Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Solid Phase Chemical Ligation Employing a Rink Amide Linker for the synthesis of Histone H2B Protein

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General:

Commercial reagents: Amino acids , coupling reagents HATU, HCTU, Resins, were purchased from Luxembourg, Novabiochem, Aapptec and Chem-Impex. DMF was purchased from biotech grade.

Fmoc-SPPS was carried out manually in syringes with filters or on automated peptide synthesizer (Liberty 1, CSBIO). All reactions were carried out at RT.

Analytical HPLC was performed on a Thermo instrument (Spectra System P4000) using an analytical column (Jupiter 5 micron, C18/C4 300 Å 150 x 4.6 mm) at a flow rate of 1.2 mL/min.

Preparative HPLC was performed on a Waters instrument using preparative column (Jupiter 10 micron, C18/C4 300 Å, 250 x 22.4 mm) at a flow rate of 15 mL/min.

Buffer B: 0.1% TFA in acetonitrile, Buffer A: 0.1% TFA in water.

Mass spectrometry analysis was carried out using LCQ Fleet Ion Trap (Thermo Scientific).

Synthesis of model peptide, Thz-LYRAGLYRL-NBZ, 1:

The synthesis of the model peptide **1** was carried out using Fmoc-SPPS on Rink amide resin (0.27 mmol/g, 0.1 mmol scale). The resin was treated with 20% piperidine (5min X3) washed with DMF and coupled to 4 eq Fmoc-Dbz (Mono-Fmoc-3,4-diaminobenzoic acid), using HATU/DIEA (4 eq/8 eq) for 50 min (X2). The remaining peptide was synthesized on an automated peptide synthesizer using 4 eq of amino acid, 8 eq of DIEA and 4 eq of HCTU. The coupling was performed for 60 min, and Fmoc-deprotection was achieved using 20% piperidine (5min X3). Finally the peptide bound resin was washed with DMF and DCM and was treated with a solution of 50 eq of p-nitrophenylchloroformate in DCM, for 30 min (X3). The resin was washed with DCM and DMF and treated with a solution of 0.5M DIEA in DMF and shaken for 40 min at RT. Finally the resin was washed with DMF/DCM and kept to dry. Mixture of TFA, H_2O, TIS (95:2.5:2.5) was added to the resin which was shaken for 2 h at RT. The peptide was precipitated in cold ether and collected by centrifugation followed by dissolving the peptide in 25% acetonitrile/H_2O, followed by lyophilization. HPLC purification affording the product in 60% yield.

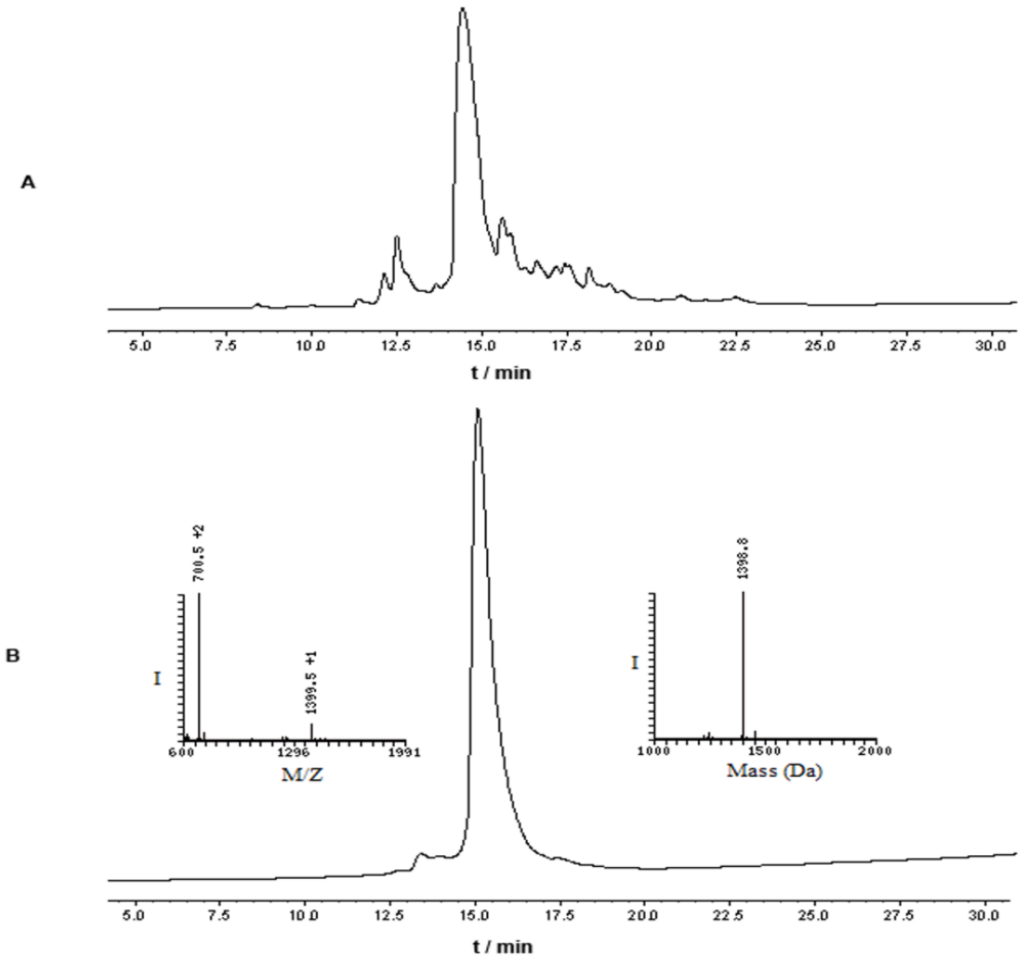


Figure 1: Analytical HPLC and ESI-MS traces of the crude (A) and pure (B) peptide **1** with the observed mass of 1398.8 Da (calculated 1399 Da).

Synthesis of Thz-H2B(97-124)-Nbz, Thz-H2B(58-96)-Nbz, Thz-H2B(21-57)-Nbz and HA-H2B(1-20)-Nbz:

HA-H2BSequence:

YPYDVPDYAPEPAKSAPAPKKGSKKAVTKAQKKDGKKRKRSRKESYSVYVYKVLKQV HPDTGISSKAMGIMNSFVNDIFERIAGEASRLAHYNKRSTITSREIQTAVRLLLPGELAKH AVSEGTKAVTKYTSSK

The Ala residues in positions 97, 58, 21 were mutated to Cys to allow for NCL. Met residues in positions 62 and 59 were mutated to Norleucine to prevent oxidation, Lys in positions 57 and 20 were protected with the photolabile Nvoc protecting group to prevent lactamization during ligation, the Nvoc protecting group can be removed by UV or during the final desulfurization step.

SPPS: The synthesis was carried out on the Rink amide (0.27mmol/g, 0.1 mmol scale). The resin was treated with 20% piperidine (5min X3) washed with DMF and coupled to 4 eq Fmoc-Dbz, using HATU/DIEA (4 eq/8 eq) for 60 min (X2). The remaining amino acids were coupled using an automated peptide synthesizer. Linker activation was achieved as described for peptide **1**. Cleavage and purification were carried out as described above affording the products in 30-50% yield.

1. **Thz-H2B(97-124)-Nbz**:

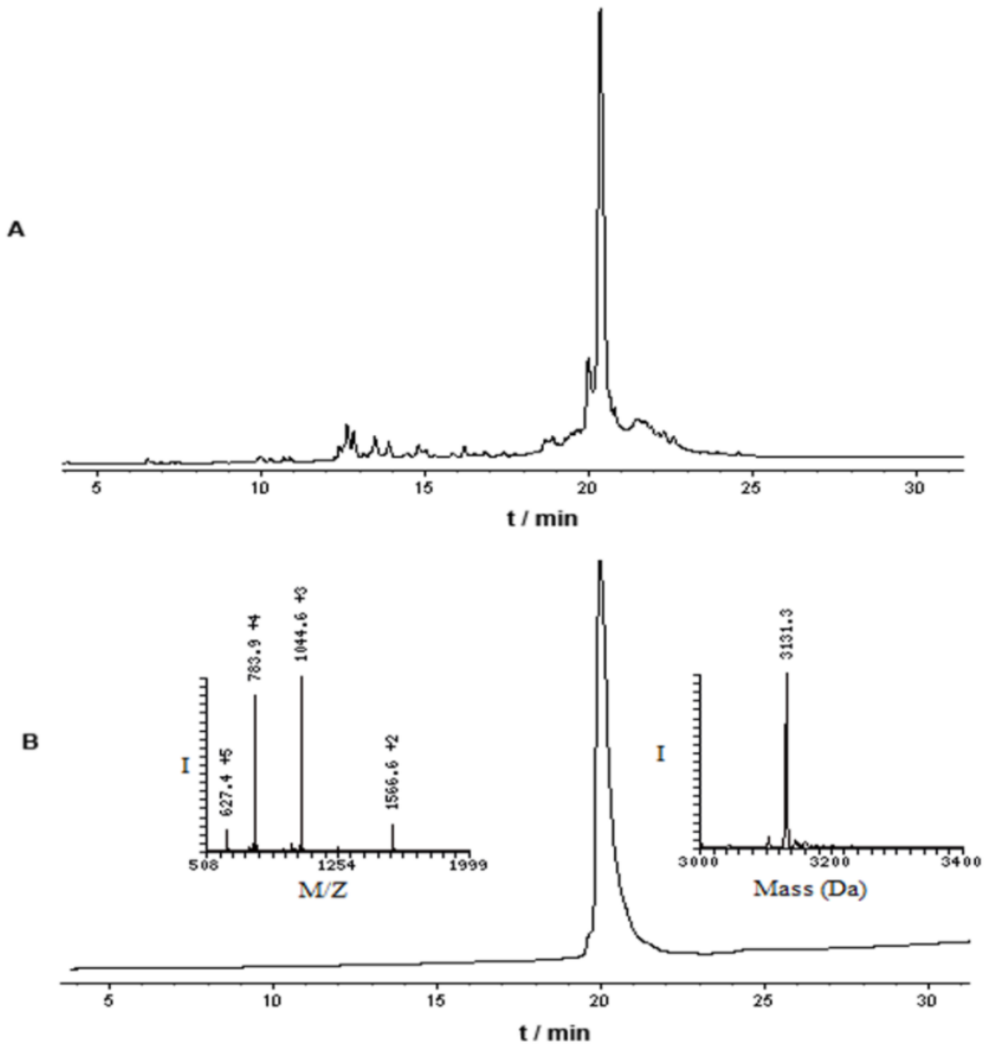


Figure 2: Analytical HPLC and ESI-MS traces of the crude (A) and pure (B) Thz-H2B(58-96)-Nbz with the observed mass of 3131.3 Da (calculated 3131 Da).

2. Thz-H2B(58-96)-Nbz:

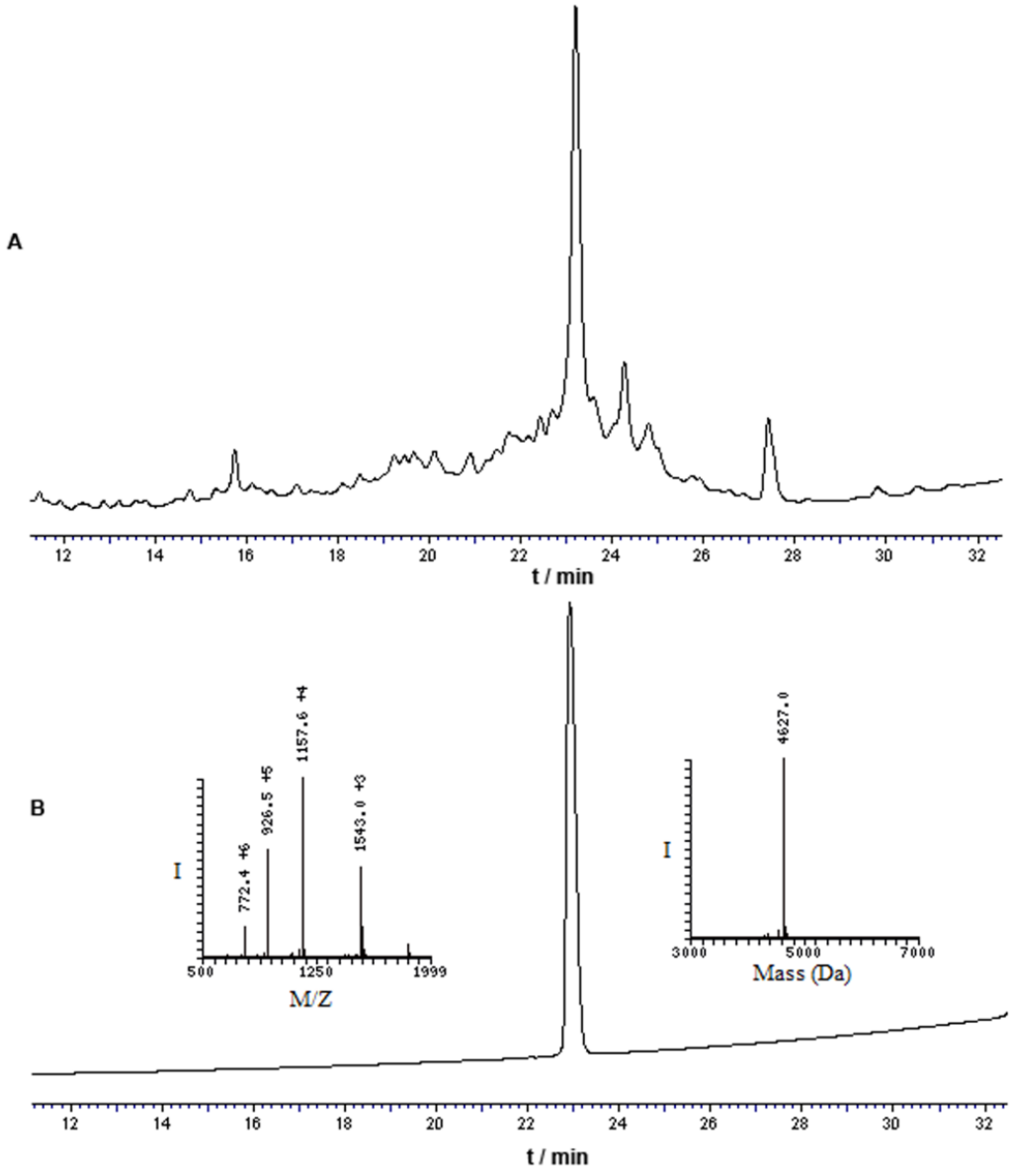


Figure 3: Analytical HPLC and ESI-MS traces of the crude (A) and pure (B) Thz-H2B(97-124)-Nbz with the observed mass of 4627.0 Da (calculated 4626 Da).

3. Thz-H2B(21-57)-Nbz:

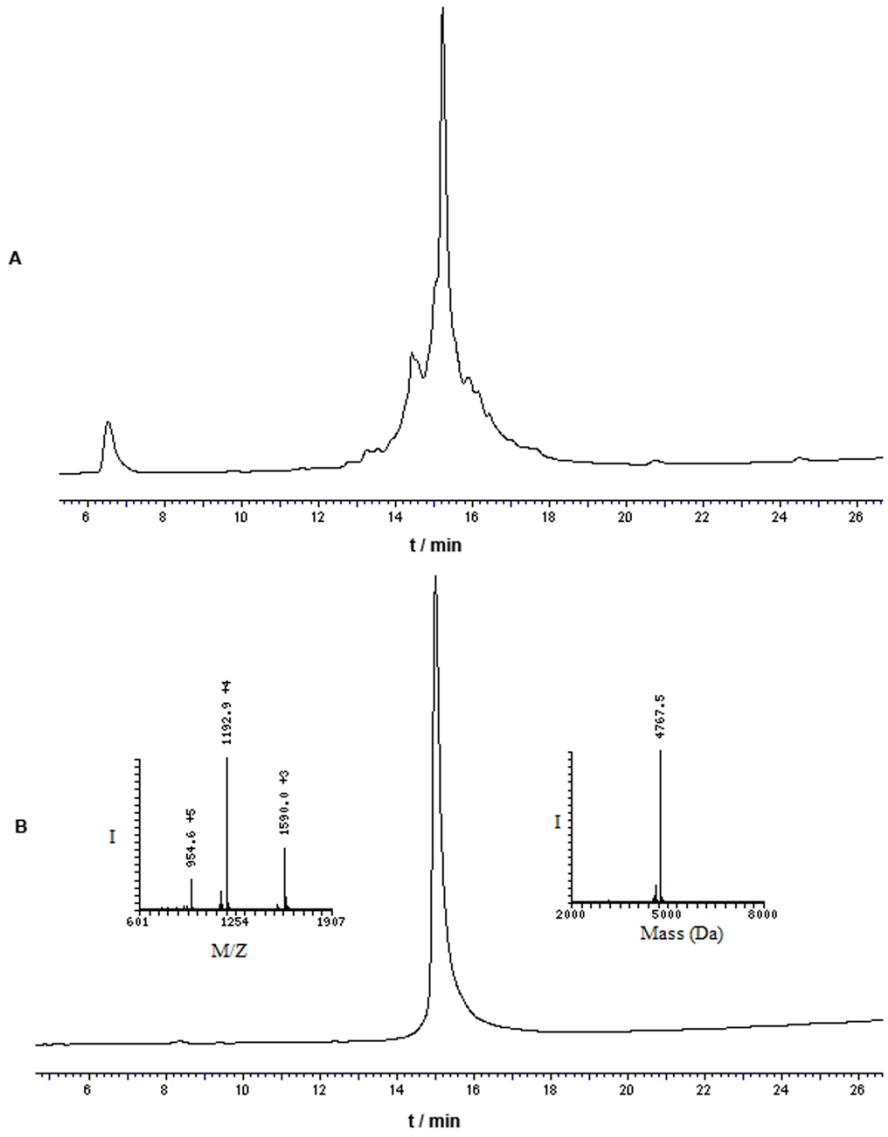


Figure 4: Analytical HPLC and ESI-MS traces of the crude (A) and pure (B) Thz-H2B(21-57)-Nbz with the observed mass of 4767.5 Da (calculated 4768 Da).

4. HA-H2B(1-20)-Nbz:

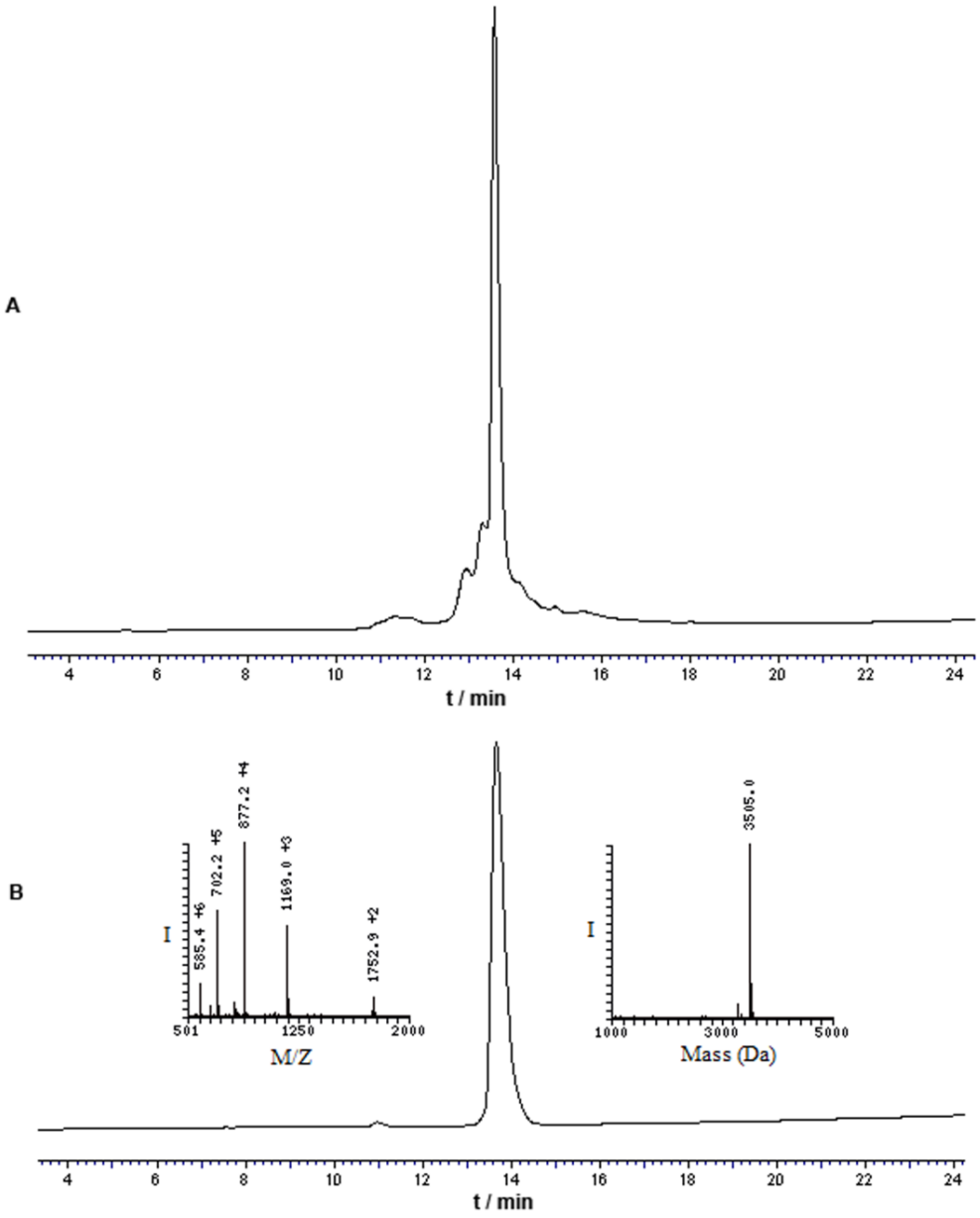
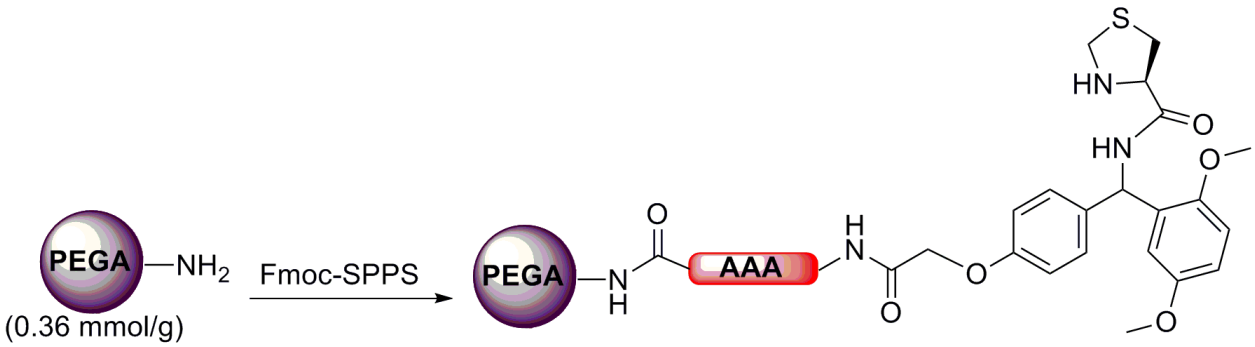


Figure 5: Analytical HPLC and ESI-MS traces of the crude (A) and pure (B) HA-H2B(1-20)-Nbz with the observed mass of 3505.0 Da (calculated 3505 Da).

Resin preparation: PEGA resin (0.72 mmol,0.36 mmol/g) was swelled in DMF, then Fmoc-Ala-OH X3 (spacer), Fmoc-Rink amide, Fmoc-THz-OH, were coupled using 4 eq HATU, 8 eq DIEA, for 1 h for each coupling. Fmoc was removed using 20% piperidine. Finally the resin was washed with DMF ,DCM and 6M Gn·HCl, 200 mM phosphate buffer, pH ~7.



Thz to Cys conversion: The PEGA resin after Thz coupling was treated with a solution of 0.2 M methoxylamine and 20 eq TCEP (6 M Gn·HCl, 200 mM phosphate buffer, pH=4) at 37C°, for 4 h. The progress of the reaction was monitored using analytical C4 column with a gradient of 5-60% B over 40 min.

SPCL: All the ligation reactions were performed at 3-4 mM concentration. Ligation of the first fragment Thz-H2B(97-124)-Nbz (1.5 eq) was carried out in presence of 30 eq MPAA, 20 eq TCE, in 6 M Gn·HCl, 200 mM phosphate buffer, pH ~7.1, 37C° for 15 h. The ligation of the other 3 fragments Thz-H2B(58-96)-Nbz, Thz-H2B(21-57)-Nbz and HA-H2B(1-20)-Nbz was carried out using 1.2 eq of each peptide in similar manner, however for 10 h ligation time for each fragment. The progress of reaction was monitored by analytical cleavage, using C4 column and a gradient of 5-60% B over 40 min.

Desulfurization: The polypeptide bound-PEGA resin after washing was treated with a solution of 20 eq VA-044 per thiol, 0.1 M TCEP and 10% v/v t-butyl mercaptan in 6 M Gn·HCl, 200 mM phosphate buffer, pH 7, for 14 h at 40C°. The progress of reaction was monitored by analytical cleavage, using analytical C4 column with a gradient of 5-60% B for 40 min.

Photolysis: to remove the Nvoc protecting group**,** the polypeptide bound-PEGA resin was treated with a solution of 0.2 M of methoxylamin and 30 eq of DTT in 6 M Gn·HCl, 200 mM phosphate buffer, pH 7, the mixture was irradiated with a UV lamp at 350 nm for 2 h. The progress of the reaction was monitored by analytical cleavage, using analytical C4 column with a gradient of 5-60% B for 40 min.

Cleavage: The polypeptide was cleaved form the resin after washing with 6 M Gn·HCl (3X) and water (3X), with (TFA: TIS: H_2O) 95:2.5:2.5, for 1 h. The peptide was precipitated in cold ether, followed by centrifugation and dissolving in 50% acetonitrile/H_2O, followed by lyophilization. HPLC purification affording the product in 10 % yield.

Circular dichroism analysis (CD) : Sample of the protein were dissolved 25 mM Tris buffer (pH 7.66). Exact concentration of the protein solution was determined using Pierce® BCA Protein Assay Kit (Thermo scientific) and diluted to a final concentration of 20 μM. The CD measurements were carried out on a Jasco-815 CD spectropolarimeter, at 28 °C, by using a quartz cell with 1.0 mm path length and 16 second averaging S18 times. Data was converted to ellipticity (θ in deg*cm2*dmol-1) according to the equation: [θ]molar = θobs / (nlc), where θobs is the CD signal in degrees, n is the number of peptide bonds, l is the path length in cm, and c is the concentration in decimoles per cm3.

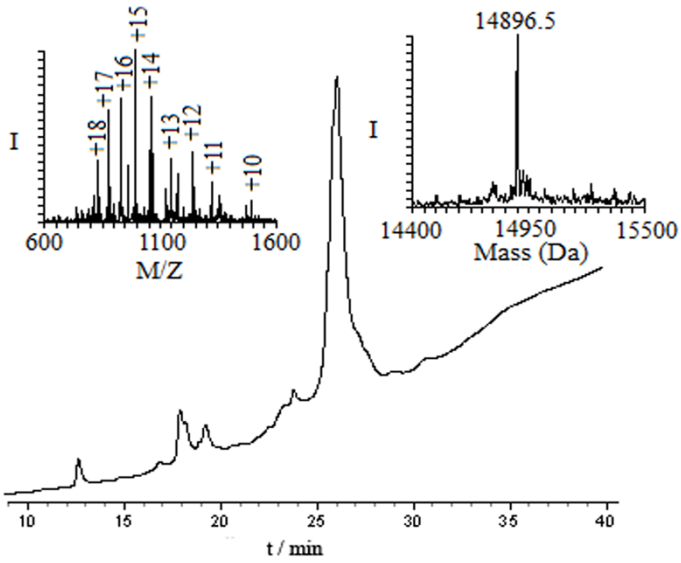


Figure 6: Analytical HPLC and ESI-MS traces of HA-H2B after UV, with the observed mass of 14896.5 Da (calculated 14895 Da).