This article can be cited before page numbers have been issued, to do this please use: R. de Vries and G. Roelfes, Chem. Commun., 2020, DOI: 10.1039/D0CC05026A.

This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
COMMUNICATION

Received 00th January 20xx, Accepted 00th January 20xx
DOI: 10.1039/x0xx00000x

Cu(II)-Catalysed β-Silylation of Dehydroalanine Residues in Peptides and Proteins

Reinder H. de Vries* and Gerard Roelfes*

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 acids13 which occur naturally in ribosomally synthesized and post-translationally modified peptides (RiPPs).14 They are uniquely reactive as electrophiles, which makes them attractive chemical handles for late-stage modification of such complex natural products.15-27 Cu(II)-catalyzed β-silyl conjugate additions to α,β-unsaturated carbonyl substrates using PinBSiMe₂Ph (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 thiopptide thiostrepton was investigated (Figure 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 PinBSiMe₂Ph and 1 mol% CuSO₄ · 5H₂O, >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 (Figure 1B). Splitting of the peaks of both the singly and doubly silylated peptide indicated a mixture of diastereomers (Figure 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 solution30, the base was omitted.

To demonstrate the chemoselectivity of this approach for the different Dha residues within thiostrepton, the doubly modified products (2a and 2b) were isolated using preparative HPLC (SI-4) and characterized via HRMS (SI-5) and 2D NMR spectroscopy (SI-6-8). 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 Dha8 were conserved (Figure 1C, see SI-6-8 for a detailed explanation), meaning that our method is not only very fast, but also highly selective.

Scheme 1. Cu(II)-catalysed β-silylation of Dha (blue) in peptides and proteins using Suginome’s Reagent (PinBSiMe₂Ph).

---

*Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands. Email: J.G.Roelfes@rug.nl

Electronic Supplementary Information (ESI) available: [Detailed experimental procedures and data analysis]. See DOI: 10.1039/x0xx00000x
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 (Figure 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 (Figure 3A, SI-10). 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). 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 (+H2O) and cleavage of the two C-terminal amino acids (-CTerm.))31 (Figure 3B). In contrast to thiostrepton, in which Dha3 is buried in a macrocycle and Dhb8 does not have carbonyl substituent, 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.

Next, the scope of the reaction on different Dha-containing peptides was studied, starting with the thiopeptide nosiheptide (Figure 2A, SI-9). Similar conditions were used, except a higher catalyst loading (2 eq.) and addition of 10 equivalents 4-picoline were necessary to achieve efficient...
In conclusion, the Cu(II)-catalysed β-silylation of Dha residues is a straightforward, fast and selective method for the 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.

The authors wish to thank Roos van Lier for providing SUMO_G98Dha. This project was supported by the Netherlands Organisation for Scientific Research (NWO) (Vici grant 724.013.003). G.R. acknowledges support from the Ministry of Education Culture and Science (Gravitation programme no. 024.001.035).

Conflicts of interest
There are no conflicts to declare.

Notes and references

E. Rémond, C. Martin, J. Martinez and F. Cavelier, Chem. Rev., 2016, 116, 11654–11684.
2 B. Weidmann, Chimia, 1992, 46, 312–313.
3 R. Tacke, M. Merget, R. Bertermann, M. Bernd, T. Beckers and T. Reissmann, Organometallics, 2000, 19, 3486–3497.
4 F. Cavelier, D. Marchand, J. Martinez and S. Sagan, J. Pept. Res., 2004, 63, 290–296.
5 M. Mortensen, R. Husmann, E. Veri and C. Bolm, Chem. Soc. Rev., 2009, 38, 1002–1010.
6 S. Pujals, J. Fernández-Carneado, M. J. Kogan, J. Martinez, F. Cavelier and E. Giralt, J. Am. Chem. Soc., 2006, 128, 8479–8483.
7 G. K. Min, D. Hernández and T. Skrydstrup, Acc. Chem. Res., 2013, 46, 457–470.
8 F. Bartoccini, S. Bartolucci, S. Lucarini and G. Piersanti, European J. Org. Chem., 2015, 2015, 3352–3360.
9 J. Y. L. Chung, M. Shevlin, A. Klapars and M. Journet, J. Am. Chem. Soc., 2016, 138, 13859–13862.
10 B. B. Zhan, J. Fan, L. Jin and B. F. Shi, ACS Catal., 2019, 9, 3298–3303.
11 D. J. Newman and G. M. Cragg, J. Nat. Prod., 2016, 79, 629–661.
12 D. Siodlak, Amino Acids, 2015, 47, 1–17.
13 P. G. Arnison, M. J. Bibb, G. Bierbaum, A. A. Bowers, T. S. Bugni, G. Bulaj, J. A. Camarero, D. J. Campopiano, G. L. Challis, J. Clardy, P. D. Cotter, D. J. Craik, M. Dawson, E. Fischbach, J. S. Garavelli, U. Göransson, C. W. Gruber, D. H. Haft, T. K. Hem scheidt, C. Hertweck, C. Hill, A. R. Horswill, M. Jaspars, W. L. Kelly, J. P. Klinman, O. P. Kuipers, A. J. Link, W. Liu, M. A. Marahiel, D. A. Mitchell, G. N. Moll, B. S.
Moore, R. Müller, S. K. Nair, I. F. Nes, G. E. Norris, B. M.
Olivera, H. Onaka, M. L. Patchett, J. Piel, M. J. T. Reaney, S.
Rebuffat, R. P. Ross, H. G. Sahli, E. W. Schmidt, M. E.
Selsted, K. Severinov, B. Shen, K. Sivonen, L. Smith, T. Stein,
R. D. Süßmuth, J. R. Tagg, G. L. Tang, A. W. Truman, J. C.
Vederas, C. T. Walsh, J. D. Walton, S. C. Wenzel, J. M.
Wille and W. A. Van Der Donk, Nat. Prod. Rep., 2013, 30,
108–160.

15 J. M. Chalker, S. B. Gunnoo, O. Boutureira, S. C.
Gerstberger, M. Fernández-González, G. J. L. Bernardes, L.
Griffin, H. Hallu, C. J. Schofield and B. G. Davis, Chem. Sci.,
2011, 2, 1666–1676.

16 J. Dadová, S. R. Galan and B. G. Davis, Curr. Opin. Chem.
Biol., 2018, 46, 71–81.

17 K. Maruyama and M. Kanai, Chem. Lett., 2019, 48, 1421–
1432.

18 J.-A. Shin, J. Kim, H. Lee, S. Ha and H.-Y. Lee, J. Org. Chem.,
2019, 84, 4558–4565.

19 R. J. Scamp, E. deRamon, E. K. Paulson, S. J. Miller and J. A.
Ellman, Angew. Chem. Int. Ed., 2020, 59, 890–895.

20 E. A. Hoyt, P. M. S. D. Cal, B. L. Oliveira and G. J. L.
Bernardes, Nat. Rev. Chem., 2019, 3, 147–171.

21 J. W. Bogart and A. A. Bowers, Org. Biomol. Chem., 2019,
17, 3653–3669.

22 M. R. Aronoff, B. Gold and R. T. Raines, Org. Lett., 2016, 18,
1538–1541.

23 H. M. Key and S. J. Miller, J. Am. Chem. Soc., 2017, 139,
15460–15466.

24 J. G. Gober, S. V. Ghodge, J. W. Bogart, W. J. Wever, R. R.
Watkins, E. M. Brustad and A. A. Bowers, ACS Chem. Biol.,
2017, 12, 1726–1731.

25 A. D. de Bruijn and G. Roelfes, Chem. Eur. J., 2018, 24,
12728–12733.

26 A. D. De Bruijn and G. Roelfes, Chem. Eur. J., 2018, 24,
11314–11318.

27 R. H. de Vries, J. H. Viel, R. Oudshoorn, O. P. Kuipers and G.
Roelfes, Chem. Eur. J., 2019, 25, 12698–12702.

28 T. Kitano, N. L. Zhu, C. Liu, P. Xu and S. Kobayashi, J. Am.
Chem. Soc., 2015, 137, 15422–15425.

29 J. A. Calderone and W. L. Santos, Org. Lett., 2012, 14,
2090–2093.

30 S. Schoof, S. Baumann, B. Ellinger and H. D. Arndt,
ChemBioChem, 2009, 10, 242–245.

31 H. S. Rollema, J. W. Metzger, P. Both, O. P. Kuipers and R. J.
Siezen, Eur. J. Biochem., 1996, 241, 716–722.