

Supporting Information

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Convergent Chemical Synthesis of Histone H2B Protein for the Site-Specific Ubiquitination at Lys34**

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Experimental Methods

General

SPPS was carried out manually in syringes, equipped with teflon filters, purchased from Torviq or by using an automated peptide synthesizer (Liberty 1, CSBIO). If it is not differently described, all reactions were carried out at room temperature.

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 a semi-preparative column (Jupiter 10 micron, C4 300 Å, 250 x 10 mm and a flow rate of 5 mL/min or a preparative column (Jupiter 10 micron, C18/C4 300 Å, 250 x 22.4 mm) at a flow rate of 20 mL/min.

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

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

Commercial reagents were used without further purification. Resins, protected amino acids and HBTU, HCTU, HATU, were purchased from Novabiochem, Aapptec and Chem-Impex. DMF was purchased in biotech grade.

SPPS of H2B fragments

HA-P¹EPAKSAPAP¹⁰ KKGSKKAVTK²⁰ AQKKDGKKRK³⁰ RSRKESYSVY⁴⁰ VYKVLKQVHP⁵⁰ DTGISSKAMG⁶⁰ IMNSFVNDIF⁷⁰
ERIAGEASRL⁸⁰ AHYNKRSTIT⁹⁰ SREIQTAVRL¹⁰⁰ LLPGELAKHA¹⁰⁰ VSEGTKAVTK¹²⁰ YTSSK

Figure S1: Sequence of HA-H2B showing the pseudoproline or Asp(Dmp)Gly dipeptide that were used to achieve efficient synthesis. The corresponding dipeptide was coupled manually using 2.5 equiv of dipeptide 2.5 equiv of HATU and 5 equiv of DIEA.

Synthesis of H2B(97-125): The synthesis was carried out using Fmoc-SPPS on Rink amide resin (0.2mmol/g, 0.1 mmol scale). The peptide synthesis was performed on peptide synthesizer in presence of 4 equiv of amino acid (AA), 8 equiv of DIEA and 4 equiv of HBTU/HOBt to the initial loading of the resin.

Cleavage from the resin: A mixture of TFA, triisopropylsilane (TIS) and water (95:2.5:2.5) was added to the dried peptide-resin and the reaction mixture was shaken for 2 h at RT. The resin was removed by filtration and was washed with TFA (2 x 2 mL). To precipitate the peptide, the combined filtrate was added drop-wise to 10-fold volume of cold ether followed by centrifugation, decanting of ether and by dissolution of residue in acetonitrile-water for the HPLC purification step.

Synthesis of HA-H2B(1-20)-Nbz, H2B(21-57)-Nbz and H2B(58-96)-Nbz: The synthesis was carried out using the N-acylurea method on Rink amide resin (0.2 mmol/g, 0.1 mmol scale). Amino acids and HOBt/HBTU were used in 4 folds excess of the initial loading of the resin. DIEA was used in 8 folds excess. The first two amino acids were double coupled for 1h. Fmoc deprotection was achieved by treatment of the resin with 20% piperidine (3 x 3 min). The remaining amino acids were coupled using peptide synthesizer as described above. After completion of peptide synthesis, the resin was washed with DCM and a solution of *p*-nitrophenylchloroformate (100 mg, 5 eq) in 5 mL of DCM was added and shaken for 1 h at RT. The resin was washed with DCM (3 x 5 mL). Following these steps, a solution of 0.5 M DIEA in DMF (5 mL) was added and shaken for additional 30 min. The resin was washed using DMF (3 x 5 mL). The activation of **H2B(97-125)** peptides was carried out in DMF as was previously described.¹ Cleavage and purification were carried out as described above for **HA-H2B(1-20)** and **H2B(97-125)** affording the product in 40% and 50% yield, respectively.

H2B(21-57)-NHNH₂: The crude peptide **H2B(21-57)-Nbz** was dissolved in 200 mM of hydrazine solution in of 6 M Gn·HCl, 200 mM phosphate buffer pH 6.5 and left to react for 2 h. Thereafter, the reaction was purified using preparative column to give **H2B(21-57)-NHNH₂** in 30% yield.

General procedure for NCL: The ligation reaction was performed at 2 mM concentration and 1.1 equiv of peptide thioester was taken relatively to the cysteinal peptide. Both peptides were dissolved in argon purged buffer of 6M Gn.HCl, 200 mM Na₂HPO₄ with pH ~7.3. Progress of the reaction was monitored using analytical HPLC using C4 analytical column with a gradient of 5-55% buffer B. The ligation product was purified using preparative column.

Thz to Cys conversion: Following the ligation reaction, the mixture was diluted to 1 mM concentration with a solution of 0.4 M methoxylamine containing 30 equiv TCEP, pH 4, and incubated at 37 °C. The progress of reaction was monitored using analytical C4 column with a gradient of 5-50% B over 40 min.

In-situ Oxidation-ligation reaction: Peptide hydrazide was dissolved in Gn.HCl buffer, pH 3. The solution was cooled in a freezing mixture (-15 °C) for 15 min, thereafter, 15 equiv of 200 mM sodium nitrite solution (NaNO₂) were added and left to react for 20 minutes. Subsequently, 10 equiv of 200 mM of MPAA in 6M Gn.HCl solution (pH 6.0) were added and the mixture was left to react at -15 °C. Two additional portions of MPAA were added at room temperature and at 37 °C. After the second addition of MPAA solution, this solution was added to the cysteinal peptide **5**. After 20 minutes, 20 equiv of 200 mM TCEP solution (pH 8.0) were added and the pH adjusted to 6.5. The reaction was left to react for another 6 h for completion.

Acm deprotection: The protected peptide was dissolved at 0.6 mM concentration, in 10% AcOH (pH 4.0) containing 30 equiv of Hg(OAc)₂. The reaction mixture was mixed well and left to react at room temperature for 3 h. Thereafter, 120 equiv of DTT were added and the mixture was allowed to react for 12 h to precipitate all Hg(OAc)₂. The black precipitate was spun down and the supernatant was collected for HPLC purification.

Desulfurization: The purified peptide was dissolved in 6 M Gn.HCl, 200 mM phosphate buffer pH 7.4, purged under argon, to a concentration of 2 mM. To this solution, the same volume of the initial solution of 0.5 M solution of TCEP was added followed by the addition of 4% (v/v) of *t*-butyl mercaptan and 8 equiv per thiol of 0.1 M VA-044 dissolved in Gn.HCl. The reaction was left at 37 °C for 6-8 h. The progress of reaction was monitored using analytical C4 column and a gradient of 5% B-55% over 30 min.

Photolysis: To remove the photolabile protecting groups, the purified peptide was dissolved in 6 M Gn.HCl, 200 mM phosphate buffer pH 7.4, purged under argon, containing 0.2 M of methoxylamine.HCl and 30 eq. of DTT. Thereafter, the mixture was irradiated with a UV lamp at 365 nm, 28 °C for 2 h. The progress of reaction was monitored using analytical C4 column using a gradient of 5% B-55% over 30 min. Purification and lyophilization afforded the desired product.

Analytical data of peptide fragments

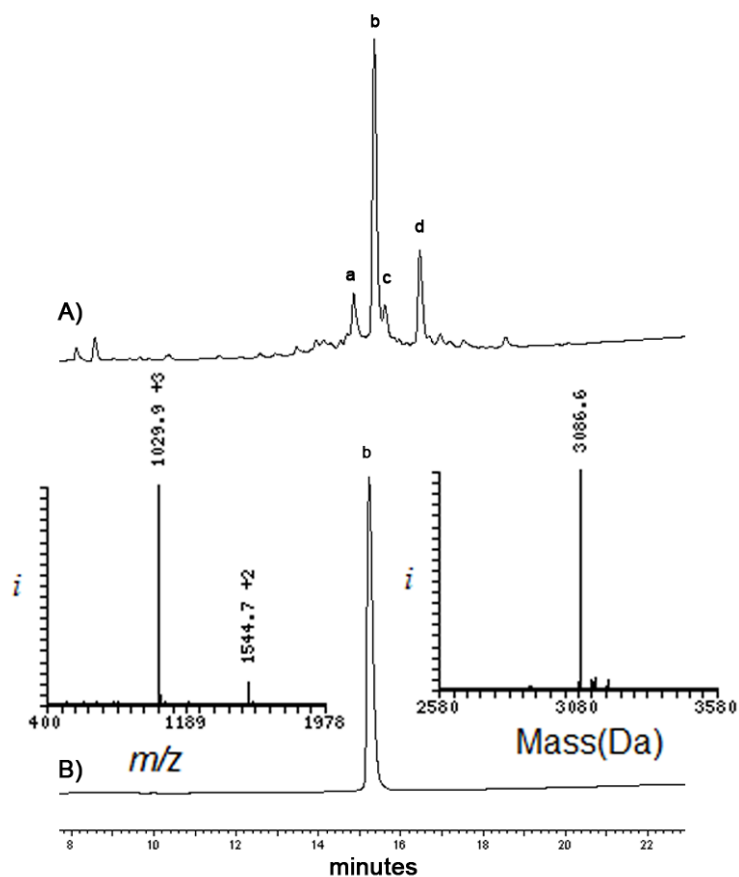


Figure S2: Synthesis of fragment H2B(97-125), **1**, crude (A) and pure (B). A) Peak a corresponds to a deletion of Leu with the observed mass 2973.3 Da (calcd 2973.4 Da); Peak b corresponds to the desired peptide, **1** with the observed mass 3086.5 Da (calcd 3086.5 Da); Peak c corresponds to the addition of Leu with the observed mass 3199.3 Da (calcd 3199.8 Da); Peak d corresponds to the addition of Val with the observed mass 3186.0 Da (calcd 3185.7 Da). B) Purified peptide **1**.

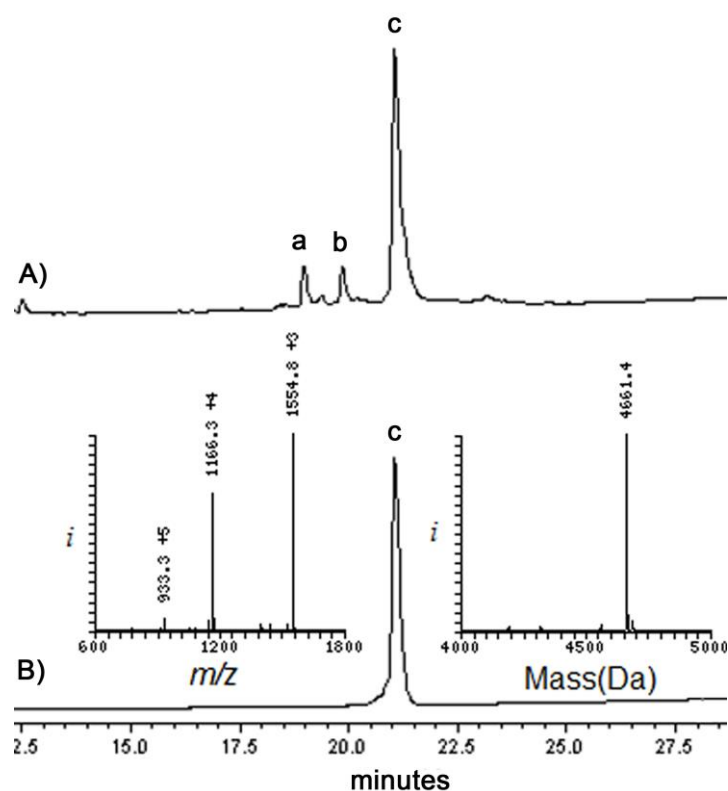


Figure S3: Synthesis of fragment H2B(58-96), **2**, crude (A) and pure (B). A) Peak a corresponds to unidentified product with a deletion of 408 Da; Peak b corresponds to a deletion of Val with the observed mass 4561.5 Da (calcd 4560.7 Da); Peak c corresponds to the desired peptide, **2**, with the observed mass 4661.4 Da (calcd 4659.9 Da). B) Purified peptide **2**.

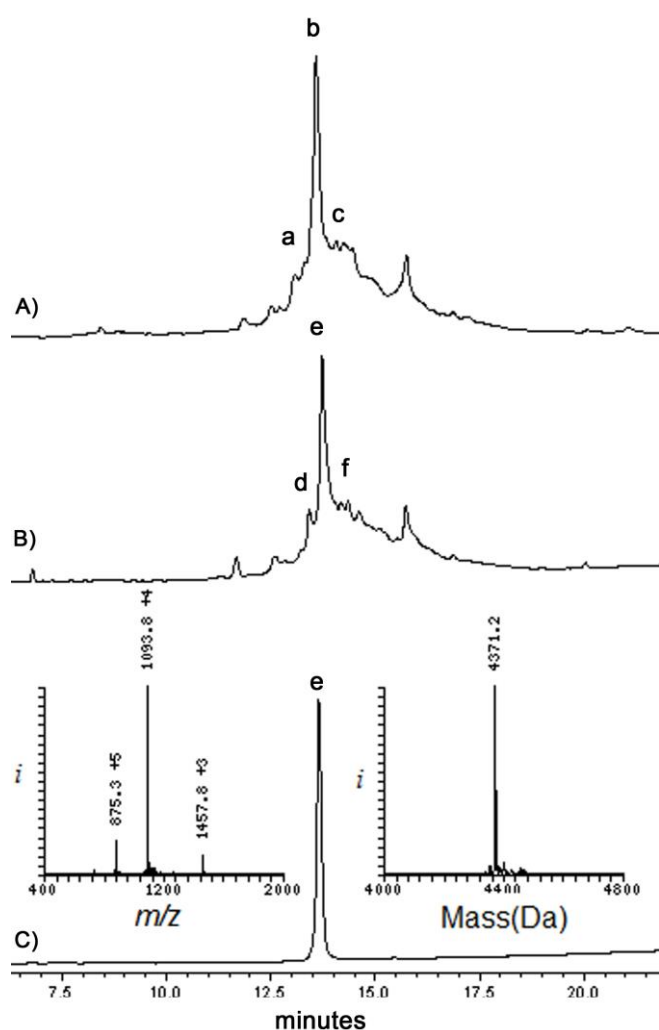


Figure S4: Synthesis of fragment H2B(21-57), **3**. A) the crude peptide bearing C-terminal N-acylurea. Peak a unidentified product; Peak b corresponds to the desired peptide with the observed mass 4513.8 Da (calcd 4513.0 Da); Peak c corresponds to a deletion of Gln with the observed mass 4386.1 Da (calcd 4384.9 Da). B) The crude peptide bearing C-terminal hydrazide after 2 h of reaction with hydrazine. Peak d unidentified product; Peak e corresponds to the desired peptide with the observed mass 4371.2 Da (calcd 4370.0 Da); Peak c corresponds to a deletion of Gln with the observed mass 4242.4 Da (calcd 2441.9 Da). C) Purified peptide **3**.

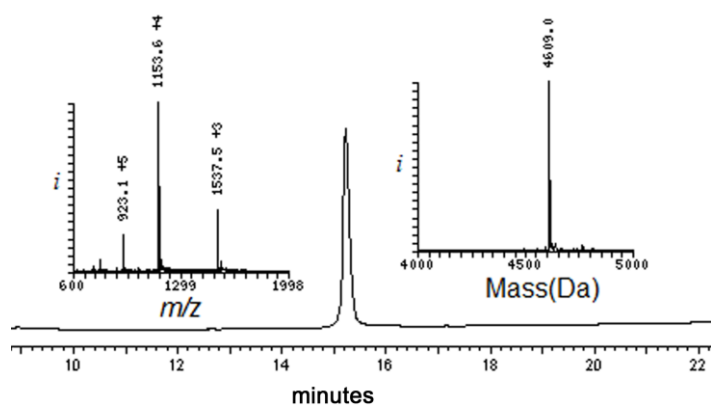


Figure S5: Synthesis of fragment H2B(21-57), **3a**, bearing Nvoc protected Lys57. The Peak corresponds to the purified peptide, **3a**, with the observed mass 4609.0 Da (calcd 4609.0 Da).

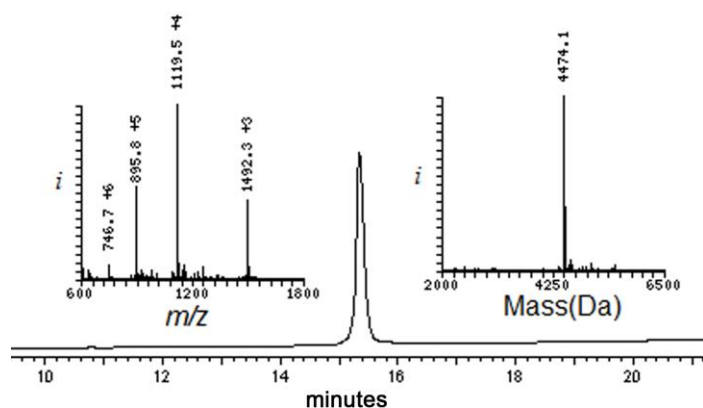


Figure S6: Synthesis of fragment H2B(21-57), **9**, bearing thiolysine at position 34 and Cys(Acm) at N-terminal. The Peak corresponds to the purified peptide with the mass of 4474.1 Da (calcd 4473.0 Da).

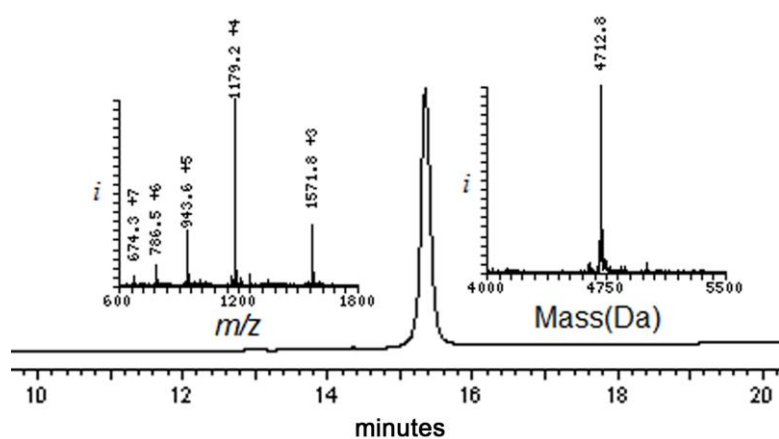


Figure S7: Synthesis of fragment **9a** bearing mLys at position 34, Nvoc protected Lys57 and Cys(Acm) at N-terminal. The Peak corresponds to the desired peptide with the mass of 4712.8 Da (calcd 4712.0 Da).

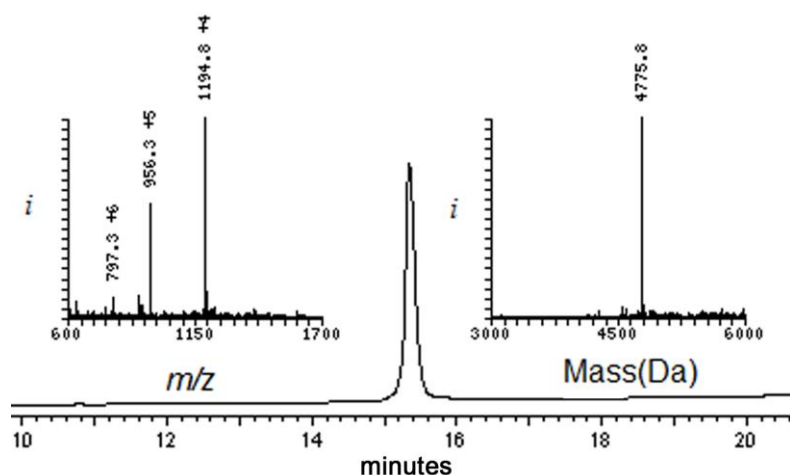


Figure S8: Synthesis of fragment H2B(21-57), **14**, bearing thiolysine at position 34 protected with o-nitrobenzyl and Lys57 protected with Nvoc. The Peak corresponds to the desired peptide with the observed mass 4775.8 Da (calcd 4776.0 Da).

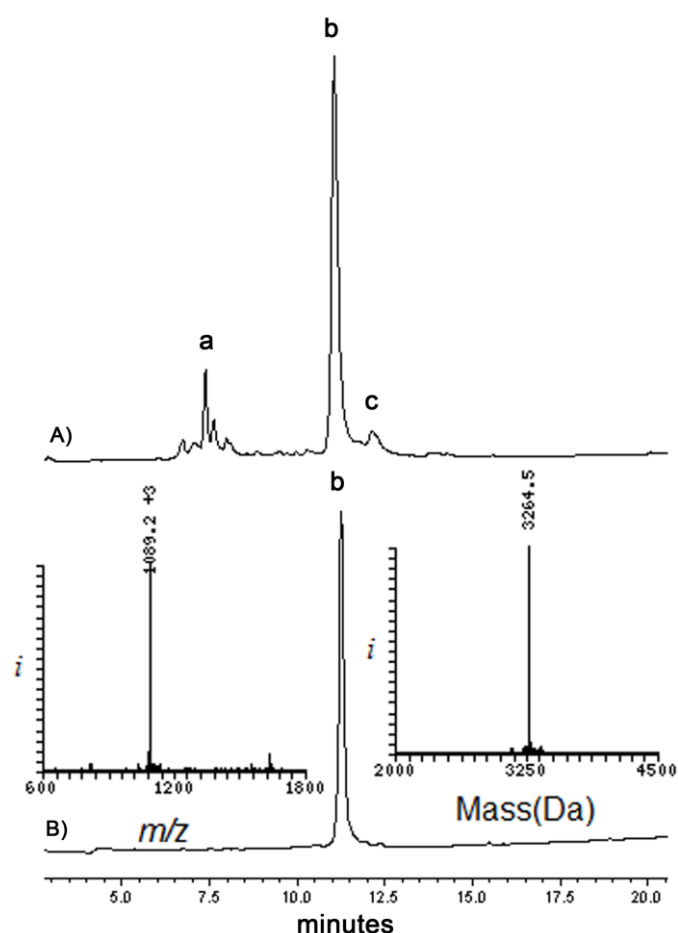


Figure S9: Synthesis of fragment HA-H2B(1-20), **4**, crude (A) and pure (B). A) Crude peptide **4** bearing C-terminal N-acylurea. Peak a corresponds to unidentified mass; Peak b corresponds to the desired peptide, **4**, with the observed mass 3264.5 Da (calcd 3262.5 Da); Peak c corresponds to a deletion of Pro

with the observed mass 3163.1 Da (calcd 3164.4 Da). B) Purified peptide **4** bearing C-terminal N-acylurea. Peak b corresponds to the desired peptide, **4**.

Analytical data of ligation reactions

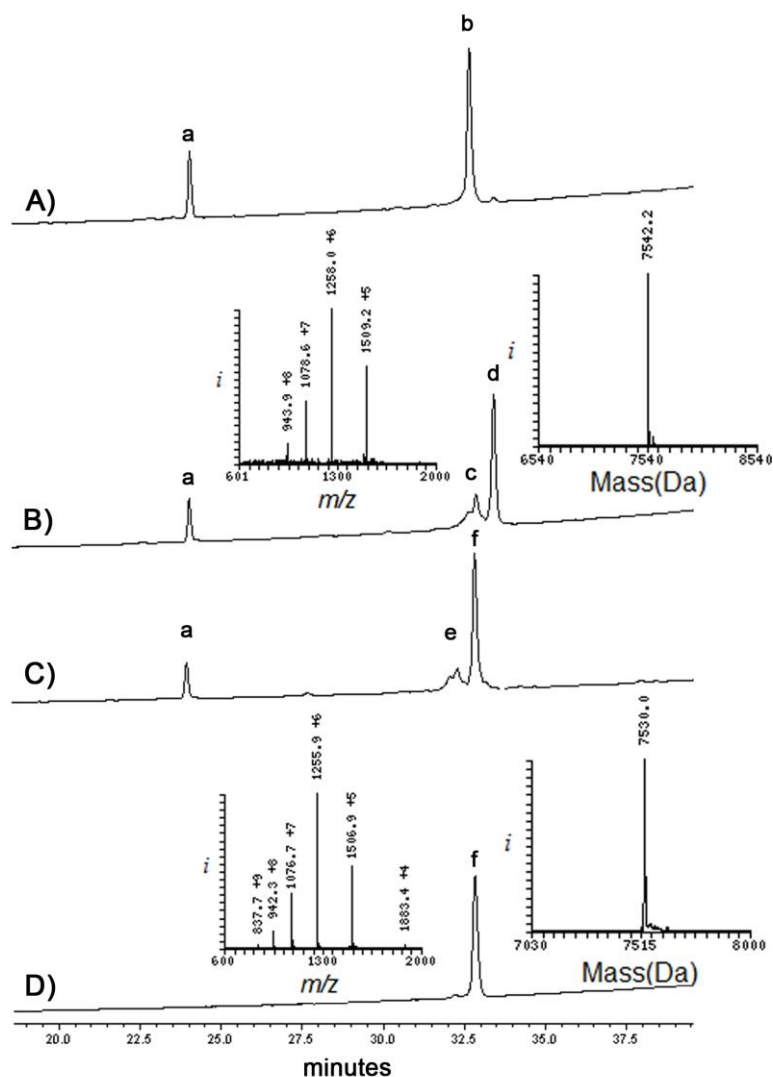


Figure S10: Ligation of peptides **2** and **1** followed by Thz-Cys conversion. A) HPLC trace of ligation course at time zero. Peak a corresponds to peptide **1** with the observed mass 3086.5 Da (calcd 3086.5 Da); Peak b corresponds to peptide **2** with the observed mass 4661.4 Da (calcd 4659.9 Da). B) HPLC trace of ligation of peptides **2** and **1** after 6 h. Peak a corresponds to peptide **1** with the observed mass 3086.5 Da (calcd 3086.5 Da); Peak b corresponds to **2** hydrolysis with the observed mass 4473.5 Da (calcd 4472.9 Da); Peak d corresponds to ligation product with the observed mass 7542.2 Da (calcd 7541.5 Da). C) HPLC trace of Thz-Cys conversion after overnight. Peak a corresponds to **1** with the observed mass 3086.5 Da (calcd 3086.5 Da); Peak e corresponds to **2** hydrolysis and Thz-Cys conversion; Peak f

corresponds to the Thz-Cys conversion product, **5**, with the observed mass 7530.0 Da (calcd 7529.7 Da).

D) Purified peptide **5**.

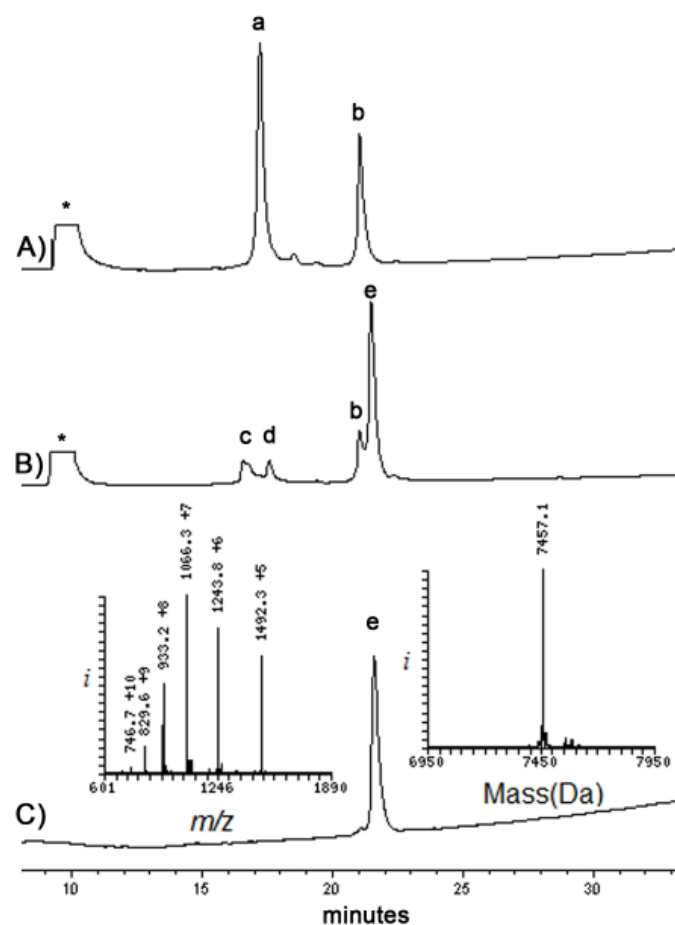


Figure S11: Ligation of peptides **4** with **3**. A) HPLC trace of ligation course at time zero. Peak **a** corresponds to peptide **4** with the observed mass 3264.5 Da (calcd 3262.5 Da); Peak **b** corresponds to peptide **3** with the observed mass 4371.2 Da (calcd 4370.0 Da). B) HPLC trace of ligation of peptides **4** and **3** after 6 h. Peak **c** corresponds to lactamization product of peptide **4** with the observed mass 3086.7 Da (calcd 3087.5 Da); Peak **d** corresponds to peptide **4** hydrolysis with the observed mass 3105.0 Da (calcd 3104.5 Da); Peak **b** corresponds to peptide **3** with the observed mass 4371.2 Da (calcd 4370.0 Da); Peak **e** corresponds to ligation product **6** with the observed mass 7457.1 Da (calcd 7456.6 Da). C) HPLC trace of purified ligation product **6**.

Peak c corresponds to ligation product **7** with the observed mass 14957.4 Da (calcd 14955.3 Da); Peak g corresponds to peptide **5** with the observed mass 7530.0 Da (calcd 7529.7 Da). D) HPLC trace of purified ligation product **7**. Peak c corresponds to ligation product **7** with the observed mass 14957.4 Da (calcd 14955.3 Da).

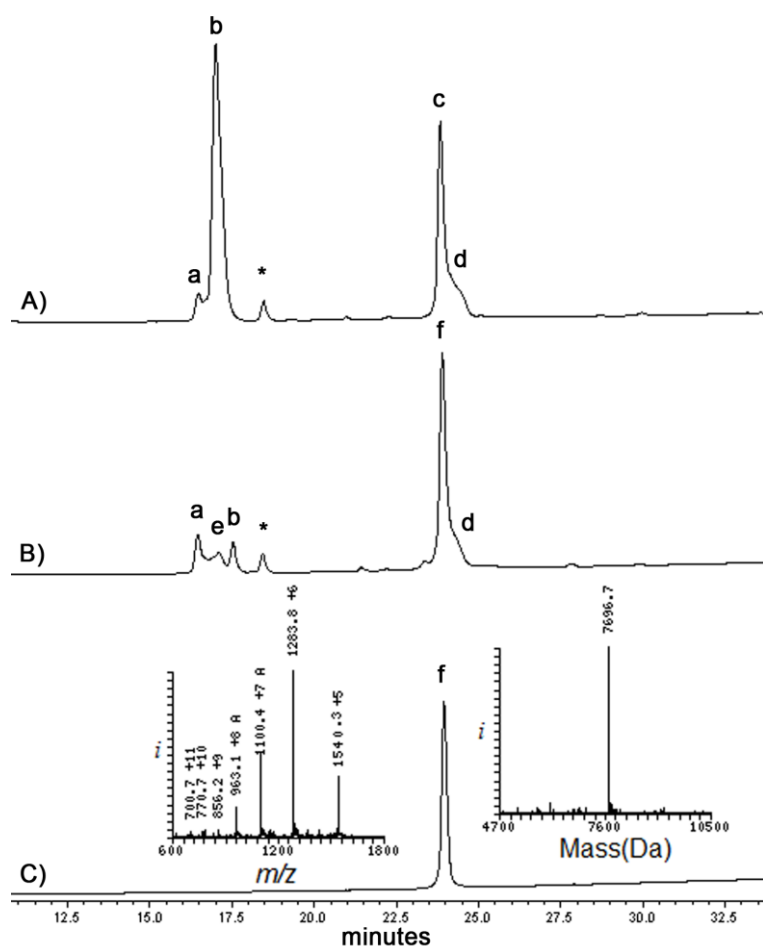


Figure S13: Ligation of peptides **4** and **3a**. A) HPLC trace of ligation course at time zero. Peak a corresponds to lactamization product of peptide **4** with the observed mass 3086.7 Da (calcd 3087.5 Da); Peak b corresponds to peptide **4** with the observed mass 3264.5 Da (calcd 3262.5 Da); Peak c corresponds to peptide **3a** with the observed mass 4609.0 Da (calcd 4609.0 Da); Peaks * and d corresponds to unidentified mass. B) HPLC trace of ligation of **4** and **3a** after 6 h. Peak a corresponds to lactamization product of peptide **4** with the observed mass 3086.7 Da (calcd 3087.5 Da); Peak b corresponds to peptide **4** with the observed mass 3264.5 Da (calcd 3262.5 Da); Peak e corresponds to peptide **4** hydrolysis with the observed mass 3105.0 Da (calcd 3104.5 Da); Peak f corresponds to ligation product **6a** with the observed mass 7696.7 Da (calcd 7695.6Da); Peaks * and d corresponds to unidentified mass. C) HPLC trace of purified ligation product **6a**.

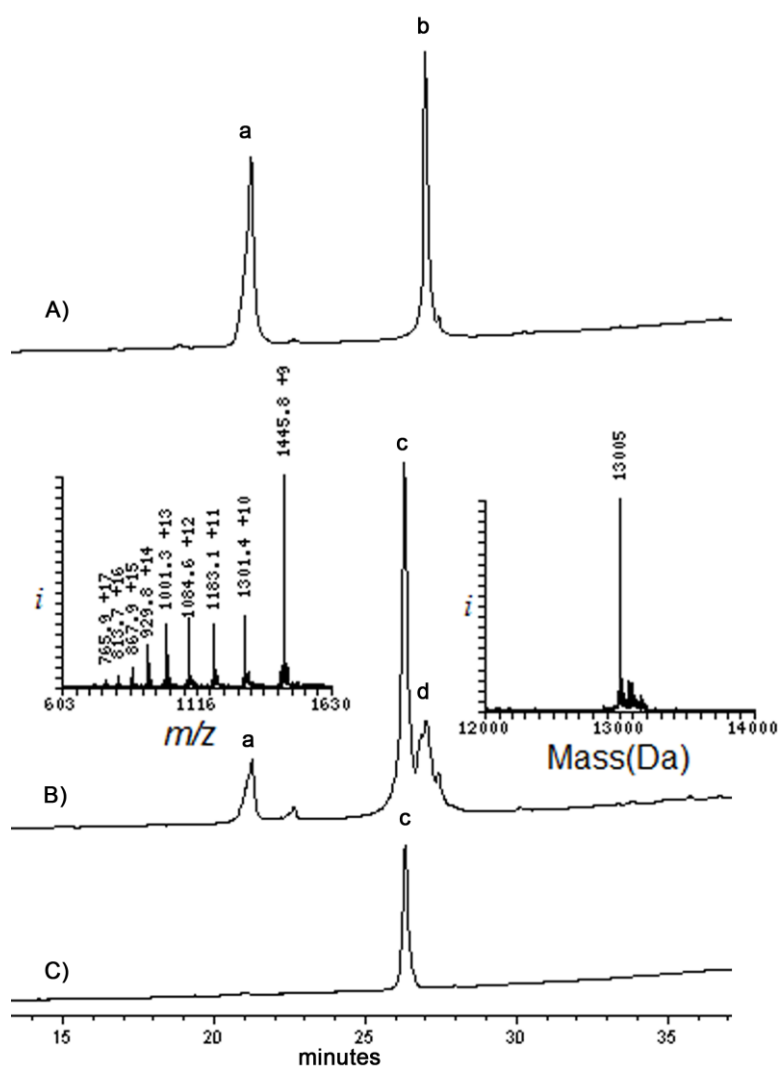


Figure S14: Ligation of peptide **9** and Ub-thioester. A) HPLC trace of ligation course at time zero. Peak a corresponds to peptide **9** with the observed mass 4474.1 Da (calcd 4473.0 Da); Peak b corresponds to Ub-MMP thioester with the observed mass 8649.9 Da (calcd 8648.8 Da). B) HPLC trace of ligation of peptide **9** with Ub-MMP thioester after 5 h. Peak a corresponds to peptide **9** with the observed mass 4474.1 Da (calcd 4473.0 Da); Peak c corresponds to ligation product with the observed mass 13005.0 Da (calcd 13002.8 Da); Peak d corresponds to Ub hydrolysis and Ub-MPAA thioester with the observed masses 8546.0 Da (calcd 8546.8 Da) and 8696.9 Da (calcd 8696.8 Da), respectively. C) HPLC trace of purified ligation product with the observed mass 13005.0 Da (calcd 13002.8 Da)

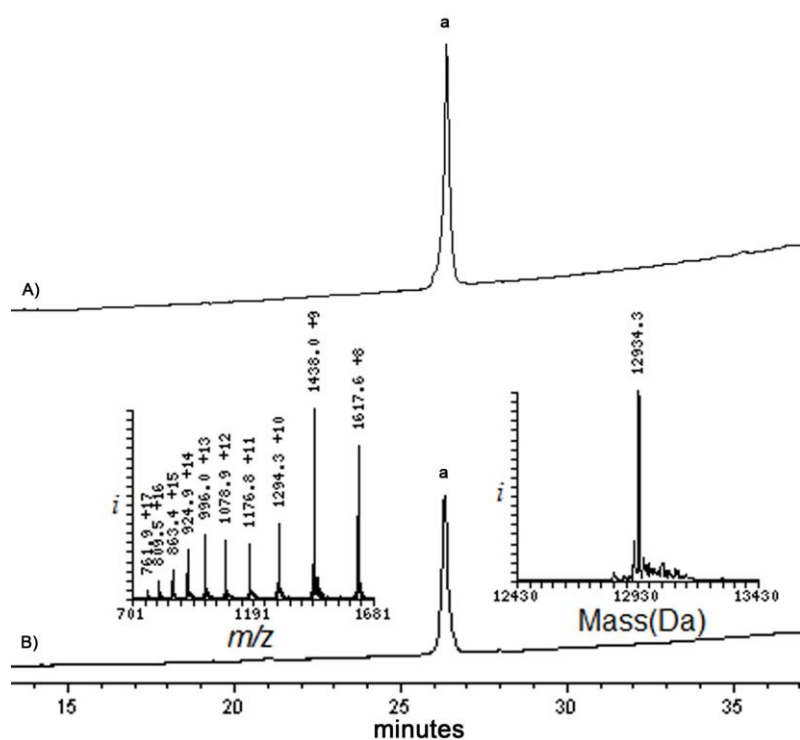


Figure S15: Acm removal from ligation product of previous reaction. A) HPLC trace of Acm deprotection after treatment with DTT for overnight. Peak a corresponds to Acm deprotection peptide **10** with the observed mass 12934.3 Da (calcd 12930.8 Da) B) HPLC trace of purified ligation product **10**.

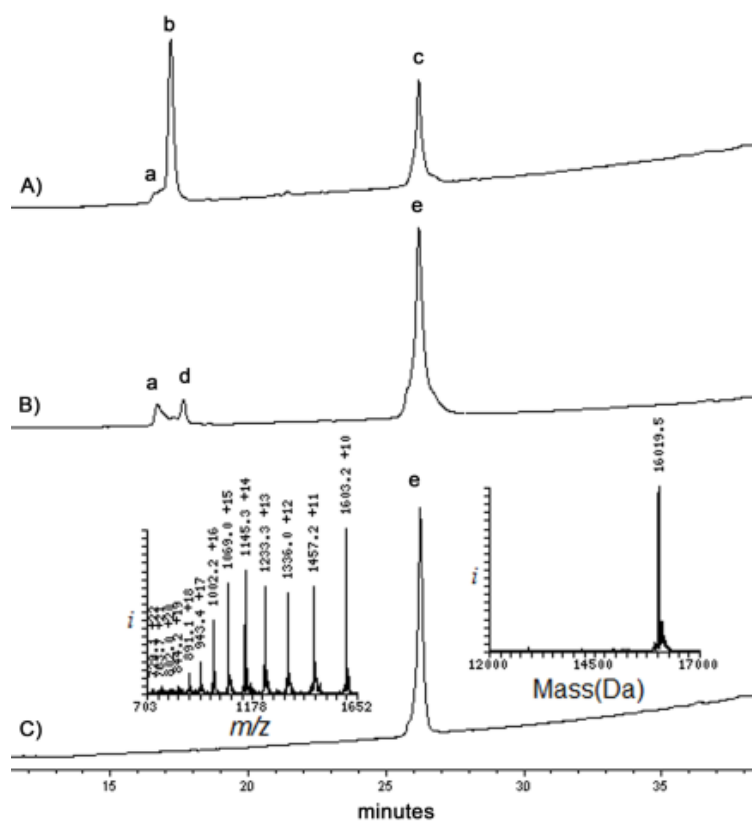


Figure S16: Ligation of peptides **4** and **10**. A) HPLC trace of ligation course at time zero. Peak a corresponds to lactamization product of peptide **4** with the observed mass 3086.7 Da (calcd 3087.5 Da); Peak b corresponds to peptide **4** with the observed mass 3264.5 Da (calcd 3262.5 Da); Peak c corresponds to peptide **10** with the observed mass 12934.3 Da (calcd 12930.8 Da). B) HPLC trace of ligation of peptides **4** and **10** after 6 h. Peak a corresponds to lactamization product of **4** with the observed mass 3086.7 Da (calcd 3087.5 Da); Peak d corresponds to peptide **4** hydrolysis with the observed mass 3105.0 Da (calcd 3104.5 Da); Peak e corresponds to ligation product **11** with the observed mass 16019.5 Da (calcd 16017.4 Da). C) HPLC trace of purified ligation product **11**.

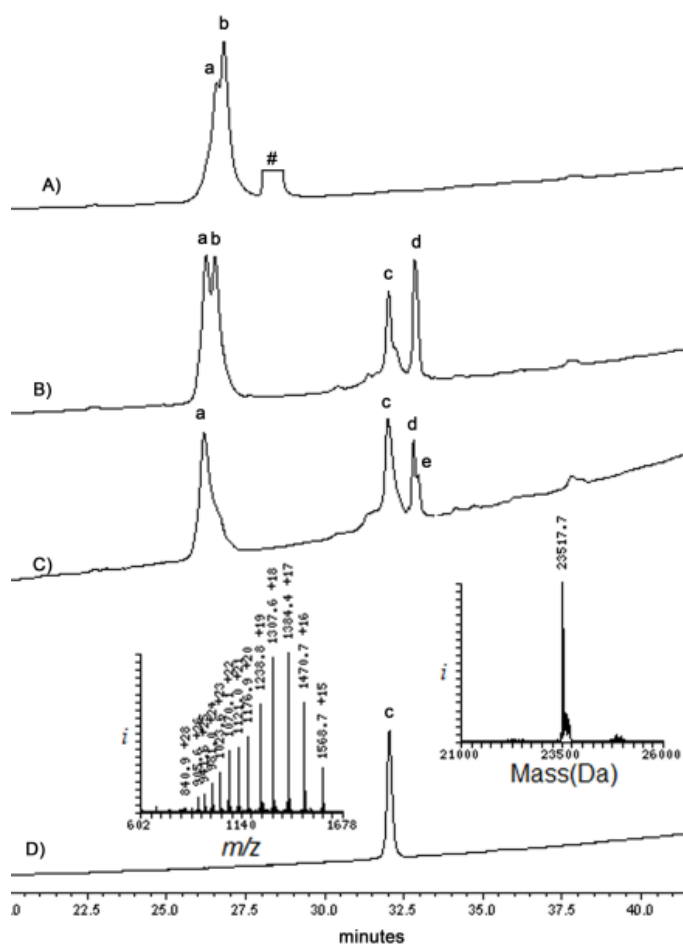


Figure S17: Oxidation of peptide **11** followed by ligation of with peptide **5**. A) Oxidation of peptide **11** followed by thioesterification using MPAA. Peak a corresponds to hydrolysis of peptide **11** with the observed mass 16002.8 Da (calcd 16003.4 Da) and lactamization of **11** bearing one/two oxidized MPAA on the peptide thiol side chains with the masses 16320.20 Da (calcd 16320 Da) and 16488.3 Da (calcd 16487.4 Da) respectively; Peak b corresponds to peptide **11** thioester with the observed mass 16155.4 Da (calcd 16153.4 Da); Peak # corresponds to oxidized MPAA. B) Ligation of peptide **11** thioester with **5** after 2 h. Peak a corresponds to hydrolysis of peptide **11** with the observed mass 16002.8 Da (calcd 16003.4 Da).

16003.4 Da) and lactamization of **11** bearing one/two oxidized MPAA on the peptide thiol handles with the masses 16320.20 Da (calcd 16320 Da) and 16488.3 Da (calcd 16487.4 Da) respectively; Peak b corresponds to peptide **11** thioester with the observed mass 16155.4 Da (calcd 16153.4 Da); Peak c corresponds to ligation product **12** with the observed mass 23517.7 Da (calcd 23515.1 Da); Peak d corresponds to peptide **5** with the observed mass 7530.0 Da (calcd 7529.7 Da). C) Ligation of peptide **6** thioester with **5** after 6 h . Peak a corresponds to hydrolysis of peptide **11** with the observed mass 16002.8 Da (calcd 16003.4 Da) and lactamization of **11** bearing one/two oxidized MPAA on the peptide thiol side chains with the masses 16320.20 Da (calcd 16320 Da) and 16488.3 Da (calcd 16487.4 Da) respectively; Peak c corresponds to ligation product **12** with the observed mass 23517.7 Da (calcd 23515.1 Da); Peak d corresponds to peptide **5** with the observed mass 7530.0 Da (calcd 7529.7 Da); Peak e corresponds to peptide **5** with bearing disulfide bond with MPAA moieties with observed mass 7697.6 Da (calcd 7696.7 Da). D) HPLC trace of purified ligation product **12**. Peak c corresponds to ligation product **12** with the observed mass 23517.7 Da (calcd 23515.1 Da).

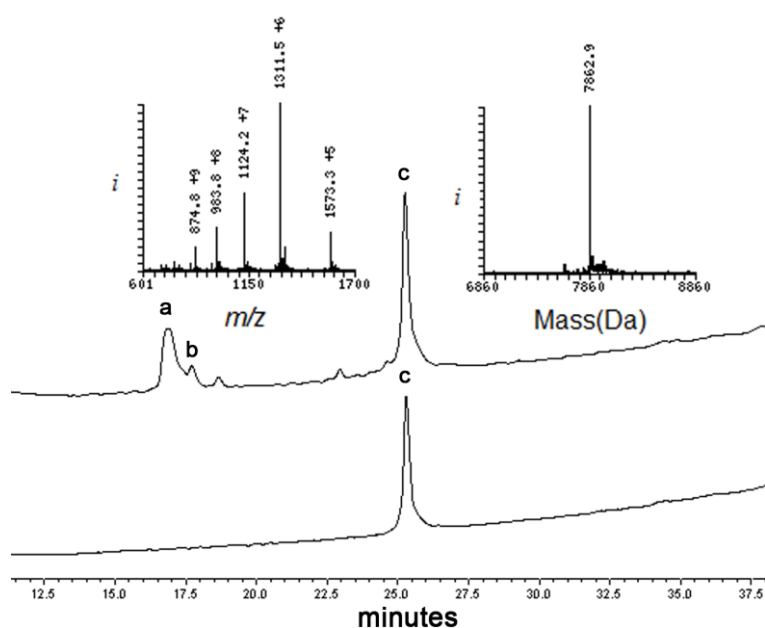


Figure S18: Ligation of peptides **4** and **14**. A) HPLC trace of ligation of peptides **4** and **14** after 6 h. Peak a corresponds to lactamization product of **4** with the observed mass 3086.7 Da (calcd 3087.5 Da); Peak b corresponds to **4** thioester hydrolysis with the observed mass 3105.0 Da (calcd 3104.5 Da); Peak c corresponds to ligation product **15** with the observed mass 7862.9 Da (calcd 7862.6 Da). B) HPLC trace of purified ligation product **15**.

References:

- 1) P. Siman, O. Blatt, T. Moyal, T. Danieli, M. Lebendiker, H. A. Lashuel, A. Friedler and A. Brik, *ChemBioChem*, **2011**, *12*, 1097-1104.