SYNTHESIS OF NON-CODED AMINO ACIDS WITH A SALICYLALDEHYDE TAG FOR REVERSIBLE-COVALENT PEPTIDES

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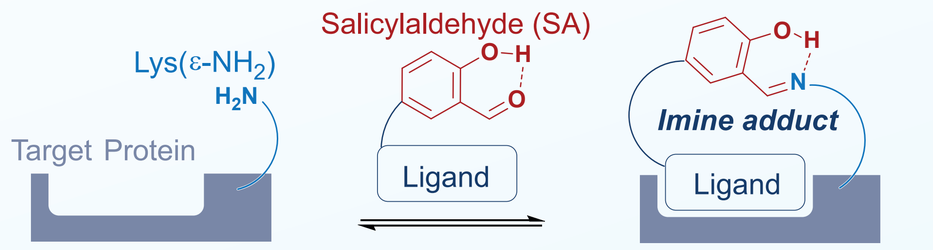
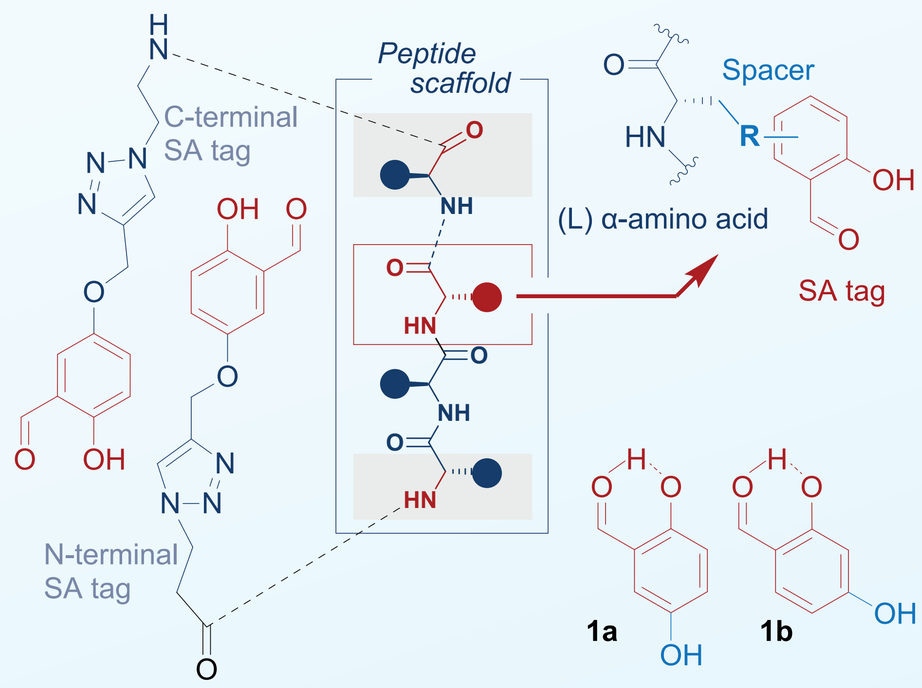
1. Peptides are advancing as tools to inhibit pharmacological targets and protein interactions, leveraging recent progress in synthesis and screening. Modern peptides offer the benefits of both biologic drugs (like specificity and modularity) and small molecules (targeting inside cells and potential 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.[1] 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.[2]

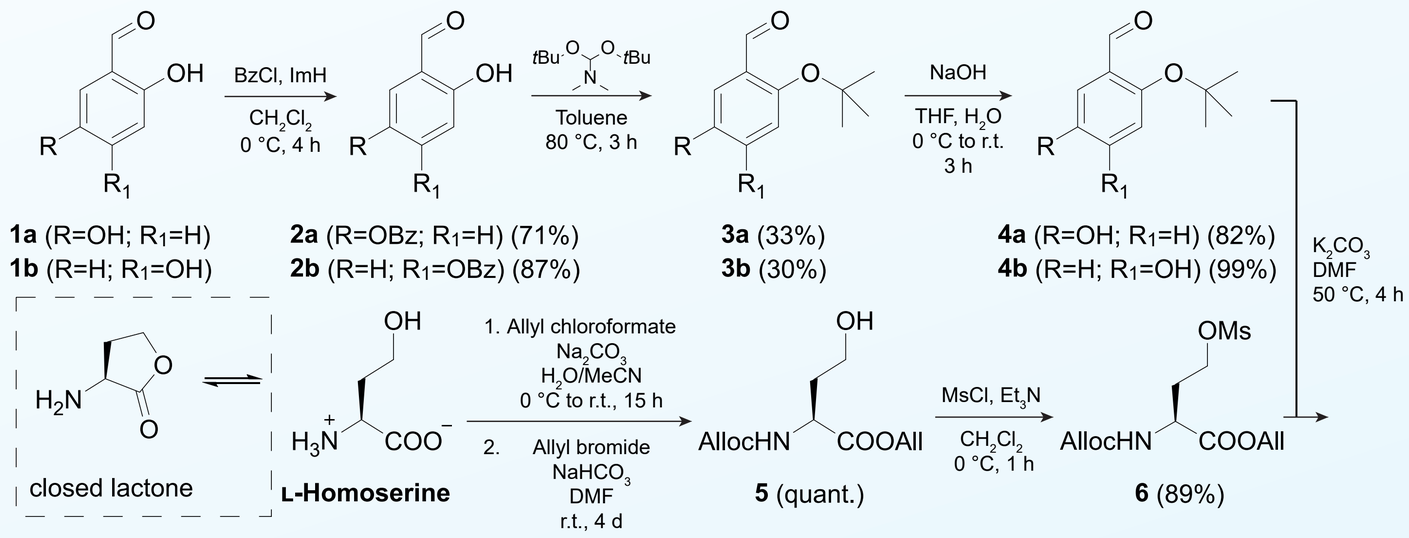


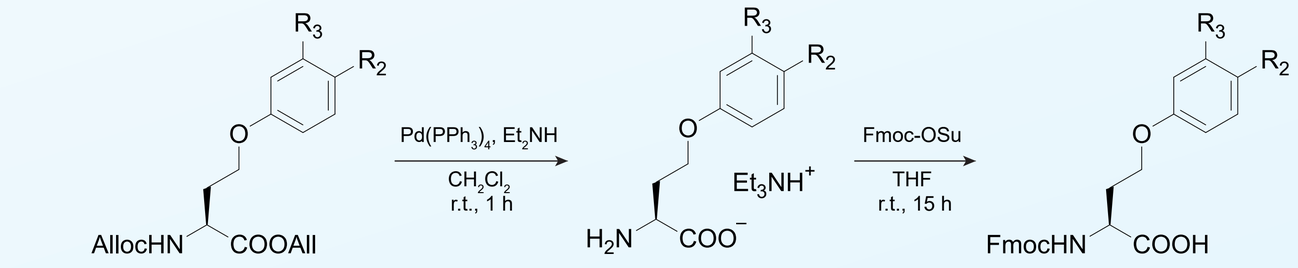
Previous Work:[2]
SA tag at N or C termini

This Work:[3]
SA tag on α-amino acid side chain

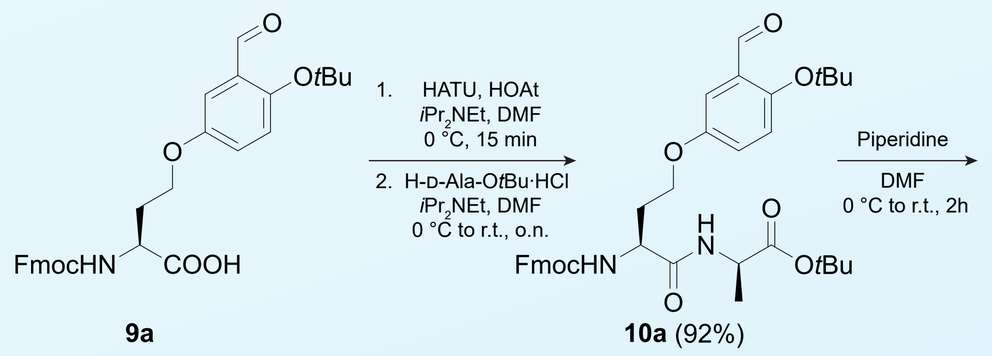


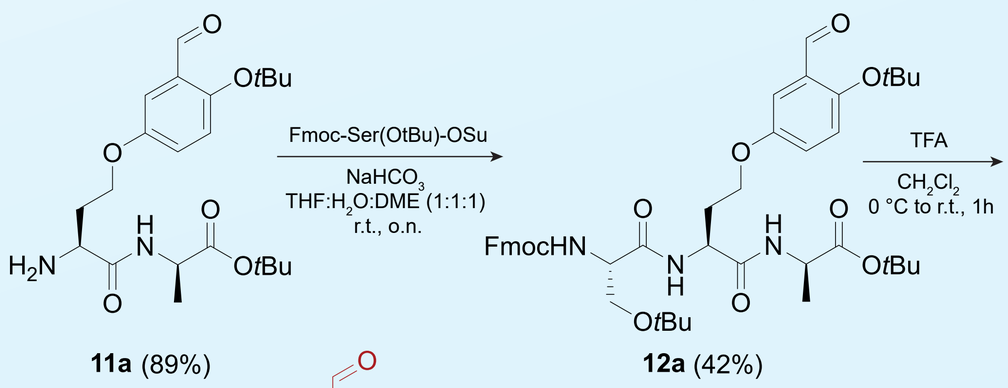
Synthetic pathway (1st generation SA-bearing ncAA)

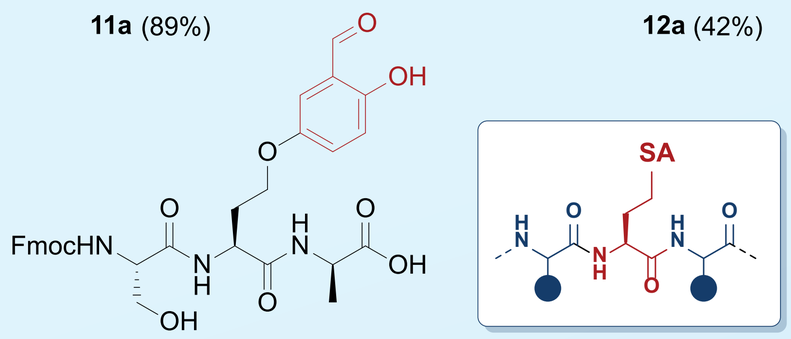




2. We describe herein a synthetic approach to synthetize a new **non-canonical amino acid** (ncAA) bearing a SA-tag, which can be installed at suitable positions of peptide sequences to favor reversible-covalent interactions with the target protein.[3]

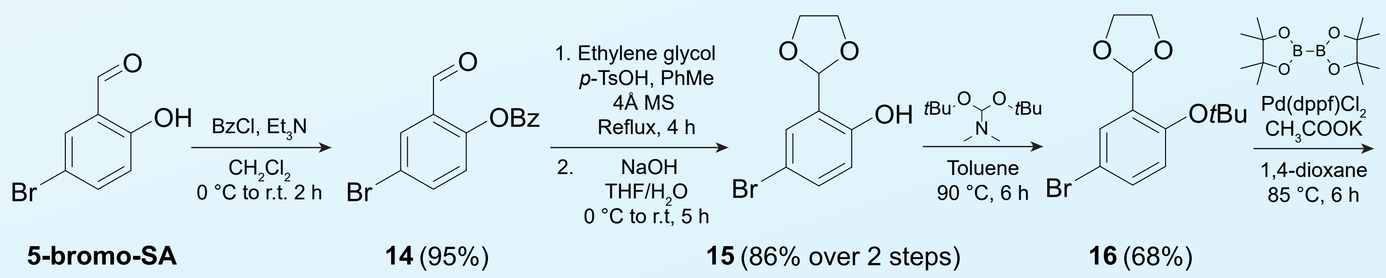


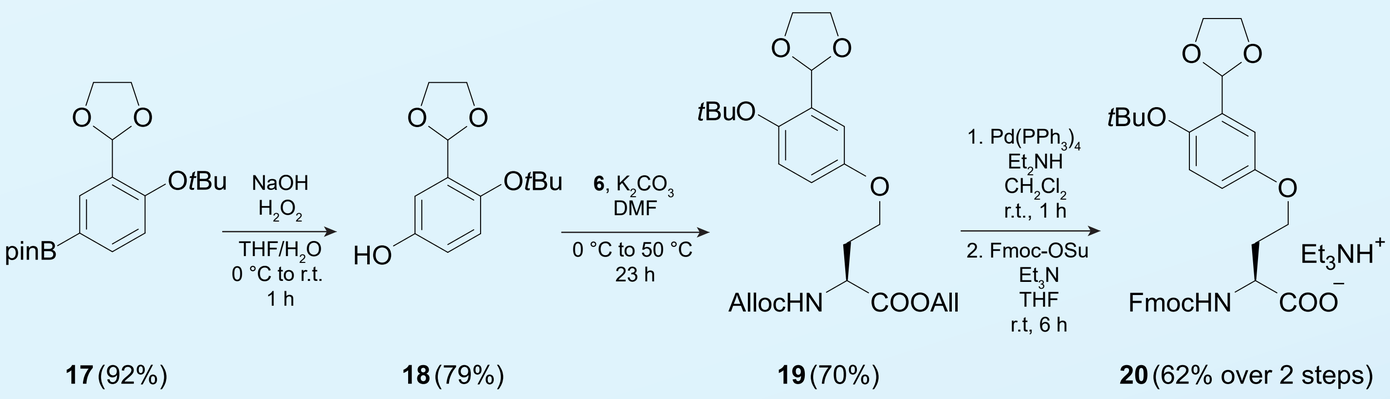




3. Unfortunately, this protection strategy proved ineffective during SPPS protocols, as the free aldehyde group interfered with peptide formation on solid phase. Consequently, we developed what we refer to as the 2nd generation SA-bearing ncAA, specifically designed for SPPS applications. In this new 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 (2nd generation SA-bearing ncAA)





4. In conclusion, we have developed a reliable synthetic method to obtain ncAAs with a unique side chain containing the SA tag and demonstrated its practicality for generating **reversible covalent** (RC) **peptides** in solution.[3] This work provides the basis for future innovations in SA-bearing AAs, expanding the toolkit of aldehyde building blocks to enhance RC peptide capabilities.

References:

1. For recent reviews on the use of aldehydes in drug discovery, see: a) M. Mason, L. Belvisi, L. Pignataro, A. Dal Corso, *ChemBioChem* **2023**, e202300743; b) C. Gampe, V. A. Verma, *J. Med. Chem.* **2020**, *63*, 14357-14381.
2. G. Sacco, S. Stammwitz, L. Belvisi, L. Pignataro, A. Dal Corso, C. Gennari, *Eur. J. Org. Chem.* **2021**, *2021*, 1763.
3. M. Mason, B. Nava, L. Belvisi, L. Pignataro, A. Dal Corso, *Eur. J. Org. Chem.* **2024**, *27*, e202400229.