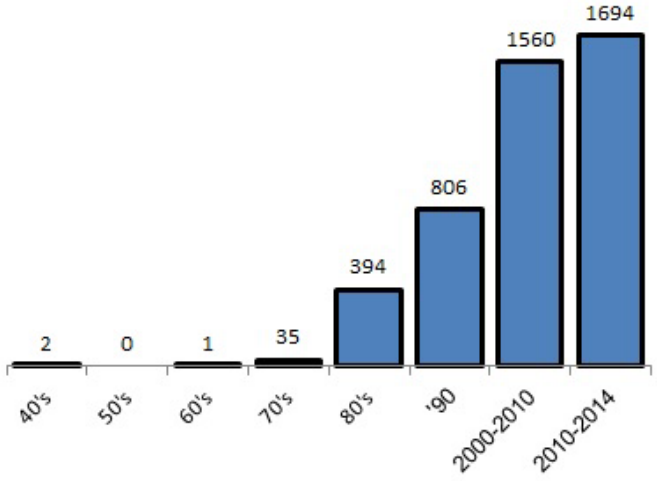
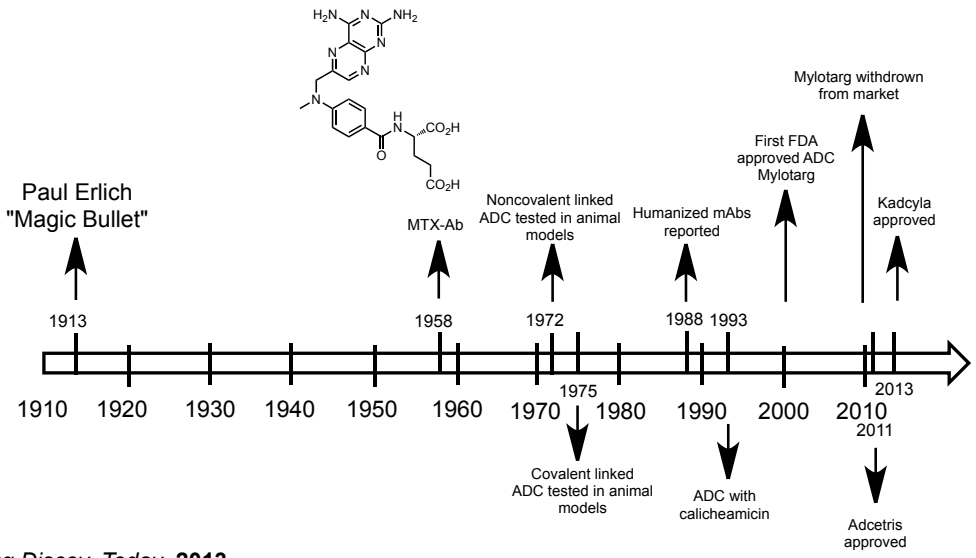
Antibody-Drug Conjugation (ADC)

- Taking the advantage of the specificity of mAb (monoclonal antybodies) to deliver potent cytotoxic drug selectively to antigen-expressing tumor cells.
  Chemistry allows to use highly potent drugs that could not be used alone.

- No. of publication with the concept "antibody drug conjugated" over ther years:



Antibody-Drug Conjugates - History



Drug Discov. Today, **2013**

Dominant Investigator

- Peter D. Senter, Ph.D. - B.A Biotechnology (Berkeley), Ph.D. Chemistry (University of Illinois), Postdoctoral in the Max-Planck Institute for Experimental Medicine.
  Worked at Cytokine Networks, Bristol-Myers-Squibb Research Institute.
  Current position: Vice president, Seattle Genetic.
  (Development of potent drugs, novel linker systems and conjugation methodology)
  Senior editor of Molecular Cancer Therapeutics
  Affilate Professor (University of Washington)

Antibody-Drug Conjugates - Clinical Reports (clinicaltrials.gov 2012)

Agensys - 3 ADCs in Phase I (auristatine) - carcinoma and prostate cancer

Bayer HealthCare - 2 ADCs in Phase I (Auristatine/maytansinoid)

Biogen - 1 ADC in Phase I (DM4) - breast cancer

Biotest - 1 ADC in Phase II (DM4) - myeloma

BMS - 1 ADC in Phase I (duocarmycin) - non-Hodgkin's lymphoma

Celldex - 1 ADC in Phase II (auristatine)

Immunogen - 2 ADCs in Phase I (DM1 and DM4)

Pfizer - 1 ADC in Phase III - non-Hodgkin's lymphoma

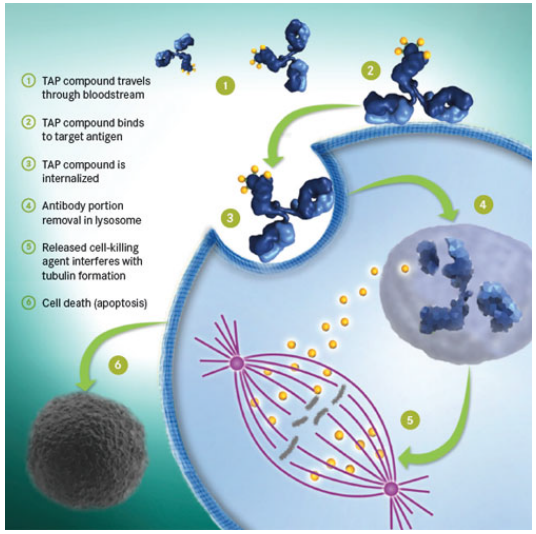
Progenics - 1 ADC in Phase I (auristatine) - prostate cancer

Roche - 1 ADC in Phase III and 2 ADCs in Phase I (DM1) - breast cancer

Sanofi - 2 ADCs in Phase II (DM4) - lymphoma and leukemia

Seattle Genetics - 1 ADC in Phase III and 1 ADC in Phase I (auristatine) - non-Hosdgkin's lymphoma and carcinoma

Mode of Action

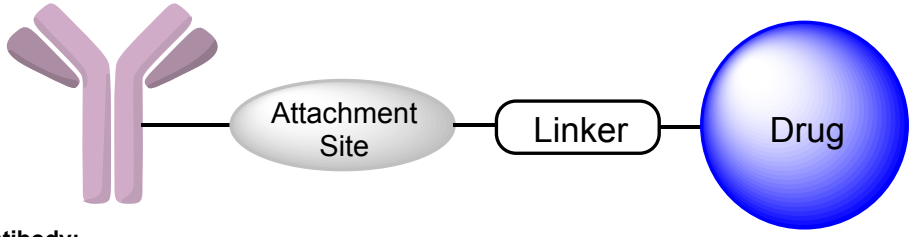


ImmunoGen Inc.

- Use of highly potent drugs (Especially negative-charged drugs)
- Ab can be also anti-cancer
- Drug is stable and usually inactive until it reaches the cancer cells

ADC with a specific drug is usually more potent than the drug alone

Antibody-Drug Conjugates - Components & Demands



Antibody:
1. Targets a well-characterized antigen with high tumor expression
2. Maintain all of it's properties upon conjugation to the drug
3. Minimal non specific binding

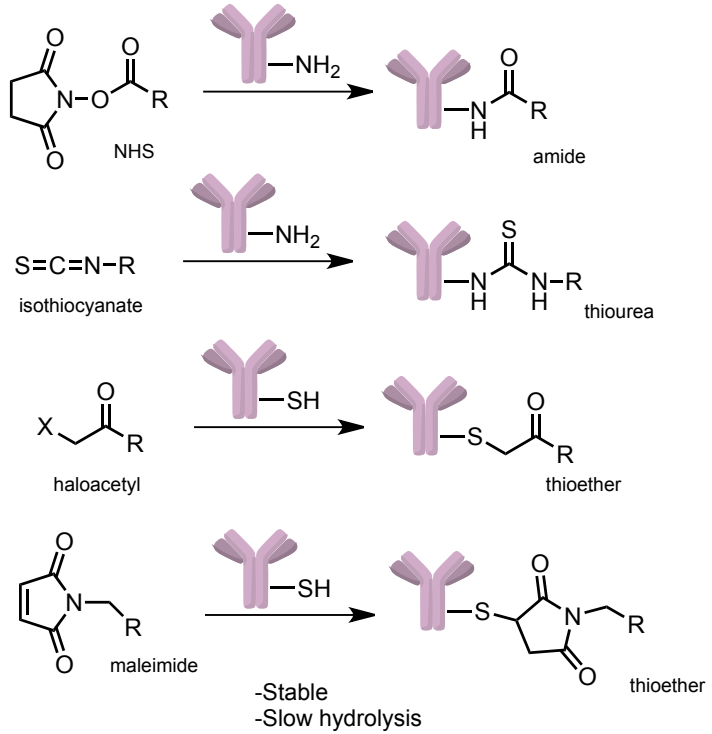
Attechment site:
1. Typically through cysteine or lysine residues on the Ab
2. Variable drug:Ab ratio or site-selective conjugation

Linker:
1. Cleavable or non cleavable
2. Stable with selective release of the drug

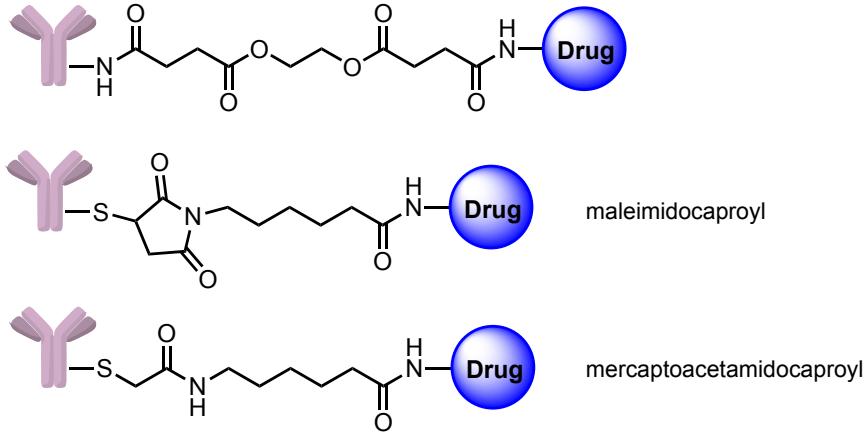
Drug:
1. Highly potent (usually 2-4 drugs per mAb)
2. Non-immunogenic
3. Could be linked to the Ab
4. Defined mechanism of action

Current Opinion in Chemical Biology, **2010**, 14, 529

General Linkers

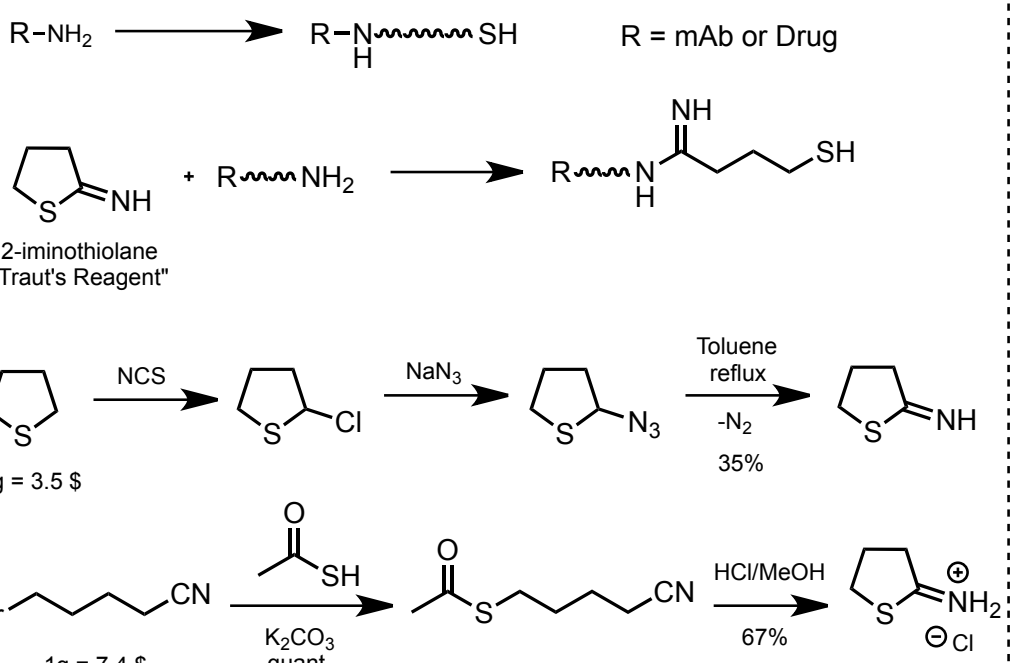


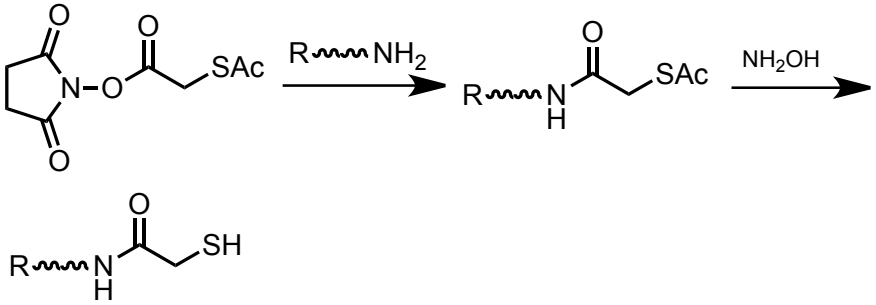
Simple Conjugation



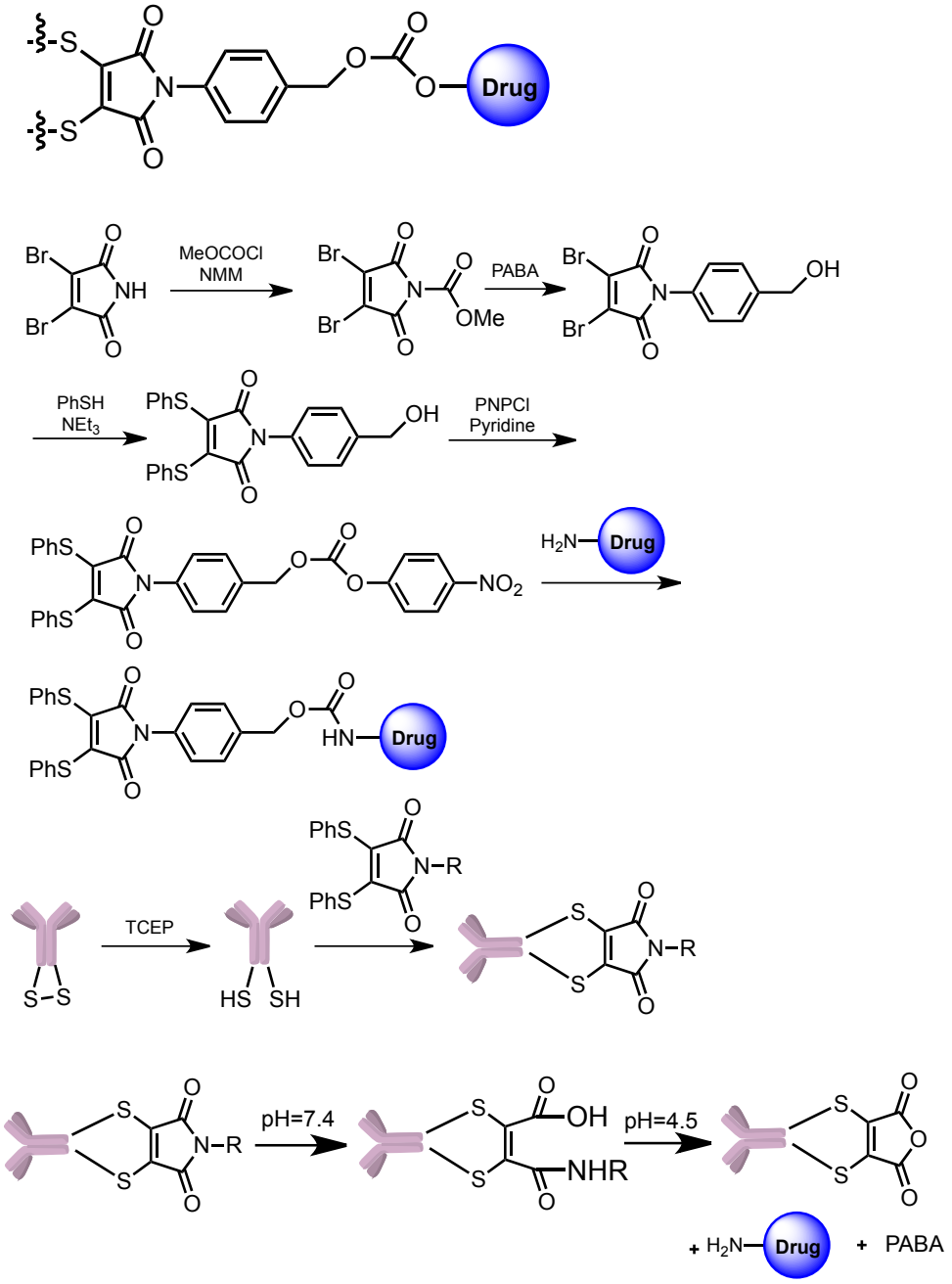
Thiolation Reagents

Canadian J. Org. Chem., **1984**, 62(3), 586
Chem. Abstr., **1968**, 68

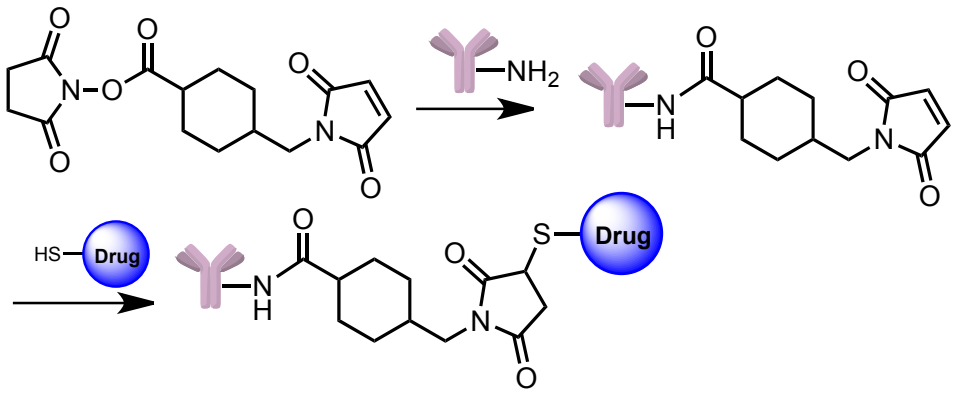




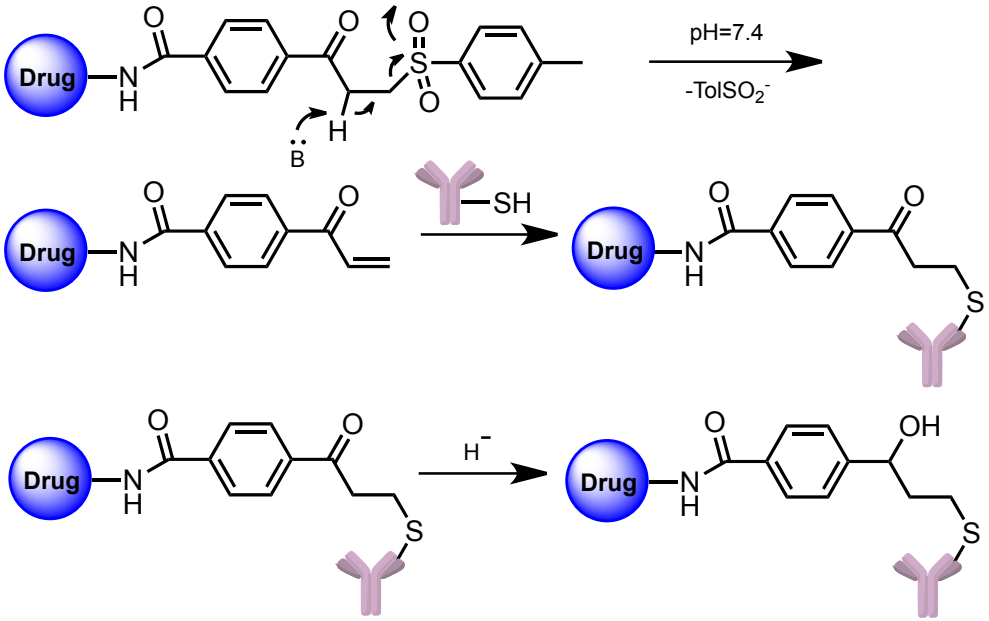
Chem. Commun., **2013,** 49, 8187



Bifunctional Linkers

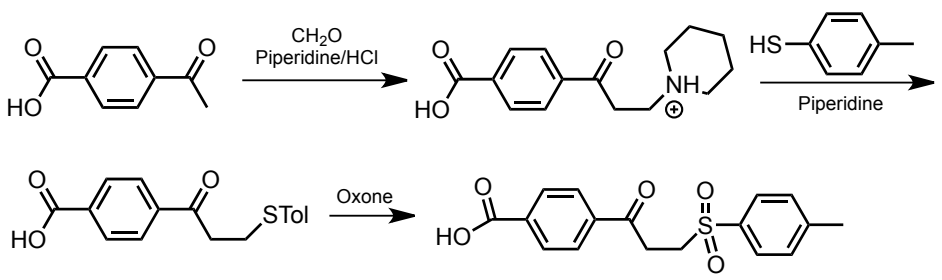


Bioconjugate Chem., **2014**, 25, 460



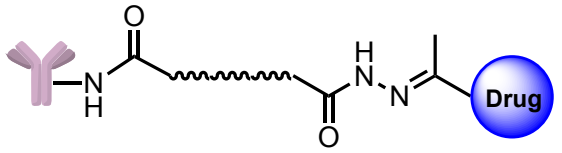
Can deconjugate
(retro-michael)

Can't deconjugate

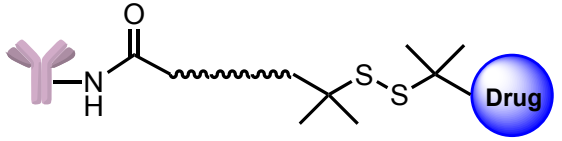


Misc. Drug Linking with Specific Release Mechanism

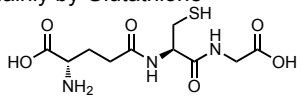
J. Controlled Release, **2012**, 161, 422

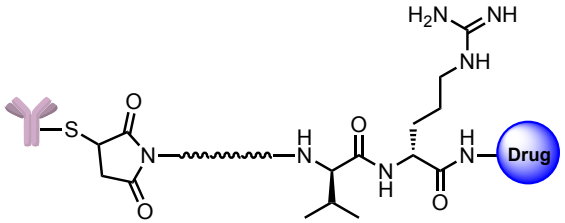


Hydrazone (Rapid hydrolysis in the lysosome)



Disulfide (Reduction in lysosome)
Mainly by Glutathione



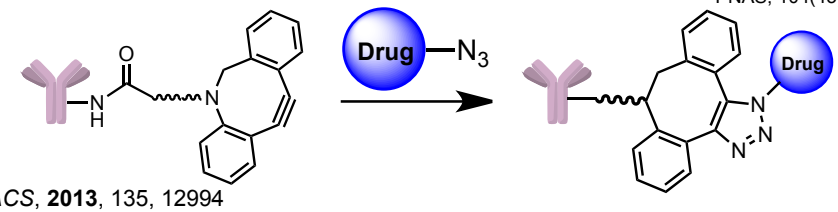


Val-Cit dipeptide
(site-specific proteolysis)

Sometimes also Phe-Lys

Drug Linking by Click

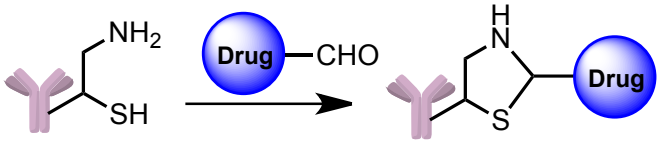
copper-free click - developed by Bertozzi (2007) PNAS, 104(43), 16793



JACS, **2013**, 135, 12994

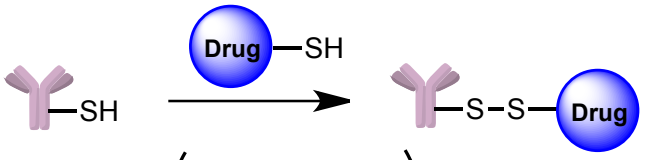
The Chemistry Behind Antibody-Drug Conjugation

Traceless Linkers

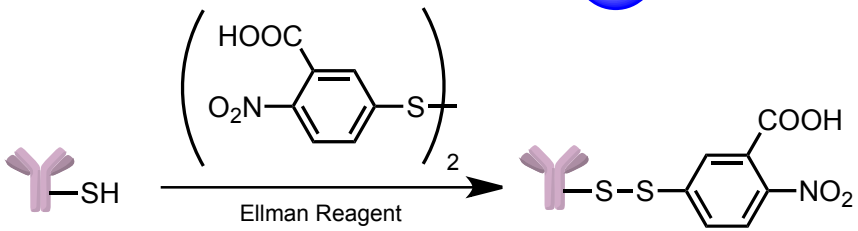


(Hydrolysis)

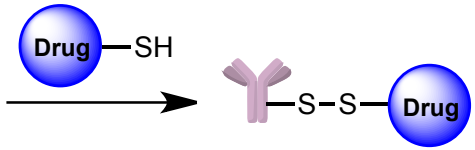
thiazolidine



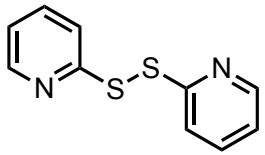
(Reduction)



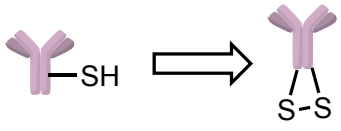
Ellman Reagent

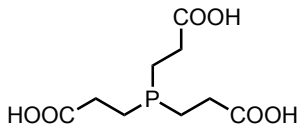


Nature Protocols, **2013,** 8, 2079



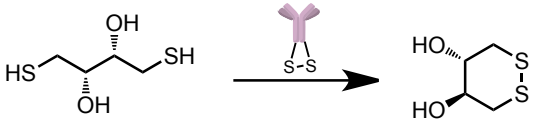
2,2'-dithiopyridine



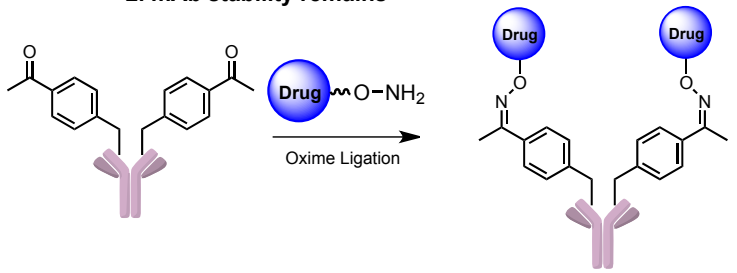


TCEP

DTT
(dithiothreitol)
(Cleland's Reagent)



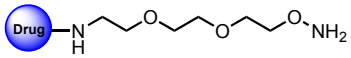
Precise Controll on Conjugation to Synthesize homogeneous ADCs
Advantages: 1. Known mAb:Drug ratio.
2. mAb stability remains



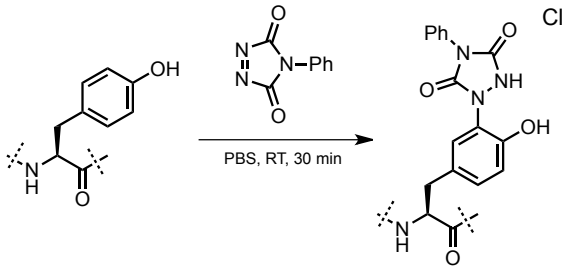
Genetically encoded unnatural amino acid with ortogonal chemical reactivity

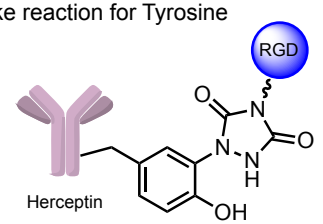
Schultz, *PNAS,* **2012,** 92(3), 13

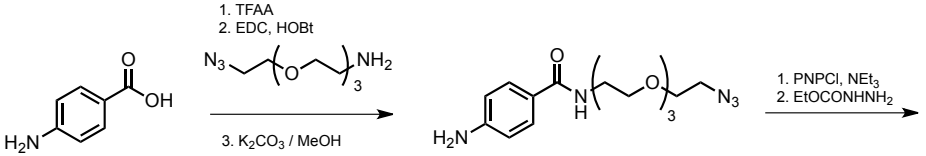
Drug = auristatine

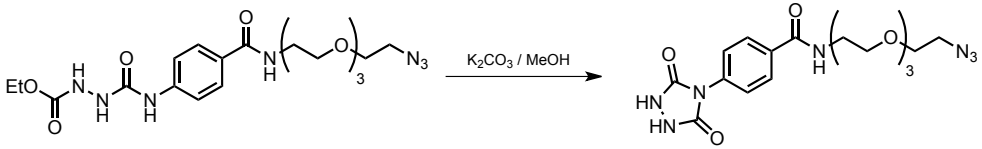


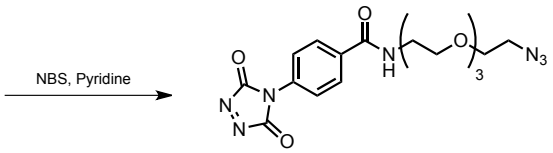
Click-like reaction for Tyrosine





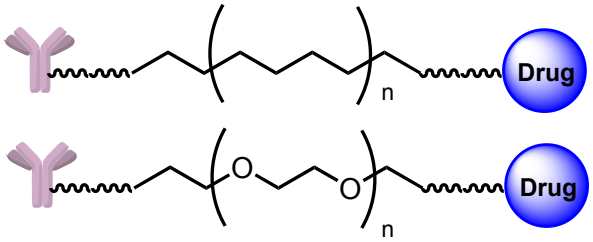




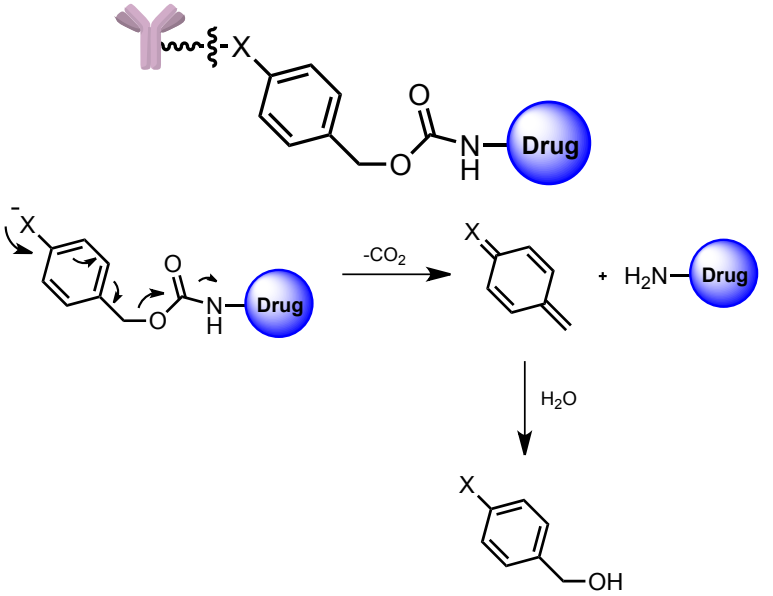


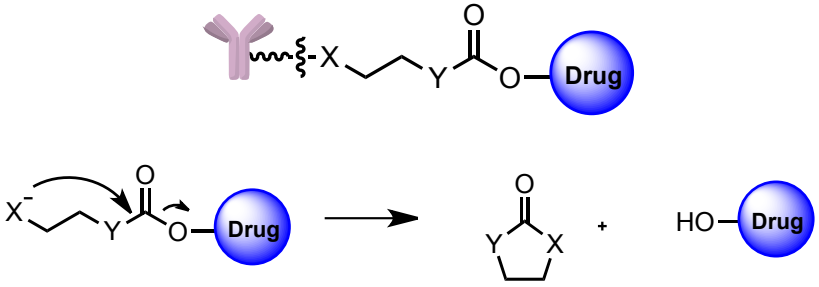
Barbas, *JACS,* **2010**, 132, 1523

Spacers

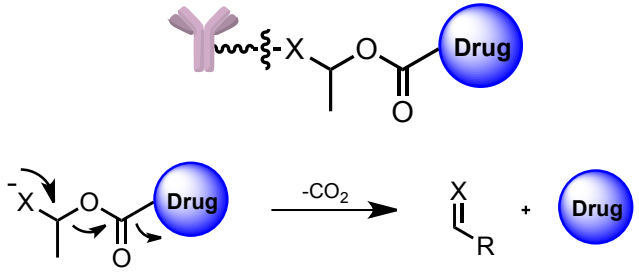


Self-Immolative Spacers

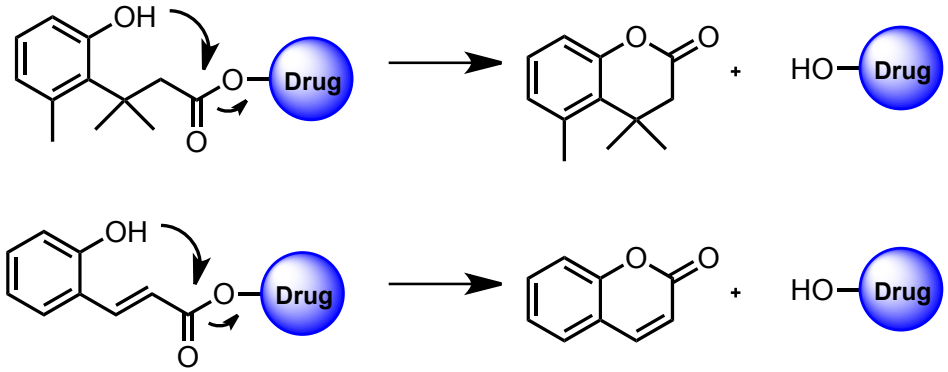


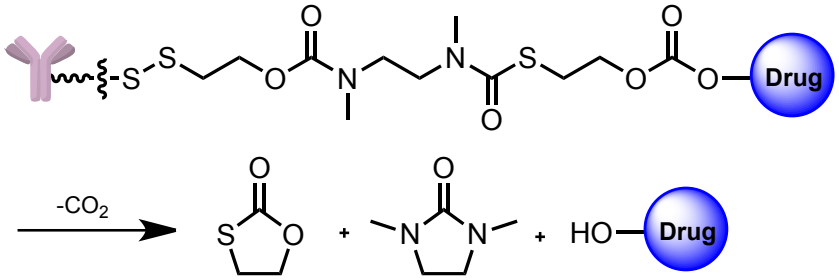


Self-Immolative Spacers (Cont.)



Examples





Adcetris (Brentuximab Vedotin)

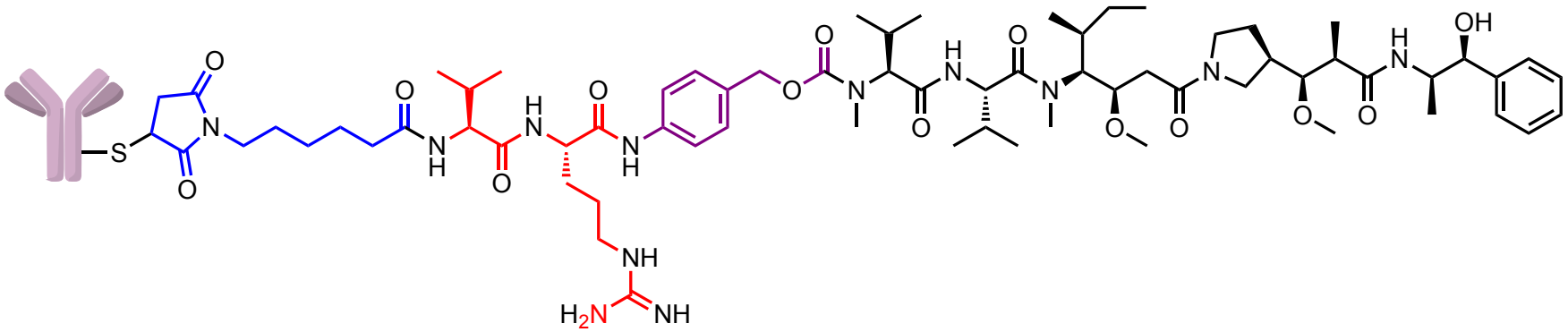
mAb - Brentuximab, directs to protein CD30 (expressed in Hodgkin's lymphoma and large-cell lymphoma)

Attachment Site - thiomaleimido caproyl

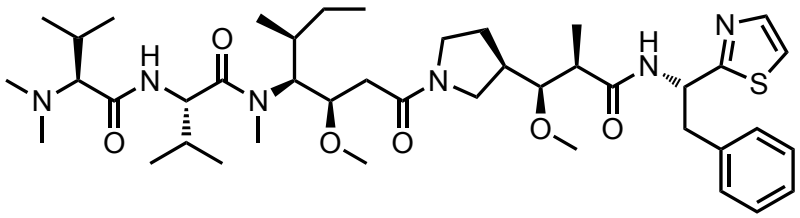
Linker - Cathepsin (protease) cleavable linker (Val-Cit)

Spacer - PABA

Drug - Monomethyl auristatine E (MMAE)



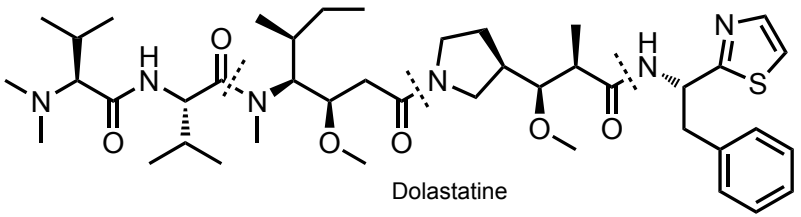
MMAE -
- antimitotic drug (inhibits mitosis, cell division), from the marine shell less *Dolabella Auricularia*
- Blocking the polymerisation of tubulin
- 200 times more potent than vinblastine

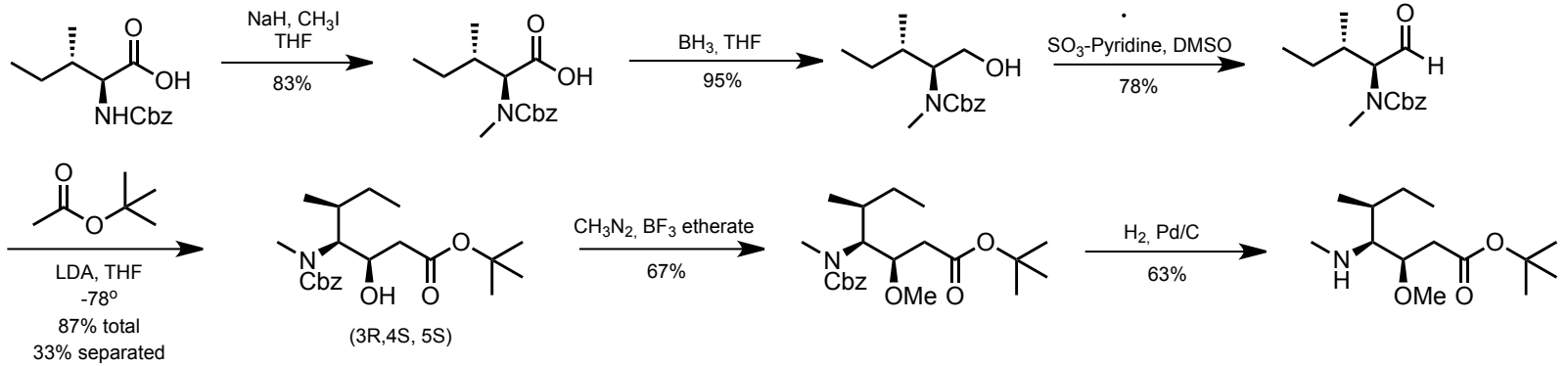


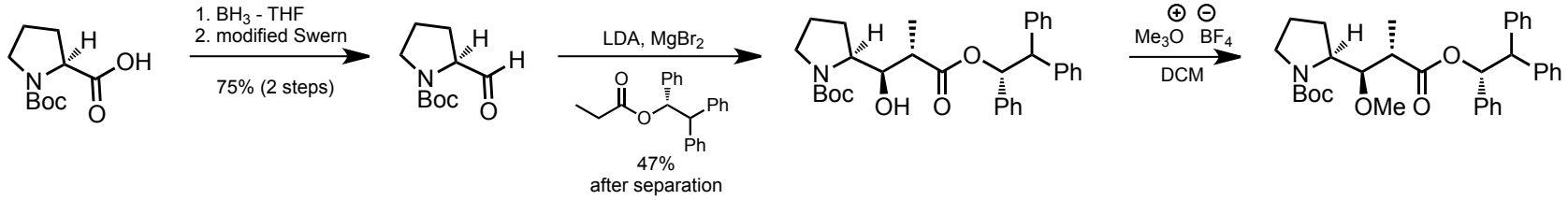
Dolastatine

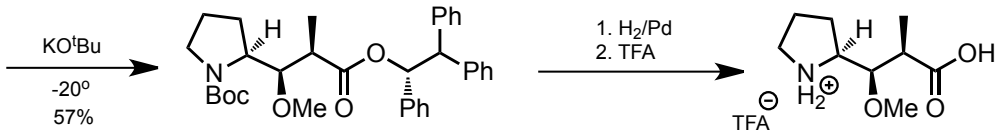
Adcetris (Brentuximab Vedotin)

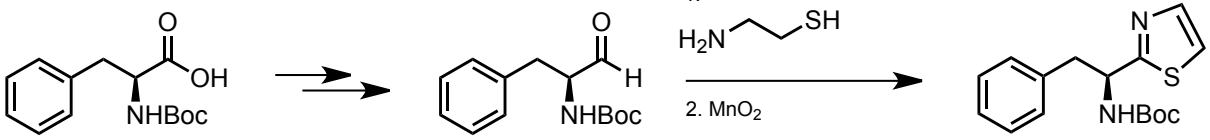
Pettit, *JACS*, **1989,** 111(14), 5463





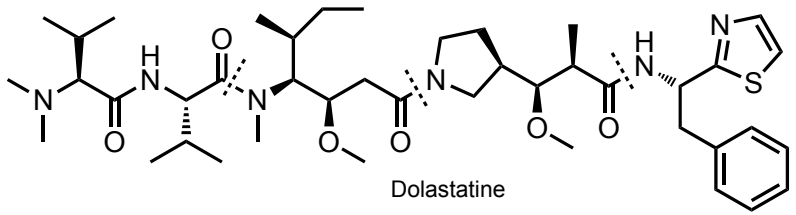




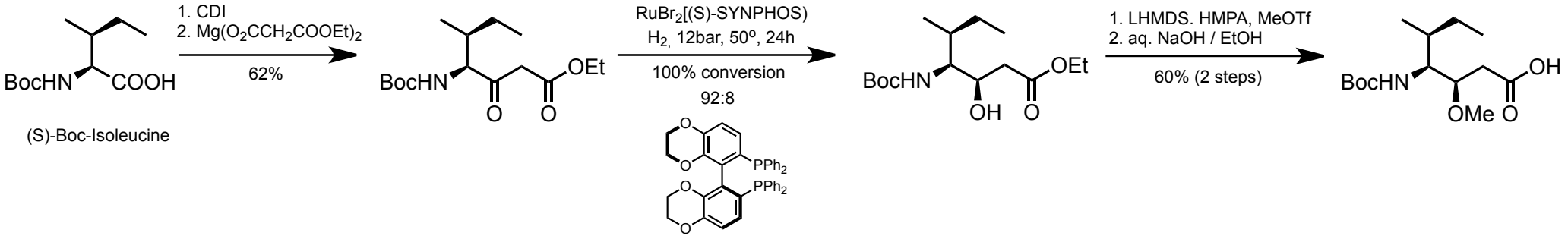


Adcetris (Brentuximab Vedotin)

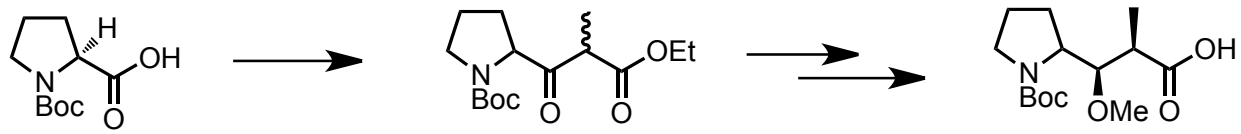
Tetrahedron, **2007**, 63, 6115



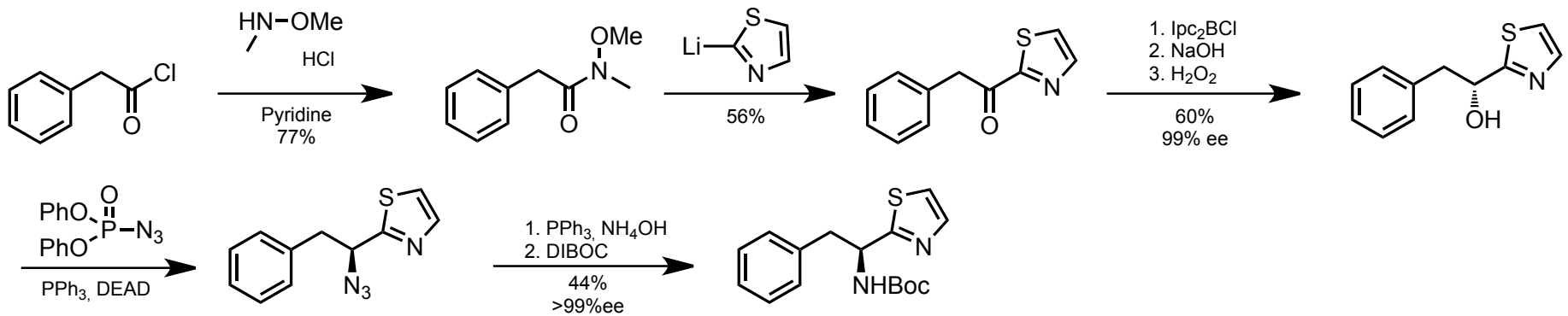
Dolastatine



(S)-Boc-Isoleucine



(S)-Boc-Proline

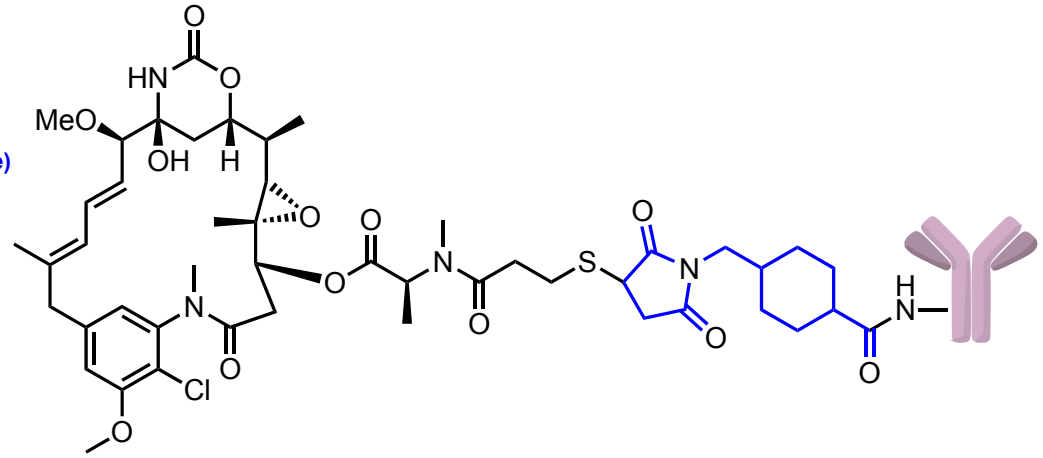


Kadcyla (Trastuzumab emtansine)

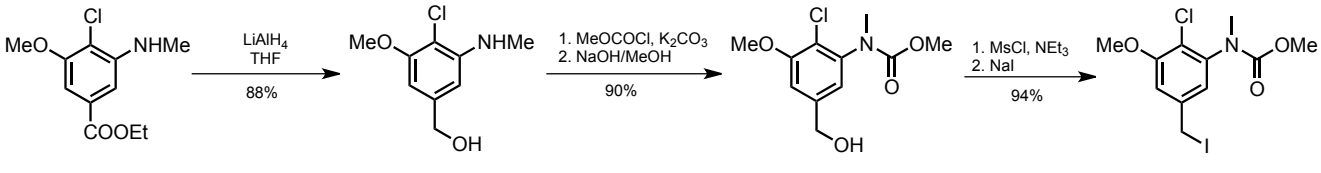
mAb - Trastuzumab, targets HER2 receptor, also stop cell groth alone

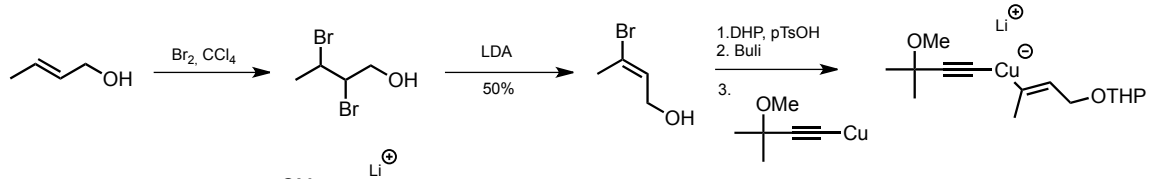
Attachment Site - SMCC (succinimidyl trans-4-(maleimidylmethyl)cyclohexane-1-carboxylate)

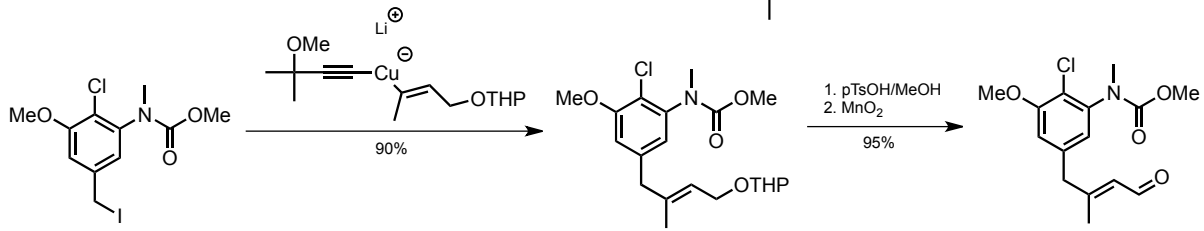
Drug - Mertansine (3.5 units/mAb in avarage)

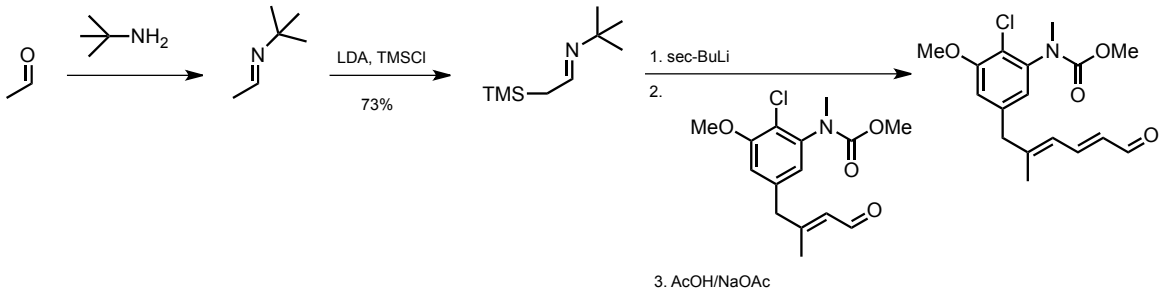


Mertansine (DM1)
- Thiol derivative of Maytansine
- Antimitotic



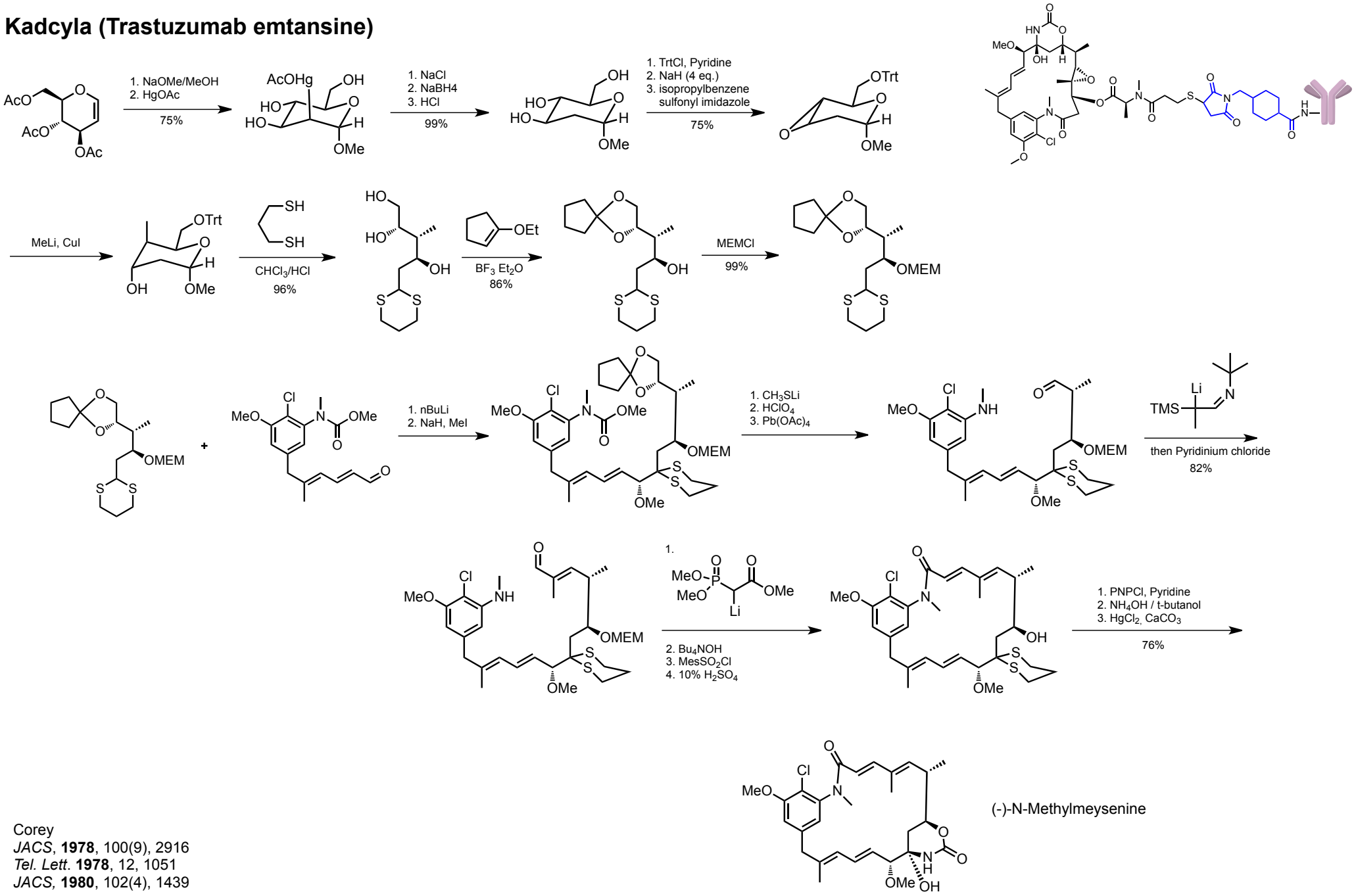






Corey
JACS, **1978**, 100(9), 2916
Tel. Lett. **1978**, 12, 1051
JACS, **1980**, 102(4), 1439

Kadcyla (Trastuzumab emtansine)



(-)-N-Methylmeysenine

Corey
JACS, **1978**, 100(9), 2916
Tel. Lett. **1978**, 12, 1051
JACS, **1980**, 102(4), 1439

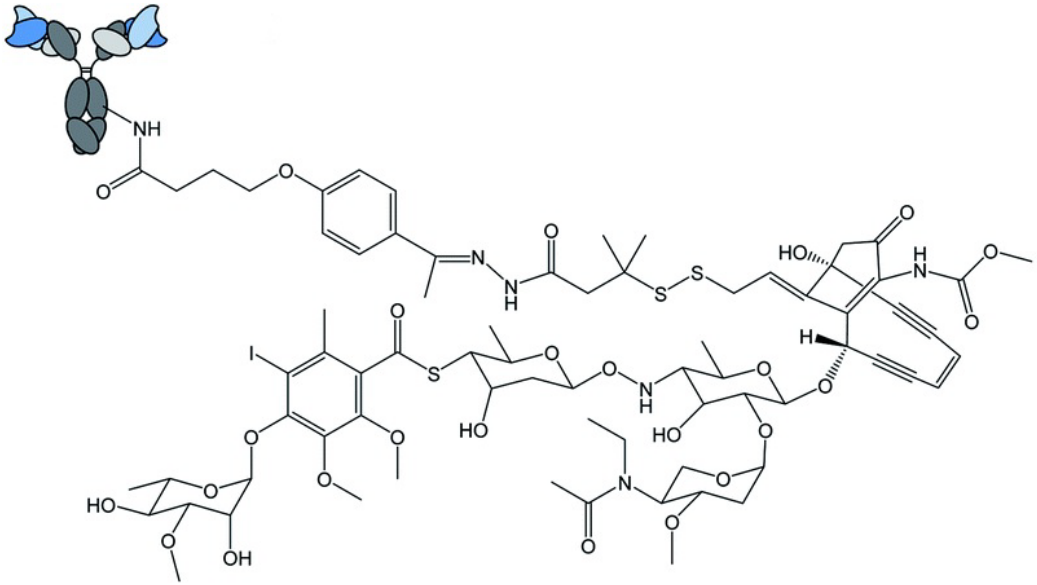
Mylotarg (Gentuzumab ozogamicin)

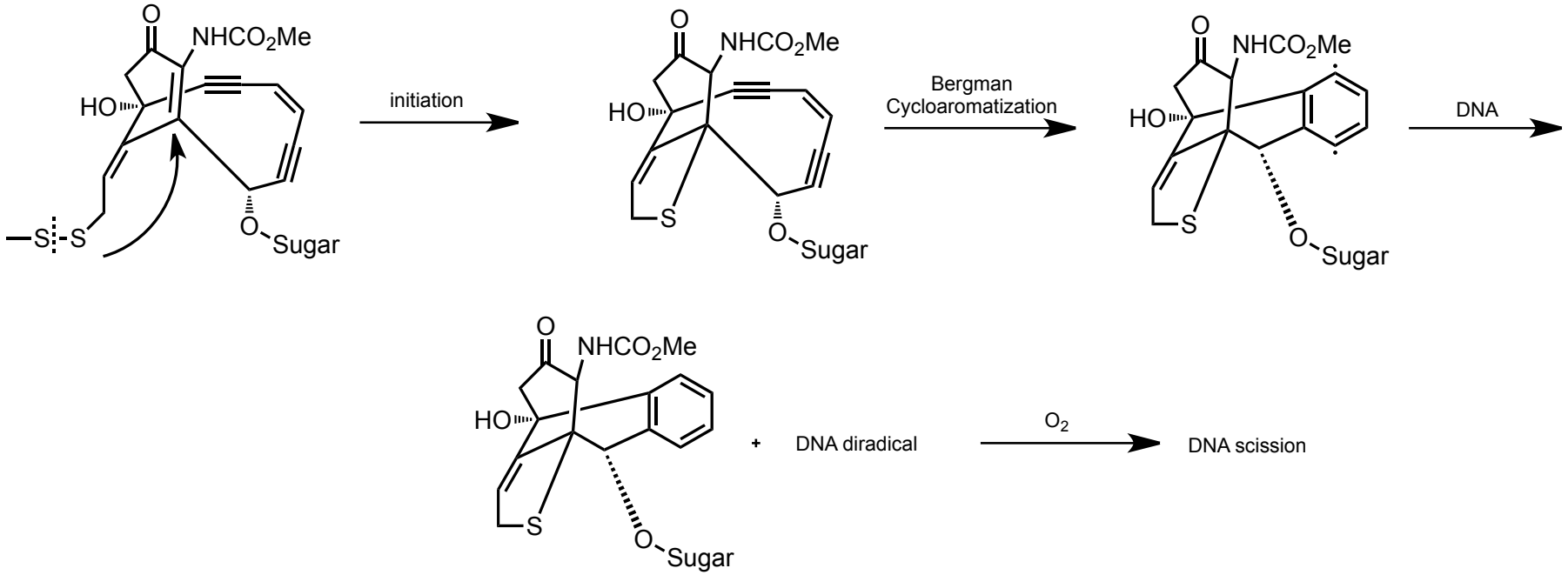
mAb - Gentuzumab (antibody to CD33 expressed in leukemia cells)

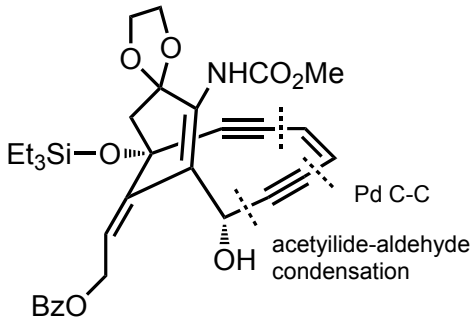
Linker - includes Hydrazone and stable S-S linkage

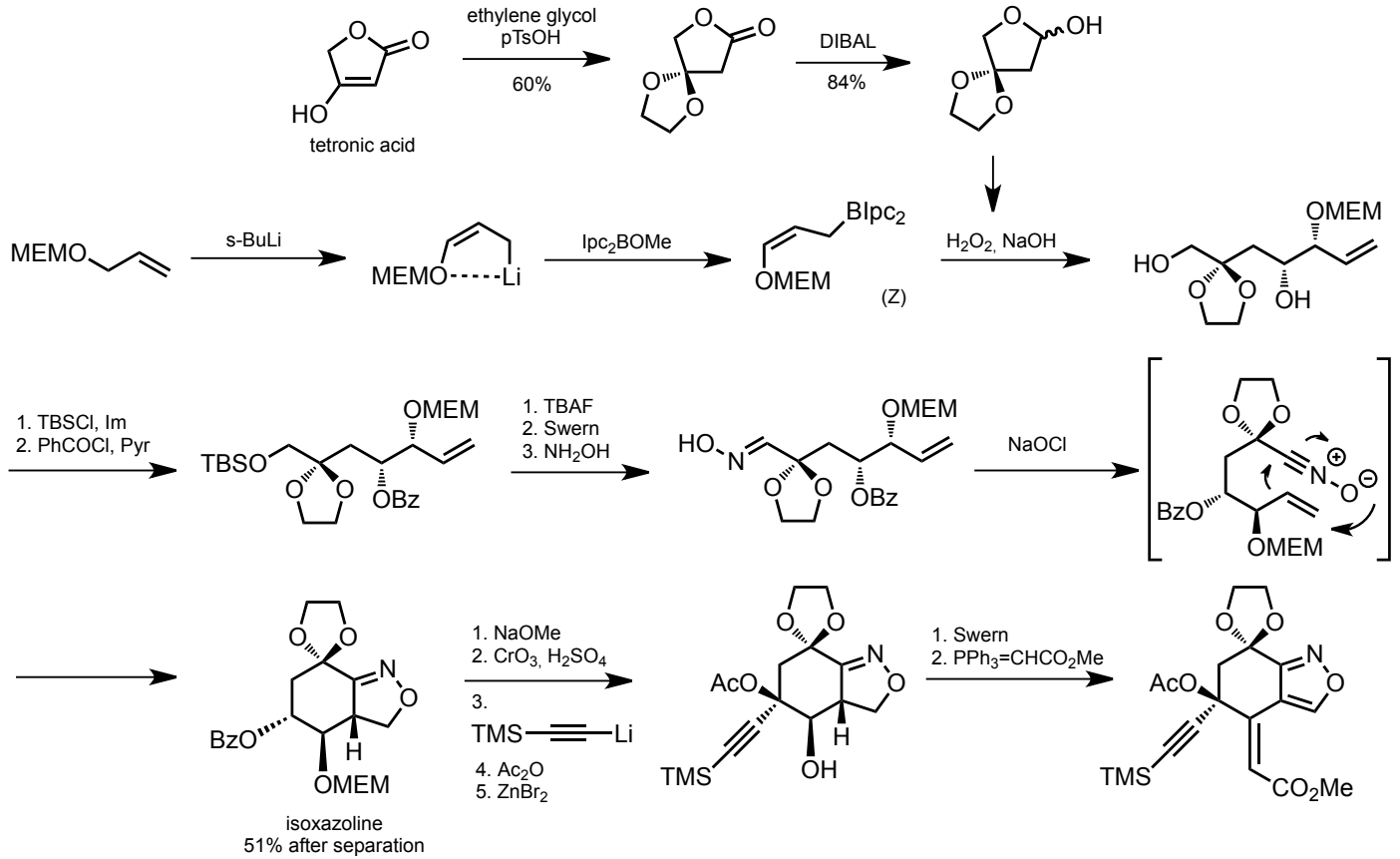
Drug - Calicheamicin

Calicheamicin
- from Micromonospora echinospora, Texas, 1980
- Causes DNA strand scission

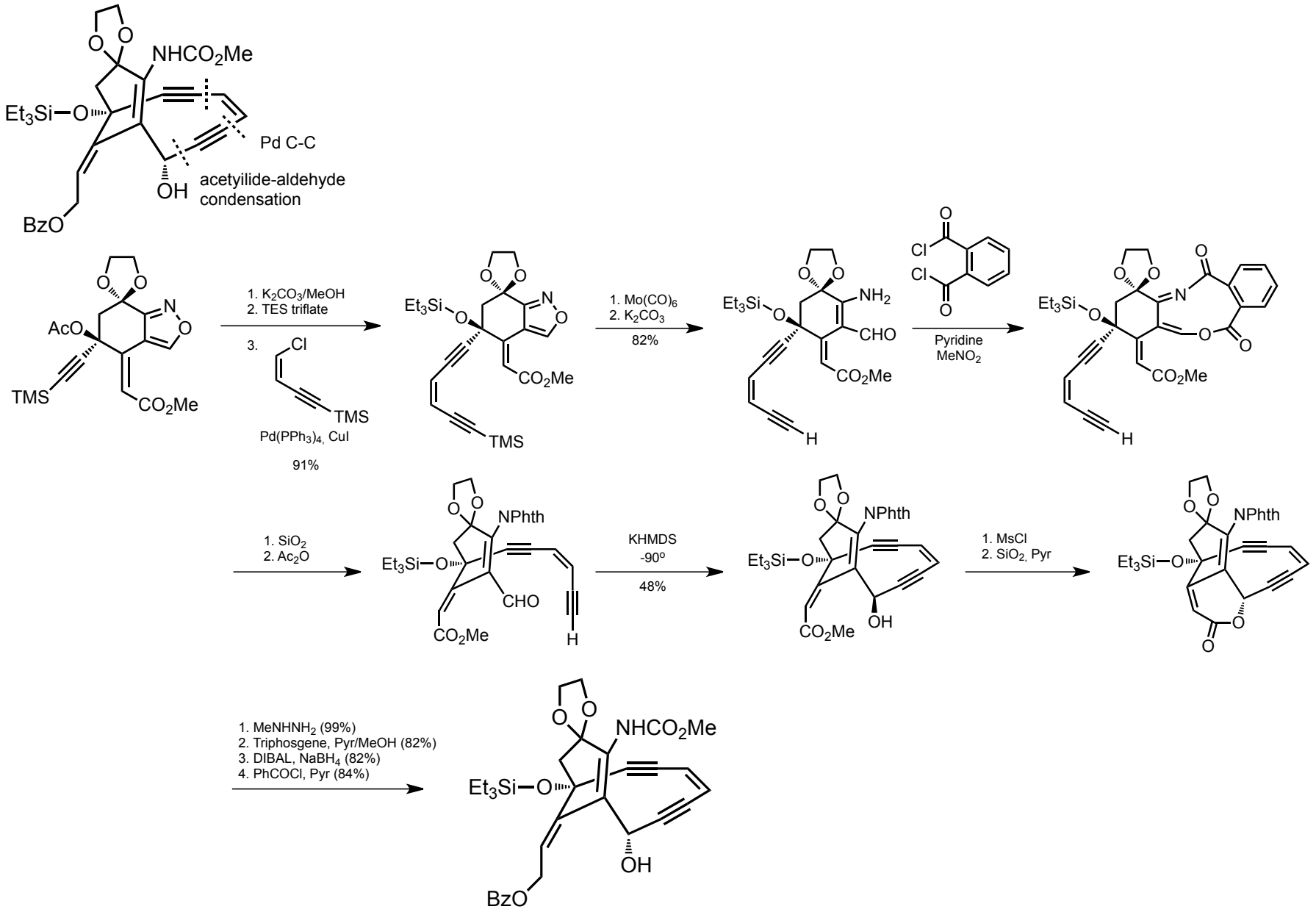








Nicolaou, *JACS*, **1992**, 114, 10082



Nicolaou, *JACS*, **1992**, 114, 10082