Bioorthogonal Prodrug Activation Driven by a Strain-Promoted 1,3-Dipolar Cycloaddition

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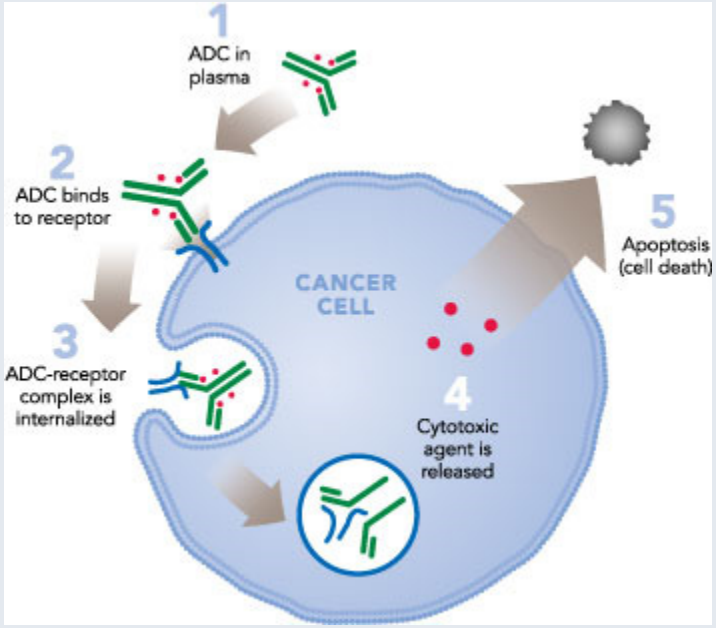
A. Manos-Turvey,
Wipf Group Current Literature
February 28th, 2015

Prodrugs for Cancer Therapies

- Non-selectivity in cancer treatments leads to off-target side-effects
- Prodrug activation is seen as a viable method allowing for direct drug delivery
  - Cleavage of a deactivating linker, leading to activation
  - Can react with off-target sources due to hydrolysis

- Antibody-Drug Conjugates (ADCs)
  - ADCs can elicit an immune system response
  - The linkers need to be fine tuned between stability and “cleavability”
  - Drugs become diluted as this is dependant on cell surface receptors, leading to < potent

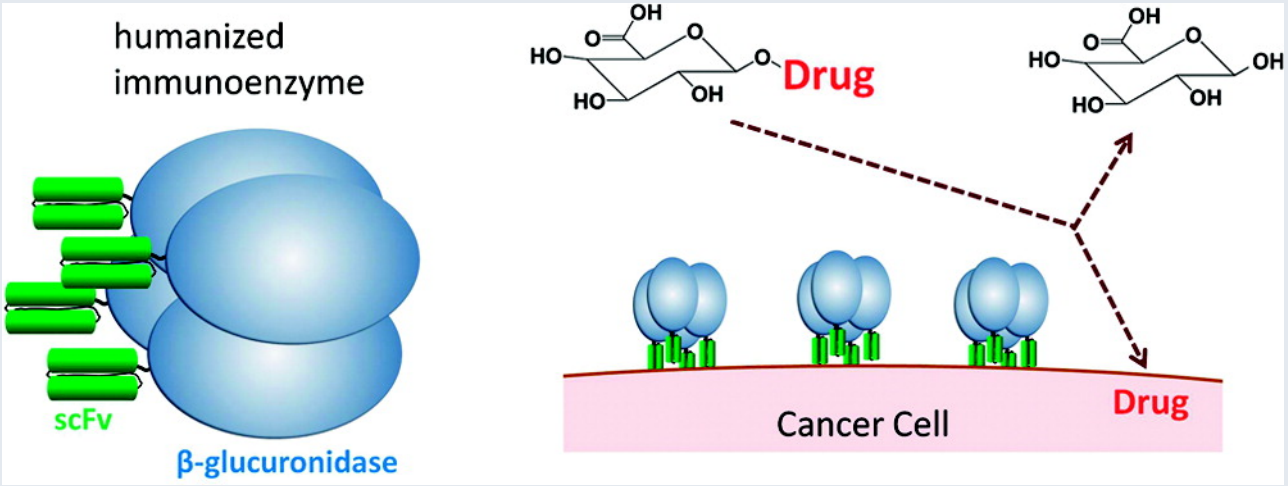


R.V.J. Chari, M.L. Miller, W.C. Widdison, *Angew. Chem.*, **2014**, *53*, 3796-3827

Fig: http://static.cdn-seekingalpha.com/uploads/2014/1/19447671_13889572897936_1.jpg

Prodrugs for Cancer Therapies

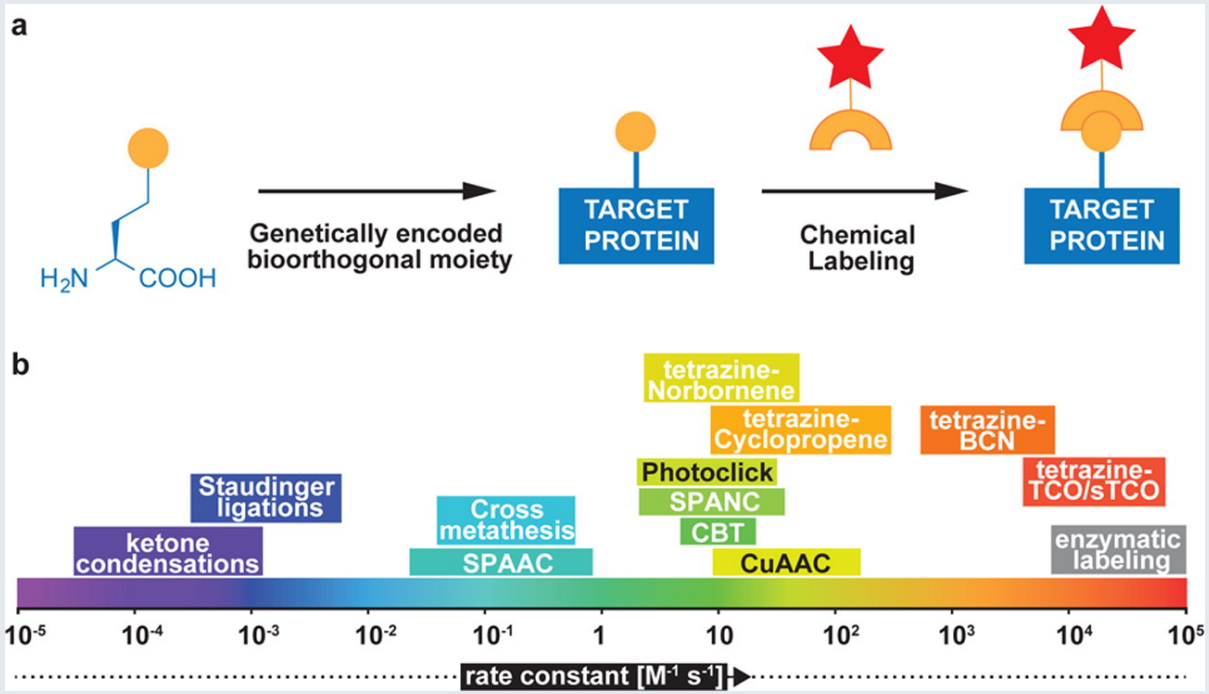
- Antibody-Directed Enzyme Prodrug Therapy (ADEPT)
  - Targets an antibody-enzyme conjugate to a cancer cell
  - Limited to human enzymes, to avoid anti-enzyme immune responses



K.D. Bagshawe, S.K. Sharma, R.H.J. Begent, *Expert Opin. Biol. Ther.*, **2004**, *4*, 1777-1789
K.-C. Chen, S.-Y. Wu, Y.-L. Leu, Z.M. Prijovich, B.-M. Chen, H.-E. Wang, T.-L. Cheng, S.R. Roffler, *Bioconjugate Chem.*, **2011**, *22*, 938-948

Prodrugs for Cancer Therapies

- Bioorthogonal Chemistry
  - Not many examples for *in vitro* prodrug activation

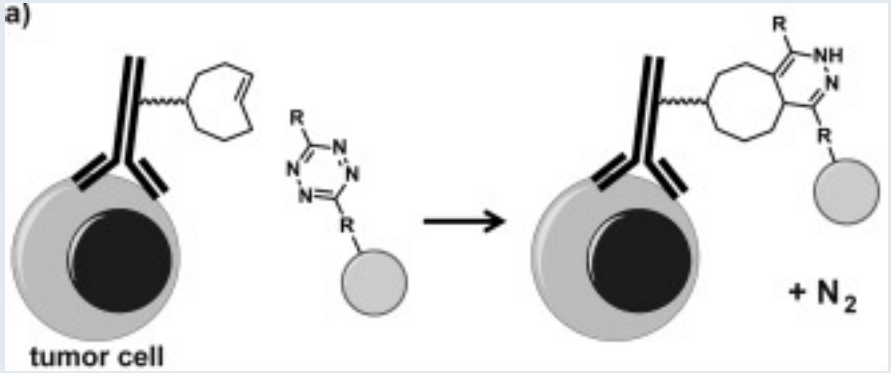


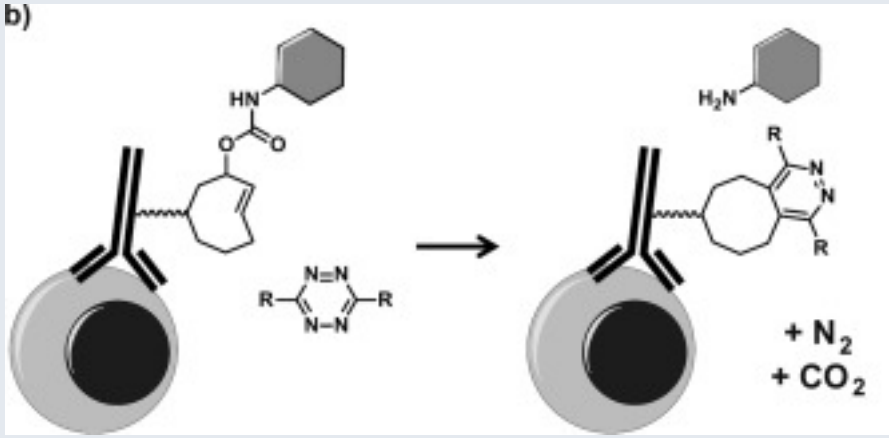
  - Staudinger and tetrazine-TCO (Inverse-Electron-Demand Diels-Alder Cycloadditions) reactions have been used.

K. Lang, J.W. Chin, *Chem. Rev.*, **2014**, 4764-4806

Prodrugs for Cancer Therapies

- Bioorthogonal Chemistry





  - TCO/tetrazine prodrug activation difficulties:
    - Lower TCO-conjugate activity
    - Low tumour-to-background ADC ratio, leading to off target effects
    - TCO-prodrug variant may isomerise to *cis* form if administered separately

R.M. Versteegen, R. Rossin, W. ten Hoeve, H.M. Janssen, M.S. Robillard, *Angew. Chem. Int. Ed.*, **2013**, *52*, 14112-14116

Prodrugs for Cancer Therapies: 1,3-Dipolar Click Reaction with TCO and Azide

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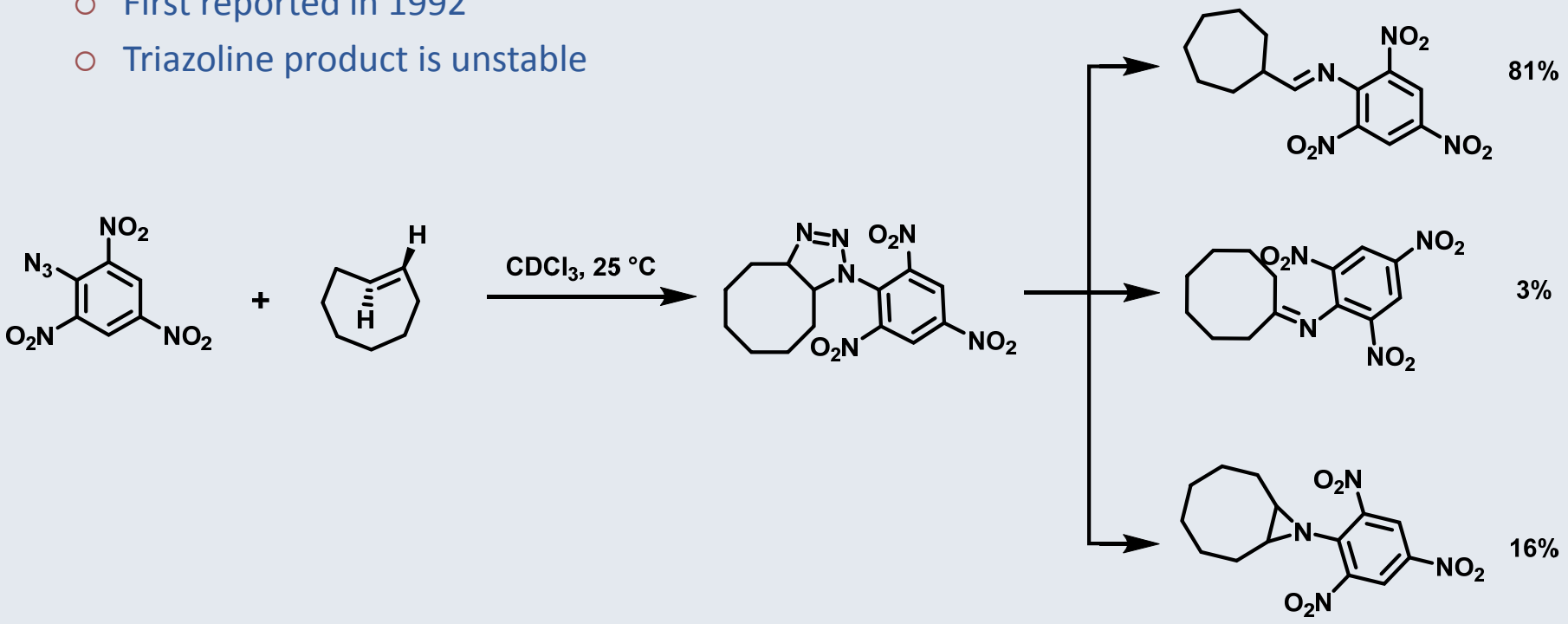
- Bioorthogonal Chemistry: TCO and Azide
  - First reported in 1992
  - Triazoline product is unstable



K.J. Shea, J.-S. Kim, *J. Am. Chem. Soc.*, **1992**, 4846-485

Prodrugs for Cancer Therapies: 1,3-Dipolar Click Reaction with TCO and Azide

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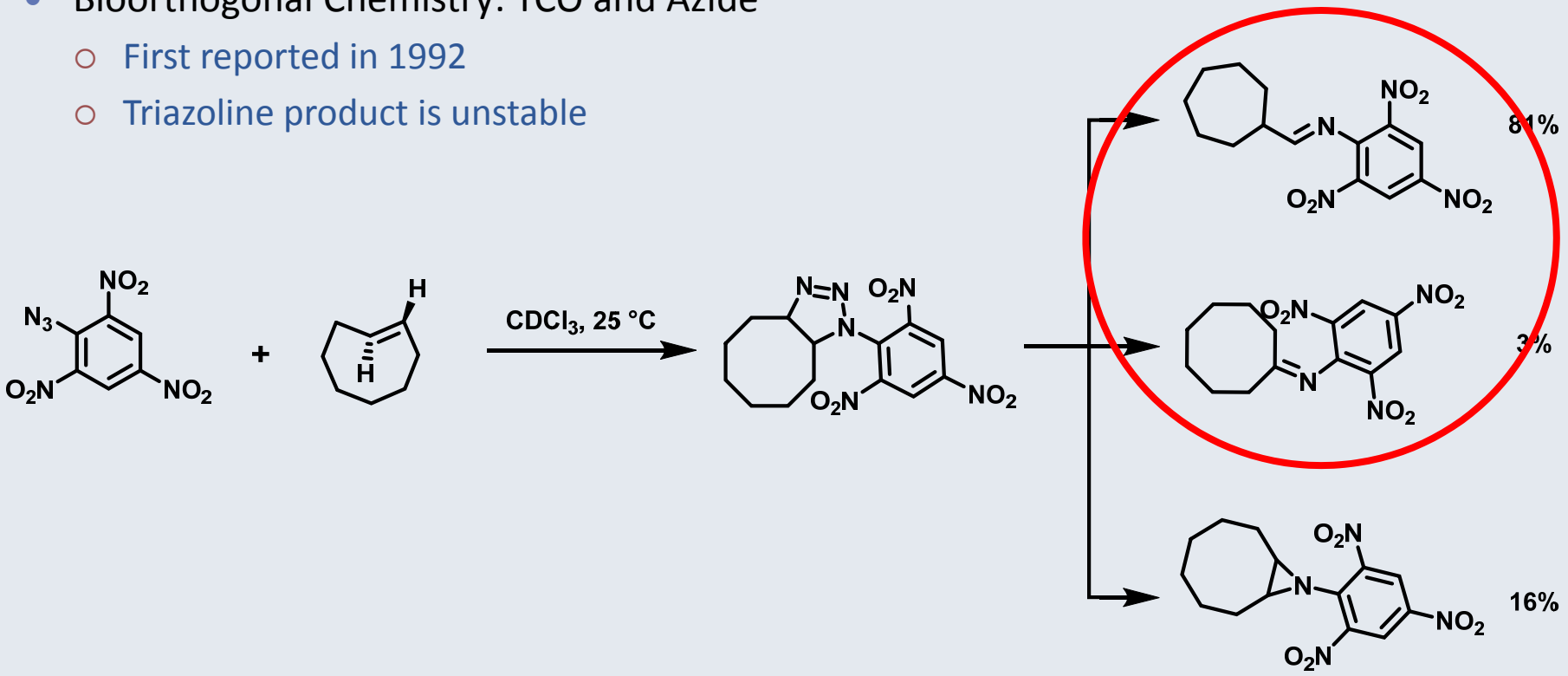

K.J. Shea, J.-S. Kim, *J. Am. Chem. Soc.*, **1992**, 4846-485

Prodrugs for Cancer Therapies: 1,3-Dipolar Click Reaction with TCO and Azide

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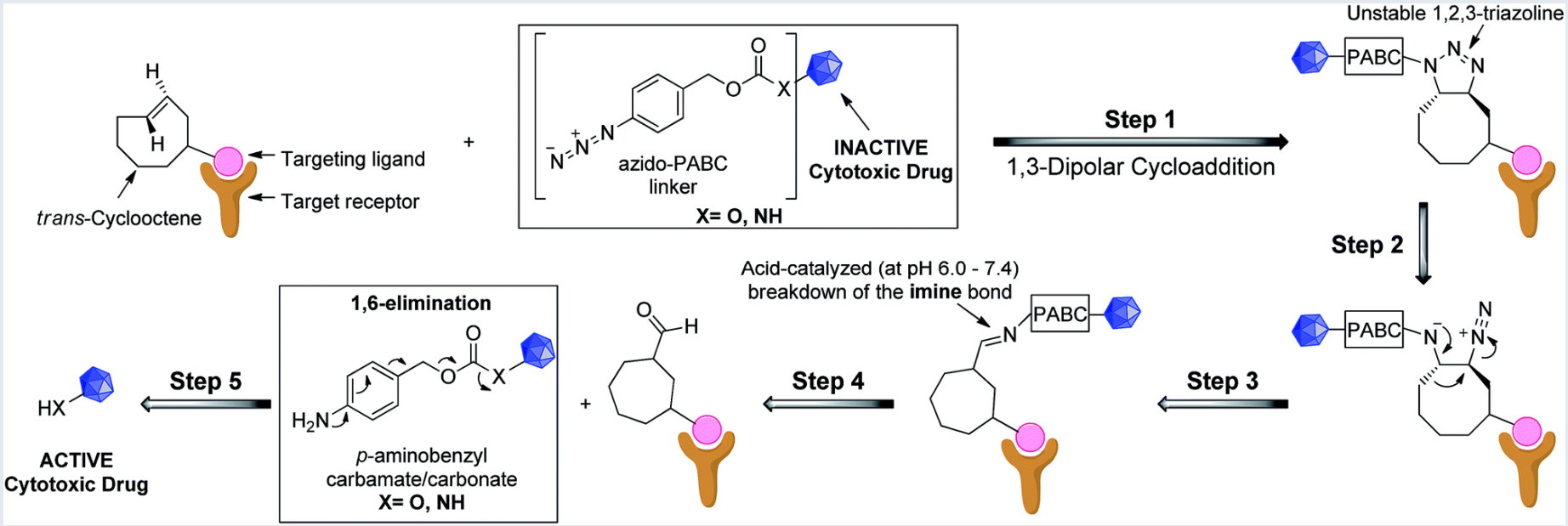
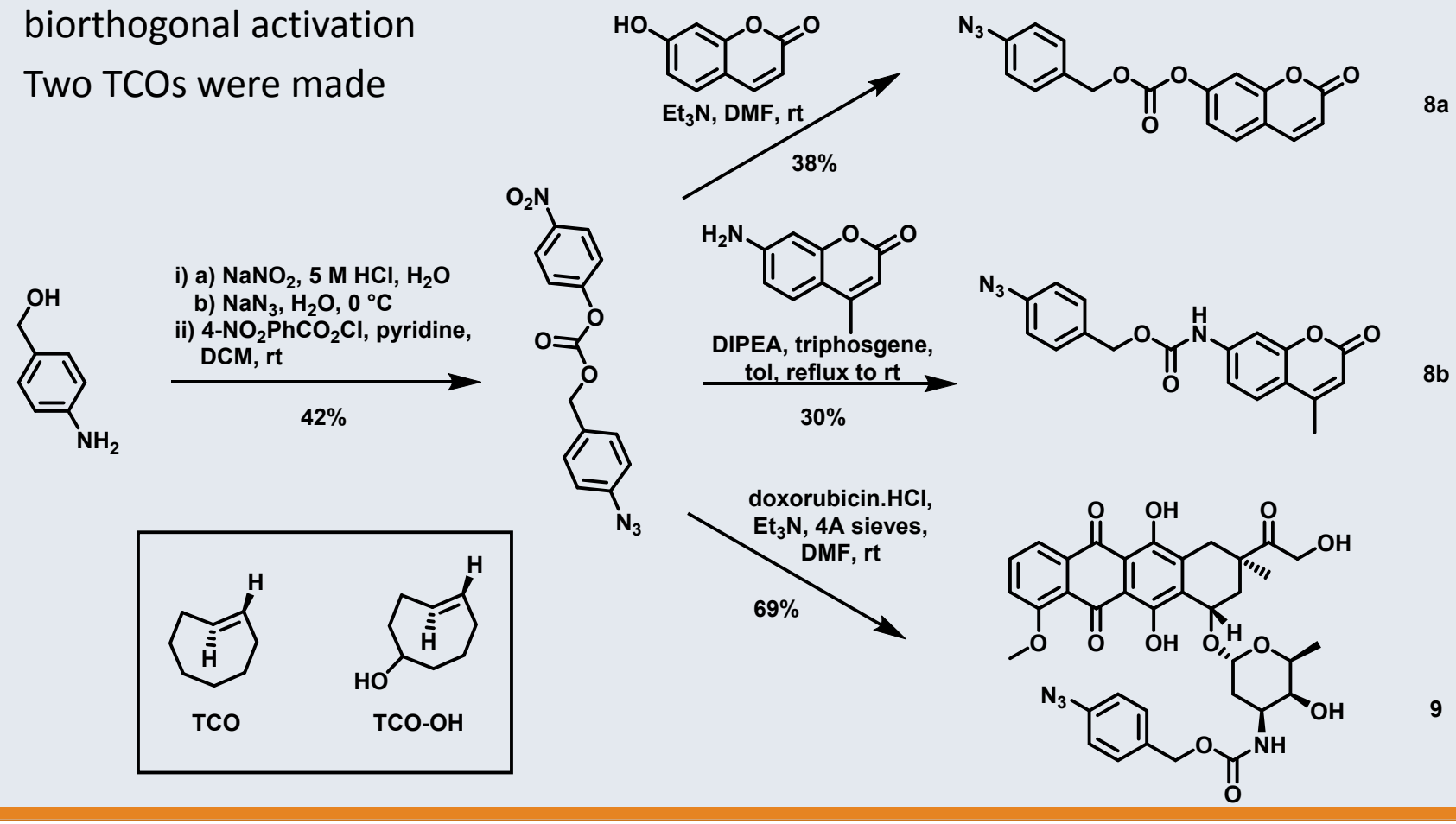
- TCO and Azide for Prodrug Activation!
  - Attach electron-deficient linker to inactive drug
  - TCO identifies target
  - Aqueous environment is key



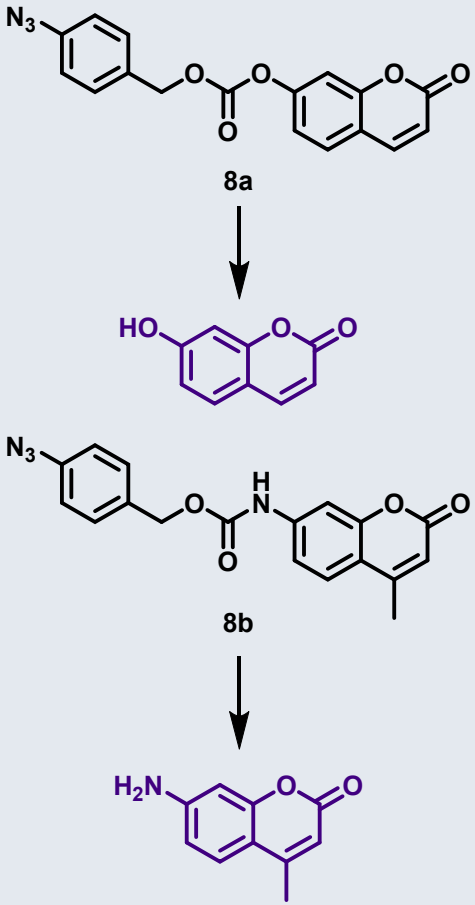
Proof of Concept

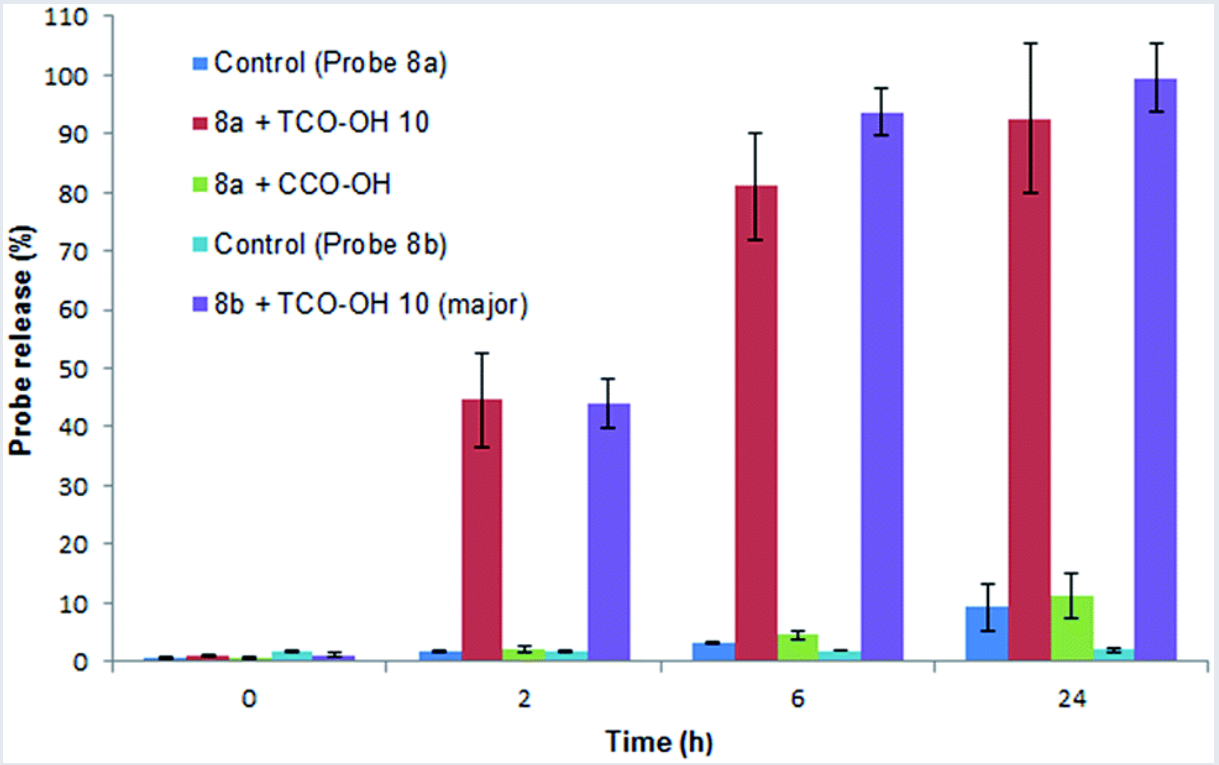
- Coumarin probes were synthesised to investigate rate and mechanism of reaction
- A doxorubicin azido-PABC prodrug was synthesized to investigate in vitro biorthogonal activation
- Two TCOs were made



Proof of Concept

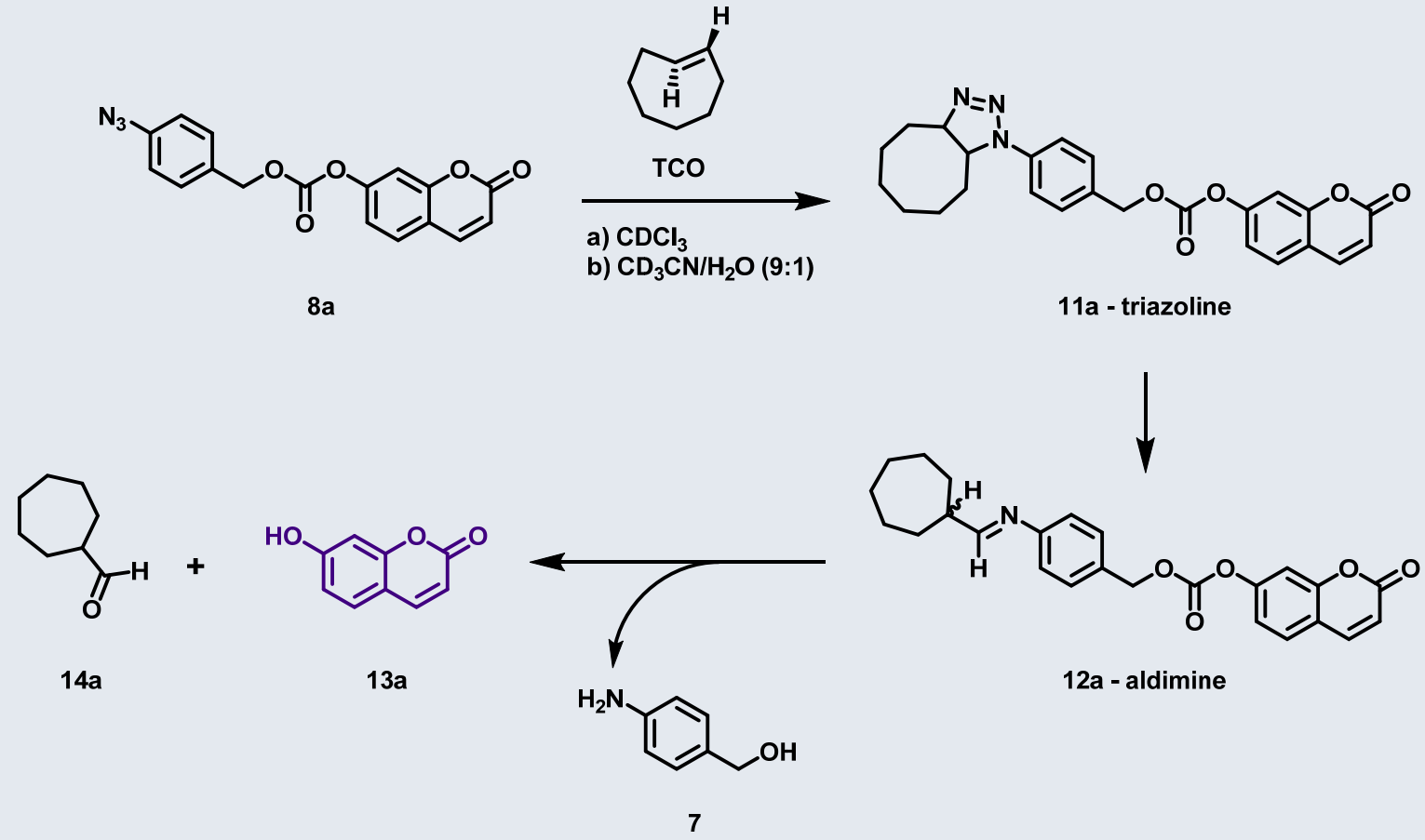
- Tested by spectrofluorometry, measuring fluorescence (ex 360 nm, em 455 nm)
  - in PBS:MeCN (1:1) n=3





Mechanism

- A series of 1H NMR experiments were then carried out
  - **8a** carbonate was used at 6.7 mM, TCO at 18.7 mM



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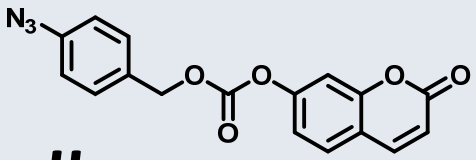
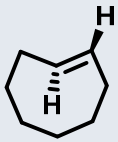
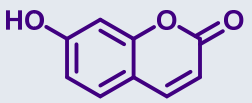


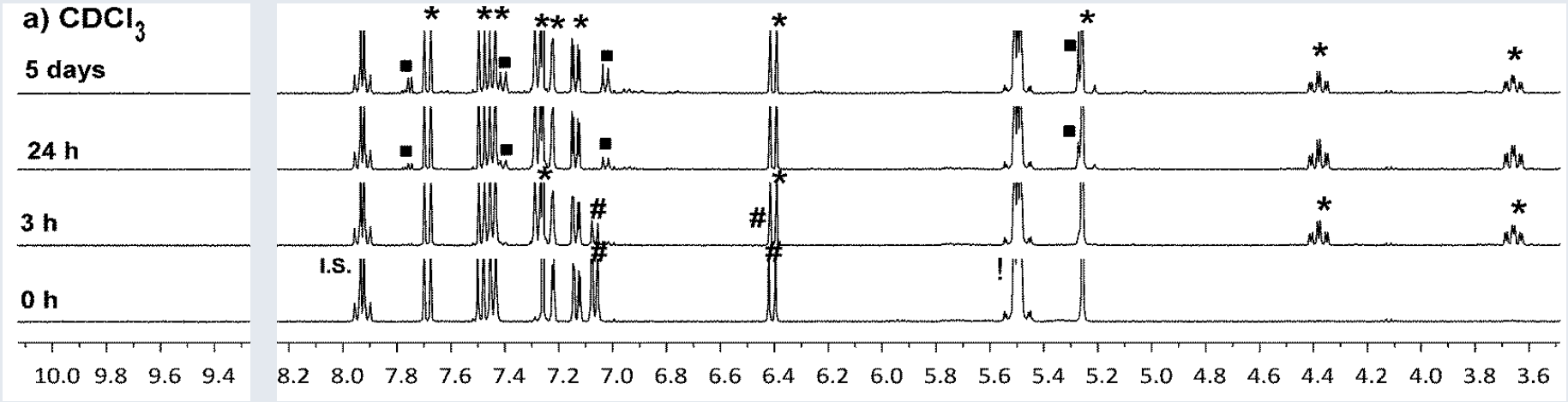
\# 8a - SM carbonate

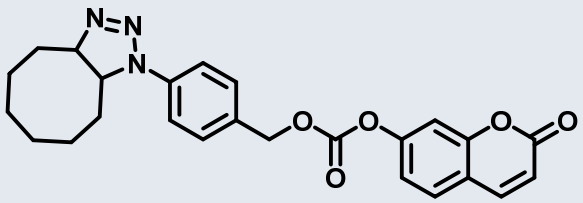


! TCO

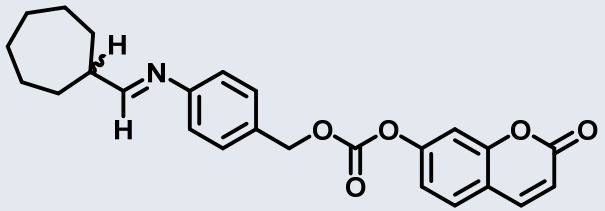


● 13a - coumarin



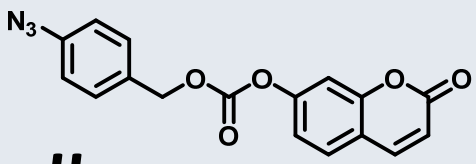


* 11a - triazoline



■ 12a - aldimine

Mechanism



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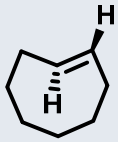
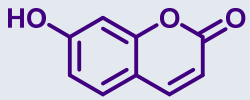


! TCO



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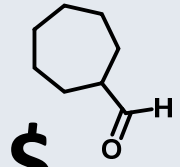
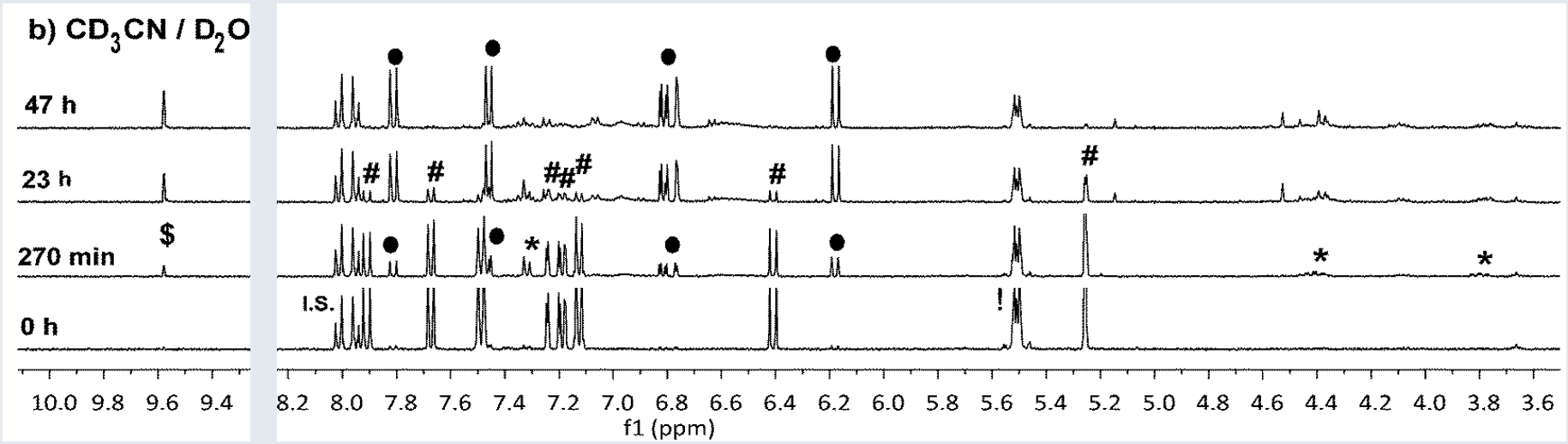
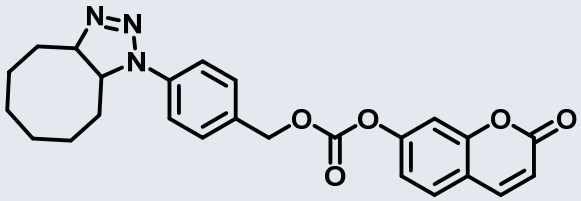
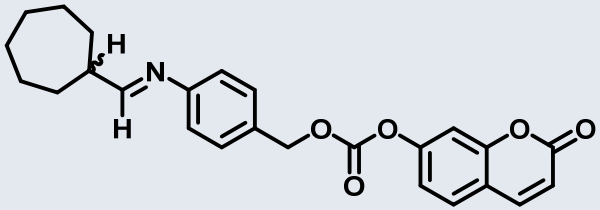


$ 14a





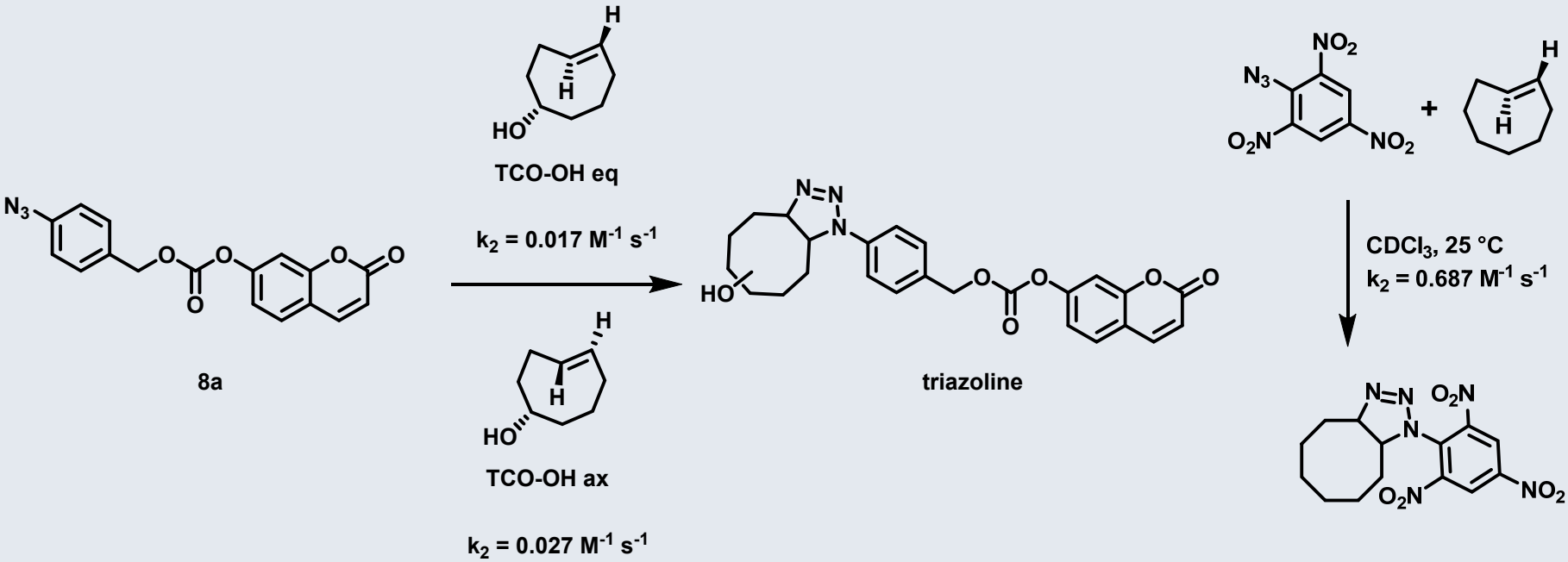
* 11a - triazoline



■ 12a - aldimine

Rate of Reaction of 1,3-Dipolar Cycloaddition

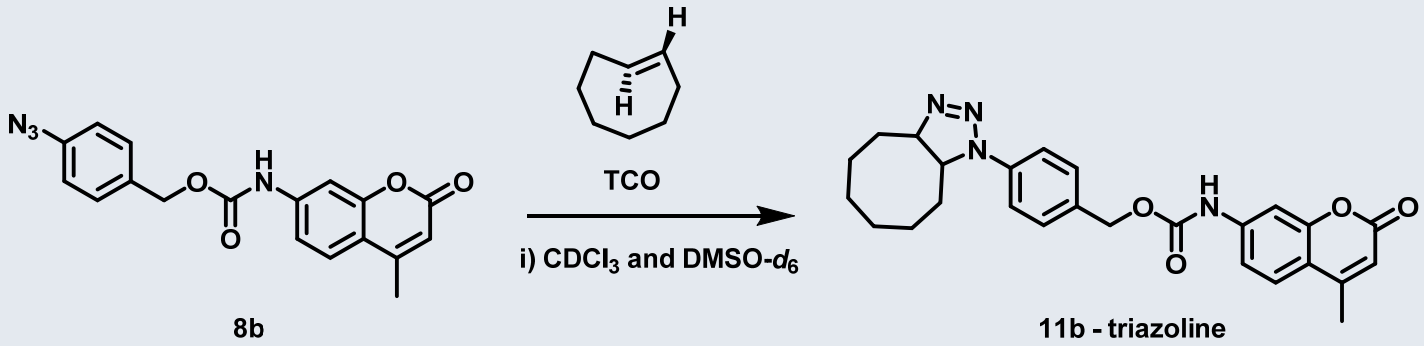
- TCO/TCO-OH activation results in coumarin release
- RP-HPLC was used to determine the second order rate of the initial 1,3-dipolar cycloaddition
  - MeCN:PBS (1:1, 37 °C), measuring disappearance of SM at 254 nm
  - Comparable rates to those seen with first generation SPAAC (10^{0}-10^{-3} M^{-1} s^{-1})



K.J. Shea, J.-S. Kim, *J. Am. Chem. Soc.*, **1992**, 4846-485

Rate of Reaction for Coumarin Release

- **8b** and TCO were reacted in CD_3CN and DMSO-d_6 for 19 h to give **11b**
- An aliquot of the NMR was then diluted into PBS and the rate of triazoline and imine degradation was monitored spectroscopically by the appearance of **13b**



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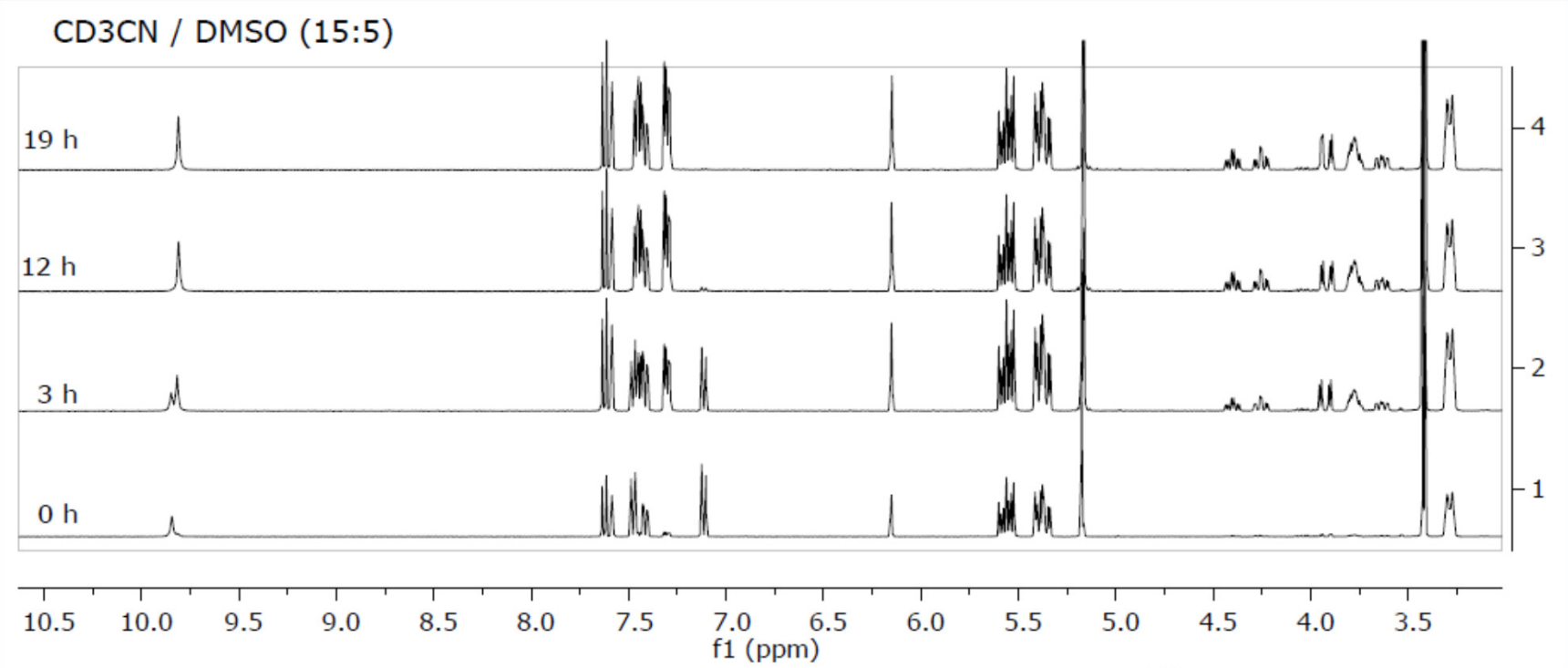

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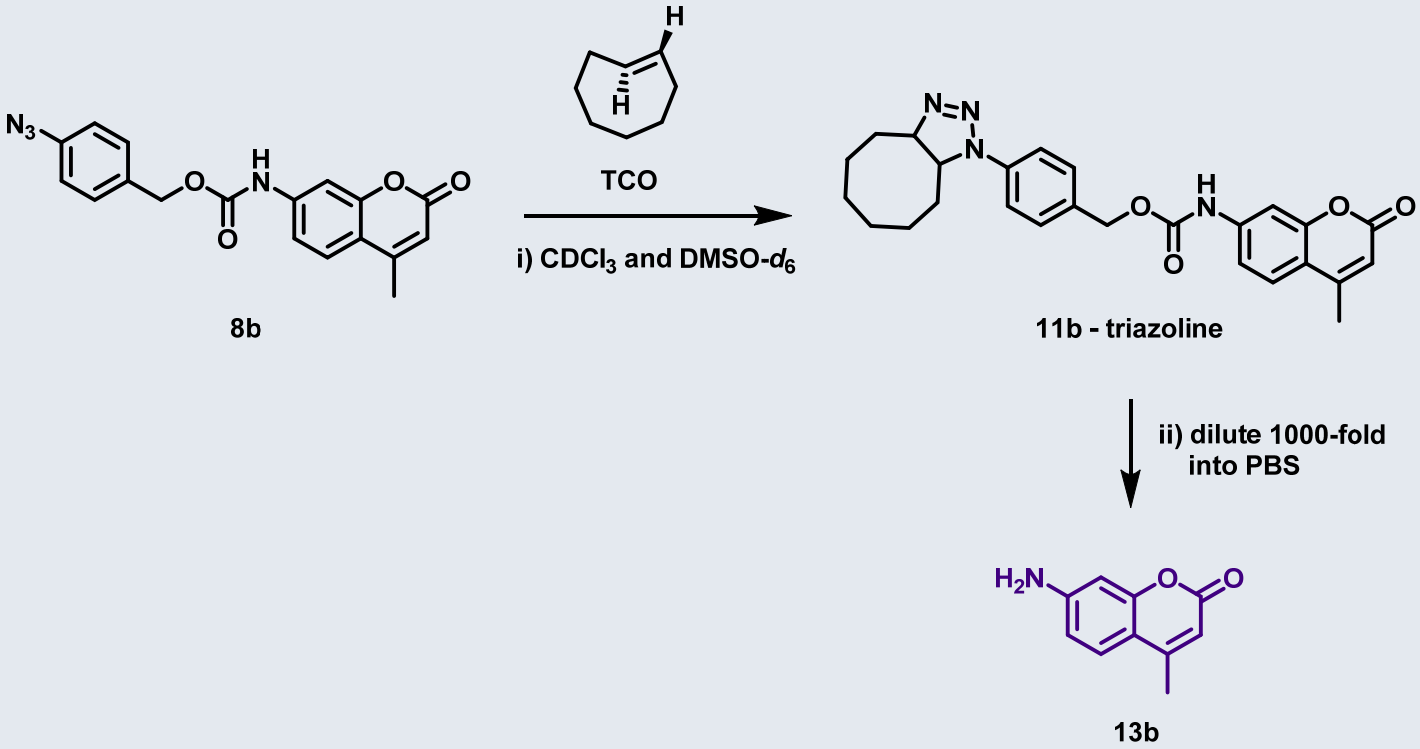

Rate of Reaction for Coumarin Release

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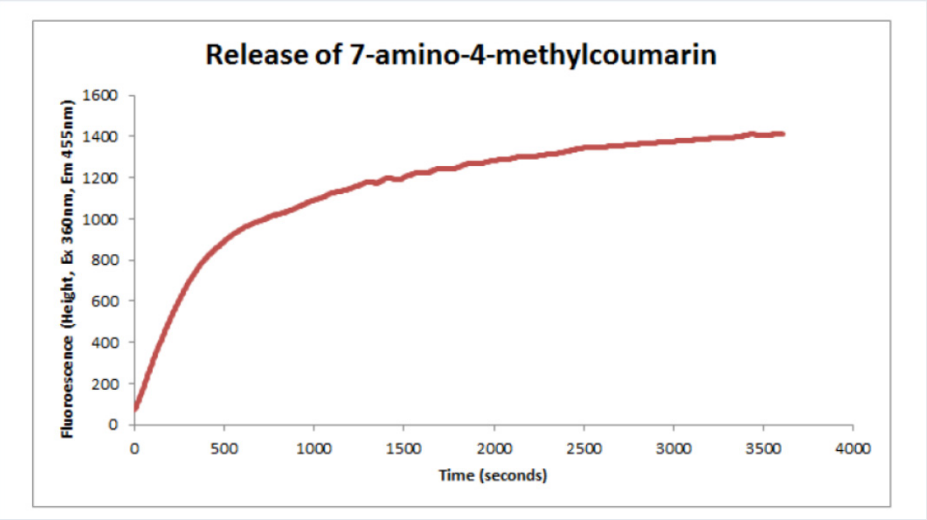
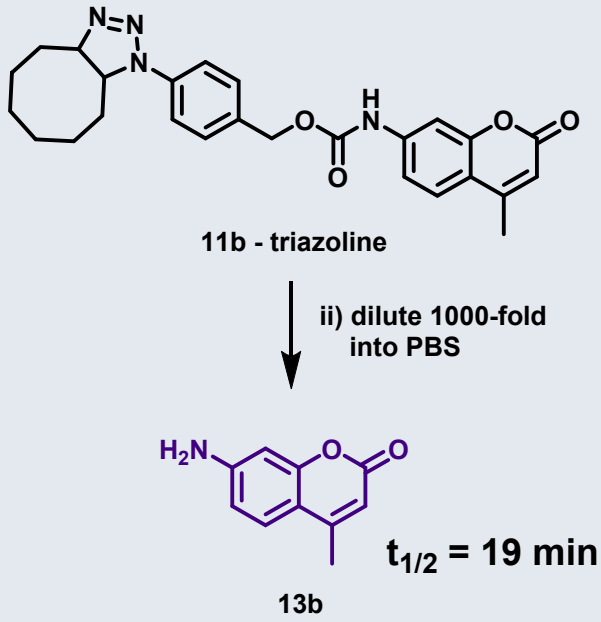
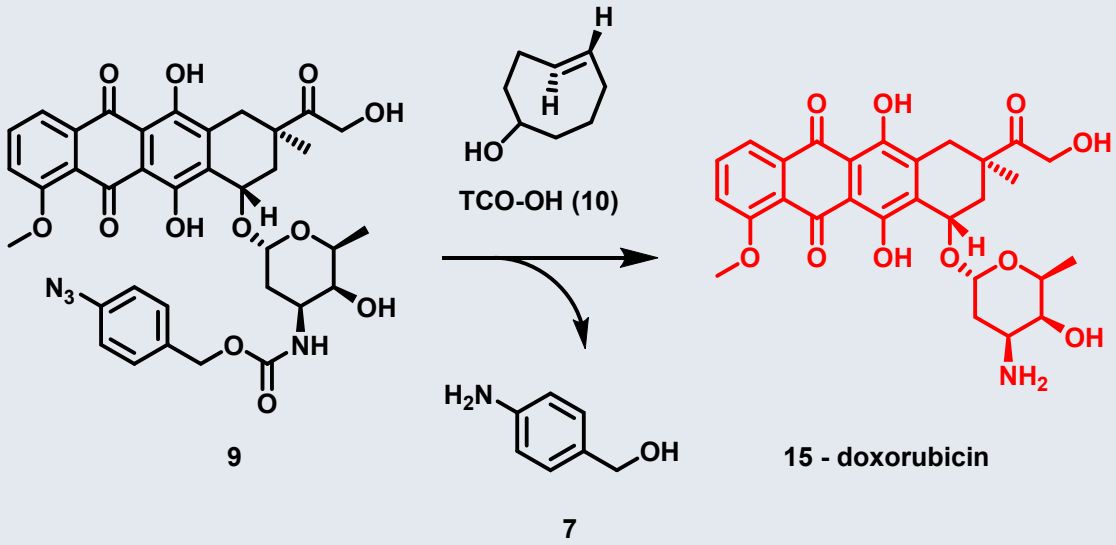




- Degradation and release (3 steps) follows pseudo first-order kinetics in polar protic solvents
  - Assumption that either triazoline degradation or imine hydrolysis is the rate limiting step
  - *In vivo* this rate is less significant as both intermediates will be fixed to a cancer cell surface

Bioorthogonal Potential



Compound	IC_{50} (μM)
doxorubicin (**15**)	0.71
9 pro-drug	49.9
9 + CCO-OH (100 μM)	55.0
9 + TCO-OH (**mix**) (100 μM)	0.96
9 + TCO-OH (**eq**) (100 μM)	1.47
9 + TCO-OH (**ax**) (100 μM)	1.34
9 + TCO-OH (**eq**) (10 μM)	4.98

- Using a model murine melanoma cell line (B16-OVA), the reaction strategy was evaluated *in vitro* following 72 h incubation with **9** and **10** at 37 °C
  - **9** cytotoxicity alone is low
- The authors propose that **15** is released outside of targeted tumour cells and then diffuses into the closely located cancer cells

Bioorthogonal Potential

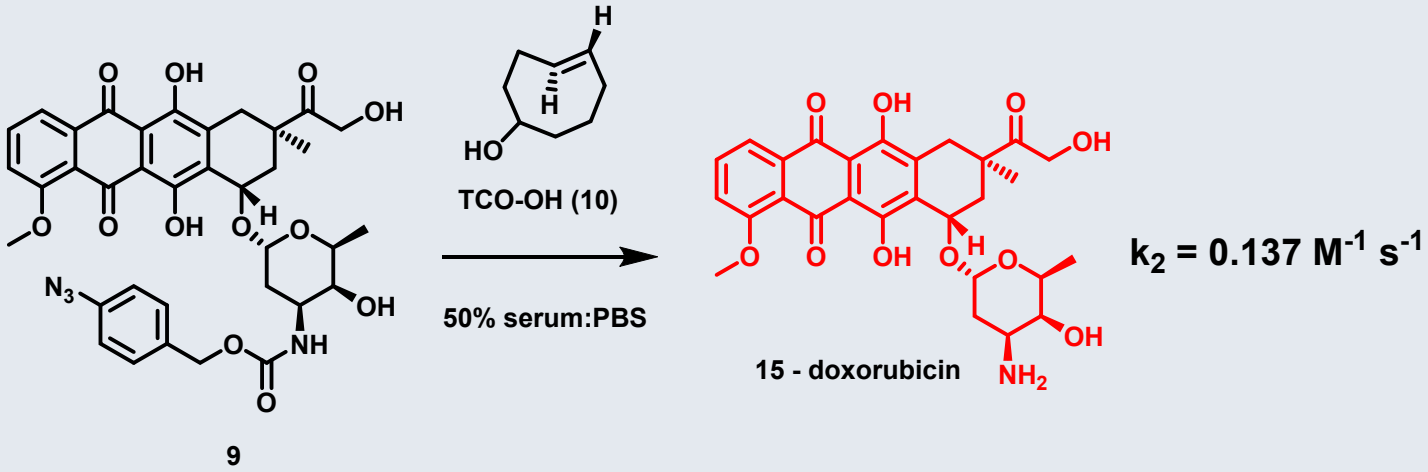
18

- Attachment of 1-6 TCOs to each monoclonal antibody with binding with 10^5 cell surface receptors, [TCO] = 0.4-2.5 µM on tumour cell surfaces
  - **9** rate of reaction still needs improvement
  - Need to overcome rapid clearance rates from mice ($t_{1/2}$ = mins) for similar compounds
- *In vivo* suitability was evaluated by monitoring stability and activation of **9** in 50% mouse serum:PBS and PBS only (HPLC analysis)

Time (h)	Degradation of 9 (15 release) PBS only	PBS + MS	Degradation of 9 (15 release) + TCO-OH eq PBS only	PBS + MS
0	100% (0%)	100% (0%)	100% (0%)	100% (0%)
4	106% (0%)	95% (0%)	84% (34%)	47% (51%)
24	121% (0%)	68% (0%)	39% (77%)	12% (59%)
48	112% (0%)	56% (6%)	12% (79%)	2% (5%)

Bioorthogonal Potential

- No reaction deactivation from serum derived byproducts
  - a problem seen with Staudinger prodrug activations
- Investigated if serum protein interactions may reduce effective [**9**] and therefore activity
  - SPAAC cyclooctyne reactants (added after tumour targeting) interact with serum and show reduced *in vivo* reaction rates
- The reaction proceeds faster in the presence of serum than the model systems of **8a** and **8b** in MeCN:PBS (k_2 = 0.017-0.027 M^{-1} s^{-1})



Conclusions and Scope

- Successfully use a 1,3-dipolar cycloaddition between TCO-OH and an azide, to facilitate prodrug activation
  - Activation is 1-2 orders of magnitude faster than the Staudinger reaction variants (and faster still in serum:PBS mixtures
  - TCO-OH could isomerise, but will be modified with antibody linkers known to stabilise isomerization
  - Still room to expand on reactivity through modification of the azido-prodrug
  - Good stability in mouse serum (min to h)
- Next step: need to move from the hypothetical to the actual prodrug antibody system