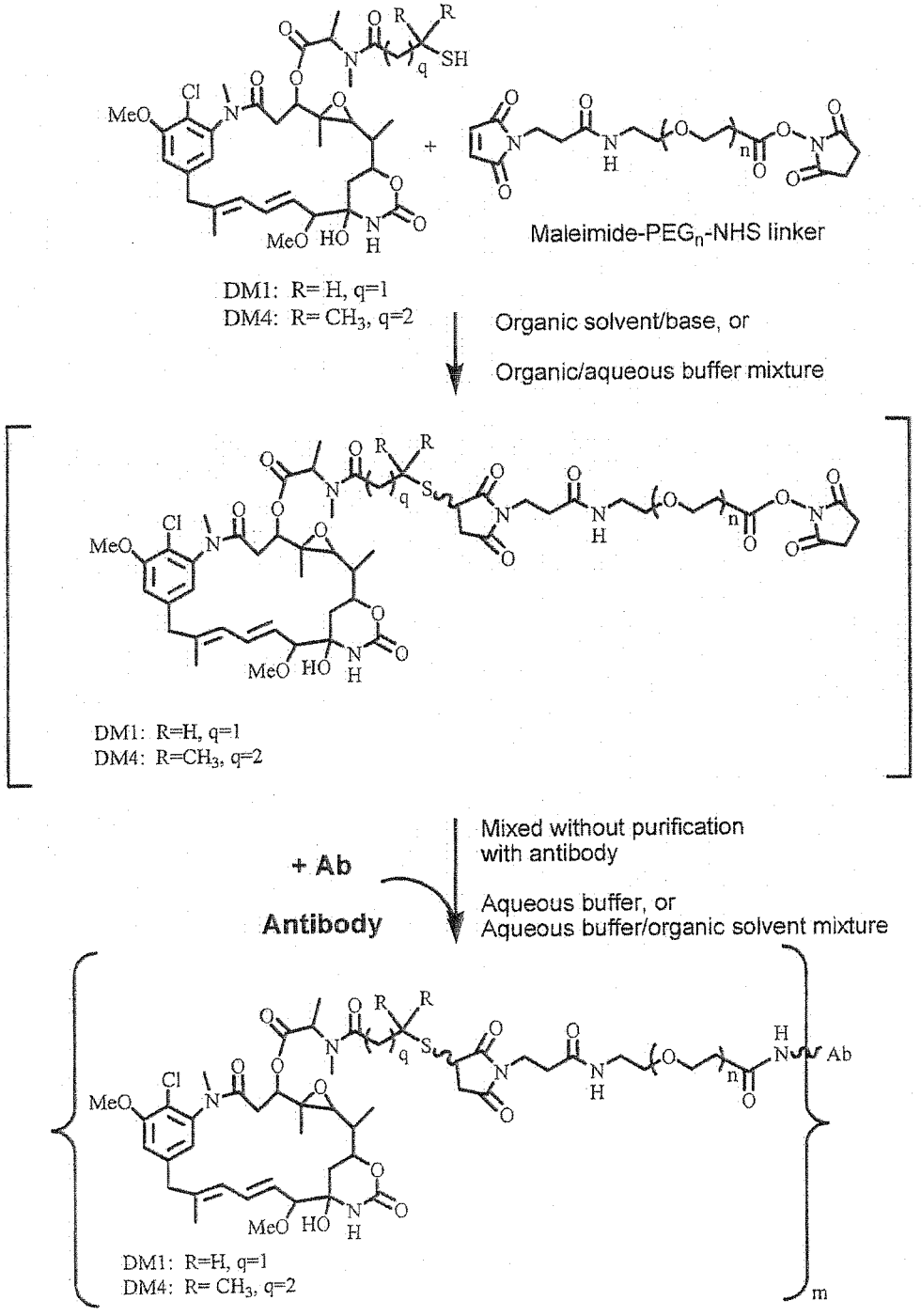
FIG. 1

Conjugation of antibody with a reaction mixture of DM1 (or DM4) and Maleimide-PEG_n-NHS linker



Antibody-maytansinoid conjugate (Ab-PEG_n-Mal-DMx)

FIG. 2

Reducing SDS-PAGE of Ab-(PEG_4-Mal)-DM4 conjugates prepared using the method described in this invention versus conjugates prepared using the traditional 2-step method.

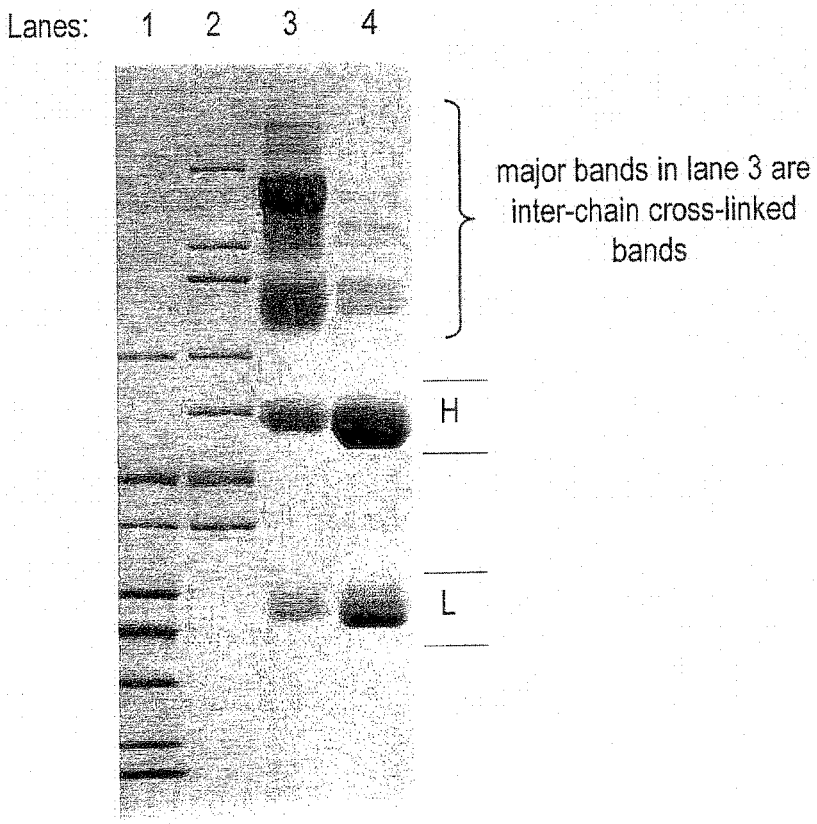


FIG. 3A

Protein LabChip electrophoresis of Antibody-(PEG_4-Mal)-DM4 conjugate prepared using the method described in this invention versus conjugate prepared using the traditional 2-step method.

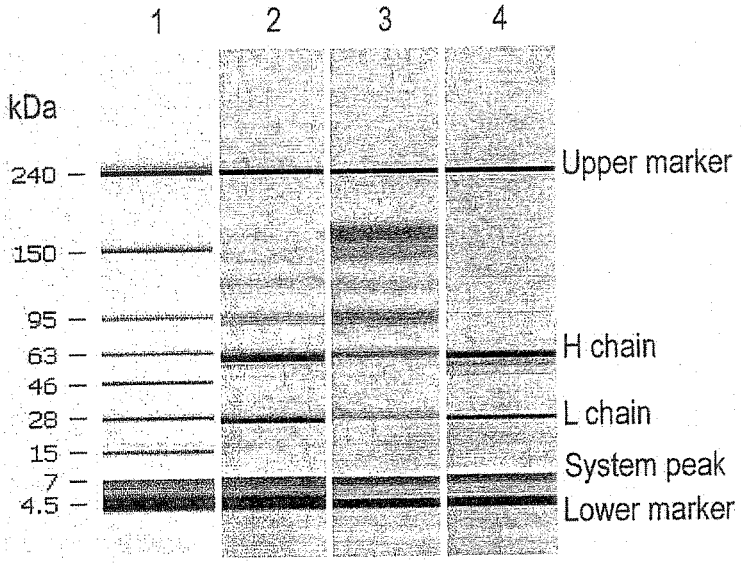


FIG. 3B

		% of Total Protein Mass Loaded		
	Band MW (kDa)	Unconjugated Ab	Ab-PEG4-Mal-DM4 This method	Ab-PEG4-Mal-DM4 2 Step
	17	1	2	1
L Chain	**27**	**30**	**30**	**8**
	56	4	2	-
H Chain	**61**	**65**	**58**	**16**
	94	-	6	26
	122	-	2	7
	147	-	-	12
	169	-	-	31

FIG. 4A

MS of Antibody-(PEG4-Mal)-DM4 conjugate prepared using the method described in this invention versus conjugate prepared using the traditional 2-step method (deglycosylated conjugate samples were analyzed by MS).

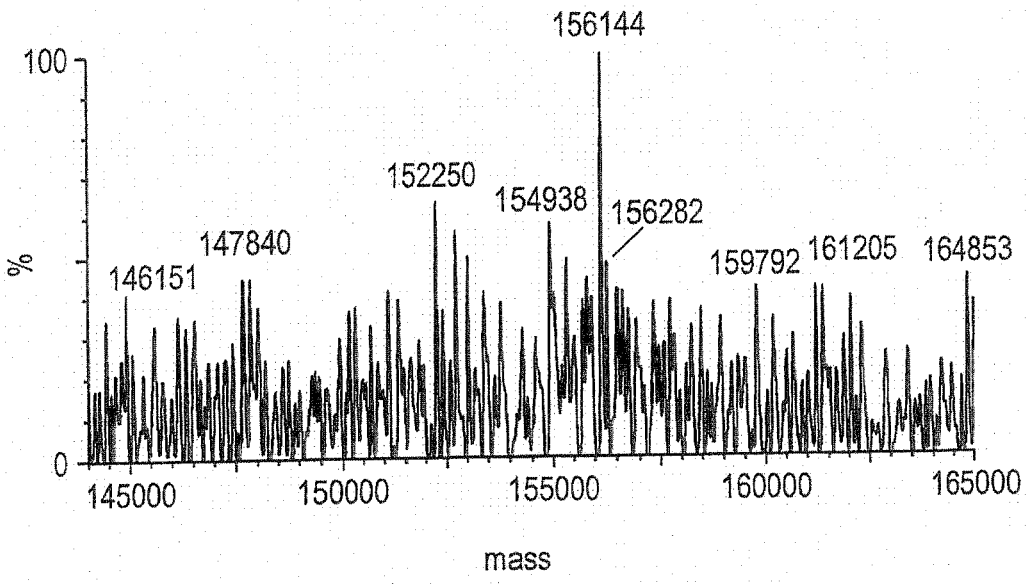


FIG. 4B

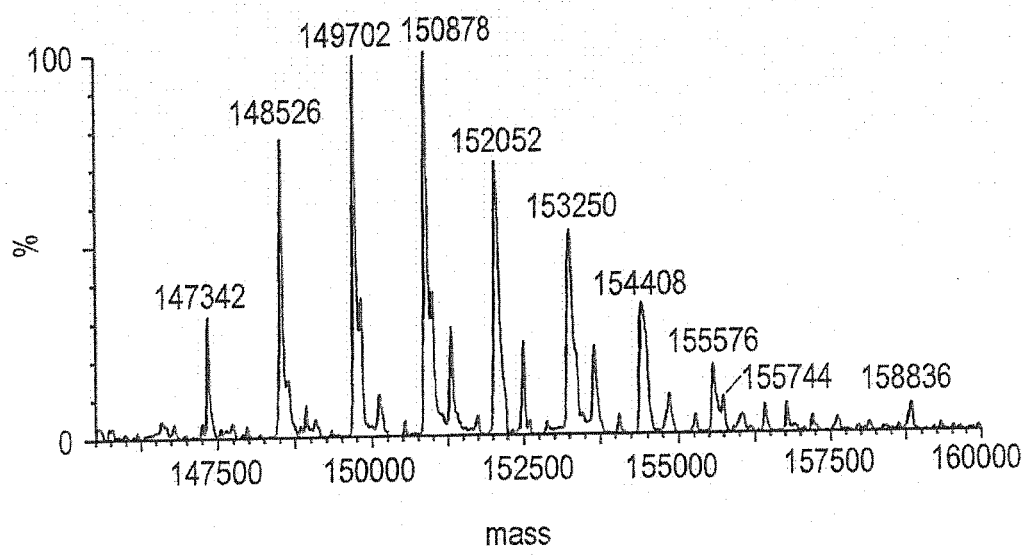


FIG. 5

Binding of an anti-CanAg antibody-PEG_4-Mal-DM1 conjugate with 6.7 DM1 per antibody (prepared using the method described in this invention) versus binding of unmodified antibody toward CanAg antigen-expressing COLO205 cells

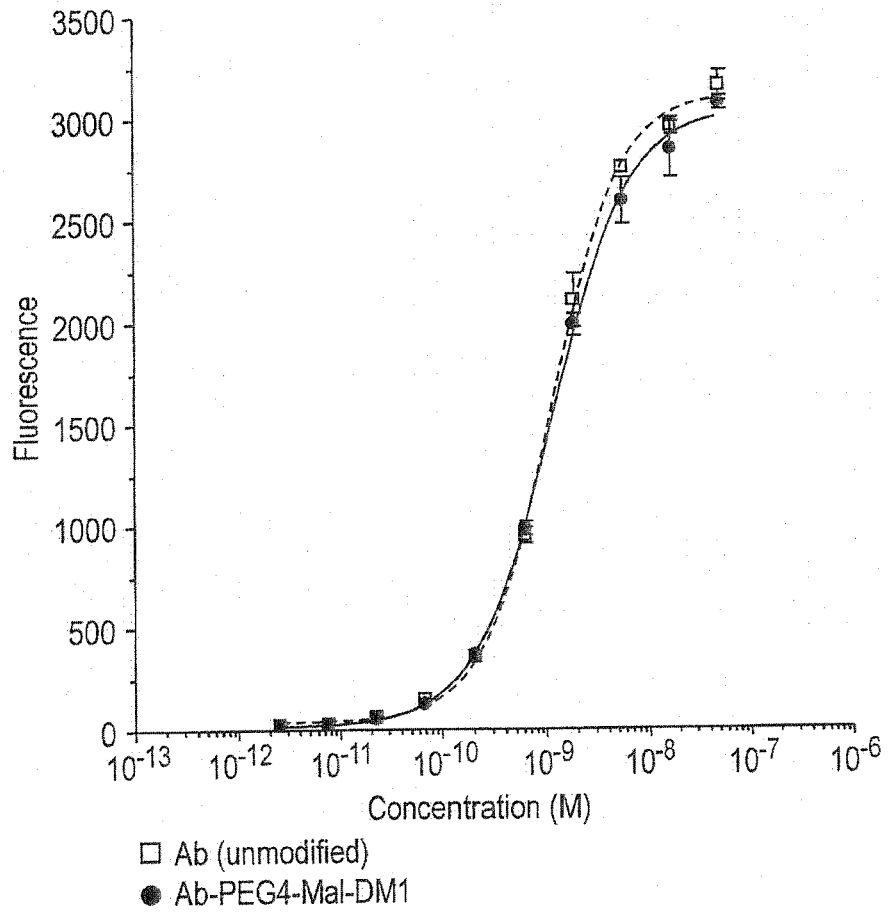


FIG. 6

In vitro cytotoxicity of an anti-CanAg Antibody-PEG_4-Mal-DM1 conjugate with 6.7 DM1 per antibody (prepared using the method described in this invention) toward CanAg antigen-expressing COLO205 cells.

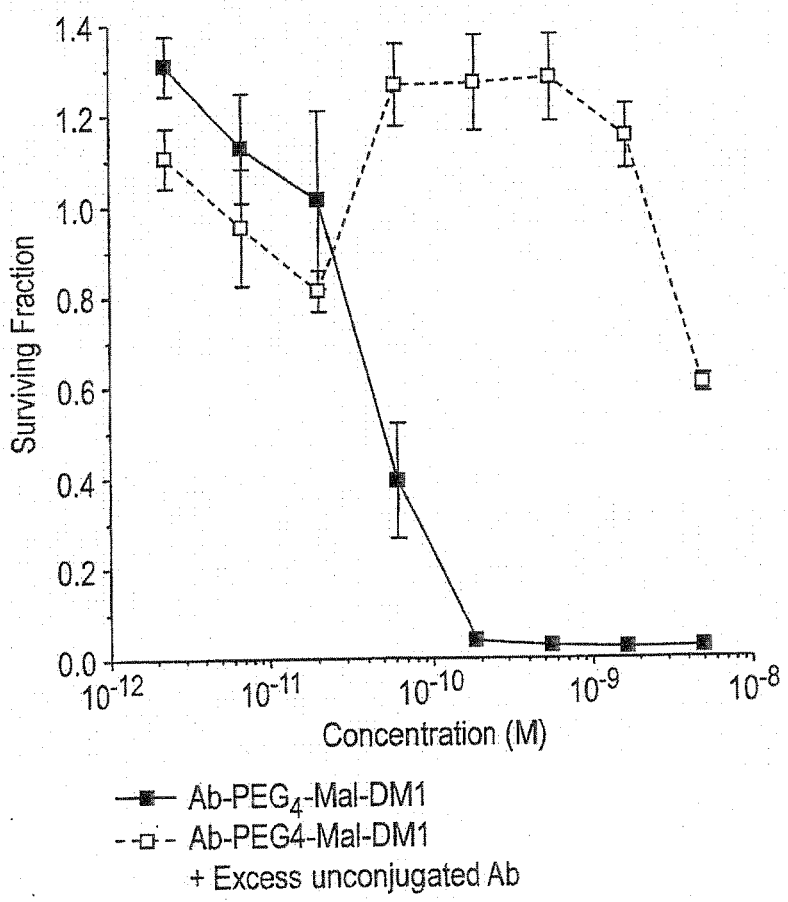
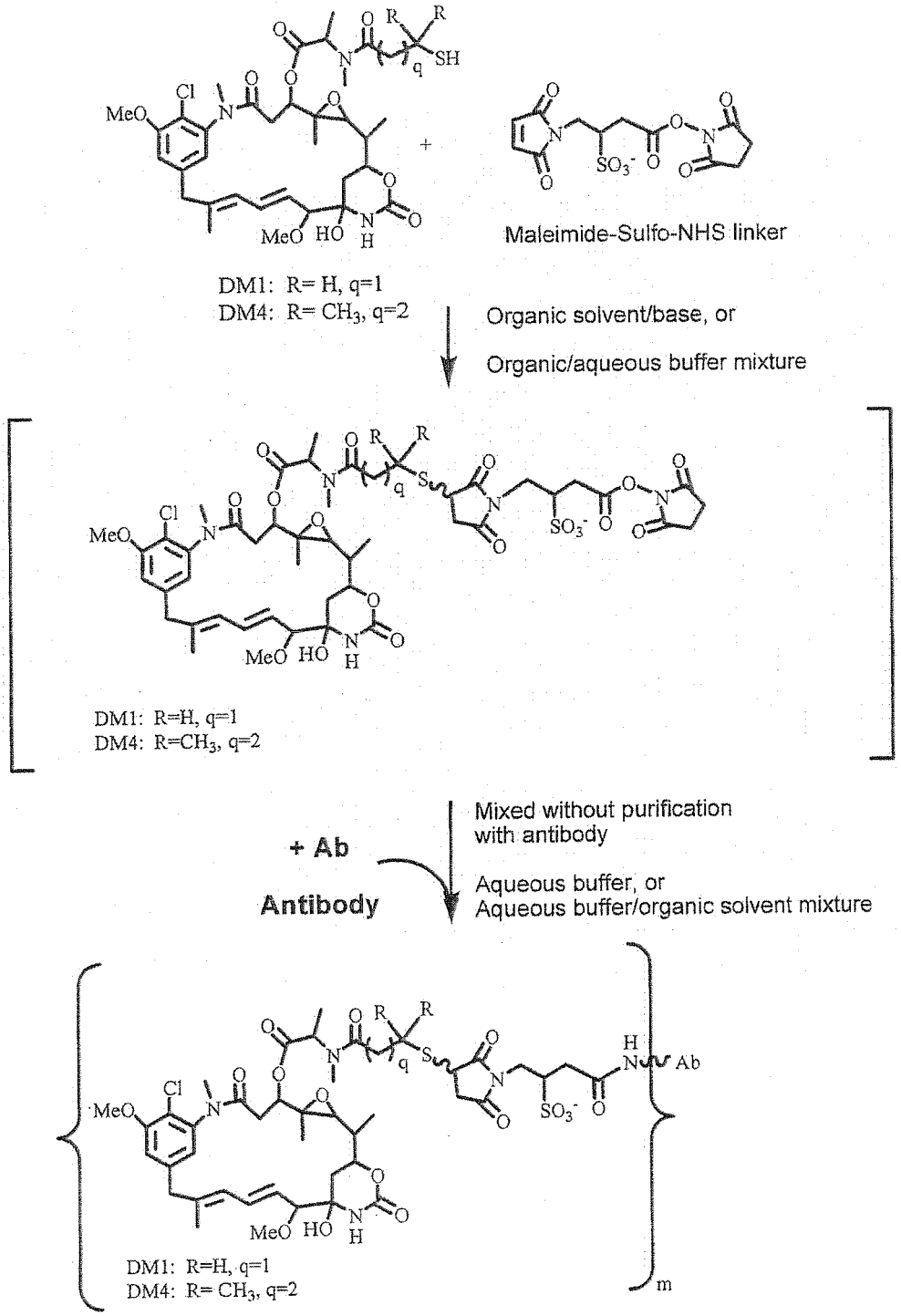


FIG. 7

Conjugation of antibody with a reaction mixture of DM1 (or DM4) and Maleimide-Sulfo-NHS linker



Antibody-maytansinoid conjugate (Ab-Sulfo-Mal-DMx)

FIG. 8

Reducing SDS-PAGE of Ab-(Sulfo-Mal)-DM1 conjugates prepared using the method described in this invention versus conjugates prepared using the traditional 2-step method.

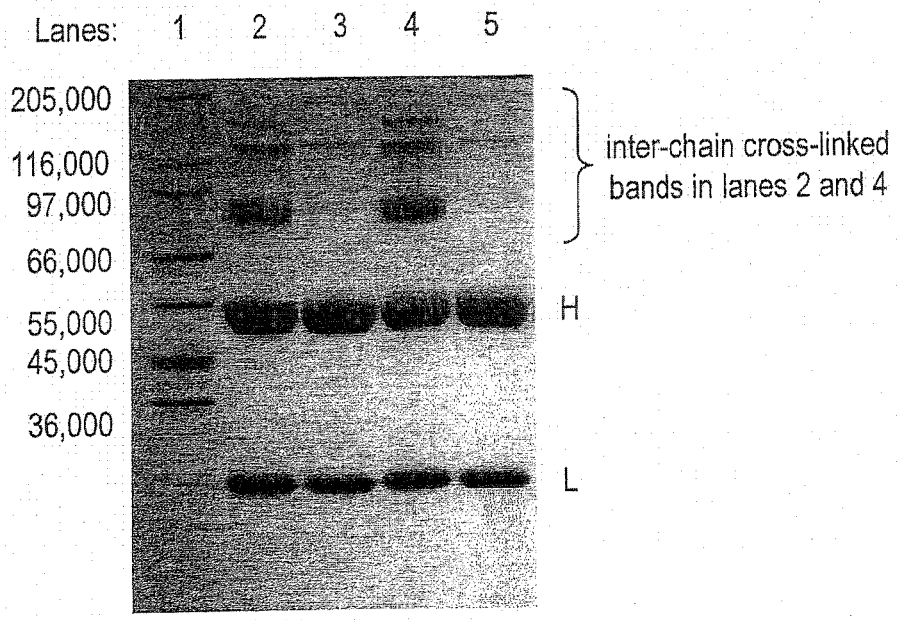


FIG. 9A

Protein LabChip electrophoresis of Antibody-(Sulfo-Mal)-DM1 conjugate prepared using the method described in this invention versus conjugate prepared using the traditional 2-step method.

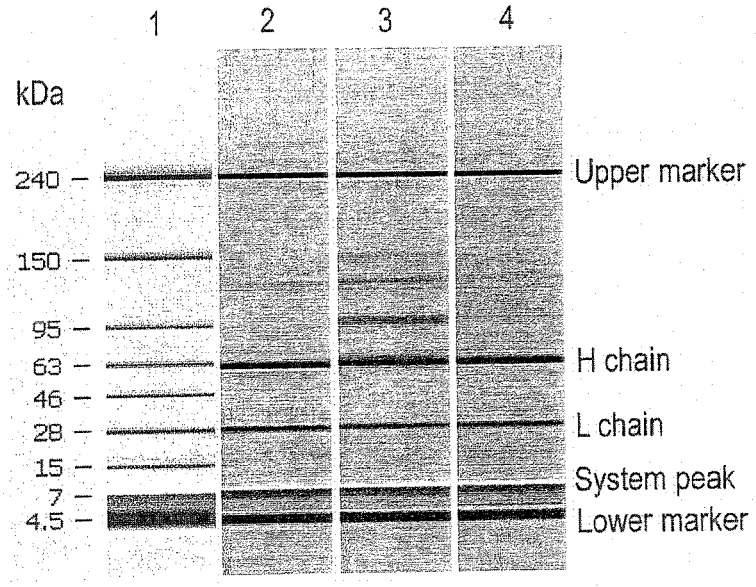


FIG. 9B

		% Total Protein Mass Loaded		
	Band MW (kDa)	Unconjugated Ab	Ab-Sulfo-Mal-DM1 2 Step	Ab-Sulfo-Mal-DM1 This method
	18	-	-	1
L Chain	**29**	**30**	**23**	**28**
H Chain	**62**	**70**	**53**	**70**
	99	-	15	1
	130	1	7	1
	152	-	3	-

FIG. 10A

Ab-(3-Sulfo-Mal)-DM1 conjugate (3.6 D/Ab)
prepared using the method described in this invention

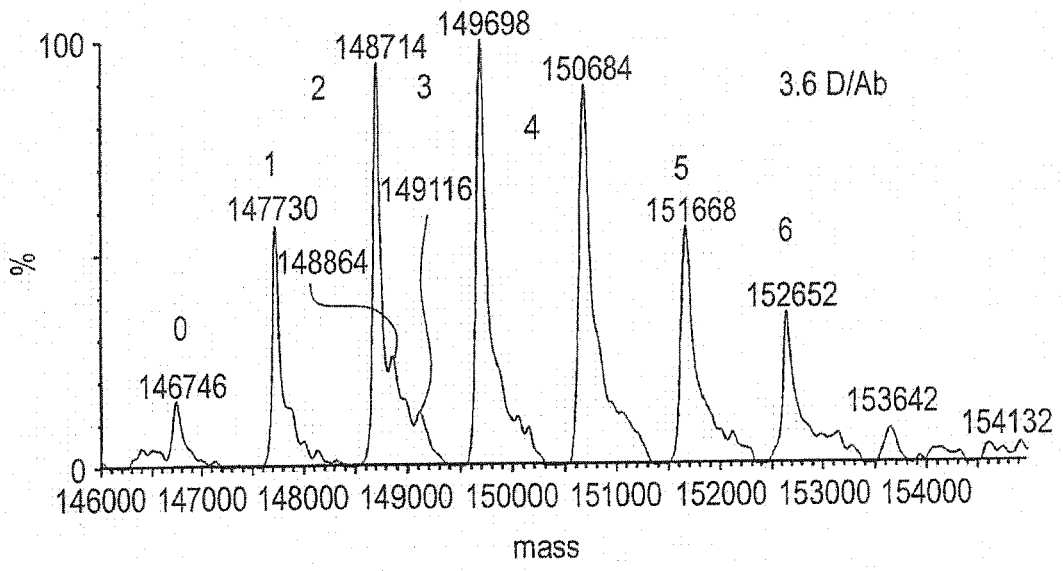


FIG. 10B

Ab-(3-Sulfo-Mal)-DM1 Conjugate (4.0 D/Ab)
traditional two-step conjugation method

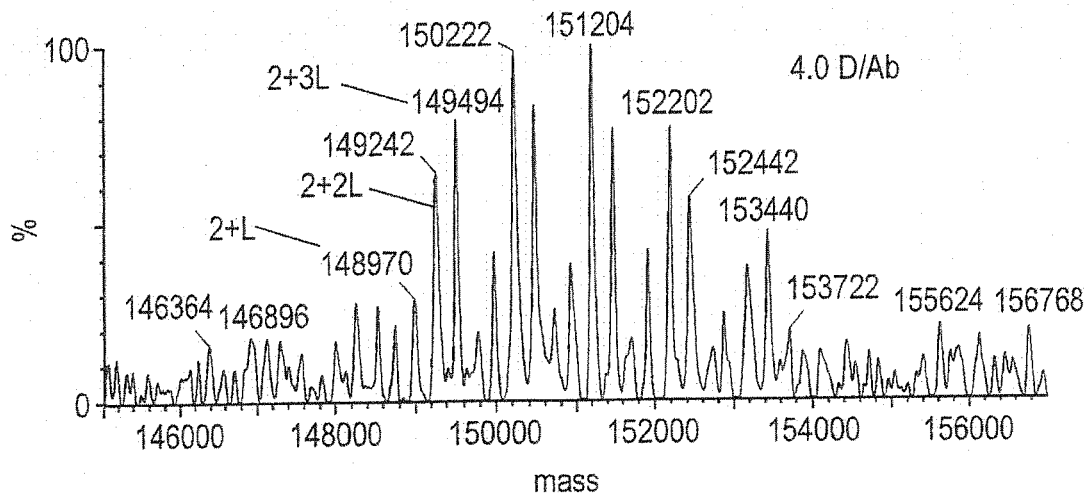


FIG. 11

Binding of an anti-CanAg antibody-Sulfo-Mal-DM1 conjugate with 5.6 DM1 per antibody (prepared using the method described in this invention) versus binding of unmodified antibody toward CanAg antigen-expressing COLO205 cells

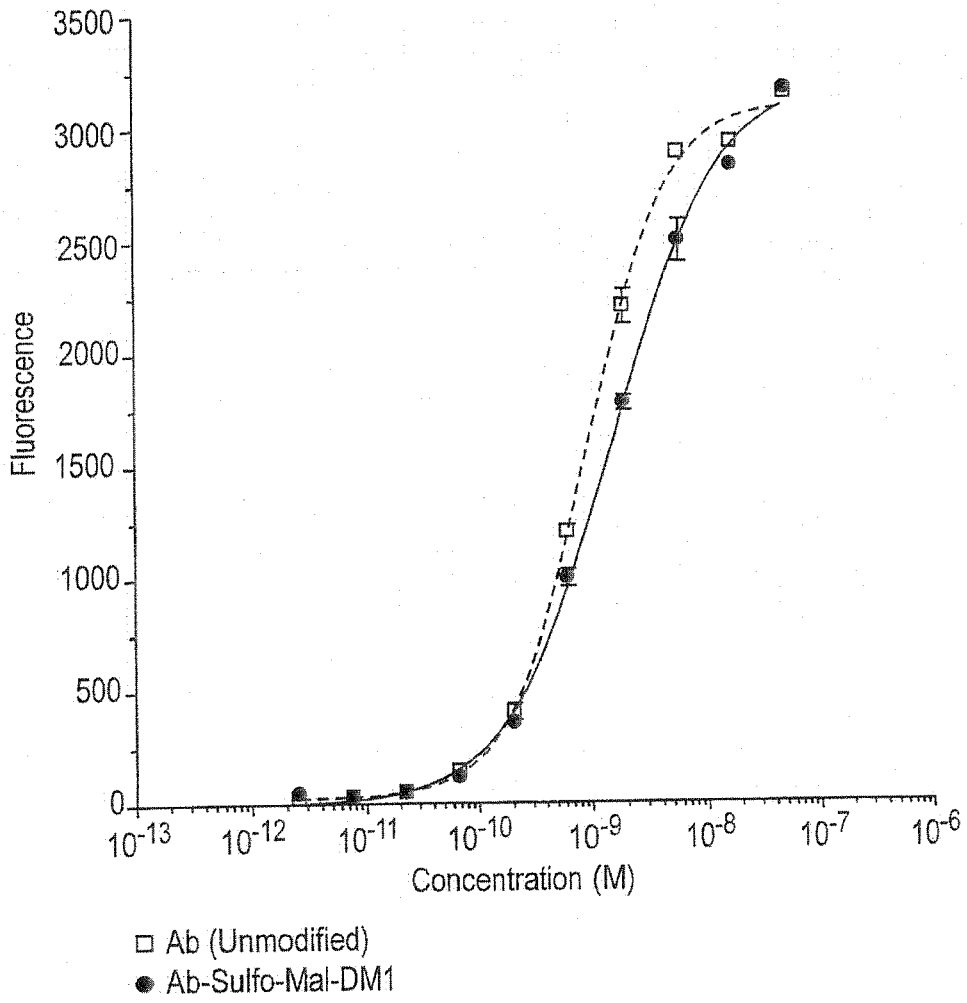


FIG. 12

In vitro cytotoxicity of an anti-CanAg Antibody-Sulfo-Mal-DM1 conjugate with 5.6 DM1 per antibody (prepared using the method described in this invention) toward CanAg antigen-expressing COLO205 cells.

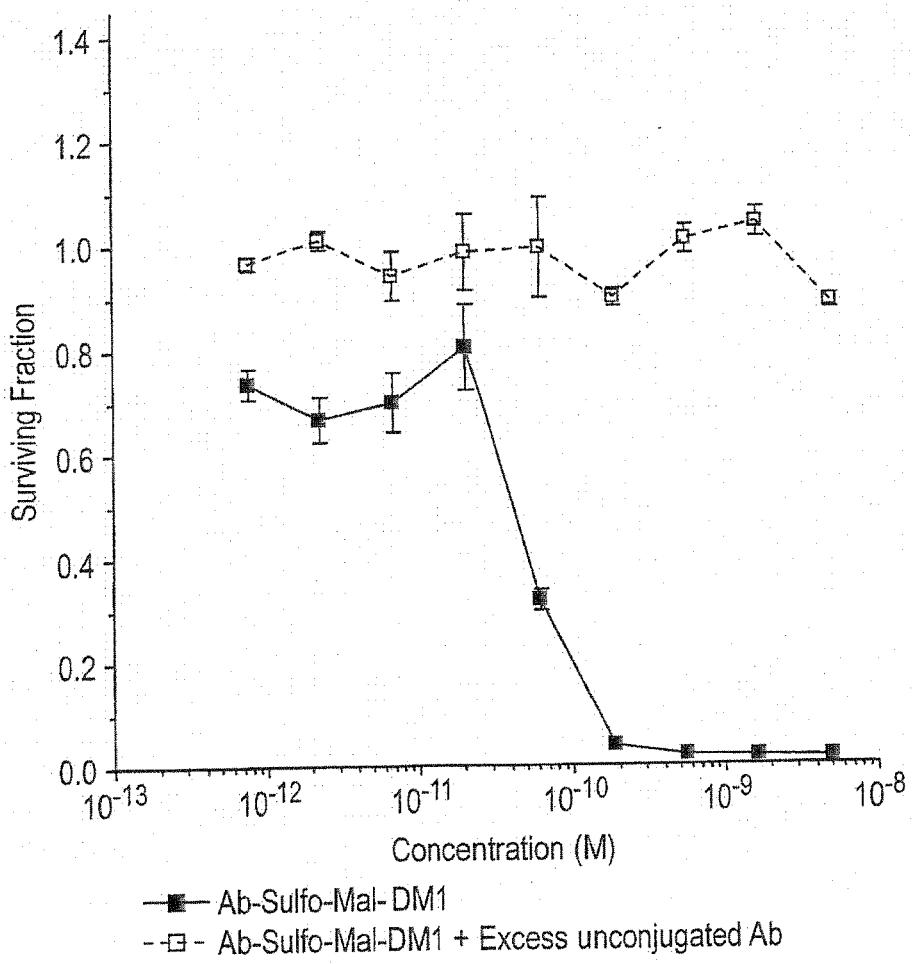
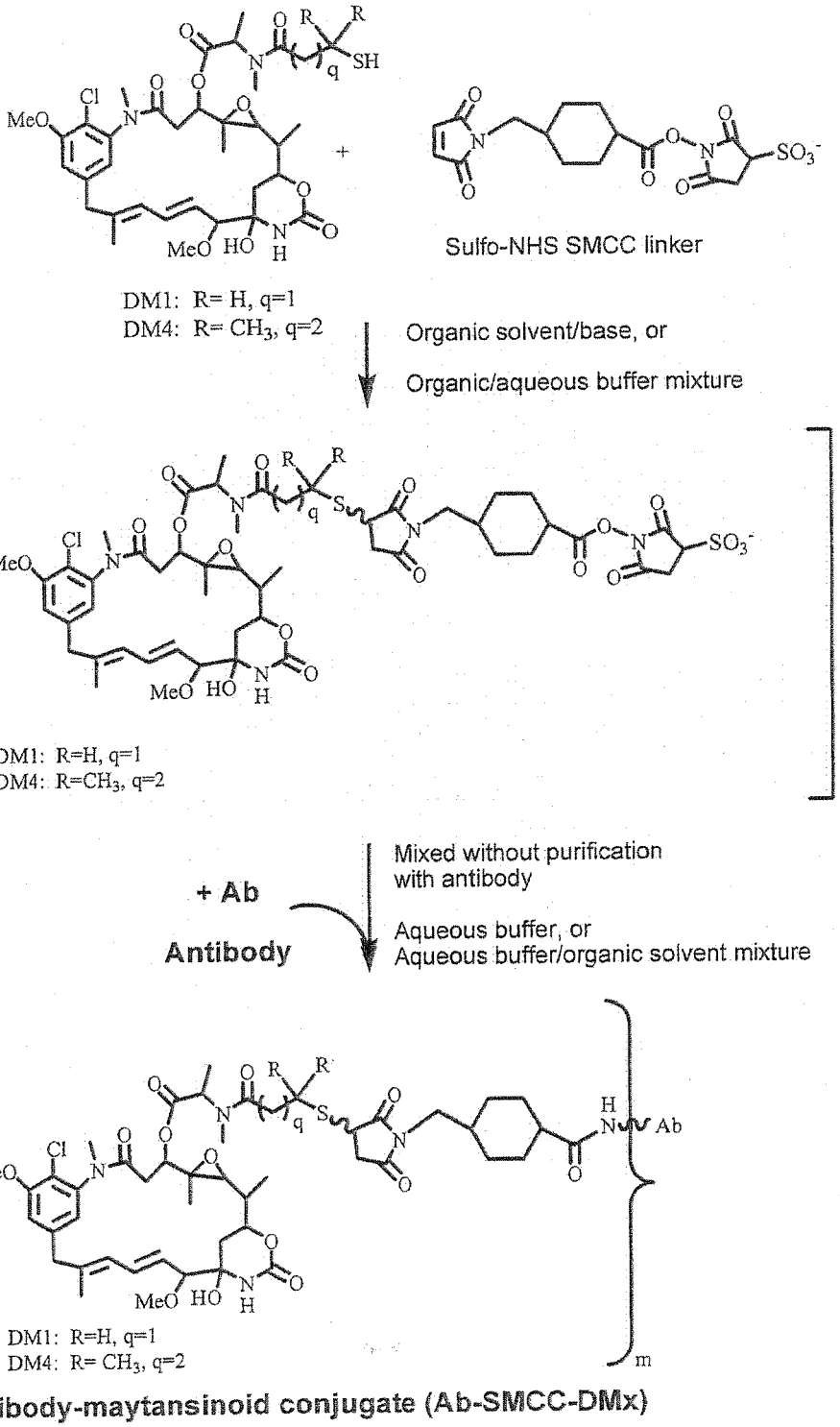


FIG. 13

Conjugation of antibody with a reaction mixture of DMI (or DM4) and Sulfo-NHS SMCC linker



Antibody-maytansinoid conjugate (Ab-SMCC-DMx)

FIG. 14

Reducing SDS-PAGE of Ab-(SMCC)-DM1 conjugate prepared using the method described in this invention versus conjugate prepared using the traditional 2-step method.

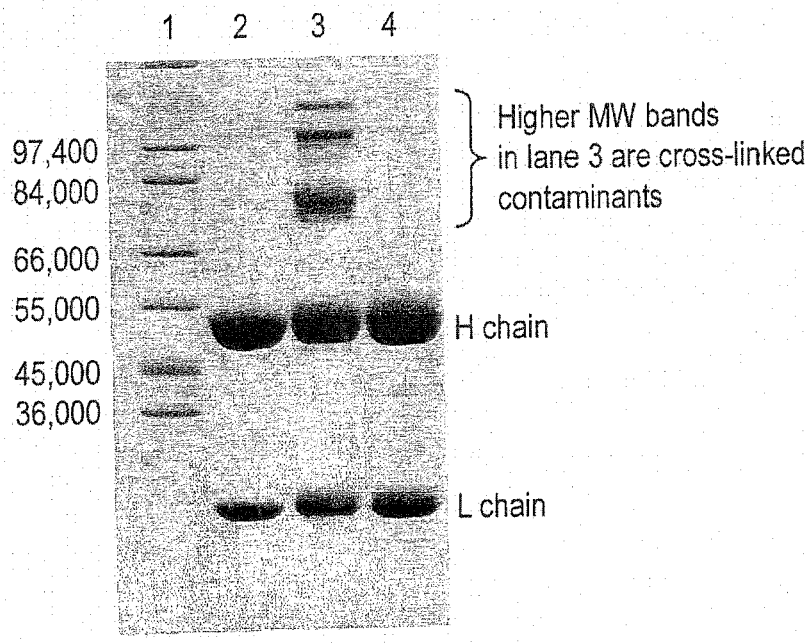


FIG. 15A

Protein LabChip electrophoresis of Antibody-(SMCC)-DM1 conjugate prepared using the method described in this invention versus conjugate prepared using the traditional 2-step method.

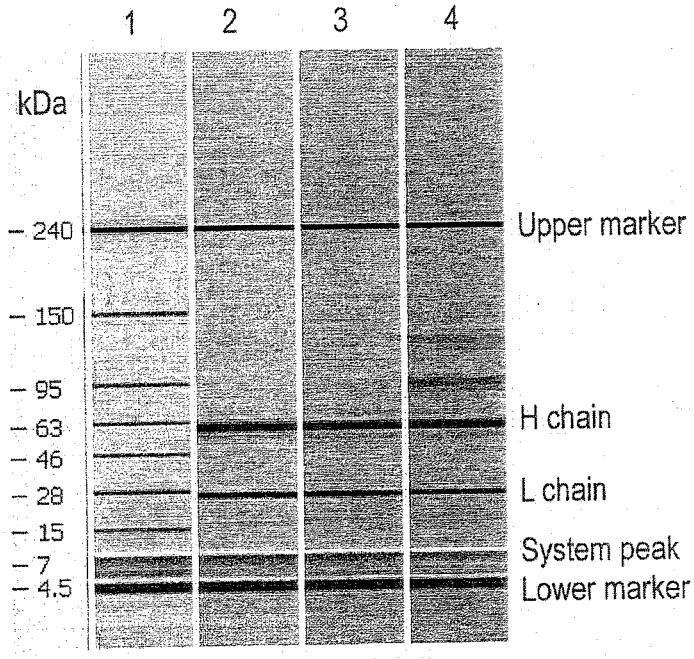


FIG. 15B

		% of Total Protein Mass Loaded		
	Band MW (kDa)	Ab-SMCC-DM1 This Method	Unconjugated Ab	Ab-SMCC-DM1 2-Step
L Chain	**27**	**30**	**30**	**24**
	56	2	2	2
H Chain	**61**	**67**	**68**	**54**
	96	1	-	12
	129	1	-	6
	148	-	-	2

FIG. 16A

Ab SMCC DM1, 3.1 D/Ab, prepared
with traditional 2 step conjugation method

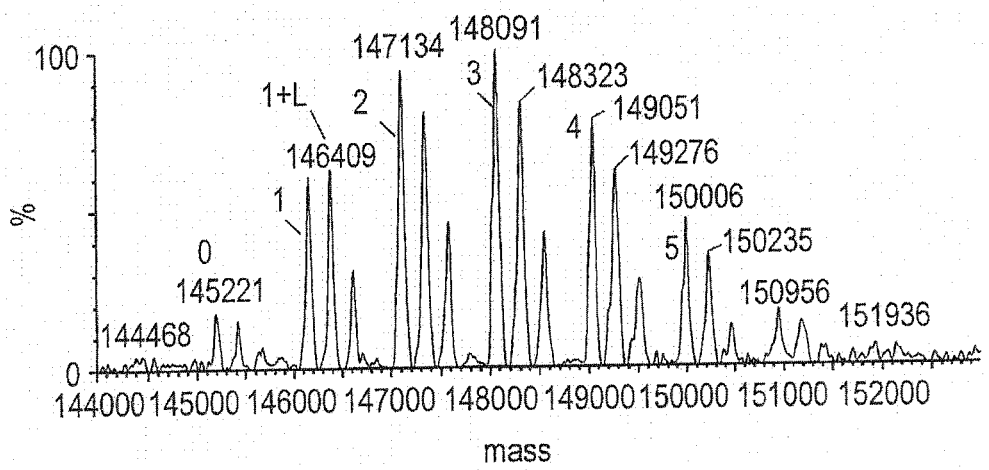


FIG. 16B

Ab SMCC-DM1, 3.1 D/Ab, prepared using
the method described in this invention

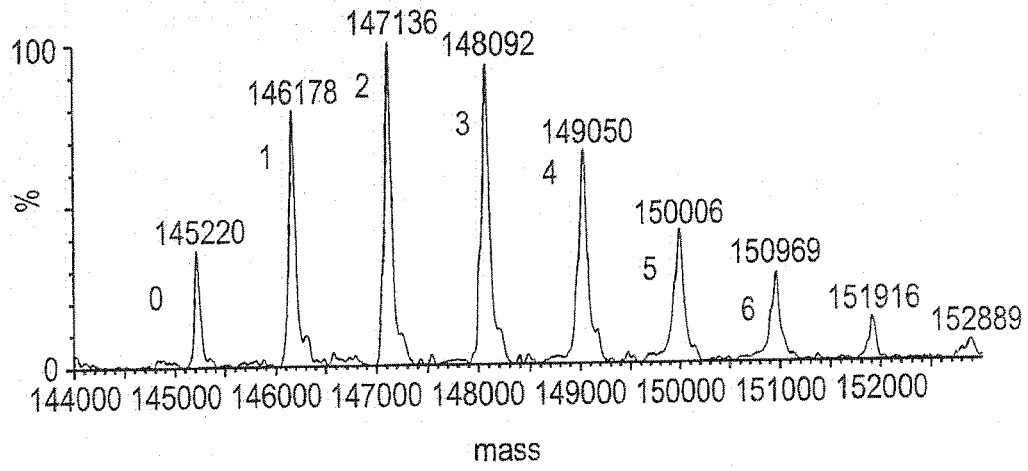


FIG. 17

Proposed mechanism for inter-chain cross-linking and maleimide inactivation during conjugation by the traditional 2-step method

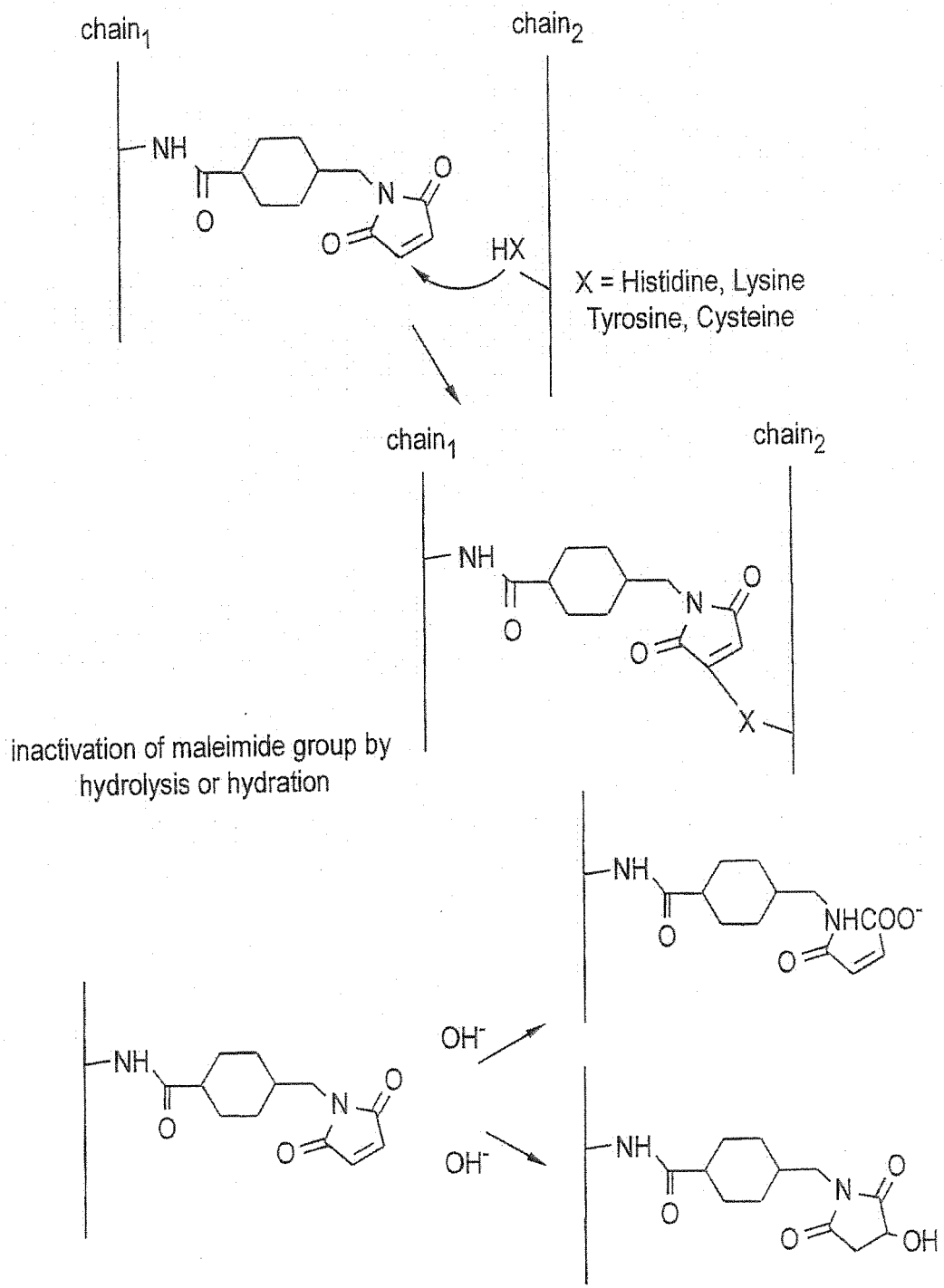
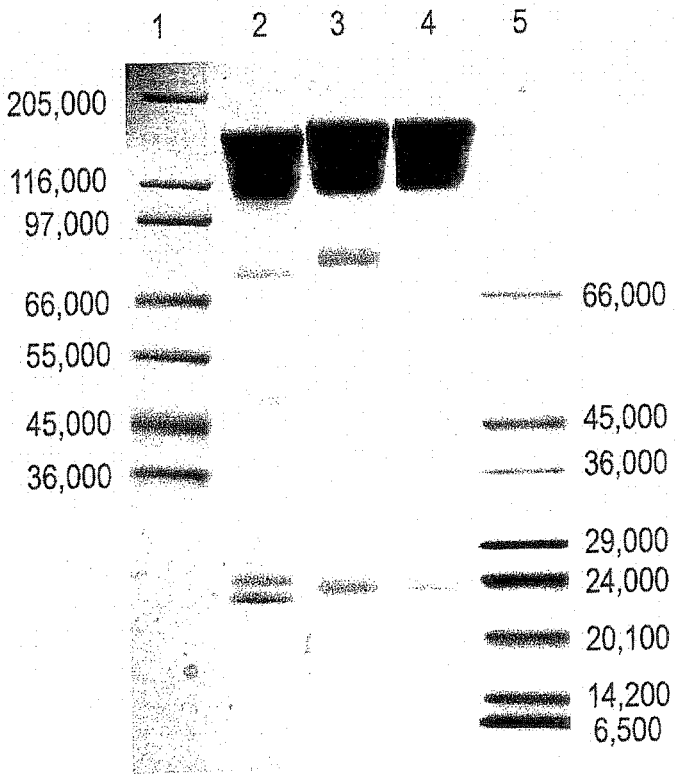


FIG. 18

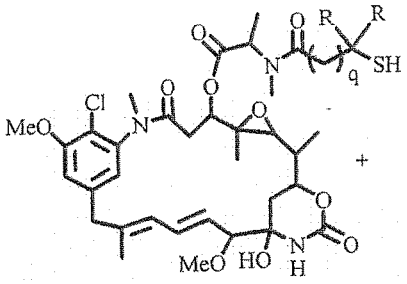
Non-reducing SDS PAGE of Ab-(Sulfo-Mal)-DM4 conjugate prepared using the method described in this invention and quenching of free DM4 thiol (after the initial DM4 + NHS-Sulfo-Mal heterobifunctional reagent coupling reaction) using 4-maleimidobuytric acid prior to the antibody conjugation reaction.



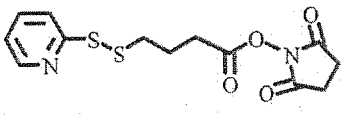
19/25

FIG. 19

Preparation of disulfide-linked conjugate of antibody using a reaction mixture of DM1 (or DM4) and SPDB linker



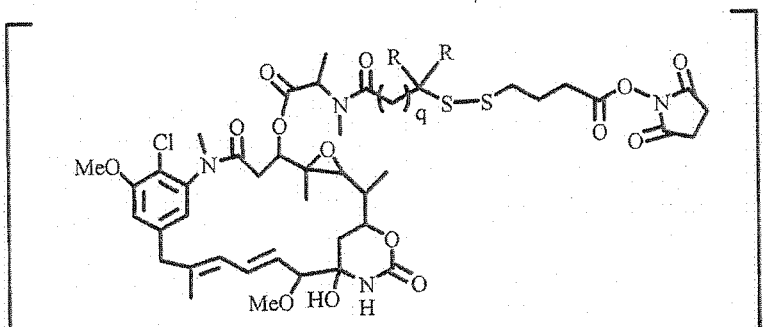
DM1: R= H, q=1
DM4: R= CH_3, q=2



SPDB linker

Organic solvent/base, or

Organic/aqueous buffer mixture



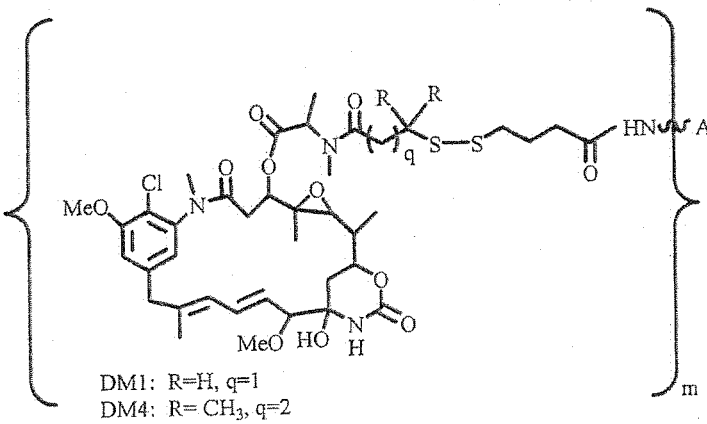
DM1: R=H, q=1
DM4: R=CH_3, q=2

Mixed without purification with antibody

+ Ab

Antibody

Aqueous buffer, or
Aqueous buffer/organic solvent mixture

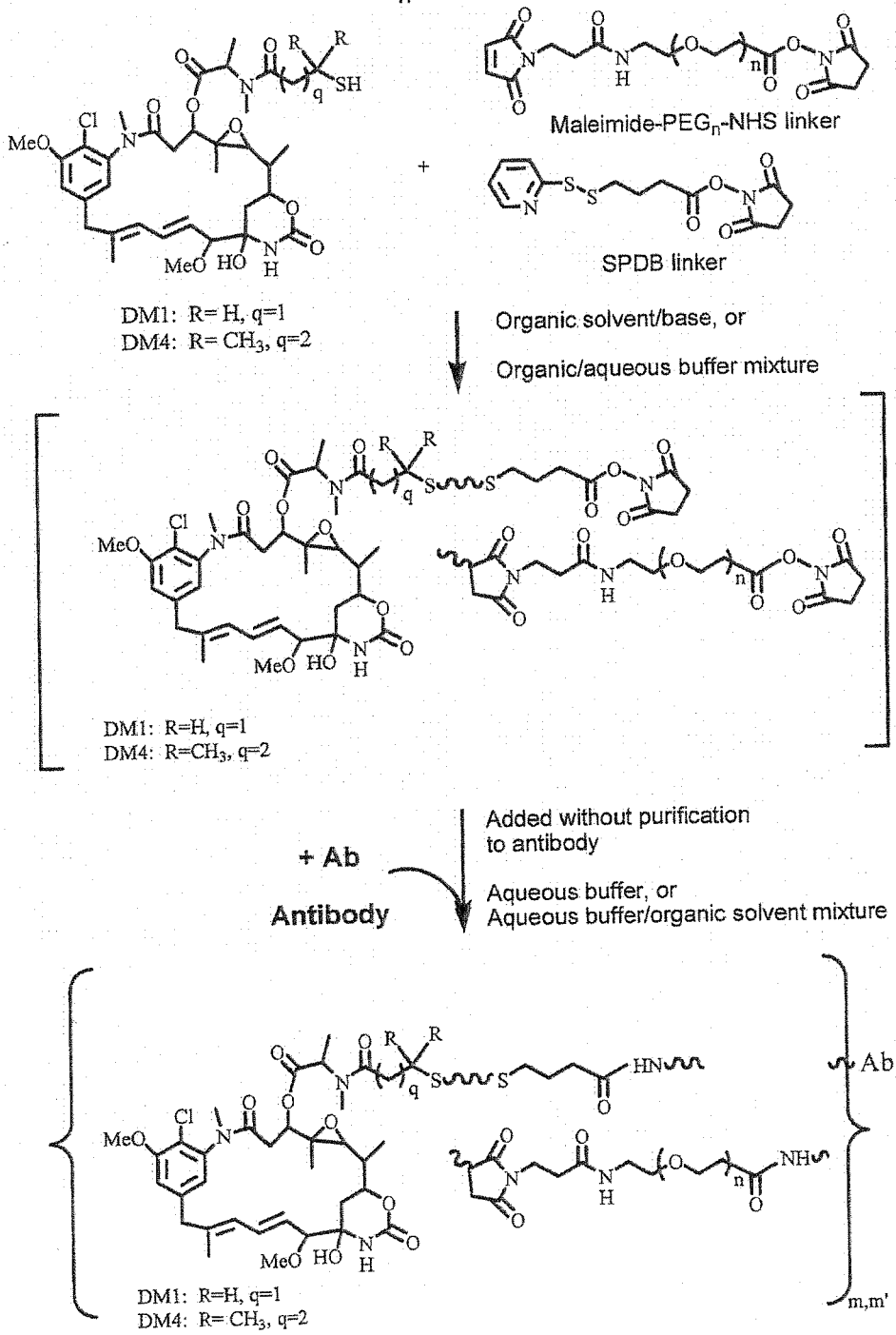


DM1: R=H, q=1
DM4: R= CH_3, q=2

Antibody-maytansinoid conjugate with cleavable, disulfide

FIG. 20

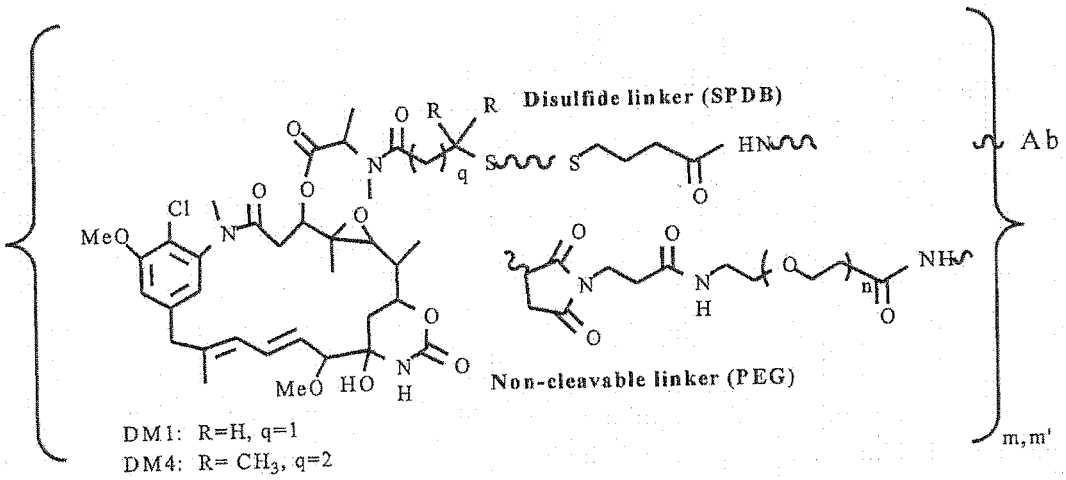
Preparation of antibody-maytansinoid conjugate with both disulfide- and non-cleavable PEG_n-Mal linkers via antibody conjugation with an unpurified reaction mixture of DM1 (or DM4) and both SPDB and NHS-PEG_n-Mal linkers



Antibody-maytansinoid conjugate linked via both non-cleavable and cleavable linkers

FIG. 21

MS of antibody-maytansinoid conjugate with both disulfide- and non-cleavable PEG4-Mal linkers (prepared by antibody conjugation with an unpurified reaction mixture of DM1 (or DM4) and both SPDB and NHS-PEG_n-Mal linkers). The conjugate had an average load of 3.5 maytansinoid molecules per antibody molecule.



Antibody-maytansinoid conjugate linked via both non-cleavable and cleavable linkers

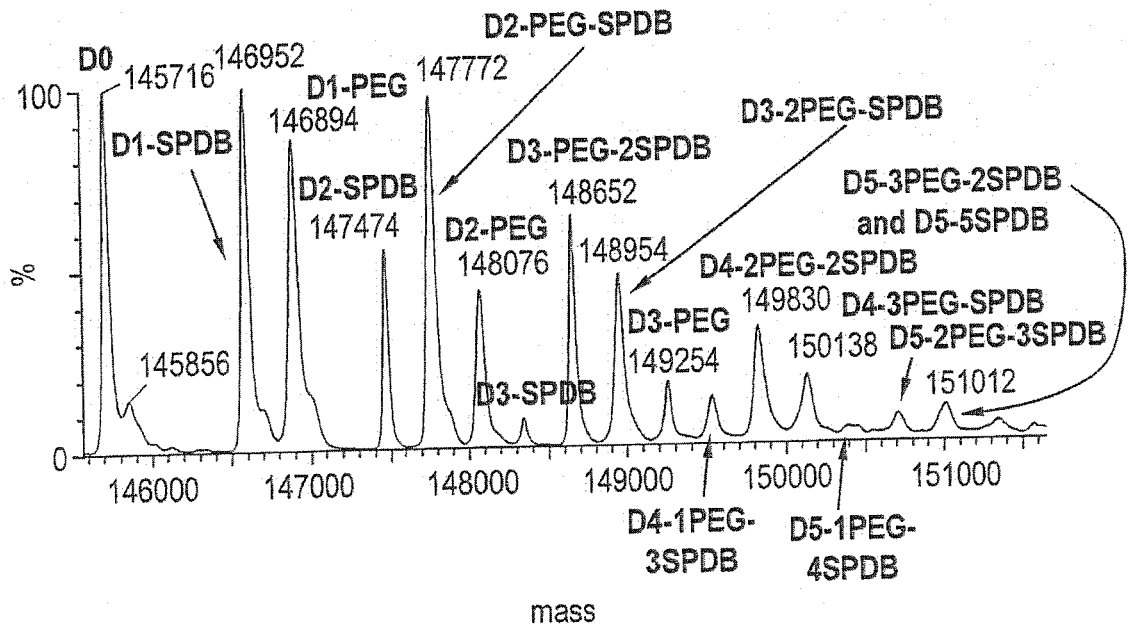
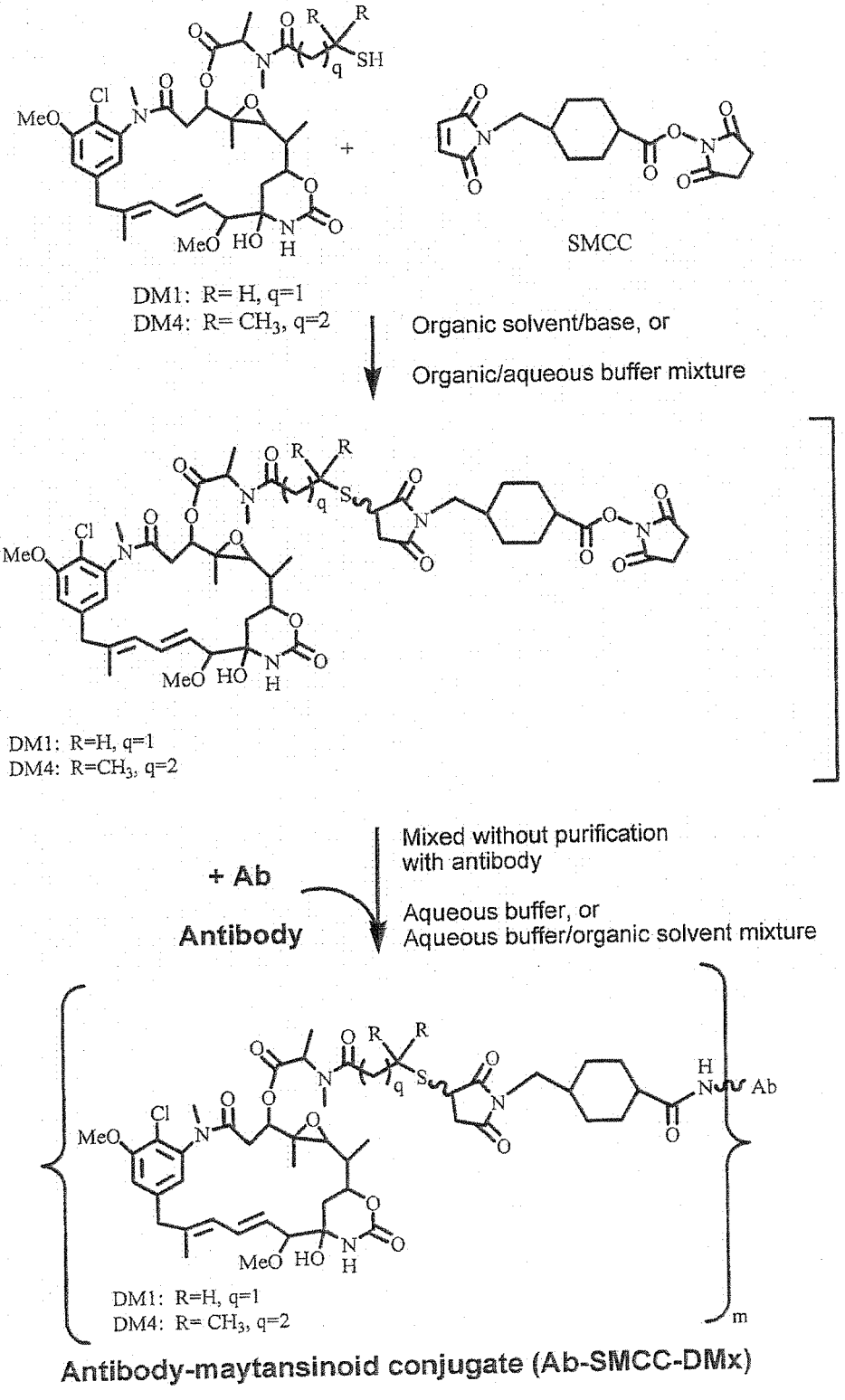


FIG. 22

Conjugation of antibody with a reaction mixture of DM1 (or DM4) and SMCC linker



Antibody-maytansinoid conjugate (Ab-SMCC-DMx)

FIG. 23

MS of Antibody-SMCC-DM1 conjugate prepared using SMCC by the method described in this invention, containing average 3.1 DM1 per antibody

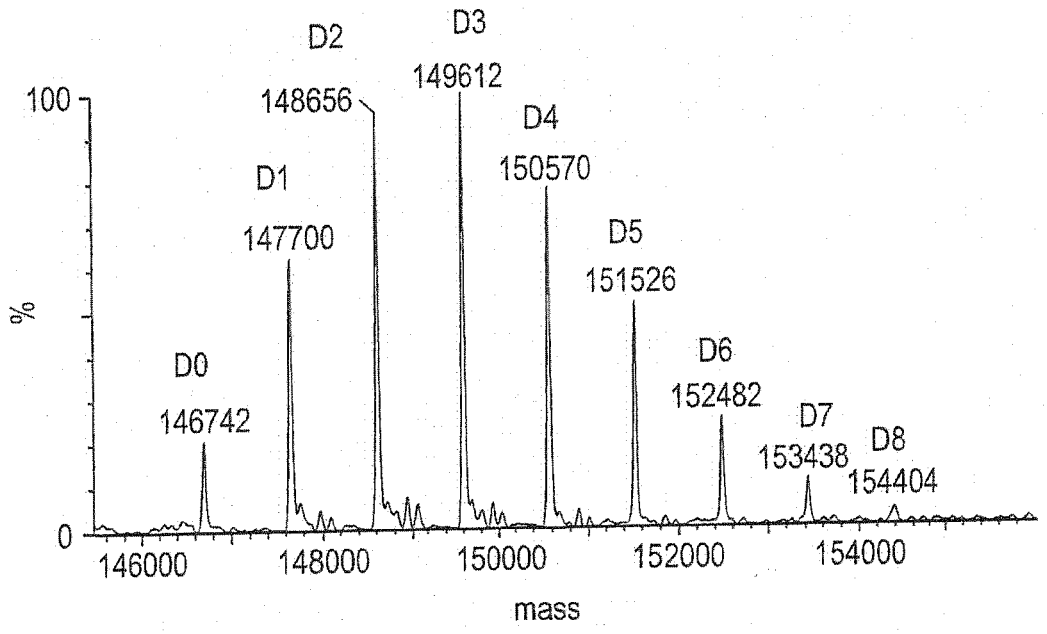
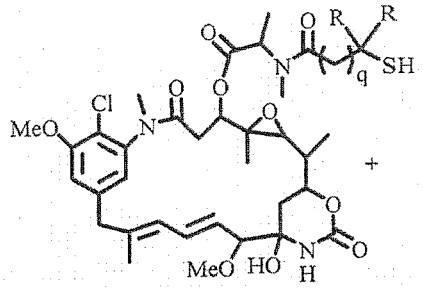
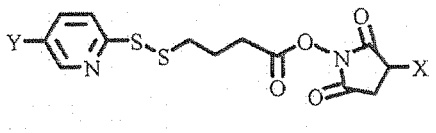


FIG. 24

Preparation of disulfide-linked conjugate of antibody using a reaction mixture of DM1 (or DM4) and SSNPB linker



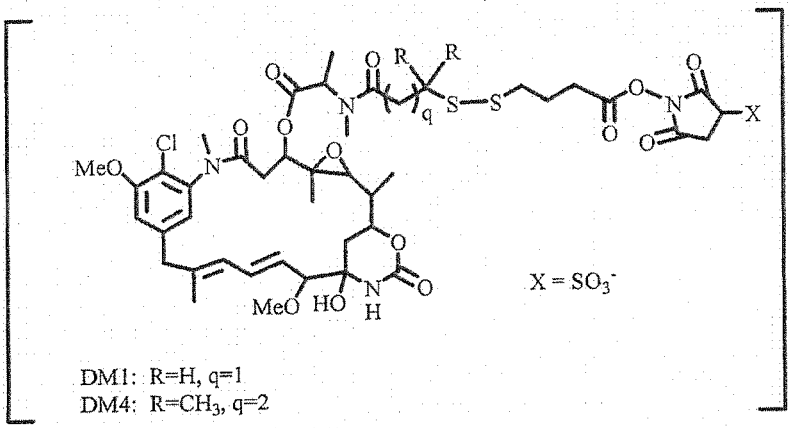
DM1: R= H, q=1
DM4: R= CH_3, q=2



SSNPB linker: X = SO_3^-, Y = NO_2

Organic solvent/base, or

Organic/aqueous buffer mixture



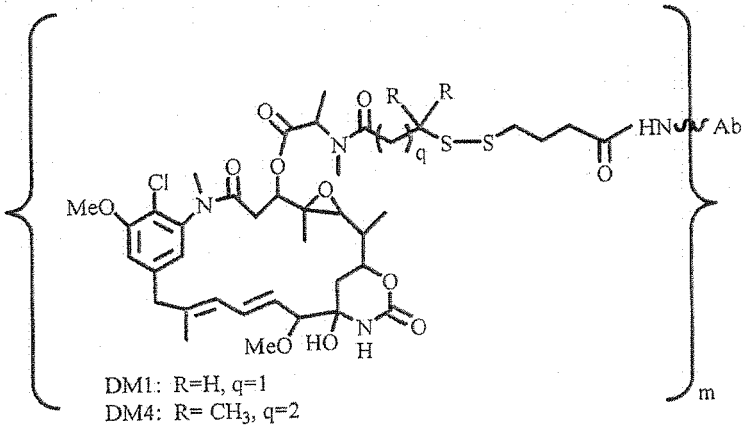
DM1: R=H, q=1
DM4: R=CH_3, q=2

Mixed without purification with antibody

+ Ab

Antibody

Aqueous buffer, or
Aqueous buffer/organic solvent mixture



DM1: R=H, q=1
DM4: R= CH_3, q=2

Antibody-maytansinoid conjugate with cleavable, disulfide linker

FIG. 25

Conjugation of antibody with a reaction mixture of DM1 (or DM4) and heterobifunctional linker with aliphatic linear carbon chain

