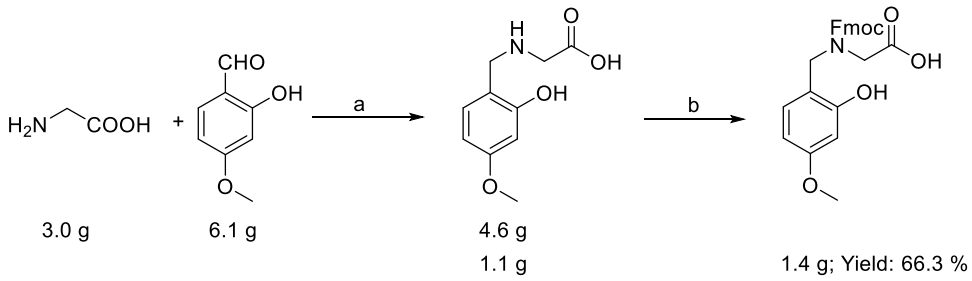
Synthesis procedures and spectrums for all proteins and peptides in this study

TABLE OF CONTENTS

1. Preparation of Fmoc-Gly(Hmb)-OH



3.0 g 6.1 g 4.6 g

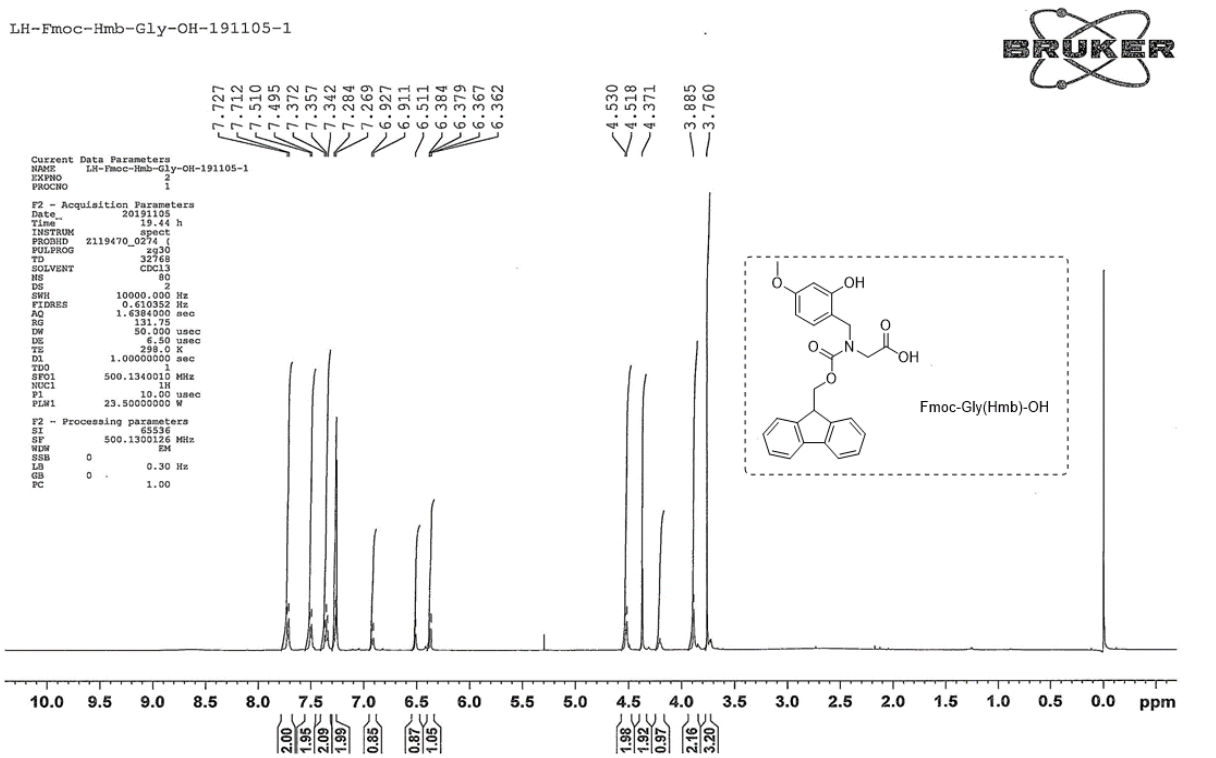
1.1 g

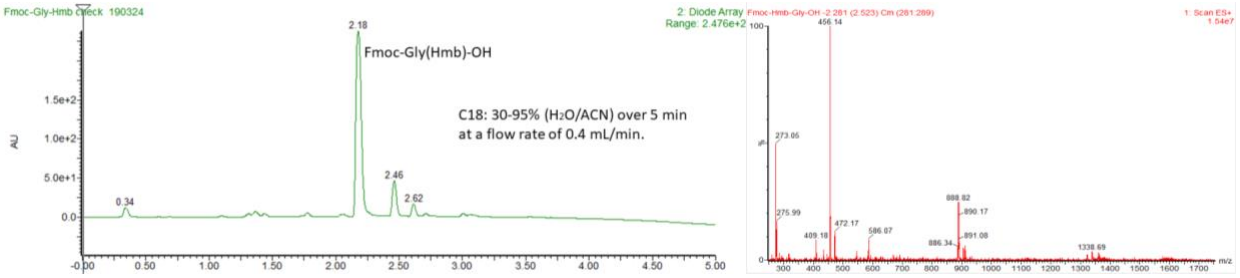
1.4 g; Yield: 66.3 %

a) $NaBH_4$, KOH, H_2O/EtOH; b) Fmoc-OSu; TEA; CH_3CN/H_2O (3:1)

Glycine (3.0 g; 40 mmol; 1.0 equiv.) was dissolved in 20 mL 2 M KOH aqueous solution and stirred for 20 mins. 2-Hydroxy-4-methoxybenzaldehyde (6.1 g; 40 mmol; 1.0 equiv.) was dissolved in 8 mL ethanol, which was then poured into the aqueous solution. The mixture was stirred at room temperature for 1 hour. $NaBH_4$ (1.6 g; 44 mmol; 1.1 equiv.) in 10 mL H_2O was then added slowly and stirred for 20 mins at room temperature and another 20 mins at 60 ^oC. After cooling down, the solution was acidified by concentrated HCl to pH around 4, which was then stored in 4 ^oC fridge for overnight precipitation. The solid part was washed with ice-cold water and 50% MeOH. The crude solid was dried over at 80 ^oC oven for 8 hours to give 4.6 g crude N-(2-Hydroxyl-4-methoxybenzyl)-L-glycine.

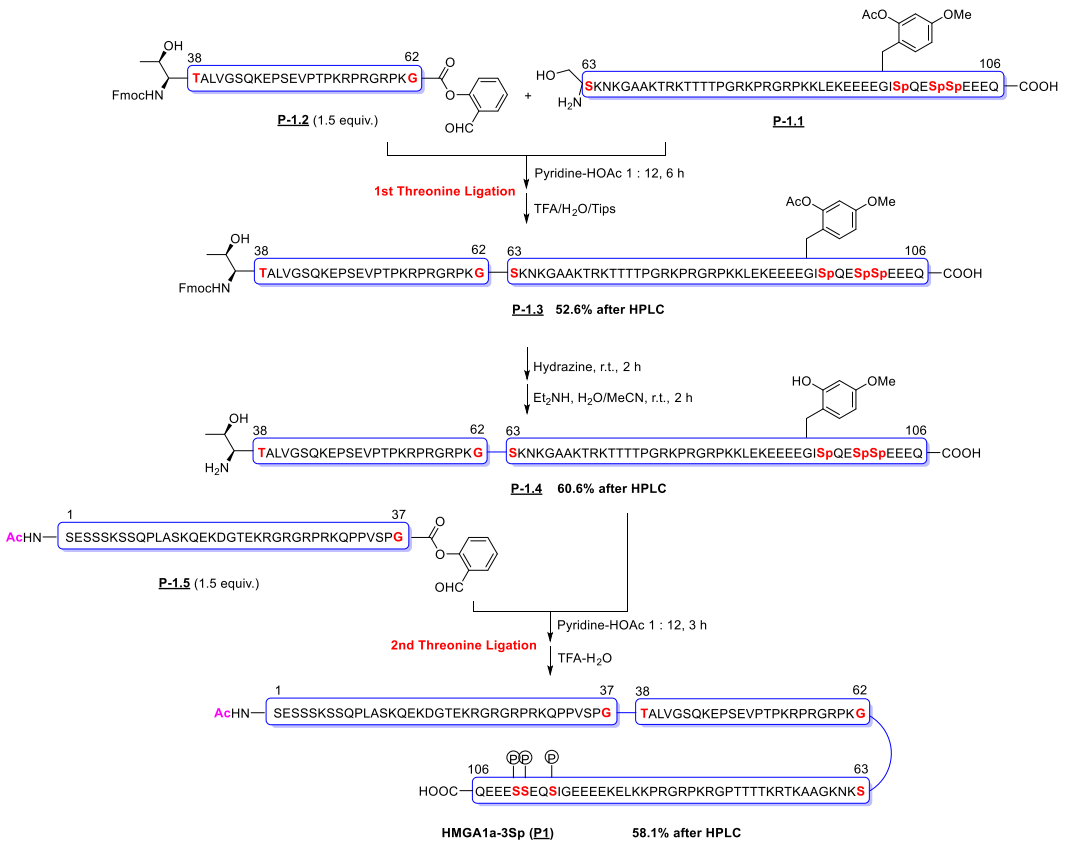
N-(2-Hydroxyl-4-methoxybenzyl)-L-glycine (1.1 g; 5 mmol; 1.0 equiv.) was suspended in 40 mL of CH_3CN/H_2O. After the addition of 2.1 mL of TEA (2.1 mL; 15 mmol; 3.0 equiv.), the solution became clear. 3.37 g (10 mmol; 2.0 equiv.) of Fmoc-OSu was then added into the mixture. After stirring for 90 min, the reaction was evaporated to approximately half of the volume. The mixture was then poured into a mixture of 200 mL of 5% citric acid and ethyl acetate (1:1). The organic part was collected, and the aqueous phase was extracted twice more with ethyl acetate. The combined ethyl acetate was washed by saturated sodium chloride solution, then dried over anhydrous sodium sulfate and evaporated in vacuum to give the crude product as a yellow oil. The crude products were purified by chromatography over silica gel with the gradient DCM/MeOH = 30:1 and 1.4 g pure Fmoc-Hmb-Gly-OH can be obtained with the yield around 66.3%. ^1H NMR (500 MHz, CDCl3) δ 7.72 (d, J = 7.5 Hz, 2H), 7.49 (dd, J = 15.8, 6.1 Hz, 2H), 7.36 (t, J = 7.4 Hz, 2H), 7.30 – 7.26 (m, 1H), 6.92 (d, J = 8.2 Hz, 1H), 6.51 (s, 1H), 6.37 (dd, J = 8.3, 2.5 Hz, 1H), 4.52 (t, J = 5.9 Hz, 2H), 4.37 (s, 2H), 4.21 (t, J = 5.8 Hz, 1H), 3.89 (s, 2H), 3.76 (s, 3H).



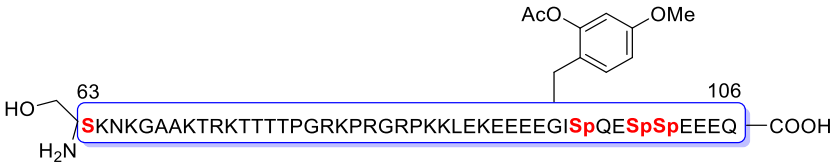


Calc. m/z for $[M+Na]^+$ 456.14, found 456.14.

2. Preparation of HMGA1a-3pSer (P1)

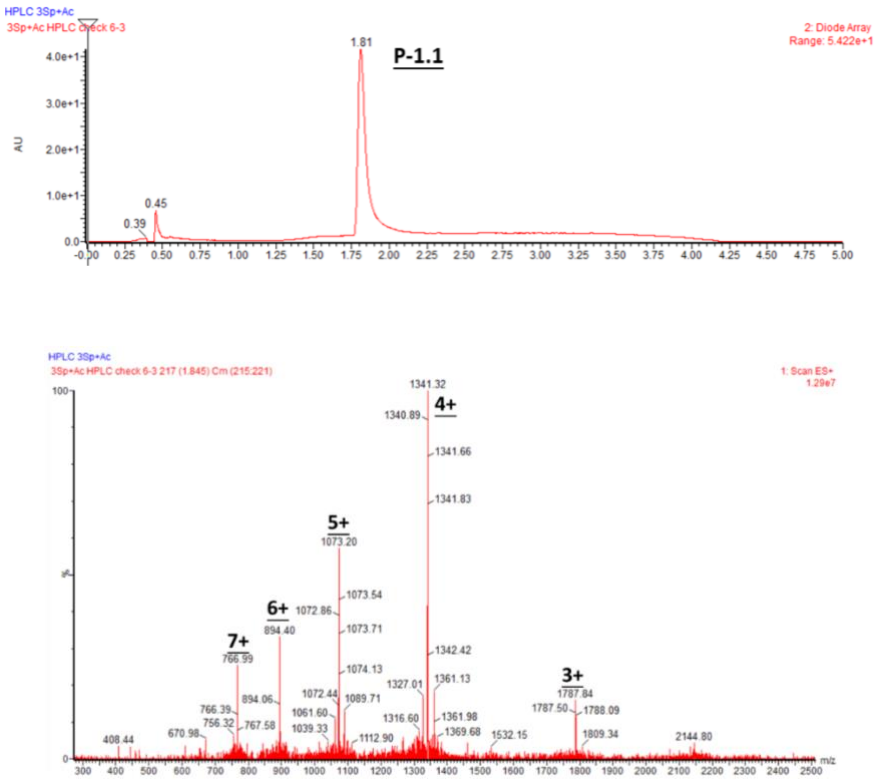


Synthesis of peptide P-1.1



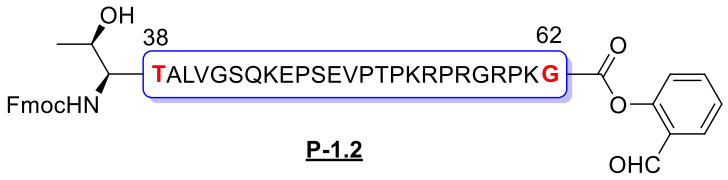
P-1.1

The preparation of peptide **P-1.1** was following **general procedure 1** via Fmoc-SPPS. In the end, the resin was treated with TFA/H_2O/Tips/Phenol (92.5/2.5/2.5/2.5) for 3 h to remove the side-chain protection group on the peptide as well as Bn group on the phosphate. After HPLC purification (gradient: 5-40% CH_3CN/H_2O over 40 min) and lyophilization, 95 mg pure product can be obtained from 0.5 g trityl chloride resin (11.8% yield based on resin loading).



P-1.1 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{214}H_{364}N_{67}O_{87}P_3$ molecular weight: 5360.57. P_1 $[M+3H]^{3+}$ m/z = 1787.86; P_2 $[M+4H]^{4+}$ m/z = 1341.14; P_3 $[M+5H]^{5+}$ m/z = 1073.11; P_4 $[M+6H]^{6+}$ m/z = 894.43; P_5 $[M+7H]^{7+}$ m/z = 766.80. Found: 1787.84; 1341.32; 1073.20; 894.40; 766.99.

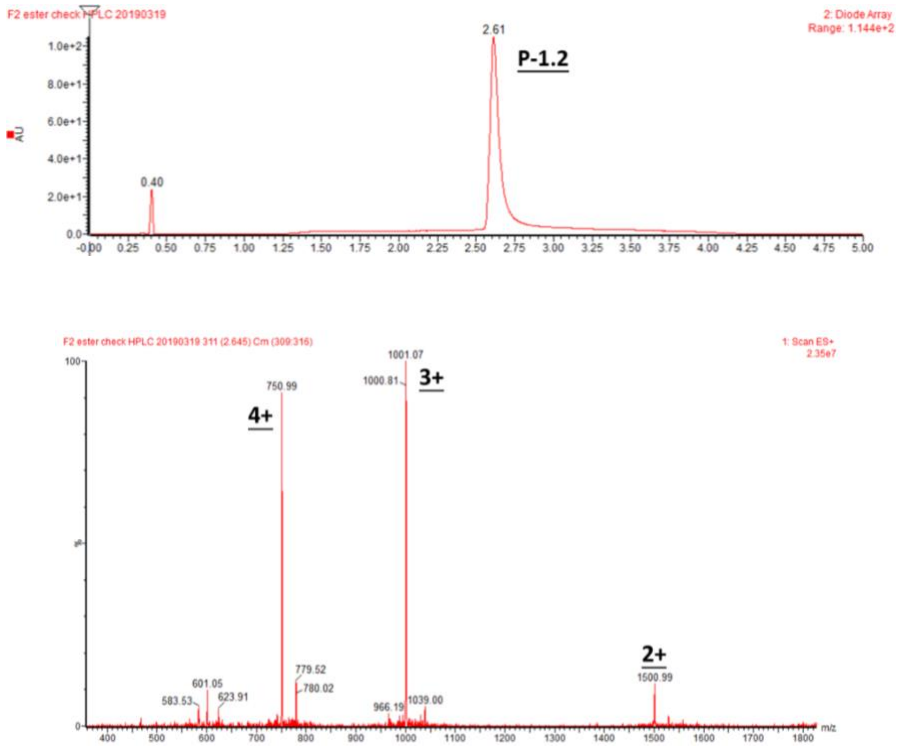
Synthesis of peptide P-1.2



P-1.2

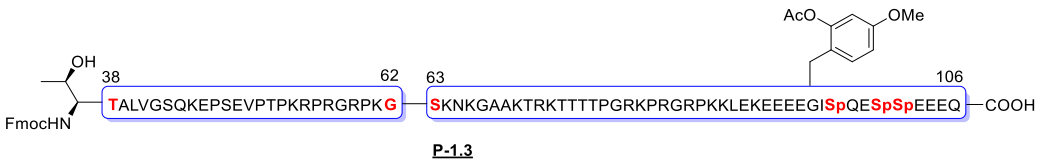
The preparation of **P-1.2** was following **general procedure 2**.

150 mg crude peptide (1.0 equiv., 0.034 mmol) together with PyBOP (3.0 equiv., 0.102 mmol) and DIEA (10.0 equiv. 65 µL) was dissolved in 4mL anhydrous DCM and stirred for 10 min at room temperature. α, α-dimethoxy-xx (20.0 equiv.; 0.69 mmol) was added to the mixture and reacted for 12 h, followed by TFA/phenol/H_2O to remove peptide side-chain protecting group and afford crude peptide SAL ester. TFA was removed under a stream of condensed air. Crude products were precipitated by cold ether to give white solid peptides, which were centrifuged to remove ether in the supernatant. The obtained solid was purified via HPLC (20-50% CH_3CN/H_2O over 40 min) and lyophilized to give 37.6 mg pure product with yield 39.4%



P-1.2 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{137}H_{212}N_{38}O_{38}$ molecular weight: 2999.43. P_1 $[M+2H]^{2+}$ m/z = 1500.72; P_2 $[M+3H]^{3+}$ m/z = 1000.81; P_3 $[M+4H]^{4+}$ m/z = 750.86; P_4 $[M+5H]^{5+}$ m/z = 600.89. Found: 1500.99; 1001.07; 750.99; 601.05.

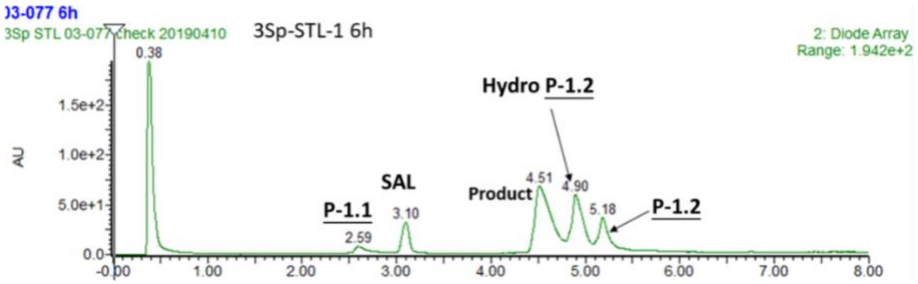
Synthesis of peptide P-1.3

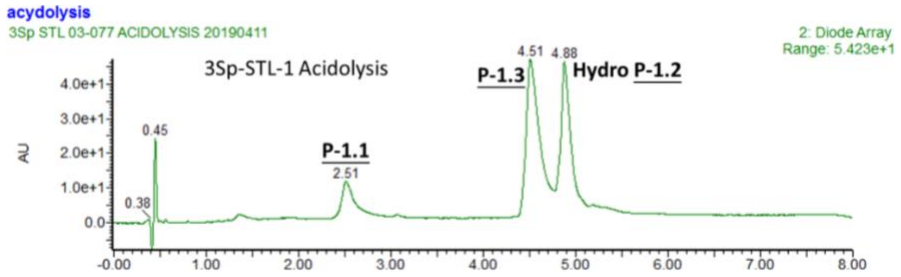


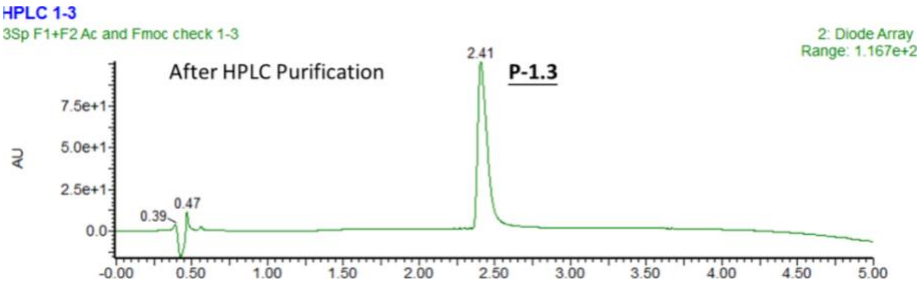
P-1.3

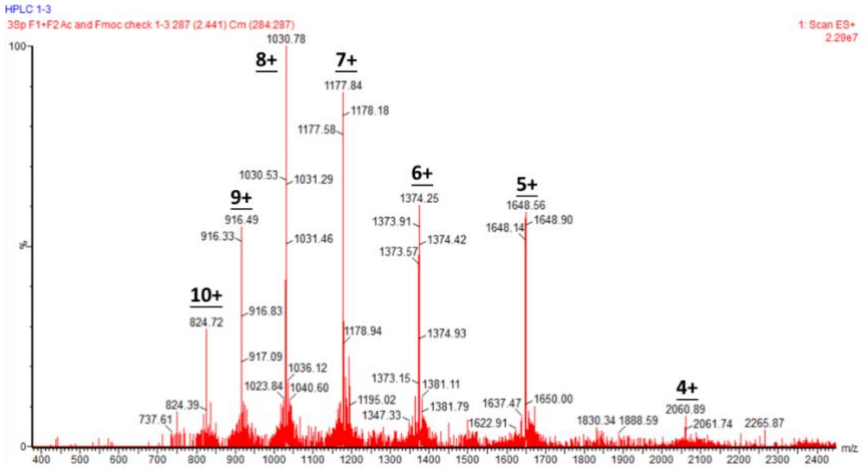
The preparation of peptide **P-1.3** was following **general procedure 4**.

21 mg (0.0039 mmol, 1 equiv.) of **P-1.1** and 18 mg (0.0060 mmol, 1.5 equiv.) of **P-1.2** were mixed together, which were further dissolved in 400 µl pyridine/AcOH (1/12, mol/mol) to make the concentration of **P-1.1** at 10 mM. The reaction was conducted at room temperature and stirred for 6 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 17 mg pure product (yield 52.6%) were obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



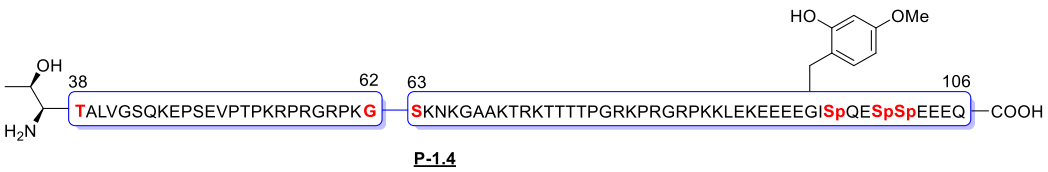






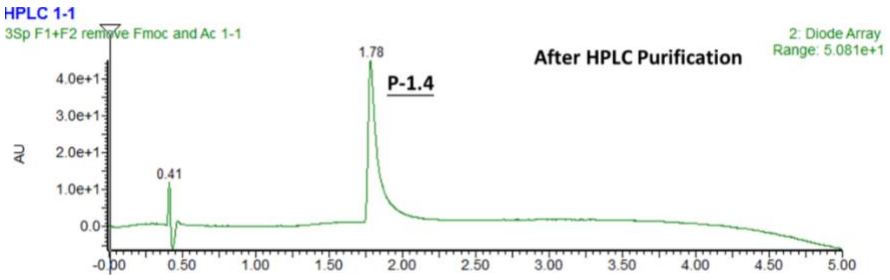
P-1.3 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{344}H_{570}N_{105}O_{123}P_3$ molecular weight: 8237.88. P_1 $[M+4H]^{4+}$ m/z = 2060.48; P_2 $[M+5H]^{5+}$ m/z = 1648.58; P_3 $[M+6H]^{6+}$ m/z = 1373.98; P_4 $[M+7H]^{7+}$ m/z = 1177.84; P_5 $[M+8H]^{8+}$ m/z = 1030.74; P_6 $[M+9H]^{9+}$ m/z = 916.32; P_7 $[M+10H]^{10+}$ m/z = 824.79. Found: 2060.89; 1648.56; 1374.25; 1177.84; 1030.78; 916.49; 824.72.

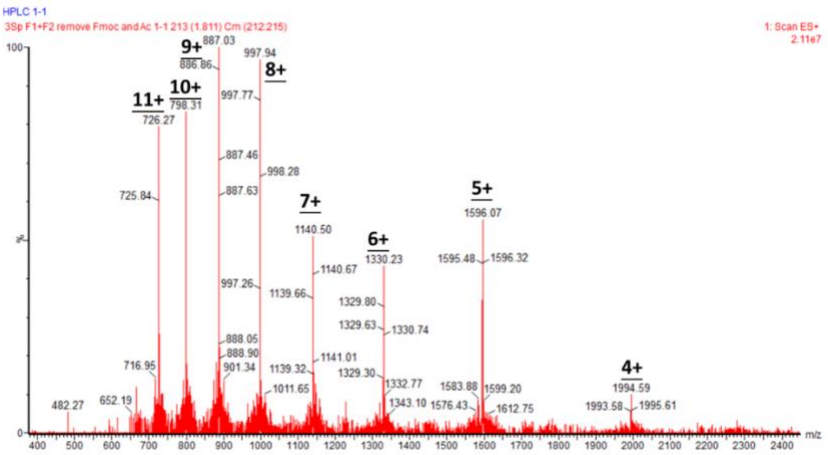
Synthesis of peptide P-1.4



The preparation of peptide **P-1.4** was following general procedure 5.

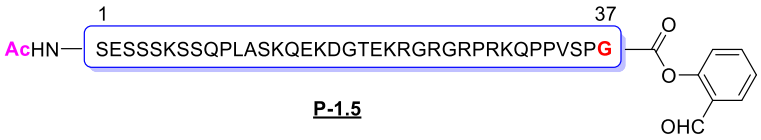
To make the concentration of peptide around 5 mM, 17 mg (0.0021 mmol, 1 equvi.) of **P-1.3** was dissolved in 420 µl H_2O. 3.4 µl of Hydrazine monohydrate (0.062 mmol, 30 equiv.) was then added into the solution and stirred for 2 h at room temperature. 42 µl DEA in 378 µl of H_2O/CH_3CN (1/1, v/v) was poured into the solution and stirred for another 2 h at room temperature. The product was purified by HPLC (gradient 5-40% CH_3CN/H_2O over 40 min) and 9.8 mg of the pure product can be obtained with 60.6% yield.





P-1.4 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{327}H_{558}N_{105}O_{120}P_3$ molecular weight: 7973.60. P_1 $[M+4H]^{4+}$ m/z = 1994.40; P_2 $[M+5H]^{5+}$ m/z = 1595.72; P_3 $[M+6H]^{6+}$ m/z = 1329.93; P_4 $[M+7H]^{7+}$ m/z = 1140.09; P_5 $[M+8H]^{8+}$ m/z = 997.70; P_6 $[M+9H]^{9+}$ m/z = 886.96; P_7 $[M+10H]^{10+}$ m/z = 798.36; P_8 $[M+11H]^{11+}$ m/z = 725.87. Found: 1994.85; 1595.98; 1330.23; 1140.50; 997.94; 887.03; 798.48; 726.27.

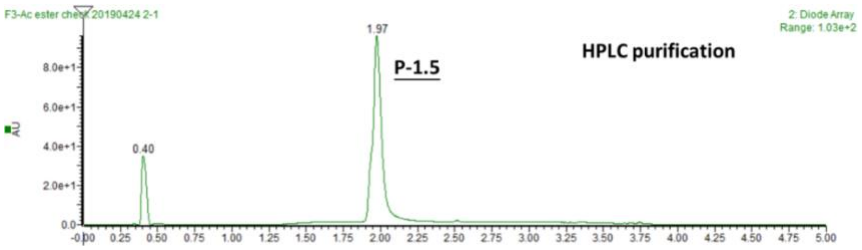
Synthesis of peptide P-1.5

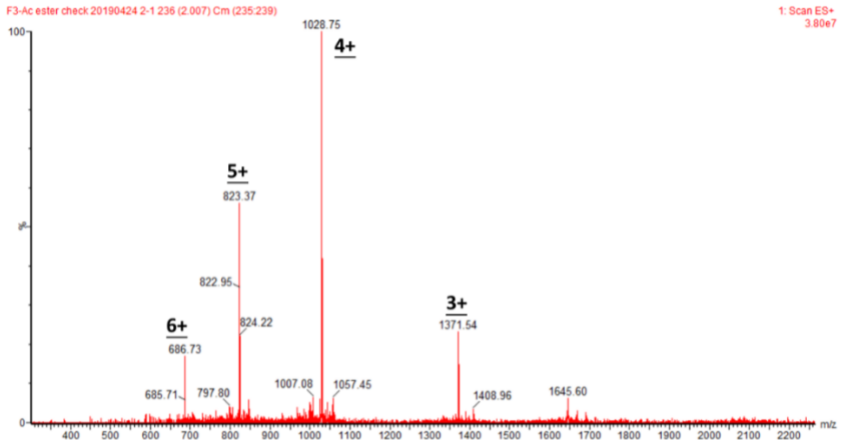


P-1.5

The preparation of **P-1.5** was following **general procedure 3**.

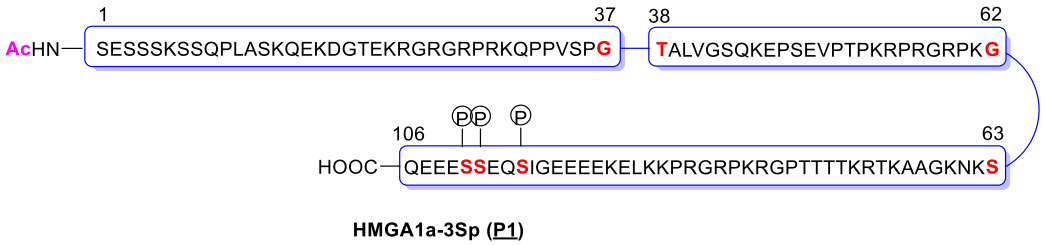
The N-terminal of last amino acid Ser was protected by the Fmoc protection group, which was further removed and give out the free amino group. The final acetyl group was installed to the N-terminus free amine by using Ac_2O/TEA (1 mol/1 mol) in 5 mL DCM, with DMAP as the catalyst. 150 mg crude peptide (1.0 equiv., 0.023 mmol) together with PyBOP (3.0 equiv., 0.069 mmol) and DIEA (10.0 equiv. 46 µL) was dissolved in 4mL anhydrous DCM and stirred for 10 min at room temperature. α, α-dimethoxy-salicylaldehyde (20.0 equiv., 0.46 mmol) was added to the mixture and reacted for 12 h, followed by TFA/phenol/H_2O to remove peptide side-chain protecting group and afford crude peptide SAL ester. TFA was removed under a stream of condensed air. Crude products were precipitated by cold ether to give white solid peptides, which were centrifuged to remove ether in the supernatant. The obtained solid was purified via HPLC (10-40% CH_3CN/H_2O over 40 min) and the pure product was lyophilized to give 37.8 mg pure product with yield 35.9%





P-1.5 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{172}H_{285}N_{57}O_{60}$ molecular weight: 4111.51. P_1 $[M+3H]^{3+}$ m/z = 1371.50; P_2 $[M+4H]^{4+}$ m/z = 1028.89; P_3 $[M+5H]^{5+}$ m/z = 823.30; P_4 $[M+6H]^{6+}$ m/z = 686.25; Found: 1371.54; 1028.75; 823.37; 686.75.

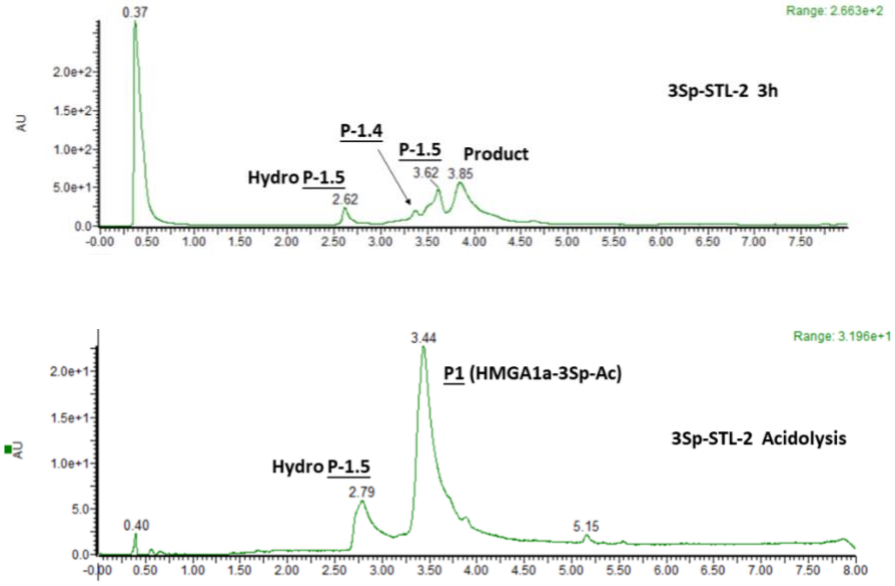
Synthesis of HMGA1a-3pSer

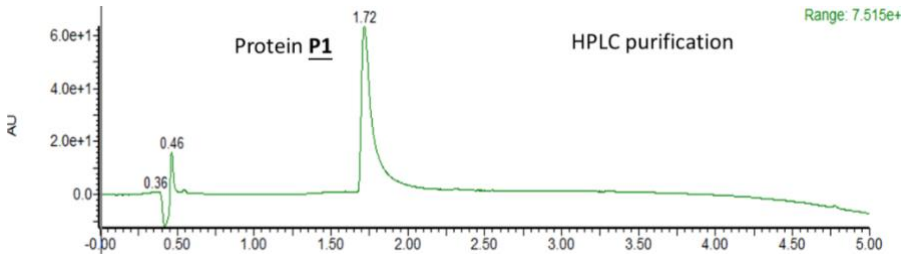


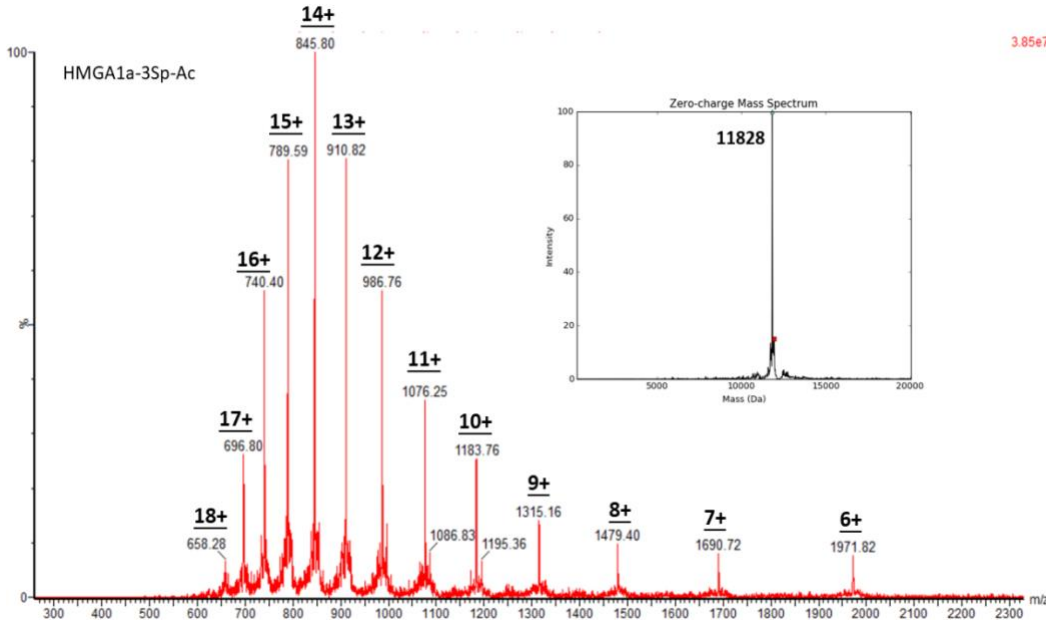
HMGA1a-3Sp (P1)

The preparation of protein **P1** was following general procedure 6.

5.0 mg (0.00063 mmol, 1 equiv.) of **P-1.4** and 4.5 mg (0.0011 mmol, 1.5 equiv.) of **P-1.5** were mixed together, which were further dissolved in 100 µl pyridine/AcOH (1/12, mol/mol). The reaction was conducted at room temperature and stirred for 3 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 4.3 mg pure product (yield 58.1%) were obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).

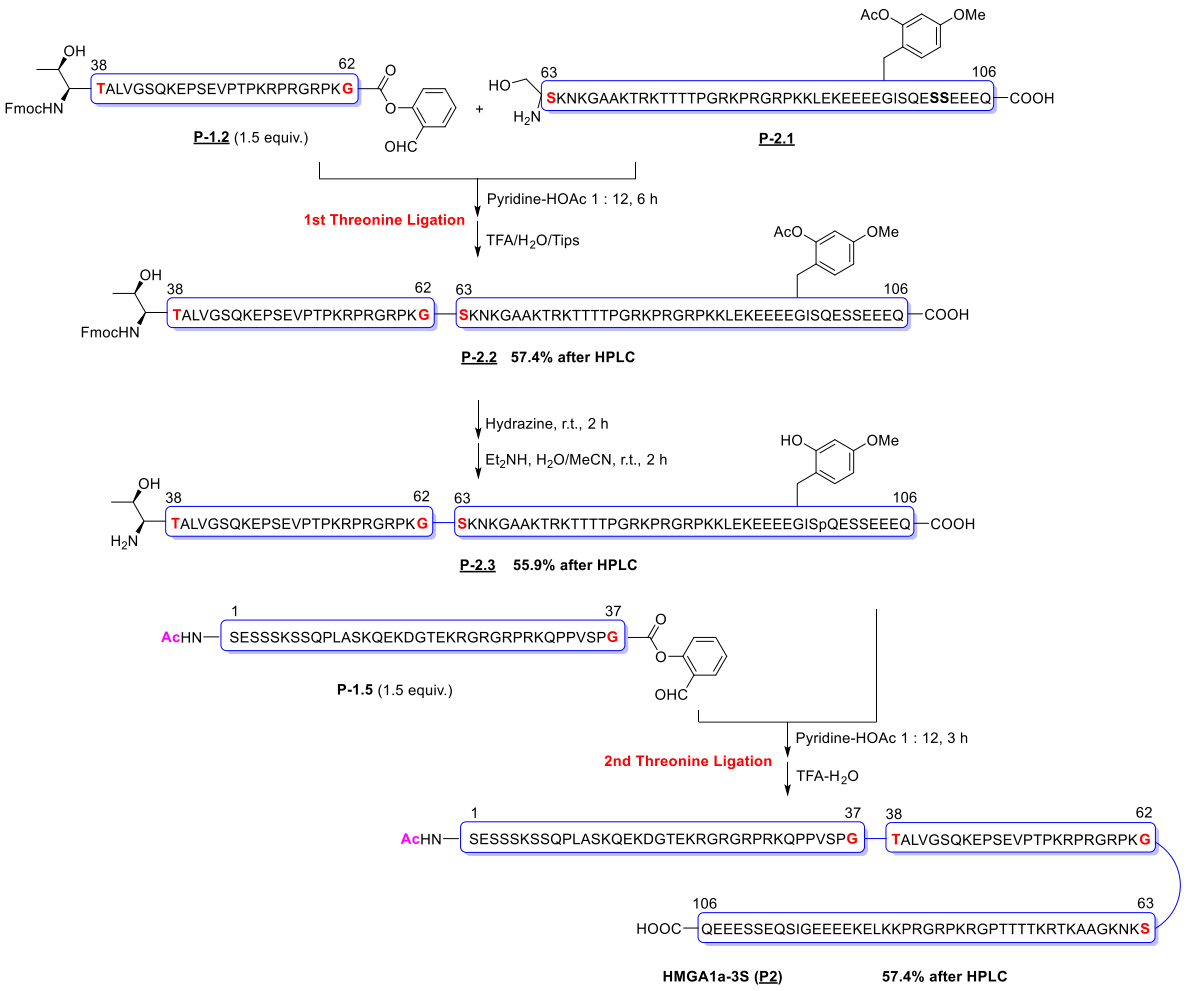




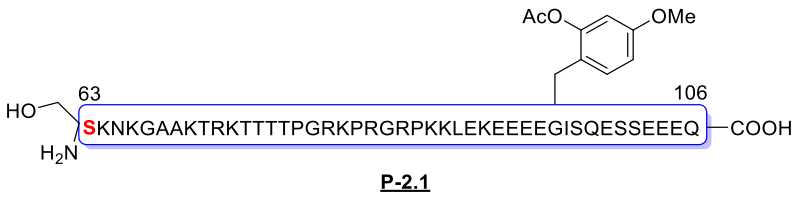


HMGA1a-3pSer (P1) was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{484}H_{829}N_{162}O_{176}P_3$ molecular weight: 11826.84. P_1 $[M+6H]^{6+}$ m/z = 1972.11; P_2 $[M+7H]^{7+}$ m/z = 1690.52; P_3 $[M+8H]^{8+}$ m/z = 1479.33.39; P_4 $[M+9H]^{9+}$ m/z = 1315.07; P_5 $[M+10H]^{10+}$ m/z = 1183.67; P_6 $[M+11H]^{11+}$ m/z = 1076.15; P_7 $[M+12H]^{12+}$ m/z = 986.55; P_8 $[M+13H]^{13+}$ m/z = 910.74; P_9 $[M+14H]^{14+}$ m/z = 845.76; P_{10} $[M+15H]^{15+}$ m/z = 789.44; P_{11} $[M+16H]^{16+}$ m/z = 740.17; P_{12} $[M+17H]^{17+}$ m/z = 696.69. Found: 1971.82; 1690.72; 1479.40; 1315.16; 1183.76; 1076.25; 986.76; 910.82; 845.80; 789.59; 740.40; 696.80.

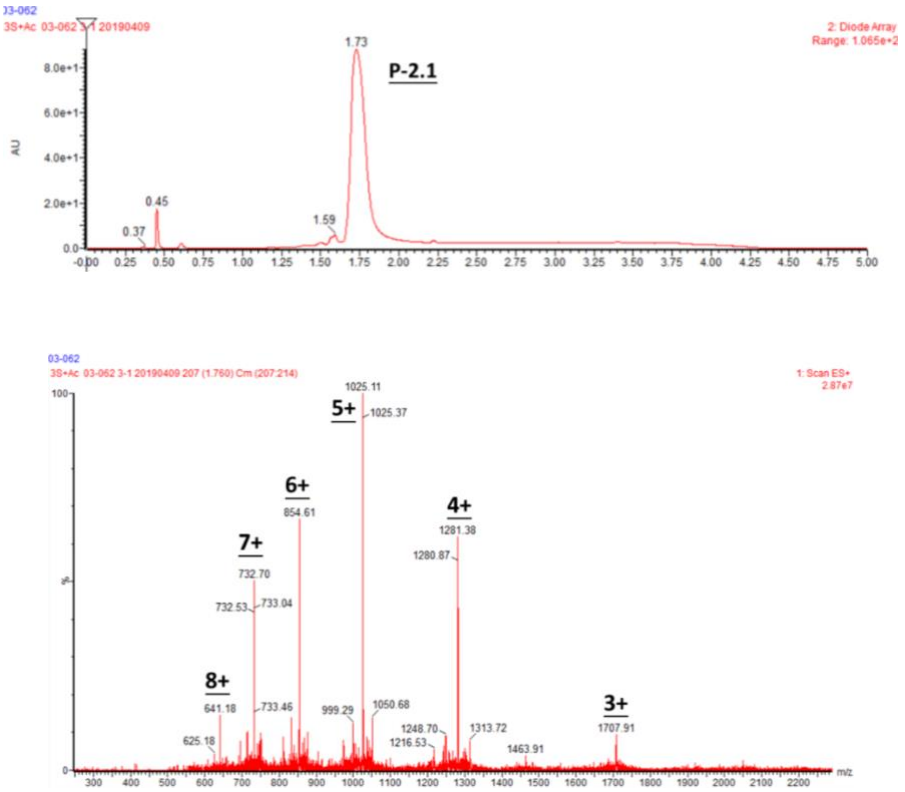
3. Preparation of HMGA1a-3S (P2)



Synthesis of peptide P-2.1

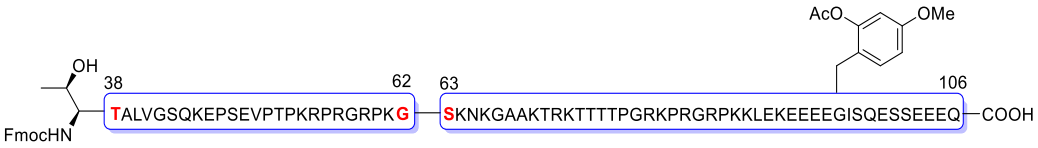


The preparation of peptide **P-2.1** was following **general procedure 1** via Fmoc-SPPS. After HPLC purification (gradient: 5-40% CH_3CN/H_2O over 40 min) and lyophilization, 80 mg pure product can be obtained from 0.5 g trityl chloride resin (10.4% yield based on resin loading).



P-2.1 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{214}H_{361}N_{67}O_{78}$ molecular weight: 5120.63. P_1 $[M+3H]^{3+}$ m/z = 1707.87; P_2 $[M+4H]^{4+}$ m/z = 1281.16; P_3 $[M+5H]^{5+}$ m/z = 1025.13; P_4 $[M+6H]^{6+}$ m/z = 854.44; P_5 $[M+7H]^{7+}$ m/z = 732.52; P_6 $[M+8H]^{8+}$ m/z = 641.08. Found: 1707.91; 1281.38; 1025.11; 854.61; 732.70; 641.18.

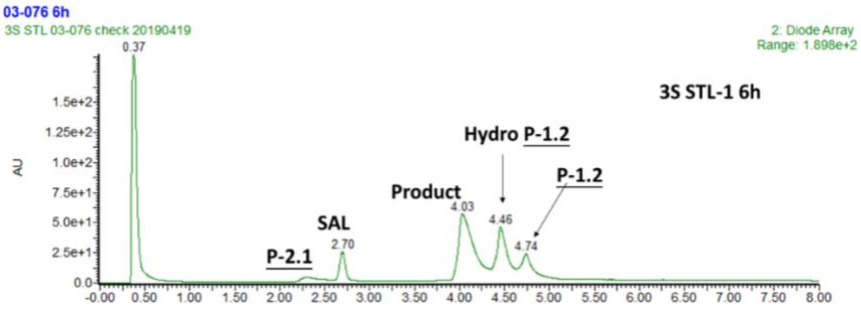
Synthesis of peptide P-2.2

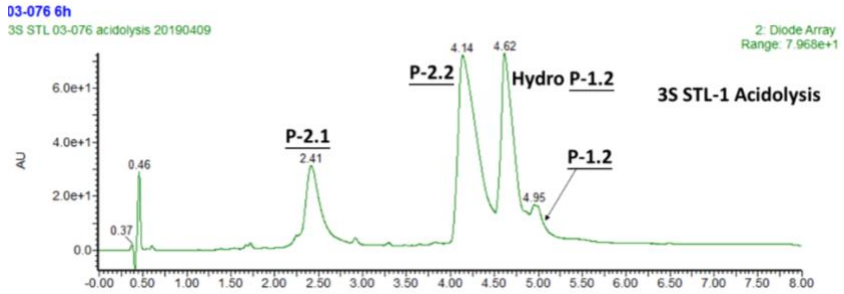


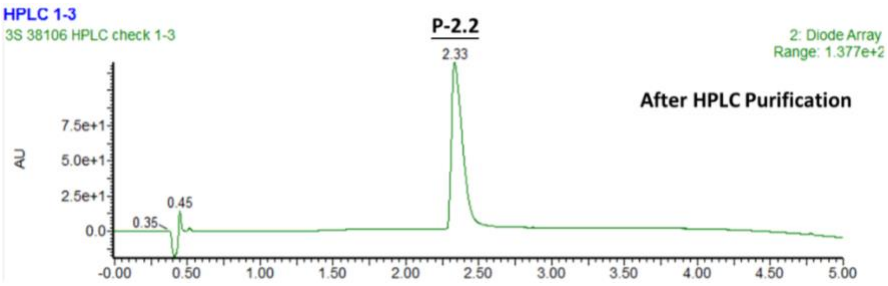
P-2.2

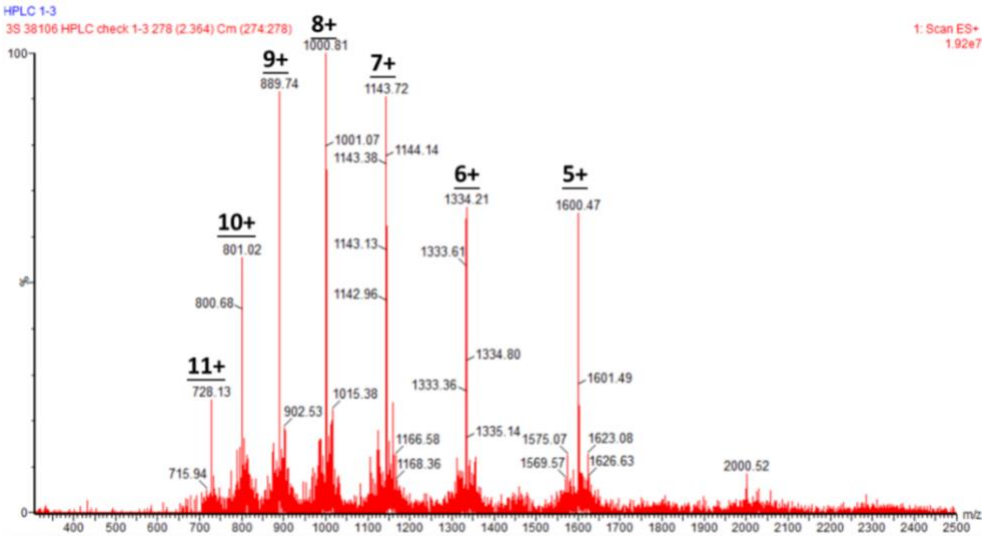
The preparation of peptide **P-2.2** was following general procedure 4.

21 mg (0.0039 mmol, 1 equiv.) of **P-2.1** and 18 mg (0.0060 mmol, 1.5 equiv.) of **P-1.2** were mixed together, which were further dissolved in 400 µl pyridine/AcOH (1/12, mol/mol) to make the concentration of **P-2.1** at 10 mM. The reaction was conducted at room temperature and stirred for 6 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 18.5 mg pure product (yield 57.4%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).





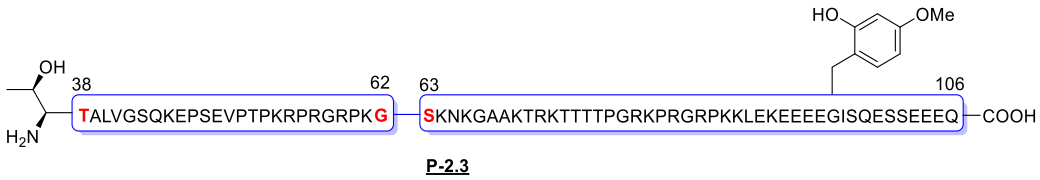




P-2.2 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{344}H_{567}N_{105}O_{114}$ molecular weight: 7997.94. P_1 $[M+5H]^{5+}$ m/z = 1600.59; P_2 $[M+6H]^{6+}$ m/z = 1333.99; P_3 $[M+7H]^{7+}$ m/z = 1143.56; P_4 $[M+8H]^{8+}$ m/z = 1000.74. P_5 $[M+9H]^{9+}$ m/z = 889.66; P_5 $[M+10H]^{10+}$ m/z = 800.79;

P_6 $[M+11H]^{11+}$ m/z = 728.09. Found: 1600.47; 1334.21; 1143.72; 1000.81; 899.74; 801.02; 728.13.

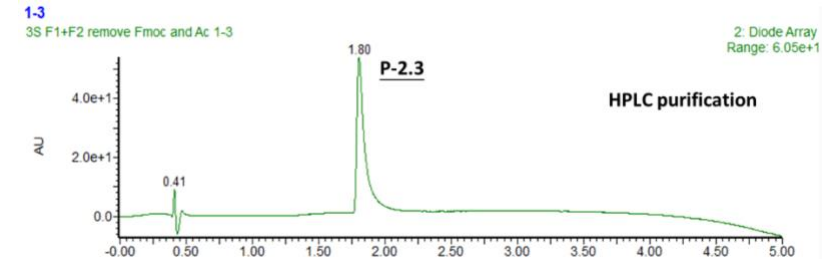
Synthesis of peptide P-2.3

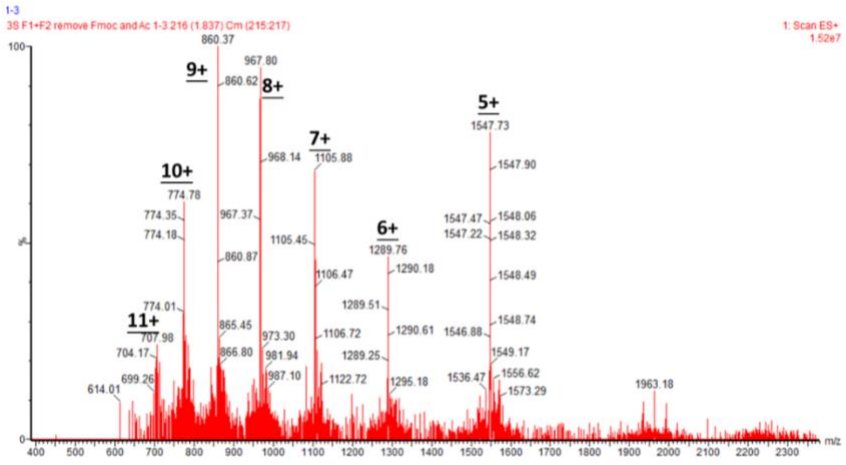


P-2.3

The preparation of peptide **P-2.3** was following general procedure 5.

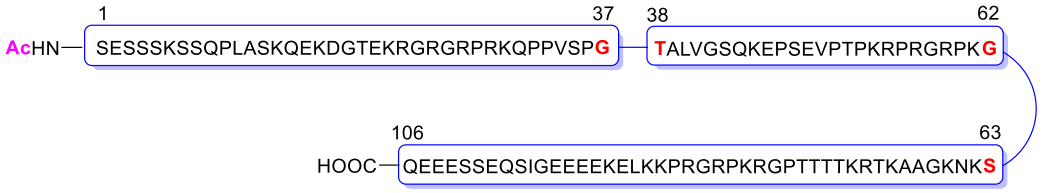
To make the concentration of peptide around 5 mM, 18.5 mg (0.0023 mmol, 1 equiv.) of **P-2.2** was dissolved in 460 µl H_2O. 3.5 µl of Hydrazine monohydrate (0.069 mmol, 30 equiv.) was then added into the solution and stirred for 2 h at room temperature. 46 µl DEA in 414 µl of H_2O/CH_3CN (1/1, v/v) was poured into the solution and stirred for another 2 h at room temperature. The product was purified by HPLC (gradient 5-40% CH_3CN/H_2O over 40 min) and 11 mg of the pure product was obtained with 58.6% yield.





P-2.3 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{327}H_{555}N_{105}O_{111}$ molecular weight: 7733.66. P_1 $[M+5H]^{5+}$ m/z = 1547.73; P_2 $[M+6H]^{6+}$ m/z = 1289.94; P_3 $[M+7H]^{7+}$ m/z = 1105.81; P_4 $[M+8H]^{8+}$ m/z = 967.71. P_5 $[M+9H]^{9+}$ m/z = 860.30; P_6 $[M+10H]^{10+}$ m/z = 774.37; P_7 $[M+11H]^{11+}$ m/z = 704.06. Found: 1547.73; 1289.76; 1105.88; 967.80; 860.37; 774.78; 704.17.

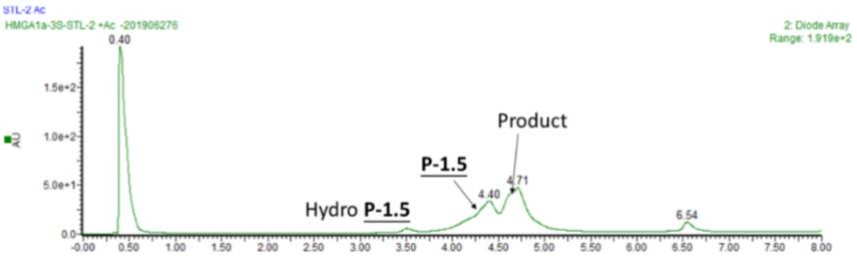
Synthesis of protein P2

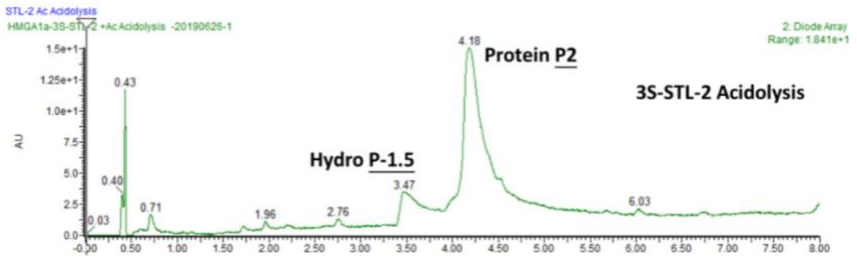


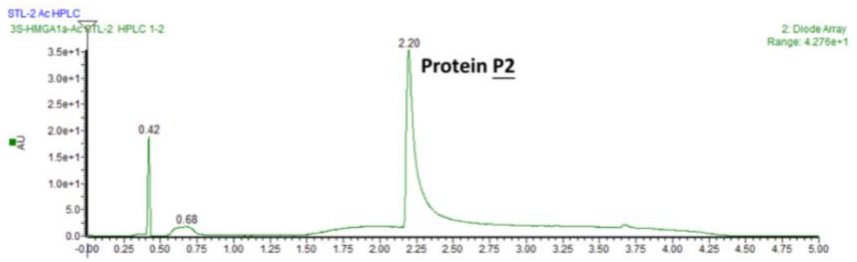
protein P2 (HMGA1a-3S-Ac)

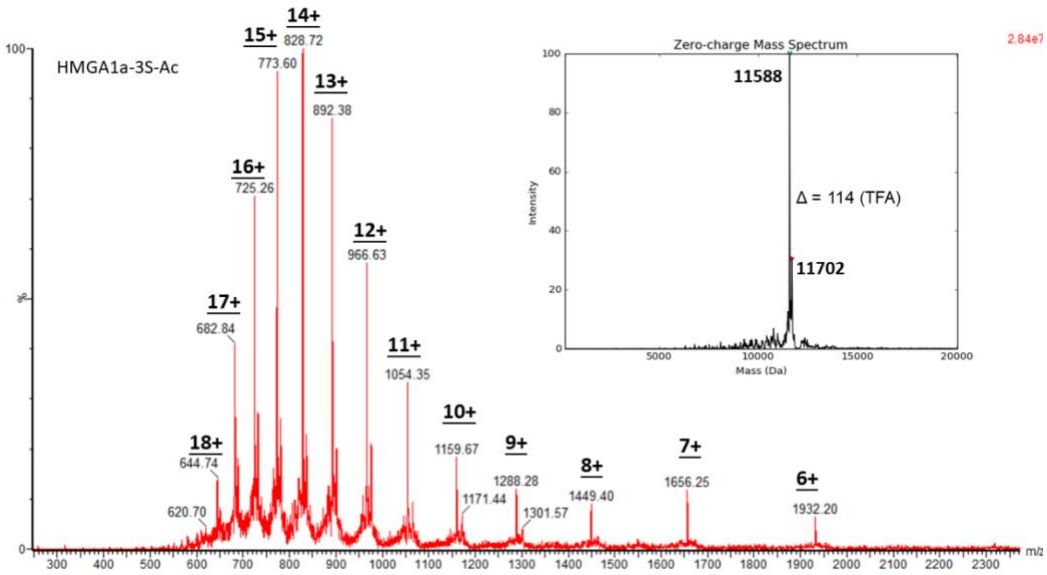
The preparation of protein **P2** was following general procedure 6.

5.13 mg (0.00066 mmol, 1 equiv.) of **P-2.3** and 4.1 mg (0.001 mmol, 1.5 equiv.) of **P-1.5** were mixed together, which were further dissolved in 100 µl pyridine/AcOH (1/12, mol/mol). The reaction was conducted at room temperature and stirred for 3 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 4.1 mg pure product (yield 55.9%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



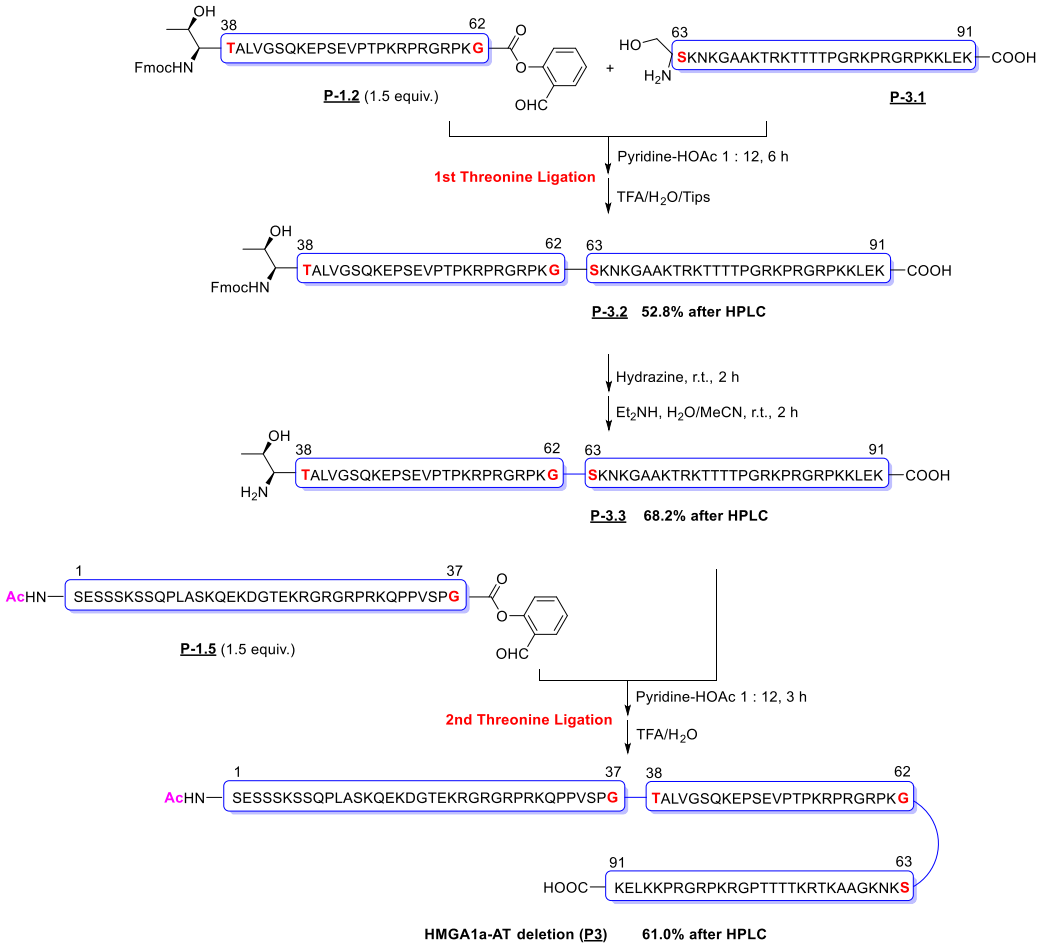




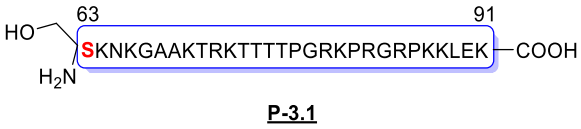


HMGA1a-3S (**P2)** was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{484}H_{826}N_{162}O_{167}$ molecular weight: 11586.90. P_1 $[M+6H]^{6+}$ m/z = 1932.15; P_2 $[M+7H]^{7+}$ m/z = 1656.27; P_3 $[M+8H]^{8+}$ m/z = 1449.36; P_4 $[M+9H]^{9+}$ m/z = 1288.43; P_5 $[M+10H]^{10+}$ m/z = 1159.69; P_6 $[M+11H]^{11+}$ m/z = 1054.35; P_7 $[M+12H]^{12+}$ m/z = 966.58; P_8 $[M+13H]^{13+}$ m/z = 892.30; P_9 $[M+14H]^{14+}$ m/z = 828.64; P_{10} $[M+15H]^{15+}$ m/z = 773.46; P_{11} $[M+16H]^{16+}$ m/z = 725.18; P_{12} $[M+17H]^{17+}$ m/z = 682.58; P_{13} $[M+18H]^{18+}$ m/z = 644.72. Found: 1932.20; 1656.25; 1449.40; 1288.28; 1159.67; 1054.35; 966.63; 892.38; 828.72; 773.60; 725.26; 682.84; 644.74.

4. Preparation of HMGA1a-AT deletion (P3)

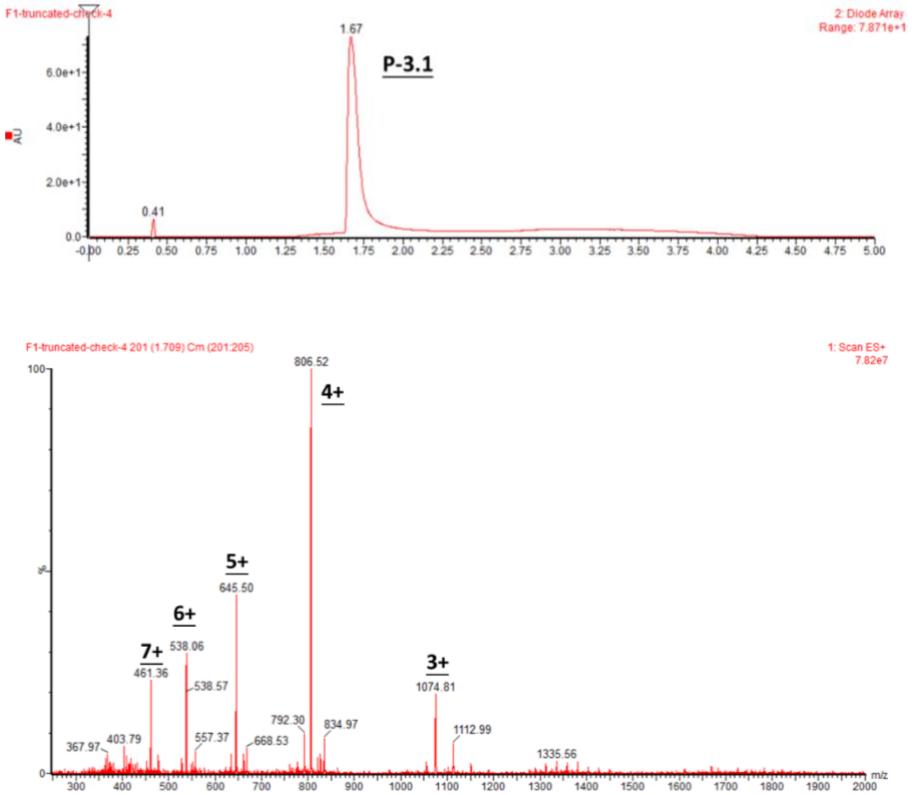


Synthesis of peptide P-3.1



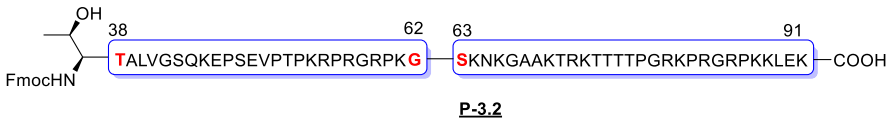
P-3.1

The preparation of peptide **P-3.1** was following **general procedure 1** via Fmoc-SPPS. After HPLC purification (gradient: 5-40% CH_3CN/H_2O over 40 min) and lyophilization, 128 mg pure product can be obtained from 0.4 g trityl chloride resin (20.8% yield based on resin loading).



P-3.1 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{137}H_{251}N_{51}O_{38}$ molecular weight: 3220.83. P_1 $[M+3H]^{3+}$ m/z = 1074.61; P_2 $[M+4H]^{4+}$ m/z = 806.21; P_3 $[M+5H]^{5+}$ m/z = 645.17; P_4 $[M+6H]^{6+}$ m/z = 537.81; P_5 $[M+7H]^{7+}$ m/z = 461.12. Found: 1074.81; 806.52; 645.50; 538.06; 461.36.

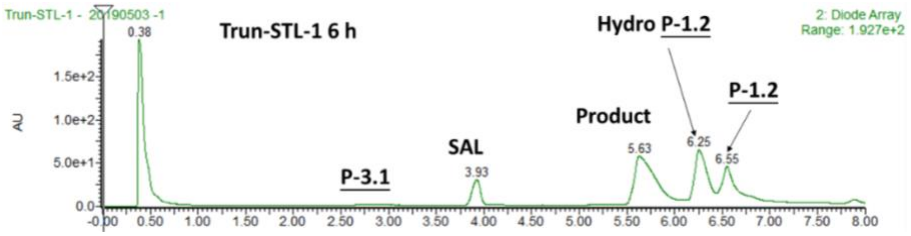
Synthesis of peptide P-3.2

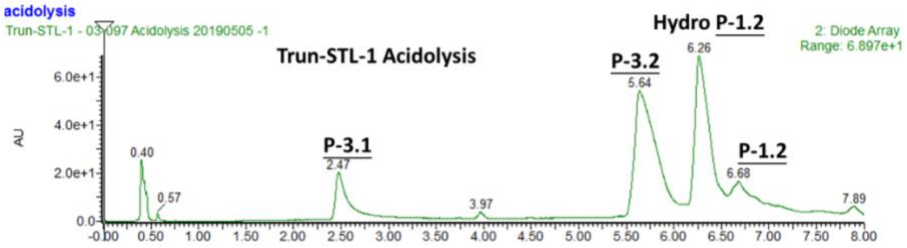


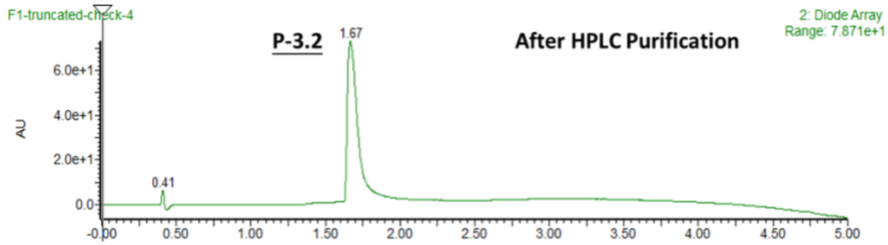
P-3.2

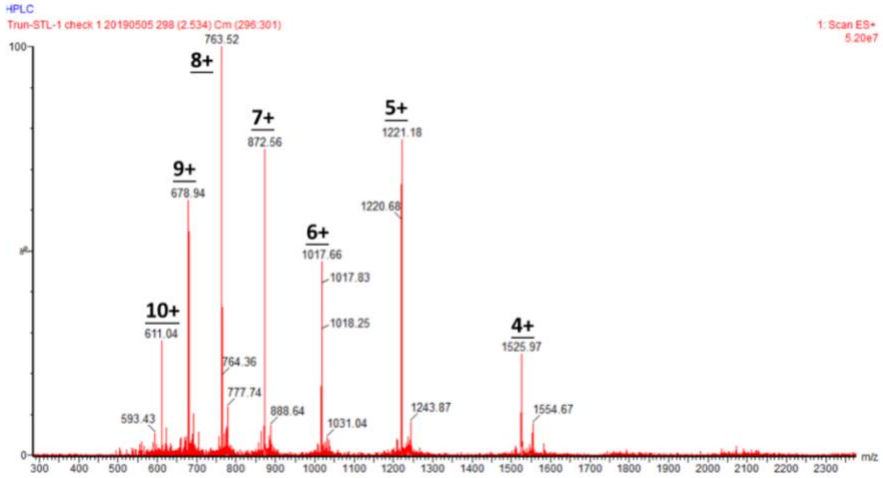
The preparation of peptide **P-3.2** was following **general procedure 4**.

23 mg (0.0072 mmol, 1 equiv.) of **P-2.1** and 31.6 mg (0.0107 mmol, 1.5 equiv.) of **P-1.2** were mixed together, which were further dissolved in 715 µl pyridine/AcOH (1/12, mol/mol) to make the concentration of **P-2.1** at 10 mM. The reaction was conducted at room temperature and stirred for 6 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 23.0 mg pure product (yield 52.8%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



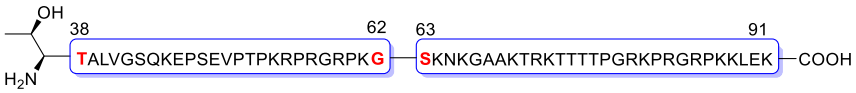






P-3.2 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{267}H_{457}N_{89}O_{74}$ molecular weight: 6098.14. P_1 $[M+4H]^{4+}$ m/z = 1525.54; P_2 $[M+5H]^{5+}$ m/z = 1220.63; P_3 $[M+6H]^{6+}$ m/z = 1017.36; P_4 $[M+7H]^{7+}$ m/z = 872.16; P_5 $[M+8H]^{8+}$ m/z = 763.27; P_6 $[M+9H]^{9+}$ m/z = 678.57; P_7 $[M+10H]^{10+}$ m/z = 610.82. Found: 1525.97; 1221.18; 1017.66; 872.56; 763.52; 678.94; 611.04.

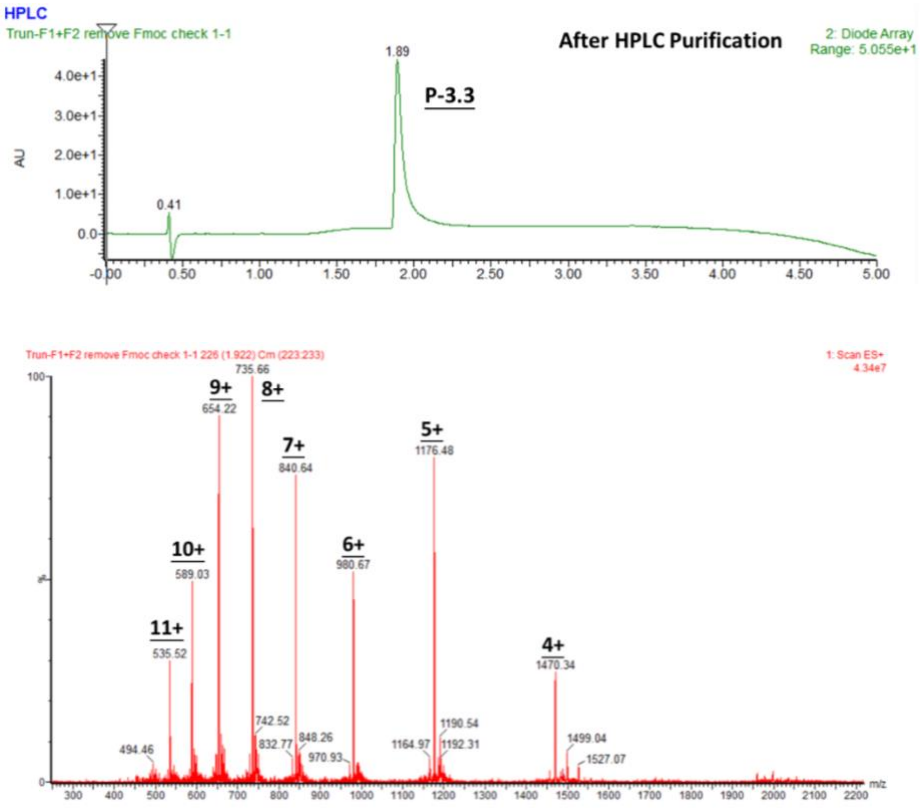
Synthesis of P-3.3



P-3.3

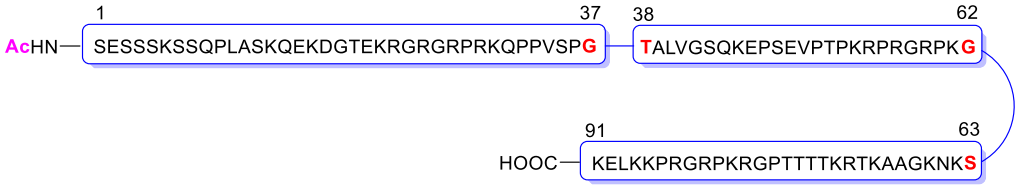
The preparation of peptide **P-3.3** was following **general procedure 5**.

To make the concentration of peptide around 5 mM, 23.2 mg (0.0038 mmol, 1 equiv.) of **P-3.2** was dissolved in 675 µl H_2O. 75 µl DEA added into the solution and stirred for another 2 h at room temperature. The product was purified by HPLC (gradient 5-40% CH_3CN/H_2O over 40 min) and 15.1 mg of the pure product was obtained with 68.2% yield.



P-3.3 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{252}H_{447}N_{89}O_{72}$ molecular weight: 5875.90. P_1 $[M+4H]^{4+}$ m/z = 1469.98; P_2 $[M+5H]^{5+}$ m/z = 1176.18; P_3 $[M+6H]^{6+}$ m/z = 980.32; P_4 $[M+7H]^{7+}$ m/z = 840.42; P_5 $[M+8H]^{8+}$ m/z = 735.49; P_6 $[M+9H]^{9+}$ m/z = 653.88; P_7 $[M+10H]^{10+}$ m/z = 588.59; P_8 $[M+11H]^{11+}$ m/z = 535.17. Found: 1470.34; 1176.48; 980.67; 840.64; 735.66; 654.22; 589.03; 535.52.

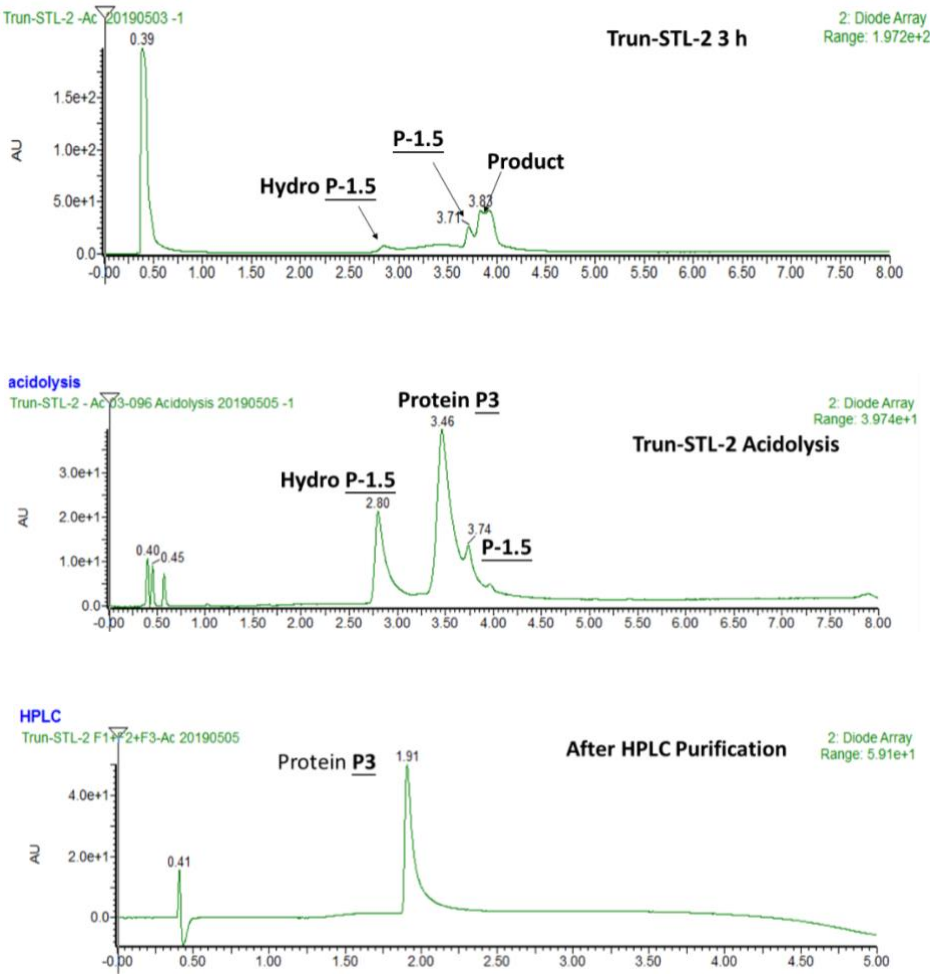
Synthesis of protein P3

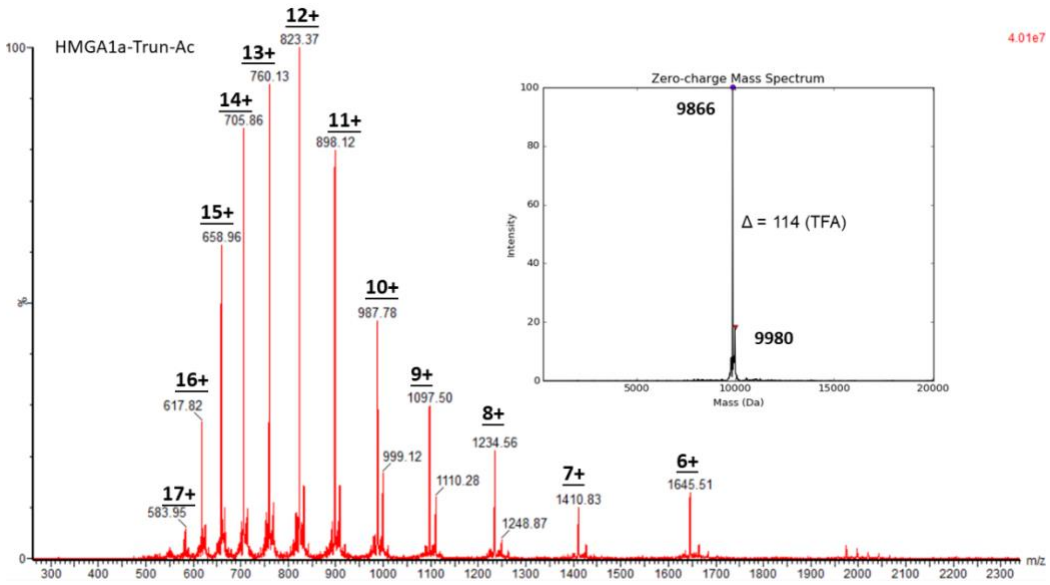


HMGA1a-AT deletion (P3)

The preparation of protein **P3** was following **general procedure 6**.

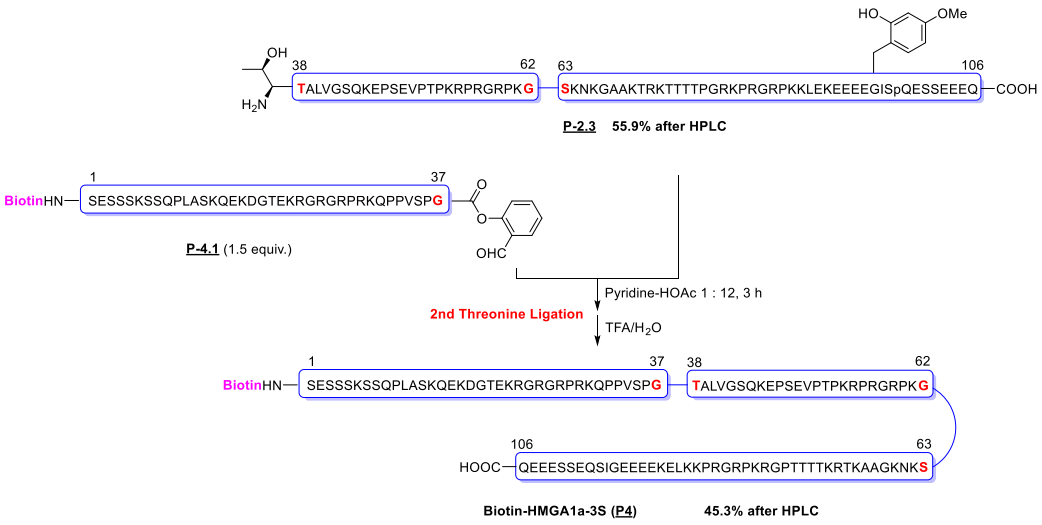
8.5 mg (0.0015 mmol, 1 equiv.) of **P-3.3** and 9.3 mg (0.0023 mmol, 1.5 equiv.) of **P-1.5** were mixed together, which were further dissolved in 145 µl pyridine/AcOH (1/12, mol/mol). The reaction was conducted at room temperature and stirred for 3 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 8.8 mg pure product (yield 61.0%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



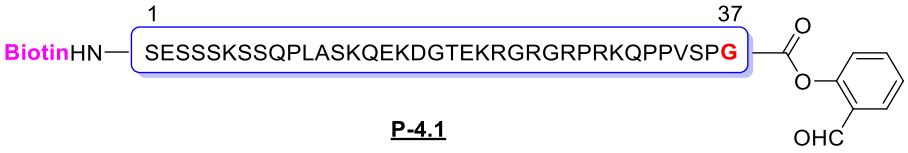


HMGA1a-AT deletion (**P3**) was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{417}H_{726}N_{146}O_{130}$ molecular weight: 9865.29. P_1 $[M+5H]^{5+}$ m/z = 1974.06; P_2 $[M+6H]^{6+}$ m/z = 1645.22; P_3 $[M+7H]^{7+}$ m/z = 1410.33; P_4 $[M+8H]^{8+}$ m/z = 1234.16; P_5 $[M+9H]^{9+}$ m/z = 1097.14; P_6 $[M+10H]^{10+}$ m/z = 987.53; P_7 $[M+11H]^{11+}$ m/z = 897.85; P_8 $[M+12H]^{12+}$ m/z = 823.11; P_9 $[M+13H]^{13+}$ m/z = 759.87; P_{10} $[M+14H]^{14+}$ m/z = 705.66; P_{11} $[M+15H]^{15+}$ m/z = 658.69; P_{12} $[M+16H]^{16+}$ m/z = 617.58. Found: 1974.53; 1645.51; 1410.83; 1234.56; 1097.50; 987.78; 898.12; 823.37; 760.13; 705.96; 658.96; 617.82.

5. Preparation of Biotin-HMGA1a-3S (P4)

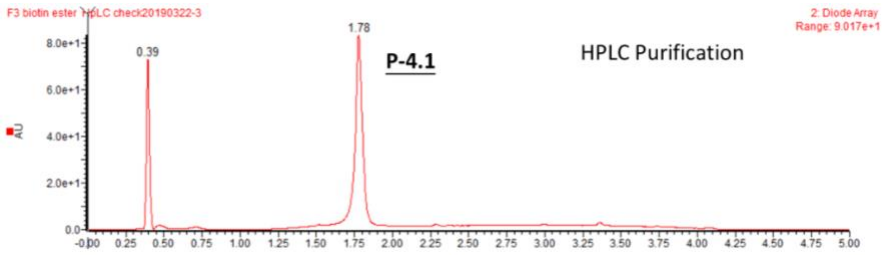


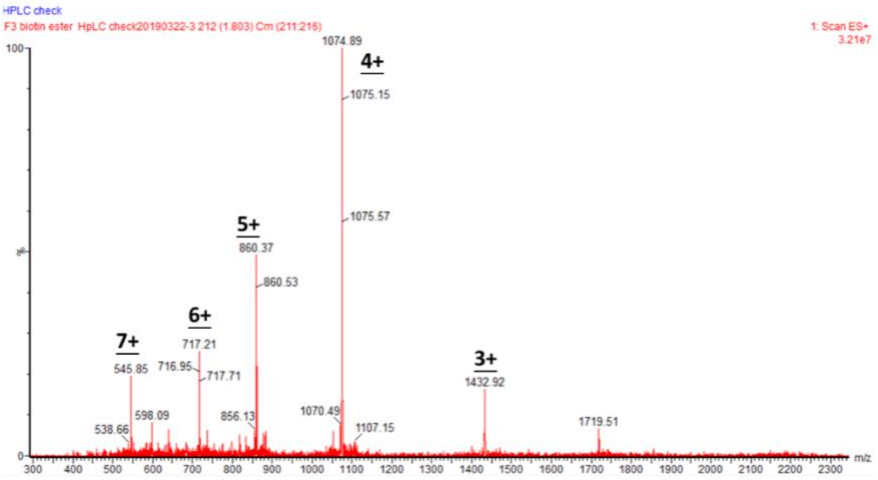
Synthesis of **P-4.1**



The preparation of **P-4.1** was following **general procedure 3**.

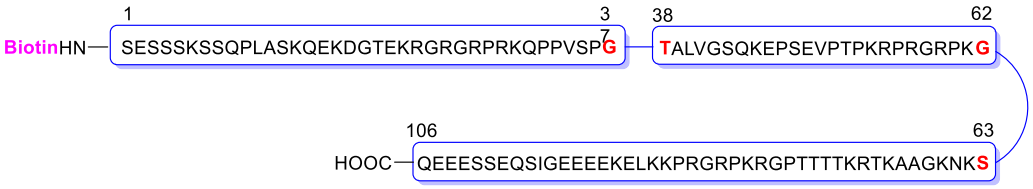
The N-terminal of last amino acid Ser was protected by the Fmoc protection group, which was further removed and give a free amino group for the installing of biotin. After finished SPPS, the resin was treated with AcOH/TFE/DCM (1/1/8, v/v/v) for 1.5 h to obtain crude peptide with all side-chain protected. A mixture of crude peptide/PyBOP/DIEA (1/3/3 mol/mol/mol) was then dissolved in DCM and stirred 10 min for the activation of carboxylic acid on the C-terminal of the crude peptide. Twenty equivalent of dimethyl acetal salicylaldehyde was then added for an overnight reaction. After removing DCM, the mixture was treated with TFA/H_2O/Phenol (95/2.5/2.5, v/v/v) for 1.5 h. The product was purified by HPLC (gradient: 10-40% CH_3CN/H_2O over 40 min) and lyophilization. 48 mg pure product was obtained from 200 mg crude peptide with the yield 34.6%.





P-4.1 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{180}H_{297}N_{59}O_{61}$ molecular weight: 4295.77. P_1 $[M+3H]^{3+}$ m/z = 1432.92; P_2 $[M+4H]^{4+}$ m/z = 1074.94; P_3 $[M+5H]^{5+}$ m/z = 860.15; P_4 $[M+6H]^{6+}$ m/z = 716.96; Found: 1432.92; 1074.89; 860.37; 717.21.

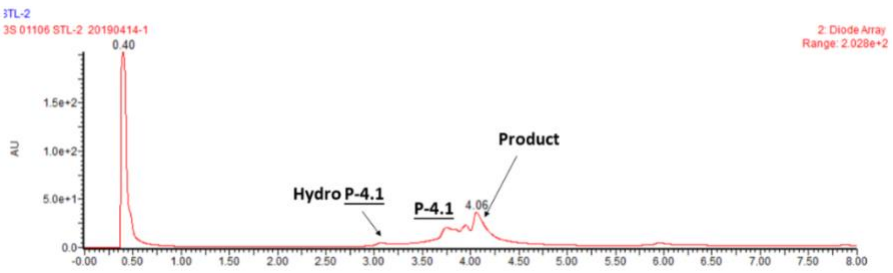
Synthesis of protein P4

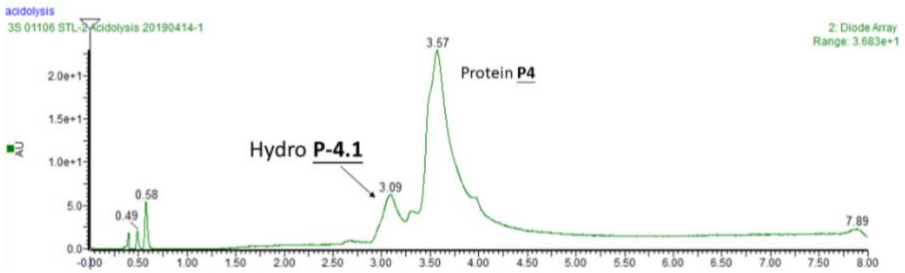


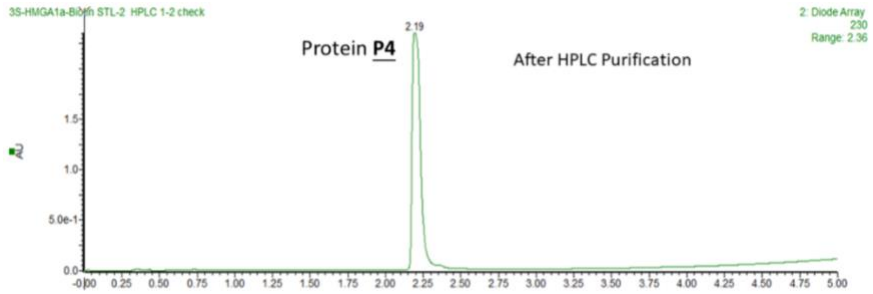
protein P4 (HMGA1a-3S-Biotin)

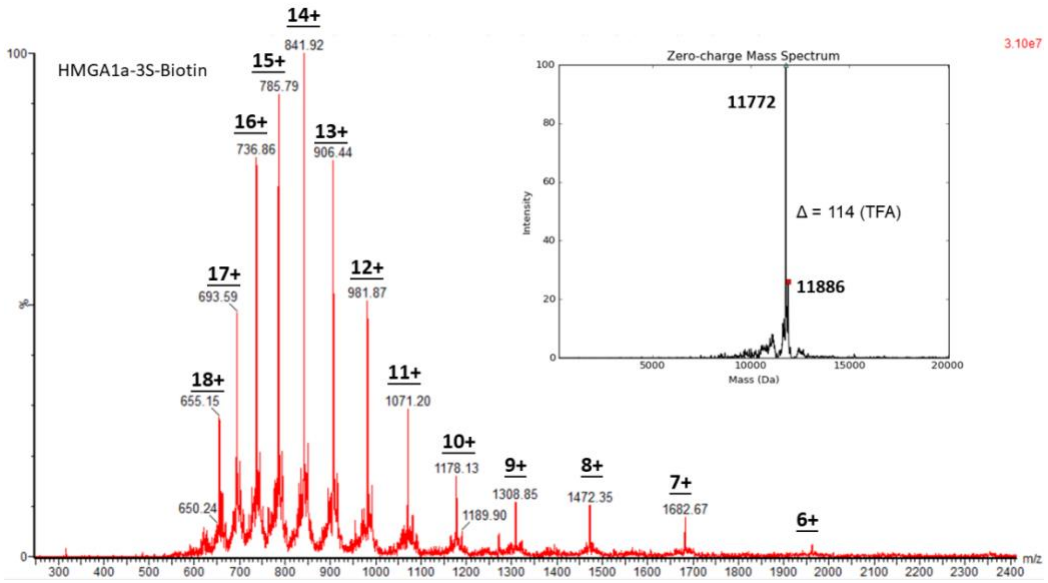
The preparation of protein **P4** was following **general procedure 6**.

10.0 mg (0.0013 mmol, 1 equiv.) of **P-2.3** and 8.5 mg (0.0019 mmol, 1.5 equiv.) of **P-4.1** were mixed together, which were further dissolved in 130 µl pyridine/AcOH (1/12, mol/mol). The reaction was conducted at room temperature and stirred for 3 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 6.9 mg pure product (yield 45.3%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



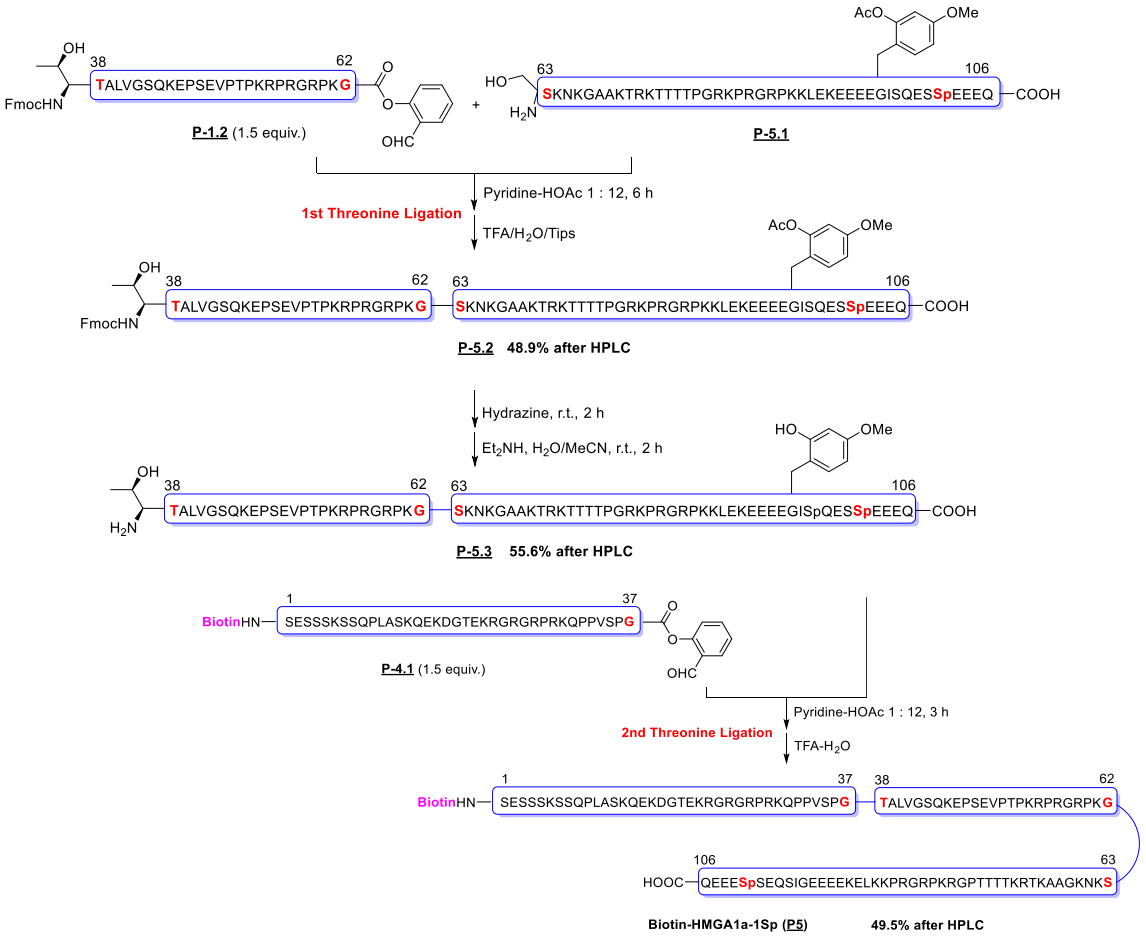




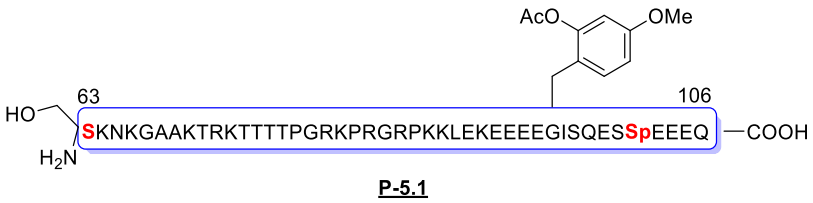


Biotin-HMGA1a-3S (P4) was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{492}H_{838}N_{164}O_{168}S$ molecular weight: 11771.16. P_1 $[M+7H]^{7+}$ m/z = 1682.59; P_2 $[M+8H]^{8+}$ m/z = 1472.40; P_3 $[M+9H]^{9+}$ m/z = 1308.91; P_4 $[M+10H]^{10+}$ m/z = 1178.12; P_5 $[M+11H]^{11+}$ m/z = 1071.11; P_6 $[M+12H]^{12+}$ m/z = 981.93; P_7 $[M+13H]^{13+}$ m/z = 906.47; P_8 $[M+14H]^{14+}$ m/z = 841.80; P_9 $[M+15H]^{15+}$ m/z = 785.74; P_{10} $[M+16H]^{16+}$ m/z = 736.70; P_{11} $[M+17H]^{17+}$ m/z = 693.42; P_{12} $[M+18H]^{18+}$ m/z = 653.95. Found: 1682.67; 1472.35; 1308.85; 1178.13; 1071.20; 981.87; 906.44; 841.92; 785.79; 736.86; 693.59; 655.15.

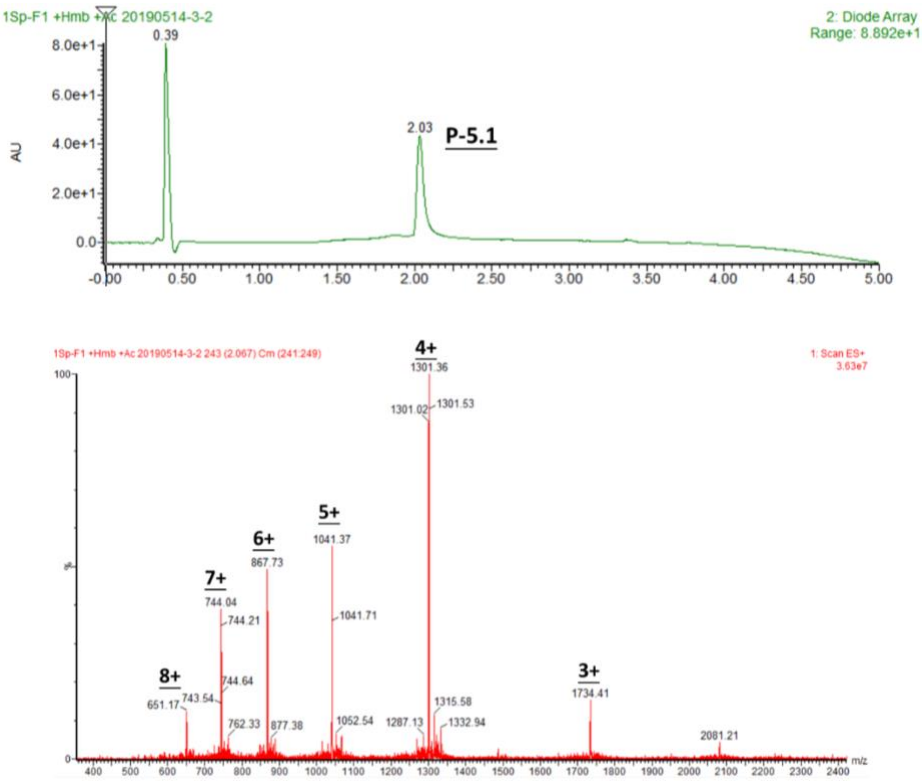
6. Preparation of Biotin-HMGA1a-1pSer (P5)



Synthesis of P-5.1

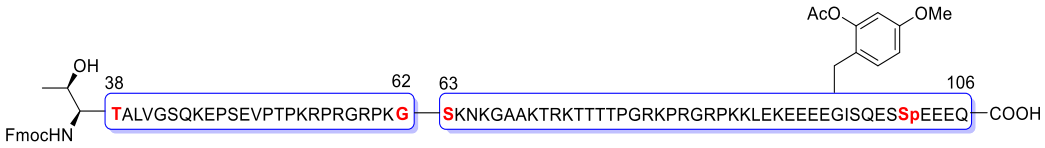


The preparation of peptide **P-5.1** was following **general procedure 1** via Fmoc-SPPS. All problems should be noticed has been described in the synthesis of peptide **P-1.1**. After HPLC purification (gradient: 5-40% CH_3CN/H_2O over 40 min) and lyophilization, 93 mg pure product can be obtained from 0.5 g trityl chloride resin (11.9% yield based on resin loading).



P-5.1 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{214}H_{362}N_{67}O_{81}P$ molecular weight: 5200.61. P_1 $[M+3H]^{3+}$ m/z = 1734.54; P_2 $[M+4H]^{4+}$ m/z = 1301.15; P_3 $[M+5H]^{5+}$ m/z = 1041.12; P_4 $[M+6H]^{6+}$ m/z = 867.77; P_5 $[M+7H]^{7+}$ m/z = 743.95; P_6 $[M+8H]^{8+}$ m/z = 651.08. Found: 1734.41; 1301.36; 1041.37; 867.73; 744.04; 651.17.

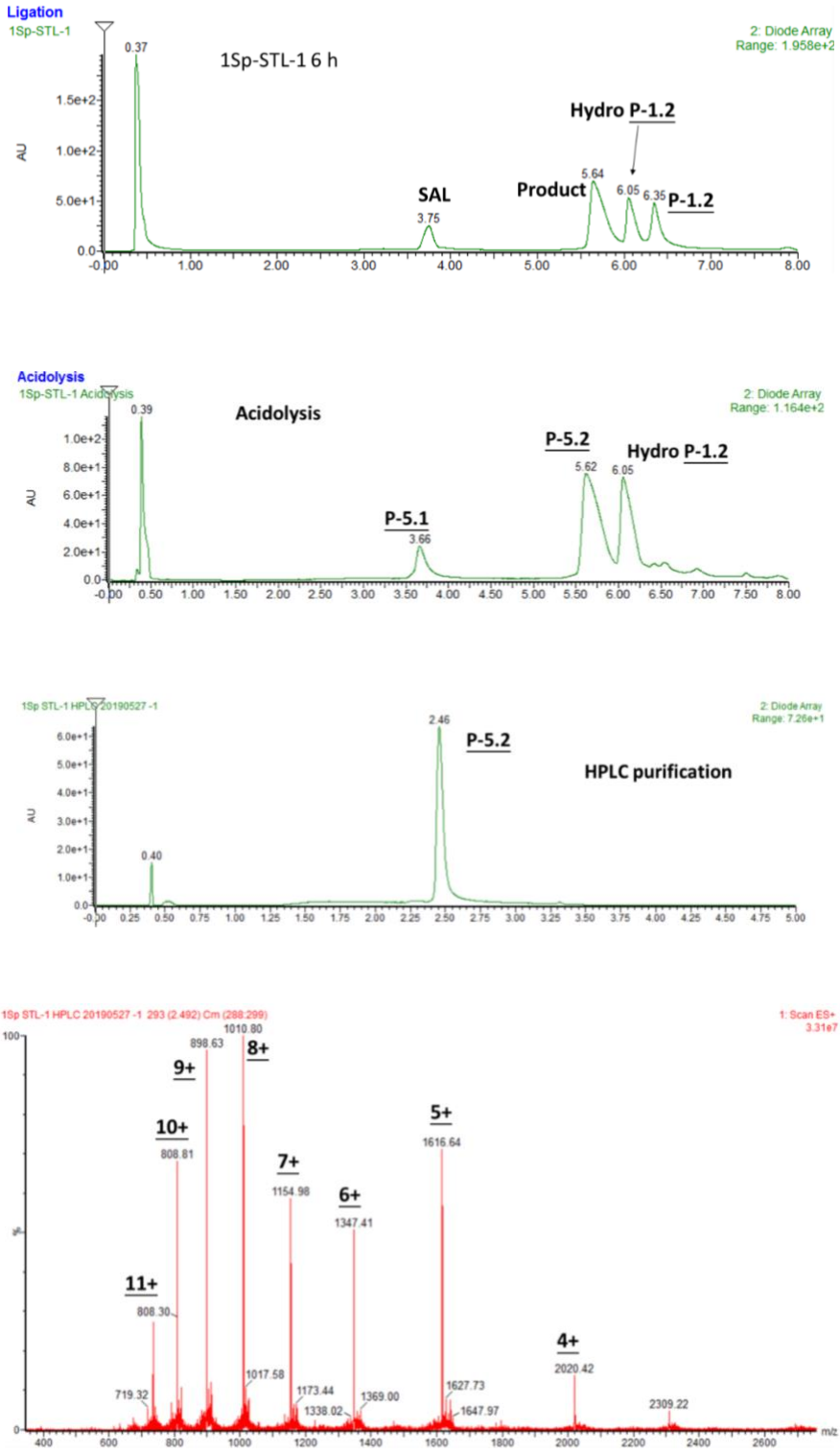
Synthesis of P-5.2



P-5.2

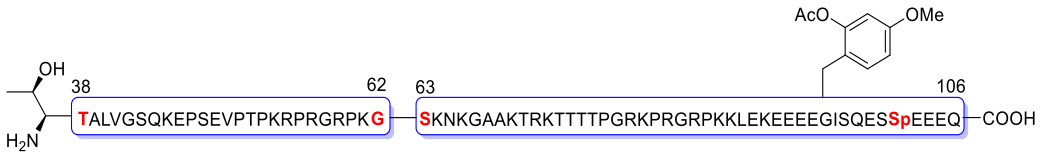
The preparation of peptide **P-5.2** was following **general procedure 4**.

29 mg (0.0056 mmol, 1 equiv.) of **P-5.2** and 25.5 mg (0.0084 mmol, 1.5 equiv.) of **P-1.2** were mixed together, which were further dissolved in 560 µl pyridine/AcOH (1/12, mol/mol) to make the concentration of **P-5.2** at 10 mM. The reaction was conducted at room temperature and stirred for 6 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 22.3 mg pure product (yield 48.9%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



P-5.2 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{344}H_{568}N_{105}O_{117}P$ molecular weight: 8077.92. P_1 $[M+4H]^{4+}$ m/z = 2020.48; P_2 $[M+5H]^{5+}$ m/z = 1616.58; P_3 $[M+6H]^{6+}$ m/z = 1347.32; P_4 $[M+7H]^{7+}$ m/z = 1154.99; P_5 $[M+8H]^{8+}$ m/z = 1010.74; P_6 $[M+9H]^{9+}$ m/z = 898.55; P_7 $[M+10H]^{10+}$ m/z = 808.79; P_8 $[M+11H]^{11+}$ m/z = 735.36; Found: 2020.42; 1616.64; 1347.41; 1154.98; 1010.80; 898.63; 808.81; 735.42.

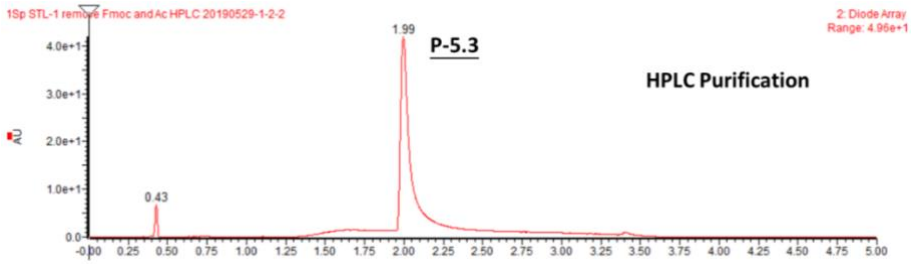
Synthesis of P-5.3

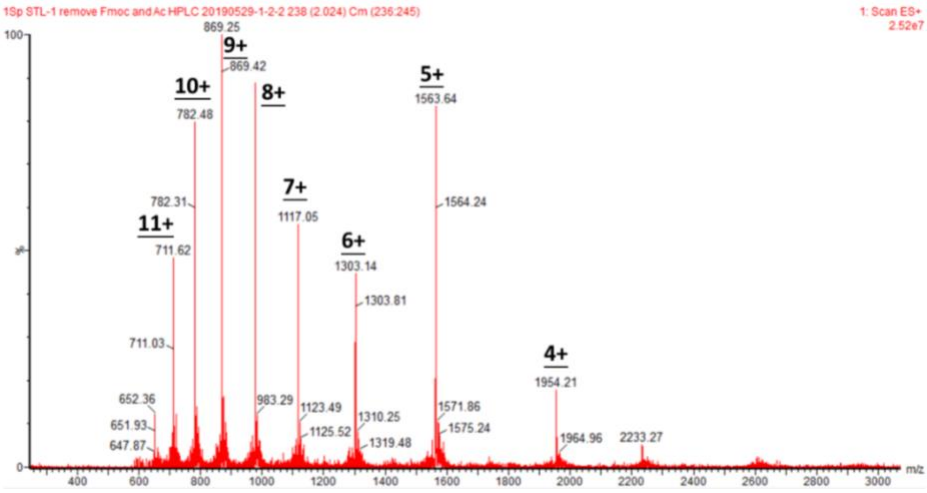


P-5.3

The preparation of peptide **P-5.3** was following **general procedure 5**.

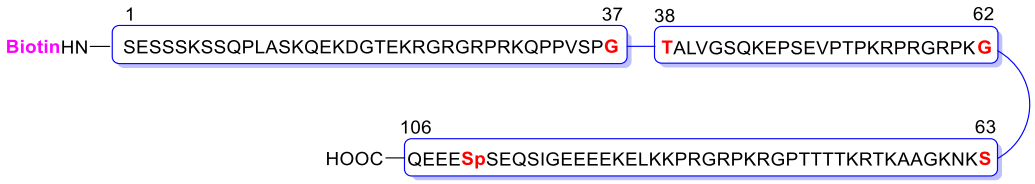
To make the concentration of peptide around 5 mM, 22.3 mg (0.0027 mmol, 1 equiv.) of **P-5.2** was dissolved in 540 µl H_2O. 4.3 µl of Hydrazine monohydrate (0.082 mmol, 30 equiv.) was then added into the solution and stirred for 2 h at room temperature. 54 µl DEA in 486 µl of H_2O/CH_3CN (1/1, v/v) was poured into the solution and stirred for another 2 h at room temperature. The product was purified by HPLC (gradient 5-40% CH_3CN/H_2O over 40 min) and 12.1 mg of the pure product can be obtained with 55.6% yield.





P-5.3 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{327}H_{556}N_{105}O_{114}P$ molecular weight: 7813.64. P_1 $[M+4H]^{4+}$ m/z = 1954.41; P_2 $[M+5H]^{5+}$ m/z = 1563.73; P_3 $[M+6H]^{6+}$ m/z = 1303.27; P_4 $[M+7H]^{7+}$ m/z = 1117.23; P_5 $[M+8H]^{8+}$ m/z = 977.71; P_6 $[M+9H]^{9+}$ m/z = 869.18; P_7 $[M+10H]^{10+}$ m/z = 782.36; P_8 $[M+11H]^{11+}$ m/z = 711.33; P_9 $[M+12H]^{12+}$ m/z = 652.14. Found: 1954.21; 1563.64; 1303.14; 1117.05; 977.53; 869.25; 782.48; 711.62; 652.36.

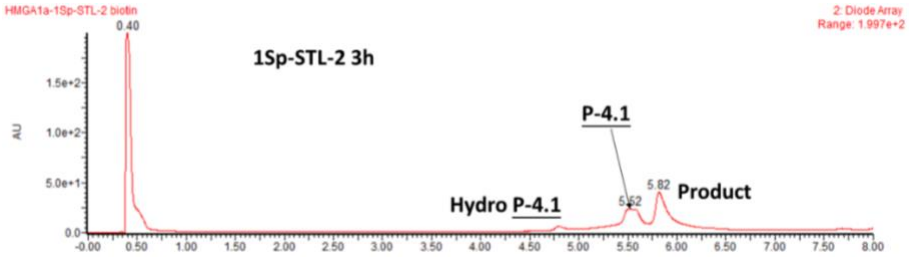
Synthesis of Biotin-HMGA1a-1pSer (P5)

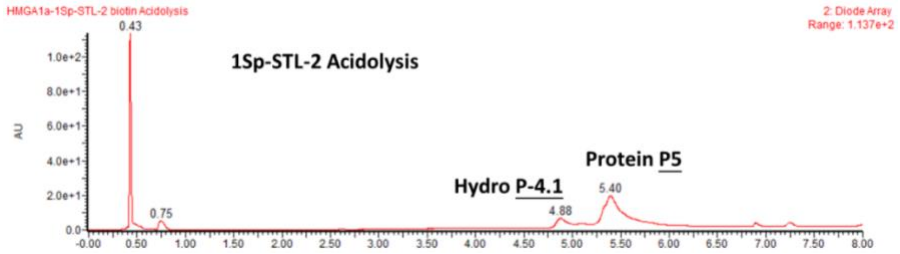


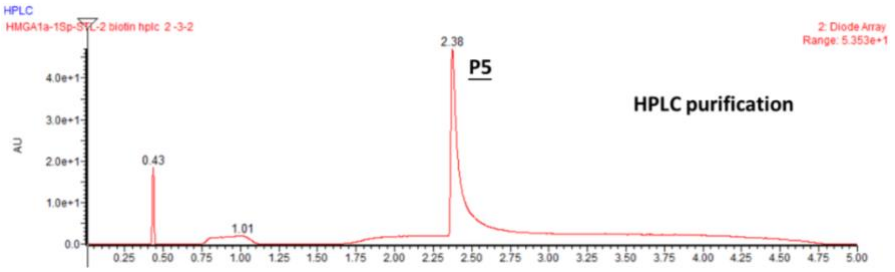
Biotin-HMGA1a-1Sp (P5)

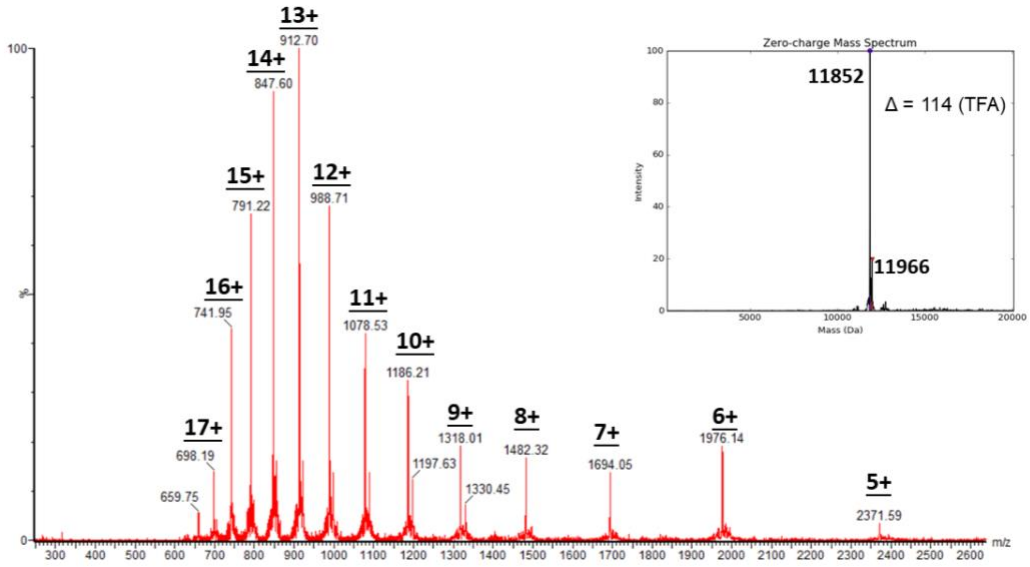
The preparation of protein **P5** was following **general procedure 6**.

6.3 mg (0.00081 mmol, 1 equiv.) of **P-5.3** and 5.5 mg (0.0019 mmol, 1.5 equiv.) of **P-4.1** were mixed together, which were further dissolved in 100 µl pyridine/AcOH (1/12, mol/mol). The reaction was conducted at room temperature and stirred for 3 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 4.8 mg pure product (yield 49.5%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



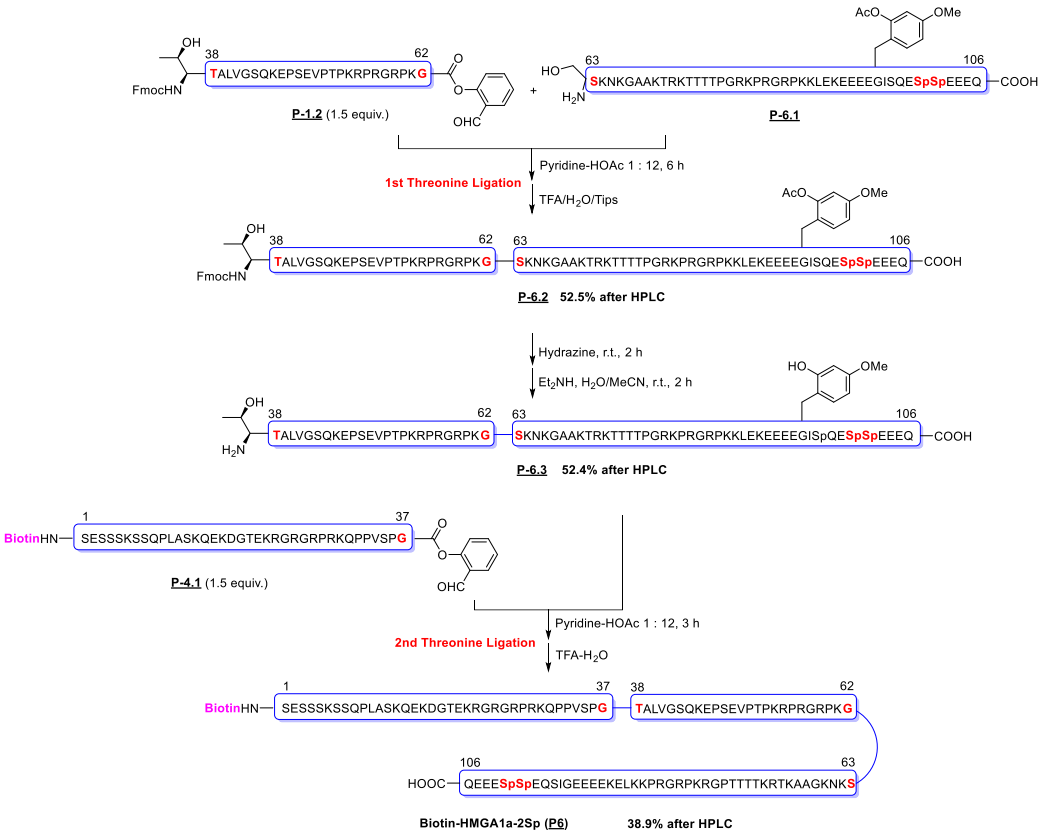




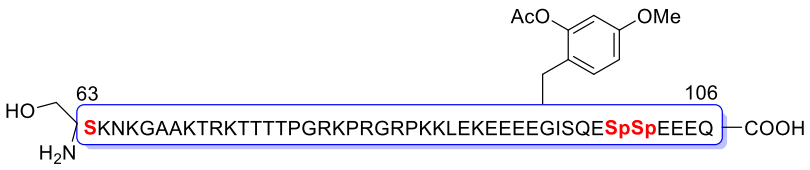


Biotin-HMGA1a-1pSer (P5) was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{492}H_{839}N_{164}O_{171}PS$ molecular weight: 11851.13. P_1 $[M+5H]^{5+}$ m/z = 2371.23; P_2 $[M+6H]^{6+}$ m/z = 1976.19; P_3 $[M+7H]^{7+}$ m/z = 1694.02; P_4 $[M+8H]^{8+}$ m/z = 1482.39; P_5 $[M+9H]^{9+}$ m/z = 1317.79; P_6 $[M+10H]^{10+}$ m/z = 1186.11; P_7 $[M+11H]^{11+}$ m/z = 1078.37; P_8 $[M+12H]^{12+}$ m/z = 988.60; P_9 $[M+13H]^{13+}$ m/z = 912.63; P_{10} $[M+14H]^{14+}$ m/z = 847.51; P_{11} $[M+15H]^{15+}$ m/z = 791.08; P_{12} $[M+16H]^{16+}$ m/z = 741.70; P_{13} $[M+17H]^{17+}$ m/z = 698.12. Found: 2371.59; 1976.14; 1694.05; 1482.32; 1318.01; 1186.21; 1078.53; 988.71; 912.70; 847.60; 791.22; 741.95; 698.19.

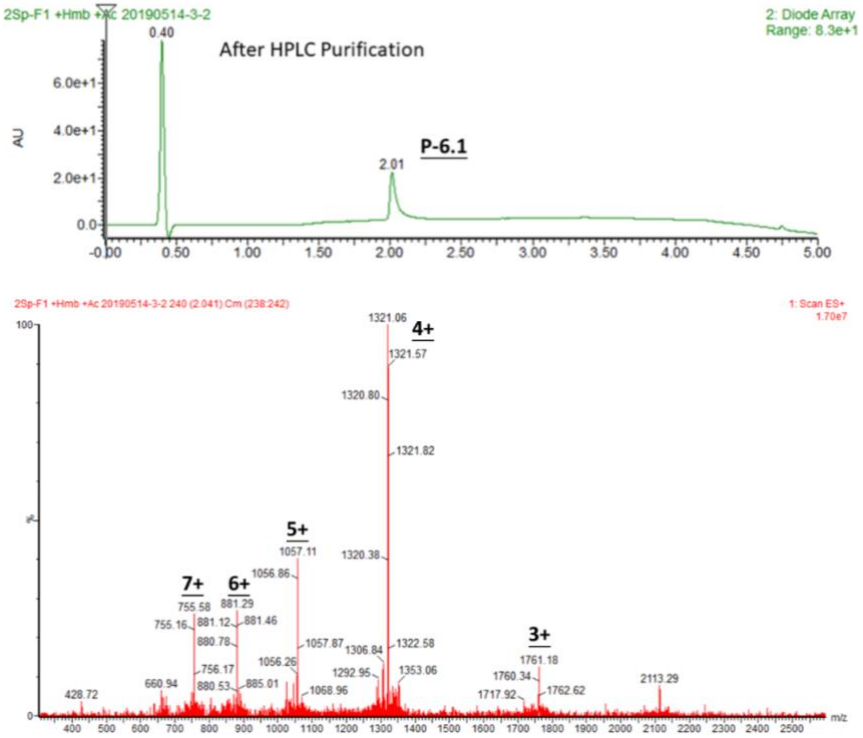
7. Preparation of Biotin-HMGA1a-2pSer (P6)



Synthesis of P-6.1

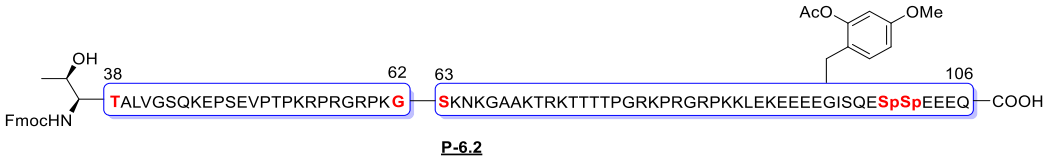


The preparation of peptide **P-6.1** was following **general procedure 1** via Fmoc-SPPS. All problems should be noticed has been described in the synthesis of peptide **P-1.1**. After HPLC purification (gradient: 5-40% CH_3CN/H_2O over 40 min) and lyophilization, 89.7 mg pure product can be obtained from 0.5 g trityl chloride resin (10.5% yield based on resin loading).



P-6.1 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{214}H_{363}N_{67}O_{84}P_2$ molecular weight: 5280.59. P_1 $[M+3H]^{3+}$ m/z = 1761.20; P_2 $[M+4H]^{4+}$ m/z = 1321.15; P_3 $[M+5H]^{5+}$ m/z = 1057.12; P_4 $[M+6H]^{6+}$ m/z = 881.10; P_5 $[M+7H]^{7+}$ m/z = 755.37; P_6 $[M+8H]^{8+}$ m/z = 661.07. Found: 1761.18; 1321.06; 1057.11; 881.29; 755.58; 660.94.

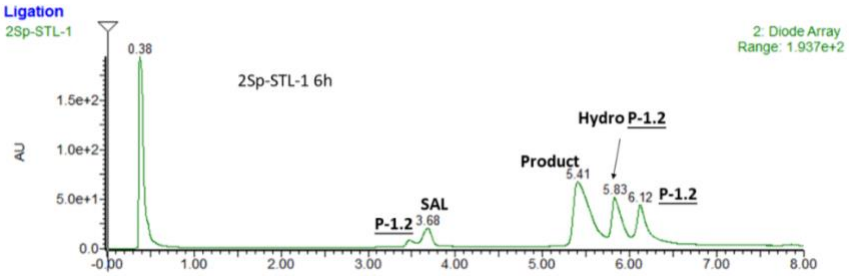
Synthesis of P-6.2

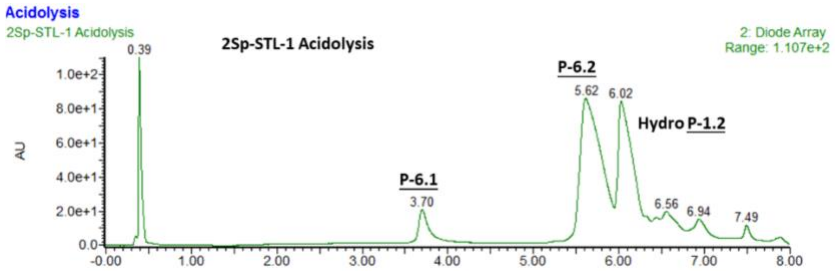


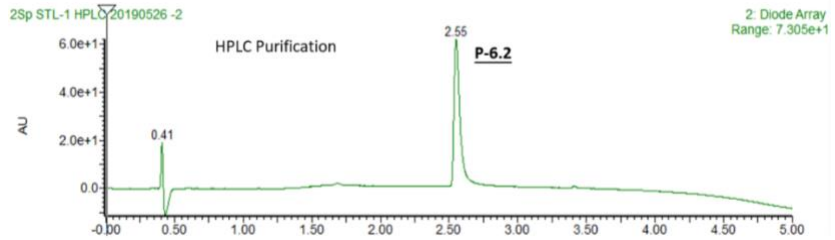
P-6.2

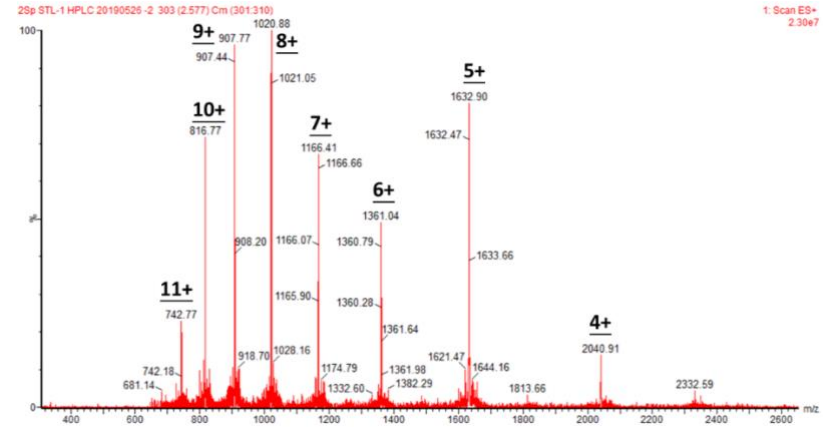
The preparation of peptide **P-6.2** was following **general procedure 4**.

26 mg (0.0050 mmol, 1 equiv.) of **P-6.1** and 23.0 mg (0.0074 mmol, 1.5 equiv.) of **P-1.2** were mixed together, which were further dissolved in 500 µl pyridine/AcOH (1/12, mol/mol) to make the concentration of **P-6.1** at 10 mM. The reaction was conducted at room temperature and stirred for 6 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 21.3 mg pure product (yield 52.5%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



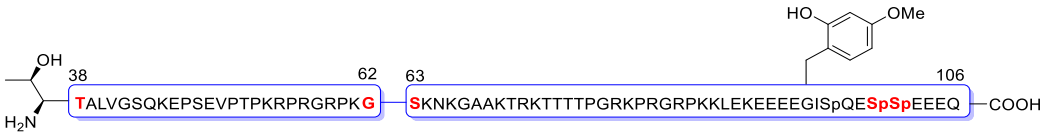






P-6.2 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{344}H_{569}N_{105}O_{120}P_2$ molecular weight: 8157.90. P_1 $[M+4H]^{4+}$ m/z = 2040.48; P_2 $[M+5H]^{5+}$ m/z = 1632.58; P_3 $[M+6H]^{6+}$ m/z = 1360.65; P_4 $[M+7H]^{7+}$ m/z = 1166.41; P_5 $[M+8H]^{8+}$ m/z = 1020.74; P_6 $[M+9H]^{9+}$ m/z = 907.43; P_7 $[M+10H]^{10+}$ m/z = 816.79; P_8 $[M+11H]^{11+}$ m/z = 742.63; Found: 2040.91; 1632.90; 1361.04; 1166.41; 1020.88; 907.77; 816.77; 742.77.

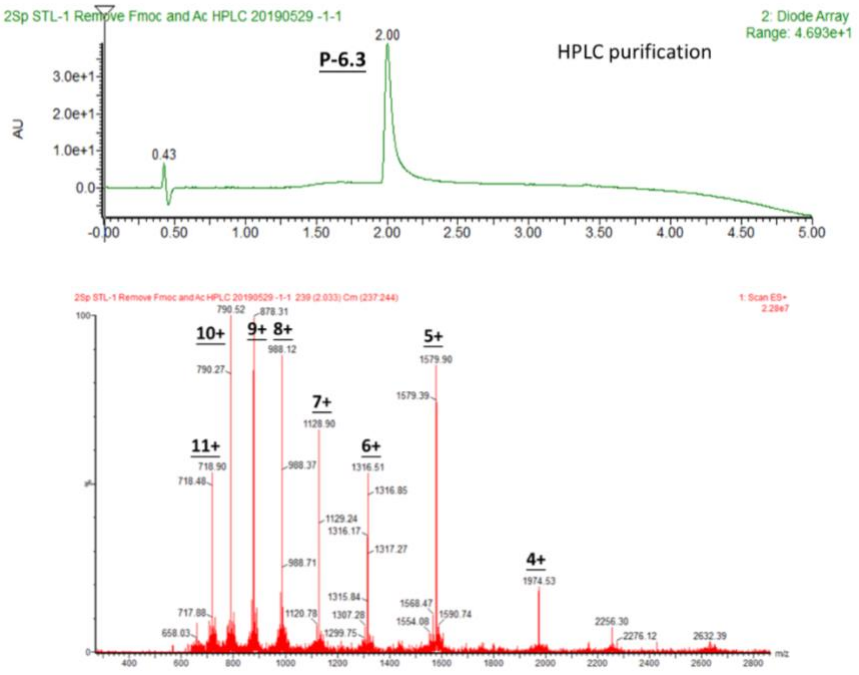
Synthesis of P-6.3



P-6.3

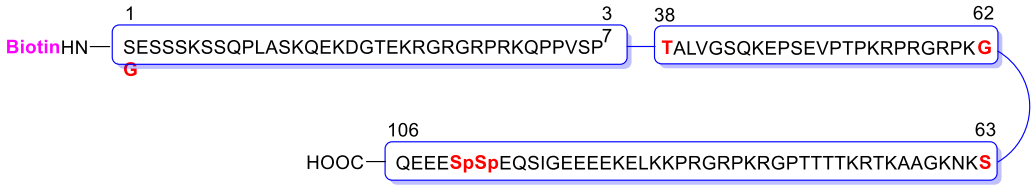
The preparation of peptide **P-6.3** was following general procedure 5.

To make the concentration of peptide around 5 mM, 21.3 mg (0.0026 mmol, 1 equiv.) of **P-6.2** was dissolved in 510 µl H_2O. 4.0 µl of Hydrazine monohydrate (0.078 mmol, 30 equiv.) was then added into the solution and stirred for 2 h at room temperature. 51 µl DEA in 459 µl of H_2O/CH_3CN (1/1, v/v) was poured into the solution and stirred for another 2 h at room temperature. The product was purified by HPLC (gradient 5-40% CH_3CN/H_2O over 40 min) and 10.7 mg of the pure product can be obtained with 52.4% yield.



P-6.3 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{327}H_{557}N_{105}O_{117}P_2$ molecular weight: 7893.62. P_1 $[M+3H]^{3+}$ m/z = 2632.21; P_2 $[M+4H]^{4+}$ m/z = 1974.41; P_3 $[M+5H]^{5+}$ m/z = 1579.72; P_4 $[M+6H]^{6+}$ m/z = 1316.60; P_5 $[M+7H]^{7+}$ m/z = 1128.66; P_6 $[M+8H]^{8+}$ m/z = 987.70; P_7 $[M+9H]^{9+}$ m/z = 878.07; P_8 $[M+10H]^{10+}$ m/z = 790.52; P_9 $[M+11H]^{11+}$ m/z = 718.60; P_{10} $[M+12H]^{12+}$ m/z = 658.88. Found: 1973.53; 1579.90; 1316.51; 1128.90; 988.12; 878.31; 790.52; 718.90.

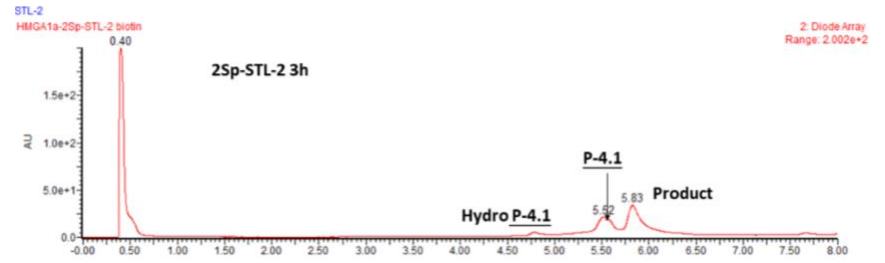
Synthesis of Biotin-HMGA1a-2pSer (P6)

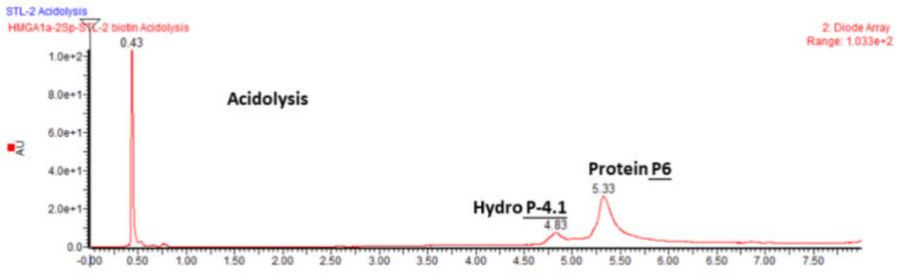


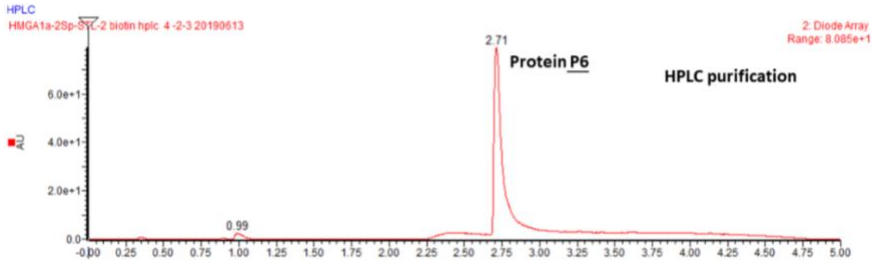
Biotin-HMGA1a-2Sp (P6)

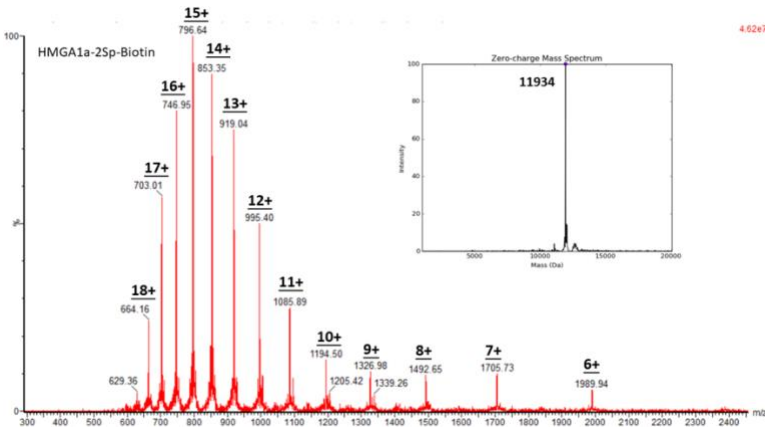
The preparation of protein **P6** was following general procedure 6.

6.0 mg (0.00076 mmol, 1 equiv.) of **P-6.3** and 5.0 mg (0.0011 mmol, 1.5 equiv.) of **P-4.1** were mixed together, which were further dissolved in 100 µl pyridine/AcOH (1/12, mol/mol). The reaction was conducted at room temperature and stirred for 3 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 3.5 mg pure product (yield 38.9%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



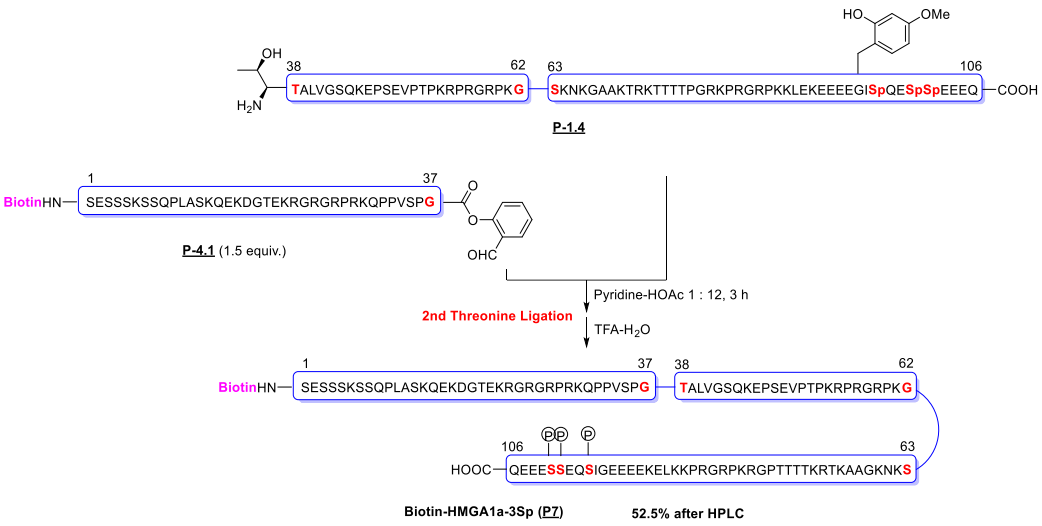






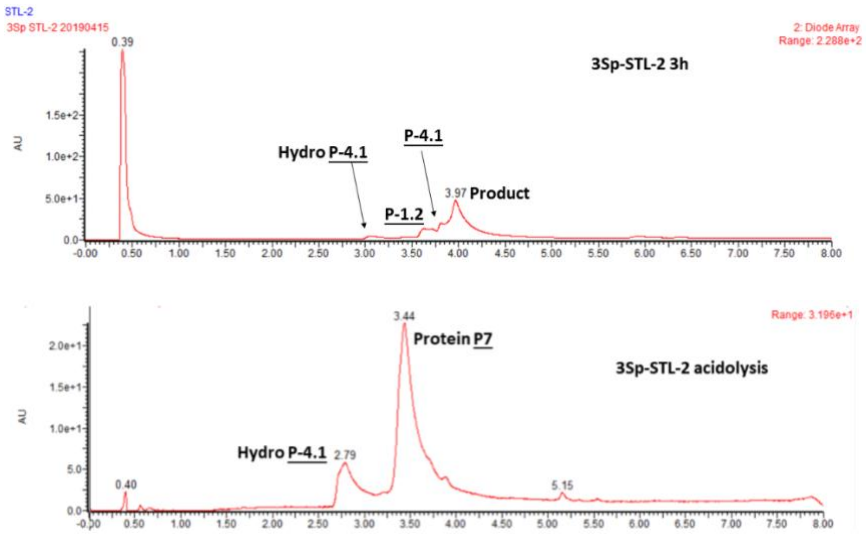
Biotin-HMGA1a-2pSer (P6) was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{492}H_{840}N_{164}O_{174}P_2S$ molecular weight: 11931.11. P_1 $[M+6H]^{6+}$ m/z = 1989.52; P_2 $[M+7H]^{7+}$ m/z = 1705.44; P_3 $[M+8H]^{8+}$ m/z = 1492.39; P_4 $[M+9H]^{9+}$ m/z = 1326.68; P_5 $[M+10H]^{10+}$ m/z = 1194.11; P_6 $[M+11H]^{11+}$ m/z = 1085.65; P_7 $[M+12H]^{12+}$ m/z = 995.26; P_8 $[M+13H]^{13+}$ m/z = 917.78; P_9 $[M+14H]^{14+}$ m/z = 853.22; P_{10} $[M+15H]^{15+}$ m/z = 796.41; P_{11} $[M+16H]^{16+}$ m/z = 746.70; P_{12} $[M+17H]^{17+}$ m/z = 702.83; P_{13} $[M+18H]^{18+}$ m/z = 663.84. Found: 1989.94; 1705.73; 1492.65; 1326.98; 1194.50; 1085.89; 995.40; 919.04; 853.35; 796.64; 746.95; 703.01; 664.16.

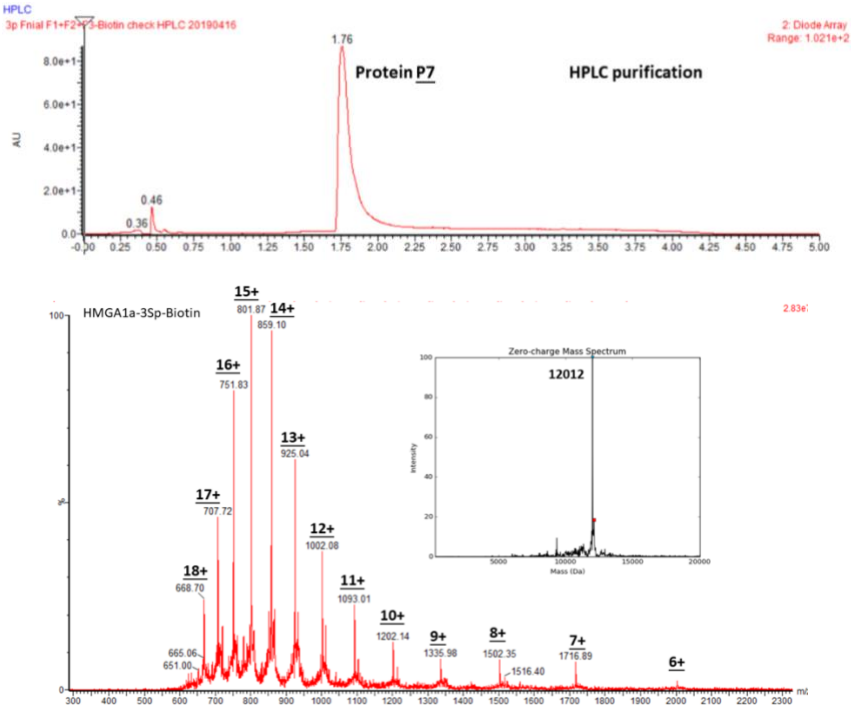
8. Preparation of Biotin-HMGA1a-3pSer (P7)



The preparation of protein **P7** was following general procedure 6.

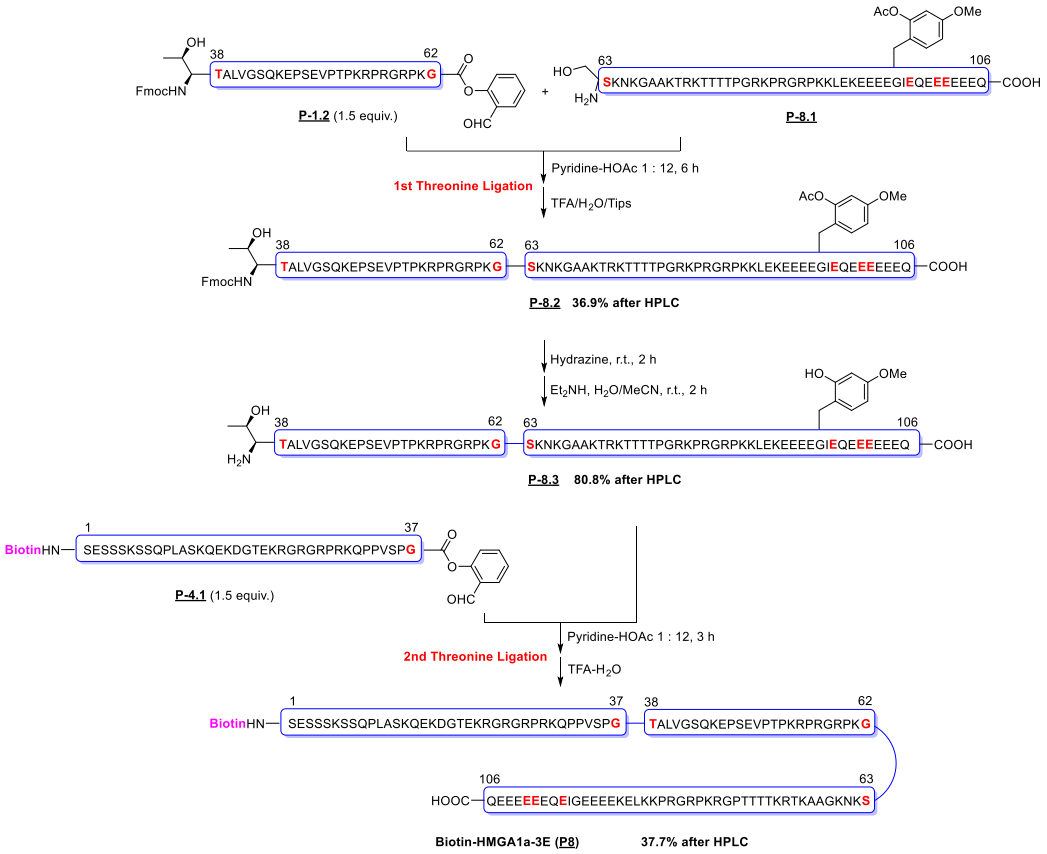
5.3 mg (0.00066 mmol, 1 equiv.) of **P-1.4** and 4.5 mg (0.0011 mmol, 1.5 equiv.) of **P-4.1** were mixed together, which were further dissolved in 100 µl pyridine/AcOH (1/12, mol/mol). The reaction was conducted at room temperature and stirred for 3 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 4.1 mg pure product (yield 52.5%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



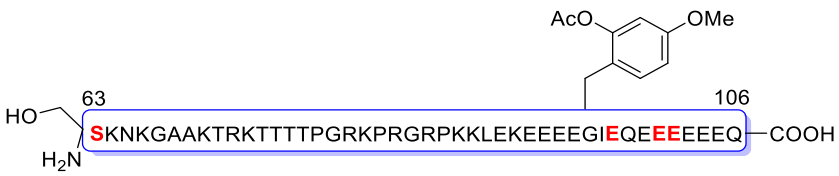


Biotin-HMGA1a-3pSer (P7) was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{492}H_{841}N_{164}O_{177}P_3S$ molecular weight: 12011.09. P_1 $[M+6H]^{6+}$ m/z = 2002.85; P_2 $[M+7H]^{7+}$ m/z = 1716.87; P_3 $[M+8H]^{8+}$ m/z = 1502.39; P_4 $[M+9H]^{9+}$ m/z = 1335.57; P_5 $[M+10H]^{10+}$ m/z = 1202.11; P_6 $[M+11H]^{11+}$ m/z = 1092.92; P_7 $[M+12H]^{12+}$ m/z = 1001.92; P_8 $[M+13H]^{13+}$ m/z = 924.93; P_9 $[M+14H]^{14+}$ m/z = 858.94; P_{10} $[M+15H]^{15+}$ m/z = 801.74; P_{11} $[M+16H]^{16+}$ m/z = 750.69; P_{12} $[M+17H]^{17+}$ m/z = 707.54; P_{13} $[M+18H]^{18+}$ m/z = 668.28. Found: 2003.15; 1716.89; 1502.26; 1336.07; 1202.14; 1093.01; 1002.08; 925.04; 859.10; 801.87; 751.75; 707.72; 668.70.

9. Preparation of Biotin-HMGA1a-3E (P8)

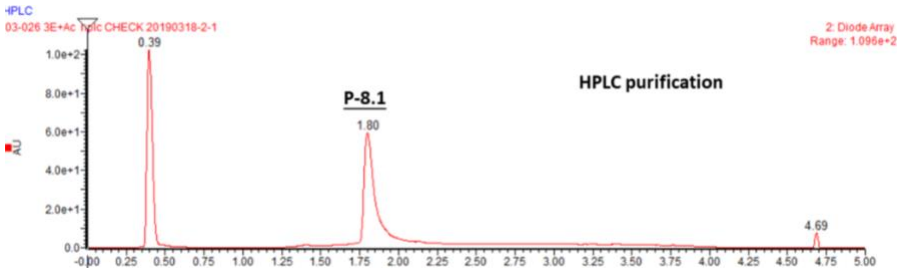


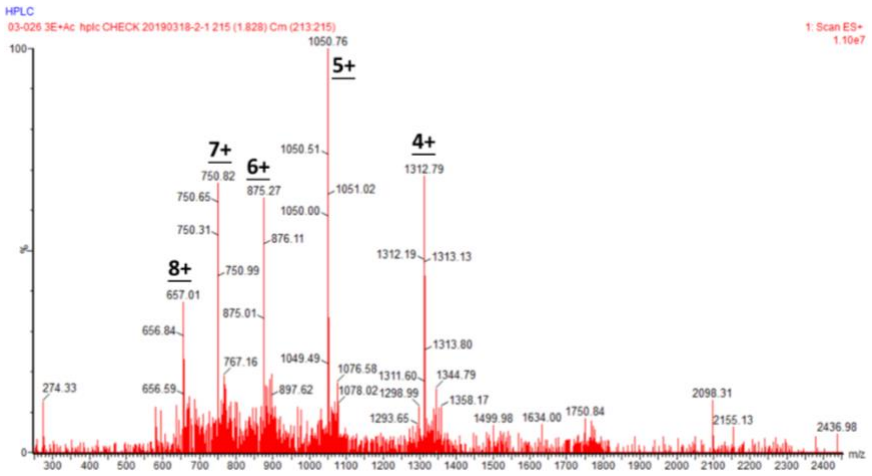
Synthesis of P-8.1



P-8.1

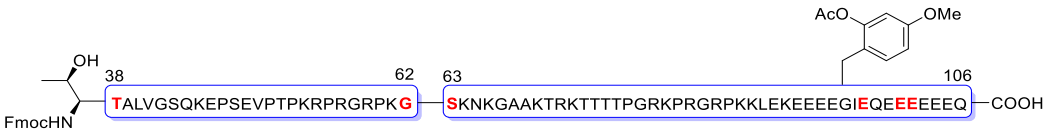
The preparation of peptide **P-8.1** was following **general procedure 1** via Fmoc-SPPS. All problems should be noticed has been described in the synthesis of peptide **P-1.1**. After HPLC purification (gradient: 5-40% CH_3CN/H_2O over 40 min) and lyophilization, 38.2 mg pure product was obtained from 0.3 g trityl chloride resin (8.1% yield based on resin loading).





P-8.1 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{220}H_{367}N_{67}O_{81}$ molecular weight: 5246.74. P_1 $[M+3H]^{3+}$ m/z = 1749.91; P_2 $[M+4H]^{4+}$ m/z = 1312.69; P_3 $[M+5H]^{5+}$ m/z = 1050.35; P_4 $[M+6H]^{6+}$ m/z = 875.46; P_5 $[M+7H]^{7+}$ m/z = 750.54; P_6 $[M+8H]^{8+}$ m/z = 656.84. Found: 1750.84; 1312.79; 1050.76; 875.27; 750.82; 657.01.

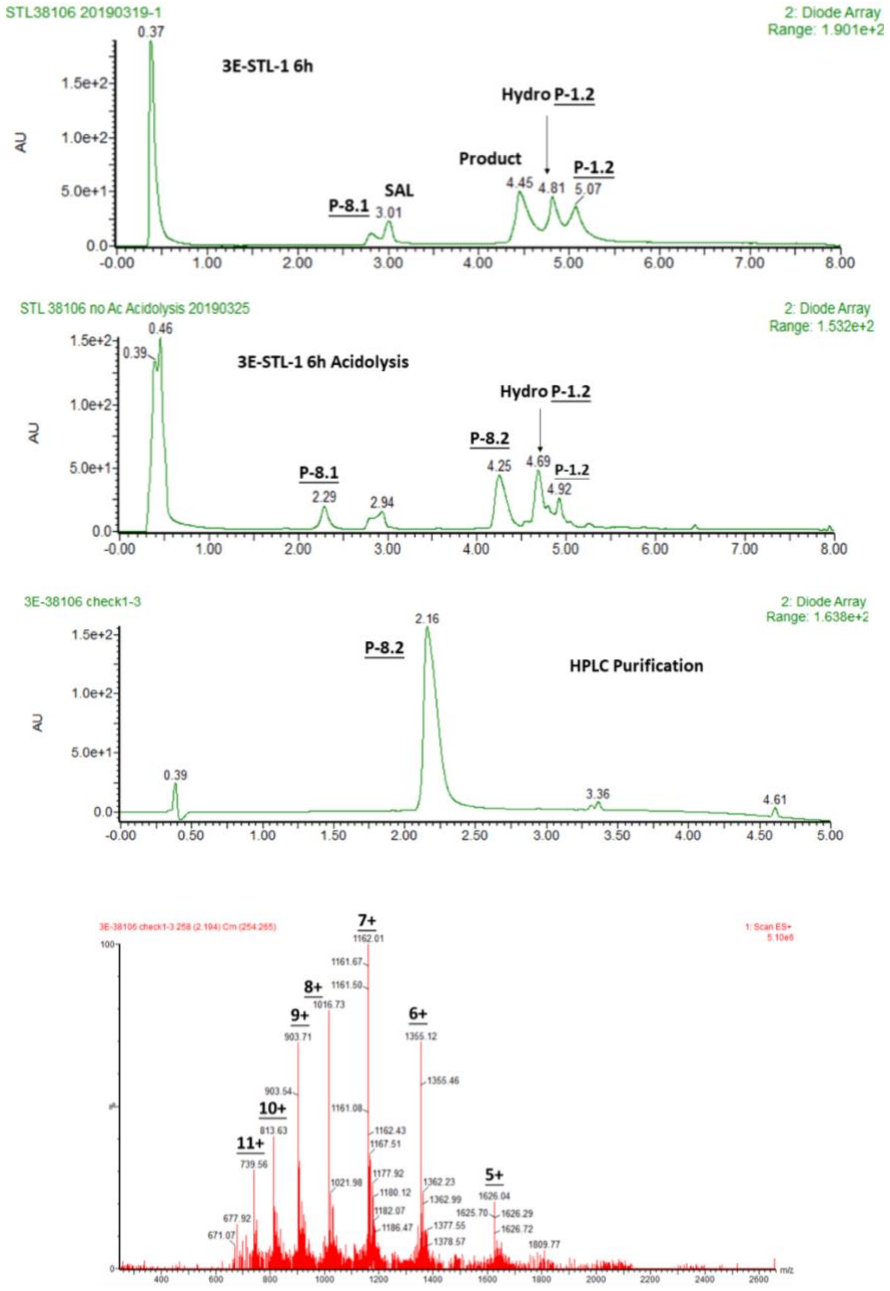
Synthesis of P-8.2



P-8.2

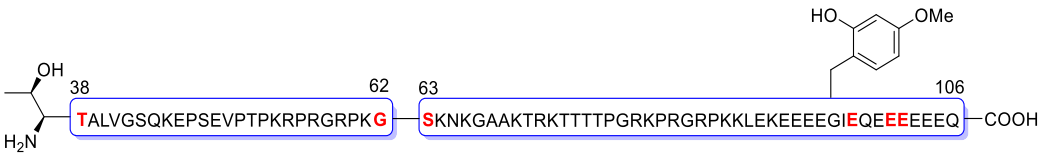
The preparation of peptide **P-8.2** was following **general procedure 4**.

20.2 mg (0.0038 mmol, 1 equiv.) of **P-8.1** and 18.3 mg (0.0058 mmol, 1.5 equiv.) of **P-1.2** were mixed together, which were further dissolved in 390 µl pyridine/AcOH (1/12, mol/mol) to make the concentration of **P-8.1** at 10 mM. The reaction was conducted at room temperature and stirred for 6 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 12.3 mg pure product (yield 36.9%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



P-8.2 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{350}H_{573}N_{105}O_{117}$ molecular weight: 8124.05. P_1 $[M+5H]^{5+}$ m/z = 16245.81; P_2 $[M+6H]^{6+}$ m/z = 1355.01; P_3 $[M+7H]^{7+}$ m/z = 1161.58; P_4 $[M+8H]^{8+}$ m/z = 1016.51; P_5 $[M+9H]^{9+}$ m/z = 903.67; P_5 $[M+10H]^{10+}$ m/z = 813.41; P_6 $[M+11H]^{11+}$ m/z = 739.55. Found: 1626.04; 1354.12; 1162.01; 1016.73; 903.71; 813.63; 739.56.

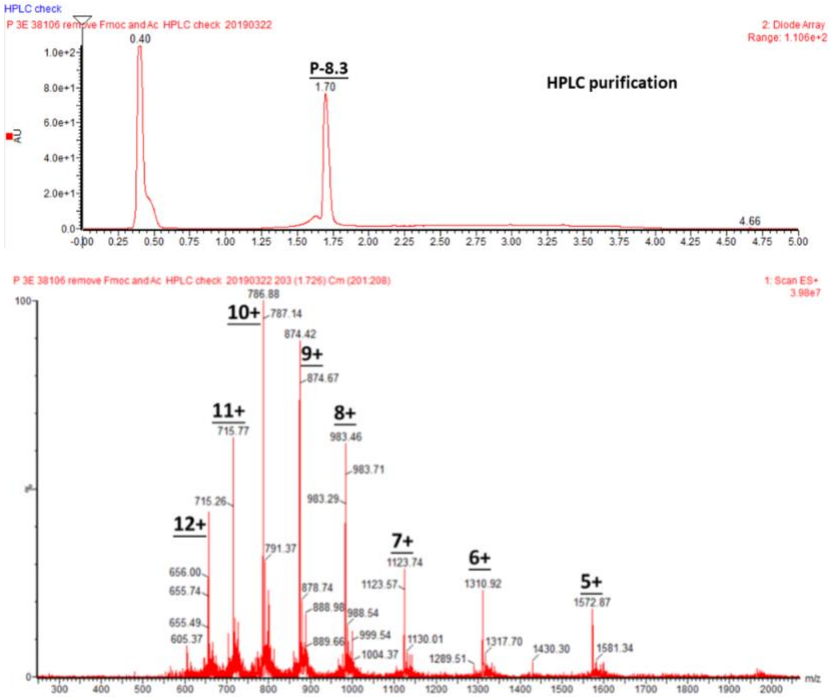
Synthesis of P-8.3



P-8.3

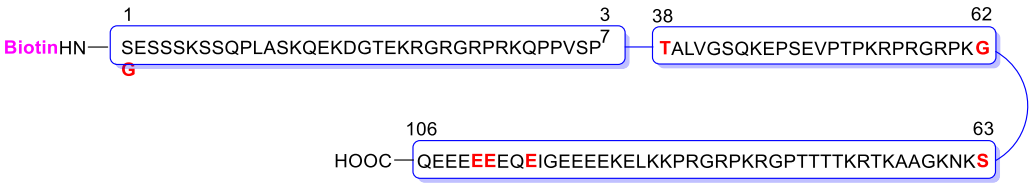
The preparation of peptide **P-8.3** was following **general procedure 5**.

To make the concentration of peptide around 5 mM, 11.2 mg (0.0014 mmol, 1 equiv.) of **P-8.2** was dissolved in 270 µl H_2O. 2.0 µl of Hydrazine monohydrate (0.042 mmol, 30 equiv.) was then added into the solution and stirred for 2 h at room temperature. 27 µl DEA in 243 µl of H_2O/CH_3CN (1/1, v/v) was poured into the solution and stirred for another 2 h at room temperature. The product was purified by HPLC (gradient 5-40% CH_3CN/H_2O over 40 min) and 8.6 mg of the pure product was obtained with 80.8% yield.



P-8.3 was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{333}H_{561}N_{105}O_{114}$ molecular weight: 7859.77. P_1 $[M+5H]^{5+}$ m/z = 1572.95; P_2 $[M+6H]^{6+}$ m/z = 1310.96; P_3 $[M+7H]^{7+}$ m/z = 1123.83; P_4 $[M+8H]^{8+}$ m/z = 983.47; P_5 $[M+9H]^{9+}$ m/z = 874.31; P_5 $[M+10H]^{10+}$ m/z = 786.98; P_6 $[M+11H]^{11+}$ m/z = 715.53; P_7 $[M+12H]^{12+}$ m/z = 655.98. Found: 1572.87; 1310.92; 1123.74; 983.46; 874.42; 786.88; 715.77; 656.17.

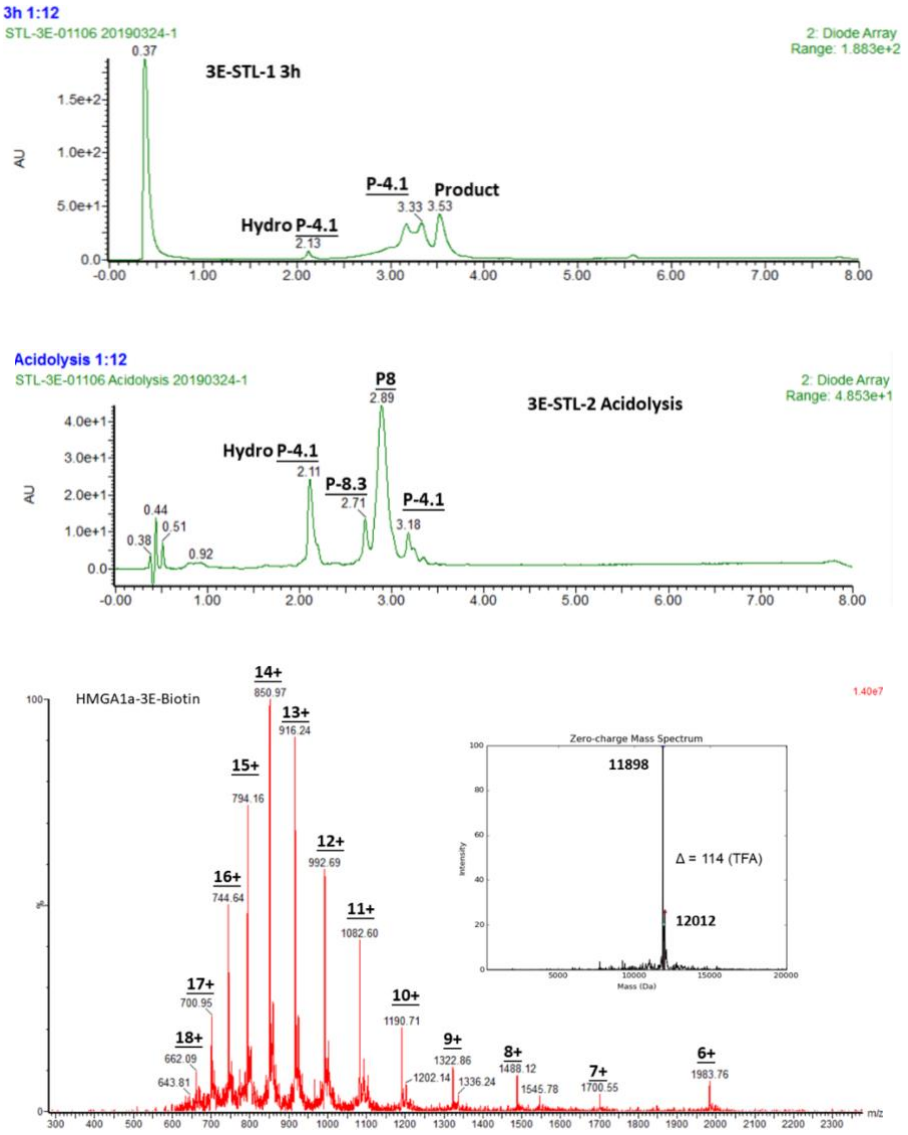
Synthesis of Biotin-HMGA1a-3E (P8)



Biotin-HMGA1a-3E (P8)

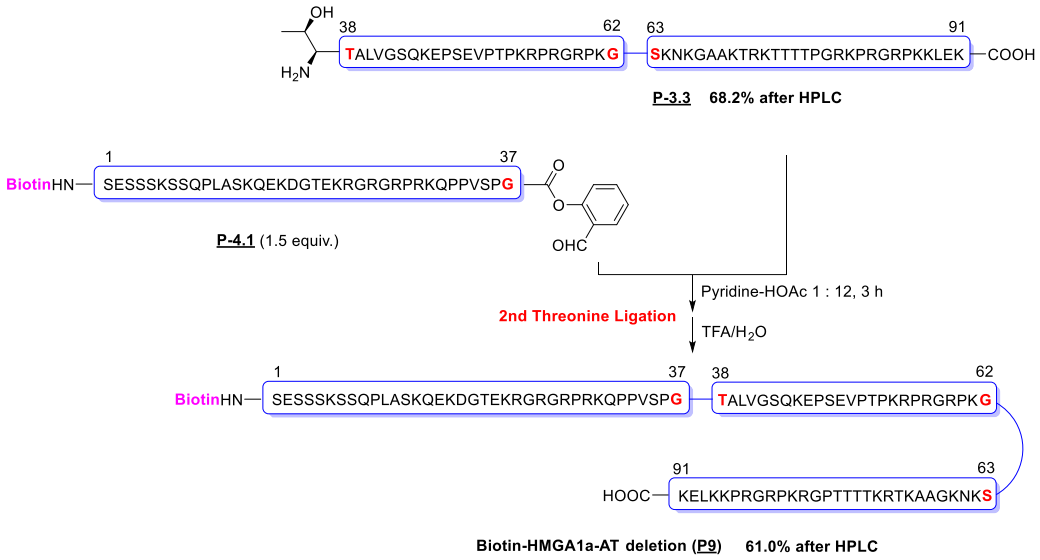
The preparation of protein **P8** was following **general procedure 6**.

8.6 mg (0.0011 mmol, 1 equiv.) of **P-8.3** and 7.1 mg (0.0017 mmol, 1.5 equiv.) of **P-4.1** were mixed together, which were further dissolved in 110 µl pyridine/AcOH (1/12, mol/mol). The reaction was conducted at room temperature and stirred for 3 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 4.9 mg pure product (yield 37.7%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



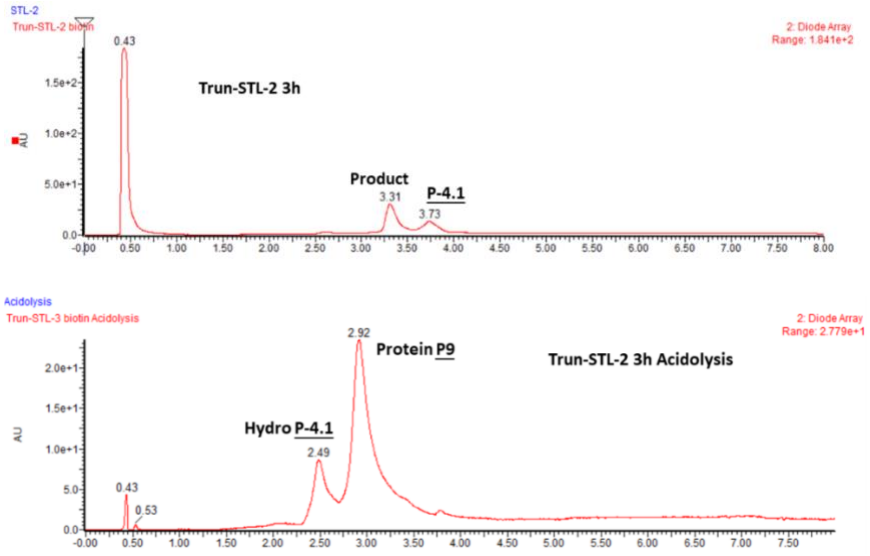
Biotin-HMGA1a-3E (P8) was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{498}H_{844}N_{164}O_{171}S$ molecular weight: 11897.27. P_1 $[M+6H]^{6+}$ m/z = 1983.88; P_2 $[M+7H]^{7+}$ m/z = 1700.61; P_3 $[M+8H]^{8+}$ m/z = 1488.16; P_4 $[M+9H]^{9+}$ m/z = 1322.92; P_5 $[M+10H]^{10+}$ m/z = 1190.73; P_6 $[M+11H]^{11+}$ m/z = 1082.57; P_7 $[M+12H]^{12+}$ m/z = 992.44; P_8 $[M+13H]^{13+}$ m/z = 916.17; P_9 $[M+14H]^{14+}$ m/z = 850.81; P_{10} $[M+15H]^{15+}$ m/z = 794.15; P_{11} $[M+16H]^{16+}$ m/z = 744.58; P_{12} $[M+17H]^{17+}$ m/z = 700.84. Found: 1983.76; 1700.55; 1488.12; 1322.86; 1190.71; 1082.60; 992.69; 916.24; 850.97; 794.16; 744.64; 700.95.

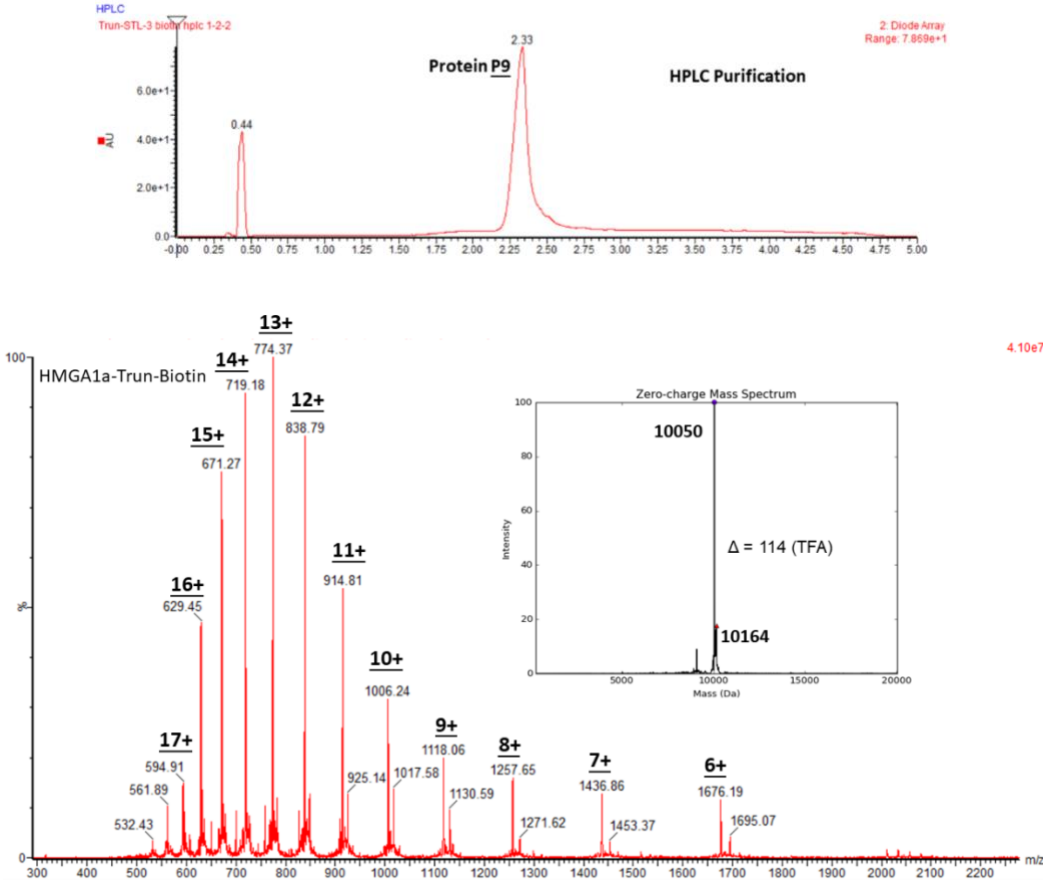
10. Preparation of Biotin-HMGA1a-AT deletion (P9)



The preparation of protein **P9** was following general procedure 6.

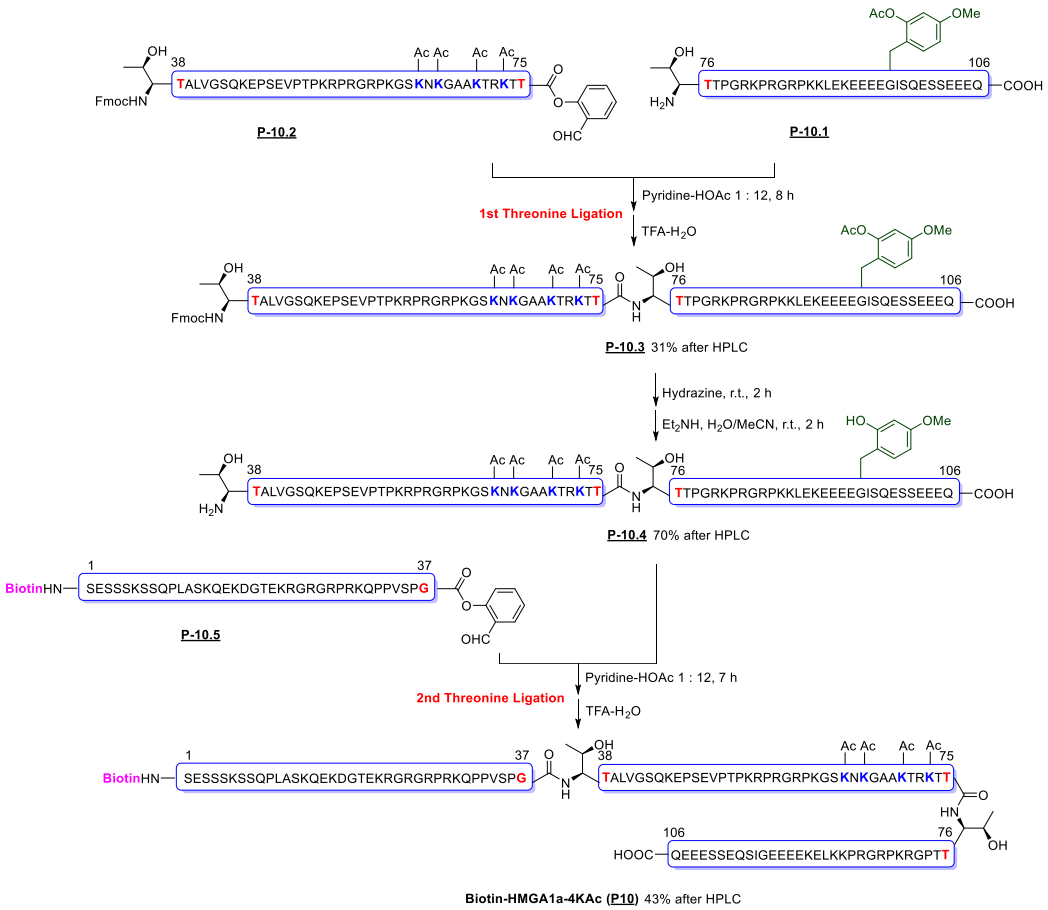
6.5 mg (0.0011 mmol, 1 equiv.) of **P-3.3** and 7.2 mg (0.0017 mmol, 1.5 equiv.) of **P-4.1** were mixed together, which were further dissolved in 110 µl pyridine/AcOH (1/12, mol/mol). The reaction was conducted at room temperature and stirred for 3 h. Then 40 mL cold Diethyl Ether was used to quench ligation and peptides were precipitated out as white solid. Peptides were further washed twice with cold Diethyl Ether to remove out AcOH and pyridine. After that, peptides were treated with TFA/H_2O/Tips (95/2.5/2.5, v/v/v) for acidolysis. 8.8 mg pure product (yield 61.0%) was obtained after HPLC purification (gradient 5-40% CH_3CN/H_2O over 40 min).



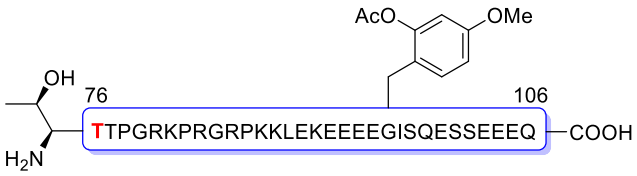


Biotin-HMGA1a-AT deletion (P9) was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{425}H_{738}N_{148}O_{131}S$ molecular weight: 10049.54. P_1 $[M+6H]^{6+}$ m/z = 1675.93; P_2 $[M+7H]^{7+}$ m/z = 1436.65; P_3 $[M+8H]^{8+}$ m/z = 1257.19; P_4 $[M+9H]^{9+}$ m/z = 1117.62; P_5 $[M+10H]^{10+}$ m/z = 1005.96; P_6 $[M+11H]^{11+}$ m/z = 914.59; P_7 $[M+12H]^{12+}$ m/z = 838.46; P_8 $[M+13H]^{13+}$ m/z = 774.04; P_9 $[M+14H]^{14+}$ m/z = 718.83; P_{10} $[M+15H]^{15+}$ m/z = 670.97; P_{11} $[M+16H]^{16+}$ m/z = 629.10; P_{12} $[M+17H]^{17+}$ m/z = 593.16. Found: 1676.19; 1436.86; 1257.65; 1118.06; 1006.24; 914.81; 838.79; 774.37; 719.18; 671.27; 629.45; 594.91.

11. Preparation of Biotin-HMGA1a-4AcLys (P10)

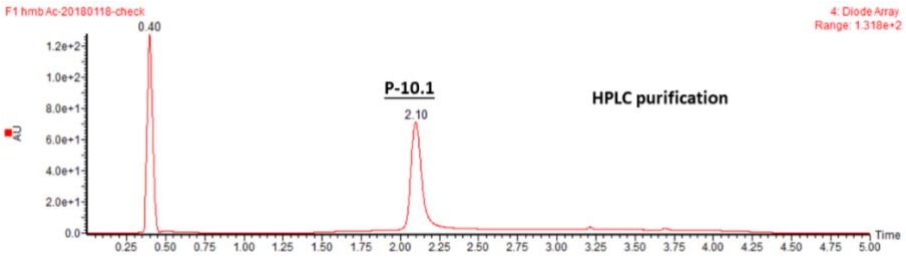


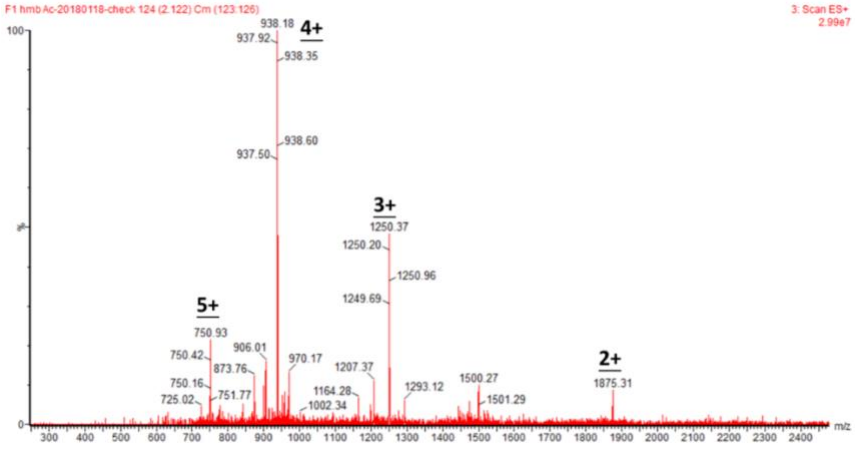
Synthesis of P-10.1



P-10.1

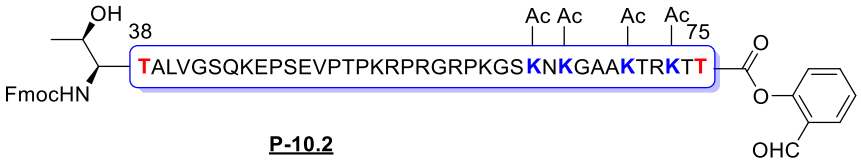
The preparation of peptide **P-10.1** was following **general procedure 1** via Fmoc-SPPS. All problems should be noticed has been described in the synthesis of peptide **P-1.1**. After HPLC purification (gradient: 5-40% CH_3CN/H_2O over 40 min) and lyophilization, 105.3 mg pure product can be obtained from 0.4 g trityl chloride resin (19.2% yield based on resin loading).





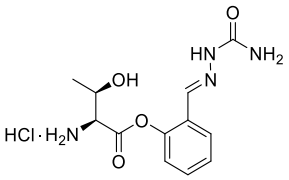
P-10.1was characterized under analytical condition (10-95% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 ml/min. ESI calcd for $C_{157}H_{256}N_{46}O_{60}$ $[M+2H]^{2+}$ m/z = 1875.02 $[M+3H]^{3+}$ m/z = 1250.35 $[M+4H]^{4+}$ m/z = 938.08 $[M+5H]^{5+}$ m/z = 750.6, found: 1875.31; 1250.37; 938.18; 750.93.

Preparation of P-10.2



P-10.2

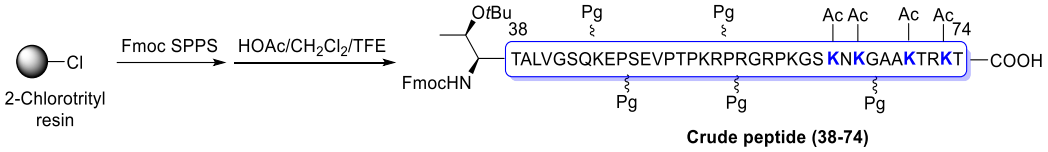
Synthesis of C-terminal '1' unit threonine salicylaldehyde semicarbazone ester hydrochloride



The target compound was prepared following the procedure we reported before.[51] To a solution of BocHN-Thr(*t*Bu)-COOH (1.0 equiv.) in CH_2Cl_2 at room temperature, *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDCI, 3.0 equiv.) and 4-dimethylaminopyridine (DMAP, 0.1 equiv.) were added, followed by salicylaldehyde

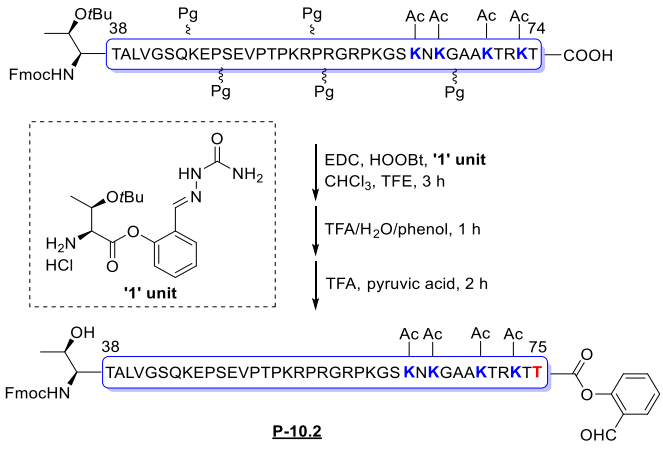
semicarbazone (1.0 equiv.). The reaction mixture was stirred overnight at room temperature and concentrated in vacuo. Purification by silica gel chromatography (CH_2Cl_2/EA, 1:1 *v*/*v*) gave the BocHN-Thr(*t*Bu)-CO-SAL^{off} as a white solid. This compound was treated with a solution of HCl in dioxane (4 M) and stirred for 2 h. The solvent was blown off by a stream of condensed air and the residue was triturated with diethyl ether and dried under vacuum to afford HCl·H_2N-Thr-CO-SAL^{off} as a white solid. This salt was subjected to the 'n+1' reaction without further purification.

Synthesis of the side-chain protected crude peptide (38-74)



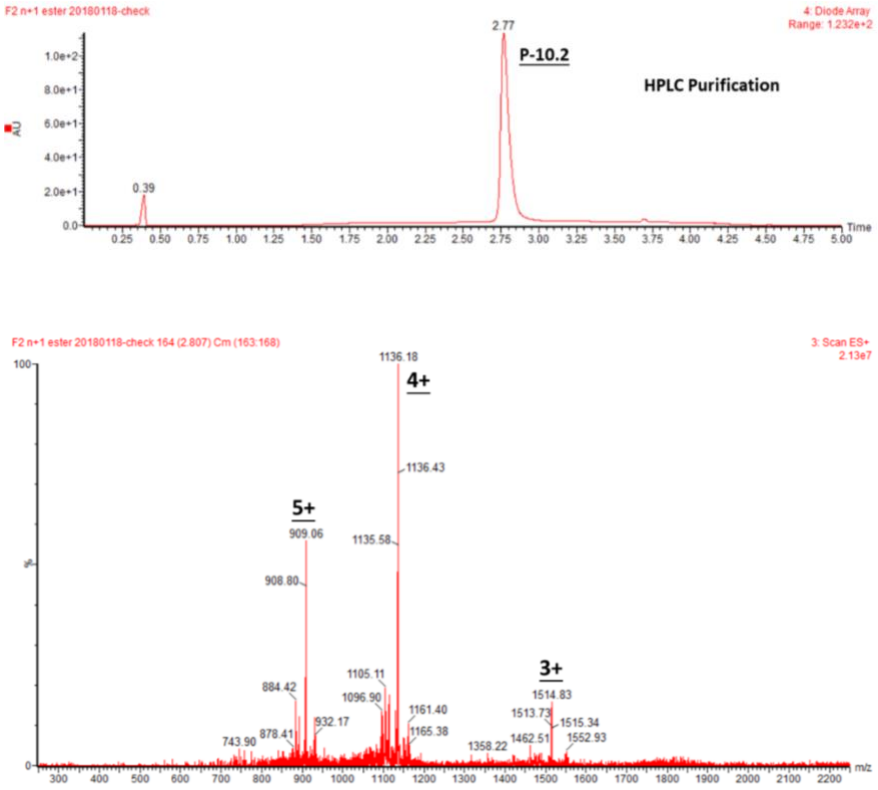
The peptide was assembled by the Fmoc-SPPS strategy from 2-chlorotrityl resin following the general procedure described above. The coupling of Fmoc-Lys(Ac)-OH was carried out under standard coupling protocols. Crude peptide product from Fmoc-SPPS with protected side chains was obtained by treating with a mild acidic cleavage cocktail CH_2Cl_2/TFE/AcOH (8/1/1, *v*/*v*/*v*) for 1 hour. After filtration, the resulting cleavage solution was combined and concentrated to give the residue containing crude peptide. The acetic acid and TFE in the residue were thoroughly removed by repeated co-evaporation with CH_2Cl_2/hexane (five times) under reduced pressure. The desired crude peptide was obtained as white solid, which was directly used for the C-terminal salicylaldehyde ester preparation.

Synthesis of <u>P-10.2</u>



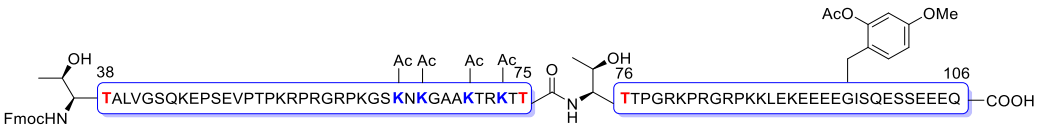
The side-chain protected crude peptide (300 mg, 1.0 equiv) obtained in the former step was dissolved in CHCl3/TFE (3.3 mL, 3/1, *v*/*v*, 15 mM,), then *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC) (22.8 mg, 3.0 equiv.) and 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine (HOOBt) (24.0 mg, 3.0 equiv.) were added. After 5 min, the

threonine salicylaldehyde semicarbazone ester hydrochloride (HCl·H2N-Thr-CO-SAL^{off}, 3.0 equiv.), obtained as described in the previous section, was added, and the reaction mixture was stirred for 3 hours to form the crude protected C-terminal peptide SAL^{off} ester. All protecting groups were removed by the global deprotection as mentioned above for 1 hour, followed by adding 100 equiv. of pyruvic acid to the TFA cocktail. After another 2 hours, the cocktail was blown off by compress air stream, and the residue was triturated with cold diethyl ether. Preparative HPLC purification (25-45% CH_3CN/H_2O over 35 min) followed by lyophilization gave the desired product **P-10.2**. (33.2 mg, 14% yield).



P-10.2 was characterized under analytical condition (10-95% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 ml/min. ESI calcd for $C_{202}H_{325}N_{59}O_{60}$ $[M+3H]^{3+}$ m/z = 1514.40 $[M+4H]^{4+}$ m/z = 1136.05 $[M+5H]^{5+}$ m/z =909.04 found: 1514.83; 1136.18; 909.06.

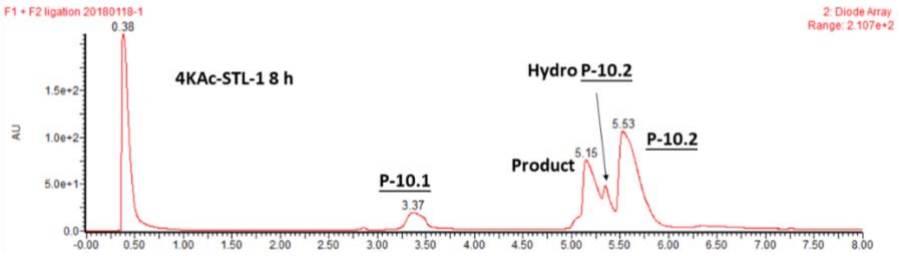
Synthesis of P-10.3

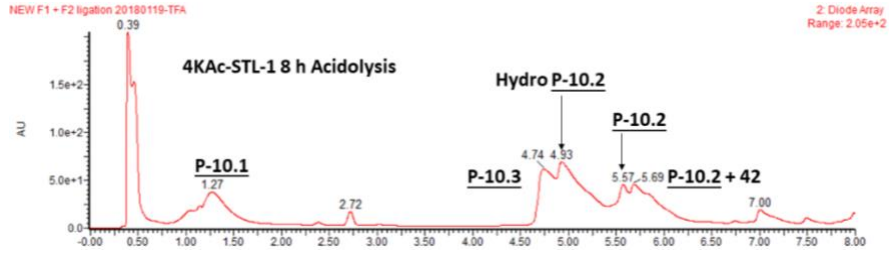


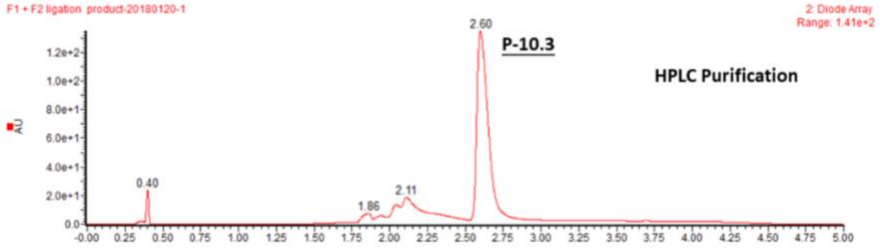
P-10.3

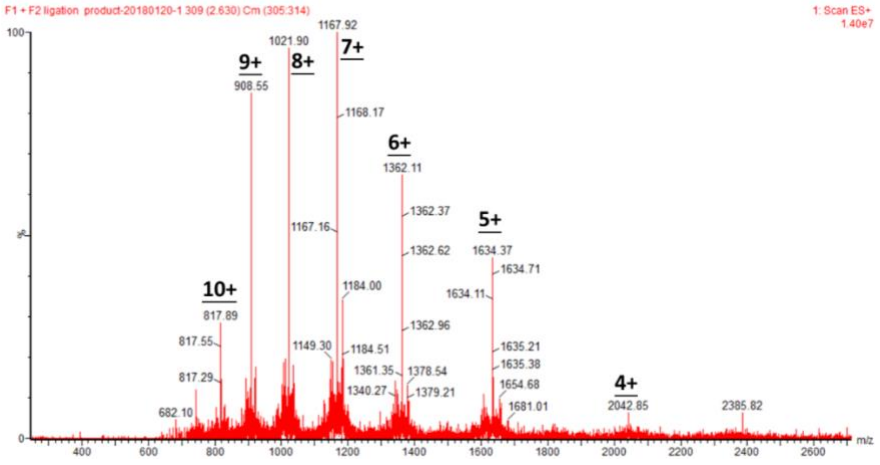
P-10.1 (20.0 mg, 1.0 equiv.) and 32.3 mg **P-10.2** (32.3 mg, 1.0 equiv.) were incubated in pyridine/acetic acid buffer (534 µL, 1/12 mole/mole) at a concentration of 10 mM at room temperature. The reaction was monitored on a C18

analytical column with a gradient of 20-50% CH_3CN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. After the completion of the reaction within 8 h, the solvent was blown off by a compressed air stream. The residue was then treated with TFA/H_2O/TIPS cocktail (95/2.5/2.5, *v*/*v*/*v*) to hydrolyze the *N*-peptidyl *N*,*O*-benzylidene acetal intermediate. Preparative HPLC purification (20-50% CH_3CN/H_2O containing 0.1% TFA over 30 min) followed by lyophilization afforded the product **P-10.3** (13.5 mg, 31% yield) as a white powder.





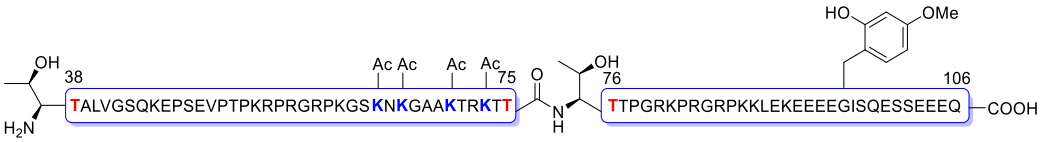




P-10.3 was characterized under analytical condition (10-95% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 ml/min.

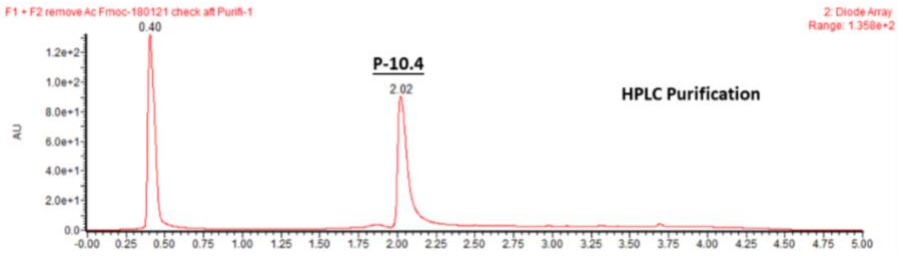
ESI calcd for $C_{353}H_{575}N_{105}O_{118}$ $[M+4H]^{4+}$ m/z = 2042.53 $[M+5H]^{5+}$ m/z = 1634.22 $[M+6H]^{6+}$ m/z = 1362.01 $[M+7H]^{7+}$ m/z = 1167.59 $[M+8H]^{8+}$ m/z = 1021.76 $[M+9H]^{9+}$ m/z = 908.34 $[M+10H]^{10+}$ m/z = 817.61 found: 2042.85; 1634.37; 1362.11; 1167.92; 1021.90; 908.55; 817.89.

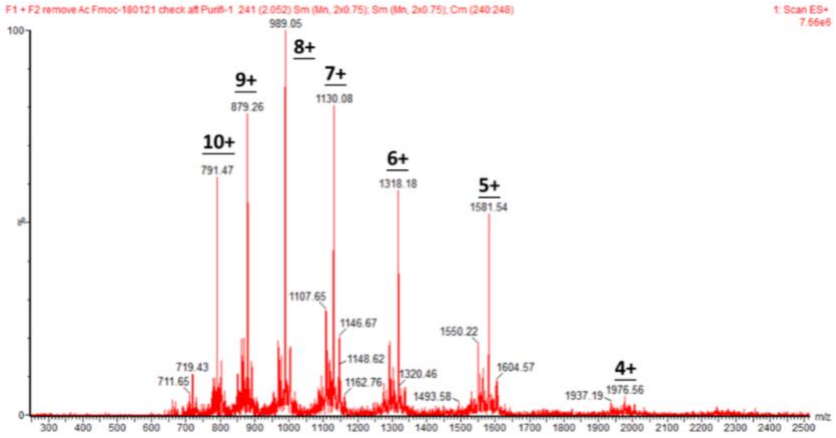
Synthesis of <u>P-10.4</u>



<u>P-10.4</u>

Peptide **<u>P-10.3</u>** (13.5 mg) was treated with N_2H_4 monohydrate (10 equiv.) in H_2O at a concentration of 5 mM. The reaction was monitored on a C18 analytical column with a gradient of 10-95% CH_3CN/H_2O containing 0.1% TFA over 5 min at a flow rate of 0.4 mL/min. After the full conversion of the material within 2 hours, 10% Et_2NH solution in CH_3CN/H_2O (1/1, *v*/*v*) was added to this mixture, rendering the peptide at a concentration of 2.5 mM. After incubation for another 2 hours at room temperature, the reaction was quenched with 1% aqueous TFA. Preparative HPLC purification (10–50% CH_3CN/H_2O containing 0.1% TFA over 30 min) followed by lyophilization afforded peptide **<u>P-10.4</u>** (9.0 mg, 70% yield) as a white powder.

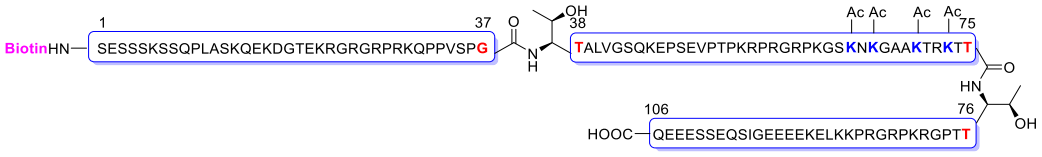




Peptide <u>P-10.4</u> was characterized under analytical condition (10-95% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 ml/min. ESI calcd for $C_{335}H_{563}N_{105}O_{115}$ $[M+4H]^{4+}$ m/z = 1976.45 $[M+5H]^{5+}$ m/z = 1581.36 $[M+6H]^{6+}$ m/z = 1317.97

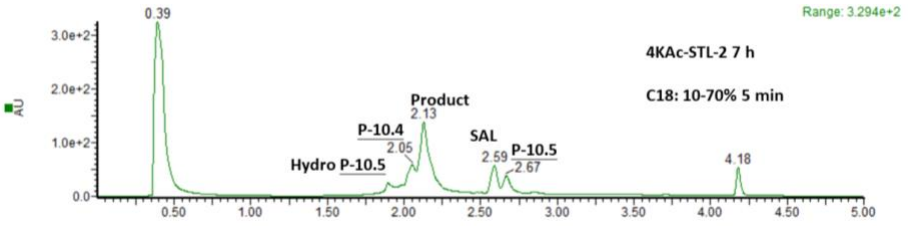
[M+7H]$^{7+}$ m/z = 1129.83 [M+8H]$^{8+}$ m/z = 988.72 [M+9H]$^{9+}$ m/z = 878.98 [M+10H]$^{10+}$ m/z = 791.18 found: 1976.56; 1581.54; 1318.18; 1130.08; 989.05; 879.26; 791.47.

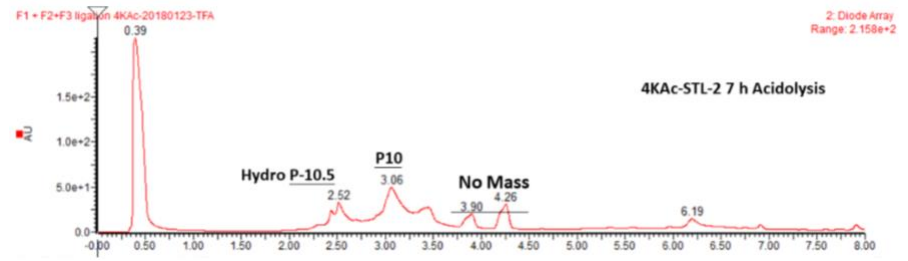
Synthesis of Biotin-HMGA1a-4AcLys (P10)

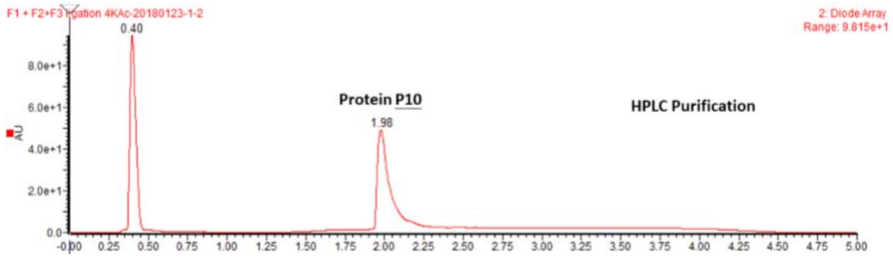


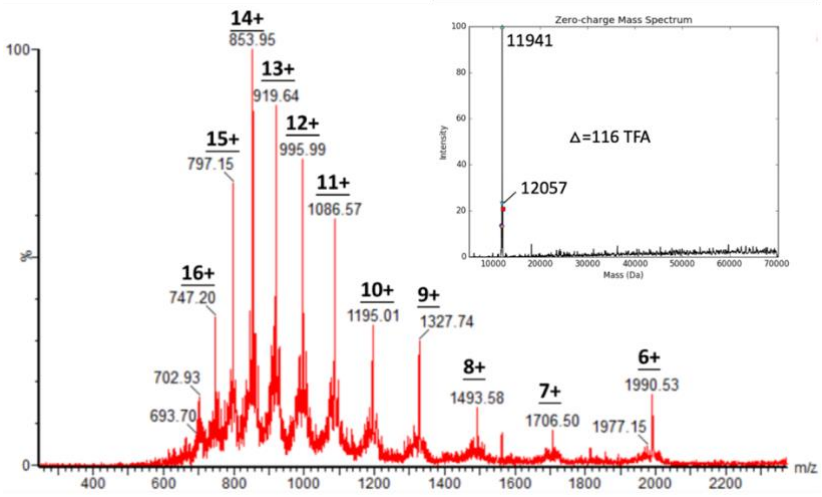
Biotin-HMGA1a-4KAc (P10)

Peptide **P-4.1** (10.0 mg, 2.0 equiv.) and peptide **P-10.4** (8.7 mg, 1.0 equiv.) were incubated in pyridine/acetic acid buffer (110 µL, 1/12 mole/mole) at a concentration of 10 mM at room temperature. The reaction was monitored on a C18 analytical column with a gradient of 10-90% CH_3CN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. After the completion of the reaction within 7 hours, the solvent was blown off by a compressed air stream. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, *v*/*v*/*v*) to hydrolyze the *N*-peptidyl *N*,*O*-benzylidene acetal intermediate. The reaction time was prolonged to 1 hour to allow complete deprotection of Hmb which rendered native Gly96 concomitantly, as analyzed on a C18 analytical column with a gradient of 10-90% CH_3CN/H_2O containing 0.1% TFA over 8 min at a flow rate of 0.4 mL/min. Preparative HPLC purification (10-40% CH_3CN/H_2O over 30 min) followed by lyophilization afforded the desired full-length HMGA1a-4Ac protein (**P10**, 5.6 mg, 43% yield) as a white powder.





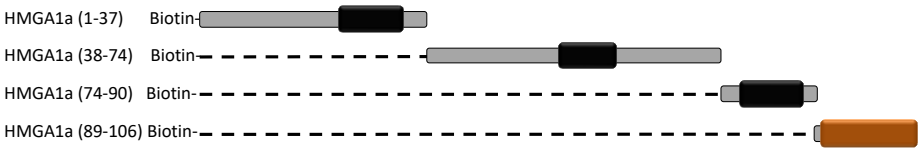




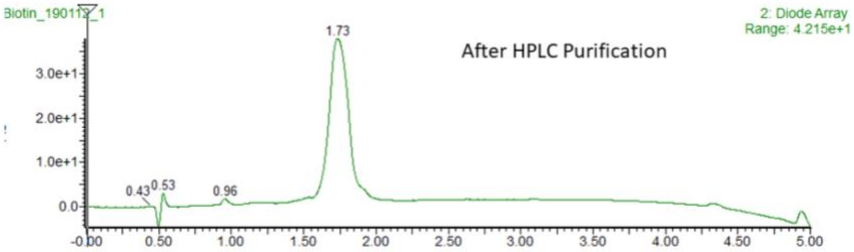
Biotin-HMGA1a-4AcLys (P10) was characterized under analytical condition (10-95% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 ml/min. ESI calcd for $C_{500}H_{846}N_{164}O_{172}S$ molecular weight: 11939.09. $[M+6H]^{6+}$ m/z = 1990.88 $[M+7H]^{7+}$ m/z = 1706.61 $[M+8H]^{8+}$ m/z = 1493.41 $[M+9H]^{9+}$ m/z = 1327.59 $[M+10H]^{10+}$ m/z = 1194.93 $[M+11H]^{11+}$ m/z = 1086.39 $[M+12H]^{12+}$ m/z = 995.94 $[M+13H]^{13+}$ m/z = 919.41 $[M+14H]^{14+}$ m/z = 853.81 $[M+15H]^{15+}$ m/z = 796.95 $[M+16H]^{16+}$ m/z = 747.21 found: 1990.53; 1706.50; 1493.58; 1327.74; 11945.01; 1086.57; 995.99; 919.64; 853.95; 797.15; 747.20.

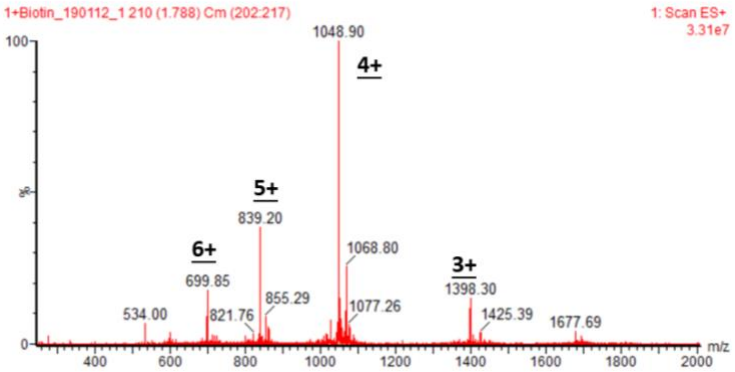
12. Synthesis of HMGA1a peptide probes

Synthesis of Biotin-HMGA1a Peptide Fragments



Preparation of HMGA1a (1-37)

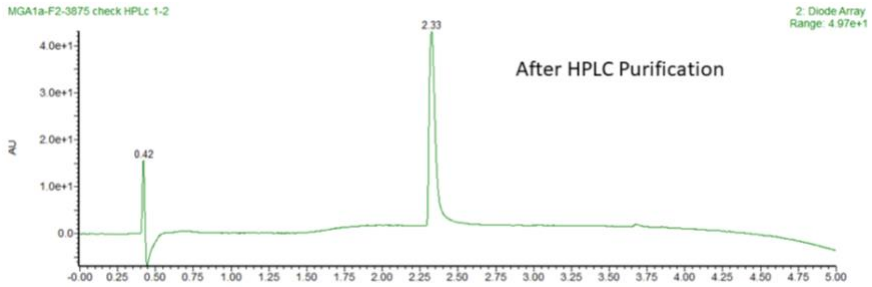


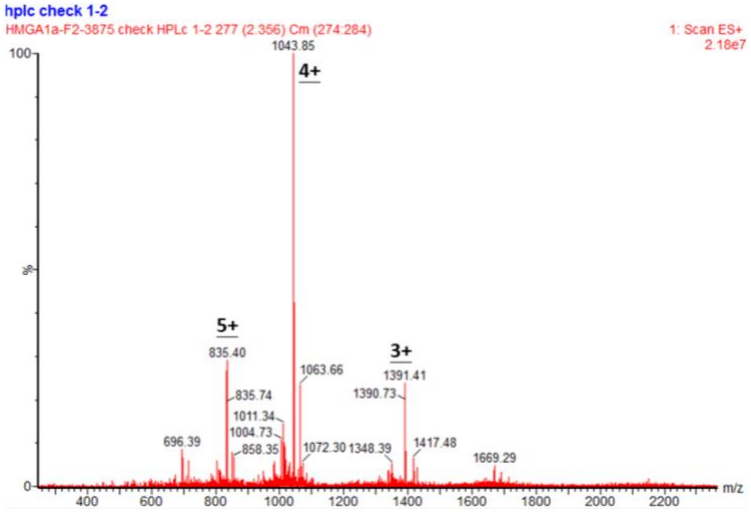


After HPLC and lyophilization, 25.3 mg pure product was obtained from 100 mg 2-Chlorotrityl chloride resin with 12.1% yield. **HMGA1a (1-37)** was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{173}H_{293}N_{59}O_{60}S$ molecular weight: 4191.66. P_1 $[M+3H]^{3+}$ m/z = 1398.22; P_2 $[M+4H]^{4+}$ m/z = 1048.92; P_3 $[M+5H]^{5+}$ m/z = 839.33; P_4 $[M+6H]^{6+}$ m/z = 699.61. Found: 1398.30; 1048.90; 839.20;

699.85.

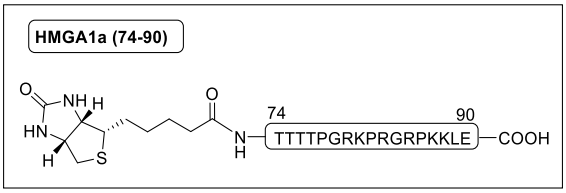
Preparation of HMGA1a (38-74)

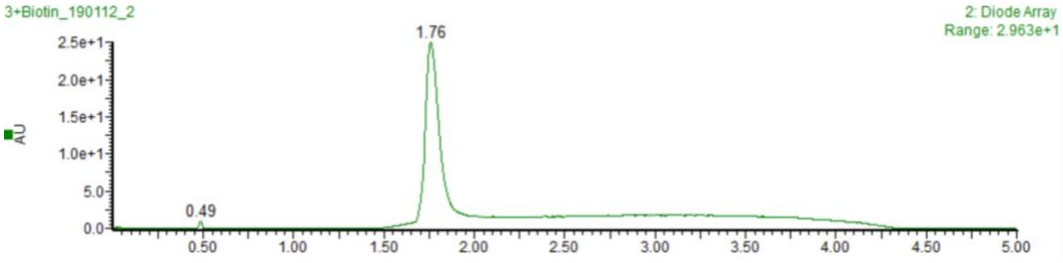


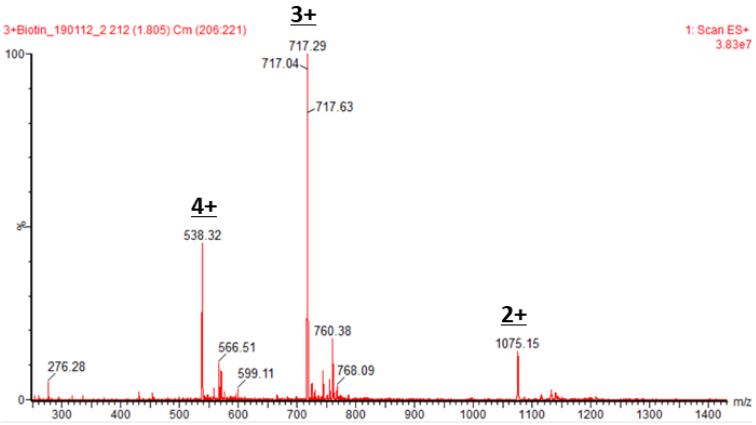


After HPLC and lyophilization, 33.8 mg pure product was obtained from 100 mg 2-Chlorotrityl chloride resin with 16.1% yield. **HMGA1a (38-74)** was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{178}H_{310}N_{60}O_{53}S$ molecular weight: 4170.88. P_1 $[M+3H]^{3+}$ m/z = 1391.29; P_2 $[M+4H]^{4+}$ m/z = 1043.72; P_3 $[M+5H]^{5+}$ m/z = 835.18; P_4 $[M+6H]^{6+}$ m/z = 696.15; P_5 $[M+7H]^{7+}$ m/z = 596.84. Found: 1391.1; 1043.7; 835.2; 696.2; 597.1.

Preparation of HMGA1a (74-90)



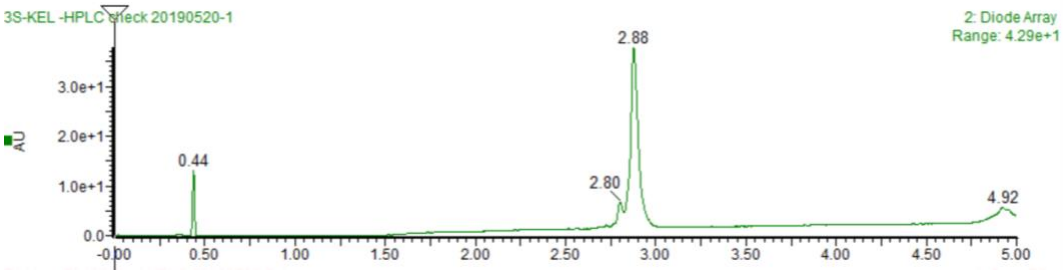


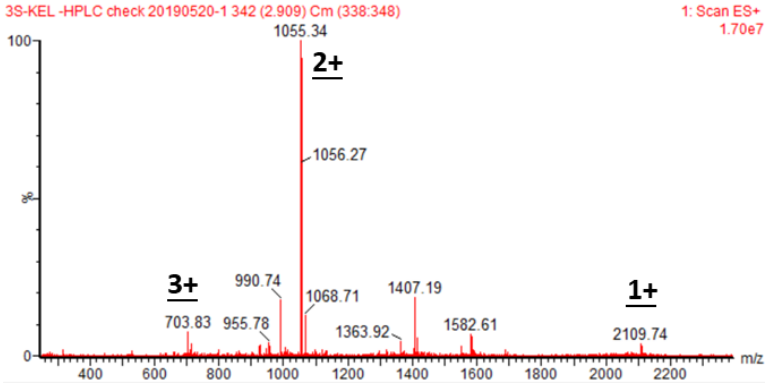


After HPLC and lyophilization, 18.3 mg pure product was obtained from 200 mg 2-Chlorotrityl chloride resin with 8.4% yield. **HMGA1a (74-90)** was characterized under analytical condition (10-90% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{92}H_{161}N_{31}O_{26}S$ molecular weight: 2148.55. P_1 $[M+2H]^{2+}$ m/z = 1075.28; P_2 $[M+3H]^{3+}$ m/z = 717.18; P_3 $[M+4H]^{4+}$ m/z = 538.14. Found: 1075.15; 717.29; 538.32.

Preparation of acidic tail peptide fragments (AT, HMGA1a (89-106)).

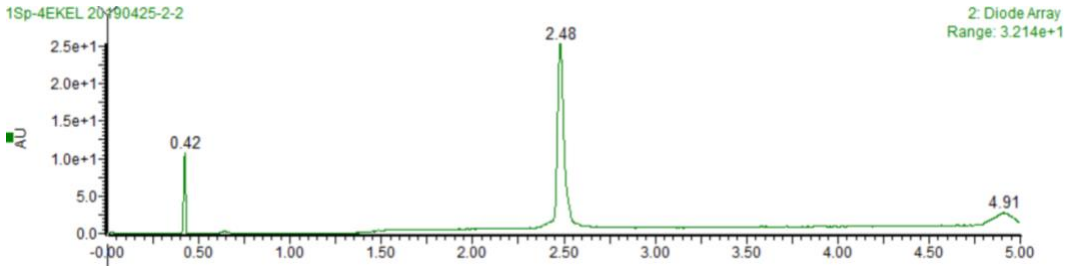
Preparation of AT

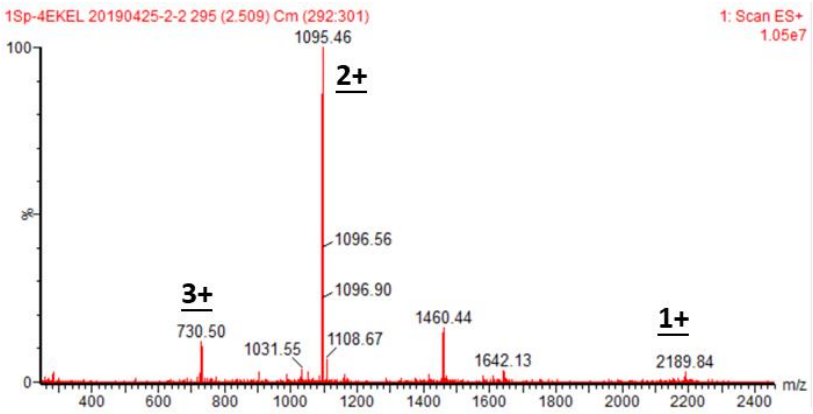




AT was characterized under analytical condition (0-30% CH_3CN/H_2O over 5 min) at a flow rate 0.4 mL/min. ESI calcd. for $C_{84}H_{133}N_{21}O_{42}$ molecular weight: 2109.09. P_1 $[M+1H]^{1+}$ m/z = 2110.09; P_2 $[M+2H]^{2+}$ m/z = 1055.55; P_3 $[M+3H]^{3+}$ m/z = 704.03. Found: 2109.74; 1055.34; 703.83.

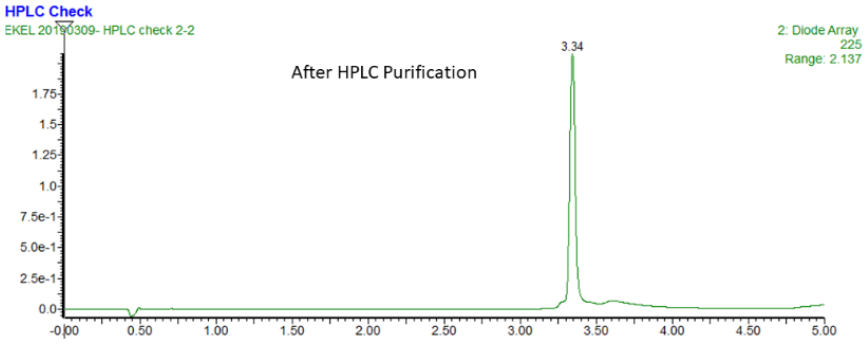
Preparation of AT-pSer102

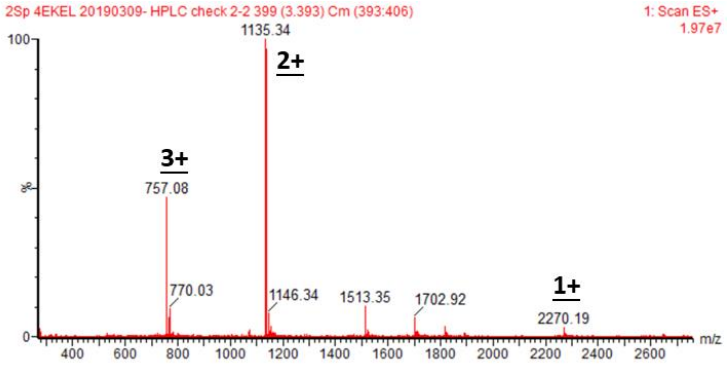




After HPLC and lyophilization, 13.2 mg pure product was obtained from 100 mg 2-Chlorotrityl chloride resin with 24.2% yield. **AT-pSer102** was characterized under analytical condition (2-30% CH_3CN/H_2O over 5 min) at a flow rate 0.4 mL/min. ESI calcd. for $C_{84}H_{134}N_{21}O_{45}P$ molecular weight: 2189.07. P_1 $[M+1H]^{1+}$ m/z = 2190.07; P_2 $[M+2H]^{2+}$ m/z = 1095.54; P_3 $[M+3H]^{3+}$ m/z = 730.69. Found: 2189.84; 1095.46; 730.50.

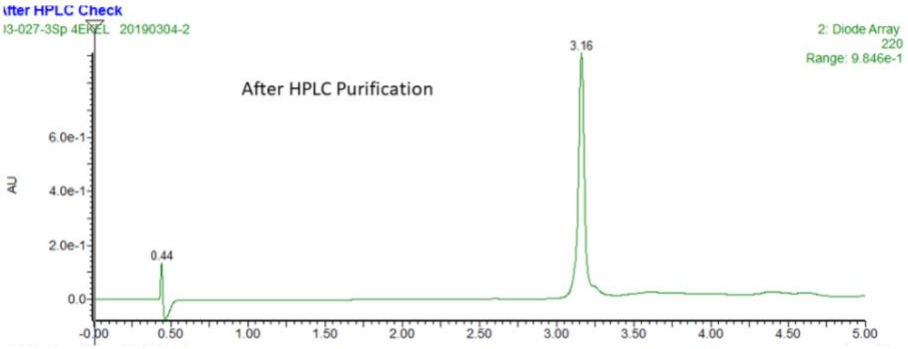
Preparation of AT-2pSer (pSer101, pSer102)

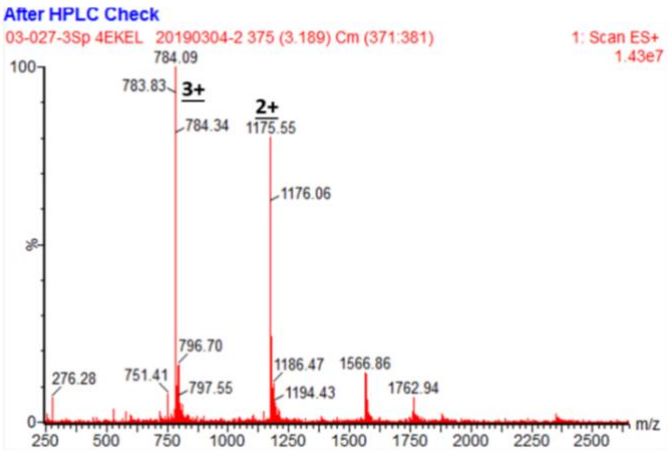




After HPLC and lyophilization, 16.7 mg pure product was obtained from 100 mg 2-Chlorotrityl chloride resin with 29.3% yield. **AT-2pSer** was characterized under analytical condition (2-15% CH_3CN/H_2O over 5 min) at a flow rate 0.4 mL/min. ESI calcd. for $C_{84}H_{135}N_{21}O_{48}P_2$ molecular weight: 2269.05. P_1 $[M+1H]^{1+}$ m/z = 2270.05; P_2 $[M+2H]^{2+}$ m/z = 1135.53; P_3 $[M+3H]^{3+}$ m/z = 757.35. Found: 2270.19; 1135.34; 757.08.

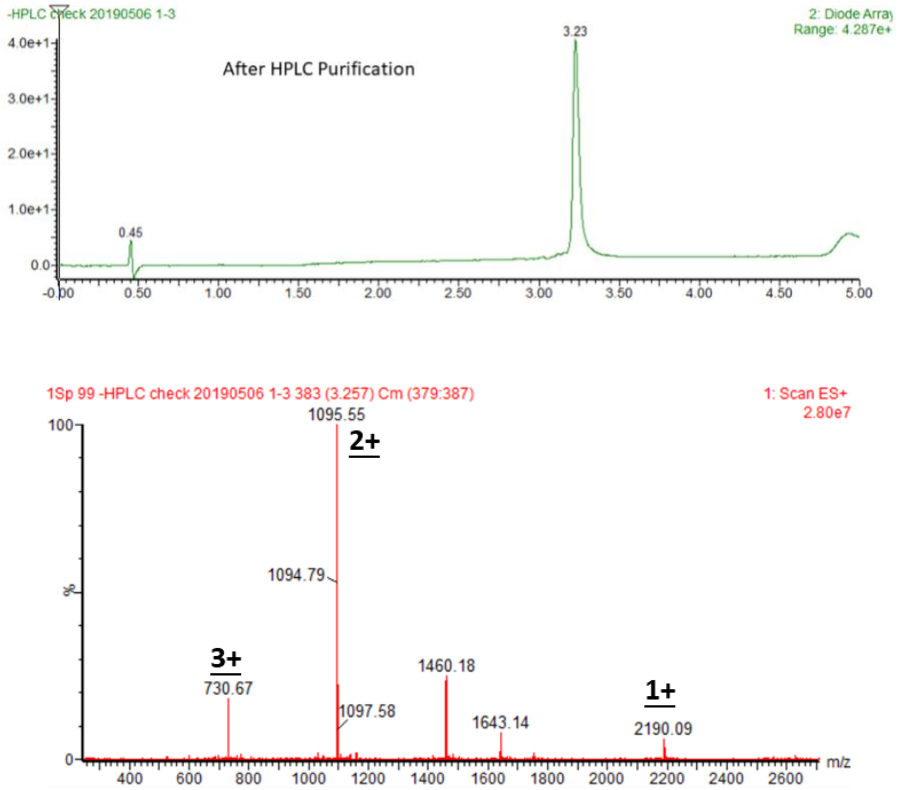
Preparation of AT-3pSer (pSer98, pSer101, pSer102)





After HPLC and lyophilization, 18.7 mg pure product was obtained from 100 mg 2-Chlorotrityl chloride resin with 31.5% yield. **AT-3pSer** was characterized under analytical condition (2-15% CH_3CN/H_2O over 5 min) at a flow rate 0.4 mL/min. ESI calcd. for $C_{84}H_{136}N_{21}O_{51}P_3$ molecular weight: 2349.03. P_1 $[M+2H]^{2+}$ m/z = 1175.52; P_2 $[M+3H]^{3+}$ m/z = 784.01. Found: 1175.55; 784.09.

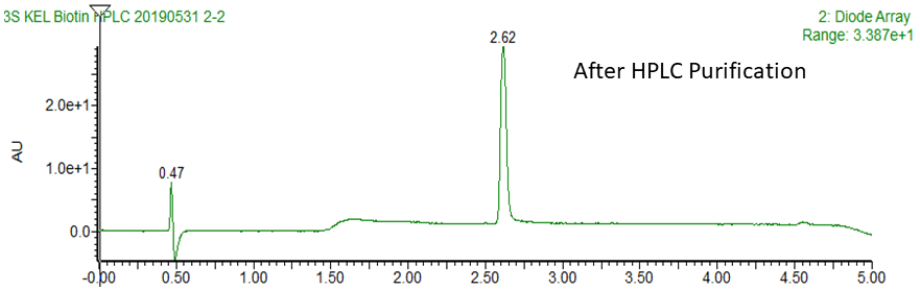
Preparation of AT-pSer98

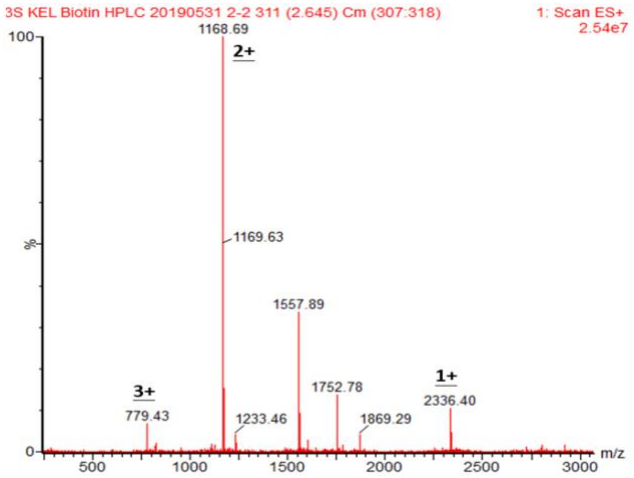


AT-pSer98 was characterized under analytical condition (0-20% CH_3CN/H_2O over 5 min) at a flow rate 0.4 mL/min. ESI calcd. for $C_{84}H_{134}N_{21}O_{45}P$ molecular weight: 2189.07. P_1 $[M+1H]^{1+}$ m/z = 2190.07; P_2 $[M+2H]^{2+}$ m/z = 1095.54; P_3 $[M+3H]^{3+}$ m/z = 730.69. Found: 2190.09; 1095.55; 730.67.

Synthesis of Biotinylated HMGA1a acidic tail probes

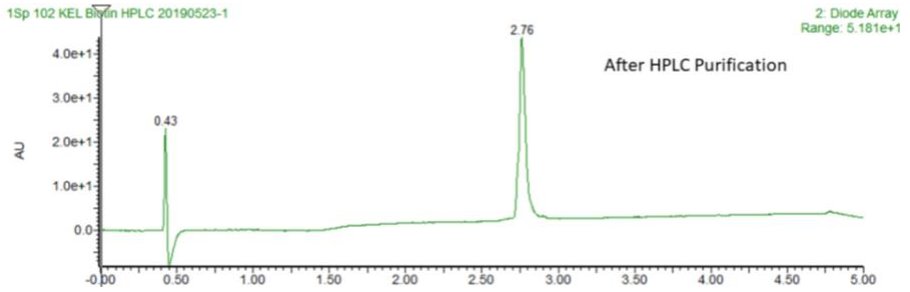
Preparation of Biotin-AT

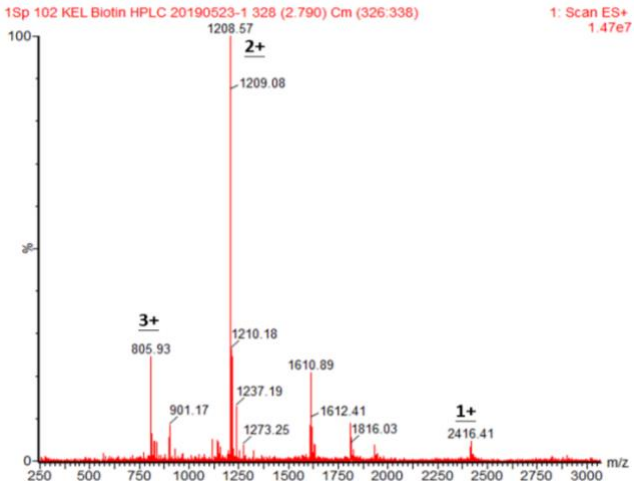




After HPLC and lyophilization, 24.8 mg pure product was obtained from 200 mg 2-Chlorotrityl chloride resin with 17.7% yield. **Biotin-AT** was characterized under analytical condition (0-50% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{94}H_{147}N_{23}O_{44}S$ molecular weight: 2335.39. P_1 $[M+1H]^{1+}$ m/z = 2336.39; P_2 $[M+2H]^{2+}$ m/z = 1168.70; P_3 $[M+3H]^{3+}$ m/z = 779.46. Found: 2336.40; 1168.69; 779.43.

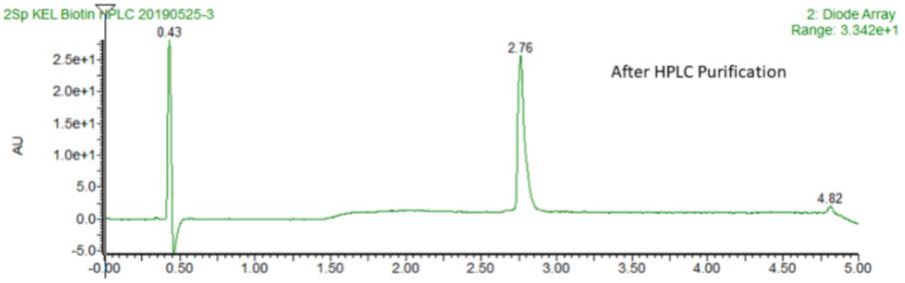
Preparation of Biotin-AT-pSer102

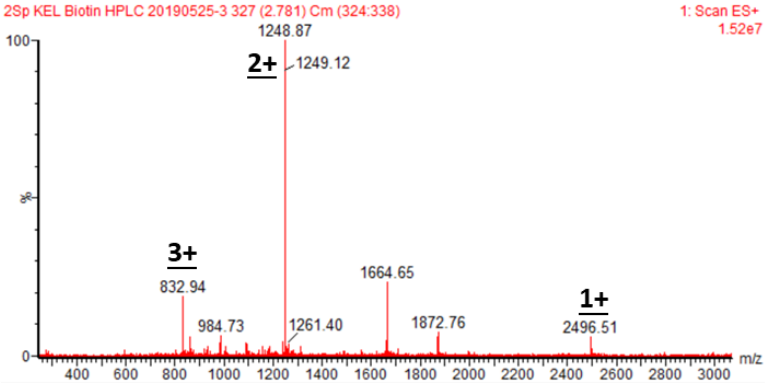




After HPLC and lyophilization, 23.4 mg pure product was obtained from 200 mg 2-Chlorotrityl chloride resin with 16.1% yield. **Biotin-AT-pSer102** was characterized under analytical condition (0-50% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{94}H_{148}N_{23}O_{47}PS$ molecular weight: 2415.37. P_1 $[M+1H]^{1+}$ m/z = 2416.37; P_2 $[M+2H]^{2+}$ m/z = 1208.69; P_3 $[M+3H]^{3+}$ m/z = 806.12. Found: 2416.41; 1208.57; 805.93.

Preparation of Biotin-AT-2pSer (pSer101, pSer102)

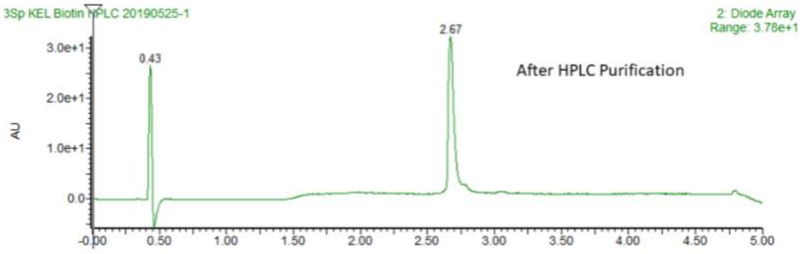


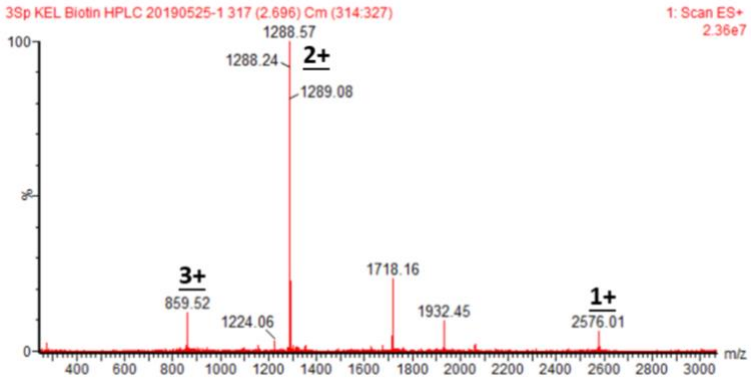


After HPLC and lyophilization, 33.6 mg pure product was obtained from 200 mg 2-Chlorotrityl chloride resin with 22.8% yield. **Biotin-AT-2pSer** was characterized under analytical condition (0-50% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{94}H_{149}N_{23}O_{50}P_2S$ molecular weight: 2495.34. P_1 $[M+1H]^{1+}$ m/z = 2496.34;

P_2 $[M+2H]^{2+}$ m/z = 1248.67; P_3 $[M+3H]^{3+}$ m/z = 832.78. Found: 2496.51; 1248.87; 832.94.

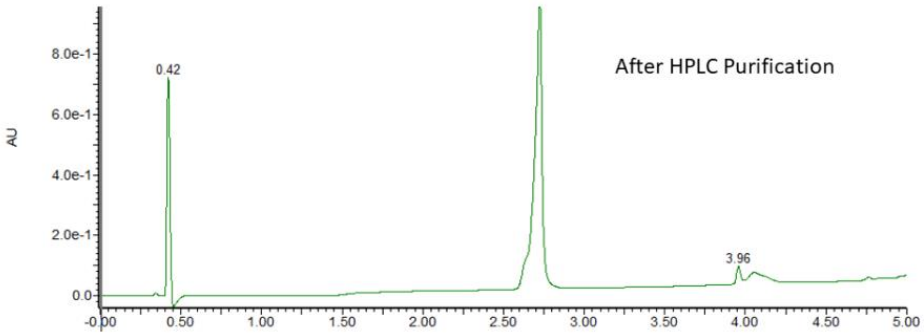
Preparation of Biotin-AT-3pSer (pSer98, pSer101, pSer102)

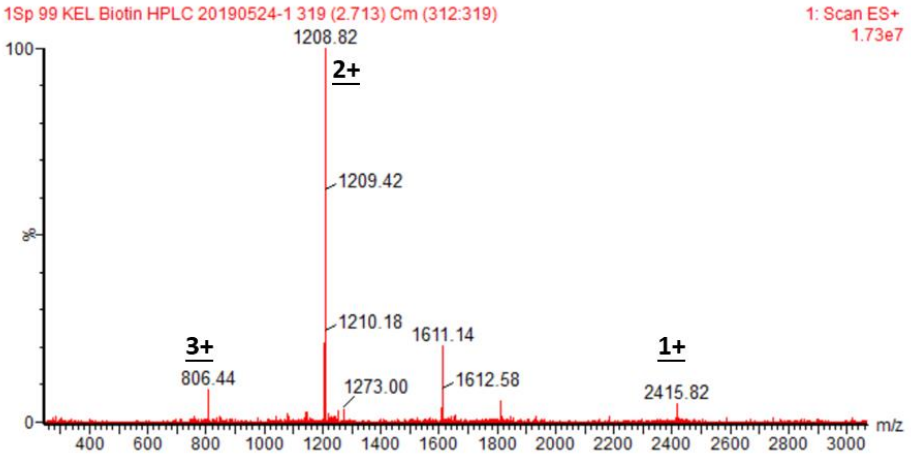




After HPLC and lyophilization, 19.7 mg pure product was obtained from 200 mg 2-Chlorotrityl chloride resin with 12.8% yield. **Biotin-AT-3pSer** was characterized under analytical condition (0-50% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{94}H_{150}N_{23}O_{53}P_3S$ molecular weight: 2575.32. P_1 $[M+1H]^{1+}$ m/z = 2576.32; P_2 $[M+2H]^{2+}$ m/z = 1288.66; P_3 $[M+3H]^{3+}$ m/z = 859.44. Found: 2576.01; 1288.57; 859.52.

Preparation of Biotin-AT-pSer98





After HPLC and lyophilization, 28.8 mg pure product was obtained from 200 mg 2-Chlorotrityl chloride resin with 19.9% yield. **Biotin-AT-pSer98** was characterized under analytical condition (0-50% CH_3CN/H_2O over 5 min) at a flow rate of 0.4 mL/min. ESI calcd. for $C_{94}H_{148}N_{23}O_{47}PS$ molecular weight: 2415.37. P_1 $[M+1H]^{1+}$ m/z = 2416.37; P_2 $[M+2H]^{2+}$ m/z = 1208.69; P_3 $[M+3H]^{3+}$ m/z = 806.12. Found: 2415.82; 1208.82; 806.44.