

# SALICYLALDEHYDE-TAGGED PEPTIDES AS LYSINE-REACTIVE, REVERSIBLE-COVALENT PROTEIN BINDERS

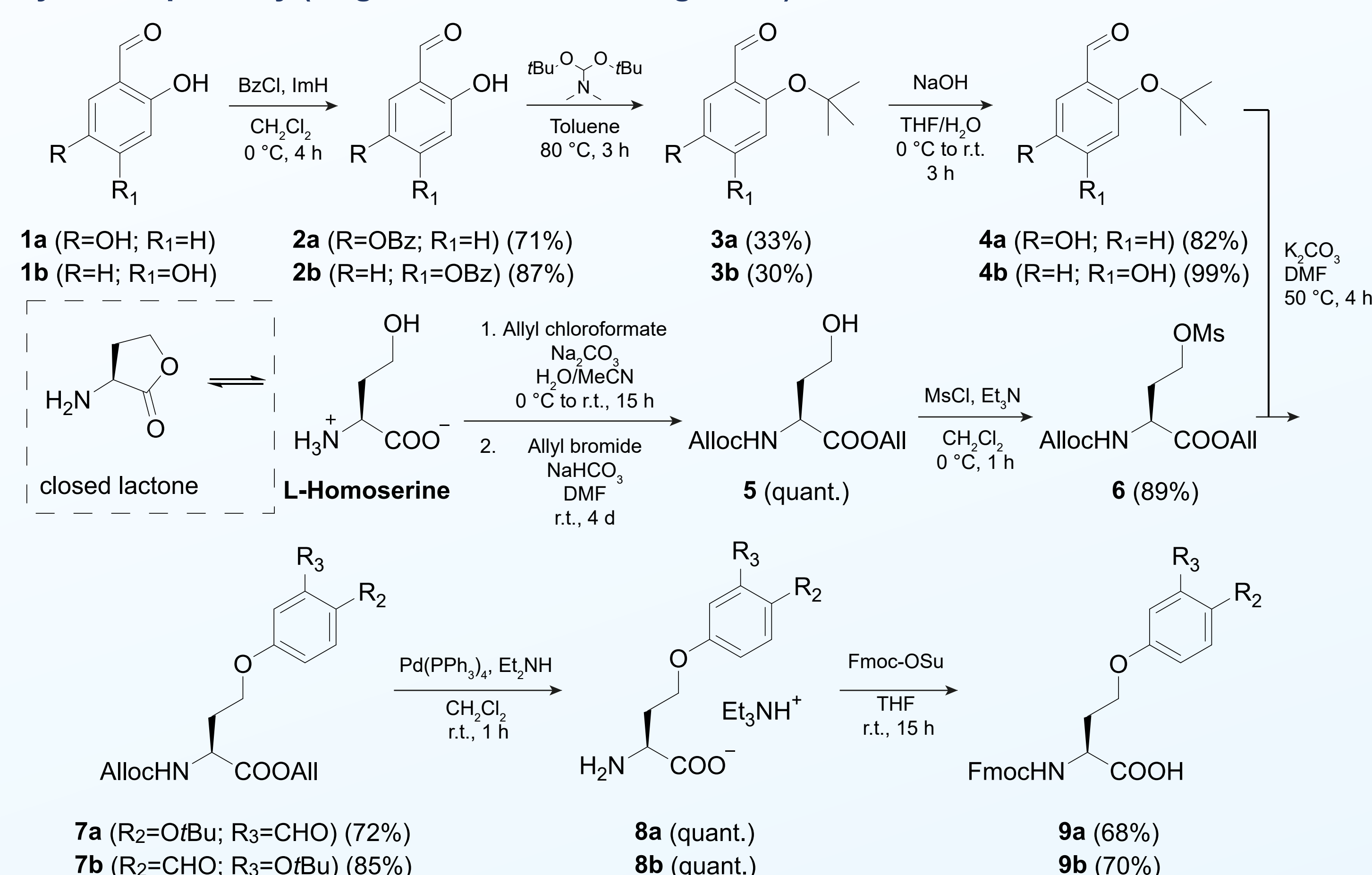
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**1.** Peptides are advancing as tools to inhibit pharmacological targets and protein interactions, leveraging recent progress in synthesis and screening. Modern peptides offer the benefits of both biologic drugs (like specificity and modularity) and small molecules (targeting inside cells and potential oral availability), promising future medical applications.

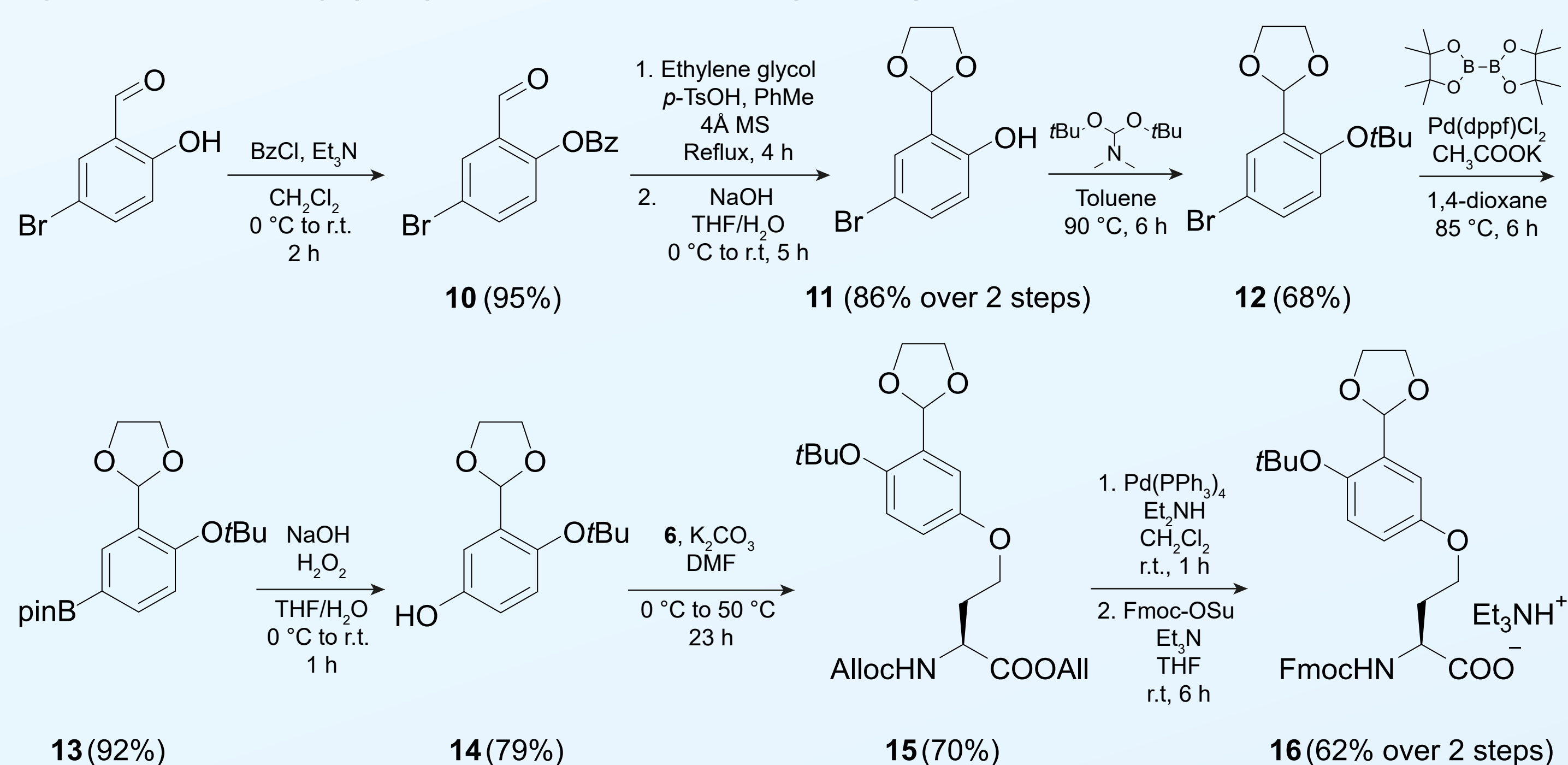
To enhance pharmacological effects, peptides can be derivatized with electrophilic groups that form covalent bonds with target proteins, stabilizing the interaction. For instance, salicylaldehyde (SA) derivatives can form imine bonds with primary amines in aqueous media, stabilized by an intramolecular H-bond between the imine N atom and the proximal phenolic proton. The SA tag has been recently installed as aminophilic warhead in multiple classes of protein targets to target lysine (Lys) residues on proteins.<sup>[1]</sup> Our research group has explored its integration into peptides, such as at the N or C termini of model peptides, to effectively engage Lys residues without disrupting peptide-protein binding.<sup>[2]</sup>

## Synthetic pathway (1<sup>st</sup> generation SA-bearing ncAA)



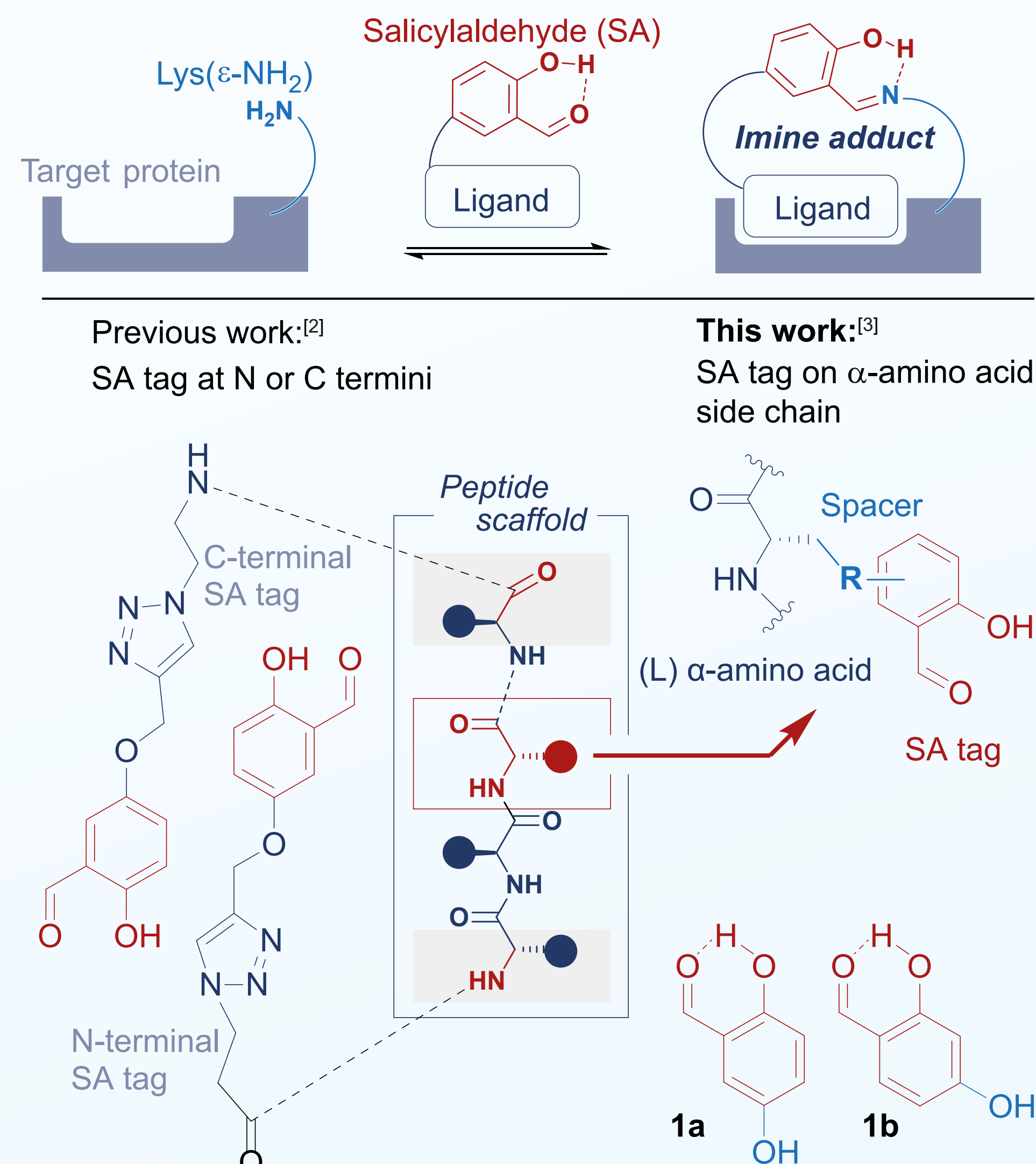
**3.** Unfortunately, this protection strategy proved ineffective during SPPS protocols, as the free aldehyde group interfered with peptide formation on solid phase. Consequently, we developed what we refer to as the 2<sup>nd</sup> generation SA-bearing ncAA, specifically designed for SPPS applications. In this new structure, the aldehyde group is also protected with an acid-labile group (acetal), ideally allowing simultaneous removal of protecting groups at both SA's phenol and aldehyde units following peptide treatment with acids.

## Synthetic pathway (2<sup>nd</sup> generation SA-bearing ncAA)

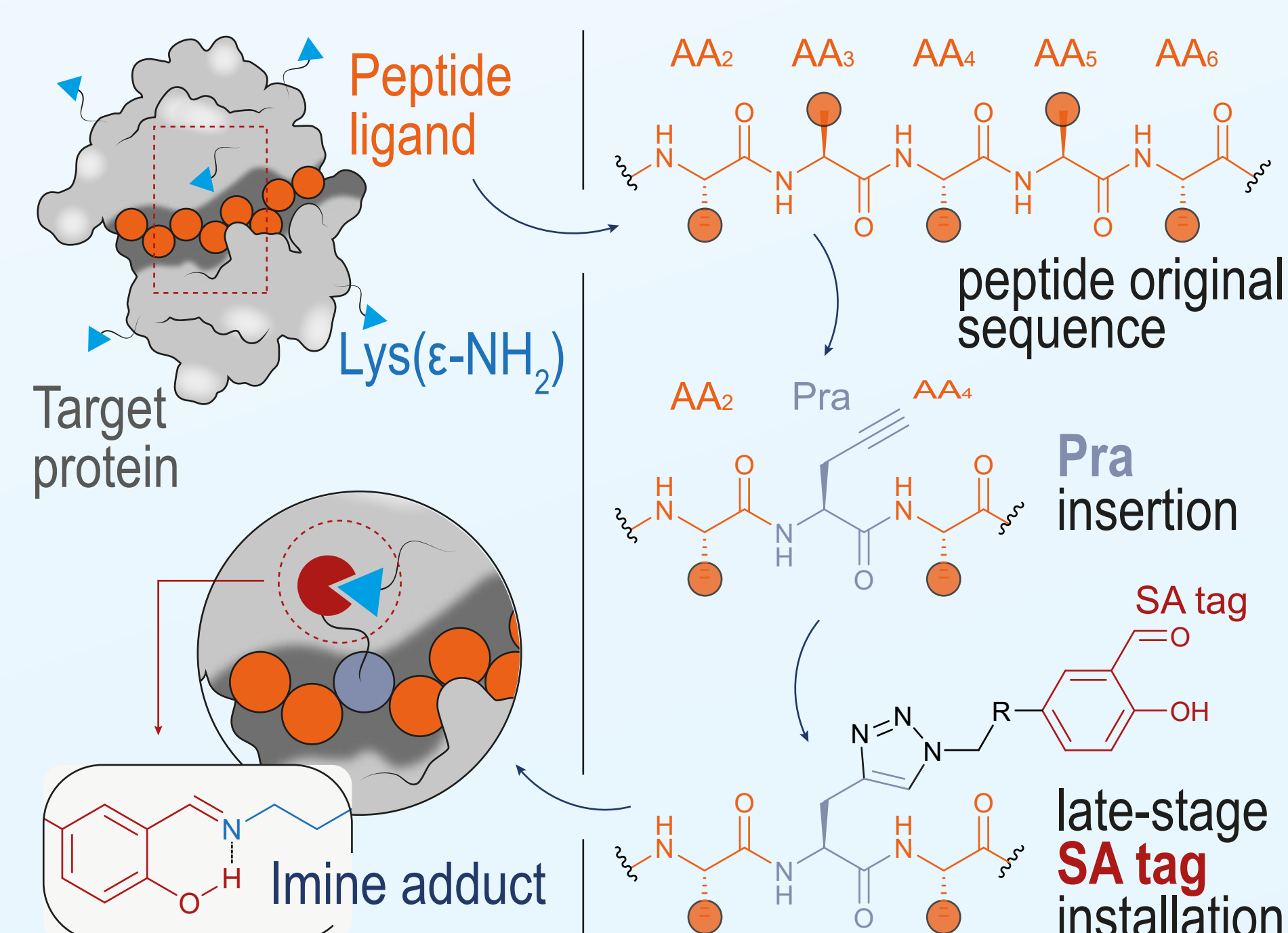


## References:

- For recent reviews on the use of aldehydes in drug discovery, see: a) M. Mason, L. Belvisi, L. Pignataro, A. Dal Corso, *ChemBioChem* **2023**, 25, e202300743; b) C. Gampe, V. A. Verma, *J. Med. Chem.* **2020**, 63, 14357-14381.
- G. Sacco, D. Arosio, M. Paolillo, A. Gloger, J. Scheuermann, L. Pignataro, L. Belvisi, A. Dal Corso, C. Gennari, *Chem. Eur. J.* **2023**, e202203768.
- M. Mason, B. Nava, L. Belvisi, L. Pignataro, A. Dal Corso, *Eur. J. Org. Chem.* **2024**, 27, e202400229.
- M. Mason, K. Pegini, F. Uggeri, D. Rondelli, S. Sattin, L. Pignataro, S. Pellegrino, L. Belvisi, E. Scarpa, R. Bucci, A. Dal Corso, *Manuscript submitted*.



**2.** We describe herein a synthetic approach to synthesize a new non-canonical amino acid (ncAA) bearing a SA tag, which can be installed at suitable positions of peptide sequences to favor reversible-covalent (RC) interactions with the target protein.<sup>[3]</sup>



**4.** In order to reduce synthetic efforts we looked at a more practical and versatile design consisting in the late-stage peptide functionalization with the SA tag. Copper-catalysed azide-alkyne cycloaddition (CuAAC) proved a robust approach to connect the azido group in biomolecules to alkyne-bearing SA derivatives, devoid of protecting groups on the aldehyde and phenol units. Inspired by this, we designed a strategy for the synthesis of RC peptide binders through tailored insertion of a propargylglycine residue (Pra) at suitable positions of the peptide original sequence, followed by installation of the SA tag through CuAAC.<sup>[4]</sup>

**5.** In conclusion, we developed a reliable synthetic method to obtain ncAAs with a unique side chain containing the SA tag and demonstrated its practicality for generating RC peptides in solution.<sup>[3]</sup> We also designed a new CuAAC approach for the late-stage SA installation.<sup>[4]</sup> This work establishes a foundation for future innovations in SA-bearing amino acids, expanding the toolkit of aldehyde building blocks to enhance RC peptide capabilities.