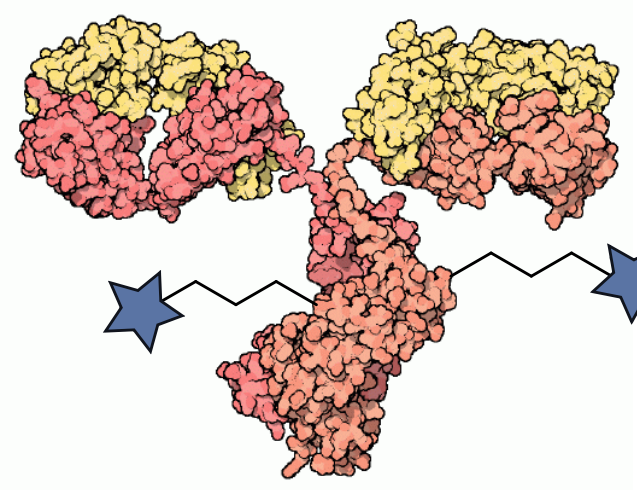
Antibody-Drug Conjugates

Design, Development, and FDA Approval of a New Drug Class



Elaine Tsui
Knowles Group
Department of Chemistry, Princeton University
July 9, 2021

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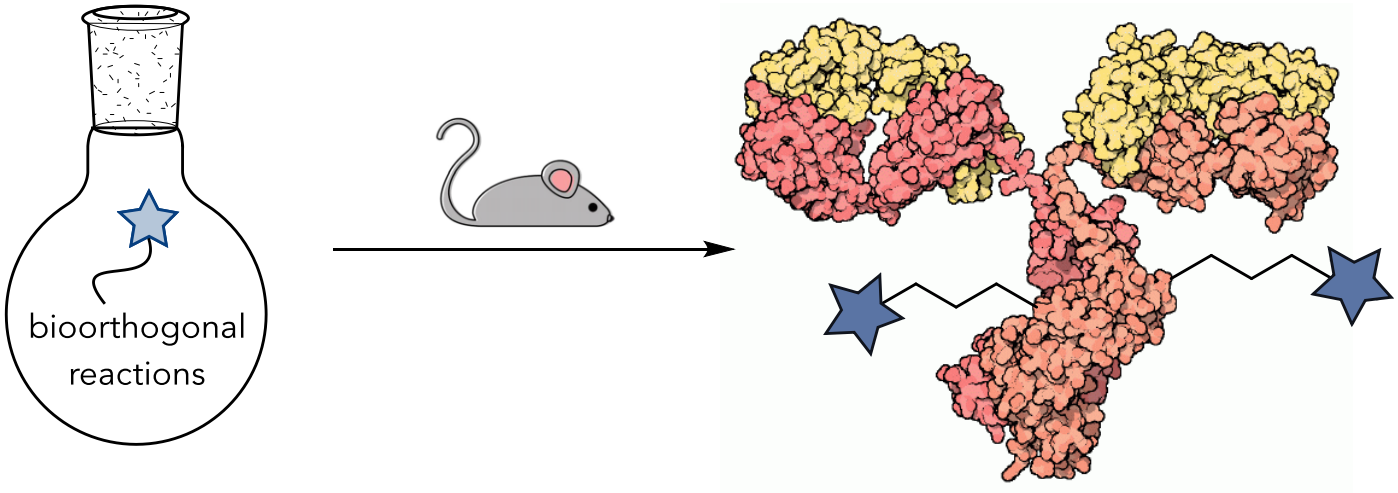

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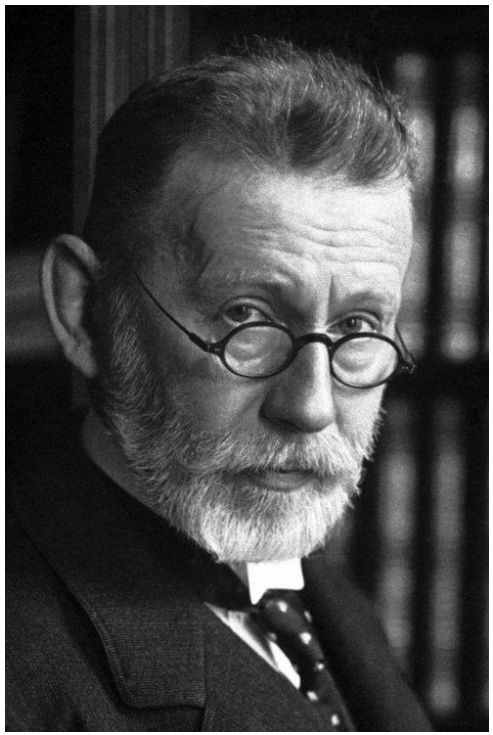
Ehrlich's Magic Bullet



Paul Ehrlich
1908 Nobel Prize in
Physiology or Medicine

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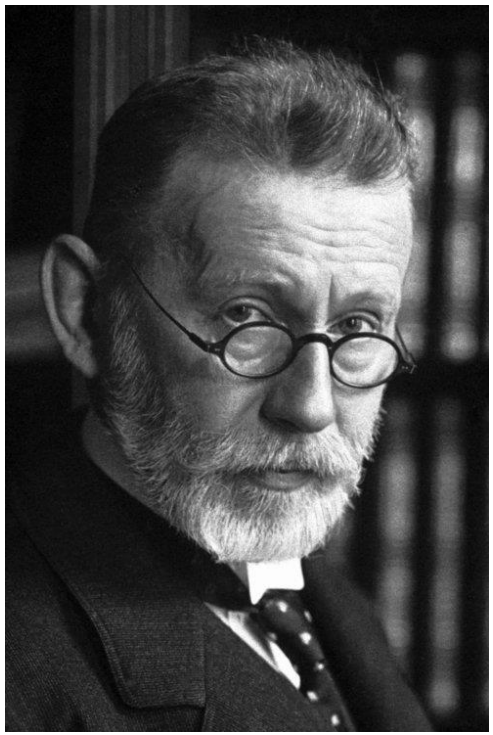

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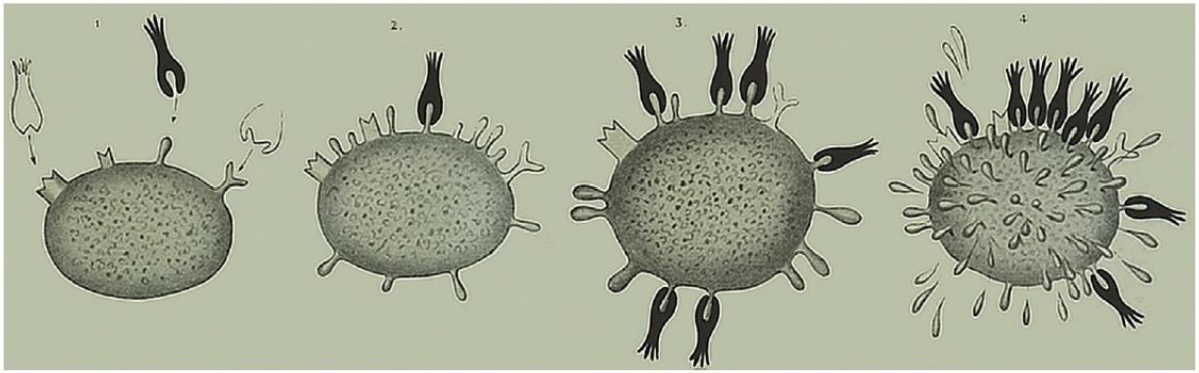

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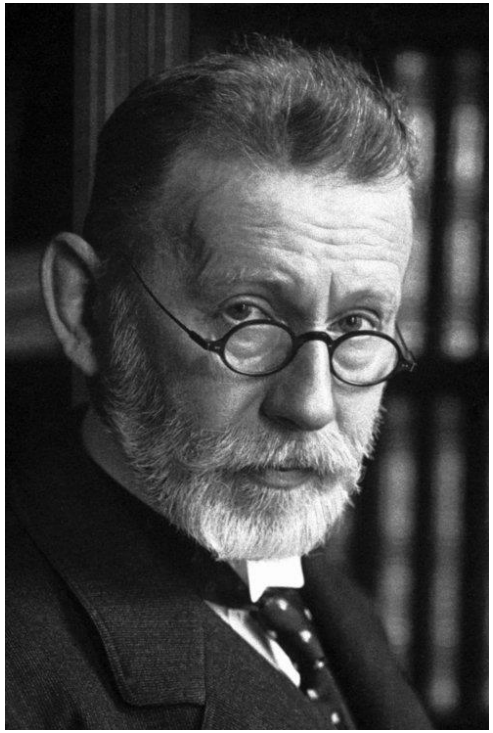

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1908 Nobel Prize in
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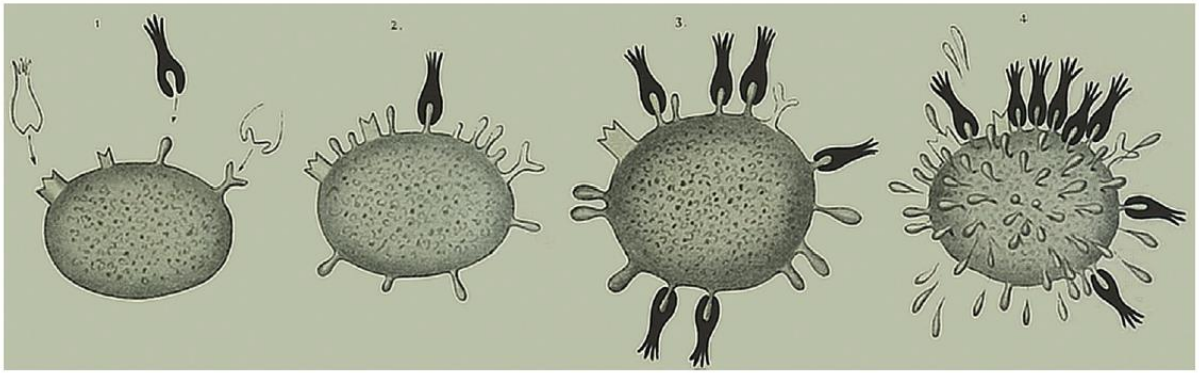
"Now, an essential task of the new Institute will be to find substances and chemical groups that have a special relationship to certain organs. It will be of particular importance, however, to equip such substances, acting as trucks so to speak, with chemical groups possessing pharmacological or toxicological effects, so that at the same time they convey the potent load commissioned to them to the appropriate places."



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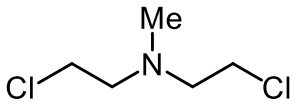
Evolution of Cancer Therapies

Development of Cancer Therapies

Nitrogen Mustards



chlormethine

for DNA alkylation

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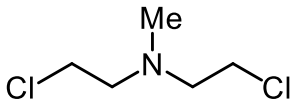
Evolution of Cancer Therapies

Development of Cancer Therapies

Nitrogen Mustards → Anti-Folates



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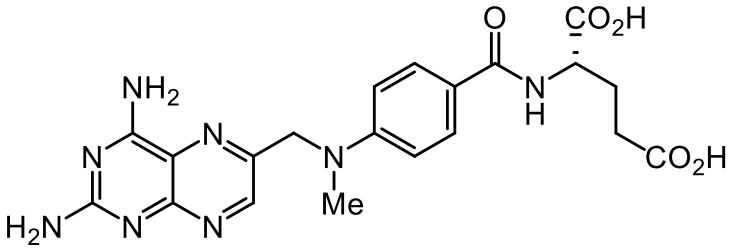

methotrexate

for blocking tumor growth

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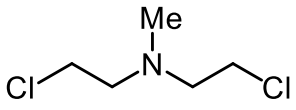
Evolution of Cancer Therapies

Development of Cancer Therapies

Nitrogen Mustards → Anti-Folates → Vinca Alkaloids



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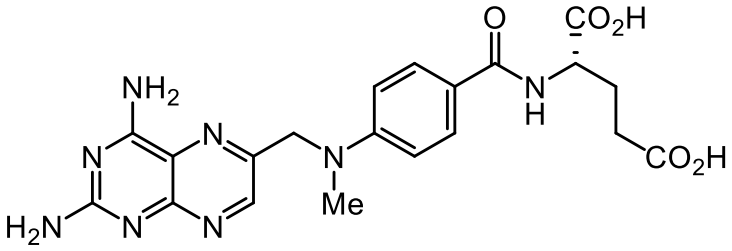

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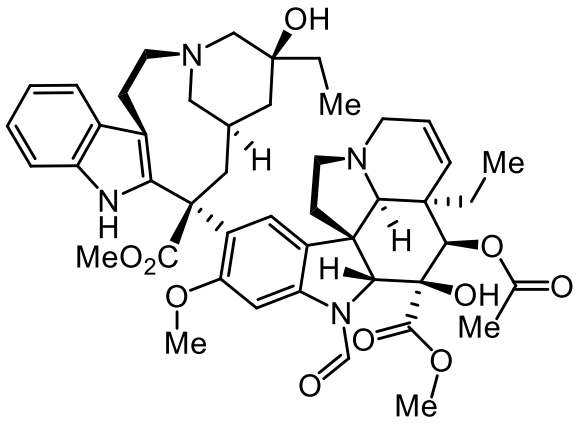

vincristine

for inhibiting tubulin polymerization

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Cancer Therapies

Development of Cancer Therapies

Chemotherapy

cytotoxic agents: cisplatin, nucleoside analogues, cyclophosphamide, Taxol, anthracyclines

lack of selectivity, toxicity to normal cells, limited efficacy

99% of tumor cells have to be killed to achieve remission

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angiogenesis inhibitors, signal transduction inhibitors, proteasome inhibitors (all agents targeting cancer cell machinery)

monoclonal antibodies: rituximab (Rituxan), trastuzumab (Herceptin)

low efficacy

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garnered significant attention but still in early stages of research and development

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Outline

I. What is an ADC?

II. History of the Development of ADCs

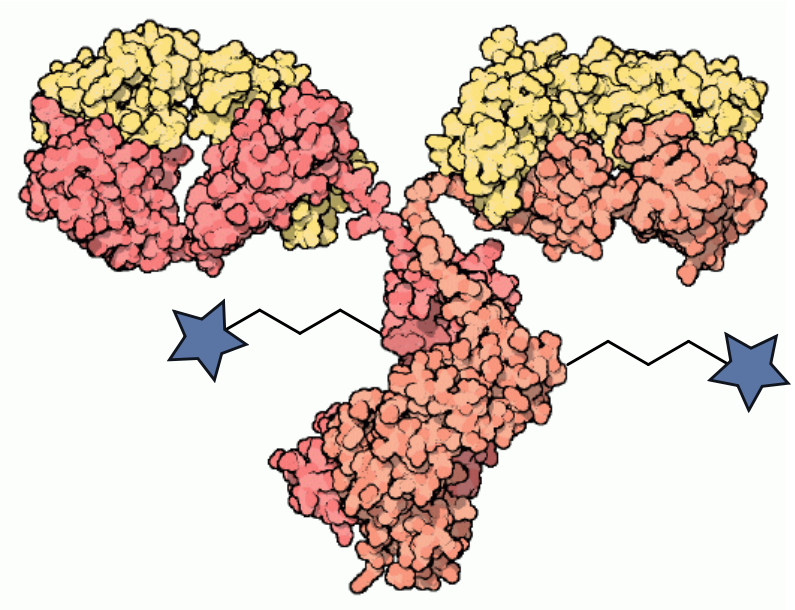
III. FDA-Approved ADCs

IV. Outlook and Conclusion

What is an ADC?

Antibody-Drug Conjugate (ADC)

- cytotoxic agent + monoclonal antibody



1) Binds selectively to cancer cell antigen

2) Internalizes through endocytosis

3) Releases payload/warhead/drug

4) Kills cancer cell

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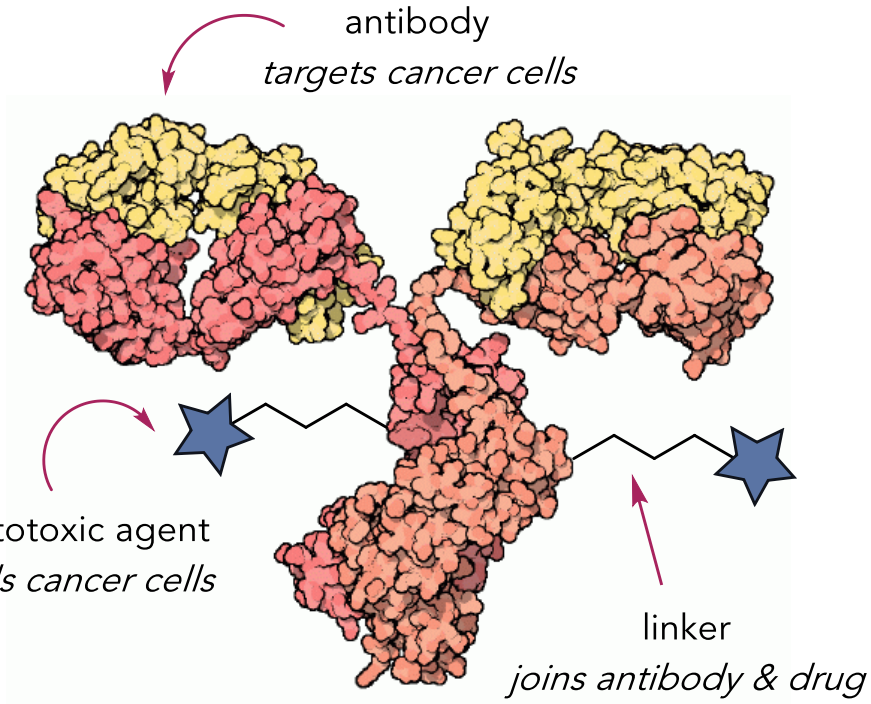

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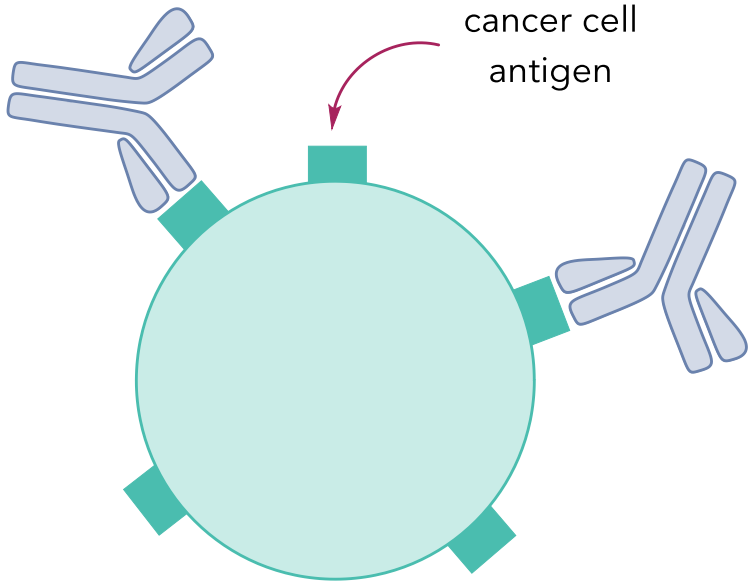
ADC Optimization

Antigen Selection

- highly and selectively expressed on the surface of tumor cells with minimal expression in normal cells
- internalizing antigen that can transport drug into cell



Beck, A.; Goetsch, L.; Dumontet, C.; Corvaia, N. *Nat. Rev. Drug Discov.* **2017**, *16*, 315–337.

ADC Optimization

Monoclonal Antibody (mAb)

- an antibody that targets a specific antigen
- relies on hybridoma technology developed by Kohler and Milstein (1975) for mass production
- considered a key breakthrough
- mAbs themselves do not need to exhibit functional activity in an ADC

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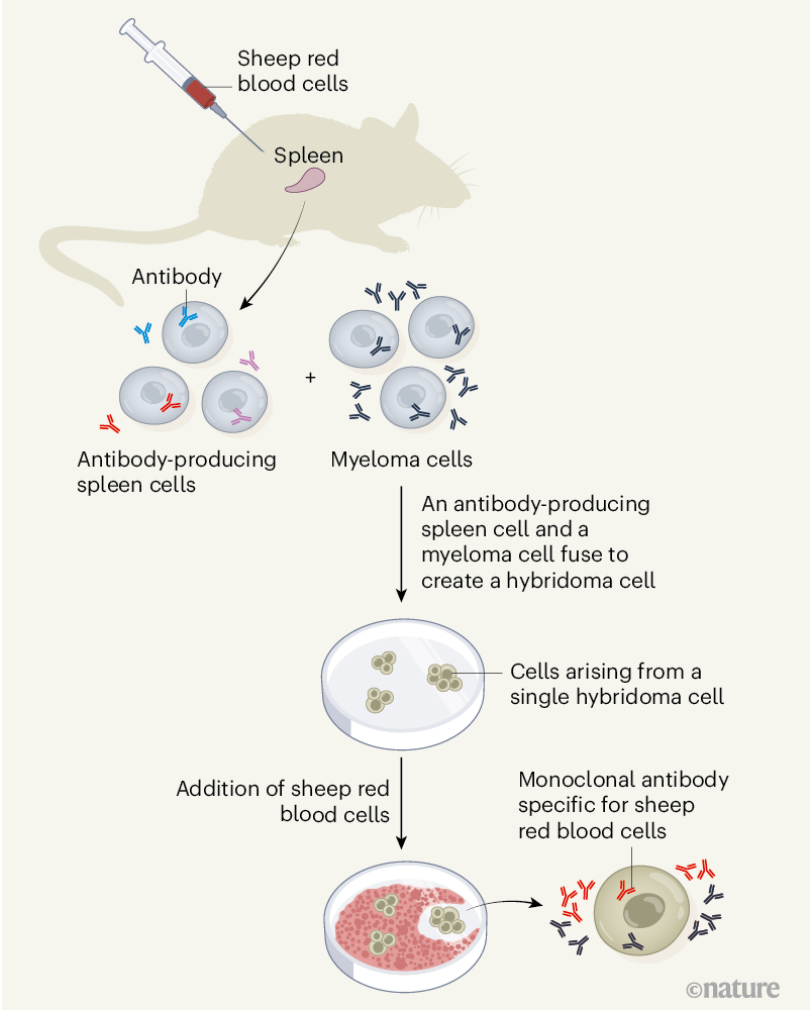

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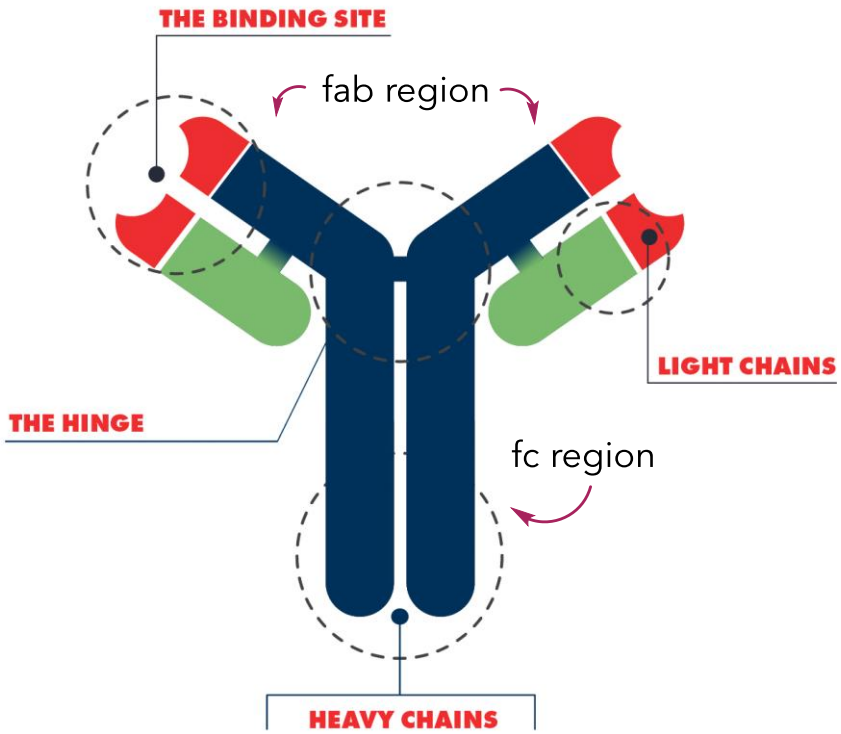

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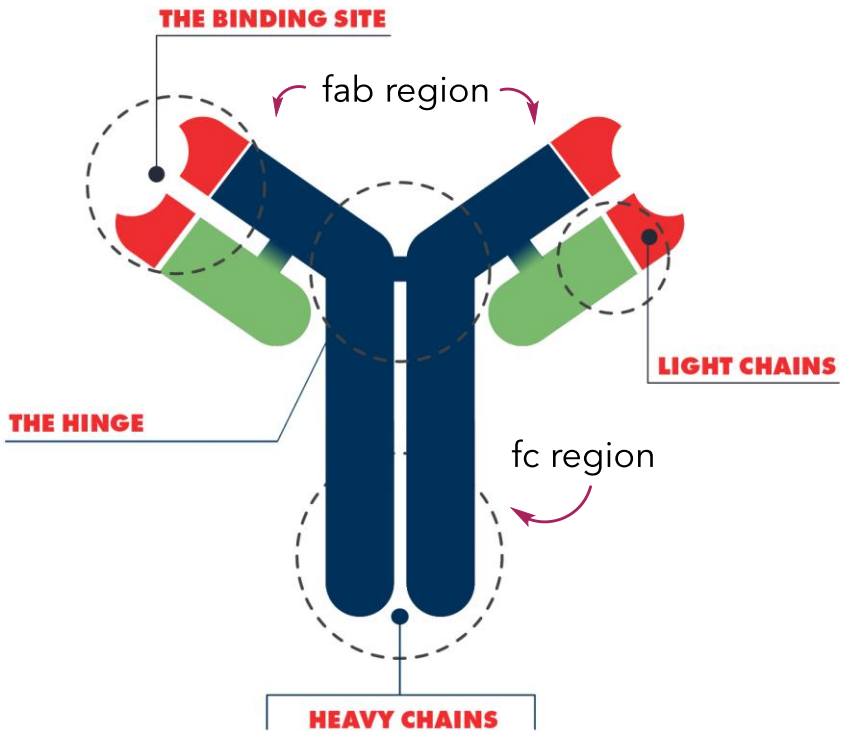
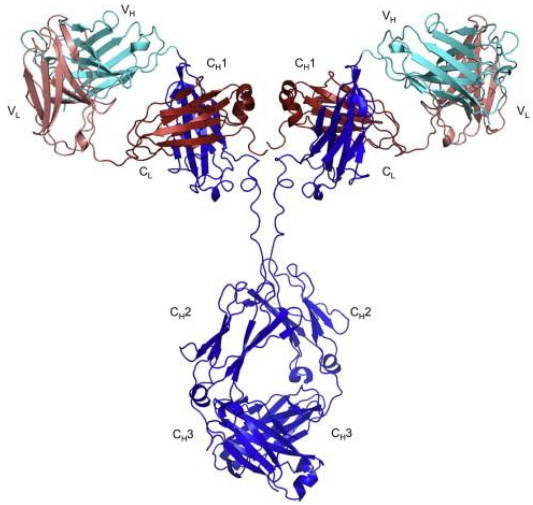


- all ADCs use immunoglobulin G (IgG) antibodies
- different isotypes exist based on heavy chain amino acid sequences
- isotypes determine clearance rates, immune activation, number of disulfide bonds available for modification



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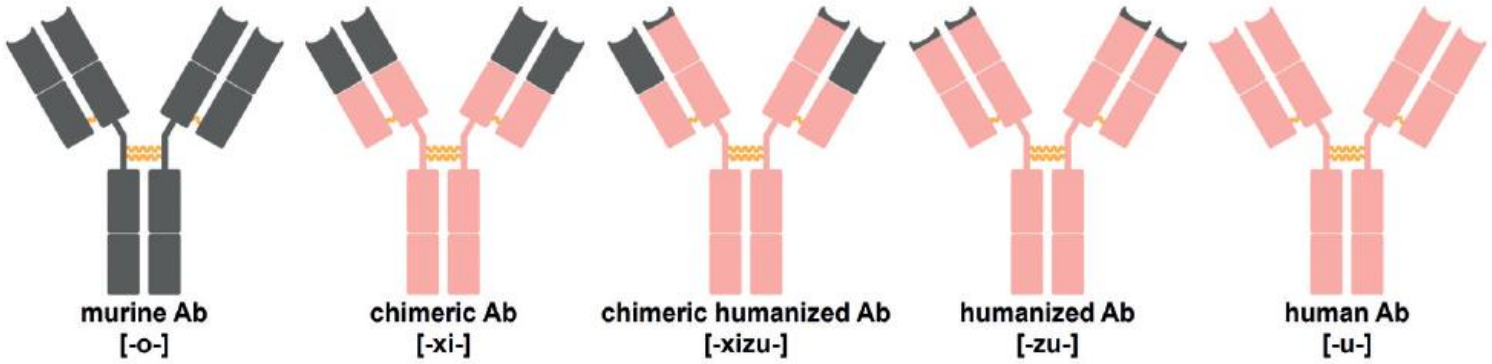
ADC Optimization

Monoclonal Antibody (mAb)



- interspecies usage of antibodies provoke harmful immunogenic responses
- humanize antibodies by replacing non-human domains with protein sequences occurring naturally in humans
- advantage: eliminate immune response and longer circulation half-life

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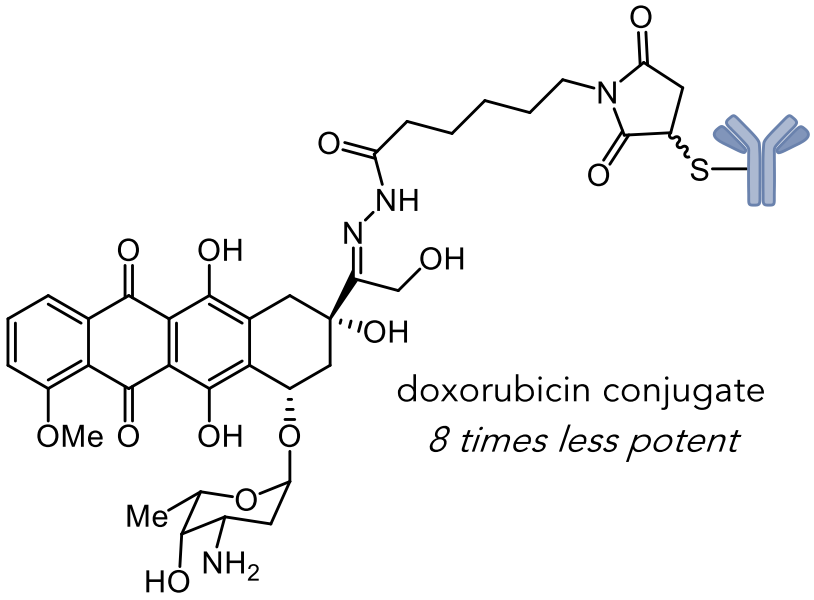
ADC Optimization

Cytotoxic Small Molecule



doxorubicin conjugate

8 times less potent

- picomolar potency required
- conjugated drug has decreased potency compared to free drug (e.g. why methotrexate and taxoids don't work)
- drug has to be stable and soluble in aqueous environment of antibody and has to avoid antibody aggregation. Can be easily modified to allow for conjugation

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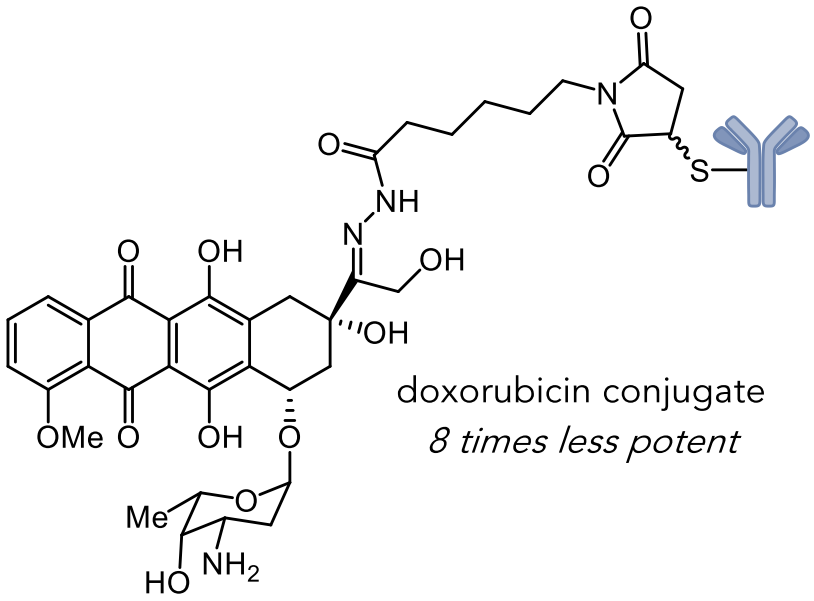

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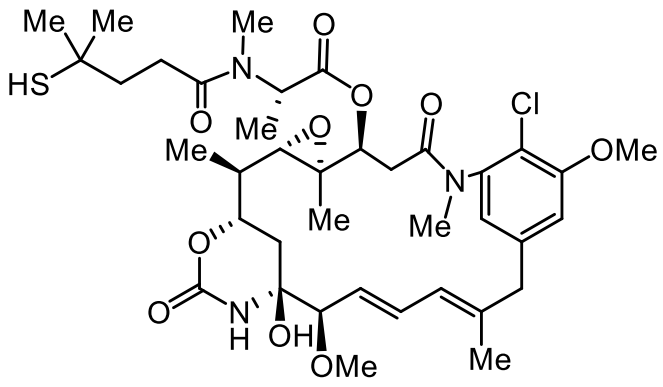

maytansine analogue

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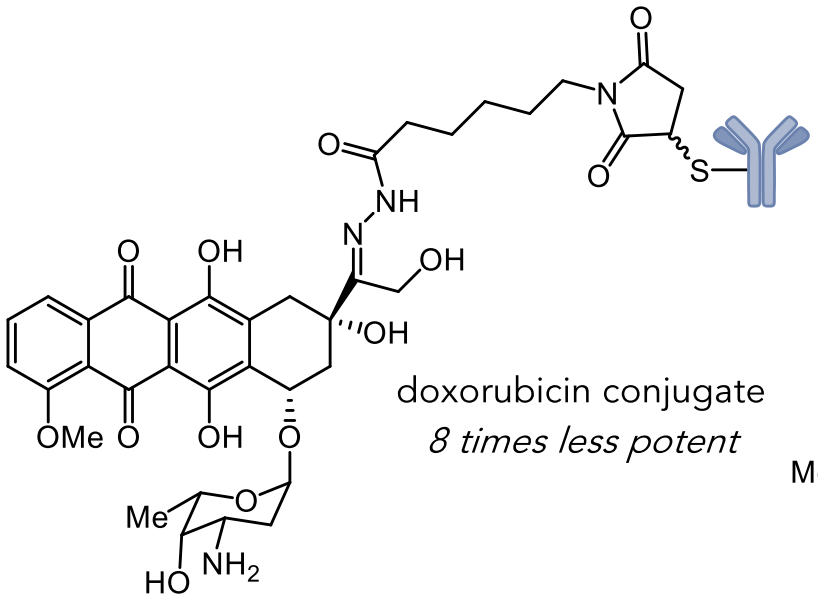

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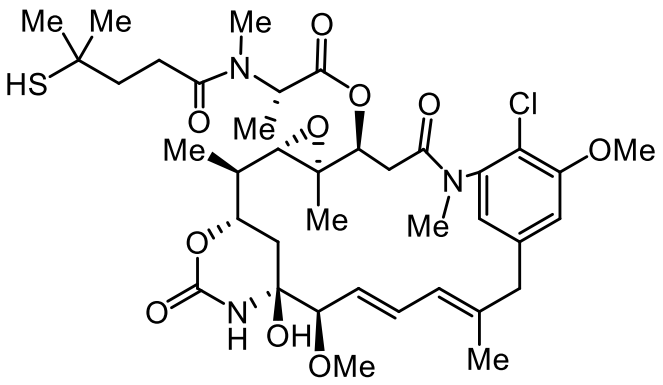

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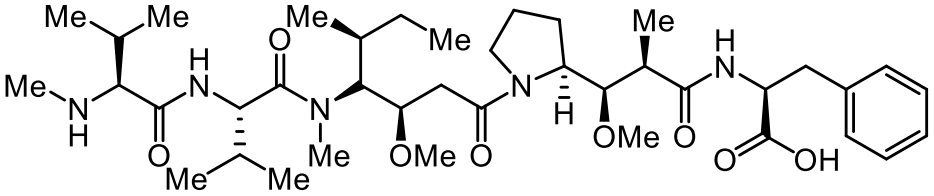

monomethyl auristatin F

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- drug has to be stable and soluble in aqueous environment of antibody and has to avoid antibody aggregation. Can be easily modified to allow for conjugation

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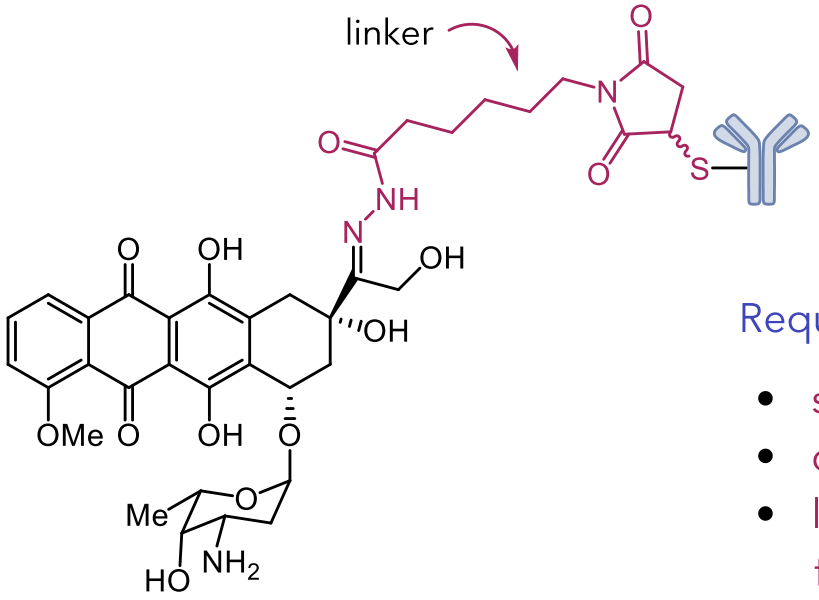
ADC Optimization

Design and Optimization of Linkers



Requirements:

- stable for several days in circulation
- cleaved upon internalization to release drug
- location of linker should not interfere with function of antibody
- solubilize hydrophobic drug
- drug-to-antibody ratio must be optimized for potency without compromising safety

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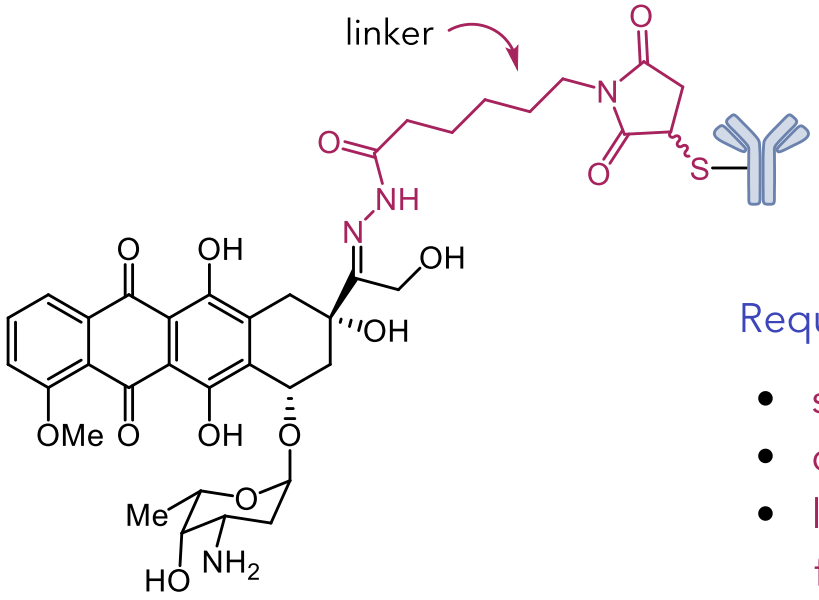

cleavage occurs *via* lysosomal proteases, disulfide reduction, hydrolysis under acidic conditions

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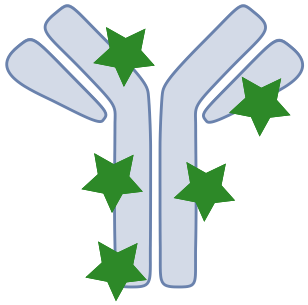
ADC Optimization

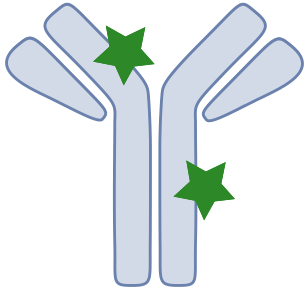
Drug-to-Antibody Ratio (DAR) Considerations



decreasing DAR

increasing DAR

most commonly 2-4



increased potency,
but protein aggregation,
increased ADC clearance,
toxicity

balance between
potency of drug and
level of efficacy and toxicity

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ADC Optimization

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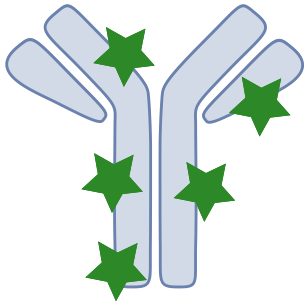

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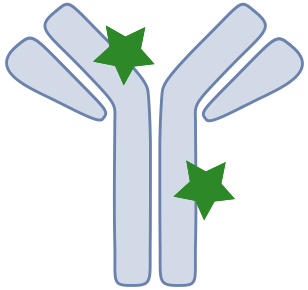
most commonly 2-4



increased potency,
but protein aggregation,
increased ADC clearance,
toxicity

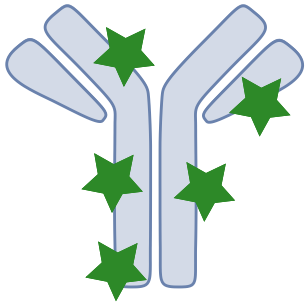
balance between
potency of drug and
level of efficacy and toxicity

heterogeneous DAR and drug attachment sites leads to unpredictable pharmacokinetic properties and lower efficacy

Spring, D. R. *et. al. Chem. Rev. Soc.* **2021**, *50*, 1305–1353.

ADC Optimization

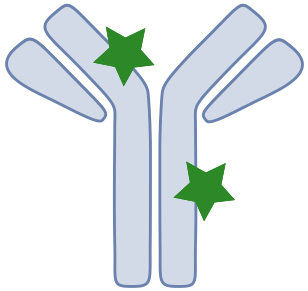
Drug-to-Antibody Ratio (DAR) Considerations



decreasing DAR

increasing DAR

most commonly 2-4



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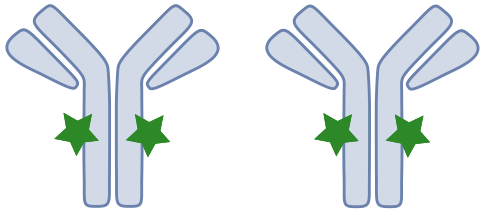


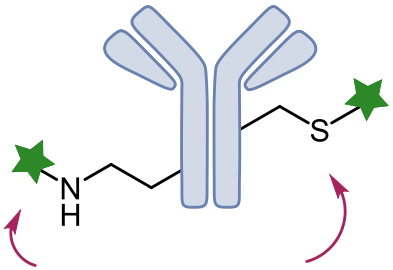
ideally, homogeneous
ADCs

Spring, D. R. *et. al. Chem. Rev. Soc.* **2021**, *50*, 1305–1353.

ADC Optimization

Bioconjugation Strategies

- Goal: to achieve site-selective protein modification and conjugation



often through hydrazone formation or addition to maleimide

Amino Acid Conjugation

natural or engineered residues

Agarwal, P.; Bertozzi, C. R. *Bioconjugate Chem.* 2015, *26*, 176–192.
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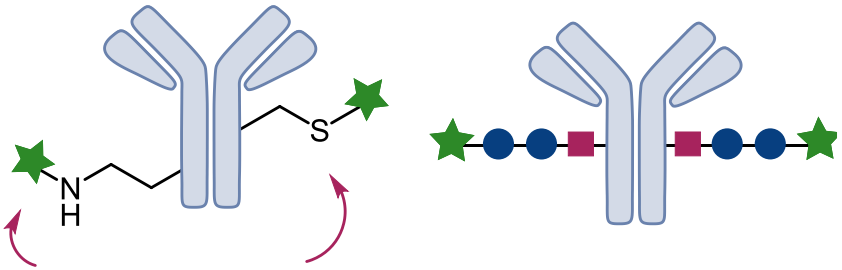

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Glycan Modification

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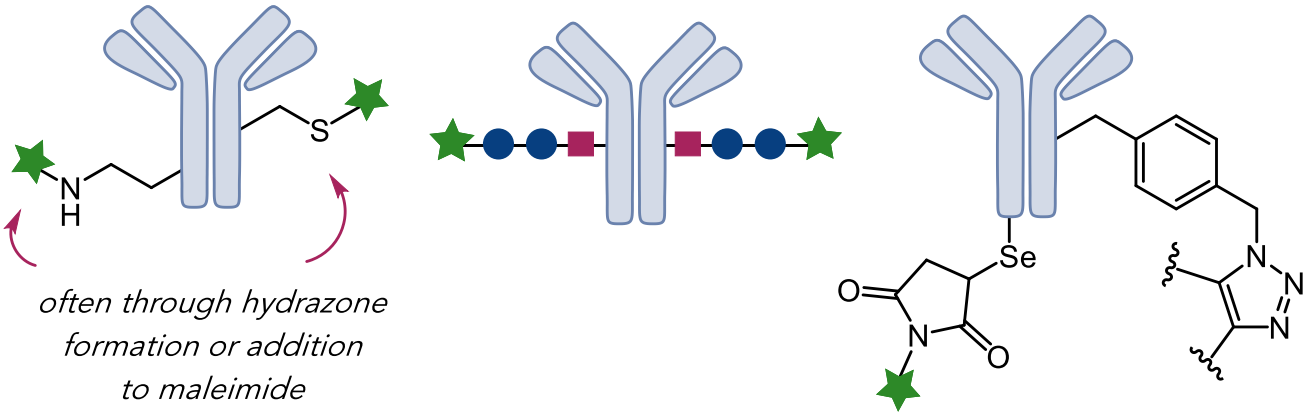

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Amino Acid Conjugation	Glycan Modification	Unnatural Amino Acid Incorporation

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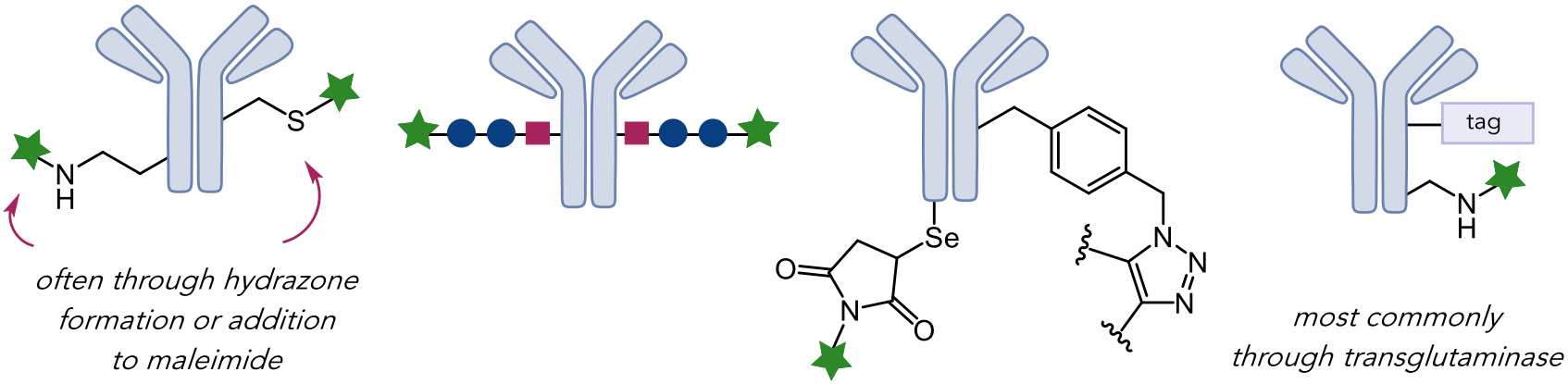

often through hydrazone formation or addition to maleimide

most commonly through transglutaminase

Amino Acid Conjugation	Glycan Modification	Unnatural Amino Acid Incorporation	Peptide Tags

natural or engineered residues

enzymatic modification of amino acids

Agarwal, P.; Bertozzi, C. R. *Bioconjugate Chem.* 2015, *26*, 176–192.
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Development of ADCs

Early Experimental ADCs

- grew out of a need to improve tumor selectivity
- first ADC reported by Mathe (1958)

Mathe, G.; Loc, T.; Bernard, J. *C. R. Hebd. Seances Acad. Sci.* **1958**, *246*, 1626–1628.
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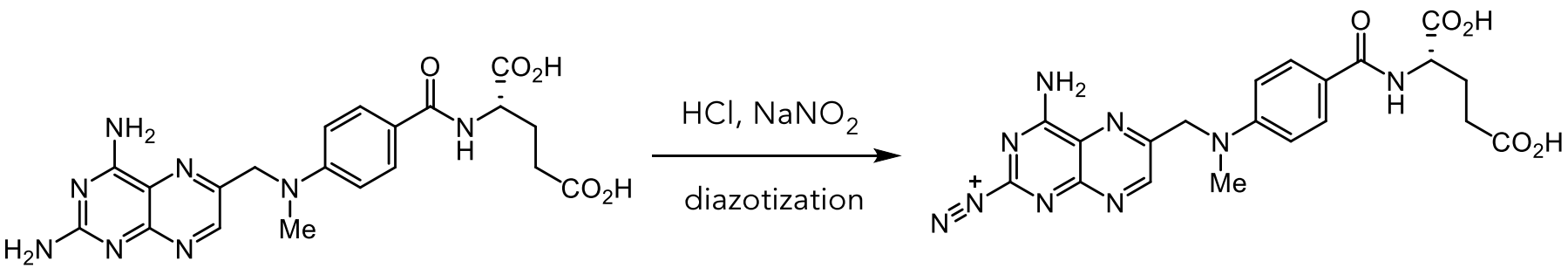

methotrexate

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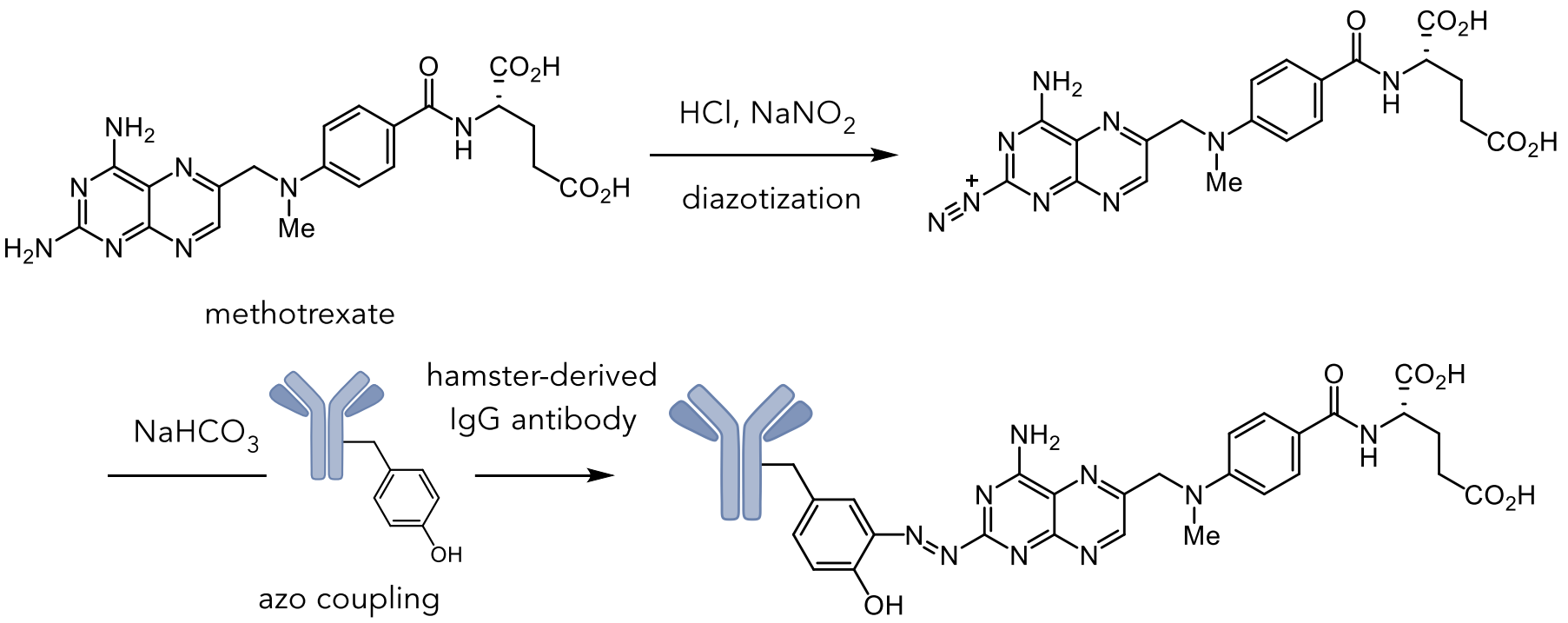

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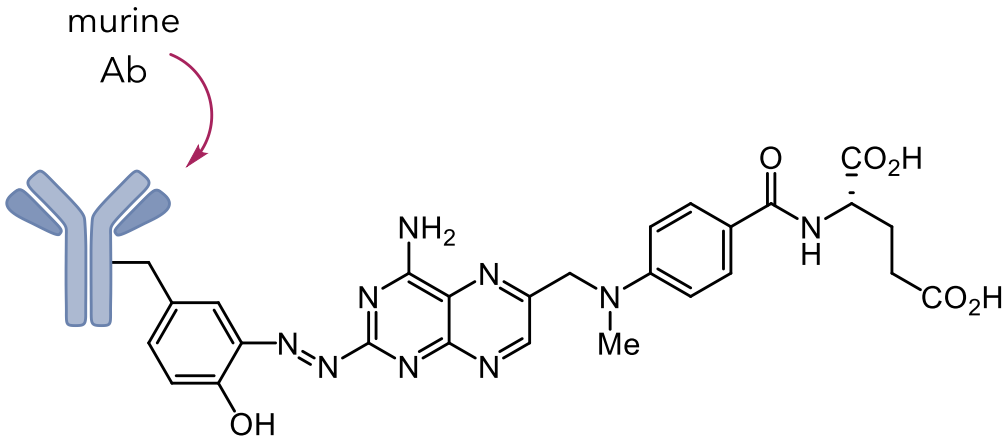

demonstrated superior activity against leukemia in mice compared to methotrexate or antibody alone

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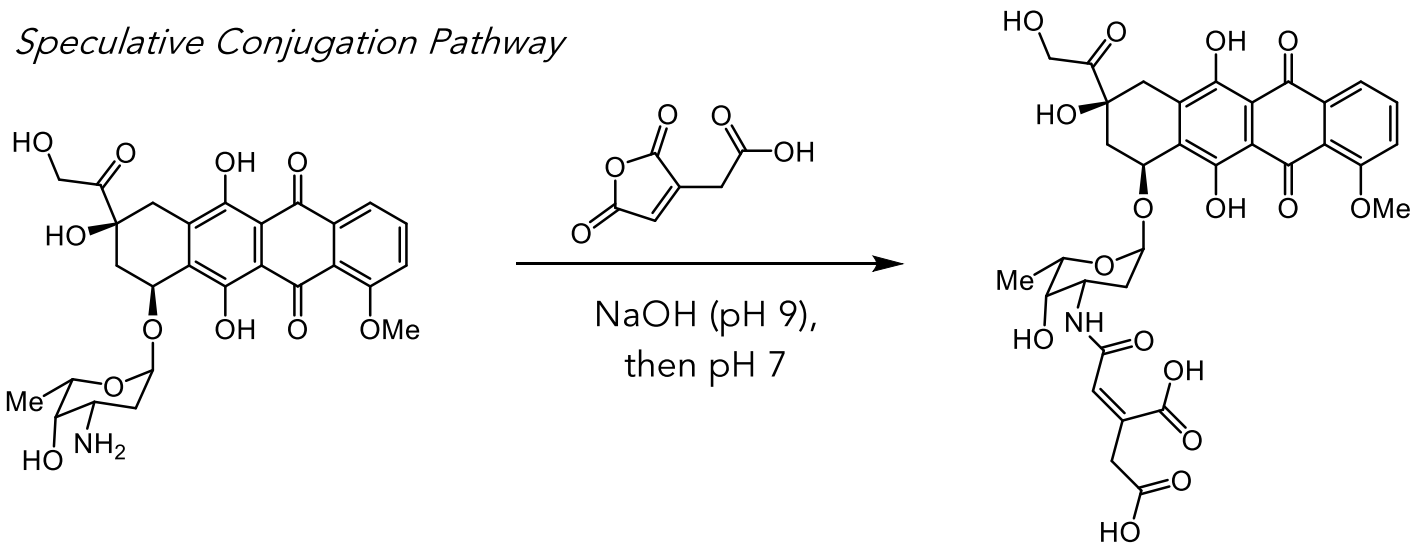
Early Experimental ADCs

Yang & Reisfeld (1988)

- first conjugation of intact doxorubicin

Speculative Conjugation Pathway



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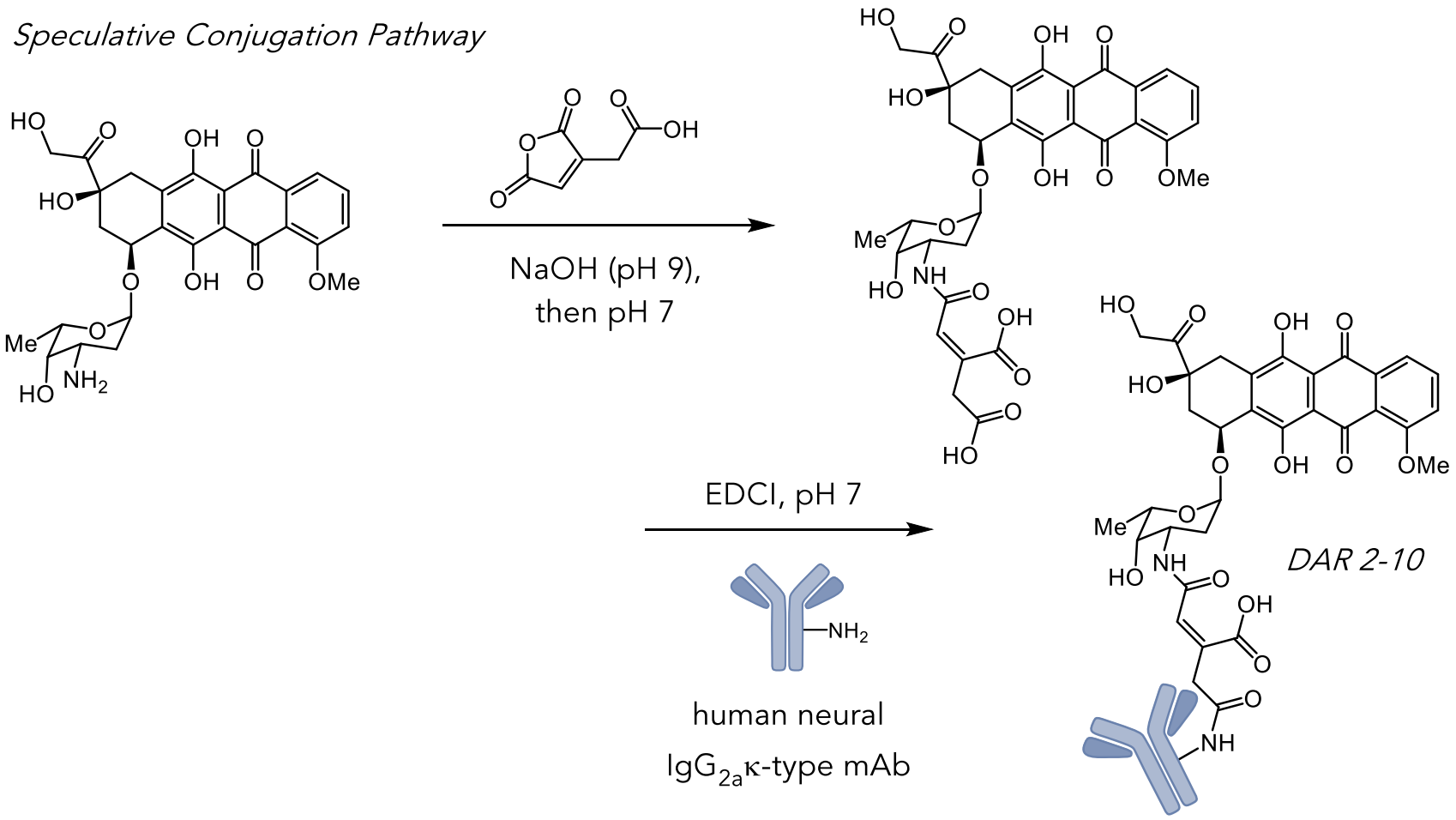

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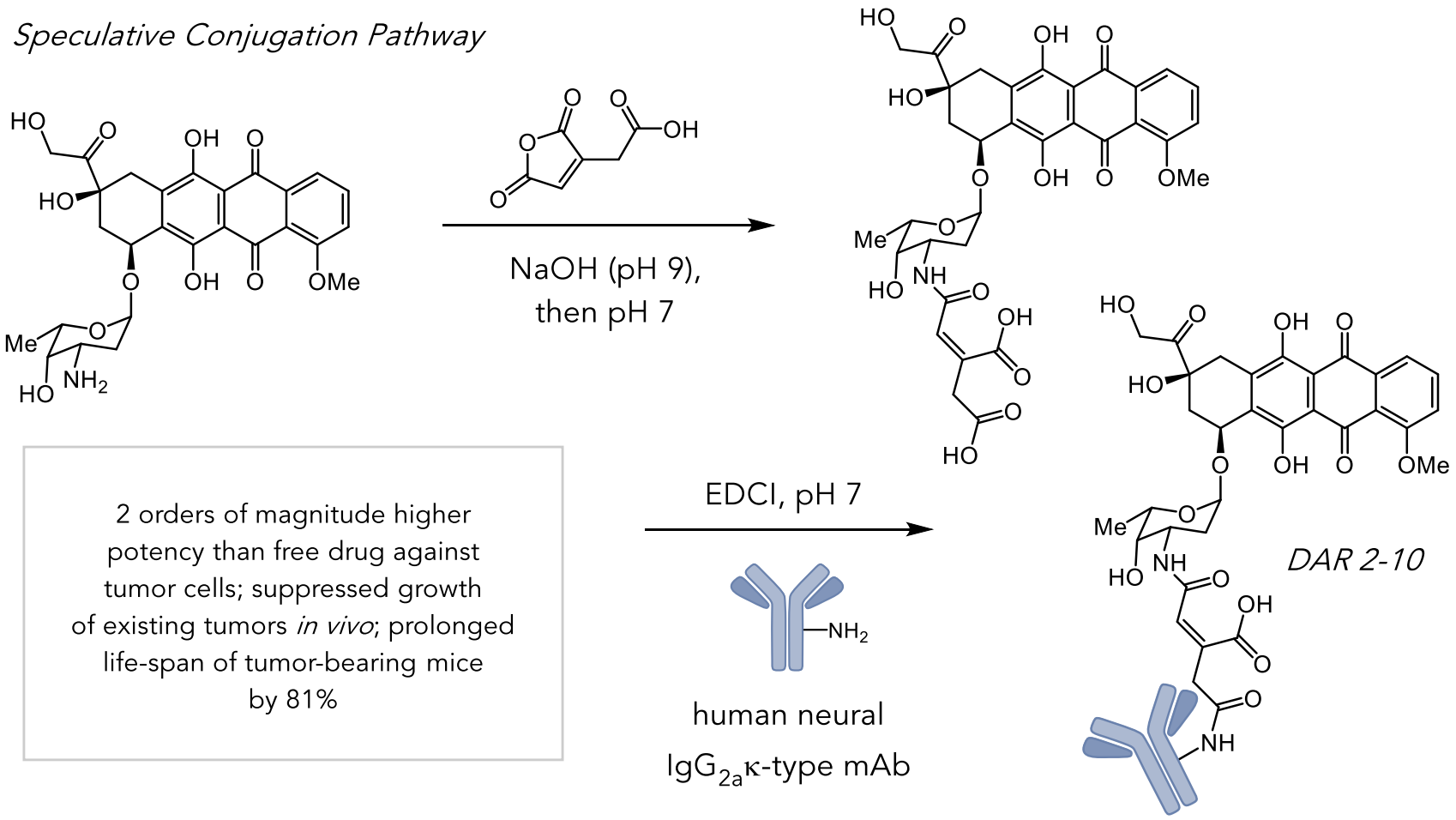

2 orders of magnitude higher potency than free drug against tumor cells; suppressed growth of existing tumors *in vivo*; prolonged life-span of tumor-bearing mice by 81%

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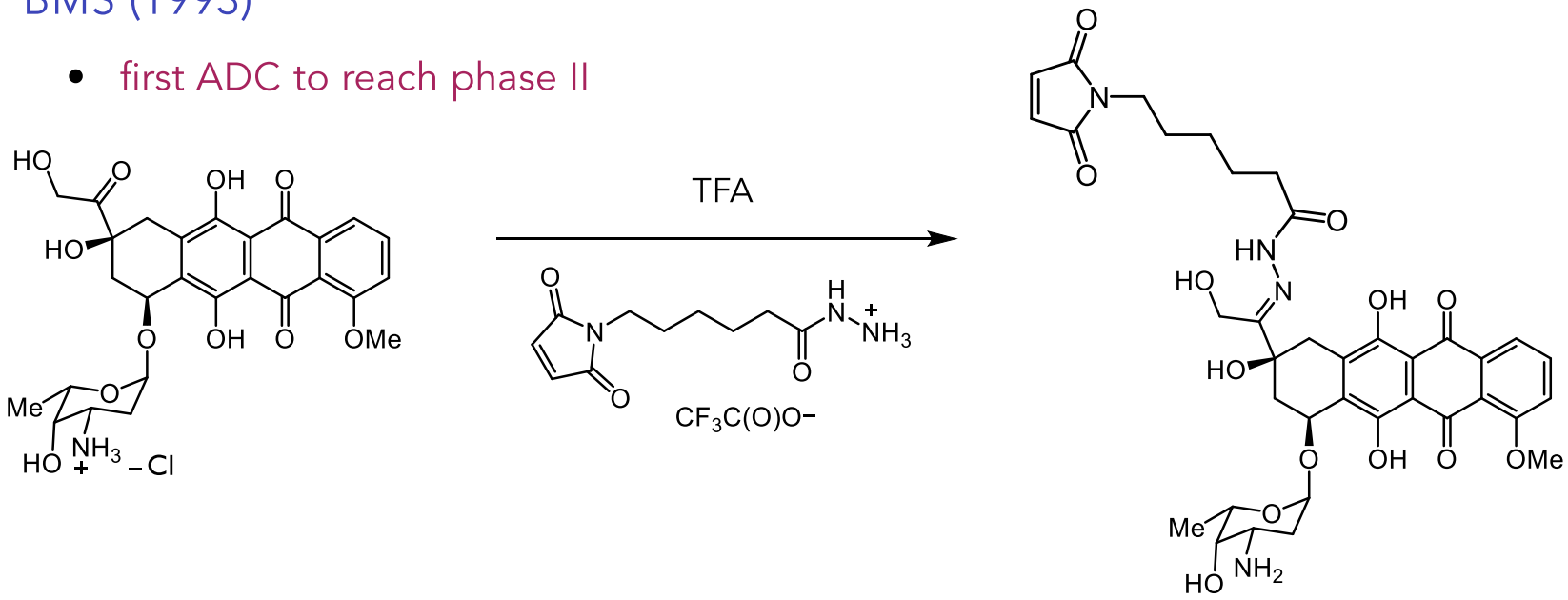
Early Experimental ADCs

BMS (1993)

- first ADC to reach phase II



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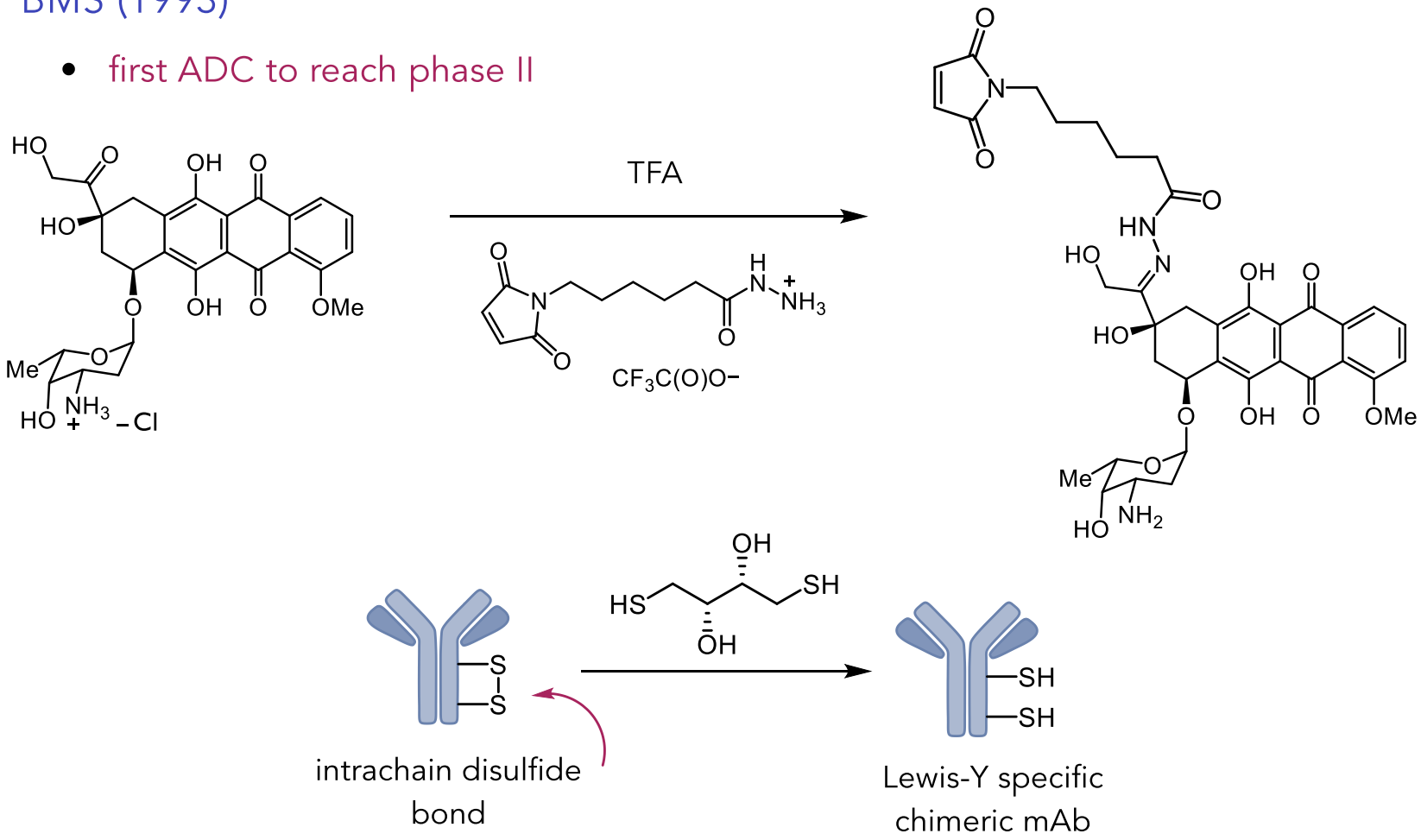

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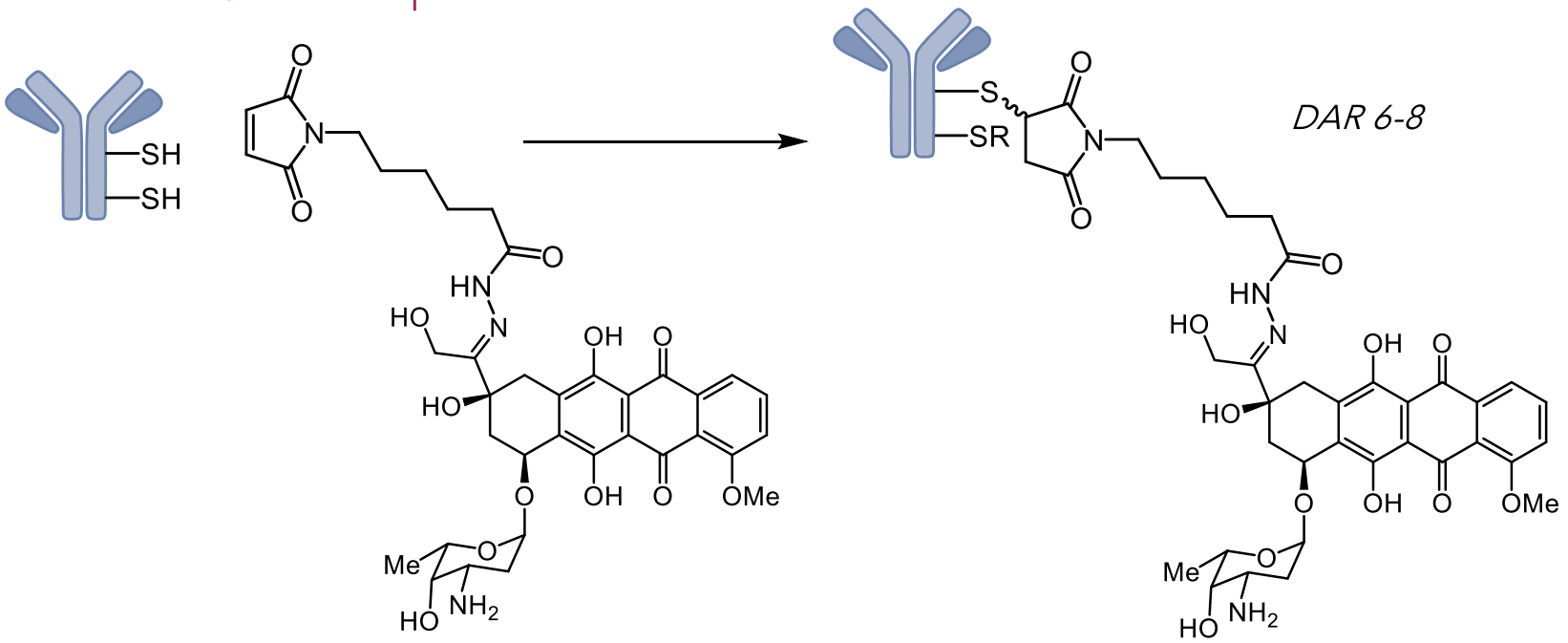

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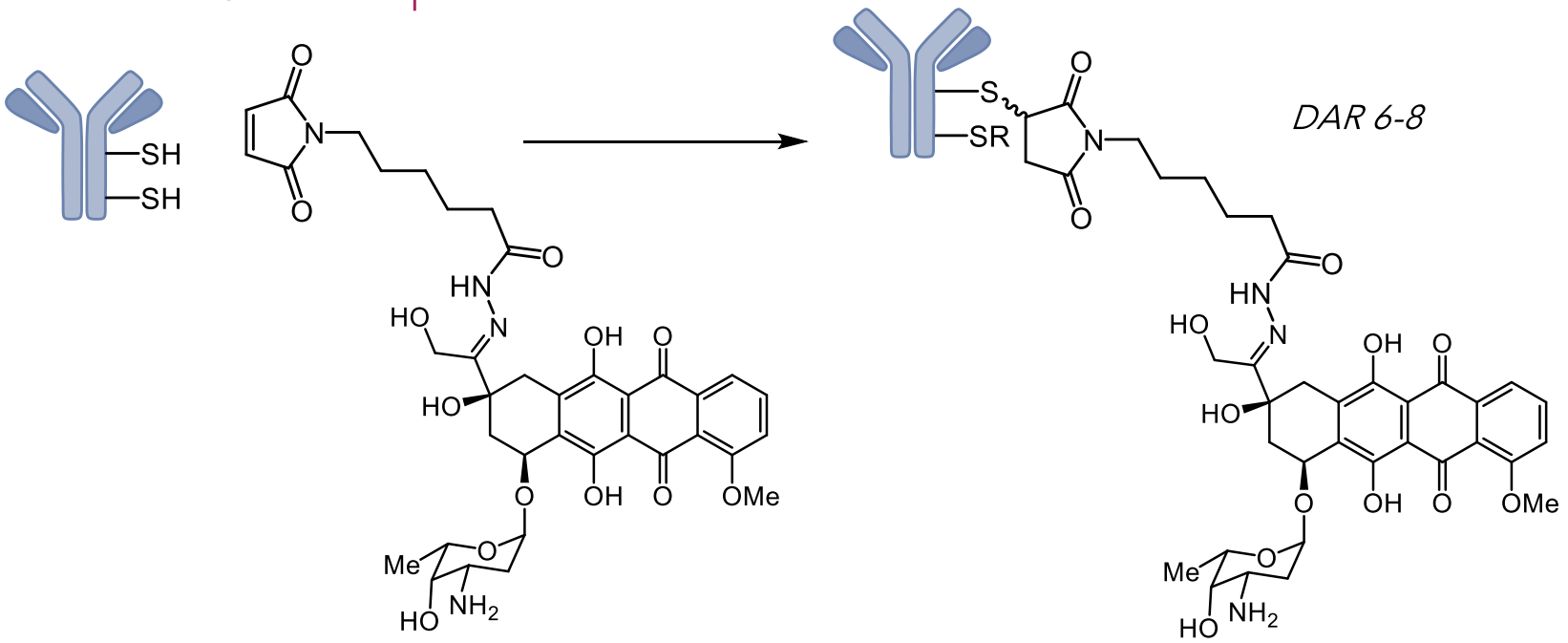


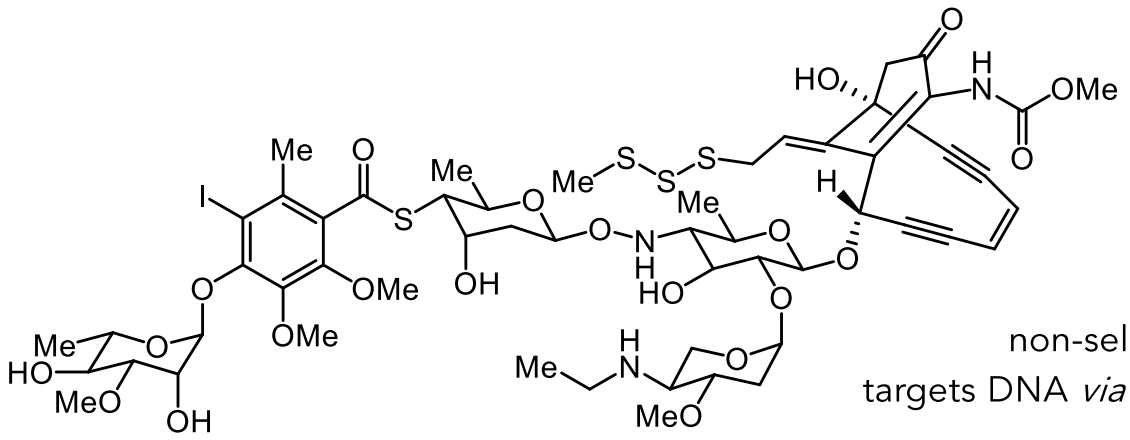
regression and cures of human lung, breast, and colon carcinomas in mouse model; cured 70% mice bearing metastases of human lung carcinoma and 94% of rats with subcutaneous human lung carcinoma

failed to gain FDA approval due to low efficacy in humans

Trail, P. A. *et al. Science* **1993**, *261*, 212–215.

Nicolaou, K. C.; Rigol, S. *Angew. Chem. Int. Ed.* **2019**, *58*, 11206–11241.

Introduction of Calicheamicin γ_1^I



non-selective
targets DNA *via* strand scission

discovered in 1987 from *Micromonospora chinospora calichensis*
first synthesis by Nicolaou in 1992
second synthesis by Danishefsky in 1994

component of first FDA approved ADC

Nicolaou, K.C. *et al. J. Am. Chem. Soc.* **1992**, *114*, 10082–10084.
Danishefsky, S. J. *Angew. Chem. Int. Ed.* **1994**, *33*, 858–862.
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FDA-Approved ADCs

Mylotarg

gemtuzumab ozogamicin
(Pfizer)
DNA cleavage
approved 2000
withdrew 2010

Adcetris

brentuximab vedotin
(Seattle Genetics)
microtubule inhibitor
approved 2011

Kadcyla

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Lumoxiti

moxetumomab pasudotox
(AstraZeneca)
elongation factor-2 inhibitor
approved 2018

Polivy

polatuzumab vedotin
(Genentech/Roche)
microtubule inhibitor
approved 2019

Padcev

enfortumab vedotin
(Astellas/Seattle Genetics)
microtubule inhibitor
approved 2019

Enhertu

trastuzumab deruxtecan
(AstraZeneca/Daiichi Sankyo)
TOPO1 inhibitor
approved 2019

Trodelvy

sacituzumab govitecan
(Immunomedics)
TOPO1 inhibitor
approved 2020

Blenrep

belantamab mafodotin
(GlaxoSmithKline)
microtubule inhibitor
approved 2020

Zynlonta

loncastuximab tesirine
(ADC Therapeutics)
DNA crosslinking
approved 2021

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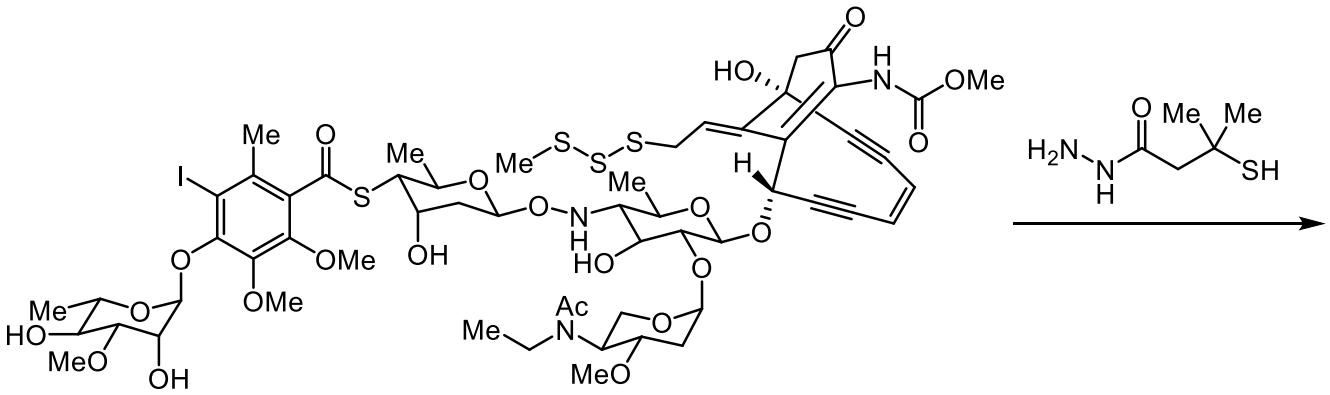
Mylotarg: gemtuzumab ozogamicin

First-Generation ADC



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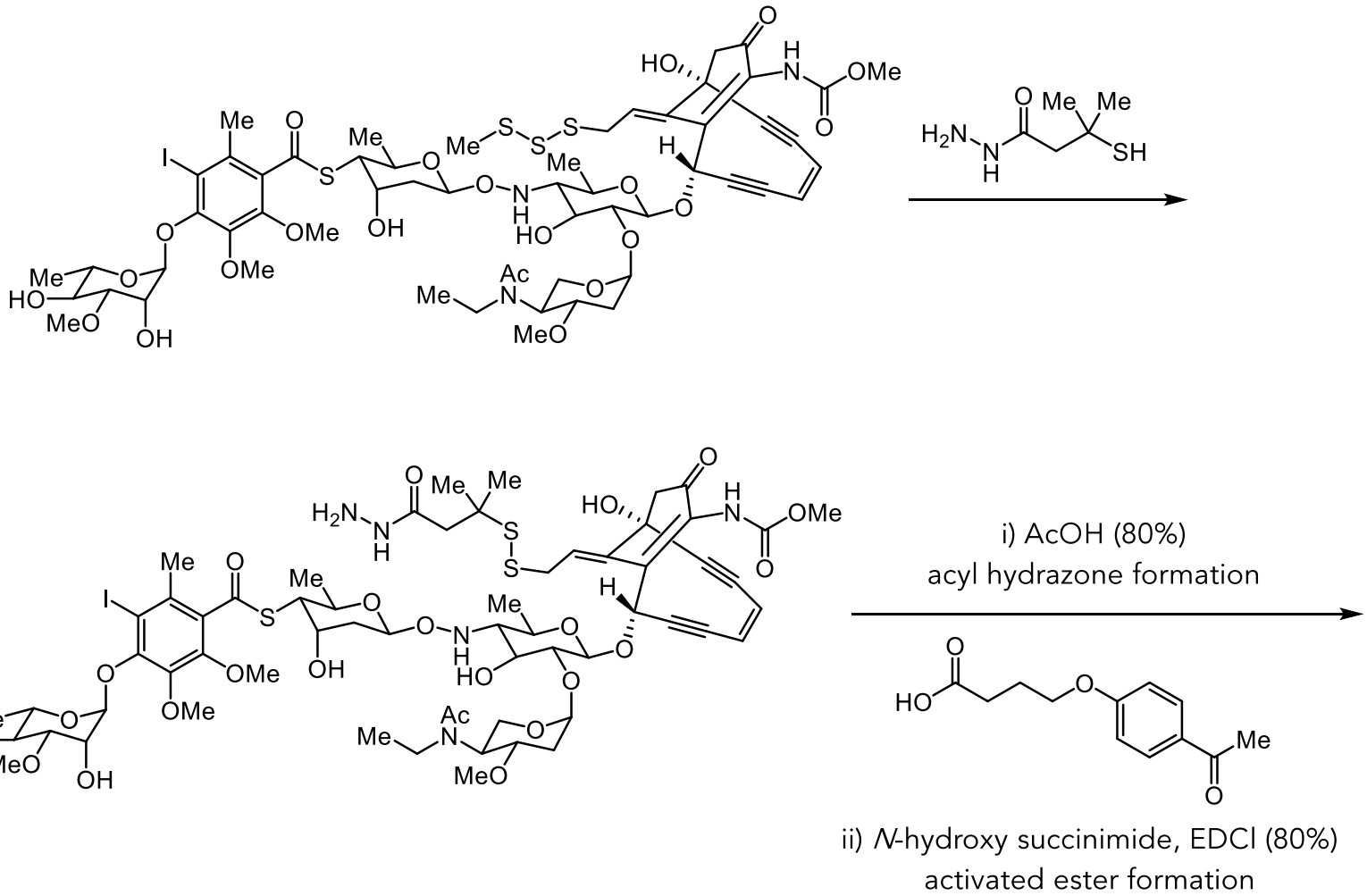

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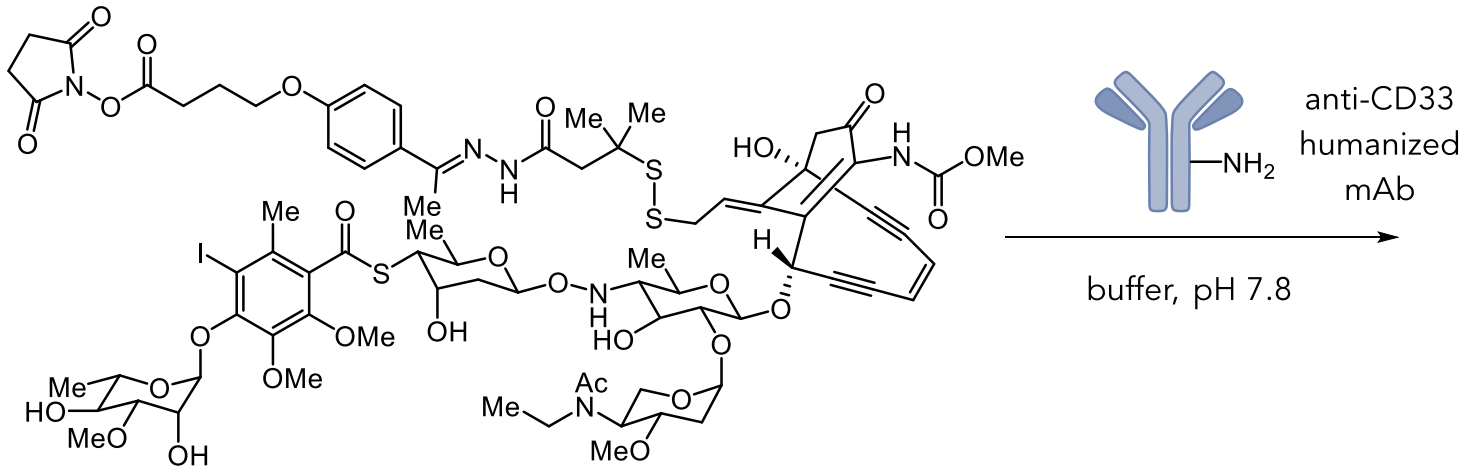

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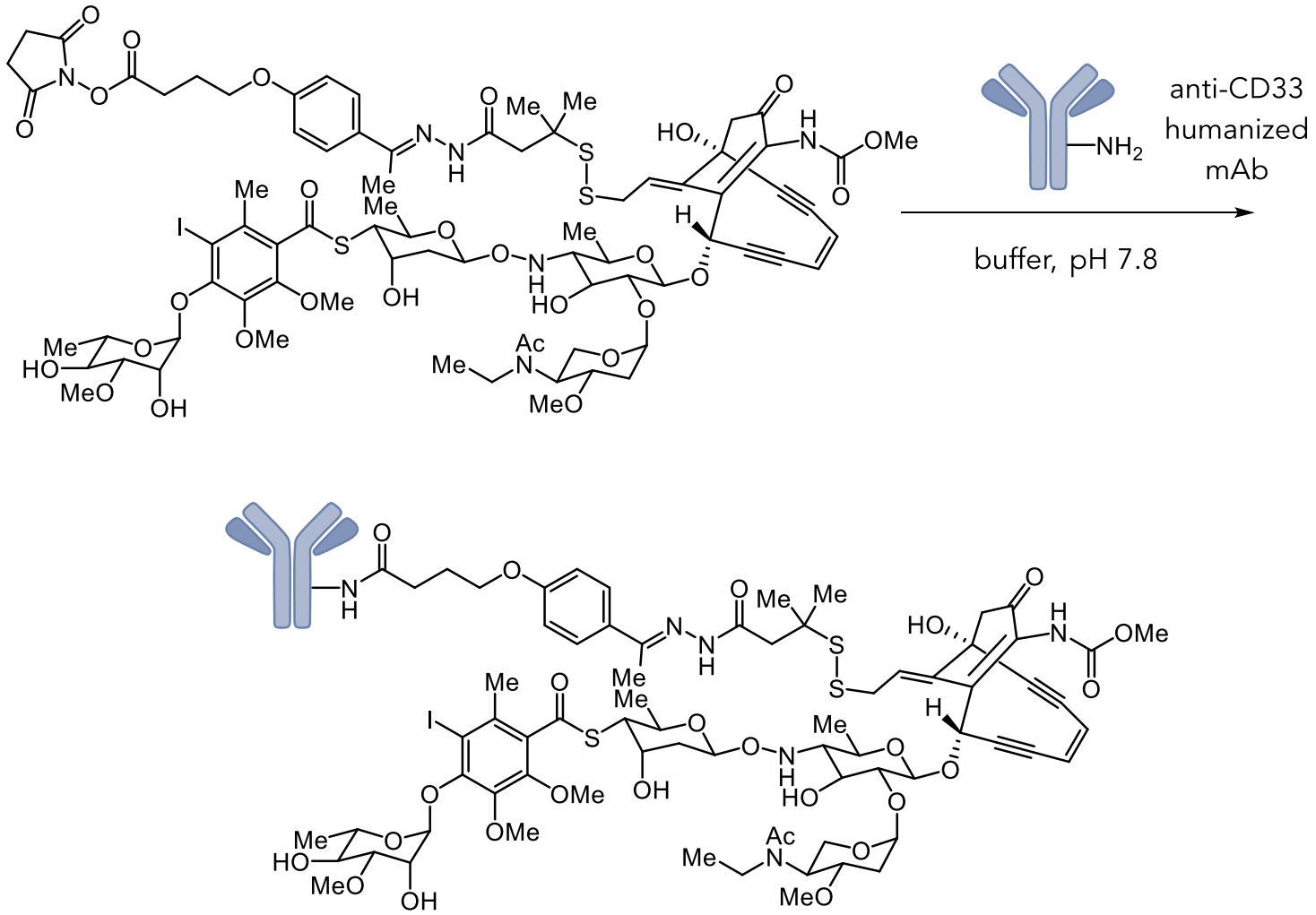

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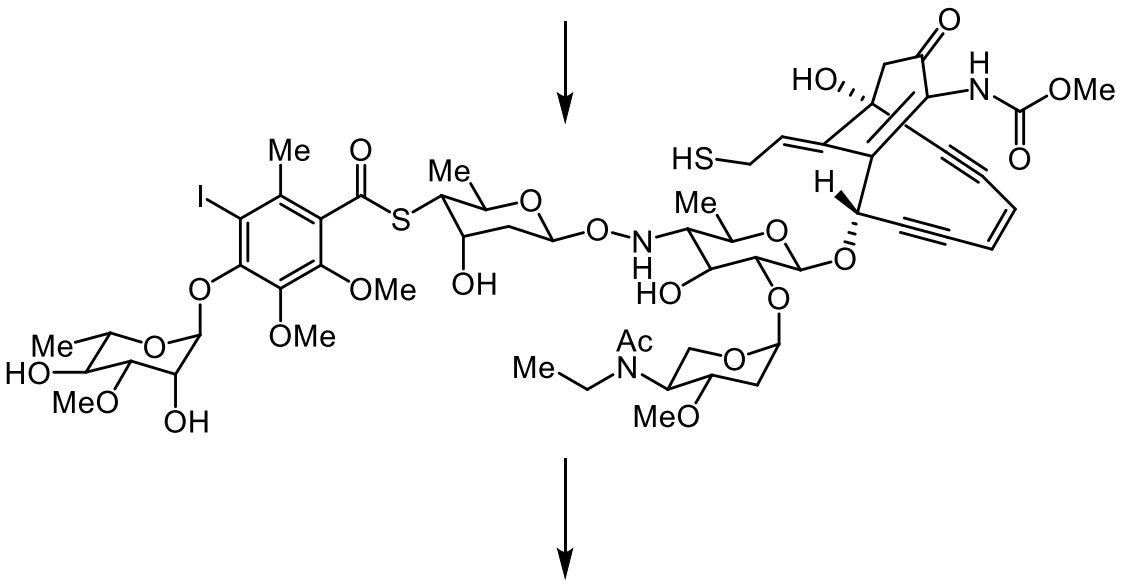
Mylotarg: gemtuzumab ozogamicin

First-Generation ADC

payload release *via* endosomal hydrazone cleavage/
disulfide exchange with glutathione



1,4-conjugate addition
Bergman cyclization

Nicolaou, K. C.; Rigol, S. *Angew. Chem. Int. Ed.* **2019**, *58*, 11206–11241.

Besponsa: inotuzumab ozogamicin

Second-Generation ADC

- Mylotarg withdrew from market in 2010
- no improvement in survival and higher fatal toxicity rate
- two different internalization mechanisms and off-target effects
- highly heterogeneous mixture with 50% unconjugated antibody
- linker labile toward hydrolysis

Beck, A.; Goetsch, L.; Dumontet, C.; Corvaia, N. *Nat. Rev. Drug Discov.* 2017, *16*, 315–337.
Nicolaou, K. C.; Rigol, S. *Angew. Chem. Int. Ed.* 2019, *58*, 11206–11241.

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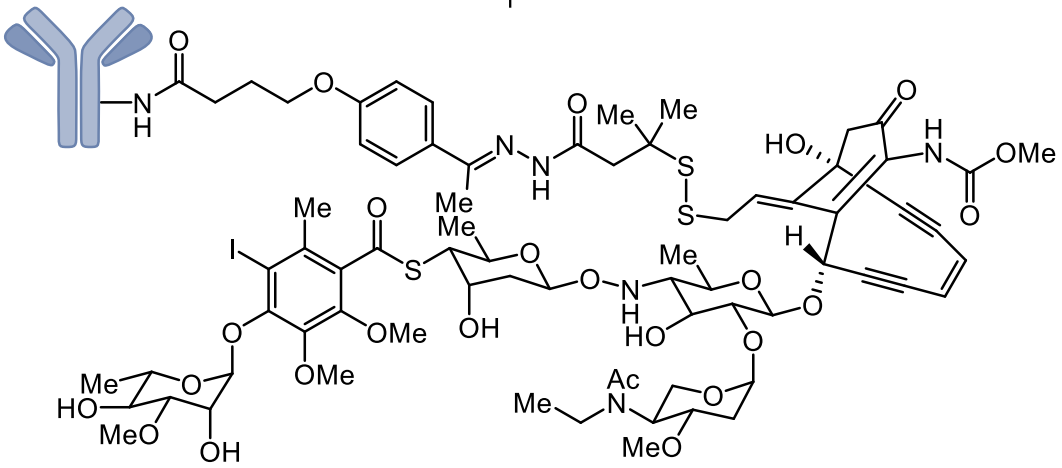

mAb targets CD22
higher average DAR = 6

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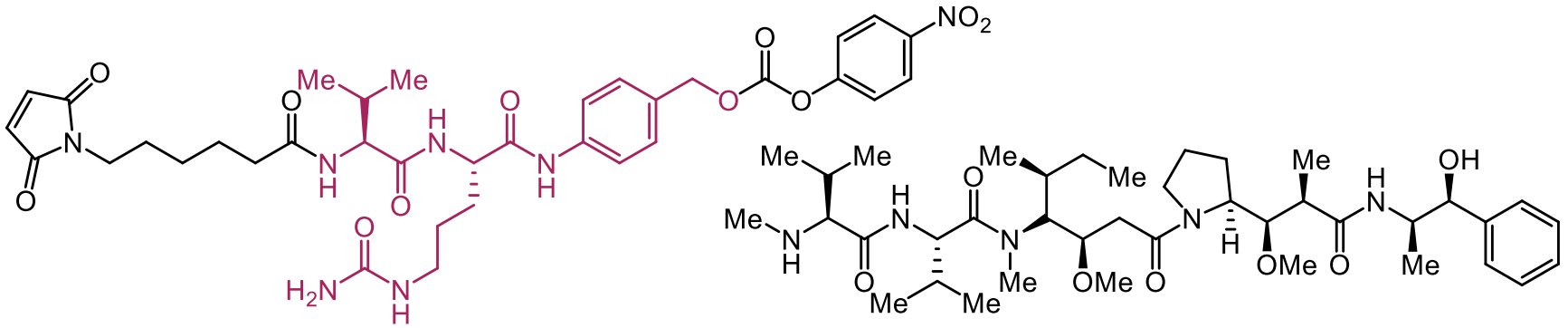
Adcetris: brentuximab vedotin



cathepsin cleavable
valine-citrulline-PAB

monomethyl auristatin E

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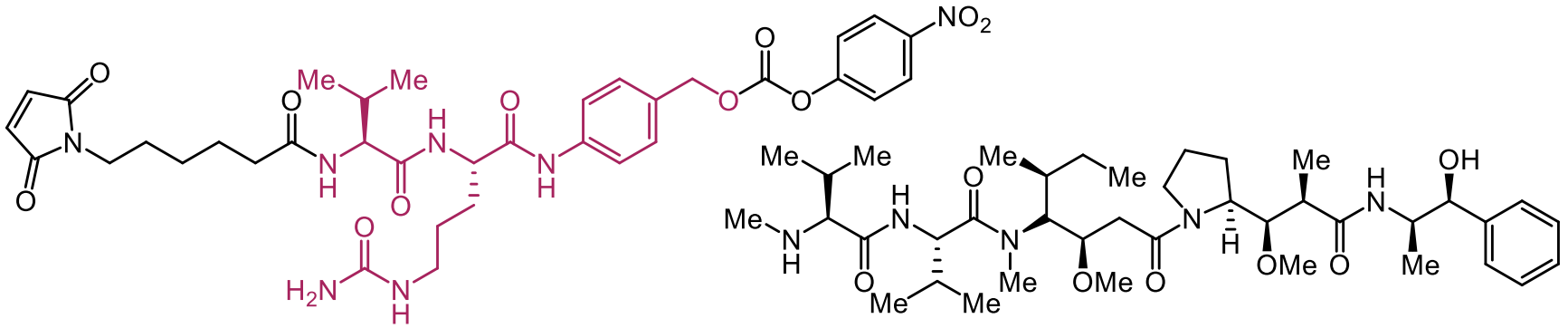

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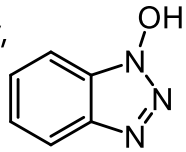

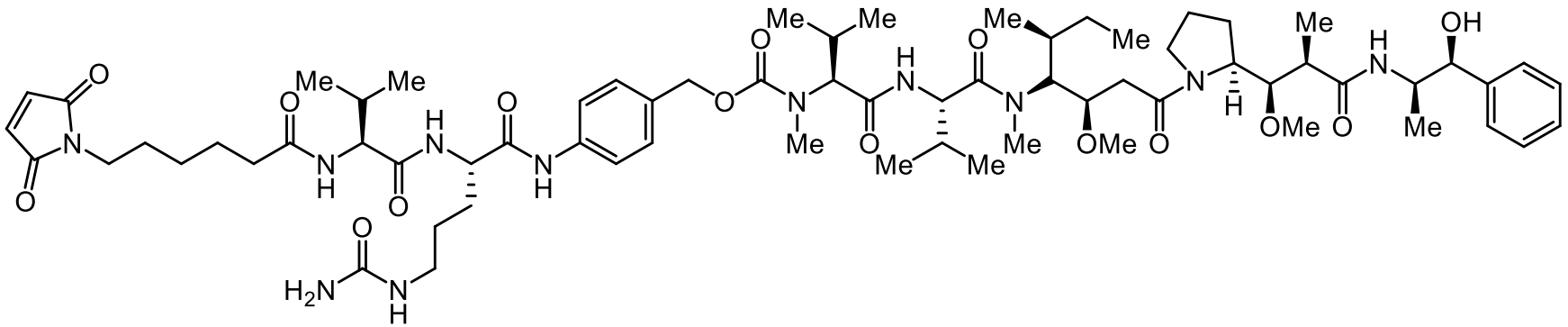

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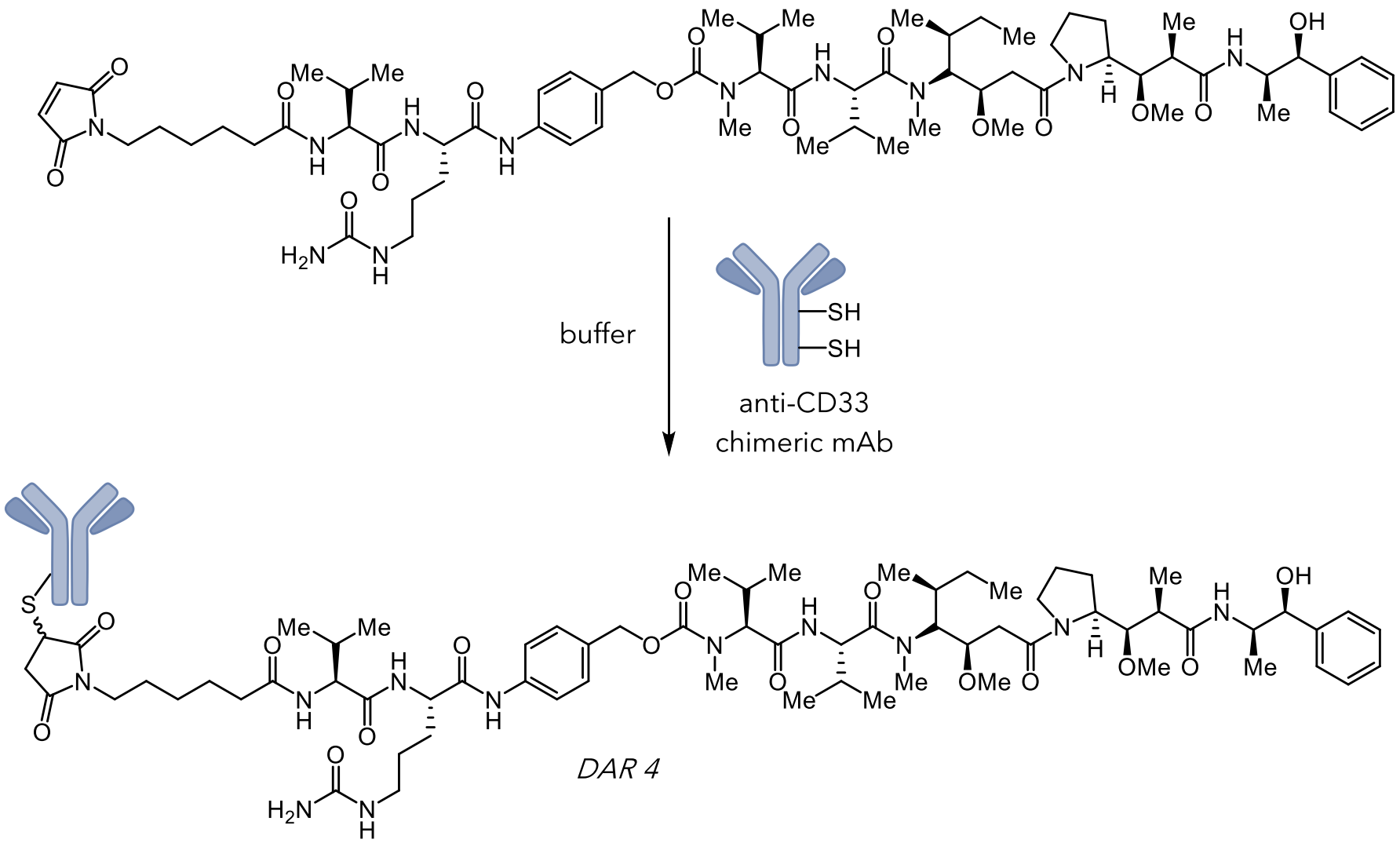

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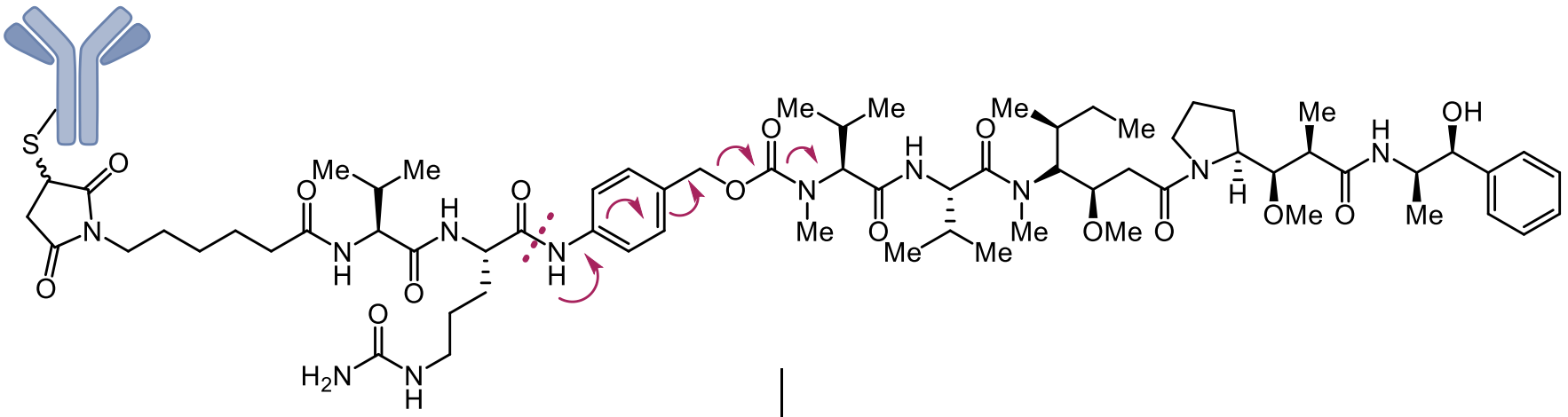
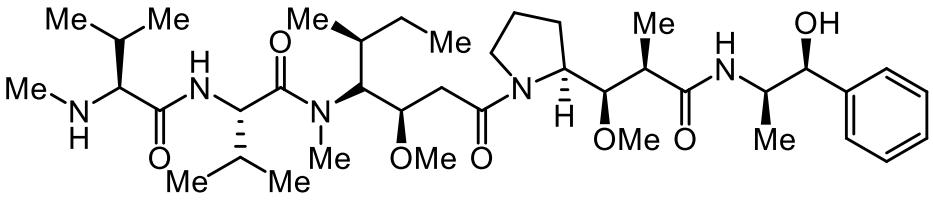


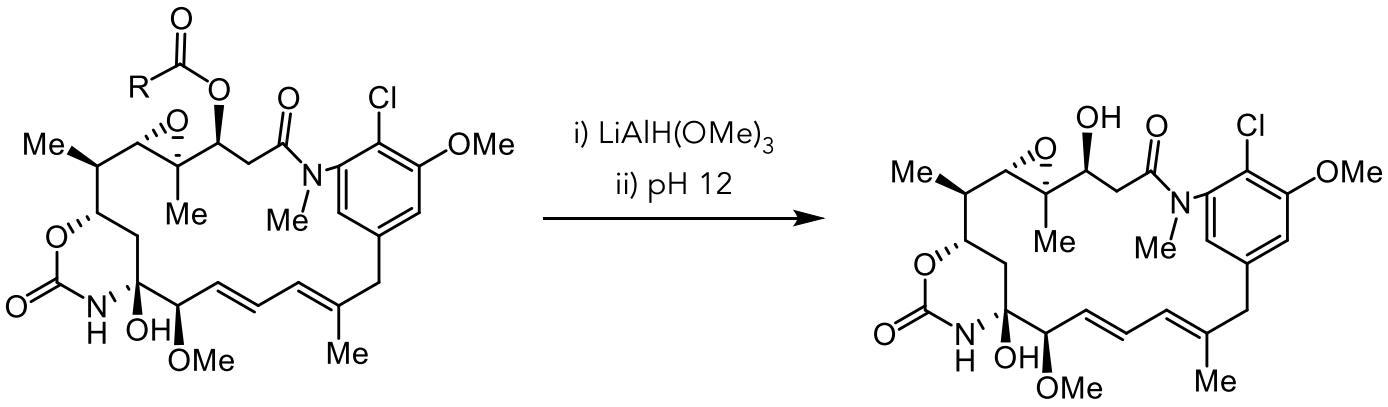
cathepsin B protease
cleavage



monomethyl auristatin E
blocks tubulin polymerization

Nicolaou, K. C.; Rigol, S. *Angew. Chem. Int. Ed.* **2019**, *58*, 11206–11241.

Kadcyla: trastuzumab emtansine



mixture of ansamitocins

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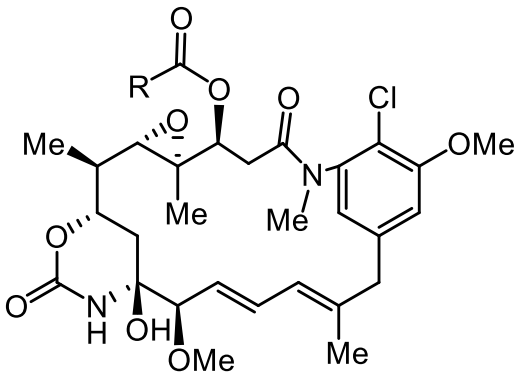
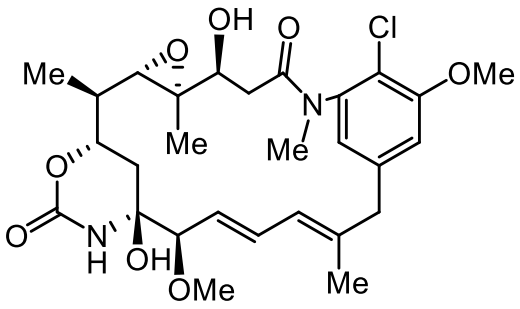


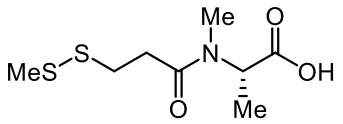
mixture of ansamitocins

i) $LiAlH(OMe)_3$

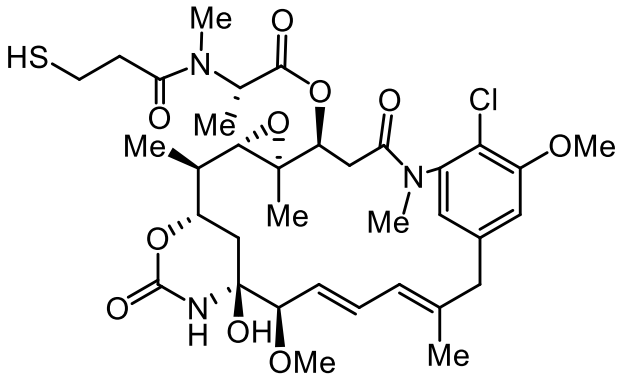
ii) pH 12



i) EDCI, $ZnCl_2$



ii) DTT, buffer pH 7.5



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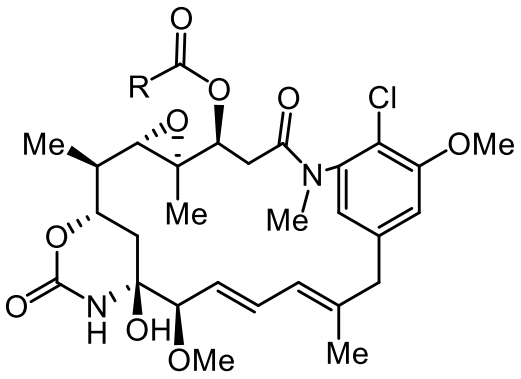
Kadcyla: trastuzumab emtansine



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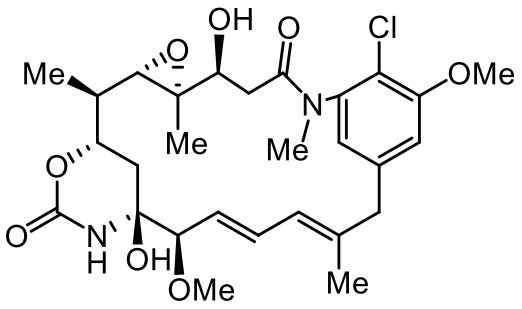

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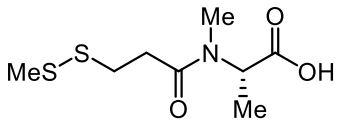

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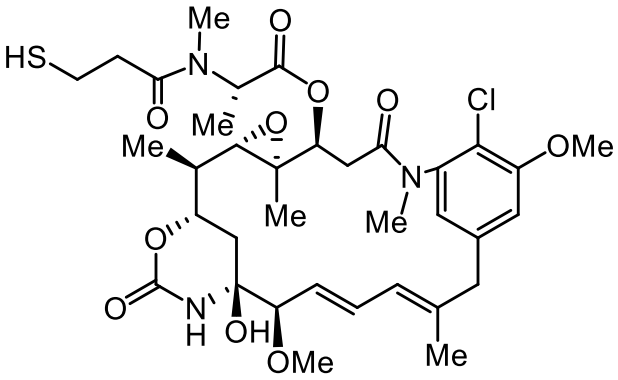
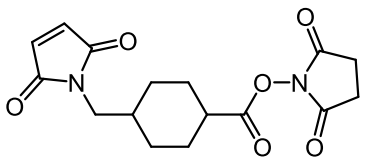


doubly-activated



phosphate buffer pH 6

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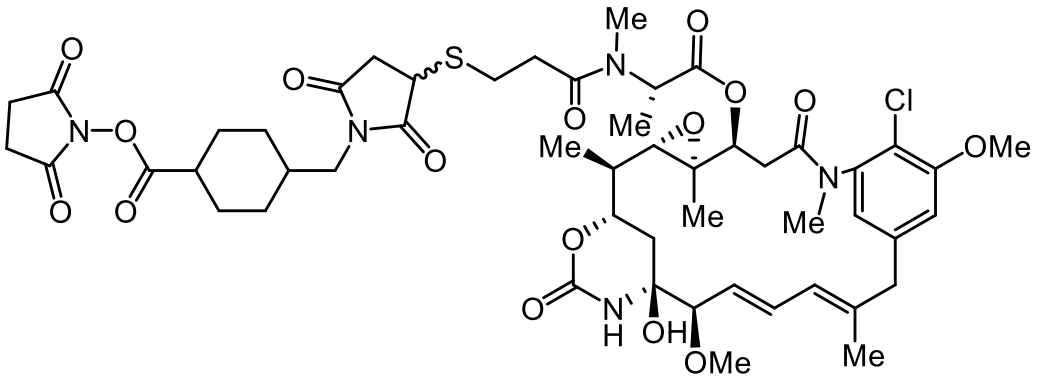
Kadcyla: trastuzumab emtansine



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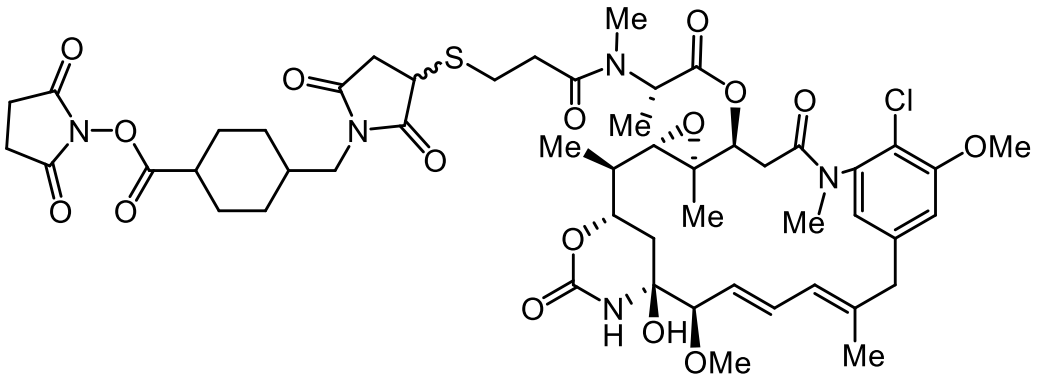

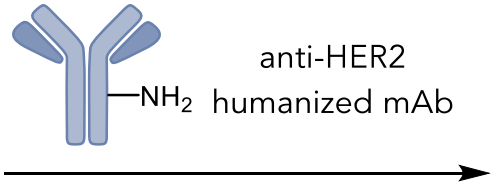

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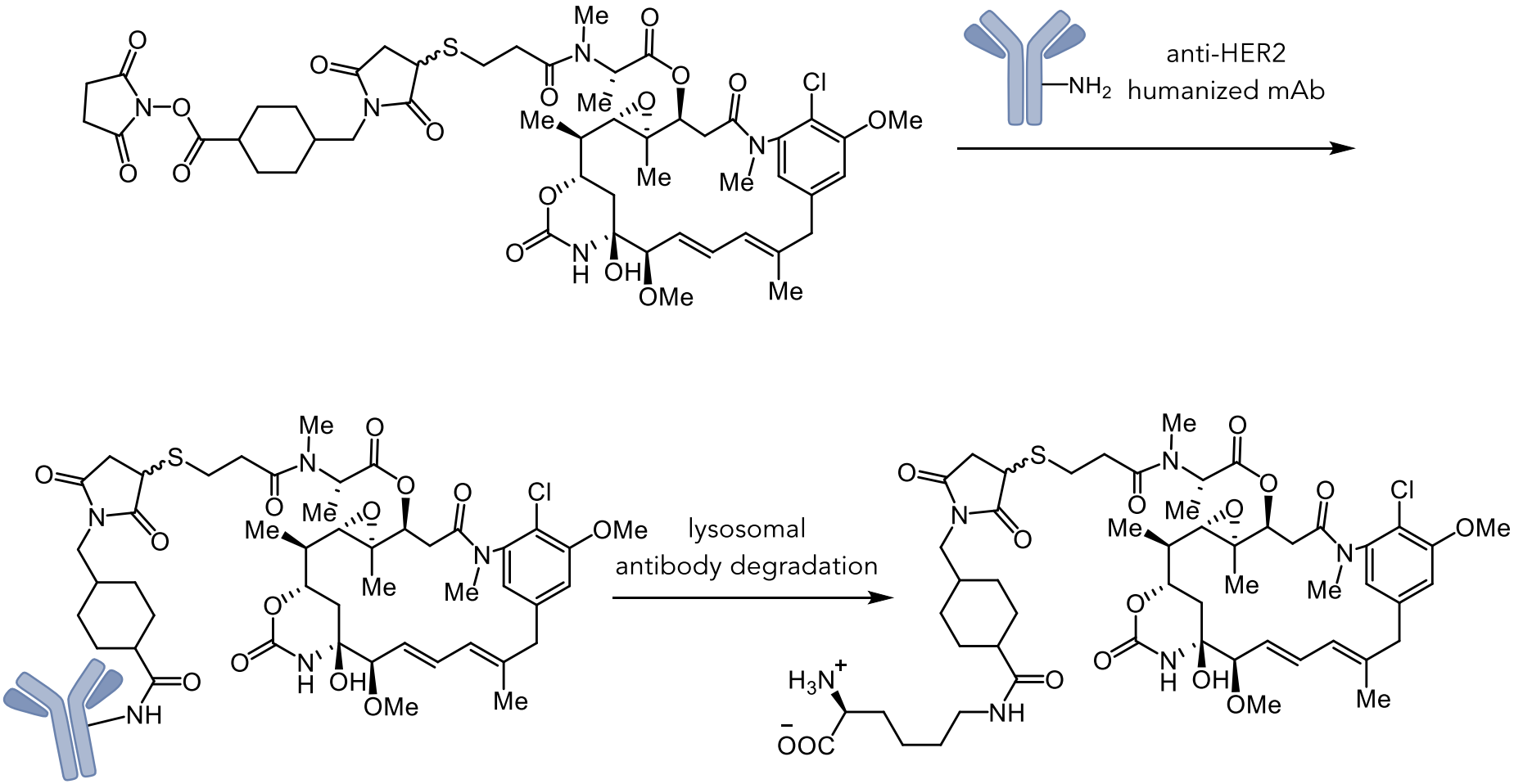


Nicolaou, K. C.; Rigol, S. *Angew. Chem. Int. Ed.* **2019**, *58*, 11206–11241.

Recent Revival

Mylotarg

gemtuzumab ozogamicin
(Pfizer)
DNA cleavage
approved 2000
withdrew 2010

Adcetris

brentuximab vedotin
(Seattle Genetics)
microtubule inhibitor
approved 2011

Kadcyla

trastuzumab emtansine
(Genentech/Roche)
microtubule inhibitor
approved 2013

Besponsa

inotuzumab ozogamicin
(Pfizer)
DNA cleavage
approved 2017

Lumoxiti

moxetumomab pasudotox
(AstraZeneca)
elongation factor-2 inhibitor
approved 2018

Polivy

polatuzumab vedotin
(Genentech/Roche)
microtubule inhibitor
approved 2019

Padcev

enfortumab vedotin
(Astellas/Seattle Genetics)
microtubule inhibitor
approved 2019

Enhertu

trastuzumab deruxtecan
(AstraZeneca/Daiichi Sankyo)
TOPO1 inhibitor
approved 2019

Trodelvy

sacituzumab govitecan
(Immunomedics)
TOPO1 inhibitor
approved 2020

Blenrep

belantamab mafodotin
(GlaxoSmithKline)
microtubule inhibitor
approved 2020

Zynlonta

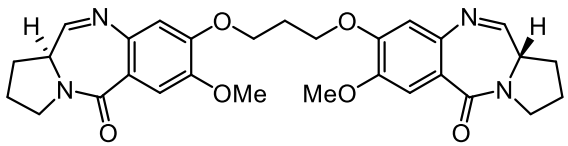
loncastuximab tesirine
(ADC Therapeutics)
DNA crosslinking
approved 2021

Future Directions

New Drug Agents

potent small molecule agents with different mechanisms of action

Zynlonta™
loncastuximab tesirine-lpyl
for injection, for intravenous use



DNA crosslinking with guanine

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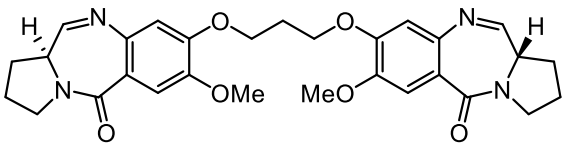
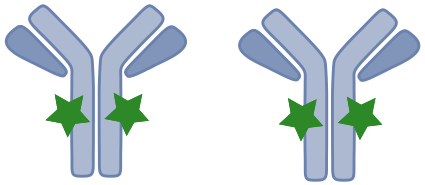


DNA crosslinking with guanine

Homogeneous ADCs

homogeneous ADCs require improved site-selectivity and better conjugation methods



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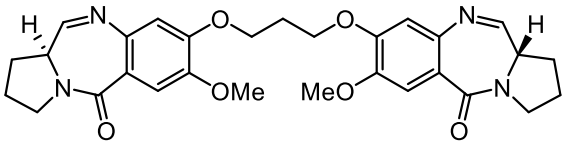

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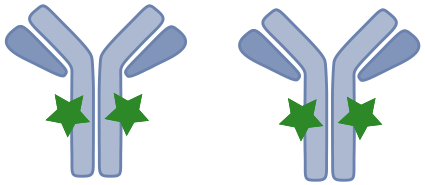
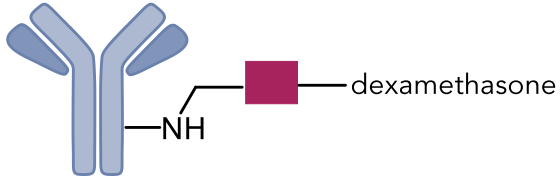


Beyond Oncology

ADCs explored for the treatment of inflammatory disorders and as an antibiotic



targeted delivery of glucocorticoid

Conclusion

A New Era in the Development of ADCs

- Despite struggling in the clinic for most of the last 20 years, ADCs are seeing a comeback. 6 ADCs approved in just the past three years (now 10 total on the market) and >60 in clinical trials

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"Now, an essential task of the new Institute will be to find substances and chemical groups that have a special relationship to certain organs. It will be of particular importance, however, to equip such substances, acting as trucks so to speak, with chemical groups possessing pharmacological or toxicological effects, so that at the same time they convey the potent load commissioned to them to the appropriate places."

Paul Ehrlich (1906)