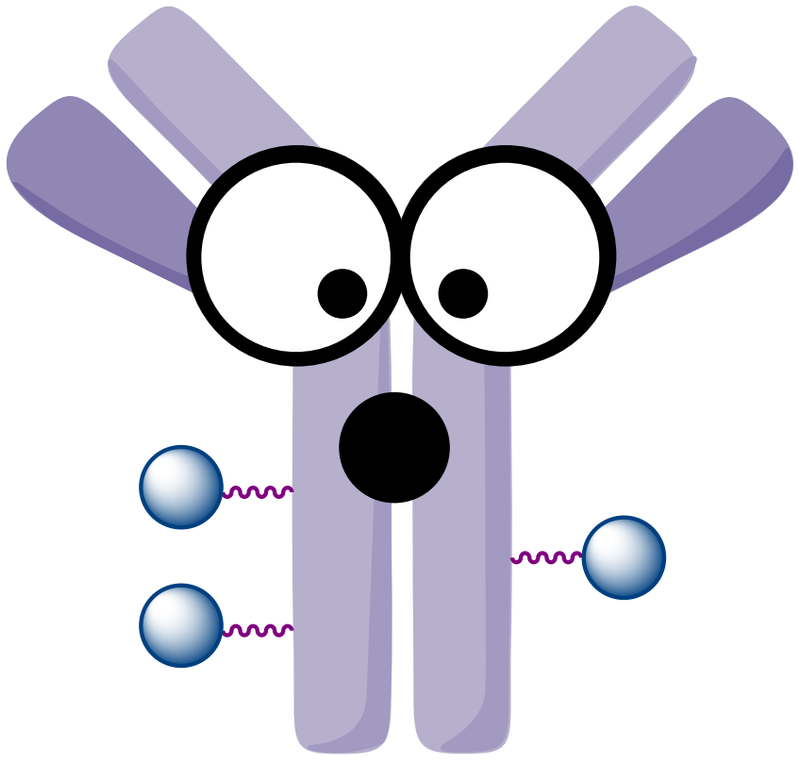
The Advent of Antibody-Drug Conjugates



MacMillan Group Meeting

4 June 2015

Tracy Liu

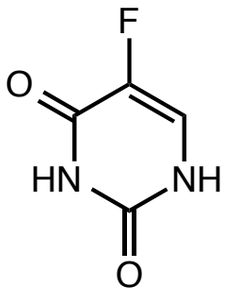
Traditional Cancer Therapy

The Double Edged Sword

Anti-cancer treatments should be as aggressive as possible to fully eradicate the tumor...

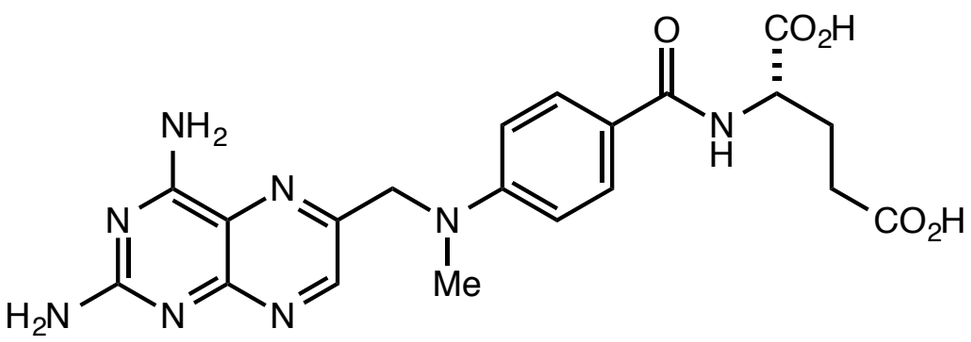
...but it is precisely this aggressiveness that often causes severe side effects

Common Chemotherapeutic Agents



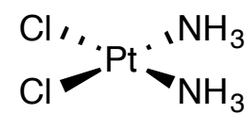
5-fluorouracil

thymidylate synthase inhibitor



methotrexate

anti-folate



cisplatin

DNA crosslinking agent

- lack of tumor selectivity - killing of proliferating normal cells
- requires administration at near the maximum tolerated dosage
  - 99% of cells in a tumor must be killed to achieve complete remission

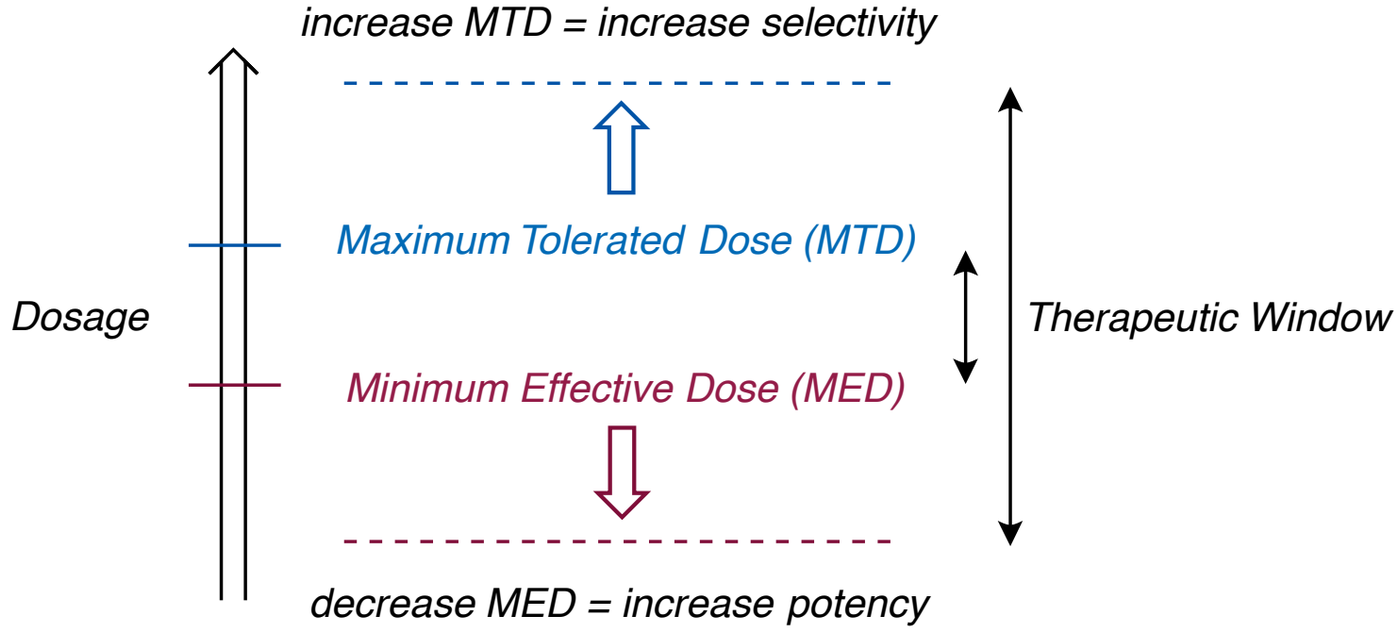
Traditional Cancer Therapy

Maximizing the Therapeutic Window

Limited clinical efficacy of chemotherapeutics is due to an insufficient ***therapeutic window*** *-*

lack of ability to kill enough cancel cells without causing toxicity to normal cells

Current most critical need: Maximization of the Therapeutic Window



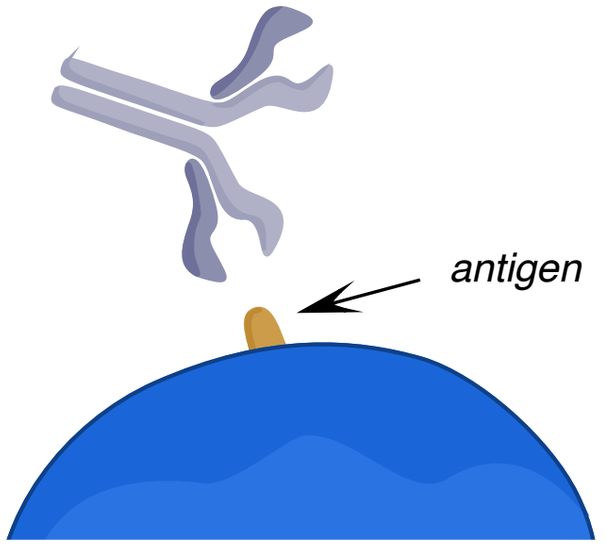
Angew. Chem. Int. Ed. **2014**, *53*, 3796.

Targeted Cancer Therapy

Direct Approaches

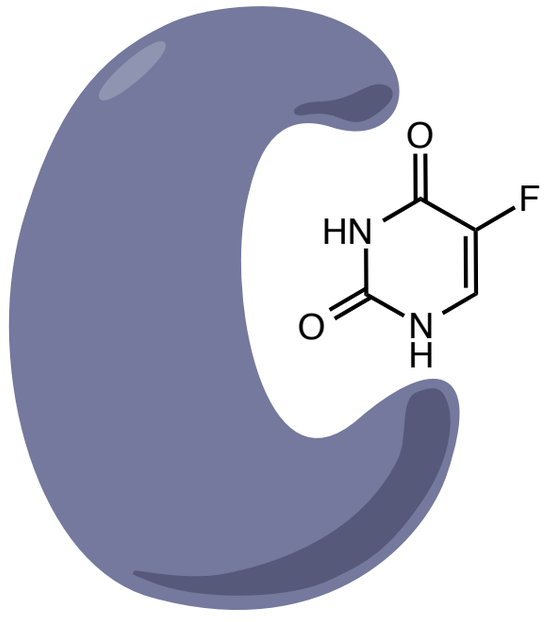
Targeting tumor-associated or specific proteins to directly alter their signaling by:

monoclonal antibody



tumor cell

- direct binding of monoclonal antibody to antigen expressed on tumor cell surface to induce immune responses



protein

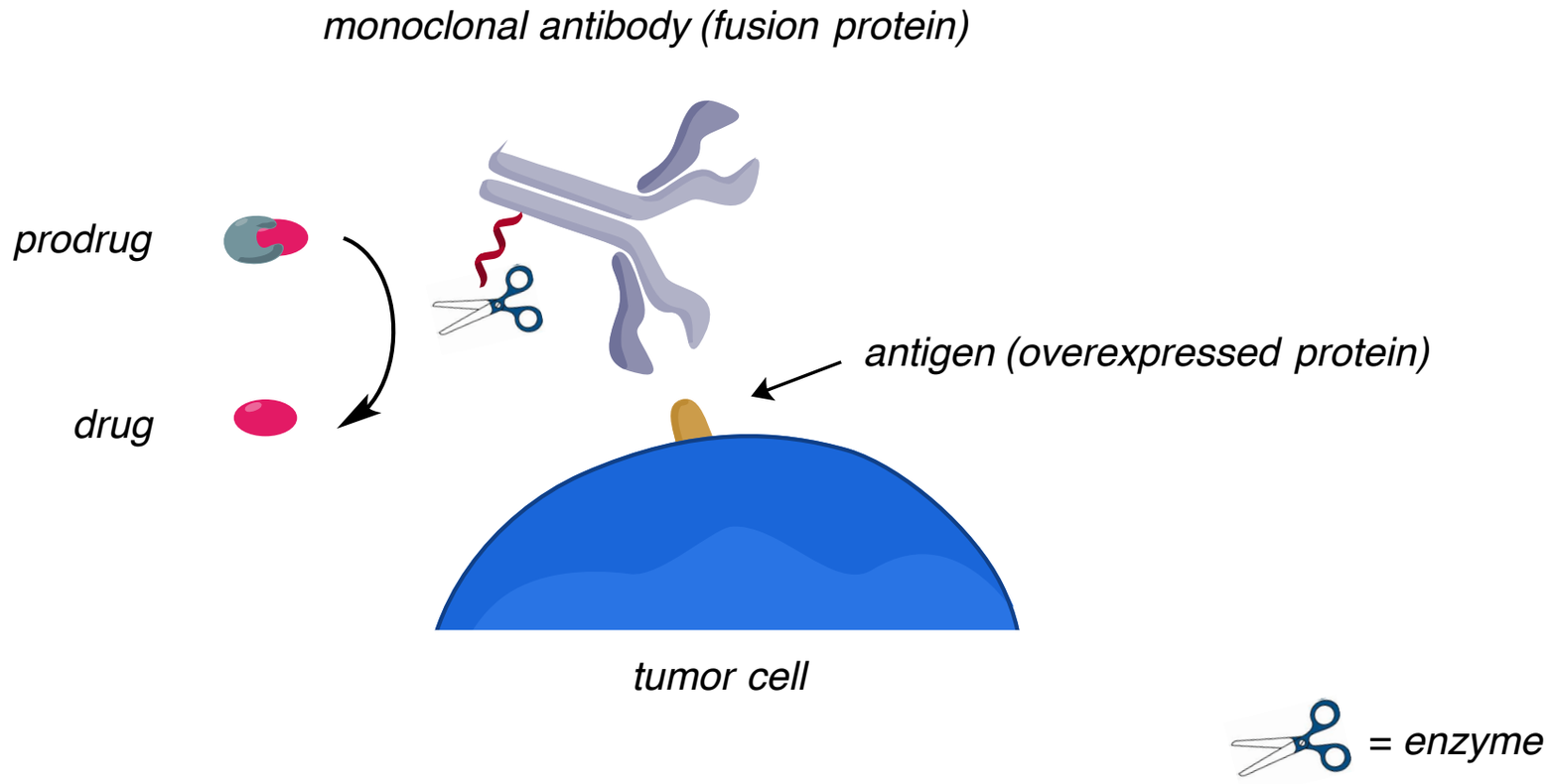
- binding of small molecule drugs to active site of a protein to disrupt normal function

Nature Rev. **2006**, *5*, 147.

Targeted Cancer Therapy

Indirect Approaches

Reliance on proteins specifically expressed or overexpressed on tumor cell surfaces that function as a targeting platform for fusion proteins bearing different effector molecules



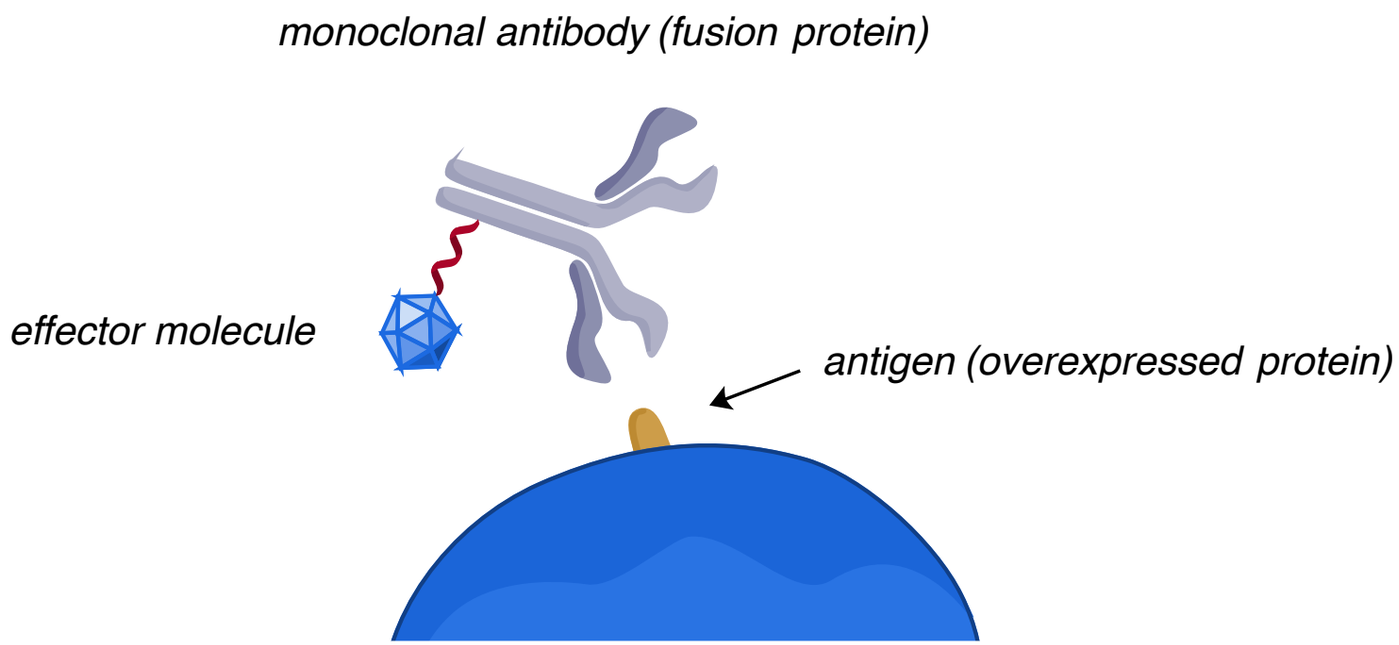
- antibody-directed enzyme prodrug therapy
  - seletive activation of a mildly toxic prodrug to a toxic drug at the tumor site through conjugation of an enzyme to a tumor-specific antibody

Nature Rev. **2006**, *5*, 147.

Targeted Cancer Therapy

Indirect Approaches

Reliance on proteins specifically expressed or overexpressed on tumor cell surfaces that function as a targeting platform for fusion proteins bearing different effector molecules



effector molecule

small molecule drug (antibody-drug conjugate)

toxin (immunotoxins)

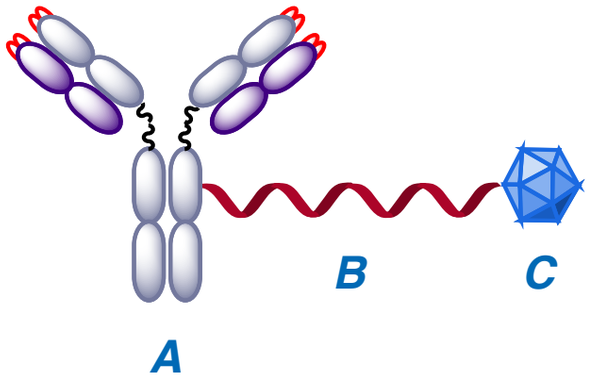
radionucleotides (radioimmuno conjugate)

immunoregulatory cytokines (antibody-cytokine fusion protein)

Nature Rev. **2006**, *5*, 147.

Antibody-Drug Conjugates

A Brief Introduction and History



Anatomy of an Antibody-Drug Conjugate

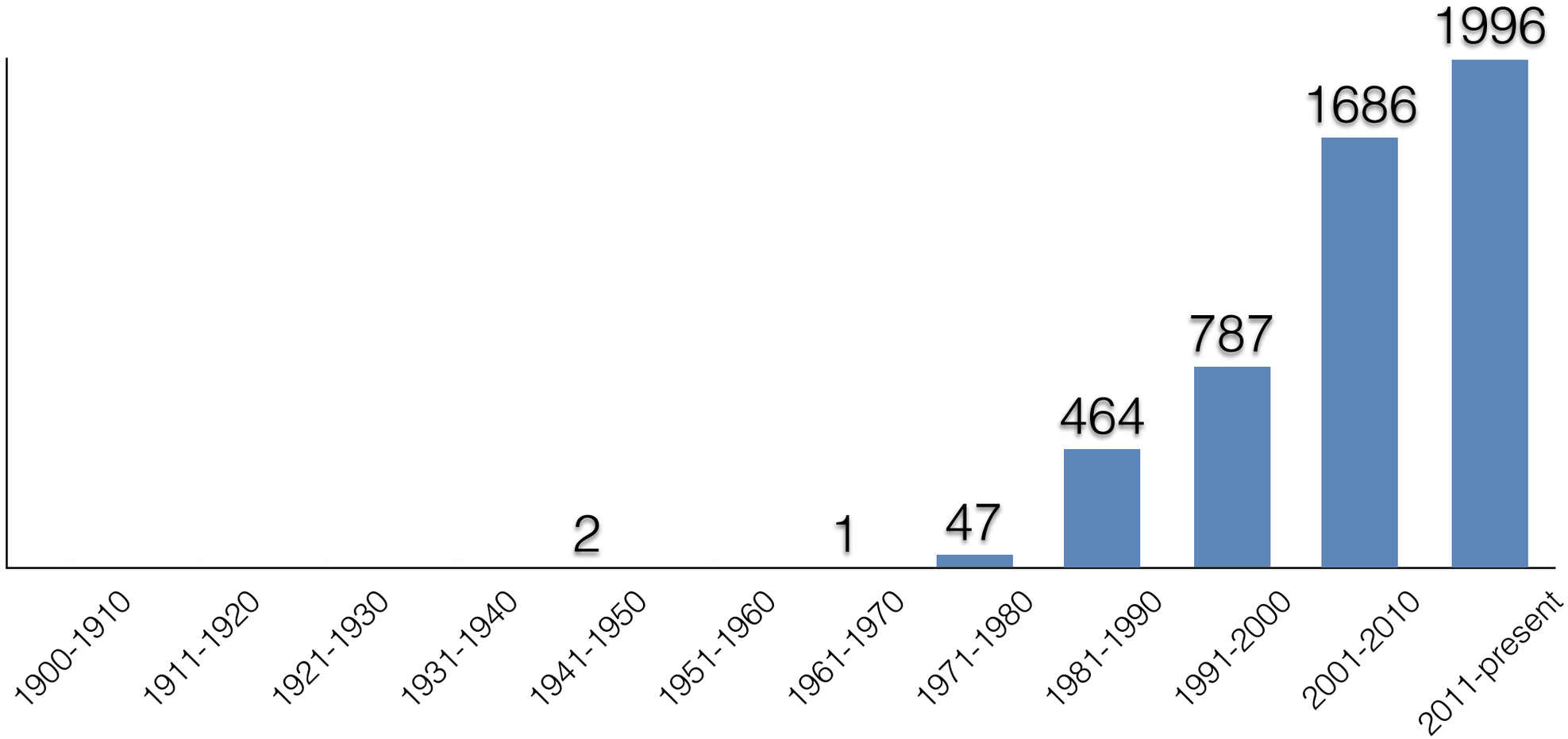
A. Antibody

B. Linker

C. Small-molecule drug warhead

Number of Publications in Antibody-Drug Conjugate Research

1900 - present day



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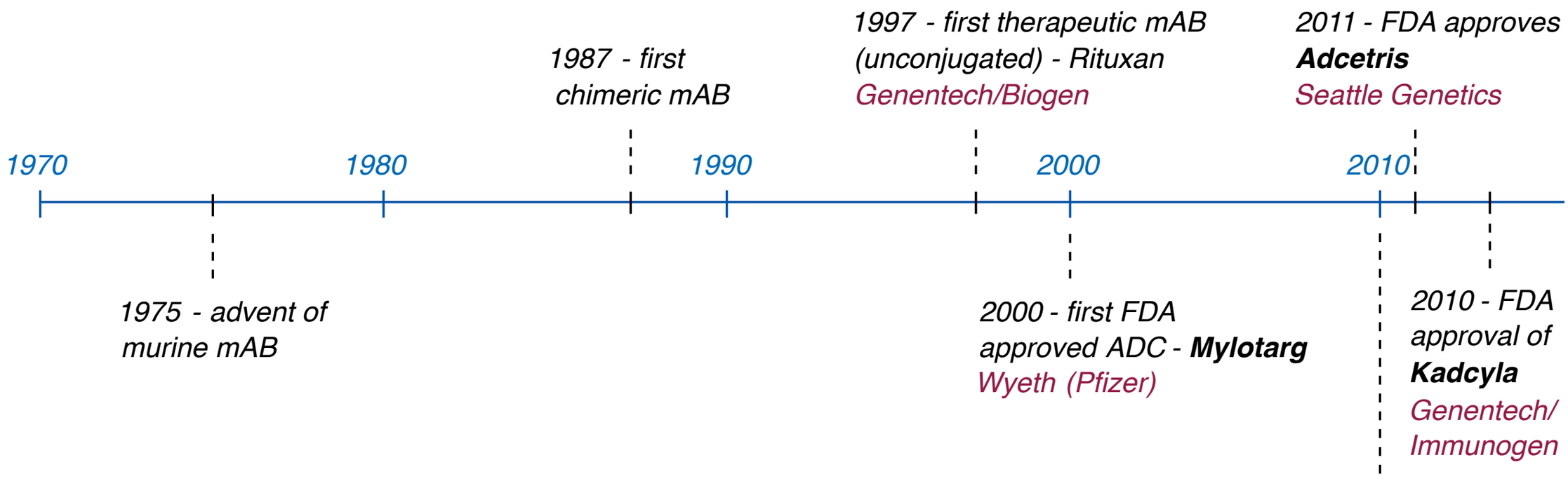

mAB = monoclonal antibody
ADC = antibody-drug conjugate

Part I.
First Generation Antibody-Drug Conjugates and Lessons Learned

Part II.
Second Generation Antibody-Drug Conjugates and Their Improvements

Part III.
Current Challenges and Overview of Clinical Performance

Nature Rev. **2006**, *5*, 147.; *Bioconjugate Chem.* **2015**, *26*, 176.

Antibody-Drug Conjugates

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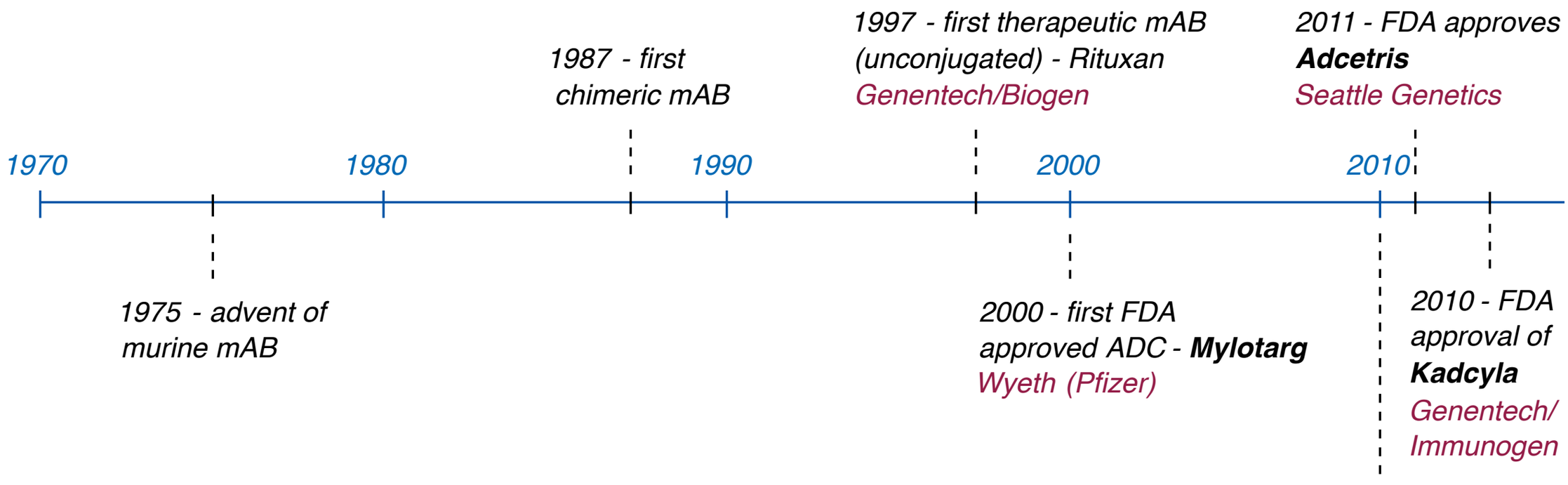

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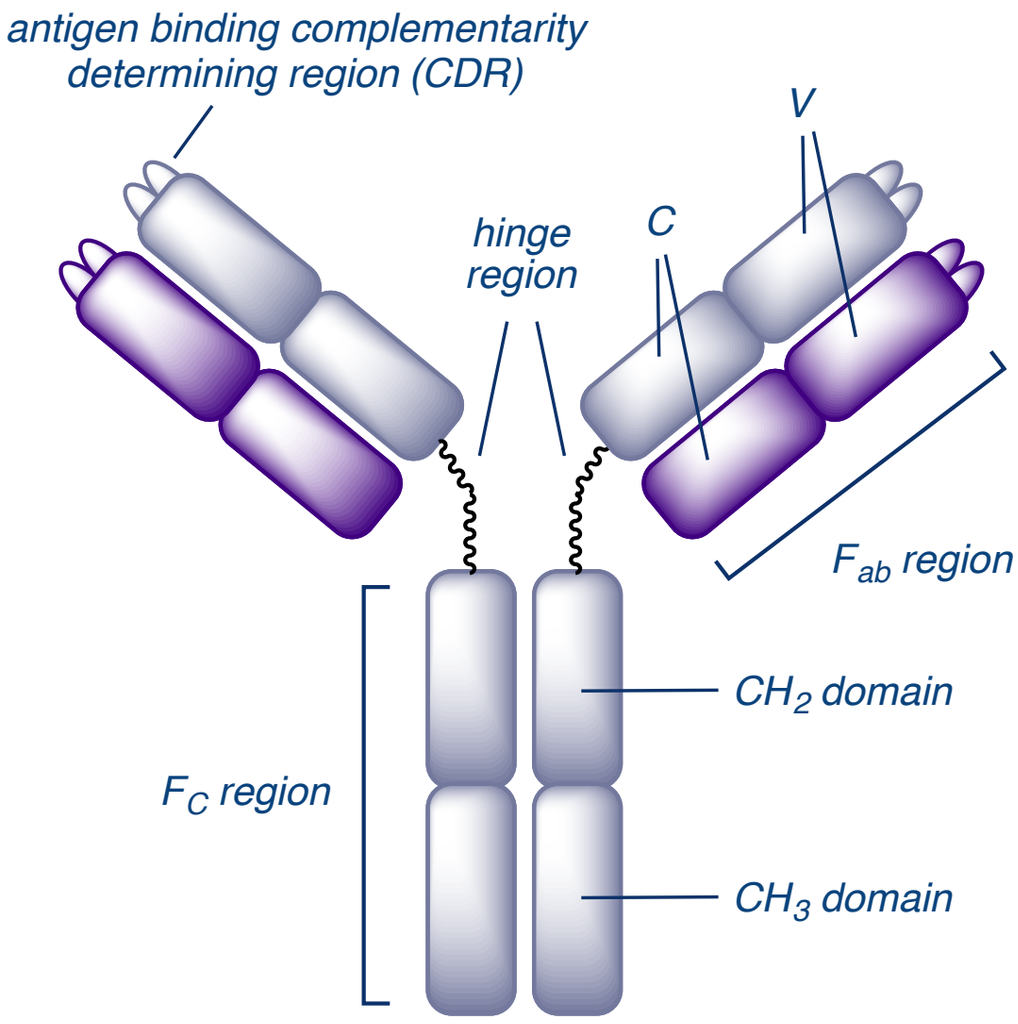
Nature Rev. **2006**, *5*, 147.; *Bioconjugate Chem.* **2015**, *26*, 176.

Deconstruction of Antibody-Drug Conjugates

Key Domains of the Immunoglobulin G Antibody Scaffold

The majority of antibody-drug conjugates are built upon the immunoglobulin G (IgG) scaffold



Domains of a Typical IgG Antibody

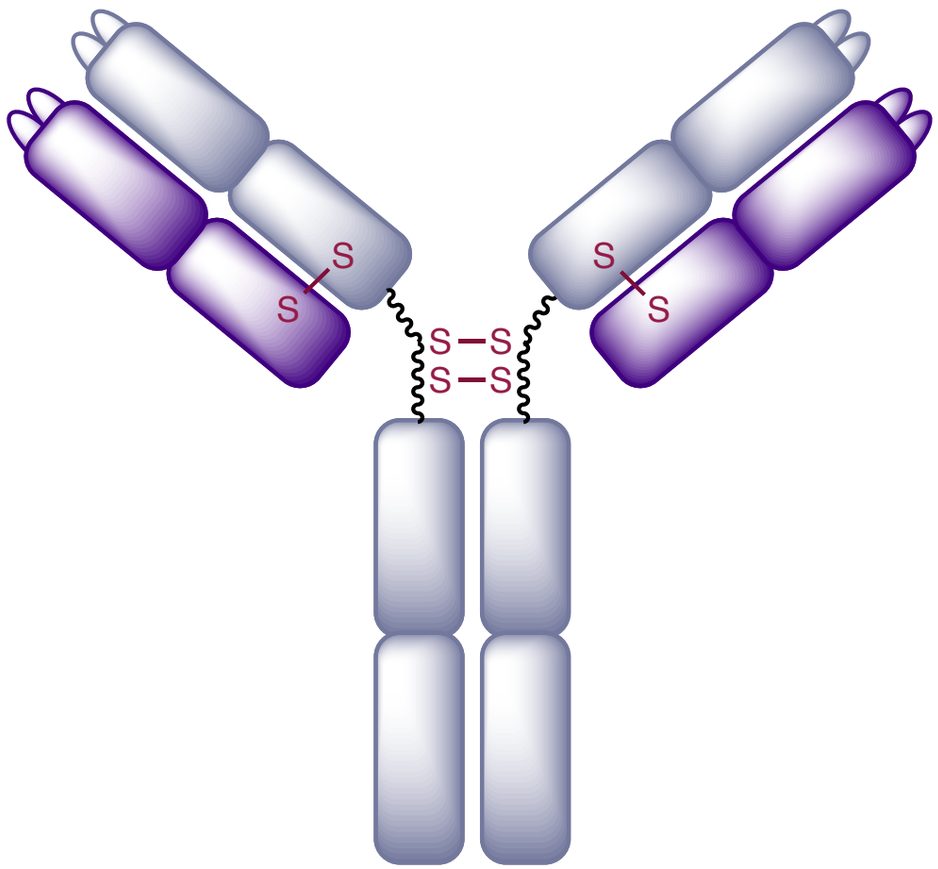
- represents 75% of serum antibodies in humans
- protein complex of 4 peptide chains in a Y shape
  - *2 identical heavy chains (light purple)*
  - *2 identical light chains (dark purple)*
- F_{ab} = Fragment antigen-binding domain
  - *Consists of variable (V) and constant (C) domains*
  - *Antigen binding CDR domains found at termini*
- F_c = Fragment crystallizable/constant domain
  - *Ideal location for drug conjugation - far from CDR*
  - *Consists of a CH_2 domain and a CH_3 domain*

Angew. Chem. Int. Ed. **2014**, *53*, 3796.

Deconstruction of Antibody-Drug Conjugates

Key Domains of the Immunoglobulin G Antibody Scaffold Relevant to Drug Conjugation

Nature of the chemistry between antibody and linker is primarily determined by the naturally occuring functional groups present on the surface of the antibody



Domains of a Typical IgG Antibody

- Linking through native **cysteine** residues

Bioconj. Chem. **2015**, *26*, 176.

Deconstruction of Antibody-Drug Conjugates

Traditional Methods of Linker Conjugation to Antibody

Linking through native Cysteine residues

Pros

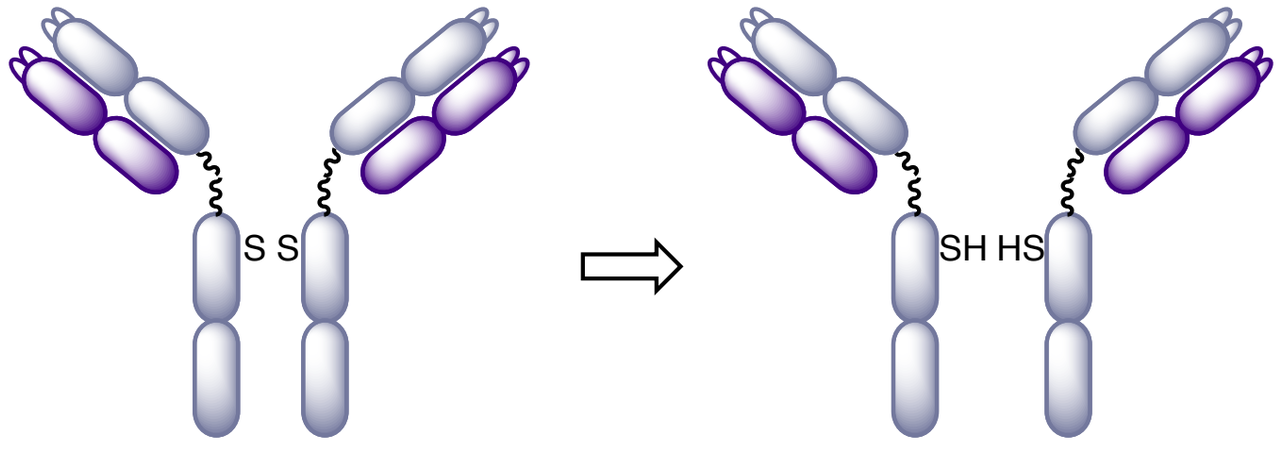
- High nucleophilicity of sulfur - naturally high reactivity for conjugation chemistry
- Low abundance of cysteine in primary sequence - easier control of *drug to antibody ratio (DAR)*

  4 interchain disulfide bridges - easier to reduce

  12 intrachain disulfide bridges - harder to reduce

Cons

- No free thiols naturally present - partial reduction required
- Selective reduction of the 4 interchain disulfide bridges is most common, but this partial reduction can result in a destabilized antibody



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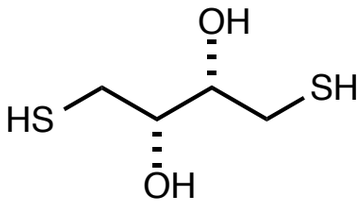
Deconstruction of Antibody-Drug Conjugates

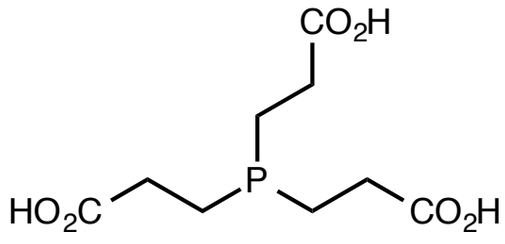
Traditional Methods of Linker Conjugation to Antibody

Linking through native Cysteine residues

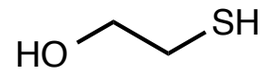
Common disulfide bridge reducing agents



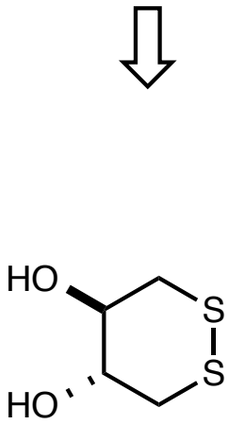
dithiothreitol (DTT)

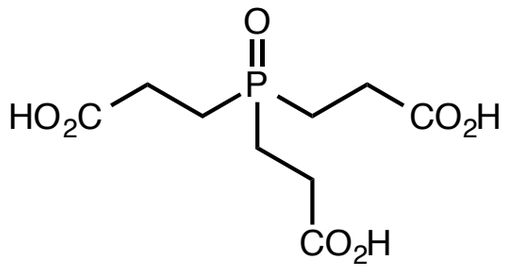


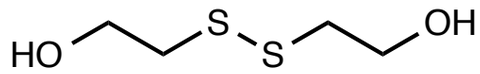
tris(2-carboxyethyl)phosphine (TCEP)



2-mercaptoethanol





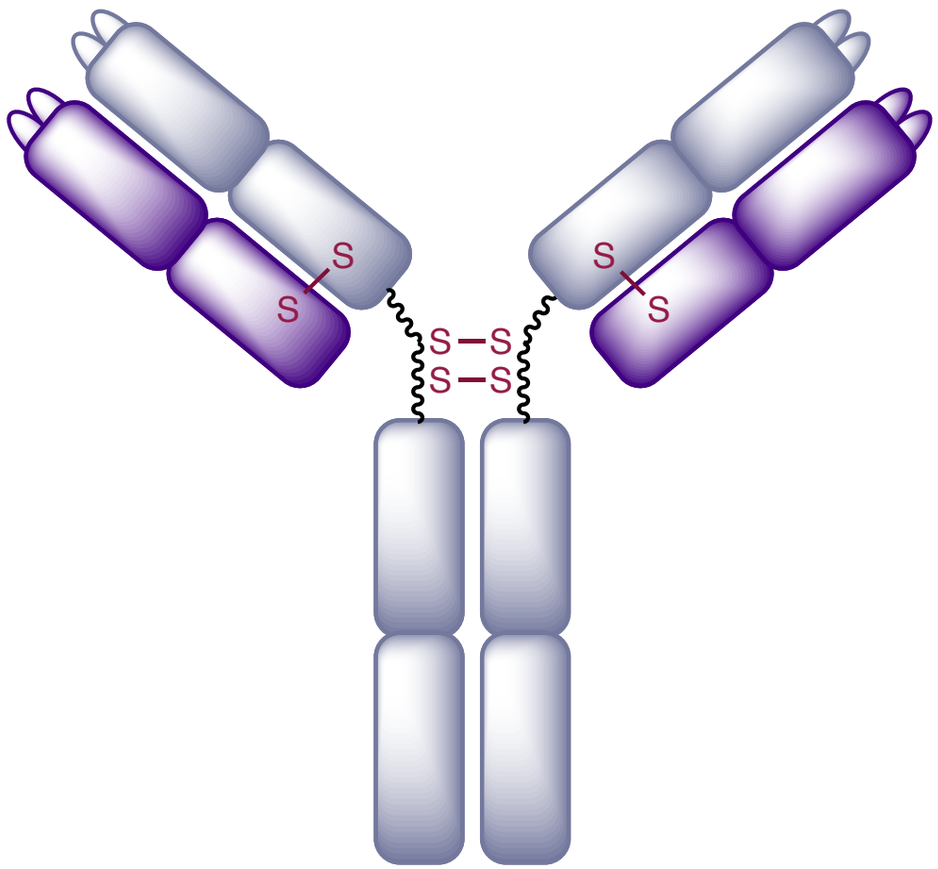


Corresponding oxidized byproducts

Deconstruction of Antibody-Drug Conjugates

Key Domains of the Immunoglobulin G Antibody Scaffold Relevant to Drug Conjugation

Nature of the chemistry between antibody and linker is primarily determined by the naturally occuring functional groups present on the surface of the antibody



Domains of a Typical IgG Antibody

- Linking through native **cysteine** residues
  - *Requires reduction to access free thiol*
  - *4 interchain disulfide bridges - easier to reduce*
  - *12 intrachain disulfide bridges - harder to reduce*

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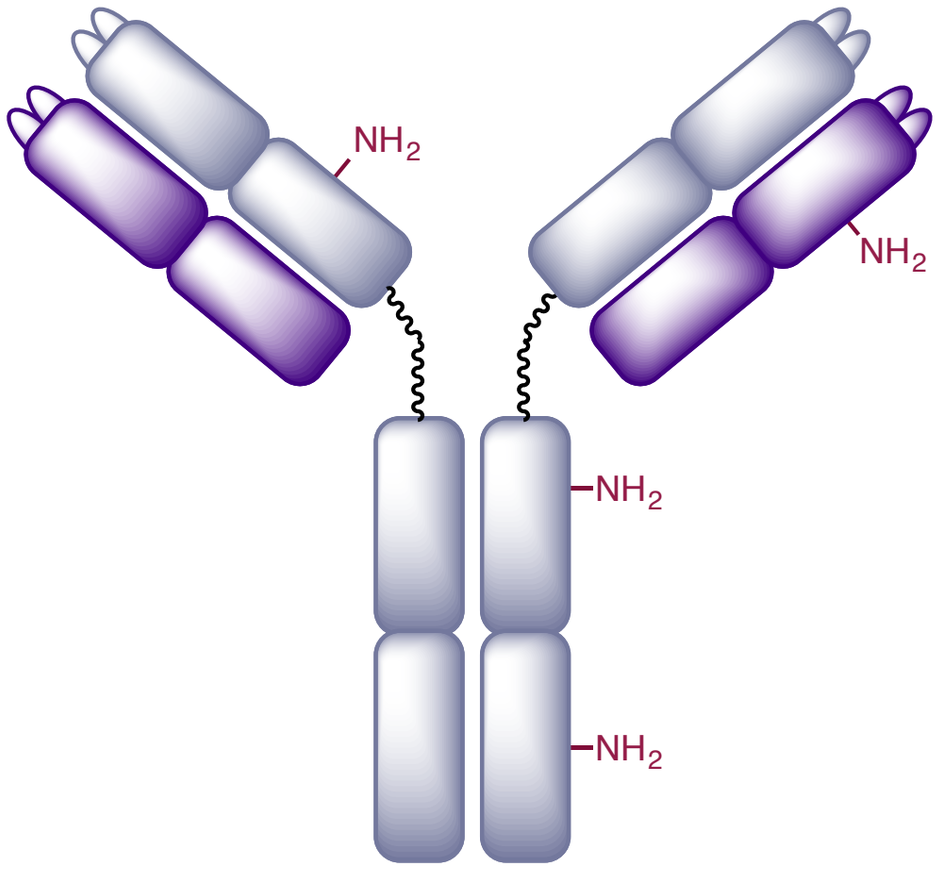

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- Linking through native **lysine** residues

Bioconj. Chem. **2015**, *26*, 176.

Deconstruction of Antibody-Drug Conjugates

Traditional Methods of Linker Conjugation to Antibody

Linking through native Lysine residues

Pros

- Naturally nucleophilic functional handle
- No requirement for pre-functionalization prior to conjugation with linker

Cons

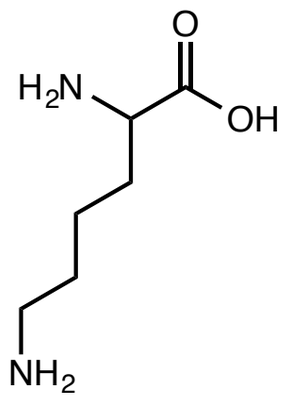
- Greater natural abundance of lysine - control of drug to antibody ratio significantly more difficult

  ~86 lysine residues total spanning all domains

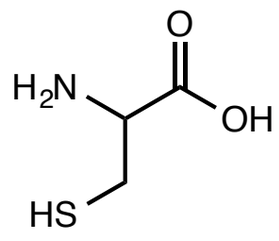
  ~20 accessible for functionalization

- Low levels of competitive cysteine and tyrosine conjugation observed in some cases

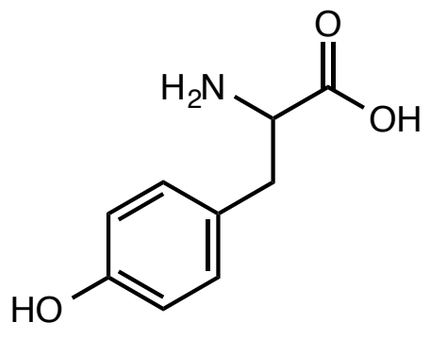
Lysine



Cysteine



Tyrosine



Garbaccio, R. M. Chemistry of Antibody-Small Molecule Drug Conjugates. From *Comprehensive Organic Synthesis II*, **2014**, *9*, 438.

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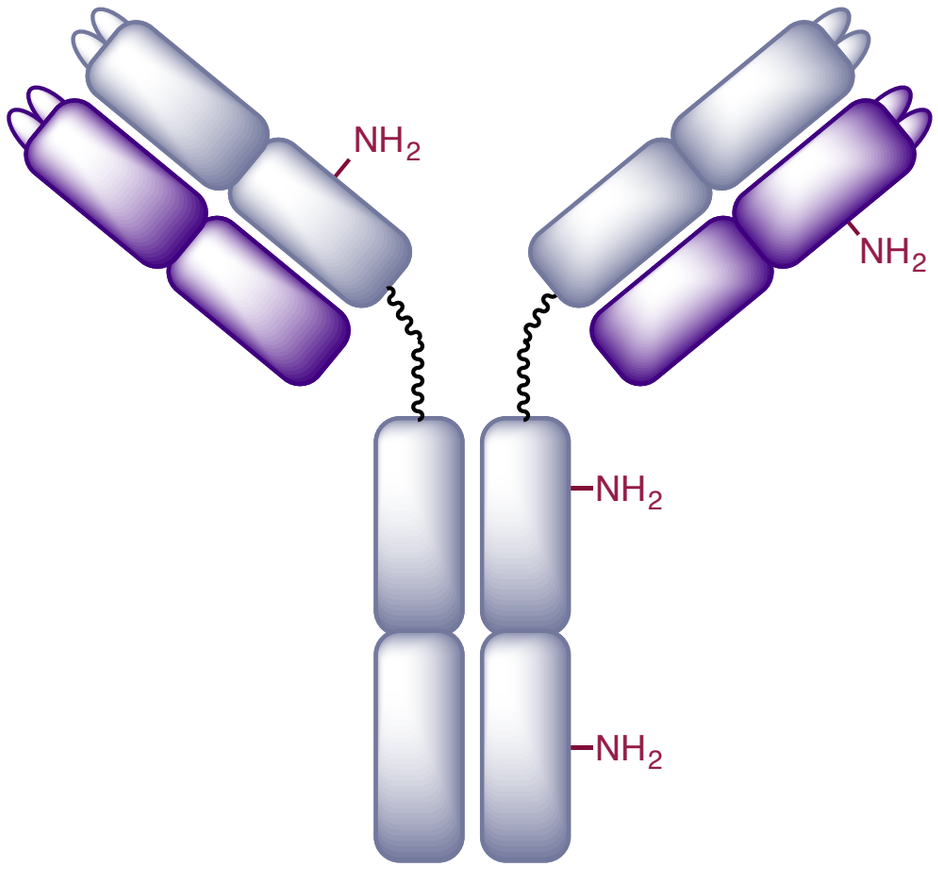

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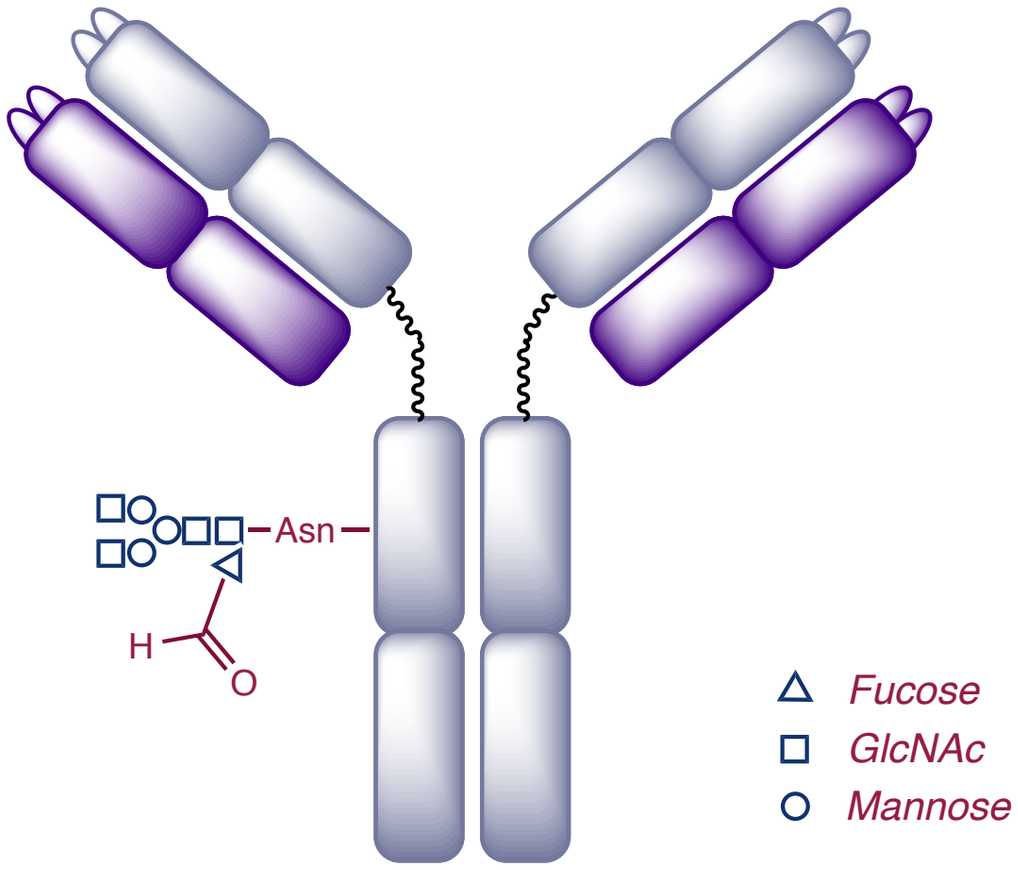

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- Linking through conserved **glycans** in CH_2 domain

Bioconj. Chem. **2015**, *26*, 176.

Deconstruction of Antibody-Drug Conjugates

Traditional Methods of Linker Conjugation to Antibody

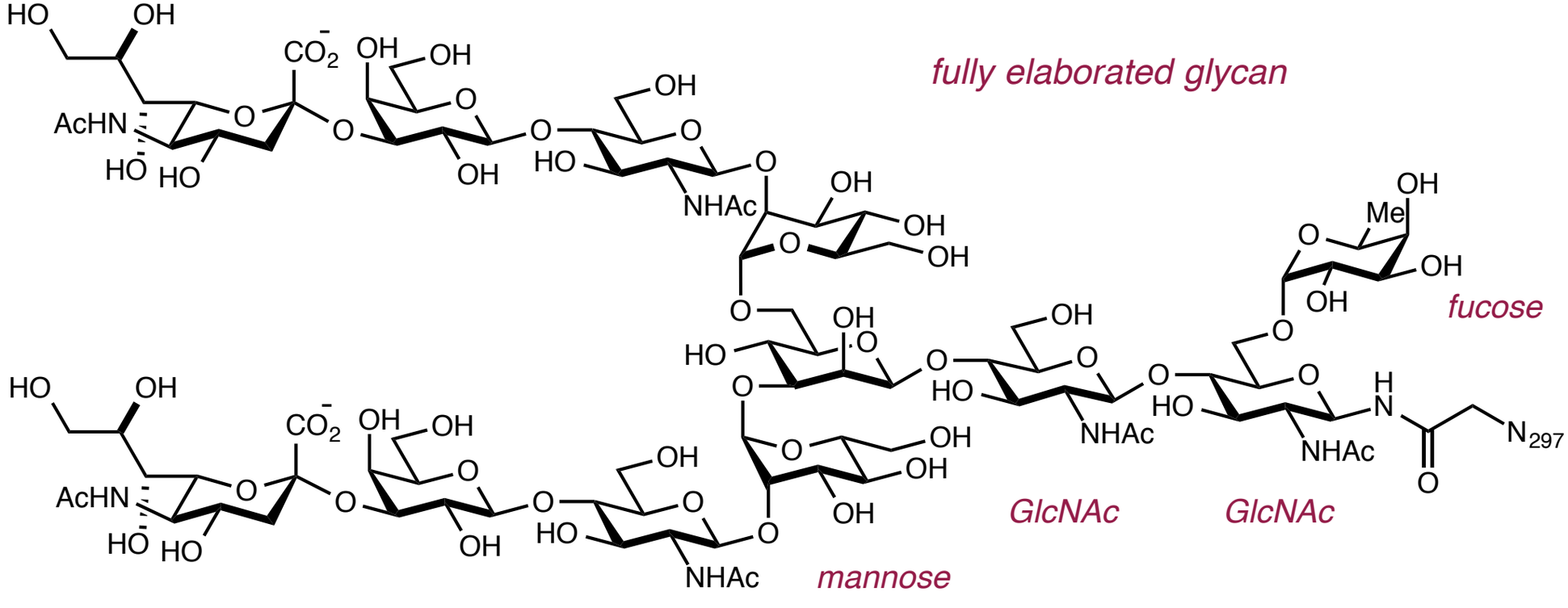
Linking through native Glycan residues

Pros

- Post-translational glycosylation of N297 (Asparagine) residue provides facile site-specific conjugation
- Glycosylation site is in CH_2 domain - well removed from antigen binding domain

Cons

- Glycosylation is a heterogenous post-translational modification - difficult to control drug to antibody ratio
- Requires pre-oxidation of vicinal diol moiety on glycan to access the bioorthogonal aldehyde handle



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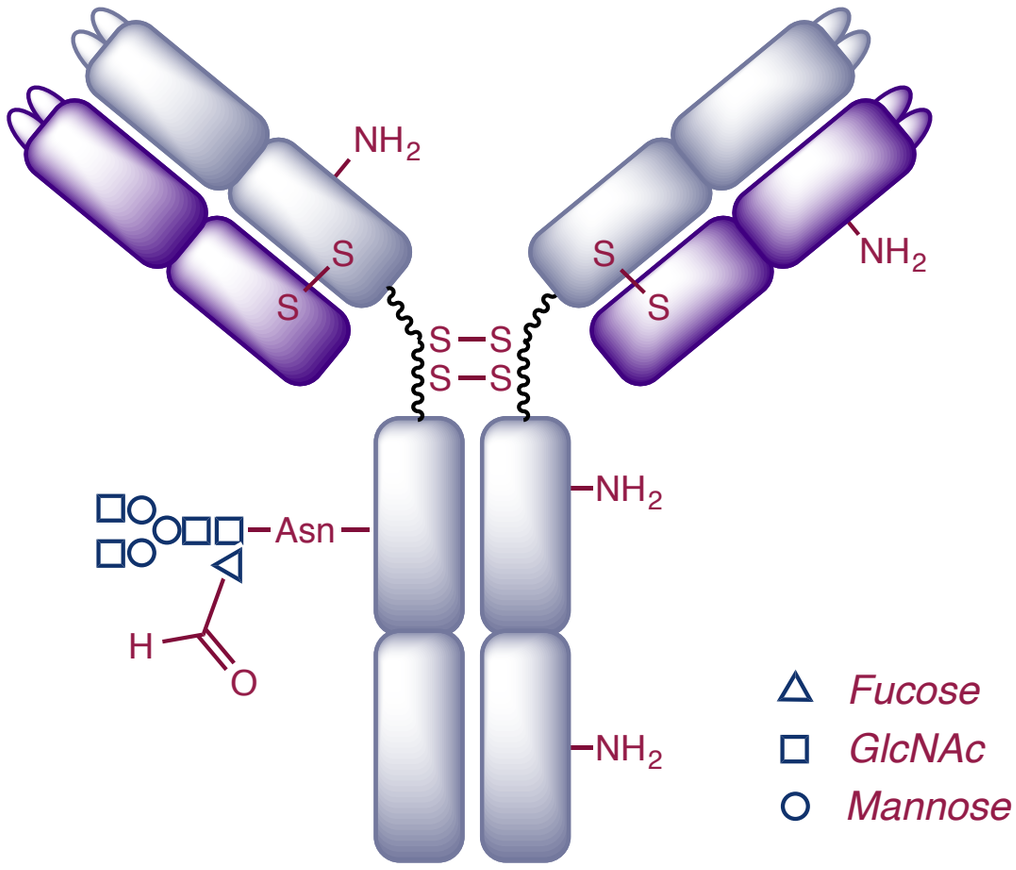

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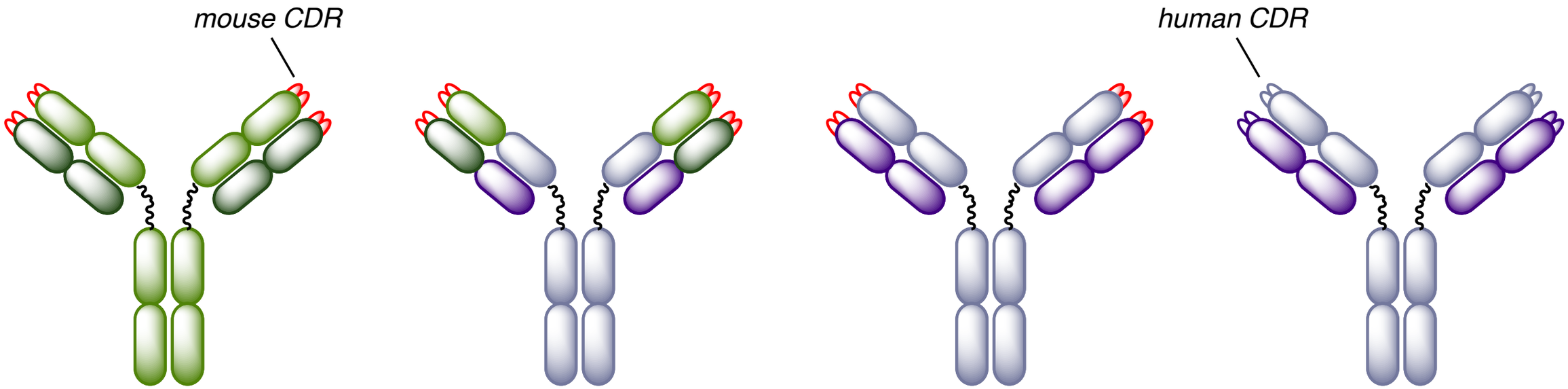
- Linking through conserved **glycans** in CH_2 domain
  - *Post translational modification glycosylates N297*
  - *Oxidation of terminal sugar furnishes aldehyde*

Bioconj. Chem. **2015**, *26*, 176.

Deconstruction of Antibody-Drug Conjugates

More on the Antibody

Advances in recombinant DNA technology have enabled the generation of engineered antibodies



murine mAB | ***chimeric mAB*** | ***humanized mAB*** | ***human mAB***

F_c region of murine mAB replaced with corresponding human constant domain

only the essential murine CDR regions are retained

increasing humanization

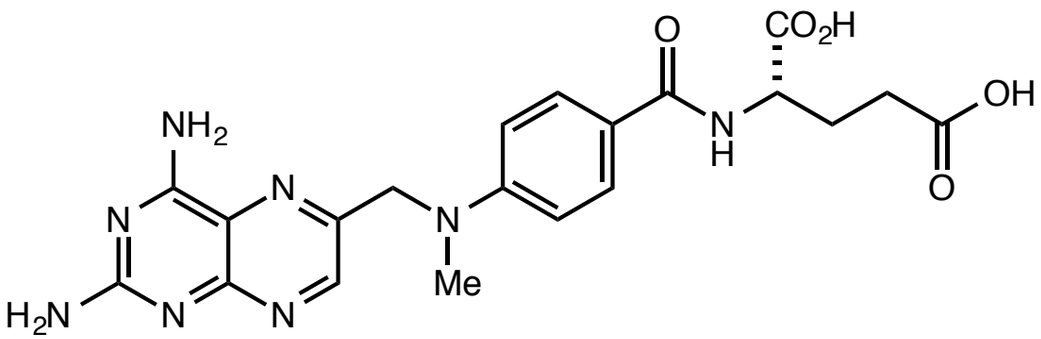
replacement of protein sequences of a mouse antibody with naturally occuring sequences in humans significantly reduces undesired immune responses

Angew. Chem. Int. Ed. **2014**, *53*, 3796.

First Generation Antibody-Drug Conjugates

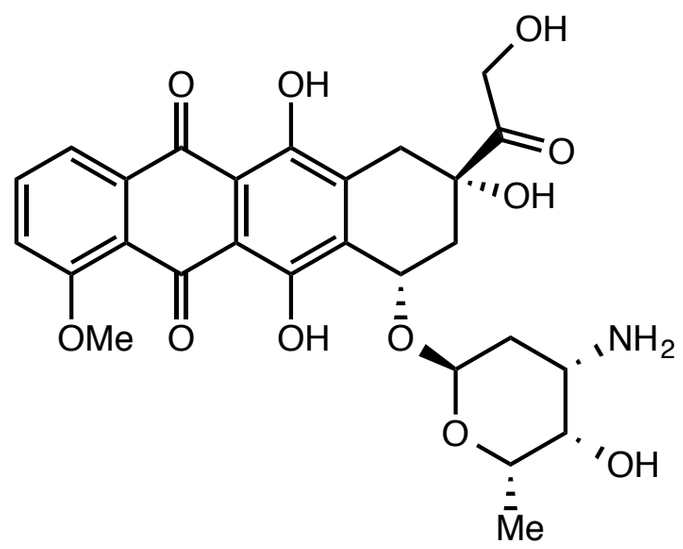
Transition from Chemotherapeutics

In an attempt to achieve greater selectivity for chemotherapy drugs, first generation antibody-drug conjugates took clinically established cancer drugs as warheads



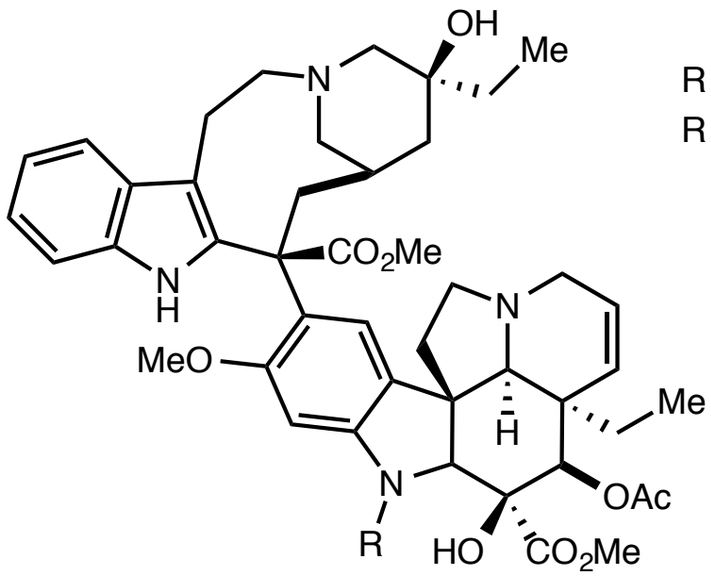
methotrexate

anti-folate



doxorubicin

antibiotic



R = Me
R = CHO

vinca alkaloids

anti-mitotic/microtubule agent

Survey of 4 clinically evaluated first generation antibody-drug conjugates

---

KS1/4 - methotrexate

KS1/4 - DAVLB

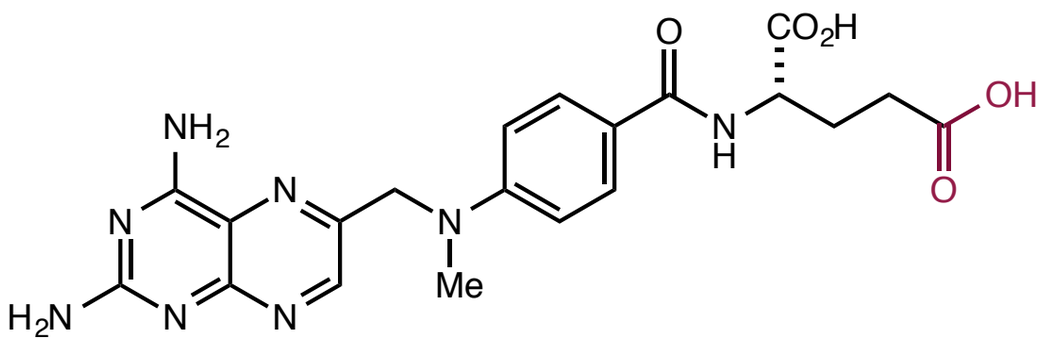
KS1/4 - DAVLBHYD

BR96 - doxorubicin

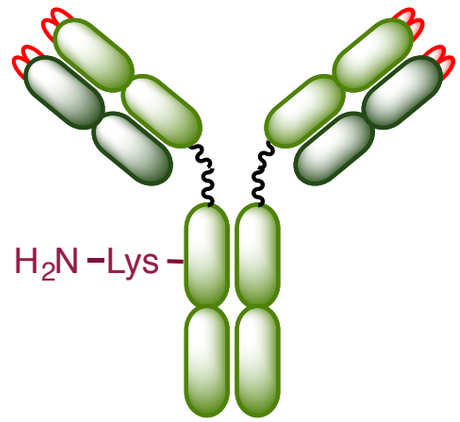
Adv. Drug Delivery Rev., **1998**, *31*, 89.

First Generation Antibody-Drug Conjugates

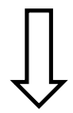
KS1/4S2 - Methotextrate Conjugate



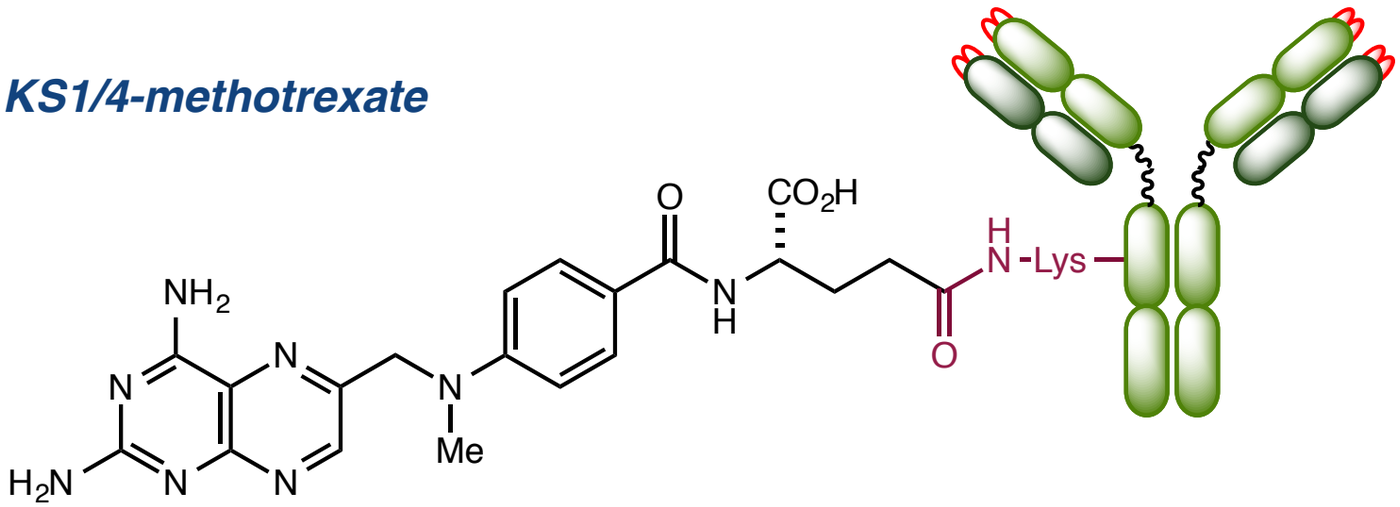
methotrexate



KS1/4S2 murine mAB



KS1/4-methotrexate

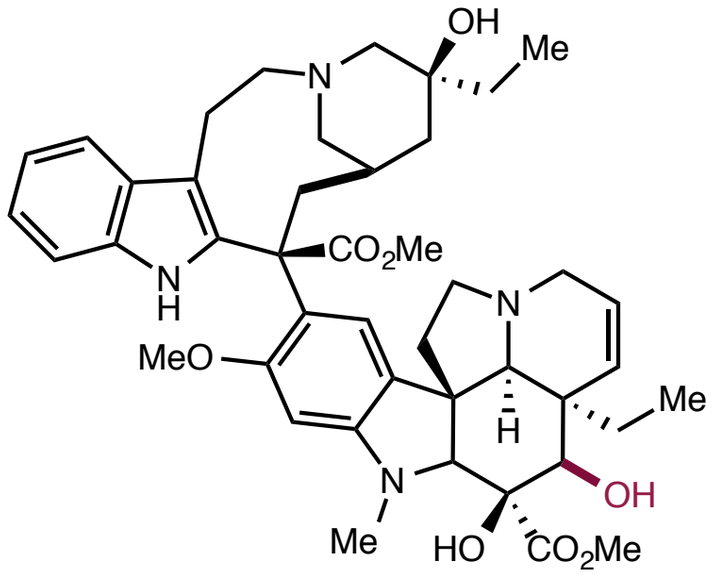


- non-cleavable amide linker formed through non-selective EDC coupling
- significant localization to tumor
- Phase I clinical trials revealed little therapeutic benefit potentially due to non-cleavable linker
- murine mAB illicited a human anti-murine antibody (HAMA) response in patients

Am. J. Respir. Crit. Care Med., **1994**, *150*, 1114.

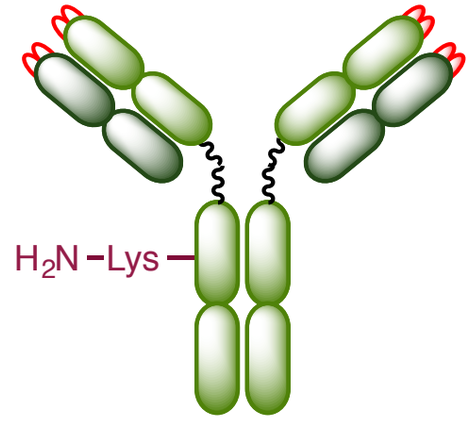
First Generation Antibody-Drug Conjugates

KS1/4 – 4-Desacetylvinblastine Conjugates



4-desacetylvinblastine

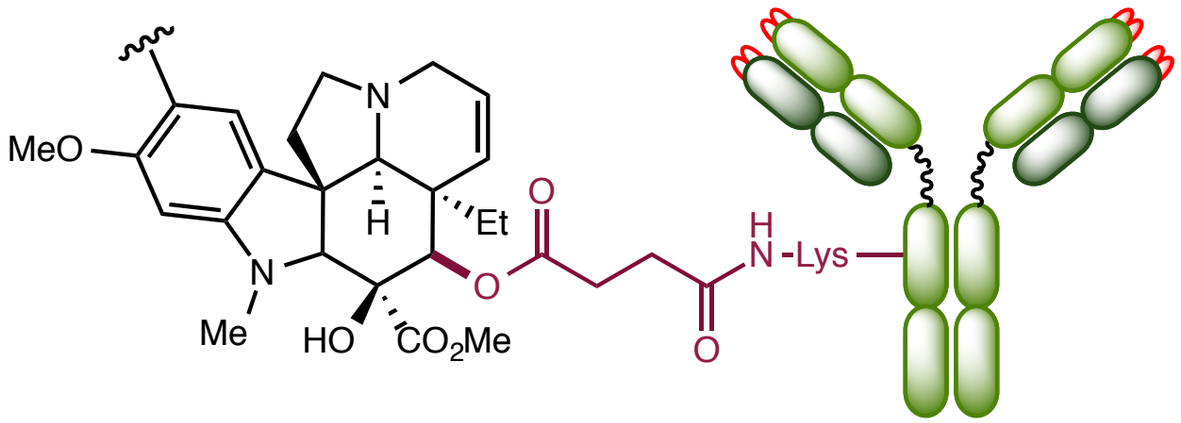
succinic anhydride



KS1/4S2 murine mAB

⇩

KS1/4-DAVLB

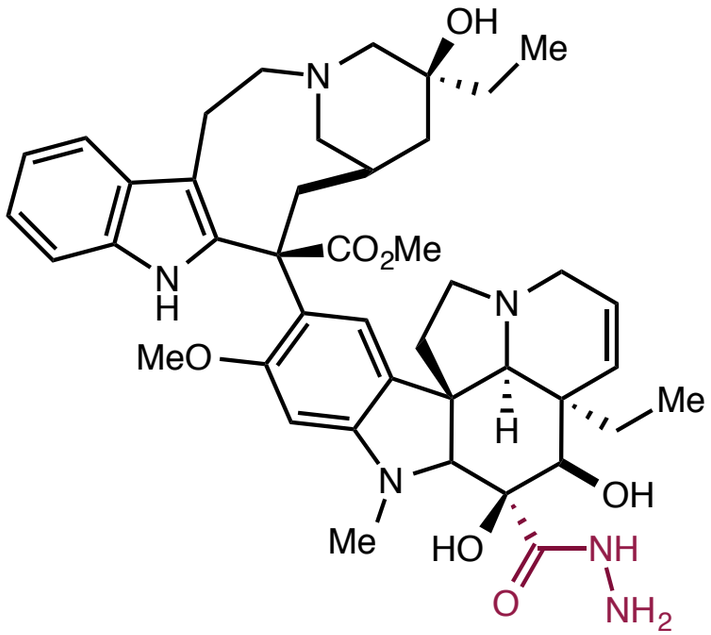


- esterase-labile hemisuccinate linker
- highly potent *in vivo* activity with greater efficacy than unconjugated drug
- Phase I clinical trials using radiolabeled conjugate indicates localization of drug to tumor cells
- no increased therapeutic effect
- patients developed immune responses to both the antibody and vinca alkaloid

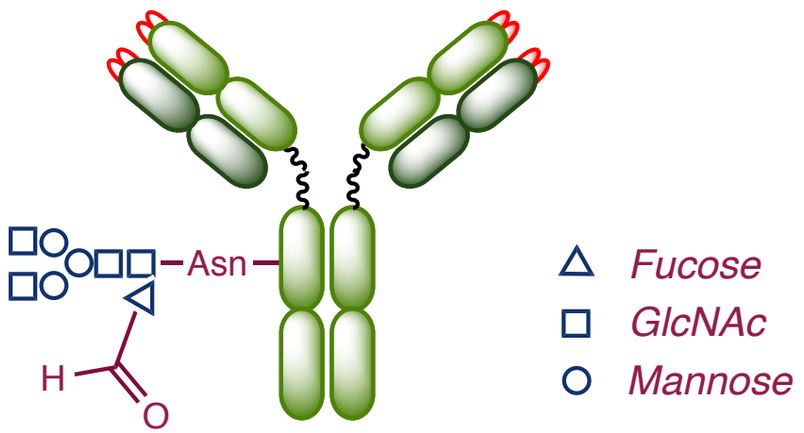
Clin. Pharmacol. Ther., **1990**, *47*, 36.
Cancer Res., **1991**, *51*, 2286.

First Generation Antibody-Drug Conjugates

KS1/4 – 4-Desacetylvinblastine Conjugates



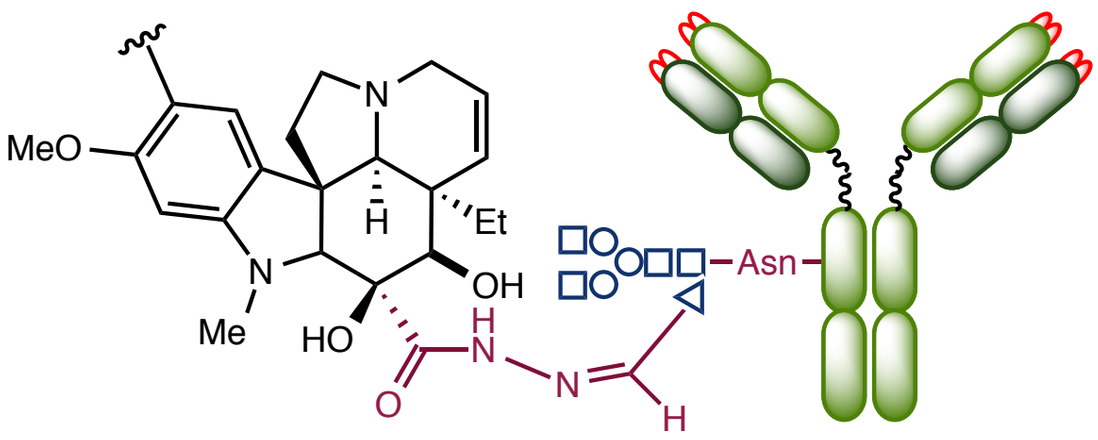
4-desacetylvinblastine derivative



KS1/4S2 murine mAB

treated with $NaIO_4$

KS1/4-DAVLBHYD

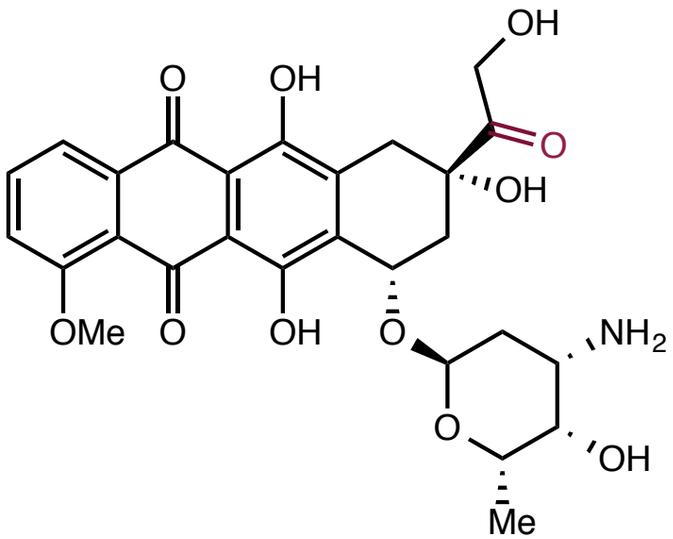


- cleavable acid-labile hydrazone linker
- highly potent *in vivo* activity with greater efficacy than unconjugated drug
- Phase I clinical trials indicates localization drug to tumor cells
- no increased therapeutic effect - premature cleavage of hydrazone
- patients developed immune responses to both the antibody and vinca alkaloid

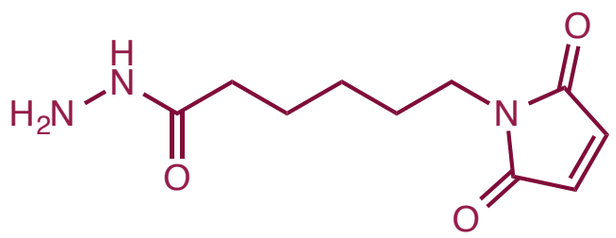
Cancer Res., **1991**, *51*, 2286.

First Generation Antibody-Drug Conjugates

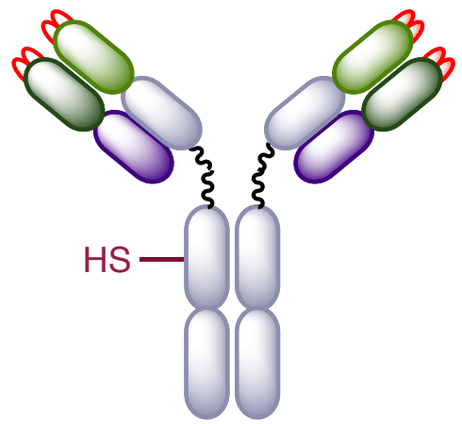
BR96 - Doxorubicin Conjugate



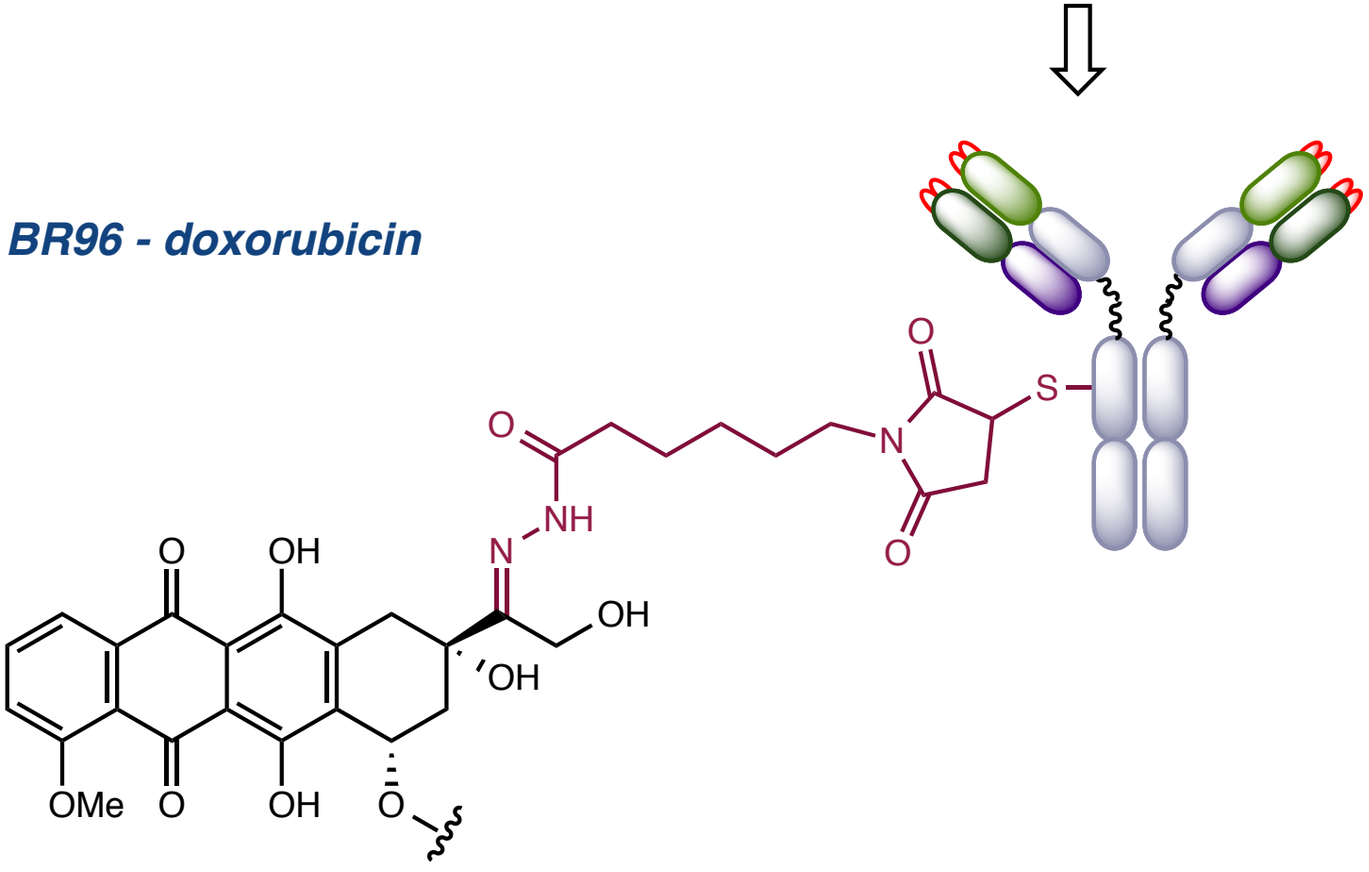
doxorubicin



linker with hydrazide and maleimide



BR96 chimeric mAB



- acid-labile hydrazone linker
- highly potent *in vivo* activity with greater efficacy than unconjugated drug
- advanced to Phase II clinical trials
- significant gastrointestinal toxicity - target antigen expression on normal tissue cells
- 50% of patients developed immune responses despite chimeric mAB

J. Clin. Oncol., **1999**, *17*, 478.

First Generation Antibody-Drug Conjugates

Universal Shortcomings and Lessons Learned

All four case studies successfully demonstrated localization of drug payload to tumor sites, but in all cases no significant improvement in therapeutic activity was observed...

I. Low *in vitro* potency - conjugation results in decreased cytotoxicity compared to free drug

- Different mechanisms of cellular uptake
  - Free drugs can diffuse through cell membrane
  - Conjugated drugs require efficient internalization after binding to antigen
- Need 10^6 molecules/cell of a *moderately* potent cytotoxic drug to effect cell kill
- Limited expression of antigen - tumor cells typically express 1 x 10^5 receptors/cell

II. Stability of the linker was inadequately tuned

- Hydrazone linkers were too labile - prone to cleavage prior to cellular uptake
- Amide linker not labile enough - no cleavage to release drug after internalization

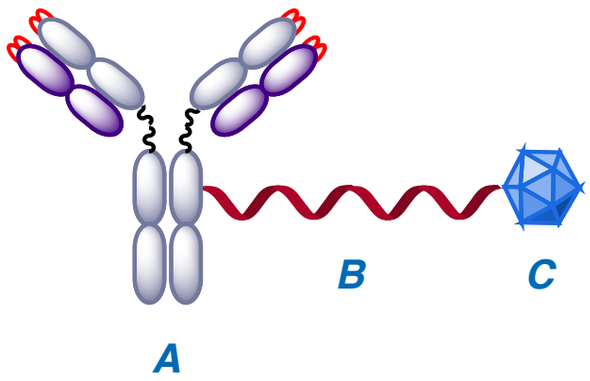
III. Antibodies of murine or chimeric origin illicited undesired immune response

- Generation of human anti-murine antibodies
- Rapid clearance of antibody-drug conjugate upon repeat dosing

Acc. Chem. Res., **2008**, *41*, 98.

Improving Antibody-Drug Conjugates

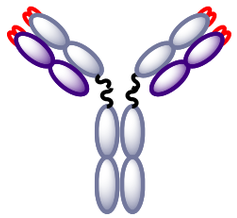
Ideal Characteristics of an Antibody-Drug Conjugate



A. Antibody

B. Linker

C. Small-molecule drug



- selective for antigens with high copy numbers (>10^5/cell) on target cell
- selective for antigens uniquely expressed on tumor cell
- homogeneous expression of antigen on tumor cell
- induces minimal immunogenic response

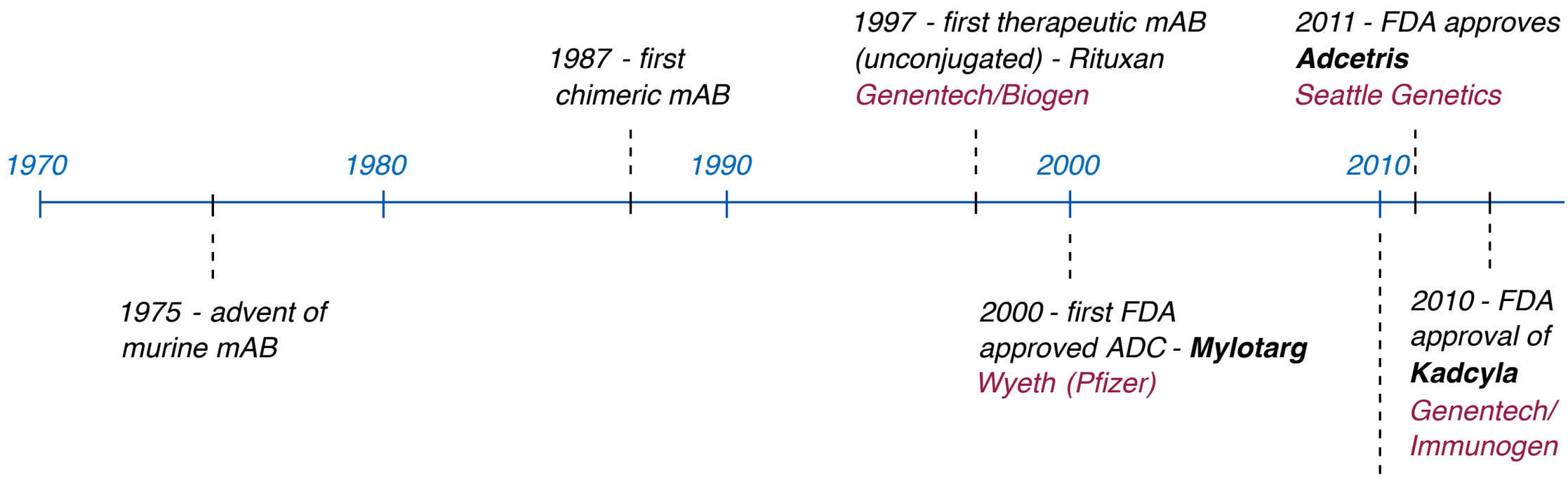
- Stable to circulation *in vivo*
- Selectively cleaved only once internalized inside target cell
  - *disulfide linkers*
  - *protease labile linkers*
- Designed to release drug in its active form (without linkers)
  - *self immolative linkers*
- Stable to long-term storage in aqueous environments

- highly potent *in vitro* - potency in picomolar range required
- sensitive to the ideal mechanism of action for specific tumor types
- amenable to introduction of functional groups for linking
- water soluble

Acc. Chem. Res., **2008**, *41*, 98.

Antibody-Drug Conjugates

A Brief Introduction and History



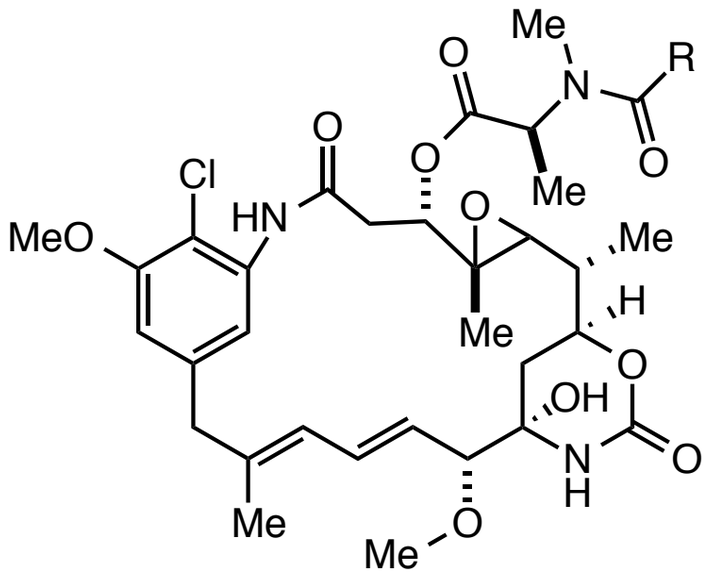
mAB = monoclonal antibody
ADC = antibody-drug conjugate

Part I. First Generation Antibody-Drug Conjugates and Lessons Learned	***Part II.*** *Second Generation Antibody-Drug Conjugates and Their Improvements*	*Part III.* Current Challenges and Overview of Clinical Performance

Nature Rev. **2006**, *5*, 147.; *Bioconjugate Chem.* **2015**, *26*, 176.

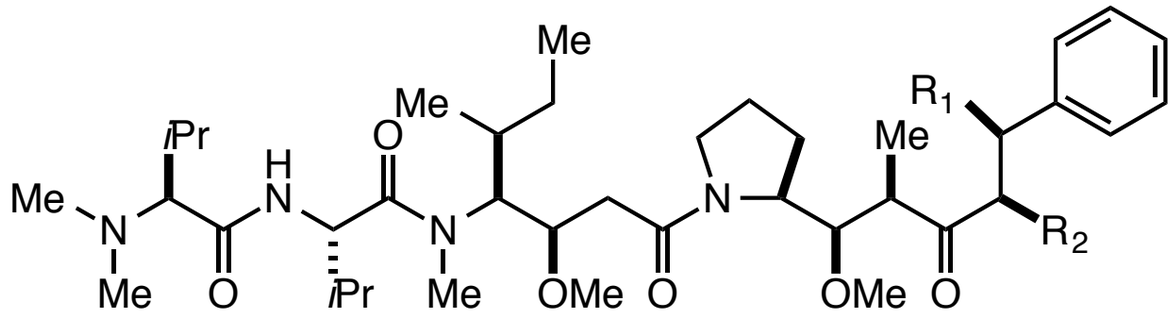
Second Generation Antibody-Drug Conjugates

Most Commonly Used Drug Payloads



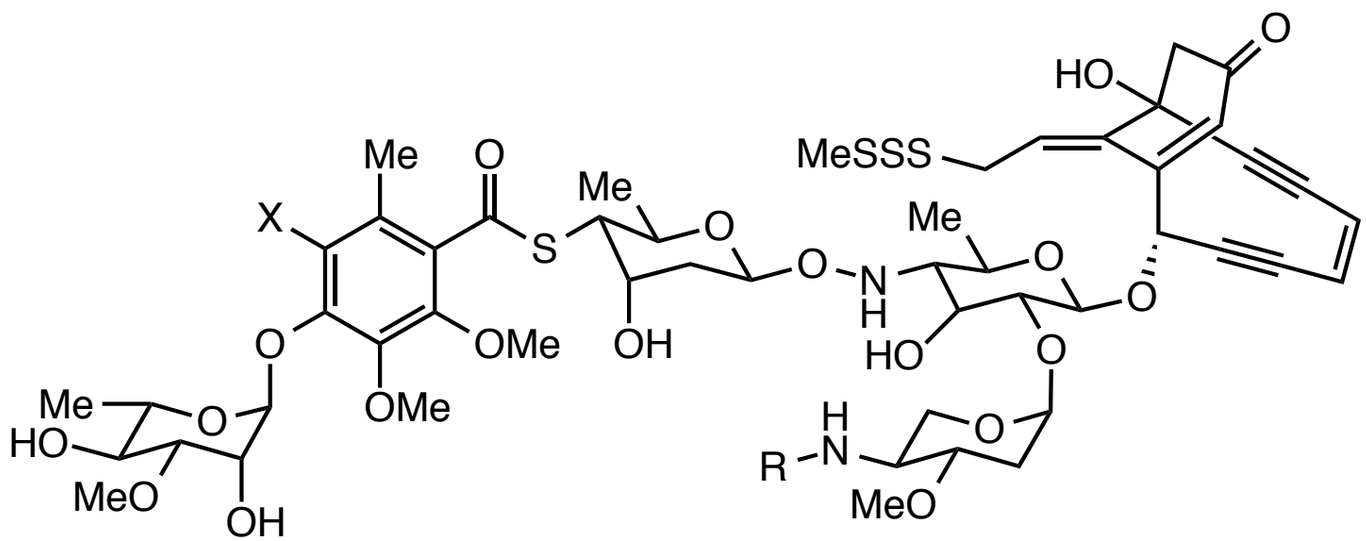
maytansanoids

anti-mitotic agent



auristatins

anti-mitotic agent



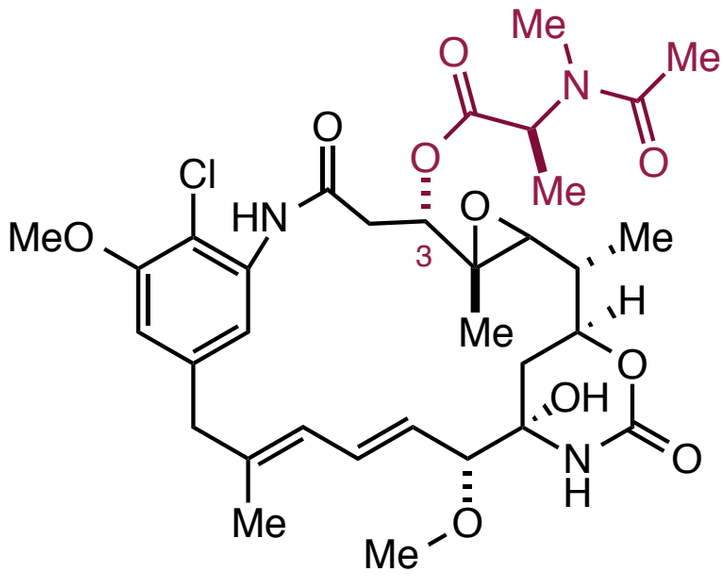
calicheamicins

DNA damaging agent

Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

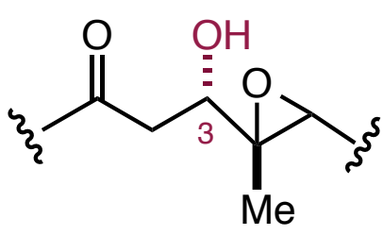
Maytansanoid Antibody-Drug Conjugates



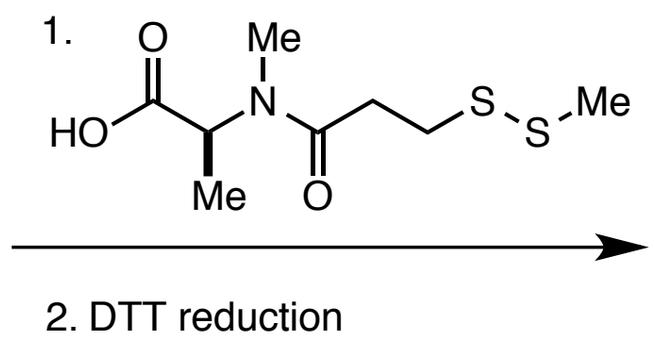
maytansine

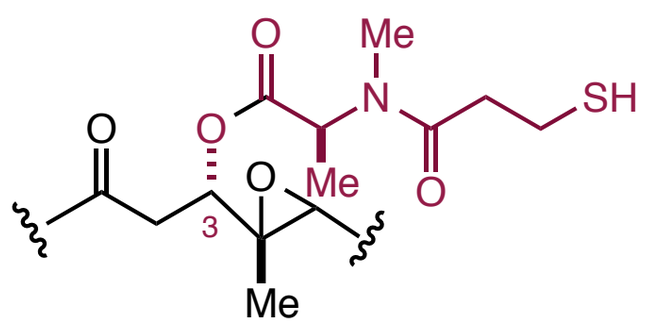
anti-mitotic agent

- 1000 fold more cytotoxic than first generation payloads
- Binds tubulin to suppress microtubule dynamics, resulting in cell arrest in G2/M phase
- Good aqueous solubility
- SAR activity indicates that the ester at C_3 can be derivatized for linker conjugation without impacting drug activity



maytansinol

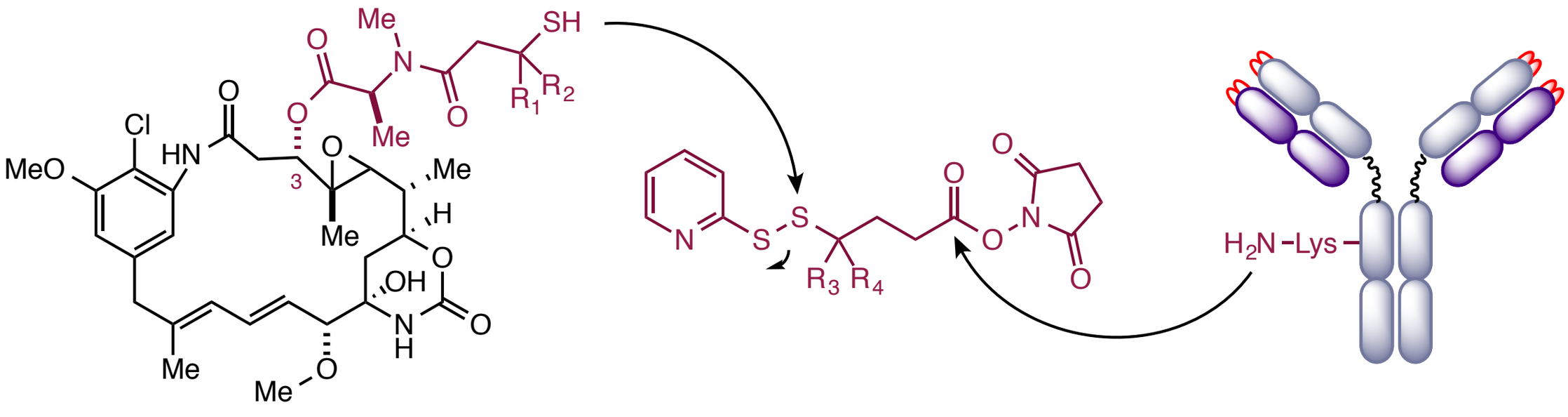




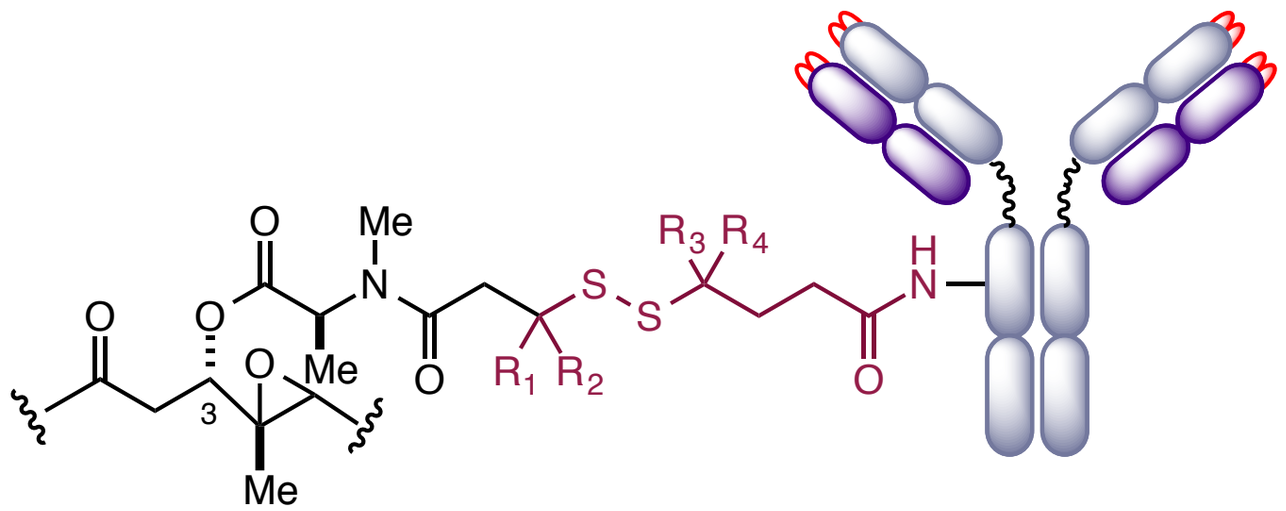
Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

Maytansanoid Antibody-Drug Conjugates



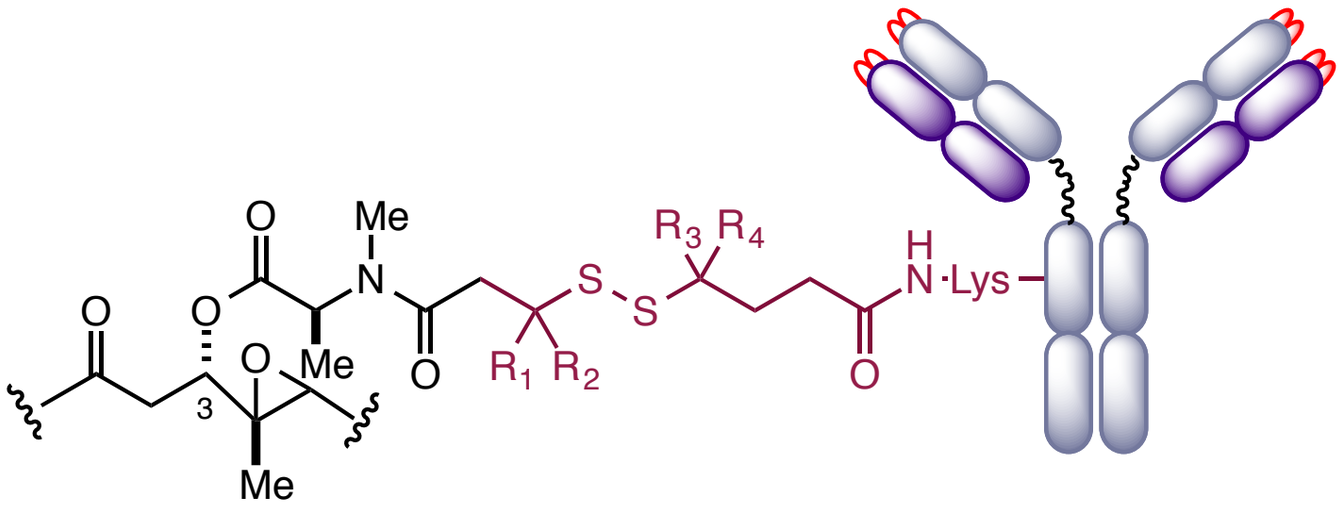
maytansine *heterobifunctional linker* *humanized C242 mAB*



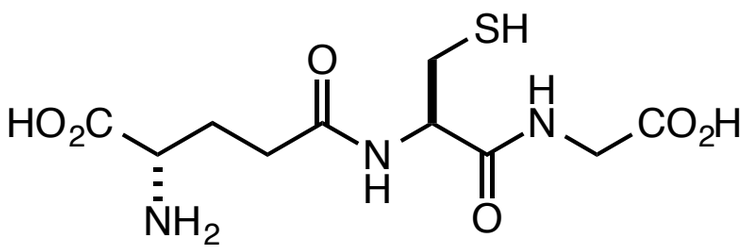
Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

Maytansanoid Antibody-Drug Conjugates



Improved mechanism of selective drug release



glutathione
disulfide bond reducing agent

- glutathione naturally present in high concentration inside tumor cells (millimolar range), but exceptionally low (micromolar range) in blood stream - ***selective cleavage upon internalization***
- disulfide linkage is stable under physiological pH
- stability of the antibody-drug conjugate can be tuned by varying the sterics of the R groups flanking the disulfide bond

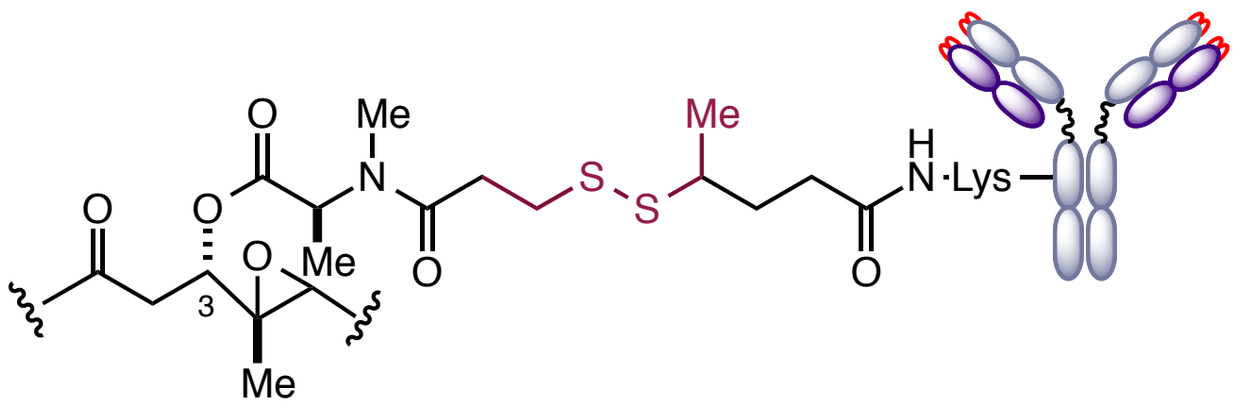
Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

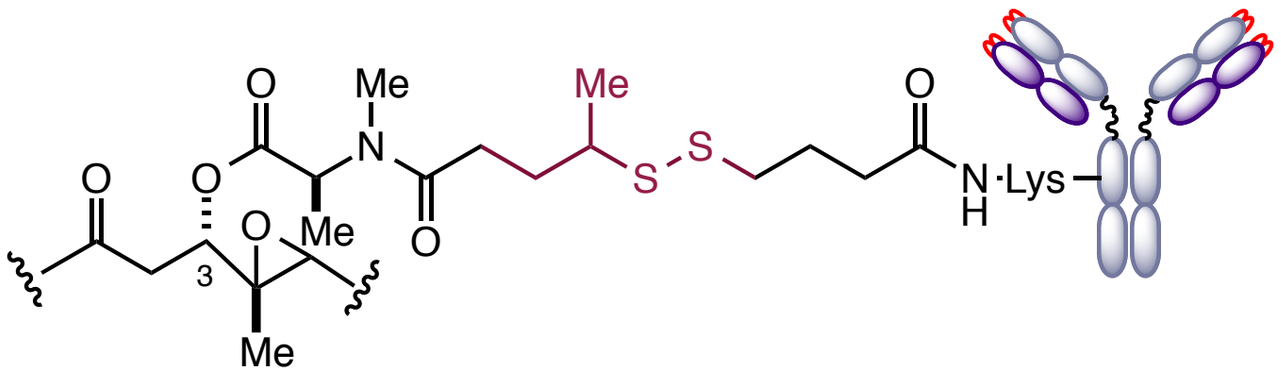
Maytansanoid Antibody-Drug Conjugates

Study on effect of linker stability to therapeutic efficacy

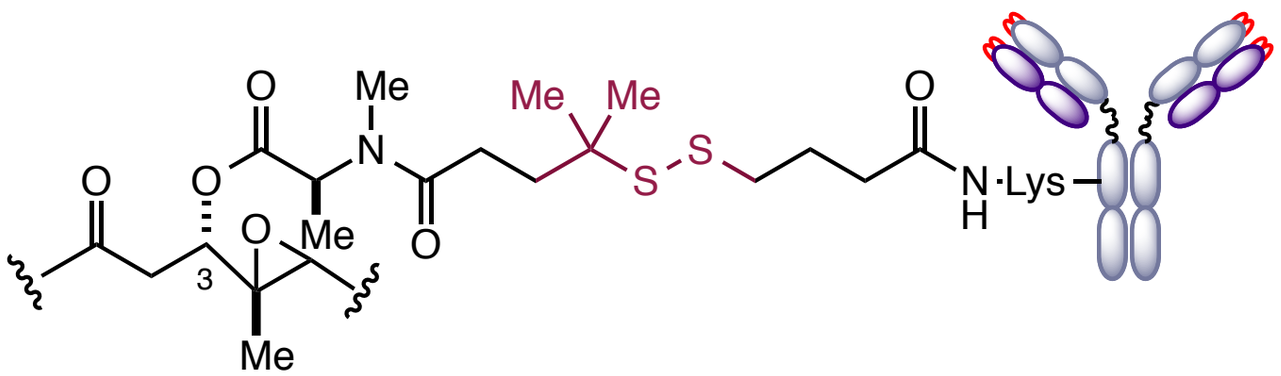
HuC242-DM1



HuC242-DM3



HuC242-DM4



increased stability in blood stream

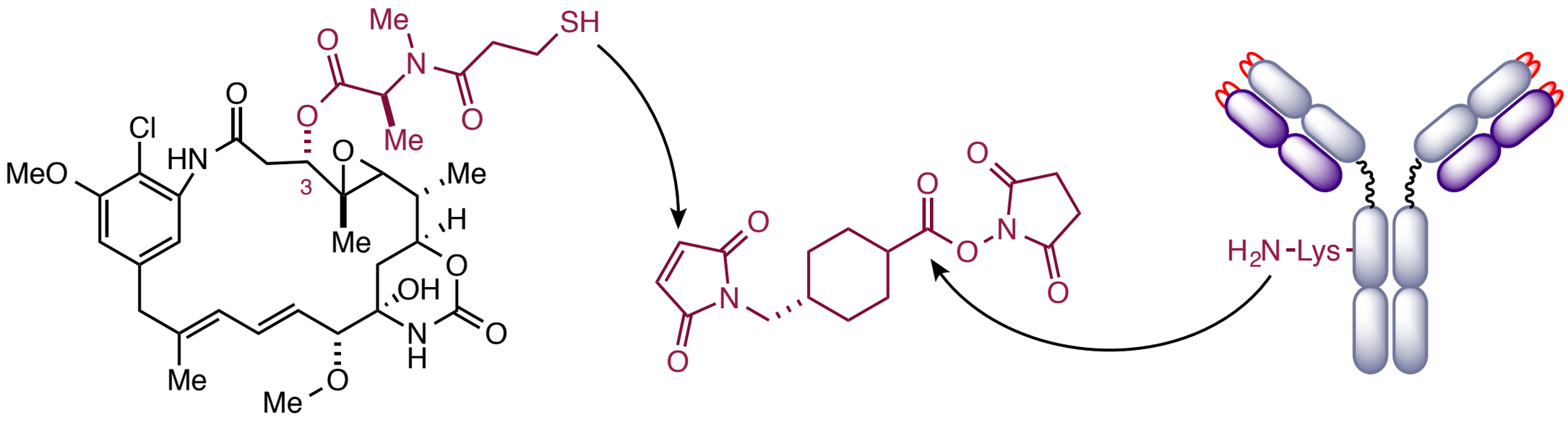
increasing therapeutic efficacy

Extreme case - synthesis of a non-cleavable conjugate?

Acc. Chem. Res., **2008**, *41*, 98.

Second Generation Antibody-Drug Conjugates

Maytansanoid Antibody-Drug Conjugates



maytansine

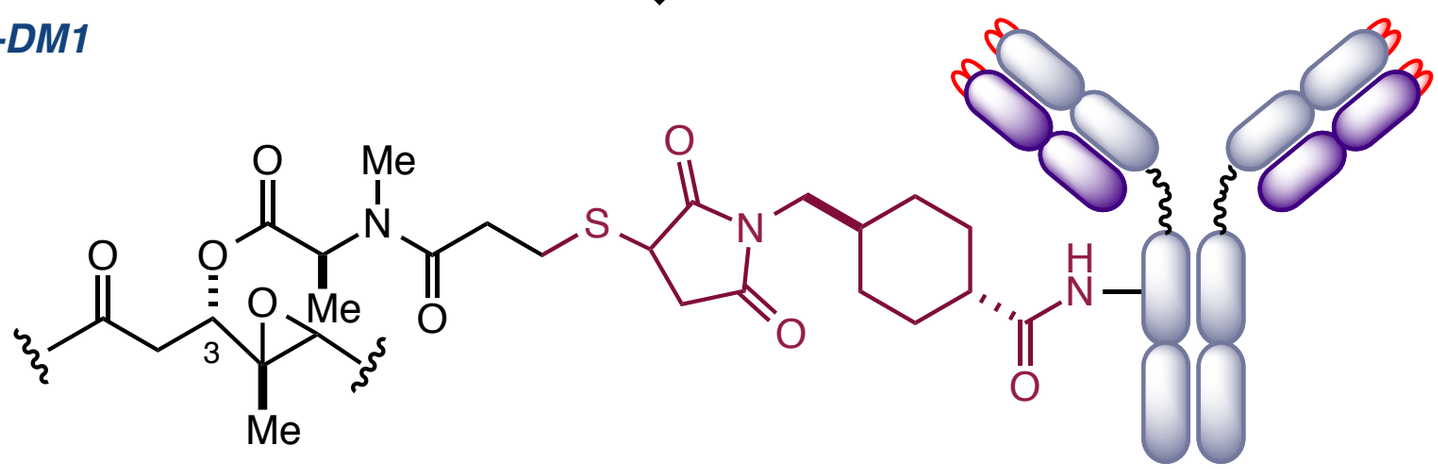
SMCC (non-cleavable) linker

humanized C242 mAB

succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate

⇩

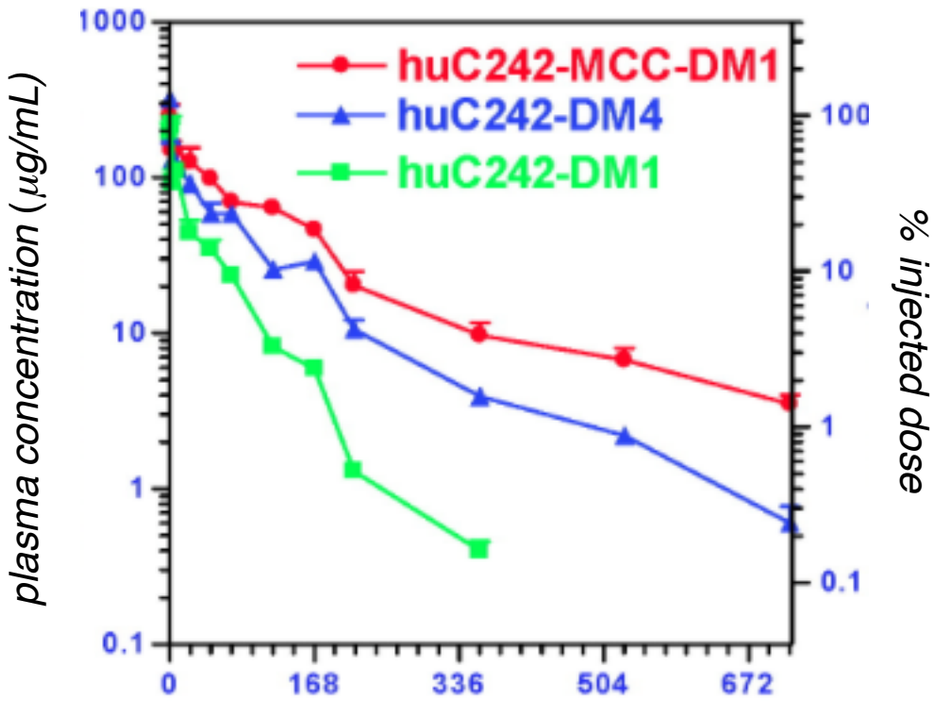
HuC242-MCC-DM1



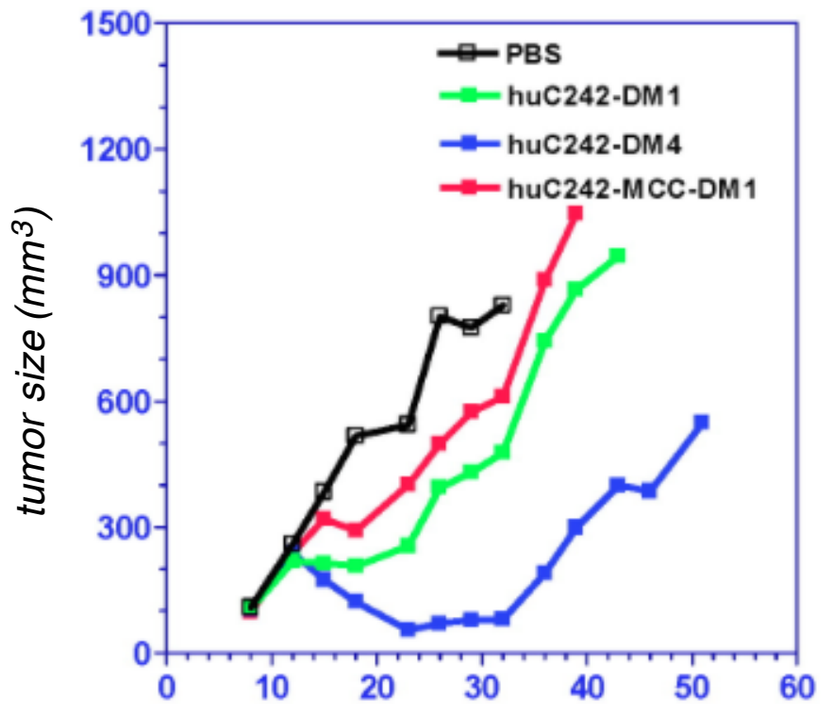
Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

Maytansanoid Antibody-Drug Conjugates



time (hrs) post administration



days post tumor inoculation

- HuC242-MCC-DM1 shows greatest stability
  (*in vivo* half life of 134 hrs)

- HuC242-DM4 shows greatest cytotoxicity

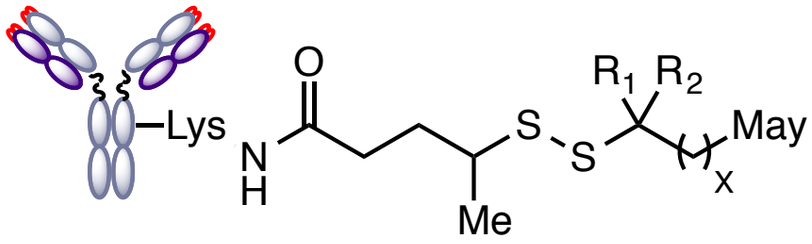
What is the mechanism of action of these maytansanoid antibody-drug conjugates?

Acc. Chem. Res., **2008**, *41*, 98.

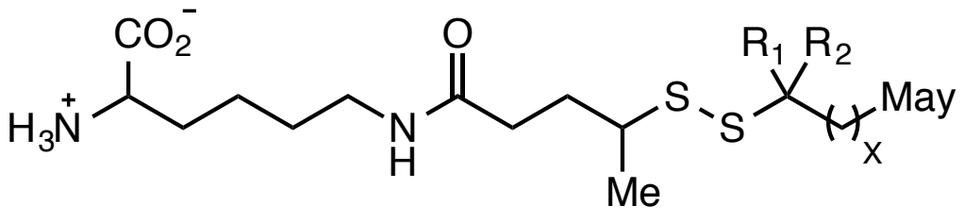
Second Generation Antibody-Drug Conjugates

Maytansanoid Antibody-Drug Conjugates

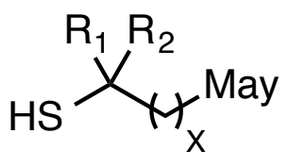
Cellular Processing of Disulfide Linked Maytansanoids



lysosomal processing

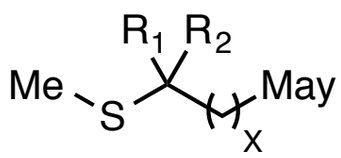


disulfide reduction

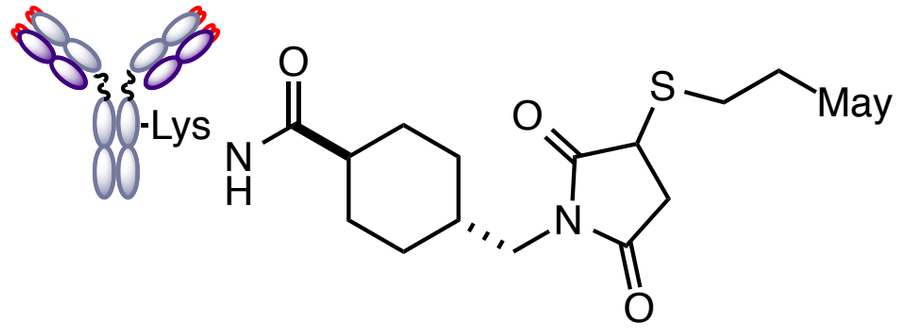


good bystander killing

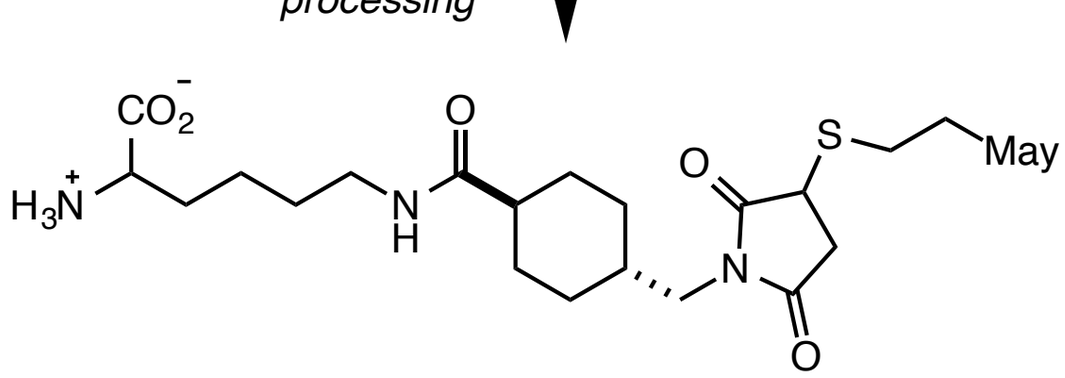
S-methylation



excellent bystander killing



lysosomal processing



poor bystander killing

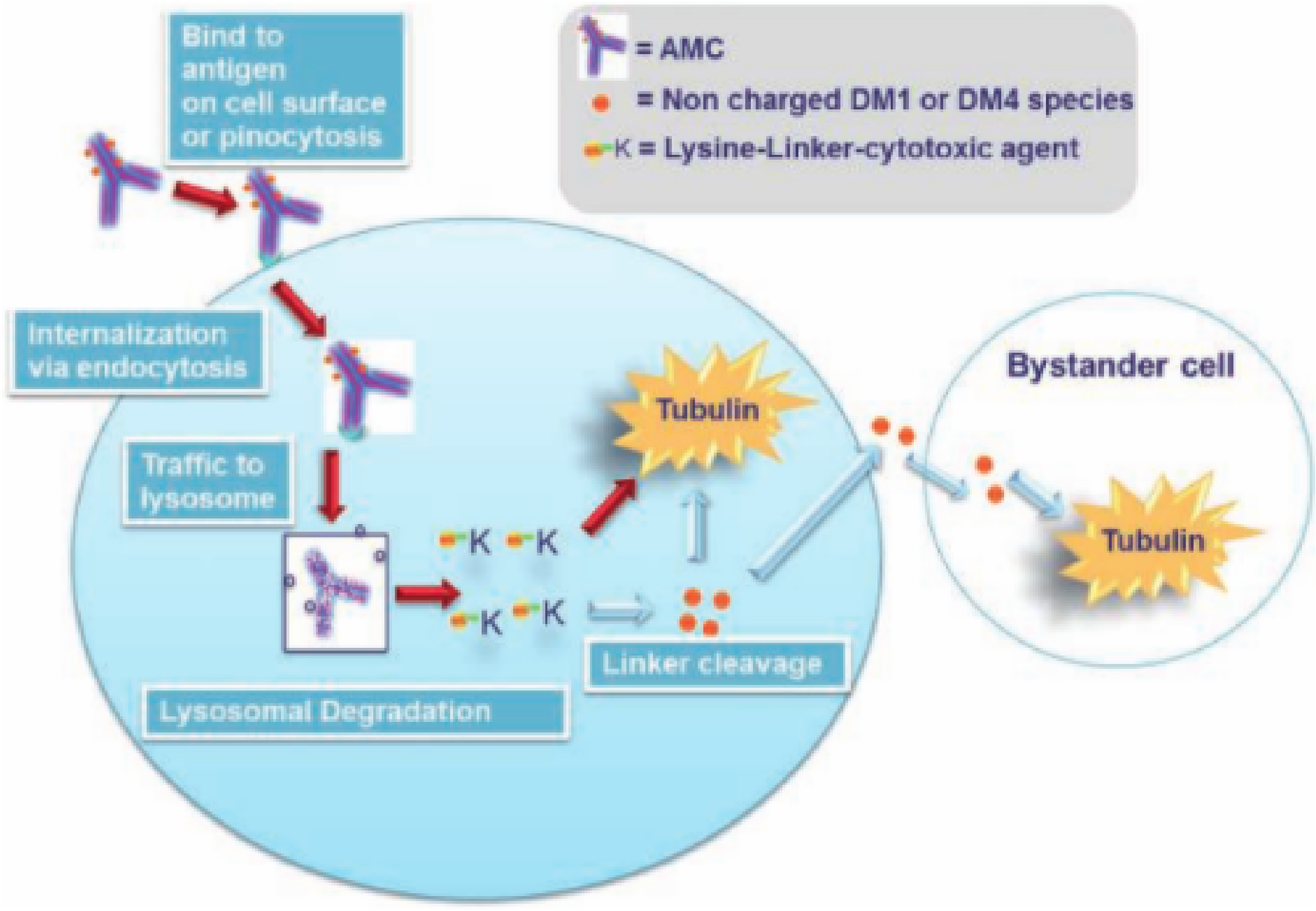
charged nature prevents diffusion into neighboring cells

Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

Maytansanoid Antibody-Drug Conjugates

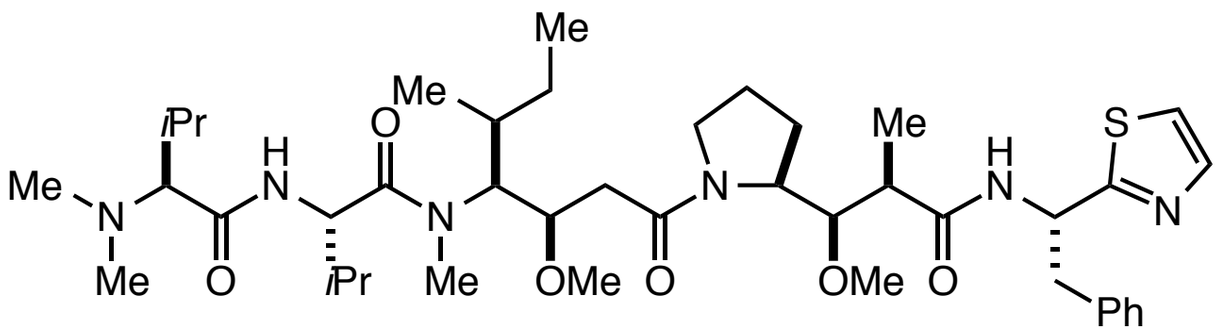
Enhanced Therapeutic Efficacy of Cleavable Disulfide Linkers is Due to Bystander Cell Killing



Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

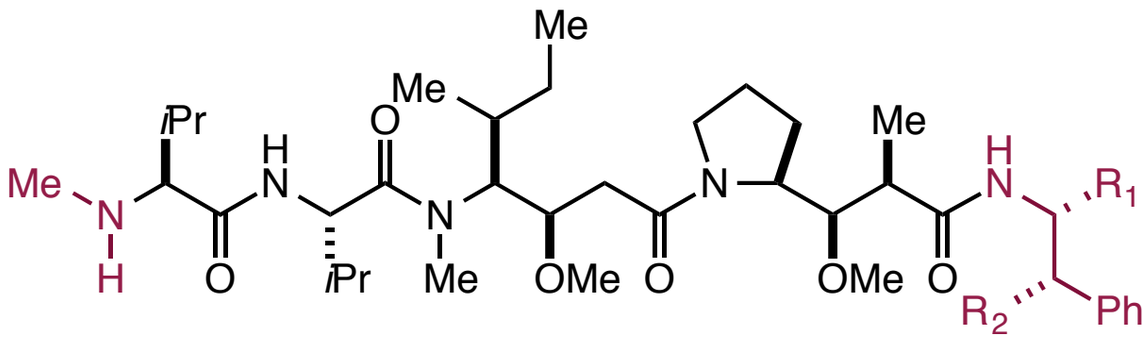
Auristatin Antibody-Drug Conjugates



dolastatin 10

anti-mitotic agent

- inhibits tubulin-dependent GTP binding and microtubule dynamics
- fully synthetic series of highly potent anti-mitotic agents based on SAR studies on dolastatin 10
- inhibits tubulin-dependent GTP binding and
- SAR indicates terminal 3º amine can be derivatized for conjugation AND terminal phenethyl amine can be changed without loss of efficacy



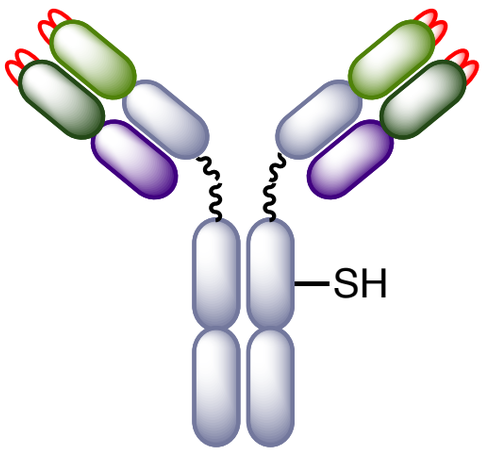
monomethyl auristatin E (MMAE) (R_1 = Me, R_2 = OH)

monomethyl auristatin F (MMAF) (R_1 = CO_2H, R_2 = H)

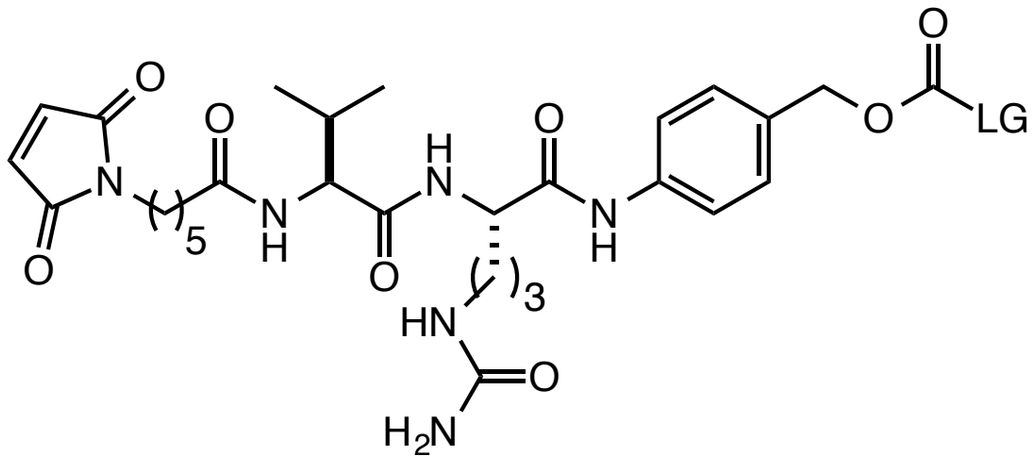
Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

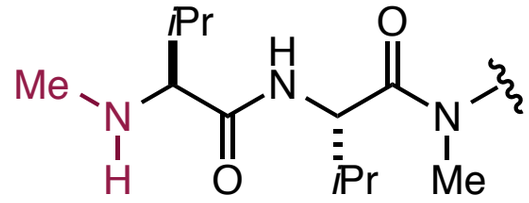
Auristatin Antibody-Drug Conjugates



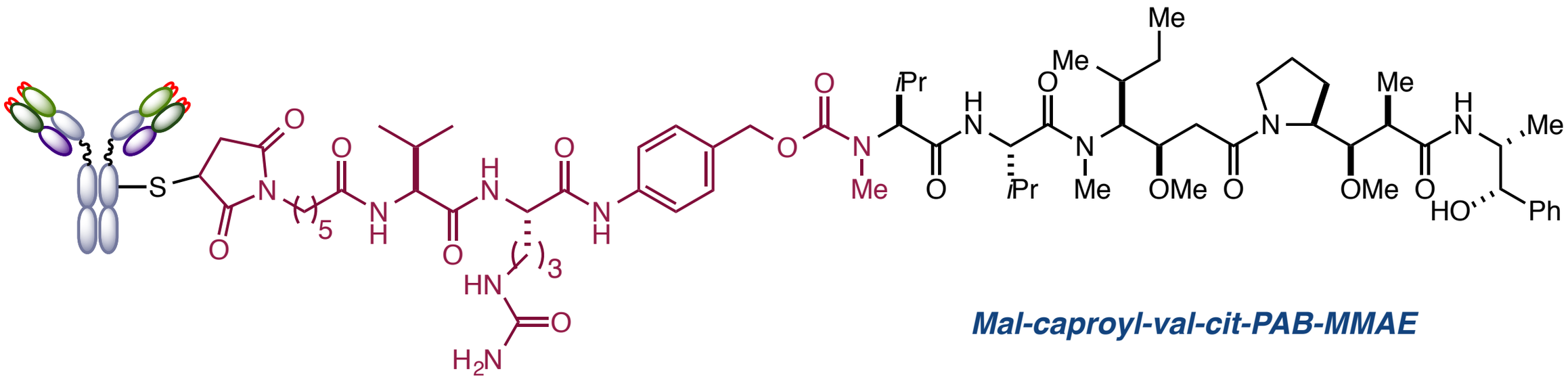
chimeric cAC10 mAB



mal-caproyl-val-cit-PAB linker



monomethyl auristatin

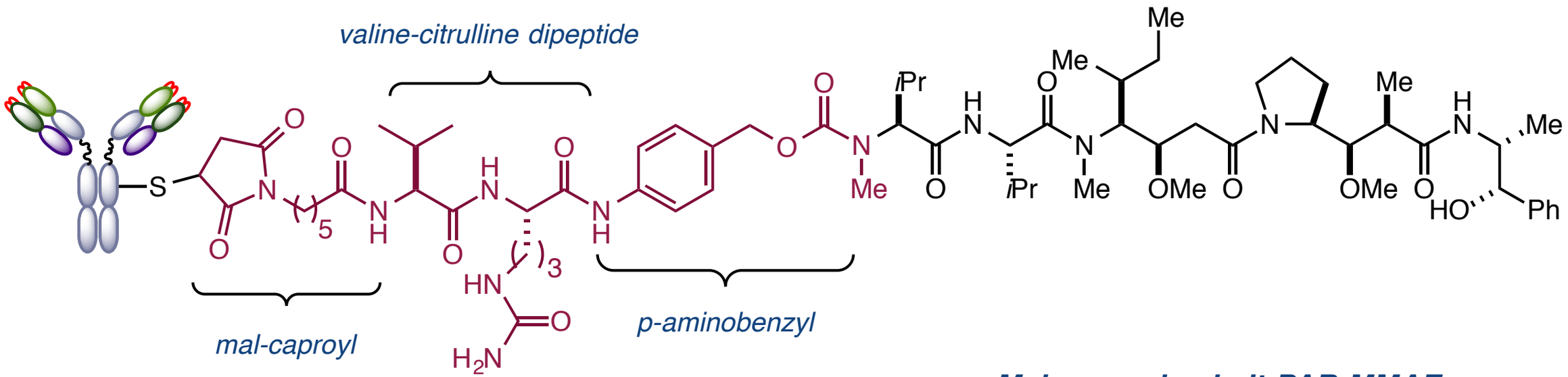


Mal-caproyl-val-cit-PAB-MMAE

Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

Auristatin Antibody-Drug Conjugates



Mal-caproyl-val-cit-PAB-MMAE

Improved mechanism of selective drug release

- Valine-citrulline dipeptide moiety is known to be selectively cleaved by the protease cathepsin B
- *p*-aminobenzyl group is self immolating - fragments to release the MMAE drug without any residual groups

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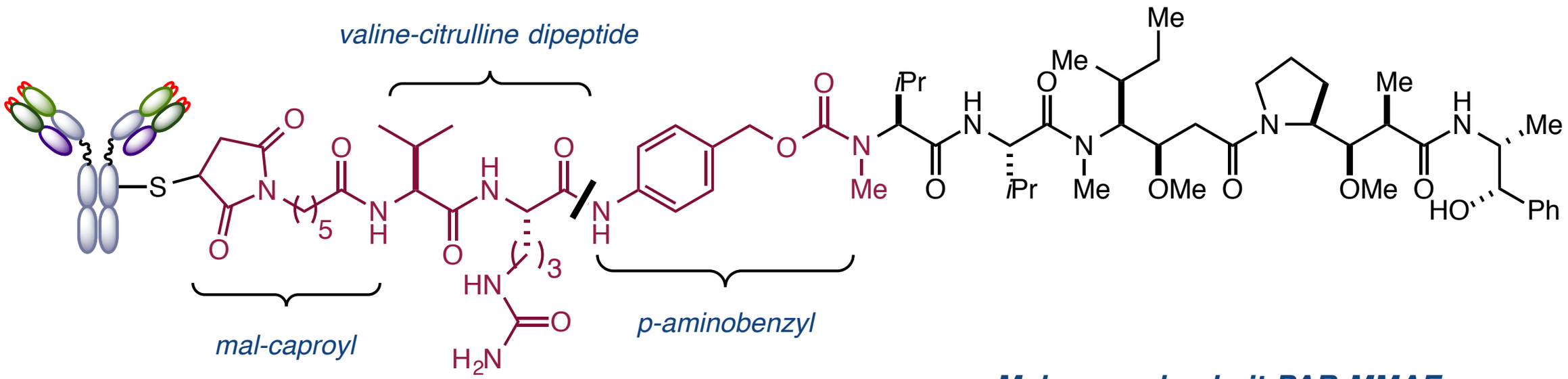
Second Generation Antibody-Drug Conjugates

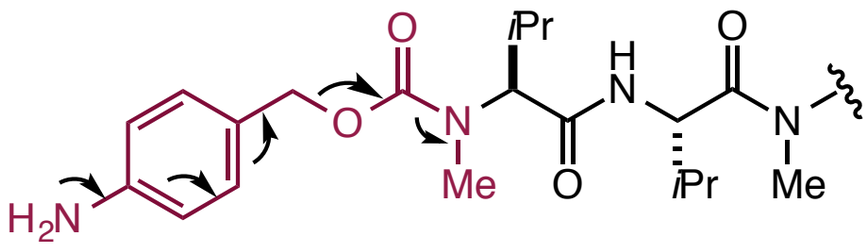
Auristatin Antibody-Drug Conjugates

Mechanism of cellular processing of mal-caproyl-val-cit-MMAE conjugate



Mal-caproyl-val-cit-PAB-MMAE

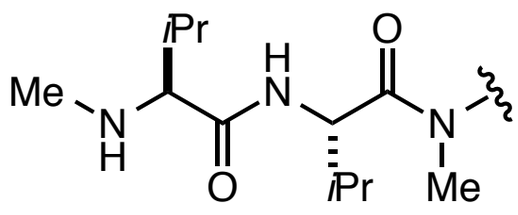
cathepsin B mediated peptide cleavage



self immolation

-PAB

-CO_2



free MMAE

good bystander killing

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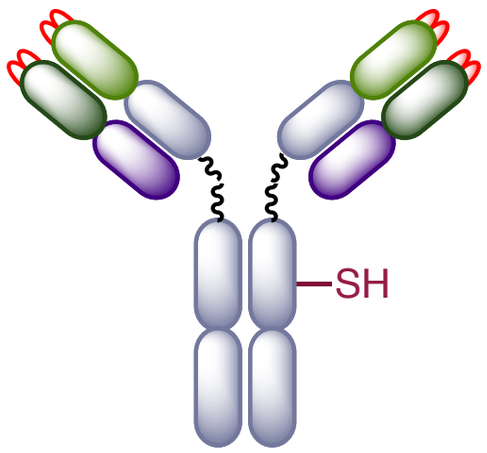
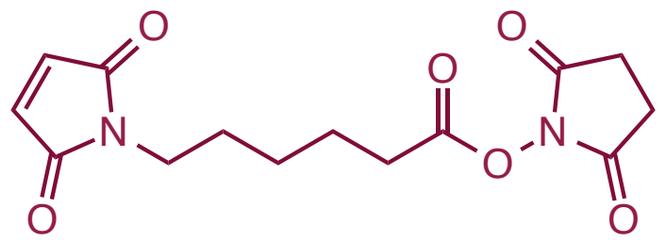
Second Generation Antibody-Drug Conjugates

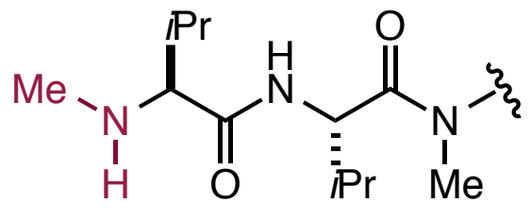
Auristatin Antibody-Drug Conjugates



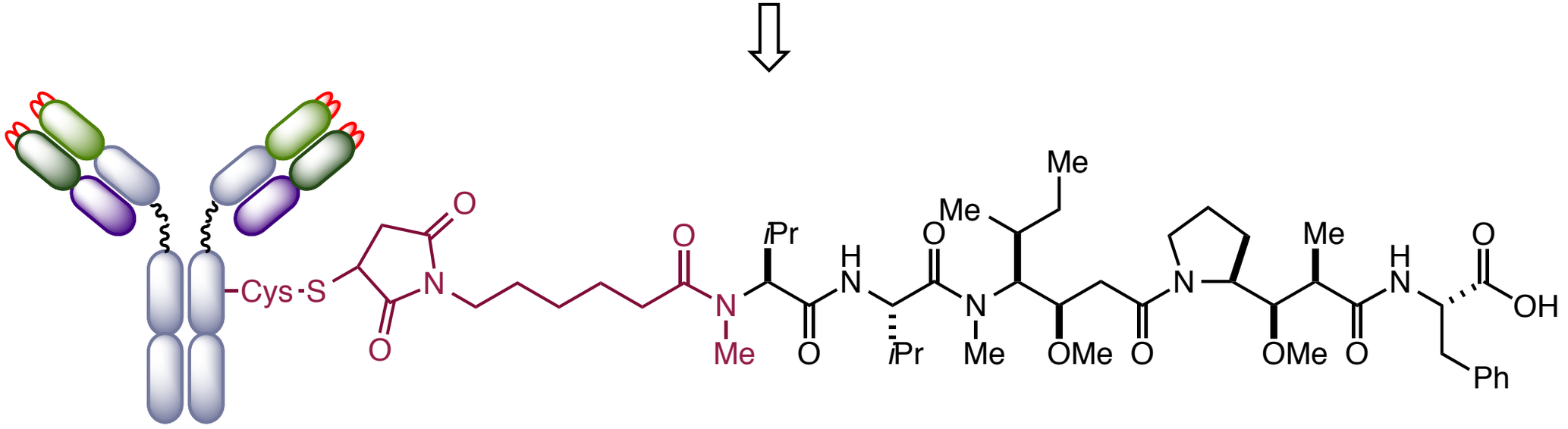
chimeric cAC10 mAB



mal-caproyl (non-cleavable) linker



monomethyl auristatin



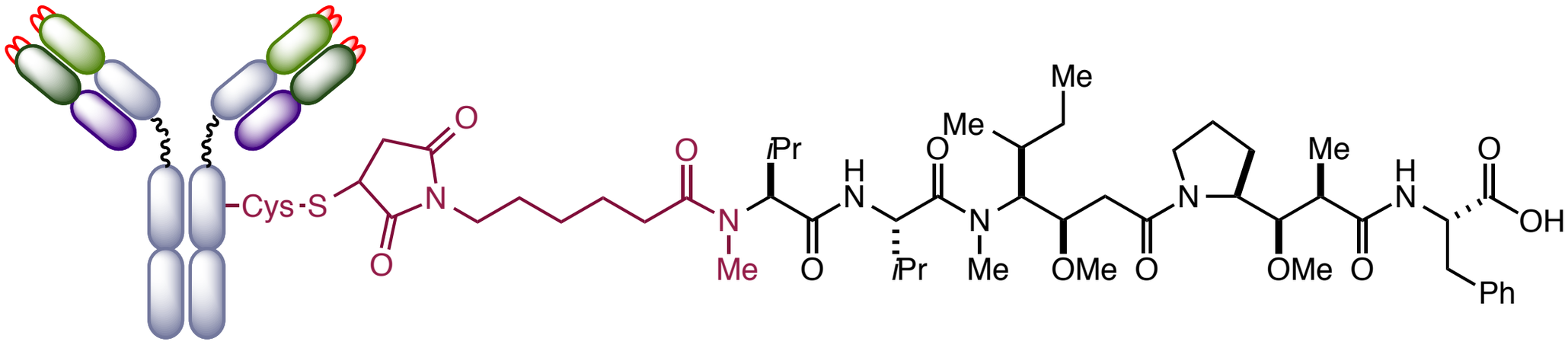
Mal-caproyl-MMAF

Angew. Chem. Int. Ed., **2014**, *53*, 3796.

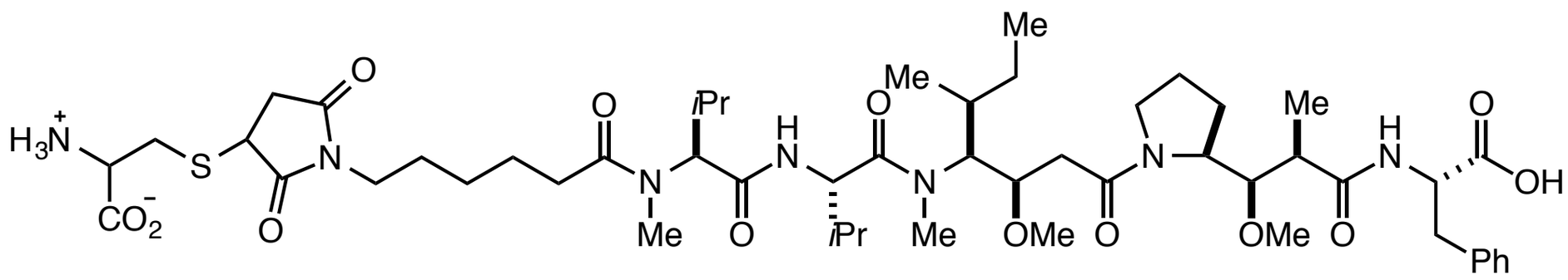
Second Generation Antibody-Drug Conjugates

Auristatin Antibody-Drug Conjugates

Cellular Processing of Non-Cleavable Mal-caproyl-MMAF Conjugates



lysosomal processing



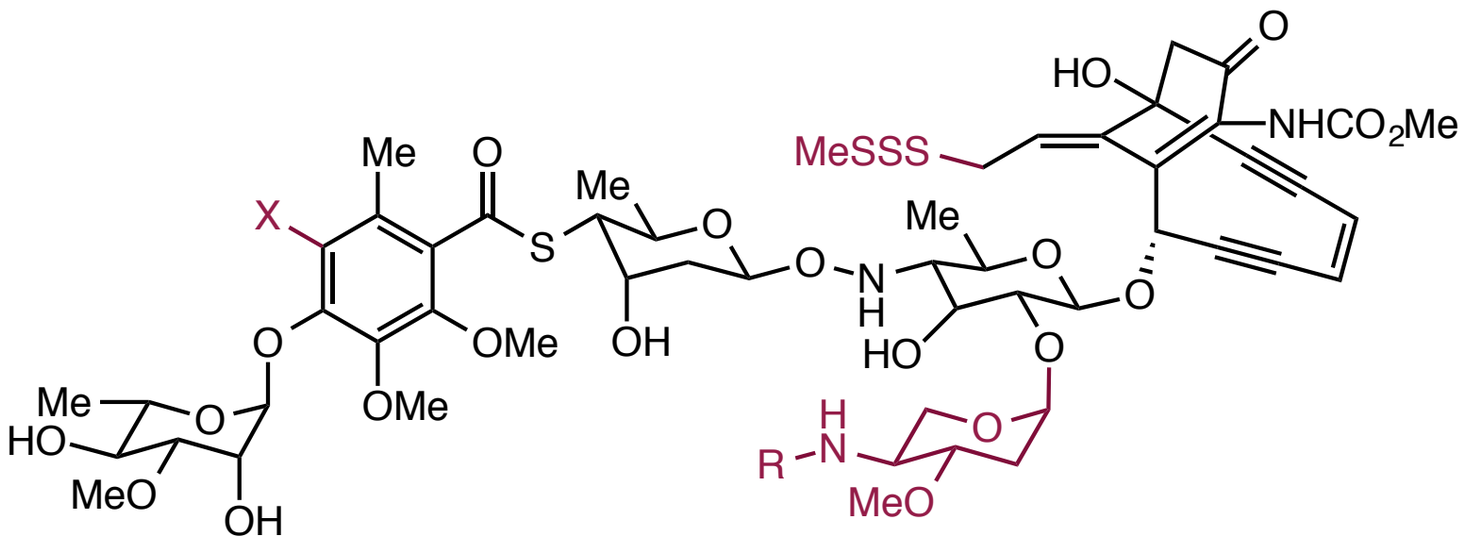
poor bystander killing

charged nature prevents diffusion into neighboring cells

Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

Calicheamicin Antibody-Drug Conjugates



calicheamicins

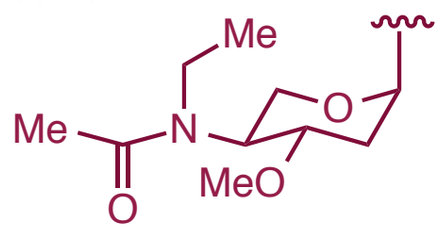
DNA damaging agent

calicheamicin γ_1^{Br} - X = Br, R = *i*Pr

calicheamicin γ_1^{Br} - X = Br, R = Et

calicheamicin γ_1^{I} - X = I, R = Et

N-acetyl calicheamicin γ_1^{I} - X = I



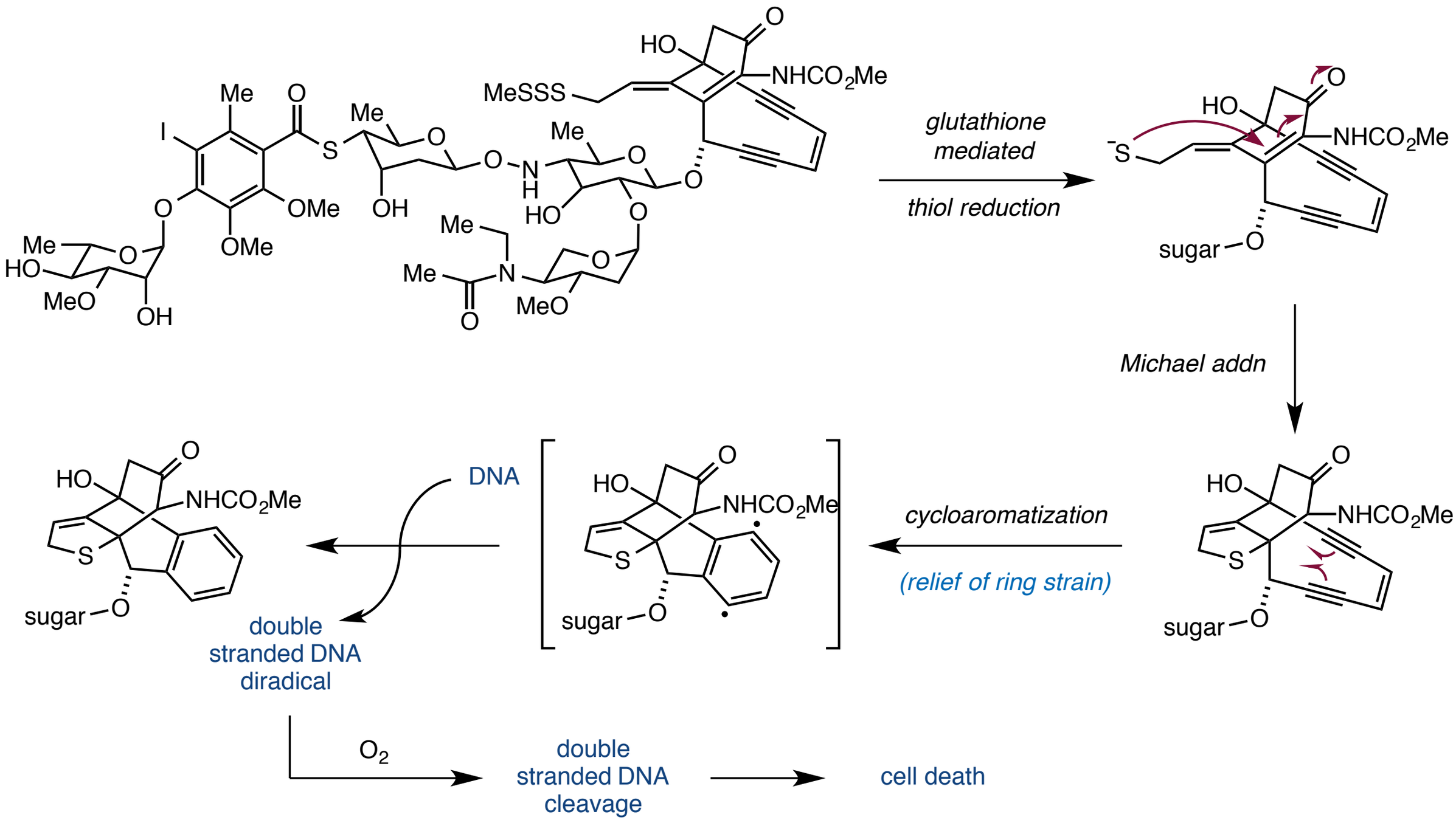
- insanely cytotoxic class of anti-tumor antibiotics (0.15μg/kg dose)
- aryl tetrasaccharide moiety binds in minor groove of DNA, placing ene-diyne warhead within double helix
- too toxic for use as drug warhead - 20 fold less potent *N*-acetyl analogue developed for applications to ADCs
- trisulfide converted to disulfide - provides a handle for conjugation

Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

Calicheamicin Antibody-Drug Conjugates

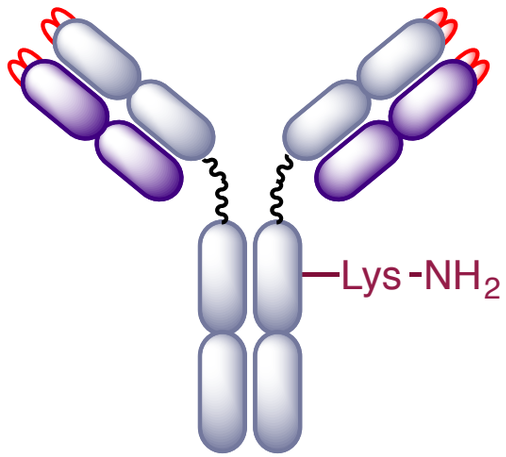
Mechanism of Action of the Calicheamicins



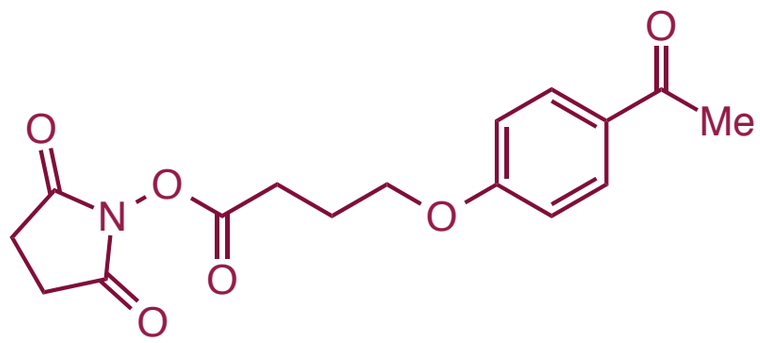
Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

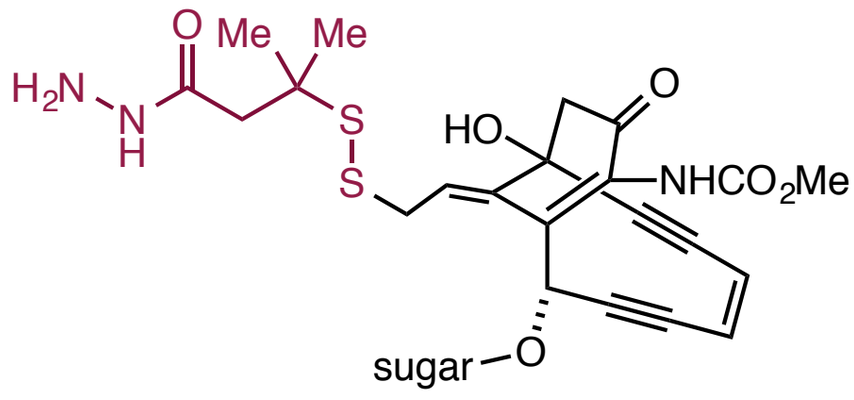
Calicheamicin Antibody-Drug Conjugates



hP67.6 humanized mAB

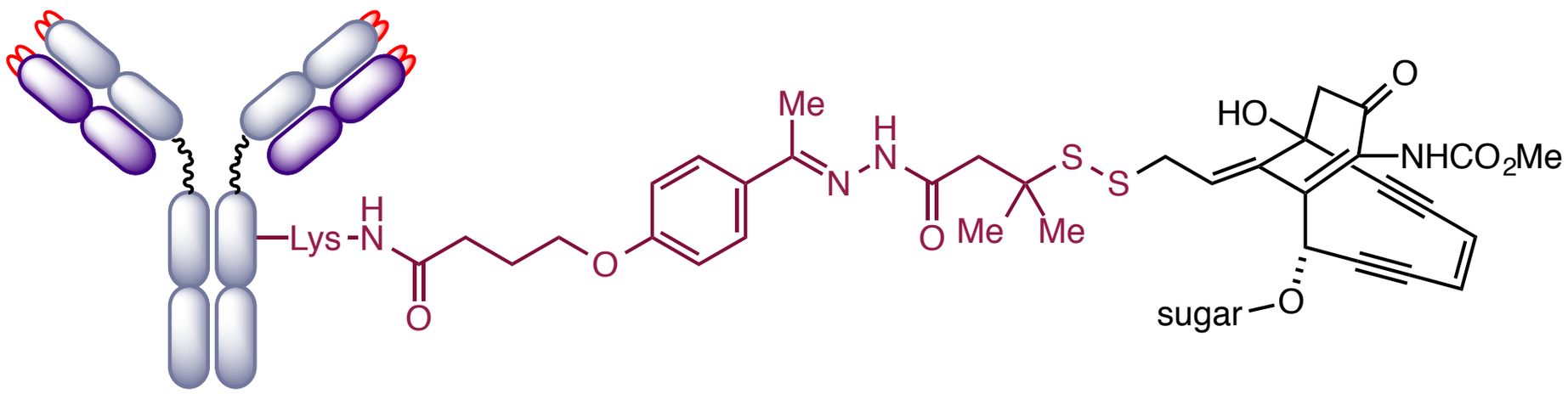


AcBut linker
(4-(4'acetylphenyl)butanoic acid)



N-acetyl calicheamicin

⇩

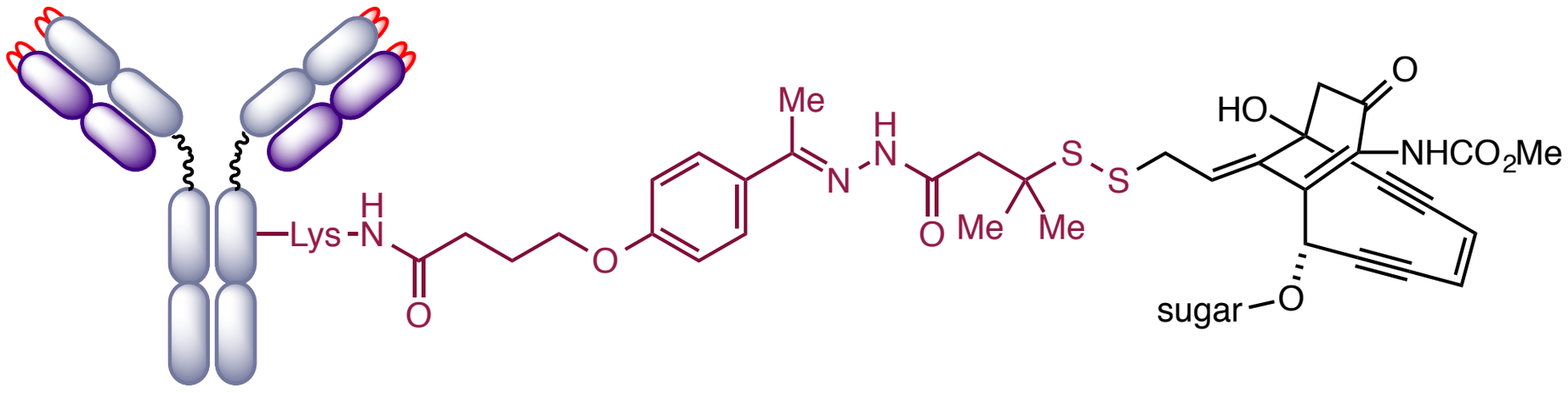


hP67.6 N-Ac-γ-calicheamicin DMH AcBut

Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Second Generation Antibody-Drug Conjugates

Calicheamicin Antibody-Drug Conjugates



hP67.6 N-Ac-γ-calicheamicin DMH AcBut

Improved mechanism of selective drug release

- Linker specifically designed to provide high stability prior to internalization into tumor cells, but is readily cleaved once inside the lysosome - hydrazone formed from ketone rather than aldehyde

  only 6% hydrolysis observed at pH = 7.4

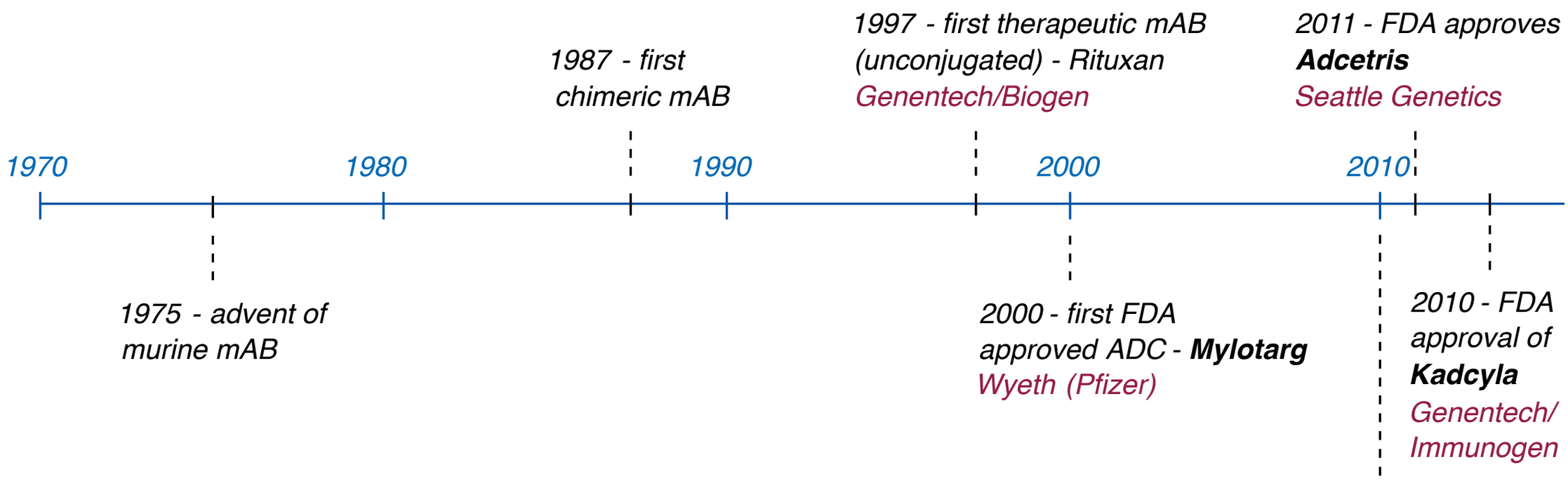
  97% hydrolysis observed at pH = 4.5 at 37ºC over 24 hrs

- Inclusion of a hindered disulfide moiety in the linker provides a second handle for selective drug cleavage via glutathione reduction upon internalization inside cell

Bioconj. Chem., **2002**, *13*, 47.

Antibody-Drug Conjugates

A Brief Introduction and History



mAB = monoclonal antibody
ADC = antibody-drug conjugate

Part I.
First Generation Antibody-Drug Conjugates and Lessons Learned

Part II.
Second Generation Antibody-Drug Conjugates and Their Improvements

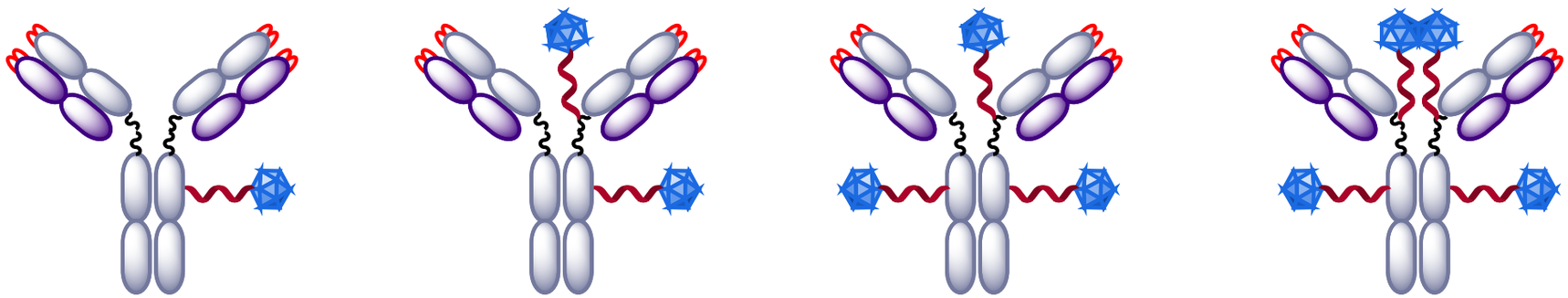
Part III.
Current Challenges and Overview of Clinical Performance

Nature Rev. **2006**, *5*, 147.; *Bioconjugate Chem.* **2015**, *26*, 176.

Current Trends in Research in Antibody-Drug Conjugates

Controlling the Drug to Antibody Ratio (DAR) and Achieving Site-Specific Conjugation

Though the underlying protein scaffold is constant in a heterogeneous population of antibody-drug conjugates, each conjugate has its own set of pharmacokinetic, toxicity, aggregation, antigen affinity, and drug release properties



Forefront of research in this field currently lies in achieving:

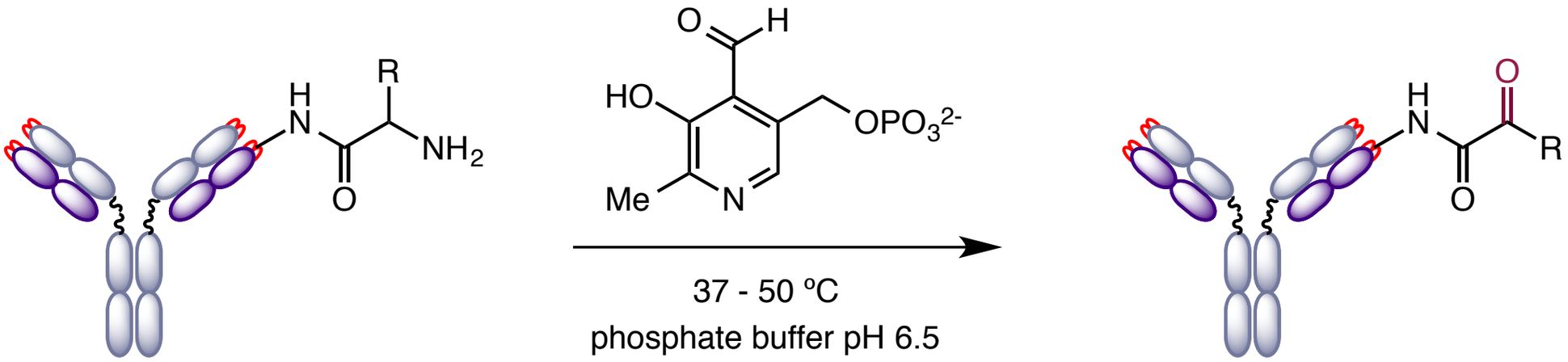
1. Populations of antibody-drug conjugates with a homogenous DAR
2. Site-specific conjugation of drugs on a given antibody

Bioconj. Chem., **2015**, *26*, 176.

Current Trends in Research in Antibody-Drug Conjugates

Methods of Homogeneous Conjugation Using Natural Antibodies

- *N*-terminal conjugation leveraging differences in pK_a between terminal and internal amino acids



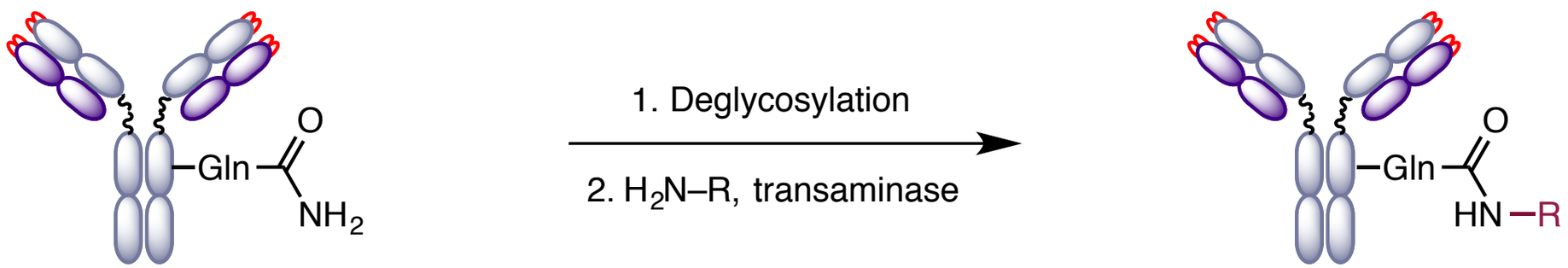
- Though conjugation via this method is in the antigen binding domain, drug conjugation here does not seem to impact antigen recognition and binding
- Resulting ketone product can be easily further functionalized through reaction with oximes bearing a linker or drug
- Limitation: Reaction sensitive to nature of terminal amino acid - works best for alanine, glycine, aspartate glutamate, and asparagine
- Limitation: Some antibodies may not be able to tolerate elevated temperatures required for transamination

Garbaccio, R. M. Chemistry of Antibody-Small Molecule Drug Conjugates. From *Comprehensive Organic Synthesis II*, **2014**, *9*, 438.

Current Trends in Research in Antibody-Drug Conjugates

Methods of Homogeneous Conjugation Using Natural Antibodies

- Site-specific functionalization of glutamines through enzymatic conjugation

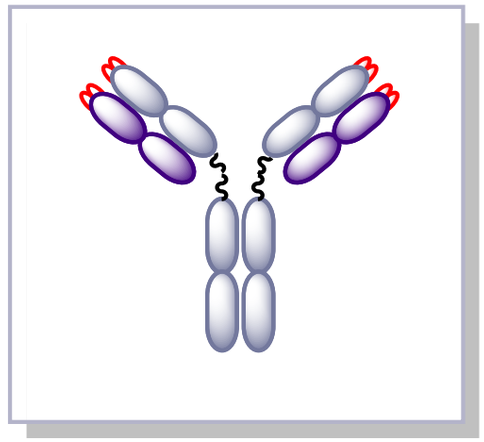


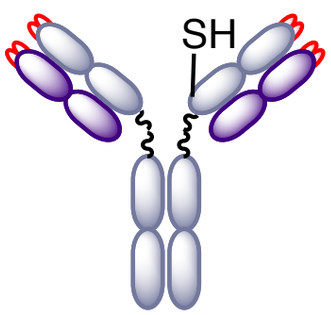
- Selectively functionalizes only Q295 residue (flanked by a consensus recognition sequence for a bacterial transaminase)
- Q295 residue is distant from antigen binding domain
- Limitation: Requires deglycosylation in the CH_2 domain prior to functionalization, which may impact function and properties of the antibody-drug conjugate

Garbaccio, R. M. Chemistry of Antibody-Small Molecule Drug Conjugates. From *Comprehensive Organic Synthesis II*, **2014**, *9*, 438.

Current Trends in Research in Antibody-Drug Conjugates

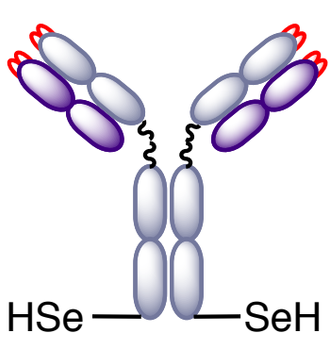
Methods of Homogeneous Conjugation Using Engineered Antibodies





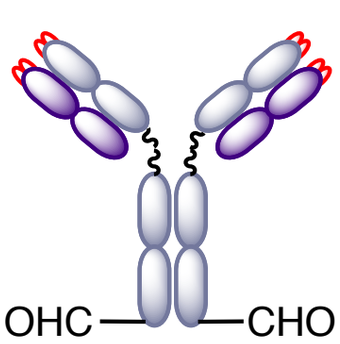
THIOMABs

engineered Cys at positions
with minimal interference
to antibody function



***C*-terminal selenocysteine**

engineered selenocysteines
(more nucleophilic)
selectively at *C*-terminus



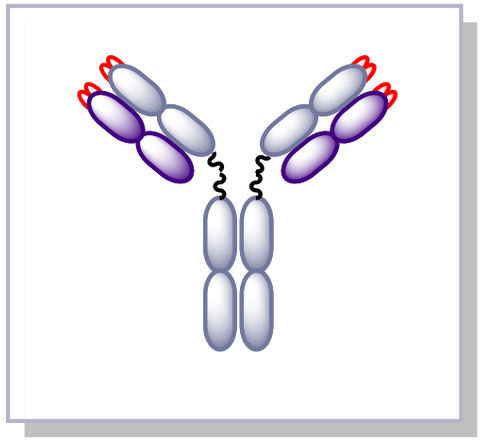
aldehyde tagging

engineered recognition
sequence for formylglycine
generating enzyme (FGE)

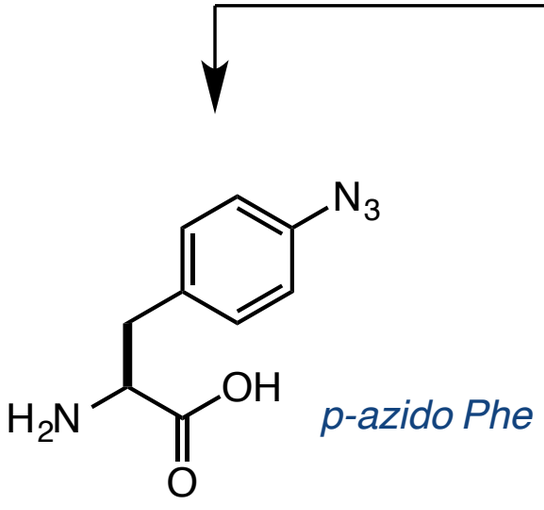
Garbaccio, R. M. Chemistry of Antibody-Small Molecule Drug Conjugates. From *Comprehensive Organic Synthesis II*, **2014**, *9*, 438.

Current Trends in Research in Antibody-Drug Conjugates

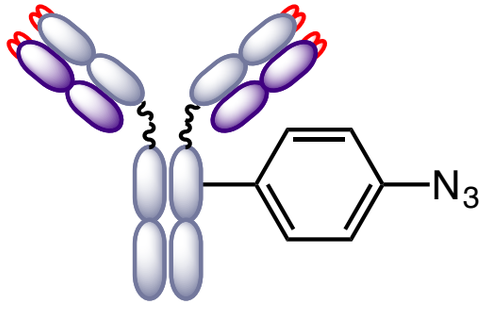
Methods of Homogeneous Conjugation Using Engineered Antibodies

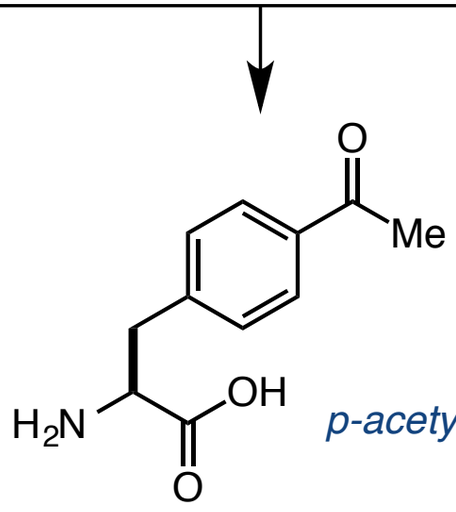


Protein Engineering to Incorporate Unnatural Amino Acids

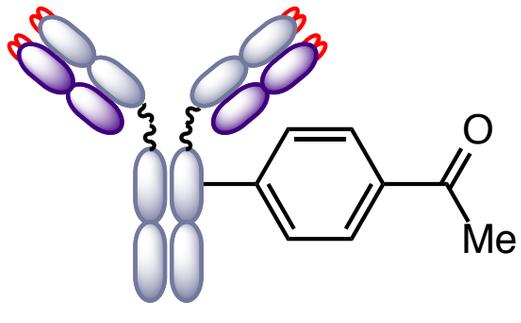


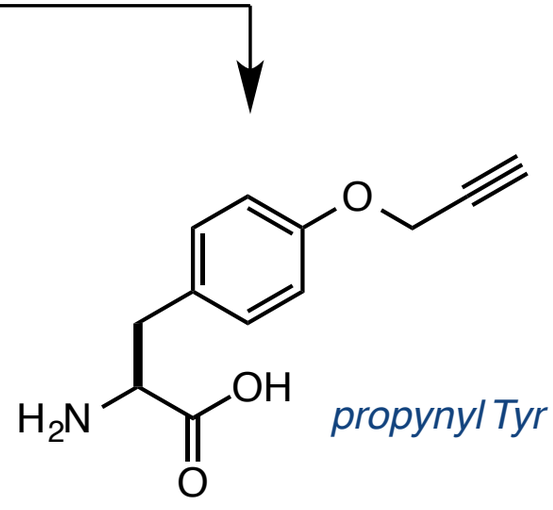
p-azido Phe



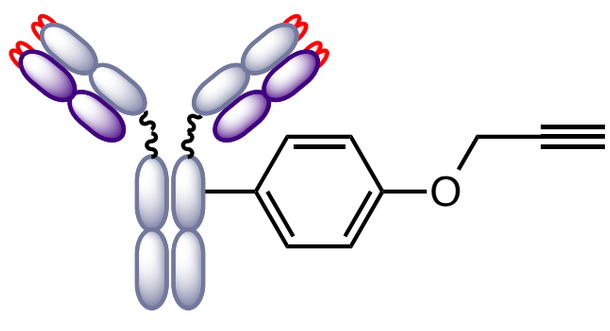


p-acetyl Phe





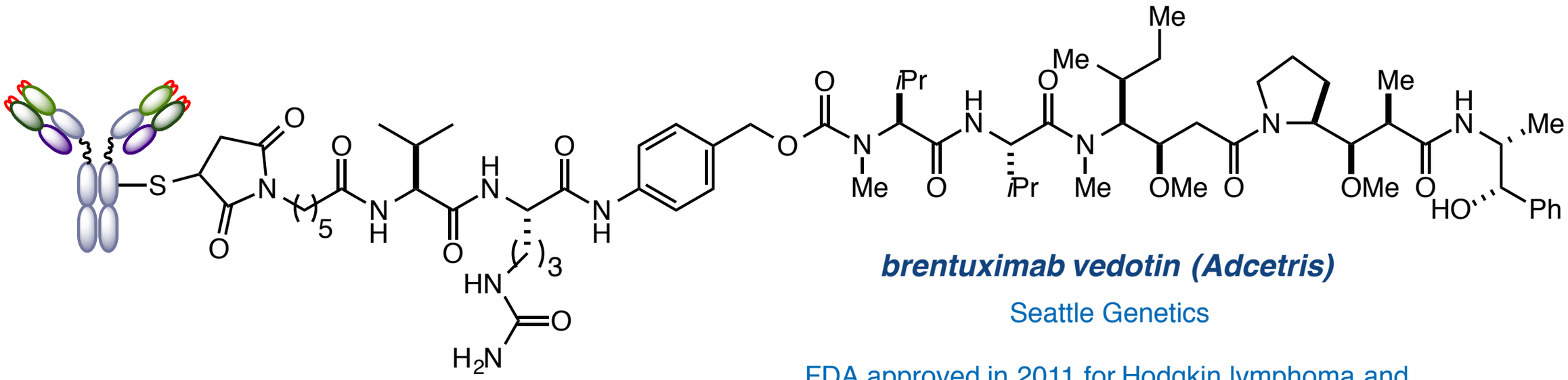
propynyl Tyr



Garbaccio, R. M. Chemistry of Antibody-Small Molecule Drug Conjugates. From *Comprehensive Organic Synthesis II*, **2014**, *9*, 438.

Clinically Successful Antibody-Drug Conjugates

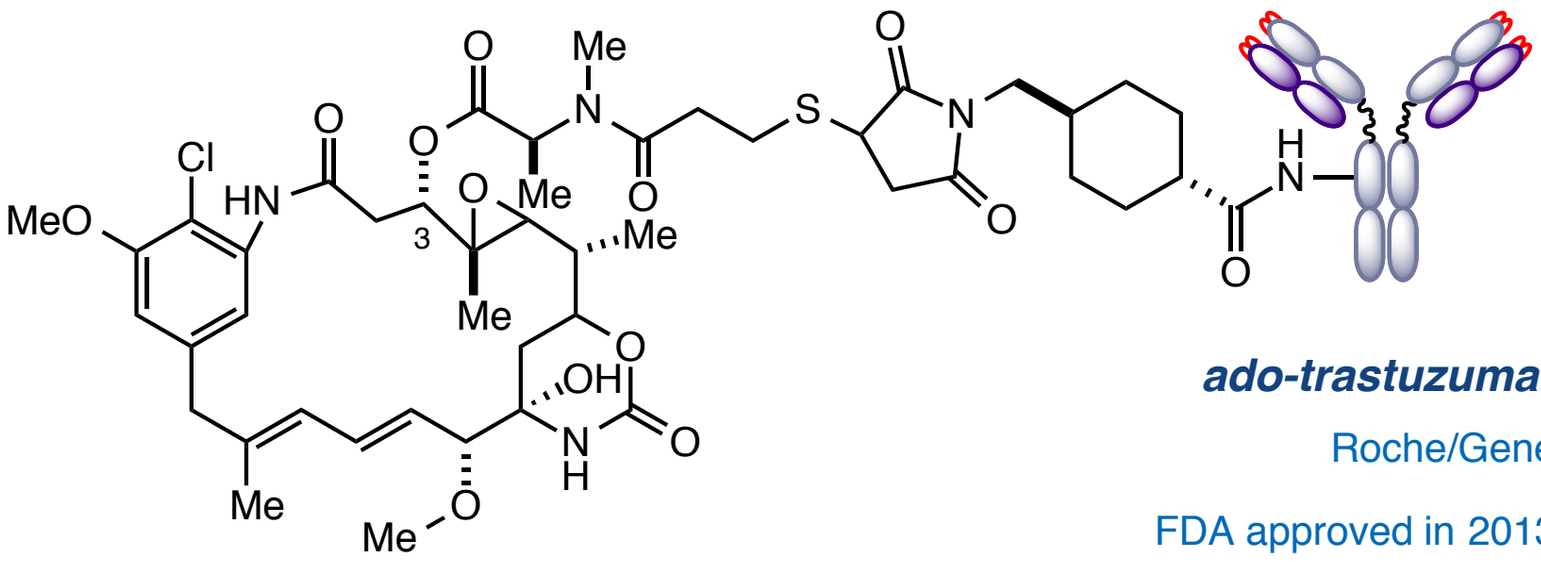
FDA Approved Antibody-Drug Conjugates



brentuximab vedotin (Adcetris)

Seattle Genetics

FDA approved in 2011 for Hodgkin lymphoma and anaplastic large cell lymphoma (ALCL)



ado-trastuzumab emtansine (Kadycla)

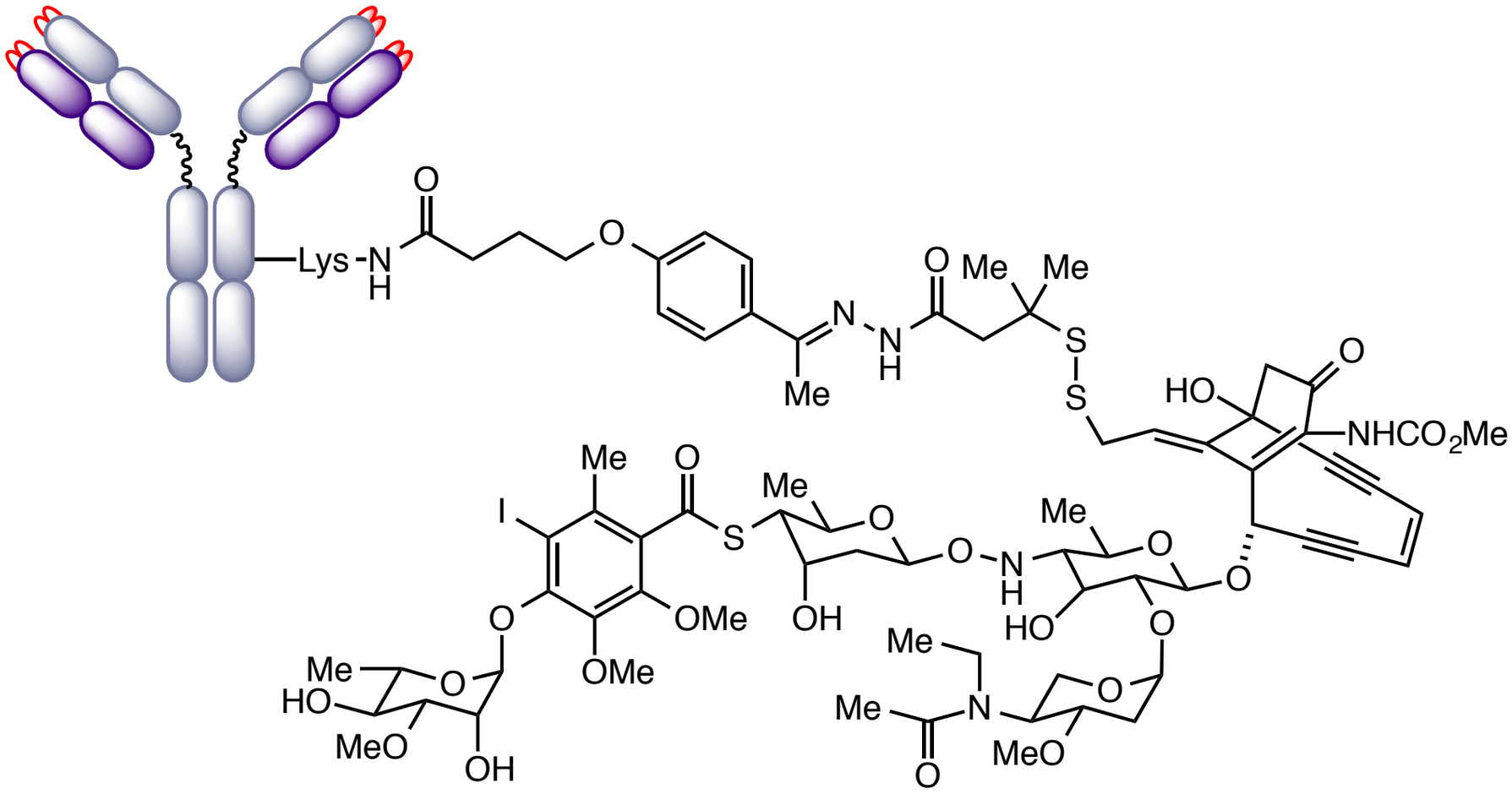
Roche/Genentech/ImmunoGen

FDA approved in 2013 for metastatic breast cancer

Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Clinically Successful Antibody-Drug Conjugates

FDA Approved Antibody-Drug Conjugates



Gemtuzumab ozogamicin (Mylotarg)

Wyeth/Pfizer

FDA approved in 2000 for acute lymphoblastic lukemia

Withdrawn in 2010 due to toxicity concerns
and lack of improvement in patient survival time

Angew. Chem. Int. Ed., **2014**, *53*, 3796.

Clinically Successful Antibody-Drug Conjugates

Antibody-Drug Conjugates Currently in Clinical Evaluations

Candidate	Drug	Antigen	Lead Indicator	Developer/Partner
Phase III				
Inotuzumab ozogamicin (CMC-544)	Calicheamicin	CD22	ALL	Pfizer
Gemtuzumab ozogamicin (CMA-676)	Calicheamicin	CD33	AML	Pfizer
Phase II				
SAR3419	DM4	CD19	B-Cell malignancies	Sanofi/ImmunoGen
RG7593	MMAE	CD22	B-Cell malignancies	Roche/Genentech/Seattle Genetics
RG7596	MMAE	CD79b	B-Cell malignancies	Roche/Genentech/Seattle Genetics
Glembatumumab vedotin (CDX-011)	MMAE	GPNMB	Breast Cancer, Melanoma	Celldex Therapeutics/Seattle Genetics
PSMA-ADC	MMAE	PSMA	Prostate Cancer	Progenics Pharma/Seattle Genetics
Phase I				
Lorvotuzumab mertanisine	DM1	CD56	SCLC	ImmunoGen
IMGN529	DM1	CD37	B-Cell malignancies	ImmunoGen
IMGN853	DM4	FRα	Solid Tumors	ImmunoGen
IMGN289	DM1	EGFR	Solid Tumors	ImmunoGen
SAR566658	DM4	CA6	Solid Tumors	Sanofi/ImmunoGen
BT-062	DM4	CD138	Multiple Myeloma	Biotest/ImmunoGen
BAY 94-9343	DM4	mesothelin	Solid Tumors	Bayer/ImmunoGen
AMG 595	DM1	EGFRvIII	Gliomas	Amgen/ImmunoGen
AMG 172	DM1	CD27L	ccRCC	Amgen/ImmunoGen
SGN-CD19A	MMAF	CD19	NHL/ALL	Seattle Genetics
AGS-22ME	MMAE	Nectin 4	Solid Tumors	Astellas Pharma/Seattle Genetics
RG7450	MMAE	STEAP1	Prostate Cancer	Roche/Genentech/Seattle Genetics
RG7458	MMAE	MUC16	Ovarian Cancer	Roche/Genentech/Seattle Genetics
RG7599	MMAE	NaPi2b	NSCLC, Ovarian Cancer	Roche/Genentech/Seattle Genetics
MLN0264	MMAE	GCC	GI Malignancies	Takeda/Seattle Genetics
SGN-CD33A	PBD	CD33	AML	Seattle Genetics
MDX-1203	Duocarmycin	CD70	NHL, RCC	Bristol-Myers Squibb
Labetuzumab-SN-38	SN-38	CD66e	CRC	Immunomedics
IMMU-132	SN-38	Trop-2	Epithelial Cancers	Immunomedics
Milatuzumab Doxorubicin	Doxorubicin	CD74	Multiple Myeloma	Immunomedics
RG7598, RG7600, RG7636	Undisclosed	Undisclosed	Various	Roche/Genentech/Seattle Genetics

Angew. Chem. Int. Ed., **2014**, *53*, 3796.