## General Experimental

Hydrogen peroxide (30% w/v in water), N-ethyl maleimide, and 5,5-dimethyl-1,3-cyclohexanedione (dimedone) were purchased from Sigma. Antibody drug conjugates (Ab-SMCC-DM1, Ab-PEG$_4$-mal-DM1, Ab-PEG$_4$-mal-DM4) were prepared according to published methods (Kovtun, et al, *Cancer Research*, 2010, **70**, 2528-37). A Bruker ESQUIRE™ 3000 ion trap mass spectrometer was used to obtain mass spectra for all reaction products and was used in line with a Water 2695 series HPLC. Samples were analyzed using the analytical reverse phase HPLC method described below:

Column: Vydac C8 (208TP104), 250 x 4.6 mm, 5 micron. Temperature: 25 $^{o}$C
Flow rate: 1.0 mL/ min Injection volume: 50 microliters
Absorbance detection: 252 nm
Linear gradient of 80% A → 80% B over 30 min
Solvent **A**: water (0.025% formic acid)
Solvent **B**: acetonitrile (0.024% formic acid)

### Mass spectrometer conditions

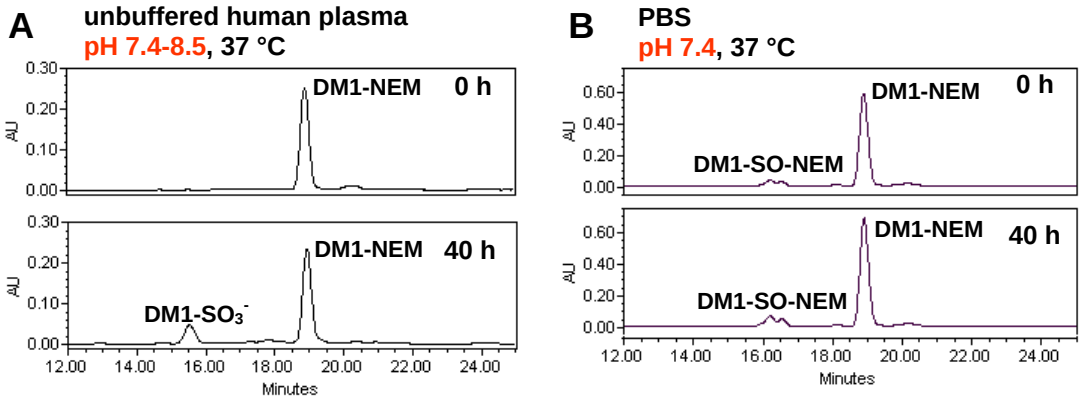
Ionization method: Electrospray; Nebulizer gas flow: 25 psi;
Drying temperature: 350$^{o}$C; Heating gas flow: 8.0 L/min;
Mass range: 50 – 2000

MS detection in alternating positive and negative ion modes or in MS$^2$ mode

### DM1-NEM human plasma incubations

DM1-NEM (8.6 μM in 3% v/v dimethylacetamide) was incubated with fresh unbuffered human plasma (5 mg/mL protein; Celsis, Inc) or PBS pH 7.4 at 37°C. Aliquots were removed at 0, 16, 20, 36, and 40 h and protein precipitated with 6 volumes of ice-cold acetone and held for 1 h at -20°C. The resulting suspension was pelleted (15,000 g, 15 min) and the pellet was then washed 3x with acetone. Combined supernatants were evaporated to dryness using a speedvac. Residues were reconstituted in 125 μL 20% acetonitrile/ 80% water containing 0.025% acetic acid. 100 μL of each sample was analyzed by LC-MS.

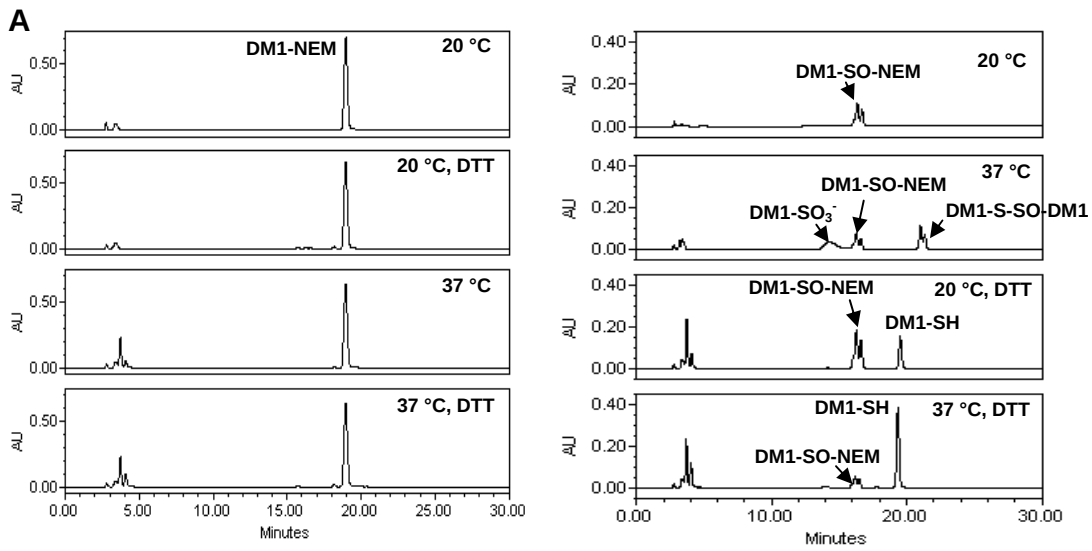
**S1.** Reaction products of DM1-NEM (40 h, 37°C) when incubated in human plasma (A) or PBS pH 7.4 (B). HPLC analysis of acetone extracts shown at time 0 h and 40 h. Thioether succinimide cleavage to form DM1-$SO_3^-$ occurs only in the unbuffered plasma incubation.

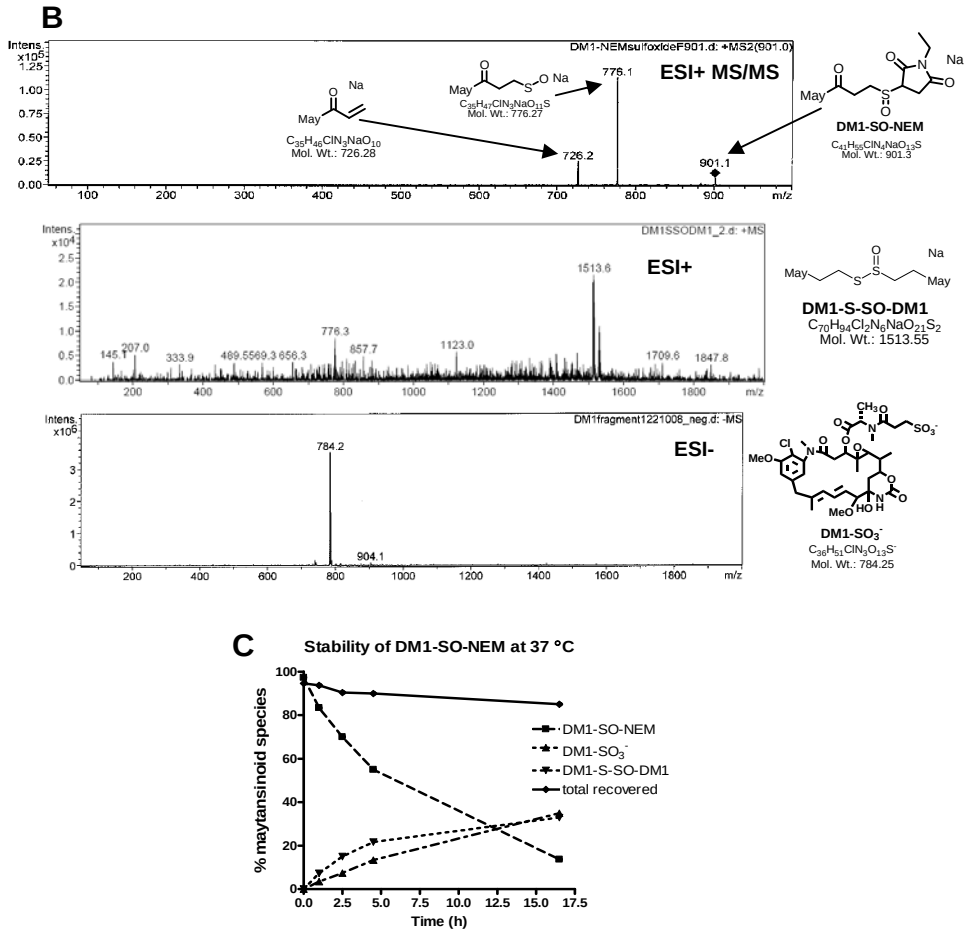



**Thermal and reductive stability of DM1-NEM and DM1-SO-NEM**

Synthetic DM1-NEM (8.8 µM in PBS, pH 7.4, 5% DMA) or DM1-SO-NEM (5.4 µM in PBS, pH 7.4, 5% DMA) were incubated at 20°C or 37°C, +/- 10 mM DTT (dithiothreitol) for 18 h. Reaction aliquots were removed and analyzed by LC-MS. Maytansinoid products were quantified by measuring integrated area under the curve at 252 nm and comparison to a calibration curve with known standards.

**S2.** A) LC-MS analysis of thermal and reductive stability of DM1-NEM and DM1-SO-NEM. B) ESI-MS spectra for DM1-SO-NEM, DM1-S-SO-DM1 and DM1-$SO_3^-$ C) Rate data for sulfoxide elimination from DM1-SO-NEM.

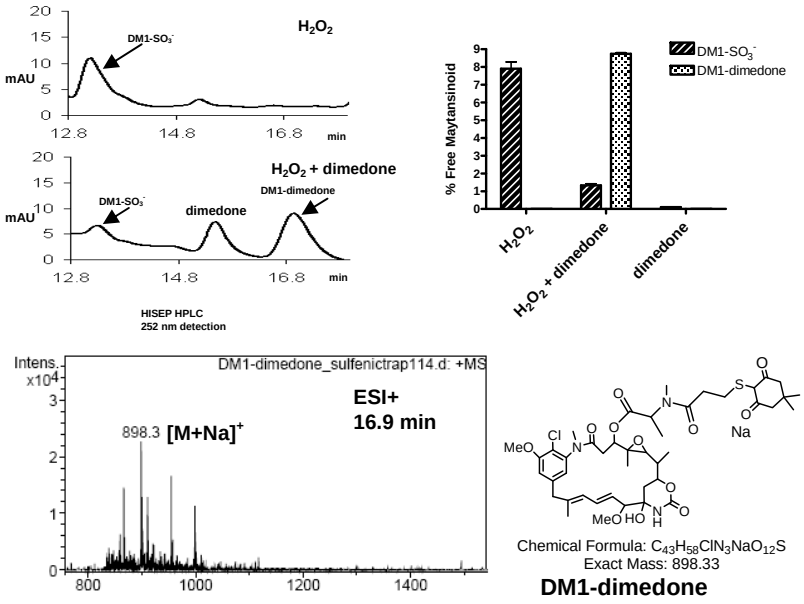





## Dimedone trapping of sulfenic acid intermediate:

Conjugates were assayed for extent of thioether oxidation to sulfoxide by kinetic trapping of the released sulfenic acid intermediate. Sulfoxide linker conjugate **8** (36 μM) was incubated with 10 mM dimedone (12 h, 37°C, PBS, pH 7.4), a quenching agent for sulfenic acid. Free maytansinoid species were acetone extracted and identified by LC-MS and quantified (by integrated area at 252 nm and comparison to a standard curve). Presence of maytansinoid-dimedone adduct (i.e. DM1-dimedone) was evidence of oxidation of the thioether ether linkage selectively to the sulfoxide.

**S3.** HPLC and MS data showing formation of DM1-dimedone upon free maytansinoid release from oxidized Ab-SMCC-DM1 conjugate **8** after 12 h at 37 °C.



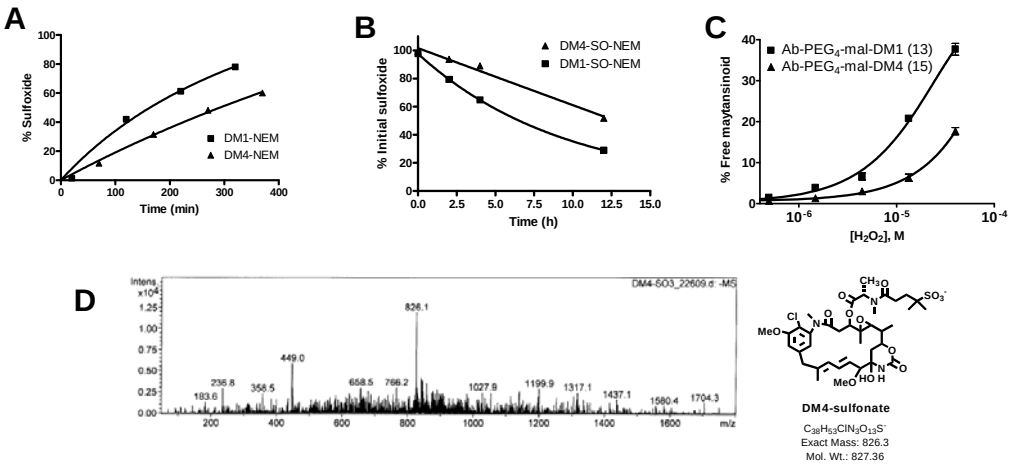

**Oxidation promoted release of maytansinoids from thioether linker Ab –PEG$_4$-mal-DMx:**

Ab-PEG$_4$-mal-DM1 **13** and Ab-PEG$_4$-mal-DM4 **15** (30 μM, average of 4 maytansinoids per antibody) with no detectable unconjugated maytansinoid was treated with hydrogen peroxide (0.1 μM – 50 μM final concentration) at pH 7.4 (PBS) for 12 h at 37 °C. Increase in free maytansinoids was quantified using a mixed-mode chromatography method ( Fleming, et al, *Analytical Biochemistry*, 2005, **340**, 272). Briefly, a HISEP shielded hydrophobic phase column (5 μm particle size, 4.6 × 250 mm length, Supelco, Bellefonte, PA, USA) was used for analyzing Ab–DM1 conjugates. Detection was at 252 and 280 nm (extracted from PDA spectra). The flow rate was 0.7 mL/min. Mobile phase A consisted of 100 mM ammonium acetate (pH 7.0). Mobile phase B was 100% acetonitrile. The column was equilibrated at 25% B followed by a linear gradient over 25 min to 40% B after sample injection. Intact conjugate elutes between 2-5 min while released maytansinoid is detected between 10-25 min.

**Rate of oxidation of DM1-NEM and DM4-NEM:**
10 μM DM1-NEM or DM4-NEM (95% PBS pH 7.4, 5% dimethylacetamide) were treated at ambient temperature with 1 mM hydrogen peroxide and reaction aliquots (30 μL) were injected directly onto RP-HPLC at various time points from 0-400 min. Maytansinoid products were quantified by measuring integrated area under the curve at 252 nm and comparison to a calibration curve with known standards.

**Rate of sulfoxide elimination of DM1-SO-NEM and DM4-SO-NEM:**
8 μM DM1-SO-NEM or DM4-SO-NEM (95% PBS pH 7.4, 5% dimethylacetamide) were incubated at 37 °C and reaction aliquots (50 μL) were injected directly onto RP-HPLC at various time points from 0-12 h. Maytansinoid products were quantified by measuring integrated area under the curve at 252 nm and comparison to a calibration curve with known standards.

**S4.** Rate comparison of model thioether oxidation (A), model sulfoxide elimination (B) and free maytansinoid formation from Ab-PEG$_4$-mal-DMx conjugate (C) for unhindered (DM1) and hindered (DM4) maytansinoid thioethers. Thioether derived from hindered thiol DM4 oxidizes 2-fold slower and undergoes sulfoxide elimination 3-fold slower than corresponding thioether formed from unhindered DM1 thiol. The observation that Ab-PEG$_4$-mal-DM4 is ~5-fold more resistant to oxidation-promoted free maytansinoid release than Ab-PEG$_4$-mal-DM1 (panel C) is consistent with the observed rates of sulfoxide formation and beta-elimination. Panel D shows MS data (ESI$^-$) for DM4-sulfonate (16) formed from Ab-PEG$_4$-mal-DM4 oxidation at 37°C, pH 7.4.

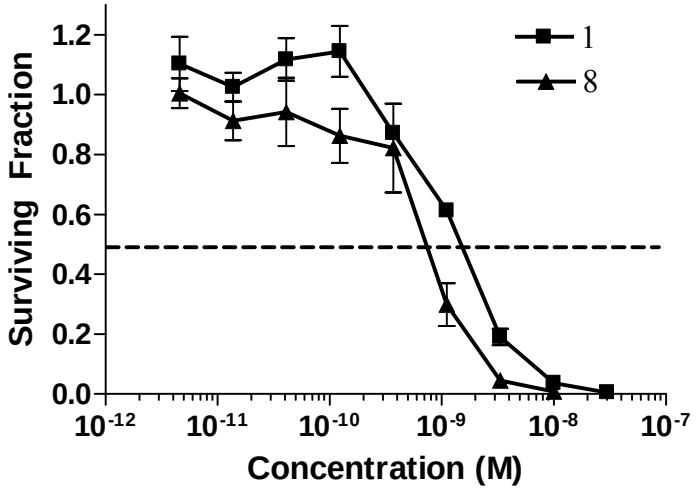



***In Vitro* cytotoxicity assays comparing activity of conjugate 1 (Ab-SMCC-DM1) and selectively oxidized conjugate 8**

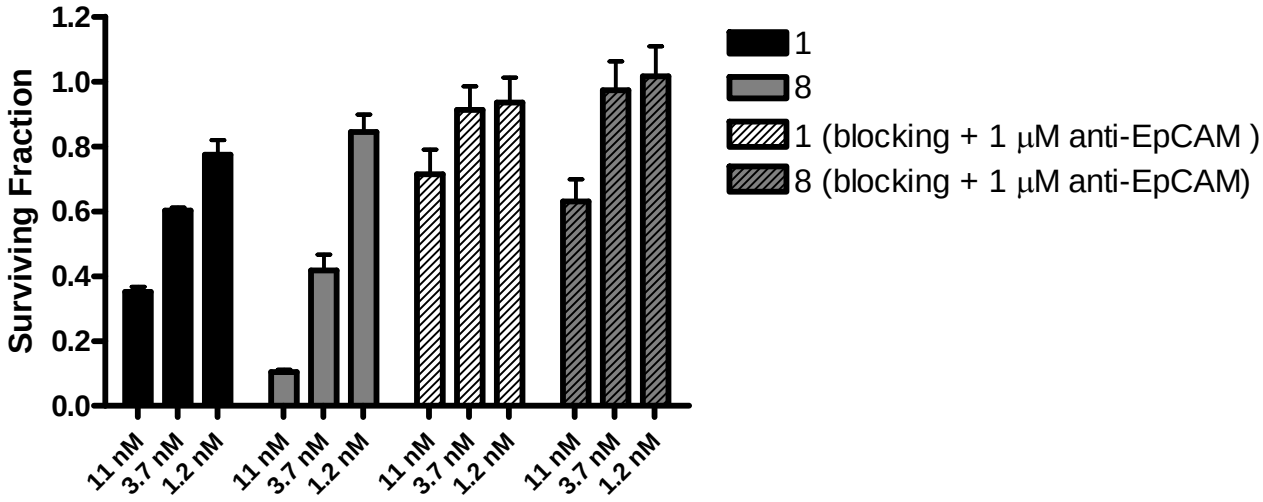
PC9 cells (for anti-EpCAM-SMCC-DM1) and A431 cells (for anti-EGFR-SMCC-DM1) grown in growth medium (DMEM (Invitrogen) containing 10% fetal bovine serum (ATCC), 2 mM L-glutamine (Invitrogen), and penicillin-streptomycin (Invitrogen)) were seeded at 1000 and 2000 cells/well in 96-well flat-bottom tissue culture plates (Falcon) in growth media and allowed to adhere overnight at 37 $^{o}$C. All cells were obtained from ATCC. After 1 day, cells were continuously exposed to anti-EpCAM or anti-EGFR

AMCs (**1** and **8**) for 96 h at 37 $^{o}$C at concentrations of 2 pM to 30 nM (3-fold serial dilutions). Blocking controls were performed in parallel where cells were treated for 1 h at 37 $^{o}$C with 1 μM unconjugated antibody in fresh growth media prior to conjugate exposure. Each test condition was performed in triplicate. After conjugate treatment, 20 μl WST-8 reagent (Dojindo Molecular) was added to each well and incubated at 37 $^{o}$C for 1-5 h for color development. The plate absorbance was measured at 450 nm and used to calculate the surviving cell fraction (compared with untreated control cells). The $IC_{50}$ values were estimated from plots of surviving cell fraction versus conjugate concentration (M).

**S5.** Anti-EGFR-SMCC-DM1 **1** and anti-EGFR-sulfoxide-DM1 **8** cell killing activity against A431 squamous cell carcinoma in vitro. Conjugate **8** (thioether partially oxidized selectively to the sulfoxide) is ~ 2-fold more potent than the parent conjugate **1**.



**S6.** Anti-EpCAM-SMCC-DM1 **1** and anti-EpCAM-sulfoxide-DM1 **8** cell killing activity against PC9 lung carcinoma in vitro. Conjugate **8** (thioether partially oxidized selectively to the sulfoxide) is ~ 3-fold more potent than the parent conjugate **1**. Preincubation of PC9 cells with 1 μM unconjugated anti-EpCAM antibody (to fully block antigen binding sites) leads to significantly reduced cell killing activity for conjugates **1** and **8**. Therefore, the additional activity of **8** over **1** is antigen dependant.




**S7.** Mechanistic scheme explaining all observed products from mono-oxidized maytansinoid succinimide thioethers.