

Thiol-yne Mediated Peptide Macrocyclisation and Derivatisation Strategies

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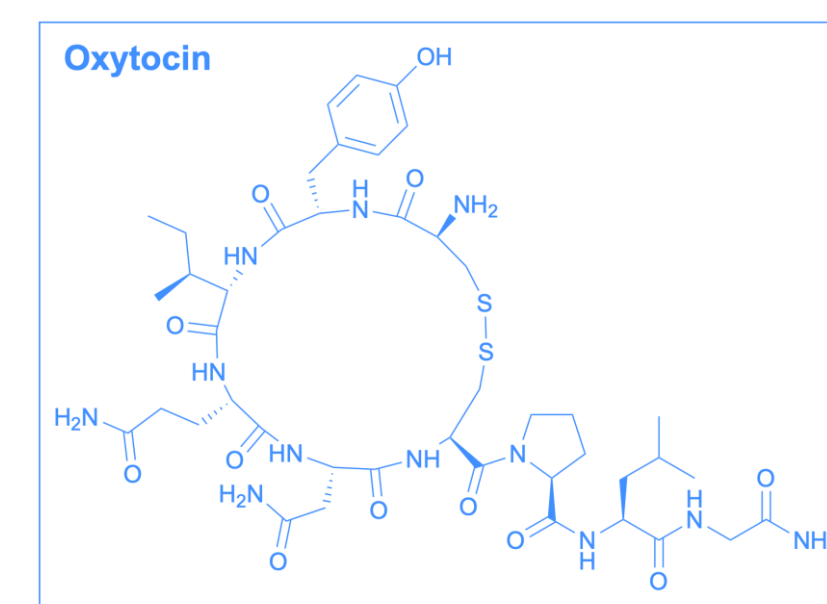
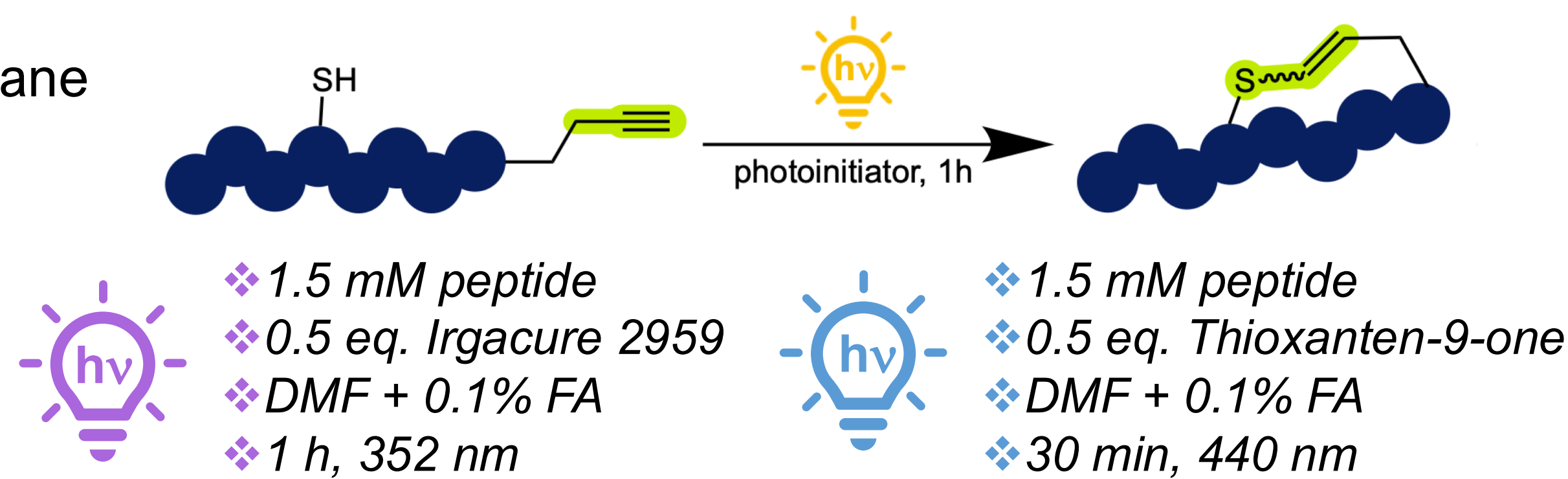


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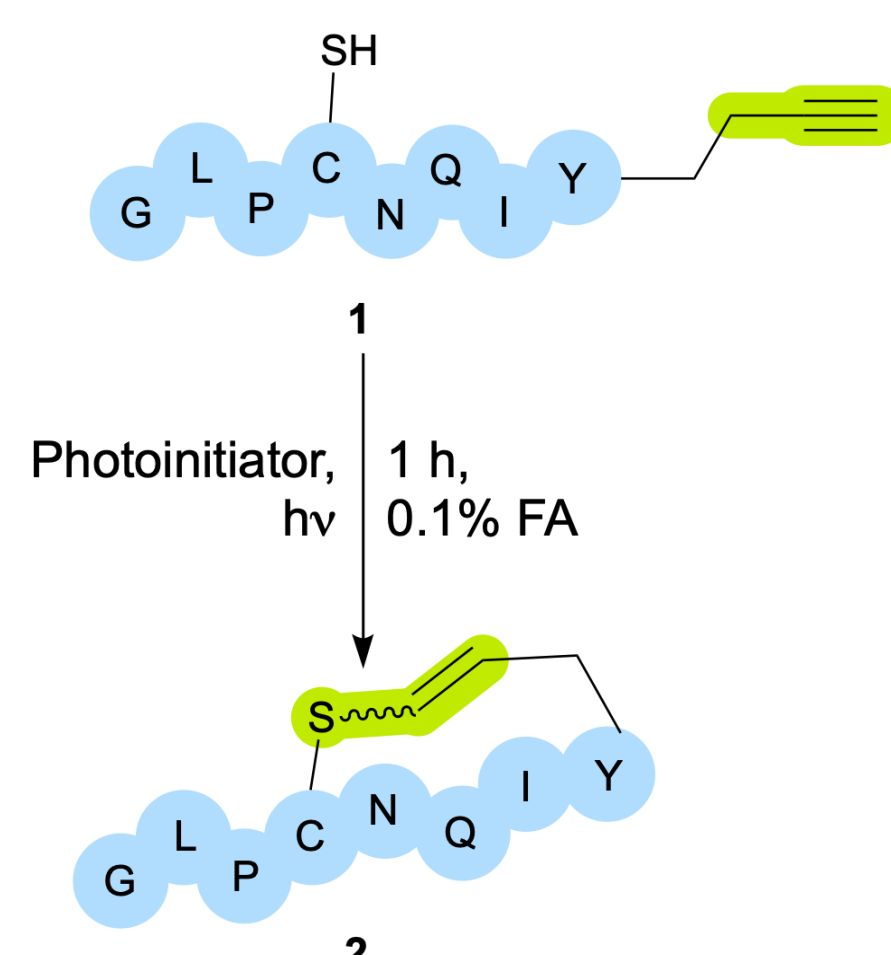
Introduction

- Peptides offer significant potential as therapeutics. However, poor membrane permeability and susceptibility to peptidases are of concern ¹
- Replacement of disulfide bonds offers a solution to increase stability ^{2,3}
- Thiol-yne 'click' cyclisation has been poorly explored ⁴
 - Mild, metal free, selective and light mediated solution
 - Vinyl sulfide adds rigidity with minimal perturbation of native sequence



Thiol-yne Optimisation

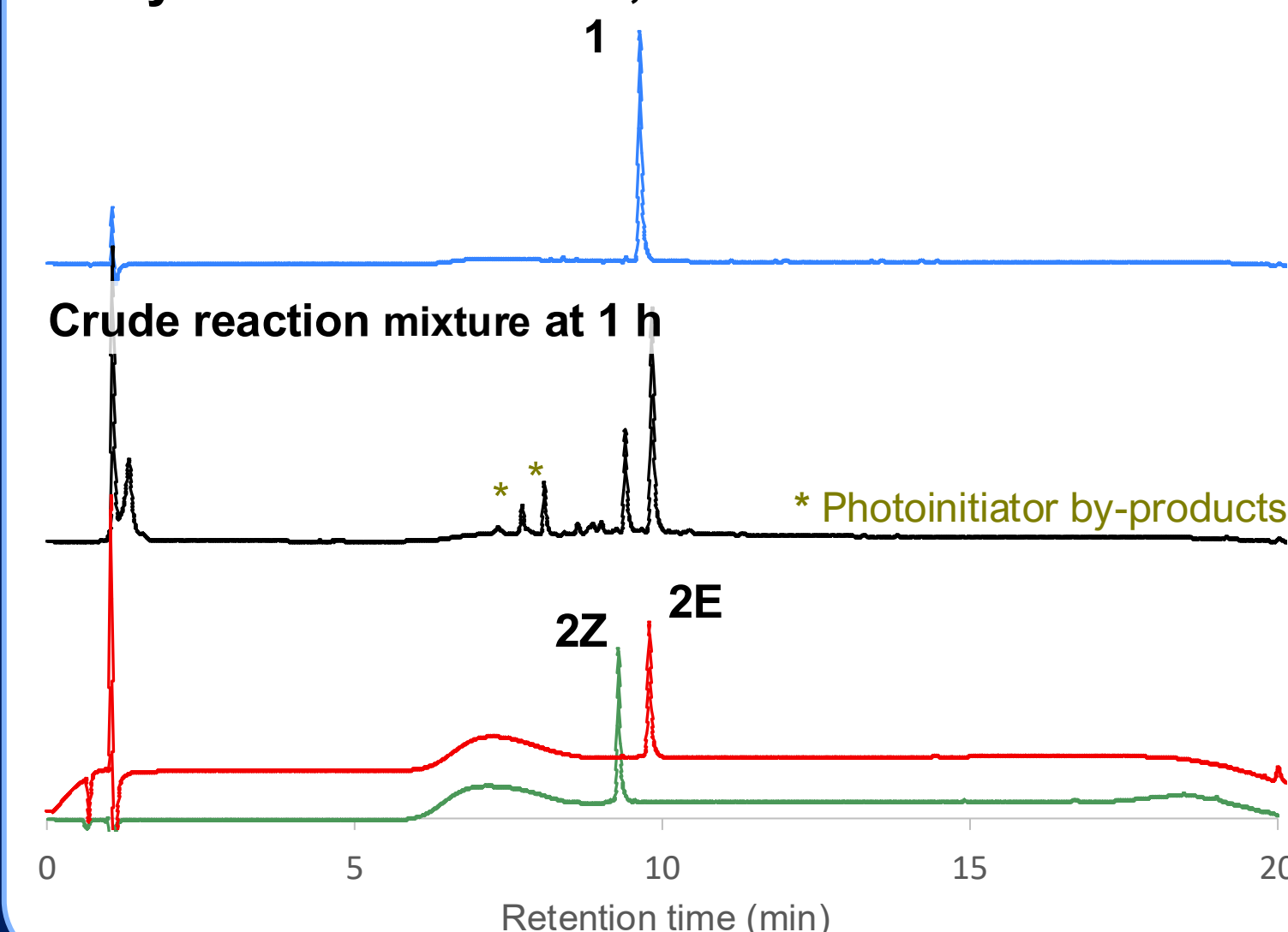
- Alkyne containing peptides synthesised through incorporation of an alkyne amino acid or carboxylic acid during SPPS
- Optimisation carried out exploring photoinitiator, pH, concentration and solvent effects



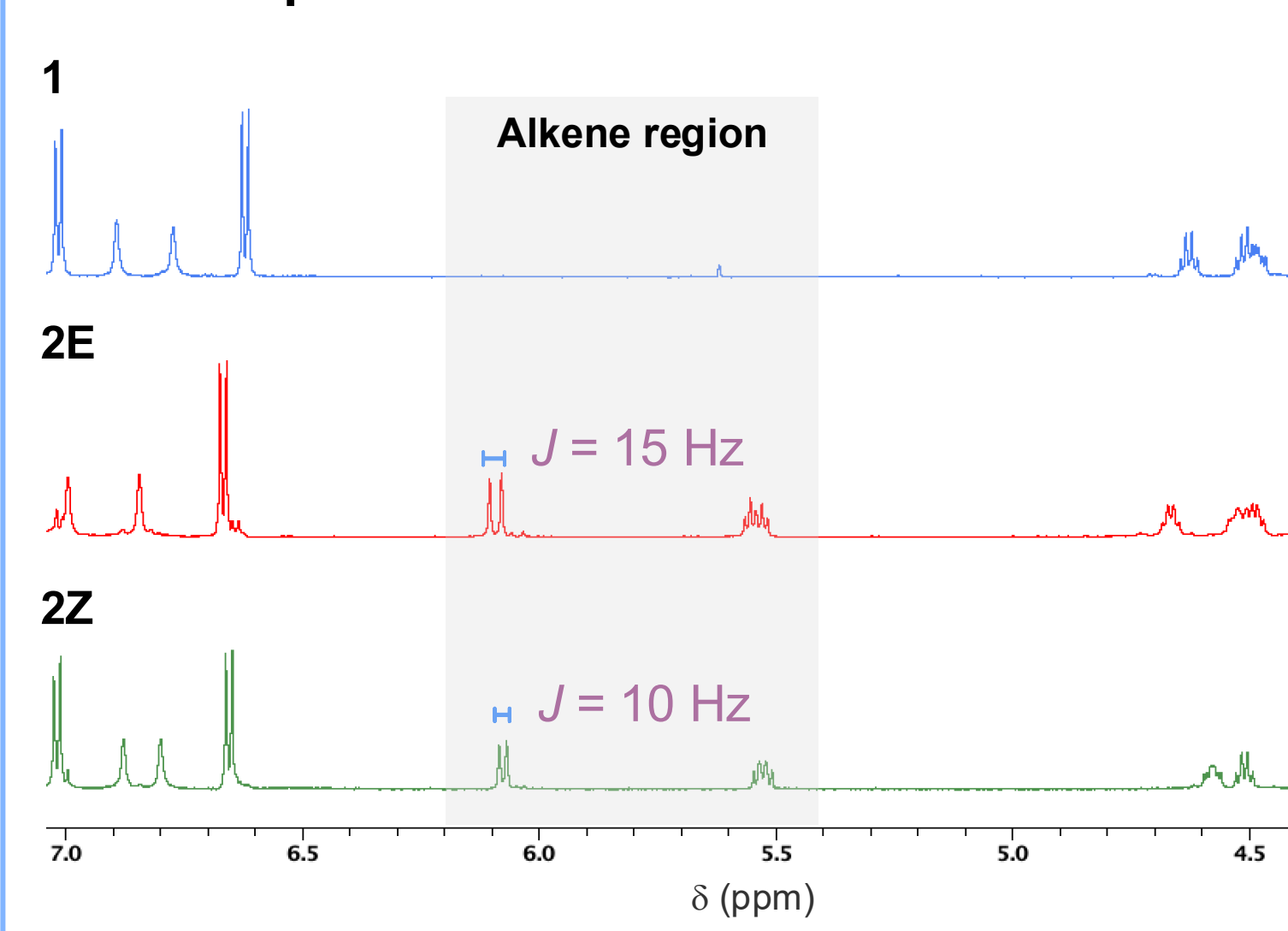
Photoinitiator	eq.	Conc.	Solvent	Additive	Conversion
DPAP	1.0	1.7 mM	H ₂ O : AcN	0.1% TFA, MAP	N.R.
DPAP	0.5	0.5 mM	DMF	–	26%
Irgacure 2959	0.5	0.5 mM	DMF	–	31%
Irgacure 2959	0.5	0.5 mM	DMF	0.1% TFA	16%
Irgacure 2959	0.5	0.5 mM	DMF	0.1% FA	60%
Irgacure 2959	0.5	1.0 mM	DMF	0.1% FA	81%
Irgacure 2959	0.5	1.5 mM	DMF	0.1% FA	>95%
Irgacure 2959	0.5	2.5 mM	DMF	0.1% FA	89%

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

Analytical HPLC traces, 214 nm

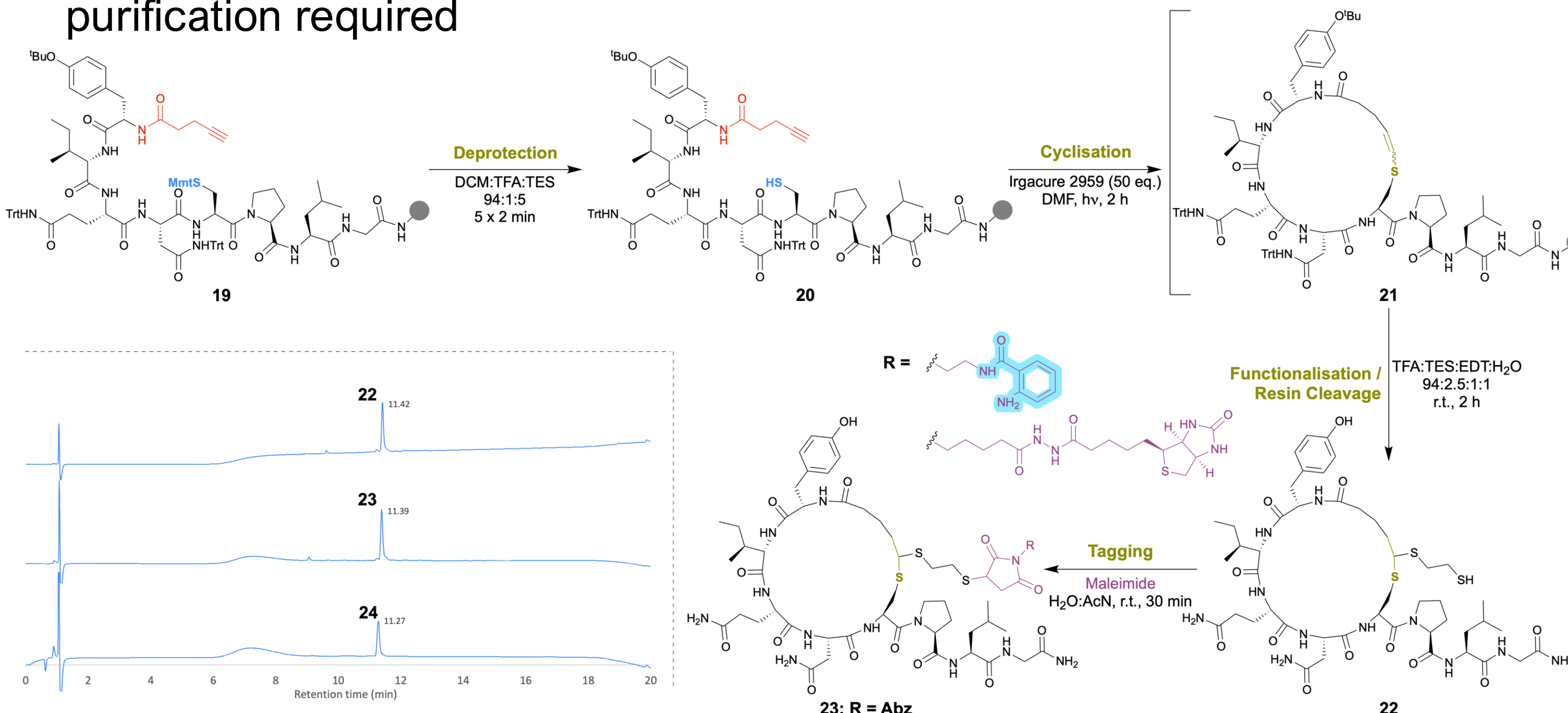


¹H NMR Spectra



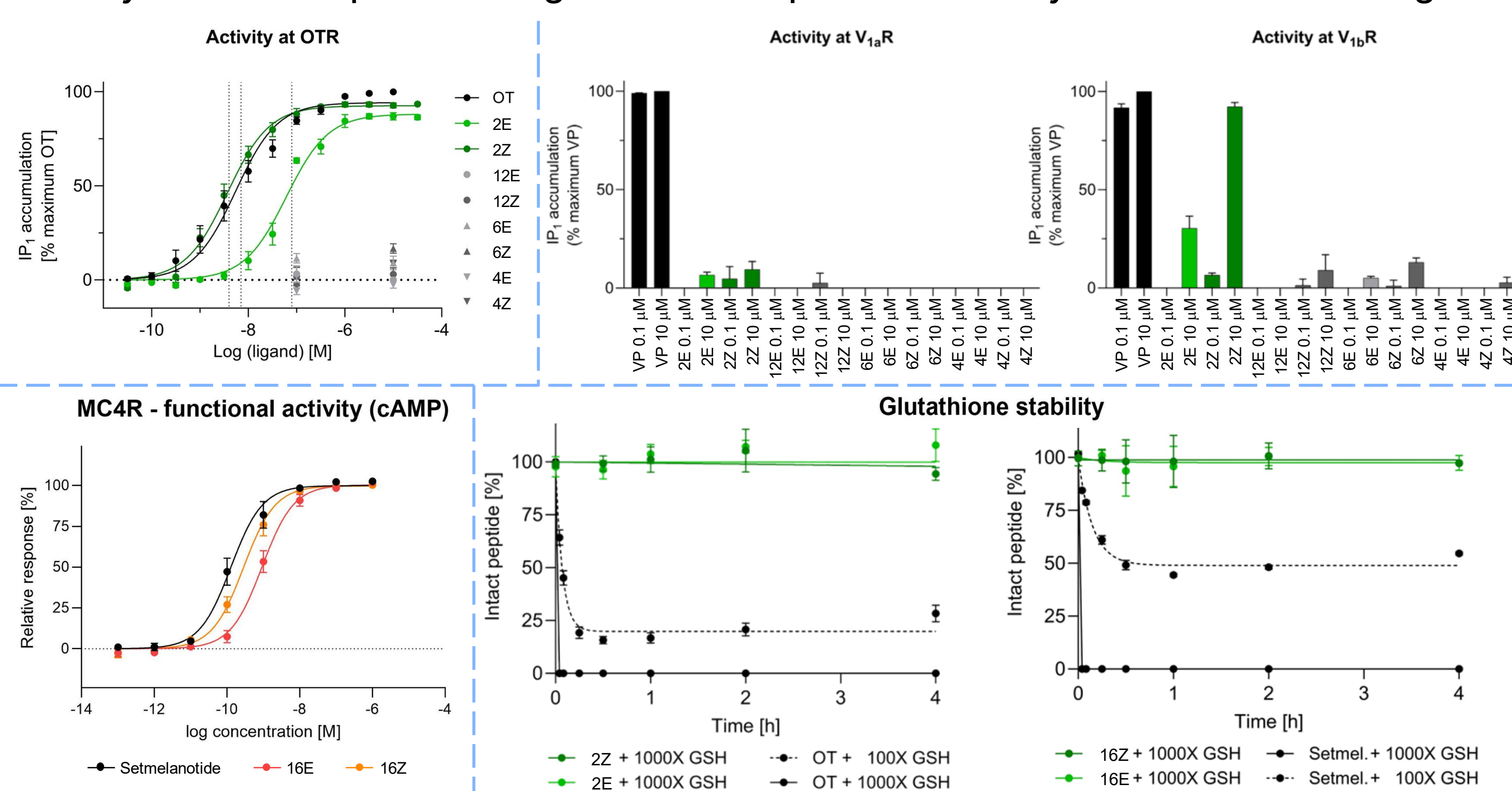
On-resin Cyclisation & Modification

- On-resin thiol-yne reaction, followed by concomitant ionic hydrothiolation during peptide cleavage yields a cyclic peptide with a free thiol handle
- Suitable for functionalisation with maleimide tags
- Fluorophore and biotin tagged cyclic peptides synthesised with minimal purification required



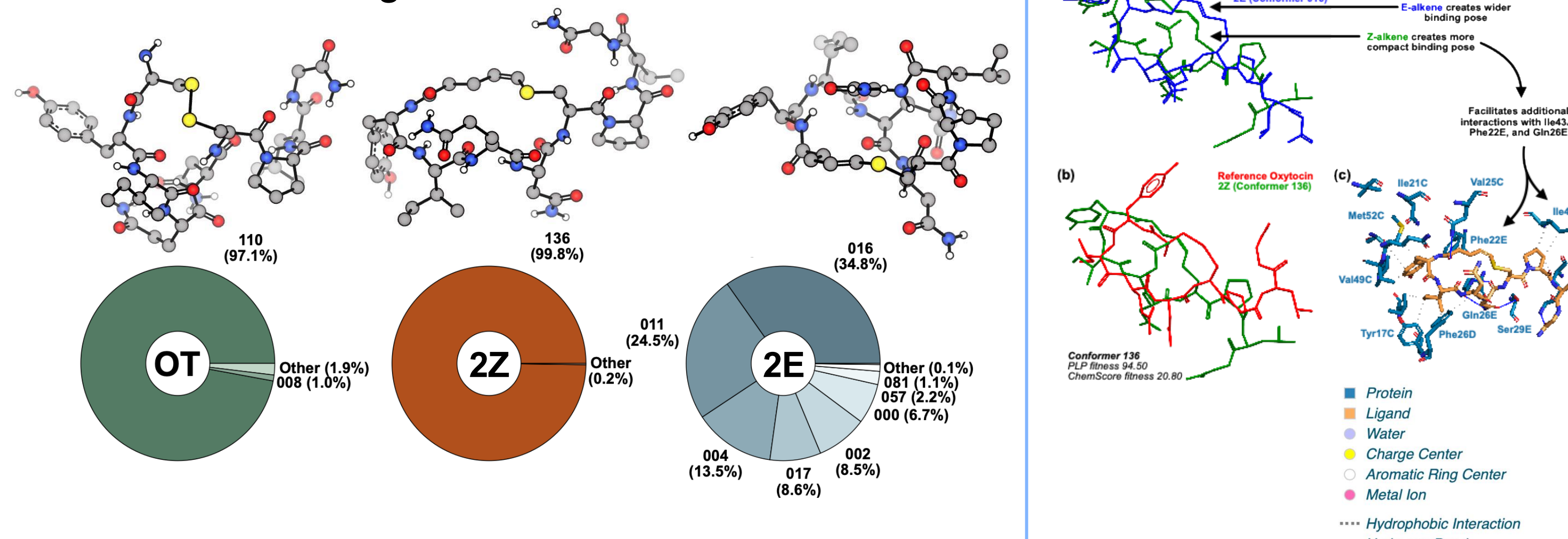
Biological Assays

- Biological activity assays of isolated neuropeptide analogues were obtained ⁵
- Peptides analogues **2Z** and **16Z** demonstrate EC₅₀ values with equivalent or improved activity relative to their respective parent compounds
- Oxytocin analogues do not show crossover activity at vasopressin receptors
- Vinyl sulfide stapled analogues have improved stability over disulfide bridges



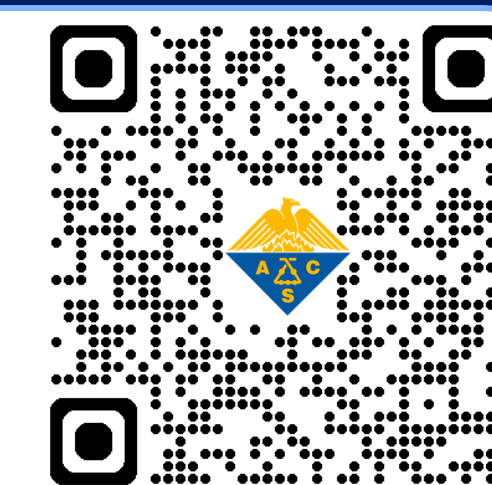
Conformational Analysis

- Analysis of **2Z** and **2E** shows (*Z*) vinyl sulfide has a single dominant conformer while (*E*) has 8 major conformers
- (*E*) vinyl sulfide has a wider ring size, (*Z*) more compact which better facilitates binding site interactions



Conclusions

- ✓ 'Click' type reaction
 - ✓ Mild, rapid, photo-radical conditions
 - ✓ Late stage on-resin modification
 - Investigating methodology for medium sized ring synthesis
- ✓ Increased rigidity & stability
 - ✓ Promising biological activity
 - ✓ (*E*) and (*Z*) isomers available



<https://doi.org/10.1021/jacs.6c08113>

References

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