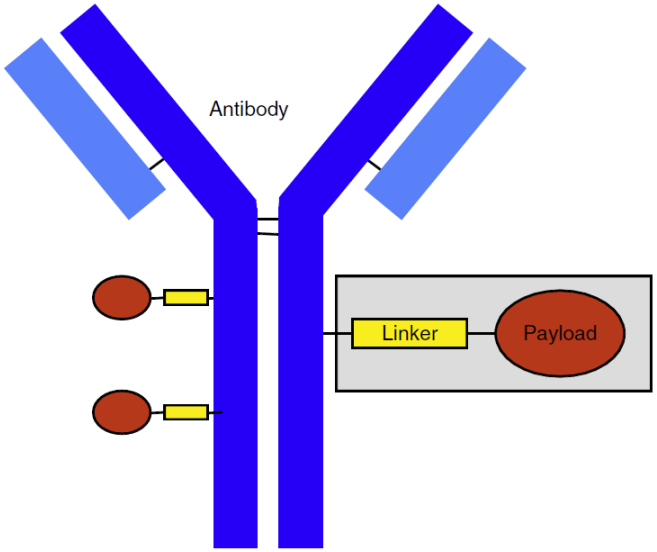
Antibody-Drug Conjugates

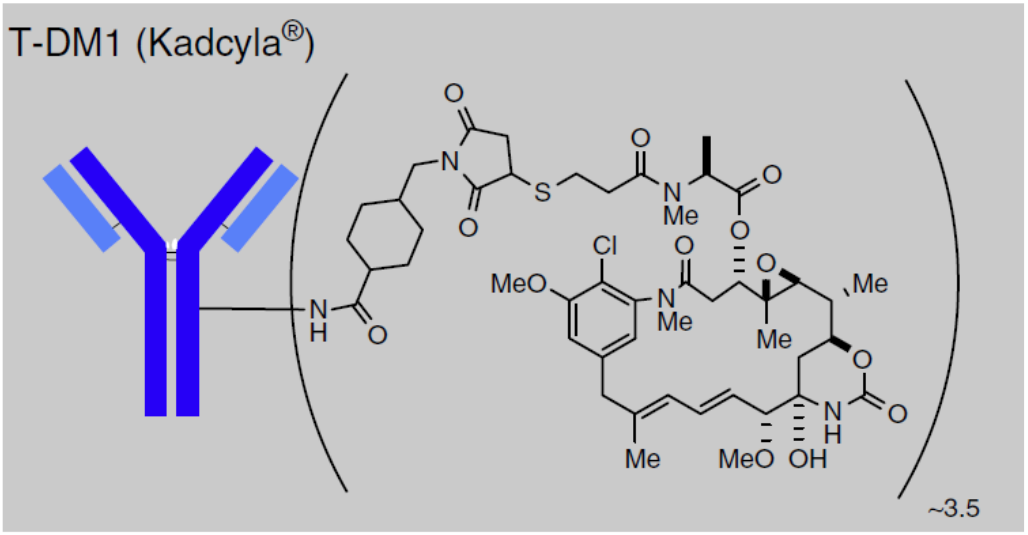
Literature Seminar (2016/11/15)

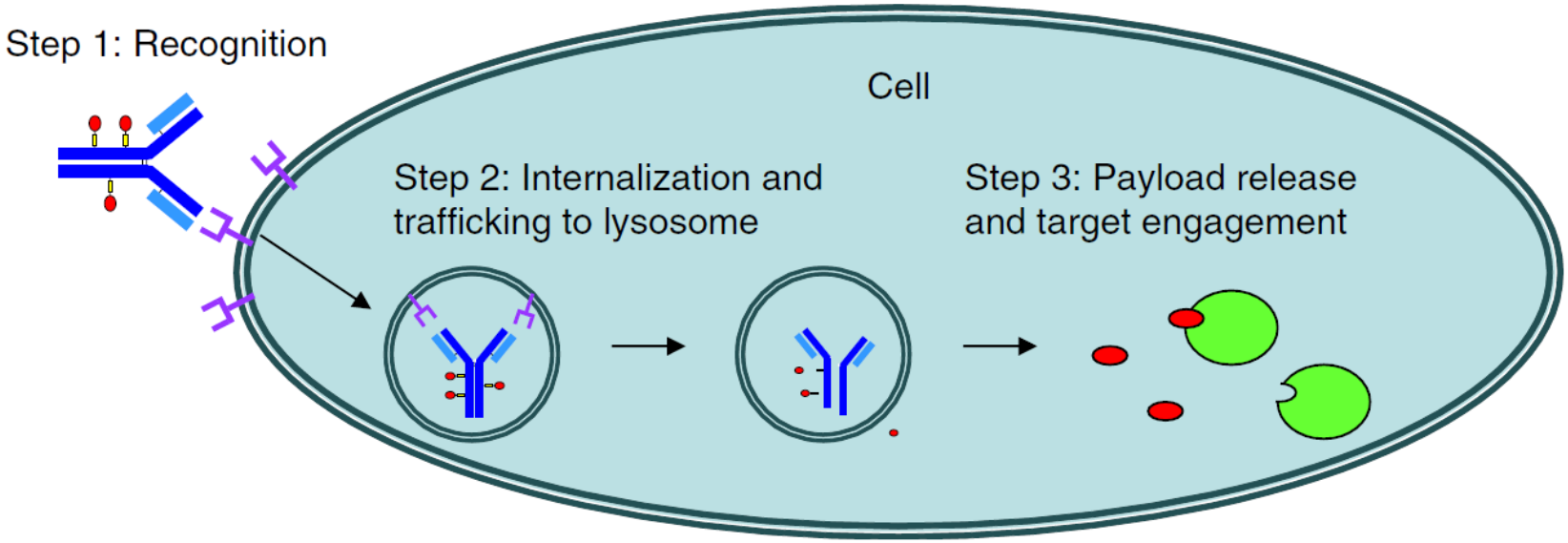
D2 Satoshi Hashimoto

Contents

Antibody-Drug Conjugates (ADCs)

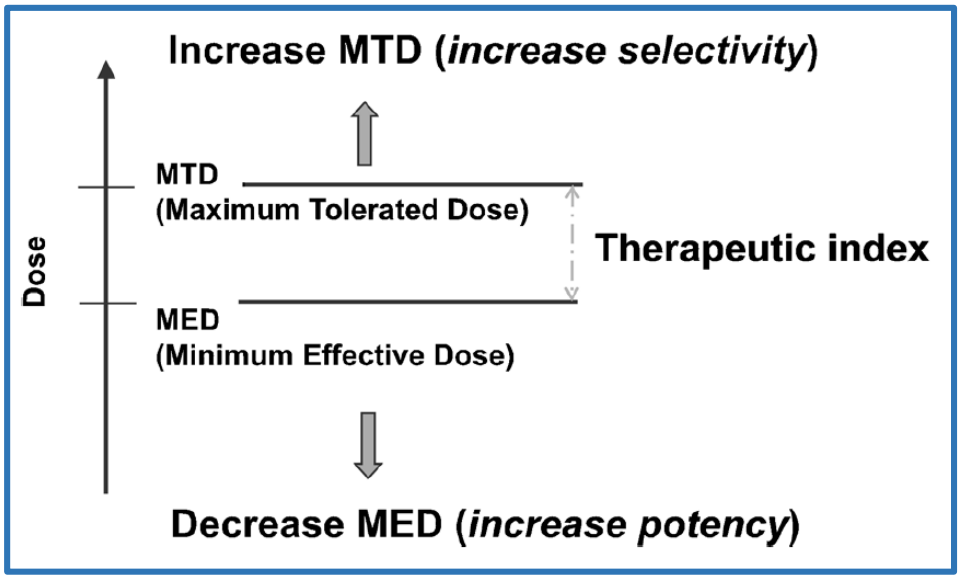


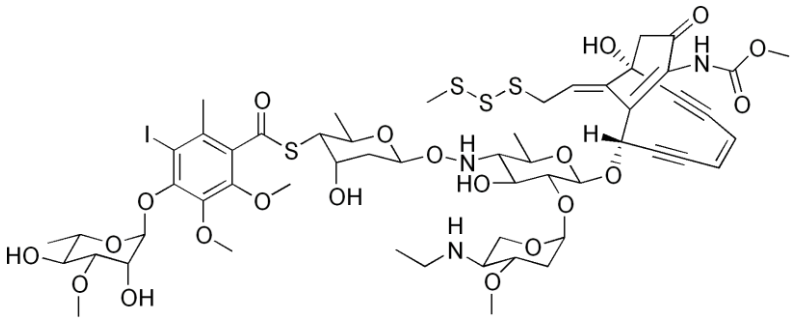




Comprehensive Organic Synthesis II, Volume 9 , 438-462.

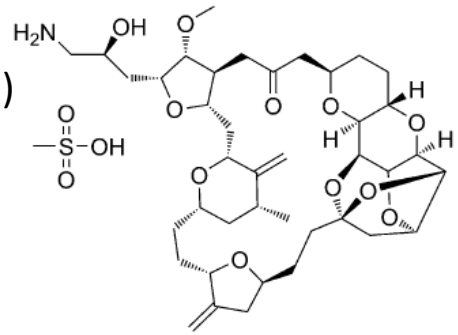
Anti-Cancer Agents

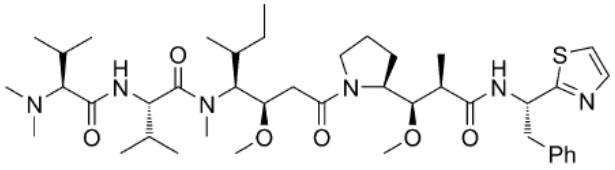




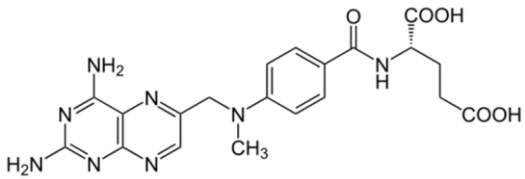
Carcheamicin ɣ1 (DNA cleavage)

Eribulin mesylate
(microtubule inhibition)





Dolastatin 10 (microtubule inhibition)



Methotrexate (antimeabolation of folate)

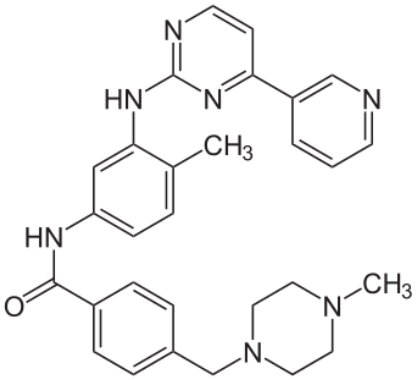
- Low therapeutic index caused systemic cytotoxicities
- Multidrug therapy is standard method today

Improvement of therapeutic index is needed.

Chari, R. V. J.; Miller, M. L.; Widdison, W. C. *Angew. Chem. Int. Ed.* **2014**, *53*, 3796.

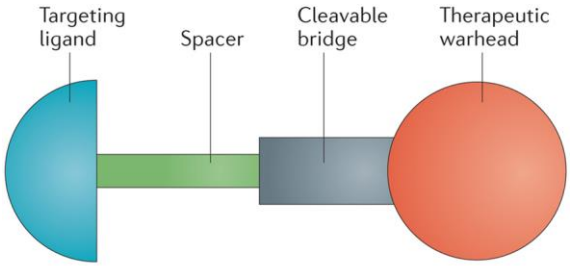
Targeted Therapies

- Inhibitors of receptor tyrosine kinases



Imatinib

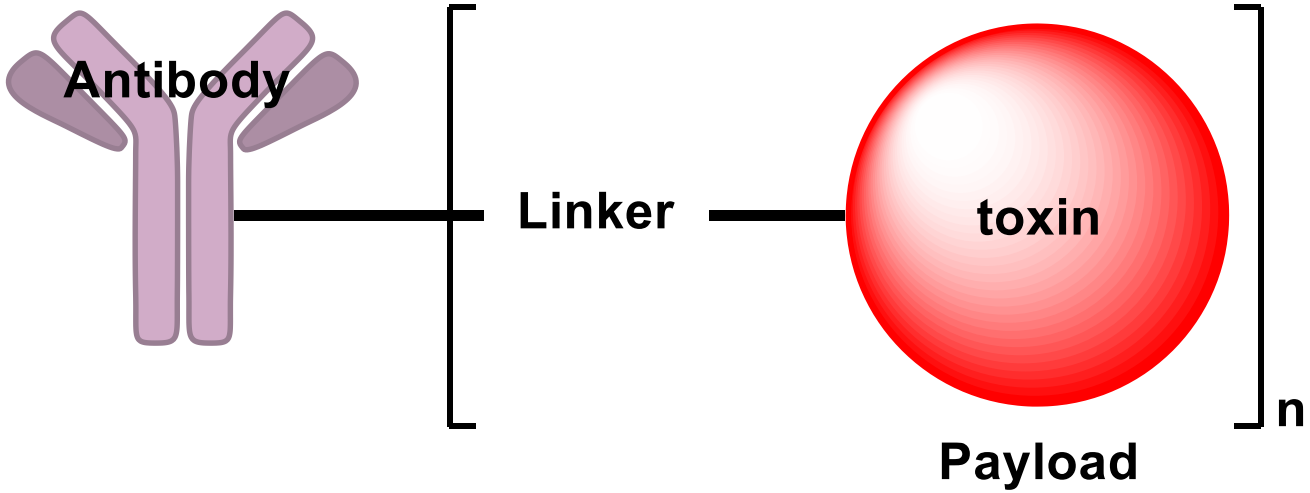
- Monoclonal antibodies
- Antibody-drug conjugates
- Small targeting molecule-drug conjugates



- Antisense method

- Drug container
  (151120_LS_Akinori_YAMAGUCHI)

Key Requirements of ACDs



Antibody

- High binding affinity
- Internizable
- Humanized/Human mAb

Linker

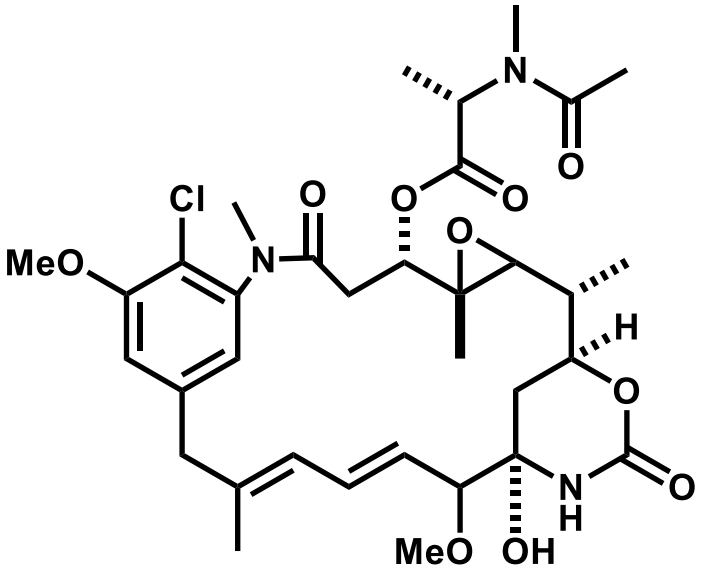
- Stable
- Efficient cleavage in cell

Payload

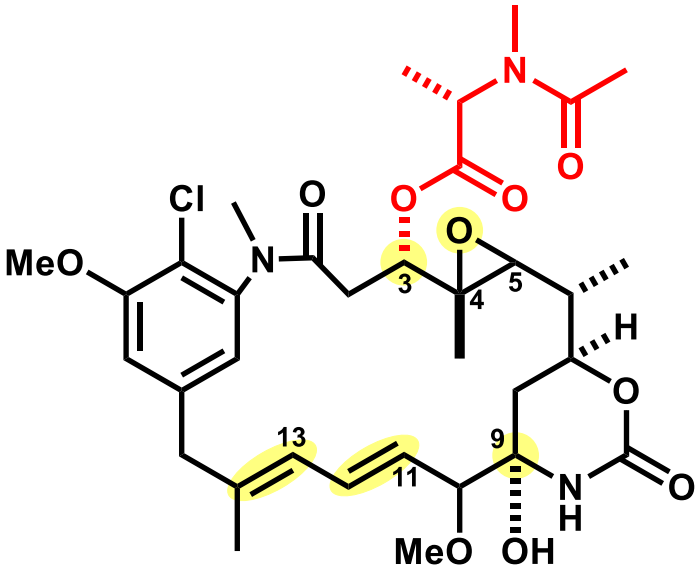
- High potency [pM]
- Stable
- Soluble
- Chemically modification
- Tumor selectivity

Contents

Maytansinoids



Maytansine



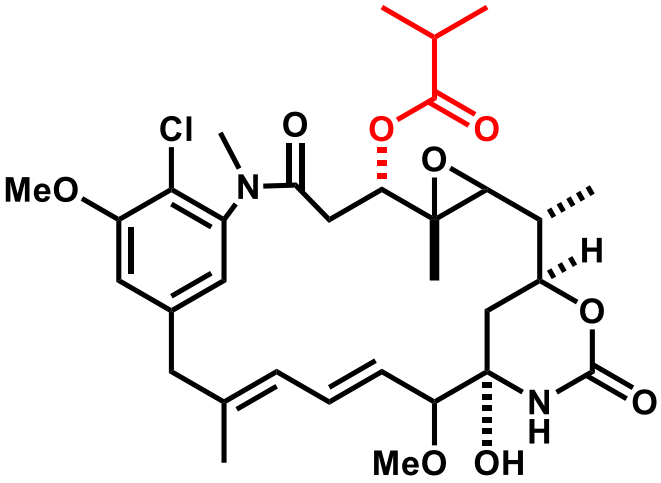
SAR of maytansine

Isolation: from the bark of *Maytenus ovatys* [1)]
Biological activity: microtubule inhibition [2)]
Structural features: 19-membered macrolactam
Total syntheses of Maytansine: [3)]
Corey (1980), Gao (1988),
Total syntheses of Maytansinol: [4)]
Meyers (1980), Isobe (1984), Khuong-Huu (1996)

Required:
Carbamate at C9
Double bonds at C11 & C13
Epoxide
Ester at C3 but structure could be varied.

1) Kupchan, S. M. *et al. J. Am. Chem. Soc.* **1972**, *94*, 1354.
2) Kupchan, S. M. *et al. Science* **1975**, *189*, 1002.
3) (a) Corey, E. J. *et al. J. Am. Chem. Soc.* **1980**, *102*, 6613. (b) Gao, Y. *Sci. Sin. Ser. B (Engl. Ed.)* **1988**, *31*, 1342.
4) (a) Meyers, A. I. *et al. J. Am. Chem. Soc.* **1980**, *102*, 6597. (b) Isobe, M. *et al. J. Am. Chem. Soc.* **1984**, *106*, 3252. (c) Khuong-Huu, F. *et al. J. Org. Chem.* **1996**, *61*, 7133.

Preparation of Antibody-Maytansinoid Conjugates (AMCs)

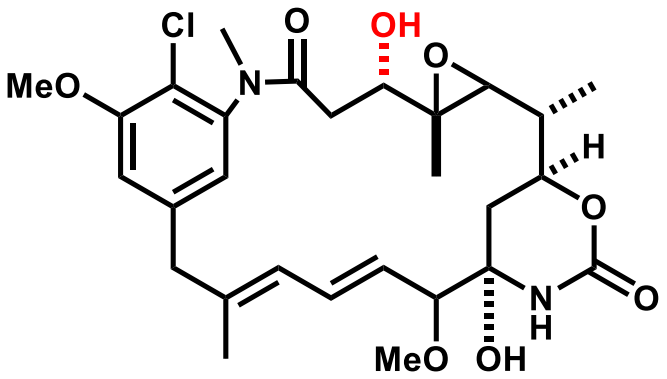


Ansamitocin P-3

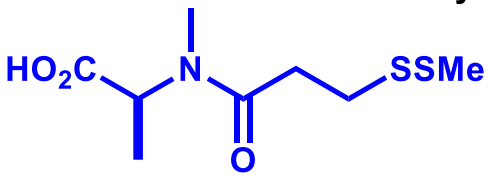
$LiAl(OMe)_3H$

THF

81%

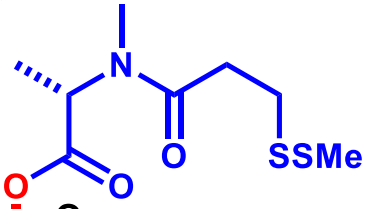


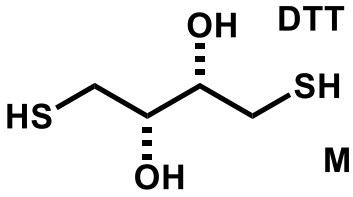
Maytansinol



DCC, $ZnCl_2$

CH_2Cl_2, 32%

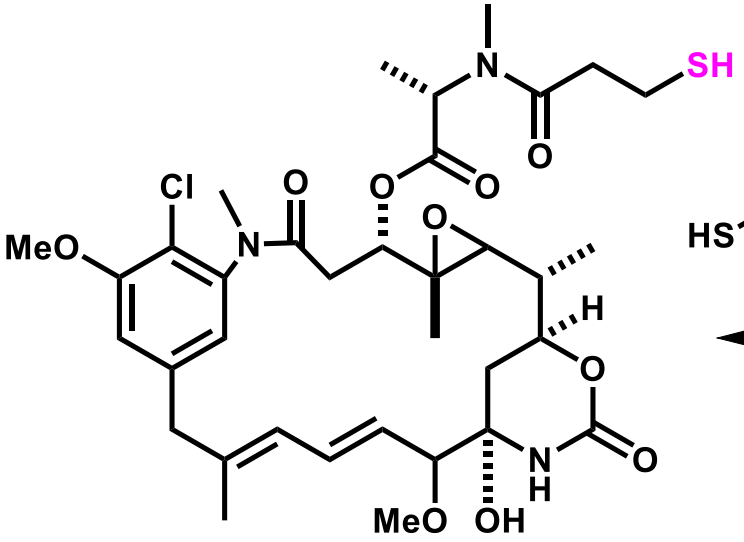




DTT

reduction

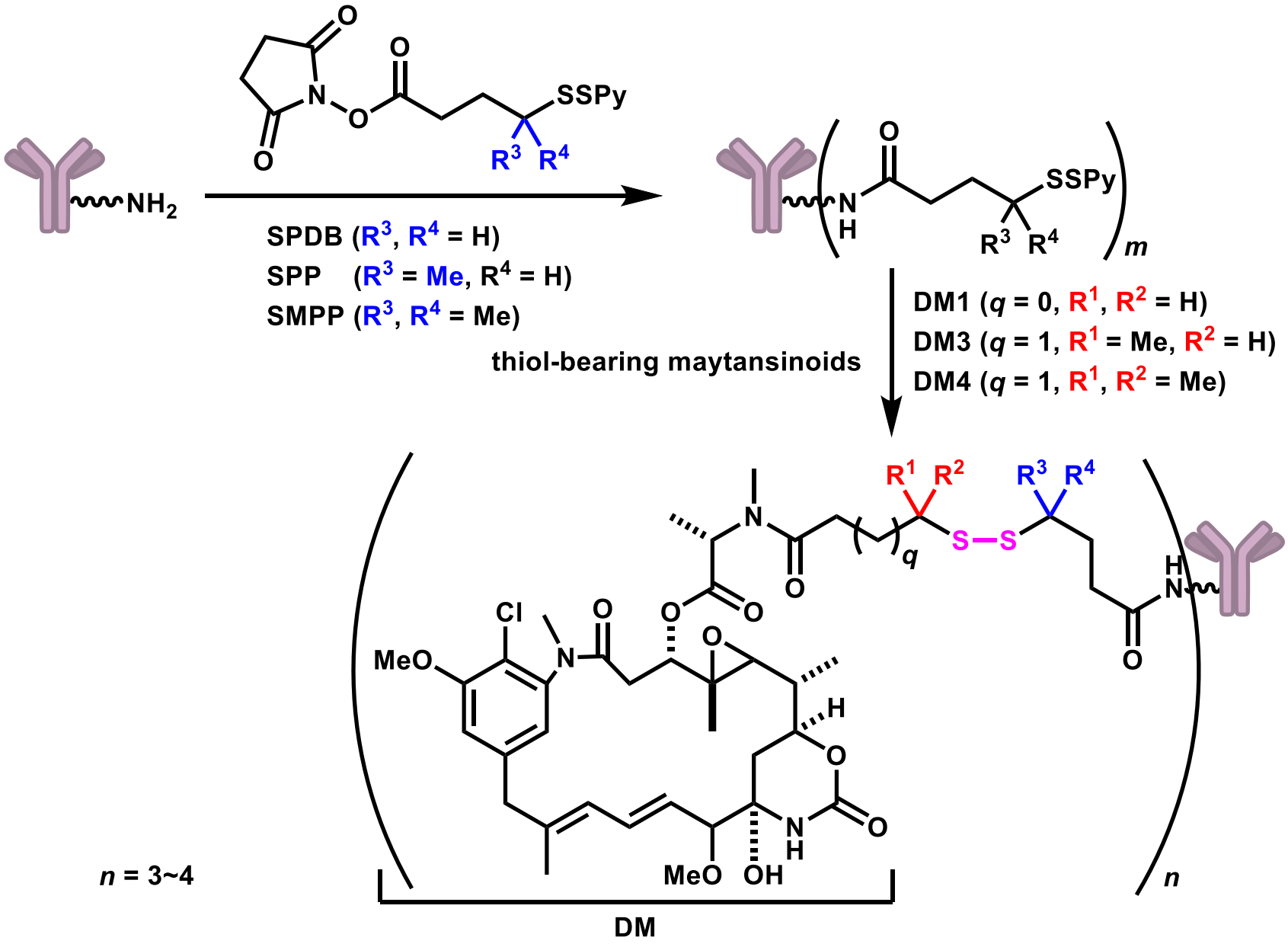
76%



DM1 (Drug Maytansine)

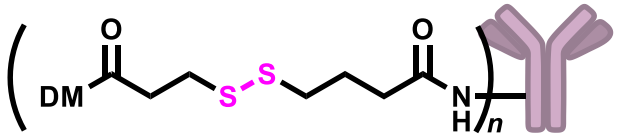
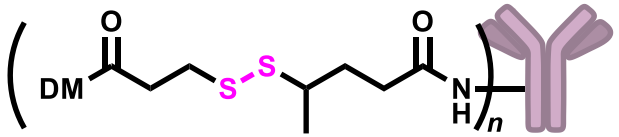
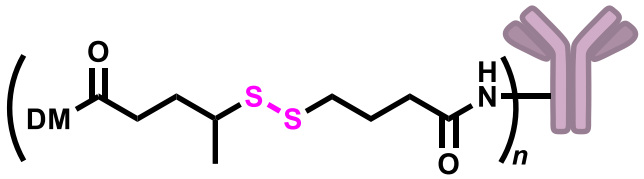
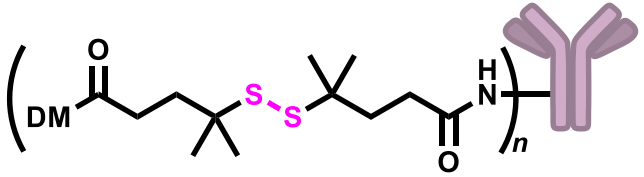
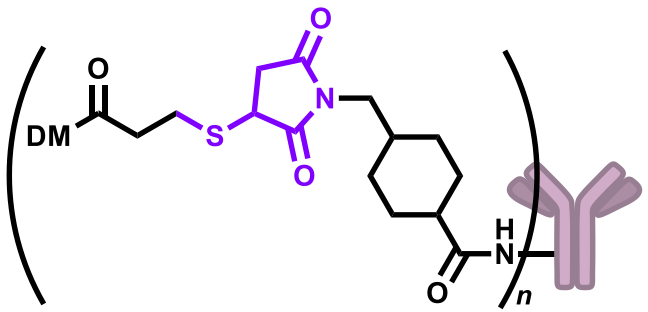
Chari, R. V. J. *et al. J. Med. Chem.* **2006**, *49*, 4392.

Preparation of Antibody-Maytansinoid Conjugates (AMCs)



Chari, R. V. J. *et al. J. Med. Chem.* **2006**, *49*, 4392.

Effect of Different Linkers on Stability and Cytotoxicity

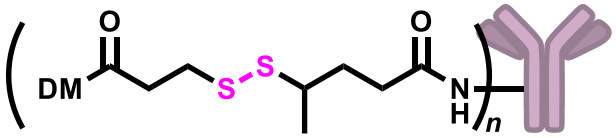
	Structure	Disulfide reduction rate[a] (k [$M^{-1}min^{-1}$])	Relative stability	EC_{50} (pM)
huC242-SPDB-DM1		14	1	15
huC242-SPP-DM1		2	7	15
huC242-SPDB-DM3		1.0	14	5.0
huC242-SMPP-DM4		<0.00064	>22000	5
huC242-SMCC-DM1 (non-cleavable thioether linker)		n.d.	n.d.	3.5

[a] disulfide reduction by DTT at pH 6.5, 37 °C.

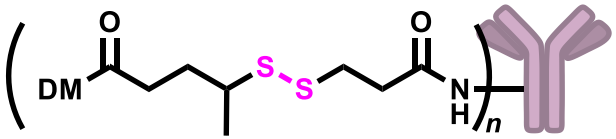
Kellogg, B. A. *et al. Bioconhugate Chem.* **2011**, *22*, 717.

Bystander Effect of AMCs

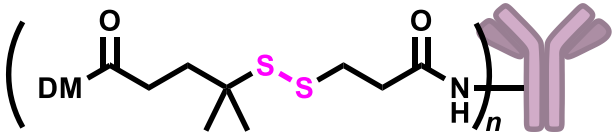
huC242-SPP-DM1



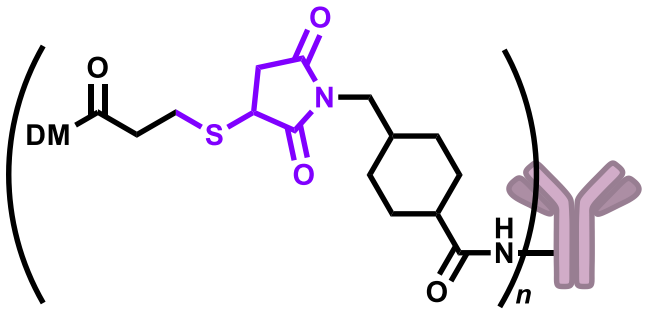
huC242-SPDP-DM3

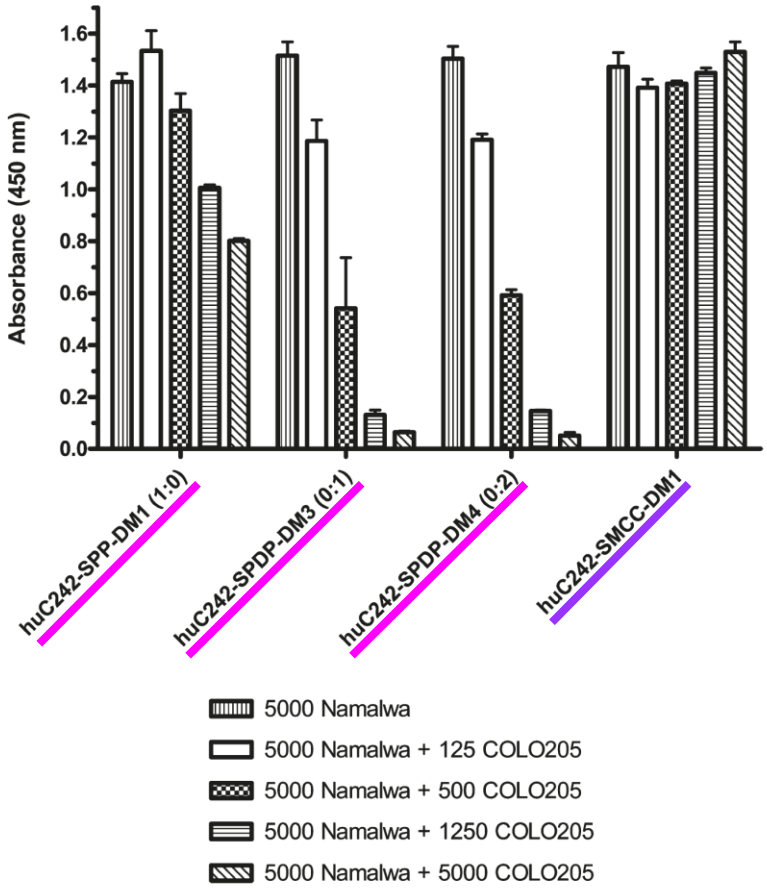


huC242-SPDP-DM4



huC242-SMCC-DM1
(non-cleavable thioether linker)



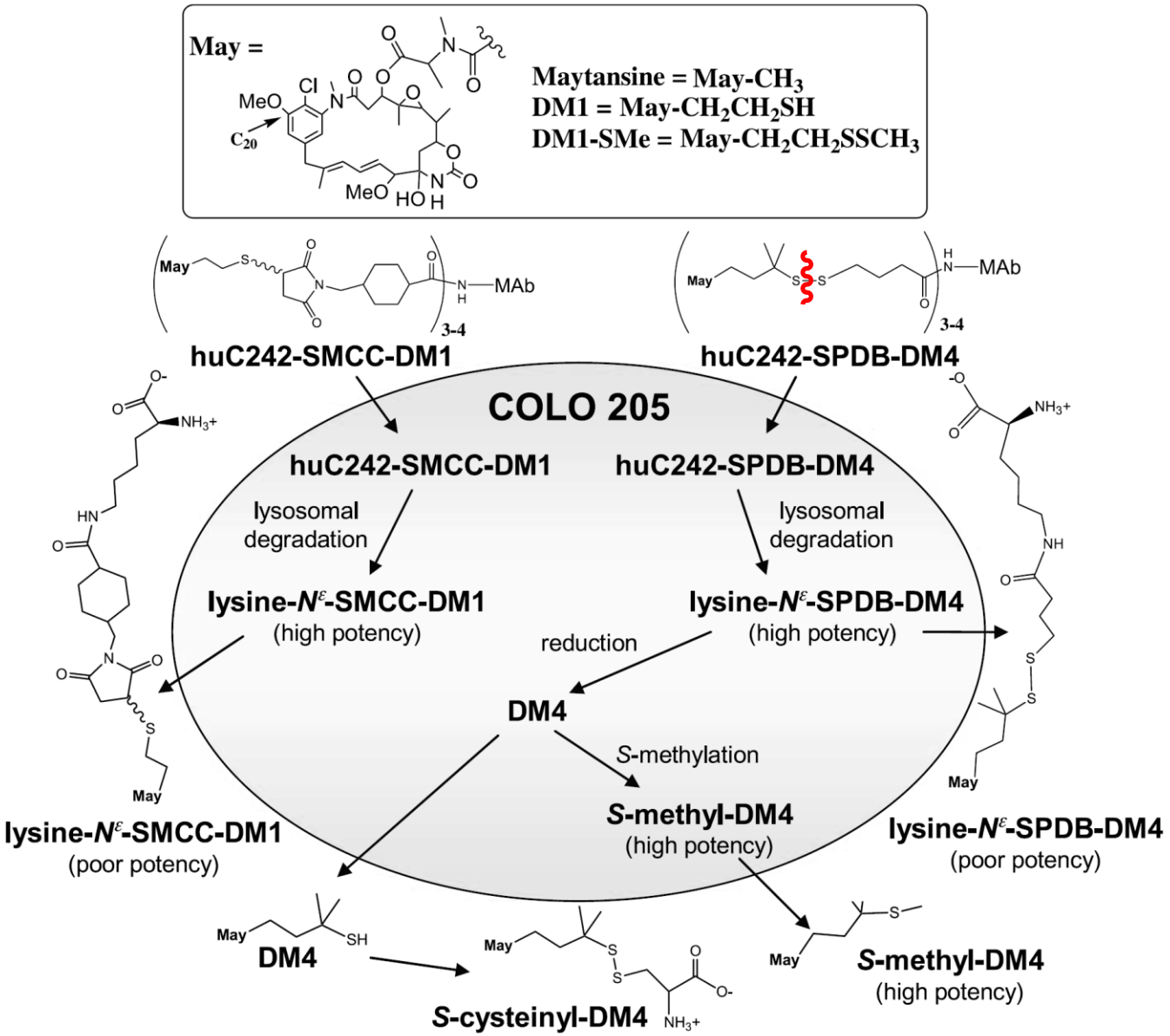


In vitro bystander killing of Ag(-) Namalwa cells by Ag(+) COLO 205 cells treated with huC242 AMCs.Cells were exposed to 1 nM conjugate for 4 days. All conhugates had ~4 DMx/mAb. Absorbance (450 nm) is a measure of cell viability.

Only AMC with the noncleavable linker had no bystander killing.

Kellogg, B. A. *et al. Bioconhugate Chem.* **2011**, *22*, 717.

Cellular Catabolism of AMCs

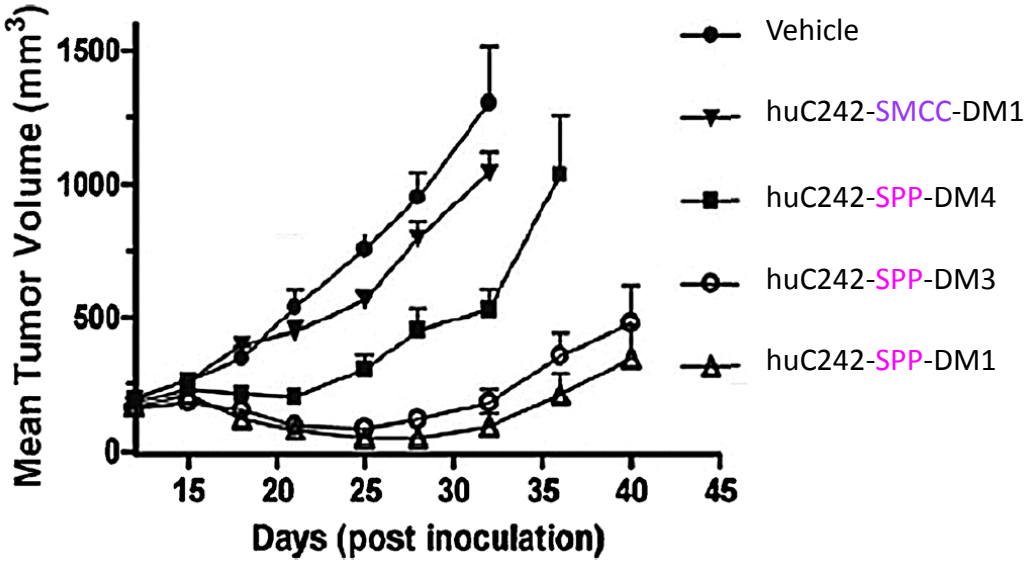


Erickson, H. K. *et al. Cancer Res.* 2006, *66*, 4426.

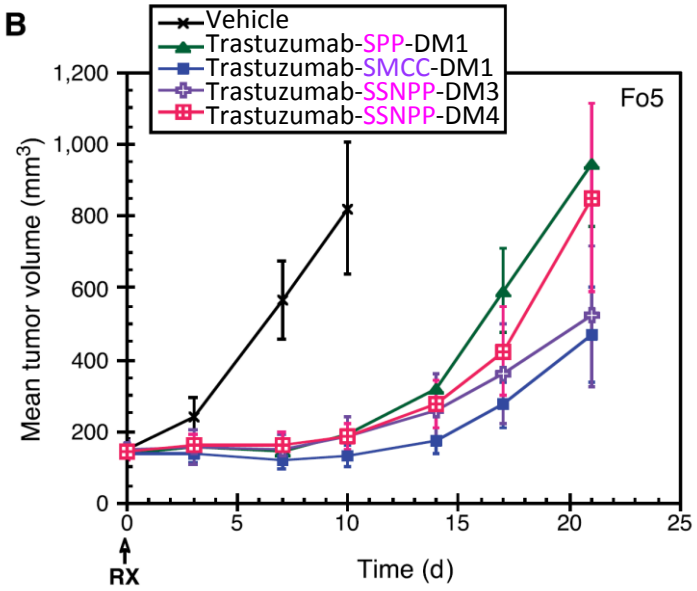
Effect of Linkers on Different Antibodies

Comparison of antitumor activities

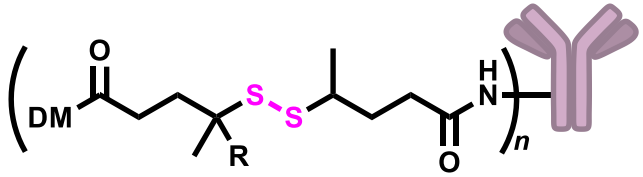
huC242 AMCs targeted CanAg [1)]



Tmab AMCs targeted HER2 [2)]



Cleavable disulfide linker ADC has a better drug release. Whereas **non-cleavable** thioether linker ADC has a improved stability.
There may be some target-antigen dependency.



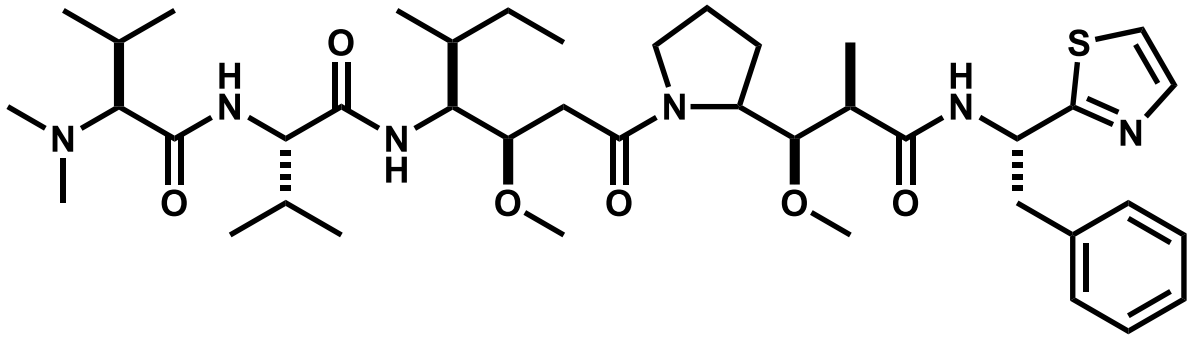
trastuzumab-SSNPP-DM3 (R = H)
trastuzumab-SSNPP-DM4 (R = Me)

Tmab-SMCC-DM1 was clinically approved by FDA as Kadcyla.

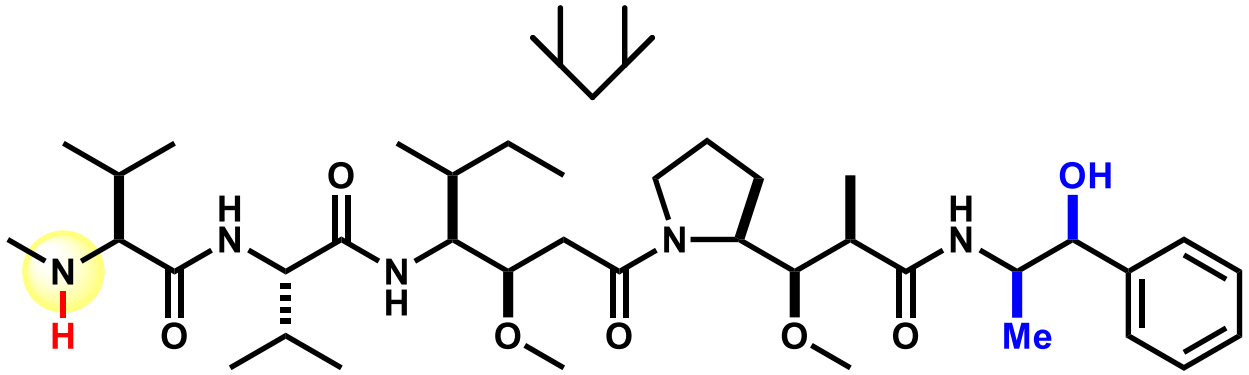
1) Kellogg, B. A. *et al. Bioconjugate Chem.* **2011**, *22*, 717. 2) Phillips, G. D. L. *et al. Cancer Res.* **2008**, *68*, 9280.

Contents

Auristatin Analogues



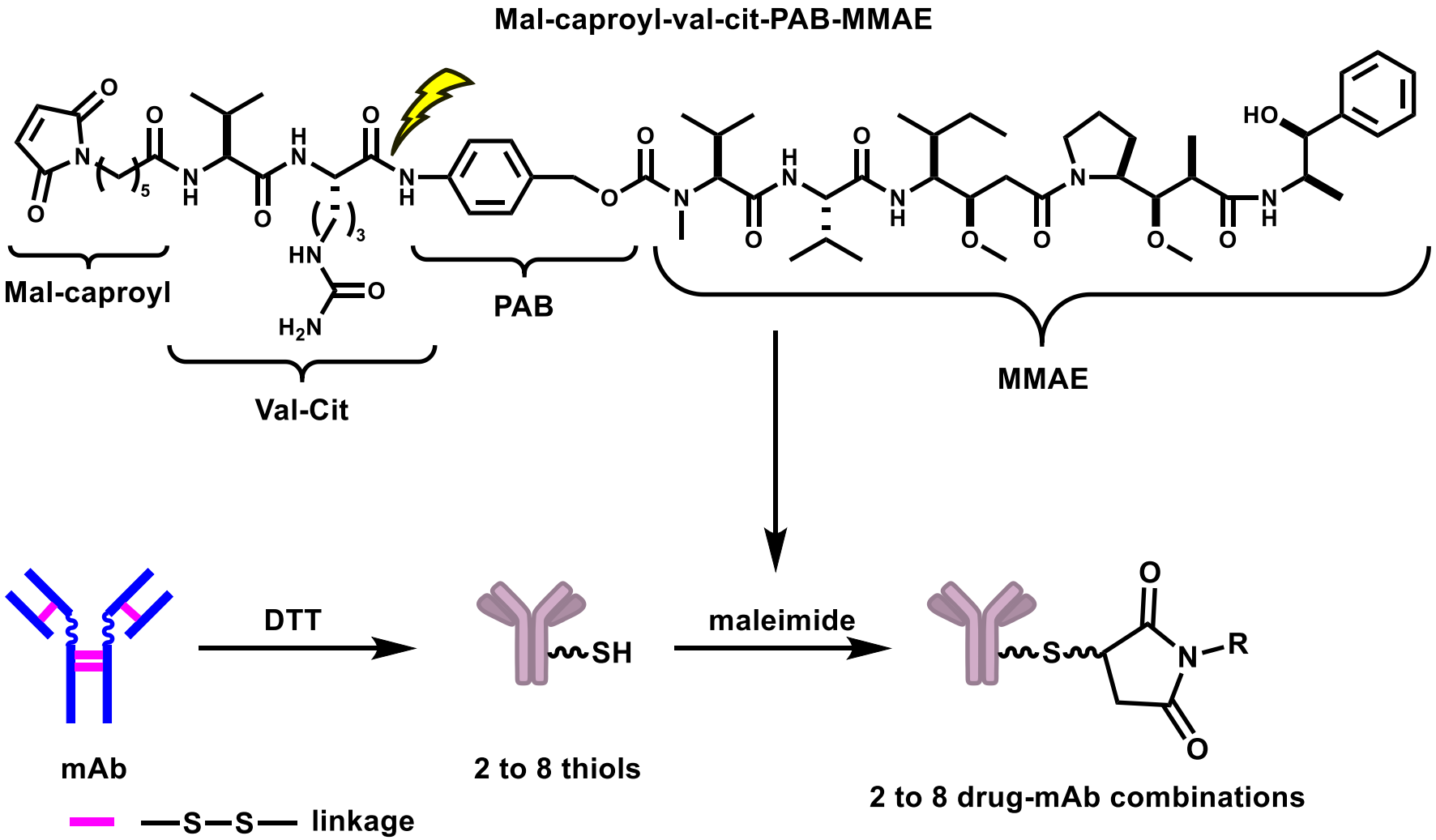
Dolastatin 10: highly potent antimitotic agent, IC_{50} = 0.1 (nM)
no appropriate site to bind antibody



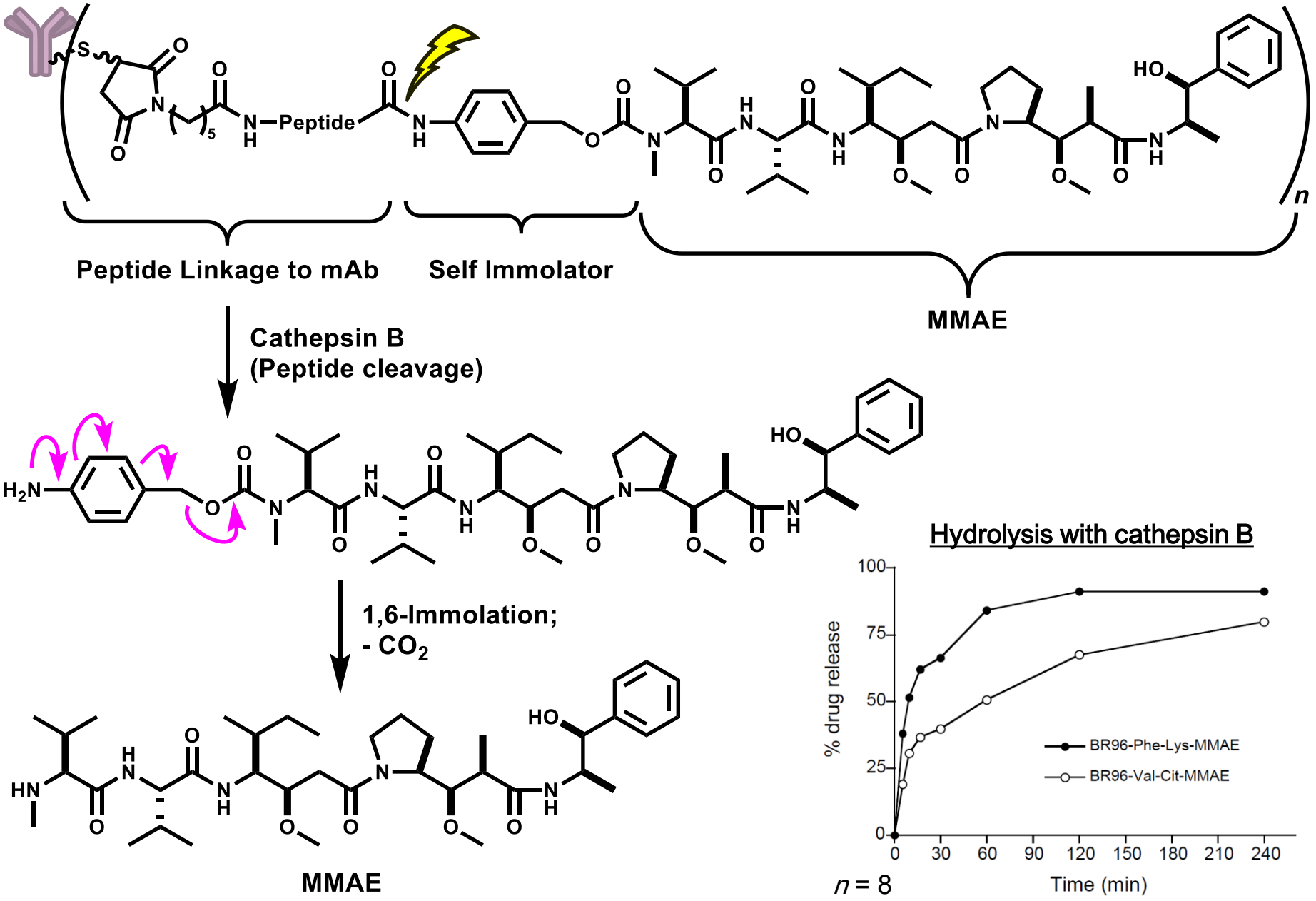
Monomethyl Auristatin E (MMAE): IC_{50} = 0.1 (nM) [2)]

1) Pettit, G. R. *et al. J. Am. Chem. Soc.* **1987**, *109*, 6883.
2) Doronina, S. O. *et al. Bioconjugate Chem.* **2006**, *17*, 114.

Linker Design of Auristatin ADCs

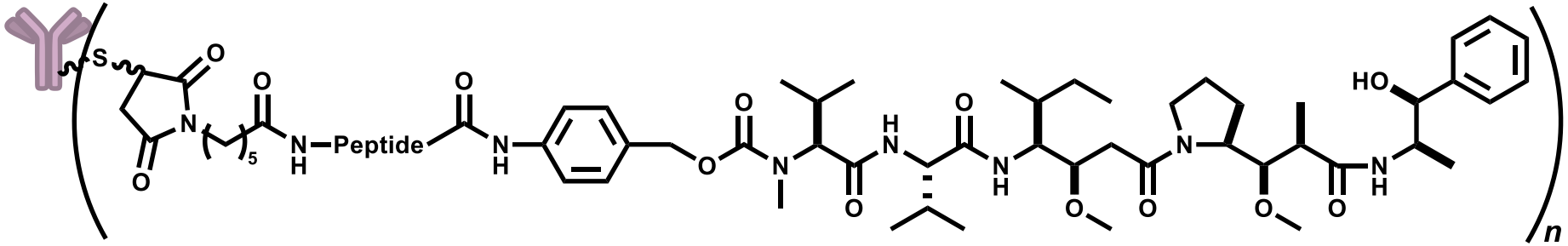


Cellular Catabolism of Auristatin ADCs

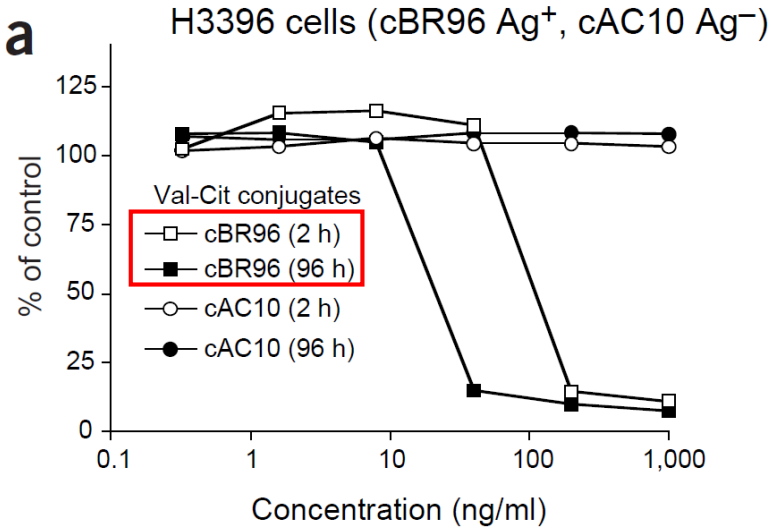


Doronina, S. O. *et al. Nat. Biotechnol.* **2013**, *21*, 778

Citotoxicity of Auristatin ACD

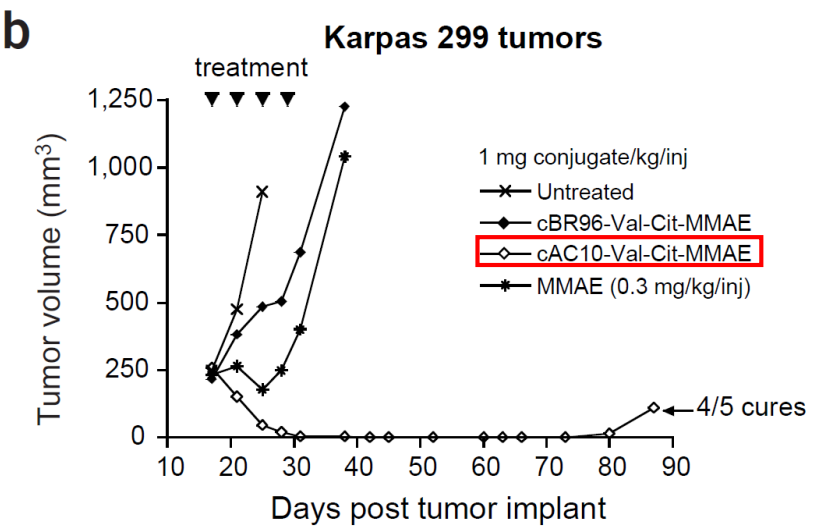


In Vitro



Cytotoxic effects on H3396 human breast carcinoma cells (cBR96 Ag+, cAC10 Ag-).

In Vivo

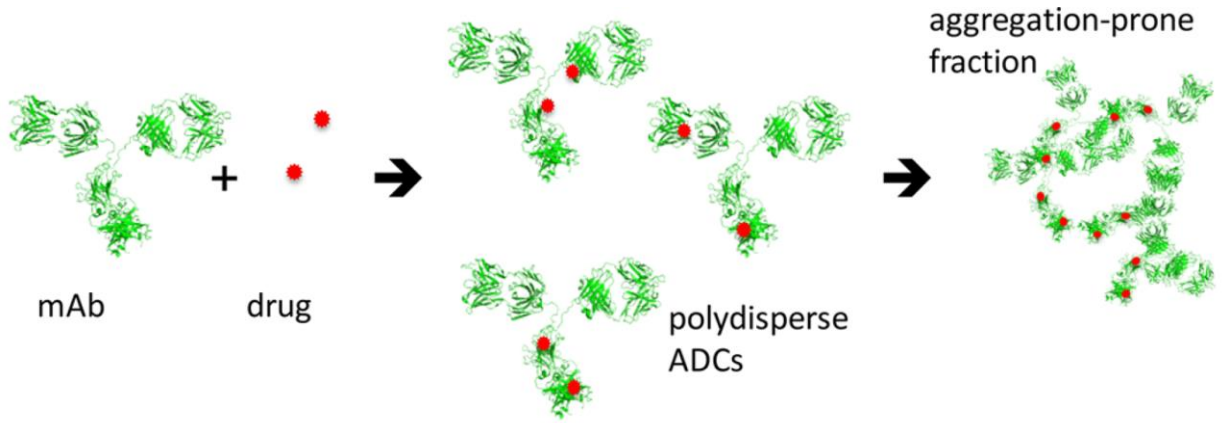


SCID mice with Karpas 299 human ALCL tumors (cAC10 Ag+, cBR96 Ag-) were treated with MMAE or mAb-Val-Cit-MMAE (1/30th of MTD).

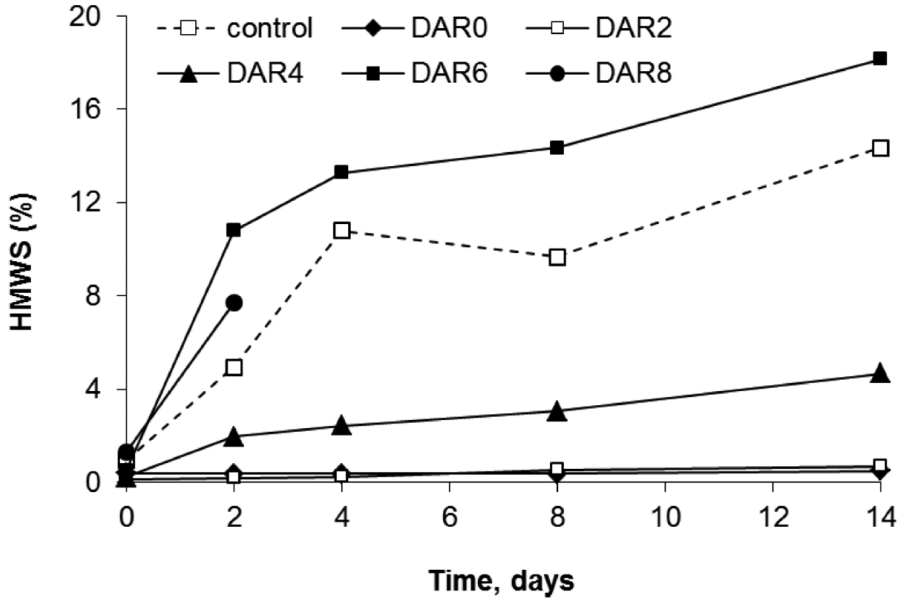
cAC10-Val-Cit-MMAE was clinically approved by FDA as Brentuximab vedotin (Adcetris).

Contents

Potential Aggregation



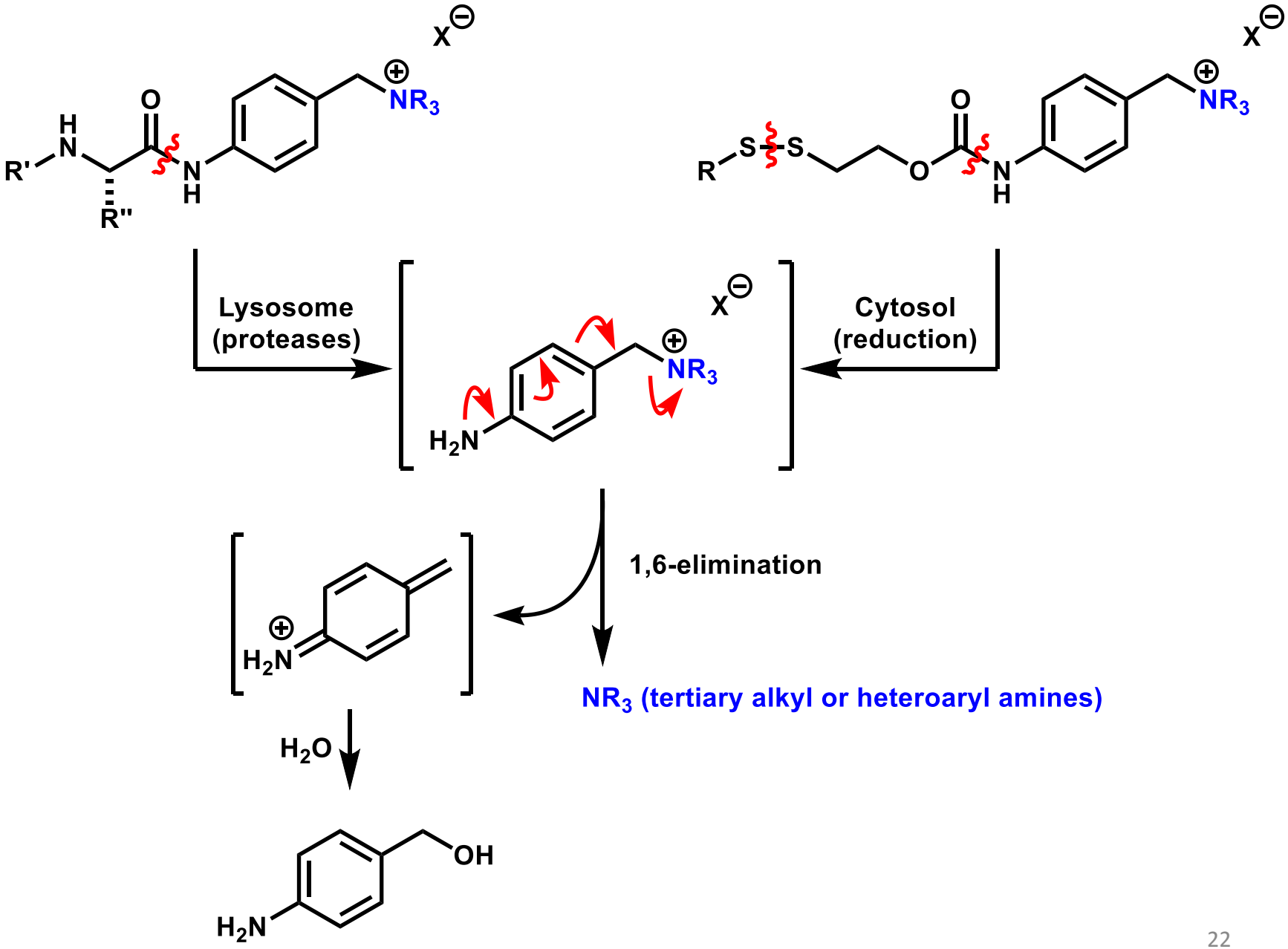
Thermal Stabilities of isolated mAb-Val-Cit-MMAE



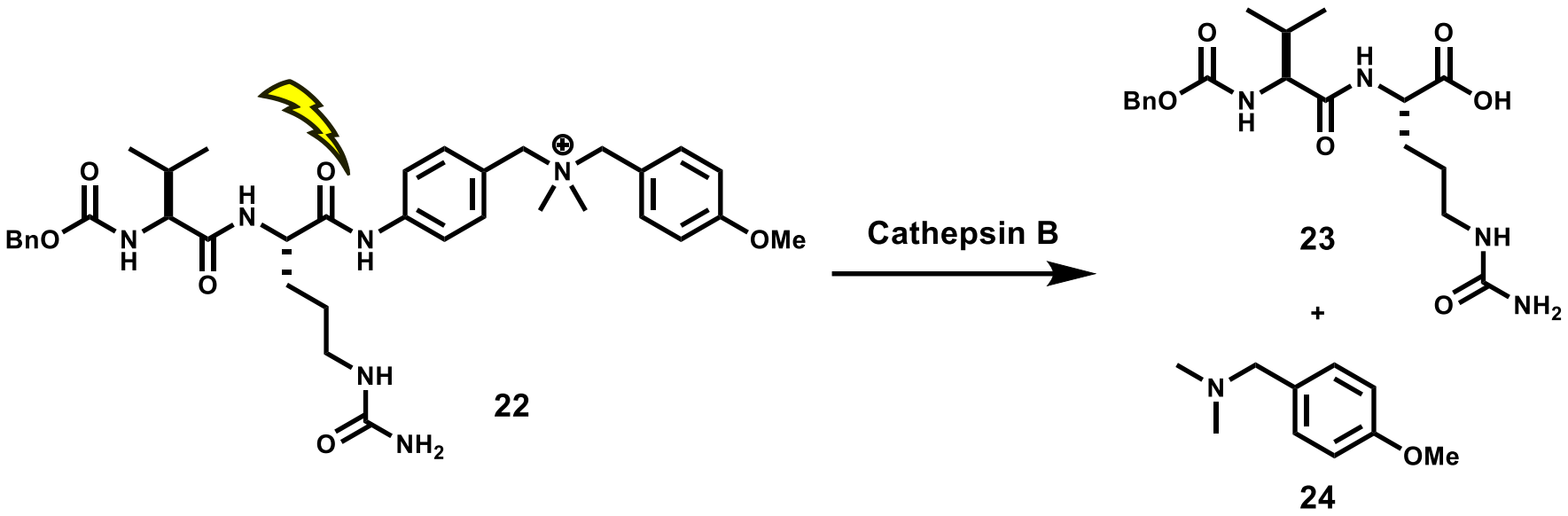
mAb-ADCs were incubated in buffer and HMWS was determined by size-exclusion chromatography. The control was mAb-ADC containing a mixture of 0-8 drug molecules per mAb (average DAR = 3.6).
(DAR = drug to antibody ratio, HMWS = high molecular-weight species)

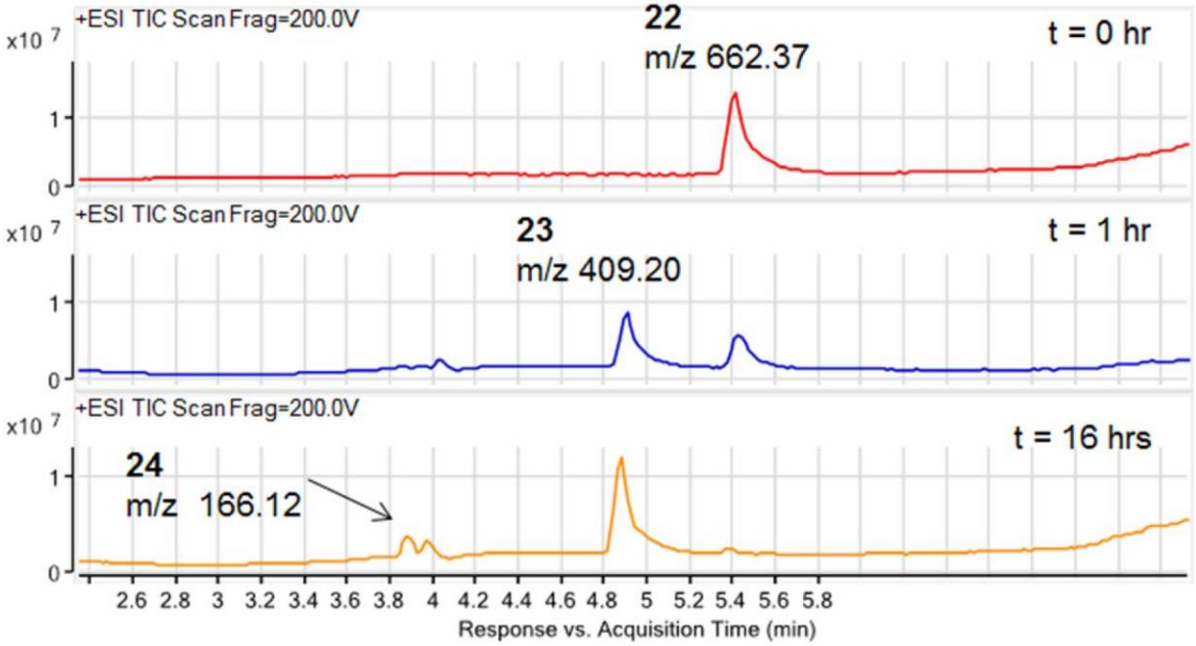
Adem, Y. T. *et al. Bioconhugate Chem.* **2014**, *25*, 656

Mechanism of *p*-Aminobenzyl Quaternary Ammonium Salt

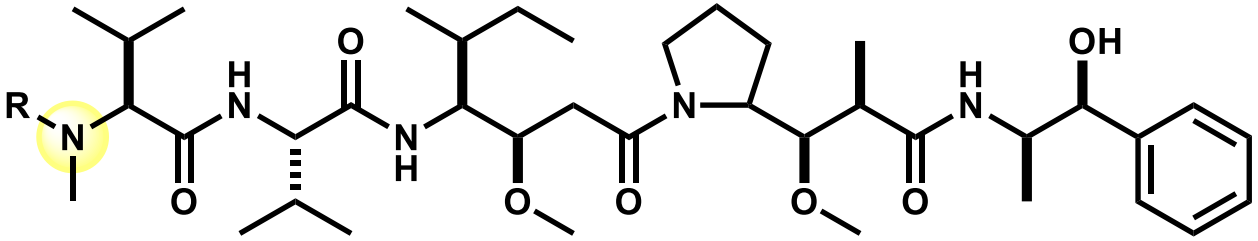


Characterization of Quaternary-Ammonium-Linked System

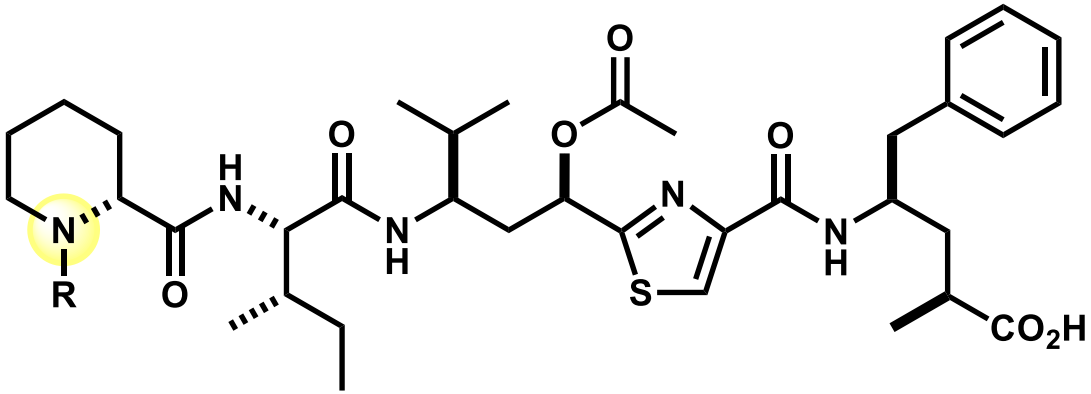




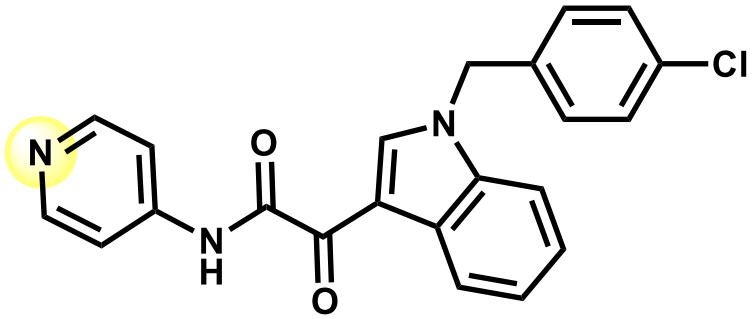
Selection and Evaluation of Amines



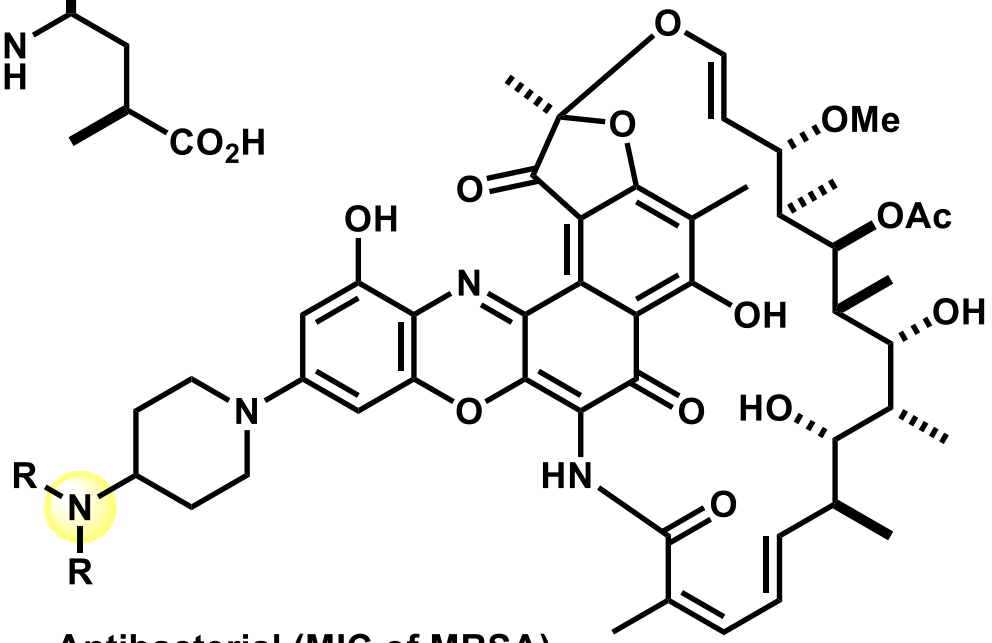
Antitumor (IC_{50})
Auristatin E: R = Me (0.12 nM)
MMAE: R = H (0.23 nM)



Antitumor (IC_{50})
Tubulysin: R = Me (0.048 nM)
Tubulysin: R = H (1.2 nM)

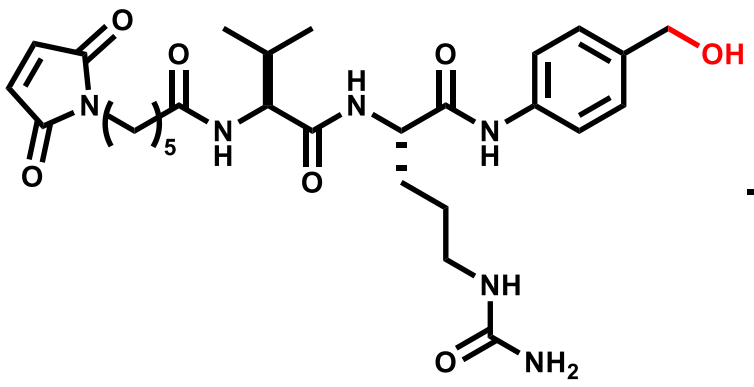


Antitumor (IC_{50})
Indibulin (59.1 nM)



Antibacterial (MIC of MRSA)
dmDNA31: R = Me (3.9 nM)
DNA31: R = H (10 nM)

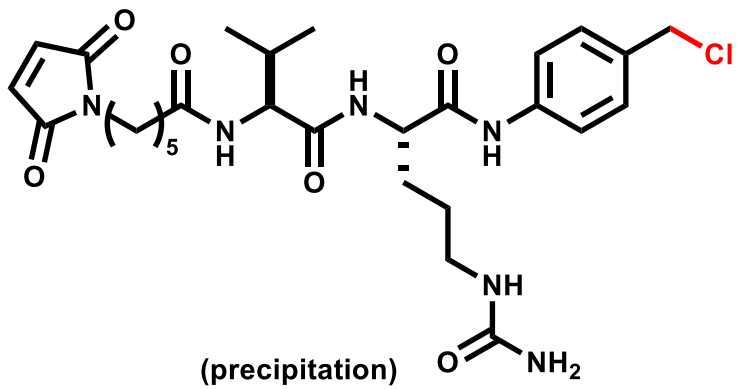
Synthesis of a Library of PABQ-Linked Amines



$SOCl_2$

DMF

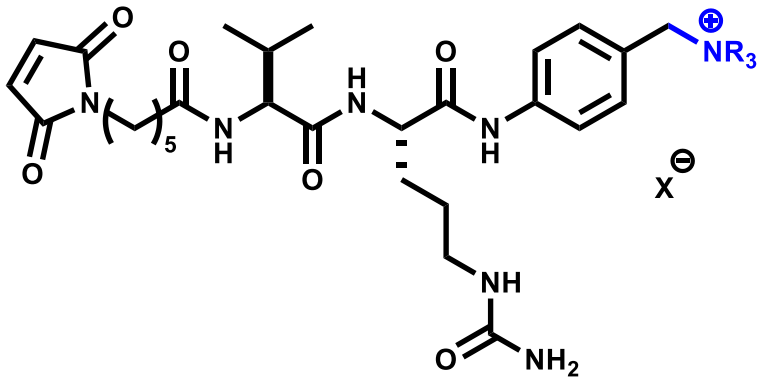
77%



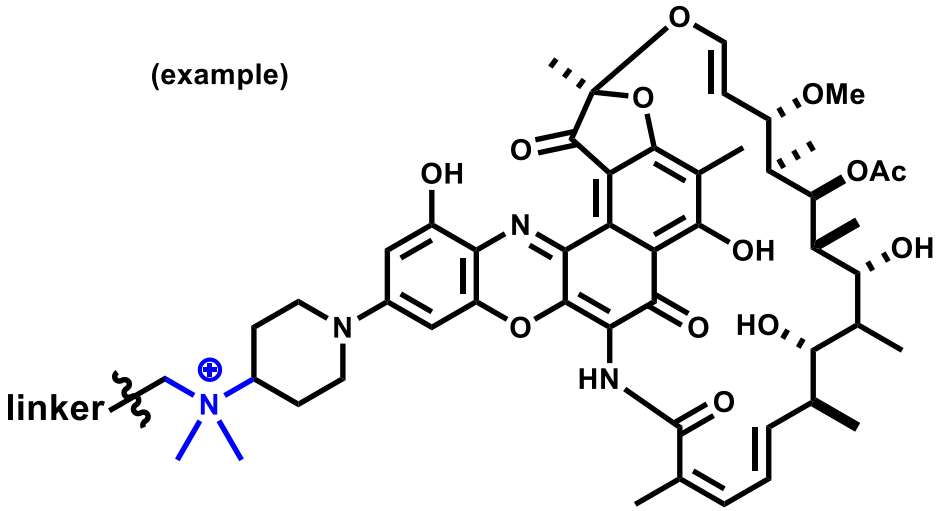
(precipitation)

NR_3, TBAI
DIPEA, DMF

11-60%
(10 compounds)

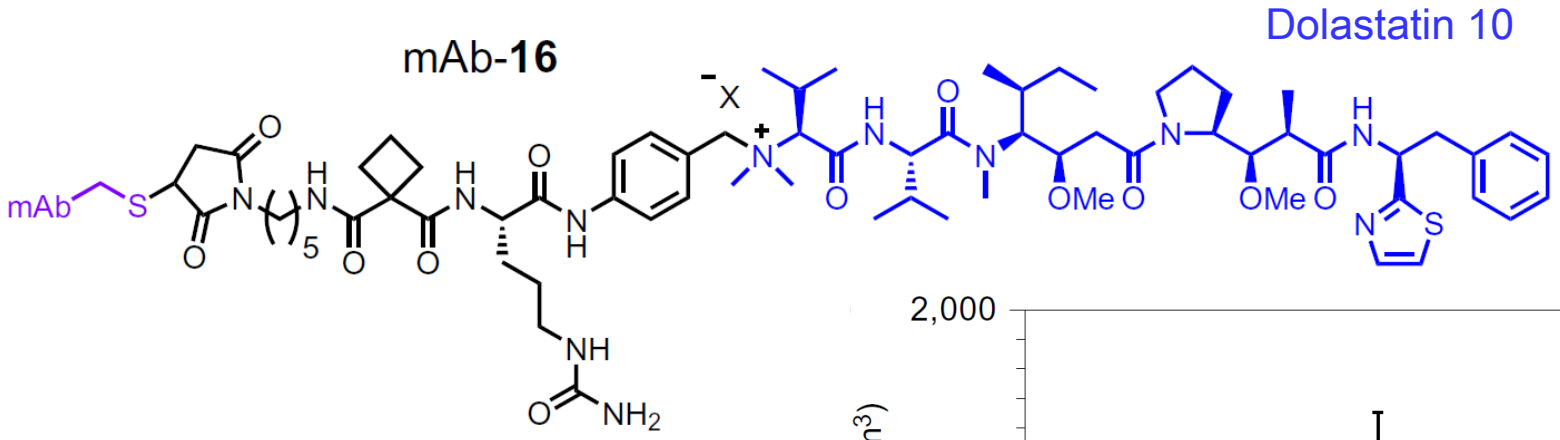


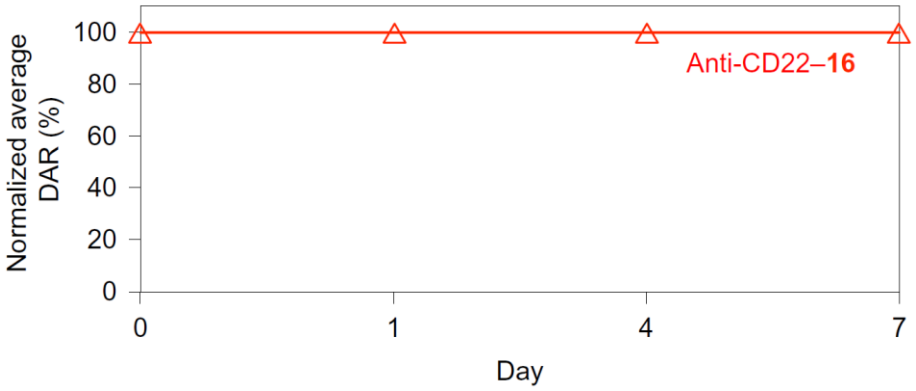
(example)



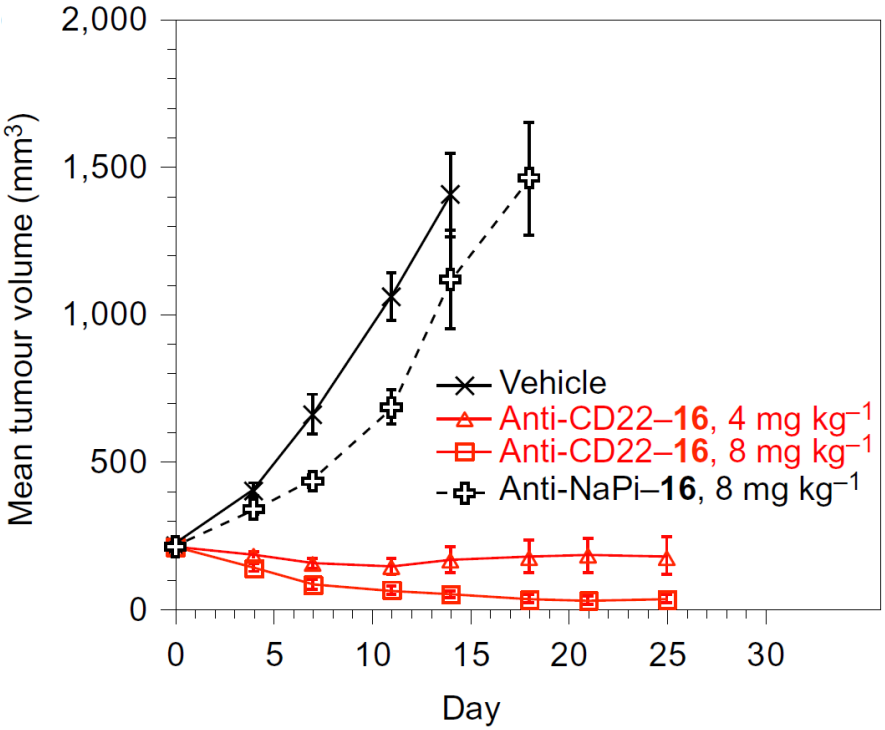
MC-Val-Cit-PAB-dimethylDNA31
(60%, 2.54 g scale)

In Vivo Stability and Efficacy





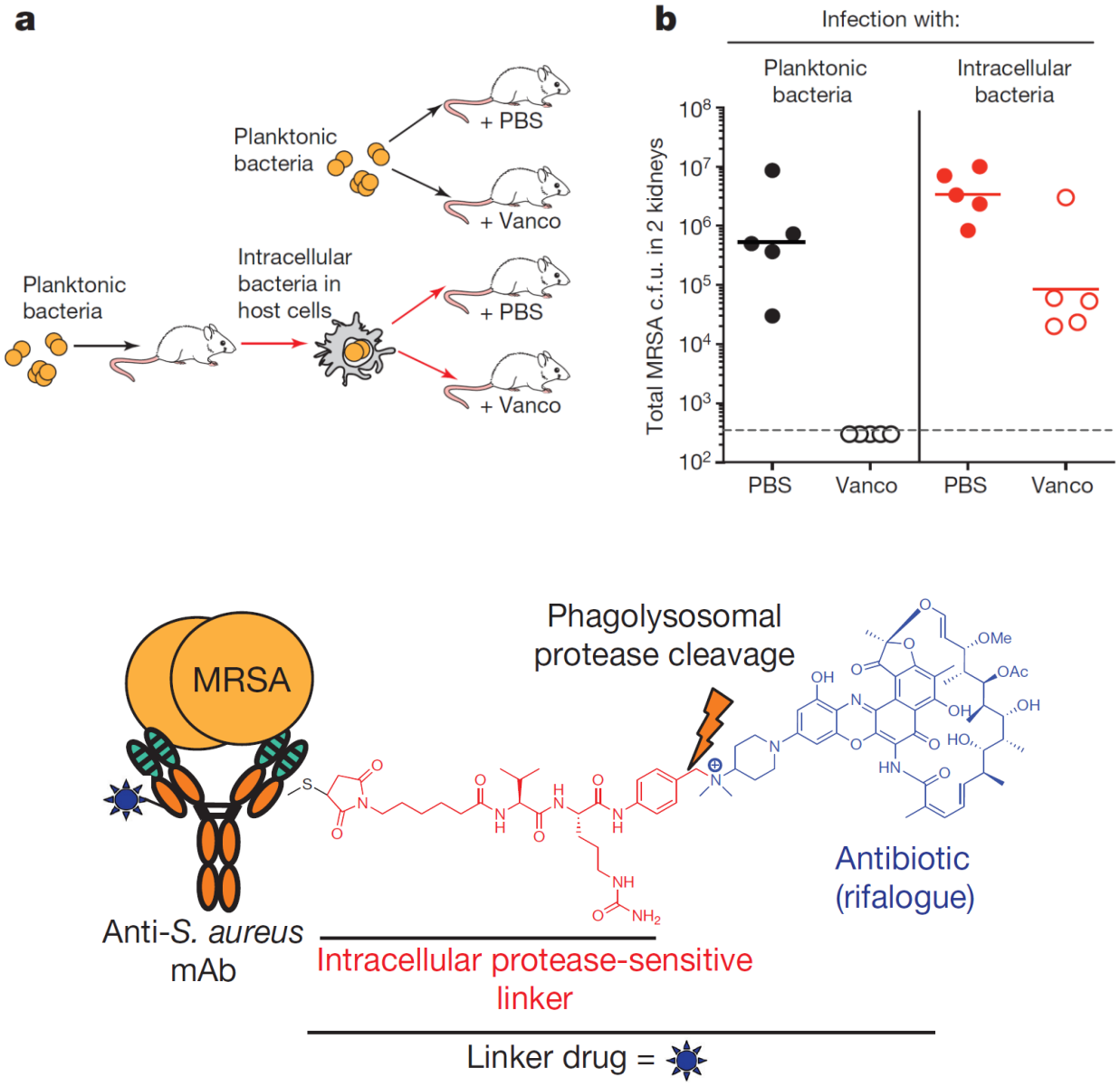
In vivo stability of mAb-**16** in mice. SSID mice were dosed intravenously with 4 mg/kg of mAb-**16**. DAR was determined using an affinity-capture LC/MS.



In vivo efficacy of mAb-**16**. anti-NaPi (negative control)

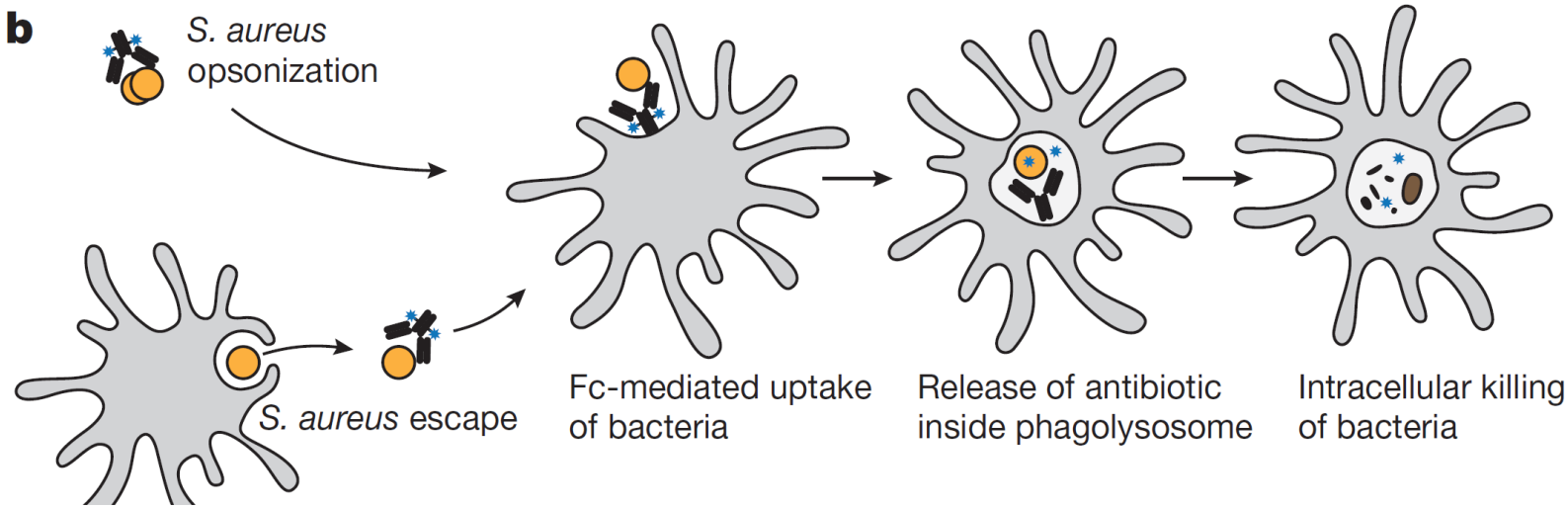
mAb-16 showed good stability in blood circulation and complete regression of tumor (8 mg/kg).

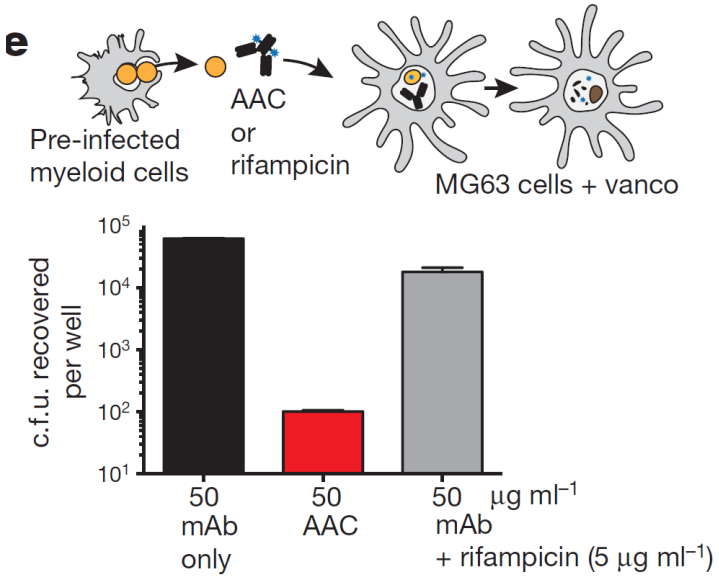
Application to Antibiotic ACDs

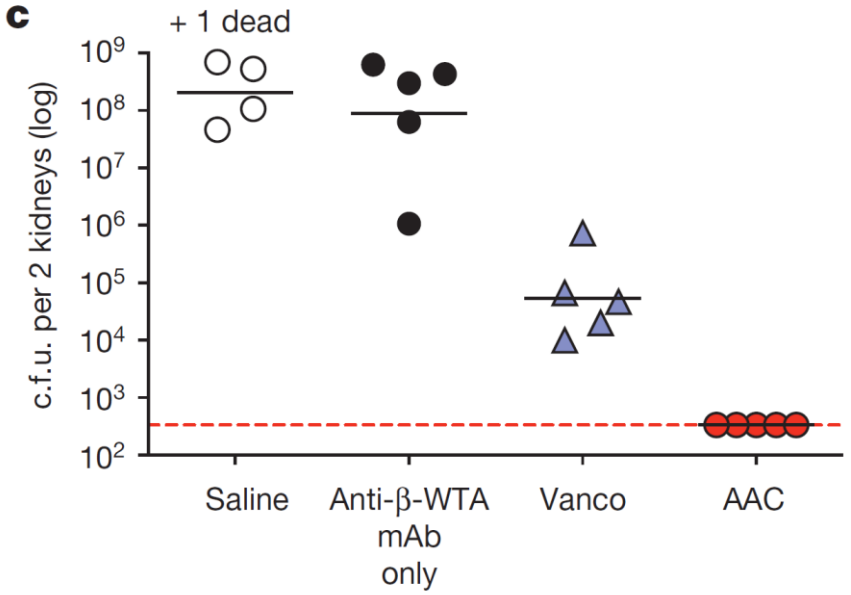


Lehar, S. M. *et al. Nature* **2015**, *527*, 323.

Application to Antibiotic ACDs

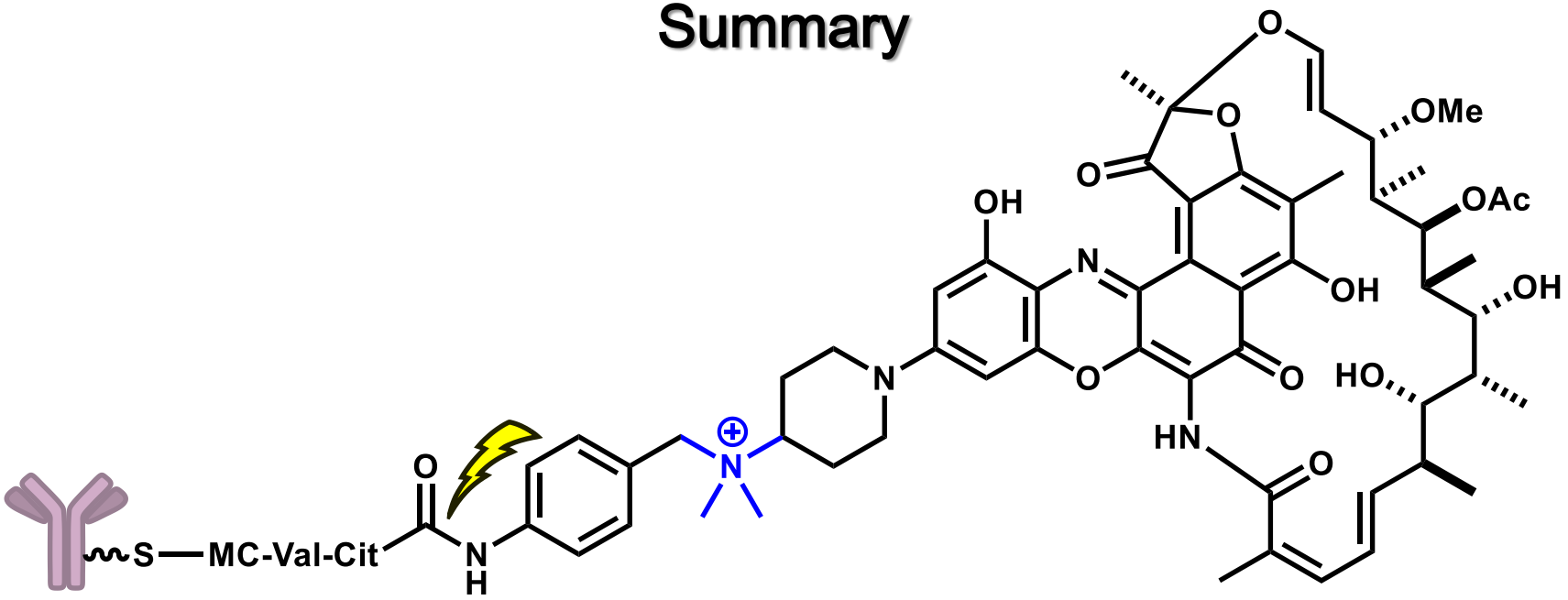






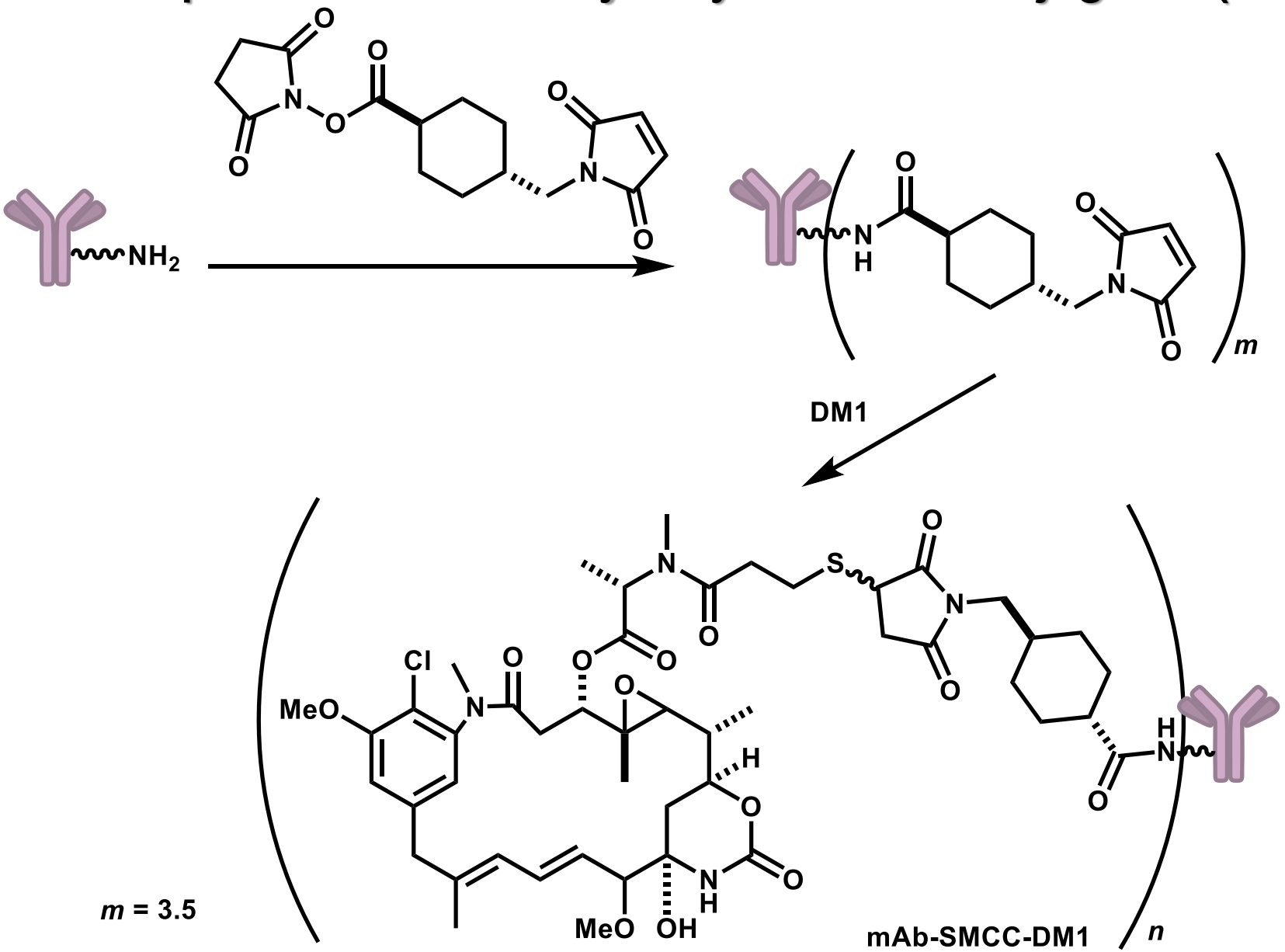
Lehar, S. M. *et al. Nature* **2015**, *527*, 323.

Summary



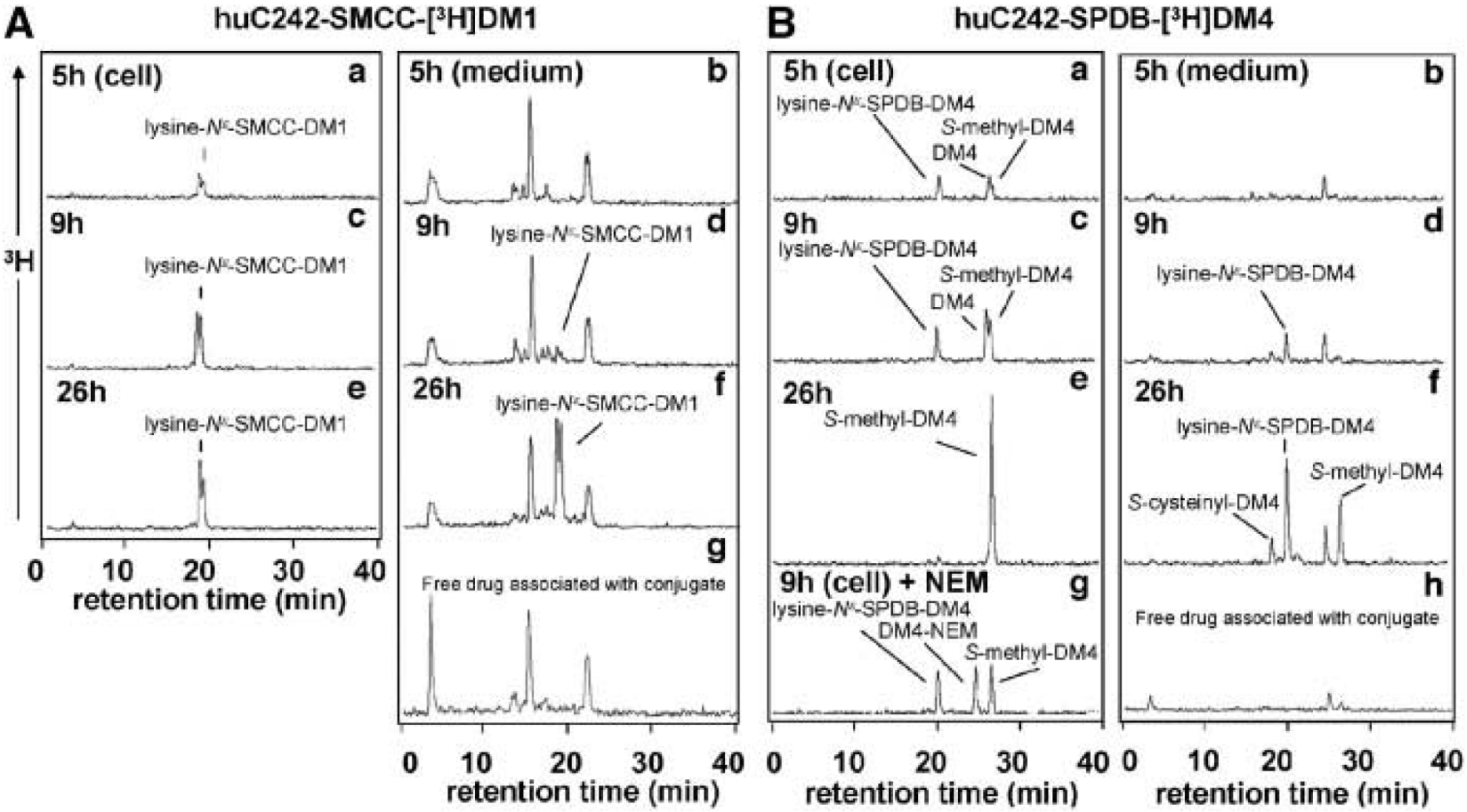
- **Modification of tertialy and heteroaryl amines**
- **Increased water solubility**
- **Efficient synthesis for assessing new drug classes**
- **Appication to other therapeutic areas**

A1. Preparation of Antibody-Maytansinoid Conjugates (AMCs)



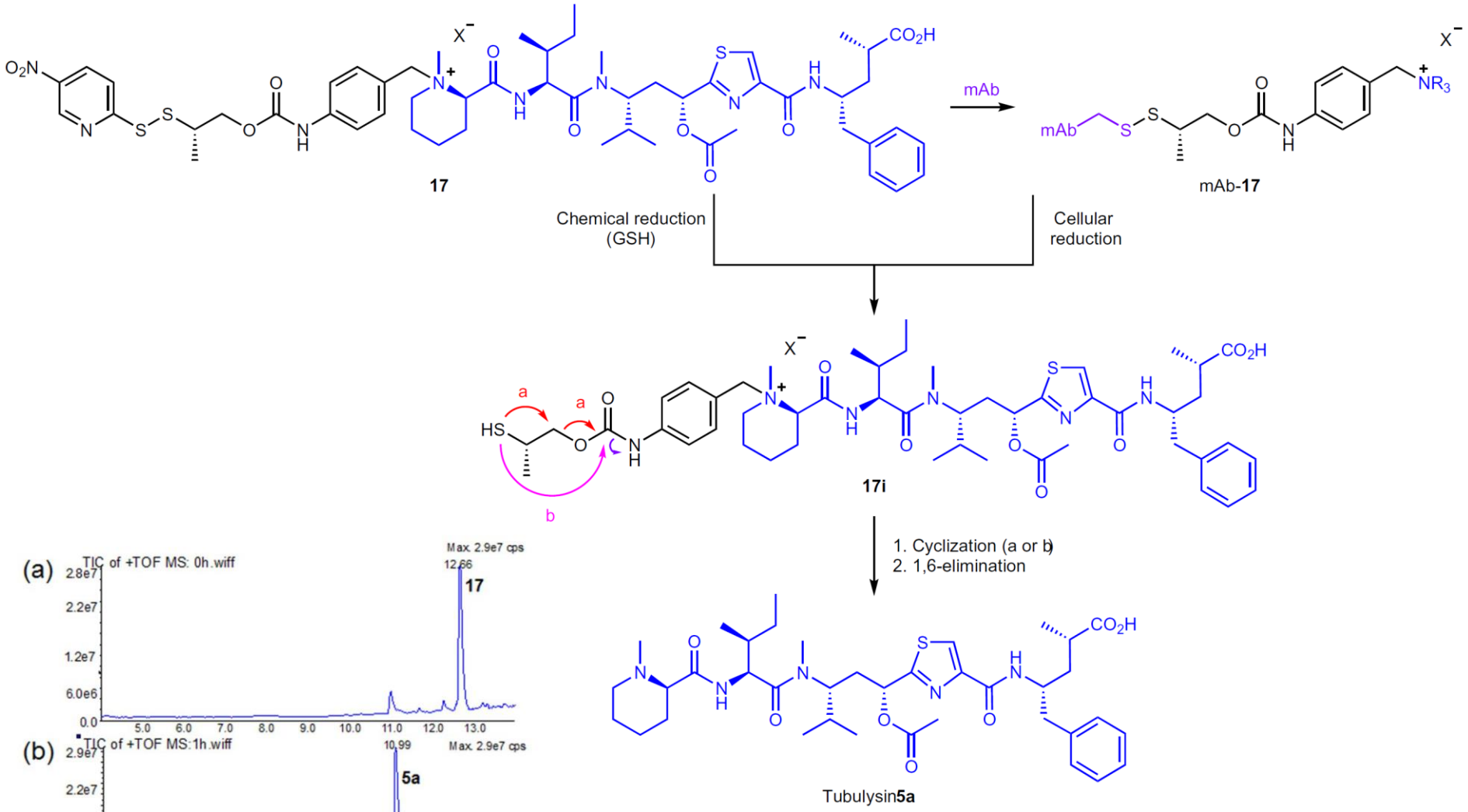
Kellogg, B. A. *et al. Bioconjugate Chem.* **2011**, *22*, 717.

A2. Maytansinoid Catabolites Detected Inside and Outside Cells



g(A) and h(B), chromatograms obtained from the acetone extract of the ADC samples before exposure to cells (control). Metabolites were analyzed by HPLC.

A3. Quaternary-Ammonium-Linked Disulfide Conjugates



Total ion chromatograms of **17** (a) before (b) 1 h after glutathione (GSH) reduction.