Convergent Synthesis

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Convergent Versus Sequential Protein Synthesis: The Case of Ubiquitinated and Glycosylated H2B**

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Abstract: *The chemical synthesis of a protein from four fragments or more applying native chemical ligation could be achieved stepwise, in one-pot, convergently, or on a solid support. With the increasing demands of applying protein synthesis to highly complex targets, examining these approaches becomes essential to achieve highly efficient synthesis. Different chemical synthetic strategies are compared for the preparation of the H2B protein having different post-translational modifications. The analogues include H2B that is ubiquitinated at Lys34, Lys120, glycosylated at Ser112, and doubly modified with ubiquitin and N-acetylglucosamine. This study demonstrates that the applied convergent strategy for the synthesis of most of these complex targets was better than the one-pot approach in terms of yield and purity. Some guidelines are offered for future synthetic endeavors of similar challenging proteins.*

The chemical synthesis of a protein target from four peptide fragments or more by applying native chemical ligation (NCL)[1] could be achieved sequentially, in one-pot,[2] convergently[3] or on a solid support.[4] With the increasing demands of applying protein synthesis to highly complex targets in terms of size and molecular composition,[5] examining these different approaches becomes essential to achieve highly efficient synthesis and deliver the target protein in high homogeneity and workable quantities. In such synthetic endeavors, the most efficient approach must be sought and the most suitable reactive C-terminal functionality found for some of the peptide building blocks, which should be tuned or controlled depending on the adopted strategy. This is also true for the orthogonal protecting groups, which are employed along the synthesis to control the site of the attachment with other peptide fragments and/or a specific modification such as glycosylation. Herein we present the challenges and the different solutions in the synthesis of singly or doubly modified H2B with ubiquitin (Ub) or/and *N*-acetylglucosamine (GlcNAc) applying stepwise, one-pot, and convergent approaches.

Post-translational modifications (PTMs) of histones play important roles in regulating chromatin structure, transcription, and DNA damage repair.[6] For example, ubiquitination of H2B at Lys120 (H2BK120Ub) activates H3K79 methylation by DOT1 histone methyl transferase.[7] Interestingly, recent studies revealed that H2BK34Ub can directly stimulates histone H3K4 and K79 for methylation in vitro and in cells.[8] On the other hand GlcNAcylation of histone H2B at Ser112 (H2BS112GlcNAc) was recently found to promote ubiquitination of K120 to generate doubly modified histone (H2BS112GlcNAc-K120Ub), and its fate and interacting proteins still unclear.[9] To better understand how these modifications achieve their function at the molecular level there is a need to prepare these analogues in high homogeneity and workable quantities.[10] For example, our group reported the semisynthesis of H2BK120Ub and the chemical synthesis of H2BK34Ub, which were instrumental for a variety of biochemical and biological studies.[3c,11] Nevertheless, the challenges that we faced during the total synthesis of H2BK34Ub and the needs to prepare other analogues of this histone triggered us to examine alternative synthetic strategies to find the optimal synthetic approach for the specific site of modification. Herein we report on the total chemical synthesis of histone H2BK34Ub by sequential ligation, H2BS112GlcNAc, H2BK120Ub, and H2BS112GlcNAc-K120Ub by a convergent and one-pot approach. This enabled the direct comparison between these different strategies for future scale-up preparation of these analogues and should offer some guidelines for future synthetic endeavors of similar challenging proteins.

Our previous chemical synthesis of HA-H2BK34Ub employing the convergent strategy[3c] has the limitations to achieve workable quantities owing to the low yield of some of the peptide fragments, which required special protection of the δ-mercaptolysine[12] as well as poor yield in the oxidation-ligation[13] step, which led to the overall yield of this target of about 1%. To circumvent these problems, we decided to examine a sequential synthesis with as many as ligation in one-pot manner. The polypeptide was divided into four segments, HA tag H2B(1-20), H2B(21-57), H2B(58-96), and H2B(97-125), (Scheme 1). The HA tag was included to enable detection of these constructs in our ongoing biochemical and biophysical studies. The fragments HA-H2B(1-20) and H2B-(21-57) were prepared with Nvoc-protected at ε-amine of C-terminal Lys to minimize lactamization during the ligation step.[3c,14] To accomplish isopeptide chemical ligation (ICL) with the Ub-thioester at K34, we incorporated δ-mercaptolysine[3c] in the H2B(21-57) fragment. The N-terminal Ala

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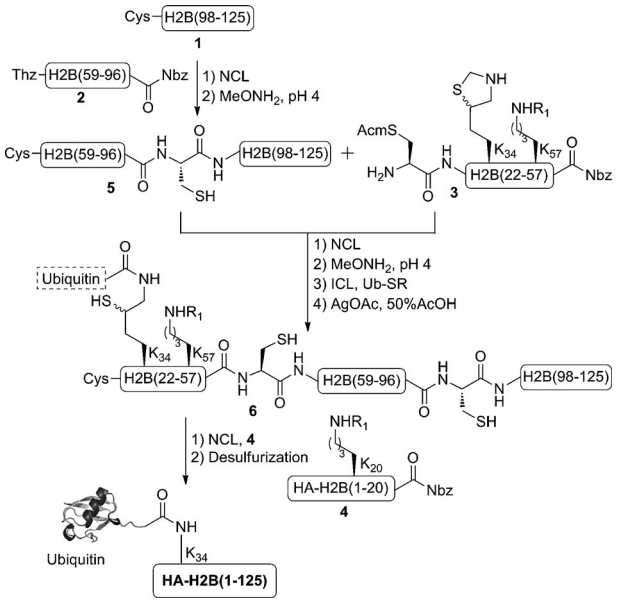


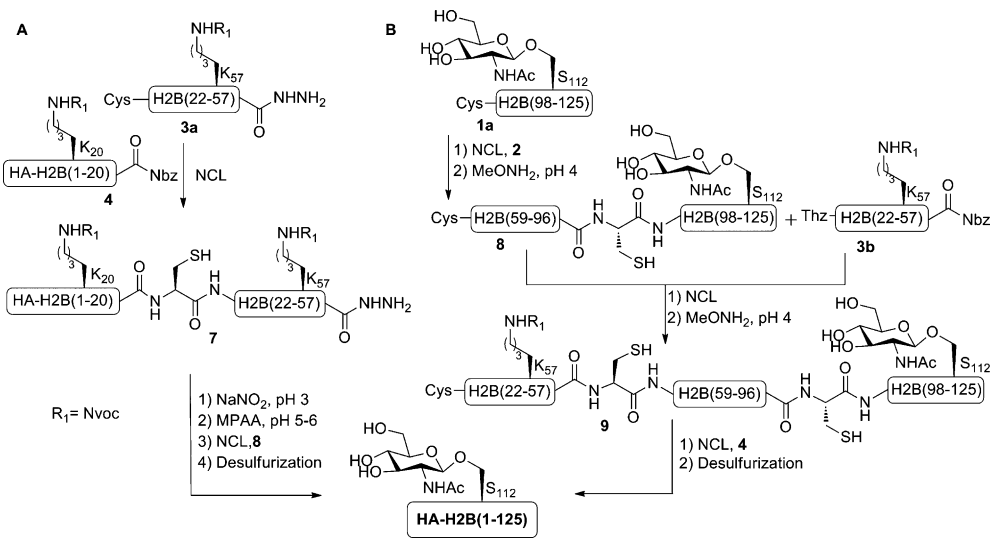
Scheme 1. Synthesis of HA-H2BK34Ub using a sequential strategy.

residues in peptide H2B(21-57) and H2B(97-125) were substituted with Cys to give peptide **3** and **1**, respectively, and in H2B(58-96) with Thz to give peptide **2**. Fragments **2**, **3**, and **4**, which carry activated C-termini, were synthesized using the N-acylurea method.[15] To minimize the lactamization side product in fragment **4**, which we observed during previous synthesis, we protected the side chain Lys with Nvoc group. Overall the synthesis of all the fragments through Fmoc-SPPS was straightforward and gave the pure peptides in very good yields (Supporting Information). The sequential synthesis of HA-H2BK34Ub started with the ligation of **1** and **2** in the presence of mercaptophenylacetic acid (MPAA) and 2-carboxy(ethyl phosphine) hydrochloride (TCEP·HCl). After the completion of the reaction, the crude mixture was subjected with $MeONH_2{\cdot}HCl$ at pH ≈ 4 for 8 h, which underwent Thz ring opening to furnish peptide **5**, H2B(58-125). Prior to the next ligation the pH was adjusted to about 7 by using 4 M NaOH. Freshly prepared solution of fragment **3** in Gn·HCl buffer was added to this reaction mixture for the second ligation, which proceeded smoothly.

Subsequently, the δ-mercaptolysine at K34 was opened with $MeONH_2{\cdot}HCl$ at pH ≈ 4 for 12 h to give H2B(21-125), followed by pH adjustment to about 7 to enable ICL with ubiquitin thioester in one pot. However, under standard ligation condition, we did not observe any formation of the desired product. Instead, after 8 h we observed the hydrolysis of Ub-MPAA to Ub-COOH as well as a major product of Ub-NHOMe, owing to the excess of $MeONH_2$, while H2B(21-125) remains intact.[16]

To overcome this problem we decided first to deprotect N-terminal Cys bearing the Acm group instead of performing Thz opening. However under these conditions and in presence of AgOAc, 50% AcOH, we observed δ-mercaptolysine thiazolidine opening along with Acm deprotection. This has prompted us to purify H2B(21-125) after unmasking δ-mercaptolysine at K34 to furnish peptide H2B(21-125) in about 40% yield. The peptide Cys(Acm)-H2B(22-125) and Ub-thioester were reacted for 8 h under standard ligation conditions to afford the Cys(Acm)-H2B(22-125)K34Ub in about 50% yield of isolated product (Supporting Information, Figure S9). Subsequently the peptide underwent smooth Acm deprotection to afford peptide **6** in 60% yield. Final ligation of fragments **4** and **6** for 5 h resulted in the formation of HA-H2B(1-125)K34Ub. One-pot desulfurization[17] followed by Nvoc deprotection furnished the full length HA-H2BK34Ub in 32% yield of isolated product over two steps (Supporting Information, Figure S10). Through this sequential synthesis we achieved full-length protein HA-H2BK34Ub in about 4% overall yield, which is four-fold higher than our previous approach.

We were then curious to compare the convergent and one-pot approaches to synthesize HA-H2BS112GlcNAc (Scheme 2). The required fragments **1a, 2, 3a**, and **4** were synthesized by Fmoc-SPPS in good yields. For example, the synthesis of fragment **1a** (Supporting Information, Figure S2) bearing the glycosylated Ser was achieved in circa 45% yield on Rink amide resin by using Fmoc-Ser(GlcNAc)-OH.[18] After having these fragments in hand, we initiated the synthesis of HA-H2BS112GlcNAc through convergent strategy (Scheme 2 A) by ligating peptide **1a** and **2** for 4 h. Subsequently, Thz-opening furnished peptide **8** in 62% yield.

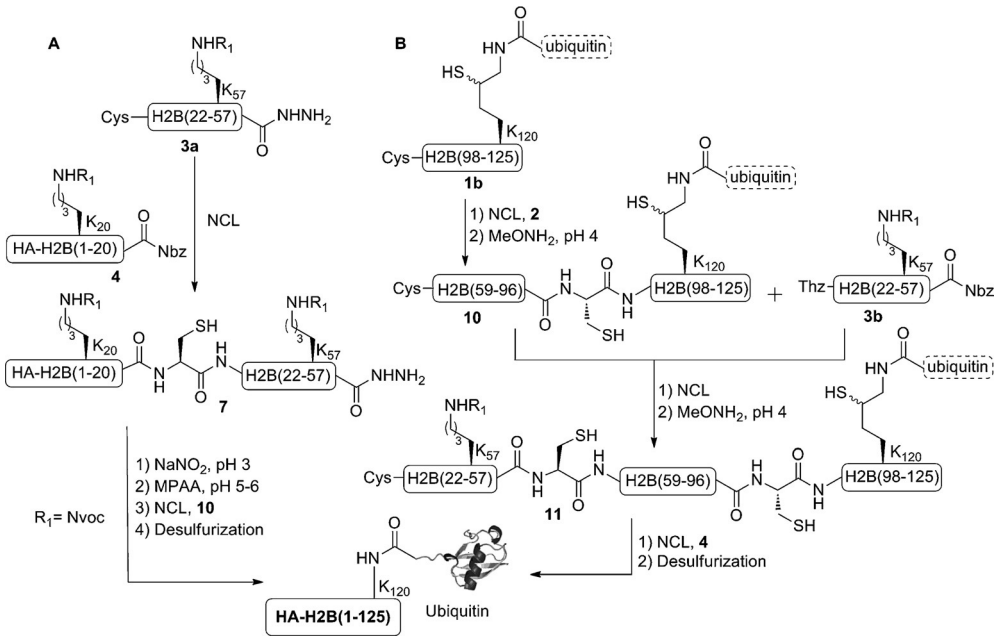


Scheme 2. A) Synthesis of HA-H2BS112GlcNAc applying a convergent strategy. B) Synthesis of HA-H2BS112GlcNAc in one pot.

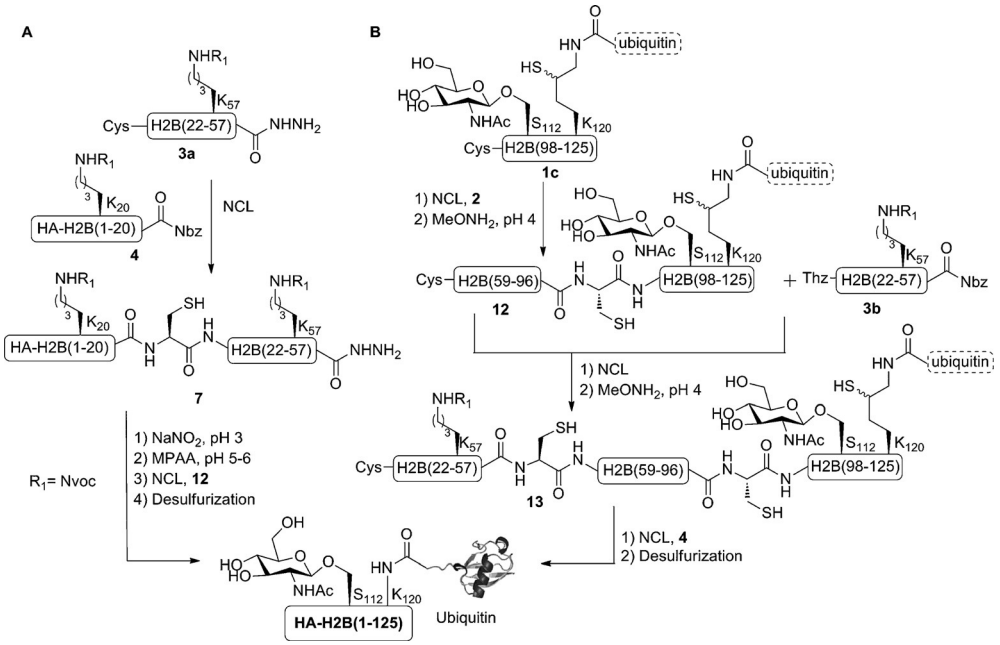
Peptide **7** was prepared in 56% yield by ligating fragments **4** and **3a**, for 6 h. Subsequent oxidation with $NaNO_2$ in Gn·HCl buffer at pH 3 gave the corresponding acyl azide. Further switching was facilitated with MPAA to give HA-H2B(1-57)-MPAA. Prior to the next ligation, we adjusted the pH to 6.5 using 4 M NaOH. Ligation with peptide **8** for 8 h furnished the desired product HA-H2B(1-125)S112GlcNAc in 52% yield, In this case we also observed lactam formation as a side product in less than 8% at K57, despite Nvoc protection, possibly because of partial deprotection of this group during the oxidation step. Final desulfurization and Nvoc deprotection gave the desired product HA-H2BS112GlcNAc in overall circa 17% yield of isolated product (Supporting Information, Figure S11).

We then turned our attention towards the one-pot synthesis of this analogue (Scheme 2B). The required four segments for this strategy (**1a, 2, 3b, 4**) were synthesized using Fmoc-SPPS in about 50% yield (Supporting Information). As with the convergent synthesis, the ligation of segment **2** and **1a** proceeded smoothly to furnish the desired product Thz-H2B(59-125) followed by Thz conversion to Cys. After adjustment of the pH from 4 to 7, peptide **3b** was added to the reaction mixture to afford Thz-H2B(22-125). After the completion of ligation (5 h), Thz was converted into Cys to enable the last ligation step. Peptide thioester **4** was then added to the crude reaction mixture of **9**.

After 6 h, the desired polypeptide, HA- H2B-(1-125)S112GlcNAc was obtained. This crude reaction mixture was then subjected to Nvoc deprotection and desulfurization, in one-pot manner, to give final protein HA-H2BS112GlcNAc in 16% yield of isolated product, with only one purification step (Supporting Information, Figure S12). Next we attempted the synthesis of HA-H2BK120Ub applying the one-pot and convergent approaches. The required four building blocks (**1b, 2, 3b, 4**) were synthesized by Fmoc-SPPS in about 50% yield. To obtain the HA-H2BK120Ub applying the one-pot approach (Scheme 3B), the first ligation reaction was carried out between **1b** and **2**, which proceeded smoothly to give the desired peptide Thz-H2B(59-125)K120Ub within 4 h. This was followed by Thz deprotection to give Cys-H2B(59-



Scheme 3. A) Synthesis of HA-H2BK120Ub applying a convergent strategy. B) synthesis of HA-H2BK120Ub in a one-pot strategy.



Scheme 4. A) Synthesis of HA-H2BS112GlcNAc-K120Ub applying a convergent strategy. B) Synthesis of HA-H2BS112GlcNAc-K120Ub in a one-pot strategy.

125)K120Ub, **10**. To this reaction mixture and after pH adjustment, peptide **3b** was added to enable a second ligation reaction, which gave the Thz-H2B(22-125)K120Ub intermediate. Subsequently, treatment with $MeONH_2$ yielded Cys-H2B(22-125)K120Ub **11**, to which peptide fragment **4** was added for final ligation. After 6 h, the desired ligated product HA-H2B(1-125)K120Ub was obtained. This crude reaction mixture was then dialyzed and subjected to Nvoc deprotection and desulfurization, in one-pot manner to give final product HA-H2BK120Ub in 14% yield of isolated product applying only one purification step (Supporting Information, Figure S15).

To compare the one-pot synthesis of the HA-H2BK120Ub with the convergent approach, we then undertook the synthesis of HA-H2BK120Ub in convergent fashion (Scheme 3A). The ligation reaction between peptides **1b** and **2** was followed by deprotection of Thz to furnish peptide **10** in 61% yield. On the other hand, fragment **3a** was ligated with **4** in presence of MPAA and TCEP to give the desired peptide hydrazide **7**. Subsequent oxidation of **7** with $NaNO_2$ furnished the acyl azide intermediate, to which peptide **10** along with MPAA was added to give the HA-H2B(1-125)K120Ub in 51% yield. Desulfurization and Nvoc deprotection of this ligation product by radical method afforded the target protein HA-H2BK120Ub in overall 17% yield (Supporting Information, Figure S16).

We then turned our attention towards the more challenging target HA-H2BS112GlcNAc-K120Ub, which consists of 210 amino acids and two PTMs. To achieve the synthesis of this target, we incorporated the GlcNAc and Ub in fragment **1c**, (Supporting Information, Figure S18). We initiated the one-pot synthesis (Scheme 4B) by ligating fragment **2** with fragment **1c**, to get the desired peptide Thz-H2B(59-125)S112GlcNAc-K120Ub. After N-terminal Thz conversion to Cys the third fragment Thz-H2B(22-57)-Nbz **3b** was added to the reaction mixture and the ligation proceeded under standard conditions to furnish Thz-H2B(22-125)S112GlcNAc-K120Ub, which upon Thz conversion to Cys afforded Cys-H2B(22-125) **13**, ready for the final ligation. The pH of the crude reaction mixture was adjusted to 7, and the fourth building block **4** was added under standard ligation conditions. To our delight, the reaction proceeded smoothly to furnish the HA-H2B(1-125)S112GlcNAc-K120Ub. Subsequent one-pot desulfurization and Nvoc deprotection gave the final protein HA-H2BS112GlcNAc-K120Ub in about 11% yield of isolated product, after only one purification (Supporting Information, Figure S19).

Next we attempted the synthesis of this analogue using the convergent approach (Scheme 4A). The required four fragments (**1c, 2, 3a, 4**) were synthesized by employing Fmoc-SPPS in about 50% yield. The synthesis started by ligating peptide **1c** and peptide **2** for 4 h, followed by conversion of Thz to Cys for 8 h to furnish peptide **12** in 60% yield. On the other hand, fragment **3a** was ligated with **4**, to afford the desired peptide hydrazide **7**, which after oxidation gave the corresponding acyl azide intermediate. To this we directly added a mixture of peptide **12** and MPAA to enable NCL reaction to obtain HA-H2B(1-125)S112GlcNAc-K120Ub in 55% yield. Simultaneous desulfurization and Nvoc gave the final protein HA-H2BS112GlcNAc-K120Ub in overall circa 16% yield (Supporting Information, Figure S20). The final yields of all the different analogues were calculated based on fragment one used in each synthesis.

Next, we further tested the purity of the H2B analogues by SDS-PAGE to validate the purity determined by HPLC-MS. Proteins that were synthesized in a convergent manner exhibited higher purity than that of one-pot approach (Supporting Information, Figure S21). This could be due to the formation of side reactions such as cross-ligation, which are accumulated with the desired product and were difficult to purify by HPLC, thus resulting in relatively less pure material. These proteins were further purified using size exclusion chromatography and also analyzed by western blot with anti-

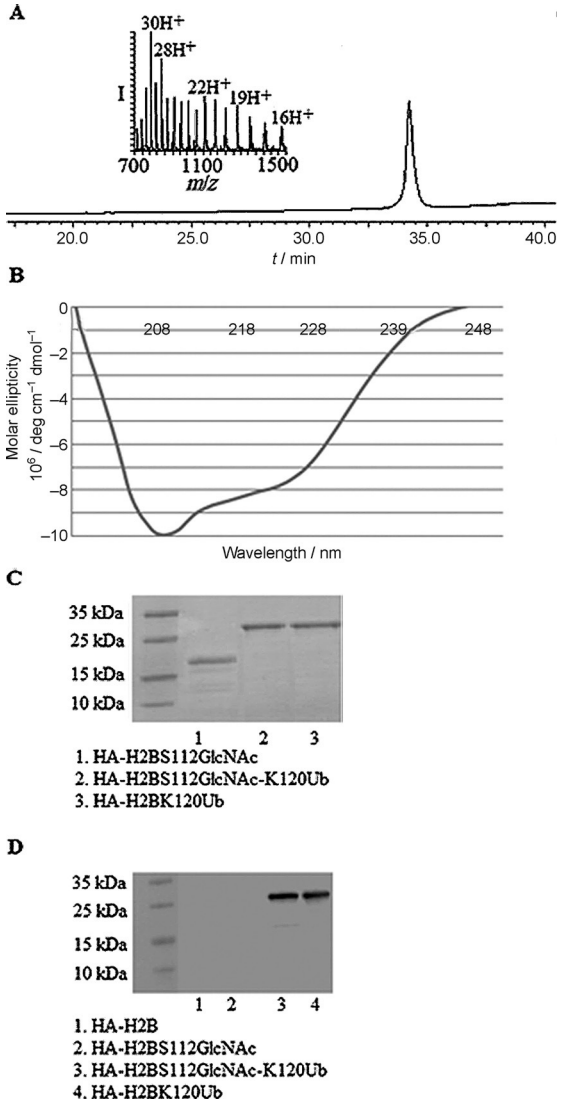


Figure 1. A) Analytical HPLC and mass analysis of purified protein HA-H2BS112GlcNAc-K120Ub with the observed mass 23556.7 ± 1.8 Da, calcd 23554.7 Da, (average isotopes). B) CD analysis of HA-H2BS112GlcNAc-K120Ub, C) SDS PAGE of purified proteins of HA-H2BS112GlcNAc, HA-H2BS112GlcNAC-K120Ub and HA-H2BK120Ub, D) western blot analysis of HA-H2B, HA-H2BS112GlcNAc, HA-H2BS112GlcNAc-K120Ub, and HA-H2BK120Ub proteins using anti-H2BK120Ub antibody.

H2BK120Ub. Interestingly, the doubly modified HA-H2BS112GlcNAc-K120Ub was recognized in a similar manner of the singly modified H2BK120Ub. Additionally, the CD spectrum of HA-H2BS112GlcNAc-K120Ub exhibited similar CD signature to the singly modified H2BK120Ub (Figure 1).

In summary, we successfully accomplished the total chemical synthesis of four different analogues of H2B from four peptide fragments by convergent and one-pot approaches. Based on repeating the synthesis of each analogue three times, our results show that the convergent strategy when applied to complex proteins gave better results in term of yields and purity of the final target compared to the one-pot approach.[19] Our study is the first example of exploring different synthetic routes for protein analogues derived from the same sequence and bearing various modifications. Such a study should give some guideline in selecting the most efficient approach for preparing challenging protein targets for biochemical and biophysical studies.

Keywords: convergent synthesis · H2B protein · native chemical ligation · one-pot synthesis · post-translational modifications

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[1] P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. Kent, *Science* **1994**, *266*, 776 – 779.

[2] a) D. Bang, S. B. Kent, *Angew. Chem. Int. Ed.* **2004**, *43*, 2534 – 2538; *Angew. Chem.* **2004**, *116*, 2588 – 2592; b) N. Ollivier, J. Vicogne, A. Vallin, H. Drobecq, R. Desmet, O. El Mahdi, B. Leclercq, G. Goormachtigh, V. Fafeur, O. Melnyk, *Angew. Chem. Int. Ed.* **2012**, *51*, 209 – 213; *Angew. Chem.* **2012**, *124*, 213 – 217; c) B. Fauvet, S. M. Butterfield, J. Fuks, A. Brik, H. A. Lashuel, *Chem. Commun.* **2013**, *49*, 9254 – 9256; d) S. Tang, Y. Y. Si, Z. P. Wang, K. R. Mei, X. Chen, J. Y. Cheng, J. S. Zheng, L. Liu, *Angew. Chem. Int. Ed.* **2015**, DOI: 10.1002/anie.201500051; *Angew. Chem.* **2015**, DOI: 10.1002/ange.201500051.

[3] a) S. Liu, B. L. Pentelute, S. B. Kent, *Angew. Chem. Int. Ed.* **2012**, *51*, 993 – 999; *Angew. Chem.* **2012**, *124*, 1017 – 1023; b) G. M. Fang, J. X. Wang, L. Liu, *Angew. Chem. Int. Ed.* **2012**, *51*, 10347 – 10350; *Angew. Chem.* **2012**, *124*, 10493 – 10496; c) P. Siman, S. V. Karthikeyan, M. Nikolov, W. Fischle, A. Brik, *Angew. Chem. Int. Ed.* **2013**, *52*, 8059 – 8063; *Angew. Chem.* **2013**, *125*, 8217 – 8221; d) D. Bang, B. L. Pentelute, S. B. Kent, *Angew. Chem. Int. Ed.* **2006**, *45*, 3985 – 3988; *Angew. Chem.* **2006**, *118*, 4089 – 4092; e) K. Sato, A. Shigenaga, K. Kitakaze, K. Sakamoto, D. Tsuji, K. Itoh, A. Otaka, *Angew. Chem. Int. Ed.* **2013**, *52*, 7855 – 7859; *Angew. Chem.* **2013**, *125*, 8009 – 8013; f) T. Durek, V. Y. Torbeev, S. B. Kent, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 4846 – 4851.

[4] a) L. E. Canne, P. Botti, R. J. Simon, Y. Chen, E. A. Dennis, S. B. H. Kent, *J. Am. Chem. Soc.* **1999**, *121*, 8720 – 8727; b) A. Brik, E. Keinan, P. E. Dawson, *J. Org. Chem.* **2000**, *65*, 3829 – 3835; c) D. Bang, S. B. Kent, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5014 – 5019; d) L. Raibaut, H. Adihou, R. Desmet, A. F. Delmas, V. Aucagne, O. Melnyk, *Chem. Sci.* **2013**, *4*, 4061 – 4066; e) I. E. Decostaire, D. Lelievre, V. Aucagne, A. F. Delmas, *Org. Biomol. Chem.* **2014**, *12*, 5536 – 5543; f) M. Jbara, M. Seenaiah, A. Brik, *Chem. Commun.* **2014**, *50*, 12534 – 12537; g) O. Reimann, C. Smet-Nocca, C. P. Hackenberger, *Angew. Chem. Int. Ed.* **2015**, *54*, 306 – 310; *Angew. Chem.* **2015**, *127*, 311 – 315.

[5] a) K. S. Kumar, S. N. Bavikar, L. Spasser, T. Moyal, S. Ohayon, A. Brik, *Angew. Chem. Int. Ed.* **2011**, *50*, 6137 – 6141; *Angew. Chem.* **2011**, *123*, 6261 – 6265; b) M. T. Weinstock, M. T. Jacobsen, M. S. Kay, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 11679 – 11684; c) F. Wintermann, S. Engelbrecht, *Angew. Chem. Int. Ed.* **2013**, *52*, 1309 – 1313; *Angew. Chem.* **2013**, *125*, 1347 – 1351; d) C. Unverzagt, Y. Kajihara, *Chem. Soc. Rev.* **2013**, *42*, 4408 – 4420; e) P. Wang, S. W. Dong, J. A. Brailsford, K. Iyer, S. D. Townsend, Q. Zhang, R. C. Hendrickson, J. Shieh, M. A. S. Moore, S. J. Danishefsky, *Angew. Chem. Int. Ed.* **2012**, *51*, 11576 – 11584; *Angew. Chem.* **2012**, *124*, 11744 – 11752.

[6] a) J. Kim, S. B. Hake, R. G. Roeder, *Mol. Cell* **2005**, *20*, 759 – 770; b) S. L. Berger, *Nature* **2007**, *447*, 407 – 412; c) V. W. Zhou, A. Goren, B. E. Bernstein, *Nat. Rev. Genet.* **2011**, *12*, 7 – 18.

[7] R. K. McGinty, J. Kim, C. Chatterjee, R. G. Roeder, T. W. Muir, *Nature* **2008**, *453*, 812 – 816.

[8] a) L. Wu, B. M. Zee, Y. Wang, B. A. Garcia, Y. Dou, *Mol. Cell* **2011**, *43*, 132 – 144; b) Z. W. Sun, C. D. Allis, *Nature* **2002**, *418*, 104 – 108.

[9] R. Fujiki, W. Hashiba, H. Sekine, A. Yokoyama, T. Chikanishi, S. Ito, Y. Imai, J. Kim, H. H. He, K. Igarashi, J. Kanno, F. Ohtake, H. Kitagawa, R. G. Roeder, M. Brown, S. Kato, *Nature* **2011**, *480*, 557 – 560.

[10] M. M. Müller, T. W. Muir, *Chem. Rev.* **2015**, *115*, 2296 – 2349.

[11] a) G. Fuchs, E. Shema, R. Vesterman, E. Kotler, Z. Wolchinsky, S. Wilder, L. Golomb, A. Pribluda, F. Zhang, M. Haj-Yahya, E. Feldmesser, A. Brik, X. Yu, J. Hanna, D. Aberdam, E. Domany, M. Oren, *Mol. Cell* **2012**, *46*, 662 – 673; b) L. Long, J. P. Thelen, M. Furgason, M. Haj-Yahya, A. Brik, D. Cheng, J. Peng, T. Yao, *J. Biol. Chem.* **2014**, *289*, 8916 – 8930; c) E. Shema-Yaacoby, M. Nikolov, M. Haj-Yahya, P. Siman, E. Allemand, Y. Yamaguchi, C. Muchardt, H. Urlaub, A. Brik, M. Oren, W. Fischle, *Cell. Rep.* **2013**, *4*, 601 – 608.

[12] K. S. Ajish Kumar, M. Haj-Yahya, D. Olschewski, H. A. Lashuel, A. Brik, *Angew. Chem. Int. Ed.* **2009**, *48*, 8090 – 8094; *Angew. Chem.* **2009**, *121*, 8234 – 8238.

[13] G. M. Fang, Y. M. Li, F. Shen, Y. C. Huang, J. B. Li, Y. Lin, H. K. Cui, L. Liu, *Angew. Chem. Int. Ed.* **2011**, *50*, 7645 – 7649; *Angew. Chem.* **2011**, *123*, 7787 – 7791.

[14] F. K. Deng, L. Zhang, Y. T. Wang, O. Schneewind, S. B. Kent, *Angew. Chem. Int. Ed.* **2014**, *53*, 4662 – 4666; *Angew. Chem.* **2014**, *126*, 4750 – 4754.

[15] J. B. Blanco-Canosa, P. E. Dawson, *Angew. Chem. Int. Ed.* **2008**, *47*, 6851 – 6855; *Angew. Chem.* **2008**, *120*, 6957 – 6961.

[16] C. Piontek, D. Varon Silva, C. Heinlein, C. Pohner, S. Mezzato, P. Ring, A. Martin, F. X. Schmid, C. Unverzagt, *Angew. Chem. Int. Ed.* **2009**, *48*, 1941 – 1945; *Angew. Chem.* **2009**, *121*, 1974 – 1978.

[17] a) Q. Wan, S. J. Danishefsky, *Angew. Chem. Int. Ed.* **2007**, *46*, 9248 – 9252; *Angew. Chem.* **2007**, *119*, 9408 – 9412; b) L. Z. Yan, P. E. Dawson, *J. Am. Chem. Soc.* **2001**, *123*, 526 – 533; c) T. Moyal, H. P. Hemantha, P. Siman, M. Refua, A. Brik, *Chem. Sci.* **2013**, *4*, 2496 – 2501.

[18] S. A. Mitchell, M. R. Pratt, V. J. Hruby, R. Polt, *J. Org. Chem.* **2001**, *66*, 2327 – 2342.

[19] J. B. Hendrickson, *J. Am. Chem. Soc.* **1977**, *99*, 5439 – 5450.

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