

**CPPC
2021**

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Polyfluorinated aromatic porphyrin as a photoactive scaffold for peptide cyclisation

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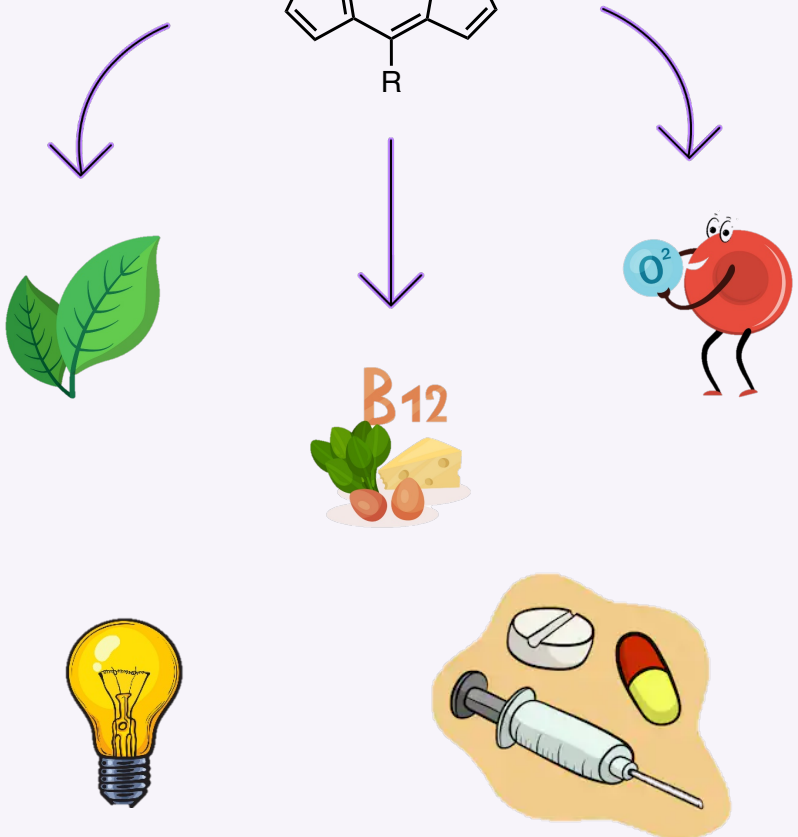
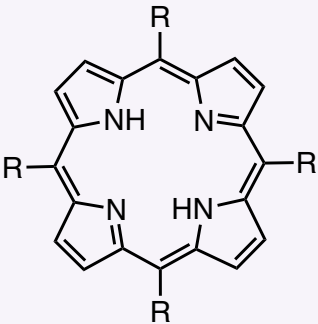
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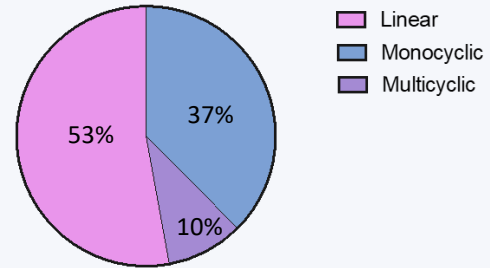
Introduction – conjugation of peptides and porphyrins

Porphyrins

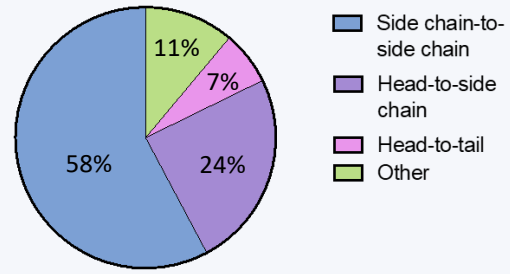


Peptides

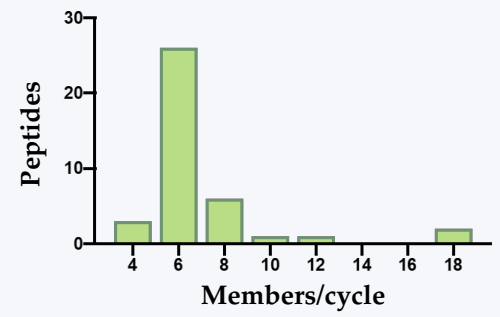
Peptide structure



Bond to form macrocycle



Macrocycle size



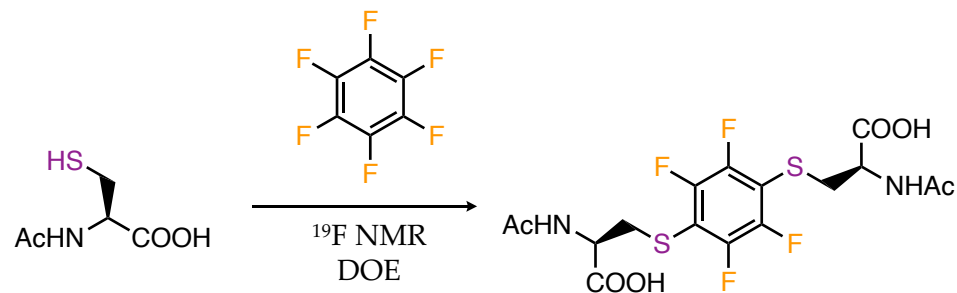
Vera D'Aloisio's Poster at CPPC2021: Structural composition analysis of approved peptide therapeutics and diagnostics as a guide for future peptide drug candidates

D'Aloisio, et al. PepTherDia: database and structural composition analysis of approved peptide therapeutics and diagnostics, *Drug Discovery Today*, 2021, ISSN 1359-6446.



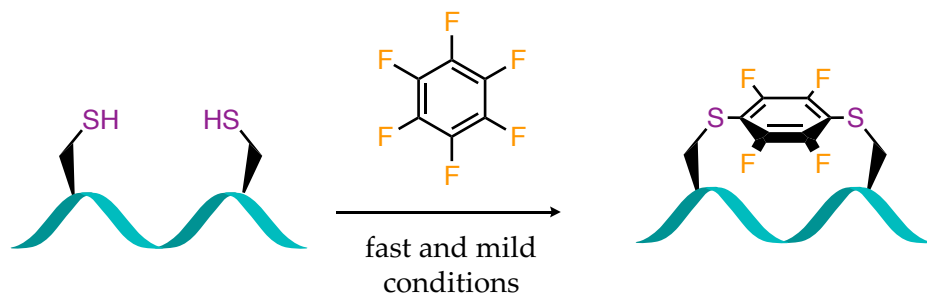
Introduction – from hexafluorobenzene to fluorinated porphyrins

Model reaction

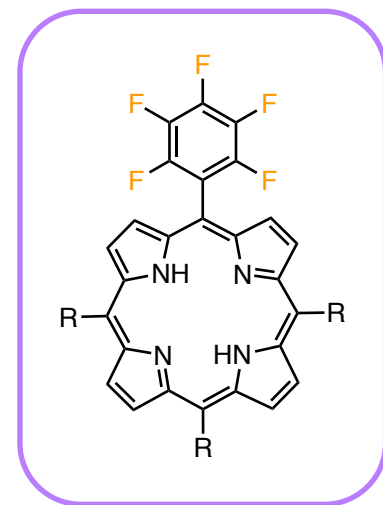


Base – Thiol – Solvent – Time – Temperature

Peptide system



New photoactive scaffold for peptide conjugation and cyclisation

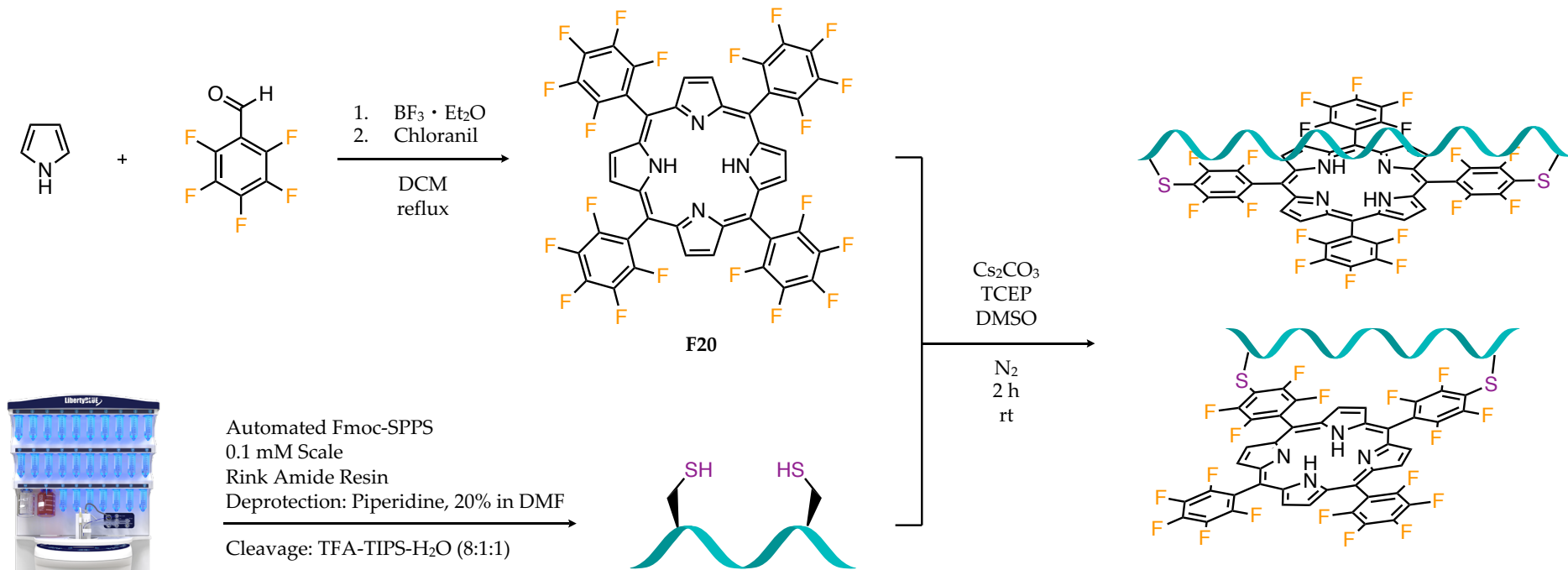


Spokoyny, A. M. et al. A perfluoroaryl-cysteine $\text{S}_{\text{N}}\text{Ar}$ chemistry approach to unprotected peptide stapling, *Journal of the American Chemical Society*, 2013; 135(16), 5946–5949.

Verhoorck, S. J. M. et al. Tuning the binding affinity and selectivity of perfluoroaryl-stapled peptides by cysteine-editing, *Chemistry - A European Journal*, 2019; 25, 177.

Dognini, P. et al. Using ^{19}F NMR and two-level factorial design to explore thiol-fluoride substitution in hexafluorobenzene and its application in peptide stapling and cyclisation, *Peptide Science*, 2021; 113:e24182.

Results and discussion – tetra(pentafluorophenyl)porphyrin (F20)



Entry	Inter-thiol distance	Peptide sequence	Cyclisation
1	i, i+10	ACATGSTQHQACG	Yes
2	i, i+9	ACATGSTQHQCG	Yes
3	i, i+8	ACTGSTQHQCG	Yes
4	i, i+8	ACTHGQTQSCG	Yes
5	i, i+7	ACTSTQHQCG	Yes
6	i, i+6	ACSTQHQCG	Yes
7	i, i+4	ACQHQCG	Yes
8	i, i+2	ACACG	Yes
9	i, i+1	ACCG	No
10	i, i+9	NCVVG YIGERCQ	Yes *

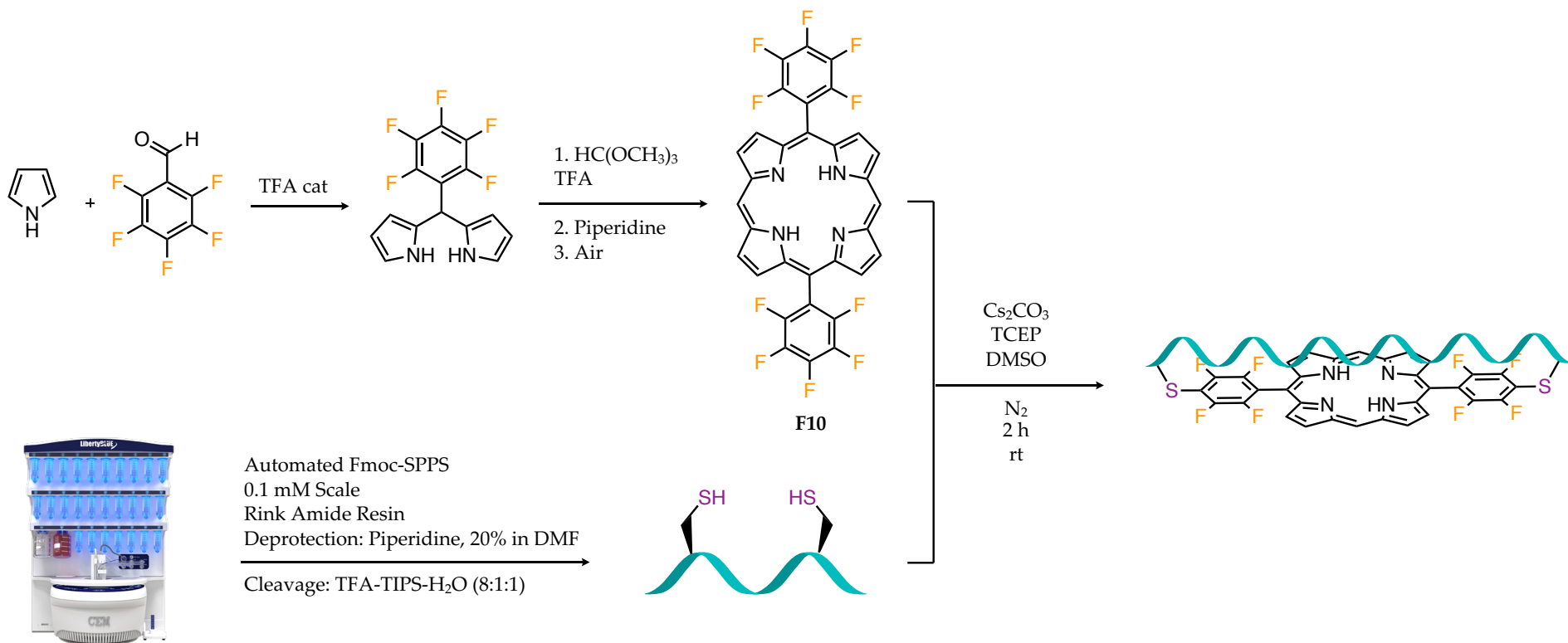
Skin Penetrating And Cell Entering (SPACE) Peptide

SPACE control

* The reaction is slower.

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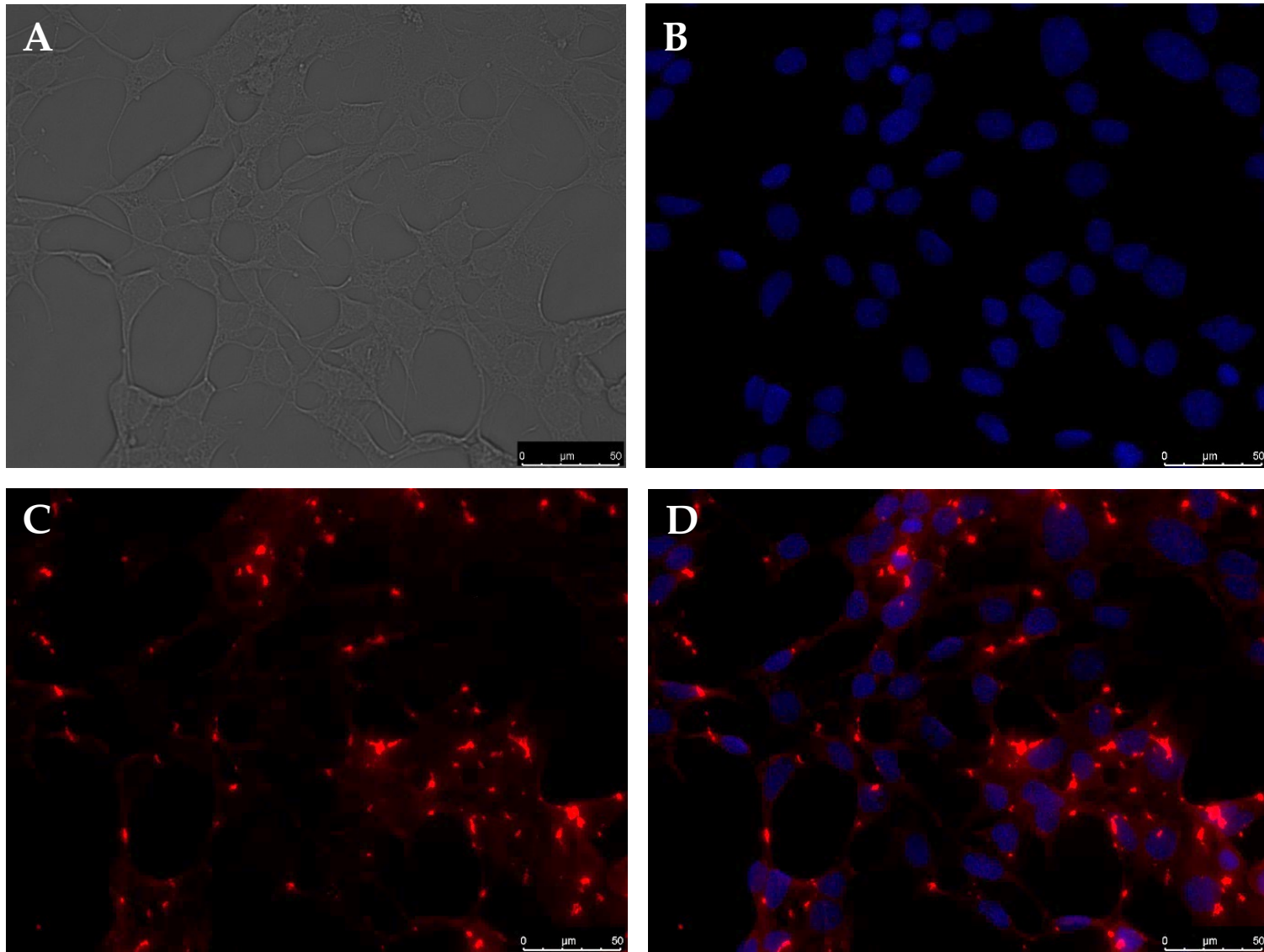
Results and discussion – *bis(pentafluorophenyl)porphyrin (F10)*



Entry	Inter-thiol distance	Peptide sequence	Cyclisation
11	i, i+8	ACTGSTQHCCG	Yes
12	i, i+6	ACSTQHCCG	Yes
13	i, i+5	ACTQHCCG	No
14	i, i+4	ACQHCCG	No

Skin Penetrating And Cell Entering (SPACE) Peptide

Results and discussion – F20-SPACE peptide conjugate skin cell uptake



SCCIC8 cells were treated in complete media with F20-SPACE peptide conjugate (10 μ M) and incubated for 24h in the dark. Images were captured at 40X magnification with a Leica Live cell imaging microscope. A) Bright Field image of cells. B) Nuclei stained with DAPI. C) Uptake of the F20-SPACE peptide conjugate. D) Collated image of conjugate uptake with DAPI stained nuclei.

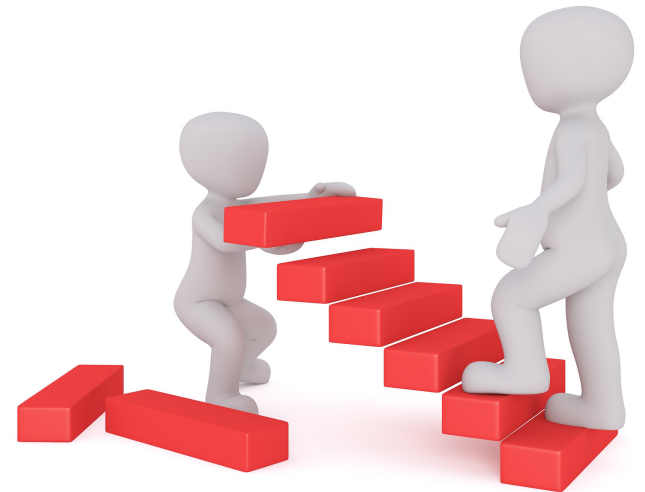
Conclusions

- The cyclisation is **not sequence dependent**.
- **$i, i + 6$** is the minimal inter-thiol distance for **trans**-cyclisation.
- **$i, i + 2$** is the minimal inter-thiol distance for **cis**-cyclisation.
- Both SPACE peptide and F20 porphyrin retain their properties after conjugation.



Next steps

- **^{19}F NMR** to confirm substitution pattern.
- Cyclisation of different peptides.
- Tetra functionalisation of F20.
- Investigation of the **uptake mechanism** of the conjugate and comparison with the uptake of SPACE peptide alone.



Acknowledgments



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Thank you!

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