J|A|C|S COMMUNICATIONS

Published on Web 03/22/2008

Facile Conversion of Cysteine and Alkyl Cysteines to Dehydroalanine on Protein Surfaces: Versatile and Switchable Access to Functionalized Proteins

Gonçalo J. L. Bernardes, Justin M. Chalker, James C. Errey, and Benjamin G. Davis*

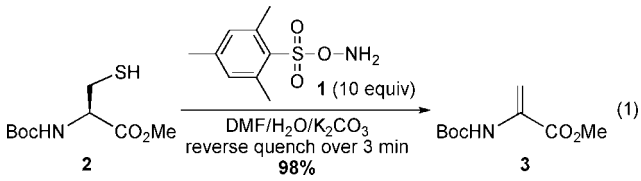
Chemistry Research Laboratory, Dept. of Chemistry, University of Oxford, 12 Mansfield Road, Oxford OX1 3TA, U.K.

Received February 10, 2008; E-mail: ben.davis@chem.ox.ac.uk

Site-selective chemical modification of proteins has emerged as a versatile strategy for modulating macromolecular function and understanding post-translational processing.[1] Dehydroalanine (Dha) is a unique chemical handle for such modifications. Synthetically, Dha can be accessed by oxidative elimination of alkylated derivatives of cysteine (Cys)[2] and selenocysteine.[3] Recently, biosynthetic incorporation of selenocysteine derivatives into peptides[4] and proteins[5] and conversion to Dha was reported. These strategies, however, rely on peroxide-induced oxidative elimination that compromises sensitive side chains such as methionine (Met).[4] Direct chemical conversion of a natural amino acid to Dha would be generally useful in protein modification provided such a reaction proceeded rapidly in aqueous media while preserving all other amino acid side chains. Cys is an attractive precursor to Dha because of the unique reactivity of the side chain thiol. While the β-elimination of dialkylated sulfonium derivatives of Cys is known, their use on proteins is limited by competing oxazoline formation, long reaction times, and nonselective alkylation.[6] Instead we sought other Cys derivatives that could undergo similar elimination yet avoid such problems of selectivity and efficiency.

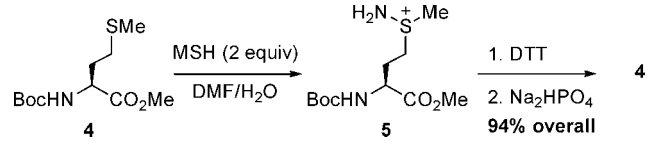
We explored *O*-mesitylenesulfonylhydroxylamine (MSH, **1**)[7] as a potential reagent in the oxidative elimination of Cys to Dha. While the transformation of sulfenamides to alkenes has not been reported, our motivation derived from the base-induced elimination of sulfilimines formed from thioethers using MSH.[8] It might seem that the reaction of MSH with thioethers does not bode well for preserving Met in the proposed oxidative elimination, but it is known that sulfilimines can undergo reversion to the native thioether in the presence of certain bases.[8b,9] Furthermore, oxidative elimination of Cys was expected to be faster than Met since the former proceeds through the removal of an acidic α proton while the latter through the less labile β proton.

A simple amino acid model was investigated to test the strategy outlined above. Treatment of Boc-Cys methyl ester **2** with MSH resulted in conversion to the Dha derivative **3** and the disulfide of **2** in 48 and 45% yield, respectively. Remarkably, the reaction was complete in less than a minute at room temperature in aqueous DMF and could be conducted in open air. Isolated disulfide did not react with MSH, ruling out disulfide as an intermediate, and was avoided by a reverse addition (**2** to excess MSH). Under such conditions, Boc-Dha methyl ester **3** is obtained in near quantitative yield (eq 1).



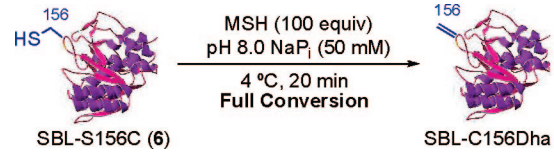
(1)

Scheme 1. Methionine Recovery from Iminosulfonium Salt



To ascertain whether MSH could be used in the presence of Met, **4** was treated with MSH. TLC indicated rapid, complete conversion of thioether, potentially to **5**, but subsequent treatment with thiol (DTT) and K_2HPO_3—conditions encountered in the conjugate addition of a thiol to Dha, an ultimate goal of this study—regenerated the Met derivative **4** in excellent yield (Scheme 1). No sulfoxide or sulfone formation was detected, even in aqueous media.[10,11] With a Met-compatible conversion of Cys to Dha in hand, we explored its application to protein modification.

The serine protease mutant subtilisin *Bacillus lentus* (SBL) S156C contains a single, surface-exposed Cys and was selected as a model for the use of MSH on protein surfaces.[12] Much to our delight, treatment of this protein with MSH at 4 °C resulted in rapid and complete conversion of Cys156 to Dha156 (eq 2). Elimination was verified by ESI-MS (34 Da decrease from **6**; **7**: 26681 calculated, 26681 found); absence of free thiol in **7** was verified by modified Ellman's assay.[10] Phosphate buffer (50 mM) at pH 8.0 was optimal for the elimination. Organic and carbonate buffers led to incomplete conversion, reflecting MSH sensitivity to certain bases.[10] Importantly, no oxidation at any of three Met residues was observed.[13]

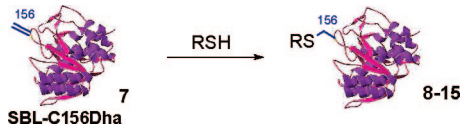


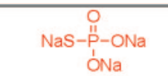
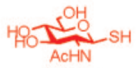
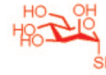
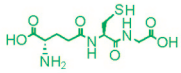
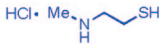
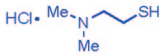
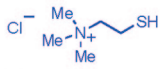
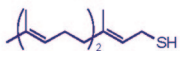
(2)

The presence of Dha was further corroborated by conversion to several thioether derivatives by conjugate addition of thiol nucleophiles (Table 1). Phosphorylation[14] and glycosylation[15] are the most prevalent post-translational modifications, mediating a wide range of cellular processes. Phospho- and glycocysteine derivatives were accessed by the efficient addition of the corresponding thiols to Dha protein **7** (entries 1–3). Importantly, SBL remained catalytically active after two-step modification to thioether protein (k_{cat} = 5.7 s^{-1}; K_M = 0.72 mM for **9**).[10,16] Peptide conjugation (entry 4), of relevance to synthetic vaccine design,[17] and methyl lysine analogues[18] (entries 5–7) were also readily accessed. The latter provide efficient access to the full series of a poorly understood post-translational modification of histone proteins involved in encoding cellular epigenetic status.[19] Finally, the natural (*S*)-farnesyl linkage was installed (entry 8). The incorporation of farnesyl and

10.1021/ja800800p CCC: $40.75

Table 1. Synthesis of Post-Translationally Modified Proteins[a]



Entry	RSH	Product	Conversion[b]	Calcd. mass	Observed mass
1		**8**	>95	26795	26794
2		**9**	>95	26918	26918
3		**10**	>95	26877	26878
4		**11**	>95	26988	26987
5		**12**	>95	26772	26773
6		**13**	>95	26786	26787
7		**14**	>95	26801	26801
8		**15**	>95	26941	26940[c]

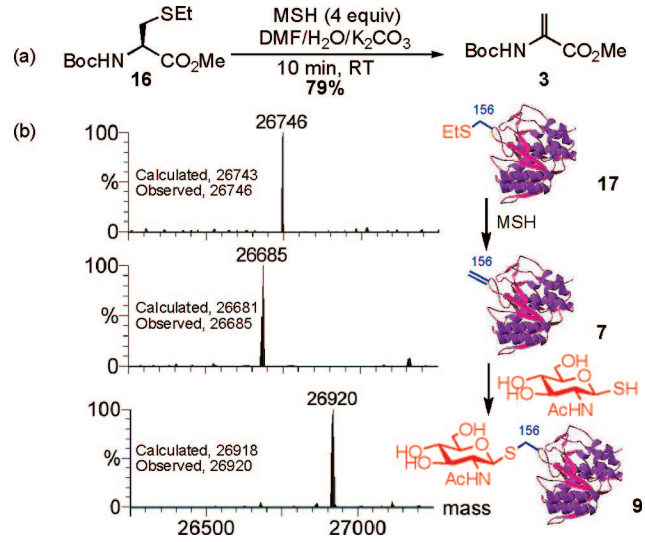
[a] See Supporting Information for full details. [b] Determined by ESI-MS analysis. [c] Na^+ adduct.

other hydrophobic polyprene units onto proteins is critical in membrane localization and protein−protein interactions.[20] While diastereoselectivity of thiol conjugate addition in simple Dha peptides is typically low,[21] the outcome on a protein surface will be highly dependent on sequence[22] and local geometry of the protein. Furthermore, diastereoselectivity may be inconsequential when only a reliable, well-defined conjugation strategy is required or when one modified protein diastereoisomer interacts stereospecifically with enzyme or receptor.

To demonstrate thioether stability, SBL-S-GlcNAc **9** was subjected to 10 mM glutathione, the natural cellular redox buffer. Whereas the disulfide counterpart underwent rapid reduction, thioether **9** exhibited the expected resilience (S23, Supporting Information).[10] This stability, however, does not imply permanence. The same method for eliminating Cys to Dha was used for converting such (*S*)-alkyl cysteine thioethers to Dha; the mechanism is likely a *syn*-elimination of the sulfilimine. This strategy was applied to (*S*)-ethyl cysteine **16** as well as its protein counterpart **17** (Scheme 2). Regeneration of Dha allows different modifications in a subsequent step, in this case glycosylation of **7** with GlcNAc-thiol. This alkyl cysteine nonpermanence is distinguished from other temporary protein modifications in that it is not due to inherent reversibility or instability of the linkage, but rather a consequence of unique reactivity of both cysteine and (*S*)-alkyl cysteine to MSH. This "functional switch" allows rapid, controlled conversion between desired protein modifications.

In conclusion, we have presented a new transformation of Cys to Dha that is useful on protein surfaces. The Dha handle provides easy access to several important post-translational modifications. The linkage is stable, yet not permanent, and will likely find wide use in covalent protein modification. Finally, while the discovery and development of MSH-mediated conversion of Cys to Dha was driven by the need for selective methods in protein modification,

Scheme 2. MSH-Induced Oxidative Elimination Of Cys Thioethers: A Functional Switch on Protein Surfaces



its utility in peptide synthesis has not escaped our attention. Applications to this end, expanded chemistry at Dha, and mechanistic studies are currently under investigation.

Acknowledgment. We gratefully acknowledge FCT, Portugal (G.J.L.B.) and the Rhodes Trust (J.M.C.) for financial support. We thank Professor Steven Ley for helpful discussions.

Supporting Information Available: Full experimental details including ^1H and ^{13}C NMR data for new compounds and ESI-MS spectra for all protein modifications. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Davis, B. G. *Science* **2004**, *303*, 480–482. (b) van Kasteren, S. I.; Kramer, H. B.; Jensen, H. H.; Campbell, S. J.; Kirkpatrick, J.; Oldham, N. J.; Anthony, D. C.; Davis, B. G. *Nature* **2007**, *446*, 1105–1109. (c) Foley, T. L.; Burkart, M. D. *Curr. Opin. Chem. Biol.* **2007**, *11*, 12–19.
(2) Rich, D. H.; Mathiaparanam, T. P.; Grant, J. A.; Mabuni, C. *J. Chem. Soc., Chem. Commun.* **1974**, 897–898.
(3) (a) Hashimoto, K.; Sakai, M.; Okuno, T.; Shirahama, H. *Chem. Commun.* **1996**, 1139–1140. (b) Okeley, N.; Zhu, Y.; van der Donk, W. A. *Org. Lett.* **2000**, *2*, 3603–3606.
(4) Seebeck, F. P.; Szostak, J. W *J. Am. Chem. Soc.* **2006**, *128*, 7150–7151.
(5) Wang, J.; Schiller, S. M.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2007**, *46*, 6849–6851.
(6) Holmes, T. J., Jr.; Lawton, R. G. *J. Am. Chem. Soc.* **1977**, *99*, 1984–1986.
(7) (a) Tamura, Y.; Minamikawa, J.; Sumoto, K.; Fujii, S.; Ikeda, M. *J. Org. Chem.* **1973**, *38*, 1239–1241. (b) Johnson, C. R.; Kirchhoff, R. A.; Corkins, H. G. *J. Org. Chem.* **1974**, *39*, 2458–2459.
(8) (a) Franek, W.; Claus, P. K. *Monatsh. Chem.* **1990**, *121*, 539–547. (b) Matsuo, J.; Kozai, T.; Ishibashi, H. *Org. Lett.* **2006**, *8*, 6095–6098.
(9) Furukawa, N.; Omata, T.; Yoshimura, T.; Aida, T.; Oae, S. *Tetrahedron Lett.* **1972**, *16*, 1619–1622.
(10) See Supporting Information for full details.
(11) For sulfilimine conversion to sulfides and sulfoxides, see refs 8b and 9.
(12) DeSantis, G.; Berglund, P.; Stabile, M. R.; Gold, M.; Jones, J. B. *Biochemistry* **1998**, *37*, 5968–5973.
(13) In addition to the selective Met recovery shown in Scheme 1, differential accessibility of C156, M119, M175, and M222 may also play a role.
(14) (a) Hunter, T.; Karin, M. *Cell* **1992**, *70*, 375–387. (b) Cohen, P. *Nat. Cell Biol.* **2002**, *4*, E127–E130.
(15) (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720. (b) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579–601. (c) Ohtsubo, K.; Marth, J. D. *Cell* **2006**, *126*, 855–867.
(16) DeSantis, G.; Paech, C.; Jones, J. B. *Bioorg. Med. Chem.* **2000**, *8*, 563–570.
(17) (a) Danishefsky, S. J.; Allen, J. A. *Angew. Chem., Int. Ed.* **2000**, *39*, 836–863. (b) Ouerfelli, O.; Warren, J. D.; Wilson, R. M.; Danishefsky, S. J. *Exp. Rev. Vaccines* **2005**, *4*, 677–685. (c) Wittrock, S.; Becker, T.; Kunz, H. *Angew. Chem., Int. Ed.* **2007**, *46*, 5226–5230.
(18) Simon, M. D.; Chu, F.; Racki, L. R.; de la Cruz, C. C.; Burlingame, A. L.; Panning, B.; Narlikar, G. J.; Shokat, K. M. *Cell* **2007**, *128*, 1003–1012.
(19) Trojer, P.; Reinberg, D. *Cell* **2006**, *125*, 213–217.
(20) (a) Zhang, F. L.; Casey, P. J. *Annu. Rev. Biochem.* **1996**, *65*, 241–269. (b) Marshall, C. J. *Science* **1993**, *259*, 1865–1866.
(21) (a) Zhu, Y.; van der Donk, W. A. *Org. Lett.* **2001**, *3*, 1189–1192. (b) Galonic, D. P.; van der Donk, W. A.; Gin, D. Y. *Chem.—Eur. J.* **2003**, *9*, 5997–6006.
(22) Schmidt, U.; Öhler, E. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 42.

JA800800P