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Cu(II)-Catalysed β-silylation of dehydroalanine residues in peptides and proteins†

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We report the efficient and selective Cu(II)-catalysed β-silylation of naturally occurring dehydroalanine (Dha) residues in various ribosomally synthesized and post-translationally modified peptides (RiPPs). The method is also applicable to proteins, as was shown by the modification of a Dha residue that was chemically introduced into Small Ubiquitin-like Modifier (SUMO).

The introduction of silylated amino acids into biologically active peptides is an attractive approach in medicinal chemistry to overcome some of the poor pharmacological properties associated with such substances.[1] The substitution of carbon residues for silicon isosteres hampers the recognition by proteolytic enzymes and thus improves resistance against degradation.[2–5] Additionally, silyl groups greatly increase the lipophilicity of peptides, which can be an important factor for enhancing cellular uptake.[6]

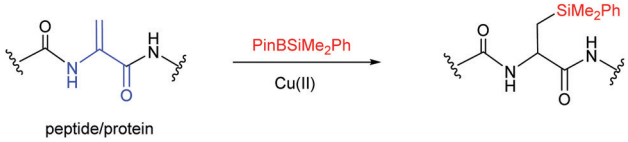
Methods for the silylation of amino acids and peptides have shown to be effective, yet often require harsh conditions or multiple synthetic steps.[7–11] Currently, a mild approach that is suitable for the chemical incorporation of silyl groups onto large peptides and proteins is lacking despite the growing popularity of such compounds in medicine.[12] Here we report the rapid and selective Cu(II)-catalysed β-silylation of dehydroalanine (Dha) residues in peptides and proteins (Scheme 1).

Dehydroalanines are α,β-unsaturated amino acids[13] which occur naturally in ribosomally synthesized and post-translationally modified peptides (RiPPs)[14] and can also be introduced chemically into other peptides and proteins.[15] They are uniquely reactive as electrophiles, which makes them attractive chemical handles for late-stage modification of such complex natural products.[16–27] Cu(II)-Catalyzed β-silyl conjugate additions to α,β-unsaturated carbonyl substrates using $PinBSiMe_2Ph$ (also known as Suginome's reagent) have been performed at room temperature, in aqueous media and open to the air.[28,29] Therefore, we envisioned this method to be an excellent starting point for exploring the silylation of Dha in peptides and proteins.

First, the β-silylation of Dha acceptors in the thiopeptide thiostrepton was investigated (Fig. 1A). Due to the poor water solubility of thiostrepton 2,2,2-trifluoroethanol (TFE) was used as a co-solvent in our initial screening conditions. Using 10 equivalents of $PinBSiMe_2Ph$ and 1 mol% $CuSO_4$, >95% conversion of thiostrepton was achieved within 1 hour, which was accompanied by the rapid formation of the singly and doubly silylated peptide as detected with LC-MS (Fig. 1B). Splitting of the peaks of both the singly and doubly silylated peptide indicated a mixture of diastereomers (Fig. 1B). In contrast to previous studies,[29] efficient silylation was achieved without the use of 4-picoline as a Brønsted base. Since the base was not required for the reaction and the presence of amine bases is known to be detrimental for the stability of thiostrepton in solution,[30] the base was omitted.

To demonstrate the chemo- and site-selectivity of this approach for the different Dha residues within thiostrepton, the doubly modified products (**2a** and **2b**) were isolated using preparative HPLC (SI-4, ESI†) and characterized *via* HRMS (SI-5, ESI†) and 2D NMR spectroscopy (SI-6–8, ESI†). It was found that the two terminal Dha residues in the tail region of thiostrepton (Dha16 and Dha17) were modified. Unfortunately, the different diastereomers could not be assigned by 1D and 2D NMR. Finally, the signals of Dha3 and Dhb8 were conserved (Fig. 1C, see SI-6–8, ESI† for a detailed explanation), meaning that our method is not only very fast, but also highly selective.

Next, the scope of the reaction on different Dha-containing peptides was studied, starting with the thiopeptide nosiheptide



Scheme 1 Cu(II)-Catalysed β-silylation of Dha (blue) in peptides and proteins using Suginome's reagent ($PinBSiMe_2Ph$).

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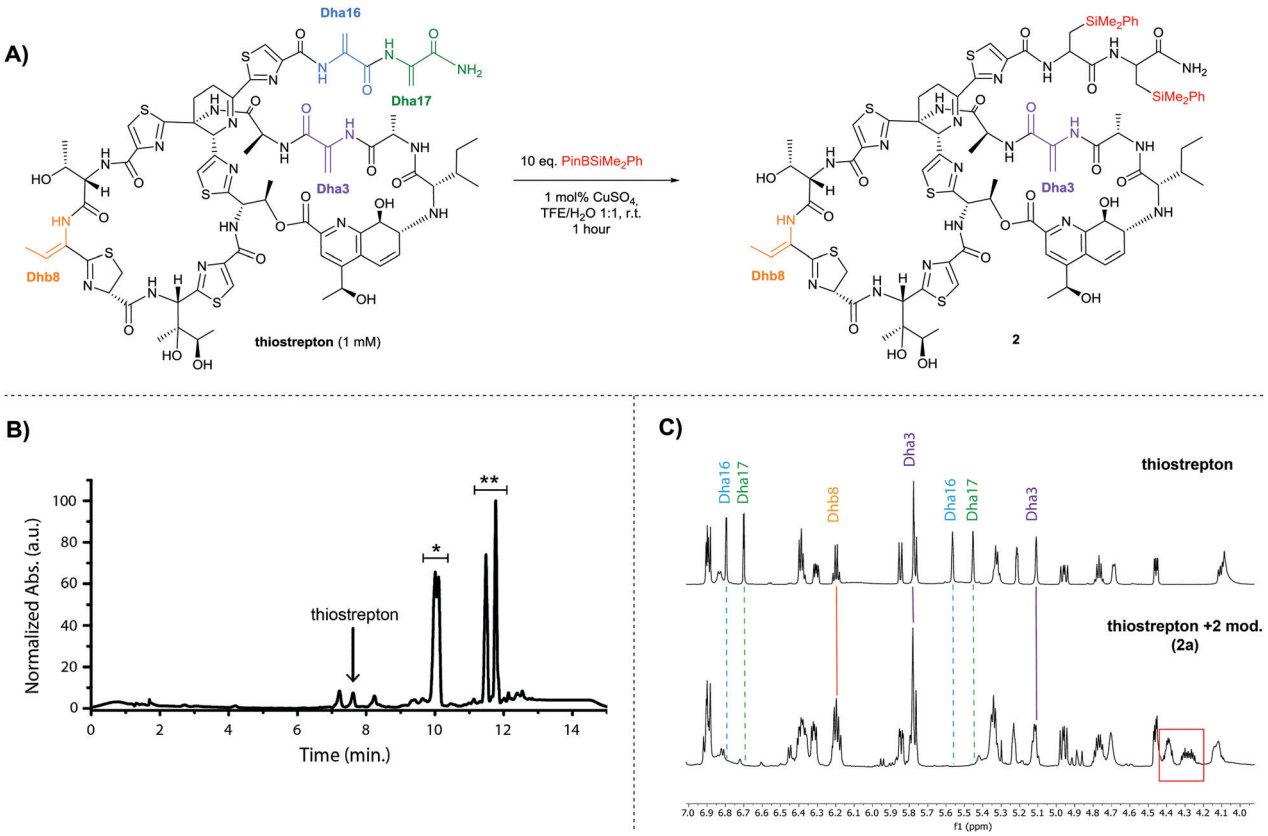

Fig. 1 (A) β-Silylation of thiostrepton. (B) LC-MS UV (280 nm) chromatogram of the crude reaction mixture after 1 hour (* single silylation, ** double silylation). (C) Stacked ^{1}H NMR spectra of thiostrepton and **2a**.

(Fig. 2A and SI-9, ESI†). Similar conditions were used, except a higher catalyst loading (2 eq.) and addition of 10 equivalents 4-picoline were necessary to achieve efficient silylation. In the analysis of the crude product *via* LC-MS 80% conversion of nosiheptide to its singly silylated derivative was detected after 1 hour of reaction time (Fig. 2B). Modification of nosiheptide that lacks the terminal Dha, which is present as an impurity in the commercially available nosiheptide, as well as any double modification were not observed. This indicates that, similar to thiostrepton, the reaction is completely selective for the terminal Dha over the internal Dhb.

Nisin Z, a member of the lanthipeptide family of RiPPs, was also investigated as a substrate (Fig. 3A and SI-10, ESI†). In contrast to thiopeptides, nisin Z has a high aqueous solubility, avoiding the need for TFE as a co-solvent and allowing us to conduct the silylation in pure water. It was found that also here the addition of 4-picoline was necessary to achieve efficient silylation. After 1 hour of reaction at room temperature the mixture was analyzed by LC-MS and the mass spectrum of the crude product containing the nisin species was deconvoluted (SI-10, ESI†). A small amount of nisin Z was found, as well as singly and doubly silylated nisin Z products (including the known degradation products of nisin due to water addition (+H_2O) and cleavage of the two C-terminal amino acids (–CTerm.))[31] (Fig. 3B). In contrast to thiostrepton, in which Dha3 is buried in a macrocycle and Dhb8 does not have carbonyl substituent,

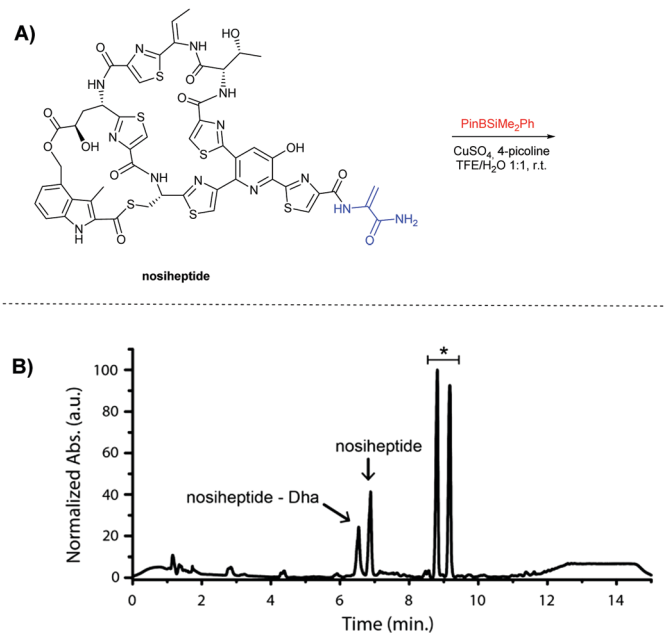

Fig. 2 (A) β-Silylation of nosiheptide. (B) LC-MS UV (280 nm) of the crude reaction mixture after 1 hour (* single silylation).

the dehydroamino acids in nisin Z are all reactive and accessible resulting in a mixture of regioisomers that could not be resolved using UPLC-MS.

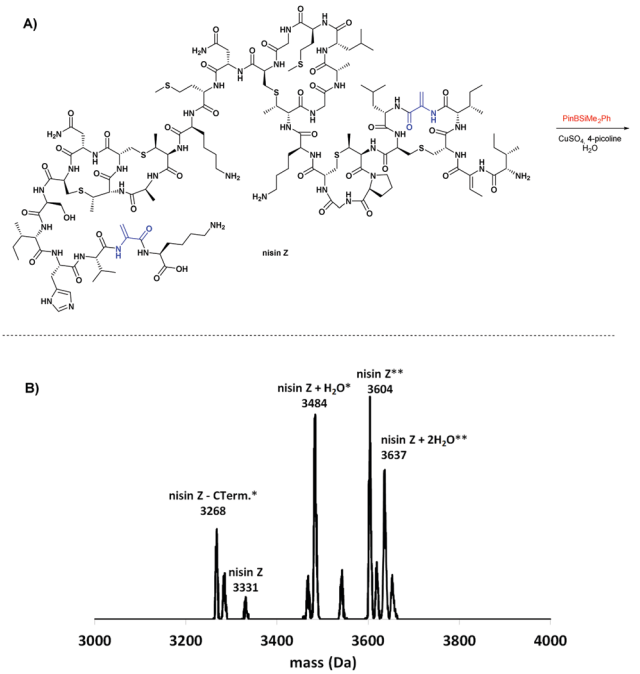

Fig. 3 (A) β-Silylation of the lanthipeptide nisin Z. (B) Deconvolution result of the raw mass spectrum of the crude reaction mixture after 1 hour (* single silylation, ** double silylation).

The scope of the reaction was extended to proteins. For this purpose, we performed the reaction on the 12.5 kDa protein Small Ubiquitin-like Modifier (SUMO). SUMO_G98Dha, a mutant with a Dha that was chemically introduced near the C-terminus, was subjected to the silylation conditions (Fig. 4A) and after 1 hour at room temperature the reaction mixture was analysed by LC-MS (SI-11, ESI†). Upon deconvolution of the mass spectra the singly silylated protein was observed, together with a small amount of unreacted SUMO_G98Dha (Fig. 4B).

In conclusion, the Cu(II)-catalysed β-silylation of Dha residues is a straightforward, fast and selective method for the

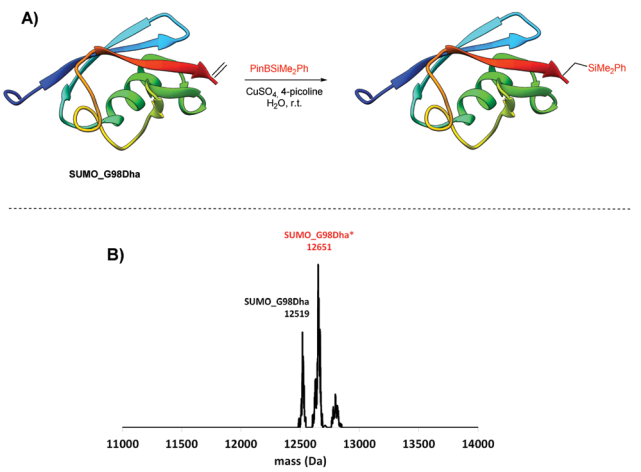

Fig. 4 (A) Silylation of SUMO_G98Dha. (B) Deconvolution result of the raw mass spectrum of the crude reaction mixture after 1 hour (* single silylation, small peak at 12 796 Da is an unidentified minor byproduct).

silylation of bioactive peptides and proteins. The reaction is robust and efficient in aqueous media both with and without added co-solvent, enabling the silylation of a variety of natural products. Moreover, the modification of chemically incorporated Dha residues demonstrates that the method is generally applicable in the silylation of peptides and proteins.

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Conflicts of interest

There are no conflicts to declare.

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