ThioBridge™ as a Tool for the Design, Optimization & Manufacture of ADCs

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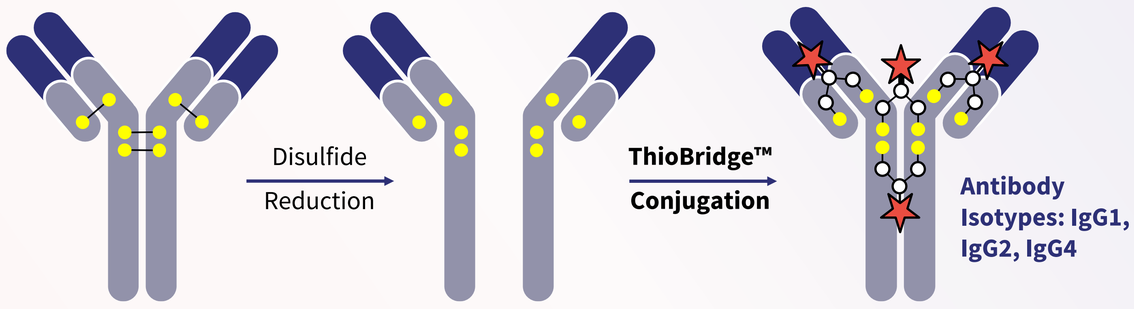
Abstract

The **ThioBridge™** conjugation technology makes use of naturally occurring interchain disulfide bonds of an antibody to generate antibody drug conjugates (ADCs).

ThioBridge™ offers several key features including homogeneity with high conversion to a single DAR species, stability as the linker does not deconjugate or cross-conjugate and site-specificity due to conserved location of conjugation.

Thiobridge™ Conjugation – Key Features



- No engineering of antibody required.
- Stable attachment of the linker to the antibody via a 3-carbon bridge.
- Optimized PK properties.
- Efficient conjugation achieving 70-90% target DAR.
- One-pot process.

Homogeneous DAR 2, 4 & 8 ADCs With ThioBridge™

Abzena has developed its ThioBridge™ technology to conjugate drugs to antibodies efficiently to create more homogeneous ADCs with improved stability.

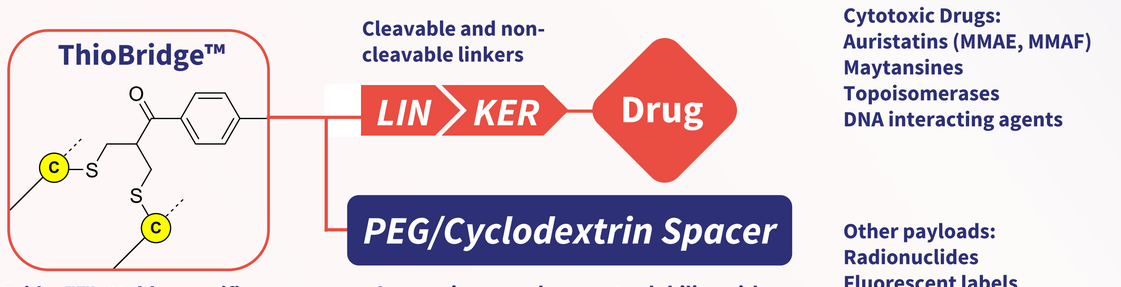


Figure 1: The ThioBridge™ linker incorporates a spacer, linker and drug and can conjugate to different antibody isotypes

Adcetris® - maleimide conjugation Av. DAR 4 **ThioBridge conjugation** - Av. DAR 4 **Kadcyla®** - lysine conjugation Av. DAR 3.5

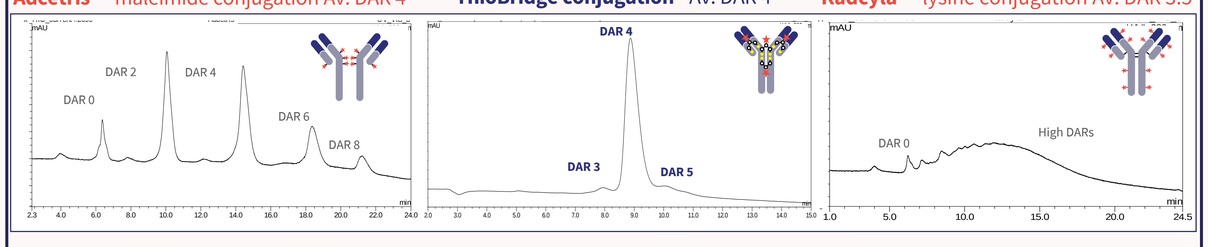


Figure 2: Hydrophobic Interaction Chromatography (HIC) reveals the homogeneity of ThioBridge™ ADCs in contrast with maleimide and lysine conjugation technologies.

Many cytotoxic drug-spacer combinations are highly hydrophobic, resulting in low conjugation efficiency and poor solubility.

- ThioBridge™ reagents offer the option of using PEG sulfonyl leaving groups, resulting in improved conversion rates and a faster conjugation process.
- ThioBridge™ formats can improve solubility when using hydrophobic linker-drug combinations. A range of linear, cyclodextrin and cyclic PEG spacer options are available, which can result in improved conjugation and efficacy in animal models.

Trastuzumab linker-monomethyl auristatin E (MMAE) ADCs

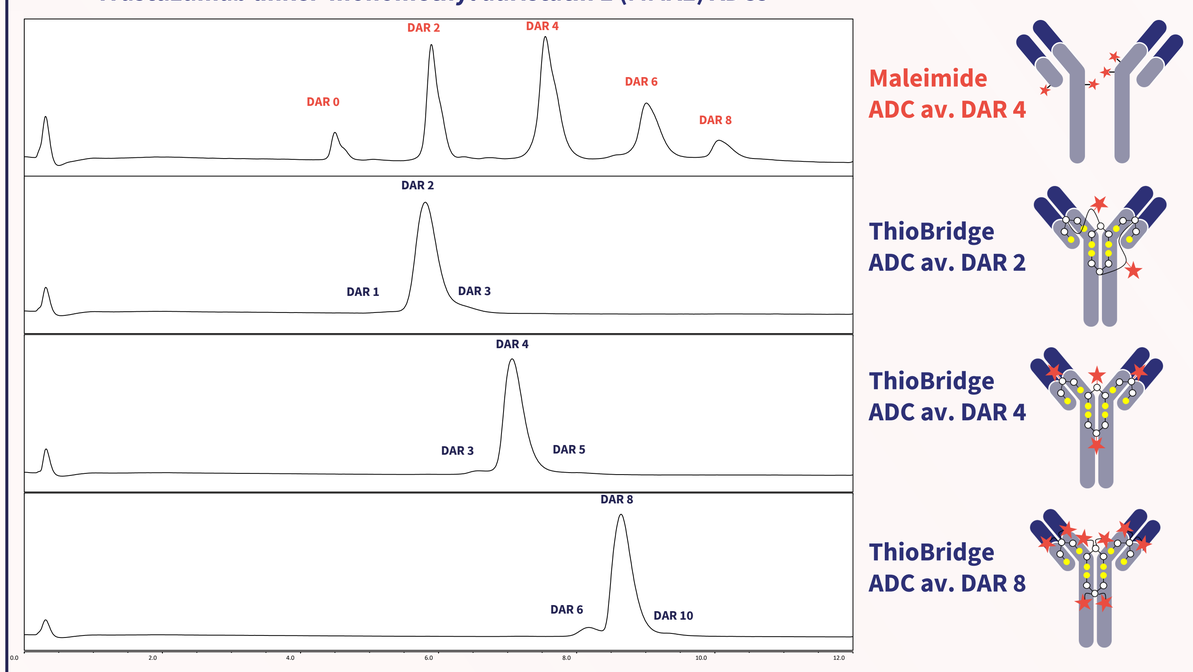


Figure 3: Comparative HIC of purified Trastuzumab linker-monomethyl auristatin (MMAE) ADCs. ThioBridge™ formats can be used to generate more homogeneous DAR 2, DAR 4 and DAR 8 conjugates, leading to higher yields of ADCs with a desired target DAR. ThioBridge ™ conjugates can also display potentially beneficial properties compared to maleimide counterparts e.g. greater stability, less hydrophobicity.

Modulation of Drug to antibody ratio (DAR) is paramount in designing a successful ADC

- The ThioBridge™ format can produce homogeneous ADCs with different DARs through site-specific conjugation at interchain disulfide bonds.

[1] (a) Pabst, M., *et al.* Modulation of drug-linker design to enhance in vivo potency of homogeneous antibody-drug *J. Control. Release*, **2017**, *253*, 160-164. (b) Godwin A, Kyle A, Evans N (2017) Conjugates and conjugating reagents comprising a linker that includes at least two (-CH2-CH2-O-) units in a ring. WO 2017/178828 A1. (c) Godwin, A., *et al.* Conjugates and conjugating reagents. WO 2017/199046 A1. (d) Evans, N *et al*. Incorporation of Hydrophilic Macrocycles Into Drug-Linker Reagents Produces Antibody-Drug Conjugates With Enhanced in vivo Performance. Front. Pharmacol. 13:764540. doi: 10.3389/fphar.2022.764540

ThioBridge™ ADCs exhibit higher *in vitro* stability than maleimide conjugates

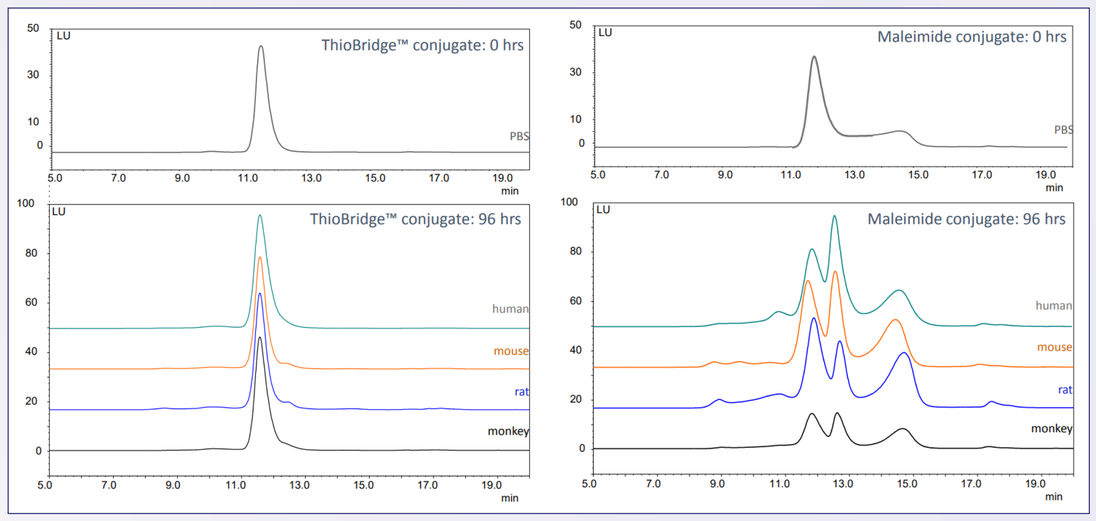


Figure 4: Alexa Fluor 488 conjugated to Trastuzumab using ThioBridge™ or maleimide chemistry with average of 2 reduced disulfides. Conjugates incubated in sera at 37 °C for 96 hrs; analysed by SEC.

ThioBridge™ brentuximab ADCs show efficacy *in vivo*

A DAR 4 ThioBridge™ brentuximab-MMAE ADC demonstrated improved *in vivo* efficacy vs Adcetris.

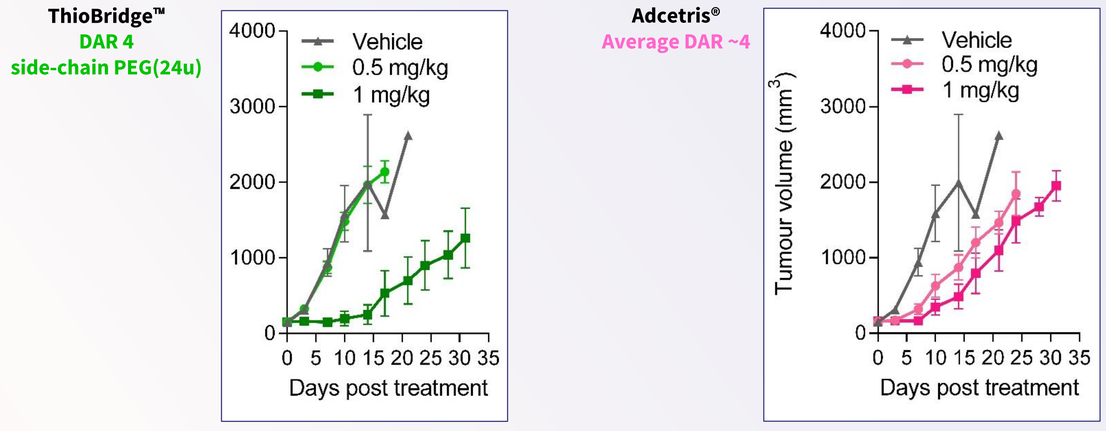


Figure 5: ThioBridge™ brentuximab ADC (DAR 4) and Adcetris® (~DAR 4) were tested in a Karpas299 CD30 +ve xenograft model at 0.5 mg/kg and 1 mg/kg, single dose i.v. administration at d0, with the ThioBridge™ ADC demonstrating improved efficacy.

ThioBridge™ Conjugation ADC Toolbox

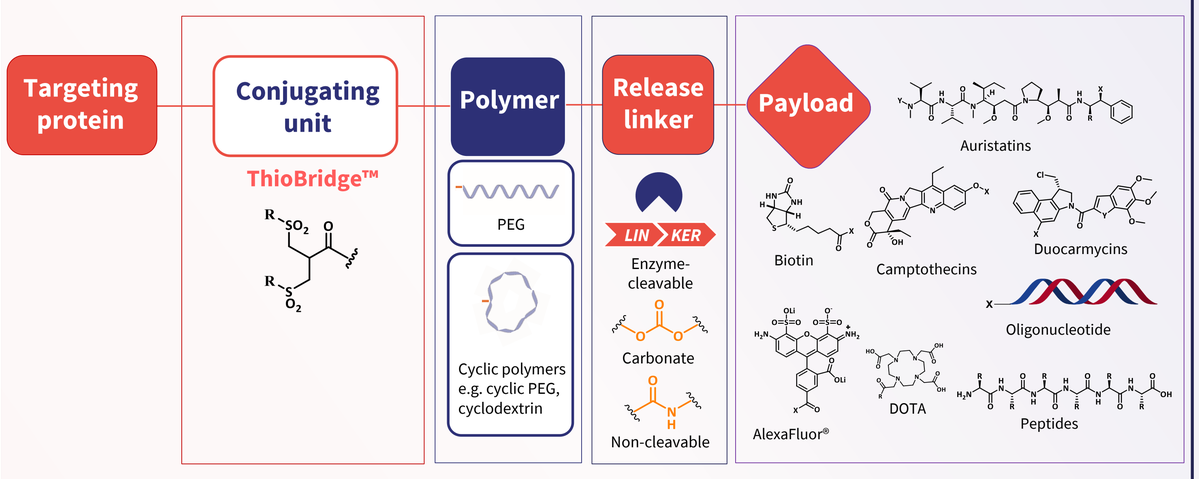


Figure 6: Example of the ThioBridge™ ADC toolbox that can be tailored for use with different biomolecules and linker-drug moieties, enabling the generation of custom bioconjugates.

ThioBridge™ is compatible across a variety of modalities and can incorporate novel linker architecture allowing further optimization of ADCs across a broader range of antibody:drug combinations. Spacer design plays a critical role in the successful development of an ADC and can significantly affect potency, PK and efficacy. A range of linear, cyclodextrin and cyclic PEG spacer options are available, which can result in improved solubility, conjugation and efficacy in animal models. [1]

Summary

ThioBridge™ conjugation offers advantages over other linker technologies including:

- **Homogeneity** – high conversion to a single DAR species
- **Stability** – linker does not deconjugate or cross-conjugate
- **Site-specificity** – conserved location of conjugation

The ThioBridge™ linker technology forms a stable and homogeneous conjugate at the naturally occurring interchain disulfide bonds of an antibody. The location proximal to the hinge region can sterically shield the Fcγ receptor binding sites thereby widening the TI by reducing the off-target toxicities that are observed in the marketed ADCs.