

FIG. 1A

Mass spectrograms of deglycosylated huMy9-6-2 conjugates prepared without and with bisulfite, containing 1.4 DAR (A) and 3.1 DAR (B), respectively.

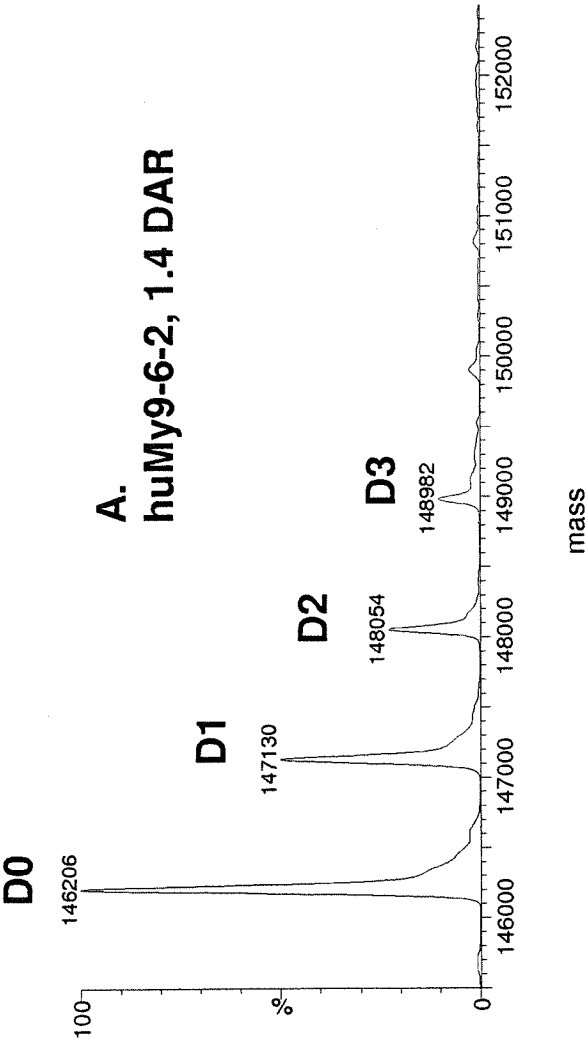


FIG. 1B

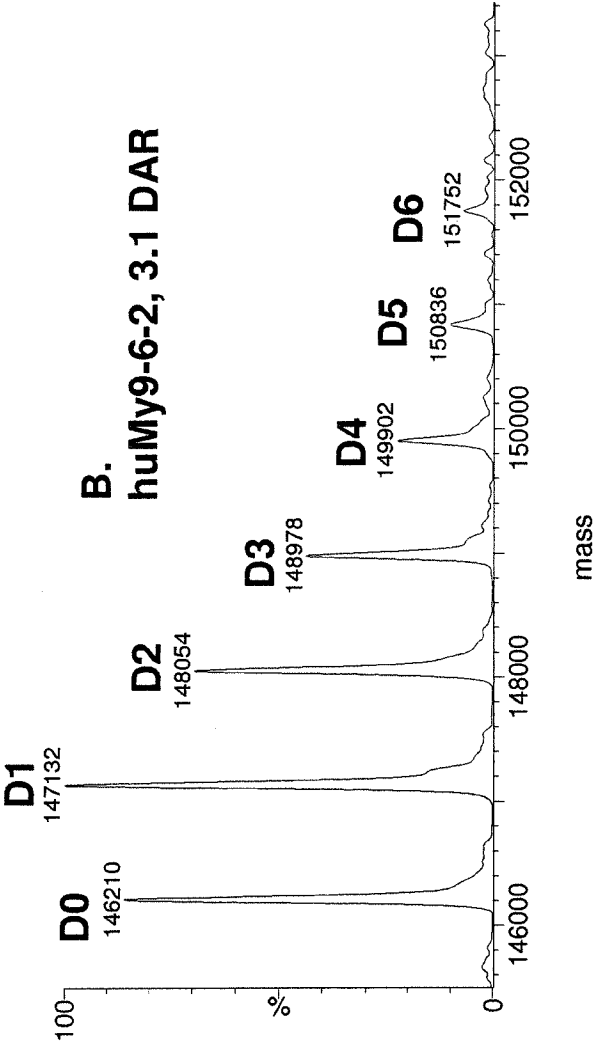


FIG. 2

HuMy9-6-2 conjugates prepared without and with sodium bisulfite show similar *in vitro* cytotoxicity toward CD33-antigen expressing HL60 cells

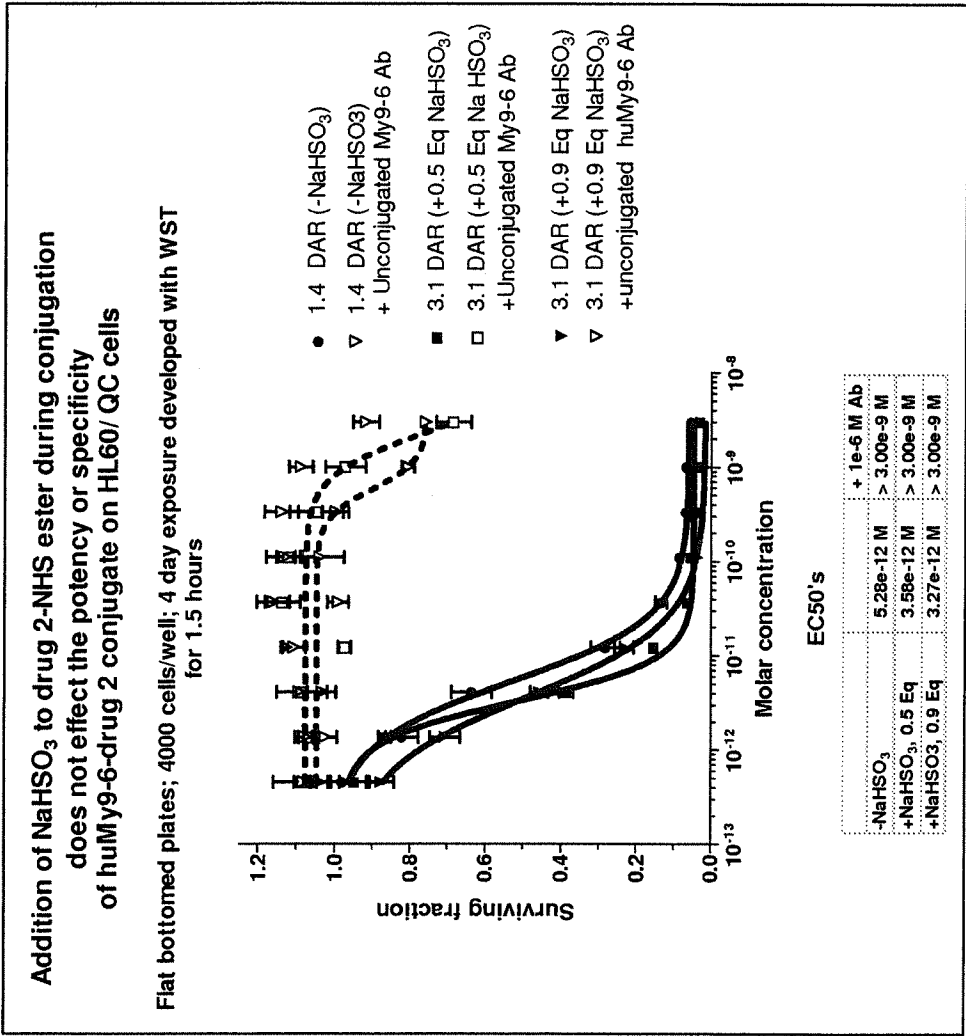


FIG. 3

Anti-CD22 Ab-2 conjugates prepared without and with sodium bisulfite show similar *in vitro* cytotoxicity toward CD22-antigen expressing BJAB cells.

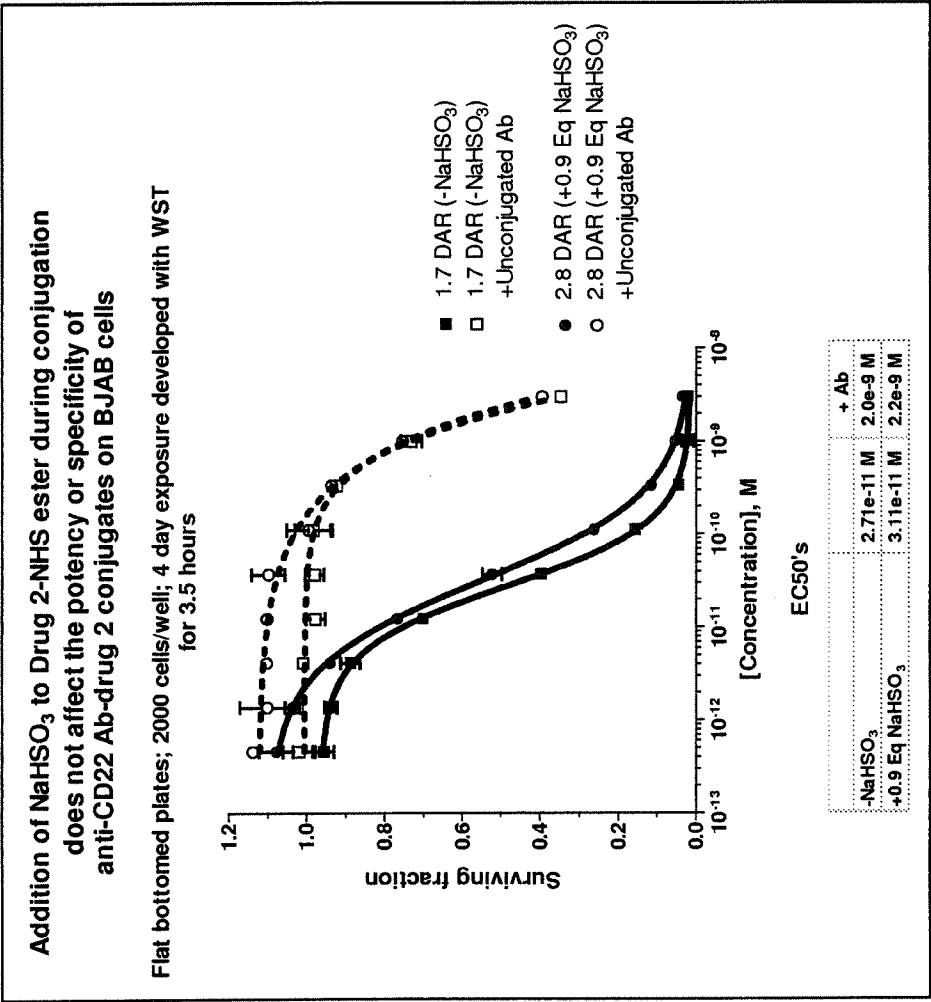


FIG. 4
Reverse phase HPLC analysis of 2-NHS and sodium bisulfite-treated 2-NHS

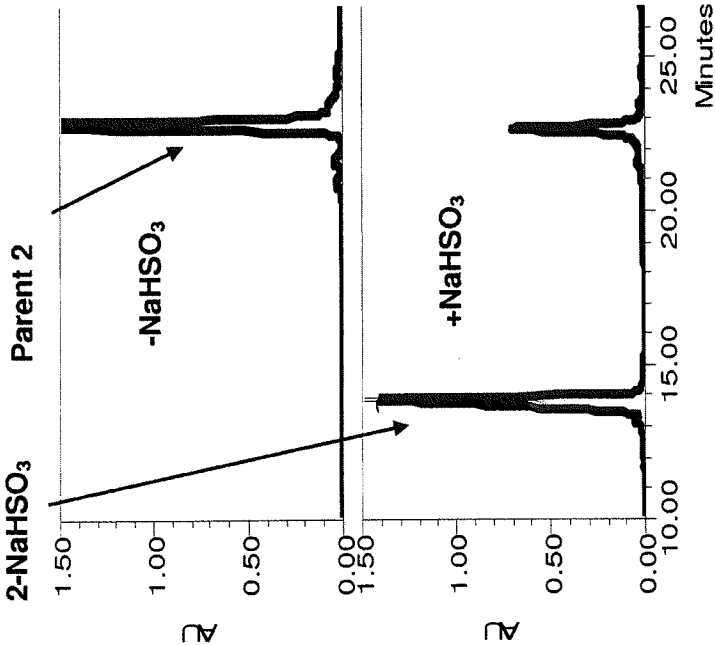


FIG. 5

MS analysis of deglycosylated huMy9-6-SPDB-1 prepared with and without sodium bisulfite using 7 molar equivalents of 1-SPDB-NHS per antibody. A. Conjugate prepared without sodium bisulfite with average 1.4 1/Ab and antibody species with up to three linked 1 molecules. B. Conjugate prepared with sodium bisulfite with average of 2.5 1/Ab and antibody species with up to seven linked 1 molecules.

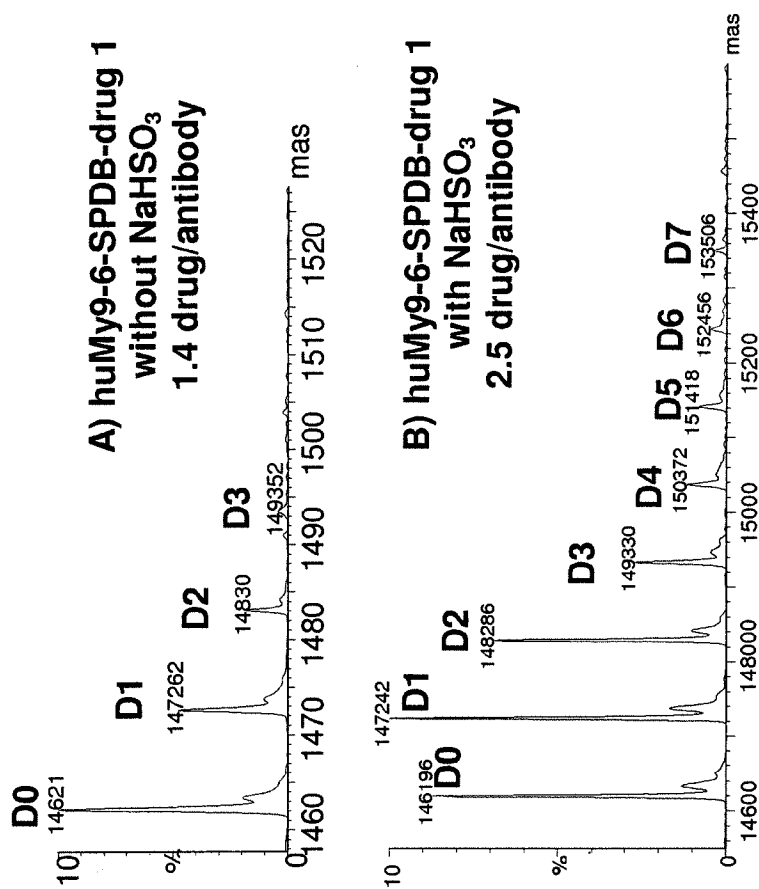


FIG. 6

Addition of sodium bisulfite in 1-SPDB-NHS conjugation reaction
did not result in fragmentation of antibody (non-reducing SDS-PAGE; gel chip analysis)

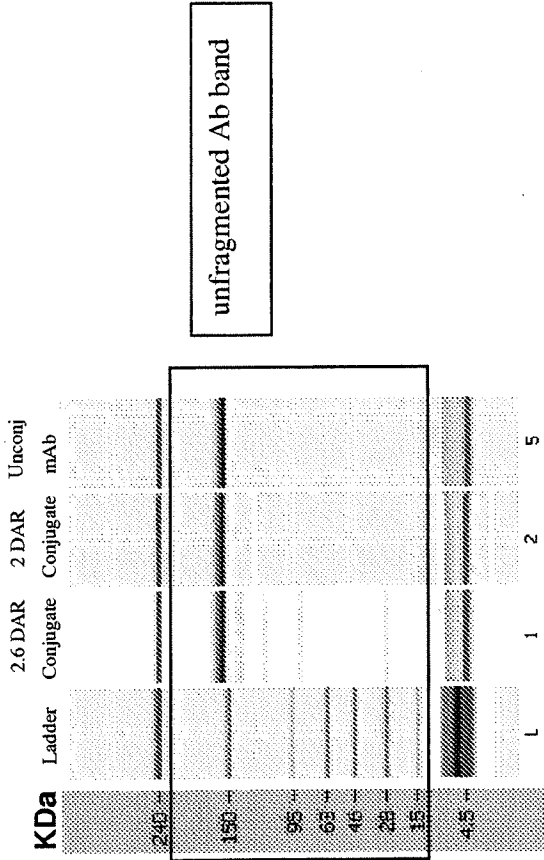


FIG. 7

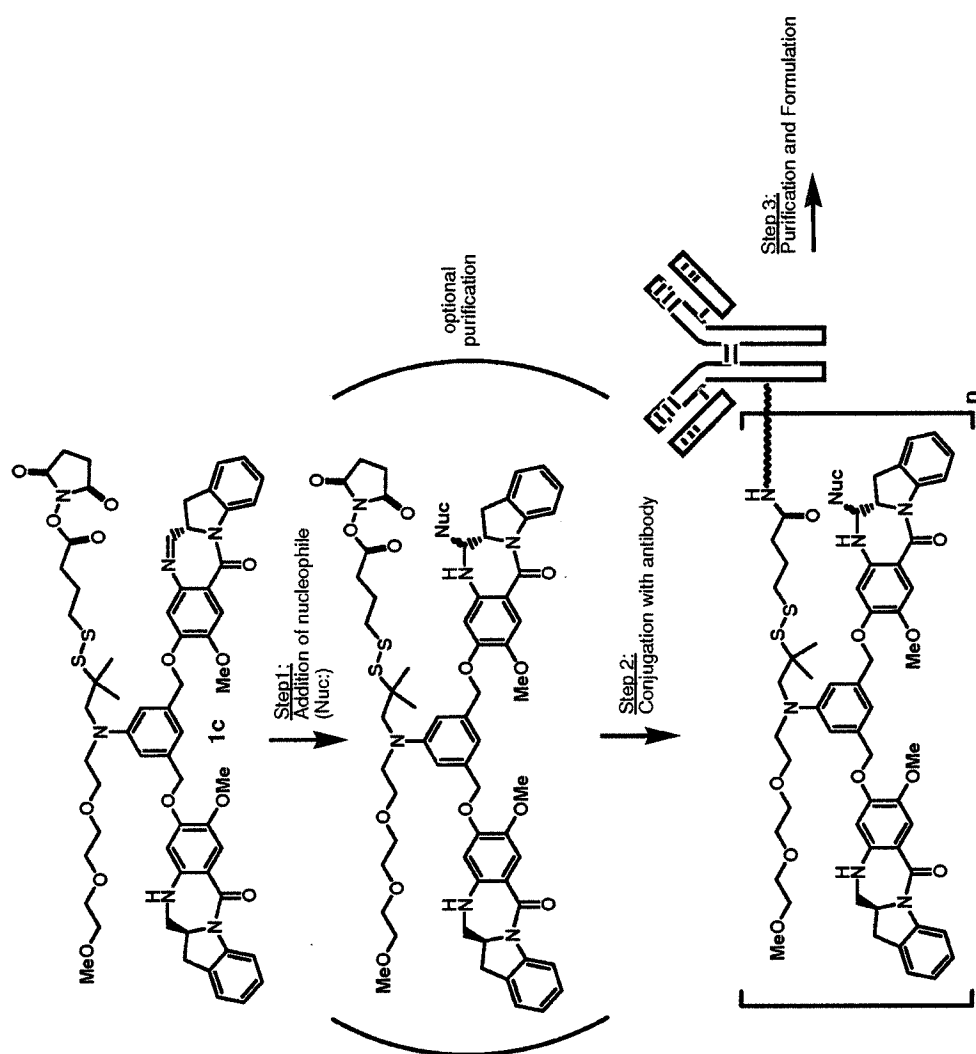
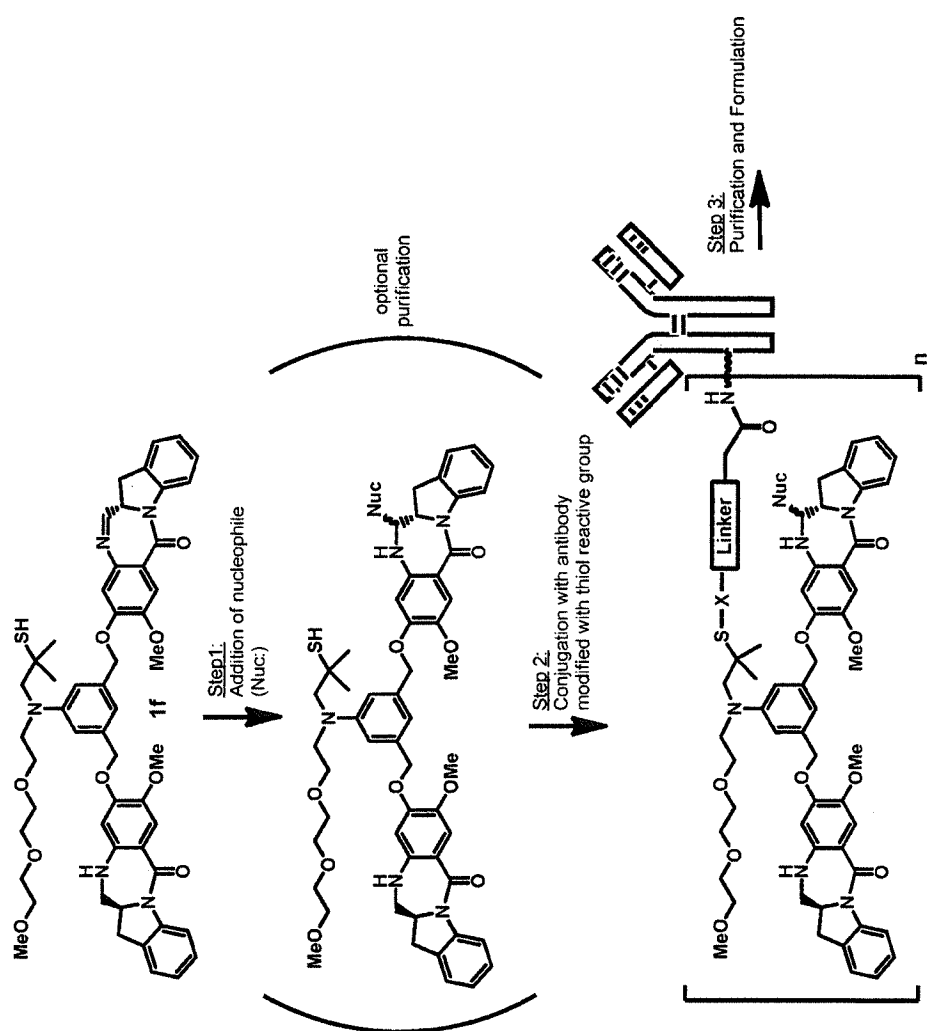


FIG. 8



Chemical reaction scheme for the synthesis of a nucleoside-antibody conjugate:

Step 1: Addition of nucleophile (Nuc-) to precursor molecule (6a or 6b) to form a thiol intermediate.

Step 2: Conjugation with antibody containing thiol groups to form a conjugate.

Step 3: Purification and Formulation of the final conjugate.

FIG. 10

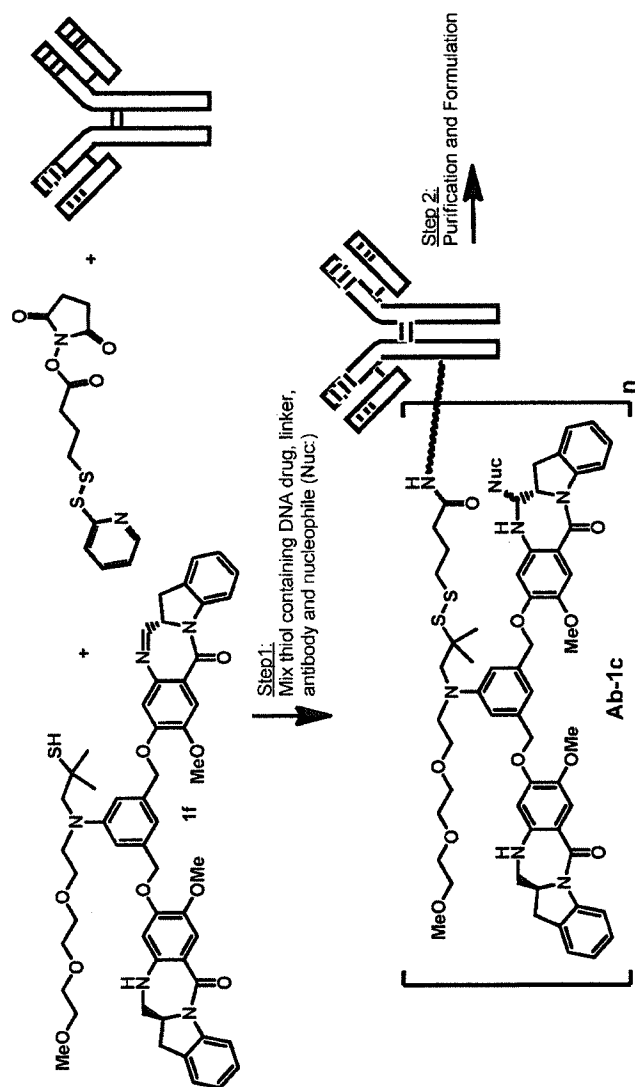


FIG. 11

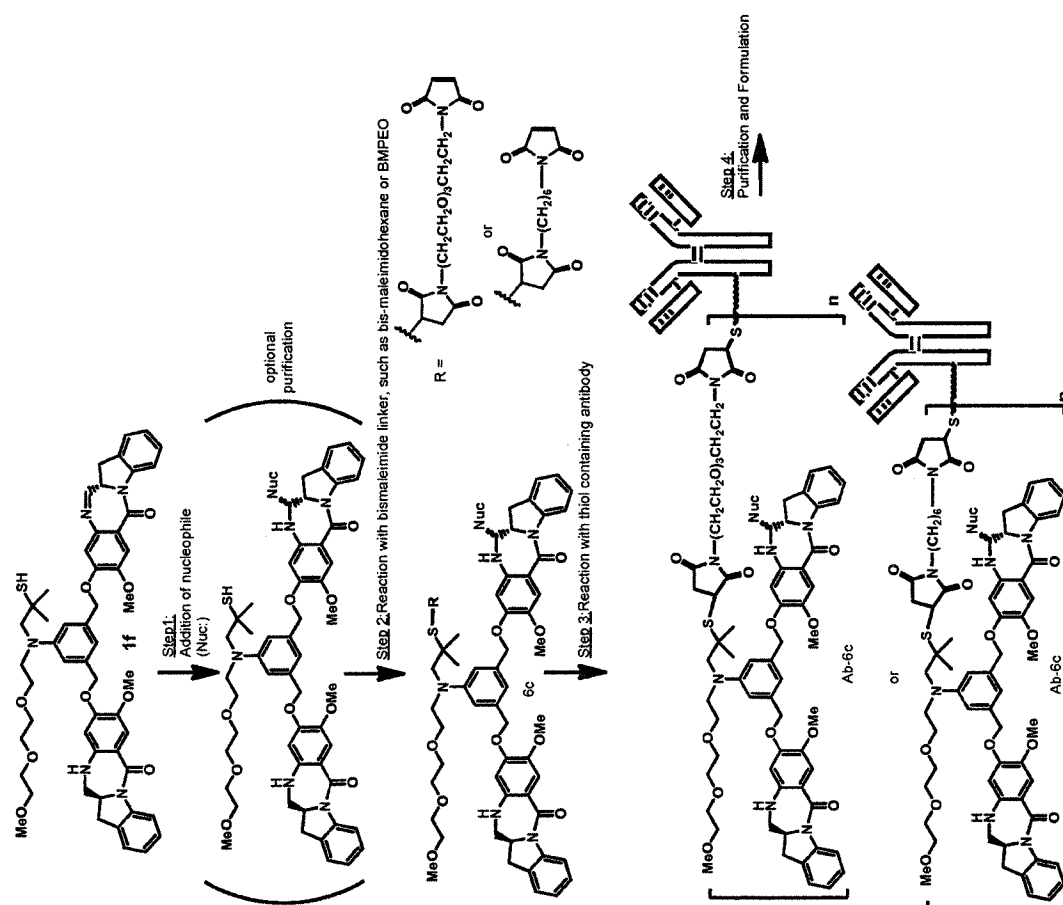


FIG. 12

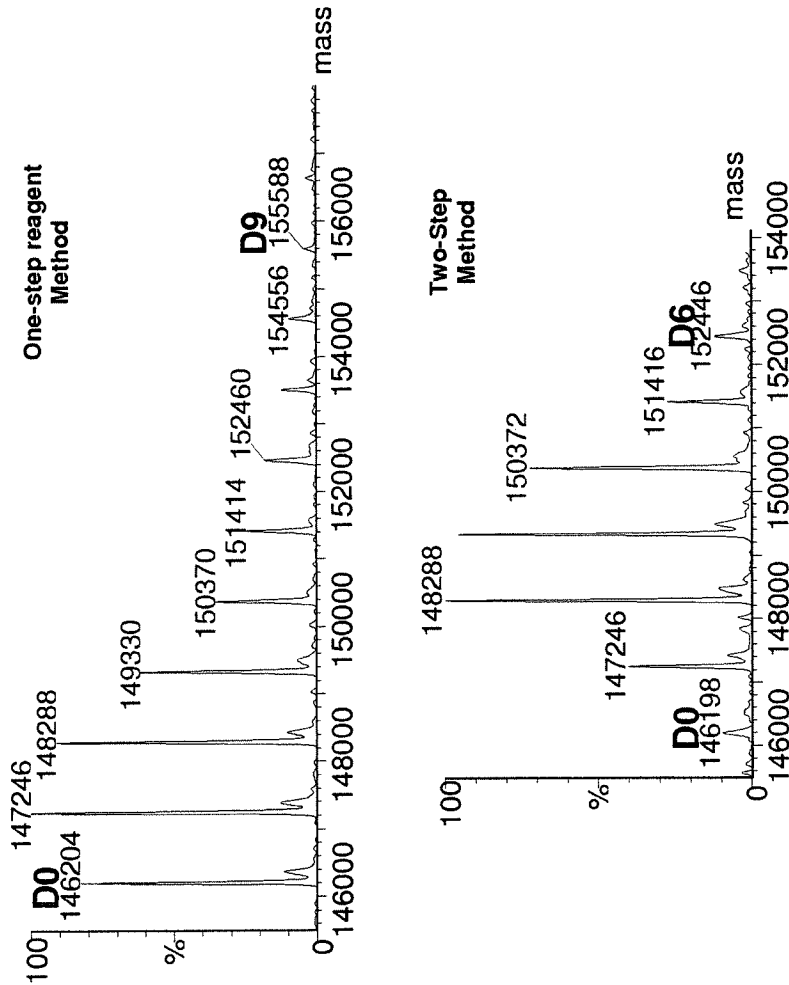


FIG. 13

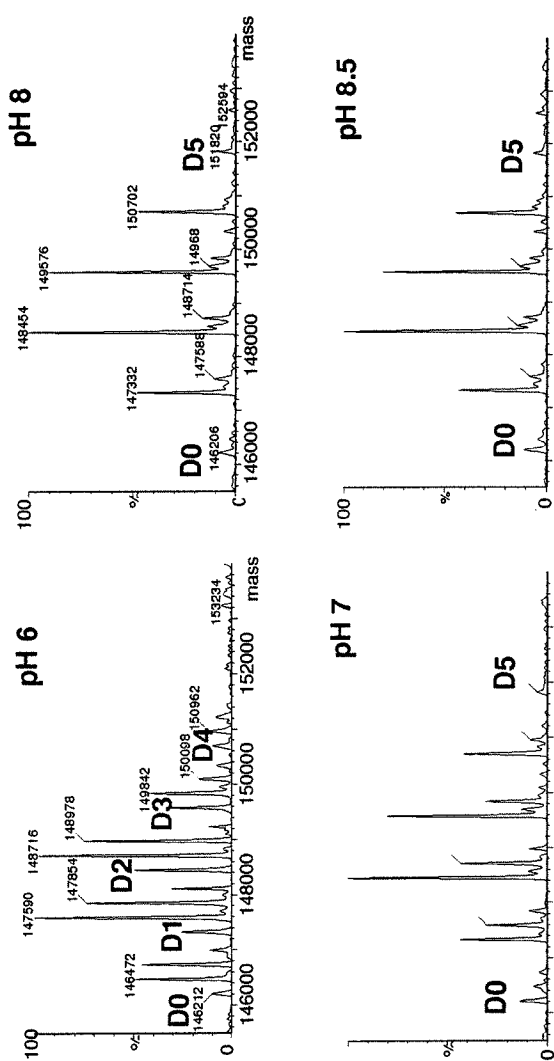


FIG. 14

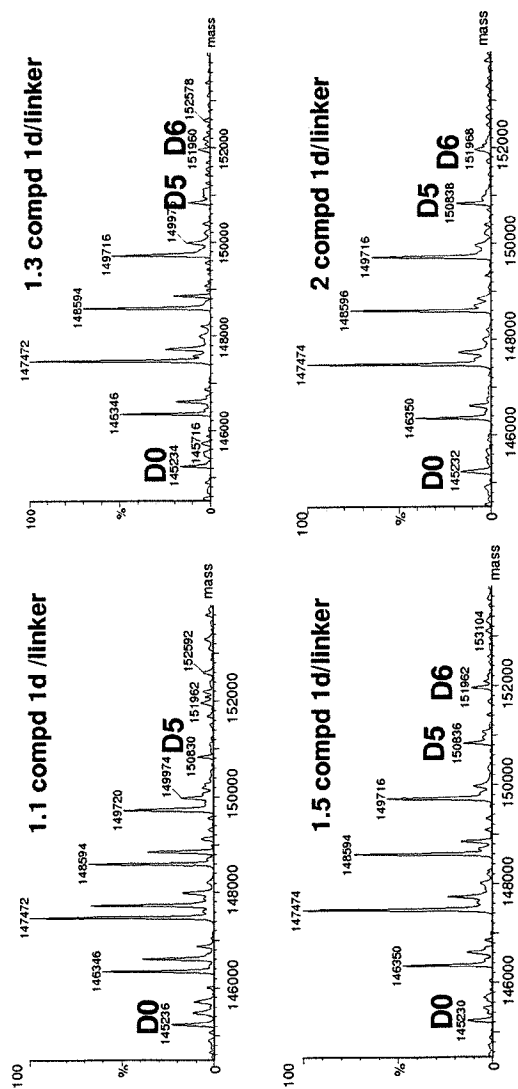
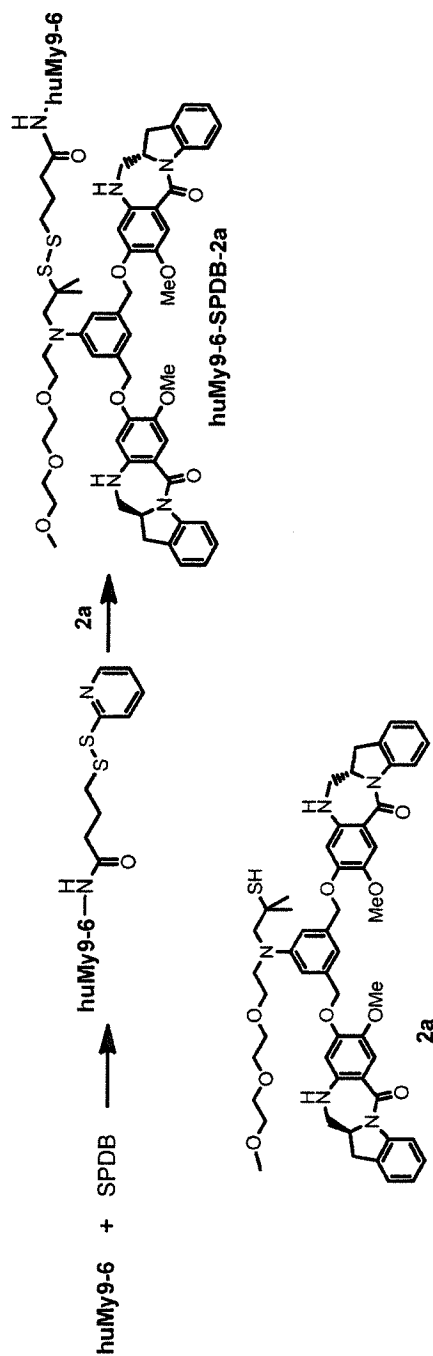


FIG. 15

Reactant	Drug/Ab (UV)	% monomer (SEC)	% drug on monomer
Sodium Hydrosulfite	2.6	88	82
Sodium Bisulfite	2.6	88	83
Sodium Metabisulfite	2.7	88	82
No additive	0.1	98	94

FIG. 16
Two-step Conjugation Scheme



Representative thiol reactive linkers:

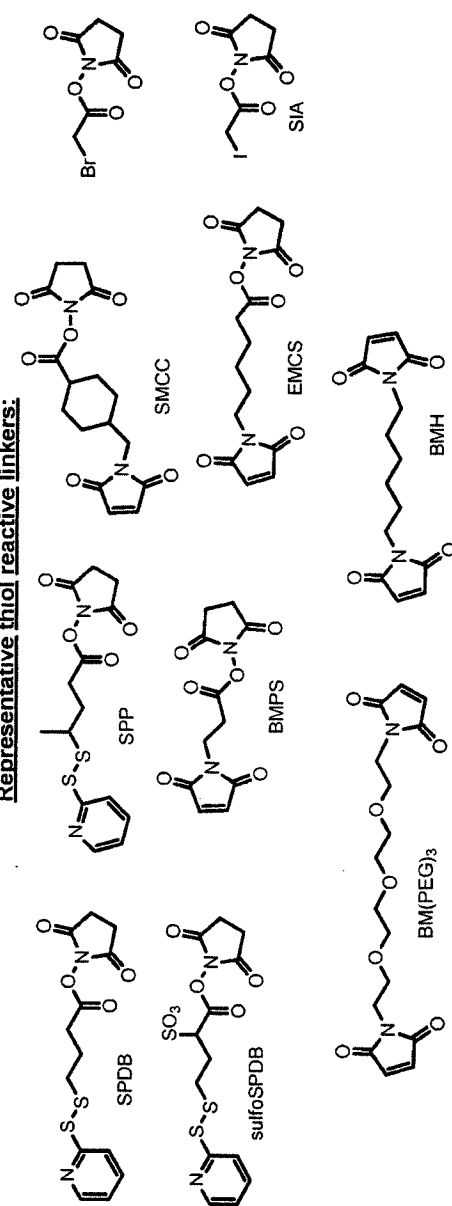
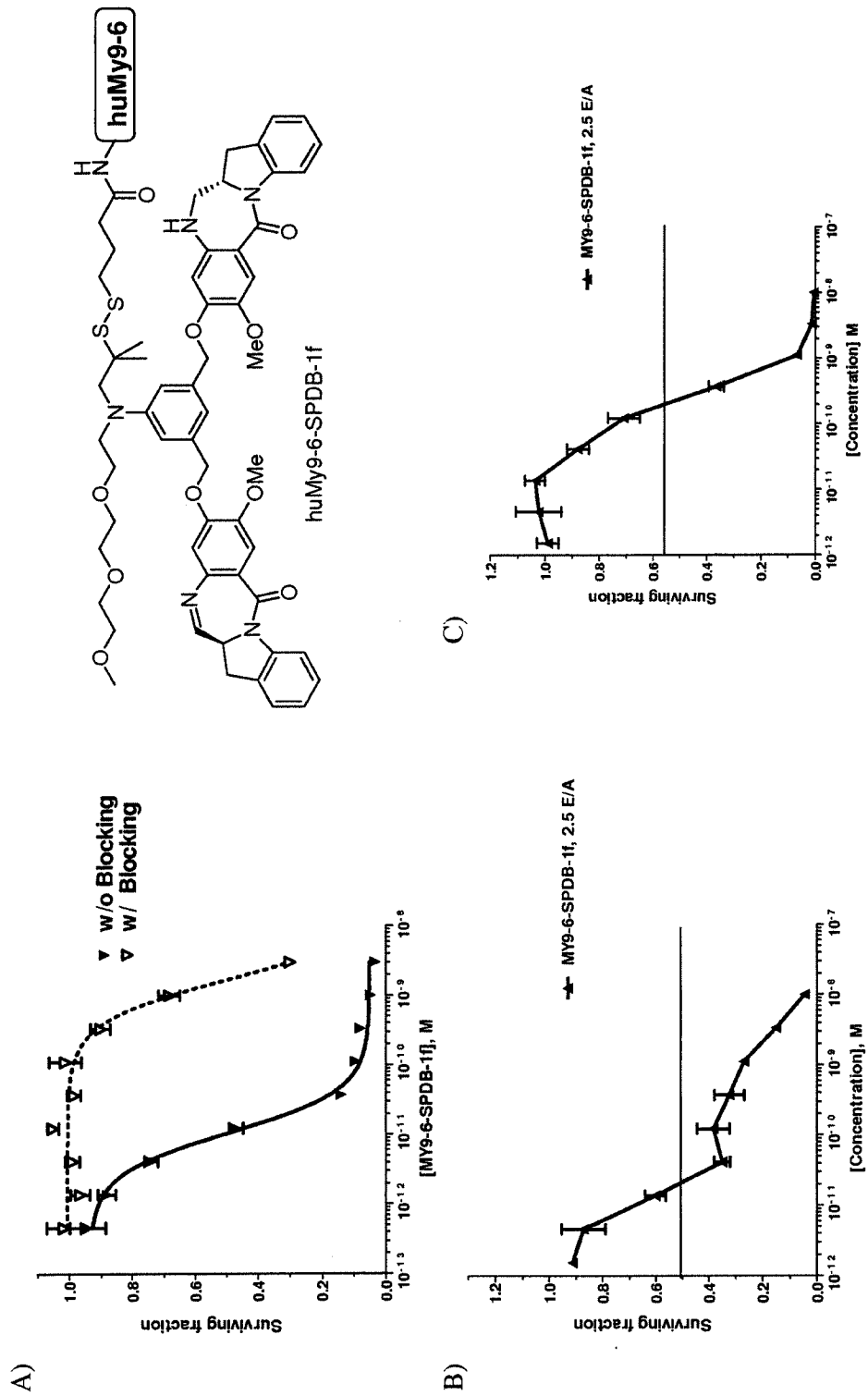


FIG. 17

Antiproliferative Activity of huMY9-6-SPDB-1f against (A) HL60/QC (Ag^+) cells with and without blocking of antigen binding sites, (B) HL60/ATCC (Ag^+) cells, and (C) NB-4 (Ag^+) cells



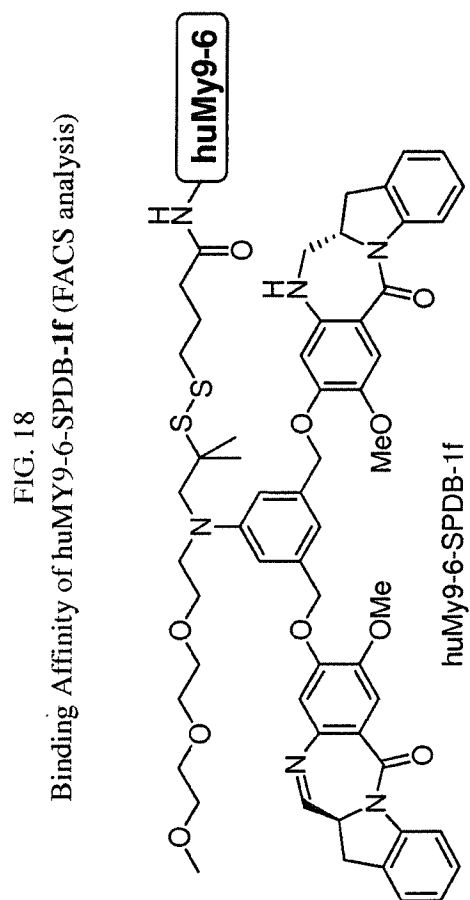
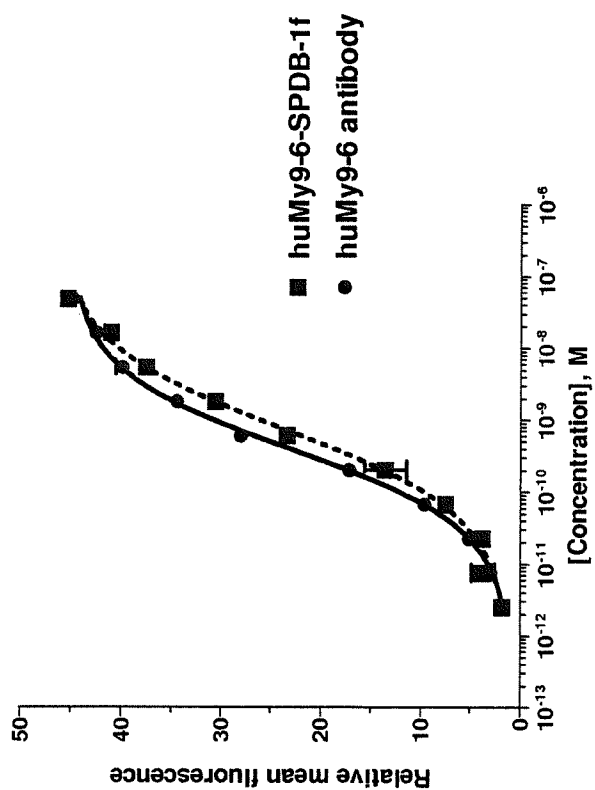


FIG. 19
In vivo Efficacy of huMy9-6-SPDB-1f in HL60/QC Tumor Bearing Nude Mice

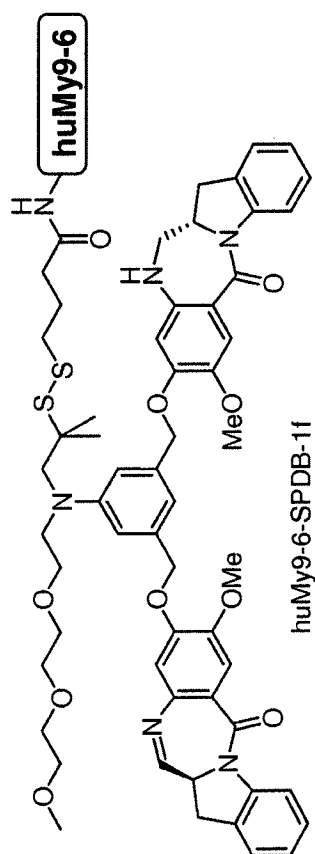
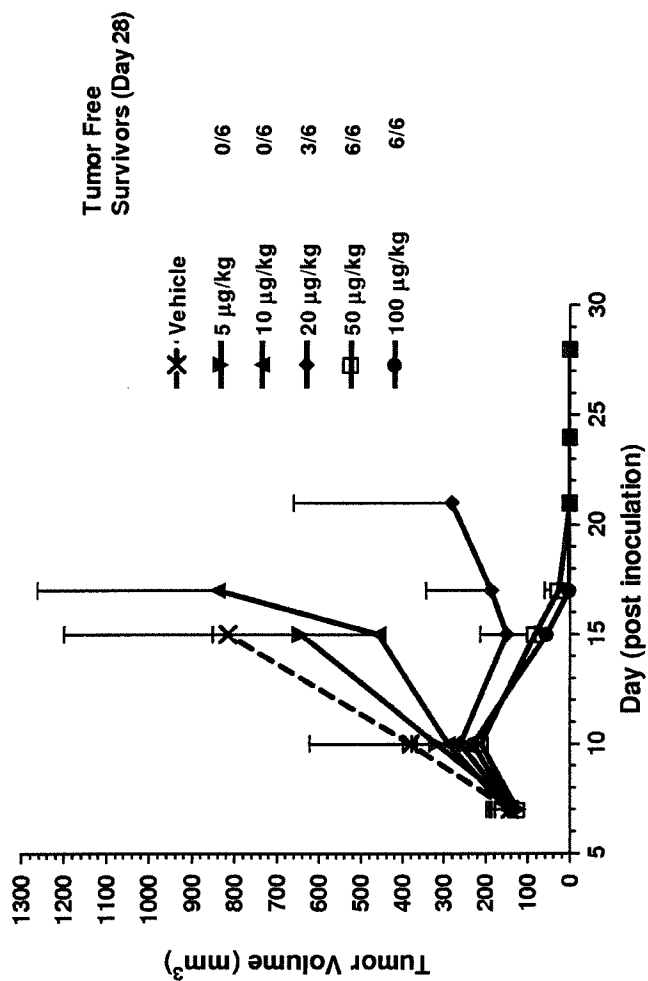
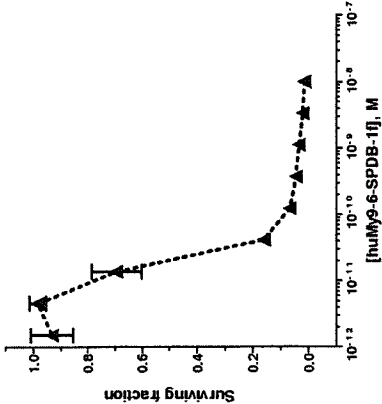
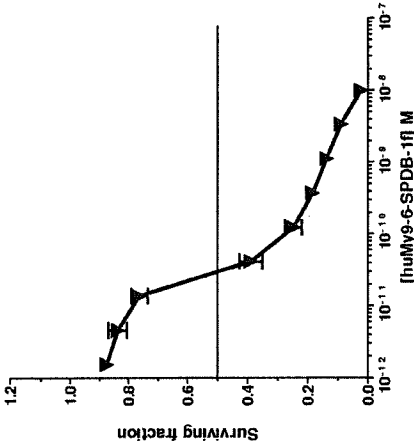


FIG. 20
Antiproliferative Activity of huMY9-6-SPDB-1f against Antigen Positive Cell Line (A) HL60/QC cells, (B) HL60/ATCC cells, (C) NB-4 cells, and HEL 92.1.7 cells (D)

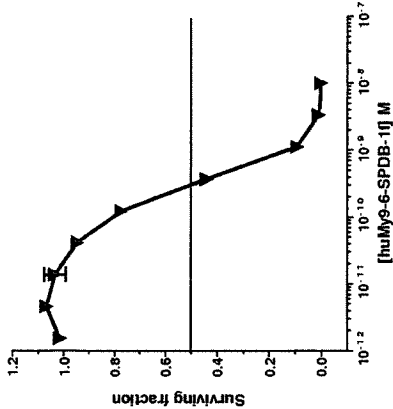
A)



B)



C)



D)

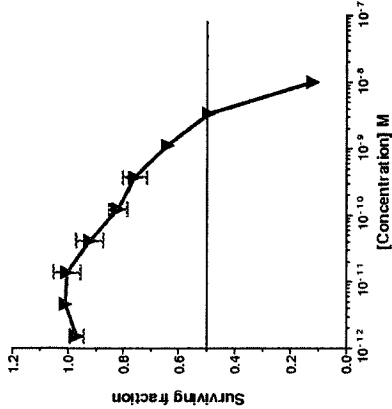
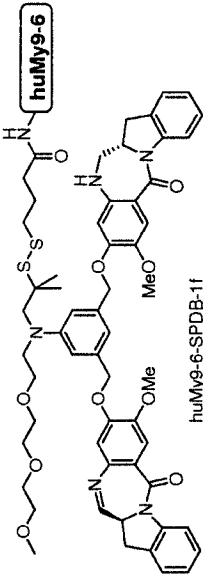


FIG. 21
Antiproliferative Activity Comparing (A) huMy9-6-SPDB-1f and (B) huMy9-6-sulfoSPDB-1f, and (C) huMy9-6-BMPS-1f against HL60/QC (Ag⁺) Cells with and without Blocking of Antigen Binding Sites

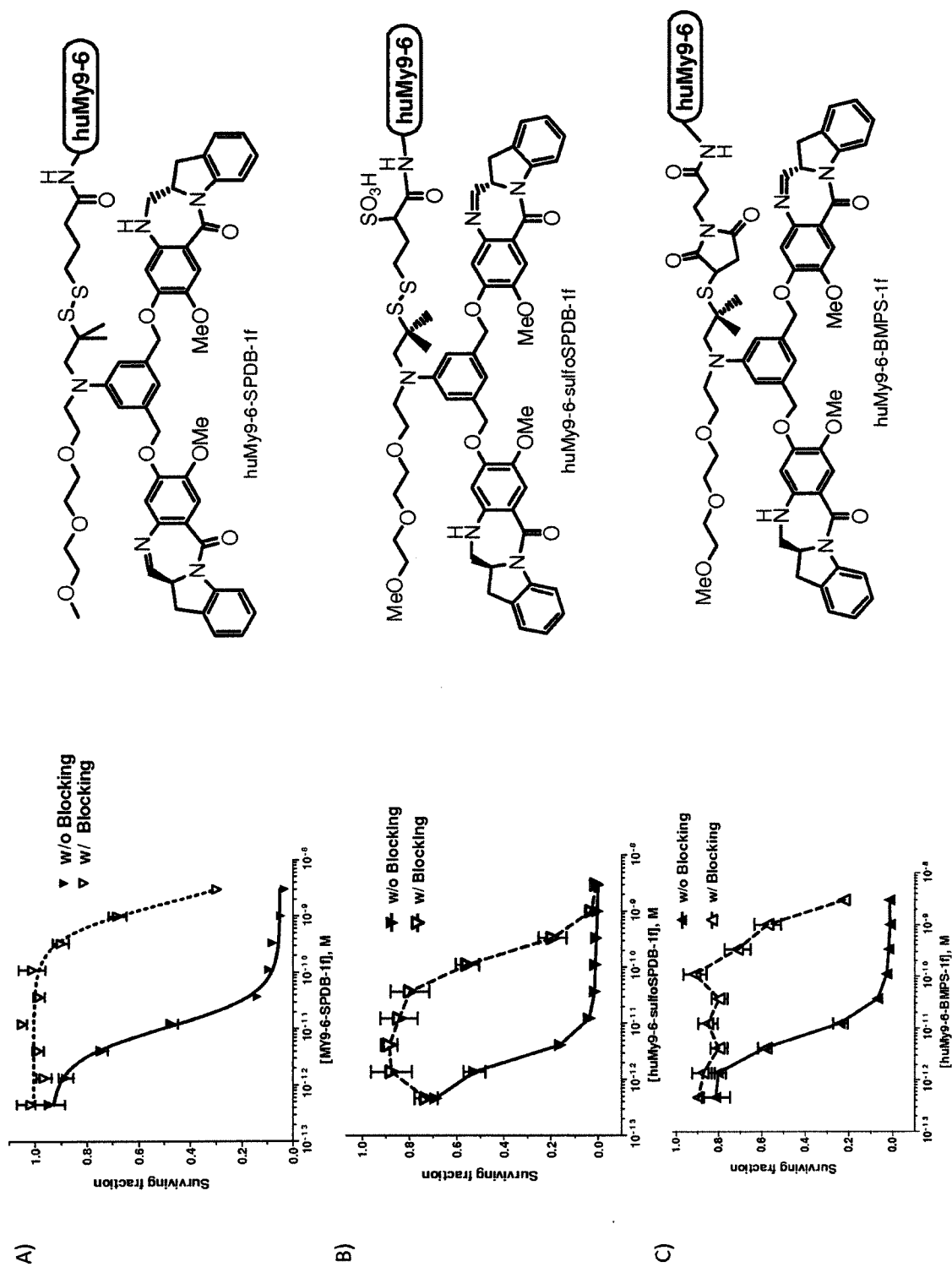


FIG. 22
Antiproliferative Activity Comparing (A) chB38.1-SPDB-1f and (B) chB38.1-sulfoSPDB-1f against COLO205 (Ag^+) cells

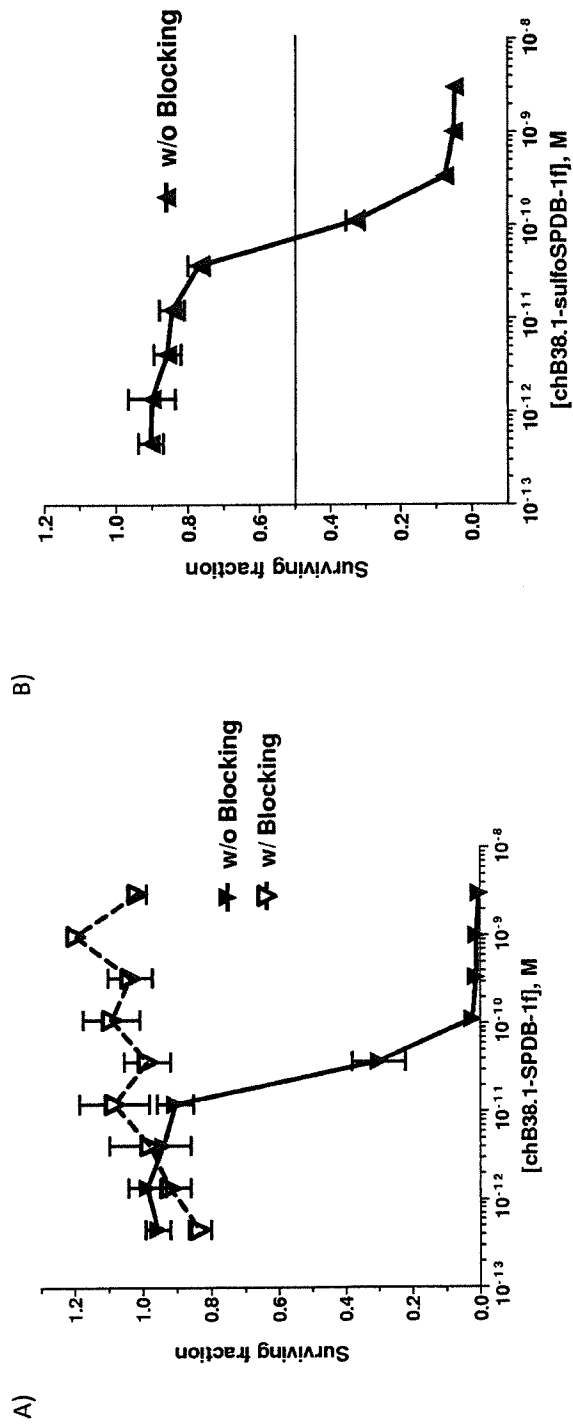


FIG. 23
In vivo Efficacy of huMy9-6-SPDB-1f in HL60/QC Tumor Bearing Mice

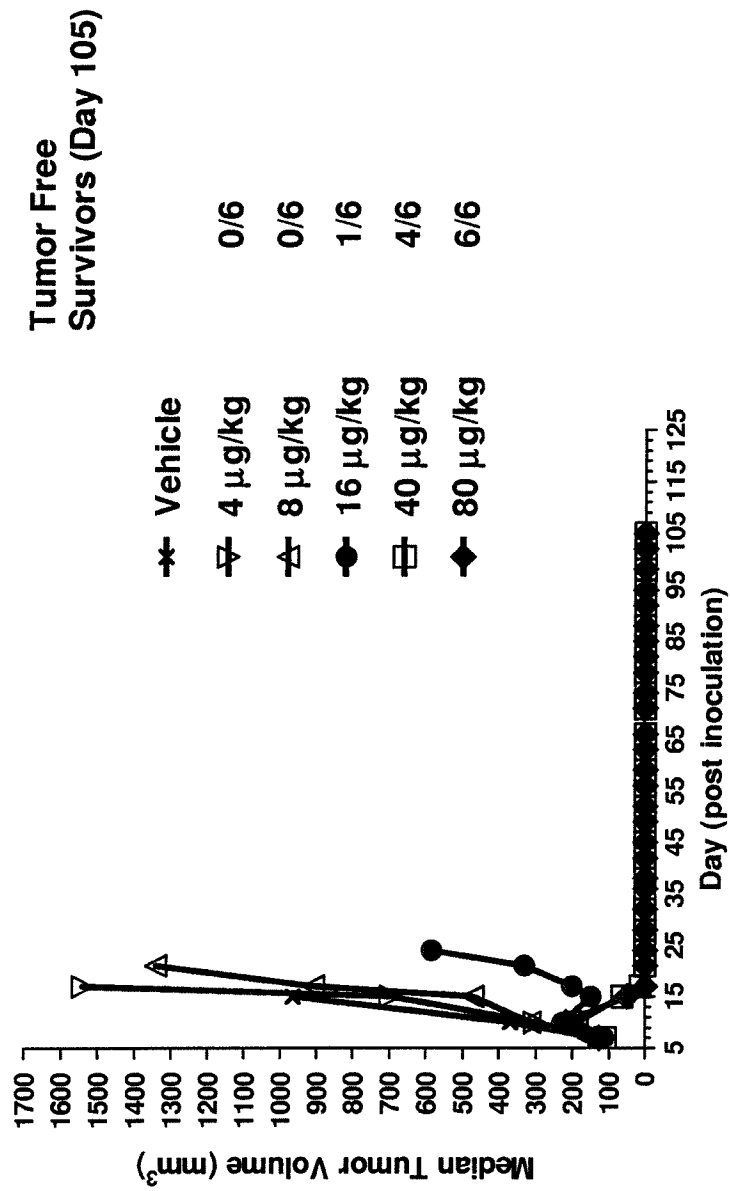
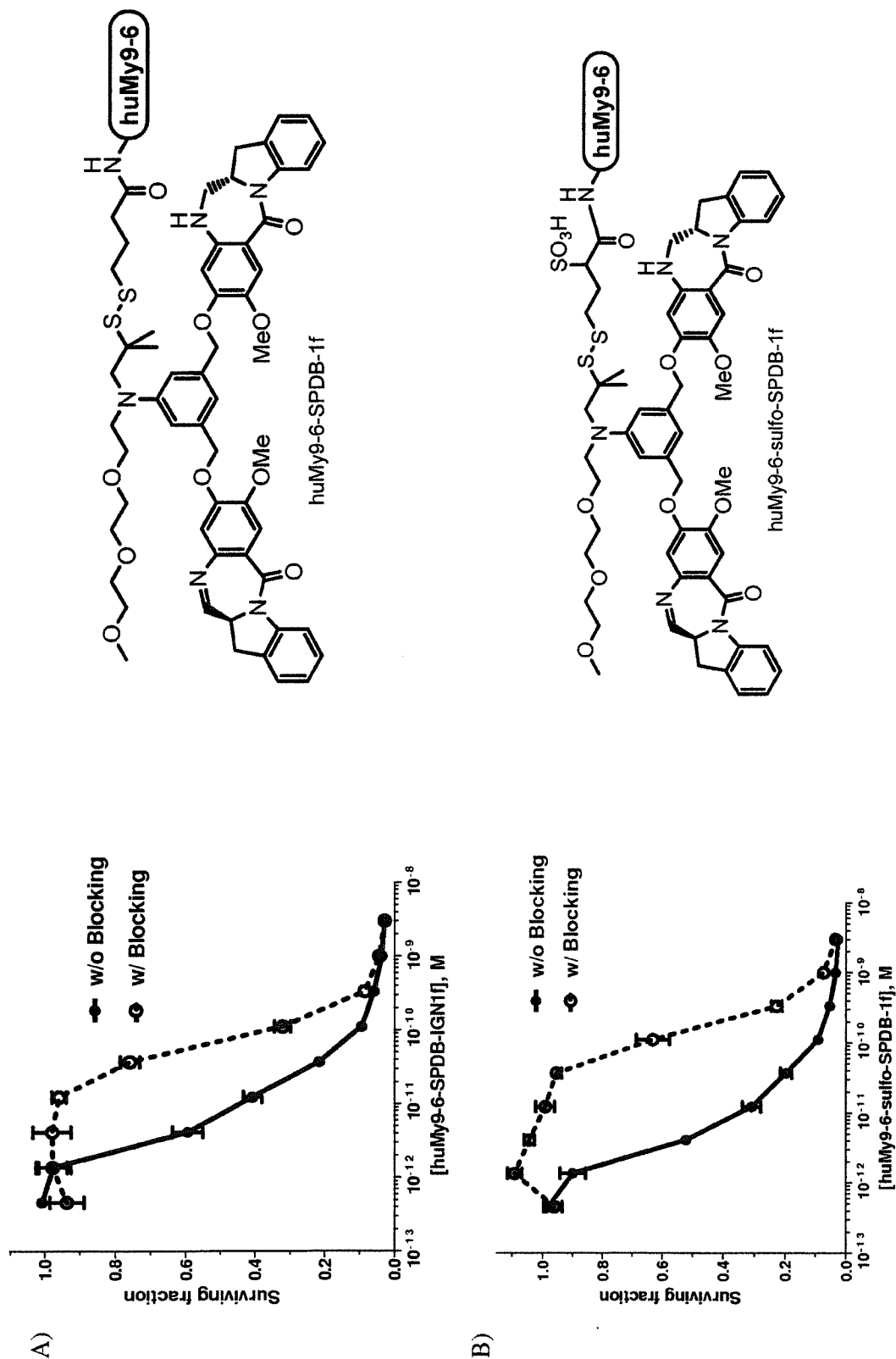


FIG. 24

Antiproliferative Activity comparing (A) huMy9-6-SPDB-1f, (B) huMy9-6-sulfoSPDB-1f, and, (C) huMy9-6-BMPS-1f against OCI-AML3 (Ag⁺) cells with and without blocking of antigen binding sites



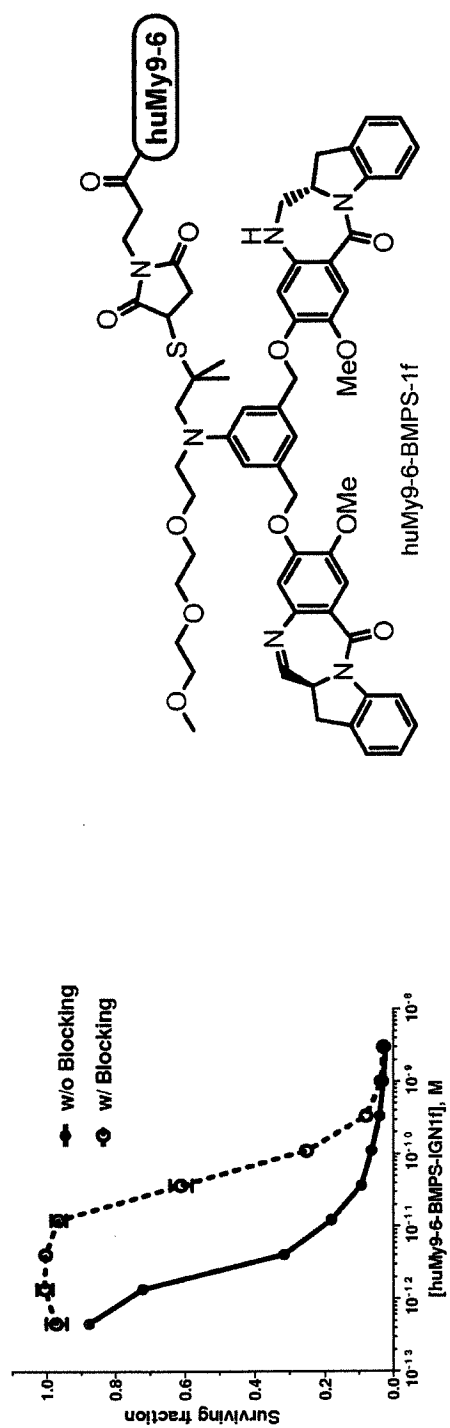


FIG. 25
In vivo Efficacy of huMy9-6-BMPS-1f in MOLM-13 Tumor Bearing Mice

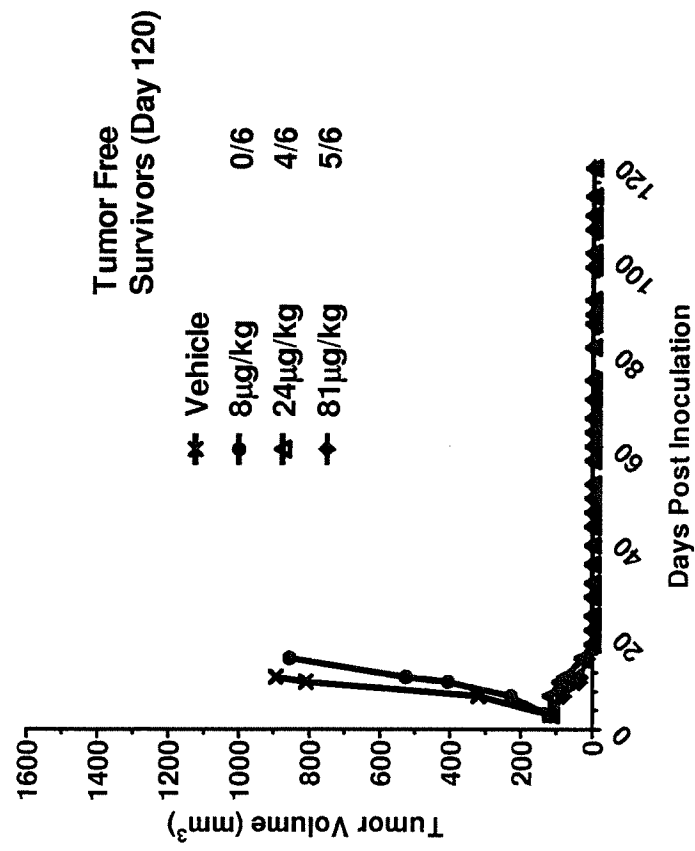


FIG. 26
Representative Synthesis Scheme for a Sulfonated Folate / Cytotoxic Compound Conjugate

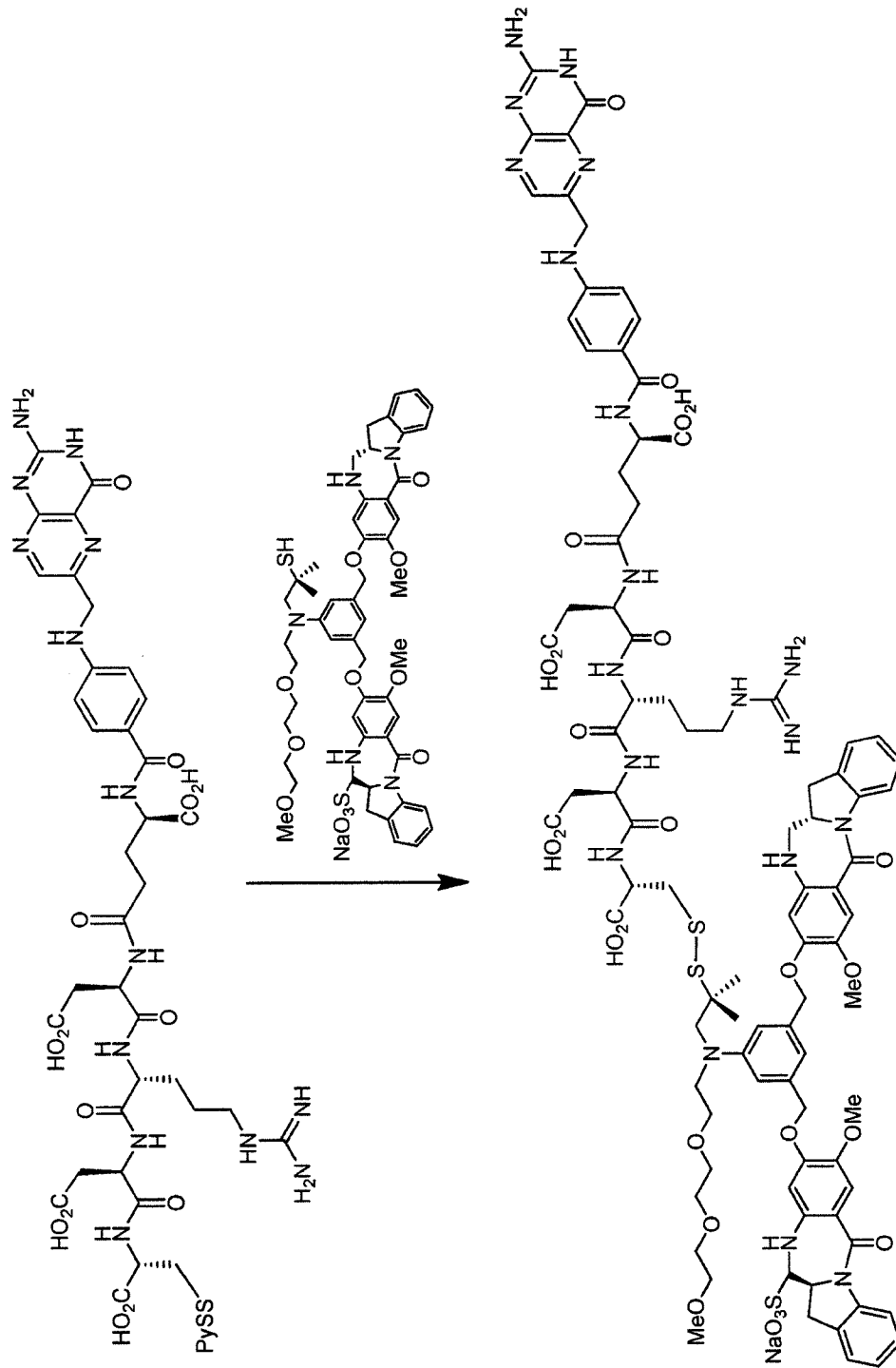


FIG. 27
In vivo Efficacy of huMy9-6-**drug 2** in MOLM-13 Tumor Bearing Mice

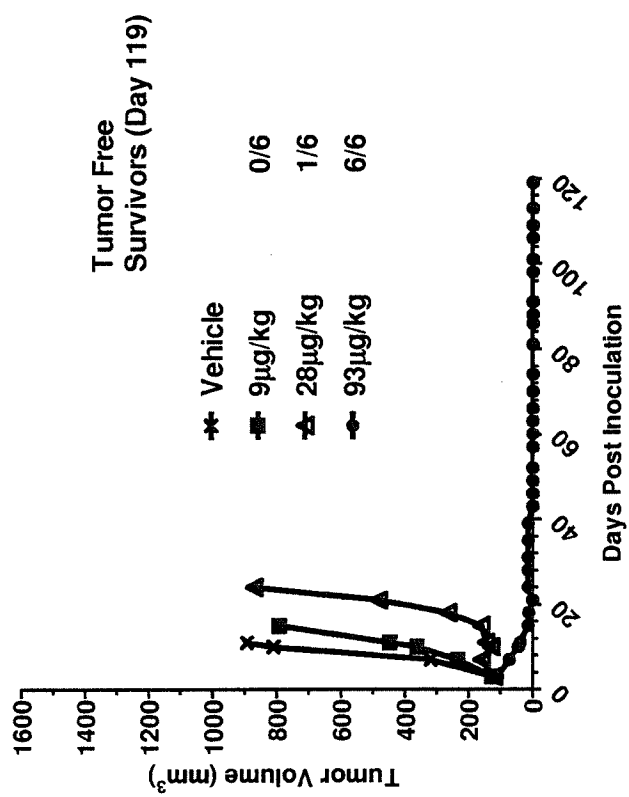


FIG. 28

