

Supplementary Information

Convergent Synthesis of *N*-Linked Glycopeptides *via* Solid-Phase Aspartylation

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General procedures

¹H NMR spectra were recorded using a Bruker Avance DPX 400 at a frequency of 400.2 MHz. The spectra are reported as parts per million (ppm) downfield shift using the solvent peak as an internal reference. The data are reported as chemical shift (δ), multiplicity, relative integral, coupling constant (J Hz) and assignment where possible. The presence of rotamers was confirmed by saturation transfer NMR experiments. Low resolution mass spectra were recorded on a Finnigan LCQ Deca ion trap mass spectrometer (ESI). High resolution mass spectra were recorded on a Bruker 7T Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR)

Analytical reverse-phase HPLC was performed on a Waters System 2695 separations module with an Alliance series column heater at 30 °C and 2996 photodiode array detector. A Waters Sunfire 5 μ m, 2.1 x 150 mm column was used at a flow rate of 0.2 mL min⁻¹ using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) using a linear gradient of 2-50% B over 30 min for glycopeptides **6a-6l** and **23** and 30-100% B over 40 min for dipeptides **11a-11e** and **16a-16c**. Results were analysed with Waters Empower software. Preparative reverse-phase HPLC was performed using a Waters 600 Multisolvant Delivery System and Waters 500 pump with 2996 photodiode array detector or Waters 490E Programmable wavelength detector operating at 254 and 280 nm. A Waters Sunfire 5 μ m, 19 x 150 mm column was used at a flow rate of 7 mL min⁻¹ using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) using a linear gradient of 2-50% B over 30 min for glycopeptides **6a-6l** and **23** and 0-100% B over 45 min for dipeptides **11a-11e** and **16a-16c**.

LC-MS was performed on a Thermo Separation Products: Spectra System consisting of P400 Pump and a UV6000LP Photodiode array detector on a Phenomenex Jupiter 5 μ m, 2.1 x 150 mm column at a flow rate of 0.2 mL min⁻¹ coupled to a Thermoquest Finnigan LCQ Deca mass spectrometer (ESI) operating in positive mode. Separations involved a mobile phase of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) using a linear gradient of 2-50% B over 30 min.

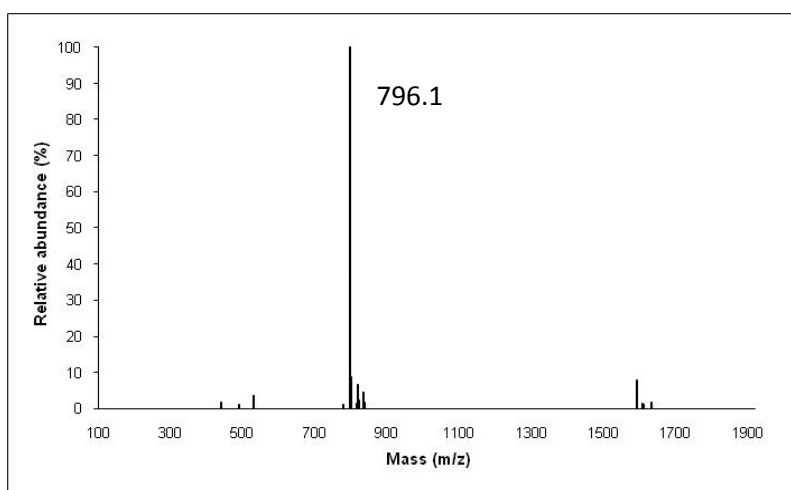
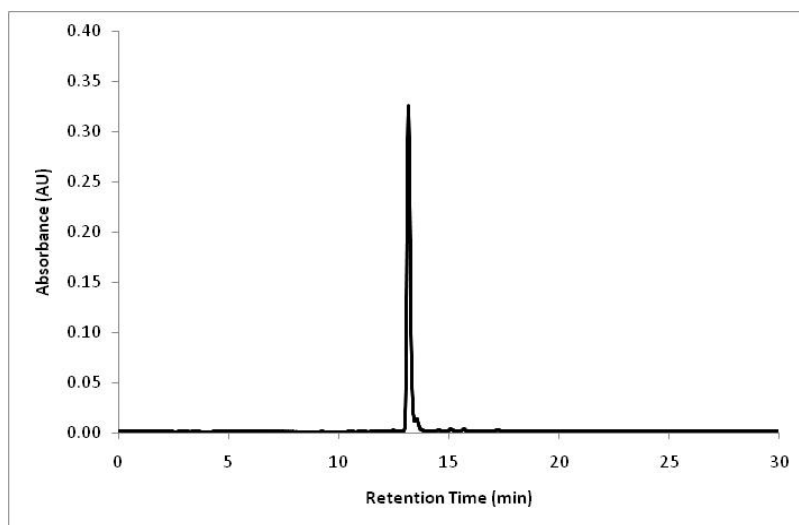
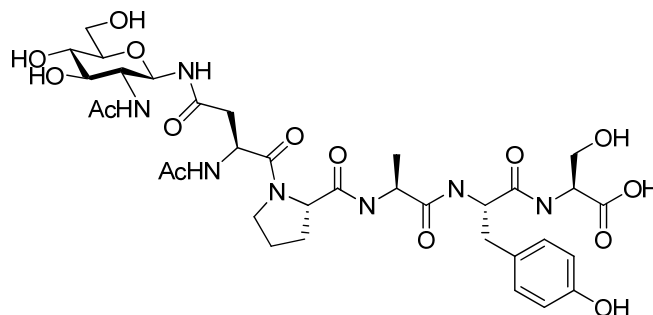
Materials

Analytical thin layer chromatography (TLC) was performed on commercially prepared silica plates (Merck Kieselgel 60 0.25 mm F254). Flash column chromatography was performed using 230-400 mesh Kieselgel 60 silica eluting with distilled solvents as described.

Commercial materials were used as received unless otherwise noted. Amino acids, coupling reagents and resins were obtained from Novabiochem. DCM and methanol were distilled from calcium hydride. DMF was obtained as peptide synthesis grade from Auspep or Labscan.

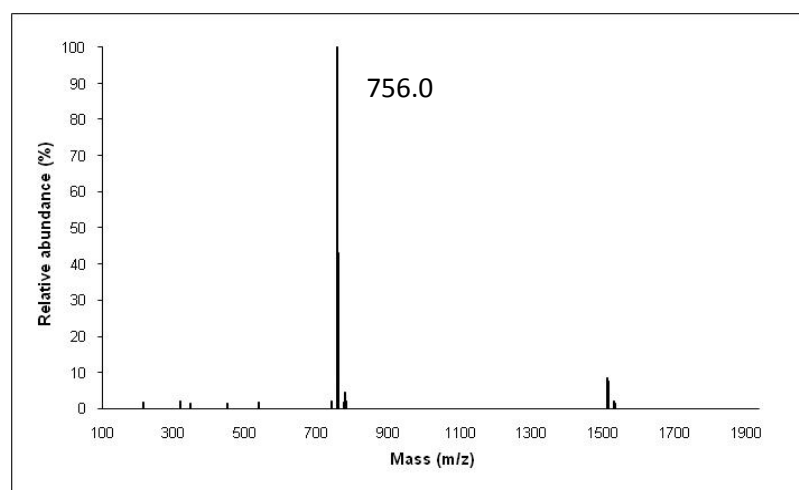
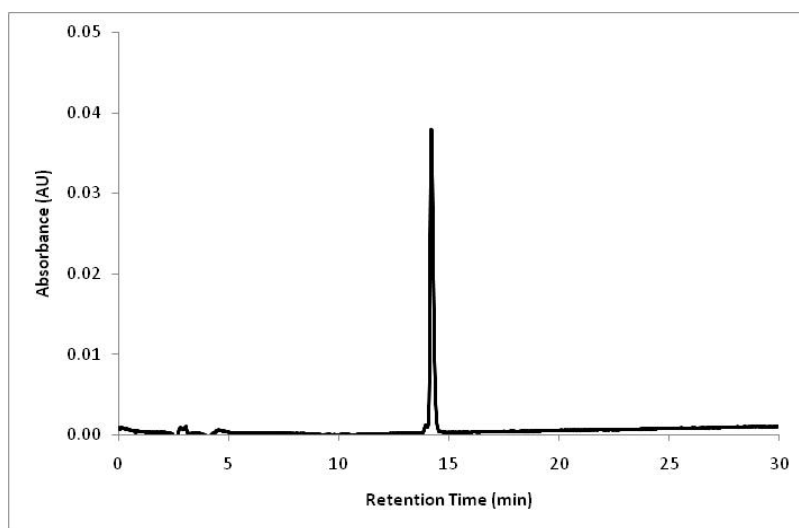
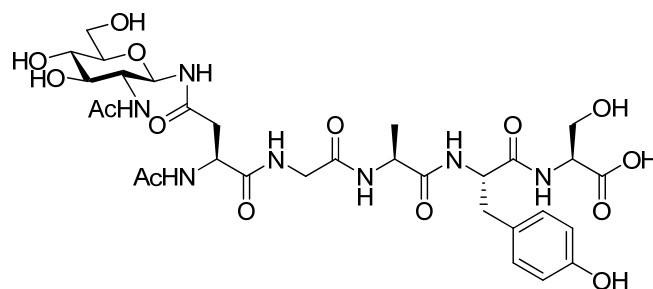
Analytical Data for *N*-linked glycopeptides 6a-6l

Ac-Asn(β -GlcNAc)-Pro-Ala-Tyr-Ser-OH (6a)



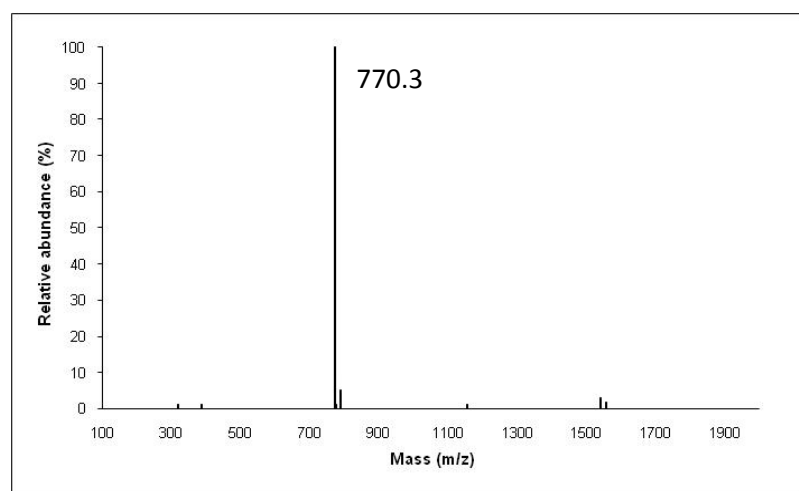
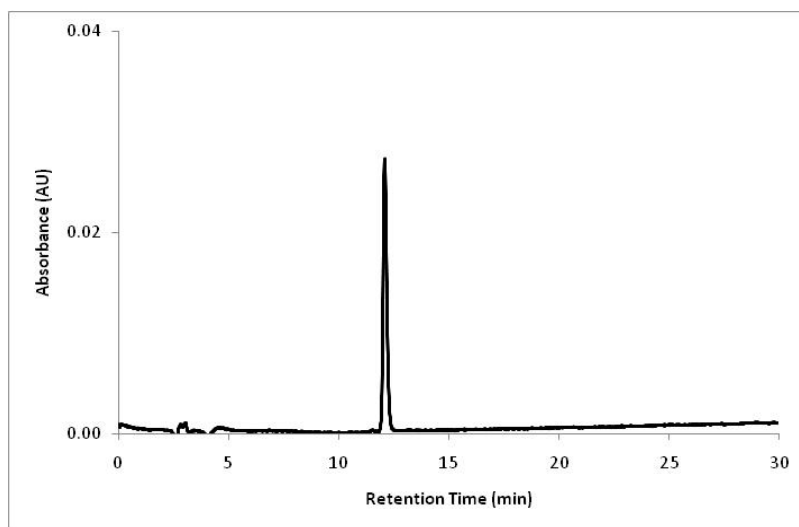
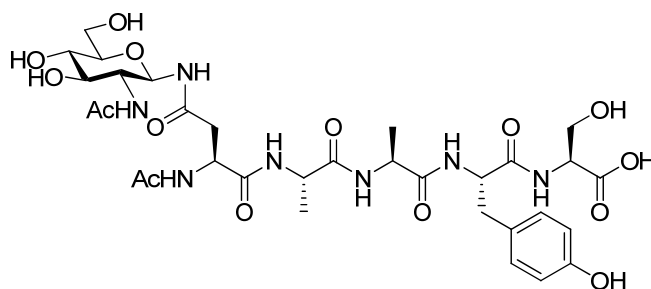
Analytical HPLC: $R_t = 13.4$ min (2-50% B over 30 min, $\lambda = 280$ nm); LC-MS: $R_t = 12.8$ min (Gradient A) MS (ESI) m/z 796.1 [(M+H)⁺, 100%]; HRMS Calcd for C₃₄H₄₉N₇O₁₅Na: MNa⁺, 818.3179 found MNa⁺, 818.3178.

Ac-Asn(β -GlcNAc)-Gly-Ala-Tyr-Ser-OH (6b)



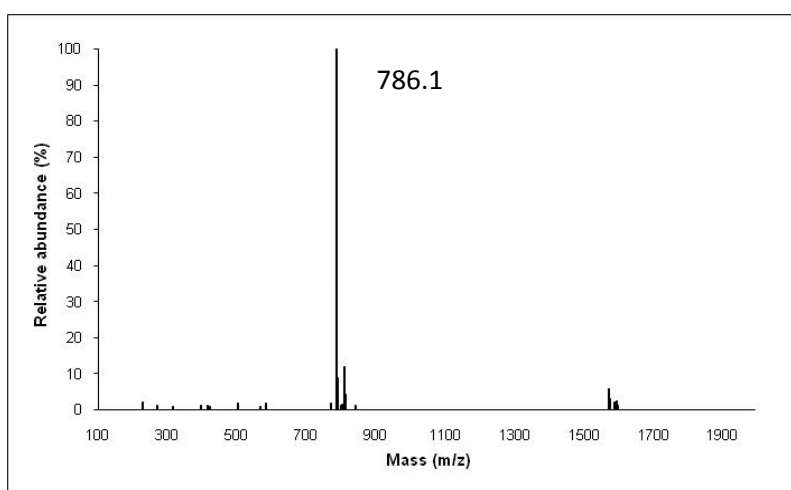
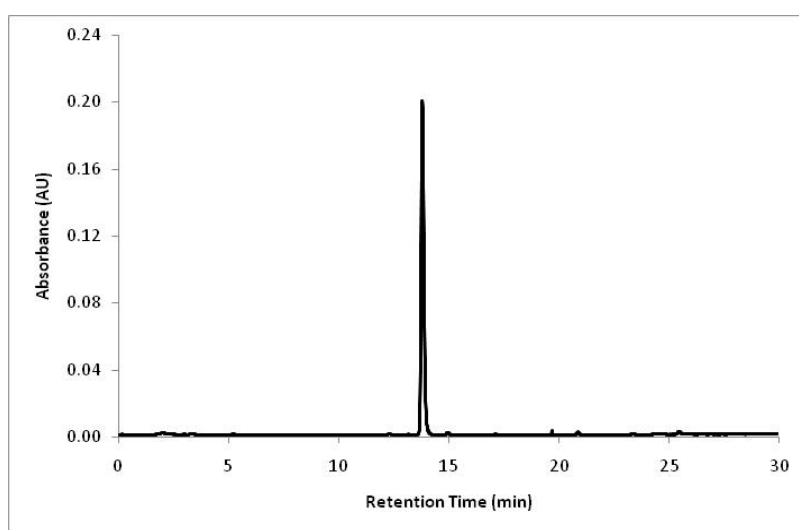
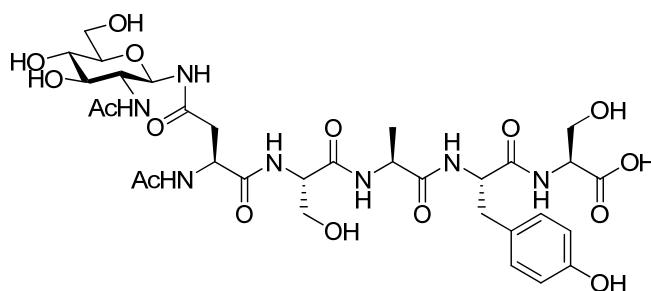
Analytical HPLC: $R_t = 14.3$ min (2-50% B over 30 min, $\lambda = 280$ nm), LC-MS: $R_t = 13.6$ min (Gradient A); MS (ESI) m/z 756.0 $[(M+H)^+]$, 100%; HRMS Calcd for $C_{31}H_{45}N_7O_{15}Na$: MNa^+ , 778.2866 found MNa^+ , 778.2867.

Ac-Asn(β -GlcNAc)-Ala-Ala-Tyr-Ser-OH (6c)



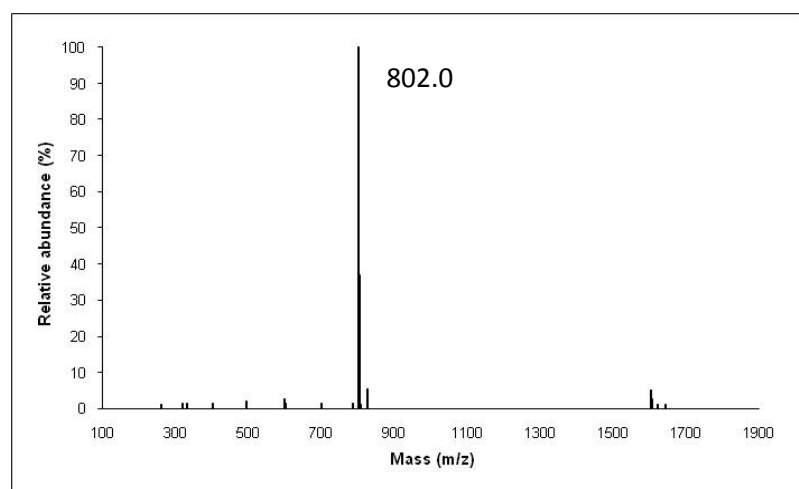
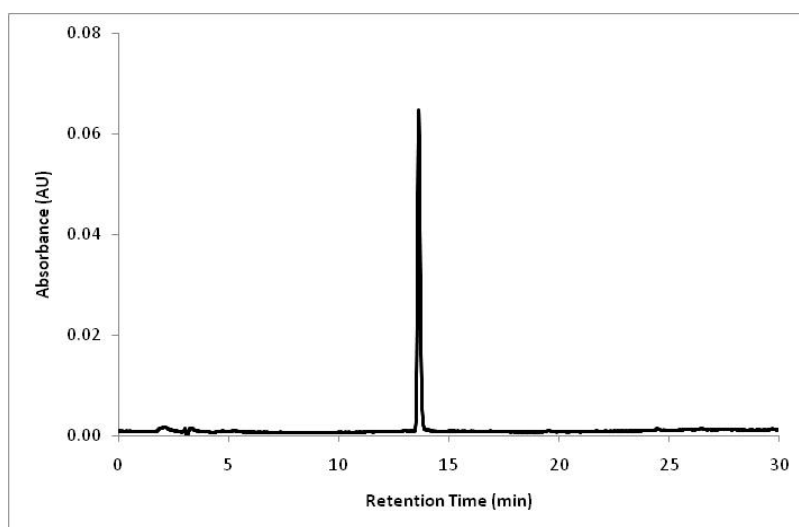
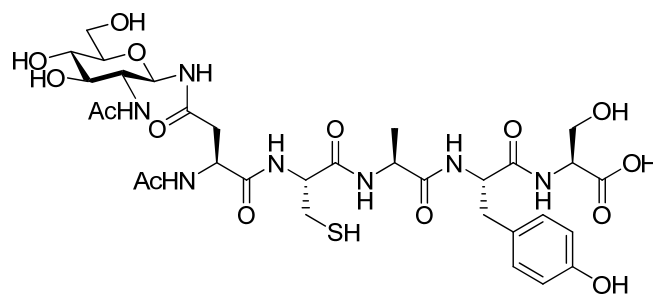
Analytical HPLC: $R_t = 12.1$ min (2-50% B over 30 min, $\lambda = 280$ nm); LC-MS: $R_t = 12.0$ min (Gradient A); MS (ESI) m/z 770.3 $[(M+H)^+]$, 100%; HRMS Calcd for $C_{32}H_{47}N_7O_{15}Na$: MNa^+ , 792.3028 found MNa^+ , 792.3025.

Ac-Asn(β -GlcNAc)-Ser-Ala-Tyr-Ser-OH (6d)



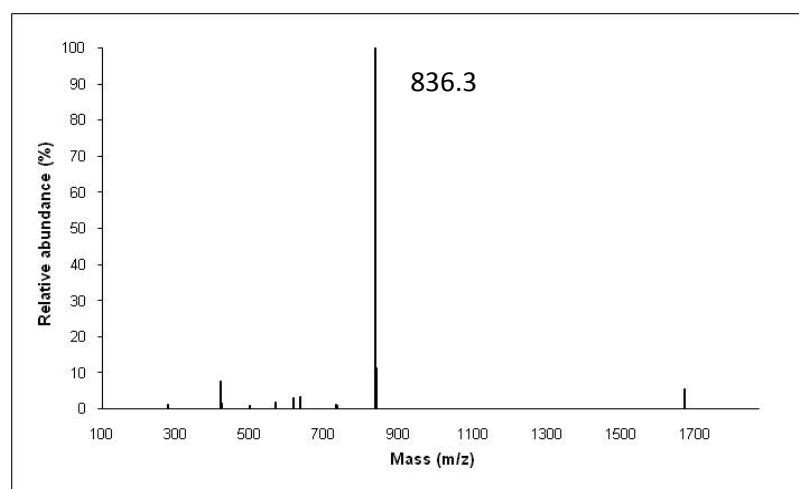
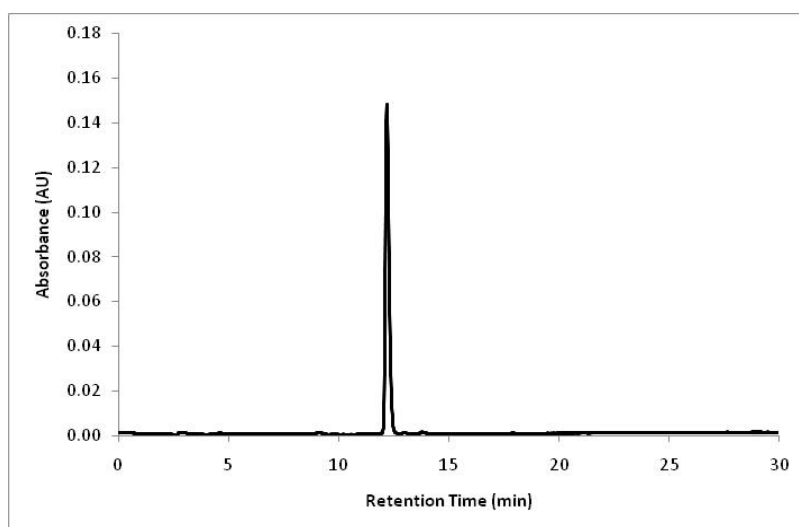
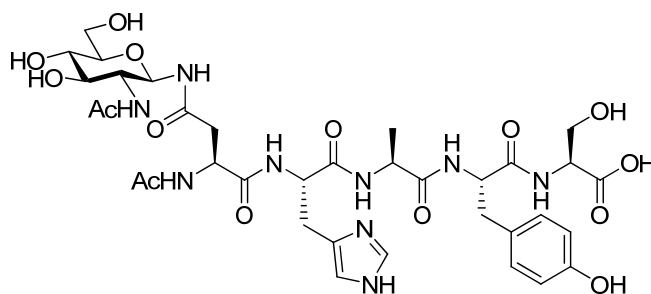
Analytical HPLC: R_t ; 14.2 min (2-50% B over 30 min, $\lambda = 280$ nm); LC-MS: R_t ; 13.6 min (Gradient A); MS (ESI) m/z 786.1 [(M+H)⁺, 100%]; HRMS Calcd for C₃₂H₄₇N₇O₁₆Na: MNa⁺, 808.2972 found MNa⁺, 808.2958.

Ac-Asn(β -GlcNAc)-Cys-Ala-Tyr-Ser-OH (6e)



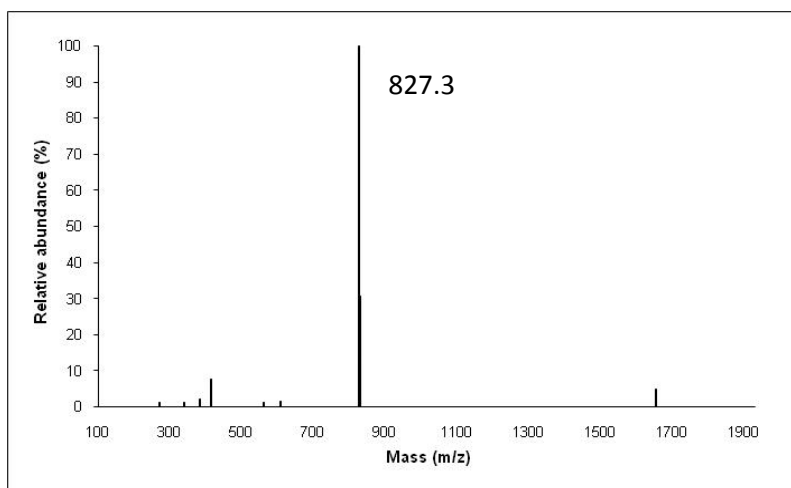
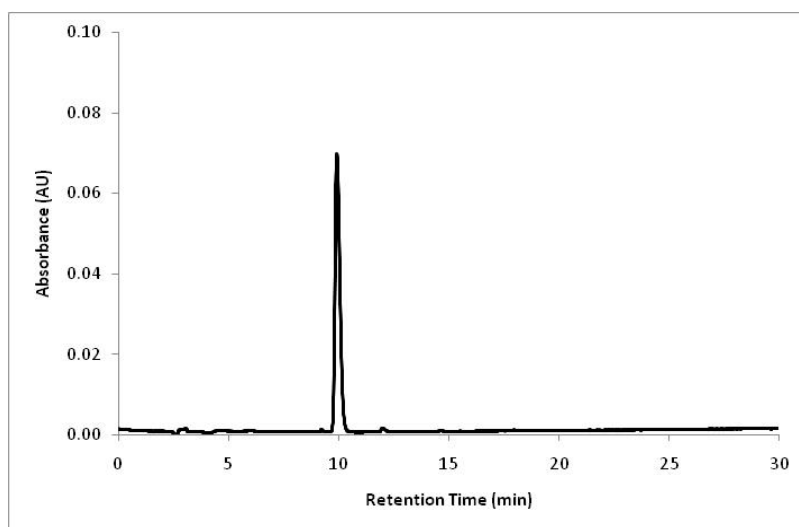
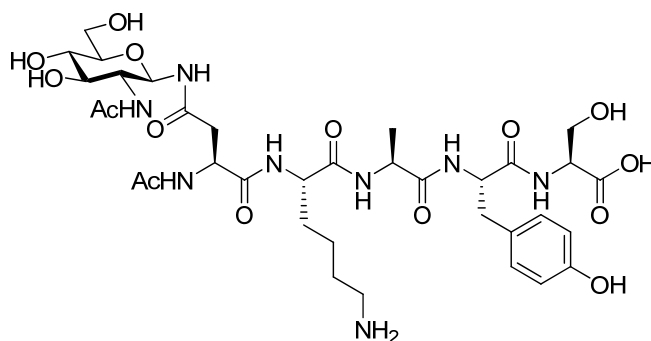
Analytical HPLC: $R_t = 14.1$ min (2-50% B over 30 min, $\lambda = 280$ nm); LC-MS: $R_t = 13.5$ min (Gradient A); MS (ESI) m/z 802.0 $[(M+H)^+]$, 100%; HRMS Calcd for $C_{32}H_{47}N_7O_{15}SNa$: MNa^+ , 824.2743 found MNa^+ , 822.2735.

Ac-Asn(β -GlcNAc)-His-Ala-Tyr-Ser-OH (6f)



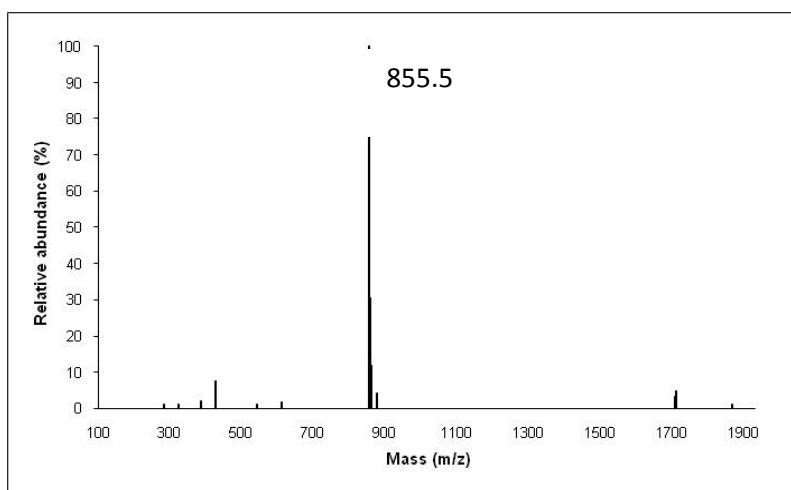
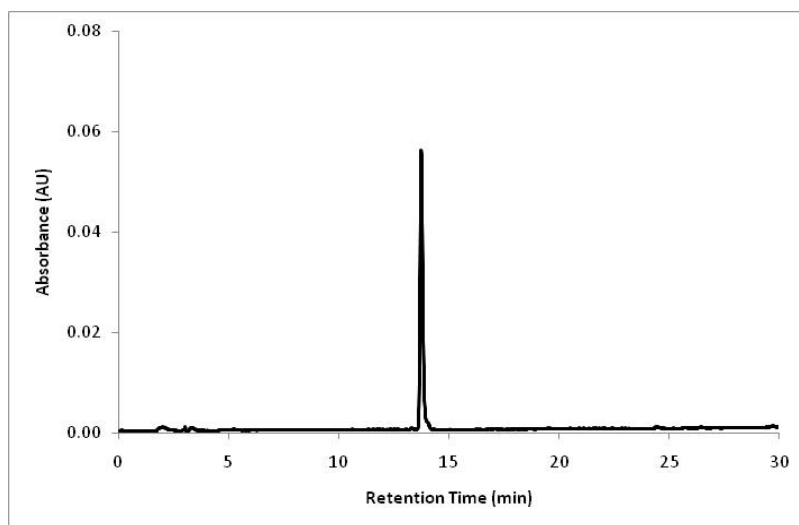
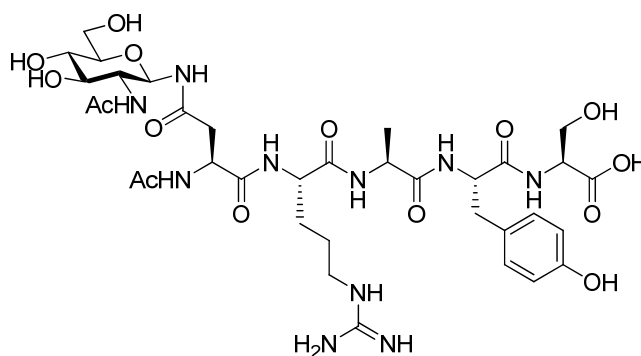
Analytical HPLC: $R_t = 12.2$ min (2-50% B over 30 min, $\lambda = 280$ nm); LC-MS: $R_t = 11.1$ min (Gradient A); MS (ESI) m/z 836.3 $[(M+H)^+]$, 100%; HRMS Calcd for $C_{35}H_{50}N_9O_{15}$: MH^+ , 836.3421 found MH^+ , 836.3417.

Ac-Asn(β -GlcNAc)-Lys-Ala-Tyr-Ser-OH (6g)



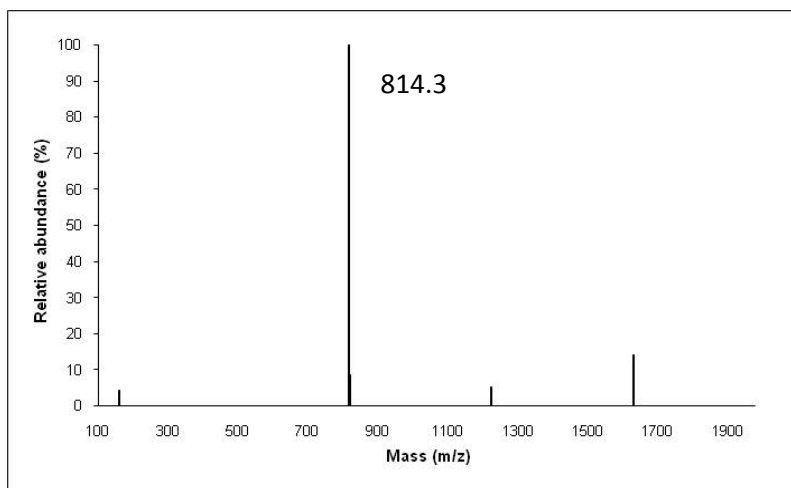
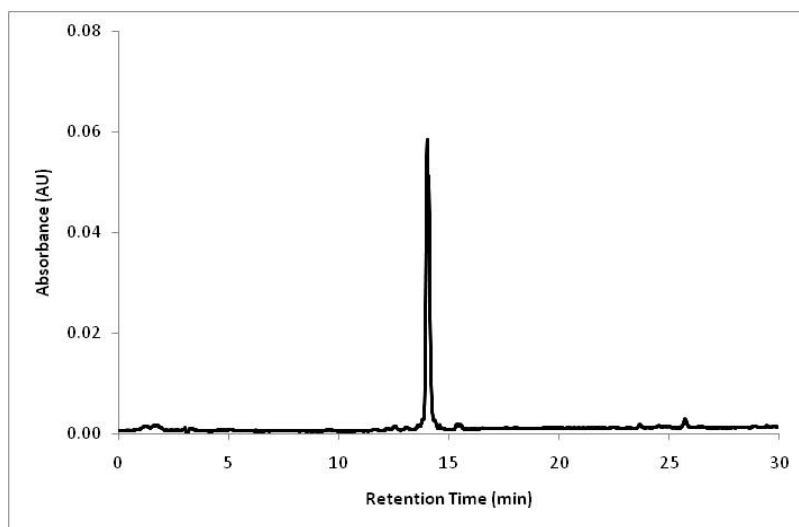
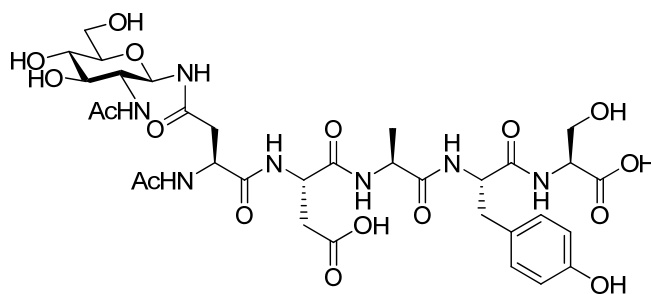
Analytical HPLC: $R_t = 9.9$ min (2-50% B over 30 min, $\lambda = 280$ nm); LC-MS: $R_t = 9.1$ min (Gradient A); MS (ESI) m/z 827.3 $[(M+H)^+]$, 100%; HRMS Calcd for $C_{32}H_{47}N_7O_{15}Na$: MNa^+ , 849.3601 found MNa^+ , 849.3611.

Ac-Asn(β -GlcNAc)-Arg-Ala-Tyr-Ser-OH (6h)



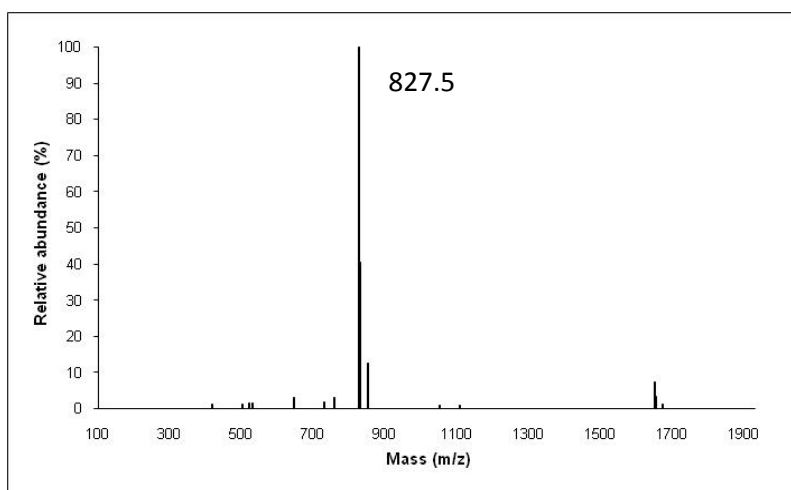
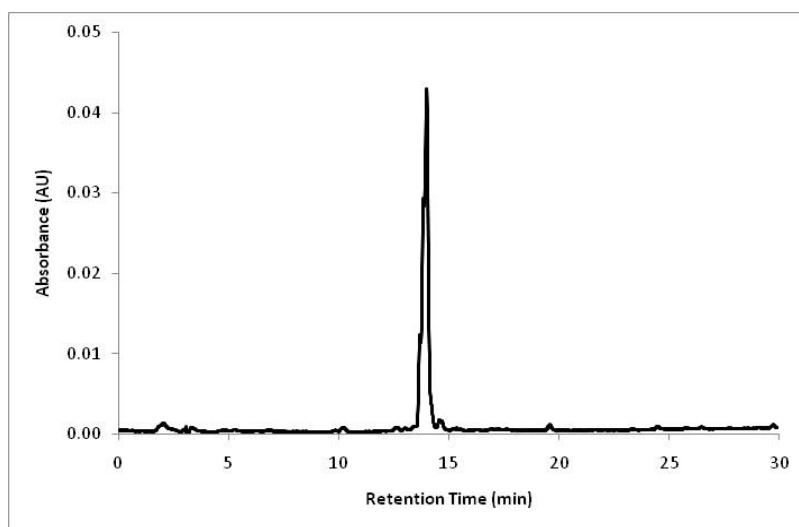
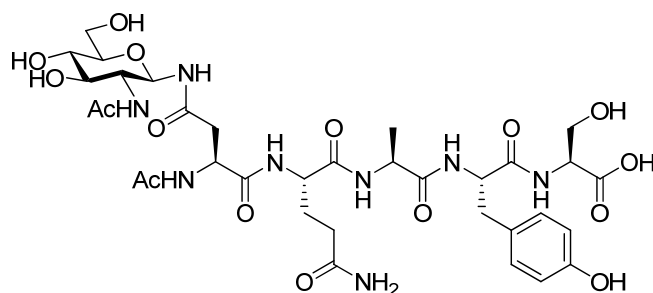
Anal HPLC: R_t = 13.9 min (2-50% B over 30 min, λ = 280 nm); LC-MS: R_t = 13.1 min (Gradient A); MS (ESI) m/z 855.5 [(M+H)⁺, 100%]; HRMS Calcd for C₃₅H₅₅N₁₀O₁₅: MH⁺, 855.3843 found MH⁺, 855.3833.

Ac-Asn(β -GlcNAc)-Asp-Ala-Tyr-Ser-OH (6i)



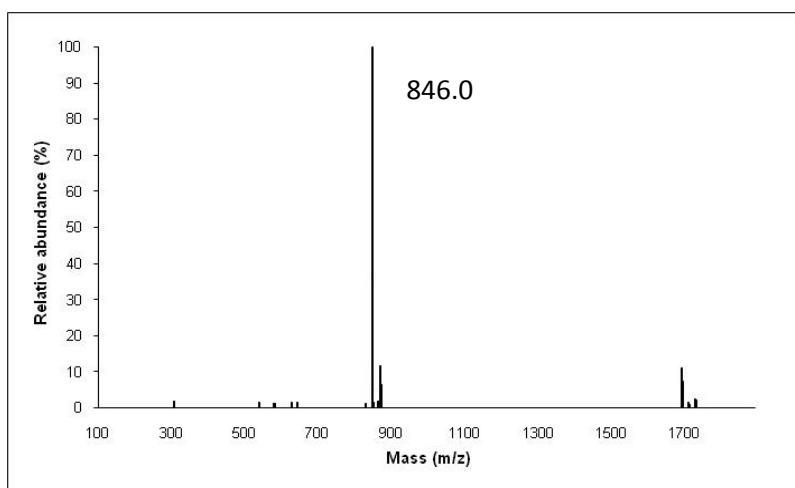
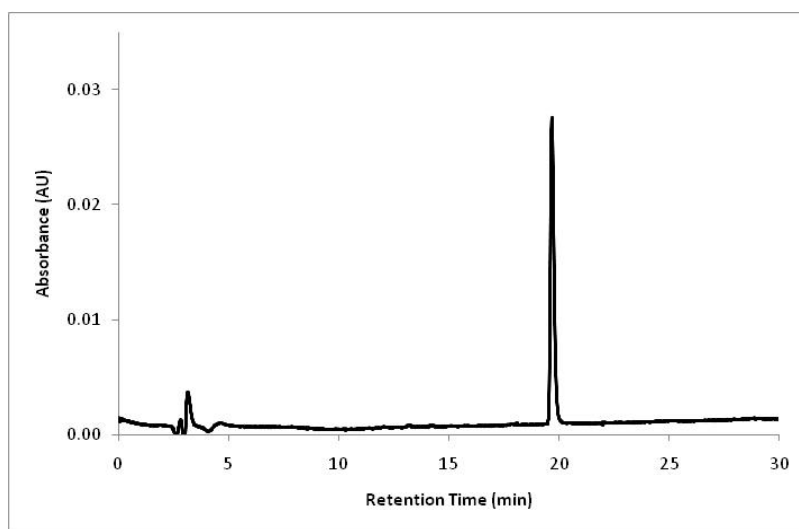
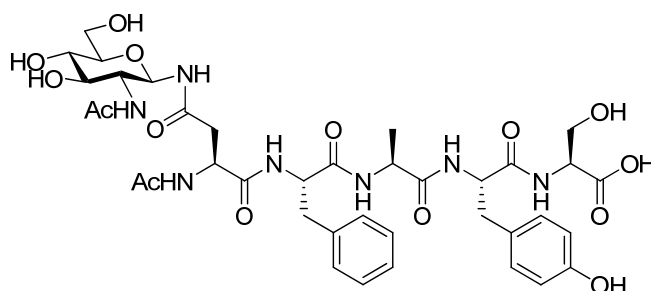
Analytical HPLC: $R_t = 14.5$ min (2-50% B over 30 min, $\lambda = 280$ nm); LC-MS: $R_t = 13.7$ min (Gradient A); MS (ESI) m/z 814.3 $[(M+H)^+]$, 100%; HRMS Calcd for $C_{33}H_{47}N_7O_{17}Na$: MNa^+ , 836.2921 found MNa^+ , 836.2918.

Ac-Asn(β -GlcNAc)-Gln-Ala-Tyr-Ser-OH (6j)



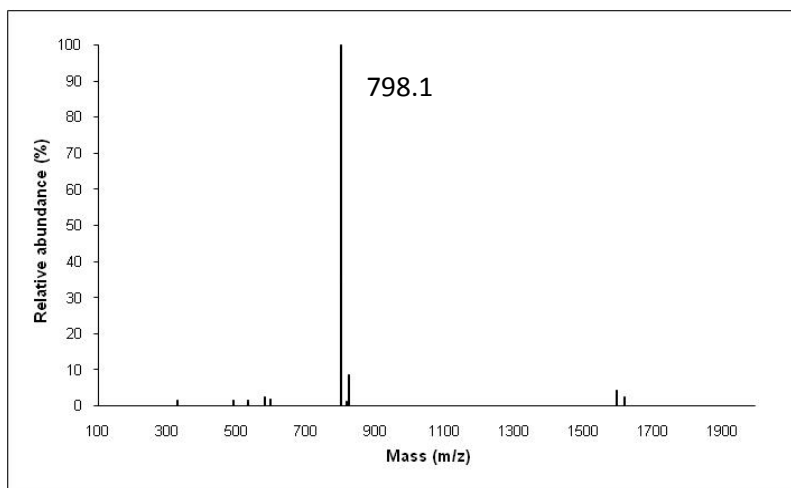
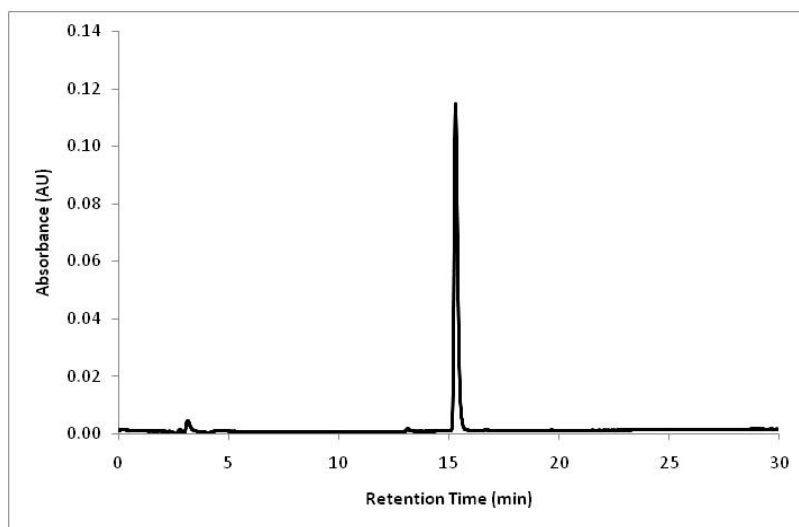
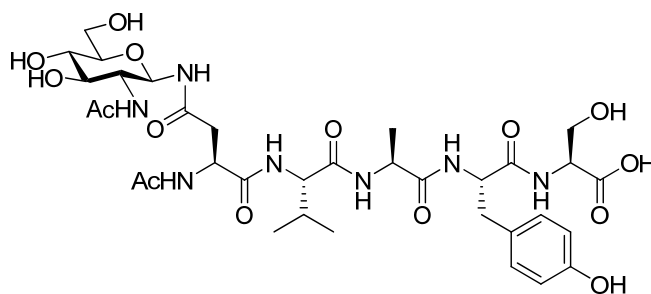
Analytical HPLC: $R_t = 14.2$ min (2-50% B over 30 min, $\lambda = 280$ nm), LC-MS: $R_t = 13.5$ min (Gradient A); MS (ESI) m/z 827.5 $[(M+H)^+]$, 100%; HRMS Calcd for $C_{35}H_{55}N_{10}O_{15} MH^+$, 827.3418 found MH^+ , 827.3416.

Ac-Asn(β -GlcNAc)-Phe-Ala-Tyr-Ser-OH (6k)



Analytical HPLC: $R_t = 19.7$ min (2-50% B over 30 min, $\lambda = 280$ nm); LC-MS: $R_t = 17.8$ min (Gradient A); MS (ESI) m/z 846.0 $[(M+H)^+]$, 100%; HRMS Calcd for $C_{38}H_{51}N_7O_{15}Na$: MNa^+ , 868.3335 found MNa^+ , 868.3336.

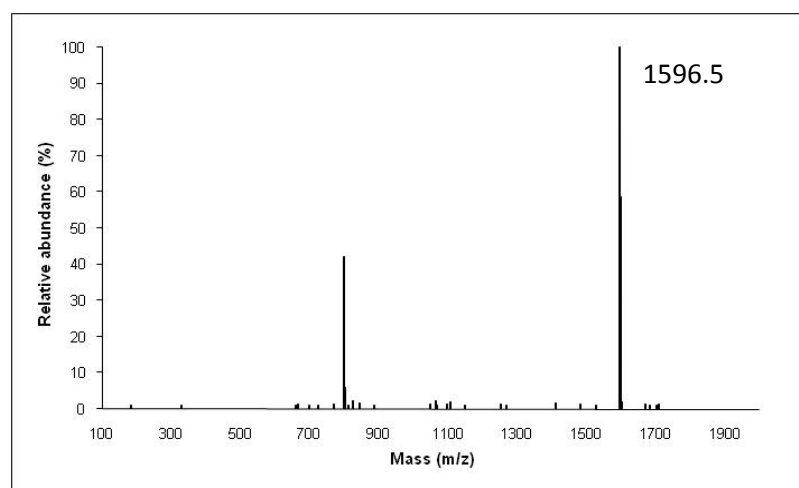
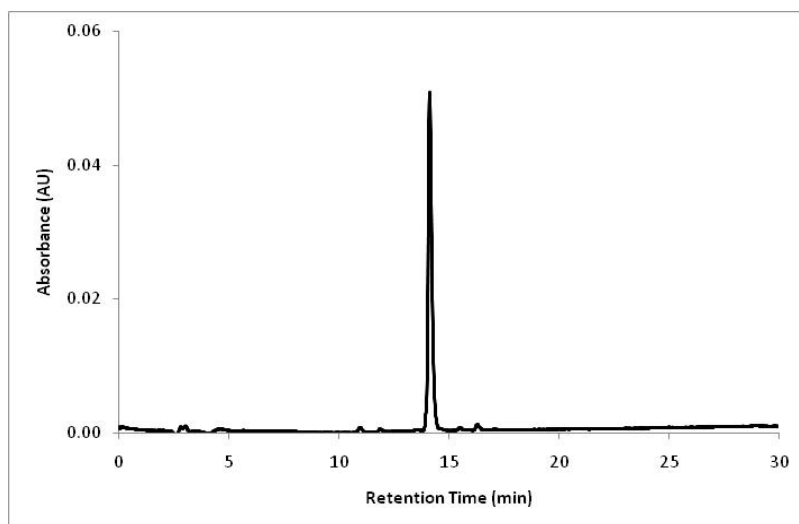
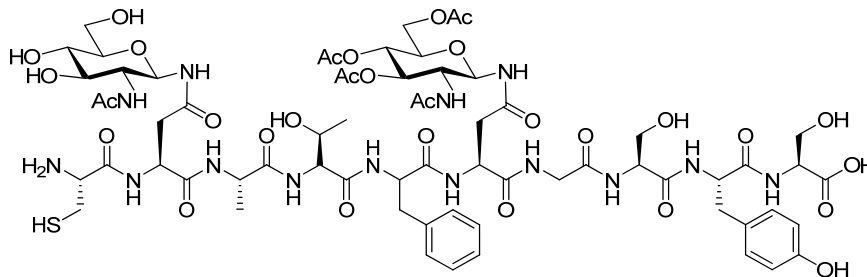
Ac-Asn(β -GlcNAc)-Val-Ala-Tyr-Ser-OH (6l)



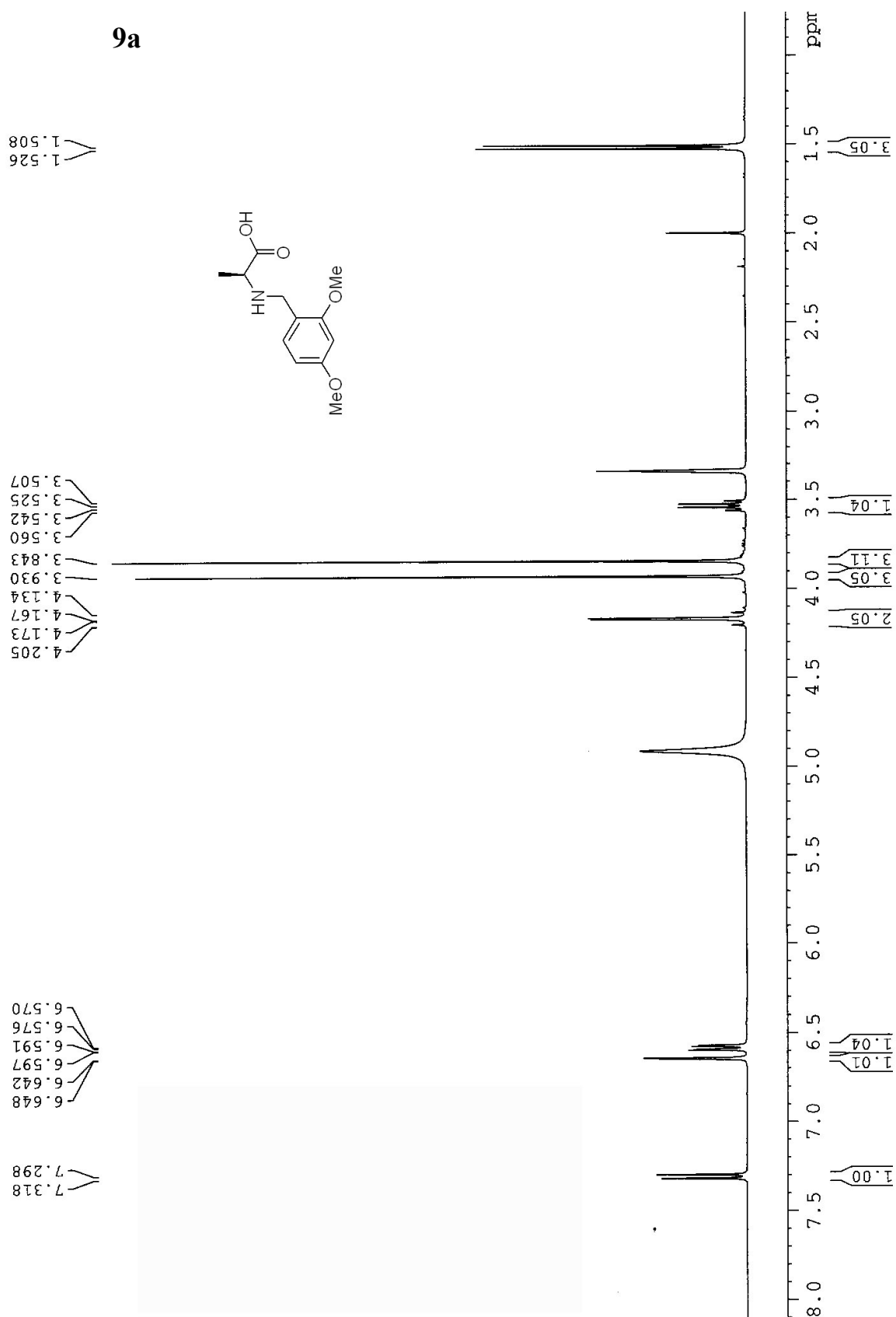
Analytical HPLC: $R_t = 15.4$ min (2-50% B over 30 min, $\lambda = 280$ nm), LC-MS: $R_t = 14.3$ min (Gradient A); MS (ESI) m/z 798.1 $[(M+H)^+]$, 100%; HRMS Calcd for $C_{34}H_{51}N_7O_{15}Na$: MNa^+ , 820.3354 found MNa^+ , 820.3342.

Analytical Data for glycodecapeptide 23

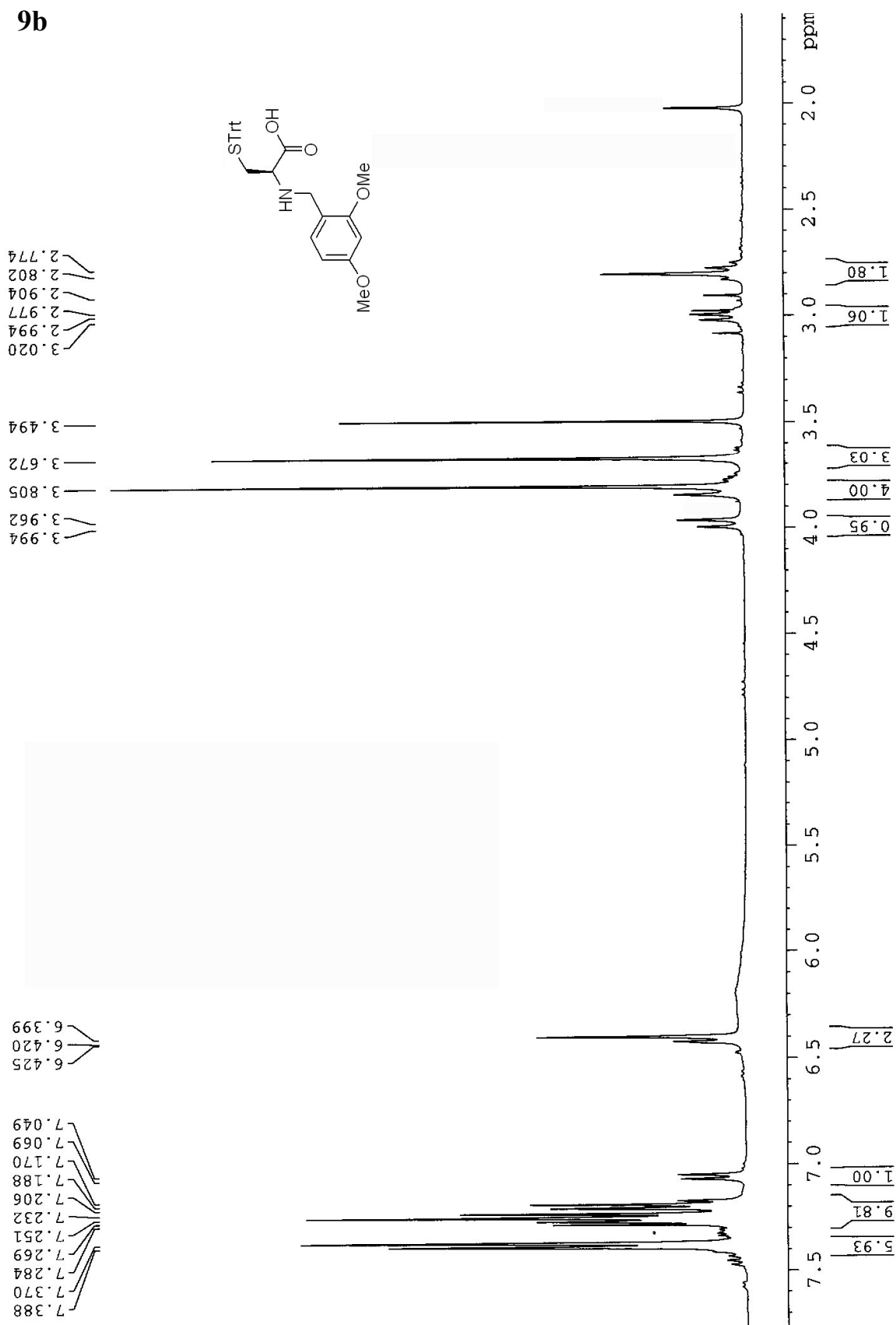
Ac-Cys-Asn(β -GlcNAc)-Ala-Thr-Phe- Asn(β -GlcNAc)-Gly-Ser-Tyr-Ser-OH (23)

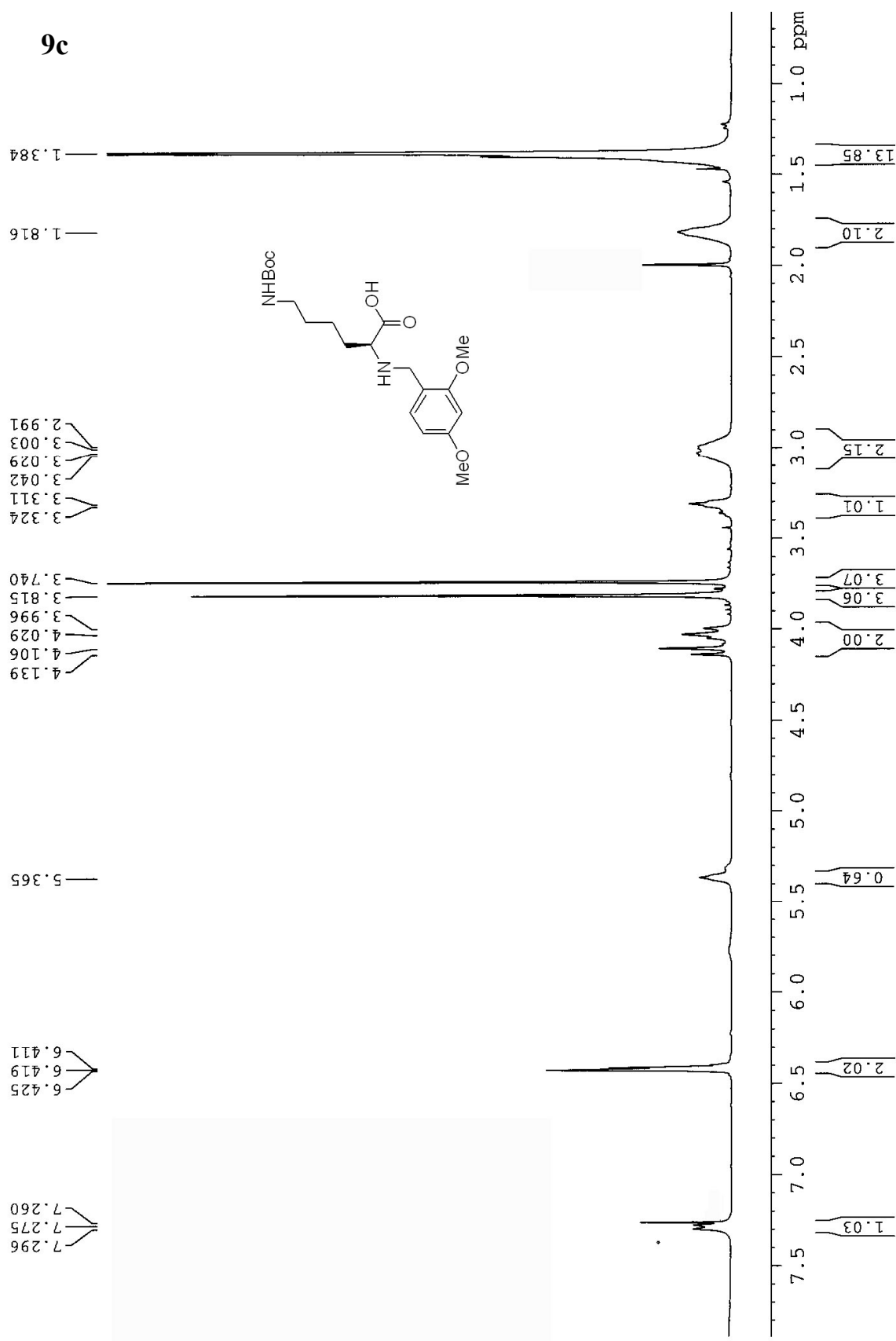


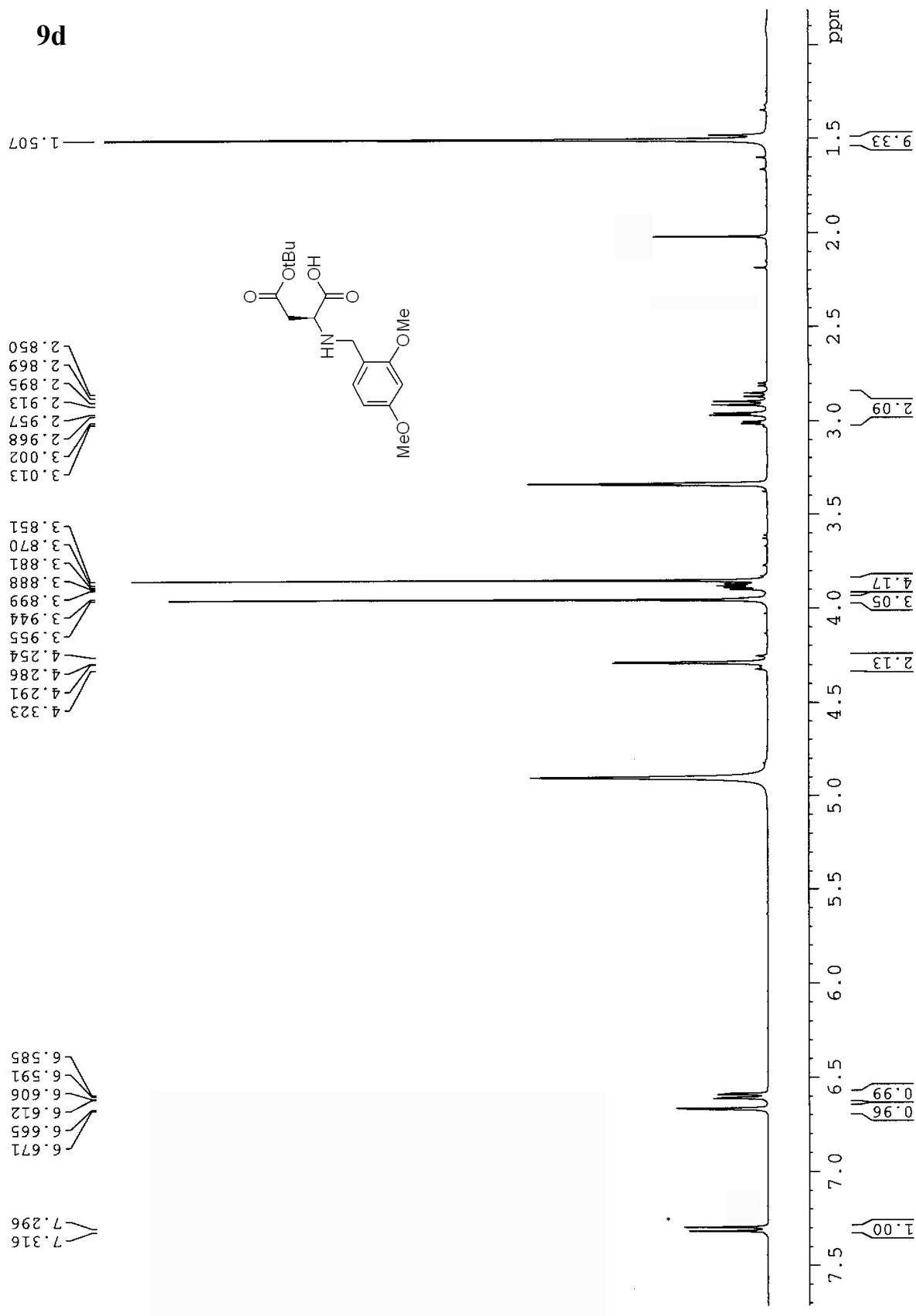
Anal HPLC: $R_t = 14.2$ min (2-50% B over 30 min, $\lambda = 280$ nm); LC-MS: $R_t = 13.9$ min (Gradient A); MS (ESI) m/z 1596.5 [$(M+H)^+$, 100%].



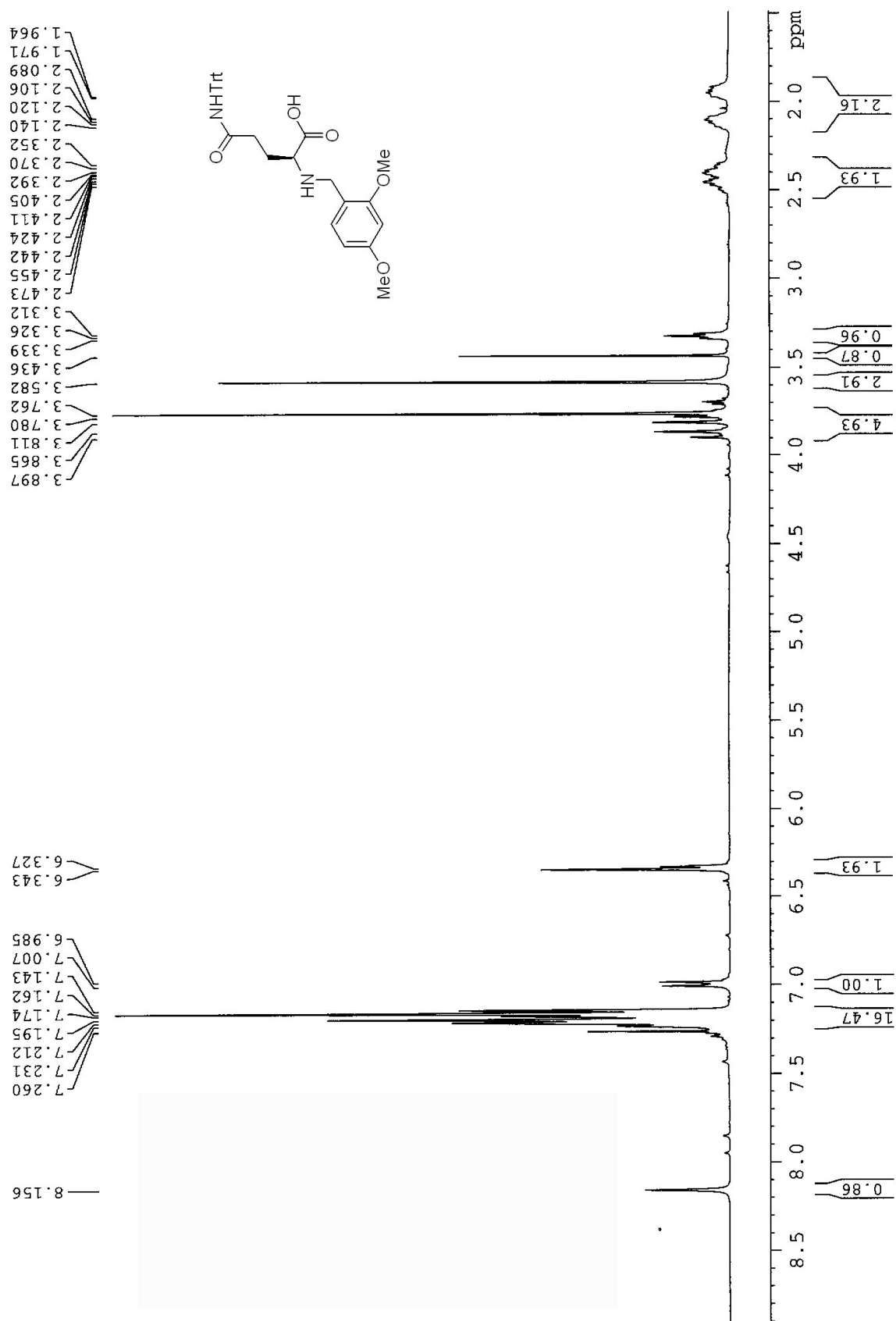
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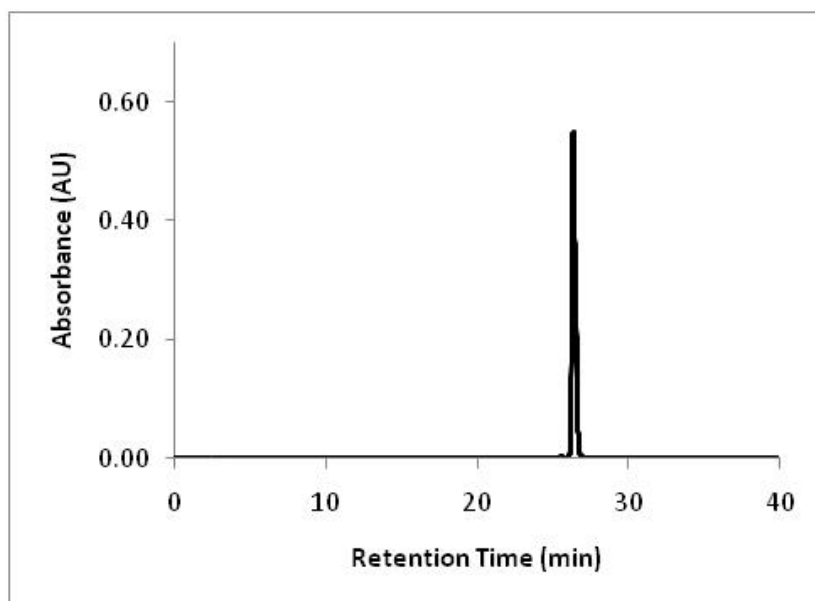
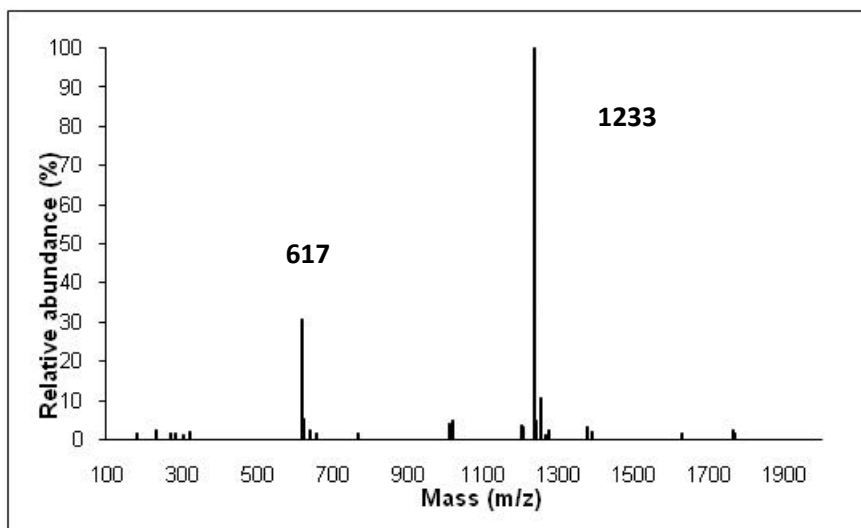


9e



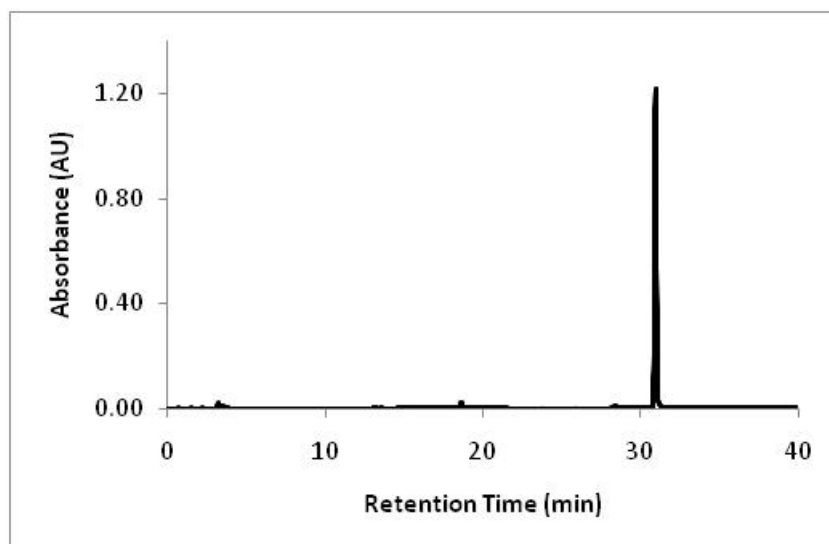
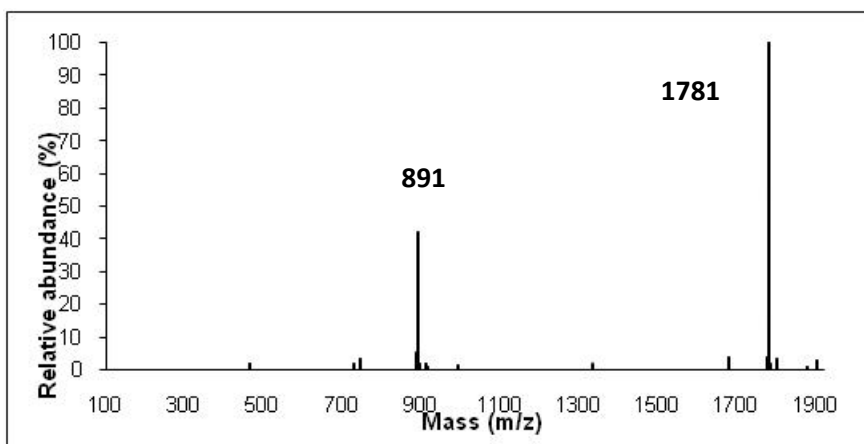
Analytical Data for dipeptides 11a-e and 16a-c

Fmoc-Asp(OAll)-(Dmb)Ala-OH (11a)



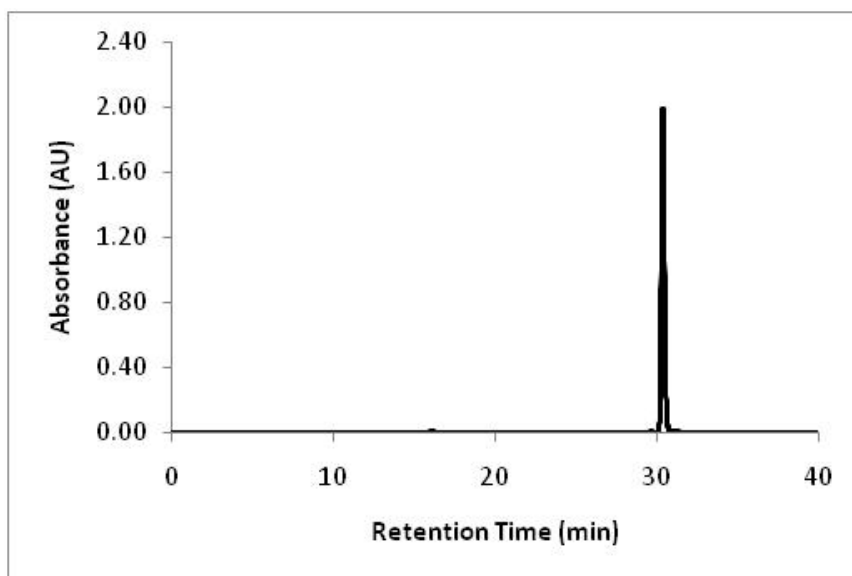
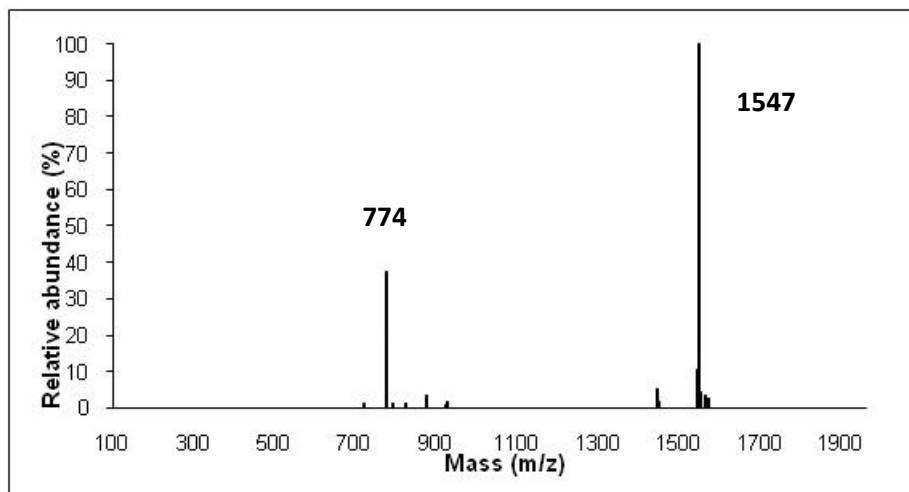
Analytical HPLC: $R_t = 26.4$ min (30-100% B over 30 min, $\lambda = 280$ nm); MS (ESI) m/z 1233 [(2M+H)⁺, 100%]; HRMS Calcd for C₃₄H₃₆N₂O₉Na: MNa⁺, 639.2313 found MNa⁺, 639.2301

Fmoc-Asp(OAll)-(Dmb)Cys(Trt)-OH (11b)



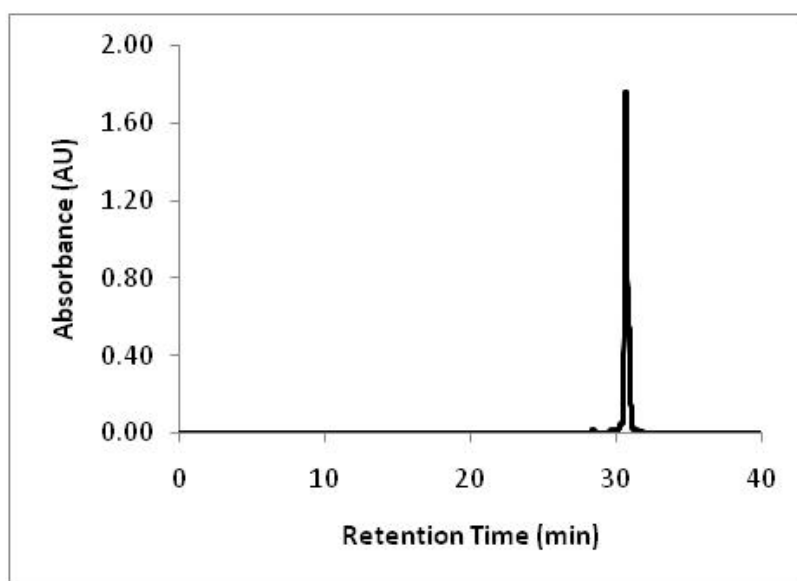
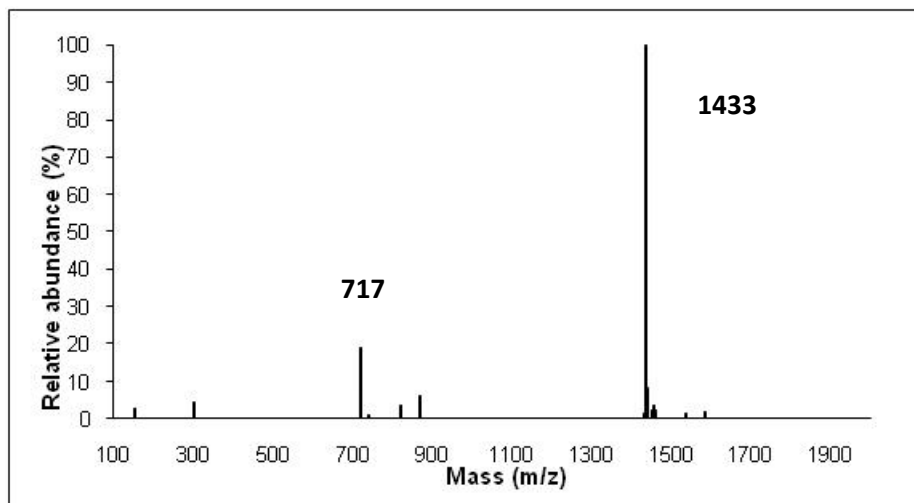
Analytical HPLC: $R_t = 31.2$ min (30-100% B over 30 min, $\lambda = 280$ nm); MS (ESI) m/z 1781 $[(2M+H)^+]$, 100%; HRMS Calcd for $C_{53}H_{50}N_2O_9SNa$: MNa^+ , 913.3129 found MNa^+ , 913.3126

Fmoc-Asp(OAll)-(Dmb)Lys(Boc)-OH (11c)



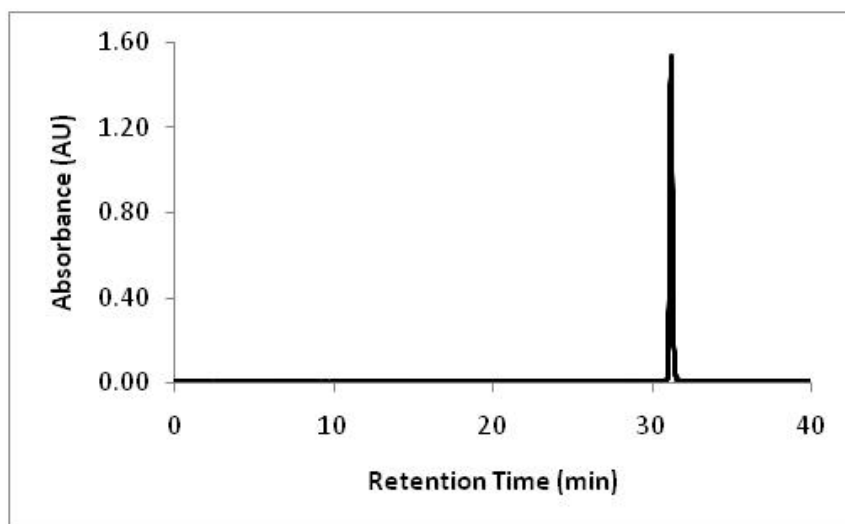
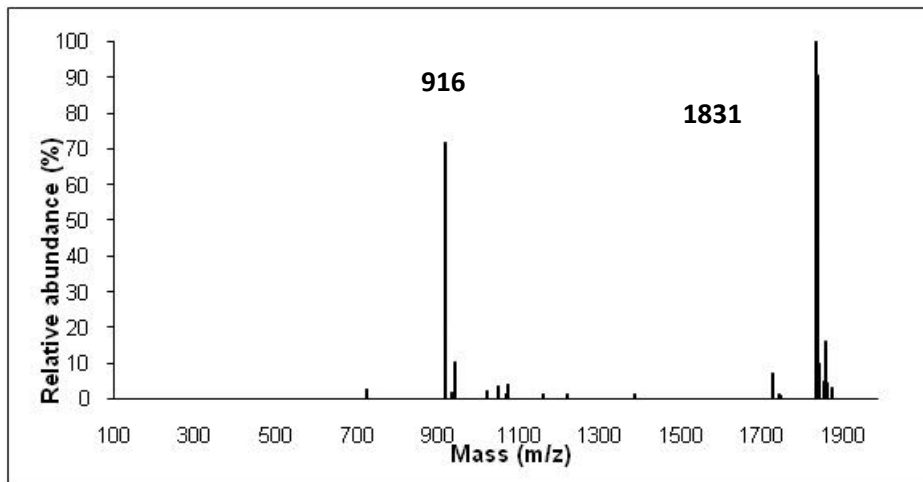
Analytical HPLC: $R_t = 30.7$ min (30-100% B over 30 min, $\lambda = 280$ nm); MS (ESI) m/z 1547 [(2M+H)⁺, 100%]; HRMS Calcd for C₄₂H₅₁N₃O₁₁Na: MNa⁺, 796.3416 found MNa⁺, 796.3407

Fmoc-Asp(OAll)-(Dmb)Asp(OtBu)-OH (11d)



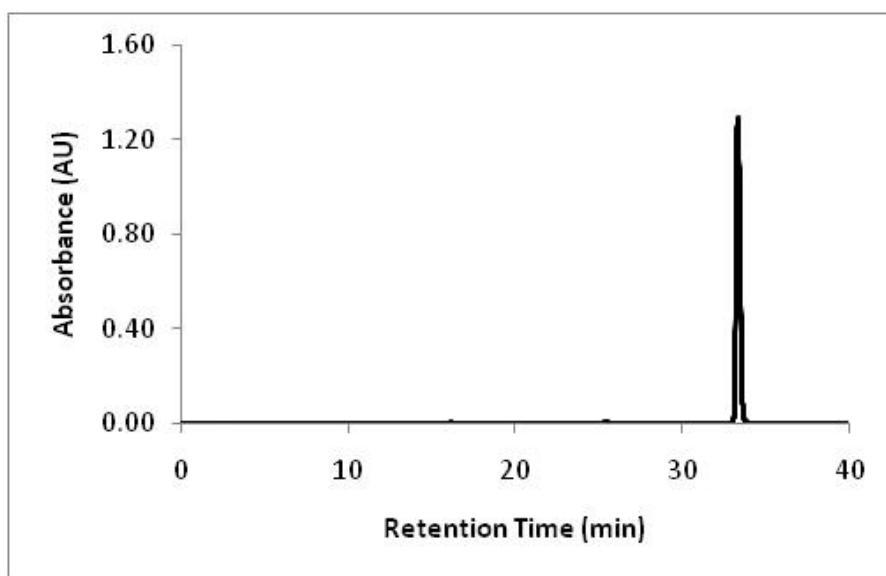
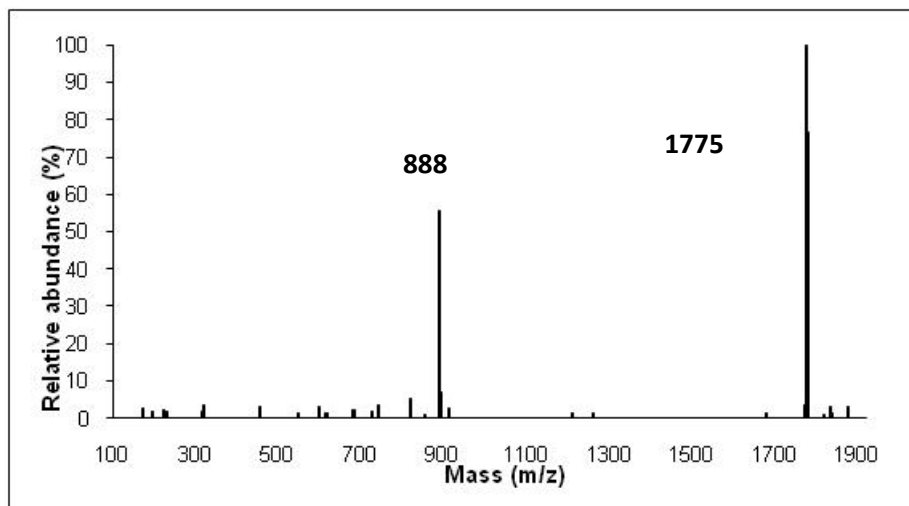
Analytical HPLC: $R_t = 31.1$ min (30-100% B over 30 min, $\lambda = 280$ nm); MS (ESI) m/z 1433 $[(2M+H)^+]$, 100%; HRMS Calcd for $C_{39}H_{44}N_2O_{11}Na$: MNa^+ , 739.2837 found MNa^+ , 739.2836

Fmoc-Asp(OAll)-(Dmb)Gln(Trt)-OH (11e)



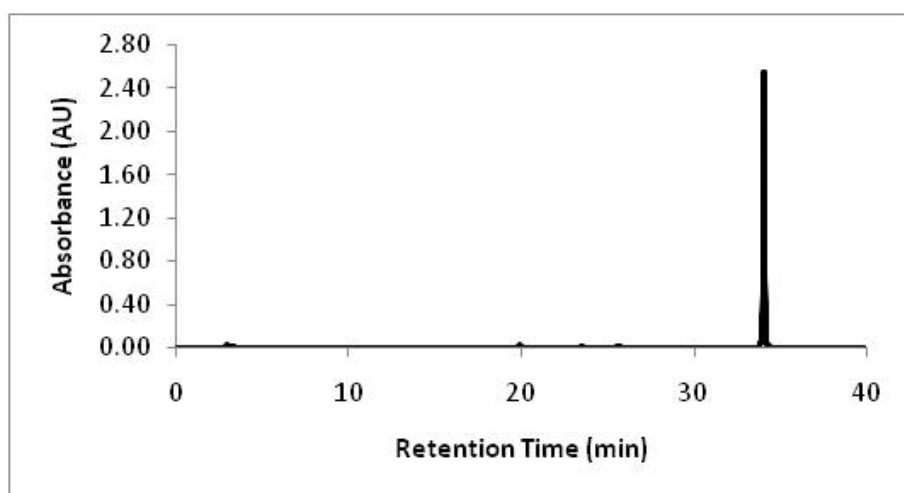
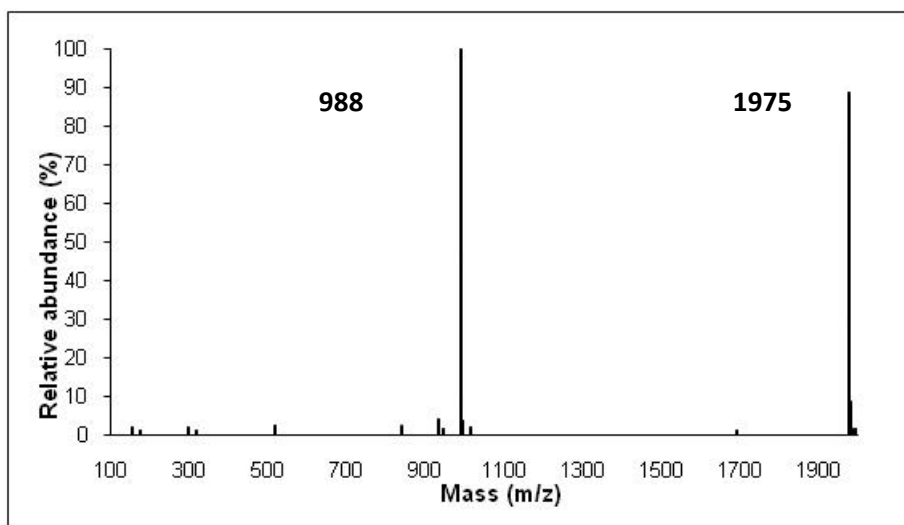
Analytical HPLC: $R_t = 32.1$ min (30-100% B over 30 min, $\lambda = 280$ nm); MS (ESI) m/z 1831 $[(2M+H)^+]$, 100%; HRMS Calcd for $C_{55}H_{53}N_3O_{10}Na$: MNa^+ , 938.3623 found MNa^+ , 938.3620.

Fmoc-Asp(ODmab)-(Dmb)Ala-OH (16a)



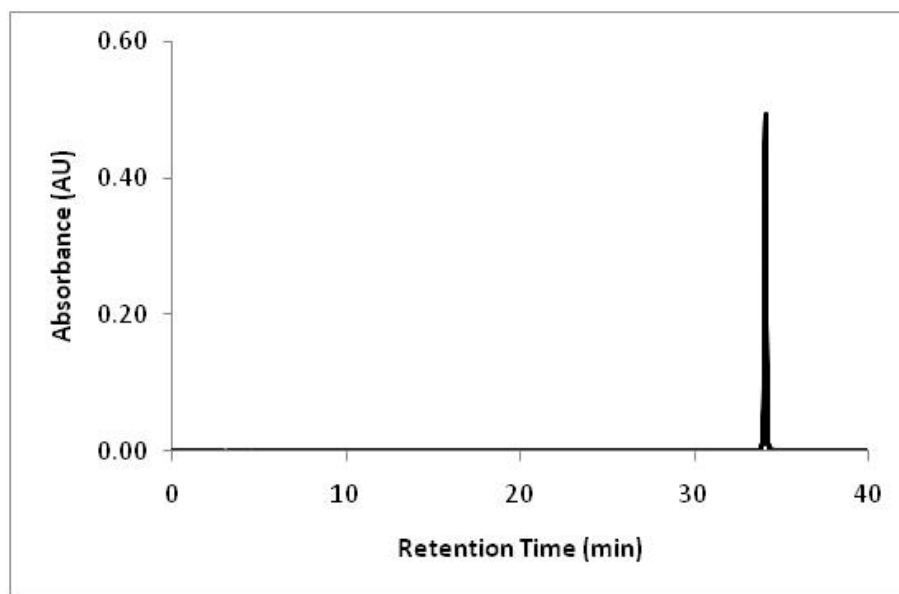
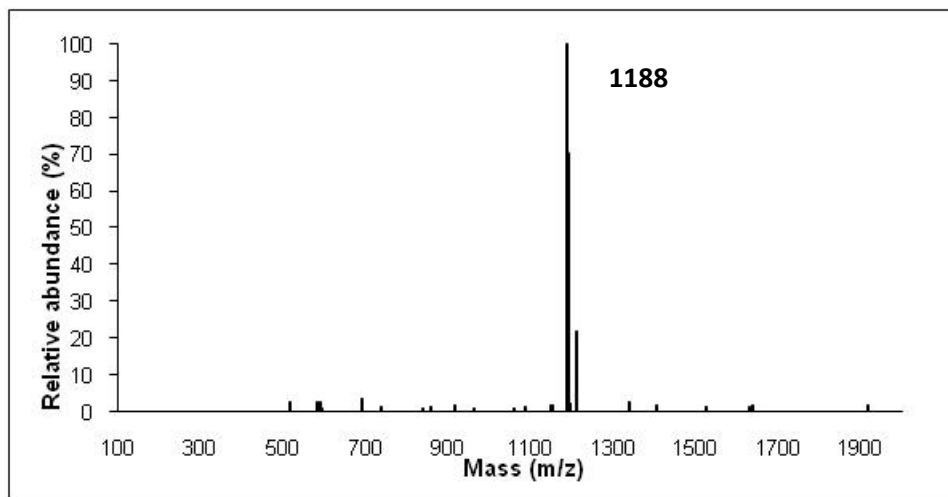
Analytical HPLC: $R_t = 32.1$ min (30-100% B over 30 min, $\lambda = 280$ nm); MS (ESI) m/z 1775 $[(2M+H)^+]$, 100%; HRMS Calcd for $C_{51}H_{57}N_3O_{11}Na$: MNa^+ , 910.3885 found MNa^+ , 910.3884.

Fmoc-Asp(ODmab)-(Dmb)Asp(OtBu)-OH (16b)



Analytical HPLC: $R_t = 34.2$ min (30-100% B over 30 min, $\lambda = 280$ nm); MS (ESI) m/z 988 [(M+H)⁺, 100%]; HRMS Calcd for C₅₆H₆₆N₃O₁₃: MH⁺, 988.4590 found MH⁺, 988.4589.

Fmoc-Asp(ODmab)-(Dmb)Gln(Trt)-OH (16c)



Analytical HPLC: $R_t = 34.2$ min (30-100% B over 30 min, $\lambda = 280$ nm); MS (ESI) m/z 1188 $[(M+H)^+$, 100%]; HRMS Calcd for $C_{72}H_{74}N_4O_{12}Na$: MNa^+ , 1209.5201 found MNa^+ , 1209.5193.