SIMPLE ONE-POT PEPTIDE CLEAVAGE AND SPONTANEOUS MACROCYCLIZATION THROUGH PAAL-KNORR-LIKE REACTIONS USING 2,5-DIALKYLFURAN DERIVATIVES

Alex Manicardi,[a,b*] Atiruj Theppawong,[b] Marleen Van Troys,[c] Annemieke Madder[b]

[a] Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze, 17/A, Parma, I-43124, Italy
[b] Department of Organic and Macromolecular Chemistry, Organic and Biomimetic Chemistry Research Group, Ghent University, Krijgslaan 281 S4, Ghent, B-9000, Belgium
[b] Department of Biomedical Molecular Biology, Ghent University, Technologiepark-Zwijnaarde 71, B-9052 Ghent, Belgium

alex.manicardi@unipr.it

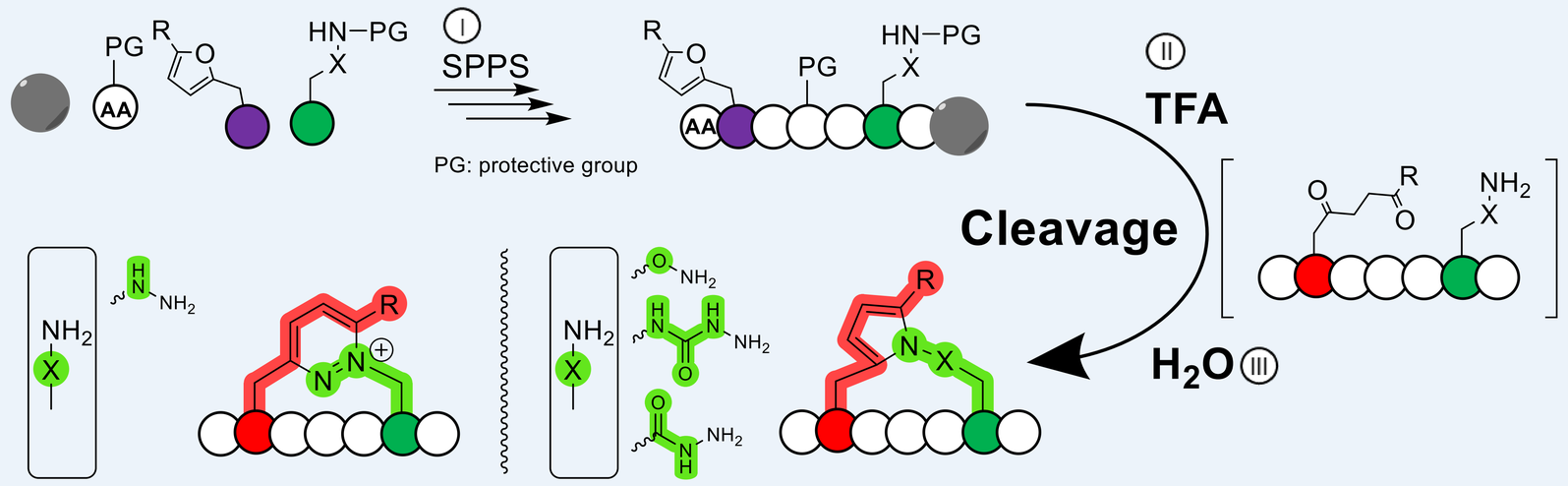
https://doi.org/10.17952/37EPS.2024.P2049

1 INTRODUCTION
WHAT'S THE PROBLEM?

Macrocyclic peptides show enhanced properties as compared to linear peptides (e.g., enhanced target affinity, resistance toward degradation), making them attractive candidates for therapeutic applications and for interfering with protein-protein interactions (PPIs). Synthetic techniques have revolutionized peptide macrocyclizations, relying on the use of both natural and unnatural amino acids to achieve chemoselective ligations on protected or unprotected peptides. Recently, research has been oriented toward one-pot chemistries that rely on simple reaction conditions or reagent-less approaches. Despite the notable progress in peptide stapling techniques, challenges persist in existing methodologies, particularly in controlling the reaction order at multiple sites and achieving precise positional selectivity.

Our idea: use **2,5-disubstituted furans** as **stable** handles to generate **γ-diketones** that can be exploited for the one-pot peptide macrocyclization in the presence of **α-effect nucleophilic** moieties.

2 THE STRATEGY
A RESUSPEND-AND-WAIT PROTOCOL FOR PEPTIDE CYCLIZATION

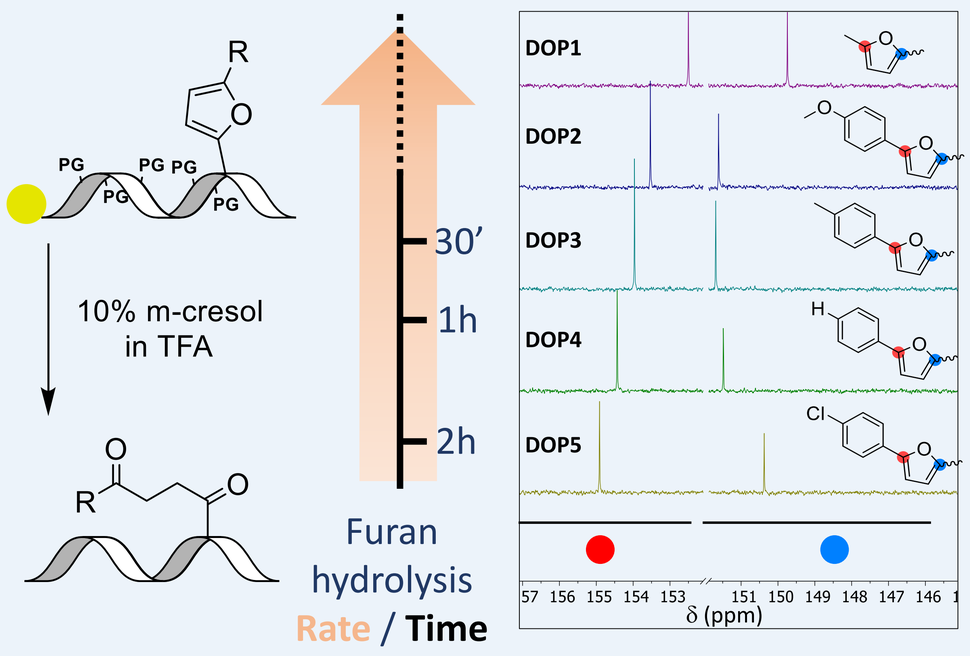


I Standard SPPS using Fmoc-protected monomers

II Standard peptide cleavage

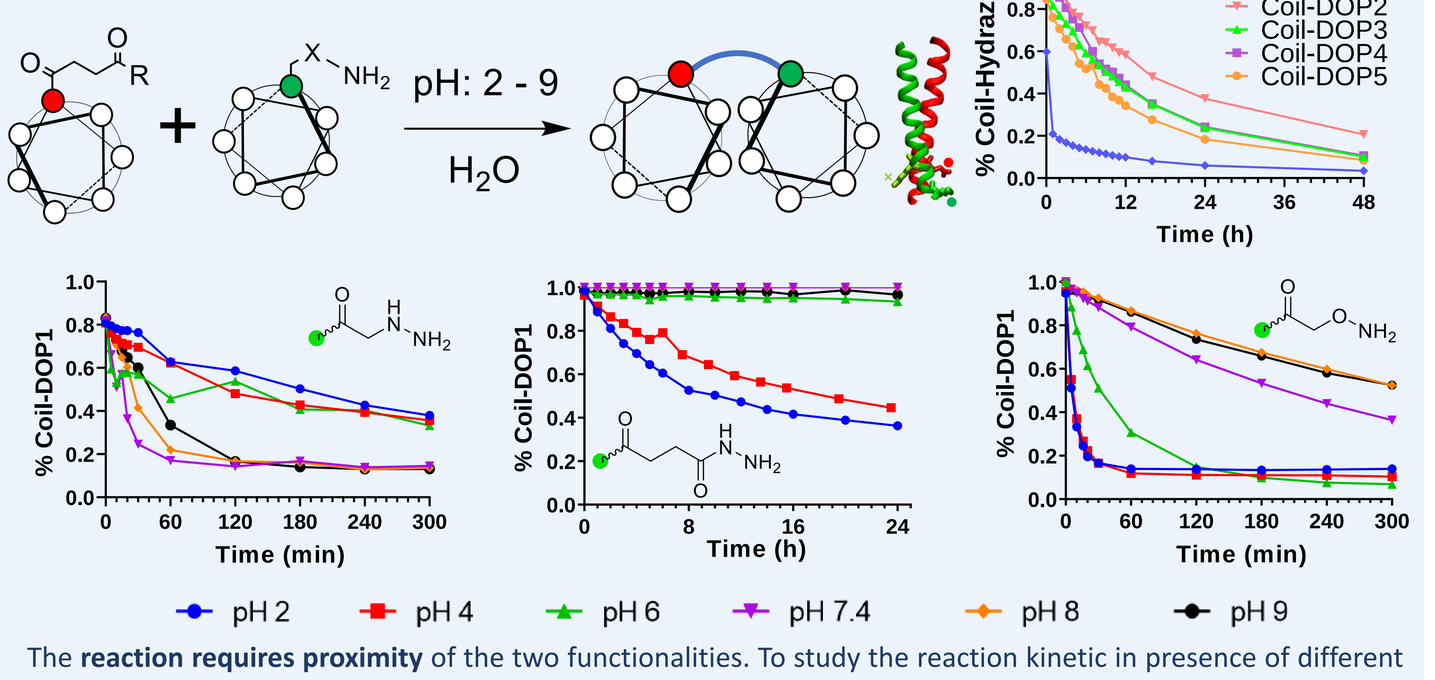
III Crude resuspension, waiting for reaction, HPLC purification

3 FURAN OPENING
PRO-ELECTROPHILE ACTIVATION



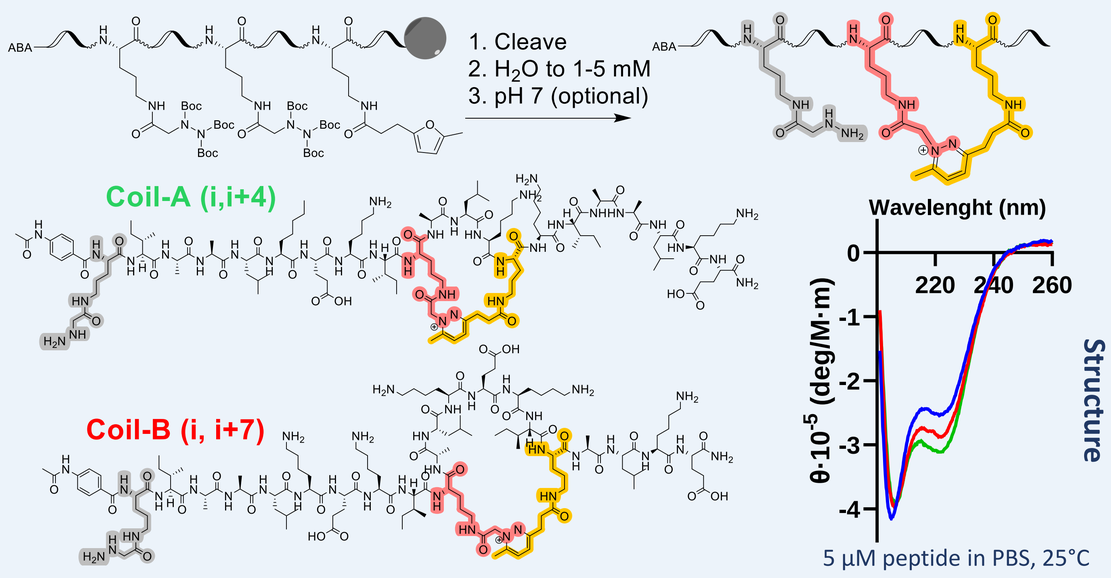
Furan hydrolysis rate is correlated to aromatic ring electron density. Cleavage duration can be predicted by simple ^{13}C-NMR (●).

4 REACTION KINETICS
PROXIMITY-INDUCED BIORTHOGONAL REACTION

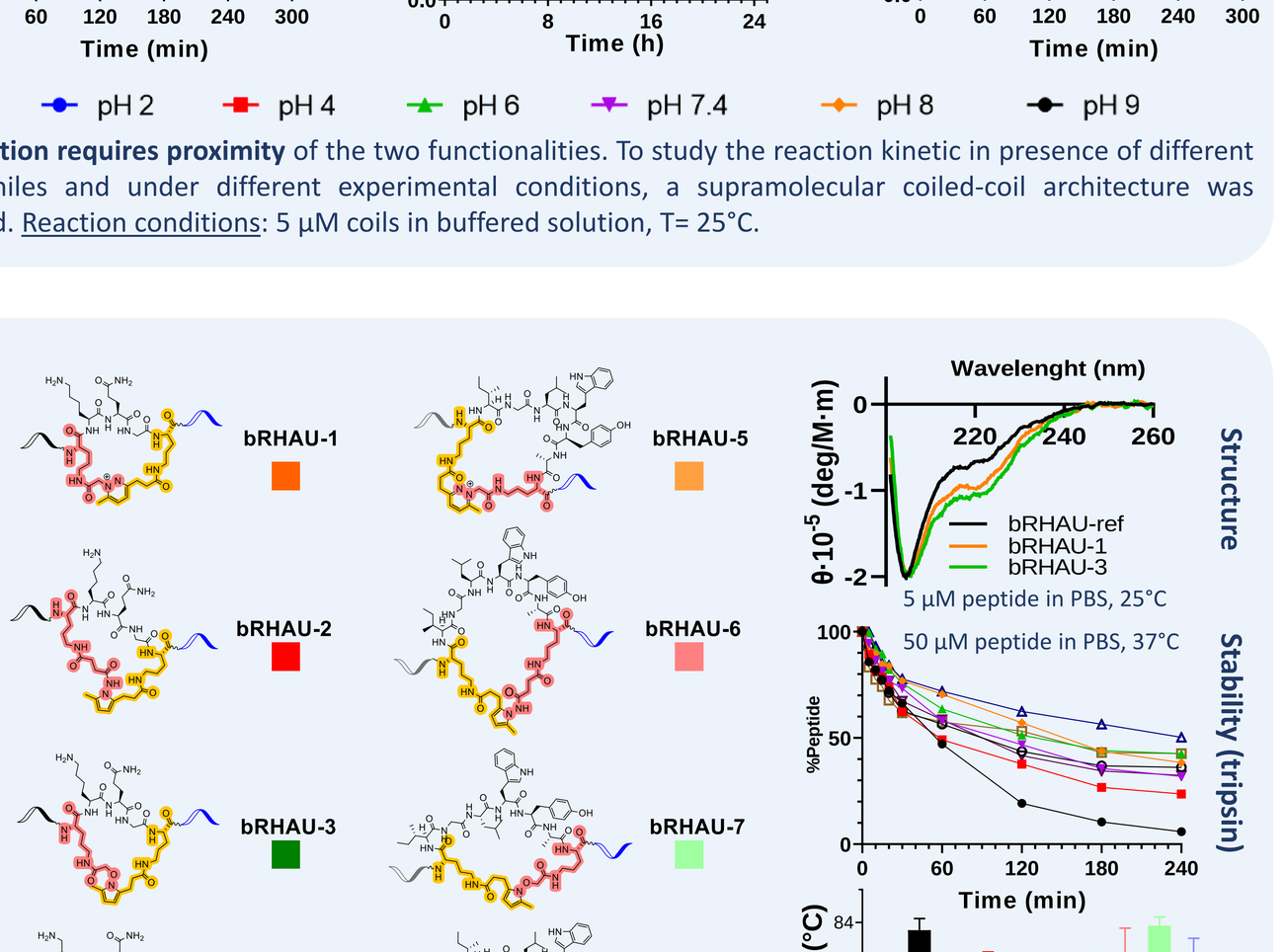


The **reaction requires proximity** of the two functionalities. To study the reaction kinetic in presence of different nucleophiles and under different experimental conditions, a supramolecular coiled-coil architecture was exploited. Reaction conditions: 5 µM coils in buffered solution, T= 25°C.

5 SCOPE
2 COILED SYSTEMS. 8 G4-BINDERS. 8 CYCLIC RGD MODELS



Structures confirmed by trypsin digestion



6 CONCLUSIONS
AND ACKNOWLEDGEMENTS

- Pro-electrophile and nucleophile can be included using standard SPPS protocols
- Pro-electrophile is activated during peptide cleavage
- Reaction after simple peptide resuspension in water/buffer
- Stable bond formation, orthogonal to other available chemistries

This work was supported by UGent Industrieel OnderzoeksFonds projects F2019/IOF-ConcepTT/188, F2020/IOF-ConcepTT/111, and by the University of Parma through the action 'Bando di Ateneo 2021 per la ricerca' co-funded by MUR-Italian Ministry of Universities and Research - D.M. 737/2021 - PNR - PNRR - NextGenerationEU (Al.M). A.T. is indebted to Research Foundation Flanders (FWO) for support of his postdoctoral fellowship (1273521N). MVT is supported by a concerted research action grant from Ghent University (GOA-028-19).
This work was partially supported by a cascade call issued within the framework of the "National Center for Gene Therapy and Drugs based on RNA Technology (CN RNA & Gene Therapy)" program, using the resources of the National Recovery and Resilience Plan (PNRR), Mission 4 "Education and Research", Component 2 "From research to business", Investment Line 1.4 "Strengthening research structures and creation of national R&D champions on some Key Enabling Technologies", Call Code 00000041_1, CUP [C93C22002780006]; funded by the European Union – NextGenerationEU.Project CASN_A_24_PNRR_BAC-HERMES_01 PNRR–M4C2–BAC–Notice, Title "Highly Efficient and selective RNA delivery systems for the treatment of MEtabolic and cardiovascular diseaseS – HERMES" codeCN00000041 – CUP CUPC93C22002780006.