Synthesis and Structural Characterisation of Trastuzumab-MMAE Antibody Drug Conjugate

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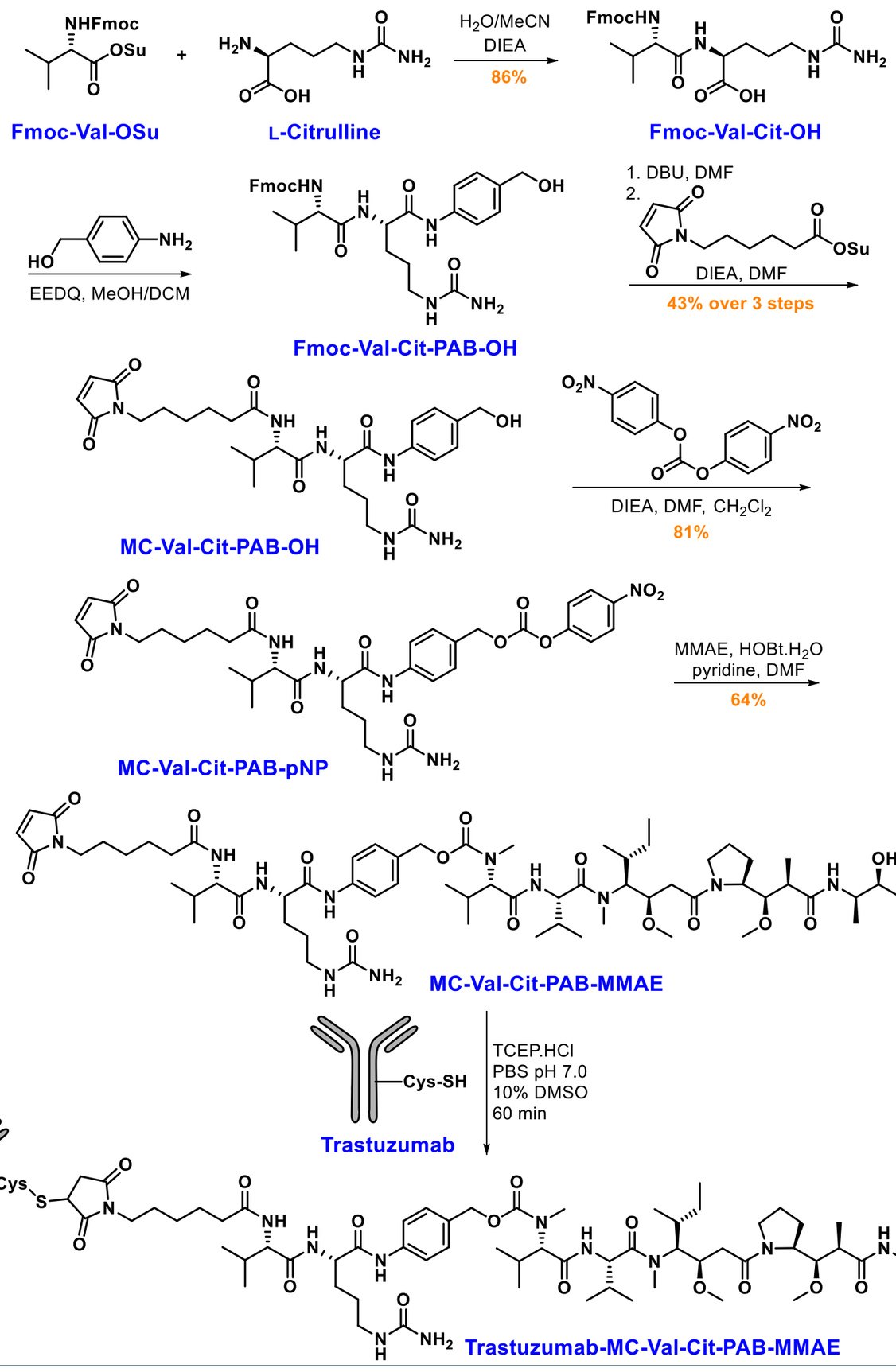
INTRODUCTION

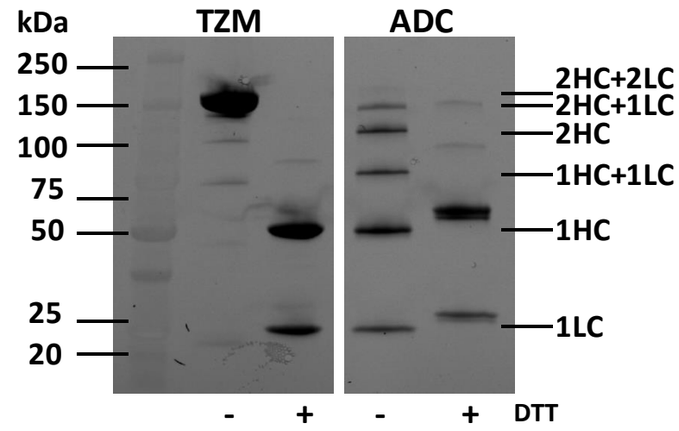
Antibody drug conjugates (ADCs) are attracting a tremendous amount of attention from both academia and industry because they have the potential to revolutionise cancer treatment. Monoclonal antibodies allow the selective delivery of cytotoxic agents to tumour cells leaving healthy surrounding tissue unharmed thereby potentially mitigating some of the damaging side-effects of cancer chemotherapy. This concept is not only the realisation of Paul Ehrlich's *magic bullet*s but may one day be personalised for each individual patient.

ADC SYNTHESIS

- Non-proprietary ADC is a combination of Kadcyla® and Adcetris®.
- Trastuzumab (Herceptin®) is a monoclonal antibody which targets the extracellular domain of the human epidermal growth factor receptor 2 (HER2).
- Cathepsin B (lysomal cysteine protease) cleavable linker.
- MMAE is a microtubule destabiliser.
- ADC purification via buffer exchange and centrifugal ultrafiltration.

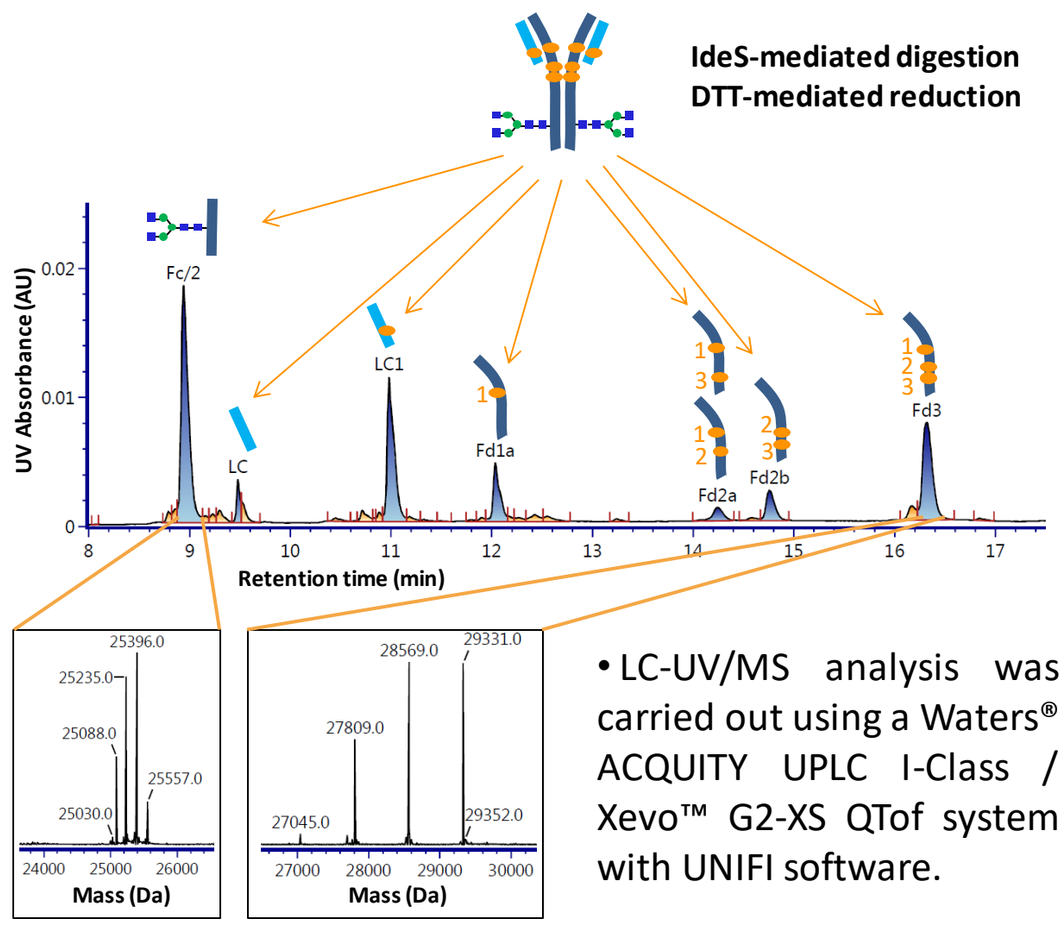


SDS-PAGE



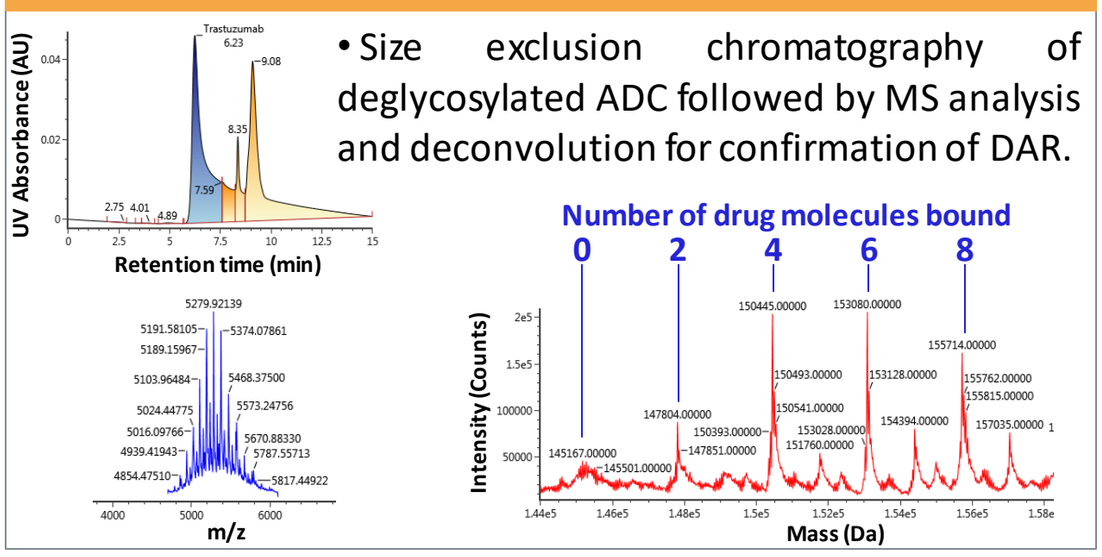
- Multiple bands of ADC under non-reducing conditions.
- No evidence of aggregation and oligomeric species.

SUBUNIT ANALYSIS



- LC-UV/MS analysis was carried out using a Waters® ACQUITY UPLC I-Class / Xevo™ G2-XS QTof system with UNIFI software.
- Calculated drug-antibody ration (DAR) of 6.4.
- Characterisation of ADC positional isomers demonstrated reproducibility.
- No Fd or Fd1b fragments due to saturation.
- Representative deconvolutions (MaxEnt1) shown for Fc/2, with glycoform profile, and for Fd3, with in-source fragmentation of conjugated drug.

NATIVE ANALYSIS

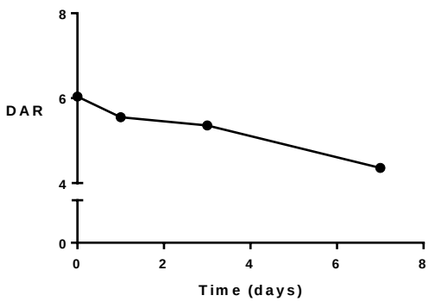


- Size exclusion chromatography of deglycosylated ADC followed by MS analysis and deconvolution for confirmation of DAR.

REFERENCES

General reviews 1) R. V. Chari *et al.*, *Angew. Chem., Int. Ed.*, 2014, **53**, 3796-3827. 2) P. D. Senter, *Curr. Opin. Chem. Biol.*, 2009, **13**, 235-244. **Synthesis** 1) Firestone *et al.*, US Pat., 6 214 345, 2001. 2) B. Wei *et al.*, *J. Med. Chem.*, 2018, **61**, 989-1000. **Workflow** X. Yao *et al.*, *Breast Cancer Res. Treat.*, 2015, **53**, 123-133. **Subunit analysis** M-C. Janin-Bussat *et al.*, *J. Chromatogr. B*, 2015, 9-13.

IN VITRO METABOLIC STABILITY



- ADC incubated in, and then immunoaffinity purified out of mouse serum.
- Decrease in DAR from 6.0 to 4.4 over seven days.
- Site-specific rates of deconjugation observed.