FIGURE 1. Structural representation of representative PEG-containing non-cleavable thiosuccinimyl-linked conjugates of the present invention

(mAb = monoclonal antibody; n = 1-2000; m = 2-15)

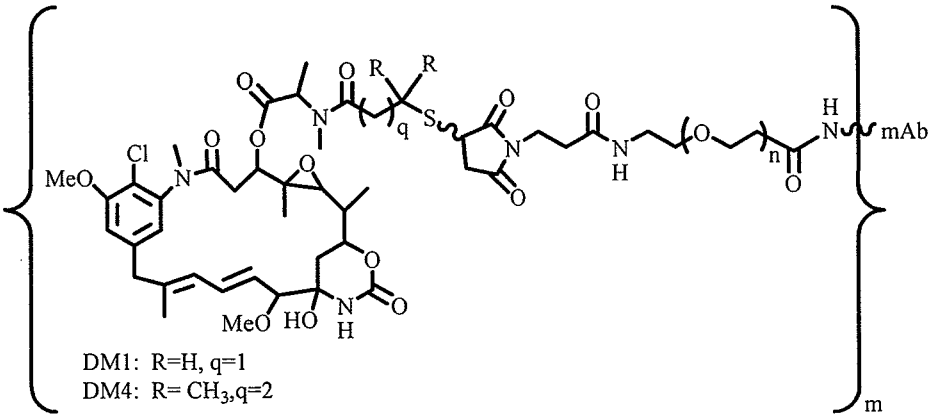


FIGURE 2.

Structural representation of representative PEG-containing non-cleavable thioacetamidyl-linked conjugates of the present invention (n = 1-2000; m = 2-15)

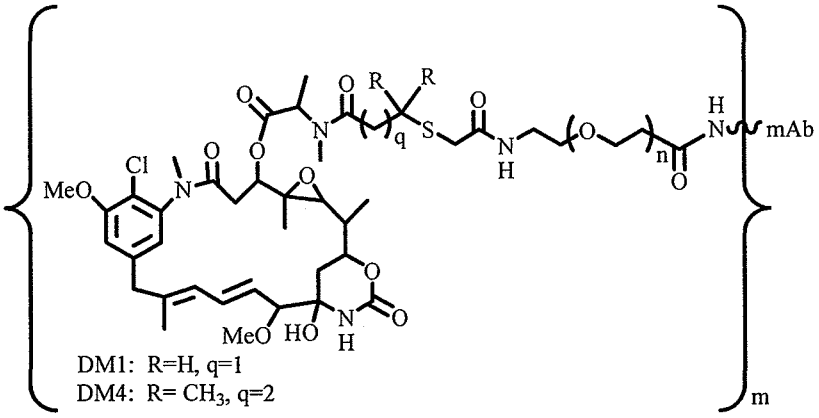


FIGURE 3. Structural representation of representative PEG-containing disulfide linked compounds of the present invention (n = 1-14; m = 3-8)

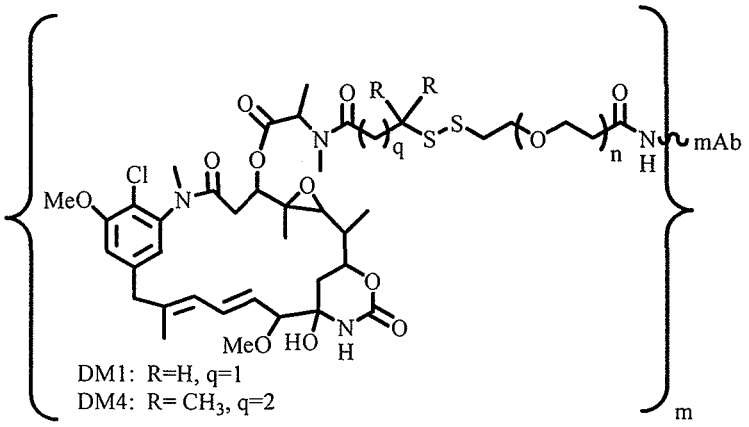


FIGURE 4. Synthetic scheme for PEG-containing compounds (non-cleavable thiosuccinimidyl-linked) of the present invention (n = 1-2000)

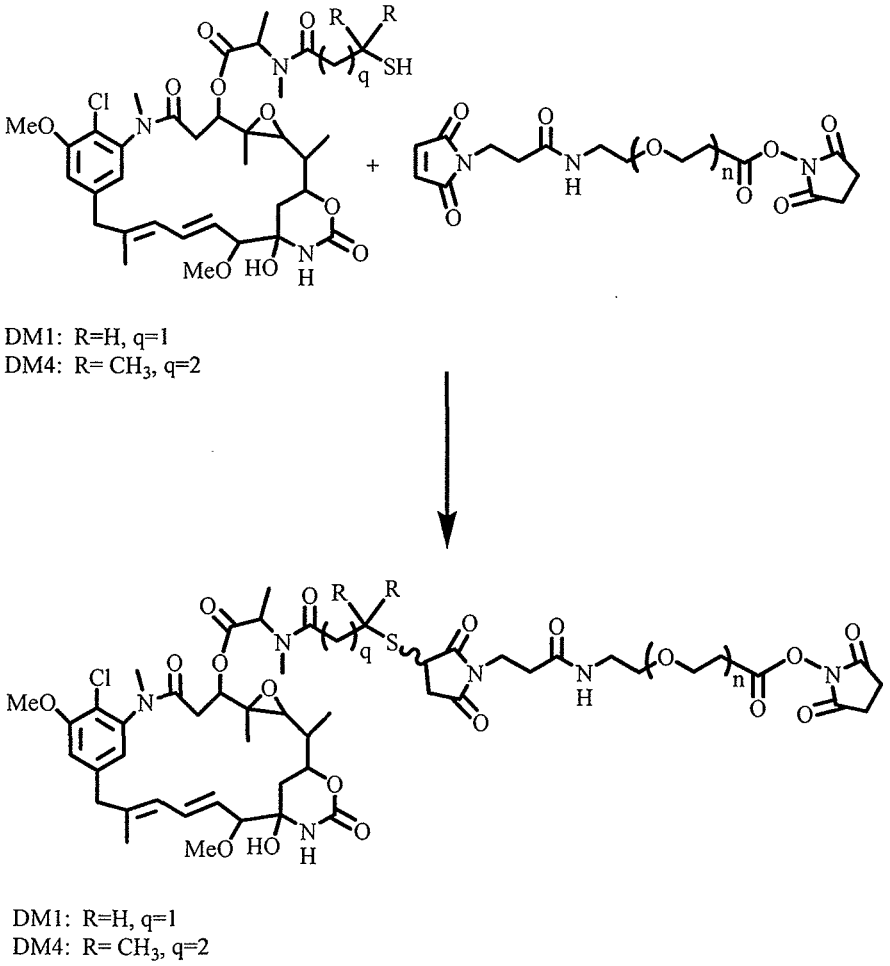


FIGURE 5. Synthetic scheme for PEG-containing compounds (non-cleavable thioacetamidyl-linked) of the present invention (n = 1-2000)

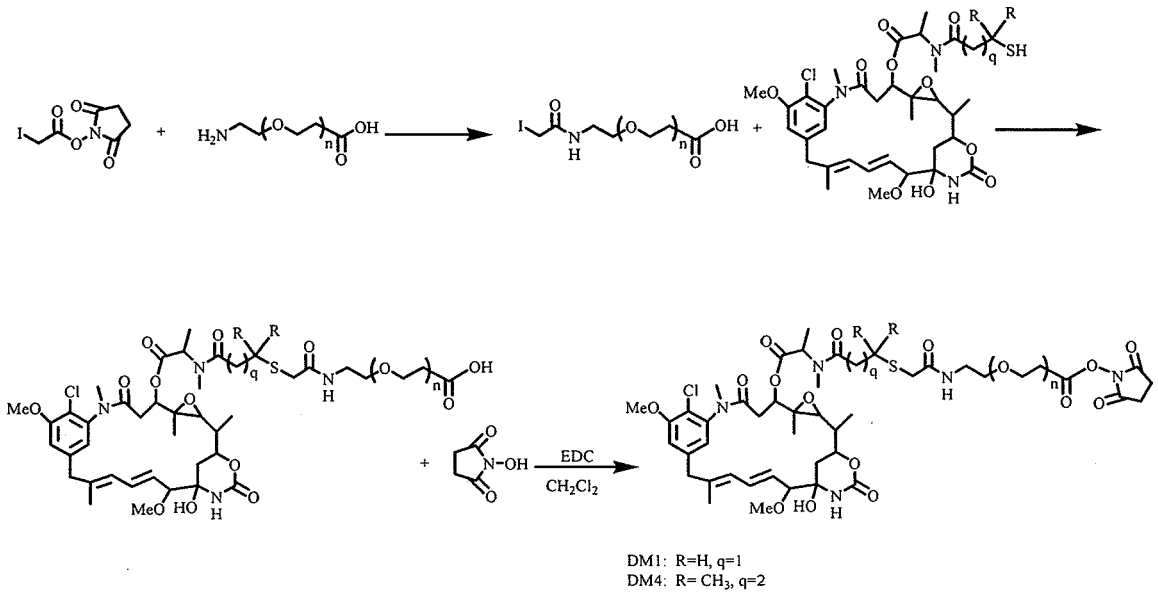
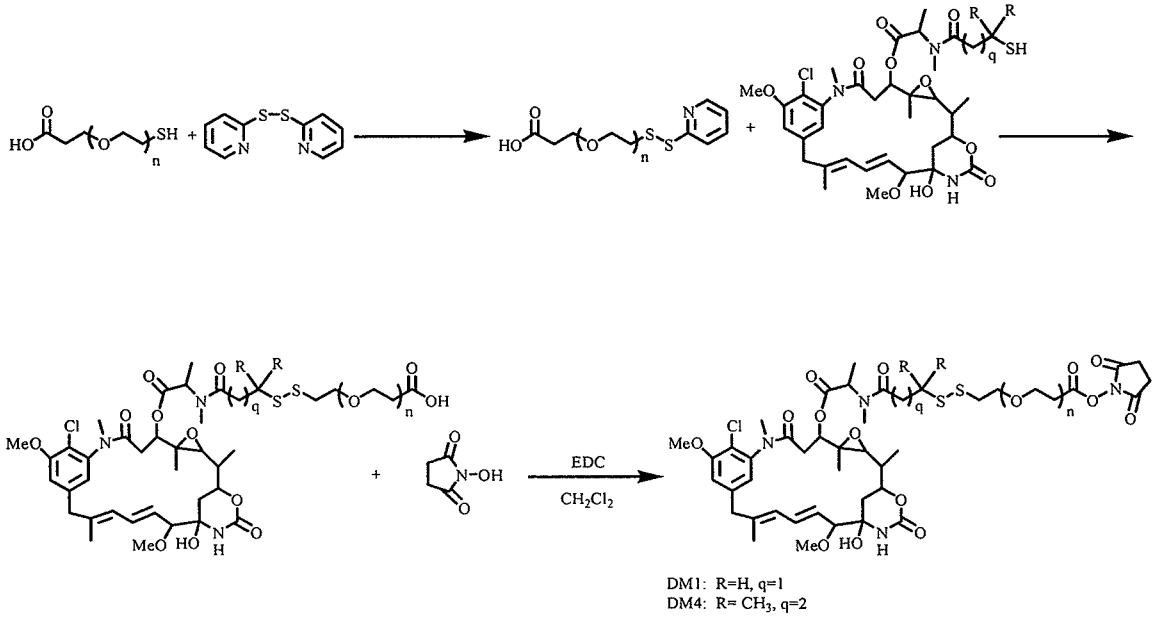


FIGURE 6. Synthetic scheme for PEG-containing disulfide linked compounds of the present invention (n = 1-14)

a.) Synthesis of the PEG-containing disulfide linked compound for 1-step conjugation to cell-binding agent



b.) Synthesis of the heterobifunctional PEG-containing disulfide linked crosslinking compound

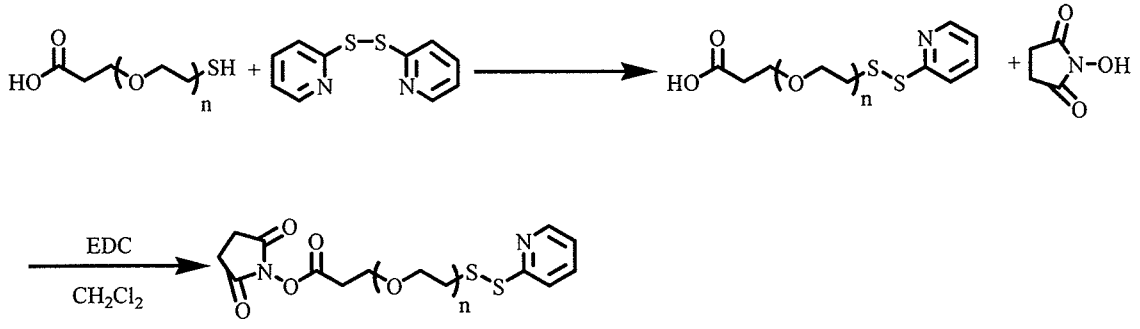


FIGURE 7. Conjugation procedure for PEG-containing non-cleavable thiosuccinimidyl-linked conjugate of the present invention (one-step conjugation; n = 1-2000; m = 2-15)

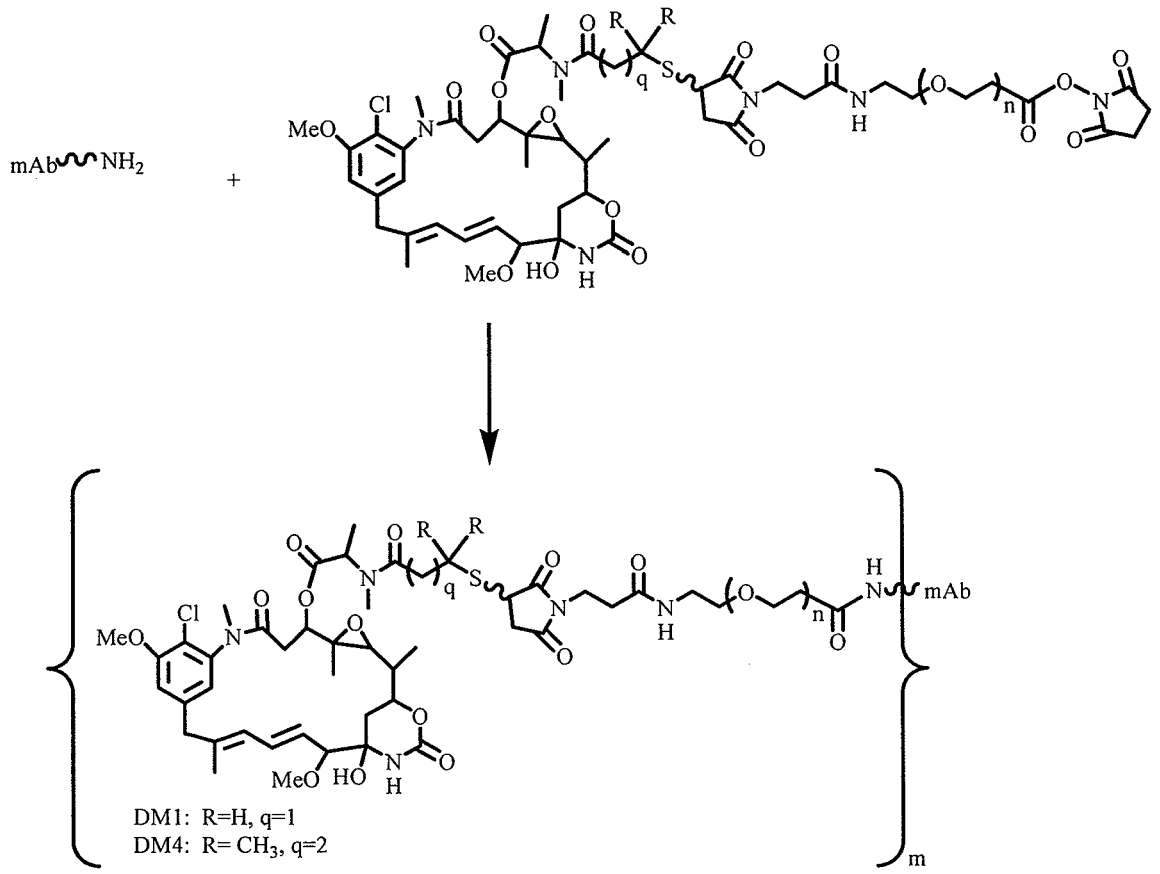


FIGURE 8. Conjugation procedure for PEG-containing non-cleavable conjugate of the present invention (2-step conjugation; n = 1-2000; m = 2-15)

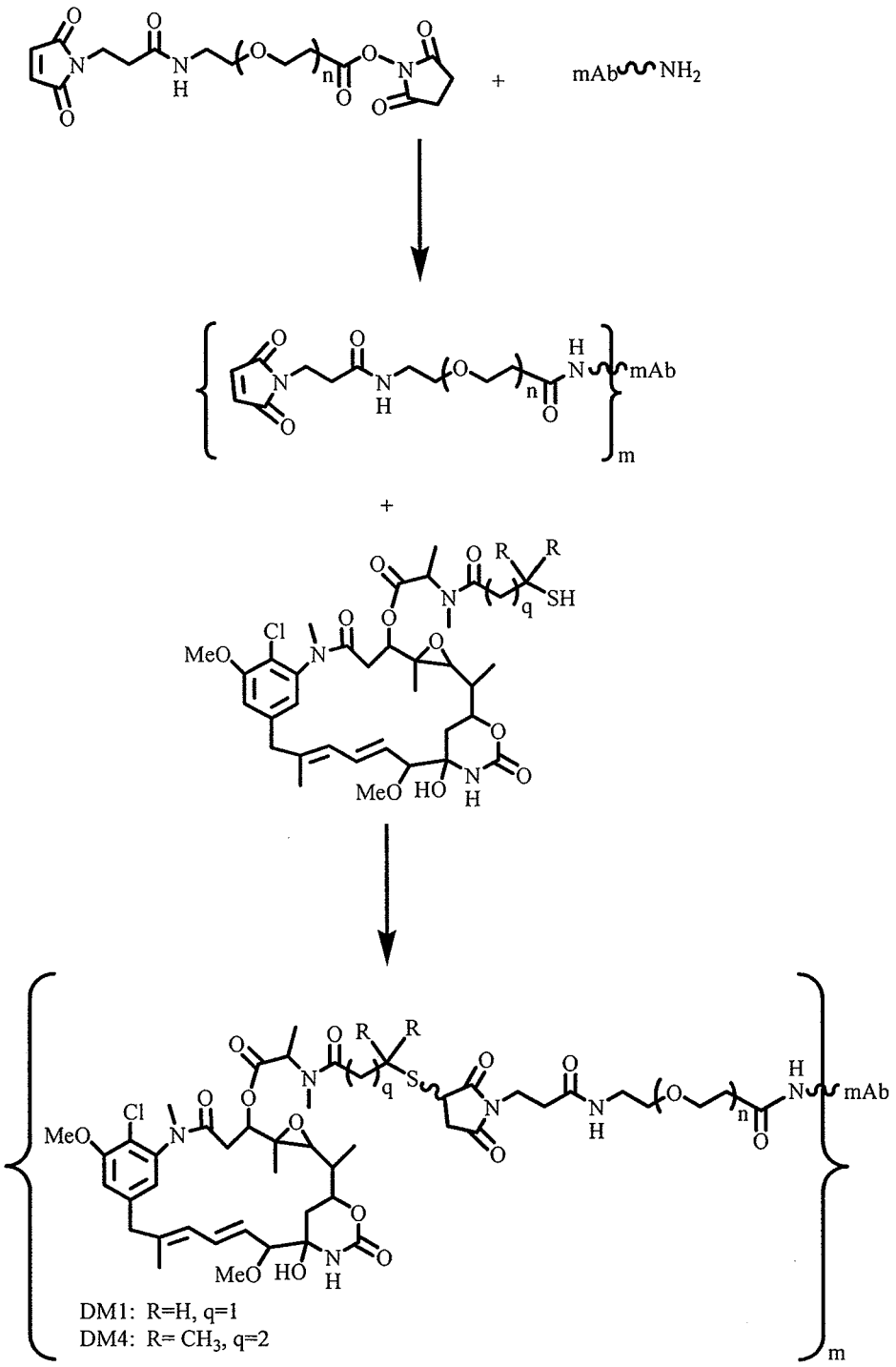


FIGURE 9. Conjugation procedure for PEG-containing non-cleavable thioether-linked (thioacetamidyl-linked) conjugate of the present invention (1-step conjugation; n = 1-2000; m = 2-15)

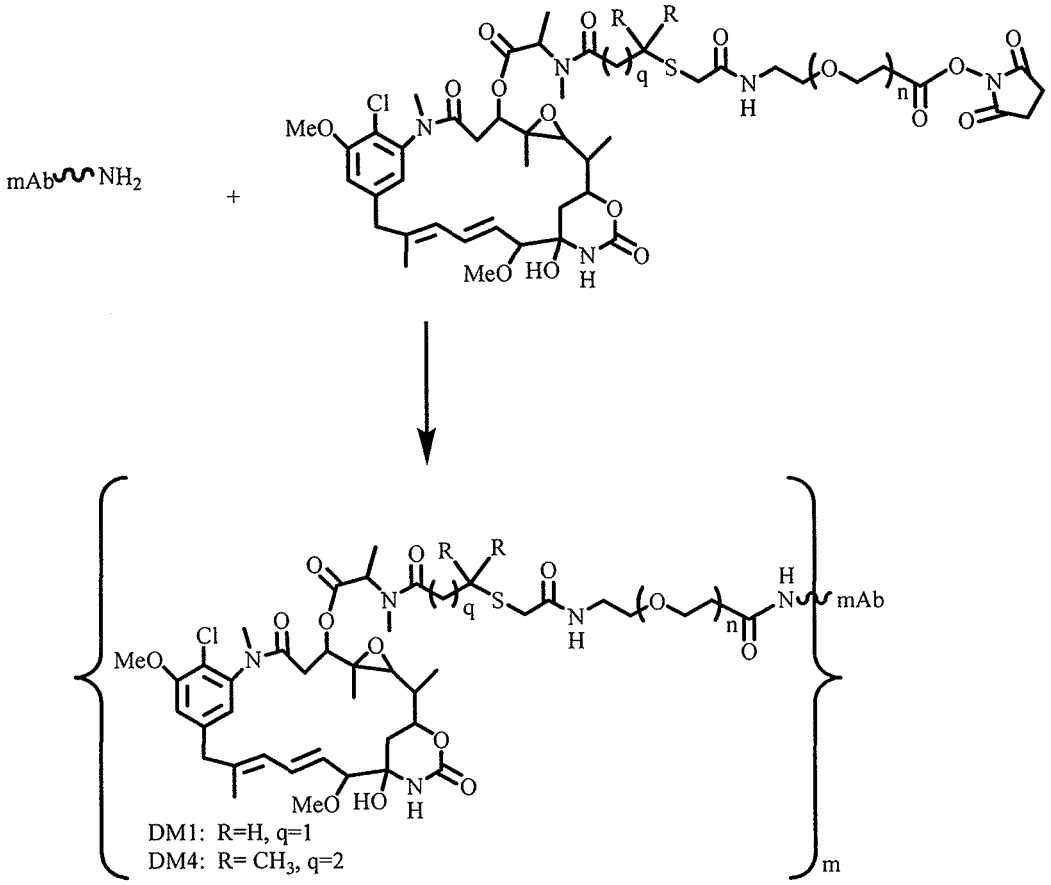


FIGURE 10. Conjugation procedure for PEG-containing non-cleavable thioether-linked (thioacetamidyl-linked) conjugate of the present invention (2-step conjugation; n = 1-2000; m = 2-15)

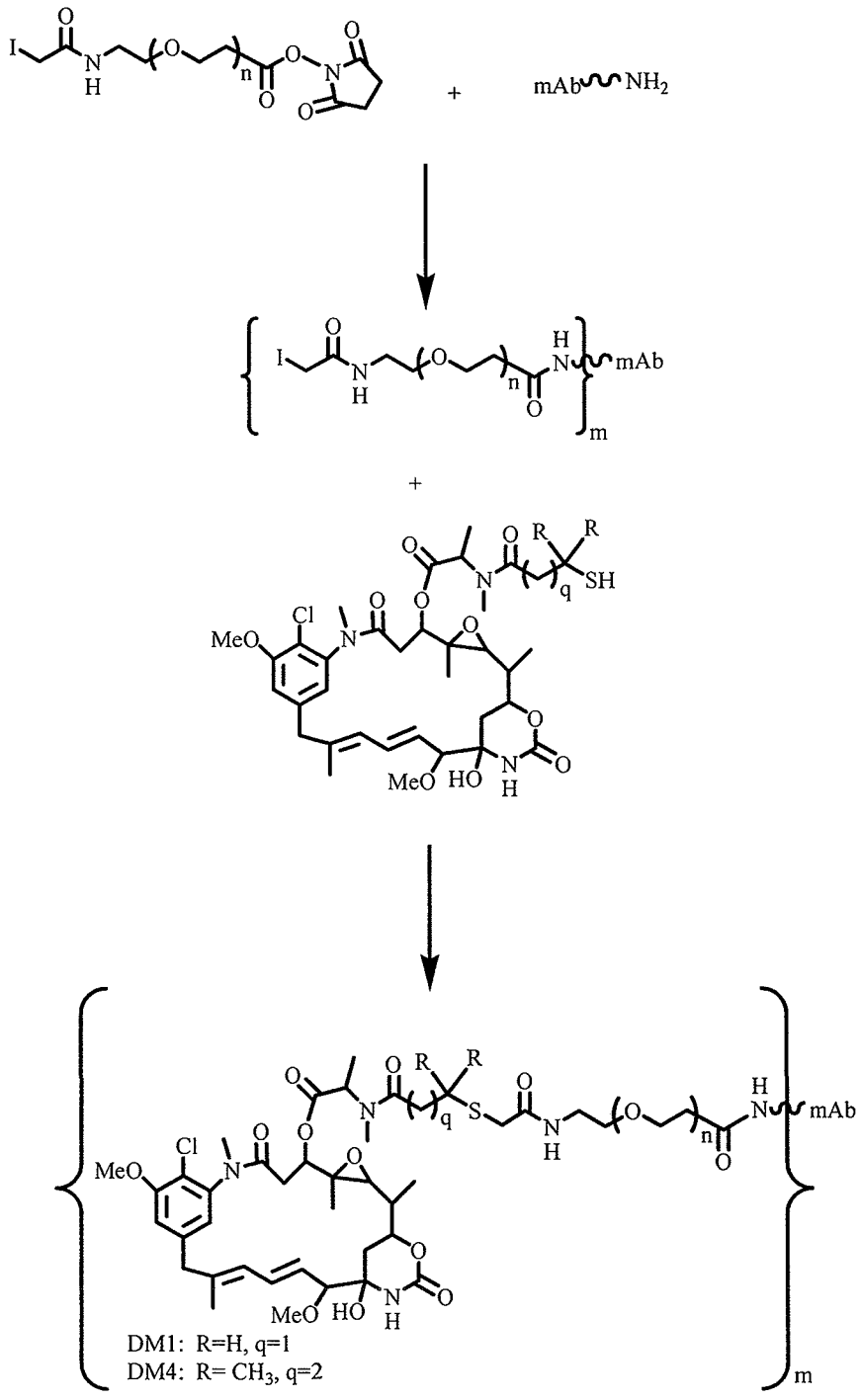


FIGURE 11. Conjugation procedure for PEG-containing disulfide linked conjugate of the present invention (1-step conjugation; n = 1-14; m = 3-8)

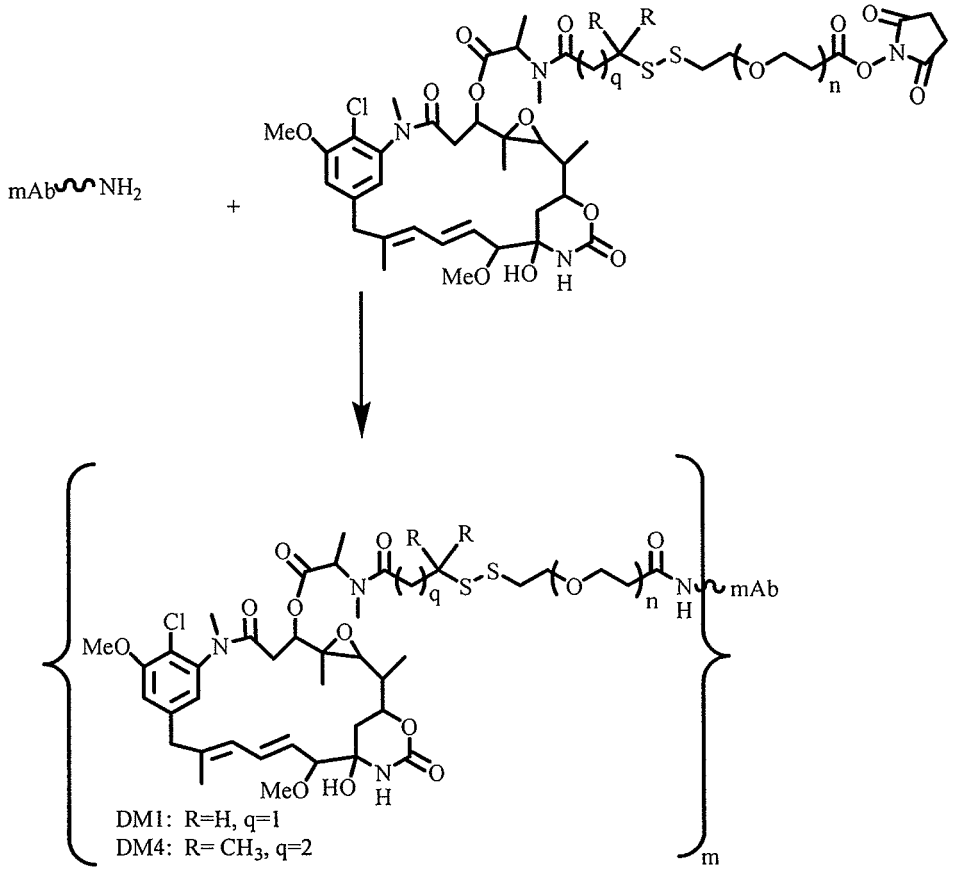


FIGURE 12. Conjugation procedure for PEG-containing disulfide linked conjugate of the present invention (2-step conjugation; n = 1-14; m = 3-8)

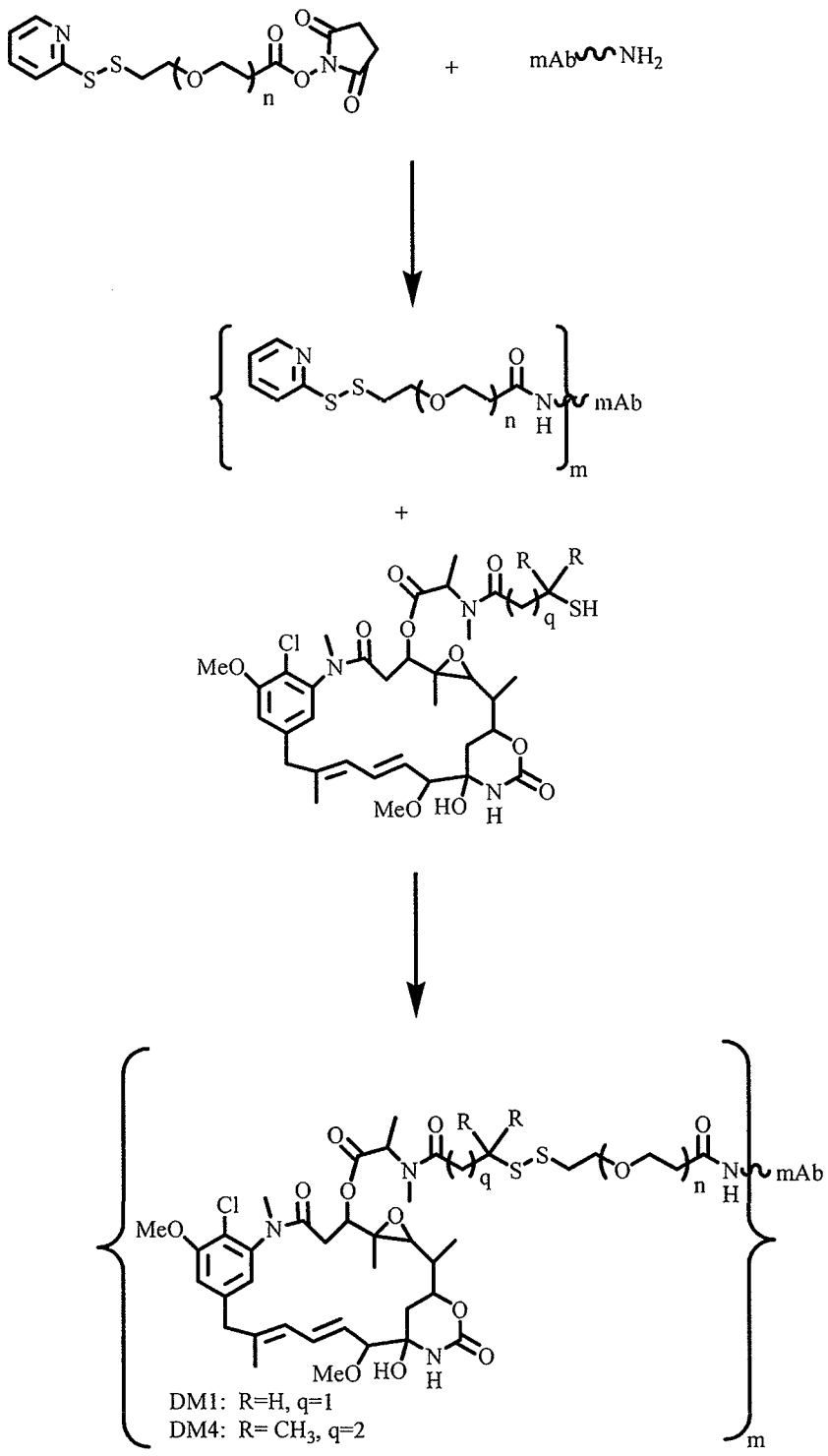
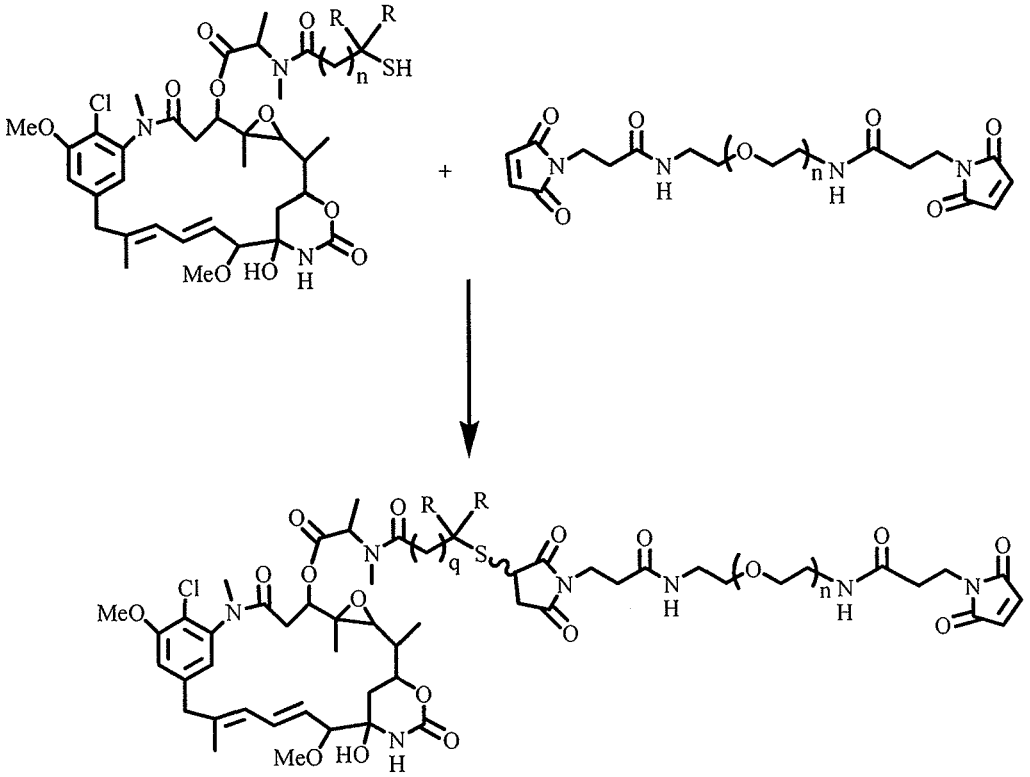


FIGURE 13. Synthetic scheme for PEG-containing, sulfhydryl-reactive compounds (non-cleavable thiosuccinimidyl-linked) of the present invention (n=1-2000)



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

FIGURE 14. Conjugation procedure for PEG-containing non-cleavable thiosuccinimidyl-linked conjugate of the present invention (1-step conjugation; n = 1-2000; m = 2-15)

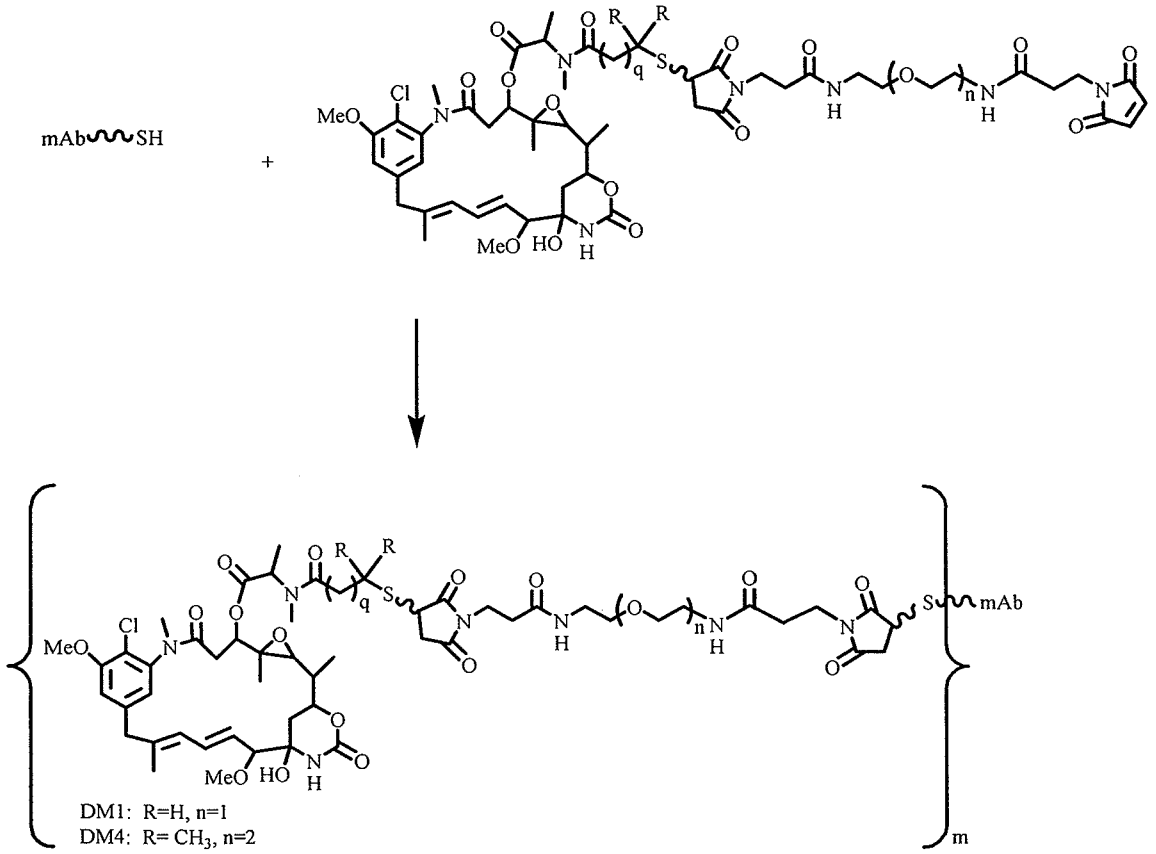


FIGURE 15. Conjugation procedure for PEG-containing non-cleavable, thiosuccinimidyl-linked conjugate of the present invention (2-step conjugation; n = 1-2000; m = 2-15)

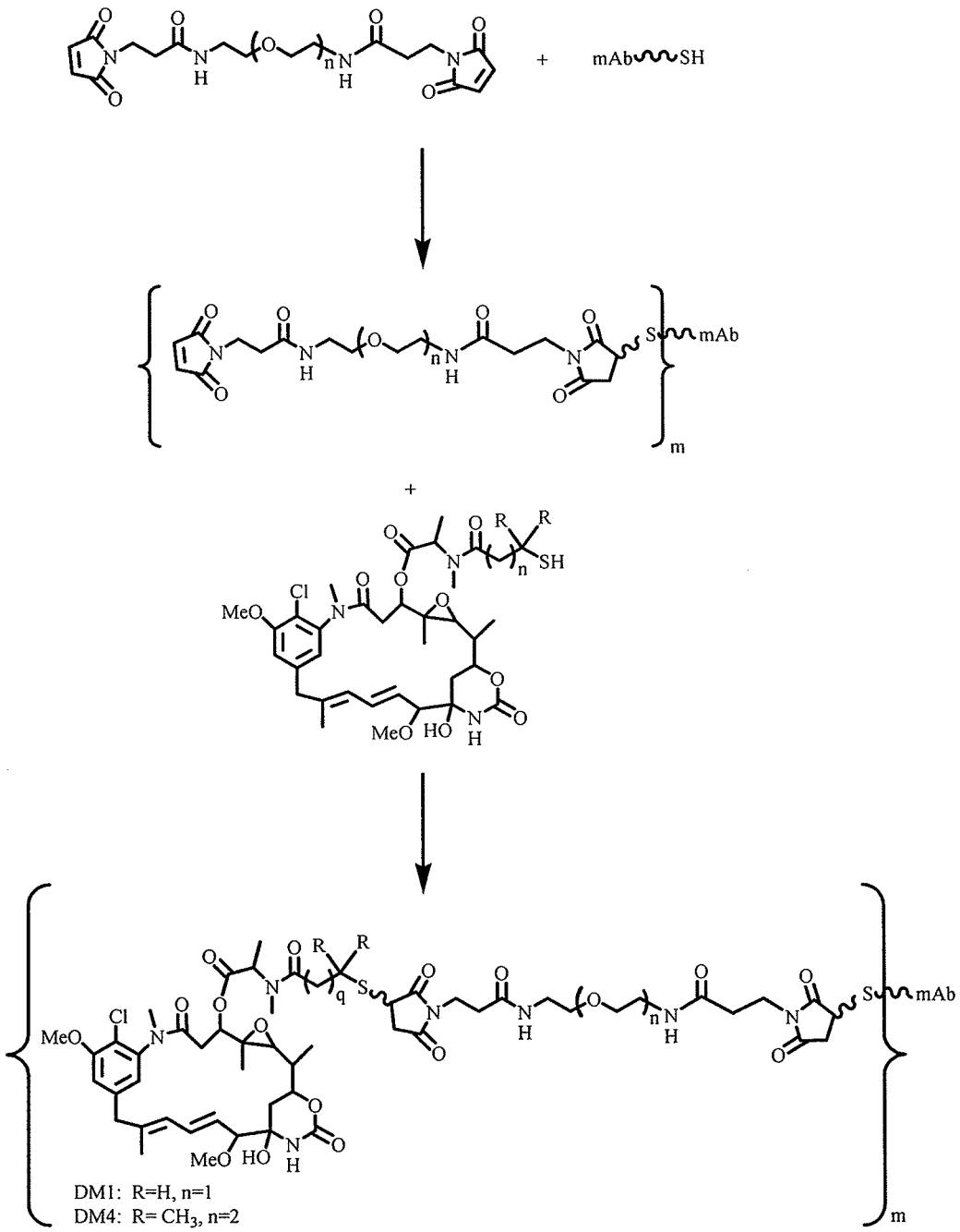
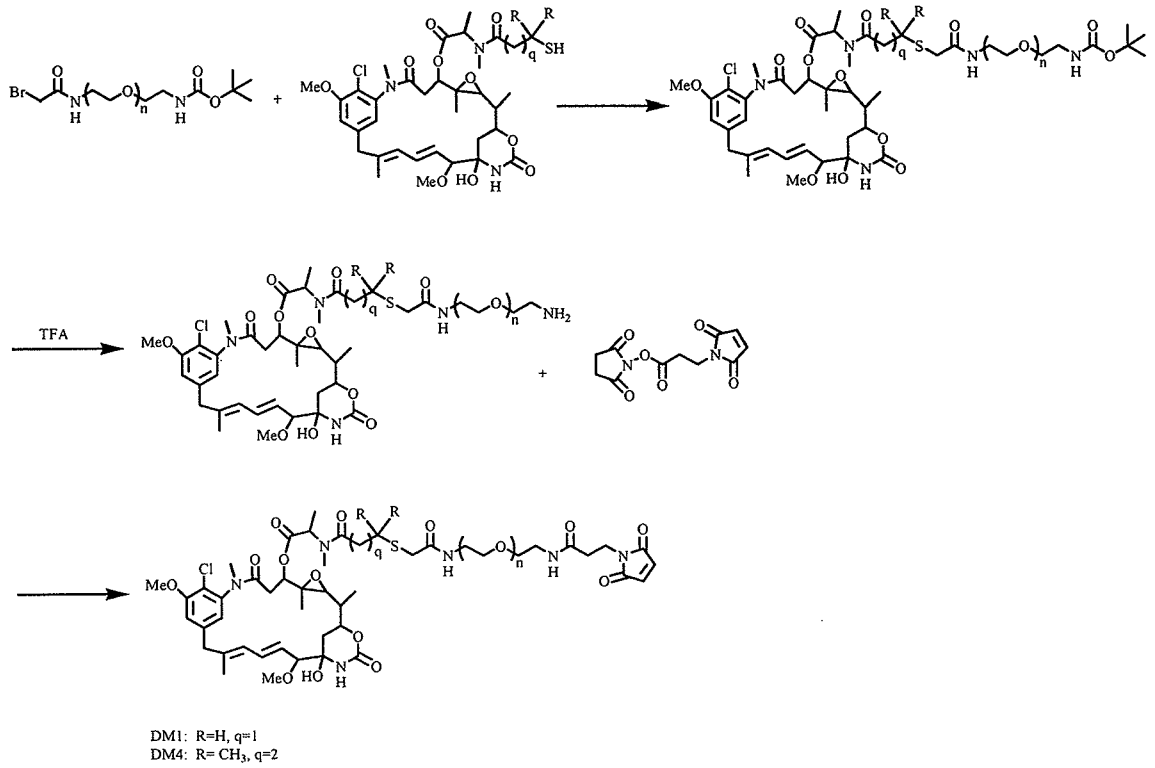


FIGURE 16. Synthetic scheme for PEG-containing, sulfhydryl-reactive, non-cleavable thioacetamidyl-linked compounds of the present invention (n = 1-2000)

a.) Synthesis of the PEG-containing, sulfhydryl-reactive, thioacetamide linked compound for 1-step conjugation



b.) Synthesis of the heterobifunctional PEG-containing, sulfhydryl-reactive crosslinking compound for 2-step conjugation

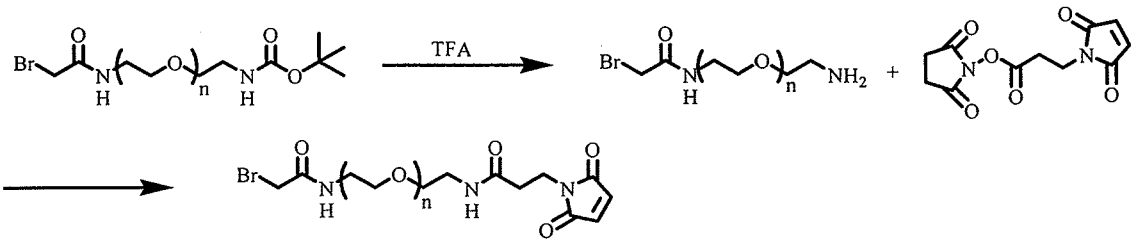


FIGURE 17. Conjugation procedure for PEG-containing non-cleavable thioacetamidyl-linked conjugate of the present invention (1-step conjugation; n = 1-2000; m = 2-15)

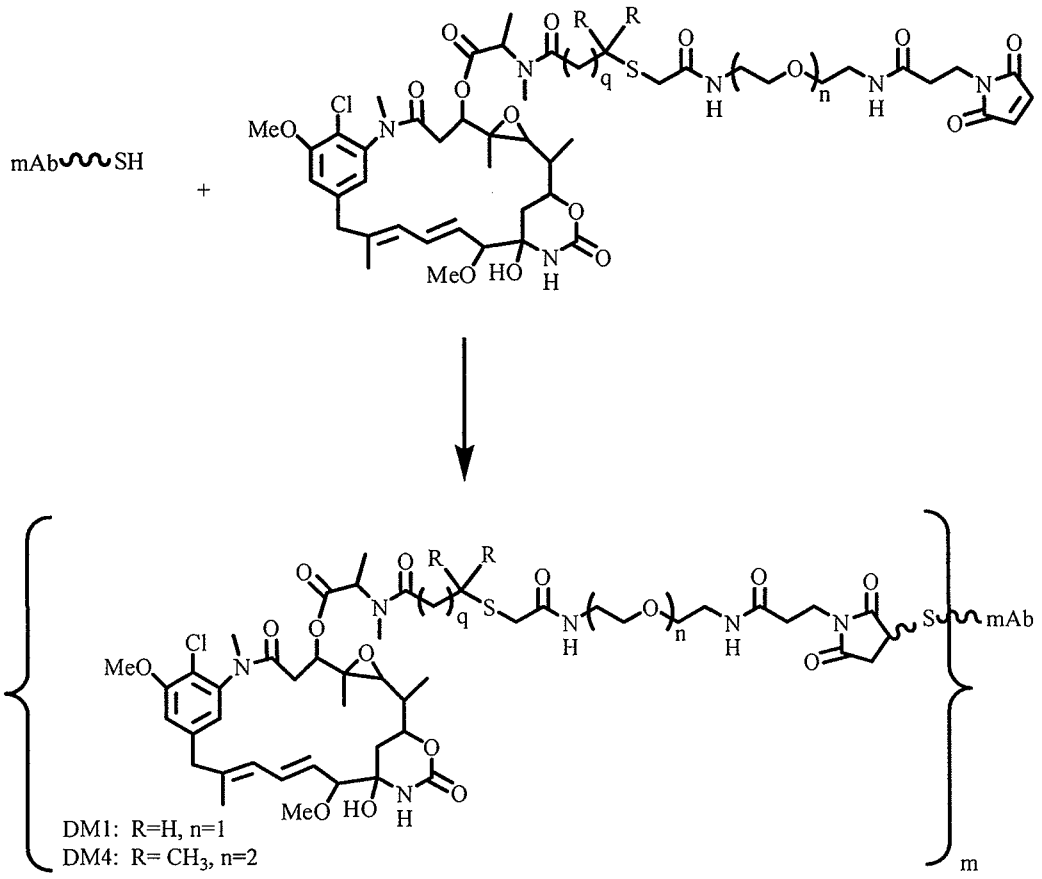


FIGURE 18. Conjugation procedure for PEG-containing non-cleavable thioacetamidyl-linked conjugate of the present invention (2-step conjugation; n = 1-2000; m = 2-15)

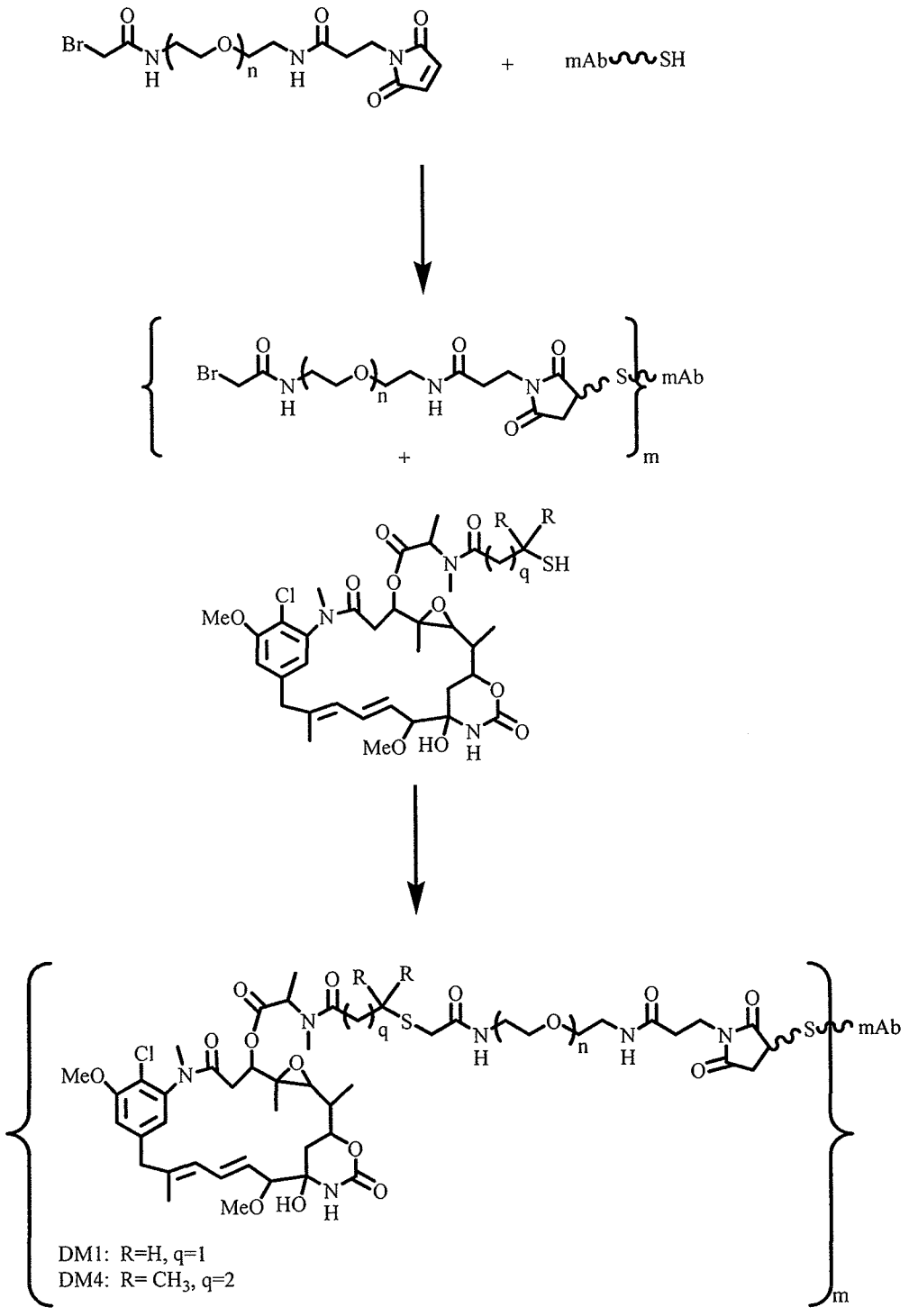
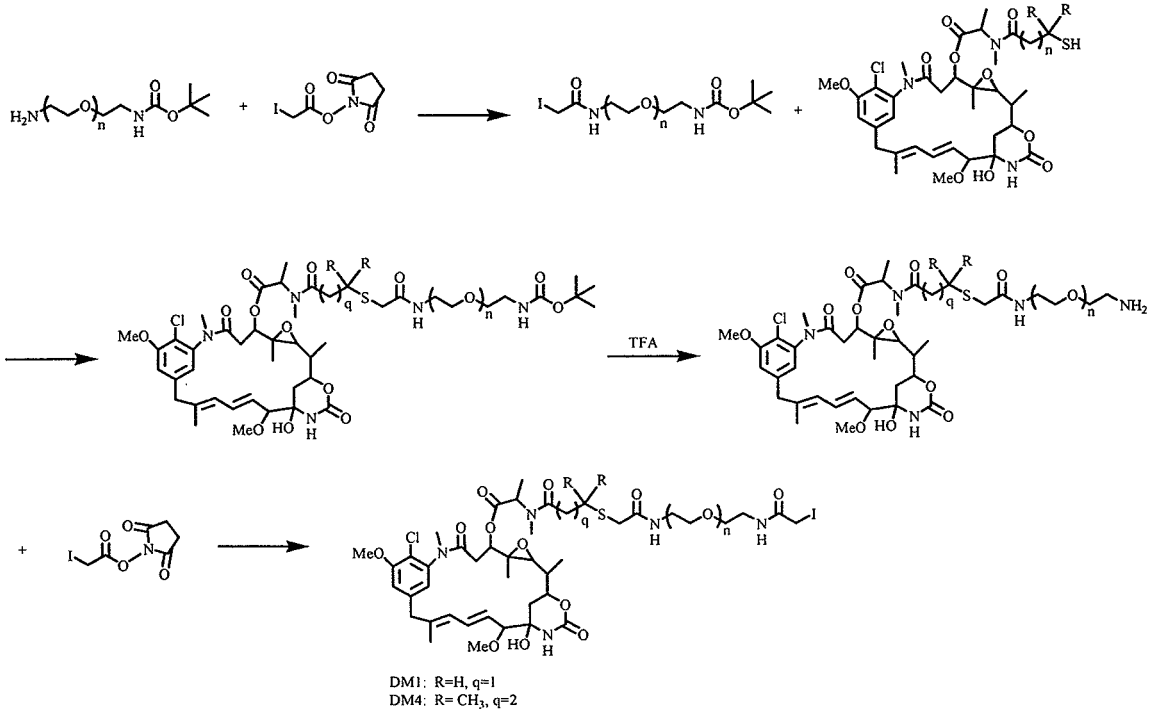


FIGURE 19. Synthetic scheme for the PEG-containing, sulfhydryl-reactive, non-cleavable, thioether-linked compounds of the present invention (n = 1-2000)

a.) Synthesis of the PEG-containing, sulfhydryl-reactive, non-cleavable thioacetamidyl-linked compound for 1-step conjugation



b.) Synthesis of the homobifunctional PEG-containing, sulfhydryl-reactive crosslinking compound for 2-step conjugation

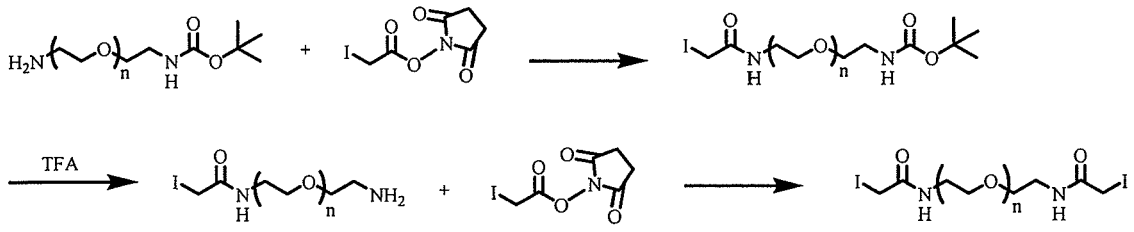


FIGURE 20. Conjugation procedure for PEG-containing non-cleavable thioacetamidyl-linked conjugate of the present invention (1-step conjugation; n = 1-2000; m = 2-15)

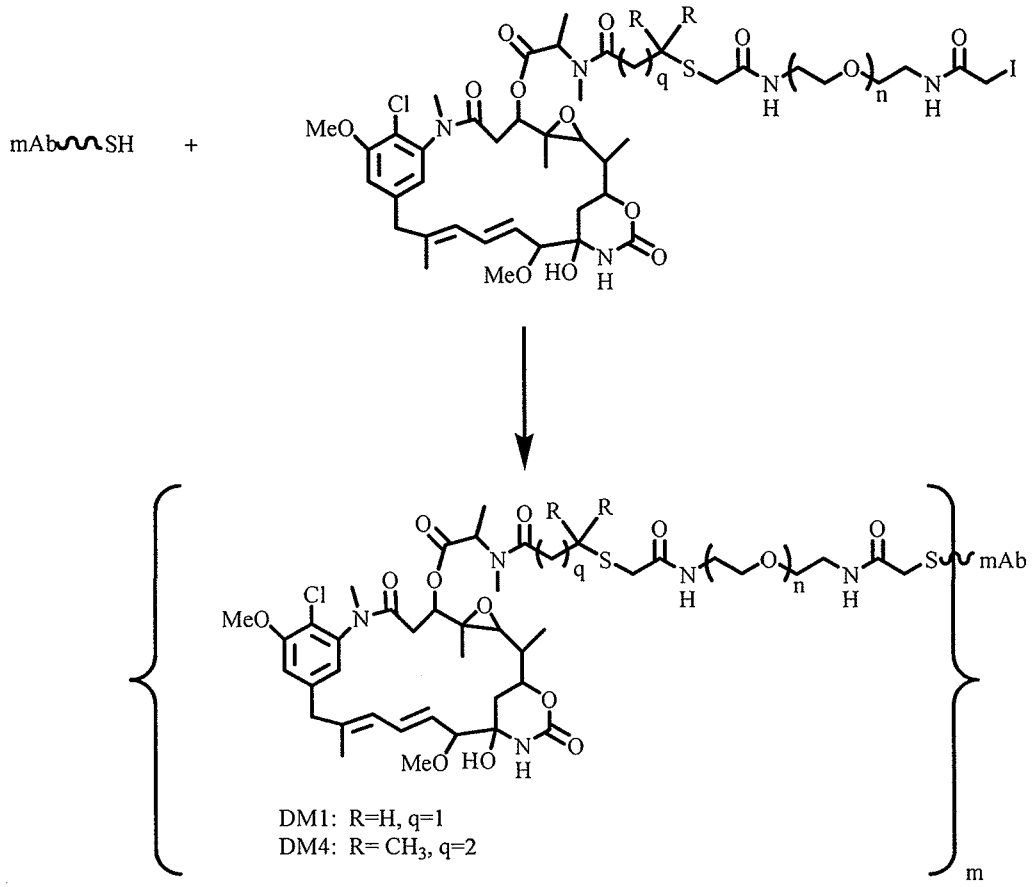


FIGURE 21. Conjugation procedure for PEG-containing non-cleavable thioacetamidyl-linked conjugate of the present invention (2-step conjugation; n = 1-2000; m = 2-15)

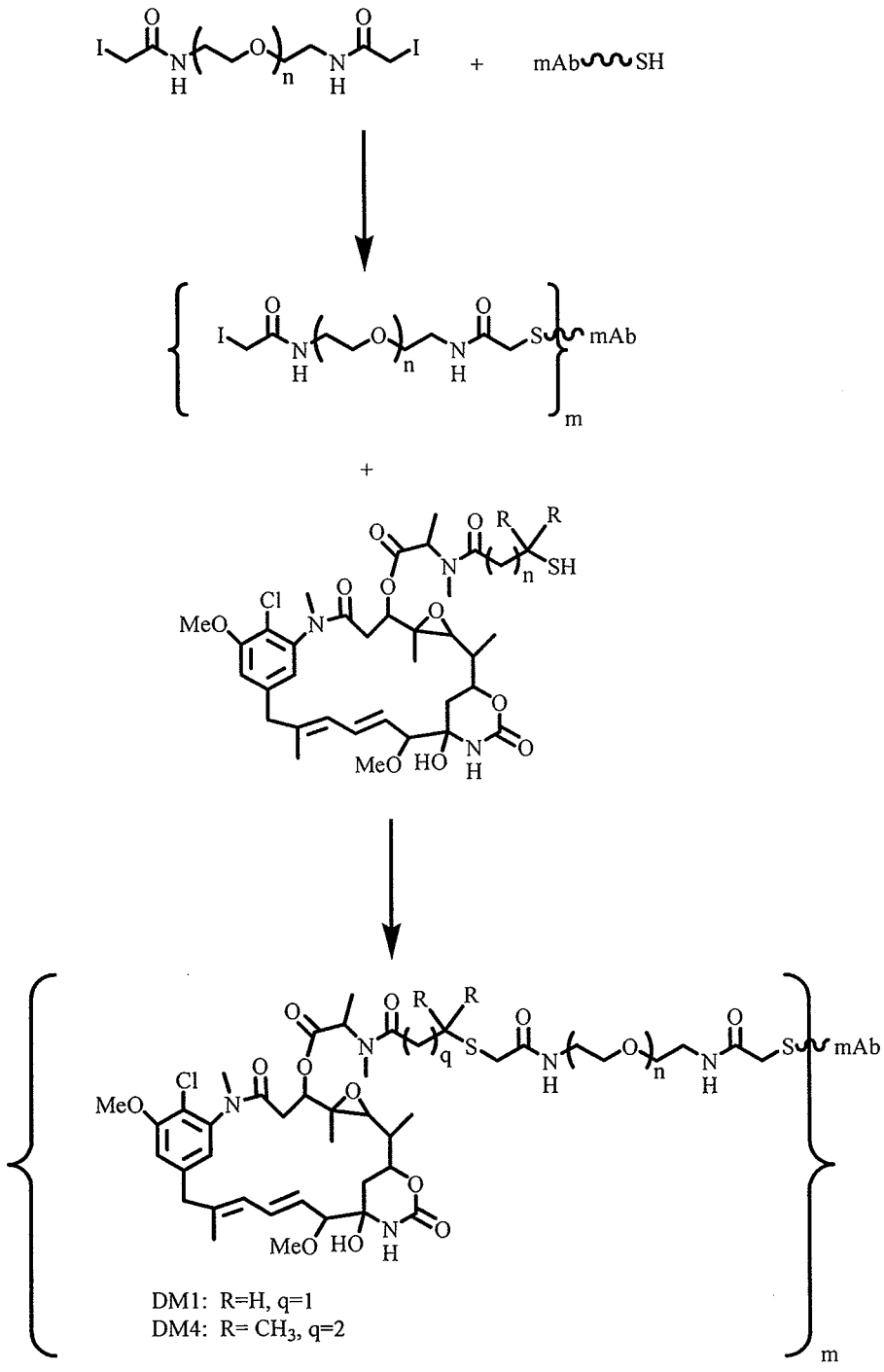


FIGURE 22

Mass spectrum (MS) of deglycosylated

HuAb-PEG$_4$Mal-DM1 conjugate (10.7 DM1/Ab, average)

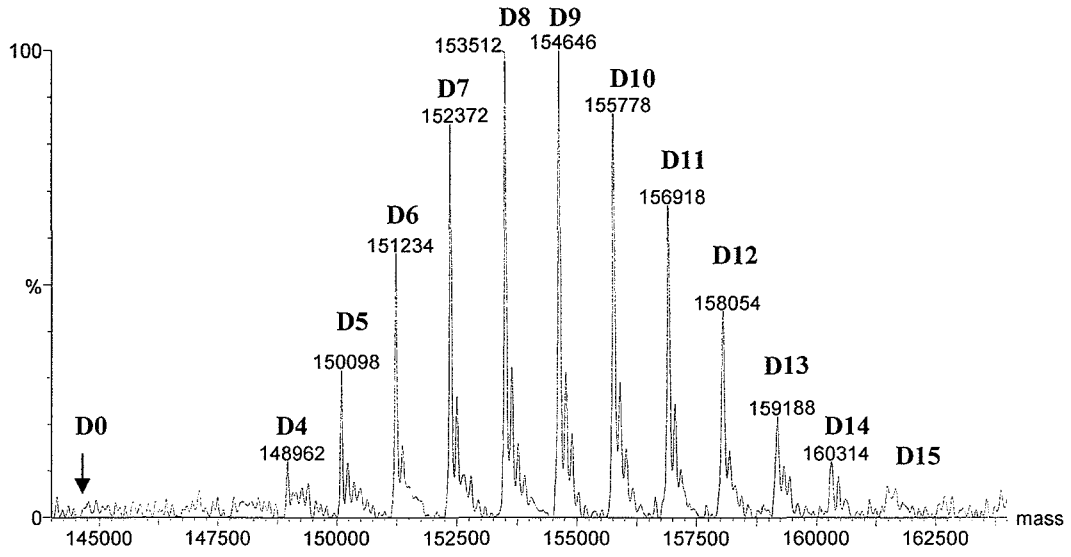


FIGURE 23

Size exclusion chromatography (SEC) of HuAb-PEG$_4$Mal-DM1 conjugate (10.7 DM1/Ab, average)

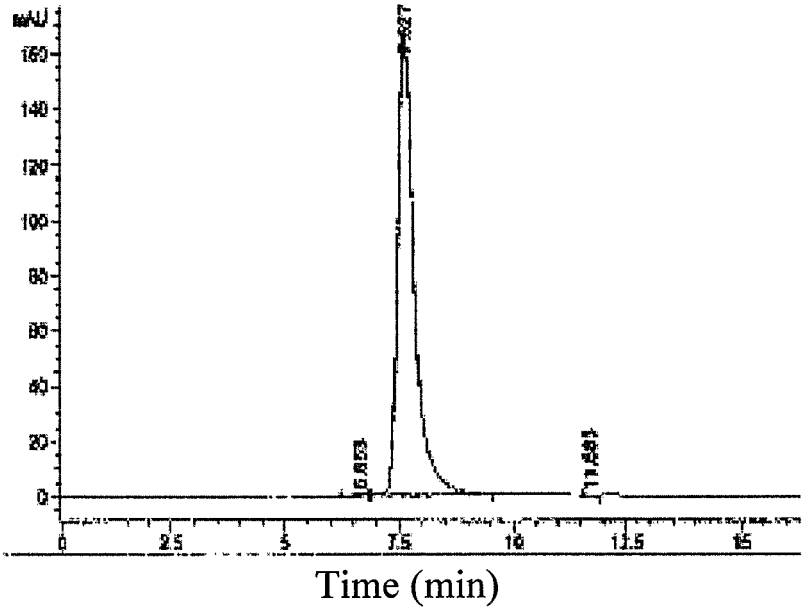


FIGURE 24

FACS binding of HuAb-PEG4Mal-DM1 conjugate (10.7 maytansinoid/antibody) is similar to that of unmodified
antibody

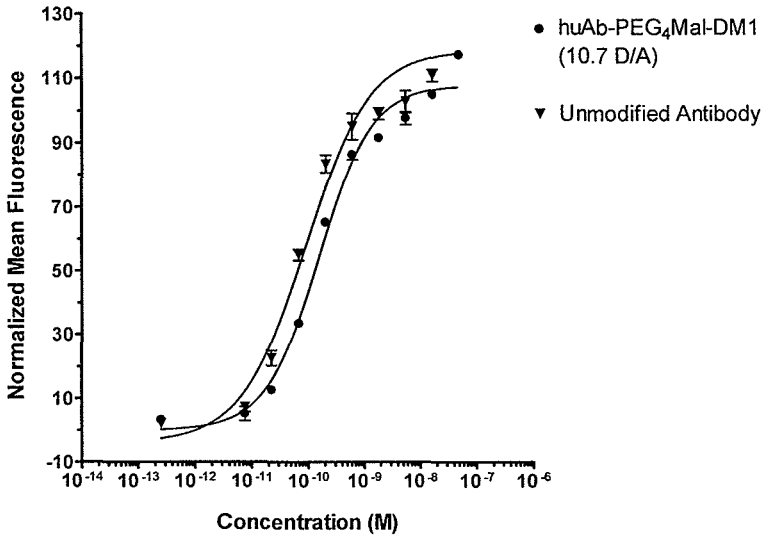


FIGURE 25

Cytotoxicity of anti-EpCAM antibody-maytansinoid conjugates on multi-drug resistant COLO205-MDR cells

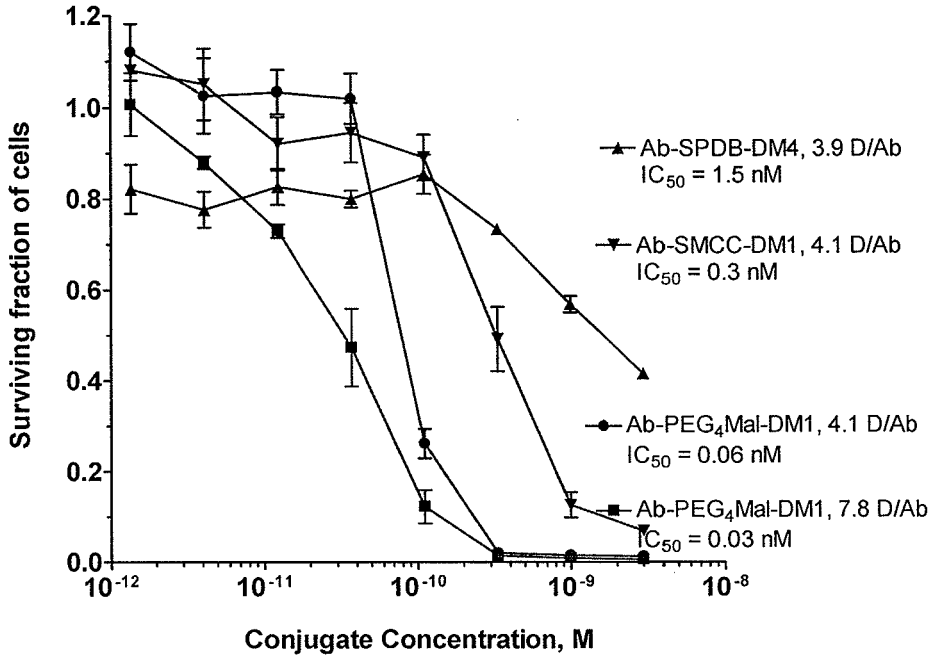


FIGURE 26

Cytotoxicity of Anti-CanAg antibody-maytansinoid conjugates on multi-drug resistant COLO205-MDR cells

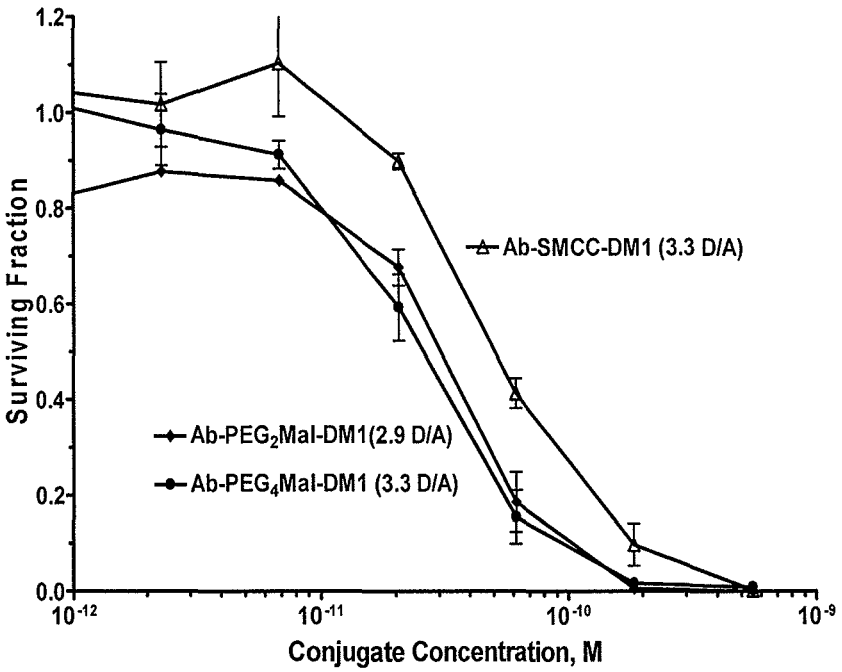


FIGURE 27

Cytotoxicity of Anti-CD56 antibody-maytansinoid conjugates

on Molp-8 multiple myeloma cells

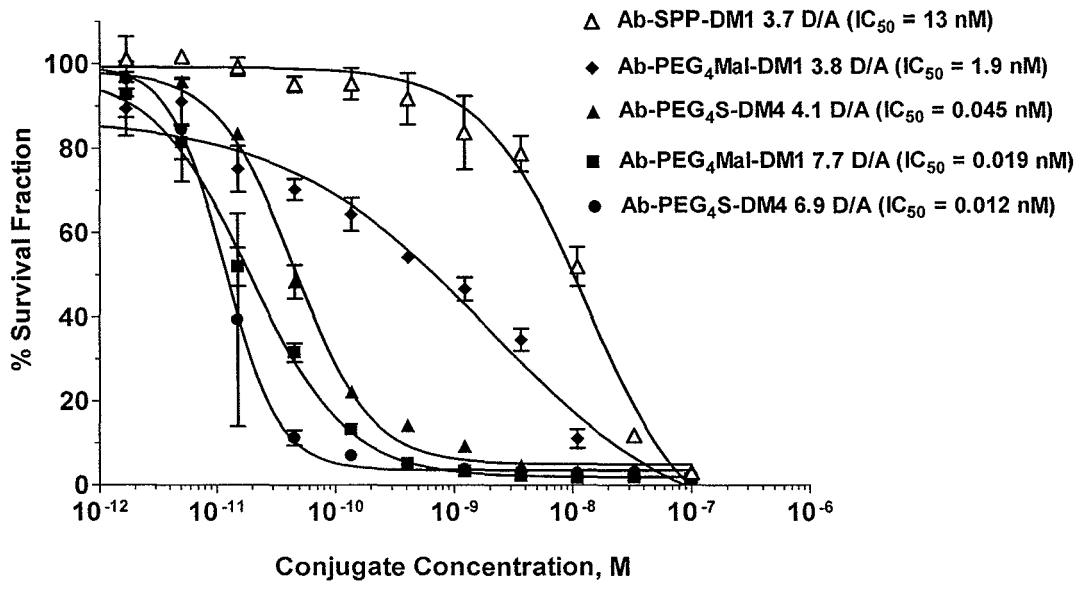


FIGURE 28

Cytotoxicity of anti-EpCAM antibody-maytansinoid conjugates on HCT15 cells

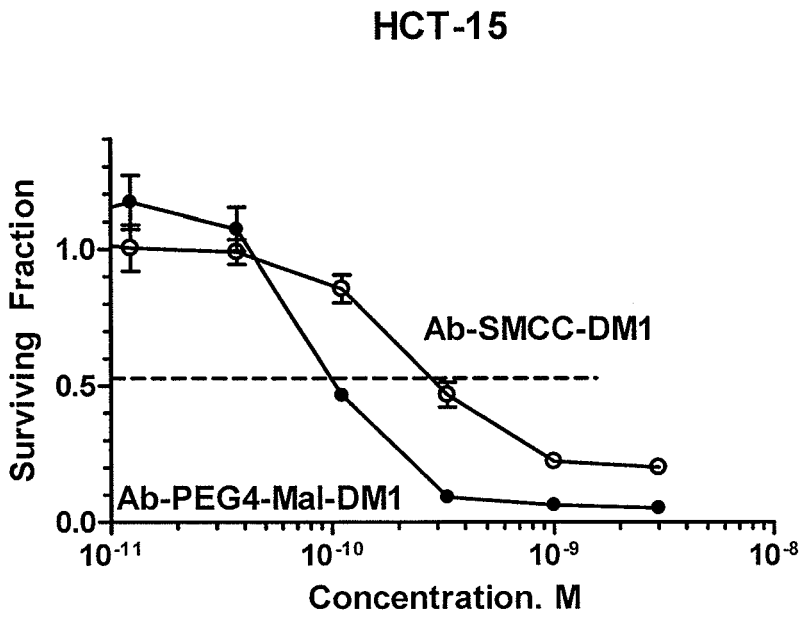


FIGURE 29

Cytotoxicity of anti-EpCAM antibody-maytansinoid conjugates on COLO 205 mdr cells

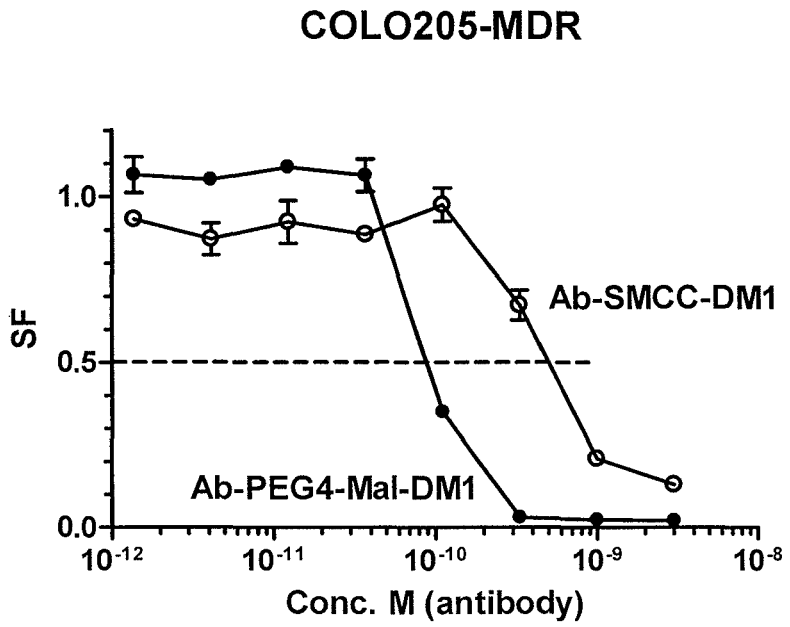


FIGURE 30

In vivo anti-tumor activity of anti-EpCAM antibody-maytansinoid conjugates on HCT15 xenografts

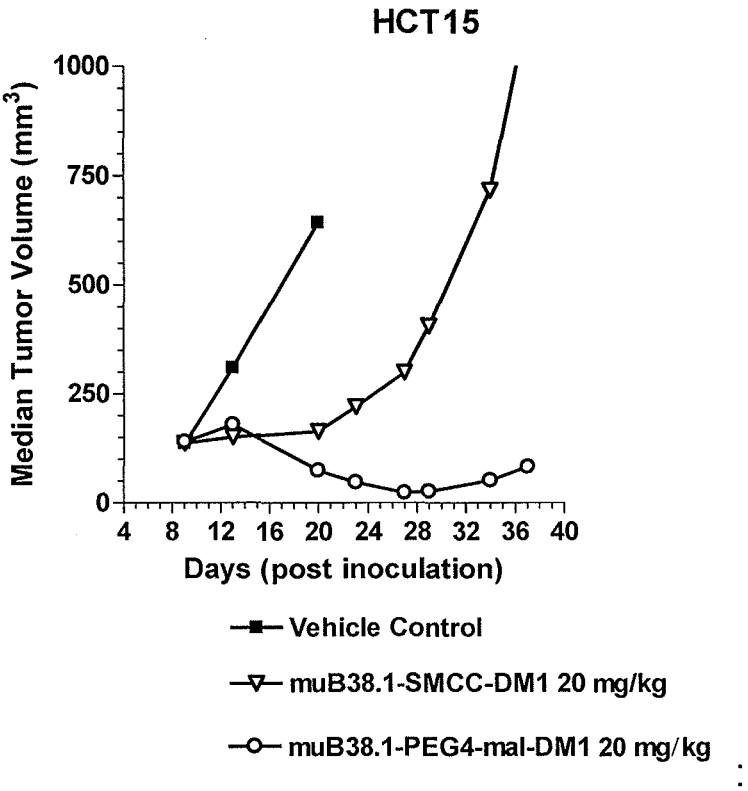


FIGURE 31

In vivo anti-tumor activity of anti-EpCAM antibody-maytansinoid conjugates on COLO205 *mdr* xenografts

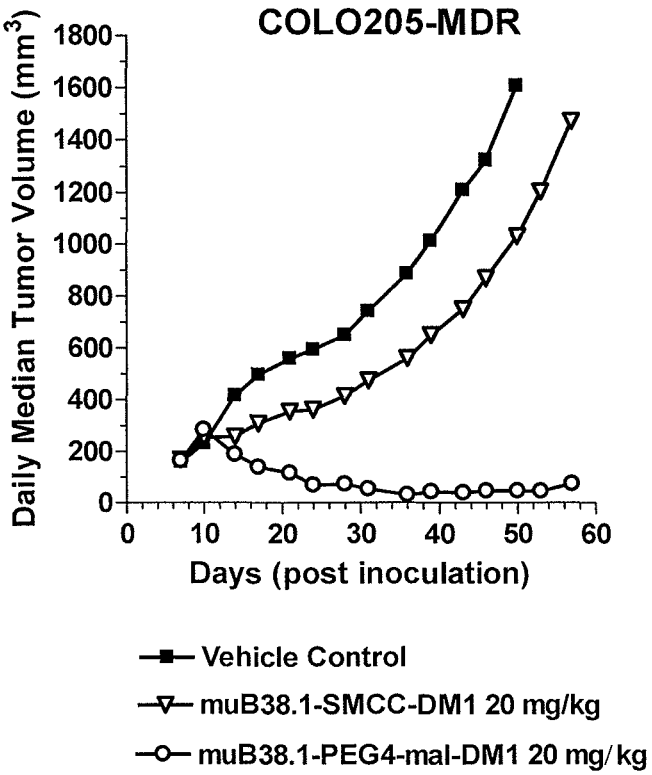


FIGURE 32. In vivo anti-tumor activity of anti-EpCAM antibody-maytansinoid conjugates on COLO 205 xenografts

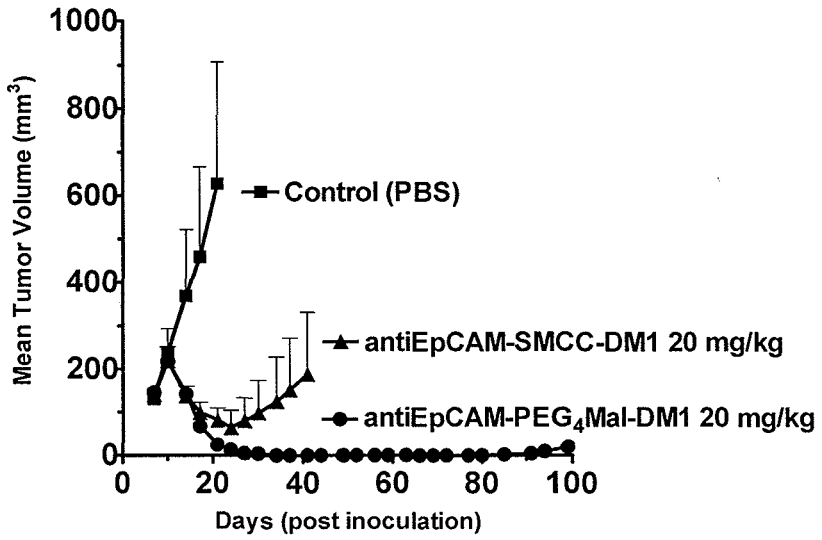


FIGURE 33

In vivo anti-tumor activity of anti-CanAg antibody-maytansinoid conjugates on COLO205 *mdr* xenografts

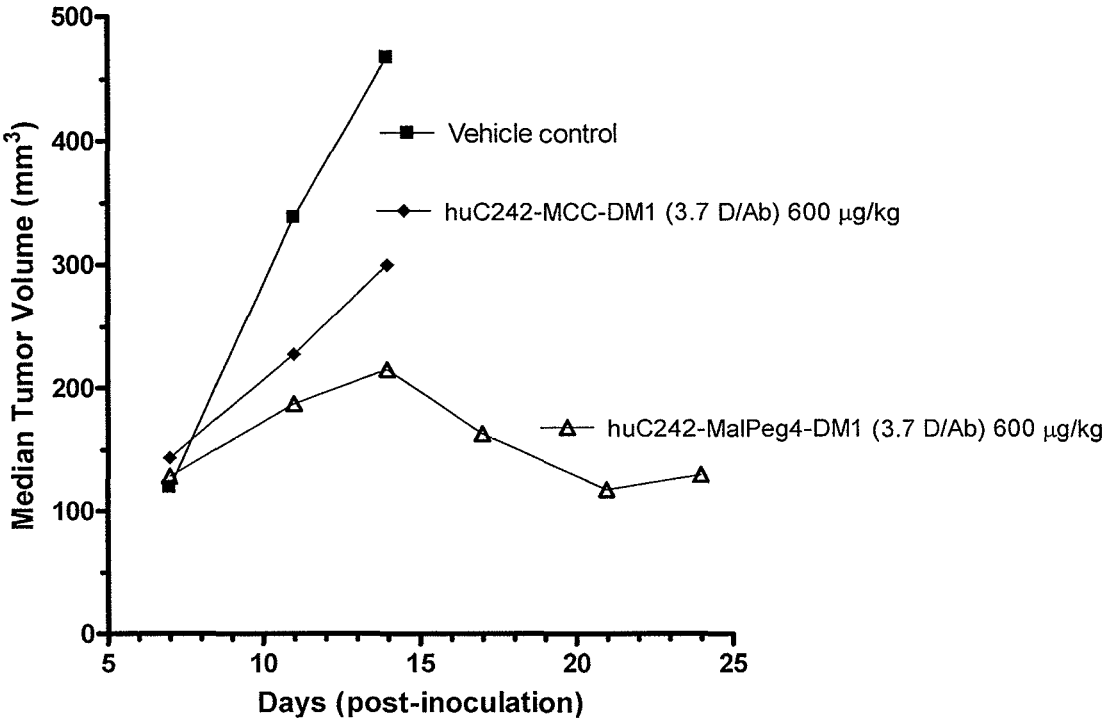


FIGURE 34

Binding of anti-CanAg antibody (huC242)-PEG24-Mal-DM1 conjugate with up to 17 D/A

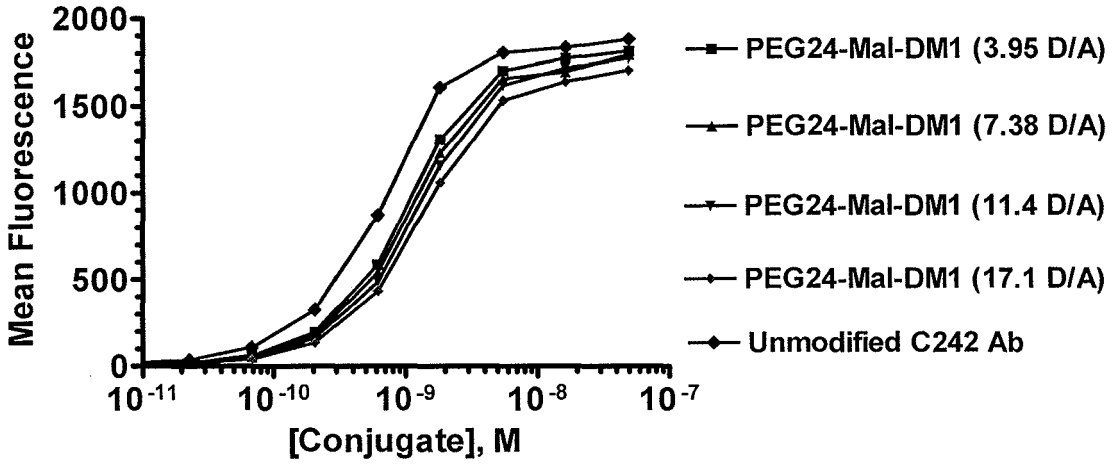


FIGURE 35. In vitro potency of Anti-CanAg antibody (huC242)-PEG24-Mal-DM1 conjugates with 4 to 17 D/A toward COLO205 cells

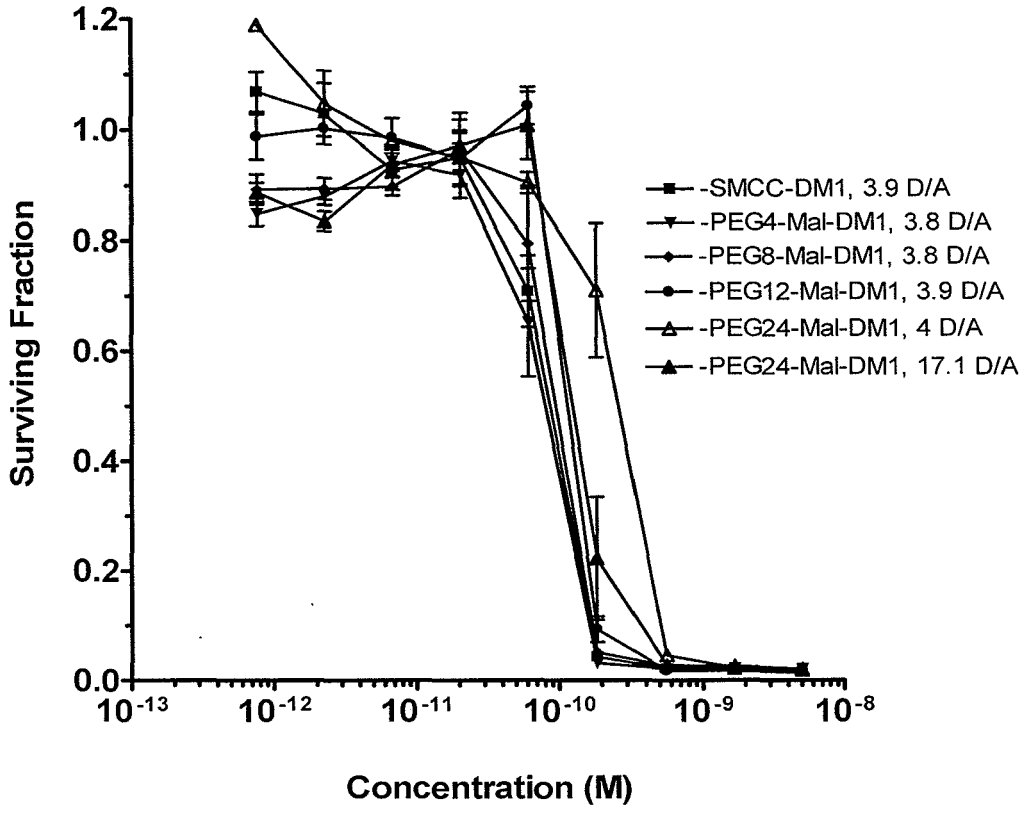


FIGURE 36. In vitro potency of anti-CanAg antibody (huC242)-PEG24-Mal-DM1 conjugates with 4 to 17 D/A toward multi-drug resistant (pgp+) COLO205-MDR cells

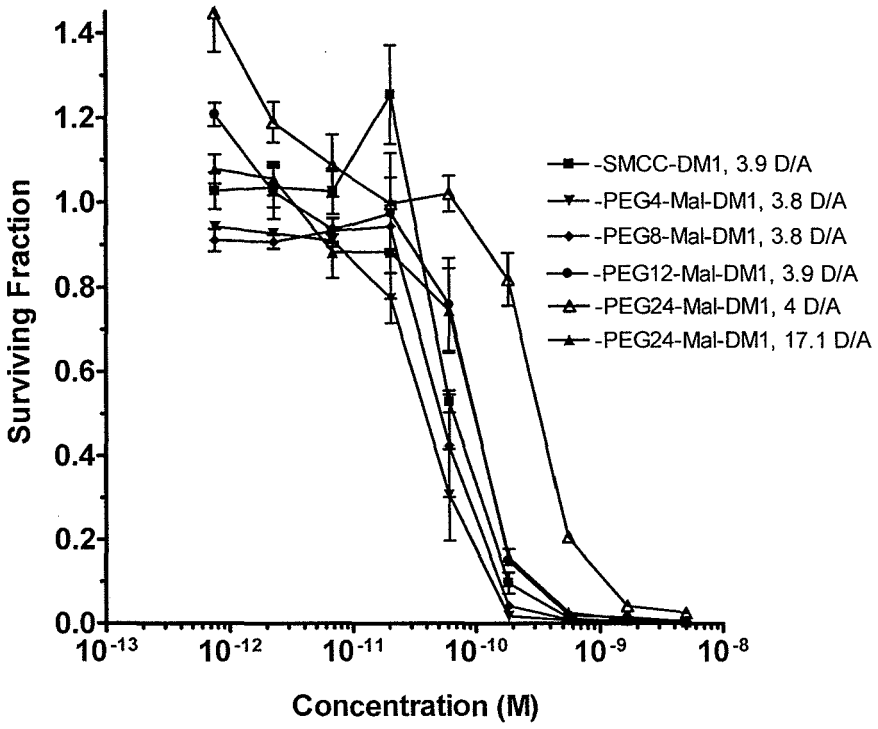


FIGURE 37. Cytotoxicity of Anti-EGFR Antibody-Maytansinoid conjugates on UO-31 Cells

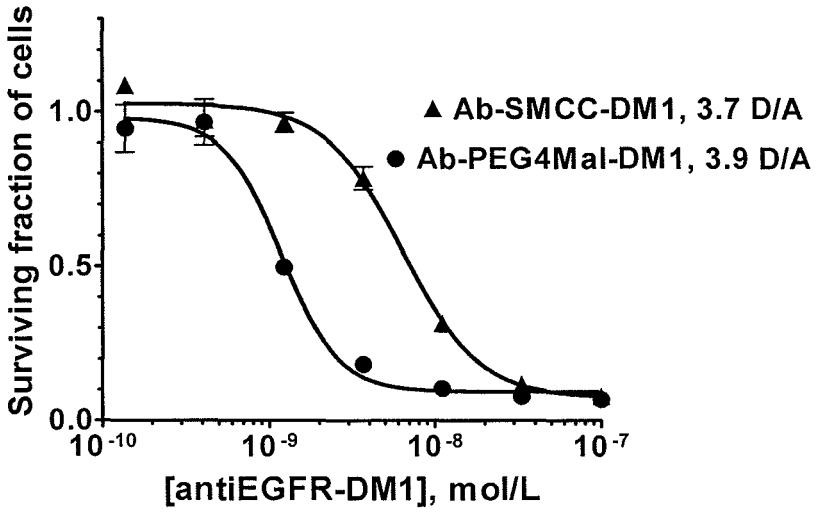
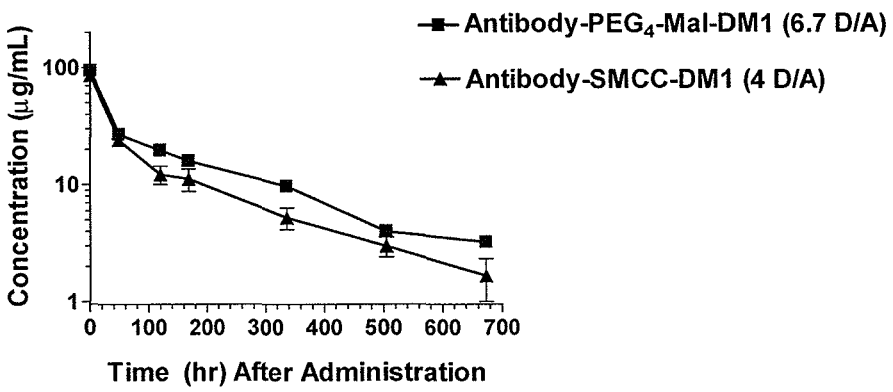


FIGURE 38

Plasma pharmacokinetics in CD-1 mice: A. Antibody-PEG4-Mal-DM1 conjugate with 6.7 D/A compared to Antibody-SMCC-DM1 conjugate with 4 D/A (initial i.v. dose 5 mg/kg); B. Antibody-PEG4-Mal-(3H-labeled)DM1 conjugate with 3.3 D/A compared with unmodified antibody and Antibody-SMCC-(3H-labeled)DM1 conjugate with 4.2 D/A (initial i.v. dose 10-12 mg/kg)

A.



B.

