

ULTRAFILTRATION / DIAFILTRATION PURIFICATION METHODS

BACKGROUND

[0001] Antibody drug conjugates (ADCs) have emerged as a new class of biotherapeutics in areas such as oncology, infectious diseases, and immunological indications. ADCs generally comprise an antigen-targeting monoclonal antibody (mAb) and at least one payload (e.g., a drug or other active agent) that are covalently attached via chemical linkages (i.e., linkers). Most ADCs rely on chemical methods to attach the linkers and payloads (e.g., a drug or other active agent) to residues (such as lysine groups or cysteine groups) of the antibody. The chemical processes involved in ADC production generate a mixture of reagents, solvents, reaction byproducts, side products, and other small molecule impurities that are not typically associated with traditional mAb production. It is known that some impurities such as residual free drugs, residual drug-linkers and residual linkers may be especially difficult to efficiently remove from ADCs using various techniques, such as conventional Ultrafiltration / diafiltration (UF/DF) processes.

[0002] UF/DF is a commonly used method for performing purification and buffer exchange on biopharmaceutical products. As illustrated in FIGs. 1 and 2, the UF/DF process often involves continuously filtering a crude product contained within an aqueous buffer through a membrane, while simultaneously adding an exchange medium, in a manner such that the desired product is retained in the retentate chamber by the membrane while smaller compounds (such as unwanted solvents, reaction intermediates, residual reagents, byproducts, side products, and/or other impurities) are removed within the filtrate.

[0003] Although UF/DF has been shown to be effective in removing smaller compounds or impurities from certain classes of biopharmaceutical products, in some instances UF/DF is not particularly effective in removing certain impurities. For example, as indicated above, some residual free drugs, residual drug-linkers, residual linker, byproducts and/or side products associated with the synthesis of ADCs and other conjugates can be difficult to remove using conventional UF/DF techniques. It is theorized that conventional UF/DF is not effective in removing certain impurities due to non-covalent bond formation and/or other non-covalent interactions that can form between impurities and the desired product—thus preventing these impurities from being filtered away from the desired product. This technical challenge can be especially problematic when UF/DF is used to purify some antibody drug conjugates (ADCs), e.g., camptothecin-based ADCs and ADCs of camptothecin-derivatives, such as exatecan-based ADCs

[0004] There is a need to discover new separation methods for removing small molecule impurities (such as residual free drugs, residual drug-linkers, residual linkers and/or byproducts associated with synthesis of conjugate compounds) from antibody drug conjugates and other conjugates. Surprisingly, the UF/DF methods of the present disclosure address this need.

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SUMMARY

[0005] Provided herein are purification methods that are highly effective in removing organic impurities (e.g., residual free drug compounds, residual drug-linker compounds, residual linker compounds, reaction byproducts, residual side products, and/or combinations thereof), including product-bound impurities that are difficult to remove using conventional aqueous UF/DF processes.

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[0006] In one embodiment, the present invention provides a purification method comprising:

(a) subjecting a sample comprising at least one conjugate compound and at least one impurity to an organic ultrafiltration/diafiltration (UF/DF) process such that the sample is filtered through a semi-permeable membrane having a pore size that allows the at least one impurity to pass through the semi-permeable membrane while retaining the at least one conjugate compound as a retentate, wherein the organic UF/DF process uses a first exchange medium and gives rise to a retentate comprising the at least one conjugate compound; and

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(b) subjecting the retentate product of (a) to an aqueous UF/DF process such that it is filtered through a semi-permeable membrane having a pore size that allows the at least one impurity to pass through the semi-permeable membrane while retaining the conjugate compound in a retentate, wherein the aqueous UF/DF process uses a second exchange medium and results in a retentate comprising the at least one conjugate compound,

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wherein:

the at least one impurity comprises a residual free drug compound, a residual drug-linker compound, a residual linker compound, a residual byproduct compound, a residual side product compound, or any combination thereof;

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the first exchange medium comprises at least one organic solvent and a first buffer system; and

the first exchange medium and the second exchange medium are different.

[0007] In some embodiments, provided are purification methods including the steps of: (a) combining a first exchange medium with a crude composition to obtain a crude mixture, wherein the crude composition comprises a conjugate compound and at least one impurity; (b) subjecting the crude mixture to an organic UF/DF process such that the crude mixture is filtered through a

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first semi-permeable membrane having a first pore size that allows the impurity to pass through the membrane in a first filtrate while retaining the conjugate compound in a first retentate, and liquid volume filtered from the crude mixture is replaced at least in part with additional first exchange medium in the first retentate, to obtain a separated composition; and (c) subjecting the separated composition to an aqueous ultrafiltration/ diafiltration process such that the separated composition is filtered through a second semi-permeable membrane having a second pore size that allows the impurity to pass through the membrane while retaining the conjugate compound in a second retentate, and liquid volume filtered from the separated composition is replaced at least in part with a second exchange medium comprising a second buffer system, to obtain a purified composition in the second retentate, wherein the first exchange medium comprises at least one organic solvent and a first buffer system, and wherein the first exchange medium and the second exchange medium are different, and wherein the impurity may include a residual free drug compound, a residual drug-linker compound, a residual linker compound, a residual byproduct compound, a residual side product compound, or any combination thereof.

5 [0008] In some embodiments, the conjugate compound comprises an antibody drug conjugate (ADC). In other embodiments, the conjugate compound comprises a conjugate other than an ADC. Hence, the invention may be also applied to a non-antibody drug conjugate.

10 [0009] It was surprisingly discovered that addition of at least one organic solvent to the first exchange medium enables the inventive methods to more effectively remove small-molecule impurities (e.g., residual free drug compounds, residual drug linker compounds, residual linker compounds, residual byproduct compounds, etc.) that cannot be efficiently removed using conventional UF/DF and/or TFF processes. Thus, a first exchange medium employed in the present invention will comprise at least one organic solvent. As illustrated in Examples 1-6, the inventive methods can be used to efficiently remove small molecule impurities, such as residual

20 drug compounds and drug-linker compounds, from ADCs and other conjugates.

25 [0010] In some embodiments, the separated composition is substantially free of small molecule impurities, such as for example product-bound impurities. In some embodiments, the purified composition is substantially free of both small molecule impurities (e.g., product-bound impurities) and the organic solvent. In some embodiments, the separated compositions resulting from the purification methods of the present invention lack the free linker form of a linker present in the ADC. In some embodiments, the purified drug conjugate will comprise either no impurities or only impurities at a level which is not significant for administration to a subject.

30 [0011] In some embodiments, provided are methods for preparing purified conjugate compounds using separately prepared drug-linker compounds, including the steps of: (i) reducing a targeting

agent by contacting the targeting agent with a reducing agent to obtain a crude reduced targeting agent; (ii) conjugating the reduced targeting agent with a drug-linker compound to obtain a crude composition comprising a conjugate compound; (iii) optionally adding a quenching agent to the crude composition; and (iv) purifying the crude composition using a UF/DF method of the present disclosure, wherein the first exchange medium comprises at least one organic solvent, to obtain a purified conjugate compound. In some embodiments, the targeting agent is an antibody. In some embodiments, the targeting agent comprises a non-antibody scaffold.

[0012] In some embodiments, provided are methods for preparing purified conjugate compounds using separately prepared drug-linker compounds, including the steps of:

- 10 (i) reducing a targeting agent by contacting the targeting agent with a reducing agent to obtain a reduced targeting agent;
- (ii) conjugating the reduced targeting agent with a drug-linker compound to obtain a crude composition comprising a conjugate compound comprising a conjugate; and
- (iii) purifying the crude composition using a UF/DF method of the present disclosure wherein

15 the first exchange medium comprises at least one organic solvent. In some embodiments, the targeting agent is an antibody. In some embodiments, the targeting agent comprises a non-antibody scaffold.

[0013] In some embodiments, provided are methods for preparing purified conjugate compounds by sequential attachment of linking group(s) and drug compound(s), including the

- 20 steps of: (i) reducing a targeting agent by contacting the targeting agent with a reducing agent to obtain a crude reduced targeting agent; (ii) attaching at least one linking group to the reduced targeting agent to obtain a crude composition comprising a targeting agent-linker intermediate;
- (iii) attaching at least one drug compound to the linker of the targeting agent-linker intermediate to obtain a crude composition comprising a conjugate compound; (iv) optionally adding a

25 quenching agent to the crude composition; and (v) purifying the crude composition using a UF/DF method of the present disclosure, wherein the first exchange medium comprises at least one organic solvent, to obtain a purified conjugate compound. In some embodiments, the targeting agent is an antibody. In some embodiments, the targeting agent comprises a non-antibody scaffold.

30 **[0014]** In some embodiments, the targeting agent is an antibody or an antigen binding fragment, and the purified conjugate compound is a purified antibody-drug conjugate (ADC). In other embodiments, the targeting agent comprises a non-antibody scaffold, and the purified conjugate compound is a purified non-ADC conjugate.

[0015] In some embodiments, any of the methods of the present invention may further comprise formulating the purified product obtained comprising the drug conjugate with a pharmaceutical carrier or excipient to give a pharmaceutical composition.

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FIGURES

[0016] Embodiments are illustrated by way of example and not by way of limitation in all of the accompanying figures, wherein:

[0017] FIG. 1 illustrates the basic process of a conventional ultrafiltration/diafiltration (UF/DF) using an exemplary apparatus;

10 [0018] FIG. 2 is a schematic diagram of one embodiment of a UF/DF apparatus that can be used to carry out a UF/DF process of the present disclosure;

[0019] FIG. 3 shows plots of Residual Free Drug versus diafiltration volume for the UF/DF studies 4.1-4.3 of Example 4; and

15 [0020] FIG. 4 is a flowchart summarizing one embodiment of a synthetic process for preparing ADC1 including a UF/DF method of the present disclosure.

DETAILED DESCRIPTIONS

[0021] Provided herein are purification methods that are highly effective in removing small-molecule impurities (e.g., residual free drug compounds, residual drug-linker compounds and/or residual linker compounds) that are often difficult to remove using conventional UF/DF or TFF processes. It was surprisingly discovered that addition of at least one organic solvent to the first exchange medium enables the inventive methods to effectively remove small-molecule impurities that cannot be efficiently removed using conventional UF/DF or TFF processes.

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Definitions

[0022] For convenience, certain terms in the specification, examples and claims are defined here.

25 Unless stated otherwise, or implicit from context, the following terms and phrases have the meanings provided below. The definitions below are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention, because the scope of the invention is limited only by the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art

30 to which this invention belongs.

[0023] As used herein and unless otherwise indicated, the terms "a" and "an" are taken to mean "one", "at least one" or "one or more". Unless otherwise required by context, singular terms used herein shall include pluralities and plural terms shall include the singular.

[0024] Other than in the examples, or where otherwise indicated, all numbers expressing quantities of ingredients or process conditions used herein should be understood as modified in all instances by the term "about." Ranges and amounts can be expressed as "about" a particular value or range. The term "about" also includes the exact amount. Hence, the phrase "about 30%" means "about 30%" and also means "30%." In some embodiments, "about" means within $\pm 10\%$ of the value (e.g., the phrase "about 30%" means 27-33%). In some embodiments, "about" means within $\pm 5\%$ of the value. In some embodiments, about means within 4% of the value. In some embodiments, about means within 3% of the value. In some embodiments, about means within 2% of the value. In some embodiments, about means within 1% of the value. Generally, the term "about" includes an amount that would be expected to be within experimental error.

[0025] As used herein, unless specifically indicated otherwise, the word "or" means "either/or," but is not limited to "either/or." Instead, "or" may also mean "and/or."

[0026] Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

[0027] As used herein, the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment. Where embodiments are set out herein using comprising language also provided are embodiments that consist essentially of what is set out.

[0028] As used herein, the term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment. Where embodiments are set out herein using comprising language also provided are embodiments that consist of what is set out.

[0029] The terms "decreased," "reduce," "reduced", "reduction", "decrease," and "inhibit" in the context of a numerical value are all used herein generally to mean a decrease by a statistically significant amount relative to a reference.

[0030] The term "plurality" means "two or more", unless expressly specified otherwise. For example, "plurality" may simply refer to a multiple number of injections carried out into esophageal tissue.

[0031] The terms "statistically significant" or "significantly" refer to statistical significance and generally mean a two-standard deviation (2SD) difference, above or below a reference value.

[0032] The term "substantially free" means that the referred-to component is not contained in the composition, or is contained in the composition with a proportion of less than 10% by weight or
5 by volume, or less than 5% by weight or by volume, or less than 3% by weight or by volume, or less than 2% by weight or by volume, or less than 1% by weight or by volume, or less than 0.5% by weight or by volume, relative to a total weight or volume of the composition.

[0033] As used herein, the term "targeting agent" refers to an antibody (including an antigen-binding fragment) or other non-antibody scaffold that specifically binds to a target molecule, such
10 as a protein, carbohydrate, glycoprotein or the like.

[0034] As used herein, the term "conjugate compound" refers to an ADC or other conjugate that comprises an antibody or non-antibody scaffold as the targeting agent that is covalently linked to at least one drug moiety by at least one linking group (linker).

[0035] As used herein, the term "conjugation reaction" refers to the chemical process of attaching
15 an antibody or other non-antibody protein scaffold to at least one linker and at least one drug to form a conjugate compound.

[0036] As used herein, the term "conjugation mixture" refers to a crude product mixture resulting from a conjugation reaction.

[0037] As used herein, the term "antibody drug conjugate" or "ADC" refers to a conjugate
20 compound comprising a targeting antibody that is covalently linked to at least one drug moiety by at least one linking group (linker).

[0038] As used herein, the term "non-ADC conjugate" or "conjugate other than ADC" refers to a conjugate compound comprising at least one non-antibody-containing targeting agent (i.e., targeting moiety) that is covalently linked to at least one payload moiety (such as a drug payload)
25 by at least one linking group (linker).

[0039] As used herein, the term "drug-linker compound" refers to a compound comprising a drug moiety and a linker moiety covalently attached to the drug moiety, wherein the drug-linker compound is capable of covalently linking to a protein (e.g., antibody or other binding agent) or other targeting agents. As used herein, the term "binding agent" refers to a protein that includes
30 an antibody or non-antibody scaffold which can covalently link to the drug-linker compound. The term antibody also includes antigen-binding antibody fragments.

[0040] As used herein, the term "drug moiety" refers to a drug, a prodrug or other active agent that is attached to the linker of a drug-linker compound or a conjugate compound.

[0041] As used herein, the term “protein-linker compound” refers to a compound comprising a protein moiety and a linker moiety covalently attached to the protein moiety, wherein the protein-linker compound is capable of covalently linking to a drug compound.

[0042] As used herein, the term “drug-antibody ratio” or “DAR” refers to the average number of drugs or drug moieties attached to an antibody or other targeting agent. For example, a DAR of 1:4 refers to an average of 4 drugs or drug moieties per antibody or other targeting agent.

[0043] As used herein, the term “small molecules impurities” include impurities from a conjugation reaction that remain present upon termination of the conjugation reaction, including residual free drug compounds, residual drug linker compounds, residual linker compounds, reaction byproducts, and/or side products.

[0044] As used herein, the term “product-bound impurities” refers to small-molecule impurities from a conjugation reaction that tend to associate with conjugate compounds due to non-covalent bonds and other non-covalent interactions or associations that can form between the impurities and the conjugates. Product-bound impurities may include residual free drug compounds, residual drug-linker compounds and/or residual linker compounds. Product-bound impurities may be protein-bound impurities that tend to associate with the protein-moieties of ADCs or other conjugates.

[0045] The term “organic solvent” as used herein refers to a non-reactive organic compound which contains carbon, is at least partially soluble in water, and facilitates the removal of small molecule impurities from a crude composition. The term “non-reactive” as used herein means that the organic solvent does not react with, denature or decompose an ADC or other conjugate compound at a temperature below 50°C when the concentration of the organic solvent is equal to or less than 30% (w/v) relative to a total volume of an exchange medium containing the organic solvent. In some embodiments, the organic solvent includes carbon and at least one atom selected from oxygen, nitrogen and sulfur. In some embodiments, the organic solvent is miscible in water. In some embodiments, the term “organic solvent” is used in accordance with its ordinary meaning in the field of chemistry. Suitable organic solvents may include, for example, alcohol compounds, polar aprotic compounds, and combinations thereof. In some embodiments, suitable organic solvents do not include sugar alcohols.

[0046] As used herein, the term “alcohol compounds” refers to non-reactive, carbon-containing organic compounds that contain at least one hydroxyl (–OH) group. Alcohol compounds may include monohydric alcohols (containing 1 OH group), dihydric alcohols (containing 2 OH groups) and polyhydric alcohols (containing 3 or more OH groups).

[0047] “Monohydric alcohols” may include, for example, aliphatic monohydric alcohols (e.g., paraffinic alcohols, olefinic alcohols) which may be straight chain, branched and/or cyclic; aromatic monohydric alcohols (e.g., phenolic alcohols, benzylic alcohols); heterocyclic monohydric alcohols having 1 to 20 carbon atoms (e.g., furfuryl alcohol); polycyclic monocyclic alcohols (e.g., sterols), and the like, or combinations thereof.

[0048] “Monohydric alcohols” may include, for example, methanol, ethanol, n-propyl alcohol, isopropyl alcohol, 1-butanol, 2-butanol, tert-butanol, iso-butanol, 1-pentanol, 2-pentanol, 3-pentanol, iso-pentanol, tert-pentanol, a hexanol (straight chain, branched and/or cyclic), ethylene glycol monomethyl ether, cis-3-hexen-1-ol, trans-2-hexen-1-ol, 5-hexen-1-ol, a phenol, a benzyl alcohol, and the like, or combinations thereof.

[0049] “Dihydric alcohols” may include, for example, 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,2-pentanediol, 1,5-pentanediol, 2,2-dimethylpropane-1,3-diol, 2-butyl-2-ethylpropane-1,3-diol, 2-methyl-2,4-pentane diol, 2-ethyl-1,3-hexane diol, 2-methyl-1,3-propane diol, 1,2-hexanediol, 1,5-hexanediol, 1,6-hexanediol, 1,8-octanediol, 1,10-decanediol, 2,2,4,4-tetramethylcyclobutane-1,3-diol, 1,3-cyclopentanediol, 1,2-cyclohexanediol, 1,3-cyclohexanediol, 1,4-cyclohexanediol, 1,2-cyclohexanedimethanol, 1,3-cyclohexanedimethanol, 1,4-cyclohexanedimethanol, 1,4-cyclohexanediethanol, isosorbide, a glycerol monoester, a glycerol monoether, a trimethylolpropane monoester, a trimethylolpropane monoether, a pentaerythritol diester, a pentaerythritol diether, dipropylene glycol, diethylene glycol, triethylene glycol, 2-methyl-1,3-propanediol, 2,2-dimethyl-1,3-propanediol, neopentyl glycol, xylylene glycol, bis(p-hydroxy)diphenyl, bis(p-hydroxy)diphenyl propane, 2,2'-bis[4-(2-hydroxyethoxy)phenyl]propane, bis[4-(2-hydroxyethoxy)phenyl]sulfone, 1,1-bis[4-(2-hydroxyethoxy)phenyl]cyclohexane, and the like, or combinations thereof.

[0050] “Polyhydric alcohols” may include, for example, alditol, 2-ethyl-2-hydroxymethyl-1,3-propanediol, 1,2,4-butanetriol, 1,2,5-pentanetriol, 1,2,6-hexanetriol, 1,2,3,6-hexanetetrol, glycerin (aka. Glycerol), diglycerin, triglycerin, tetraglycerin, pentaglycerin, hexaglycerin, triethanolamine, trimethylol ethane, trimethylol propane, ditrimethylol propane, tri-trimethylol propane, 2-methylpropanetriol, 2-methyl-1,2,4-butanetriol, pentaerythritol, dipentaerythritol, tripentaerythritol, methylglucoside, sorbitol, mannitol, sucrose, 1,3,5-trihydroxybenzene, 1,2,4-trihydroxybenzene, a (poly)oxyethylene glycerin, a (poly)oxypropylene glycerin, a (poly)oxyethylene diglycerin, a (poly)oxypropylene diglycerin, a (poly)oxyethylene trimethylolpropane, a (poly)oxypropylene trimethylolpropane, a (poly)oxyethylene ditrimethylolpropane, a (poly)oxypropylene ditrimethylolpropane, erythritol, inositol, threitol, arabitol, xylitol, ribitol, galactitol, mannitol, sorbitol, a (poly)oxyethylene pentaerythritol, a (poly)oxypropylene pentaerythritol, a

(poly)oxyethylene dipentaerythritol, a (poly)oxypropylene dipentaerythritol, and the like, and combinations thereof.

[0051] “Sugar alcohols” are typically derived from carbohydrates and include at least one hydroxyl group (–OH), or hydroxyl-derived ether group (–O–), attached to each carbon atom.

5 Sugar alcohols may include, for example, arabinitol, erythritol, fructose, galactitol, glucose, iditol, isomalt, lactitol, lactose, mannitol, maltitol, maltose, maltotriose, myoinisitol, perseitol, ribitol, sorbitol, sucrose, threitol, trehalose, volemitol, xylitol, xylitose, and the like, and combinations thereof.

[0052] The term “polar aprotic compound” refers to an organic compound having a high dielectric constant and high dipole moment and that lacks an acidic hydrogen. Polar aprotic compounds may include, for example, acetone (ACE), acetonitrile (I), 4-acetyl morpholine, N-cyclohexyl-2-pyrrolidone (CHP), 1,2-dimethoxyether (DME), N,N-dimethylacetamide (DMAc), N,N-diethylacetamide, dimethylformamide (DMF), diethylformamide, N,N-dimethylpropionamide, 3-methoxy-N,N-dimethylpropionamide, 3-methoxy-N,N-diethylpropionamide, 3-methoxy-N,N-methylethylpropionamide, 3-ethoxy-N,N-dimethylpropionamide, 1,3-dimethyl-2 imidazolidinone (DMI), dimethyl sulfoxide (DMSO), 1,4-dioxane, 1,3-dioxolane (DN), N,N'-dimethylpropyleneurea (DMPU), hexamethylphosphoramide (HMPA), N-methyl-2-pyrrolidone (NMP), N-ethyl-2-pyrrolidone, N-methyl-ε-caprolactam, 2-methyltetrahydrofuran (Me-THF), 2,5-dimethyl tetrahydrofuran, 4-propionyl morpholine, sulfolane, tetrahydrofuran (THF), tris(N,N-tetra
15 methylene)phosphoric acid triamide, alcohol ethoxylate, diethyl ester dimethyl ammonium chloride, linear alkylbenzene sulfonate, and the like, or combinations thereof.
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[0053] As used herein, the term “miscible” refers to the property wherein two substances (i.e., liquids) form a homogeneous mixture when mixed together at room temperature. For example, an organic solvent is miscible with water if it forms a homogeneous mixture with water (with no
25 phase separation) at room temperature.

[0054] As used herein, the term “partially soluble” means that at least 1% by mass of a solute dissolves in a solvent, or that two substances form a homogeneous mixture (with no phase separation) only if the mixture is heated to at least 30°C.

[0055] As used herein, the term “polarity index” refers to the Burdick & Jackson polarity index (see, e.g., Przybitek (1980) “High Purity Solvent Guide,” Burdick and Jackson Laboratories, Inc.). The polarity indices of a variety of solvents are found in “High Purity Solvent Guide,” Burdick and Jackson Laboratories, Inc., distributed by American Scientific Products.

[0056] In the present disclosure, a “logP” value means that the value is determined by the partitioning of the small-molecule impurities between a first exchange medium and a first buffer

system at 25°C with reference to “logP” value as described in Dearden et al. (Molecular Informatics, Vol 7(3), page 133-134, 1988). “P” stands for partition coefficient.

[0057] As used herein, the term “residual free drug compound” refers to a drug compound (e.g., payload) that remains in a conjugation reaction used to form an ADC or other conjugate.

5 **[0058]** As used herein, the term “residual drug-linker compound” refers to a drug-linker compound that remains in a conjugation reaction used to form an ADC or other conjugate.

[0059] As used herein, the term “residual linker compound” refers to a linker compound that remains in conjugation reaction used to form an ADC or other conjugate.

10 **[0060]** As used herein, the term “residual reagent” refers to a reagent (such as a reducing agent and/or quenching agent) that remains in a crude ADC composition following a conjugation reaction used to form an ADC.

[0061] As used herein, the term “residual byproduct” refers to a product of a desired conjugation reaction (e.g., reaction used to form an ADC or other conjugate compound), but that is not the desired product (i.e., conjugate compound) of the desired reaction.

15 **[0062]** The term “residual side product” refers to a product that results from a side reaction, which is not the main or desired reaction (i.e., the conjugation reaction).

[0063] As used herein, the recitation of a numerical range for a variable is intended to convey that the disclosure may be practiced with the variable equal to any of the values within that range. Thus, for a variable which is inherently discrete, the variable can be equal to any integer value within the numerical range, including the end-points of the range. Similarly, for a variable which is inherently continuous, the variable can be equal to any real value within the numerical range, including the end-points of the range. As an example, and without limitation, a variable which is described as having values between 0 and 2 can take the values 0, 1 or 2 if the variable is inherently discrete, and can take the values 0.0, 0.1, 0.01, 0.001, or any other real values ≥ 0 and ≤ 2 if the variable is inherently continuous.

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Purification Methods

[0064] In one embodiment, the present invention provides a purification method comprising:

30 (a) subjecting a sample comprising at least one conjugate compound and at least one impurity to an organic ultrafiltration/diafiltration (UF/DF) process such that the sample is filtered through a semi-permeable membrane having a pore size that allows the at least one impurity to pass through the semi-permeable membrane while retaining the at least one

conjugate compound as a retentate, wherein the organic UF/DF process uses a first exchange medium and gives rise to a retentate comprising the at least one conjugate compound; and

(b) subjecting the retentate product of (a) to an aqueous UF/DF process such that it is filtered through a semi-permeable membrane having a pore size that allows the at least one
5 impurity to pass through the semi-permeable membrane while retaining the conjugate compound in a retentate, wherein the aqueous UF/DF process uses a second exchange medium and results in a retentate comprising the at least one conjugate compound,

wherein:

the at least one impurity comprises a residual free drug compound, a residual drug-linker
10 compound, a residual linker compound, a residual byproduct compound, a residual side product compound, or any combination thereof;

the first exchange medium comprises at least one organic solvent and a first buffer system; and

the first exchange medium and the second exchange medium are different. In some
15 embodiments prior to (a) the sample is formed by combining a drug conjugate with the first exchange medium.

[0065] In some embodiments, provided are purification methods comprising the steps of: (a) combining a first exchange medium with a crude composition, comprising a conjugate compound and at least one impurity, to obtain a crude mixture; (b) subjecting the crude mixture to an organic
20 UF/DF process such that the crude mixture is filtered through a first semi-permeable membrane having a first pore size that allows the impurity to pass through the membrane in a first filtrate while retaining the conjugate compound in a first retentate, and liquid volume filtered from the crude mixture is replaced at least in part with additional first exchange medium in the first retentate, to obtain a separated composition; and (c) subjecting the separated composition to an
25 aqueous ultrafiltration/ diafiltration process such that the separated composition is filtered through a second semi-permeable membrane having a second pore size that allows the impurity to pass through the membrane while retaining the conjugate compound in a second retentate, and liquid volume filtered from the separated composition is replaced at least in part with a second exchange medium comprising a second buffer system, to obtain a purified composition in the second
30 retentate, wherein: the impurity comprises a residual free drug compound, a residual drug-linker compound, a residual linker compound, a residual byproduct compound, a residual side product compound, or any combination thereof; the first exchange medium comprises at least one organic solvent and a first buffer system; and the first exchange medium and the second exchange medium are different.

[0066] In some embodiments, the separated composition is substantially free of product-bound impurities. In some embodiments, the separated composition is substantially free of small molecule impurities. In some embodiments, the separated composition is substantially free of residual free drug compounds, residual drug-linker compounds, residual linker compounds, residual byproduct compounds, or any combination thereof. In some embodiments, the purified composition is substantially free of the organic solvent.

[0067] As illustrated in the experimental section, it was surprisingly discovered that the presence of at least one organic solvent in the first exchange medium enables the disclosed methods to effectively separate small-molecule impurities from crude compositions containing conjugate compounds. For example, as illustrated in Table 5, the presence of the organic solvent (even in relatively small quantities) can reduce the amount of residual free drug, drug-linker compound and/or residual linker in the purified composition by a factor of twenty or more. In some embodiments, the organic solvent can be in a solid form before being added to the first buffer system. In some embodiments, the first exchange medium is aqueous. The separation of the impurities in the first exchange medium is better than in the organic solvent alone and better than the first buffer system alone.

[0068] In some embodiments, the organic solvent comprises an alcohol compound, a polar aprotic compound, or combinations thereof. In some embodiments, the organic solvent comprises an alcohol compound. In some embodiments, the organic solvent comprises a polar aprotic compound. In some embodiments, the organic solvent comprises an alcohol compound and a polar aprotic compound. Alcohol compounds may include monohydric alcohols, dihydric alcohols, polyhydric alcohols, or combinations thereof. In some embodiments, the organic solvent comprises a monohydric alcohol. In some embodiments, the organic solvent comprises a dihydric alcohol. In some embodiments, the organic solvent comprises a polyhydric alcohol. In some embodiments, the organic solvent comprises at least two of a monohydric alcohol, a dihydric alcohol and polyhydric alcohol.

[0069] In certain embodiments, the organic solvent comprises a monohydric alcohol selected from methanol, ethanol, n-propyl alcohol, iso-propyl alcohol, 1-butanol, 2-butanol, tert-butanol, iso-butanol, 1-pentanol, 2-pentanol, 3-pentanol, iso-pentanol, tert-pentanol, a hexanol (straight chain, branched and/or cyclic), ethylene glycol monomethyl ether, cis-3-hexen-1-ol, trans-2-hexen-1-ol, 5-hexen-1-ol, a phenol, a benzyl alcohol, and the like, as well as combinations thereof. For example, in some embodiments, the organic solvent is selected from a lower alcohol such as methanol, ethanol, n-propyl alcohol, iso-propyl alcohol, 1-butanol and/or 2-butanol.

[0070] In certain embodiments, the organic solvent comprises a monohydric alcohol selected from methanol, ethanol, n-propanol, isopropanol, n-butanol, sec-butanol, tert-butanol, and a combination thereof.

[0071] In certain embodiments, the organic solvent comprises a monohydric alcohol is methanol, ethanol, or a combination thereof.

[0072] In some embodiments, the organic solvent comprises at least one monohydric alcohol in combination with at least one of a dihydric alcohol, a polyhydric alcohol and/or a polar aprotic compound as described herein.

[0073] In certain embodiments, the organic solvent comprises a dihydric alcohol selected from 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,2-pentanediol, 1,5-pentanediol, 2,2-dimethylpropane-1,3-diol, 2-butyl-2-ethylpropane-1,3-diol, 2-methyl-2,4-pentane diol, 2-ethyl-1,3-hexane diol, 2-methyl-1,3-propane diol, 1,2-hexanediol, 1,5-hexanediol, 1,6-hexanediol, 1,8-octanediol, 1,10-decanediol, 2,2,4,4-tetramethylcyclobutane-1,3-diol, 1,3-cyclopentanediol, 1,2-cyclohexanediol, 1,3-cyclohexanediol, 1,4-cyclohexanediol, 1,2-cyclohexanedimethanol, 1,3-cyclohexanedimethanol, 1,4-cyclohexanedimethanol, 1,4-cyclohexanediethanol, isosorbide, a glycerol monoester, a glycerol monoether, a trimethylolpropane monoester, a trimethylolpropane monoether, a pentaerythritol diester, a pentaerythritol diether, dipropylene glycol, diethylene glycol, triethylene glycol, 2-methyl-1,3-propanediol, 2,2-dimethyl-1,3-propanediol, neopentyl glycol, xylene glycol, bis(p-hydroxy)diphenyl, bis(p-hydroxy)diphenyl propane, 2,2'-bis[4-(2-hydroxyethoxy)phenyl]propane, bis[4-(2-hydroxyethoxy)phenyl]sulfone, 1,1-bis[4-(2-hydroxy ethoxy)phenyl]cyclohexane, and the like, as well as combinations thereof. For example, in some embodiments, the organic solvent is selected from a lower-molecular weight dihydric alcohol such as 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,2-pentanediol, 1,5-pentanediol, 2,2-dimethylpropane-1,3-diol, 2-butyl-2-ethylpropane-1,3-diol, 2-methyl-2,4-pentane diol, 2-ethyl-1,3-hexane diol, 2-methyl-1,3-propane diol, 1,2-hexanediol, 1,5-hexanediol and/or 1,6-hexanediol.

[0074] In certain embodiments, the organic solvent comprises a dihydric alcohol selected from 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,2-pentanediol, 1,5-pentanediol, and any combination thereof.

[0075] In certain embodiments, the organic solvent comprises a dihydric alcohol selected from 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, and any combination thereof. In certain embodiments, the organic solvent comprises 1,2-propanediol.

[0076] In some embodiments, the organic solvent comprises at least one dihydric alcohol in combination with at least one of a monohydric alcohol, a polyhydric alcohol and/or a polar aprotic compound as described herein.

[0077] In certain embodiments, the organic solvent comprises a polyhydric alcohol selected from the group consisting of alditol, 2-ethyl-2-hydroxymethyl-1,3-propanediol, 1,2,4-butanetriol, 1,2,5-pentanetriol, 1,2,6-hexanetriol, 1,2,3,6-hexanetetrol, glycerin (aka. glycerol), diglycerin, triglycerin, tetraglycerin, pentaglycerin, hexaglycerin, triethanolamine, trimethylol ethane, trimethylol propane, ditrimethylol propane, tri-trimethylol propane, 2-methylpropanetriol, 2-methyl-1,2,4-butanetriol, pentaerythritol, dipentaerythritol, tripentaerythritol, methylglucoside, sorbitol, mannitol, sucrose, 1,3,5-trihydroxybenzene, 1,2,4-trihydroxybenzene, erythritol, inositol, threitol, arabitol, xylitol, ribitol, galactitol, mannitol, sorbitol, and the like, as well as combinations thereof. For example, in some embodiments, the organic solvent is selected from a lower-molecular weight polyhydric alcohol such as alditol, 1,2,3-propanetriol, 2-ethyl-2-hydroxymethyl-1,3-propanediol, 1,2,4-butanetriol, 1,2,5-pentanetriol, 1,2,6-hexanetriol, 1,2,3,6-hexanetetrol, glycerin (aka. glycerol) and/or diglycerin.

[0078] In certain embodiments, the organic solvent comprises a polyhydric alcohol selected from glycerin (aka. glycerol), diglycerin, triethanolamine, trimethylol ethane, trimethylol propane, ditrimethylol propane, tri-trimethylol propane, 2-methylpropanetriol, 2-methyl-1,2,4-butanetriol, and any combination thereof.

[0079] In certain embodiments, the organic solvent comprises a polyhydric alcohol selected from glycerin (aka. glycerol), diglycerin, trimethylol ethane, trimethylol propane, and any combination thereof.

[0080] In some embodiments, the organic solvent comprises at least one polyhydric alcohol in combination with at least one of a monohydric alcohol, a dihydric alcohol and/or a polar aprotic compound as described herein.

[0081] In some embodiments, the organic solvent does not include a sugar alcohol. In some embodiments, the organic solvent does not include one or any combination of the following sugar alcohols: arabinitol, erythritol, fructose, galactitol, glucose, iditol, isomalt, lactitol, lactose, mannitol, maltitol, maltose, maltotriose, myoinisitol, perseitol, ribitol, sorbitol, sucrose, threitol, trehalose, volemitol, xylitol, xylitose, and the like. In some embodiments, the organic solvent does not include glycerol.

[0082] In certain embodiments, the organic solvent comprises a polar aprotic compound selected from acetone (ACE), acetonitrile (ACN), 4-acetyl morpholine, N-cyclohexyl-2-pyrrolidone (CHP), 1,2-dimethoxyether (DME), N,N-dimethylacetamide (DMA), N,N-diethylacetamide,

dimethylformamide (DMF), diethylformamide, N,N-dimethylpropionamide, 3-methoxy-N,N-dimethylpropionamide, 3-methoxy-N,N-diethylpropionamide, 3-methoxy-N,N-methylethylpropionamide, 3-ethoxy-N,N-dimethylpropionamide, 1,3-dimethyl-2 imidazolidinone (DMI), dimethyl sulfoxide (DMSO), 1,4-dioxane, 1,3-dioxolane (DN), N,N'-dimethylpropyleneurea (DMPU),
 5 hexamethylphosphoramide (HMPA), N-methyl-2-pyrrolidone (NMP), N-ethyl-2-pyrrolidone, N-methyl-ε-caprolactam, 2-methyltetrahydrofuran (Me-THF), 2,5-dimethyltetrahydrofuran, 4-propionyl morpholine, sulfolane, tetrahydrofuran (THF), tris(N,N-tetramethylene)phosphoric acid triamide, alcohol ethoxylate, diethyl ester dimethyl ammonium chloride, linear alkylbenzene sulfonate, and the like, as well as combinations thereof. For example, in some embodiments, the
 10 organic solvent is selected from acetone (ACE), acetonitrile (ACN), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), N,N'-dimethylpropyleneurea (DMPU), hexamethylphosphoramide (HMPA), N-methyl-2-pyrrolidone (NMP) and/or tetrahydrofuran (THF).

[0083] In certain embodiments, the organic solvent comprises a polar aprotic compound selected from acetone (ACE), acetonitrile (ACN), 1,2-dimethoxyether (DME), N,N-dimethylacetamide
 15 (DMA), N,N-diethylacetamide, dimethylformamide (DMF), N,N-dimethylpropionamide, 3-methoxy-N,N-dimethylpropionamide, dimethyl sulfoxide (DMSO), N,N'-dimethylpropyleneurea (DMPU), hexamethylphosphoramide (HMPA), N-methyl-2-pyrrolidone (NMP), N-ethyl-2-pyrrolidone, N-methyl-ε-caprolactam, tetrahydrofuran (THF), and any combination thereof.

[0084] In certain embodiments, the organic solvent comprises a polar aprotic compound selected from acetone (ACE), acetonitrile (ACN), N,N-dimethylacetamide (DMA), dimethylformamide
 20 (DMF), dimethyl sulfoxide (DMSO), N,N'-dimethylpropyleneurea (DMPU), hexamethylphosphoramide (HMPA), N-methyl-2-pyrrolidone (NMP), tetrahydrofuran (THF), and any combination thereof.

[0085] In some embodiments, the organic solvent comprises at least one polar aprotic compound in combination with at least one of a monohydric alcohol, a dihydric alcohol and/or a polyhydric
 25 alcohol as described herein.

[0086] In certain embodiments, the organic solvent is selected from methanol (MeOH), ethanol (EtOH), 1,2-propanediol (PG), glycerin, acetone (ACE), acetonitrile (ACN), N,N-dimethylacetamide (DMA), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), N,N'-
 30 dimethylpropyleneurea (DMPU), hexamethylphosphoramide (HMPA), N-methyl-2-pyrrolidone (NMP), tetrahydrofuran (THF), and combinations thereof. For example, in some embodiments, the organic solvent is selected from DMSO, DMA, PG, and any combination thereof.

[0087] In certain embodiments, the first exchange medium employed is one of those set out in Table 5 comprising at least one organic solvent. In one embodiment, the at least one organics

solvent employed is at least one of DMA, acetone, acetonitrile, THF, ethanol and 1,2 propanediol. In one embodiment, the at least one organic solvent is acetonitrile. In one embodiment, it is 15 to 25% acetonitrile. In another embodiment it is about 20% acetonitrile. In one embodiment, the at least one organic solvent is THF. In one embodiment, the at least one organic solvent is 10 to 15% THF. In one embodiment the at least one organic solvent is about 15% THF. In one embodiment, the at least one organic solvent is 15 to 25% ethanol. In one embodiment, it is about 20% ethanol. In one embodiment a combination of acetonitrile and 1,2 propanediol is used for organic solvent. In one embodiment the buffer used with such amounts of organic solvent comprises about 50 mM PBS and a pH 7.2. In another embodiment, the buffer used is 20 mM histidine pH 5.5. In certain embodiments, the first exchange medium employed is one of those set out in Table 6. In certain embodiments, the first exchange medium employed is one of those set out in Table 7.

[0088] In certain embodiments, the amount of impurities in the purified product is less than 5%, less than 4%, less than 3%, less than 2%, or less than 1%. In certain embodiments, the amount of impurities in the purified product is 0.1%-5%, 0.1%-4%, 0.1%-3%, 0.1%-2%, or 0.1%-1%.

[0089] In certain embodiments, the organic solvent is miscible with water at room temperature. In some embodiments, the organic solvent is miscible with water when the resulting mixture is heated to at least 30°C. In some embodiments, the organic solvent is miscible with water when the resulting mixture is cooled to a temperature ranging from 5°C to 15°C. In some embodiments, at least two organic solvents are miscible with water when used in combination. In some embodiments, at least two organic solvents are miscible with water when used in combination and when the resulting mixture is heated to at least 30°C. In some embodiments, at least two organic solvents are miscible with water when used in combination and when the resulting mixture is cooled to a temperature ranging from 5°C to 15°C. In some embodiments, the organic solvent is partially soluble in water. In some embodiments, the organic solvent is partially soluble in water when the resulting mixture is heated to at least 30°C. In some embodiments, the organic solvent is partially soluble in water when the resulting mixture is heated to at least 30°C.

[0090] In certain embodiments, the organic solvent has a boiling point of at least 150°C. In some embodiments, the organic solvent has a boiling point of at least 160°C. In some embodiments, the organic solvent has a boiling point of at least 170°C. In some embodiments, the organic solvent has a boiling point of at least 180°C. In some embodiments, the organic solvent has a boiling point ranging from about 50°C to about 300°C, from about 80°C to about 275°C, or from about 100°C to about 250°C, or from about 150°C to about 225°C. In some embodiments, the organic solvent is a solid at room temperature in its pure form.

[0091] In certain embodiments, the organic solvent has a density of at least 0.7 g/cm³. In some embodiments, the organic solvent has a density of at least 0.8 g/cm³. In some embodiments, the organic solvent has a density of at least 0.9 g/cm³. In some embodiments, the organic solvent has a density of at least 1.0 g/cm³. In some embodiments, the organic solvent has a density of at least 1.1 g/cm³. In some embodiments, the organic solvent has a density of at least 1.2 g/cm³. In some embodiments, the organic solvent has a density ranging from about 0.6 g/cm³ to about 1.5 g/cm³, or from about 0.7 g/cm³ to about 1.4 g/cm³, or from about 0.8 g/cm³ to about 1.3 g/cm³, or from about 0.9 g/cm³ to about 1.3 g/cm³, or from about 1.0 g/cm³ to about 1.3 g/cm³.

[0092] In certain embodiments, the organic solvent has a polarity index of at least 3.0. In some embodiments, the organic solvent has a polarity index of at least 4.0. In some embodiments, the organic solvent has a polarity index of at least 5.0. In some embodiments, the organic solvent has a polarity index of at least 6.0. In some embodiments, the organic solvent has a polarity index of at least 7.0. In some embodiments, the organic solvent has a polarity index of at least 8.0. In some embodiments, the organic solvent has a polarity index of at least 9.0. In some embodiments, the organic solvent has a polarity index ranging from about 3.0 to about 9.0, or from about 4.0 to about 8.5, or from about 4.5 to about 8.5, or from about 5.0 to about 8.5, or from about 5.0 to about 8.0, or from about 5.5 to about 8.0.

[0093] In certain embodiments, the organic solvent has a logP of less than 0 as measured at 25°C. In some embodiments, the organic solvent has a logP of less than -0.1 as measured at 25°C. In other embodiments the organic solvent has a logP ranging from about -0.04 to -1.5 as measured at 25°C.

[0094] In certain embodiments, the first exchange medium comprises at least two organic solvents. In some embodiments, the first exchange medium comprises at least three organic solvents. In some embodiments, the first exchange medium comprises an alcohol compound and a polar aprotic compound. In some embodiments, the first exchange medium comprises a monohydric alcohol and a polar aprotic compound. In some embodiments, the first exchange medium comprises a dihydric alcohol and a polar aprotic compound. In some embodiments, the first exchange medium comprises a polyhydric alcohol and a polar aprotic compound. In some embodiments, the first exchange medium comprises a plurality of alcohol compounds. In some embodiments, the first exchange medium comprises a plurality of polar aprotic compounds.

[0095] In certain embodiments, a proportion of the organic solvent in the first exchange medium ranges from about 1% to about 30%, based on a mass amount of the organic solvent relative to a total volume of the first exchange medium. In some embodiments, a proportion of the organic solvent in the first exchange medium ranges from about 1% to about 30%, or from about 2% to

about 28%, or from about 3% to about 27%, or from about 4% to about 26%, or from about 5% to about 25%, or from about 6% to about 24%, or from about 7% to about 23%, or from about 8% to about 22%, or from about 9% to about 21%, or from about 10% to about 20%, based on a mass amount of the organic solvent relative to a total volume of the first exchange medium. In some
 5 embodiments, a proportion of the organic solvent in the first exchange medium ranges from about 5% to about 20%, based on a mass amount of the organic solvent relative to a total volume of the first exchange medium. In certain embodiments, the first exchange medium comprises at least two organic solvents such that a total proportion of the at least two organic solvents in the first exchange medium ranges from about 1% to about 30%, based on a total mass amount of the at
 10 least two organic solvents relative to a total volume of the first exchange medium. In some embodiments, a total proportion of the at least one organic solvent in the first exchange medium is less than about 30% based on a total mass of the at least one organic solvent relative to a total volume of the first exchange medium.

[0096] In certain embodiments, the first buffer system and the second buffer system are
 15 independently selected from an acetate buffer system, an ADA ((N-(2-acetamido)iminoacetic acid) buffer system, an ACES (N-(2-acetamido)-2-amino ethanesulfonic acid) buffer system, an AMPD (2-amino-2-methyl-1,3-propanediol) buffer system, an AMPSO (3-(1,1-dimethyl-2-hydroxyethyl)amino-2-hydroxypropanesulfonic acid) buffer system, a BES (N,N-bis-(2-hydroxyethyl)-2-aminoethanesulfonic Acid) buffer system, a bicarbonate buffer system, a bicine (N,N-
 20 Bis(2-hydroxyethyl) glycine) buffer system, a Bis-Tris (2-bis(2-hydroxyethyl)amino-2-(hydroxy methyl)-1,3-propanediol) buffer system, a BTP (1,3-bis(tris(hydroxymethyl)methyl amino) propane) buffer system, a CABS (4-(cyclohexylamino)-1-butanefulfonic acid) buffer system, a CAPS (N-cyclohexyl-3-aminopropanesulfonic acid) buffer system, a CAPSO (3-(cyclohexyl amino)-2-hydroxypropanesulphonic Acid) buffer system, a citrate buffer system, a DIPSO (3-
 25 (N,N-bis[2-hydroxyethyl] amino)-2-hydroxypropanesulfonic acid) buffer system, an EDTA (ethylenediaminetetraacetic acid) buffer system, a Gly-Gly buffer system, a His-Glu buffer system, a HEPBS (N-(2-hydroxyethyl)piperazine-N'-(4-butanefulfonic acid)) buffer system, a HEPES (4-(2-hydroxy ethyl)-1-piperazineethanesulfonic acid) buffer system, a HEPPSO ((2-hydroxyethyl)-piperazine-N-2-hydroxypropanesulfonic acid) buffer system, a histidine buffer system, a MES (2-
 30 (N-morpholino)ethanesulfonic acid) buffer system, a MOBS (4-(4-morpholinyl)butanesulfonic acid) buffer system, a MOPS (3-(N-morpholino)propanesulfonic acid) buffer system, a MOPSO (3-morpholino-2-hydroxypropane sulfonic acid) buffer system, a PB (phosphate buffer) buffer system, a PBS (phosphate buffered saline) buffer system, a PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)) buffer system, a POPSO (piperazine-N,N'-bis(2-hydroxypropanesulfonic

acid)) buffer system, a succinate buffer system, a TES (2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethane-1-sulfonic acid) buffer system, a TAPSO (N-[tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonic acid) buffer system, a TAE (tris-acetate-EDTA) buffer system, a Tricine (N-(tri(hydroxymethyl)methyl)glycine) buffer system, and a TAPS
5 ([tris(hydroxymethyl)methylamino]propanesulfonic acid) buffer system.

[0097] In certain embodiments, the first buffer system and the second buffer system are independently selected from an ADA buffer system, an acetate buffer system, a bicarbonate buffer system, a Bis-Tris buffer system, a CABS buffer system, a citrate buffer system, an EDTA buffer system, a Gly-Gly buffer system, a HIS (histidine) buffer system, a His-Glu buffer system,
10 a His-Gly buffer system a PB (phosphate buffer) buffer system, a PBS (phosphate buffered saline) buffer system, a succinate buffer system, and a Tricine buffer system.

[0098] In certain embodiments, the first buffer system and the second buffer system are independently selected from an acetate buffer system, a bicarbonate buffer system, a citrate buffer system, a Gly-Gly buffer system, a HIS (histidine) buffer system, a PB (phosphate buffer)
15 buffer system, a PBS (phosphate buffered saline) buffer system, and a succinate buffer system.

[0099] In some embodiments, the first buffer system and the second buffer system are the same buffer. In other embodiments the first buffer system and the second buffer system are different buffers. In some embodiments, the first exchange medium and the second exchange medium only differ in respect of the first exchange medium comprising the at least one organic solvent
20 which is absent from the second exchange medium. In other embodiments, the two exchange mediums differ in other respects.

[0100] In some embodiments, the concentration of first and second aqueous buffers is the same. In other embodiments the concentration of first and second aqueous buffers is different.

[0101] In some embodiments, the organic UF/DF and the aqueous UF/DF are carried out in the same retentate volume, for example in the same apparatus. In other embodiments the organic UF/DF and the aqueous UF/DF are carried out in different retentate volumes, for example in different apparatuses. In certain embodiments, the organic UF/DF process and the aqueous UF/DF process are performed as a tangential flow filtration process.
25

[0102] In certain embodiments, a pH of the first and second exchange media independently ranges from about 4.0 to about 10.0, or from about 4.5 to about 9.5, from about 5.0 to about 9.5, from about 5.0 to about 9.0, or from about 5.0 to about 8.5, or from about 5.0 to about 8.0, or from about 5.5 to about 8.0, or from about 5.5 to about 7.5. In some embodiments, the first and second exchange media have the same pH. In other embodiments the first and second exchange media has different pHs.
30

[0103] In certain embodiments, the organic UF/DF and the aqueous UF/DF are independently carried out at a flux rate ranging from about 50 L/hr/m² to about 800 L/hr/m², or from about 100 L/hr/m² to about 700 L/hr/m², or from about 150 L/hr/m² to about 600 L/hr/m², or from about 200 L/hr/m² to about 600 L/hr/m², or from about 200 L/hr/m² to about 550 L/hr/m², or from about 200 L/hr/m² to about 500 L/hr/m², or from about 250 L/hr/m² to about 500 L/hr/m², or from about 250 L/hr/m² to about 450 L/hr/m², or from about 250 L/hr/m² to about 400 L/hr/m². In some embodiments, the organic UF/DF and the aqueous UF/DF are carried out at the same flux rate. In other embodiments the organic UF/DF and the aqueous UF/DF are carried out different flux rates.

[0104] In certain embodiments, the organic UF/DF and the aqueous UF/DF are independently carried out at a transmembrane pressure ranging from about 2 psi to about 50 psi, or from about 5 psi to about 40 psi, or from about 5 psi to about 30 psi, or from about 10 psi to about 30 psi, or from about 10 psi to about 25 psi, or from about 15 psi to about 25 psi. In some embodiments, the organic UF/DF and the aqueous UF/DF are each carried out at the same transmembrane pressure. In other embodiments the organic UF/DF and the aqueous UF/DF are carried out at different transmembrane pressures.

[0105] In certain embodiments, the organic UF/DF and the aqueous UF/DF are independently carried out such that a temperature in a retentate chamber containing the compound ranges from about 5°C to about 60°C, or from about 5°C to about 55°C, or from about 5°C to about 50°C, or from about 5°C to about 45°C, or from about 5°C to about 40°C, or from about 5°C to about 35°C, or from about 5°C to about 30°C, or from about 5°C to about 25°C, or from about 5°C to about 20°C, or from about 5°C to about 15°C, or from about 5°C to about 10°C. In some embodiments, the organic UF/DF and the aqueous UF/DF are carried out at the same temperature in a retentate chamber. In other embodiments the organic UF/DF and the aqueous UF/DF are carried out at different temperature in a retentate chamber.

[0106] In certain embodiments, a diafiltration volume (DV) of the organic UF/DF and the aqueous UF/DF independently range from about 2 to about 50, or from about 2 to about 35, or from about 2 to about 30, or from about 2 to about 25, from about 2 to about 20, or from about 2 to about 18, or from about 2 to about 17, or from about 2 to about 16, or from about 2 to about 15, or from about 2 to about 14, or from about 2 to about 13, or from about 2 to about 12, or from about 2 to about 11, or from about 2 to about 10, or from about 2 to about 9, or from about 2 to about 8, or from about 2 to about 7, or from about 2 to about 6. In some embodiments, the DV is at least 5. In some embodiments, the DV for the organic UF/DF and the DV for the aqueous UF/DF are the same. In other embodiments the DV of the organic UF/DF and the DV of the aqueous UF/DF are

different. In some embodiments, the DV of the organic UF/DF ranges from about 12 to 16, or from about 12 to 15, or from about 12 to 14.

[0107] In certain embodiments, the first semi-permeable membrane and the second semi-permeable membrane are independently selected from a polyolefin, a polystyrene, a polysulfone, a polyester, a polyamide, a polyacrylate, a polycarbonate, and the like, as well as copolymers and combinations thereof. In some embodiments, the first semi-permeable membrane and the second semi-permeable membrane are independently selected from a polyethersulfone (PES), a polytetrafluoroethylene (PTFE), a polypropylene, a sulphonated polysulfone, a polyvinylidene fluoride (PVDF), a polyacrylonitrile (PAN), a cellulosic polymer, a polyimide, a polyether imide (PEI), an aliphatic polyamide, a polyetheretherketone (PEEK), a polyphenylene oxide (PPO), a polysulfone (PSf), and the like, as well as mixtures and copolymers thereof. In some embodiments, the first and second semi-permeable membranes are the same semi-permeable membrane. In other embodiments the first and second semi-permeable membranes are different semi-permeable membranes. In some embodiments, the first and second semi-permeable members are both neutral membranes that are uncharged. In other embodiments only one of the first and second semi-permeable members are a neutral membrane that is uncharged.

[0108] In some embodiments, the first and second pore sizes independently range from about 0.2 kDa MWCO to about 500 kDa MWCO, or from about 0.2 kDa MWCO to about 1 kDa MWCO, or from about 1 kDa MWCO to about 10 kDa MWCO, or from about 10 kDa MWCO to about 50 kDa MWCO, or from about 50 kDa MWCO to about 100 kDa MWCO, or from about 75 kDa MWCO to about 125 kDa MWCO, or from about 100 kDa MWCO to about 200 kDa MWCO, or from about 200 kDa MWCO to about 500 kDa MWCO.

[0109] In certain embodiments, the organic UF/DF process and the aqueous UF/DF process are performed as a tangential flow filtration (TFF) process.

[0110] In certain embodiments, a concentration of the ADC or other conjugate compound in the crude composition ranges from about 0.5 mg/mL to about 500 mg/mL, or from about 0.5 mg/mL to about 200 mg/mL, or from about 1 mg/mL to about 200 mg/mL, or from about 2 mg/mL to about 150 mg/mL, or from about 1 mg/mL to about 100 mg/mL, or from about 5 mg/mL to about 100 mg/mL, or from about 5 mg/mL to about 50 mg/mL, or from about 5 mg/mL to about 25 mg/mL.

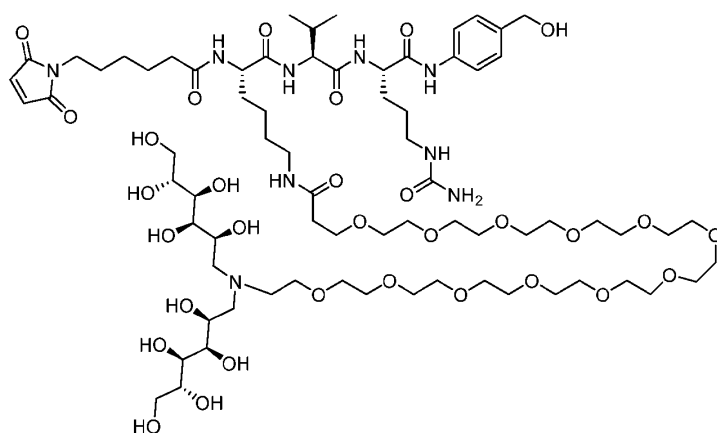
[0111] In some embodiments, the amount of ADC (or other conjugate compound) in the crude composition which is to be purified ranges from about 1 gram to about 1 kilogram. In some embodiments, the amount of ADC (or other conjugate compound) in the crude composition ranges from about 10 grams to about 1 kilogram. In some embodiments, the amount of ADC (or other conjugate compound) in the crude composition ranges from about 10 grams to about 1

kilogram. In some embodiments, the amount of ADC (or other conjugate compound) in the crude composition ranges from about 100 grams to about 1 kilogram. In some embodiments, the amount of ADC (or other conjugate compound) in the crude composition ranges from about 100 grams to about 10 kilograms. In some embodiments, the amount of ADC (or other conjugate compound) in the crude composition ranges from about 1 kilogram to about 10 kilograms.

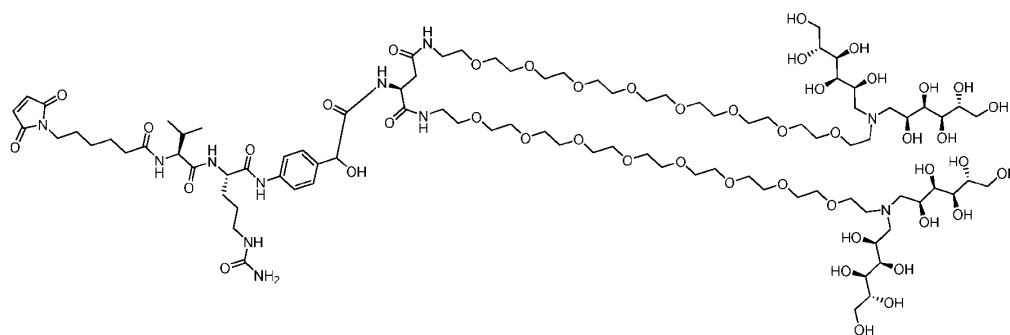
[0112] In certain embodiments, the crude mixture comprises at least one of a residual free drug compound, a residual drug-linker compound, and/or residual linker compound in addition to an ADC or conjugate other than an ADC. In certain embodiments, the crude mixture comprises at least one of a residual free drug compound, a residual drug-linker compound, residual linker compound and/or residual byproduct in addition to an ADC or conjugate other than an ADC.

[0113] In certain embodiments, the impurity comprises a drug compound selected from a topoisomerase I (TOP1) inhibitor, a topoisomerase II (TOP2) inhibitor, a microtubule disruptor, a DNA damaging agent (such as an alkylator), a protein degrader, a TLR7 agonist, a TLR8 agonist, a STING agonist, an XPO1 inhibitor, and the like, and the derivatives thereof. For example, the crude mixture may contain at least one drug compound selected from exetecan, DXd, camptothecin, SN-38 (7-ethyl-10-hydroxy-camptothecin), a microtubule disruptor such as eribulin, DM1 (mertansine), a DM1 derivative, DM4 (mertansine), MMAE (monomethyl auristatin E), monomethyl auristatin F (MMAF), a DNA intercalator such as a pyrrolobenzodiazepine (PBD), a DNA alkylating agent such as a duocarmycin, and others such as leptomysin B, and derivatives thereof. In certain embodiments, the crude mixture may contain exetecan or a derivative thereof.

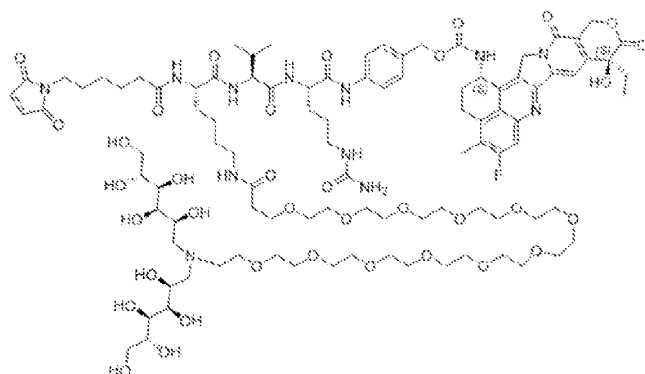
[0114] In some embodiments, the impurity comprises a residue linker compound, such as the linkers or derivatives thereof described in PCT/CN2021/104174 (Publication No. WO 2022/217022) and PCT/CN2024/071901 (Publication No. WO2024149345A1), the entirety of all of which are incorporated herein by reference. In certain embodiments, the linker compound has the following structure:



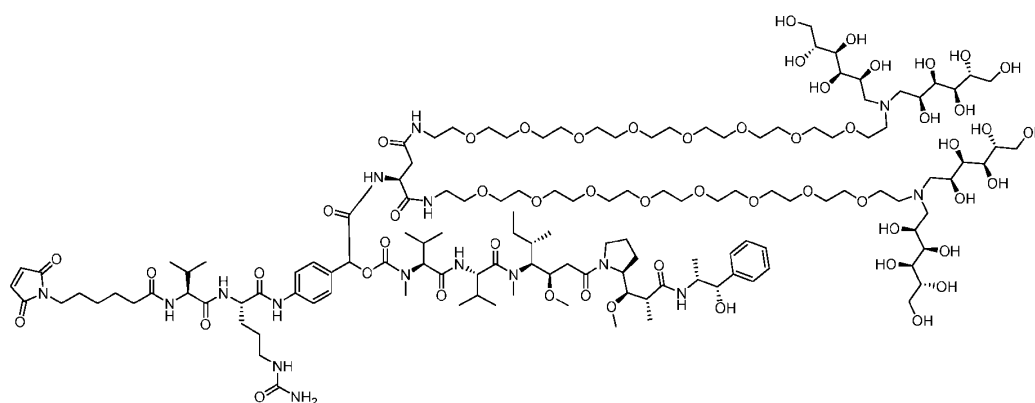
In certain embodiments, the linker compound has the following structure:



- [0115]** In some embodiments, the impurity comprises a drug-linker compound containing at least one drug moiety derived from one of the drug compounds enumerated above as a payload. In some embodiments, the impurity comprises a drug-linker compound containing at least one linker moiety derived from one of the linker compounds noted above. In some embodiments, the drug-linker compound, in a free form, is one of the drug-linker compounds or derivatives thereof described in PCT/CN2021/104174 (Publication No. WO 2022/217022) and PCT/CN2024/071901 (Publication No. WO2024149345A1), the entirety of all of which are incorporated herein by reference. In certain embodiments, the drug-linker compound is the LD038 compound provided in Example 1 of the present disclosure and has the following structure:



In certain embodiments, the drug-linker compound is an LD343 compound and has the following structure:



- 5 **[0116]** In certain embodiments, the crude mixture comprises an ADC. In some embodiments, the ADC comprises a humanized, fully human, chimeric, bispecific, trispecific or poly specific antibody. In some embodiments, the ADC comprises a monoclonal antibody, a Fab, a Fab', an F(ab'), an Fv, a disulfide linked Fc, a scFv, a single domain antibody, a diabody, a bi-specific antibody, or a multi-specific antibody. In some embodiments, the ADC comprises a scFv1-ScFv2, a ScFv12-Fc-scFv22, a IgG-scFv, a DVD-Ig, a triomab/quadroma, a two-in-one IgG, a scFv2-Fc, a TandAb, and an scFv-HSA-scFv.
- 10

- [0117]** In some embodiments, the ADC comprises a drug-linker moiety, in a form that is covalently bound to an antibody, such as the drug-linker moieties or derivatives described in, WO2022/217022 and WO2024/092067, the entirety of all of which are incorporated herein by reference. In certain embodiments, the drug-linker moiety is a derivative of the LD038 compound provided in Example 1 of the present disclosure. In some embodiments, the drug-linker moiety is a moiety formed based on a linker, such as mc-VC-PAB, CL2, CL2A or
- 15

(Succinimid-3-yl-N)-(CH₂)_n-C(=O)-Gly-Gly-Phe-Gly-NH-CH₂-O-CH₂-(C=O)-, and a drug, such as a camptothecin or camptothecin derivative, such as exatecan.

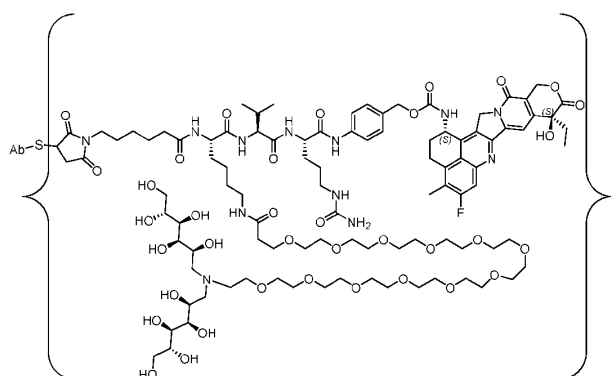
[0118] In certain embodiments, the crude mixture comprises a conjugate other than an ADC. In some embodiments, the conjugate other than an ADC comprises a non-antibody protein scaffold.

- 5 Such non-antibody scaffolds include, for example, Affibodies, Affilins, Anticalins, Atrimers, Avimers, Bicyclic peptides, Cys-knots, DARPs, FN3 scaffolds (e.g., Adnectins, Centyrins, Pronectins, and Tn3), Fynomers, Kunitz domains and OBodies. (See, e.g., Vazquez-Lombardi et al., Drug Discovery Today 20(10):1271 (2015) and the references cited therein.) Such Non-antibody protein scaffolds include, for example, Affibodies, Affilins, Anticalins, Atrimers, Avimers,
- 10 Bicyclic peptides, Cys-knots, DARPs, FN3 scaffolds (e.g., Adnectins, Centyrins, Pronectins, and Tn3), Fynomers, Kunitz domains and OBodies. (See, e.g., Vazquez-Lombardi et al., Drug Discovery Today 20(10):1271 (2015) and the references cited therein.)

[0119] In some embodiments, the ADC (or conjugate other than an ADC) has a molecular weight of at least 25 kilodaltons (kDa), at least 50 kDa, at least 75 kDa or at least 100 kDa.

- 15 **[0120]** In some embodiments, the crude mixture comprises an antibody-drug conjugate (ADC), such as the ADCs described in PCT/CN2021/104174 (Publication No. WO 2022/217022), PCT/US2023/077814 (Publication No. WO2024/092067), and CN202310035642.4 (Publication No. CN116036303A), the entirety of all of which are incorporated herein by reference. In some embodiments, the crude mixture comprises an antibody-drug conjugate (ADC) containing at least
- 20 one drug moiety derived from a drug compound selected from the group consisting of exetecan, camptothecin, eribulin, SN-38 (7-ethyl-10-hydroxy-camptothecin), DM1 (mertansine), leptomysin B, and MMAE (monomethyl auristatin E). In some embodiments, the drug moiety is a camptothecin selected from the group consisting of 10-hydroxycamptothecin, topotecan, irinotecan, 9-aminocamptothecin, 9-nitrocamptothecin, SN-38, lurtotecan, BMS422461, CMMD-Gly, morpholino-CPT, exatecan, belotecan, DB-67, karenitecin, ST1481, and chimmitecan.
- 25

[0121] In some embodiments, the ADC has the following structure:



ⁿ. In certain embodiments, n is 8 and Ab represents an antibody F131 comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1 having an amino acid sequence of SEQ ID NO: 1, HCDR2 having an amino acid sequence of SEQ ID NO: 2, and HCDR3 having an amino acid sequence of SEQ ID NO: 3, and the VL region comprising LCDR1 having an amino acid sequence of SEQ ID NO: 4, LCDR2 having an amino acid sequence of SEQ ID NO: 5, and LCDR3 having an amino acid sequence of SEQ ID NO: 6. In certain embodiments, n is 8 and Ab represents an antibody 2E7 comprising a heavy chain variable (VH) region and a light chain variable (VL) region, the VH region comprising complementarity determining regions HCDR1 having an amino acid sequence of SEQ ID NO: 7, HCDR2 having an amino acid sequence of SEQ ID NO: 8, and HCDR3 having an amino acid sequence of SEQ ID NO: 9, and the VL region comprising LCDR1 having an amino acid sequence of SEQ ID NO: 10, LCDR2 having an amino acid sequence of SEQ ID NO: 11, and LCDR3 having an amino acid sequence of SEQ ID NO: 12. In certain embodiments, n is 8 and Ab represents trastuzumab.

[0122] In some embodiments the conjugate compound is an ADC having a drug-antibody ratio ranging from about 1:1 to about 30:1, or from about 1:1 to about 25:1, or from about 1:1 to about 24:1, or from about 1:1 to about 20:1, or from about 1:1 to about 16:1, or from about 1:1 to about 10:1, or from about 1:1 to about 9:1, or from about 1:1 to about 8:1, or from about 1:1 to about 7:1, or from about 1:1 to about 6:1, or from about 1:1 to about 5:1, or from about 1:1 to about 4:1.

[0123] In some embodiments, the crude composition is a conjugation mixture comprising the antibody-drug conjugate (ADC) (or conjugate other than an ADC) and at least one of a residual free drug compound, a residual linker compound, a residual free drug-linker compound, and/or a residual byproduct. In some embodiments, a concentration of residual free drug compound, a residual linker compound, a residual drug-linker compound, or any combination thereof, in the purified composition is less than 200 µg/mL, or is less than 150 µg/mL, or is less than 100 µg/mL,

or is less than 75 µg/mL, or is less than 50 µg/mL, or is less than 40 µg/mL, based on a total volume of the purified conjugate compound. A concentration of the purified conjugate compound herein is in a range from 1 mg/mL - 200 mg/mL, 2 mg/mL - 150 mg/mL, 3 mg/mL - 120 mg/mL, 4 mg/mL - 80 mg/mL, 5 mg/mL - 50 mg/mL, 8 mg/mL - 40 mg/mL, 10 mg/mL - 35 mg/mL, or 12 mg/mL - 30 mg/mL. In some embodiments, the concentration of the purified conjugate compound herein is 15 mg/mL, 18 mg/mL, 20 mg/mL, 25 mg/mL, or 30 mg/mL.

Methods of Preparing Antibody-Drug Conjugates (ADCs) and Conjugates Other Than ADCs

[0124] In some embodiments, provided are methods for preparing purified conjugate compounds using at least one separately-prepared drug-linker compound, including the steps of: (i) reducing a targeting agent by contacting the targeting agent with a reducing agent to obtain a crude reduced targeting agent; (ii) conjugating the reduced targeting agent with a drug-linker compound to obtain a crude composition comprising a conjugate compound; (iii) optionally adding a quenching agent to the crude composition; and (iv) purifying the crude composition using a UF/DF method of the present disclosure, wherein the first exchange medium comprises at least one organic solvent.

[0125] In some embodiments, provided are methods for preparing purified conjugate compounds by sequential attachment of linking group(s) and drug compound(s), including the steps of: (i) reducing a targeting agent by contacting the targeting agent with a reducing agent to obtain a crude reduced targeting agent; (ii) attaching at least one linking group to the reduced targeting agent to obtain a crude composition comprising a targeting agent-linker intermediate; (iii) attaching at least one drug compound to the linker of the targeting agent-linker intermediate to obtain a crude composition comprising a conjugate compound; (iv) optionally adding a quenching agent to the crude composition; and (v) purifying the crude composition using a UF/DF method of the present disclosure, wherein the first exchange medium comprises at least one organic solvent, to obtain a purified conjugate compound.

[0126] In some embodiments, a conjugation reaction is performed as generally described in WO 2023/280227, WO 2005/081711, WO 2005/077090, WO 2012/135517, WO 2006/086733, WO 2006/034488 and WO 2013/055993.

[0127] In some embodiments, the targeting agent is an antibody or an antigen binding fragment, and the purified conjugate compound is a purified antibody-drug conjugate (ADC). In other embodiments, the targeting agent is a non-antibody-containing targeting agent, and the purified conjugate compound is a purified conjugate other than an ADC.

[0128] In some embodiments, suitable reducing agents include sulfide reducing agents which break disulfide bonds such as tris(2-carboxyethyl)phosphine (TCEP), 2-mercaptoethanol (BME), dithiothreitol (DTT), dithioerythritol (DTE), sodium borohydride, sodium cyanoborohydride, 3,3',3"-phosphanetriyltris(benzenesulfonic acid) trisodium (TPPTS), cysteine hydrochloride, and cysteine.

5 In some embodiments, the reducing agent is TCEP.

[0129] In some embodiments, suitable quenching agents include cysteine, TCEP, ketone quenching agents such as acetone, alcohol quenching agents such as tert-butanol, amine quenching agents such as ethanolamine, azide quenching agents such as penta-PEG azide (as described in Kantner et al., ACS Omega (2017), 2, 5785-5791), and maleimide or haloacetamide
10 quenching agents, such as 4-maleimidobutyric acid, 3-maleimidopropionic acid, N-ethylmaleimide, iodoacetamide, or iodoacetamidopropionic acid, and the like, or combinations thereof.

[0130] In some embodiments, the methods for preparing purified conjugate compounds further comprises purifying the crude targeting agent by performing an initial aqueous UF/DF process on
15 the crude reduced targeting agent, prior to converting the reduced targeting agent into the conjugate compound. The initial aqueous UF/DF may be carried out in a manner analogous to the step (c) aqueous UF/DF of the methods disclosed herein, or the initial aqueous UF/DF may be carried out using a conventional UF/DF process.

[0131] In some embodiments, the conjugate compound comprises at least one drug moiety
20 derived from a drug compound selected from the group consisting of a topoisomerase I (TOP1) inhibitor, a topoisomerase II (TOP2) inhibitor, a microtubule disruptor, a DNA damaging agent, a DNA intercalating agent, a protein degrader, a TLR7 agonist, a TLR8 agonist, a STING agonist, and an XPO1 inhibitor. In some embodiments, the drug compound is selected from the group consisting of exetecan, eribulin, DXd, camptothecin, SN-38 (7-ethyl-10-hydroxy-camptothecin),
25 DM1 (mertansine), a DM1 derivative, DM4 (mertansine), MMAE (monomethyl auristatin E), MMAF, a pyrrolbenzodiazepine (PBD) and a leptomysin B.

[0132] In some embodiments, the drug moiety is a camptothecin selected from the group consisting of 10-hydroxycamptothecin, topotecan, irinotecan, 9-aminocamptothecin, 9-nitrocamptothecin, SN-38, lurtotecan, BMS422461, CMMD-Gly, morpholino-CPT, exatecan,
30 belotecan, DB-67, karenitecin, ST1481, and chimmitecan.

Methods of preparing pharmaceutical compositions

[0133] Any of the methods of the present invention may involve a step of formulating the purified drug conjugate with a pharmaceutical carrier or excipient to give a pharmaceutical composition. Hence, the invention also provides a method of producing a pharmaceutical composition comprising performing a purification method of the present invention and then formulating the purified product with a pharmaceutical carrier or excipient to give a pharmaceutical composition comprising the drug conjugate. Examples of appropriate formulations can be found in: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

EMBODIMENTS

[0134] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize.

The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. These and other changes can be made to the disclosure in light of the detailed description.

[0135] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

[0136] All patents and other publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are

provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the
5 applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[0137] The following numbered embodiments represent further embodiments of the invention:

1. A purification method, comprising:
 - (a) combining a first exchange medium with a crude composition to obtain a crude
10 mixture, wherein the crude composition comprises a conjugate compound and at least one impurity;
 - (b) subjecting the crude mixture to an organic ultrafiltration/diafiltration (UF/DF) process such that the crude mixture is filtered through a first semi-permeable membrane having a first pore size that allows the impurity to pass through the first semi-permeable membrane in a
15 first filtrate while retaining the conjugate compound in a first retentate, and liquid volume filtered from the crude mixture is replaced at least in part with additional first exchange medium in the first retentate, to obtain a separated composition; and
 - (c) subjecting the separated composition to an aqueous UF/DF process such that the separated composition is filtered through a second semi-permeable membrane having a
20 second pore size that allows the impurity to pass through the second semi-permeable membrane while retaining the conjugate compound in a second retentate, and liquid volume filtered from the separated composition is replaced at least in part with a second exchange medium comprising a second buffer system, to obtain a purified composition in the second retentate,
- 25 wherein:
 - the impurity comprises a residual free drug compound, a residual drug-linker compound, a residual linker compound, a residual byproduct compound, a residual side product compound, or any combination thereof;
 - the first exchange medium comprises at least one organic solvent and a first buffer
30 system; and
 - the first exchange medium and the second exchange medium are different.
2. The method according to 1, wherein the separated composition is substantially free of product-bound impurities.

3. The method according to 1 or 2, wherein the purified composition is substantially free of the organic solvent.

4. The method according to any one of 1-3, wherein the organic solvent comprises an alcohol compound, a polar aprotic compound, or a combination thereof.

5 5. The method according to any one of 1-4, wherein the organic solvent comprises a monohydric alcohol, a dihydric alcohol, a polyhydric alcohol, or a combination thereof.

6. The method according to any one of 1-5, wherein the organic solvent comprises a monohydric alcohol selected from the group consisting of methanol, ethanol, n-propyl alcohol, iso-propyl alcohol, 1-butanol, 2-butanol, tert-butanol, iso-butanol, 1-pentanol, 2-pentanol, 3-pentanol, iso-pentanol, tert-pentanol, a hexanol (straight chain, branched and/or cyclic), ethylene glycol monomethyl ether, cis-3-hexen-1-ol, trans-2-hexen-1-ol, 5-hexen-1-ol, a phenol, a benzyl alcohol, and the like, and combinations thereof.

7. The method according to any one of 1-6, wherein the organic solvent comprises a dihydric alcohol selected from the group consisting of 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,2-pentanediol, 1,5-pentanediol, 2,2-dimethylpropane-1,3-diol, 2-butyl-2-ethylpropane-1,3-diol, 2-methyl-2,4-pentane diol, 2-ethyl-1,3-hexane diol, 2-methyl-1,3-propane diol, 1,2-hexanediol, 1,5-hexanediol, 1,6-hexanediol, 1,8-octanediol, 1,10-decanediol, 2,2,4,4-tetramethylcyclobutane-1,3-diol, 1,3-cyclopentanediol, 1,2-cyclohexanediol, 1,3-cyclohexanediol, 1,4-cyclohexanediol, 1,2-cyclohexanedimethanol, 1,3-cyclohexanedimethanol, 1,4-cyclohexanedimethanol, 1,4-cyclohexanediethanol, isosorbide, a glycerol monoester, a glycerol monoether, a trimethylolpropane monoester, a trimethylolpropane monoether, a pentaerythritol diester, a pentaerythritol diether, dipropylene glycol, diethylene glycol, triethylene glycol, 2-methyl-1,3-propanediol, 2,2-dimethyl-1,3-propanediol, neopentyl glycol, xylylene glycol, bis(p-hydroxy)diphenyl, bis(p-hydroxy)diphenyl propane, 2,2'-bis[4-(2-hydroxyethoxy)phenyl]propane, bis[4-(2-hydroxyethoxy)phenyl]sulfone, 1,1-bis[4-(2-hydroxy ethoxy)phenyl]cyclohexane, and combinations thereof.

8. The method according to any one of 1-7, wherein the organic solvent comprises a polyhydric alcohol selected from the group consisting of alditol, 1,2,3-propanetriol, 2-ethyl-2-hydroxymethyl-1,3-propanediol, 1,2,4-butanetriol, 1,2,5-pentanetriol, 1,2,6-hexanetriol, 1,2,3,6-hexanetetrol, glycerin (aka. glycerol), diglycerin, triglycerin, tetraglycerin, pentaglycerin, hexaglycerin, triethanolamine, trimethylol ethane, trimethylol propane, ditrimethylol propane, tri-trimethylol propane, 2-methylpropanetriol, 2-methyl-1,2,4-butanetriol, pentaerythritol, dipentaerythritol, tripentaerythritol, methylglucoside, sorbitol, mannitol, sucrose, 1,3,5-

trihydroxybenzene, 1,2,4-trihydroxybenzene, erythritol, inositol, threitol, arabitol, xylitol, ribitol, galactitol, and combinations thereof.

9. The method according to any one of 1-8, wherein the organic solvent comprises a polar aprotic compound selected from the group consisting of acetone (ACE), acetonitrile (ACN), 4-acetyl morpholine, N-cyclohexyl-2-pyrrolidone (CHP), 1,2-dimethoxyether (DME), N,N-dimethylacetamide (DMA), N,N-diethylacetamide, dimethylformamide (DMF), diethylformamide, N,N-dimethylpropionamide, 3-methoxy-N,N-dimethylpropionamide, 3-methoxy-N,N-diethylpropionamide, 3-methoxy-N,N-methylethylpropionamide, 3-ethoxy-N,N-dimethylpropionamide, 1,3-dimethyl-2 imidazolidinone (DMI), dimethyl sulfoxide (DMSO), 1,4-dioxane, 1,3-dioxolane (DN), N,N'-dimethylpropyleneurea (DMPU), hexamethylphosphoramide (HMPA), N-methyl-2-pyrrolidone (NMP), N-ethyl-2-pyrrolidone, N-methyl- ϵ -caprolactam, 2-methyltetrahydrofuran (Me-THF), 2,5-dimethyltetrahydrofuran, 4-propionyl morpholine, sulfolane, tetrahydrofuran (THF), tris(N,N-tetramethylene)phosphoric acid triamide, alcohol ethoxylate, diethyl ester dimethyl ammonium chloride, linear alkylbenzene sulfonate, and combinations thereof.

10. The method according to any one of 1-9, wherein the organic solvent is selected from the group consisting of methanol, ethanol, 1,2-propanediol, glycerin, acetone (ACE), acetonitrile (ACN), N,N-dimethylacetamide (DMA), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), N,N'-dimethylpropyleneurea (DMPU), hexamethylphosphoramide (HMPA), N-methyl-2-pyrrolidone (NMP), tetrahydrofuran (THF), and combinations thereof.

11. The method according to any one of 1-10, wherein the organic solvent is not a sugar alcohol.

12. The method according to any one of 1-11, wherein the organic solvent is miscible with water.

13. The method according to any one of 1-12, wherein the organic solvent has a boiling point of at least 150°C.

14. The method according to any one of 1-13, wherein the organic solvent has a density of at least 0.9 g/cm³.

15. The method according to any one of 1-14, wherein the organic solvent has a polarity index of at least 6.0.

16. The method according to any one of 1-15, wherein the organic solvent has a logP of less than 0 as measured at 25°C.

17. The method according to any one of 1-16, wherein the organic solvent has a logP ranging from about -0.04 to -1.5 as measured at 25°C.

18. The method according to any one of 1-17, wherein the first exchange medium comprises at least two organic solvents.

19. The method according to any one of 1-18, wherein the organic solvent comprises a dihydric alcohol and a polar aprotic compound.

5 20. The method according to any one of 1-19, wherein a proportion of the organic solvent in the first exchange medium ranges from about 1% to about 30%, based on a mass amount of the organic solvent relative to a total volume of the first exchange medium.

21. The method according to any one of 1-19, wherein a total proportion of the at least one organic solvent in the first exchange medium is less than about 30%, based on a total
10 mass of the at least one organic solvent relative to a total volume of the first exchange medium.

22. The method according to any one of 1-21, wherein the first buffer system and the second buffer system are independently selected from the group consisting of an acetate buffer system, an ADA ((N-(2-acetamido)iminoacetic acid) buffer system, an ACES (N-(2-acetamido)-2-amino ethanesulfonic acid) buffer system, an AMPD (2-amino-2-methyl-1,3-propanediol) buffer system, an AMPSO (3-(1,1-dimethyl-2 hydroxyethyl) amino-2-hydroxypropanesulfonic acid) buffer system, a BES (N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic Acid) buffer system, bicarbonate buffer system, a bicine (N,N-bis(2-hydroxyethyl)glycine) buffer system, a Bis-Tris (2-bis(2-hydroxyethyl)amino-2-(hydroxymethyl)-1,3-propanediol) buffer system, a BTP (1,3-bis(tris(hydroxymethyl)methylamino)propane) buffer system, a CABS (4-(cyclohexylamino)-1-butanefulfonic acid) buffer system, a CAPS (N-cyclohexyl-3-aminopropanesulfonic acid) buffer system, a CAPSO (3-(cyclohexylamino)-2-hydroxypropanesulphonic Acid) buffer system, a citrate buffer system, a DIPSO (3-(N,N-Bis[2-hydroxyethyl]amino)-2-hydroxypropanesulfonic acid) buffer system, an EDTA (ethylenediaminetetraacetic acid) buffer system, a Gly-Gly buffer system, a His-Glu buffer system, a HEPBS (N-(2-Hydroxyethyl)piperazine-N'-(4-butanefulfonic acid)) buffer system, a HEPES (4-(2-hydroxy ethyl)-1-piperazineethanesulfonic acid) buffer system, a HEPPSO ((2-hydroxyethyl)-piperazine-N-2-hydroxypropanesulfonic acid) buffer system, a histidine buffer system, a MES (2-(N-morpholino)ethanesulfonic acid) buffer system, a MOBS (4-(4-morpholinyl)butanesulfonic acid) buffer system, a MOPS (3-(N-morpholino)propanesulfonic acid) buffer system, a MOPSO (3-morpholino-2-hydroxypropanesulfonic acid) buffer system, a PB (phosphate buffer) buffer system, a PBS (phosphate buffered saline) buffer system, a PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)) buffer system, a POPSO (piperazine-N,N'-bis(2-hydroxypropanesulfonic acid)) buffer system, a succinate buffer system, a TES (2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethane-1-sulfonic acid) buffer system, a TAPSO (N-[tris (hydroxymethyl)methyl]-3-amino-2-

hydroxypropanesulfonic acid) buffer system, a TAE (tris-acetate-EDTA) buffer system, a tricine (N-(tri(hydroxymethyl)methyl)glycine) buffer system, and a TAPS ([tris(hydroxymethyl)methylamino]propanesulfonic acid) buffer system.

23. The method according to any one of 1-22, wherein a pH of the first exchange
5 medium ranges from about 5.0 to about 8.0.

24. The method according to any one of 1-23, wherein a pH of the second exchange medium ranges from about 5.0 to about 8.0.

25. The method according to any one of 1-24, wherein the organic UF/DF process is carried out at a flux rate ranging from about 200 L/hr/m² to about 500 L/hr/m².

10 26. The method according to any one of 1-25, wherein the aqueous UF/DF process is carried out at a flux rate ranging from about 200 L/hr/m² to about 500 L/hr/m².

27. The method according to any one of 1-26, wherein the organic UF/DF process is carried out at a transmembrane pressure ranging from about 5 psi to about 30 psi.

15 28. The method according to any one of 1-27, wherein the aqueous UF/DF process is carried out at a transmembrane pressure ranging from about 5 psi to about 30 psi.

29. The method according to any one of 1-28, wherein the organic UF/DF process is carried out such that a temperature in a retentate chamber containing the conjugate compound ranges from about 5°C to about 40°C.

20 30. The method according to any one of 1-29, wherein the aqueous UF/DF process is carried out such that a temperature in a retentate chamber containing the conjugate compound ranges from about 5°C to about 40°C.

31. The method according to any one of 1-30, wherein a diafiltration volume of the organic UF/DF process ranges from about 2 to about 20.

25 32. The method according to any one of 1-31, wherein a diafiltration volume of the aqueous UF/DF process ranges from about 2 to about 20.

33. The method according to any one of 1-32, wherein the first semi-permeable membrane and the second semi-permeable membrane are independently selected from the group consisting of a polyolefin, a polystyrene, a polysulfone, a polyester, a polyamide, a polyacrylate, a polycarbonate, and mixtures and copolymers thereof.

30 34. The method according to any one of 1-33, wherein the first semi-permeable membrane and the second semi-permeable membrane are independently selected from the group consisting of a polyethersulfone (PES), a polytetrafluoroethylene (PTFE), a polypropylene, a sulphonated polysulfone, a polyvinylidene fluoride (PVDF), a polyacrylonitrile (PAN), a cellulosic polymer, a polyimide, a polyether imide (PEI), an aliphatic polyamide, a

polyetheretherketone (PEEK), a polyphenylene oxide (PPO), a polysulfone (PSf), and mixtures and copolymers thereof.

35. The method according to any one of 1-34, wherein the first and second semi-permeable membranes are the same semi-permeable membrane.

5 36. The method according to any one of 1-35, wherein the first and second semi-permeable members are both neutral membranes that are uncharged.

37. The method according to any one of 1-6, wherein the organic UF/DF process and the aqueous UF/DF process are performed as a tangential flow filtration (TFF) process.

10 38. The method according to any one of 1-37, wherein the first and second pore sizes independently range from about 0.2 kDa MWCO to about 500 kDa MWCO .

39. The method according to any one of 1-38, wherein the conjugate compound comprises an antibody drug conjugate (ADC).

40. The method according to any one of 1-38, wherein the conjugate compound comprises a conjugate other than an antibody drug conjugate (ADC).

15 41. The method according to any one of 1-40, wherein a concentration of the conjugate compound in the crude composition ranges from about 1 mg/mL to about 100 mg/mL.

42. The method according to any one of 1-41, wherein the impurity comprises the residual free drug compound, the residual drug-linker compound, or a combination thereof.

20 43. The method according to any one of 1-38, wherein the impurity comprises the residual free drug compound.

44. The method according to 43, wherein the residual free drug compound is selected from the group consisting of a topoisomerase I (TOP1) inhibitor, a topoisomerase II (TOP2) inhibitor, a microtubule disruptor, a DNA damaging agent, a DNA intercalator, a protein degrader, a TLR7 agonist, a TLR8 agonist, a STING agonist, an XPO1 inhibitor, and any
25 combination thereof.

45. The method according to 43 or 44, wherein the residual free drug compound is selected from the group consisting of exetecan, DXd, camptothecin, SN-38 (7-ethyl-10-hydroxycamptothecin), DM1 (mertansine), a DM1 derivative, DM4 (mertansine), MMAE (monomethyl auristatin E), MMAF (monomethyl auristatin F), a leptomysin B, and any combination thereof.

30 46. The method according to any one of 1-45, wherein the impurity comprises the residual drug-linker compound.

47. The method according to 46, wherein the residual drug-linker compound comprises at least one drug moiety derived from a drug compound selected from the group consisting of a topoisomerase I (TOP1) inhibitor, a topoisomerase II (TOP2) inhibitor, a

microtubule disruptor, a DNA damaging agent, a DNA intercalator, a protein degrader, a TLR7 agonist, a TLR8 agonist, a STING agonist, an XPO1 inhibitor, and any combination thereof.

48. The method according to 47, wherein the drug compound is selected from the group consisting of exatecan, DXd, camptothecin, SN-38 (7-ethyl-10-hydroxy-camptothecin),
5 DM1 (mertansine), a DM1 derivative, DM4 (mertansine), MMAE (monomethyl auristatin E), MMAF, a DNA alkylator, a pyrrolobenzodiazepine (PBD), a leptomysin B, and any combination thereof.

49. The method according to any one of c1-48, wherein the conjugate compound comprises an antibody-drug conjugate (ADC).

10 50. The method according to 49, wherein the ADC comprises at least one drug moiety derived from a drug compound selected from the group consisting of a topoisomerase I (TOP1) inhibitor, a topoisomerase II (TOP2) inhibitor, a microtubule disruptor, a DNA damaging agent, a DNA intercalator, a protein degrader, a TLR7 agonist, a TLR8 agonist, a STING agonist, an XPO1 inhibitor, and any combination thereof.

15 51. The method according to 50, wherein the drug compound is selected from the group consisting of exetecan, DXd, camptothecin, SN-38 (7-ethyl-10-hydroxy-camptothecin), DM1 (mertansine), a DM1 derivative, DM4 (mertansine), MMAE (monomethyl auristatin E), MMAF, a pyrrolobenzodiazepine (PBD), a leptomysin B, and any combination thereof.

20 52. The method according to any one of 49-51, wherein a drug-antibody ratio of the antibody-drug conjugate (ADC) ranges from about 1:1 to about 10:1.

53. The method according to any one of 1-52, wherein the crude mixture is a conjugation mixture.

25 54. The method according to any one of 1-53, wherein a concentration of the at least one impurity in the purified composition is less than 50 µg/mL, based on a total volume of the purified composition.

55. A method for preparing a purified conjugate compound, the method comprising:

(i) reducing a targeting agent by contacting the targeting agent with a reducing agent to obtain a crude reduced targeting agent;
(ii) conjugating the reduced targeting agent with a drug-linker compound to obtain a
30 crude composition comprising a conjugate compound;
(iii) optionally adding a quenching agent to the crude composition; and
(iv) purifying the crude composition using a method according to any one of 1-54 to obtain the purified conjugate compound.

56. A method for preparing a purified conjugate compound, the method comprising:

- (i) reducing a targeting agent by contacting the targeting agent with a reducing agent to obtain a crude reduced targeting agent;
- (ii) attaching at least one linking group to the reduced targeting agent to obtain a crude composition comprising a targeting agent-linker intermediate;
- 5 (iii) attaching at least one drug compound to the linker of the targeting agent-linker intermediate to obtain a crude composition comprising a conjugate compound;
- (iv) optionally adding a quenching agent to the crude composition; and
- (v) purifying the crude composition using a UF/DF method according to any one of 1-54 to obtain the purified conjugate compound.
- 10 57. The method according to 55 or 56, wherein the targeting agent is an antibody or an antigen binding fragment, and the purified conjugate compound is a purified ADC.
58. The method according to 55 or 56, wherein the targeting agent is a non-antibody-containing targeting agent, and the purified conjugate compound is a purified conjugate other
- 15 than an ADC.
59. The method according to any one of 55-58, further comprising purifying the crude reduced targeting agent by performing an initial aqueous UF/DF process on the crude reduced targeting agent, prior to conjugating the reduced targeting agent with the drug-linker compound to obtain the conjugate compound.
- 20 60. The method according to any one of 55-59, wherein the conjugate compound comprises at least one drug moiety derived from a drug compound selected from the group consisting of a topoisomerase I (TOP1) inhibitor, a topoisomerase II (TOP2) inhibitor, a microtubule disruptor, a DNA damaging agent, a DNA intercalating agent, a protein degrader, a TLR7 agonist, a TLR8 agonist, a STING agonist, an XPO1 inhibitor, and any combination
- 25 thereof.
61. The method according to 60, wherein the drug compound is selected from the group consisting of exetecan, DXd, camptothecin, SN-38 (7-ethyl-10-hydroxy-camptothecin), DM1 (mertansine), a DM1 derivative, DM4 (mertansine), MMAE (monomethyl auristatin E), MMAF, a pyrrolobenzodiazepine (PBD), a leptomysin B, and any combination thereof.
- 30 62. The method according to any one of 55-61, wherein a concentration of residual free drug compound, residual linker compound, residual drug-linker compound, or any combination thereof, in the purified conjugate compound is less than 200 µg/mL, based on a total volume of the purified conjugate compound.

[0138] The disclosure is illustrated by the following examples, which are not intended to be limiting.

EXAMPLES

A. Abbreviations

- 5 [0139] *Ab*: antibody;
[0140] *ACE*: acetone;
[0141] *ACN*: acetonitrile;
[0142] *ADC1*: antibody drug conjugate formed from Drug-Linker Compound (LD038);
[0143] *DAD*: diode array detector;
10 [0144] *DAR*: drug-antibody ratio;
[0145] *DCM*: dichloromethane;
[0146] *DIPEA*: N,N-Diisopropylethylamine;
[0147] *DMA*: dimethylacetamide;
[0148] *DMF*: dimethylformamide;
15 [0149] *DMPU*: N,N'-Dimethylpropyleneurea;
[0150] *DMSO*: dimethylsulfoxide;
[0151] *DV*: diafiltration volume;
[0152] *EDCI*: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide;
[0153] *EDTA*: Ethylenediaminetetraacetic acid;
20 [0154] *EtOH*: ethanol;
[0155] *GLY*: glycerol;
[0156] *HATU*: 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate;
[0157] *HIS*: histidine buffer;
25 [0158] *HMPA*: Hexamethylphosphoramide;
[0159] *HOSu*: N-hydroxysuccinimide
[0160] *HPLC*: high-pressure liquid chromatography;
[0161] *LCMS*: liquid chromatography mass spectrometry;
[0162] *LMH*: L/hr/m²;
30 [0163] *MeOH*: methanol;
[0164] *MWCO*: molecular weight cut off
[0165] *MWD*: multiple wavelength detector;
[0166] *NMP*: N-methyl-2-pyrrolidone;

[0167] *LD038*: Drug-Linker compound prepared in Example 1;

[0168] *PBS*: phosphate-buffered saline;

[0169] *PD*: 1,2-propanediol;

[0170] *RCF*: relative centrifugal force;

5 [0171] *RP*: reverse phase;

[0172] *TCEP*: Tris(2-carboxyethyl) phosphine

[0173] *TFA*: trifluoroacetic acid;

[0174] *THF*: tetrahydrofuran;

[0175] *TMP*: trans membrane pressure;

10 [0176] *UPLC*: ultra performance liquid chromatography.

B. Equipment and Instruments

[0177] Table 1 below summarizes the equipment and instruments used in the following studies.

[0178] Table 1: Equipment and Instruments

15

Device Name	Manufacturer/ Model
UPLC	Agilent /1290
Electronic Balance	Mettler Toledo/XSR205DU/A
RP Chromatography Column	Agilent Poroshell 120SB-C18, 2.7 μ m, 2.1*150 mm /683775-902T
Centrifuge	Eppendorf/5424R
Milli-Q Water System	Millipore/Milli-Q IQ 7000
Pipette	Eppendorf/1-10 μ L, 2-20 μ L, 10-100 μ L, 20-200 μ L, 100-1000 μ L, 1-5 mL
Vortex Mixer	Shanghai Huxi/WH-2

C. HPLC-grade Reagents and Solvents

[0179] Table 2 below summarizes the HPLC-grade reagents and solvents used in the following studies.

20 [0180] Table 2: HPLC-grade Reagents and Solvents

Chemical Name	Grade	Manufacturer/Product Number
Trifluoroacetic acid	HPLC	Thermo scientific / 28904
Isopropanol	HPLC	J.T Baker / 9095-89

Chemical Name	Grade	Manufacturer/Product Number
Acetonitrile	HPLC	Merck / 1.00030.4008
Methanol	HPLC grade	Merck/1.06007.4008

D. Chromatography Standards

[0181] Table 3 below summarizes the chromatography standards used in the following studies.

5 [0182] Table 3: Chromatography Standards

Reference Standard	Comments
Exatecan Mesylate Standard	Use the actual strength and assay recorded in the sample management system
ADC1-TFA Standard	Use the actual strength and assay recorded in the sample management system

E. Preparation of Chromatography Solutions

10

[0183] The volumes of the chromatography solutions described below can be scaled proportionally according to requirements.

Preparation of Protein Precipitator:

15 [0184] Weigh 0.50±0.1 g of NaCl to 50 mL MeOH, mix well, and allow the solution to stand for at least 1 hour before use. Use the supernatant as the Protein Precipitator. Expiration: 3 months when stored at 10 - 30°C.

Preparation of Diluent:

[0185] Thoroughly Mix 3 mL of the Protein Precipitator described above with 1 mL of Milli-Q water.

Preparation of Mobile Phase A: 0.1% TFA/water:

20 [0186] Add 1 mL of TFA into 1000 mL of Milli-Q water and mix well by inversion. Transfer the resulting mixture to a labeled 1L solvent bottle. Preparation for Commonly-used-solution in Quality Control Laboratory for detailed instructions on preparation of this solution. Expiration: 14 days, when stored at room temperature.

25

Preparation of Mobile phase B: 0.1% TFA/CAN:

[0187] Add 1 mL of TFA into 1000 mL ACN and mix well by inversion. Transfer the resulting mixture to a labeled 1L solvent bottle. Preparation for Commonly-used-solution in Quality Control Laboratory for detailed instruction on preparation of this solution. Expiration: 1 month, when stored at room temperature.

5

F. Procedure for Chromatographic Analyses

Preparation of Blank Control:

[0188] Transfer at least 1000 µL of the Diluent described above to an HPLC vial as a Blank Control, 10 µL per injection.

10

Preparation of Exatecan standard curve stock solutions:

[0189] Weigh 50±5 mg of exatecan mesylate into a 50mL volumetric flask, then add 40mL of a 30% acetonitrile solution in water to fully dissolve the exatecan mesylate. Adjust the solution volume to 50mL with more of the 30% acetonitrile solution to prepare a bulk exatecan mesylate standard solution. Aliquot the bulk exatecan mesylate standard solution to 250µL per vial as the stock solution. Expiration: six month when storing at -80 °C. The concentration of the exatecan mesylate stock solution may be calculated using the Equation (I) below:

15

$$[\text{Exatecan Mesylate stock solution}] = \frac{\text{Exatecan Mesylate weighed weight}}{\text{Exatecan Mesylate Assay result in CoA} \times 435.46 \div 531.51} \times \text{volume} \quad (\text{I})$$

20

Preparation of ADC1 standard curve stock solutions:

[0190] Weigh 50±5 mg LD038·TFA into a 50mL volumetric flask, then add 40mL of 30% acetonitrile solution to fully dissolve the exatecan mesylate. Adjust the solution volume to 50mL with the 30% acetonitrile solution. Aliquot the LD038 solution to 250µL per vial as stock solutions. Expiration: twelve month when storing at -80 °C. The concentration of LD038 stock solution may be calculated using the Equation (II) below:

25

$$[\text{LDLD038 stock solution}] = \frac{\text{Net of LDLD038·TFA}}{\text{LD038·TFA COA} \div 2204.34 \times 2090.32} \div \text{solution volume} \times \text{Assay result of LD038} \quad (\text{II})$$

30

Preparation of standard curve solutions:

[0191] Exatecan mesylate and LD038 mixed standard curve solutions are prepared such that the exatecan concentration ranges from 0.5 µg/mL to 4.8 µg/mL, and the LD038 concentration ranges from 1.0 µg/mL to 20.0 µg/mL. The samples are thoroughly mixed and transferred to HPLC vials, such that individual samples are prepared for each concentration level.

35

Preparation of sample solution:

[0192] 100 μ L of ADC sample is added into a 1.5 mL Eppendorf tube, and then 300 μ L of the Protein Precipitator described above is added and the mixture is vortexed for 5 min at 2000 rpm using a mixer at 10 - 30°C. The resulting solution is centrifuged for 30 minutes at 20,000 RCF at 4°C maintaining the temperature $\leq 15^\circ\text{C}$. The resulting supernatant is then transferred immediately into an HPLC vial. Sample solutions can be stored at 2 - 8°C for 24 hours in the autosampler prior to performing chromatography.

Table 4 below summarizes the chromatography parameters for the chromatographic analyses described in the studies below.

[0193] Table 4: Chromatography Parameters

Column	Agilent Poroshell 120SB-C18, 2.7 μm , 2.1*150 mm /683775-902T Agilent/Eclipse Plus C18 Column, 4.6*100 mm, 3.5 μm /959961-902		
Detection wavelength	362 nm / 4 nm for DAD or MWD (Suggestion)		
Injection Volume	10 μL		
Column Temp	$45 \pm 2^\circ\text{C}$		
Autosampler thermostat	$5 \pm 3^\circ\text{C}$		
Mobile phase A	0.1% TFA/water		
Mobile phase B	0.1% TFA/ACN		
Flow Rate	0.6 mL/min		
Gradient program	Time (min)	% Mobile phase A	% Mobile phase B
	0.0	90	10
	2.0	90	10
	5.0	68	32
	20.0	58	42
	22.0	10	90
	25.0	10	90
	25.1	90	10
	30.0	90	10

15 *Calibration Curves:*

[0194] Calibration curves for exatecan and LD038 are generated by plotting total peak area of the respective peaks along the y axis versus sample concentration along the x axis—such that the linear relationship is based upon the Equation (III) below wherein k is the slope of the line and B is the intercept of the line with the y axis.

$$y = k \times x + B \quad (III)$$

Normalize Measured Concentrations Based on Dilution Factor:

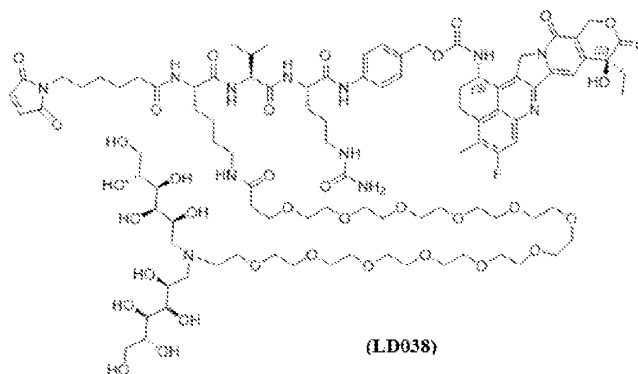
[0195] Normalized concentrations of exatecan and LD038 are calculated based on the relevant dilution factor using the Equations (IV) and (V) below, wherein “C_{Exatecan}” is the normalized concentration of exatecan in the original sample (μg/mL), “C_{calculated Exatecan}” is the measured concentration of exatecan based on the relevant calibration curve (“C_{LD038}” and “C_{calculated LD038}” are the corresponding parameters for LD038), and “dilution factor” is based on the dilution during preparation of the relevant sample solution.

$$C_{\text{Exatecan}} = C_{\text{calculated Exatecan}} \times \text{dilution factor}$$

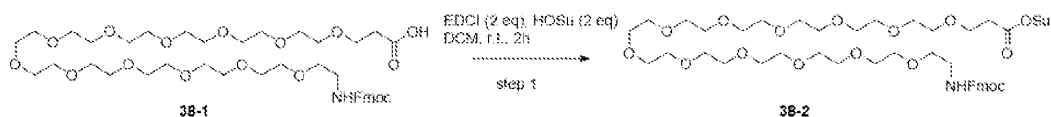
$$C_{\text{LD038}} = C_{\text{calculated LD038}} \times \text{dilution factor} \quad (V)$$

G. Preparation of Drug-Linker Compound and ADC

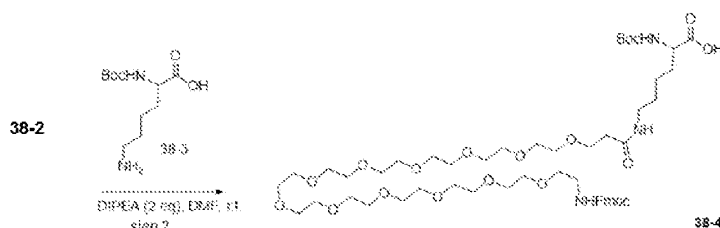
[0196] **Example 1:** Preparation of the Drug-Linker compound LD038 containing a PEG unit and a cleavable linker attached to exatecan



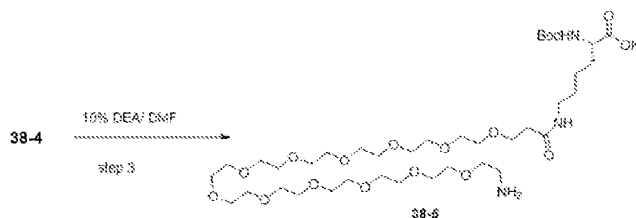
[0197] The preparation of the Drug-Linker compound LD038 is also described below.

Step 1:

A solution of compound 38-1 (650 mg, 0.774 mmol) and HOSu (177.98 mg, 1.548 mmol) in anhydrous DCM (8 mL) was stirred at room temperature, and then EDCI (296.69 mg, 1.548 mmol) was added. The resulting solution was stirred for another 1 hr at r.t. until LCMS indicated that all starting amine had disappeared and the desired product was detected. The resulting solution was washed with water, the organic layer was collected, and then the water phase was extracted with DCM (10 mL *2). The combined organic layer was dried over sodium sulfate and filtered, concentrated to dryness to give compound 38-2 (552 mg, 0.589 mmol, 76.12%) as colorless oil and used as such in the next step. LCMS: $m/z = 959.4$ ($M+Na$)⁺.

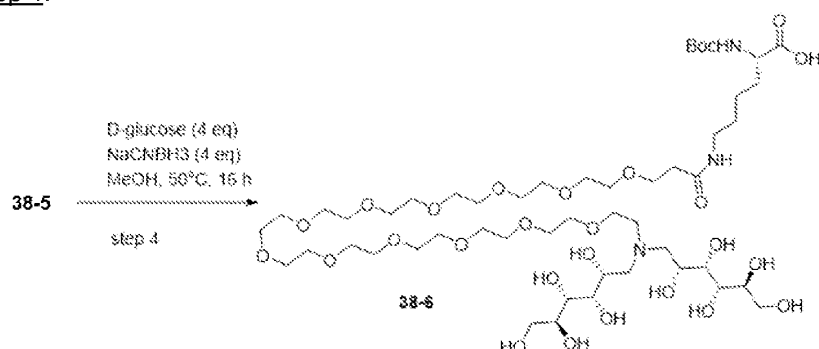
Step 2:

[0198] A solution of compound 38-2 (300 mg, 0.357 mmol) and DIPEA (138.22 mg, 1.071 mmol) in anhydrous DMF (2 mL) was stirred at room temperature, and then compound 38-3 (87.97 mg, 0.357 mmol) was added and the starting amine was suspended in the solution. The resulting mixture was kept stirring at r.t. for another 6 hrs. The starting amine dissolved gradually during this period, and the suspension turned into clear light yellow solution. The reaction solution was terminated and purified directly by reverse phase liquid chromatography (40g C18 column, eluting with 0-100% acetonitrile in water with 0.01% TFA over 15 min) to give compound 38-4 (260 mg, 0.243 mmol, 68.14%) as pale yellow oil, LCMS ($(M-100)/2+H$)⁺ = 484.9.

Step 3:

A solution of compound 38-4 (260 mg, 0.243 mmol) in acetonitrile (1.8 mL) was stirred at r.t. and diethyl amine anhydrous (0.2 mL, 1.941 mmol) was added. The resulting solution was stirred at r.t. for 2h until the LCMS of the solution showed that most of starting material was consumed. Then the solution was concentrated to dryness and the residue was purified by reverse phase column chromatography (12 g C18 column, eluting with 0-50% acetonitrile in water with 0.01% TFA) to give expected fractions of compound 38-5 (170 mg, 0.201 mmol, 82.54%) as pale yellow oil. LCMS, ESI m/z = 846.6 (M+H)⁺; Retention time (0.01% TFA) = 1.451min; no UV.

Step 4:

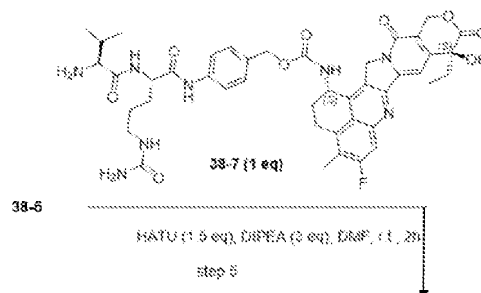


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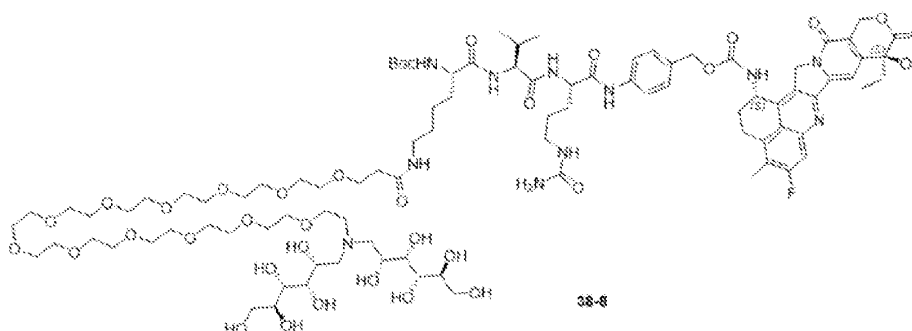
[0199] A clear reaction solution of 38-5 (170 mg, 0.201 mmol), D-glucose (217.08 mg, 1.206 mmol) and acetic acid (1.21 mg, 0.020 mmol) in methanol (5 mL) was heated at 50°C for 30 min, and then NaCNBH₃ (75.98 mg, 1.206 mmol) was added. The resulting solution was stirred at 50 °C under N₂ for 4 hr. Then additional NaCNBH₃ (75.98 mg, 1.206 mmol) and D-glucose (217.08 mg, 1.206 mmol) were added and kept stirring at 50 °C for overnight. After stirring for 20 hr, LCMS indicated the reaction was complete. The solvents were evaporated, and the residue was purified by C18 reversed-phase chromatography to give the desired product 38-6 (106 mg, 0.090 mmol, 44.92%). LCMS, ESI m/z = 537.9 ((M-100)/2+H)⁺.

15

Step 5:

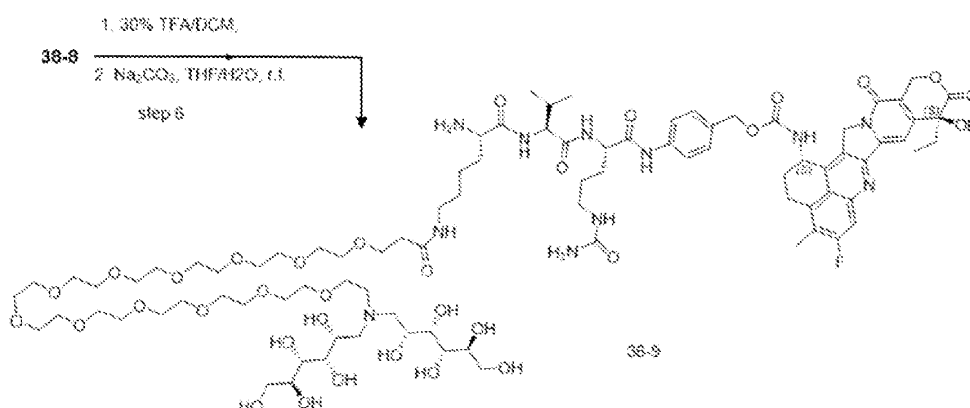


20



[0200] A solution compound 38-6 (250 mg, 0.213 mmol), HATU (121.45 mg, 0.319 mmol) and DIPEA (82.41 mg, 0.639 mmol) in anhydrous DMF (2 mL) was stirred at room temperature for 5 min, and then compound 38-7 (178.88 mg, 0.213 mmol) was added. The resulting solution was stirred for another 2 hr at r.t. until LCMS indicated a complete reaction. The reaction solution was purified directly by reverse phase liquid chromatography (40g C18 column, eluting with 0-70% acetonitrile in water with 0.01% TFA over 15 min) to give compound 38-8 (270 mg, 0.135 mmol, 63.48%) as a white solid. LCMS, ESI m/z = 666.6 ($M/3+H$)⁺, 999.2 ($M/2+H$)⁺.

10 **Step 6:**

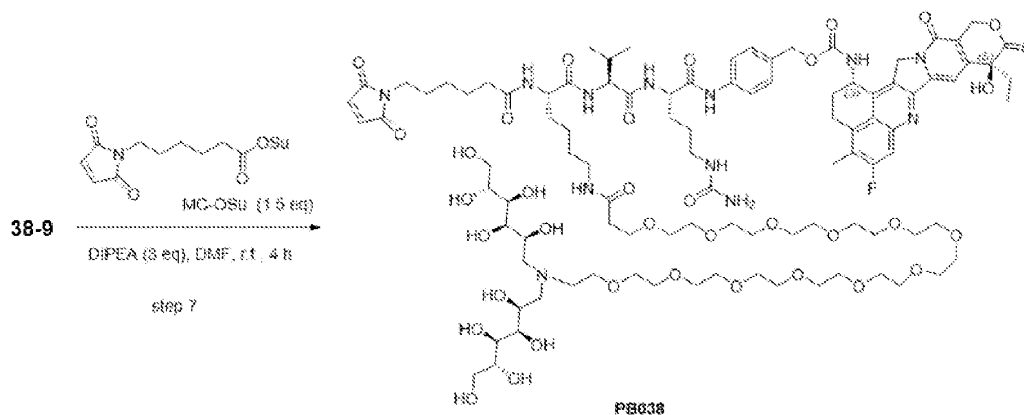


[0201] A solution of compound 38-8 (120 mg, 0.060 mmol) in TFA (2 mL) was stirred at r.t. for 1 hr. The LCMS of the mixture showed that the reaction was completed, all starting material was consumed, and the desired product (m/z = 633 = 1896/3+H, R.T. 1.501 min) along with the sugar-esterification product (TFA was condensed with hydroxy group in sugar unit, mono-ester with m/z = (1896+96)/2+H=665, R.T. 1.58 min) were formed. The completed reaction solution was condensed to dryness and then redissolved in THF (4 mL) and water (2 mL), and treated with saturated aqueous sodium carbonate solution to adjust pH to 8-9. The resulting solution was

15

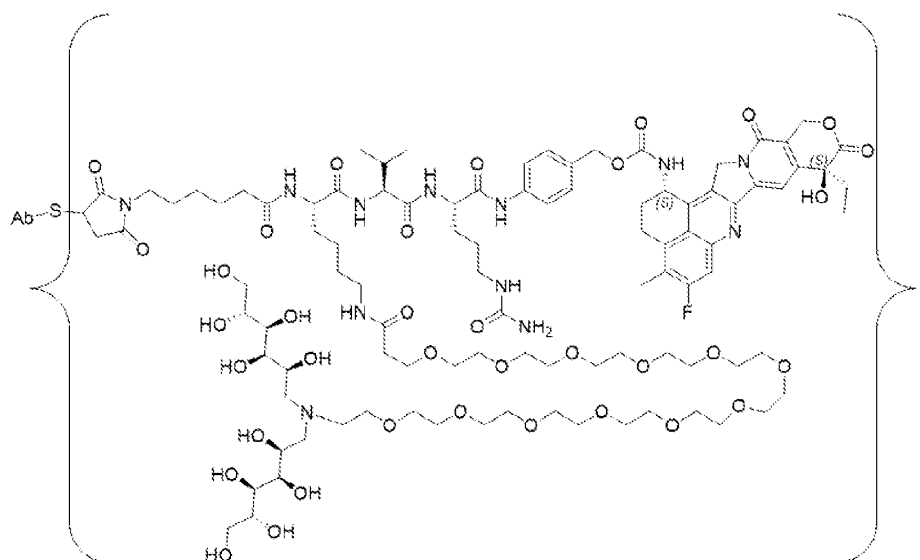
stirred at room temperature for 1h to achieve complete hydrolysis. The solution was then neutralized with diluted TFA and condensed. The residue was purified by reverse phase liquid chromatography (C18 column, eluting with 0-25% acetonitrile in water with 0.01% TFA for 15 min) to give the expected product 38-9 (80 mg, 0.042 mmol, 70.19%) as a white solid after lyophilization. LCMS, ESI $m/z = 633.2 (M/3+H)^+$, 949.2 ($M/2+H$).

Step 7:



[0202] A solution of compound 38-9 (20 mg, 0.011 mmol) and DIPEA (4.08 mg, 0.032 mmol) in anhydrous DMF (1 mL) was stirred at room temperature for 5 min, and then a solution compound 38-10 (4.88 mg, 0.016 mmol) in anhydrous DMF (1 mL) was added dropwise by syringe over 2min. The resulting solution was stirred for another 4hr at r.t. until all starting amine was disappeared and the mass of desired product was detected. The resulting solution was neutralized with formic acid to adjust pH6-7. Then the reaction solution was purified by prep. HPLC (eluting with gradient with 0.01% TFA over 20 min) to give LD038 (11 mg, 0.005 mmol, 49.91%) as a white solid (TFA salt). LCMS, $m/z = 697.7 (M/3+H)^+$; $^1\text{H NMR}$ (400MHz, DMSO- d_6): δ 10.03 (s, 1H), 8.19-8.11 (m, 2H), 8.07 (d, $J = 8.8$ Hz, 1H), 7.96 (d, $J = 7.6$ Hz, 1H), 7.82-7.77 (m, 2H), 7.66 (d, $J = 8.4$ Hz, 1H), 7.60 (d, $J = 8.4$ Hz, 2H), 7.36 (d, $J = 8.0$ Hz, 2H), 7.32 (s, 1H), 7.00 (s, 2H), 6.53 (s, 1H), 5.99 (t, $J = 5.6$ Hz, 1H), 5.45-5.43 (m, 6H), 5.30-5.24 (m, 3H), 5.08 (s, 2H), 4.84-4.74 (m, 2H), 4.65-4.49 (m, 4H), 4.45-4.35 (m, 3H), 4.27-4.17 (m, 2H), 4.04-3.95 (m, 2H), 3.80-3.77 (m, 2H), 3.71-3.67 (m, 2H), 3.62-3.55 (m, 9H), 3.53-3.43 (m, 44H), 3.27-3.21 (m, 2H), 3.16-3.07 (m, 2H), 3.07-2.93 (m, 6H), 2.38 (s, 3H), 2.29 (t, $J = 6.4$ Hz, 2H), 2.23-2.13 (m, 2H), 2.13-2.08 (m, 2H), 2.00-1.82 (m, 4H), 1.73-1.54 (m, 4H), 1.54-1.40 (m, 7H), 1.40-1.30 (m, 4H), 1.30-1.14 (m, 5H), 0.90-0.81 (m, 9H) ppm.

[0203] Example 2: Preparation of Crude Antibody-Drug Conjugate 1 (ADC1) formed from a mAb and the Drug-Linker compound LD038



[0204] 2 mL of antibody (10 mg/mL) in 50 mM sodium phosphate buffer containing 5 mM EDTA (pH = 6.9) was added to the aqueous of 10 mM TCEP HCl at the molar ratio of TCEP:mAb = 8.0. The resulting reduction reaction proceeded for 2 hours at 25°C to obtain reduced mAb in crude form. The excess TCEP and its byproduct were removed by ultrafiltration using a 50 mM sodium phosphate buffer (pH = 6.9) as the exchange medium. LD038 (as a TFA salt from Example 1) was dissolved in water at a concentration of 20 mg/mL, and the resulting solution was added to the reduced mAb at a molar ratio of 7.7 (LD038: mAb). The coupling reaction was stirred for 2 hours at 25°C. The excess LD038 and its impurities were removed by ultrafiltration with 50mM sodium phosphate buffer. The resulting crude ADC was stored in 20 mM histidine buffer containing 6% sucrose and 0.02% (w/v) Tween 20. The purity of the resulting crude ADC ("ADC1") as determined by HPLC was 97.5% and DAR value was 7.6 as determined by LC-MS using the Procedure for Chromatographic Analysis described below. The crude ADC1 was stored in different buffer systems for subsequent analyses – standard solutions in the following buffer systems were prepared.

- 50 mM PBS (pH = 7.2)
- 20 mM HIS (pH = 5.7)
- 10 mM HIS (pH = 5.6)

[0205] Example 3: The effects of different organic solvents on the UF/DF Process (Free Drug-Linker (DL) / Total DL)

[0206] A series of studies were carried out using the crude ADC1 prepared in Example 2, in which different organic solvents were used during the first UF/DF in the Studies (Std.) 3.1-3.16, as compared to the Comparative Studies (Comp. Std.) 3.1 and 3.2 performed without an organic solvent. Each study used the same UF/DF device (see Figure 2), where the membrane filter used was an Amicon Ultra-4 (UFC500396), under the process parameters shown below.

[0207] The crude ADC1 and different buffer listed in table 5 were added to Amicon Ultra-4 tube. For the inventive Studies 3.1-3.15, an UF/DF was carried out using 8 DV of a first exchange medium containing organic solvent (or mixture thereof). For the Comparative Studies 3.1 and 3.2, the first exchange medium did not include an organic solvent. Then, an aqueous UF/DF was carried out using 8 DV of exchange medium containing the same buffer system contained in the first exchange medium twice. The resulting purified ADC1, which was retained in the Amicon Ultra-4 tube, was then analysed to determine molar ratios of Free DL/Total DL. The Free DL herein included residual drug-linker compound and the Total DL included the residual drug-linker compound and a drug-linker compound in the ADC1.

[0208] Table 5 summarizes the experimental data of Example 3.

[0209] Table 5: Effect of Different Solvents on Free DL/Total DL

Std. (No.)	Organic co-solvent	Buffer	(Molar ratio: Free DL/total DL*100%)			UF/DF speed (normal, slow, very slow)
			Crude ADC1	After 3 times	After 5 times	
Comp. Std. 3.1	NA	50mM PBS Ph7.2	10.10%	4.10%	1.48%	normal
Std. 3.1	10%DMSO	50mM PBS Ph7.2	10.10%	3.83%	0.78%	normal
Std. 3.2	10%DMA	50mM PBS Ph7.2	10.10%	2.43%	0.48%	normal
Std. 3.3	20%Acetone	50mM PBS Ph7.2	10.10%	0.66%	0.46%	normal
Std. 3.4	20%Acetonitrile	50mM PBS Ph7.2	10.10%	2.41%	0.17%	normal
Std. 3.5	10%DMF	50mM PBS Ph7.2	10.10%	2.00%	0.60%	normal
Std. 3.6	15%THF	50mM PBS Ph7.2	10.10%	1.00%	0.10%	very slow
Std. 3.7	20%1,2-Propanediol	50mM PBS Ph7.2	10.10%	2.56%	0.57%	slow

Std. 3.8	20%methanol	50mM PBS Ph7.2	10.10%	1.00%	0.51%	very slow
Std. 3.9	20%Ethanol	50mM PBS Ph7.2	10.10%	0.51%	0.11%	normal
Std. 3.10	5%Acetonitrile	50mM PBS Ph7.2	10.10%	3.53%	1.55%	normal
Std. 3.11	10%Acetonitrile	50mM PBS Ph7.2	10.10%	2.42%	0.92%	normal
Std. 3.12	15%Acetonitrile	50mM PBS Ph7.2	10.10%	1.31%	0.17%	normal
Std. 3.13	15%1,2-Propanediol,5 %Acetonitrile	50mM PBS Ph7.2	10.10%	2.15%	0.61%	normal
Std. 3.14	15%1,2-Propanediol,10 %Acetonitrile	50mM PBS Ph7.2	10.10%	1.18%	0.23%	slow
Comp. Std. 3.2	NA	20mM Histine pH5.5	10.10%	3.00%	1.48%	normal
Std. 3.15	20%1,2-Propanediol	20mM Histine pH5.5	10.10%	3.10%	0.78%	normal
Std. 3.16	10%1,2-Propanediol,5 %DMSO	20mM Histine pH5.5	10.10%	3.02%	0.62%	normal

[0210] The data in Table 5 showed that the presence of the organic solvent significantly reduced free DL levels in the ADC1 samples purified using the inventive process, as shown in Studies 3.1-3.16. In particular, with the same starting free DL/total DL level at 10.10%, all the samples that employed DMSO (Std. 3.1), DMA (Std. 3.2), Acetone (Std. 3.3), Acetonitrile (Std. 3.4, 3.10-3.12), DMF (Std. 3.5), THF (Std. 3.6), PD (1,2-propanediol) (Std. 3.7, 3.13, 3.15), methanol (Std. 3.8), ethanol (Std. 3.9), and organic solvent combinations (Std. 3.13, 3.14, 3.16) in the UF/DF process demonstrated decreases in free DL/total DL levels after 3 or 5 cycles.

[0211] These three UF/DF speeds were categorized according to the time duration of conducting the UF/DF process. Generally, the “normal” speed was well known to a skilled person as the generally accepted speed for performing UF/DF; accordingly, a duration corresponding to the “slow” speed was about twice the duration corresponding to the “normal” speed; a duration corresponding to the “very slow” speed was more than twice the duration corresponding to the “slow” speed. It should also be noted that a “very slow” speed could damage the UF/DF tubes.

[0212] **Examples 4:** Studying Effect of Diafiltration Volume (DV) on the Organic UF/DF Step

[0213] A series of studies were carried out using the crude ADC1 prepared in Example 2, in which three different first exchange mediums were used to perform the inventive UF/DF process to understand how the diafiltration volume (DV) of organic UF/DF step affects the amount of

Residual Free Drug (LD038 and/or exatecan) in the retentate. Each study used the same UF/DF device (see Figure 2), where the membrane filter used was an Amicon Ultra-4 (UFC500396), under the process parameters shown below.

- Feed Flux: 300 LMH (flux value, average flow, L/hr/m²)
- TMP: 10-20 PSI
- ADC concentration: 20 mg/mL
- Loading Capacity: 300 g/cm²

[0214] Table 6 below summarizes the UF/DF process conditions of Studies (Std.) 4.1-4.3, where the three different first exchange mediums were used to perform the organic UF/DF step, and the organic UF/DF step was carried out from 0 DV to 16 DV with continual measurements being taken of the amount of Residual Free Drug in the retentate.

[0215] Table 6: Summary of UF/DF Process Conditions of Studies 5.1-5.3

Process Step	Std. 4.1	Std. 4.2	Std. 4.3	TMP (psi)	DV
	Exchange Media				
Sanitation	0.1 M NaOH			10-20	> 20 L/m ²
Equilibration before loading crude ADC1	30% PG in 10 nM HIS (pH = 5.6)	20% PG in 10 nM HIS (pH = 5.6)	10% DMSO in 50 nM PB (pH = 7.2)		Equilibrate to Stable pH
UF/DF-1					0 Dv to 16 DV
UF/DF-2	10 nM HIS (pH = 5.6)				

[0216] Table 7 below summarizes the Residual Free Drug data that was measured at different time periods throughout the organic UF/DF step—corresponding to diafiltration volumes (DVs) 0 through 16. Plots of this data are shown in Figure 3.

[0217] Table 7: Residual Free Drug Concentration as a Function of Diafiltration Volume (DV)

Diafiltration Volume (DV) of Organic UF/DF (UF/DF-1)	Std. 4.1	Std. 4.2	Std. 4.3
	30% PG in 10 nM HIS (pH = 5.6)	20% PG in 10 nM HIS (pH = 5.6)	10% DMSO in 50 nM PB (pH = 7.2)
	Residual Free Drug (µg/mL)		
0	136	118	135
2	108	99	108

4	78	79	96
6	49	58	73
8	46	52	68
10	39	49	63
12	33	39	42
14	27	36	40
16	27	35	38

[0218] As illustrated Figure 3, it was observed for each of Studies 4.1-4.3 that the amount of Residual Free Drug in the retentate during the organic UF/DF (UF/DF-1) seemed to plateau after approximately DV 14. It was also seen that the use of 30% PG in 10 nM HIS (pH = 5.6), in the first exchange medium of Study 4.1, enabled a slightly more effective removal of Residual Free Drug from the crude ADC1.

[0219] **Examples 5:** Scale-Up Purifications of ADC1

[0220] A series of studies were carried out using the crude ADC1 prepared in Example 2, in which the three different first exchange mediums were used to perform the inventive UF/DF process on a larger scale. Each study used the same UF/DF device (see Figure 2), where the membrane filter used was an Amicon Ultra-4 (UFC500396), under the process parameters shown below.

- Feed Flux: 300 LMH (flux value, average flow, L/hr/m²)
- TMP: 10-20 PSI
- Crude ADC1 concentration: 20 mg/mL
- Concentration of Residual Free Drug in Crude ADC1: ~120-140 µg/mL
- Loading Capacity: 300 g/cm²
- DV of UF/DF-1 = 16
- DV of UF/DF-2 = 8

[0221] Table 8 below summarizes experimental results for the scale-up purifications of ADC1 of Studies 5.1-5.3. Each purified ADC1 product was analyzed to determine the final concentration of ADC1 (mg/mL); the drug-antibody ratio (measured using both UV and MS detection); the percentages of high-molecular weight (HMW%), low-molecule weight (LMW%) and monomers (Monomer %) based on size exclusion chromatography (SEC); and the concentration of Residual Free Drug (LD038 and/or exatecan) in the retentate.

[0222] **Table 8:** Data Summary for Scaled-Up Purifications of ADC1

	First Exchange Medium (UF/DF-1)	Second Exchange Medium (UF/DF-2)	Conc ADC1 (mg/ml)	DAR (UV)	DAR (MS)	SEC HMW %	SEC Monomer %	SEC LMW %	Residual Free Drug (ug/ml)
Std. 5.1	30% PG in 10 nM HIS (pH=5.6)	10 nM HIS (pH=5.6)	21.0	7.2	8.0	1.3	97.6	1.2	21.3
Std. 5.2	20% PG in 10 nM HIS (pH=5.6)		22.5	7.2	8.0	1.2	97.4	1.4	30.8
Std. 5.3	10% DMSO in 50 nM PB (pH=7.2)		21.2	7.3	8.0	2.0	96.7	1.3	35.8

[0223] The data in Table 8 illustrates that the inventive UF/DF process can be used to perform scaled-up purifications of ADC1 in a manner that significantly reduces the concentration of Residual Free Drug without leading to significant amounts of degradation or denaturing of ADC1.

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[0224] **Example 6:** Synthesis of Purified ADC1

[0225] A purified ADC1 was prepared using the synthetic process summarized in Figure 4, which includes the inventive UF/DF method (labelled as “UFDF-2 Purification” in Figure 4). The purified ADC1 has a concentration of 20g/L and pH of 6.0.

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SEQUENCE LISTING

- SEQ ID NO: 1 F131 HCDR1
SYGMH
- 5 SEQ ID NO: 2 F131 HCDR2
VISYDGSNKYYADSVKG
SEQ ID NO: 3 F131 HCDR3
PRAYYGAYGSSFDY
SEQ ID NO: 4 F131 LCDR1
- 10 RASQGISSWLA
SEQ ID NO: 5 F131 LCDR2
AASSLQS
SEQ ID NO: 6 F131 LCDR3
QQSYSTPLT
- 15 SEQ ID NO: 7 2E7 HCDR1
SSDYYYWS
SEQ ID NO: 8 2E7 HCDR2
YIYYSGSTNYPNPSLKS
SEQ ID NO: 9 2E7 HCDR3
- 20 GDGDFLGVCFDY
SEQ ID NO: 10 2E7 LCDR1
RASQSVSSYLA
SEQ ID NO: 11 2E7 LCDR2
DASNRAT
- 25 SEQ ID NO: 12 2E7 LCDR3
QQRSNWPLT

CLAIMS

1. A purification method comprising:
 - (a) subjecting a sample comprising at least one conjugate compound and at least one impurity to an organic ultrafiltration/diafiltration (UF/DF) process such that the sample is filtered through a semi-permeable membrane having a pore size that allows the at least one impurity to pass through the semi-permeable membrane while retaining the at least one conjugate compound as a retentate, wherein the organic UF/DF process uses a first exchange medium and gives rise to a retentate comprising the at least one conjugate compound; and
 - (b) subjecting the retentate product of (a) to an aqueous UF/DF process such that it is filtered through a semi-permeable membrane having a pore size that allows the at least one impurity to pass through the semi-permeable membrane while retaining the conjugate compound in a retentate, wherein the aqueous UF/DF process uses a second exchange medium and results in a retentate comprising the at least one conjugate compound, wherein:
 - the at least one impurity comprises a residual free drug compound, a residual drug-linker compound, a residual linker compound, a residual byproduct compound, a residual side product compound, or any combination thereof;
 - the first exchange medium comprises at least one organic solvent and a first buffer system; and
 - the first exchange medium and the second exchange medium are different.
2. The method of claim 1, wherein prior to step (a) the method further comprises combining a crude composition comprising the at least one conjugate compound and at least one impurity with the first exchange medium to produce the sample for step (a).
3. The method of claim 1 or 2, wherein step (b) further comprises adding first exchange medium to the retentate of (a) prior to performing the aqueous UF/DF process of (b) in order to replace at least part of the volume lost in step (a).
4. The method of any one of claims 1 to 3, wherein the second exchange medium:
 - (i) does not comprise the at least one organic solvent present in the first exchange medium; or
 - (ii) does not comprise any organic solvent.

5. The method of any one of claims 1 to 4, wherein step (a) and/or (b) is/are performed more than once.
6. The method of claim 5, wherein step (b) is performed at least 3 times.
7. The method of any one of claims 1 to 6, wherein step (b) is performed at least 5 times.
8. The method according to any one of claims 1-7, wherein the separated composition is substantially free of product-bound impurities.
9. The method according to any one of claims 1-8, wherein the purified composition is substantially free of the organic solvent.
10. The method according to any one of claims 1-9, wherein the organic solvent comprises an alcohol compound, a polar aprotic compound, or a combination thereof.
11. The method according to any one of claims 1-10, wherein the organic solvent comprises a monohydric alcohol, a dihydric alcohol, a polyhydric alcohol, or a combination thereof.
12. The method according to any one of claims 1-11, wherein the organic solvent comprises a monohydric alcohol selected from the group consisting of methanol, ethanol, n-propyl alcohol, iso-propyl alcohol, 1-butanol, 2-butanol, tert-butanol, iso-butanol, 1-pentanol, 2-pentanol, 3-pentanol, iso-pentanol, tert-pentanol, a hexanol (straight chain, branched and/or cyclic), ethylene glycol monomethyl ether, cis-3-hexen-1-ol, trans-2-hexen-1-ol, 5-hexen-1-ol, a phenol, a benzyl alcohol, and the like, and combinations thereof.
13. The method according to any one of claims 1-12, wherein the organic solvent comprises a dihydric alcohol selected from the group consisting of 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 1,2-pentanediol, 1,5-pentanediol, 2,2-dimethylpropane-1,3-diol, 2-butyl-2-ethylpropane-1,3-diol, 2-methyl-2,4-pentane diol, 2-ethyl-1,3-hexane diol, 2-methyl-1,3-propane diol, 1,2-hexanediol, 1,5-hexanediol, 1,6-hexanediol, 1,8-octanediol, 1,10-decanediol, 2,2,4,4-tetramethylcyclobutane-1,3-diol, 1,3-

cyclopentanediol, 1,2-cyclohexanediol, 1,3-cyclohexanediol, 1,4-cyclohexanediol, 1,2-cyclohexanedimethanol, 1,3-cyclohexanedimethanol, 1,4-cyclohexanedimethanol, 1,4-cyclohexanediethanol, isosorbide, a glycerol monoester, a glycerol monoether, a trimethylolpropane monoester, a trimethylolpropane monoether, a pentaerythritol diester, a pentaerythritol diether, dipropylene glycol, diethylene glycol, triethylene glycol, 2-methyl-1,3-propanediol, 2,2-dimethyl-1,3-propanediol, neopentyl glycol, xylylene glycol, bis(p-hydroxy)diphenyl, bis(p-hydroxy)diphenyl propane, 2,2'-bis[4-(2-hydroxyethoxy)phenyl]propane, bis[4-(2-hydroxyethoxy)phenyl]sulfone, 1,1-bis[4-(2-hydroxy ethoxy)phenyl]cyclohexane, and combinations thereof.

14. The method according to any one of claims 1-13, wherein the organic solvent comprises a polyhydric alcohol selected from the group consisting of alditol, 1,2,3-propanetriol, 2-ethyl-2-hydroxymethyl-1,3-propanediol, 1,2,4-butanetriol, 1,2,5-pentanetriol, 1,2,6-hexanetriol, 1,2,3,6-hexanetetrol, glycerin (aka. glycerol), diglycerin, triglycerin, tetraglycerin, pentaglycerin, hexaglycerin, triethanolamine, trimethylol ethane, trimethylol propane, ditrimethylol propane, tri-trimethylol propane, 2-methylpropanetriol, 2-methyl-1,2,4-butanetriol, pentaerythritol, dipentaerythritol, tripentaerythritol, methylglucoside, sorbitol, mannitol, sucrose, 1,3,5-trihydroxybenzene, 1,2,4-trihydroxybenzene, erythritol, inositol, threitol, arabitol, xylitol, ribitol, galactitol, and combinations thereof.

15. The method according to any one of claims 1-14, wherein the organic solvent comprises a polar aprotic compound selected from the group consisting of acetone (ACE), acetonitrile (ACN), 4-acetyl morpholine, N-cyclohexyl-2-pyrrolidone (CHP), 1,2-dimethoxyether (DME), N,N-dimethylacetamide (DMA), N,N-diethylacetamide, dimethylformamide (DMF), diethylformamide, N,N-dimethylpropionamide, 3-methoxy-N,N-dimethylpropionamide, 3-methoxy-N,N-diethylpropionamide, 3-methoxy-N,N-methylethylpropionamide, 3-ethoxy-N,N-dimethylpropionamide, 1,3-dimethyl-2 imidazolidinone (DMI), dimethyl sulfoxide (DMSO), 1,4-dioxane, 1,3-dioxolane (DN), N,N'-dimethylpropyleneurea (DMPU), hexamethylphosphoramide (HMPA), N-methyl-2-pyrrolidone (NMP), N-ethyl-2-pyrrolidone, N-methyl-ε-caprolactam, 2-methyltetrahydrofuran (Me-THF), 2,5-dimethyltetrahydrofuran, 4-propionyl morpholine, sulfolane, tetrahydrofuran (THF), tris(N,N-tetramethylene)phosphoric acid triamide, alcohol ethoxylate, diethyl ester dimethyl ammonium chloride, linear alkylbenzene sulfonate, and combinations thereof.

16. The method according to any one of claims 1-15, wherein the organic solvent is selected from the group consisting of methanol, ethanol, 1,2-propanediol, glycerin, acetone (ACE), acetonitrile (ACN), N,N-dimethylacetamide (DMA), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), N,N'-dimethylpropyleneurea (DMPU), hexamethylphosphoramide (HMPA), N-methyl-2-pyrrolidone (NMP), tetrahydrofuran (THF), and combinations thereof.

17. The method according to any one of claims 1-16, wherein the organic solvent is not a sugar alcohol.

18. The method according to any one of claims 1-17, wherein the organic solvent is miscible with water.

19. The method according to any one of claims 1-18, wherein the organic solvent has a boiling point of at least 150°C.

20. The method according to any one of claims 1-19, wherein the organic solvent has a density of at least 0.9 g/cm³.

21. The method according to any one of claims 1-20, wherein the organic solvent has a polarity index of at least 6.0.

22. The method according to any one of claims 1-21, wherein the organic solvent has a logP of less than 0 as measured at 25°C.

23. The method according to any one of claims 1-22, wherein the organic solvent has a logP ranging from about -0.04 to -1.5 as measured at 25°C.

24. The method according to any one of claims 1-23, wherein the first exchange medium comprises at least two organic solvents.

25. The method according to any one of claims 1-24, wherein the organic solvent comprises a dihydric alcohol and a polar aprotic compound.

26. The method according to any one of claims 1-25, wherein the proportion of the organic solvent in the first exchange medium ranges from about 1% to about 30%, based on a mass amount of the organic solvent relative to a total volume of the first exchange medium.

27. The method according to any one of claims 1-26, wherein a total proportion of the at least one organic solvent in the first exchange medium is less than about 30%, based on a total mass of the at least one organic solvent relative to a total volume of the first exchange medium.

28. The method according to any one of claims 1-27, wherein the buffer system of the first exchange medium and the buffer system of the second exchange medium are independently selected from the group consisting of an acetate buffer system, an ADA ((N-(2-acetamido)iminoacetic acid) buffer system, an ACES (N-(2-acetamido)-2-amino ethanesulfonic acid) buffer system, an AMPD (2-amino-2-methyl-1,3-propanediol) buffer system, an AMPSO (3-(1,1-dimethyl-2 hydroxyethyl) amino-2-hydroxypropanesulfonic acid) buffer system, a BES (N,N-bis-(2-hydroxyethyl)-2-aminoethanesulfonic Acid) buffer system, bicarbonate buffer system, a bicine (N,N-bis(2-hydroxyethyl)glycine) buffer system, a Bis-Tris (2-bis(2-hydroxyethyl)amino-2-(hydroxymethyl)-1,3-propanediol) buffer system, a BTP (1,3-bis(tris(hydroxymethyl)methylamino)propane) buffer system, a CABS (4-(cyclohexylamino)-1-butanefulfonic acid) buffer system, a CAPS (N-cyclohexyl-3-aminopropanesulfonic acid) buffer system, a CAPSO (3-(cyclohexylamino)-2-hydroxypropanesulphonic Acid) buffer system, a citrate buffer system, a DIPSO (3-(N,N-Bis[2-hydroxyethyl]amino)-2-hydroxypropanesulfonic acid) buffer system, an EDTA (ethylenediaminetetraacetic acid) buffer system, a Gly-Gly buffer system, a His-Glu buffer system, a HEPBS (N-(2-Hydroxyethyl)piperazine-N'-(4-butanefulfonic acid)) buffer system, a HEPES (4-(2-hydroxy ethyl)-1-piperazineethanesulfonic acid) buffer system, a HEPPSO ((2-hydroxyethyl)-piperazine-N-2-hydroxypropanesulfonic acid) buffer system, a histidine buffer system, a MES (2-(N-morpholino)ethanesulfonic acid) buffer system, a MOBS (4-(4-morpholinyl)butanesulfonic acid) buffer system, a MOPS (3-(N-morpholino)propanesulfonic acid) buffer system, a MOPSO (3-morpholino-2-hydroxypropanesulfonic acid) buffer system, a PB (phosphate buffer) buffer system, a PBS (phosphate buffered saline) buffer system, a PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)) buffer system, a POPSO (piperazine-N,N'-bis(2-hydroxypropanesulfonic acid)) buffer system, a succinate buffer system, a TES (2-([1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino)ethane-1-sulfonic acid) buffer system, a TAPSO (N-[tris (hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonic acid) buffer system, a TAE (tris-acetate-EDTA) buffer system, a tricine

(N-(tri(hydroxymethyl)methyl)glycine) buffer system, and a TAPS ([tris(hydroxymethyl)methylamino]propanesulfonic acid) buffer system.

29. The method according to any one of claims 1-28, wherein the buffer system of the first exchange medium and the buffer system of the second exchange medium are: (i) the same; or (ii) different.

30. The method according to any one of claims 1-29, wherein a pH of the first exchange medium ranges from about 5.0 to about 8.0.

31. The method according to any one of claims 1-30, wherein a pH of the second exchange medium ranges from about 5.0 to about 8.0.

32. The method according to any one of claims 1-31, wherein the organic UF/DF process of (a) and/or the aqueous UF/DF of (b) is/are carried out in a continuous flux system.

33. The method according to any one of claims 1-32, wherein the organic UF/DF process is carried out at a flux rate ranging from about 200 L/hr/m² to about 500 L/hr/m².

34. The method according to any one of claims 1-33, wherein the aqueous UF/DF process is carried out at a flux rate ranging from about 200 L/hr/m² to about 500 L/hr/m².

35. The method according to any one of claims 1-34, wherein the organic UF/DF process is carried out at a transmembrane pressure ranging from about 5 psi to about 30 psi.

36. The method according to any one of claims 1-35, wherein the aqueous UF/DF process is carried out at a transmembrane pressure ranging from about 5 psi to about 30 psi.

37. The method according to any one of claims 1-36, wherein the organic UF/DF process is carried out such that a temperature in a retentate chamber containing the conjugate compound ranges from about 5°C to about 40°C.

38. The method according to any one of claims 1-37, wherein the aqueous UF/DF process is carried out such that a temperature in a retentate chamber containing the conjugate compound ranges from about 5°C to about 40°C.

39. The method according to any one of claims 1-38, wherein a diafiltration volume of the organic UF/DF process ranges from about 2 to about 20.

40. The method according to any one of claims 1-39, wherein a diafiltration volume of the aqueous UF/DF process ranges from about 2 to about 20.

41. The method according to any one of claims 1-40, wherein the first semi-permeable membrane and the second semi-permeable membrane are independently selected from the group consisting of a polyolefin, a polystyrene, a polysulfone, a polyester, a polyamide, a polyacrylate, a polycarbonate, and mixtures and copolymers thereof.

42. The method according to any one of claims 1-41, wherein the first semi-permeable membrane and the second semi-permeable membrane are independently selected from the group consisting of a polyethersulfone (PES), a polytetrafluoroethylene (PTFE), a polypropylene, a sulphonated polysulfone, a polyvinylidene fluoride (PVDF), a polyacrylonitrile (PAN), a cellulosic polymer, a polyimide, a polyether imide (PEI), an aliphatic polyamide, a polyetheretherketone (PEEK), a polyphenylene oxide (PPO), a polysulfone (PSf), and mixtures and copolymers thereof.

43. The method according to any one of claims 1-42, wherein the first and second semi-permeable membranes are the same semi-permeable membrane.

44. The method according to any one of claims 1-43, wherein the first and second semi-permeable members are both neutral membranes that are uncharged.

45. The method according to any one of claims 1-44, wherein the organic UF/DF process and the aqueous UF/DF process are performed as a tangential flow filtration (TFF) process.

46. The method according to any one of claims 1-45, wherein the first and second pore sizes independently range from about 0.2 kDa MWCO to about 500 kDa MWCO.

47. The method according to any one of claims 1-46, wherein the conjugate compound comprises an antibody drug conjugate (ADC).

48. The method according to any one of claims 1-47, wherein the conjugate compound comprises a conjugate other than an antibody drug conjugate (ADC).

49. The method according to any one of claims 1-48, wherein a concentration of the conjugate compound in the crude composition ranges from about 1 mg/mL to about 100 mg/mL.

50. The method according to any one of claims 1-49, wherein the at least one impurity comprises the residual free drug compound, the residual drug-linker compound, or a combination thereof.

51. The method according to any one of claims 1-50, wherein the at least one impurity comprises the residual free drug compound.

52. The method according to claim 51, wherein the residual free drug compound is selected from the group consisting of a topoisomerase I (TOP1) inhibitor, a topoisomerase II (TOP2) inhibitor, a microtubule disruptor, a DNA damaging agent, a DNA intercalator, a protein degrader, a TLR7 agonist, a TLR8 agonist, a STING agonist, an XPO1 inhibitor, and any combination thereof.

53. The method according to claim 51 or 52, wherein the residual free drug compound is selected from the group consisting of exetecan, DXd, camptothecin, SN-38 (7-ethyl-10-hydroxycamptothecin), DM1 (mertansine), a DM1 derivative, DM4 (mertansine), MMAE (monomethyl auristatin E), MMAF (monomethyl auristatin F), a leptomysin B, and any combination thereof.

54. The method according to any one of claims 1-53, wherein the impurity comprises the residual drug-linker compound.

55. The method according to claim 54, wherein the residual drug-linker compound comprises at least one drug moiety derived from a drug compound selected from the group consisting of a topoisomerase I (TOP1) inhibitor, a topoisomerase II (TOP2) inhibitor, a

microtubule disruptor, a DNA damaging agent, a DNA intercalator, a protein degrader, a TLR7 agonist, a TLR8 agonist, a STING agonist, an XPO1 inhibitor, and any combination thereof.

56. The method according to claim 55, wherein the drug compound is selected from the group consisting of exatecan, DXd, camptothecin, SN-38 (7-ethyl-10-hydroxy-camptothecin), DM1 (mertansine), a DM1 derivative, DM4 (mertansine), MMAE (monomethyl auristatin E), MMAF, a DNA alkylator, a pyrrolobenzodiazepine (PBD), a leptomysin B, and any combination thereof.

57. The method according to any one of claims 1-56, wherein the conjugate compound comprises an antibody-drug conjugate (ADC).

58. The method according to claim 57, wherein the ADC comprises at least one drug moiety derived from a drug compound selected from the group consisting of a topoisomerase I (TOP1) inhibitor, a topoisomerase II (TOP2) inhibitor, a microtubule disruptor, a DNA damaging agent, a DNA intercalator, a protein degrader, a TLR7 agonist, a TLR8 agonist, a STING agonist, an XPO1 inhibitor, and any combination thereof.

59. The method according to claim 58, wherein the drug compound is selected from the group consisting of exetecan, DXd, camptothecin, SN-38 (7-ethyl-10-hydroxy-camptothecin), DM1 (mertansine), a DM1 derivative, DM4 (mertansine), MMAE (monomethyl auristatin E), MMAF, a pyrrolobenzodiazepine (PBD), a leptomysin B, and any combination thereof.

60. The method according to any one of claims 57-59, wherein a drug-antibody ratio of the antibody-drug conjugate (ADC) ranges from about 1:1 to about 10:1.

61. The method according to any one of claims 1-60, wherein the sample is a conjugation mixture.

62. The method according to any one of claims 1-61, wherein a concentration of the at least one impurity in the purified composition is less than 50 µg/mL, based on a total volume of the purified composition.

63. The method according to any one of claims 1-62, wherein the method further comprises:

(c) formulating the retentate product of step (b) to produce a pharmaceutical composition that is suitable for administration to a subject, optionally a human subject.

64. A method for preparing a purified conjugate compound, the method comprising:

(i) reducing a targeting agent by contacting the targeting agent with a reducing agent to obtain a crude reduced targeting agent;

(ii) conjugating the reduced targeting agent with a drug-linker compound to obtain a crude composition comprising a conjugate compound;

(iii) optionally adding a quenching agent to the crude composition to give a sample; and

(iv) purifying the sample of (iii) using a method according to any one of claims 1-62 to obtain the purified conjugate compound.

65. A method for preparing a purified conjugate compound, the method comprising:

(i) reducing a targeting agent by contacting the targeting agent with a reducing agent to obtain a crude reduced targeting agent;

(ii) attaching at least one linking group to the reduced targeting agent to obtain a crude composition comprising a targeting agent-linker intermediate;

(iii) attaching at least one drug compound to the linker of the targeting agent-linker intermediate to obtain a sample comprising a conjugate compound;

(iv) optionally adding a quenching agent to the sample; and

(v) purifying the sample using a UF/DF method according to any one of claims 1-62 to obtain the purified conjugate compound.

66. The method according to claim 64 or 65, wherein the targeting agent is an antibody or an antigen binding fragment, and the purified conjugate compound is a purified ADC.

67. The method according to claim 64 or 65, wherein the targeting agent is a non-antibody-containing targeting agent, and the purified conjugate compound is a purified conjugate other than an ADC.

68. The method according to any one of claims 64-67, further comprising purifying the crude reduced targeting agent by performing an initial aqueous UF/DF process on the crude reduced targeting agent, prior to conjugating the reduced targeting agent with the drug-linker compound to obtain the conjugate compound.

69. The method according to any one of claims 64-68, wherein the conjugate compound comprises at least one drug moiety derived from a drug compound selected from the group consisting of a topoisomerase I (TOP1) inhibitor, a topoisomerase II (TOP2) inhibitor, a microtubule disruptor, a DNA damaging agent, a DNA intercalating agent, a protein degrader, a TLR7 agonist, a TLR8 agonist, a STING agonist, an XPO1 inhibitor, and any combination thereof.

70. The method according to claim 69, wherein the drug compound is selected from the group consisting of exetecan, DXd, camptothecin, SN-38 (7-ethyl-10-hydroxy-camptothecin), DM1 (mertansine), a DM1 derivative, DM4 (mertansine), MMAE (monomethyl auristatin E), MMAF, a pyrrolobenzodiazepine (PBD), a leptomysin B, and any combination thereof.

71. The method according to any one of claims 64-70, wherein a concentration of residual free drug compound, residual linker compound, residual drug-linker compound, or any combination thereof, in the purified conjugate compound is less than 200 µg/mL, based on a total volume of the purified conjugate compound.

72. A method of producing a pharmaceutical composition comprising:
(a) performing a method according to any one of claims 64 to 71; and
(b) formulating the purified conjugate compound produced in (a) to give a pharmaceutical composition comprising the purified conjugate compound and a pharmaceutically acceptable carrier or excipient.

73. A purified conjugate compound obtainable by a method according to any one of claims 1-62 or 64 to 71.

74. A pharmaceutical composition obtainable by a method of claim 63 or 72.