Supplementary Information

Synthetic glycopeptides reveal the glycan specificity of HIV-neutralizing antibodies

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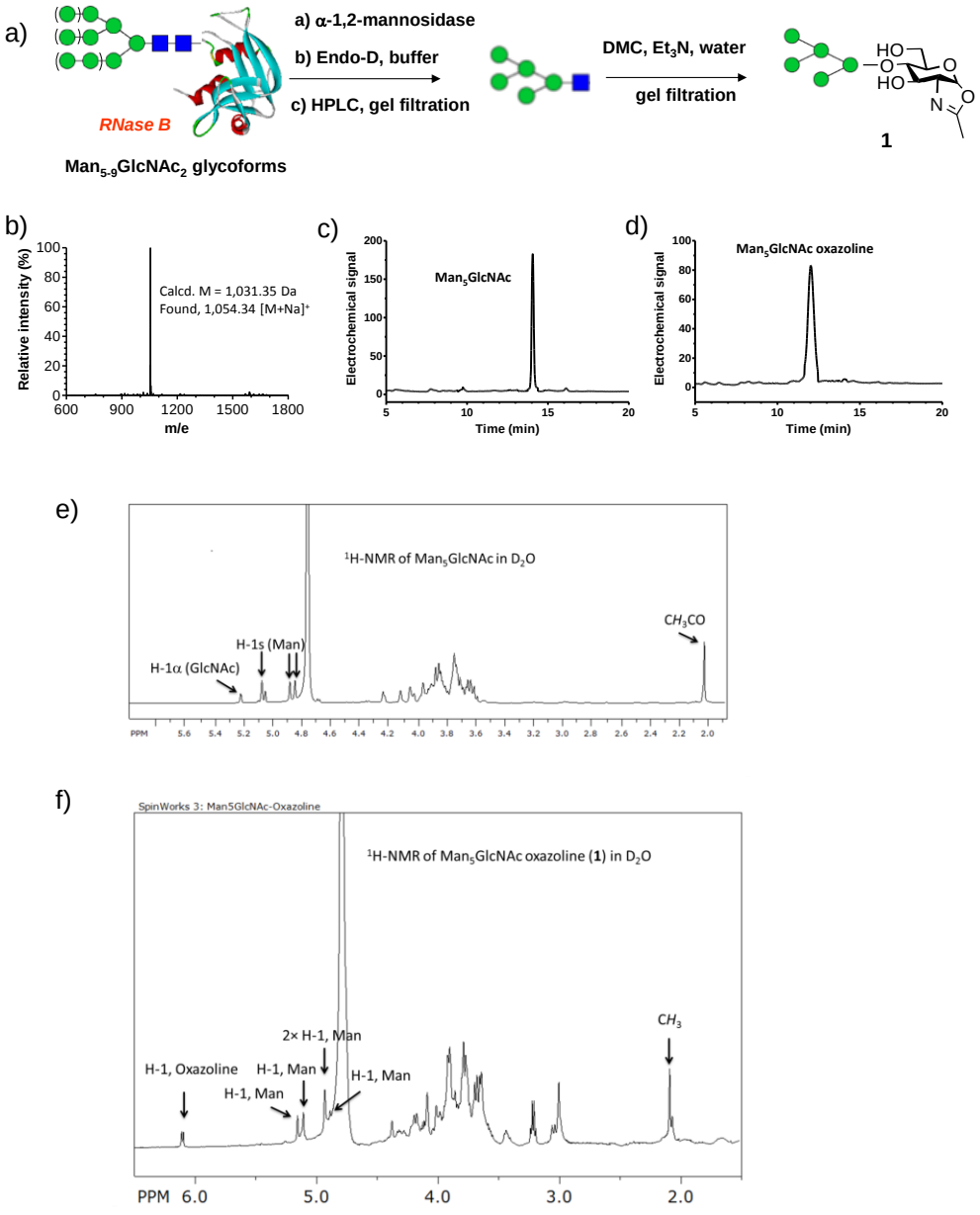
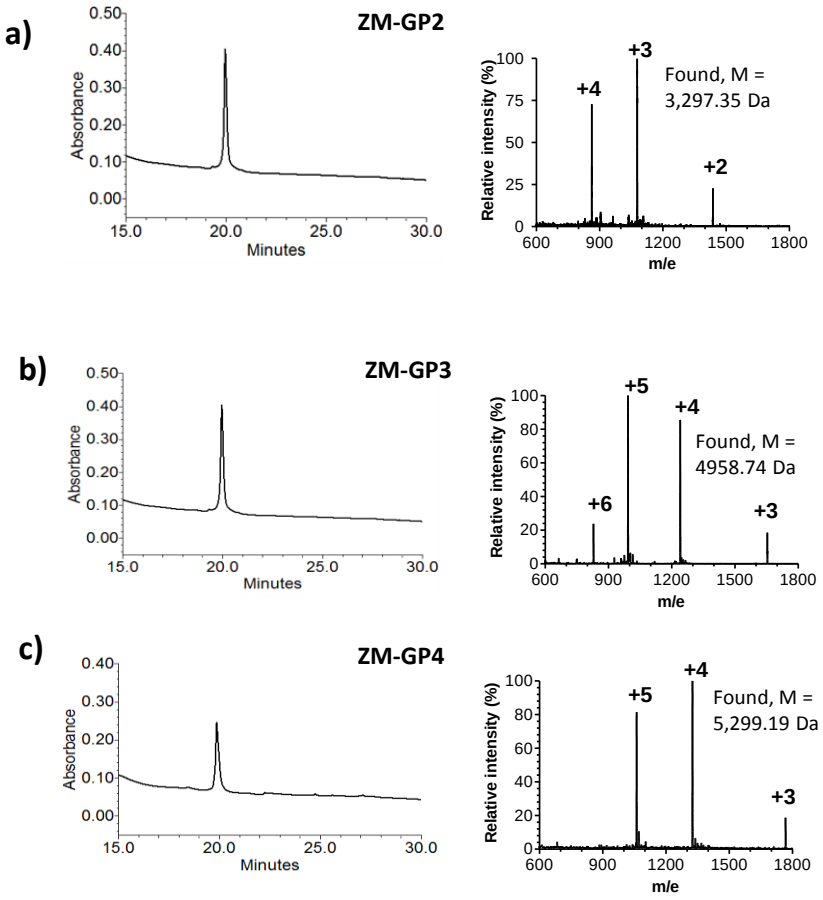
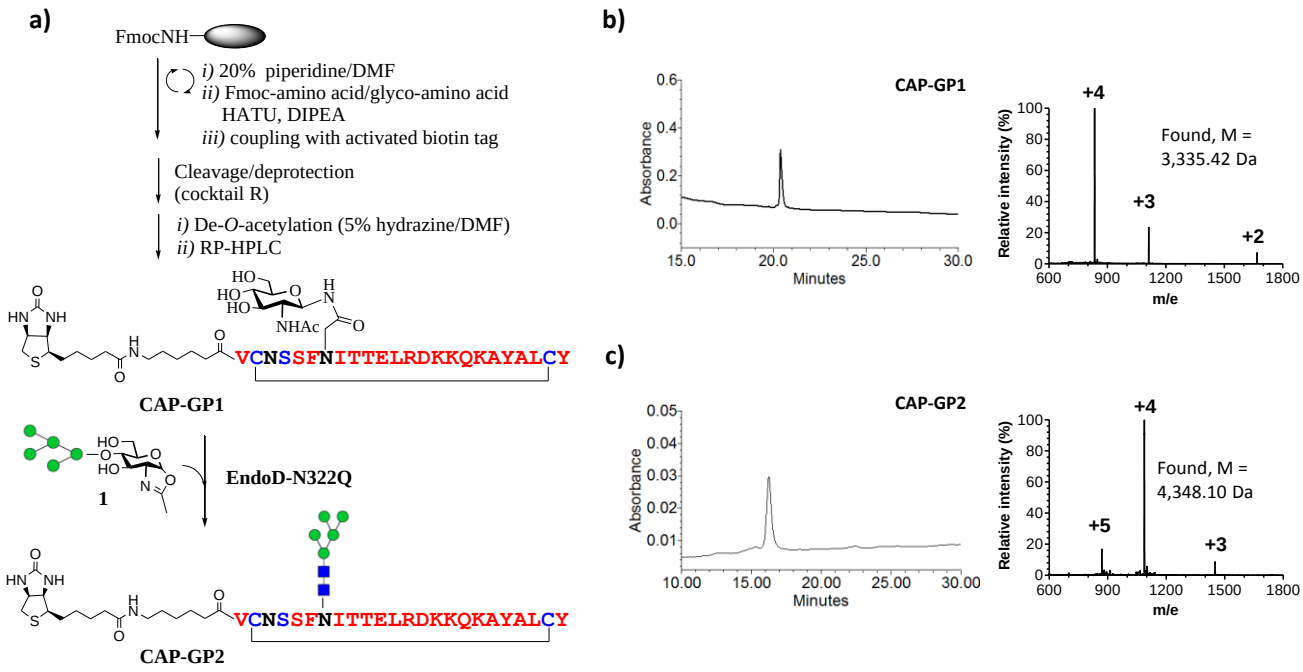
Supplementary Results



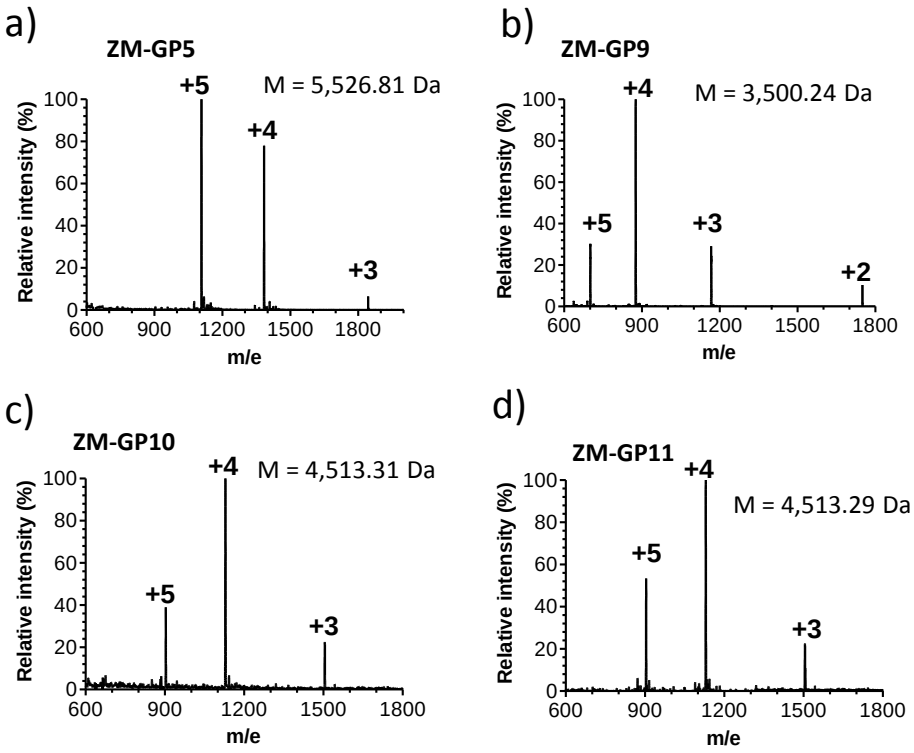
Supplementary Figure 1. Synthesis and characterization of $Man_5GlcNAc$ oxazoline (1). a) Synthesis of Man5GlcNAc oxazoline (**1**); b) MALDI-TOF MS of $Man_5GlcNAc$; c) HPAEC (Dionex) of $Man_5GlcNAc$; d) HPAEC (Dionex) of $Man_5GlcNAc$ oxazoline; e) ^{1}H-NMR of $Man_5GlcNAc$ in D_2O; f) ^{1}H-NMR of $Man_5GlcNAc$ oxazoline (**1**) in D_2O.



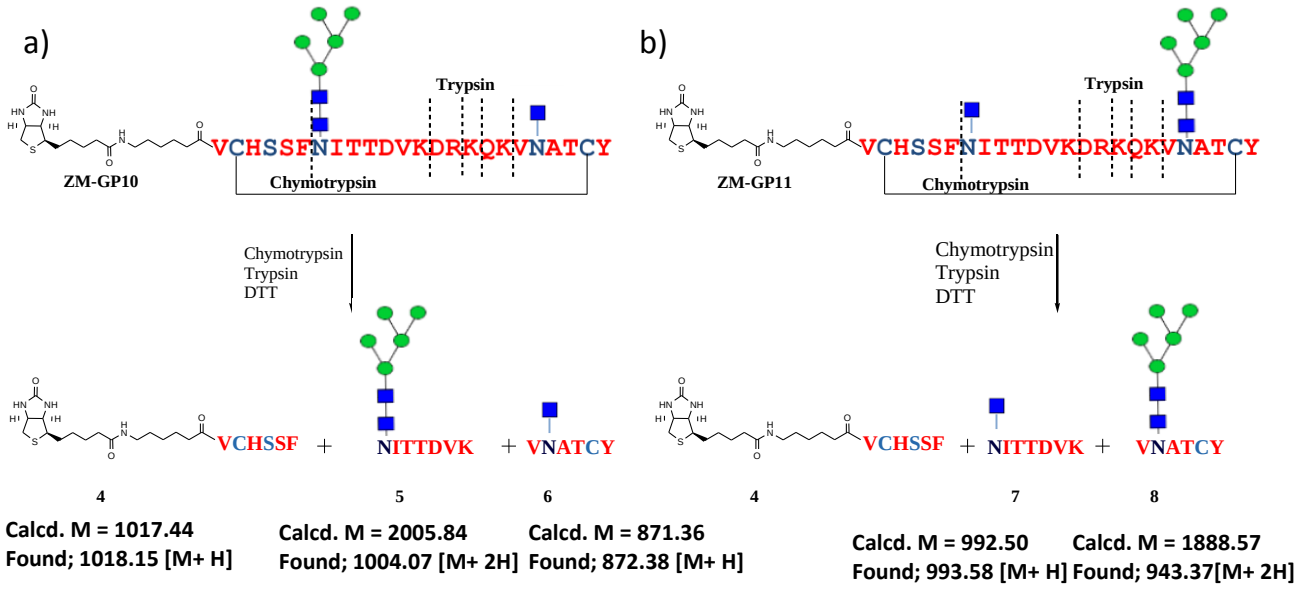
Supplementary Figure 2. HPLC and ESI-MS profile of the synthetic ZM109 V1V2 glycopeptides carrying an N-glycan at N160 position. The HPLC was performed on a C18 column with a linear gradient of 0-90% MeCN in 30 min. a) ZM-GP2; b) ZM-GP3; and c) ZM-GP4.



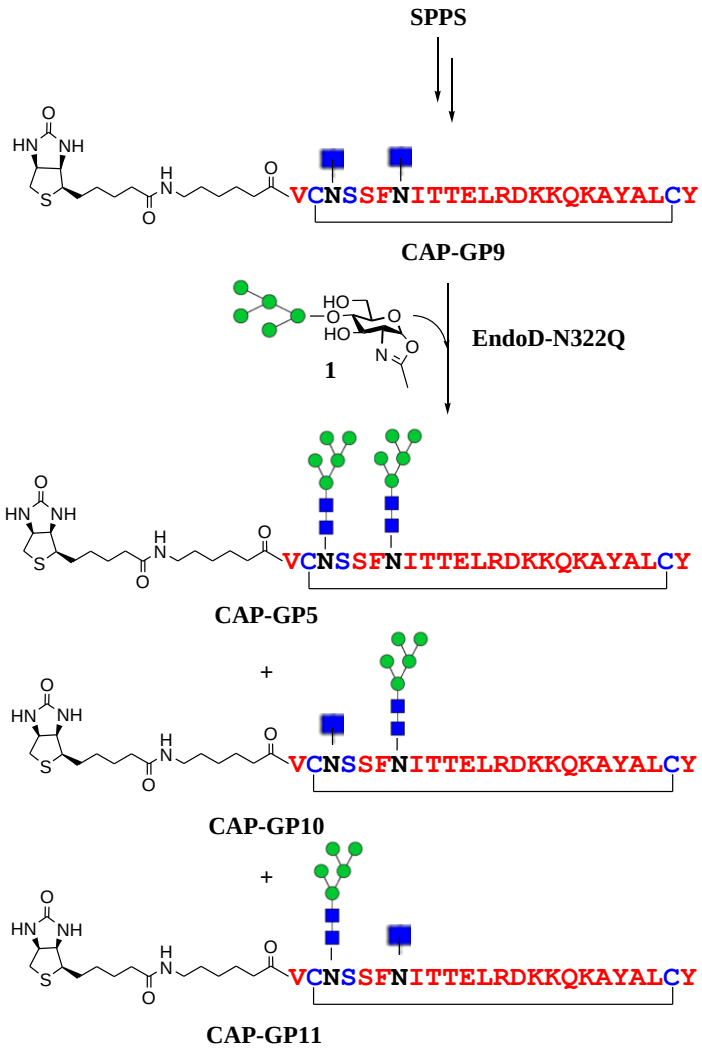
Supplementary Figure 3. Synthesis and characterization of N160-glycosylated CAP-GP2. a) Synthesis scheme; b) HPLC and ESI-MS profiles of synthetic CAP-GP1; c) HPLC and ESI-MS profiles of synthetic CAP-GP2 (The HPLC was performed on a C18 column with a linear gradient of 0-90% MeCN in 30 min.).



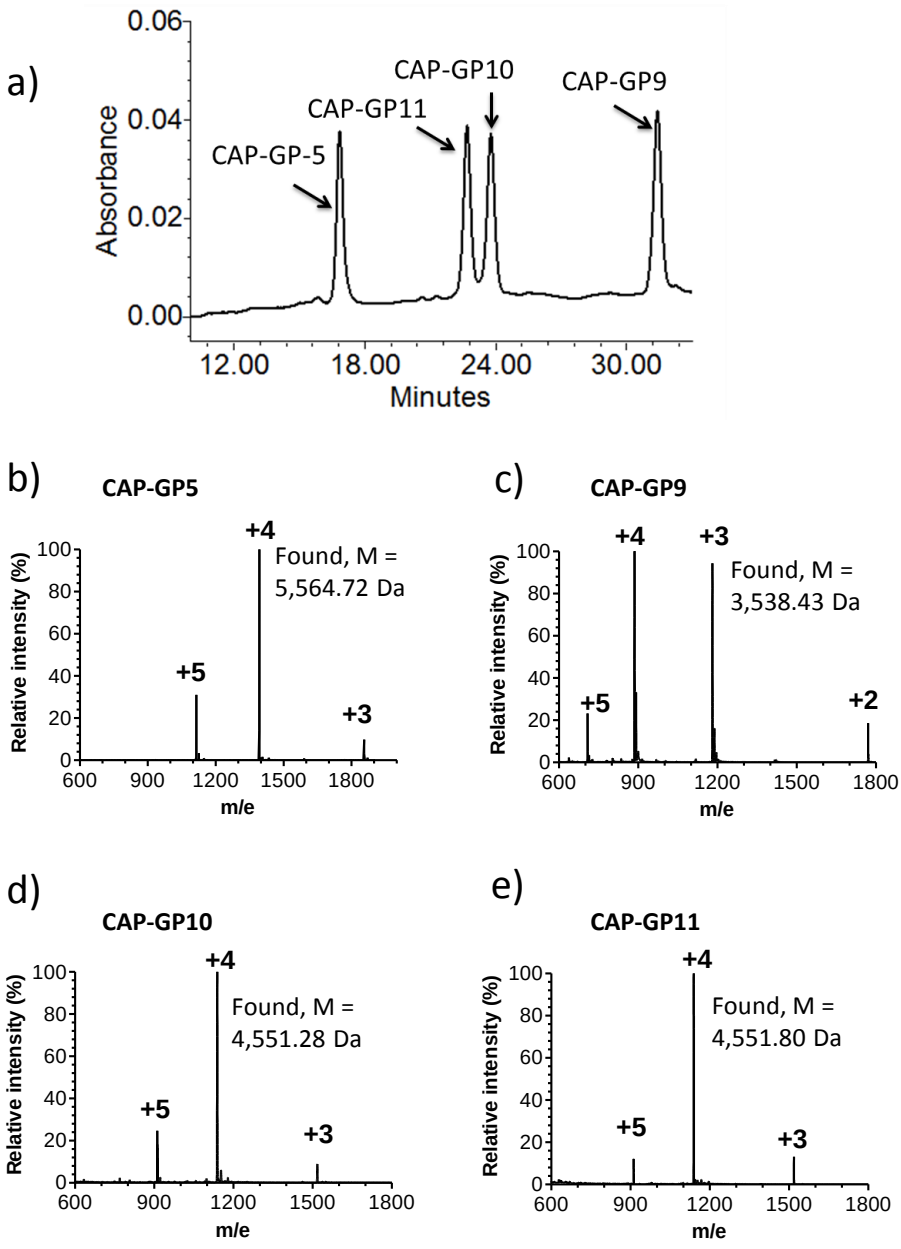
Supplementary Figure 4. The ESI-MS profiles of selectively glycosylated ZM109 V1V2 glycopeptides. a) ZM-GP5; b) ZM-GP9; c) ZM-GP10; and d) ZM-GP11.



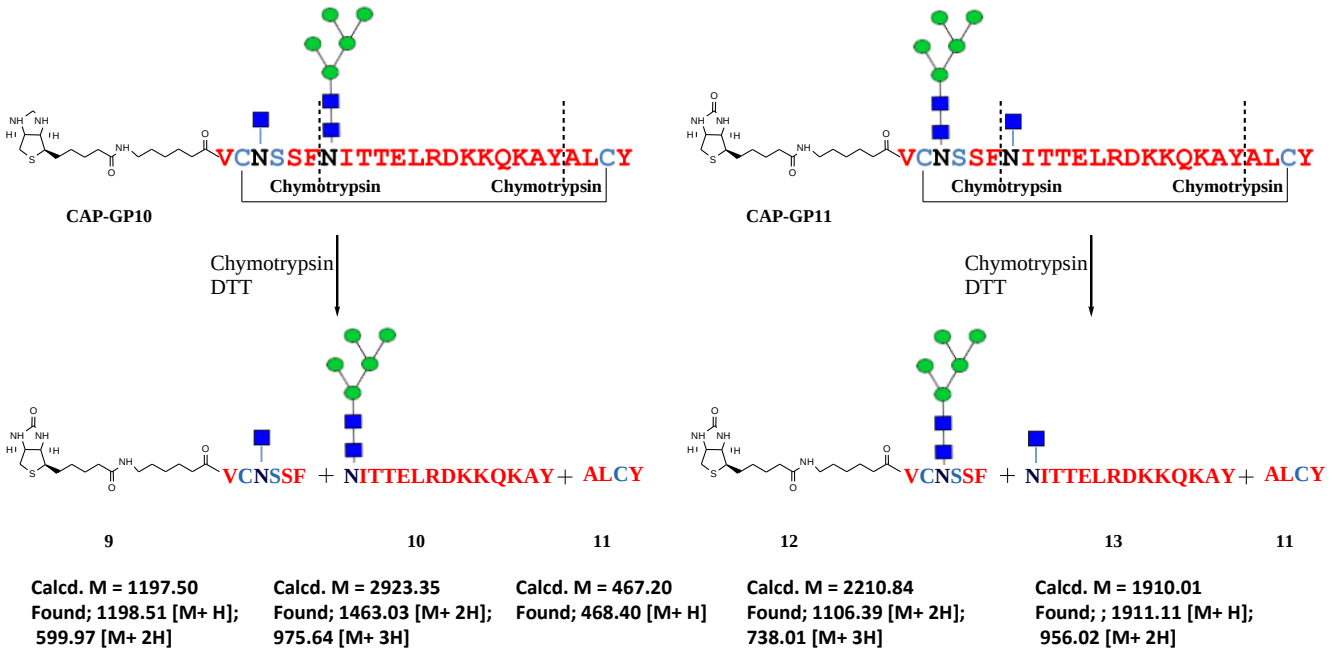
Supplementary Figure 5. Characterization of site-selective glycosylation products of ZM-GP10 and ZM-GP11. Treatment of ZM-GP10 with a mixture of chymotrypsin (specific for cleaving phenylalanine amide bond) and trypsin (specific for cleaving lysine and arginine amide linkage), followed by dithiothreitol (DTT) to cleave the disulfide bond, gave three major peptide/glycopeptide fragments: the biotinylated N-terminal fragment (**4**) (calcd M = 1016.44 Da; found (m/z), 1017.66 $[M + H]^+$), the internal fragment (**5**) carrying a $Man_5GlcNAc_2$ glycan (calcd M = 2005.84 Da; found (m/z), 1004.07 $[M+ 2H]^{2+}$), and the C-terminal fragment carrying only a GlcNAc moiety (calcd M = 871.28 Da; found (m/z), 872.38 $[M + H]^+$). These data suggest that the transferred N-glycan was attached at the N160 position in ZM-GP10. Similarly, treatment of the other isomer (ZM-GP11) with chymotrypsin/trypsin and then DTT gave fragments **4**, **7**, and **8**. LC-MS analysis of **7** (calcd M = 992.50 Da; found (m/z); 993.58 $[M + H]^+$) and **8** (calcd M = 1888.57 Da; found (m/z), 943.37 $[M + 2H]^{2+}$) indicated that fragment **7** carried only a GlcNAc moiety and fragment **8** carried a $Man_5GlcNAc_2$ glycan. These results confirm that in ZM-GP11, the $Man_5GlcNAc_2$ is attached at the N173 site while a GlcNAc is attached at the N160 site.



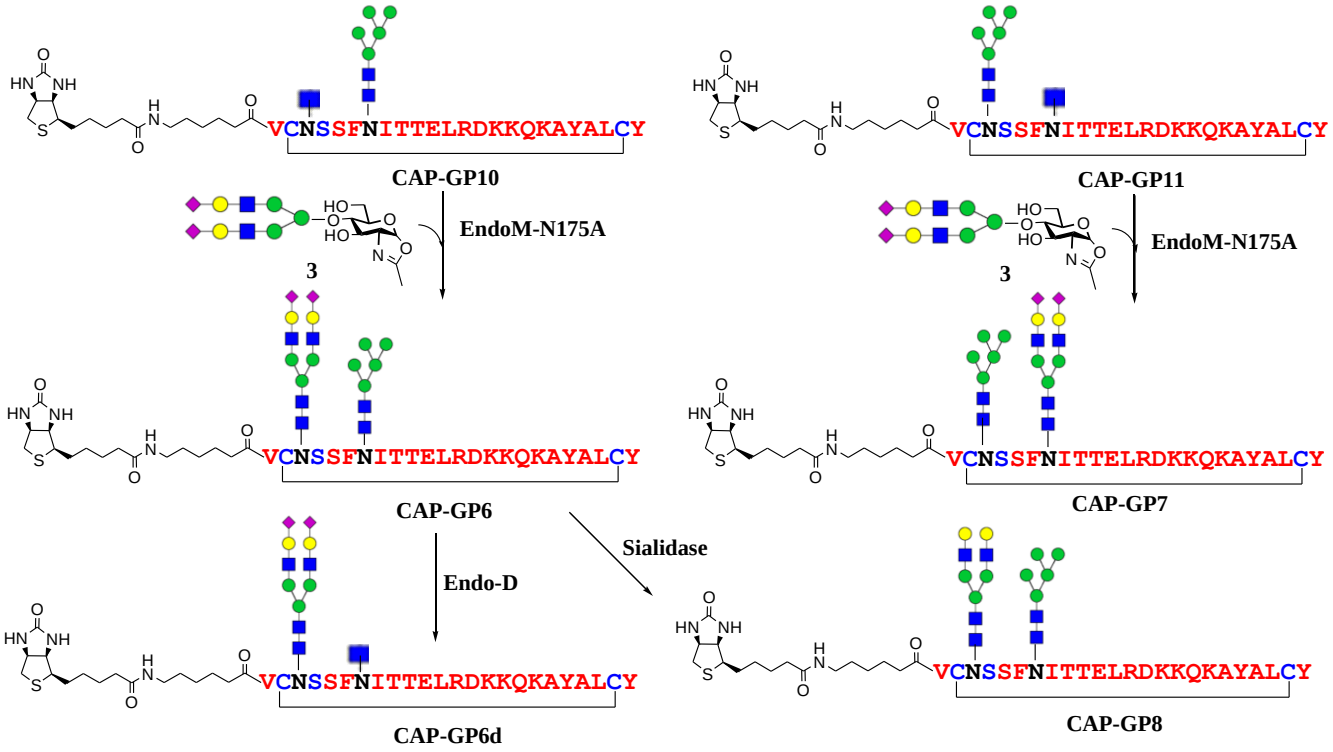
Supplementary Figure 6. Controlled glycosylation of the CAP glycopeptides



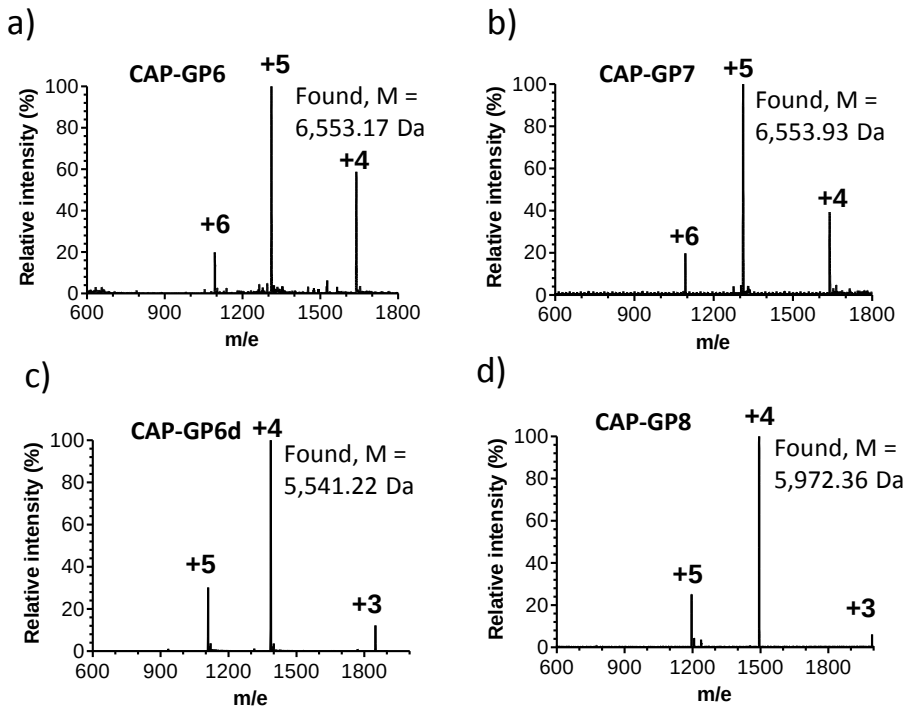
Supplementary Figure 7. ESI-MS profile of selectively glycosylated CAP45 V1V2 glycopeptides. a) CAP-GP5; b) CAP-GP9; c) CAP-GP10; and d) CAP-GP11.



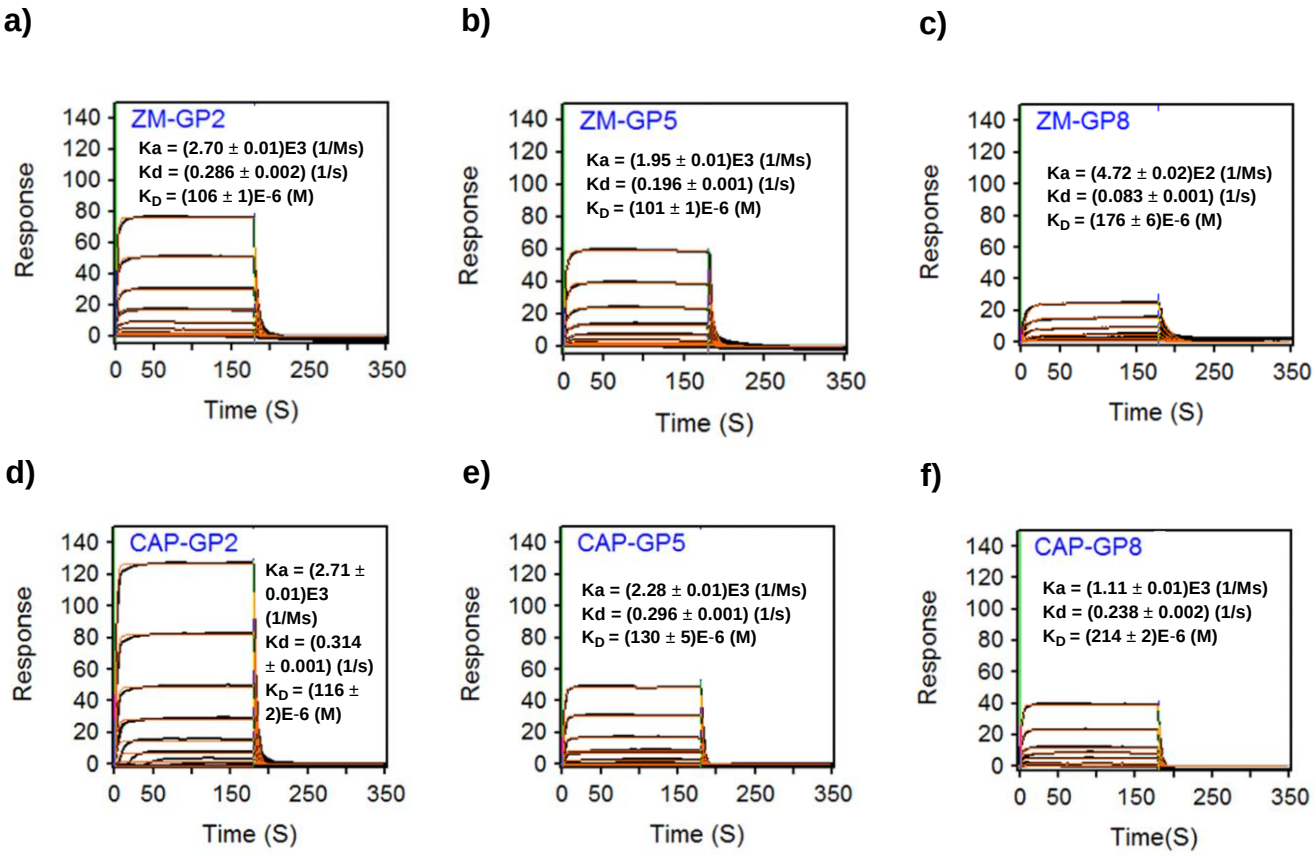
Supplementary Figure 8. Characterization of glycosylation sites of CAP-GP10 and CAP-GP11. The assignment was performed in the similar way as demonstrated for ZM-GP10 and ZM-GP11. Briefly, treatment of CAP-GP10 or CAP-GP11 with Chymotrypsin and DTT, followed by LC-MS analysis of the digestion fragments (**9**, **10**, **11**, **12**, and **13**) confirmed that the $Man_5GlcNAc_2$ glycan was attached at the N160 position in CAP-GP10 while this glycan was attached at N173 in CAP-GP11.



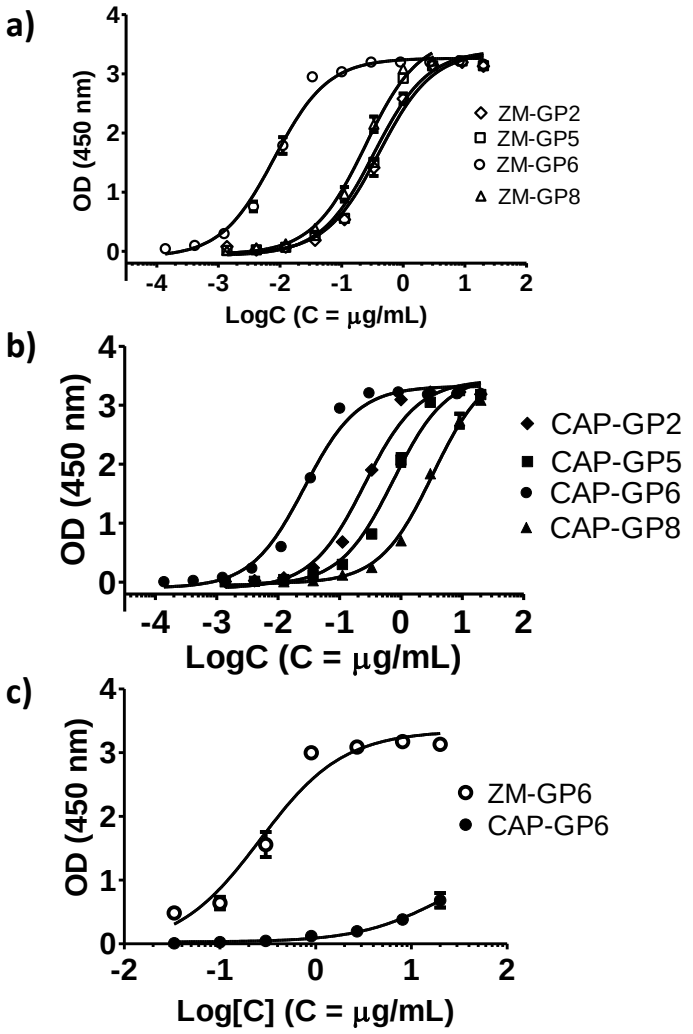
Supplementary Figure 9. Chemoenzymatic synthesis of distinctly double-glycosylated CAP45 glycopeptides



Supplementary Figure 10. ESI-MS profile of the double-glycosylated CAP45 V1V2 glycopeptides. a) CAP-GP6; b) CAP-GP7; c) CAP-GP6d; and d) CAP-GP8.



Supplementary Figure 11. Antibody PG9 Fab binding to synthetic ZM- and CAP-glycopeptides. Biotinylated glycopeptides were immobilized on streptavidin chips and antibody PG9 FAB flowed through as analytes. The surface-plasmon resonance sensorgrams were recorded with 2-fold serial dilutions starting at the highest concentration of 100 μM. The respective fitted curves were shown in orange and the kinetic data were shown as inserts. a) ZM-GP2; b) ZM-GP5; c) ZM-GP8; d) CAP-GP2; e) CAP-GP5; f) CAP-GP8. The binding of ZM-GP6 and CAP-GP6 with PG9 Fab was shown in Fig. 5 in the text. The following ZM- and CAP-glycopeptides did not show apparent binding responses at up to 100 μM: ZM-GP1, ZM-GP3, ZM-GP4, ZM-GP7, ZM-GP9; CAP-GP1; CAP-GP7, CAP-GP9; and those non-glycosylated peptides.



Supplementary Figure 12. ELISA binding of PG9 and PG16 to synthetic V1V2 glycopeptides. a) PG9 with ZM109 glycopeptides; b) PG9 with CAP45 glycopeptides; and c) PG16 with ZM109 and CAP45 glycopeptides.

Supplementary Table 1. K_D values (μM) of PG9 and PG16 Fab binding with the synthetic ZM109/CAP45 glycopeptides and the scaffolded V1V2 domains

Fab	ZM-GP1	ZM-GP2	ZM-GP2a	ZM-GP3	ZM-GP4	ZM-GP5	ZM-GP6	ZM-GP7	ZM-GP8	IJO8 ZM109 V1V2	IFD6 ZM109 V1V2	ZM109 gp120
PG9	--	106 ± 8	319 ± 9	--	--	101 ±1	5.1 ± 0.5	--	176 ± 4	0.32*	5.6*	0.09*
PG16	--	--	nd	--	--	--	18 ± 0.3	--	--	nd	nd	nd

Fab	CAP-GP1	CAP-GP2	CAP-GP2a	CAP-GP3	CAP-GP4	CAP-GP5	CAP-GP6	CAP-GP7	CAP-GP8	IJO8 CAP45 V1V2	IFD6 CAP45 V1V2	CAP45 gp120
PG9	--	116 ± 2	nd	--	--	130 ±5	6.9 ± 0.4	--	214 ± 2	nd	nd	nd
PG16	--	--	nd	--	--	--	130 ± 2	--	--	nd	nd	nd

"--", no binding at 100 μM; "nd", not determined; *adapted from ref. 13.

Supplementary Notes

Characterization data for synthetic glycopeptides:

Nonglycosylated cyclic ZM109 peptide. Yield, 36% (based on solid-phase peptide synthesis, SPPS, on 0.2 mmol scale); analytical RP-HPLC, t_R = 24.2 min (gradient, 15-30% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 3094.34; found 619.73 $[M + 5H]^{5+}$, 774.40 $[M + 4H]^{4+}$, 1032.45 $[M + 3H]^{3+}$, 1548.17 $[M + 2H]^{2+}$. Deconvolution mass, 3094.25 ± 0.29.

Glycopeptide ZM-GP1. Yield, 38% (based on solid-phase peptide synthesis, SPPS, on 0.2 mmol scale); analytical RP-HPLC, t_R = 14.2 min (gradient, 20-30% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 3297.35; found 660.53 $[M + 5H]^{5+,}$ 825.40 $[M + 4H]^{4+}$, 1099.68 $[M + 3H]^{3+}$. Deconvolution mass, 3297.14 ± 0.32.

Glycopeptide ZM-GP5. Yield, 87%; analytical RP-HPLC, t_R = 25.1 min (gradient, 15-30% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 5526.93; found 1106.45 $[M + 5H]^{5+}$, 1382.73 $[M + 4H]^{4+}$, 1843.14 $[M + 3H]^{3+}$. Deconvolution mass, 5526.81 ± 0.15.

Glycopeptide ZM-GP6. Yield, 95%; analytical RP-HPLC, t_R = 17.4 min (gradient, 20-22% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 6517.30; found 1304.88 $[M + 5H]^{5+}$, 1630.59 $[M + 4H]^{4+}$. Deconvolution mass, 6516.74 ± 1.23.

Glycopeptide ZM-GP6d. Obtained from ZM-GP6 by treatment with Endo-D.; Yield, 86%; analytical RP-HPLC, t_R = 22.9 min (gradient, 20-22% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 5503.96; found 1101.80 $[M + 5H]^{5+}$, 1377.14 $[M + 4H]^{4+}$. Deconvolution mass, 5504.0 ± 0.56.

Glycopeptide ZM-GP7. Yield, 89%; analytical RP-HPLC, t_R = 17.3 min (gradient, 20-22% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 6517.30; found 1087.10 $[M + 6H]^{6+}$, 1304.18 $[M + 5H]^{5+}$, 1629.90 $[M + 4H]^{4+}$. Deconvolution mass, 6516.60 ± 0.89.

Glycopeptide ZM-GP8. From sialidase treatment of ZM-GP6.; Yield, 93%; analytical RP-HPLC, t_R = 24.6 min (gradient, 20-22% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 5933.01; found 1187.92 $[M + 5H]^{5+}$, 1484.56 $[M + 4H]^{4+}$. Deconvolution mass, 5933.59 ± 0.56.

Glycopeptide ZM-GP9. From solid phase peptide synthesis (SPPS); Yield, 37%; analytical RP-HPLC, t_R = 28.8 min (gradient, 15-30% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 3500.36; found 701.45 $[M + 5H]^{5+}$, 876.55 $[M + 4H]^{4+}$, 1168.42 $[M + 3H]^{3+}$. Deconvolution mass, 3500.24 ± 0.21.

Glycopeptide ZM-GP10. From control transglycosylation reaction; Yield, 24%; analytical RP-HPLC, t_R = 23.5 min (gradient, 20-22% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 4513.70; found 903.78 $[M + 5H]^{5+}$, 1129.33 $[M + 4H]^{4+}$, 1505.58 $[M + 3H]^{3+}$. Deconvolution mass, 4513.31 ± 0.43.

Glycopeptide ZM-GP11. From control transglycosylation reaction; Yield, 24%; analytical RP-HPLC, t_R = 25.5 min (gradient, 20-22% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 4513.70; found 903.79 $[M + 5H]^{5+}$, 1129.29 $[M + 4H]^{4+}$, 1505.59 $[M + 3H]^{3+}$. Deconvolution mass, 4513.95 ± 0.43.

Nonglycosylated cyclic CAP45 peptide. Yield, 34% (based on solid-phase peptide synthesis, SPPS); analytical RP-HPLC, t_R = 18.8 min (gradient, 25-40% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 3132.41; found 784.02 $[M + 4H]^{4+}$, 1044.98 $[M + 3H]^{3+}$, 1567.37 $[M + 2H]^{2+}$. Deconvolution mass, 3132.41 ± 0.07

Glycopeptide CAP-GP1. Yield, 39% (based on solid-phase peptide synthesis, SPPS); analytical RP-HPLC, t_R = 21.3 min (gradient, 0-90% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 3335.42; found 834.79 $[M + 4H]^{4+}$, 1112.36 $[M + 3H]^{3+}$, 1168.51 $[M + 2H]^{2+}$. Deconvolution mass, 3335.12 ± 0.24.

Glycopeptide CAP-GP2. Yield, 82%; analytical RP-HPLC, t_R = 16.7 min (gradient, 0-90% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 4348.76; found 870.69 $[M + 5H]^{5+}$, 1088.00 $[M + 4H]^{4+}$, 1450.30 $[M + 3H]^{3+}$. Deconvolution mass, 4348.10 ± 0.64.

Glycopeptide CAP-GP5. Yield, 83%; analytical RP-HPLC, t_R = 16.2 min (gradient, 23-25% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 5565.12; found 1113.98 $[M + 5H]^{5+}$, 1392.08 $[M + 4H]^{4+}$, 1855.67 $[M + 3H]^{3+}$. Deconvolution mass, 5564.72 ± 0.66.

Glycopeptide CAP-GP6. Yield, 83%; analytical RP-HPLC, t_R = 16.9 min (gradient, 23-25% aq. MeCN containing 0.1% TFA for 35 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 6553.46; found 1093.33 $[M + 6H]^{6+}$, 1311.62 $[M + 5H]^{5+}$, 1639.14 $[M + 4H]^{4+}$. Deconvolution mass, 6553.17 ± 0.56.

Glycopeptide CAP-GP6d. Obtained from CAP-GP6 by treatment with Endo-D.; Yield, 87%; analytical RP-HPLC, t_R = 21.0 min (gradient, 23-25% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 5541.22; found 1109.86 $[M + 5H]^{5+}$, 1386.75 $[M + 4H]^{4+}$. Deconvolution mass, 5543.0 ± 1.30.

Glycopeptide CAP-GP7. Yield, 87%; analytical RP-HPLC, t_R = 20.4 min (gradient, 0-90% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 6553.46; found 1311.83 $[M + 5H]^{5+}$, 1639.35 $[M + 4H]^{4+}$, 2185.43 $[M + 3H]^{3+}$, Deconvolution mass, 6554.93 ± 1.33.

Glycopeptide CAP-GP8. From sialidase treatment of CAP-GP6.; Yield, 83%; analytical RP-HPLC, t_R = 24.8 min (gradient, 20-30% aq. MeCN containing 0.1% TFA for 30 min; flow rate, 0.5 mL/min); ESI-

MS: calcd M = 5972.60; found 1195.51 $[M + 5H]^{5+}$, 1494.06 $[M + 4H]^{4+}$, 1992.65 $[M + 3H]^{3+}$. Deconvolution mass, 5972.36 ± 0.14.

Glycopeptide CAP-GP9. From solid phase peptide synthesis (SPPS); Yield, 36%; analytical RP-HPLC, t_R = 31.5 min (gradient, 23-25% aq. MeCN containing 0.1% TFA for 35 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 3538.43; found 708.61 $[M + 5H]^{5+}$, 885.59 $[M + 4H]^{4+}$, 1180.27 $[M + 3H]^{3+}$, 1770.39 $[M + 2H]^{2+}$. Deconvolution mass, 3538.43 ± 1.00.

Glycopeptide CAP-GP10. From the controlled transglycosylation reaction: Yield, 24%; analytical RP-HPLC, t_R = 23.8 min (gradient, 23-25% aq. MeCN containing 0.1% TFA for 35 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 4551.77; found 912.44 $[M + 5H]^{5+}$, 1138.96 $[M + 4H]^{4+}$, 1518.09 $[M + 3H]^{3+}$. Deconvolution mass, 4551.80 ± 0.0.

Glycopeptide CAP-GP11. From controlled transglycosylation reaction: Yield, 23%; analytical RP-HPLC, t_R = 22.2 min (gradient, 23-25% aq. MeCN containing 0.1% TFA for 35 min; flow rate, 0.5 mL/min); ESI-MS: calcd M = 4551.77; found 912.36 $[M + 5H]^{5+}$, 1138.89 $[M + 4H]^{4+}$, 1518.01 $[M + 3H]^{3+}$. Deconvolution mass 4551.28 ± 0.27.