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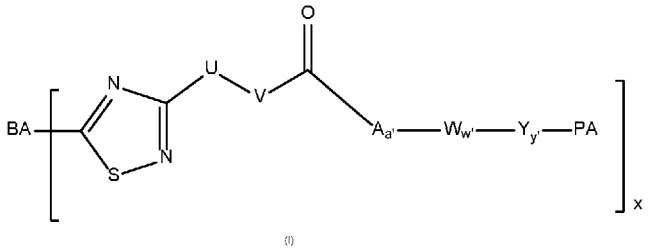

(I)

(57) Abstract: The present disclosure provides antibody drug conjugate platforms comprising a conjugator assembly component, and antibody drug conjugates comprising platform-derived linker-payloads and antibodies or antigen-binding fragments thereof. In some embodiments, an antibody drug conjugate is of the following formula (I) or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, wherein values for the variables (e.g., BA, U, V, A, a', W, w', Y, y', PA, x) are as described herein.

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BIOACTIVE CONJUGATE, PREPARATION METHOD THEREFOR AND USE THEREOF

1. CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to International Application No. PCT/CN2023/083522, filed March 23, 2023, the disclosure of which is hereby incorporated by reference in its entirety.

2. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing, which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on March 14, 2024, is named "01368-0070-00PCT-ST26", and is 5,851 bytes in size.

3. FIELD

[0003] Provided herein are antibody drug conjugate platforms and antibody drug conjugates (ADCs) comprising the platforms plus an antibody, or antigen-binding fragment thereof, as well as uses of the ADC platforms and ADCs.

4. BACKGROUND

[0004] Antibody drug conjugates (ADCs) combine the targeting effect of antibodies with the cell-killing activity of bioactive molecules, making them "biological missiles." An ADC is guided by an antibody to bind target cells, and then is internalized by cells to release bioactive molecule payloads, thereby treating relevant diseases. The bioactive molecule is covalently coupled to the antibody via a linker.

[0005] Lysine is the most common linking site in antibodies, and ε-amino groups thereof can react with activated carboxyl groups of linkers to form amide bonds. Techniques for site-specific coupling are currently available, that is, carboxyl groups of linkers are activated and then form amide bonds with specific lysine ε-amino groups in antibodies to complete the coupling. However, such amide bonds are prone to hydrolysis under the action of enzymes in vivo. As a result, bioactive molecules and antibodies dissociate before reaching target cells, resulting in off-target toxicity.

[0006] Thiol groups of antibody cysteine residues usually exist in the form of disulfide bonds. The disulfide bonds in the antibody can be broken to provide multiple free sulfhydryl groups as coupling sites. One method of coupling with the antibody sulfhydryl groups is a Michael addition reaction between the free sulfhydryl groups and an electrophilic maleimide functional group, or two Michael addition reactions between a specific substrate and free sulfhydryl groups of the antibody to

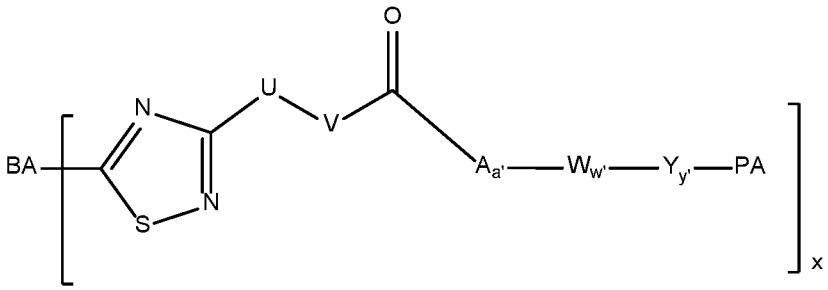
form a sulfur bridge bond in a unique structure. WO2016142049 discloses amatoxins as bioactive molecules, and structures comprising amatoxins bound to methylsulfonyl-substituted oxadiazole-based linkers, but details of coupling with antibodies are not specifically described. As has been noted by multiple investigators in the bioconjugate field, the thiol-substituted product of the reaction between the electrophilic maleimide functional group and a free thiol of an antibody is subject to slow elimination, thus reversing the reaction.

[0007] When this reversible reaction occurs in a purified preparation of an ADC, the reaction is largely undetectable because the maleimide and thiol that are regenerated through the elimination process simply react again, thus reforming the intact conjugate. However, when other thiols are present, the net effect can be the transfer of the maleimide from the antibody of the ADC onto any other available thiol. This process has been documented to occur in plasma, in which the maleimide of an ADC transfers to cysteine 34 of serum albumin (Alley et al., Bioconjugate Chem. 2008, 19, 759-765). This process has also been reported when an ADC is incubated in the presence of excess cysteine or glutathione (Jununtula et al., Nature Biotech, 2012). The present disclosure is directed to, inter alia, bioconjugates that do not undergo this transfer reaction.

5. BRIEF SUMMARY

[0008] Provided herein are antibody drug conjugate platforms and antibody drug conjugates (ADCs). Also provided are uses of the ADC platforms to prepare ADCs.

[0009] In some embodiments, provided herein are ADC compounds of Formula (I):



(I)

or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, wherein:

BA is a binding agent selected from a humanized, chimeric, or human antibody or an antigen binding fragment thereof;

U is arylene, heteroarylene, or a bond;

V is a bond or $-C\equiv C-(CH_2)_n-$;

n is an integer between 0 and 10;

A is a Stretcher unit residue;

subscript a' is 0 or 1;

W is a Cleavable unit;

subscript w' is 0 or 1;

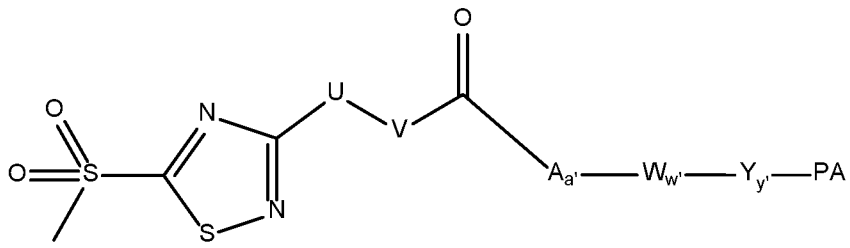
Y is a Spacer unit;

subscript y' is 0 or 1;

PA is a payload residue; and

subscript x is from 1 to 15.

[0010] In some embodiments, the platform is linker-payload compound of Formula (II):



(II)

or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, wherein:

U is arylene, heteroarylene, or a bond;

V is a bond or $-C\equiv C-(CH_2)_n-$;

n is an integer between 0 and 10;

A is a Stretcher unit residue;

subscript a' is 0 or 1;

W is a Cleavable unit;

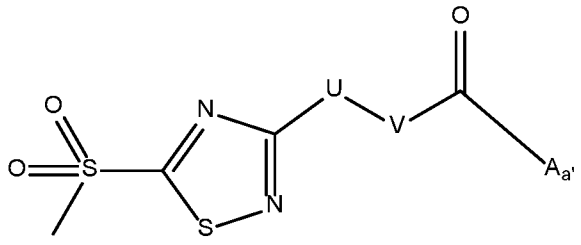
subscript w' is 0 or 1;

Y is a Spacer unit;

subscript y' is 0 or 1; and

PA is a payload residue.

[0011] In some embodiments, the platform is a linker compound of Formula (III):



(III)

or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, wherein:

U is arylene, heteroarylene, or a bond;

V is a bond or -C≡C-$(CH_2)_n$-;

n is an integer between 0 and 10;

A is a Stretcher unit; and

subscript a' is 0 or 1.

[0012] Additional objects and advantages will be set forth in part in the description that follows, and in part will be understood from the description, or may be learned by practice. The objects and advantages will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[0013] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the claims.

[0014] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate embodiments and together with the description serve to explain the principles described herein.

6. BRIEF DESCRIPTION OF FIGURES

[0015] Figure 1 depicts stability data of conjugator-antibody conjugate 3-1 in pH 7.4 and 8.0 buffer with and without GSH.

[0016] Figure 2 depicts stability data of conjugator-antibody conjugate 3-2 in pH 7.4 and 8.0 buffer with and without GSH.

[0017] Figure 3 depicts stability data of conjugator-antibody conjugate 3-3 in pH 7.4 and 8.0 buffer with and without GSH.

[0018] Figure 4 depicts stability data of conjugator-antibody conjugate 3-4 in pH 7.4 and 8.0 buffer with and without GSH.

[0019] Figure 5 depicts stability data of conjugator-antibody conjugate 3-5 in pH 7.4 and 8.0 buffer with and without GSH.

[0020] Figure 6 depicts stability data of conjugator-antibody conjugate 3-1 in pH 5.5 histidine buffer for 168 h.

[0021] Figure 7 depicts stability data of conjugator-antibody conjugate 3-2 in pH 5.5 histidine buffer for 168 h.

[0022] Figure 8 depicts stability data of conjugator-antibody conjugate 3-3 in pH 5.5 histidine buffer for 168 h.

[0023] Figure 9 depicts stability data of conjugator-antibody conjugate 3-4 in pH 5.5 histidine buffer for 168 h.

[0024] Figure 10 depicts stability data of conjugator-antibody conjugate 3-5 in pH 5.5 histidine buffer for 168 h.

[0025] Figure 11 depicts stability data of ADC 4-1 in pH 7.4 or 8.0 GSH buffer.

[0026] Figure 12 depicts stability data of ADC 4-2 in pH 7.4 or 8.0 GSH buffer.

[0027] Figure 13 depicts stability data of ADC 4-3 in pH 7.4 or 8.0 GSH buffer.

[0028] Figure 14 depicts stability data of ADC 4-1 in formulation buffer.

[0029] Figure 15 depicts stability data of ADC 4-2 in formulation buffer.

[0030] Figure 16 depicts stability data of ADC 4-3 in formulation buffer.

[0031] Figure 17 depicts ADC direct killing activity on HL60 cells.

[0032] Figure 18 depicts ADC direct killing activity on U937 cells.

[0033] Figure 19 depicts ADC direct killing activity on TF1 cells.

[0034] Figure 20 depicts ADC direct killing activity on a B7H3 high-expression cell line (H1650).

[0035] Figure 21 depicts ADC direct killing activity on a B7H3 low-expression cell line (Capan-1).

[0036] Figure 22 depicts ADC direct killing activity on a B7H3(-) cell line (MB-453).

[0037] Figure 23 depicts ADC direct killing activity on a B7H3 high-expression cell line (H1650).

[0038] Figure 24 depicts ADC direct killing activity on a B7H3 low-expression cell line (Capan-

1).

[0039] Figure 25 depicts ADC direct killing activity on a B7H3(-) cell line (MB-453).

[0040] Figure 26 depicts the ADC bystander killing on NCI-H358/MDA-MB-453 (nano-Luc) in a co-culture assay.

[0041] Figure 27 depicts the ADC bystander killing on NCI-H358/MDA-MB-453 (nano-Luc) in a co-culture assay.

[0042] Figure 28 depicts the ADC bystander killing on NCI-H358/MDA-MB-453 (nano-Luc) in a co-culture assay.

[0043] Figure 29 depicts the ADC bystander killing on NCI-H358/MDA-MB-453 (nano-Luc) in a co-culture assay.

[0044] Figure 30 is a line graph showing ADC anti-tumor activity in an H1650 xenograft model.

[0045] Figure 31 is a line graph showing dose-dependent ADC anti-tumor activity in an H1650 xenograft model.

[0046] Figure 32 is a line graph showing payload release rate from ADCs in mouse plasma.

[0047] Figure 33 is a line graph showing payload release rate from ADCs in human plasma.

[0048] Figure 34 is a line graph showing payload release rate from ADCs in mouse plasma.

[0049] Figure 35 is a line graph showing payload release rate from ADCs in human plasma.

[0050] Figure 36 is a line graph showing the PK profiles of ADCs in tumor-bearing mice.

[0051] Figure 37 is a line graph showing the PK profiles of ADCs in non-tumor-bearing mice.

7. DETAILED DESCRIPTION

[0052] Provided herein are antibody drug conjugates (ADCs) and covalent linkers and linker-payloads (platforms) for making ADCs. The ADCs may be used to treat a disease or disorder, such as cancer, such as by providing a composition comprising an ADC. The presently disclosed ADCs are more stable than known ADCs.

[0053] Some conjugators are known to be difficult to conjugate with binding agents, such as requiring harsh reaction conditions that can detrimentally modify the binding agents, or requiring multiple reaction steps. The presently disclosed conjugator assemblies are efficiently conjugated to cysteine residues on antibodies without the need for, e.g., a separate hydrolysis step. Some ADCs are known to undergo deconjugation under physiological conditions. The presently disclosed conjugator-antibody conjugates are more resistant to premature deconjugation in plasma due to

improved coupling of the disclosed conjugator assemblies to binding agents and payloads.

7.1. Definitions

[0054] In the present disclosure, the following terms have the following meanings unless indicated otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this disclosure pertains. In the event that there is a plurality of definitions for a term provided herein, these Definitions prevail unless stated otherwise.

[0055] The term "antibody" herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, monospecific antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments that exhibit the desired biological activity. An intact antibody has primarily two regions: a variable region and a constant region. The variable region binds to and interacts with a target antigen. The variable region includes a complementary determining region (CDR) that recognizes and binds to a specific binding site on a particular antigen. The constant region may be recognized by and interact with the immune system (see, e.g., Janeway et al., 2001, Immuno. Biology, 5th Ed., Garland Publishing, New York). An antibody can be of any type (e.g., IgG, IgE, IgM, IgD, and IgA), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) or subclass. The antibody can be derived from any suitable species. In some embodiments, the antibody is of human or murine origin. An antibody can be, for example, human, humanized, or chimeric.

[0056] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method.

[0057] An "intact antibody" is one that comprises an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2, CH3, and CH4, as appropriate for the antibody class. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variant thereof.

[0058] An "antibody fragment" comprises a portion of an intact antibody, comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab',

$F(ab')_2$, and Fv fragments, diabodies, triabodies, tetrabodies, linear antibodies, single-chain antibody molecules, scFv, scFv-Fc, multispecific antibody fragments formed from antibody fragment(s), a fragment(s) produced by a Fab expression library, or an epitope-binding fragment of any of the above which immunospecifically binds to a target antigen (e.g., a cancer cell antigen, a viral antigen or a microbial antigen).

[0059] An "antigen" is an entity to which an antibody specifically binds.

[0060] The terms "specific binding" and "specifically binds" mean that the antibody or antibody derivative will bind, in a highly selective manner, to its corresponding target antigen and not with the multitude of other antigens. Typically, the antibody or antibody derivative binds with an affinity of at least about 1×10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M and binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely related antigen.

[0061] The term "inhibit" or "inhibition of" means to reduce by a measurable amount, or to prevent entirely.

[0062] The term "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of a drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent or stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent or stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may inhibit growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

[0063] The term "substantial" or "substantially" refers to a majority, i.e. >50% of a population, of a mixture or a sample, such as more than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of a population.

[0064] The terms "intracellularly cleaved" and "intracellular cleavage" refer to a metabolic process or reaction inside a cell on a ligand drug conjugate (e.g., an antibody drug conjugate (ADC)), whereby the covalent attachment, e.g., the linker, between the drug moiety (D) and the ligand unit (e.g., an antibody (BA or Ab)) is broken, resulting in the free drug, or another metabolite

of the conjugate dissociated from the antibody inside the cell. The cleaved moieties of the drug-linker-ligand conjugate are thus intracellular metabolites.

[0065] The term "cytotoxic activity" refers to a cell-killing, a cytostatic or an anti-proliferative effect of a drug-linker-ligand conjugate compound or an intracellular metabolite of a drug-linker-ligand conjugate. Cytotoxic activity may be expressed as the IC50 value, which is the concentration (molar or mass) per unit volume at which half the cells survive.

[0066] The term "cytotoxic agent" as used herein refers to a substance that inhibits the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., 211At, 131I, 125I, 90Y, 186Re, 188Re, 153Sm, 212Bi, 32P, 60C, and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant, or animal origin, including synthetic analogs and derivatives thereof.

[0067] The terms "cancer" and "cancerous" refer to or describe the physiological condition or disorder in mammals that is typically characterized by unregulated cell growth. A "tumor" comprises one or more cancerous cells. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g., epithelial squamous cell cancer); lung cancer including small-cell lung cancer, non-small cell lung cancer ("NSCLC"), adenocarcinoma of the lung, and squamous carcinoma of the lung; cancer of the peritoneum; hepatocellular cancer; gastric or stomach cancer including gastrointestinal cancer; pancreatic cancer; glioblastoma; cervical cancer; ovarian cancer; liver cancer; bladder cancer; hepatoma; breast cancer; colon cancer; rectal cancer; colorectal cancer; endometrial or uterine carcinoma; salivary gland carcinoma; kidney or renal cancer; prostate cancer; vulval cancer; thyroid cancer; hepatic carcinoma; anal carcinoma; penile carcinoma; as well as head and neck cancer..

[0068] An "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or proteins.

[0069] Examples of a "patient" include, but are not limited to, mammals such as a human, rat, mouse, guinea pig, monkey, pig, goat, cow, horse, dog, or cat, and birds or fowl. In an embodiment, the patient is a human.

[0070] The terms "treat" or "treatment," unless otherwise indicated by context, refer to therapeutic treatment and prophylactic measures to prevent relapse, wherein the object is to inhibit or slow down (lessen) an undesired physiological change or disorder, such as the development or

spread of cancer. For purposes of this disclosure, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder.

[0071] In the context of cancer, the term "treating" includes any or all of inhibiting growth of tumor cells, cancer cells, or of a tumor, inhibiting replication of tumor cells or cancer cells, lessening of overall tumor burden or decreasing the number of cancerous cells, and ameliorating one or more symptoms associated with the disease.

[0072] In the context of an autoimmune disease, the term "treating" includes any or all of inhibiting replication of cells associated with an autoimmune disease state including, but not limited to, cells that produce an autoimmune antibody, lessening the autoimmune-antibody burden, and ameliorating one or more symptoms of an autoimmune disease.

[0073] As used herein, and in the specification and the accompanying claims, the indefinite articles "a" and "an" and the definite article "the" include the plural as well as single referents, unless the context clearly indicates otherwise.

[0074] As used herein, and unless otherwise specified, the terms "about" and "approximately," when used in connection with amounts, or weight percentage of ingredients of a composition, mean an amount or weight percent that is recognized by one of ordinary skill in the art to provide a pharmacological effect equivalent to that obtained from the specified amount or weight percent. In certain embodiments, the terms "about" and "approximately," when used in this context, contemplate an amount or weight percent within 30%, within 20%, within 15%, within 10%, or within 5%, of the specified amount or weight percent.

[0075] As used herein, and unless otherwise specified, the terms "about" and "approximately," when used in connection with a numeric value or range of values that is provided to characterize a particular solid form, e.g., a specific temperature or temperature range, such as, for example, that describes a melting, dehydration, desolvation, or glass transition temperature; a mass change, such as, for example, a mass change as a function of temperature or humidity; a solvent or water content, in terms of, for example, mass or a percentage; or a peak position, such as, for example, in analysis

by, for example, IR or Raman spectroscopy or XRPD; indicate that the value or range of values may deviate to an extent deemed reasonable to one of ordinary skill in the art while still describing the solid form. Techniques for characterizing crystal forms and amorphous solids include, but are not limited to, thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), single-crystal X-ray diffractometry, vibrational spectroscopy, e.g., infrared (IR) and Raman spectroscopy, solid-state and solution nuclear magnetic resonance (NMR) spectroscopy, optical microscopy, hot stage optical microscopy, scanning electron microscopy (SEM), electron crystallography and quantitative analysis, particle size analysis (PSA), surface area analysis, solubility studies, and dissolution studies. In certain embodiments, the terms "about" and "approximately," when used in this context, indicate that the numeric value or range of values may vary within 30%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1.5%, 1%, 0.5%, or 0.25% of the recited value or range of values. For example, in some embodiments, the value of an XRPD peak position may vary by up to ±0.2° 2θ while still describing the particular XRPD peak.

[0076] An "alkyl" group is a saturated, partially saturated, or unsaturated straight chain or branched non-cyclic hydrocarbon having from 1 to 10 carbon atoms, typically from 1 to 8 carbons or, in some embodiments, from 1 to 6, 1 to 4, or 2 to 6 carbon atoms. Representative alkyl groups include -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, and n-hexyl; saturated branched alkyls include -isopropyl, -*sec*-butyl, -isobutyl, -*tert*-butyl, -isopentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2,3-dimethylbutyl and the like. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, allyl, $CH{=}CH(CH_3)$, $-CH{=}C(CH_3)_2$, $-C(CH_3){=}CH_2$, $-C(CH_3){=}CH(CH_3)$, $C(CH_2CH_3){=}CH_2$, $C{\equiv}CH$, $-C{\equiv}C(CH_3)$, $-C{\equiv}C(CH_2CH_3)$, $-CH_2C{\equiv}CH$, $-CH_2C{\equiv}C(CH_3)$, and $CH_2C{\equiv}C(CH_2CH_3)$, among others. An alkyl group can be substituted or unsubstituted. In certain embodiments, when the alkyl groups described herein are said to be "substituted," they may be substituted with any substituent or substituents as those found in the compounds and embodiments disclosed herein, as well as halogen (chloro, iodo, bromo, or fluoro); hydroxyl; alkoxy; alkoxyalkyl; amino; alkylamino; carboxy; nitro; cyano; thiol; thioether; imine; imide; amidine; guanidine; enamine; aminocarbonyl; acylamino; phosphonato; phosphine; thiocarbonyl; sulfonyl; sulfone; sulfonamide; ketone; aldehyde; ester; urea; urethane; oxime; hydroxyl amine; alkoxyamine; aralkoxyamine; N-oxide; hydrazine; hydrazide; hydrazone; azide; isocyanate; isothiocyanate; cyanate; thiocyanate; $B(OH)_2$; or O(alkyl)aminocarbonyl.

[0077] An "alkenyl" group is a straight chain or branched non-cyclic hydrocarbon having from 2 to 10 carbon atoms, typically from 2 to 8 carbon atoms, and including at least one carbon-carbon

double bond. Representative straight chain and branched (C_2-C_8)alkenyls include -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, -1-hexenyl, 2-hexenyl, -3-hexenyl, -1-heptenyl, -2-heptenyl, -3-heptenyl, -1-octenyl, -2-octenyl, 3-octenyl and the like. The double bond of an alkenyl group can be unconjugated or conjugated to another unsaturated group. An alkenyl group can be unsubstituted or substituted.

[0078] A "cycloalkyl" group is a saturated or a partially saturated cyclic alkyl group of from 3 to 10 carbon atoms having a single cyclic ring or multiple condensed or bridged rings which can be optionally substituted with from 1 to 3 alkyl groups. In some embodiments, the cycloalkyl group has 3 to 8 ring members, whereas in other embodiments the number of ring carbon atoms ranges from 3 to 5, 3 to 6, or 3 to 7. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, 1-methylcyclopropyl, 2-methylcyclopentyl, 2-methylcyclooctyl, and the like, or multiple or bridged ring structures such as adamantyl and the like. Examples of unsaturated cycloalkyl groups include cyclohexenyl, cyclopentenyl, cyclohexadienyl, butadienyl, pentadienyl, and hexadienyl, among others. A cycloalkyl group can be substituted or unsubstituted. Such substituted cycloalkyl groups include, by way of example, cyclohexanone and the like.

[0079] An "aryl" group is an aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (*e.g.*, phenyl) or multiple condensed rings (*e.g.*, naphthyl or anthryl). In some embodiments, aryl groups contain 6 to 14 carbons, and in others from 6 to 12 or even 6 to 10 carbon atoms in the ring portions of the groups. Particular aryls include phenyl, biphenyl, naphthyl and the like. An aryl group can be substituted or unsubstituted. The phrase "aryl groups" also includes groups containing fused rings, such as fused aromatic-aliphatic ring systems (e.g., indanyl, tetrahydronaphthyl, and the like).

[0080] An "arylene" group is a bivalent aryl group as defined herein.

[0081] A "heteroaryl" group is an aryl ring system having one to four heteroatoms as ring atoms in a heteroaromatic ring system, wherein the remainder of the atoms are carbon atoms. In some embodiments, heteroaryl groups contain 5 to 6 ring atoms, and in others from 6 to 9 or 6 to 10 atoms in the ring portions of the groups. Suitable heteroatoms include oxygen, sulfur, and nitrogen. In certain embodiments, the heteroaryl ring system is monocyclic or bicyclic. Non-limiting examples include, but are not limited to, groups such as pyrrolyl, pyrazolyl, imidazolyl, triazolyl, tetrazolyl,

oxazolyl, isoxazolyl, thiazolyl, pyrrolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, thiophenyl, benzothiophenyl, furanyl, benzofuranyl (for example, isobenzofuran-1,3-diimine), indolyl, azaindolyl (for example, pyrrolopyridyl or 1H-pyrrolo[2,3-b]pyridyl), indazolyl, benzimidazolyl (for example, 1H-benzo[d]imidazolyl), imidazopyridyl (for example, azabenzimidazolyl, 3H-imidazo[4,5-b]pyridyl or 1H-imidazo[4,5-b]pyridyl), pyrazolopyridyl, triazolopyridyl, benzotriazolyl, benzoxazolyl, benzothiazolyl, benzothiadiazolyl, isoxazolopyridyl, thianaphthalenyl, purinyl, xanthinyl, adeninyl, guaninyl, quinolinyl, isoquinolinyl, tetrahydroquinolinyl, quinoxalinyl, and quinazolinyl groups.

[0082] A "heteroarylene" group is a bivalent heteroaryl group as defined herein.

[0083] A "heterocyclyl" is an aromatic (also referred to as heteroaryl) or non-aromatic cycloalkyl in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N. In some embodiments, heterocyclyl groups include 3 to 10 ring members, whereas other such groups have 3 to 5, 3 to 6, or 3 to 8 ring members. Heterocyclyls can also be bonded to other groups at any ring atom (*i.e.*, at any carbon atom or heteroatom of the heterocyclic ring). A heterocyclyl group can be substituted or unsubstituted. Heterocyclyl groups encompass unsaturated, partially saturated, and saturated ring systems, such as, for example, imidazolyl, imidazolinyl, and imidazolidinyl groups. The term "heterocyclyl" includes fused ring species, including those comprising fused aromatic and non-aromatic groups, such as, for example, benzotriazolyl, 2,3-dihydrobenzo[l,4]dioxinyl, and benzo[l,3]dioxolyl. The term also includes bridged polycyclic ring systems containing a heteroatom such as, but not limited to, quinuclidyl. Representative examples of a heterocyclyl group include, but are not limited to, aziridinyl, azetidinyl, pyrrolidyl, imidazolidinyl, pyrazolidinyl, thiazolidinyl, tetrahydrothiophenyl, tetrahydrofuranyl, dioxolyl, furanyl, thiophenyl, pyrrolyl, pyrrolinyl, imidazolyl, imidazolinyl, pyrazolyl, pyrazolinyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, thiazolyl, thiazolinyl, isothiazolyl, thiadiazolyl, oxadiazolyl, piperidyl, piperazinyl, morpholinyl, thiomorpholinyl, tetrahydropyranyl (for example, tetrahydro-2H-pyranyl), tetrahydrothiopyranyl, oxathiane, dioxyl, dithianyl, pyranyl, pyridyl, pyrimidinyl, pyridazinyl, pyrazinyl, triazinyl, dihydropyridyl, dihydrodithiinyl, dihydrodithionyl, homopiperazinyl, quinuclidyl, indolyl, indolinyl, isoindolyl, azaindolyl (pyrrolopyridyl), indazolyl, indolizinyl, benzotriazolyl, benzimidazolyl, benzofuranyl, benzothiophenyl, benzthiazolyl, benzoxadiazolyl, benzoxazinyl, benzodithiinyl, benzoxathiinyl, benzothiazinyl, benzoxazolyl, benzothiazolyl, benzothiadiazolyl, benzo[l,3]dioxolyl, pyrazolopyridyl, imidazopyridyl (azabenzimidazolyl; for example, 1H-imidazo[4,5-b]pyridyl, or

1H-imidazo[4,5-b]pyridin-2(3H)-onyl), triazolopyridyl, isoxazolopyridyl, purinyl, xanthinyl, adeninyl, guaninyl, quinolinyl, isoquinolinyl, quinolizinyl, quinoxalinyl, quinazolinyl, cinnolinyl, phthalazinyl, naphthyridinyl, pteridinyl, thianaphthalenyl, dihydrobenzothiazinyl, dihydrobenzofuranyl, dihydroindolyl, dihydrobenzodioxinyl, tetrahydroindolyl, tetrahydroindazolyl, tetrahydrobenzimidazolyl, tetrahydrobenzotriazolyl, tetrahydropyrrolopyridyl, tetrahydropyrazolopyridyl, tetrahydroimidazopyridyl, tetrahydrotriazolopyridyl, and tetrahydroquinolinyl groups. Representative substituted heterocyclyl groups may be mono-substituted or substituted more than once, such as, but not limited to, pyridyl or morpholinyl groups, which are 2-, 3-, 4-, 5-, or 6-substituted, or disubstituted with various substituents such as those listed below.

[0084] A "cycloalkylalkyl" group is a radical of the formula -alkyl-cycloalkyl, wherein alkyl and cycloalkyl are defined above. Substituted cycloalkylalkyl groups may be substituted at the alkyl, the cycloalkyl, or both the alkyl and the cycloalkyl portions of the group. Representative cycloalkylalkyl groups include but are not limited to cyclopentylmethyl, cyclopentylethyl, cyclohexylmethyl, cyclohexylethyl, and cyclohexylpropyl. Representative substituted cycloalkylalkyl groups may be mono-substituted or substituted more than once.

[0085] An "aralkyl" group is a radical of the formula -alkyl-aryl, wherein alkyl and aryl are defined above. Substituted aralkyl groups may be substituted at the alkyl, the aryl, or both the alkyl and the aryl portions of the group. Representative aralkyl groups include, but are not limited to, benzyl and phenethyl groups and fused (cycloalkylaryl)alkyl groups such as 4-ethyl-indanyl.

[0086] A "heterocyclylalkyl" group is a radical of the formula -alkyl-heterocyclyl, wherein alkyl and heterocyclyl are defined above. Substituted heterocyclylalkyl groups may be substituted at the alkyl, the heterocyclyl, or both the alkyl and the heterocyclyl portions of the group. Representative heterocyclylalkyl groups include, but are not limited to, 4-ethyl-morpholinyl, 4-propylmorpholinyl, furan-2-yl methyl, furan-3-yl methyl, pyrdine-3-yl methyl, (tetrahydro-2H-pyran-4-yl)methyl, (tetrahydro-2H-pyran-4-yl)ethyl, tetrahydrofuran-2-yl methyl, tetrahydrofuran-2-yl ethyl, and indol-2-yl propyl.

[0087] A "halogen" is chloro, iodo, bromo, or fluoro.

[0088] A "hydroxyalkyl" group is an alkyl group as described above substituted with one or more hydroxy groups.

[0089] An "alkoxy" group is O(alkyl), wherein alkyl is defined above.

[0090] An "alkoxyalkyl" group is (alkyl)O(alkyl), wherein alkyl is defined above.

[0091] As used herein, "alkynyl" refers to a monovalent hydrocarbon radical moiety containing at least two carbon atoms and one or more carbon-carbon triple bonds. Alkynyl is optionally substituted and can be linear, branched, or cyclic. Alkynyl includes, but is not limited to, those radicals having 2-20 carbon atoms, i.e., C_{2-20} alkynyl; 2-12 carbon atoms, i.e., C_{2-12} alkynyl; 2-8 carbon atoms, i.e., C_{2-8} alkynyl; 2-6 carbon atoms, i.e., C_{2-6} alkynyl; and 2-4 carbon atoms, i.e., C_{2-4} alkynyl. Examples of alkynyl moieties include, but are not limited to, ethynyl, propynyl, and butynyl.

[0092] As used herein, "haloalkyl" refers to alkyl, as defined above, wherein the alkyl includes at least one substituent selected from a halogen, for example, fluorine (F), chlorine (Cl), bromine (Br), or iodine (I). Examples of haloalkyl include, but are not limited to, $-CF_3$, $-CH_2CF_3$, $-CCl_2F$, and $-CCl_3$.

[0093] As used herein, "haloalkoxy" refers to alkoxy, as defined above, wherein the alkoxy includes at least one substituent selected from a halogen, e.g., F, Cl, Br, or I.

[0094] As used herein, "arylalkyl" refers to a monovalent moiety that is a radical of an alkyl compound, wherein the alkyl compound is substituted with an aromatic substituent, i.e., the aromatic compound includes a single bond to an alkyl group and wherein the radical is localized on the alkyl group. An arylalkyl group bonds to the illustrated chemical structure via the alkyl group. An arylalkyl can be represented by the structure, e.g., $B\text{-}CH_2\text{-}$, $B\text{-}CH_2\text{-}CH_2\text{-}$, $B\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}$, $B\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}$, $B\text{-}CH(CH_3)\text{-}CH_2\text{-}CH_2\text{-}$, $B\text{-}CH_2\text{-}CH(CH_3)\text{-}CH_2\text{-}$, wherein B is an aromatic moiety, e.g., phenyl. Arylalkyl is optionally substituted, i.e., the aryl group and/or the alkyl group, can be substituted as disclosed herein. Examples of arylalkyl include, but are not limited to, benzyl.

[0095] As used herein, "alkylaryl" refers to a monovalent moiety that is a radical of an aryl compound, wherein the aryl compound is substituted with an alkyl substituent, i.e., the aryl compound includes a single bond to an alkyl group and wherein the radical is localized on the aryl group. An alkylaryl group bonds to the illustrated chemical structure via the aryl group. An alkylaryl can be represented by the structure, e.g., $-B\text{-}CH_3$, $-B\text{-}CH_2\text{-}CH_3$, $-B\text{-}CH_2\text{-}CH_2\text{-}CH_3$, $-B\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_3$, $-B\text{-}CH(CH_3)\text{-}CH_2\text{-}CH_3$, $-B\text{-}CH_2\text{-}CH(CH_3)\text{-}CH_3$, wherein B is an aromatic moiety, e.g., phenyl. Alkylaryl is optionally substituted, i.e., the aryl group and/or the alkyl group, can be substituted as disclosed herein. Examples of alkylaryl include, but are not limited to, toluyl.

[0096] As used herein, "aryloxy" refers to a monovalent moiety that is a radical of an aromatic

compound wherein the ring atoms are carbon atoms and wherein the ring is substituted with an oxygen radical, i.e., the aromatic compound includes a single bond to an oxygen atom and wherein the radical is localized on the oxygen atom, e.g., C_6H_5-O-, for phenoxy. Aryloxy substituents bond to the compound which they substitute through this oxygen atom. Aryloxy is optionally substituted. Aryloxy includes, but is not limited to, those radicals having 6 to 20 ring carbon atoms, i.e., C_{6-20} aryloxy; 6 to 15 ring carbon atoms, i.e., C_{6-15} aryloxy, and 6 to 10 ring carbon atoms, i.e., C_{6-10} aryloxy. Examples of aryloxy moieties include, but are not limited to phenoxy, naphthoxy, and anthroxy.

[0097] An "amino" group is a radical of the formula NH_2.

[0098] A "hydroxyl amine" group is a radical of the formula $N(R^{\#})OH$ or NHOH, wherein $R^{\#}$ is a substituted or unsubstituted alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heterocyclyl or heterocyclylalkyl group as defined herein.

[0099] An "alkoxyamine" group is a radical of the formula $-N(R^{\#})O$-alkyl or -NHO-alkyl, wherein $R^{\#}$ is as defined above.

[0100] An "aralkoxyamine" group is a radical of the formula $N(R^{\#})O$-aryl or NHOaryl, wherein $R^{\#}$ is as defined above.

[0101] An "alkylamine" group is a radical of the formula NHalkyl or $N(alkyl)_2$, wherein each alkyl is independently as defined above.

[0102] An "aminocarbonyl" group is a radical of the formula $-C(=O)N(R^{\#})_2$, $-C(=O)NH(R^{\#})$, or $C(=O)NH_2$, wherein each $R^{\#}$ is as defined above.

[0103] An "acylamino" group is a radical of the formula $NHC(=O)(R^{\#})$ or $N(alkyl)C(=O)(R^{\#})$, wherein each alkyl and $R^{\#}$ are independently as defined above.

[0104] An "O(alkyl)aminocarbonyl" group is a radical of the formula $-O(alkyl)C(=O)N(R^{\#})_2$, $-O(alkyl)C(=O)NH(R^{\#})$, or $-O(alkyl)C(=O)NH_2$, wherein each $R^{\#}$ is independently as defined above.

[0105] An "N-oxide" group is a radical of the formula $-N^{+}$-O^{-}.

[0106] A "carboxy" group is a radical of the formula C(=O)OH.

[0107] A "ketone" group is a radical of the formula $C(=O)(R^{\#})$, wherein $R^{\#}$ is as defined above.

[0108] An "aldehyde" group is a radical of the formula -CH(=O).

[0109] An "ester" group is a radical of the formula $C(=O)O(R^{\#})$ or $OC(=O)(R^{\#})$, wherein $R^{\#}$ is as defined above.

[0110] A "urea" group is a radical of the formula -N(alkyl)C(=O)N($R^{\#}$)$_2$, -N(alkyl)C(=O)NH($R^{\#}$), -N(alkyl)C(=O)NH_2, -NHC(=O)N($R^{\#}$)$_2$, -NHC(=O)NH($R^{\#}$), or NHC(=O)$NH_2^{\#}$, wherein each alkyl and $R^{\#}$ are independently as defined above.

[0111] An "imine" group is a radical of the formula -N=C($R^{\#}$)$_2$ or -C($R^{\#}$)=N($R^{\#}$), wherein each $R^{\#}$ is independently as defined above.

[0112] An "imide" group is a radical of the formula -C(=O)N(R#)C(=O)($R^{\#}$) or N((C=O)($R^{\#}$))$_2$, wherein each $R^{\#}$ is independently as defined above.

[0113] A "urethane" group is a radical of the formula -OC(=O)N($R^{\#}$)$_2$, -OC(=O)NH($R^{\#}$), -N($R^{\#}$)C(=O)O($R^{\#}$), or -NHC(=O)O($R^{\#}$), wherein each $R^{\#}$ is independently as defined above.

[0114] An "amidine" group is a radical of the formula -C(=N($R^{\#}$))N($R^{\#}$)$_2$, -C(=N($R^{\#}$))NH($R^{\#}$), -C(=N($R^{\#}$))NH_2, -C(=NH)N($R^{\#}$)$_2$, -C(=NH)NH($R^{\#}$), -C(=NH)NH_2, -N=C($R^{\#}$)N($R^{\#}$)$_2$, -N=C($R^{\#}$)NH($R^{\#}$), -N=C($R^{\#}$)NH_2, -N($R^{\#}$)C($R^{\#}$)=N($R^{\#}$), -NHC($R^{\#}$)=N($R^{\#}$), -N($R^{\#}$)C($R^{\#}$)=NH, or -NHC($R^{\#}$)=NH, wherein each $R^{\#}$ is independently as defined above.

[0115] A "guanidine" group is a radical of the formula -N($R^{\#}$)C(=N($R^{\#}$))N($R^{\#}$)$_2$, -NHC(=N($R^{\#}$))N($R^{\#}$)$_2$, -N($R^{\#}$)C(=NH)N($R^{\#}$)$_2$, -N($R^{\#}$)C(=N($R^{\#}$))NH($R^{\#}$), -N($R^{\#}$)C(=N($R^{\#}$))NH_2, -NHC(=NH)N($R^{\#}$)$_2$, -NHC(=N($R^{\#}$))NH($R^{\#}$), -NHC(=N($R^{\#}$))NH_2, -NHC(=NH)NH($R^{\#}$), -NHC(=NH)NH_2, -N=C(N($R^{\#}$)$_2$)$_2$, -N=C(NH($R^{\#}$))$_2$, or -N=C(NH_2)$_2$, wherein each $R^{\#}$ is independently as defined above.

[0116] An "enamine" group is a radical of the formula -N($R^{\#}$)C($R^{\#}$)=C($R^{\#}$)$_2$, -NHC($R^{\#}$)=C($R^{\#}$)$_2$, -C(N($R^{\#}$)$_2$)=C($R^{\#}$)$_2$, -C(NH($R^{\#}$))=C($R^{\#}$)$_2$, -C(NH_2)=C($R^{\#}$)$_2$, -C($R^{\#}$)=C($R^{\#}$)(N($R^{\#}$)$_2$), C($R^{\#}$)=C($R^{\#}$)(NH($R^{\#}$)) or -C($R^{\#}$)=C($R^{\#}$)(NH_2), wherein each $R^{\#}$ is independently as defined above.

[0117] An "oxime" group is a radical of the formula -C(=NO($R^{\#}$))($R^{\#}$), -C(=NOH)($R^{\#}$), -CH(=NO($R^{\#}$)), or -CH(=NOH), wherein each $R^{\#}$ is independently as defined above.

[0118] A "hydrazide" group is a radical of the formula -C(=O)N($R^{\#}$)N($R^{\#}$)$_2$, -C(=O)NHN($R^{\#}$)$_2$, -C(=O)N($R^{\#}$)NH($R^{\#}$), -C(=O)N($R^{\#}$)NH_2, -C(=O)NHNH($R^{\#}$)$_2$, or -C(=O)NHNH_2, wherein each $R^{\#}$ is independently as defined above.

[0119] A "hydrazine" group is a radical of the formula -N($R^{\#}$)N($R^{\#}$)$_2$, -NHN($R^{\#}$)$_2$, -N($R^{\#}$)NH($R^{\#}$), -N($R^{\#}$)NH_2, -NHNH($R^{\#}$)$_2$, or -NHNH_2, wherein each $R^{\#}$ is independently as defined above.

[0120] A "hydrazone" group is a radical of the formula -C(=N-N($R^{\#}$)$_2$)($R^{\#}$)$_2$, -C(=NNH($R^{\#}$))($R^{\#}$)$_2$, -C(=N-NH_2)($R^{\#}$)$_2$, -N($R^{\#}$)(N=C($R^{\#}$)$_2$), or -NH(N=C($R^{\#}$)$_2$), wherein each $R^{\#}$ is

independently as defined above.

[0121] An "azide" group is a radical of the formula $-N_3$.

[0122] An "isocyanate" group is a radical of the formula N=C=O.

[0123] An "isothiocyanate" group is a radical of the formula N=C=S.

[0124] A "cyanate" group is a radical of the formula OCN.

[0125] A "thiocyanate" group is a radical of the formula SCN.

[0126] A "thioether" group is a radical of the formula $-S(R^{\#})$, wherein $R^{\#}$ is as defined above.

[0127] A "thiocarbonyl" group is a radical of the formula $-C(=S)(R^{\#})$, wherein $R^{\#}$ is as defined above.

[0128] A "sulfinyl" group is a radical of the formula $-S(=O)(R^{\#})$, wherein $R^{\#}$ is as defined above.

[0129] A "sulfone" group is a radical of the formula $-S(=O)_2(R^{\#})$, wherein $R^{\#}$ is as defined above.

[0130] A "sulfonylamino" group is a radical of the formula $-NHSO_2(R^{\#})$ or $-N(alkyl)SO_2(R^{\#})$, wherein each alkyl and $R^{\#}$ are defined above.

[0131] A "sulfonamide" group is a radical of the formula $-S(=O)_2N(R^{\#})_2$, $-S(=O)_2NH(R^{\#})$, or $-S(=O)_2NH_2$, wherein each $R^{\#}$ is independently as defined above.

[0132] A "phosphonate" group is a radical of the formula $-P(=O)(O(R^{\#}))_2$, $-P(=O)(OH)_2$, $-OP(=O)(O(R^{\#}))(R^{\#})$, or $-OP(=O)(OH)(R^{\#})$, wherein each $R^{\#}$ is independently as defined above.

[0133] A "phosphine" group is a radical of the formula $-P(R^{\#})_2$, wherein each $R^{\#}$ is independently as defined above.

[0134] When the groups described herein, with the exception of alkyl groups, are said to be "substituted," they may be substituted with any appropriate substituent or substituents. Illustrative examples of substituents are those found in the compounds and embodiments disclosed herein, as well as halogen (chloro, iodo, bromo, or fluoro); alkyl; hydroxyl; alkoxy; alkoxyalkyl; amino; alkylamino; carboxy; nitro; cyano; thiol; thioether; imine; imide; amidine; guanidine; enamine; aminocarbonyl; acylamino; phosphonate; phosphine; thiocarbonyl; sulfinyl; sulfone; sulfonamide; ketone; aldehyde; ester; urea; urethane; oxime; hydroxyl amine; alkoxyamine; aralkoxyamine; N-oxide; hydrazine; hydrazide; hydrazone; azide; isocyanate; isothiocyanate; cyanate; thiocyanate; oxygen (=O); $B(OH)_2$, O(alkyl)aminocarbonyl; cycloalkyl, which may be monocyclic or fused or non-fused polycyclic (*e.g.*, cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl), or a heterocyclyl,

which may be monocyclic or fused or non-fused polycyclic (*e.g.*, pyrrolidyl, piperidyl, piperazinyl, morpholinyl, or thiazinyl); monocyclic or fused or non-fused polycyclic aryl or heteroaryl (*e.g.*, phenyl, naphthyl, pyrrolyl, indolyl, furanyl, thiophenyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, triazolyl, tetrazolyl, pyrazolyl, pyridinyl, quinolinyl, isoquinolinyl, acridinyl, pyrazinyl, pyridazinyl, pyrimidinyl, benzimidazolyl, benzothiophenyl, or benzofuranyl) aryloxy; aralkyloxy; heterocyclyloxy; and heterocyclyl alkoxy.

[0135] As used herein, the term "pharmaceutically acceptable salt(s)" refers to a salt prepared from a pharmaceutically acceptable non-toxic acid or base including an inorganic acid or base and an organic acid or base.

[0136] As used herein and unless otherwise indicated, the term "solvate" means a compound, or a salt thereof, that further includes a stoichiometric or non-stoichiometric amount of a solvent bound by non-covalent intermolecular forces. In one embodiment, the solvate is a hydrate.

[0137] As used herein and unless otherwise indicated, the term "hydrate" means a compound, or a salt thereof, that further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.

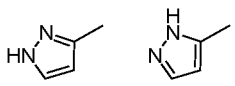
[0138] As used herein and unless otherwise indicated, the term "prodrug" means a compound derivative that can hydrolyze, oxidize, or otherwise react under biological conditions (*in vitro* or *in vivo*) to provide an active compound. Examples of prodrugs include, but are not limited to, derivatives and metabolites of a compound that include biohydrolyzable moieties such as biohydrolyzable amides, biohydrolyzable esters, biohydrolyzable carbamates, biohydrolyzable carbonates, biohydrolyzable ureides, and biohydrolyzable phosphate analogues. In certain embodiments, prodrugs of compounds with carboxyl functional groups are the lower alkyl esters of the carboxylic acid. The carboxylate esters may be formed by esterifying any of the carboxylic acid moieties present on the molecule. Prodrugs can typically be prepared using well-known methods, such as those described by *Burger's Medicinal Chemistry and Drug Discovery* 6th ed. (Donald J. Abraham *ed.*, 2001, Wiley) and *Design and Application of Prodrugs* (H. Bundgaard *ed.*, 1985, Harwood Academic Publishers Gmfh).

[0139] As used herein and unless otherwise indicated, the term "stereoisomer" or "stereomerically pure" means one stereoisomer of a compound that is substantially free of other stereoisomers of that compound. For example, a stereomerically pure compound having one chiral center will be substantially free of the opposite enantiomer of the compound. A stereomerically

pure compound having two chiral centers will be substantially free of other diastereomers of the compound. A typical stereomerically pure compound comprises greater than about 80% by weight of one stereoisomer of the compound and less than about 20% by weight of other stereoisomers of the compound, greater than about 90% by weight of one stereoisomer of the compound and less than about 10% by weight of the other stereoisomers of the compound, greater than about 95% by weight of one stereoisomer of the compound and less than about 5% by weight of the other stereoisomers of the compound, or greater than about 97% by weight of one stereoisomer of the compound and less than about 3% by weight of the other stereoisomers of the compound. The compounds can have chiral centers and can occur as racemates, individual enantiomers or diastereomers, and mixtures thereof. All such isomeric forms are included within the embodiments disclosed herein, including mixtures thereof. The use of stereomerically pure forms of such compounds, as well as the use of mixtures of those forms, are encompassed by the embodiments disclosed herein. For example, mixtures comprising equal or unequal amounts of the enantiomers of a particular compound may be used in methods and compositions disclosed herein. These isomers may be asymmetrically synthesized or resolved using standard techniques such as chiral columns or chiral resolving agents. *See, e.g.*, Jacques, J., *et al., Enantiomers, Racemates and Resolutions* (WileyInterscience, New York, 1981); Wilen, S. H., *et al., Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGrawHill, NY, 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN, 1972).

[0140] It should also be noted that the compounds can include E and Z isomers, or a mixture thereof, and cis and trans isomers, or a mixture thereof. In certain embodiments, the compounds are isolated as either the cis or trans isomer. In other embodiments, the compounds are a mixture of the cis and trans isomers.

[0141] "Tautomers" refers to isomeric forms of a compound that are in equilibrium with each other. The concentrations of the isomeric forms will depend on the environment the compound is found in and may be different depending upon, for example, whether the compound is a solid or is in an organic or aqueous solution. For example, in an aqueous solution, pyrazoles may exhibit the following isomeric forms, which are referred to as tautomers of each other:



[0142] As readily understood by one skilled in the art, a wide variety of functional groups and

other structures may exhibit tautomerism and all tautomers of the compounds are within the scope of the present disclosure.

[0143] It should also be noted the compounds can contain unnatural proportions of atomic isotopes at one or more of the atoms. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I), sulfur-35 (^{35}S), or carbon-14 (^{14}C), or may be isotopically enriched, such as with deuterium (^{2}H), carbon-13 (^{13}C), or nitrogen-15 (^{15}N). As used herein, an "isotopologue" is an isotopically enriched compound. The term "isotopically enriched" refers to an atom having an isotopic composition other than the natural isotopic composition of that atom. "Isotopically enriched" may also refer to a compound containing at least one atom having an isotopic composition other than the natural isotopic composition of that atom. The term "isotopic composition" refers to the amount of each isotope present for a given atom. Radiolabeled and isotopically enriched compounds are useful as therapeutic agents, e.g., cancer and inflammation therapeutic agents, research reagents, e.g., binding assay reagents, and diagnostic agents, e.g., in vivo imaging agents. All isotopic variations of the compounds as described herein, whether radioactive or not, are intended to be encompassed within the scope of the embodiments provided herein. In some embodiments, there are provided isotopologues of the compounds, for example, the isotopologues are deuterium, carbon-13, or nitrogen-15 enriched compounds.

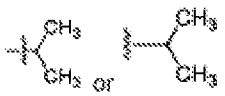
[0144] It should be noted that if there is a discrepancy between a depicted structure and a name for that structure, the depicted structure is to be accorded more weight It should be noted that if there is a discrepancy between a depicted structure and a name for that structure, the depicted structure is to be accorded more weight.

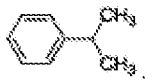
[0145] As used herein, the term "residue" refers to the chemical moiety within a compound that remains after a chemical reaction. For example, the term "amino acid residue" or "N-alkyl amino acid residue" refers to the product of an amide coupling or peptide coupling of an amino acid or a N-alkyl amino acid to a suitable coupling partner; wherein, for example, a water molecule is expelled after the amide or peptide coupling of the amino acid or the N-alkylamino acid, resulting in the product having the amino acid residue or N-alkyl amino acid residue incorporated therein.

[0146] As used herein, "sugar" or "sugar group" or "sugar residue" refers to a carbohydrate moiety which may comprise 3-carbon (those) units, 4-carbon (tetrose) units, 5-carbon (pentose) units, 6-carbon (hexose) units, 7-carbon (heptose) units, or combinations thereof, and may be a

monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, an oligosaccharide, or any other polysaccharide. In some instances, a "sugar" or "sugar group" or "sugar residue" comprises furanoses (e.g., ribofuranose, fructofuranose) or pyranoses (e.g., glucopyranose, galactopyranose), or a combination thereof. In some instances, a "sugar" or "sugar group" or "sugar residue" comprises aldoses or ketoses, or a combination thereof. Non-limiting examples of monosaccharides include ribose, deoxyribose, xylose, arabinose, glucose, mannose, galactose, and fructose. Non-limiting examples of disaccharides include sucrose, maltose, lactose, lactulose, and trehalose. Other "sugars" or "sugar groups" or "sugar residues" include polysaccharides and/or oligosaccharides, including, but not limited to, amylose, amylopectin, glycogen, inulin, and cellulose. In some instances, a "sugar" or "sugar group" or "sugar residue" is an amino-sugar. In some instances, a "sugar" or "sugar group" or "sugar residue" is a glucamine residue (1-amino-1-deoxy-D-glucitol) linked to the rest of molecule via its amino group to form an amide linkage with the rest of the molecule (i.e., a glucamide).

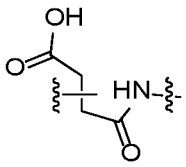
[0147] Certain groups, moieties, substituents, and atoms are depicted with a wiggly line, e.g., that intersects a bond or bonds, to indicate the atom through which the groups, moieties, substituents, atoms are bonded. For example, a phenyl group that is substituted with a propyl group depicted as:

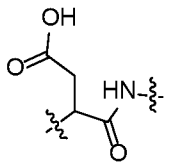


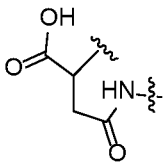
has the following structure: 

.

[0148] Illustrations showing substituents bonded to a non-cyclic group through a bond between two atoms are meant to indicate, unless specified otherwise, that the substituent may be bonded to either atom of the bond through which the substituent bond passes, according to techniques set forth herein or which are known in the field to which the instant disclosure pertains. Thus, for example,



encompasses 

and 

.

[0149] As used herein, "binding agent" refers to any molecule, e.g., antibody, capable of binding with specificity to a given binding partner, e.g., antigen.

[0150] As used herein, the term "amino acid" refers to an organic compound that contains amino ($-NH_2$) and carboxyl (-COOH) functional groups, along with a side chain (R group), which is

specific to each amino acid. Amino acids may be proteinogenic or non-proteinogenic. By "proteinogenic" is meant that the amino acid is one of the twenty naturally occurring amino acids found in proteins. The proteinogenic amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. By "non-proteinogenic" is meant that either the amino acid is not found naturally in protein or is not directly produced by cellular machinery (e.g., is the product of post-translational modification). Non-limiting examples of non-proteinogenic amino acids include gamma-aminobutyric acid (GABA), taurine (2-aminoethanesulfonic acid), theanine (L-γ-glutamylethylamide), hydroxyproline, beta-alanine, ornithine, and citrulline.

[0151] As used herein "peptide", in its various grammatical forms, is defined in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs, or other peptidomimetics. The subunits may be linked by peptide bonds or by other bonds, for example, ester, ether, and the like. As used herein, the term "amino acid" refers to either natural and/or unnatural, proteinogenic or non-proteinogenic, or synthetic amino acids, including glycine and both the D and L optical isomers, and amino acid analogs and peptidomimetics. If the peptide chain is short, e.g., two, three or more amino acids, it is commonly called an oligopeptide. If the peptide chain is longer, the peptide is typically called a polypeptide or a protein. Full-length proteins, analogs, mutants, and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, acetylation, phosphorylation, and the like. Furthermore, as ionizable amino and carboxyl groups are present in the molecule, a particular peptide may be obtained as an acidic or basic salt, or in neutral form. A peptide may be obtained directly from the source organism or may be recombinantly or synthetically produced.

[0152] The amino acid sequence of an antibody can be numbered using any known numbering schemes, including those described by Kabat et al., ("Kabat" numbering scheme); Al-Lazikani et al., 1997, J. Mol. Biol., 273:927-948 ("Chothia" numbering scheme); MacCallum et al., 1996, J. Mol. Biol. 262:732-745 ("Contact" numbering scheme); Lefranc et al., Dev. Comp. Immunol., 2003, 27:55-77 ("IMGT" numbering scheme); and Honegge and Pluckthun, J. Mol. Biol., 2001, 309:657-70 ("AHo" numbering scheme). Unless otherwise specified, the numbering scheme used herein is the Kabat numbering scheme. However, selection of a numbering scheme is not intended to imply differences in sequences where they do not exist, and one of skill in the art can readily confirm a

sequence position by examining the amino acid sequence of one or more antibodies. Unless stated otherwise, the "EU numbering scheme" is generally used when referring to a residue in an antibody heavy chain constant region (e.g., as reported in Kabat et al., supra).

[0153] As used herein, the term "anti-HER2 antibody" refers to an antibody selectively binding to the HER2 receptor, e.g., trastuzumab (Herceptin). In one embodiment, trastuzumab can be made and used as described in US6407213 and US5821337, the entire disclosures of which are incorporated herein by reference.

[0154] As used herein, the term "ifinatamab" refers to an antibody selectively binding to the B7H3 receptor. In one embodiment, ifinatamab can be made and used as described in US10117952 or WO2022102695, the entire disclosures of which are incorporated herein by reference.

[0155] As used herein, the term "cell-killing activity" refers to the activity that decreases or reduces the cell viability of the tested cell line.

[0156] In the claims that follow and in the preceding description, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e., to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments.

7.2. Conjugates

[0157] In embodiments, a conjugate, or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, includes a protein linked to at least one payload or payload residue (also referred to herein as a Drug unit) and linked to at least one hydrophilic moiety via a covalent linker. The covalent linker is bonded directly or indirectly to each of the protein, the payload residue, and the hydrophilic moiety. In some embodiments, the protein is a binding agent, such as an antibody or antigen binding fragment thereof.

[0158] In some embodiments, the protein is bonded directly to a covalent linker, such as a linker set forth herein. In such cases, the binding agent is one bond position away from the covalent linker. The covalent linker may also be bonded directly to a payload residue such that the covalent linker is one bond position away from a payload residue. The payload may be any payload set forth herein. In some embodiments, the covalent linker is also bonded directly to a hydrophilic moiety such that the covalent linker is one bond position away from a hydrophilic moiety. The hydrophilic moiety may be any hydrophilic moiety (HG) set forth herein.

[0159] In some embodiments, the binding agent is bonded indirectly to a covalent linker such that the binding agent is more than one bond position away from the covalent linker. In such cases, the binding agent is bonded through another moiety to the covalent linker. For example, the binding agent may be bonded to a maleimide group which is bonded to a polyethylene glycol group which is bonded to the covalent linker.

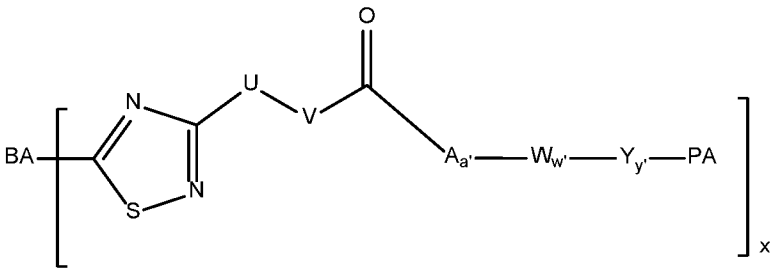
[0160] In some examples, the covalent linker is also bonded indirectly to a payload residue such that the covalent linker is more than one bond position away from a payload residue. The covalent linker is bonded through another moiety to the payload. For example, the covalent linker may be bonded to a dipeptide, such as but not limited to Val-Ala or Val-Cit, which may be bonded to PAB which may be bonded to the payload residue.

[0161] In some embodiments, the covalent linker is bonded indirectly to a hydrophilic moiety such that the covalent linker is more than one bond position away from a hydrophilic moiety. The covalent linker is bonded through another moiety to the hydrophilic moiety.

7.2.1. Aspect 1

[0162] Provided herein are ADCs, e.g., for use in therapy, such as cancer therapy

[0163] One embodiment is an ADC compound of Formula (I):



(I)

or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, wherein:

BA is a binding agent selected from a humanized, chimeric, or human antibody or an antigen binding fragment thereof;

U is arylene, heteroarylene, or a bond;

V is a bond or $-C\equiv C-(CH_2)_n-$;

n is an integer between 0 and 10;

A is a Stretcher unit residue;

subscript a' is 0 or 1;

W is a Cleavable unit;

subscript w' is 0 or 1;

Y is a Spacer unit;

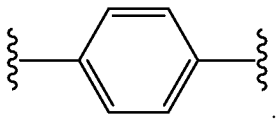
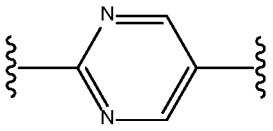
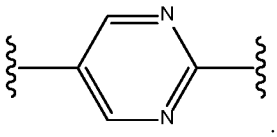
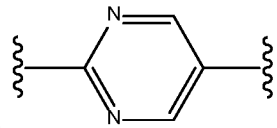
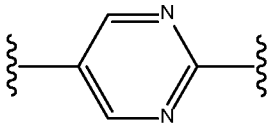
subscript y' is 0 or 1;

PA is a payload residue; and

subscript x is from 1 to 15.

[0164] In one embodiment, subscript x is from 1 to 15. In one embodiment, subscript x is from 1 to 12. In one embodiment, subscript x is from 1 to 10. In one embodiment, subscript x is from 2 to 10. In one embodiment, subscript x is from 3 to 10. In one embodiment, subscript x is from 4 to 10. In one embodiment, subscript x is from 4 to 9. In one embodiment, subscript x is from 4 to 8.

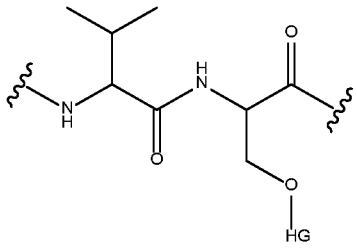
[0165] In some embodiments, U and V are not each a bond at the same time.

[0166] In one embodiment, U is a bond. In one embodiment, U is arylene. In one embodiment, U is phenylene. In some embodiments, U is  . In one embodiment, U is heteroarylene. In one embodiment, U is a bivalent pyrimidine ring. In one embodiment, U is  or  . In one embodiment, U is  . In one embodiment, U is  .

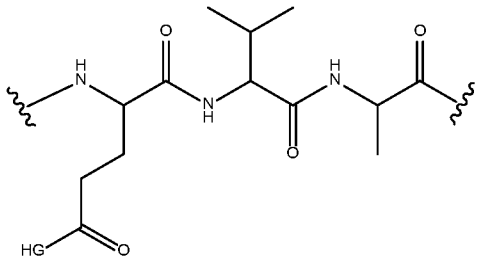
[0167] In one embodiment, V is a bond. In one embodiment, V is -C≡C-$(CH_2)_n$-. In one embodiment, V is -C≡C-$(CH_2)_3$-.

[0168] In some embodiments, A is -$(CH_2)_m$-C(=O)-, -CH_2-C(=O)-NH-$(CH_2)_m$-C(=O)-, -$(CH_2CH_2O)_m$-CH_2CH_2-C(=O)-, -CH[-$(CH_2)_m$-COOH]-C(=O)-, -CH_2-C(=O)-NH-$(CH_2)_m$-C(=O)-NH-$(CH_2)_m$-C(=O)-, -C(=O)-$(CH_2)_m$-C(=O)-, -NH-$(CH_2)_m$-C(=O)-, or -NH-$(CH_2CH_2O)_m$-CH_2CH_2-C(=O)-, wherein each m independently represents an integer of 1, 2, 3, 4, or 5.

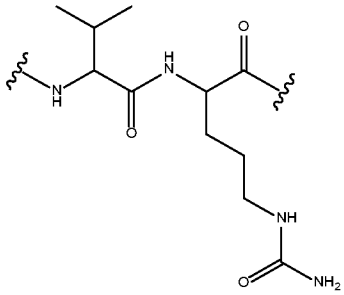
[0169] In some embodiments, W is one of the following formulas:



Formula (Ia),



Formula (Ib), or

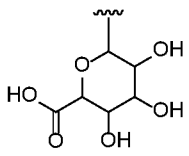
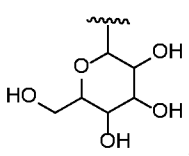
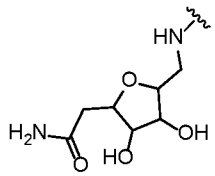
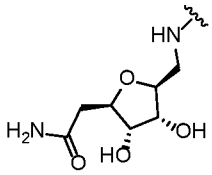
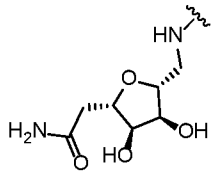


Formula (Ic),

wherein HG is a hydrophilic moiety or hydrogen.

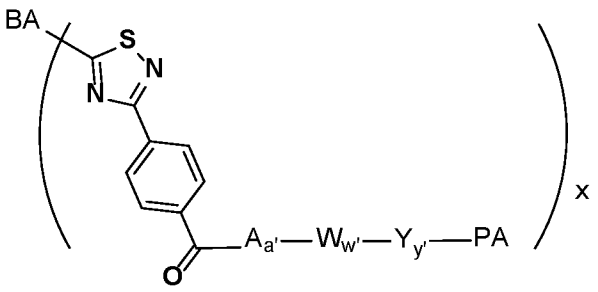
[0170] In some embodiments, HG is a saccharide, phosphate ester, sulfate ester, phosphodiester, or phosphonate.

[0171] In some embodiments, HG is a saccharide selected from β-D-galactose, N-acetyl-P-D-galactosamine, N-acetyl-a-D-galactosamine, N-acetyl-P-D-glucosamine, β-D-glucuronic acid, a-L-iduronic acid, a-D-galactose, a-D-glucose, β-D-glucose, a-D-mannose, β-D-mannose, a-L-fucose, β-D-xylose, a neuraminic acid or any analogue or modification thereof, or sulfate, phosphate, carboxyl, amino, or O-acetyl modification thereof.

[0172] In some embodiments, HG is  ,  , or  , or a stereoisomer thereof. In some embodiments, HG is  or  .

[0173] In some embodiments, Y is -NH-CH_2-O- or -NH-(*p*-C_6H_4)-CH_2-O-.

[0174] In some embodiments, the ADC compound of Formula (I) has one of the following formulas:



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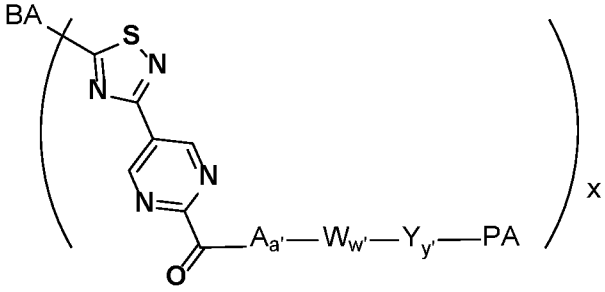
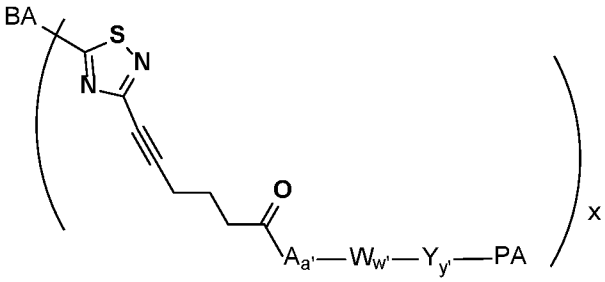


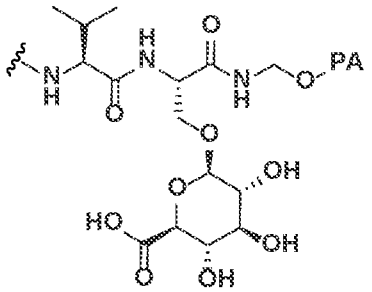
, or



,

wherein values for the variables (e.g., BA, PA, A, W, Y, a', w', y', x) are as described above.

[0175] In some embodiments, the fraction $\S\text{—}A_{a'}\text{—}W_{w'}\text{—}Y_{y'}\text{—}PA$ is one of the following formulas:



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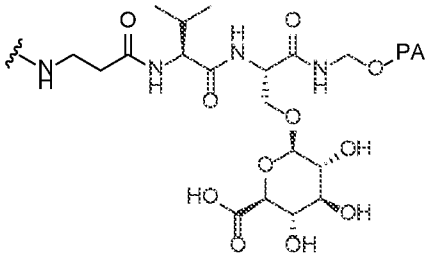

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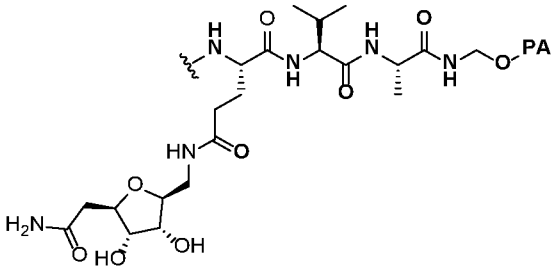
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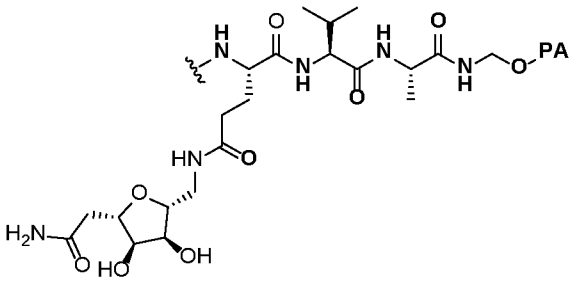
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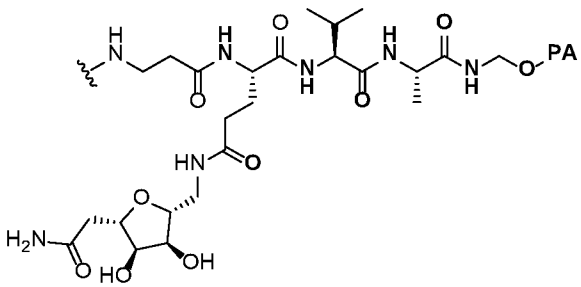
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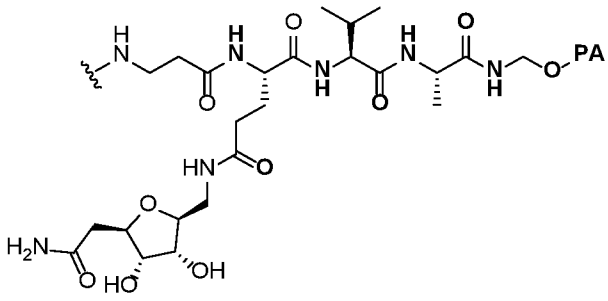
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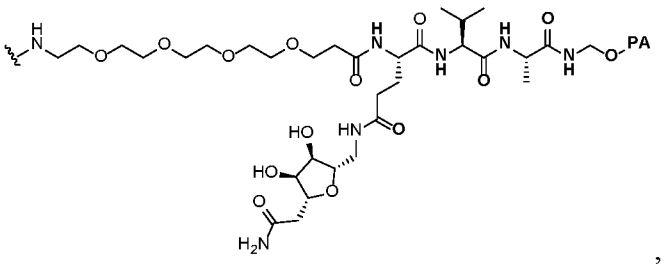

,

wherein PA is as described below.

[0176] In some embodiments, the Stretcher unit (-A-) is present and extends the framework of the covalent linker to provide more distance between a conjugator assembly and a Drug unit

(payload or payload residue). The conjugator assembly may include the components of Formula (I) other than the binding agent (BA), Stretcher unit (A), Cleavable unit (W), Spacer unit (Y), and payload residue (PA). The conjugator assembly may include the components of Formula (II) other than the Stretcher unit (A), Cleavable unit (W), Spacer unit (Y), and payload residue (PA). The conjugator assembly may include the components of Formula (III) other than the Stretcher unit (A). In embodiments, the conjugator includes the methylsulfonyl group, thiadiazole group, U, V, and carbonyl group of Formulas (I), (II), and/or (III). A Stretcher unit may link the conjugator assembly to the Cleavable unit when the Cleavable unit is present, the conjugator assembly to the Spacer unit when the Cleavable unit is absent and the Spacer unit is present, or the conjugator assembly to the Drug unit when both the Cleavable unit and the Spacer unit are absent. A Stretcher unit may attach to more than one Cleavable unit, Spacer unit, and/or Drug unit. The conjugator assembly, Cleavable unit, Spacer unit, and Drug unit may be any conjugator assembly, Cleavable unit, Spacer unit, and Drug unit, respectively, described herein.

[0177] The Stretcher unit may alter the physiochemical properties of the Drug-Linker depending on the components of the Stretcher unit. In some implementations, the Stretcher unit may increase the solubility of the Drug-Linker and may include one or more solubility-enhancing groups such as ionic groups or water-soluble polymers. Water-soluble polymers may be soluble in water at room temperature and may include poly(ethylene)glycol groups as well as other polymers such as polyethyleneimines.

[0178] A Stretcher unit may comprise one or more stretcher groups. Examples of stretcher groups include, for example, -C_{1-10} alkylene-C(O)-, -C_{1-10} alkylene-C(O)-NH-C_{1-10} alkylene-C(O)-, -$(CH_2CH_2O)_u$-CH_2CH_2-C(O)-, -CH[-C_{1-10} alkylene-COOH]-C(O)-, -C_{1-10} alkylene-C(O)-NH-C_{1-10} alkylene-C(O)-NH-C_{1-10} alkylene-C(O)-, -C(O)-C_{1-10} alkylene-C(O)-, -NH-C_{1-10} alkylene-C(=O)-, -NH-$(CH_2CH_2O)_u$-CH_2CH_2-C(=O)-, -NH-C_{1-10}alkylene-, -NH-C_{1-10} alkylene-NH-C(O)-C_{1-10} alkylene-, -NH-C_{1-10}alkylene-C(O)-NH-C_{1-10}alkylene-, -NH-$(CH_2CH_2O)_u$-, -NH-$(CH_2CH_2O)_u$-CH_2-, -NH-$(CH_2CH_2NH)_u$-$(CH_2)_u$-NH-$(CH_2CH_2NH)_u$-$(CH_2)_u$-NH-C(O)-$(CH_2)_u$-NH-(C_3-C_8 carbocyclo)-, -NH-(arylene)-, and -NH-(C_3-C_8 heterocyclo-)-, wherein each u is independently 1-10.

[0179] In some embodiments, the Cleavable unit (-$W_{w'}$-) is present and may link the conjugator assembly to the Spacer unit when the Spacer unit is present or link the conjugator assembly to the Drug unit when the Spacer unit is absent. The linkage from the conjugator assembly to the Spacer unit or to the Drug unit can be directly from the conjugator assembly when the Stretcher unit is

absent or via the Stretcher unit if the Stretcher unit is present.

[0180] In some embodiments, the Cleavable unit is directly conjugated to the conjugator assembly on one end and to the Drug unit on the other end. In some embodiments, the Cleavable unit is directly conjugated to the Stretcher unit on one end and to the Drug unit on the other end. In further embodiments, the Cleavable unit is directly conjugated to the Stretcher unit on one end and to the Spacer unit on the other end. In still further embodiments, the Cleavable unit is directly conjugated to the conjugator assembly on one end and to the Spacer unit on the other end. In embodiments, the Stretcher Unit and/or Spacer unit may be absent.

[0181] The Cleavable unit may form a cleavable bond with a Drug unit (PA) or a Spacer unit. Reactive groups for forming cleavable bonds can include, for example, sulfhydryl groups to form disulfide bonds; aldehyde, ketone, or hydrazine groups to form hydrazone bonds; carboxylic or amino groups to form peptide bonds; and carboxylic or hydroxy groups to form ester bonds.

[0182] The Cleavable unit may include a disulfide-containing linker cleavable through disulfide exchange, an acid-labile linker at acidic pH, or a linker cleavable by an enzyme such as a hydrolase, peptidase, esterase, or glucoronidase. The Cleavable unit may include one or more cleavage sites.

[0183] In some embodiments, the Cleavable unit includes one or more amino acids, such as 1 to 12. The Cleavable unit can include, for example, a monopeptide, dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide, or dodecapeptide unit.

[0184] Each amino acid may be a natural or unnatural amino acid, and/or a D- or L-isomer of the same, provided that a cleavable bond is available. In some embodiments, the Cleavable unit includes only natural amino acids. Each amino acid may be a proteinogenic or non-proteinogenic amino acid.

[0185] In some embodiments, each amino acid is independently selected from the group consisting of alanine, arginine, aspartic acid, asparagine, histidine, glycine, glutamic acid, glutamine, phenylalanine, lysine, leucine, serine, tyrosine, threonine, isoleucine, proline, tryptophan, valine, cysteine, methionine, selenocysteine, ornithine, penicillamine, β-alanine, aminoalkanoic acid, aminoalkynoic acid, aminoalkanedioic acid, aminobenzoic acid, amino-heterocyclo-alkanoic acid, heterocyclo-carboxylic acid, citrulline, statine, diaminoalkanoic acid, and derivatives thereof. In some embodiments, each amino acid is independently selected from the group consisting of alanine, arginine, aspartic acid, asparagine, histidine, glycine, glutamic acid, glutamine, phenylalanine, lysine, leucine, serine, tyrosine, threonine, isoleucine, proline, tryptophan, valine,

cysteine, methionine, and selenocysteine. In some embodiments, each amino acid is independently selected from the group consisting of alanine, arginine, aspartic acid, asparagine, histidine, glycine, glutamic acid, glutamine, phenylalanine, lysine, leucine, serine, tyrosine, threonine, isoleucine, proline, tryptophan, and valine.

[0186] In some embodiments, each amino acid is independently selected from an L isomer of the following natural amino acids: alanine, arginine, aspartic acid, asparagine, histidine, glycine, glutamic acid, glutamine, phenylalanine, lysine, leucine, serine, tyrosine, threonine, isoleucine, tryptophan and valine. In some embodiments, each amino acid is a D isomer of the following natural amino acids: alanine, arginine, aspartic acid, asparagine, histidine, glycine, glutamic acid, glutamine, phenylalanine, lysine, leucine, serine, tyrosine, threonine, isoleucine, tryptophan and valine.

[0187] In embodiments, the Cleavable unit is the dipeptide -Val-Cit-, -Phe-Lys-, or -Val-Ala.

[0188] In some embodiments, the Cleavable unit includes one or two terminal amino acids and is linked to the Drug unit and/or Spacer unit through functional units present in a terminal amino acid, e.g., its carboxylic acid or amino termini.

[0189] In some embodiments, the bond between the Cleavable unit and the Drug unit can be enzymatically cleaved by one or more enzymes, including a tumor-associated protease, to liberate the Drug unit (-PA), which may be protonated in vivo upon release to provide a Drug (PA).

[0190] Useful Cleavable units may be designed to optimize their selectivity for enzymatic cleavage by a particular enzyme, such as a tumor-associated protease. In one embodiment, a linkage between the Cleavable unit and the Drug unit or Spacer unit is one for which cleavage is catalyzed by cathepsin B, C, and/or D, or a plasmin protease.

[0191] In some embodiments, the Spacer unit (-$Y_{y'}$-) is present and extends the framework of the covalent linker. A Spacer unit may link a Cleavable unit to the Drug unit or a Stretcher unit to the Drug unit or a conjugator assembly to a Drug unit. The Spacer unit may include one or more self-immolative or non-self-immolative groups. In some embodiments, the Spacer unit includes one or more self-immolative groups. In this context, the term "self-immolative group" refers to a bifunctional chemical moiety that is capable of covalently linking together two spaced chemical moieties into a normally stable tripartite molecule. The self-immolative group will spontaneously separate from the second chemical moiety if its bond to the first moiety is cleaved. In other embodiments, the Spacer unit is not self-immolative. In such embodiments, part or all of the Spacer unit remains attached to the Drug unit.

[0192] In some embodiments, -$Y_{y'}$- is a self-immolative group and is linked to a Cleavable unit via a methylene carbon atom of the self-immolative group, and linked directly to the Drug unit via a carbonate, carbamate, or ether group.

[0193] In some embodiments, -$Y_{y'}$- is a p-aminobenzyl alcohol (PAB) unit (e.g., -NH-(C_6H_4)-CH_2-O-C(=O)-). The phenylene portion of the PAB unit is optionally substituted with -C_1-C_8 alkyl, -O-(C_1-C_8 alkyl), -halogen, -nitro, or -cyano.

[0194] In some embodiments, -$Y_{y'}$- is -NH-C_{1-10}alkylene-O- or -NH-(*p*-C_6H_4)- C_{1-10}alkylene-O-.

[0195] In another embodiment, -$Y_{y'}$- is a carbonate group.

[0196] Other examples of self-immolative groups include, but are not limited to, aromatic compounds that are electronically similar to the PAB unit such as 2-aminoimidazol-5-methanol derivatives (see, e.g., Hay et al., 1999, *Bioorg. Med. Chem. Lett.* 9:2237) and ortho- or para-aminobenzylacetals. Suitable Spacer units include those that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (see, e.g., Rodrigues et al., 1995, *Chemistry Biology* 2:223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (see, e.g., Storm et al., 1972, *J. Amer. Chem. Soc.* 94:5815), and 2-aminophenylpropionic acid amides (see, e.g., Amsberry et al., 1990, *J. Org. Chem.* 55:5867). Elimination of amine-containing drugs that are substituted at the α-position of glycine (see, e.g., Kingsbury et al., 1984, *J. Med. Chem.* 27:1447) are also examples of suitable self-immolative groups.

[0197] Other suitable Spacer units are disclosed in U.S. Publication No. 2005-0238649, the disclosure of which is incorporated by reference herein.

[0198] Suitable Stretcher units, Cleavable units, and Spacer units for use with the presently disclosed linkers, platforms, and ADCs care described in WO 2004/010957, WO 2007/038658, WO 2005/112919, U.S. Patent Nos. 6,214,345, 7,659,241, 7,498,298, 7,968,687, and 8,163,888, and U.S. Publication Nos. 2009-0111756, 2009-0018086, and 2009-0274713, each of which is incorporated herein by reference in its entirety and for all purposes.

Binding agents

[0199] Provided herein are binding agents (BA or Ab), e.g., for use in an ADC described herein.

[0200] Compounds of Formula (I) may include any BA described herein.

[0201] In some embodiments, BA is an antibody or antigen binding fragment thereof, e.g., a humanized, chimeric, or human antibody or an antigen binding fragment thereof.

[0202] In some embodiments, the antibody or antigen binding fragment thereof specifically binds human B7H3. In some embodiments, the antibody or antigen binding fragment thereof is

ifinatamab.

[0203] In some embodiments, the antibody or antigen binding fragment thereof specifically binds HER2. In some embodiments, the antibody or antigen binding fragment thereof is trastuzumab.

[0204] In some embodiments, the antibody or antigen binding fragment thereof specifically binds CLL1. In some embodiments, the antibody or antigen binding fragment thereof is 6E7.

[0205] In some embodiments, the antibody or antigen-binding fragment thereof is a monoclonal antibody, a chimeric antibody, a humanized antibody, a human engineered antibody, a single chain antibody (scFv), a Fab fragment, a Fab' fragment, or a F(ab')2 fragment.

Payloads

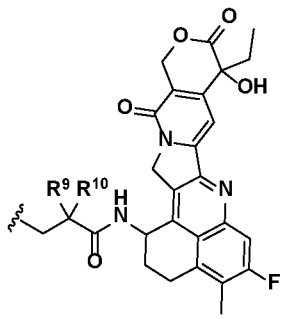
[0206] Provided herein are payloads (PA), e.g., for use in a platform and/or ADC described herein.

[0207] Compounds of Formula (I) may include any PA described herein

[0208] In some embodiments, each PA is independently a cytotoxic agent.

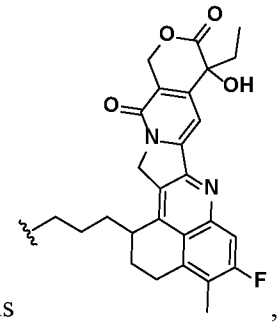
[0209] In some embodiments, each PA is independently selected from the group consisting of DXd, 7-ethyl-10-hydroxy-camptothecin (SN-38), and monomethyl auristatin E (MMAE).

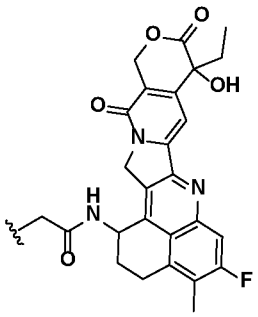
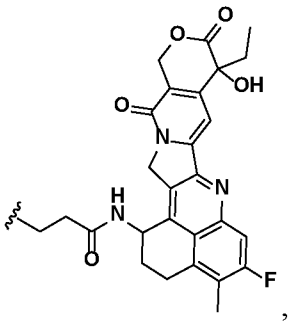
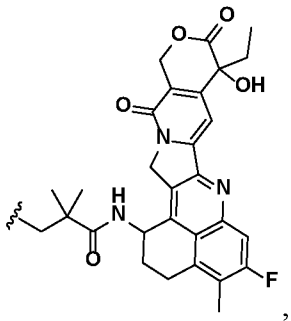
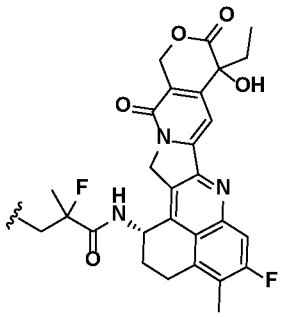
[0210] In some embodiments, each PA independently is a compound of formula (VI):



(VI),

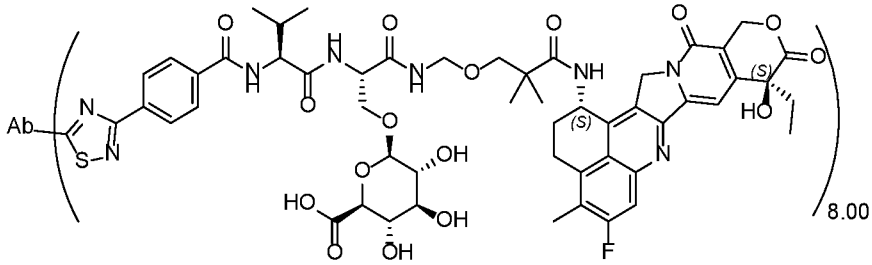
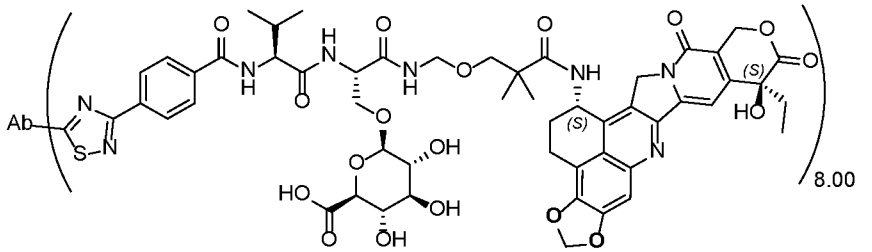
and each of R^9 and R^{10} is independently hydrogen, halogen, or substituted or unsubstituted C_{1-4} alkyl.

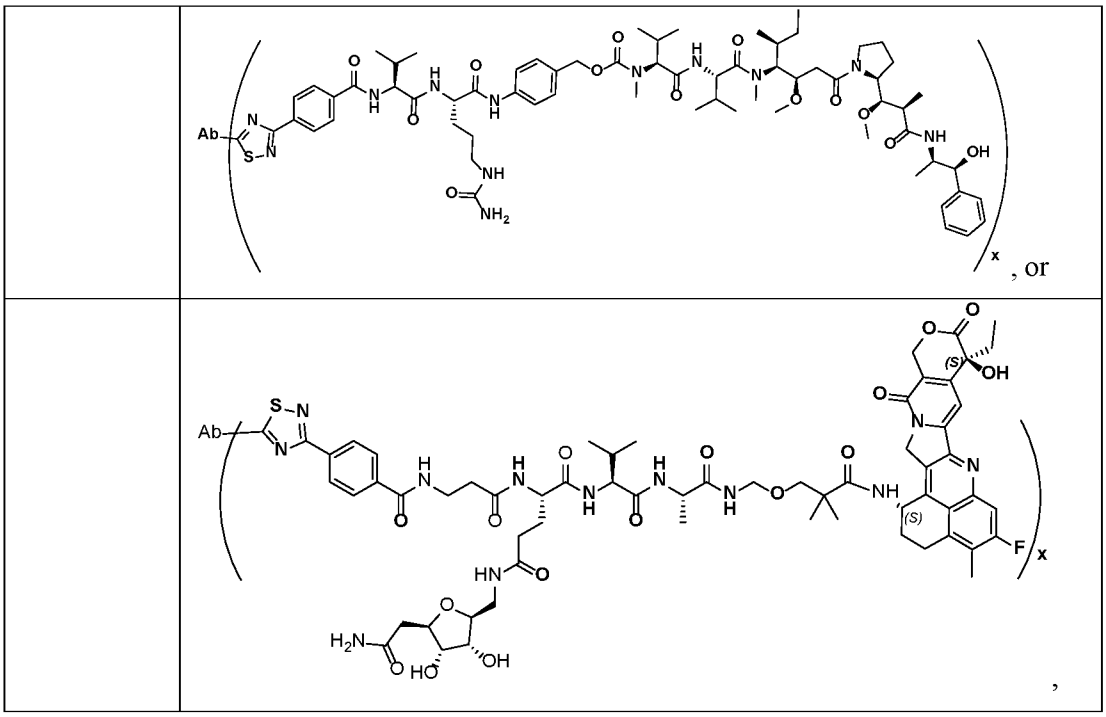


[0211] In some embodiments, each PA independently is  ,  ,  , or  .

7.2.2. Aspect 2

[0212] In some embodiments, the ADC compound is represented by one of the following formulas, or a pharmaceutically acceptable salt, solvate, and/or stereoisomer.

No.	Structure
	 ,
	 ,

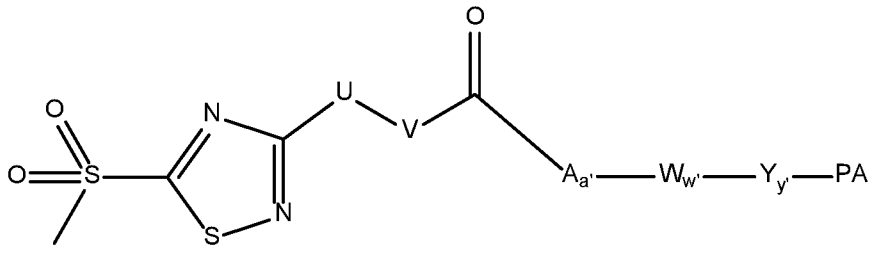


wherein Ab is a humanized, chimeric, or human antibody or an antigen binding fragment thereof and subscript x is from 1 to 15. Alternative values for Ab are as set forth herein, e.g., with respect to Aspect 1. Alternative values for variable subscript x are as set forth herein, e.g., with respect to compounds of Formula (I).

7.2.3. Aspect 3

[0213] Also provided herein are platforms, e.g., for use in preparing an ADC, such as an ADC described herein.

[0214] In some embodiments, the platform is a linker-payload compound of Formula (II):



(II)

or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, wherein:

U is arylene, heteroarylene, or a bond;

V is a bond or $-C\equiv C-(CH_2)_n-$;

n is an integer between 0 and 10;

A is a Stretcher unit residue;

subscript a' is 0 or 1;

W is a Cleavable unit;

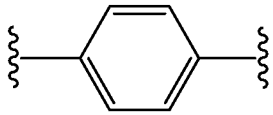
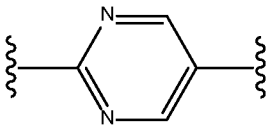
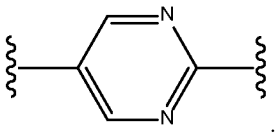
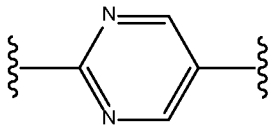
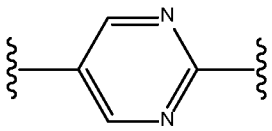
subscript w' is 0 or 1;

Y is a Spacer unit;

subscript y' is 0 or 1; and

PA is a payload residue.

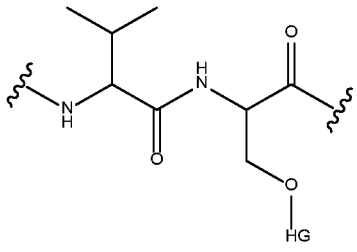
[0215] In some embodiments, U and V are not each a bond at the same time.

[0216] In one embodiment, U is a bond. In one embodiment, U is arylene. In one embodiment, U is phenylene. In some embodiments, U is  . In one embodiment, U is heteroarylene. In one embodiment, U is a bivalent pyrimidine ring. In one embodiment, U is  or  . In one embodiment, U is  . In one embodiment, U is  .

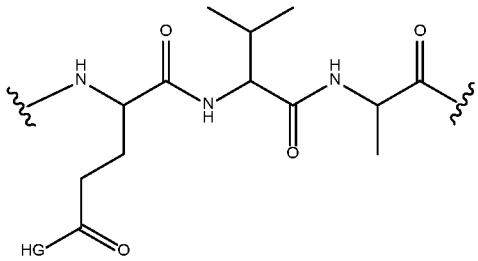
[0217] In one embodiment, V is a bond. In one embodiment, V is -C≡C-$(CH_2)_n$-. In one embodiment, V is -C≡C-$(CH_2)_3$-.

[0218] In some embodiments, A is -$(CH_2)_m$-C(=O)-, -CH_2-C(=O)-NH-$(CH_2)_m$-C(=O)-, -$(CH_2CH_2O)_m$-CH_2CH_2-C(=O)-, -CH[-$(CH_2)_m$-COOH]-C(=O)-, -CH_2-C(=O)-NH-$(CH_2)_m$-C(=O)-NH-$(CH_2)_m$-C(=O)-, -C(=O)-$(CH_2)_m$-C(=O)-, -NH-$(CH_2)_m$-C(=O)-, or -NH-$(CH_2CH_2O)_m$-CH_2CH_2-C(=O)-, wherein each m independently represents an integer of 1, 2, 3, 4, or 5.

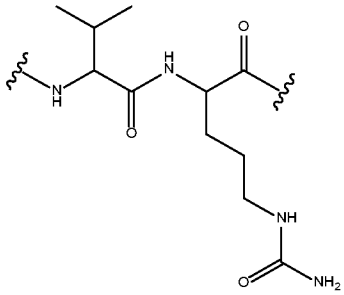
[0219] In some embodiments, W is one of the following formulas:



Formula (Ia),



Formula (Ib), or

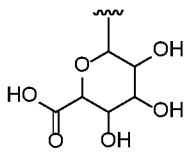
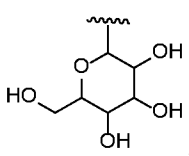
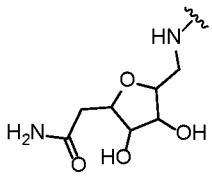
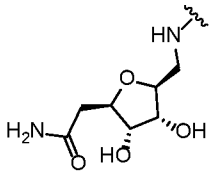
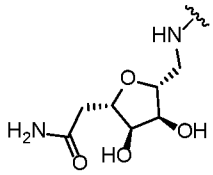


Formula (Ic),

wherein HG is a hydrophilic moiety or hydrogen.

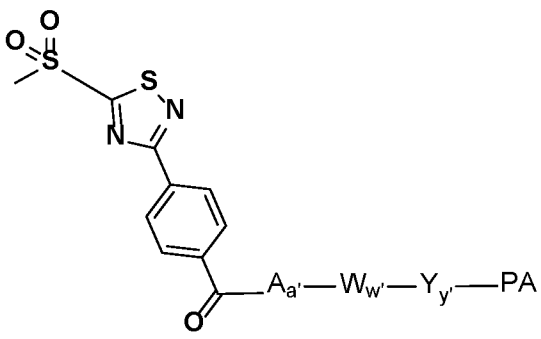
[0220] In some embodiments, HG is a saccharide, phosphate ester, sulfate ester, phosphodiester, or phosphonate.

[0221] In some embodiments, HG is a saccharide selected from β-D-galactose, N-acetyl-P-D-galactosamine, N-acetyl-a-D-galactosamine, N-acetyl-P-D-glucosamine, β-D-glucuronic acid, a-L-iduronic acid, a-D-galactose, a-D-glucose, β-D-glucose, a-D-mannose, β-D-mannose, a-L-fucose, β-D-xylose, a neuraminic acid or any analogue or modification thereof, or sulfate, phosphate, carboxyl, amino, or O-acetyl modification thereof.

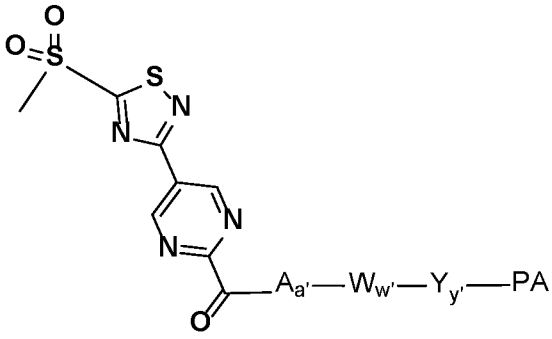
[0222] In some embodiments, HG is  ,  , or  , or a stereoisomer thereof. In some embodiments, HG is  or  .

[0223] In some embodiments, Y is -NH-CH_2-O- or -NH-(*p*-C_6H_4)-CH_2-O-.

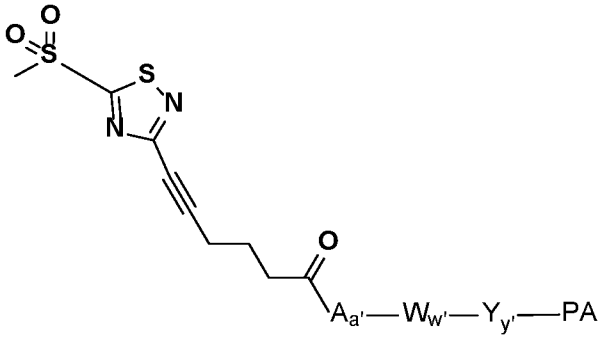
[0224] In some embodiments, the compound has one of the following formulas:



,

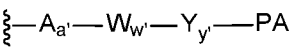


, or

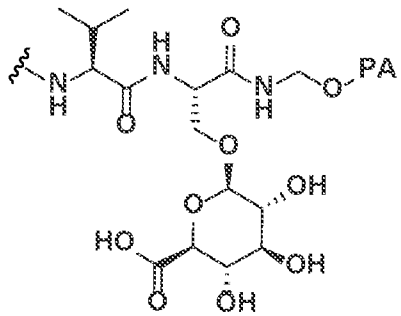


,

wherein values for the variables (e.g., PA, A, W, Y, a', w', y', x) are as described above.

[0225] In some embodiments, the fraction 

is one of the following formulas:



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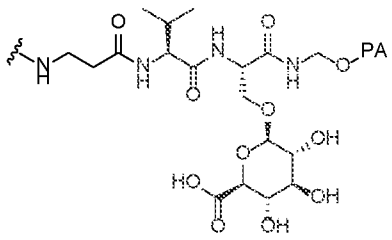

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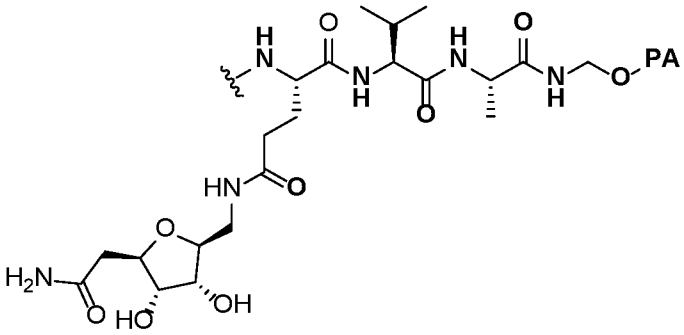

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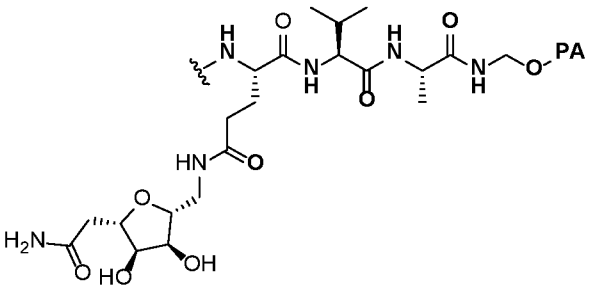

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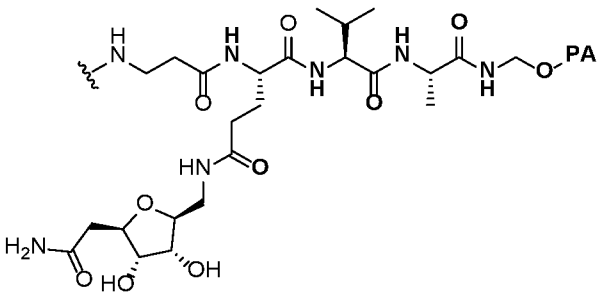

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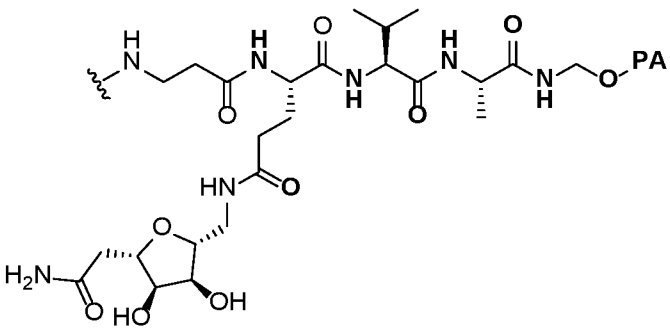
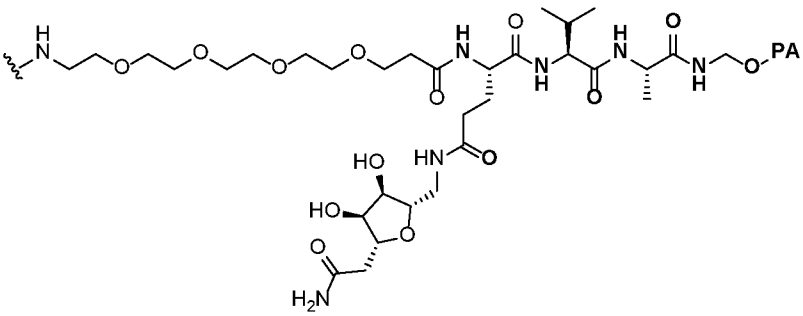


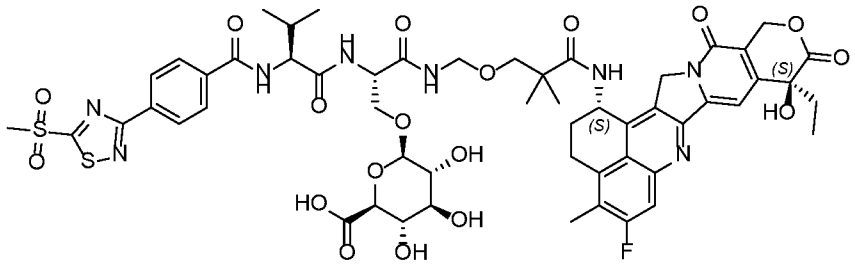
, or



,

wherein PA is as described above.

[0226] In some embodiments, the compound is



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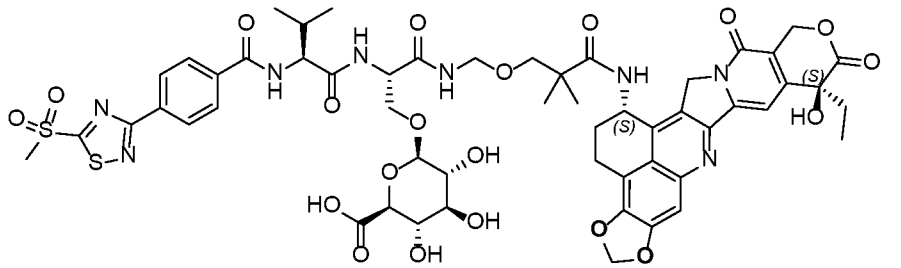

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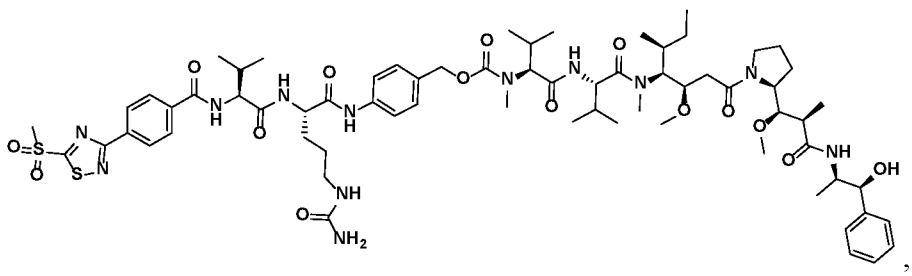

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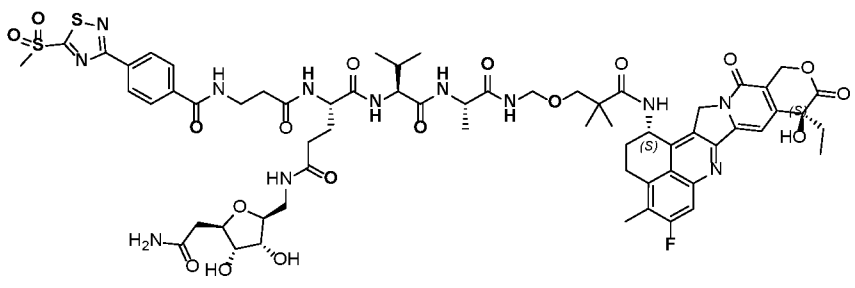

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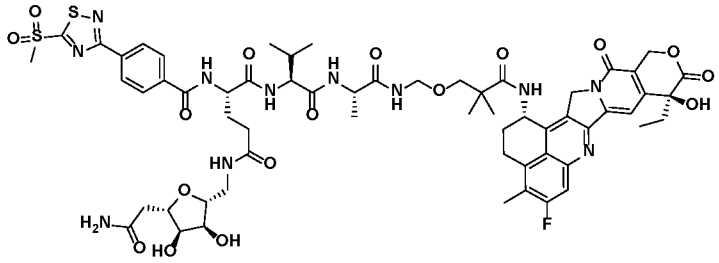
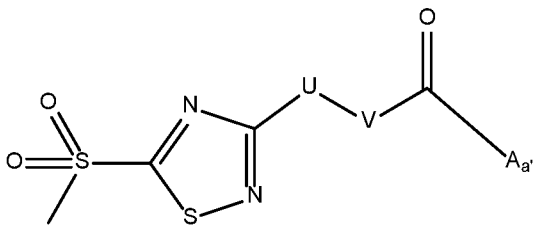


, or a pharmaceutically acceptable salt, tautomer, or solvate thereof.

7.2.4. Aspect 4

[0227] Also provided herein are covalent linkers, e.g., for use in a platform and/or ADC described herein.

[0228] In some embodiments (e.g., of a linker for use in a platform), the linker is a compound of Formula (III):



(III)

or a pharmaceutically acceptable salt, tautomer, solvate, or thereof, wherein:

U is arylene, heteroarylene, or a bond;

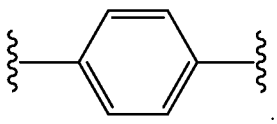
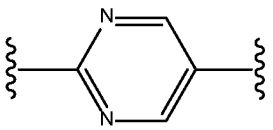
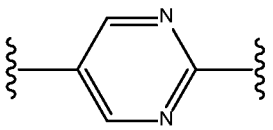
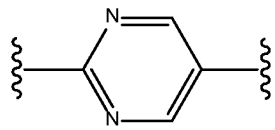
V is a bond or -C≡C-$(CH_2)_n$-;

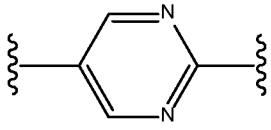
n is an integer between 0 and 10;

A is a Stretcher unit; and

subscript a' is 0 or 1.

[0229] In some embodiments, U and V are not each a bond at the same time.

[0230] In one embodiment, U is a bond. In one embodiment, U is arylene. In one embodiment, U is phenylene. In some embodiments, U is  . In one embodiment, U is heteroarylene. In one embodiment, U is a bivalent pyrimidine ring. In one embodiment, U is  or  . In one embodiment, U is  . In

one embodiment, U is  .

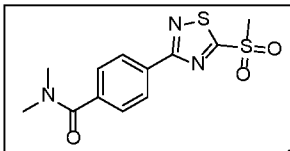
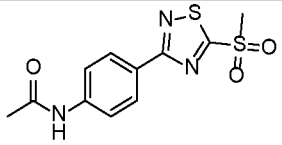
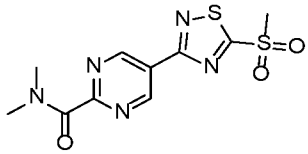
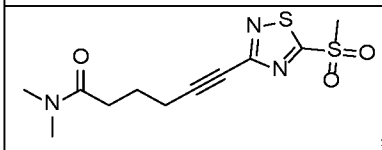
[0231] In one embodiment, V is a bond. In one embodiment, V is -C≡C-$(CH_2)_n$-. In one embodiment, V is -C≡C-$(CH_2)_3$-.

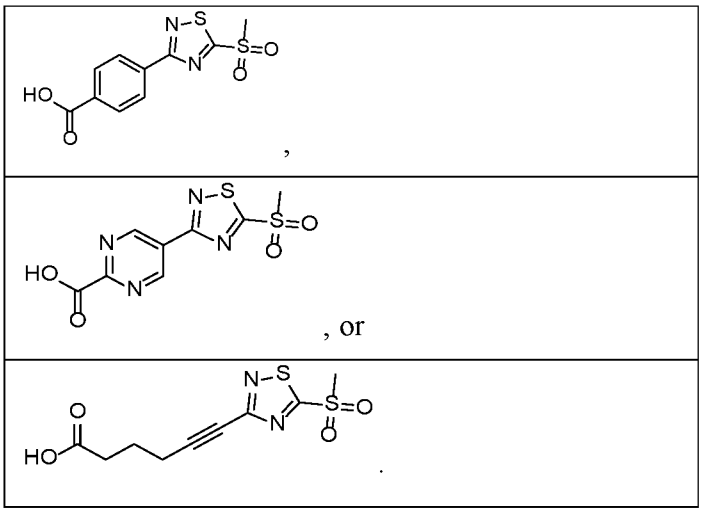
[0232] In some embodiments, A is a bond, -OH, -CH_3, -$N(CH_3)_2$, -$(CH_2)_m$-C(=O)R^7, -CH_2-C(=O)-NH-$(CH_2)_m$-C(=O)R^7, -$(CH_2CH_2O)_m$-CH_2CH_2-C(=O)R^7, -CH[-$(CH_2)_m$-COOH]-C(=O)R^7, -CH_2-C(=O)-NH-$(CH_2)_m$-C(=O)-NH-$(CH_2)_m$-C(=O)R^7, -C(=O)-$(CH_2)_m$-C(=O)R^7, -NH-$(CH_2)_m$-C(=O)R^7, or -NH-$(CH_2CH_2O)_m$-CH_2CH_2-C(=O)R^7, wherein each m independently represents an integer of 1, 2, 3, 4, or 5; R^7 is OH or $NR^{8a}R^{8b}$; and each of R^{8a} and R^{8b} is, independently, H; substituted or unsubstituted C_{1-4} alkyl; substituted or unsubstituted C_{3-5} cycloalkyl; or R^{8a} and R^{8b} together with the atom to which they are attached form a substituted or unsubstituted C_{3-5} cycloalkyl.

[0233] In some embodiments, R^7 is OH, NH_2, $NHCH_3$, or $N(CH_3)_2$.

7.2.5. Aspect 5

[0234] In some embodiments, the linker compound is any one of the following, or a pharmaceutically acceptable salt and/or solvate thereof

 ,
 ,
 ,
 ,



7.3. Methods or Processes of Making the Conjugates

[0235] Provided herein are methods of preparing a conjugate by contacting a binding agent (Ab) with a linker-payload compound under conditions suitable for forming a bond between the binding agent and the linker-payload compound. The reaction conditions may be any suitable reaction conditions known in the art. The binding agent may be an antibody and the bond may form an antibody-drug conjugate.

[0236] Examples of such reactions are provided in the Examples below.

[0237] In some embodiments, methods of making a conjugate including treating or contacting a compound with a binding agent under coupling conditions. The compound may include a reactive linker bonded to at least one payload. The compound may be any of the linker or platform compounds disclosed herein.

7.4. Pharmaceutical Compositions

[0238] Also provided herein are compositions, including pharmaceutical compositions, comprising an ADC set forth herein. In some embodiments, the compositions (e.g., pharmaceutical compositions) further comprise a pharmaceutically acceptable excipient.

[0239] Pharmaceutical compositions in accordance with the present disclosure can be prepared by mixing an antibody drug conjugate having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to, buffers such as phosphate, citrate, and other organic acids;

antioxidants including ascorbic acid and methionine; preservatives (such as octadecyl dimethyl benzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3- pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Nos. US 7,871,607 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0240] Exemplary lyophilized formulations are described in US Patent No. 6,267,958. Aqueous formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0241] Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody drug conjugate, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

[0242] The formulations to be used for in vivo administration are generally sterile. Sterility can be readily accomplished, e.g., by filtration through sterile filtration membranes.

7.5. Methods of Using

[0243] In some embodiments, set forth herein is a method of treating a disease or disorder (e.g., a cancer) in a subject (e.g., patient) in need thereof, comprising administering to the patient an effective amount of an ADC disclosed herein.

[0244] The antibody drug conjugates disclosed herein can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial,

intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g., by injection, such as intravenous or subcutaneous injection, depending in part on whether the administration is brief or chronic. Various dosing schedules, including but not limited to, single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0245] Antibody drug conjugates of the disclosure can be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

8. EXAMPLES

[0246] The examples below are intended to be exemplary and should not be considered limiting in any way. Unless otherwise specified, the experimental methods in the Examples described below are conventional methods. Unless otherwise specified, the reagents and materials are commercially available. All solvents and chemicals employed were of analytical grade or chemical purity. Solvents were redistilled before use. Anhydrous solvents were prepared according to standard methods or reference methods. Silica gel (100-200 meshes) for column chromatography and silica gel (GF254) for thin-layer chromatography (TLC) are commercially available from Tsingdao Haiyang Chemical Co., Ltd. or Yantai Chemical Co., Ltd. of China; all were eluted with petroleum ether (60-90 °C)/ethyl acetate (v/v) and visualized by iodine or the solution of molybdphosphoric acid in ethanol unless otherwise specified. All extraction solvents, unless otherwise specified, were dried over anhydrous Na_2SO_4. ^1H NMR spectra were recorded on Bruck-400, Varian 400MR nuclear magnetic resonance spectrometer with TMS (tetramethylsilane) as the internal standard. Coupling constants were given in hertz. Peaks were reported as singlet (s), doublet (d), triplet (t), quartet (q), quintet (p), sextet (h), septet (hept), multiplet (m), or a combination thereof; br stands for broad. LC/MS data was recorded by using Agilent1100,1200 High Performance Liquid Chromatography-Ion Trap Mass Spectrometer (LC-MSD Trap) equipped with a diode array detector (DAD) detected at 214 nm and 254 nm, and an ion trap (ESI source). All compound names except the reagents were generated by ChemDraw® 18.0.

[0247] For the sake of conciseness, certain abbreviations are used herein. One example is the single letter abbreviation to represent an amino acid. The amino acids and their corresponding three

letter and single letter abbreviations are as follows:

alanine	Ala	(A)
arginine	Arg	(R)
asparagine	Asn	(N)
aspartic acid	Asp	(D)
cysteine	Cys	(C)
glutamic acid	Glu	(E)
glutamine	Gln	(Q)
glycine	Gly	(G)
histidine	His	(H)
isoleucine	Ile	(I)
leucine	Leu	(L)
lysine	Lys	(K)
methionine	Met	(M)
phenylalanine	Phe	(F)
proline	Pro	(P)
serine	Ser	(S)
threonine	Thr	(T)
tryptophan	Trp	(W)
tyrosine	Tyr	(Y)
valine	Val	(V)

[0248] In the following examples, the following abbreviations are used:

TEA	Triethyl amine
THF	Tetrahydrofuran

MeOH	Methanol
HOBt	1-Hydroxybenzotriazole
DIPEA	N,N-Diisopropylethylamine
DMF	N,N-Dimethylformamide
LiOH	Lithium hydroxide
HCl	Hydrochloric acid
TFA	Trifluoroacetic acid
K_2CO_3	Potassium carbonate
Na_2SO_4	Sodium sulfate
EtOAc	Ethyl acetate
prep-HPLC	Preparative high performance liquid chromatography
$Cu(OAc)_2$	Anhydrous cupric acetate
$Pb(OAc)_4$	Lead tetraacetate
Et_2N	Diethylamine
Et_3N	triethylamine
r.t.	Room temperature
MS	Mass spectrometry
ESI	Electron spray ionization
FA	Formic acid
HPLC	High performance liquid chromatography
DMSO	Dimethylsulfoxide
DCM	Dichloromethane
EDCI	1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide
PE	Polyethylene
EA	Ethyl acetate
m-CPBA	Meta-chloroperoxybenzoic acid
FCC	Flash column chromatography
$Sc(OTf)_3$	Scandium(III) trifluoromethanesulfonate
$Pd(dppf)Cl_2$	(1,1'-Bis(diphenylphosphine)ferrocene)palladium(II)dichloride

UPLC analysis methods

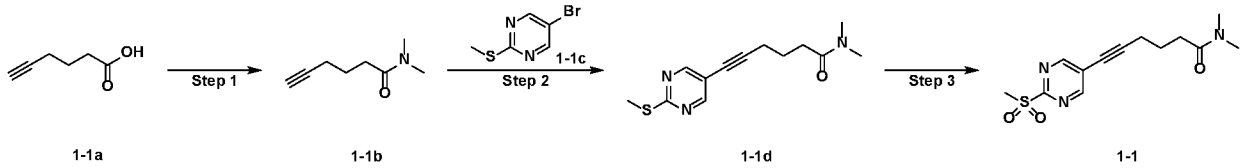
[0249] **Method A**: Mobile phase A: 0.1% FA in water, B: MeCN; Gradient: 10%B maintain 0.2 min, 10% - 95%B, 5.8 min, 95%B maintain 0.5 min; Flow rate: 0.6 mL/min; Column: ACQUITY UPLC® BEH

C18 1.7μm.

[0250] **Method B**: Mobile phase A: 0.1% FA in water, B: MeCN; Gradient: 10%B maintain 0.5 min, 10% - 90%B, 2.5 min, 90%B maintain 0.2 min; Flow rate: 0.6 mL/min; Column: ACQUITY UPLC® BEH C18 1.7μm.

[0251] **Method C**: Mobile phase A: 0.1% FA in water, B: MeCN; Gradient: 10%B maintain 0.2 min, 10% - 90%B, 1.3 min, 90%B maintain 0.3 min; Flow rate: 0.6 mL/min; Column: ACQUITY UPLC® BEH C18 1.7μm.

Example 1-1



[0252] Step 1: N,N-dimethylhex-5-ynamide (**1-1b**)

[0253] To a solution of compound **1-1a** (500 mg, 4.46 mmol) in DCM (10 mL) were added dimethylamine hydrochloride (545 mg, 6.69 mmol), EDCI (1110 mg, 5.8 mmol), HOBt (783 mg, 5.8 mmol) and Et_3N (1350 mg, 13.4 mmol). The mixture was stirred at r.t. for 16 h. The mixture was diluted with EA (200 mL), washed with 1 N HCl (50 mL*3), sat. $NaHCO_3$ (50 mL*3) and brine (50 mL*3). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to give the crude product **1-1b** (458 mg, crude) as a light-yellow solid. MS (ESI) m/z: 140.2 $[M+H]^+$.

[0254] Step 2: N,N-dimethyl-6-(2-(methylthio)pyrimidin-5-yl)hex-5-ynamide (**1-1d**)

[0255] To a solution of compound **1-1b** (100 mg, 0.72 mmol) in DMF (4 mL) were added compound **1-1c** (162 mg, 0.79 mmol), CuI (14 mg, 0.072 mmol), $Pd(PPh_3)_2Cl_2$ (36 mg, 0.072 mmol), and Et_3N (1 mL). The mixture was stirred at 90 °C for 6 h under N_2 atmosphere. The mixture was filtered through a pad of celite, diluted with EA (100 mL), washed with brine (50 mL*4). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography (eluted with PE:EA=0-40%). Compound **1-1d** (115 mg, 60.8% yield) was obtained as a light-yellow solid. MS (ESI) m/z: 264.1 $[M+H]^+$.

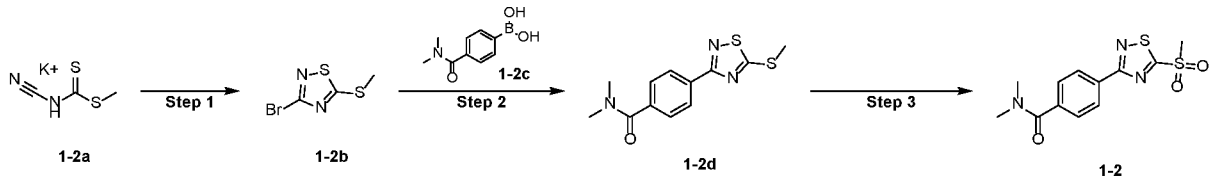
[0256] Step 3: N,N-dimethyl-6-(2-(methylsulfonyl)pyrimidin-5-yl)hex-5-ynamide (**1-1**)

[0257] To a solution of compound **1-1d** (100 mg, 0.38 mmol) in DCM (4 mL) was added m-CPBA (262 mg, 1.52 mmol). The mixture was stirred at r.t. for 30 min. The mixture was diluted with EA (150 mL), washed with sat. $NaHCO_3$ (100 mL) and brine (100 mL*3). The organic layer

was dried over anhydrous Na_2SO_4, filtered, and concentrated, The residue was purified by prep-HPLC (Method: column：XBridge Prep C18 OBD 5um 19*250 mm; Mobile phase: A-water (0.1% TFA): B-acetonitrile; Flow rate: 20 mL/min).

[0258] Compound **1-1** (45.8 mg, 99% purity) was obtained as a white solid. MS (ESI) m/z: 296.2 $[M+H]^+$.

Example 1-2



[0259] Step 1: 3-bromo-5-(methylthio)-1,2,4-thiadiazole (**1-2b**)

[0260] To a solution of compound **1-2a** (5 g, 29.4 mmol) in dichloromethane (100 mL) was added dropwise Br_2 (5.2 g, 32.9 mmol) at 0 °C. After addition, the resulting mixture was stirred at room temperature overnight. To the reaction mixture was added excess Na_2SO_3 and water (50 mL) to decompose the excess Br_2. The mixture was separated, and the separated organic layer was washed with brine (50 mL x 3), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (eluted with PE:EA = 0%-40%) to give compound **1-2b** (4.2 g, 68.2% yield) as a light-yellow solid. MS (ESI) m/z: 212.9 $[M+H]^+$.

[0261] Step 2: N,N-dimethyl-4-(5-(methylthio)-1,2,4-thiadiazol-3-yl)benzamide (**1-2d**)

[0262] To a solution of compound **1-2b** (100 mg, 0.47 mmol) in DMF (4 mL) and H_2O (1 mL) were added compound **1-2c** (110 mg, 0.57 mmol), K_3PO_4 (201 mg, 0.95 mmol), and $Pd(PPh_3)_4$ (36 mg, 0.072 mmol). The mixture was stirred at 90 °C for 6 h under N_2 atmosphere. The mixture was filtered through a pad of celite, diluted with EA (100 mL), washed with brine (50 mL*4). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography (eluted with PE:EA=0-40%). Compound **1-2d** (35 mg, 26.4% yield) was obtained as white solid. MS (ESI) m/z: 280.2 $[M+H]^+$.

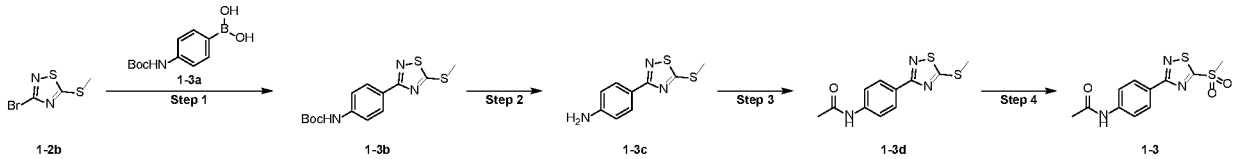
[0263] Step 3: N,N-dimethyl-4-(5-(methylsulfonyl)-1,2,4-thiadiazol-3-yl)benzamide (**1-2**)

[0264] To a solution of compound **1-2d** (30 mg, 0.11 mmol) in DCM (4 mL) were added m-CPBA (74 mg, 0.43 mmol). The mixture was stirred at room temperature for 3 h. The mixture was concentrated and purified by prep-HPLC (Method: column：XBridge Prep C18 OBD 5um 19*250 mm; Mobile phase: A-water (0.1% TFA): B-acetonitrile; Flow rate: 20 mL/min). Compound **1-2**

(4.0 mg, 12% yield) was obtained as a white solid.

[0265] MS (ESI) m/z: 312.3 $[M+H]^+$.

Example 1-3



[0266] Step 1: tert-butyl (4-(5-(methylthio)-1,2,4-thiadiazol-3-yl)phenyl)carbamate (**1-3b**)

[0267] To a solution of compound **1-2b** (150 mg, 0.71 mmol) in toluene/EtOH (v:v=7:3, 5 mL) were added **1-3a** (202 mg, 0.85 mmol), Na_2CO_3 (150 mg, 1.42 mmol) in H2O (1 mL) and $Pd(PPh_3)_4$ (82 mg, 0.071 mmol). The mixture was heated to refluxing for 3 hr under N_2. After, the reaction mixture was cooled to room temperature and then diluted with 5.0 ml ethyl acetate. Then the aqueous layer was separated, the organic layer was washed with sat. NaCl solution, dried with Na_2SO_4, concentrated and then the residue was purified by flash column chromatography (eluted with PE:EA=0%-40%) to give compound **1-3b** (75 mg, 32.6% yield).

[0268] MS (ESI) m/z: 324.1 $[M+H]^+$.

[0269] Step 2: 4-(5-(methylthio)-1,2,4-thiadiazol-3-yl)aniline (**1-3c**)

[0270] To a solution of compound **1-3b** (75 mg, 0.23 mmol) in DCM (4 mL) was added TFA (1 mL) dropwise. The mixture was stirred at room temperature for 1 h. The mixture was concentrated and co-evaporated with toluene (3 mL*3). Compound **1-3b** (55 mg, crude) was obtained as a white solid, which was used for the next step without further purification.

[0271] MS (ESI) m/z: 224.1 $[M+H]^+$.

[0272] Step 3: N-(4-(5-(methylthio)-1,2,4-thiadiazol-3-yl)phenyl)acetamide (**1-3d**)

[0273] To a solution of compound **1-3c** (55 mg, 0.25 mmol) in THF (4 mL) was added acetic anhydride (50.3 mg, 0.49 mmol) and Et_3N (50 mg, 0.49 mmol). The mixture was stirred at room temperature for 2 h. The mixture was filtered, the filtrate was diluted with EA (50 mL), washed by sat. $NaHCO_3$ (50 mL*3), brine (50 mL*3), respectively. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography (eluted with PE:EA=0%-40%). Compound **1-3d** (50 mg, 76.5% yield) was obtained as a white solid.

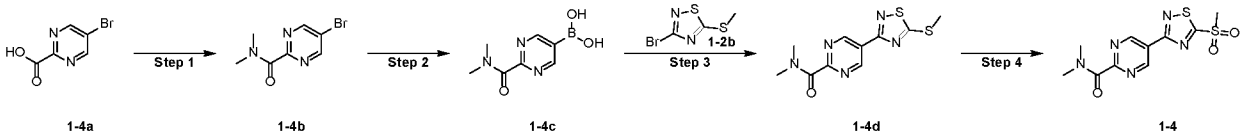
[0274] MS (ESI) m/z: 266.1 $[M+H]^+$.

[0275] Step 4: N-(4-(5-(methylsulfonyl)-1,2,4-thiadiazol-3-yl)phenyl)acetamide (**1-3**)

[0276] To a solution of compound **1-3d** (50 mg, 0.19 mmol) in DCM (4 mL) was added compound m-CPBA (130 mg, 0.75 mmol). The mixture was stirred at room temperature for 3 h. The mixture was concentrated and purified by prep-HPLC (Method: column：XBridge Prep C18 OBD 5um 19*250 mm; Mobile phase: A-water (0.1% TFA): B-acetonitrile; Flow rate: 20 mL/min). Compound **1-3** (12 mg, 12.7% yield) was obtained as a white solid.

[0277] MS (ESI) m/z: 298.1 $[M+H]^+$.

Example 1-4



[0278] Step 1: 5-bromo-N,N-dimethylpyrimidine-2-carboxamide (**1-4b**)

[0279] To a solution of compound **1-4a** (2000 mg, 9.85 mmol) in DMF (40 mL) were added dimethylamine hydrochloride (1210 mg, 14.78 mmol), HATU (5620 mg, 14.78 mmol), and DIPEA (2550 mg, 19.70 mmol). The mixture was stirred at rt for 3 h under N_2 atmosphere. The mixture was concentrated. The crude product was purified by flash column chromatography (eluted with DCM : MeOH=0-20%). Compound **1-4b** (1500 mg, 66.1% yield) was obtained as an off-white solid.

[0280] MS (ESI) m/z: 232.0 $[M+H]^+$.

[0281] Step 2: (2-(dimethylcarbamoyl)pyrimidin-5-yl)boronic acid (**1-4c**)

[0282] To a solution of compound **1-4b** (1.4 g, 6.09 mmol) in 1,4-dioxane (30 mL) and H_2O (3 mL) were added 4,4,4',4',5,5,5',5'-octamethyl-2,2'-Bi-(1,3,2-dioxaborolane) (1.85 g, 7.3 mmol) and potassium acetate (1.19 g, 12.2 mmol) under nitrogen atmosphere. The reaction mixture was stirred for 5 minutes and $Pd(dppf)Cl_2$.DCM (495 mg, 0.60 mmol) was added to it. The reaction mixture was refluxed for 16 h. After total consumption of starting material, the reaction mixture was diluted with water and extracted with ethyl acetate (3×100 mL). The combined organic layer was dried over Na_2SO_4, filtered, and purified by flash column chromatography (eluted with CH_2Cl_2:MeOH=0%-20%) to give compound **1-4c** (1.05 g, 88.3% yield) as white solid.

[0283] MS (ESI) m/z: 196.0 $[M+H]^+$.

[0284] Step 3: N,N-dimethyl-5-(5-(methylthio)-1,2,4-thiadiazol-3-yl)pyrimidine-2-carboxamide (**1-4d**)

[0285] To a solution of compound **1-2c** (150 mg, 0.71 mmol) in toluene/EtOH (v:v=7:3, 5 mL)

were added **1-2b** (166 mg, 0.85 mmol), Na_2CO_3 (150 mg, 1.42 mmol) in H_2O (1 mL) and $Pd(PPh_3)_4$ (82 mg, 0.071 mmol). The mixture was heated to refluxing for 3 hr under N_2. After, the reaction mixture was cooled to room temperature and then diluted with 5.0 ml ethyl acetate. Then the aqueous layer was separated, the organic layer was washed with sat. NaCl solution, dried with Na_2SO_4, concentrated and then the residue was purified by flash column chromatography (eluted with PE:EA=0%-40%) to give compound **1-4d** (45 mg, 22.5% yield).

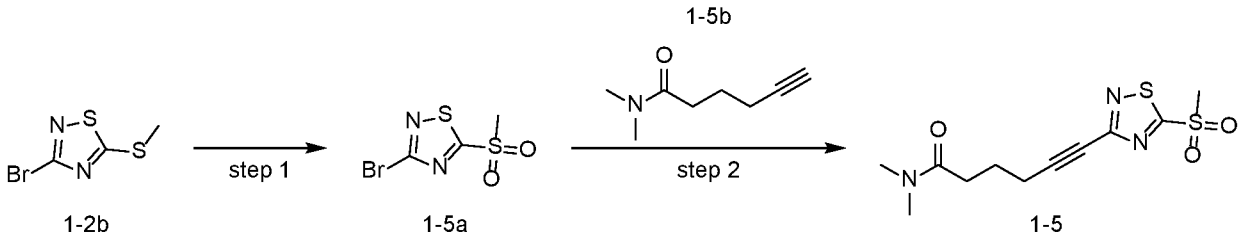
[0286] MS (ESI) m/z: 282.0 $[M+H]^+$.

[0287] Step 4: N,N-dimethyl-5-(5-(methylsulfonyl)-1,2,4-thiadiazol-3-yl)pyrimidine-2-carboxamide (**1-4**)

[0288] To a solution of compound **1-4d** (40 mg, 0.14 mmol) in DCM (3 mL) was added m-CPBA (123 mg, 0.71 mmol). The mixture was stirred at room temperature for 2 h.

[0289] The mixture was concentrated and purified by prep-HPLC (Method: column：XBridge Prep C18 OBD 5um 19*250 mm; Mobile phase: A-water (0.1% TFA): B-acetonitrile; Flow rate: 20 mL/min). Compound **1-4** (3.7 mg, 9.3 yield) was obtained as a white solid. MS (ESI) m/z: 314.1 [M+H]+.

Example 1-5



[0290] Step 1: (3-bromo-5-(methylsulfonyl)-1,2,4-thiadiazole (**1-5a**)

[0291] To a solution of **1-2b** (200 mg, 0.947 mmol) in DCM (3 mL) was added m-CPBA (817.5 mg, 4.737 mmol). The white suspension was stirred at r.t. for 5 hr. The mixture was quenched with sat.$Na_2S_2O_3$ (50 mL), extracted with DCM (30 mL * 2). Combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered and the filtrate was concentrated under vacuum to give a residue. It was purified by FCC (EA/PE=0%~20%), fraction was concentrated under vacuum to give **1-5a** (239 mg, 95% yield) as a white solid.

[0292] ^1H NMR (400 MHz, $CDCl_3$) δ 2.76 (s, 1H).

[0293] Step 2: N,N-dimethyl-6-(5-(methylsulfonyl)-1,2,4-thiadiazol-3-yl)hex-5-ynamide (**1-5**)

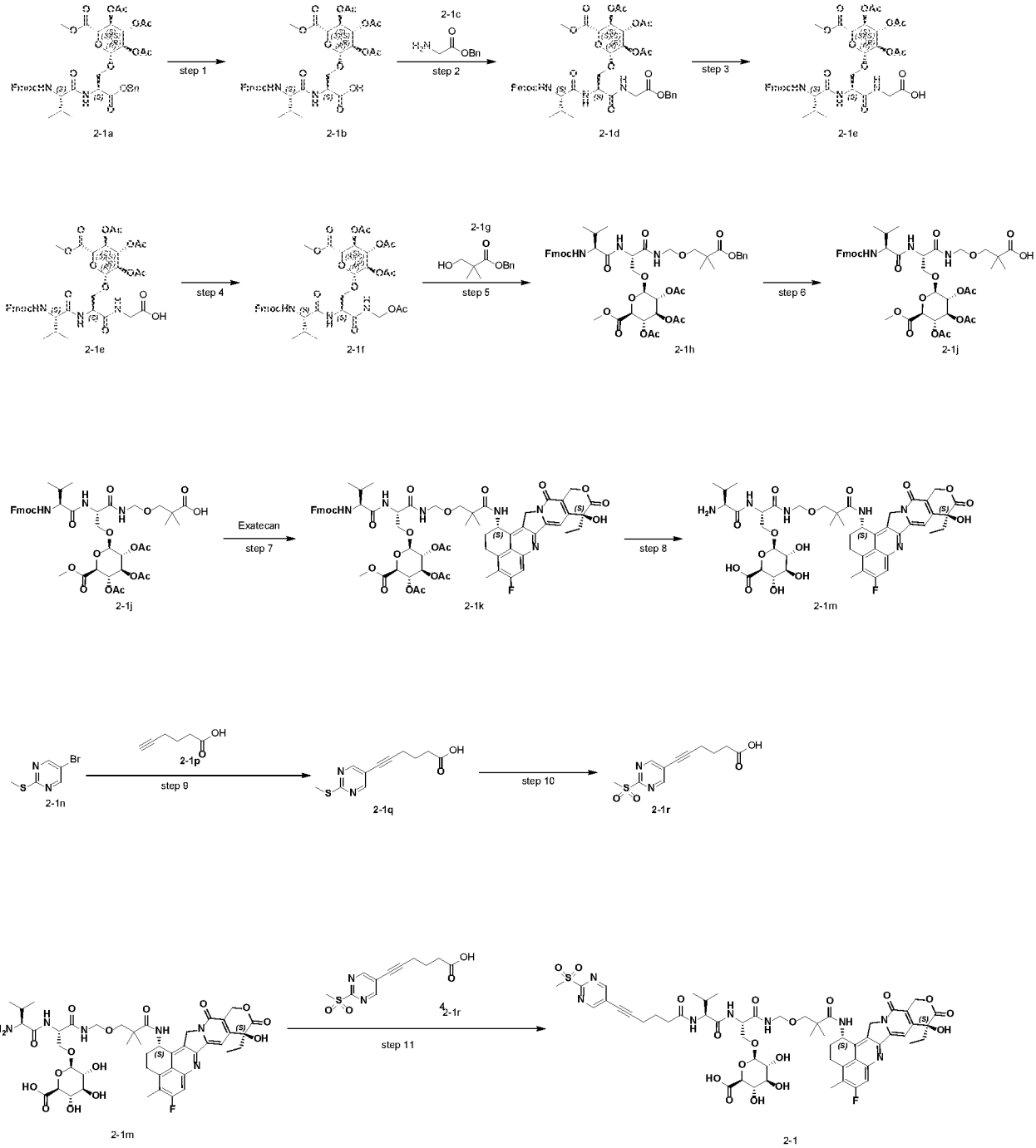
[0294] To a mixture of **1-5a** (239.0 mg, 0.983 mmol) and **1-5b** (150.5 mg, 1.081 mmol) in DMF (4.0 mL) were added CuI (37.4 mg, 0.197 mmol), $Pd(PPh_3)Cl_2$ (69.0 mg, 0.098 mmol), and DIPEA (205 μL, 152.5 mg, 1.18 mmol) then degassed with nitrogen atmosphere three times. The mixture was stirred at r.t. overnight. The mixture was filtered and purified by prep-HPLC (0.1% FA), fraction was freeze-dried to give **1-5** (13.6 mg, 4% yield) as a yellow oil.

[0295] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.38 (s, 3H), 3.01 (s, 3H), 2.95 (s, 3H), 2.60 (t, J = 6.8 Hz, 2H), 2.50 (t, J = 7.2 Hz, 1H), 2.00 (p, J = 7.0 Hz, 2H).

[0296] MS (ESI) m/z: 302.0 $[M+H]^+$.

[0297] ^{1}H NMR (400 MHz, DMSO) δ 7.09 (s, 2H), 6.99 (t, J = 6.4 Hz, 1H), 4.60 (dd, J = 10.8, 4.0 Hz, 1H), 3.59-3.53 (m, 1H), 3.46-3.38 (m, 1H), 1.32 (s, 9H).

Example 2-1



[0298] Step 1: N-((((9H-fluoren-9-yl)methoxy)carbonyl)-L-valyl)-O-((2R,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)-L-serine (**2-1b**)

[0299] To a mixture of **2-1a** (4.1 g, 4.92 mmol, purchased from MCE) in MeOH (50 mL), THF (100 mL) and DCM (20 mL) was added wet Pd/C (400 mg, 10% purity). The black suspension was

purged with H_2 balloon three times and then stirred at r.t. for 1 hr. The black suspension was filtered through a pad of celite, washed with MeOH (200 mL). Organic layers were combined and concentrated under vacuum to give **2-1b** (3650 mg, 99.8% yield) as an off-white solid.

[0300] MS (ESI) m/z: 743.6 $[M+H]^+$.

[0301] Step 2: (2R,3R,4S,5S,6S)-2-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-3-((2-(benzyloxy)-2-oxoethyl)amino)-3-oxopropoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**2-1d**)

[0302] To a solution of **2-1b** (3.65 g, 4.92 mmol) and **2-1c** (1.66 g, 4.92 mmol) in DMF (50 mL) were added HATU (1.87 g, 4.92 mmol) and DIPEA (1.59 g, 12.29 mmol). The mixture was stirred at r.t. for 30 min. The mixture was purified by FCC (MeOH/DCM=0~10%), and the fraction was concentrated under vacuum to give **2-1d** (3.8 g, 86.9% yield) as an off-white foamed solid.

[0303] MS (ESI) m/z: 890.7 $[M+H]^+$.

[0304] Step 3: N-((((9H-fluoren-9-yl)methoxy)carbonyl)-L-valyl)-O-((2R,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)-L-serylglycine (**2-1e**)

[0305] To a mixture of **2-1d** (3.8 g, 4.27 mmol) in MeOH (150 mL) and DCM (50 mL) was added wet Pd/C (400 mg, 10% purity). The black suspension was purged with H_2 balloon three times and then stirred at r.t. for 40 min. The black suspension was filtered through a pad of celite, washed with MeOH (150 mL). Organic layers were combined and concentrated under vacuum to give **2-1e** (3.3 g, 96.6% yield) as an off-white solid.

[0306] MS (ESI) m/z: 800.7 $[M+H]^+$.

[0307] Step 4: (2R,3R,4S,5S,6S)-2-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-3-((acetoxymethyl)amino)-3-oxopropoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**2-1f**)

[0308] To a solution of **2-10e** (3.3 g, 4.13 mmol) in DMF (30 mL) were added $Pb(OAc)_4$ (2.74 g, 6.19 mmol), Cu(OAc)2 (74.9 mg, 0.41 mmol), and HOAc (247.8 mg, 4.13 mmol). The resulting dark-colored mixture was purged with N_2 balloon three times and then stirred at 65 °C for 40 min, turning the mixture to deep blue. The mixture was diluted with EtOAc (300 mL), washed with brine (100 mL * 3), dried over Na_2SO_4, filtered, and concentrated under vacuum to give a residue. It was purified by FCC (MeOH/DCM=0~10%), and the fraction was concentrated under vacuum to give **2-1f** (2.8 g, 83.4% yield) as a pale-yellow solid.

[0309] MS (ESI) m/z: 836.6 $[M+Na]^+$.

[0310] Step 5: (2R,3R,4S,5S,6S)-2-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-3-(((3-(benzyloxy)-2,2-dimethyl-3-oxopropoxy)methyl)amino)-3-oxopropoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**2-1h**)

[0311] A white suspension mixture of **2-1f** (300 mg, 0.37 mmol), **2-1g** (153.7 mg, 0.74 mmol) and 4Å molecular sieve (200 mg) in anhydro THF (10 mL) was stirred at r.t. for 10 min. $Sc(OTf)_3$ (217.9 mg, 0.44 mmol) was added and the resulting yellow suspension was stirred at r.t. for 4 hr. The yellow suspension mixture was filtered through a pad of celite and washed with EA. Combined organic layers were washed with sat.$NaHCO_3$ (30 mL) and brine (30 mL), dried over Na_2SO_4, filtered, and the filtrate was concentrated under vacuum to give a residue. It was purified by silica gel column (MeOH/DCM=0%~5%), and the fraction was concentrated under vacuum to give **2-1h** (275 mg, 77.5% yield) as a white foam solid.

[0312] MS (ESI) m/z: 984.8 $[M+Na]^+$.

[0313] Step 6: (5S,8S)-1-(9H-fluoren-9-yl)-5-isopropyl-14,14-dimethyl-3,6,9-trioxo-8-((((2R,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)-2,12-dioxa-4,7,10-triazapentadecan-15-oic acid (**2-1j**)

[0314] To a solution of **2-1h** (275 mg, 0.29 mmol) in MeOH (10 mL) was added wet Pd/C (55 mg, 10% purity). The black suspension was purged with H_2 balloon three times, then stirred at r.t. for 2 hr. The mixture was filtered through syringe head and washed with MeOH (15 mL), concentrated under vacuum to give **2-1j** (230 mg, crude) as a white foam solid.

[0315] MS (ESI) m/z: 894.6 $[M+Na]^+$.

[0316] Step 7: (2R,3R,4S,5S,6S)-2-((S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-3-(((3-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2,2-dimethyl-3-oxopropoxy)methyl)amino)-3-oxopropoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**2-1k**)

[0317] To a mixture of **2-10j** (230 mg, crude), exatecan mesylate (139.9 mg, 0.26 mmol) and HATU (100.3 mg, 0.26 mmol) in DMF (5 mL) was added DIPEA (102.3 mg, 0.79 mmol). The resulting brown mixture was stirred at r.t. for 1 hr. The mixture was diluted with EtOAc (20 mL), washed with brine (20 mL * 3), dried over Na_2SO_4, filtered, and concentrated under vacuum to give a residue. It was purified by FCC (MeOH/DCM = 0%~3%), and concentrated under vacuum to give **2-1k** (325 mg, 95.6% yield) as an off-white foam solid.

[0318] MS (ESI) m/z: 1289.9 $[M+H]^+$.

[0319] Step 8: (2S,3S,4S,5R,6R)-6-((S)-2-((S)-2-amino-3-methylbutanamido)-3-(((3-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2,2-dimethyl-3-oxopropoxy)methyl)amino)-3-oxopropoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**2-1m**)

[0320] To a solution of **2-1k** (325 mg, 0.25 mmol) in DMF (5 mL) was added Et_2N (523.2 mg, 5.06 mmol). The mixture was stirred at r.t. for 20 min. After LCMS showed the reaction was completed, it was concentrated under vacuum to give a crude product. The crude product was dissolved in MeOH (6 mL). K_2CO_3 (174.7 mg, 1.26 mmol) was added and stirred at r.t. for 10 min, then H_2O (2 mL) was added to the mixture and stirred at r.t. for 30 min. The mixture was acidified with sat.$KHSO_4$ at 0 °C to pH=3, filtered, and purified by prep-HPLC (0.1% FA). The fraction was lyophilized to give **2-1m** (140 mg, 59.7% yield) as a pale yellow solid.

[0321] MS (ESI) m/z: 927.4 $[M+H]^+$.

[0322] ^1H NMR (400 MHz, d_6-DMSO) δ 9.56 (s, 1H), 8.39 (s, 1H), 8.07 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 11.2 Hz, 1H), 7.31 (s, 1H), 6.52 (s, 1H), 5.54 (dd, J = 13.2, 7.2 Hz, 1H), 5.43 (s, 2H), 5.18 (dd, J = 41.6, 18.8 Hz, 2H), 5.09 – 5.02 (m, 1H), 4.96 (s, 1H), 4.62 (dd, J = 10.0, 6.8 Hz, 1H), 4.56 – 4.44 (m, 2H), 4.19 (d, J = 7.6 Hz, 1H), 3.82 (dd, J = 10.8, 6.8 Hz, 1H), 3.61 (dd, J = 11.6, 6.4 Hz, 2H), 3.17-3.05 (m, 4H), 2.94 (t, J = 8.0 Hz, 1H), 2.39 (s, 3H), 2.11 (dt, J = 21.3, 7.6 Hz, 2H), 2.03 – 1.93 (m, 2H), 1.92 – 1.78 (m, 3H), 1.12 (d, J = 8.0 Hz, 6H), 0.87 (dd, J = 13.0, 6.6 Hz, 9H).

[0323] Step 9: 6-(2-(methylthio)pyrimidin-5-yl)hex-5-ynoic acid (**2-1q**)

[0324] To a mixture of **2-1n** (2.0 g, 9.751 mmol) and **2-1p** (1.31 g, 11.702 mmol) in DMF (30 mL) were added CuI (185.7 mg, 0.975 mmol), Pd(PPh3)Cl2 (684.4 mg, 0.975 mmol) and TEA (4.1 mL, 2.960 g, 29.254 mmol). The mixture was degassed with nitrogen atmosphere three times, then stirred at 95 °C for 2 hr. The mixture was diluted with EtOAc (30 mL), washed with brine (30 mL * 2), dried over Na_2SO_4, and the filtrate was concentrated under vacuum to give a residue. It was purified by FCC (MeOH/DCM = 0% ~3%), and the fraction was concentrated under vacuum to give **2-1q** (1.6 g, ~65% yield) as a yellow solid.

[0325] MS (ESI) m/z: 237.1 $[M+H]^+$.

[0326] Step 10: 6-(2-(methylsulfonyl)pyrimidin-5-yl)hex-5-ynoic acid (**2-1r**)

[0327] To a solution of **2-1q** (520 mg, 1.613 mmol) in DCM (10 mL) was added m-CPBA (1113.4

mg, 6.452 mmol). The resulting yellow suspension was stirred at r.t. for 1.5 hr. The mixture was diluted with EA (50 mL) and quenched by addition of sat.$Na_2S_2O_3$ (30 mL), stirred at r.t for 10 min, extracted with DCM (30 mL * 3). Organic layers were combined and washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum to give crude product as a yellow solid. It was purified by prep-HPLC (FA), and the fraction was freeze-dried to give **2-1r** (78.0 mg, 18% yield) as a white solid.

[0328] MS (ESI) m/z: 269.1 $[M+H]^+$.

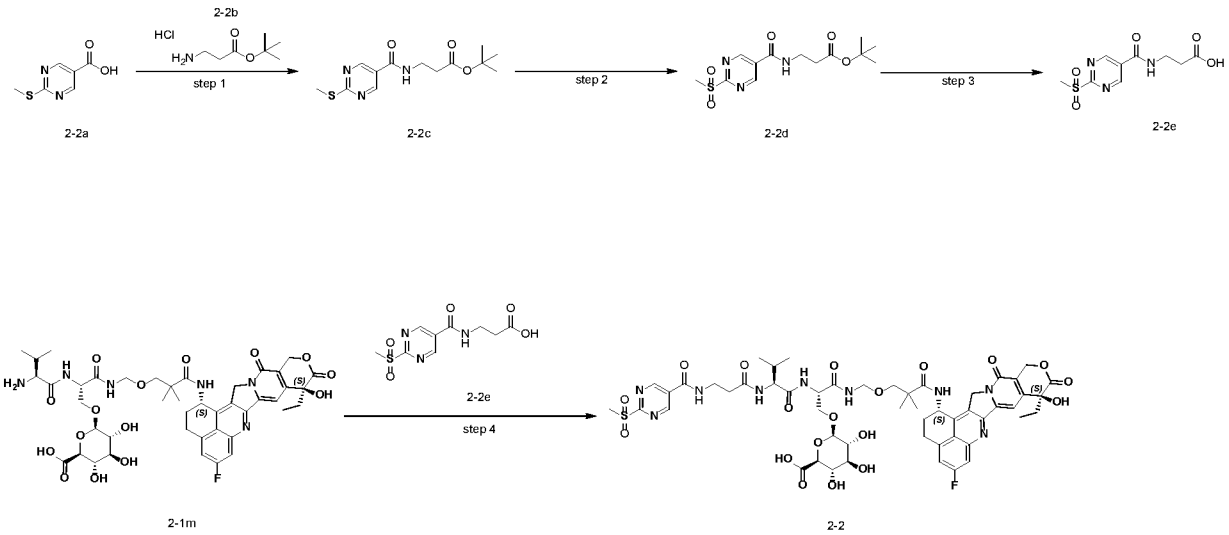
[0329] ^1H NMR (400 MHz, d_6-DMSO) δ 12.17 (s, 1H), 9.12 (s, 2H), 3.41 (s, 3H), 2.60 (t, J = 7.2 Hz, 2H), 2.41 (t, J = 7.2 Hz, 2H), 1.82 (p, J = 6.8 Hz, 2H).

[0330] Step 11: (2S,3S,4S,5R,6R)-6-((S)-3-(((3-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2,2-dimethyl-3-oxopropoxy)methyl)amino)-2-((S)-3-methyl-2-(6-(2-(methylsulfonyl)pyrimidin-5-yl)hex-5-ynamido)butanamido)-3-oxopropoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**2-1**)

[0331] To a solution of **2-1r** (5.2 mg, 0.019 mmol) and **2-1m** (15.0 mg, 0.016 mmol) in DMF (2 mL) were added HATU (8.6 mg, 0.024 mmol) and DIPEA (7 μL, 5.2 mg, 0.04 mmol). The mixture was stirred at r.t. for 30 min. The mixture was purified by prep-HPLC (FA 0.1%), and the fraction was freeze-dried to give **2-1** (7.1 mg, 37.3% yield) as a white solid.

[0332] MS (ESI) m/z: 1177.9 $[M+H]^+$.

Example 2-2



[0333] Step 1: tert-butyl 3-(2-(methylthio)pyrimidine-5-carboxamido)propanoate (**2-2c**)

[0334] To a mixture of **2-2a** (600 mg, 3.525 mmol) in DMF (15 mL) were added HATU (1621.9 mg, 4.266 mmol) and DIPEA (1.9 mL, 1503.5 mg, 11.633 mmol). The mixture was stirred at 45 °C for 10 min, **2-2b** (704.6 mg, 3.878 mmol) was added and stirred at 45 °C for 40 min. The mixture was diluted with EtOAc (50 mL), washed with brine (30 mL * 3), dried over Na_2SO_4, filtered and concentrated under vacuum to give a residue. It was purified by FCC (MeOH/DCM) to give **2-1c** (1.3 g crude) as a brown oil.

[0335] MS (ESI) m/z: 298.4 $[M+H]^+$.

[0336] Step 2: tert-butyl 3-(2-(methylsulfonyl)pyrimidine-5-carboxamido)propanoate (**2-2d**)

[0337] To a solution of **2-2c** (500 mg, crude) in DCM (10 mL) was added m-CPBA (1160.5 mg, 6.725 mmol). The white suspension was stirred at r.t. for 1 hr. The mixture was diluted with EA (50 mL) and quenched by addition of sat.$Na_2S_2O_3$ (30 mL), stirred at r.t for 10 min, extracted with DCM (30 mL * 3). Organic layers were combined and washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum to give crude product as a yellow solid. It was purified by FCC (MeOH/DCM), and the fraction was concentrated under vacuum to give **2-2d** (303 mg, ~50% yield) as a white solid.

[0338] MS (ESI) m/z: 330.3 $[M+H]^+$.

[0339] ^1H NMR (400 MHz, $cdcl_3$) δ 9.27 (s, 2H), 7.38 (s, 1H), 3.74 (dd, J = 11.6, 6.0Hz, 1H), 3.40 (s, 2H), 2.60 (t, 1H), 1.47 (s, 5H).

[0340] Step 3: 3-(2-(methylsulfonyl)pyrimidine-5-carboxamido)propanoic acid (**2-2e**)

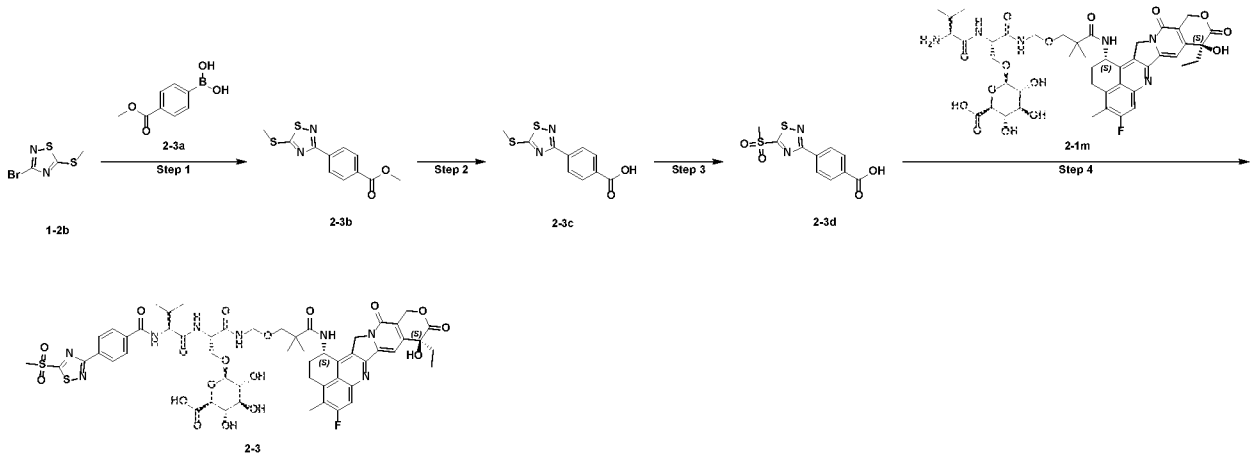
[0341] A mixture of **2-2d** (303.0 mg, 0.92 mmol) in TFA (1 mL) and DCM (2 mL) was stirred at r.t. for 1.5 hr. The mixture was concentrated under vacuum and co-evaporated with toluene three time to give **2-2e** (255 mg) as an off-white solid. MS (ESI) m/z: 274.1 $[M+H]^+$.

[0342] Step 4: (2S,3S,4S,5R,6R)-6-((S)-3-(((3-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2,2-dimethyl-3-oxopropoxy)methyl)amino)-2-((S)-3-methyl-2-(3-(2-(methylsulfonyl)pyrimidine-5-carboxamido)propanamido)butanamido)-3-oxopropoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**2-2**)

[0343] To a solution of **2-2e** (7.1 mg, 0.026 mmol) and **2-1m** (20.0 mg, 0.022 mmol) in DMF (2 mL) were added HATU (11.5 mg, 0.030 mmol) and DIPEA (9 μL, 7.0 mg, 0.054 mmol). The mixture was stirred at r.t. for 30 min. The mixture was purified by prep-HPLC (FA 0.1%), and the fraction was freeze-dried to give **2-2** (13.4 mg, ~52.5% yield) as a white solid. MS (ESI) m/z:

1182.8 $[M+H]^+$.

Example 2-3



[0344] Step 1: methyl 4-(5-(methylthio)-1,2,4-thiadiazol-3-yl)benzoate (**2-3b**)

[0345] To a solution of compound **1-2b** (100 mg, 0.47 mmol) in toluene (4 mL) and H_2O (1 mL) were added compound **2-3a** (109.72 mg, 0.568 mmol), K_2CO_3 (168 mg, 0.947 mmol) and $Pd(dppf)Cl_2$.DCM (34.6 mg, 0.047 mmol). The mixture was stirred at 110 °C for 3 h under N_2 atmosphere. The mixture was filtered through a pad of celite, diluted with EA (100 mL), washed with brine (50 mL*4). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography (eluted with PE:EA=0-40%). Compound **2-3b** (56 mg, 44.4% yield) was obtained as an off-white solid.

[0346] MS (ESI) m/z: 267.1 $[M+H]^+$.

[0347] Step 2: 4-(5-(methylthio)-1,2,4-thiadiazol-3-yl)benzoic acid (**2-3c**)

[0348] To a solution of compound **2-3b** (54 mg, 0.20 mmol) in MeOH (3 mL) and H_2O (1 mL) was added LiOH (17 mg, 0.41 mmol). The mixture was stirred at r.t. for 2 h. The mixture was adjusted to pH 7 and purified by prep-HPLC (FA condition) to give compound **2-3c** (36 mg, 70.3% yield) as a white solid.

[0349] MS (ESI) m/z: 253.1 $[M+H]^+$.

[0350] Step 3: 4-(5-(methylsulfonyl)-1,2,4-thiadiazol-3-yl)benzoic acid (**2-3d**)

[0351] To a solution of compound **2-3c** (35 mg, 0.14 mmol) in DCM (3 mL) and THF (3 mL) was added m-CPBA (96 mg, 0.55 mmol). The mixture was stirred at room temperature for 16 h. The mixture was concentrated and purified by prep-HPLC (Method: column：XBridge Prep C18 OBD 5um 19*250 mm; Mobile phase: A-water (0.1% TFA): B-acetonitrile; Flow rate: 20 mL/min).

Compound **2-3d** (12 mg, 99% purity) was obtained as a white solid.

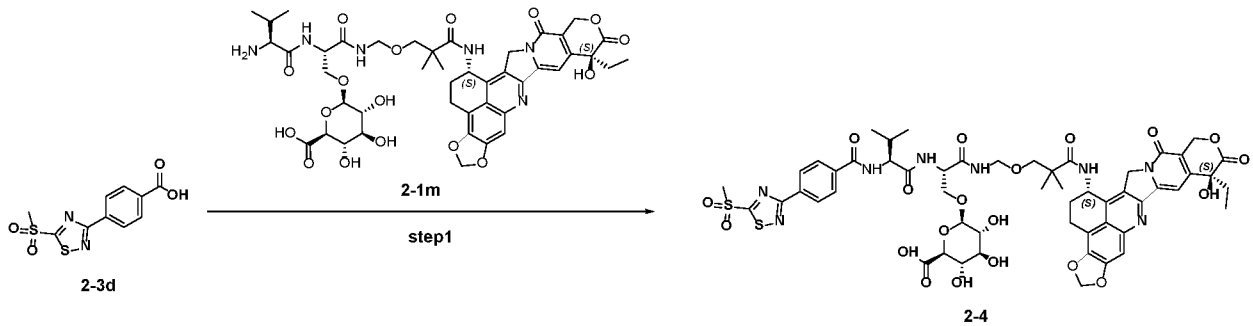
[0352] MS (ESI) m/z: 284.8 $[M+H]^+$.

[0353] Step 4: (2S,3S,4S,5R,6R)-6-((S)-3-(((3-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2,2-dimethyl-3-oxopropoxy)methyl)amino)-2-((S)-3-methyl-2-(4-(5-(methylsulfonyl)-1,2,4-thiadiazol-3-yl)benzamido)butanamido)-3-oxopropoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**2-3**)

[0354] To a solution of compound **2-3d** (7.36 mg, 0.026 mmol) in DMF (2 mL) were added HATU (9.02 mg, 0.024 mmol) and DIPEA (5.58 mg, 0.043 mmol). The mixture was stirred at r.t. for 30 min. Compound **2-1m** (20 mg, 0.022 mmol) was added to the mixture and stirred at r.t. for 15 min. The reaction was purified by prep-HPLC (Method: column：XBridge Prep C18 OBD 5um 19*250 mm; Mobile phase: A-water (0.1% TFA): B-acetonitrile; Flow rate: 20 mL/min) to give compound **2-3** (7.6 mg, 29.5% yield) as a white solid.

[0355] MS (ESI) m/z: 1193.5 $[M+H]^+$.

Example 2-4



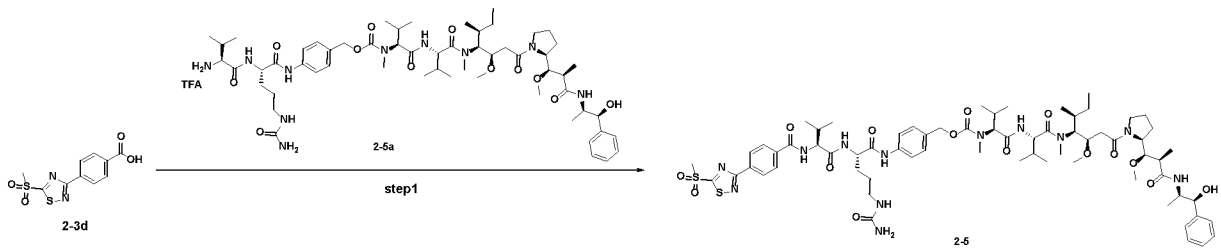
[0356] Step 1: (2*S*,3*S*,4*S*,5*R*,6*R*)-6-((*S*)-3-(((3-(((1*S*,10*S*)-10-ethyl-10-hydroxy-11,14-dioxo-2,3,10,11,14,16-hexahydro-1*H*,13*H*-benzo[*de*][1,3]dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2,2-dimethyl-3-oxopropoxy)methyl)amino)-2-((*S*)-3-methyl-2-(4-(5-(methylsulfonyl)-1,2,4-thiadiazol-3-yl)benzamido)butanamido)-3-oxopropoxy)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylic acid (**2-4**)

[0357] To a solution of **2-3d** (5.9 mg, 0.021 mmol) in dry DMF (0.5 mL) were added HATU (7.9 mg, 0.021 mmol) and DIPEA (0.008 mL, 0.043 mmol), stirred at r.t. for 15 min. Then to the above mixture was added **2-1m** (trifluoroacetate) (18.0 mg, 0.017 mmol), stirred at r.t. for 10 min. The resulting solution was purified by prep-HPLC (Method: column XBridge Prep C18 OBD 5um

19*150 mm; Mobile phase: A-water (0.1% TFA): B-acetonitrile; Flow rate: 20 mL/min), and the fraction was lyophilized to give **3-1** (5.5 mg, 26.7% yield) as a yellow solid.

[0358] MS (ESI) m/z: 1205.6 $[M+H]^+$.

Example 2-5

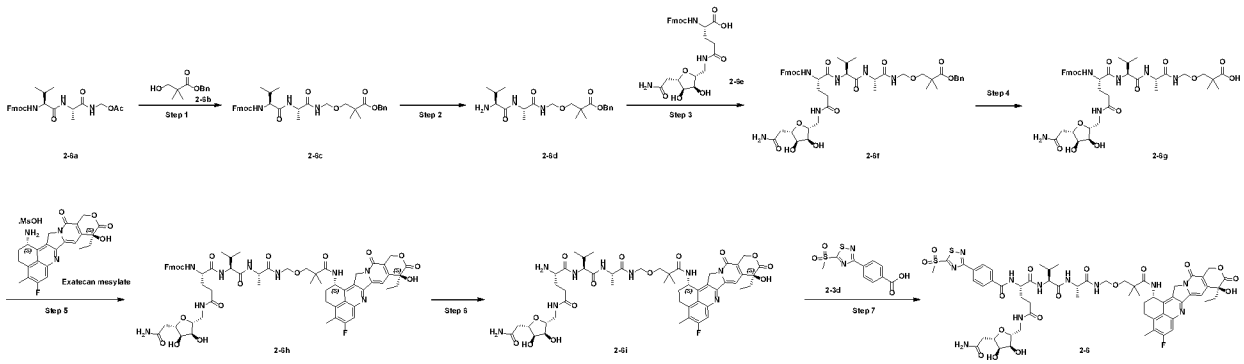


[0359] 4-((S)-2-((S)-3-methyl-2-(4-(5-(methylsulfonyl)-1,2,4-thiadiazol-3-yl)benzamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (**2-5**)

[0360] To a solution of **2-3d** (10 mg, 0.035 mmol) in dry DMF (1 mL) were added HATU (15 mg, 0.040 mmol) and DIPEA (9.1 mg, 0.070 mmol). The mixture was stirred at r.t. for 15 min. Then to the above mixture was added **2-5a** (TFA salt, commercially available) (36 mg, 0.032 mmol) and further stirred at r.t. for 10 min. The resulting solution was purified by prep-HPLC (Method: column XBridge Prep C18 OBD 5um 19*150 mm; Mobile phase: A-water (0.1% TFA): B-acetonitrile; Flow rate: 20 mL/min), and the fraction was lyophilized to give **2-5** (14 mg, 31.8% yield) as a white solid.

[0361] MS (ESI) m/z: 1389.8 $[M+H]^+$.

Example 2-6



[0362] Step 1: benzyl (5S,8S)-1-(9H-fluoren-9-yl)-5-isopropyl-8,14,14-trimethyl-3,6,9-trioxo-2,12-

dioxa-4,7,10-triazapentadecan-15-oate (**2-6c**)

[0363] A white suspension mixture of **2-6a** (300 mg, 0.623 mmol), **2-6b** (259.6 mg, 1.246 mmol), and 4Å molecular sieves in anhydro THF (10 mL) was stirred at r.t. for 10 min. $Sc(OTf)_3$ (368.0 mg, 0.748 mmol) was added and the resulting yellow suspension was stirred at r.t. for 4 hr. The yellow suspension mixture was filtered through a pad of celite and washed with EtOAc (30 mL). Combined organic layers were washed with sat.$NaHCO_3$ (30 mL) and brine (30 mL), dried over Na_2SO_4, filtered, and the filtrate was concentrated under vacuum to give a residue. It was purified by silica gel column (MeOH/DCM=0%~5%), and the fraction was concentrated under vacuum to give **2-6c** (274 mg, 69.8% yield) as a white solid.

[0364] MS (ESI) m/z: 652.6 $[M+Na]^+$.

[0365] Step 2: benzyl 3-(((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)methoxy)-2,2-dimethylpropanoate (**2-6d**)

[0366] To a solution of **2-6c** (274.0 mg, 0.44 mmol) in DMF (5 mL) was added Et2N (477.3 mg, 5.53 mmol). The mixture was stirred at r.t. for 20 min. The reaction mixture was concentrated under vacuum and co-evaporated with toluene two times to give **2-6d** (275.3 mg, crude) as a brown oil,

[0367] MS (ESI) m/z: 430.4 $[M+Na]^+$.

[0368] Step 3: benzyl (5S,8S,11S)-5-(3-((((2R,3S,4R,5S)-5-(2-amino-2-oxoethyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)-3-oxopropyl)-1-(9H-fluoren-9-yl)-8-isopropyl-11,17,17-trimethyl-3,6,9,12-tetraoxo-2,15-dioxa-4,7,10,13-tetraazaoctadecan-18-oate (**2-6f**)

[0369] To a solution of **2-6d** (275.3 mg, crude) and **2-6e** (282.3 mg, 0.52 mmol) in DMF (5 mL) were added HATU (198.2 mg, 0.52 mmol) and DIPEA (168.4 mg, 1.30 mmol). The mixture was stirred at r.t. for 10 min. The mixture was purified by reverse phase (C18, 60 g, 30%~70%), and the fraction was freeze-dried to give **2-6f** (370 mg, 91.5% yield) as a brown solid.

[0370] MS (ESI) m/z: 953.8 $[M+Na]^+$.

[0371] Step 4: (5S,8S,11S,17R)-5-(3-((((2R,3S,4R,5S)-5-(2-amino-2-oxoethyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)-3-oxopropyl)-1-(9H-fluoren-9-yl)-17-fluoro-8-isopropyl-11,17-dimethyl-3,6,9,12-tetraoxo-2,15-dioxa-4,7,10,13-tetraazaoctadecan-18-oic acid (**2-6g**)

[0372] To a solution of compound **2-6f** (3.0 g, 3.21 mmol) in co-solvent DMF-MeOH (40 mL, 1:1, *v:v*) was added Pd/C (10%, 600 mg). The mixture was stirred at H_2 atmosphere (15 psi) for 7 h. The mixture was filtered through a pad of celite, and concentrated to give compound **2-6g** (2.5 g, crude)

as a white solid.

[0373] MS (ESI) m/z: 863.7 $[M+Na]^+$.

[0374] Step 5: (9H-fluoren-9-yl)methyl ((6S,9S,12S)-1-((2R,3S,4R,5S)-5-(2-amino-2-oxoethyl)-3,4-dihydroxytetrahydrofuran-2-yl)-19-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-9-isopropyl-12,18,18-trimethyl-3,7,10,13,19-pentaoxo-16-oxa-2,8,11,14-tetraazanonadecan-6-yl)carbamate (**2-6h**)

[0375] To a solution of exatecan mesylate (1000 mg, 1.18 mmol) in DMF (20 mL) were added compound **2-6g** (692 mg, 1.3 mmol), HATU (675 mg, 1.78 mmol), and DIPEA (459 mg, 3.55 mmol). The mixture was stirred at r.t. for 30 min. The mixture was concentrated and purified by a silica gel column chromatography (eluent: DCM/MeOH = 100/0 to 20/80) to give the title compound **2-6h** (1320 mg, 88.6% yield) as an off-white solid.

[0376] MS (ESI) m/z: 1282.1 $[M+Na]^+$.

[0377] Step 6: (S)-2-amino-N5-(((2R,3S,4R,5S)-5-(2-amino-2-oxoethyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-N1-((S)-1-(((S)-1-(((3-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2,2-dimethyl-3-oxopropoxy)methyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)pentanediamide (**2-6i**)

[0378] To a solution of compound **2-6h** (1000 mg, 0.793 mmol) in DMF (20 mL) was added Et_2NH (580 mg, 7.93 mmol). The mixture was stirred at r.t. for 30 min. The mixture was concentrated under high vacuum to give compound **2-6i** (824.6 mg, crude) as an off-white solid, which was used directly without further purification.

[0379] MS (ESI) m/z: 1036.9 $[M+H]^+$.

[0380] Step 7: (S)-N5-(((2R,3S,4R,5S)-5-(2-amino-2-oxoethyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-N1-((S)-1-(((S)-1-(((3-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)amino)-2,2-dimethyl-3-oxopropoxy)methyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)-2-(4-(5-(methylsulfonyl)-1,2,4-thiadiazol-3-yl)benzamido)pentanediamide (**2-6**)

[0381] To a solution of **2-3d** (15.2 mg, 0.053 mmol) in dry DMF (1.0 mL) were added HATU (22.2 mg, 0.058 mmol) and DIPEA (0.017 mL, 0.097 mmol), stirred at r.t. for 15 min. Then to the above

mixture was added **2-6i** (50.0 mg, 0.048 mmol), stirred at r.t. for 10 min. The resulting solution was purified by prep-HPLC (Method: column XBridge Prep C18 OBD 5um 19*150 mm; Mobile phase: A-water (0.1% TFA): B-acetonitrile; Flow rate: 20 mL/min), and the fraction was lyophilized to give **2-6** (32 mg, 50.9% yield) as a yellow solid.

[0382] MS (ESI) m/z: 1303.0 $[M+H]^+$.

[0383] The compounds of Examples 1-1 to 1-5 are shown below in Table 1. The compounds of Examples 2-1 to 2-6 are shown below in Table 2.

Conjugator-antibody conjugate and antibody drug conjugate preparation

[0384] **DAR8 antibody drug conjugate/conjugator-antibody conjugate preparation.** Antibody in conjugation buffer (with concentration 0.5-25 mg/mL, PBS buffer pH 6.0-8.5) was incubated under reduction temperature (0-40 °C) for 10 min. 8-15 eq. TECP solution (5 mM stock in PBS buffer) was added to the reaction mixture and the reduction reaction was left for 1-8 hours at reduction temperature. Organic solvent (e.g., DMSO, DMF, DMA, PG, acetonitrile, 0-25% v/v) and conjugator-linker-payload (see Table 2) or conjugator (see Table 1) stock (10-25 eq, 10 mM stock in organic solvent) were added stepwise after the reduction mixture was cooled to 0-25 °C. Conjugation solution was left for 1-3 h at 0-25 °C, and the reaction was quenched with N-acetyl cysteine (1 mM stock). The solution was submitted to buffer exchange (spin desalting column, ultrafiltration, and dialysis) into storage buffer (for example, pH 5.5- 6.5 histidine acetate buffer, with optional additive such as sucrose, trehalose, tween 20, 60, 80).

[0385] Conjugator-antibody conjugates and ADCs prepared according to the foregoing method are shown in Tables 3 and 4, respectively.

[0386] **ADC characterization.** ADCs were characterized using the following analytical methods. Drug to antibody ratios (DAR) of the ADCs were determined by LCMS method or HIC method. SEC purity of ADCs made were all > 95 % purity.

[0387] *LCMS method:* LC-MS analysis was carried out under the following measurement conditions:

LC-MS system: Vanquish Flex UHPLC and Orbitrap Exploris 240 Mass Spectrometer

Column: MAbPac™ RP, 2.1*50mm, 4μm, 1,500 Å, Thermo Scientific™

Column temperature: 80 °C

Mobile phase A: 0.1 % formic acid (FA) aqueous solution

Mobile phase B: Acetonitrile solution containing 0.1 % formic acid (FA)

Gradient program: 25 %B-25 %B (0 min-2 min), 25 %B-50 %B (2 min-18 min), 50 %B-90 %B (18 min-18.1 min), 90 %B-90 %B (18.1 min-20 min), 90 %B-25 %B (20 min-20.1 min) , 25 %B-25 %B (20.1 min-25 min)

Injected sample amount: 1 μg

MS parameters: Intact and denaturing MS data were acquired in HMR mode at setting of R=15k and deconvolved using the ReSpect™ algorithm and Sliding Window integration in Thermo Scientific™ BioPharma Finder™ 4.0 software.

[0388] *HIC method:* HPLC analysis was carried out under the following measurement conditions:

Method 1

HPLC system: Waters ACQUITY ARC HPLC System

Detector: measurement wavelength: 280 nm

Column: Tosoh Bioscience 4.6 μm ID×3.5 cm, 2.5 μm butyl-nonporous resin column

Column temperature: 25 °C

Mobile phase A: 1.5 M ammonium sulfate, 50 mM phosphate buffer, pH 7.0

Mobile phase B: 50 mM phosphate buffer, 25% (V/V) isopropanol, pH 7.0

Gradient program: 0%B-0%B (0 min-2 min), 0%B-100%B (2 min-15 min), 100%B-100%B (15 min-16 min), 100%B-0%B (16 min-17 min), 0%B-0%B (17 min-20 min)

Injected sample amount: 20 μg

Method 2

HPLC system: Waters ACQUITY ARC HPLC System

Detector: measurement wavelength: 280 nm

Column: MABPac HIC-10, 5 μm, 4.6×10 mm (Thermo)

Column temperature: 25 °C

Mobile phase A: 1.5 M ammonium sulfate, 50 mM sodium phosphate, pH 7.0

Mobile phase B: 50 mM sodium phosphate, pH 7.0

Gradient program: 20 % B- 20 % B (0 min-1 min), 0 % B-0 % B (1 min-35 min), 20 % B-20 % B (35 min-40 min)

Flow rate: 0.5 mL/min

Sample preparation: The sample was diluted with initial mobile phase to 0.5 mg/mL.

[0389] *SEC method to determine ADC purity*: HPLC analysis was carried out under the following measurement conditions:

HPLC system: Waters H-Class UPLC System

Detector: measurement wavelength: 280 nm

Column: ACQUITY UPLC BEH200 SEC 1.7um 4.6x150mm, Waters

Column temperature: room temperature

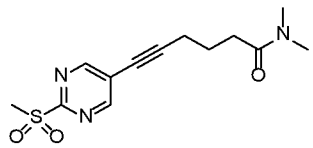
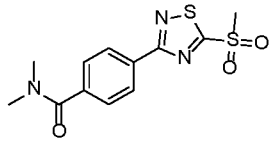
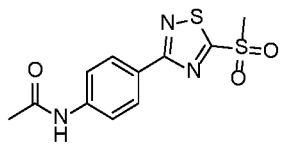
Mobile phase A: 200 mM phosphate buffer, 250 mM potassium chloride, 15 % isopropyl alcohol, pH 7.0

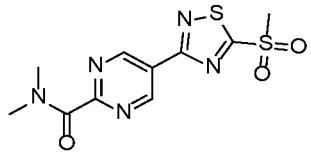
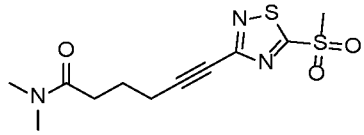
Gradient program: under 10 min isocratic elutions with the flow rate of 0.3 mL/min

Injected sample amount: 20 μg

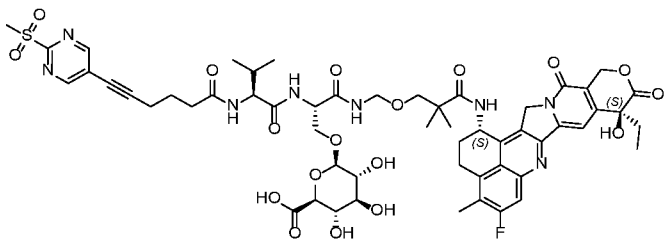
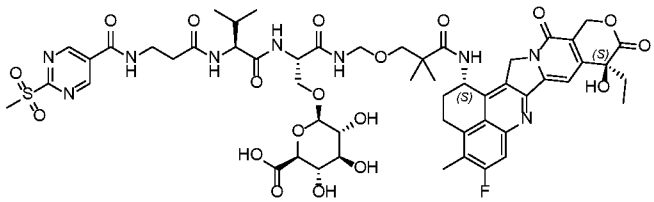
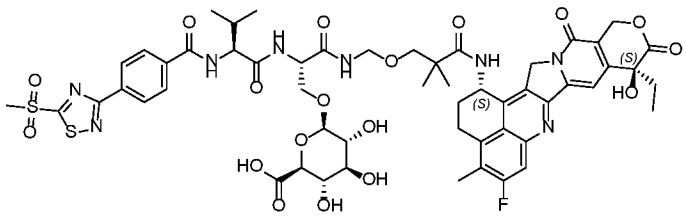
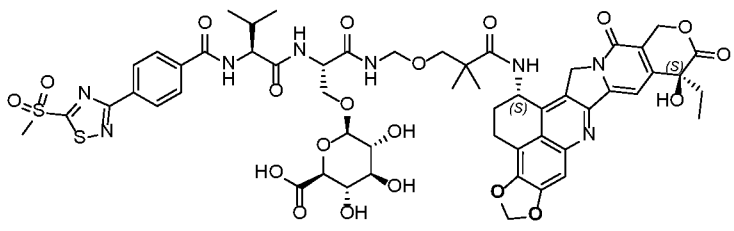
[0390] *ADC hydrophobicity evaluation:* ADC hydrophobicity was evaluated using the HIC (hydrophobicity interaction column) chromatography method described above. An ADC with a higher hydrophobic property would appear with a later retention time from HIC. Results are presented in Table 4 using the DAR8 peak as a reference

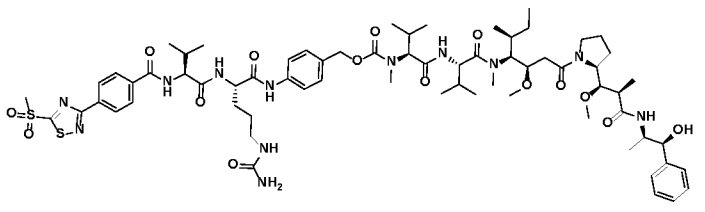
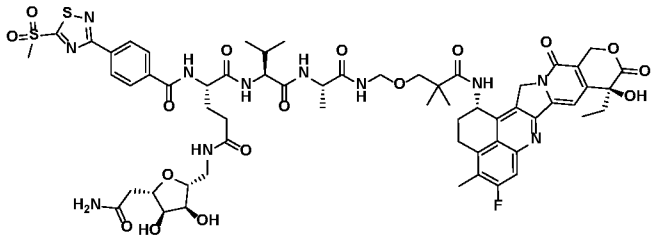
[0391] Table 1: Conjugators

Compound No.	**Conjugator Structure**
1-1	
1-2	
1-3	

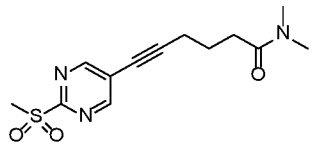
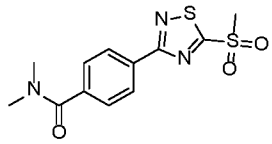
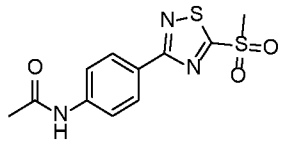
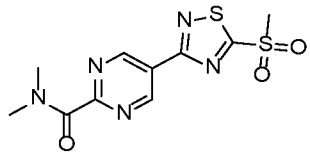
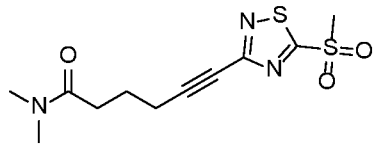
1-4	
1-5	

[0392] Table 2: Conjugator-linker-payloads

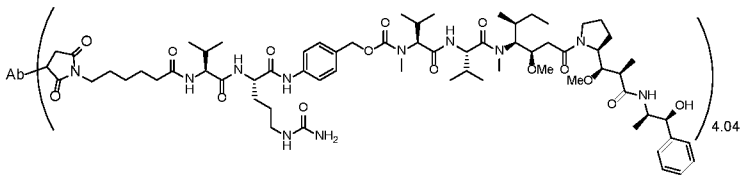
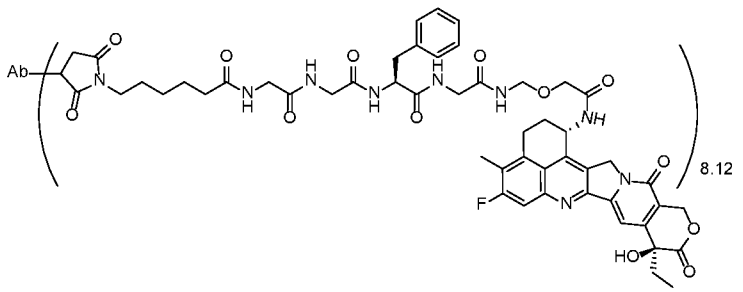
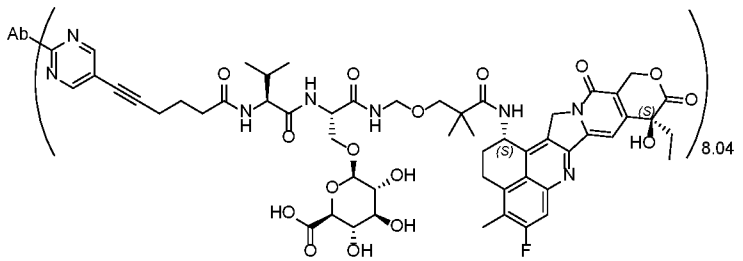
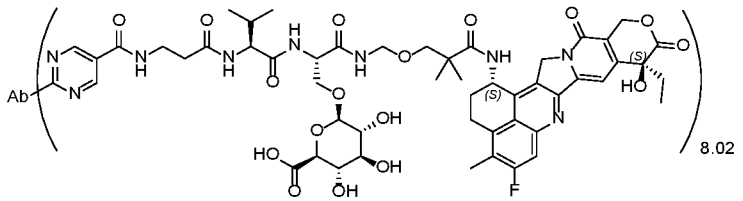
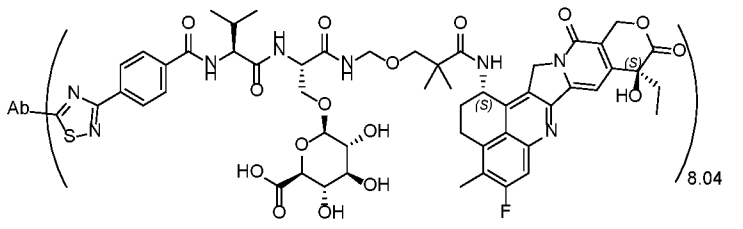
Compound No.	**Conjugator-Linker-Payload Structure**
2-1	
2-2	
2-3	
2-4	

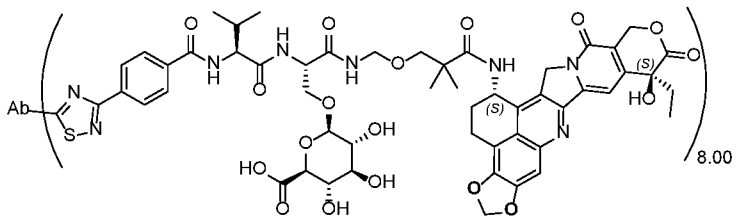
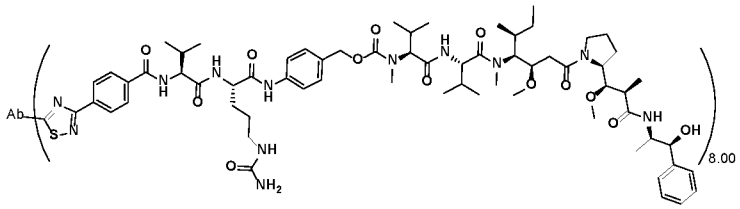
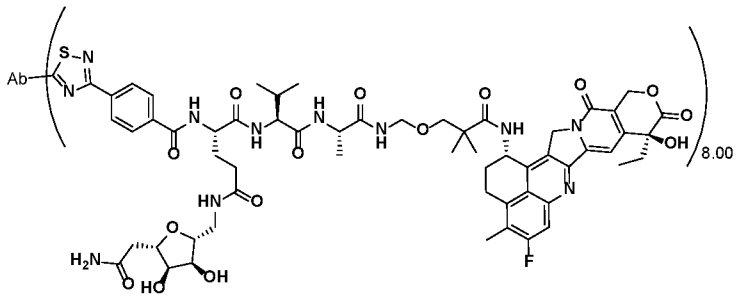
2-5	
2-6	

[0393] Table 3: Conjugator-antibody conjugates

Conjugate-Antibody Conjugate No.	Antibody	Conjugator Structure	MS DAR
3-1	Herceptin		8.00
3-2	Herceptin		8.62
3-3	Herceptin		
3-4	Herceptin		
3-5	Herceptin		

[0394] Table 4: ADCs

ADC No.	Antibody	ADC Structure	DAR8 Species HIC Retention Time (min) (Method 1)
4-A	6E7		14.466
4-B	Ifinatamab		10.860
4-1	Ifinatamab		9.765
4-2	Ifinatamab		9.704
4-3	Ifinatamab		9.756

4-4	Ifinatamab		9.487
4-5	6E7		14.167
4-6	Ifinatamab		9.217

Antibody information

[0395] Ifinatamab (anti-B7H3 antibody)

[0396] Light Chain sequence (SEQ ID NO: 1)

EIVLTQSPATLSLSPGERATLSCRASSRLIYMHWYQQKPGQAPRPLIYATSNLASGIPARFSG
SGSGTDFTLTISSLEPEDFAVYYCQQWNSNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC

[0397] Heavy Chain sequence (SEQ ID NO: 2)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTNYVMHWVRQAPGQGLEWMGYINPYNDDV
KYNEKFKGRVTITADESTSTAYMELSSLRSEDTAVYYCARWGYYGSPLYYFDYWGQGTLV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG

NVFSCSVMHEALHNHYTQKSLSLSPGK

[0398] 6E7 (anti-CLL antibody)

[0399] Light Chain sequence (SEQ ID NO: 3)

DIQMTQSPSSLSASVGDRVTITCRASQSVSTSSYNYMHWYQQKPGKPPKLLIKYASNLESGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQHSWEIPLTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK
ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0400] Heavy Chain sequence (SEQ ID NO: 4)

EVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYMHWVRQAPGQGLEWIGRINPYAGAAF
YSQNFKDRVTLTVDTSTSTAYLELSSLRSEDTAVYYCAIERGADLEGYAMDYWGQGTLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

Conjugator-Antibody Conjugates Stability Study: ADC storage buffer and GSH solution

[0401] In these investigations, the stabilities of conjugator-antibody conjugates in a typical ADC acidic storage buffer were investigated, as were deconjugation events when the conjugates were incubated in glutathione (GSH) solutions. Specifically, after the conjugation steps described above (see "**DAR8 antibody drug conjugate/conjugator-antibody conjugate preparation**"), conjugator-antibody conjugates were incubated in formulation buffer (pH 5.5 20 mM histidine buffer) or GSH buffer (pH 7.4 or 8.0), and the solutions were left at 22 or 37 °C for 1~168 h. Results are presented in Tables 5 and 6 and Figures 1-10).

Stability with GSH				
Conjugate (mM)	GSH (mM)	pH	Time (h)	Temp. (°C)
0.001-0.03	10	7.4/8.0	18	22/37

[0402] Table 5: Conjugator-antibody stability in GSH buffer (pH 7.4 and 8.0)

Conjugator-Antibody Conjugate No.	Incubation Time (h)	pH	Temp. (°C)	T0 MS DAR	MS DAR after incubation
3-1	18	7.4	22	8.00	8.04
			37	8.00	8.04
		8.0	22	8.00	8.02
3-2	120	7.4	22	8.66	8.32
			37	8.66	8.30
		8.0	22	8.66	8.20
3-3	168	7.4	22	8.42	8.14
			37	8.42	8.16
		8.0	22	8.42	8.14
3-4	168	7.4	22	8.70	8.00
			37	8.70	8.32
		8.0	22	8.70	8.12
3-5	96	7.4	22	9.54	8.52
			37	9.54	8.36
		8.0	22	9.54	8.50

[0403] The results demonstrate that DAR values of all conjugates remained almost the same as T0 after incubation in GSH buffers (pH 7.4 at 22/37 °C and pH 8.0 at 22 °C) for at least 18 h.

[0404] Table 6: Conjugator-antibody stability in formulation buffer (pH 5.5 20 mM histidine buffer)

Conjugator-Antibody Conjugate No.	Incubation Time (h)	Temp. (°C)	MS DAR-T0	MS DAR-T=168 h
3-1	168	22	8.00	8.04
3-2	168	22	8.66	8.86
3-3	168	22	8.42	8.42
3-4	168	22	8.70	8.82

3-5	168	22	9.54	9.40

[0405] The results demonstrate that DAR values of all conjugates remained almost the same as T0 after incubation in formulation buffer for 168 h.

[0406] Results of stability assessments of ADCs in GSH solution or formulation buffer are presented in Tables 7 and 8, respectively, and Figures 11-16.

[0407] Table 7: ADC stability in GSH buffer (pH 7.4 and 8.0)

ADC No.	Incubation Time (h)	pH	Temp. (°C)	T0 MS DAR	MS DAR after incubation
4-1	24	7.4	22	8.04	8.02
			37	8.04	8.00
		8.0	22	8.04	8.00
4-2	168	7.4	22	8.02	7.94
			37	8.02	7.98
		8.0	22	8.02	7.86
4-3	20	7.4	22	8.04	8.06
			37	8.04	8.02
		8.0	22	8.04	8.06

[0408] The results presented in Table 7 demonstrate that no deconjugation events were observed for ADC 4-1 to 4-3 after incubation in GSH buffers (pH 7.4 at 22/37 °C and pH 8.0 at 22 °C).

[0409] Table 8: ADC stability in formulation buffer (pH 5.5 20 mM histidine buffer)

ADC No.	Incubation Time (h)	Temp. (°C)	MS DAR-T0	MS DAR-T=168 h
4-1	168	22	8.04	8.04
4-2	168	22	8.02	8.00
4-3	168	22	8.04	8.06

[0410] The results presented in Table 8 demonstrate that no deconjugation events were observed for ADC 4-1 to 4-3 after storage in formulation buffer for one week.

Cell lines

[0411] **NCI-H1650 (ATCC, CRL-5883).** NCI-H1650 is a cell line exhibiting epithelial morphology that was isolated in 1987 from the lung tissue of a 27-year-old male smoker with stage 3, bronchoalveolar carcinoma, and NCI-H1650 was purchased from ATCC. The base medium for

NCI-H1650 is ATCC-formulated RPMI-1640 Medium, ATCC 30-2001. To make the complete growth medium, fetal bovine serum to a final concentration of 10% (Gibco, 10099-141C) was added to the base medium. The cell line was grown in a humidified 5% CO_2 atmosphere at 37 °C, and was regularly tested for the presence of mycoplasma with MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, LT07-710).

[0412] Capan-1 (ATCC, HTB-79). Capan-1 is a cell line with epithelial morphology that was isolated from the pancreas of a 40-year-old white male with pancreatic adenocarcinoma, and Capan-1 was purchased from ATCC. The base medium for Capan-1 is ATCC-formulated Iscove's Modified Dulbecco's Medium, Catalog No. 30-2005. To make the complete growth medium, fetal bovine serum to a final concentration of 20% (Gibco, 10099-141C) was added to the base medium. The cell line was grown in a humidified 5% CO_2 atmosphere at 37 °C, and was regularly tested for the presence of mycoplasma with MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, LT07-710).

[0413] MDA-MB-453 (SIBS). MDA-MB-453 was derived from an effusion of a 48-year-old female patient with metastatic carcinoma of the breast, involving the nodes, brain, and both pleural and pericardial cavities, and MDA-MB-453 was purchased from SIBS. The base medium for MDA-MB-453 is RPMI 1640 Medium, HEPES (Gibco, 22400105). To make the complete growth medium, fetal bovine serum to a final concentration of 10% (Gibco, 10099-141C) was added to the base medium. The cell line was grown in a humidified 5% CO_2 atmosphere at 37 °C, and was regularly tested for the presence of mycoplasma with MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, LT07-710).

[0414] U937 (ATCC, CRL-1593.2). U-937 is a cell line exhibiting monocyte morphology that was derived in 1974 from malignant cells obtained from the pleural effusion of a 37-year-old white male with histiocytic lymphoma. U937 was purchased from ATCC. The base medium for U937 is ATCC-formulated RPMI-1640 Medium (ATCC 30-2001). To make the complete growth medium, fetal bovine serum to a final concentration of 10% (Gibco, 10099-141C) was added to the base medium. The cell line was grown in a humidified 5% CO_2 atmosphere at 37 °C, and was regularly tested for the presence of mycoplasma with MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, LT07-710).

[0415] HL60 (ATCC, CCL-240). HL-60 cells are promyeoloblasts isolated from the peripheral blood by leukopheresis from a 36-year-old white female with acute promyelocytic leukemia. HL60 was purchased from ATCC. The base medium for HL60 is ATCC-formulated Iscove's Modified

Dulbecco's Medium, Catalog No. 30-2005. To make the complete growth medium, fetal bovine serum to a final concentration of 20% (Gibco, 10099-141C) was added to the base medium. The cell line was grown in a humidified 5% CO_2 atmosphere at 37 °C, and was regularly tested for the presence of mycoplasma with MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, LT07-710).

[0416] TF1 (ATCC, CRL-2003). TF-1 erythroblasts were isolated in 1987 from bone marrow derived from a 35-year-old Asian male with severe pancytopenia. TF-1 was purchased from ATCC. The base medium for TF-1 is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, fetal bovine serum to a final concentration of 10% (Gibco, 10099-141C) was added to the base medium. The cell line was grown in a humidified 5% CO_2 atmosphere at 37 °C, and was regularly tested for the presence of mycoplasma with MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, LT07-710).

[0417] NCI-H358 (ATCC, CRL-5807). NCI-H358 cells are epithelial-like cells isolated from the bronchiole of a male patient with bronchioalveolar carcinoma. NCI-H358 was purchased from ATCC. The base medium for NCI-H358 is ATCC-formulated RPMI-1640 Medium, ATCC 30-2001. To make the complete growth medium, fetal bovine serum to a final concentration of 10% (Gibco, 10099-141C) was added to the base medium. The cell line was grown in a humidified 5% CO_2 atmosphere at 37 °C, and was regularly tested for the presence of mycoplasma with MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, LT07-710).

Table 10A: B7H3 expression level

Cell line	Molecular No. (QSC kit)
NCI-H1650	505998
Capan-1	63062
MDA-MB-453	0

Table 10B: CLL1 expression level

Cell line	FACS (Emax MFI, 100 nM)	Molecular No. (QSC kit)
U937	1607	84858
HL60	846	NA
TF-1	50	<5806

ADC direct killing in U937, HL60, TF1, NCI-H1650, Capan-1, and MDA-MB-453 cancer cell lines

[0418] ADC direct killing was assessed in U937, HL60, and TF1 cancer lines. Cells were seeded (U937 or HL60 (3E3/well) or TF1 (6E3/well)) into 2D 96-well plates (Greiner: 655090), 100 µl/well (including 150 µg/ml Fc blocker), and incubated at 37 °C, 5% CO_2, overnight. Fresh growth medium was added containing varying concentrations of ADCs, 50 µl/well, and incubated at 37 °C, 5% CO_2, for 6 days. The cell viability was detected by Cell Titer-Glo (Promega, G7573), 70 µl/well. The 2D plates were allowed to incubate at room temperature for 10 minutes to stabilize the luminescent signal. The plates were analyzed with a Microplate Reader.

[0419] ADC direct killing was assessed in NCI-1650, Capan-1, and MDA-MB-453 cancer lines. Cells were seeded (NCI-1650 or MDA-MB-453 (2E3/well) or Capan-1 (4E3/well)) into 3D 96-well plates (Corning: 4520), 80 µl/well, and incubated at 37 °C, 5% CO_2, overnight. Fresh growth medium was added containing varying concentrations of ADCs, 40 µl/well, and incubated at 37 °C, 5% CO_2, for 6 days. The cell viability was detected by 3D reagent (Promega, G9683), 100 µl/well. The 3D plates were allowed to incubate at room temperature for 30 minutes to stabilize the luminescent signal. The plates were analyzed with a Microplate Reader.

[0420] Data are summarized in Figures 17-25 and Tables 11-13.

Table 11: ADC direct killing potency

ADC	U937		HL60	
	Emax (%)	EC50 (nM)	Emax (%)	EC50 (nM)
4-A	99.9	0.8	89.0	6.3
4-5	99.5	0.2	55.9	8.2

Table 12: ADC direct killing potency on B7H3 high- and low-expression cell lines

ADCs	NCI-H1650 (High), 3D		CAPAN-1 (Low), 3D	
	Emax (%)	EC50 (nM)	Emax (%)	EC50 (nM)
4-B	75.3	3.9	84.9	0.5
4-1	83.6	2.8	66.4	1.1
4-2	83.0	2.9	62.8	0.8

Table 13: ADC direct killing potency on B7H3 high- and low-expression cell lines

ADCs	NCI-H1650 (High), 3D		CAPAN-1 (Low), 3D	
	Emax (%)	EC50 (nM)	Emax (%)	EC50 (nM)
4-B	78.9	1.7	81.7	0.4
4-3	78.1	1.1	66.9	0.4
4-4	85.2	0.9	70.4	0.4

ADC bystander killing in NCI-H358 co-culture with MDA-MB-453-nanoLuc

[0421] Method: MDA-MB-453-nanoLuc cell line construction. PT67-nanoLuc cells were cultured, then the cell-culture medium (containing the virus (nano-Luc gene)) was collected and filtered. MDA-MB-453 cells were seeded in 6-well plates at 1E5 cells/well, and incubated at 37 °C, 5% CO_2, overnight. The PT67-nanoLuc cell medium and 8 μg/ml polybrene were added. The infection was repeated 3 times, every one day. Then the MDA-MB-453-nanoLuc cells were cultured with the addition of 1 mg/ml Geneticin for 5 days. The MDA-MB-453-nanoLuc cells were collected, and Nano-Glo reagent (Promega: N1120) was added to test the nano-Luc transfection efficiency.

[0422] Method: ADC bystander killing. NCI-H358 & MDA-MB-453-nanoLuc (10:1), or MDA-MB-453-nanoLuc cells alone were seeded into 3D-96-well plates (Corning: 4520) at 80 μl/well, and incubated at 37°C, 5% CO_2, overnight. Fresh growth medium containing the varying concentrations of ADCs was added at 40 μl/well. The cells were incubated at 37°C, 5% CO_2, 6 days. The 3D-plates were centrifuged at 1500 rpm, 25 °C, 5 min, then the supernatant was discarded.

[0423] The Calu-6-nanoLuc cell viability was detected by Nano-Glo reagent (Promega: N1120), 150 μl/well. The 3D-plates were allowed to incubate at room temperature for 10 minutes to stabilize the luminescent signal. Then the plates were analyzed with a microplate reader.

[0424] Results are presented in Tables 14-15 and Figures 26-29.

Table 14: ADC bystander killing effect on NCI-H358/MDA-MB-453 (nano-Luc) co-culture

ADC No.	NCI-H358: MDA-MB-453-nanoLuc = 10 : 1, 3D	
	Emax (%)	EC50 (nM)
4-B	88.1	0.8
4-1	83.7	1.0

4-2	83.8	0.6

Table 15: ADC bystander killing effect on NCI-H358/MDA-MB-453 (nano-Luc) co-culture

ADC No.	NCI-H358 : MDA-MB-453-nanoLuc = 10 : 1, 3D	
	Emax (%)	EC50 (nM)
4-B	89.2	0.7
4-3	86.9	0.6
4-4	85.6	0.8

ADC in vivo efficacy study in H1650 xenograft model

[0425] Female BALB/c Nude mice were subcutaneously implanted with 3×10^6 H1650 cells per 200 μL PBS/matrigel in the right flank. After inoculation, tumor volumes were determined twice weekly in two dimensions using a caliper and were expressed in mm^3 using the formula $V = 0.5(a \times b^2)$ where a and b are the long and short diameters of the tumor, respectively. When tumors reached a mean volume of approximately 200 mm^3 in size, mice were randomly allocated into 3 groups with 7 animals in each group, and were intravenously treated with vehicle, or ADC 4-B, 4-2, 4-3 or 4-4 at 1 mpk or 3 mpk QW*2. Partial regression (PR) was defined as tumor volume smaller than 50% of the starting tumor volume on the first day of dosing in three consecutive measurements and complete regression (CR) was defined as tumor volume less than 14 mm^3 in three consecutive measurements. Data are presented as mean tumor volume ± standard error of the mean (SEM). Tumor growth inhibition (TGI) is calculated using the following formula :

$$\%\ growth\ inhibition = 100 \times \left(1 - \left(\frac{(treated\ t) - (treated\ to)}{(placebo\ t) - (placebo\ to)}\right)\right)$$

treated t = treated tumor volume at time t
treated t_0 = treated tumor volume at time 0
placebo t = placebo tumor volume at time t
placebo t_0 = placebo tumor volume at time 0

[0426] Results are presented in Figures 30 and 31. The results demonstrate that each of ADC 4-2, 4-3, and 4-4 showed good anti-tumor activity (Figures 30 and 31). ADC 4-2 exhibited better anti-tumor activity than did reference ADC 4-B (Figure 30). ADC 4-3 and 4-4 exhibited comparable

anti-tumor activity (Figure 31). ADC 4-3 showed dose-dependent efficacy (Figure 31).

ADC plasma stability

[0427] Incubation of ADC with plasma: ADCs were diluted into mouse or human plasma to yield a final solution of 100 μg/mL ADC in plasma. The samples were incubated at 37 °C. Aliquots (100 μL) are taken at five time points (0, 2 (4), 24, 72, or 168 h). Samples were frozen at – 80 °C until analysis.

[0428] Plasma payload concentrations were carried out under the following measurement conditions:

Instrument: LC-MS/MS (Triple Quad 6500 plus)

Monitor: MRM

Column: Advanced Materials Technology, HALO AQ-C18 2.7μm 90Å, 50*2.1 mm

Column temperature: 40 °C

Mobile phase A: H_2O-0.1%FA

Mobile phase B: ACN-0.1%FA

Gradient program for MMAE: 15%B-15%B (0 min-0.4 min), 15%B-30%B (0.4 min-0.8 min), 30%B-30%B (0.8 min-1.8 min), 30%B-90%B (1.8 min-1.9 min), 90%B-90%B (1.9 min-2.4 min), 90%B-15%B (2.4 min-2.5 min), 15%B-15%B (2.5 min-3.0 min)

Gradient program for Dxd: 2%B-2%B (0 min-0.2 min), 2%B-98%B (0.2 min-1.2 min), 98%B-98%B (1.2 min-2.0 min), 98%B-2%B (2.0 min-2.01 min), 2%B-2%B (2.01 min-4.0 min)

Injected sample amount: 10 μL (DXd or DXd analogues or MMAE)

[0429] Results are presented in Figures 32-35. ADC 4-1 to 4-4 showed lower payload release % than did ADC-4-B in both mouse plasma (Figure 32) and human plasma (Figure 33). ADC 4-5 showed comparable payload release % to ADC 4-A in mouse plasma (Figure 34) and higher payload release% to ADC 4-A in human plasma (Figure 35).

In mice PK study 1

[0430] After one dose of intravenously administered ADC (3 mg/kg) to H1650 tumor-bearing mice, blood samples were collected 0.0833, 2, 24, 72, 120, and 168 h later, followed by centrifugation (4 °C, 3000 ×g, 7 min) to separate plasma. The concentrations of ADCs were measured by in-house developed Meso Scale Discovery (MSD) ligand binding methods. Briefly, a His-tagged B7H3 extracellular domain fusion protein was used as a capture reagent, and biotin-

labelled anti-payload Ab was used as the detection reagent for ADCs. Plasma concentration of payload was measured using the same method as described above.

[0431] Results are presented in Figure 36. The results demonstrate that ADC 4-2 to 4-4 showed comparable PK profiles to ADC 4-B in an H1650 efficacy model.

In mice PK study 2

[0432] After one dose of intravenously administered ADC (5 mg/kg) to non-tumor-bearing mice, blood samples were collected 0.5, 2, 8, 24, 72 and 168 h later, followed by centrifugation (4 °C, 3000 ×g, 7 min) to separate plasma. The concentrations of ADCs were measured by in-house developed Meso Scale Discovery (MSD) ligand binding methods. Briefly, a His-tagged CLL1 extracellular domain fusion protein was used as a capture reagent, and biotin-labelled anti-payload Ab was used as the detection reagent for ADCs. Plasma concentration of payload was measured using the same method as described above.

[0433] Results are presented in Figure 37. The results demonstrate that ADC 4-5 showed comparable PK profiles to ADC 4-A in non-tumor-bearing mice.

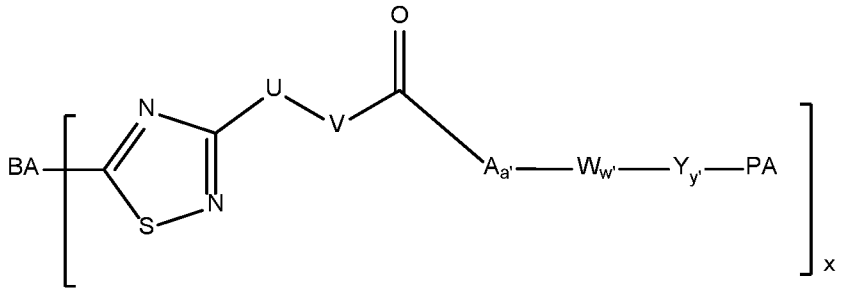
[0434] Although the foregoing disclosure has been presented in some detail by way of illustration and example for purposes of clarity of understanding, it is apparent to those skilled in the art that certain minor changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting.

[0435] It is to be understood that, if any publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art in any country.

[0436] The disclosures of all non-patent publications, patents, patent applications, and published patent applications referred to herein are hereby incorporated herein by reference in their entireties.

What is claimed is:

1. A compound of Formula (I):



(I)

or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, wherein:

BA is a binding agent selected from a humanized, chimeric, or human antibody or an antigen binding fragment thereof;

U is arylene, heteroarylene, or a bond;

V is a bond or $-C\equiv C-(CH_2)_n-$;

n is an integer between 0 and 10;

A is a Stretcher unit residue;

subscript a' is 0 or 1;

W is a Cleavable unit;

subscript w' is 0 or 1;

Y is a Spacer unit;

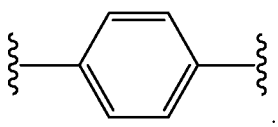
subscript y' is 0 or 1;

PA is a payload residue; and

subscript x is from 1 to 15.

2. The compound of claim 1, wherein U is arylene.

3. The compound of claim 2, wherein U is phenylene.

4. The compound of claim 3, wherein U is  .

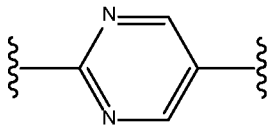
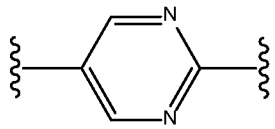
5. The compound of any one of claims 1-4, wherein V is a bond.

6. The compound of any one of claims 1-4, wherein V is -C≡C-$(CH_2)_n$-.

7. The compound of claim 6, wherein V is -C≡C-$(CH_2)_3$-.

8. The compound of claim 1, wherein U is heteroarylene.

9. The compound of claim 8, wherein U is a bivalent pyrimidine ring.

10. The compound of claim 9, wherein U is  or  .

11. The compound of any one of claims 8-10, wherein V is a bond.

12. The compound of any one of claims 8-10, wherein V is -C≡C-$(CH_2)_n$-.

13. The compound of claim 12, wherein V is -C≡C-$(CH_2)_3$-.

14. The compound of claim 1, wherein U is a bond.

15. The compound of claim 14, wherein V is -C≡C-$(CH_2)_n$-.

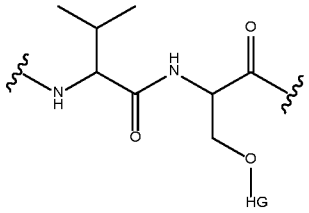
16. The compound of claim 15, wherein V is -C≡C-$(CH_2)_3$-.

17. The compound of any one of claims 1-16, wherein A is -$(CH_2)_m$-C(=O)-, -CH_2-C(=O)-NH-$(CH_2)_m$-C(=O)-, -$(CH_2CH_2O)_m$-CH_2CH_2-C(=O)-, -CH[-$(CH_2)_m$-COOH]-C(=O)-, -CH_2-C(=O)-NH-$(CH_2)_m$-C(=O)-NH-$(CH_2)_m$-C(=O)-, -C(=O)-$(CH_2)_m$-C(=O)-, -NH-$(CH_2)_m$-C(=O)-, or -NH-$(CH_2CH_2O)_m$-CH_2CH_2-C(=O)-, and each m independently represents an integer of 1, 2, 3, 4, or 5.

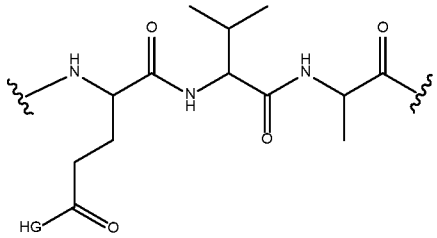
18. The compound of any one of claims 1-17, wherein subscript a' is 0.

19. The compound of any one of claims 1-17, wherein subscript a' is 1.

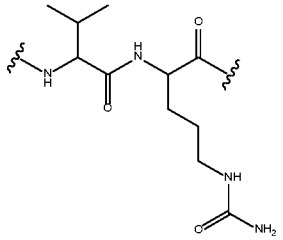
20. The compound of any one of claims 1-19, wherein W is one of the following formulas:



Formula (Ia),



Formula (Ib), or

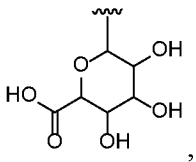
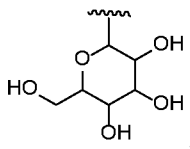
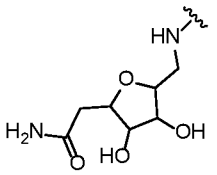


Formula (Ic),

wherein HG is a hydrophilic moiety or hydrogen.

21. The compound of claim 20, wherein HG is a saccharide, phosphate ester, sulfate ester, phosphodiester, or phosphonate.

22. The compound of claim 21, wherein HG is a saccharide and the saccharide is β-D-galactose, N-acetyl-P-D-galactosamine, N-acetyl-a-D-galactosamine, N-acetyl-P-D-glucosamine, β-D-glucuronic acid, a-L-iduronic acid, a-D-galactose, a-D-glucose, β-D-glucose, a-D-mannose, β-D-mannose, a-L-fucose, β-D-xylose, a neuraminic acid, sulfate, phosphate, carboxyl, amino, or O-acetyl modification thereof.

23. The compound of claim 20, wherein HG is  ,  , or  .

24. The compound of any one of claims 1-23, wherein subscript w' is 1.

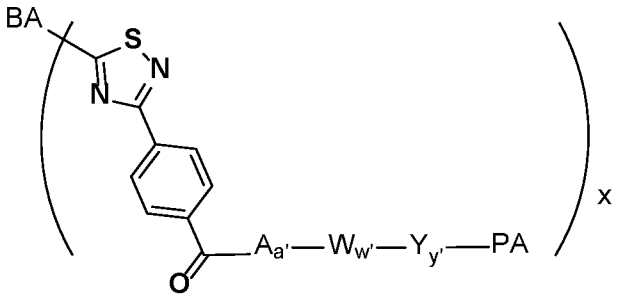
25. The compound of any one of claims 1-23, wherein subscript w' is 0.

26. The compound of any one of claims 1-25, wherein Y is -NH-CH_2-O- or -NH-(*p*-C_6H_4)-CH_2-O-.

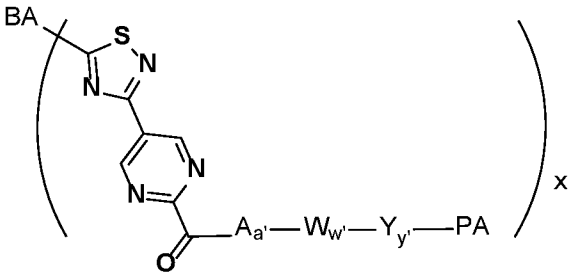
27. The compound of any one of claims 1-26, wherein subscript y' is 1.

28. The compound of any one of claims 1-26, wherein subscript y' is 0.

29. The compound of any one of claims 1-28, wherein the compound is one of the following formulas:

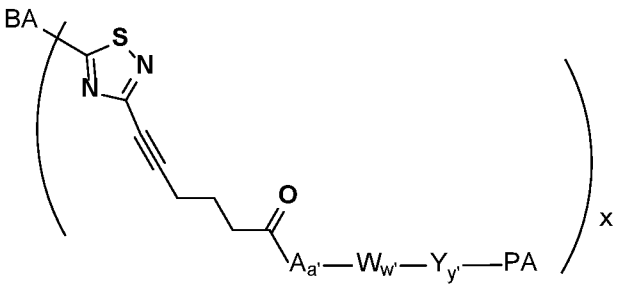


,



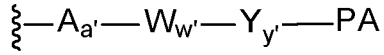
, or

,

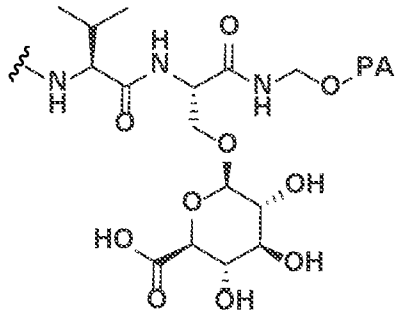


.

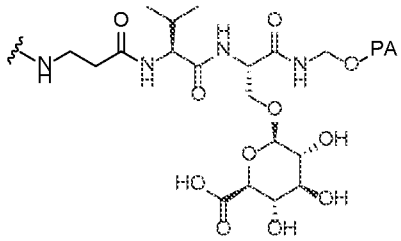
30. The compound of any one of claims 1-16 and 29, wherein the fraction



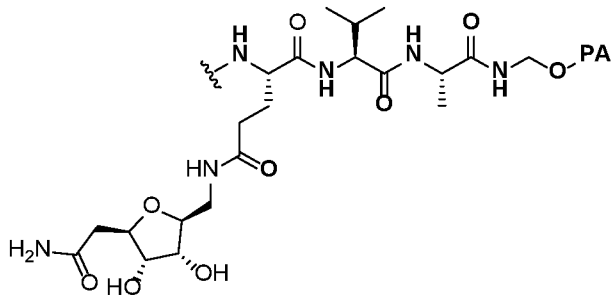
is one of the following formulas:



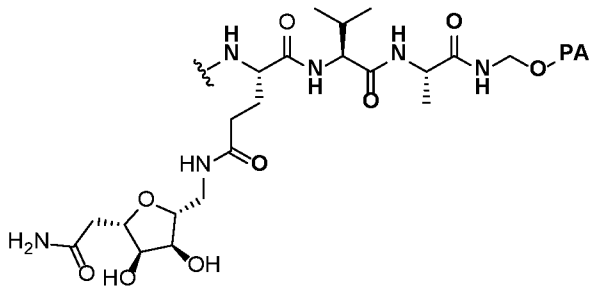
,



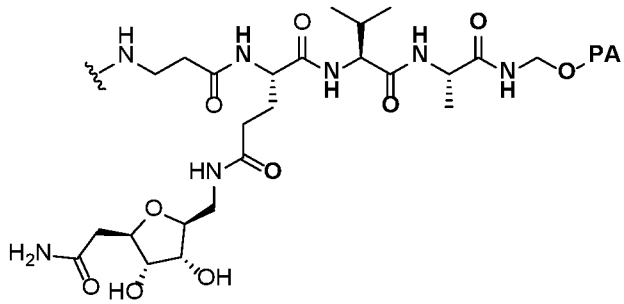
,



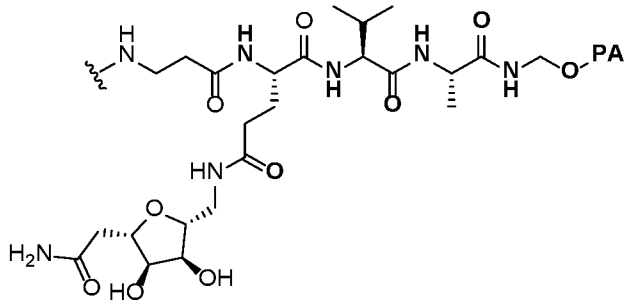
,



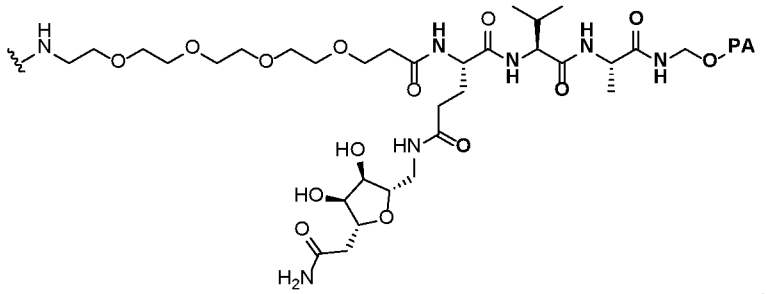
,



,



, or

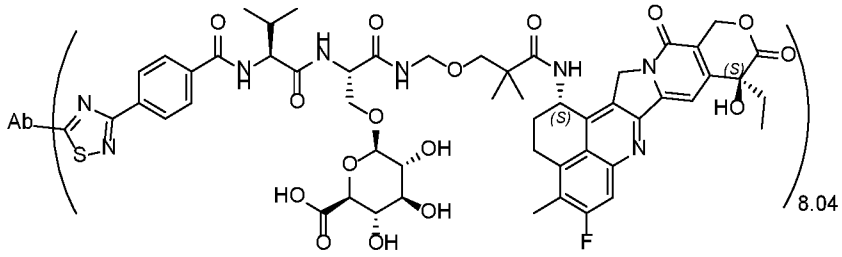


.

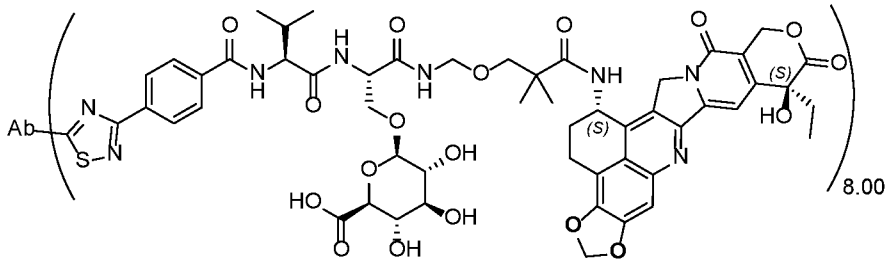
31. The compound of any one of claims 1-30, wherein BA is ifinatamab, 6E7, or trastuzumab, or an antigen binding fragment of ifinatamab, 6E7, or trastuzumab.

32. The compound of any one of claims 1-30, wherein BA is a humanized, chimeric, or human antibody or the antigen binding fragment thereof which binds to one or more of receptors chosen from HER2, CLL1, or B7H3.

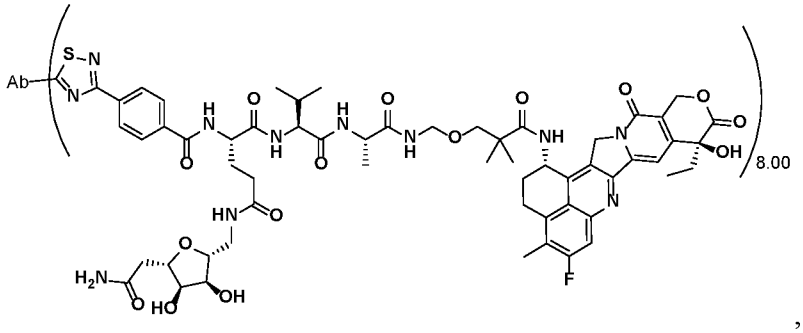
33. The compound of claim 1, wherein the compound is



,



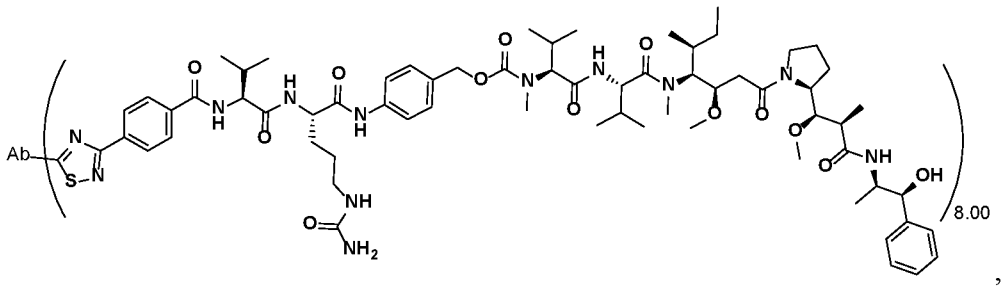
, or



,

or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein Ab is ifinatamab.

34. The compound of claim 1, wherein the compound is

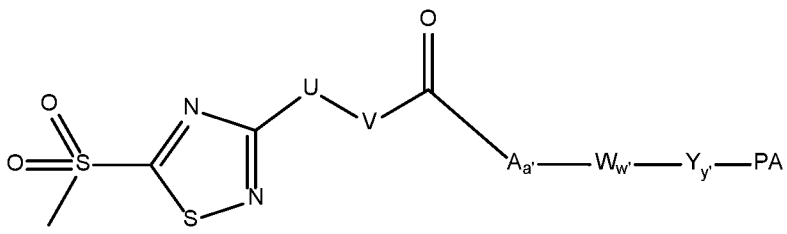


,

or a pharmaceutically acceptable salt, tautomer, or solvate thereof, wherein Ab is 6E7.

35. A pharmaceutical composition comprising a compound of any one of claims 1-34, or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, and a pharmaceutically acceptable excipient.

36. A compound of Formula (II):



(II)

or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, wherein:

U is arylene, heteroarylene, or a bond;

V is a bond or $-C\equiv C-(CH_2)_n-$;

n is an integer between 0 and 10;

A is a Stretcher unit residue;

subscript a' is 0 or 1;

W is a Cleavable unit;

subscript w' is 0 or 1;

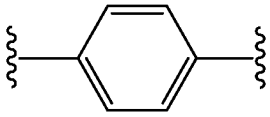
Y is a Spacer unit;

subscript y' is 0 or 1; and

PA is a payload residue.

37. The compound of claim 36, wherein U is arylene.

38. The compound of claim 37, wherein U is phenylene.

39. The compound of claim 38, wherein U is  .

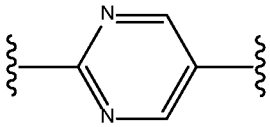
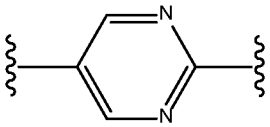
40. The compound of any one of claims 36-39, wherein V is a bond.

41. The compound of any one of claims 36-39, wherein V is -C≡C-$(CH_2)_n$-.

42. The compound of claim 41, wherein V is -C≡C-$(CH_2)_3$-.

43. The compound of claim 36, wherein U is heteroarylene.

44. The compound of claim 43, wherein U is a bivalent pyrimidine ring.

45. The compound of claim 44, wherein U is  or  .

46. The compound of any one of claims 43-45, wherein V is a bond.

47. The compound of any one of claims 43-45, wherein V is -C≡C-$(CH_2)_n$-.

48. The compound of claim 47, wherein V is -C≡C-$(CH_2)_3$-.

49. The compound of claim 36, wherein U is a bond.

50. The compound of claim 49, wherein V is -C≡C-$(CH_2)_n$-.

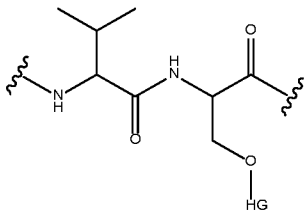
51. The compound of claim 50, wherein V is -C≡C-$(CH_2)_3$-.

52. The compound of any one of claims 36-51, wherein A is
–$(CH_2)_m$-C(=O)-, -CH_2-C(=O)-NH-$(CH_2)_m$-C(=O)-,
-$(CH_2CH_2O)_m$-CH_2CH_2-C(=O)-, -CH[-$(CH_2)_m$-COOH]-C(=O)-,
-CH_2-C(=O)-NH-$(CH_2)_m$-C(=O)-NH-$(CH_2)_m$-C(=O)-, -C(=O)-$(CH_2)_m$-C(=O)-,
-NH-$(CH_2)_m$-C(=O)-, or -NH-$(CH_2CH_2O)_m$-CH_2CH_2-C(=O)-, and each m independently represents an integer of 1, 2, 3, 4, or 5.

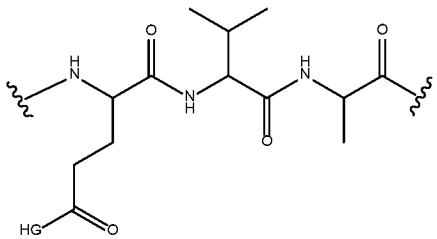
53. The compound of any one of claims 36-52, wherein subscript a' is 0.

54. The compound of any one of claims 36-52, wherein subscript a' is 1.

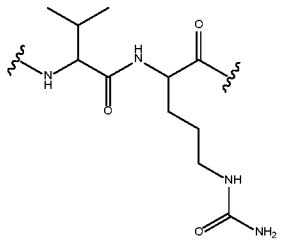
55. The compound of any one of claims 36-54, wherein W is one of the following formulas:



Formula (Ia),



Formula (Ib), or

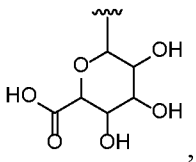
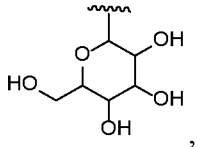
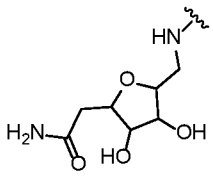


Formula (Ic),

and wherein HG is a hydrophilic moiety or hydrogen.

56. The compound of claim 55, wherein HG is a saccharide, phosphate ester, sulfate ester, phosphodiester, or phosphonate.

57. The compound of claim 56, wherein HG is a saccharide and the saccharide is β-D-galactose, N-acetyl-P-D-galactosamine, N-acetyl-a-D-galactosamine, N-acetyl-P-D-glucosamine, β-D-glucuronic acid, a-L-iduronic acid, a-D-galactose, a-D-glucose, β-D-glucose, a-D-mannose, β-D-mannose, a-L-fucose, β-D-xylose, a neuraminic acid, sulfate, phosphate, carboxyl, amino, or O-acetyl modification thereof.

58. The compound of claim 55, wherein HG is  ,  , or  .

59. The compound of any one of claims 36-58, wherein subscript w' is 1.

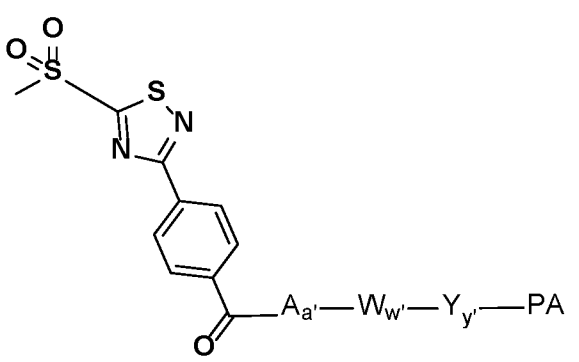
60. The compound of any one of claims 36-58, wherein subscript w' is 0.

61. The compound of any one of claims 36-60, wherein Y is -NH-CH_2-O- or -NH-(*p*-C_6H_4)-CH_2-O-.

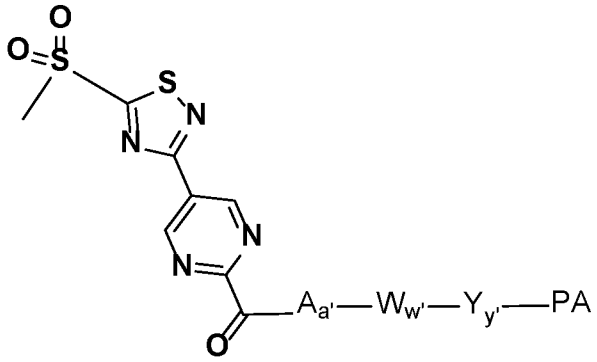
62. The compound of any one of claims 36-61, wherein subscript y' is 1.

63. The compound of any one of claims 36-61, wherein subscript y' is 0.

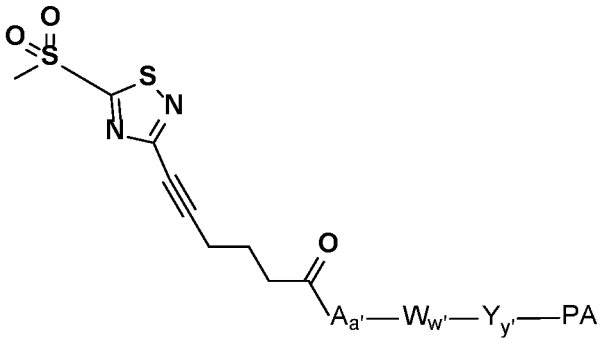
64. The compound of any one of claims 36-63, wherein the compound is one of the following formulas:



,

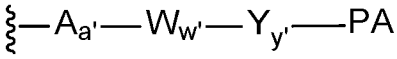


, or

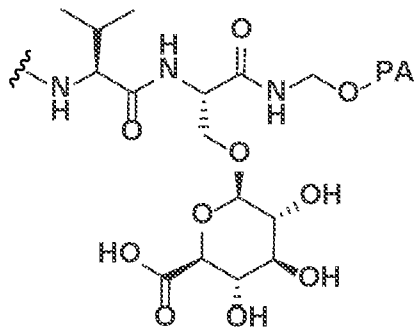


.

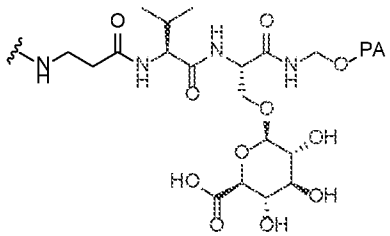
65. The compound of any one of claims 36-51 and 64, wherein the fraction



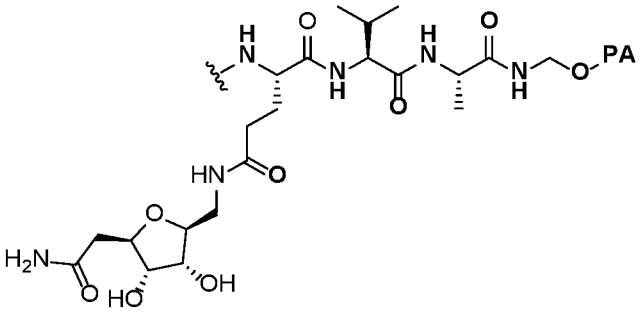
is one of the following formulas:



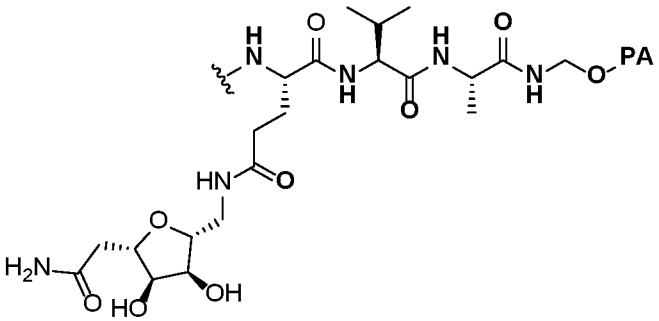
,



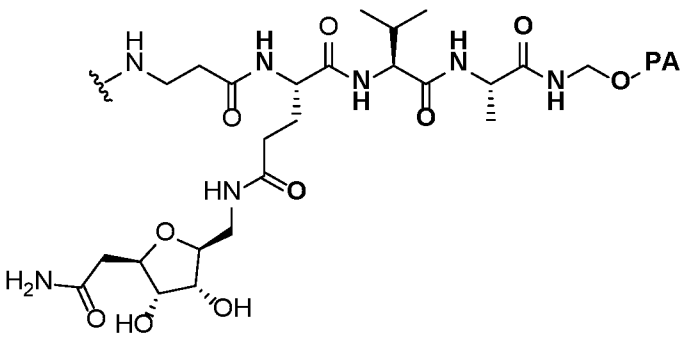
,


H
N
O
N
H
O
H
N
O
N
H
O
PA
HN
O
O
H2N
O
HO
OH

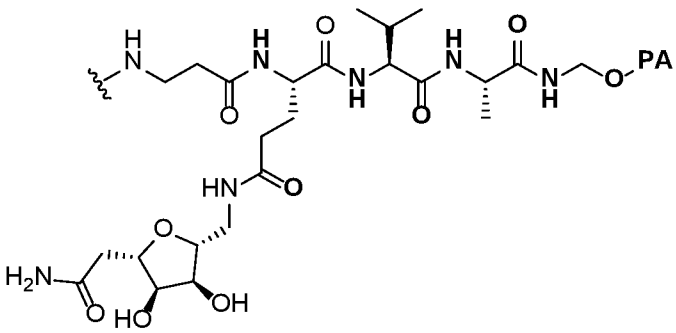
,


H
N
O
N
H
O
H
N
O
N
H
O
PA
HN
O
O
H2N
O
HO
OH

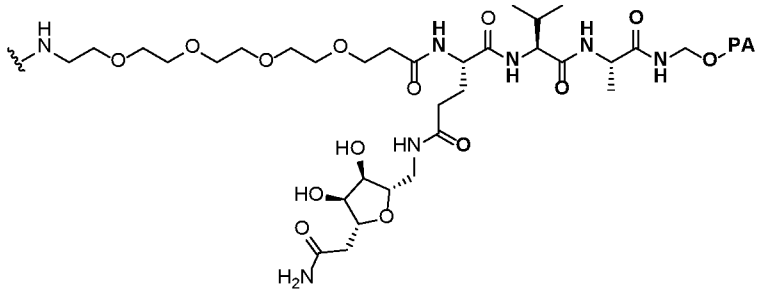
,


H
N
H
N
O
O
N
H
O
H
N
O
N
H
O
PA
HN
O
O
H2N
O
HO
OH

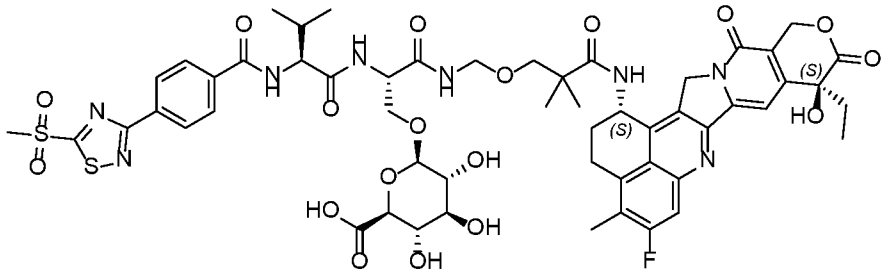
,


H
N
H
N
O
O
N
H
O
H
N
O
N
H
O
PA
HN
O
O
H2N
O
HO
OH

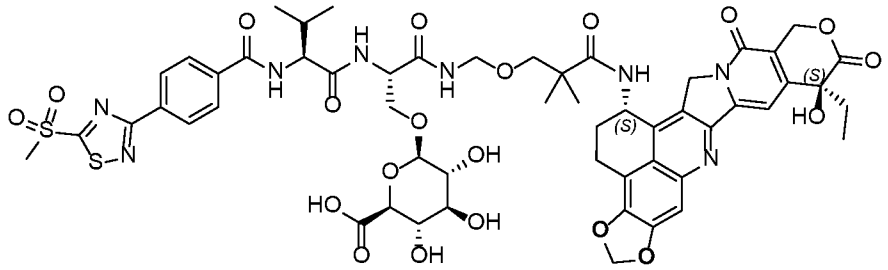
, or



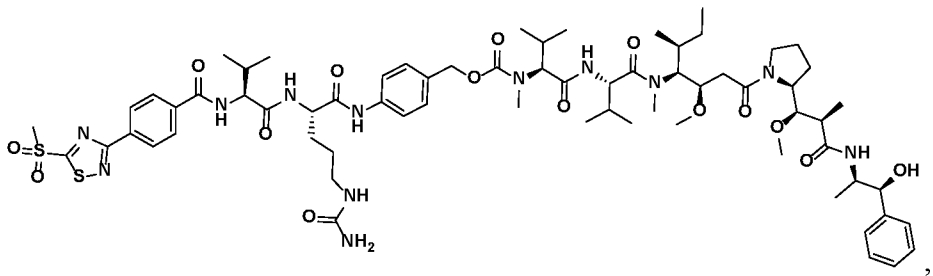
66. The compound of claim 65, wherein the compound is



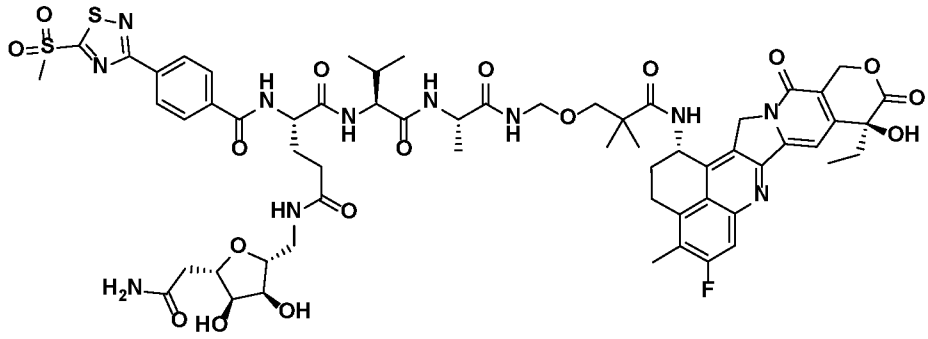
,



,



, or



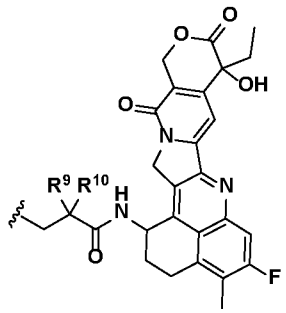
,

or a pharmaceutically acceptable salt, tautomer, or solvate thereof.

67. The compound of any one of claims 1-32 or 36-65, wherein each PA is independently a cytotoxic agent.

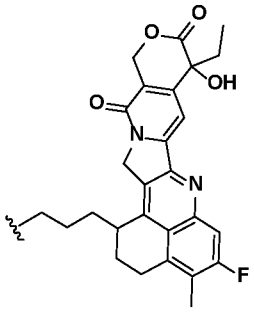
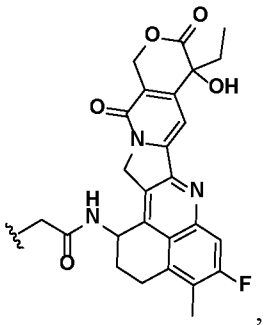
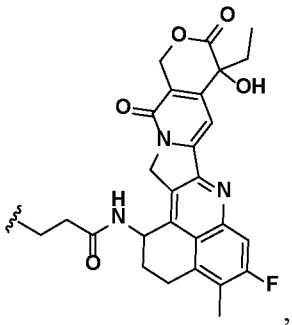
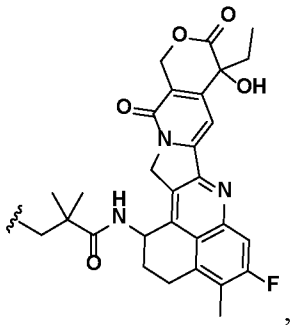
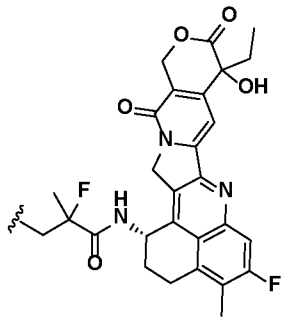
68. The compound of claim 67, wherein each PA is independently selected from a residue of the group consisting of DXd, 7-ethyl-10-hydroxy-camptothecin (SN-38), and monomethyl auristatin E (MMAE).

69. The compound of claim 68, wherein each PA independently is a compound of formula (VI):

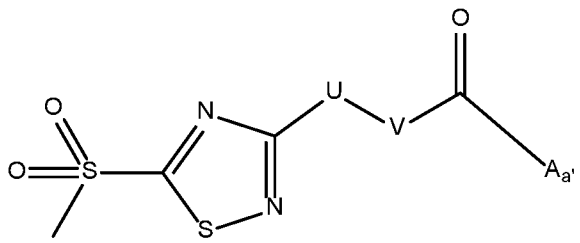


(VI),

wherein each of R^9 and R^{10} is independently hydrogen, halogen, or substituted or unsubstituted C_{1-4} alkyl.

70. The compound of claim 69, wherein each PA is independently  ,  ,  ,  , or  .

71. A compound of Formula (III):



(III)

or a pharmaceutically acceptable salt, tautomer, solvate, or stereoisomer thereof, wherein:

U is arylene, heteroarylene, or a bond;

V is a bond or -C≡C-$(CH_2)_n$-;

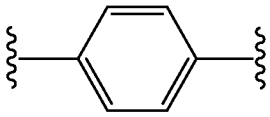
n is an integer between 0 and 10;

A is a Stretcher unit; and

subscript a' is 0 or 1.

72. The compound of claim 71, wherein U is arylene.

73. The compound of claim 72, wherein U is phenylene.

74. The compound of claim 73, wherein U is  .

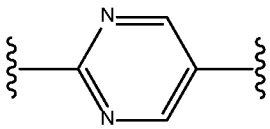
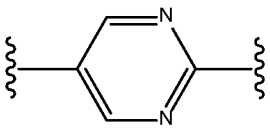
75. The compound of any one of claims 71-74, wherein V is a bond.

76. The compound of any one of claims 71-74, wherein V is -C≡C-$(CH_2)_n$-.

77. The compound of claim 76, wherein V is -C≡C-$(CH_2)_3$-.

78. The compound of claim 71, wherein U is heteroarylene.

79. The compound of claim 78, wherein U is a bivalent pyrimidine ring.

80. The compound of claim 79, wherein U is  or  .

81. The compound of any one of claims 78-80, wherein V is a bond.

82. The compound of any one of claims 78-80, wherein V is -C≡C-$(CH_2)_n$-.

83. The compound of claim 82, wherein V is -C≡C-$(CH_2)_3$-.

84. The compound of claim 71, wherein U is a bond.

85. The compound of claim 84, wherein V is -C≡C-$(CH_2)_n$-.

86. The compound of claim 85, wherein V is -C≡C-$(CH_2)_3$-.

87. The compound of any one of claims 71-86, wherein:

A is a bond, -OH, $-CH_3$, $-N(CH_3)_2$, $-(CH_2)_m-C(=O)R^7$,
$-CH_2-C(=O)-NH-(CH_2)_m-C(=O)R^7$, $-(CH_2CH_2O)_m-CH_2CH_2-C(=O)R^7$,
$-CH[-(CH_2)_m-COOH]-C(=O)R^7$,
$-CH_2-C(=O)-NH-(CH_2)_m-C(=O)-NH-(CH_2)_m-C(=O)R^7$, $-C(=O)-(CH_2)_m-C(=O)R^7$,
$-NH-(CH_2)_m-C(=O)R^7$, or $-NH-(CH_2CH_2O)_m-CH_2CH_2-C(=O)R^7$;

each m independently represents an integer of 1, 2, 3, 4, or 5;

R^7 is OH or $NR^{8a}R^{8b}$; and

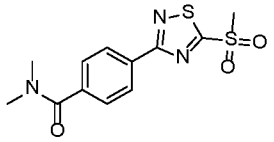
each of R^{8a} and R^{8b} is, independently, H; substituted or unsubstituted C_{1-4} alkyl; substituted or unsubstituted C_{3-5} cycloalkyl; or R^{8a} and R^{8b} together with the atom to which they are attached form a substituted or unsubstituted C_{3-5} cycloalkyl.

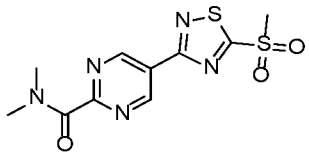
88. The compound of claim 87, wherein R^7 is OH, NH_2, $NHCH_3$, or $N(CH_3)_2$.

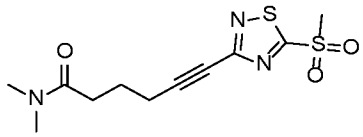
89. The compound of any one of claims 71-88, wherein subscript a' is 0.

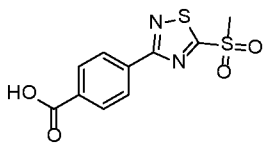
90. The compound of any one of claims 71-88, wherein subscript a' is 1.

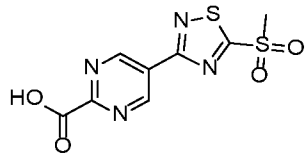
91. The compound of claim 88, wherein the compound is


,

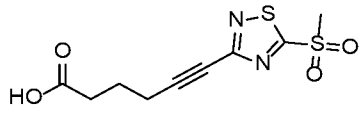

,


,


,



, or



,

or a pharmaceutically acceptable salt, tautomer, or solvate thereof.

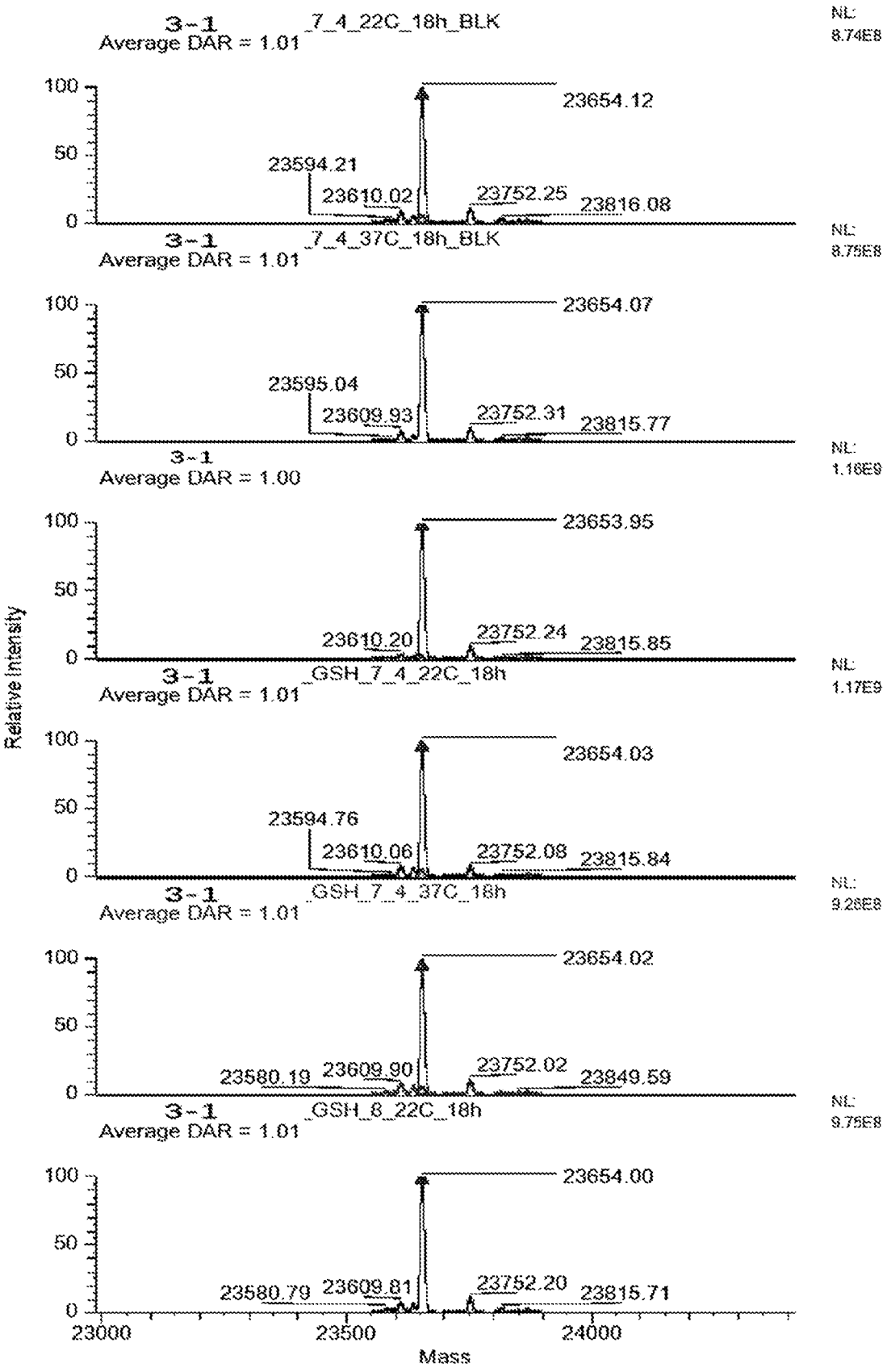

3-1 _7_4_22C_18h_BLK
Average DAR = 1.01
NL: 8.74E8
23654.12
23594.21
23610.02
23752.25
23816.08
3-1 _7_4_37C_18h_BLK
Average DAR = 1.01
NL: 8.75E8
23654.07
23595.04
23609.93
23752.31
23815.77
3-1
Average DAR = 1.00
NL: 1.18E9
23653.95
23610.20
23752.24
23815.85
3-1 _GSH_7_4_22C_18h
Average DAR = 1.01
NL: 1.17E9
23654.03
23594.76
23610.06
23752.08
23815.84
3-1 _GSH_7_4_37C_18h
Average DAR = 1.01
NL: 9.26E8
23654.02
23580.19
23609.90
23752.02
23849.59
3-1 _GSH_8_22C_18h
Average DAR = 1.01
NL: 9.75E8
23654.00
23580.79
23609.81
23752.20
23815.71
Relative Intensity
100
50
0
23000
23500
24000
Mass

Fig. 1

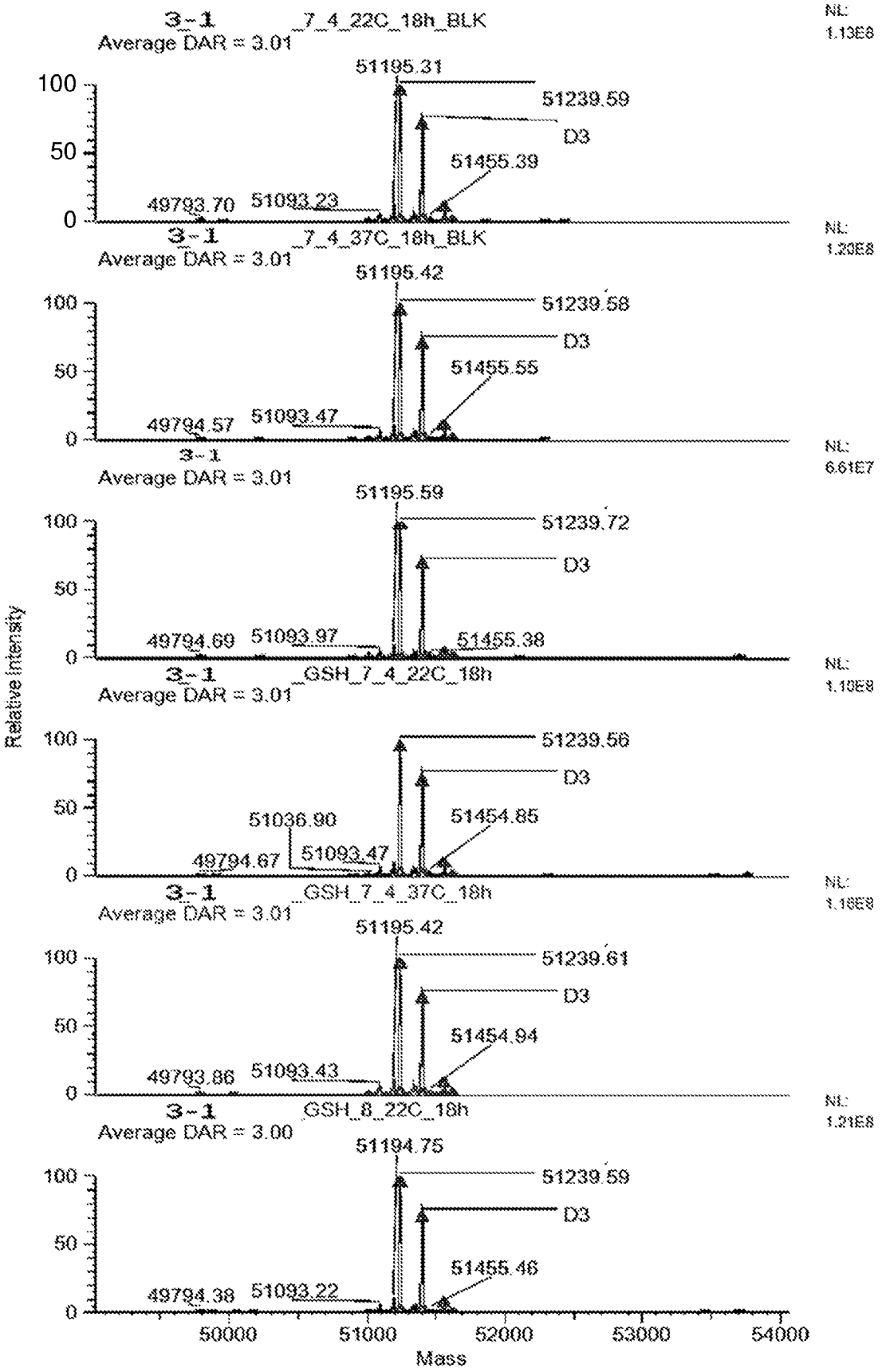

3-1 _7_4_22C_18h_BLK
Average DAR = 3.01
51195.31
51239.59
D3
51455.39
49793.70
51093.23
NL: 1.13E8
3-1 _7_4_37C_18h_BLK
Average DAR = 3.01
51195.42
51239.58
D3
51455.55
49794.57
51093.47
NL: 1.23E8
3-1
Average DAR = 3.01
51195.59
51239.72
D3
49794.69
51093.97
51455.38
NL: 6.61E7
3-1 _GSH_7_4_22C_18h
Average DAR = 3.01
51239.56
D3
51036.90
51454.85
49794.67
51093.47
NL: 1.10E8
3-1 _GSH_7_4_37C_18h
Average DAR = 3.01
51195.42
51239.61
D3
51454.94
49793.86
51093.43
NL: 1.16E8
3-1 _GSH_8_22C_18h
Average DAR = 3.00
51194.75
51239.59
D3
51455.46
49794.38
51093.22
NL: 1.21E8
Relative Intensity
100
50
0
50000
51000
52000
53000
54000
Mass

Fig. 1 (cont.)

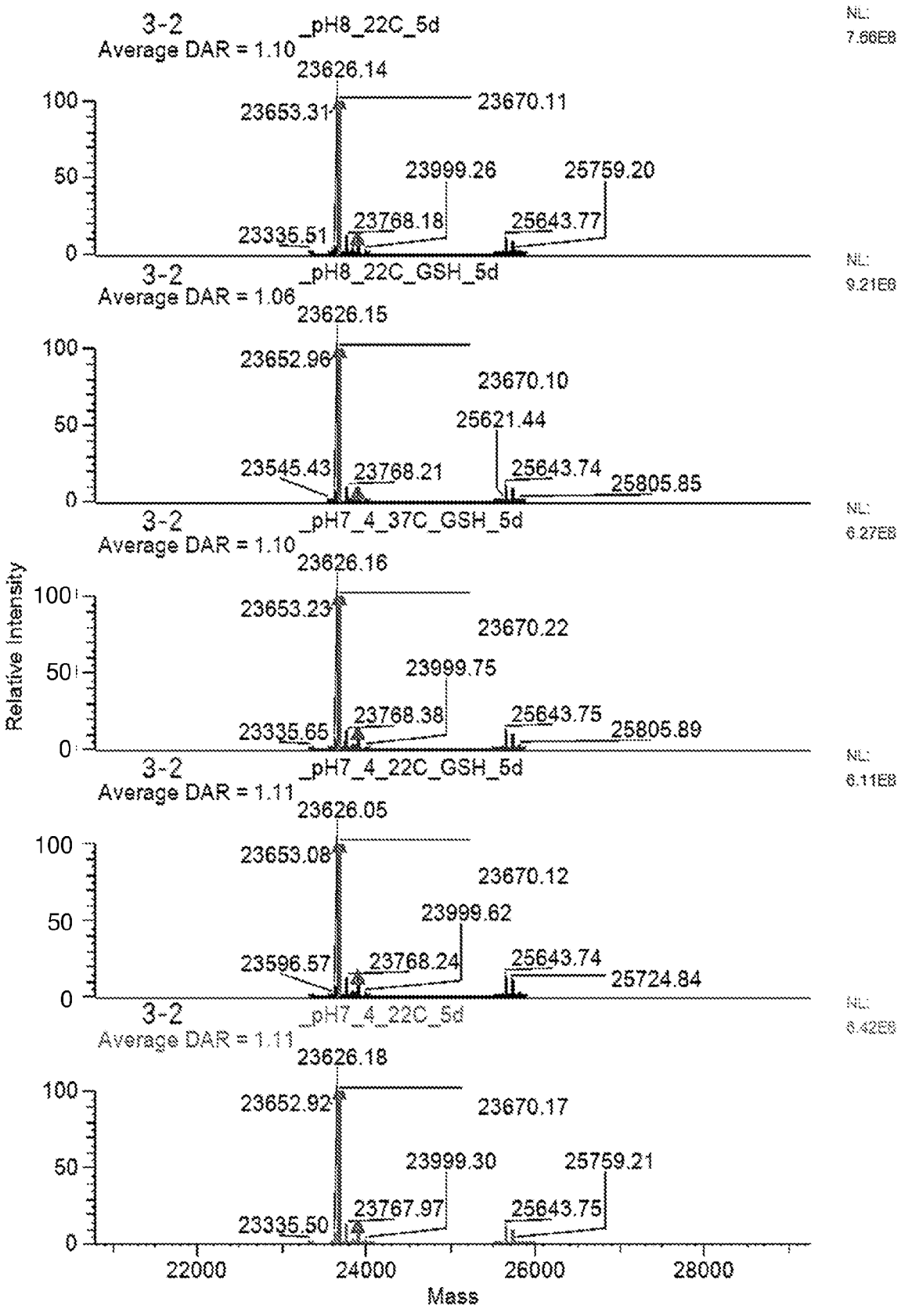

3-2 _pH8_22C_5d
Average DAR = 1.10
NL: 7.66E8
23626.14
23653.31
23670.11
23999.26
25759.20
23335.51
23768.18
25643.77
3-2 _pH8_22C_GSH_5d
Average DAR = 1.06
NL: 9.21E8
23626.15
23652.96
23670.10
25621.44
23545.43
23768.21
25643.74
25805.85
3-2 _pH7_4_37C_GSH_5d
Average DAR = 1.10
NL: 6.27E8
23626.16
23653.23
23670.22
23999.75
23335.65
23768.38
25643.75
25805.89
3-2 _pH7_4_22C_GSH_5d
Average DAR = 1.11
NL: 6.11E8
23626.05
23653.08
23670.12
23999.62
23596.57
23768.24
25643.74
25724.84
3-2 _pH7_4_22C_5d
Average DAR = 1.11
NL: 8.42E8
23626.18
23652.92
23670.17
23999.30
25759.21
23335.50
23767.97
25643.75
Relative Intensity
Mass
22000
24000
26000
28000

Fig. 2

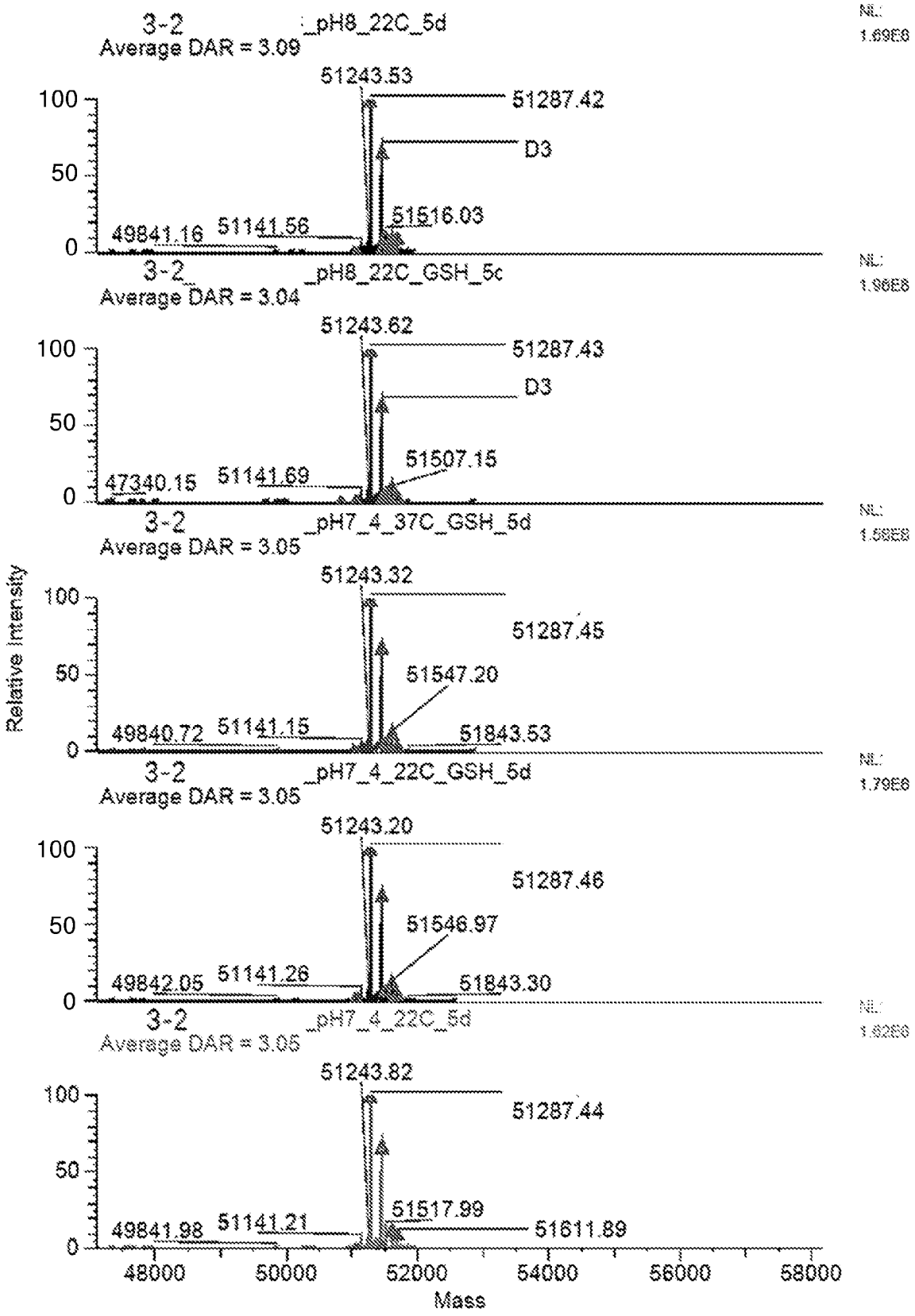

3-2 _pH8_22C_5d
Average DAR = 3.09
NL: 1.69E8
51243.53
51287.42
D3
51516.03
49841.16
51141.56
3-2 _pH8_22C_GSH_5c
Average DAR = 3.04
NL: 1.96E8
51243.62
51287.43
D3
51507.15
47340.15
51141.69
3-2 _pH7_4_37C_GSH_5d
Average DAR = 3.05
NL: 1.58E8
51243.32
51287.45
51547.20
49840.72
51141.15
51843.53
3-2 _pH7_4_22C_GSH_5d
Average DAR = 3.05
NL: 1.79E8
51243.20
51287.46
51546.97
49842.05
51141.26
51843.30
3-2 _pH7_4_22C_5d
Average DAR = 3.05
NL: 1.62E8
51243.82
51287.44
51517.99
49841.98
51141.21
51611.89
Relative Intensity
100
50
0
48000
50000
52000
54000
56000
58000
Mass

Fig. 2 (cont.)

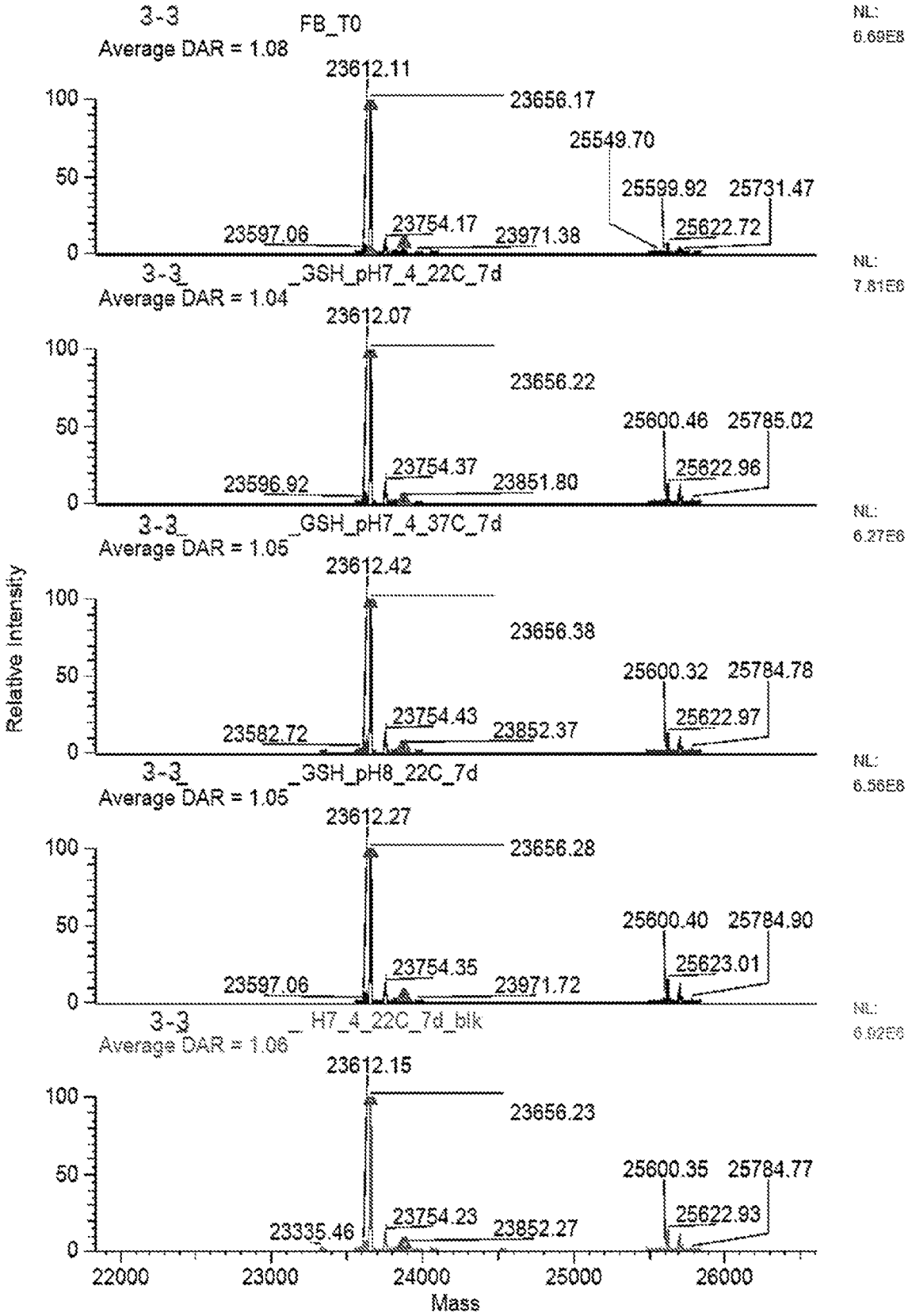

3-3 FB_T0
Average DAR = 1.08
23612.11
23656.17
25549.70
25599.92
25731.47
23597.06
23754.17
23971.38
25622.72
NL: 6.69E8
3-3_ _GSH_pH7_4_22C_7d
Average DAR = 1.04
23612.07
23656.22
25600.46
25785.02
23596.92
23754.37
23851.80
25622.96
NL: 7.81E8
3-3_ _GSH_pH7_4_37C_7d
Average DAR = 1.05
23612.42
23656.38
25600.32
25784.78
23582.72
23754.43
23852.37
25622.97
NL: 6.27E8
3-3_ _GSH_pH8_22C_7d
Average DAR = 1.05
23612.27
23656.28
25600.40
25784.90
23597.06
23754.35
23971.72
25623.01
NL: 6.56E8
3-3 _ H7_4_22C_7d_blk
Average DAR = 1.06
23612.15
23656.23
25600.35
25784.77
23335.46
23754.23
23852.27
25622.93
NL: 6.82E8
Relative Intensity
100
50
0
22000
23000
24000
25000
26000
Mass

Fig. 3

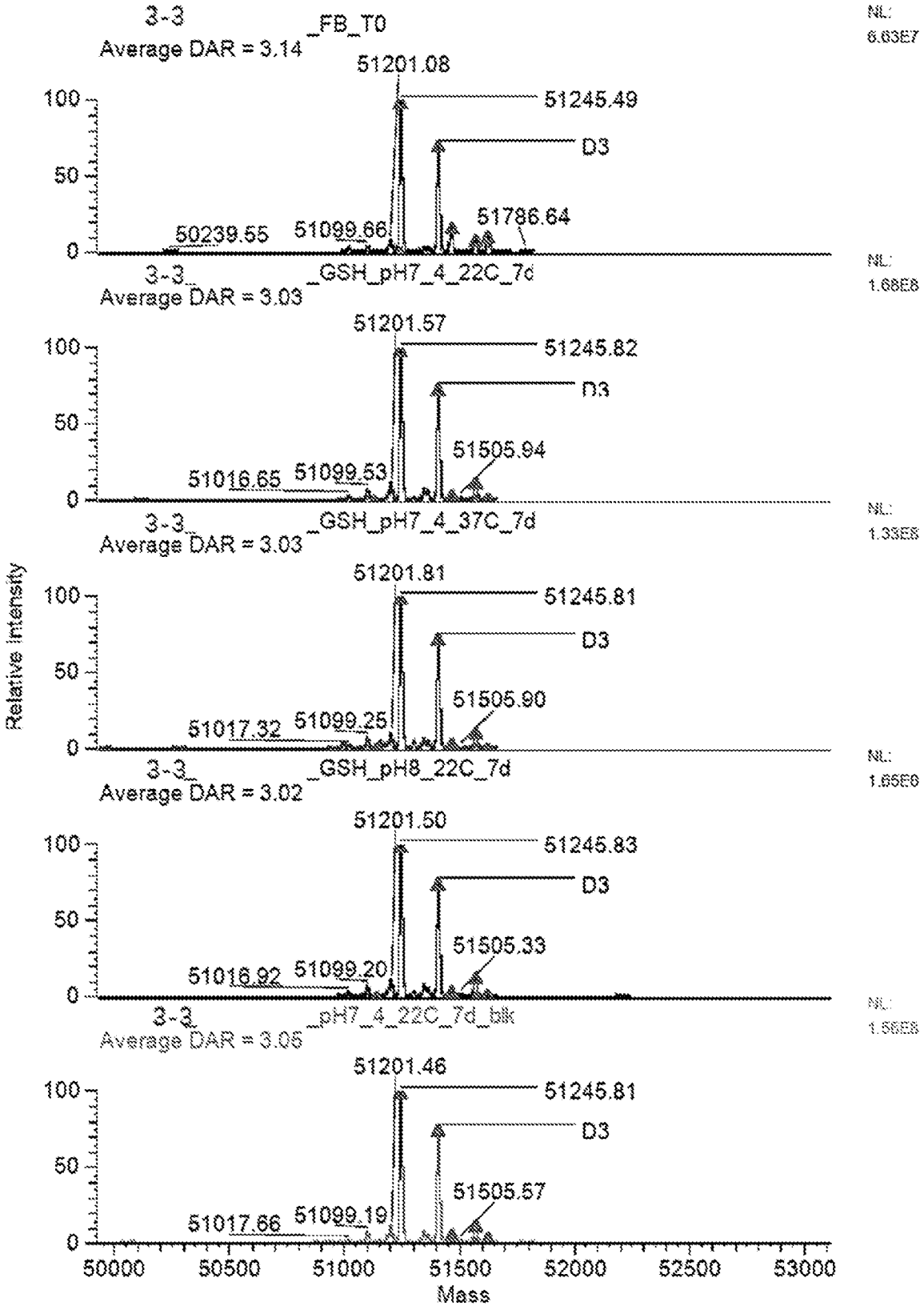

3-3 _FB_T0
Average DAR = 3.14
51201.08
51245.49
D3
51786.64
50239.55
51099.66
NL: 6.63E7
3-3 _GSH_pH7_4_22C_7d
Average DAR = 3.03
51201.57
51245.82
D3
51505.94
51016.65
51099.53
NL: 1.68E8
3-3 _GSH_pH7_4_37C_7d
Average DAR = 3.03
51201.81
51245.81
D3
51505.90
51017.32
51099.25
NL: 1.33E8
3-3 _GSH_pH8_22C_7d
Average DAR = 3.02
51201.50
51245.83
D3
51505.33
51016.92
51099.20
NL: 1.65E8
3-3 _pH7_4_22C_7d_blk
Average DAR = 3.05
51201.46
51245.81
D3
51505.57
51017.66
51099.19
NL: 1.56E8
Relative Intensity
100
50
0
50000
50500
51000
51500
52000
52500
53000
Mass

Fig. 3 (cont.)

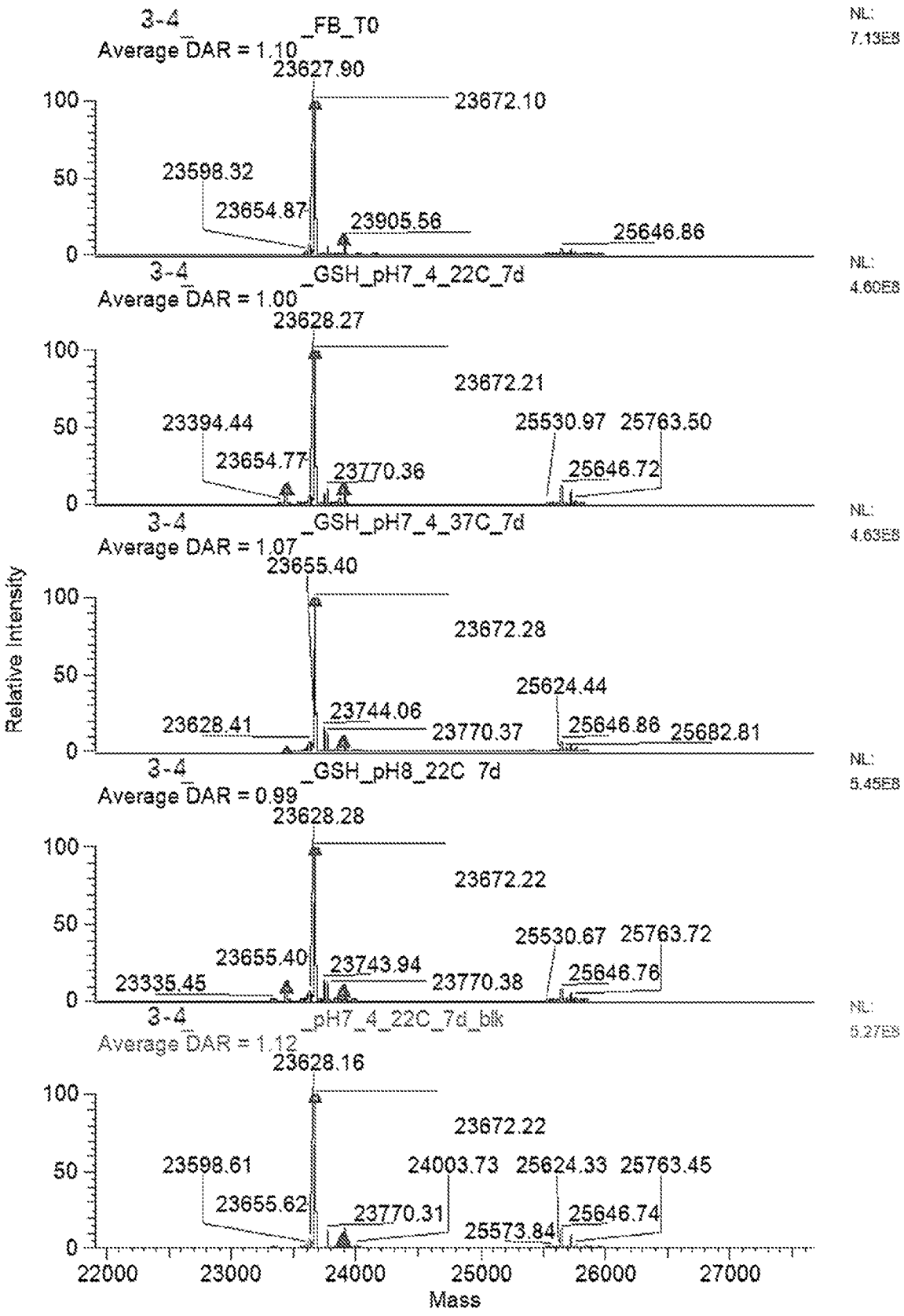

3-4_FB_T0
Average DAR = 1.10
23627.90
23672.10
23598.32
23654.87
23905.56
25646.86
NL: 7.13E8
3-4_GSH_pH7_4_22C_7d
Average DAR = 1.00
23628.27
23672.21
23394.44
25530.97
25763.50
23654.77
23770.36
25646.72
NL: 4.60E8
3-4_GSH_pH7_4_37C_7d
Average DAR = 1.07
23655.40
23672.28
25624.44
23628.41
23744.06
23770.37
25646.86
25682.81
NL: 4.63E8
3-4_GSH_pH8_22C 7d
Average DAR = 0.99
23628.28
23672.22
25530.67
25763.72
23655.40
23743.94
23335.45
23770.38
25646.76
NL: 5.45E8
3-4_pH7_4_22C_7d_blk
Average DAR = 1.12
23628.16
23672.22
23598.61
24003.73
25624.33
25763.45
23655.62
23770.31
25573.84
25646.74
NL: 5.27E8
Relative Intensity
100
50
0
22000
23000
24000
25000
26000
27000
Mass

Fig. 4

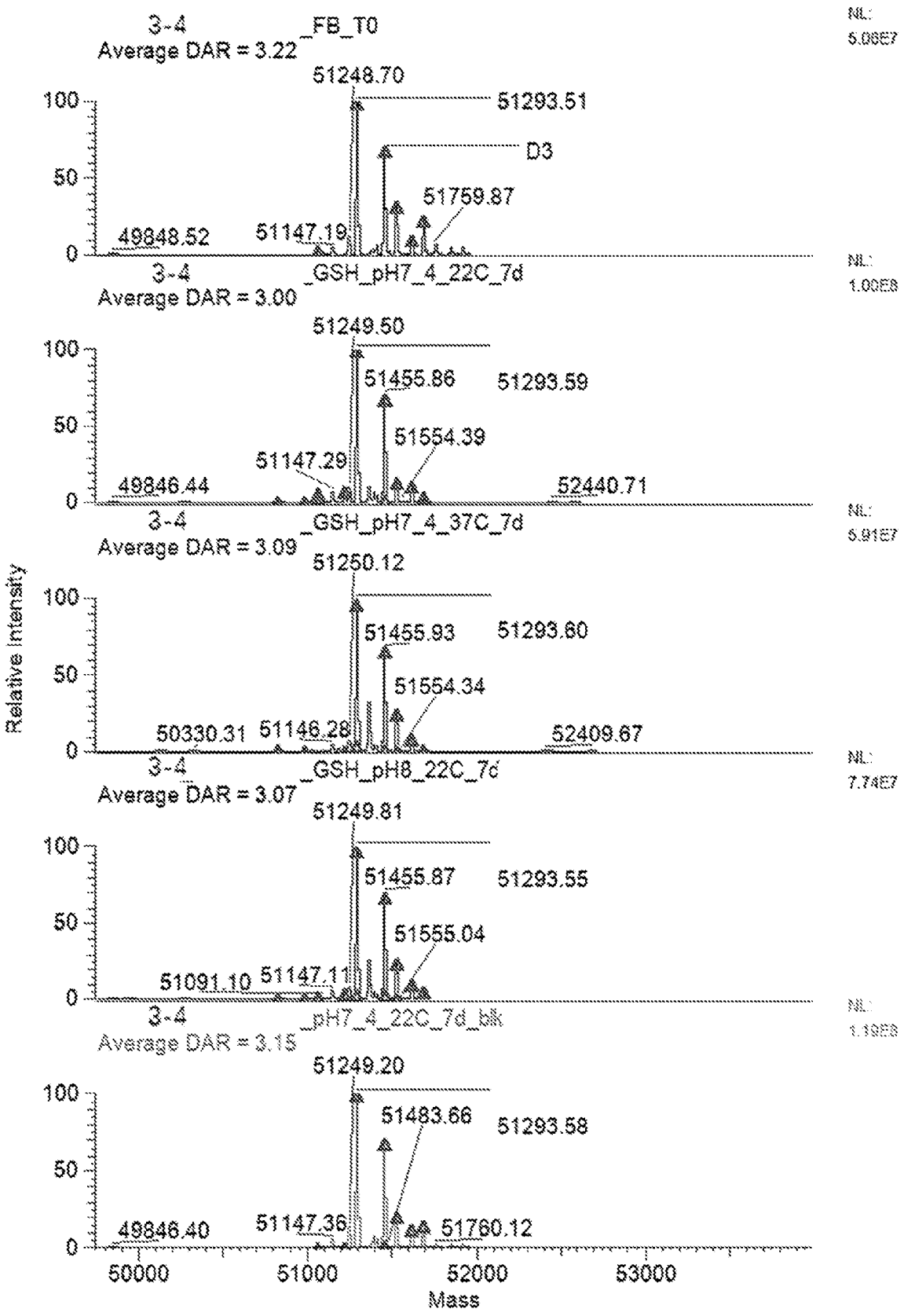

3-4 _FB_T0
Average DAR = 3.22
NL: 5.06E7
51248.70
51293.51
D3
51759.87
49848.52
51147.19
3-4 _GSH_pH7_4_22C_7d
Average DAR = 3.00
NL: 1.00E8
51249.50
51455.86
51293.59
51554.39
51147.29
49846.44
52440.71
3-4 _GSH_pH7_4_37C_7d
Average DAR = 3.09
NL: 5.91E7
51250.12
51455.93
51293.60
51554.34
50330.31
51146.28
52409.67
3-4 _GSH_pH8_22C_7d
Average DAR = 3.07
NL: 7.74E7
51249.81
51455.87
51293.55
51555.04
51091.10
51147.11
3-4 _pH7_4_22C_7d_blk
Average DAR = 3.15
NL: 1.19E8
51249.20
51483.66
51293.58
49846.40
51147.36
51760.12
Relative Intensity
100
50
0
50000
51000
52000
53000
Mass

Fig. 4 (cont.)

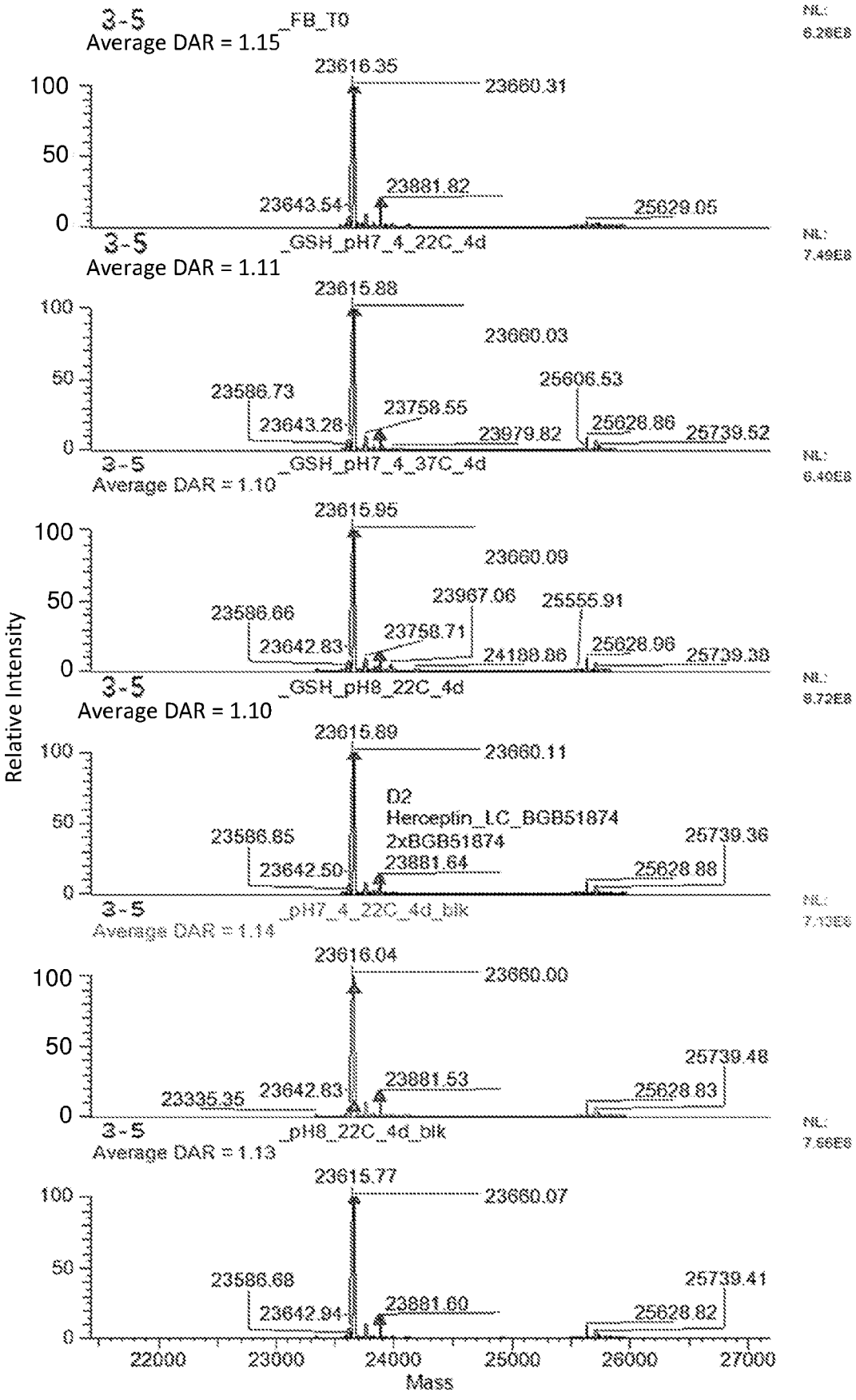

3-5 _FB_T0
Average DAR = 1.15
23616.35
23680.31
23881.82
23643.54
25628.05
3-5 _GSH_pH7_4_22C_4d
Average DAR = 1.11
23615.88
23680.03
25606.53
23586.73
23758.55
23643.28
23979.82
25628.86
25739.52
3-5 _GSH_pH7_4_37C_4d
Average DAR = 1.10
23615.95
23680.09
23967.06
25555.91
23586.66
23758.71
23642.83
24188.86
25628.96
25739.38
3-5 _GSH_pH8_22C_4d
Average DAR = 1.10
23615.89
23680.11
D2
Heroeplin_LC_BGB51874
2xBGB51874
23586.65
25739.36
23642.50
23881.64
25628.88
3-5 _pH7_4_22C_4d_blk
Average DAR = 1.14
23616.04
23680.00
25739.48
23881.53
23335.35
23642.83
25628.83
3-5 _pH8_22C_4d_blk
Average DAR = 1.13
23615.77
23680.07
23586.68
25739.41
23642.94
23881.60
25628.82
Relative Intensity
Mass
22000
23000
24000
25000
26000
27000

Fig. 5

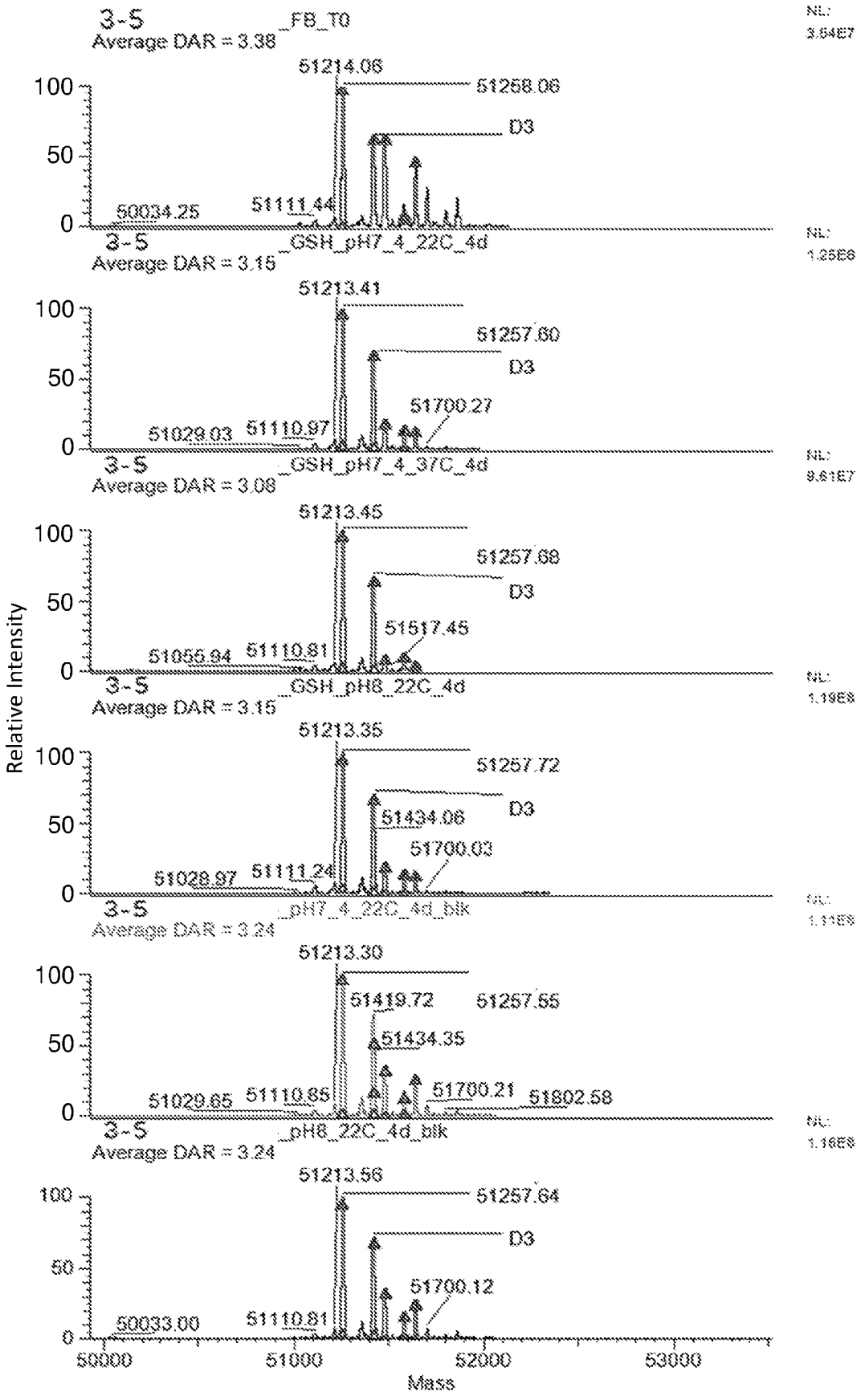

3-5 _FB_T0
Average DAR = 3.38
NL: 3.64E7
51214.06
51258.06
D3
50034.25
51111.44
3-5 _GSH_pH7_4_22C_4d
Average DAR = 3.15
NL: 1.25E6
51213.41
51257.60
D3
51700.27
51029.03
51110.97
3-5 _GSH_pH7_4_37C_4d
Average DAR = 3.08
NL: 9.61E7
51213.45
51257.68
D3
51517.45
51056.94
51110.81
3-5 _GSH_pH8_22C_4d
Average DAR = 3.15
NL: 1.19E8
51213.35
51257.72
D3
51434.06
51700.03
51028.97
51111.24
3-5 _pH7_4_22C_4d_blk
Average DAR = 3.24
NL: 1.11E8
51213.30
51419.72
51257.55
51434.35
51029.65
51110.85
51700.21
51802.58
3-5 _pH8_22C_4d_blk
Average DAR = 3.24
NL: 1.16E8
51213.56
51257.64
D3
51700.12
50033.00
51110.81
Relative Intensity
Mass
50000
51000
52000
53000

Fig. 5 (cont.)

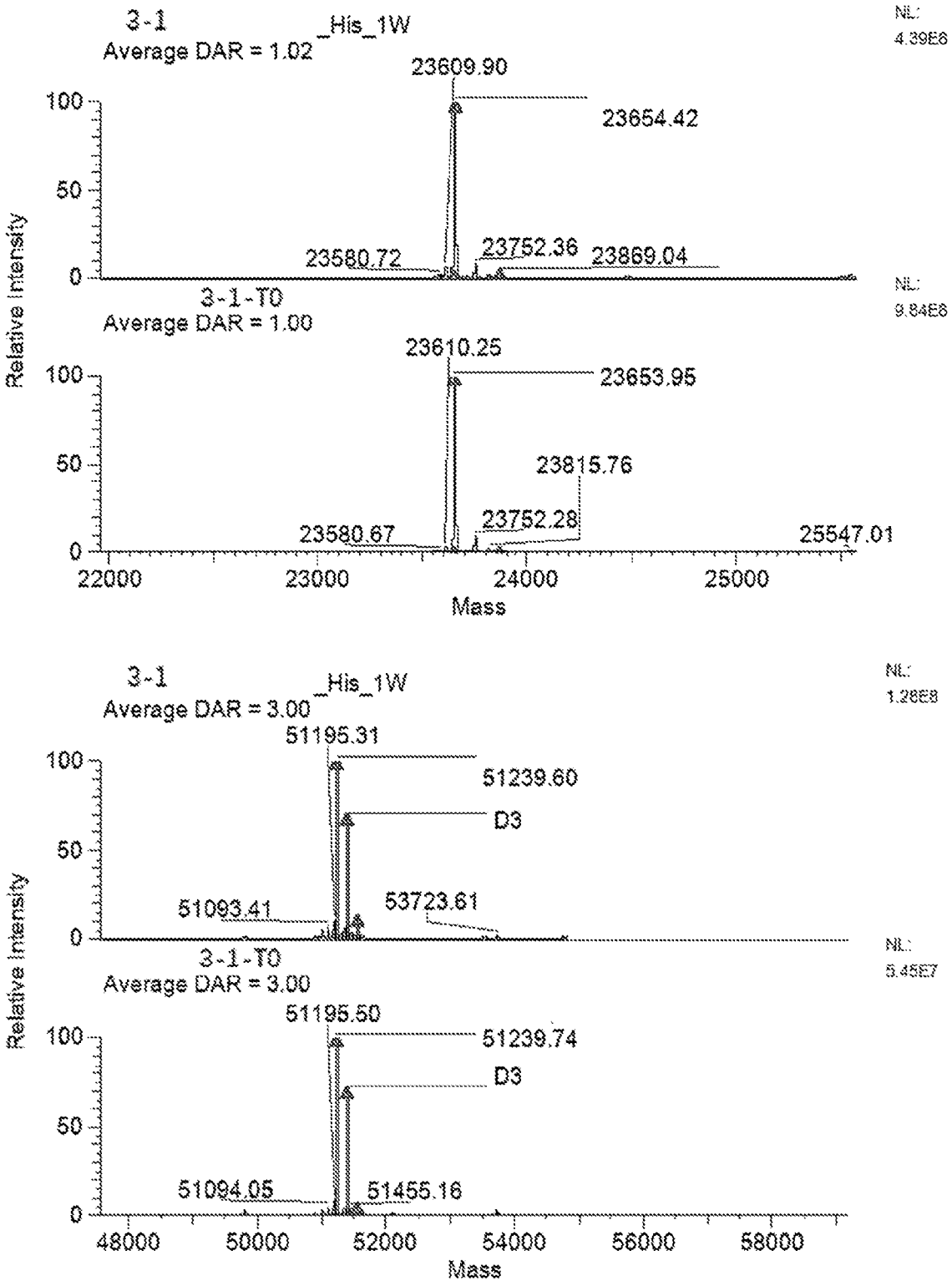

3-1 _His_1W
Average DAR = 1.02
23609.90
23654.42
23580.72
23752.36
23869.04
NL: 4.39E8
3-1-T0
Average DAR = 1.00
23610.25
23653.95
23815.76
23580.67
23752.28
25547.01
NL: 9.84E8
Relative Intensity
Mass
3-1 _His_1W
Average DAR = 3.00
51195.31
51239.60
D3
51093.41
53723.61
NL: 1.26E8
3-1-T0
Average DAR = 3.00
51195.50
51239.74
D3
51094.05
51455.16
NL: 5.45E7
Relative Intensity
Mass

Fig. 6

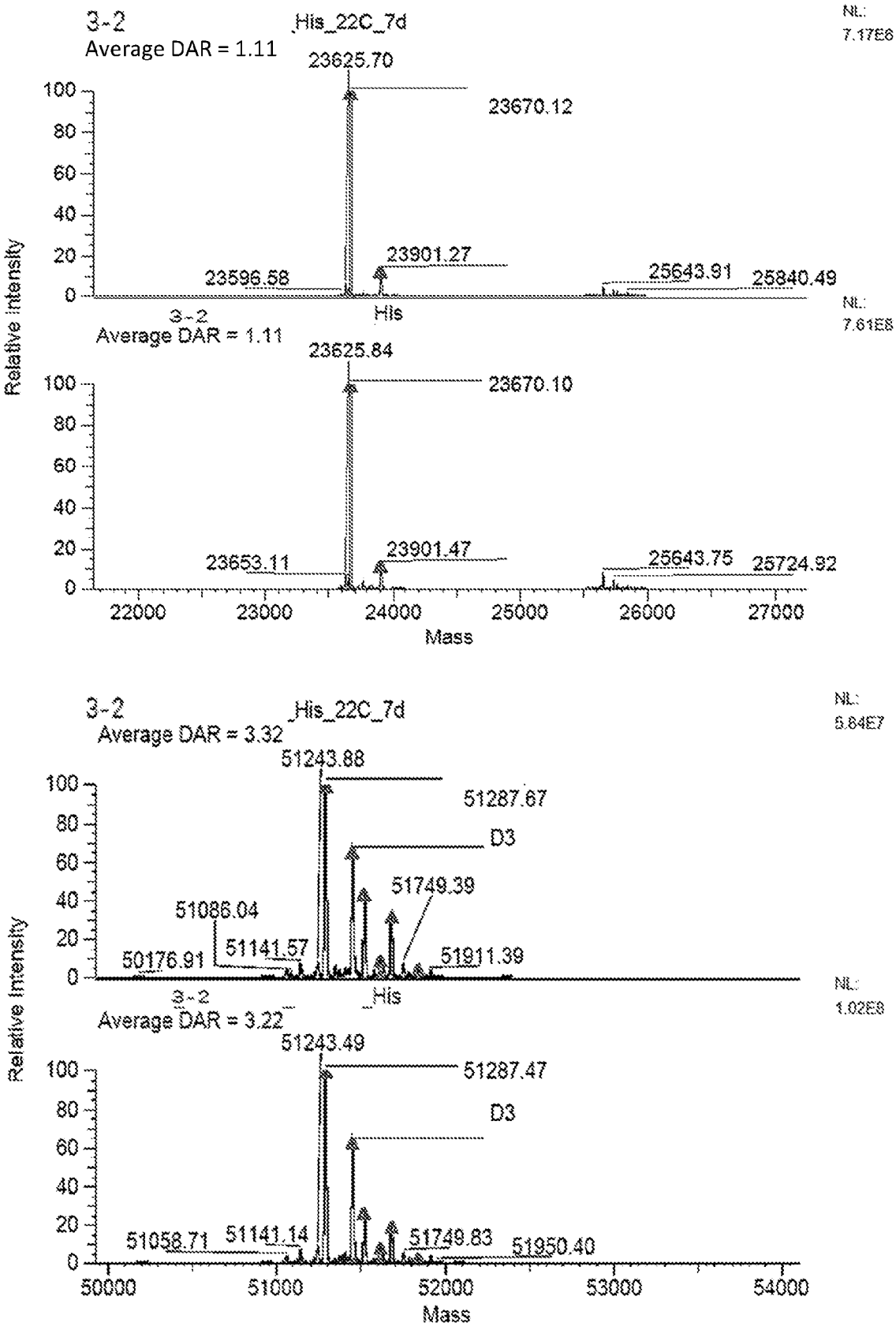

3-2
Average DAR = 1.11
His_22C_7d
NL:
7.17E6
23625.70
23670.12
23901.27
23596.58
25643.91
25840.49
3-2
Average DAR = 1.11
His
NL:
7.61E8
23625.84
23670.10
23901.47
23653.11
25643.75
25724.92
Relative Intensity
Mass
3-2
Average DAR = 3.32
His_22C_7d
NL:
5.84E7
51243.88
51287.67
D3
51749.39
51086.04
51141.57
50176.91
51911.39
3-2
Average DAR = 3.22
His
NL:
1.02E8
51243.49
51287.47
D3
51058.71
51141.14
51749.83
51950.40
Relative Intensity
Mass

Fig. 7

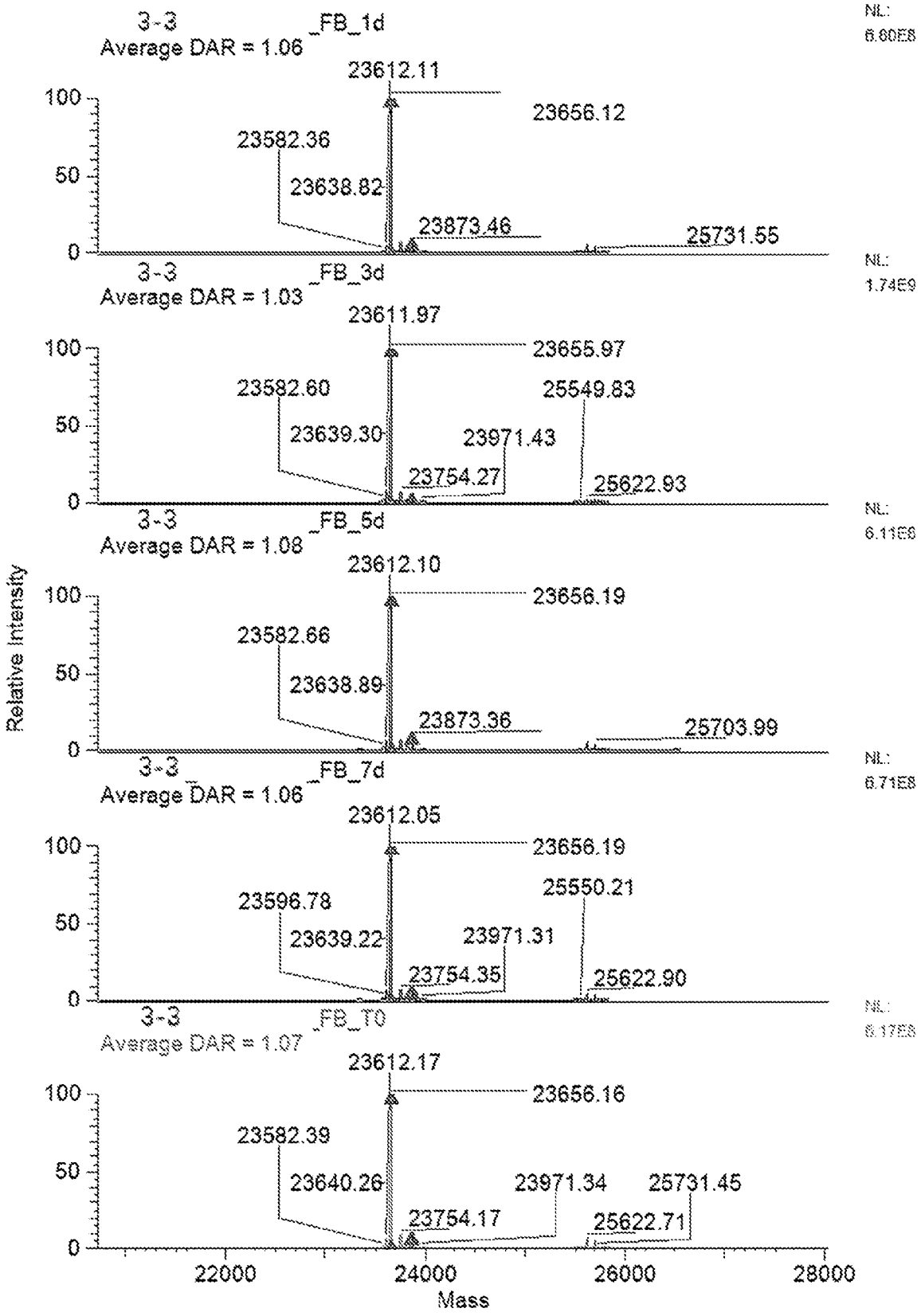

3-3 _FB_1d
Average DAR = 1.06
23612.11
23656.12
23582.36
23638.82
23873.46
25731.55
NL: 6.60E8
3-3 _FB_3d
Average DAR = 1.03
23611.97
23655.97
23582.60
25549.83
23639.30
23971.43
23754.27
25622.93
NL: 1.74E9
3-3 _FB_5d
Average DAR = 1.08
23612.10
23656.19
23582.66
23638.89
23873.36
25703.99
NL: 6.11E8
3-3 _FB_7d
Average DAR = 1.06
23612.05
23656.19
25550.21
23596.78
23639.22
23971.31
23754.35
25622.90
NL: 6.71E8
3-3 _FB_T0
Average DAR = 1.07
23612.17
23656.16
23582.39
23640.26
23971.34
25731.45
23754.17
25622.71
NL: 6.17E8
Relative Intensity
100
50
0
22000
24000
26000
28000
Mass

Fig. 8

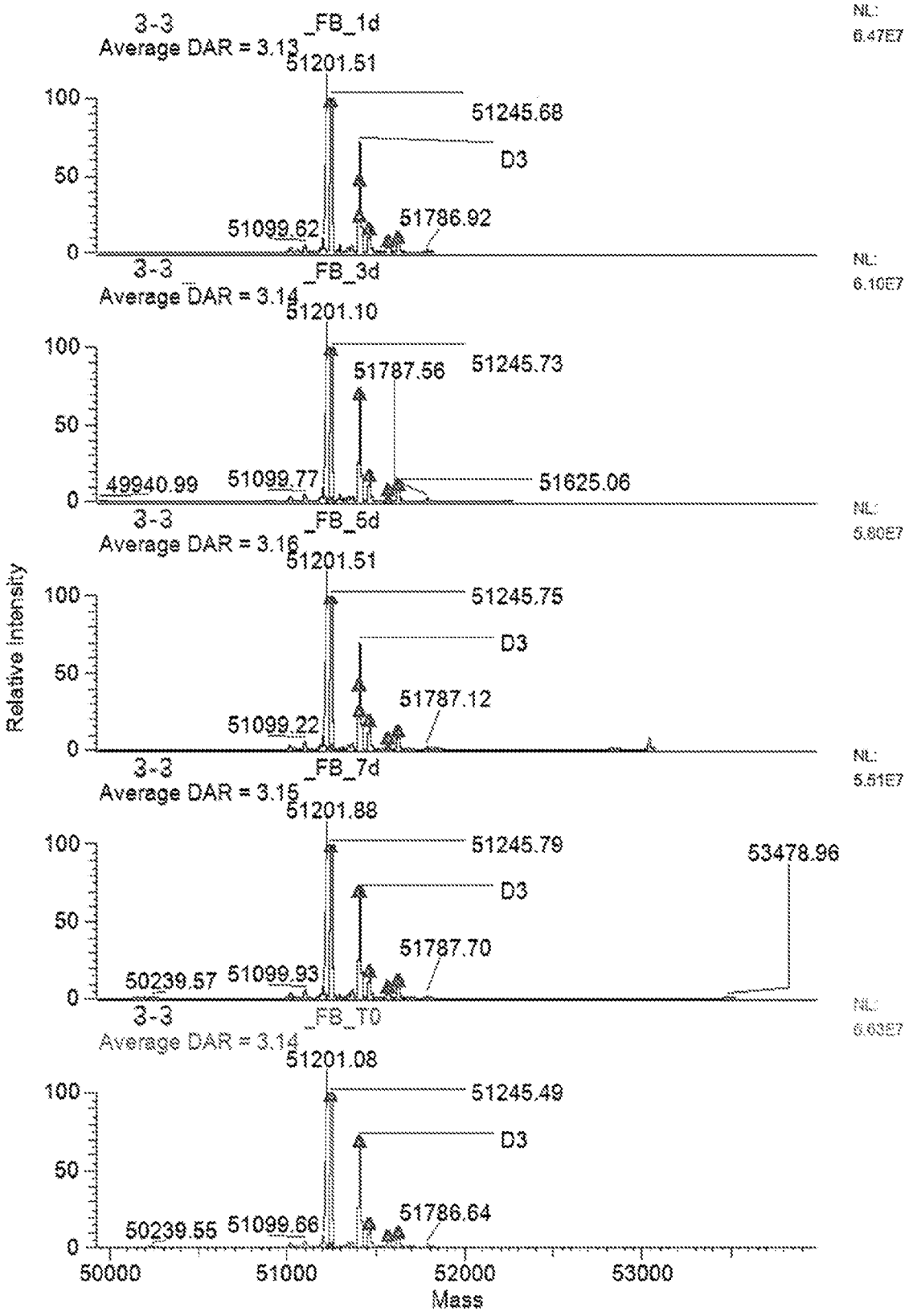

3-3_FB_1d
Average DAR = 3.13
51201.51
51245.68
D3
51786.92
51099.62
NL: 6.47E7
3-3_FB_3d
Average DAR = 3.14
51201.10
51787.56
51245.73
49940.99
51099.77
51625.06
NL: 6.10E7
3-3_FB_5d
Average DAR = 3.16
51201.51
51245.75
D3
51787.12
51099.22
NL: 5.80E7
3-3_FB_7d
Average DAR = 3.15
51201.88
51245.79
53478.96
D3
51787.70
50239.57
51099.93
NL: 5.51E7
3-3_FB_T0
Average DAR = 3.14
51201.08
51245.49
D3
51786.64
50239.55
51099.66
NL: 6.63E7
Relative Intensity
100
50
0
50000
51000
52000
53000
Mass

Fig. 8 (cont.)

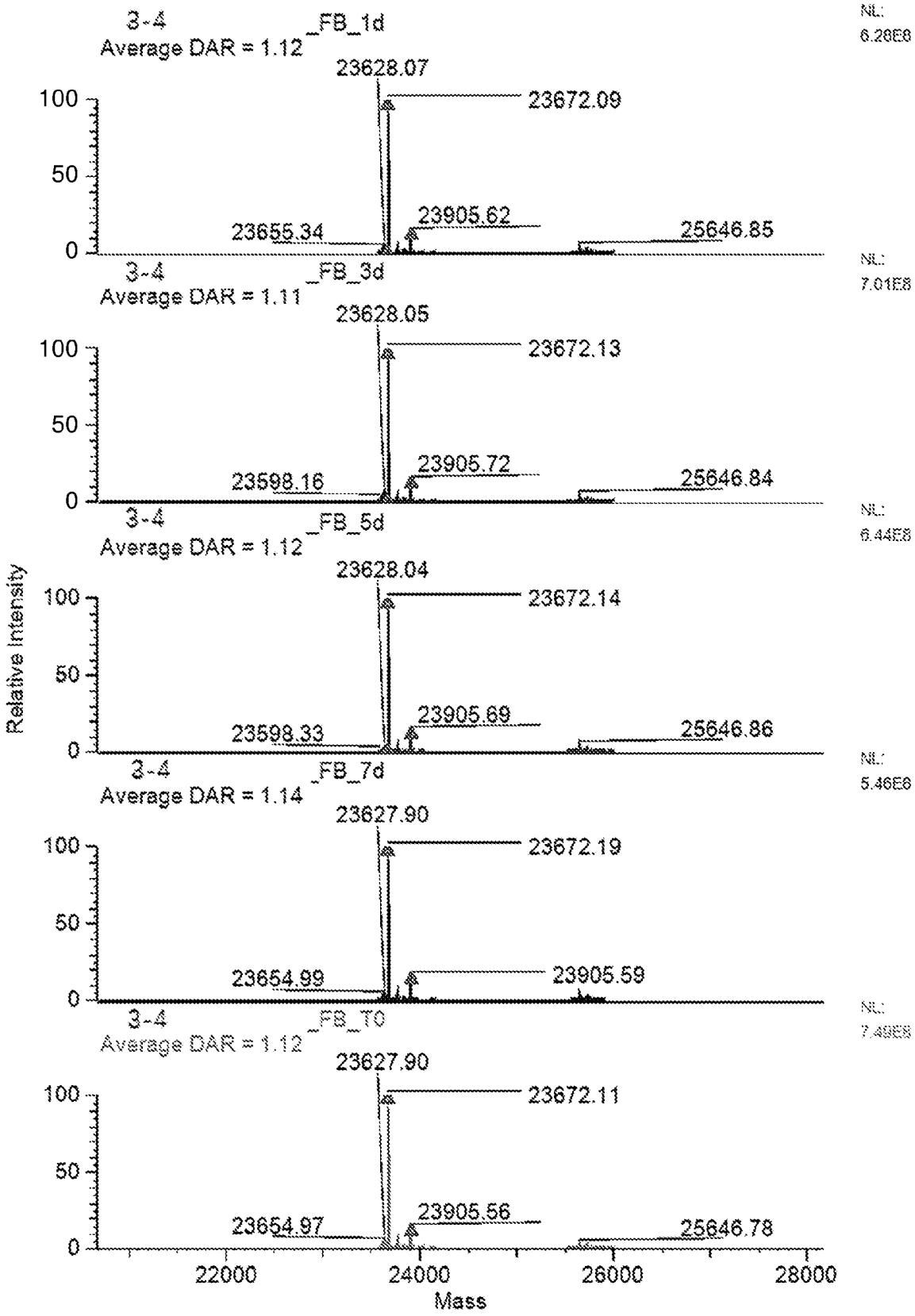

3-4 _FB_1d
Average DAR = 1.12
23628.07
23672.09
23655.34
23905.62
25646.85
NL: 6.28E8
3-4 _FB_3d
Average DAR = 1.11
23628.05
23672.13
23598.16
23905.72
25646.84
NL: 7.01E8
3-4 _FB_5d
Average DAR = 1.12
23628.04
23672.14
23598.33
23905.69
25646.86
NL: 6.44E8
3-4 _FB_7d
Average DAR = 1.14
23627.90
23672.19
23654.99
23905.59
NL: 5.46E8
3-4 _FB_T0
Average DAR = 1.12
23627.90
23672.11
23654.97
23905.56
25646.78
NL: 7.49E8
Relative Intensity
100
50
0
22000
24000
26000
28000
Mass

Fig. 9

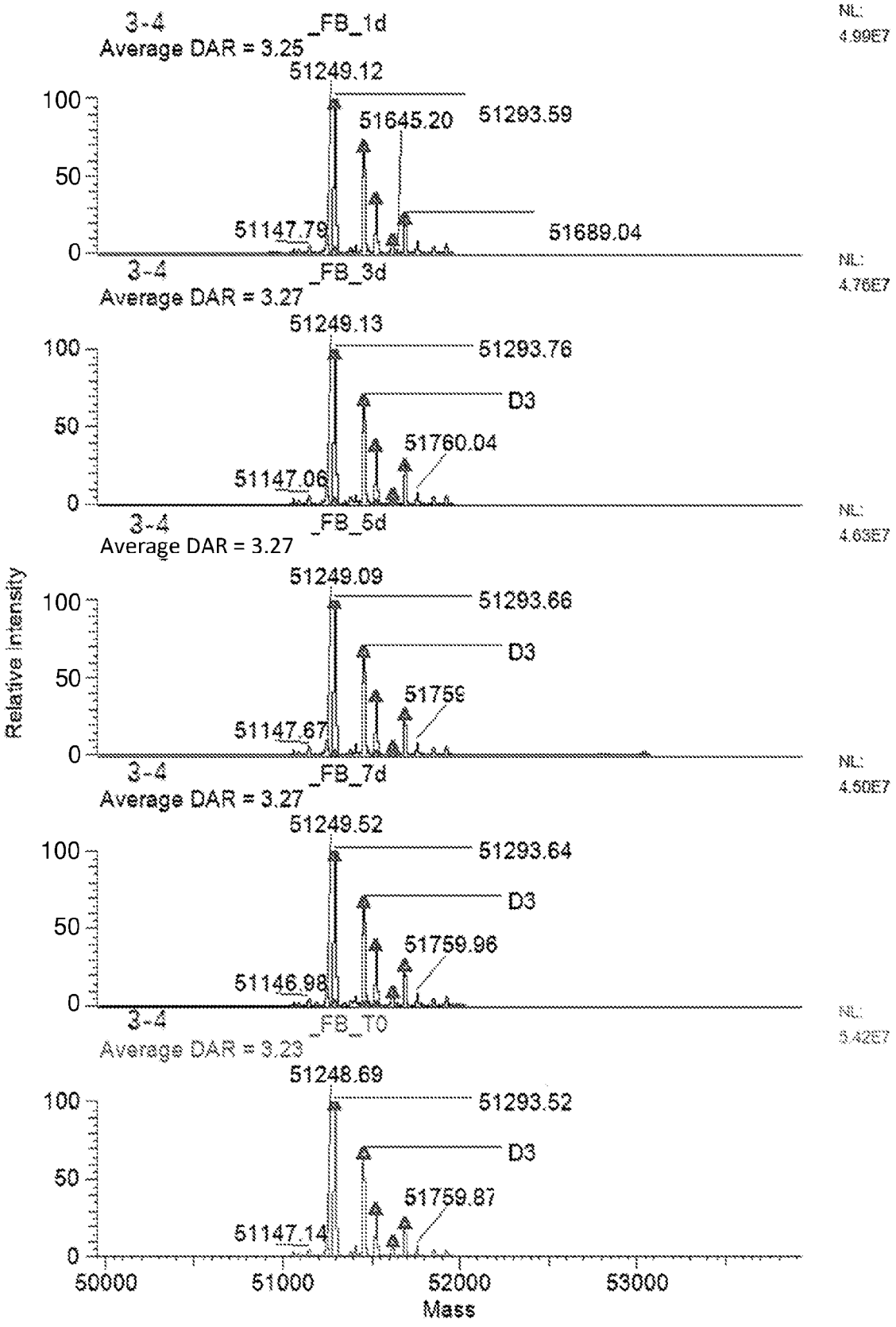

3-4 _FB_1d
Average DAR = 3.25
51249.12
51645.20
51293.59
51147.79
51689.04
NL: 4.99E7
3-4 _FB_3d
Average DAR = 3.27
51249.13
51293.76
D3
51760.04
51147.06
NL: 4.76E7
3-4 _FB_5d
Average DAR = 3.27
51249.09
51293.66
D3
51759
51147.67
NL: 4.63E7
3-4 _FB_7d
Average DAR = 3.27
51249.52
51293.64
D3
51759.96
51146.98
NL: 4.60E7
3-4 _FB_T0
Average DAR = 3.23
51248.69
51293.52
D3
51759.87
51147.14
NL: 5.42E7
Relative intensity
100
50
0
50000
51000
52000
53000
Mass

Fig. 9 (cont.)

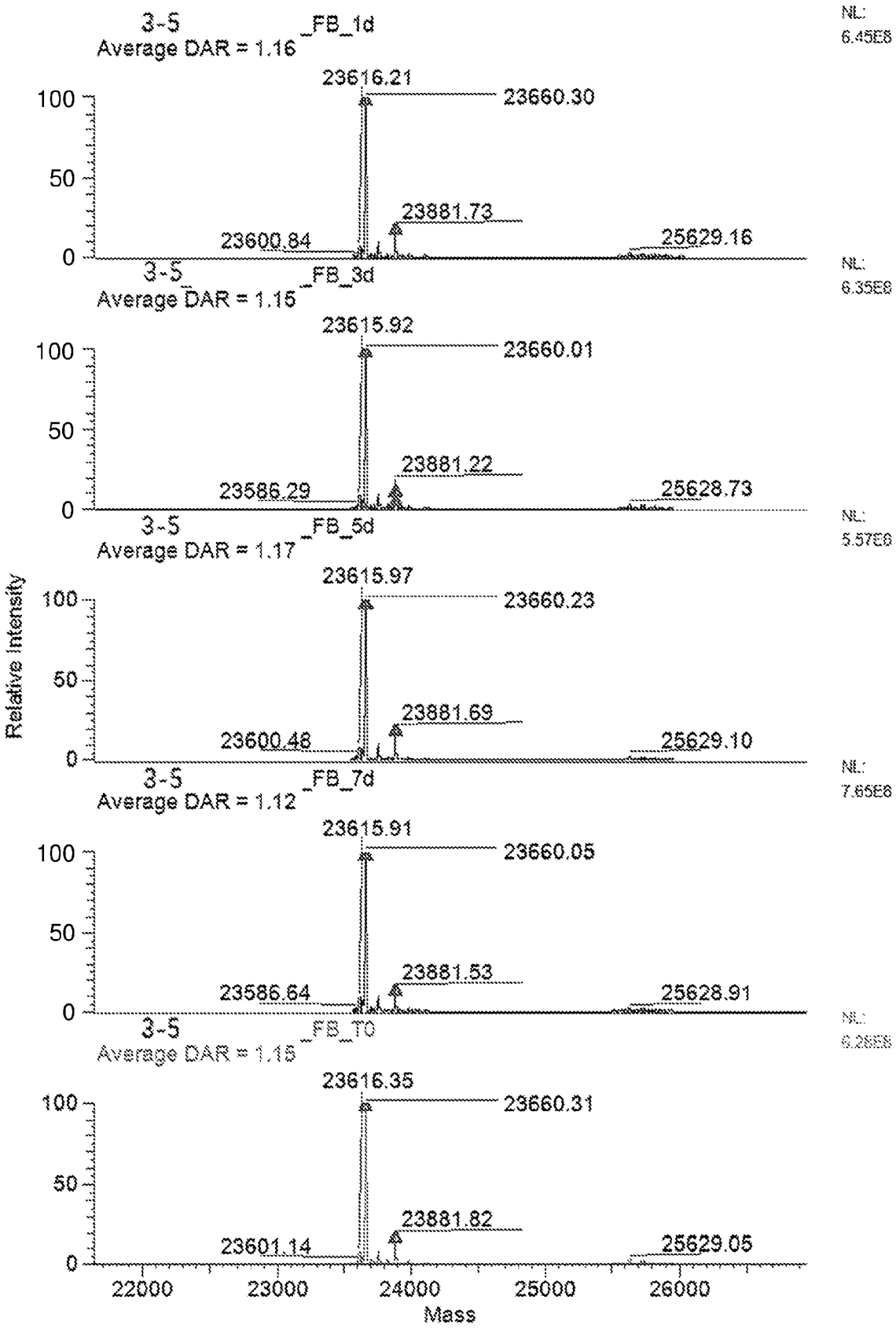

3-5_FB_1d
Average DAR = 1.16
23616.21
23660.30
23881.73
23600.84
25629.16
NL: 6.45E8
3-5_FB_3d
Average DAR = 1.15
23615.92
23660.01
23881.22
23586.29
25628.73
NL: 6.35E8
3-5_FB_5d
Average DAR = 1.17
23615.97
23660.23
23881.69
23600.48
25629.10
NL: 5.57E8
3-5_FB_7d
Average DAR = 1.12
23615.91
23660.05
23881.53
23586.64
25628.91
NL: 7.65E8
3-5_FB_T0
Average DAR = 1.15
23616.35
23660.31
23881.82
23601.14
25629.05
NL: 6.26E8
Relative Intensity
100
50
0
22000
23000
24000
25000
26000
Mass

Fig. 10

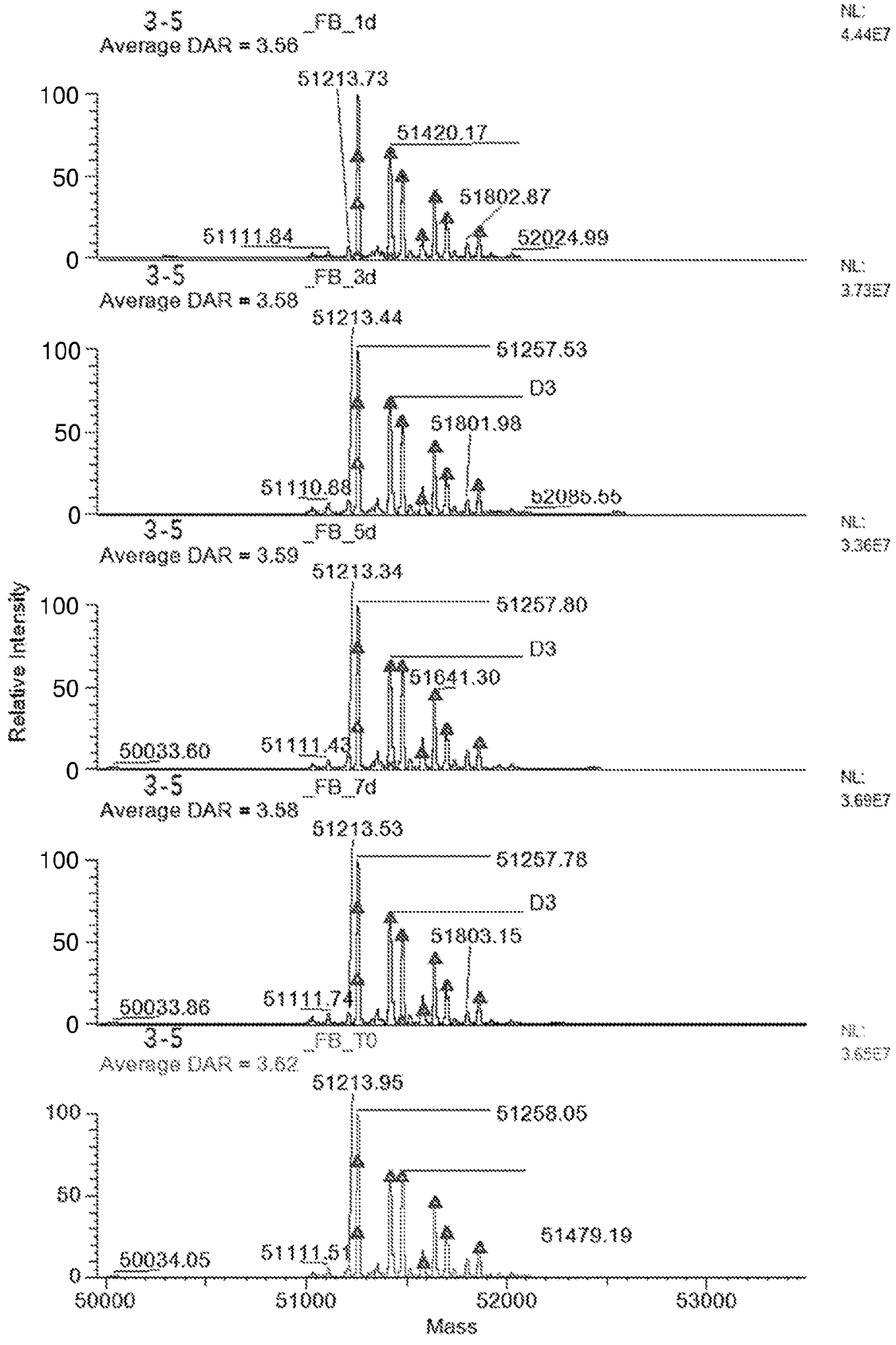

3-5 _FB_1d
Average DAR = 3.56
NL: 4.44E7
51213.73
51420.17
51802.87
51111.84
52024.99
3-5 _FB_3d
Average DAR = 3.58
NL: 3.73E7
51213.44
51257.53
D3
51801.98
51110.88
52085.55
3-5 _FB_5d
Average DAR = 3.59
NL: 3.36E7
51213.34
51257.80
D3
51641.30
50033.60
51111.43
3-5 _FB_7d
Average DAR = 3.58
NL: 3.69E7
51213.53
51257.78
D3
51803.15
50033.86
51111.74
3-5 _FB_T0
Average DAR = 3.62
NL: 3.65E7
51213.95
51258.05
51479.19
50034.05
51111.51
Relative Intensity
Mass
50000
51000
52000
53000

Fig. 10 (cont.)

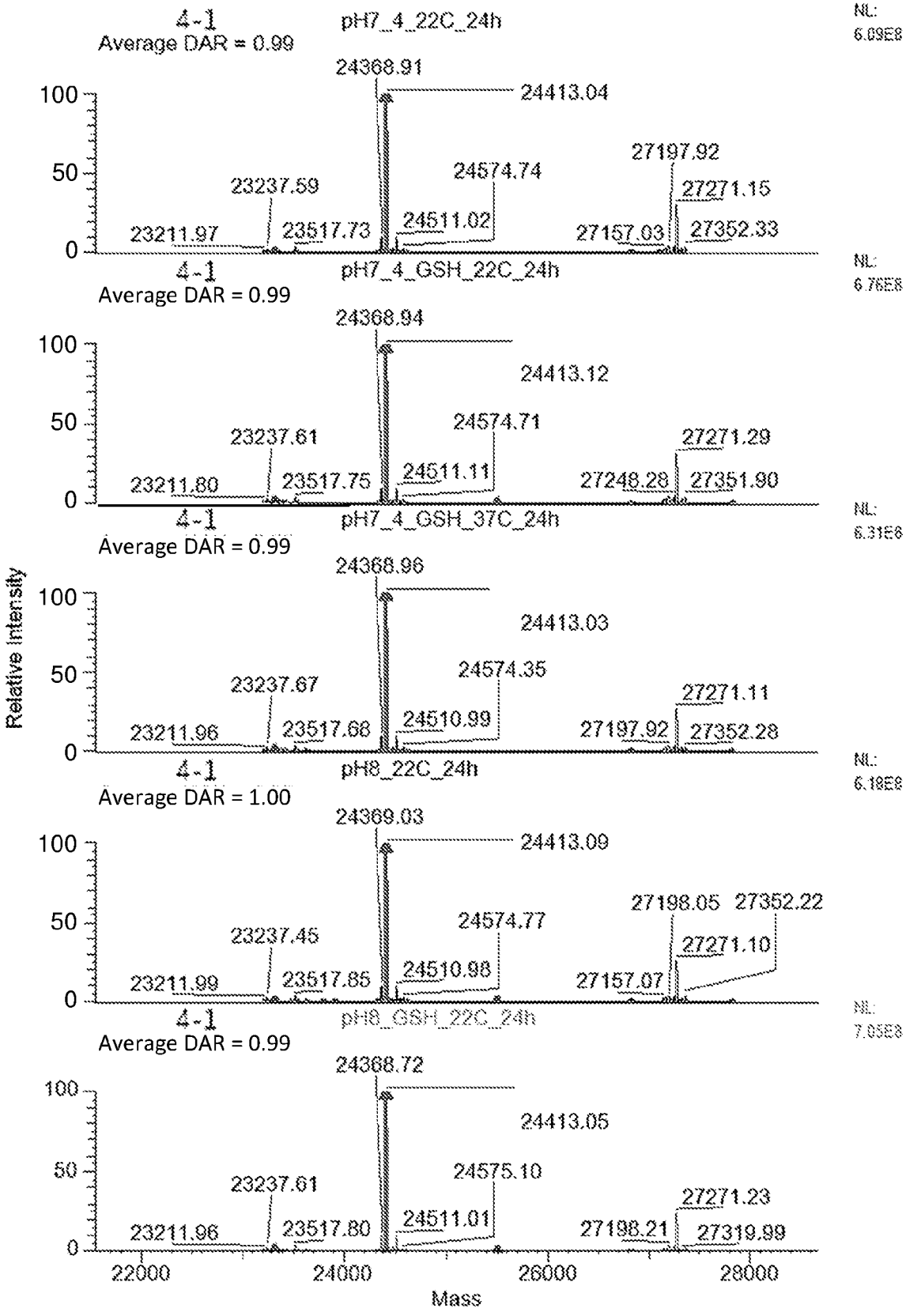

4-1
Average DAR = 0.99
pH7_4_22C_24h
NL:
6.09E8
24368.91
24413.04
27197.92
24574.74
23237.59
27271.15
23211.97
23517.73
24511.02
27157.03
27352.33
4-1
Average DAR = 0.99
pH7_4_GSH_22C_24h
NL:
6.76E8
24368.94
24413.12
24574.71
23237.61
27271.29
23211.80
23517.75
24511.11
27248.28
27351.90
4-1
Average DAR = 0.99
pH7_4_GSH_37C_24h
NL:
6.31E8
24368.96
24413.03
24574.35
23237.67
27271.11
23211.96
23517.68
24510.99
27197.92
27352.28
4-1
Average DAR = 1.00
pH8_22C_24h
NL:
6.18E8
24369.03
24413.09
27198.05
27352.22
24574.77
23237.45
27271.10
23211.99
23517.85
24510.98
27157.07
4-1
Average DAR = 0.99
pH8_GSH_22C_24h
NL:
7.05E8
24368.72
24413.05
24575.10
23237.61
27271.23
23211.96
23517.80
24511.01
27198.21
27319.99
Relative Intensity
100
50
0
22000
24000
26000
28000
Mass

Fig. 11

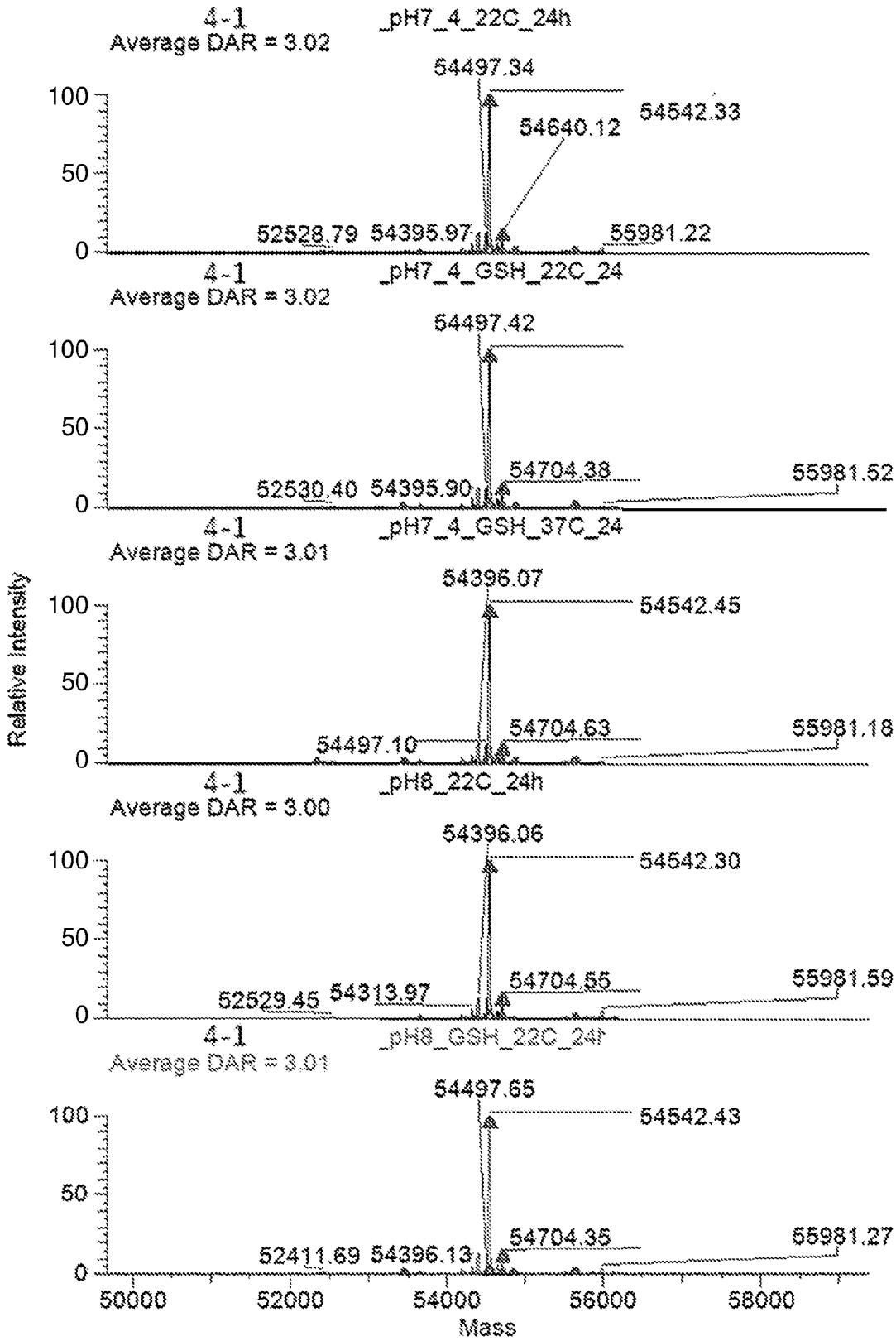

4-1
_pH7_4_22C_24h
Average DAR = 3.02
54497.34
54542.33
54640.12
52528.79
54395.97
55981.22
4-1
_pH7_4_GSH_22C_24
Average DAR = 3.02
54497.42
54704.38
55981.52
52530.40
54395.90
4-1
_pH7_4_GSH_37C_24
Average DAR = 3.01
54396.07
54542.45
54704.63
55981.18
54497.10
4-1
_pH8_22C_24h
Average DAR = 3.00
54396.06
54542.30
54704.55
55981.59
52529.45
54313.97
4-1
_pH8_GSH_22C_24h
Average DAR = 3.01
54497.65
54542.43
54704.35
55981.27
52411.69
54396.13
Relative Intensity
100
50
0
50000
52000
54000
56000
58000
Mass

Fig. 11 (cont.)

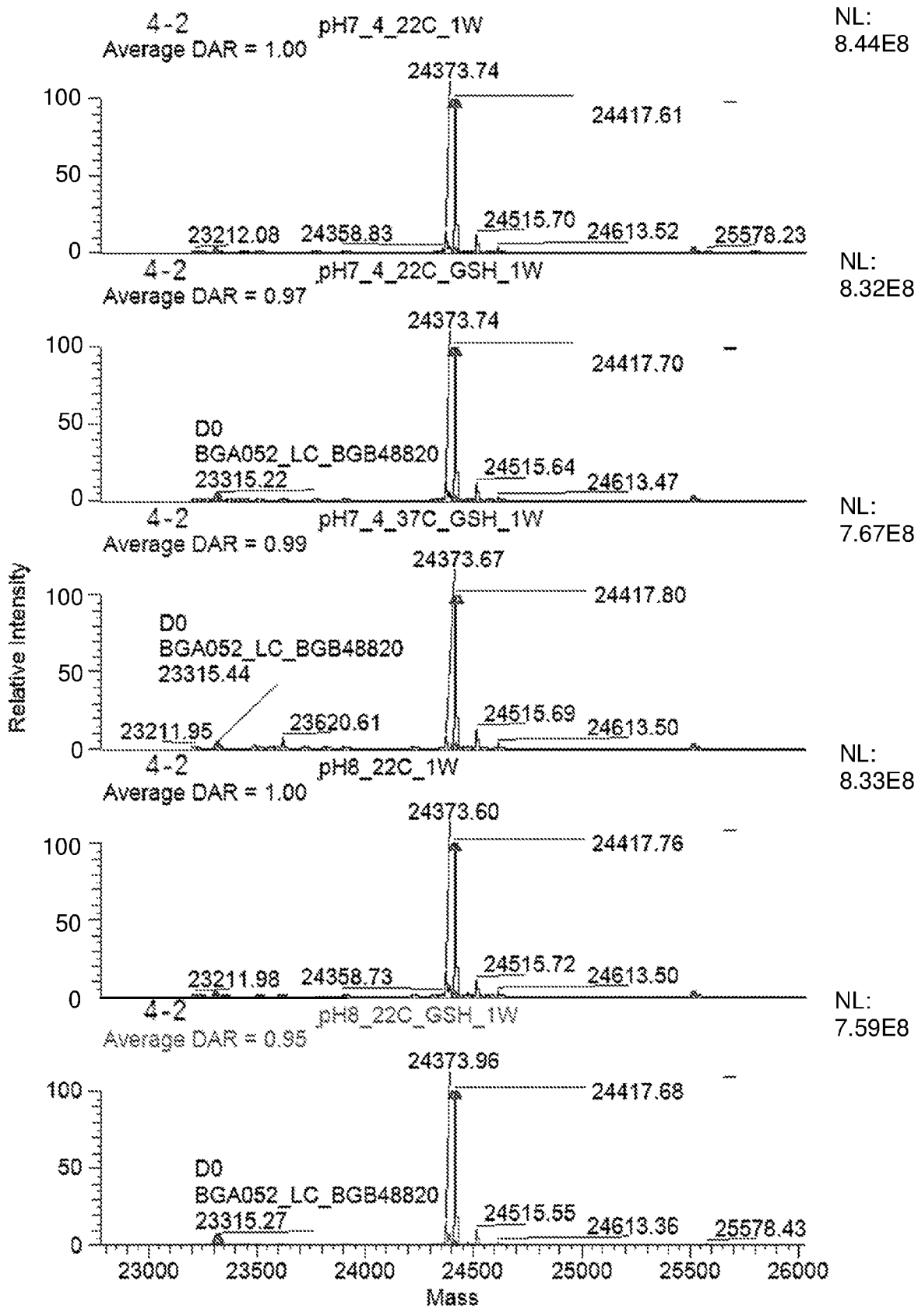

4-2
Average DAR = 1.00
pH7_4_22C_1W
NL:
8.44E8
24373.74
24417.61
23212.08
24358.83
24515.70
24613.52
25578.23
4-2
Average DAR = 0.97
pH7_4_22C_GSH_1W
NL:
8.32E8
24373.74
24417.70
D0
BGA052_LC_BGB48820
23315.22
24515.64
24613.47
4-2
Average DAR = 0.99
pH7_4_37C_GSH_1W
NL:
7.67E8
24373.67
24417.80
D0
BGA052_LC_BGB48820
23315.44
23211.95
23620.61
24515.69
24613.50
4-2
Average DAR = 1.00
pH8_22C_1W
NL:
8.33E8
24373.60
24417.76
23211.98
24358.73
24515.72
24613.50
4-2
Average DAR = 0.95
pH8_22C_GSH_1W
NL:
7.59E8
24373.96
24417.68
D0
BGA052_LC_BGB48820
23315.27
24515.55
24613.36
25578.43
Relative Intensity
Mass
100
50
0
23000
23500
24000
24500
25000
25500
26000

Fig. 12

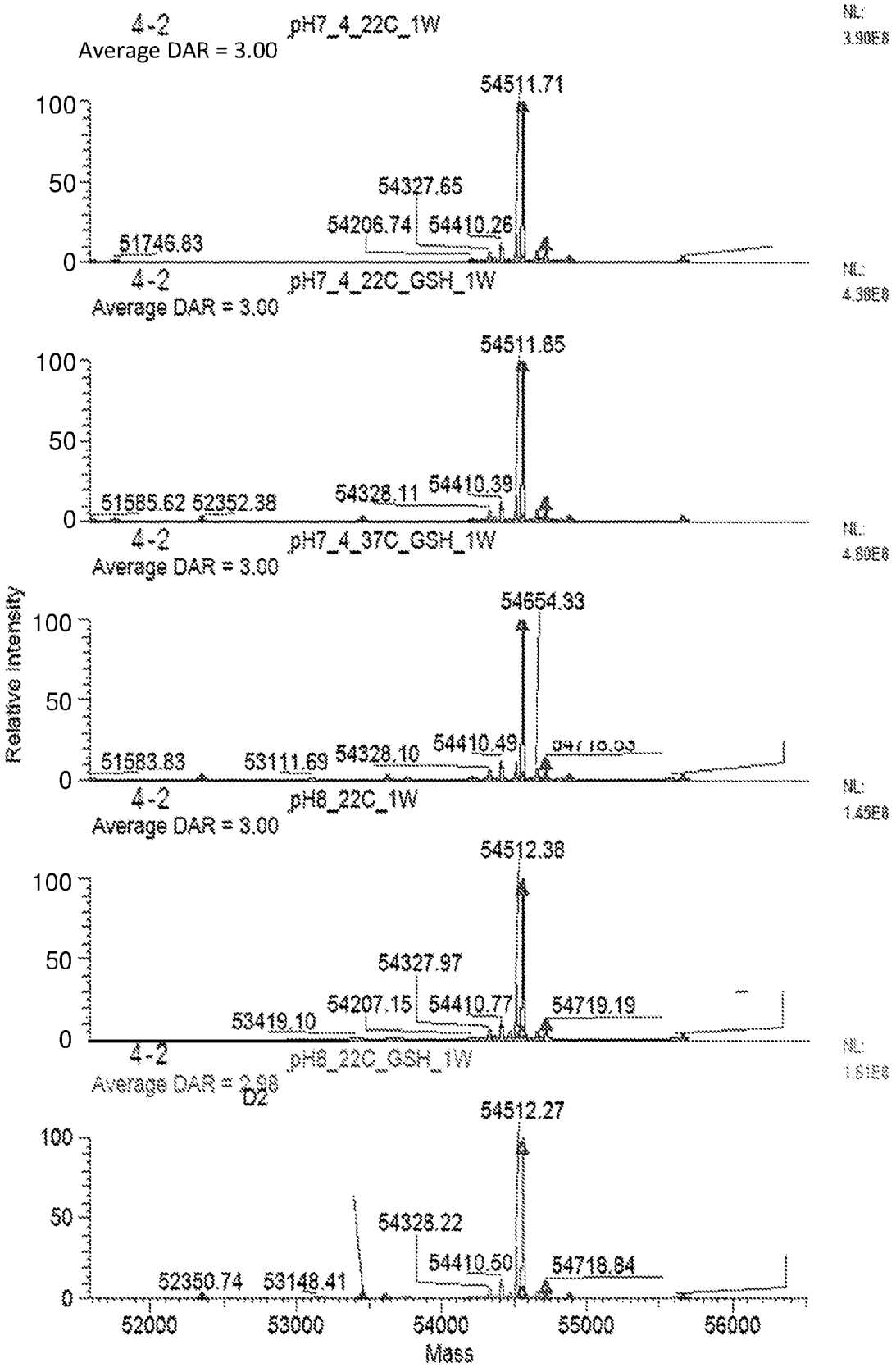

4-2
Average DAR = 3.00
pH7_4_22C_1W
NL:
3.90E8
54511.71
54327.65
54206.74
54410.26
51746.83
4-2
Average DAR = 3.00
pH7_4_22C_GSH_1W
NL:
4.38E8
54511.85
51585.62
52352.38
54328.11
54410.39
4-2
Average DAR = 3.00
pH7_4_37C_GSH_1W
NL:
4.80E8
54654.33
51583.83
53111.69
54328.10
54410.49
4-2
Average DAR = 3.00
pH8_22C_1W
NL:
1.43E8
54512.38
54327.97
54207.15
54410.77
54719.19
53419.10
4-2
Average DAR = 2.98
D2
pH8_22C_GSH_1W
NL:
1.61E8
54512.27
54328.22
54410.50
54718.84
52350.74
53148.41
Relative Intensity
Mass
52000
53000
54000
55000
56000

Fig. 12 (cont.)

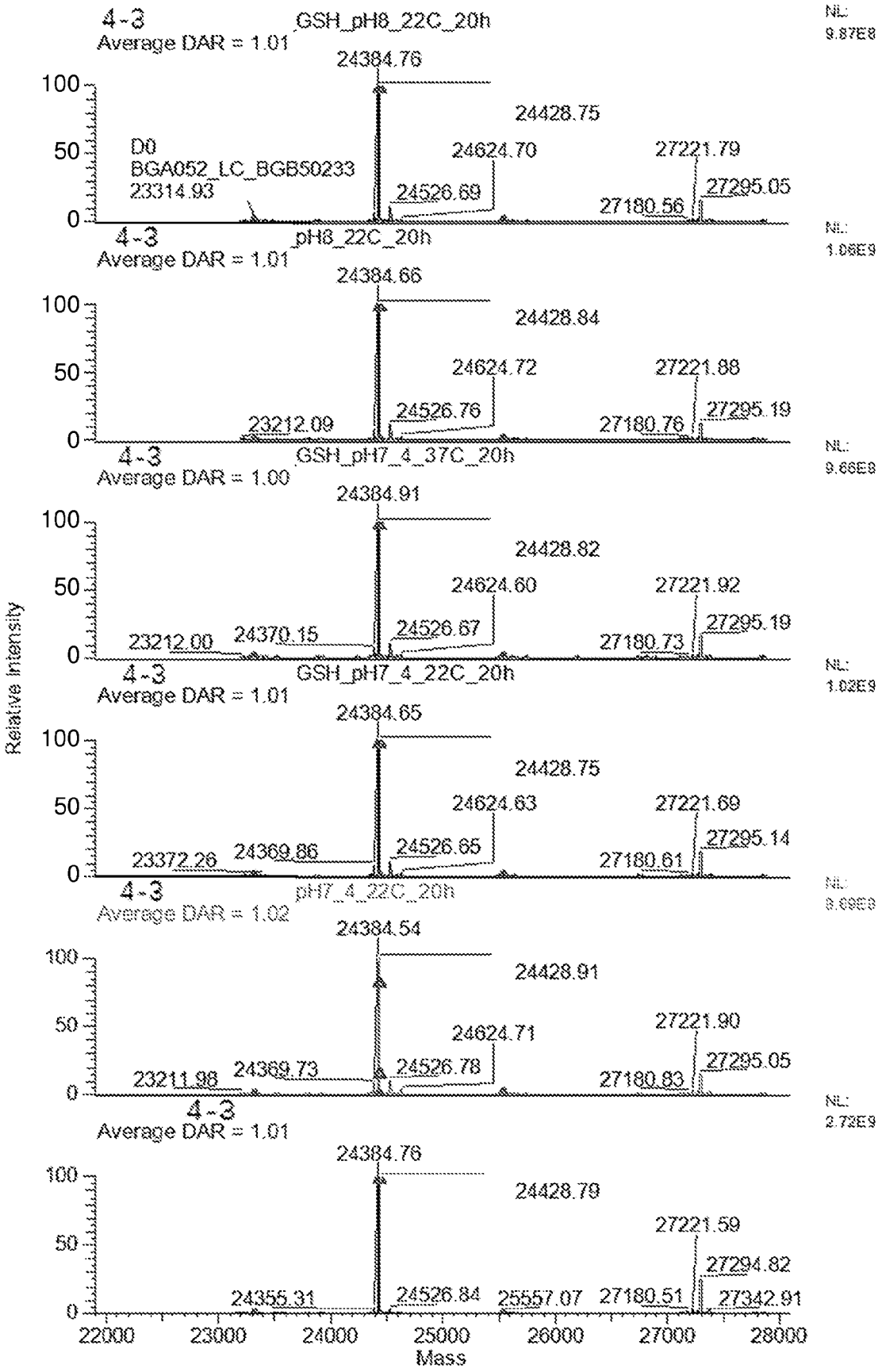

4-3
Average DAR = 1.01
GSH_pH8_22C_20h
NL:
9.87E8
24384.76
24428.75
24624.70
27221.79
27295.05
24526.69
27180.56
D0
BGA052_LC_BGB50233
23314.93
4-3
Average DAR = 1.01
pH8_22C_20h
NL:
1.08E9
24384.66
24428.84
24624.72
27221.88
27295.19
24526.76
23212.09
27180.76
4-3
Average DAR = 1.00
GSH_pH7_4_37C_20h
NL:
9.66E8
24384.91
24428.82
24624.60
27221.92
27295.19
24526.67
23212.00
24370.15
27180.73
4-3
Average DAR = 1.01
GSH_pH7_4_22C_20h
NL:
1.02E9
24384.65
24428.75
24624.63
27221.69
27295.14
24526.65
23372.26
24369.86
27180.61
4-3
Average DAR = 1.02
pH7_4_22C_20h
NL:
9.69E8
24384.54
24428.91
24624.71
27221.90
27295.05
24526.78
23211.98
24369.73
27180.83
4-3
Average DAR = 1.01
NL:
2.72E9
24384.76
24428.79
27221.59
27294.82
24526.84
24355.31
25557.07
27180.51
27342.91
Relative Intensity
100
50
0
22000
23000
24000
25000
26000
27000
28000
Mass

Fig. 13

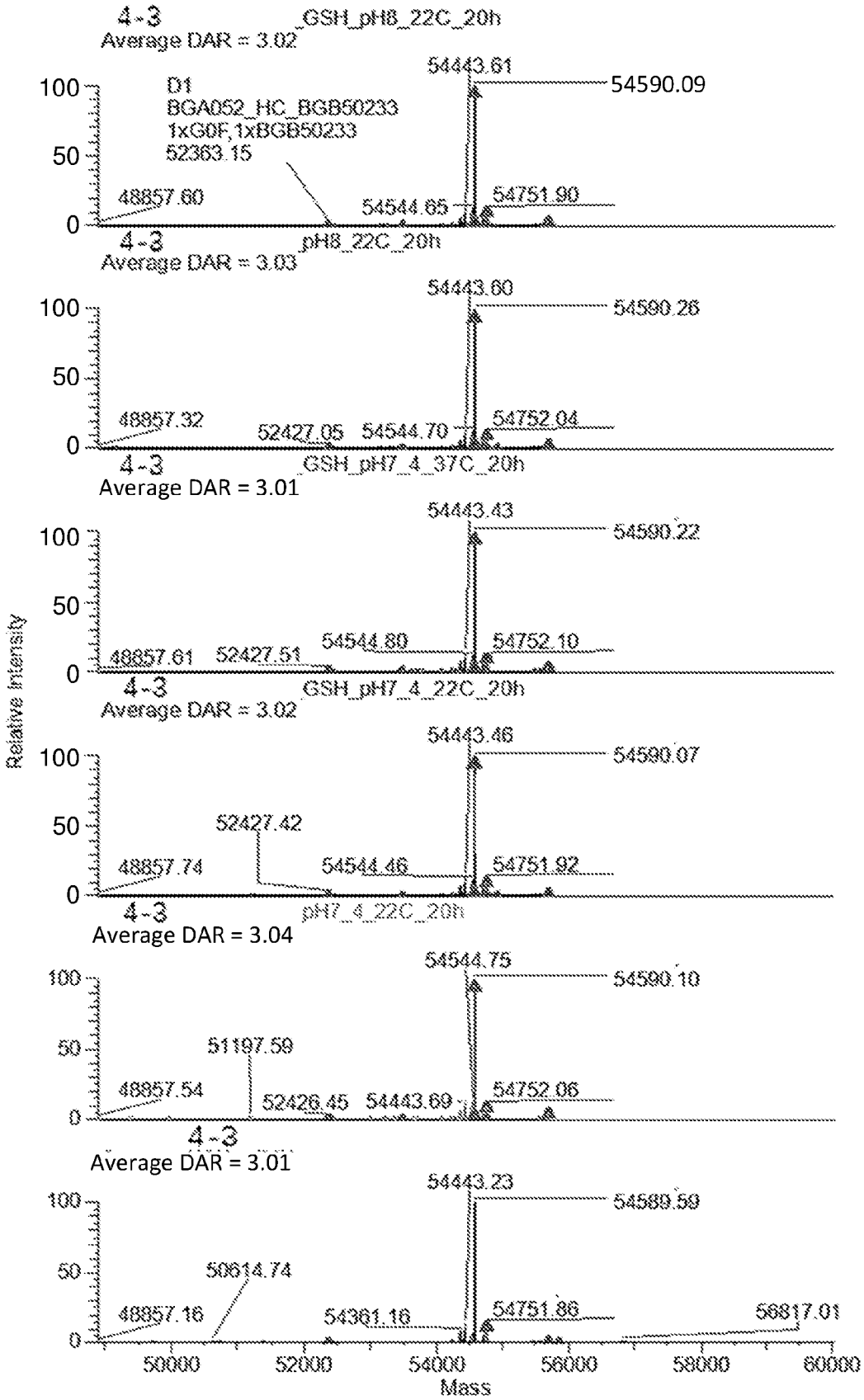

4-3 _GSH_pH8_22C_20h
Average DAR = 3.02
54443.61
54590.09
D1
BGA052_HC_BGB50233
1xG0F,1xBGB50233
52363.15
48857.60
54544.65
54751.90
4-3 pH8_22C_20h
Average DAR = 3.03
54443.60
54590.26
48857.32
52427.05
54544.70
54752.04
4-3 _GSH_pH7_4_37C_20h
Average DAR = 3.01
54443.43
54590.22
48857.61
52427.51
54544.80
54752.10
4-3 _GSH_pH7_4_22C_20h
Average DAR = 3.02
54443.46
54590.07
52427.42
48857.74
54544.46
54751.92
4-3 pH7_4_22C_20h
Average DAR = 3.04
54544.75
54590.10
51197.59
48857.54
52426.45
54443.69
54752.06
4-3
Average DAR = 3.01
54443.23
54589.59
50614.74
48857.16
54361.16
54751.86
56817.01
Relative Intensity
100
50
0
50000
52000
54000
56000
58000
60000
Mass

Fig. 13 (cont.)

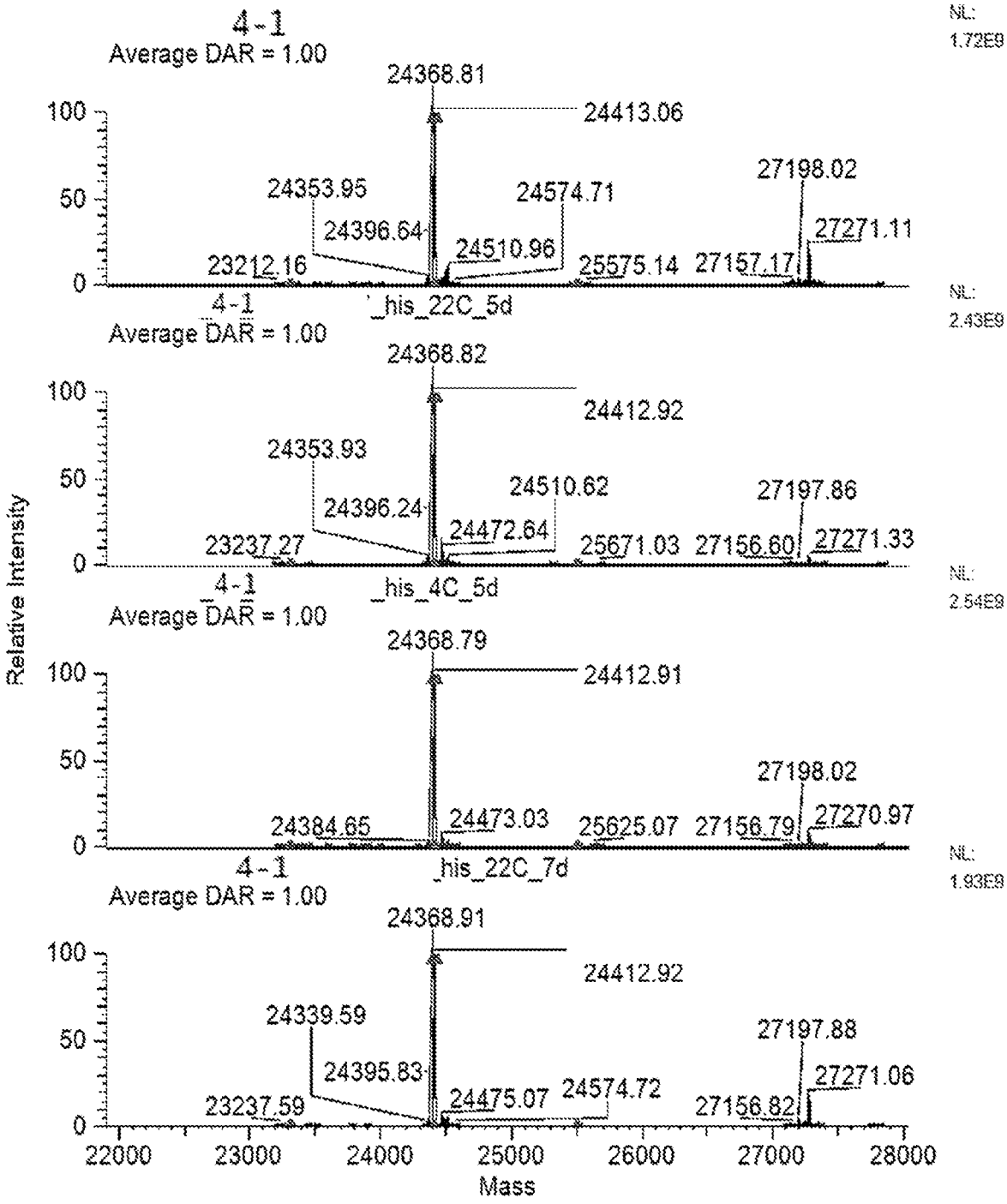

4-1
Average DAR = 1.00
24368.81
24413.06
24353.95
24574.71
27198.02
24396.64
24510.96
27271.11
23212.16
25575.14
27157.17
NL:
1.72E9
4-1
_his_22C_5d
Average DAR = 1.00
NL:
2.43E9
24368.82
24412.92
24353.93
24510.62
27197.86
24396.24
24472.64
23237.27
25671.03
27156.60
27271.33
4-1
_his_4C_5d
Average DAR = 1.00
NL:
2.54E9
24368.79
24412.91
27198.02
24384.65
24473.03
25625.07
27156.79
27270.97
4-1
_his_22C_7d
Average DAR = 1.00
NL:
1.93E9
24368.91
24412.92
24339.59
27197.88
24395.83
27271.06
23237.59
24475.07
24574.72
27156.82
Relative Intensity
100
50
0
22000
23000
24000
25000
26000
27000
28000
Mass

Fig. 14

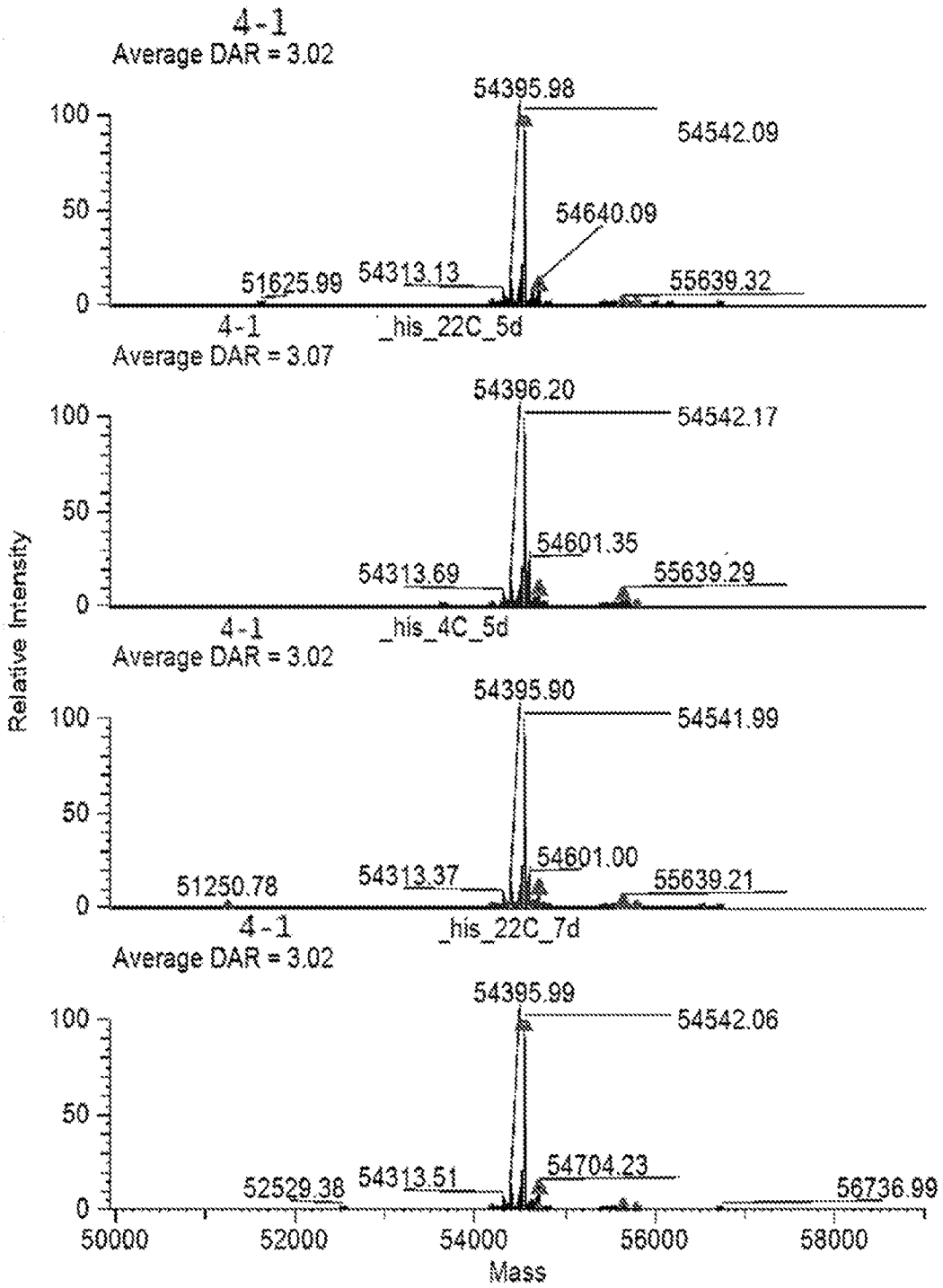

4-1
Average DAR = 3.02
54395.98
54542.09
54640.09
51625.99
54313.13
55639.32
4-1
_his_22C_5d
Average DAR = 3.07
54396.20
54542.17
54601.35
54313.69
55639.29
4-1
_his_4C_5d
Average DAR = 3.02
54395.90
54541.99
54601.00
51250.78
54313.37
55639.21
4-1
_his_22C_7d
Average DAR = 3.02
54395.99
54542.06
54704.23
52529.38
54313.51
56736.99
Relative Intensity
100
50
0
50000
52000
54000
56000
58000
Mass

Fig. 14 (cont.)

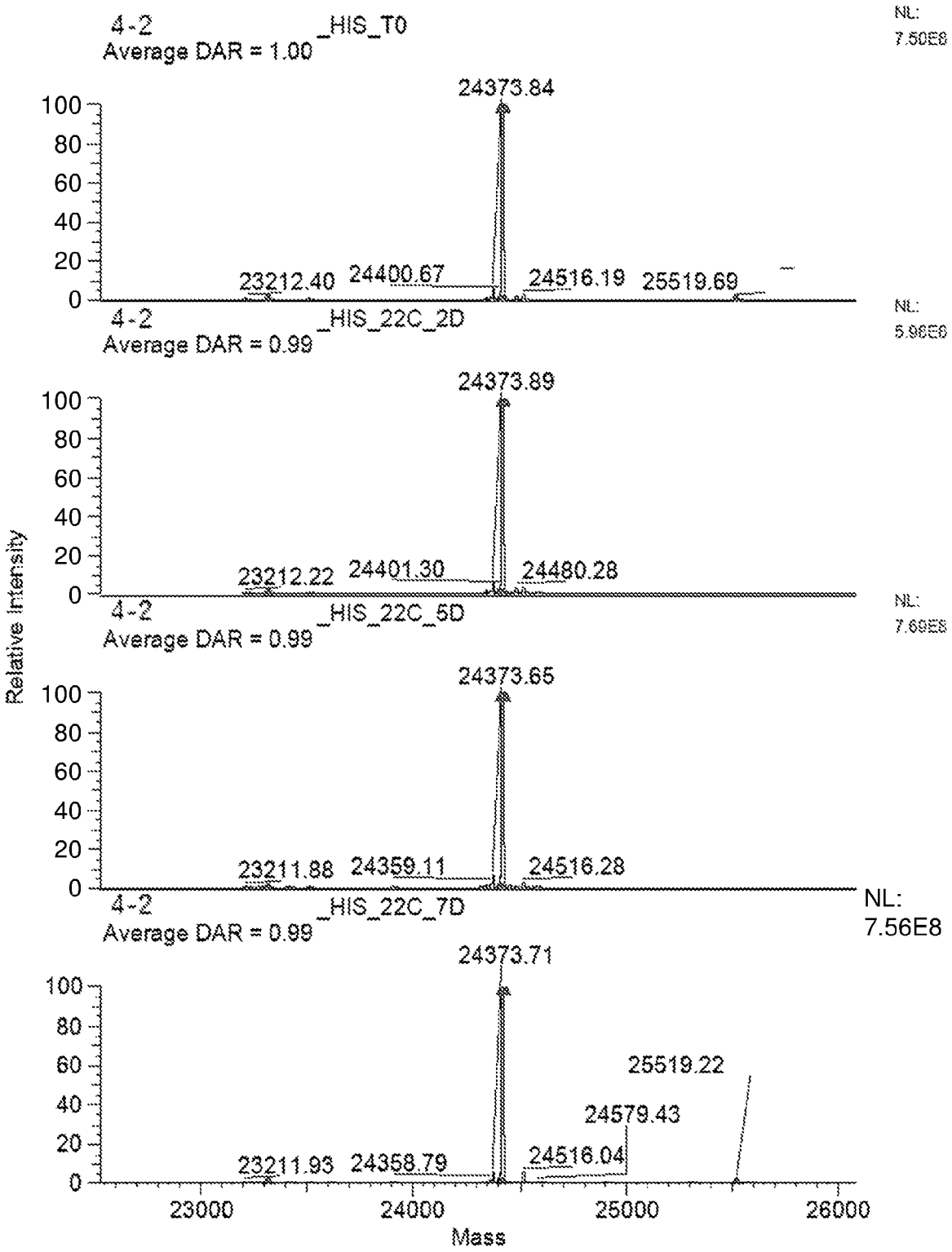

4-2 _HIS_T0
Average DAR = 1.00
NL: 7.50E8
24373.84
23212.40
24400.67
24516.19
25519.69
4-2 _HIS_22C_2D
Average DAR = 0.99
NL: 5.96E8
24373.89
23212.22
24401.30
24480.28
4-2 _HIS_22C_5D
Average DAR = 0.99
NL: 7.69E8
24373.65
23211.88
24359.11
24516.28
4-2 _HIS_22C_7D
Average DAR = 0.99
NL: 7.56E8
24373.71
25519.22
24579.43
23211.93
24358.79
24516.04
Relative Intensity
Mass
23000
24000
25000
26000

Fig. 15

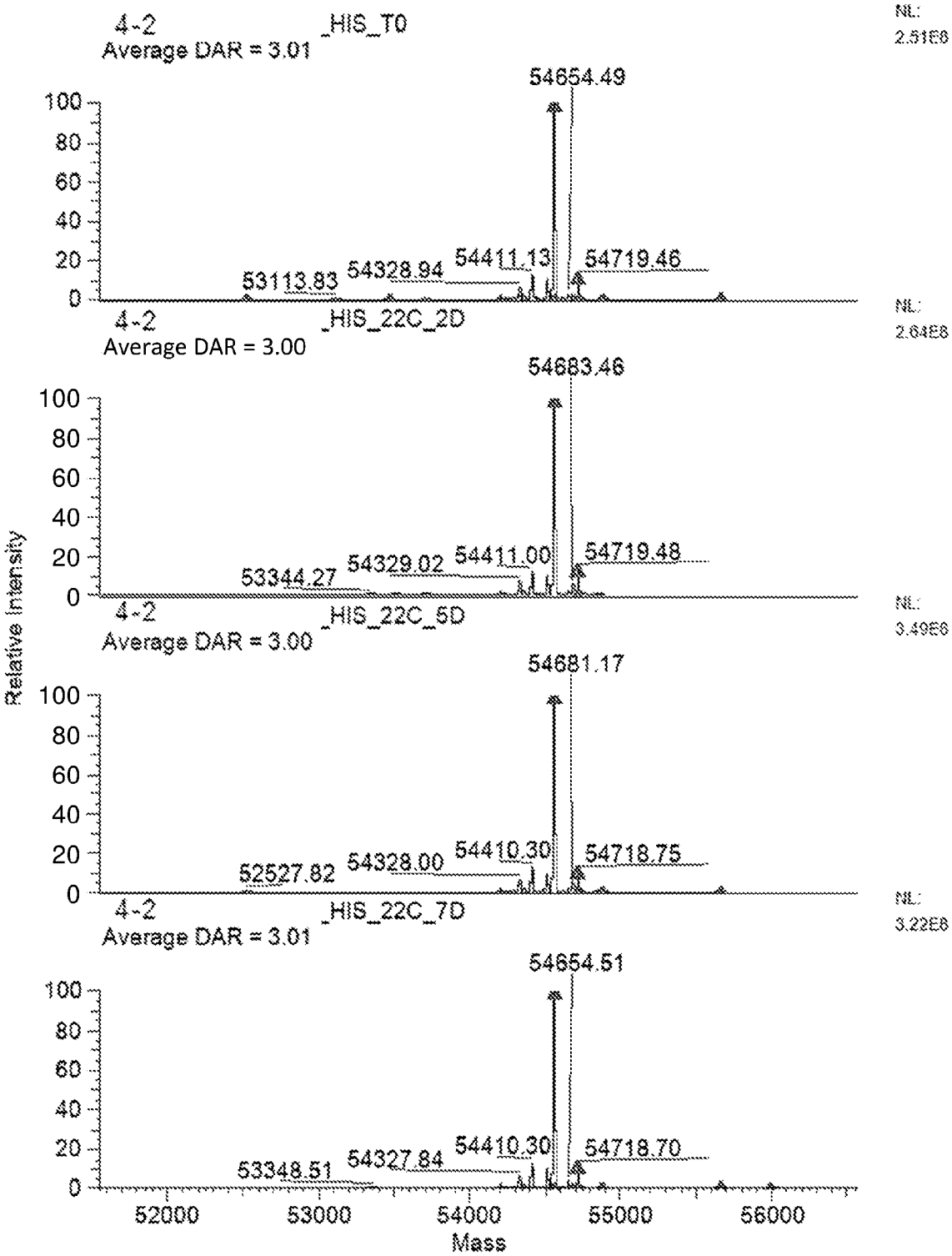

4-2 _HIS_T0
Average DAR = 3.01
54654.49
53113.83
54328.94
54411.13
54719.46
NL: 2.51E8
4-2 _HIS_22C_2D
Average DAR = 3.00
54683.46
53344.27
54329.02
54411.00
54719.48
NL: 2.64E8
4-2 _HIS_22C_5D
Average DAR = 3.00
54681.17
52527.82
54328.00
54410.30
54718.75
NL: 3.49E8
4-2 _HIS_22C_7D
Average DAR = 3.01
54654.51
53348.51
54327.84
54410.30
54718.70
NL: 3.22E8
Relative Intensity
Mass
52000
53000
54000
55000
56000

Fig. 15 (cont.)

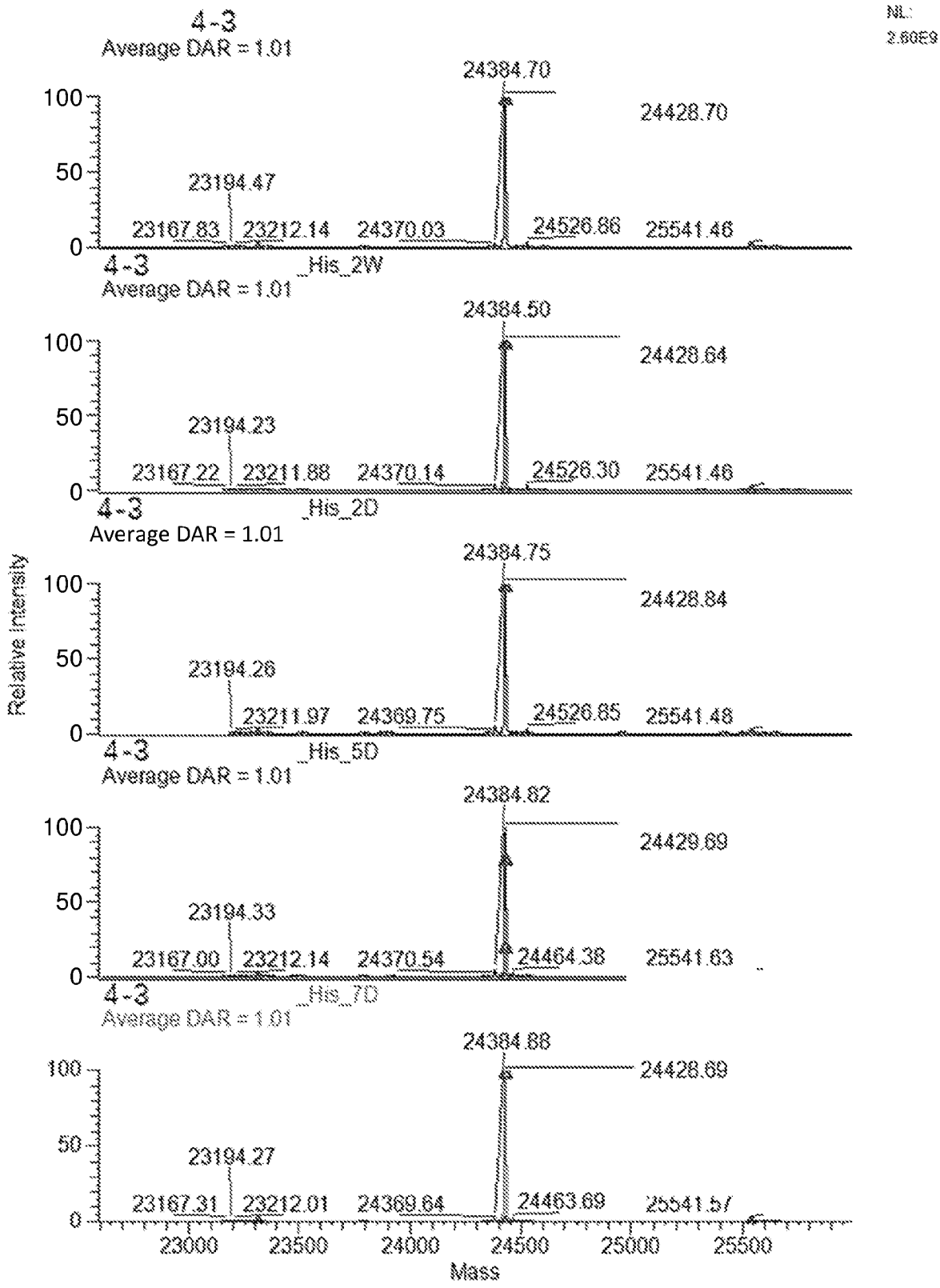

4-3
Average DAR = 1.01
NL:
2.60E9
24384.70
24428.70
23194.47
23167.83
23212.14
24370.03
24526.86
25541.46
4-3 _His_2W
Average DAR = 1.01
24384.50
24428.64
23194.23
23167.22
23211.88
24370.14
24526.30
25541.46
4-3 _His_2D
Average DAR = 1.01
24384.75
24428.84
23194.26
23211.97
24369.75
24526.85
25541.48
4-3 _His_5D
Average DAR = 1.01
24384.82
24429.69
23194.33
23167.00
23212.14
24370.54
24464.38
25541.63
4-3 _His_7D
Average DAR = 1.01
24384.88
24428.69
23194.27
23167.31
23212.01
24369.64
24463.69
25541.57
Relative Intensity
Mass
100
50
0
23000
23500
24000
24500
25000
25500

Fig. 16

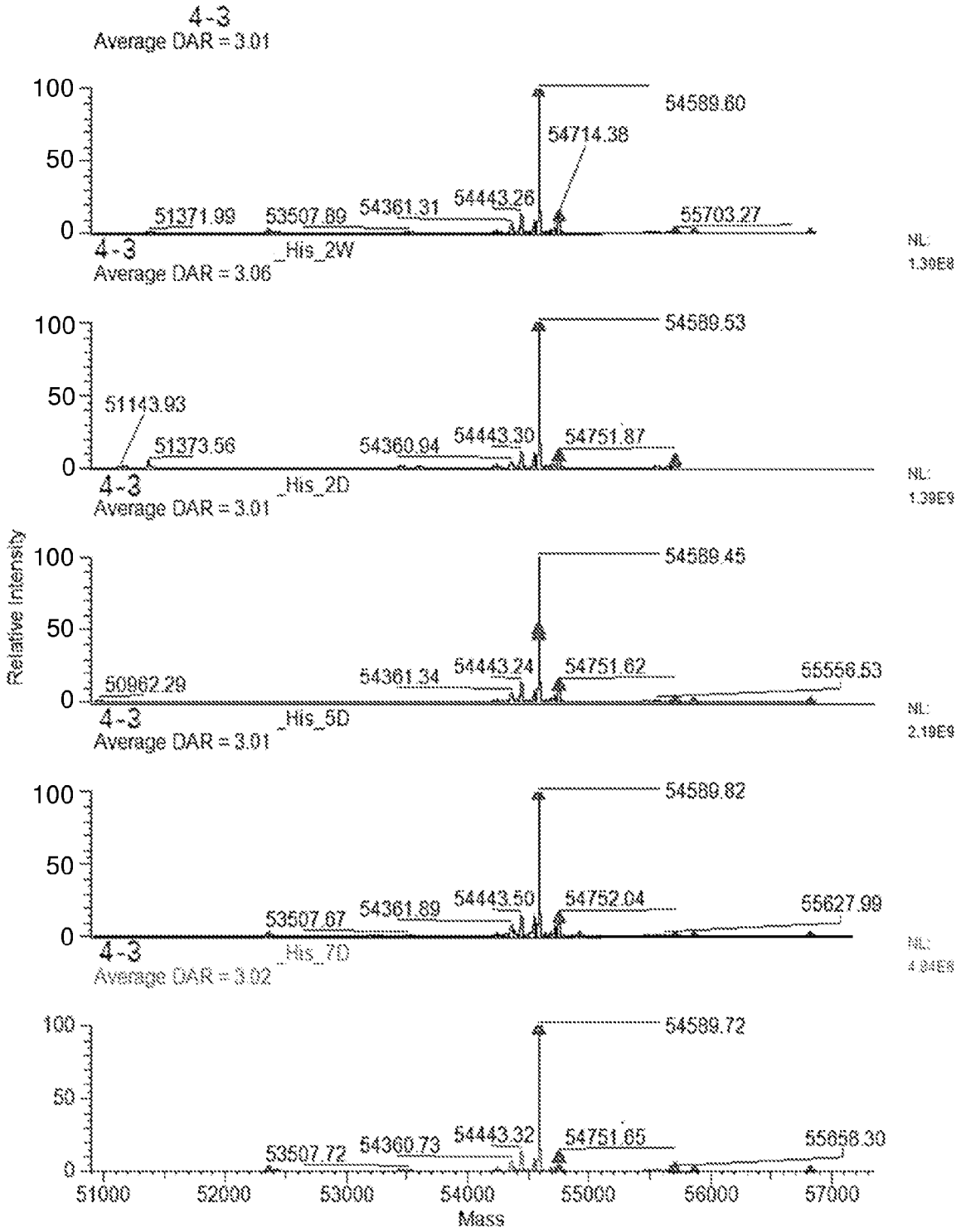

4-3
Average DAR = 3.01
54589.60
54714.38
51371.99
53507.69
54361.31
54443.26
55703.27
4-3
_His_2W
Average DAR = 3.06
54589.53
51143.93
51373.56
54360.94
54443.30
54751.87
4-3
_His_2D
Average DAR = 3.01
54589.45
50962.29
54361.34
54443.24
54751.62
55556.53
4-3
_His_5D
Average DAR = 3.01
54589.82
53507.67
54361.89
54443.50
54752.04
55627.99
4-3
_His_7D
Average DAR = 3.02
54589.72
53507.72
54360.73
54443.32
54751.65
55656.30
Relative Intensity
100
50
0
51000
52000
53000
54000
55000
56000
57000
Mass
NL:
1.39E8
NL:
1.39E8
NL:
2.19E8
NL:
4.84E8

Fig. 16 (cont.)

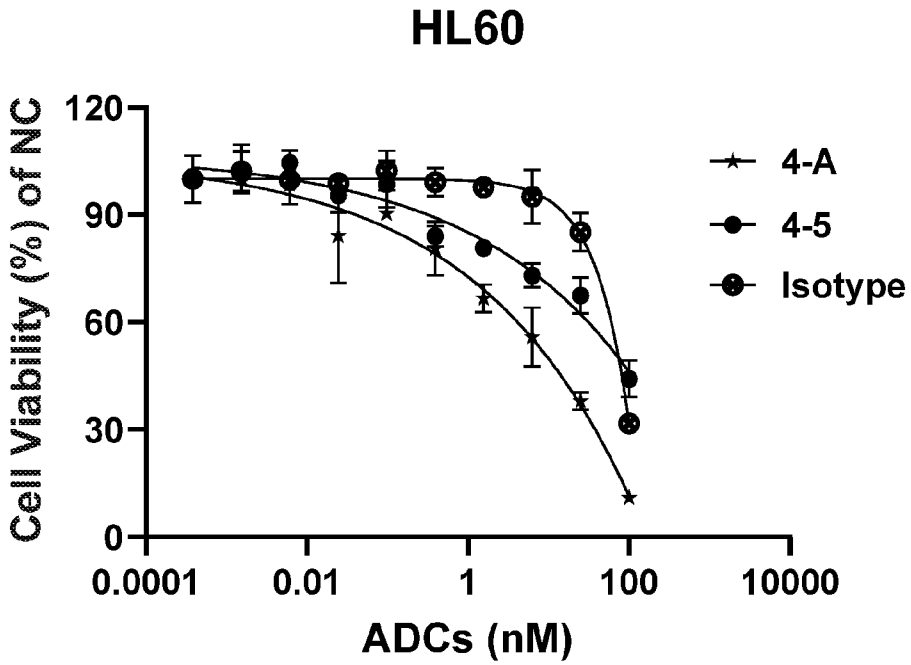

HL60
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-A
4-5
Isotype

Fig. 17

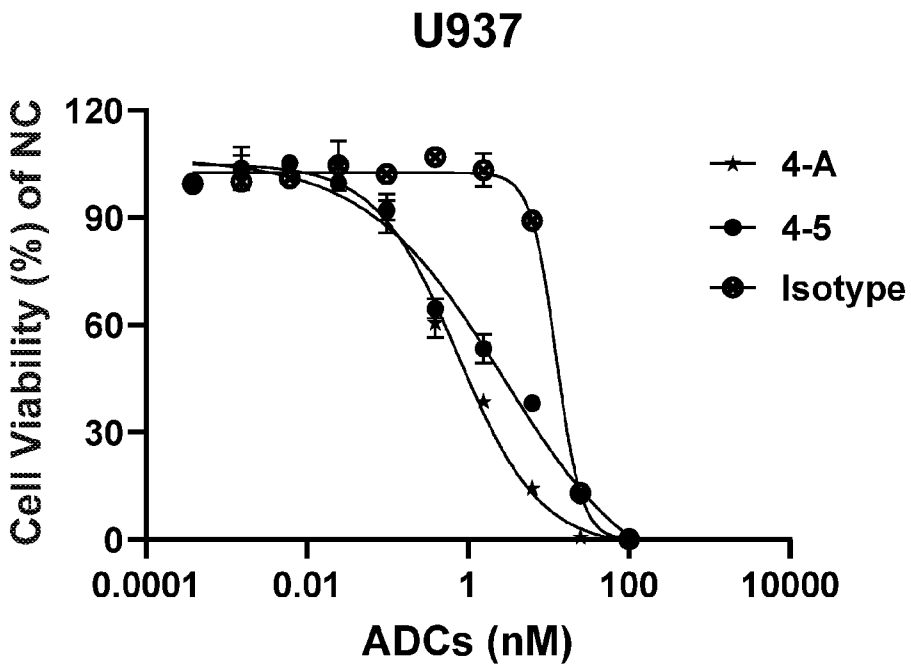

U937
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-A
4-5
Isotype

Fig. 18

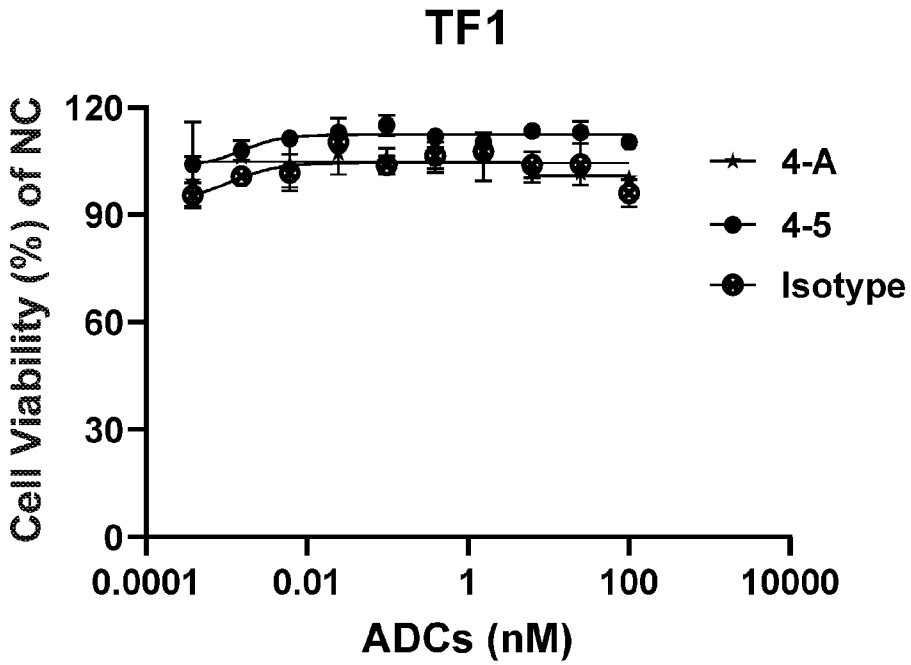

TF1
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-A
4-5
Isotype

Fig. 19

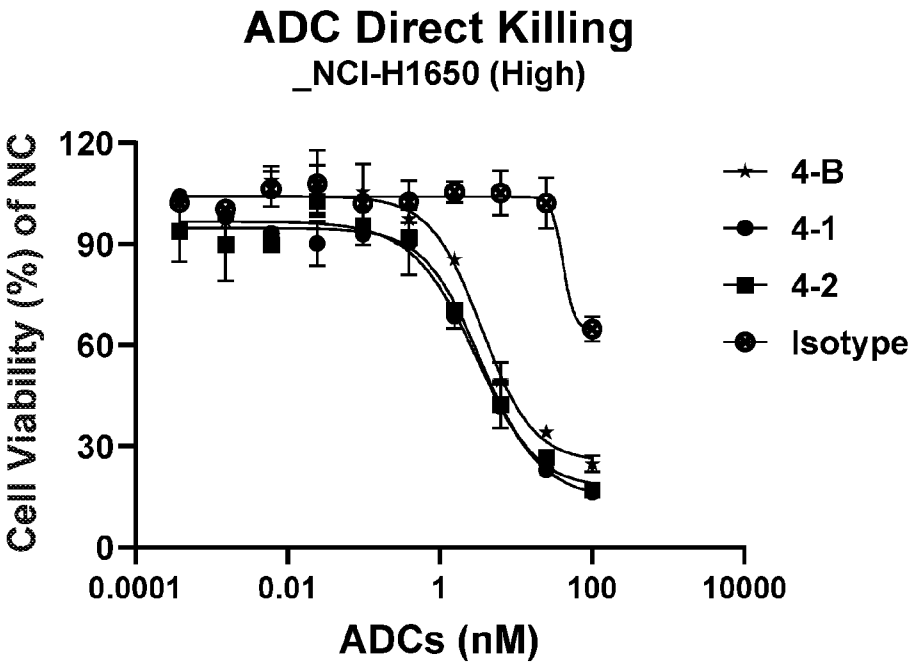

ADC Direct Killing
_NCI-H1650 (High)
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-B
4-1
4-2
Isotype

Fig. 20

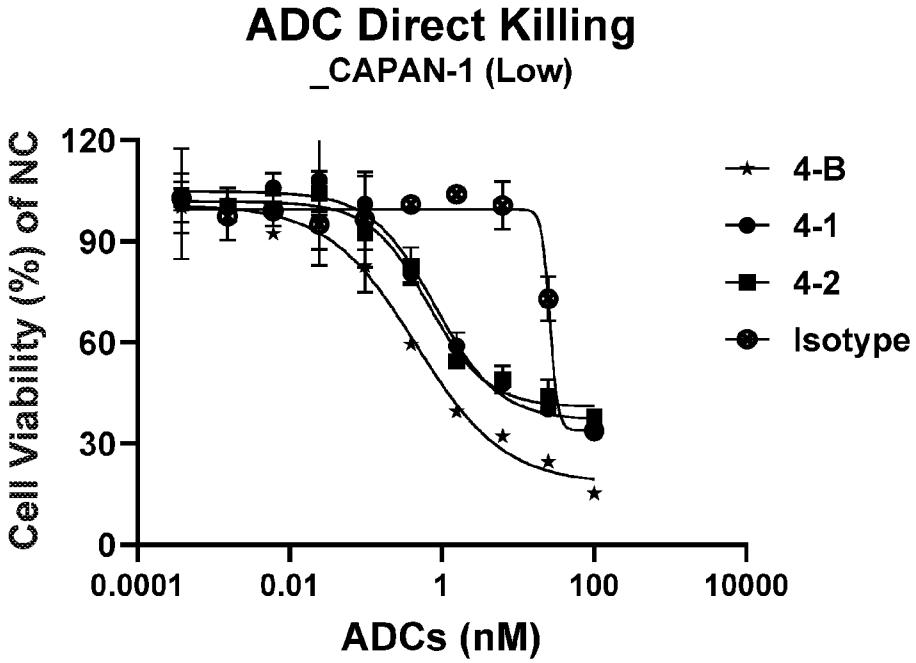

ADC Direct Killing
_CAPAN-1 (Low)
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-B
4-1
4-2
Isotype

Fig. 21

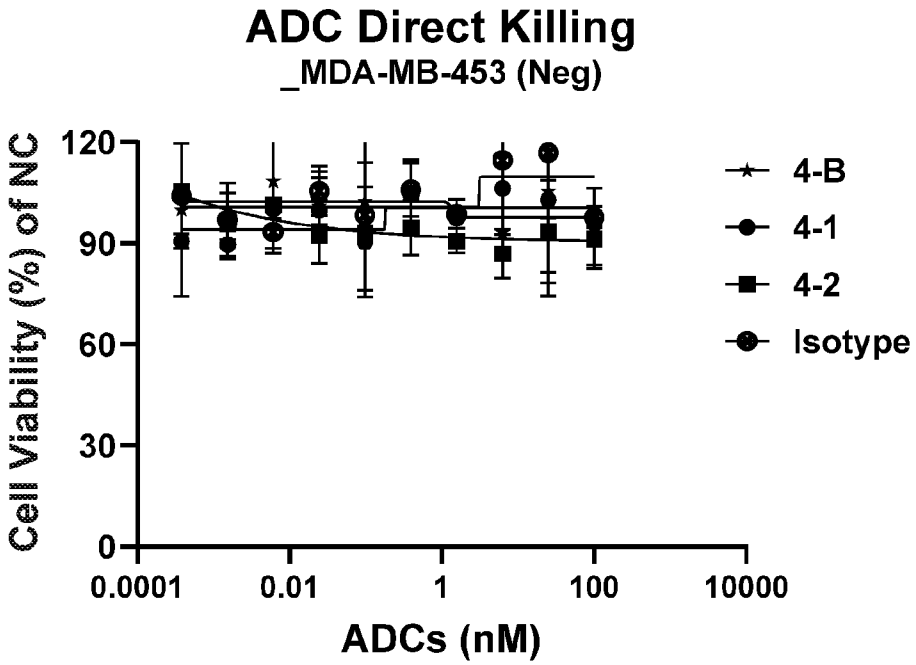

ADC Direct Killing
_MDA-MB-453 (Neg)
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-B
4-1
4-2
Isotype

Fig. 22

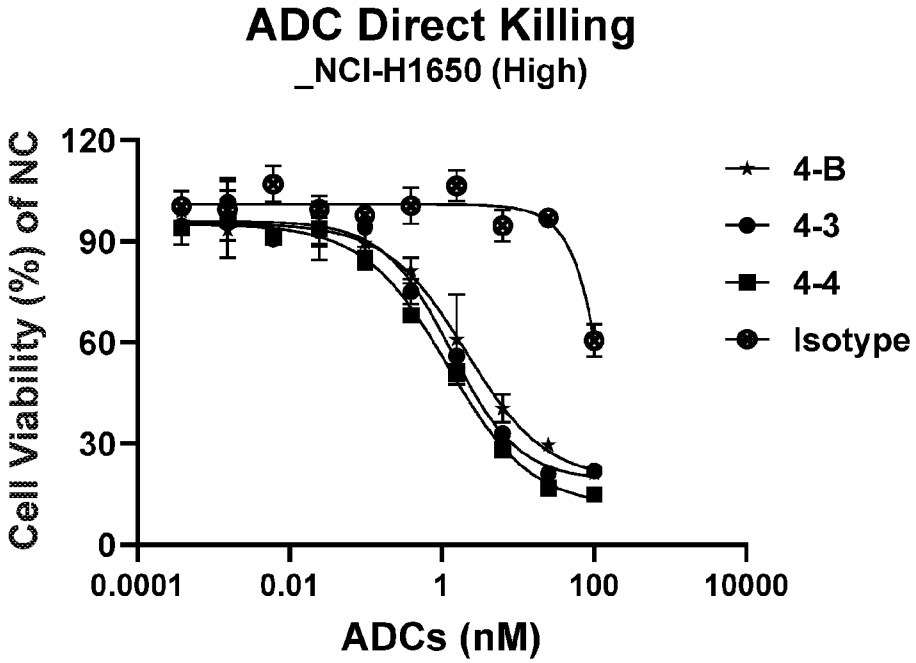

ADC Direct Killing
_NCI-H1650 (High)
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-B
4-3
4-4
Isotype

Fig. 23

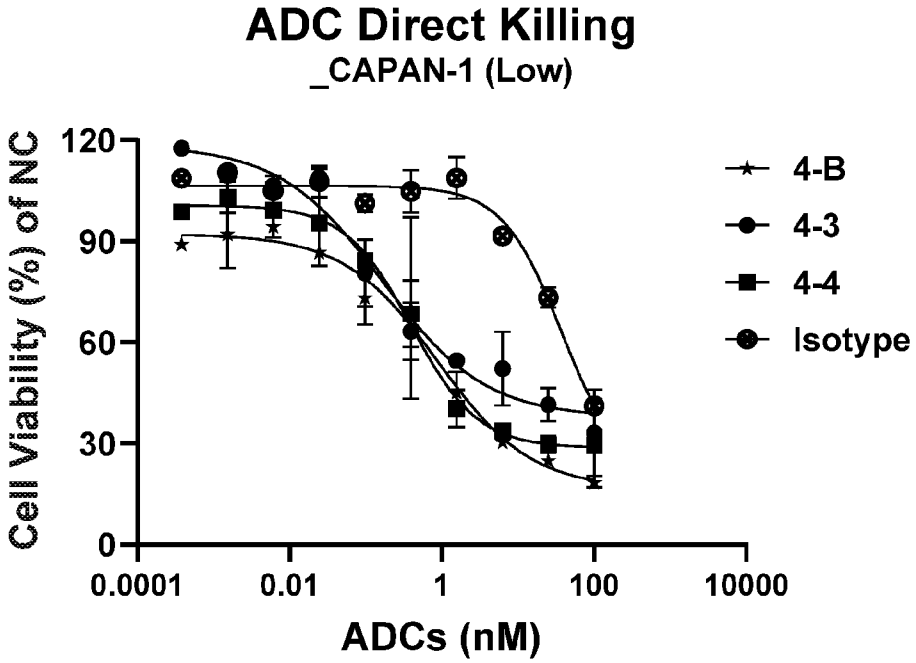

ADC Direct Killing
_CAPAN-1 (Low)
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-B
4-3
4-4
Isotype

Fig. 24

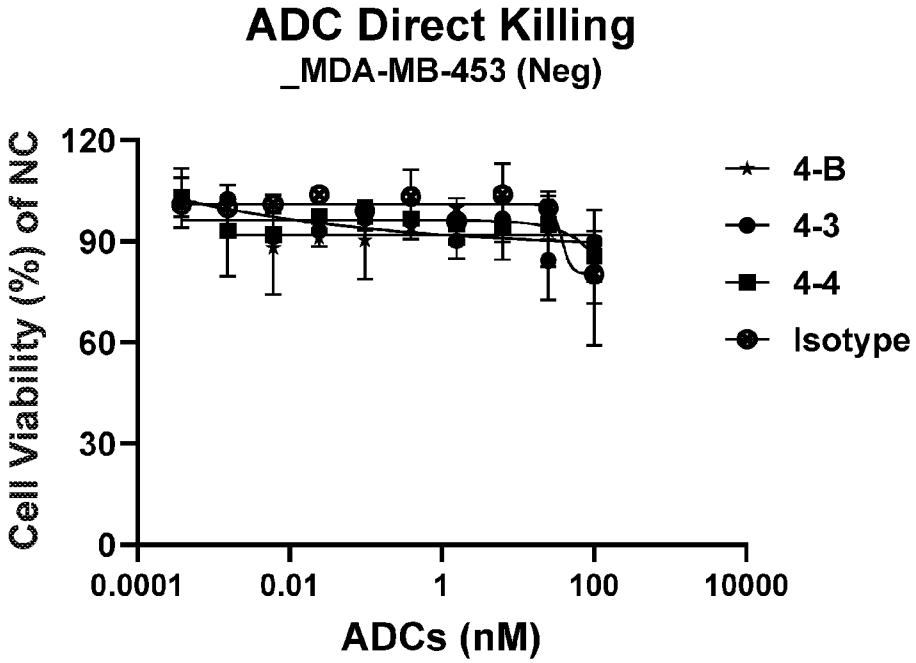

ADC Direct Killing
_MDA-MB-453 (Neg)
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-B
4-3
4-4
Isotype

Fig. 25

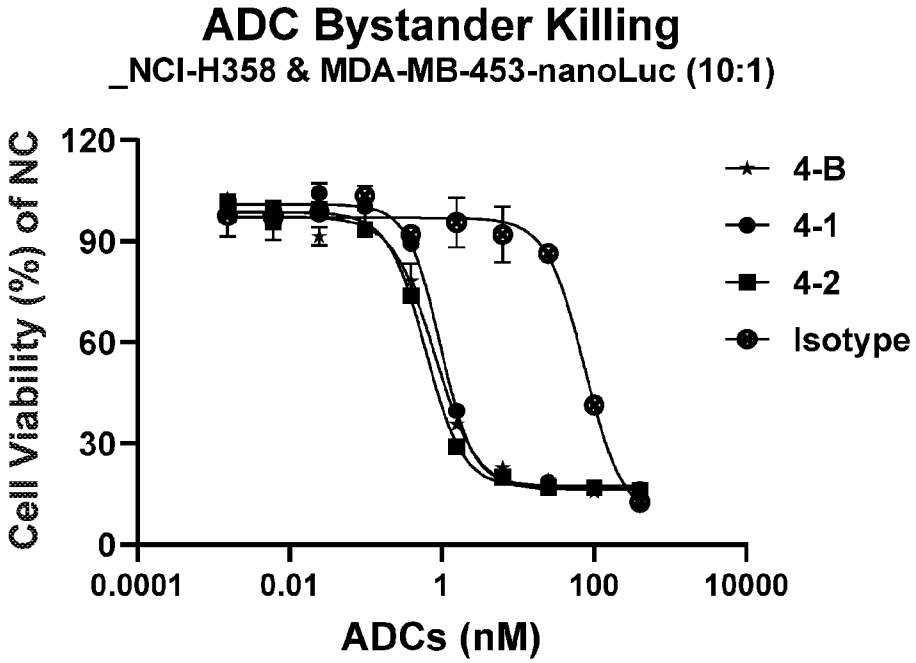

ADC Bystander Killing
_NCI-H358 & MDA-MB-453-nanoLuc (10:1)
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-B
4-1
4-2
Isotype

Fig. 26

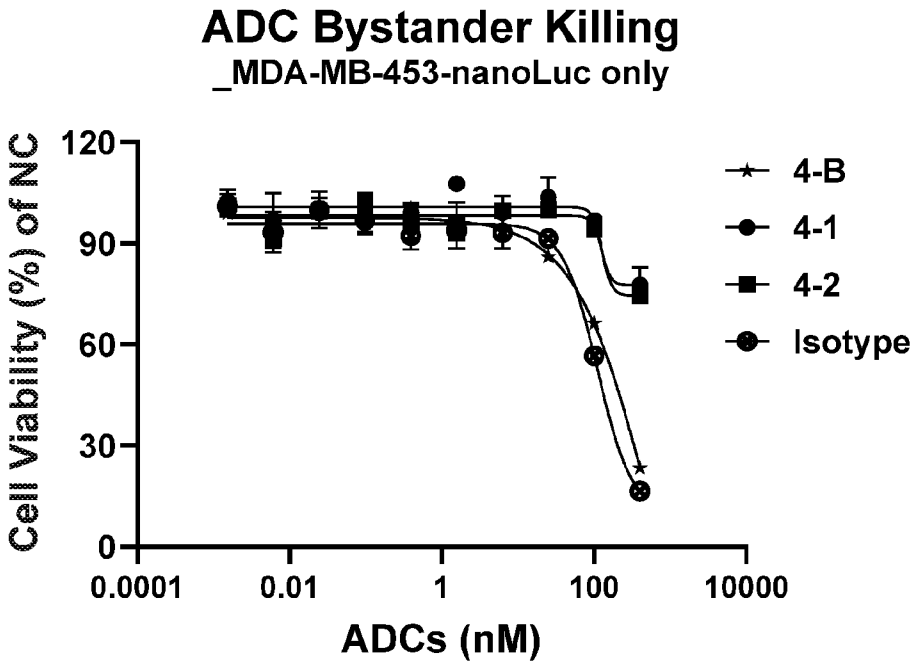

ADC Bystander Killing
_MDA-MB-453-nanoLuc only
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-B
4-1
4-2
Isotype

Fig. 27

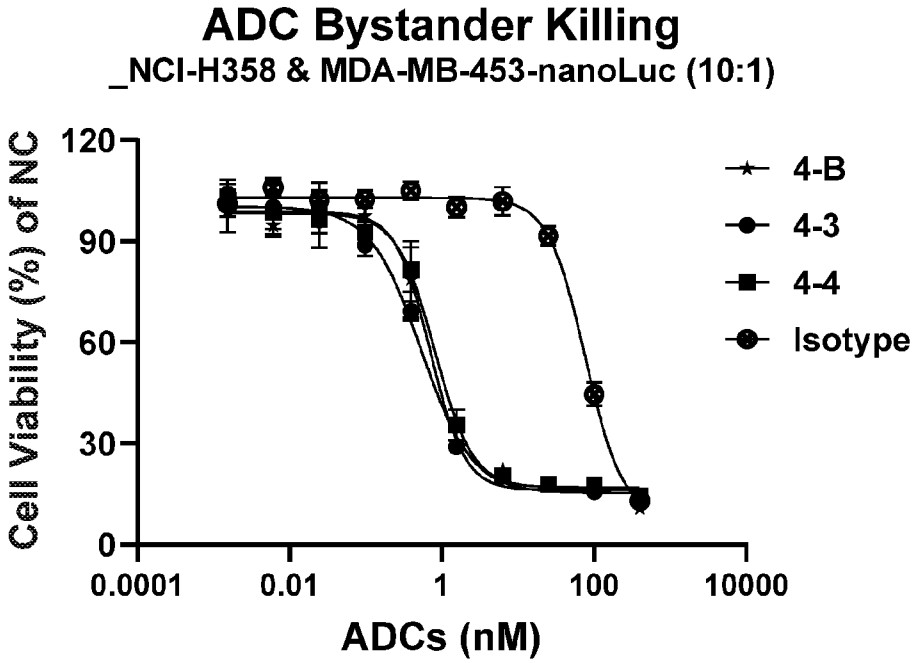

ADC Bystander Killing
_NCI-H358 & MDA-MB-453-nanoLuc (10:1)
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-B
4-3
4-4
Isotype

Fig. 28

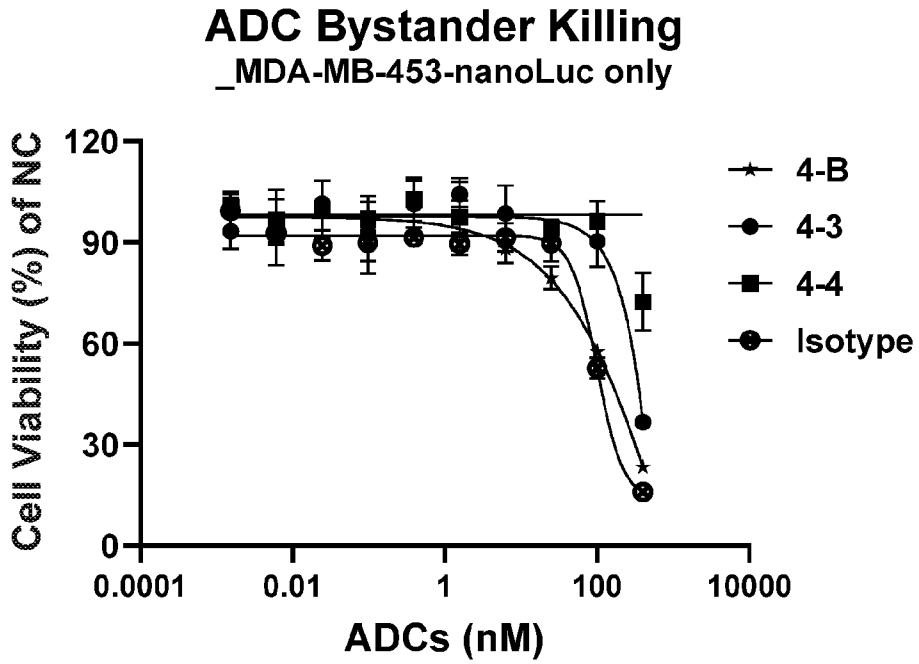

ADC Bystander Killing
_MDA-MB-453-nanoLuc only
Cell Viability (%) of NC
120
90
60
30
0
0.0001
0.01
1
100
10000
ADCs (nM)
4-B
4-3
4-4
Isotype

Fig. 29

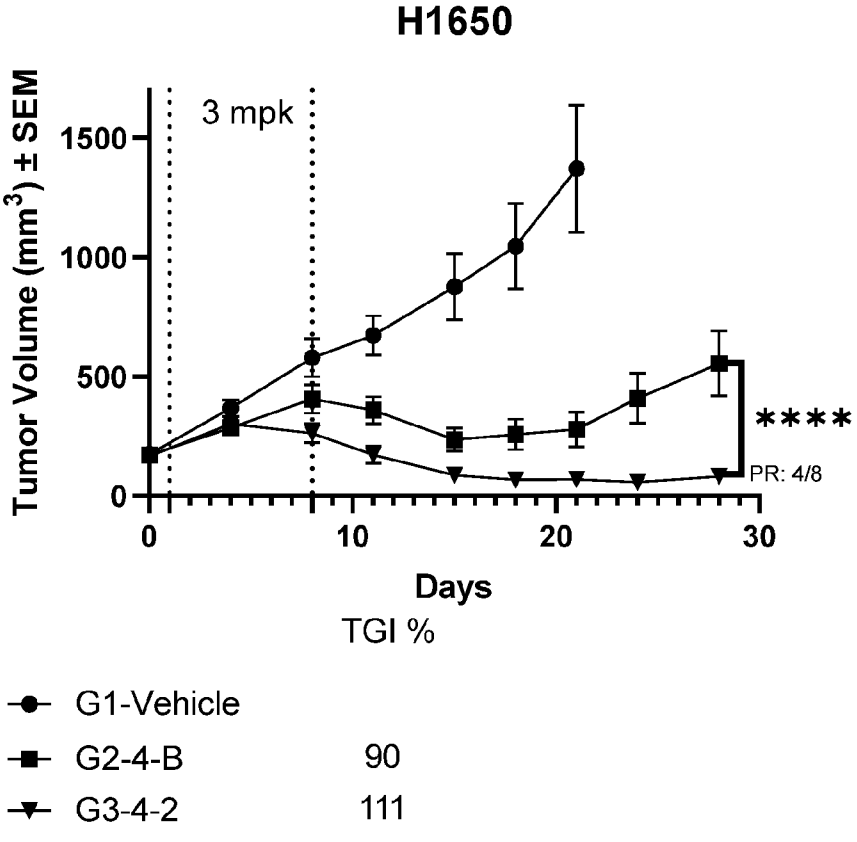

H1650
Tumor Volume (mm3) ± SEM
3 mpk
1500
1000
500
0
0
10
20
30
Days
****
PR: 4/8
TGI %
G1-Vehicle
G2-4-B 90
G3-4-2 111

Fig. 30

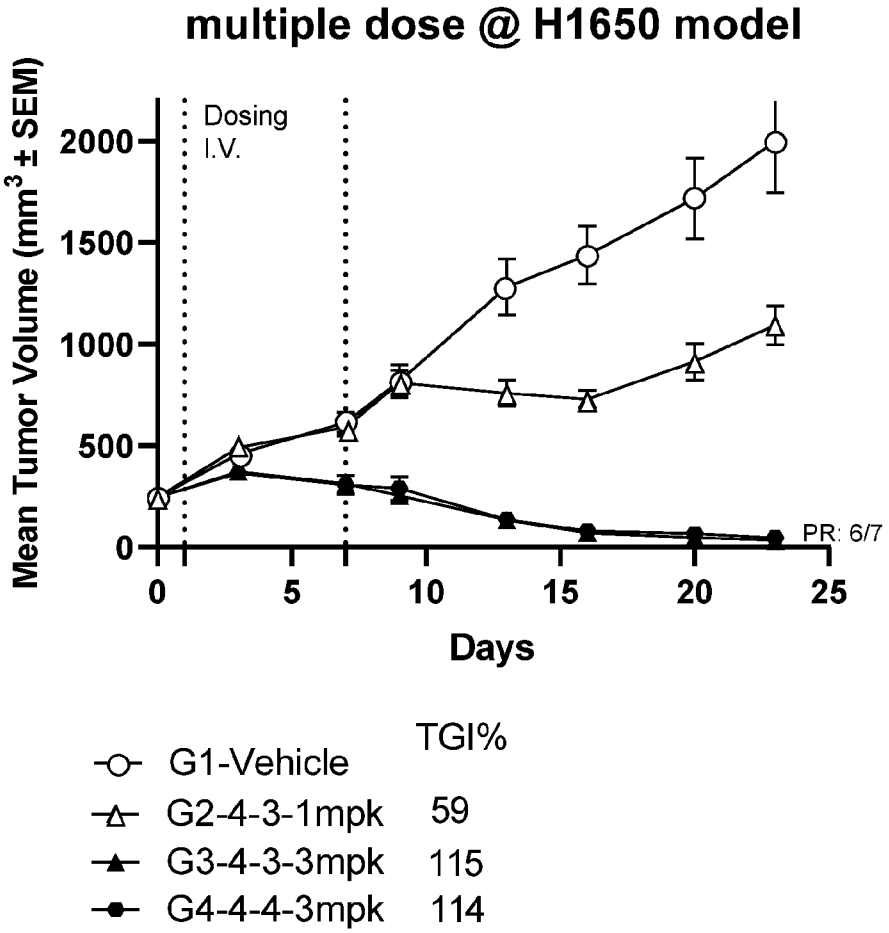

multiple dose @ H1650 model
Mean Tumor Volume (mm3 ± SEM)
2000
1500
1000
500
0
Dosing
I.V.
PR: 6/7
0
5
10
15
20
25
Days
TGI%
G1-Vehicle
G2-4-3-1mpk 59
G3-4-3-3mpk 115
G4-4-4-3mpk 114

Fig. 31

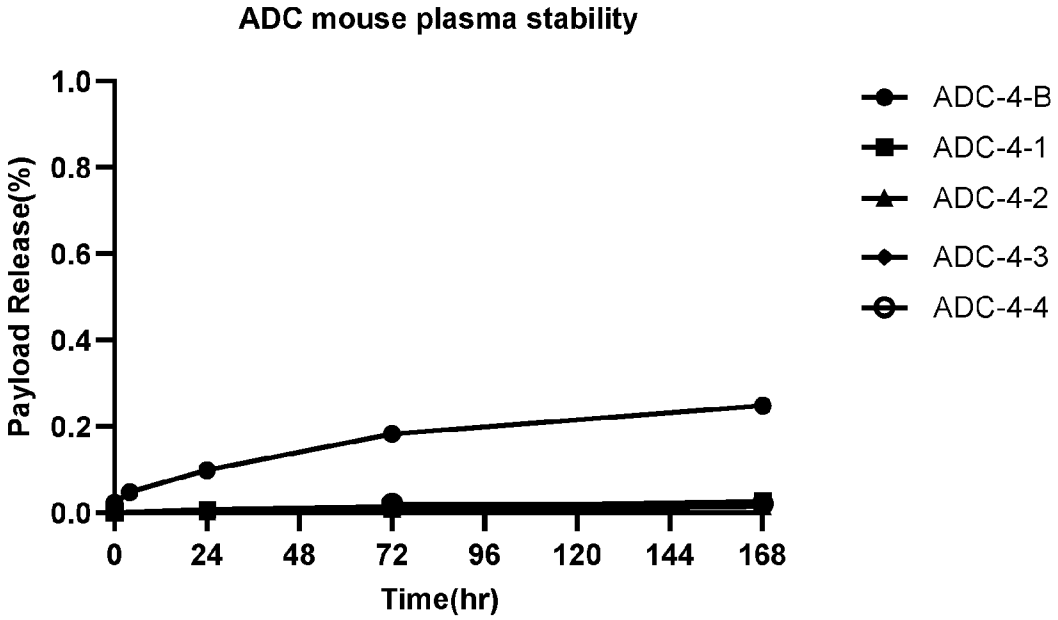

ADC mouse plasma stability
Payload Release(%)
1.0
0.8
0.6
0.4
0.2
0.0
0
24
48
72
96
120
144
168
Time(hr)
ADC-4-B
ADC-4-1
ADC-4-2
ADC-4-3
ADC-4-4

Fig. 32

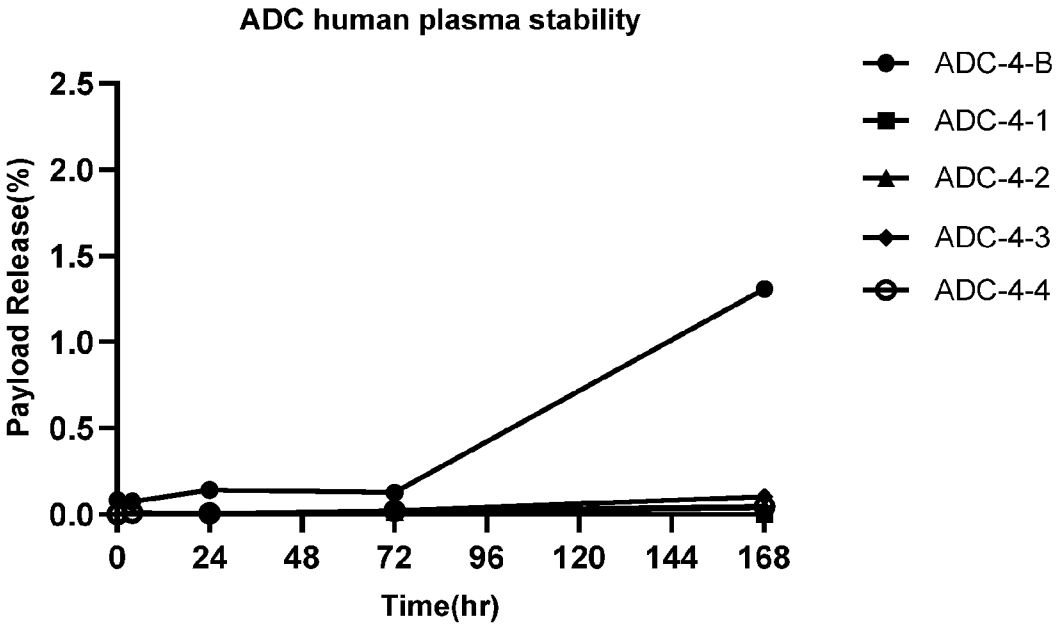

ADC human plasma stability
Payload Release(%)
2.5
2.0
1.5
1.0
0.5
0.0
0
24
48
72
96
120
144
168
Time(hr)
ADC-4-B
ADC-4-1
ADC-4-2
ADC-4-3
ADC-4-4

Fig. 33

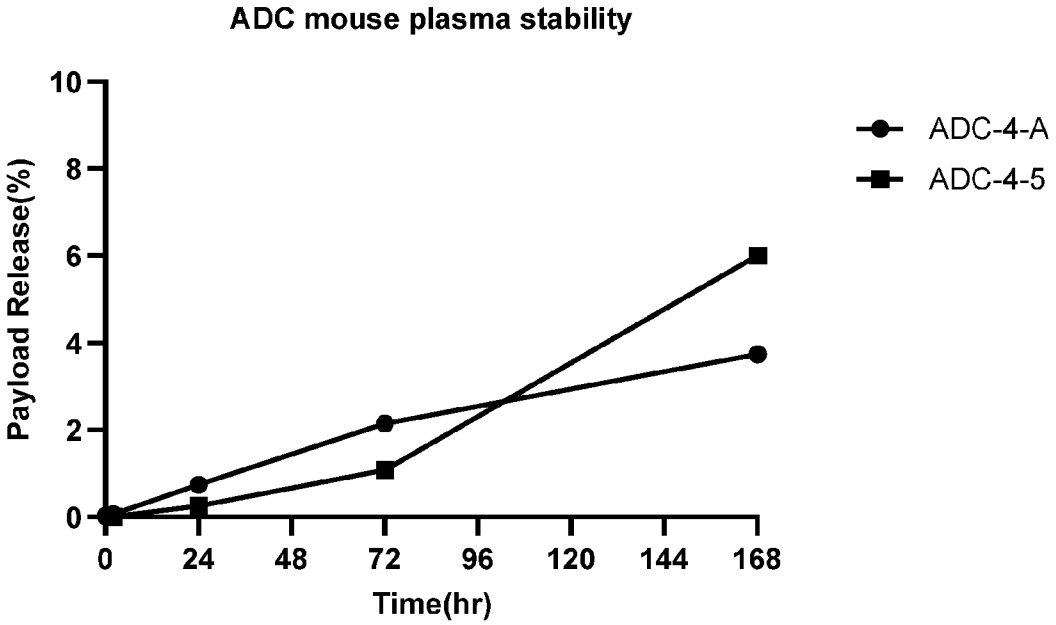

ADC mouse plasma stability
Payload Release(%)
10
8
6
4
2
0
0
24
48
72
96
120
144
168
Time(hr)
ADC-4-A
ADC-4-5

Fig. 34

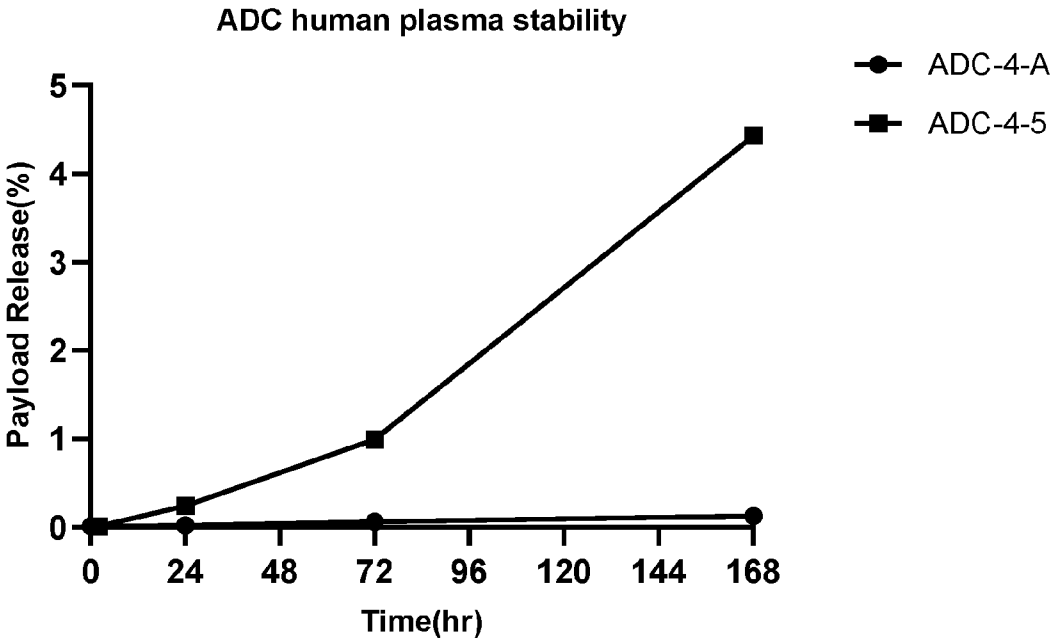

ADC human plasma stability
Payload Release(%)
5
4
3
2
1
0
0
24
48
72
96
120
144
168
Time(hr)
ADC-4-A
ADC-4-5

Fig. 35

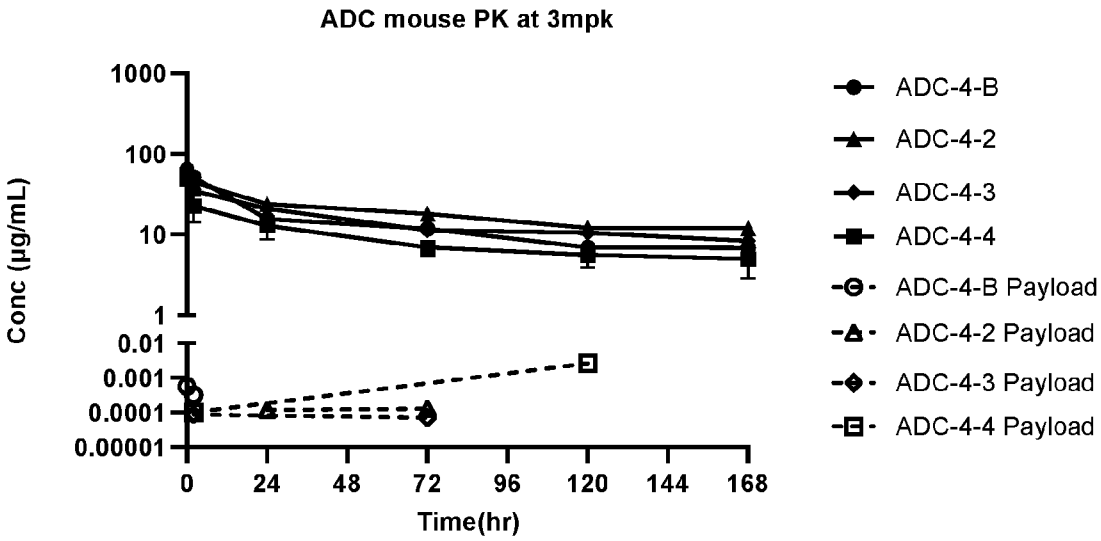

ADC mouse PK at 3mpk
Conc (µg/mL)
1000
100
10
1
0.01
0.001
0.0001
0.00001
0
24
48
72
96
120
144
168
Time(hr)
ADC-4-B
ADC-4-2
ADC-4-3
ADC-4-4
ADC-4-B Payload
ADC-4-2 Payload
ADC-4-3 Payload
ADC-4-4 Payload

Fig. 36

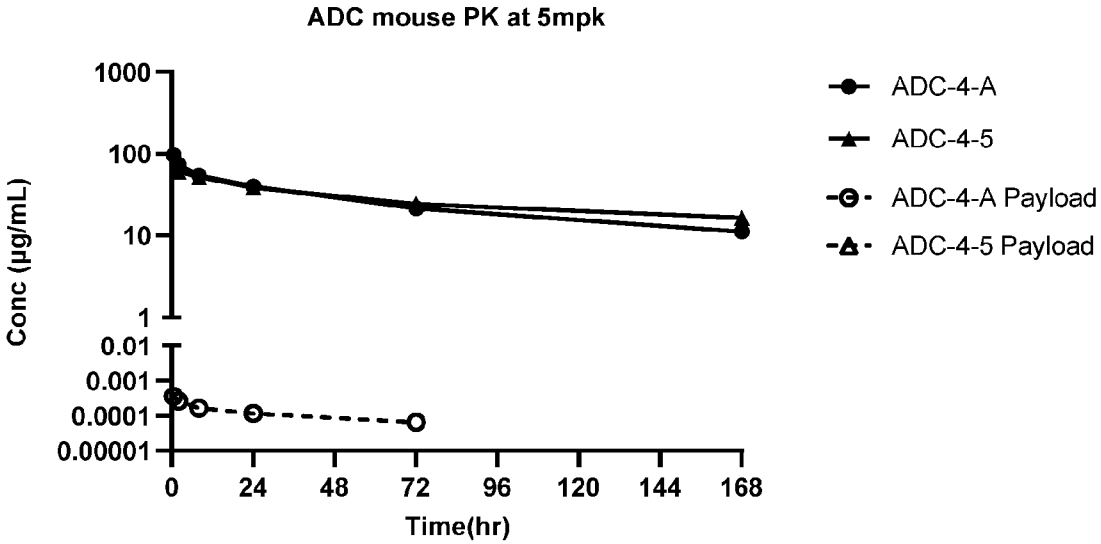

ADC mouse PK at 5mpk
Conc (µg/mL)
1000
100
10
1
0.01
0.001
0.0001
0.00001
0
24
48
72
96
120
144
168
Time(hr)
ADC-4-A
ADC-4-5
ADC-4-A Payload
ADC-4-5 Payload

Fig. 37

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/052794

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/68 A61P35/00 C07D285/125 C07D417/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07D A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2018/025168 A1 (PFIZER [US]) 8 February 2018 (2018-02-08) page 3, line 19 - page 4, line 11 page 11, line 22 - page 15, line 6 page 108, line 11 - page 118, line 10 page 6, line 5 - page 8, line 5	1-91
A	WO 2022/170971 A1 (MEDILINK THERAPEUTICS SUZHOU CO LTD [CN]) 18 August 2022 (2022-08-18) page 176 - page 339	1-91
A	EP 3 632 471 A1 (DAIICHI SANKYO CO LTD [JP]) 8 April 2020 (2020-04-08) paragraph [0332] - paragraph [0605]	1-91

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 June 2024	05/07/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Monami, Amélie

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/052794

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2024/110905 A1 (BEIGENE LTD; BEIGENE SWITZERLAND GMBH [CH]) 30 May 2024 (2024-05-30) paragraph [0357] - paragraph [0360] tables 17, 18 page 189 - page 193 paragraph [0028] paragraph [0032] paragraph [0045] paragraph [0056] -----	1-5,18, 20-24, 26-30, 35-40, 53, 55-59, 61-75, 87,89-91

1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2024/052794

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/052794

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2024/052794

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