Protein Modifications

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Conversion of Cysteine into Dehydroalanine Enables Access to Synthetic Histones Bearing Diverse Post-Translational Modifications**

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The eukaryotic genome is packaged into a complex of DNA and proteins called chromatin. The fundamental repeating unit of chromatin, the nucleosome, consists of an octamer of conserved histone proteins and approximately 147 base pairs of DNA. Linker DNA and linker histones string together each nucleosome (Figure 1).[1,2]

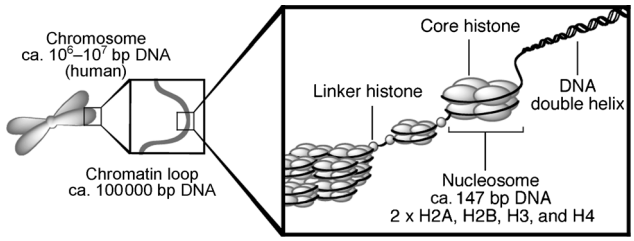


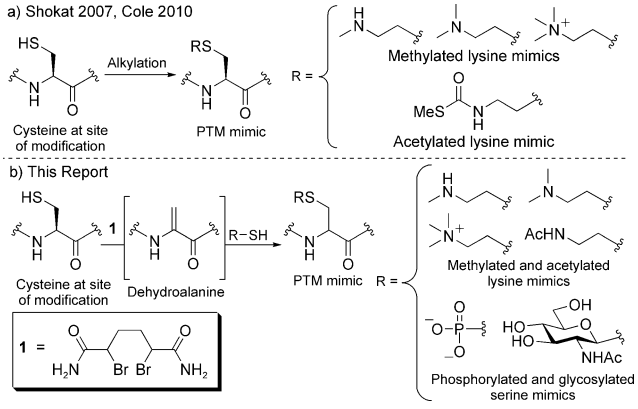
Figure 1. Schematic representation of chromatin packaging.

Histones undergo many post-translational modifications (PTMs) at multiple sites and residue types.[3,4] Lysine methylation, acetylation, ubiquitination, and several other acylations (including succinylation and crotonylation), serine and threonine phosphorylation and glycosylation, as well as arginine methylation and deimination are PTMs common to histones.[3–6] Many are dynamic and regulate chromatin structure, transcription, and DNA replication and repair.[3,4] A biochemical understanding of how histone PTMs regulate such function is a current frontier in biology.[7–10] To enable these studies, access to histones bearing PTMs is required.[9,11,12]

The precision of chemical synthesis is presently unrivaled in accessing purified histones with multiple PTMs, which may be especially valuable when the associated modifying enzyme or co-substrates are unknown or hard to acquire. Native chemical ligation (NCL)[13] and expressed protein ligation (EPL)[14,15] are two powerful methods.[11,12,16] However, when PTMs lie outside of the first 50 residues of either terminus, or are present at both termini, multiple ligations may be required—a significant challenge.[17,18]

Amber codon suppression technology can also provide modified histones. Acetyllysine and latent forms of mono- and dimethyllysine can be incorporated into histones using evolved pyrrolysyl-tRNA synthetase/tRNA pairs.[19–22] This route to modified histones is promising, but several chemical steps are sometimes required after expression to access the final protein,[22] and it is difficult to apply to multiple modifications. Moreover, many histone modifications—lysine trimethylation, serine and threonine phosphorylation and glycosylation, for example—are not generally accessible using this technology.[23]

Site-selective modification of a preformed, full-length histone scaffold is an alternative strategy. This approach would allow, in principle, rapid, diverse elaboration of full-length proteins as common synthetic intermediates. Divergence of late-stage intermediates is common practice (because of advantages in strategy and efficiency) in small-molecule synthetic chemistry, but explored less frequently in synthetic biology. Notably, other than Cys110 of histone H3, the core human histones do not contain cysteines.[1] A unique Cys can therefore be positioned at desired sites of modification. Cys alkylation can then provide methylated lysine mimics[24] or a nonhydrolyzable thiocarbamate analogue of acetylated lysine[25] (Scheme 1a). These aminoethylation protocols, which target protein nucleophiles, benefit from operational simplicity, thus enabling the study of lysine modifications in several contexts.[11,12] However, whilst



Scheme 1. Cysteine and dehydroalanine as chemical precursors to histones bearing post-translational modifications.

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useful for these mimics, the thiocarbamate acetyllysine mimic is not a substrate for histone deacetylase enzymes.[25] Moreover, there are no reported chemical methods for the introduction of mimics of other important histone PTMs, including serine phosphorylation and glycosylation. Indeed, the use of chemical (e.g., glycosyl or phosphoryl) electrophiles to target protein nucleophiles under appropriate aqueous conditions would be difficult because of high hydrolytic susceptibility of such electrophiles.

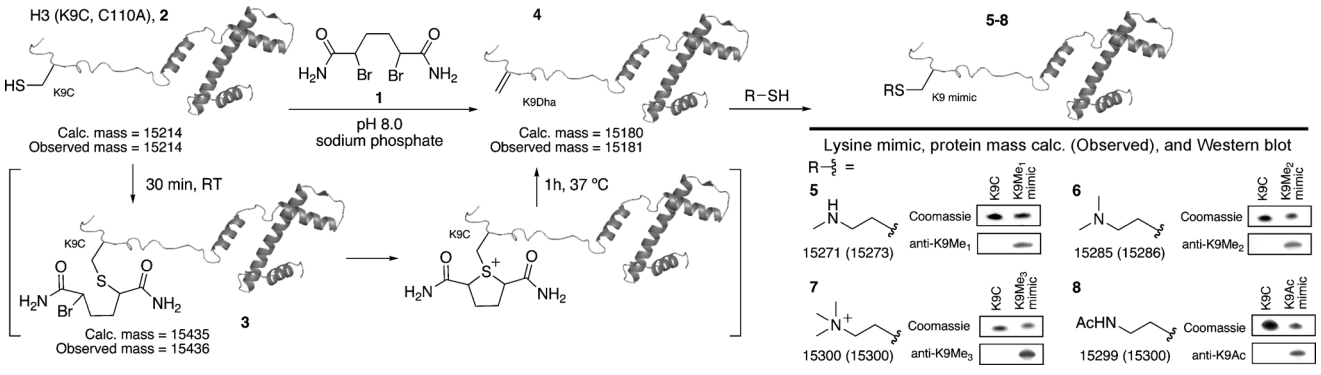
Herein we report that from single dehydroalanine (Dha) precursor sites, six chemically diverse histone PTMs can be installed site-selectively in histones (Scheme 1b). Previously, we have found Dha to be a useful precursor to PTMs. However, its use was limited by a lack of general methods for introduction into full-length proteins.[26,27] Dha has been previously used in histone modification, but the method used to generate Dha (oxidative elimination of a phenylselenocysteine residue using H_2O_2 after incorporation using amber codon suppression) resulted in nonselective oxidation of other residues such as methionine, thus causing the formation of heterogeneous product/byproduct mixtures.[28] In a recent study we investigated the effectiveness of various reagents in forming Dha from Cys (a natural residue that can be positioned using standard mutagenesis techniques) and found that the dibromide **1** was the most efficient of reagents tested on enzymes and antibodies.[27] We were therefore interested to test whether **1** could be applied cleanly to histones, to what extent Dha could be used to install chemically diverse PTM mimics, and whether such mimics are functionally competent with respect to biological recognition and processing. Additionally, for all the histone modifications proposed in Scheme 1b, we desired operationally simple and scalable protocols so that multi-milligram quantities could be prepared rapidly and readily.

As a testing ground, we chose histone H3, which is extensively modified on its N terminus.[3,4,11,12,29] H3 has a single Cys110 that can be mutated to alanine without discernable effects on structure or function.[24] Cys can therefore be genetically installed at the desired modification site. Accordingly, single-Cys mutant H3 (K9C,C110A) (**2**) was prepared: Lys9 is naturally acetylated and methylated,[3,4,11,12,29] which are key modifications in chromatin function.[30,31]

We found that treatment of H3 (K9C, C110A) with reagent **1** gave the alkylated, uncyclized intermediate **3** as the major product after 30 minutes at room temperature, as determined by LC/MS (Scheme 2).[32] To complete the clean conversion of **3** into Dha, the reaction was simply incubated at 37 °C for an additional hour. The product protein **4** did not react with Ellman's reagent, which was consistent with the desired conversion of Cys into Dha at position 9. The formation of Dha was additionally corroborated by addition of thiols (Scheme 2).

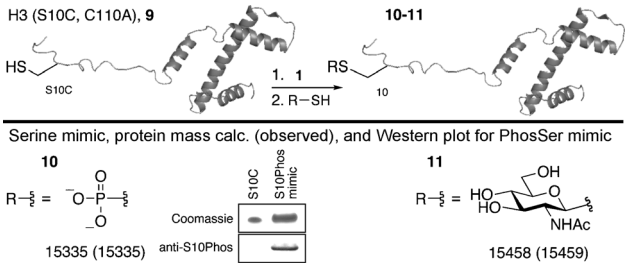
The Dha residue generated in this way in protein **4** was then efficiently converted into mimics of all three methylation states of Lys as well as an acetylated Lys mimic by addition of the appropriate thiol (Scheme 2). The sequential formation of Dha and its conversion into these PTM mimics could be carried out in a simple one-pot operation; full conversions were observed in all cases (>95% by LC/MS).[33] The modified proteins were easily purified using a desalting column or dialysis, and protein recovery was typically 80–96%.[33] Although thiol additions likely result in epimeric mixtures, these PTM mimics were recognized by primary antibodies raised to the native modification at position 9 of H3 (see the Western blot in Scheme 2). Moreover, good selectivity was shown for the methylation state; there was no cross-reactivity with mimics bearing other methylation states (see the Supporting Information). While a similar analysis has been carried out on methylated lysine mimics obtained by cysteine aminoethylation,[24] this is the first demonstration that antibodies raised to acetyllysine at position 9 of H3 recognize a corresponding thialysine analogue in protein **8**. Interestingly, relative selectivity over peptide binding varied with the antibody source used, thus highlighting key differences in reagents that are routinely used to probe histones.

With **1** now established as a selective reagent for the incorporation of Dha, the synthesis of other PTMs was tested. To explore another site a different H3 mutant, H3 (S10C, C110A) (**9**), was prepared with a single Cys at position 10. Serine 10 is naturally phosphorylated and is involved in a variety of processes in cell division and transcription.[34–36] Access to phosphorylated histones will help clarify their specific roles. Dha was first installed on **9** by reagent **1** using the same protocol. Again, Dha was formed efficiently, as monitored by LC/MS.[33] The product did not react with



Scheme 2. Dehydroalanine is a precursor to methylated and acetylated lysine mimics. Western blot analysis of these modifications, installed at position 9 of H3, demonstrates that antibodies raised to the natural modifications recognize the corresponding mimics.

Ellman's reagent, consistent with reaction of the single Cys10. Addition of 2-mercaptoethanol to product protein corroborated the formation of Dha.[33] Next, to install a mimic of phosphoserine, sodium thiophosphate was added to the Dha generated at position 10. Full conversion into phosphocysteine in **10** was observed by LC/MS after 1 hour at 37 °C at pH 8.0 (Scheme 3).[33] The Western blot of **10** demonstrated



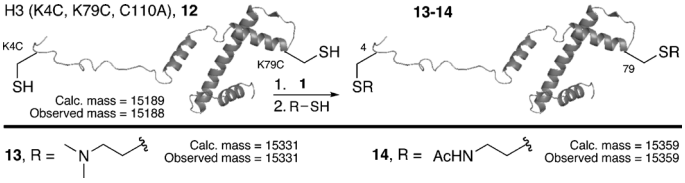
Scheme 3. One-pot installation of a phosphoserine mimic and a GlcNAc-serine mimic at position 10 of H3. Western blot analysis demonstrates that antibodies raised to phosphoserine at position 10 of H3 bind to phosphocysteine at the same position.

that phosphocysteine is sufficiently similar to phosphoserine such that antibodies raised to natural H3 pSer10 protein recognized our synthetically produced **10** (Scheme 3). This mimic also showed no sign of degradation over several hours (see the Supporting Information).

Although phosphorylated serine residues and related analogues have been installed in proteins through NCL and EPL,[37] this is the first demonstration that antibody recognition of phosphoserine can be mimicked by phosphocysteine. Notably, enhanced binding over a peptide mimic was observed (see the Supporting Information). Moreover, there are currently no alternative methods for site-selective chemical phosphorylation of Cys or Ser residues in full-length proteins.[23] We therefore propose the use of Dha and its conversion into phosphocysteine to chemically access functional mimics of proteins containing pSer residues. Moreover, the facile generation of a PTM mimic (pS10) at another site in H3 highlighted the flexibility of our approach. To test this hypothesis we also examined the interaction of the synthetic phosphoH3 **10** with a so-called chromatin 'reader' protein, 14-3-3ζ,[38] using phosphopeptide displacement in an amplified (chemi)luminescent proximity homogeneous assay[39] (ALPHA, see the Supporting Information). Consistent with known modulations and interactions of naturally derived H3 and phosphoH3,[38,40] both H3 and **10** interacted with 14-3-3ζ; the interaction of **10** was strongest.

Recently, Hart and co-workers have shown that O-linked β-*N*-acetyl glucosamine (*O*-GlcNAc) is found at serine and threonine residues in histones.[5] While modification sites have not yet been identified, it has been shown that H3 bears *O*-GlcNAc.[5] Since this modification is a relatively recent addition to known histone PTMs, its influence on chromatin is unexplored. The synthesis of histones bearing *O*-GlcNAc or appropriate mimics will help elucidate roles. Since phosphorylation and GlcNAc often occur at the same residue and impart reciprocal function,[41,42] the installation of GlcNAc at position 10 provides a modified histone that may be useful. Accordingly, addition of GlcNAc glycosyl thiol to Dha provided the GlcNAc-modified protein **11** (Scheme 3) containing a mimic of GlcNAc-Ser that we have validated in assays on several proteins.[43]

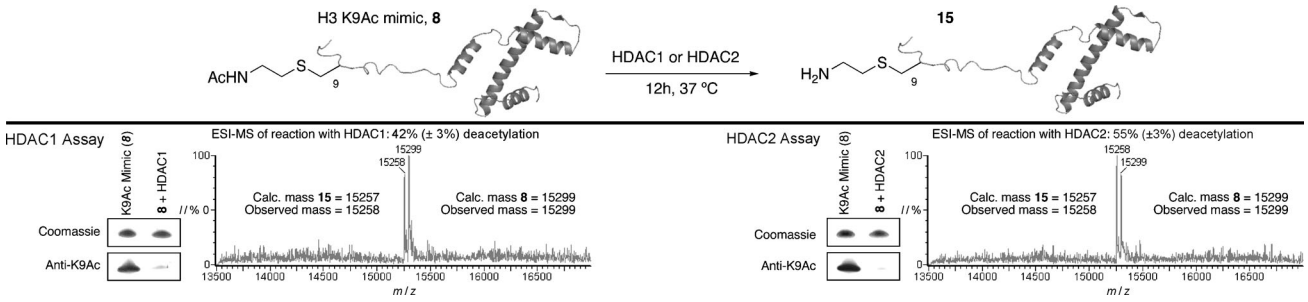
All modifications discussed so far were carried out at single sites. Most histones, however, bear multiple PTMs,[3,4,11,12] so we next tested our strategy for installation of modifications at more than one site. Two alternative PTM sites were explored; another H3 mutant (**12**) was prepared with two Lys→Cys mutations at positions 4 and 79, two other sites of Lys methylation and acetylation.[3,4,44] Reagent **1** readily converted both Cys residues into Dha, thus it was the first demonstration that it can efficiently modify multiple Cys residues. Both Dha residues could then be converted into PTM mimics by addition of appropriate thiols. H3 derivatives bearing two dimethyllysine mimics (**13**) or two acetyllysine mimics (**14**) were prepared by this protocol (Scheme 4).



Scheme 4. Double modification of H3 at position 4 and position 79.

Proteins containing multiple PTMs separated by more than 50 residues are not easily prepared by NCL and EPL since they require multiple ligations. Amber codon suppression is complicated by competing termination of translation, especially when compounded by the need for suppression of multiple stop codons. The chemistry in Scheme 4, however, is comparatively simple from an operational standpoint. Moreover, this was performed on 10 mg scale, thus providing ample quantities in short-order from recombinant protein.[33,45]

To demonstrate how these modified histones might be used in the study of chromatin-modifying enzymes ("writers/erasers"), the H3K9Ac mimic protein **8** was used as a substrate in a direct histone deacetylase (HDAC) assay. After incubation with either HDAC1 or HDAC2, Western blot analysis showed only trace amounts of acetylated histone, but LC/MS analysis of the reactions revealed 42% (±3%) and 55% (±3%) deacetylation by HDAC1 and HDAC2, respectively (Scheme 5).[46] When **8** was incubated under the same conditions without HDAC, clear binding was observed by Western blot analysis and no reaction observed by LC/MS, thus ruling out adventitious hydrolysis of the natural L epimer of **8** as well as suggesting selective binding to the L epimer by the primary antibody used in the blot. While kinetic data might be difficult to extract from such an assay, nominal activity can be determined unambiguously. In fact, the Western blot and LC/MS analysis shown in Scheme 5 are the first direct evidence for HDAC activity on the acetyllysine mimic in **8**.[47] The only other acetyllysine mimic available, the thiocarbamate analogue introduced by Cole and co-workers (Scheme 1a), is not a substrate for HDAC enzymes.[25]



Scheme 5. HDAC assay using an acetyllysine mimic prepared at position 9 of H3.

Therefore, use of Dha and its conversion into acetyllysine mimics such as **8** is currently the most convenient strategy for investigating HDAC activity on full-length histones. Notably, the deacetylation of mimic **8** by both HDAC1 and HDAC2 was inhibited by the clinically approved HDAC inhibitor suberoylanilide hydroxamic acid[48] (SAHA/Vorinostat; see the Supporting Information).

In conclusion, we have shown that Dha is readily installed in histones by the reaction of reagent **1** with genetically incorporated Cys residues. Dha, in turn, is a chemical precursor to mimics of six distinct PTMs: mono-, di-, and trimethylated lysine, acetylated lysine, phosphorylated serine, and glycosylated serine. Through their combined use we have shown ready switching of site and PTM type. It is important to note that the PTM mimics introduced through Dha are likely formed as C_α epimeric mixtures. Nevertheless, despite this isomerism these PTM mimics were of functional use in both immunoblot and enzymatic assays. Indeed, the partial processing by some enzymes, observed here, creates direct implications regarding selectivity-determining features of protein substrates (i.e., HDAC1/2 are both clearly sensitive to D/L-configuration at C_α of K9Ac in H3). The same modification could also be installed at multiple sites on a multi-milligram scale. To illustrate the potential of this technology, the first validation of phosphocysteine as a mimic of phosphoserine in any protein was described, the first access to a glycosylated histone was enabled, and the first direct observation of HDAC activity on an acetylated lysine mimic was reported for a full-length histone.

The demonstrated interaction of our synthetic modified histones with both readers and writer/erasers shown here is a key step. Currently, we are exploring the use of the modified histones reported here as substrates for other chromatin-modifying enzymes. Additionally, we are coordinating methods described in this report with orthogonal chemistry for the installation of multiple, distinct PTMs. These studies are designed to complement the many efforts to synthesize modified histones and study the complex roles of PTMs in chromatin biology.[9–12]

Experimental Section

Representative procedure for histone modification: A 250 μL aliquot of a solution of H3 (K9C, C110A, 2.9 mg mL^{-1} aq.) was diluted with 250 μL of sodium phosphate buffer (pH 8.0, 50 mM). DTT (11 mg) was added as a solid and the solution shaken at RT for 15 min to ensure reduction. The protein solution was passed through a PD minitrap (GE Healthcare), eluting with 50 mM sodium phosphate (1.0 mL, pH 8.0). An 89 μL aliquot of a stock solution of the dibromide **1** (30 mg in 250 μL DMF) was added to the resulting protein solution. The reaction was shaken at RT for 30 min and then 37 °C for 1 h. Solids were removed by centrifugation (1 min, 14000*g*) and the reaction analyzed by LC/MS. Full conversion into Dha was observed (calculated mass = 15180; observed mass = 15180). *N*-acetyl cysteamine (46 μL neat) was added to the mixture and shaken at RT for 1 h. The reaction was analyzed directly by LC/MS, thus revealing full conversion (> 95%) to the H3K9Ac mimic **8** (calculated mass = 15299; observed mass = 15300). Small molecules were removed by dialysis in 4 L of water (Slide-A-Lyzer, 10 K MWCO, Pierce). Protein recovery was measured by BCA assay (0.7 mg, 96% recovery). The modified histone was greater than 95% pure (SDS/PAGE, LC/MS) and used directly in Western blot and HDAC assays.

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[1] K. Luger, A. W. Mäder, R. K. Richmond, D. F. Sargent, T. J. Richmond, *Nature* **1997**, *389*, 251.
[2] R. D. Kornberg, Y. Lorch, *Cell* **1999**, *98*, 285.
[3] T. Kouzarides, *Cell* **2007**, *128*, 693.
[4] A. J. Bannister, T. Kouzarides, *Cell Res.* **2011**, *21*, 381.
[5] K. Sakabe, Z. Wang, G. W. Hart, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 19915.
[6] M. Tan, H. Luo, S. Lee, F. Jin, J. S. Yang, E. Montellier, T. Buchou, Z. Cheng, S. Rousseaux, N. Rajagopal, Z. Lu, Z. Ye, Q. Zhu, J. Wysocka, Y. Ye, S. Khochbin, B. Ren, Y. Zhao, *Cell* **2011**, *146*, 1016.
[7] B. D. Strahl, C. D. Allis, *Nature* **2000**, *403*, 41.
[8] T. Jenuwein, C. D. Allis, *Science* **2001**, *293*, 1074.
[9] C. D. Allis, T. W. Muir, *ChemBioChem* **2011**, *12*, 264.
[10] N. R. Rose, C. J. Schofield, T. D. Heightman, *New Front. Chem. Biol.* **2011**, 151.
[11] C. Chatterjee, T. W. Muir, *J. Biol. Chem.* **2010**, *285*, 11045.
[12] A. Dhall, C. Chatterjee, *ACS Chem. Biol.* **2011**, *6*, 987.
[13] P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776.
[14] T. C. Evans, J. Benner, M.-Q. Xu, *Protein Sci.* **1998**, *7*, 2256.
[15] T. W. Muir, D. Sondhi, P. A. Cole, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6705.
[16] R. R. Flavell, T. W. Muir, *Acc. Chem. Res.* **2009**, *42*, 107.
[17] B. L. Nilsson, M. B. Soellner, R. T. Raines, *Annu. Rev. Biophys. Biomol. Struct.* **2005**, *34*, 91.

[18] S. B. H. Kent, *Chem. Soc. Rev.* **2009**, *38*, 338.
[19] H. Neumann, S. M. Hancock, R. Buning, A. Routh, L. Chapman, J. Somers, T. Owen-Hughes, J. van Noort, D. Rhodes, J. W. Chin, *Mol. Cell* **2009**, *36*, 153.
[20] D. P. Nguyen, M. M. Garcia Alai, P. B. Kapadnis, H. Neumann, J. W. Chin, *J. Am. Chem. Soc.* **2009**, *131*, 14194.
[21] H.-w. Ai, J. W. Lee, P. G. Schultz, *Chem. Commun.* **2010**, *46*, 5506.
[22] D. P. Nguyen, M. M. Garcia Alai, S. Virdee, J. W. Chin, *Chem. Biol.* **2010**, *17*, 1072.
[23] In a notable exception, phosphoserine has recently been incorporated into proteins using *E. coli* translational machinery. Protein yields for this method, however, remain low. H.-S. Park, M. J. Hohn, T. Umehara, L.-T. Guo, E. M. Osborned, J. Benner, C. J. Noren, J. Rinehart, D. Söll, *Science* **2011**, *333*, 1151.
[24] M. D. Simon, F. Chu, L. R. Racki, C. C. de La Cruz, A. L. Burlingame, B. Panning, G. J. Narlikar, K. M. Shokat, *Cell* **2007**, *128*, 1003.
[25] R. Huang, M. A. Holbert, M. K. Tarrant, S. Curtet, D. R. Colquhoun, B. M. Dancy, B. C. Dancy, Y. Hwang, Y. Tang, K. Meeth, R. Marmorstein, R. N. Cole, S. Khochbin, P. A. Cole, *J. Am. Chem. Soc.* **2010**, *132*, 9986.
[26] G. J. L. Bernardes, J. M. Chalker, J. C. Errey, B. G. Davis, *J. Am. Chem. Soc.* **2008**, *130*, 5052.
[27] J. M. Chalker, S. B. Gunnoo, O. Boutureira, S. C. Gerstberger, M. Fernández-González, G. J. L. Bernardes, L. Griffin, H. Hailu, C. J. Schofield, B. G. Davis, *Chem. Sci.* **2011**, *2*, 1666.
[28] J. Guo, J. Wang, J. S. Lee, P. G. Schultz, *Angew. Chem.* **2008**, *120*, 6499; *Angew. Chem. Int. Ed.* **2008**, *47*, 6399.
[29] S. S. Oliver, J. M. Denu, *ChemBioChem* **2011**, *12*, 299.
[30] C. Martin, Y. Zhang, *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 838.
[31] A. Kimura, K. Matsubara, M. Horikoshi, *J. Biochem.* **2005**, *138*, 647.
[32] Reaction conversions were determined by relative peak height of all protein material in the LC/MS trace. For full details of LC/MS analysis, including method validation, see the Supporting Information.
[33] See the Supporting Information for full experimental details.
[34] F. Hans, S. Dimitrov, *Oncogene* **2001**, *20*, 3021.
[35] S. J. Nowak, V. G. Corces, *Trends Genet.* **2004**, *20*, 214.
[36] M. H. Dyson, S. Thomson, L. C. Mahadevan, *Cell Cycle* **2005**, *4*, 13.
[37] M. K. Tarrant, P. A. Cole, *Annu. Rev. Biochem.* **2009**, *78*, 797.
[38] N. Macdonald, J. P. I. Welburn, M. E. M. Noble, A. Nguyen, M. B. Yaffe, D. Clynes, J. G. Moggs, G. Orphanides, S. Thomson, J. W. Edmunds, A. L. Clayton, J. A. Endicott, L. C. Mahadevan, *Mol. Cell* **2005**, *20*, 199.
[39] Y. Du, F. R. Khuri, H. Fu, *Curr. Chem. Genomics* **2008**, *2*, 40.
[40] S. Winter, W. Fischle, C. Seiser, *Cell Cycle* **2008**, *7*, 1336.
[41] N. E. Zachara, G. W. Hart, *Chem. Rev.* **2002**, *102*, 431.
[42] Z. Wang, M. Gucek, G. W. Hart, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13793.
[43] M. Fernández-González, O. Boutureira, G. J. L. Bernardes, J. M. Chalker, M. A. Young, J. C. Errey, B. G. Davis, *Chem. Sci.* **2010**, *1*, 709.
[44] B. A. Garcia, S. B. Hake, R. L. Diaz, M. Kauer, S. A. Morris, J. Recht, J. Shabanowitz, N. Mishra, B. D. Strahl, C. D. Allis, D. F. Hunt, *J. Biol. Chem.* **2007**, *282*, 7641.
[45] Denaturing conditions (3 M guanidinium hydrochloride, pH 8.0) were used to maintain solubility of **12** at the high concentrations used in these modifications (10 to 20 $mg\,mL^{-1}$).
[46] The error in MS peak height was estimated from the standard deviation of multiple measurements of a standard protein mixture.
[47] A related HDAC3 assay was reported in Ref. [28], but deacetylation was only inferred indirectly from an Aurora kinase B assay that is affected by acetylation at lysine 9 of H3. In Ref. [28], no MS data was provided for the deacetylated histone and no Western blot was provided for the modification at lysine 9 of H3.
[48] P. A. Marks, R. Breslow, *Nat. Biotechnol.* **2007**, *25*, 84.
[49] An alternative method for accessing an acetyllysine mimic through a thio-ene reaction with cysteine was reported while this manuscript was under review: F. Li, A. Allahverdi, R. Yang, G. B. J. Lua, X. Zhang, Y. Cao, N. Korolev, L. Nordenskiöld, C.-F. Lui. *Angew. Chem.* **2011**, *123, 9785; Angew. Chem. Int. Ed.* **2011**, 50, 9611.; *Angew. Chem. Int. Ed.* **2011**, *50*, 9611.

