



Thiol-yne Mediated Macrocyclisation and Derivatisation Strategies

Conor Williams, Dearbhla Tully, Susannah H. Calvert, Joanna F. McGouran and Eoin M. Scanlan

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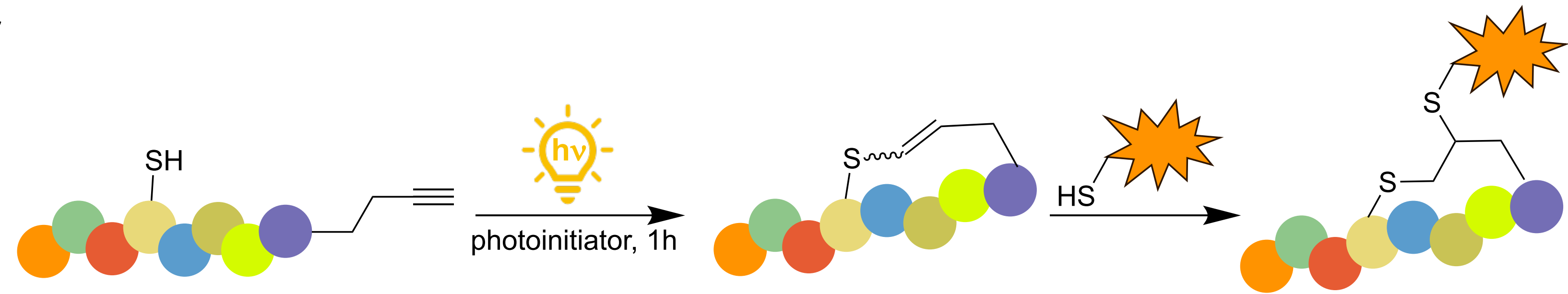
Trinity Biomedical Sciences Institute, Trinity College Dublin, 152-160 Pearse St., Dublin, Ireland

Contact: cowillia@tcd.ie, scanlae@tcd.ie

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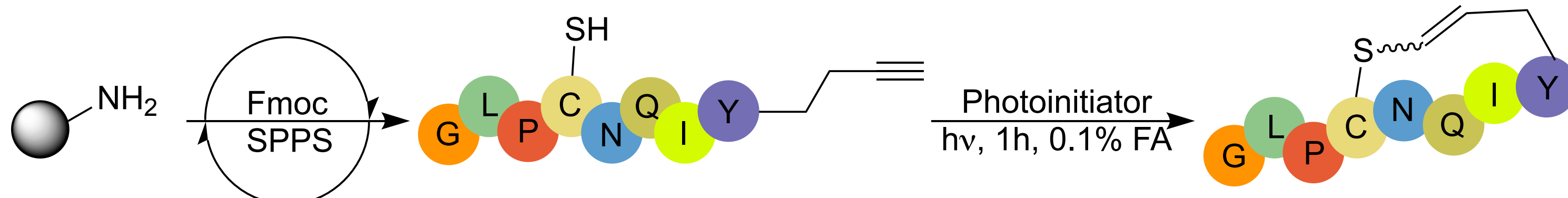
Introduction

- Peptides offer significant potential as therapeutics. However, poor membrane permeability and susceptibility to peptidases are of concern¹
- Replacement of disulfide bonds offers a solution to increase stability²
- Thiol-yne 'click' cyclisation has been poorly explored³
 - Mild, metal free, selective and light mediated solution
 - Provides a new functional handle for further functionalisation



Thiol-yne Optimisation

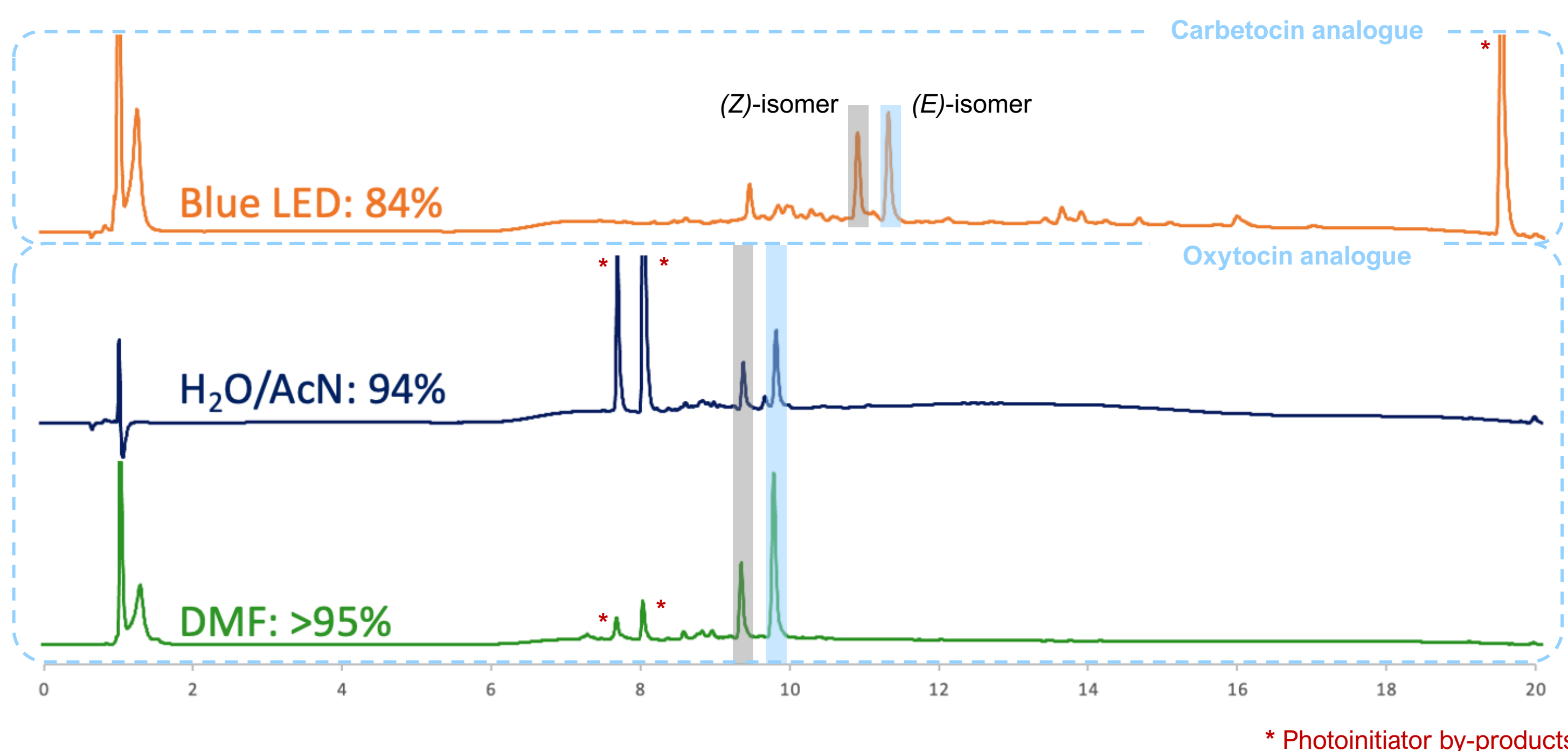
- Alkyne containing peptides synthesised through incorporation of an alkyne amino acid or carboxylic acid



- Optimisation carried out exploring photoinitiator, pH, concentration and solvent effects

| Photoinitiator | Photoinitiator eq. | Concentration (mM) | Solvent | Additive(s) | Conversion (%) |
|----------------------|--------------------|--------------------|------------------------|----------------|----------------|
| DPAP | 1 | 1.7 | H ₂ O : AcN | 0.1% TFA, MAP | N.R. |
| DPAP | 0.5 | 0.5 | DMF | – | 26% |
| Irgacure 2959 | 0.5 | 0.5 | DMF | – | 31% |
| Irgacure 2959 | 0.5 | 0.5 | DMF | 0.1% TFA | 16% |
| Irgacure 2959 | 0.5 | 0.5 | DMF | 0.1% FA | 60% |
| Irgacure 2959 | 0.5 | 1 | DMF | 0.1% FA | 81% |
| Irgacure 2959 | 0.5 | 1.5 | DMF | 0.1% FA | >95% |
| Irgacure 2959 | 0.5 | 2 | DMF | 0.1% FA | 89% |

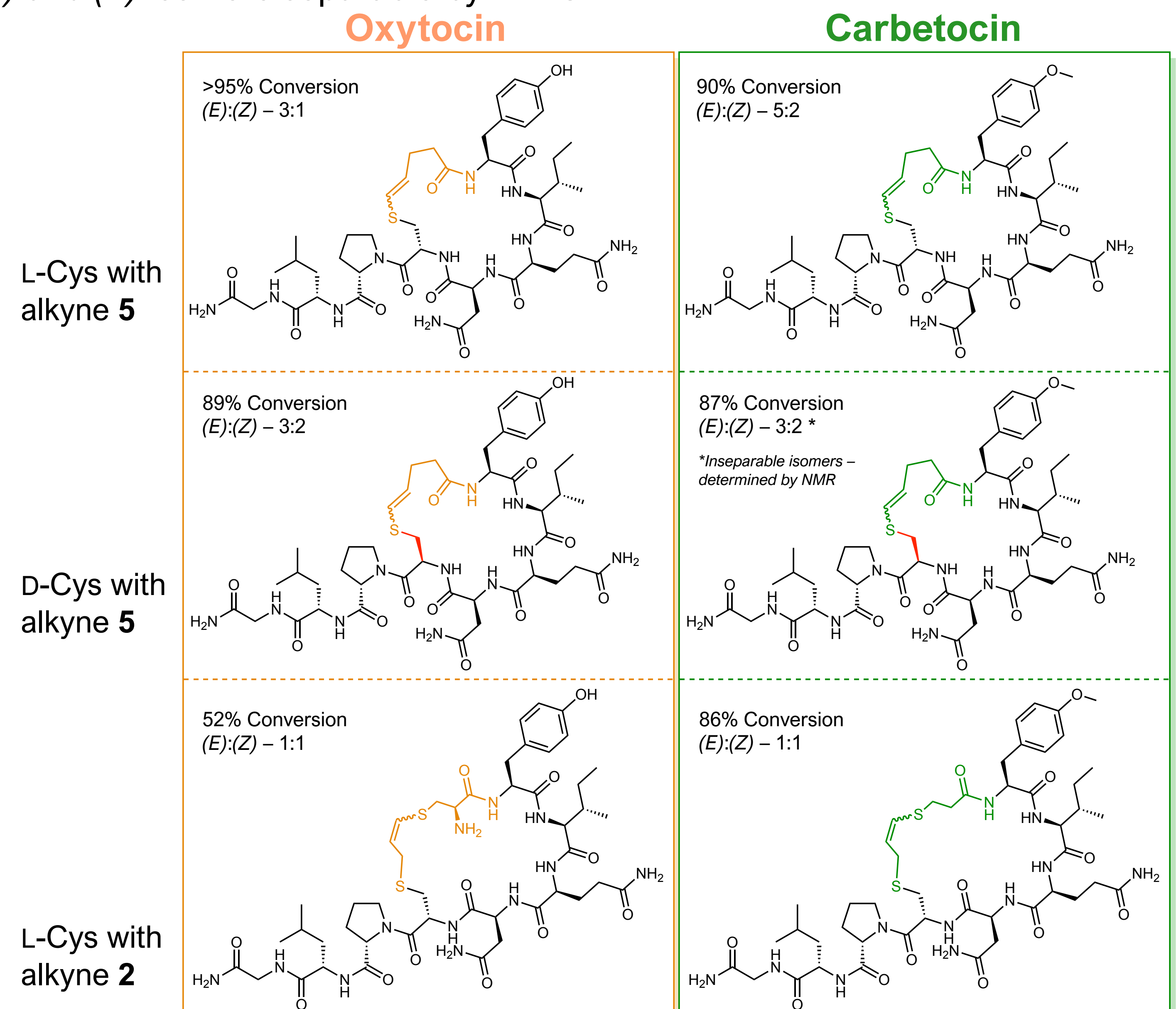
- Reaction can be modified for use in aqueous conditions or with Blue LED initiation, for compatibility with biological applications



Cyclic Neuropeptide Analogues

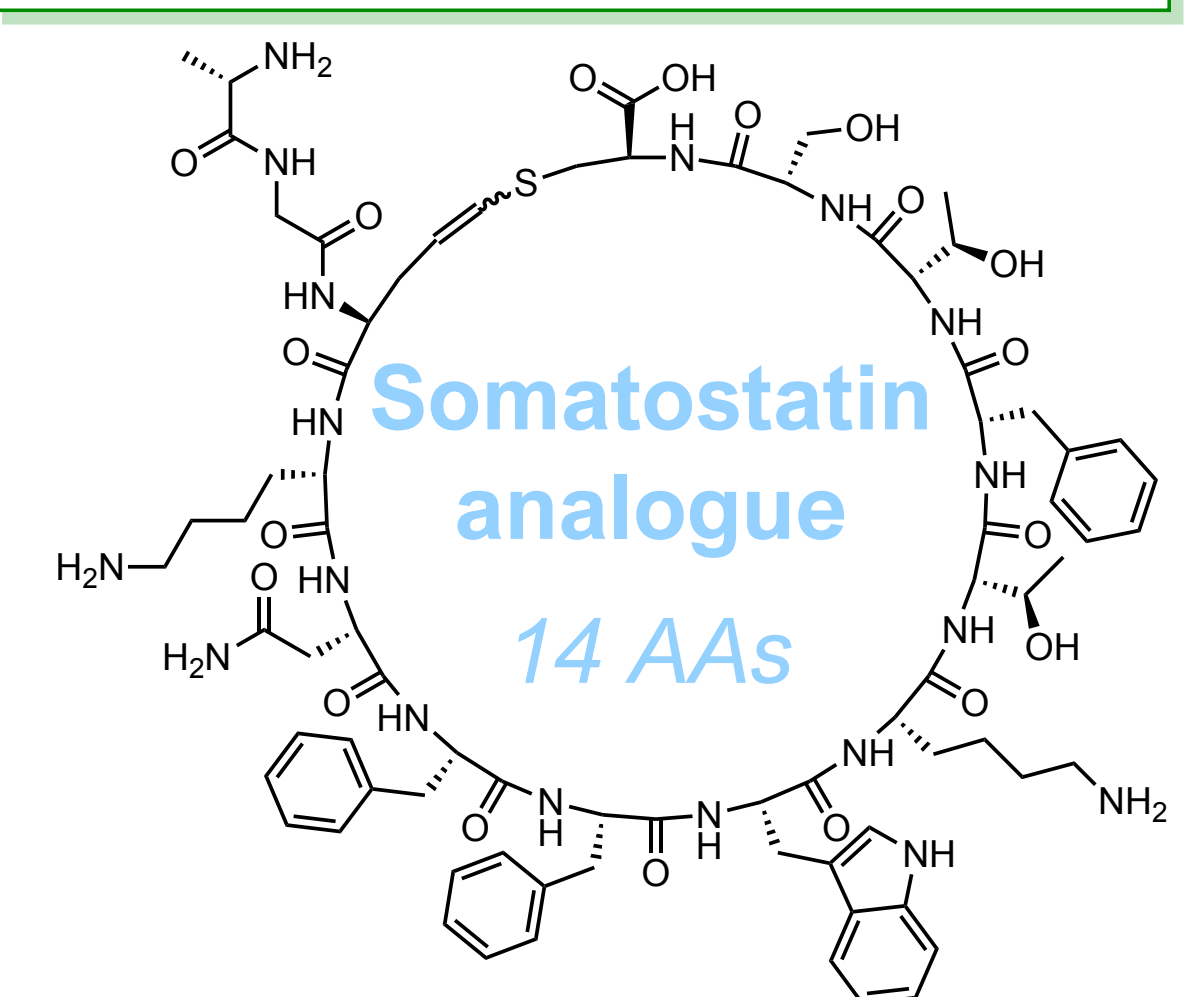
- Variety of Oxytocin, Carbetocin and Somatostatin analogues synthesised by SPPS and solution phase thiol-yne cyclisation

- (E) and (Z) isomers separable by HPLC



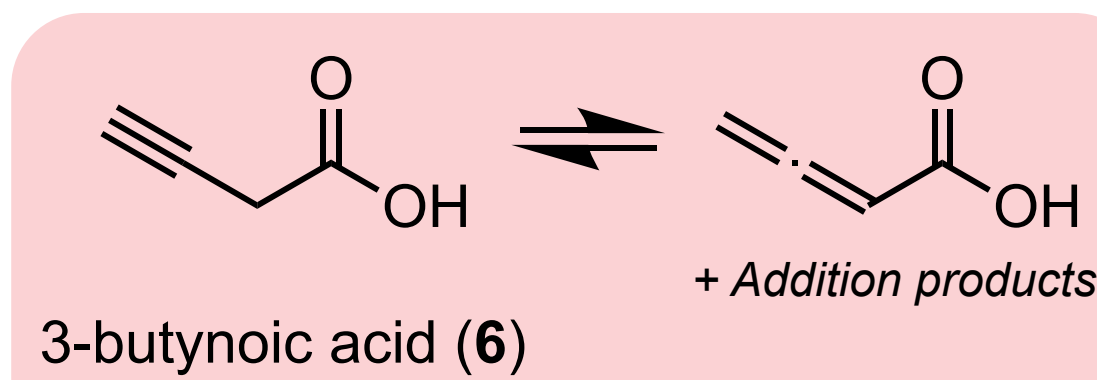
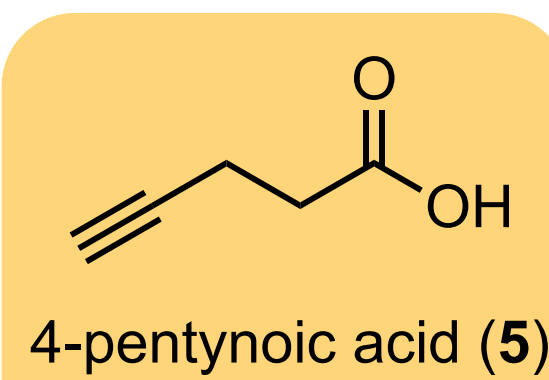
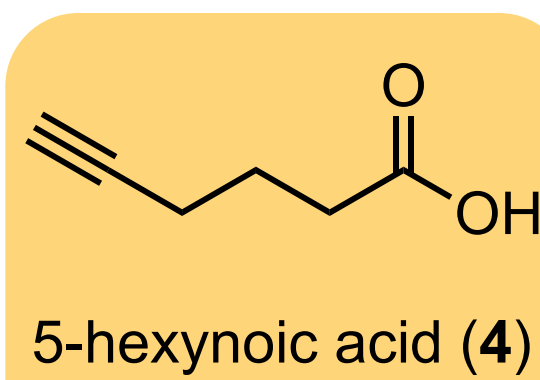
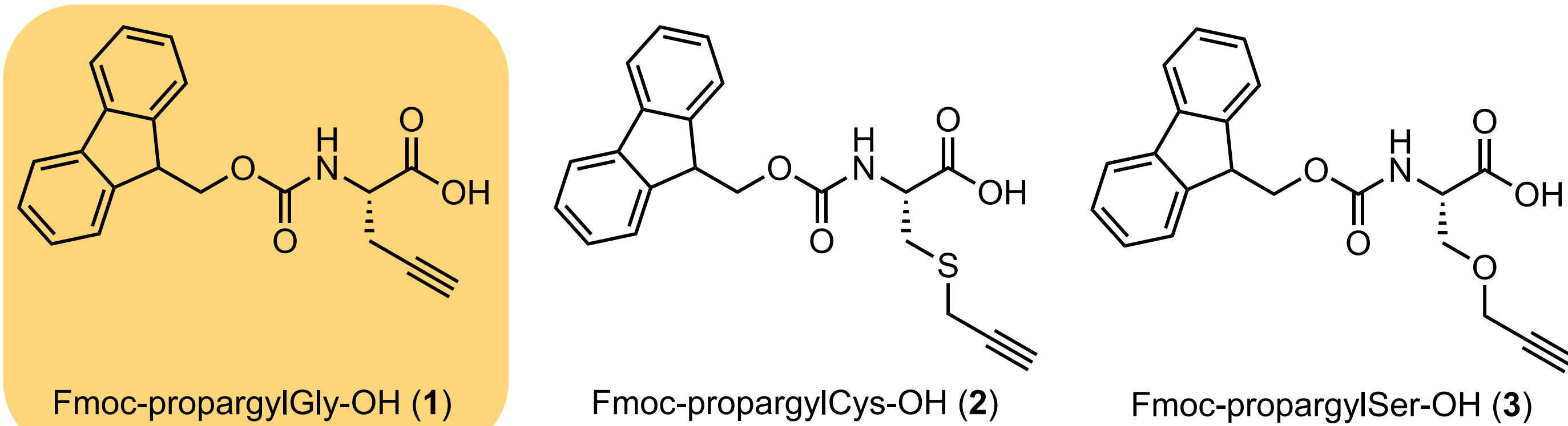
- Additional Somatostatin and small ring peptides currently in preparation

- Biological activity assays of neuropeptide analogues are being carried out in collaboration with the Muttenthaler group, University of Vienna⁶



Propargylated Amino Acids

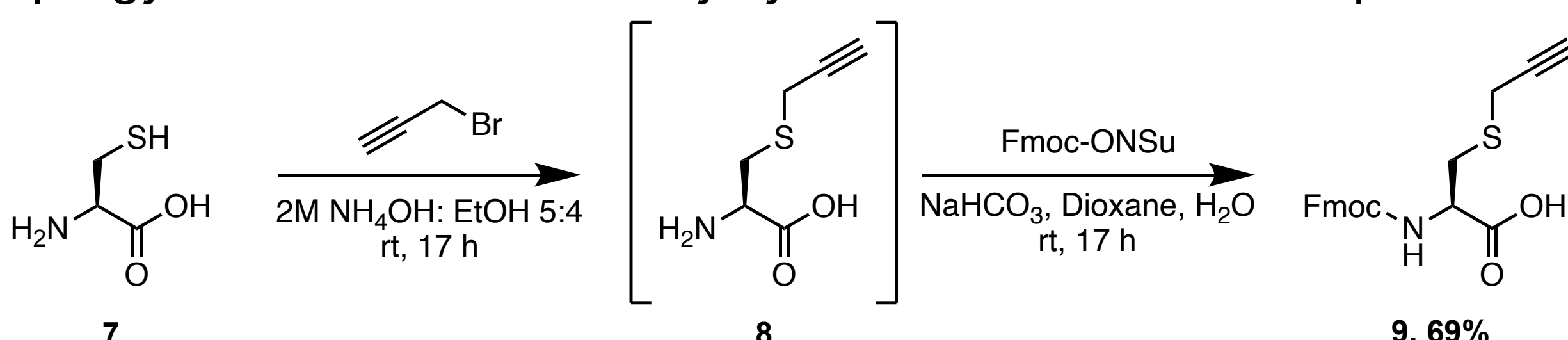
- Variety of SPPS compatible alkyne handles available



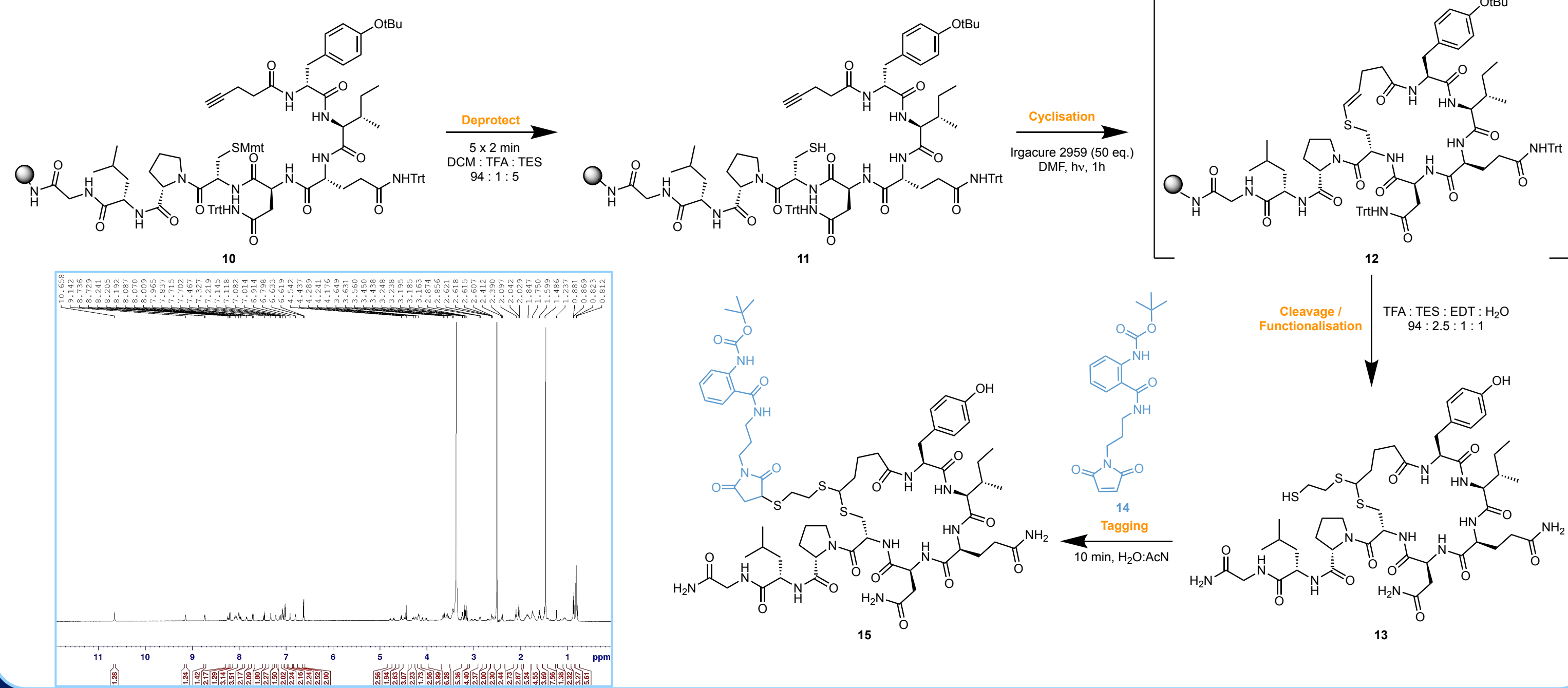
Commercially Available

SPPS incompatible⁴

- Propargylated Fmoc AAs readily synthesised with minimal purification⁵



On-resin Cyclisation & Modification



Conclusions and Future Work

- ✓ SPPS Compatible
- ✓ "Click" type reaction
- ✓ Compatible with biological systems
- ✓ Mild, photo-radical conditions
- ✓ Rapid 1h reaction
- ✓ Utilises Cys handle

- Exploration into thiol-ene functionalisation of alkyne handle currently underway

References

- L. Wang, N. Wang, W. Zhang, X. Cheng, Z. Yan, G. Shao, X. Wang, R. Wang and C. Fu, *Sig. Transduct. Target Ther.*, 2022, 7, 48.
- S. Tomassi, A. M. Trotta, C. Ierani, F. Merlino, A. Messere, G. Rea, F. Santoro, D. Brancaccio, A. Carotenuto, V. M. D'Amore, F. S. Di Liava, E. Novellino, S. Cosconati, L. Marinelli, S. Scala and S. Di Marco, *Chem. Eur. J.*, 2020, 26, 10113–10125.
- Y. Tian, J. Li, H. Zhao, X. Zeng, D. Wang, Q. Liu, X. Niu, X. Huang, N. Xu and Z. Li, *Chem. Sci.*, 2016, 7, 3325–3330.
- A. J. Cameron, P. W. R. Harris and M. A. Brimble, *Angew. Chem. Int. Ed.*, 2020, 59, 18054–18061.
- G. Fumagalli, R. J. Carbajo, J. W. M. Nissink, J. Tart, R. Dou, A. P. Thomas and D. R. Spring, *J. Med. Chem.*, 2021, 64, 17287–17303.
- A. D. De Araujo, M. Mobil, J. Castro, A. M. Harrington, I. Vetter, Z. Dekan, M. Muttenthaler, J. Wan, R. J. Lewis, G. F. King, S. M. Brierley and P. F. Alewood, *Nat. Commun.*, 2014, 5, 3165.