Green Synthesis of a Novel Primary Amine Protecting Group for Solid-phase Peptide Synthesis

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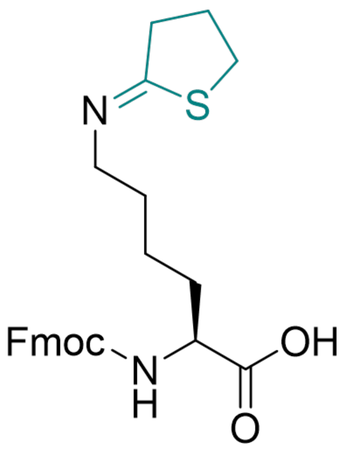
INTRODUCTION

Orthogonal protecting groups are vital in the chemo-selective modification of biologically active peptides and proteins, especially in solid-phase peptide synthesis (SPPS). Existing protecting groups for the temporary protection of the reactive ε-amino group of lysine sidechain, such as Alloc, mtt, iNoc, Dde, and ivDde, often require harsh conditions for their removal and involve complex synthesis with suboptimal yields.

Our new protecting group (**Dty**) offers a more efficient and environmentally friendly alternative for the temporary masking of reactive side-chain functionalities in amino acids, particularly lysine's ε-amino group (sidechain). We present a novel primary amine protecting group, developed via a one-pot, two-step reaction in water using a known cross-linking agent. This method offers high yields, good atom economy, and minimal waste, addressing the need for greener synthetic pathways.

Dty protecting group



- Water-based synthesis
- Small and polar group
- No amino scrambling
- Gram-scale synthesis
- Orthogonal to Fmoc

SYNTHESIS

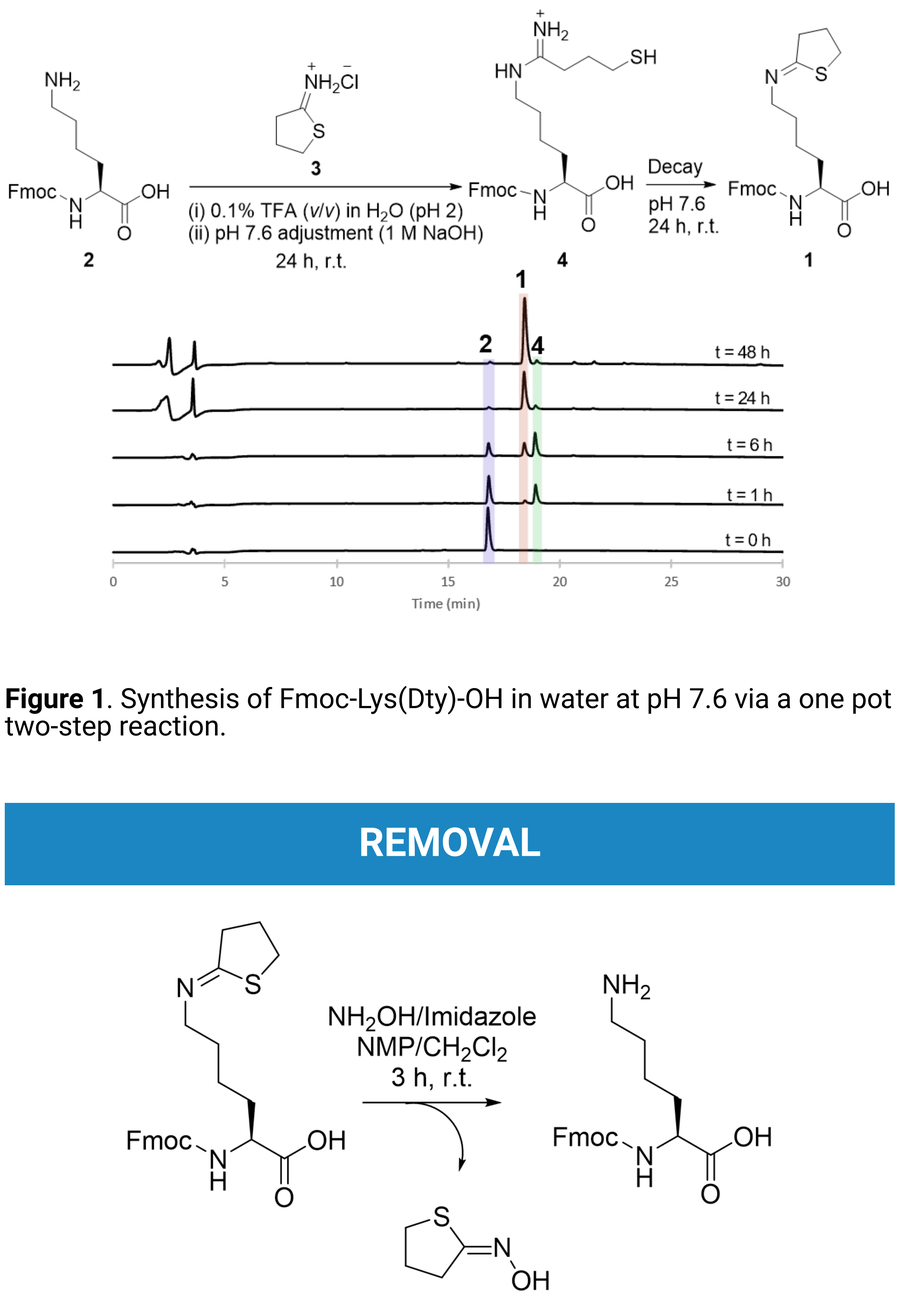


Figure 1. Synthesis of Fmoc-Lys(Dty)-OH in water at pH 7.6 via a one pot two-step reaction.

REMOVAL

Figure 2. Removal of the Dty protecting group using a mixture of NH_2OH and imidazole.

APPLICATIONS IN SPPS

Scenario 1: Compared to Dde, the Dty group does not exhibit intramolecular migration between ε-amino groups of lysine side-chains.

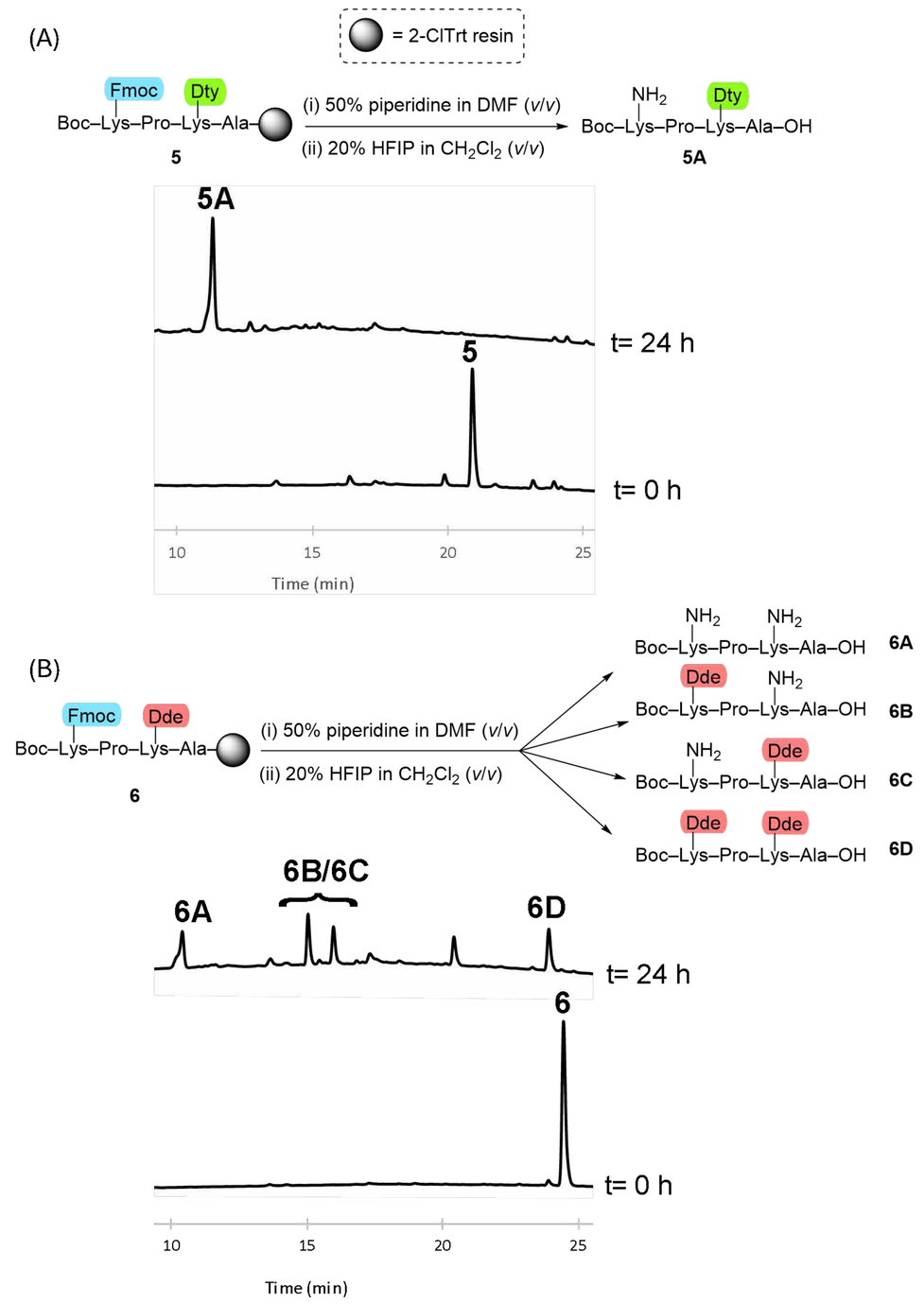


Figure 3. **(A)** Testing Dty migration after removal of Fmoc from a neighbouring lysine sidechain using 50% piperidine in DMF (*v*/*v*) for 24 h. **(B)**Testing the migration of Dde after removal of Fmoc from a neighbouring lysine sidechain using 50% piperidine in DMF (*v*/*v*) for 24 h

Scenario 2: Selective removal of the Dty protecting group through the use of NH_2OH, allows for the site-selective modification of biologically relevant peptides.

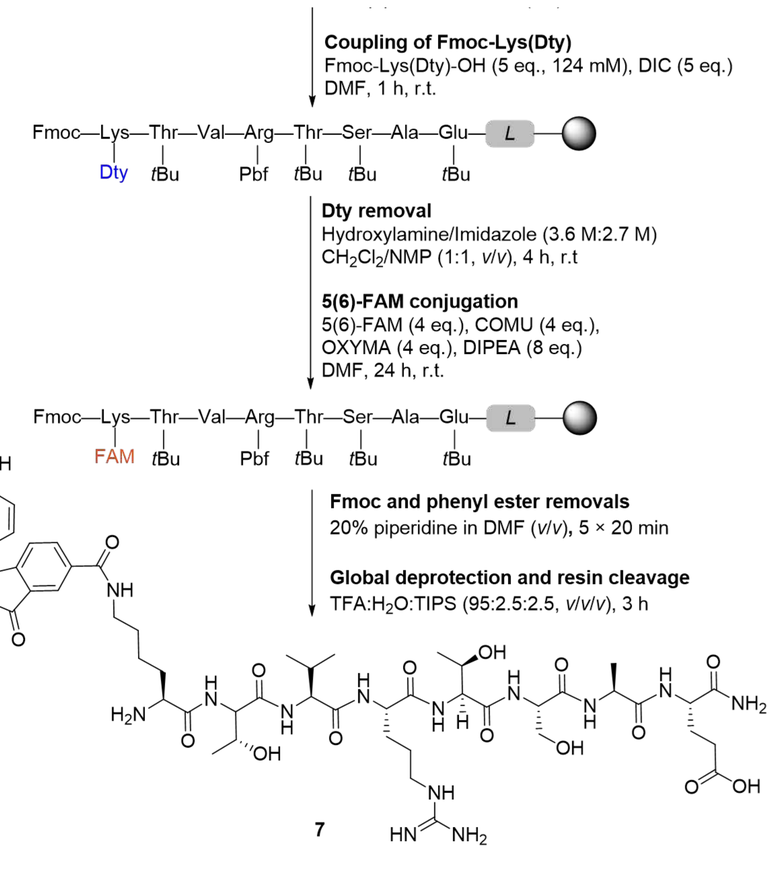
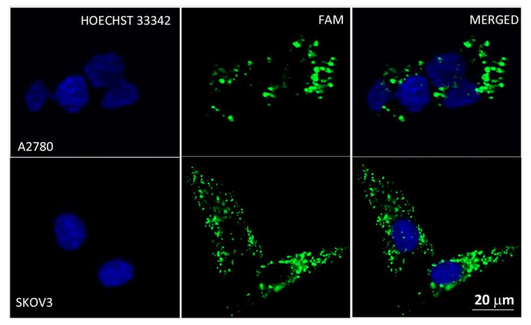
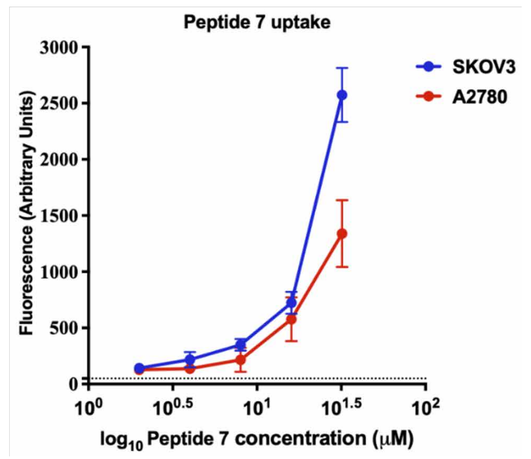


Figure 4. Synthesis and site-selective modification of folate binding peptide TVRTSAE. Dty protected lysine is coupled at the *N*-terminus and the Dty protecting group is selectively removed and was subsequently conjugated with a fluorophore, 5(6)-FAM.

BIOLOGICAL RESULTS





CONCLUSION

In summary, we herein introduce the Dty amino protecting group and demonstrate its suitability for conducting Fmoc SPPS. Our findings indicate that Dty meets the criteria as an ideal lysine ε-amino protecting group, displaying excellent compatibility with standard peptide synthesis procedures and efficient removal under mild nucleophilic conditions.

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