, the reaction solution was concentrated under reduced pressure, and the residue was purified by ISCO (mobile phase: MeOH : H_2O = 0 : 100-100 : 0) to obtain compound-13 as a white solid (29 mg, yield: 51%). MS(m/z) = 1897.8 $[M+H]^+$

^1H NMR (400 MHz, DMSO) δ 10.04 (s, 1H), 8.41 – 8.28 (m, 2H), 8.06 (d, J = 8.6 Hz, 0.5H), 7.88 (d, J = 8.8 Hz, 1H), 7.78 (d, J = 8.9 Hz, 1H), 7.73 (t, J = 5.7 Hz, 1H), 7.62 (d, J = 8.2 Hz, 0.5H), 7.54 (d, J = 7.2 Hz, 2H), 7.33 – 7.21 (m, 7H), 7.16 (d, J = 7.2 Hz, 1H), 6.98 (s, 2H), 5.47 – 5.31 (m, 2H), 5.09-4.93 (m, 2H), 4.76 – 4.56 (m, 1H), 4.48-4.34 (m, 3H), 4.29 – 4.21 (m, 2H), 4.10 – 3.93 (m, 3H), 3.83 (s, 2H), 3.53-3.45 (m, 36H), 3.41-3.37 (m, 5H), 3.22-3.19 (m, 7H), 3.18-3.14 (m, 3H), 3.11-3.07 (m, 3H), 2.95 (s, 1H), 2.88 – 2.79 (m, 3H), 2.65 – 2.52 (m, 3H),

2.43 – 2.29 (m, 6H), 2.28-2.18 (m, 3H), 2.16 – 2.03 (m, 2H), 2.02 – 1.89 (m, 2H), 1.80 – 1.64 (m, 3H), 1.53-1.40 (m, 8H), 1.24-1.13 (m, 4H), 1.04-0.91 (m, 8H), 0.87-0.72 (m, 25H).

Preparation of compound-14

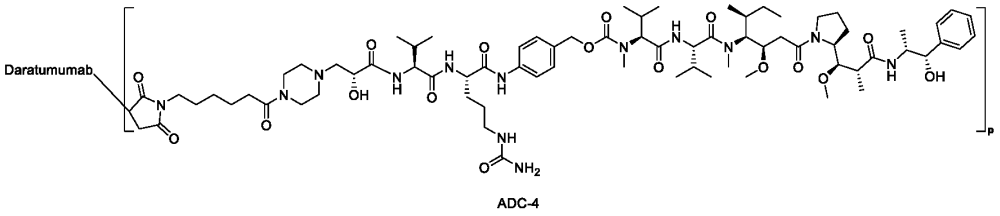


Under nitrogen protection, to a solution of intermediate-11 (48 mg, 0.037 mmol) and intermediate-20 (51 mg, 0.076 mmol) in DMF (1.5 mL) were added DIPEA (14 mg, 0.10 mmol) and HATU (40 mg, 0.10 mmol). The reaction was stirred at room temperature for 3 hours. The reaction solution was concentrated to remove the solvent, and the residue was purified by ISCO (mobile phase: MeOH : H_2O (0.1%HCOOH) = 0 : 100-100 : 0) to obtain compound-14 as a colorless viscous substance (20 mg, yield: 27.7%). MS(m/z) = 1925.6 $[M+H]^+$

^1H NMR (400 MHz, DMSO) δ 8.60 – 8.52 (m, 1H), 8.44 (d, J = 8.3 Hz, 0.5H), 8.30 (t, J = 5.0 Hz, 1H), 8.12 (t, J = 6.6 Hz, 1H), 8.06 (t, J = 5.6 Hz, 1H), 7.99 (t, J = 5.2 Hz, 1H), 7.95-7.85 (m, 1H), 7.61 (d, J = 9.0 Hz, 0.5H), 7.30 – 7.21 (m, 8H), 7.18-7.13 (m, 2H), 6.97 (s, 2H), 4.76 – 4.38 (m, 6H), 4.33 – 3.87 (m, 3H), 3.80 – 3.51 (m, 8H), 3.31-3.26 (m, 3H), 3.22 – 2.94 (m, 12H), 2.88-2.74 (m, 4H), 2.59-2.51 (m, 1H), 2.41 – 2.18 (m, 2H), 2.08 (t, J = 7.4 Hz, 3H), 2.03-1.88 (m, 1H), 1.82 – 1.61 (m, 2H), 1.54 – 1.39 (m, 6H), 1.32 – 1.11 (m, 4H), 1.06 – 0.92 (m, 7H), 0.91-0.70(m, 20H).

Example 3 Preparation of ADC

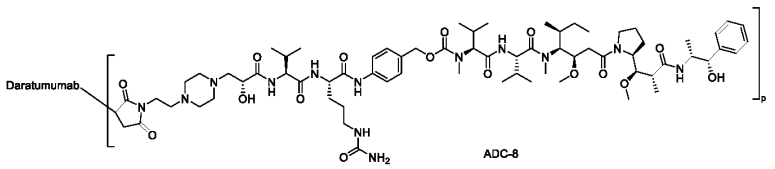
Preparation of ADC-4



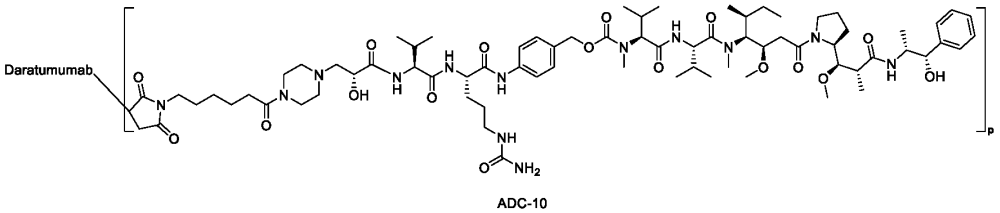
A TCEP solution (20 μL, 10 mmol/L ddH_2O, 6.0 eq) was added to a daratumumab solution with a concentration of 6 mg/mL (0.84 mL, 5 mg) prepared with a PBS-D buffer (10 mmol/L, pH 7.0), and the resulting solution was reacted at 37°C for 120 min. A solution of compound 3 in DMSO : H_2O (volume ratio: 3 : 1) (100 μL, 5 mmol/L, 15 eq) was added to the system, and further reacted overnight at 4°C. 20 equivalents of L-Cysteine were added, and the reaction was quenched at 4°C for 1 h. The reaction solution was transferred to a Slide-A-Lyzer®Dislysis Cassette, and 10 mmol/L PBS (pH 7.0) was added for solution exchange, so that a product was obtained. As detected by HIC-HPLC, the drug/antibody ratio (measured DAR value) was calculated as 7.59.

The following control compounds were prepared according to the preparation procedure of compound ADC-4 using corresponding Linker-Payload (e.g., see example 2) under appropriate conditions that will be recognized by a person skilled in the art.

Table 1

ADC number	Structure	Measured DAR
ADC-8		7.10

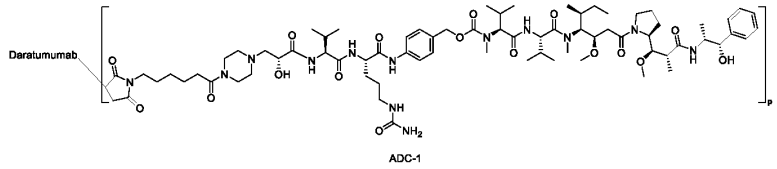
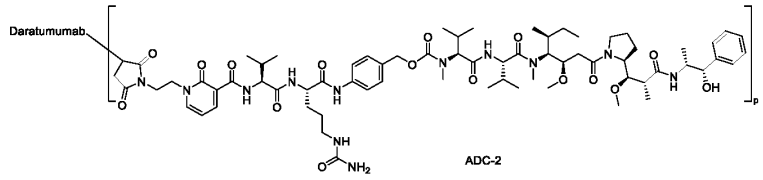
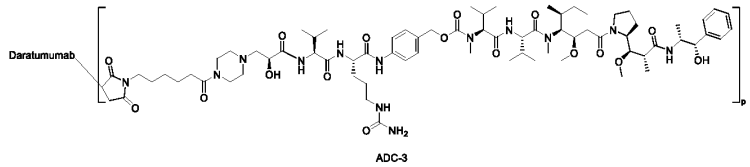
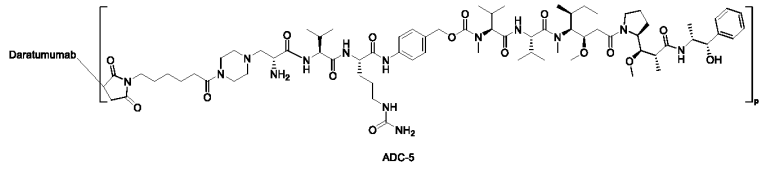
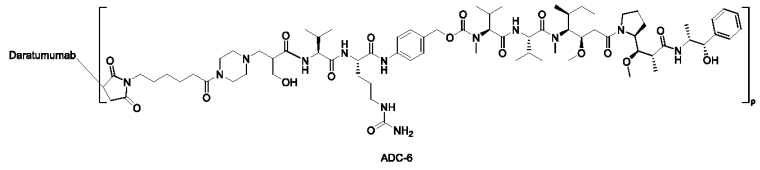
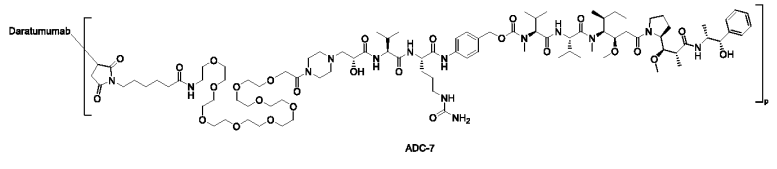
Preparation of ADC-10

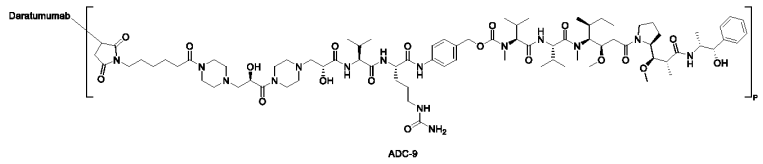
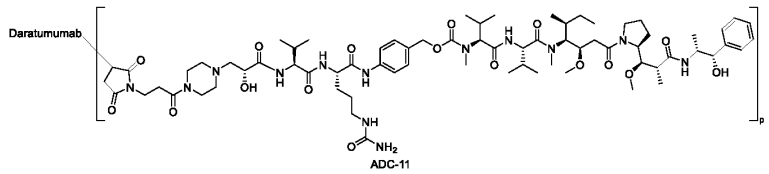
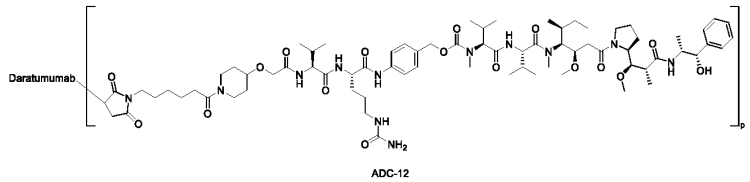
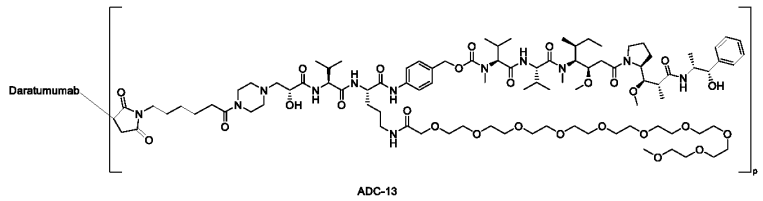
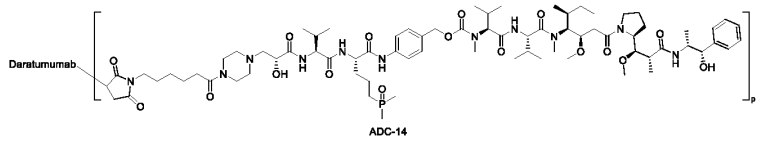
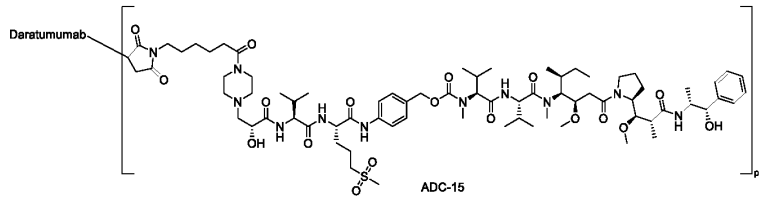
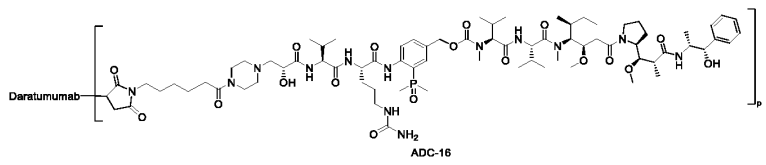


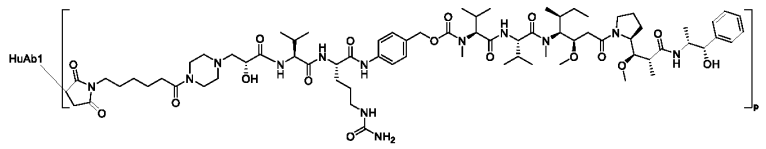
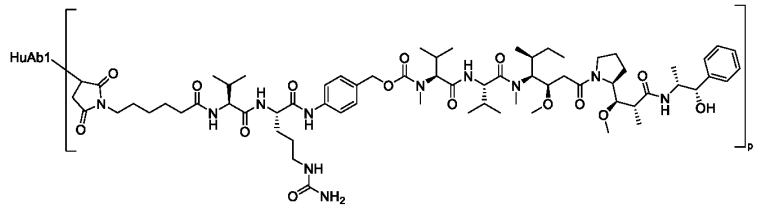
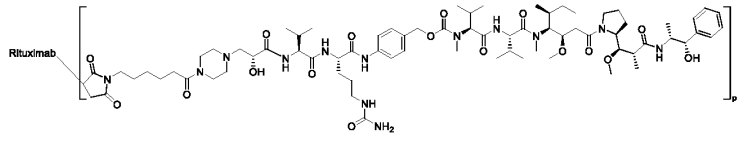
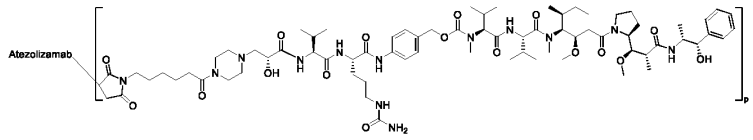
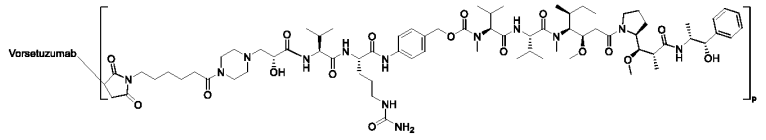
His buffer (725 μL), TCEP solution (10 μL, 10 mmol/L in His buffer, 2.5 eq) and DTPA solution (15 μL, 10 mmol/L in His buffer) were successively added to 8 mg/mL daratumumab solution (0.75 mL, 6 mg) prepared with His buffer (10 mmol/L, pH 7.0). The resulting solution was reacted at 22°C for 60 min, and then a solution of compound 3 in DMSO : H_2O (volume ratio: 3 : 1) (80 μL, 5 mmol/L, 10 eq) was added. The mixture was further reacted at 22°C for 60 min. The reaction solution was transferred to an ultrafiltration centrifuge tube (Millipore Amicon® Ultra), and a His/His·HCl buffer (10

mmol/L, pH 6.2) was added for solution exchange, so that a target product was obtained. As detected by HIC-HPLC, the drug/antibody ratio (DAR value) was calculated as 3.80.

The following antibody-drug conjugates were prepared according to the preparation procedure of ADC-10 using corresponding Linker-Payload (e.g., see example 2) under appropriate conditions that will be recognized by a person skilled in the art.

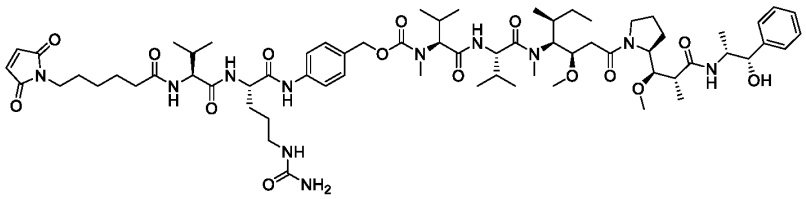
ADC number	Structure	Measured DAR
ADC-1		5.05
ADC-2		4.20
ADC-3		5.38
ADC-5		4.04
ADC-6		3.58
ADC-7		4.01

ADC-9		4.00
ADC-11		3.98
ADC-12		4.08
ADC-13		4.33
ADC-14		4.09
ADC-15		4.09
ADC-16		3.99

ADC-17		4.18
ADC-18		4.23
ADC-19		3.57
ADC-20		4.0
ADC-21		4.0

Preparation of control ADC

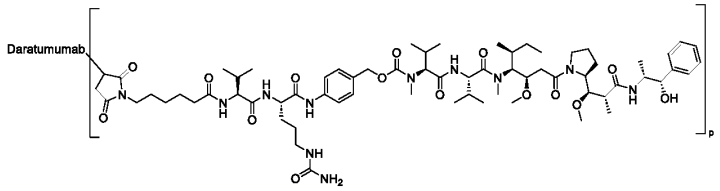
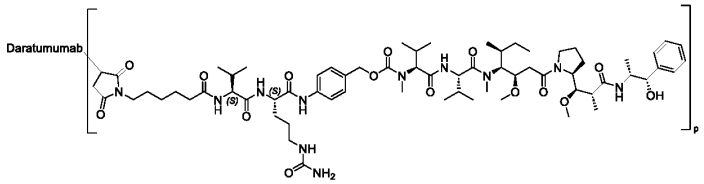
The Linker-payload structure used in the control ADC is as follows:



The following control ADC-1 was prepared according to the preparation procedure of the above ADC-4, and the following control ADC-2 was prepared according to the preparation procedure of the above ADC-10, using corresponding Linker-Payload (MC-

VC-PAB-MMAE) under appropriate conditions that will be recognized by a person skilled in the art.

Table 3

Control ADC number	Structure	Measured DAR
Control ADC-1	 Control ADC-1	7.64
Control ADC-2	 Control ADC-2	4.56

Example 4: Generation of antibody HuAb1

In brief, mice were immunized by using the recombinant protein CD38-His (ACRO Biosystems, Cat: CD8-H5224), which contains a human CD38 extracellular domain with a His tag, as an antigen, and positive mouse plasma cells (binding CD38 and expressing IgG) were separated and screened by the beacon single-cell photoconductive system. Furthermore, the single-cell sequencing was used to obtain a sequence encoding a variable region of an anti-CD38 antibody in the positive mouse plasma cells. Appropriate candidate antibodies were selected by analyzing the positive binding of the antibody secreted by each cell to CD38 and the gene encoding the antibody sequence for the antibody thereof.

The variable region sequences of the chimeric antibody were compared with the PDB_Antibody database by Blast, and the human backbone sequences with the highest similarity were selected for humanization. The heavy chain variable region had relatively high sequence homology with human germline IGHV1-2, and the light chain variable region had relatively high sequence homology with human germline IGKV1-33. Then,

the amino acid sequence of the variable region CDR and the accurate boundary thereof were defined by the Kabat numbering system. Then, the CDR segments of the variable region of the murine antibody were grafted into the human backbone sequence to obtain the humanized antibody. Furthermore, in order to maintain the activity of the antibody, the framework amino acid sequences of the variable region and its surrounding region were also analyzed by using computer simulation technology and molecular docking, and their spatial stereoscopic binging mode was investigated. By calculating electrostatic force, van der Waals force, hydrophilicity and entropy, the key amino acid individuals that may interact with CD38 and maintain the spatial framework in the candidate antibody gene sequence were analyzed and grafted back to the selected human antibody framework. Meanwhile, the amino acid sites in the framework region that must be reserved were marked on this basis, and the humanized antibody is synthesized. Some key sites in the antibody framework region were back mutated into the antibody framework region sequence of the chimeric antibody.

The exemplary candidate chimeric antibody Ab1 had a heavy chain variable region with an amino acid sequence as set forth in SEQ ID NO: 7, and a light chain variable region with an amino acid sequence as set forth in SEQ ID NO: 8. The exemplary humanized antibody was HuAb1, which had a heavy chain variable region with an amino acid sequence as set forth in SEQ ID NO: 9, and a light chain variable region with an amino acid sequence as set forth in SEQ ID NO: 10. The CDRs of variable regions of Ab1 and HuAb1 (defined by the Kabat numbering system) had amino acid sequences of SEQ ID NO: 1 for HCDR1, SEQ ID NO: 2 for HCDR2, SEQ ID NO: 3 for HCDR3, SEQ ID NO: 4 for LCDR1, SEQ ID NO: 5 for LCDR2, and SEQ ID NO: 6 for LCDR3.

Candidate antibody Ab1 and humanized antibody HuAb1 were prepared as follows:

The genes encoding variable region sequences of the candidate antibody or humanized antibody were cloned into expression vectors (PCDNA 3.4, purchased from Life technologics, ThermoFisher) containing genes encoding the human IgG1 heavy chain constant region (with an amino acid sequence as set forth in SEQ ID NO: 13) and kappa constant region (with an amino acid sequence as set forth in SEQ ID NO: 14). The antibody expression was achieved by a transient transfection method in Expi293 cells, and the antibodies in the expression supernatant were purified by the protein A affinity

purification method, so as to obtain the chimeric antibody (such as Ab1) or humanized antibody (such as HuAb1).

Table 4 Sequence listing

SEQ ID NO	Domain	Amino acid sequence
1	HCDR1	NYWIT
2	HCDR2	DIYPGSTFTNYNEMFKS
3	HCDR3	LWILALDY
4	LCDR1	HASQNIHIWLS
5	LCDR2	KASNLHT
6	LCDR3	QQGQTYPYT
7	Ab1 heavy chain variable region (VH)	QVQLQQPGAELVKPGASVKMSCKASGYTFTNYWITWVKQRPGQ GLEWIGDIYPGSTFTNYNEMFKSKATLTVDTSSSTAYMQLSTLTS DDSAVYYCARLWILALDYWGQGTSVTVSS
8	Ab1 light chain variable region (VL)	DIQMNQSPSSLSASLGDTITITCHASQNIHIWLSWYQQKPGNIPKLL IYKASNLHTGVPSRFSGSGSGTGFTLTISSLQPEDIAAYYCQQGQT YPYTFGGGTKLEMK
9	HuAb1 heavy chain variable region (VH)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWITWVRQAPGQ GLEWIGDIYPGSTFTNYNEMFKSRATLTVDTSISTAYMELSRLRSD DTVVYYCARLWILALDYWGQGTTVTVSS
10	HuAb1 light chain variable region (VL)	DIQMTQSPSSLSASVGDRVTITCHASQNIHIWLSWYQQKPGKAPK LLIYKASNLHTGVPSRFSGSGSGTGFTFTISSLQPEDIATYYCQQGQ TYPYTFGGGTKVEIK
11	Daratumumab heavy chain (HC)	EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKG LEWVSAISGSGGGTYYADSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYFCAKDKILWFGEPVFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK
12	Daratumumab light chain (LC)	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRL LIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSN WPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
13	Human IgG1 heavy chain-1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS

	constant region-1	RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
14	kappa constant region	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
15	Daratumumab heavy chain variable region	EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKG LEWVSAISGSGGGTYYADSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYFCAKDKILWFGEPVFDYWGQGTLVTVSS
16	Daratumumab light chain variable region	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRL LIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSN WPPTFGQGTKVEIK
17	HCDR1	SFAMS
18	HCDR2	AISGSGGGTYYADSVKG
19	HCDR3	DKILWFGEPVFDY
20	LCDR1	RASQSVSSYLA
21	LCDR2	DASNRAT
22	LCDR3	QQRSNWPPT
23	Human IgG1 heavy chain constant region-2	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK

Example 5: Detection of binding activity of CD38 antibody to CD38 by ELISA

Recombinant human CD38-His protein (ACRO Biosystems, Cat: CD8-H5224) or monkey CD38-His protein (ACRO Biosystems, Cat: CD8-C5223) was coated onto a 96-well plate and incubated overnight at 4°C. After the plate was washed 3 times with PBST (PBS containing 0.5% Tween-20), PBST containing 2% BSA was added for blocking the plate for 2 hours. Then, the plate was washed three times with PBST, and the serially diluted (initial concentration: 33.3 nM, 4-fold dilution) test antibodies (daratumumab, Ab1, HuAb1 and hIgG1) were added and incubated at 37°C for 1 hour. The plate was

washed three times with PBST, the HRP-labeled anti-human IgG-Fc secondary antibody (Sigma, Cat: A0170) was added, and the plate was incubated at 37°C for 30 minutes. After the plate was washed three times with PBST, the plates were developed with TMD (Surmodics, Cat: TMBW-1000-01). After incubation in the dark for 15 minutes, the color development reaction was terminated by adding 2N H_2SO_4, and OD450 was read on a microplate reader. The hIgG1 mentioned here and below was purchased from Biolegend (Cat: 403502).

The results were shown in Figures 1 and 2. Ab1 and HuAb1 showed relatively high binding activity to human CD38, with EC_{50} of 0.091 nM and 0.099 nM, respectively. In the same experiment, daratumumab showed EC_{50} of 0.099 nM, which was comparable to that of Ab1 and HuAb1. Moreover, Ab1 and HuAb1 were positive for binding to monkey CD38 protein, with EC_{50} of 0.16 nM and 0.15 nM, respectively, and daratumumab did not bind to monkey CD38.

Example 6: Detection of binding activity of anti-CD38 antibody to human CD38 by Biacore

Biacore was used to determine the binding kinetic parameters by measuring surface plasmon resonance (SPR). This technology was used to detect the microscopic rate constants of the binding (ka) and dissociation (kd) of an antibody and an antigen, and the affinity value of the antibody and the antigen can be obtained by the calculation. Both Biacore instrument (Biacore T200) and reagents were purchased from GE Healthcare.

The purified antibodies (daratumumab, Ab1 and HuAb1) were diluted in 1X sample dilution buffer (Cytiva, BR-1006-69) (antibody concentration: 1 μg/ml) and flowed over a CM5 chip (Cytiva 29-1275-56) pre-immobilized with protein A. Then, a serially diluted (initial concentration: 100 nM, 2-fold dilution) human CD38-His (ACROBiosystems, Cat: CD8-H5224) fusion protein was flowed over the above detection chip to measure the binding of the antigen and the antibody, and then 1X mobile phase buffer (Cytiva, BR-1006-69) was flowed over the chip to detect the dissociation of the antigen and the antibody. The binding and dissociation signal data of the antigen and the antibody were collected at different concentrations, and fitted at 1: 1 by a Langmuir model to calculate the affinity of the antigen and the antibody.

As shown in Table 5, the anti-CD38 antibodies Ab1 and HuAb1 showed high affinity to human CD38, with KD values of 1.068E-8 (M) and 9.535E-9 (M), respectively, which were significantly higher than 1.543E-7 (M) for daratumumab.

Table 5 Detection of kinetic constants of anti-CD38 antibody binding to human CD38 by Biacore

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
Daratumumab	1.317E+5	2.032E-2	1.543E-7
Ab1	4.581E+5	4.894E-3	1.068E-8
HuAb1	4.086E+5	3.896E-3	9.535E-9

Example 7: Detection of binding activity of anti-CD38 antibody to CD38 on cell surface by FACS

Human Burkitt's lymphoma cell line Daudi (ATCC, Cat: CCL-213) and human myeloma cell line HCI-H929 cells (ATCC, Cat: CRL-3580) both endogenously express human CD38 on the surface thereof. The antibodies (Ab1, HuAb1 and hIgG1 (Biolegend, Cat: 403502)) were serially diluted (initial concentration: 100 nM, 3-fold dilution) in PBS containing 2% fetal bovine serum (FBS, Gibco, Cat: 10100147). The diluted antibodies were mixed with the test cells ($5*10^5$ cells/well) and then incubated at 4°C for 1 hour. After the cells were washed three times with PBS containing 2% fetal bovine serum (FBS), a PE-labeled mouse anti-human IgG Fc antibody (Biolegend, Cat: 409304) was added and incubated at 4°C in the dark for 1 hour. The cells were washed three times with PBS containing 2% fetal bovine serum (FBS), and then the fluorescence signal was detected by the CantoII (BD Biosciences) flow cytometer, and according to the mean fluorescence intensity (MFI) of the signal, GraphPad was used for fitting a concentration dependent curve, and the EC_{50} was calculated.

As shown in Figures 3 and 4, the anti-CD38 antibodies Ab1 and HuAb1 bind to CD38 on Daudi cells with EC_{50} of 0.87 nM and 0.82 nM, respectively, and bind to CD38 on HCI-H929 cells with EC_{50} of 1.09 nM and 0.77 nM, respectively.

Example 8: Effect of anti-CD38 antibody on phagocytosis of tumor cells by human macrophages

The ability of the anti-CD38 antibody of the present invention to promote phagocytosis of tumor cells by macrophages was detected by flow cytometry. Human

blood was freshly collected from donors, and the peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation with Ficoll-Paque PLUS (GE Healthcare, Cat: 17-1440-02). Monocytes were further isolated and obtained by using the human total monocyte Isolation Kit (Miltenyi biotec, cat: 130-096-537). To induce the monocytes to differentiate into macrophages, macrophage colony stimulating factor (M-CSF, R & D Systems, Cat: 216-MC) was added and the monocytes were subjected to adherent culture for 7 consecutive days. On the day of the phagocytosis experiment, the above-mentioned differentiated macrophages were starved in a serum-free medium for 2 hours. At the same time, target tumor cells Daudi were fluorescently labeled with CFSE (eBioscience, Cat: 65-0850-85) according to the experimental instructions. The labeled tumor cells and macrophages were mixed at a ratio of 4 : 1, and the experimental antibodies (Ab1, HuAb1 and hIgG1 (Biolegend, Cat: 403502)) at test concentrations (initial concentration: 10 ug/ml, 10-fold dilution) were added and incubated at 37°C for 2 hours. Then, the cells were washed twice with PBS, and then digested with trypsin (Gibco, Cat: 25200072); an APC-labeled anti-CD14 antibody (Biolegend, Cat: 325608) was added and incubated in the dark on ice in PBS containing 2% fetal bovine serum for 30 minutes. The cells were washed twice and analyzed by flow cytometry.

The percentage of CFSE positive cells in CD14 positive macrophage populations was calculated. As shown in Figure 5, the anti-CD38 antibodies Ab1 and HuAb1 can effectively promote the phagocytosis of tumor cells by macrophages.

Example 9: Anti-CD38 antibody mediating complement killing of Daudi cells

Complement-mediated cell killing was once considered to be an important component of antibody-mediated cell killing functions. The C1q molecule in the complement system binds to the antigen-antibody complex on the cell surface, which leads to the activation. Then, through the classical pathway of the complement activation pathway and a series of cascade enzymatic reaction processes, a membrane attack complex is formed on the surface of target cells, which eventually leads to the lysis and death of the target cells. However, recent studies have shown that when antibodies mediate complement killing of target cells, the complement activation caused thereby is closely related to the infusion reactions that occur in patients during treatment. (*See Antibodies (Basel). 2018 Mar; 7(1): 14*). During the treatment with daratumumab, the infusion reaction is a common treatment-related side effect, which greatly affects the

application of daratumumab and is closely related to the development of its drug resistance mechanism. An in vitro complement killing experiment was performed to evaluate the killing effect of the complement mediated by the anti-CD38 antibody of the present invention on the tumor cell Daudi expressing CD38.

Specifically, Daudi cells were washed twice with a cell culture medium free of FBS and added to the test plate at a density of 1E4/well. The test antibodies (daratumumab, Ab1, HuAb1, and hIgG1 (Biolegend, Cat: 403502)) were added at a concentration of 100 nM, and incubated in a cell culture incubator for 30 minutes, and then the serum containing the complement (Quidel Corporation, Cat: A113) was added to achieve a working concentration of 10%. The test plate was incubated in the cell culture incubator for 5 hours, and then 50 μl of Cell Titer-Glo was added to each well of the plate and mixed on a shaker for 2 minutes to induce cell lysis. The plate was incubated at room temperature for 10 minutes. Chemiluminescence was determined using the Envision reader.

The cytotoxicity% was calculated using the formula: "cytotoxicity% = 100* [maximum killing - sample]/[maximum killing - background]". As shown in Figure 6, the experimental results showed that daratumumab exhibited a strong effect of mediating the complement killing of CD38-expressing cells, and the effects of Ab1 and HuAb1 in mediating the complement killing were very weak. This indicated that in clinical applications, the risk of causing infusion reactions in patients was relatively low.

Example 10: Binding of anti-CD38 antibody to NK cells

In the clinical application of the anti-CD38 antibody daratumumab, a decrease in the number of NK cells is often seen in the test patients, and is considered to be closely related to the failure of daratumumab treatment. Since CD38 is also expressed on the surface of NK cells, the ADCC, CDC, ADCP and other effects produced by the binding of the anti-CD38 antibody may be one of the reasons for the decrease in the number of NK cells. The binding of anti-CD38 antibody to NK cells in human peripheral blood was detected by FACS.

Human blood was freshly collected from donors and separated to obtain peripheral blood mononuclear cells (PBMCs). The experimental antibodies were serially diluted (initial concentration: 100 nM, 10-fold dilution) with PBS containing 2% fetal bovine serum (FBS, Gibco, Cat: 10100147). The diluted antibodies were mixed with PBMCs (8

* 10^5 cells per well), and then the mixture was incubated at 4°C for 30 minutes. After the cells were washed three times with PBS containing 2% fetal bovine serum (FBS), a PE-labeled anti-human IgG Fc antibody (Biolegend, Cat: 366904), and a Pacific Blue-labeled anti-human CD56 antibody (Biolegend, Cat: 304629) for labeling NK cells were added and incubated at 4°C in the dark for 30 minutes. The cells were washed three times with PBS containing 2% fetal bovine serum (FBS), and the fluorescence signal was detected by the CantoII (BD Biosciences) flow cytometer. As shown in Figure 7, all test anti-CD38 antibodies bound to human NK cells, but the binding of antibodies Ab1 and HuAb1 to NK cells was significantly weaker than that of daratumumab, which indicated a weaker toxic effect on NK cells.

Example 11: SEC-HPLC detection

The ADC samples prepared in example 3 were filtered through the Millex® 0.22 μm hydrophilic durapore (PVDF) membrane and sampled for analysis.

Instrument: Agilent 1260 Infinity II

Chromatographic column: Waters XBridge®BEH200Å SEC 3.5 μm, 7.8×300 mm

Mobile Phase: A: 10 mmol/L PBS+15% IPA

Condition: 0.7 ml/min 100%A, 20 min; detection wavelength: 280 nm

The aggregation degree of each ADC can be obtained by the SEC-HPLC detection, with the data summarized in Table 6.

Table 6

Target conjugate	Monomer rate	Aggregation rate
Control ADC-1	91.3%	8.70%
Control ADC-2	97.2%	2.80%
ADC-1	100.00%	0.00%
ADC-2	97.01%	2.99%
ADC-3	98.72%	0.61%
ADC-4	97.28%	2.72%
ADC-5	100.00%	0.0%
ADC-6	100.0%	0.0%
ADC-7	98.02%	1.98%
ADC-8	92.29%	7.71%
ADC-9	98.73%	1.27%
ADC-10	98.81%	1.19%

ADC-11	97.44%	2.56%
ADC-12	99.01%	0.99%
ADC-13	100.0%	0.0%
ADC-14	100.0%	0.0%
ADC-15	99.97%	0.03%
ADC-16	100.0%	0.0%
ADC-17	100.0%	0.0%

Conclusion: The ADC products introduced with the linker of the present invention has a relatively low aggregation rate.

Example 12: Test of binding of antibody or antibody-drug conjugate of the present invention to CD38 positive cells

Experimental method

First, Ramos cells (Cat. No. CRL-1596), H929 cells (Cat. No. CRL-9068) or RPMI-8226 cells (Cat. No. CCL-155) (all purchased from the cell bank of American Type Culture Collection, ATCC) were cultured normally in an RPMI-1640 medium (Gibco, Cat. No. A1049101) and 10% heat-inactivated fetal bovine serum FBS (Gibco, Cat. No. C20012500BT) in a cell culture incubator at 5% CO_2 and 37°C. The suspended Ramos cells were collected and centrifuged, and the cells were resuspended in a 4°C staining buffer (PBS (Gibco, Cat. No. C20012500BT) + 2% FBS) and added to a V-bottom 96-well plate (BD falcon) at 1.0×10^5 cells/well and 50 μL per well. The antibody or the antibody-drug conjugate was diluted with a staining buffer in 4-fold gradient to the corresponding concentration, and then the diluted antibody or antibody-drug conjugate at different concentrations (final concentrations: 150, 37.5, 9.38, 2.34, 0.586, 0.146, 0.037 and 0.0092 nM) or the human IgG1 isotype reference antibody (Biolegend, 403502) were added to the corresponding cell wells at 50 μL/well. The plate was incubated at 4°C for 1.0 hour. After the centrifugation at 300 g for 5 minutes, the supernatant was aspirated, and the pellet was resuspended in a staining buffer at 200 μL/well and washed twice. The PE-labeled anti-human IgG Fc antibody (Biolegend, 410708) diluted in a staining buffer was added at 100 μL/well and incubated in the dark at 4°C for 45 minutes. After the centrifugation at 300 g for 5 minutes, the supernatant was aspirated, and the pellet was resuspended in a staining buffer at 200 μL/well and washed twice. Then, the cells were resuspended in an FACS fixation buffer (PBS + 2% FBS + 1% tissue cell fixation buffer

(Beijing Dingguo Changsheng Biotechnology Co., Ltd., AR-0211)) at 200 μL/well and fixed at room temperature for 15 minutes or at 4°C overnight.

Detection and data analysis

Fluorescence signals were detected on a BD FACSCanto™ II flow cytometer (BD Biosciences) to obtain the mean fluorescence intensity (MFI) of the binding of the antibody or antibody-drug conjugate to cells, and the EC_{50} values were obtained using the Log (agonist) vs. response equation in the GraphPad Prism software. (See Tables 7-1 to 7-3).

Table 7-1

Compound number	Ramos ($CD38^+$) EC_{50}, nM
Daratumumab	0.34
ADC-1	0.35
ADC-2	0.37
ADC-3	0.43
ADC-4	0.21
ADC-6	0.35
ADC-7	0.20
ADC-8	0.28
ADC-9	0.33
ADC-10	0.31
ADC-11	0.26
ADC-12	0.46
ADC-15	0.52
ADC-16	0.31
ADC-17	0.31

Table 7-2

Compound number	H929 ($CD38^+$) EC_{50}, nM
ADC-4	0.51
ADC-1	0.72
ADC-2	0.53
ADC-8	0.71

Table 7-3

Compound number	RPMI-8226 (EC_{50}, nM)

ADC-1	0.37
ADC-3	0.61
ADC-11	0.58

Conclusion: It can be seen from the data in Table 7-1, Table 7-2 and Table 7-3 that the ADC products of the present invention exhibit similar cell binding activity to the daratumumab antibody in the Ramos (CD38+) cell line, H929 (CD38+) cell line and RPMI-8226 cell line.

Example 13: Internalization experiment of antibody or antibody-drug conjugate of the present invention on CD38-positive Ramos cells

Experimental method

First, Ramos cells (ATCC, Cat. No. CRL-1596) were cultured normally in an RPMI-1640 medium (Gibco, Cat. No. A1049101) and 10% heat-inactivated fetal bovine serum FBS (Gibco, Cat. No. 10100147) in a cell culture incubator at 5% CO2 and 37°C. The suspended Ramos cells were collected and centrifuged, and the cells were resuspended in a 4°C staining buffer (PBS (Gibco, Cat. No. C2001200CP) + 2% FBS) and added to a V-bottom 96-well plate (BD falcon) at 1.0 x 10^5 cells/well and 50 μL per well. The antibody or the antibody-drug conjugate was diluted with a staining buffer to the corresponding concentration (20 μg/ml), and then the diluted antibody or antibody-drug conjugates at a final concentration of 10 μg/mL or the human IgG1 isotype reference antibody (Biolegend, 403502) were added to the corresponding cell wells at 50 μL/well. The plate was incubated at 4°C for 1.0 hour, After the centrifugation at 300 g for 5 minutes, the supernatant was aspirated, and the pellet was resuspended in a staining buffer at 200 μL/well and washed twice. The PE-labeled anti-human IgG Fc antibody (Biolegend, 410708) diluted in a staining buffer was added at 100 μL/well and incubated in the dark at 4°C for 45 minutes. After the centrifugation at 300 g for 5 minutes, the supernatant was aspirated, and the pellet was resuspended in a staining buffer at 200 μL/well and washed twice. After the centrifugation, the cells were resuspended in a complete culture medium at 100 μL/well and incubated in a cell culture incubator at 5% CO_2 and 37°C or at 4°C for 4 hours. After the centrifugation at 300 g for 5 minutes, the supernatant was aspirated, and the pellet was resuspended by adding a staining buffer (pH = 2.8) acidified with hydrochloric acid (Sinopharm, Shanghai) at 200 μL/well, and incubated at 4°C for 5 minutes. After the centrifugation at 300 g for 5 minutes, the

supernatant was aspirated, and the pellet was resuspended in a staining buffer at 200 μL/well and washed twice. Then, the cells were resuspended in an FACS fixation buffer (PBS + 2% FBS + 1% tissue cell fixation buffer (Beijing Dingguo Changsheng Biotechnology Co., Ltd., AR-0211)) at 200 μL/well and fixed at room temperature for 15 minutes or at 4°C overnight.

Detection and data analysis

Fluorescence signals were detected on a BD FACSCanto™ II flow cytometer (BD Biosciences) to obtain the mean fluorescence intensity (MFI) of the binding of the antibody or antibody-drug conjugate to cells. The internalization signal value of the antibody or antibody-drug conjugate was expressed as the MFI obtained by incubating the labeled cells at 37°C and performing acid washing; and the control internalization signal value (non-internalization signal value) was expressed as the MFI obtained by incubating the labeled cells at 4°C and performing acid washing. The internalization signal values of each ADC product are shown in Figure 8.

Conclusion: As can be seen from the data in Figure 8, the ADC products of the present invention exhibit good internalization.

Example 14: Cell proliferation inhibition experiment

Experimental method

Ramos (CD38 positive) (Cat. No. CRL-1596), RPMI-8226 (CD38 positive) (Cat. No. CCL-155) and SU-DHL-2 (CD38 negative) (Cat. No. CRL-2956) cells (all purchased from the cell bank of American Type Culture Collection, ATCC) were normally cultured in an RPMI-1640 medium (Gibco, Cat. No. A1049101) and 10% heat-inactivated fetal bovine serum (FBS) (Gibco, Cat. No. 10100147) in a cell culture incubator at 5% CO_2 and 37°C. The cells were added to a 96-well plate at 1.0 x 10^4 cells/well and 100 μL/well and incubated in a cell culture incubator at 5% CO_2 and 37°C. The test antibody drugs (such as antibodies or ADCs) were diluted with PBS (Gibco, Cat. No. C20012500BT) in 4-fold gradient to the corresponding concentration, and then the diluted test antibody drugs at different concentrations (final concentrations: 200, 50.0, 12.5, 3.13, 0.781, 0.195, 0.049 and 0.012 nM) or the control solution (PBS) were added at 10 μL/well to a 100 μL/well cell culture system. The cells were incubated in a cell culture incubator at 5% CO_2 and 37°C for 72 hours.

After 72 hours of the culture, the CellTiter-Glo® reagent (Promega, Cat. No. G9243) was added to the wells at 50 μL/well, mixed evenly with shaking and incubated for 10 minutes.

Detection and data analysis

The luminescent signal was read on an EnVision™ multi-function microplate reader (PerkinElmer), and the inhibition rate (%) of the compound was calculated. Then, the half maximal inhibitory concentration (IC_{50}) values were obtained using the XLfit5 software (ID Business Solutions Limited). All samples were tested in duplicate wells.

$$Inhibition\% = (1-(Lum_{antibody\ drug} - Lum_{background})/(Lum_{cell} - Lum_{background}))\times 100\%$$

wherein

$Lum_{antibody\ drug}$ represents the luminescent signal in the well of the test antibody drug;

Lum_{cell} represents the luminescent signal in the cell control well;

$Lum_{background}$ represents the luminescent signal in the cell-free control well.

Other cell sources and catalog numbers are as follows: OPM-2 cells (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Cat. No. ACC 50/G), SU-DHL-6 (ATCC, Cat. No. CRL-2959), SU-DHL-4 (ATCC, Cat. No. CRL-2957), Daudi (ATCC, Cat. No. CCL-213), Mino (ATCC, Cat. No. CRL-3000), MAVER-1 (ATCC, Cat. No. CRL-3008), H1975 (ATCC, Cat. No. CRL-5908), HCC4006 (ATCC, Cat. No. CRL-2871), MFE-280 (European Collection of Authenticated Cell Cultures (ECACC), Cat. No. 98050131) and Caki-1 (CD70+) (cell bank of Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences).

The resulting data are shown in Tables 8-1 to 8-5 below.

Table 8-1

ADC number	Cell proliferation inhibitory activity (IC_{50}, nM)		
	Ramos ($CD38^+$)	RPMI-8226 ($CD38^+$)	SU-DHL-2 ($CD38^-$)
Control ADC-1	0.13	0.23	74
Control ADC-2	0.072	0.21	33.8
ADC-1	0.11	0.38	77.8
ADC-2	0.1	-	48.1
ADC-3	0.16	0.35	135.7
ADC-4	0.063	0.11	66.8
ADC-5	0.085	1	5.9
ADC-6	0.1	1.30	186.2

ADC-7	0.053	0.31	>200
ADC-8	0.06		12.2
ADC-9	0.11	0.31	184.5
ADC-10	0.054	0.39	>200
ADC-11	0.056	1.3	151.1
ADC-12	0.074	0.34	>200
ADC-13	0.11	104.2	> 200
ADC-14	0.3	2.3	>200
ADC-15	0.18	0.27	>200
ADC-16	0.27	0.55	>200
ADC-17	0.14	0.32	>200

Table 8-2

ADC number	Cell proliferation inhibitory activity (IC_{50}, nM)				
	SU-DHL-6	SU-DHL-4	Daudi	Mino	MAVER-1
ADC-10	0.060	0.30	0.23	0.18	0.35

Table 8-3

ADC number	Cell proliferation inhibitory activity (IC_{50}, nM)		
	Ramos ($CD20^+$)	MAVER-1 ($CD20^+$)	OPM-2 ($CD20^-$)
ADC-19	0.43	0.33	46.7

Table 8-4

ADC number	Cell proliferation inhibitory activity (IC_{50}, nM)		
	H1975 ($PDL1^+$)	HC4006 ($PDL1^+$)	MFE-280 ($PDL1^-$)
ADC-20	31.8	3.20	>200

Table 8-5

ADC number	Cell proliferation inhibitory activity (IC_{50}, nM)		
	MAVER-1 ($CD70^+$)	Caki-1 ($CD70^+$)	OPM-2 ($CD70^-$)
ADC-21	0.074	1.20	72.4

Conclusion: The ADC products of the present invention exhibit in vitro proliferation inhibitory activity dependent on CD38 expression of cells (Table 8-1). According to the above method, the inhibitory activities of ADC-10 and control ADC-2 were measured in

parallel in the OPM-2 proliferation inhibition assay, wherein the ADC-10 had an IC_{50} of 0.69 nM and the control ADC-2 had an IC_{50} of 1.2 nM. ADC-10 has significantly higher activity than the control ADC-2 (p value: 0.00033), but has weaker activity on CD38-negative SU-DHL-2 cells (Table 8-1), suggesting that ADC-10 is less toxic to CD38-negative cells. In addition to multiple myeloma (MM) cells, ADC10 also exhibits strong proliferation inhibitory activity against other CD38-positive B-cell lymphoma (such as diffuse large B-cell lymphoma, Burkitt's lymphoma and mantle cell lymphoma) cells (Table 8-2).

The data in Tables 8-3 to 8-5 indicate that the linker of the present invention is still compatible with other antibodies and can exhibit excellent activity in positive cells and relatively low cytotoxicity in negative cell lines.

Example 15 Plasma stability of ADC of the present invention

Experimental method:

To mouse, rat and human plasma were added control ADC-1 and control ADC-2 at a final concentration of 100 ug/ml, ADC-1 and ADC-4 at a final concentration of 10 ug/ml, and ADC-7, ADC-9, ADC-10, ADC-12, ADC-16 and ADC-17 at a final concentration of 100 nM. After mixing evenly, a specified volume of a sample were immediately taken out and frozen in a -60°C ~ -80°C refrigerator as a 0 h sample. The remaining samples were incubated in a cell culture incubator at 37°C and 5% CO_2. At 96 h, 50 μL of the sample was transferred, and detected for free toxins by LC-MS/MS.

200 μL of acetonitrile solution containing internal standard working solution was added to 50 μL of the sample for precipitation. The mixture was subjected to vortex mixing for 3 minutes and then centrifuged at 4400 rpm for 10 minutes. 50 μL of the supernatant was appropriately diluted and analyzed by LC/MS/MS (AB SCIEX).

Free toxin release rate% = concentration of free toxin/theoretical incubation concentration of antibody-conjugated toxin × 100%

Experimental result:

The toxin release rates of all ADCs after 96 h (4 days) of incubation in mouse plasma were less than 25%, among which the control ADC-2 showed the highest release rate (21.6%). No release of free toxin was detected (below the detection limit) after ADC-16 was incubated in mouse plasma for 96 h. The toxin release rates of all ADCs after 96 h of incubation in rat plasma were significantly lower than those in mouse

plasma. No free toxin release was detected after ADC-1 and ADC-16 were incubated in rat plasma for 96 h. Except for control ADC-1, control ADC-2 and ADC-7, which showed a free toxin release rate of ~ 0.2% after 96 h of incubation in human plasma, no free toxin release was detected in the remaining ADCs after 96 h of incubation in human plasma. Specific results are shown in Table 9.

Table 9

ADC	Incubation time (hour)	Toxin release rate (%, mean±SD)		
		Mouse plasma	Rat plasma	Human plasma
Control ADC-1	0	1.55±0.0456	ND	ND
	96	15.0±0.294	6.77±0.119	0.213±0.00521
Control ADC-2	0	2.60±0.0727	0.0130±0.0113	0.00986±0.00908
	96	21.6±0.880	5.01±0.0645	0.210±0.00839
ADC-4	0	ND	ND	ND
	96	2.29±0.174	0.895±0.0773	ND
ADC-1	0	ND	ND	ND
	96	3.27±0.0828	ND	ND
ADC-10	0	ND	ND	ND
	96	3.63±0.0948	0.281±0.0227	ND
ADC-7	0	ND	ND	ND
	96	14.6±0.383	0.810±0.0547	0.190±0.00729
ADC-9	0	ND	ND	ND
	96	3.28±0.0363	0.197±0.0225	ND
ADC-12	0	ND	ND	ND
	96	6.57±0.0177	0.376±0.0390	ND
ADC-17	0	ND	ND	ND
	96	10.1±0.0430	0.315±0.000186	ND
ADC-16	0	ND	ND	ND
	96	ND	ND	ND

ND: Not detectable

Example 16 In vivo antitumor activity of antibody-drug conjugate (ADC-10) of the present invention

Objective: To evaluate the anti-tumor activity of the antibody-drug conjugate (ADC-10) of the present invention in Daudi subcutaneous tumor model in BALB/c nude mice.

Method: Human B-cell lymphoma cells Daudi (ATCC® CRL-213™) were cultured and proliferated in an RPMI1640 medium containing 10% fetal bovine serum. Tumor cells in the exponential growth phase were suspended in an RPMI1640 containing

Matrigel, and implanted subcutaneously into the right flank of female BALB/c nude mice (Shanghai Lingchang Biotechnology Co., Ltd.) at 5×10^6 cells per mouse.

After inoculation of tumor cells, the mice were randomly divided into 4 groups according to tumor volume, and Daratumumab and antibody-drug conjugate ADC-10 were diluted with normal saline and administered according to the regimen shown in Table 10. The antibody Daratumumab was purchased from Johnson & Johnson Pharmaceutical Ltd., and the antibody-drug conjugate ADC-10 was prepared according to the method of example 3.

Table 10. Dosage regimen of Daratumumab and ADC-10

Test substance	Group	Dosage (mg/kg/time×time/ week)	Total weekly dose×week (mg/kg)	Dosage regimen	Number of mice
Saline	Vehicle group	-×2	-	Administration for a total of two weeks, via tail vein on day 1, day 5, day 8 and day 12	7
Daratumumab	Daratumumab	10×2	20×2	Administration for a total of two weeks, via tail vein on day 1, day 5, day 8 and day 12	7
ADC-10	ADC-10-2.5 mg/kg	2.5×2	5×2	Administration for a total of two weeks, via tail vein on day 1, day 5, day 8 and day 12	7
	ADC-10-10.0 mg/kg	10×2	20×1	Administration for a total of one week, via tail vein on day 1 and day 5	7

Note: The grouping day is defined as day 0, and the next day for drug administration is day 1.

The tumor volumes (tumor volume = 0.5 × long diameter × short diameter^2), and body weights of the mice were measured regularly. The tumor growth inhibition rate and tumor regression rate of each treatment group after administration were statistically analyzed.

The calculation formula of tumor growth inhibition rate of each treatment group is: 1-[(D_t tumor volume-D_0 tumor volume)$_{\text{treatment group}}$/($D_t$ tumor volume-D_0 tumor volume)$_{\text{vehicle group}}$] × 100%.

If the tumor volume after treatment is smaller than the tumor volume before treatment, the tumor regression rate is calculated. The calculation formula is: [(D_0 tumor volume-D_t tumor volume)/D_0 tumor volume] × 100%.

D_t tumor volume represents the tumor volume on day t, and D_0 tumor volume represents the tumor volume on day 0.

The t-test was used for pairwise comparison between each administration group and the vehicle control group, and between the administration groups. The p value was calculated, and $p < 0.05$ indicated a significant statistical difference between two groups. The mice were weighed regularly and their relative weights were calculated using the formula: (weight of mice on the day of measurement/weight of mice at the time of grouping) × 100%.

Results:

After 26 days of administration, Daratumumab (10 mg/kg, administered on days 1, 5, 8 and 12) can significantly inhibit the growth of Daudi subcutaneous tumors, with a tumor growth inhibition rate of 96.8%; ADC-10 (2.5 mg/kg, administered on days 1, 5, 8, and 12) showed an anti-tumor effect similar to that of the Daratumumab administration group and had a tumor growth inhibition rate of 95.9%. The ADC-10 (administered at 10 mg/kg on days 1 and 5) administration group achieved the complete tumor regression (tumor regression rate of 100%) in all animals when measured on day 16, and no tumor reappeared or grew until day 26, as shown in Figure 9. Tumor-bearing mice tolerated the above test substances well. On day 26, the mean relative body weight of mice in the ADC-10 administration group (2.5 mg/kg and 10 mg/kg) ranged from 105% to 111%.

Based on the above data, ADC-10 showed a strong and dose-dependent anti-tumor effect on the Daudi nude mouse subcutaneous transplanted tumor model, and the efficacy

of administration of ADC-10 at 10 mg/kg for one week was significantly stronger than administration of Daratumumab at the same dose for two weeks ($p<0.01$).

Example 17 In vivo antitumor activity of antibody-drug conjugate (ADC-10) of the present invention

Objective: To evaluate the anti-tumor activity of the antibody-drug conjugate (ADC-10) of the present invention in KMS-11 subcutaneous tumor model in BALB/c nude mice.

Method: Human multiple myeloma cells KMS-11 (HSRRB® JCRB1179) were cultured and proliferated in an RPMI1640 medium containing 10% fetal bovine serum. Tumor cells in the exponential growth phase were implanted subcutaneously into the right flank of female BALB/c nude mice (Shanghai Lingchang Biotechnology Co., Ltd.) at 1×10^7 cells per mouse.

After inoculation of tumor cells, the mice were randomly divided into 5 groups according to tumor volume, and Daratumumab and antibody-drug conjugate ADC-10 were diluted with normal saline and administered according to the regimen shown in Table 12. The antibody Daratumumab was purchased from Johnson & Johnson Pharmaceutical Ltd., and the antibody-drug conjugate ADC-10 was prepared according to the method of example 3.

Table 12. Dosage regimen of Daratumumab and ADC-10

Test substance	Group	Dosage (mg/kg/time×time/ week)	Total weekly dose (mg/kg)×week	Dosage regimen	Number of mice
Saline	Vehicle group	-×2	-	Administration via tail vein injection on days 1, 5, 8 and 12	8
Daratumumab	Daratumumab	10×2	20×2	Administration via tail vein injection on days 1, 5, 8 and 12	8
ADC-10	ADC-10-2.5 mg/kg	2.5×2	5×2	Administration via tail vein injection on days 1, 5, 8 and 12	8

ADC-10-5.0 mg/kg	5.0×2	10×2	Administration via tail vein injection on days 1, 5, 8 and 12	8
ADC-10-10.0 mg/kg	10×1	10×1	Administration via tail vein injection on day 1	8

Note: The grouping day is defined as day 0, and the next day for drug administration is day 1.

The tumor volumes (tumor volume = 0.5 × long diameter × short diameter^2), and body weights of the mice were measured regularly. The tumor growth inhibition rate and tumor regression rate of each treatment group after administration were statistically analyzed.

The calculation formula of tumor growth inhibition rate of each treatment group is: 1-[(D_t tumor volume-D_0 tumor volume)$_{\text{treatment group}}$/($D_t$ tumor volume-D_0 tumor volume)$_{\text{vehicle group}}$] × 100%.

If the tumor volume after treatment is smaller than the tumor volume before treatment, the tumor regression rate is calculated. The calculation formula is: [(D_0 tumor volume-D_t tumor volume)/D_0 tumor volume] × 100%.

D_t tumor volume represents the tumor volume on day t, and D_0 tumor volume represents the tumor volume on day 0.

The t-test was used for pairwise comparison between each administration group and the vehicle control group, and between the administration groups. The p value was calculated, and $p < 0.05$ indicated a significant statistical difference between two groups. The mice were weighed regularly and their relative weights were calculated using the formula: (weight of mice on the day of measurement/weight of mice at the time of grouping) × 100%.

Results:

After 25 days of administration, daratumumab (10 mg/kg, administered on days 1, 5, 8 and 12) can significantly inhibit the growth of KMS-11 subcutaneous tumors, with a tumor growth inhibition rate of 89.8%. The 2.5 mg/kg ADC-10 and 5.0 mg/kg ADC-10 (both administered on days 1, 5, 8 and 12) and ADC-10 (administered at 10 mg/kg on day 1) administration groups achieved the complete tumor regression (tumor regression

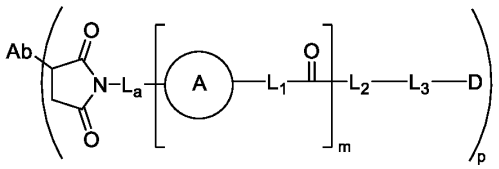
rate of 100%) in all animals when measured on days 13, 11, and 11, respectively, and no tumor reappeared or grew until day 25, as shown in Figure 10. Tumor-bearing mice tolerated the above test substances well. On day 25, the mean relative body weight of the ADC-10 group (2.5 mg/kg, 5.0 mg/kg, and 10.0 mg/kg) was in the range of 105% to 111%.

Based on the above data, ADC-10 showed a strong anti-tumor effect on the KMS-11 mouse subcutaneous transplanted tumor model, and the efficacy of administration of ADC-10 at a single dose of 10 mg/kg was significantly stronger than administration of Daratumumab at the same dose for two weeks ($p < 0.01$).

The present invention has been illustrated by various specific embodiments. However, a person of ordinary skill in the art will appreciate that the present invention is not limited to various specific embodiments. A person of ordinary skill can make various modifications or variations within the scope of the present invention. The various technical features mentioned throughout the description can be combined with each other without departing from the spirit and scope of the present invention. Such modifications and variations are all within the scope of the present invention.

CLAIMS

1. A drug conjugate represented by formula (III),



(III)

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof,

wherein Ab is a targeting moiety such as an antibody or an antigen-binding fragment thereof;

p is an integer selected from 1-16, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 12;

D is a drug active molecule;

L_a is selected from -C_{1-12} alkylene-, -C_{1-12} alkylene-C(O)-, -C_{1-12} alkylene-CONH-$(CH_2CH_2O)_n$-CH_2-C(O)- or -$(CH_2CH_2O)_n$-CH_2-C(O)-; n is an integer selected from 1-18, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

"ring A" is an optionally substituted 3- to 8-membered heterocycloalkylene or 6- to 10-membered heteroarylene;

L_1 is selected from -CH_2-CHR_1- or -O-CH_2-, or is absent; R_1 is -OH, -NH_2, -C_{1-4} alkylene-OH or -C_{1-4} alkylene-NH_2;

L_2 is a linking unit selected from short peptide residues consisting of 2-10 amino acids, wherein the amino acids are natural amino acids or non-natural amino acids;

L_3 is a spacer unit; and

m is 1, 2, 3 or 4.

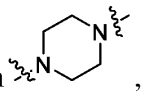
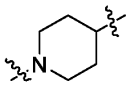
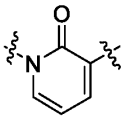
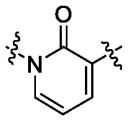
2. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 1, wherein

L_a is selected from -C_{1-6} alkylene-, -C_{1-6} alkylene-C(O)-, -C_{1-6} alkylene-CONH-$(CH_2CH_2O)_n$-CH_2-C(O)- or -$(CH_2CH_2O)_n$-CH_2-C(O)-; n is an integer selected from 1-12;

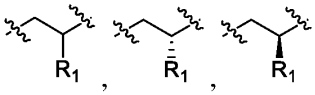
"ring A" is 5- or 6-membered nitrogen-containing saturated heterocycloalkylene, or 5- or 6-membered nitrogen-containing unsaturated heterocycloalkylene substituted with

oxo (=O); and when ring A is 5- or 6-membered nitrogen-containing unsaturated heterocycloalkylene substituted with oxo, L_1 is absent.

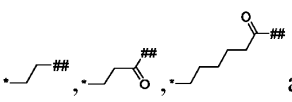
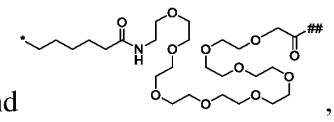
3. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to either claim 1 or claim 2, wherein

ring A is selected from ,  and , and when ring A is , L_1 is absent.

4. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 3, wherein

L_1 is selected from , and-O-CH_2-; R_1 is -OH, -NH_2, -C_{1-4} alkylene-OH or -C_{1-4} alkylene-NH_2.

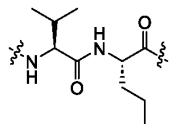
5. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 4, wherein

L_a is selected from  and , wherein * represents a position connected to a maleimide group, and ## represents a position connected to ring A.

6. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 5, wherein

L_2 is a linking unit selected from short peptide residues consisting of 2-10 amino acid residues, wherein the amino acid residues can be independently selected from L- or D-isomers of the following amino acids: alanine (including β-alanine), arginine, aspartic acid, asparagine, cysteine, histidine, glycine, glutamic acid, valine, glutamine, phenylalanine, lysine, leucine, isoleucine, methionine, serine, tyrosine, threonine, tryptophan, proline, ornithine, penicillamine, aminoacetylenic acid, aminoalkanedioic acid, heterocyclyl-carboxylic acid, citrulline, selenocysteine [1-2], pyrrolysine, diaminoalkanoic acid, and a derivative thereof; preferably L_2 is selected from -Phe-Lys-, -Val-Lys-, -Phe-Phe-Lys-, -D-Phe-Phe-Lys-, -Gly-Phe-Lys-, -Ala-Lys-, -Val-Cit-, -Phe-Cit-, -Leu-Cit-, -Iie-Cit-, -Trp-Cit-, -Phe-Ala-, -Gly-Phe-Leu-Gly-, -Ala-Leu-Ala-Leu-, -Val-Ala-, -Gly-Gly-Phe-Gly, -Gly-Cit-, -Cit-Val-, -Cit-Ala-, -Lys-Val-, -Val-Lys(Ac)-, -Phe-Lys(Ac)-, -Ala-Ala-, -Ala-Ala-Ala-, -Ala-Ala-Asn-, -Ala-Ala-Asp-, -Lys-Ala-Ala-Asn-, -Lys-Ala-Ala-Asp-, -D-Val-Leu-Lys-, -Gly-Gly-Arg-, -Gly-Gly-Phe-, -Val-Lys-Gly-, -Val-Lys-Gly-Gly- and -Lys-Ala-Asn- or a derivative thereof; more preferably, L_2 is -Val-Cit- or a derivative thereof.

7. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 6, wherein L_2 has a structure represented by formula (I-4):



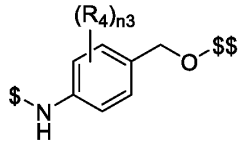
Formula (I-4)

wherein R_3 is selected from $-NHCONH_2$, $-NHCOCH_2(OCH_2CH_2)_{n1}OCH_3$, $-(CH_2)_{n2}SO_2CH_3$ or $-(CH_2)_{n2}P(O)(CH_3)_2$, wherein n1 is an integer selected from 1-18, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12; n2 is 0, 1, 2, 3, 4, 5, 6, 7 or 8;

preferably, R_3 is selected from $-NHCONH_2$, $-NHCOCH_2(OCH_2CH_2)_{n1}OCH_3$, $-SO_2CH_3$ or $-P(O)(CH_3)_2$, wherein n1 is an integer selected from 6-12.

8. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 7, wherein

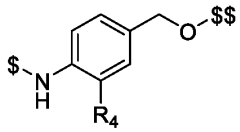
L_3 has a structure represented by formula (II-5):



Formula (II-5) ,

wherein R_4 is selected from $-(CH_2)_{n2}SO_2CH_3$, $-(CH_2)_{n2}P(O)(CH_3)_2$ or hydrogen, wherein n2 is 0, 1, 2, 3, 4, 5, 6, 7 or 8; n3 is 1 or 2; $ is an active site connected to L_2, and $$ is a site connected to the drug active molecule;

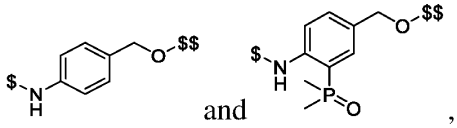
preferably, L_3 has a structure represented by formula (II-6)



Formula (II-6) ,

wherein each symbol is as defined in formula (II-5).

9. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 8, wherein L_3 has a structure selected from:

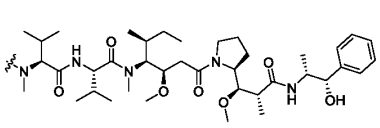


and ,

wherein $ represents a connection site to L_2, and $$ represents a connection site to the drug active molecule.

10. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 9,

wherein D is Dolastatin 10 or a Dolastatin 10 derivative, preferably selected from:



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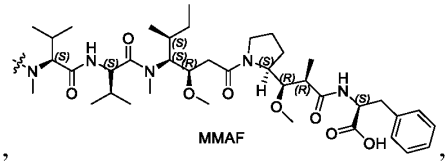

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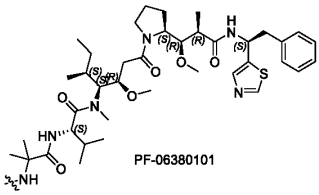

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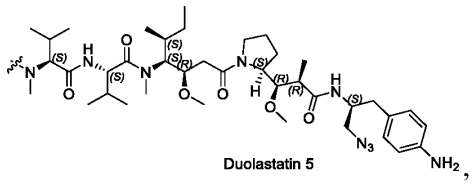

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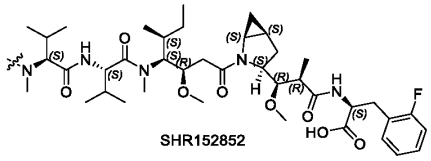

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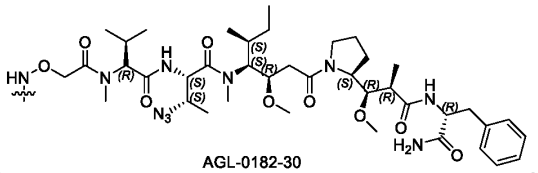

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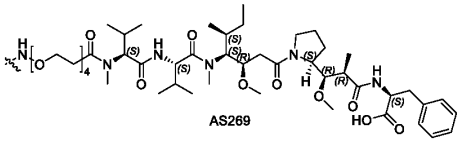

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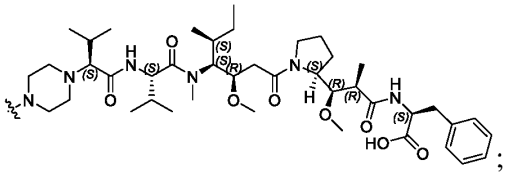
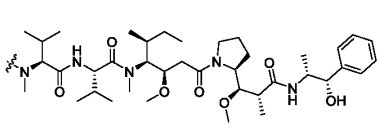

;

preferably, D is a Dolastatin 10 derivative selected from:



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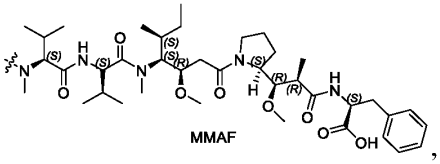

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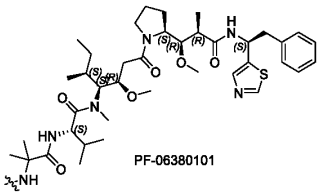
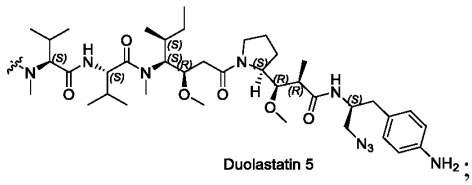
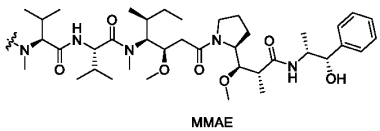


and


;

more preferably, D is selected from:



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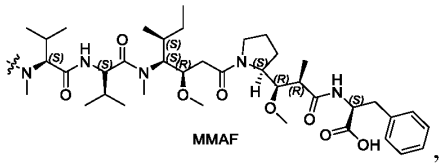


,

wherein the wavy line represents a connection site to L_3.

11. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claims 1 to 10, wherein p is selected from 2, 3, 4, 5, 6, 7 and 8.

12. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 11, wherein Ab is an anti-CD38 antibody or an antigen-binding fragment thereof, an anti-CD20 antibody or an antigen-binding fragment thereof, an anti-CD70 antibody or an antigen-binding fragment thereof, or a PD-L1 antibody or an antigen-binding fragment thereof.

13. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 12, wherein the anti-CD20 antibody is selected from Rituximab, Ibritumomab, Tositumomab, Ofatumumab, Ocrelizumab, Obinutuzumab, Ripertamab, Ublituximab or Zuberitamab; the anti-CD70 antibody is Vorsetuzumab; the PD-L1 antibody is selected from Adebrelimab, Sugemalimab, Envafolimab, Durvalumab, Avelumab or Atezolizumab.

14. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 12, wherein the Ab is an anti-CD38 antibody or an antigen-binding fragment thereof.

15. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 14, wherein the Ab is an anti-CD38 antibody or an antigen-binding fragment thereof comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the heavy chain variable region comprises three heavy chain complementarity determining regions (HCDRs) in SEQ ID NO: 7 or 9, and the light chain variable region comprises three light chain complementarity determining regions (LCDRs) in SEQ ID NO: 8 or 10; or

the Ab is an anti-CD38 antibody or an antigen-binding fragment thereof comprising a heavy chain variable region (VH) and a light chain variable region (VL); the VH

comprises HCDR1, HCDR2 and HCDR3, and the VL comprises LCDR1, LCDR2 and LCDR3, wherein

the HCDR1 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 2, and the HCDR3 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 3; the LCDR1 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 6; or

the HCDR1 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 17, the HCDR2 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 18, and the HCDR3 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 19; the LCDR1 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 20, the LCDR2 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 21, and the LCDR3 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 22.

16. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 14 or 15, wherein the VH comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 7 or 9, and/or the VL comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 8 or 10;

or the VH comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 15, and/or the VL comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 16;

preferably, the VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 7, and/or the VL comprises or consists of an amino acid sequence as set

forth in SEQ ID NO: 8; or the VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 9, and/or the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 10; or

the VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 15, and/or the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 16.

17. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 14 to 16, wherein the anti-CD38 antibody or the antigen-binding fragment thereof is of IgG subtype, such as IgG1, IgG2, IgG3 or IgG4 subtype.

18. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 14 to 17, wherein the anti-CD38 antibody or the antigen-binding fragment thereof further comprises a heavy chain constant region, such as a heavy chain constant region of IgG1, IgG2, IgG3 or IgG4, such as a heavy chain constant region of human IgG1, IgG2, IgG3 or IgG4; preferably,

the heavy chain constant region comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 13 or SEQ ID NO: 23; and/or

the anti-CD38 antibody or the antigen-binding fragment thereof further comprises a light chain constant region, such as a Kappa light chain constant region or a Lambda light chain constant region, such as a human Kappa light chain constant region or a human Lambda light chain constant region; preferably,

the light chain constant region comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 14; preferably,

the anti-CD38 antibody or the antigen-binding fragment thereof further comprises a heavy chain constant region and a light chain constant region, wherein the heavy chain constant region comprises or consists of an amino acid sequence as set forth in SEQ ID

NO: 13 or SEQ ID NO: 23, and the light chain constant region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 14.

19. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 14 to 18, wherein the anti-CD38 antibody or the antigen-binding fragment thereof comprises a heavy chain and a light chain,

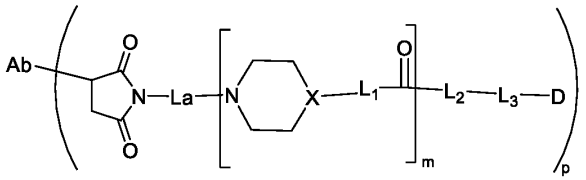
wherein the heavy chain comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 11; and/or the light chain comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 12;

optionally, the heavy chain comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 11, and the light chain comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 12; optionally, the Ab is Daratumumab.

20. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 14 to 19, wherein the anti-CD38 antibody or the antigen-binding fragment thereof is a murine antibody, a chimeric antibody, or a humanized antibody; and/or

the anti-CD38 antibody or the antigen-binding fragment thereof is a full-length antibody, a disulfide-stabilized Fv fragment (dSFv), a single-domain antibody such as VHH, a Fab antibody (such as a Fab' antibody and Fab'-SH), a $(Fab')_2$ antibody, a single-chain antibody such as scFv, Fv and dAb (domain antibody) or a multispecific antibody such as a bispecific antibody.

21. A drug conjugate represented by formula (III-2),

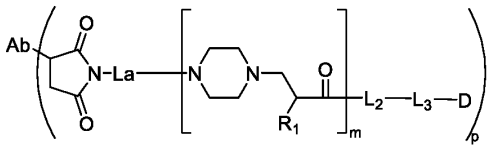


Formula (III-2)

,

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein X is N or CH, and Ab, L_a, L_1, L_2, L_3, D, m and p are as defined in formula (III) of any one of claims 1 to 20.

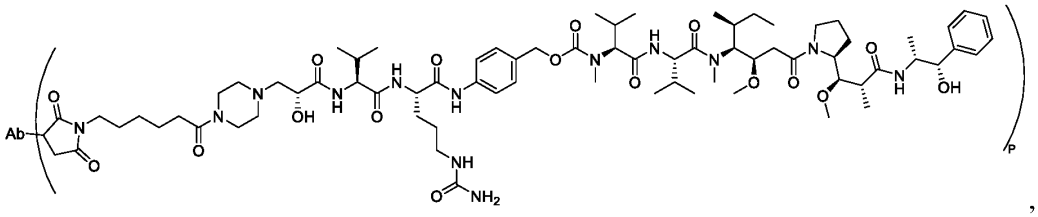
22. A drug conjugate represented by formula (III-3),



(III-3)

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein Ab, L_a, R_1, L_2, L_3, D, m and p are as defined in formula (III) of any one of claims 1 to 20.

23. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 22, wherein the drug conjugate is selected from:



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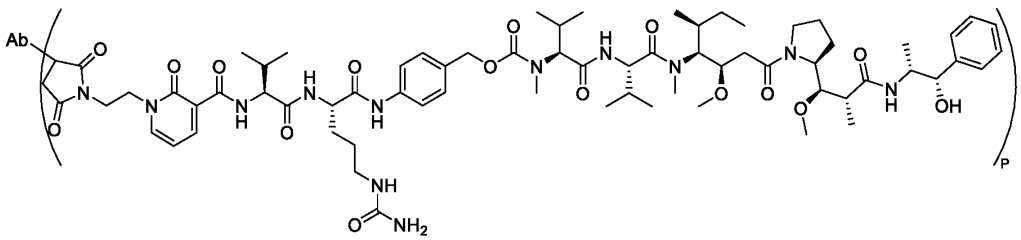

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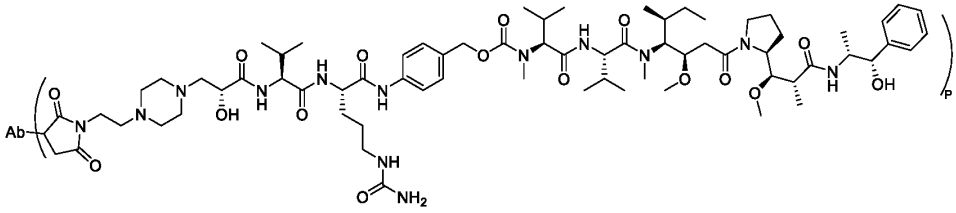

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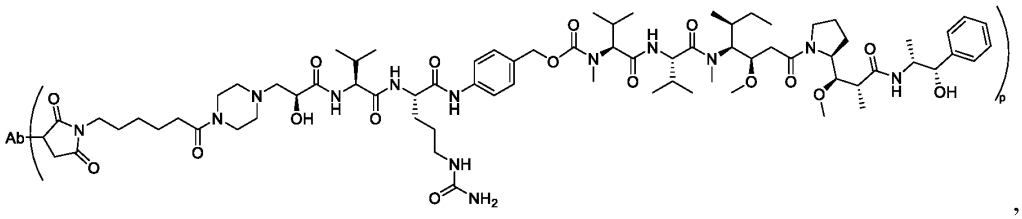

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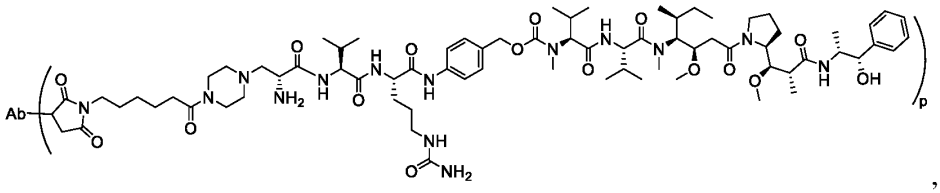

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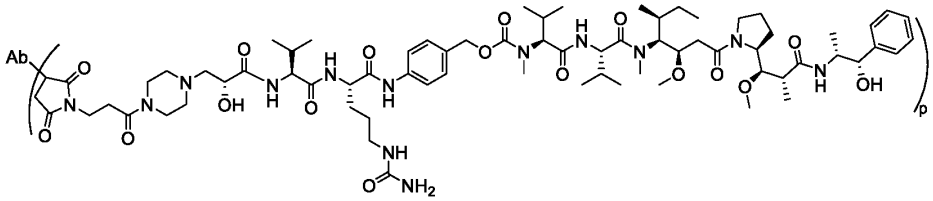

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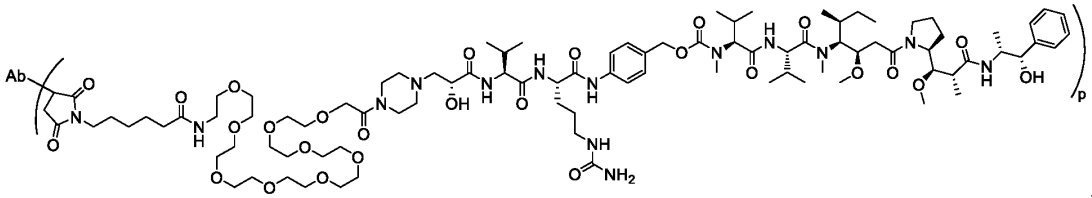

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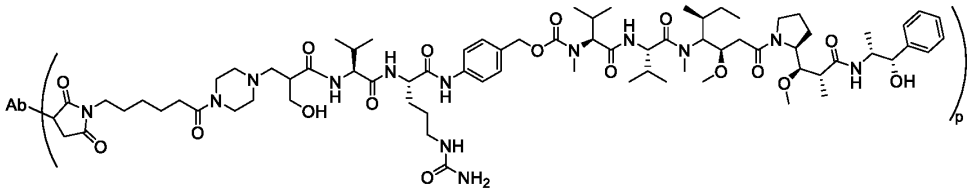

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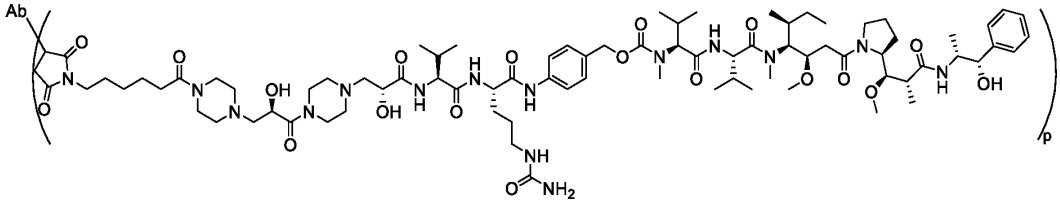

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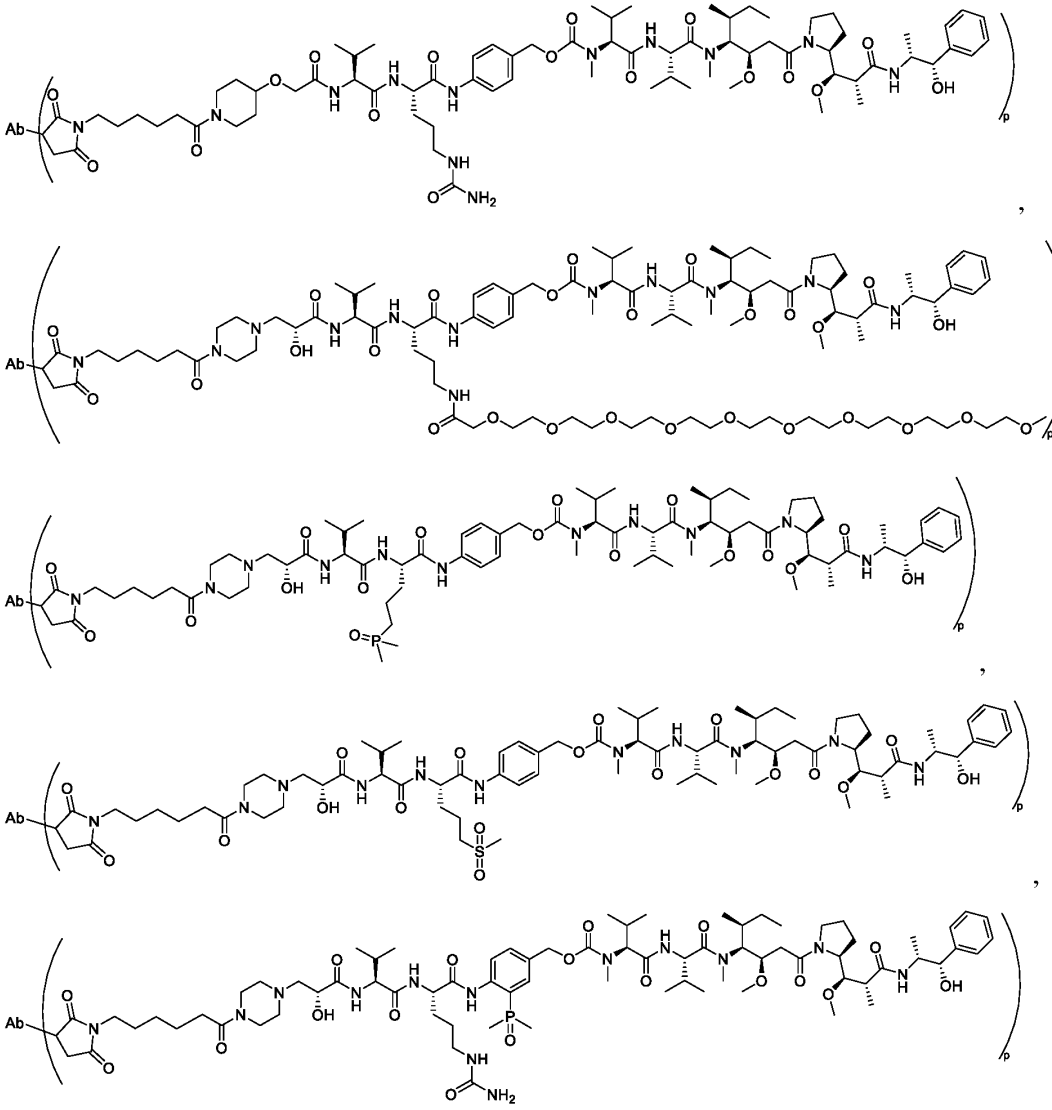
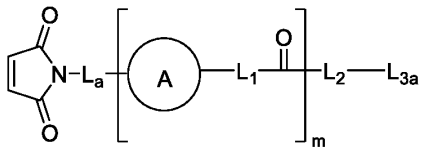


wherein Ab and p are as defined in any one of claims 1 to 22.

24. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 23, wherein the Ab is Daratumumab, and p is 2, 3, 4, 5, 6, 7 or 8, or wherein the Ab is Ab1 or huAb1; p is 2, 3, 4, 5, 6, 7 or 8.

25. The drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 24, having an average DAR of 2-8, such as an average DAR of 3-5 or 6-8.

26. A compound represented by formula (I):



(I)

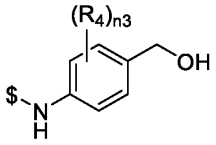
,

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein

L_a, ring A, L_1, L_2 and m are as defined in any one of claims 1 to 23;

L_{3a} is a spacer unit.

27. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 26, wherein L_{3a} has a structure represented by formula (I-5):

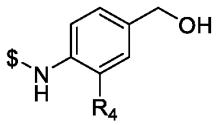


Formula (I-5)

,

wherein R_4 is selected from $-(CH_2)_{n2}SO_2CH_3$, $-(CH_2)_{n2}P(O)(CH_3)_2$ or hydrogen, wherein n2 is 0, 1, 2, 3, 4, 5, 6, 7 or 8; n3 is 1 or 2; $ is an active site connected to L_2;

preferably, L_{3a} has a structure represented by formula (I-6)

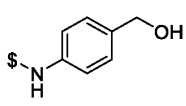


Formula (I-6)

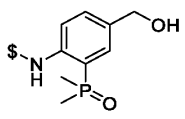
,

wherein each symbol is as defined in formula (I-5).

28. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 27, wherein L_{3a} has a structure selected from:



and



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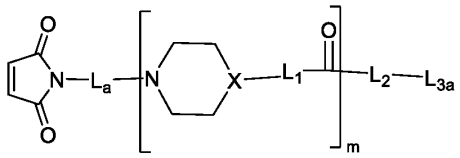
wherein $ represents a connection site to L_2.

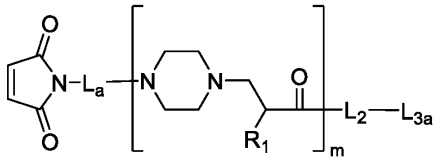
29. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 26 to 28, wherein the compound is a compound represented by formula (I-2):



Formula (I-2)

wherein X is N or CH; L_a, L_1, L_2, L_{3a} and m are as defined in any one of claims 26 to 28.

30. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 26 to 29, wherein the compound is a compound represented by formula (I-3):

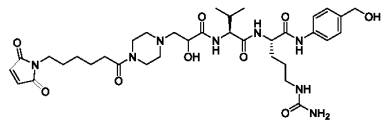


(I-3)

wherein R_1 is as defined in claim 4, and the remaining variables are as defined in any one of claims 26 to 29;preferably,

R_1 is -OH, $-NH_2$, $-CH_2$-OH or $-CH_2-NH_2$.

31. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 26, wherein the compound is selected from: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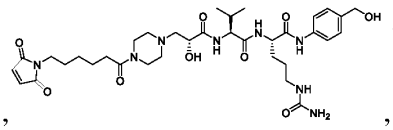
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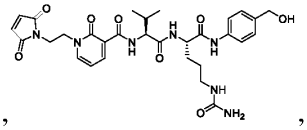
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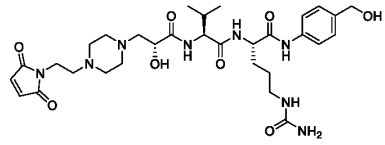
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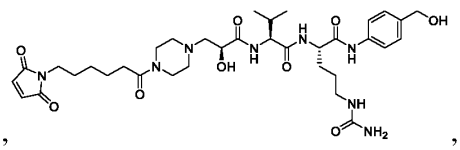
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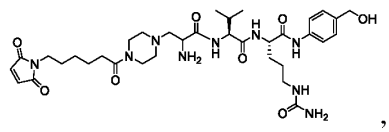
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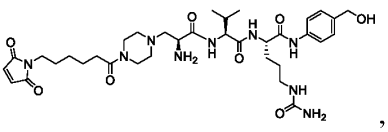
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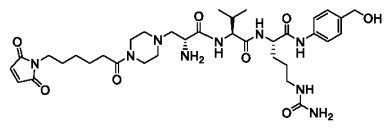
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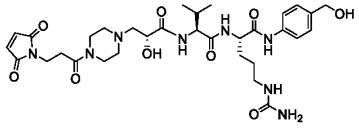
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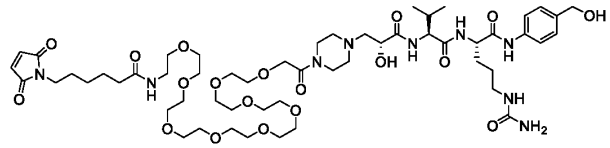
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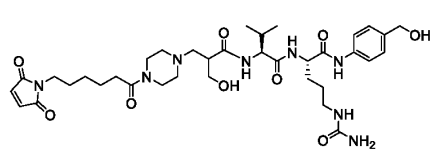
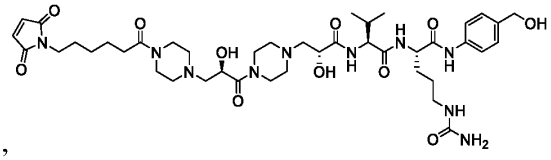
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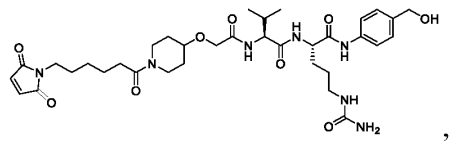
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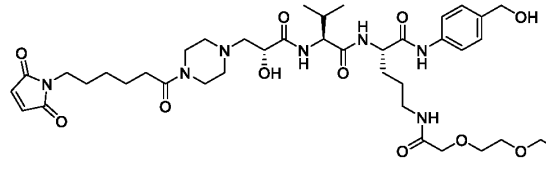
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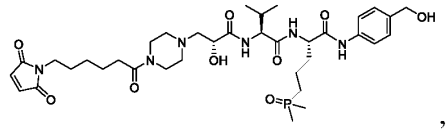
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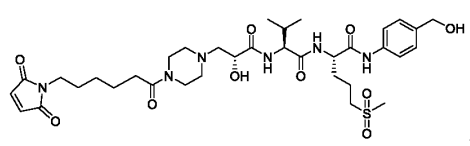
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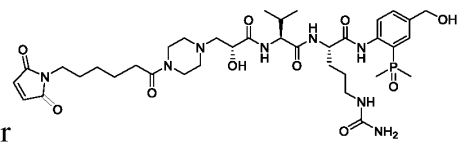
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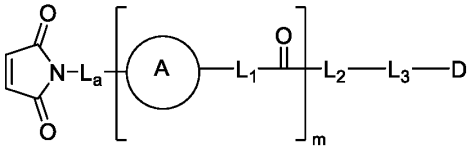
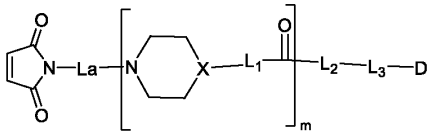
32. A compound represented by formula (II):



(II)

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof, wherein D, L_a, ring A, L_1, L_2, L_3 and m are as defined in any one of claims 1 to 24.

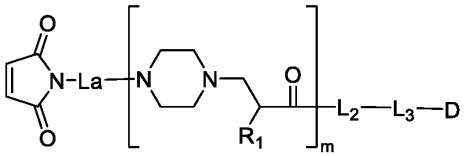
33. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 32, wherein the compound is a compound represented by formula (II-2):



Formula (II-2)

wherein each variable is as defined in claim 21;

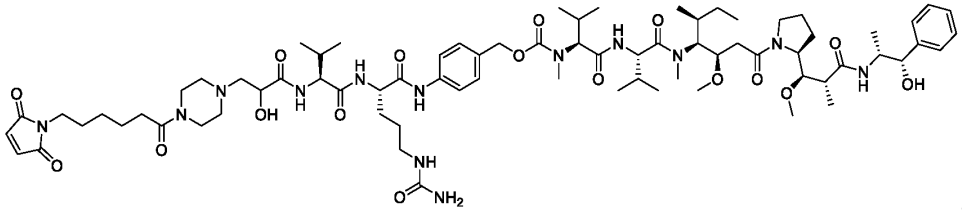
preferably, the compound represented by formula (II-3):



(II-3)

wherein each variable is as defined in claim 22.

34. The compound or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to claim 32, wherein the compound is selected from: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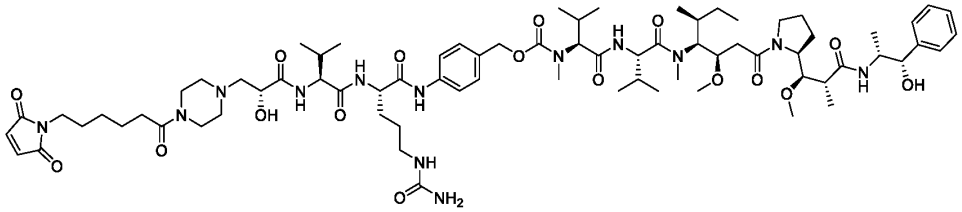
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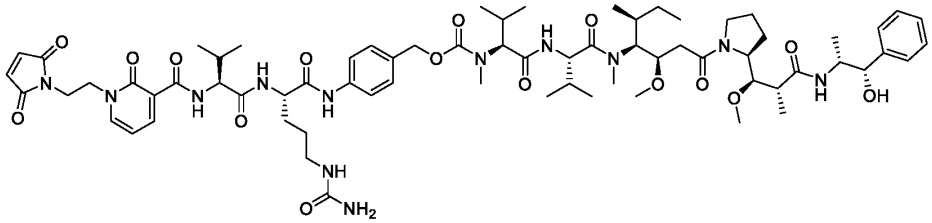
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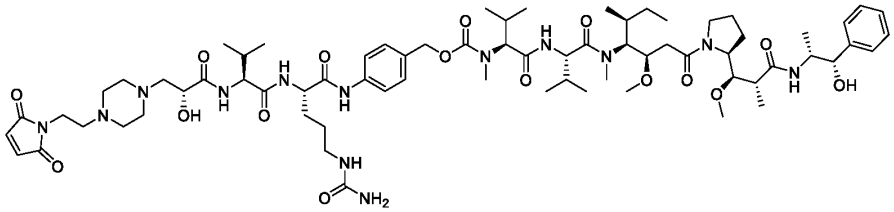
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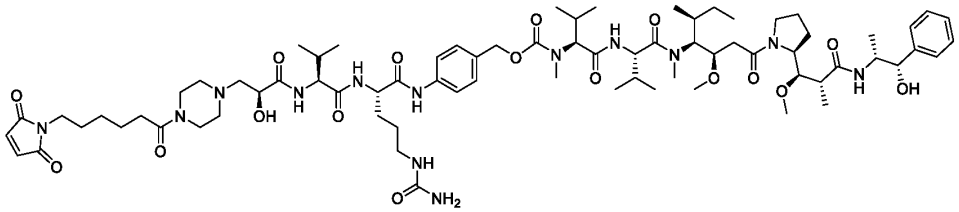
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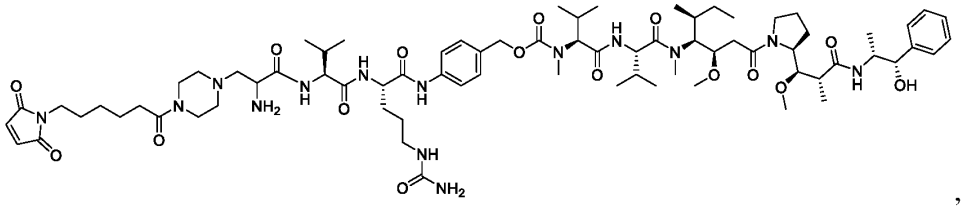
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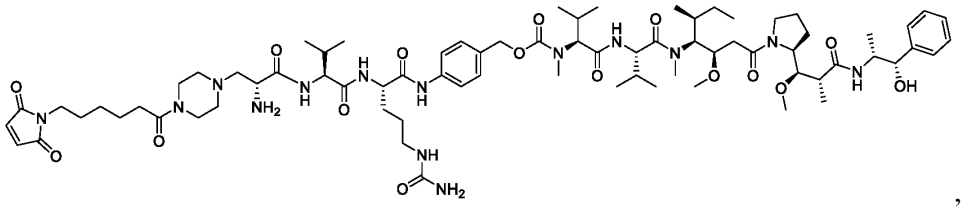
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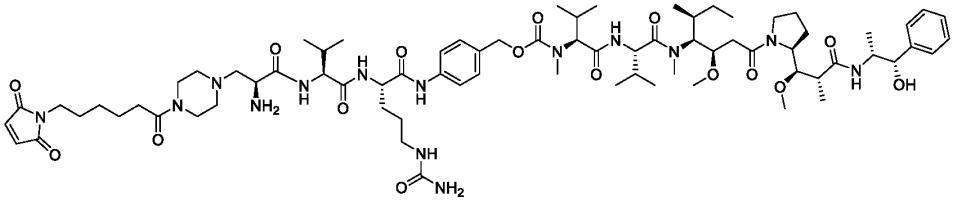

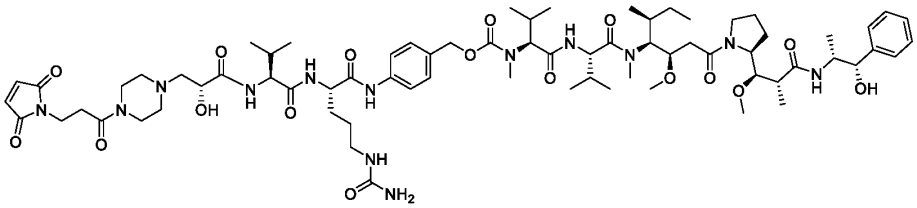

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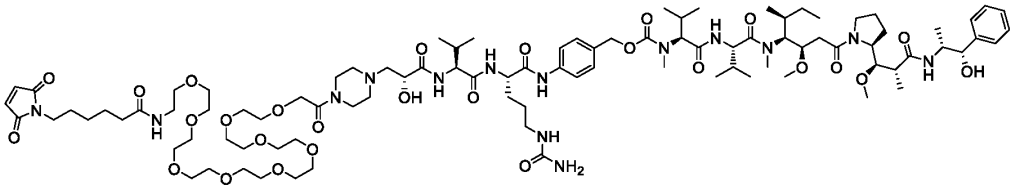

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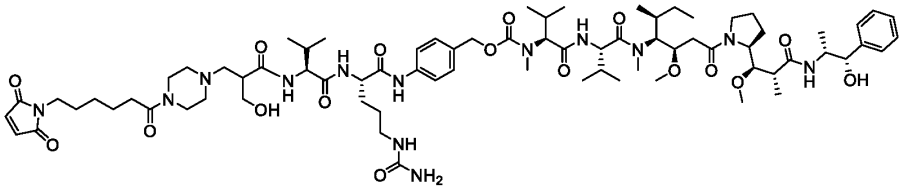

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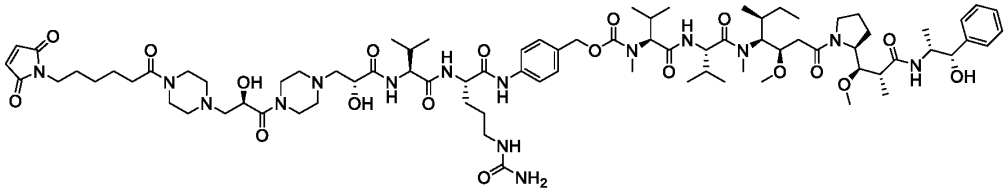

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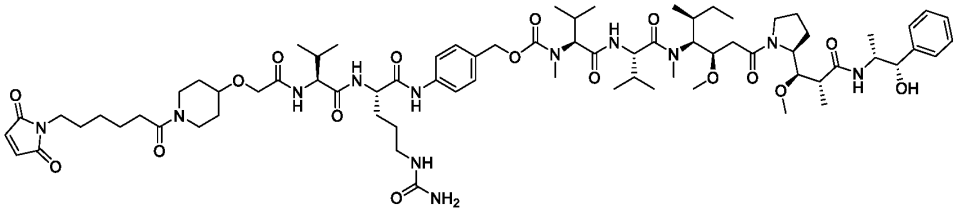

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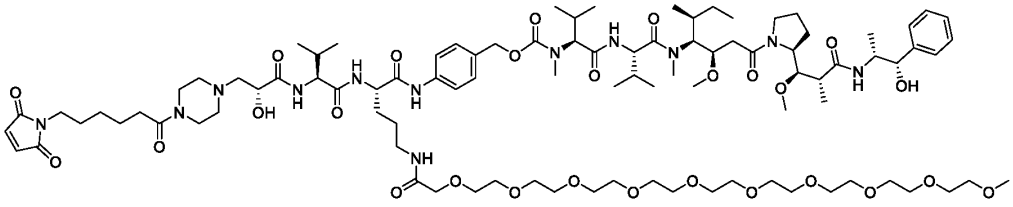

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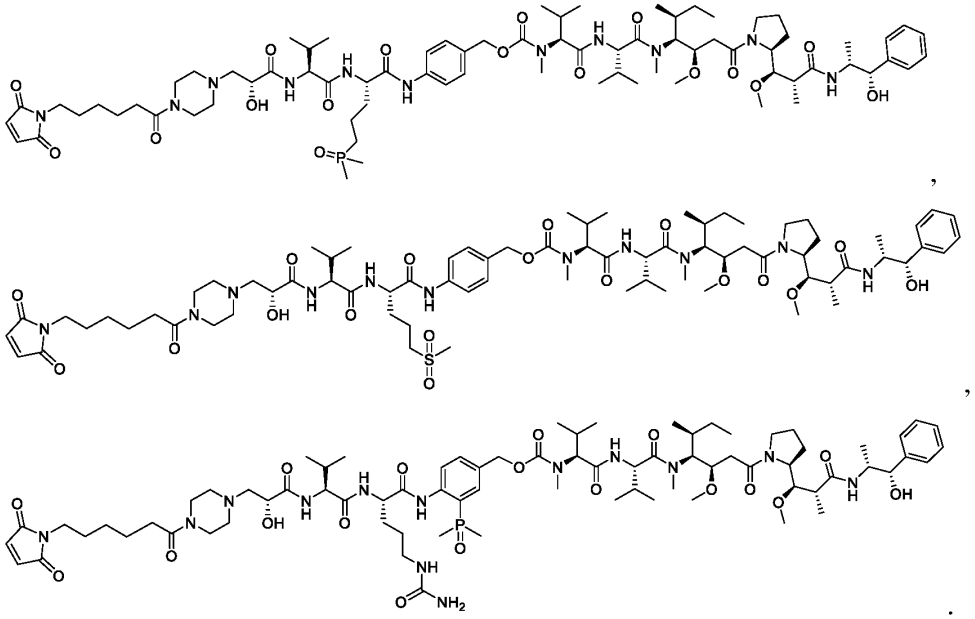


35. An isolated anti-CD38 antibody or an antigen-binding fragment thereof, wherein the anti-CD38 antibody or the antigen-binding fragment thereof comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the heavy chain variable region comprises three heavy chain complementarity determining regions (HCDRs) in SEQ ID NO: 7 or 9, and the light chain variable region comprises three light chain complementarity determining regions (LCDRs) in SEQ ID NO: 8 or 10;

optionally, the anti-CD38 antibody or the antigen-binding fragment comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises HCDR1, HCDR2 and HCDR3, and the VL comprises LCDR1, LCDR2 and LCDR3, wherein

the HCDR1 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 1, the HCDR2 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 2, and the HCDR3 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 3; the LCDR1 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 4, the LCDR2 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 5, and the LCDR3 comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 6;

optionally, the anti-CD38 antibody or the antigen-binding fragment comprising a VH and VL,

wherein the VH comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 7 or 9, and/or the VL comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 8 or 10;

optionally, the anti-CD38 antibody or the antigen-binding fragment comprising a VH and VL,

wherein the VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 7, and/or the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 8; or the VH comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 9, and/or the VL comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 10;

optionally, the anti-CD38 antibody or the antigen-binding fragment thereof is of IgG subtype, such as IgG1, IgG2, IgG3 or IgG4 subtype;

optionally, the anti-CD38 antibody or the antigen-binding fragment thereof further comprises a heavy chain constant region, such as a heavy chain constant region of IgG1, IgG2, IgG3 or IgG4, for example, a heavy chain constant region of human IgG1, IgG2, IgG3 or IgG4;

preferably, the heavy chain constant region comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 13 or SEQ ID NO: 23; optionally,

the anti-CD38 antibody or the antigen-binding fragment thereof further comprises a light chain constant region, such as a Kappa light chain constant region or a Lambda light chain constant region, such as a human Kappa light chain constant region or a human Lambda light chain constant region; preferably,

the light chain constant region comprises or consists of an amino acid sequence identical to or having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence as set forth in SEQ ID NO: 14; optionally,

the anti-CD38 antibody or the antigen-binding fragment thereof further comprises a heavy chain constant region and a light chain constant region, wherein the heavy chain

constant region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 13 or SEQ ID NO: 23, and the light chain constant region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 14; optionally,

the anti-CD38 antibody or the antigen-binding fragment thereof comprises a heavy chain and a light chain, wherein the heavy chain comprises or consists of a heavy chain variable region and a heavy chain constant region; and/or the light chain comprises or consists of a light chain variable region and a light chain constant region; optionally,

the anti-CD38 antibody or the antigen-binding fragment thereof is a murine antibody, a chimeric antibody or a humanized antibody; optionally,

the anti-CD38 antibody or the antigen-binding fragment thereof is a full-length antibody, a disulfide-stabilized Fv fragment (dSFv), a single-domain antibody such as VHH, a Fab antibody (such as a Fab' antibody and Fab'-SH), a $(Fab')_2$ antibody, a single-chain antibody such as scFv, Fv and dAb (domain antibody) or a multispecific antibody such as a bispecific antibody.

36. An isolated nucleic acid, wherein the isolated nucleic acid encodes the anti-CD38 antibody or the antigen-binding fragment thereof according to claim 35; further, the nucleic acid encodes a heavy chain and/or a light chain of the anti-CD38 antibody or the antigen-binding fragment thereof according to claim 35; further, the nucleic acid encodes a heavy chain variable region and/or a light chain variable region of the anti-CD38 antibody or the antigen-binding fragment thereof according to claim 35.

37. A recombinant vector or expression vector, wherein the recombinant vector or expression vector comprises one or more nucleic acids according to claim 36; further, the vector is suitable for recombinant production of the anti-CD38 antibody or the antigen-binding fragment thereof according to claim 35, and for example, the vector is a pcDNA vector, such as pCDNA3.1 and pCDNA3.4.

38. A host cell comprising one or more nucleic acids according to claim 36 or the recombinant vector or expression vector according to claim 37, wherein the host cell is, for example, a CHO cell or a 293 cell, such as Expi293.

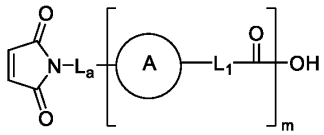
39. A method of producing the anti-CD38 antibody or the antigen-binding fragment thereof according to claim 35, wherein the method comprises

culturing a host cell containing the encoding for a polypeptide chain of the antibody under the condition suitable for expression of the polypeptide chain, and optionally, the method further comprises assembling the polypeptide chain to produce a molecule under the condition suitable for assembly of the polypeptide chain into the molecule; or

culturing a host cell comprising the nucleic acid molecule according to claim 36 or the recombinant vector or expression vector according to claim 37 or culturing the host cell according to claim 38 under the condition suitable for expression of a chain of the antibody, and optionally recovering the antibody from the host cell (or a culture medium for the host cell).

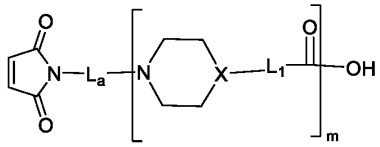
40. An immunoconjugate or immune fusion, wherein the immunoconjugate or immune fusion comprises the anti-CD38 antibody or the antigen-binding fragment thereof according to claim 35; further, the immunoconjugate or immune fusion comprises other active molecules, such as the Dolastatin 10 and Dolastatin 10 derivative as defined in claim 10.

41. A compound of formula (IV-1), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof:



Formula (IV-1) ;

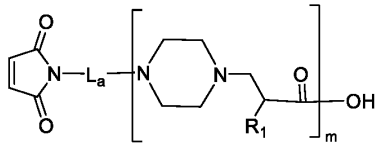
wherein L_a, ring A, L_1 and m are as defined in any one of claims 1 to 5; preferably, the compound is a compound represented by formula (IV-2):



Formula (IV-2)

wherein X is N or CH, and L_a, L_1 and m are as defined in any one of claims 1 to 5; preferably,

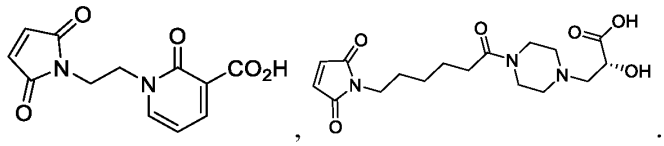
the compound is a compound represented by formula (IV-3):



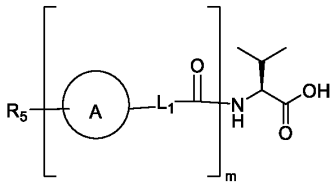
Formula (IV-3)

wherein L_a, R_1, L_1 and m are as defined in claim 22; preferably,

the compound is selected from:

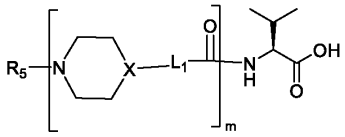


42. A compound represented by formula (V-1), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof:



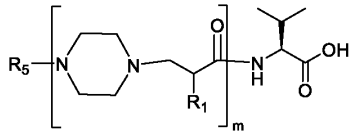
Formula (V-1)

wherein R_5 is an amino protecting group; and ring A, L_1 and m are as defined in any one of claims 1 to 5; or a compound represented by formula (V-2), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof,



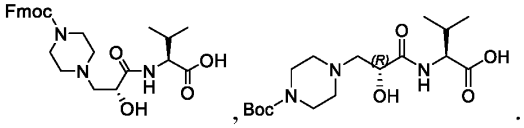
Formula (V-2)

wherein R_5 is an amino protecting group; and X, L_1 and m are as defined in claim 41; or a compound represented by formula (V-3), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof,



Formula (V-3)

wherein R_5 is an amino protecting group; and the remaining variables are as defined in claim 41; preferably, the compound is selected from:



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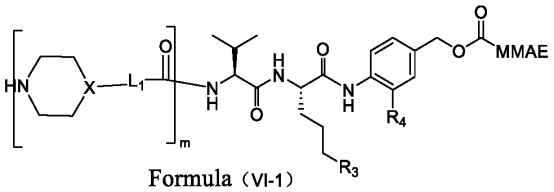
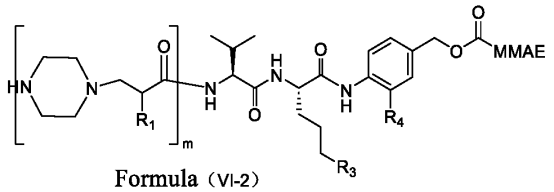
43. A compound represented by formula (VI-1), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof,



Formula (VI-1)

,

wherein X is N or CH, and L_1, R_3, R_4 and m are as defined in claim 32; or a compound represented by formula (VI-2), or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof:

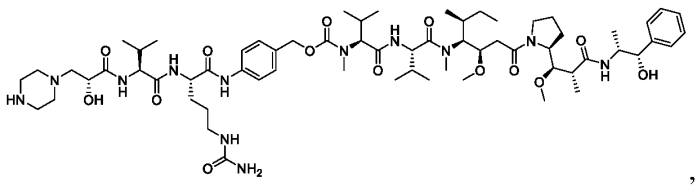


Formula (VI-2)

,

wherein R_1 is as defined in claim 30 or 31, and R_3, R_4 and m are as defined in claim 32.

44. The compound according to claim 43, selected from:



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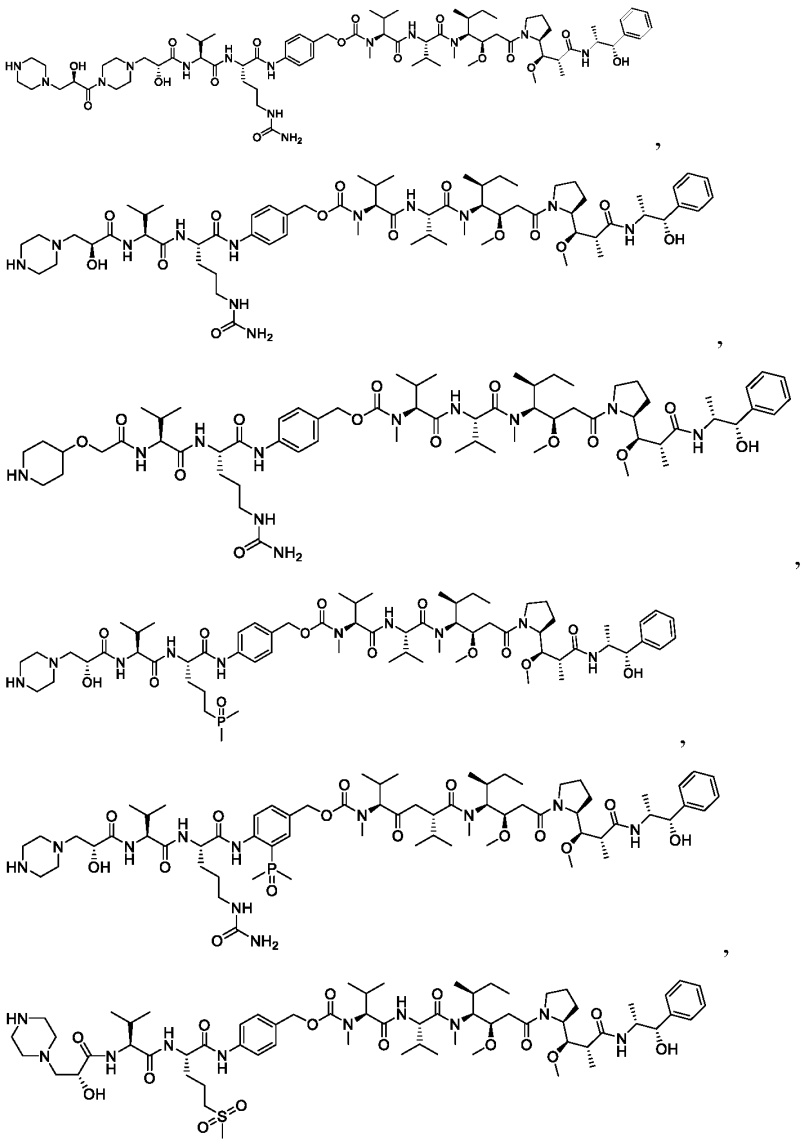

or a pharmaceutically acceptable salt thereof, or a solvate, a racemic mixture, an enantiomer, a diastereomer or a tautomer thereof.

45. A pharmaceutical composition comprising the drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 25, the drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 32 to 34, the anti-CD38 antibody or the antigen-binding fragment thereof according to claim 35, the nucleic acid according to claim 36, the recombinant

vector or expression vector according to claim 37, the host cell according to claim 38, or the immunoconjugate or immune fusion according to claim 40, and optionally a pharmaceutically acceptable adjuvant material such as a pharmaceutical carrier or a pharmaceutical excipient.

46. A pharmaceutical combination comprising the drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 25, the drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 32 to 34, the anti-CD38 antibody or the antigen-binding fragment thereof according to claim 35, the nucleic acid according to claim 36, the recombinant vector or expression vector according to claim 37, the host cell according to claim 38, or the immunoconjugate or immune fusion according to claim 40, and one or more additional therapeutic agents.

47. Use of the drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 1 to 25, the drug conjugate or the pharmaceutically acceptable salt thereof, or the solvate, the racemic mixture, the enantiomer, the diastereomer or the tautomer thereof according to any one of claims 32 to 34, the anti-CD38 antibody or the antigen-binding fragment thereof according to claims 35, the nucleic acid according to claim 36, the recombinant vector or expression vector according to claim 37, the host cell according to claim 38, the immunoconjugate or immune fusion according to claim 40, the pharmaceutical composition according to claim 45, or the pharmaceutical combination according to claim 46 in the preparation of a medicament for treating tumor, such as cancer.

48. The use according to claim 47, wherein the tumor is selected from hematopoietic and lymphoid tissue tumors, such as non-Hodgkin's lymphoma, myeloma and leukemia; preferably, the tumor is selected from B-cell lymphoma, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma, Burkitt's lymphoma, multiple myeloma

(MM), chronic myeloid leukemia, chronic lymphocytic leukemia and acute lymphocytic leukemia.