Supporting Information

Convergent chemo-enzymatic synthesis of mannosylated glycopeptides; targeting of putative vaccine candidates to antigen presenting cells

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Experimental

General methods

All reactions were carried out in oven-dried, nitrogen-purged glassware under an atmosphere of nitrogen. Melting points were recorded on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Polarimeter 341 with a path length of 1 dm. Concentrations are given in g / 100 mL. Infrared spectra were recorded on a Perkin-Elmer Spectrum One. Proton and carbon nuclear magnetic resonance (δ_H, δ_C) spectra were recorded on Agilent Technologies 400 MR (400 MHz) or Varian VNMR500 (500 MHz) spectrometers. All chemical shifts are quoted on the δ-scale in ppm using residual solvent as an internal standard. High-resolution mass spectra were recorded with a Bruker maXis 3G UHR-TOF mass spectrometer. Thin Layer Chromatography (t.l.c.) was carried out on Merck silica gel $60F_{254}$ aluminium-backed plates. Visualisation of the plates was achieved using a UV lamp (λ_{max} = 254 or 365 nm), and/or 5% w/v ammonium molybdate in 2 M sulfuric acid. Flash column chromatography was carried out using Sorbsil C60 40/60 silica. Unless preparative details are provided, all reagents were commercially available or made following literature procedures. "Petrol" refers to the fraction of light petroleum ether boiling in the range of 40-60 °C.

Glycopeptide synthesis, purification and analysis

Fmoc SPPS was performed on a Liberty 1 Microwave Peptide Synthesiser (CEM Corporation, Mathews, NC) using the Fmoc/*t*Bu strategy as previously described.[1] The Fmoc-[GlcNAc$(OAc)_3$β1-]Asn-OH glycosyl amino acid 8 was synthesised as previously described, and was pre-activated before placement in the synthesiser as follows; 8 (2.0 eq) was dissolved in DMF, and HATU (1.9 eq) was then added and the mixture shaken until all solids had dissolved. The solution was then transferred to the reaction vessel, 2,4,6-trimethylpyridine (6.0 eq) was added, and the mixture was then subjected to microwave irradiation for 15 min at 75 °C (20 W). 5(6)-Carboxyfluorescein was coupled using microwave enhanced Fmoc SPPS using slightly modified conditions as previously described; HCTU, was used in place of HBTU as the coupling reagent, and the reaction time was decreased from 30 min to 20 min. The progress of coupling reactions using both Fmoc-[GlcNAc$(OAc)_3$β1-]Asn-OH 8 and 5(6)-carboxyfluorescein was monitored by the Kaiser test.[2] The resulting glycopeptides were cleaved from the resin with simultaneous side chain protecting group removal by treatment with TFA/*i*Pr_3SiH/DODT/H_2O (94/1/2.5/2.5; v/v/v/v) for 2 h at room temperature. Crude peptides were precipitated and triturated with cold diethyl ether, isolated (centrifugation), and then dissolved in 50% acetonitrile (aq) containing 0.1% TFA and lyophilized. Analytical reverse phase high-performance liquid chromatography (RP-HPLC) was performed using a Dionex P680 system using Waters XTerra® column (MS C_{18}, 150 mm x 4.6 mm; 5 μm), or using an Agilent Technologies 1120 Compact LC system using an Agilent Zorbax

column (C_{18}, 150 mm x 3.0 mm, 3.5 μm), using gradient systems as indicated below. The solvent systems used were A (0.1% TFA in H_2O) and B (0.1% TFA in acetonitrile (MeCN)) with detection at 210 nm, 214 nm, 254 nm, and 280 nm. The ratio of products was determined by integration of spectra recorded at 210 nm or 214 nm. Peptide masses were confirmed by a Bruker micrOTOF-Q II mass spectrometer or a Hewlett Packard (HP) 1100 series mass spectrometer, or an inline Thermo Finnegan MSQ mass spectrometer using ESI in the positive mode. Peptide purification was performed using a Waters 600E system using a semi-preparative Phenomenex Gemini column (C_{18}, 250 mm x 10 mm; 5μm) or a Waters XTerra® column (C_{18}, 300 mm × 19 mm; 10 μm). Gradients were adjusted according to the elution profiles and peak profiles obtained from the analytical RP-HPLC chromatograms, with detection at 210 nm. Fractions were collected, analysed by either RP-HPLC or ESI-MS, pooled and lyophilised.

Enzymatic Glycosylation

The tetrasaccharide oxazoline **12** was prepared by total synthesis, following literature procedures.[3] The decasaccharide oxazoline **13** was prepared from soybean flour following the reported procedures.[4] The Endo-A E173H mutant enzyme was produced using the pET23d-Endo-A plasmid, originally supplied by Professor Kaoru Takegawa (Kyushu University), as previously described. The Endo-M N175Q mutant enzyme was from purchased Tokyo Chemical Industry Co., Ltd. Reverse phase high performance liquid chromatography was performed on a Dionex P680 HPLC instrument with a Phenomenex Jupiter 5μ C18 300A column (5.0 μm, 4.6× 250 mm) at 40 °C. The column was eluted with a linear gradient of MeCN at a flow rate of 1 mL/min using one of the following two methods, depending on the properties of the compounds to be separated: method A, 30–40% MeCN containing 0.05% trifluoroacetic acid for 14 min; method B, 25–40% MeCN containing 0.05% trifluoroacetic acid for 14 min. High resolution mass spectra were recorded with a Bruker maXis 3G UHR-TOF mass spectrometer.

Monocyte-derived dendritic cell culture and binding assay

Peripheral blood mononuclear cells (PBMC) were purified from healthy human volunteer blood, collected according to a protocol approved by the University of Auckland Human Participants Ethics Committee, New Zealand. Monocytes were isolated from the PBMC using the Pan Mono Isolation Kit (Miltenyi Biotec) according to the manufacturer's protocol. Following purification, monocytes were cultured for 6 days in RPMI (Invitrogen) containing 10% foetal bovine serum (Invitrogen), 100ng/ml GM-CSF and 50ng/ml IL-4 (both from Peprotech). The resulting monocyte-derived dendritic cells are hereafter referred to as APC (antigen presenting cells). There are typically a small number of lymphocytes (<10%) that remain in culture which were analysed as

MR-low/negative control cells. The culture medium as refreshed every other day with RPMI/10% foetal bovine serum containing GM-CSF and IL-4.

Cells (50,000 per sample) were incubated with 1µM of the indicated compounds for 1 hour at 37°C. Following incubation, excess antigen was removed by washes and the samples analysed on a Becton Dickinson FACS Aria II SORP. Data were analysed using FlowJo V10 and the APC and lymphocyte populations were distinguished based on size. To determine expression of MR (CD206) and DC-SIGN (CD209), cells were stained with CD206-APC (clone 19.2; BD Biosciences) and CD209-FITC (clone DCN47.5; Miltenyi Biotec) and incubated on ice for 30 min. Excess antibody was removed by washing and DAPI (1:5,000) was added prior to analysis to determine cell viability.

T Cell activation assay

Day 6-MoDCs from HLA A2-positive donors were pulsed with 10 µM of the indicated construct overnight at 37 °C. Excess antigen was removed by washing and the antigen-loaded MoDC were incubated (at a 1:1 ratio) with a T cell clone directed against the immunodominant epitope derived from the CMV protein pp65 (epitope sequence: NLVPMVATV). Golgistop (BD Biosciences) was added to the culture and the cells incubated for 6 hours at 37 °C. Expression of TNFα (using TNFα -Alexa Fluor® 488; clone Mab11; Biolegend) was determined by intracellular staining of the NLV-specific T cell clone using the CytoFix/CytoPerm^{TM} Method (BD Biosciences) according to manufacturer's instructions. Samples were analysed on a Becton Dickinson FACS Aria II SORP and the data was analyzed using FlowJo V10. To ensure only NLV-specific T cell clones were assessed, the NLV-specific T cell clone was stained with 0.5µM Cell Tracker Violet (Molecular Probes) prior to the assay and the cells analysed for cytokine secretion were gated on Cell Tracker Violet-positive cells.

Peptide Synthesis

5(6)-CF-CMV 1

5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-Asn-Leu-Lys

Microwave enhanced Fmoc SPPS was used for the synthesis of 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-Asn-Leu-Lys (**9**) using procedures as described in general section and starting from "*in house*" prepared aminomethyl polystyrene resin (0.100 g, 0.1 mmol)[3,4] to which Fmoc-Lyc(Boc)-HMPP (129.4 mg, 0.2 mmol) was initially attached using DIC (30.9 µL, 0.2 mmol) in CH_2Cl_2 (1.5 mL) for 2 h, following published procedures.[1] Synthesis afforded **9** as a regioisomeric mixture of 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-Asn-Leu-Lys (**9**) (217.1 mg); $R_{t\ 5(CF)\ and\ 6(CF)}$ 17.65 min; *m/z* (ESI-MS) 814.0 ($[M + 3H]^{3+}$ requires 813.8), Figure S 1.

The reduction of methionine sulfoxide was performed according to the procedure by Giralt *et al.*[5] Thus, crude glycopeptide **9** (217.1 mg, 89.0 x 10^{-3} mmol) was dissolved in neat TFA (110.0 mL) and the solution cooled on ice bath (0 ºC). TBAI (0.987 g, 2.67 mmol) and DMS (196 µL, 2.67 mmol) were then added and the mixture was vigorously stirred for 1 h, during which time most of the starting material had disappeared as judged by analytical RP-HPLC. Glycopeptide was precipitated and triturated with cold diethyl ether, isolated (centrifugation), dissolved in 50% acetonitrile (aq) containing 0.1% TFA and lyophilized to afford a crude regioisomeric mixture 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-Asn-Leu-Lys (**1**) (92.0 mg). The crude product **1** was purified by RP-HPLC using a semi-preparative Waters XTerra® column at a flow rate of 10 mL min^{-1}, using a linear gradient of 5%B to 85%B over 80 min (*ca.* 1%B per minute) and lyophilised to afford a regioisomeric mixture of 5(6)-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-Asn-Leu-Lys (**1**) as a yellow amorphous solid (19.2 mg, 8% overall yield based on resin loading of 0.1 mmol/g); $R_{t\ 5(CF)\ and\ 6(CF)}$ 25.36 min; *m/z* (ESI-MS) 808.9 ($[M + 3H]^{3+}$ requires 808.5), Figure S 2.

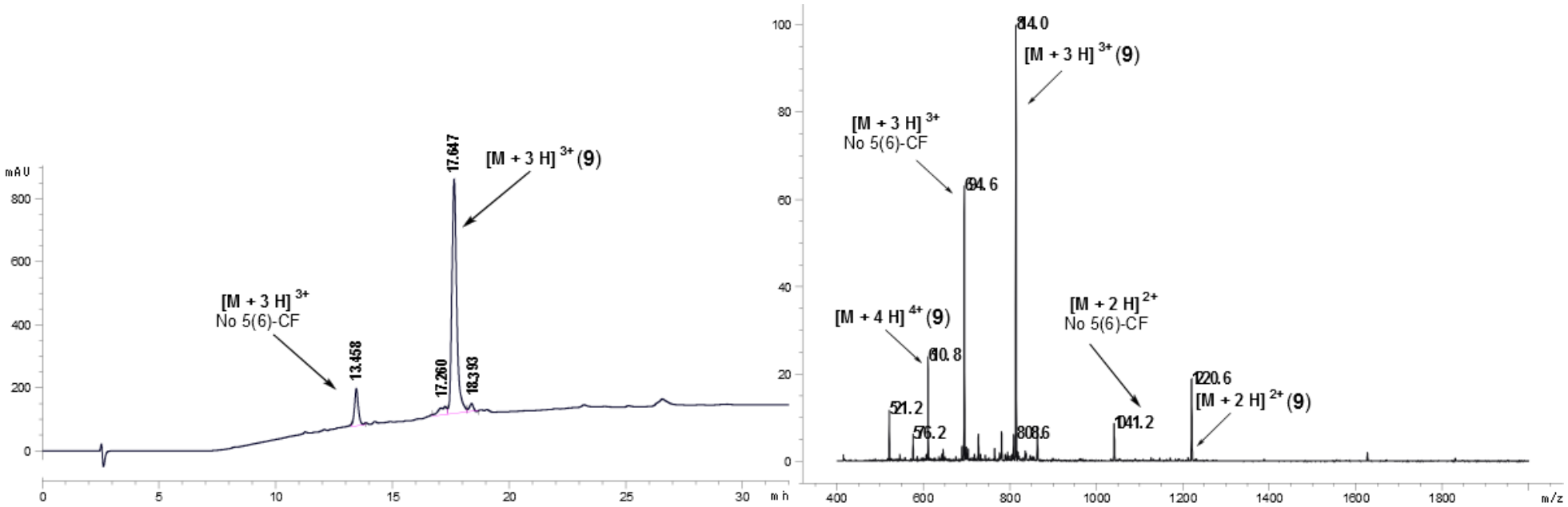


Figure S 1 RP-HPLC (Dionex P680) and ESI-MS (HP 1100) traces of crude 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-Asn-Leu-Lys (**9**) (*ca.* 76% as judged by peak area of RP-HPLC at 214 nm); Agilent Zorbax column, linear gradient of 1%B to 100%B over 30 min, *ca.* 3%B per minute (0.3 mL/min).

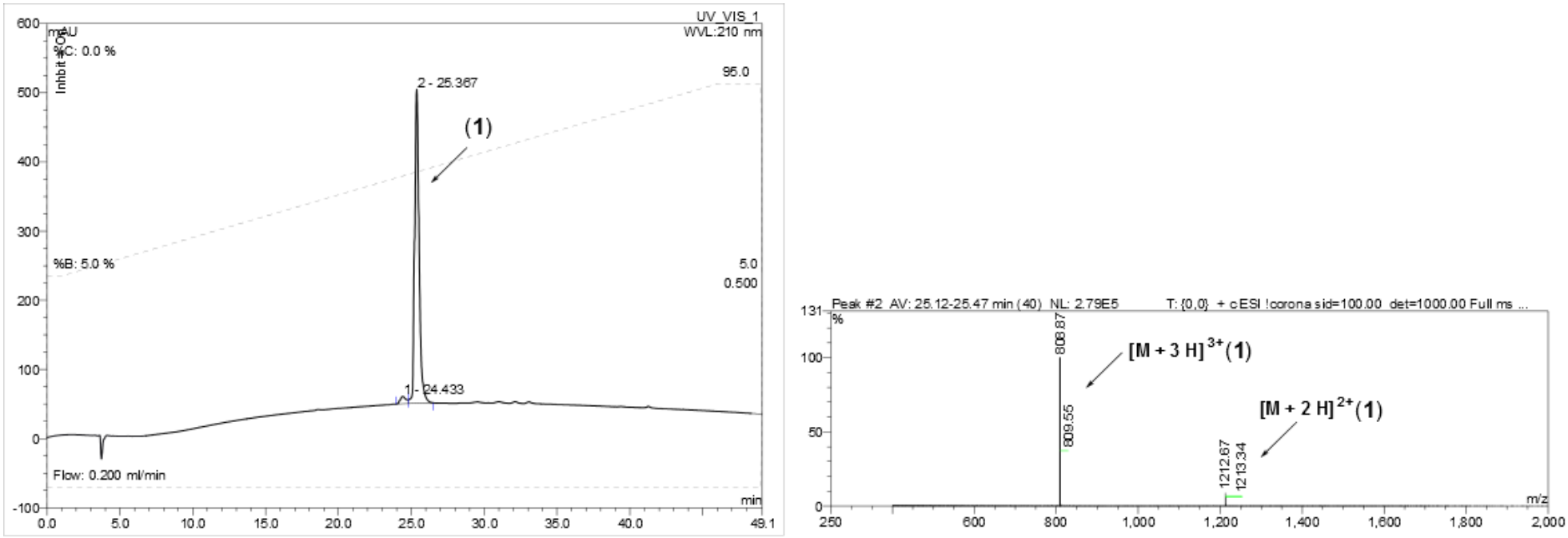
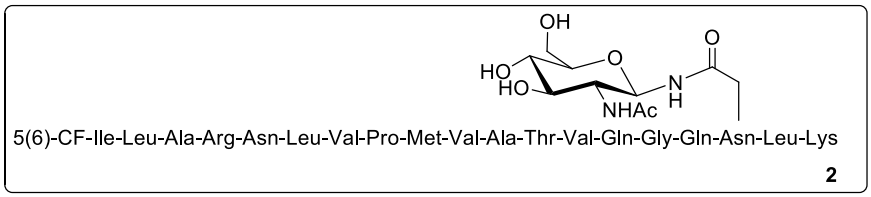


Figure S 2 RP-HPLC (Dionex P680) and ESI-MS (Thermo Finnegan MSQ) traces of purified 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-Asn-Leu-Lys (**1**) (*ca.* 97% as judged by peak area of RP-HPLC at 210 nm); Agilent Zorbax column, linear gradient of 5%B to 95%B over 45 min, *ca.* 2%B per minute.

5(6)-CF-[(GlcNAc)Asn^{17}]CMV 2



Microwave enhanced Fmoc SPPS was used for the synthesis of 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAc(OAc)$_3$β1-]Asn-Leu-Lys (**10**) using procedures as described in general section and starting from "*in house*" prepared aminomethyl polystyrene resin (0.100 g, 0.1 mmol)[5,6] to which Fmoc-Lyc(Boc)-HMPP (129.4 mg, 0.2 mmol) was initially attached using DIC (30.9 µL, 0.2 mmol) in CH_2Cl_2 (1.5 mL) for 2 h, following published procedures.[3] Synthesis afforded **10** as a regioisomeric mixture of 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAc(OAc)$_3$β1-]Asn-Leu-Lys (**10**) (200.9 mg); $R_{t\ 5(CF)\ and\ 6(CF)}$ 18.19 min; *m/z* (ESI-MS) 923.8 ([M + 3H]$^{3+}$ requires 923.5), Figure S 3. Crude glycopeptide **10** (51.9 mg, 18.6 x 10^{-3} mmol) was dissolved in methanol (45.0 mL) and 1 M NaOMe solution in methanol was added to adjust the pH to 12.2 (pH meter). When the starting material had disappeared as judged by analytical RP-HPLC (1 h 30 min), the solution was neutralised with a portion of dry ice and the solvent was removed *in vacuo* to afford a crude regioisomeric mixture of 5(6)-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]-Asn-Leu-Lys (49.0 mg); $R_{t\ 5(CF)\ and\ 6(CF)}$ 17.66 min; *m/z* (ESI-MS) 881.8 ([M + 3H]$^{3+}$ requires 881.5), Figure S 4. The reduction of methionine sulfoxide was performed according to the procedure by Giralt *et al.*[7] Thus, crude glycopeptide produced above (49.0 mg, 18.4 x 10^{-3} mmol) was dissolved in neat TFA (35.0 mL) and the solution cooled on ice bath (0 ºC). TBAI (0.218 g, 0.59 mmol) and DMS (43 µL, 0.59 mmol) were then added and the mixture was vigorously stirred for 1 h 30 min, during which time most of the starting material had disappeared as judged by analytical RP-HPLC. Glycopeptide was precipitated and triturated with cold diethyl ether, isolated (centrifugation), dissolved in 50% acetonitrile (aq) containing 0.1% TFA and lyophilized to afford a crude regioisomeric mixture 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]Asn-Leu-Lys (**2**) (45.0 mg); $R_{t\ 5(CF)\ and\ 6(CF)}$ 17.88 min; *m/z* (ESI-MS) 876.4 ([M + 3H]$^{3+}$ requires 876.2). The crude product **2** was purified by RP-HPLC using a semi-preparative Gemini C18 column (Phenomenex), at a flow rate of 5 mL min^{-1}, using a linear gradient of 1%B to 81%B over 80 min (*ca.* 1%B per minute) and lyophilised to afford a regioisomeric mixture of 5(6)-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]Asn-Leu-Lys (**2**) as a yellow amorphous solid (20.8 mg, 32% overall yield based on resin loading of 0.1 mmol/g); $R_{t\ 5(CF)\ and\ 6(CF)}$ 19.02 min; *m/z* (ESI-MS) 876.4 ([M + 3H]$^{3+}$ requires 876.2), Figure S 5.

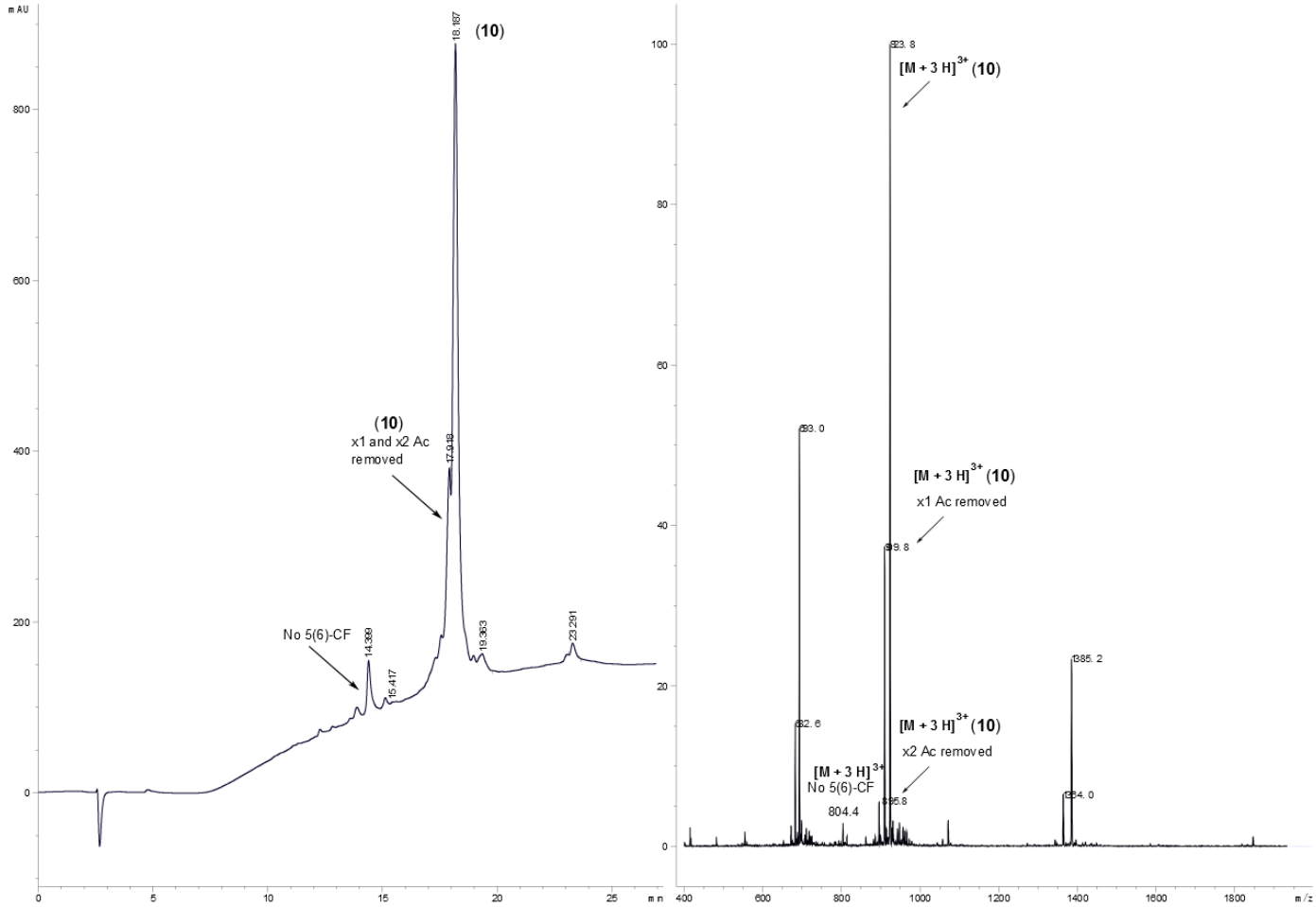


Figure S 3 RP-HPLC (Agilent Technologies) and ESI-MS (HP 1100) traces of crude 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAc(OAc)$_3$β1-]Asn-Leu-Lys (**10**) (*ca.* 75% as judged by peak area of RP-HPLC at 214 nm); Agilent Zorbax column, linear gradient of 1%B to 100%B over 30 min, *ca.* 3%B per minute (0.3 mL/min).

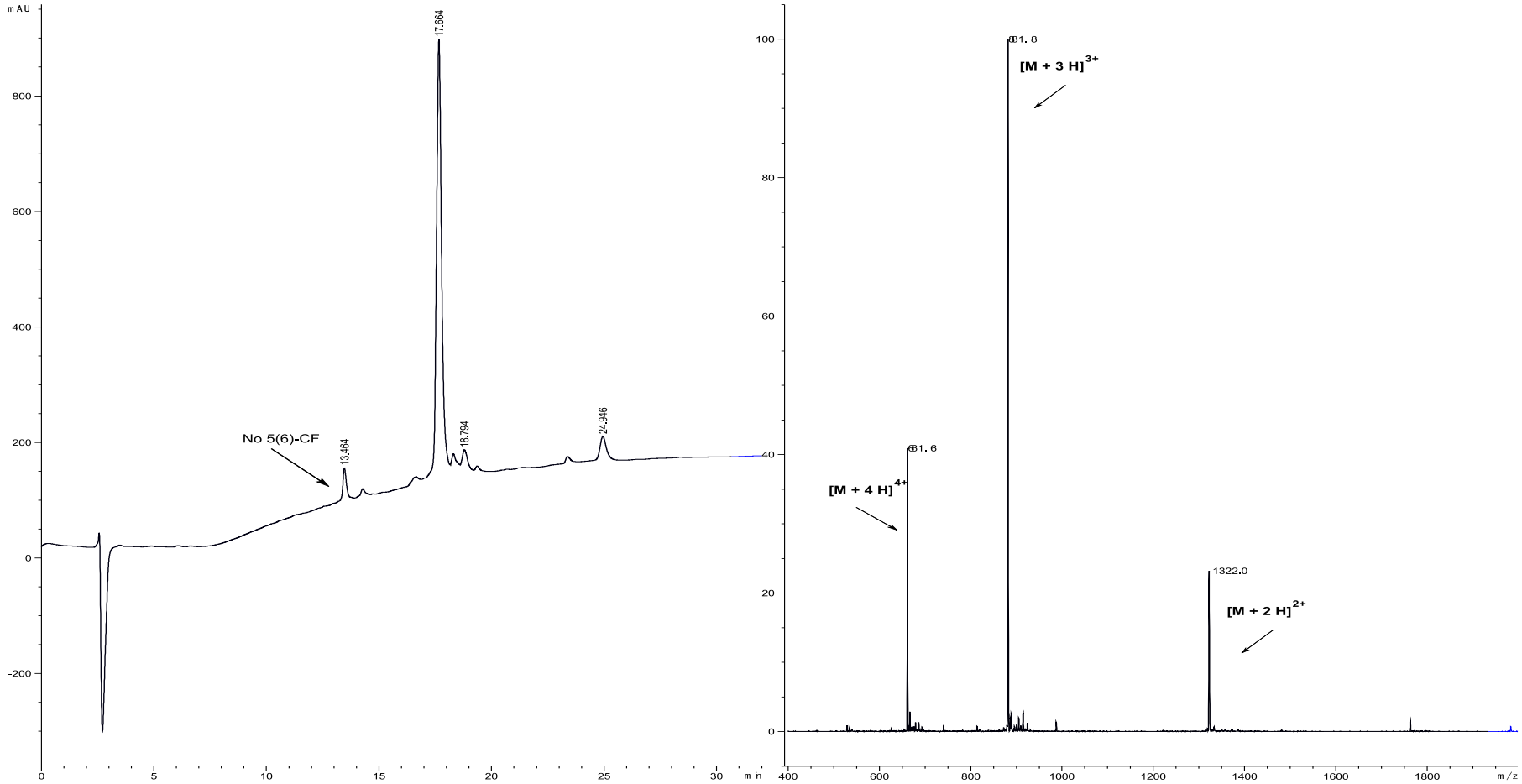


Figure S 4 LC-MS (Agilent Technologies and HP 1100) traces of crude 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]Asn-Leu-Lys (*ca.* 84% as judged by peak area of RP-HPLC at 214 nm); Agilent Zorbax column, linear gradient of 1%B to 100%B over 30 min, *ca.* 3%B per minute (0.3 mL/min).

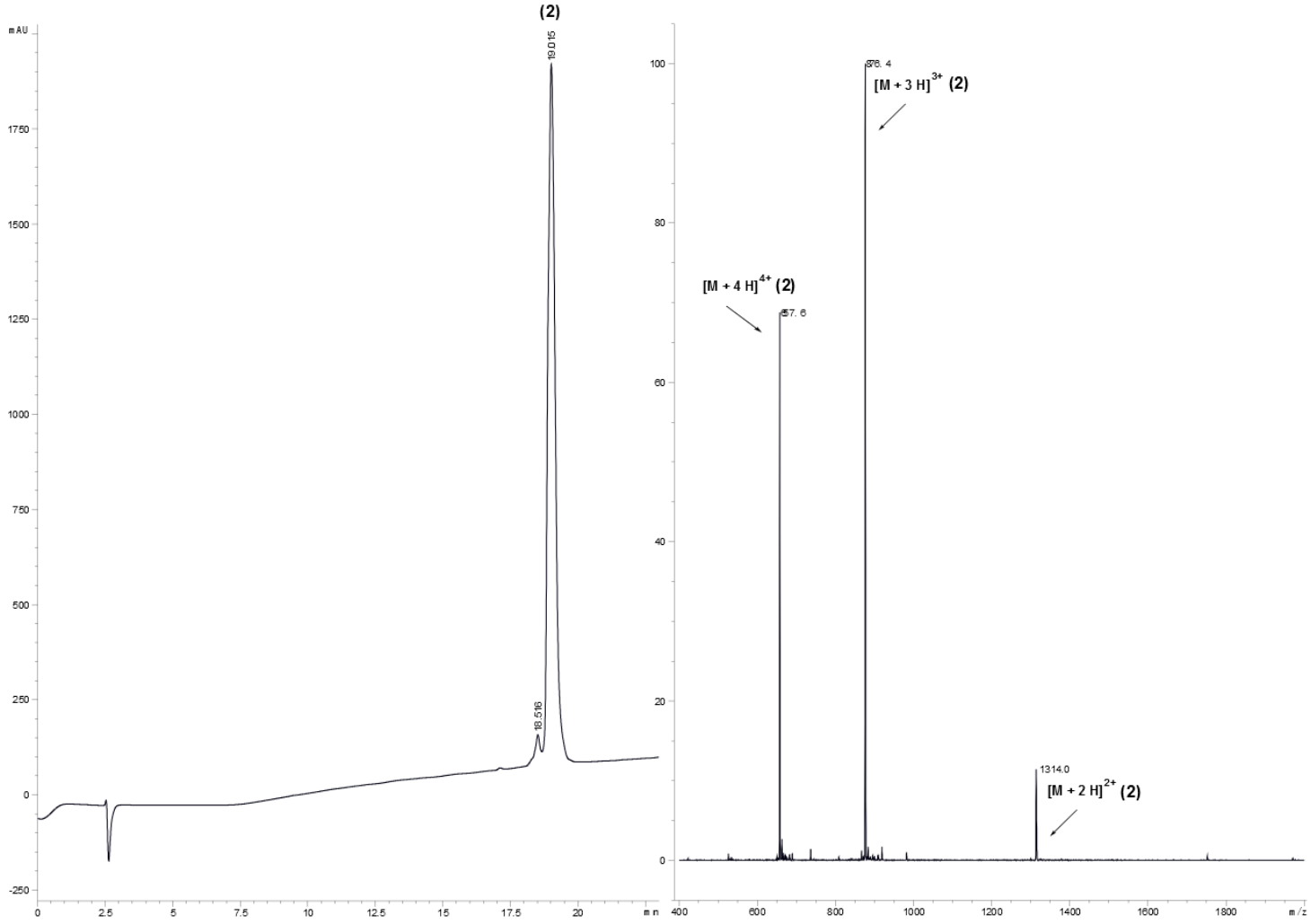
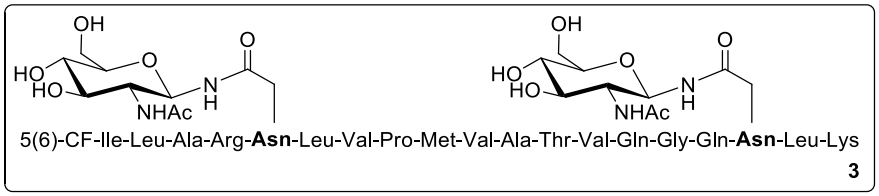


Figure S 5 LC-MS (Agilent Technologies and HP 1100) traces of purified 5(6)-CF-Ile-Leu-Ala-Arg-Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]Asn-Leu-Lys (**2**) (*ca.* 99% as judged by peak area of RP-HPLC at 214 nm); Agilent Zorbax column, linear gradient of 1%B to 61%B over 20 min, *ca.* 3%B per minute.

5(6)-CF-[(GlcNAc)Asn^{5,17}]CMV 3



Microwave enhanced Fmoc SPPS was used for the synthesis of 5(6)-CF-Ile-Leu-Ala-Arg-[GlcNAc(OAc)$_3$β1-]Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAc(OAc)$_3$β1-]Asn-Leu-Lys (**11**) using procedures as described in general section and starting from "*in house*" prepared aminomethyl polystyrene resin (0.050 g, 0.05 mmol)[3,4] to which Fmoc-Lyc(Boc)-HMPP (64.7 mg, 0.1 mmol) was initially attached using DIC (15.5 µL, 0.1 mmol) in CH_2Cl_2 (1.5 mL) for 2 h, following published procedures.[1] Synthesis afforded **11** as a regioisomeric mixture of 5(6)-CF-Ile-Leu-Ala-Arg-[GlcNAc(OAc)$_3$β1-]Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAc(OAc)$_3$β1-]Asn-Leu-Lys (**11**) (74.5 mg); $R_{t\ 5(CF)\ and\ 6(CF)}$ 16.23 min; *m/z* (ESI-MS) 1033.52 ($[M + 3H]^{3+}$ requires 1033.18), Figure S 6.

Crude glycopeptide **11** (74.5 mg, 2.40 x 10^{-2} mmol) was dissolved in methanol (10.0 mL) and 1 M NaOMe solution in methanol was added to adjust the pH to 10.4 (pH meter). When the starting material had disappeared as judged by analytical RP-HPLC (3 h), the solution was neutralised with a portion of dry ice and the solvent was removed *in vacuo* to afford a crude regioisomeric mixture of 5(6)-CF-Ile-Leu-Ala-Arg-[GlcNAcβ1-]Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]Asn-Leu-Lys (79.3 mg); $R_{t\ 5(CF)\ and\ 6(CF)}$ 14.57 min; *m/z* (ESI-MS) 949.43 ($[M + 3H]^{3+}$ requires 949.15), Figure S 7. The crude product was purified by RP-HPLC using a semi-preparative Gemini C18 column (Phenomenex), at a flow rate of 5 mL min^{-1}, using a linear gradient of 1%B to 61%B over 60 min (*ca.* 1%B per minute) and lyophilised to afford 5(6)-CF-Ile-Leu-Ala-Arg-[GlcNAcβ1-]Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]Asn-Leu-Lys as a yellow amorphous solid (35.1 mg); $R_{t\ 5(CF)\ or\ 6(CF)}$ 15.20 min (*ca*. 39% by RP-HPLC at 210 nm) and $R_{t\ 5(CF)\ or\ 6(CF)}$ 15.56 min (*ca*. 61% by RP-HPLC at 210 nm); *m/z* (ESI-MS) 949.49 ($[M + 3H]^{3+}$ requires 949.15).

The reduction of methionine sulfoxide was performed according to the procedure by Giralt *et al.*[5] Thus, purified glycopeptide (33.0 mg, 11.60 x 10^{-3} mmol) was dissolved in neat TFA (15.0 mL) and the solution cooled on ice bath (0 ºC). TBAI (0.129 g, 0.35 mmol) and DMS (26 µL, 0.35 mmol) were then added and the mixture was vigorously stirred for 9 h 30 min, during which time most of the starting material had disappeared as judged by analytical RP-HPLC. Glycopeptide was precipitated and triturated with cold diethyl ether, isolated (centrifugation), dissolved in 50% acetonitrile (aq) containing 0.1% TFA and lyophilized to afford a crude regioisomeric mixture of

5(6)-CF-Ile-Leu-Ala-Arg-[GlcNAcβ1-]Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]Asn-Leu-Lys (**3**) (28.2 mg); $R_{t\ 5(CF)\ and\ 6(CF)}$ 16.15 min; *m/z* (ESI-MS) 944.0 ([M + 3H]$^{3+}$ requires 943.9).

The crude product **3** was purified by RP-HPLC using a semi-preparative Gemini C18 column (Phenomenex), at a flow rate of 5 mL min^{-1}, using a linear gradient of 1%B to 81%B over 80 min (*ca.* 1%B per minute) and lyophilised to afford 5(6)-Ile-Leu-Ala-Arg-[GlcNAcβ1-]Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]Asn-Leu-Lys (**3**) as a yellow amorphous solid (11.0 mg, 9% overall yield based on resin loading of 0.05 mmol/g); $R_{t\ 5(CF)\ or\ 6(CF)}$ 14.90 min (*ca*. 32% by RP-HPLC at 210 nm) and $R_{t\ 5(CF)\ or\ 6(CF)}$ 14.98 min (*ca*. 66% by RP-HPLC at 210 nm) *m/z* (ESI-MS) 944.2 ([M + 3H]$^{3+}$ requires 943.9), Figure S 8.

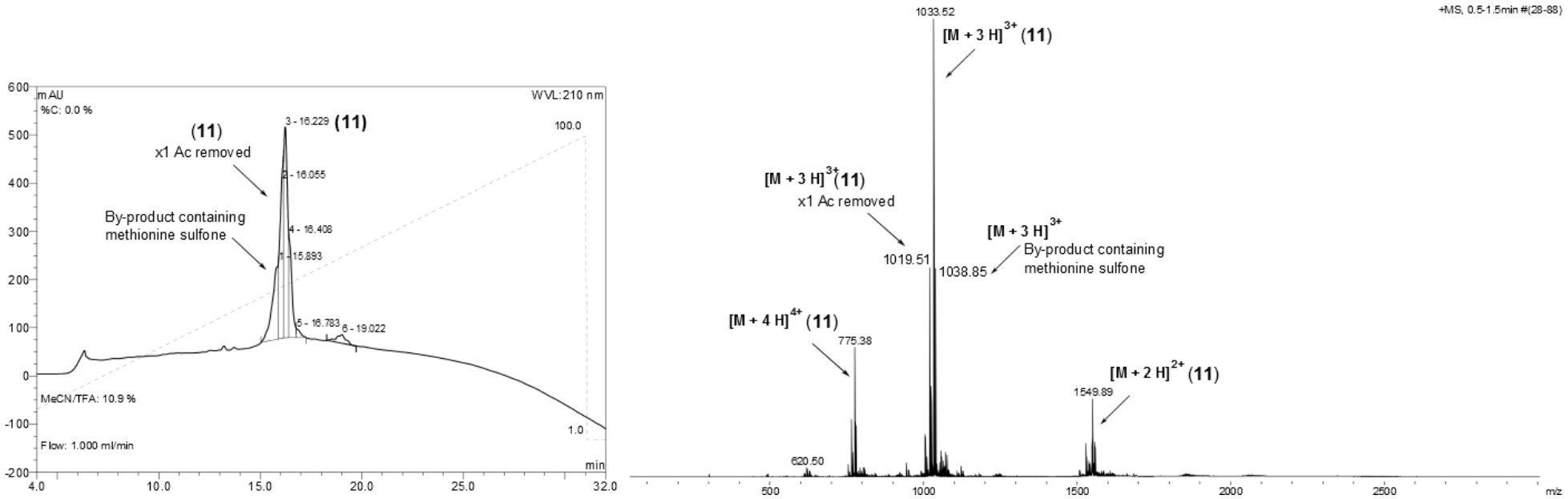


Figure S 6 RP-HPLC (Dionex P680) and ESI-MS (Bruker micrOTOF-Q II) traces of crude 5(6)-CF-Ile-Leu-Ala-Arg-[GlcNAc(OAc)$_3$β1-]Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAc(OAc)$_3$β1-]Asn-Leu-Lys (**11**) (*ca.* 35% as judged by peak area of RP-HPLC at 210 nm); Waters XTerra® column, linear gradient of 1%B to 100%B over 30 min, *ca.* 3%B per minute.

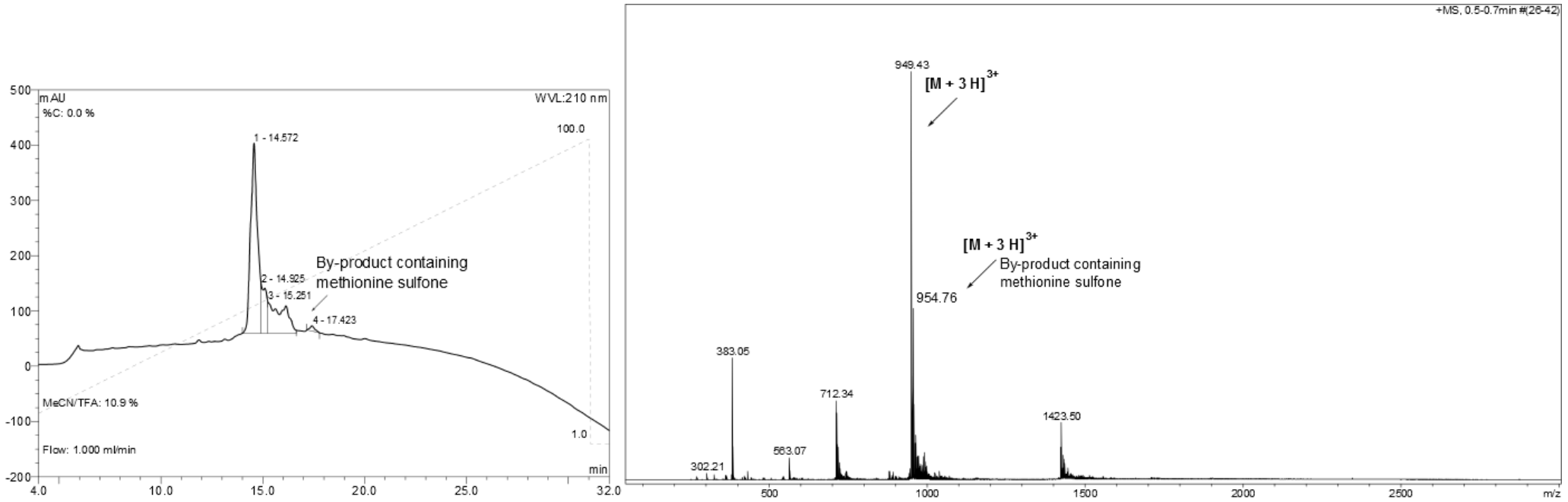


Figure S 7 RP-HPLC (Dionex P680) and ESI-MS (Bruker micrOTOF-Q II) traces of crude 5(6)-CF-Ile-Leu-Ala-Arg-[GlcNAcβ1-]Asn-Leu-Val-Pro-Met(O)-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]Asn-Leu-Lys (ca. 64% as judged by peak area of RP-HPLC at 210 nm); Waters XTerra® column, linear gradient of 1%B to 100%B over 30 min, ca. 3%B per minute.

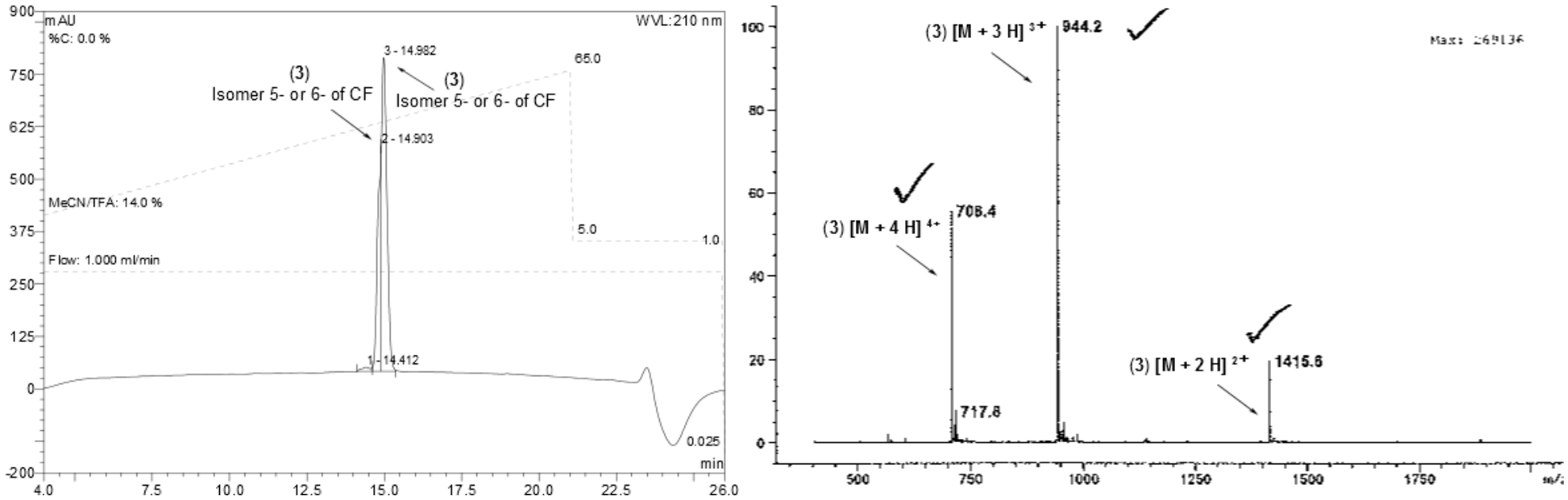


Figure S 8 RP-HPLC (Dionex P680) and ESI-MS (HP 1100) traces of purified 5(6)-CF-Ile-Leu-Ala-Arg-[GlcNAcβ1-]Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val-Gln-Gly-Gln-[GlcNAcβ1-]Asn-Leu-Lys (3) (ca. 98% as judged by peak area of RP-HPLC at 210 nm); Waters XTerra® column, linear gradient of 5%B to 65%B over 20 min, ca. 3%B per minute.

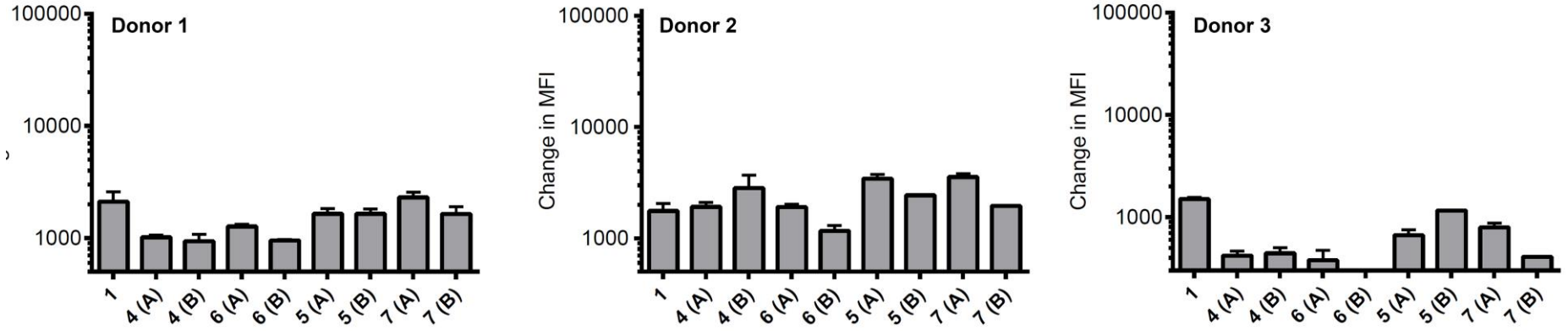


Figure S 9. Analysis of binding of peptide **1** and glycopeptides **4-7** to lymphocytes as MR-low/negative control cells. Results are given as the change in measured fluorescence intensity (MFI, calculated as the MFI of sample minus the MFI of the untreated control); each sample was tested in duplicate. Lymphocytes cultured from three individual donors are shown.

Enzyme catalyzed Glycosylation

[($Man_3GlcNAc_2$)Asn^{17}]CMV 4

A solution of the tetrasaccharide oxazoline **12** (0.622 mg, 0.902 □mol) and [(GlcNAc)Asn^{17}]CMV **2** (0.735 mg, 0.280 □mol) was incubated with 7.1 μg of Endo-A E173H in 45 μL of sodium phosphate buffer (100 mM, pH 6.5) at 23 °C. After 7 h, RP-HPLC (method A) indicated the formation of a new product, which was then purified directly by RP-HPLC to give **[($Man_3GlcNAc_2$)Asn^{17}]CMV 4** (68%, HPLC yield based on acceptor) as a white powder (1.0 mg); analytical HPLC: t_R = 10.2 min(A), 10.8 min(B); ESI-MS: calculated for $C_{146}H_{227}N_{29}O_{56}S$: 3314.5527. Found: 3315.5612(A), 3315.5625(B) $(M+H)^+$.

[($Man_9GlcNAc_2$)Asn^{17}]CMV 5

A solution of the decasaccharide oxazoline **13** (3.80 mg, 2.29 □mol) and [(GlcNAc)Asn^{17}]CMV **2** (1.00 mg, 0.381 □mol) was incubated with 9.9 □U of Endo-M N175Q in 63 μL of sodium phosphate buffer (100 mM, pH 6.5) at 23 °C. After 7 h, RP-HPLC (method A) indicated the formation of a new product, which was then purified directly by RP-HPLC to give **[($Man_9GlcNAc_2$)Asn^{17}]CMV 5**: (48%, HPLC yield based on acceptor) as a white powder (1.6 mg); analytical HPLC: t_R = 9.7 min(A), 10.3 min(B); ESI-MS: calculated for $C_{182}H_{287}N_{29}O_{86}S$: 4286.8697. Found: 4287.8791(A), 4287.8791(B) $(M+H)^+$.

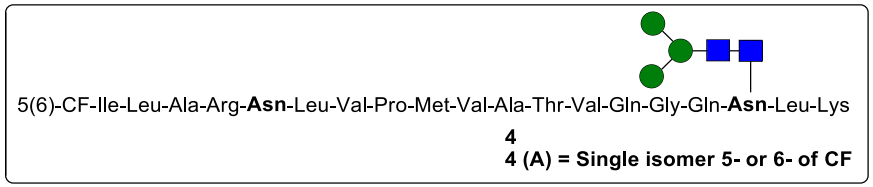
[($Man_3GlcNAc_2$)$Asn^{5,17}$]CMV 6

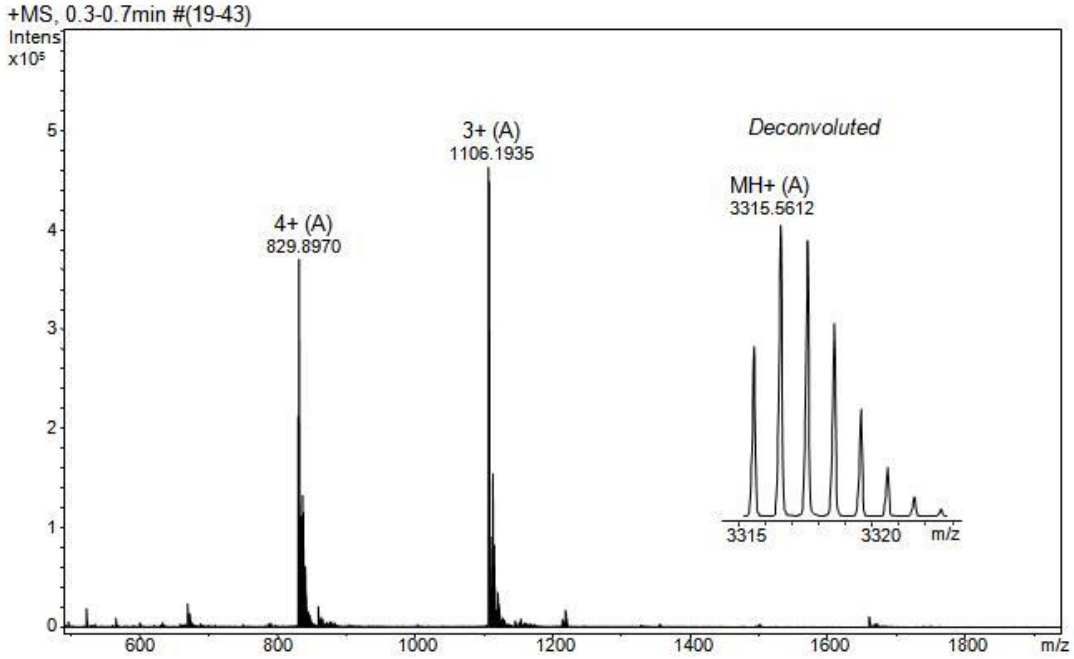
A solution of the tetrasaccharide oxazoline **12** (0.429 mg, 0.622 □mol) and [(GlcNAc)$Asn^{5,17}$]CMV **3** (0.286 mg, 0.101 □mol) was incubated with 4.9 μg of Endo-A E173H in 17 μL of sodium phosphate buffer (100 mM, pH 6.5) at 23 °C. After 4.5 h, RP-HPLC (method B) indicated the formation of a new product, which was then purified directly by RP-HPLC to give [($Man_3GlcNAc_2$)$Asn^{5,17}$]CMV **6** (89%, HPLC yield based on acceptor) as a white powder (1.9 mg); analytical HPLC: t_R = 11.8 min(A), 12.2 min(B); ESI-MS: calculated for $C_{180}H_{283}N_{31}O_{81}S$: 4206.8699. Found: 4207.8785(A), 4207.8796(B) $(M+H)^+$.

[($Man_9GlcNAc_2$)$Asn^{5,17}$]CMV 7

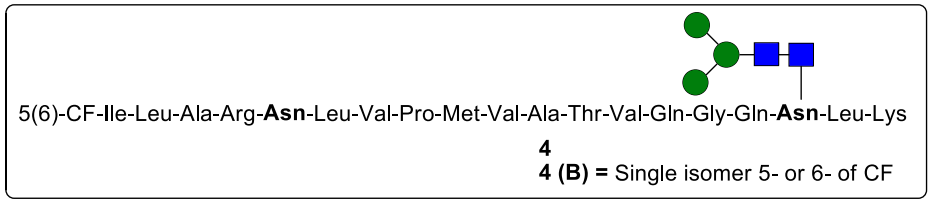
A solution of the decasaccharide oxazoline **13** (1.00 mg, 0.604 □mol) and [(GlcNAc)$Asn^{5,17}$]CMV **3** (0.286 mg, 0.101 □mol) was incubated with 4.9 □U of Endo-M N175Q in 18 μL of sodium phosphate buffer (100 mM, pH 6.5) at 23 °C. After 6 h, RP-HPLC (method B) indicated the formation of a new product, which was then purified directly by RP-HPLC to give **[($Man_9GlcNAc_2$)$Asn^{5,17}$]CMV 7**: (54%, HPLC yield based on acceptor) as a white powder (1.1 mg); analytical HPLC: t_R = 11.0 min(A), 11.3 min(B); ESI-MS: calculated for $C_{252}H_{403}N_{31}O_{141}S$: 6151.5038. Found: 6152.5172(A), 6152.5157(B) $(M+H)^+$.

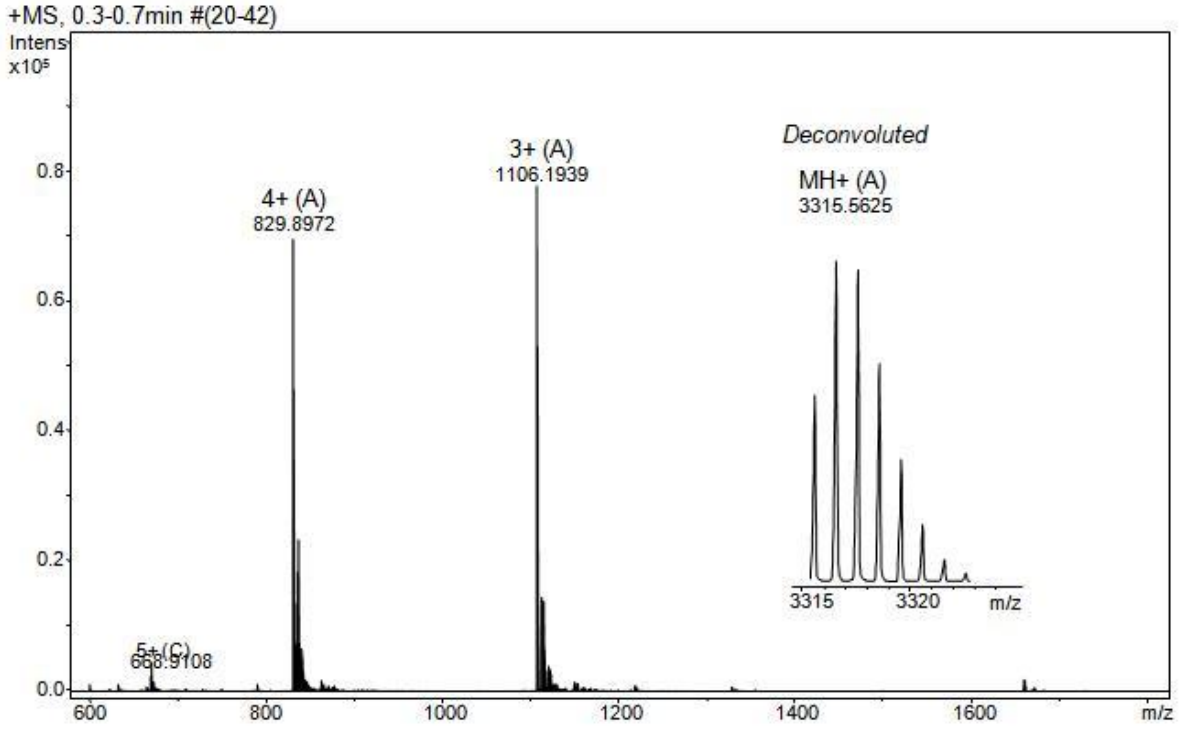
[($Man_3GlcNAc_2$)Asn^{17}]CMV 4(A)



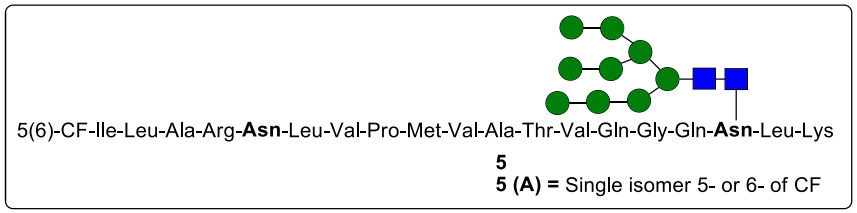


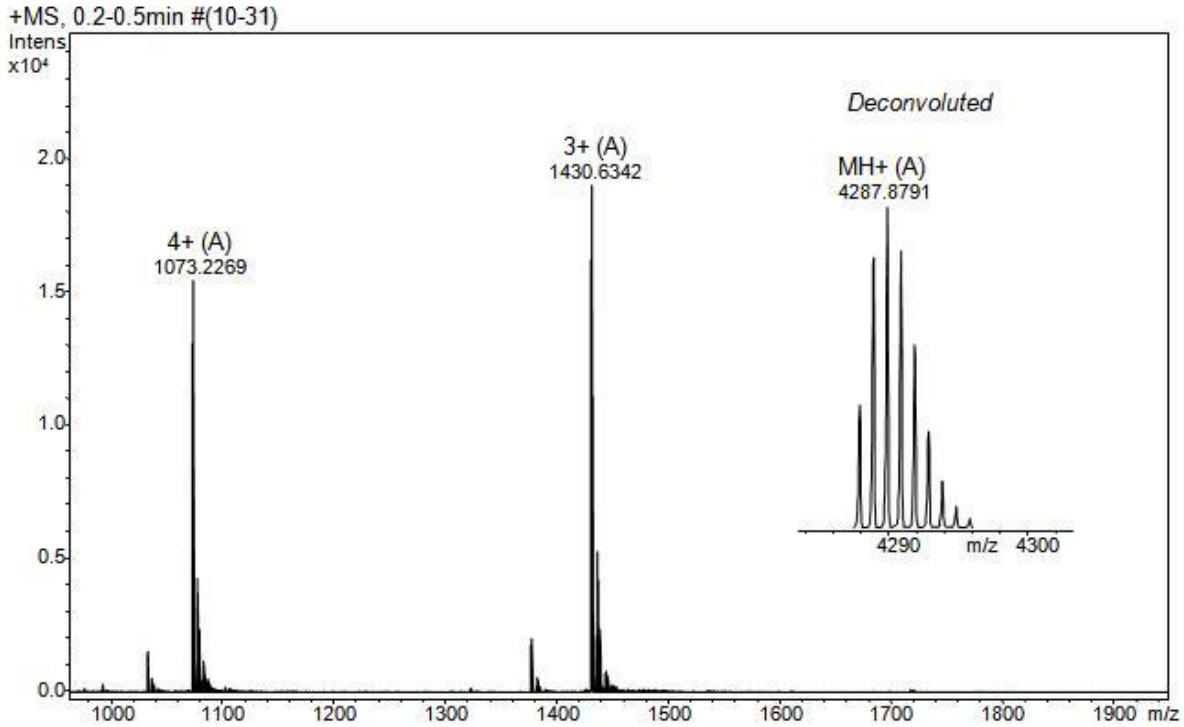
[($Man_3GlcNAc_2$)Asn^{17}]CMV 4(B)



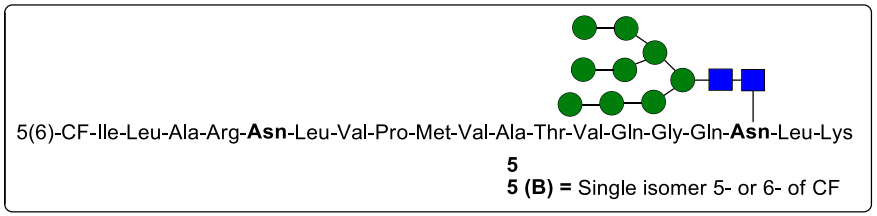


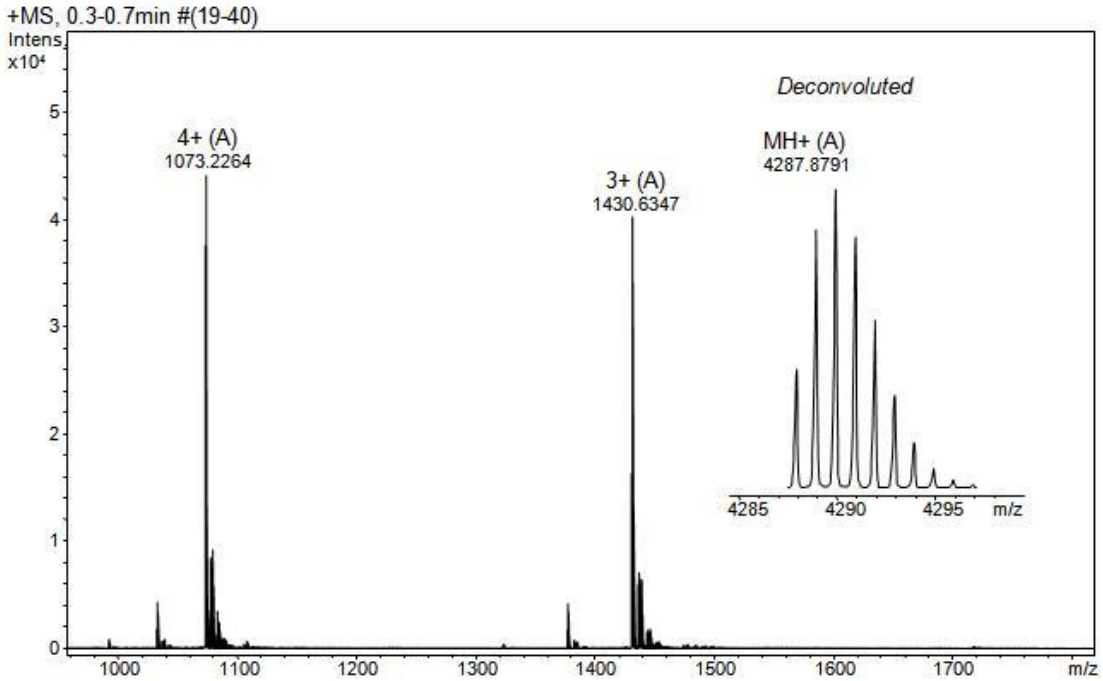
[($Man_9GlcNAc_2$)Asn^{17}]CMV 5(A)



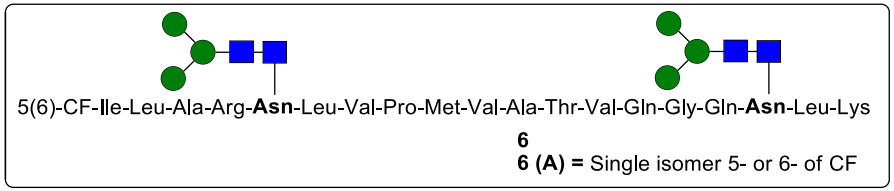


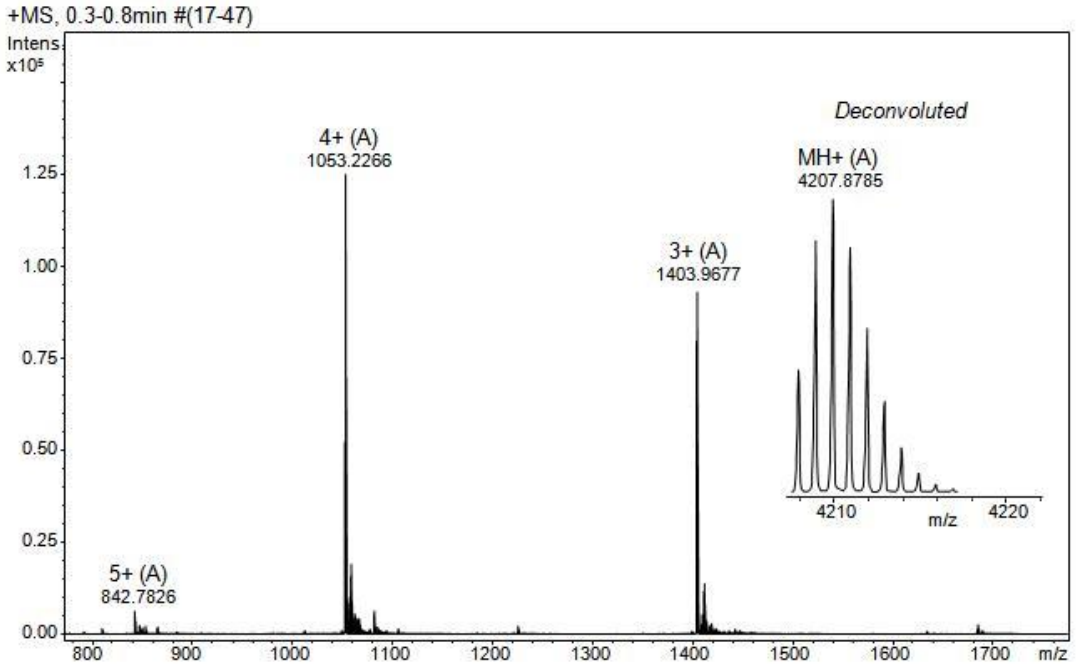
[($Man_9GlcNAc_2$)Asn^{17}]CMV 5(B)



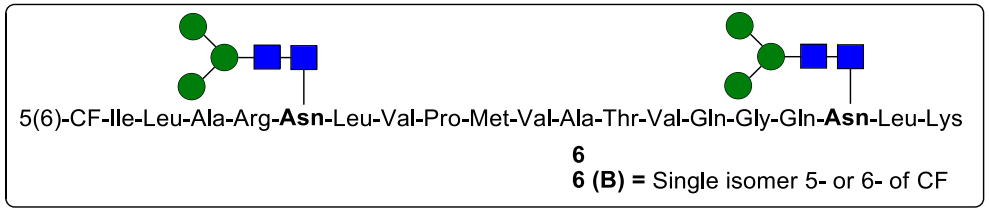


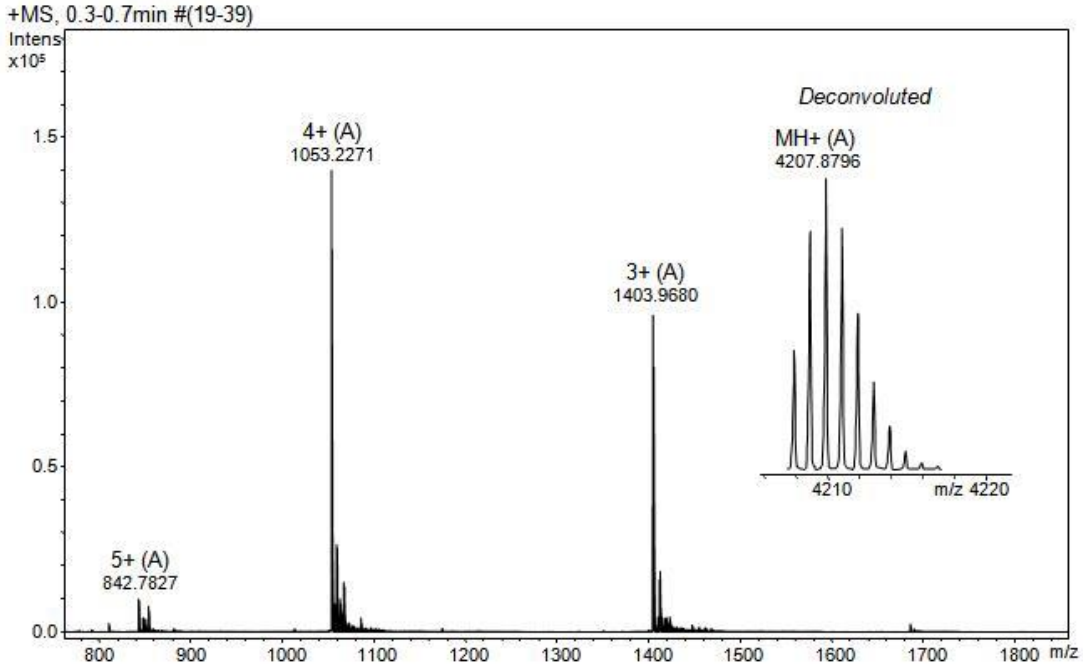
[($Man_3GlcNAc_2$)$Asn^{5,17}$]CMV 6(A)



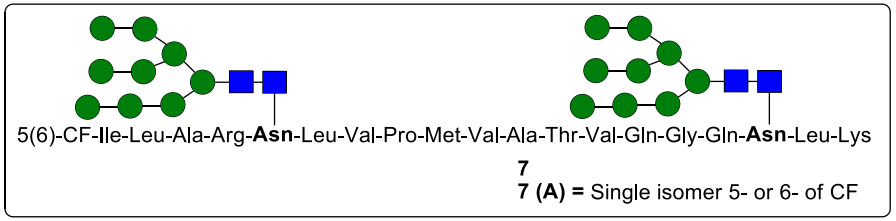


[($Man_3GlcNAc_2$)$Asn^{5,17}$]CMV 6(B)

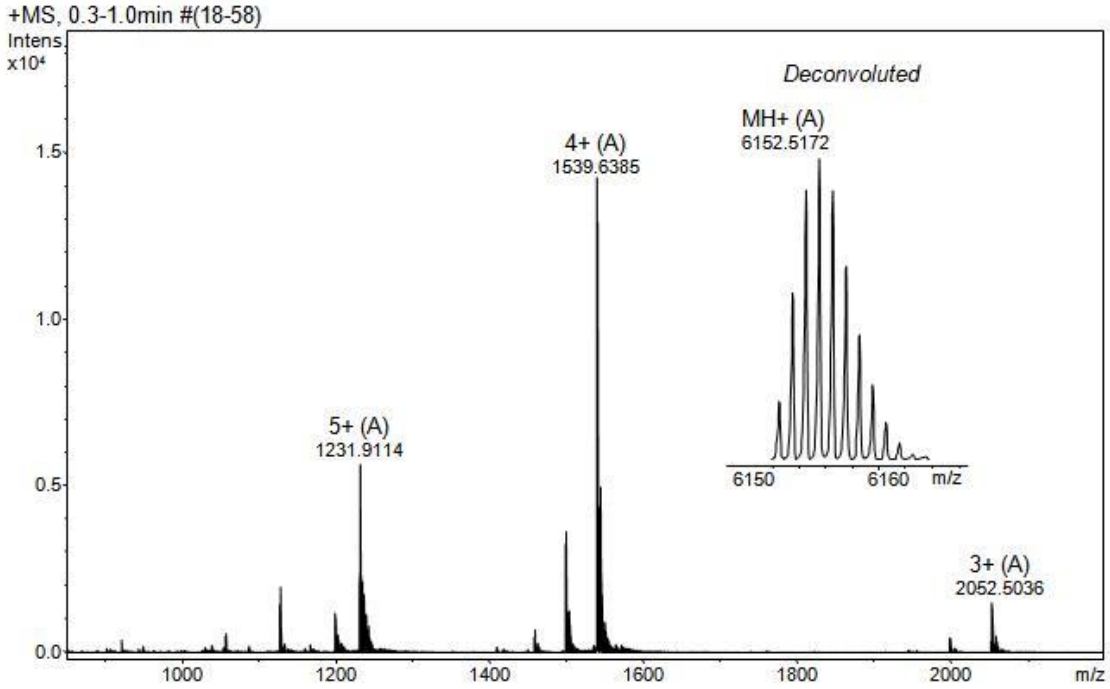




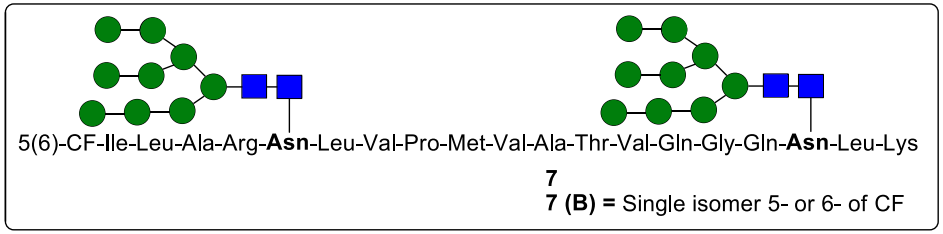
[($Man_9GlcNAc_2$)$Asn^{5,17}$]CMV 7(A)



7
7 (A) = Single isomer 5- or 6- of CF

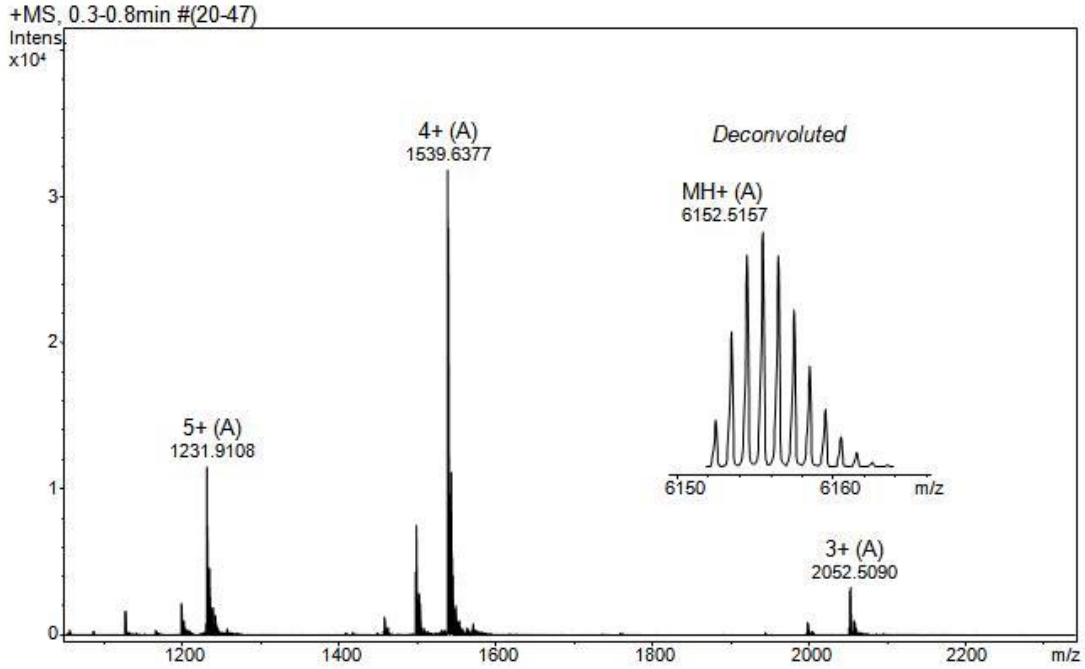


[(Man$_9$GlcNAc$_2$)Asn^{5,17}]CMV 7(B)



7

7 (B) = Single isomer 5- or 6- of CF



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