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

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Supporting Information

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1.1. Materials

The resins, amino acid derivatives, coupling reagents and cleavage reagents were purchased from GL Biochem (Shanghai) Ltd. Nova-PEG Wang Resin was purchased from Novabiochem. DMF, DCM, Et_2O, MeOH, THF, Ac_2O, NH_2NH_2 (85%) and other common reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. 4-(4,4,5,5–Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl methanol and 4-nitrophenyl chloroformate were purchased from Alfa Aesar. Tris(2-carboxyethyl)phosphine (TCEP), DL-dithiothreitol (DTT) and 4-mercapto-benzeneacetic acid (MPAA) were purchased from Sigma-Aldrich. All reagents and solvents were purified when necessary.

1.2. General procedure of Fmoc SPPS

The desired resin was swelled in DMF/DCM = 1:1 for about 2 h. The SPPS procedure was performed on an automated peptide synthesizer (C.S. Bio, Co. CS136XT). The amino acid residues were coupled by 4 equiv of protected amino acid (AA), 8 equiv of DIEA and 3.8 equiv of HCTU to the initial loading of resin. For peptide hydrazides, the first amino acid was double coupled for 1h by 4 equiv of AA, 16 equiv of DIEA and 3.8 equiv of HCTU. The coupling was performed once (60 min) or twice (10 min + 60 min) and the Fmoc was deprotected with 20% piperidine/DMF (5 min + 10 min). Then, the finished peptide was cleaved from the resin with a mixture of 87.5% TFA, 5% water, 5% phenol and 2.5% TIPS. After 2.5 h, the resin was removed by filtration and washed with neat TFA. The combined solution was concentrated under N_2 flowing. Then, the crude peptide was obtained by precipitating in cold Et_2O and centrifugation for three times. Finally, the crude peptide was dissolved in H_2O/CH_3CN and purified by preparative HPLC.

1.3. HPLC

Analytical HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using an analytical column (Grace Vydac “Protein C18”, 150 × 4.6 mm or Grace Vydac “Protein C4”, 250 × 4.6 mm, 5 um particle size, flow rate 1.0 mL/min, rt). Semi-preparative HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using a semi-preparative column (Grace Vydac “Protein C18”, 150 × 22 mm, 10 um particle size, flow rate 8.0 mL/min, rt) used for purification of crude peptides or a semi-preparative column (Grace Vydac “Protein C18”, 250 × 10 mm, 10 um particle size, flow rate 4.0 mL/min, rt). Solvent A was 0.1 % TFA in acetonitrile and solvent B was 0.1 % TFA in water.

1.4. Mass spectrometry and NMR spectrometry

Peptides were confirmed by ESI-MS (Agilent 6210 Time of Flight Mass Spectrometer), MALDI-TOF (ABI 4800 plus MALDI-TOF-TOF mass spectrometer), MS-MS analysis (Thermo Scientific Q Exactive LC-MS/MS) and isotope envelope (Waters Xevo G2). ^{1}H and ^{13}C NMR spectra of small molecular were recorded on an

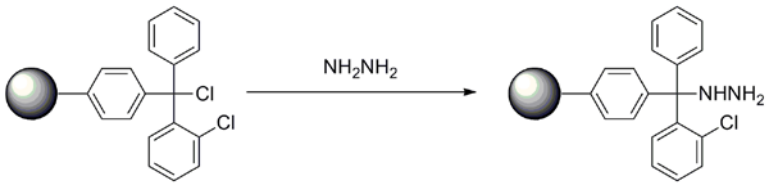
Oxford 400 MHz spectrometer in $CDCl_3$.

1.5. Solutions used in ligation reaction

Ligation buffer: 6.0 M Gn•HCl, 0.2 M Na_2HPO_4 in water;
Acidic ligation buffer: pH = 3.0;
$NaNO_2$ solution: 0.2 mM in neat water;
MPAA solution: 0.2 mM in ligation buffer;
TCEP solution: 57 mg TCEP•HCl in 1mL of ligation buffer (pH = 6.5).

2. Experimental Section……...…S3-41

2.1. Preparation of hydrazine resin



Scheme S1. Preparation of hydrazine resin

2 g 2-Chlorotrityl chloride resin was swelled in DMF (12.5mL) for 15min and cooled to 0 °C. A mixture of DIEA (1.334 mL) and hydrazine hydrate (0.4 g, 85%) in DMF (4 mL) was added slowly. Then, the suspension was stirred at room temperature. After 2 h, 0.8 mL MeOH was added and stirred for additional 15 min. Finally, the resin was washed with DMF, H_2O, DMF, MeOH, Et_2O and dried in vacuo for 2 h.

2.2 Synthesis of H3K4me3 by using N-to-C sequential ligations

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ARTKQTARKS TGGKAPRKQL ATKAARKSAP STGGVKKPHR YRPGTVALRE IRRYQKSTEL
LIRKLPFQRL VREIAQDFKT DLRFQSAAIG ALQEASEAYL VGLFEDTNLC AIHAKRVTIM
PKDIQLARRI RGERA                    Histone H3(135 amino acids)
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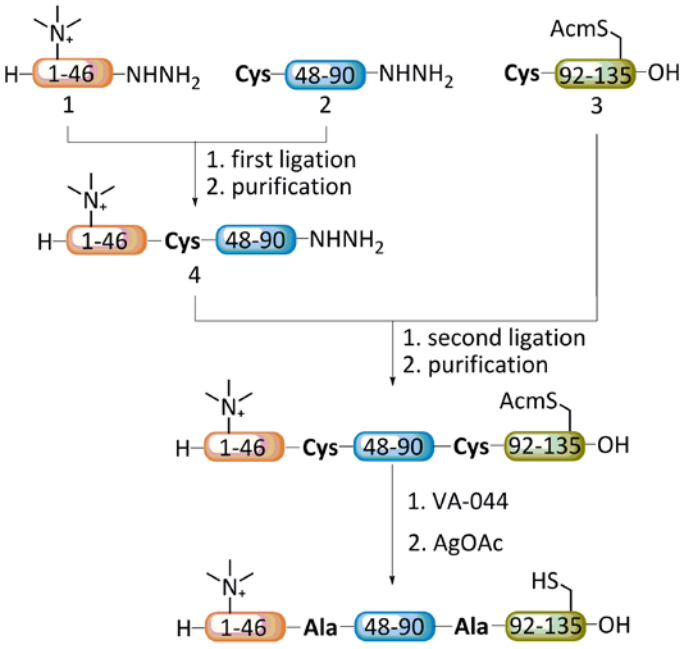


Scheme S2. N-to-C sequential strategy for the synthesis of H3K4me3

Histone H3 contains 135 amino acid residues. There is only one Cys at the site 110, which is not suitable for the ligation. The sequence was divided into three segments and desulfurization would be performed later. Thus we chose Val_{45}-Ala_{46} and Gly_{90}-Ala_{91} as the ligation sites. In order to obtain the native sequence, Cys_{110} was protected by Acm which would be removed after desulfurization. For the three segments, an N-to-C sequential strategy was performed. First, **2** was ligated to **1** and generated the **4**. Next the ligation between **4** and **3** was performed. Then desulfurization and Acm removal were carried out, generating the native H3K4me3.

Synthesis of H3K4me3[Ala_1-Val_{46}]-$NHNH_2$ (1): The synthesis was performed on the automated peptide synthesizer using hydrazine resin (400 mg, 0.25 mmol). The first amino acid was double coupled for 1 h by 4 equiv of AA, 16 equiv of DIEA and 3.8 equiv of HCTU. The Fmoc-Lys(me3)-OH was coupled manually by 2 equiv of AA, 4 equiv of DIEA, 2 equiv of HOAt and 1.9 equiv of HATU to the initial loading of resin and checked by ninhydrin test. After cleavage, the crude peptide was purified on semi-preparative C18 column using a gradient of 5-27 % solvent A over 38 min. Yield: 30.0 %.

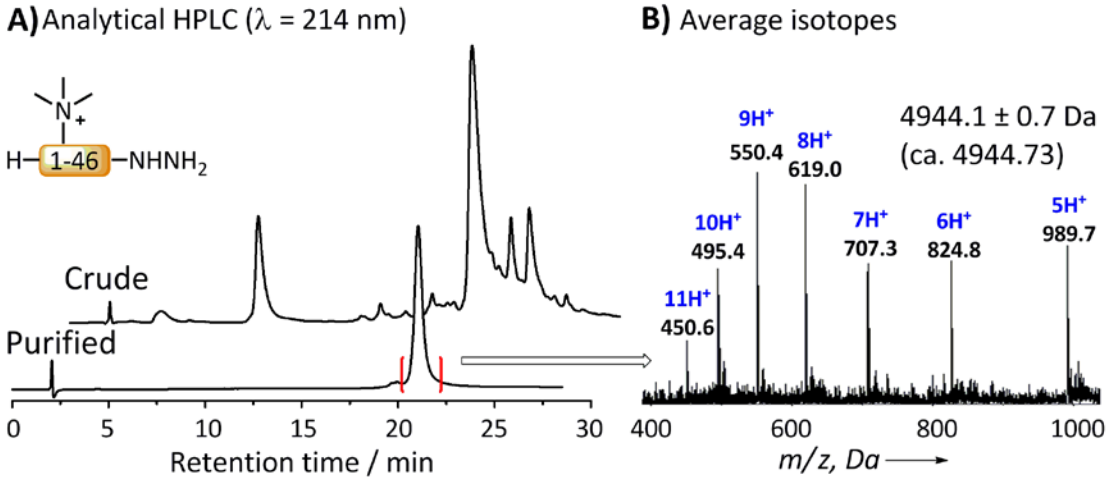


Figure S1. A): HPLC analysis (λ = 214 nm) of the crude and purified **1**. Gradient: 5-30 % solvent A over 38 min. B): ESI-MS analysis of the product. ESI-MS: Observed. 4944.1 ± 0.7 Da, Calcd. 4944.73 Da. (average isotopes).

Synthesis of H3K4me3[Cys_{47}-Gly_{90}]-$NHNH_2$ (2): The synthesis was performed on the automated peptide synthesizer using hydrazine resin (400 mg, 0.25 mmol). All the amino acids were doubly coupled. After cleavage, the crude peptide was purified on semi-preparative C18 column using a gradient of 20-55 % solvent A over 38 min. Yield: 14.5 %.

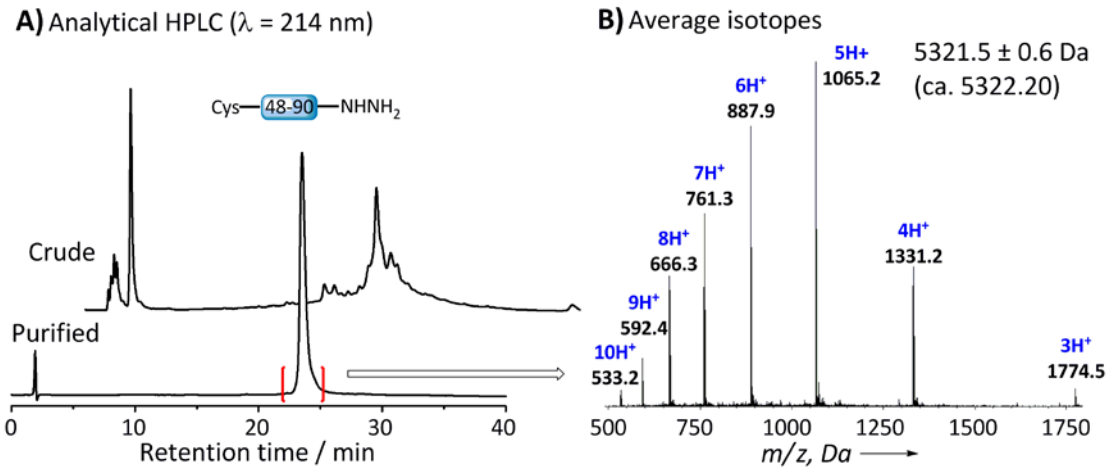


Figure S2. A): HPLC analysis (λ = 214 nm) of the crude and purified **2**. Gradient: 20-55% solvent A over 38 min. B): ESI-MS analysis of the product. ESI-MS: Observed. 5321.5 ± 0.6 Da, Calcd. 5322.20 Da. (average isotopes).

Synthesis of H3K4m3[Cys$_{91}$-Ala$_{135}$] (3): The synthesis was performed on the automated peptide synthesizer using Nova-PEG Wang Resin. The first coupling (4 equiv of amino acid, 16 equiv of DIEA, 4 equiv of HOBt, 0.1 equiv of DMAP and 3.8 equiv of HBTU) was performed overnight. Then the resin was capped with reagent (Acetic anhydride: DIEA: DMF = 1 : 1 : 8). After cleavage, the crude peptide was purified on semi-preparative C18 column using a gradient of 20-50 % solvent A over 38min. Yield: 12.5 %.

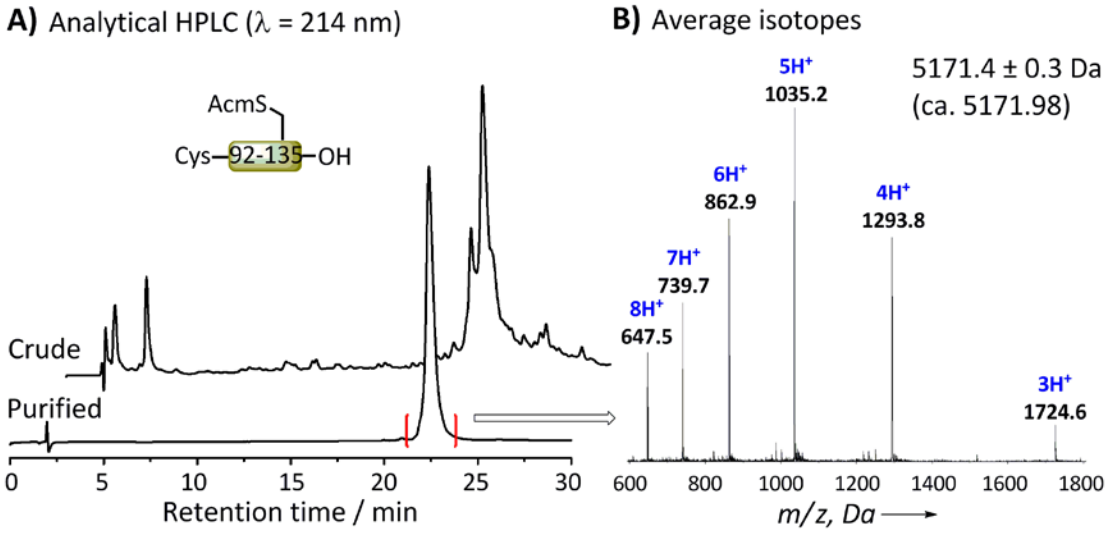


Figure S3. A): HPLC analysis (λ = 214 nm) of the crude and purified **3**. Gradient: 20-50 % solvent A over 38 min. B): ESI-MS of the product. ESI-MS: Observed. 5171.4 ± 0.3 Da, Calcd. 5171.98 Da. (average isotopes).

Ligation of 1 with 2: H3K4me3[Ala$_1$-Val$_{46}$]-NHNH$_2$ (**1**) (23 mg, 4.6 μmol, 1.5 equiv to **2**) was dissolved in 1.0 mL of acidic ligation buffer and cooled to -12 ^{o}C. Then 150 μL of $NaNO_2$ solution was added. After stirring at -12 ^{o}C for 20 min, 1.5 mL of MPAA solution was added. Next, the pH of the mixture was slowly adjusted to

6.8 with NaOH (2 M), and H3K4me3[Cys_{47}-Gly_{90}]-$NHNH_2$ (**2**) (17 mg, 3.2 μmol) was added. The ligation reaction was stirred at room temperature and monitored by analytical HPLC using a gradient of 5-55 % solvent A over 38 min. Due to the ligation site was Val and the HPLC retention time of ligation product was the same as **2**. The reaction was conducted for 36-48 hours. After completion, the ligation solution was reduced by TCEP solution and isolated by HPLC. The isolation yield was 41% approximately and afforded 13.4 mg (1.3 μmol) ligation product H3K4me3[Ala_1-Cys_{47}-Gly_{90}]-$NHNH_2$ (**4**).

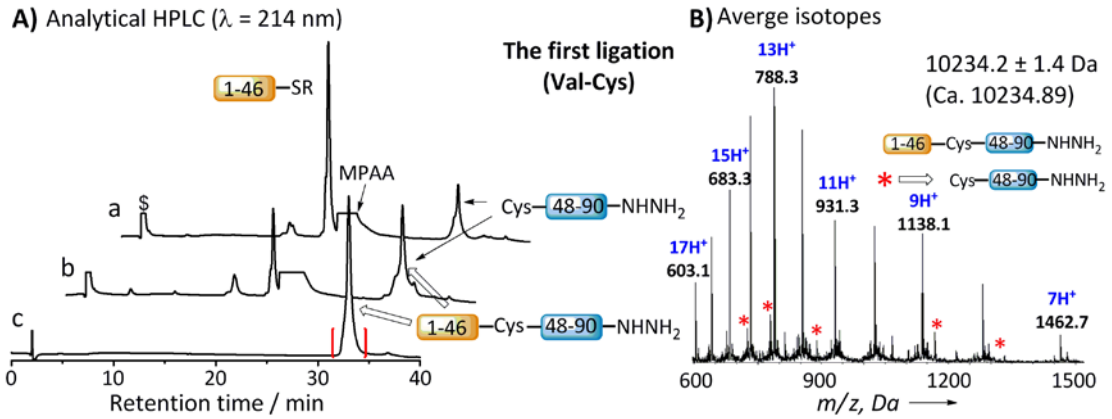


Figure S4. A): analytical HPLC traces (λ = 214 nm) of ligation between **1** and **2** at 0 min (a), 24 h (b) and purified product (c); B): ESI-MS analysis of the ligation product. Observed. 10234.2 ± 1.4 Da, Calcd. 10234.89 Da. (average isotopes). ***Note: the retention times of H3K4me3[Cys_{47}-Gly_{90}]-$NHNH_2$ and H3K4me3[Ala_1-Cys_{47}-Gly_{90}]-$NHNH_2$ were almost identical in the reverse-phase HPLC. The purification of the ligation product was very difficult and microheterogeneity was shown clearly in mass spectra.***

Ligation of 4 with 3: H3K4me3[Ala_1-Cys_{47}-Gly_{90}]-$NHNH_2$ (**4**) (13.4 mg, 1.3 μmol) was dissolved in 0.5 mL of acidic ligation buffer. 42 μL of $NaNO_2$ solution was added under -12 ^oC and kept for 20 min. Then, the mixture was treated by 420 μL of MPAA solution and its pH was slowly adjusted to 6.5 with 2.0 M NaOH solution. At the same time, H3K4me3[Cys_{91}-Ala_{135}] (**3**) (13.4 mg, 2.6 μmol) was added. The reaction was monitored by analytical HPLC using a gradient of 20-60 % solvent A over 38 min. The ligation product was isolated by semi-preparative HPLC after reduction by TCEP solution. The isolation yield was 33% approximately and afforded 6.7 mg (0.44 μmol) product H3K4me3[Ala_1-Cys_{47}-Cys_{91}-Ala_{135}].

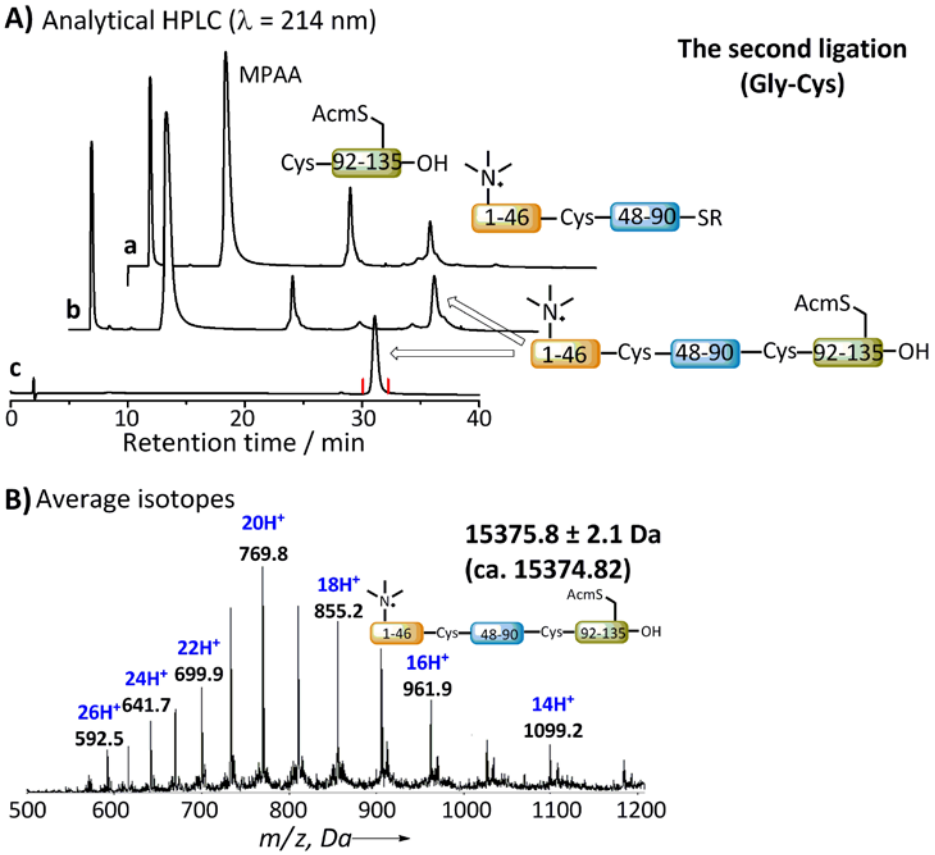


Figure S5. A): analytical HPLC traces (λ = 214 nm) of ligation between **4** and **3** at 0 min (a), 2 h (b) and purified product (c). B): ESI-MS analysis of the ligation product. Observed. 15375.8 ± 2.1 Da, Calcd. 15374.82 Da. (average isotopes). ***Note: Unreacted H3K4me3[Cys$_{47}$-Gly$_{90}$]-NHNH$_2$ in H3K4me3[Ala$_1$-Cys$_{47}$-Gly$_{90}$]-NHNH$_2$ contributed to the complexity of the second ligation. The mass spectra in Figure S5, S6, S7 show evidence of microheterogeneity. Therefore we had to give up the N-to-C sequential ligations.***

Desulfurization: 6.7 mg (0.44 μmol) H3K4me3[Ala$_1$-Cys$_{47}$-Cys$_{91}$-Ala$_{135}$] was dissolved in 1 mL solution of 6.0 M Gn•HCl, 0.2 M Na_2HPO_4 and 1.0 M TCEP (pH = 6.9). To the above solution, 60 μL ^{t}BuSH and 600 μL VA-044 solution (0.1 M in water) was added. The final pH of the solution was adjusted to 6.9 and kept at 37 ^{o}C with stirring overnight. After isolation, H3K4me3[Ala$_1$-Ala$_{47}$-Ala$_{91}$-Ala$_{135}$] was obtained, giving 5.2 mg of product with a yield of 77% .

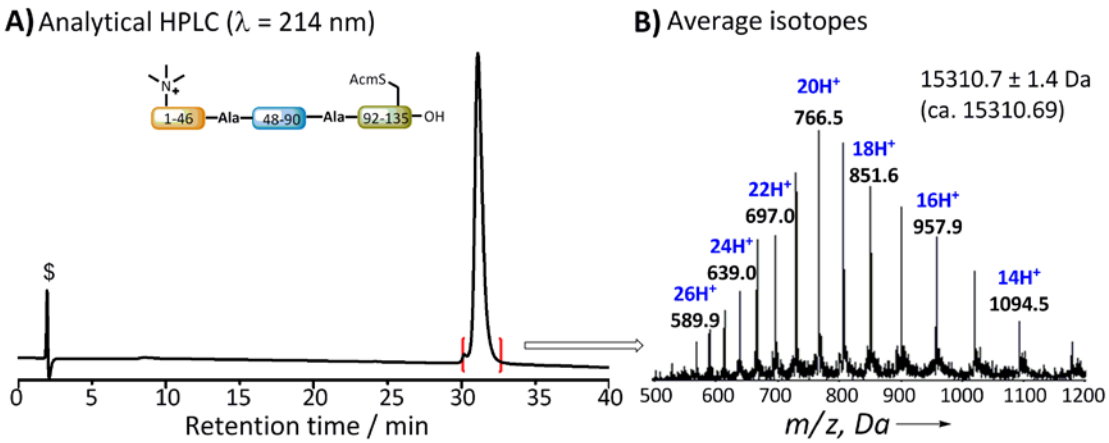


Figure S6. Analytical HPLC trace (λ = 214 nm) and ESI-MS analysis of the product after desulfurization. Observed. 15310.7 ± 1.4 Da, Calcd. 15310.69 Da. (average isotopes).

Acm removal: 5.2 mg (0.34 μmol) H3K4me3[Ala_1-Ala_{47}-Ala_{91}-Ala_{135}] was dissolved in 1.1 mL solution of 1:1 AcOH/H_2O. To the above mixture, 2.0 mg AgOAc was added and stirred at room temperature overnight. After completion, 1.1 mL solution of 1.0 M DTT in 6.0 M Gn•HCl was added and stirred for another 20 min. After centrifugation, the product was isolated by HPLC, giving 5.0 mg native H3K4me3 with a yield of 85%, and the overall isolated yeild was 10.3%.

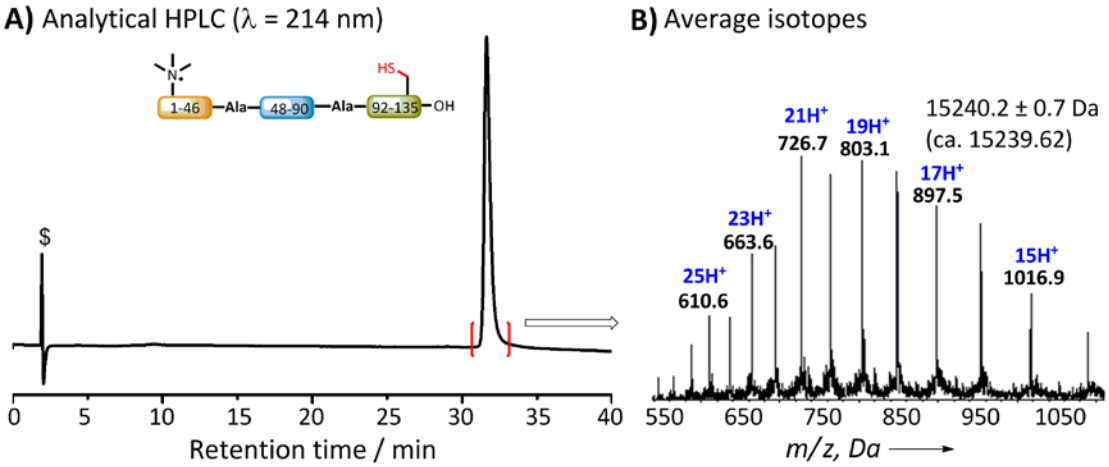
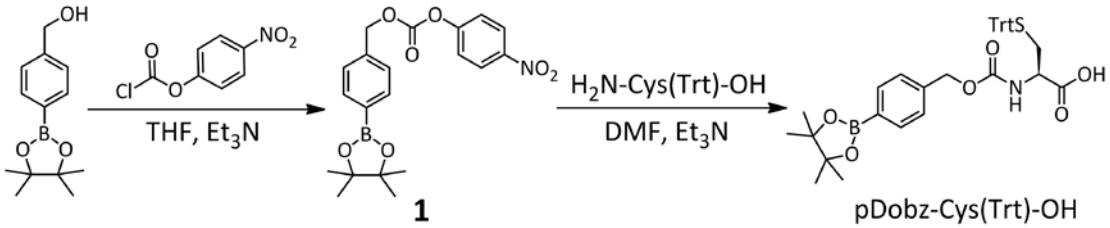


Figure S7. HPLC (λ = 214 nm) and ESI-MS analysis of the final product H3K4me3. ESI-MS: Observed. 15240.2 ± 0.7 Da, Calcd. 15239.62 Da. (average isotopes).

2.3 Tests for the use of Dobz-peptides

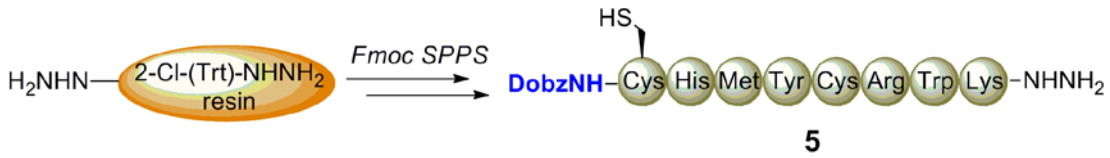
Synthesis of pDobz-Cys(Trt)-OH



Scheme S3. The synthesis of pDobz-Cys(Trt)-OH

Compound 1: 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl methanol (1.17 g, 5.0 mmol) was dissolved in dry THF (10.0 mL). Triethylamine (1.40 mL, 10.0 mmol) and 4-nitrophenyl chloroformate (1.12 g, 5.5 mmol) were added. The mixture was stirred at room temperature for 1 h and evaporated to dryness under vacuo. The residue was dissolved in ethyl acetate (100 mL). The mixture was washed with 1 M HCl, saturated sodium carbonate, brine and dried over Na_2SO_4.The crude product was further purified by column chromatography over silica (ethyl acetate/petroleum ether = 1 : 20) to give compound **1**. Yield: 1.64 g (82%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.27 (d, 2H), 7.85 (d, 2H), 7.44 (d, 2H), 7.38 (d, 2H), 5.31 (s, 2H), 1.35 (s, 12H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 155.51, 152.40, 145.38, 137.13, 135.19, 127.60, 125.26, 121.77, 83.98, 70.76, 24.87.

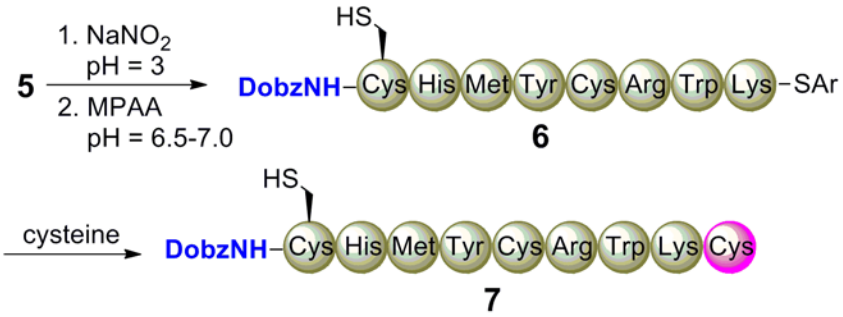
pDobz-Cys(Trt)-OH: Compound 1 (1.64 g, 4.1 mmol) was dissolved in dry DMF (83 mL). H_2N-Cys(Trt)-OH (1.90 g, 5.2 mmol) was added, followed by triethylamine (1.73 mL, 12.4 mmol). The reaction mixture was stirred at room temperature overnight and then evaporated to dryness. The residue was dissolved in ethyl acetate (100 ml). The mixture was washed with water, brine and dried over Na_2SO_4. The crude product was further purified by column chromatography over silica (ethyl acetate/petroleum = 1: 4). Finally, pDobz-Cys(Trt)-OH was obtained as light yellow solids (1.75g, 2.8 mmol, 68 %). HRMS, Calcd for $C_{36}H_{38}BNO_6S$ $[M-H]^-$: 622.5660. Observed: 622.2444. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.80 (d, 2H), 7.40-7.17 (m, 17H), 5.18-5.06 (m, 3H), 3.02-2.86 (m, 1H), 1.35 (s, 12H); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 174.56, 155.76, 144.24, 139.17, 135.02, 129.52, 128.06, 127.09, 126.92, 83.93, 67.18, 67.04, 52.84, 29.73, 24.88



Scheme S4. Synthesis of Dobz-CHMYCRWK-$NHNH_2$ (**5**)

Synthesis of Dobz-CHMYCRWK-$NHNH_2$ (5): The synthesis was performed manually using hydrazine resin. The final amino acid was coupled by 2 equiv of pDobz-Cys(Trt)-OH, 2 equiv of HOAt, 1.9 equiv of HATU and 4 equiv of DIEA and checked by ninhydrin test. After cleavage, the crude peptide was purified on semi-preparative column. ESI-MS: Observed. 1317.6 ± 0.7 Da, 1300.4 ± 0.7 Da

(M-18), Calcd. 1318.36 Da, 1300.36 Da (M-18) (Average isotopes). *Note:* M-18 and M-36 peaks corresponded to the loss of one or two water molecules between Dobz and amino acid side chains via *internal boronate ester formation*. Such internal boronate ester formation was known in other boronic acid-containing systems[1]. In water such internal boronate ester formation reaction should be *reversible*. Indeed, the dehydration peaks disappeared after deprotection of Dobz in our experiments. Therefore, the dehydration process observed in mass spectra was not harmful to the peptides.



Scheme S5. Conversion of 5 to a thioester and its ligation with Cys

Ligation of 5 with Cys: Dobz-CHMYCRWK-$NHNH_2$ (**5**) (1.6 mg, 1.2 μmol) was dissolved in 0.2 mL of ligation buffer (pH = 3.0) and cooled to -12 ^{o}C. 40 μL of $NaNO_2$ was added and kept for 20 min. Then, the reaction mixture was treated by 400 μL of MPAA solution and Cys (0.3 mg, 2.5 μmol). The pH was adjusted to 6.5 with 2.0 M NaOH. The reaction was monitored by analytical HPLC using a gradient of 10-40 % solvent A over 30 min.

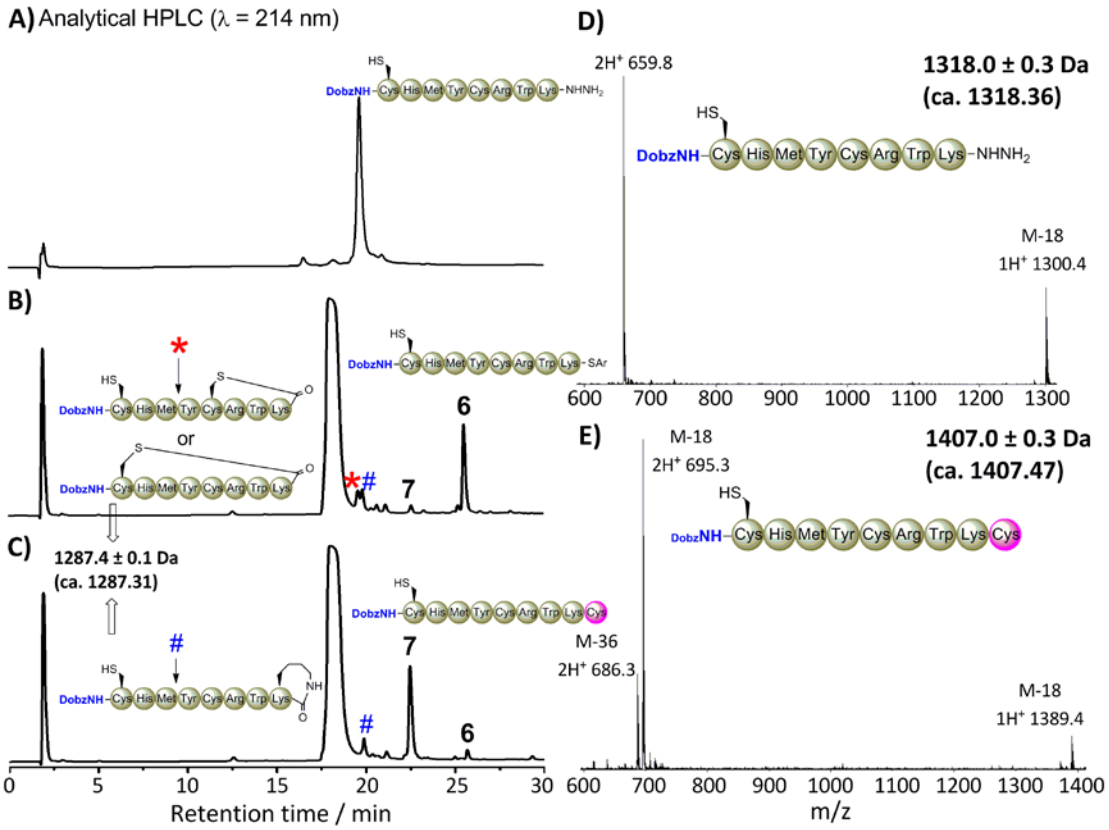


Figure S8. Conversion of **5** to a thioester and its ligation with Cys. A): analytical HPLC traces (λ = 214 nm) of **5**; B): ligation of **5** with Cys at 0 min. C) ligation of **5** with Cys at 60 min. D): ESI-MS analysis of **5**. Observed. 1318.0 ± 0.3 Da, 1300.4 ± 0.3 Da (M-18)[1], Calcd. 1318.36 Da, 1300.36 Da (M-18)[1]. (average isotopes). E): ESI-MS analysis of **7**. Observed. 1389.0 ± 0.3 Da (M-18)[1], Calcd. 1389.46. (average isotopes). ***Note: two side products (thiolactone and lactam of Lys at C-terminal, observed: 1287.4 ± 0.1 Da) were observed.***

Ligation of 8 with 9: Dobz-CRKSGGA-$NHNH_2$ (**8**) (5.0 mg, 5.15 μmol) was dissolved in 0.2 mL of acidic ligation buffer and cooled to -12 °C. 150 μL of $NaNO_2$ solution was added and kept for 20 min. Then, the pH of reaction mixture was adjusted to 6.5 after 1.5 mL of MPAA solution and H_2N-CPRQL-OH (9) (3.83 mg, 5.15 μmol) was added. The reaction was monitored by analytical HPLC using a gradient of 0-25 % solvent A over 30 min.

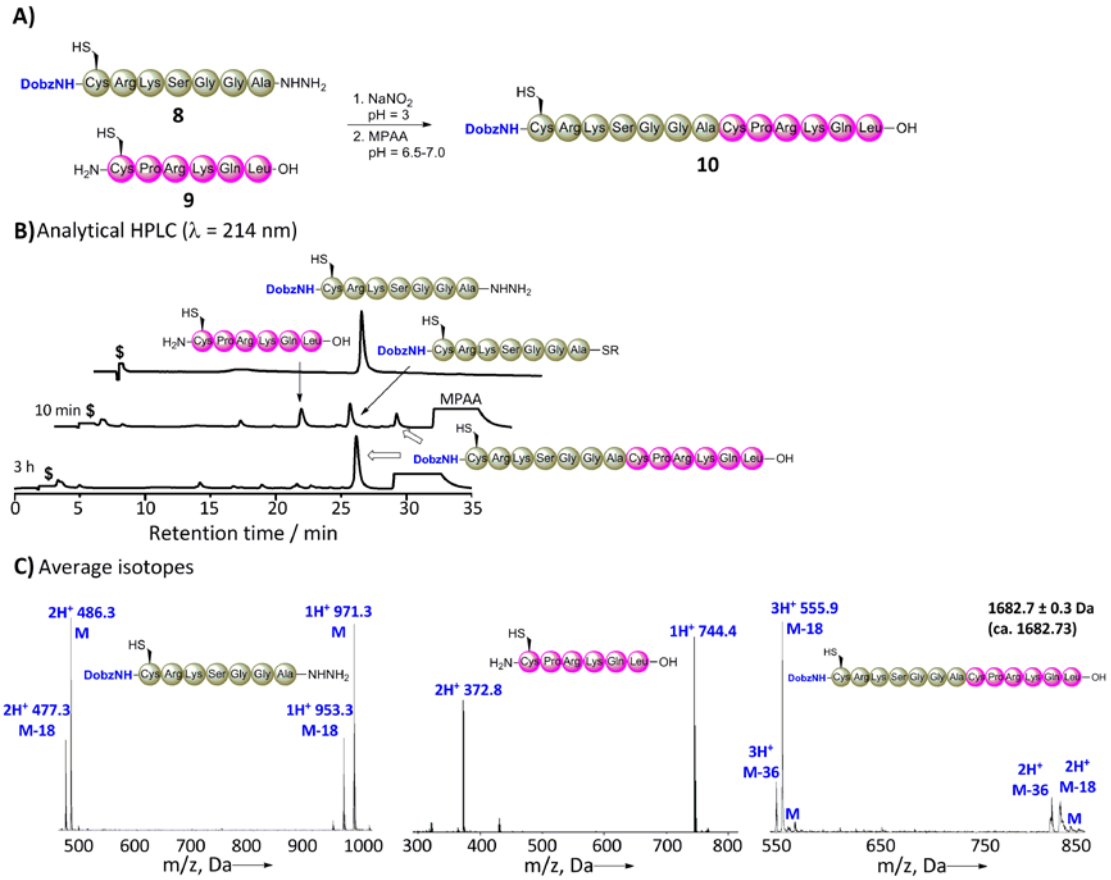
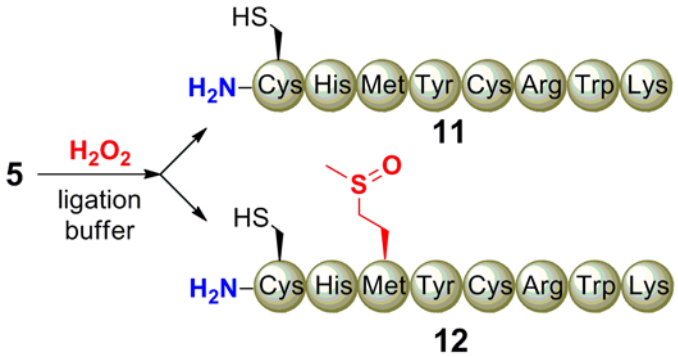


Figure S9. A) ligation of **8** with **9**. B) analytical HPLC traces (λ = 214 nm) of **8** and reaction at 10 min, 3 h. C) ESI-MS analysis of **8**: Observed. 971.0 ± 0.2 Da. Cacld. 970.86 Da. **9**: Observed. 744.0 ± 0.3 Da. Cacld. 743.92 Da. **10**: Observed. 1682.7 ± 0.3 Da. Cacld. 1682.73 Da. Peaks of M-18 and M-36 were observed[1] (average isotopes).



Scheme S6. Dobz removal by H_2O_2

Dobz removal: Dobz-CHMYCRWK-$NHNH_2$ (**5**) (1.6 mg, 1.2 μmol) was dissolved in 0.3 mL solution of ligation buffer (pH = 6.8) and MPAA solution (390 μL, 78 μmol) was added. Then the reaction mixture was treated by H_2O_2 (1 M in water). After 10 min, the reaction was monitored by analytical HPLC using a gradient of 5-50% solvent A over 30 min.

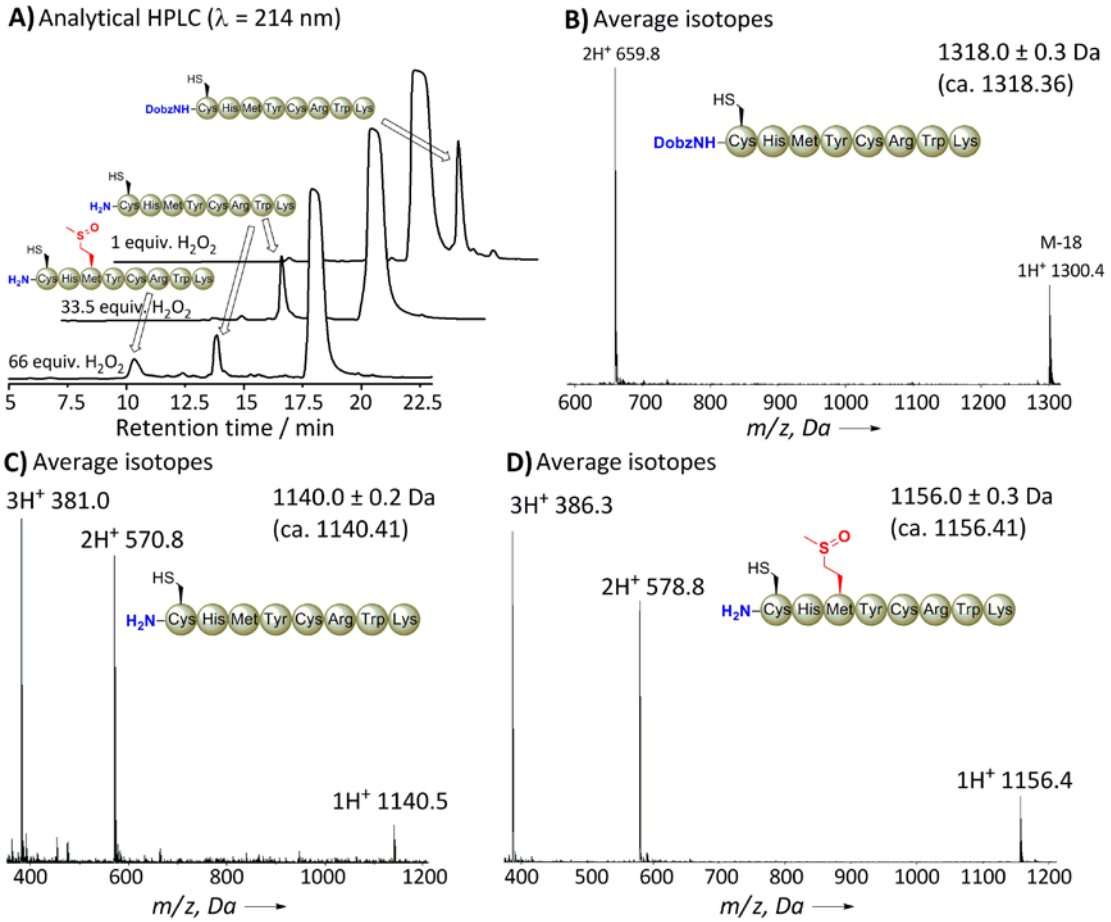
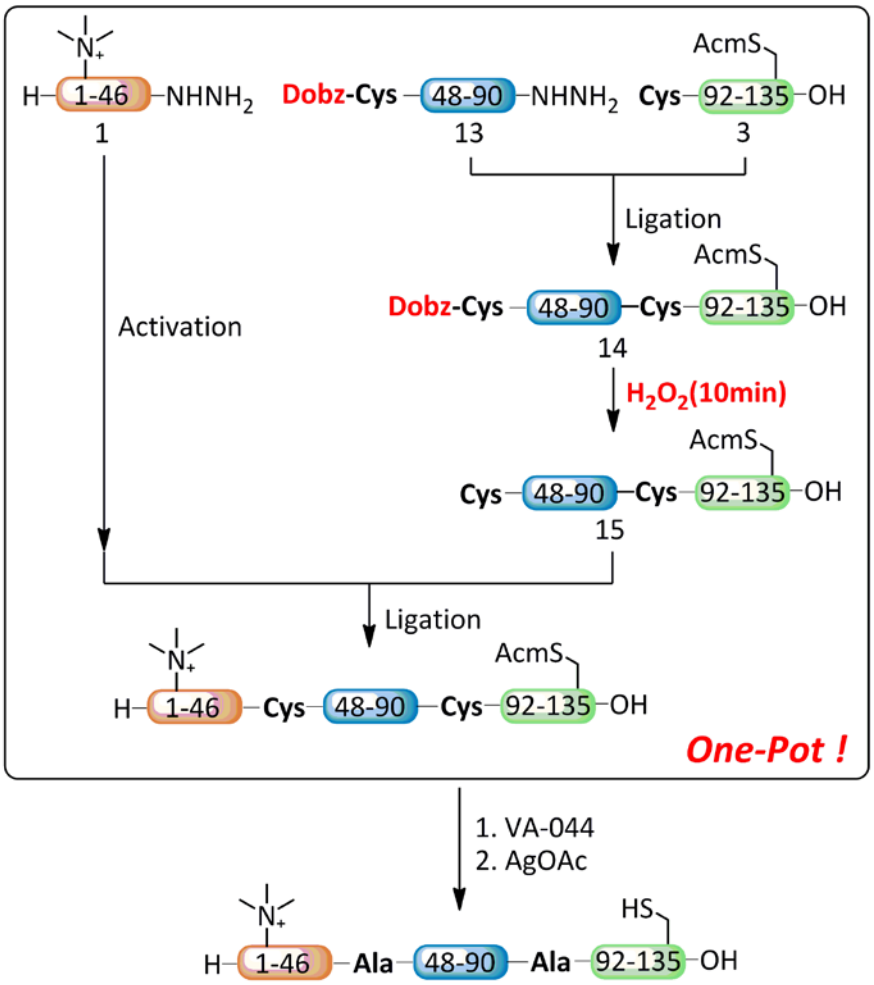


Figure S10. A): analytic HPLC traces (λ = 214 nm) of Dobz removal by H_2O_2 in model peptide. B): ESI-MS analysis of **5**. C): ESI-MS analysis of **11**. Observed. 1140.0 ± 0.2 Da, Calcd. 1140.41 Da. D): ESI-MS analysis of **12**. Observed. 1156.0 ± 0.3 Da, Calcd. 1156.41 Da. (average isotopes).

2.4 Synthesis of H3K4me3 by using H_2O_2-controlled one-pot strategy



Scheme S7. Synthesis of H3K4me3 by using H_2O_2-controlled one-pot strategy

Synthesis of Dobz-H3K4me3[Cys_{47}-Gly_{90}]-$NHNH_2$ (13): The synthesis was performed on hydrazine resin (400 mg, 0.25 mmol) using the same procedure as **2**. The last amino acid was coupled by 2 equiv of pDobz-Cys(Trt)-OH, 2 equiv of HOAt, 1.9 equiv of HATU and 4 equiv of DIEA and checked by ninhydrin test. After cleavage, the crude peptide was purified on semi-preparative C18 column using a gradient of 25-55 % solvent A over 38min. Finally, 88.8 mg of pure product was obtained with a yield of 6.5 %.

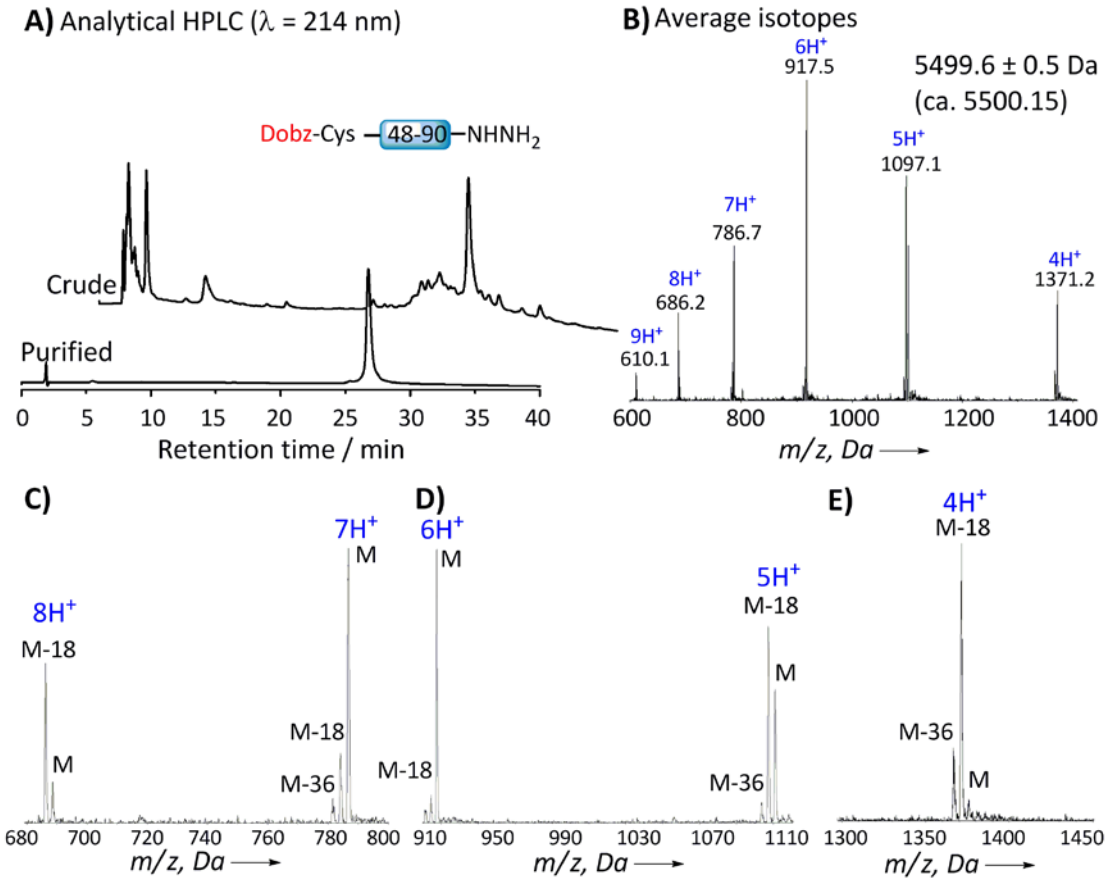


Figure S11. A): HPLC analysis (λ = 214 nm) of the crude and purified **13**. Gradient: 25-55% solvent A over 38 min. B): ESI-MS analysis of **13**. Observed. 5499.6 ± 0.5 Da, Calcd. 5500.15 Da (average isotopes). Peaks of M-18 and M-36 were observed[1]. C), D), E): the detail of the ESI-MS.

Synthesis of H3K4me3 by using one-pot strategy: Dobz-H3K4me3[Cys_{47}-Gly_{90}] -$NHNH_2$ (**13**) (20.8 mg, 3.8 μmol) was dissolved in 0.4 mL of acidic ligation buffer (6.0 M Gn•HCl, 0.2 M Na_2HPO_4, pH = 3.0). The mixture was treated by $NaNO_2$ solution (113 μL) at -12 °C and kept for 20 min. 1.25 mL of MPAA solution was added, followed by H3K4me3[Cys_{91}-Ala_{135}] (**3**) (19.5mg, 3.8 μmol) and its pH was slowly adjusted to 6.5 with 2.0 M NaOH. After about 2 h, the reaction mixture was treated by 1 M H_2O_2 (115 μL) and H3K4me3[Ala_1-Val_{46}]-$NHNH_2$ (**1**) (37 mg, 7.6 μmol) was activated at the same time in another tube. After 10 min, the activated **1** was added to the reaction mixture and its pH was slowly adjusted to 6.8 with 2.0 M NaOH. Then the reaction was kept at room temperature for 36-48 h. The procedure was monitored by analytical HPLC using a gradient of 20-55% solvent A over 38 min. The ligation product was reduced by TCEP solution and purified by HPLC. The isolation yield was 21% affording 12.0 mg H3K4me3[Ala_1-Cys_{47}-Cys_{91}-Ala_{135}].

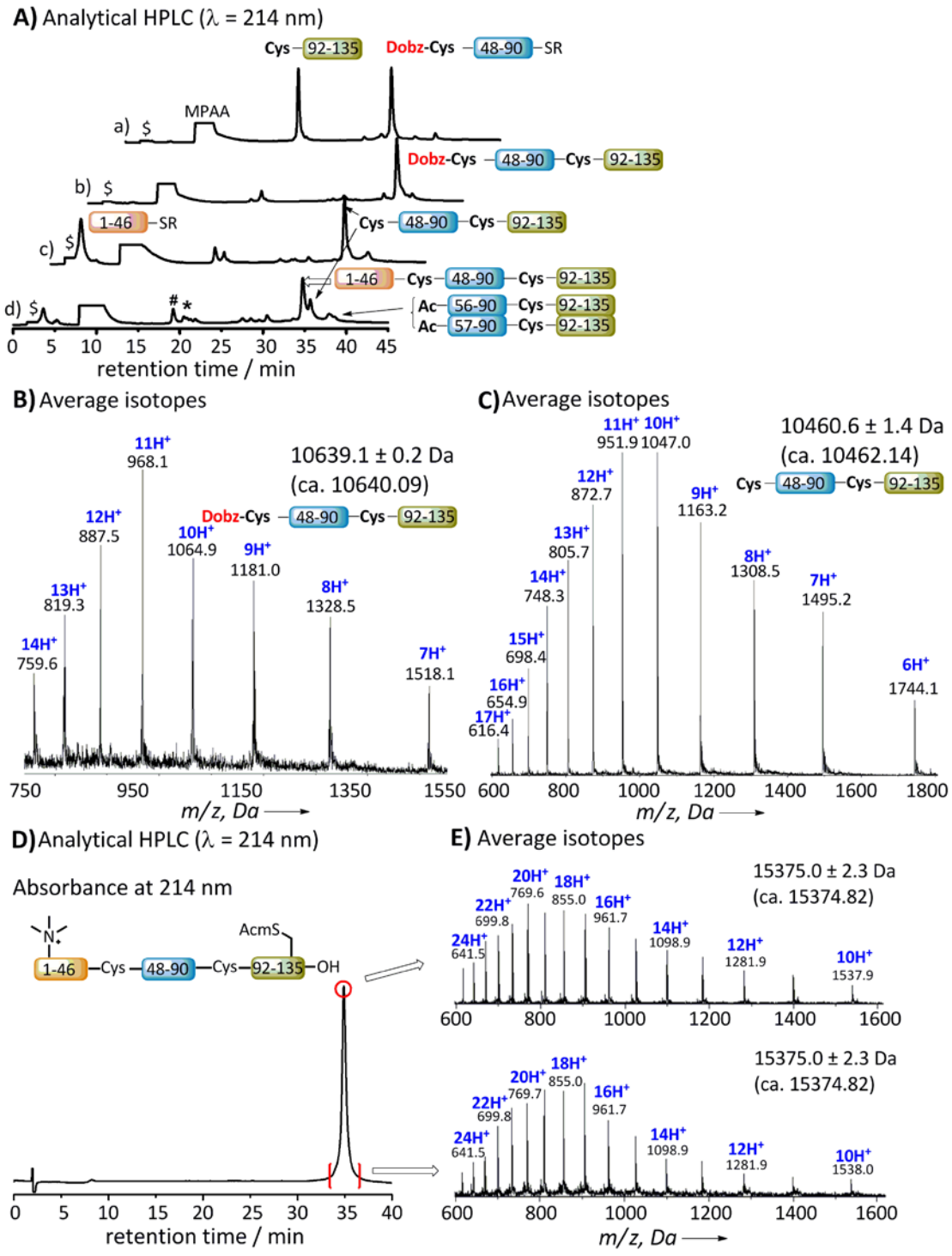


Figure S12. A) analytic HPLC traces (λ = 214 nm) of "One-Pot" synthesis of H3K4me3. a): ligation of **13** with **3** at 0 min. b): ligation of **13** with **3** at 90 min. c): analytic HPLC traces of Dobz removal. d): analytic HPLC traces of the second ligation at 45 h. * corresponds to ligation product of **1** and unreacted **3**; # was an unidentified peak with high absorption at 254 nm (possibly quinonemethides as mass spectra showed messy peaks in low MW). B) ESI-MS analysis of **14**. Observed: 10639.1 ± 0.2 Da. Calcd: 10640.09 Da. C) ESI-MS analysis of **15**. Observed: 10460.6 ± 1.4 Da. Calcd: 10462.14 Da. D) HPLC and ESI-MS analysis of the purified ligation

product. ESI-MS: Observed. 15375.0 ± 2.3 Da, Calcd. 15374.82 Da. (average isotopes).

Desulfurization and Acm removal: The same procedure as above was used. From 12.0 mg of H3K4me3[Ala_1-Cys_{47}-Cys_{91}-Ala_{135}], 8.3 mg of final product was obtained with a yield of 70 %.

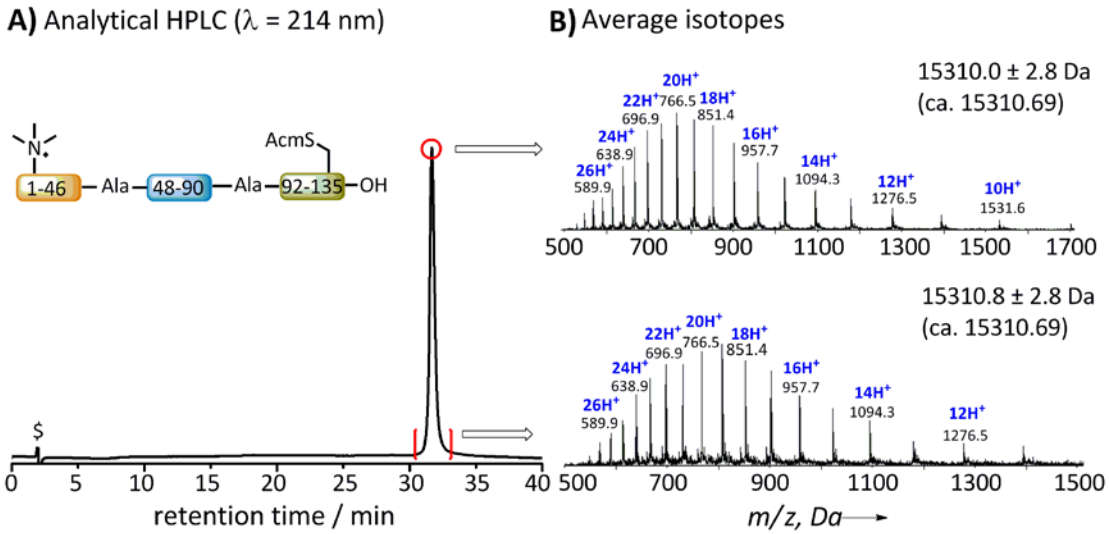


Figure S13. HPLC (A) (λ = 214 nm) and ESI-MS (B) analysis of the product after desulfurization. Observed: 15310.0 ± 2.8 Da. Calcd: 15310.69 Da (average isotopes).

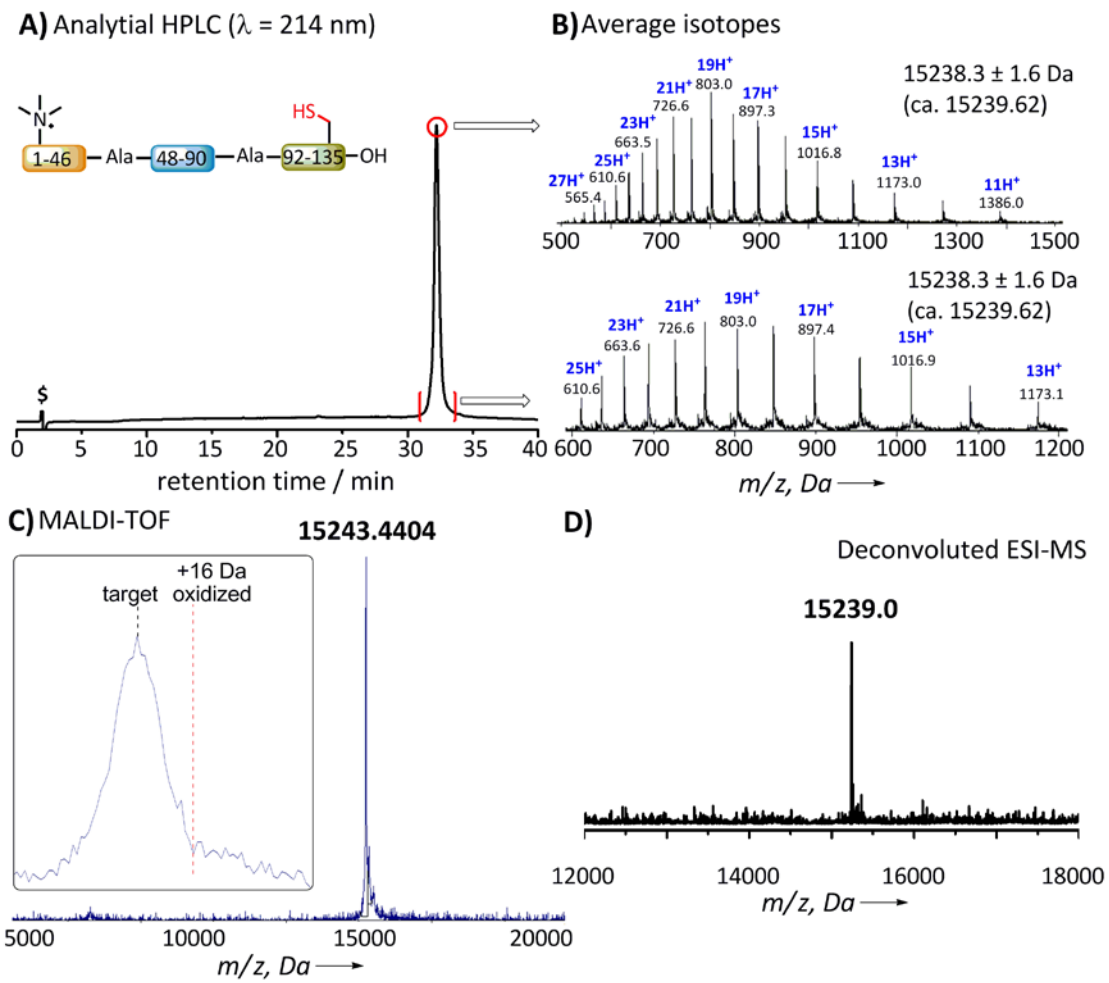


Figure S14. A) HPLC analysis (λ = 214 nm) of the product (H3K4me3) after Acm removal. B) ESI-MS analysis. Observed: 15238.3 ± 1.6 Da. Calcd: 15239.62 Da. (average isotopes). C) MALDI-TOF analysis. Observed: 15243.4404 Da. The enlarged spectrum showed that no peak was observed at the expected mass of oxidized side product. D) The deconvoluted ESI-MS.

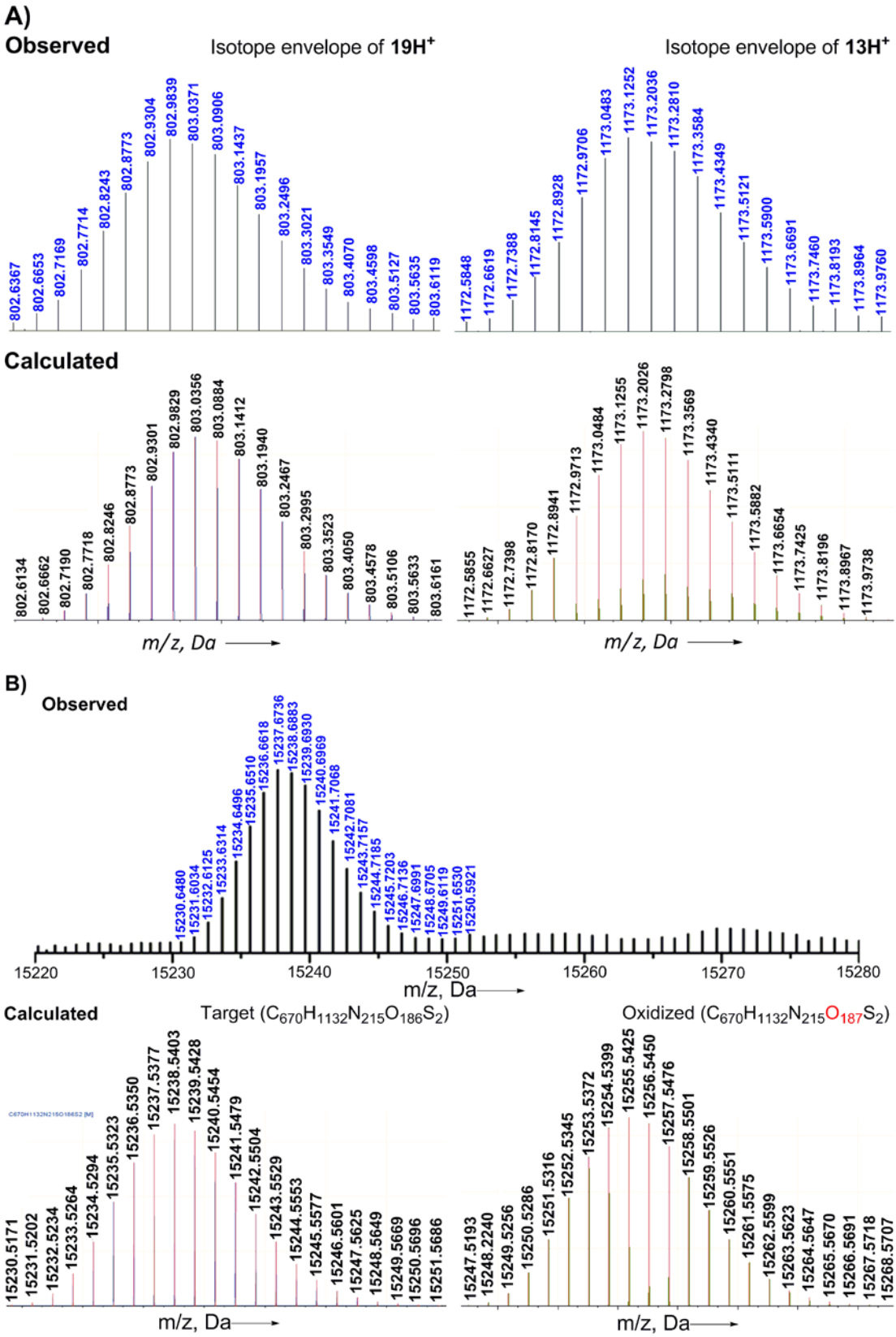


Figure S15. Isotope envelope of the final product (native H3K4me3). A) Observed and calculated isotope envelopes of different charge states ($19H^+$ and $13H^+$). B) Observed isotope envelope (doing statistics from m/z peaks in table S1) and Calculated isotope envelope of final product and oxidized side product.

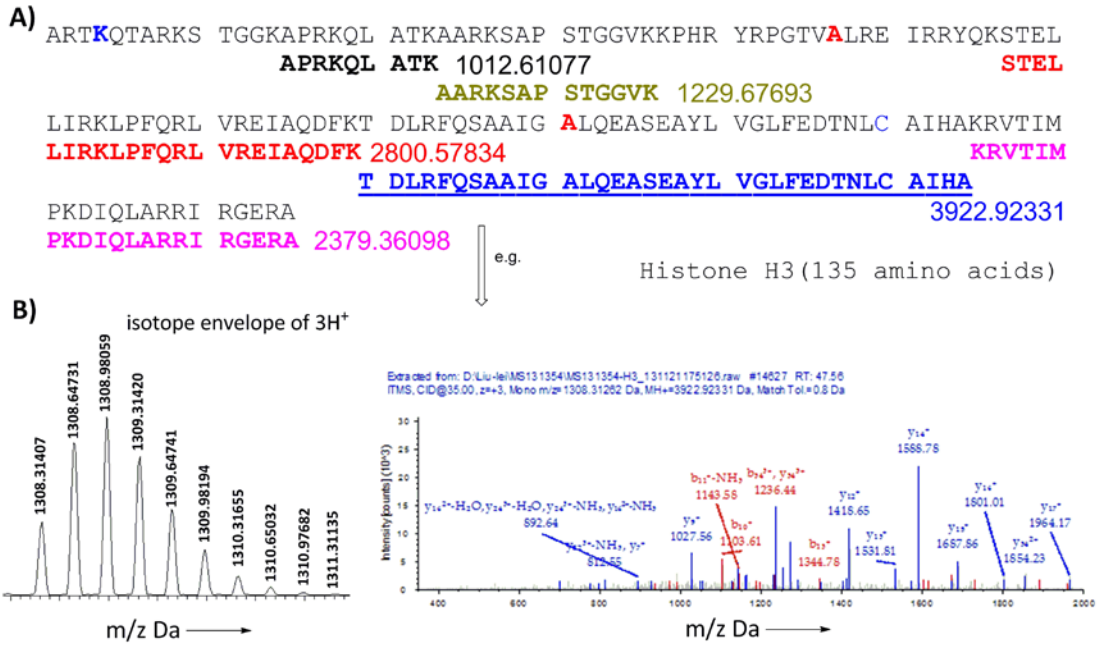


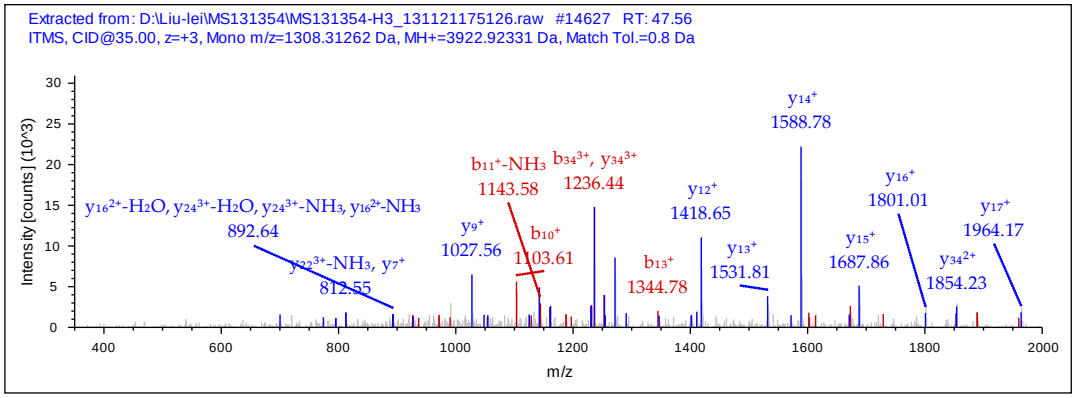
Figure S16. MS-MS analysis of the synthetic H3K4me3（and below）. A) The m/z of observed fragments was marked through the sequence of H3. B) MS-MS analysis of the underlined fragment in H3 sequence.

Details of MS-MS analysis

ARTKQTARKS TGGKAPRKQL ATKAARKSAP STGGVKKPHR YRPGTVALRE IRRYQKSTEL
LIRKLPFQRL VREIAQDFKT DLRFQSAAIG ALQEASEAYL VGLFEDTNLC AIHAKRVTIM
PKDIQLARRI RGERA Histone H3(135 amino acids)

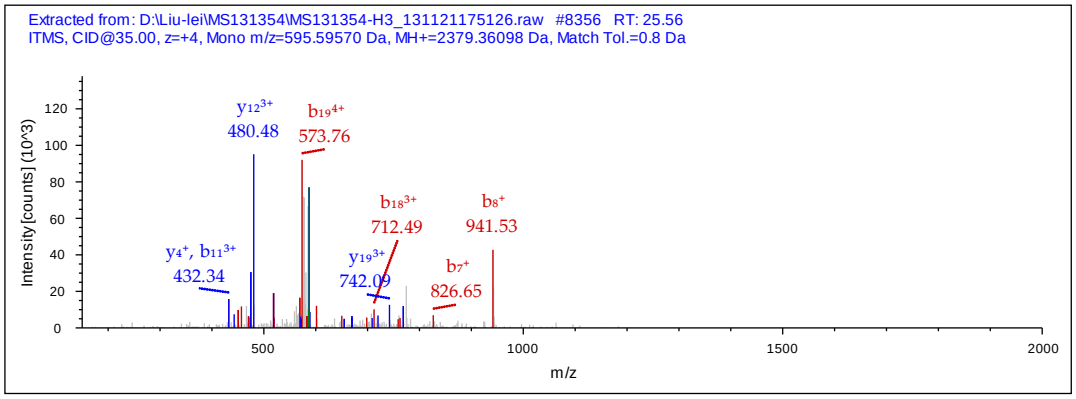
Fragment: TDLRFQSAAIGALQEASEAYLVGLFEDTNLcAIHAK, C31-Carb-amidomethyl (57.02146 Da)

(Observed. 3922.92331, Calcd. 3923.32042)

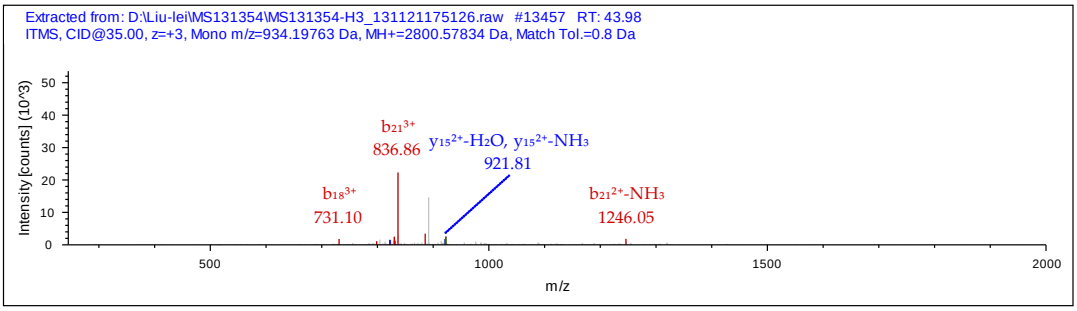


Fragment: RVTIMPKDIQLARRIRGERA

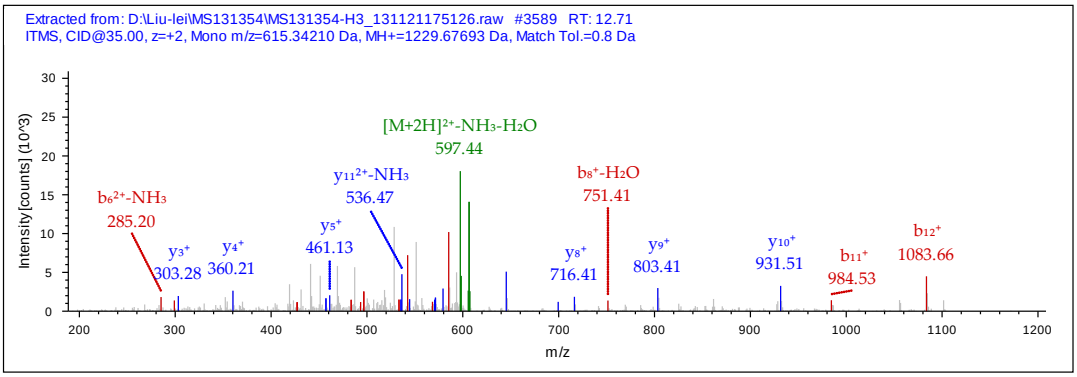
(Observed. 2379.36098, Calcd. 2379.83042)



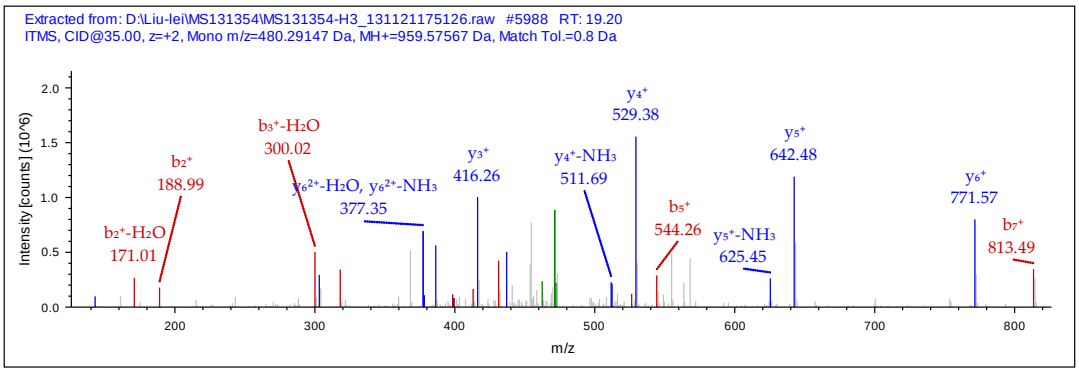
Fragment: STELLIRKLPFQRLVREIAQDFK
(Observed. 2800.57834, Calcd. 2801.28956)



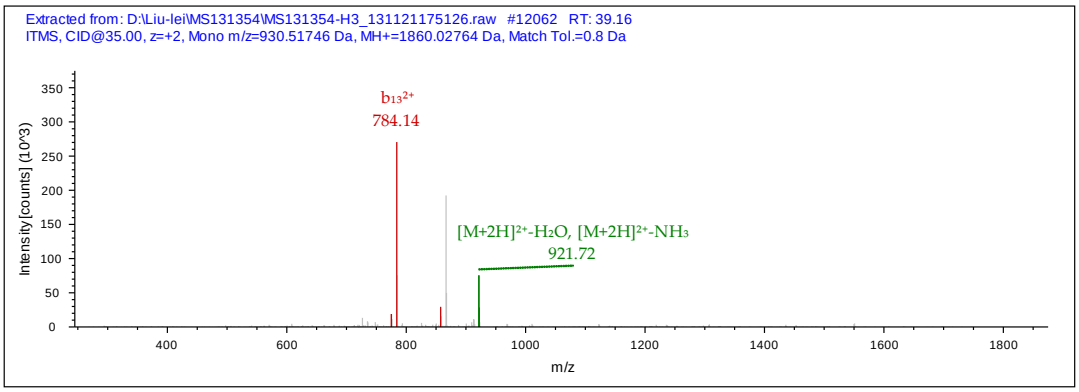
Fragment: AARKSAPSTGGVK
(Observed. 1229.67693, Calcd. 1229.38658)



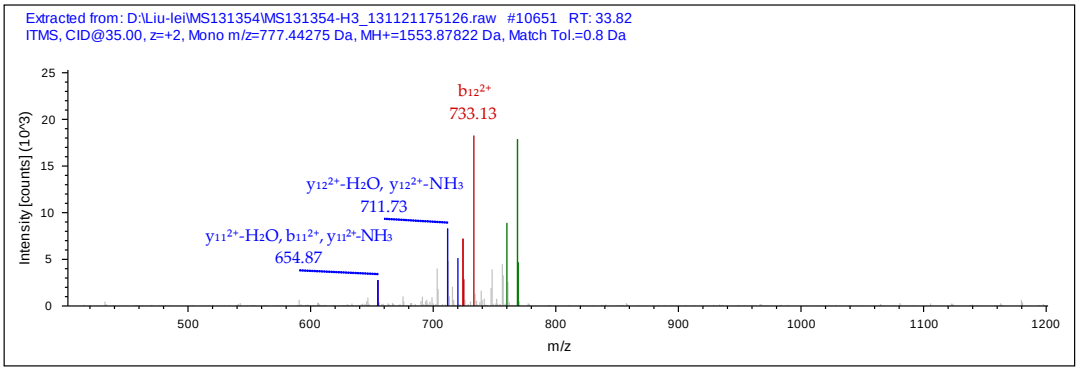
Fragment: STELLIRK
(Observed. 959.57567, Calcd. 959.14132)



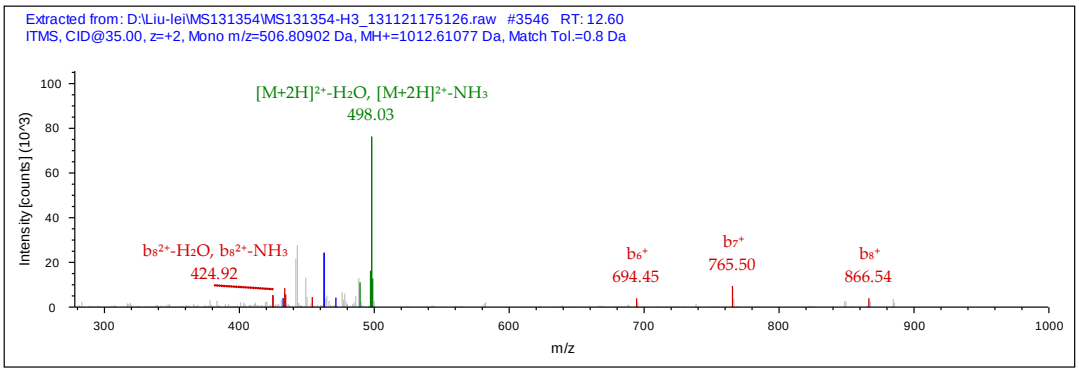
Fragment: LPFQRLVREIAQDFK
(Observed. 1860.02764, Calcd. 1860.16352)



Fragment: DIQLARRIRGERA
(Observed. 1553.87822, Calcd. 1553.76864)

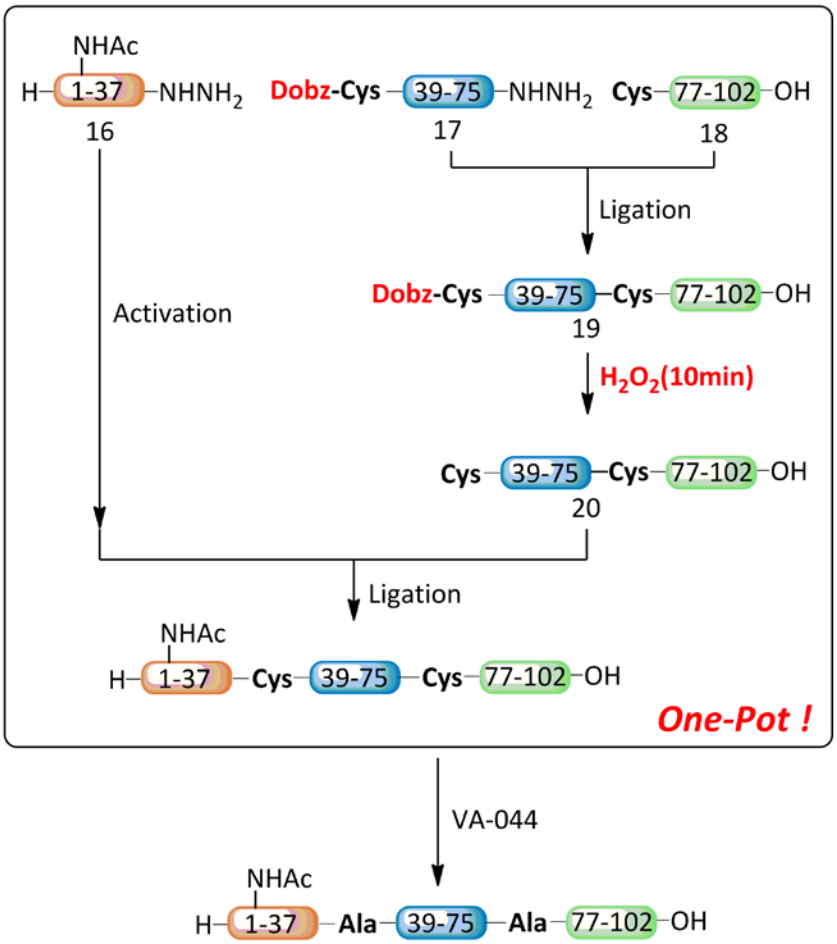


Fragment: APRKQLATK
(Observed. 1012.61077, Calcd. 1012.20724)



2.5 Synthesis of H4K16ac by using H_2O_2-controlled one-pot strategy

SGRGKGGKGL GKGGAKRHRK VLRDNIQGIT KPAIRRLARR GGVKRISGLI
YEETRGVLKV FLENVIRDAV TYTEHAKRKT VTAMDVVYAL KRQGRTLYGF
GG Histone H4 (102 amino acids)



Scheme S8. Synthesis of H3K16ac by using H_2O_2-controlled one-pot strategy

According to the sequence of histone H4, it contains 102 amino acid residues but no Cys residue. The sequence was divided into three segments and desulfurization would be performed later. Thus we chose Leu_{37}- Ala_{38} and His_{75}-Ala_{76} as the ligation sites.

Synthesis of H4K16ac[Ser$_1$-Leu$_{37}$]-NHNH$_2$ (16): The synthesis was performed on the automated peptide synthesizer using hydrazine resin (400 mg, 0.25 mmol). The Fmoc-Lys(Ac)-OH was coupled manually by 2 equiv of AA, 4 equiv of DIEA, 2 equiv of HOAt and 1.9 equiv of HATU to the initial loading of resin and checked by ninhydrin test. After cleavage, the crude peptide was purified on semi-preparative C18 column using a gradient of 5-50 % solvent over 38 min. Yeild: 275.0 mg (27.5 %).

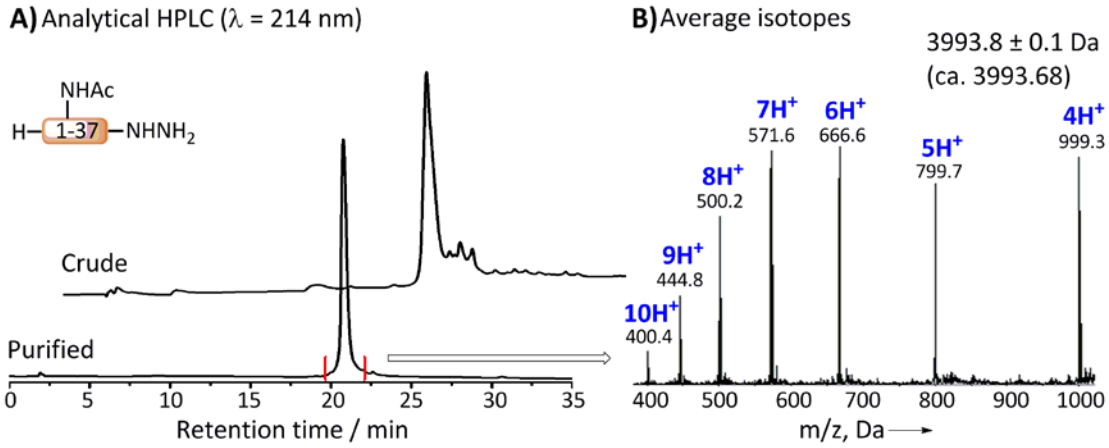


Figure S17. A) HPLC analysis (λ = 214 nm) of the crude and purified **16**. Gradient: 5-50% solvent A over 38 min. B) ESI-MS analysis of the product **16**. ESI-MS: Observed. 3993.8 ± 0.1 Da, Calcd. 3993.68 Da. (average isotopes).

Synthesis of Dobz-H4K16ac[Cys$_{38}$-His$_{75}$]-NHNH$_2$ (17): The synthesis was performed on hydrazine resin (400mg, 0.25 mmol). All the amino acid residues were double coupled. After cleavage, the crude peptide was purified on semi-preparative C18 column using a gradient of 30-70% solvent over 38min. Finally, 34.8 mg of pure product was obtained with a yield of 3.0%.

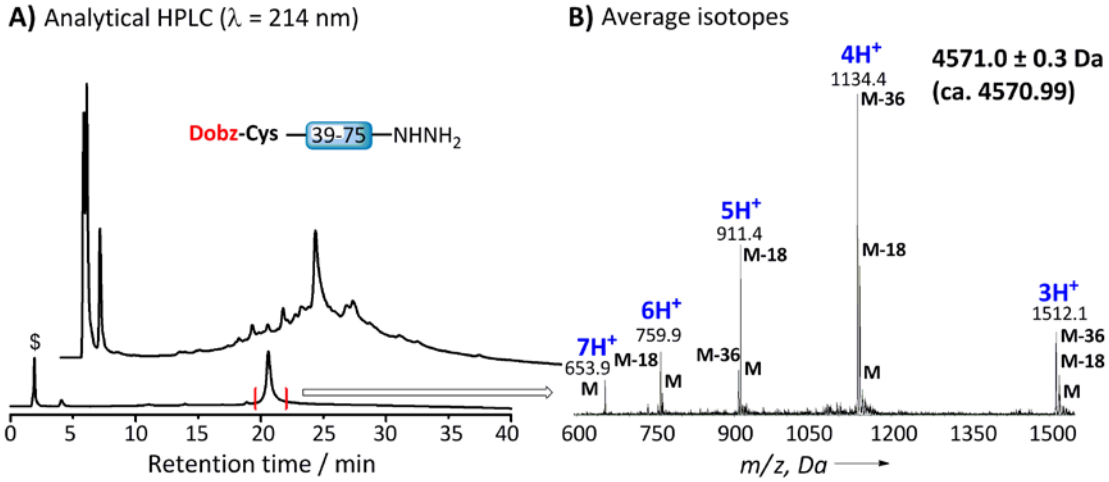


Figure S18. A) HPLC analysis (λ = 214 nm) of the crude and purified **17**. Gradient: 30-70% solvent A over 38 min. B: ESI-MS analysis of **17**. Observed. 4571.0 ± 0.3 Da, Calcd. 4570.99 Da. The peaks of M-18 and M-36 were observed.[1](average isotopes).

Synthesis of H4K16ac[Cys$_{76}$-Gly$_{102}$] (18): The synthesis was performed on the

automated peptide synthesizer using Nova-PEG Wang Resin (0.25 mmol). The first amino acid was coupled in the presence of 4 equiv of amino acid, 4 equiv of HOBt, 3.8 equiv of HBTU, 0.1 equiv of DMAP and 16 equiv of DIEA. After coupling for 12 h, the resin was capped with reagent (acetic anhydride: DIEA: DMF = 1 : 1 : 8). Then, all the amino acid residues were coupled normally. Finally, 145.0 mg of pure product was obtained with a yield of 19.2 %.

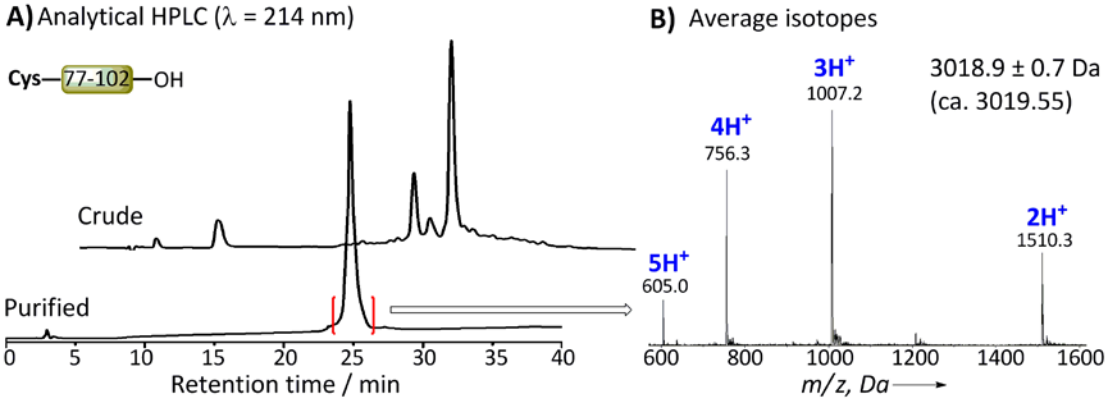


Figure S19. A) HPLC analysis (λ = 214 nm) of the crude and purified **18**. Gradient: 10-55 % solvent A over 38 min. B) ESI-MS analysis of **18**. ESI-MS: Observed. 3018.9 ± 0.7 Da, Calcd. 3019.55 Da. (average isotopes).

Synthesis of H4K16ac by using one-pot strategy: Dobz-H4K16ac[Cys_{38}-His_{75}] -$NHNH_2$ (**17**) (16.2 mg, 3.5 μmol) was dissolved in 0.4 mL of ligation buffer (6.0 M Gn•HCl, 0.2 M Na_2HPO_4, pH = 3.04) and cooled to -12 ^oC. 125 μL of $NaNO_2$ was added and kept for 20 min. The mixture was treated by 1185 μL of MPAA solution, followed by **18** (10.7 mg, 3.5 μmol) and its pH was slowly adjusted to 6.5 with 0.5 M NaOH. After 120 min, the reaction mixture was treated by 106 μL of 1 M H_2O_2. At the same time, **16** (28.3 mg, 7.0 μmol) was activated. After 20 min, activated **16** was added to the reaction mixture and its pH was slowly adjusted to 6.8 with 2.0 M NaOH. The procedure was monitored by analytical HPLC using a gradient of 10-70% solvent A over 38 min. After stirred at room temperature for 24 h, the reaction mixture was reduced by TCEP solution and isolated by HPLC. Finally, 11.0 mg of ligation product was obtained, giving an isolated yield of 27.4 %.

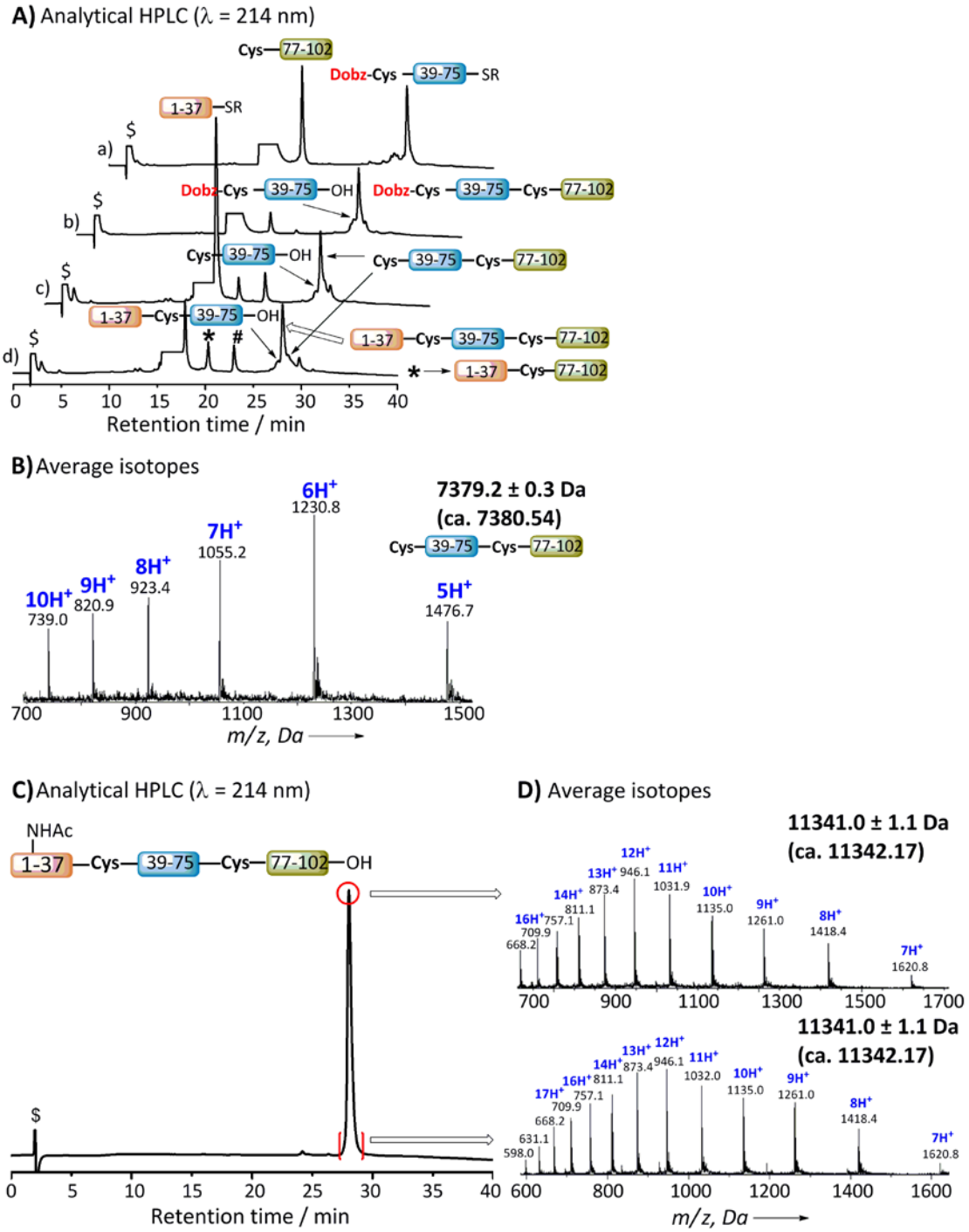


Figure S20. A): analytic HPLC traces (λ = 214 nm) of "One-Pot" synthesis of H4K16ac. a) Ligation of **17** with **18** at 0 min. b) Ligation of **17** with **18** at 120 min. c)The second ligation at 0 min (Dobz removal). d) The second ligation at 24 h. # was unidentified peak with high absorption at 254 nm (quinonemethides as mass spectra showed messy peaks in low MW).B) ESI-MS analysis of **20**. Observed: 7379.2 ± 0.3 Da, Calcd: 7380.54 Da (average isotopes). C), D): HPLC and ESI-MS analysis of the purified ligation product. ESI-MS: Observed. 11341.0 ± 1.1 Da, Calcd. 11342.17 Da (average isotopes).

Desulfurization: The same procedure as above was used. After purification, 7.3 mg of final product was obtained with a yield of 66.7 %.

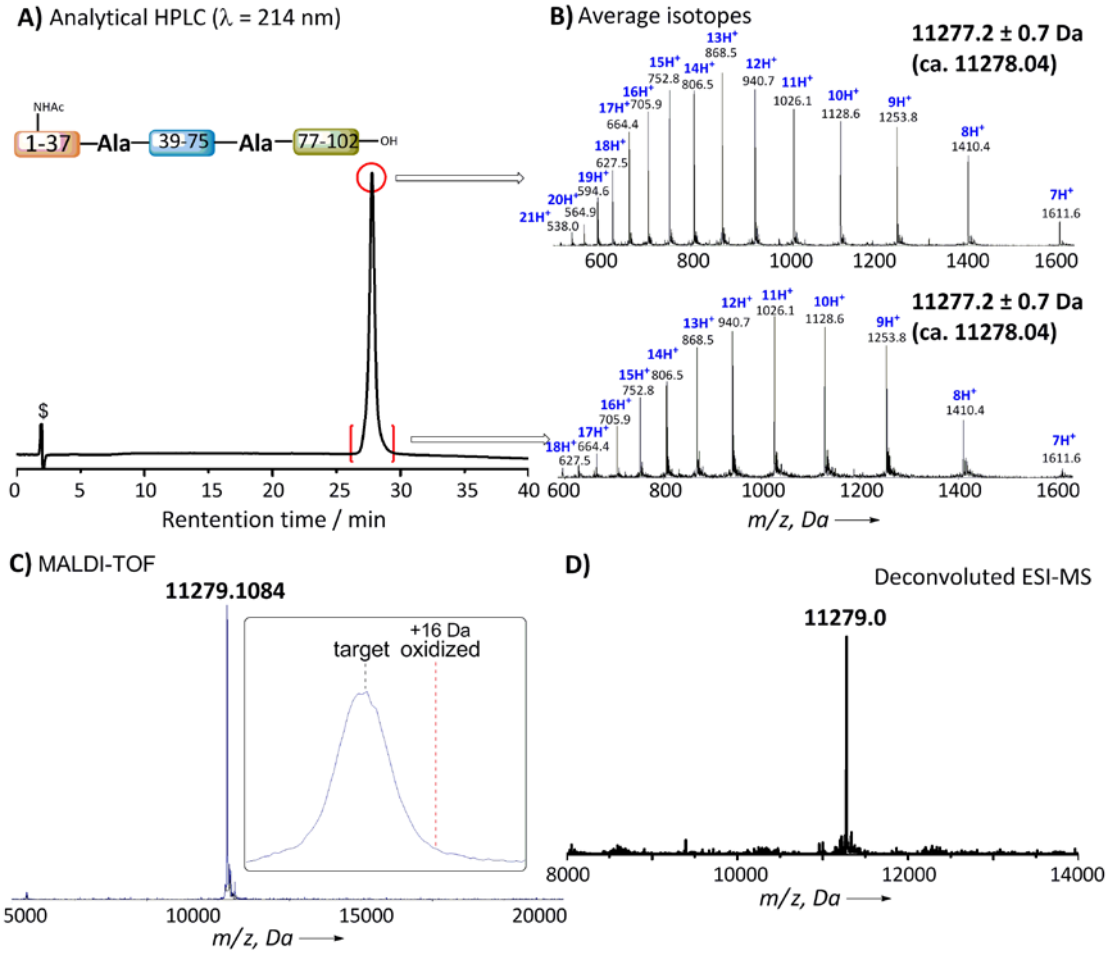


Figure S21. HPLC (A) (λ = 214 nm) and ESI-MS (B) analysis of the native H4K16ac. ESI-MS: Observed. 11277.2 ± 0.7 Da, Calcd. 11278.04 Da. (average isotopes). C) MALDI-TOF analysis. Observed: 11279.1084 Da. The enlarged spectrum showed that no peak was observed at the expected mass of oxidized side product. D) The deconvoluted ESI-MS.

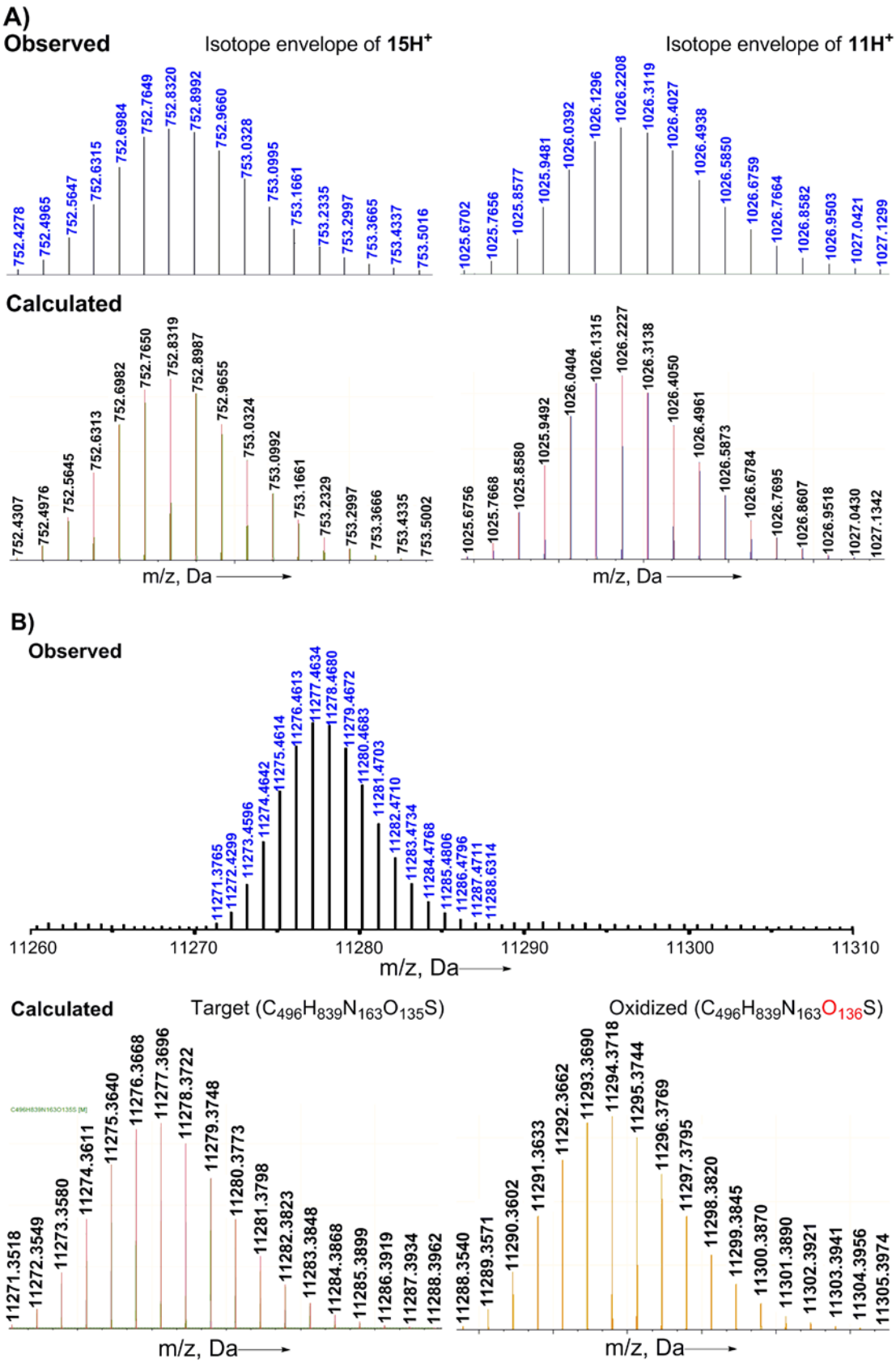


Figure S22. Isotope envelope of native H4K16ac. A) Observed and calculated isotope envelopes of different charge states ($15H^+$ and $11H^+$). B) Observed isotope envelope (doing statistics from m/z peaks in table S2) and Calculated isotope envelope of final

product and oxidized side product.

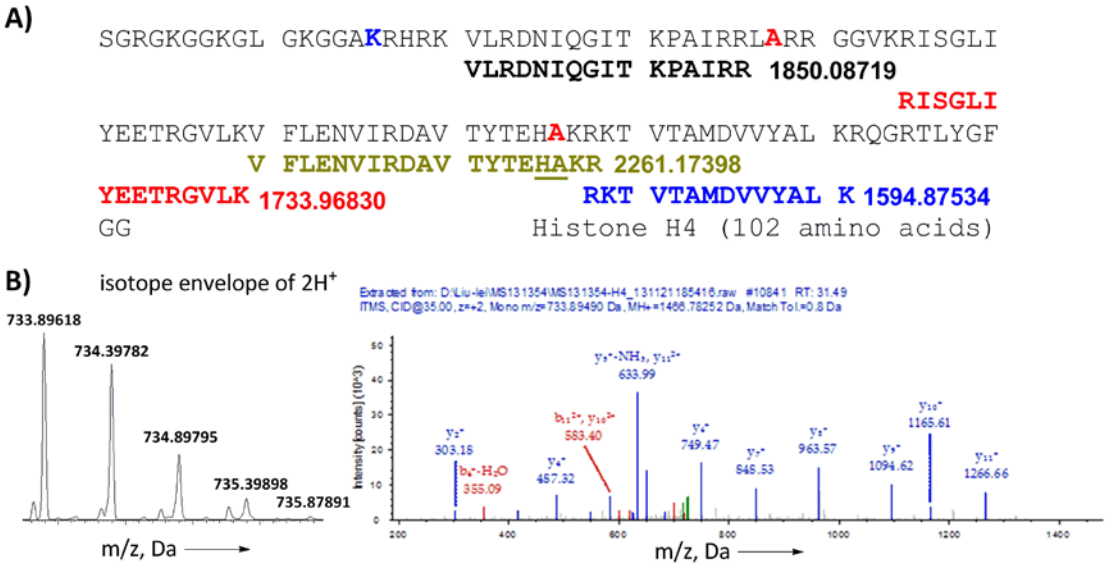
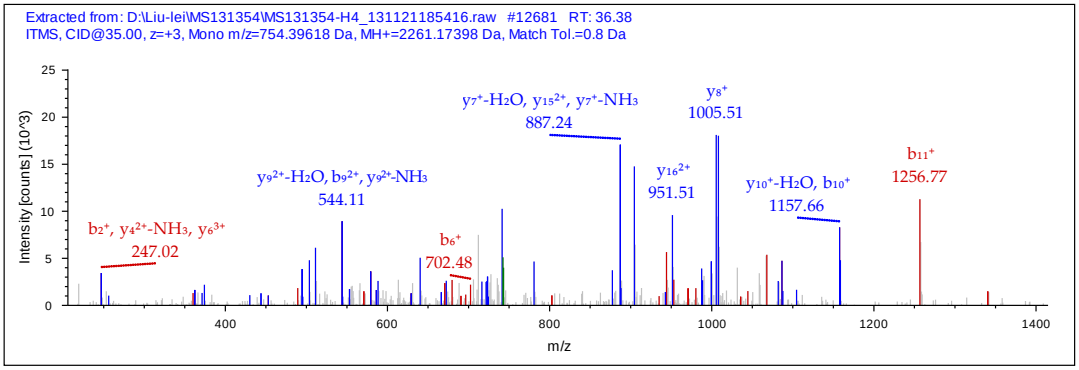


Figure S23. MS-MS analysis of the synthetic H4K16ac. A) The m/z of observed fragments was marked through the sequence of H4. B) MS-MS analysis of the underlined fragment.

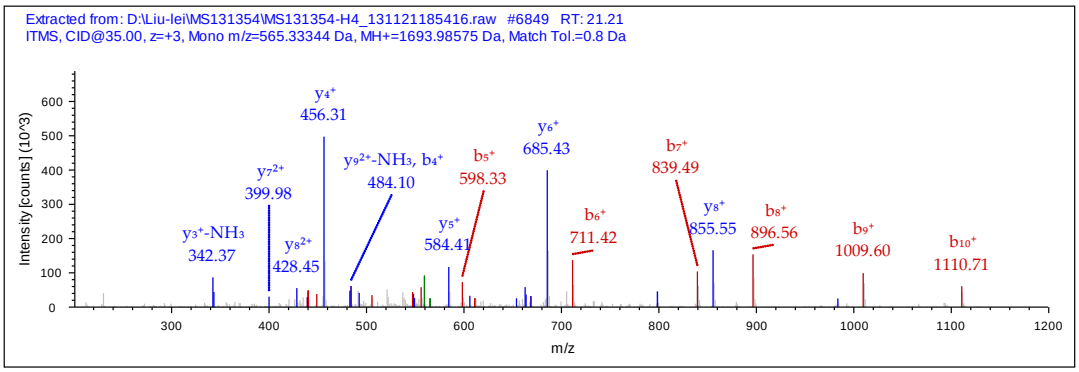
Details of MS-MS analysis:

SGRGKGGKGL GKGGAKRHRK **VLRDNIQGIT KPAIRR**LARR GGVK**RISGLI**
YEETRGVLKV FLENVIRDAV TYTEHAKRKT VTAMDVVYAL KRQGRTLYGF
GG Histone H4 (102 amino acids)

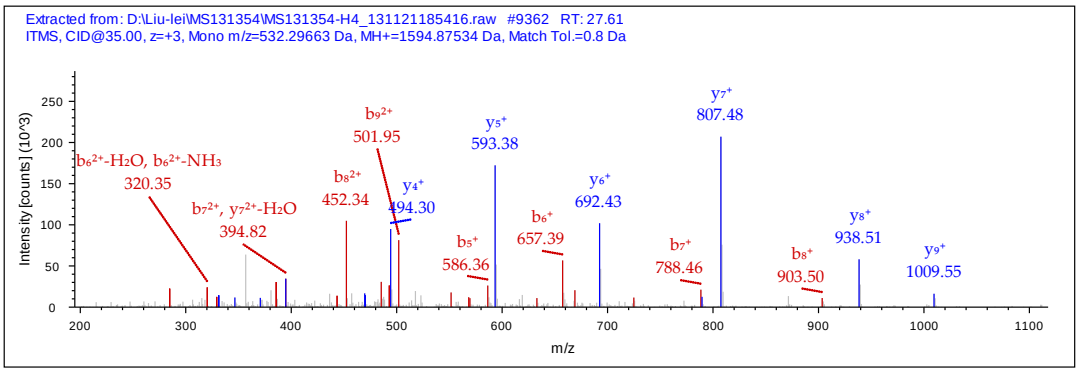
Fragment: VFLENVIRDAVTYTEHAKR
(Observed. 2261.17398, Calcd. 2261.53534)



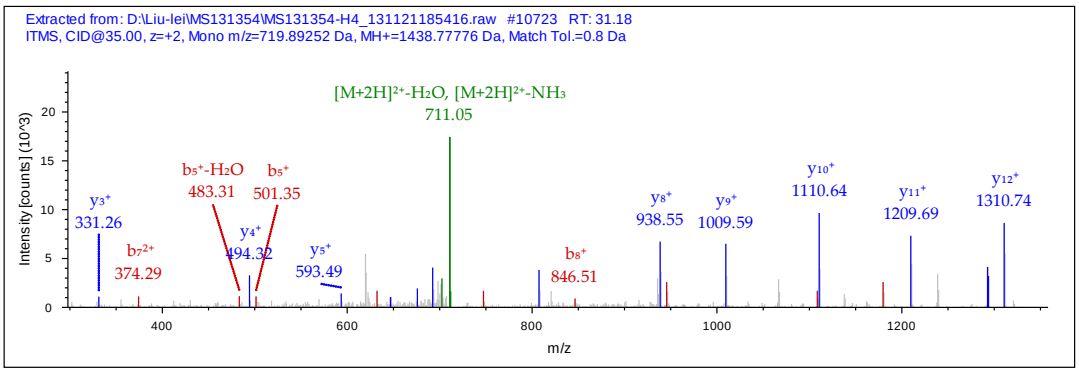
Fragment: VLRDNIQGITKPAIR
(Observed. 1693.98575, Calcd. 1893.98808)



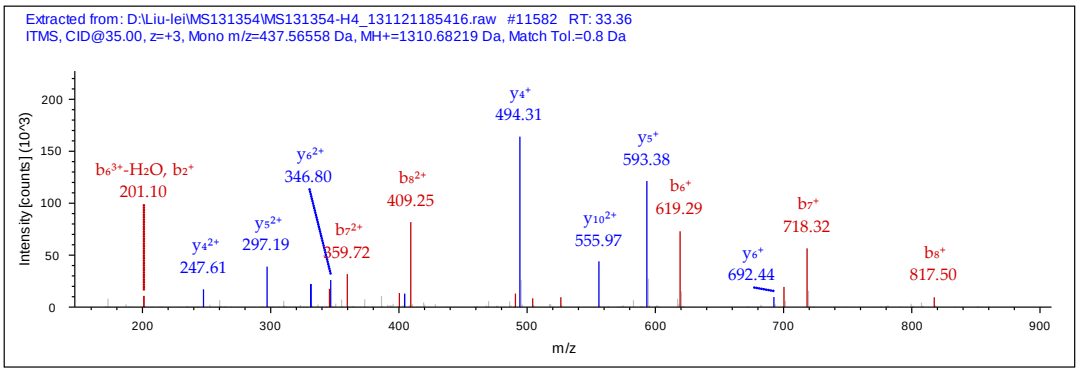
Fragment: RKTVTAMDVVYALK
(Observed. 1594.87534, Calcd. 1594.91662)



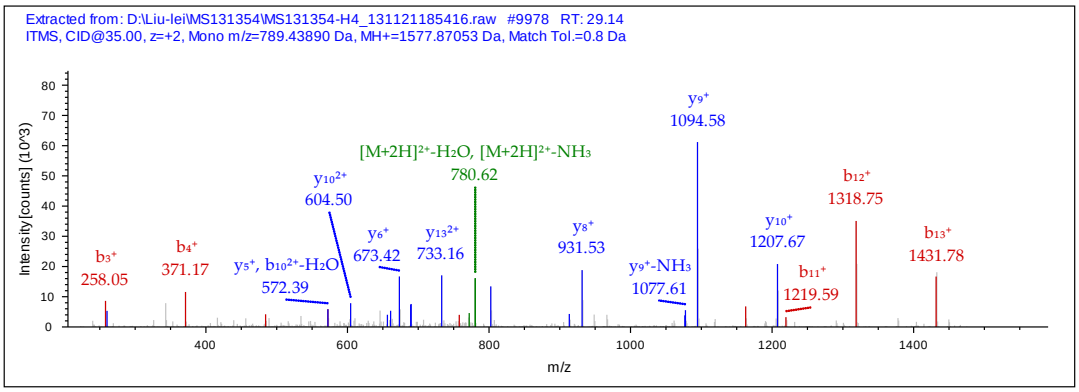
Fragment: KTVTAMDVVYALK
(Observed. 1438.77776, Calcd. 1438.73094)



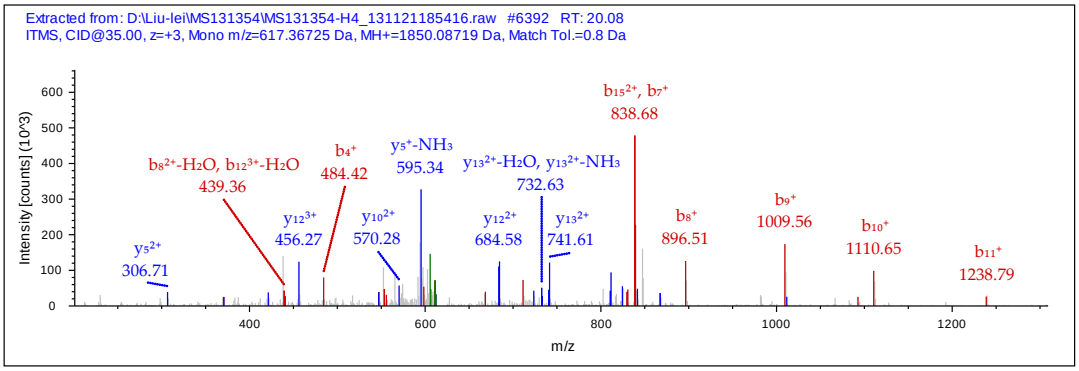
Fragment: TVTAMDVVYALK
(Observed. 1310.68219, Calcd. 1310.55866)



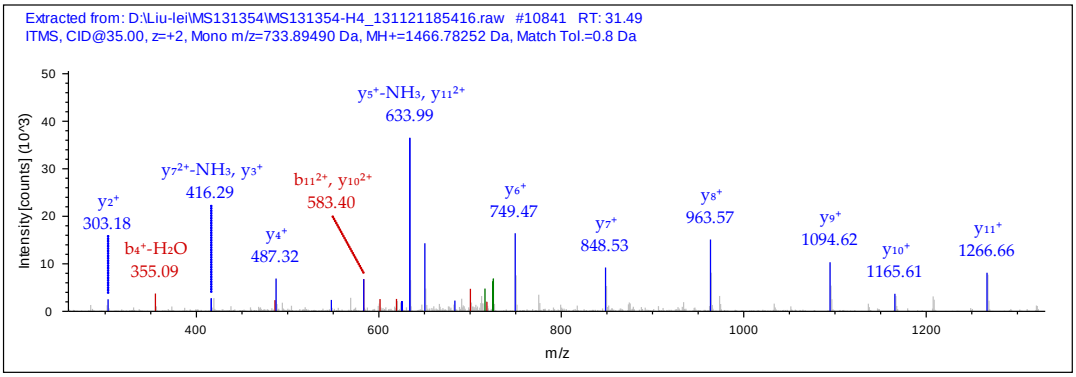
Fragment: ISGLIYEETRGVLK
(Observed. 1577.87053, Calcd. 1577.81990)



Fragment: VLRDNIQGITKPAIRR
(Observed. 1850.08719, Calcd. 1850.17376)



Fragment: TVTAMDVVYALKR
(Observed. 1466.78252, Calcd. 1466.74434)



2.6 Synthesis of H3K36me3, H4K12ac, H4K5acK8acK12acK16ac

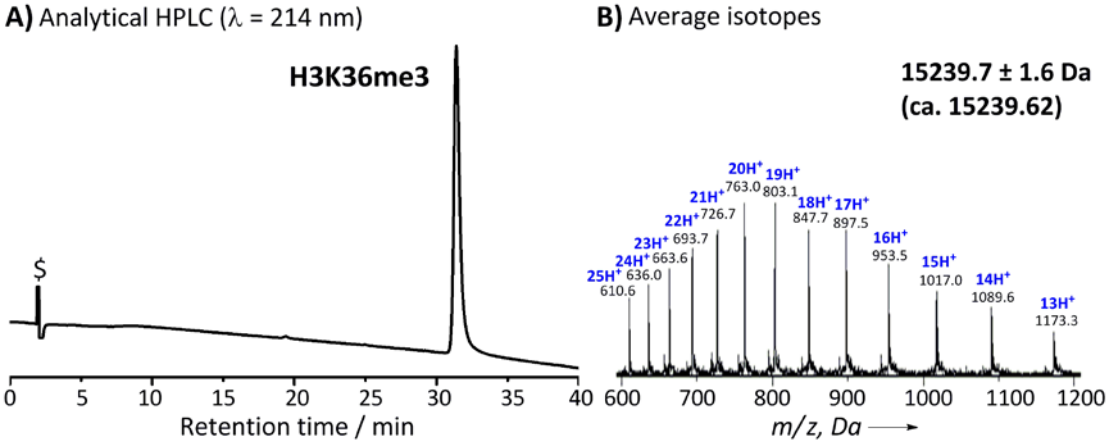


Figure S24. HPLC (A) (λ = 214 nm) and ESI-MS (B) analysis of the native H3K36me3. ESI-MS: Observed. 15239.7 ± 1.6 Da, Calcd. 15239.62 Da (average isotopes).

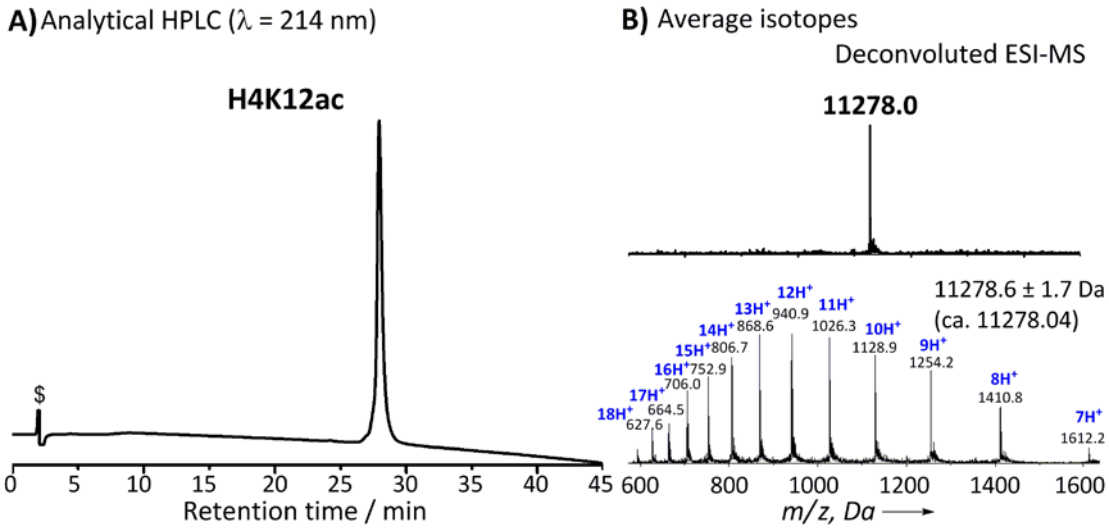


Figure S25. HPLC (A) (λ = 214 nm) and ESI-MS (B) analysis of the native H4K12ac. ESI-MS: Observed. 11278.6 ± 1.7 Da, Calcd. 11278.04 Da (average isotopes).

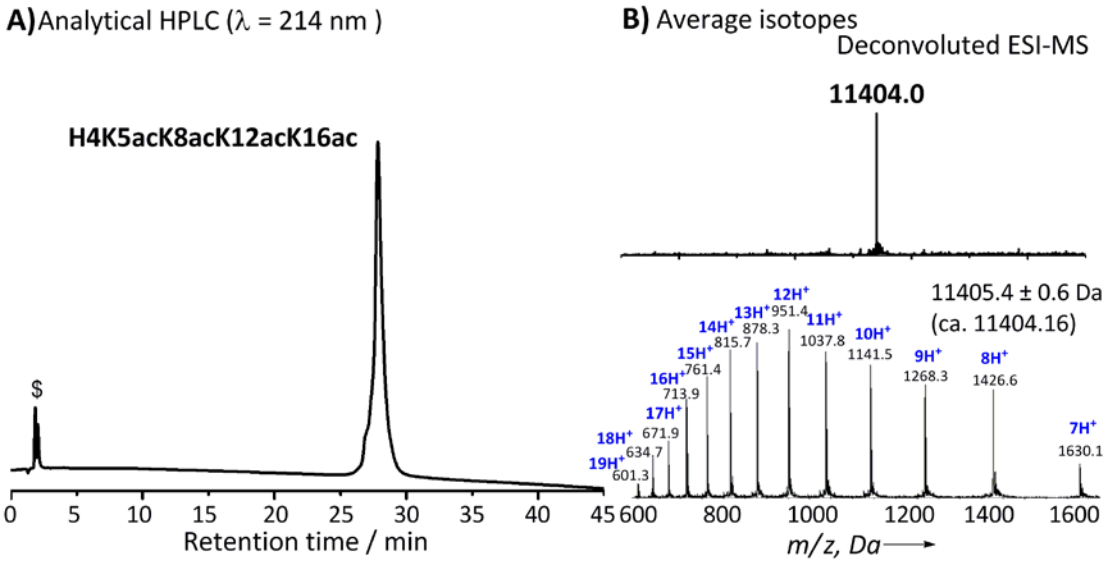
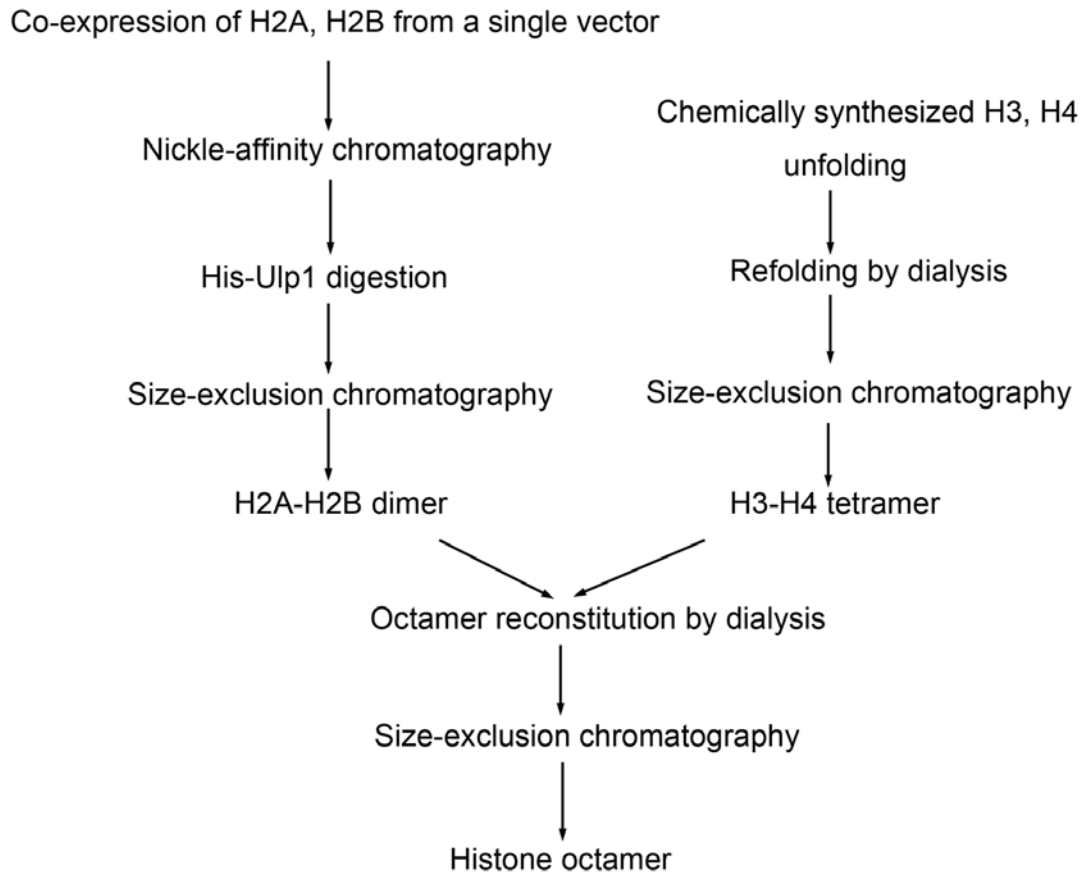


Figure S26. HPLC (A) (λ = 214 nm) and ESI-MS (B) analysis of the native H4K5acK8acK12acK16ac. ESI-MS: Observed. 11405.4 ± 0.6 Da, Calcd. 11404.16 Da (average isotopes).

2.7 Histones octamer reconstitution



Scheme S9. Histones octamer reconstitution

Histones H2A and H2B dimer purification

Histones H2A and H2B dimer was purified using a non-denaturing method according to the previous method with minor modifications. Briefly, histones H2A and H2B genes were assembled into the pRSFDuet-1 vector as a polycistronic plasmid, with a hexahistidine-SUMO tag-Ulp1 cleavage site (His6-SUMO tag) preceding the N-terminus of H2A. The resulting plasmid encoding H2A and H2B histones was transformed into BL21(DE3) cells and plated on to an LB agar plate containing kanamycin (50 μg/ml). The plate was incubated overnight (~16 h) at 37 °C. For a 1 liter scale preparation, one colony was inoculated into 50 ml LB media containing kanamycin (50 μg/ml) and was shaken at 250 rpm for 4-5 h at 37 °C, which was then amplified to 1 liter of the same media and was grown for another 3-4 h at 37 °C. When the OD600 reached ~0.4, histone coexpression was induced by adding 0.4 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 12-16 h at 25 °C. Cells were then harvested by centrifugation at 4,500 x g for 10 min at 4 °C and were processed immediately or stored at -80 °C for future purification. Cell pellets were re-suspended in lysis buffer (20 mM Tris-HCl pH 8.0, 2.0 M sodium chloride, 1 mM phenylmethanesulfonylfluoride and 1mM dithiothreitol). Re-suspended cells were lysed by EmulsiFlex-C3 high pressure homogenizer (Avestin) and clarified by centrifugation at 14,000 × g at 4 °C for 1 h. The supernatant was collected and imidazole stock solution was added to adjust the concentration to 30 mM. The clarified lysate was then loaded onto a 5 ml HisTrap FF column (GE Healthcare) pre-equilibrated in the Ni-buffer A (20 mM Tris-HCl pH 8.0, 2.0 M sodium chloride, 1mM dithiothreitol) followed by a 10 column volumes (CV) wash with Ni-buffer A containing 30 mM imidazole and then another 10 CV wash with buffer B (20 mM Tris-HCl pH 8.0, 4.0 M sodium chloride, 1mM dithiothreitol). The bound proteins were eluted by increasing the imidazole concentration from 30 mM to 500 mM linearly over 23.5 CV. Each faction was analyzed by 15% SDS-PAGE: 10 μL incubated at 98 °C for 10 min and centrifuged at 14,000 × g for 10 min, after which all supernatant was loaded on the gel. Electrophoresis was run at 180 V for 50 min.

Ulp1 digestion

Ulp1digestion was carried out by adding purified His6-Ulp1 in 25:1 mass ratio and incubating the samples at 4 °C for 3 h. The digestion was confirmed by SDS-PAGE.

Size exclusion chromatography

Ulp1-digested histones were loaded onto a 5 mL HisTrap FF column (GE Healthcare) again to remove his-Ulp1 protease and undigested his-H2A/B. The flowthrough containing native H2A/B was then concentrated to reduce sample volume to ~0.5 ml with ultrafiltration using Amicon YM50 membrane (Millipore, MWCO 30 kDa) at 4 °C. The concentrated sample was then injected onto a Superdex 200 10/300GL column (GE Healthcare) pre-equilibrated in 20 mM Tris-HCl pH 8.0, 2.0 M sodium chloride, 1mM dithiothreitol. The histone H2A/B dimer peak was eluted at an elution volume of 14.9 mL. The peak fractions were pooled and concentrated up to 8 mg/ml, aliquoted and flash-frozen in the presence of 50% glycerol for long-term storage.

Histones H3 with K4me3 and H4 with K16ac tetramer reconstitution

Chemically synthesized histones H3 with K4me3 and H4 with K16ac powder were firstly dissolved in unfolding buffer (6M Guanidine hydrochloride, 20 mM Tris-HCl pH 8.0, 5mM dithiothreitol) and left at room temperature for 1-2 h before four rounds of dialysis against refolding buffer (10 mM Tris-HCl pH 8.0, 2.0 M sodium chloride, 1mM Na-EDTA, 5mM dithiothreitol) at 4 °C for in total of about 36-48 h. Then the refolded H3-H4 sample was injected onto a Superdex 200 10/300GL column (GE Healthcare) pre-equilibrated in 20 mM Tris-HCl pH 8.0, 2.0 M sodium chloride, 1mM dithiothreitol. The histone H3-H4 tetramer peak was eluted at an elution volume of 13.6 ml. The peak fractions were pooled and concentrated up to 8 mg/ml, aliquoted and flash-frozen in the presence of 50% glycerol for long-term storage.

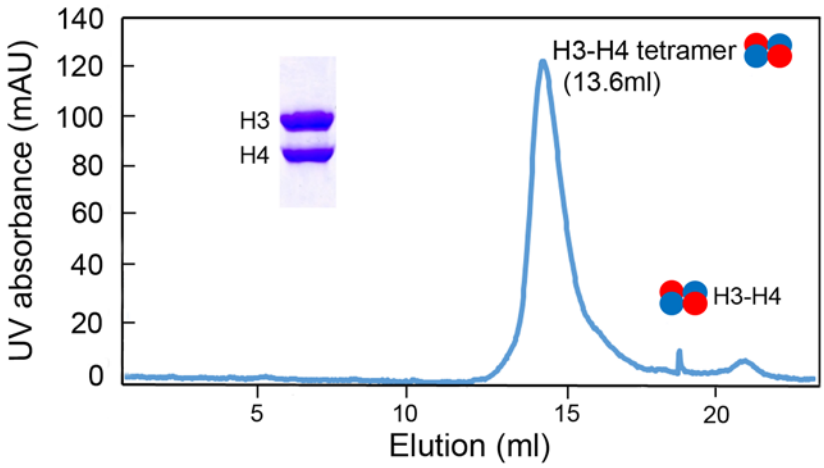


***Figure S27*:** Histone tetramer formation of synthetic H3K4me3 and H4K16ac. Blue curve: gel filtration profile of histone H3-H4 tetramer eluted over a Superdex 200 10/300 column. The peak of H3-H4 tetramer was eluted at 13.6 ml. Left to the peak: SDS-PAGE of the H3-H4 tetramer peak stained by commassie brilliant blue.

Octamer reconstitution

H2A-H2B dimer and H3-H4 tetramer were mixed at molar ratio of ~2.5:1 and then dialyzed against refolding buffer (10 mM Tris-HCl pH 8.0, 2.0 M sodium chloride, 1mM Na-EDTA, 5mM dithiothreitol) for four rounds at 4 °C for in total of about 36-48 h at 4 °C. Then the resultant product were injected onto a Superdex 200 10/300GL column (GE Healthcare) pre-equilibrated in 20 mM Tris-HCl pH 8.0, 2.0 M sodium chloride, 1mM dithiothreitol. The histone octamer peak was eluted at an elution volume of 12.8 ml. The peak fractions were pooled and concentrated up to 8 mg/ml, aliquoted and flash-frozen in the presence of 50% glycerol for long-term storage.

Western blotting

Sandwiched nitrocellulose membrane-gel-filter paper was transferred in transfer buffer (25mM Tris-base, 192mM glycine, 20% methanol) at 4 °C under 80V for 90 mins. The membrane was blocked using 5% non-fat milk at room temperature for 1 h. Rabbit anti-H3K4me3 (Millipore) and H4K16ac (Cell signalling) primary antibody

were diluted by 5000 fold using 5% non-fat milk, mixed and were then added onto the membrane for incubation overnight at 4 °C by rotation. Next day, the membrane was washed using wash buffer (TBST) at least three times, 10 mins per time before the anti-rabbit IgG secondary antibody was added for another 1 h at room temperature. Then the membrane was washed using wash buffer (TBST, 50 mM Tris 7.6, 150 mM NaCl, 0.05% Tween 20) at least three times, 10 mins per time before the freshly prepared HRP (horseradish peroxidase) chemiluminescent (TIANGEN) was added, followed by exposure and analyzed using a BioRad ChemDoc +XRS system (BioRad).

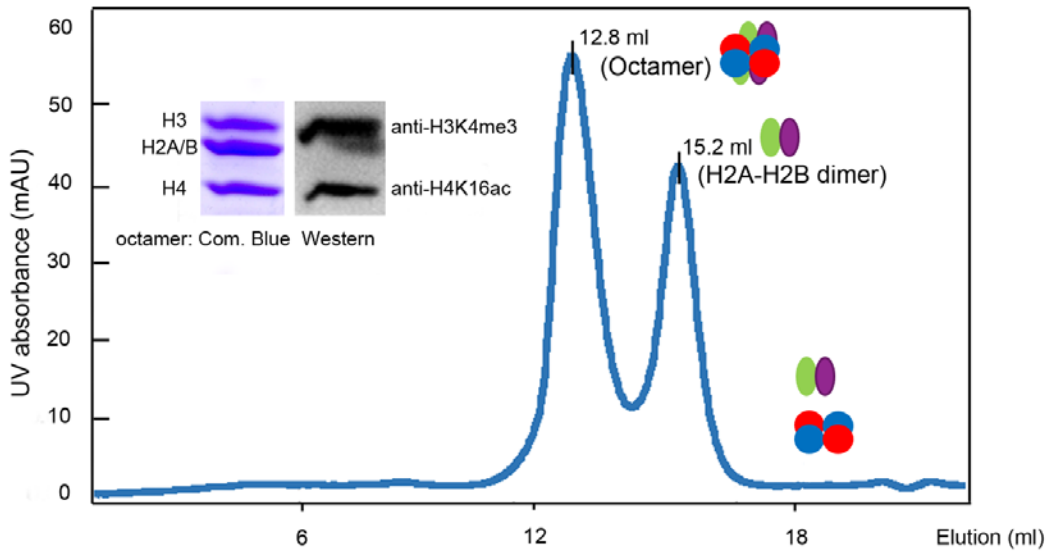


Figure S28. Synthetic H3K4me3 and H4K16ac can form histone octamer, which were purified over a Superdex 200 (10/300) column after extensive dialysis. a) The chromatogram shows that histone octamer was eluted at 12.8 ml. It was well separated from excess H2A-H2B dimer (eluted at 15.2 ml). b) The SDS–PAGE gel (left) and Western blotting (right) show that the reconstituted histone octamer from synthetic H3K4me3 and H4K16ac was intact and stoichiometric (Com. Blue: commassie brilliant blue; Western: Western blotting).

2.8 Isotope envelope of H3K4me3 and H4k16ac on different charge states

Table S1. Isotope envelope of H3K4me3

$12H^+$				$13H^+$				$14H^+$				$15H^+$			
observed		calculated		observed		calculated		observed		calculated		observed		calculated	
m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.
1270.2146	4.5	1270.2170	0.0	**1172.5848**	5.1	1172.5855	0.2	**1088.9033**	4.0	1088.9014	0.2	**1016.3698**	4.5	1016.3751	0.2
1270.2961	9.3	1270.3006	2.0	**1172.6619**	6.9	1172.6627	1.5	**1088.9669**	7.7	1088.9730	1.4	**1016.4351**	8.3	1016.4419	1.4
1270.3802	16.0	1270.3842	6.0	**1172.7388**	16.2	1172.7398	6.0	**1089.0406**	15.7	1089.0446	5.7	**1016.5049**	17.7	1016.5088	5.6
1270.4661	31.3	1270.4678	16.0	**1172.8145**	28.5	1172.8170	16.1	**1089.1143**	29.4	1089.1163	15.5	**1016.5754**	30.3	1016.5757	15.3
1270.5524	49.0	1270.5514	33.0	**1172.8928**	46.1	1172.8941	33.1	**1089.1869**	49.0	1089.1879	32.1	**1016.6417**	50.6	1016.6425	31.7
1270.6354	67.0	1270.6349	55.0	**1172.9706**	69.5	1172.9713	55.1	**1089.2579**	66.5	1089.2595	53.6	**1016.7089**	66.8	1016.7094	53.4
1270.719	87.6	1270.7185	77.0	**1173.0483**	89.2	1173.0484	76.9	**1089.3308**	83.6	1089.3312	75.4	**1016.7760**	89.2	1016.7762	75.6
1270.8026	100.0	1270.8021	93.0	**1173.1252**	100.0	1173.1255	93.1	**1089.4025**	97.9	1089.4028	92.6	**1016.8431**	100.0	1016.8431	92.5
1270.8867	99.3	1270.8856	100.0	**1173.2036**	97.9	1173.2026	100.0	**1089.4744**	100.0	1089.4744	100.0	**1016.9103**	99.2	1016.9099	100.0
1270.9703	90.7	1270.9692	96.0	**1173.2810**	93.1	1173.2798	96.6	**1089.5457**	88.2	1089.5460	97.0	**1016.9763**	93.3	1016.9768	97.3
1271.0543	77.6	1270.0527	84.0	**1173.3584**	79.9	1173.3569	84.9	**1089.6185**	73.2	1089.6176	85.4	**1017.0446**	78.6	1017.0436	86.0
1271.1398	60.5	1270.1363	68.0	**1173.4349**	61.4	1173.4340	68.7	**1089.6904**	64.6	1089.6892	68.4	**1017.1120**	60.3	1017.1104	69.4
1271.2235	46.1	1270.2198	51.0	**1173.5121**	46.0	1173.5111	52.1	**1089.7631**	43.9	1089.7608	51.3	**1017.1767**	48.4	1017.1773	52.0
1271.3074	29.3	1271.3033	35.0	**1173.5900**	33.4	1173.5882	36.2	**1089.8324**	32.7	1089.8324	35.8	**1017.2440**	32.8	1017.2441	36.2
1271.3857	24.5	1271.3869	23.0	**1173.6691**	22.4	1173.6654	23.5	**1089.9036**	21.9	1089.9040	23.4	**1017.3127**	24.1	1017.3109	23.7
1271.4744	15.0	1271.4704	14.0	**1173.7460**	13.3	1173.7425	14.3	**1089.9768**	14.2	1089.9756	14.2	**1017.3792**	14.7	1017.3777	14.4
1271.5542	13.0	1271.5539	8.0	**1173.8193**	11.9	1173.8196	8.0	**1090.0481**	10.5	1090.0473	7.9	**1017.4432**	10.8	1017.4446	8.0
1271.6399	9.9	1271.6375	4.0	**1173.8964**	8.2	1173.8967	4.0	**1090.118**	7.8	1090.1189	3.9	**1017.5083**	7.8	1017.5114	4.0
1271.7200	6.8	1271.7210	2.0	**1173.9760**	7.5	1173.9738	1.6	**1090.1875**	5.3	1090.1905	1.6	**1017.5757**	7.4	1017.5783	1.6
1271.7957	3.6	1271.8047	0.0	**1174.0444**	3.0	1174.0510	0.5	**1090.2581**	3.8	1090.2622	0.5	**1017.6421**	6.4	1017.6452	0.5

16H$^+$				17H$^+$				18H$^+$				19H$^+$			
observed		calculated		observed		calculated		observed		calculated		observed		calculated	
m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.
952.9122	5.6	952.9146	0.2	**896.9146**	5.2	896.9200	0.2	**847.1436**	5.7	847.1471	0.2	**802.6367**	1.0	802.6134	0.2
952.9737	9.1	952.9773	1.4	**896.9759**	8.9	896.9790	1.3	**847.2020**	9.3	847.2028	1.3	**802.6653**	9.3	802.6662	1.3
953.0352	16.7	953.0400	5.5	**897.0350**	17.1	897.0380	5.4	**847.2563**	18.6	847.2586	5.4	**802.7169**	16.0	802.7190	5.2
953.0988	29.3	953.1026	15.1	**897.0944**	28.8	897.0970	14.9	**847.3129**	32.8	847.3143	14.8	**802.7714**	32.0	802.7718	14.5
953.1641	49.7	953.1653	31.3	**897.1552**	49.2	897.1560	31.1	**847.3694**	50.9	847.3700	30.9	**802.8243**	51.7	802.8246	30.2
953.2266	69.8	953.2280	52.9	**897.2143**	67.5	897.2150	52.6	**847.4247**	69.0	847.4257	52.3	**802.8773**	71.8	802.8773	51.4
953.2899	88.6	953.2907	75.0	**897.2740**	86.4	897.2740	74.8	**847.4820**	92.2	847.4814	74.4	**802.9304**	87.7	802.9301	73.3
953.3532	100.0	953.3533	92.1	**897.3330**	100.0	897.3330	92.0	**847.5376**	99.4	847.5371	91.9	**802.9839**	100.0	802.9829	91.7
953.4172	97.4	953.4160	100.0	**897.3929**	98.4	897.3919	100.0	**847.5949**	100.0	847.5928	100.0	**803.0371**	97.1	803.0356	100.0
953.4797	90.5	953.4787	97.4	**897.4520**	91.6	897.4509	97.6	**847.6506**	87.7	847.6485	99.1	**803.0906**	91.7	803.0884	98.1
953.5420	77.9	953.5413	85.5	**897.5113**	81.0	897.5099	86.7	**847.7059**	78.2	847.7042	87.0	**803.1437**	75.3	803.1412	88.0
953.6052	61.2	953.6040	69.7	**897.5720**	60.2	897.5689	70.3	**847.7625**	62.8	847.7599	70.9	**803.1957**	60.5	803.1940	71.5
953.6674	45.8	953.6666	52.5	**897.6304**	43.9	897.6278	53.0	**847.8181**	48.6	847.8156	53.5	**803.2496**	47.2	803.2467	53.7
953.7324	31.8	953.7293	36.6	**897.6888**	34.9	897.6868	37.2	**847.8741**	32.0	847.8713	38.0	**803.3021**	32.5	803.2995	37.6
953.7944	22.9	953.7919	24.3	**897.7477**	23.0	897.7458	24.7	**847.9296**	23.5	847.9270	24.9	**803.3549**	22.0	803.3523	24.5
953.8560	14.8	953.8546	14.6	**897.8065**	15.5	897.8048	14.9	**847.9853**	16.4	847.9826	15.0	**803.4070**	14.9	803.4050	14.9
953.9164	9.8	953.9173	8.2	**897.8647**	10.5	897.8637	8.3	**848.0436**	10.2	848.0383	8.4	**803.4598**	11.5	803.4578	8.4
953.9805	7.2	953.9799	4.1	**897.9230**	8.1	897.9227	4.1	**848.0945**	9.0	848.0941	4.2	**803.5127**	9.7	803.5106	4.2
954.0438	7.1	954.0426	1.7	**897.9780**	7.8	897.9817	1.7	**848.1481**	7.4	848.1498	1.7	**803.5635**	6.0	803.5633	1.7
954.1035	6.3	954.1053	0.5	**898.0378**	7.2	898.0407	0.5	**848.2027**	6.3	848.2055	0.4	**803.6119**	5.0	803.6161	0.4

20H+				21H+				22H+				23H+			
observed		calculated		observed		calculated		observed		calculated		observed		calculated	
m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.	m/z	Int.
762.5301	7.0	762.5331	0.2	**726.2731**	7.2	726.2700	0.2	**693.3033**	5.4	693.3035	0.2	**663.1987**	14.8	663.2037	0.2
762.5808	9.8	762.5833	1.3	**726.3179**	8.4	726.3178	1.3	**693.3452**	11.0	693.3491	1.2	**663.2473**	13.4	663.2473	1.2
762.632	17.0	762.6334	5.2	**726.3631**	21.4	726.3655	5.2	**693.3947**	19.9	693.3947	5.0	**663.2913**	18.8	663.2909	5.0
762.6816	29.7	762.6836	14.3	**726.4139**	35.7	726.4133	14.3	**693.4405**	33.5	693.4403	13.9	**663.3337**	38.3	663.3345	13.9
762.7332	48.7	762.7337	30.0	**726.4604**	47.1	726.4610	30.1	**693.4863**	53.6	693.4858	29.3	**663.3788**	50.9	663.3781	30.3
762.784	70.6	762.7838	50.9	**726.5078**	71.5	726.5088	51.2	**693.5316**	75.2	693.5314	52.1	**663.4219**	64.8	663.4217	51.8
762.8336	82.3	762.8340	72.8	**726.5568**	90.0	726.5565	76.0	**693.5778**	88.7	693.5770	74.5	**663.4647**	85.1	663.4653	74.3
762.8859	99.2	762.8841	90.2	**726.6053**	100.0	726.6043	93.8	**693.6233**	98.4	693.6226	91.6	**663.5095**	100.0	663.5089	92.0
762.9356	100.0	762.9342	100.0	**726.6531**	95.8	726.6520	100.0	**693.6699**	100.0	693.6681	100.0	**663.5541**	98.3	663.5524	100.0
762.9863	91.1	762.9844	98.1	**726.7014**	93.0	726.6997	99.3	**693.7152**	94.8	693.7137	96.6	**663.598**	87.7	663.5960	98.4
763.0355	78.3	763.0345	87.3	**726.7498**	76.8	726.7475	88.6	**693.7606**	85.5	693.7593	86.4	**663.6406**	78.4	663.6396	88.6
763.0869	59.4	763.0846	72.2	**726.7965**	65.8	726.7952	72.5	**693.8062**	64.2	693.8049	71.3	**663.6849**	61.0	663.6832	72.6
763.1375	45.8	763.1347	54.7	**726.845**	46.4	726.8430	55.4	**693.8514**	49.3	693.8504	53.9	**663.7276**	45.7	663.7268	54.2
763.1869	32.0	763.1849	37.6	**726.8932**	33.1	726.8907	39.0	**693.8977**	34.1	693.8960	38.3	**663.7726**	37.5	663.7704	38.1
763.2378	22.3	763.2350	24.6	**726.9412**	24.6	726.9384	25.4	**693.9443**	26.3	693.9416	25.2	**663.8157**	23.0	663.8140	25.3
763.2858	16.6	763.2851	15.2	**726.9886**	16.9	726.9862	15.5	**693.9884**	16.1	693.9871	15.4	**663.8606**	20.2	663.8576	15.5
763.3373	11.1	763.3352	8.5	**727.0349**	10.9	727.0339	8.6	**694.0325**	13.7	694.0327	8.6	**663.9071**	13.4	663.9012	8.7
763.386	8.2	763.3854	4.2	**727.0804**	9.6	727.0817	4.3	**694.0791**	9.2	694.0783	4.3	**663.9483**	12.7	663.9448	4.3
763.4318	8.3	763.4355	1.7	**727.1336**	8.4	727.1294	1.7	**694.1236**	8.8	694.1239	1.7	**663.9825**	3.2	663.9883	1.7
763.4863	8.2	763.4856	0.4	**727.1748**	6.5	727.1772	0.5	**694.1681**	8.3	694.1694	0.5	**664.0132**	2.3	664.0320	0.5

Table S2. Isotope envelope of H4K16ac

19H$^+$				18H$^+$				17H$^+$				16H$^+$			
Observed		Calculated		Observed		Calculated		Observed		Calculated		Observed		Calculated	
m/z	int.	m/z	int.	m/z	int.	m/z	int.	m/z	int.	m/z	int.	m/z	int.	m/z	int.
1253.3719	3.0	1253.3797	1.7	1128.1400	2.7	1128.1425	1.6	1025.6702	2.8	1025.6756	1.5	940.2825	3.4	940.2866	1.5
1253.4913	8.7	1253.4912	9.8	1128.2410	9.2	1128.2428	9.3	1025.7656	9.2	1025.7668	8.8	940.3687	9.8	940.3702	8.6
1253.6035	23.2	1253.6026	27.9	1128.3417	23.4	1128.3431	26.9	1025.8577	23.7	1025.8580	25.6	940.4533	24.0	940.4538	25.1
1253.7140	45.2	1253.7141	54.4	1128.4424	45.6	1128.4434	52.9	1025.9481	45.9	1025.9492	51.2	940.5369	46.4	940.5373	50.3
1253.8248	69.8	1253.8255	80.8	1128.5426	70.1	1128.5437	79.3	1026.0392	71.4	1026.0404	77.7	940.6202	71.9	940.6209	76.8
1253.9366	90.7	1253.9370	97.7	1128.6421	89.8	1128.6440	96.8	1026.1296	90.8	1026.1315	96.0	940.7036	90.7	940.7045	95.4
1254.0476	100.0	1254.0484	100.0	1128.7423	100.0	1128.7442	100.0	1026.2208	100.0	1026.2227	100.0	940.7869	100.0	940.7880	100.0
1254.1589	95.5	1254.1598	89.4	1128.8425	95.2	1128.8445	90.1	1026.3119	96.8	1026.3138	90.7	940.8703	96.8	940.8716	91.0
1254.2697	82.3	1254.2712	71.2	1128.9426	81.9	1128.9448	72.1	1026.4027	84.1	1026.4050	72.9	940.9537	82.1	940.9552	73.5
1254.3810	63.9	1254.3826	51.2	1129.0427	64.0	1129.0450	52.0	1026.4938	64.1	1026.4961	52.8	941.0371	65.1	941.0387	53.5
1254.4924	45.0	1254.4939	33.7	1129.1428	45.4	1129.1452	35.1	1026.5850	45.8	1026.5873	34.9	941.1208	46.3	941.1223	35.4
1254.6038	30.1	1254.6053	20.4	1129.2428	30.0	1129.2455	21.1	1026.6759	30.8	1026.6784	21.1	941.2041	30.9	941.2058	21.5
1254.7147	19.2	1254.7167	11.3	1129.3434	18.7	1129.3458	11.8	1026.7664	19.0	1026.7695	11.8	941.2880	18.8	941.2893	12.0
1254.8269	11.3	1254.8282	5.6	1129.4437	11.2	1129.4460	5.8	1026.8582	11.1	1026.8607	5.9	941.3710	11.4	941.3729	6.0
1254.9380	6.6	1254.9395	2.5	1129.5432	6.5	1129.5463	2.5	1026.9503	6.9	1026.9518	2.6	941.4549	6.6	941.4564	2.6
1255.0474	3.7	1255.0510	0.9	1129.6423	3.9	1129.6466	0.9	1027.0421	4.0	1027.0430	0.9	941.5384	4.1	941.5400	1.0
1255.1522	2.7	1255.1624	0.1	1129.7444	2.9	1129.7468	0.2	1027.1299	3.5	1027.1342	0.1	941.6204	3.0	941.6237	0.1

15H$^+$				14H$^+$				13H$^+$				12H$^+$			
Observed		Calculated		Observed		Calculated		Observed		Calculated		Observed		Calculated	
m/z	int.	m/z	int.	m/z	int.	m/z	int.	m/z	int.	m/z	int.	m/z	int.	m/z	int.
868.0294	3.2	868.0343	1.4	806.0992	3.0	806.1038	1.4	752.4278	3.7	752.4307	1.3	705.4655	3.4	705.4668	1.3
868.1115	10.1	868.1115	8.3	806.1746	10.6	806.1755	8.2	752.4965	10.3	752.4976	8.0	705.5291	9.9	705.5295	7.8
868.1885	24.3	868.1886	24.7	806.2476	24.5	806.2471	24.2	752.5647	25.4	752.5645	23.7	705.5923	24.2	705.5921	23.5
868.2657	47.4	868.2658	49.8	806.3192	46.5	806.3188	49.2	752.6315	48.0	752.6313	48.4	705.6548	45.6	705.6548	47.9
868.3427	71.6	868.3429	76.5	806.3901	70.6	806.3904	75.8	752.6984	73.5	752.6982	74.9	705.7176	70.8	705.7175	74.5
868.4196	91.7	868.4201	94.9	806.4621	90.7	806.4620	94.7	752.7649	94.5	752.7650	94.2	705.7803	90.0	705.7802	93.9
868.4966	100.0	868.4972	100.0	806.5338	100.0	806.5336	100.0	752.8320	100.0	752.8319	100.0	705.8429	100.0	705.8428	100.0
868.5739	96.7	868.5743	91.5	806.6053	95.3	806.6052	92.5	752.8992	97.6	752.8987	92.1	705.9059	95.4	705.9055	92.3
868.6511	83.5	868.6515	74.1	806.6771	81.9	806.6769	75.2	752.9660	84.9	752.9655	75.1	705.9684	81.9	705.9681	75.5
868.7281	65.2	868.7286	53.7	806.7488	63.6	806.7485	55.0	753.0328	65.5	753.0324	55.1	706.0310	63.5	706.0308	55.4
868.8054	46.5	868.8057	35.9	806.8201	45.7	806.8201	36.3	753.0995	46.4	753.0992	36.7	706.0935	45.3	706.0935	37.1
868.8820	30.9	868.8828	21.8	806.8919	30.4	806.8917	22.1	753.1661	31.4	753.1661	22.3	706.1562	30.3	706.1561	22.4
868.9594	18.9	868.9599	12.2	806.9636	18.7	806.9633	12.4	753.2335	19.6	753.2329	12.5	706.2183	19.2	706.2188	12.6
869.0364	11.6	869.0371	6.1	807.0351	11.2	807.0349	6.2	753.2997	11.7	753.2997	6.4	706.2809	11.4	706.2815	6.4
869.1133	6.7	869.1142	2.7	807.1066	6.5	807.1066	2.7	753.3665	7.2	753.3666	2.7	706.3431	7.0	706.3441	2.8
869.1896	4.2	869.1914	1.0	807.1781	4.2	807.1782	1.0	753.4337	4.7	753.4335	1.0	706.4064	4.9	706.4068	1.0
869.2674	3.2	869.2685	0.1	807.2488	3.2	807.2499	0.1	753.5016	3.1	753.5002	0.1	706.4684	3.2	706.4694	0.1

11H$^+$				**10H$^+$**				**9H$^+$**			
Observed		Calculated		Observed		Calculated		Observed		Calculated	
m/z	int.	m/z	int.	m/z	int.	m/z	int.	m/z	int.	m/z	int.
664.0231	3.8	664.0280	1.3	627.1902	1.6	627.1935	1.3	594.2200	10.2	594.2363	1.3
664.0861	10.0	664.0870	7.7	627.2438	12.8	627.2492	7.6	594.2838	16.4	594.2891	7.5
664.1464	25.0	664.1460	23.3	627.3041	25.7	627.3049	23.1	594.3424	30.8	594.3419	22.7
664.2053	47.1	664.2050	47.6	627.3612	48.0	627.3606	47.4	594.3958	49.1	594.3947	46.7
664.2640	73.0	664.2639	74.2	627.4165	70.6	627.4163	74.0	594.4470	71.6	594.4474	73.0
664.3228	90.5	664.3229	93.8	627.4720	89.4	627.4720	93.8	594.4994	92.6	594.5002	92.6
664.3817	100.0	664.3819	100.0	627.5275	100.0	627.5277	100.0	594.5524	100.0	594.5530	100.0
664.4412	95.0	664.4409	92.6	627.5833	91.7	627.5834	93.7	594.6053	93.5	594.6057	93.1
664.4998	82.2	664.4999	75.8	627.6386	81.4	627.6391	77.0	594.6575	79.2	594.6585	76.5
664.5586	64.5	664.5588	55.8	627.6942	61.8	627.6948	56.9	594.7100	62.7	594.7113	56.5
664.6173	45.7	664.6178	37.4	627.7498	44.7	627.7506	37.7	594.7631	45.3	594.7641	38.0
664.6760	29.7	664.6768	22.9	627.8052	28.9	627.8063	23.4	594.8161	29.5	594.8168	23.3
664.7344	19.7	664.7358	12.9	627.8614	18.8	627.8620	13.1	594.8688	18.9	594.8696	13.1
664.7939	11.8	664.7947	6.5	627.9162	11.4	627.9177	6.6	594.9227	11.7	594.9224	6.6
664.8528	6.9	664.8537	2.8	627.9730	6.9	627.9734	2.9	594.9756	7.6	594.9751	2.9
664.9107	4.4	664.9127	1.0	628.0286	4.9	628.0291	1.0	595.0284	4.9	595.0279	1.0
664.9720	3.6	664.9717	0.2	628.0833	3.7	628.0848	0.2	595.0786	4.1	595.0808	0.1

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