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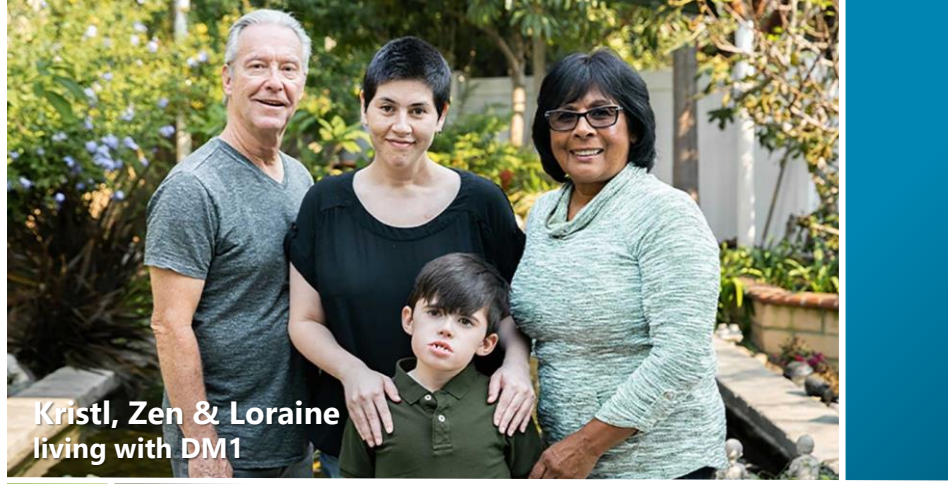
Understanding Impurities in Antibody Conjugates

Jeff Carroll

Associate Director, Avidity Biosciences

Process Development & MFG



AGENDA

I. Challenges in Characterizing ADC Impurities
II. ADC Impurities from Drug Linkers
III. ADC Impurities from mAb
IV. Summary

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Challenges in Characterizing ADC Impurities

DAR Profiles of Common Conjugation Chemistries

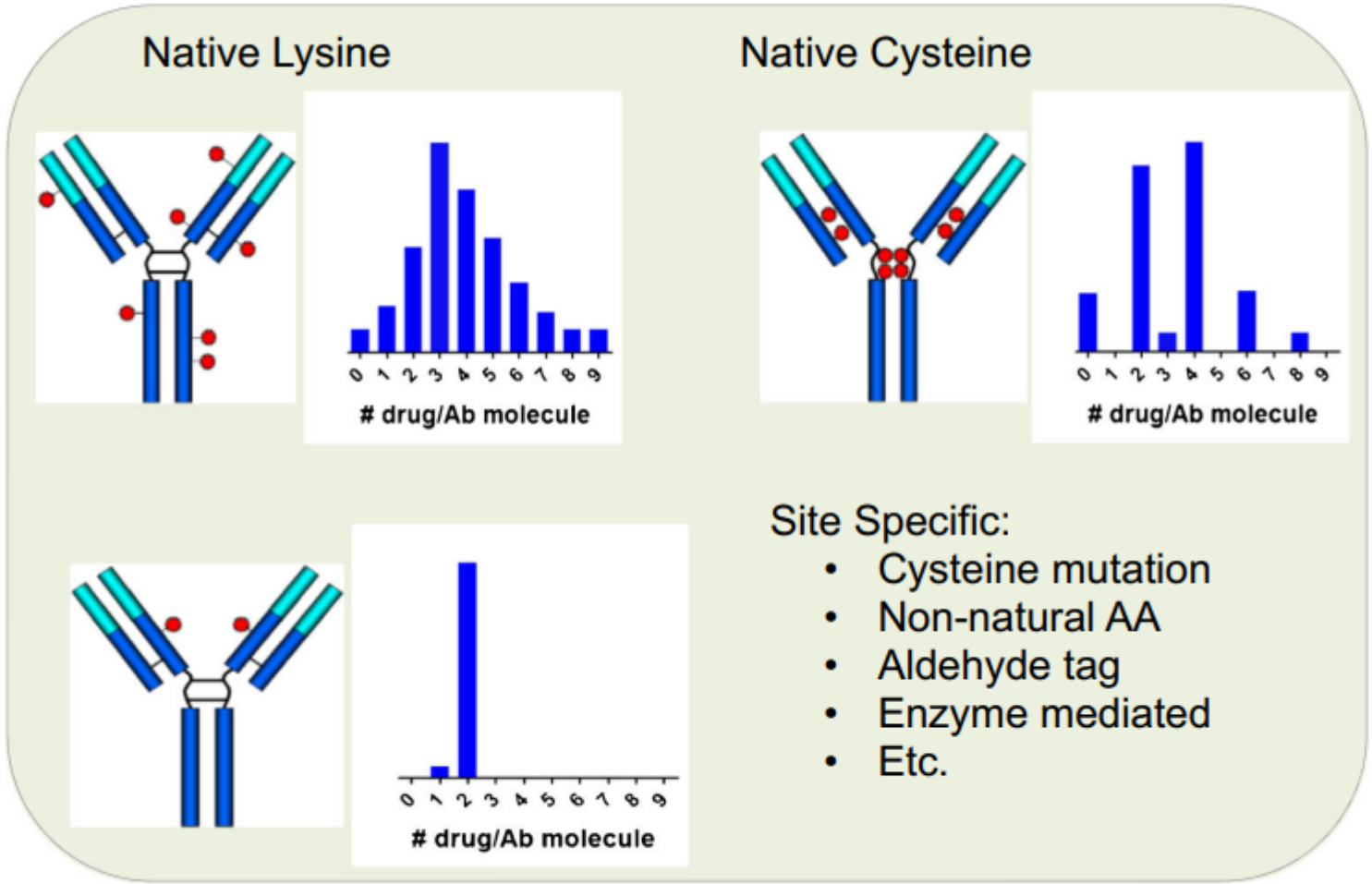
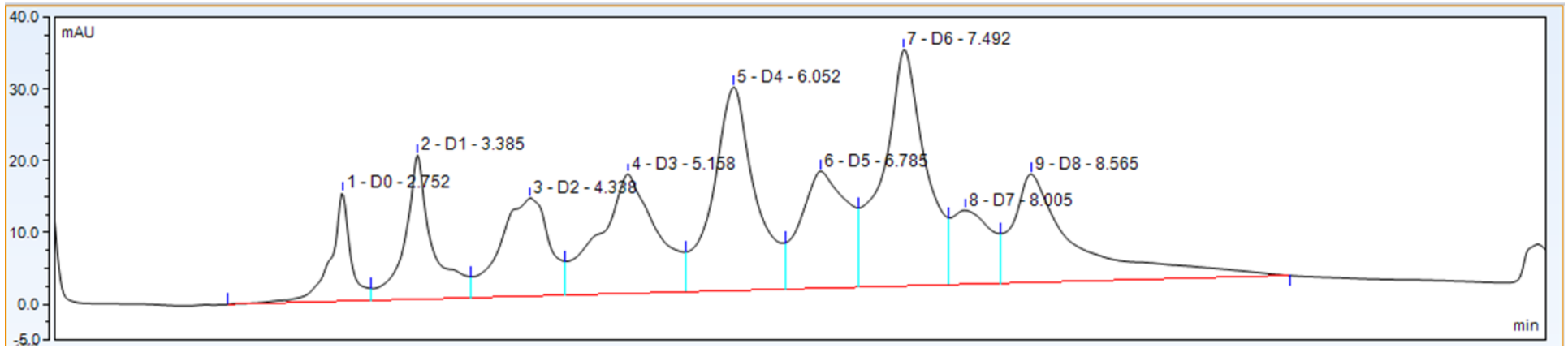


Fig. 1. A cartoon representation of different conjugation chemistries for ADCs. The linker is attached between the drug and the mAb usually by a covalent bond through a cysteine or lysine

Gang, et al AAPS PharmSciTech, Vol. 19, No. 3, April 2018

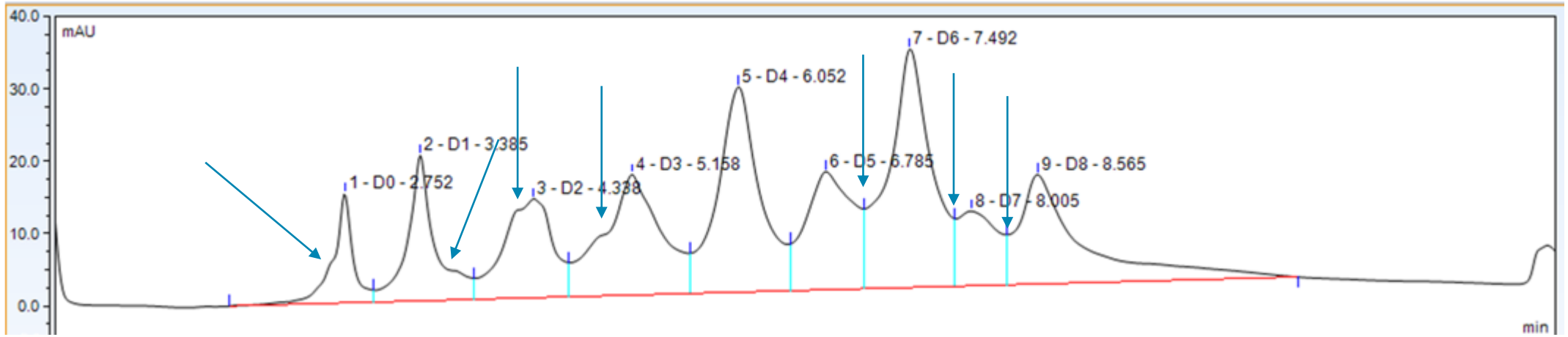
HIC Profile of Cys Conjugate

Slightly over reduced to show increase in intensities for higher species



HIC Chromatogram

Not as clean as expected



We clearly see more than the expected D0, 2, 4, 6, 8 species

The D1, D3, D5, D7 species were assigned based on UV differential of payload to mAb

But what about all the extra peaks and shoulders?

Very challenging to isolate these and characterize

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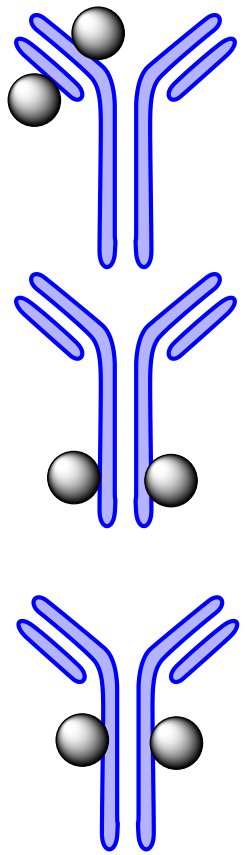
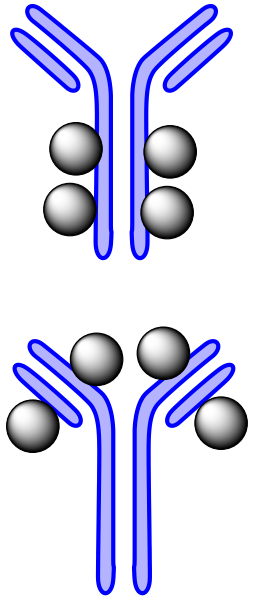
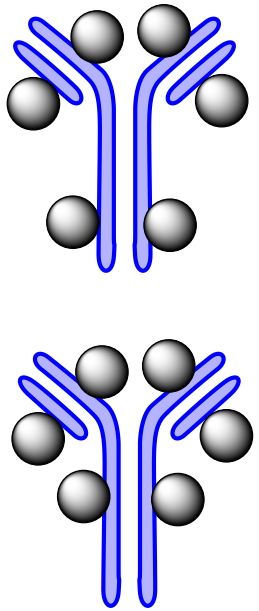
Could Expect Regio-isomers



DAR 2



DAR 4



DAR 6

Would require HIC fractionation followed by digestion and HRMS to determine what amino acids were conjugated

While some extra peaks may be isomers doubtful that all are

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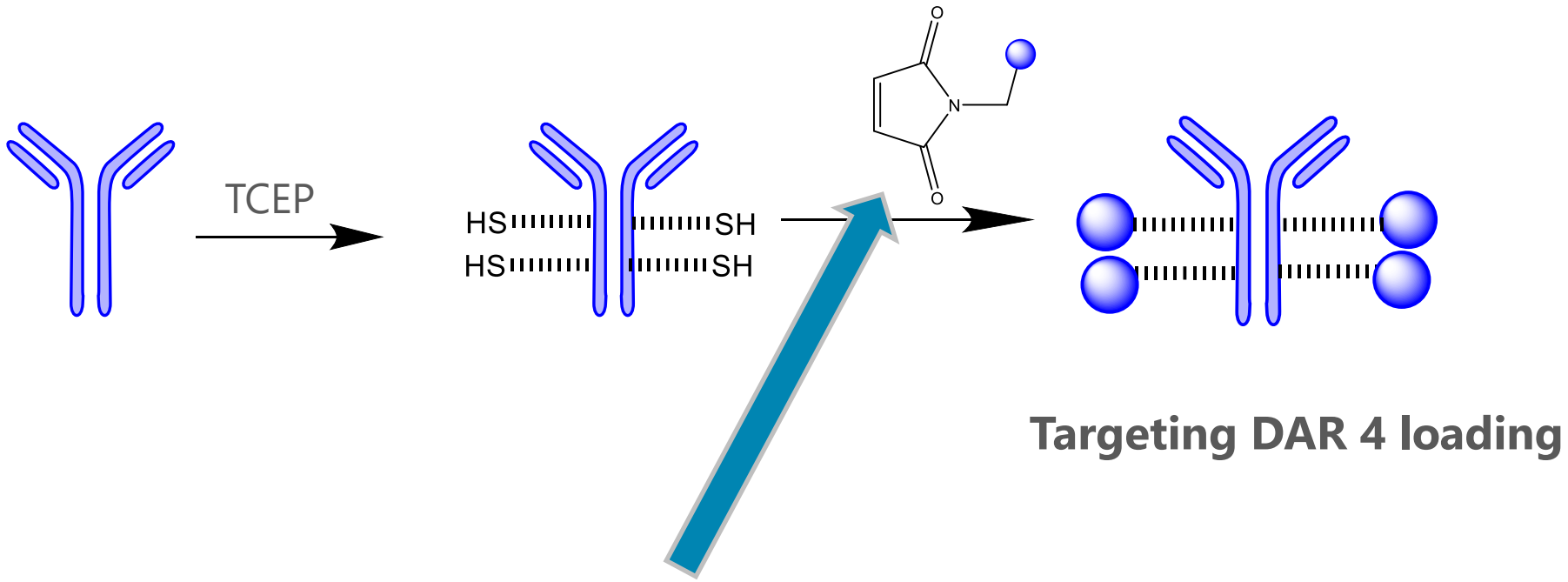
ADC Impurities from Drug Linkers

Understanding Source of Impurities

Standard Cys conjugation



HIC differentiates on lipophilicity so drug linker is likely responsible for some impurities but still challenging to isolate and characterize post conjugation

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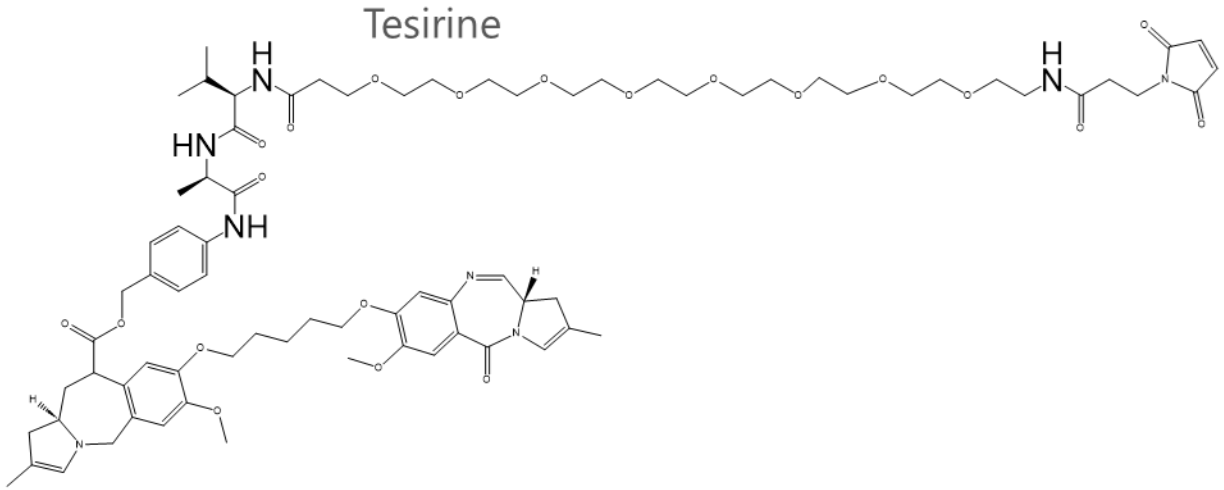
Tesirine Example



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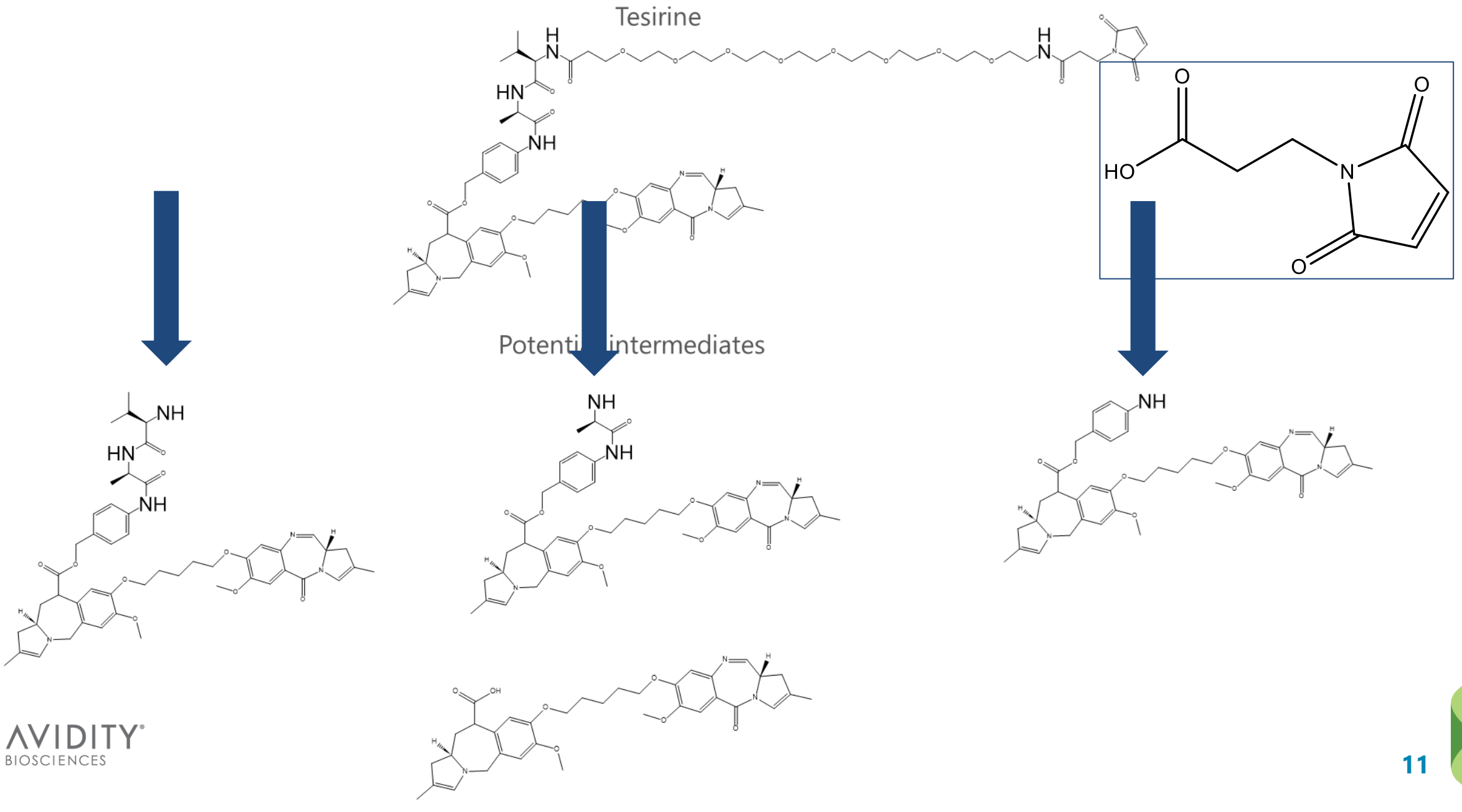
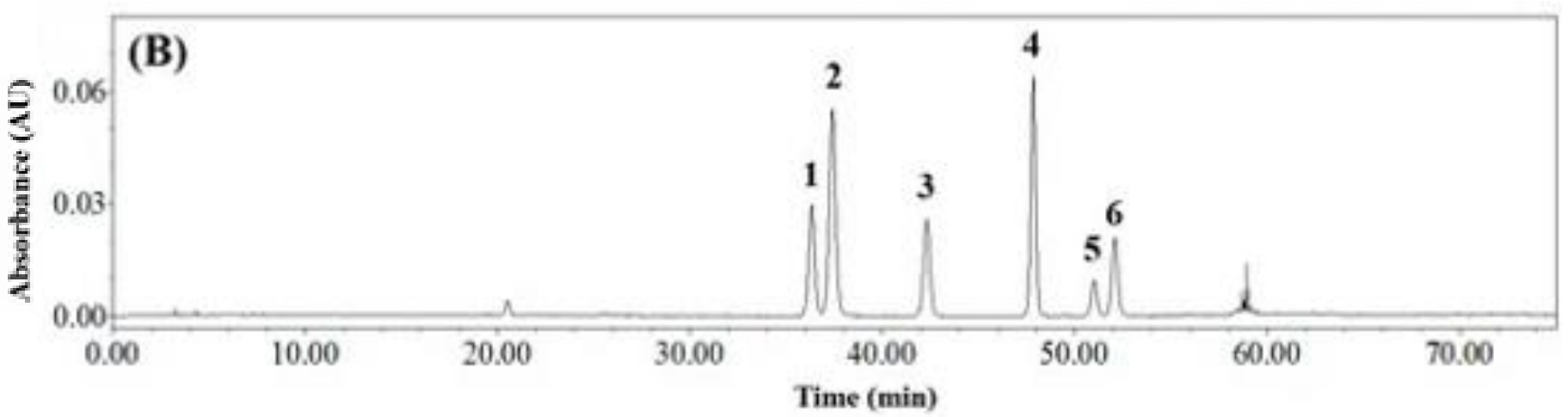
How Are Conjugatable Impurities Formed?



Take a Step Back: Hypothetical HPLC of Drug Linker



Chromatograph of development lot of drug linker

Peak 4 is the drug linker of interest

All others are impurities

While the purity needs to be better, we'd certainly like to know which ones could impact our ADC

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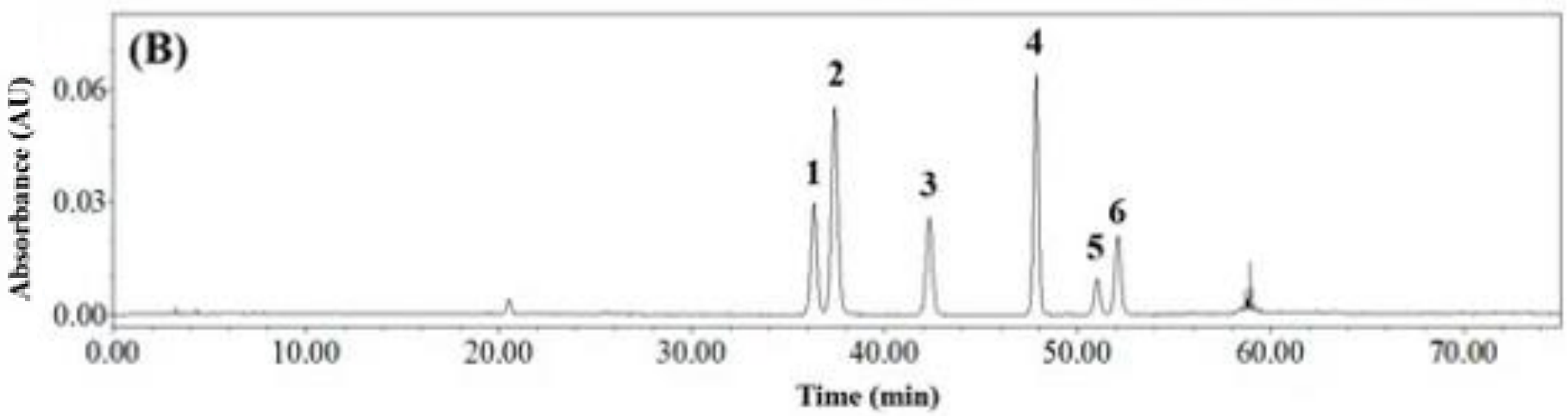
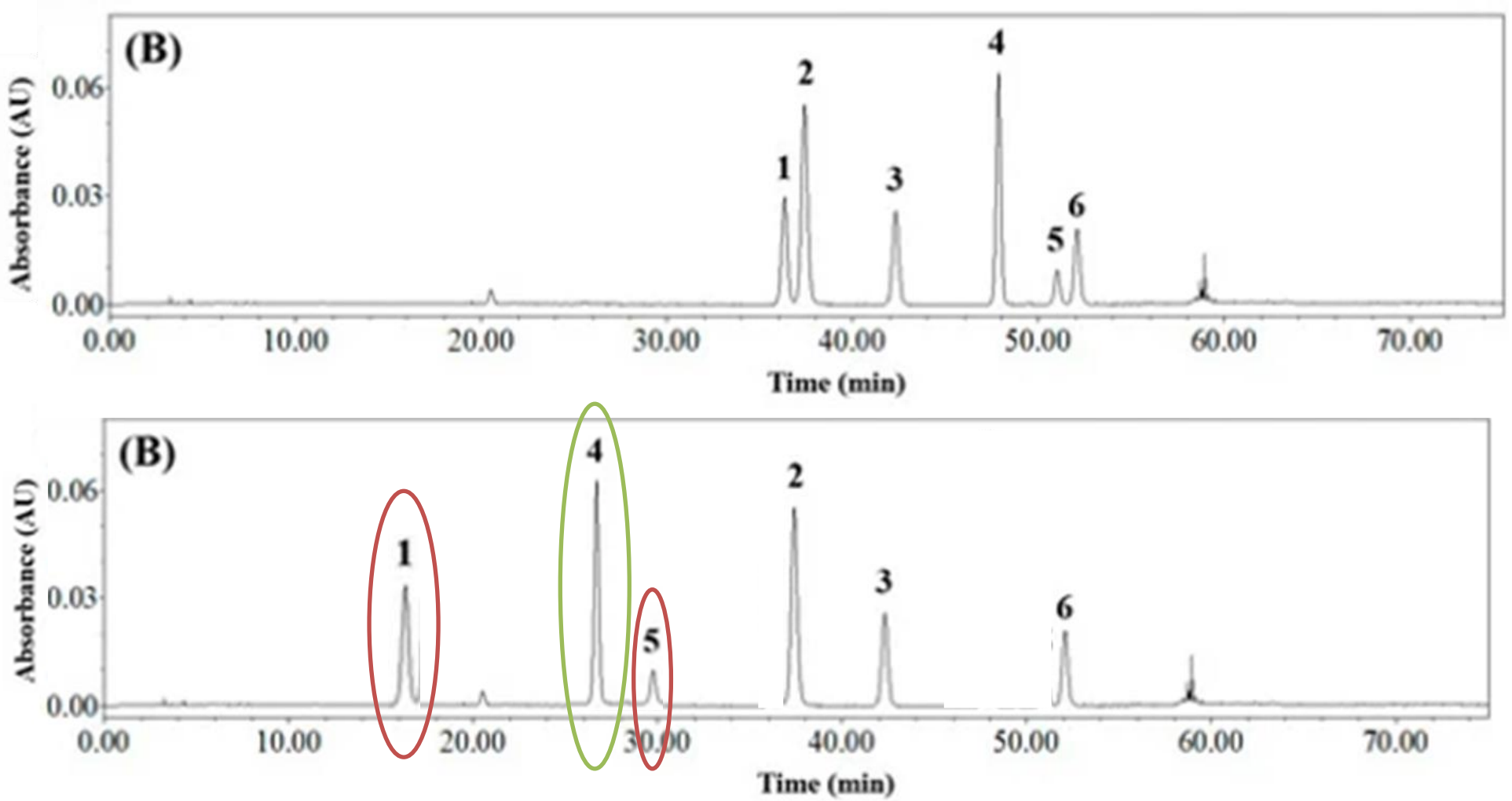
Surrogate Conjugation



If we conjugate to a surrogate thiol, we can determine which impurities are conjugatable

Drug Linker mixture + X SH → Thiol should react with any maleimide handle

HPLC Before and After Surrogate Conjugation



- Peak 4, the desired drug, linker shifts
- Peaks 1 and 5 also shift, indicating they are conjugatable impurities

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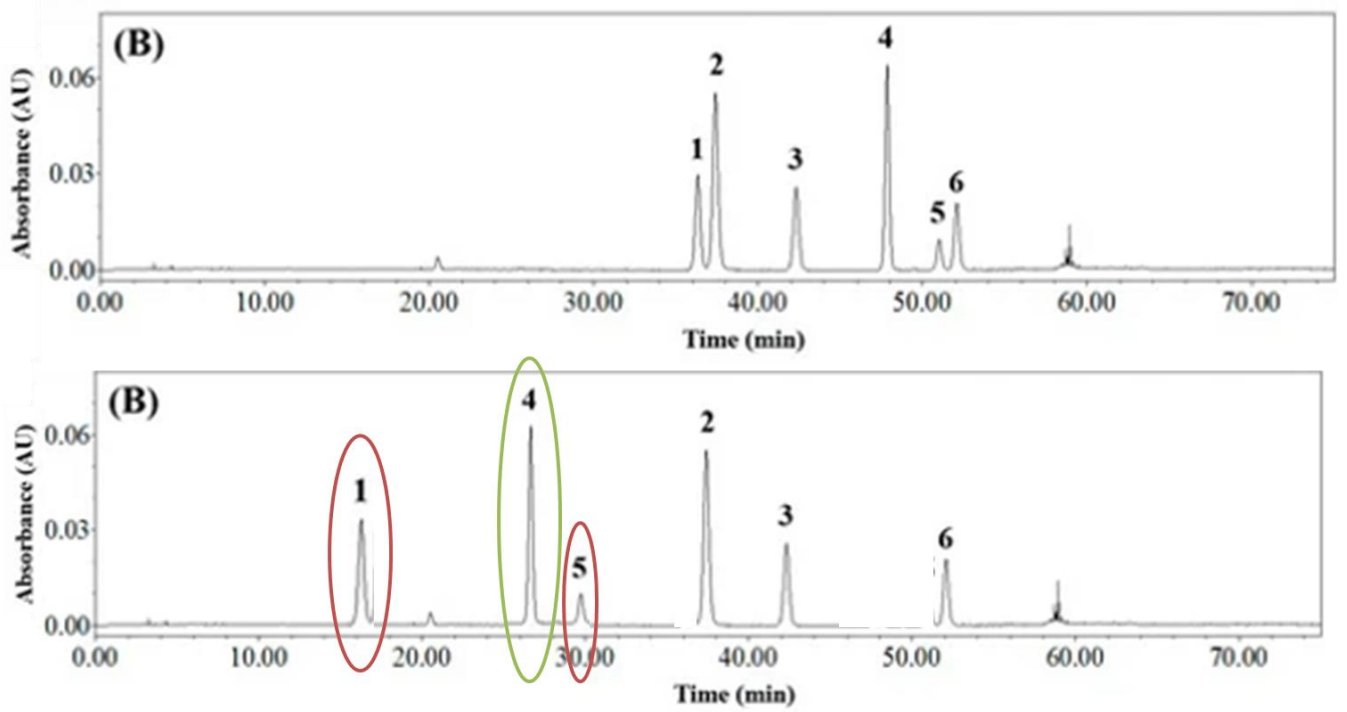
Impurity Characterization



- At the small molecule stage
- LCMS is more powerful in helping identify:
  - The impurities in the original mixture
  - The derivatized conjugatable impurity post-surrogate conjugation

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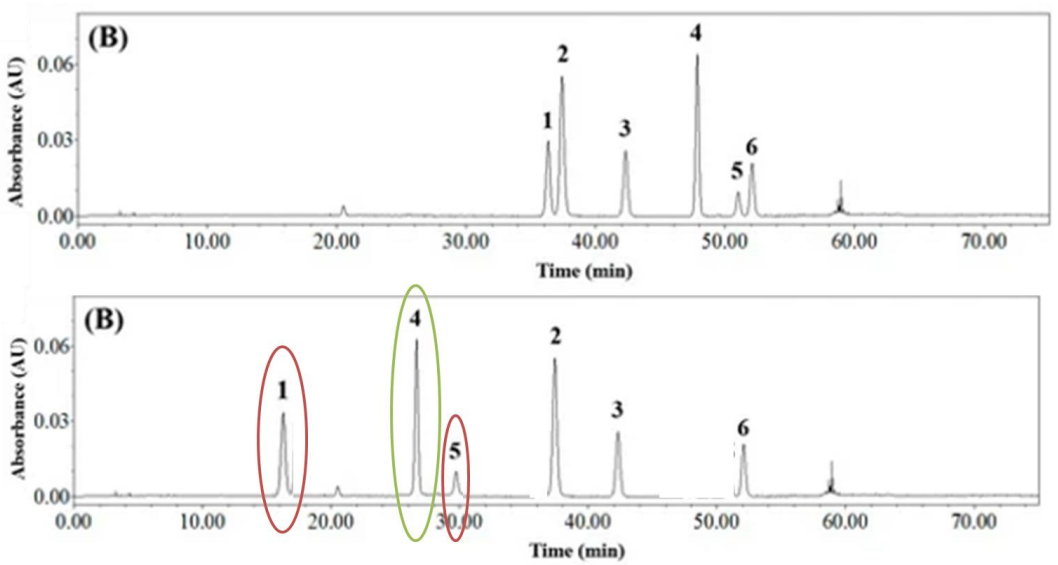
Impurity Isolation



- At the small molecule stage
- Impurities should also be easier to isolate
  - Compared to the ADC mixture

Could we react the isolated conjugatable impurity and conjugate to reduced mAb to help identify HIC impurities?

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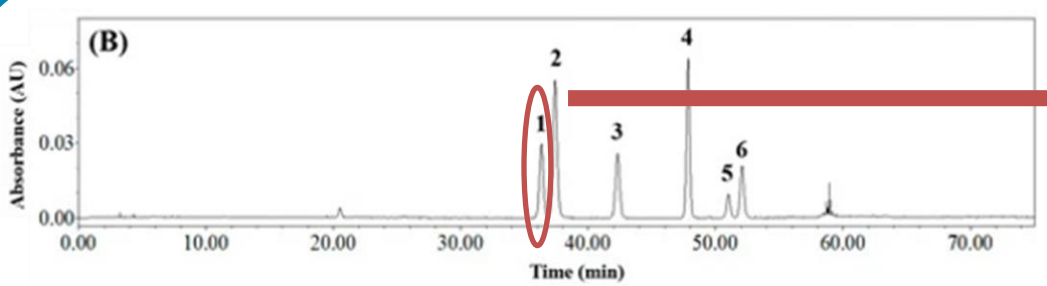
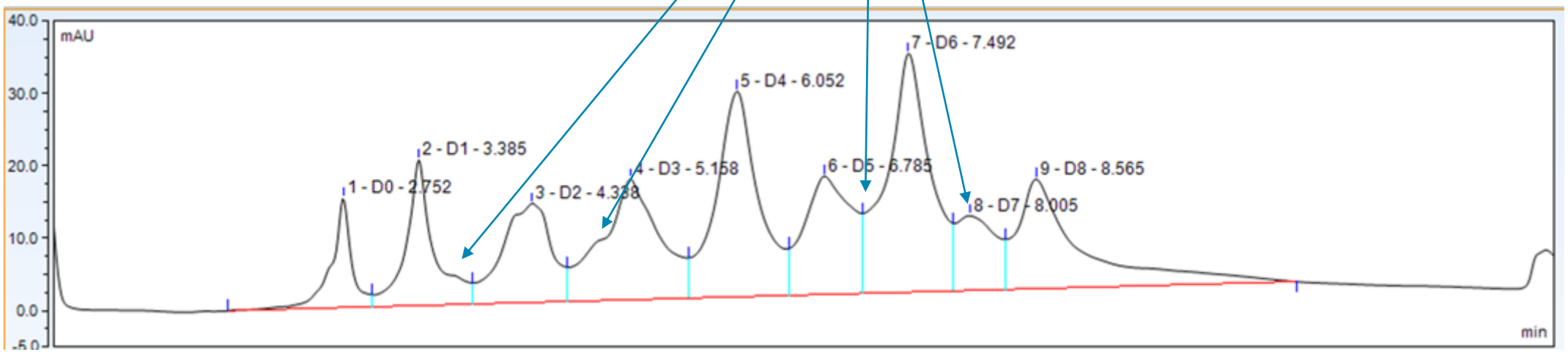
Potential Outcome



Reduced mAb

DAR 2, 4, 6, 8 of peak 1 impurity



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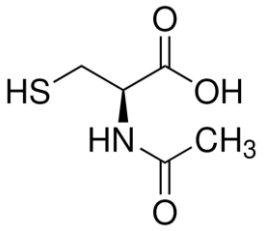
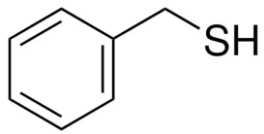
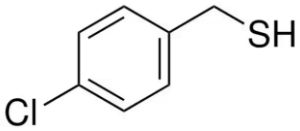
Note on Surrogate Conjugations



- For this Example, with maleimide chemistry
  - We could choose a water soluble thiol, e.g., N-acetyl cysteine
  - But for lipophilic drug linkers this could be an issue reacting in a buffer
    - We could also choose organic “friendly” surrogates
      - Benzyl mercaptan
      - thiols with a more distinct MS trace e.g., halogenated compounds (F, Cl)





    - Then run the chemistry in DMA, DMSO, or other organic solvent that offers solubility for all components

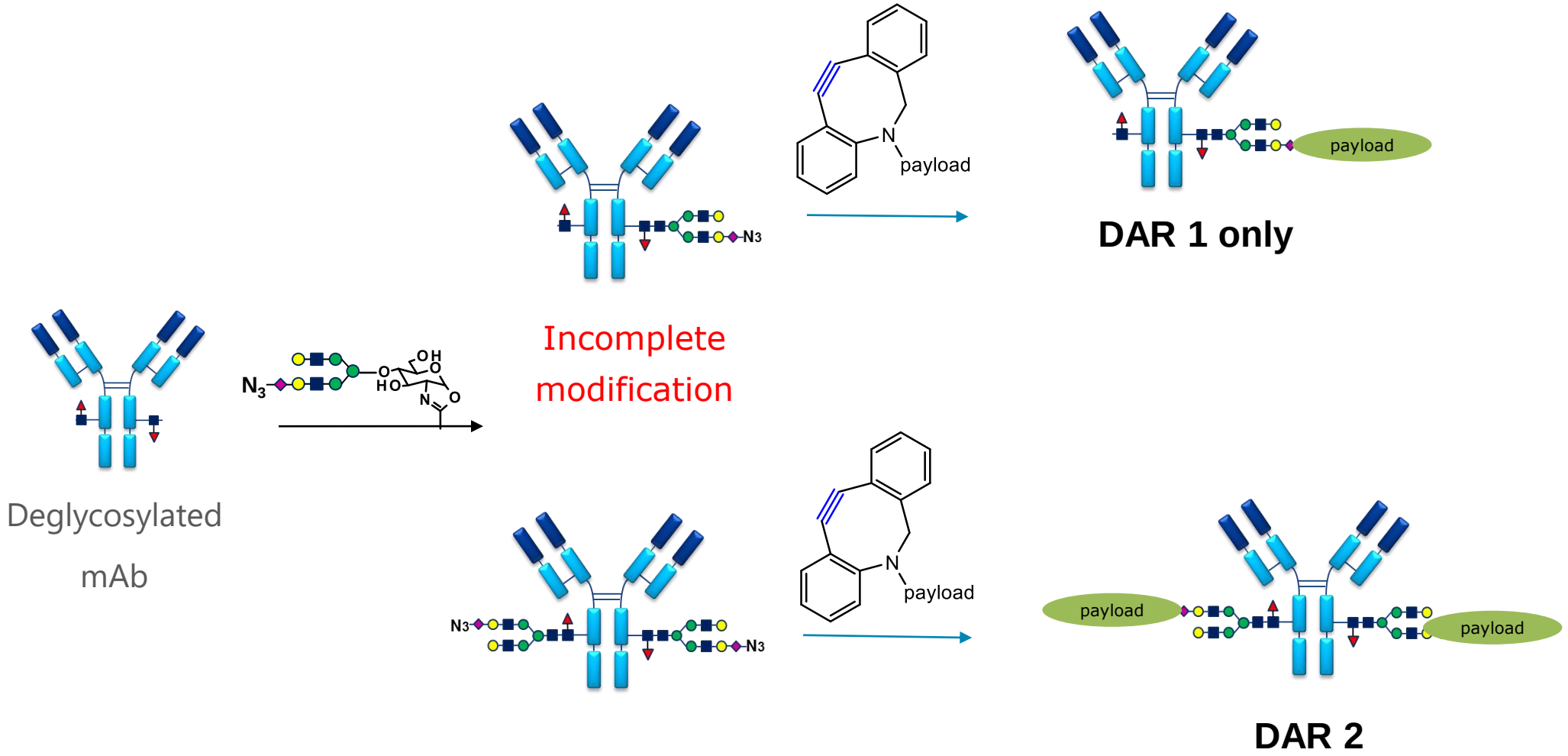
Key Take Away

- By using surrogate conjugations during Drug Linker Development
  - Programs can identify conjugatable impurities early on
    - Instead of attempting to characterize impurities in the ADC mixture
  - Focus on purification strategies to control them upstream to the Bioconjugation
    - Limit issues with the final ADC drug substance
- Non-conjugatable impurities become less of a concern and can typically be handled with UF/DF on the ADC
- This strategy can also identify non-potent conjugatable impurities
  - Allowing the program to potentially derisk their presence in the final ADC once characterized

ADC Impurities from mAb

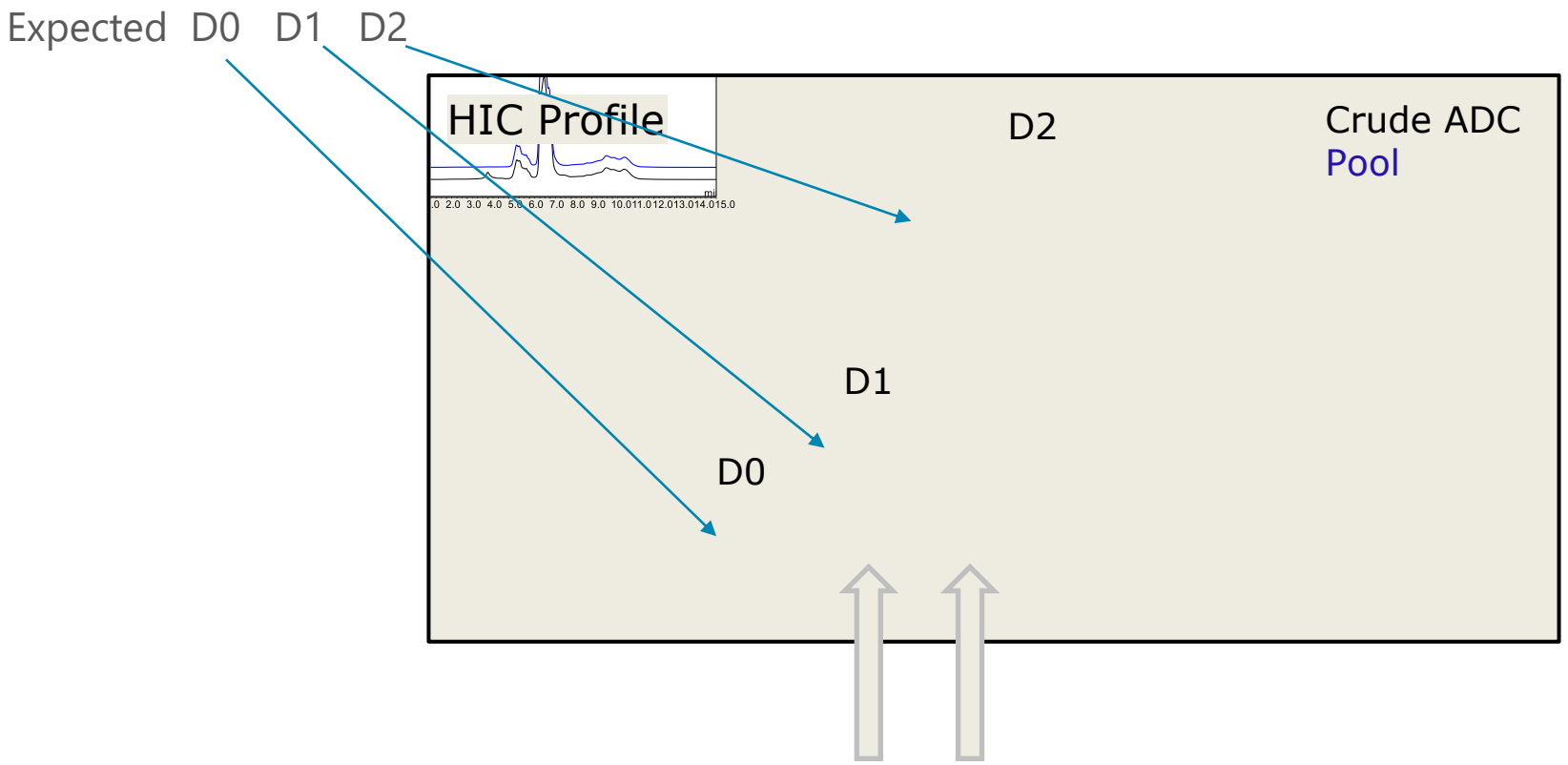
Example of Impurities from mAb

Case Study: Modified mAb + Click Conjugation



Expect DAR 0, 1, 2 in crude mixture

Case Study: Modified mAb + Click Conjugation



Examination of

- ABS 254/280 ratios
- Confirmed All peaks under D1 did in fact have 1 drug loaded
- All peaks under D2 did have 2 drugs

- But D1 and D2 peaks don't look clean, even after chromatography
- What is the source of these other species?
- The chemistry doesn't allow for multiple DAR1 species

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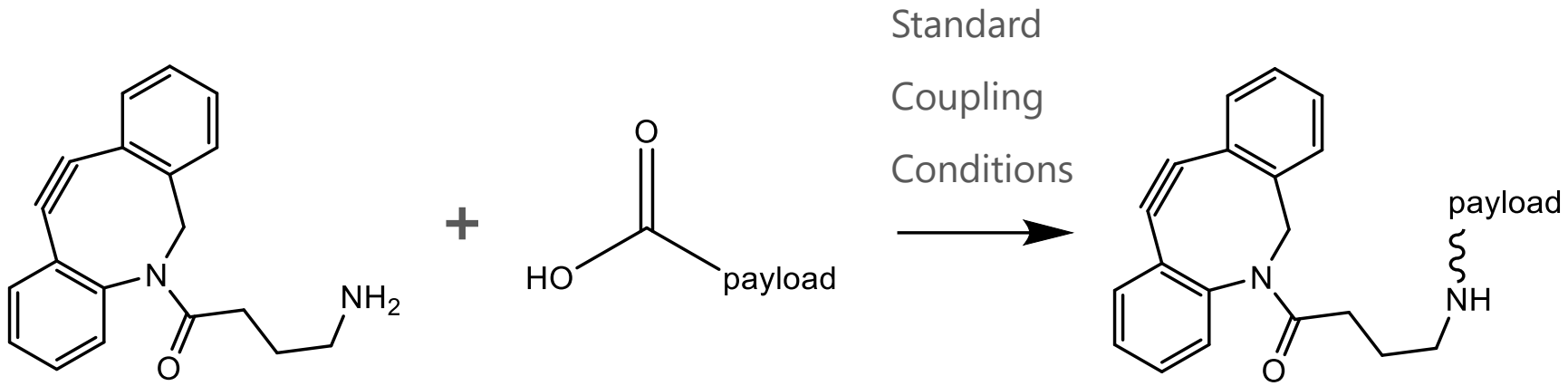
Ruling Out the Drug Linker

DL synthesis



- Final DL purity was >95% UV
  - Conjugatable impurities were <5% but free of payload

Not likely the source of the HIC impurities seen in the ADC

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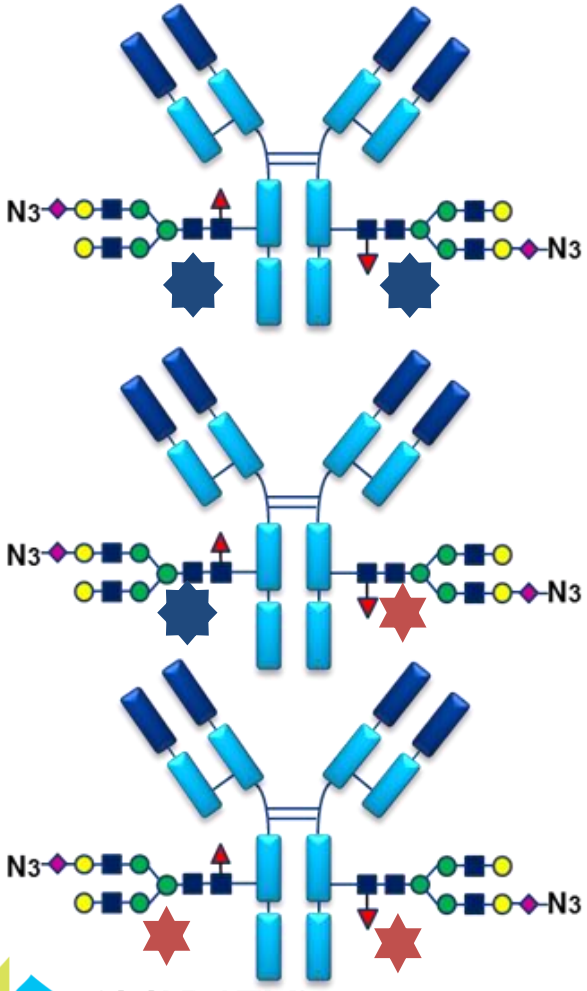
Additional Impurities from mAb



- For this modified mAb
  - Any differences in the added glycan azide will lead to a different ADC

Glycovariant A

Glycovariant B

- These glycovariants, with azide present, would still conjugate as expected
- But could resolve differently with HIC chromatography
- And maintain the same UV differential

HRMS confirmed existence of
multiple glycovariants after mAb modification

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Should We Be Concerned?

- Knowing the different HIC species were from glycovariants
  - Not likely affect mAb binding to target
  - Did not introduce a toxicity concern
  - Still allows for payload delivery

The team was able to derisk these impurities as a concern for this ADC program

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Summary

I. Drug substance of ADCs are often complex mixtures
   1. Very challenging to characterize impurities in these mixtures

II. Identify impurities during upstream development for drug linker and mAb :
   1. Take advantage of less complex mixture
   2. Utilize surrogate conjugations to identify conjugatable vs non-conjugatable impurities

III. Use this understanding of impurities to:
   1. Develop better control of conjugatable impurities prior to conjugation
   2. Derisk the impact of potential conjugatable impurities
   3. Confirm ADC purification can eliminate any non-conjugatable impurities

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Thank You!

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Questions?

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