Advances in the chemo- and regio-selective conjugation of proteins

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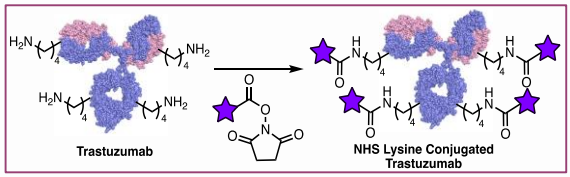
Introduction

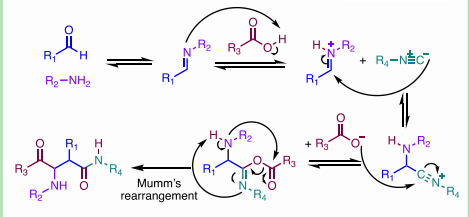
Chemo-selective strategies – i.e., targeting selectively only one family of amino acid residues – were successfully developed for the conjugation of almost all reactive amino acids. Despite major applications and a myriad of powerful methods (see picture), this approach tends to lead to heterogeneous mixtures of conjugates with variable DoC and different pharmacokinetics. This is a direct consequence of the large size of antibodies with the presence of multiple copies of the same amino acid residue at their surface.[1]



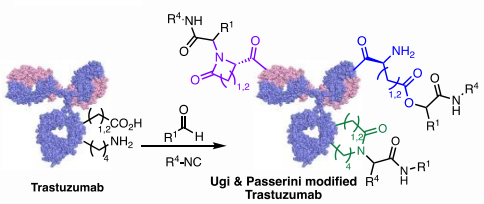
A solution to this problem was the investigation of *site-selective strategies* targeting a single copy of a precise amino acid.

The Ugi reaction

Mechanism



Previous work[2]

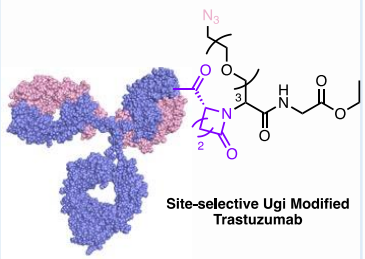


3 reaction types were detected:

- The expected **interresidue Ugi** between spatially close Lys-Glu/Asp
- A **Passerini** at the free carboxylate of *N*-terminal Glu/Asp
- An **intraresidue Ugi** between the *N*-terminal α-amine and the carboxylate side chains of *N*-terminal Glu/Asp

Towards site-selective conditions

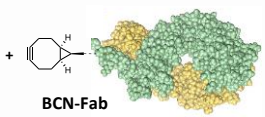
R^1-CHO						
R^4-NC						
Conversion	67%	52%	65%	44%	54%	59%
avDoC	1	0.8	1	0.5	0.8	0.9
Modification sites	E1, D1, D62-K64	E1, D1	E1	E1, D1, D185-K188	E1, D1	E1, D1, D185-K188, D224

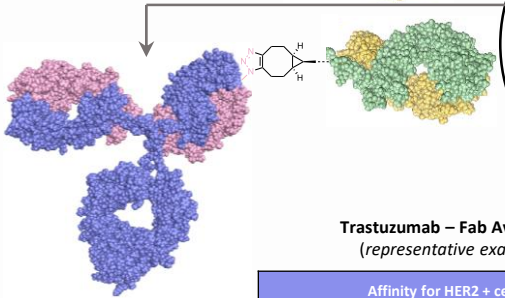


Applications

Bispecifics

ChemBioChem, 2024, e202400170

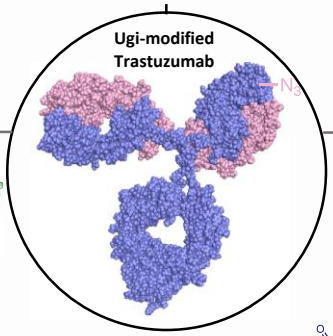




Trastuzumab – Fab Avelumab
(*representative example*)

Affinity for HER2 + cells		
Type of Antibody	**DoC**	**K_D (*p*M)**
Trastuzumab	0	6.5
Trastuzumab - Ugi	1	5.0
bsAb	1	4.9

- **7 examples of bsAbs were described**
- **Full conversion and isolation of each bsAb was achieved**
- **Retained affinity**



ADC

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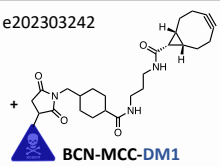

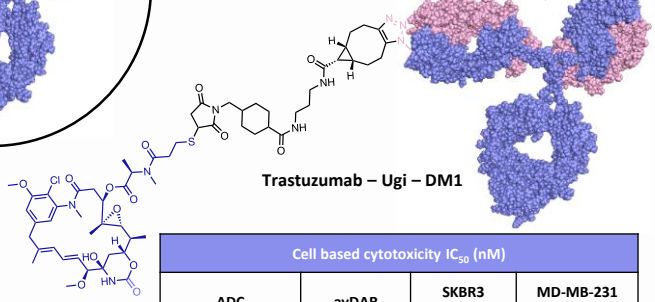

Trastuzumab – Ugi – DM1

Cell based cytotoxicity IC_{50} (nM)			
ADC	**avDAR**	**SKBR3 (HER2 +)**	**MD-MB-231 (HER2 -)**
T-Ugi-DM1	1.0	0.9	149.1
Kadcyla®	3.8	0.1	123.8

Conclusion

- Development of Ugi site-selective conditions.
- Application of Ugi reaction for the manufacturing of bispecifics.
- Synthesis of a site-selective Ugi – ADC.

References

1. Sornay, C., *et al.*, *Royal Society open access* **2022**, 9 (1).
2. Sornay, C., *et al.*, *Chem. Eur. J.* **2020**, 26 (61), 13797-13805.