https://doi.org/10.17952/37EPS.2024.P2018

Applications of Thiol-ene and Thiol-yne Chemistry for Peptide Stapling and Bioconjugation

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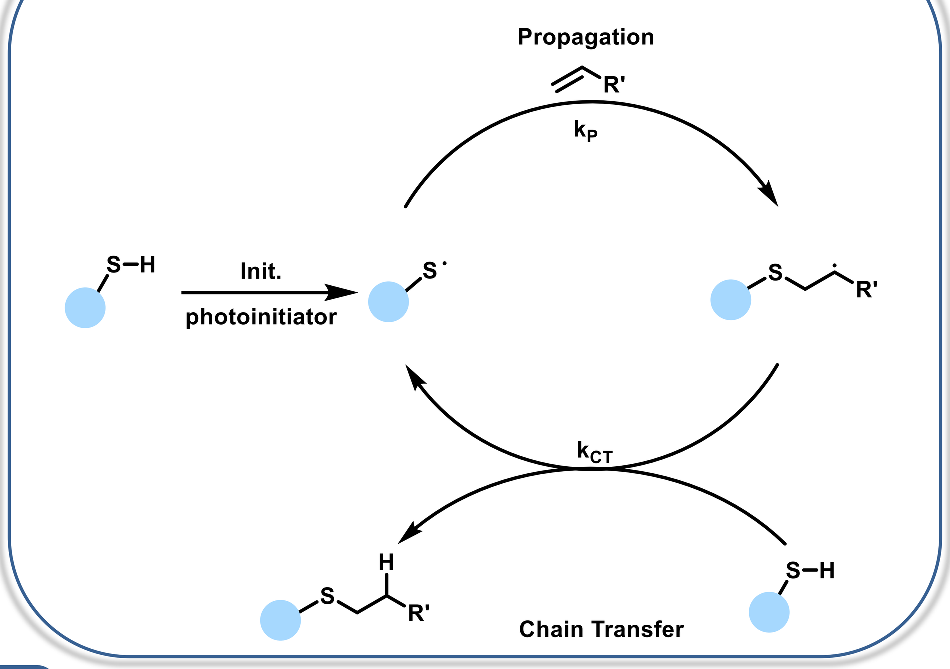
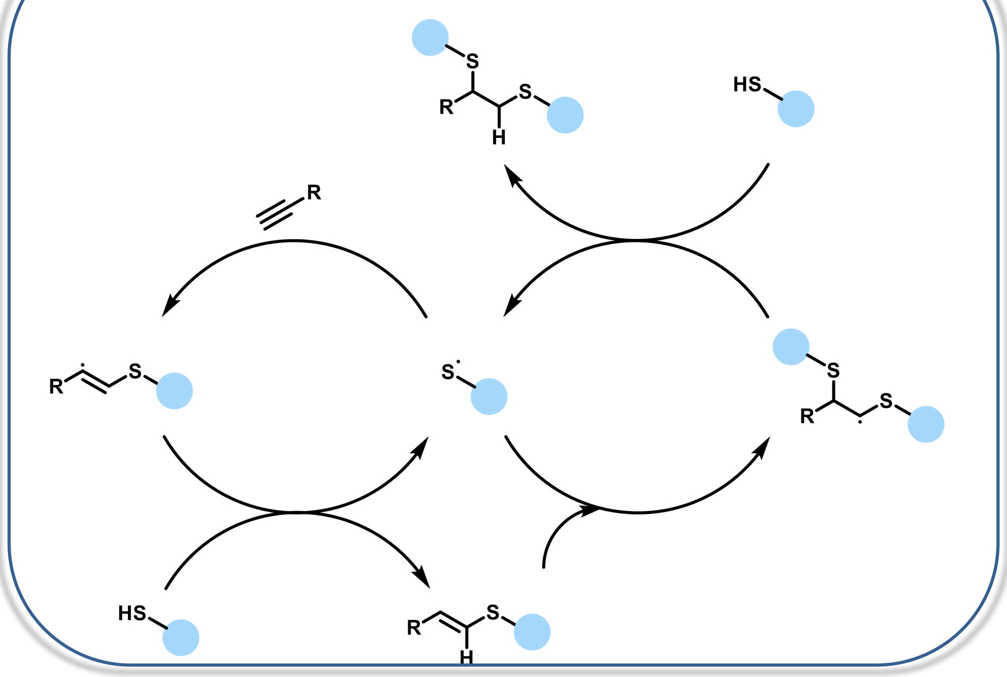
Introduction

- **Peptide modification** is gaining significance across a range of biomedical applications.
- Modified peptides have been used as **drug candidates** in the treatment of cancer, enzyme deficiency or metabolic disorders and infectious diseases.[1]
- The radical mediated **thiol-ene click (TEC) reaction** exhibits high atom economy, excellent yields and good regiocontrol.[2]
- Therefore, the **thiol-yne click (TYC) reaction** expands the chemistry of thiol-ene, providing access to a broad range of new properties.[3]

Thiol-ene click reaction

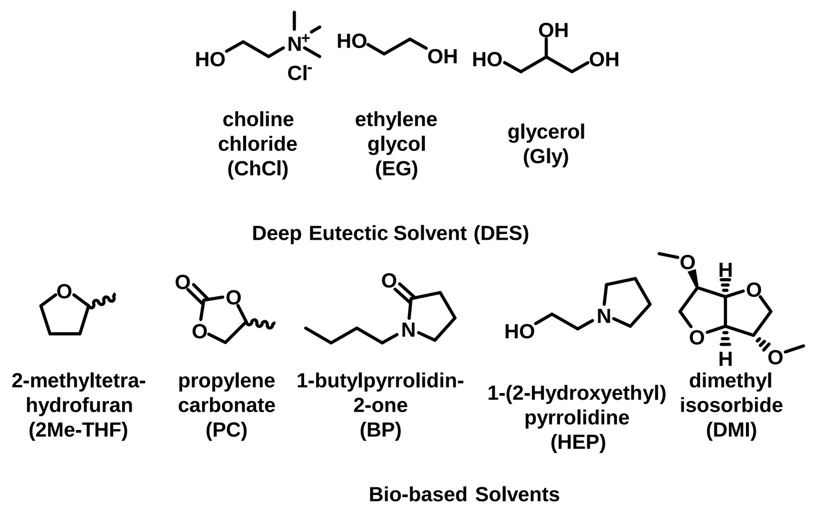


Thiol-yne click reaction



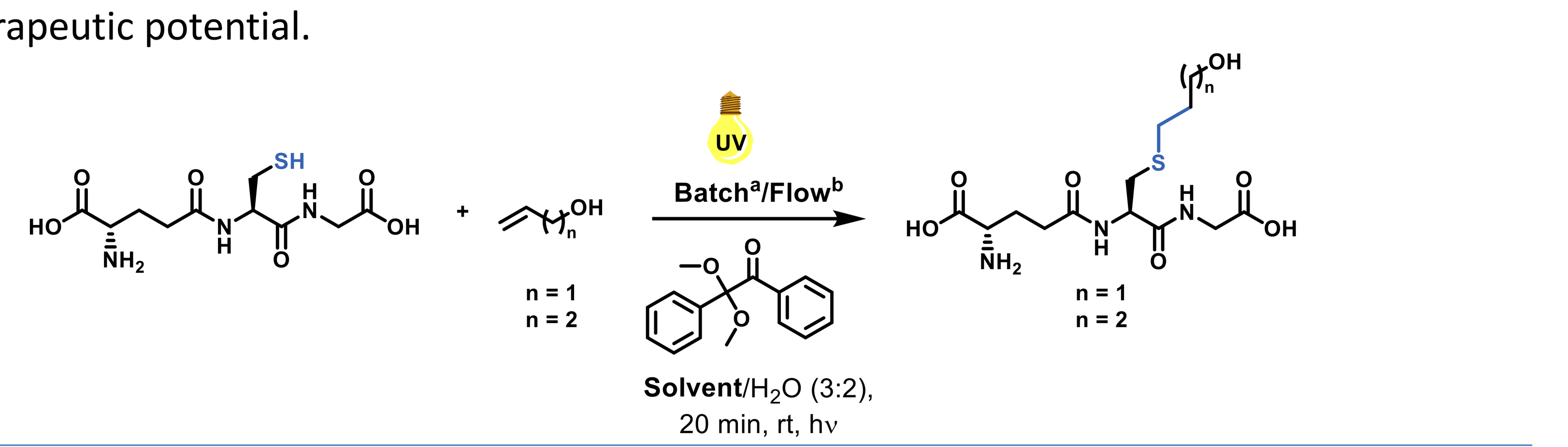
Green Continuous-Flow Bioconjugation

- **Flow chemistry** possesses a multitude of advantages, such as greater control over selectivity and reproducibility, less hazardous reaction setups and easier scale-up, and is therefore ideal for pharmaceutical and industrial applications.[4]
- **Deep Eutectic Solvents (DESs) and bio-based solvents** are promising candidates to provide significantly greener solvent options.
- The goals of flow chemistry and green chemistry are **ideally aligned** in prioritising the development of highly efficient synthetic approaches.



Green Optimisation

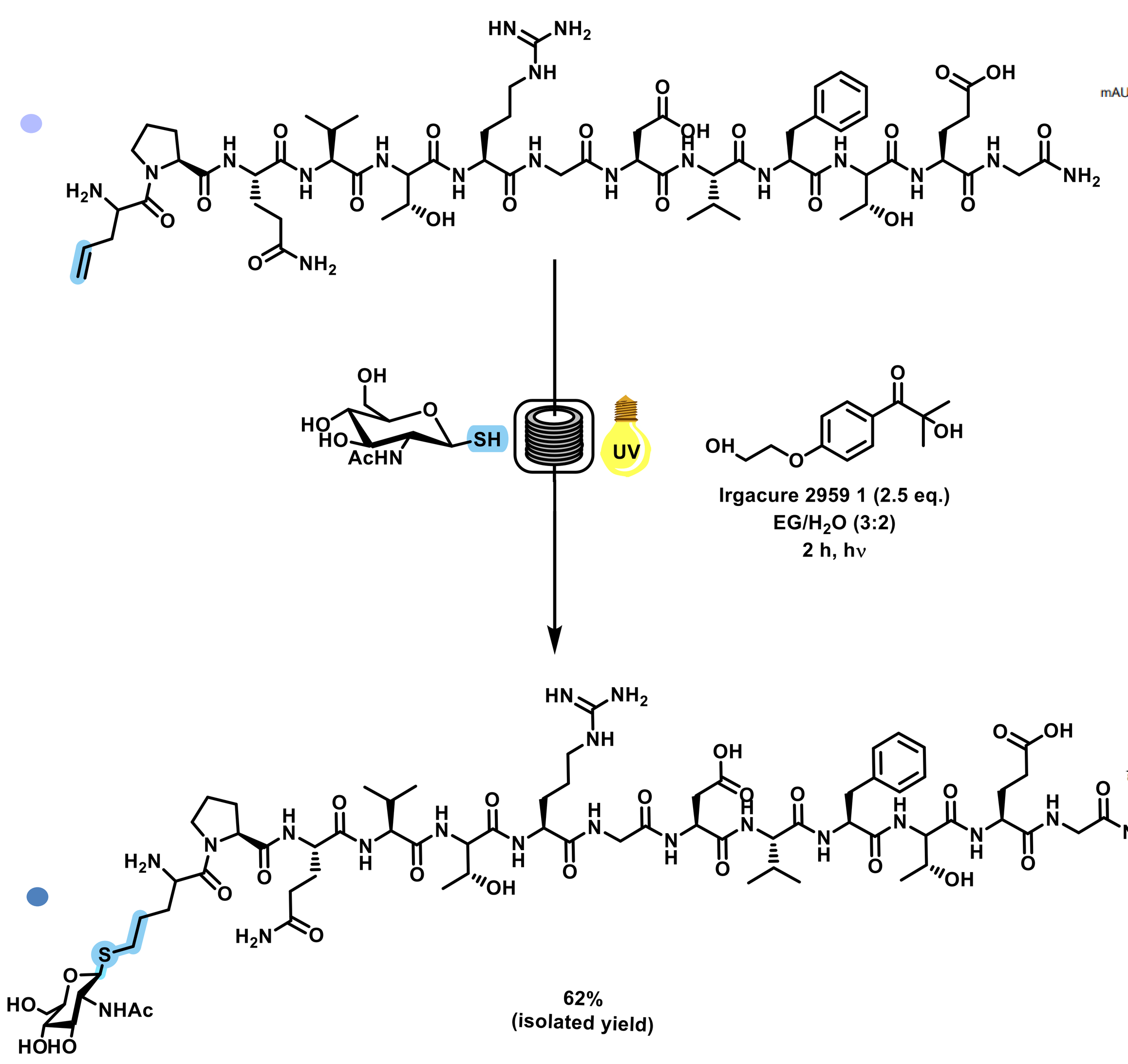
TEC was optimised with 2 different alkenes in batch and under continuous flow, utilising DES and Bio-based solvents. Glutathione (GSH) was selected as a model thiolated peptide substrate due to its therapeutic potential.

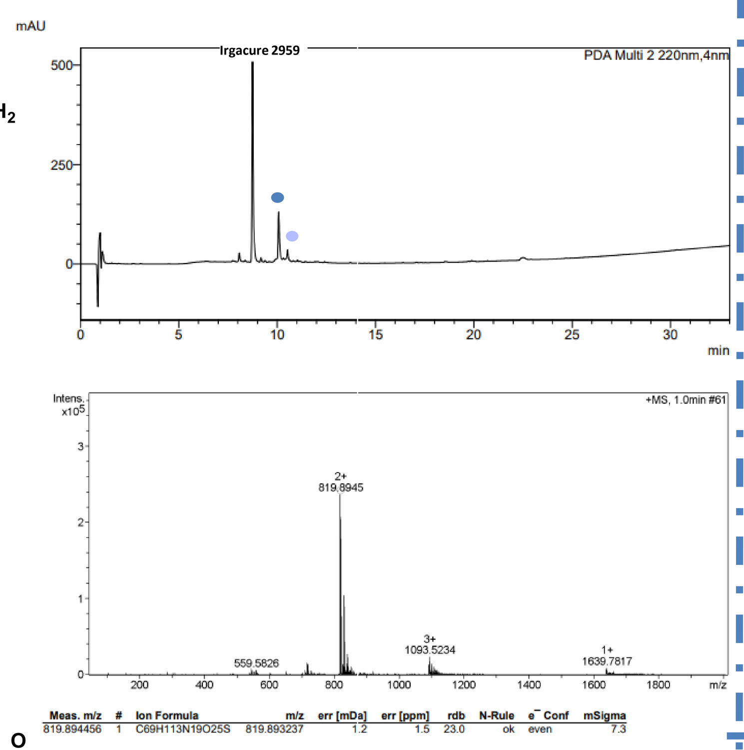


Entry	DES	n = 1 Conv.[a]	n = 1 Conv.[b]	n = 2 Conv.[a]	n = 2 Conv.[b]
I	ChCl: EG (2:1)	>99%	83%	98%	91%
II	ChCl: Gly (2:1)	Quant.	84%	>99%	Quant.
III	2Me-THF + BHT	>99%	95%	>99%	86%
IV	PC	Quant.	96%	>99%	>99%
V	BP	Quant.	93%	>99%	>99%
VI	HEP	98%	96%	Quant.	Quant.
VII	DMI	>99%	96%	Quant.	Quant.

Scale-up examples

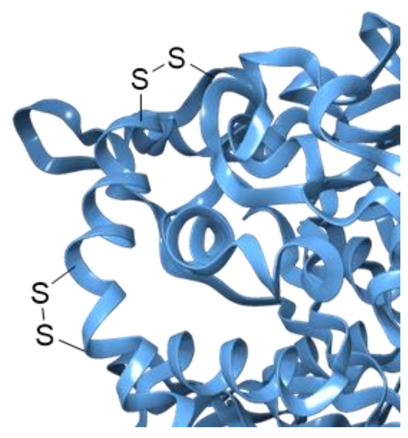
One of the peptide glycosylation reactions reported in this study was this RGD peptide with DES and water under continuous flow along with a tumour homing peptide and an AFP peptide.



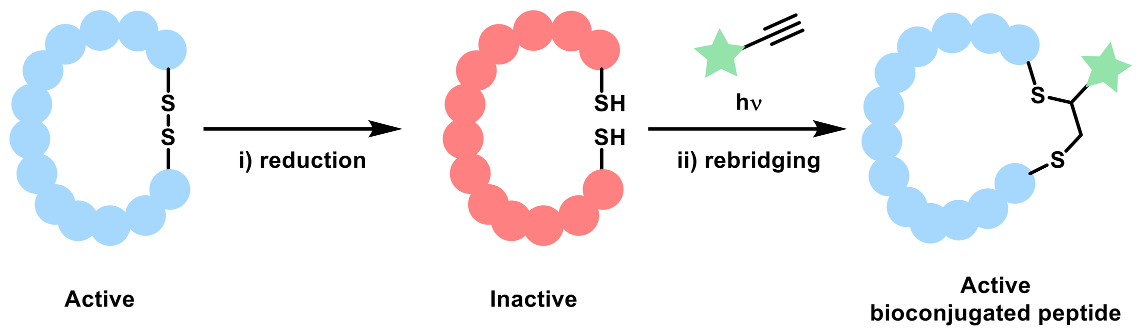


Disulfide rebridging

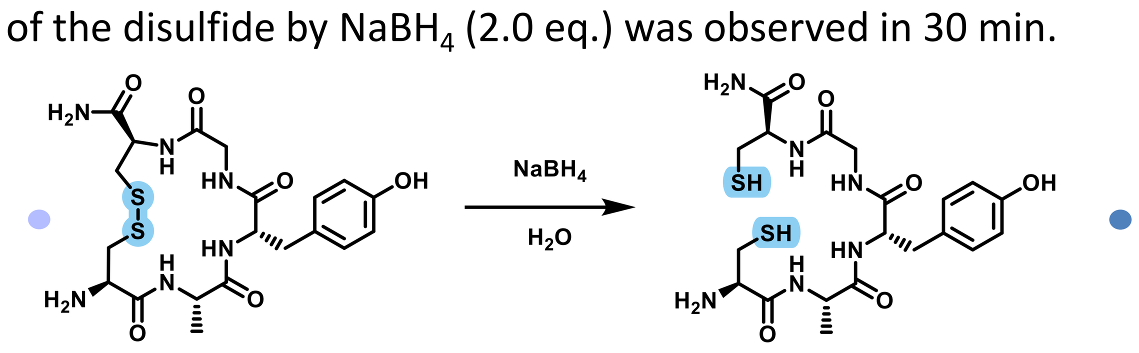
- **Essential disulfides** present a target that is known to be susceptible to topological changes upon reduction, and thus deactivation.
- Surface exposed disulfide bonds allow for the in-situ generation of unique functionalization sites through mild reduction, followed by selective chemical rebridging with unnatural linkers.[5]

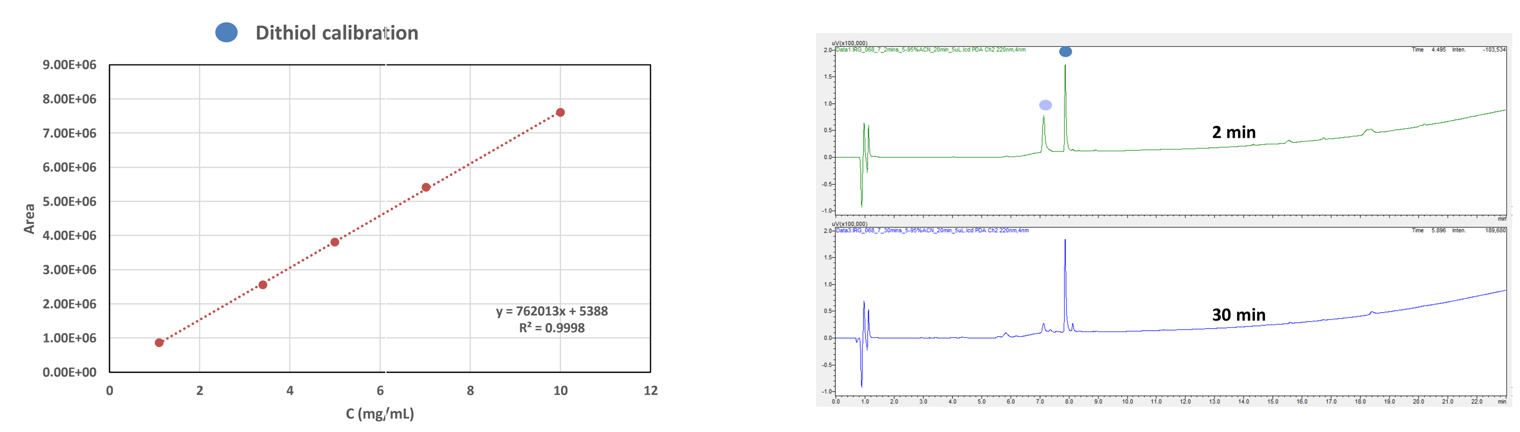


Project aim

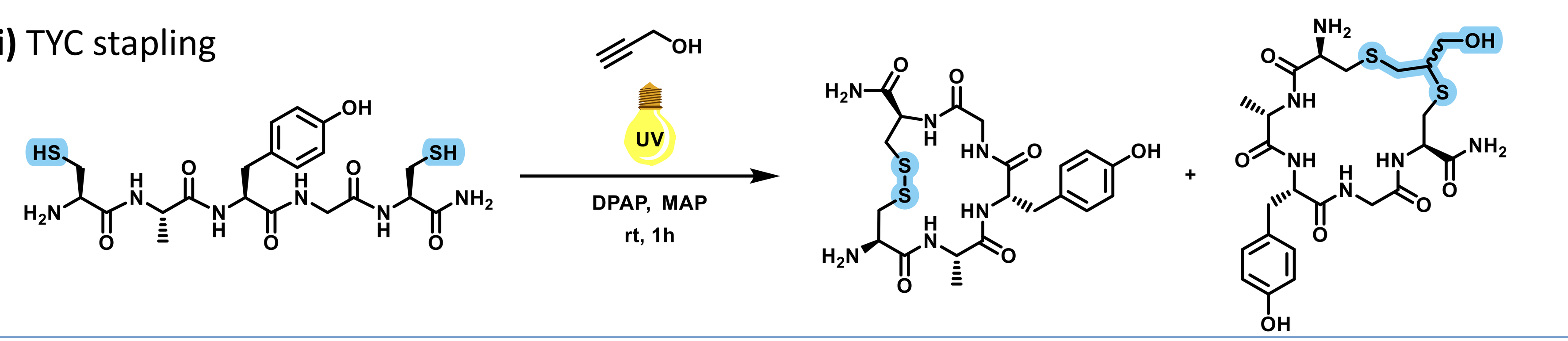


i) >99% reduction of the disulfide by $NaBH_4$ (2.0 eq.) was observed in 30 min.





ii) TYC stapling

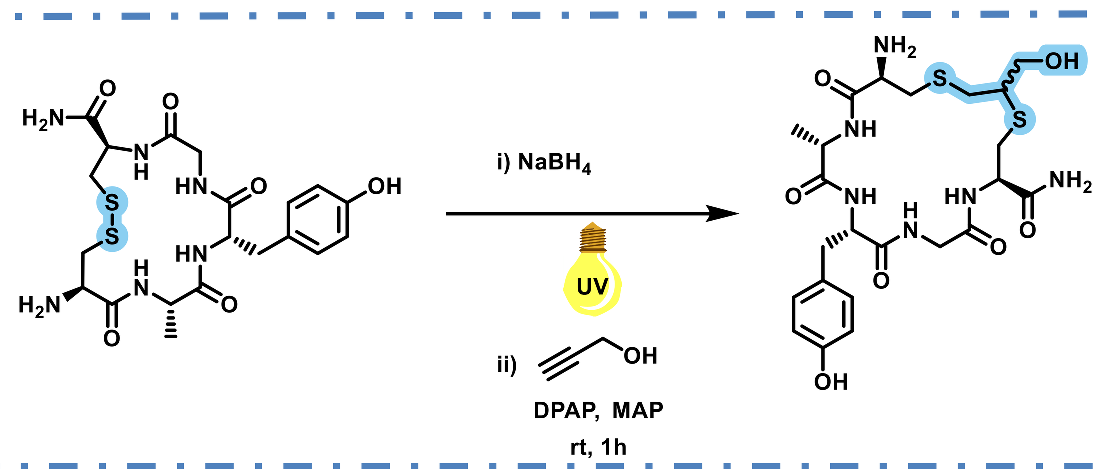


Entry	SH : alkyne (eq.)	Dithiol Concentration (mM)	Solvent	Additive	Disulfide conversion	Product conversion	Starting Dithiol
1	1 : 1	4.86	H_2O:ACN (9:1)	FA	45%	52%	>3%
2	1 : 2	4.86	H_2O:ACN (9:1)	FA	34%	58%	8%
3	1 : 4	4.86	H_2O:ACN (9:1)	FA	34%	50%	16%
4	**1 : 1**	**9.72**	**H_2O:ACN (9:1)**	**FA**	**29%**	**71%**	**0%**
5	1 : 1	19.4	H_2O:ACN (8:2)	FA	50%	25%	25%
6	1 : 1	4.86	PBS:ACN (9:1)	pH 7.4	73%	27%	0%
7	1 : 1	4.86	DMF	FA	95%	>3%	>2%
8	1 : 1	9.72	H_2O:ACN (9:1)	No additives	60%	40%	0%

*FA = Formic acid

Ongoing work

Disulfide rebridging in **one-pot reaction** will be performed to synthesise a bioconjugated active peptide for further therapeutic aims.



Conclusion

In this work, we report TEC mediated reactions under continuous-flow in both aqueous conditions, using 'green' solvents and furnishing biologically active glycopeptides in high yield. We also report a highly efficient TYC-mediated approach for peptide rebridging that can be applied to disulfide containing peptides. Following disulfide reduction, the radical-mediated crosslinking of the free thiol moieties via sequential thiol-yne ligation furnishes the covalently bound peptide macrocycle. TEC and TYC proved to be a promising and novel green strategy in the functionalisation of active peptide derivatives.

[1] I. Rabadán González, E. M. Scanlan et al., *Org. Biomol. Chem.*, 2024, **22**, 2203-2210. [2] M.D. Nolan, R. Petrarca et al., *Org. Biomol. Chem.*, 2022, **20**, 8192-8196. [3] C. N. Bowman et al., *J. Mater. Chem.*, 2010, **20**, 4745–4750. [4] F. Chen, et al., *Green Synth. Catal.*, 2022, **3**, 243-258. [5] D. A. Richards, J. R. Baker et al., *Org. Biomol. Chem.*, 2016, **14**, 455–459.