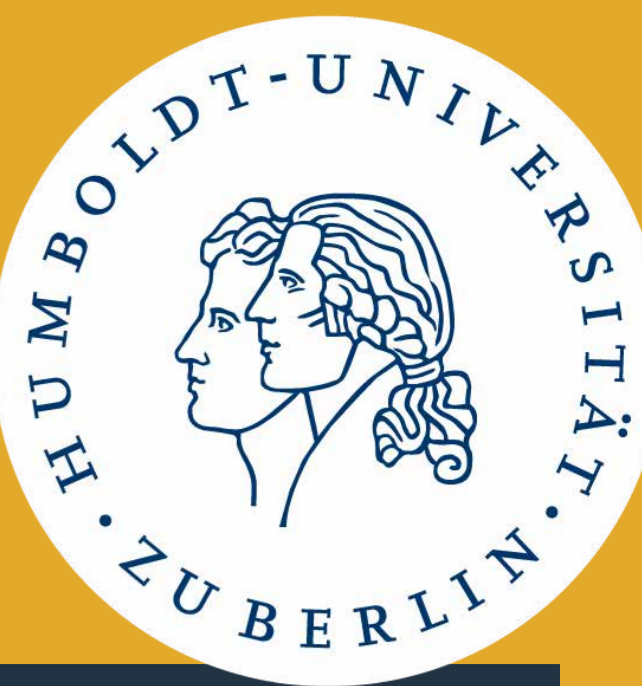


# Auxiliaries in Native Chemical Ligation: Exploring the Reactivity Landscape with new Scaffolds

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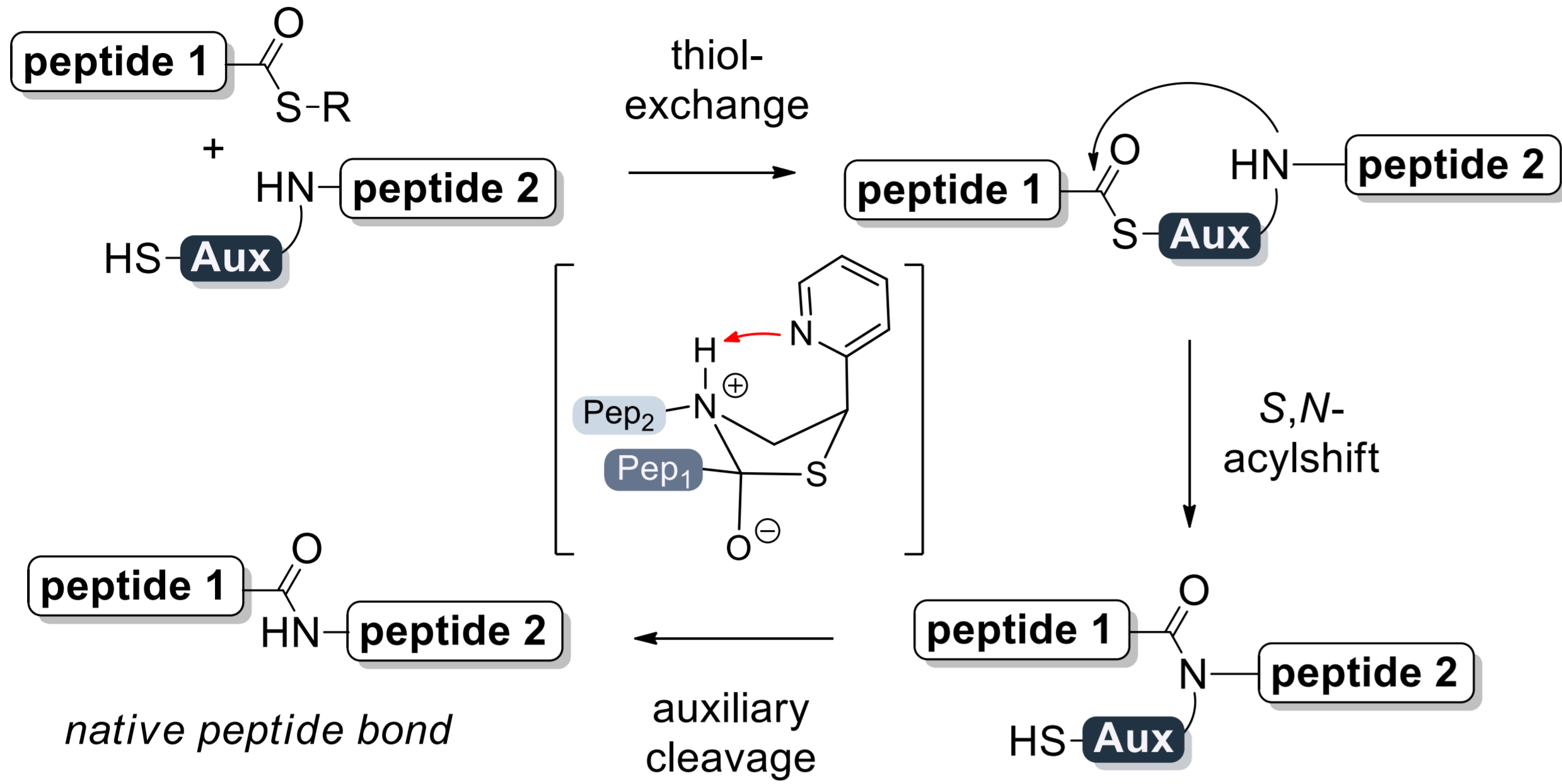
<https://doi.org/10.17952/37EPS.2024.P1023>



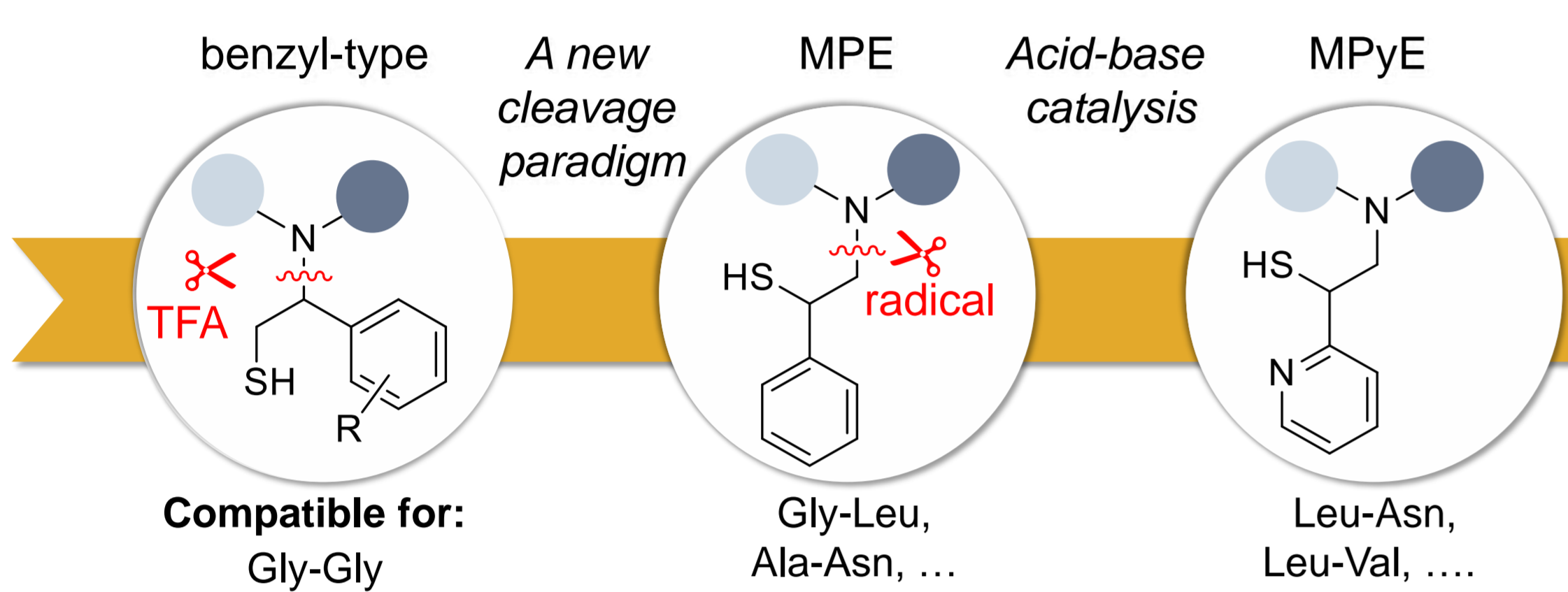
The auxiliary-mediated native chemical ligation (NCL) serves as an essential tool in expanding the scope of NCL beyond cysteine, allowing for the selection of any amide bond for retrosynthetic scission of proteins. While long conceived as limited to “easy junctions” like Gly-Gly, our newest generation of auxiliaries are capable of tackling reactions of two  $\beta$ -branched amino acids and are feasible for ligation with phosphorylated or glycosylated amino acids at pH 7 or in acidic medium.

## Base Catalysis in Auxiliary-mediated NCL

- auxiliary-mediated NCL can be enhanced via intramolecular base catalysis<sup>[1, 2, 3]</sup>

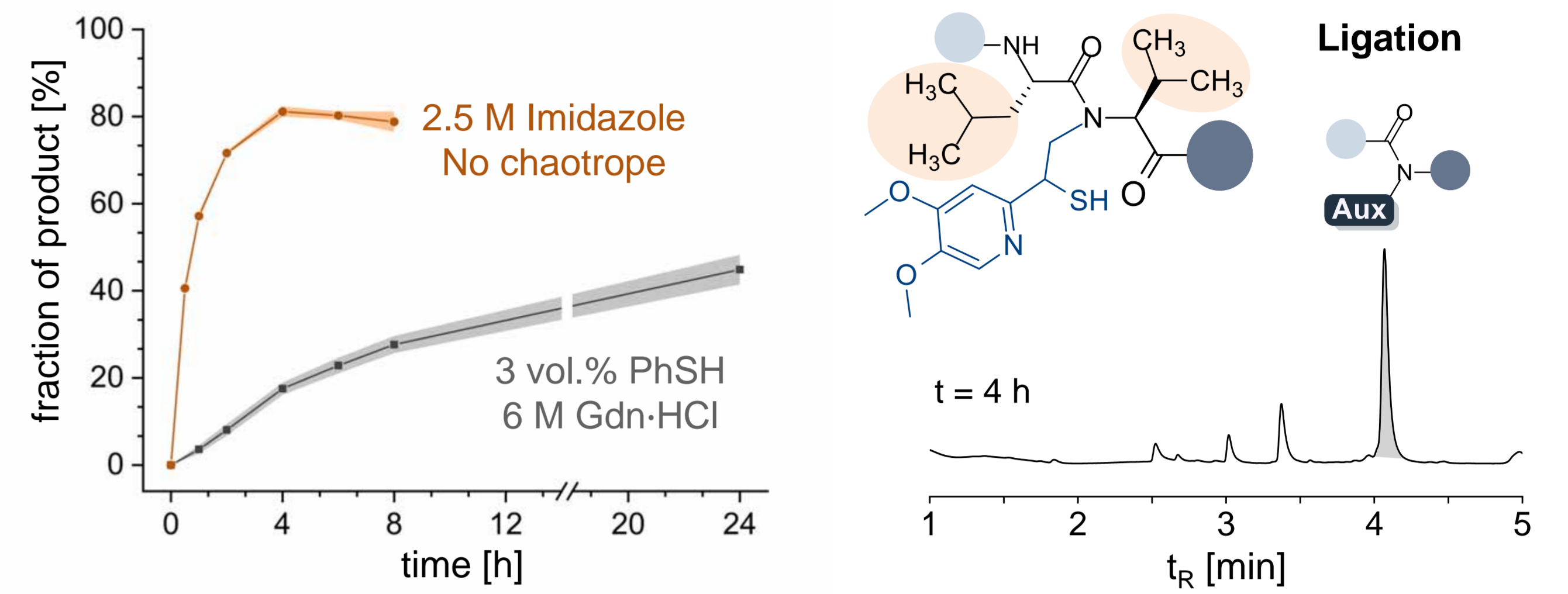


- A small history of ligation auxiliaries:

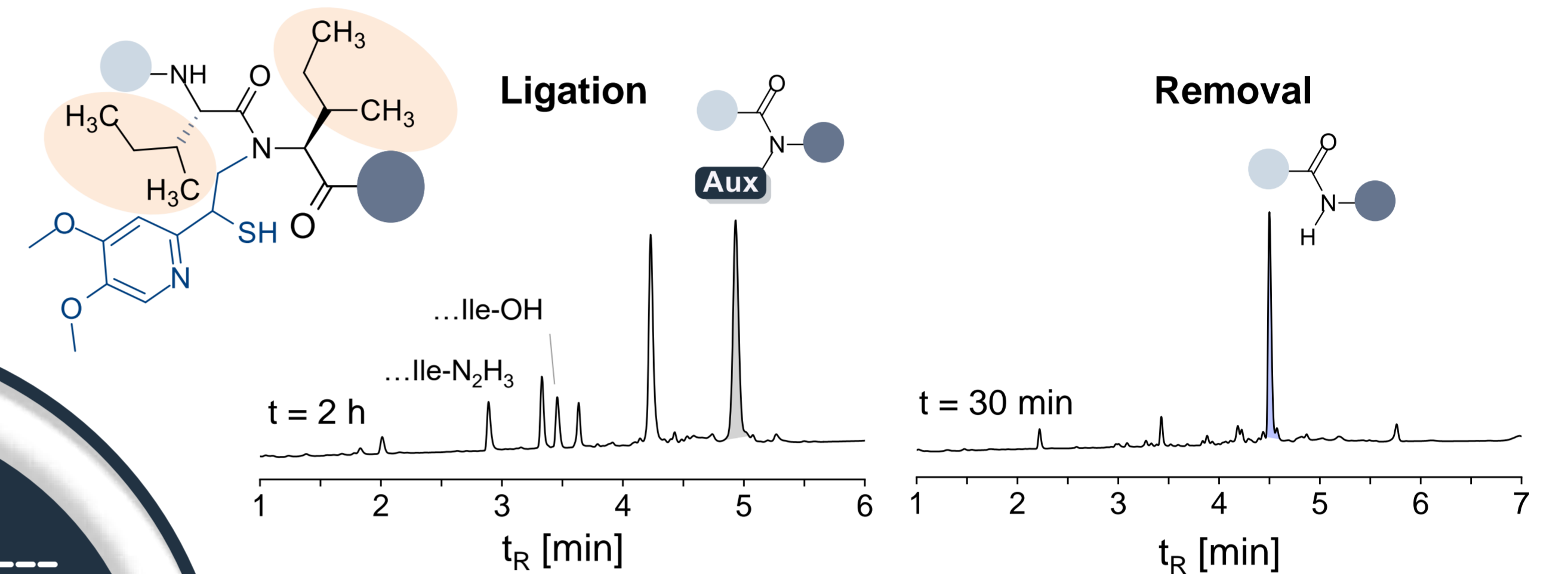


## The Holy Grail of Ligations

- exceptional ligation rate with new conditions enables Leu-Val ligation in 4 h

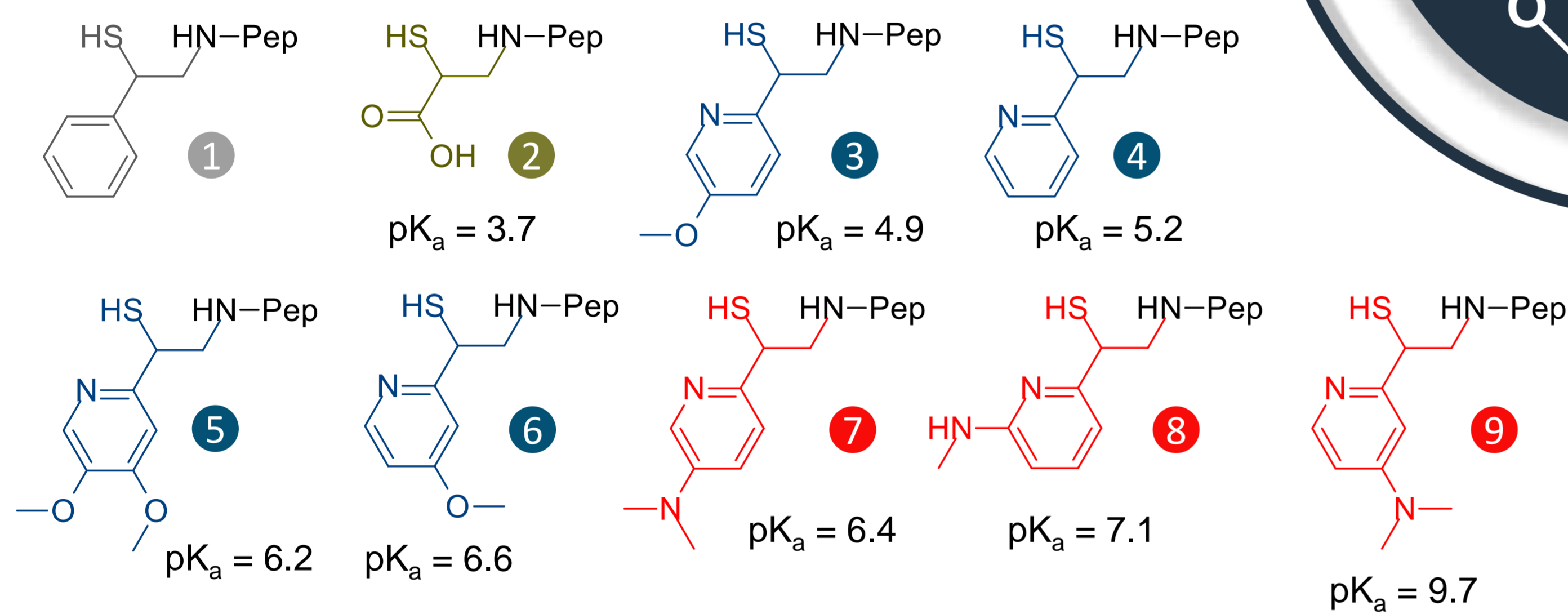


- Ligation with two  $\beta$ -branched amino acids become possible (Ile-Ile)

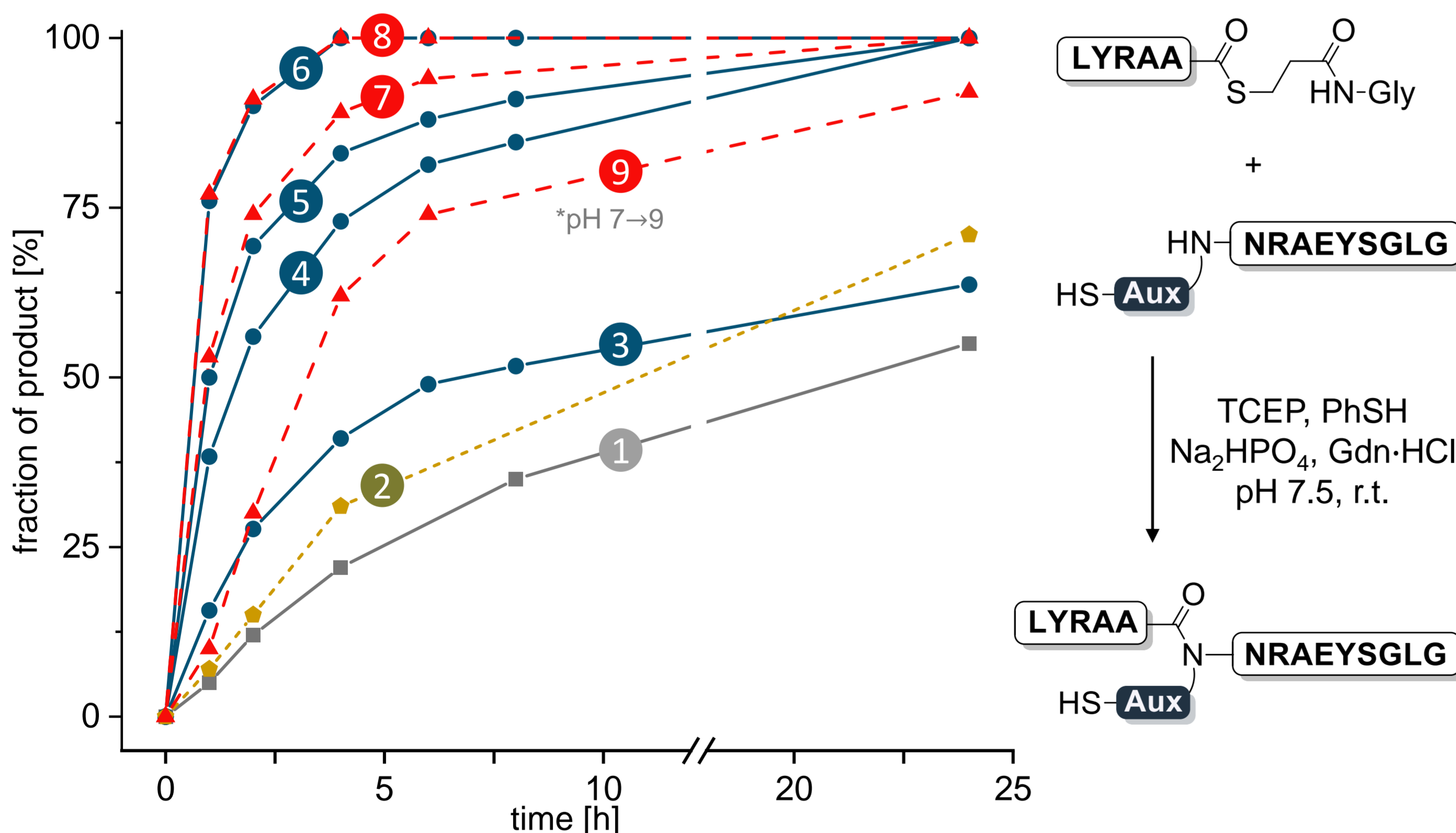


## Connecting Basicity with Reactivity

- ligation rate correlates with basicity of the auxiliary

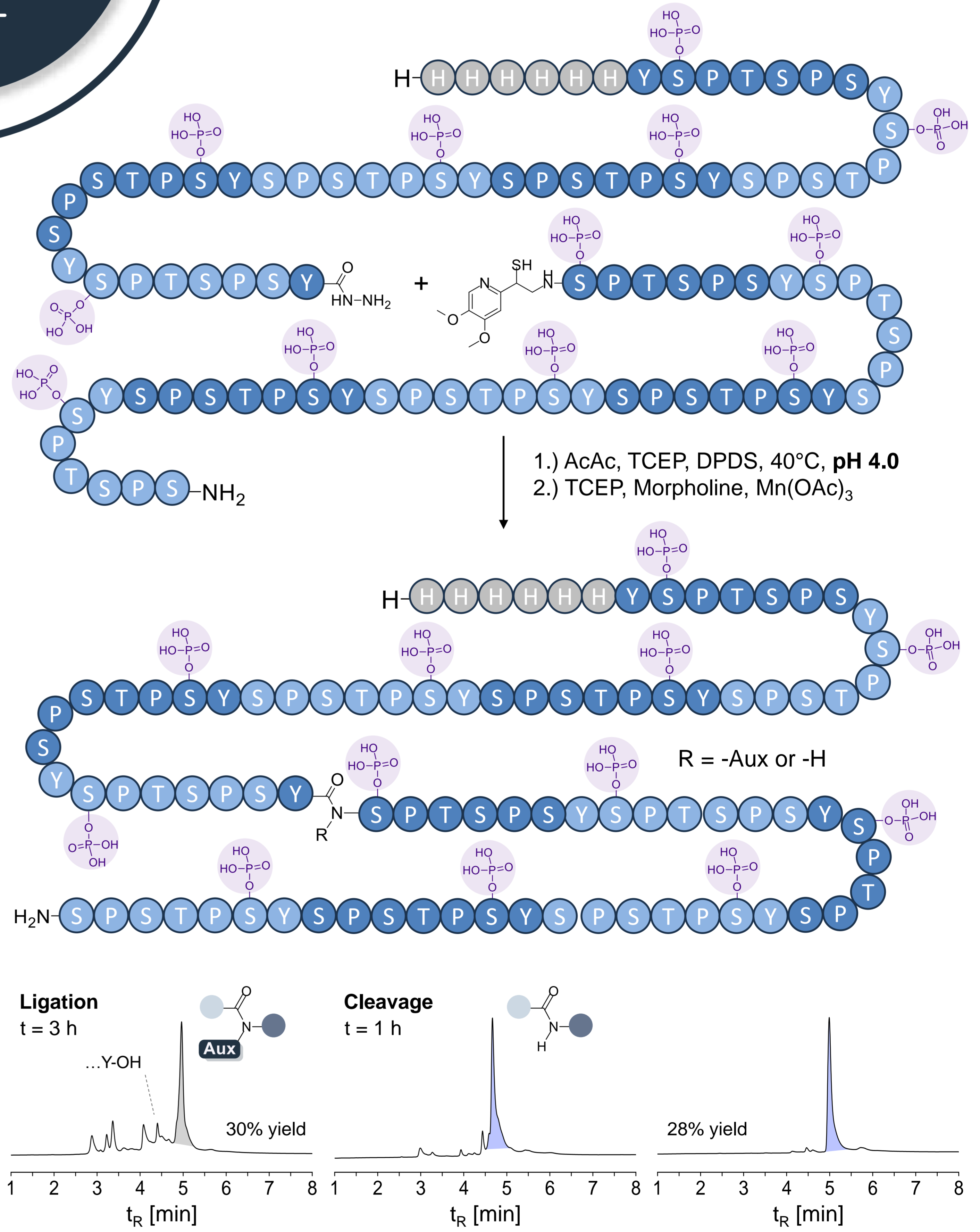


### Ala-Asn Ligation

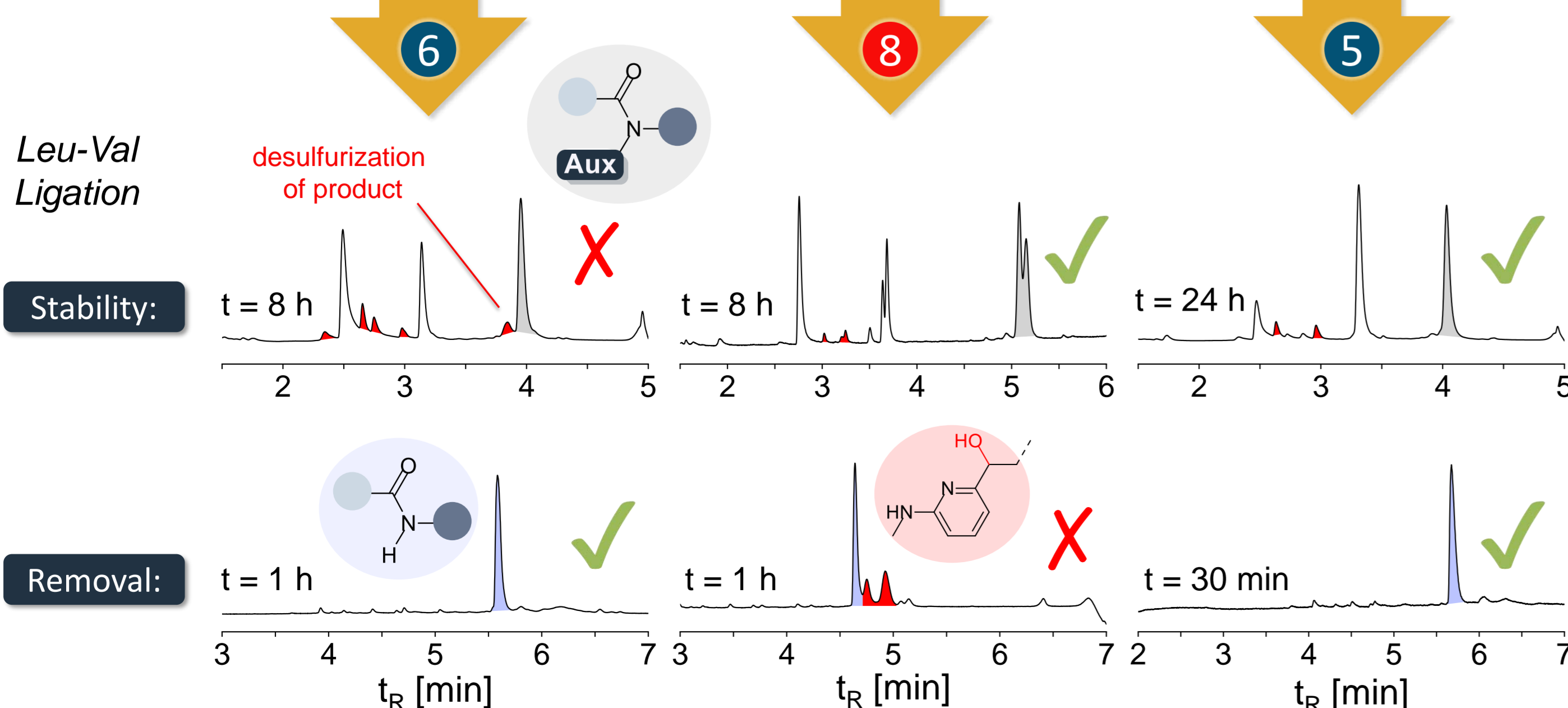


## Protein Synthesis in Acidic Medium

- auxiliaries enable protein synthesis directly from peptide hydrazides



## What Makes a Perfect Auxiliary?



## Conclusions

- crucial correlation between ligation rate and auxiliary basicity uncovered, confirming the postulated mechanism of intermediate proton abstraction
- a novel auxiliary for ligation of two amino acids with  $\beta$ -branching points
- with auxiliaries, ligation with phosphorylated and glycosylated amino acids are possible
- ligation at acidic pH feasible

[1] a) S.F. Loibl, Z. Harpaz, O. Seitz, Angew. Chem. Int. Ed., 2015, 54, 15055-15059; b) S.F. Loibl, A. Dallmann, K. Hennig, C. Judd, Chem. Eur. J., 2018, 24, 2623-3633.

[2] O. Fuchs, S. Trunschke, H. Hanebrink, M. Reimann, O. Seitz, Angew. Chem. Int. Ed., 2021, 60, 19483-19490.

[3] S. Trunschke, E. Piemontese, O. Fuchs, S. Abboud, O. Seitz, Chem. Eur. J., 2022, 28, e202202065