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A Rapid and Efficient Photochemical Modification Strategy for Late-Stage Peptide and Protein Functionalization

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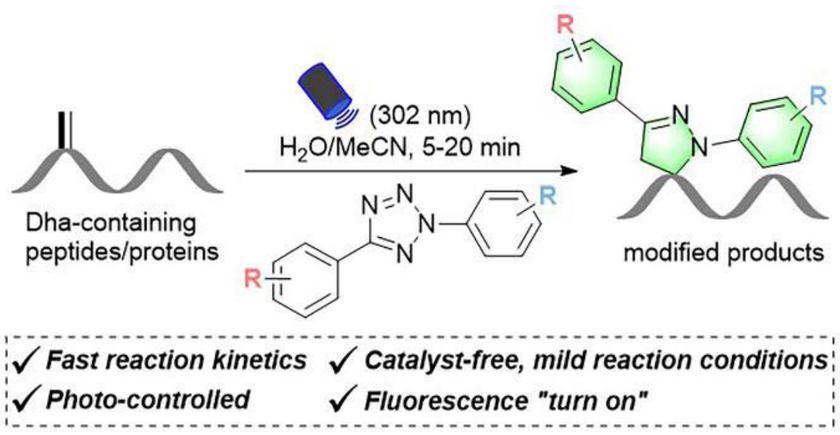
Abstract

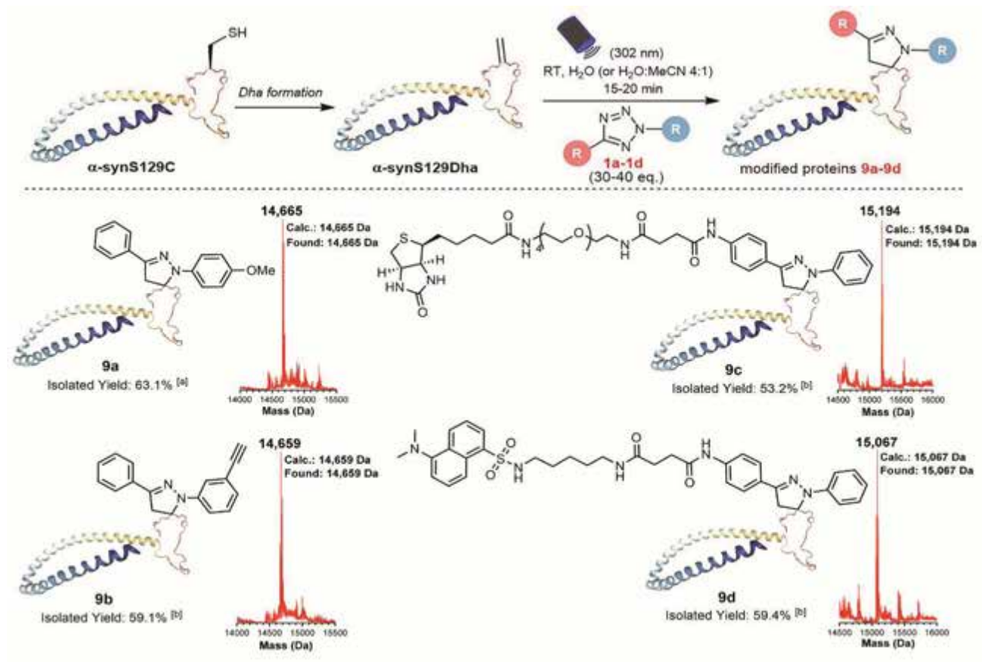
As an easily introduced noncoded amino acid with unique electrophilicity distinct from 20 natural amino acids, dehydroalanine (Dha) is a promising multifunctional labeling site for peptides and proteins. However, achieving a balance between reaction rate and mild reaction conditions has been a major challenge in developing novel Dha-modification strategies. Rapid, efficient, and mild Dha modification strategies are highly desired. Here, we present a photoinitiated 1,3-dipolar cycloaddition reaction between Dha and 2,5-diaryl tetrazoles. Under low-power UV irradiation, this reaction completes rapidly without catalysis, yielding a fluorescent pyrazoline-modified peptide or protein with exceptional Dha residue chemoselectivity. This reaction demonstrates complete site-specificity in thiostrepton modification within 20 minutes, making it the fastest Dha16-specific reaction in thiostrepton. Furthermore, this photoinitiated reaction provides a chemoselective strategy for the precise functionalization of proteins. Additionally, when combined with the fluorogenic property of the pyrazoline-modified product, this photo-controllable methodology can be applied to live cell imaging, further expanding the application scope of Dha modification techniques.

Photoinitiated 1,3-dipolar cycloaddition between dehydroalanines and tetrazoles



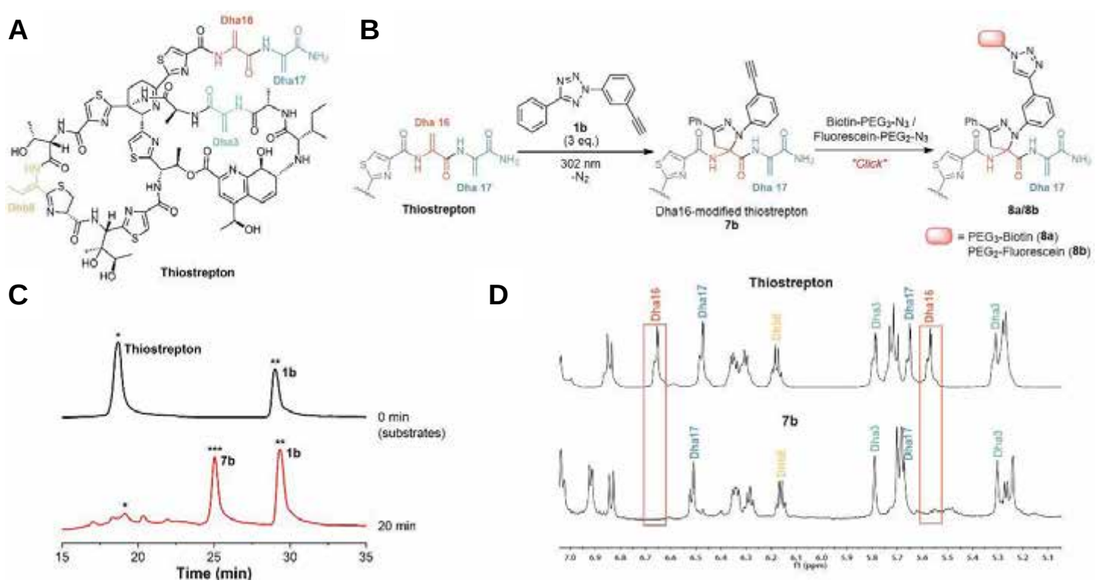
An efficient strategy for dehydroalanine cycloaddition modification was presented, which enables rapid generation of fluorescent pyrazoline-modified peptides and proteins under mild, non-catalytic reaction conditions.

Chemoselective labelling of *α*-synS129Dha



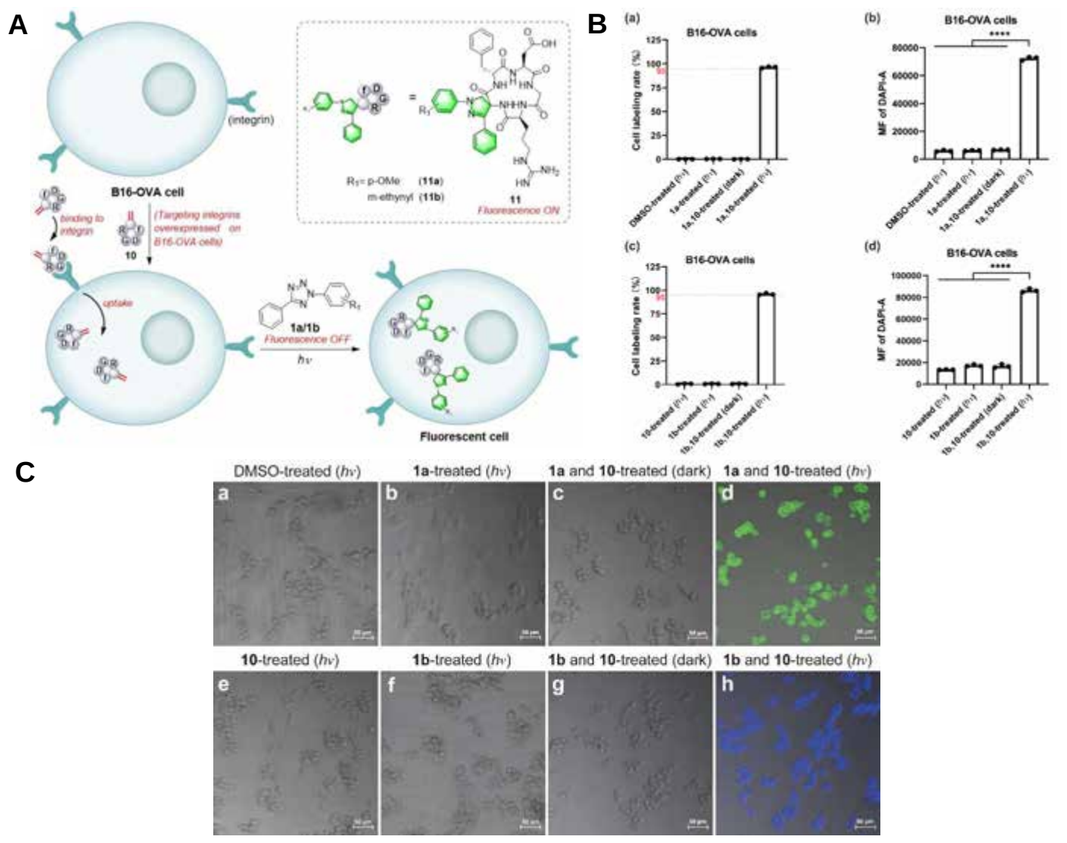
[a] The reaction was performed in H_2O. [b] The reactions were conducted in the H_2O-MeCN mixed solvent (4:1, v/v).

Site-selective labelling of thiostrepton



A) Chemical structure of thiostrepton. B) Two-step reaction for site-specific labelling of Dha16 on thiostrepton. C) HPLC analysis of the reaction mixture of thiostrepton and tetrazole **1b** at 0 min (top) and 20 min (bottom). D) ^{1}H NMR spectra of thiostrepton and Dha16-labelling product **7b**. Chemical shifts of signals from the C(sp^2)-H of Dha3, Dha16, Dha17, and Dhb8 are assigned.

In-situ imaging of tumour cells



A) Schematic illustration. B) Flow cytometry analysis of B16-OVA cells. C) Merged images of DAPI and T-PMT channels of B16-OVA cells captured by confocal laser scanning microscope.

References

1. Zhang, M.-Q.; He, P.-Y.; Li, Y.-M., Light-initiated 1,3-dipolar cycloaddition between dehydroalanines and tetrazoles: application to late-stage peptide and protein modifications, *Chem. Sci.*, **2023**, *14*, 9418-9426.