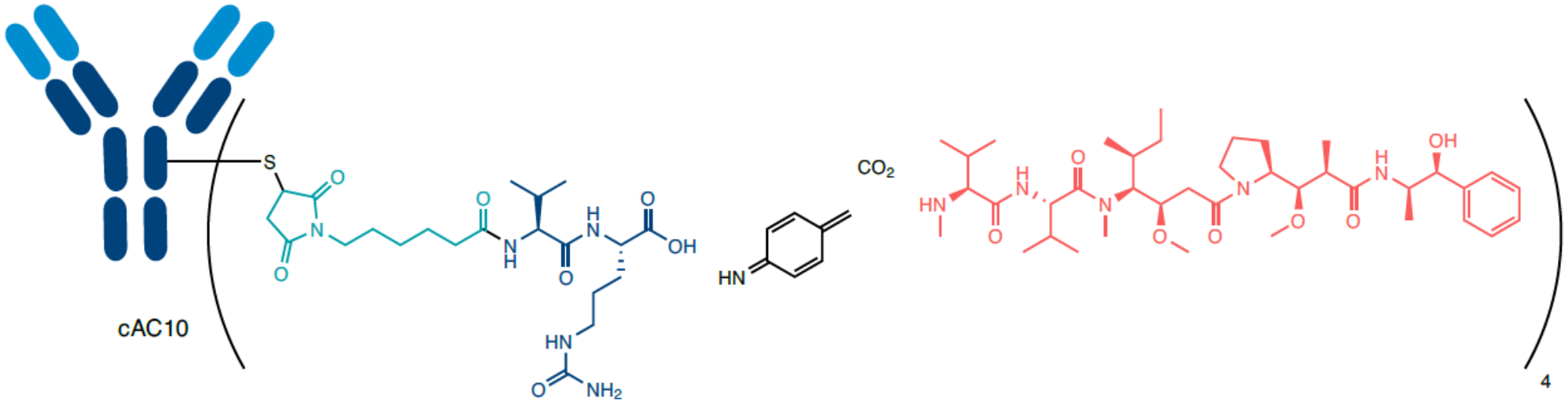
Iris Biotech

LINKEROLOGY®

ADC Pioneers



mAb targeting CD30 overexpressed in Hodgkin's lymphoma

cysteine-maleimide thioether conjugation

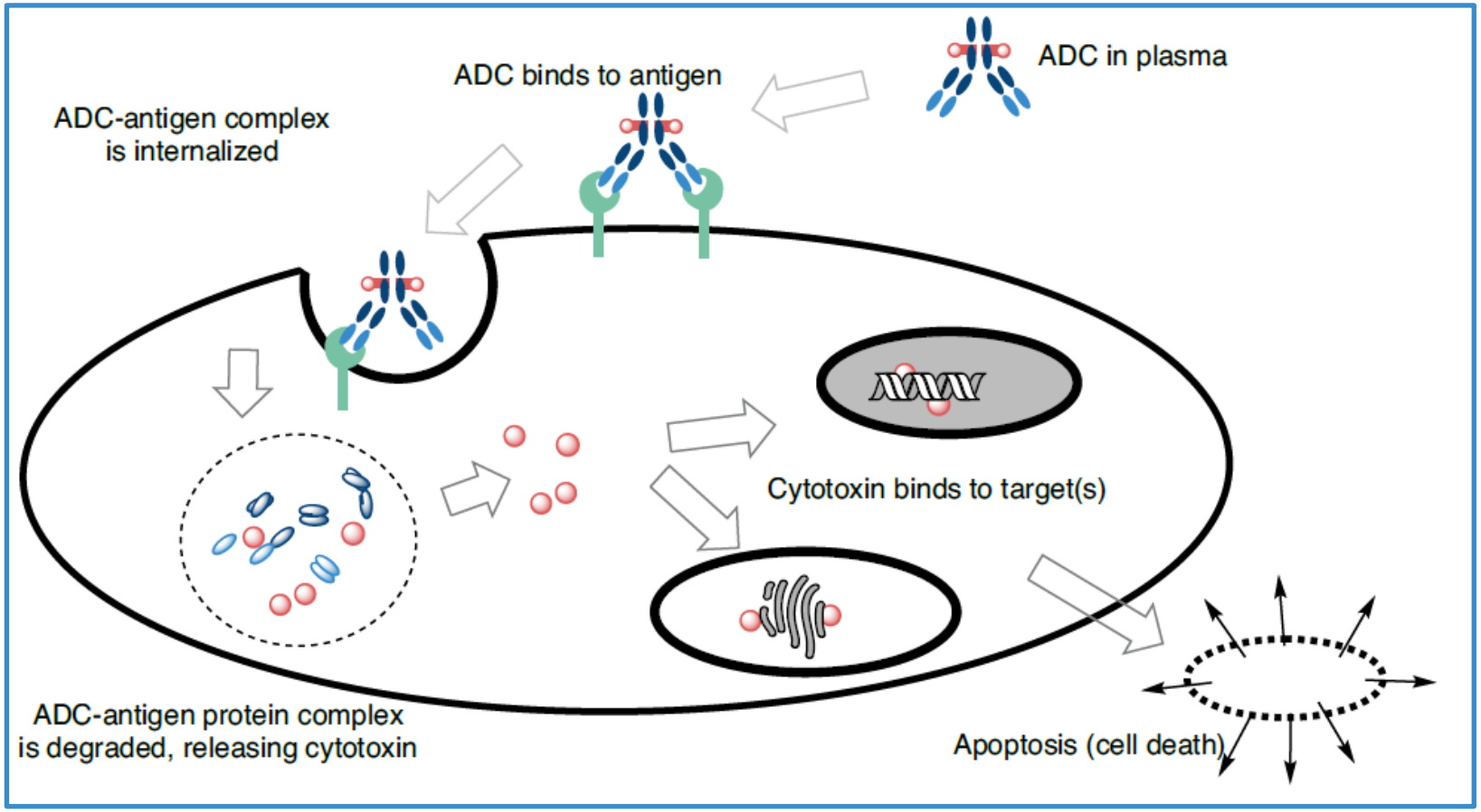
Val-Cit cathepsin cleavage site

self-immolative linker

antimitotic agent monomethyl auristatin E

Adcetris®	approved 2011	Hodgkin's lymphoma and systemic anaplastic large cell lymphoma (ALCL)	$477 million in 2018
Kadcyla®	approved 2013	HER-2 positive metastatic breast cancer	$981 million in 2018

ADC Mode of Action and Scope of Application



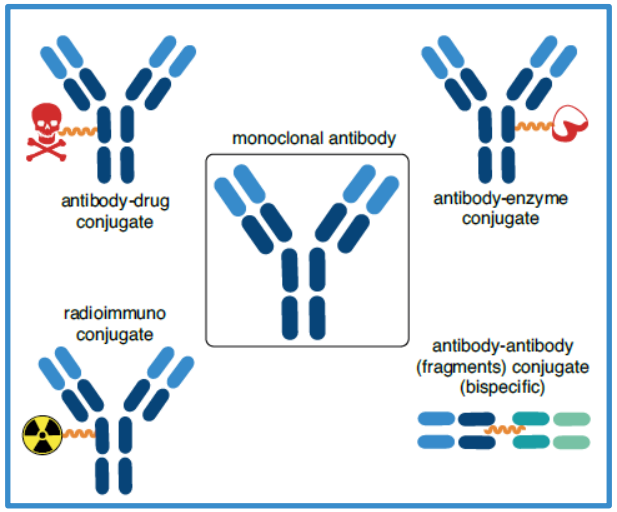
Definition and scope of application:

Conjugation of (a) potent (small) molecule(s) to a target specific vector (e.g. antibody or peptide)

Typical mode of action:

receptor binding → internalization

→ (payload release) → apoptosis



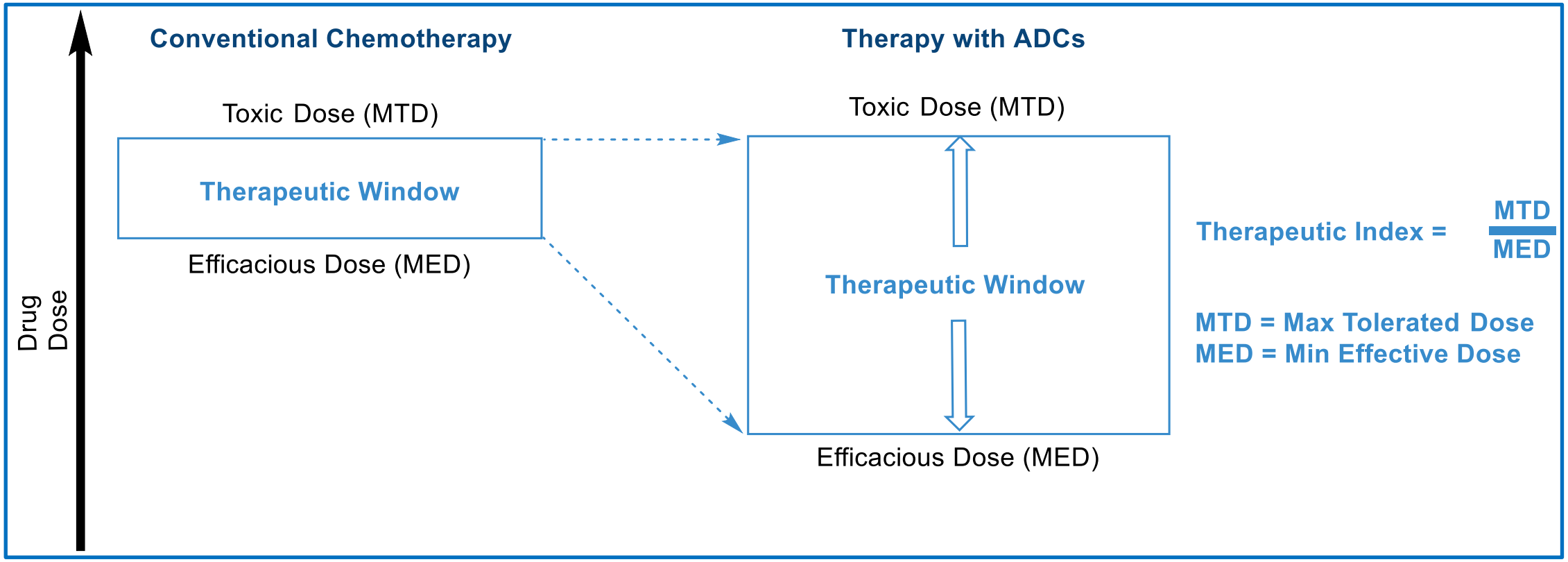
L. Anthony. 2019. ADC Landscape Review [PowerPoint slides]. Retrieved from http://worldadc-usa.com.

Design and Synthesis of Tesirine, a Clinical Antibody-Drug Conjugate Pyrrolobenzodiazepine Dimer Payload; A. C. Tiberghien, J. N. Levy, L. A. Masterson, N. V. Patel, L. R. Adams, S. Corbett, D. G. Williams, J. A. Hartley, and P. W. Howard; *ACS Med Chem Lett* 2016; **7:** 983-987. https://doi.org/10.1021/acsmedchemlett.6b00062.

*Site-specific antibody drug conjugates for cancer therapy; S. Panowski, S. Bhakta, H. Raab, P. Polakis, J. R. Junutula; MAbs 2014; **6**: 34-45. https://doi.org/10.4161/mabs.27022*

*Advances in Precision Oncology: Targeted Thorium-227 Conjugates As a New Modality in Targeted Alpha Therapy; U. B. Hagemann, K. Wickstroem, S. Hammer, R. M. Bjerke, S. Zitzmann-Kolbe, O. B. Ryan, J. Karlsson, A. Scholz, H. Hennekes, D. Mumberg , A. S. Cuthbertson; Cancer Biother Radiopharm 2020; **35(7)**: 497-510. https://doi.org/10.1089/cbr.2020.3568*

Antibody-Drug Conjugate Market



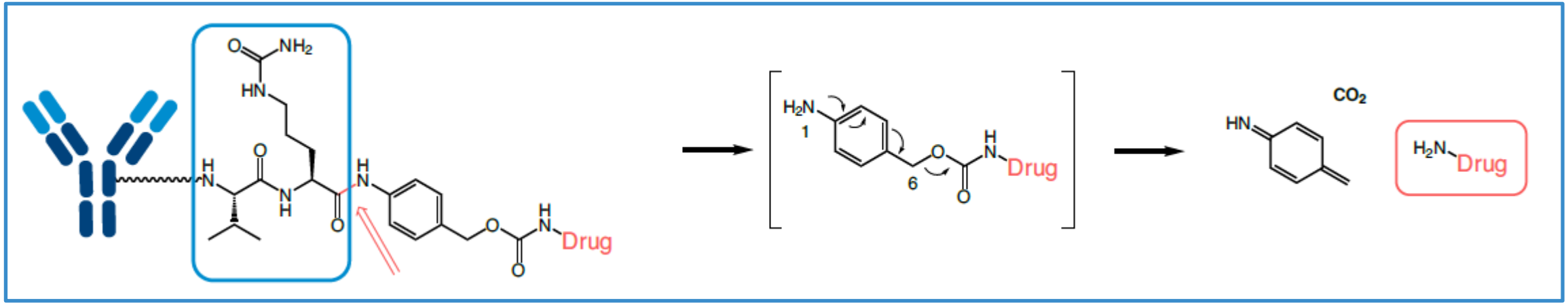
- The therapeutic window of small molecule drugs is significantly enlarged using ADCs compared to small-molecule drugs.

Antibody-Drug Conjugate Market

YEAR	USA	EU & UK	JAPAN	CHINA
2011	ADCETRIS			
2012		ADCETRIS		
2013	Kadcyla	Kadcyla		
2014			ADCETRIS, Kadcyla	
2015				
2016				
2017	BESPONSA, MYLOTARC	BESPONSA		
2018	LUMOXITI	MYLOTARC		
2019	POLIVY, ENHERTU, PADCEV			
2020	TRODELVY, BLENREP	BLENREP, POLIVY	AKALUX, ENHERTU	ADCETRIS, Kadcyla
2021	Zynlonta, Tivdak	ENHERTU		Disitamab Vedotin

- In 2019, there were approximately 100 ADCs in various phases of clinical development, of which some 20 ADCs were carrying auristatins, PBD derivatives, and maytansins, respectively.

The Concept of Self-Immolative Linkers



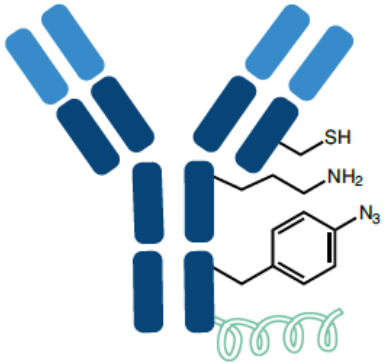
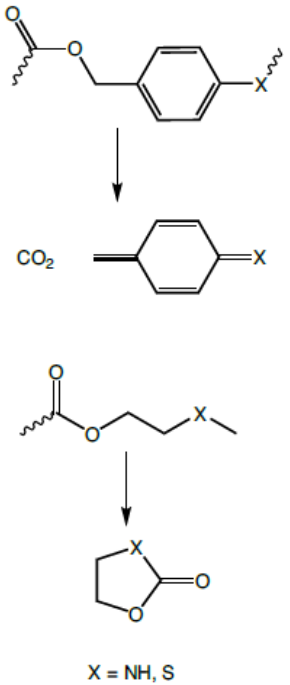
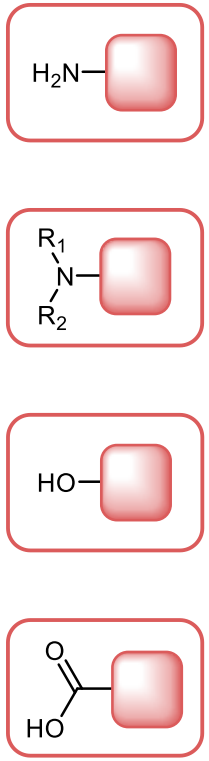
Mode of action: receptor binding → internalization → **payload release** → apoptosis

a) valyl-citrullyl dipeptide fragment serves as substrate for cathepsin B and suffers cleavage by hydrolysis
b) leading to a 1,6-elimination with fragmentation and traceless release of the drug molecule

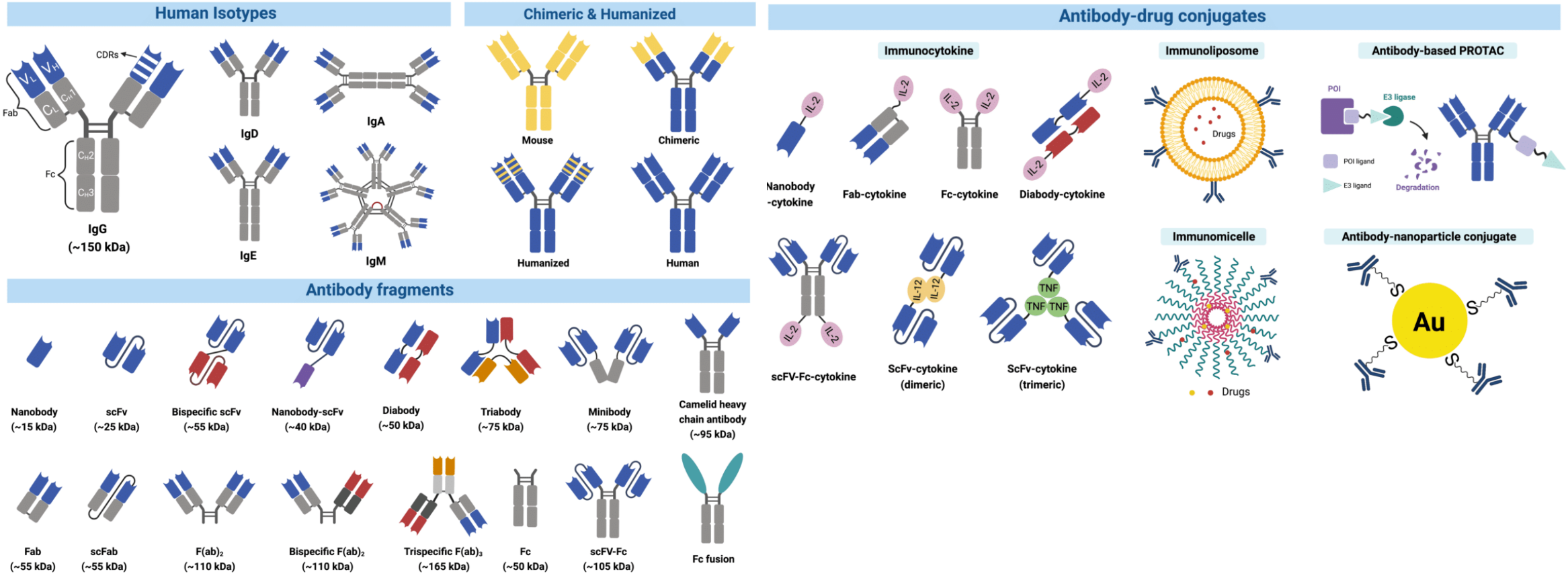
➔ Val-Cit can be substituted by Val-Ala
➔ substrates like Phe-Lys, Gly-Phe-Leu-Gly, and also Ala-Leu-Ala-Leu have been reported

Linker Technologies for Antibody–Drug Conjugates; B. Nolting; *Antibody-Drug Conjugates L. Ducry* 2013; **1045**: 71-100. https://doi.org/10.1007/978-1-62703-541-5_5

Conceptual Overview of Antibody-Drug Conjugation

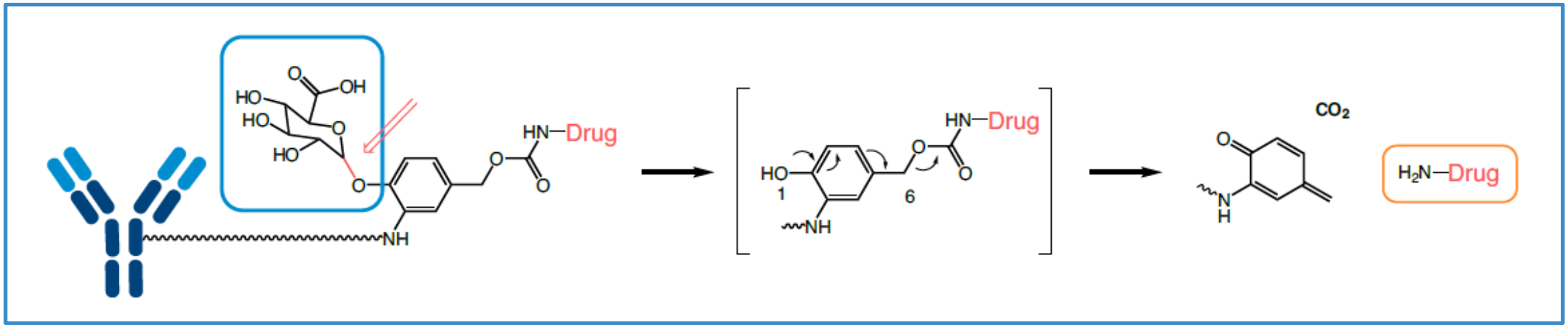
Antibody	Linker			Payload
Natural Connectivities: thiols (Cys) amines (Lys) 	Conjugation	Cleavable Part	Traceless Part	
	Chemically: maleimide disulfide acid/active ester Click tetrazine/TCO His-Tag specific acylation	***Hydrolases:*** Val-Ala Val-Cit Phe-Lys Gly-Phe-Leu-Gly Ala-Leu-Ala-Leu cyclobutyl-Ala cyclobutyl-Cit glucuronic acid		
Artificial Connectivities: azides and alkynes peptides (ligases) His-Tag	***Enzymatically:*** $(Gly)_3$-linker ligase substrate	***Oxidoreductases:*** $-CH_2-S-S-CH_2-$ $-CH_2-S-S-CHMe-$ $-CH_2-S-S-CMe_2-$		

Conceptual Overview of Antibody-Drug Conjugation



Singh, S. et al. Monoclonal Antibodies: A Review. *Curr Clin Pharmacol* 13, 85-99, doi:10.2174/1574884712666170809124728 (2018).

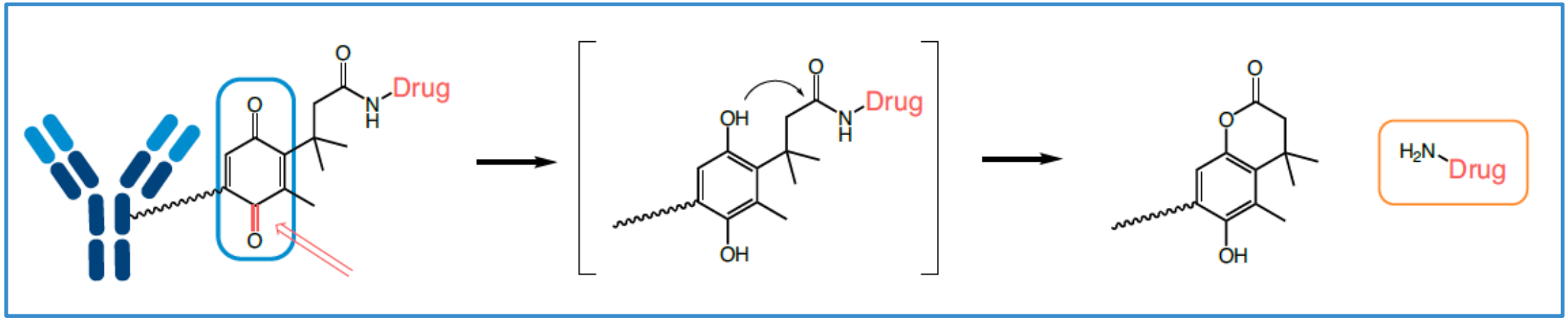
Alternative Cleavage Mechanisms - Glucuronidase



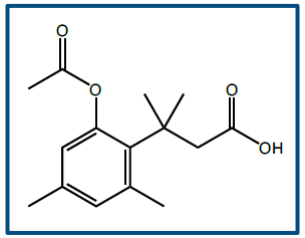
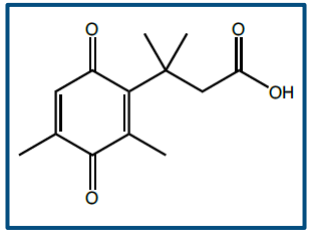
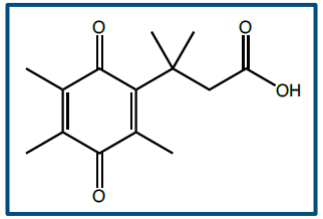
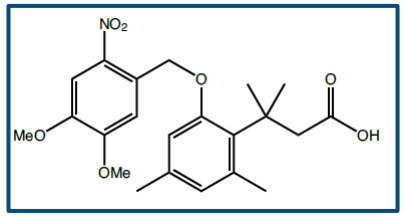
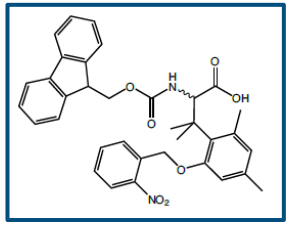
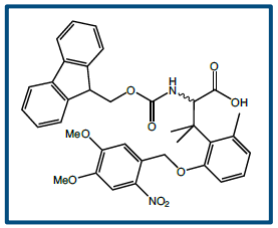
- In an extension to the peptide-based linker strategies to provide high ADC stability, β-glucuronic acid-based linkers were developed.
- Facile release of the active drug is realized through cleavage of the β-glucuronide glycosidic bond by the lysosomal enzyme β-glucuronidase.
- This enzyme is abundantly present in lysosomes and overexpressed in some tumor types, while its activity outside cells is low.
- The linker is hydrophilic, stable against circulation, and provides ADCs that are highly active both in vitro and in vivo.

Linker Technologies for Antibody–Drug Conjugates; B. Nolting; *Antibody-Drug Conjugates L. Ducry* 2013; **1045**: 71-100. https://doi.org/10.1007/978-1-62703-541-5_5

Alternative Cleavage Mechanisms – Trimethyl-Lock

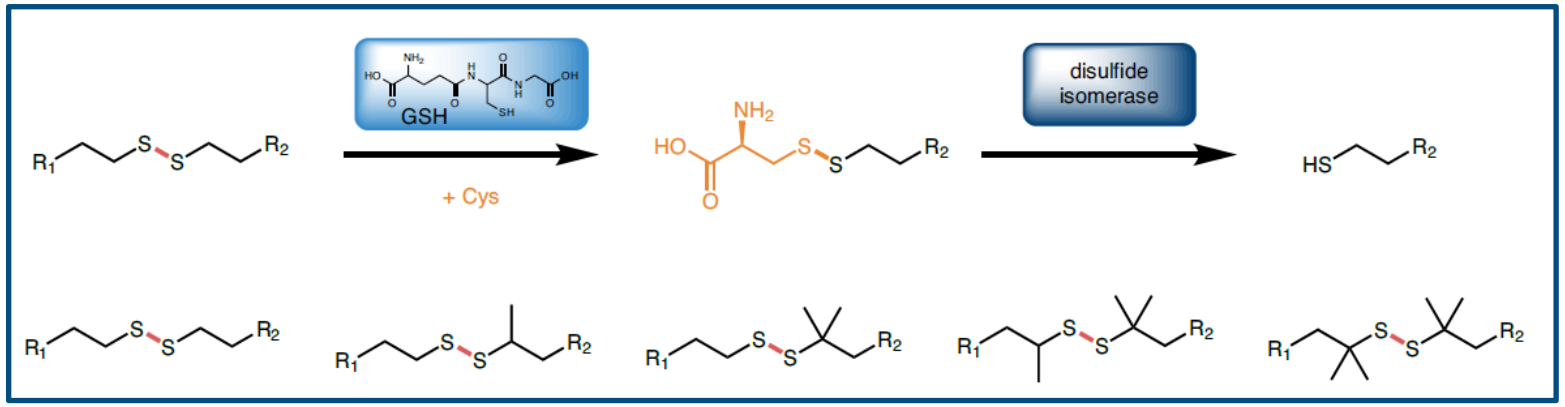


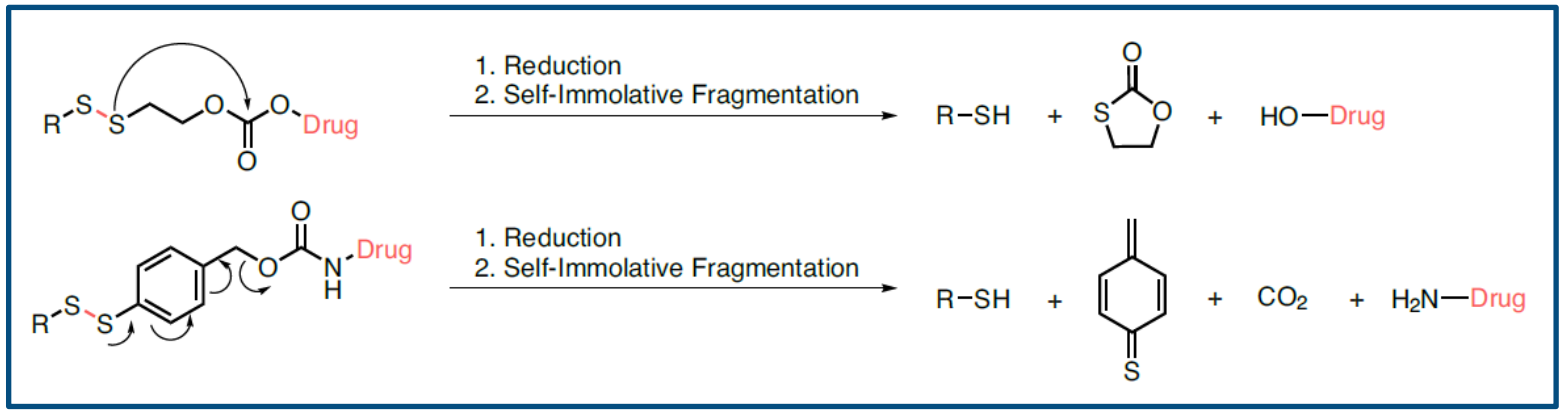
- oxidoreductases (cytochrome P450 oxidoreductase (CPR), nitroquinone oxidoreductase 1 (NQO1)) and cellular reductants such as glutathione (GSH) transform reducible fragments like chinone or disulfide toself-immolative intermediates.
- other precursors with the deprotection and cleavage mechanims hydrolysis and photo-clevage are available

*Trimethyl lock: A trigger for molecular release in chemistry, biology, and pharmacology; M. N. Levine, R. T. Raines; **Chem. Sci.** 2012; **3**: 2412-2420. https://doi.org/10.1039/C2SC20536J*
*Photo-triggered fluorescent labelling of recombinant proteins in live cells; D. Jung, K. Sato, K. Min, A. Shigenaga, J. Jung, A. Otaka, Y. Kwon; **Chem Commun** 2015; **51**: 9670-3. https://doi.org/10.1039/C2SC20536J*
*Detection of DT-diaphorase Enzyme with a ParaCEST MRI Contrast Agent; I. Daryaei, K. M. Jones, M. D. Pagel; **Chemistry** 2017; **23**: 6514-6517. https://doi.org/10.1002/chem.201700721*
*Syntheses and kinetic studies of cyclisation-based self-immolative spacers; S. Huvelle, A. Alouane, T. Le Saux, L. Jullien, F. Schmidt; **Org Biomol Chem** 2017; **15**: 3435-3443. https://doi.org/10.1039/c7ob00121e*
*Invention of stimulus-responsive peptide-bond-cleaving residue (Spr) and its application to chemical biology tools; A. Shigenaga, J. Yamamoto, T. Kohiki, T. Inokuma, A. Otaka; **J Pept Sci** 2017; **23**: 505-513. https://doi.org/10.1002/psc.2961*
*Trimethyl Lock: A Multifunctional Molecular Tool for Drug Delivery, Cellular Imaging, and Stimuli-Responsive Materials; O. A. Okoh, P. Klahn; **ChemBioChem** 2018; **19**: 1668-1694. https://doi.org/10.1002/cbic.201800269*

Alternative Cleavage Mechanisms – Reductive Cleavage of Disulfide Moieties





a) disulfide linkers are first degraded in the lysosome to generate a cysteine-disulfide catabolite

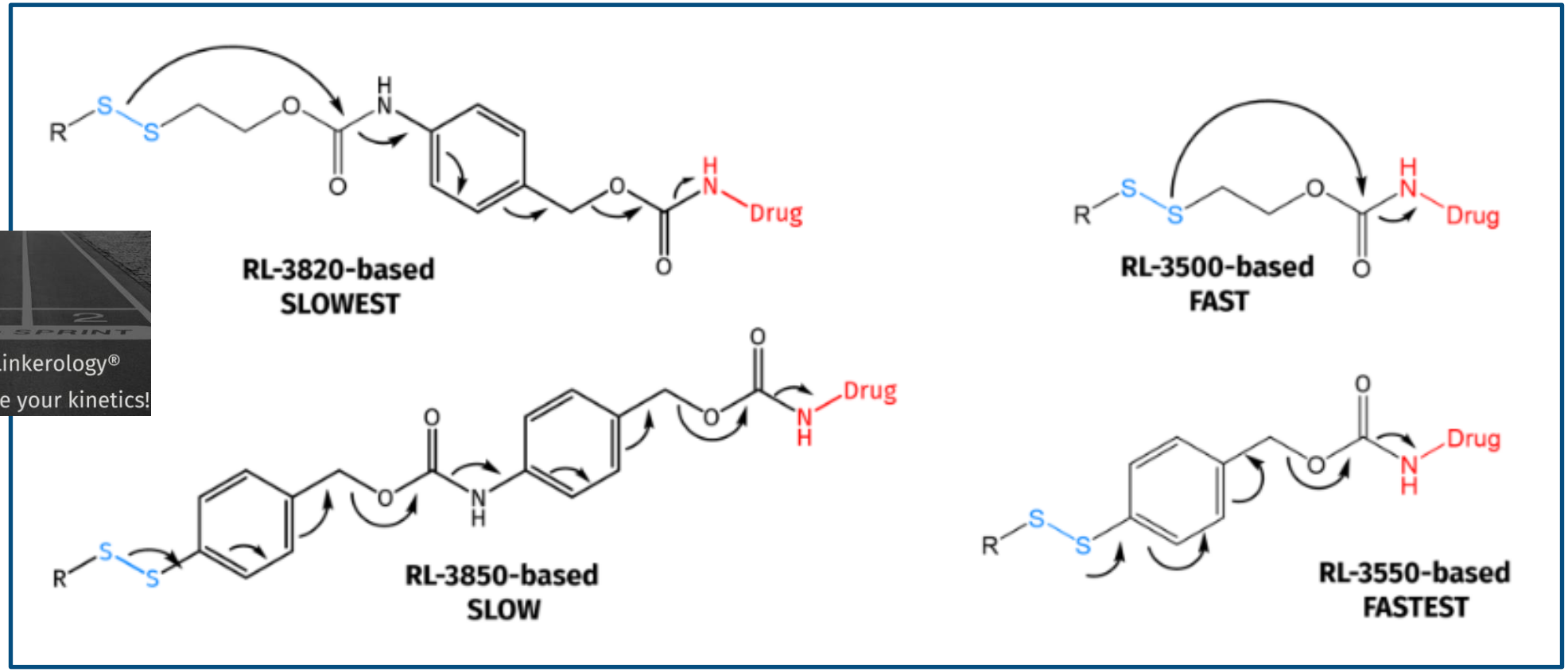
b) followed by disulfide reduction in the cytosol by cellular reductants such as GSH

c) the kinetics of reduction can be tailored by neighboring one to four methyl groups next to both sulfurs

d) Or by using either aliphatic or aromatic linkers, which undergo a different cleavage mechanism

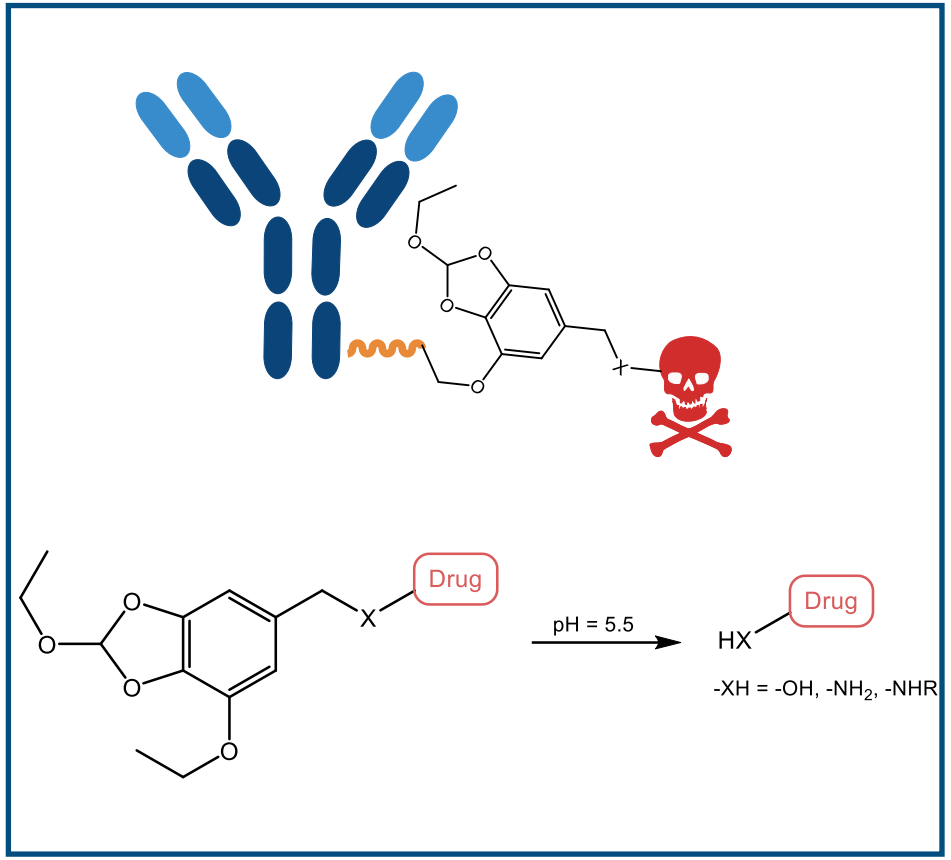
Linker Technologies for Antibody–Drug Conjugates; B. Nolting; *Antibody-Drug Conjugates L. Ducry* 2013; **1045**: 71-100. https://doi.org/10.1007/978-1-62703-541-5_5
Modulating Therapeutic Activity and Toxicity of Pyrrolobenzodiazepine Antibody-Drug Conjugates with Self-Immolative Disulfide Linkers; T. H. Pillow, M. Schutten, S. F. Yu, R. Ohri, J. Sadowsky, K. A. Poon, W. Solis, F. Zhong, G. Del Rosario, M. A. T. Go, J. Lau, S. Yee, J. He, L. Liu, C. Ng, K. Xu, D. D. Leipold, A. V. Kamath, D. Zhang, L. Masterson, S. J. Gregson, P. W. Howard, F. Fang, J. Chen, J. Gunzner-Toste, K. K. Kozak, S. Spencer, P. Polakis, A. G. Polson, J. A. Flygare and J. R. Junutula; *Mol. Cancer. Ther.* 2017; **16**: 871-878. https://doi.org/10.1158/1535-7163.MCT-16-0641.
Mechanisms of drug release in nanotherapeutic delivery systems; P. T. Wong and S. K. Choi; *Chem Rev* 2015; **115**: 3388-432. https://doi.org/10.1021/cr5004634.
Expanded Utility of the beta-Glucuronide Linker: ADCs That Deliver Phenolic Cytotoxic Agents; S. C. Jeffrey, J. De Brabander, J. Miyamoto and P. D. Senter; *ACS Med Chem Lett* 2010; **1**: 277-80. https://doi.org/10.1021/ml100039h.

Alternative Cleavage Mechanisms – Finetuning the Cleavage of Disulfide Moieties

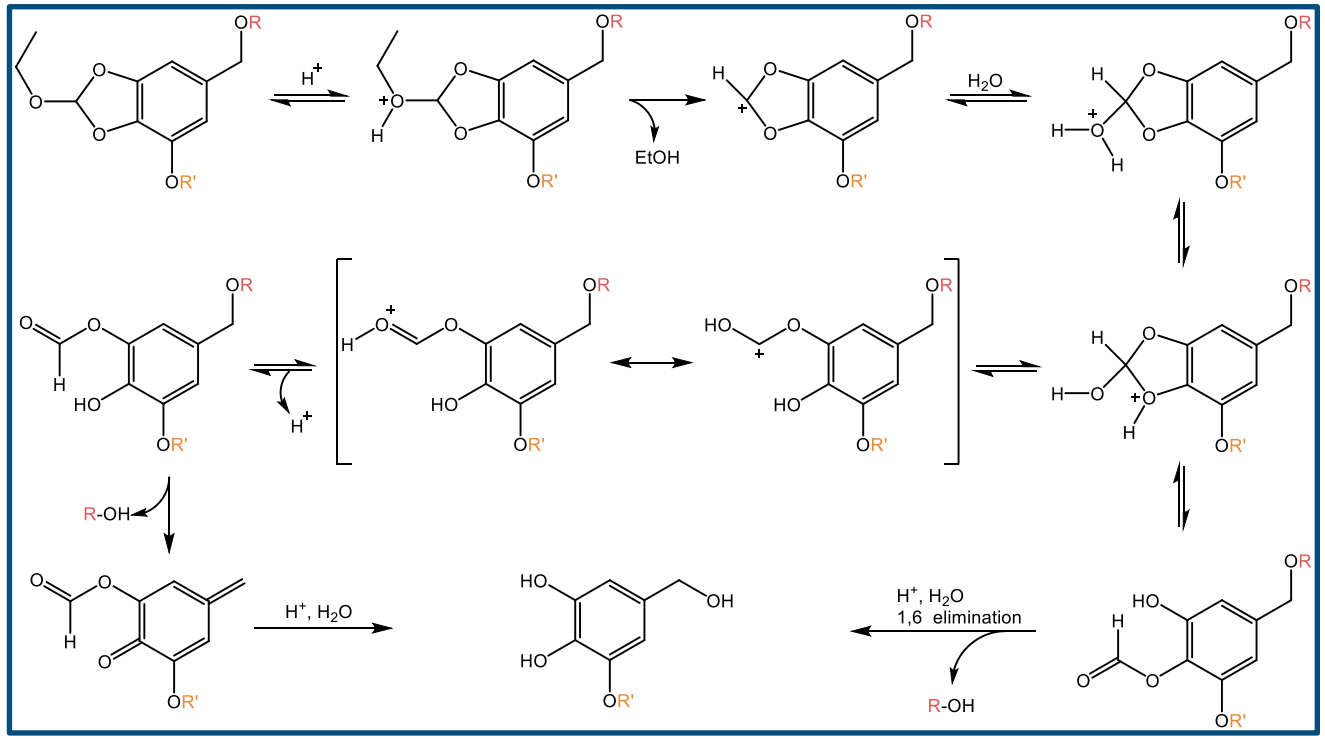


Disulfide-Based Self-Immolative Linkers and Functional Bioconjugates for Biological Applications; Z. Deng, J. Hu and S. Liu; *Macromol Rapid Commun* 2020; **41:** e1900531. https://doi.org/10.1002/marc.201900531

New pH-sensitive Linker: HMPO
5-(hydroxymethyl)pyrogallol orthoester



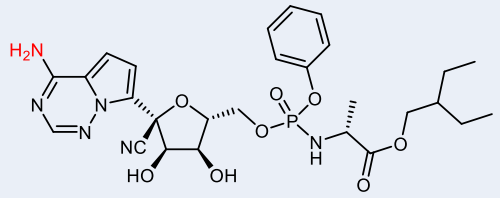
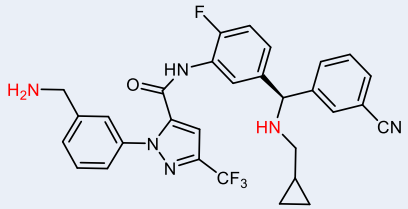
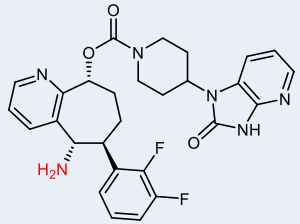
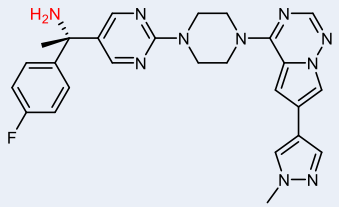
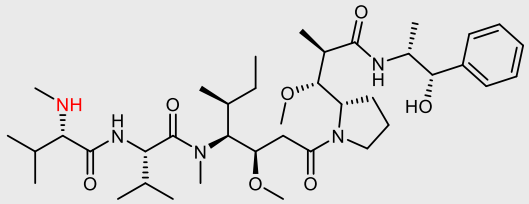
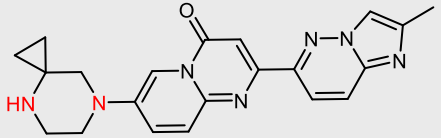
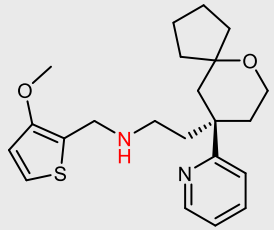
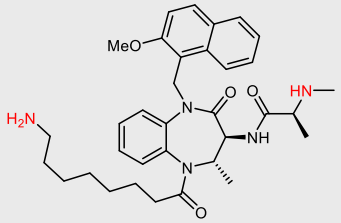
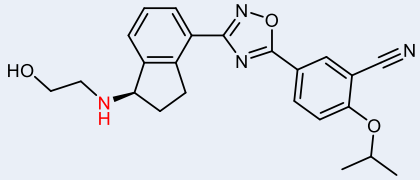
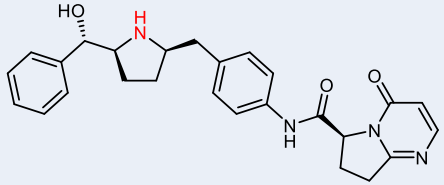
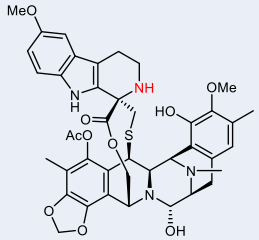
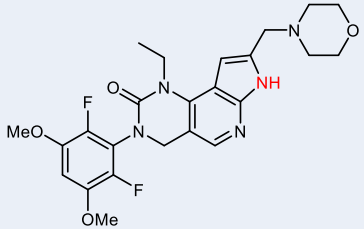
Purposed mechanism:



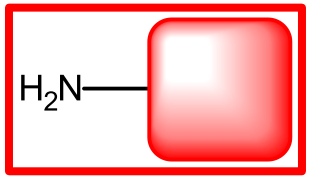
A pH-responsive crosslinker platform for antibody-drug conjugate (ADC) targeting delivery; F. Migliorini, E. Cini, E. Dreassi, F. Finetti, G. Ievoli, G. Macri, E. Petricci, E. Rango, L. Trabalzini, M. Taddei; Chem. Commun. 2022; 58(75): 10532-10535. https://doi.org/10.1039/D2CC03052G

A Self-Immolative Linker for the pH-Responsive Release of Amides; A. Petrini, G. Ievoli, F. Migliorini, M. Taddei, S. Siciliano; Molecules 2023; 28(6): 2445. https://doi.org/10.3390/molecules28062445

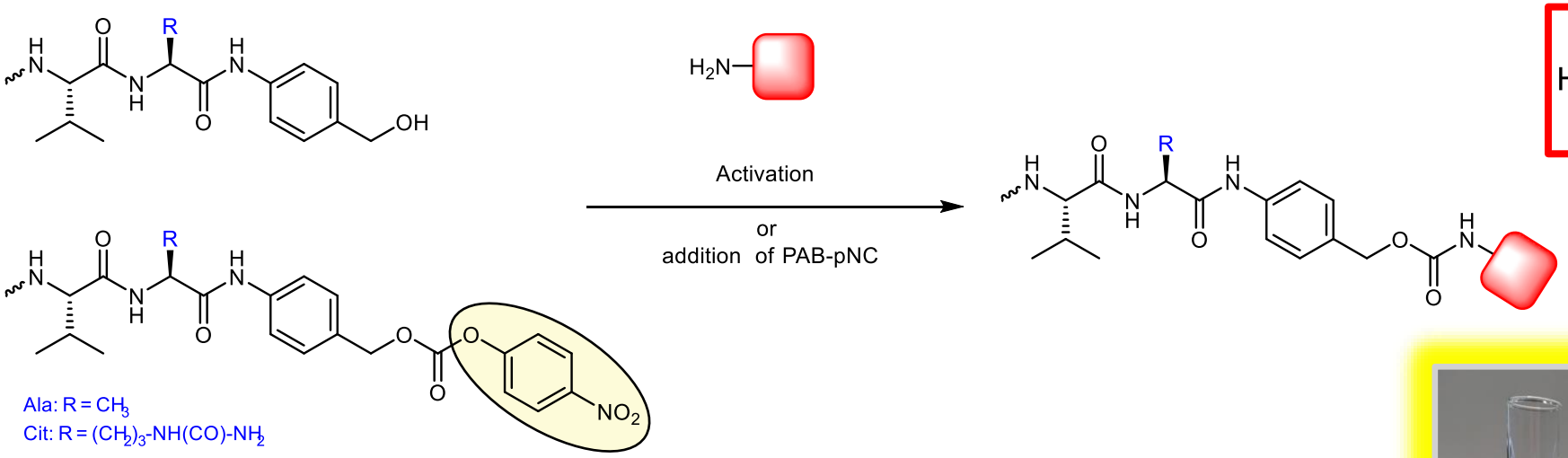
Suitable Payloads for Linker-Payload Conjugates – Primary and Secondary Amines

Remdesivir (Veklury)	Berotralstat (Orladeyo)	Rimegepant (Nurtec ODT)	Avapritinib (Ayvakit)
			
IV nucleotide RNA polymerase inhibitor infectious disease – COVID-19	Oral plasma kallikrein inhibitor hematology – hereditary angioedema	Oral CGRP GPCR antagonist neurology - migraine	Oral PDGFRA + KIT kinase inhibitor oncology – unresectable or met. GIST
Monomethyl auristatin E (MMAE)	**Risdiplam (Evrysdi)**	**Oliceridine (Olinvyk)**	
			
tubulin inhibitor ADC - oncology	oral SMN RNA splicing modifier neurology - spinal muscular atropy (SMA)	IV opioid receptor agonist neurology – acute pain incertain adults	XIAP E3-ligase degrader PROTAC
Ozanimod (Zeposia)	**Vibegron (Gemtesa)**	**Lurbinectedin (Zepzelca)**	**Pemigatinib (Pemazyre)**
			
Oral S1P(1/5) receptor modulator neurology – multiple sclerosis	Oral selective β3 adrenergic receptor agonist urology – oberactive bladder	IV DNA alkylating agent oncology – met. SCLC	Oral FGFR1/2/3 kinase inhibitor oncology – adv. or met. cholangiocarcinoma

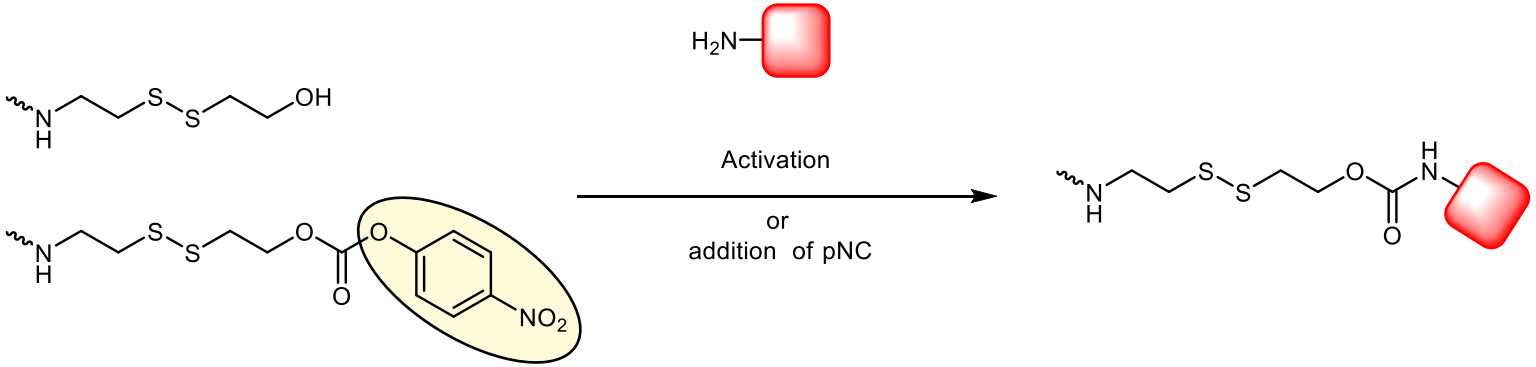
Suitable Payloads for Linker-Payload Conjugates – Primary and Secondary Amines



Peptide based linker:



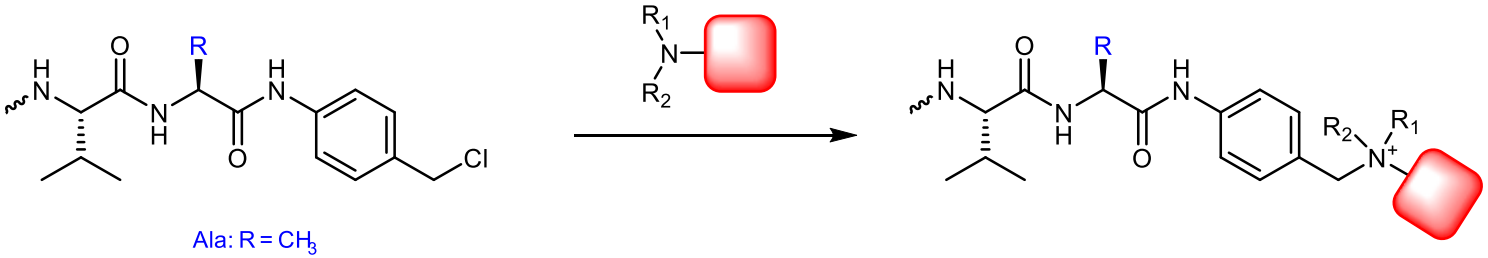
Disulfide based linker:



Linker Technologies for Antibody–Drug Conjugates; B. Nolting; *Antibody-Drug Conjugates L. Ducry* 2013; **1045**: 71-100. https://doi.org/10.1007/978-1-62703-541-5_5
Antibody–drug conjugates: Recent advances in linker chemistry; Z. Su, D. Xiao, F. Xie, L. Liu, Y. Wang, S. Fan, X. Zhou and S. Li; *Acta Pharmaceutica Sinica B* 2021. https://doi.org/10.1016/j.apsb.2021.03.042

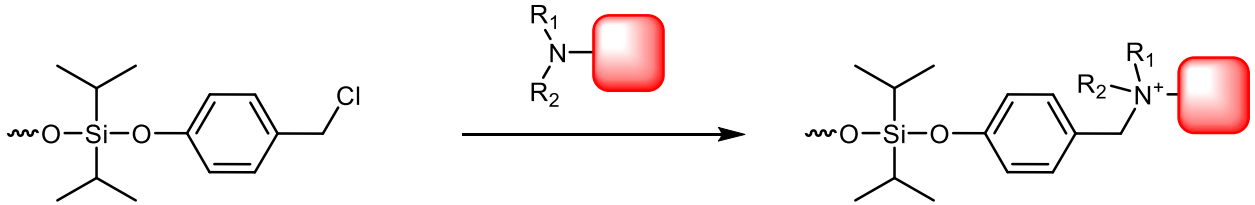
Suitable Payloads for Linker-Payload Conjugates – Tertiary Amines

Peptide based linker:

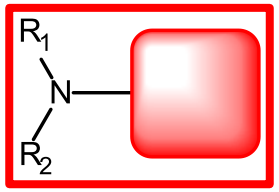


Ala: R = CH_3
Cit: R = $(CH_2)_3$-NH(CO)-NH_2

pH-sensitive linker:



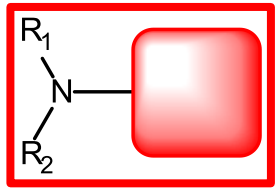
diisopropylsilyl moiety plasma half life > 7 days



Suitable Payloads for Linker-Payload Conjugates – Tertiary Amines

COVID-19 Research

University of California San Francisco (UCSF)
QBI COVID-19 Research Group (QCRG), San Francisco, CA, USA.
Institut Pasteur, Paris, France.
Novartis Institutes for BioMedical Research, Basel, Switzerland & Cambridge, MA, USA.
University of Michigan, Ann Arbor, MI, USA.
Icahn School of Medicine at Mount Sinai, New York, NY, USA.
Harvard Medical School, Boston, MA, USA.
J. David Gladstone Institutes, San Francisco, CA, USA.



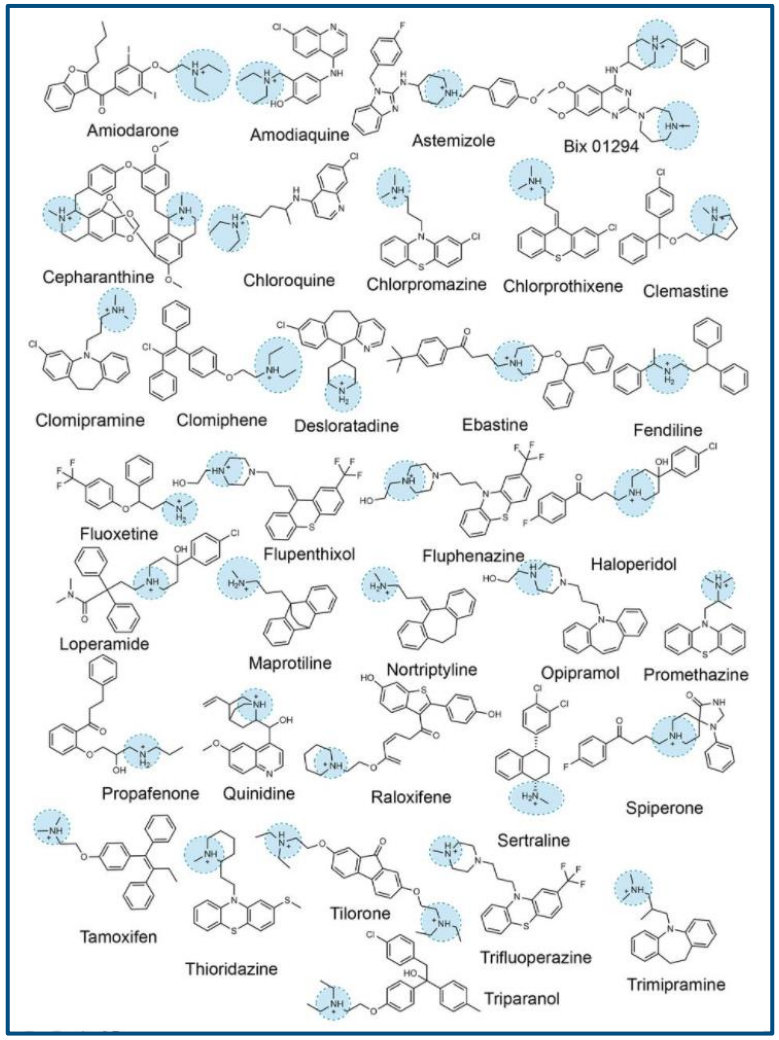
Drug-induced phospholipidosis confounds drug repurposing for SARS-CoV-2

T. A. Tummino, V. V. Rezelj, B. Fischer, A. Fischer, M. J. O'Meara, B. Monel, T. Vallet, K. M. White, Z. Zhang, A. Alon, H. Schadt, H. R. O'Donnell, J. Lyu, R. Rosales, B. L. McGovern, R. Rathnasinghe, S. Jangra, M. Schotsaert, J.-R. Galarneau, N. J. Krogan, L. Urban, K. M. Shokat, A. C. Kruse, A. García-Sastre, O. Schwartz, F. Moretti, M. Vignuzzi, F. Pognan and B. K. Shoichet;

Science 2021: eabi4708.
https://doi.org/10.1126/science.abi4708

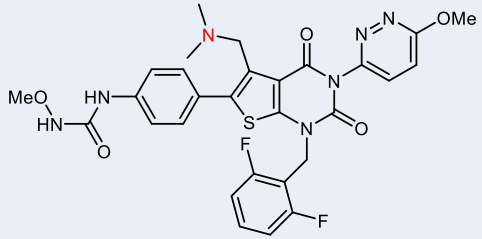
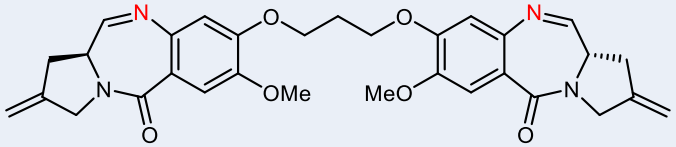
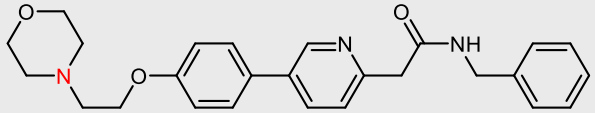
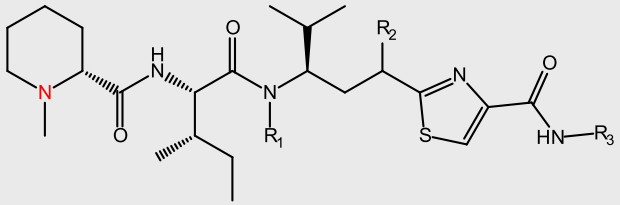
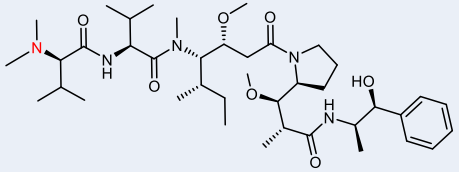
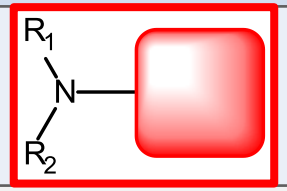
Abstract

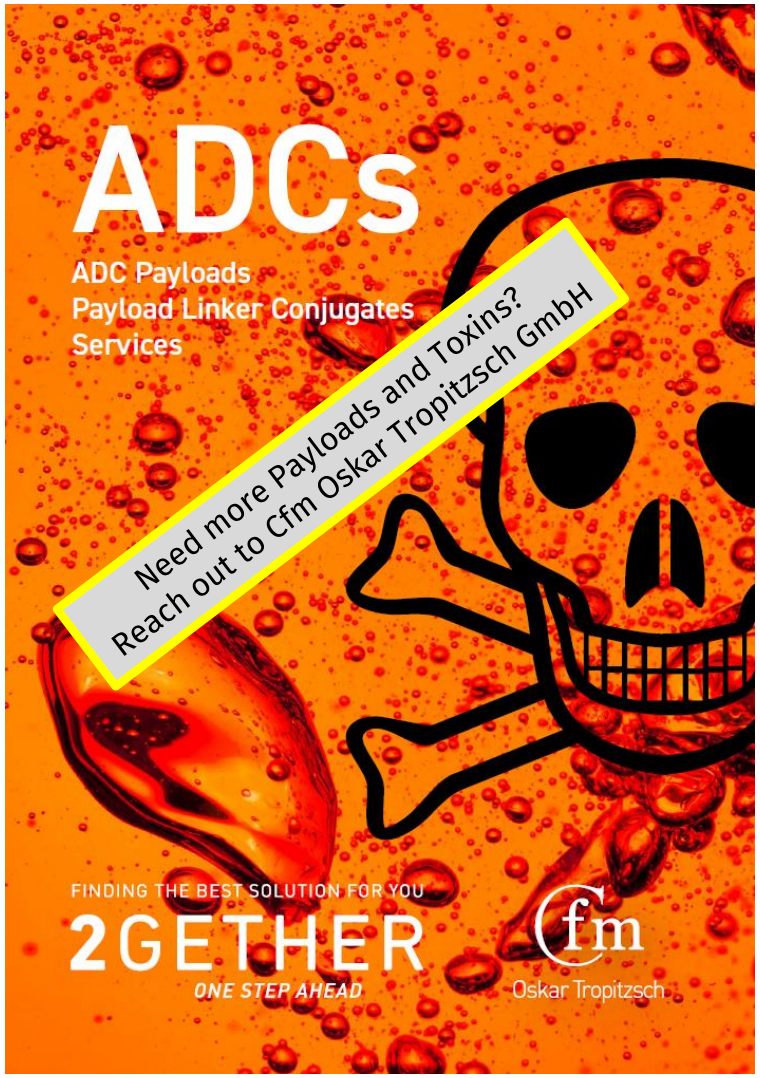
Repurposing drugs as treatments for COVID-19 has drawn much attention. Beginning with sigma receptor ligands and expanding to other drugs from screening in the field, we became concerned that phospholipidosis was a shared mechanism underlying the antiviral activity of many repurposed drugs. For all of the 23 cationic amphiphilic drugs tested, including hydroxychloroquine, azithromycin, amiodarone, and four others already in clinical trials, phospholipidosis was monotonically correlated with antiviral efficacy. Conversely, drugs active against the same targets that did not induce phospholipidosis were not antiviral. Phospholipidosis depends on the physicochemical properties of drugs, and does not reflect specific target-based activities, rather it may be considered a toxic confound in early drug discovery. Early detection of phospholipidosis could eliminate these artifacts, enabling a focus on molecules with therapeutic potential.



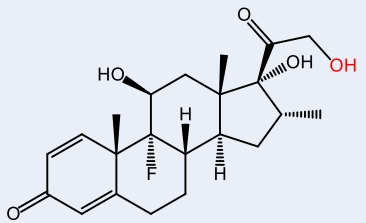
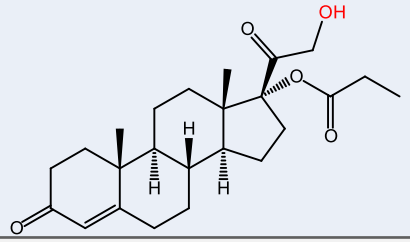
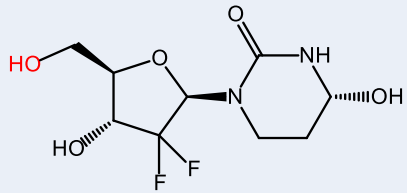
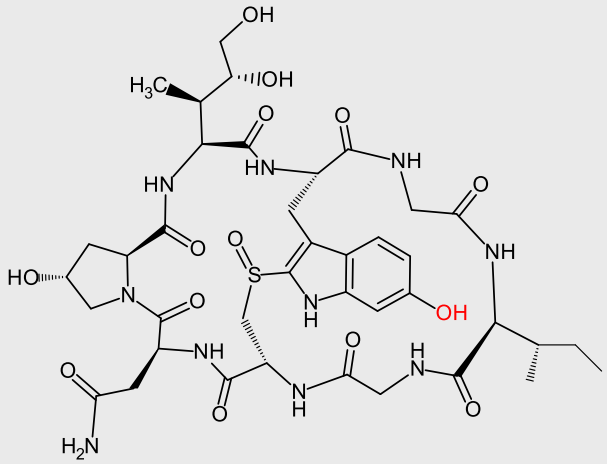
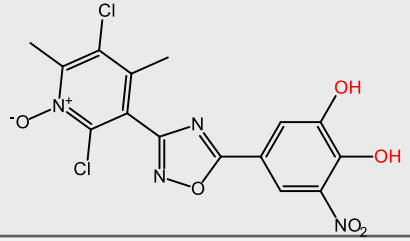
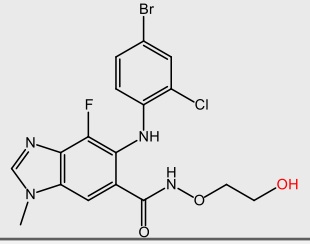
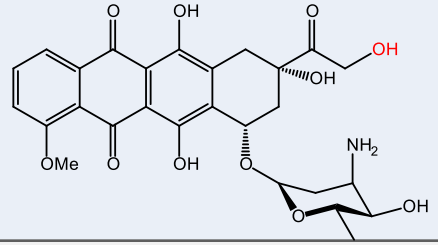
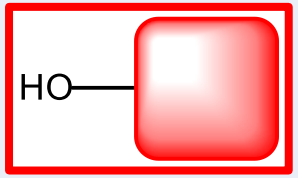
Representative examples of cationic amphiphilic drugs that are identified in SARS-CoV2 drug repurposing screens.

Suitable Payloads for Linker-Payload Conjugates – Tertiary Amines

Relugolix (Orggovyx)	Pyrrolobenzodiazepine Dimer (PBD)
	
Oral GnRH hormone receptor agonist oncology advanced prostate cancer	DNA-crosslinkage ADC - oncology
Tirbanibulin (Klisyri)	**Tubulysin derivatives**
	
topical microtubule inhibitor dermatology – actinic keratosis of face/scalp	tubulin polymerization inhibitor oncology
Auristatin E	
	
tubulin inhibitor ADC - oncology	



Suitable Payloads for Linker-Payload Conjugates – Alcohols

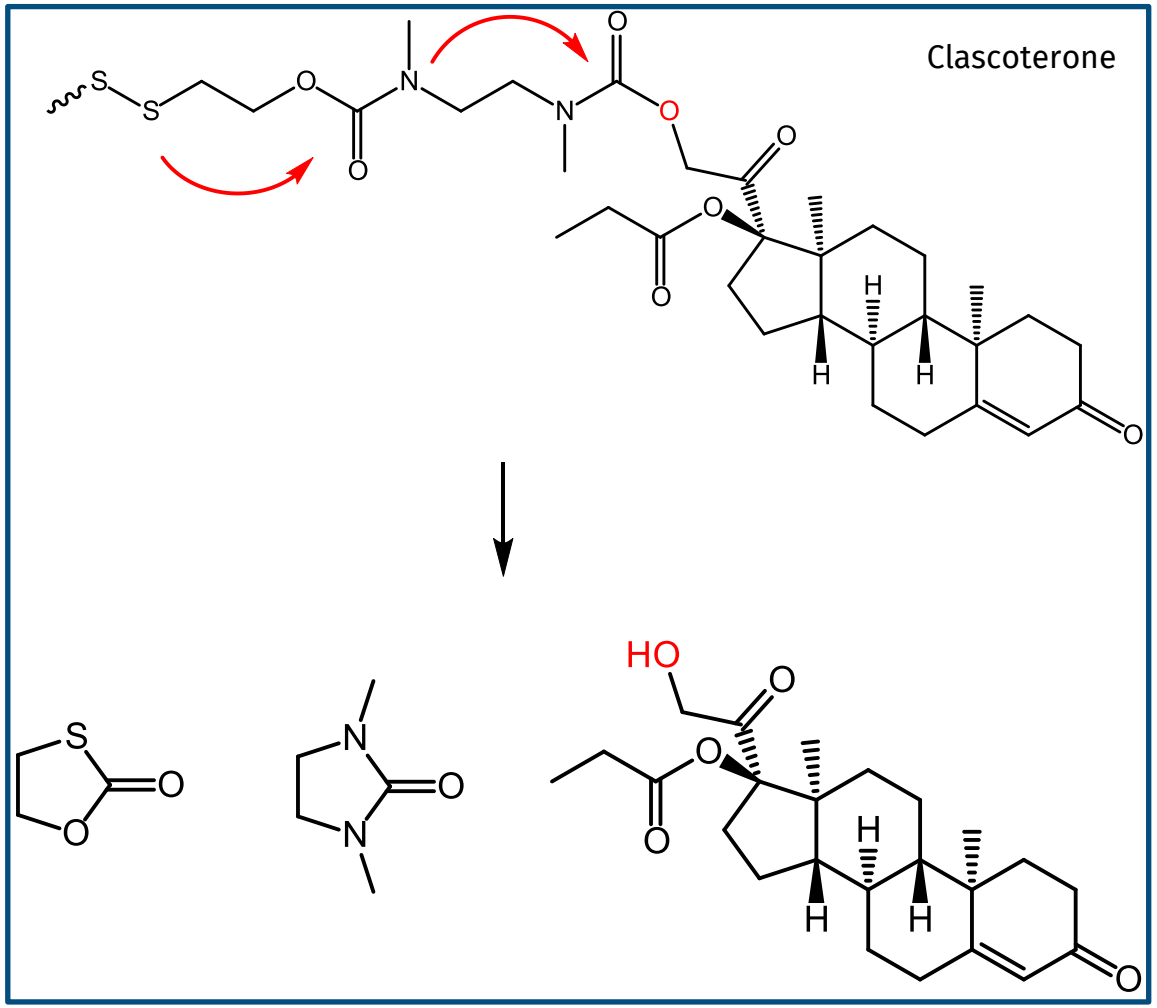
Dexamethason	Clascoterone (Winlevi)	Cedazuridine (Inqovi)
		
Glucocorticoid receptor (GR) agonist rheumatic diseases, allergies, asthma, COVID-19	Topical androgen receptor inhibitor dermatology – acne vulgaris	Oral nucleoside cytidine deaminase inhibitor oncology – myelodysplastic syndromes
α-Amanitin	**Opicapone (Ongentsys)**	**Selumetinib (Koselgo)**
		
	Oral COMT enzyme inhibitor neurology – Parkinson's	Oral MEK1/2 kinase inhibitor neuro-oncology – neurofibromatosis type 1
	Doxorubicin (Adriamycin)	
		
RNA polymerase inhibitor ADC - oncology	DNA intercalation, topoisomerase II inhibition oncology – breast, bladder, Hodgkin's lymphoma, leukemia	

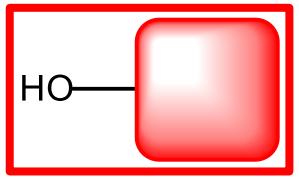
Suitable Payloads for Linker-Payload Conjugates – Alcohols

Introduction of N,N‘-dimethylaminoethyl carbamate (DMAE):

1. step: converting alcohol into amine with a self-immolative fragment

2. step: use all strategies suitable for amine payloads:
   - disulfide linker
   - peptide based linker (vc,va, etc.)
   - pH sensitive linker
   - trimethyl lock





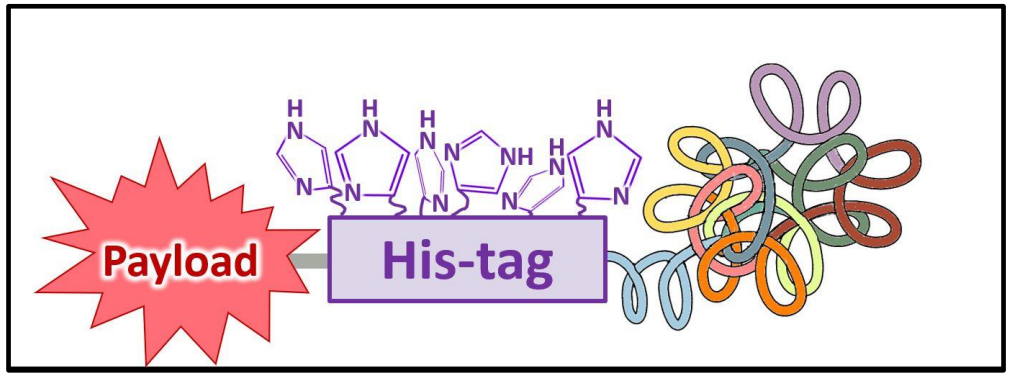
His-Tag for Specific Linker Attachment

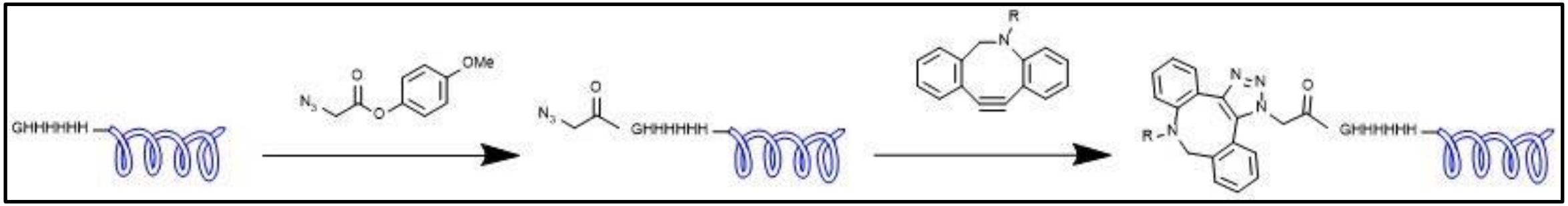
Specific His-Tag Acylation
A Broad Conjugation Methodology for many new Chemical Biology and Biopharmaceutical Applications

1. step: introduction of a His-Tag

2. step: acylation with His-Tag specific linker

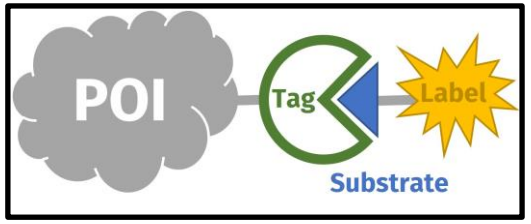
3. step: Click conjugation of small molecule



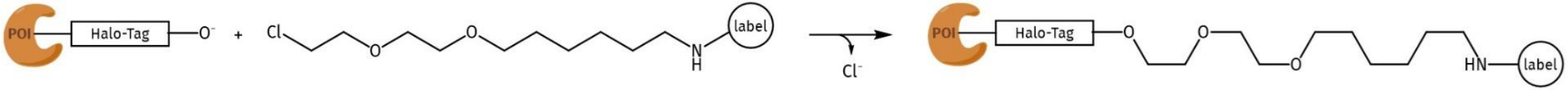


Reference:
Selective N-terminal acylation of peptides and proteins with a Gly-His tag sequence; M. C. Martos-Maldonado, C. T. Hjuler, K. K. Sorensen, M. B. Thygesen, J. E. Rasmussen, K. Villadsen, S. R. Midtgaard, S. Kol, S. Schoffelen and K. J. Jensen; *Nat Commun* 2018; **9**: 3307. https://doi.org/10.1038/s41467-018-05695-3

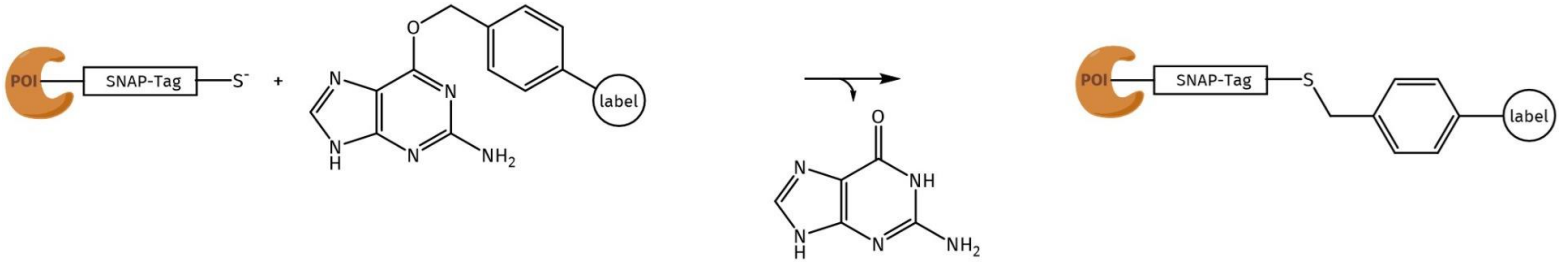
Substrates for Fusion (Halo/Snap/Clip)-Tagged Proteins



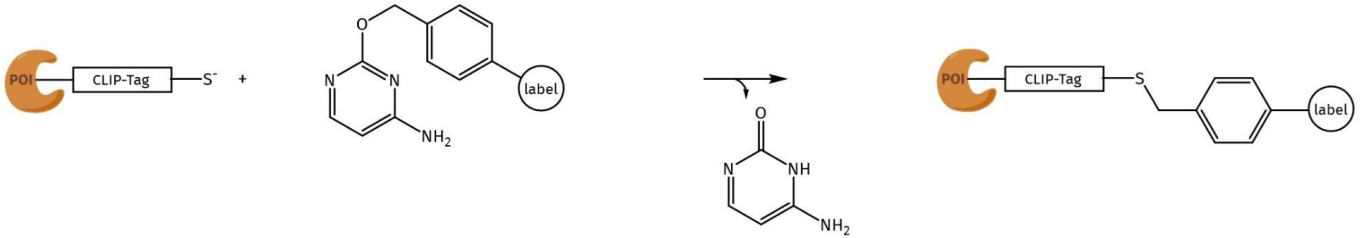
The **HaloTag®**
is a 33 kDa self-labeling protein tag derived from the haloalkane dehalogenase DhaA from *Rhodococcus rhodochrous*. Its active site reacts in a nucleophilic attack with chloroalkane linker substrates to form an irreversible bond in the case of the mutated enzyme. The chloroalkane linker can easily be functionalized with a label of choice, e.g. fluorophore, biotin. For the wild-type enzyme, this intermediate would be hydrolyzed, leading to the regeneration of the enzyme.



The **SNAP-tag®**
is a 20 kDa self-labeling protein tag based on a modified form of the human O6-alkylguanine-DNA-alkyltransferase (hAGT), a DNA repair enzyme. A cysteine residue within the SNAP-tag® undergoes an irreversible reaction with synthetic O6-benzylguanine (BG) derivatives resulting in a covalent thioether bond. The BG moiety can easily be further functionalized with a label of choice, e.g. fluorophore, biotin, generally without affecting the reaction of the substrate with the SNAP-tag®.



The **CLIP-tag™**
(20 kDa) is a modified version of the SNAP-tag, engineered to react with benzylcytosine (BC) instead of benzylguanine (BG). CLIP-tag™- and SNAP-tag®-fused proteins can be labeled simultaneously in the same cells.



SUMMARY:

- ✓ Over 100 **linkers** readily available
- ✓ Custom synthesis of **your proprietary linker**
- ✓ Custom synthesis of **your proprietary linker-payload conjugate**
- ✓ Custom synthesis of **your proprietary antibody-linker-payload conjugate**

➔ Get in contact with us, we are happy to discuss your project!

Knowledge Base