One-pot Native Chemical Ligation of Peptide Hydrazides Enables Total Synthesis of Modified Histones

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One of the rising demands in the field of protein chemical synthesis is the development of facile strategies that yield the protein in workable quantities and homogeneity with lesser handling steps. Although native chemical ligation of peptide hydrazides has recently been shown to be useful for the chemical synthesis of proteins carrying acid-sensitive modification groups, previous hydrazide-based protein synthesis studies have used sequential ligation strategies. Here we report a practical method for "one-pot" native chemical ligation of peptide hydrazides that would circumvent the need for isolation of the intermediate products. This method employed a fast and selective arylboronate oxidation reaction mediated by H_2O_2, which draws attention to the potential applications of the thus far under-exploited boron-based functionalities in protein chemical synthesis. To demonstrate the practicality and efficiency of the new one-pot method, we reported its application to scalable total synthesis of modified histones (with five analogues of H3 and H4 as examples) in multi-milligram scale with good homogeneity.

Introduction

Histone posttranslational modifications (PTMs, e.g. acetylation, methylation, and phosphorylation) play a central role in regulating the structure and dynamics of chromatin as well as DNA-driven cellular processes.[1] Understanding of how histone PTMs translate into diverse cellular events is important from both fundamental and therapeutic perspectives. Studies for this purpose need access to homogenously modified histones that unfortunately cannot be easily isolated from natural sources or obtained by classical biochemical methods.[2] To solve this problem four strategies have been used for making modified histones: 1) amber suppression mutagenesis,[3] 2) Cys-directed protein modification,[4] 3) expressed protein ligation,[5] and 4) total chemical protein synthesis.[6] In the first three approaches the main structures of histones are produced recombinantly, whereas the modification is introduced later by chemoselective reactions with small molecules or peptides. These semi-synthetic strategies have been successfully used to produce many modified histones enabling elegant structural and functional studies.[3-5] Nonetheless, total chemical synthesis of histones is needed for the generation of histones with multiple different modifications at both terminal and middle regions.

In this context Ottesen and coworkers described the first total synthesis of histone H3 through sequential native chemical ligations.[7] This important pioneering work enabled the production of fully synthetic H3 with 2-7% overall yields. A potential limitation is that the peptide segments were prepared using Boc SPPS (solid-phase peptide synthesis), whose HF cleavage conditions were unsuited for some PTMs such as phosphorylation. To overcome this problem Ottesen et al. tested the N-acylurea approach for the Fmoc SPPS of histone peptide thioesters.[8] Meanwhile, Aimoto and coworkers accomplished the first Fmoc-based total synthesis of H3 by using the Cys-Pro ester autoactivating unit as thioester precursor.[9] Notwithstanding these key advances, to meet the escalating needs in the studies of histone PTMs it is helpful to develop a more scalable and cost-effective method for the total synthesis of modified histones at multi-milligram scale.

We recently described the native chemical ligation of peptide hydrazides,[10] which can be readily converted to thioesters via $NaNO_2$ activation and subsequent thiolysis. We expected that this approach may allow for expedient synthesis of modified

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histones, as peptide hydrazides are easily synthesizable through automated Fmoc SPPS. Indeed, with the help of peptide hydrazides Brik et al. accomplished the first total synthesis of Lys34-ubiquitinated H2B.[11] However, in our synthesis of H3 and H4 using the previous sequential ligation/purification method,[10b] we encountered difficulty with separating the intermediate products, which not only complicated the experiments but also reduced the yield. To overcome this challenge we sought to use a "one-pot" strategy that would need a single final purification step.[12] However, our own tests revealed that the previously developed one-pot procedures were not readily applicable to peptide hydrazides.[13]

In the present study we described a one-pot method for the ligation of peptide hydrazides (Figure 1). We also explored the possible use of thus far under-exploited boron-based functionalities in the chemical synthesis of proteins. The practicality and efficiency of the method was demonstrated by the multi-milligram-scale synthesis of modified histone H3 and H4. Thus in terms of concept as well as technological advances, this work extends the hydrazide-based approach of native chemical ligation.

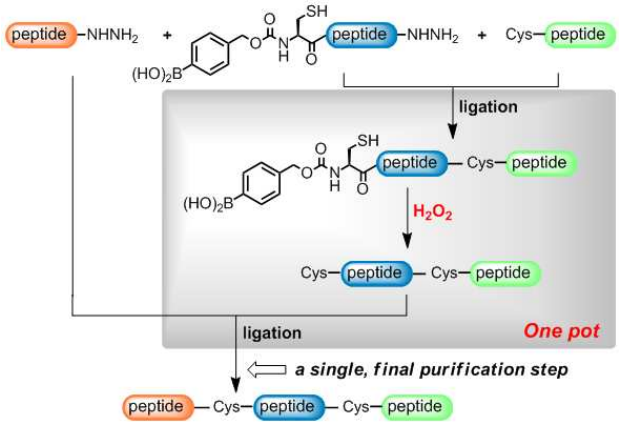

Fig. 1 One-pot ligation of peptide hydrazides

Results and discussion

Problem of sequential ligations

Before we describe the "one-pot" method, we wished to show with experimental data why a one-pot method would sometimes be practically advantageous if not obligatory for native chemical ligation of peptide hydrazides. For the synthesis of histone H3 trimethyl Lys4 (H3K4me3) we started with three synthetic peptide segments (Figure 2), i.e. H3K4me3[Ala1-Val46]-$NHNH_2$ (**1**, 46-mer), H3K4me3[Cys47-Gly90]-$NHNH_2$ (**2**, 44-mer), and H3K4me3[Cys91-Ala135] (**3**, 45-mer). Both the Ala47 and Ala91 residues were replaced by Cys to facilitate the ligations, which would be converted back to Ala after the full-length peptide was produced.[14] Furthermore, the side chain thiol of Cys110 was protected by the Acm (acetamidomethyl) group to enable selective desulfurization of Ala47 and Ala91 in the full-length polypeptide product.[15]

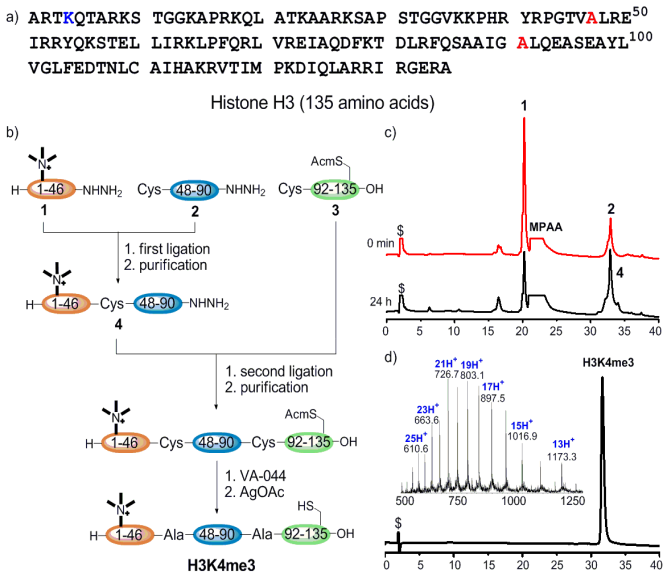

Fig. 2 Synthesis of H3K4me3 by using N-to-C sequential ligations. a) Sequence of H3. b) Procedure for the N-to-C sequential ligations. c) Analytical HPLC traces (λ = 214 nm) for the ligation between **1** and **2** after 0 min and 24 h. "$" denotes solvent fronts. d) Analytical HPLC trace (λ = 214 nm) for the final, purified H3K4me3 and its ESI-MS: observed mass 15240.2 ± 0.7 Da, calc 15239.62 Da (average isotopes).

With **1**-**3** in hand, we first condensed **1** and **2** with the help of MPAA (4-mercaptophenylacetic acid) to produce an intermediate **4**. After purification of **4** we conducted the second ligation between **4** and **3** to obtain the full-length peptide. Finally, the desired protein (i.e. H3K4me3) was generated through a free radical desulfurization reaction initiated by VA-044 (2,2'-azobis[2-(2-imidazolin-2-yl)-propane] dihydrochloride)[16] followed by Acm deprotection. As shown in Figure 2c, a tricky problem encountered in the above synthesis was that the retention times of **2** and **4** were almost identical in the reverse-phase HPLC under the conditions used. This rendered the monitoring of the first ligation reaction difficult. The purification of **4** was also challenging, which seriously increased the time cost of the synthesis while reduced the yield.

One-pot ligation of peptide hydrazides: model test

To overcome the problem of intermediate separation, we decided to use a one-pot ligation approach[12] to the native chemical ligation of peptide hydrazides. This strategy has been successfully used in many previous studies to reduce handling time and cost and increase yields in protein chemical synthesis.[12] For the ligation of peptide hydrazides the one-pot method must be compatible with the $NaNO_2$ activation step.[13] Although we previously developed the sequential procedure for the ligation of peptide hydrazides,[10b] one-pot ligation of peptide hydrazides has not been accomplished. A critical need is the development of a readily reversible protection of the N-terminal Cys residue. To compromise the operational ease and reaction efficiency, we were interested in the use of *p*-boronobenzyloxycarbonyl (Dobz) group developed by Kemp and Roberts.[18] We anticipated that Dobz-protected Cys should remain un-reactive under native

chemical ligation conditions unless it is activated by the addition of H_2O_2[17]. In the previous studies on Dobz,[18] only small peptides (up to pentamer, synthesized by the solution-phase coupling method) were tested. Thus, for the use of Dobz to accomplish one-pot native ligation of peptide hydrazides in protein total synthesis, we needed to examine a number of important issues regarding the synthesis, stability, and handling of the Dobz peptides.

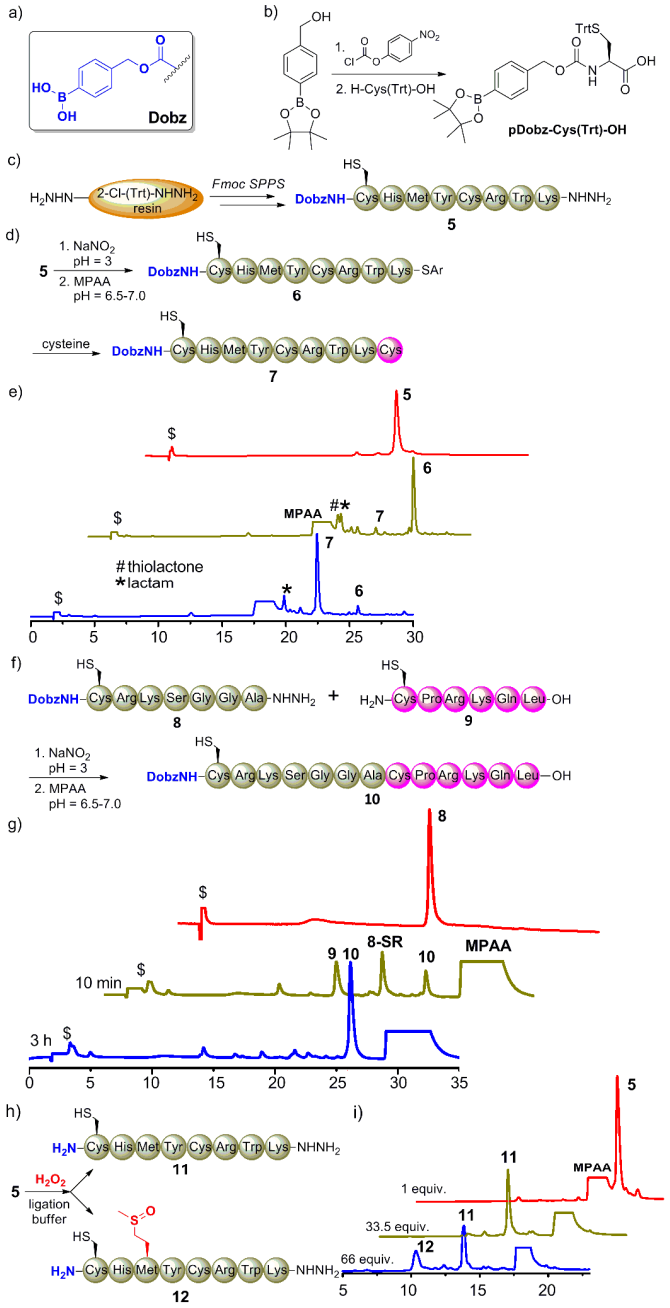

Fig. 3 Tests for the use of Dobz-peptides. a) Structure of Dobz. b) The synthesis of pDobz-Cys(Trt)-OH. c) Fmoc SPPS of Dobz-Cys-peptide-$NHNH_2$. d) Conversion of Dobz-Cys-peptide-$NHNH_2$ to a thioester and its ligation with Cys. e) Analytical HPLC traces (λ = 214 nm) for purified **5**, crude reaction mixture of **5** with $NaNO_2$ and MPAA, and crude ligation product with Cys. "$" denotes solvent fronts. f) Ligation of **8** with **9**. g) Analytical HPLC traces (λ = 214 nm) for **8**, ligation mixture of **8** and **9** after 10 min and 3 h. h) Treatment of **5** with H_2O_2 in the ligation buffer. i) Analytical HPLC traces (λ = 214 nm) after treatment of **5** (in the presence of 65 equiv. MPAA) with 1 equiv. H_2O_2 (50 min), 33.5 equiv. H_2O_2 (10 min), and 66 equiv. H_2O_2 (10 min).

First, we synthesized the pDobz protected cysteine (i.e. pDobz-Cys(Trt)-OH) in which the boronic acid was protected as a pinacol ester (Figure 3b). We then tested the use of pDobz-Cys(Trt)-OH in standard Fmoc SPPS followed by regular trifluoroacetic acid (TFA) cleavage (Figure 3c). The desired peptide (**5**) was obtained smoothly, in which the free boronic acid group was generated. The next test was the native chemical ligation of Dobz-Cys-peptide-$NHNH_2$ (**5**) under the previously described $NaNO_2$ activation and MPAA thiolysis conditions (Figure 3d).[10] The expected peptide thioester intermediate (**6**) was formed cleanly, which reacted *in situ* with subsequently added Cys to produce the ligation product **7** in almost quantitative yield after 60 min (Figure 3e). Some minor products due to formation of thiolactone with the internal Cys and lactam with C-terminal Lys[11] were observed. To further confirm the stability of the Dobz-amino protecting group to the $NaNO_2$ oxidation conditions, another test of Dobz-Cys-peptide-$NHNH_2$ was performed (Figure 3f) in which Ala was the ligation site and the middle Cys was eliminated. In this test the ligation proceeded more cleanly without the formation of any thiolactone or lactam byproducts (Figure 3g). Collectively these results showed that the Dobz group was compatible with modern Fmoc SPPS and the native chemical ligation of peptide hydrazides.

A critical test for the Dobz group was its selective removal by H_2O_2 oxidation followed by spontaneous hydrolysis. For this purpose we dissolved **5** in a ligation buffer containing 65 equiv. MPAA (pH 6.5-7.0) to simulate the conditions needed by the one-pot ligation (Figure 3h). To test whether or not the H_2O_2 treatment may cause any damage to the peptide, we incorporated all the redox sensitive amino acids (i.e. Cys, Met, Trp, Tyr, His) into **5**. When 1 equiv. H_2O_2 (as 1 M aqueous solution) was added to **5** in the ligation buffer, we observed no change of **5** after 50 min indicating that MPAA was the most susceptible to H_2O_2 oxidation in the system (Figure 3i). To fully consume MPAA and oxidize **5**, we tested the addition of 33.5 equiv. H_2O_2. Gratifyingly, under this condition we observed a complete and clean conversion of Dobz-protected **5** to the deprotected peptide product **11** in 10 min. Further increase of H_2O_2 to 50 equiv. (data not shown) and 66 equiv. was found to generate a by-product whose mass was 16 Da higher than that of **11**. According to the previous study by Schultz et al. on BoPhe-incorporated proteins, this by-product was assigned as peptide **12** with Met oxidized.[17] Nonetheless, even 66 equiv. H_2O_2 did not fully oxidize **11** to **12**. Collectively the above results suggested that H_2O_2 should oxidize MPAA first and then oxidize and remove the Dobz group selectively. Met would be oxidized if excessive H_2O_2 was added, but this side reaction could be prevented by using an accurately calculated quantity of H_2O_2.[19] Furthermore, use of norleucine to replace Met,[20] which has been a frequently employed tactic in protein chemical synthesis, would completely circumvent the Met oxidation problem. It is important to emphasize that except

for Met (and Cys that forms disulfides), we did not observe the reaction of H_2O_2 with any other amino acid residues (in particular, Tyr or Trp) under the conditions used to remove the Dobz group.[19]

Total synthesis of modified histones

The efficient and selective oxidation of the Dobz group by H_2O_2 and its removal from the N-terminal Cys allowed for the design of a H_2O_2-controlled one-pot ligation method suitable for peptide hydrazides (Figure 1). From the perspective of ease of operation, the H_2O_2 treatment did not need any pH change and it released water as the only byproduct. With this key chemistry for one-pot native chemical ligation of peptide hydrazides in hand, we carried out a new synthesis of H3K4me3.

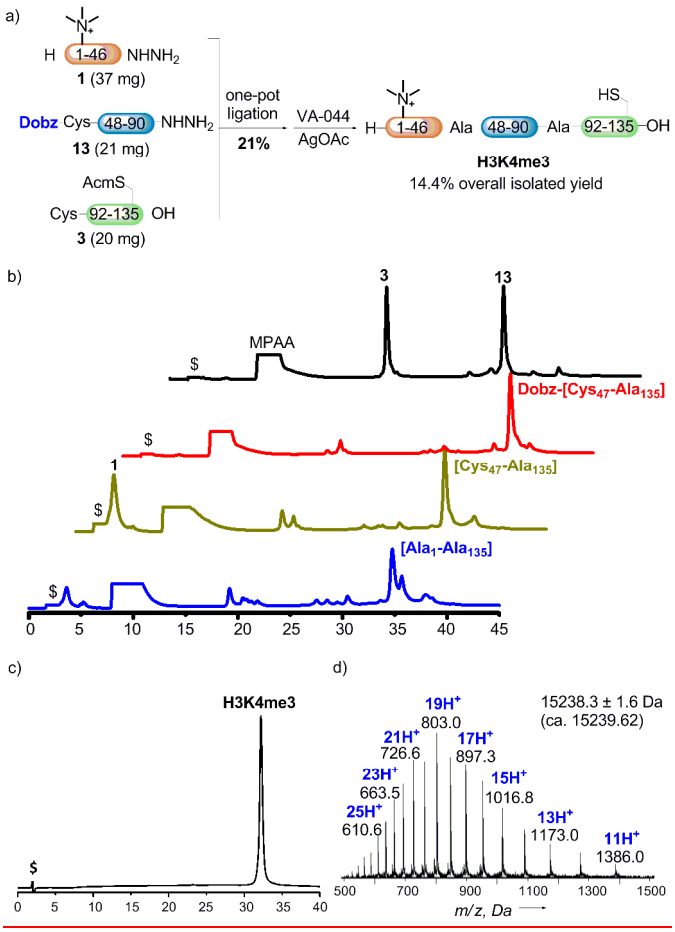

Fig. 4 Synthesis of H3K4me3 by using H_2O_2-controlled one-pot native chemical ligation of peptide hydrazides. a) The overall procedure. b) Analytical HPLC traces (λ = 214 nm) for the different steps in the one-pot ligation. Black: beginning of the whole process; Red: ligation mixture of **3** and **13** after 1 h; Olive: ligation mixture after the H_2O_2 treatment; Blue: the final ligation mixture. "$" denotes solvent fronts. For more explanation of each peak, please refer to Figure S12. c) Analytical HPLC trace (λ = 214 nm) for the final, purified H3K4me3. d) its ESI-MS: observed mass 15238.3 ± 1.6 Da, calc 15239.62 Da (average isotopes).

The three peptide segments (i.e. **1**, **13**, and **3**, Figure 4a) were prepared by using automated Fmoc SPPS and enough purified materials were obtained. However, the key segment **13** was synthesized with a low yield. After analysis of the crude **2** and **13** (Figure S2 and S11), we concluded that the low yield of **13** was due to the nature of the peptide sequence, but not due to the introduction of Dobz group. They were condensed in a one-pot fashion as follows: first, **13** (21 mg) was ligated with **3** (20 mg) at pH 6.8 for 1 hour; 2); second, the reaction mixture was treated with 115 μL of 1 M aqueous H_2O_2 for 10 minutes; and finally, **1** (37 mg, pre-activated by $NaNO_2$ and thiolyzed with MPAA) was added to the reaction mixture. Because the second ligation occurred at a Val-Cys junction, it was allowed to proceed for 48 hours. The full-length peptide was obtained through HPLC purification in 12 mg, corresponding to an isolated yield of 21% for the one-pot reactions (Figure 4b). The HPLC traces of this one-pot transformation were relatively clean, indicating that the intended reactions proceeded smoothly (For explanation of every peak in Figure 4b, please refer to Figure S12). Subsequently we carried out desulfurization and Acm deprotection on HPLC purified materials to generate 8.3 mg target product, H3K4me3, corresponding to 14.4% overall isolated yield. A repeated experiment using 14 mg **13**, 13 mg **3**, and 25 mg **1** afforded 7.7 mg of H3K4me3 corresponding to 19.8% overall isolated yield. Through analysis of the MALDI-TOF spectra (Figure S14) and every peak in ESI-MS (data was not shown), we concluded that Met_{120} in H3K4me3 was not oxidized in the H_2O_2 treatment. Thus our experiments showed that modified H3 could be practically synthesized in multi-milligram scale.

To further characterize the final synthetic H3K4me3, we conducted the isotope envelope analysis (Figure 5a and Figure S15, table S1). The results showed that the observed isotope envelope was fully consistent with the theoretically predicted pattern of target H3K4me3, but not the oxidized side product (+16 Da). Although there were peaks in the region of predicted oxidized product (Figure S15b), we could not exclude that there were caused by baseline noise. Collectively the above results of MALDI-TOF and ESI-MS, we had reason to believe that the oxidation of Met was almost prevented if not completely. In the MS-MS analysis (Figure 5b and Figure S16), the observed fragments matched well with the sequence of H3K4me3, especially that the ligation junction (Gly-Ala) was observed. These experimental results confirmed that the synthetic H3K4me3 was highly homogeneous ~~and the oxidation of Met was prevented~~.

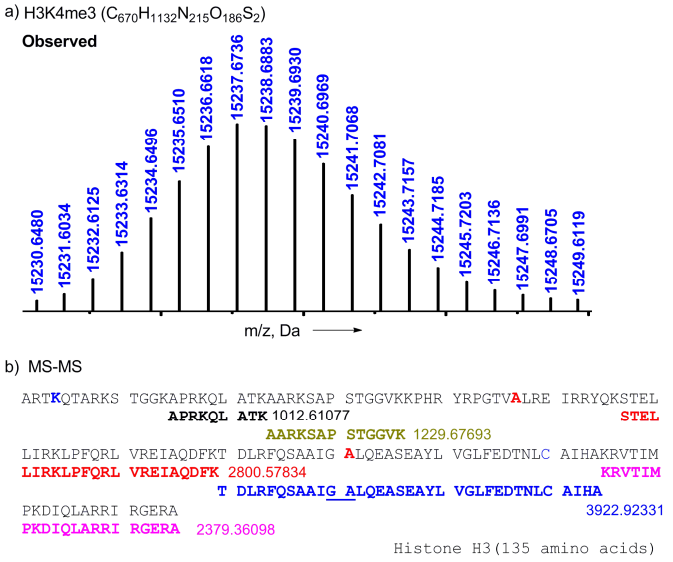

Fig. 5 Isotope envelopes and MS-MS analysis of synthetic H3K4me3. a) The observed isotope envelopes. b) The MS-MS analysis data with the observed fragments marked. For more details please refer to Figures S15 and S16.

For the application of the H_2O_2-controlled one-pot ligation method to the total synthesis of a modified H4 (i.e. H4K16ac), we also divided it into three peptide segments (Figure 6a). They were H4K16ac[Ser_1-Leu_{37}]-$NHNH_2$ (37-mer), Dobz-H4K16ac[Cys_{38}-His_{75}]-$NHNH_2$ (38-mer), and H4K16ac[Cys_{76}-Gly_{102}] (27-mer). Both Ala_{38} and Ala_{76} were changed to Cys to facilitate the ligations, which would be converted back to Ala after the full-length peptide was produced.[14] Again these three peptide segments were prepared by using automated Fmoc SPPS (Figure 6b). The middle segment was also difficult to synthesize due to its hydrophobicity (Figure S18). Comparing the HPLC trace of crude peptide H4K16ac[Cys_{38}-His_{75}]-$NHNH_2$ with a unprotected Cys at the N terminal (data was not shown), we confirmed that the low yield was not caused by the Dobz group.

The three segments of H4K16ac were condensed in a one-pot fashion from 28, 16, and 11 mg of starting materials with the control of 106 μL of 1 M aqueous H_2O_2. The full-length peptide was then obtained through HPLC purification in 11 mg, corresponding to an isolated yield of 27.4% for the one-pot ligation (Figure 6c). Subsequently we carried out desulfurization to generate the target product, H4K16ac, in 7.3 mg corresponding to 18.3% overall isolated yield. A repeated experiment using 14 mg, 8 mg, and 5.5 mg of starting materials afforded 4.0 mg of H4K16ac corresponding to 20.1% overall isolated yield. Again, the HPLC traces of this one-pot transformation (Figure 6c) were relatively clean, indicating that designed reactions proceeded smoothly (For explanation of every peak in Figure 6c, please refer to Figure S20). Note that Met_{84} in H4K16ac was not oxidized in the H_2O_2 treatment (Figure 6e and Figure S21). Besides, in the synthesis we observed that *ca.* 30% of Dobz-H4K16ac[Cys_{38}-His_{75}]-$NHNH_2$ was hydrolyzed during the ligation (Figure 6c). The hydrolysis byproduct was almost inseparable from the product of the first ligation (i.e. Dobz-H4K16ac[Cys_{38}-Gly_{102}]. Thus, the one-pot ligation also circumvented the intermediate separation problem that would be expected in the stepwise synthesis of H4K16ac. Furthermore, synthetic H4K16ac was successfully characterized by the isotope envelope and MS-MS analysis (Figure 7 and Figures S22, S23 and Table S2). Collectively the above results of characterization confirmed that synthetic H4K16ac was highly homogenous and the potential side reaction of oxidation was not a problem.

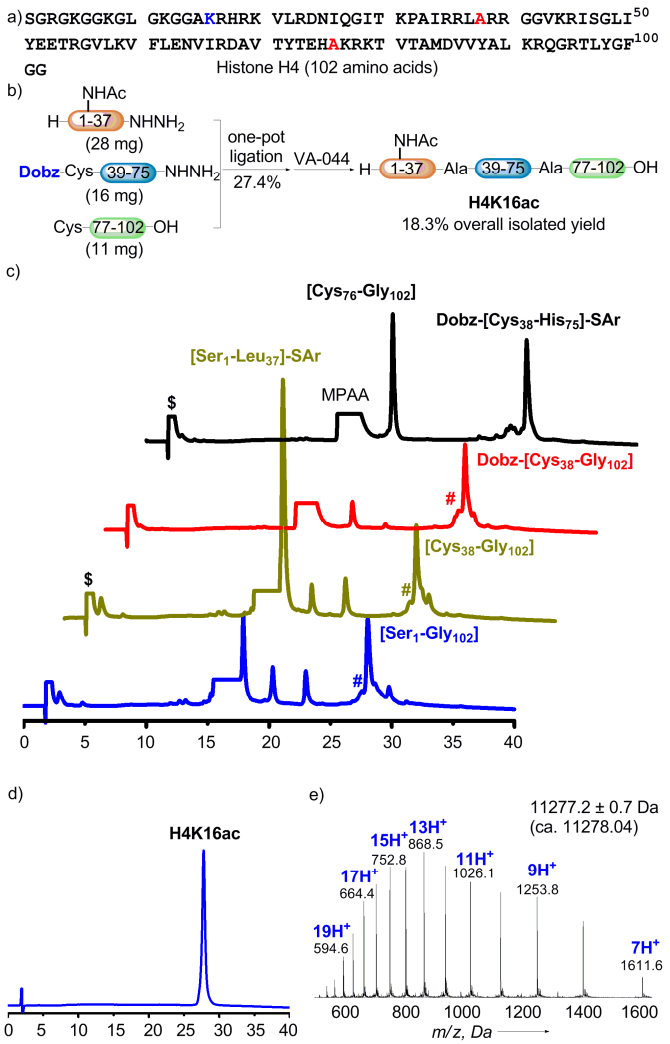

Fig. 6 Synthesis of H4K16ac by using H_2O_2-controlled one-pot ligation of peptide hydrazides. a) Sequence of H4. b) The overall procedure. c) Analytical HPLC traces (λ = 214 nm) for the different steps in the one-pot ligation. Black: beginning of the first ligation; Red: reaction mixture after the first ligation; Olive: ligation mixture after the H_2O_2 treatment; Blue: the final ligation mixture. #correspond to the hydrolysis byproduct. Red: Dobz-[Cys_{38}-His_{75}]-OH; Olive: [Cys_{38}-His_{75}]-OH; Blue: [Ser_1-His_{75}]-OH."$" denotes solvent fronts. For explanation of other peaks, please refer to Figure S20. d) Analytical HPLC trace (λ = 214 nm) for the final, purified H4K16ac. e) its ESI-MS: observed mass 11277.2 ± 0.7 Da, calc 11278.04 Da (average isotopes).

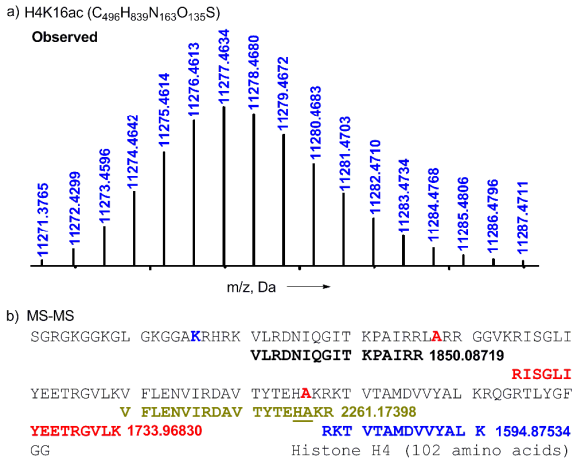

Fig. 7 Isotope envelopes and MS-MS analysis of synthetic H4K16ac. a) The observed isotope envelopes. b) The MS-MS analysis with the observed fragments marked. For more details, please see Figures S22 and S23.

Histone octamer reconstitution

The purity and identity of the synthetic H3K4me3 and H4K16ac were successfully characterized by the HPLC, MALDI-TOF, ESI-MS analysis as well as isotope envelope and MS-MS analysis (Figures 4-7). To conduct biophysical characterizations, we dissolved the synthetic H3K4me3 and H4K16ac in an unfolding buffer containing 6 M guanidine hydrochloride, 5 mM dithiothreitol, and 20 mM Tris-HCl, pH 8.0. After 2 hours the solution was subjected to four rounds of dialysis against refolding buffer containing 2.0 M sodium chloride, 1 mM Na-EDTA, 5 mM dithiothreitol, and 20 mM Tris-HCl, pH 8.0 at 4 °C for about 48 hours. Gratifyingly, we identified and isolated the refolded H3K4me3-H4K16ac tetramer through gel filtration chromatography (Figure 8).

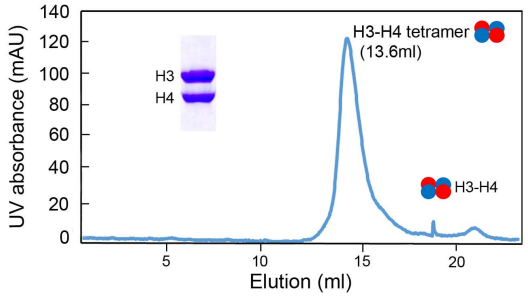

Fig. 8 Histone tetramer formation of synthetic H3K4me3 and H4K16ac. Blue curve: gel filtration profile of histone H3-H4 tetramer eluted over a Superdex 200 10/300 column. The peak of H3-H4 tetramer was eluted at 13.6 ml. Left to the peak: SDS-PAGE of the H3-H4 tetramer peak stained by commassie brilliant blue.

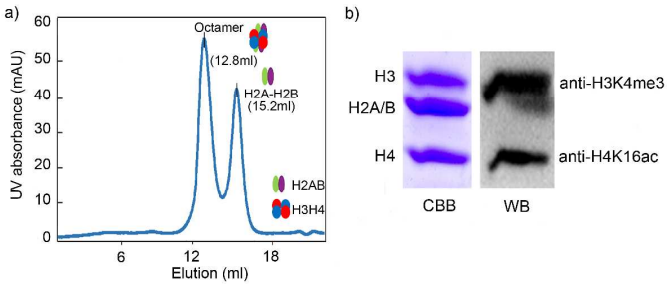

Fig. 9 Histone octamer reconstitution of synthetic H3K4me3-H4K16ac tetramer with H2A-H2B dimer. a) Gel filtration profile of histone octamer eluted over a Superdex 200 10/300 column. Histone octamer was eluted at 12.8 ml; excess H2A-H2B dimer was eluted at 15.2 ml. b) SDS–PAGE (left) and Western blotting (right) analysis of the reconstituted histone octamer. CBB: commassie brilliant blue; WB: Western blotting.

With this tetramer in hand, we next mixed it with a pre-prepared recombinant histone H2A-H2B dimer at molar ratio of 1:2.5. The mixture was then dialyzed against the refolding buffer for four rounds for 48 hours at 4 °C. Through gel filtration chromatography over a Superdex 200 10/300 column (GE Healthcare), we successfully identified and isolated the histone octamer as a single mono-dispersed peak eluted at 12.8 ml (Figure 9a), a retention volume characteristic for histone octamer.[21] Furthermore, the identities of H3K4me3 and H4K16ac in the reconstituted histone octamer were confirmed by Western blotting using rabbit anti-H3K4me3 and anti-H4K16ac antibodies (Figure 9b). Collectively, these experiments verified that our synthetic H3K4me3 and H4K16ac had correct biophysical activities that could form histone octamer smoothly and be recognized by antibodies.

Conclusion

Here we report a practical method for the one-pot native chemical ligation of peptide hydrazides. This method made use of a novel application of the Dobz protecting group, which was removable under mild conditions by a fast and selective arylboronate oxidation reaction mediated by H_2O_2, thus drawing attention to the interesting potential of using boron-based functionalities in protein chemical synthesis. Our experiments showed that the H_2O_2-based deprotection, when carefully controlled, was fast, clean and compatible with all the proteinaceous amino acids including Cys, Met, Trp, and Tyr. Thus, the present one-pot approach adds a new tool to the arsenal for the total chemical synthesis of small to medium sized proteins, which may find complementary or unique usefulness to certain protein molecules.

By using the new one-pot method we accomplished the total chemical synthesis of modified histone proteins H3K4me3, H4K16ac, and several others[22] in multi-milligram scale and good homogeneity. Our results showed that such synthesis was fast (time cost = *ca.* 2 weeks for each protein) and scalable with regular research laboratory equipments. Given the fact that the hydrazide-based ligation method is compatible with many post-

translational modifications including phosphorylation, ubiquitination, and glycosylation, we expect that the present method may find applications in the studies on histone posttranslational modifications. Application of the one-pot approach to the total synthesis of histone H2A and H2B as well as the use of fully synthetic histones to study the biology of epigenetics are ongoing in our laboratory.

Acknowledgements

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Notes and references

1 (a) B. D. Strahl and C. D. Allis, *Nature*, 2000, **456**, 41; (b) J.-S. Lee, E. Smith and A. Shilatifard, *Cell*, 2010, **142**, 682.
2 Reviews: (a) A. Dhall and C. Chatterjee, *ACS Chem. Biol.*, 2011, **6**, 987; (b) B. Fierz and T. W. Muir, *Nat. Chem. Biol*., 2012, **8**, 417.
3 Representative examples: (a) J. Guo, J. Wang, J. S. Lee and P. G. Schultz, *Angew. Chem. Int. Ed*., 2008, **47**, 6399; (b) H. Neumann, S. Y. Peak-Chew and J. W. Chin, *Nat. Chem. Biol.*, 2008, **4**, 232.
4 Representative examples: (a) M. D. Simon, F. Chu, L. R. Racki, C. C. de la Cruz, A. L. Burlingame, B. Panning, G. J. Narlikar and K. M. Shokat, *Cell*, 2007, **128**, 1003; (b) F. Li, A. Allahverdi, R. Yang, G. B. J. Lua, X. Zhang, Y. Cao, N. Korolev, L. Nordenskiöld and C. F. Liu, *Angew. Chem. Int. Ed.*, 2011, **50**, 9611.
5 Representative examples: (a) M. A. Shogren-Knaak, C. J. Fry and C. L. Peterson, *J. Biol. Chem.*, 2003, **278**, 15744; (b) S. He, D. Bauman,; J. S. Davis, A. Loyola, K. Nishioka, J. L. Gronlund, D. Reinberg, F. Meng, N. Kelleher and D. G. McCafferty, *Proc. Natl. Acad. Sci. USA.*, 2003, **100**, 12033; (c) M. Shogren-Knaak, H. Ishii, J. M. Sun, M. J. Pazin, J. R. Davie and C. L. Peterson, *Science*, 2006, **311**, 844; (d) R. K. McGinty, J. Kim, C. Chatterjee, R. G. Roeder and T. W. Muir, *Nature*, 2008, **453**, 812; (e) C. Chatterjee, R. K. McGinty, B. Fierz and T. W. Muir, *Nat. Chem. Biol.*, 2010, **6**, 267; (f) J. M. Kee, B. Villani, L. R. Carpenter and T. W. Muir, *J. Am. Chem. Soc.*, 2010, **132**, 14327; (g) B. Fierz, S. Kilic, A. R. Hieb, K. Luger and T. W. Muir, *J. Am. Chem. Soc.*, 2012, **134**, 19548; (h) X. Zhang, F. Li and C.F. Liu, *Chem. Commun.*, 2011, **47**, 1746.
6 Reviews on protein chemical synthesis: (a) S. B. H. Kent, *Chem. Soc. Rev.*, 2009, **38**, 338; (b) V. R. Pattabiraman and J. W. Bode, *Nature*, 2011, **480**, 471; (c) L. Raibaut, N. Ollivier and O. Melnyk, *Chem. Soc. Rev*., 2012, **41**, 7001.
7 (a) J. C. Shimko, J. A. North, A. N. Bruns, M. G. Poirier and J. J. Ottesen, *J. Mol. Biol.*, 2011, **408,** 187; (b) M. Simon, J. A. North, J. C. Shimko, R. A. Forties, M. B. Ferdinand, M. Manohar, M. Zhang, R. Fishel, J. J. Ottesen and M. G. Poirier, *Proc. Natl. Acad. Sci. USA*, 2011, **108**, 12711; (c) J. A. North, J. C. Shimko, S. Javaid, A. M. Mooney, M. A. Shoffner, S. D. Rose, R. Bundschuh, R. Fishel, J. J. Ottesen and M. G. Poirier, *Nucleic Acids. Res.*, 2012, **40**, 10215.
8 S. K. Mahto, C. J. Howard, J. C. Shimko and J. J. Ottesen, *ChemBioChem*, 2011, **12**, 2488.
9 T. Kawakami, Y. Akai, H. Fujimoto, C. Kita, Y. Aoki, T. Konishi, M. Waseda, L. Takemura and S. Aimoto, *Bull. Chem. Soc. Jpn.*, 2013, **86**, 690.
10 (a) G. M. Fang, Y. M. Li, F. Shen, Y. C. Huang, J. B. Li, Y. Lin, H. K. Cui and L. Liu, *Angew. Chem. Int. Ed.*, 2011, **50**, 7645; (b) G. M. Fang, J. X. Wang and L. Liu, *Angew. Chem. Int. Ed.*, 2012, **51**, 10347; (c) J. S. Zheng, S. Tang, Y. K. Qi, Z. P. Wang and L. Liu, *Nature Protoc.* 2013, **8**, 2483; (d) J. S. Zheng, S. Tang, Y. C. Huang, L. Liu, *Acc. Chem. Res.* 2013, **46**, 2475; (e) P. Wang, R. Layfield, M. Landon, R. J. Mayer and R. Ramage, *Tetrahedron Lett*., 1998, **39**, 8711; (f) P. Wang, K. T. Shaw, B. Whigham and R. Ramage, *Tetrahedron Lett.*, 1998, **39**, 8719; (g) J. S. Zheng, M. Yu, Y. K. Qi, S. Tang, F. Shen, Z. P. Wang, L. Xiao, L. H. Zhang, C. L. Tian and L. Liu, *J. Am. Chem. Soc.*, 2014, **136**, 3695; (h) Y. M. Li, Y. T. Li, M. Pan, X. Q. Kong, Y. C. Huang, Z. Y. Hong and L. Liu, *Angew. Chem. Int. Ed.*, 2014, **53**, 2198.
11 P. Siman, S. V. Karthikeyan, M. Nikolov, W. Fischle and A. Brik, *Angew. Chem. Int. Ed.*, 2013, **52**, 8059.
12 (a) D. Bang and S. B. H. Kent, *Angew. Chem. Int. Ed.*, 2004, **43**, 2534; (b) D. Bang, B. L. Pentelute and S. B. H. Kent, *Angew. Chem. Int. Ed.*, 2006, **45**, 3985; (c) Z. Tan, S. Shang and S. J. Danishefsky, *Angew. Chem. Int. Ed.*, 2010, **49**, 9500; (d) N. Ollivier, J. Vicogne, A. Vallin, H. Drobecq, R. Desmet, O. Mahdi, B. Leclercq, G. Goormachtigh, V. Fafeur and O. Melnyk, *Angew. Chem. Int. Ed.*, 2012, **51**, 209; (e) R. Okamoto,; K. Morooka and Y. Kajihara, *Angew. Chem. Int. Ed.*, 2012, **51**, 191; (f) A. Otaka, K. Sato, H. Ding, A. Shigenaga, K. Sato, A. Shigenaga, K. Kitakaze, K. Sakamoto, D. Tsuji, K. Itoh and A. Otaka, *Angew. Chem. Int. Ed*., 2013, **52**, 7855.
13 For instance, the hydrazide method is not compatible with the widely-used Thz-protected form of N-terminal Cys because the $NaNO_2$ treatment at pH 3 deprotects it (ref 10b).
14 L. Z. Yan and P. E. Dawson, *J. Am. Chem. Soc.*, 2001, **123**, 526.
15 (a) B. L. Pentelute and S. B. H. Kent, *Org. Lett.*, 2007, **9**, 687; (b) Y. Y. Yang, S. Ficht, A. Brik and C. H. Wong, *J. Am. Chem. Soc.*, 2007, **129**, 7690.
16 Q. Wan and S. J. Danishefsky, *Angew. Chem. Int. Ed.*, 2007, **46**, 9248.
17 (a) E. Brustad, M. L. Bushey, J. W. Lee, D. Groff, W. Liu and P. G. Schultz, *Angew. Chem. Int. Ed.*, 2008, **47**, 8220; (b) F. Wang, W. Niu, J. Guo and P. G. Schultz, *Angew. Chem. Int. Ed.*, 2012, **51**, 10132; (c) G. C. Van de Bittner, E. A. Dubikovskaya, C. R. Bertozzi and C. J. Chang, *Proc. Nat. Acad. Sci. USA.*, 2010, **107**, 21316.
18 D. S. Kemp and D. C. Roberts, *Tetrahedron Lett.*, 1975, **16**, 4629.
19 Kemp and Roberts reported in Ref. 18 that Met was oxidized *ca.* 400 times slower than the boronic acid, Trp 10^5 times.
20 (a) K. S. A. Kumar, S. N. Bavikar, L. Spasser, T. Moyal, S. Ohayon and A. Brik, *Angew. Chem. Int. Ed.*, 2011, **50**, 6137; (b) S. Ohayon, L. Spasser, A. Aharoni and A. Brik, *J. Am. Chem. Soc.*, 2012, **134**, 3281.
21 Y. Shim, M. R. Duan, X. Chen, M. J. Smerdon and J. H. Min, *Anal. Biochem.*, 2012, **427**, 190.
22 We have also successfully prepared H3K36me3, H4K12ac, and H4K5acK8acK12acK16ac in multi-milligram scale (Please see Supporting Information).