Chemical synthesis of HMGA1a proteins with post-translational modifications via Ser/Thr ligation

Tianlu Li, Heng Liu and Xuechen Li*

Department of Chemistry, The Key Laboratory of Synthetic Chemistry, The University of Hong Kong, Hong Kong

xuechenl@hku.hk

Contents

Materials and Methods

All commercial materials (Aldrich, Fluka and GL Biochem) were used without further purification. Amino acids, coupling reagents and resins were obtained from GL Biochem unless otherwise noted. Precast 4-12% Bis-Tris protein gels were purchased from Invitrogen part of Life Technologies. Anhydrous dichloromethane (CH_2Cl_2) was distilled from calcium hydride (CaH_2). All separations involved a mobile phase of 0.1% TFA (v/v) in acetonitrile (solvent A)/0.1% TFA (v/v) in water (Solvent B). HPLC separations were performed with a Waters HPLC system equipped with a photodiode array detector (Waters 2996) using a Vydac 218TP^{TM} C18 column (5 μm, 4.6 x 250 mm) at a flow rate of 0.6 mL/min for analytical HPLC, and Vydac 218TP^{TM} column (10 μm, 22 x 250 mm) at a flow rate of 10 mL/min for preparative HPLC. Low-resolution mass spectral analyses were performed with a Waters 3100 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DRX 300 FT-NMR Spectrometer at 300 Hz for ^{1}H NMR and 75.47 MHz for ^{13}C NMR, Bruker Avance DRX 400 FT-NMR spectrometer at 400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR and 162 MHz for ^{31}P.

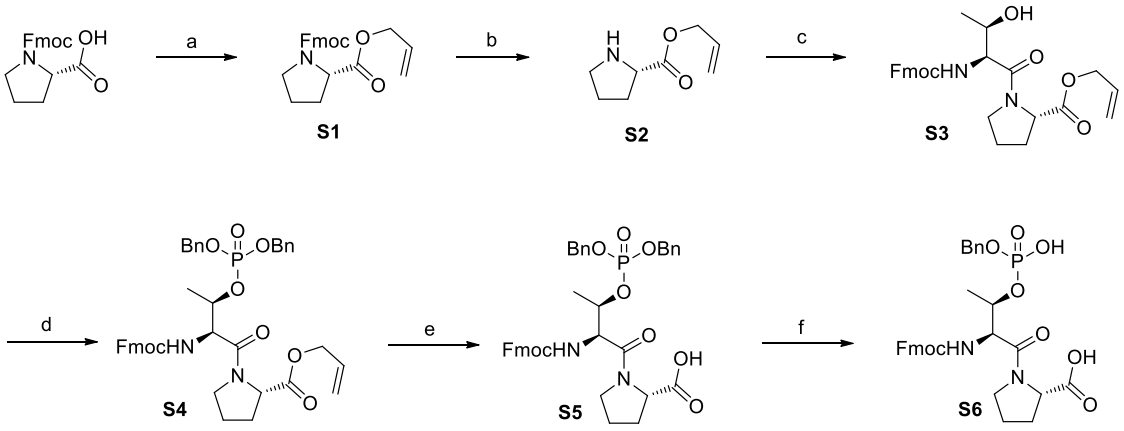
General Methods for Peptide Synthesis by Fmoc-SPPS

Synthesis was performed manually on 2-chlorotrityl chloride Resin (resin loading: 0.4 mmol/g). Peptides were synthesized under standard Fmoc/tBu protocols. The deblock solution was a mixture of 20/80 (v/v) of piperidine/DMF. The phosphate-containing dipeptide was introduced based on the known strategy.[1] Upon completion of the synthesis, global deprotection was performed by a cleavage cocktail of TFA/Phenol/H_2O (90/5/5, v/v/v). The resin was filtered and the combined filtrates were blown off under a stream of condensed air. The crude product was triturated with cold diethyl ether to give a white suspension, which was centrifuged and the ether subsequently decanted. The obtained solid was ready for HPLC purification.

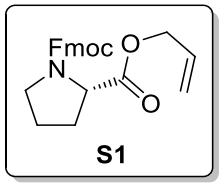
Synthesis of Fmoc-Thr(HPO_3Bn)-Pro-OH/ Fmoc-Ser(HPO_3Bn)-Pro-OH

The phosphate dipeptide was prepared based on known strategy.[2,3]

Synthesis of Fmoc-*p*TP-OH (Scheme S1)



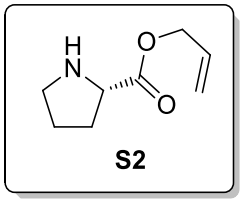
a) Allyl bromide, $KHCO_3$, DMF, 11h, 82%; b) Et_2NH/CH_2Cl_2, 2h, 81%; c) Fmoc-Thr-OH, HATU,DIEA, CH_2Cl_2, 12h, 83%; d) i)1H-tetrazole, i-$Pr_2NP(OBn)_2$, CH_2Cl_2, 12h, ii) mCPBA, -40ºC, 0.5h, 93% over two steps; e) $Pd(PPh_3)_4$, sodium tosylate, CH_2Cl_2, Ar protection, 3-5h, 79%; f) NaI, ACN, 12h, quant.



S1

Fmoc-Pro-OH (3.37 g, 10 mmol) was mixed in 5 mL anhydrous DMF, with allyl bromide (2.42 g, 1.7 mL, 20 mmol), $KHCO_3$ (2.00 g, 20 mmol). The reaction mixture was stirred at room temperature for 11 h. The reaction mixture was diluted with AcOEt (200 mL), washed with sat. NH_4Cl (100 mL x 3) and brine (100 mL x 3). The organic phase was dried over Na_2SO_4, concentrated under *vacuo* and purified by flash column chromatography on silica gel (hexane/AcOEt, 4:1) to give the titled compound **S1** (3.11 g, 82 %).[4]

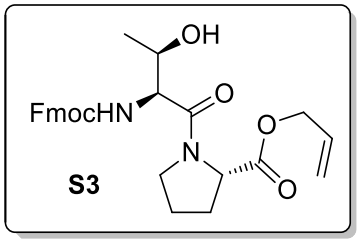
^{1}H NMR (400 MHz, $CDCl_3$, approx. 1:1 rotameric mixture) δ 7.75 and 7.74 (d, 2H, J = 7.4 Hz), 7.63-7.53 (m, 2H), 7.385 and 7.378 (t, 2H, J = 7.4 Hz), 7.30 and 7.29 (t, 2H, J = 7.4 Hz), 5.95-5.80 (m, 1H), 5.35-5.18 (m, 2H), 4.64 (d, 2H, J = 5.6 Hz), 4.55 (m, 2H), 4.47-4.41 (m, 1H), 4.37-4.24 (m, 2H), 4.16 (t, 1H, J = 6.8 Hz), 3.70-3.61 (m, 1H), 3.55-3.49 (m, 1H), 2.30-2.18 (m, 1H), 2.08-1.87 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) δ 172.31, 154.89, 154.46, 144.24, 144.19, 143.94, 143.79, 141.33, 131.89, 131.73, 127.71, 127.07, 125.24, 125.14, 119.98, 118.72, 118.43, 67.51, 65.71, 59.30, 58.87, 47.33, 47.25, 47.01, 46.51, 31.11, 29.96, 24.40, 23.40.



S2

Compound **S1** (2.97 g, 7.87 mmol) was dissolved in 16 mL of a mixture of CH_2Cl_2/Diethylamine (1/1, v/v) and stirred at room temperature for 2 h to remove the Fmoc group. The reaction mixture was concentrated under *vacuo* and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 20:1) to give compound **S2** (0.99 g, 81%).[5]

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.97-5.87 (m, 1H), 5.32 (dt, 1H, J = 1.5 Hz, J = 17.2 Hz), 5.24 (dt, 1H, J = 1.2 Hz, J = 10.4 Hz), 4.63-4.61 (m, 2H), 3.84-3.80 (m, 1H), 3.12-2.91 (m, 3H), 2.20-2.11 (m, 1H), 1.92-1.82 (m, 1H), 1.81-1.73 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.47, 131.65, 118.00, 65.09, 59.30, 46.57, 29.85, 25.06.

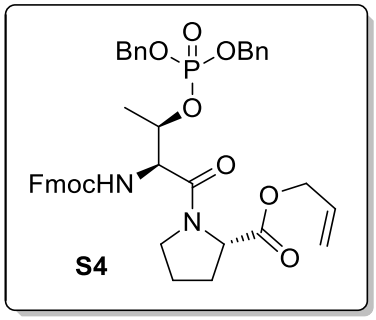


S3

Fmoc-Thr(tBu)-OH (1.95 g, 4.91 mmol), HATU (1.87 g, 4.91 mmol) and DIEA (1.52 g, 2.05 mL, 11.79 mmol) were added to the above compound **S2** in 3 mL CH_2Cl_2. Another 0.5 mL DMF was added for the sake of solubility. The reaction mixture was stirred at room temperature for 10 h; after the reaction was

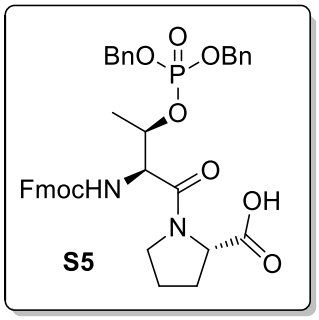
complete, it was diluted with AcOEt (200 mL), washed with 1N HCl (100 mL x 3) and brine (100 mL x 3). The organic phase was dried over Na_2SO_4, concentrated under *vacuo* and purified by flash column chromatography on silica gel (hexane/AcOEt, 2:1), which was further treated with 5 mL 95% TFA at room temperature for 30 min to remove the tBu group. The TFA was blown off under a stream of condensed air; the crude product was purified by flash column chromatography on silica gel (hexane/AcOEt, 1:1) to give compound **S3** (1.57 g, 83 % over two steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 2H, *J* = 7.5 Hz), 7.60 (d, 2H, *J* = 7.4 Hz), 7.40 (t, 2H, *J* = 7.5 Hz), 7.31 (t, 2H, *J* = 7.5 Hz), 5.96-5.85 (m, 1H), 5.74 (d, 1H, *J* = 8.8 Hz), 5.36-5.25 (m, 2H), 4.69-4.61 (m, 2H), 4.59-4.56 (m, 1H), 4.50-4.34 (m, 3H), 4.22 (t, 2H, *J* = 6.8 Hz), 3.83-3.71 (m, 2H), 3.39 (br, 1H), 2.33-2.22 (m, 1H), 2.09-1.98 (m, 3H), 1.24 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.74, 170.55, 156.75, 143.80, 141.32, 131. 63, 127.76, 127.12, 125.18, 120.03, 118.90, 67.32, 66.05, 59.05, 56.35, 47.29, 29.00, 24.96, 18.72. HRMS Calcd for $C_{27}H_{30}N_2O_6$ $[M+H]^+$ 479.2182, found 479.2135



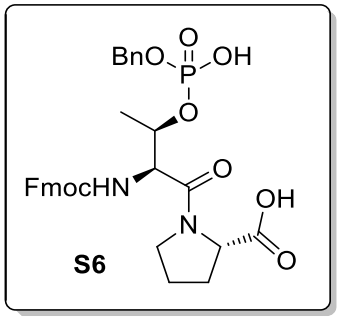
Dibenzyl N,N-diisopropyl phosphoramidite (2.64 g, 2.57 mL, 7.65 mmol) was added to a solution of compound **S3** (1.83 g, 3.82 mmol) and 1H-tetrazole (0.54 g, 7.65 mmol) in anhydrous CH_2Cl_2 under Argon protection. The reaction was initiated at 0℃ and gradually warmed to room temperature for another 5 h. It was then cooled to -40℃ and mCPBA (1.76 g, max. 75% purity, 7.65 mmol) was added there. After 30 min since mCPBA addition, it was quenched with 10% $NaHSO_3$ (aq.); it was further diluted with AcOEt (200 mL), washed with 10% $NaHSO_3$ (aq.) (100 mL x 3) and brine (100 mL x 3). The organic phase was dried over Na_2SO_4, concentrated under *vacuo* and purified by flash column chromatography on silica gel (hexane/AcOEt, 1:1) to give compound **S4** (2.64 g, 93 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, 2H, *J* = 7.5 Hz), 7.57-7.54 (m, 2H), 7.74-7.23 (m, 14H), 6.04 (d, 1H, *J* = 8.1 Hz), 5.92-5.78 (m, 1H), 5.32-5.20 (m, 2H), 5.05-5.02 (m, 4H), 4.80-4.69 (m, 1H), 4.63-4.53 (m, 4H), 4.33-4.29 (m, 1H), 4.25-4.20 (m, 1H), 4.13 (t, 1H, *J* = 7.4 Hz), 3.88-3.83 (m, 1H), 3.73-3.67 (m, 1H), 2.28-2.19 (m, 1H), 2.04-1.93 (m, 3H), 1.42 (d, 3H, *J* = 6.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.34, 168.05, 156.16, 143.80, 143.76, 141.27, 135.84, 135.75, 131.75, 128.59, 128.51, 128.00, 127.96, 127.75, 127.14, 125.28, 119.97, 118.69, 75.39, 75.33, 69.51, 67.39, 65.82, 59.13, 57.15, 57.10, 47.64, 47.02, 29.16, 24.97, 17.97; ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.11, -1.54. HRMS Calcd for $C_{41}H_{43}N_2O_9P$ $[M+H]^+$ 739.2784, found 739.2752



Compound **S4** (5.52 g, 7.47 mmol) and $Pd(PPh_3)_4$ (0.86 g, 0.75 mmol) and sodium p-toluenesulfonate (2.18 g, 11.21 mmol) were mixed in anhydrous CH_2Cl_2 under argon. Phenylsilane (1.62 g, 1.83 mL, 14.95 mmol) was added to the above solution under argon. The reaction mixture was stirred at room temperature under argon for 4 h. The reaction mixture was concentrated under *vacuo* and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 20:1, with 0.5% AcOH) to give compound **S5** (4.10 g, 79 %).

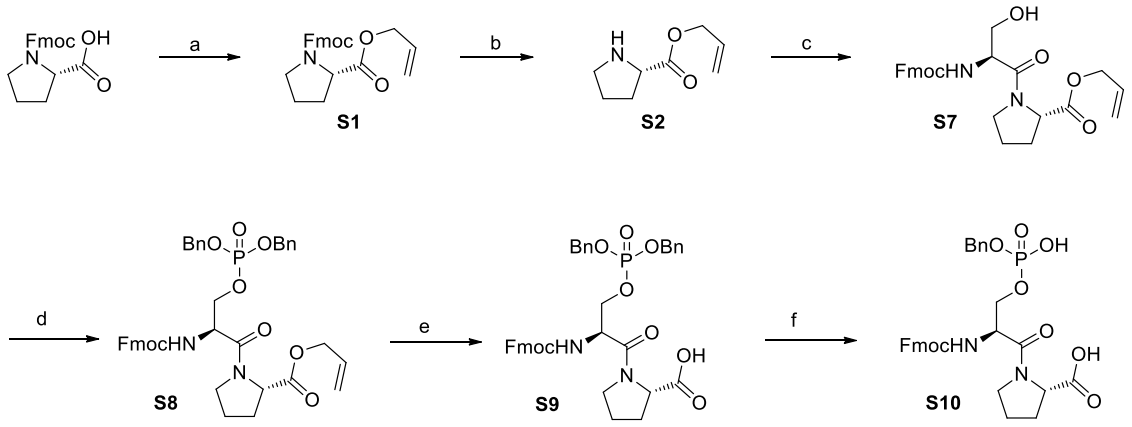
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, 2H, J = 7.5 Hz), 7.57 (d, 2H, J = 7.2 Hz), 7.37 (t, 2H, J = 7.4 Hz), 7.31-7.28 (m, 12H), 6.07 (d, 1H, J = 8.8 Hz), 5.05-4.98 (m, 4H), 4.81 (br, 1H), 4.59-4.55 (m, 2H), 4.35-4.25 (m, 2H), 4.16 (t, 1H, J = 7.2 Hz), 3.73-3.71 (m, 1H), 3.63-3.59 (m, 1H), 2.14-2.11 (m, 2H), 2.05-1.97 (m, 1H), 1.95-1.90 (m, 1H), 1.35 (d, 3H, J = 6.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.46, 168.19, 156.41, 143.80, 143.72, 141.22, 135.68, 128.57, 128.51, 128.43, 128.04, 127.95, 127.90, 127.72, 127.10, 125.28, 119.92, 75.53, 69.54, 67.51, 67.34, 59.59, 56.97, 47.58, 47.00, 28.88, 24.79, 18.21; ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.85. HRMS Calcd for $C_{38}H_{39}N_2O_9P$ $[M+H]^+$ 699.2471, found 699.2448



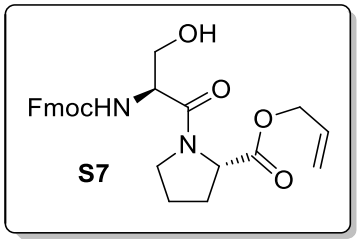
Compound **S5** (1.12 g, 1.60 mmol) was treated with sodium iodide (0.48 g, 3.21 mmol) in anhydrous CH_3CN (4.3 mL) for 11 h at room temperature. It was then concentrated under *vacuo* and precipitate with Et_2O gave the titled compound **S6** (0.95 g, *quant.*) without further purification. The quality was sufficient for SPPS.

^{1}H NMR (400 MHz, AcOH-d_4) δ 7.77 (d, 2H, J = 7.5 Hz), 7.66 (d, 2H, J = 7.4 Hz), 7.39-7.25 (m, 9H), 5.01-4.99 (m, 2H), 4.78-4.71 (m, 2H), 4.61-4.58 (m, 1H), 4.40-4.30 (m, 2H), 4.20 (t, 1H, J = 7.3 Hz), 3.93-3.91 (m, 1H), 3.85-3.82 (m, 1H), 3.55 (q, 1H, J = 7.0 Hz), 2.33-2.30 (m, 1H), 2.10-2.00 (masked in AcOH peak), 1.40 (d, 3H, J = 5.8 Hz); ^{13}C NMR (100 MHz, AcOH-d_4) δ 177.71, 172.15, 159.70, 146.20, 143.65, 139.86, 139.78, 135.40, 134.50, 134.39, 131.51, 131.38, 130.87, 130.35, 130.27, 129.92, 129.64, 127.64, 122.41, 70.16, 69.88, 66.83, 62.17, 50.08, 49.31, 33.29, 31.24, 27.08; ^{31}P NMR (162 MHz, AcOH-d_4) δ -0.68. HRMS Calcd for $C_{31}H_{33}N_2O_9P$ $[M-H]^-$ 607.1845, found 607.1862

Synthesis of Fmoc-*p*SP-OH (Scheme S2)

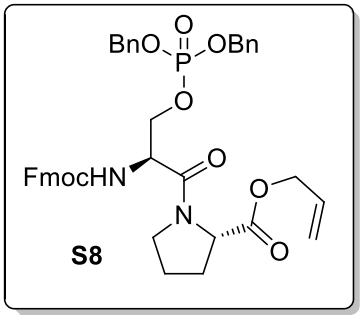


a) Allyl bromide, $KHCO_3$, DMF, 11h, 82%; b) Et_2NH/CH_2Cl_2, 2h, 81%; c) Fmoc-Ser-OH, HATU,DIEA, CH_2Cl_2, 12h, 67%; d) i)1H-tetrazole, *i*-$Pr_2NP(OBn)_2$, CH_2Cl_2, 12h, ii) mCPBA, -40ºC, 0.5h, 85% over two steps; e) $Pd(PPh_3)_4$, sodium tosylate, CH_2Cl_2, Ar protection, 3-5h, 88%; f) NaI, ACN, 12h, quant.



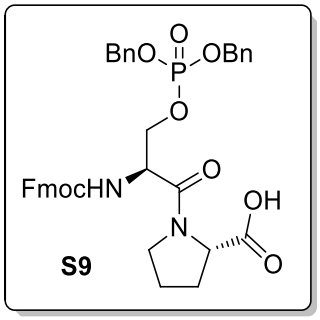
Fmoc-Ser-OH (2.77 g, 8.46 mmol), HATU (3.86 g, 10.16 mmol) and DIEA (3.28 g, 4.42 mL, 25.39 mmol) were added to compound **S2** (9.31 mmol) in 4 mL CH_2Cl_2. Another 1 mL DMF was added for the sake of solubility. The reaction mixture was stirred at room temperature for 10 h; after the reaction was complete, it was diluted with AcOEt (200 mL), washed with 1N HCl (100 mL x 3) and brine (100 mL x 3). The organic phase was dried over Na_2SO_4, concentrated under *vacuo* and purified by flash column chromatography on silica gel (hexane/AcOEt, 1:1) to give compound **S7** (2.63 g, 67.0 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 2H, *J* = 7.5 Hz), 7.59 (d, 2H, *J* = 7.4 Hz), 7.40 (t, 2H, *J* = 7.4 Hz), 7.31 (t, 2H, *J* = 7.4 Hz), 5.96-5.87 (m, 1H), 5.80 (d, 1H, *J* = 8.0 Hz), 5.37-5.26 (m, 2H), 4.71-4.61 (m, 4H), 4.38 (d, 2H, *J* = 7.1 Hz), 4.21 (t, 1H, *J* = 7.1 Hz), 3.95 (dd, 1H, *J* = 4.2 Hz, *J* = 11.4 Hz), 3.88-3.83 (m, 1H), 3.77-3.70 (m, 2H), 2.34-2.23 (m, 1H), 2.03-2.02 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.83, 169.75, 156.17, 143.67, 141.09, 131. 46, 127.57, 129.95, 125.06, 119.81, 118.68, 67.05, 65.92, 63.45, 58.96, 54.00, 47.11, 46.95, 28.76, 24.72. HRMS Calcd for $C_{26}H_{28}N_2O_6$ $[M+H]^+$ 465.2026, found 465.2058



Dibenzyl N,N-Diisopropyl phosphoramidite (3.92 g, 3.81 mL, 11.34 mmol) was added to a solution of compound **S7** (2.63 g, 5.67 mmol) and 1H-tetrazole (0.79 g, 11.34 mmol) in anhydrous CH_2Cl_2 under Argon protection. The reaction was initiated at 0 ℃ and gradually warmed to room temperature for another 5 h. It was then cooled to -40 ℃ and mCPBA (2.61 g, max. 75% purity, 11.34 mmol) was added there. After 30 min since mCPBA addition, it was quenched with 10% $NaHSO_3$ (aq.); it was further diluted with AcOEt (200 mL), washed with 10% $NaHSO_3$ (aq.) (100 mL x 3) and brine (100 mL x 3). The organic phase was dried over Na_2SO_4, concentrated under *vacuo* and purified by flash column chromatography on silica gel (hexane/AcOEt, 1:1) to give compound **S8** (3.50 g, 85%).

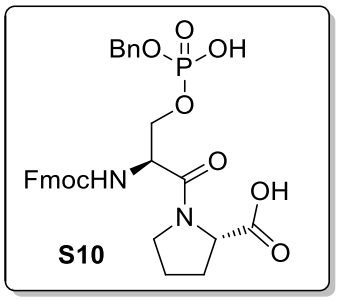
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, 2H, *J* = 7.5 Hz), 7.56 (d, 2H, *J* = 7.4 Hz), 7.38 (t, 2H, *J* = 7.5 Hz), 7.32-7.28 (m, 14H), 5.91-5.80 (m, 1H), 5.74 (d, 1H, *J* = 8.5 Hz), 5.31-5.15 (m, 2H), 5.08-5.00 (m, 4H), 4.85-4.80 (m, 1H), 4.65-4.52 (m, 4H), 4.36-4.30 (m, 2H), 4.27-4.22 (m, 1H), 4.19-4.07 (m, 2H), 3.64 (t, 1H, *J* = 6.0 Hz), 2.26-2.11 (m, 1H), 2.09-1.90 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.06, 167.43, 155.92, 143.74, 141.27, 135.72, 131.74, 128.66, 128.58, 128.55, 128.03, 127.75, 127.12, 125.18, 119.99, 118.62, 69.58, 69.53, 67.32, 66.52, 65.76, 59.18, 52.88, 52.81, 47.17, 47.02, 28.98, 24.88; ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.09, -1.29. HRMS Calcd for $C_{40}H_{41}N_2O_9P$ $[M+H]^+$ 725.2628, found 725.2601



Compound **S8** (3.44 g, 4.75 mmol) and $Pd(PPh_3)_4$ (0.45 g, 3.85 mmol) and sodium p-toluenesulfonate (1.38 g, 7.12 mmol) were mixed in anhydrous CH_2Cl_2 under argon. Phenylsilane (1.03 g, 1.17 mL, 9.50 mmol) was added to the above solution under argon. The reaction mixture was stirred at room temperature under argon for 4 h. The reaction mixture was concentrated under *vacuo* and purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH, 20:1, with 0.5% AcOH) to give compound **S9** (2.86 g, 88.0 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, 2H, *J* = 7.5 Hz), 7.56-7.53 (m, 2H), 7.36-7.32 (m, 2H), 7.27-7.22 (m, 12H), 6.37 (br, 1H), 5.03-5.01 (m, 4H), 4.82-4.81 (m, 1H), 4.51 (m, 1H), 4.28-4.26 (m, 2H), 4.18-4.11 (m, 2H), 3.58 (m, 2H), 2.07 (m, 2H), 1.93-1.82 (m, 2H);-^{13}C NMR (100 MHz, $CDCl_3$) δ 173.90, 168.05, 156.22, 143.87, 143.78, 141.27, 135.55, 128.64, 128.60, 128.08, 127.74, 127.15, 125.31, 119.97,

69.88, 67.38, 59.76, 53.15, 47.33, 47.03, 28.76, 24.94; ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.52. HRMS Calcd for $C_{37}H_{37}N_2O_9P$ $[M+H]^+$ 685.2315, found 685.2381

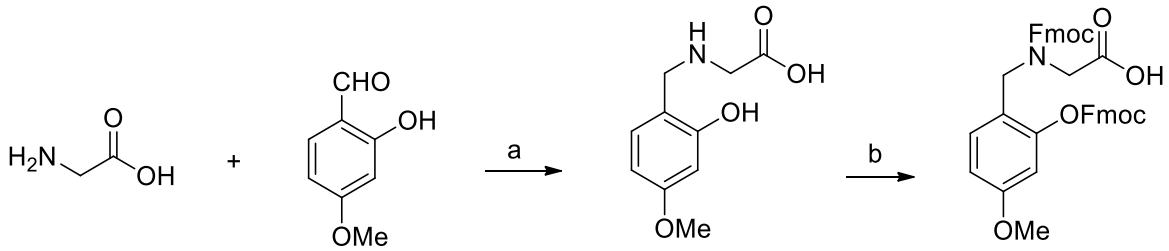


Compound **S9** (2.86 g, 4.18 mmol) was treated with sodium iodide (1.25 g, 8.35 mmol) in anhydrous CH_3CN (11.3 mL) for 11 h at room temperature. It was then concentrated under *vacuo* and precipitate with Et_2O gave the titled compound **S10** (2.42 g, *quant.*) without further purification. The quality was sufficient for SPPS.

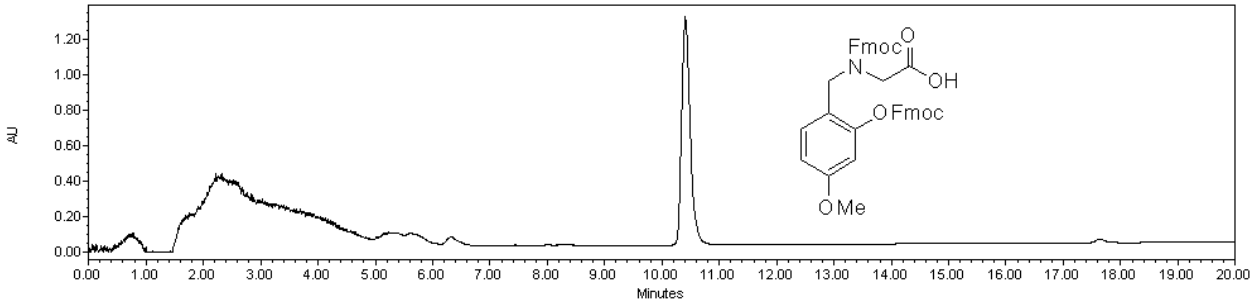
^{1}H NMR (400 MHz, AcOH-d_4) δ 7.78 (d, 2H, J = 7.4 Hz), 7.72-7.61 (m, 2H), 7.40-7.26 (m, 9H), 5.98-4.93(m, 2H), 4.60-4.58 (m, 1H), 4.42-4.29 (m, 2H), 4.26-4.15 (m, 2H), 4.10-4.04(m, 1H), 3.78 (m, 1H), 2.30-2.25 (m, 1H), 2.10-2.00 (masked in AcOH peak); ^{13}C NMR (100 MHz, AcOH-d_4) δ 171.66, 159. 07, 145.69, 143.08, 139.16, 134.57, 133.98, 133.87, 130.75, 130.63, 130.16, 129.67, 129.56, 129.31, 128.93, 127.11, 127.06, 121.73, 69.65, 69.37, 66.66, 55.10, 49.46, 48.74, 30.56, 26.41; ^{31}P NMR (162 MHz, $CDCl_3$) δ -0.52. HRMS Calcd for $C_{30}H_{31}N_2O_9P$ $[M-H]^-$ 593.1689, found 593.1692

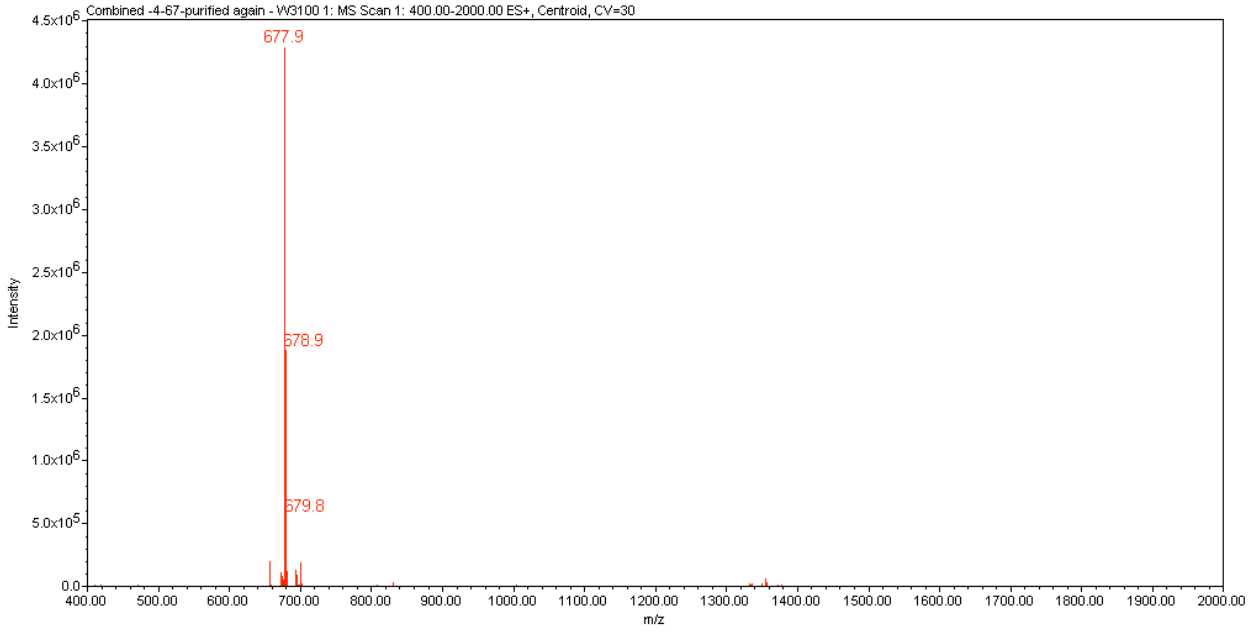
Synthesis of Fmoc-Gly(Hmb,Fmoc)-OH (Scheme S3)

Fmoc-Gly(Hmb,Fmoc)-OH was prepared as reported[6] with comparable yields, and the purity was confirmed by RP-HPLC (80-95% CH_3CN/H_2O over 15 min) at a flow rate of 0.6 mL/min.



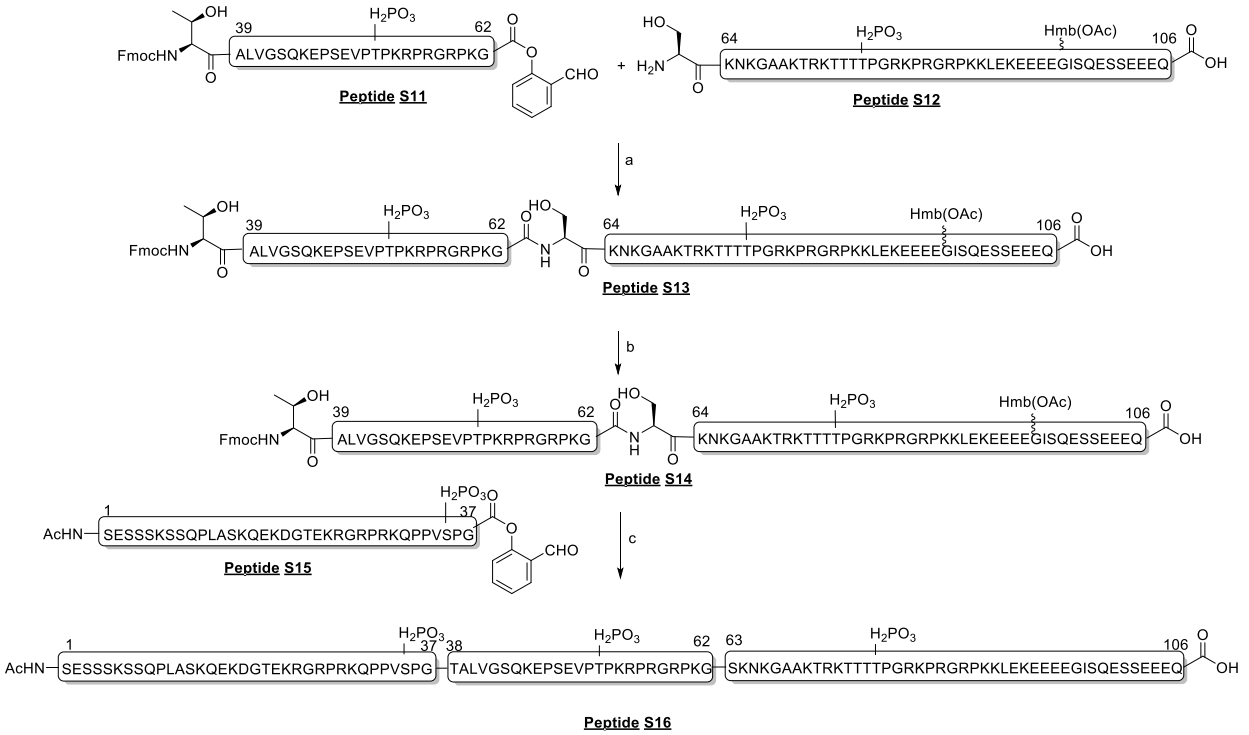
a) $NaBH_4$, KOH, H_2O/EtOH; b) Fmoc-Cl, H_2O/dioxane.





Calc. m/z for $[M+Na]^+$ 678.2, found 677.9.

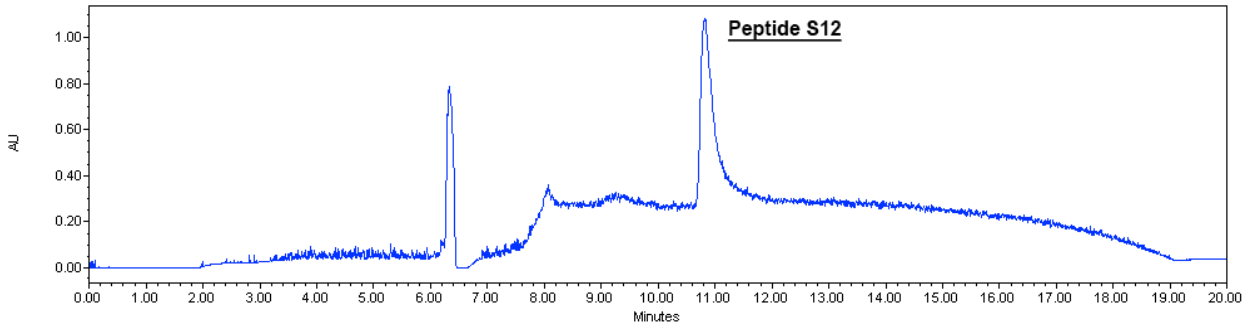
Synthesis of HMG Ala tri-phosphate Peptide S16 (Scheme S4)

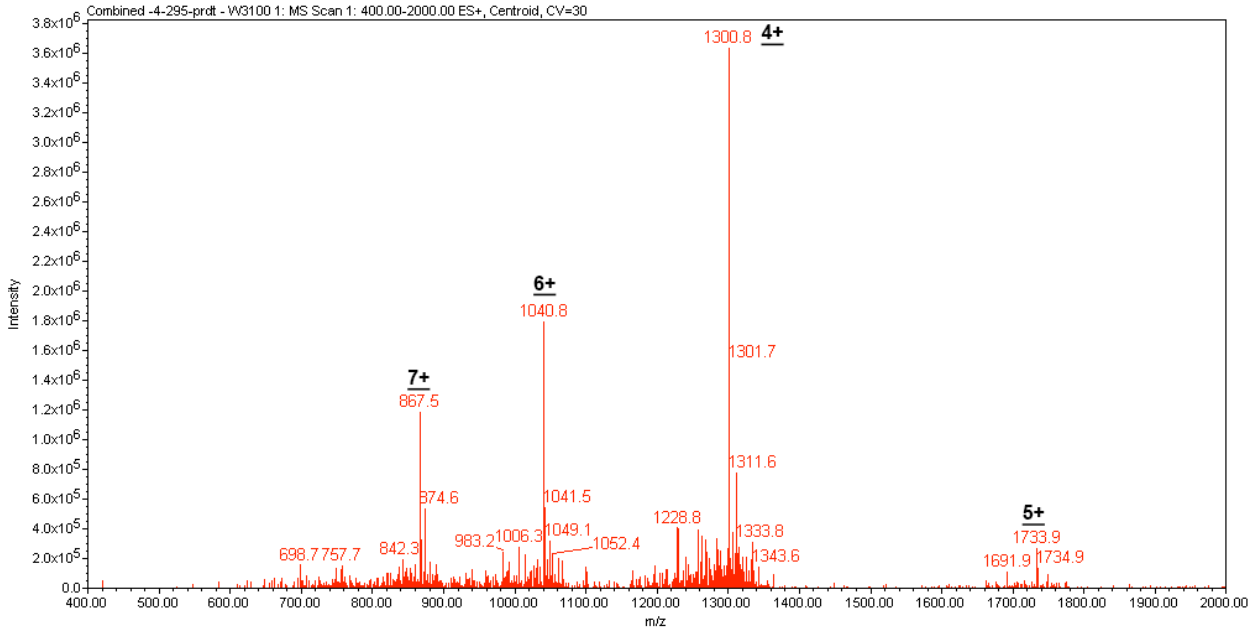


a) Pyridine/AcOH (1/12, mol/mol), 9h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 0.5 h, 38%. b) H_2NNH_2, 4 h; $Et_2NH/H_2O/CH_3CN$, 2 h, 60%. c) Pyridine/AcOH (1/12, mol/mol), 7h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 1 h, 43%.

1) Preparation of Peptide S12

The peptide was assembled according to general procedure. Fmoc-Thr(HPO_3Bn)-Pro-OH dipeptide was introduced with HATU/HOBt in DMF/NMP as indicated.[1] The incorporation of Fmoc-Gly(Hmb, Fmoc)-OH was carried out under standard coupling protocols; the acylation of this backbone secondary amine was performed in DMF/NMP(1/1, v/v) overnight as an optimized coupling condition.[7] Note that the hydroxyl group on Hmb was always subjected to acylation during the subsequent couplings; yet it fell off concomitantly once treated with piperidine/DMF (1/4, v/v) as Fmoc deblock solution. However, the N-terminal Ser63 was an exception: while Boc-Ser(tBu)-OH was introduced normally, it required up to 11h to be fully removed by the aforementioned piperidine/DMF (1/4, v/v) solution. After the hydroxyl group was liberated in this way, it was further acylated by Ac_2O/Et_3N/DMAP in anhydrous CH_2Cl_2. The global deprotection was performed as mentioned above for up to 4 h in the case of total removal of the benzyl group on phosphate. Preparative HPLC purification (10-40% CH_3CN/H_2O over 30 min) followed by concentration under *vacuo* and lyophilization gave 74 mg of **Peptide S12** out of 0.4 g of the resin (16% yield based on resin loading).



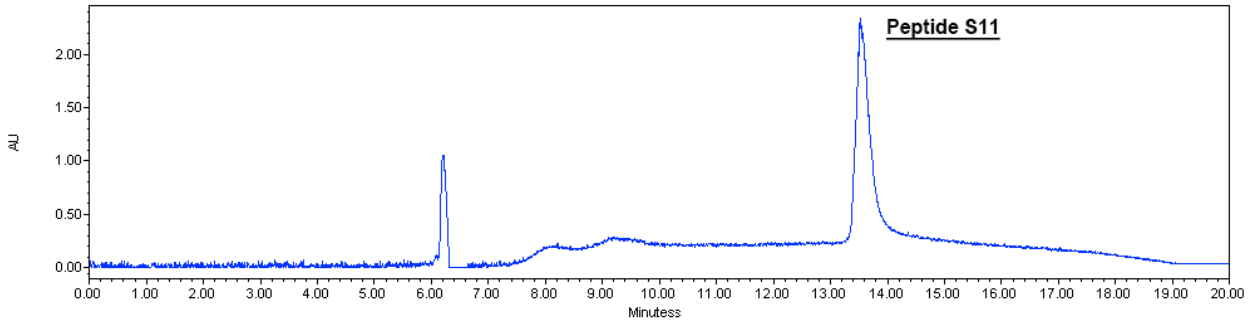


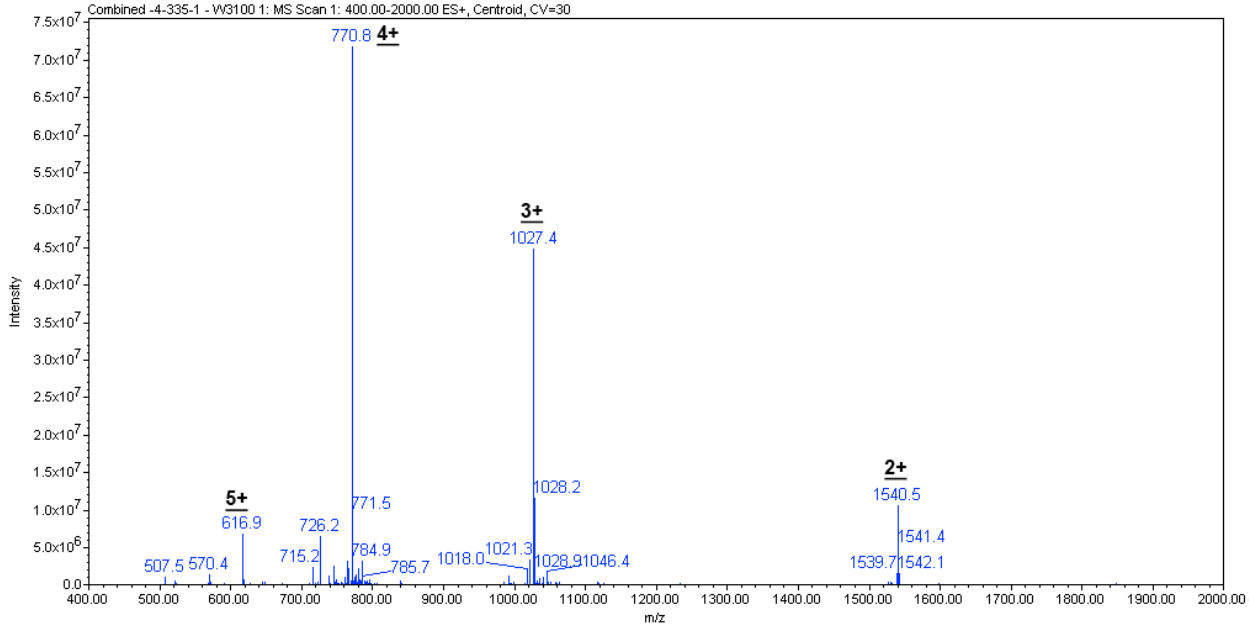
Peptide S12 was characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min) at a flow rate of 0.6 mL/min. ESI calcd for $C_{214}\ H_{362}\ N_{67}\ O_{81}\ P_1$ $[M+3H]^{3+}$ m/z = 1734.21 $[M+4H]^{4+}$ m/z = 1300.91 $[M+5H]^{5+}$ m/z = 1040.92 $[M+5H]^{5+}$ m/z = 867.61, found: 1733.9; 1300.8; 1040.8; 907.4; 867.5.

2) Preparation of Peptide S11

After the fragment was assembled following the standard protocol, it was cleaved from the resin by a cocktail of CH_2Cl_2/TFE/AcOH (8/1/1, v/v/v). The solvent was removed under reduced pressure to give the crude protected peptide.

The crude peptide (91.2 mg, 0.02 mmol) was then coupled with α, α-dimethoxy-salicylaldehyde (0.13 g, 0.79 mmol), PyBOP (0.10 g, 0.20 mmol) and DIEA (0.03 g, 0.22 mmol, 37.9 µL) in anhydrous CH_2Cl_2 (1.3 mL) at a concentration of 15 mM; after 10 h, treatment with TFA/phenol/H_2O (90/5/5, v/v/v) for up to 10-12 h allowed the complete removal of the benzyl group on the phosphate. TFA was removed under a stream of condensed air. The crude product was triturated with cold diethyl ether and centrifuged and the ether subsequently decanted. The obtained solid was purified via preparative HPLC (30-50% CH_3CN/H_2O over 30 min), followed by concentration under *vacuo* and lyophilization to give the titled fragment **Peptide S11** (21.1 mg, 35%) as a white powder.



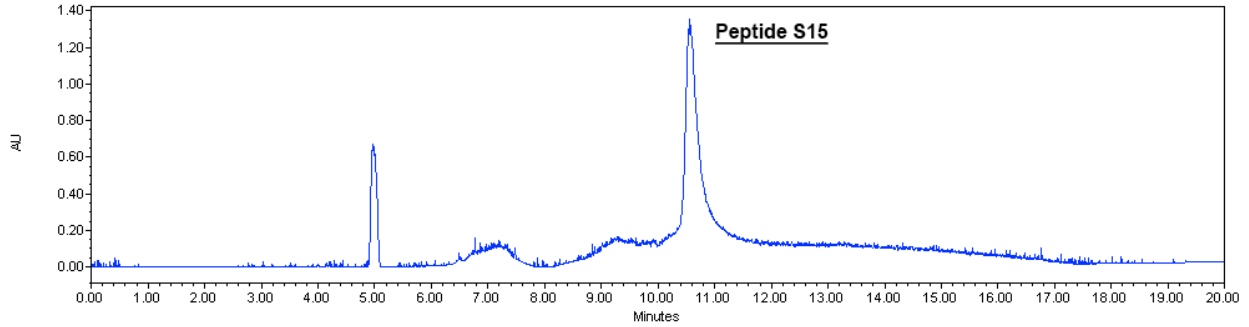


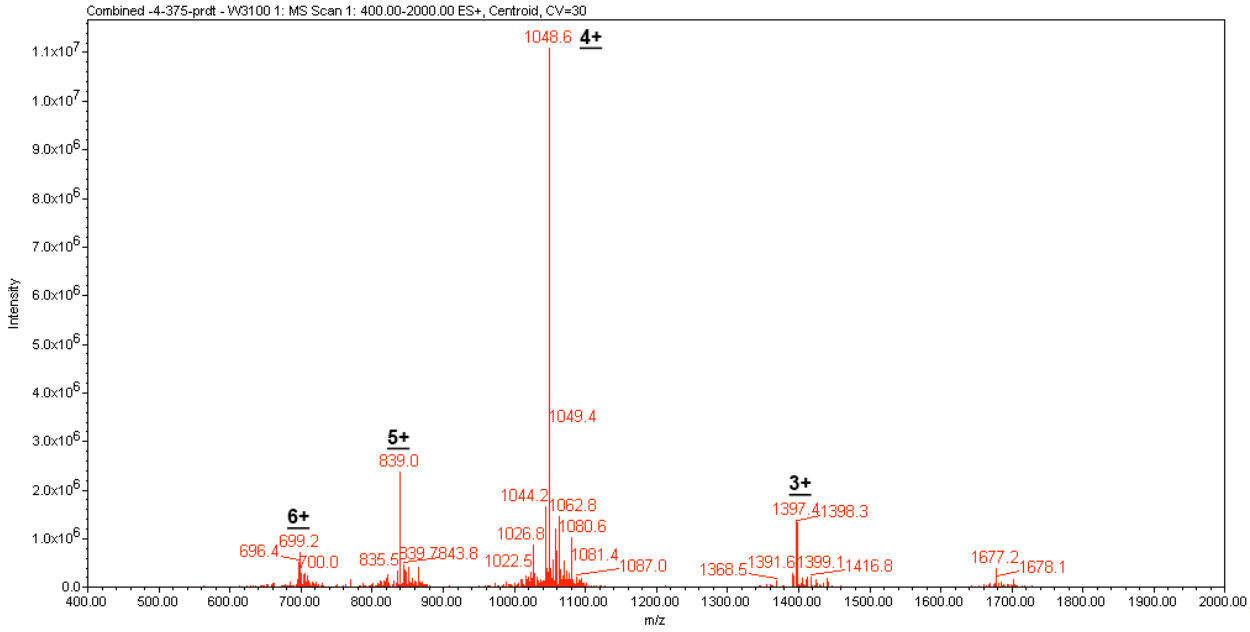
Peptide S11 was characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min). ESI calcd for $C_{137}H_{214}N_{38}O_{41}P_1$ $[M+2H]^{2+}$ m/z = 1540.28 $[M+3H]^{3+}$ m/z = 1027.19 $[M+4H]^{4+}$ m/z = 770.65 $[M+5H]^{5+}$ m/z = 616.72, found: 1540.5; 1027.4; 770.8; 616.9.

3) Preparation of Peptide S15

After the fragment was assembled following the standard protocol, along which Fmoc-Ser(HPO3Bn)-Pro-OH dipeptide was introduced with HATU/HOBt in DMF/NMP as indicated,[1] it was acetylated at the N-terminus by Ac_2O/Et_3N/DMAP in anhydrous CH_2Cl_2. It was then cleaved from the resin by a cocktail of CH_2Cl_2/TFE/AcOH (8/1/1, v/v/v). The solvent was removed under reduced pressure to give the crude protected peptide.

The crude peptide (0.13 g, 0.02 mmol) was then coupled with α, α-dimethoxy-salicylaldehyde (60.9 mg, 0.36 mmol), PyBOP (47.1 mg, 0.09 mmol) and DIEA (12.9 mg, 0.10 mmol, 17.4 µL) in anhydrous CH_2Cl_2 (1.2 mL) at a concentration of 15 mM; after 10 h, treatment with TFA/phenol/H_2O (90/5/5, v/v/v) for 3 h allowed the complete removal of the benzyl group on the phosphate. TFA was removed under a stream of condensed air. The crude product was triturated with cold diethyl ether and centrifuged and the ether subsequently decanted. The remaining solid was purified via preparative HPLC (10-50% CH_3CN/H_2O over 30 min), followed by concentration under *vacuo* and lyophilization to give the titled fragment **Peptide S15** (21.3 mg, 27%) as a white powder.

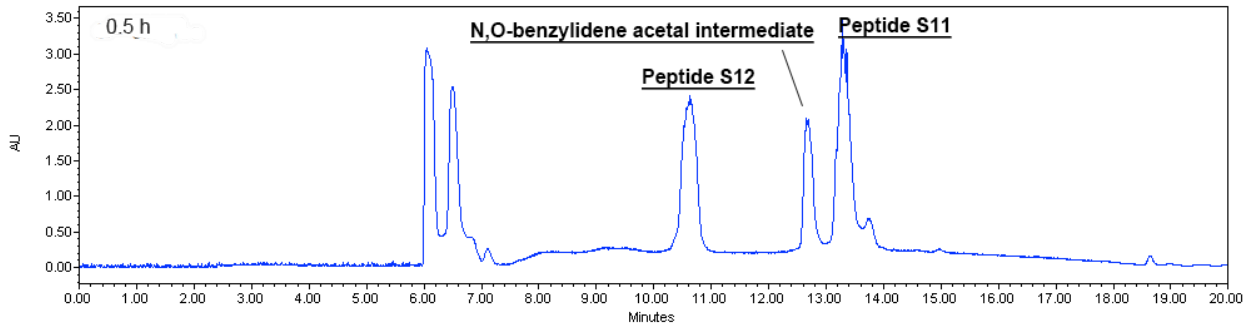


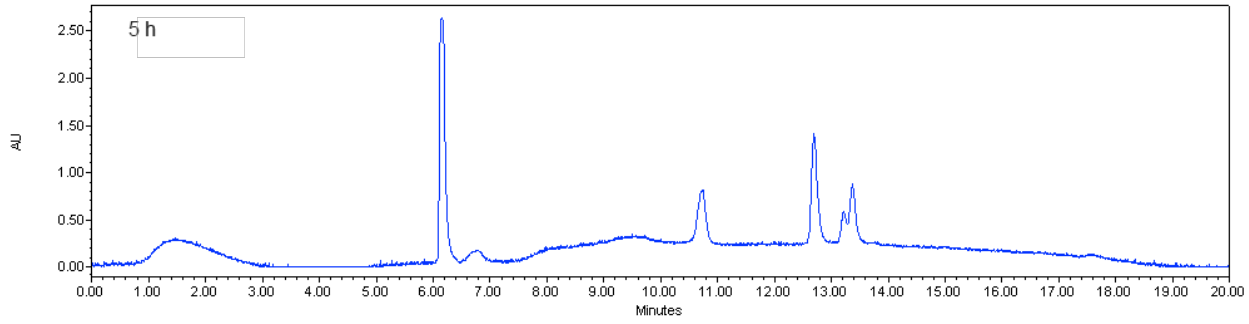


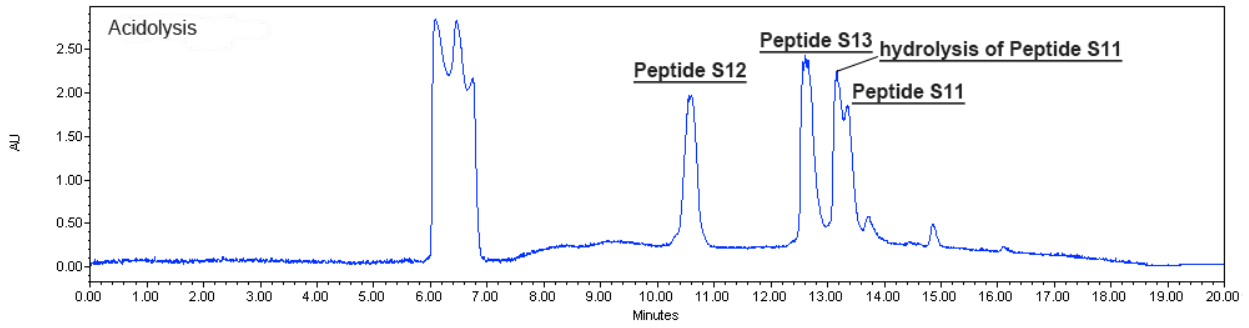
Peptide S15 was characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min). ESI calcd for $C_{172}\ H_{287}\ N_{57}\ O_{63}\ P_1$ $[M+3H]^{3+}$ m/z = 1398.03 $[M+4H]^{4+}$ m/z = 1048.78 $[M+5H]^{5+}$ m/z = 839.22 $[M+6H]^{6+}$ m/z = 699.52, found: 1398.3; 1048.6; 839.0; 699.2.

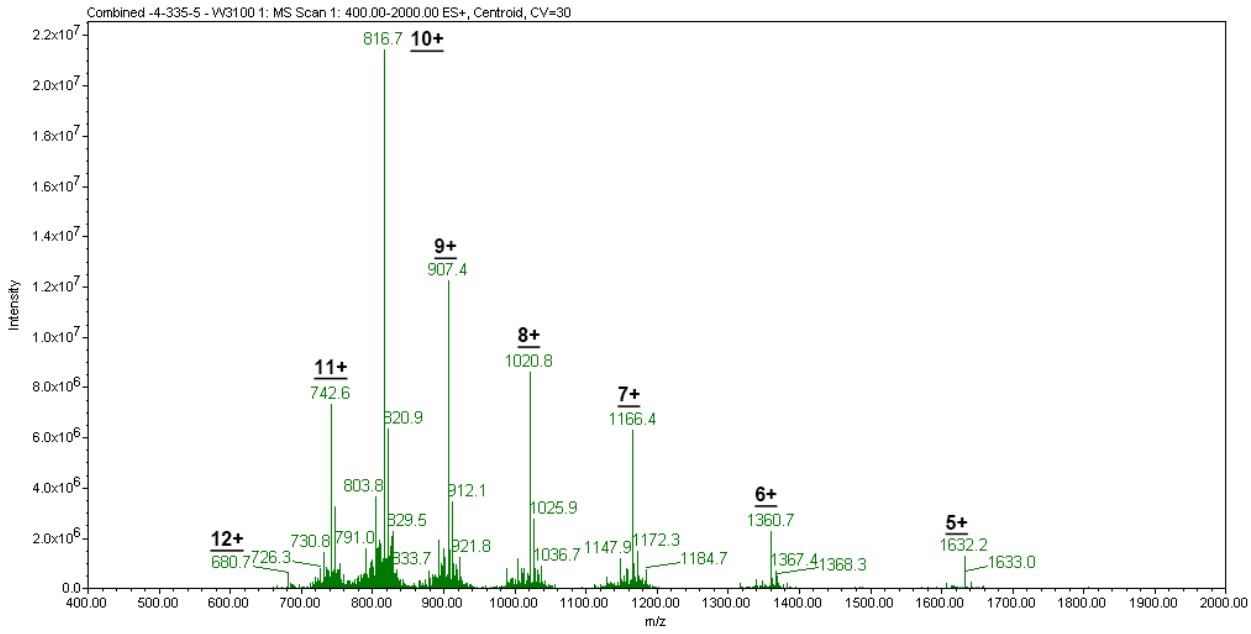
a) Synthesis of Peptide S13

18.7 mg of **Peptide S11** (1.5 equiv.) and 21.0 mg of **Peptide S12** (1.0 equiv.) were incubated in pyridine/acetic acid (202 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 10-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. After the completion of the reaction within 9 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/Triisopropylsilane (95/2.5/2.5, v/v/v). Preparative HPLC purification (10–50% CH_3CN/H_2O containing 0.1% TFA over 30 min) followed by concentration at reduced pressure and lyophilization afforded 12.4 mg (38% yield) of the titled **Peptide S13** as a white powder. (Recovery of **Peptide S11** 17%; recovery of **Peptide S12** 24%).


0.5 h
N,O-benzylidene acetal intermediate
Peptide S11
Peptide S12
AU
Minutes


5 h
AU
Minutes

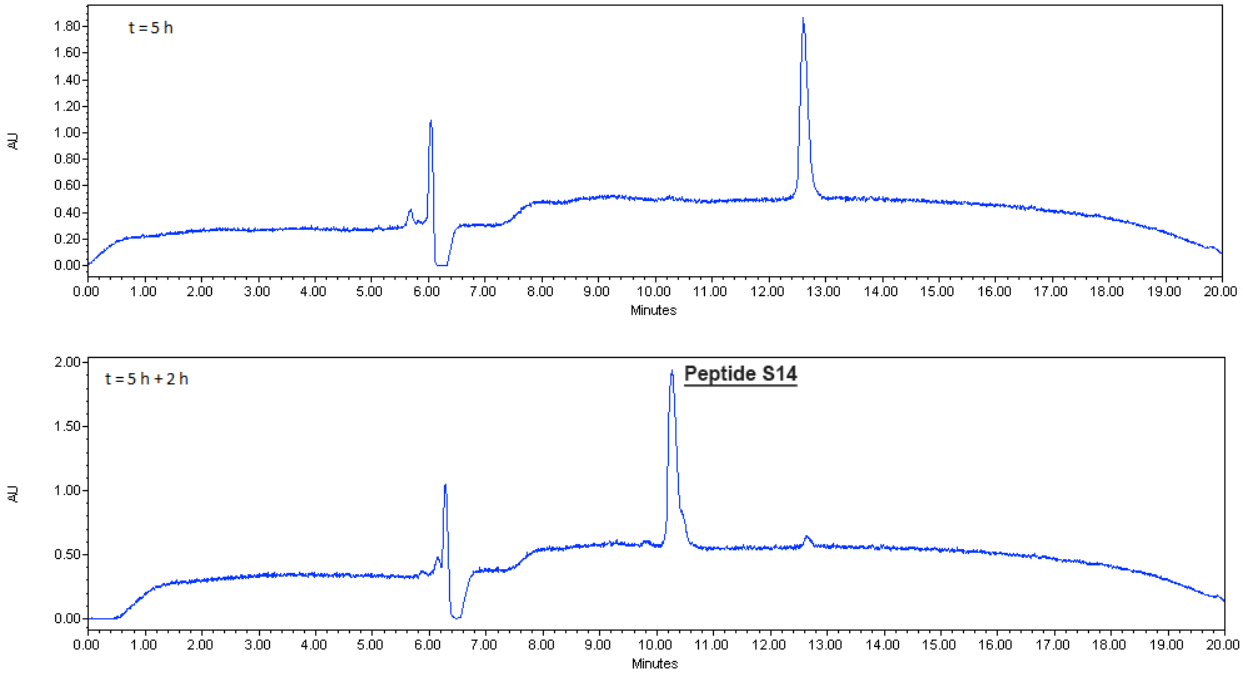

Acidolysis
Peptide S13
hydrolysis of Peptide S11
Peptide S12
Peptide S11
AU
Minutes

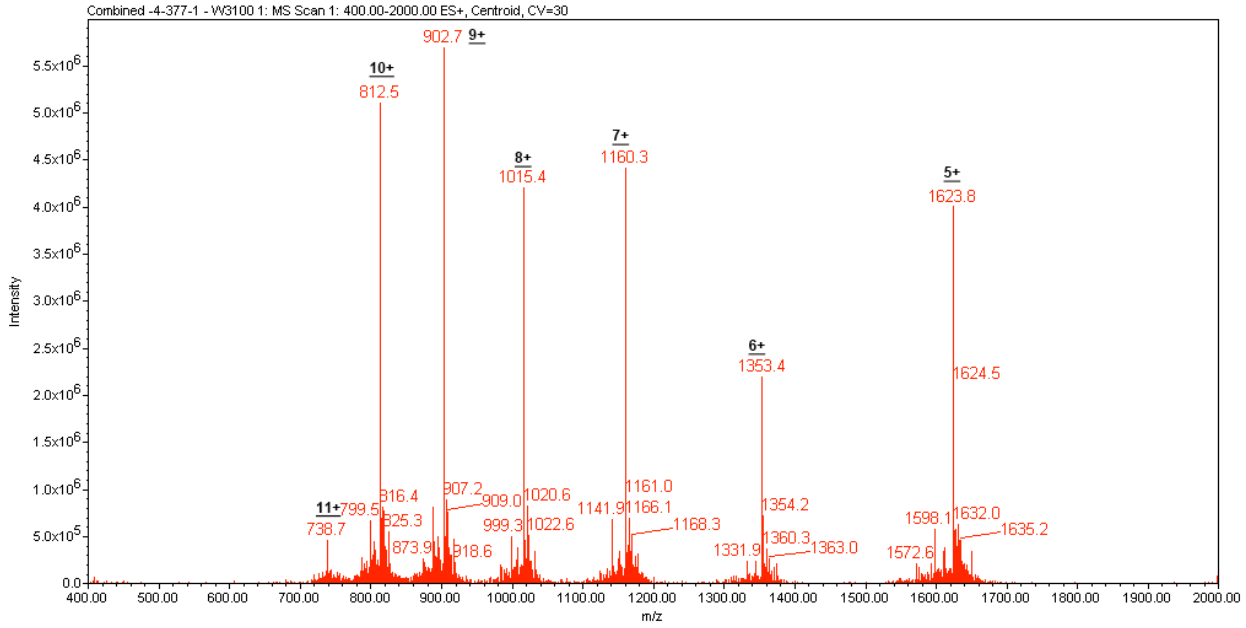

Combined -4-335-5 - W3100 1: MS Scan 1: 400.00-2000.00 ES+, Centroid, CV=30
816.7 10+
9+
907.4
8+
1020.8
11+
742.6
7+
1166.4
820.9
803.8
912.1
829.5
1025.9
6+
1360.7
12+
680.7
726.3
730.8
791.0
833.7
921.8
1036.7
1147.9
1172.3
1184.7
1367.4
1368.3
5+
1632.2
1633.0
Intensity
m/z

Peptide S13 ESI calcd for $C_{344}H_{570}N_{105}O_{120}P_2$ $[M+5H]^{5+}$ m/z = 1632.43 $[M+6H]^{6+}$ m/z = 1360.53 $[M+7H]^{7+}$ m/z = 1166.31 $[M+8H]^{8+}$ m/z = 1020.65 $[M+9H]^{9+}$ m/z = 907.35 $[M+10H]^{10+}$ m/z = 816.72 $[M+11H]^{11+}$ m/z = 742.56 $[M+12H]^{12+}$ m/z = 680.77, found: 1632.2; 1360.7; 1166.4; 1020.8; 907.4; 816.7; 742.6; 680.7.

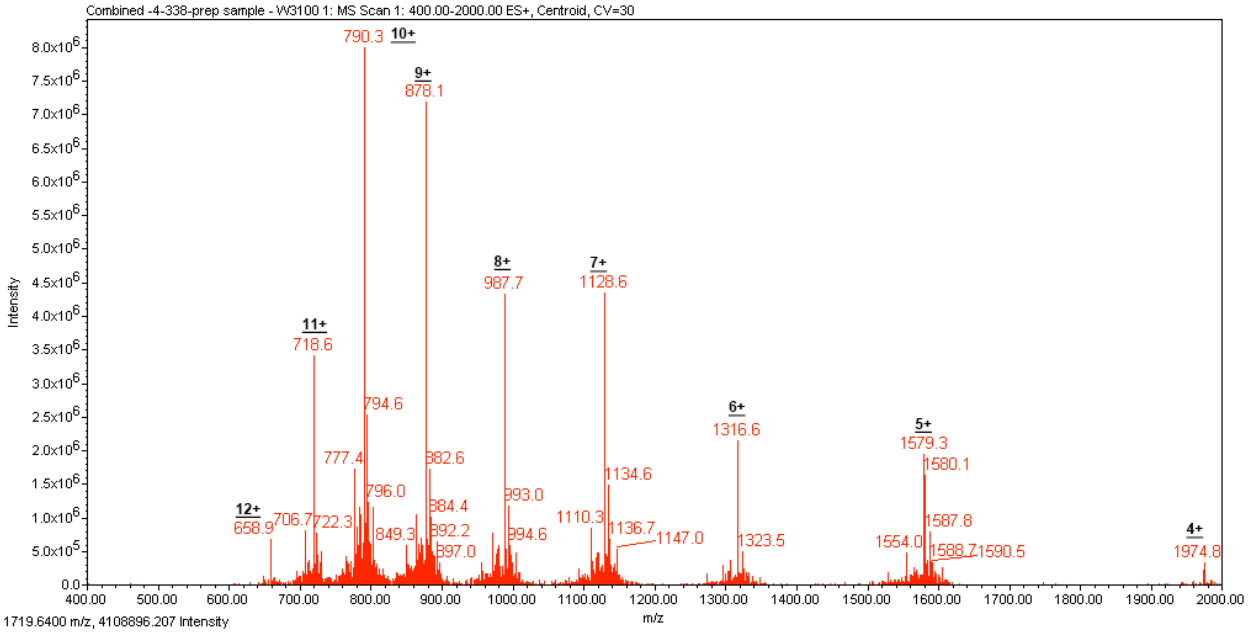
b) Synthesis of Peptide S14

12.4 mg of **Peptide S13** was treated with N_2H_4 monohydrate (10 equiv.)[8] 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 15 min at a flow rate of 0.6 mL/min. After the reaction reached completion within 4 h, 10% Et_2NH solution in CH_3CN/H_2O (1/1, v/v) was added there,[9] rendering the peptide at a concentration of 2.5 mM. After incubation for another 2 h 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 concentration at reduced pressure and lyophilization afforded 7.1 mg (60% yield) of **Peptide S14** as a white powder.





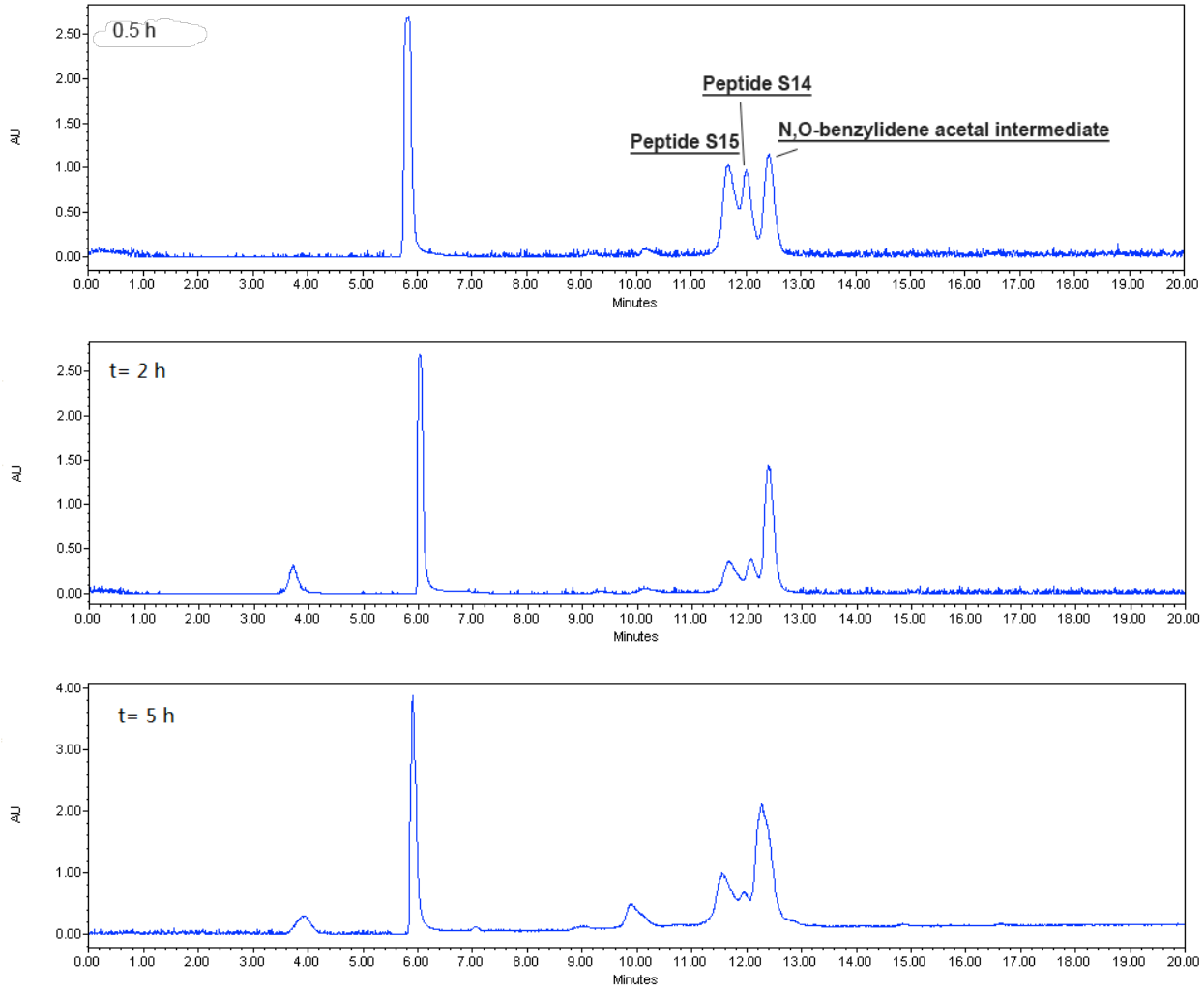
Ac removal by N_2H_4: ESI calcd for $C_{342}\ H_{568}\ N_{105}\ O_{119}\ P_2$ $[M+5H]^{5+}$ m/z = 1624.03 $[M+6H]^{6+}$ m/z = 1353.53 $[M+7H]^{7+}$ m/z = 1160.31 $[M+8H]^{8+}$ m/z = 1015.40 $[M+9H]^{9+}$ m/z = 902.69 $[M+10H]^{10+}$ m/z = 812.52 $[M+11H]^{11+}$ m/z = 738.74, found: 1623.8; 1353.4; 1160.3; 1015.4; 902.7; 812.5; 738.7.

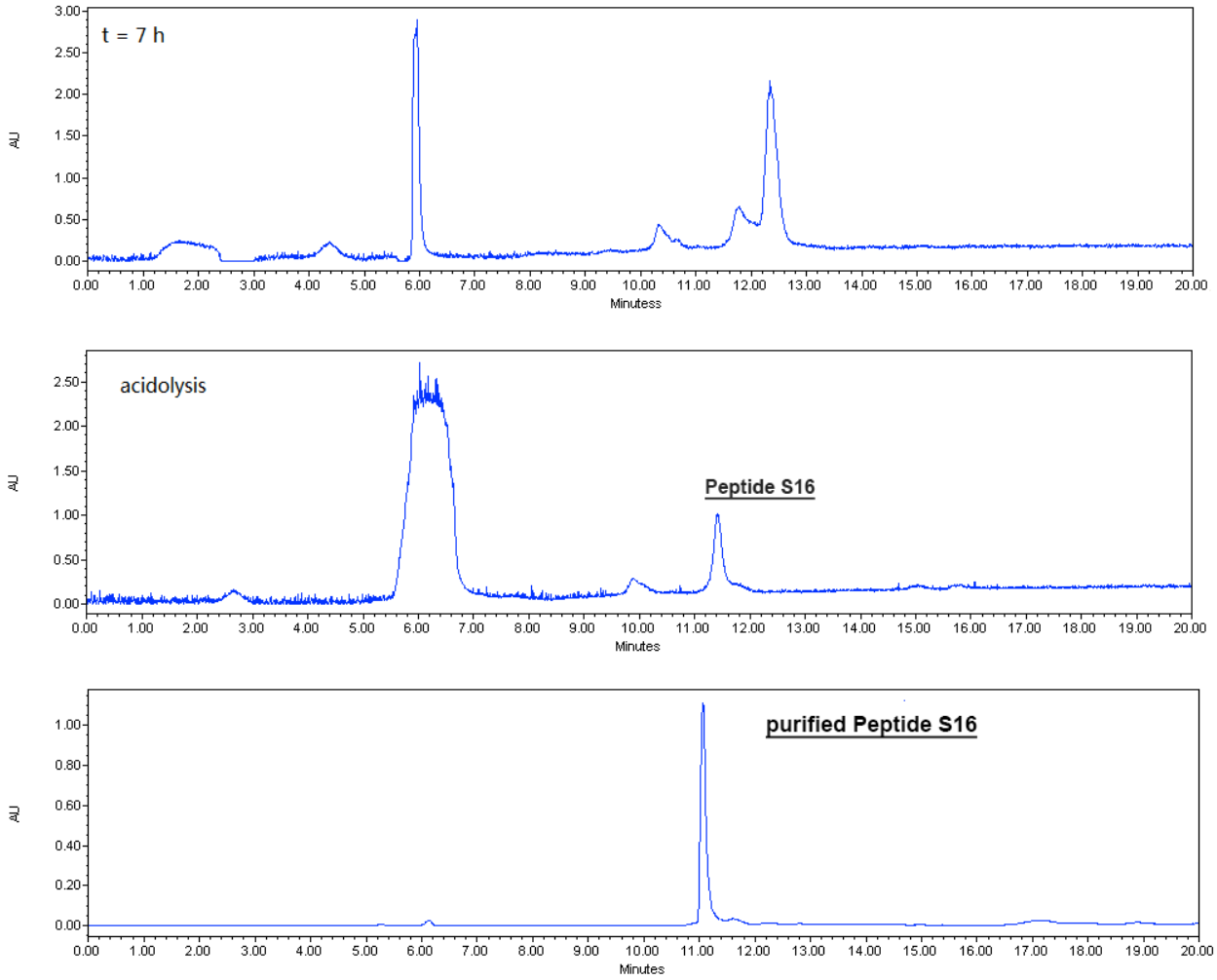


<u>Peptide S14</u> *In situ* Fmoc removal: ESI calcd for $C_{327}\ H_{558}\ N_{105}\ O_{117}\ P_2$ $[M+4H]^{4+}$ m/z = 1974.27 $[M+5H]^{5+}$ m/z = 1579.62 $[M+6H]^{6+}$ m/z = 1316.51 $[M+7H]^{7+}$ m/z = 1128.59 $[M+8H]^{8+}$ m/z = 987.64 $[M+9H]^{9+}$ m/z = 878.01 $[M+10H]^{10+}$ m/z = 790.31 $[M+11H]^{11+}$ m/z = 718.56 $[M+12H]^{12+}$ m/z = 658.76, found: 1974.8; 1579.3; 1316.6; 1128.6; 987.7; 878.1; 790.3; 718.6; 658.9.

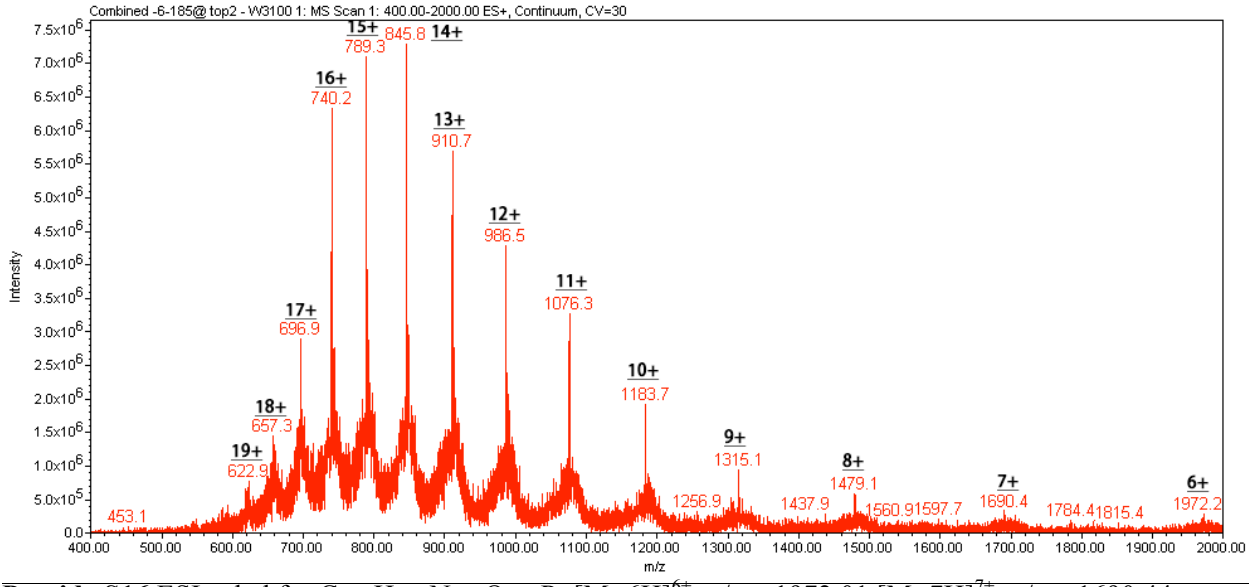
c) Synthesis of <u>Peptide S16</u>

7.7 mg of **Peptide S15** (2.0 equiv.) and 7.1 mg of **Peptide S14** (1.0 equiv.) were incubated in pyridine/acetic acid (44.9 µL, 1/12 mole/mole) at a concentration of 20 mM at room temperature. The reaction was monitored on a C4 analytical column with a gradient of 10-50% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. After the completion of the reaction within 7 h, the solvent was blown off under a stream of condensed air. The residue was then treated with $TFA/H_2O/TIPS$ (95/2.5/2.5, v/v/v) for up to 1 h to allow complete deprotection of Hmb which rendered native Gly96 concomitantly, as analyzed on a C4 analytical column with a gradient of 10-50% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. Preparative HPLC purification (10-40% CH_3CN/H_2O over 30 min) followed by concentration at reduced pressure and lyophilization afforded 4.6 mg (43% yield) of **Peptide 16** as a white powder.





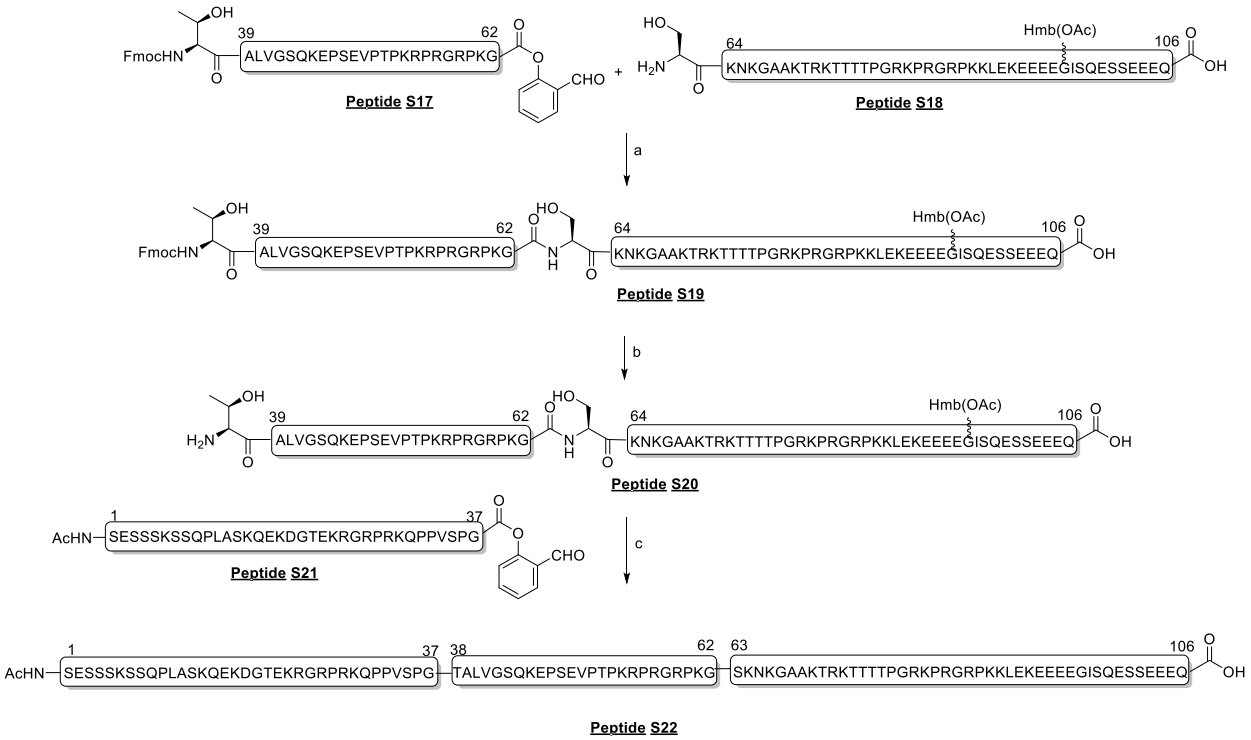
Characterization of synthetic **Peptide S16**. The analysis is run on a C18 column with a gradient of 5-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min.



Peptide S16 ESI calcd for $C_{484}H_{829}N_{162}O_{176}P_3$ $[M+6H]^{6+}$ m/z = 1972.01 $[M+7H]^{7+}$ m/z = 1690.44

$[M+8H]^{8+}$ m/z = 1479.26 $[M+9H]^{9+}$ m/z = 1315.01 $[M+10H]^{10+}$ m/z = 1183.61 $[M+11H]^{11+}$ m/z = 1076.10 $[M+12H]^{12+}$ m/z = 986.51 $[M+13H]^{13+}$ m/z = 910.70 $[M+14H]^{14+}$ m/z = 845.72 $[M+15H]^{15+}$ m/z = 789.41 $[M+16H]^{16+}$ m/z = 740.13 $[M+17H]^{17+}$ m/z = 696.66, $[M+18H]^{18+}$ m/z = 658.01, $[M+19H]^{19+}$ m/z = 623.43 found: 1972.2; 1690.4; 1479.1; 1315.1; 1183.7; 1076.3; 986.5; 910.7; 845.8; 789.3; 740.2; 696.9; 657.3; 622.9.

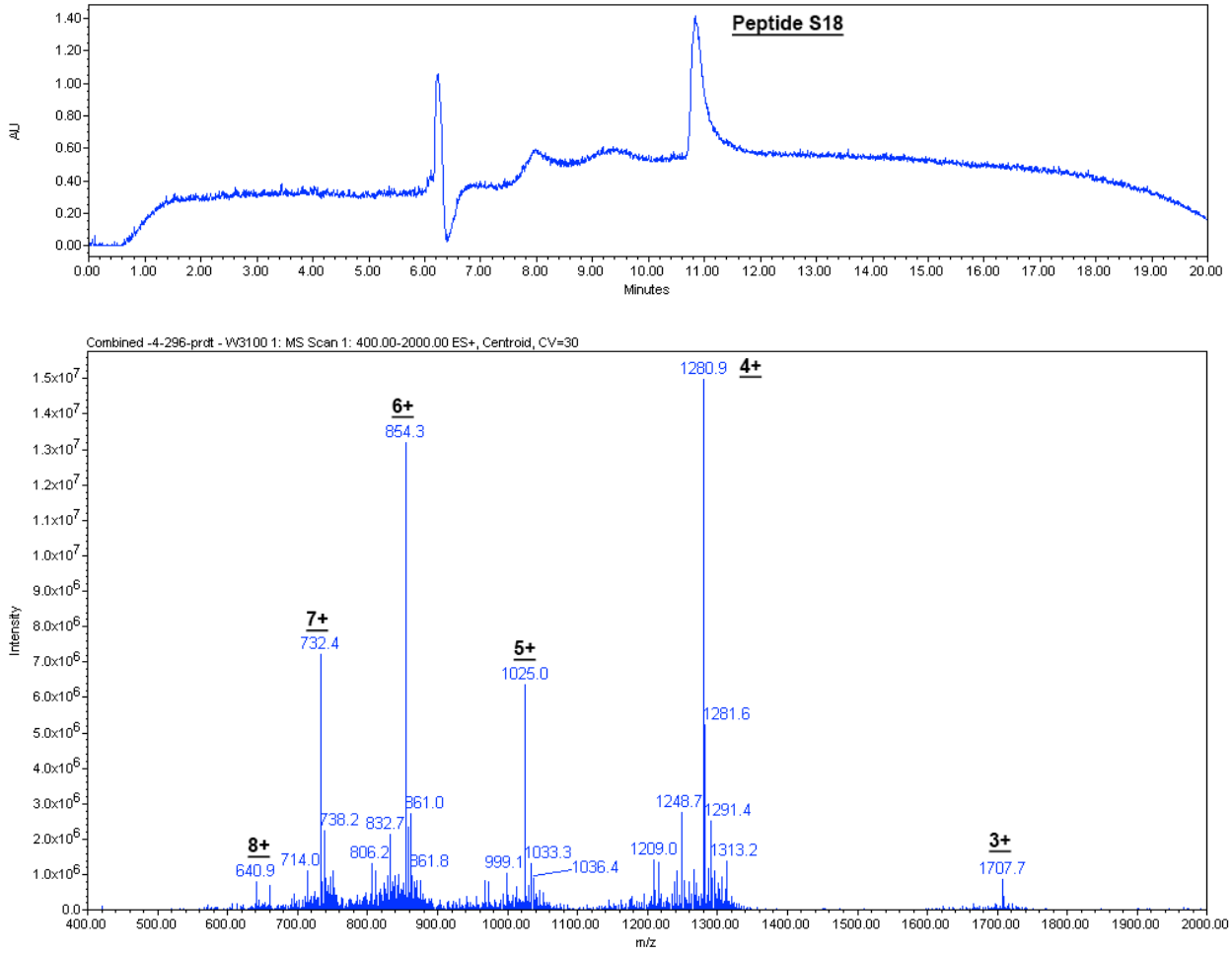
Synthesis of Peptide S22 (Scheme S5)



a) Pyridine/AcOH (1/12, mol/mol), 9h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 0.5 h, 45%. b) H_2NNH_2, 4 h; $Et_2NH/H_2O/CH_3CN$, 2 h, 57%. c) Pyridine/AcOH (1/12, mol/mol), 7h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 1 h, 57%.

1) Preparation of Peptide S18

The peptide was assembled according to general procedure. The incorporation of Fmoc-Gly(Hmb, Fmoc)-OH was carried out under standard coupling protocols; the acylation of this backbone secondary amine was performed in DMF/NMP(1/1, v/v) overnight as an optimized coupling condition.[7] Note that the hydroxyl group on Hmb were always subjected to acylation during the subsequent couplings; yet it fell off concomitantly once treated with piperidine/DMF (1/4, v/v) as Fmoc deblock solution. However, the N-terminal Ser63 was an exception: while Boc-Ser(tBu)-OH was introduced normally, it required up to 11h to be fully removed by the aforementioned piperidine/DMF (1/4, v/v) solution. After the hydroxyl group was liberated in this way, it was further acylated by Ac_2O/Et_3N/DMAP in anhydrous CH_2Cl_2. The global deprotection was performed as mentioned above for up to 4 h in the case of total removal of the benzyl group on phosphate. Preparative HPLC purification (10-40% CH_3CN/H_2O over 30 min) followed by concentration under *vacuo* and lyophilization gave 192 mg of the fragment **Peptide S18** out of 1 g of the resin (19% yield based on resin loading).



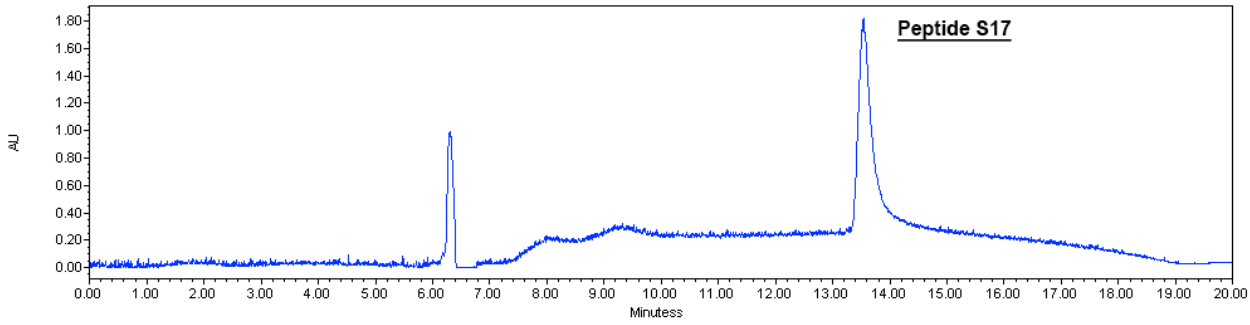
Peptide S18 was characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min). ESI calcd for $C_{214}H_{362}N_{67}O_{78}$ [M+3H] $^{3+}$ m/z = 1707.55 [M+4H] $^{4+}$ m/z = 1280.92 [M+5H] $^{5+}$ m/z = 1024.93, [M+6H] $^{6+}$ m/z = 854.28 [M+7H] $^{7+}$ m/z = 732.38 [M+8H] $^{8+}$ m/z = 640.96, found: 1707.7; 1280.9; 1025.0; 854.3; 732.4; 640.9.

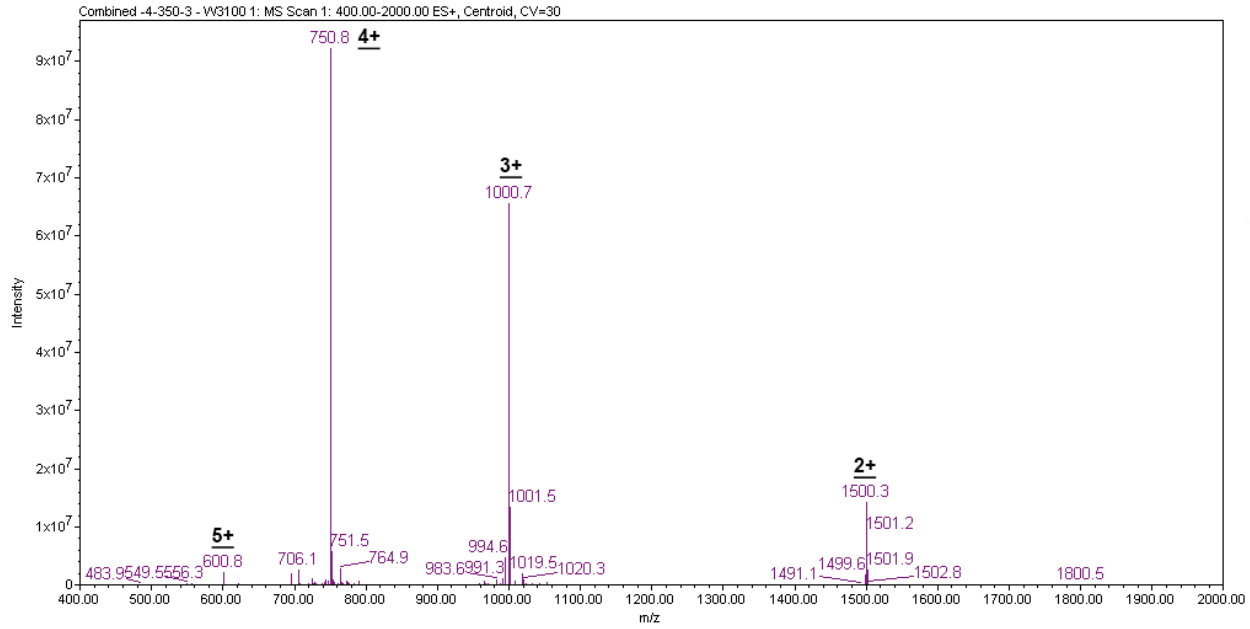
2) Preparation of Peptide S17

After the peptide fragment was assembled following the standard protocol, it was cleaved from the resin by a cocktail of CH_2Cl_2/TFE/AcOH (8/1/1, v/v/v). The solvent was removed under reduced pressure to give the crude protected peptide.

The crude peptide (95.2 mg, 0.02 mmol) was then coupled with α, α-dimethoxy-salicylaldehyde (71.2 mg, 0.42 mmol), PyBOP (55.1 mg, 0.11 mmol) and DIEA (15.0 mg, 0.12 mmol, 20.3 µL) in anhydrous CH_2Cl_2 (1.4 mL) at a concentration of 15 mM; after 10 h, it was treated with TFA/phenol/H_2O (90/5/5, v/v/v) for 2-3 h. TFA was removed under a stream of condensed air. The crude product was triturated with cold diethyl ether and centrifuged and the ether subsequently decanted. The remaining solid was purified via preparative HPLC (30-50% CH_3CN/H_2O over 30 min), followed by concentration under *vacuo* and lyophilization to give the titled fragment **Peptide S17** (22.9 mg, 36%) as a white

powder.



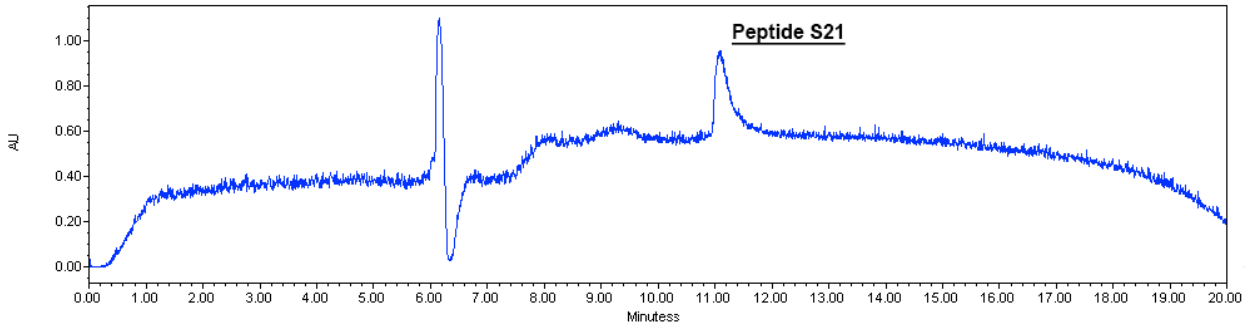


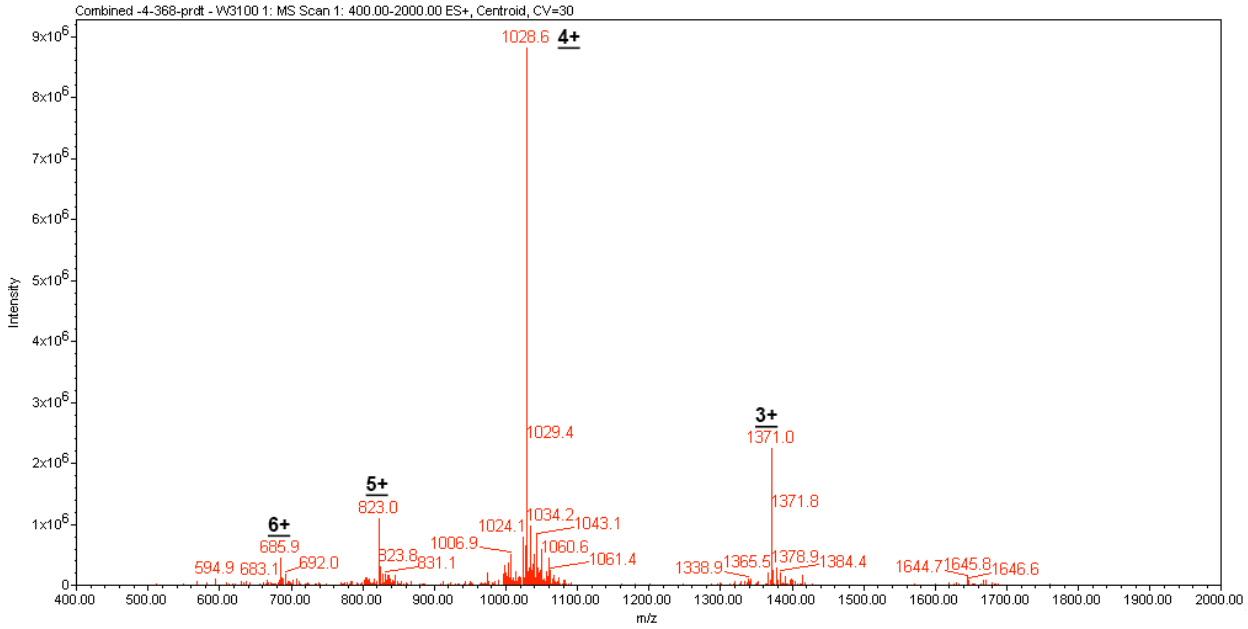
Peptide S17 Preparative HPLC purification (30-50% CH_3CN/H_2O over 30 min) followed by concentration under *vacuo* and lyophilization; characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min). ESI calcd for $C_{137}H_{218}N_{38}O_{38}$ $[M+2H]^{2+}$ m/z = 1500.30 $[M+3H]^{3+}$ m/z = 1000.54 $[M+4H]^{4+}$ m/z = 750.65 $[M+5H]^{5+}$ m/z = 600.72, found: 1500.3; 1000.7; 750.8; 600.8.

3) Preparation of Peptide S21

After the fragment was assembled following the standard protocol, it was acetylated at the N-terminus by Ac_2O/Et_3N/DMAP in anhydrous CH_2Cl_2. It was then cleaved from the resin by a cocktail of CH_2Cl_2/TFE/AcOH (8/1/1, v/v/v). The solvent was removed under reduced pressure to give the crude protected peptide.

The crude peptide (0.18 g, 0.026 mmol) was further coupled with α, α-dimethoxy-salicylaldehyde (86.5 mg, 0.52 mmol), PyBOP (66.9 mg, 0.13 mmol) and DIEA (18.3 mg, 0.40 mmol, 24.6 μL) in anhydrous CH_2Cl_2 (1.7 mL) at a concentration of 15 mM; after 10 h, it was treated with TFA/phenol/H_2O (90/5/5, v/v/v) for 2-3 h. TFA was removed under a stream of condensed air. The crude product was triturated with cold diethyl ether and centrifuged and the ether subsequently decanted. The remaining solid was purified via preparative HPLC (10-50% CH_3CN/H_2O over 30 min), followed by concentration under *vacuo* and lyophilization to give the titled fragment **Peptide S21** (17.5 mg, 17%) as a white powder.

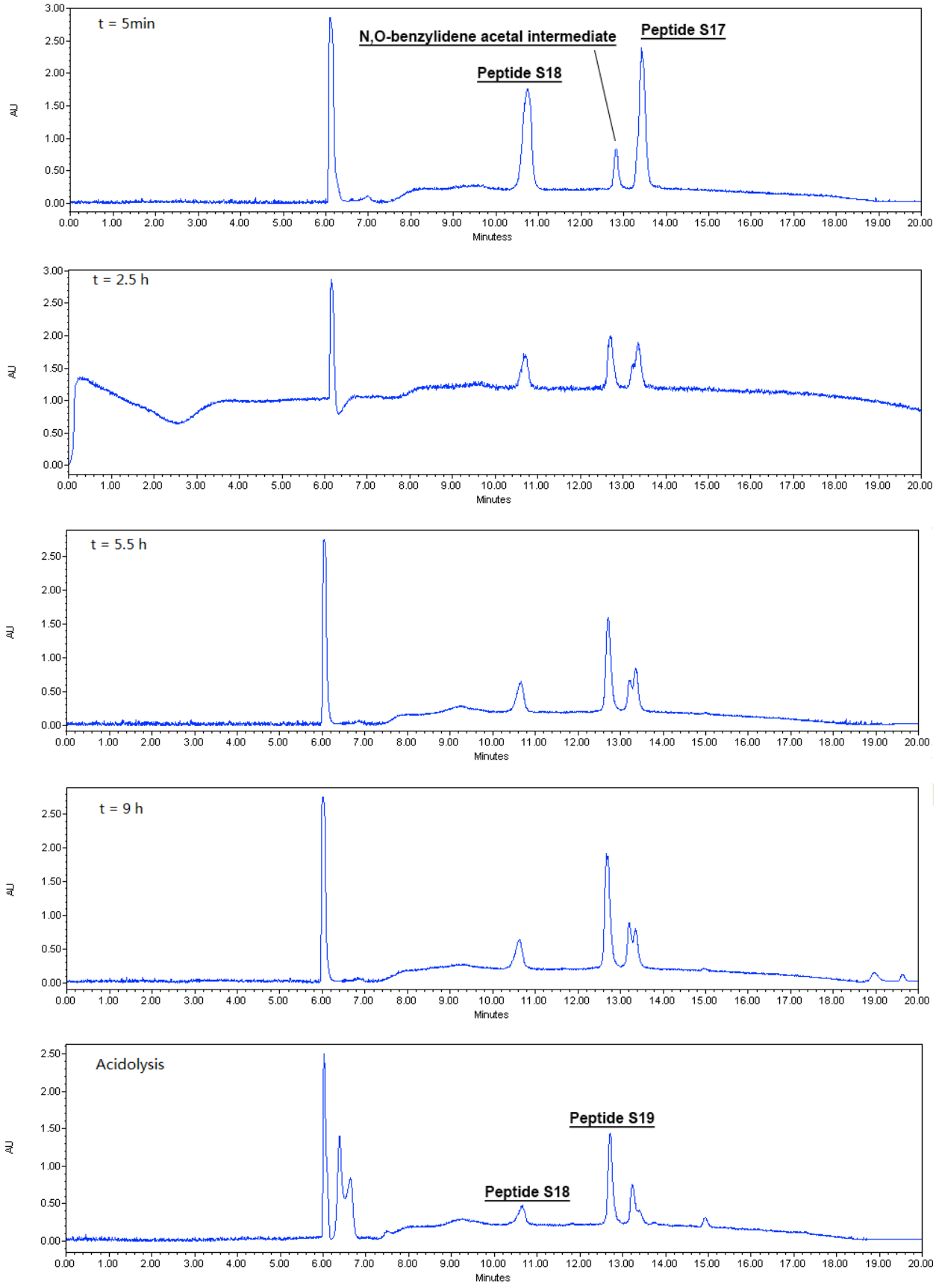


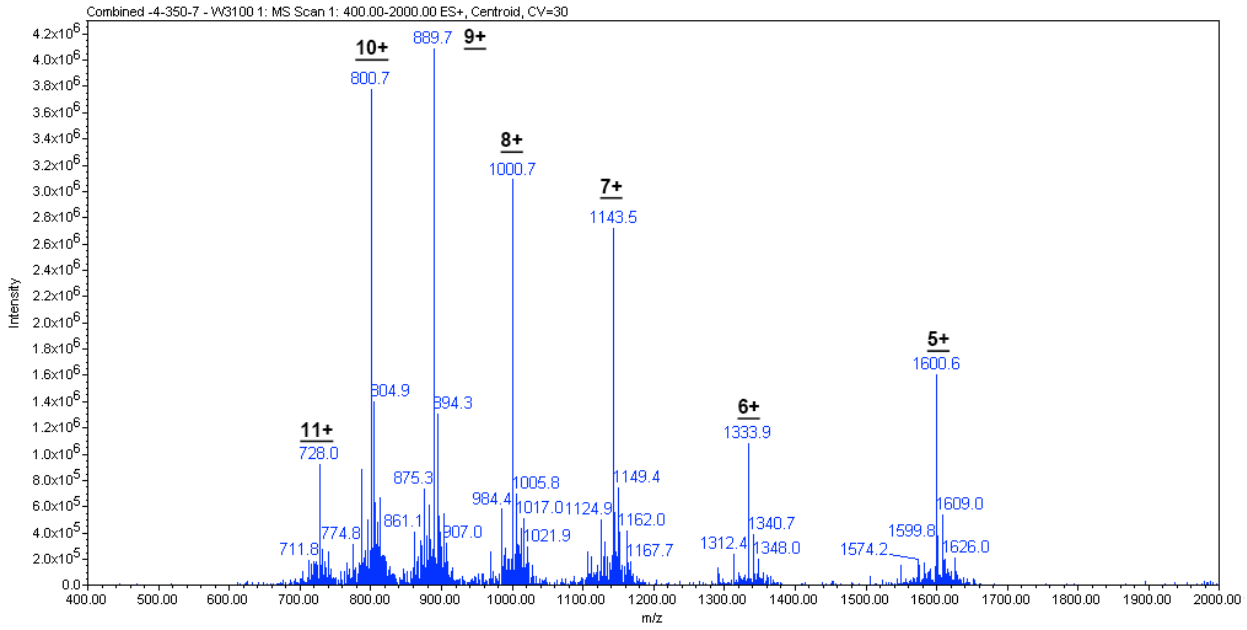


Peptide S21 was characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min). ESI calcd for $C_{172}\ H_{286}\ N_{57}\ O_{60}$ $[M+3H]^{3+}$ m/z = 1371.38 $[M+4H]^{4+}$ m/z = 1028.78 $[M+5H]^{5+}$ m/z = 823.23 $[M+6H]^{6+}$ m/z = 686.19 $[M+7H]^{7+}$ m/z = 588.31, found: 1371.0; 1028.6; 823.0; 685.9.

a) Synthesis of Peptide S19

27.2 mg of **Peptide S17** (1.5 equiv.) and 30.7 mg of **Peptide S18** (1.0 equiv.) were incubated for serine ligation as described before. The reaction was monitored on a C18 analytical column with a gradient of 10-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. After the completion of the reaction within 9 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v). Preparative HPLC purification (10–50% CH_3CN/H_2O over 30 min) followed by concentration at reduced pressure and lyophilization afforded 21.3 mg (45% yield) of **Peptide S19** as a white powder. (Recovery of **Peptide S18** 8%).

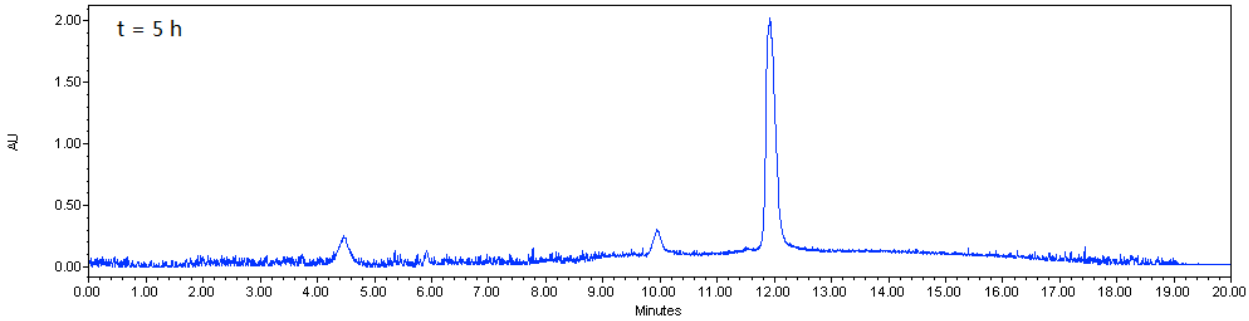

t = 5min
N,O-benzylidene acetal intermediate
Peptide S17
Peptide S18
t = 2.5 h
t = 5.5 h
t = 9 h
Acidolysis
Peptide S19
Peptide S18
AU
Minutes

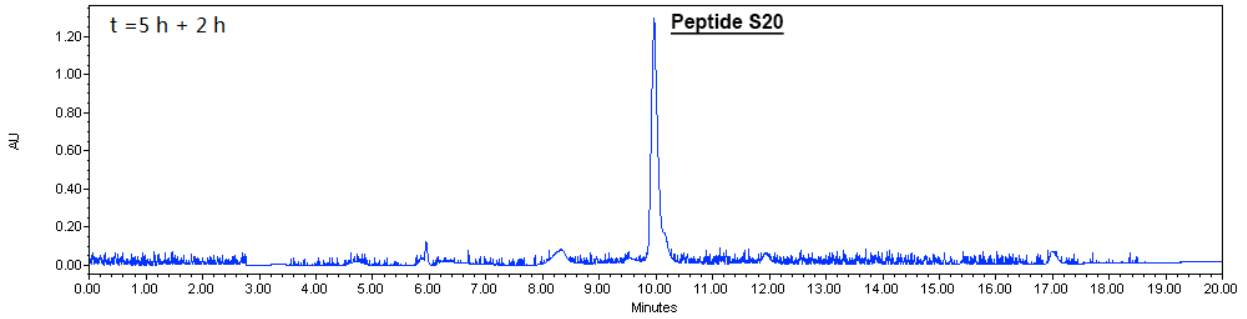


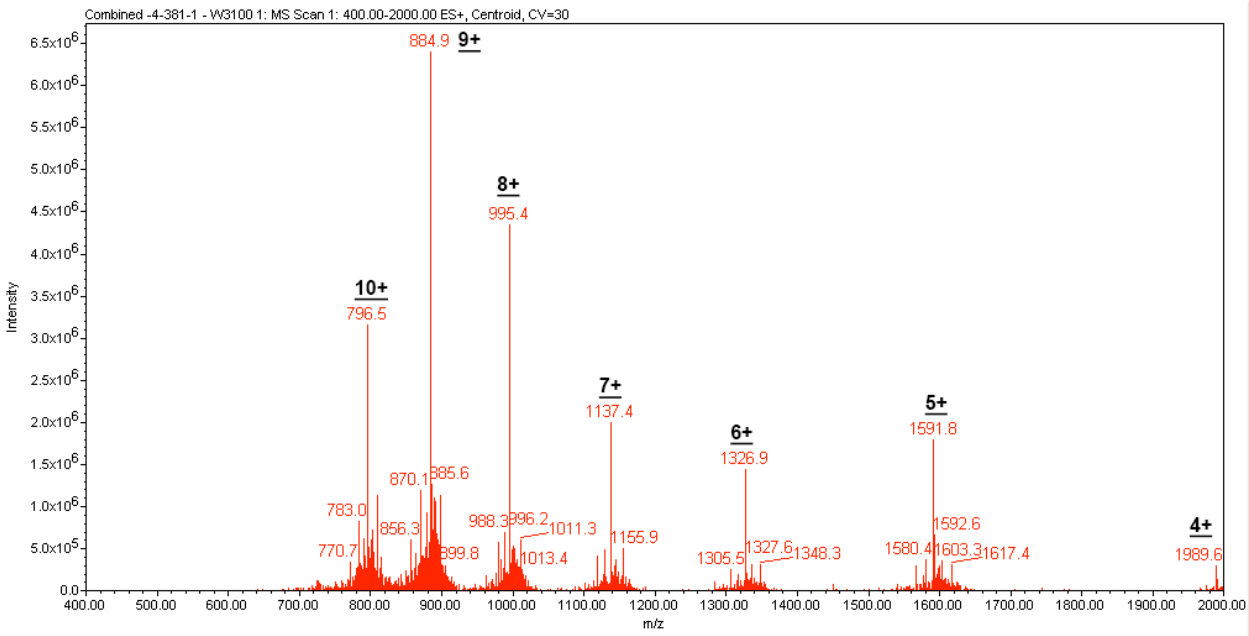
Peptide S19 ESI calcd for $C_{344}H_{568}N_{105}O_{114}$ $[M+5H]^{5+}$ m/z = 1600.45 $[M+6H]^{6+}$ m/z = 1333.87 $[M+7H]^{7+}$ m/z = 1143.46 $[M+8H]^{8+}$ m/z = 1000.66 $[M+9H]^{9+}$ m/z = 889.58 $[M+10H]^{10+}$ m/z = 800.73 $[M+11H]^{11+}$ m/z = 728.02, found: 1600.6; 1333.9; 1143.5; 1000.7; 889.7; 800.7; 728.0.

b) Synthesis of **Peptide S20**

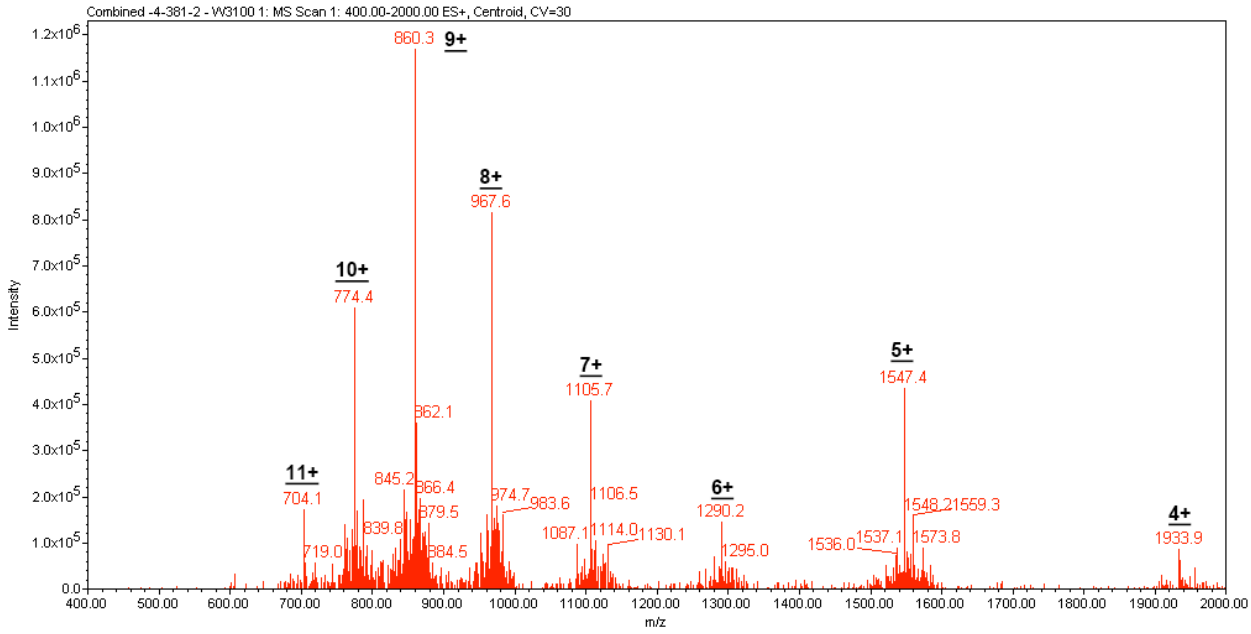
13.0 mg of **Peptide S19** was treated with N_2H_4 monohydrate (10 equiv.)[8] in H_2O at a concentration of 5 mM. The reaction was monitored on a C18 analytical column with a gradient of 10-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. After the reaction reached completion within 4-5 h, 10% Et_2NH solution in CH_3CN/H_2O (1/1, v/v) was added there,[9] rendering the peptide at a concentration of 2.5 mM. After incubation for another 2 h at room temperature, the reaction was quenched with 1% aqueous TFA. Preparative HPLC purification (10–50% CH_3CN/H_2O over 30 min) followed by concentration at reduced pressure and lyophilization afforded 7.1 mg (57% yield) of **Peptide S20** as a white powder.







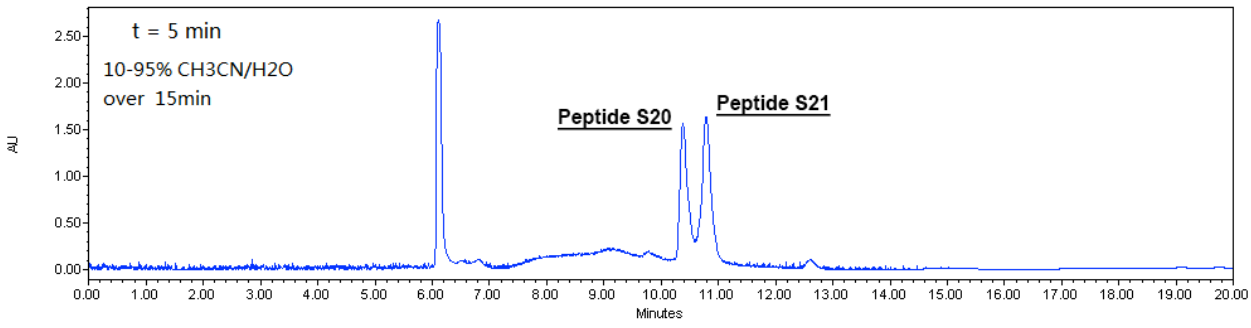
Ac removal by N_2H_4: ESI calcd for $C_{327}H_{556}N_{105}O_{111}$ $[M+4H]^{4+}$ m/z = 1989.80 $[M+5H]^{5+}$ m/z = 1592.04 $[M+6H]^{6+}$ m/z = 1326.87 $[M+7H]^{7+}$ m/z = 1137.46 $[M+8H]^{8+}$ m/z = 995.40 $[M+9H]^{9+}$ m/z = 884.92 $[M+10H]^{10+}$ m/z = 796.53, found: 1989.6; 1591.8; 1326.9; 1137.4; 995.4; 884.9; 796.5.

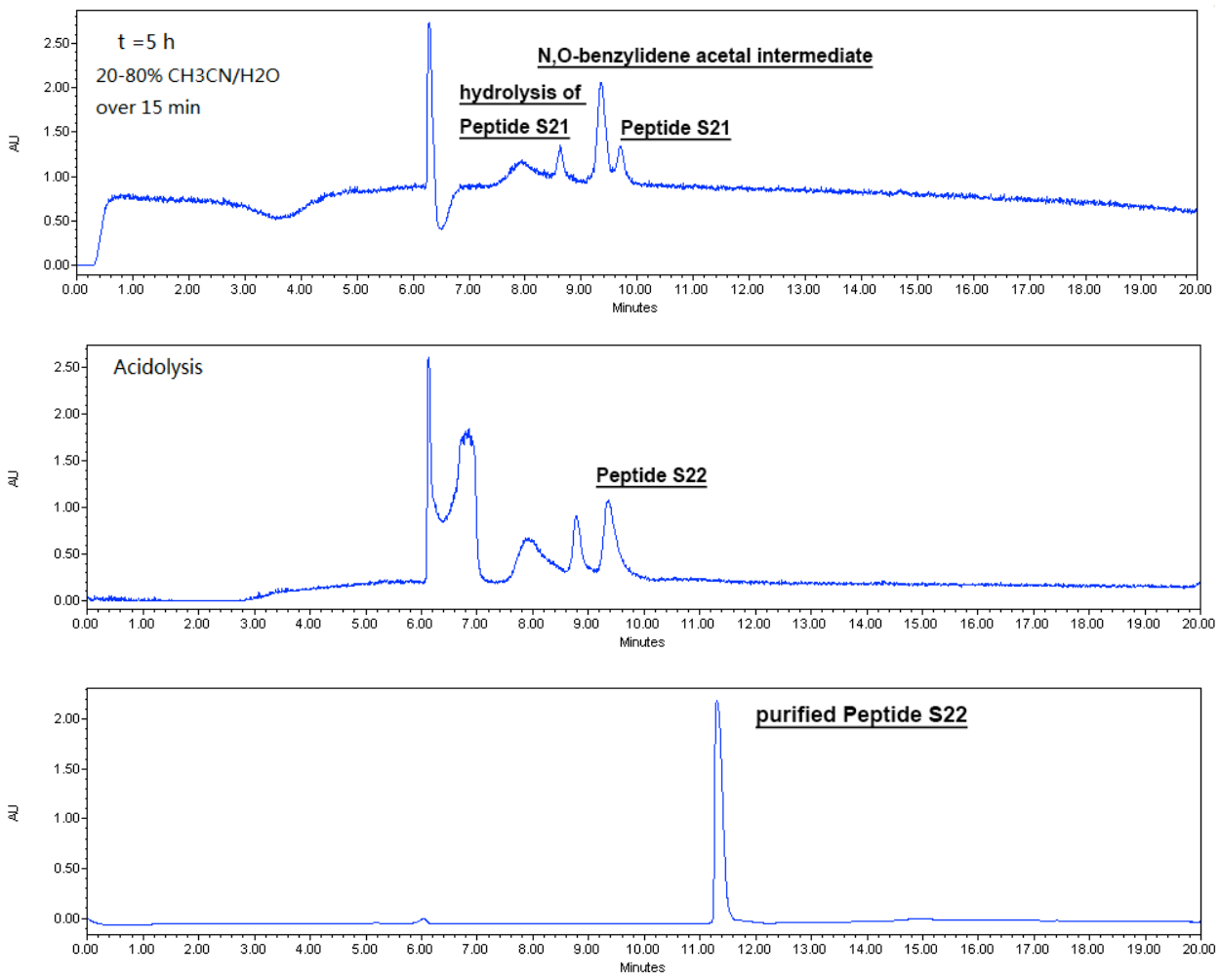


Peptide S20 *In situ* Fmoc removal: ESI calcd for $C_{327}H_{556}N_{105}O_{111}$ $[M+4H]^{4+}$ m/z = 1934.29 $[M+5H]^{5+}$ m/z = 1547.63 $[M+6H]^{6+}$ m/z = 1289.86 $[M+7H]^{7+}$ m/z = 1105.74 $[M+8H]^{8+}$ m/z = 967.65 $[M+9H]^{9+}$ m/z = 860.24 $[M+10H]^{10+}$ m/z = 774.32 $[M+11H]^{11+}$ m/z = 704.02, found: 1933.9; 1547.4; 1290.2; 1105.7; 967.6; 860.3; 774.4; 704.1.

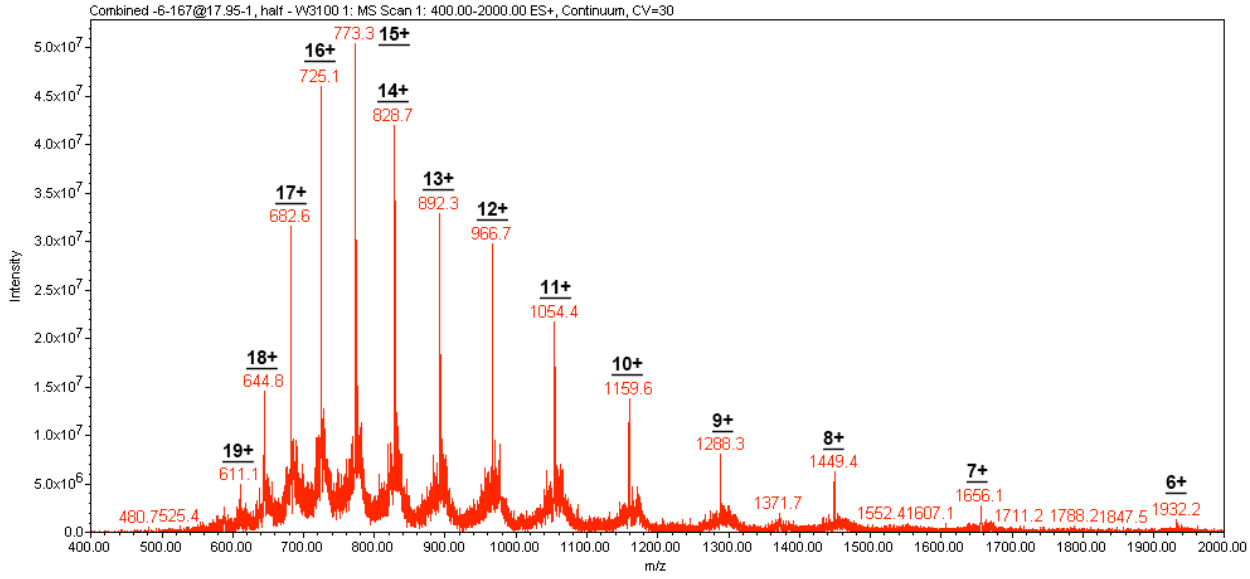
c) Synthesis of **Peptide S22**

3.1 mg of **Peptide S21** (2.0 equiv.) and 2.9 mg of **Peptide S20** (1.0 equiv.) were incubated in pyridine/acetic acid (18.6 µL, 1/12 mole/mole) at a concentration of 20 mM at room temperature. The reaction was monitored on a C4 analytical column at a flow rate of 0.6 mL/min. After the completion of the reaction within 5 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v) for up to 1 h to allow complete deprotection of Hmb which rendered native Gly96 concomitantly. Preparative HPLC purification (10-40% CH_3CN/H_2O over 30 min) followed by concentration at reduced pressure and lyophilization afforded 2.5 mg (57% yield) of **Peptide S22** as a white powder.



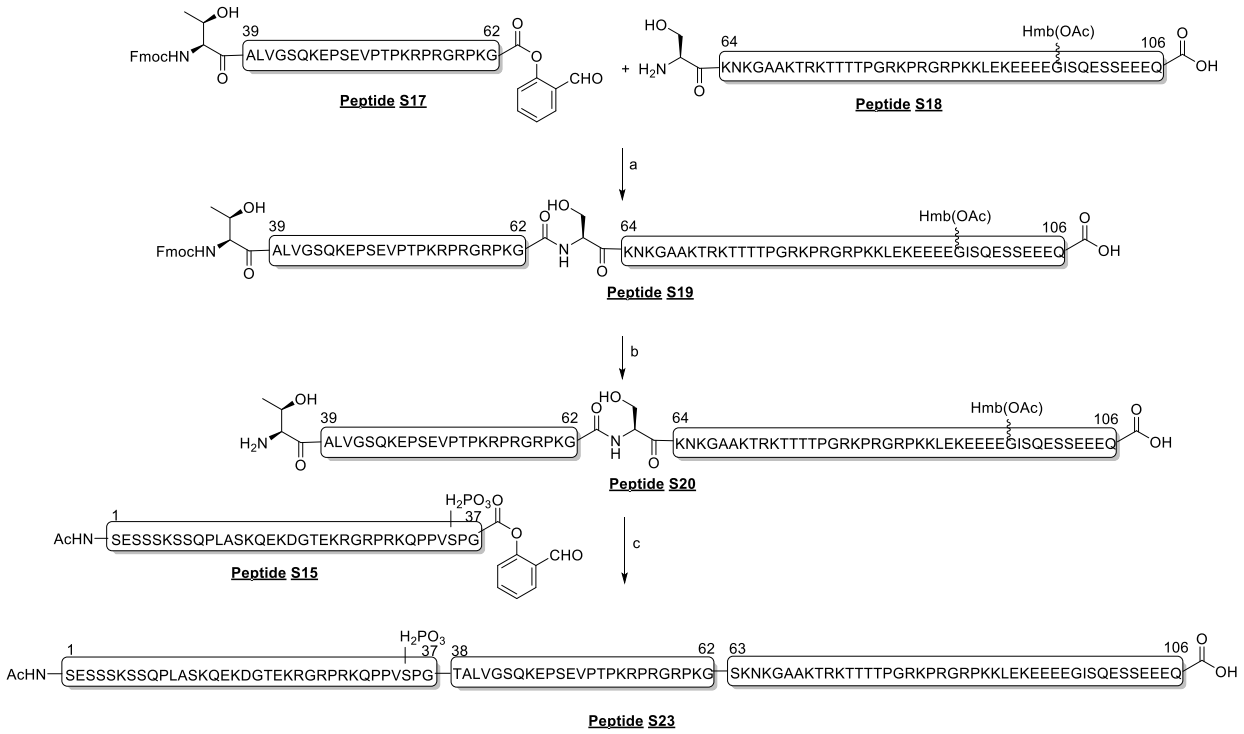


Characterization of synthetic **Peptide S22**. The analysis is run on a C18 column with a gradient of 5-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min.



Peptide S22 ESI calcd for $C_{484}H_{827}N_{162}O_{167}$ $[M+6H]^{6+}$ m/z = 1932.03 $[M+7H]^{7+}$ m/z = 1656.17 $[M+8H]^{8+}$ m/z = 1449.27 $[M+9H]^{9+}$ m/z = 1288.35 $[M+10H]^{10+}$ m/z = 1159.62 $[M+11H]^{11+}$ m/z = 1054.29 $[M+12H]^{12+}$ m/z = 966.52 $[M+13H]^{13+}$ m/z = 892.25 $[M+14H]^{14+}$ m/z = 828.59 $[M+15H]^{15+}$ m/z = 773.42 $[M+16H]^{16+}$ m/z = 725.14 $[M+17H]^{17+}$ m/z =682.54 $[M+18H]^{18+}$ m/z =644.48 $[M+19H]^{19+}$ m/z = 610.86, found: 1932.2; 1656.1; 1449.4; 1288.3; 1159.6; 1054.4; 966.7; 892.3; 828.7; 773.3; 725.1; 682.6; 644.8; 611.1.

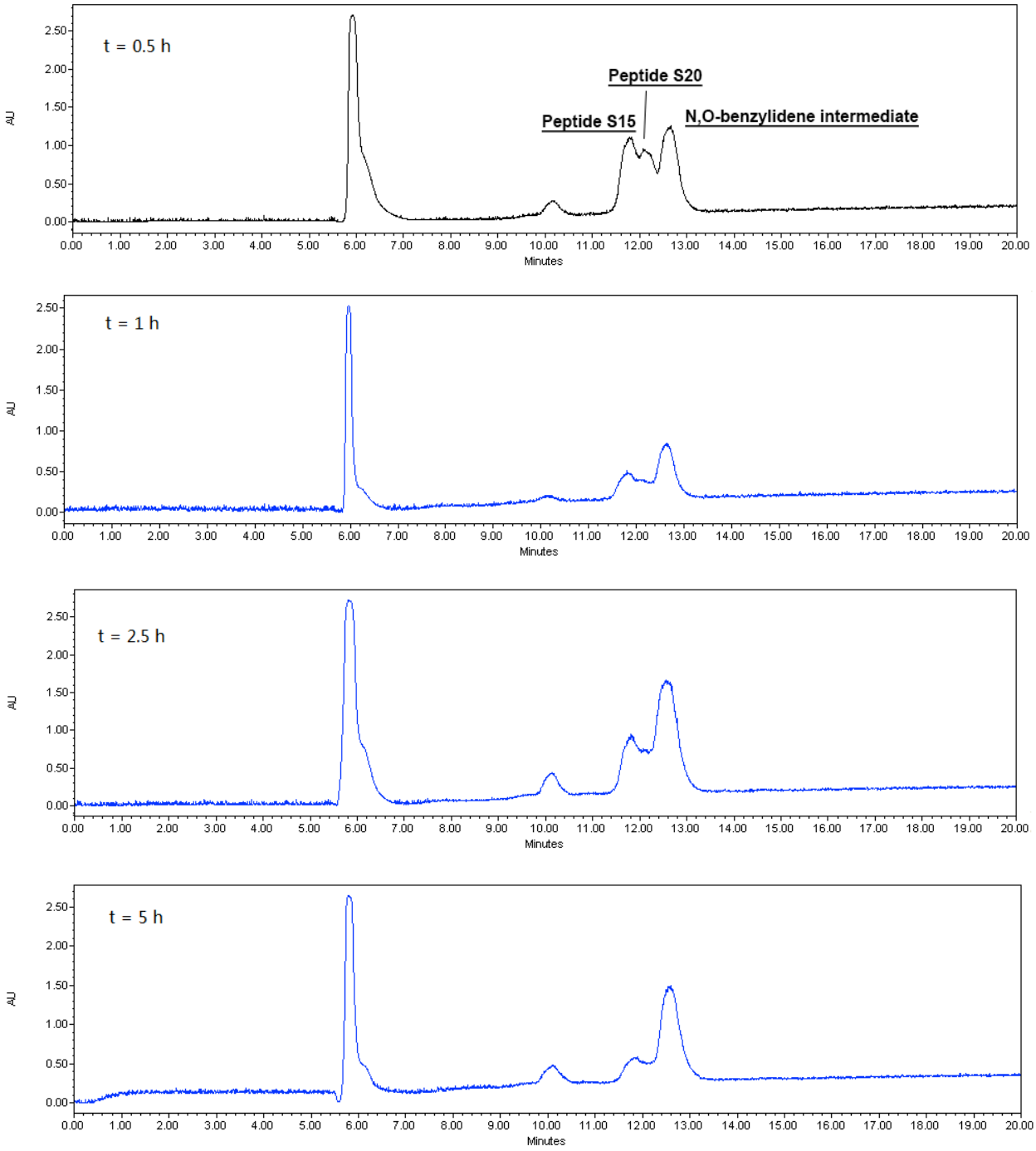
Synthesis of Peptide S23 (Scheme S6)

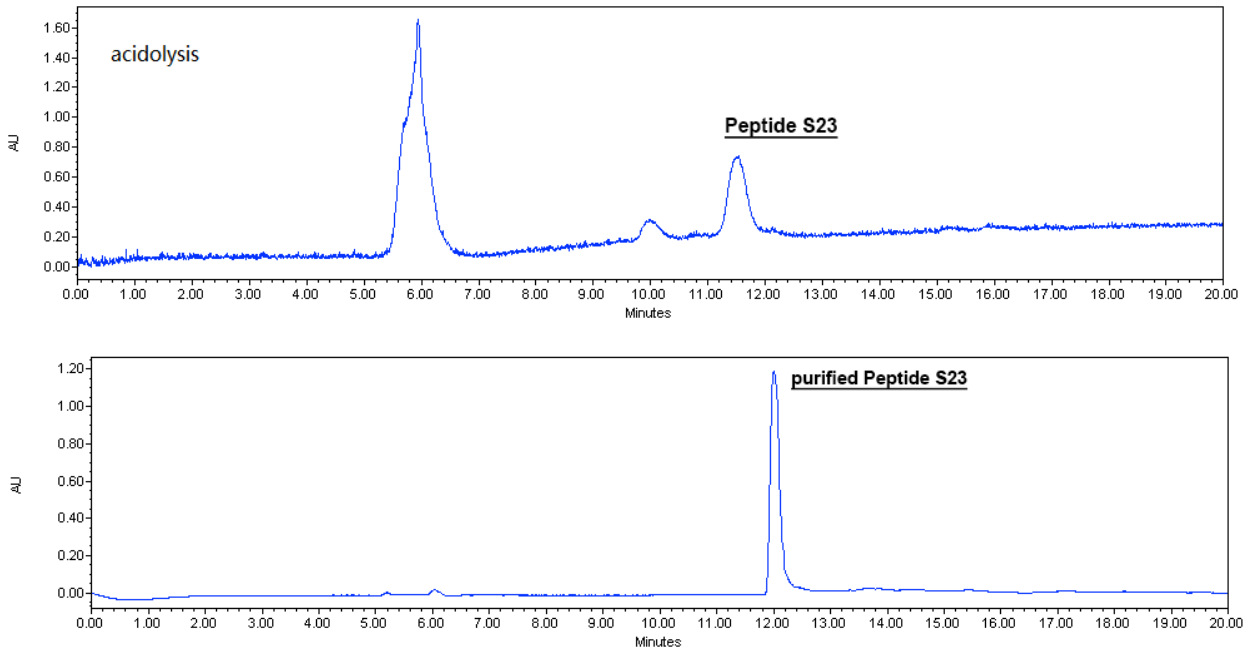


a) Pyridine/AcOH (1/12, mol/mol), 9h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 0.5 h, 45%. b) H_2NNH_2, 4 h; $Et_2NH/H_2O/CH_3CN$, 2 h, 57%. c) Pyridine/AcOH (1/12, mol/mol), 7h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 1 h, 45%.

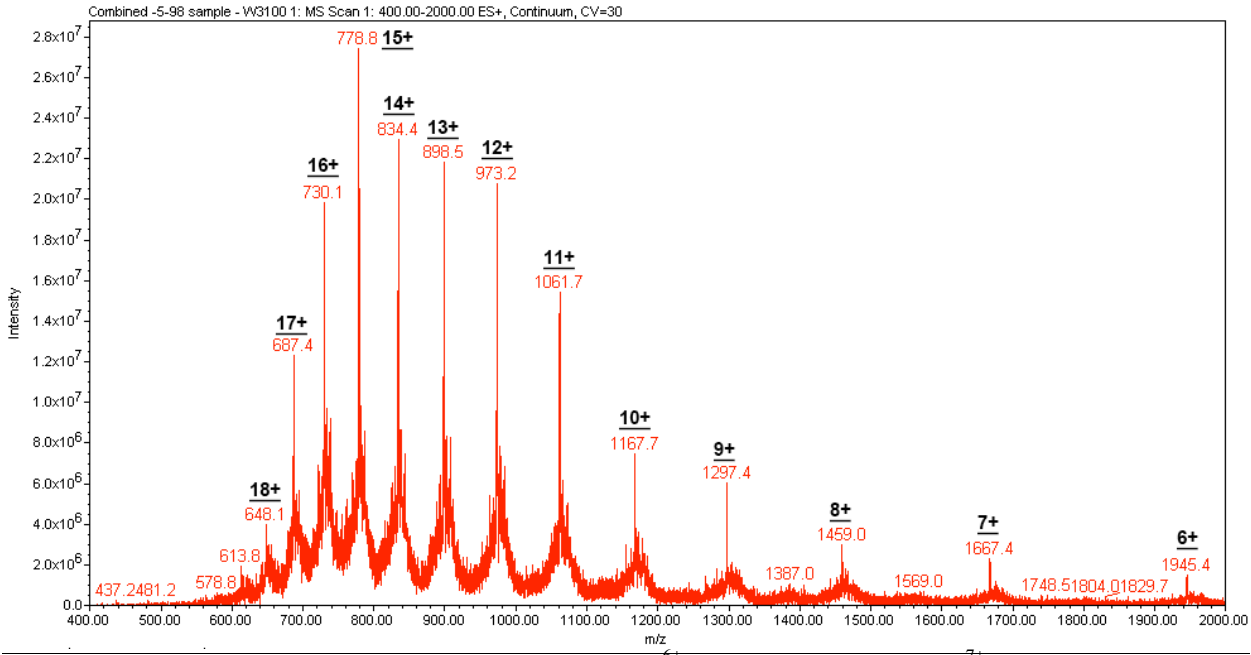
Preparation of **Peptide S15** was indicated in Scheme S4. Preparation of the fragment **Peptide S20** was indicated in Scheme S5.

2.3 mg of **Peptide S15** (2.0 equiv.) and 2.1 mg of **Peptide S20** (1.0 equiv.) were incubated in pyridine/acetic acid (13.4 µL, 1/12 mole/mole) at a concentration of 20 mM at room temperature. The reaction was monitored on a C4 analytical column with a gradient of 10-50% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. After the completion of the reaction within 5 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v) for up to 1 h to allow complete deprotection of Hmb which rendered native Gly96 concomitantly. Preparative HPLC purification (10-40% CH_3CN/H_2O containing 0.1% TFA over 30 min) followed by concentration at reduced pressure and lyophilization afforded 1.5 mg (45% yield) of **Peptide S23** as a white powder.


t = 0.5 h
Peptide S20
Peptide S15
N,O-benzylidene intermediate
t = 1 h
t = 2.5 h
t = 5 h
AU
Minutes

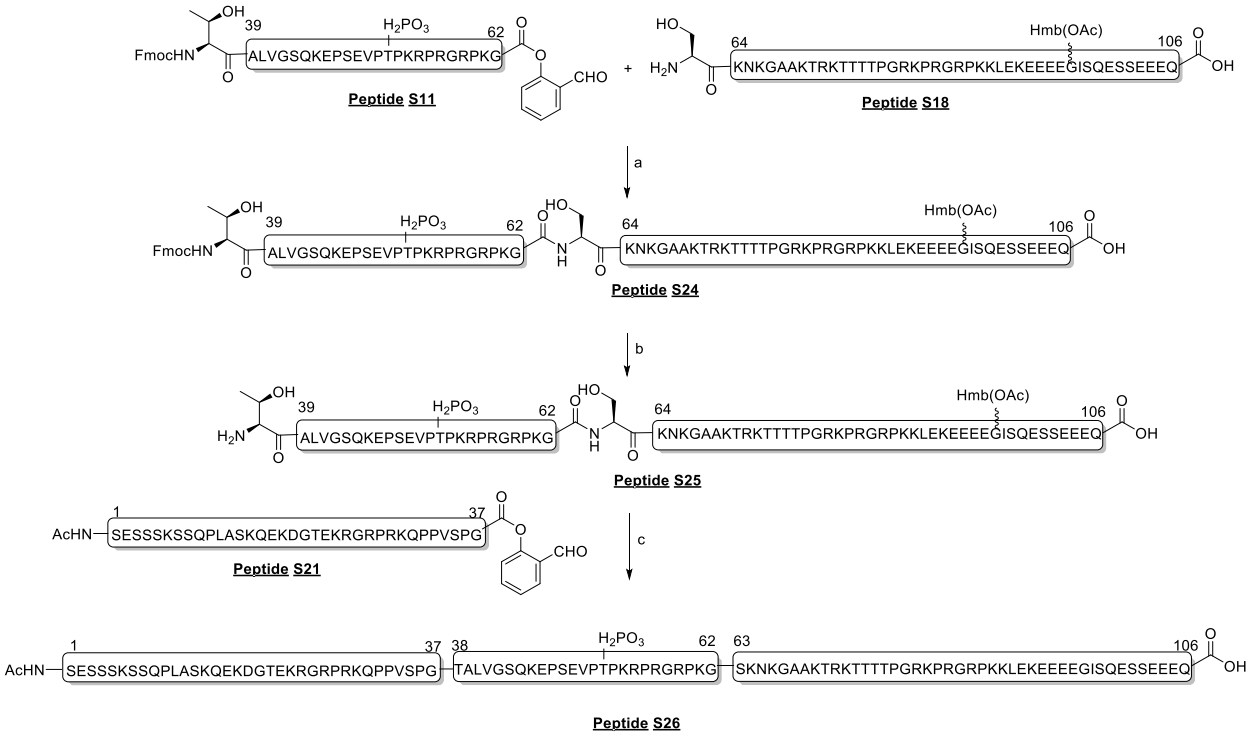


Characterization of synthetic **Peptide S23**. The analysis is run on a C18 column with a gradient of 5-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min.



Peptide S23 ESI calcd for $C_{484}H_{828}N_{162}O_{170}P$ $[M+6H]^{6+}$ m/z = 1945.36 $[M+7H]^{7+}$ m/z = 1667.59 $[M+8H]^{8+}$ m/z = 1459.27 $[M+9H]^{9+}$ m/z = 1297.24 $[M+10H]^{10+}$ m/z = 1167.62 $[M+11H]^{11+}$ m/z = 1061.56 $[M+12H]^{12+}$ m/z = 973.18 $[M+13H]^{13+}$ m/z = 898.40 $[M+14H]^{14+}$ m/z = 834.30 $[M+15H]^{15+}$ m/z = 778.75 $[M+16H]^{16+}$ m/z = 730.14 $[M+17H]^{17+}$ m/z = 687.25 $[M+18H]^{18+}$ m/z = 649.12 found: 1945.4; 1667.4; 1459.0; 1297.4; 1167.7; 1061.7; 973.2; 898.5; 834.4; 778.8; 730.1; 687.4; 648.1.

Synthesis of Peptide S26 (Scheme S7)

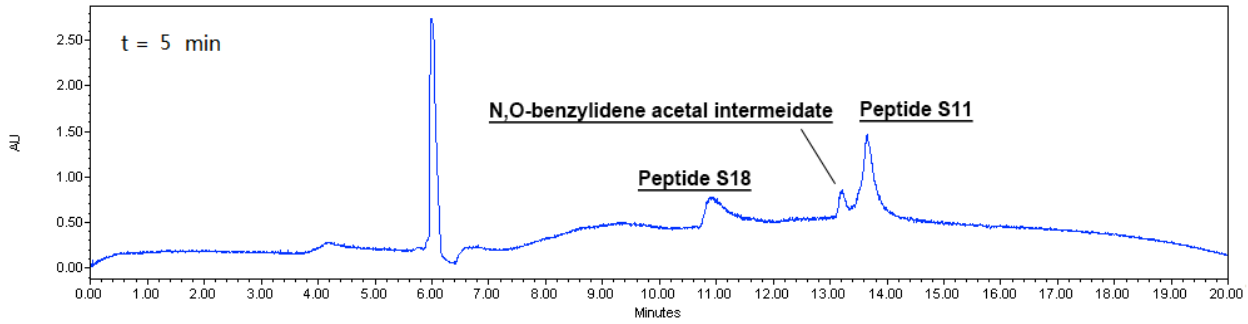


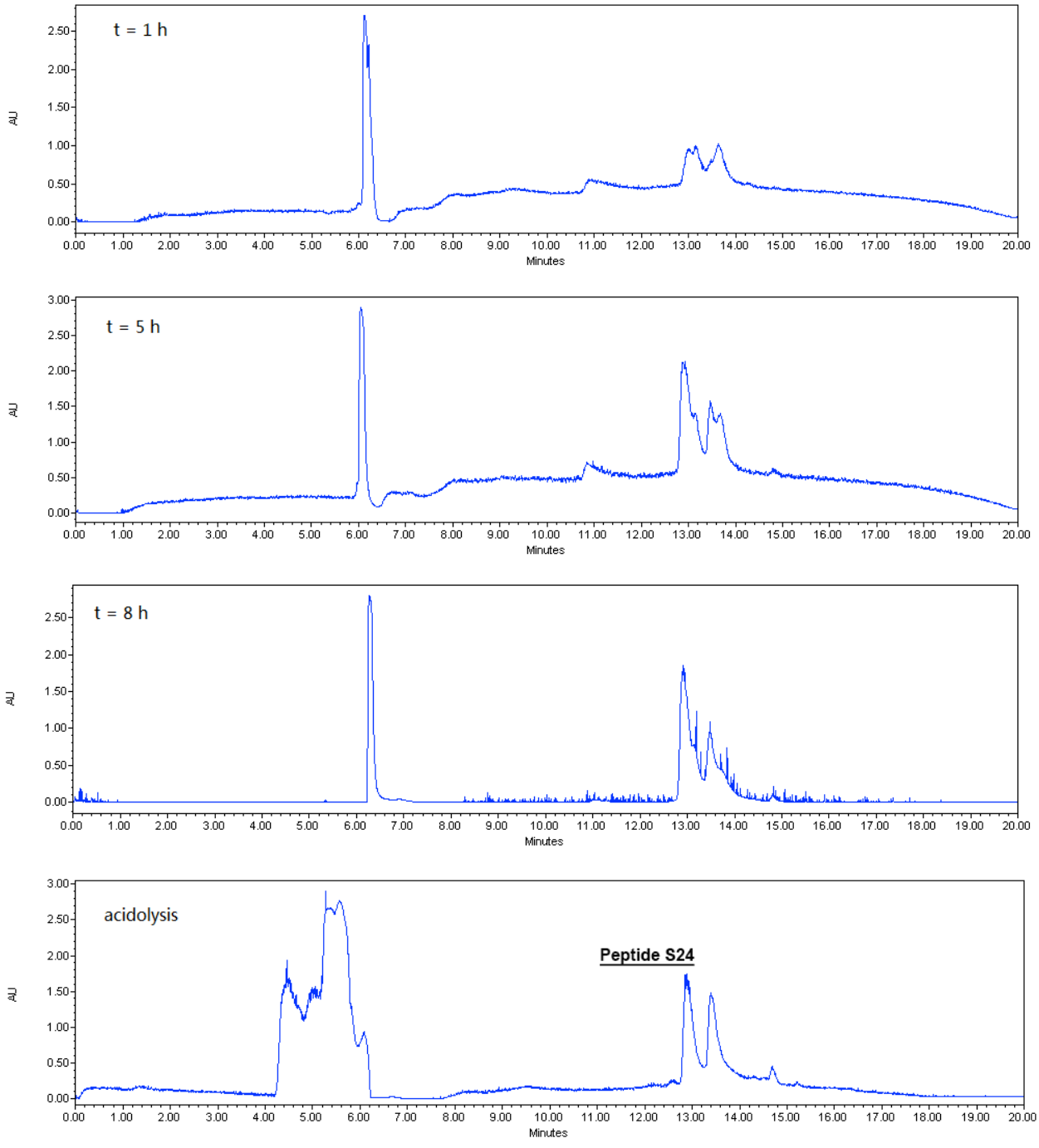
a) Pyridine/AcOH (1/12, mol/mol), 9h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 0.5 h, 45%. b) H_2NNH_2, 4 h; $Et_2NH/H_2O/CH_3CN$, 2 h, 57%. c) Pyridine/AcOH (1/12, mol/mol), 7h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 1 h, 48%.

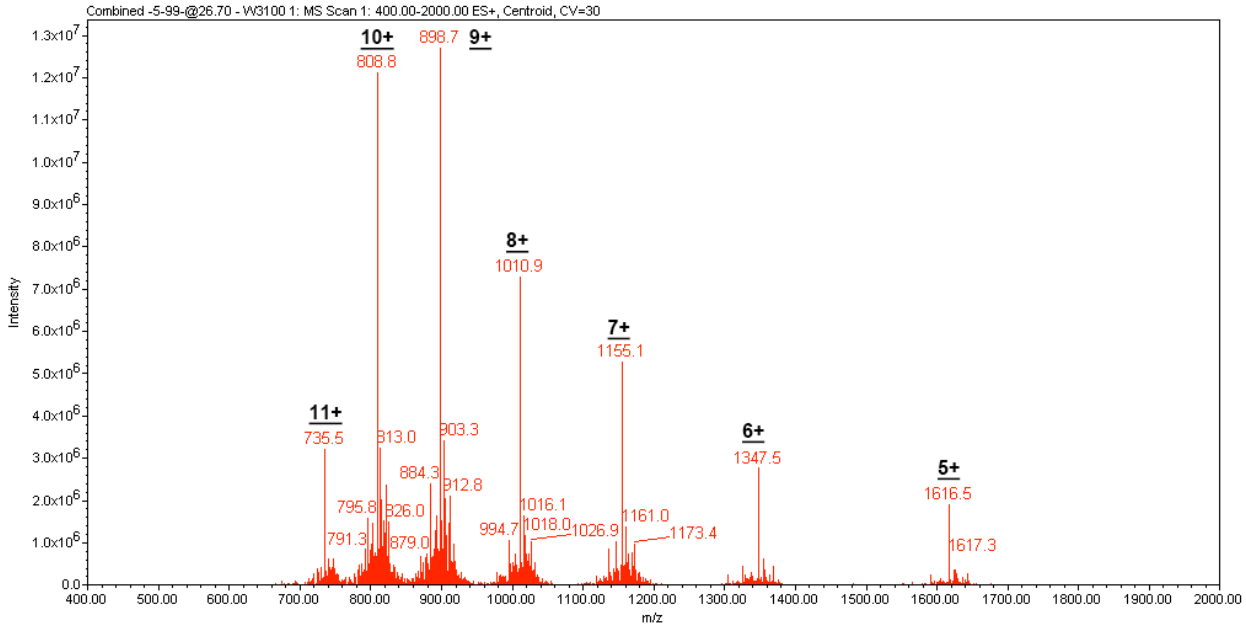
Peptide fragments are prepared as indicated above. (See Scheme S4 for **Peptide S11**; Scheme S5 for **Peptide S18** and **Peptide S21**.)

a) Synthesis of **Peptide S24**

16.5 mg of **Peptide S11** (1.5 equiv.) and 18.5 mg of **Peptide S18** (1.0 equiv.) were incubated in pyridine/acetic acid (178 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 10-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. After the completion of the reaction within 8 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v). Preparative HPLC purification (10–50% CH_3CN/H_2O containing 0.1% TFA over 30 min) followed by concentration at reduced pressure and lyophilization afforded 13.1 mg (45% yield) of **Peptide S24** as a white powder.



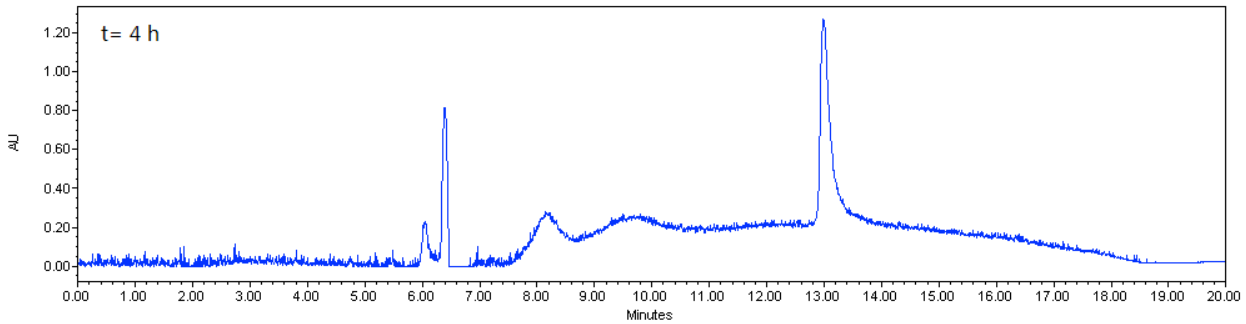

t = 1 h
t = 5 h
t = 8 h
acidolysis
Peptide S24
AU
Minutes

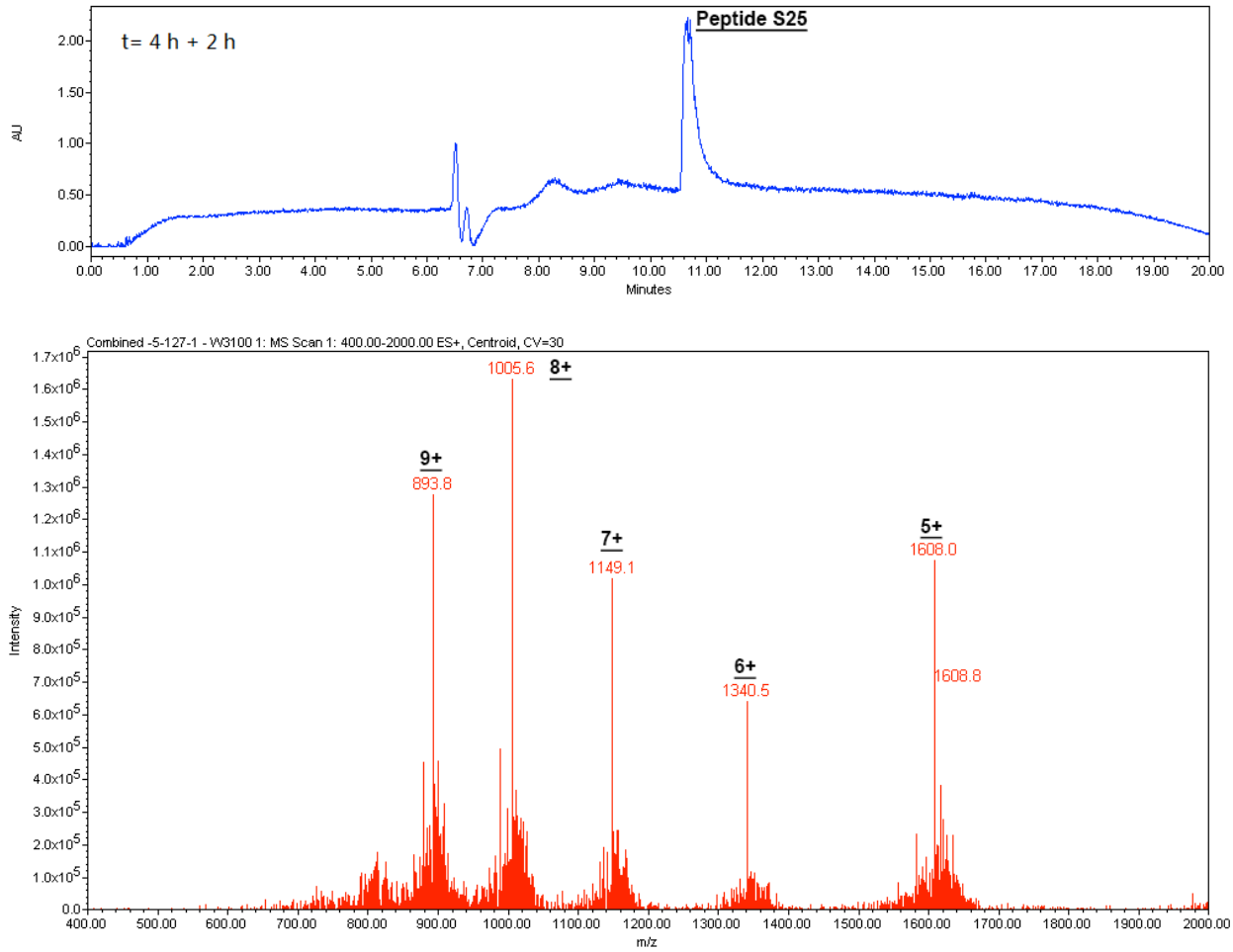


Peptide S24 ESI calcd for $C_{344}H_{569}N_{105}O_{117}P$ $[M+5H]^{5+}$ m/z = 1616.44 $[M+6H]^{6+}$ m/z = 1347.20 $[M+7H]^{7+}$ m/z = 1154.89 $[M+8H]^{8+}$ m/z = 1010.65 $[M+9H]^{9+}$ m/z = 898.47 $[M+10H]^{10+}$ m/z = 808.72 $[M+11H]^{11+}$ m/z = 735.29 found: 1616.5; 1347.5; 1155.1; 1010.9; 898.7; 808.8; 735.5.

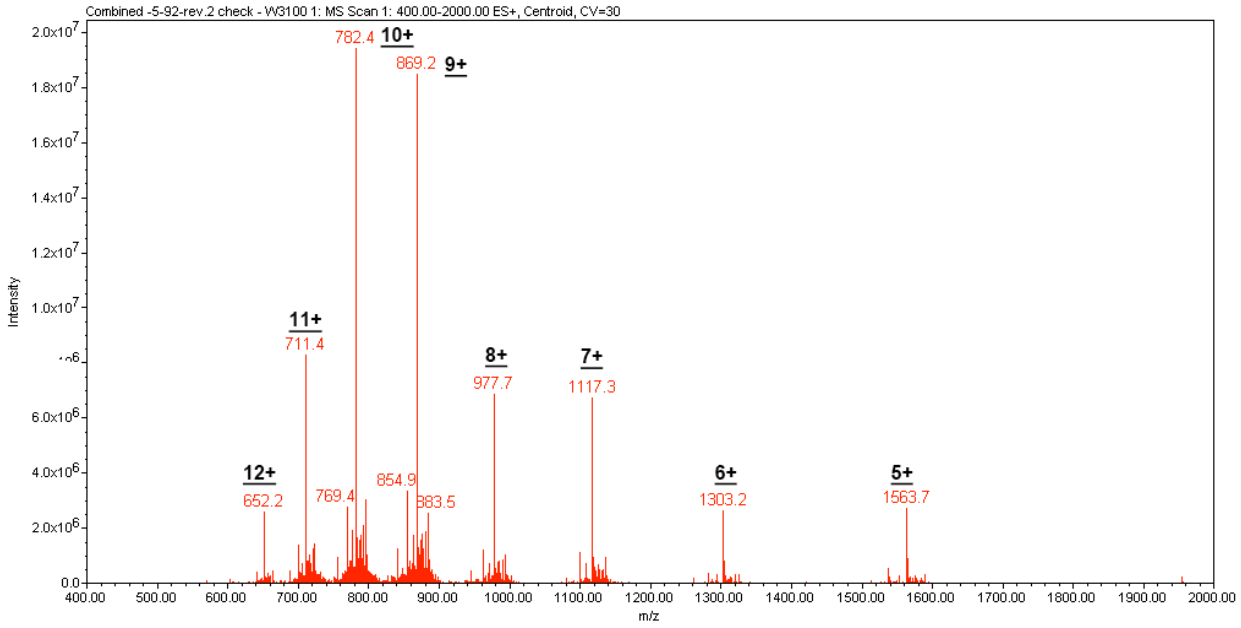
b) Synthesis of **Peptide S25**

10.7 mg of **Peptide S24** was treated with N_2H_4 monohydrate (10 equiv.)[8] 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 15 min at a flow rate of 0.6 mL/min. After the reaction reached completion within 4-5 h, 10% Et_2NH solution in CH_3CN/H_2O (1/1, v/v) was added there,[9] rendering the peptide at a concentration of 2.5 mM. After incubation for another 2 h 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 concentration at reduced pressure and lyophilization afforded 5.9 mg (57% yield) of **Peptide S25** as a white powder.





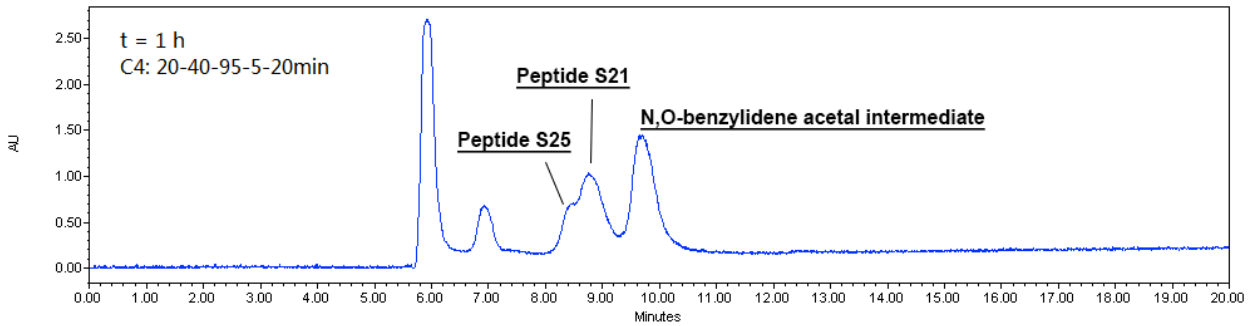
Ac removal by N_2H_4: ESI calcd for $C_{342}\ H_{567}\ N_{105}\ O_{116}P$ $[M+5H]^{5+}$ m/z = 1608.04 $[M+6H]^{6+}$ m/z = 1340.20 $[M+7H]^{7+}$ m/z = 1148.89 $[M+8H]^{8+}$ m/z = 1005.40 $[M+9H]^{9+}$ m/z = 893.80, found: 1608.0; 1340.5; 1149.1; 1005.6; 893.8.

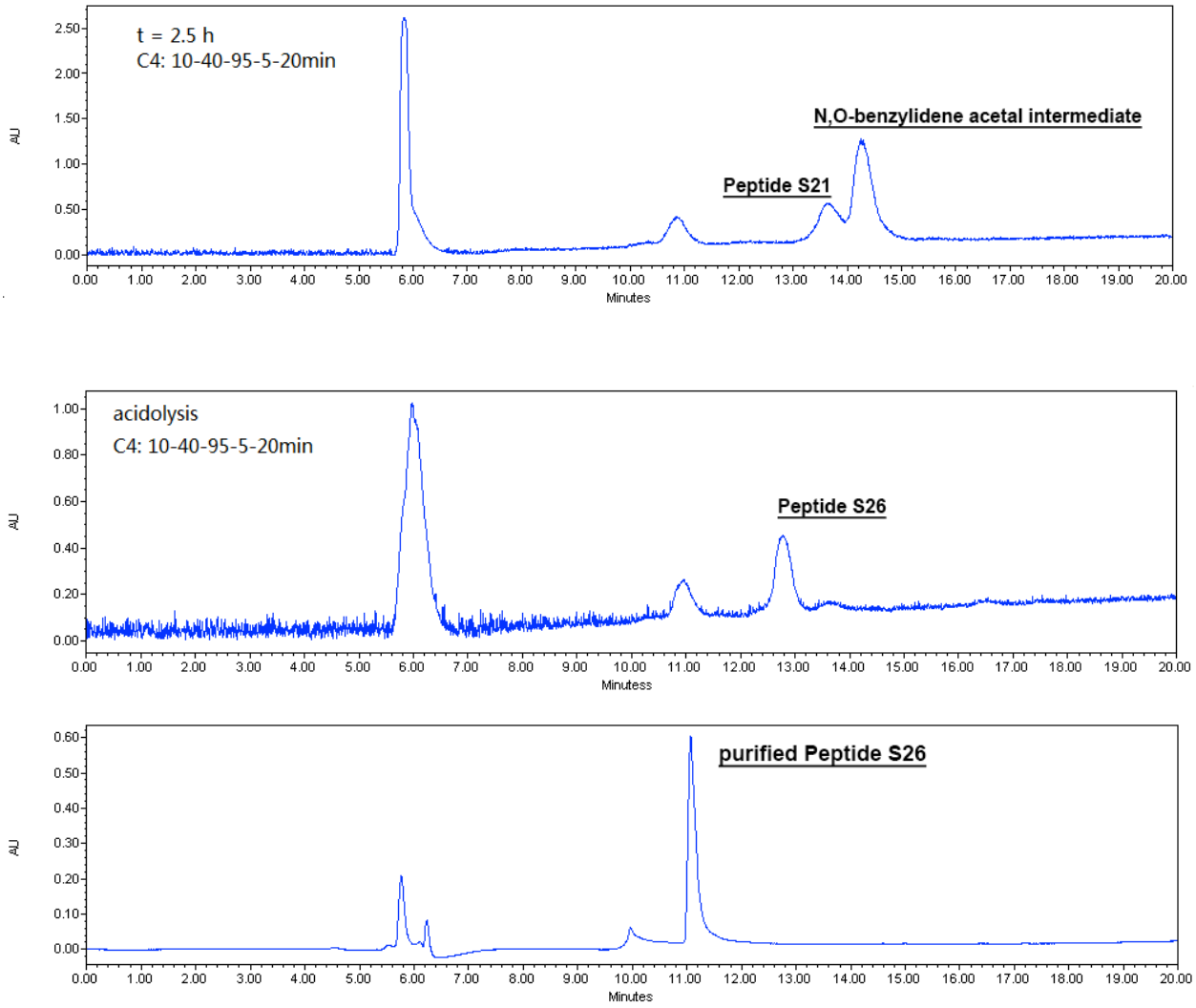


Peptide S25 *In situ* Fmoc removal: ESI calcd for for $C_{333}H_{557}N_{105}O_{114}P$ $[M+5H]^{5+}$ m/z = 1563.62 $[M+6H]^{6+}$ m/z = 1303.19 $[M+7H]^{7+}$ m/z = 1117.16 $[M+8H]^{8+}$ m/z = 977.64 $[M+9H]^{9+}$ m/z = 869.13 $[M+10H]^{10+}$ m/z = 782.32 $[M+11H]^{11+}$ m/z = 711.29, $[M+12H]^{12+}$ m/z = 652.10 found: 1563.7; 1030.2; 1117.3; 977.7; 869.2; 782.4; 711.4; 652.2.

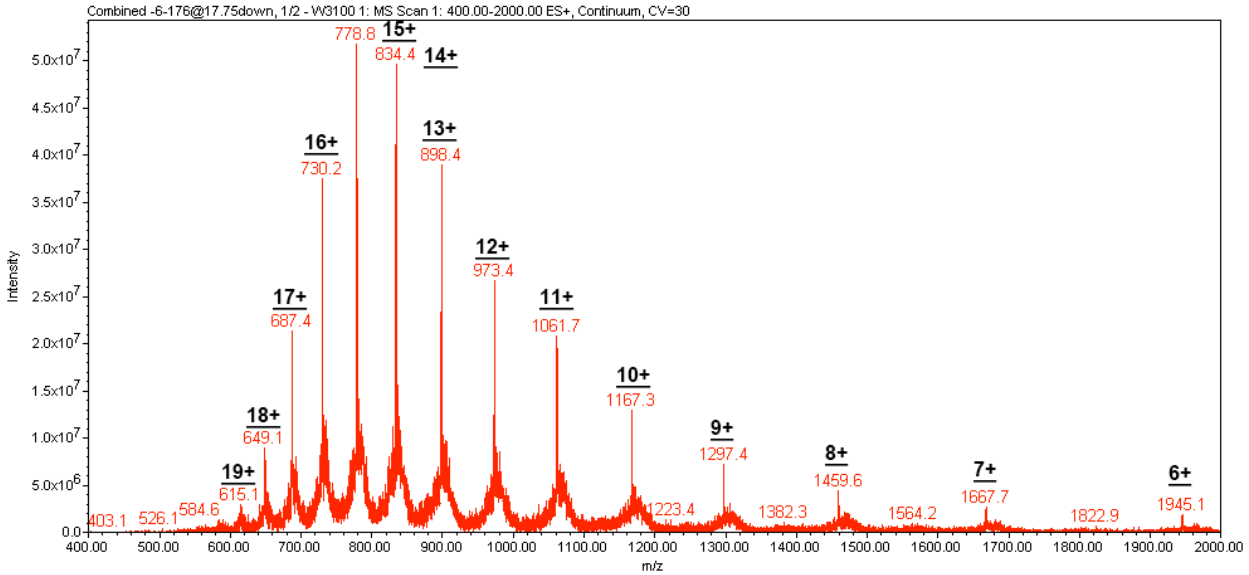
c) Synthesis of **Peptide S26**

2.2 mg of **Peptide S21** (2.0 equiv.) and 2.0 mg of **Peptide S25** (1.0 equiv.) were incubated in pyridine/acetic acid (13.0 µL, 1/12 mole/mole) at a concentration of 20 mM at room temperature. The reaction was monitored on a C4 analytical column with a variety gradient CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min, among which 10-40% was the best choice. After the completion of the reaction within 2.5 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v) for up to 1 h to allow fully deprotection of Hmb which rendered native Gly96 concomitantly, as analyzed on a C4 analytical column with a gradient of 10-40% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. Preparative HPLC purification (10-40% CH_3CN/H_2O containing 0.1% TFA over 30 min) followed by concentration at reduced pressure and lyophilization afforded 1.5 mg (48% yield) of **Peptide S26** as a white powder.





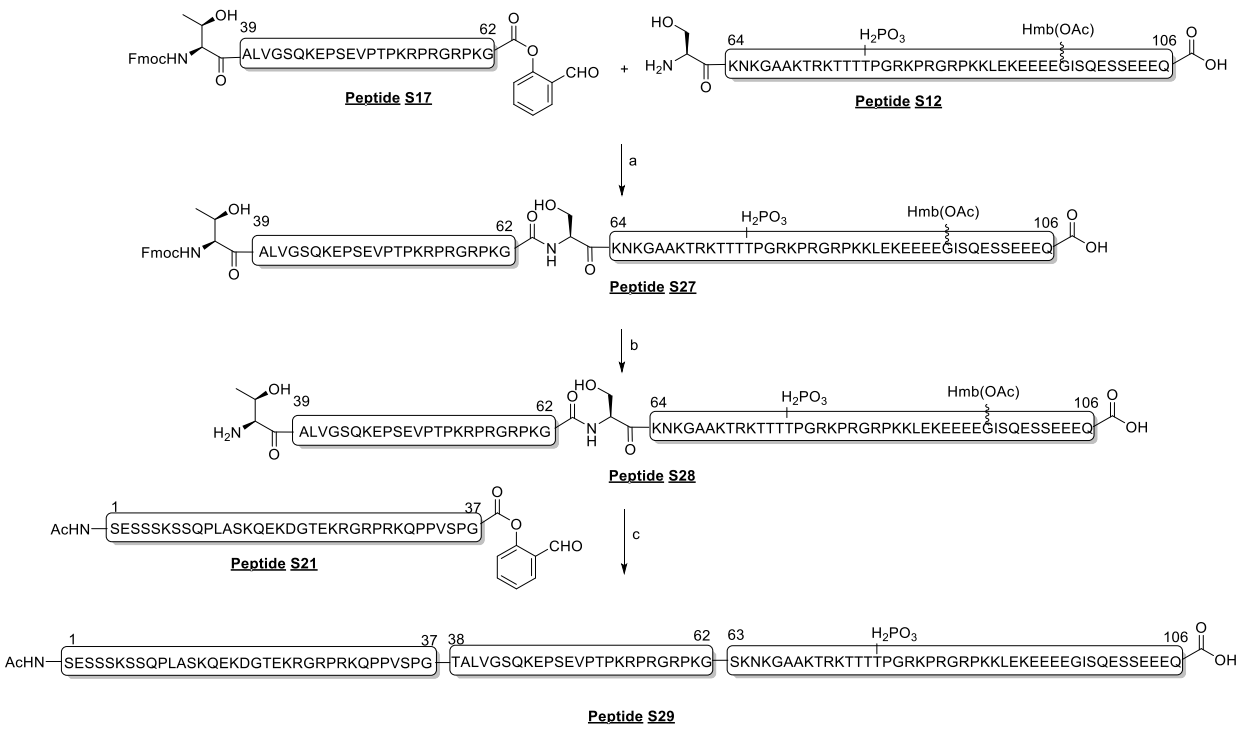
Characterization of synthetic **Peptide S26**. The analysis is run on a C18 column with a gradient of 5-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min.



Peptide S26 ESI calcd for $C_{484}H_{828}N_{162}O_{170}P$ $[M+6H]^{6+}$ m/z = 1945.36 $[M+7H]^{7+}$ m/z = 1667.59 $[M+8H]^{8+}$ m/z = 1459.27 $[M+9H]^{9+}$ m/z = 1297.24 $[M+10H]^{10+}$ m/z = 1167.62 $[M+11H]^{11+}$ m/z = 1061.56 $[M+12H]^{12+}$ m/z = 973.18 $[M+13H]^{13+}$ m/z = 898.40 $[M+14H]^{14+}$ m/z = 834.30 $[M+15H]^{15+}$ m/z = 778.75 $[M+16H]^{16+}$ m/z = 730.14 $[M+17H]^{17+}$ m/z = 687.25 $[M+18H]^{18+}$ m/z = 649.12 $[M+19H]^{19+}$ m/z = 615.01 found: 1945.1; 1667.7; 1459.6; 1297.4; 1167.3; 1061.7; 973.4; 898.4; 834.4; 778.8; 730.2; 687.4; 649.1; 615.1.

Synthesis of Peptide S29 (Scheme S8)

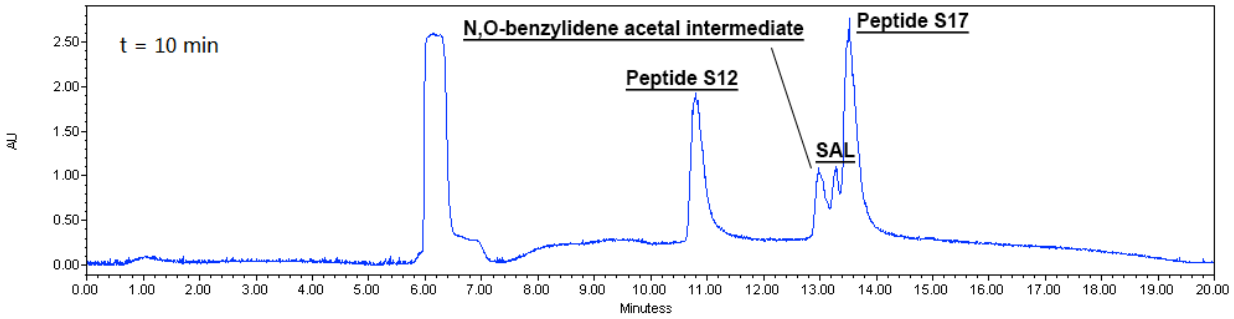
Peptide fragments are prepared as indicated above. (See Scheme S4 for **Peptide S12**; Scheme S5 for **Peptide S17** and **Peptide S21**.)

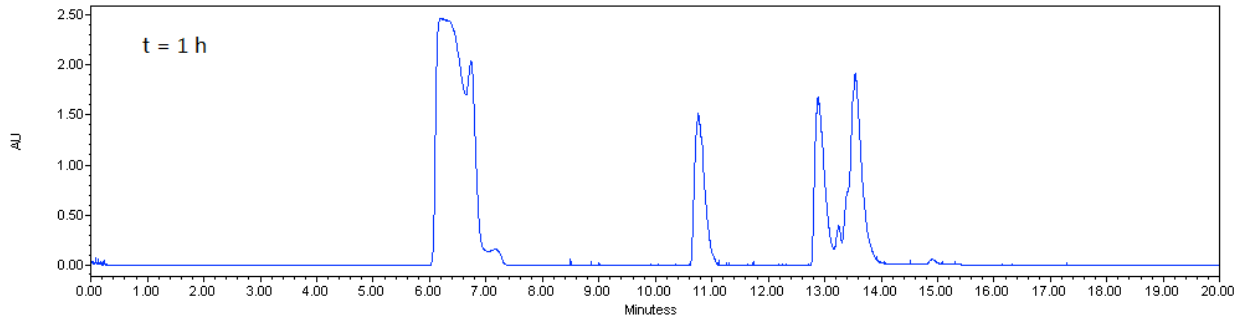


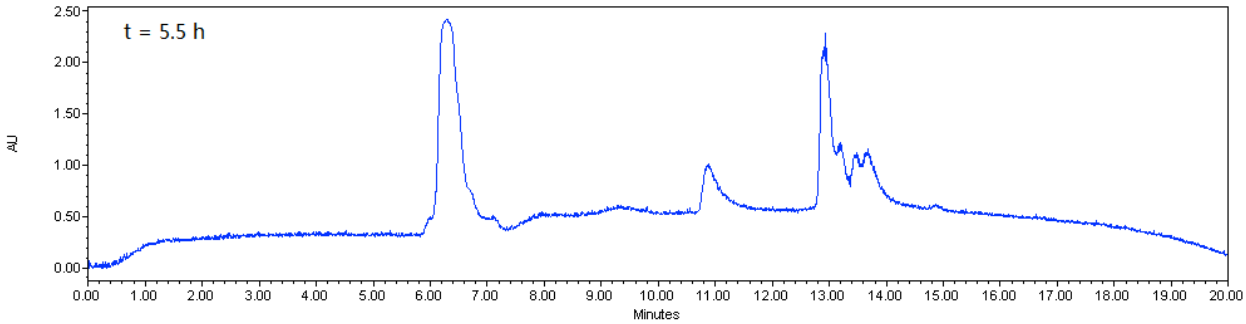
a) Pyridine/AcOH (1/12, mol/mol), 9h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 0.5 h, 40%. b) H_2NNH_2, 4 h; $Et_2NH/H_2O/CH_3CN$, 2 h, 59%. c) Pyridine/AcOH (1/12, mol/mol), 7h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 1 h, 33%.

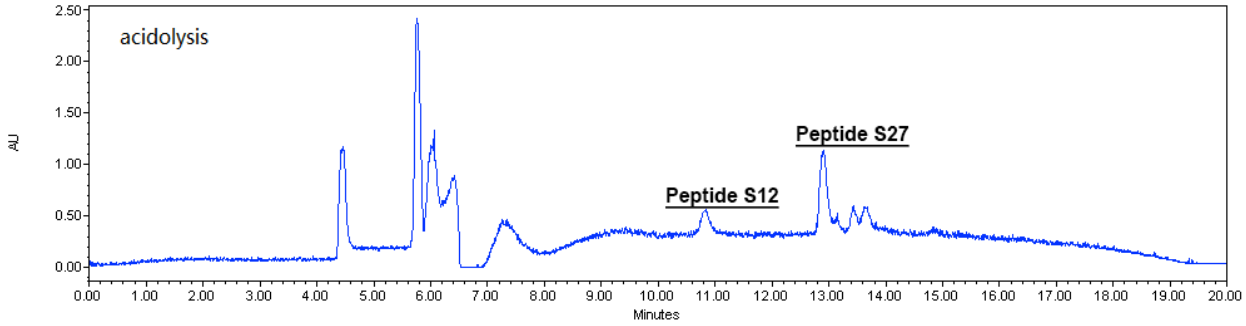
a) Synthesis of **Peptide S27**

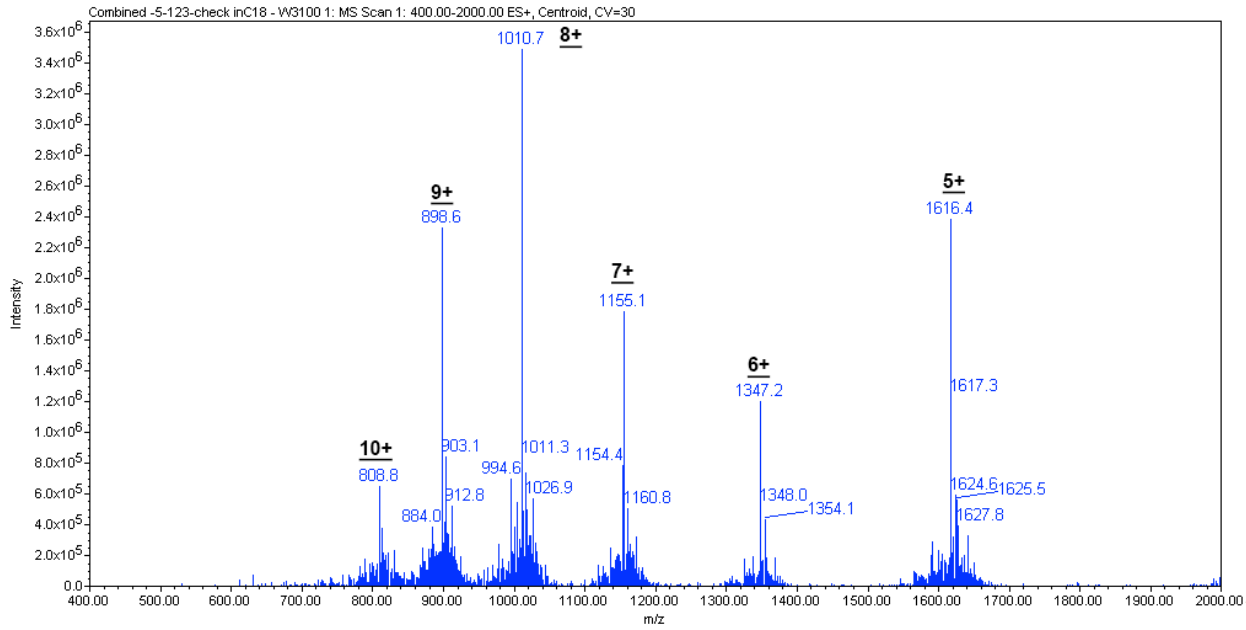
14.9 mg of **Peptide S17** (1.5 equiv.) and 17.0 mg of **Peptide S12** (1.0 equiv.) were incubated in pyridine/acetic acid (163 µL, 1/12 mole/mole) at a concentration of 10 mM at room temperature. The reaction was monitored on a C18 analytical column with a gradient of 10-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. After the completion of the reaction within 8 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v). Preparative HPLC purification (10–50% CH_3CN/H_2O containing 0.1% TFA over 30 min) followed by concentration at reduced pressure and lyophilization afforded 10.6 mg (40% yield) of **Peptide S27** as a white powder (Recovery of **Peptide S17**16%; recovery of **Peptide S12** 19%).




t = 1 h
AU
Minutes


t = 5.5 h
AU
Minutes

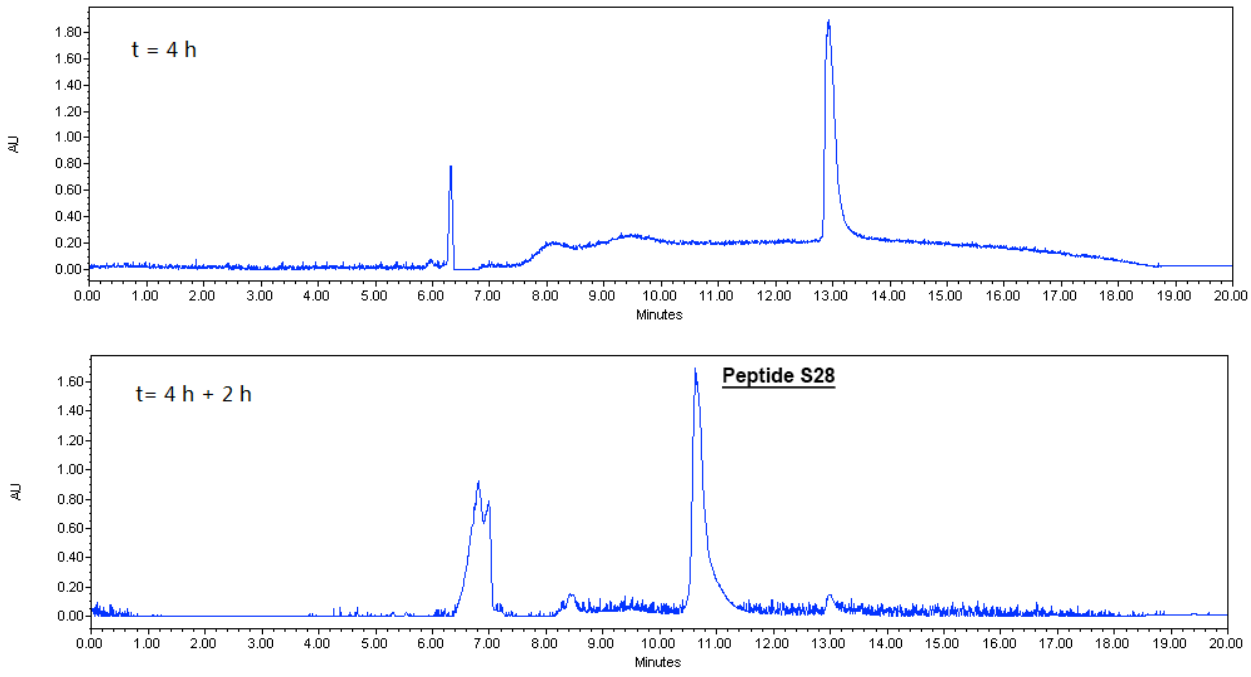

acidolysis
Peptide S27
Peptide S12
AU
Minutes

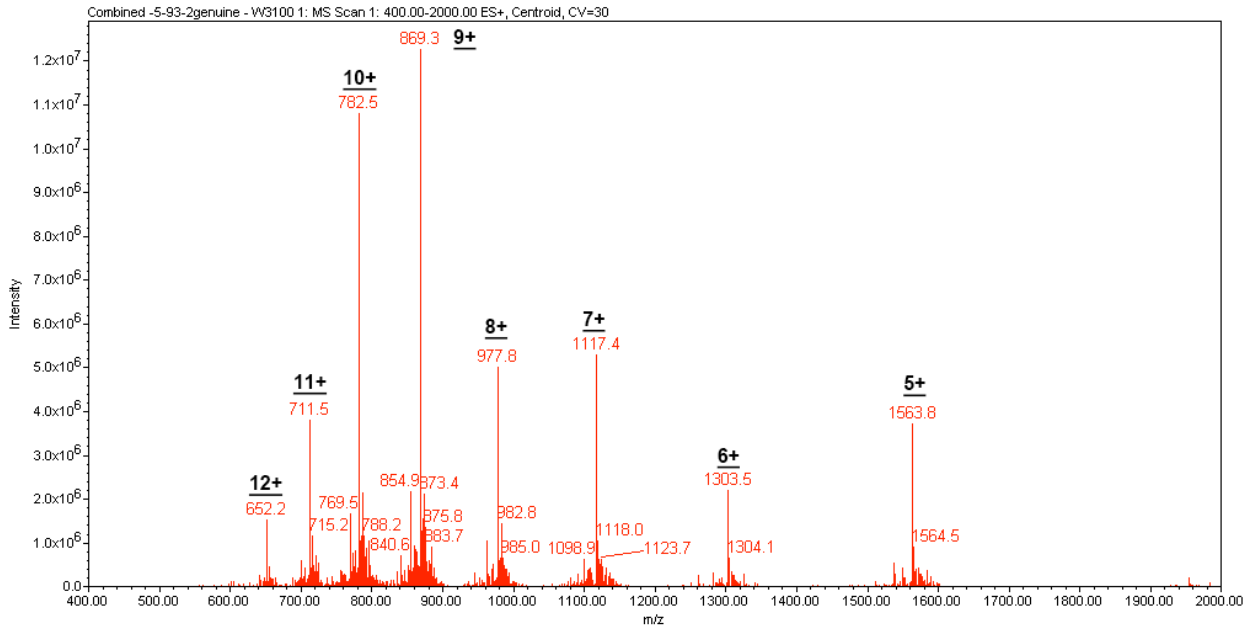

Combined -5-123-check inC18 - W3100 1: MS Scan 1: 400.00-2000.00 ES+, Centroid, CV=30
1010.7
8+
9+
898.6
5+
1616.4
7+
1155.1
6+
1347.2
10+
808.8
903.1
1011.3
1154.4
994.6
1026.9
912.8
884.0
1160.8
1348.0
1354.1
1617.3
1624.6
1625.5
1627.8
Intensity
m/z

Peptide S27 ESI calcd for $C_{344}H_{569}N_{105}O_{117}P$ $[M+5H]^{5+}$ m/z = 1616.44 $[M+6H]^{6+}$ m/z = 1347.20 $[M+7H]^{7+}$ m/z = 1154.89 $[M+8H]^{8+}$ m/z = 1010.65 $[M+9H]^{9+}$ m/z = 898.47 $[M+10H]^{10+}$ m/z = 808.72 found: 1616.4; 1347.2; 1155.1; 1010.7; 898.6; 808.8.

b) Synthesis of Peptide S28

9.8 mg of **Peptide S27** was treated with N_2H_4 monohydrate (10 equiv.)[8] 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 15 min at a flow rate of 0.6 mL/min. After the reaction reached completion within 4-5 h, 10% Et_2NH solution in CH_3CN/H_2O (1/1, v/v) was added there,[9] rendering the peptide at a concentration of 2.5 mM. After incubation for another 2 h 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 concentration at reduced pressure and lyophilization afforded 5.6 mg (59% yield) of **Peptide S28** as a white powder.

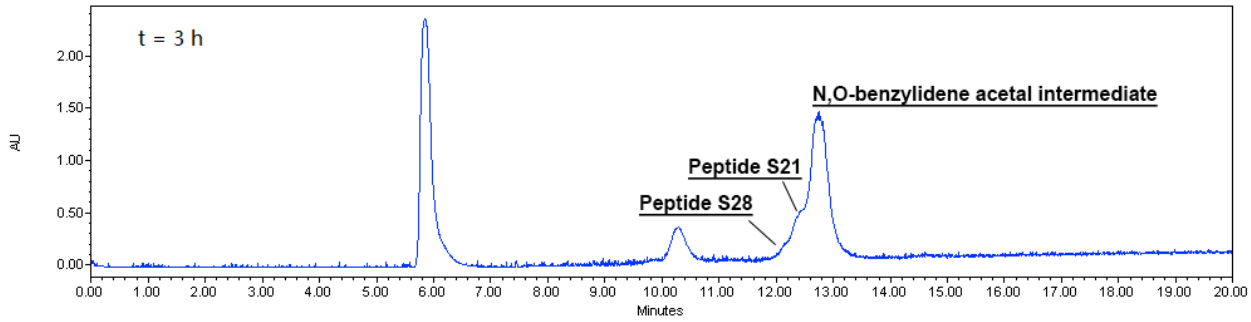


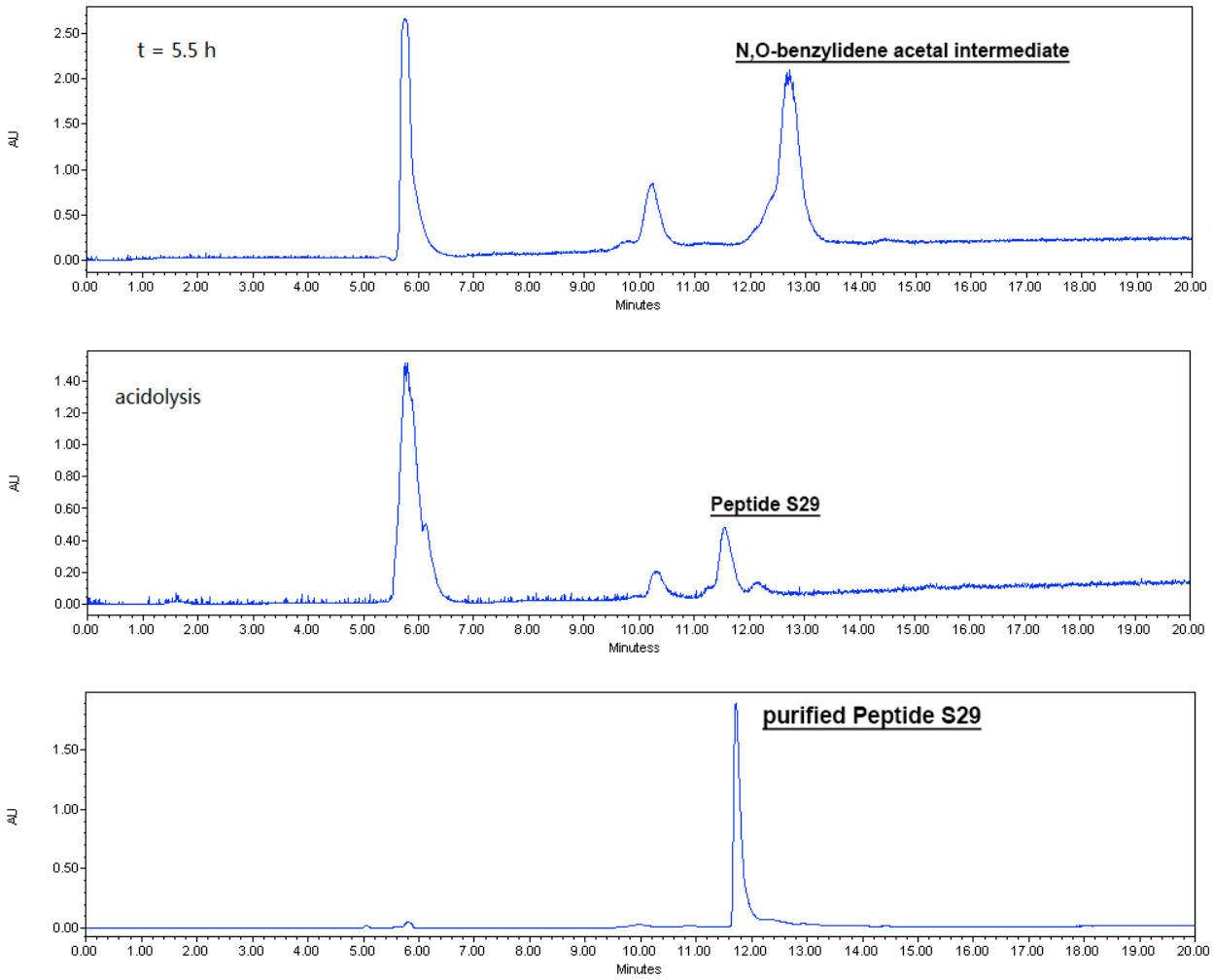


Peptide S28 Ac removal and *In situ* Fmoc removal: ESI calcd for $C_{333}H_{557}N_{105}O_{114}P$ $[M+5H]^{5+}$ m/z = 1563.62 $[M+6H]^{6+}$ m/z = 1303.19 $[M+7H]^{7+}$ m/z = 1117.16 $[M+8H]^{8+}$ m/z = 977.64 $[M+9H]^{9+}$ m/z = 869.13 $[M+10H]^{10+}$ m/z = 782.32 $[M+11H]^{11+}$ m/z = 711.29, $[M+12H]^{12+}$ m/z = 652.10 found: 1563.8; 1303.5; 1117.4; 977.8; 869.3; 782.5; 711.5; 652.2.

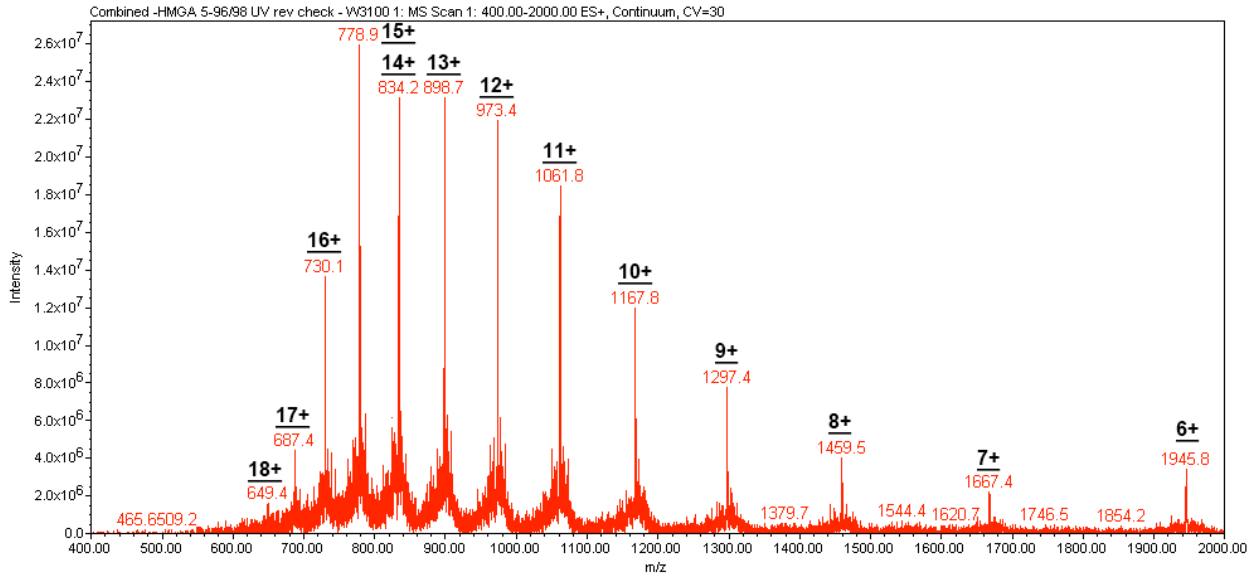
c) Synthesis of Peptide 29

2.9 mg of **Peptide S21** (2.0 equiv.) and 2.8 mg of **Peptide S28** (1.0 equiv.) were incubated in pyridine/acetic acid (45.9 µL, 1/12 mole/mole) at a concentration of 20 mM at room temperature. The reaction was monitored on a C4 analytical column with a variety gradient CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min, among which 10-50% was the best choice. After the completion of the reaction within 5 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v) for up to 1 h to allow complete deprotection of Hmb which rendered native Gly96 concomitantly. Preparative HPLC purification (10-40% CH_3CN/H_2O over 30 min) followed by concentration at reduced pressure and lyophilization afforded 1.3 mg (33% yield) of **Peptide S29** as a white powder.



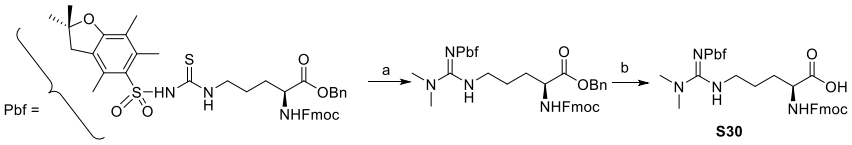


Characterization of synthetic **Peptide S29**. The analysis is run on a C18 column with a gradient of 5-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min.

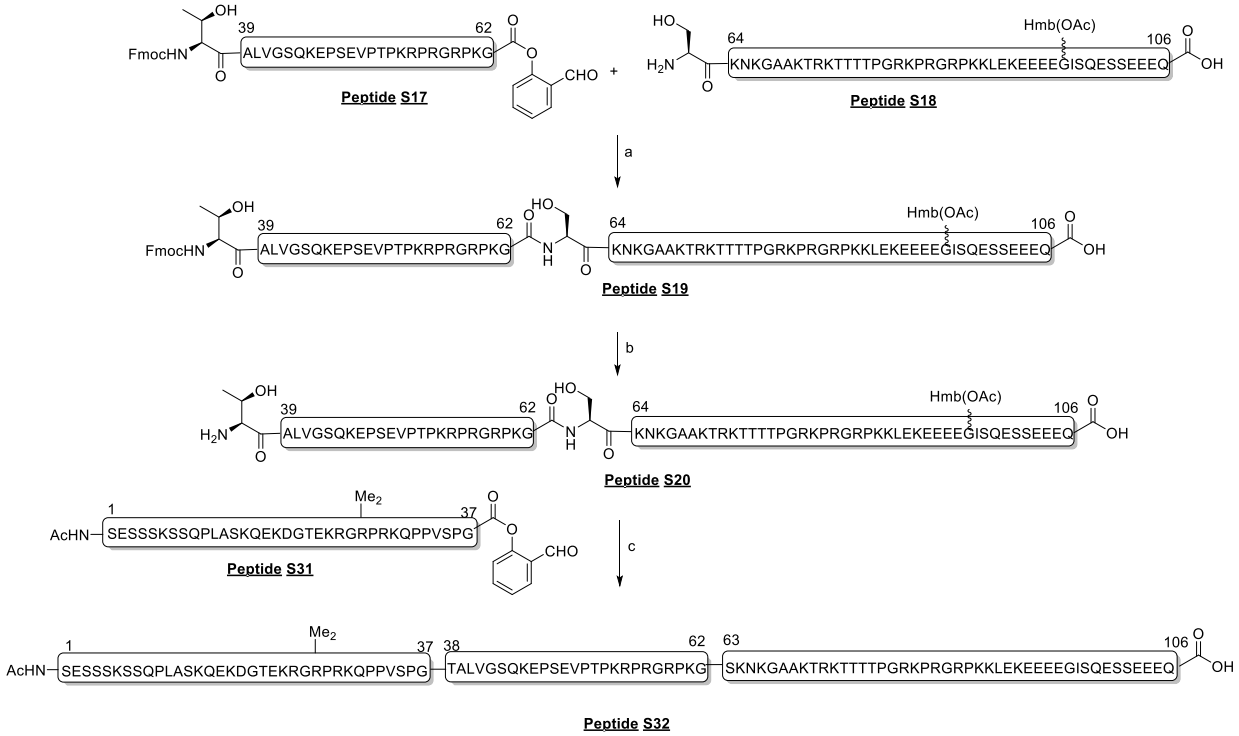


Peptide S29 ESI calcd for $C_{484}H_{828}N_{162}O_{170}P$ $[M+6H]^{6+}$ m/z = 1945.36 $[M+7H]^{7+}$ m/z = 1667.59 $[M+8H]^{8+}$ m/z = 1459.27 $[M+9H]^{9+}$ m/z = 1297.24 $[M+10H]^{10+}$ m/z = 1167.62 $[M+11H]^{11+}$ m/z = 1061.56 $[M+12H]^{12+}$ m/z = 973.18 $[M+13H]^{13+}$ m/z = 898.40 $[M+14H]^{14+}$ m/z = 834.30 $[M+15H]^{15+}$ m/z = 778.75 $[M+16H]^{16+}$ m/z = 730.14, $[M+17H]^{17+}$ m/z = 687.25 $[M+18H]^{18+}$ m/z = 649.12 found: 1945.8; 1667.4; 1459.5; 1297.4; 1167.8; 1061.8; 973.4; 898.7; 834.2; 778.9; 730.1; 687.4; 649.4.

Synthesis of **Peptide S31** **(Scheme S9)**



a) $(CH_3)_2NH_3Cl$, EDCI, CH_2Cl_2, 5min; b) H_2/Pd-C, NH_4Ac, MeOH, 2-3h.



a) Pyridine/AcOH (1/12, mol/mol), 9h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 0.5 h, 45%. b) H_2NNH_2, 4 h; $Et_2NH/H_2O/CH_3CN$, 2 h, 57%. c) Pyridine/AcOH (1/12, mol/mol), 7h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 1 h, 53%.

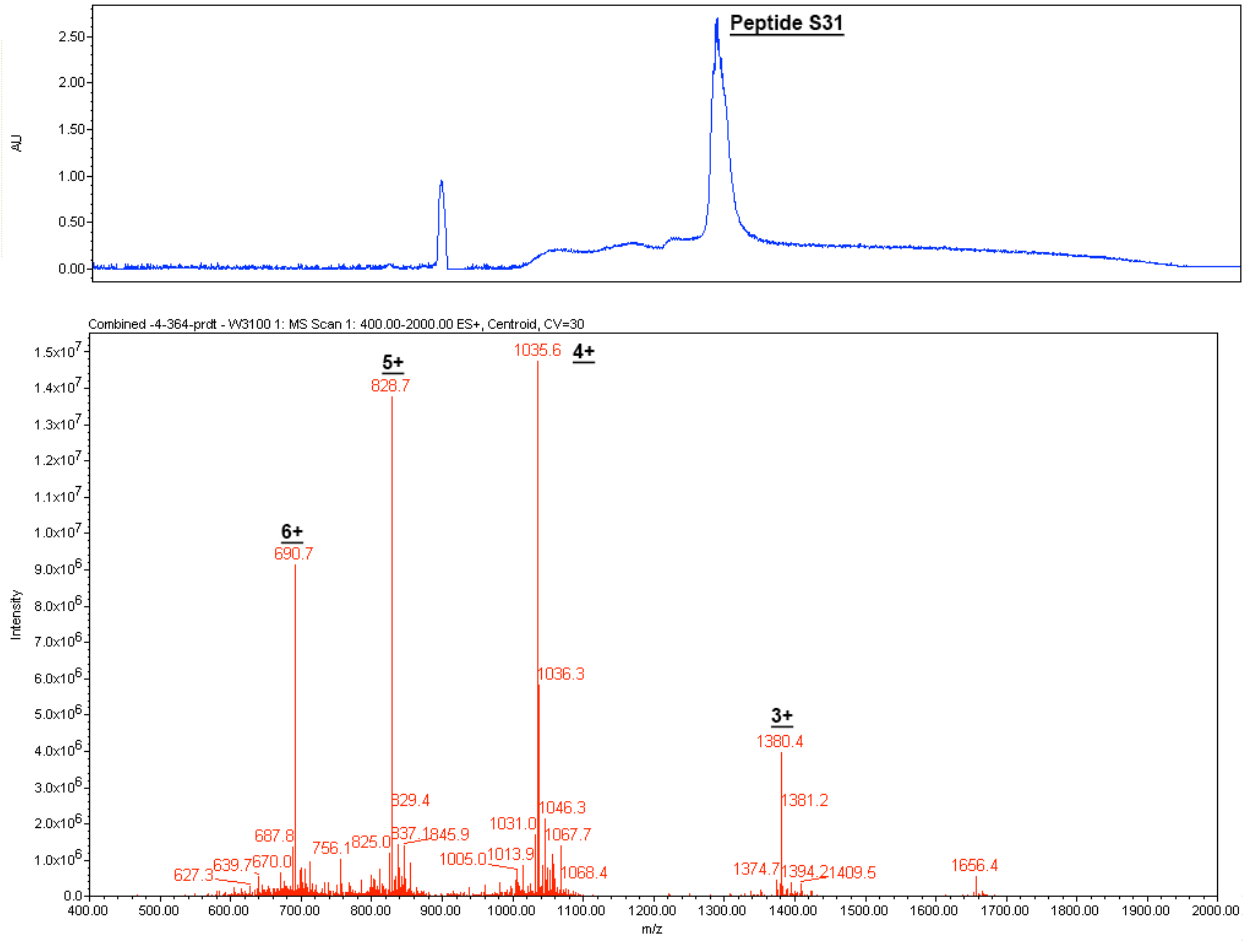
The asymmetrically di-methylated building block Fmoc-Arg(Me_2, Pbf)-OH **S30** was prepared accordingly[6,7] and characterized as white foamy solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (br s, 1H), 7.72 (d, 2H, *J* = 7.6 Hz), 7.59 (t, 2H, *J* = 8.2 Hz), 7.34 (t, 2H, *J* = 7.4 Hz), 7.25-7.20 (m, 2H), 6.53 (br t, 1H, *J* = 5.6 Hz), 6.13 (d, 1H, *J* = 7.8 Hz) 4.37-4.31 (m, 3H), 4.17 (t, 1H, *J* = 7.2 Hz), 3.22-3.10 (m, 2H), 2.89 (s, 2H), 2.86 (s, 6H), 2.58 (s, 3H), 2.50 (s, 3H), 2.06 (s, 3H), 2.04-1.92 (m, 1 H), 1.92-1.80 (m, 1H), 1.70 (br, 2H), 1.42 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.07, 160.59, 158.87, 156.66, 143.99, 143.81, 141.30, 138.50, 132.89, 132.48, 127.75, 127.18, 125.41, 124.97, 124.75, 119.97, 117.58, 86.54, 67.29, 53.60, 47.16, 45.32, 43.19, 39.46, 29.78, 29.16, 28.61, 26.41, 19.38, 18.21, 12.50 m/z Calc. for $C_{23}H_{27}N_3O_4$ $[M+H]^+$ 677.3, found 677.4.

1) Peptide fragments **Peptide S20** was prepared as described in Scheme S5.

2) **Peptide S31** was prepared and characterized as follows.

Fmoc-Arg(Me_2, Pbf)-OH was introduced following the standard protocol; N-terminal acetylation was achieved by Ac_2O/Et_3N/DMAP in anhydrous CH_2Cl_2. It was then cleaved from the resin by a cocktail of CH_2Cl_2/TFE/AcOH (8/1/1, v/v/v). The solvent was removed under reduced pressure to give the crude protected peptide.

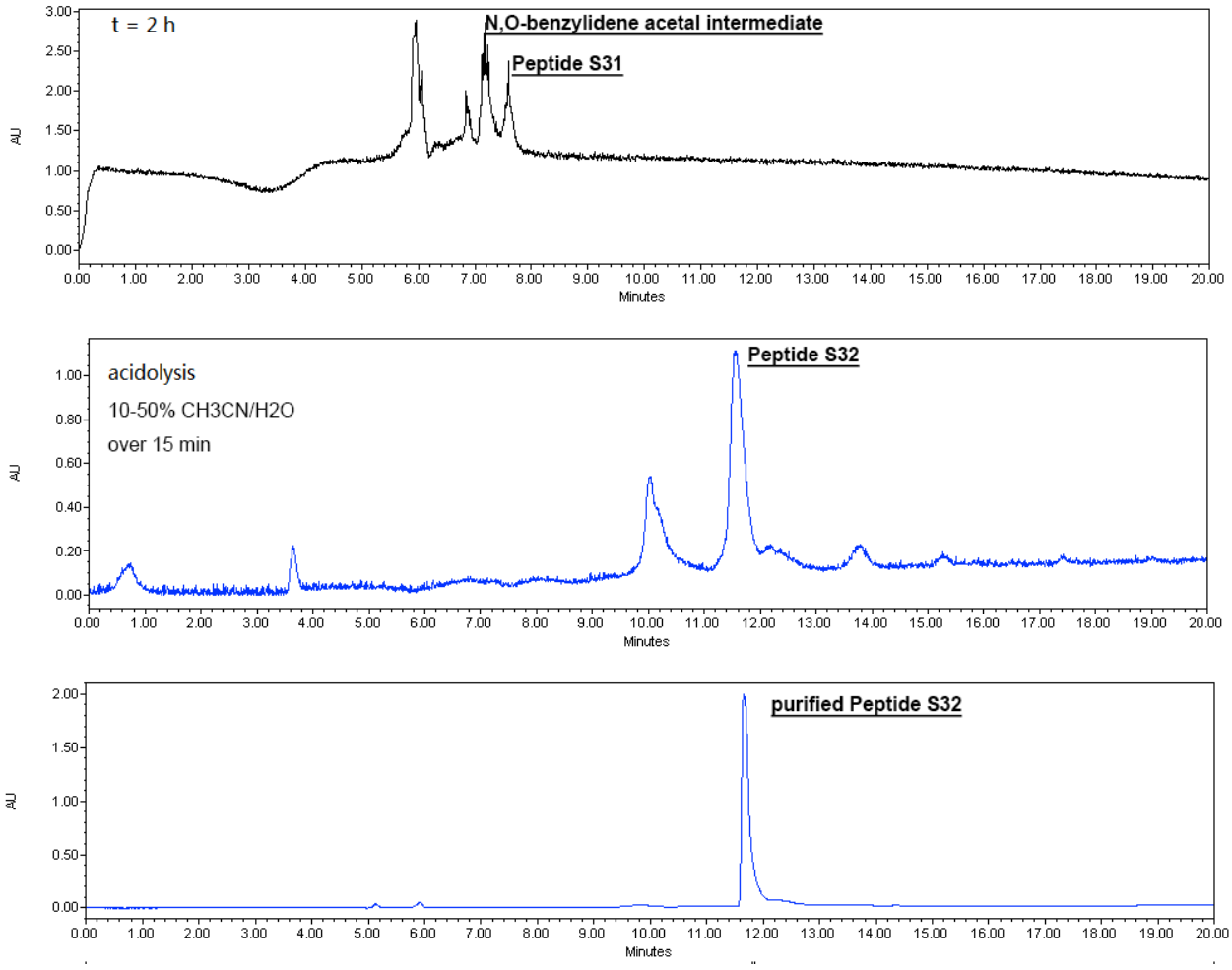
The crude peptide (0.13 g, 0.02 mmol) was then coupled with α, α-dimethoxy-salicylaldehyde (59.0 mg, 0.35 mmol), PyBOP (45.6 mg, 0.09 mmol) and DIEA (12.5 mg, 0.10 mmol, 16.8 µL) in anhydrous CH_2Cl_2 (1.2 mL) at a concentration of 15 mM; after 10 h, treatment with TFA/phenol/H_2O (90/5/5, v/v/v) for 3 h allowed the complete removal of the benzyl group on the phosphate. TFA was removed under a stream of condensed air. The crude product was triturated with cold diethyl ether and centrifuged and the ether subsequently decanted. The obtained solid was purified via preparative HPLC (10-50% CH_3CN/H_2O over 30 min), followed by concentration under *vacuo* and lyophilization to give the titled fragment **Peptide S31** (8.6 mg, 12%) as a white powder.



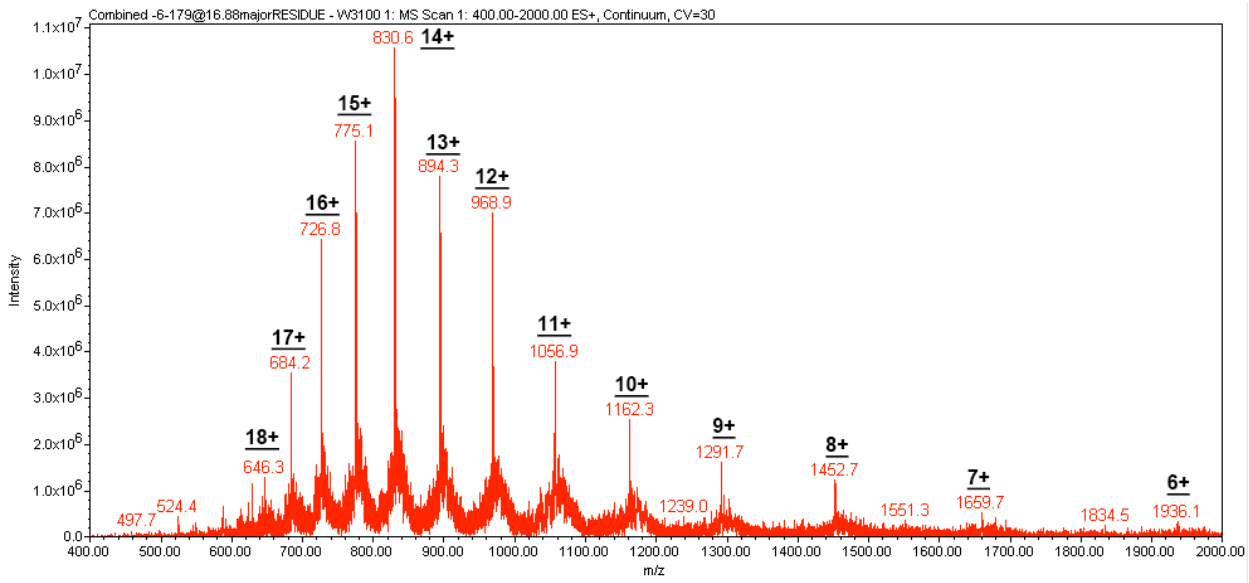
Peptide S31 Preparative HPLC purification (10-50% CH_3CN/H_2O over 30 min) followed by concentration under *vacuo* and lyophilization; characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min). ESI calcd for $C_{174}H_{290}N_{57}O_{60}$ $[M+3H]^{3+}$ m/z = 1380.72 $[M+4H]^{4+}$ m/z = 1035.79 $[M+5H]^{5+}$ m/z = 828.83 $[M+6H]^{6+}$ m/z = 690.86, found: 1380.4; 1035.6; 828.7; 690.7.

c) Synthesis of Peptide S32

7.6 mg of **Peptide S31** (2.0 equiv.) and 7.1 mg of **Peptide S20** (1.0 equiv.) were incubated in pyridine/acetic acid (45.9 µL, 1/12 mole/mole) at a concentration of 20 mM at room temperature. The reaction was monitored on a C4 analytical column column with a variety gradient CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min, among which 30-70% was the best choice. After the completion of the reaction within 5 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v) for up to 1 h to allow fully deprotection of Hmb which rendered native Gly96 concomitantly, as analyzed on a C4 analytical column with a gradient 20-40% CH_3CN/H_2O over 15 min at a flow rate of 0.6 mL/min. Preparative HPLC purification (10-40% CH_3CN/H_2O over 30 min) followed by concentration at reduced pressure and lyophilization afforded 5.6 mg (53% yield) of **Peptide S32** as a white powder.

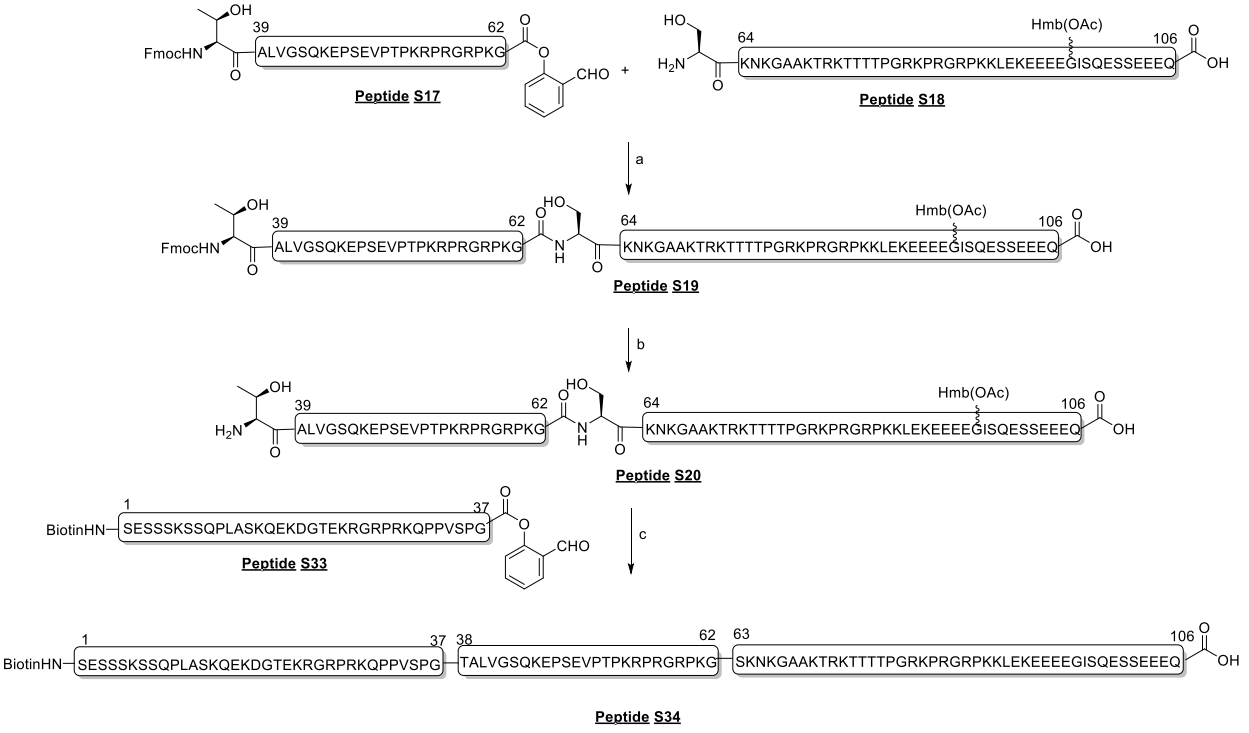


Characterization of synthetic **Peptide S32**. The analysis is run on a C18 column with a gradient of 5-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min.



Peptide S32 ESI calcd for $C_{486}H_{831}N_{162}O_{167}$ $[M+6H]^{6+}$ m/z = 1936.70 $[M+7H]^{7+}$ m/z = 1660.17 $[M+8H]^{8+}$ m/z = 1452.78 $[M+9H]^{9+}$ m/z = 1291.47 $[M+10H]^{10+}$ m/z = 1162.42 $[M+11H]^{11+}$ m/z = 1056.84 $[M+12H]^{12+}$ m/z = 968.85 $[M+13H]^{13+}$ m/z = 894.40 $[M+14H]^{14+}$ m/z = 830.59 $[M+15H]^{15+}$ m/z = 775.28 $[M+16H]^{16+}$ m/z = 726.89 $[M+17H]^{17+}$ m/z = 684.19 $[M+18H]^{18+}$ m/z = 646.24, found: 1936.1; 1659.7; 1452.7; 1291.7; 1162.3; 1056.9; 968.9; 894.3; 830.6; 775.1; 726.8; 684.2; 646.3.

Synthesis of **Peptide S34 (Scheme S10)**



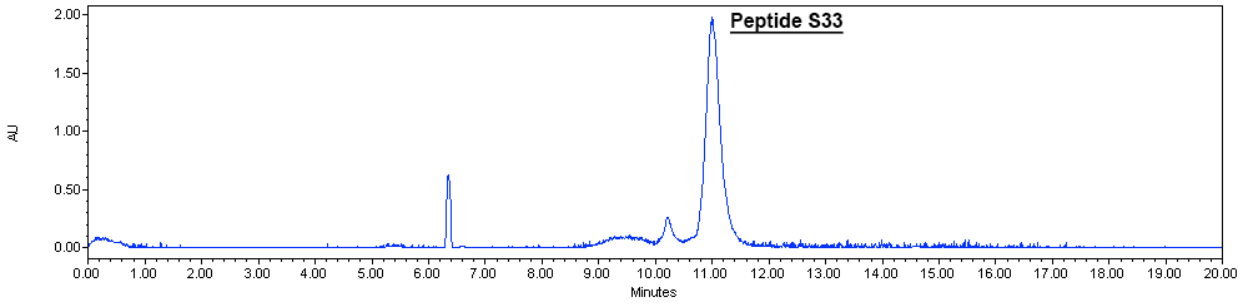
a) Pyridine/AcOH (1/12, mol/mol), 9h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 0.5 h, 45%. b) H_2NNH_2, 4 h; $Et_2NH/H_2O/CH_3CN$, 2 h, 57%. c) Pyridine/AcOH (1/12, mol/mol), 6h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 1 h, 57%.

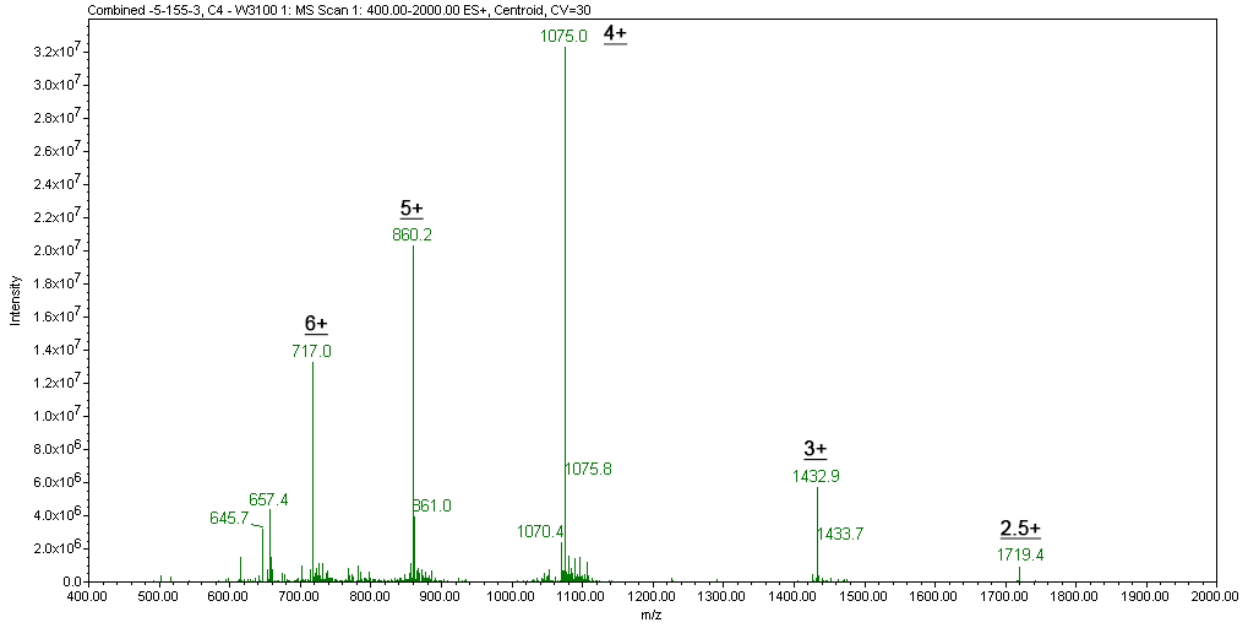
1) Peptide fragments **Peptide S20** was prepared as described in Scheme S6.

2) **Peptide S33** was prepared and characterized as follows.

N-terminal biotinylation was achieved by direct coupling of D-biotin (4 equiv.) in the presence of HATU (4 equiv.), DIEA (4 equiv.) in anhydrous DMF for 3 h at r.t. It was then cleaved from the resin by a cocktail of CH_2Cl_2/TFE/AcOH (8/1/1, v/v/v). The solvent was removed under reduced pressure to give the crude protected peptide.

The crude peptide (0.04 g, 0.005 mmol) was then coupled with α, α-dimethoxy-salicylaldehyde (18.9 mg, 0.11 mmol), PyBOP (14.6 mg, 0.03 mmol) and DIEA (4.0 mg, 0.03 mmol, 5.4 µL) in anhydrous CH_2Cl_2 (0.4 mL) at a concentration of 15 mM; after 10 h, treatment with TFA/phenol/H_2O (90/5/5, v/v/v) for 2 h yielded global deprotection. TFA was removed under a stream of condensed air. The crude product was triturated with cold diethyl ether and centrifuged and the ether subsequently decanted. The obtained solid was purified via preparative HPLC (10-50% CH_3CN/H_2O over 30 min), followed by concentration under *vacuo* and lyophilization to give the titled fragment **Peptide S33** (8.4 mg, 35 %) as a white powder.

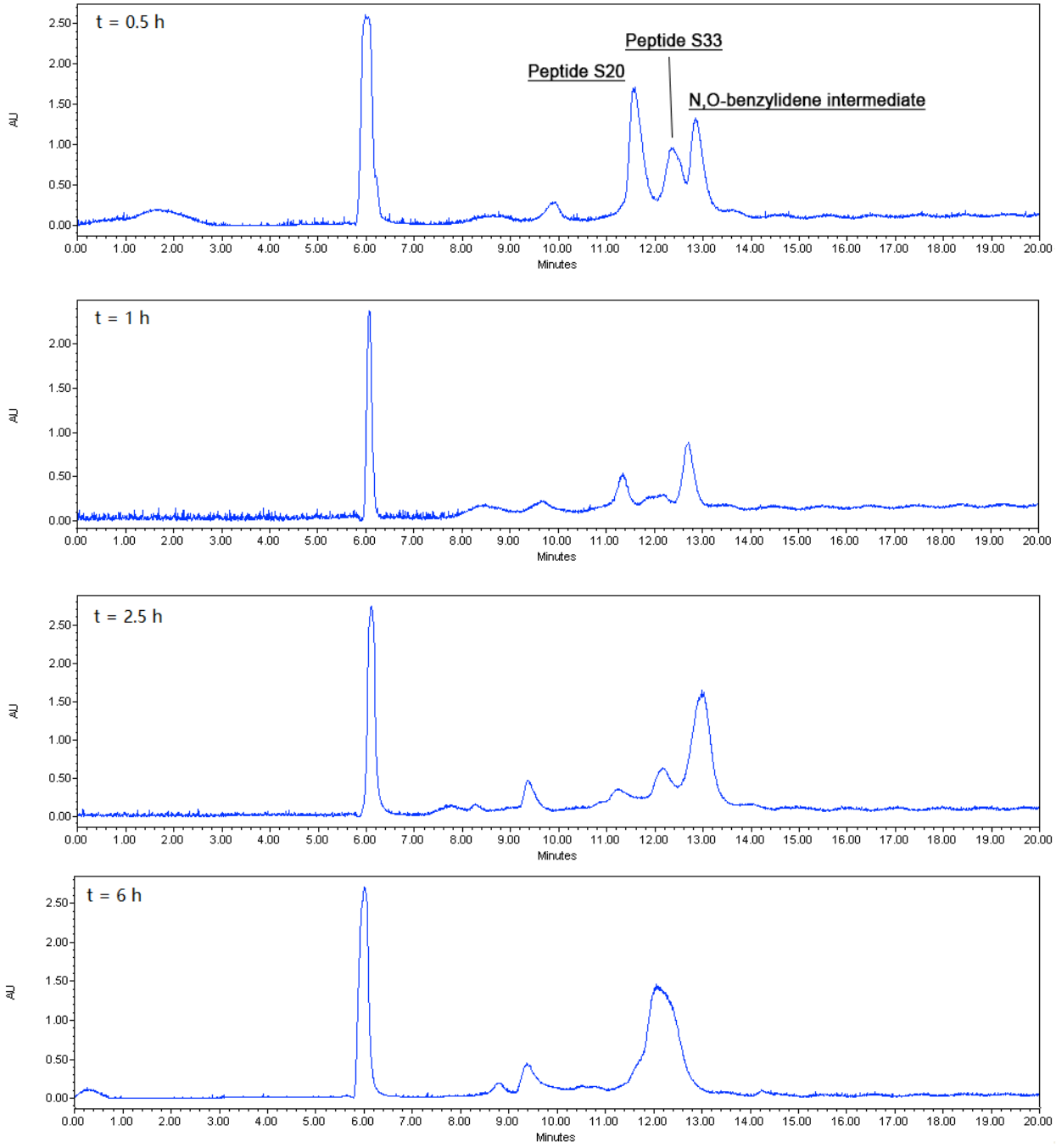


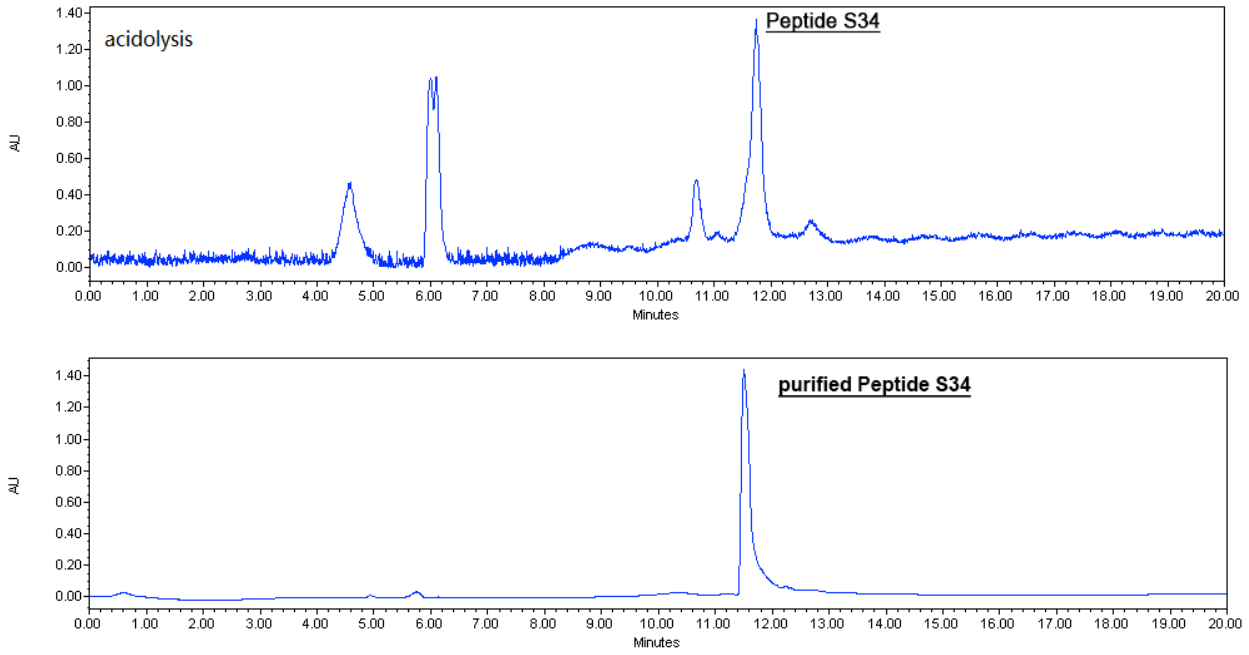


Peptide S33 Preparative HPLC purification (10-50% CH_3CN/H_2O over 30 min) followed by concentration under *vacuo* and lyophilization; characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min). ESI calcd for $C_{180}H_{298}N_{59}O_{61}S_1$ [2M+5H] $^{5+}$ m/z = 1719.68 [M+3H] $^{3+}$ m/z = 1432.73 [M+4H] $^{4+}$ m/z = 1074.80 [M+5H] $^{5+}$ m/z = 860.04 [M+6H] $^{6+}$ m/z = 716.87, found: 1719.4; 1432.9; 1075.0; 860.2; 717.0.

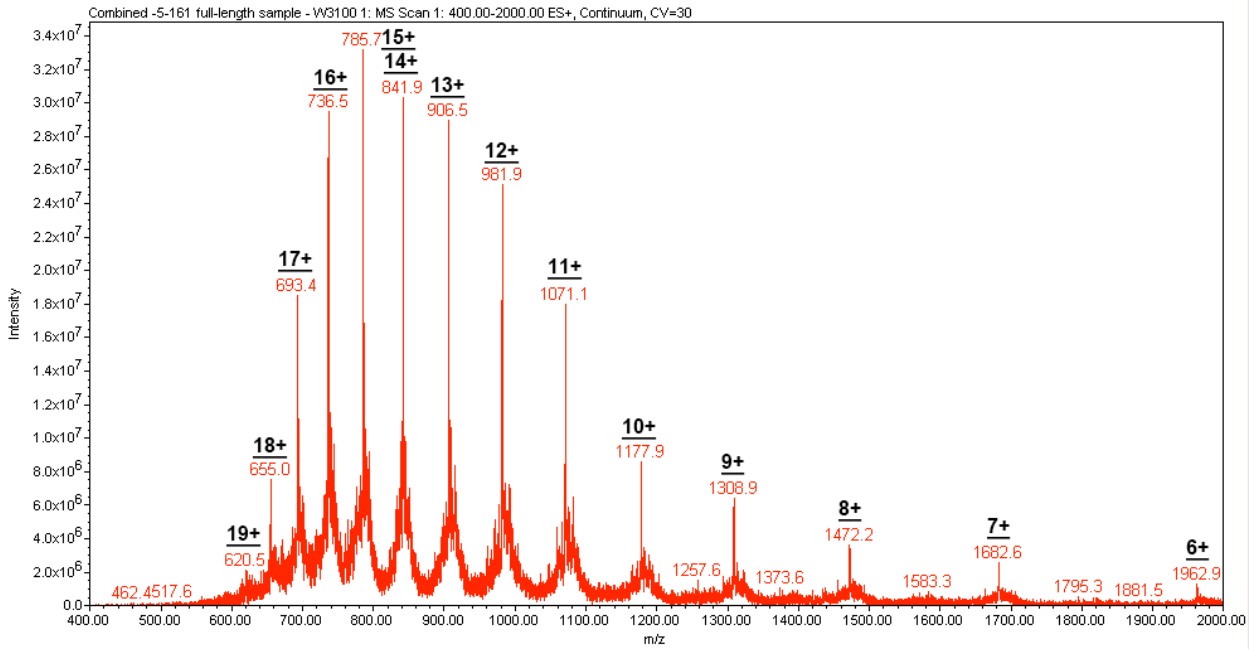
c) Synthesis of **Peptide S34**

1.4 mg of **Peptide S33** (2.0 equiv.) and 1.3 mg of **Peptide S20** (1.0 equiv.) were incubated in pyridine/acetic acid (8.4 µL, 1/12 mole/mole) at a concentration of 20 mM at room temperature. The reaction was monitored (20-40% CH_3CN/H_2O over 15 min) on a C4 analytical column at a flow rate of 0.6 mL/min. After the completion of the reaction within 6 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v) for up to 1 h to allow complete deprotection of Hmb which rendered native Gly96 concomitantly. Preparative HPLC purification (10-50% CH_3CN/H_2O over 30 min) followed by concentration at reduced pressure and lyophilization afforded 0.7 mg (57% yield) of **Peptide S34** as a white powder.



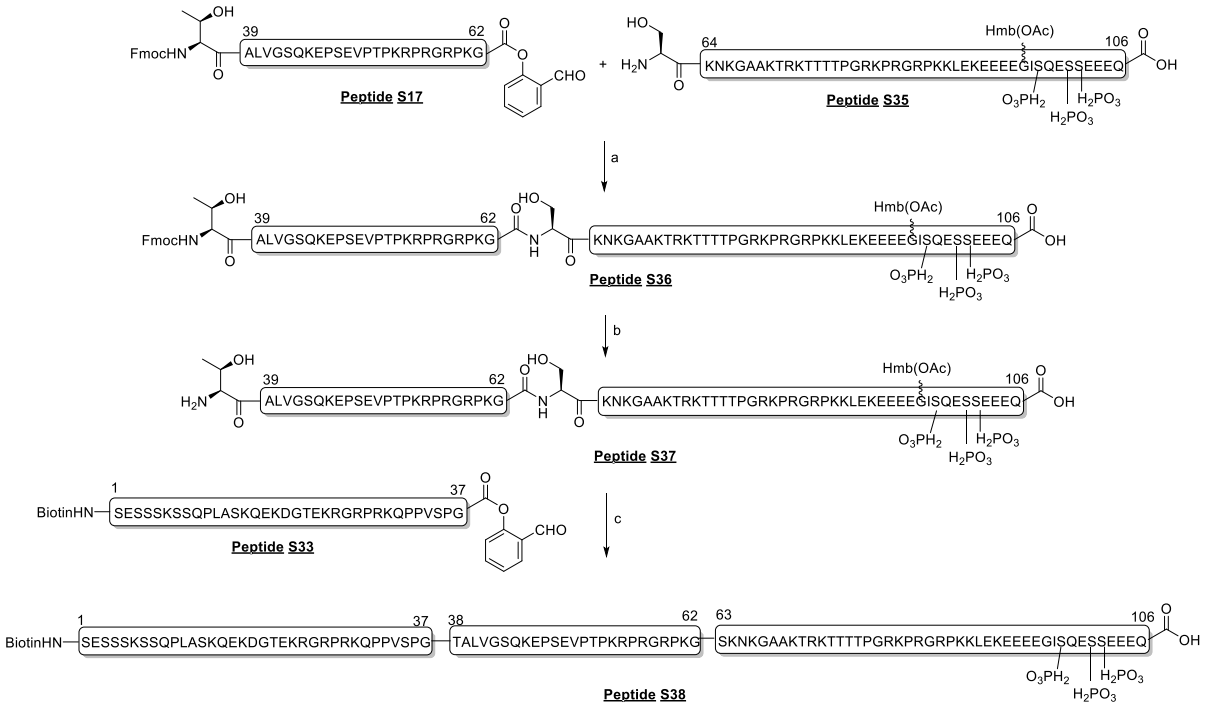


Characterization of synthetic **Peptide S34**. The analysis is run on a C18 column with a gradient of 5-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min.



Peptide S34 ESI calcd for $C_{492}H_{839}N_{164}O_{168}S_1$ $[M+6H]^{6+}$ m/z = 1962.71 $[M+7H]^{7+}$ m/z = 1682.46 $[M+8H]^{8+}$ m/z = 1472.28 $[M+9H]^{9+}$ m/z = 1308.81 $[M+10H]^{10+}$ m/z = 1178.03 $[M+11H]^{11+}$ m/z = 1071.03 $[M+12H]^{12+}$ m/z = 981.86 $[M+13H]^{13+}$ m/z = 906.41 $[M+14H]^{14+}$ m/z = 841.74 $[M+15H]^{15+}$ m/z = 785.69 $[M+16H]^{16+}$ m/z = 736.64 $[M+17H]^{17+}$ m/z = 693.37 $[M+18H]^{18+}$ m/z = 654.91 $[M+19H]^{19+}$ m/z = 620.49, found: 1962.9; 1682.6; 1472.2; 1308.9; 1177.9; 1071.1; 981.9; 906.5; 841.9; 785.7; 736.5; 693.4; 655.0; 620.5.

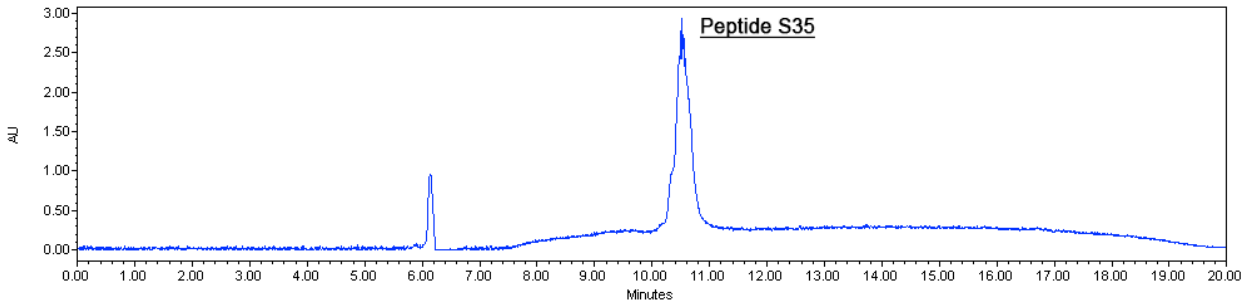
Synthesis of Peptide S38 (Scheme S11)

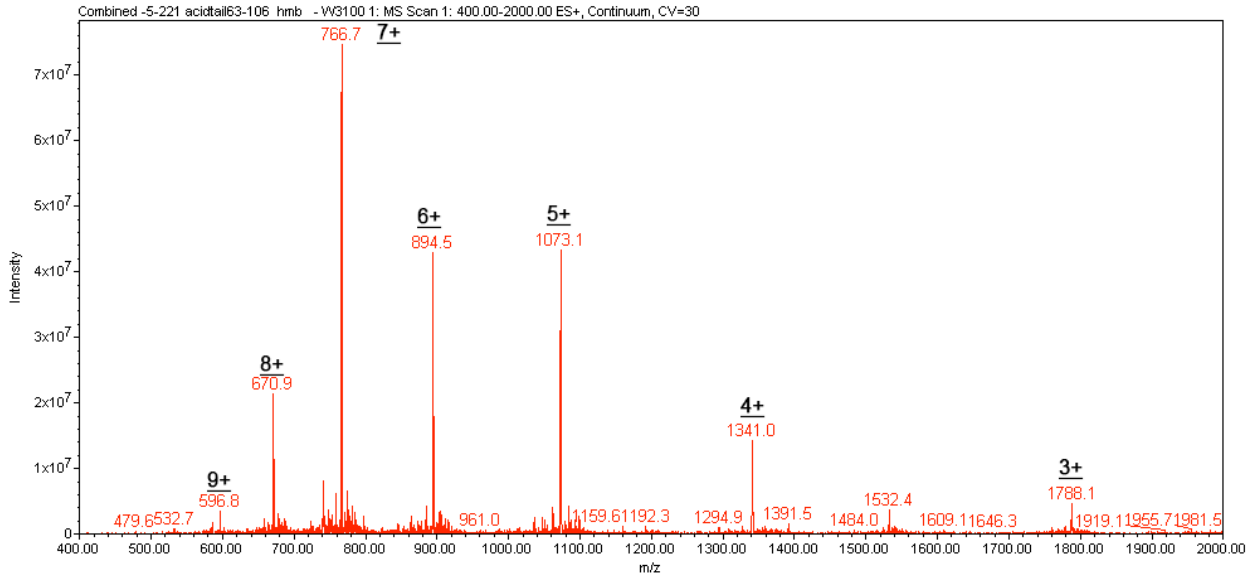


a) Pyridine/AcOH (1/12, mol/mol), 23 h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 0.5 h, 50 %. b) H_2NNH_2, 2.5 h; $Et_2NH/H_2O/CH_3CN$, 2 h, 70 %. c) Pyridine/AcOH (1/12, mol/mol), 5.5 h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 1 h, 35%.

1) Preparation of Peptide S35

The peptide was assembled according to general procedure. Fmoc-Ser(HPO3Bn)-OH was introduced by this general method, whereas the removal of its Fmoc was achieved in 2.5% (v/v) DBU and 2.5% (v/v) piperidine in DMF so as to suppress piperidinylalanine formation.[10] The incorporation of Fmoc-Gly(Hmb, Fmoc)-OH was carried out under standard coupling protocols; the acylation of this backbone secondary amine was performed in DMF/NMP(1/1, v/v) with repetitive coupling. The hydroxyl group on Hmb bearing acylation during the amino acid couplings fell off concomitantly once treated with piperidine/DMF (1/4, v/v) as Fmoc deblock solution. It was the same case with the N-terminal Ser63. The librated hydroxyl group was further acylated by Ac_2O/Et_3N/DMAP in anhydrous CH_2Cl_2. The global deprotection was performed as mentioned above for up to 4 h in the case of total removal of the benzyl group on phosphate. Preparative HPLC purification (10-50% CH_3CN/H_2O over 30 min) followed by concentration under *vacuo* and lyophilization gave 76 mg of **Peptide S35** out of 0.4 g of the resin. (yield 16% based on resin loading).





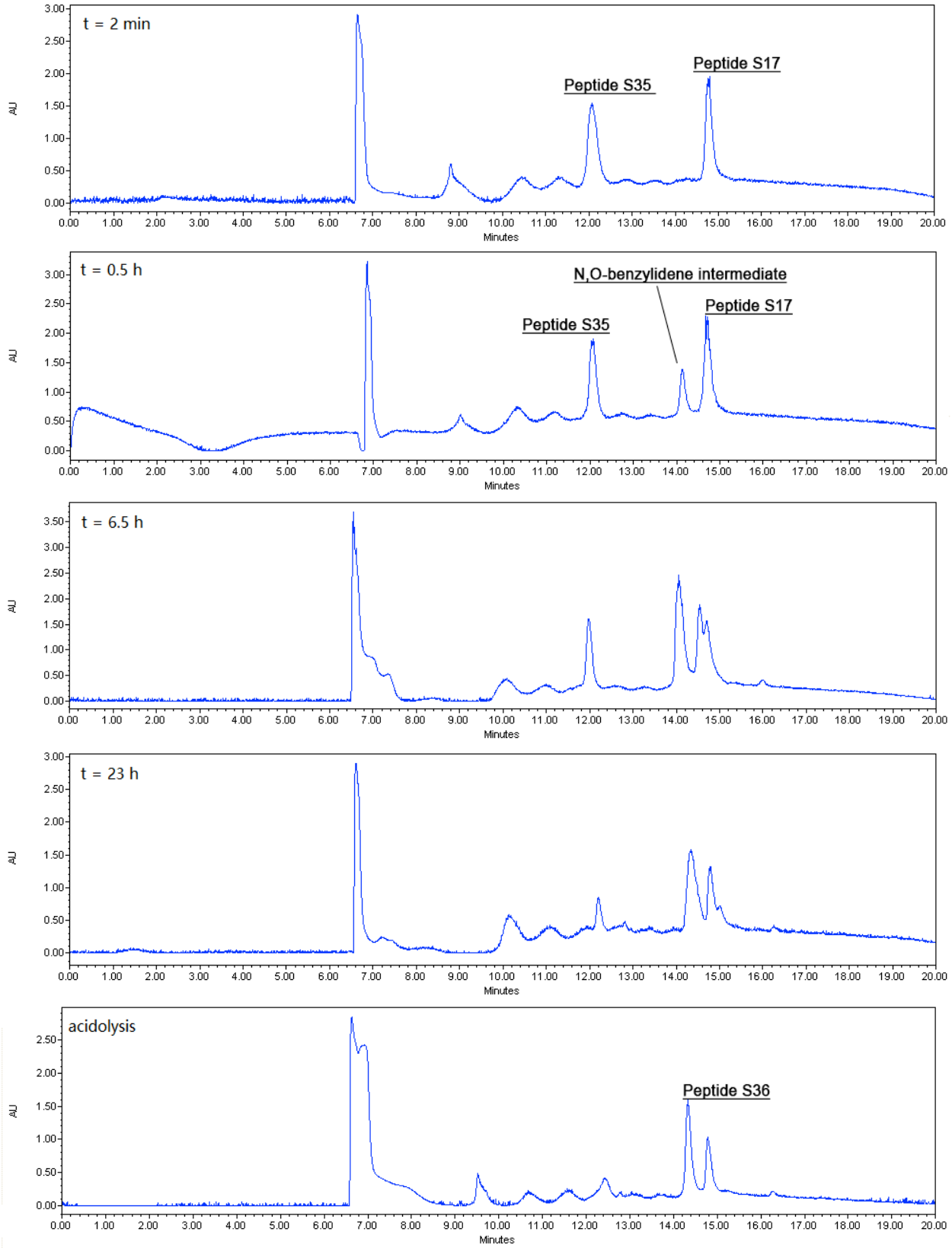
Peptide S35 was characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min) at a flow rate of 0.6 mL/min. ESI calcd for $C_{214}\ H_{364}\ N_{67}\ O_{87}\ P_3$ $[M+3H]^{3+}$ m/z = 1787.52 $[M+4H]^{4+}$ m/z = 1340.89 $[M+5H]^{5+}$ m/z = 1072.92 $[M+5H]^{5+}$ m/z = 894.26 $[M+6H]^{6+}$ m/z = 766.66 $[M+7H]^{7+}$ m/z = 670.95 $[M+8H]^{8+}$ m/z = 596.51, found: 1788.1; 1341.0; 1073.1; 894.5; 766.7; 670.9; 596.8.

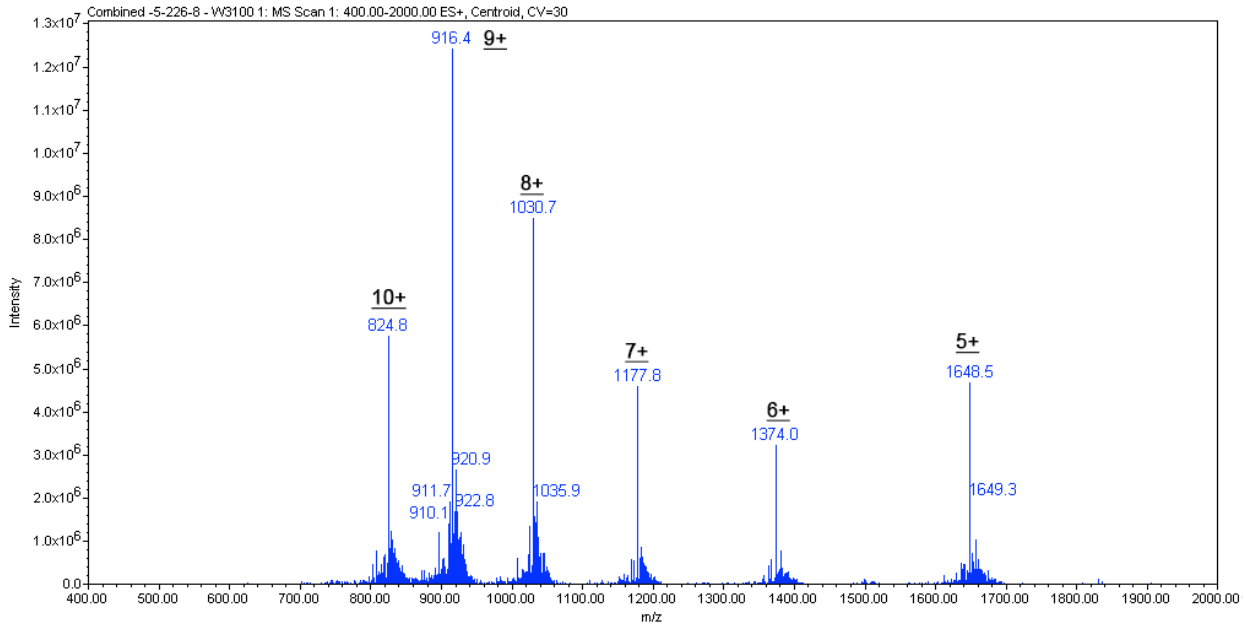
2) Preparation of Peptide S17 was indicated in Scheme S5.

3) Preparation of Peptide S33 was indicated in Scheme S10.

a) Synthesis of Peptide S36

16.4 mg of **Peptide S17** (1.6 equiv.) and 17.5 mg of **Peptide S35** (1.0 equiv.) were incubated for serine ligation as described before. The reaction was monitored on a C18 analytical column with a gradient of 10-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. After the completion of the reaction within 23 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v). Preparative HPLC purification (10–50% CH_3CN/H_2O containing 0.1% TFA over 30 min) followed by concentration at reduced pressure and lyophilization afforded 13.5 mg (50% yield) of **Peptide S36** as a white powder.

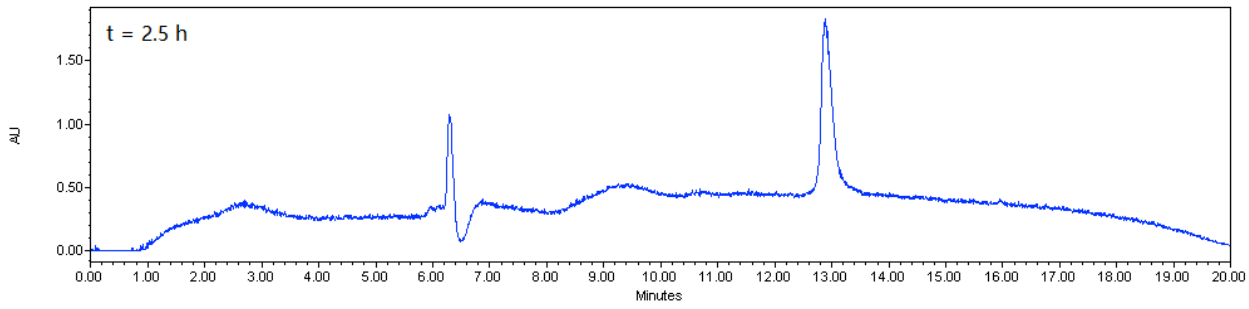

t = 2 min
Peptide S35
Peptide S17
t = 0.5 h
N,O-benzylidene intermediate
Peptide S35
Peptide S17
t = 6.5 h
t = 23 h
acidolysis
Peptide S36
AU
Minutes

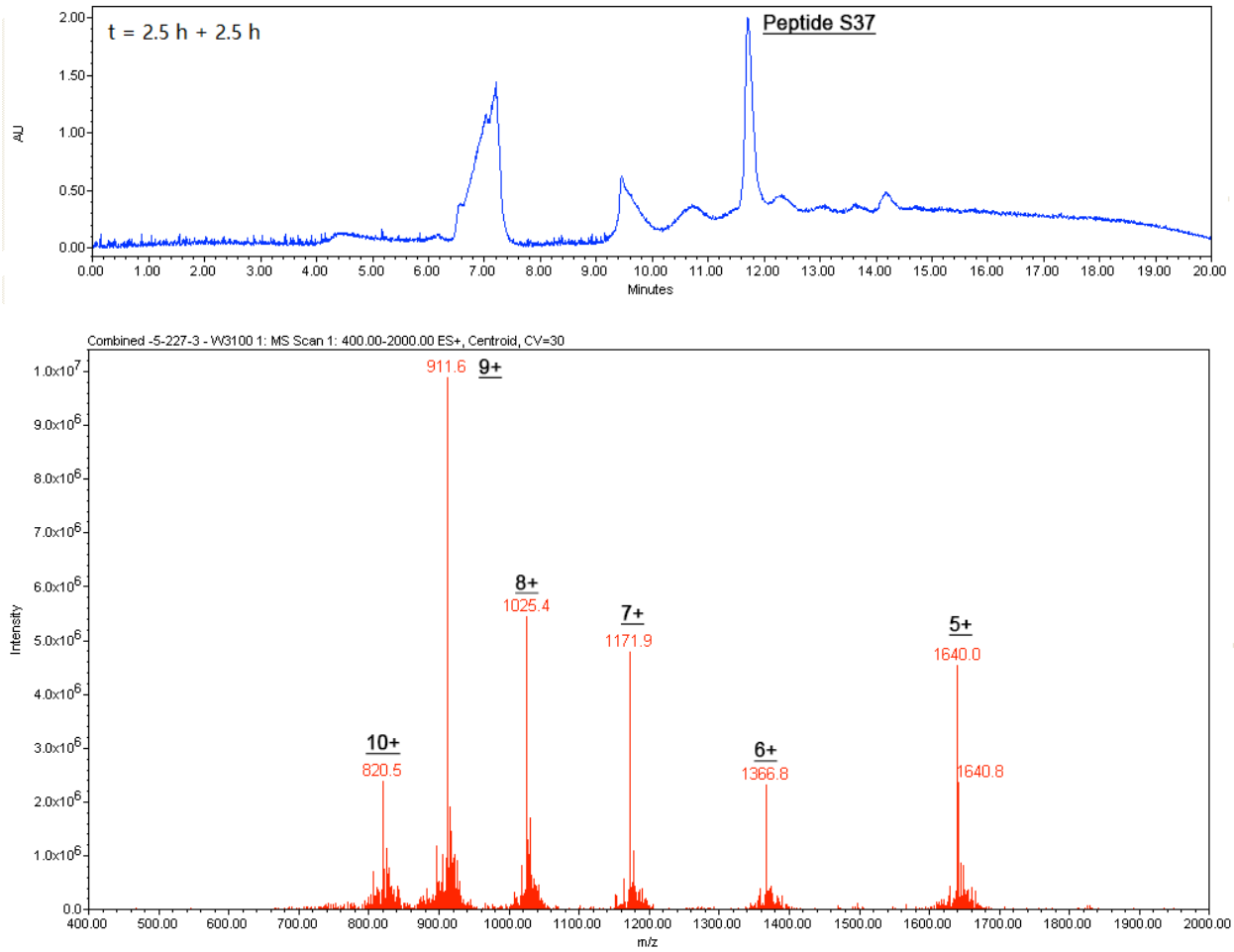


Peptide S36 was characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min). ESI calcd for $C_{344}\ H_{571}\ N_{105}\ O_{123}P_3$ $[M+5H]^{5+}$ m/z = 1648.43 $[M+6H]^{6+}$ m/z = 1373.86 $[M+7H]^{7+}$ m/z = 1177.73 $[M+8H]^{8+}$ m/z = 1030.64 $[M+9H]^{9+}$ m/z = 916.24 $[M+10H]^{10+}$ m/z = 824.72, found: 1648.5; 1374.0; 1177.8; 1030.7; 916.4; 824.8.

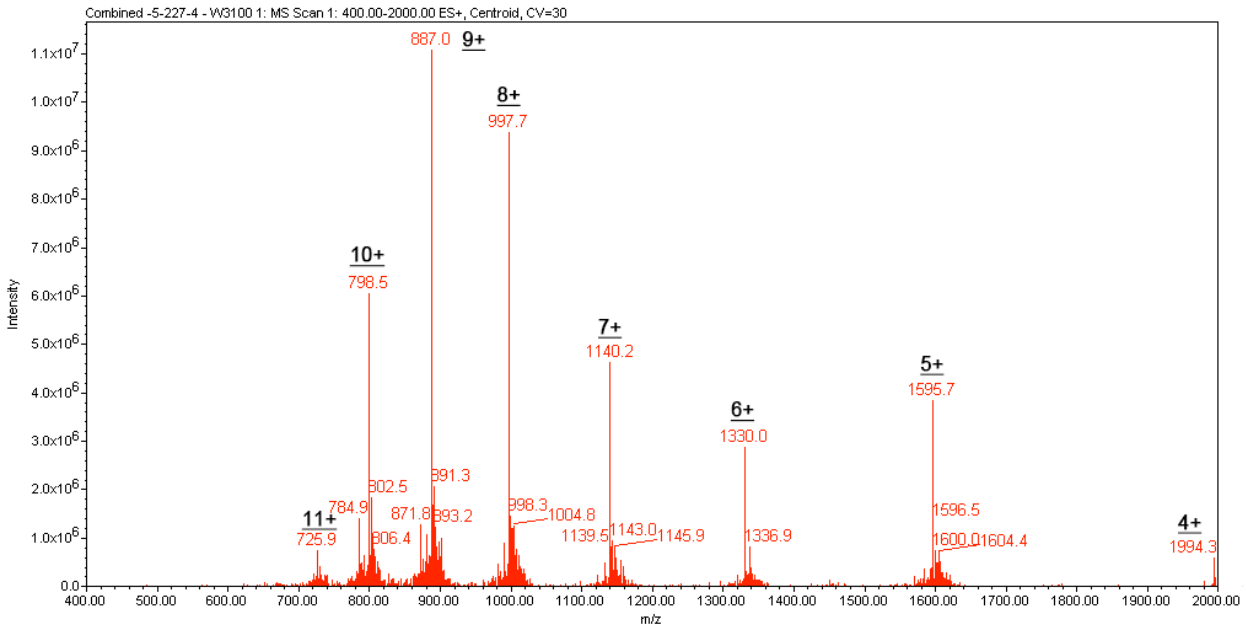
b) Synthesis of Peptide S37

6.0 mg of **Peptide S36** was treated with N_2H_4 monohydrate (10 equiv.)[8] 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 15 min at a flow rate of 0.6 mL/min. After the reaction reached completion within 2.5 h, 10% Et_2NH solution in CH_3CN/H_2O (1/1, v/v) was added there,[9] rendering the peptide at a concentration of 2.5 mM. After incubation for another 2.5 h 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 concentration at reduced pressure and lyophilization afforded 4.1 mg (70% yield) of **Peptide S37** as a white powder.





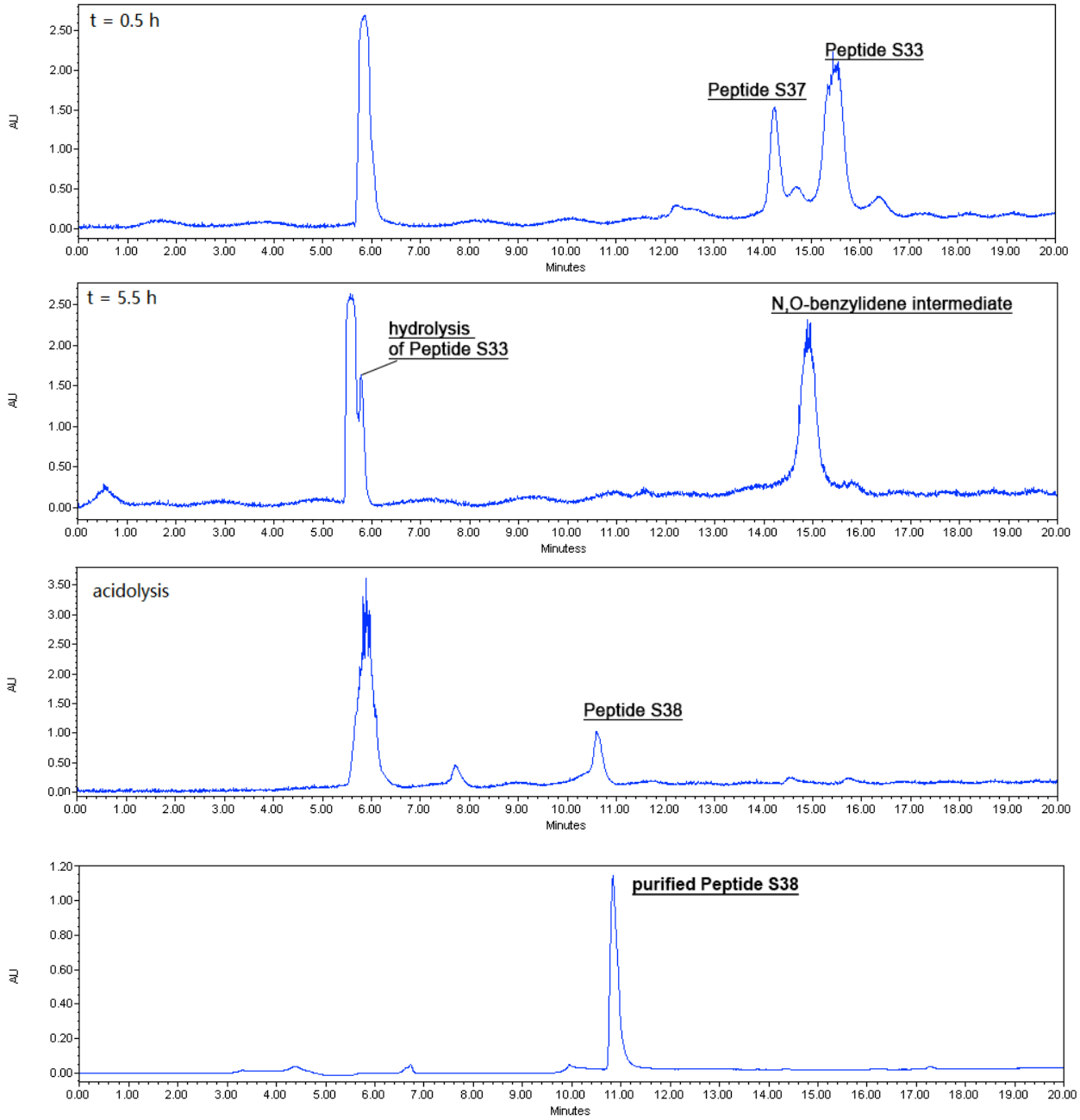
Ac removal by N_2H_4: ESI calcd for $C_{342}\ H_{569}\ N_{105}\ O_{122}P_3$ $[M+5H]^{5+}$ m/z = 1640.02 $[M+6H]^{6+}$ m/z = 1366.85 $[M+7H]^{7+}$ m/z = 1171.73 $[M+8H]^{8+}$ m/z = 1025.39 $[M+9H]^{9+}$ m/z = 911.57 $[M+10H]^{10+}$ m/z = 820.52, found: 1640.0; 1366.8; 1171.9; 1025.4; 911.6; 820.5.



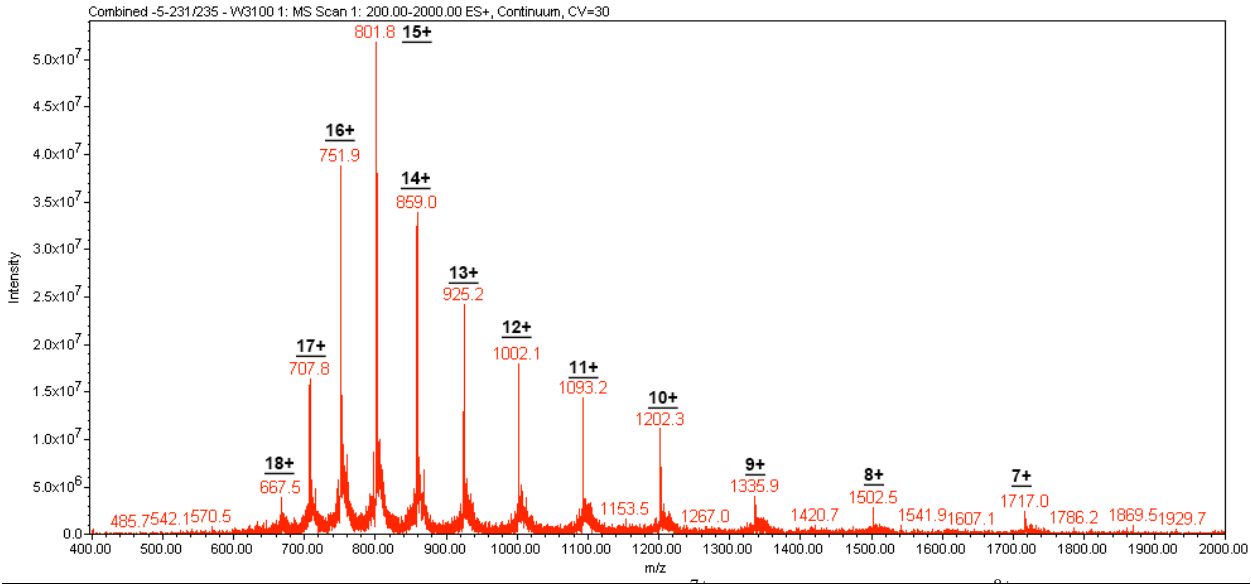
Peptide S37 *In situ* Fmoc removal: ESI calcd for $C_{327}H_{559}N_{105}O_{120}P_3$ $[M+4H]^{4+}$ m/z = 1994.26 $[M+5H]^{5+}$ m/z = 1595.61 $[M+6H]^{6+}$ m/z = 1329.84 $[M+7H]^{7+}$ m/z = 1140.01 $[M+8H]^{8+}$ m/z = 997.63 $[M+9H]^{9+}$ m/z = 886.90 $[M+10H]^{10+}$ m/z = 798.31 $[M+11H]^{11+}$ m/z = 725.83, found: 1994.3; 1595.7; 1330.0; 1140.2; 997.7; 887.0; 798.5; 725.9.

c) Synthesis of **Peptide S38**

6.1 mg of **Peptide S33** (2.1 equiv.) and 5.4 mg of **Peptide S37** (1.0 equiv.) were incubated in pyridine/acetic acid (9.6 µL, 1/12 mole/mole) at a concentration of 20 mM at room temperature. The reaction was monitored (20-40% CH_3CN/H_2O containing 0.1% TFA over 15 min) on a C4 analytical column at a flow rate of 0.6 mL/min. After the completion of the reaction within 6 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v) for up to 1 h to allow complete deprotection of Hmb which rendered native Gly96 concomitantly. Preparative HPLC purification (10-40% CH_3CN/H_2O containing 0.1% TFA over 30 min) followed by concentration at reduced pressure and lyophilization afforded 2.9 mg (35% yield) of **Peptide S38** as a white powder.

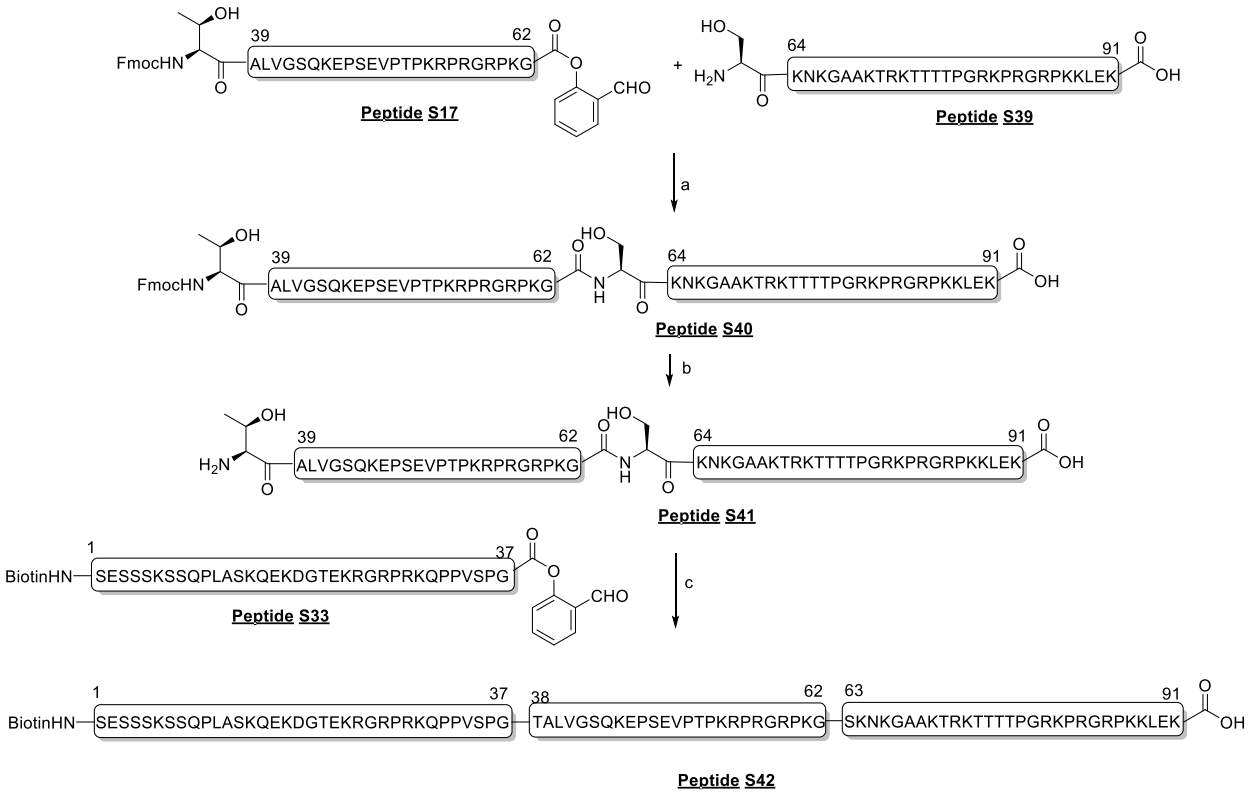


Characterization of synthetic **Peptide S38**. The analysis is run on a C18 column with a gradient of 5-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min.



Peptide S38 ESI calcd for $C_{492}H_{842}N_{164}O_{177}P_3S_1$ $[M+7H]^{7+}$ m/z = 1716.74 $[M+8H]^{8+}$ m/z = 1502.27 $[M+9H]^{9+}$ m/z = 1335.46 $[M+10H]^{10+}$ m/z = 1202.02 $[M+11H]^{11+}$ m/z = 1092.83 $[M+12H]^{12+}$ m/z = 1001.85 $[M+13H]^{13+}$ m/z = 924.86 $[M+14H]^{14+}$ m/z = 858.87 $[M+15H]^{15+}$ m/z = 801.68 $[M+16H]^{16+}$ m/z = 751.64 $[M+17H]^{17+}$ m/z = 707.48 $[M+18H]^{18+}$ m/z = 668.23, found: 1717.0; 1502.5; 1335.9; 1202.3; 1093.2;1002.1; 925.2; 859.0; 801.8; 751.9; 707.8; 667.5.

Synthesis of Peptide S42 (Scheme S12)

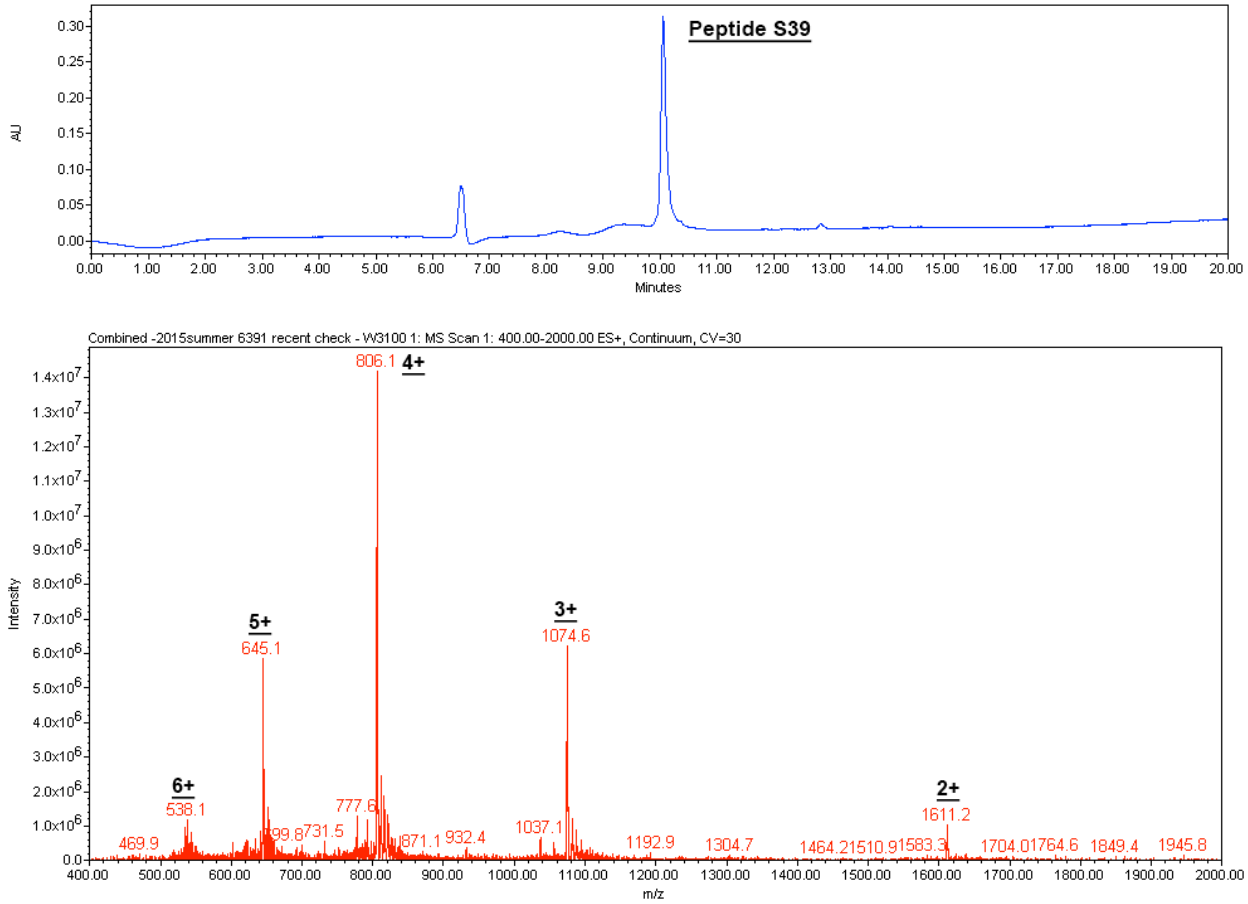


a) Pyridine/AcOH (1/12, mol/mol), 9.5 h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 0.5 h, 42 %. b) $Et_2NH/H_2O/CH_3CN$, 3.5 h, 82 %. c) Pyridine/AcOH (1/12, mol/mol), 5.5 h; TFA/H_2O/Triisopropylsilane(95/2.5/2.5, v/v/v), 0.5 h, 52%.

1) Preparation of Peptide S17 was indicated in Scheme S5.

2) Preparation of the Peptide S33 was indicated in Scheme S10.

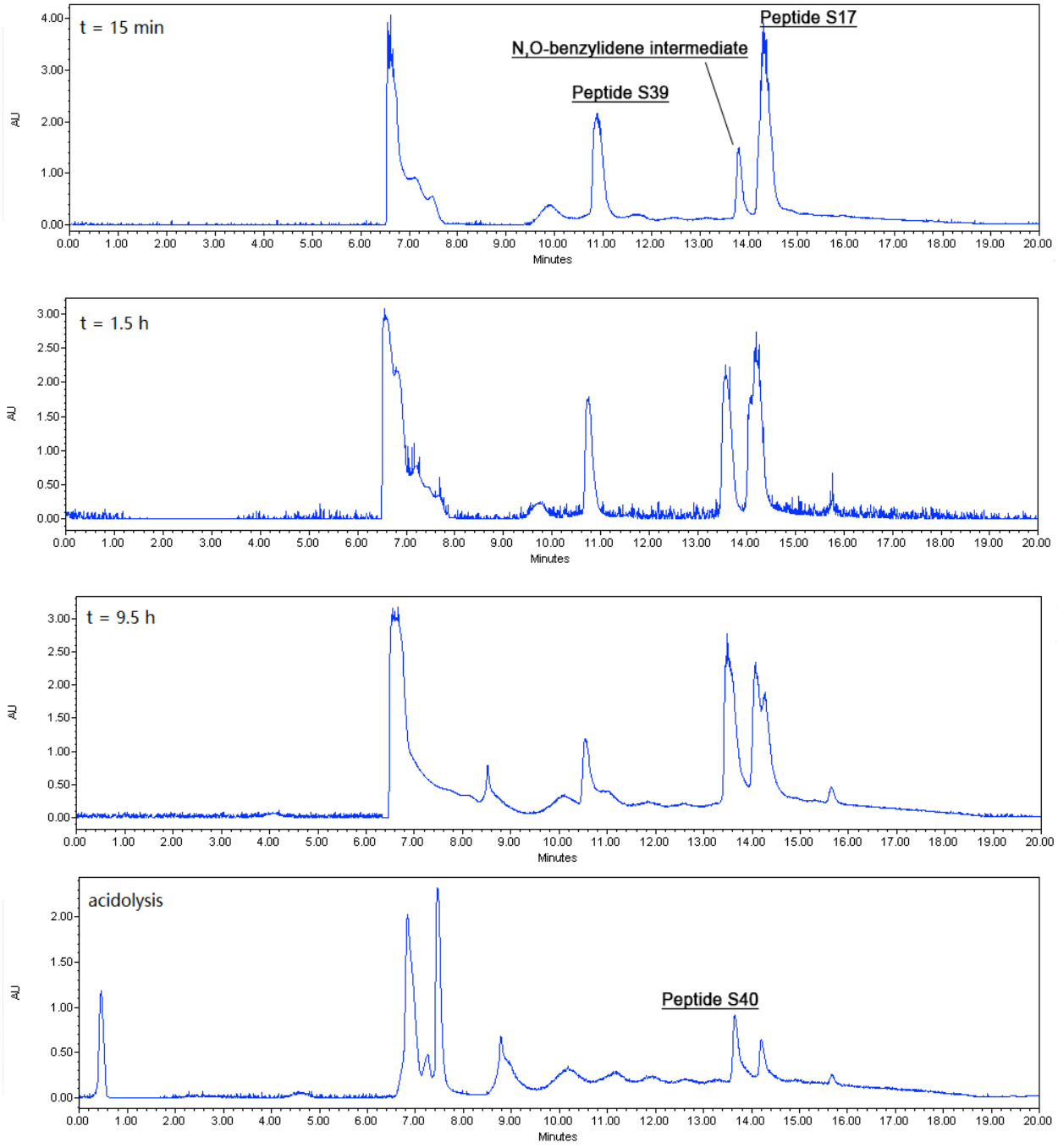
3) Preparation of Peptide S39 was achieved according to general procedure without further modification. Preparative HPLC purification (10-40% CH_3CN/H_2O over 30 min) followed by concentration under *vacuo* and lyophilization gave 40.3 mg of the fragment **Peptide S39** out of 0.1 g of the resin. (yield 15% based on resin loading).

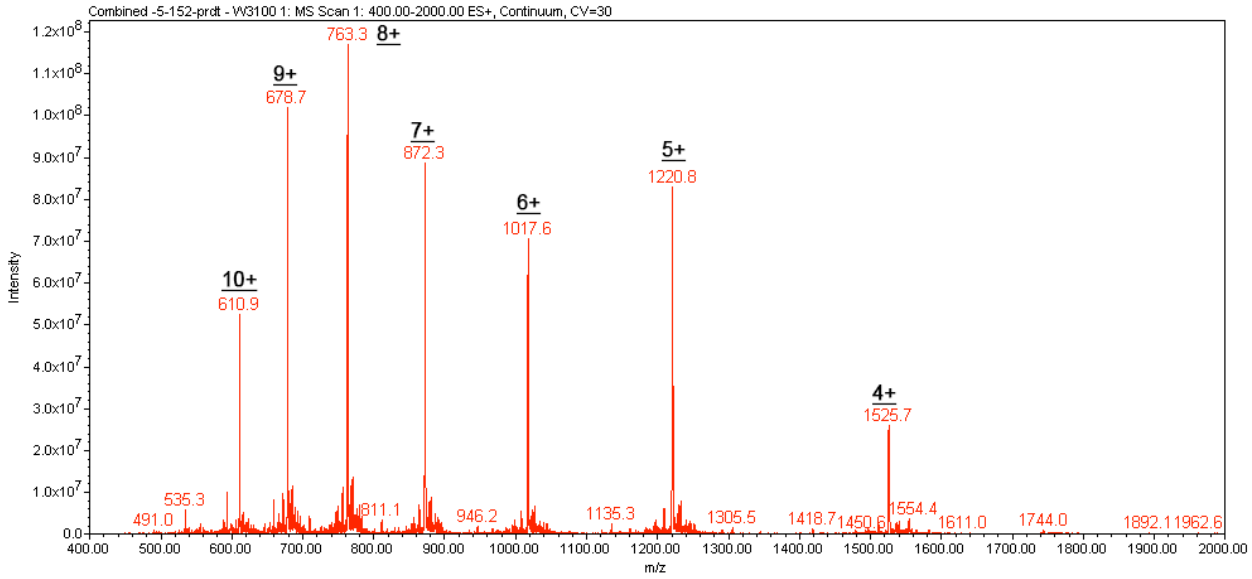


Peptide S39 was characterized under analytical condition (10-95% CH_3CN/H_2O over 15 min). ESI calcd for $C_{137}H_{251}N_{50}O_{39}$ [M+2H] $^{2+}$ m/z = 1611.46 [M+3H] $^{3+}$ m/z = 1074.65 [M+4H] $^{4+}$ m/z = 806.24 [M+5H] $^{5+}$ m/z = 645.19, [M+6H] $^{6+}$ m/z = 537.83 [M+7H] $^{7+}$ m/z = 461.14, found: 1611.5; 1074.8; 806.4; 645.4; 537.9; 461.2.

a) Synthesis of Peptide S40

32.1 mg of **Peptide S17** (1.5 equiv.) and 23.0 mg of **Peptide S39** (1.0 equiv.) were incubated for serine ligation as described before. The reaction was monitored on a C18 analytical column with a gradient of 10-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min. After the completion of the reaction within 9.5 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v). Preparative HPLC purification (10–50% CH_3CN/H_2O containing 0.1% TFA over 30 min) followed by concentration at reduced pressure and lyophilization afforded 18.5 mg (43% yield) of **Peptide S40** as a white powder.

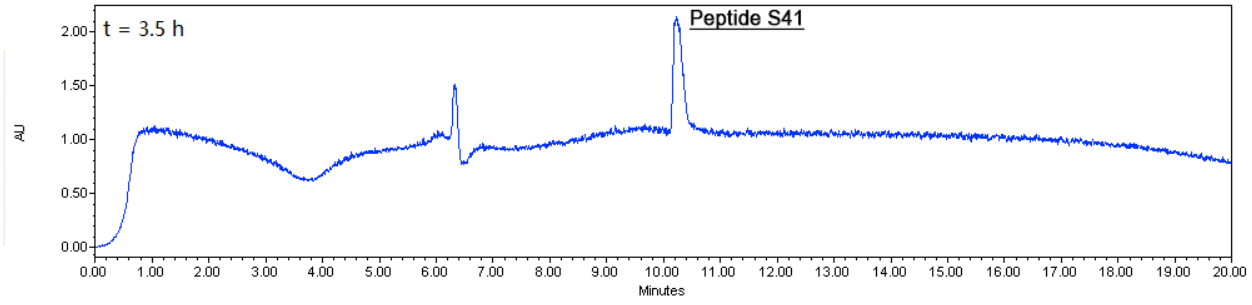

t = 15 min
Peptide S17
N,O-benzylidene intermediate
Peptide S39
t = 1.5 h
t = 9.5 h
acidolysis
Peptide S40
AU
Minutes

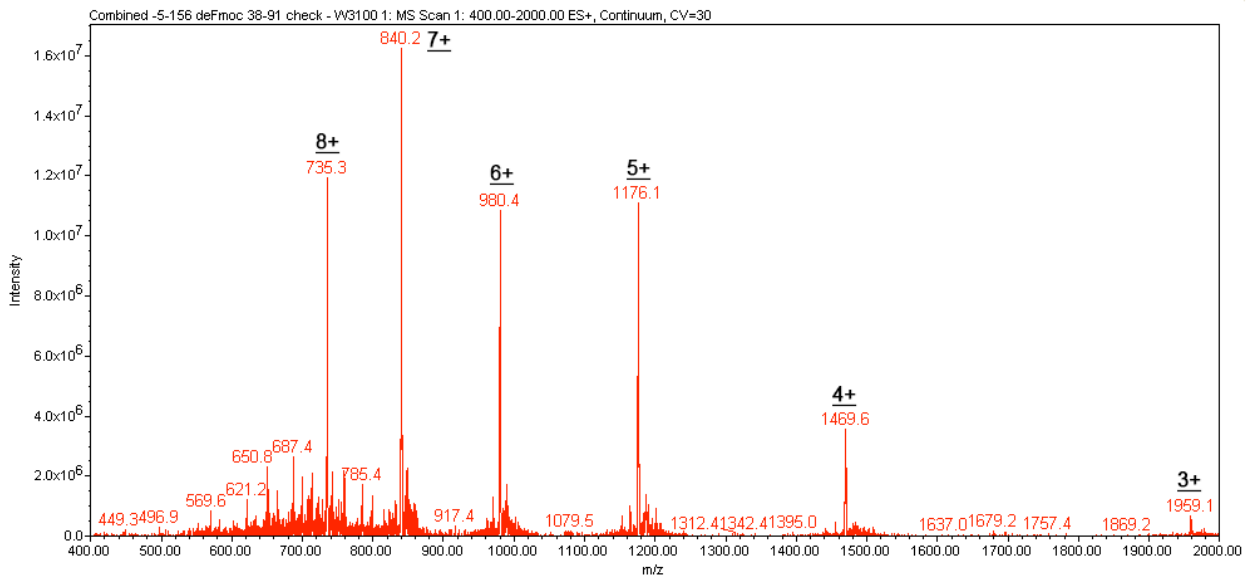


Peptide S40 ESI calcd for $C_{344}H_{568}N_{105}O_{114}$ $[M+4H]^{4+}$ m/z = 1525.62 $[M+5H]^{5+}$ m/z = 1220.70 $[M+6H]^{6+}$ m/z = 1017.42 $[M+7H]^{7+}$ m/z = 872.22 $[M+8H]^{8+}$ m/z = 763.32 $[M+9H]^{9+}$ m/z = 678.41 $[M+10H]^{10+}$ m/z = 610.85, found: 1525.7; 1220.8; 1017.6; 872.3; 763.3; 678.7; 610.9.

b) Synthesis of Peptide S41

7.6 mg of **Peptide S40** was treated with 10% Et_2NH solution in CH_3CN/H_2O (1/1, v/v)[9] at a concentration of 2.5 mM. After incubation for 3.5 h at room temperature, the reaction was quenched with 1% aqueous TFA. Preparative HPLC purification (10–50% CH_3CN/H_2O over 30 min) followed by concentration at reduced pressure and lyophilization afforded 5.6 mg (75% yield) of **Peptide S41** as a white powder.

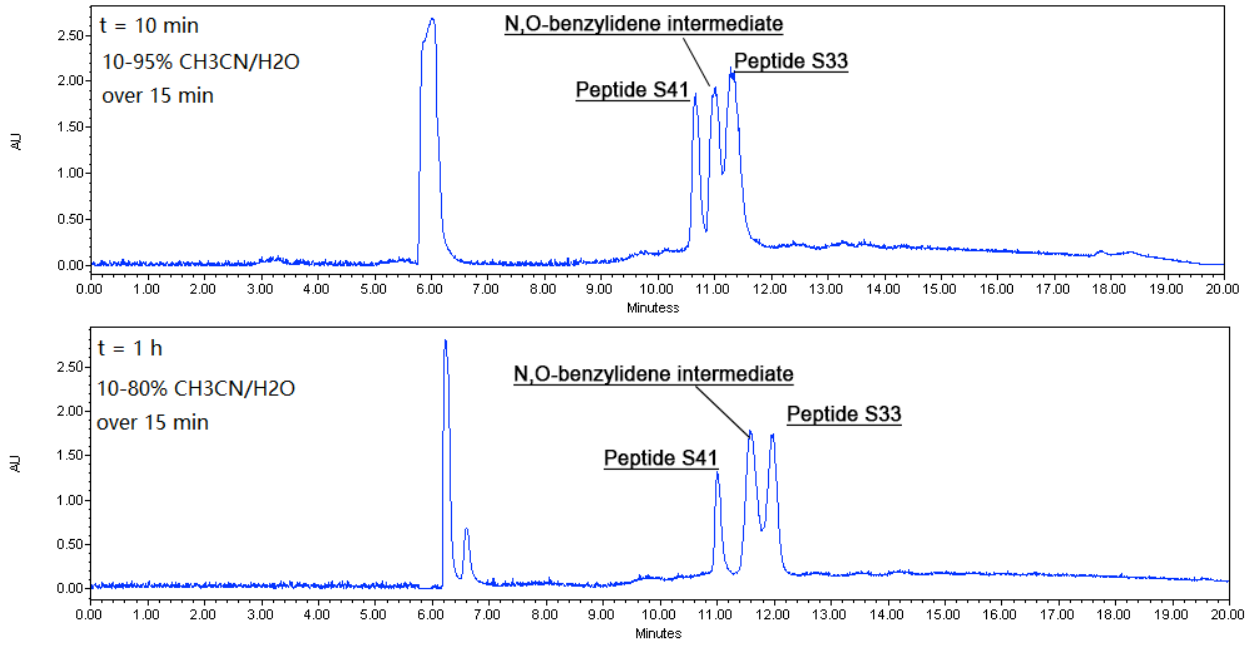


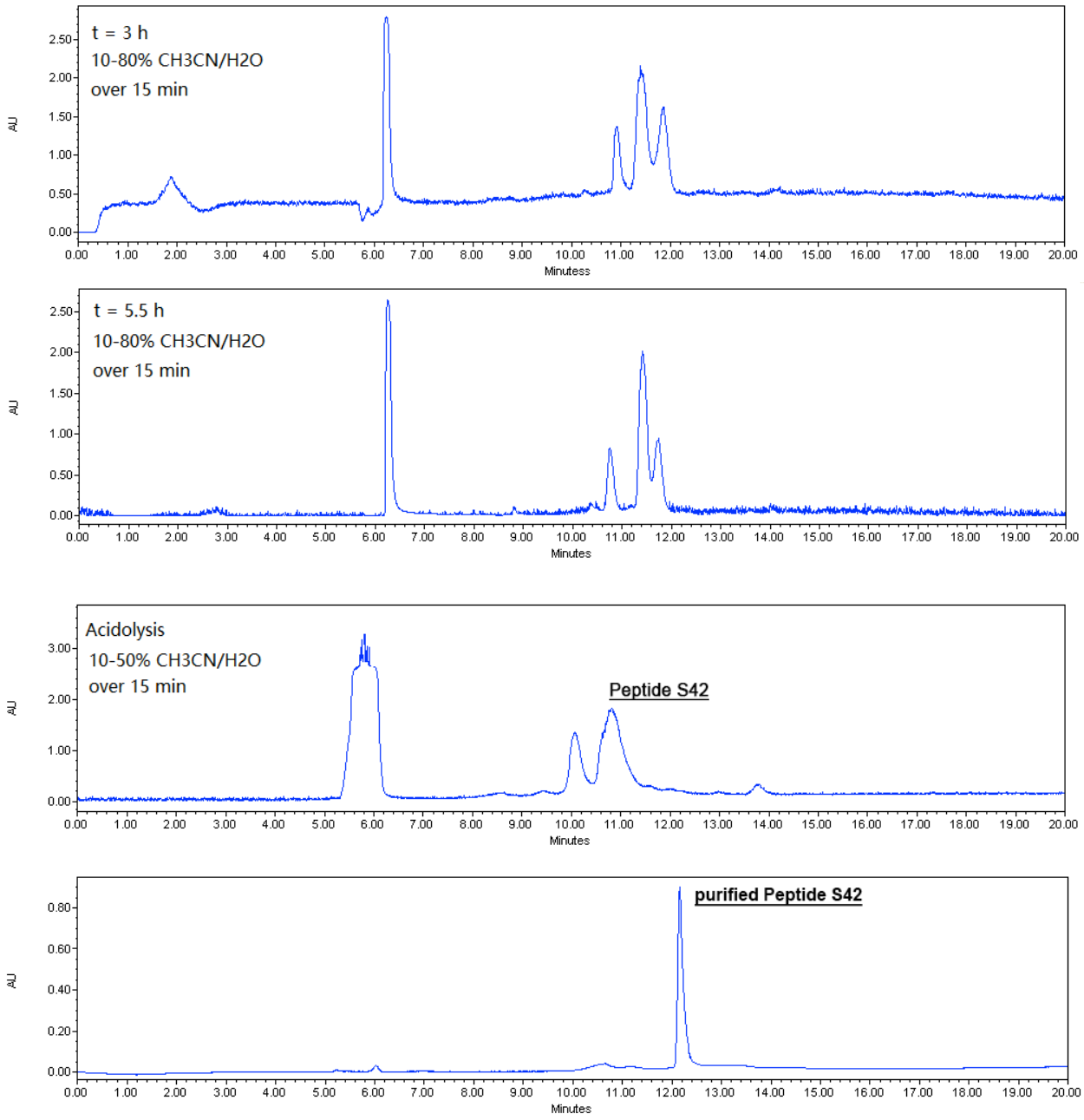


Peptide S41 ESI calcd for $C_{252}H_{447}N_{88}O_{73}$ $[M+3H]^{3+}$ m/z = 1959.81 $[M+4H]^{4+}$ m/z = 1470.11 $[M+5H]^{5+}$ m/z = 1176.29 $[M+6H]^{6+}$ m/z = 980.41 $[M+7H]^{7+}$ m/z = 840.49 $[M+8H]^{8+}$ m/z = 735.56, found: 1959.1; 1469.6; 1176.1; 980.4; 840.2; 735.3.

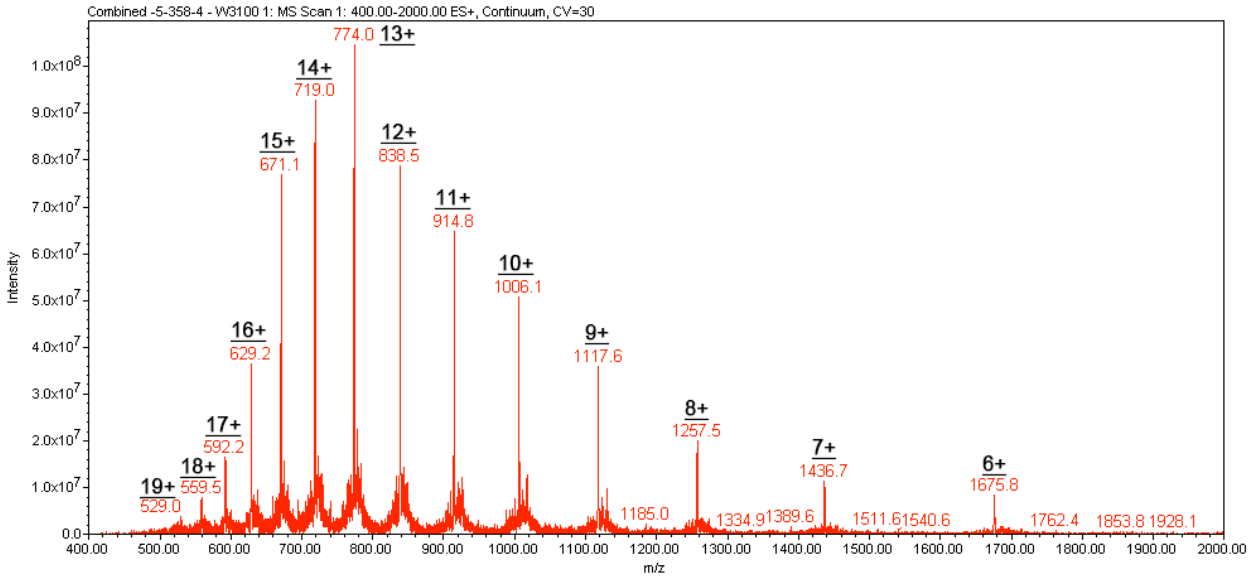
c) Synthesis of Peptide S42

4.7 mg of **Peptide S33** (2.0 equiv.) and 3.2 mg of **Peptide S41** (1.0 equiv.) were incubated in pyridine/acetic acid (27.2 µL, 1/12 mole/mole) at a concentration of 20 mM at room temperature. The reaction was monitored on a C4 analytical column at a flow rate of 0.6 mL/min. After the completion of the reaction within 5.5 h, the solvent was blown off under a stream of condensed air. The residue was then treated with TFA/H_2O/TIPS (95/2.5/2.5, v/v/v) for 0.5 h. Preparative HPLC purification (10-40% CH_3CN/H_2O over 30 min) followed by concentration at reduced pressure and lyophilization afforded 2.8 mg (52% yield) of **Peptide S42** as a white powder.





Characterization of synthetic **Peptide S42**. The analysis is run on a C18 column with a gradient of 5-95% CH_3CN/H_2O containing 0.1% TFA over 15 min at a flow rate of 0.6 mL/min.



Peptide S42 ESI calcd for $C_{425}H_{738}N_{147}O_{132}S_1$ $[M+6H]^{6+}$ m/z = 1675.93 $[M+7H]^{7+}$ m/z = 1436.65 $[M+8H]^{8+}$ m/z = 1257.20 $[M+9H]^{9+}$ m/z = 1117.62 $[M+10H]^{10+}$ m/z = 1005.96 $[M+11H]^{11+}$ m/z = 914.60 $[M+12H]^{12+}$ m/z = 838.47 $[M+13H]^{13+}$ m/z = 774.05 $[M+14H]^{14+}$ m/z = 718.83 $[M+15H]^{15+}$ m/z = 670.98 $[M+16H]^{16+}$ m/z = 629.10 $[M+17H]^{17+}$ m/z = 592.16 $[M+18H]^{18+}$ m/z = 559.31 $[M+19H]^{19+}$ m/z = 529.93, found: 1675.8; 1436.7; 1257.5; 1117.6; 1006.1; 914.8; 838.5; 774.0; 719.0; 671.1; 629.2; 592.2; 559.5; 529.0.

References

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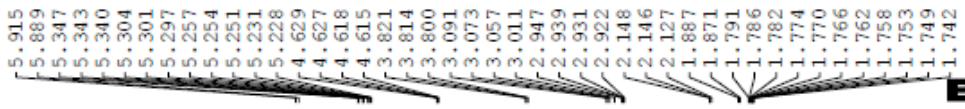

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PROCNO                        1
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Time_                     20.39
INSTRUM                   spect
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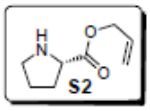
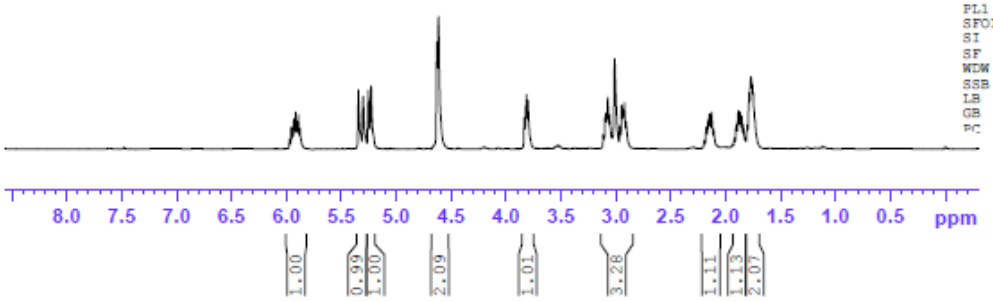
PULPROG                   zg30
TD                       32768
SOLVENT                  CDCl3
NS                          32
DS                           1
SWH                 5995.204 Hz
FIDRES              0.182959 Hz
AQ                  2.7329011 sec
RG                          18
DW                     83.400 usec
DE                      6.00 usec
TE                     295.8 K
D1                  1.00000000 sec
MCREST              0.00000000 sec
MCWRK               0.01500000 sec

======== CHANNEL f1 ========
NUC1                        1H
P1                     13.40 usec
PL1                    -4.00 dB
SFO1           400.1320007 MHz
SI                       32768
SF             400.1299234 MHz
WDW                         EM
SSB                          0
LB                      0.30 Hz
GB                           0
PC                        1.00
```





```
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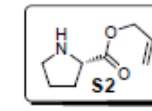
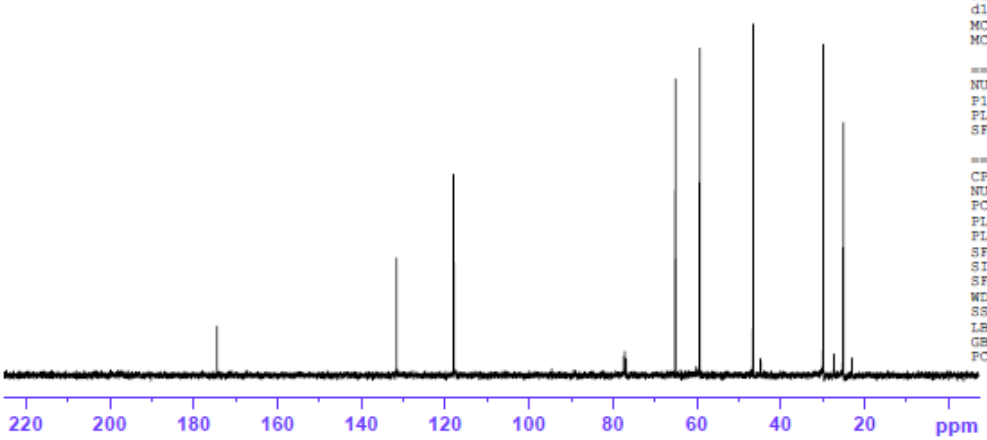
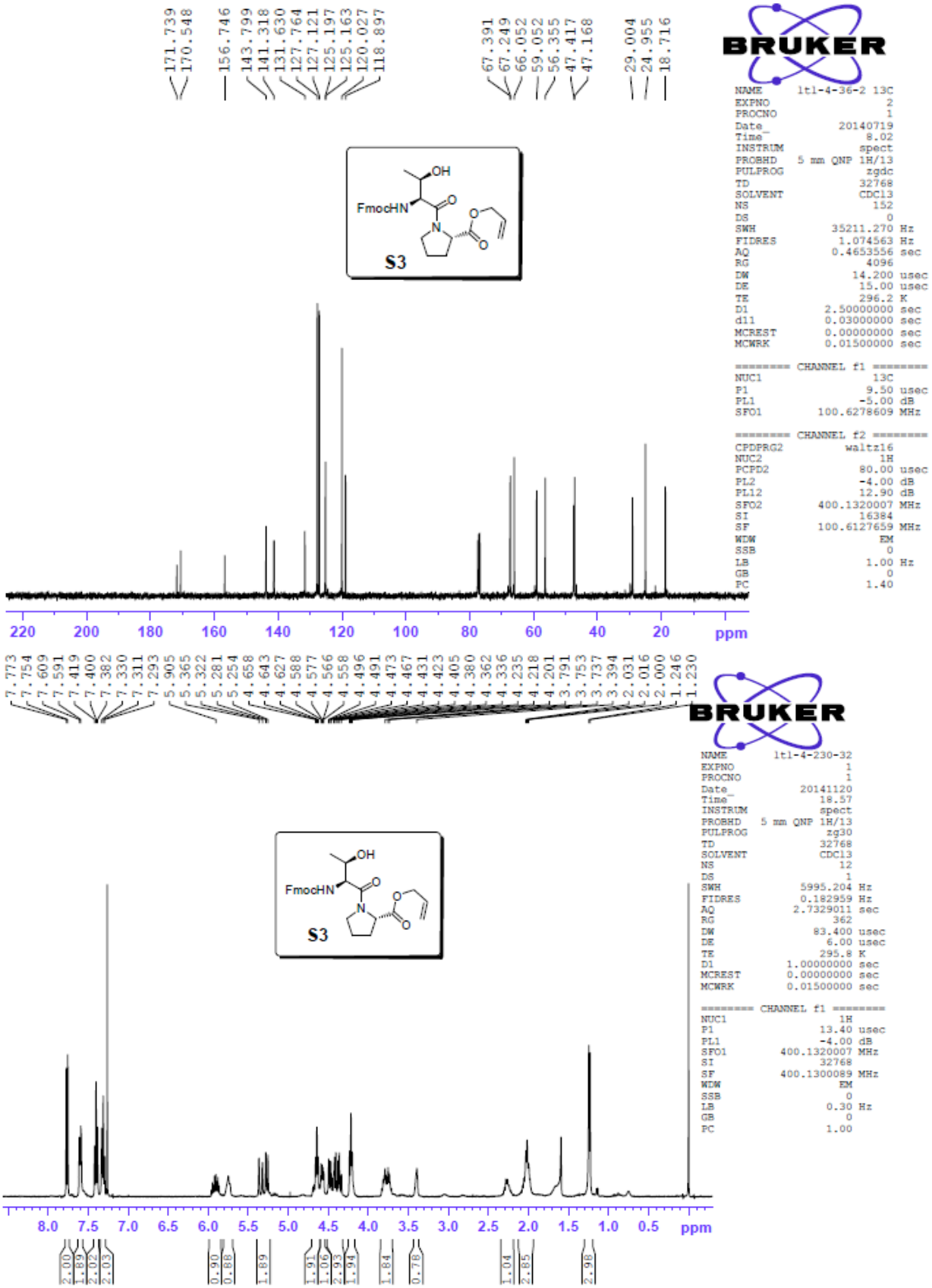
PROCNO                        1
Date_                  20141112
Time_                     20.42
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                   zgdc
TD                       32768
SOLVENT                  CDCl3
NS                          19
DS                           0
SWH                35211.270 Hz
FIDRES              1.074563 Hz
AQ                  0.4653556 sec
RG                     5792.6
DW                     14.200 usec
DE                      15.00 usec
TE                     295.9 K
D1                  2.50000000 sec
d11                 0.03000000 sec
MCREST              0.00000000 sec
MCWRK               0.01500000 sec

======== CHANNEL f1 ========
NUC1                       13C
P1                      9.50 usec
PL1                    -5.00 dB
SFO1           100.6278609 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                        1H
PCPD2                  80.00 usec
PL2                    -4.00 dB
PL12                   12.90 dB
SFO2           400.1320007 MHz
SI                       16384
SF             100.6127852 MHz
WDW                         EM
SSB                          0
LB                      1.00 Hz
GB                           0
PC                        1.40
```



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156.746
143.799
141.318
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125.197
125.163
120.027
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67.249
66.052
59.052
56.355
47.417
47.168
29.004
24.955
18.716
OH
FmocHN
O
N
O
O
S3
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NAME 1t1-4-36-2 13C
EXPNO 2
PROCNO 1
Date_ 20140719
Time_ 8.02
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT CDCl3
NS 152
DS 0
SWH 35211.270 Hz
FIDRES 1.074563 Hz
AQ 0.4653556 sec
RG 4096
DW 14.200 usec
DE 15.00 usec
TE 296.2 K
D1 2.50000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
CHANNEL f1
NUC1 13C
P1 9.50 usec
PL1 -5.00 dB
SFO1 100.6278609 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 12.90 dB
SFO2 400.1320007 MHz
SI 16384
SF 100.6127659 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
220 200 180 160 140 120 100 80 60 40 20 ppm
7.773
7.754
7.609
7.591
7.419
7.400
7.382
7.330
7.311
7.293
5.905
5.365
5.322
5.281
5.254
4.658
4.643
4.627
4.588
4.577
4.566
4.558
4.496
4.491
4.473
4.467
4.431
4.423
4.405
4.380
4.362
4.336
4.235
4.218
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3.791
3.753
3.737
3.394
2.031
2.016
2.000
1.246
1.230
OH
FmocHN
O
N
O
O
S3
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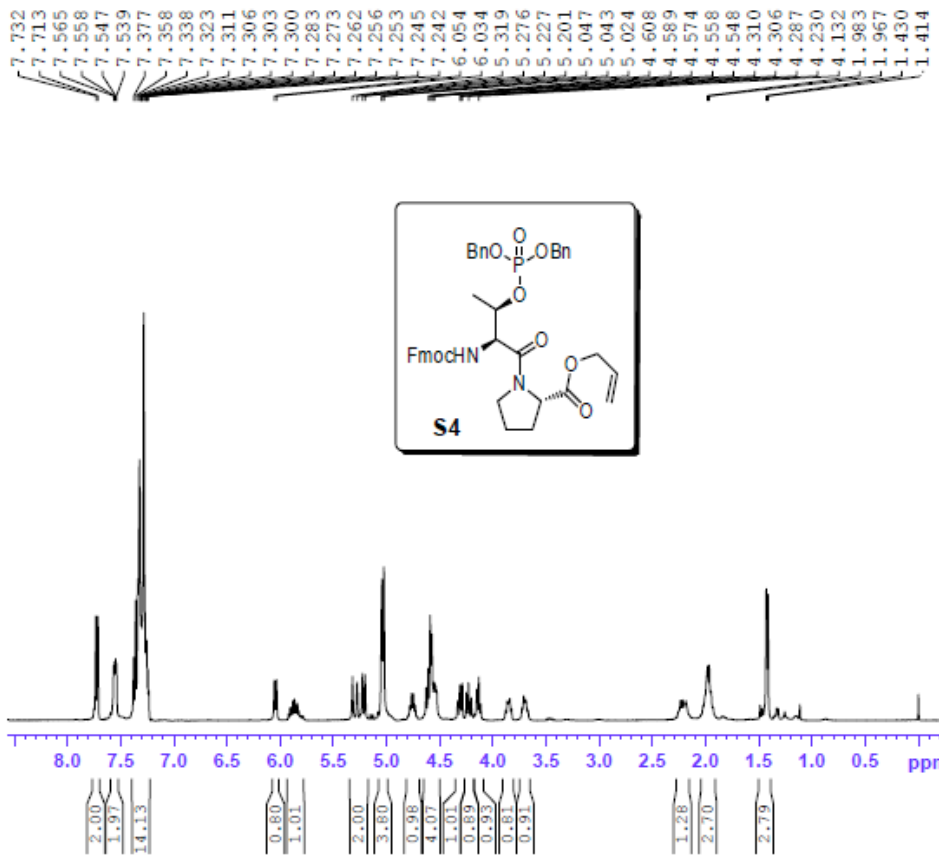
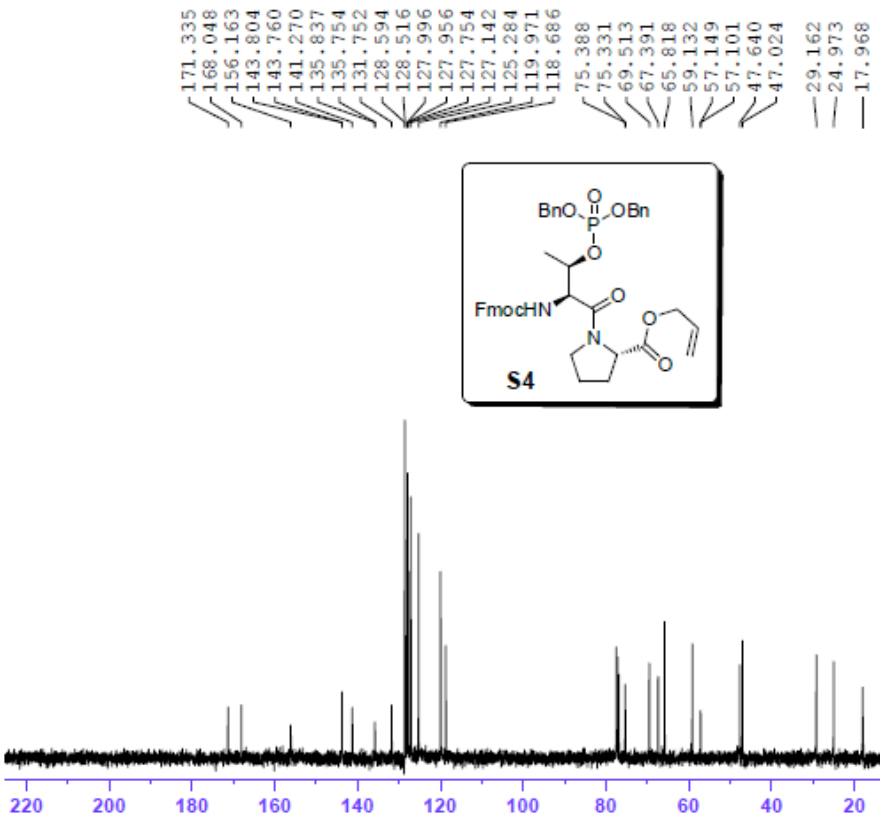
NAME 1t1-4-230-32
EXPNO 1
PROCNO 1
Date_ 20141120
Time_ 18.57
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 12
DS 1
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 362
DW 83.400 usec
DE 6.00 usec
TE 295.8 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
CHANNEL f1
NUC1 1H
P1 13.40 usec
PL1 -4.00 dB
SFO1 400.1320007 MHz
SI 32768
SF 400.1300089 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.00
1.89
2.02
2.03
0.90
0.88
1.89
1.91
1.06
2.93
1.94
1.84
0.78
1.04
2.85
2.98




```
NAME                1t1-4-232-3
EXPNO                         1
PROCNO                        1
Date_                  20141122
Time_                      8.36
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                   zg30
TD                       32768
SOLVENT                  CDCl3
NS                           11
DS                            1
SWH                   5995.204 Hz
FIDRES                0.182959 Hz
AQ                    2.7329011 sec
RG                           57
DW                       83.400 usec
DE                        6.00 usec
TE                       295.8 K
D1                    1.00000000 sec
MCREST                0.00000000 sec
MCWRK                 0.01500000 sec

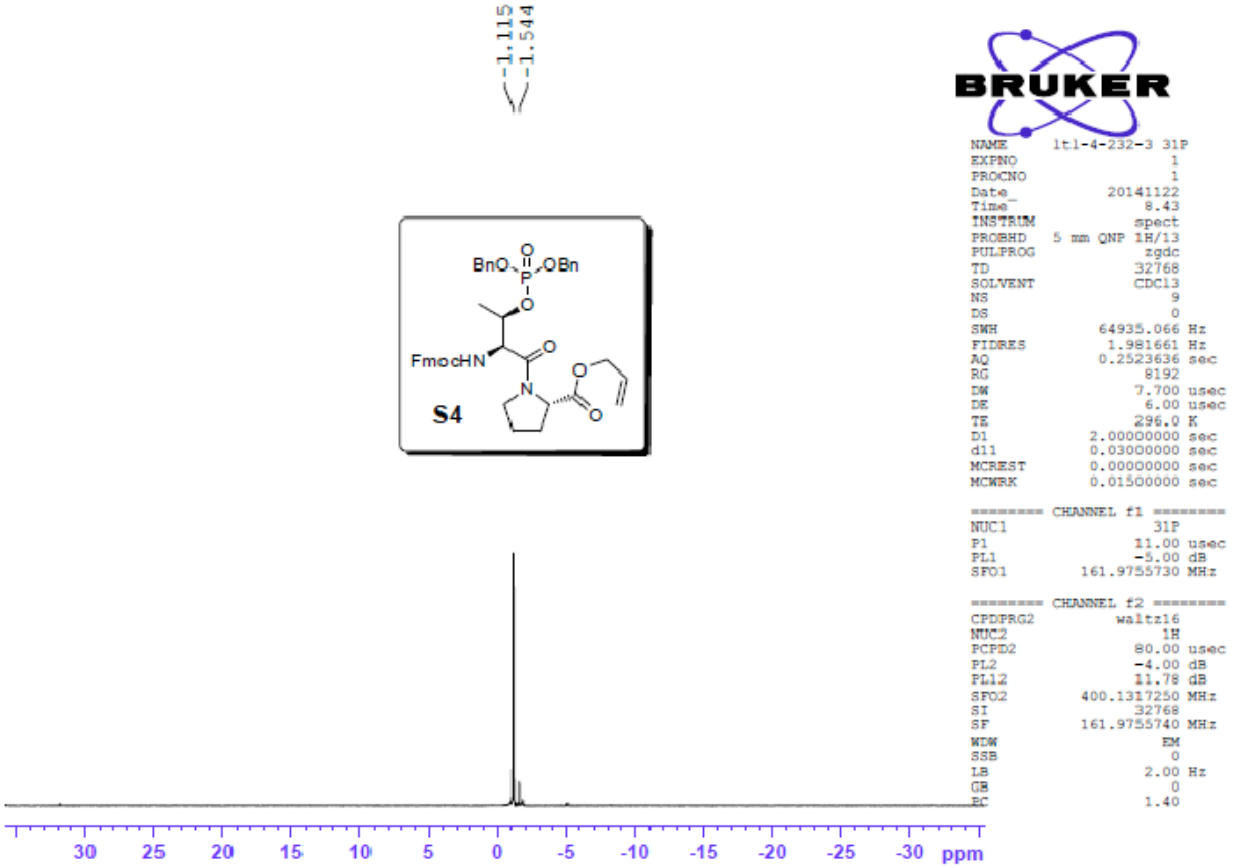
======== CHANNEL f1 ========
NUC1                         1H
P1                       13.40 usec
PL1                      -4.00 dB
SFO1                400.1320007 MHz
SI                       32768
SF                  400.1300154 MHz
WDW                         EM
SSB                          0
LB                       0.30 Hz
GB                           0
PC                        1.00
```




```
NAME                1t1-4-232-3 13C
EXPNO                         2
PROCNO                        1
Date_                  20141122
Time_                      8.40
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                   zgdc
TD                       32768
SOLVENT                  CDCl3
NS                           34
DS                            0
SWH                  35211.270 Hz
FIDRES                1.074563 Hz
AQ                    0.4653556 sec
RG                         4096
DW                       14.200 usec
DE                       15.00 usec
TE                       295.8 K
D1                    2.50000000 sec
d11                   0.03000000 sec
MCREST                0.00000000 sec
MCWRK                 0.01500000 sec

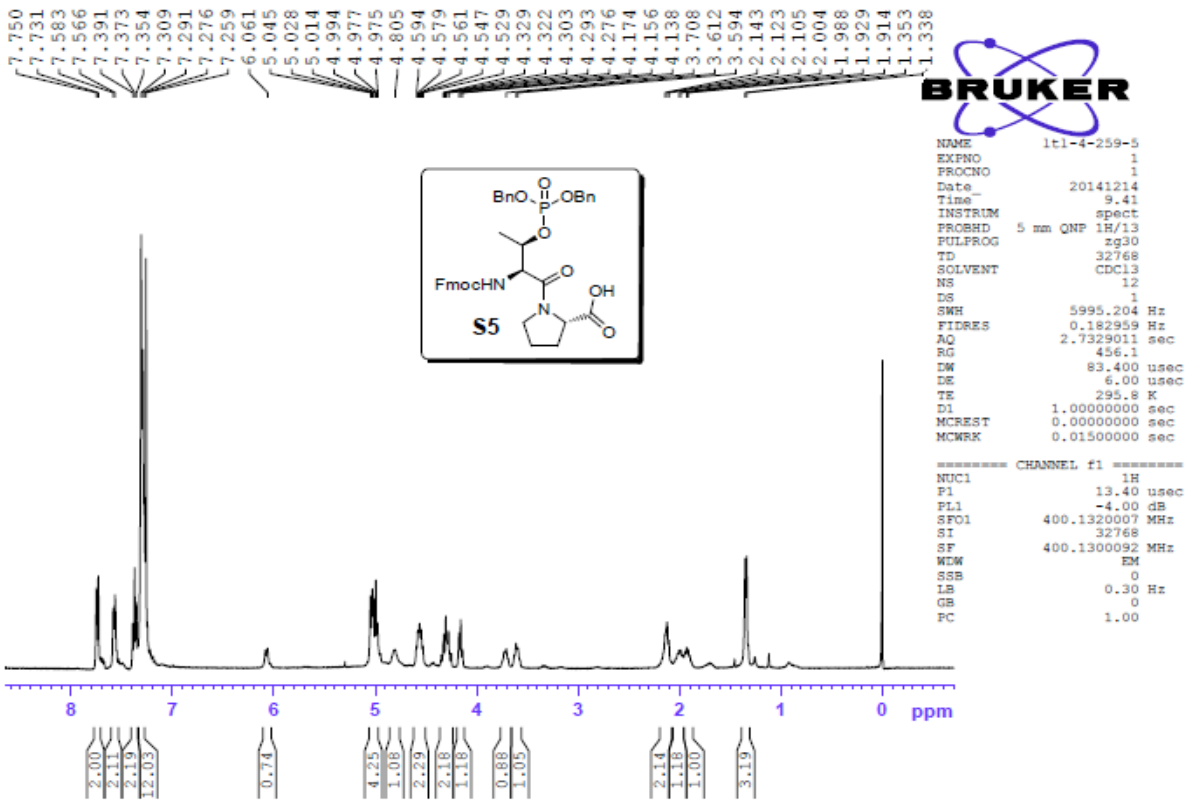
======== CHANNEL f1 ========
NUC1                        13C
P1                        9.50 usec
PL1                      -5.00 dB
SFO1                100.6278609 MHz

======== CHANNEL f2 ========
CPDPRG2                 waltz16
NUC2                         1H
PCPD2                    80.00 usec
PL2                      -4.00 dB
PL12                     12.90 dB
SFO2                400.1320007 MHz
SI                       16384
SF                  100.6127659 MHz
WDW                         EM
SSB                          0
LB                       1.00 Hz
GB                           0
                          1.40
```



BRUKER

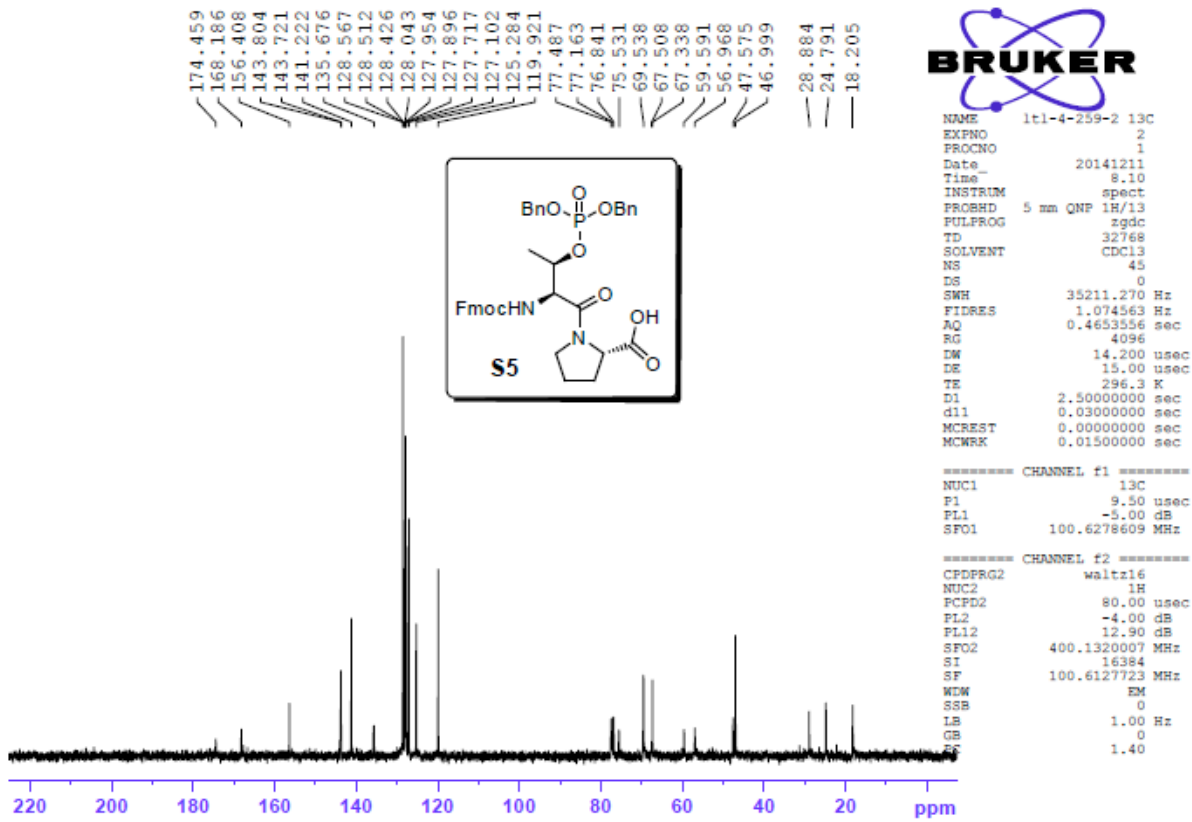
NAME	ltl-4-232-3 31P
EXPNO	1
PROCNO	1
Date_	20141122
Time_	8.43
INSTRUM	spect
PROBHD	5 mm QNP 1H/13
PULPROG	zgdc
TD	32768
SOLVENT	CDCl3
NS	9
DS	0
SWH	64935.066 Hz
FIDRES	1.981661 Hz
AQ	0.2523636 sec
RG	8192
DW	7.700 usec
DE	6.00 usec
TE	296.0 K
D1	2.00000000 sec
d11	0.03000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec
========	CHANNEL f1 ========
NUC1	31P
P1	11.00 usec
PL1	-5.00 dB
SFO1	161.9755730 MHz
========	CHANNEL f2 ========
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.00 dB
PL12	11.78 dB
SFO2	400.1317250 MHz
SI	32768
SF	161.9755740 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.40



BRUKER

```
NAME            1tl-4-259-5
EXPNO                     1
PROCNO                    1
Date_              20141214
Time_                 9.41
INSTRUM              spect
PROBHD   5 mm QNP 1H/13
PULPROG               zg30
TD                   32768
SOLVENT              CDCl3
NS                       12
DS                        1
SWH               5995.204 Hz
FIDRES            0.182959 Hz
AQ                2.7329011 sec
RG                    456.1
DW                 83.400 usec
DE                  6.00 usec
TE                  295.8 K
D1              1.00000000 sec
MCREST          0.00000000 sec
MCWRK           0.01500000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                  13.40 usec
PL1                 -4.00 dB
SFO1          400.1320007 MHz
SI                   32768
SF            400.1300092 MHz
WDW                     EM
SSB                      0
LB                  0.30 Hz
GB                       0
PC                    1.00
```

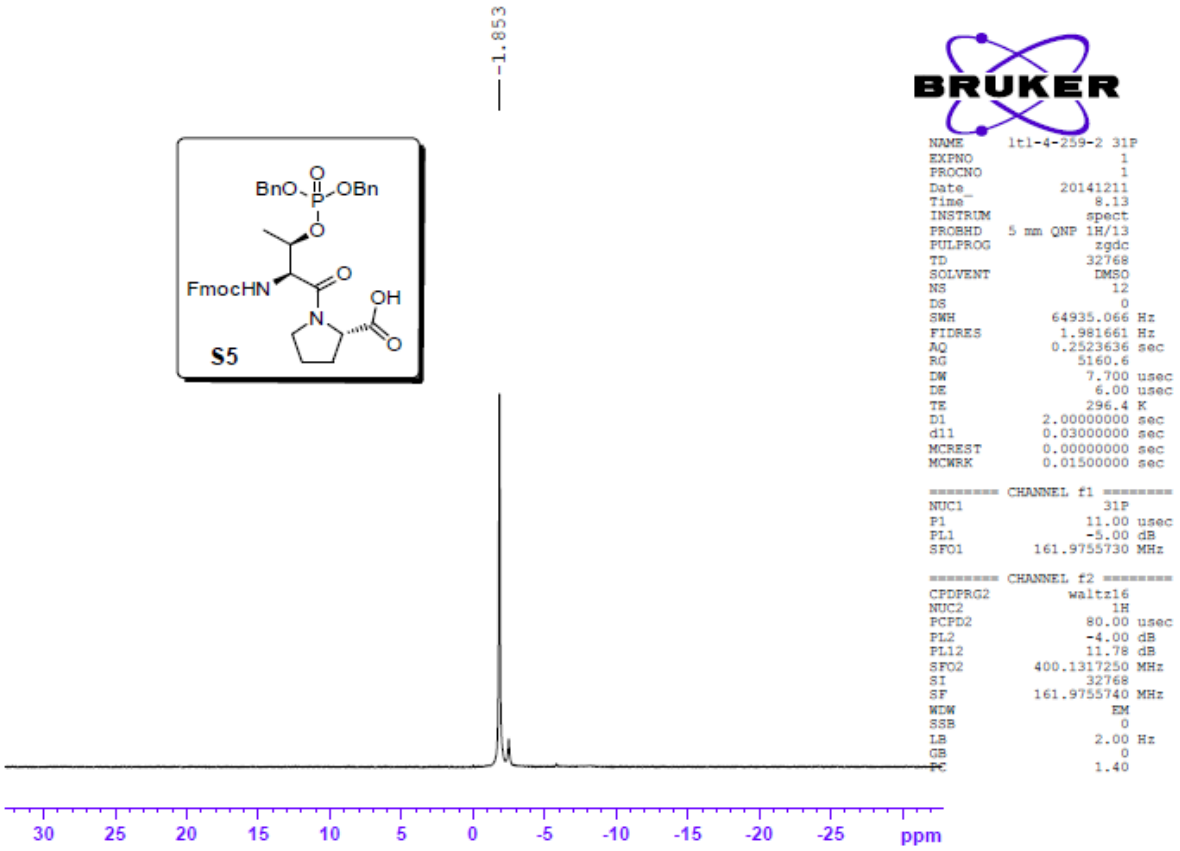


BRUKER

```
NAME          1tl-4-259-2 13C
EXPNO                     2
PROCNO                    1
Date_              20141211
Time_                 8.10
INSTRUM              spect
PROBHD   5 mm QNP 1H/13
PULPROG               zgdc
TD                   32768
SOLVENT              CDCl3
NS                       45
DS                        0
SWH              35211.270 Hz
FIDRES            1.074563 Hz
AQ                0.4653556 sec
RG                    4096
DW                 14.200 usec
DE                  15.00 usec
TE                  296.3 K
D1              2.50000000 sec
d11             0.03000000 sec
MCREST          0.00000000 sec
MCWRK           0.01500000 sec

======== CHANNEL f1 ========
NUC1                   13C
P1                  9.50 usec
PL1                -5.00 dB
SFO1          100.6278609 MHz

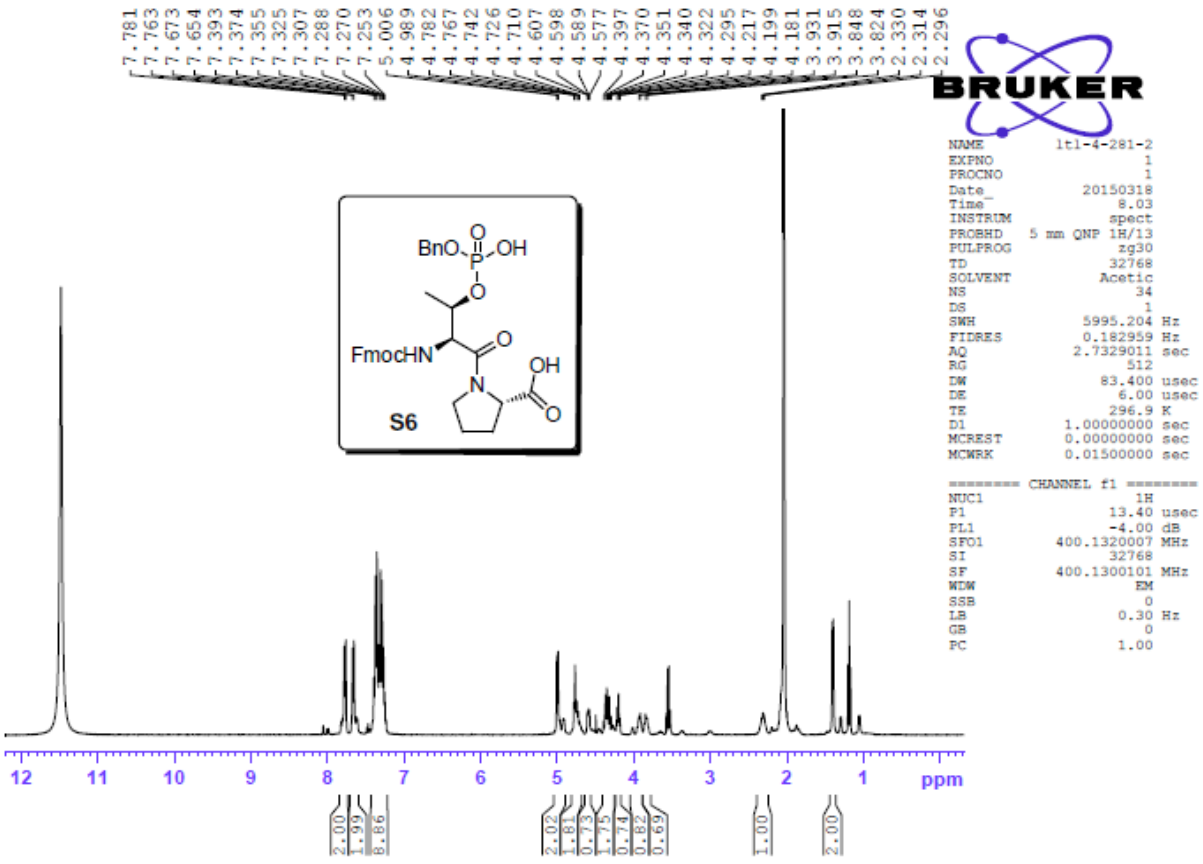
======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                    1H
PCPD2              80.00 usec
PL2                -4.00 dB
PL12               12.90 dB
SFO2          400.1320007 MHz
SI                   16384
SF            100.6127723 MHz
WDW                     EM
SSB                      0
LB                  1.00 Hz
GB                       0
PC                    1.40
```




```
NAME          1t1-4-259-2 31P
EXPNO                      1
PROCNO                     1
Date_               20141211
Time                    8.13
INSTRUM                spect
PROBHD    5 mm QNP 1H/13
PULPROG                zgdc
TD                    32768
SOLVENT                DMSO
NS                       12
DS                        0
SWH                64935.066 Hz
FIDRES             1.981661 Hz
AQ                 0.2523636 sec
RG                    5160.6
DW                     7.700 usec
DE                     6.00 usec
TE                     296.4 K
D1                 2.00000000 sec
d11                0.03000000 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                      31P
P1                     11.00 usec
PL1                    -5.00 dB
SFO1            161.9755730 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
PCPD2                  80.00 usec
PL2                    -4.00 dB
PL12                   11.78 dB
SFO2            400.1317250 MHz
SI                       32768
SF              161.9755740 MHz
WDW                        EM
SSB                         0
LB                      2.00 Hz
GB                          0
PC                      1.40
```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7.355
7.325
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7.253
5.006
4.989
4.782
4.767
4.742
4.726
4.710
4.607
4.598
4.589
4.577
4.397
4.370
4.351
4.340
4.322
4.295
4.217
4.199
4.181
3.931
3.915
3.848
3.824
2.330
2.314
2.296
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N
OH
O
S6
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EXPNO 1
PROCNO 1
Date_ 20150318
Time_ 8.03
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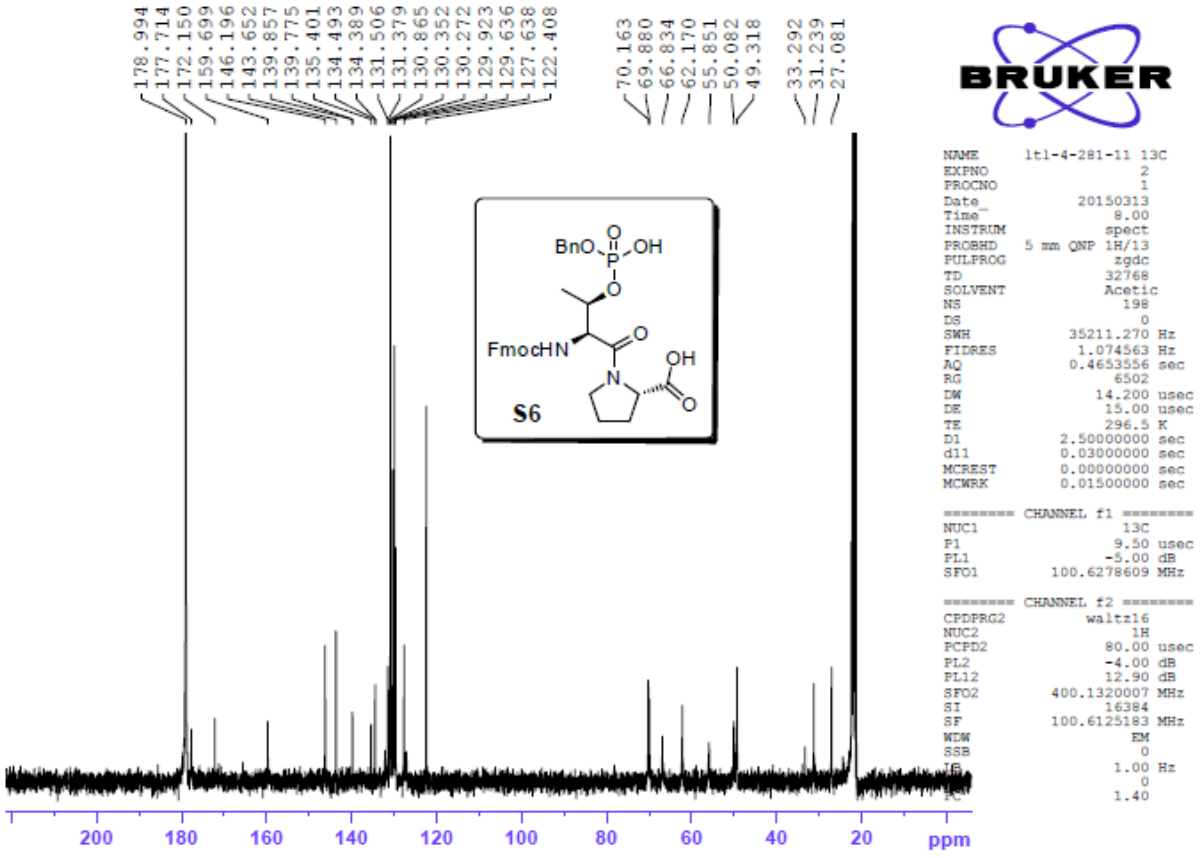
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT Acetic
NS 34
DS 1
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 512
DW 83.400 usec
DE 6.00 usec
TE 296.9 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
CHANNEL f1
NUC1 1H
P1 13.40 usec
PL1 -4.00 dB
SFO1 400.1320007 MHz
SI 32768
SF 400.1300101 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
12 11 10 9 8 7 6 5 4 3 2 1 ppm
2.00 1.99 8.86 2.02 1.81 0.73 1.75 0.74 0.82 0.69 1.00 2.00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131.379
130.865
130.352
130.272
129.923
129.636
127.638
122.408
70.163
69.880
66.834
62.170
55.851
50.082
49.318
33.292
31.239
27.081
BnO
P
OH
O
FmocHN
N
OH
O
S6
BRUKER
NAME 1tl-4-281-11 13C
EXPNO 2
PROCNO 1
Date_ 20150313
Time_ 8.00
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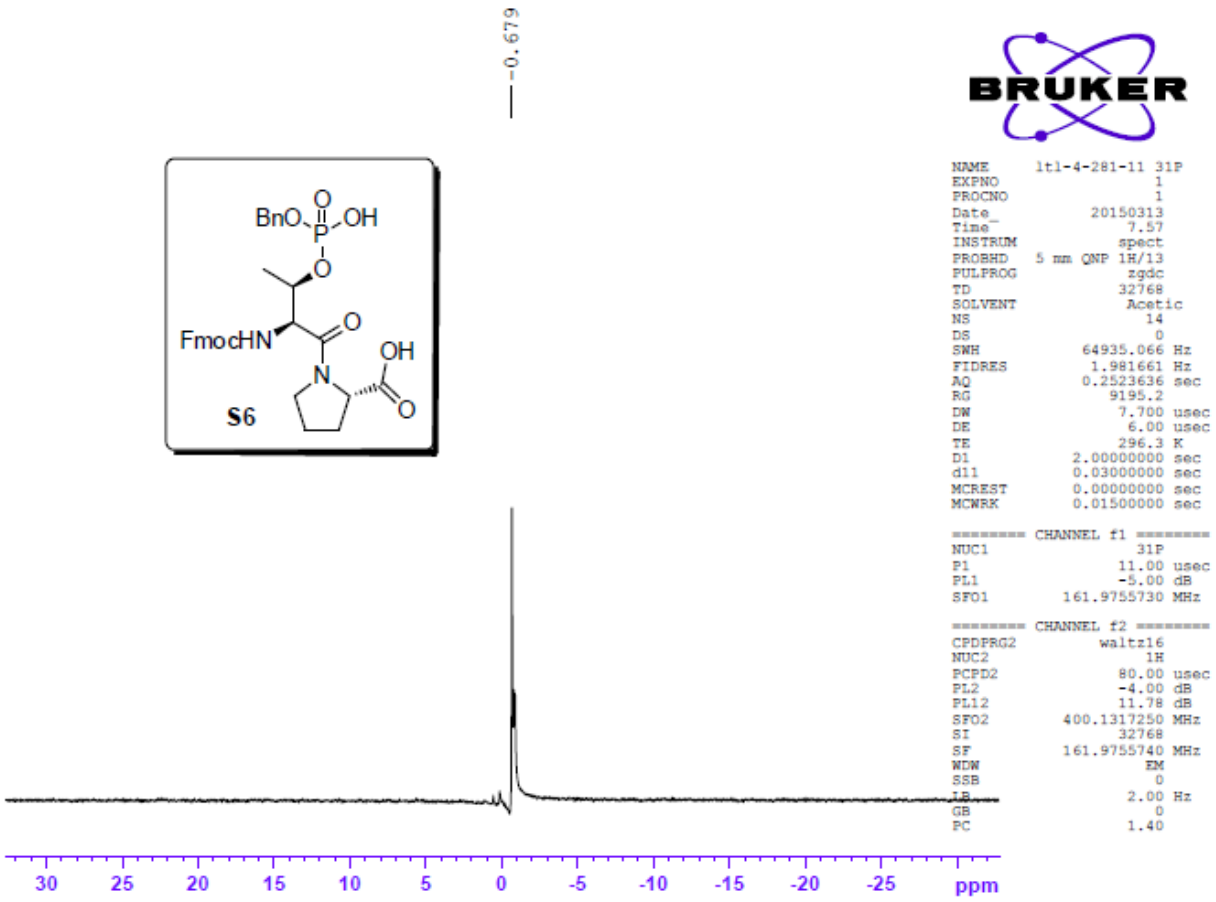
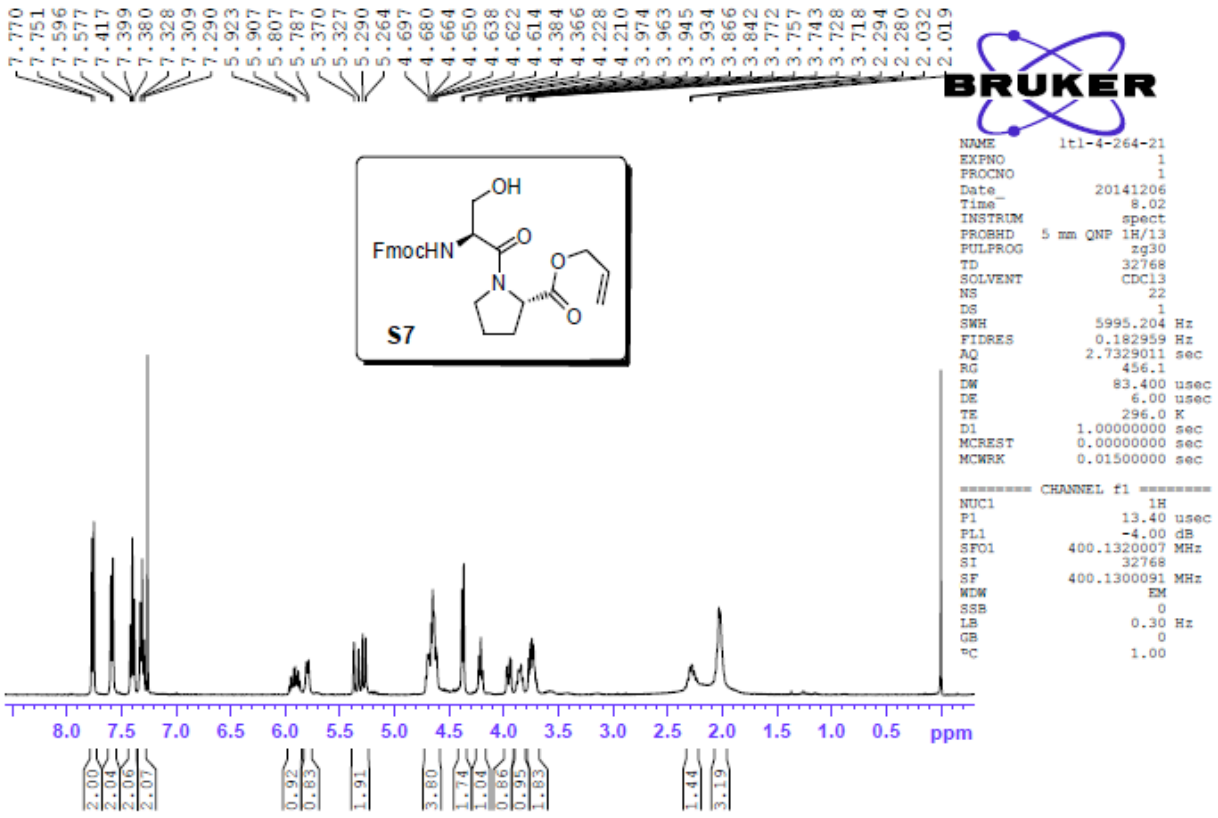
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT Acetic
NS 198
DS 0
SWH 35211.270 Hz
FIDRES 1.074563 Hz
AQ 0.4653556 sec
RG 6502
DW 14.200 usec
DE 15.00 usec
TE 296.5 K
D1 2.50000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
CHANNEL f1
NUC1 13C
P1 9.50 usec
PL1 -5.00 dB
SFO1 100.6278609 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 12.90 dB
SFO2 400.1320007 MHz
SI 16384
SF 100.6125183 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 ppm

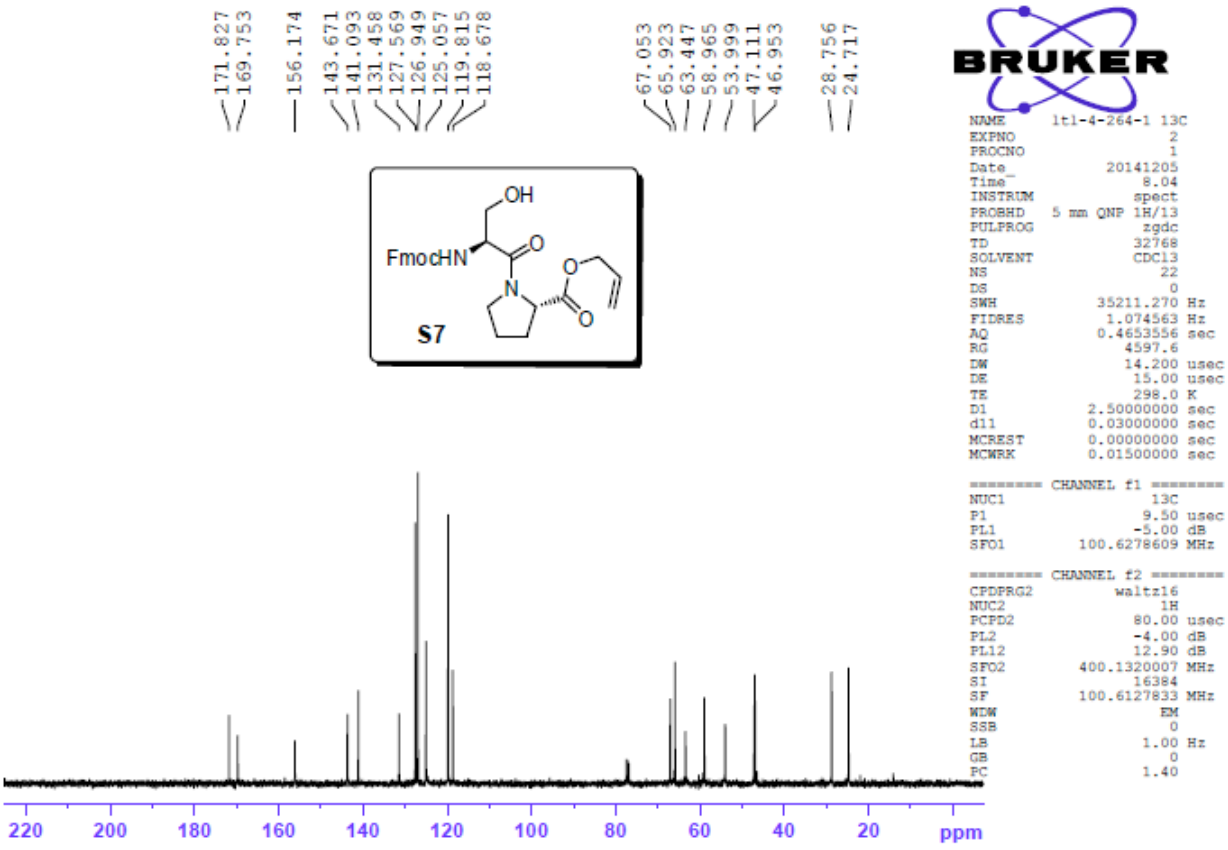


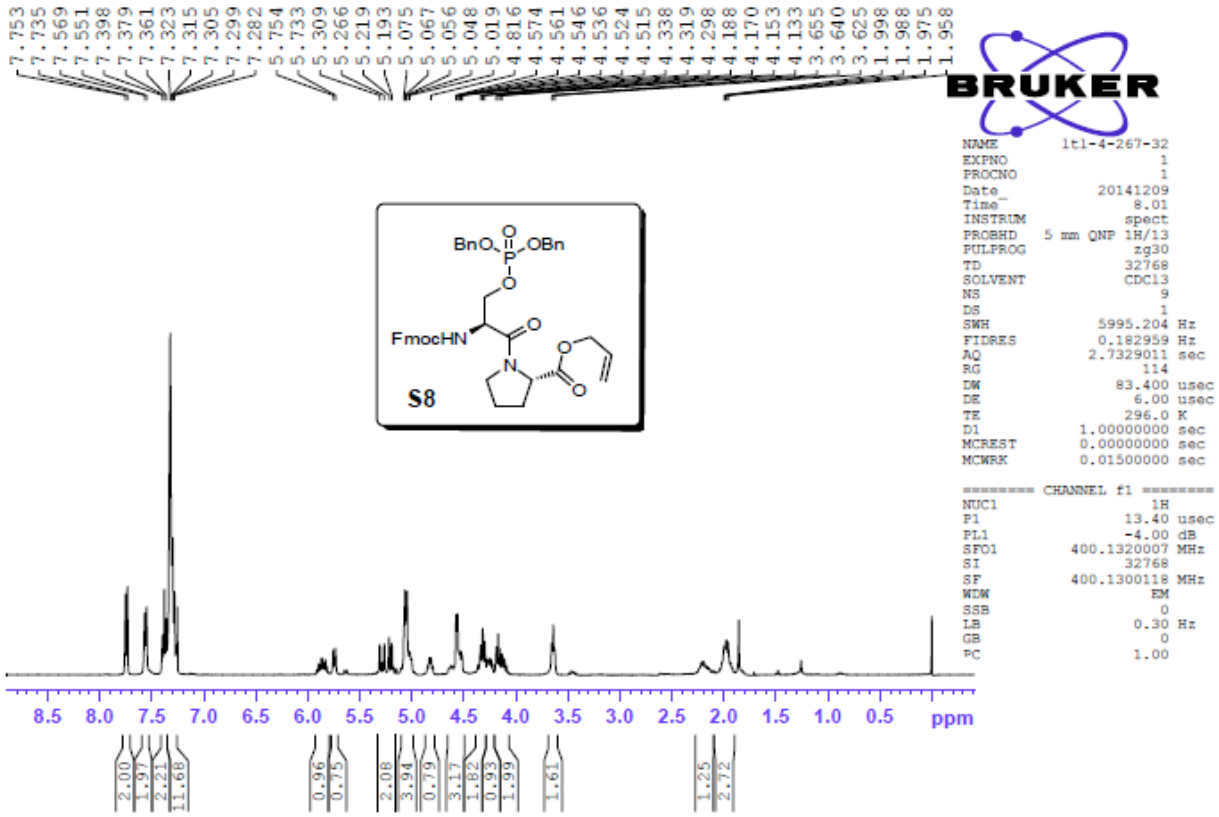

```
NAME          ltl-4-281-11 31P
EXPNO                      1
PROCNO                     1
Date_               20150313
Time_                   7.57
INSTRUM                spect
PROBHD   5 mm QNP 1H/13
PULPROG                 zgdc
TD                     32768
SOLVENT                Acetic
NS                         14
DS                          0
SWH                64935.066 Hz
FIDRES              1.981661 Hz
AQ                  0.2523636 sec
RG                     9195.2
DW                     7.700 usec
DE                     6.00 usec
TE                     296.3 K
D1                 2.00000000 sec
d11                0.03000000 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

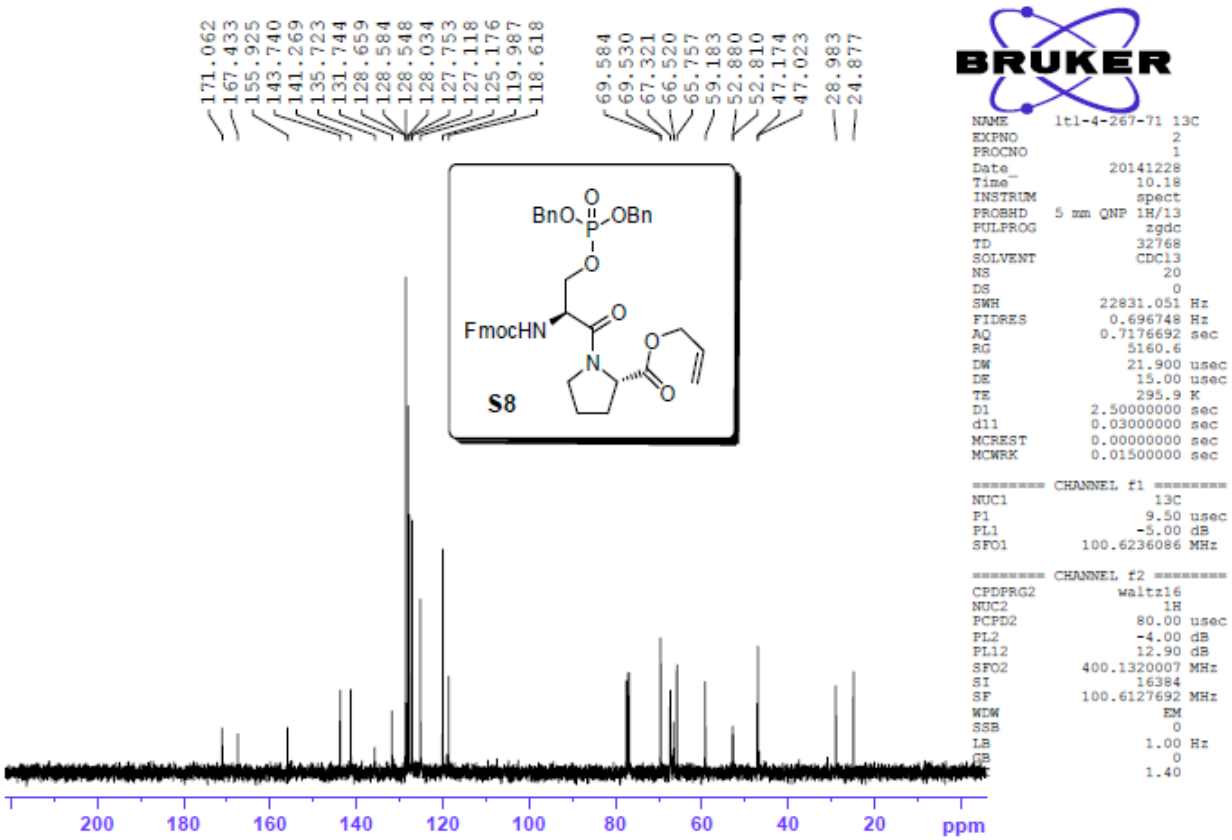
======== CHANNEL f1 ========
NUC1                      31P
P1                     11.00 usec
PL1                    -5.00 dB
SFO1              161.9755730 MHz

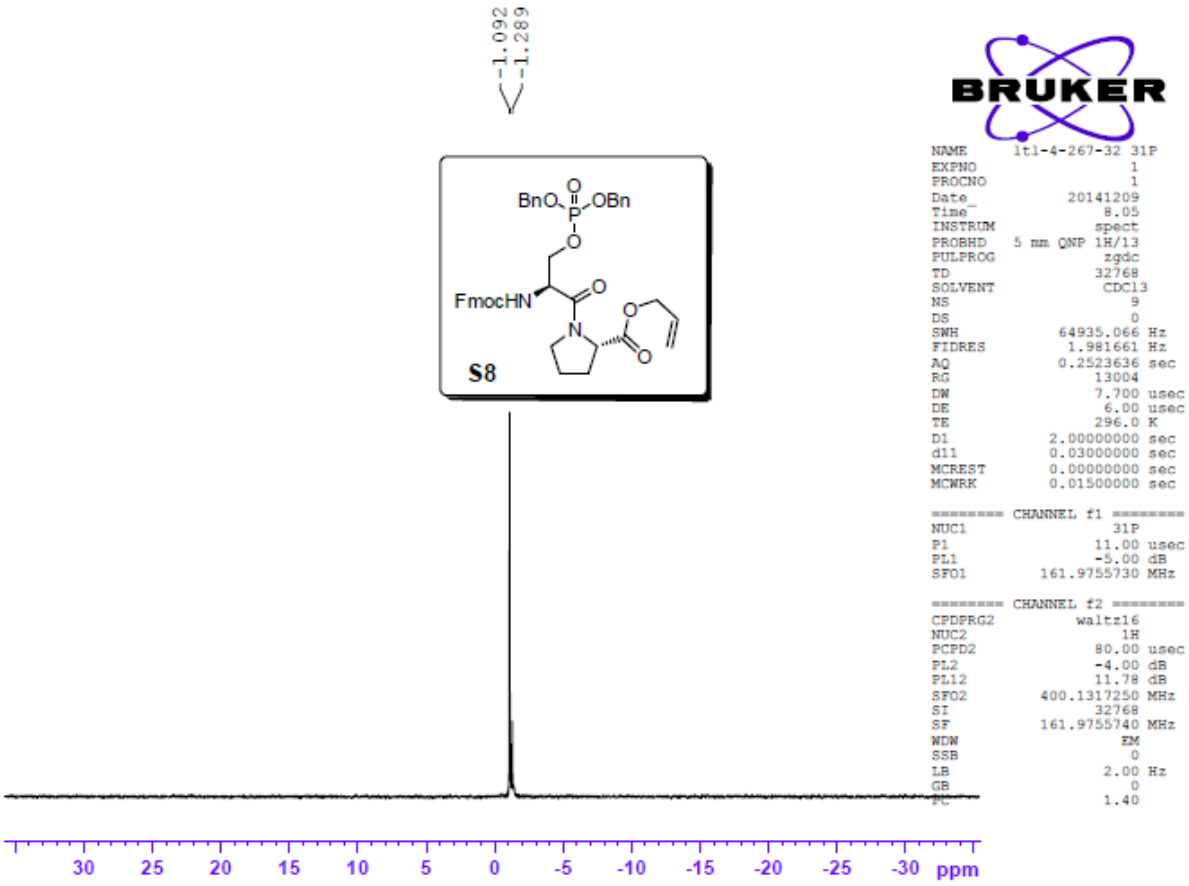
======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
PCPD2                  80.00 usec
PL2                    -4.00 dB
PL12                   11.78 dB
SFO2              400.1317250 MHz
SI                       32768
SF                161.9755740 MHz
WDW                        EM
SSB                         0
LB                     2.00 Hz
GB                          0
PC                     1.40
```


7.770
7.751
7.596
7.577
7.417
7.399
7.380
7.328
7.309
7.290
5.923
5.907
5.807
5.787
5.370
5.327
5.290
5.264
4.697
4.680
4.664
4.650
4.638
4.622
4.614
4.384
4.366
4.228
4.210
3.974
3.963
3.945
3.934
3.866
3.842
3.772
3.757
3.743
3.728
3.718
2.294
2.280
2.032
2.019
BRUKER
NAME 1tl-4-264-21
EXPNO 1
PROCNO 1
Date_ 20141206
Time_ 8.02
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 22
DS 1
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 456.1
DW 83.400 usec
DE 6.00 usec
TE 296.0 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 13.40 usec
PL1 -4.00 dB
SFO1 400.1320007 MHz
SI 32768
SF 400.1300091 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
OH
FmocHN
O
N
O
O
S7
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.00
2.04
2.06
2.07
0.92
0.83
1.91
3.80
1.74
1.04
0.86
0.95
1.83
1.44
3.19


171.827
169.753
156.174
143.671
141.093
131.458
127.569
126.949
125.057
119.815
118.678
67.053
65.923
63.447
58.965
53.999
47.111
46.953
28.756
24.717
BRUKER
NAME 1tl-4-264-1 13C
EXPNO 2
PROCNO 1
Date_ 20141205
Time_ 8.04
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT CDCl3
NS 22
DS 0
SWH 35211.270 Hz
FIDRES 1.074563 Hz
AQ 0.4653556 sec
RG 4597.6
DW 14.200 usec
DE 15.00 usec
TE 298.0 K
D1 2.50000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 9.50 usec
PL1 -5.00 dB
SFO1 100.6278609 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 12.90 dB
SFO2 400.1320007 MHz
SI 16384
SF 100.6127833 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
OH
FmocHN
O
N
O
O
S7
220 200 180 160 140 120 100 80 60 40 20 ppm

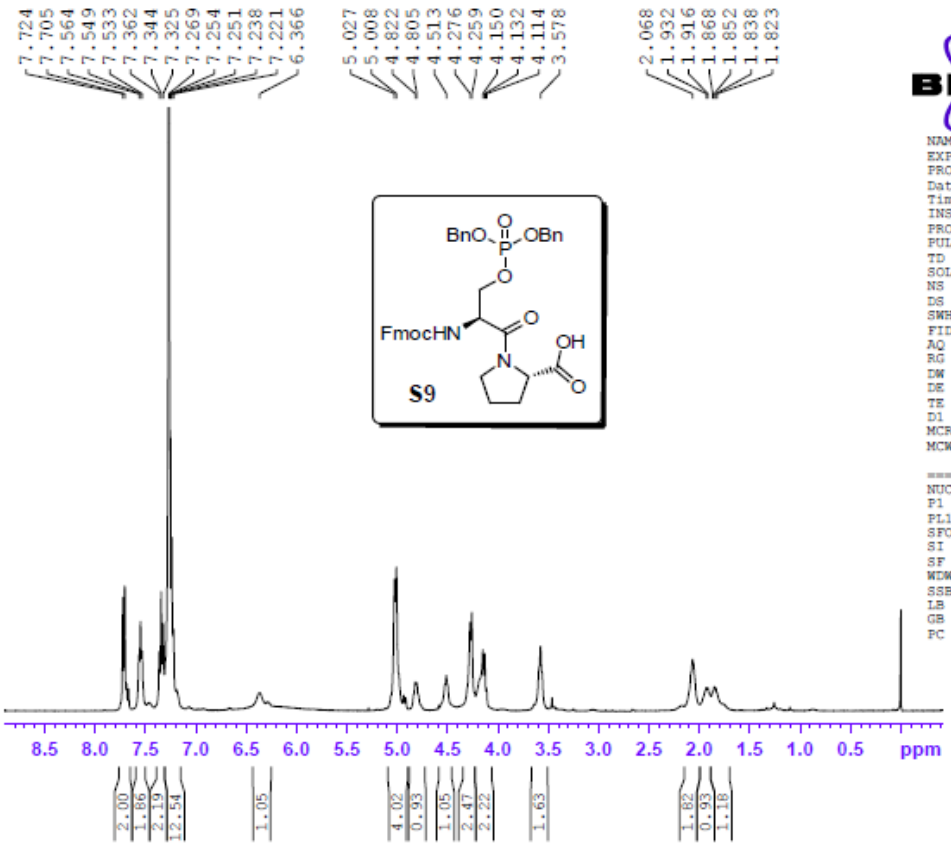

7.753 7.735 7.569 7.551 7.398 7.379 7.361 7.323 7.315 7.305 7.299 7.282 5.754 5.733 5.309 5.266 5.219 5.193 5.075 5.067 5.056 5.048 5.019 4.816 4.574 4.561 4.546 4.536 4.524 4.515 4.338 4.319 4.298 4.188 4.170 4.153 4.133 3.655 3.640 3.625 1.998 1.988 1.975 1.958
BnO OBn
FmocHN
S8
NAME ltl-4-267-32
EXPNO 1
PROCNO 1
Date_ 20141209
Time_ 8.01
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 9
DS 1
SWH 5995.204 Hz
FIDRES 0.182959 Hz
AQ 2.7329011 sec
RG 114
DW 83.400 usec
DE 6.00 usec
TE 296.0 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
CHANNEL f1
NUC1 1H
P1 13.40 usec
PL1 -4.00 dB
SFO1 400.1320007 MHz
SI 32768
SF 400.1300118 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.00 1.97 2.21 11.68 0.96 0.75 2.08 3.94 0.79 3.17 1.82 0.93 1.99 1.61 1.25 2.72

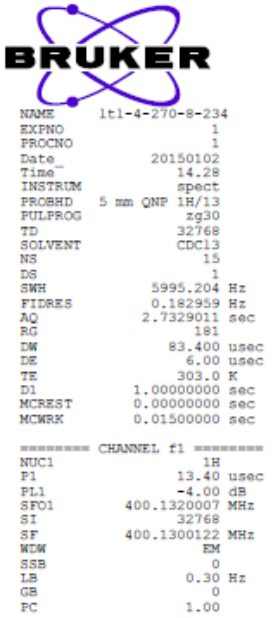

171.062 167.433 155.925 143.740 141.269 135.723 131.744 128.659 128.584 128.548 128.034 127.753 127.118 125.176 119.987 118.618
69.584 69.530 67.321 66.520 65.757 59.183 52.880 52.810 47.174 47.023 28.983 24.877
BnO OBn
FmocHN
S8
NAME ltl-4-267-71 13C
EXPNO 2
PROCNO 1
Date_ 20141228
Time_ 10.18
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgdc
TD 32768
SOLVENT CDCl3
NS 20
DS 0
SWH 22831.051 Hz
FIDRES 0.696748 Hz
AQ 0.7176692 sec
RG 5160.6
DW 21.900 usec
DE 15.00 usec
TE 295.9 K
D1 2.50000000 sec
d11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
CHANNEL f1
NUC1 13C
P1 9.50 usec
PL1 -5.00 dB
SFO1 100.6236086 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 12.90 dB
SFO2 400.1320007 MHz
SI 16384
SF 100.6127692 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
1.40
200 180 160 140 120 100 80 60 40 20 ppm



BRUKER

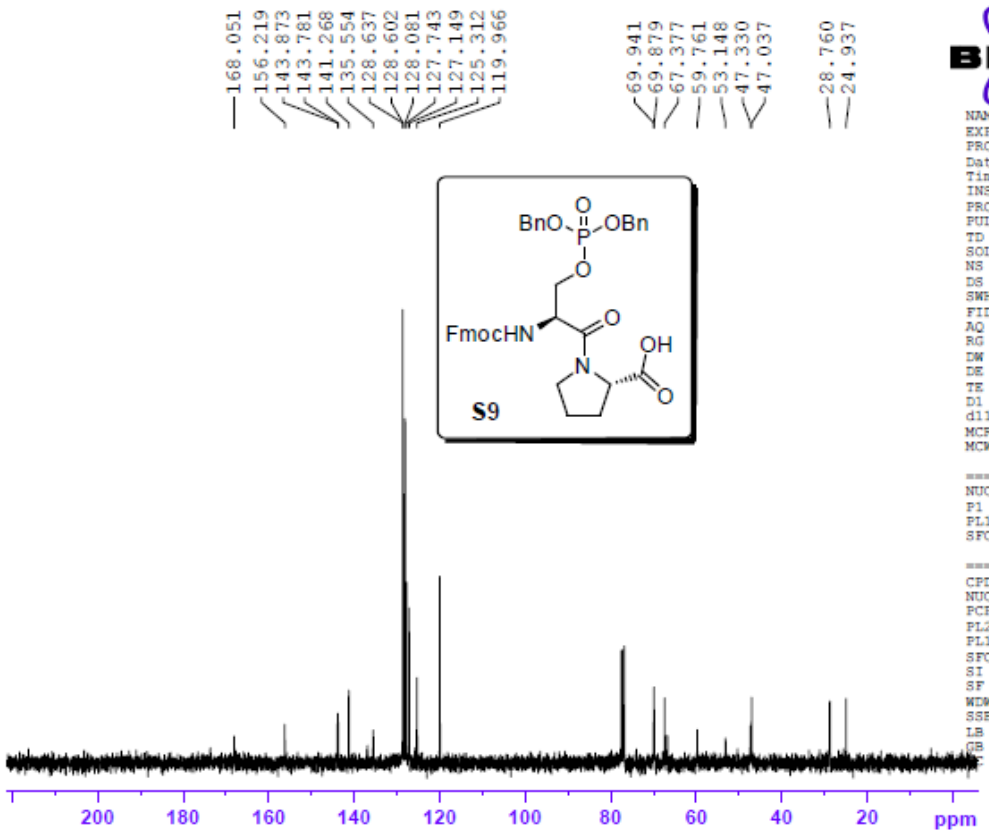
NAME	lt1-4-267-32 31P
EXPNO	1
PROCNO	1
Date_	20141209
Time_	8.05
INSTRUM	spect
PROBHD	5 mm QNP 1H/13
PULPROG	zgdc
TD	32768
SOLVENT	CDCl3
NS	9
DS	0
SWH	64935.066 Hz
FIDRES	1.981661 Hz
AQ	0.2523636 sec
RG	13004
DW	7.700 usec
DE	6.00 usec
TE	296.0 K
D1	2.00000000 sec
d11	0.03000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec
========	CHANNEL f1 ========
NUC1	31P
P1	11.00 usec
PL1	-5.00 dB
SFO1	161.9755730 MHz
========	CHANNEL f2 ========
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.00 dB
PL12	11.78 dB
SFO2	400.1317250 MHz
SI	32768
SF	161.9755740 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.40






```
NAME            lt1-4-270-8-234
EXPNO                         1
PROCNO                        1
Date_                  20150102
Time_                     14.28
INSTRUM                   spect
PROBHD   5 mm QNP 1H/13
PULPROG                   zg30
TD                       32768
SOLVENT                  CDCl3
NS                           15
DS                            1
SWH                   5995.204 Hz
FIDRES                0.182959 Hz
AQ                    2.7329011 sec
RG                          181
DW                     83.400 usec
DE                      6.00 usec
TE                      303.0 K
D1                 1.00000000 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

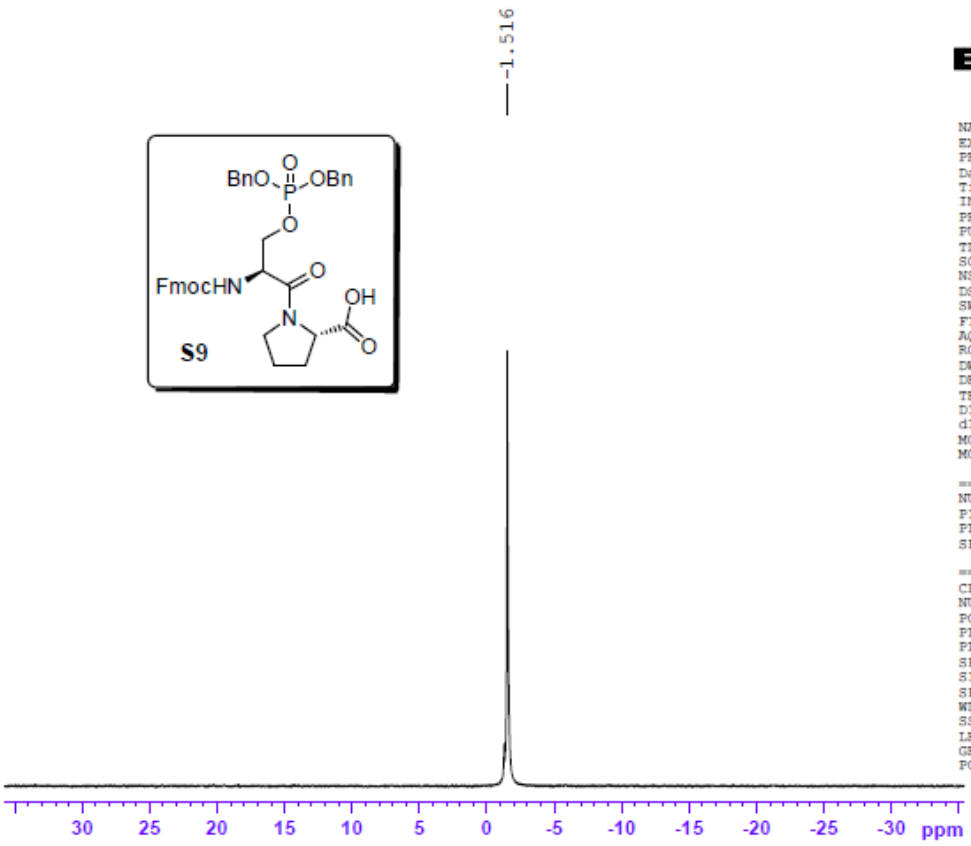
======== CHANNEL f1 ========
NUC1                        1H
P1                      13.40 usec
PL1                     -4.00 dB
SFO1           400.1320007 MHz
SI                       32768
SF             400.1300122 MHz
WDW                         EM
SSB                          0
LB                      0.30 Hz
GB                           0
PC                        1.00
```




```
NAME            lt1-4-270-1 13C
EXPNO                         2
PROCNO                        1
Date_                  20141211
Time_                     8.22
INSTRUM                   spect
PROBHD   5 mm QNP 1H/13
PULPROG                   zgdc
TD                       32768
SOLVENT                  CDCl3
NS                           24
DS                            0
SWH                  35211.270 Hz
FIDRES                1.074563 Hz
AQ                    0.4653556 sec
RG                       4597.6
DW                     14.200 usec
DE                      15.00 usec
TE                      296.2 K
D1                 2.50000000 sec
d11                0.03000000 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                       13C
P1                       9.50 usec
PL1                     -5.00 dB
SFO1           100.6278609 MHz

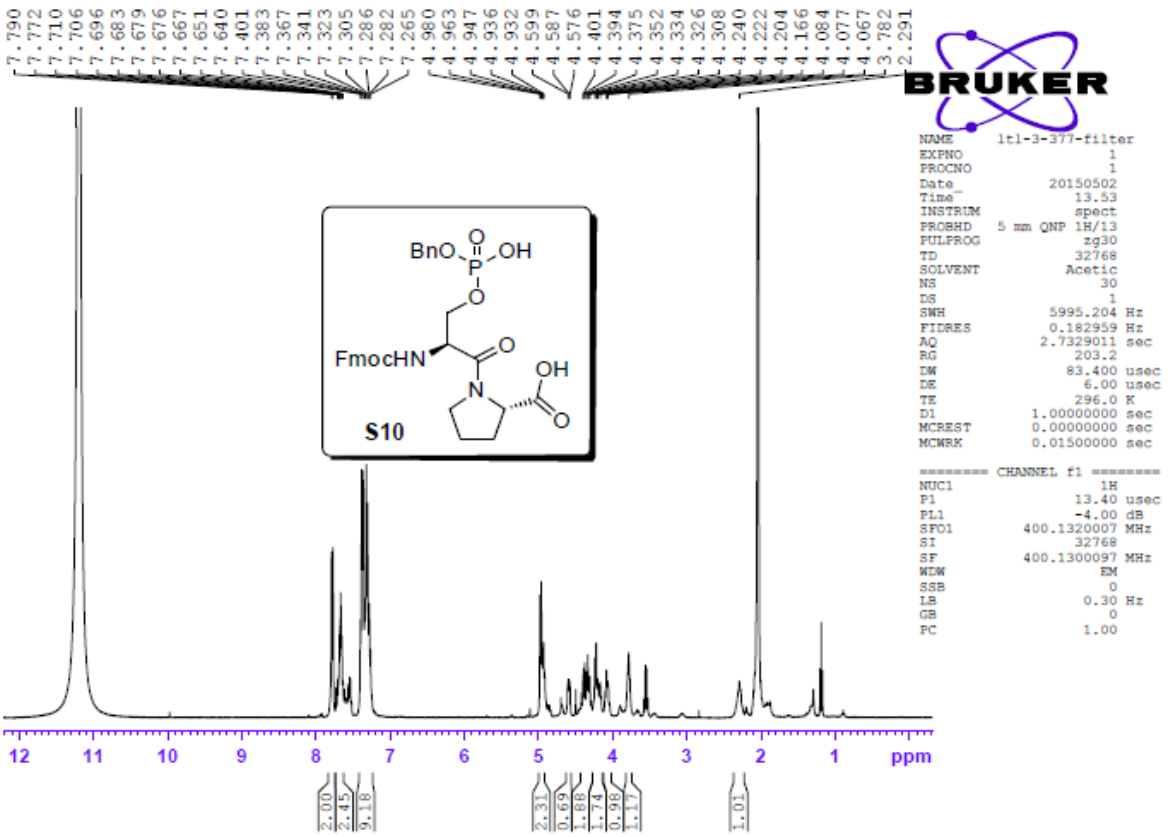
======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                        1H
PCPD2                   80.00 usec
PL2                     -4.00 dB
PL12                    12.90 dB
SFO2           400.1320007 MHz
SI                       16384
SF             100.6127659 MHz
WDW                         EM
SSB                          0
LB                      1.00 Hz
GB                           0
                        1.40
```




```
NAME          ltl-4-270-1 31P
EXPNO                     1
PROCNO                    1
Date_              20141211
Time_                  8.24
INSTRUM               spect
PROBHD   5 mm QNP 1H/13
PULPROG               zgdc
TD                   32768
SOLVENT               CDCl3
NS                       14
DS                        0
SWH              64935.066 Hz
FIDRES            1.981661 Hz
AQ                0.2523636 sec
RG                     6502
DW                   7.700 usec
DE                   6.00 usec
TE                   296.3 K
D1             2.00000000 sec
d11            0.03000000 sec
MCREST         0.00000000 sec
MCWRK          0.01500000 sec

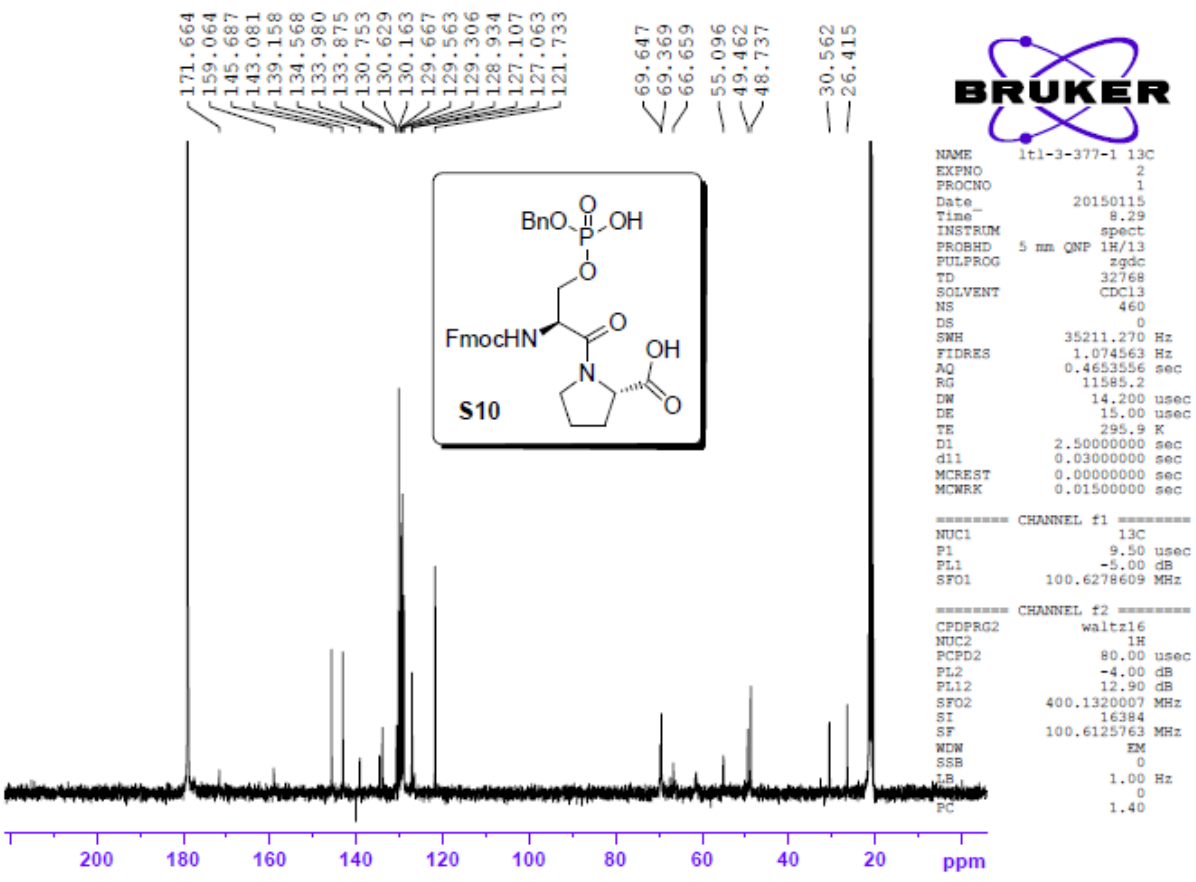
======== CHANNEL f1 ========
NUC1                   31P
P1                   11.00 usec
PL1                  -5.00 dB
SFO1          161.9755730 MHz

======== CHANNEL f2 ========
CPDPRG2             waltz16
NUC2                   1H
PCPD2                80.00 usec
PL2                  -4.00 dB
PL12                 11.78 dB
SFO2          400.1317250 MHz
SI                   32768
SF            161.9755740 MHz
WDW                    EM
SSB                     0
LB                   2.00 Hz
GB                      0
PC                   1.40
```




```
NAME          ltl-3-377-filter
EXPNO                       1
PROCNO                      1
Date_                20150502
Time_                   13.53
INSTRUM                 spect
PROBHD   5 mm QNP 1H/13
PULPROG                 zg30
TD                     32768
SOLVENT               Acetic
NS                         30
DS                          1
SWH                 5995.204 Hz
FIDRES              0.182959 Hz
AQ                  2.7329011 sec
RG                      203.2
DW                     83.400 usec
DE                      6.00 usec
TE                     296.0 K
D1                1.00000000 sec
MCREST            0.00000000 sec
MCWRK             0.01500000 sec

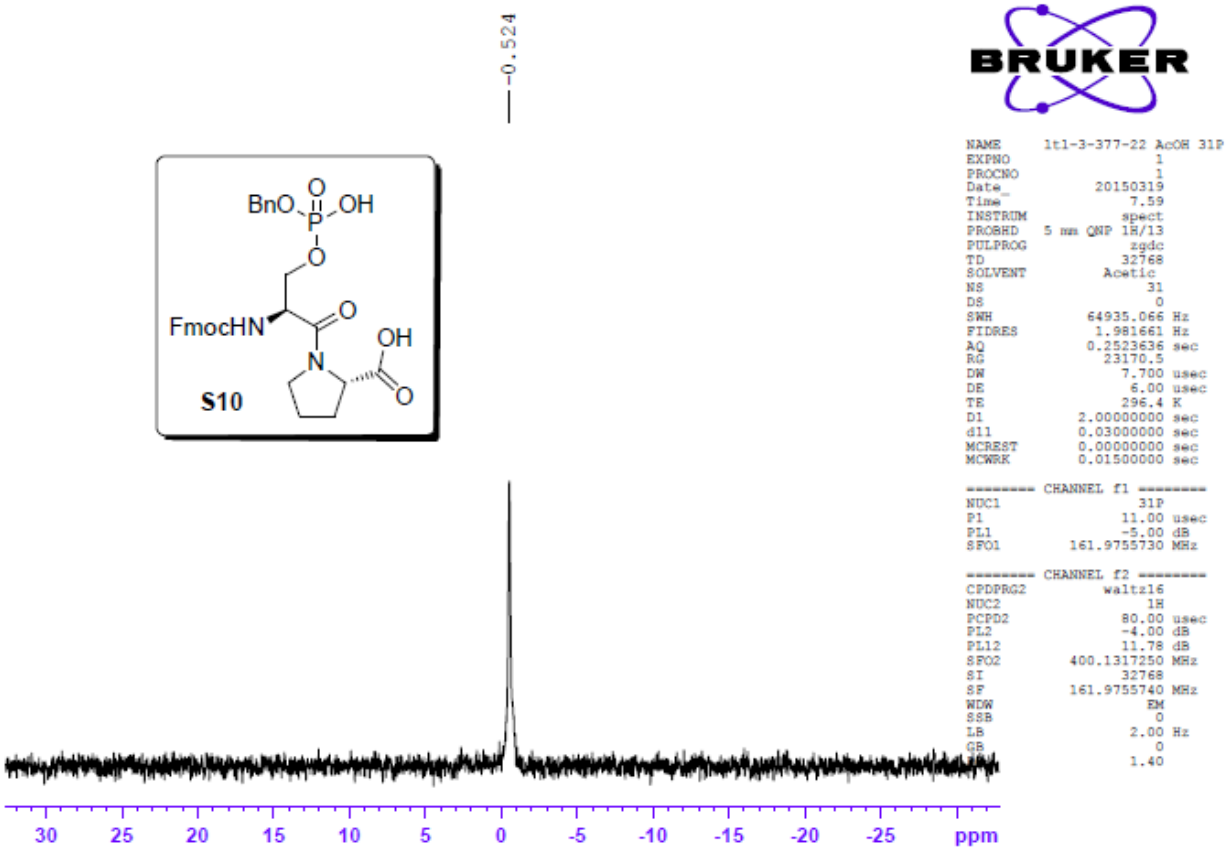
======== CHANNEL f1 ========
NUC1                        1H
P1                      13.40 usec
PL1                     -4.00 dB
SFO1           400.1320007 MHz
SI                     32768
SF             400.1300097 MHz
WDW                        EM
SSB                         0
LB                      0.30 Hz
GB                          0
PC                       1.00
```




```
NAME          ltl-3-377-1 13C
EXPNO                       2
PROCNO                      1
Date_                20150115
Time_                   8.29
INSTRUM                 spect
PROBHD   5 mm QNP 1H/13
PULPROG                 zgdc
TD                     32768
SOLVENT                CDCl3
NS                        460
DS                          0
SWH                35211.270 Hz
FIDRES              1.074563 Hz
AQ                  0.4653556 sec
RG                    11585.2
DW                     14.200 usec
DE                      15.00 usec
TE                     295.9 K
D1                2.50000000 sec
d11               0.03000000 sec
MCREST            0.00000000 sec
MCWRK             0.01500000 sec

======== CHANNEL f1 ========
NUC1                       13C
P1                      9.50 usec
PL1                     -5.00 dB
SFO1           100.6278609 MHz

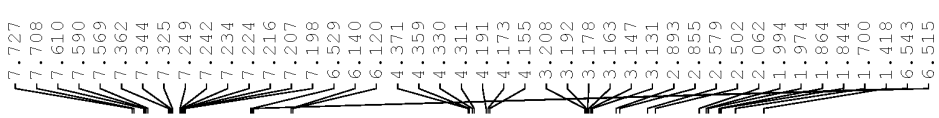
======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                        1H
PCPD2                  80.00 usec
PL2                     -4.00 dB
PL12                    12.90 dB
SFO2           400.1320007 MHz
SI                     16384
SF             100.6125763 MHz
WDW                        EM
SSB                         0
LB                      1.00 Hz
GB                          0
PC                       1.40
```

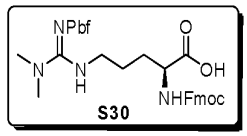



```
NAME        1t1-3-377-22 AcOH 31P
EXPNO                 1
PROCNO                1
Date_          20150319
Time_              7.59
INSTRUM           spect
PROBHD   5 mm QNP 1H/13
PULPROG           zgdc
TD               32768
SOLVENT         Acetic
NS                   31
DS                    0
SWH           64935.066 Hz
FIDRES         1.981661 Hz
AQ             0.2523636 sec
RG               23170.5
DW                7.700 usec
DE                6.00 usec
TE                296.4 K
D1           2.00000000 sec
d11          0.03000000 sec
MCREST       0.00000000 sec
MCWRK        0.01500000 sec

======== CHANNEL f1 ========
NUC1                 31P
P1                11.00 usec
PL1               -5.00 dB
SFO1        161.9755730 MHz

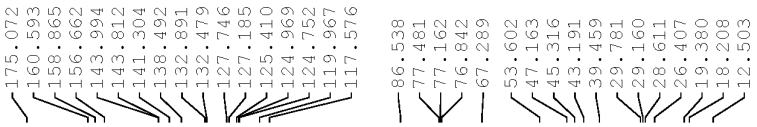
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -4.00 dB
PL12              11.78 dB
SFO2        400.1317250 MHz
SI                32768
SF          161.9755740 MHz
WDW                  EM
SSB                   0
LB                 2.00 Hz
GB                    0
                  1.40
```

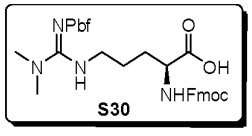





```
NAME                lt1-4-349-1
EXPNO                         1
PROCNO                        1
Date_                  20150214
Time                       9.48
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                   zg30
TD                       32768
SOLVENT                  CDCl3
NS                           19
DS                            1
SWH                   5995.204 Hz
FIDRES                0.182959 Hz
AQ                    2.7329011 sec
RG                         80.6
DW                       83.400 usec
DE                        6.00 usec
TE                       296.4 K
D1                    1.00000000 sec
MCREST                0.00000000 sec
MCWRK                 0.01500000 sec

======== CHANNEL f1 ========
NUC1                         1H
P1                       13.40 usec
PL1                      -4.00 dB
SFO1                400.1320007 MHz
SI                       32768
SF                  400.1300137 MHz
WDW                         EM
SSB                          0
LB                        0.30 Hz
GB                           0
PC                        1.00
```




```
NAME          lt1-4-349-1 13C
EXPNO                         2
PROCNO                        1
Date_                  20150214
Time                       9.54
INSTRUM                   spect
PROBHD    5 mm QNP 1H/13
PULPROG                   zgdc
TD                       32768
SOLVENT                   DMSO
NS                          121
DS                            0
SWH                  35211.270 Hz
FIDRES                1.074563 Hz
AQ                    0.4653556 sec
RG                       9195.2
DW                       14.200 usec
DE                        15.00 usec
TE                       296.7 K
D1                    2.50000000 sec
d11                   0.03000000 sec
MCREST                0.00000000 sec
MCWRK                 0.01500000 sec

======== CHANNEL f1 ========
NUC1                        13C
P1                        9.50 usec
PL1                      -5.00 dB
SFO1               100.6278609 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                         1H
PCPD2                    80.00 usec
PL2                      -4.00 dB
PL12                     12.90 dB
SFO2                400.1320007 MHz
SI                       16384
SF                  100.6127616 MHz
WDW                         EM
SSB                          0
LB                        1.00 Hz
GB                           0
PC                        1.40
```