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(54) Titre : ANTICORPS ANTI-GP73 ET IMMUNOCONJUGUES
(54) Title: ANTI-GP73 ANTIBODIES AND IMMUNOCONJUGATES

(57) **Abrégé/Abstract:**

The present invention relates to antigen-binding molecules, preferably antibodies or antigen-binding fragments thereof, that specifically bind to a GP73, or to an antigenic portion thereof, wherein the antigen-binding molecule binds to an epitope within the extracellular part of GP73 that is internalized into a cell usually subsequent to proteolytic cleavage. The invention further relates to immunoconjugates comprising the antigen-binding molecules, in particular the anti-GP73 antibodies, or antigen-binding fragments thereof. The antigen-binding molecules and immunoconjugates of the invention may be administered alone, as a therapeutic conjugate or in combination with other naked antibodies, or with therapeutic agents, or with other immunoconjugates or as a diagnostic conjugate. The present invention also relates to nucleotide sequences encoding anti GP73 antibodies, and immunoconjugates, vectors and host cells containing the nucleotide sequences, and methods of making anti-GP73-antibodies. The antigen-binding molecules, antibodies and compositions of the invention are useful in diagnostic and therapeutic applications for diseases in which expression of GP73 is altered, in particular in which GP73 is overexpressed, such as cancer.

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(57) **Abstract:** The present invention relates to antigen-binding molecules, preferably antibodies or antigen-binding fragments thereof, that specifically bind to a GP73, or to an antigenic portion thereof, wherein the antigen-binding molecule binds to an epitope within the extracellular part of GP73 that is internalized into a cell usually subsequent to proteolytic cleavage. The invention further relates to immunoconjugates comprising the antigen-binding molecules, in particular the anti-GP73 antibodies, or antigen-binding fragments thereof. The antigen-binding molecules and immunoconjugates of the invention may be administered alone, as a therapeutic conjugate or in combination with other naked antibodies, or with therapeutic agents, or with other immunoconjugates or as a diagnostic conjugate. The present invention also relates to nucleotide sequences encoding anti GP73 antibodies, and immunoconjugates, vectors and host cells containing the nucleotide sequences, and methods of making anti-GP73-antibodies. The antigen-binding molecules, antibodies and compositions of the invention are useful in diagnostic and therapeutic applications for diseases in which expression of GP73 is altered, in particular in which GP73 is overexpressed, such as cancer.

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CLAIMS

1. An antibody, or an antigen-binding fragment thereof, that specifically binds to GP73, or to an antigenic portion thereof, wherein the antibody, or the antigen-binding fragment thereof, binds to an epitope within the amino acid sequence
 - a) SSRSVDLQTRIMELEGRVRR SEQ ID NO: 30;
 - b) SSRSVELQTRIVELEGRVRR SEQ ID NO: 31;
 - c) SSRSVDLQTRIVELEGRVRR SEQ ID NO: 32; or
 - d) any combination of a) to c),

wherein the antibody, or antigen-binding fragment thereof, is internalized into a cell.
2. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the epitope is within the amino acid sequence RIMELEGRVRR (SEQ ID NO: 33).
3. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the epitope is within the amino acid sequence EGRVRR (SEQ ID NO: 34).
4. The antibody, or the antigen-binding fragment thereof, of any one of claims 1 to 3, wherein the antibody, or the antigen-binding fragment thereof, is a bispecific antibody, a designed ankyrin repeat protein (DARPIN), an aptamer or another antibody mimetic.
5. The antibody, or antigen-binding fragment thereof, of claim 3, wherein the antibody is a monoclonal antibody, a chimeric antibody, a recombinant antibody, an antigen-binding fragment of a recombinant antibody, a humanized antibody, or an antibody displayed upon the surface of a phage or displayed upon the surface of a chimeric antigen receptor (CAR) T cell.
6. The antibody, or antigen-binding fragment thereof, of claim 5, wherein the antibody is an IgG1, IgG2a or IgG2b, IgG3 or IgG4 antibody.
7. The antibody, or antigen-binding fragment thereof, of any one of claims 4 to 6, wherein the antibody comprises a variable heavy (VH) chain comprising CDR3 as defined in SEQ ID NO: 6 and a variable light (VL) chain comprising CDR3 as defined in SEQ ID NO: 9.

8. The antibody, or antigen-binding fragment thereof, of any one of claims 4 to 7, wherein the antibody
 - a) comprises a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO: 4, CDR2 as defined in SEQ ID NO: 5 and CDR3 as defined in SEQ ID NO: 6 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO: 7, CDR2 as defined in SEQ ID NO: 8 and CDR3 as defined in SEQ ID NO: 9; or
 - b) is an antibody binding to the same epitope as an antibody of (a).
9. The antibody, or antigen-binding fragment thereof, of any one of claims 4 to 8, wherein the antibody
 - a) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 35 or a sequence having 90% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 35; and
 a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 36 or a sequence having 90% sequence identity to SEQ ID NO: 3 or SEQ ID NO: 36; or
 - b) is an antibody binding to the same epitope as an antibody of (a).
10. The antibody, or antigen-binding fragment thereof, of any one of claims 4 to 8, wherein the antibody
 - a) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 35 or a sequence having 95% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 35; and
 a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 36 or a sequence having 95% sequence identity to SEQ ID NO: 3 or SEQ ID NO: 36; or
 - b) is an antibody binding to the same epitope as an antibody of (a).
11. The antibody, or antigen-binding fragment thereof, of any one of claims 4 to 6, wherein the antibody comprises a variable heavy (VH) chain comprising CDR3 as defined in SEQ ID NO: 14 and a variable light (VL) chain sequence comprising CDR3 as defined in SEQ ID NO: 17.
12. The antibody, or antigen-binding fragment thereof, of any one of claims 4 to 6

and 11, wherein the antibody

- a) comprises a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO: 12, CDR2 as defined in SEQ ID NO: 13 and CDR3 as defined in SEQ ID NO: 14 and a variable light (VL) chain sequence comprising CDR1 as defined in SEQ ID NO: 15, CDR2 as defined in SEQ ID NO: 16 and CDR3 as defined in SEQ ID NO: 17; or
 - b) is an antibody binding to the same epitope as an antibody of (a).
13. The antibody, or antigen-binding fragment thereof, of any one of claims 4 to 6, 11 and 12, wherein the antibody
- a) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 37 or a sequence having 90% sequence identity to SEQ ID NO: 10 or SEQ ID NO: 37; and
a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 38 or a sequence having 90% sequence identity to SEQ ID NO: 11 or SEQ ID NO: 38; or
 - b) is an antibody binding to the same epitope as an antibody of (a).
14. The antibody, or antigen-binding fragment thereof, of any one of claims 4 to 6, 11 and 12, wherein the antibody
- a) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 37 or a sequence having 95% sequence identity to SEQ ID NO: 10 or SEQ ID NO: 37; and
a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 38 or a sequence having 95% sequence identity to SEQ ID NO: 11 or SEQ ID NO: 38; or
 - b) is an antibody binding to the same epitope as an antibody of (a).
15. The antibody, or antigen-binding fragment thereof, of claim 4, wherein the antigen-binding fragment is a Fab fragment, a F(ab')₂ fragment or a Fv fragment.
16. A polynucleotide that encodes at least one of a variable heavy (VH) chain sequence, a variable light (VL) chain sequence or a VH and a VL chain sequences of an antibody that specifically binds to GP73, or to an antigenic

portion thereof, wherein the antibody binds to an epitope within the amino acid sequence

- a) SSRSVDLQTRIMELEGRVRR SEQ ID NO: 30;
- b) SSRSVELQTRIVELEGRVRR SEQ ID NO: 31;
- c) SSRSVDLQTRIVELEGRVRR SEQ ID NO: 32; or
- d) any combination of a) to c),

wherein the antibody is internalized into a cell.

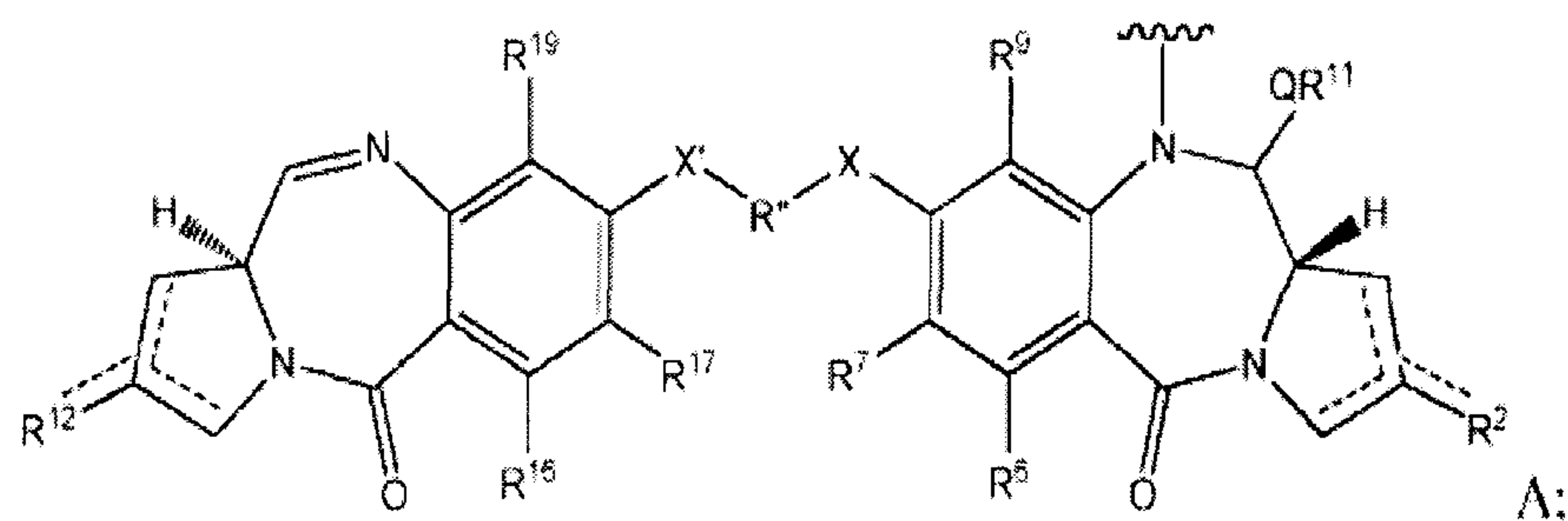
- 17. A host cell comprising the polynucleotide defined in claim 16.
- 18. A method for producing an antibody comprising culturing the host cell defined in claim 17.
- 19. A method for producing an antibody that specifically binds to a polypeptide or to an antigenic portion thereof, comprising administering to a subject a polypeptide that is

- a) SSRSVDLQTRIMELEGRVRR SEQ ID NO: 30;
- b) SSRSVELQTRIVELEGRVRR SEQ ID NO: 31; or
- c) SSRSVDLQTRIVELEGRVRR SEQ ID NO: 32,

wherein the antibody is internalized into a cell.

- 20. An immunoconjugate comprising the antibody, or the antigen-binding fragment, defined in any one of claims 1 to 15, and a cytotoxic agent or a prodrug of a cytotoxic agent.
- 21. The immunoconjugate of claim 20 having the formula Ab(-L-D)_p, wherein:
 - a) Ab is the antibody defined in any one of claims 1 to 15;
 - b) L is a linker;
 - c) D is a cytotoxic agent; and
 - d) p ranges from 1-8.
- 22. The immunoconjugate of claim 20 or 21, wherein the cytotoxic agent is a maytansinoid, a calicheamicin, a pyrrolobenzodiazepine or a nemorubicin derivative.

- 23. The immunoconjugate of claim 21, wherein D is a pyrrolobenzodiazepine of Formula A:



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

the wavy line indicates the covalent attachment site to the linker; and
the dotted lines indicate the optional presence of a double bond;

R^2 and R^{12} are each independently $-H$, $-OH$, $=O$, $=CH_2$, $-CN$, $-R$, $-OR$, $=CH-R^D$, $=C(R^D)_2$, $-O-SO_2-R$, $-CO_2R$, $-COR$, or $-halogen$,

wherein R^D is independently $-H$, $-CO_2R$, $-C(O)R$, CHO , CO_2H , $-R$, $-OH$, $-OR$, $-SH$, $-SR$, $-NH_2$, $-NHR$, $-NRR'$, $-NO_2$, Me_3Sn- or $-halogen$;

R^6 , R^9 , R^{16} and R^{19} are independently $-H$, $-R$, $-OH$, $-OR$, $-SH$, $-SR$, $-NH_2$, $-NHR$, $-NRR'$, $-NO_2$, Me_3Sn- or $-halogen$;

R^7 and R^{17} are independently $-H$, $-R$, $-OH$, $-OR$, $-SH$, $-SR$, $-NH_2$, $-NHR$, $-NRR'$, $-NO_2$, Me_3Sn- or $-halogen$;

Q is independently $-O-$, $-S-$ or $-N(H)-$;

R^{11} is either $-H$ or $-R$ or, in the case where Q is $-O-$, R^{11} may be $-SO_3M$, wherein M is an alkali metal or alkaline earth metal cation;

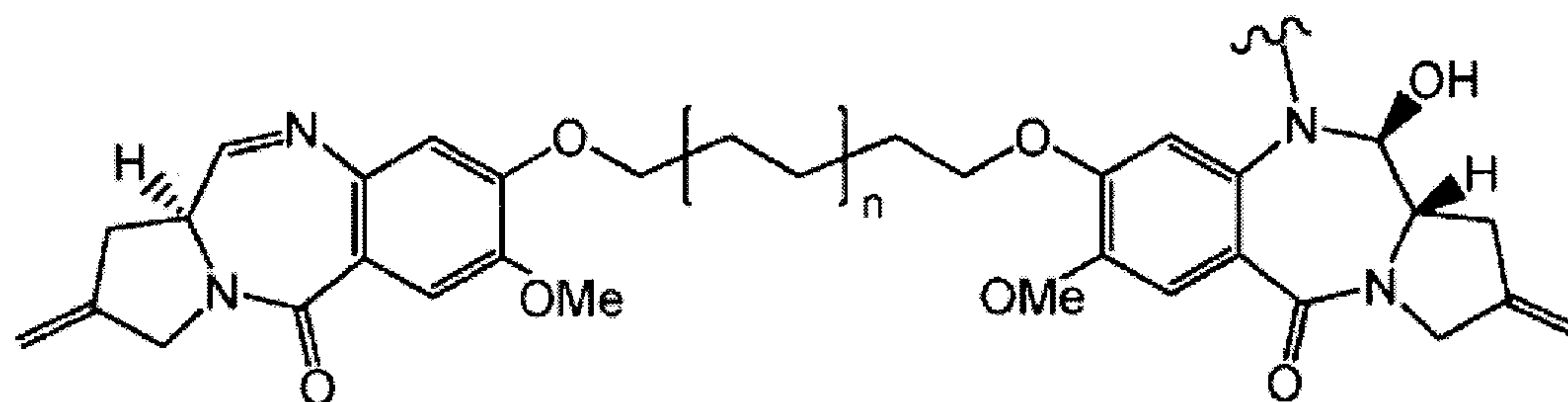
R and R' are each independently optionally substituted C_{1-12} alkyl, C_{3-8} heterocyclyl, C_{3-20} heterocycle or C_{5-20} aryl groups, or, if R and R' are bound to the same nitrogen atom, R and R' may form, together with the nitrogen atom to which they are attached, an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

R'' is a C_{3-12} alkylene group, in which one or more carbon atoms may be replaced by a heteroatom, that are independently O , NH or S , or by an aromatic ring that is optionally substituted or by a heteroatom and an aromatic ring that is optionally substituted,

wherein the aromatic rings comprise 5 or 6 carbon atoms and one or two heteroatoms that independently are N or NH ; and

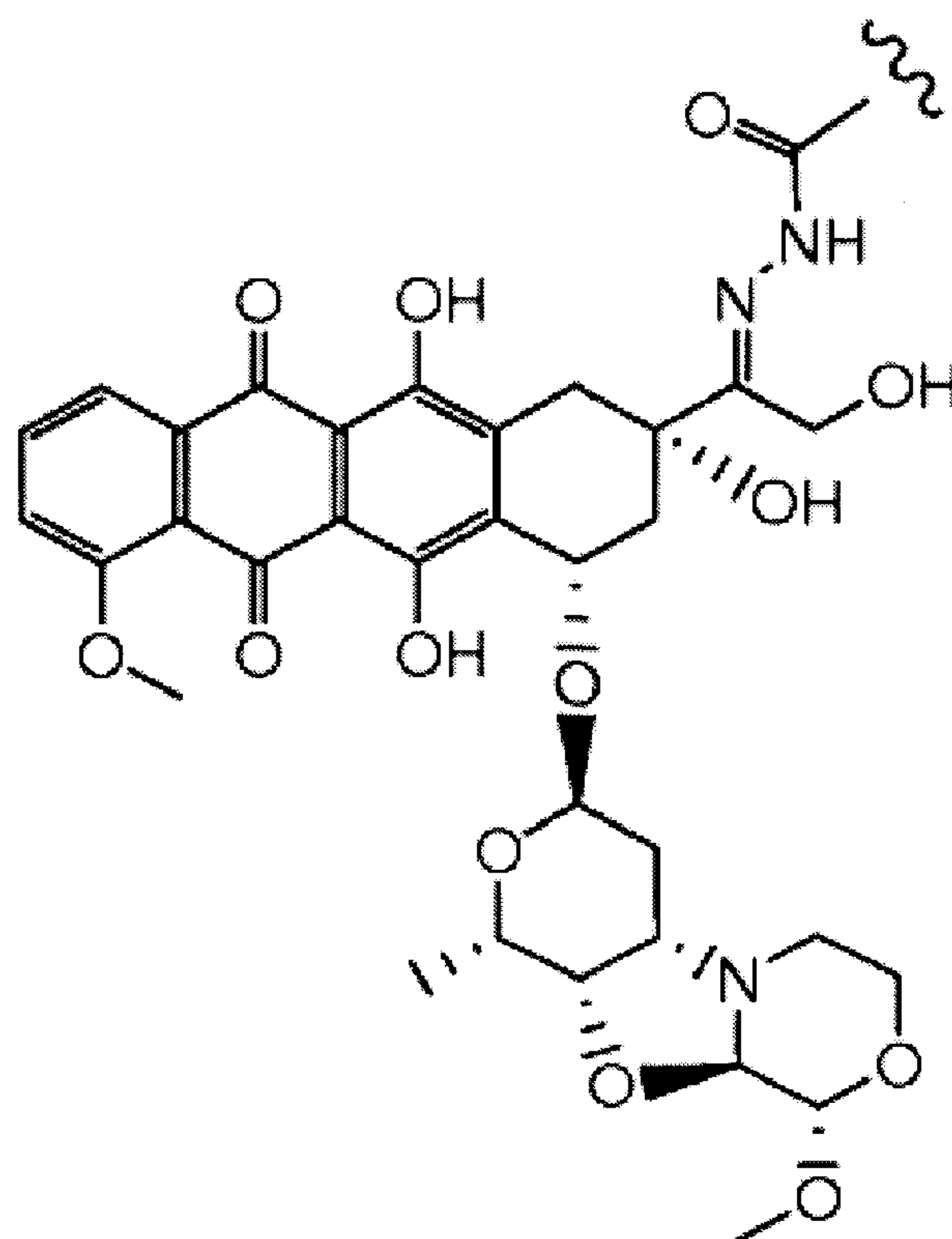
X and X' are independently O , S , or $N(H)$.

24. The immunoconjugate of claim 23, wherein D has the structure:

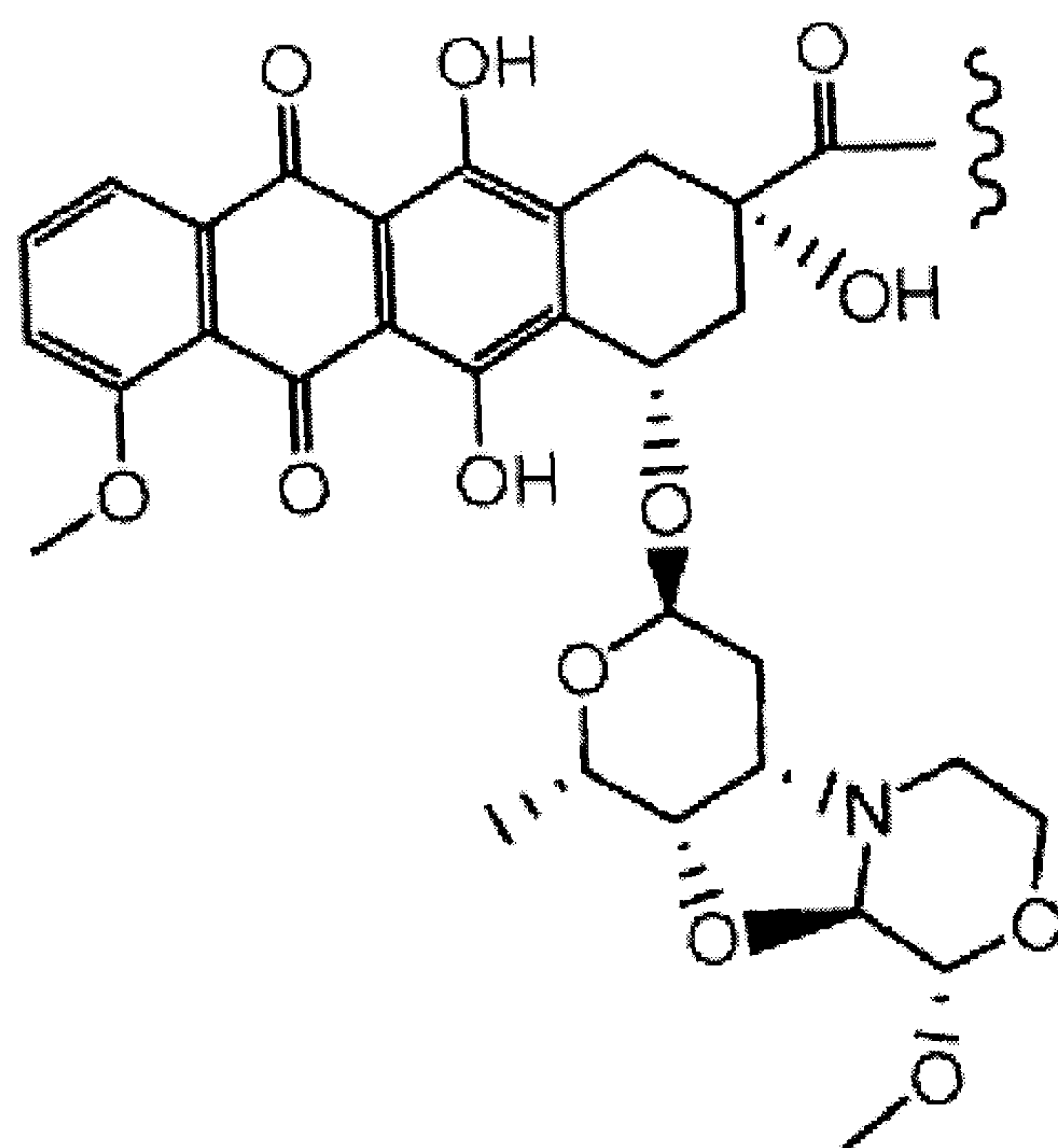


wherein n is 0 or 1.

25. The immunoconjugate of claim 21, wherein D is a nemorubicin derivative.
26. The immunoconjugate of claim 25, wherein D has a structure that is:

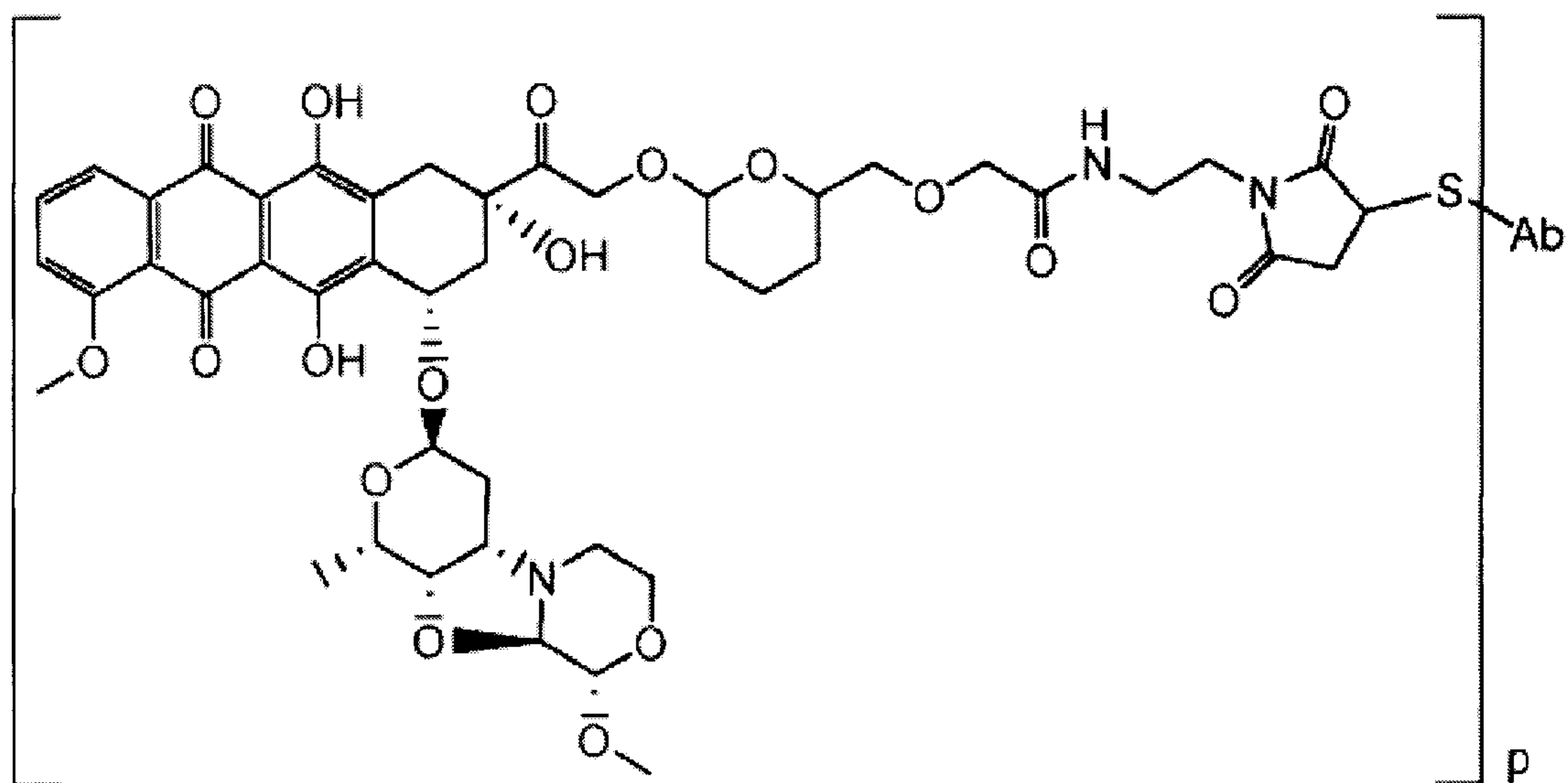


; or

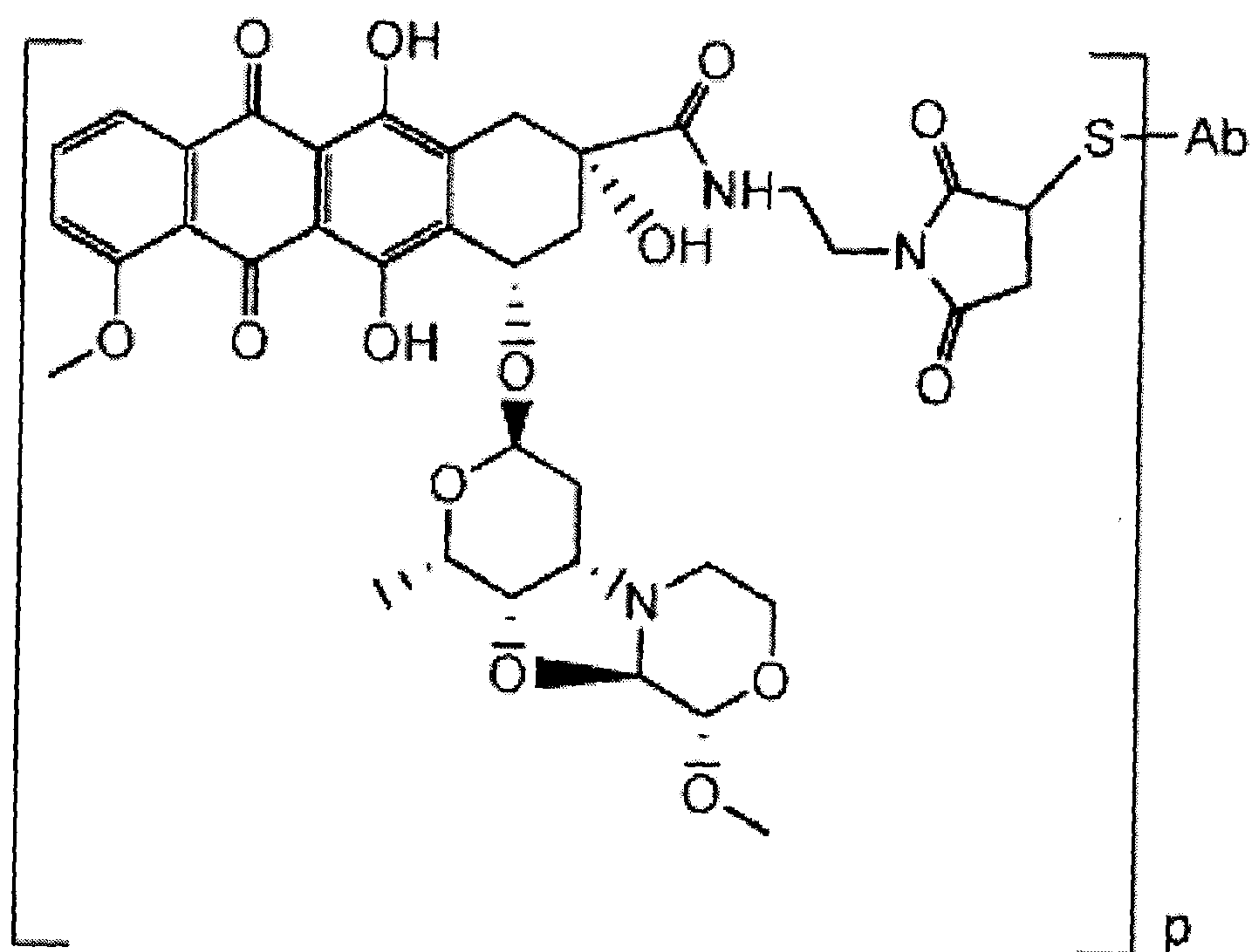


27. The immunoconjugate of any one of claims 21 to 26, wherein the linker is cleavable by a protease, is acid-labile, comprises hydrazine or any combination thereof.

28. The immunoconjugate of claim 21, having a formula that is:



; or



29. The immunoconjugate of claim 21 to 28, wherein p ranges from 2-5.
30. An immunoconjugate comprising the antibody defined in any one of claims 1 to 15 and a functional agent, wherein the functional agent is an endosomal escape domain (EED) peptide.
31. The immunoconjugate of claim 30, having the formula Ab(-EEDL-EED peptide), wherein Ab is the antibody defined in claim 1 to 15 and EEDL is an EED linker.
32. The immunoconjugate of claim 31, wherein the EED peptide is a Dengue Virus EED peptide comprising the amino acid sequence of SEQ ID NO: 42 and the EED linker comprises the amino acid sequence of SEQ ID NO: 43.
33. A pharmaceutical composition comprising the immunoconjugate defined any one of claims 20 to 32, and a pharmaceutically acceptable carrier.
34. The pharmaceutical composition of claim 33, comprising a further therapeutic agent.
35. A pharmaceutical composition comprising the antibody, or antigen-binding fragment thereof, defined in any one of claims 1 to 15, and a pharmaceutically acceptable carrier.
36. The pharmaceutical composition of claim 35, comprising a further therapeutic agent.
37. A use of an effective amount of the antibody, or antigen-binding fragment thereof, defined in any one of claims 1 to 15, of the immunoconjugate defined in any one of claims 20 to 32, or of the pharmaceutical composition defined in any one of claims 33 to 36, for treating a GP73-positive cancer.
38. The use of claim 37, wherein the GP73-positive cancer is liver cancer.
39. The use of claim 37 or 38, further comprising the use of an additional therapeutic agent.

40. The use of any one of claims 37 to 39, wherein the antibody, or antigen-binding fragment thereof, the immunoconjugate or the pharmaceutical composition is for intravenous administration, intraperitoneal administration, intramuscular administration, intrasternal administration, intratumoral administration, intravesical administration, intrauterine administration, intraarticular administration, intranasal administration, subcutaneous administration, topical administration, administration as clyster, or administration as gastric lavage.
41. An *in vitro* method of inhibiting proliferation of a GP73-positive cell, the method comprising exposing the cell to the antibody, or antigen-binding fragment thereof, defined in any one of claims 1 to 15, or the immunoconjugate defined in any one of claims 20 to 32, under conditions permissive for binding of antibody, or antigen-binding fragment thereof, or immunoconjugate to GP73 on the surface of the cell, thereby inhibiting proliferation of the cell.
42. The *in vitro* method of claim 41, wherein the cell is a liver cancer cell.
43. A use of the antibody, or antigen-binding fragment thereof, defined in any one of claims 1 to 15, or the immunoconjugate defined in any one of claims 20 to 32, for inhibiting proliferation of a GP73-positive cell, comprising exposing the cell to the antibody, antigen-binding fragment thereof, or the immunoconjugate, under conditions permissive for binding of said antibody, antigen-binding fragment thereof, or immunoconjugate, to GP73 on the surface of the cell, thereby inhibiting proliferation of the cell.
44. The use of claim 43, wherein the cell is a liver cancer cell.
45. A method of detecting human GP73 in a biological sample comprising contacting the biological sample with the antibody, or antigen-binding fragment thereof, defined in any one of claims 1 to 15, under conditions permissive for binding of the antibody, or antigen-binding fragment thereof, to a naturally occurring human GP73, and detecting whether a complex is formed between the antibody, or antigen-binding fragment thereof, and a naturally occurring human GP73 in the biological sample.
46. The method of claim 45, wherein the detecting comprises immunohistochemistry, immunofluorescence imaging, enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), Western Blot, immunoprecipitation, or radiographic imaging.
47. A method for identifying a subject as having a cancer, comprising determining the level, in a sample from the subject, of specific binding of the antibody, or

antigen-binding fragment thereof, defined in any one of claims 1 to 15 with a GP73 polypeptide or with an antigenic portion thereof, wherein detecting an altered level of the specific binding relative to a control sample identifies the subject as having a cancer.

48. The method of claim 47, wherein the cancer is liver cancer, ovarian cancer, endometrium carcinoma, malignant melanoma, prostate cancer, gastric cancer, colorectal cancer, lung cancer or leukemia.
49. A chimeric antigen receptor CAR T-cell, capable of binding to an epitope within amino acids 36 to 55 of human GP73.
50. An antigen-binding molecule that binds to a furin cleavage consensus sequence EGRVRR (SEQ ID NO: 34) and inhibits the proteolytic cleavage of GP73 proteins, and wherein the antigen-binding molecule is internalized into a cell.
51. A composition comprising the antibody, or antigen-binding fragment thereof, defined in any one of claims 1 to 15, and an EGFR antibody or small molecules inhibiting EGFR signaling for use in the treatment of cancer.
52. A bispecific antibody targeting GP73 and EGFR and blocks the EGFR signalling in susceptible cancer cells, wherein the antibody is internalized into a cell.
53. The antibody, or antigen-binding fragment thereof, of any one of claims 1 to 15, for use in treating a disease related to abnormal expression of GP73, wherein the disease is cancer.
54. The antibody, or antigen-binding fragment thereof, for use according to claim 53, wherein the cancer is liver cancer, ovarian cancer, endometrium carcinoma, melanoma, prostate cancer, colorectal carcinoma or breast cancer.
55. The antibody, or antigen-binding fragment thereof, of any one of claims 1 to 15, that reduces the level of circulating, soluble GP73 (sGP73) in the plasma.
56. The immunoconjugate of any one of claims 20 to 32, for use in treating a disease related to abnormal expression of GP73, wherein the disease is cancer.
57. The immunoconjugate for use according to claim 56, wherein the cancer is liver cancer, ovarian cancer, endometrium carcinoma, melanoma, prostate cancer, colorectal carcinoma or breast cancer.
58. The immunoconjugate of any one of claims 20 to 32, that reduces the level of circulating, soluble GP73 (sGP73) in the plasma.

59. The pharmaceutical composition of any one of claims 33 to 36, for use in treating a disease related to abnormal expression of GP73, wherein the disease is cancer.
60. The pharmaceutical composition for use according to claim 59, wherein the cancer is liver cancer, ovarian cancer, endometrium carcinoma, melanoma, prostate cancer, colorectal carcinoma or breast cancer.
61. The pharmaceutical composition of any one of claims 33 to 36 that reduces the level of circulating, soluble GP73 (sGP73) in the plasma.
62. A method for monitoring the response to treatment of a subject with the antibody, or antigen-binding fragment thereof, defined in any one of claims 1 to 15, the immunoconjugate defined in any one of claims 20 to 32, or the pharmaceutical composition defined in any one of claims 33 to 36, the method comprising measuring the level of circulating sGP73 in the plasma at one or more time points before and at one or more time points after a cancer treatment of the subject.
63. The method according to claim 62, wherein detecting a reduced level of circulating sGP73 at a time point after treatment relative to a time point before the cancer treatment in the plasma of the subject identifies the subject as being responsive to the treatment.