Application of Bioorthogonal Cleavage Reactions (BCRs) in Prodrug Activation

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Prof. Qin Chen

Oct 08, 2021

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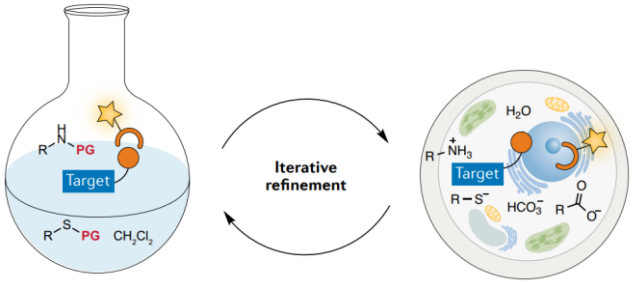
Background

01 Bioorthogonal reactions



Bioorthogonal reactions

Carolyn R. Bertozzi

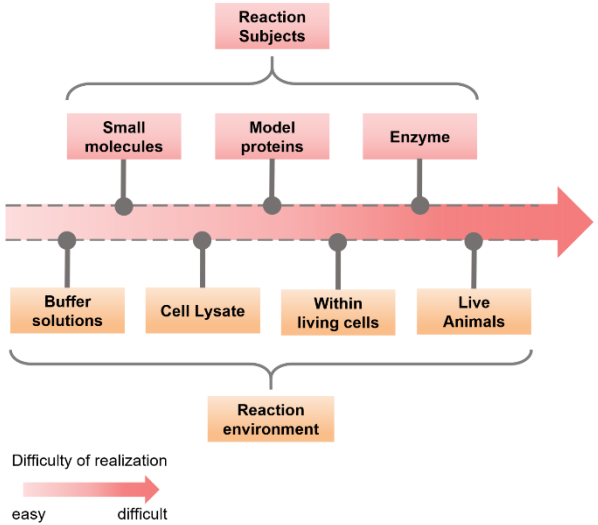
Definition:

Bioorthogonal reactions refers to any chemical reaction that proceeds rapidly and selectively in biological environments without side reactions towards endogenous functional groups.

Reaction characteristics:

- ✓ bioorthogonality
- ✓ biostability
- ✓ nontoxicity
- ✓ fast kinetics
- ✓ appropriate pharmacokinetics (in vivo)

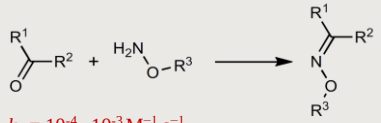
Reaction subjects and environment:



Prescher, J. A. et al. *Nat. Rev. Chem.* **2020**, *4*, 476-489.

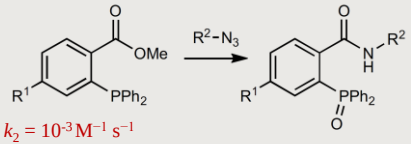
Classification of bioorthogonal reactions (in chemistry)

Oxime and hydrazone ligations 1
Enabled by ketone and aldehyde tags



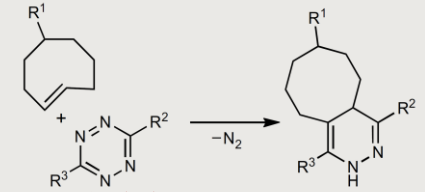
$k_2 = 10^{-4}$ - $10^{-3}\,M^{-1}\,s^{-1}$

Staudinger ligation 2
In vivo formation of amide bonds



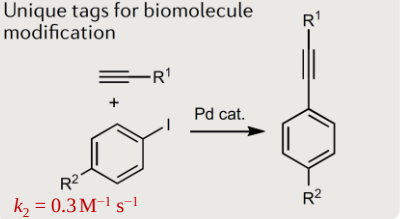
$k_2 = 10^{-3}\,M^{-1}\,s^{-1}$

Tetrazine ligation 7
Exceptionally rapid kinetics



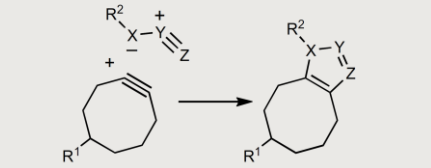
$k_2 = 1$ - $10^{6}\,M^{-1}\,s^{-1}$

Palladium and ruthenium-catalysed couplings 3
Unique tags for biomolecule modification



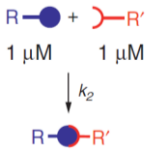
$k_2 = 0.3\,M^{-1}\,s^{-1}$

Strain-promoted [3 + 2] cycloaddition 4
Copper-free coupling with 1,3-dipoles



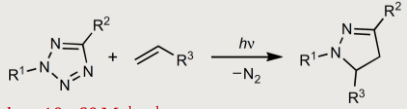
$k_2 = 10^{-2}$ - $1\,M^{-1}\,s^{-1}$

Relationship between reaction rate and half-life at a concentration of 1 μM



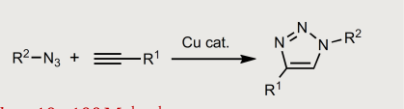
$k_2\ (M^{-1}s^{-1})$	$t_{1/2}$
10^5	10 sec
10^4	1.7 min
10^3	17 min
10^2	2.8 h
10	1.2 days
1	12 days
10^{-1}	4 months
10^{-2}	3 years

Photoinducible bioorthogonal chemistry 5
Spatio-temporal control with light



$k_2 = 10$ - $60\,M^{-1}\,s^{-1}$

Copper-catalysed azide–alkyne cycloaddition 6
Minimal size, readily accessible, broadly useful

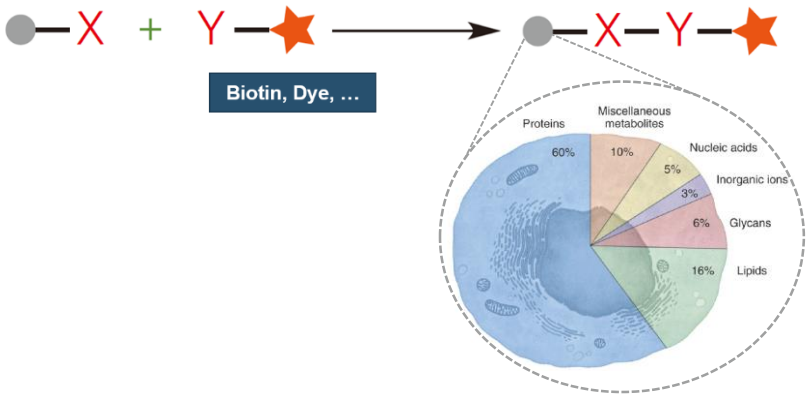


$k_2 = 10$ - $100\,M^{-1}\,s^{-1}$

1. Bertozzi, C. R. et al. *Science.* **1997**, *276*, 1125-1128.
2. Bertozzi, C. R. et al. *Science.* **2000**, *287*, 2007-2010.
3. Davis, B. G. et al. *J. Am. Chem. Soc.* **2013**, *135*, 12156-12159.
4. Bertozzi, C. R. et al. *J. Am. Chem. Soc.* **2004**, *126*, 15046-15047.
5. Lin, Q. et al. *Angew. Chem. Int. Ed.* **2012**, *124*, 10752-10756.
6. Meldal, M. et al. *J. Org. Chem.* **2002**, *67*, 3057-3064.
7. Davis, B. G. et al. *J. Am. Chem. Soc.* **2008**, *130*, 13518-13519.

Classification of bioorthogonal reactions (in application)

➢ Bioorthogonal ligation reactions

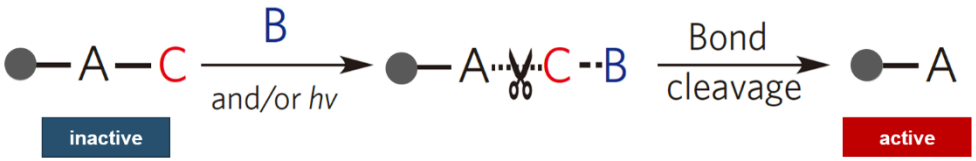


"labeling"

"capturing"

"tracing"

➢ Bioorthogonal cleavage reactions



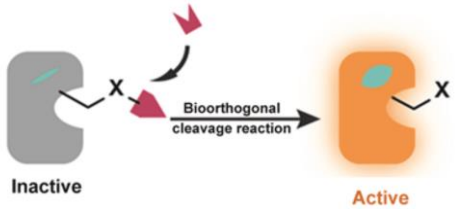
"releasing"

"activating"

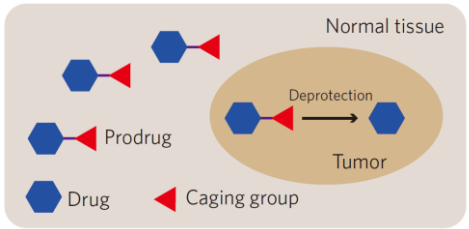
"controlling"

Devaraj, N. K. et al. *ACS Cent. Sci*. **2018**, *4*, 952-959.

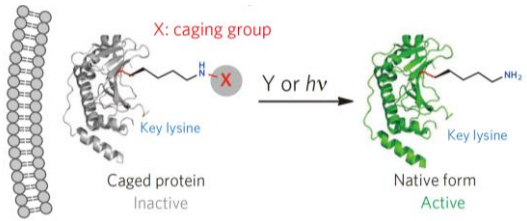
Application of bioorthogonal cleavage reactions (BCRs)



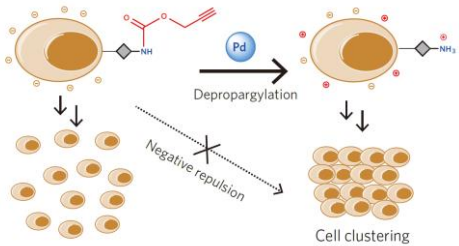
applications



Prodrug activation



Protein activation



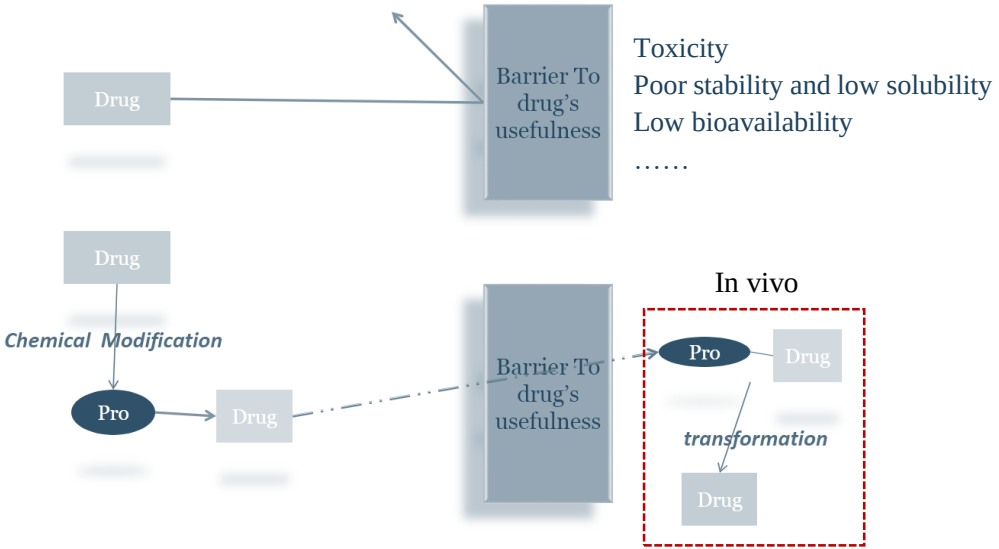
Cell surface engineering

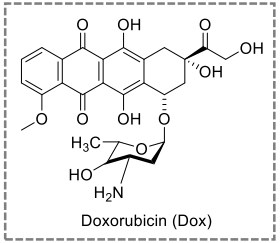
Chen, P. R. et al. *Nat. Chem. Biol.* **2016**, *12*, 129-137.

01 Introduction of prodrug

Definition:

Prodrugs are molecules with little or no pharmacological activity that are converted to the active parent drug in vivo by enzymatic or chemical reactions or by a combination of the two.



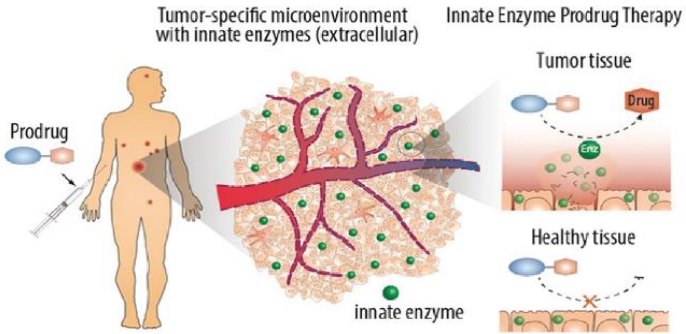


Purpose:

- To decrease undesirable toxicity
- To increase stability and solubility
- To increase therapeutic efficacy
- To improve bioavailability

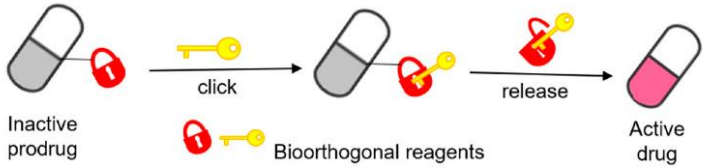
01 Advantages of BCRs in prodrug activation

Enzymatic release



Challenge: tumor microenvironment heterogeneity

Chemical release



Advantages:

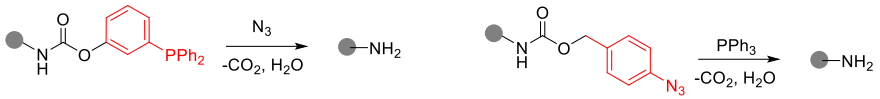
- More stability
- Selective activation at the tumor site
- Low toxicity to normal cells
- ……

Zelikin, A. N. et al. *Angew. Chem. Int. Ed.* **2020**, *132*, 7460-7466.

Classification of BCRs in prodrug activation

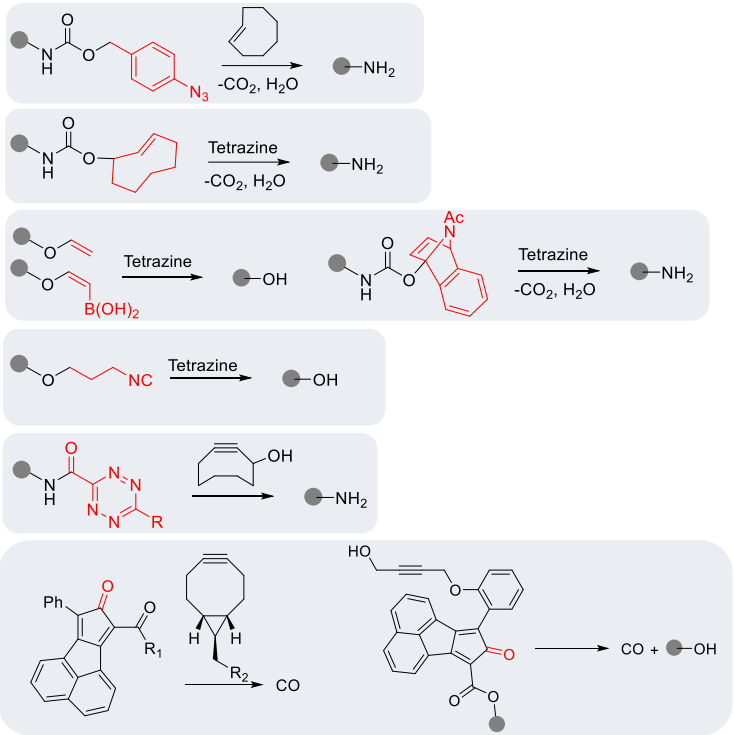
Small molecule-triggered cleavage reactions

Staudinger reaction



Cycloaddition reaction

- Trans-cyclooctene-involved reactions
- Tetrazine-involved reactions
- Alkyne-involved reactions

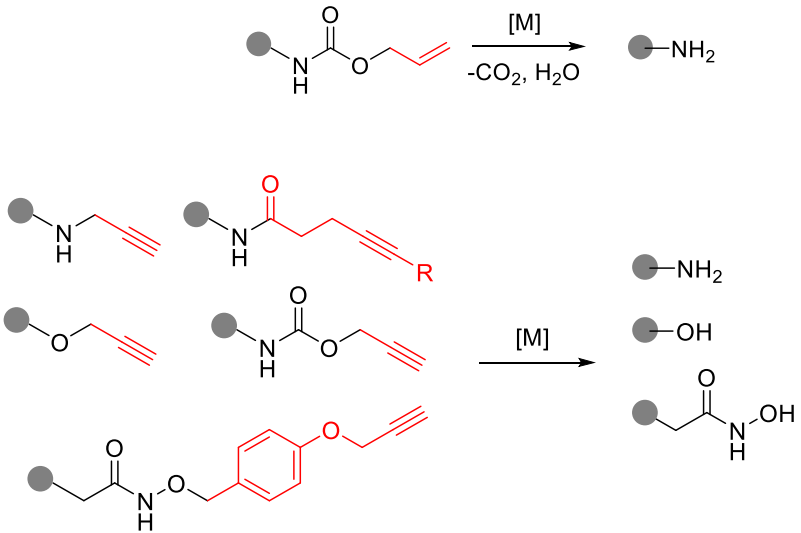


Chen, P. R. et al. *ACS Cent. Sci.* **2021**, *7*, 929–943.

Classification of BCRs in prodrug activation

Transition metal-triggered cleavage reactions

Pd Ru Au Pt

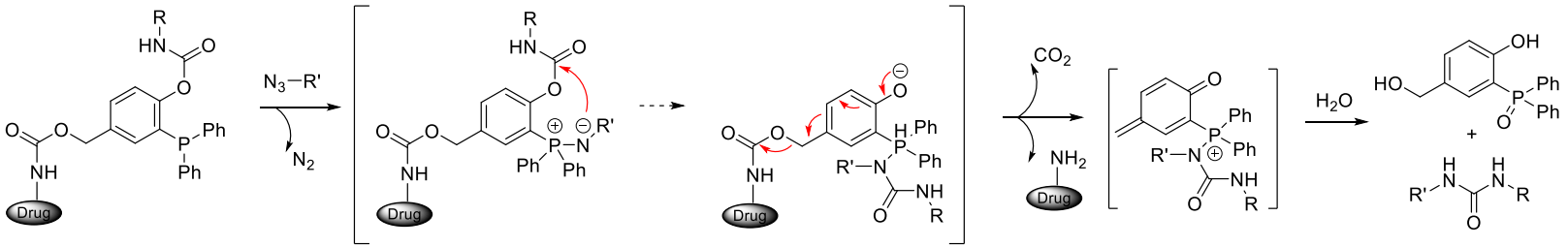


Chen, P. R. et al. *ACS Cent. Sci.* **2021**, *7*, 929–943.

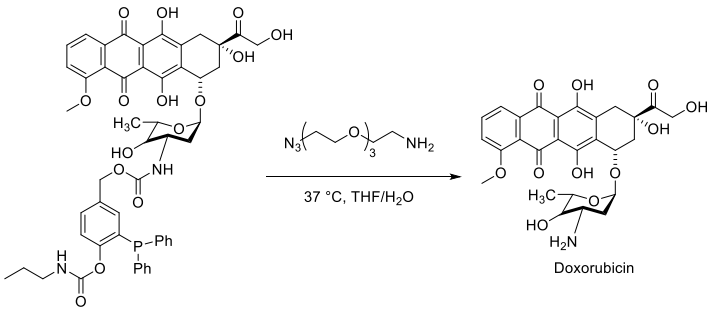
Small molecule-triggered cleavage reactions

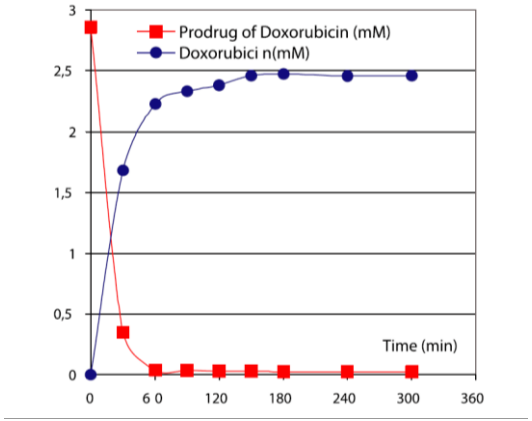
Small molecule-triggered cleavage reactions

Staudinger reaction



Triarylphosphine as the protecting group

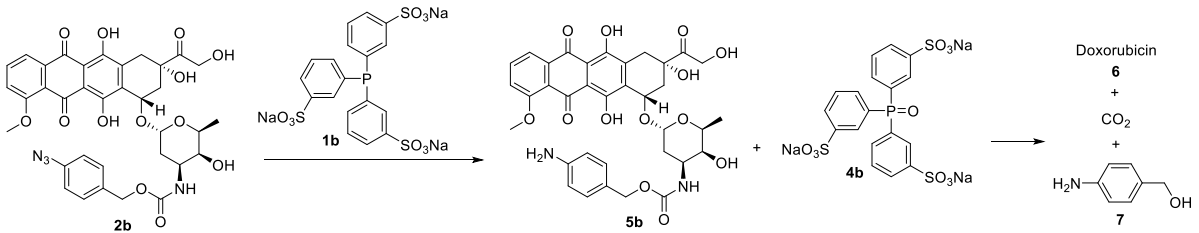




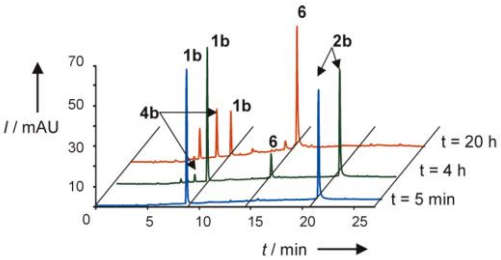
Florent, J. C. et al. *Bioorganic Med. Chem. Lett.* **2006**, *16*, 3147-3149.

2.1 Small molecule-triggered cleavage reactions

Staudinger reaction



P-azidobenzyl(PAB) as the protecting group



Activation of prodrug 2b by phosphine 1b in water followed with HPLC (260 nm)

Significance: staudinger reaction was among the first examples of BCRs being used for prodrug activation

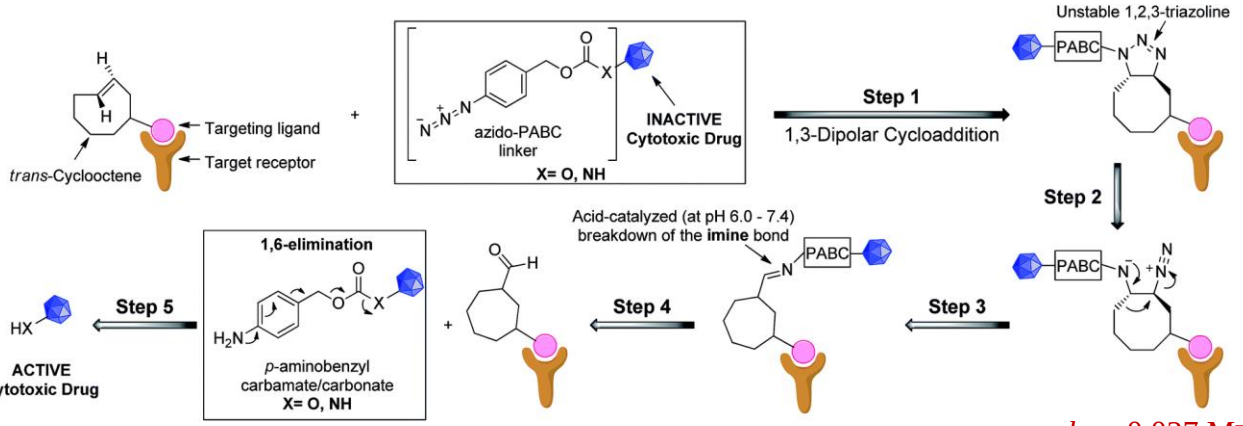
Disadvantages:

- The phosphine reagent is not stable and potentially toxic
- The reaction rate is very slow, $k_2 \approx 10^{-3}$ M^{-1} s^{-1}

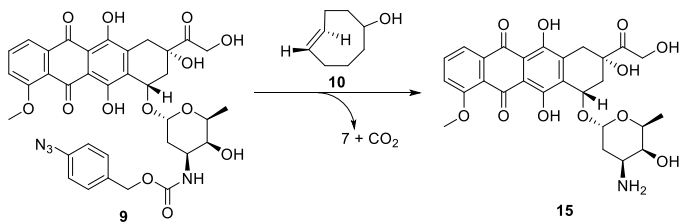
Robillard, M. S. et al. *Bioconjug. Chem.* **2008**, *19*, 714-718.

Small molecule-triggered cleavage reactions

Trans-cyclooctene-involved reactions (TCO and PAB)



$k_2 = 0.027\ M^{-1}\ s^{-1}$



Cytotoxicity assay of B16-OVA cells

Compound	IC_{50} (μM)
Doxorubicin **15**	0.71 (0.66–0.77)
Dox-prodrug **9**	49.9 (42.5–58.5)
9 + *cis*-cyclooctenol (100 μM)	55.0 (38.0–79.5)
9 + *trans*-cyclooctenol **10** (100 μM)	0.96 (0.91–1.01)

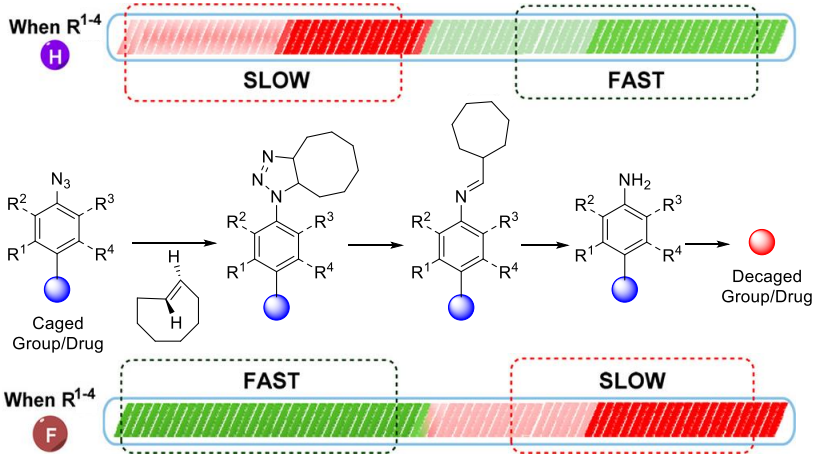
Gamble, A. B. et al. *Chem. Sci*. **2015**, *6*, 1212-1218.

Small molecule-triggered cleavage reactions

Trans-cyclooctene-involved reactions (TCO and PAB)

	Stability (% Intact)[a,b] of Prodrug 9 (100 μM)	
Time (h)	50% Serum:PBS	PBS only
0	100	100
4	95.3 ± 8.4	105.7 ± 20.6
24	68.0 ± 14.7	121.1 ± 36.0
48	55.6 ± 13.2[c]	111.9 ± 56.2

Gamble, A. B. et al. *Chem. Sci*. **2015**, *6*, 1212-1218.



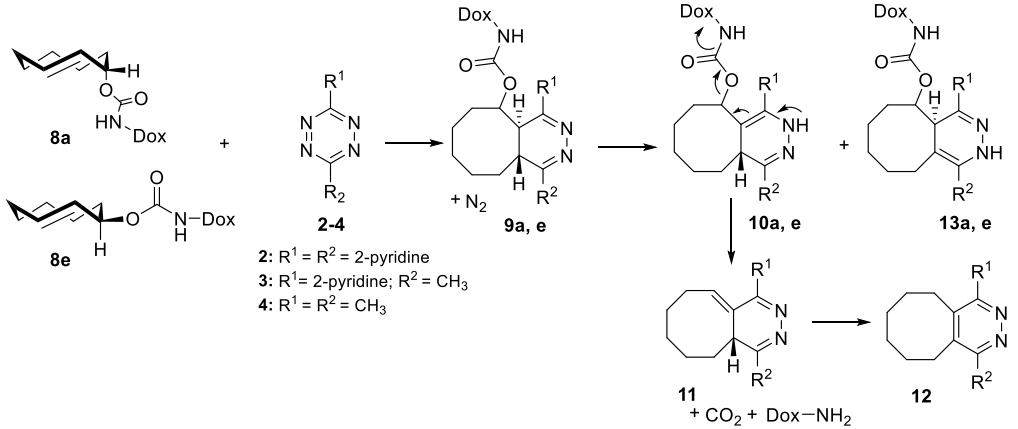
Gamble, A. B. et al. *Bioconjug. Chem.* **2018**, *29*, 324-334.

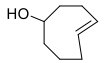
Disadvantages:

- Azide prodrug is not stable
- Slow reaction rate or slow release, $k_2 \approx 10^{-2}$ M^{-1} s^{-1}
- Optimization of the reaction is complex and difficult

2.1 Small molecule-triggered cleavage reactions

Trans-cyclooctene-involved reactions (TCO and Tz)



Probe	Tetrazine stability at 37 °C		Tetrazine reactivity with TCOs in MeCN at 20 °C: k_2 [$M^{-1}s^{-1}$]		
	in PBS: $t_{1/2}$ [h]	in serum: proportion intact at 4 h [%]	**8e-Bn**	**8a-Bn**	
2	9.6 ± 0.13	75.1 ± 4.2	0.37 ± 0.10	57.7 ± 5.0	1140 ± 85
3	141.5 ± 14.80	96.3 ± 1.9	–	5.94 ± 0.24	–
4	14.4 ± 0.35	100 ± 2.0	–	0.54 ± 0.06	–

Probe	Doxorubicin release [%]	
	in PBS/MeCN (3:1)	in serum
2	7 ± 3	12 ± 1
3	55 ± 4	46 ± 3
4	79 ± 3	75 ± 4
–[a]	0	0

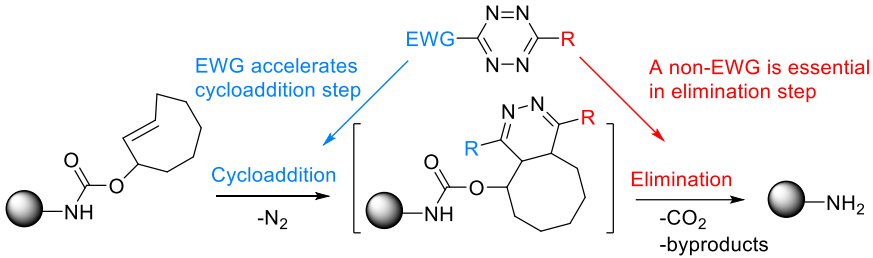
[a] No release of **14-Dox** from **8a-Dox** was observed at 37 °C in PBS (72 h) or serum (24 h).

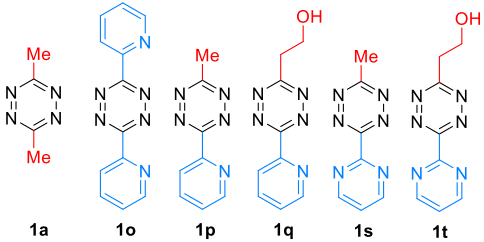
Faster reaction rates do not necessarily lead to increased drug release rates !

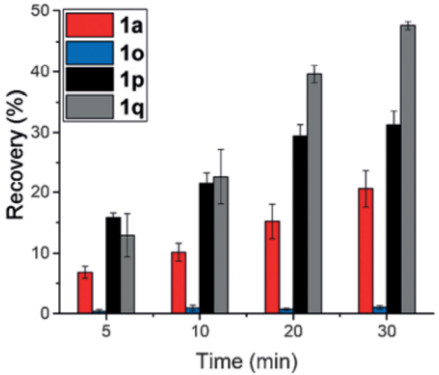
Robillard, M. S. et al. *Angew. Chem. Int. Ed.* **2013**, *125*, 14362-14366.

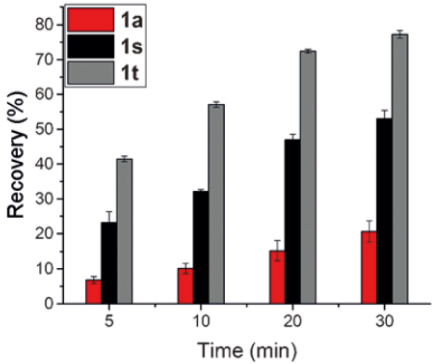
Small molecule-triggered cleavage reactions

Trans-cyclooctene-involved reactions (TCO and Tz)







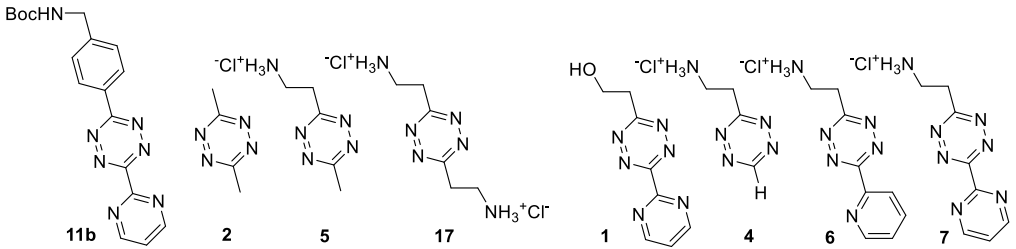


Combining EWG and non-EWG on the same tetrazine ring allows for an optimal balance of cycloaddition and degradation rates

Chen, P. R. et al. *Angew. Chem. Int. Ed.* **2016**, *128*, 14252-14256.

Small molecule-triggered cleavage reactions

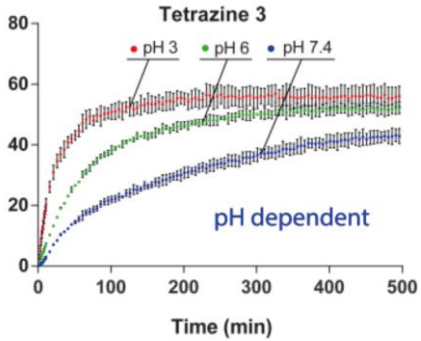
Trans-cyclooctene-involved reactions (TCO and Tz)

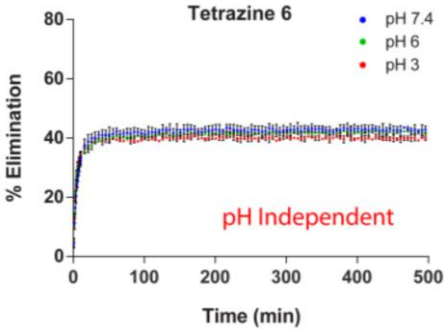


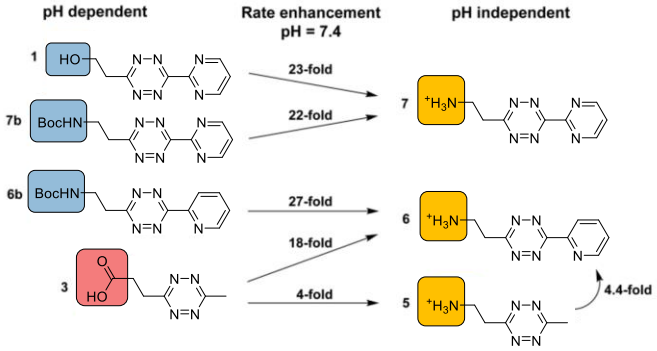
Tz	Eff (%)	k_{elim} (10^{-5} s^{-1})
11b	62	1.59
2	65	2.91
5	71	2.73
17	72	2.01

Tz	Eff (%)	k_{elim} (10^{-5} s^{-1})
1	55	8.86
4	48	>15.3*
6	53	>23.7*
7	47	>35.9*

* Estimated (minimum) K_{elim}





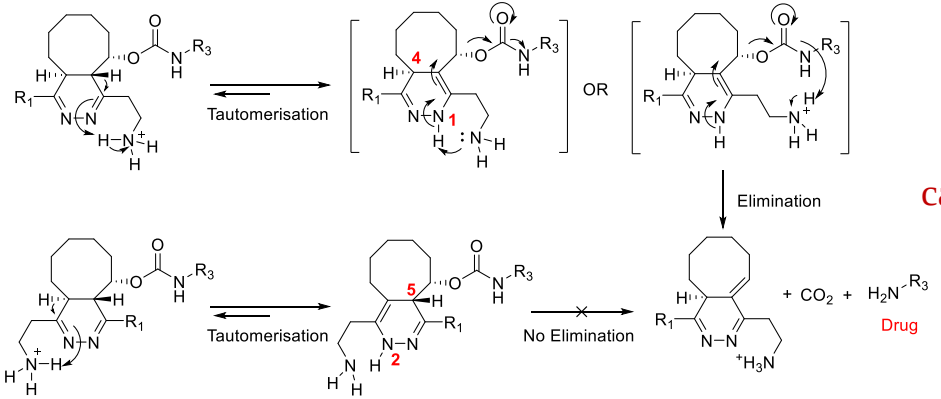


van Kasteren, S. I. et al. *Chem. Eur. J.* **2018**, *24*, 18075-18081.

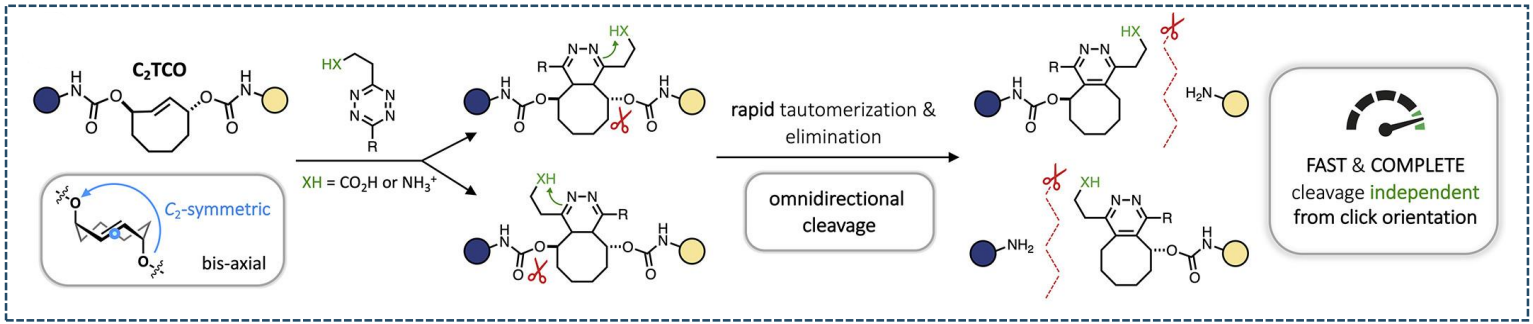
Small molecule-triggered cleavage reactions

Trans-cyclooctene-involved reactions (TCO and Tz)

Proposed mechanism:



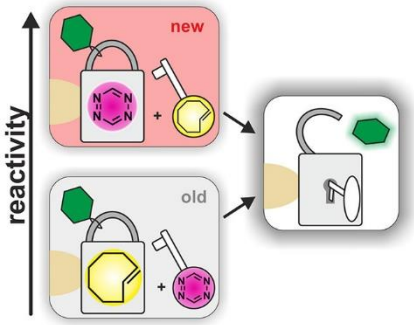
causing incomplete release!

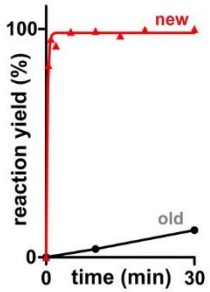


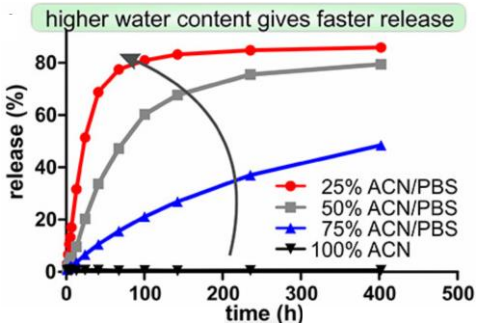
Mikula, H. et al. *J. Am. Chem. Soc.* **2020**, *142*, 19132−19141.

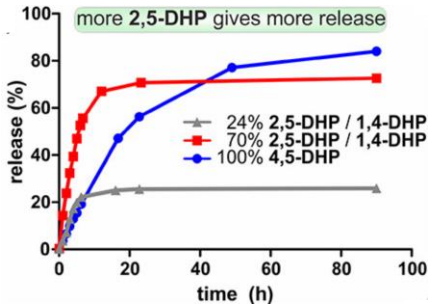
Small molecule-triggered cleavage reactions

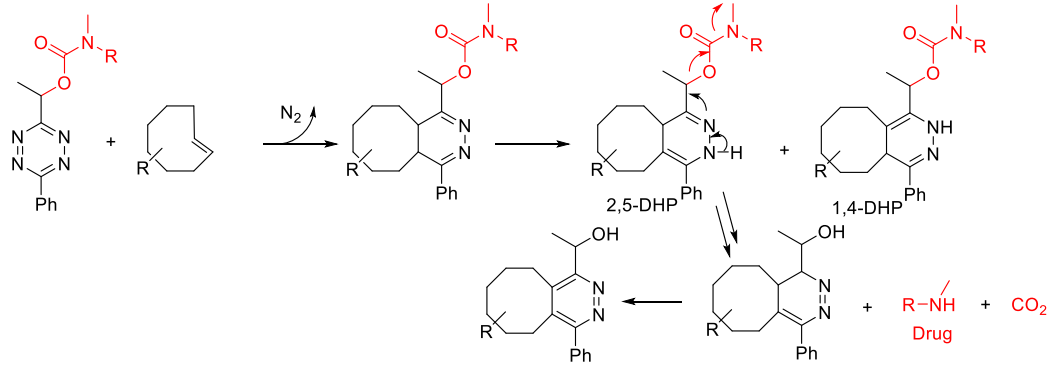
Trans-cyclooctene-involved reactions (TCO and Tz)











Robillard, M. S. et al. *J. Am. Chem. Soc.* **2020**, *142*, 10955-10963.

2.1 Small molecule-triggered cleavage reactions

Trans-cyclooctene-involved reactions (TCO and Tz)

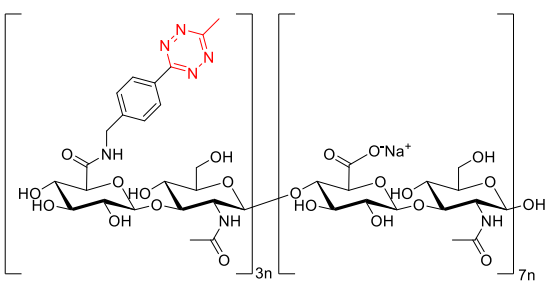
Advantages:

- Fast reaction rates and fast release rates, k_2 =1-10^4 M^{-1} s^{-1}
- Multiple compatible systems

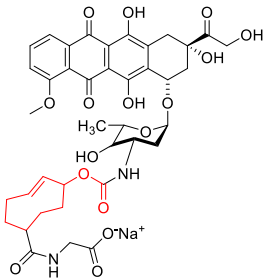
Disadvantages:

- Incomplete release
- Synthesis of tetrazine has some difficulty

Tetrazine-modified sodium hyaluronate



TCO-modified doxorubicin

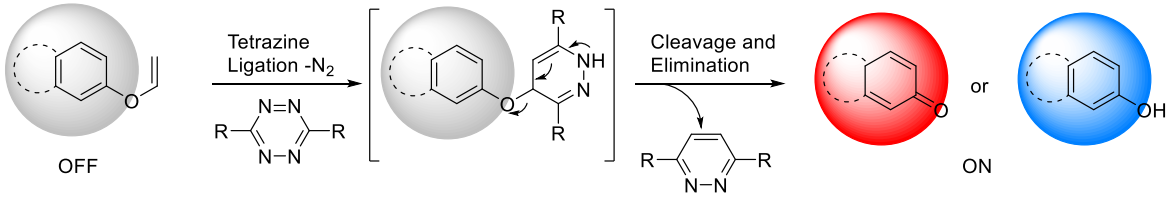


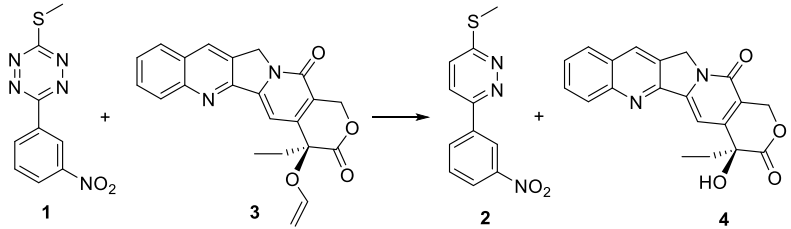
An anti-cancer therapy based on tetrazine and trans-cyclooctene has been approved for Phase I clinical trials!

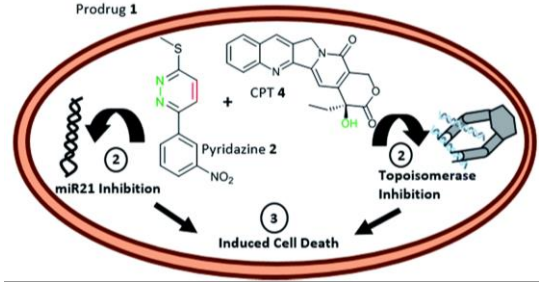
Robillard, M. S. et al. *Bioconjug. Chem.* **2016**, *27*, 1697-1706.

Small molecule-triggered cleavage reactions

Tetrazine-involved reactions (Tz and vinyl ether)



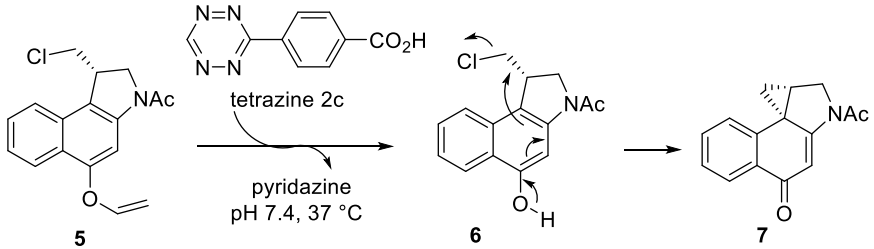




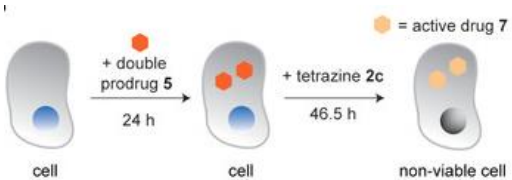
Devaraj, N. K. et al. *J. Am. Chem. Soc.* **2016**, *138*, 11429-11432.
Bradley, M. et al. *Chem. Sci.* **2018**, *9*, 7198−7203.

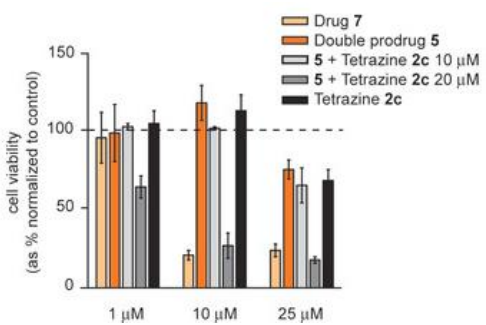
Small molecule-triggered cleavage reactions

Tetrazine-involved reactions (Tz and vinyl ether)



$k_2 = 0.00021\ M^{-1}\ s^{-1}$

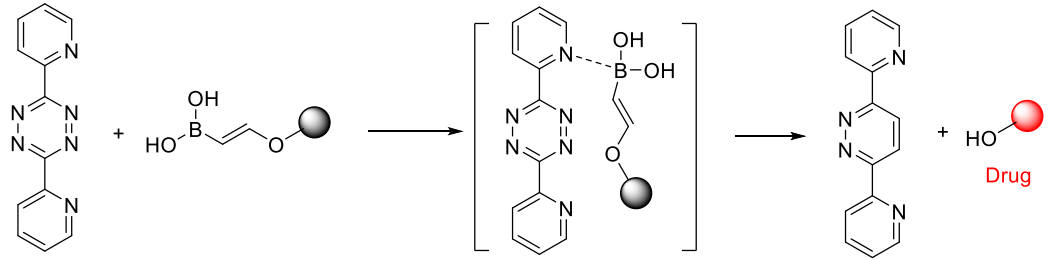




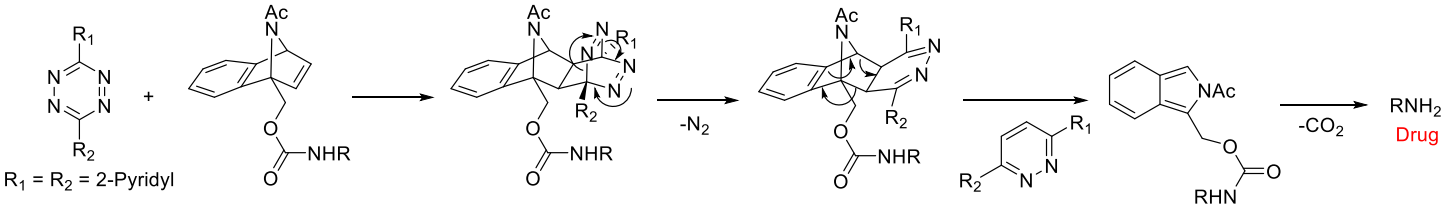
Bernardes, G. J. et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 243-247.

Small molecule-triggered cleavage reactions

Tetrazine-involved reactions (Tz and vinyl ether)



k_2 = 0.0033 M^{-1} s^{-1}



k_2 = 0.190 M^{-1} s^{-1}

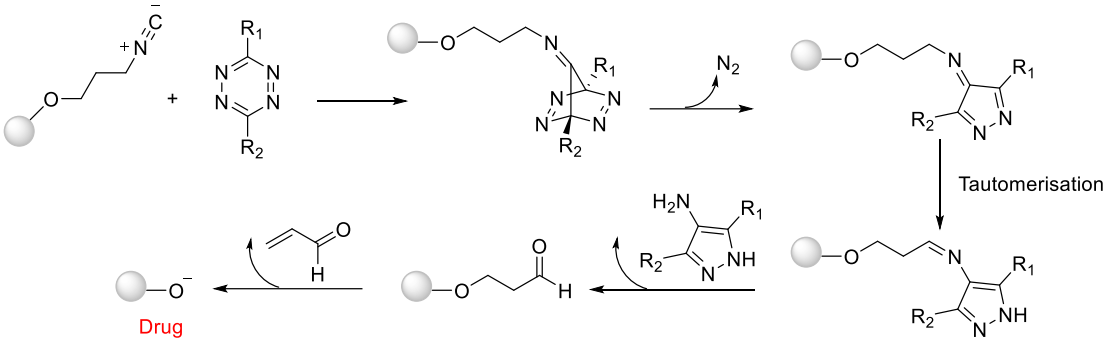
Advantages:
- Vinyl ether is stable

Disadvantages:
- Slow reaction rate or slow release, k_2 =10^{-4} ~ $10^{-1}M^{-1}$ s^{-1}

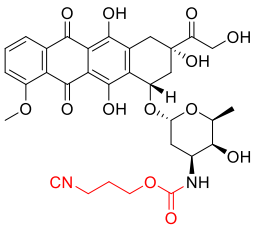
Franzini, R. M. et al. *Chem. Comm.* **2017**, *53*, 6271-6274.
Bonger, K. M. et al. *Org. Biomol. Chem.* **2019**, *17*, 8816-8821.

Small molecule-triggered cleavage reactions

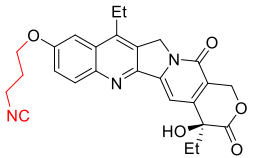
Tetrazine-involved reactions (Tz and 3-isocyanopropyl group)



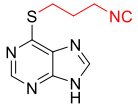
ICPrc-dox



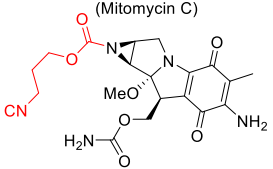
ICPr-SN-38
(SN-38)



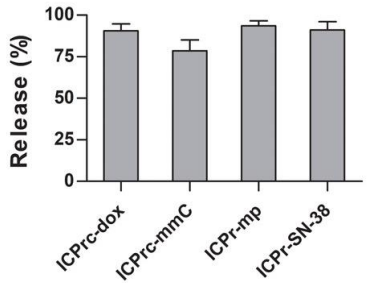
ICPr-mp
(6-mercaptopurine)

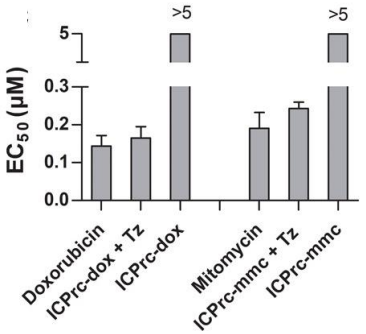


ICPrc-mmC
(Mitomycin C)



Cytotoxicity assay of A549 cells

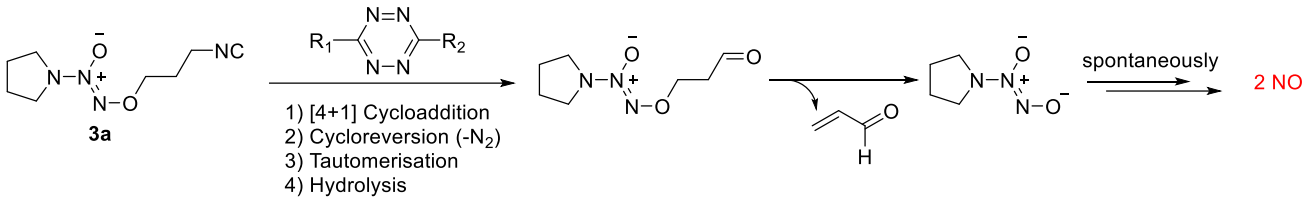




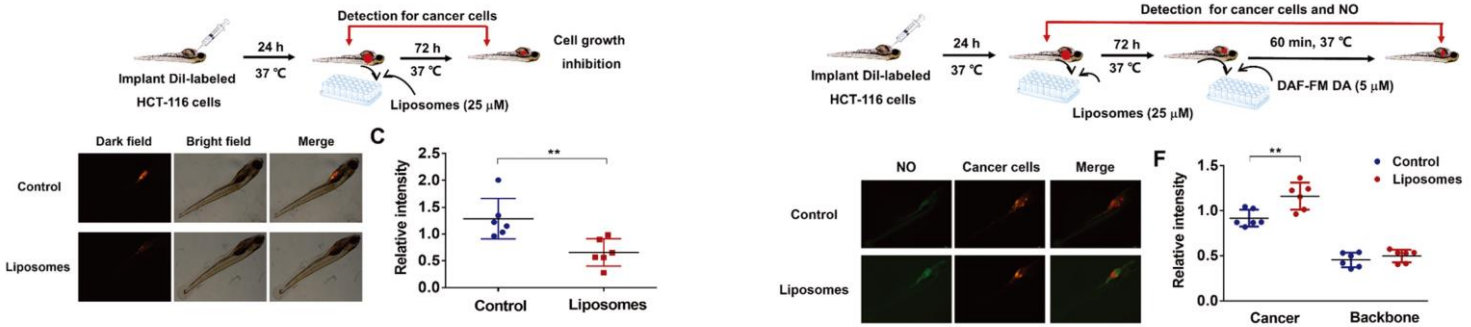
Franzini, R. M. et al. *J. Am. Chem. Soc.* **2018**, *140*, 8410-8414.

Small molecule-triggered cleavage reactions

Tetrazine-involved reactions (Tz and 3-isocyanopropyl group)



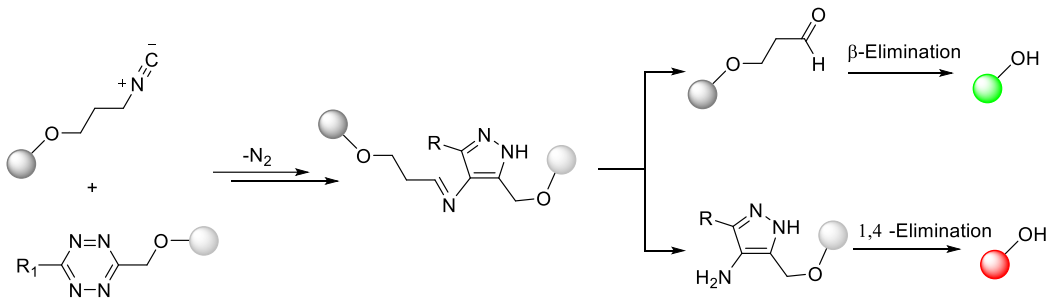
Anticancer activities and NO release behaviors of 3a and TZ liposomes in a zebrafish embryos model

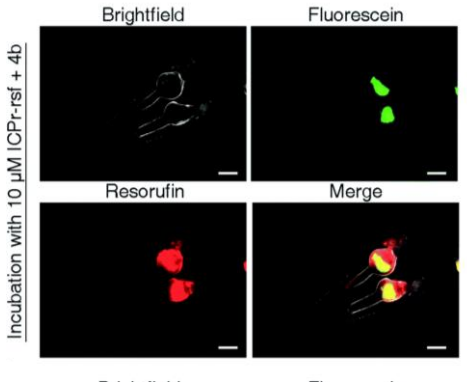


Huang, Z. et al. *Biomater. Sci.* **2021**, *9*, 1816-1825.

Small molecule-triggered cleavage reactions

Tetrazine-involved reactions (Tz and 3-isocyanopropyl group)





Advantages:

- Mutual deprotection for dual pre-drug activation
- Synthesis of isocyanide is easy

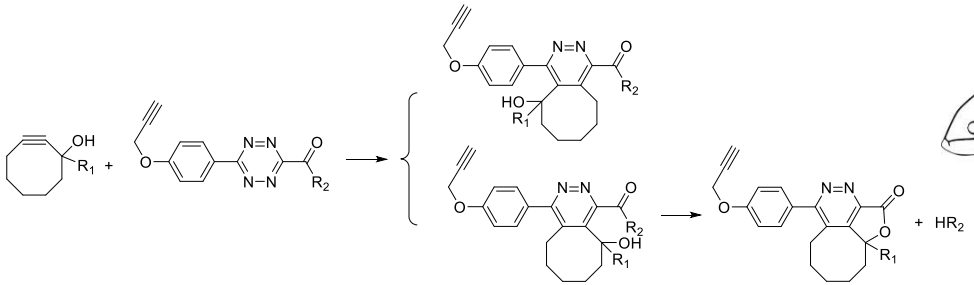
Disadvantages:

- Mild reaction rate, $k_2 \approx 1\ M^{-1}\ s^{-1}$

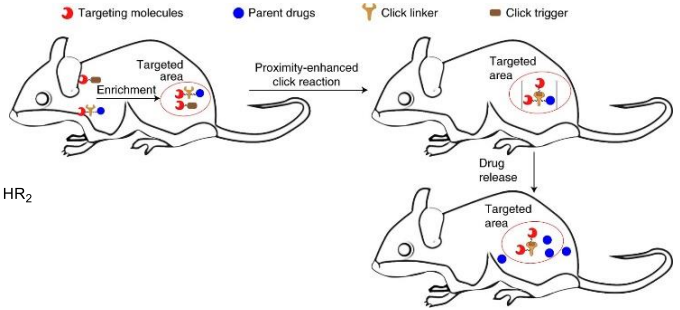
Franzini, R. M. et al. *Chem. Sci*. **2020**, *11*, 169-179.

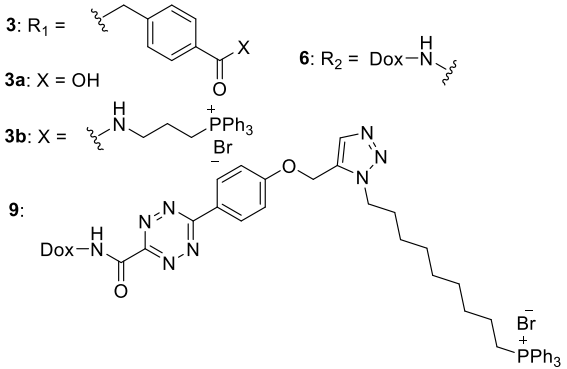
2.1 Small molecule-triggered cleavage reactions

Tetrazine-involved reactions (Tz and cyclooctyne)

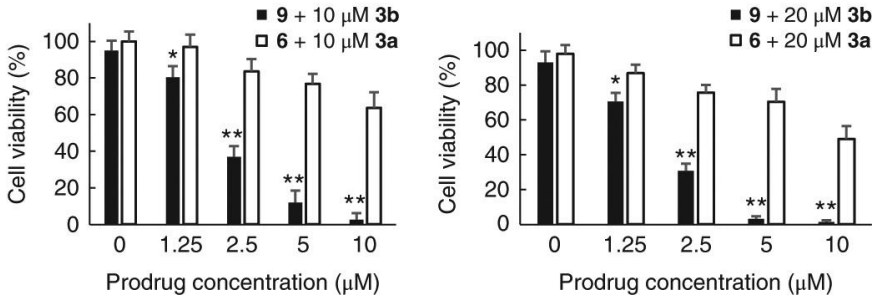


Concept of enrichment-triggered prodrug activation





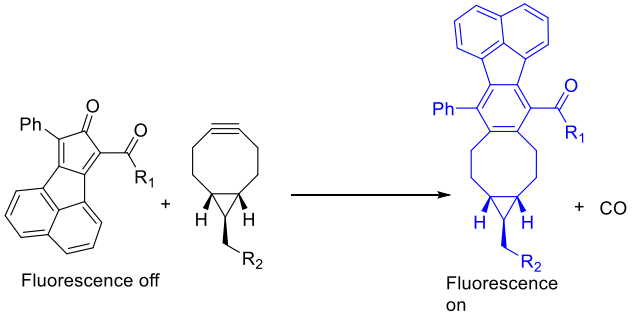
Cytotoxicity assay of HeLa cells

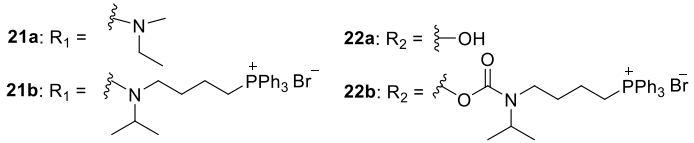


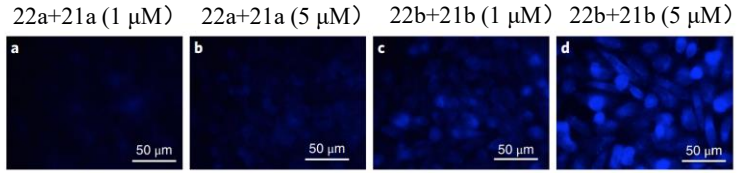
Wang, B. et al. *Nat. Chem.* **2018**, *10*, 787-794.

Small molecule-triggered cleavage reactions

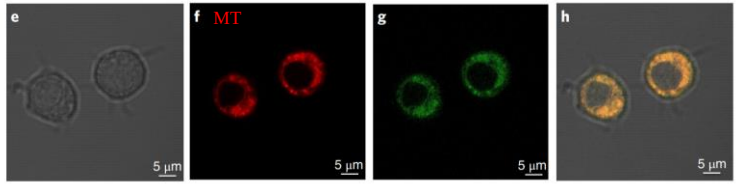
Alkyne-involved reactions (Alkyne pair and cyclopentadienone)



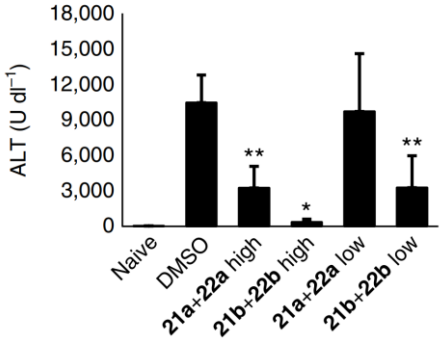




Confocal images of RAW264.7 cells treated with compounds 21b (5 μM) and 22b (2.5 μM) and MT-deep red (50 nM)



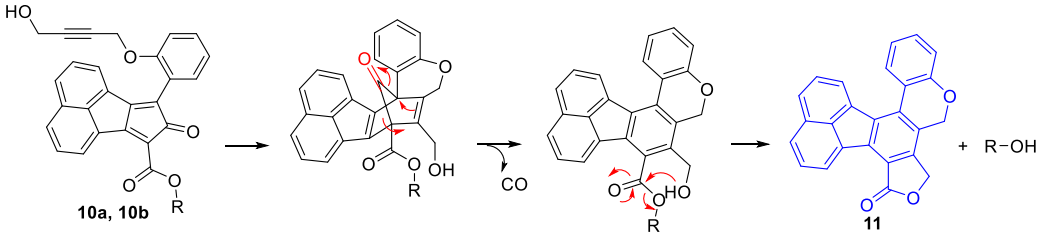
Effect of CO prodrugs on APAP-induced liver injury



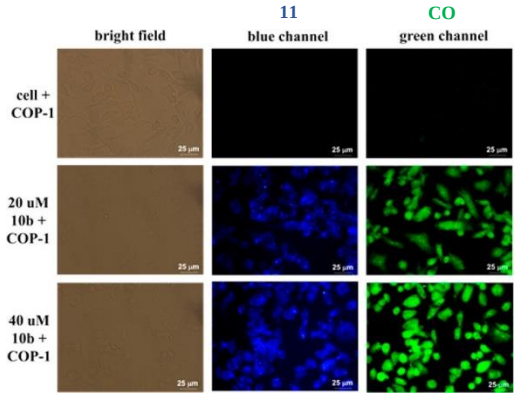
Wang, B. et al. *Nat. Chem.* **2018**, *10*, 787-794.

Small molecule-triggered cleavage reactions

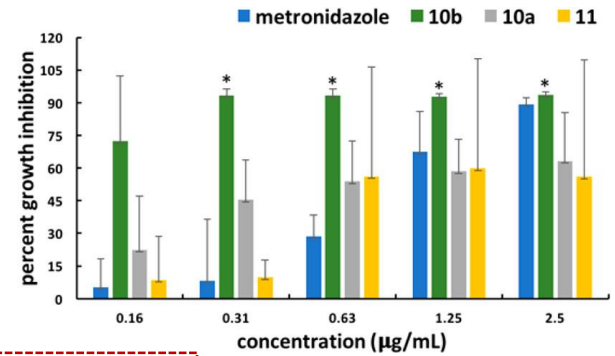
Alkyne-involved reactions (Alkyne and cyclopentadienone)



a: R = benzyl alcohol
b: R = metronidazole



Inhibition of *H. pylori* growth



Advantages:

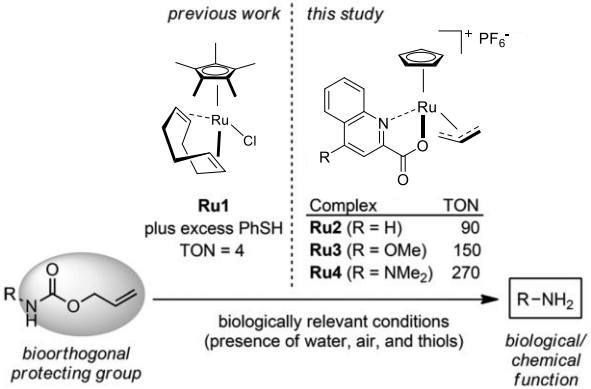
- The application of the reaction is extended to release CO

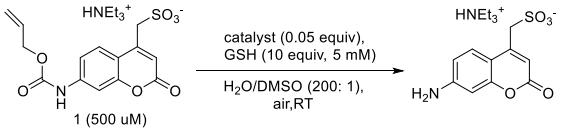
Wang, B. et al. *Org. Lett.* **2018**, *20*, 897−900

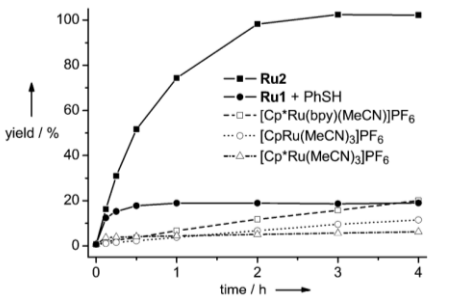
Transition metal-triggered cleavage reactions

2.2 Transition metal-triggered cleavage reactions

Ru



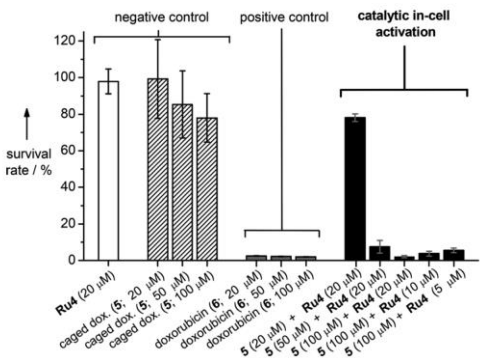




Entry		Complex	Cat. [equiv]	Yield [%][b] 1 h	4 h	24 h
1	**Ru2**	[CpRu(QA)(allyl)]PF_6	0.05	74	>99	>99
2		[CpRu(PA)(allyl)]PF_6	0.05	43	80	85
3		[Cp*Ru(QA)(allyl)]PF_6	0.05	10	38	84
4		[Cp*Ru(PA)(allyl)]PF_6	0.05	3	10	52
5	**Ru2**	[CpRu(QA)(allyl)]PF_6	0.01	16	47	65
6	**Ru3**	[CpRu(QA-OMe)(allyl)]PF_6	0.01	32	79	93
7	**Ru4**	[CpRu(QA-NMe$_2$)(allyl)]PF_6	0.01	29	91	>99

QA=2-quinolinecarboxylate
PA=2-pyridinecarboxylate

Cytotoxicity assay of HeLa cells

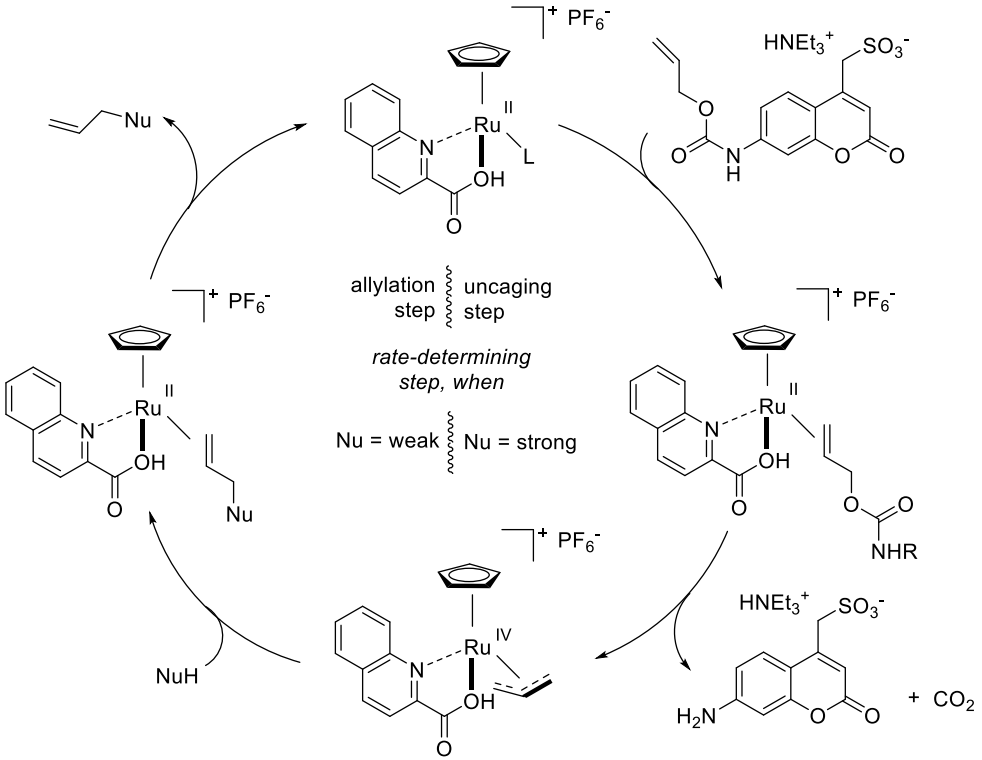


Meggers, E. et al. *Angew. Chem. Int. Ed.* **2014**, *53*, 10536-10540.

2.2 Transition metal-triggered cleavage reactions

Ru

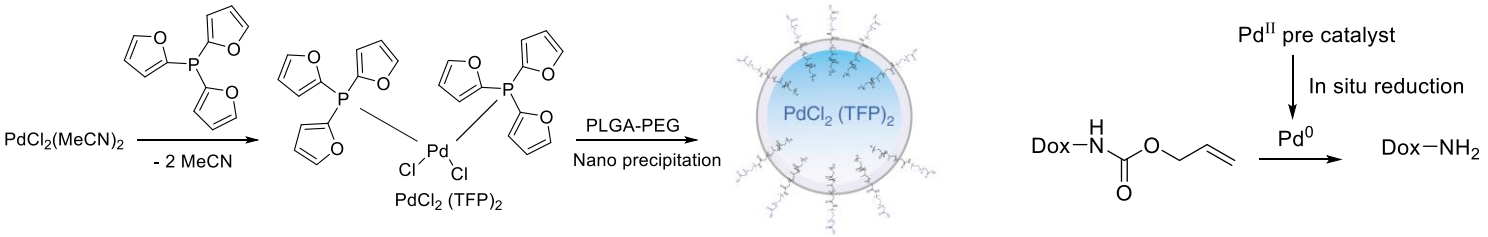
Catalytic cycle:



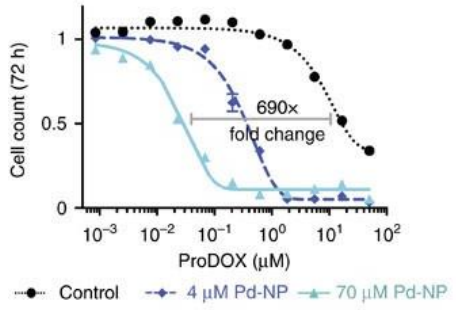
Meggers, E. et al. *Angew. Chem. Int. Ed.* **2014**, *53*, 10536-10540.

2.2 Transition metal-triggered cleavage reactions

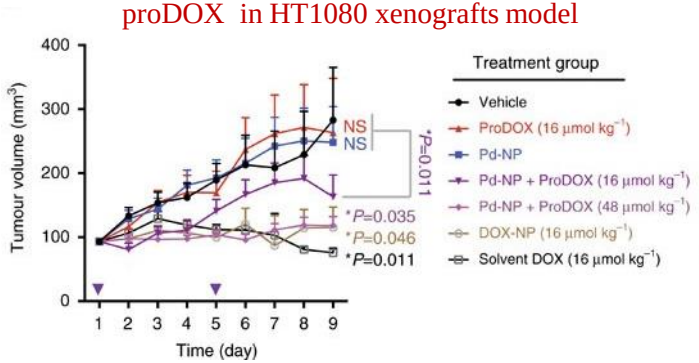
Pd



Cytotoxicity assay of HT1080 cells



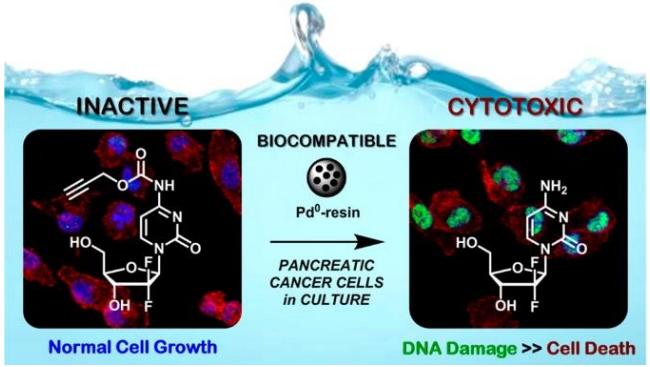
Anticancer activities of 50 mg kg^{-1} Pd-NP and proDOX in HT1080 xenografts model



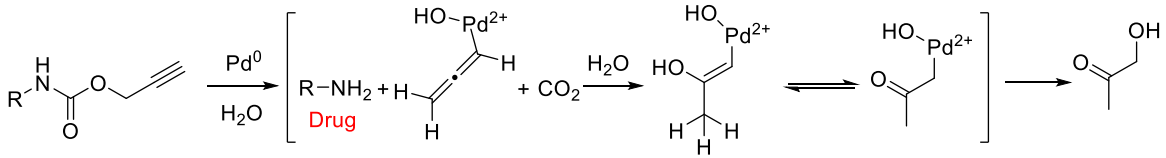
Weissleder, R. et al. *Nat. Commun.* **2017**, *8*, 15906.

2.2 Transition metal-triggered cleavage reactions

Pd



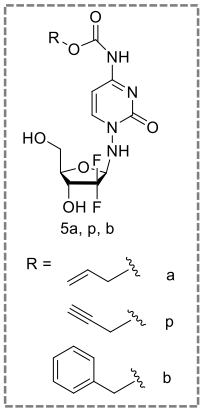
Proposed mechanism:

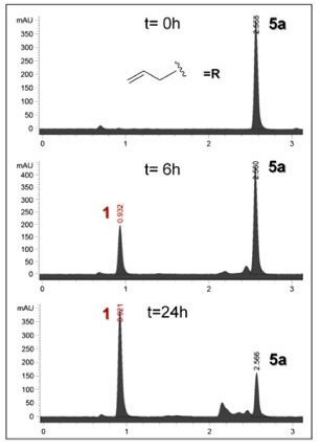


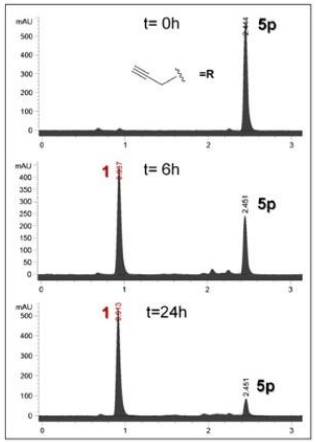
Unciti-Broceta, A. et al. *J. Med. Chem.* **2014**, *57*, 5395−5404.

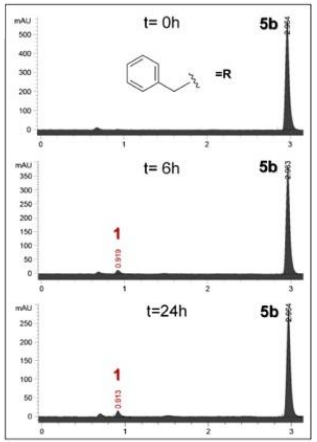
2.2 Transition metal-triggered cleavage reactions

Pd

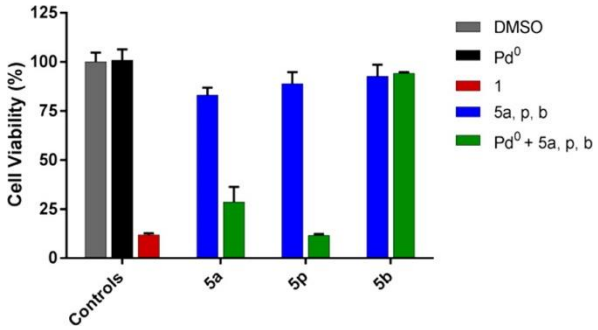








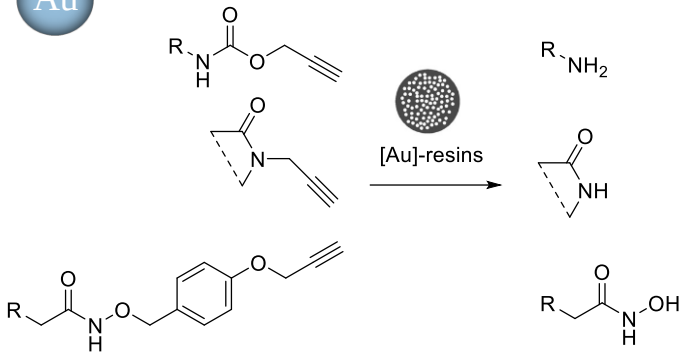
Cytotoxicity assay of BxPC-3 cells

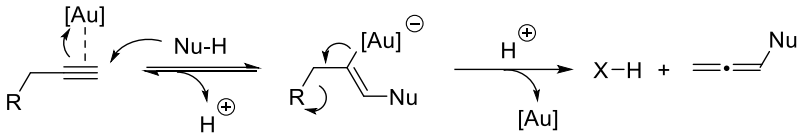


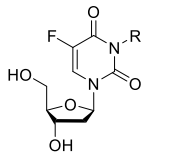
Unciti-Broceta, A. et al. *J. Med. Chem.* **2014**, *57*, 5395−5404.

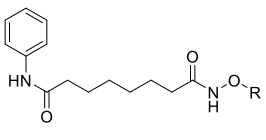
2.2 Transition metal-triggered cleavage reactions

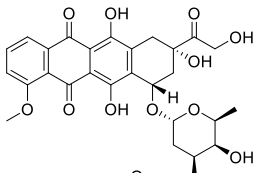
Au



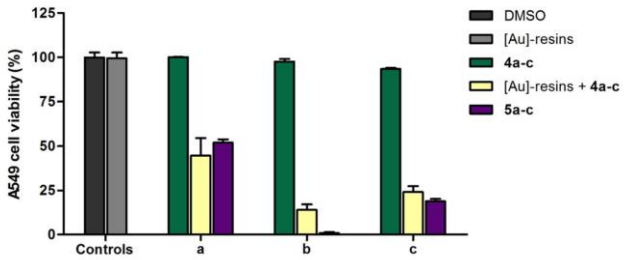








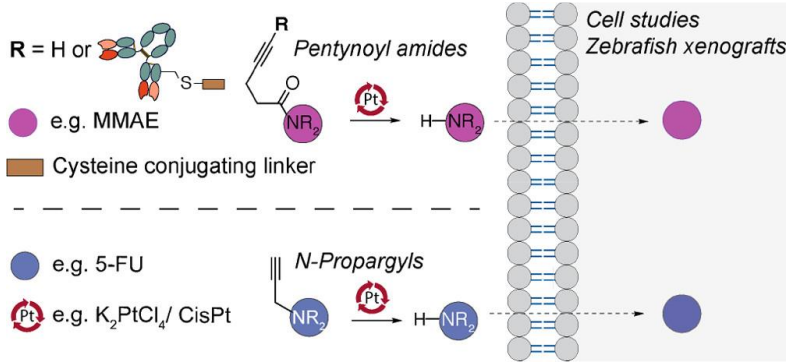
Cytotoxicity assay of A549 cells



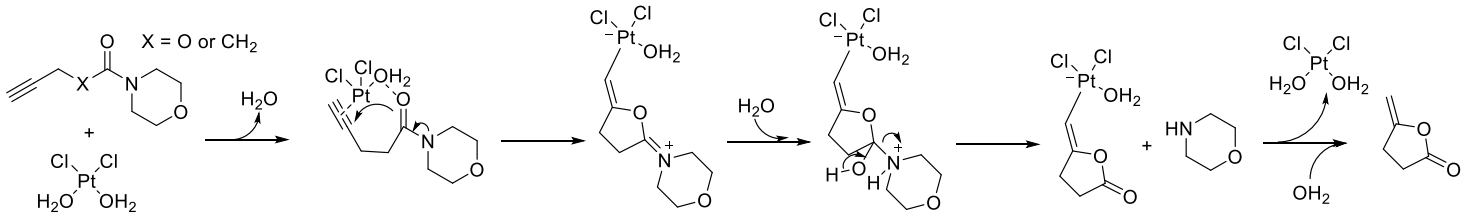
Unciti-Broceta, A. et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 12548 –12552.

2.2 Transition metal-triggered cleavage reactions

Pt



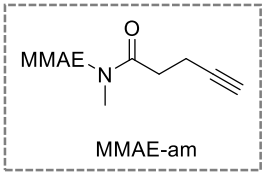
Proposed mechanism:



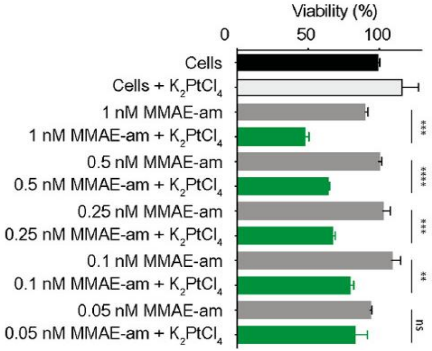
Bernardes, G. J. et al. *J. Am. Chem. Soc.* **2020**, *142*, 10869−10880.

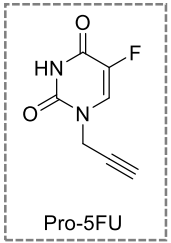
2.2 Transition metal-triggered cleavage reactions

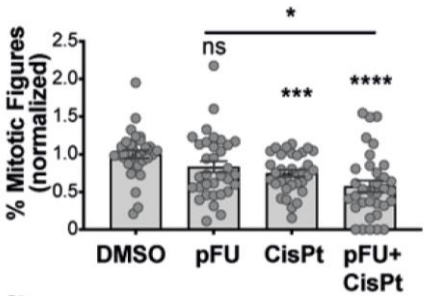
Pt

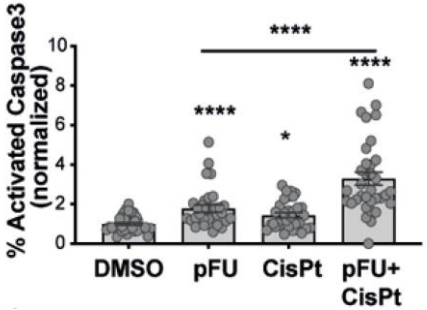


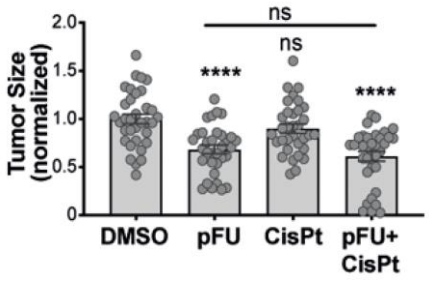
Cytotoxicity assay of HeLa cells



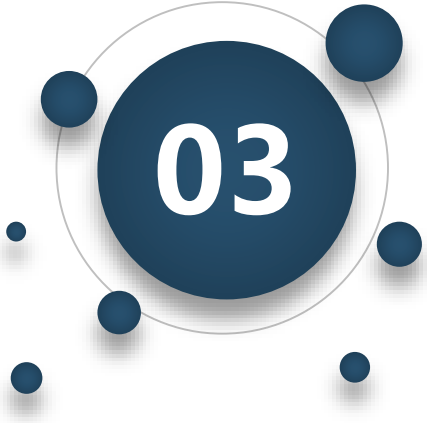








Bernardes, G. J. et al. *J. Am. Chem. Soc.* **2020**, *142*, 10869−10880.

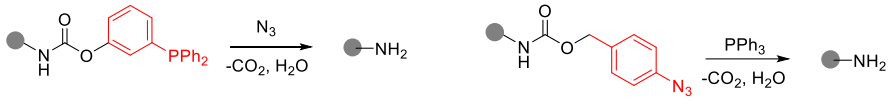


Summary and Perspective

01 Summary

Small molecule-triggered cleavage reactions

Staudinger reaction

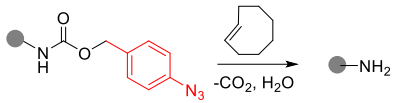


Significance: staudinger reaction was among the first examples of BCRs being used for prodrug activation

Disadvantages:

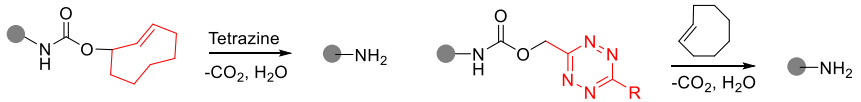
- The phosphine reagent is not stable and potentially toxic
- The reaction rate is very slow, $k_2 \approx 10^{-3}\ M^{-1}\ s^{-1}$

Trans-cyclooctene-involved reactions



Disadvantages:

- Azide prodrug is not stable
- Slow reaction rate or slow release, $k_2 \approx 10^{-2}\ M^{-1}\ s^{-1}$



Advantages:

- Fast reaction rates and fast release rates, k_2 =1-$10^4\ M^{-1}\ s^{-1}$
- Multiple compatible systems

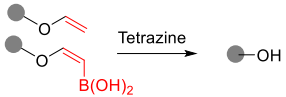
Disadvantages:

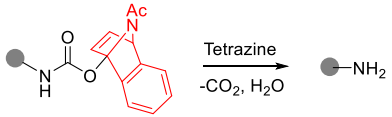
- Incomplete release
- Synthesis of tetrazine has some difficulty

01 Summary

Small molecule-triggered cleavage reactions

Tetrazine-involved reactions



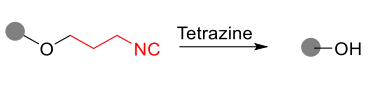


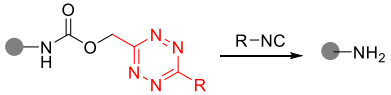
Advantages:

- Vinyl ether is stable

Disadvantages:

- Slow reaction rate or slow release, k_2 $=10^{-4} \sim 10^{-1} M^{-1}\ s^{-1}$



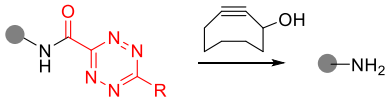


Advantages:

- Mutual deprotection for dual pre-drug activation
- Synthesis of isocyanide is easy

Disadvantages:

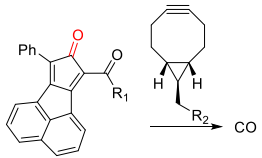
- Mild reaction rate, $k_2 \approx 1\ M^{-1}\ s^{-1}$

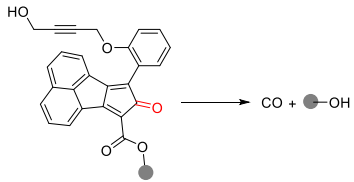


Disadvantages:

- Slow reaction rate , $k_2 = 10^{-1} \sim 1\ M^{-1}\ s^{-1}$

Alkyne-involved reactions





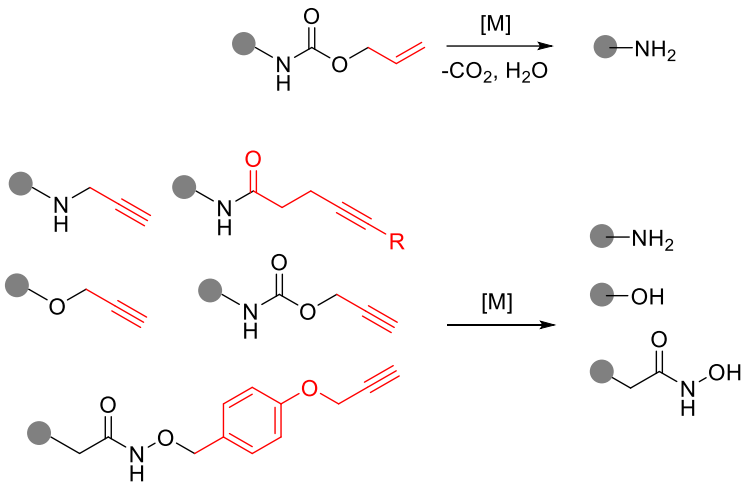
Advantages:

- The application of the reaction is extended to release CO

Summary

Transition metal-triggered cleavage reactions

Pd Ru Au Pt



Advantages:

- The transition metal can activate the prodrug as a catalyst on a continuous basis
- Transition-metal-triggered cleavage reactions can be heterogeneous reaction systems

Disadvantages:

- Mild reaction rate, $k_2 \approx$ 1-10 M^{-1} s^{-1}
- Potential cytotoxicity
- Instability of metal compounds

Prospect

This field of using bioorthogonal cleavage reactions in prodrug applications is still in its infancy

- Optimizing reaction to improve reaction rates and release rates
- Optimizing reaction to meet desired pharmacokinetic properties and safety criteria
- Increasing the stability of the trigger agent and simplifying the synthesis method
- Expanding reaction types and non-interfering reaction combination

Thanks