Supporting Information

Calorimetric Analysis of the Interplay between Synthetic Tn Antigen-presenting MUC1 Glycopeptides and Human Macrophage Galactose-type Lectin

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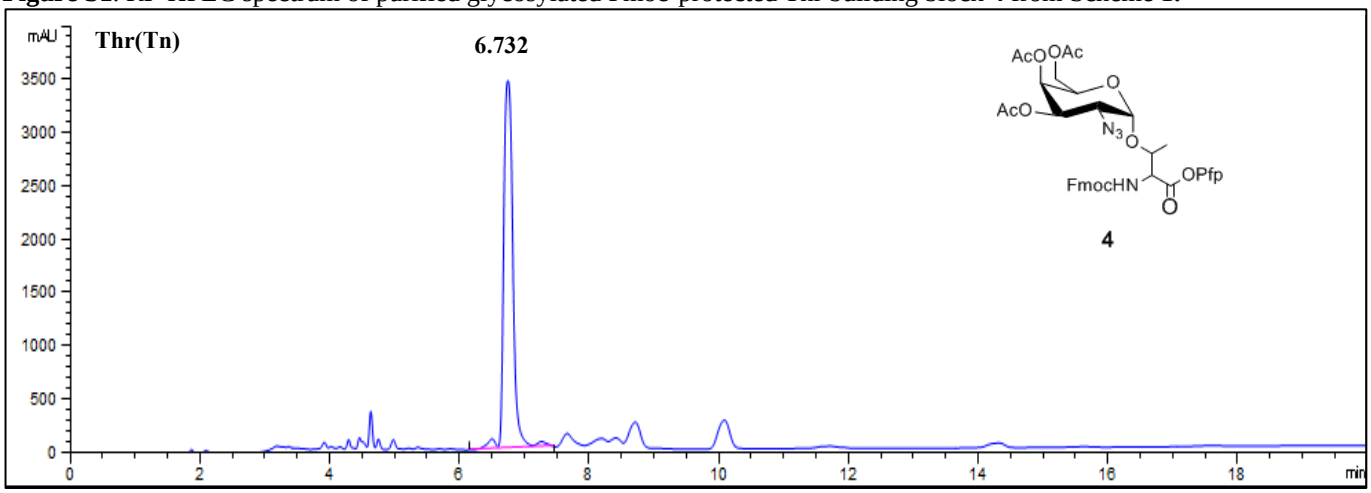
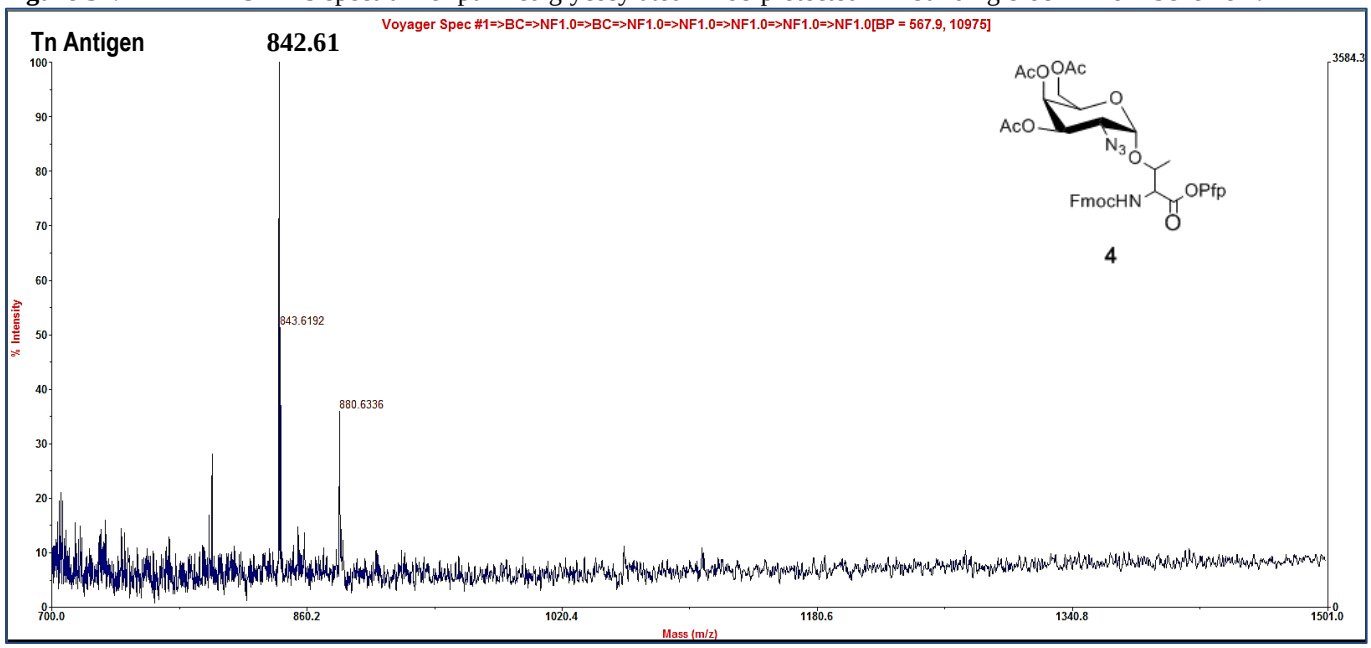
Thr-Azide

Figure S1. RP-HPLC spectrum of purified glycosylated Fmoc-protected Thr building block **4** from Scheme 1**.**



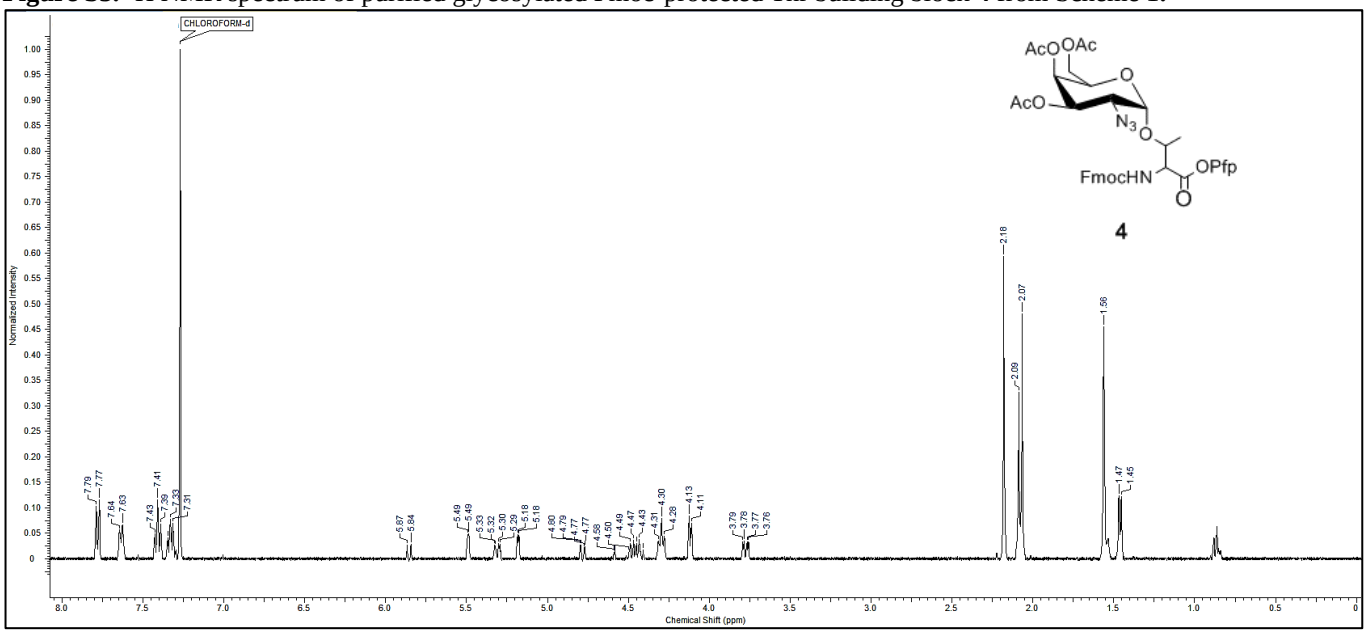
HPLC analysis of purified glycosylated Fmoc-protected Thr building block **4** derived from Scheme 1.
Eluents were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 60-100% B in 20 mins with a flow rate of 1.0 mL/min. Detection was at wavelength = 214 nm. Retention time (min) = 6.732.

Figure S2. MALDI-TOF MS spectrum of purified glycosylated Fmoc-protected Thr building block **4** from Scheme 1**.**



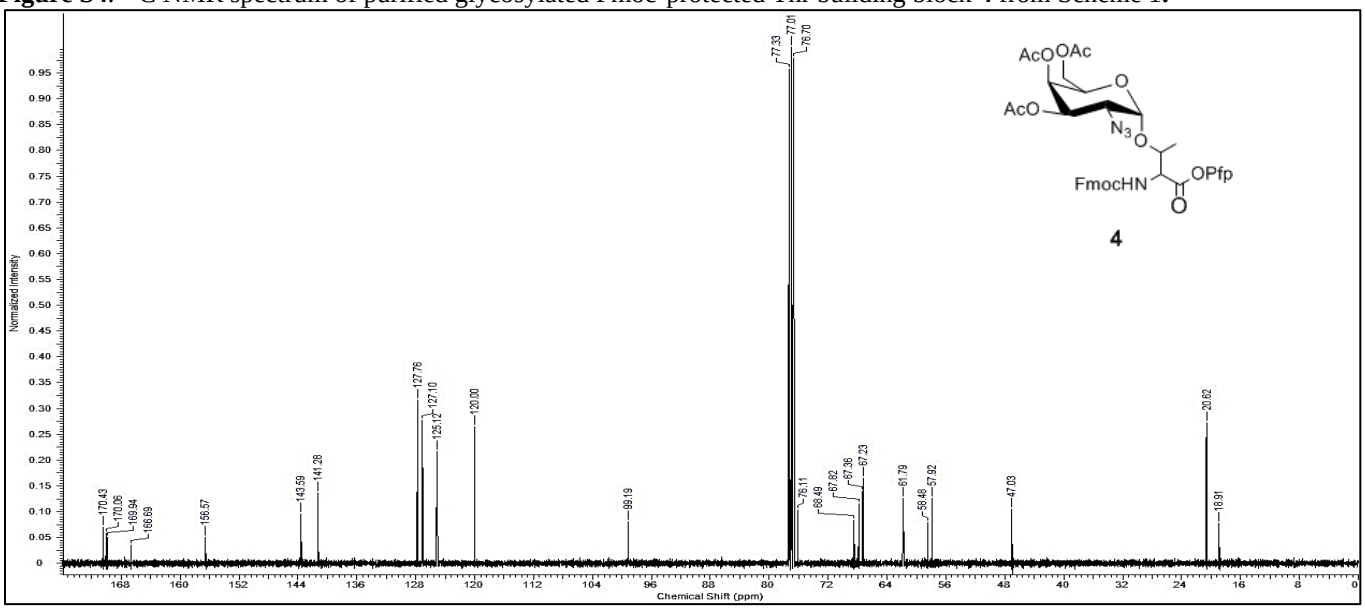
MALDI-TOF MS analysis of purified glycosylated Fmoc-protected Thr building block **4** derived from Scheme 1.
Thr-Azide: $[M + Na]^+$ = 842.61 Da (expected, 843.66 Da).

Figure S3. ^{1}H NMR spectrum of purified glycosylated Fmoc-protected Thr building block **4** from Scheme 1**.**



^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.78-7.76 (d, *J*=7.4 Hz, Ar-H Fmoc, 2H), 7.63-7.62 (d, *J*=6.5 Hz, Ar-H Fmoc, 2H), 7.42-7.38 (m, Ar-H Fmoc, 2H), 7.34-7.30 (m, Ar-H Fmoc, 2H), 5.87 -5.84 (d, *J*=9.0 Hz, HN-Thr, 1H), 5.49 (d, *J*=3.0 Hz, Gal-H_4, 1H), 5.33-5.29 (dd, *J*=11.0 Hz, Gal-H_3, 1H), 5.18 (d, *J*=3.5 Hz, Gal-αH_1, 1H), 4.80-4.77 (dd, *J*=10.7 Hz, Thr-αH, 1H), 4.59 (m, Thr-βH, 1H), 4.49-4.43 (m, Fmoc-CH_2, 2H), 4.31-4.28 (t, Fmoc-CH and Gal-H_5, 2H), 4.13-4.11 (dd, *J*=6.0, 1.5 Hz, Gal-H_{6a-6b}, 2H), 3.79-3.76 (dd, Gal-H_2, 1H), 2.17-2.07 (3s, $COCH_3$, 9H), 1.47-1.45 (d, *J*=6.0 Hz, Thr-CH_3, 3H).

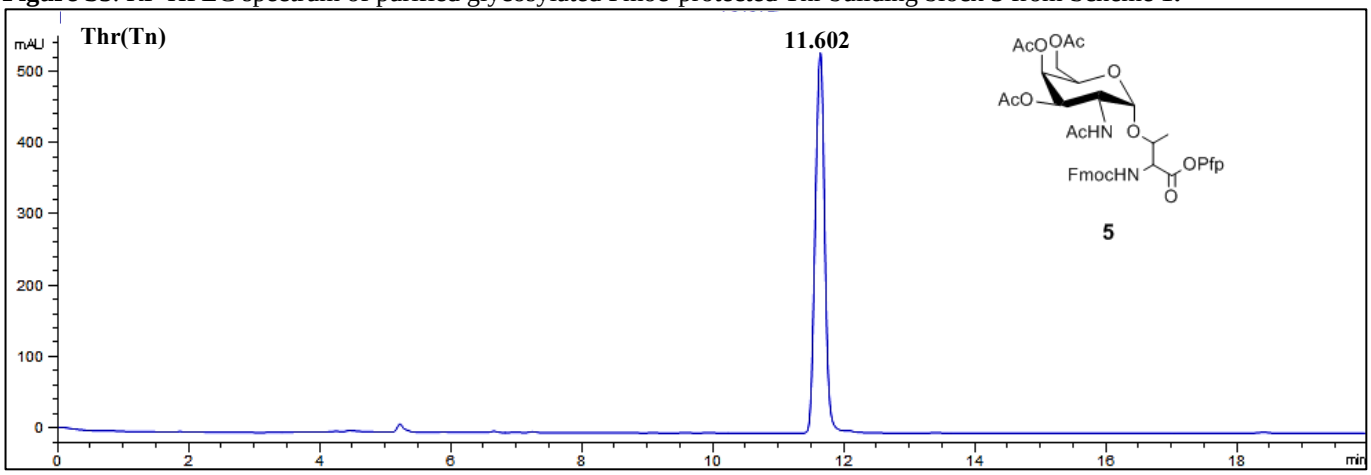
Figure S4. ^{13}C NMR spectrum of purified glycosylated Fmoc-protected Thr building block **4** from Scheme 1**.**



^{13}C NMR (400 MHz, $CDCl_3$): δ [ppm] = 170.43, 170.06, 169.94, 166.69, 156.57, 143.59, 141.28, 127.76, 127.10, 125.12, 120.00, 99.19 (C_1 Gal*N*Ac), 68.49, 67.82, 67.36, 67.23, 61.79, 58.48, 57.92, 47.03, 20.62, 18.91.

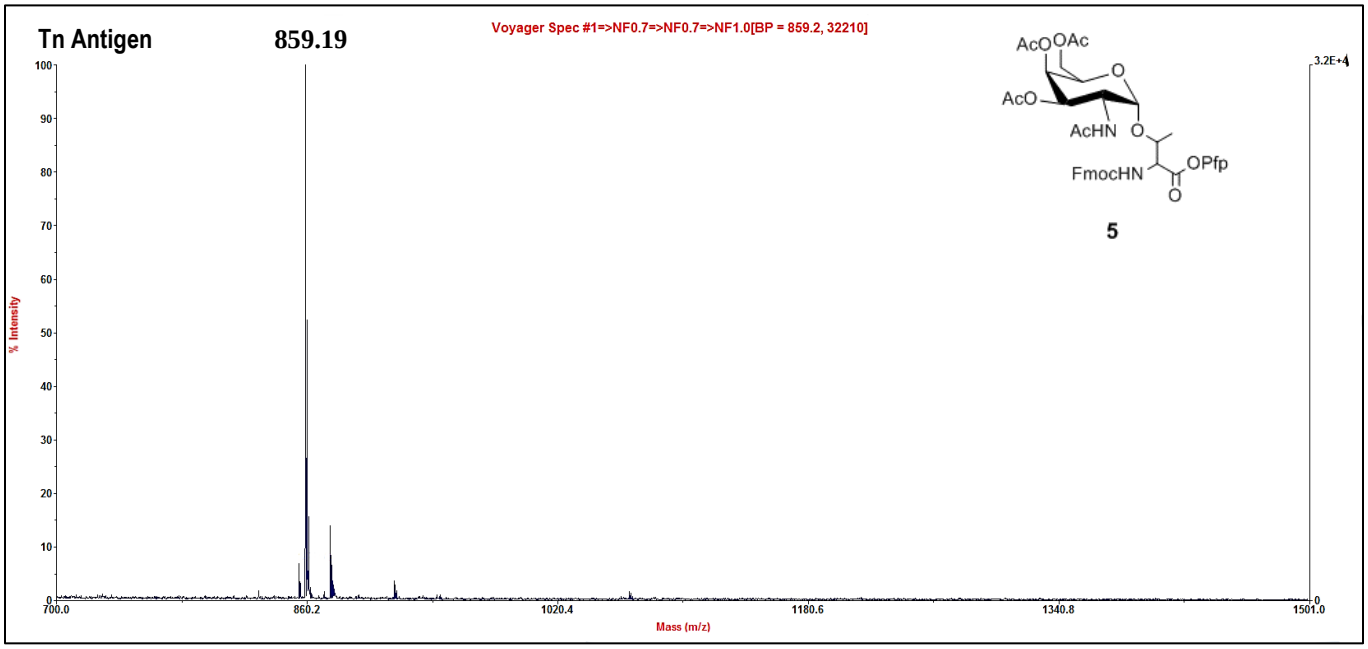
Thr(Tn)

Figure S5. RP-HPLC spectrum of purified glycosylated Fmoc-protected Thr building block **5** from Scheme 1**.**



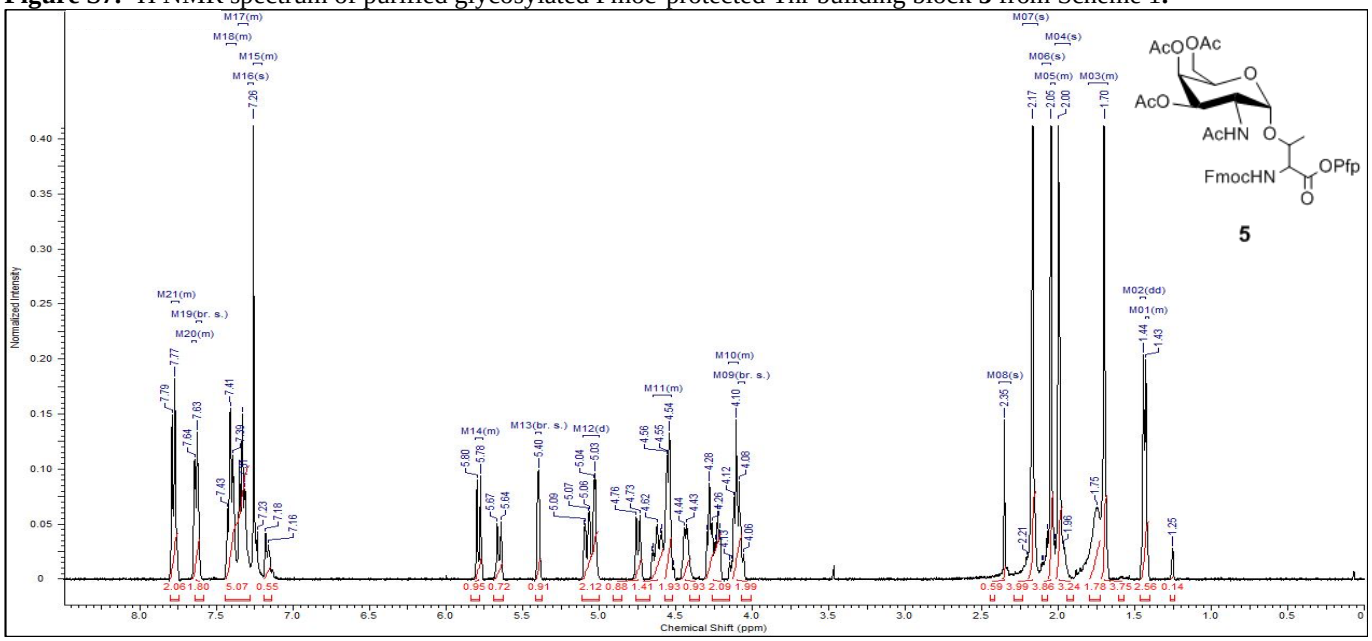
HPLC analysis of purified glycosylated Fmoc-protected Thr building block **5** derived from Scheme 1.
Eluents were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 40-100% B in 20 mins with a flow rate of 1.0 mL/min. Detection was at wavelength = 214 nm. Retention time (min) = 11.602.

Figure S6. MALDI-TOF MS spectrum of purified glycosylated Fmoc-protected Thr building block **5** from Scheme 1**.**



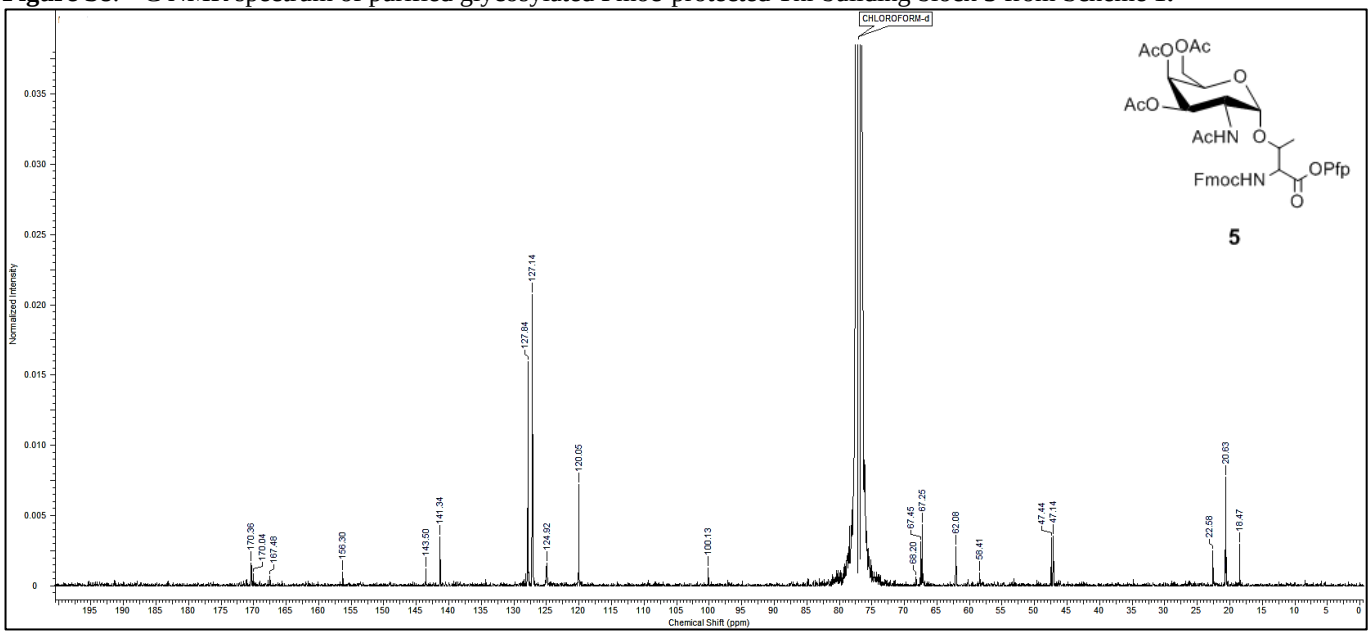
MALDI-TOF MS analysis of purified glycosylated Fmoc-protected Thr building block **5** derived from Scheme 1.
Thr(T_N): $[M + Na]^+$ = 859.19 Da (expected, 859.72 Da).

Figure S7. ^{1}H NMR spectrum of purified glycosylated Fmoc-protected Thr building block **5** from Scheme 1**.**



^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.80-7.77 (d, *J*= 7.4 Hz, Ar-H Fmoc, 2H), 7.64-7.63 (d, *J*= 7.8 Hz, Ar-H Fmoc, 2H), 7.43-7.39 (m, *J*= 7.2 Hz, Ar-H Fmoc, 2H), 7.36-7.32 (m, *J*= 7.3 Hz, Ar-H Fmoc, 2H), 7.18-7.16 (m, $NHCOCH_3$, 1H), 5.80-5.78 (d, *J*= 9.2 Hz, NH-Thr, 1H), 5.67-5.64 (d, *J*= 10.4 Hz, Gal-H_1, 1H), 5.40 (s, Gal-H_4, 1H), 5.09-5.03 (m, Gal-H_3, 1H), 4.76-4.73 (d, *J*= 8.2 Hz, αCH Thr, 1H), 4.66-4.54 (m, Gal-H_5, Fmoc CH_2, and βCH Thr , 4H), 4.44-4.43 (m, Gal-H_2, 1H), 4.31-4.22(m, Fmoc-CH, 1H) , 4.13-4.06 (m, Gal-H_{6a-6b}, 2H), 2.17 (s, $COCH_3$, 3H) 2.05 (s, $COCH_3$, 3H), 2.00 (s, $COCH_3$, 3H), 1.70 (s, $NHCOCH_3$, 3H), 1.44-1.43 (d, Thr-CH_3, 3H).

Figure S8. ^{13}C NMR spectrum of purified glycosylated Fmoc-protected Thr building block **5** from Scheme 1**.**



^{13}C NMR (400 MHz, $CDCl_3$): δ [ppm] = 170.36, 170.04,167.48, 156.30, 143.50, 141.34, 127.84, 127.14, 124.92, 120.05, 100.13 (C_1 Gal*N*Ac), 68.20, 67.45, 67.25, 62.08, 58.41, 47.44, 47.14, 22.58, 20.64, 20.63, 20.62, 18.47.

Peptide Synthesis

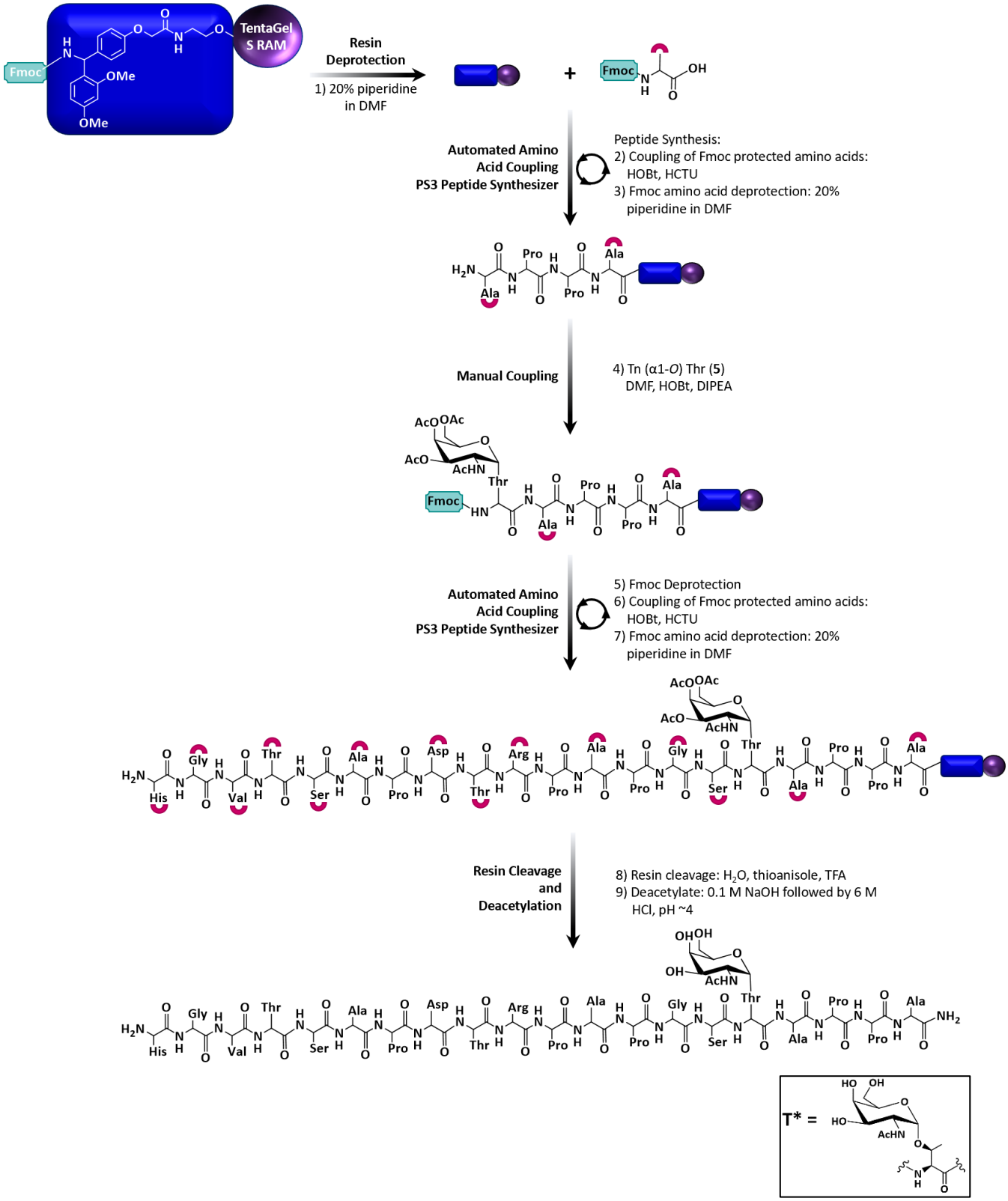
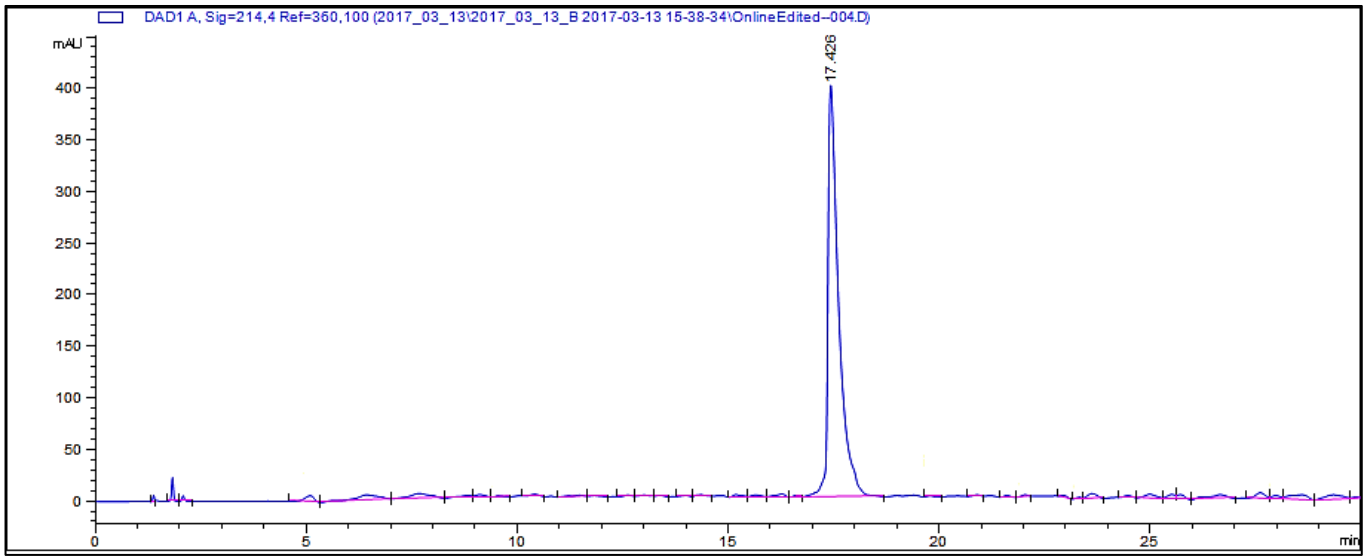


Figure S9. General synthetic scheme for glycopeptides (specifically peptide 7, MUC1: HGV**T***SAPDTRPAPGSTAPPA).

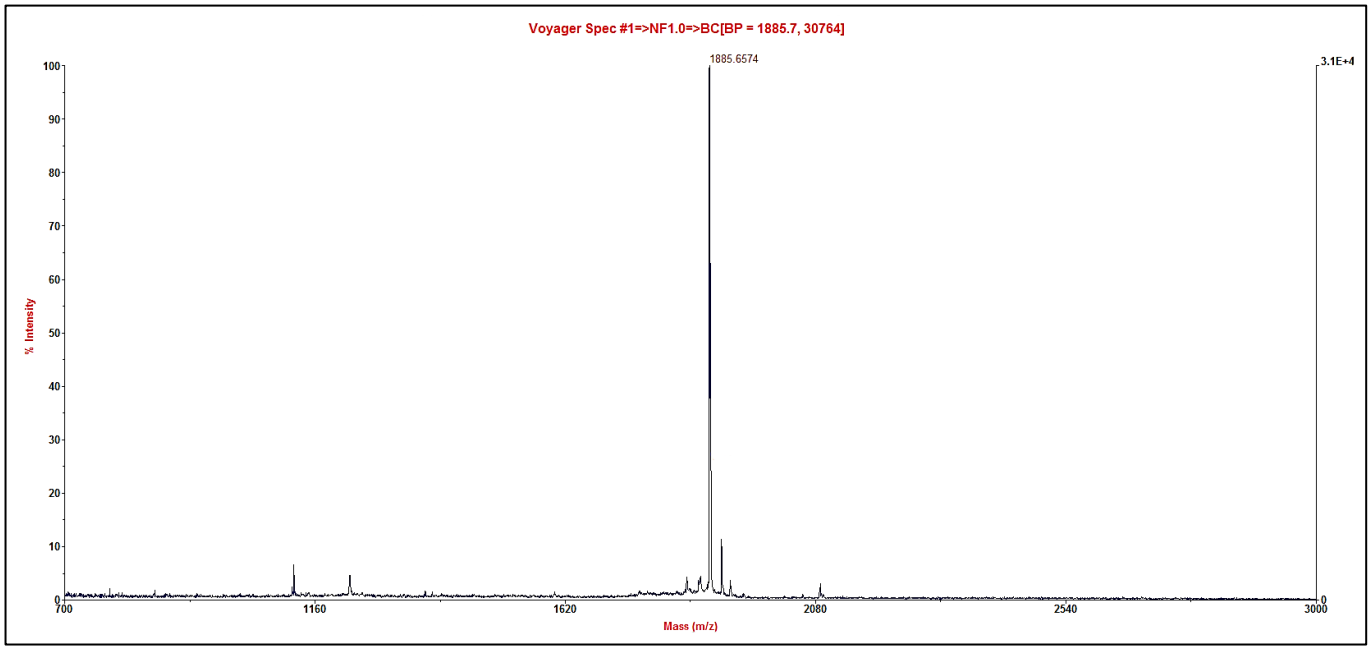
MUC1

Figure S10. RP-HPLC spectrum of purified **MUC1** peptide (**6**).
MUC1: HGVTSAPDTRPAPGSTAPPA



HPLC analysis of purified MUC1 peptide (**6**).
Eluents were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 0-30% B in 30 mins with a flow rate of 1.0 mL/min. Detection was at wavelength = 214 nm. Retention time (min) = 17.42.

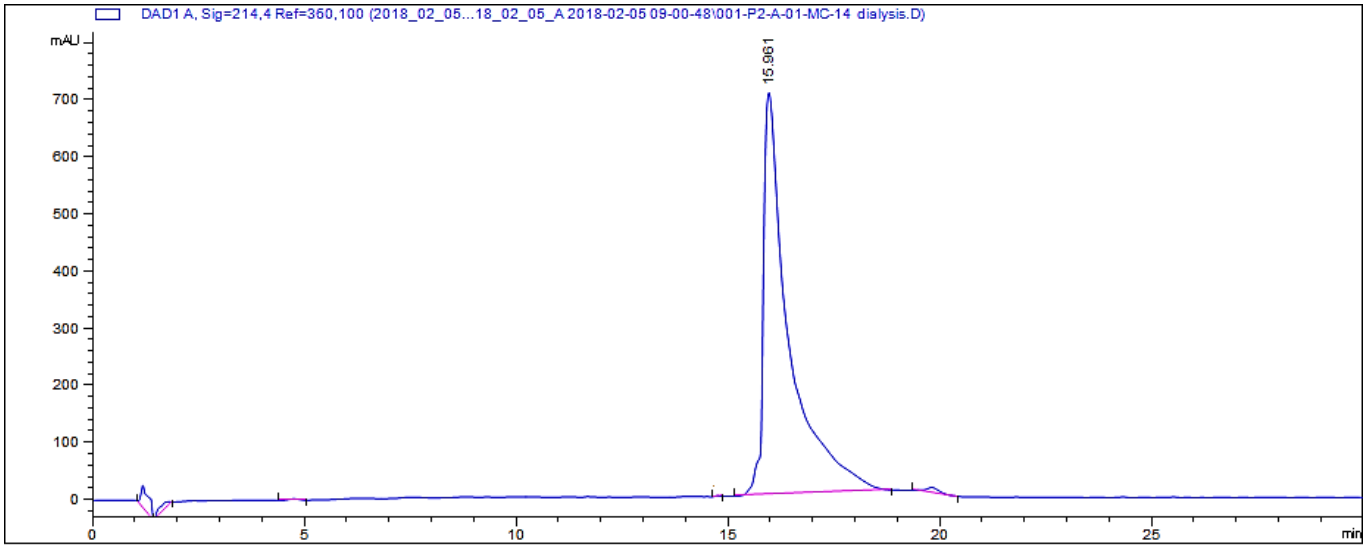
Figure S11. MALDI-TOF MS spectrum of purified **MUC1** peptide (**6**).
MUC1: HGVTSAPDTRPAPGSTAPPA



MALDI-TOF MS analysis of purified MUC1 peptide (**6**).
$[M + H]^+$ = 1885.65 Da (expected, 1884.93 Da).

MUC1-Thr4

Figure S12. RP-HPLC spectrum of purified **MUC1-Thr4** peptide (**7**).
MUC1: HGVT*SAPDTRPAPGSTAPPA



HPLC analysis of purified glycosylated MUC1-Thr4 peptide (**7**).
Eluents were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 0-30% B in 30 mins with a flow rate of 1.0 mL/min. Detection was at wavelength = 214 nm. Retention time (min) = 15.96.

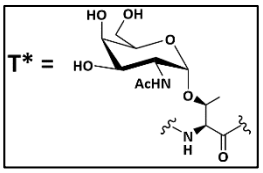
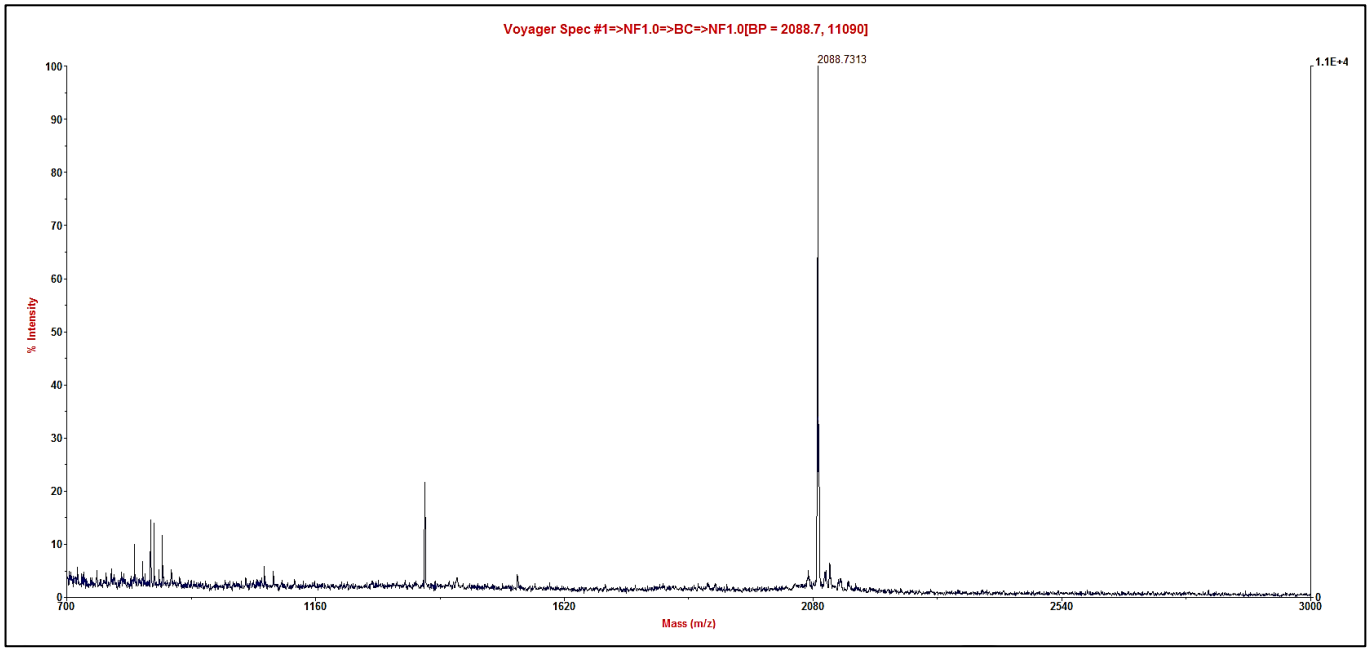


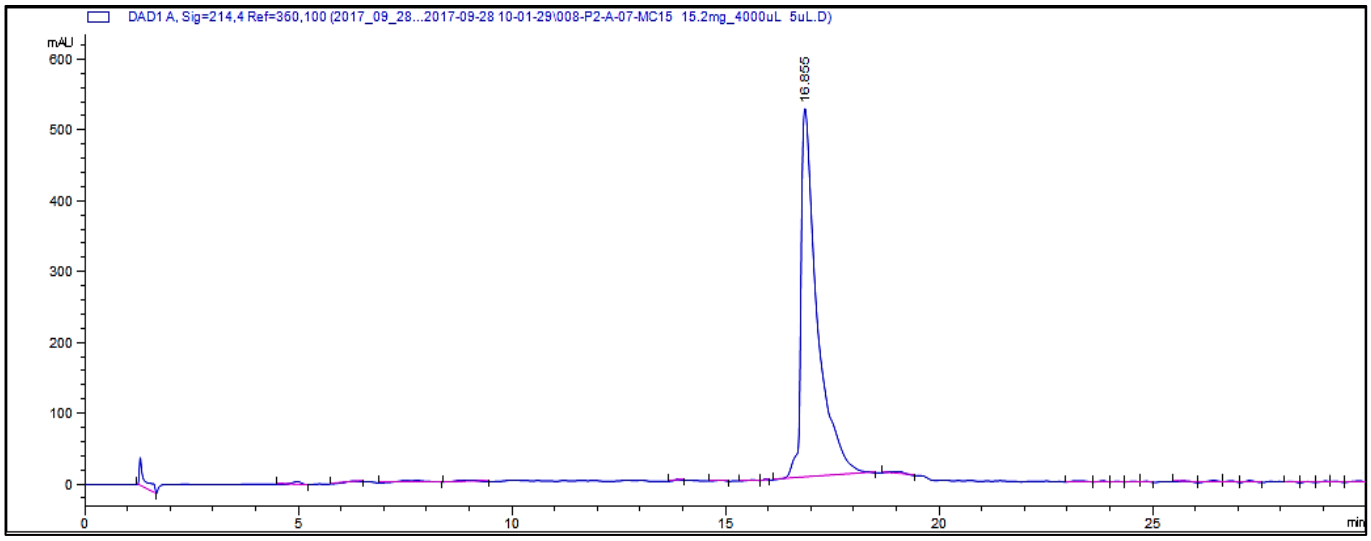
Figure S13. MALDI-TOF MS spectrum of purified **MUC1-Thr4** peptide (**7**).
MUC1: HGVT*SAPDTRPAPGSTAPPA



MALDI-TOF MS analysis of purified glycosylated MUC1-Thr4 peptide (**7**).
$[M + H]^+$ = 2088.73 Da (expected, 2089.25 Da).

MUC1-Thr9

Figure S14. RP-HPLC spectrum of purified **MUC1-Thr9** peptide (**8**).
MUC1: HGVTSAPDT*RPAPGSTAPPA



HPLC analysis of purified glycosylated MUC1-Thr9 peptide (**8**).
Eluents were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 0-30% B in 30 mins with a flow rate of 1.0 mL/min. Detection was at wavelength = 214 nm. Retention time (min) = 16.85.

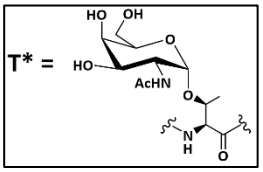
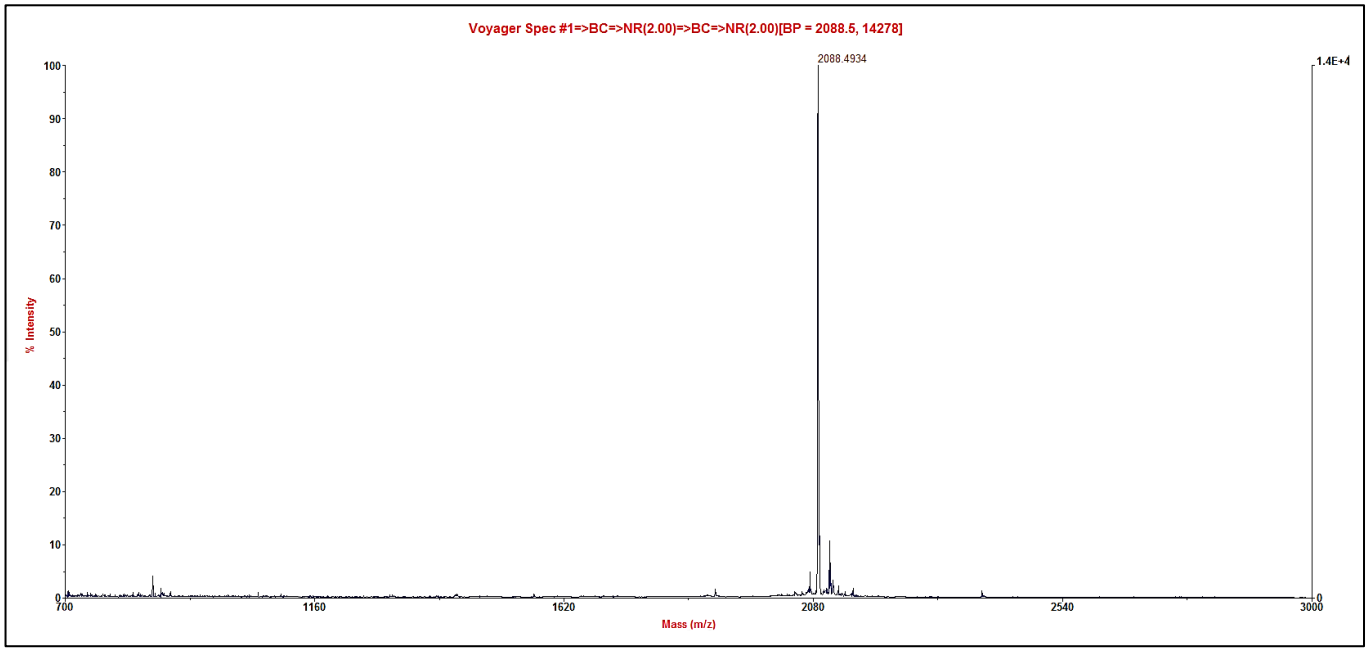


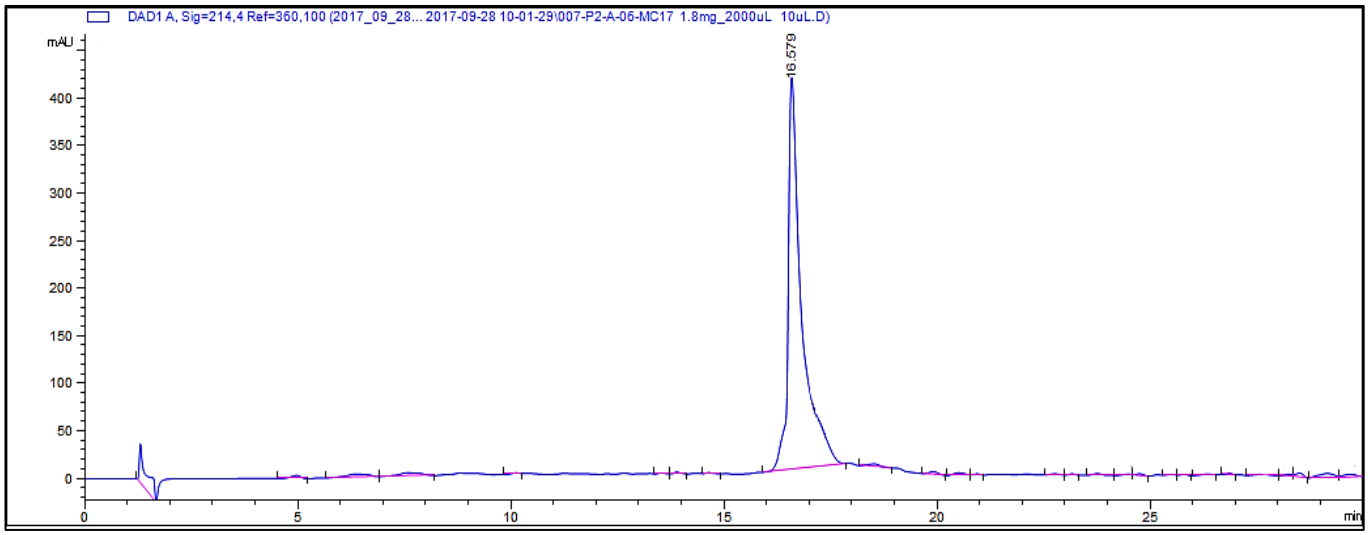
Figure S15. MALDI-TOF MS spectrum of purified **MUC1-Thr9** peptide (**8**).
MUC1: HGVTSAPDT*RPAPGSTAPPA



MALDI-TOF MS analysis of purified glycosylated MUC1-Thr9 peptide (**8**).
$[M + H]^+$ = 2088.49 Da (expected, 2089.25 Da).

MUC1-Thr16

Figure S16. RP-HPLC spectrum of purified **MUC1-Thr16** peptide (**9**).
MUC1: HGVTSAPDTRPAPGST*APPA



HPLC analysis of purified glycosylated MUC1-Thr16 peptide (**9**).
Eluents were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 0-30% B in 30 mins with a flow rate of 1.0 mL/min. Detection was at wavelength = 214 nm. Retention time (min) = 16.57.

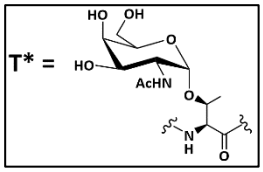
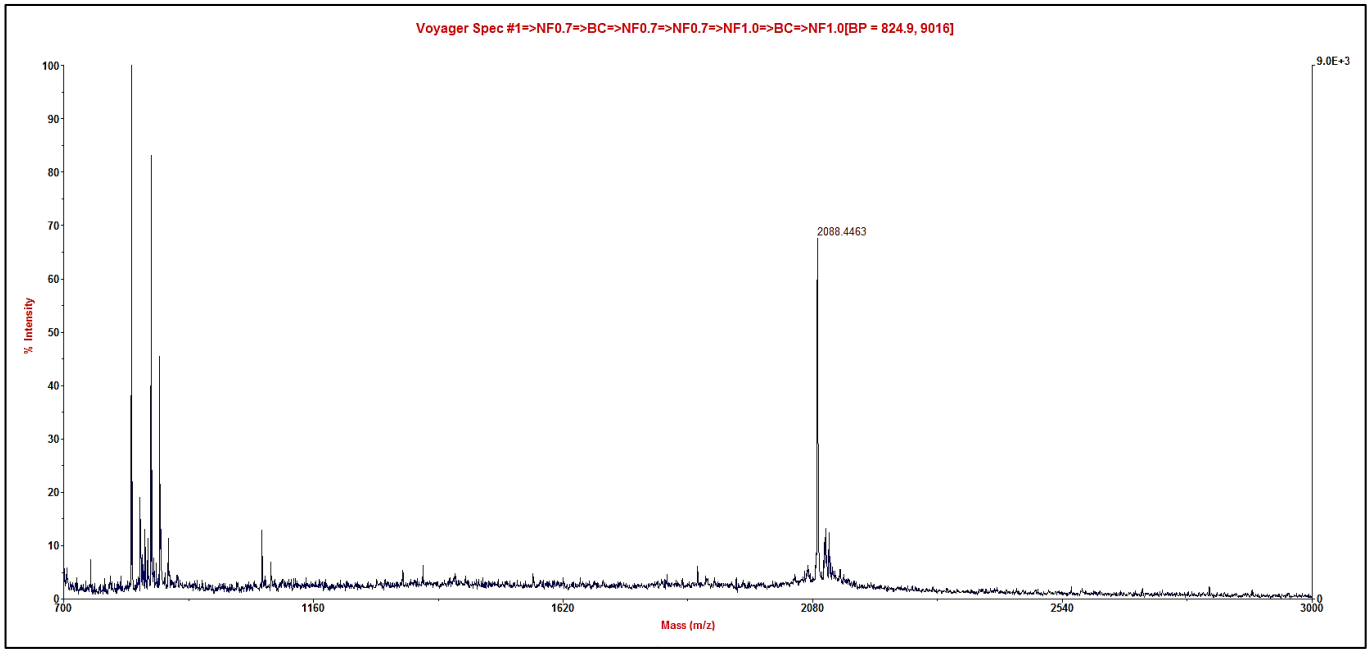


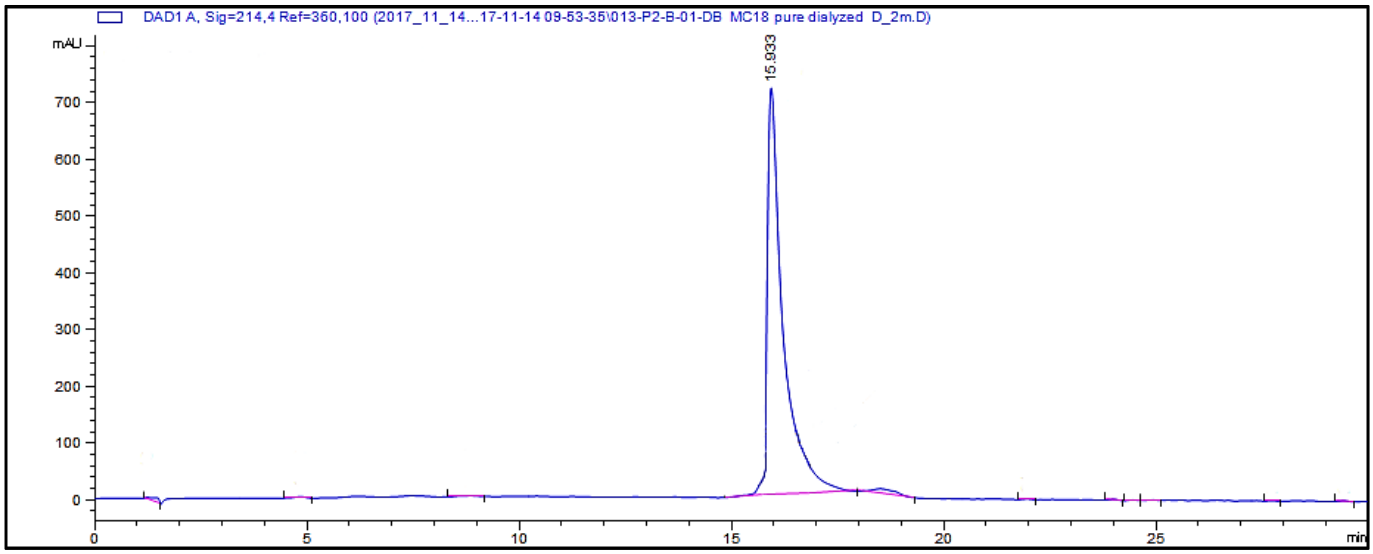
Figure S17. MALDI-TOF MS spectrum of purified **MUC1-Thr16** peptide (**9**).
MUC1: HGVTSAPDTRPAPGST*APPA



MALDI-TOF MS analysis of purified glycosylated MUC1-Thr16 peptide (**9**).
$[M + H]^+$ = 2088.44 Da (expected, 2089.25 Da).

MUC1-Thr9,16

Figure S18. RP-HPLC spectrum of purified **MUC1-Thr9,16** peptide (**10**).
MUC1: HGVTSAPDT*RPAPGST*APPA



HPLC analysis of purified glycosylated MUC1-Thr9,16 peptide (**10**).
Eluents were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 0-30% B in 30 mins with a flow rate of 1.0 mL/min. Detection was at wavelength = 214 nm. Retention time (min) = 15.93.

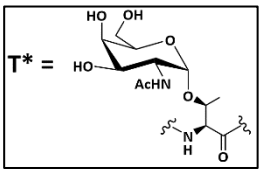
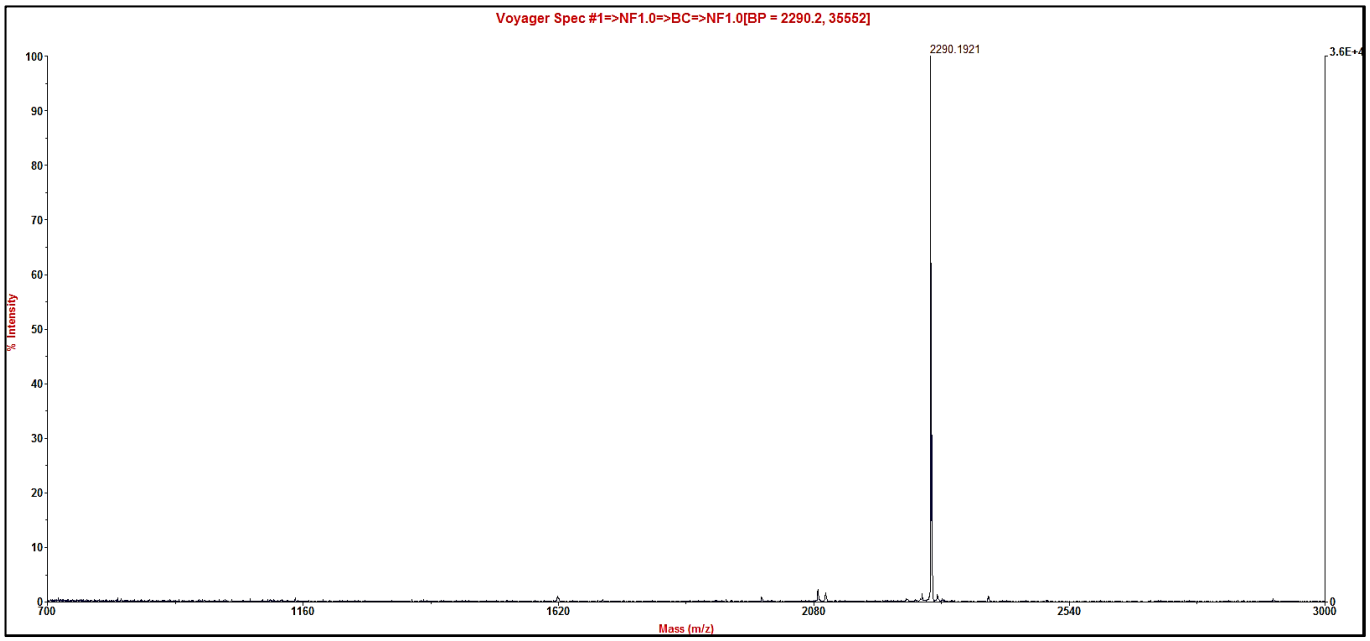


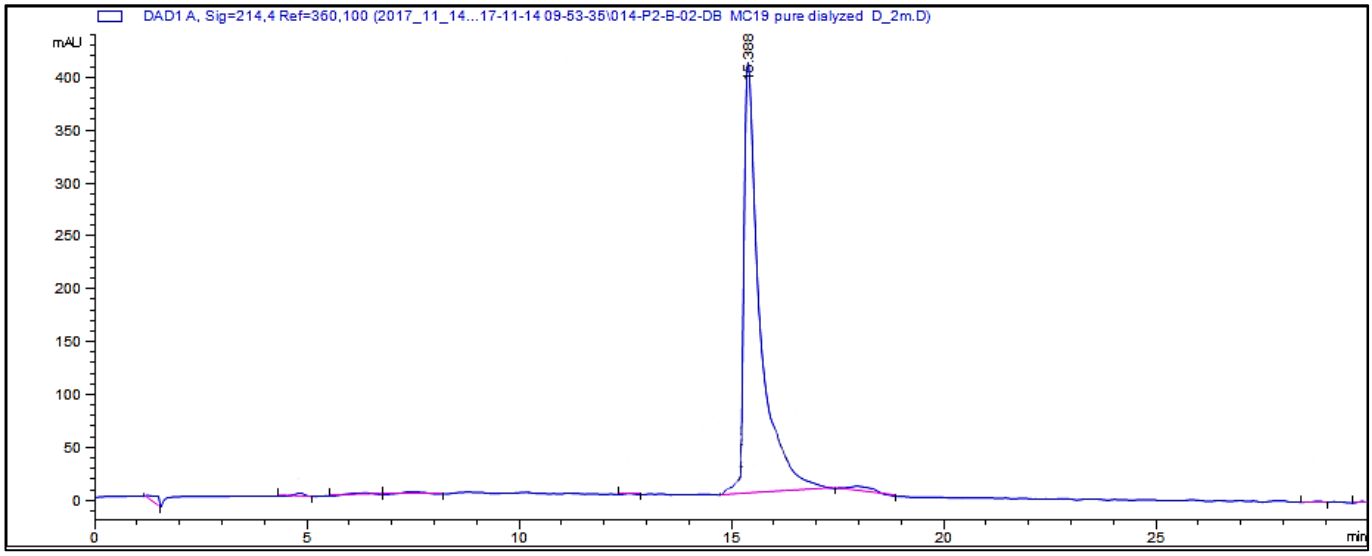
Figure S19. MALDI-TOF MS spectrum of purified **MUC1-Thr9,16** peptide (**10**).
MUC1: HGVTSAPDT*RPAPGST*APPA



MALDI-TOF MS analysis of purified glycosylated MUC1-Thr9,16 peptide (**10**).
$[M + H]^+$ = 2290.19 Da (expected, 2292.45 Da).

MUC1-Thr4,16

Figure S20. RP-HPLC spectrum of purified **MUC1-Thr4,16** peptide (**11**).
MUC1: HGVT*SAPDTRPAPGST*APPA



HPLC analysis of purified glycosylated MUC1-Thr4,16 peptide (**11**). Eluents were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 0-30% B in 30 mins with a flow rate of 1.0 mL/min. Detection was at wavelength = 214 nm. Retention time (min) = 15.38.

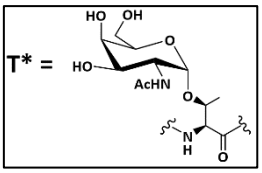
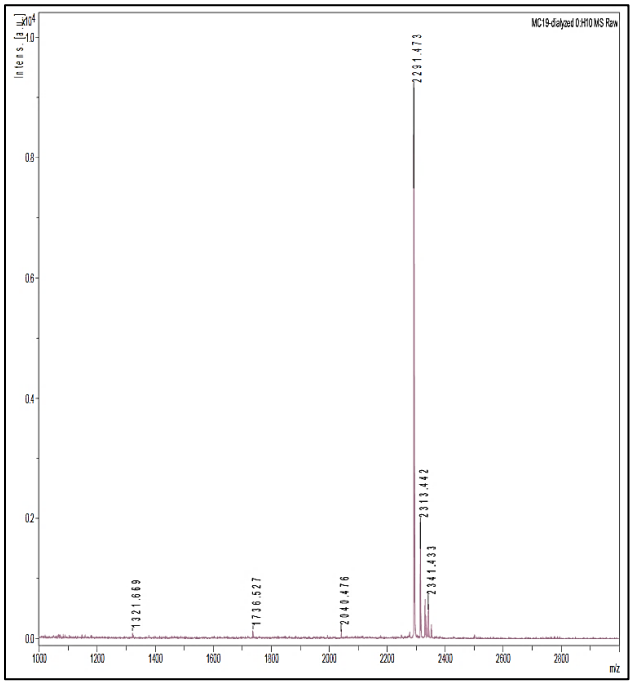


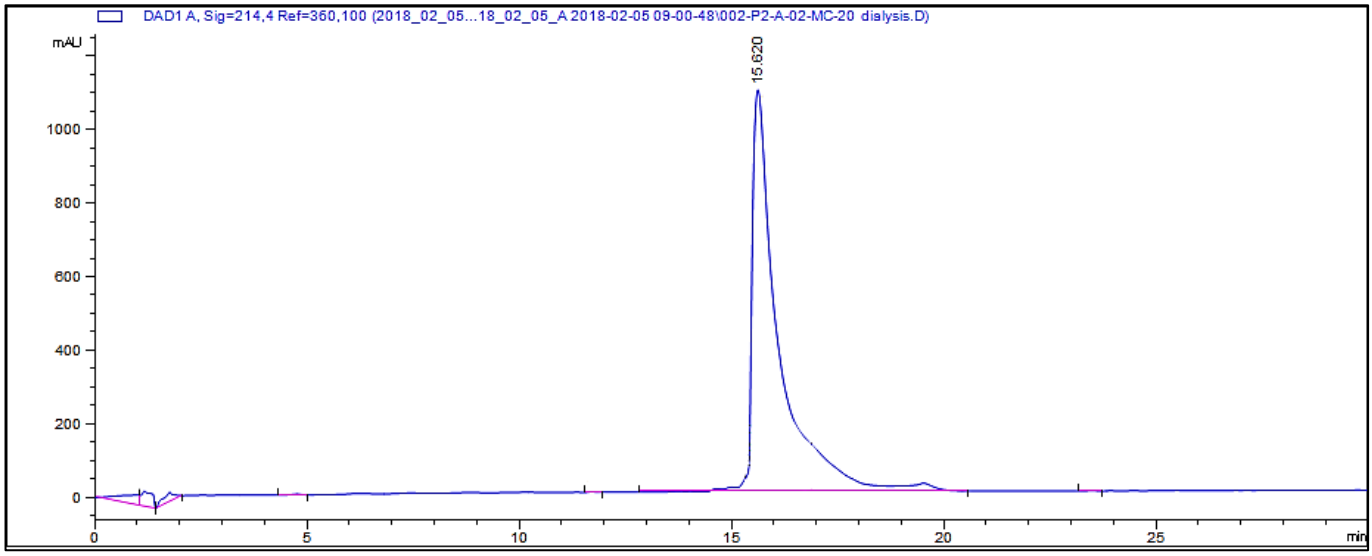
Figure S21. MALDI-TOF MS spectrum of purified **MUC1-Thr4,16** peptide (**11**).
MUC1: HGVT*SAPDTRPAPGST*APPA



MALDI-TOF MS analysis of purified glycosylated MUC1-Thr4,16 peptide (**11**) was performed with a MicroFlex LT system (Bruker). $[M + H]^+$ = 2291.47 Da (expected, 2292.45 Da).

MUC1-Thr4,9

Figure S22. RP-HPLC spectrum of purified **MUC1-Thr4,9** peptide (**12**).
MUC1: HGVT*SAPDT*RPAPGSTAPPA



HPLC analysis of purified glycosylated MUC1-Thr4,9 peptide (**12**).
Eluents were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 0-30% B in 30 mins with a flow rate of 1.0 mL/min. Detection was at wavelength = 214 nm. Retention time (min) = 15.62.

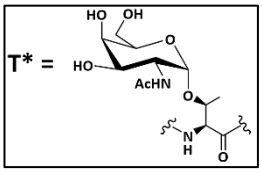
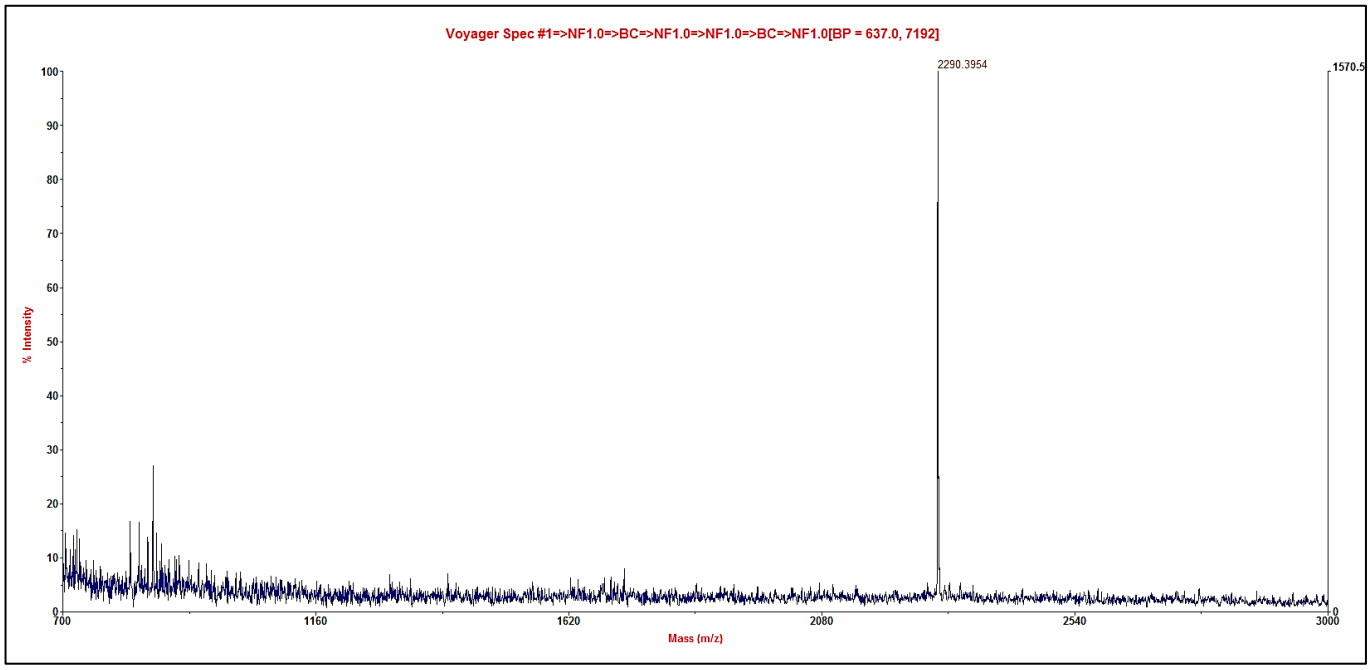


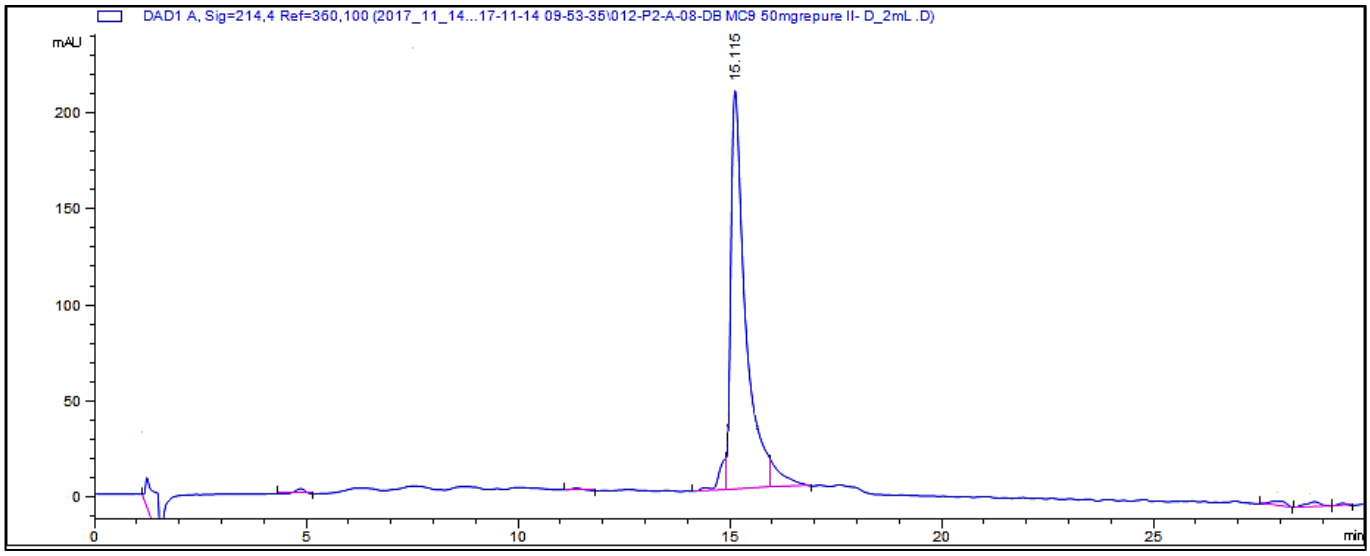
Figure S23. MALDI-TOF MS spectrum of purified **MUC1-Thr4,9** peptide (**12**).
MUC1: HGVT*SAPDT*RPAPGSTAPPA



MALDI-TOF MS analysis of purified glycosylated MUC1-Thr4,9 peptide (**12**).
$[M + H]^+$ = 2290.39 Da (expected, 2292.45 Da).

MUC1-Thr4,9,16

Figure S24. RP-HPLC spectrum of purified **MUC1-Thr4,9,16** peptide (**13**).
MUC1: HGVT*SAPDT*RPAPGST*APPA



HPLC analysis of purified glycosylated MUC1-Thr4,9,16 peptide (**13**).
Eluents were 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 0-30% B in 30 mins with a flow rate of 1.0 mL/min. Detection was at wavelength = 214 nm. Retention time (min) = 15.11.

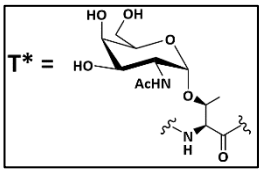
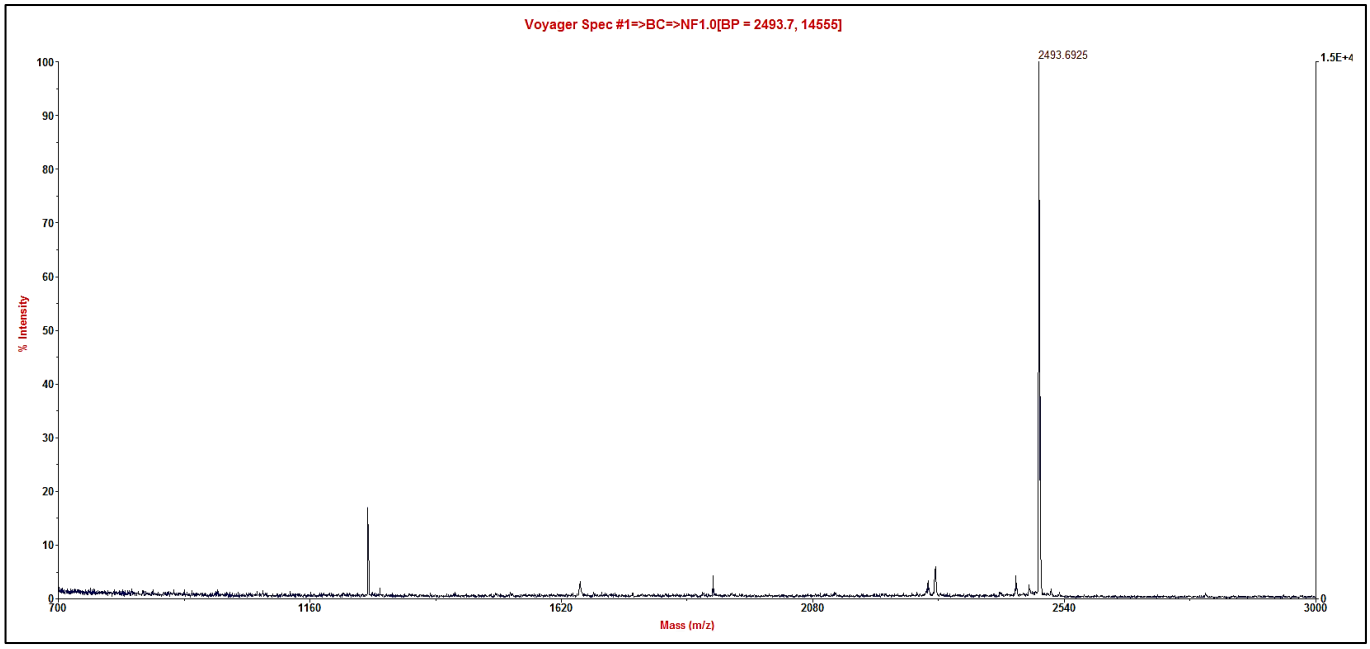


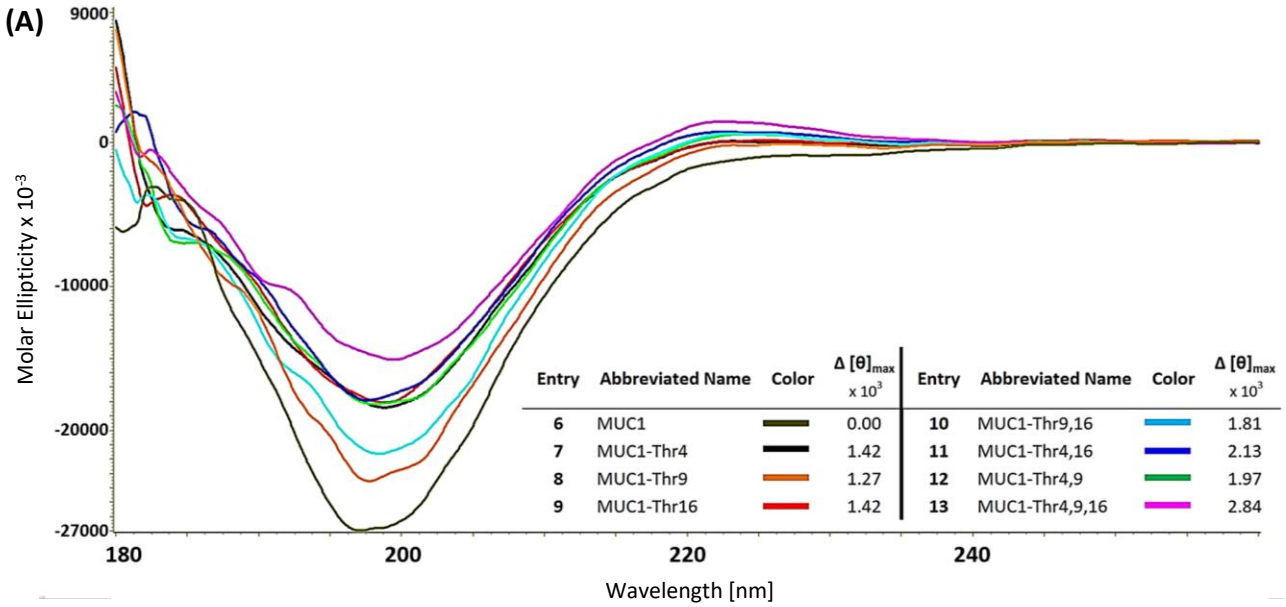
Figure S25. MALDI-TOF MS spectrum of purified **MUC1-Thr4,9,16** peptide (**13**).
MUC1: HGVT*SAPDT*RPAPGST*APPA

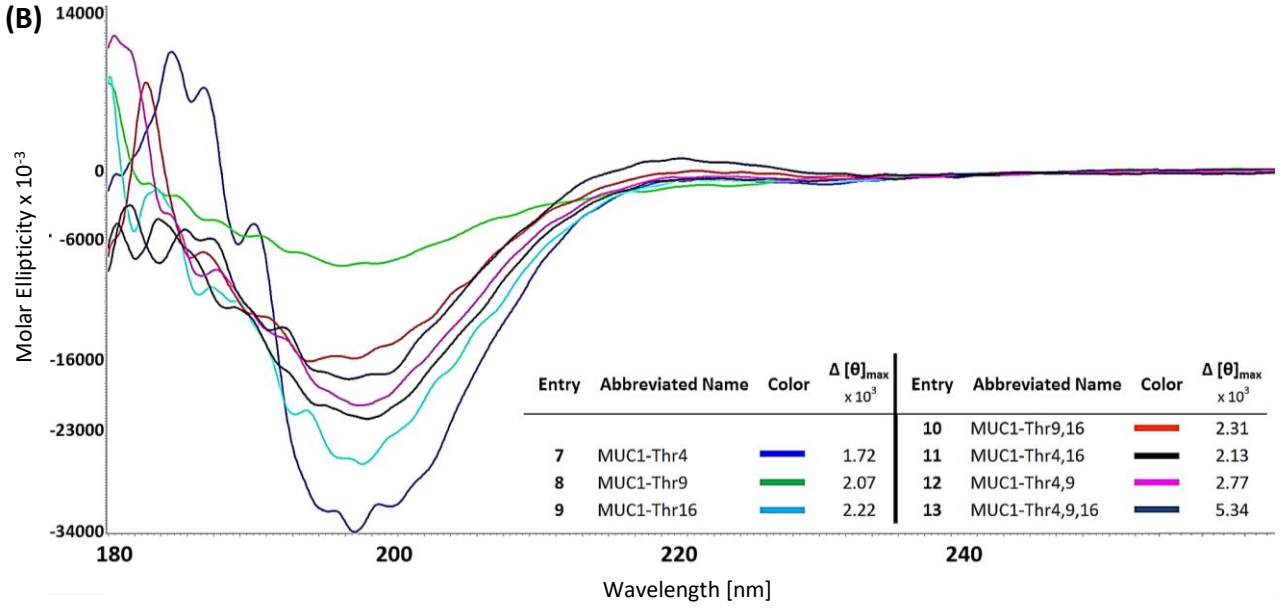


MALDI-TOF MS analysis of purified glycosylated MUC1-Thr4,9,16 peptide (**13**).
$[M + H]^+$ = 2493.69 Da (expected, 2495.28 Da).

Circular Dichroism

Figure S26. Circular dichroism spectra of MUC1 (glyco)peptides in buffered (**A**) H_2O and (**B**) D_2O. Δ[θ]max value represents each peptide molar ellipticity normalized against MUC1 [θ]max in deg cm^2 $dmol^{-1}$.





Macrophage Galactose Lectin

Figure S27A. Molecular mass determination of hMGL by MALDI-TOF MS.

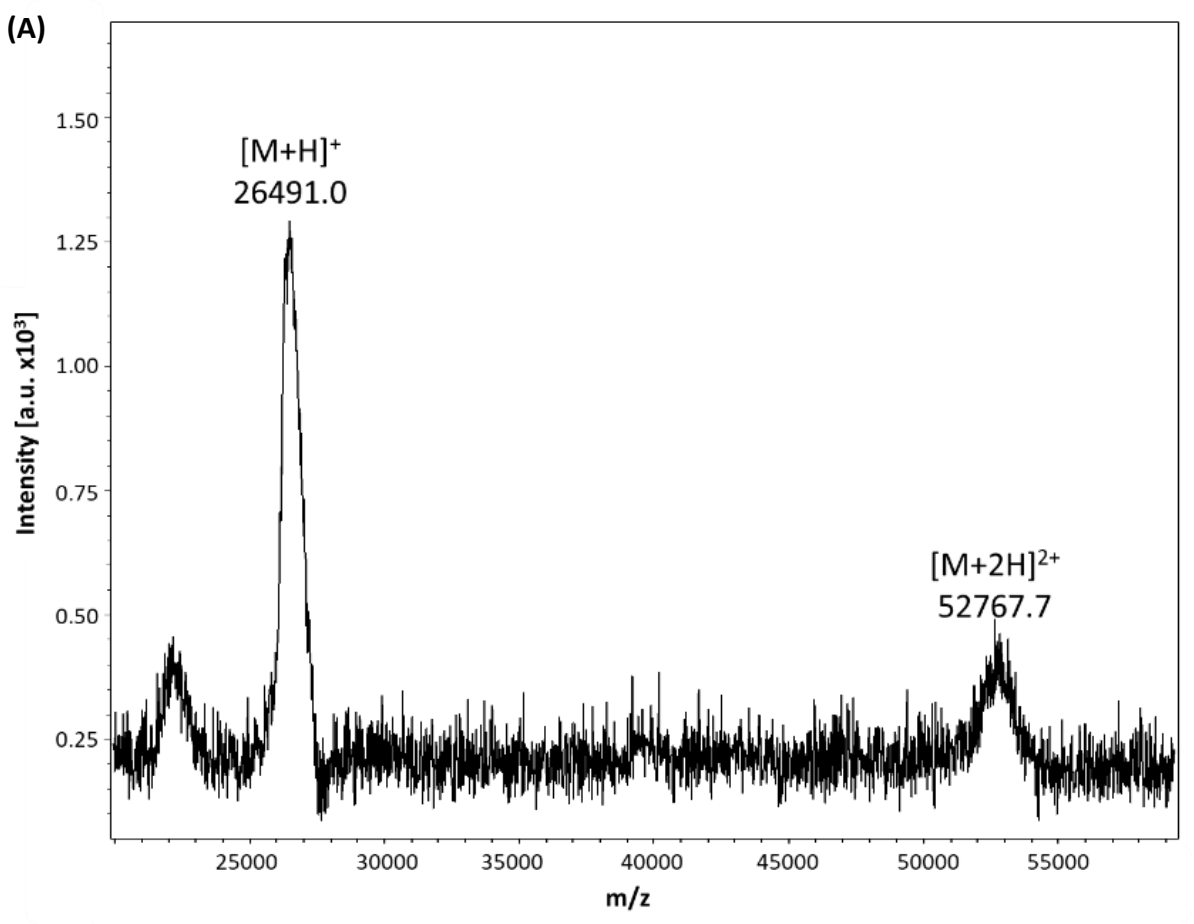
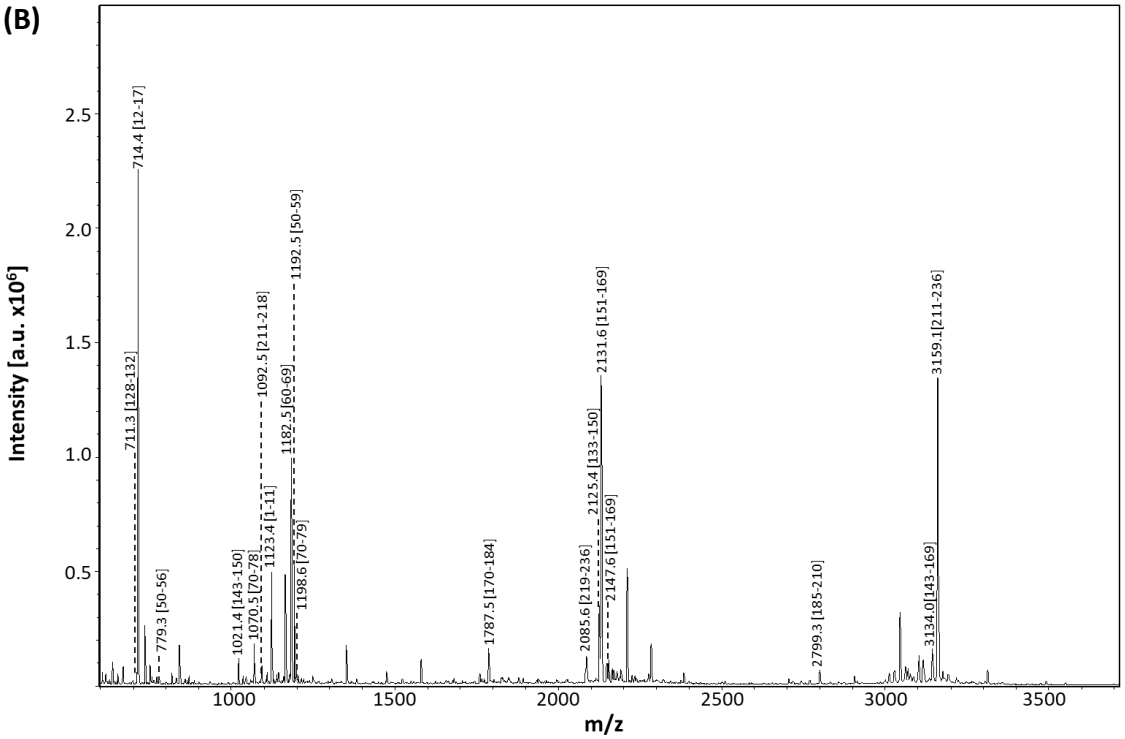


Figure S27B. Tryptic peptide mass fingerprinting of hMGL by MALDI-TOF MS.



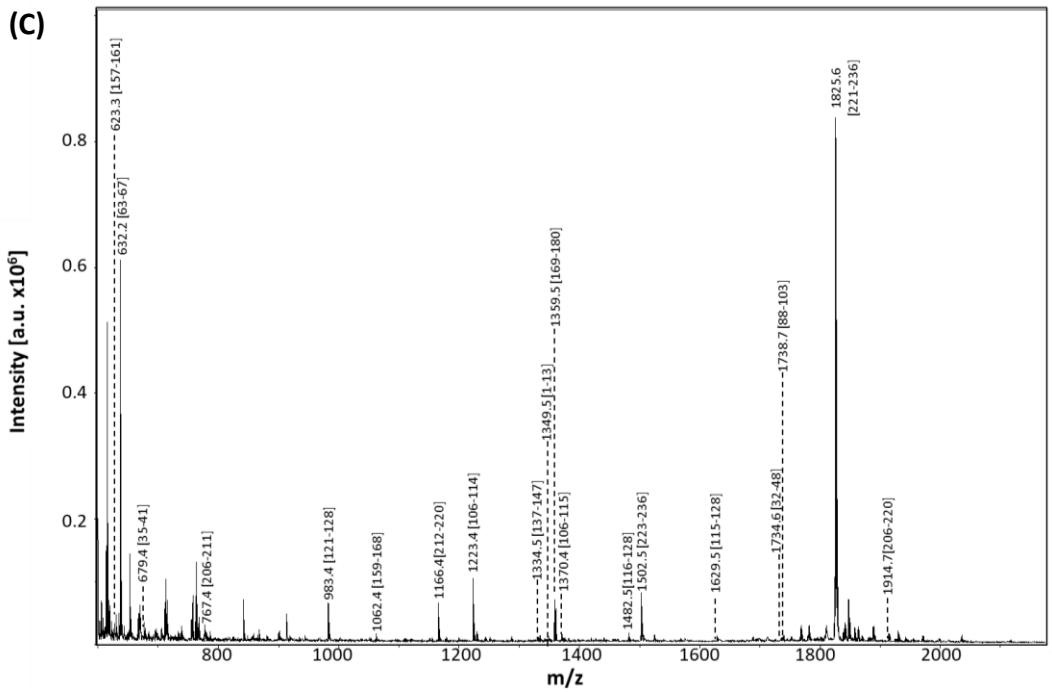
List of detected peptides with their calculated (calc) and experimentally measured (exp) mass values.

Sequence	$[MH]^+$ (mono) calc	$[MH]^+$ (mono) exp
YCQLK[1]	711.3	711.3
ILVTLR	714.5	714.4
AEVEGFK	779.4	779.3
EEQNFVQK	1021.5	1021.4
VQQLVQDLK	1070.6	1070.5
WNDDVCQR[1]	1092.5	1092.5
ASMTGGQQMGR	1123.5	1123.4
ASMTGGQQMGR[2,2]	1155.5	1155.4
QAVHSEMLLR	1183.6	1183.5
AEVEGFKQER	1192.6	1192.5
VQQLVQDLKK	1198.7	1198.6
WVDGTDYATGFQNWK	1787.8	1787.5
PYHWVCEAGLGQTSQESH[1]	2085.9	2085.6
NAHLVVINSREEQNFVQK	2125.1	2125.4
YLGSAYTWMGLSDPEGAWK	2132.0	2131.6
YLGSAYTWMGLSDPEGAWK[2]	2148.0	2147.6
PGQPDDWQGHGLGGGEDCAHFHPDGR[1]	2799.2	2799.3
EEQNFVQKYLGSAYTWMGLSDPEGAWK	3134.5	3134.0
WNDDVCQRPYHWVCEAGLGQTSQESH[1,1]	3159.3	3159.1

Sequence coverage (66.1%)

ASMTGGQQMGRILVTLRTDFSNFTSNTVAEIQALTSQGSSLEETIASLKAEVEGFKQERQAVHSEMLLRVQQLVQDLK
KLTCQVATLNNNGEEASTEGTCCPVNWVEHQDSCYWFSHSGMSWAEAEKYCQLKNAHLVVINSREEQNFVQKYLG
SAYTWMGLSDPEGAWKWVDGTDYATGFQNWKPGQPDDWQGHGLGGGEDCAHFHPDGRWNDDVCQRPYHWVCE
AGLGQTSQESH

Figure S27C. Chymotryptic peptide mass fingerprinting of hMGL by MALDI-TOF MS.



List of detected peptides with their calculated (calc) and experimentally measured (exp) mass values.

Sequence	$[MH]^+$ (mono) calc	$[MH]^+$ (mono) exp
TWMGL[2]	623.3	623.3
HSEML[2]	632.3	632.2
TSQGSSL	679.3	679.4
HPDGRW	767.4	767.4
SWAEAEKY	983.4	983.4
MGLSDPEGAW	1062.5	1062.4
NDDVCQRPY[1]	1166.5	1166.4
VEHQDSCYW[1]	1223.5	1223.4
VVINSREEQNF	1334.6	1334.5
ASMTGGQQMGRIL	1349.7	1349.5
KWVDGTDYATGF	1359.6	1359.5
VEHQDSCYWF[1]	1370.5	1370.4
SHSGMSWAEAEKY	1482.6	1482.5
VCEAGLGQTSQESH[1]	1502.7	1502.5
FSHSGMSWAEAEKY	1629.7	1629.5
QALTSQGSSLEETIASL	1734.9	1734.6
NNNGEEASTEGTCCPV	1738.7	1738.7
HWVCEAGLGQTSQESH[1]	1825.8	1825.6
HPDGRWNDDVCQRPY[1]	1914.8	1914.7

Sequence Coverage (67.8%)

ASMTGGQQMGRILVTLRTDFSNFTSNTVAEIQALTSQGSSLEETIASLKAEVEGFKQERQAVHSEMLLRVQQLVQDLK
KLTCQVATLNNNGEEASTEGTCCPVNWVEHQDSCYWFSHSGMSWAEAEKYCQLKNAHLVVINSREEQNFVQKYLG
SAYTWMGLSDPEGAWKWVDGTDYATGFQNWKPGQPDDWQGHGLGGGEDCAHFHPDGRWNDDVCQRPYHWVCE
AGLGQTSQESH

Figure S27D. Combined sequence coverage of tryptic and chymotrytic peptide mass fingerprinting of hMGL by MALDI-TOF MS.

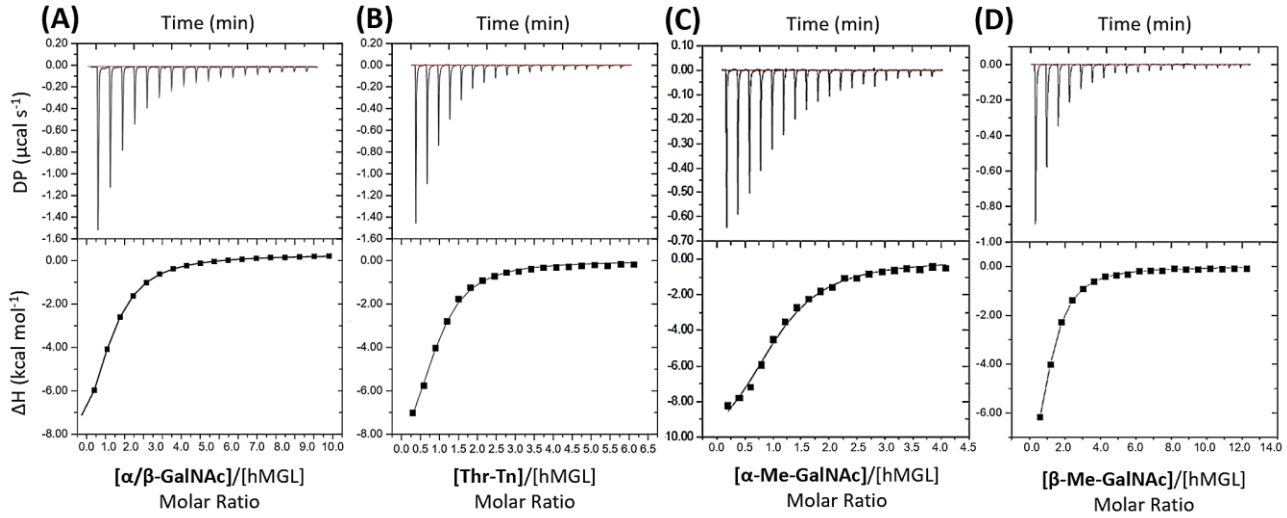
(D)

Sequence coverage (89.4%)

ASMTGGQQMGRILVTLRTDFSNFTSNTVAEIQALTSQGSSLEETIASLKAEVEGFKQERQAVHSEMLLRVQQLVQDLK
KLTCQVATLNNNGEEASTEGTCCPVNWVEHQDSCYWFSHSGMSWAEAEKYCQLKNAHLVVINSREEQNFVQKYLG
SAYTWMGLSDPEGAWKWVDGTDYATGFQNWKPGQPDDWQGHGLGGGEDCAHFHPDGRWNDDVCQRPYHWVCE
AGLGQTSQESH

Isothermal Titration Calorimetry

Figure S28. ITC binding data for free glycan-MGL interactions. Isotherms corresponding to binding of (**A**) α/β-Gal*N*Ac (1.50 mM) with hMGL (29.6 μM), (**B**) Thr-Tn (1.50 mM) with hMGL (50.0 μM), (**C**) α-Me-Gal*N*Ac (1.50 mM) with hMGL (50.0 μM), and (**D**) β-Me-Gal*N*Ac (1.50 mM) with hMGL (50.0 μM). The titrations, integrated data, and signature plots are shown in the upper, middle, and lower panels. The titrations were performed in buffered (10 mM HEPES sodium salt, 50 mM NaCl, and 2 mM $CaCl_2$ at pH 7.4) H_2O at 25˚C.



n	0.99
K_a (10^4 M^{-1})	5.65 ± 0.28
ΔH (kcal/mol)	-11.30 ± 0.13
-TΔS (kcal/mol)	4.81
K_d (μM)	**17.70 ± 0.28**

n	1.02
K_a (10^4 M^{-1})	6.17 ± 0.01
ΔH (kcal/mol)	-11.08 ± 0.49
-TΔS (kcal/mol)	4.30
K_d (μM)	**16.20 ± 0.01**

n	1.03
K_a (10^4 M^{-1})	12.90 ± 0.40
ΔH (kcal/mol)	-11.60 ± 0.48
-TΔS (kcal/mol)	4.65
K_d (μM)	**7.80 ± 0.40**

n	0.97
K_a (10^4 M^{-1})	7.10 ± 0.26
ΔH (kcal/mol)	-11.62± 0.45
-TΔS (kcal/mol)	5.01
K_d (μM)	**14.10 ± 0.26**

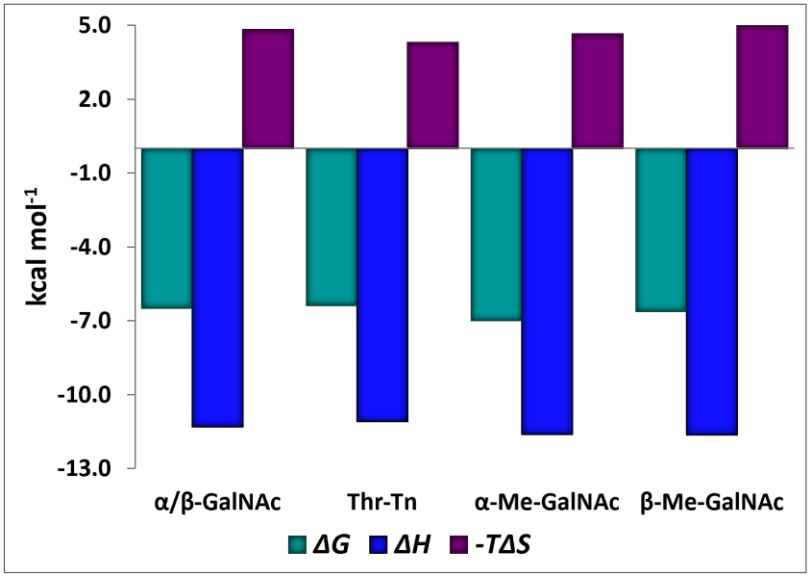


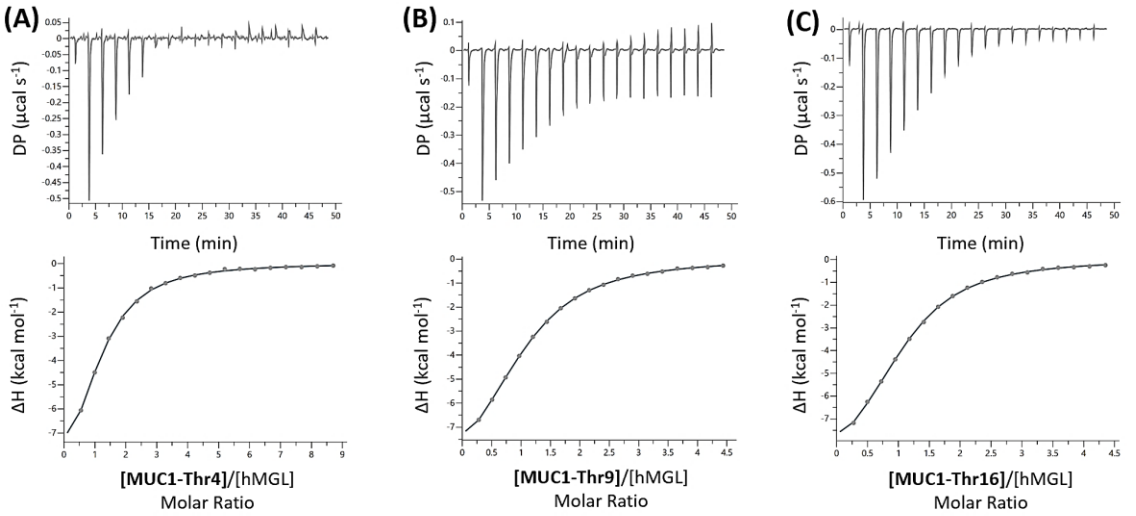
Figure S29. ITC binding data for MUC1 glycopeptide-MGL interactions. Isotherms corresponding to binding of (**A**) MUC1-Thr4 (0.50 mM) with hMGL (11.5 μM), (**B**) MUC1-Thr9 (0.44 mM) with hMGL (19.0 μM), (**C**) MUC1-Thr16 (0.50 mM) with hMGL (22.0 μM), (**D**) MUC1-Thr9,16 (0.50 mM) with hMGL (19.0 μM), (**E**) MUC1-Thr4,16 (0.50 mM) with hMGL (20.0 μM), (**F**) MUC1-Thr4,9 (0.50 mM) with hMGL (11.0 μM), and (**G**) MUC1-Thr4,9,16 (0.25 mM) with hMGL (20.0 μM). The titrations, integrated data, and signature plots are shown in the upper, middle, and lower panels. The titrations were performed in buffered (10 mM HEPES sodium salt, 50 mM NaCl, and 2 mM $CaCl_2$ at pH 7.4) H_2O at 25˚C**.**

MUC1-Thr4: **HGVT*SAPDTRPAPGSTAPPA** **MUC1-Thr9,16:** **HGVTSAPDT*RPAPGST*APPA**

MUC1-Thr9: **HGVTSAPDT*RPAPGSTAPPA** **MUC1-Thr4,16:** **HGVT*SAPDTRPAPGST*APPA**

MUC1-Thr16: **HGVTSAPDTRPAPGST*APPA** **MUC1-Thr4,9:** **HGVT*SAPDT*RPAPGSTAPPA**

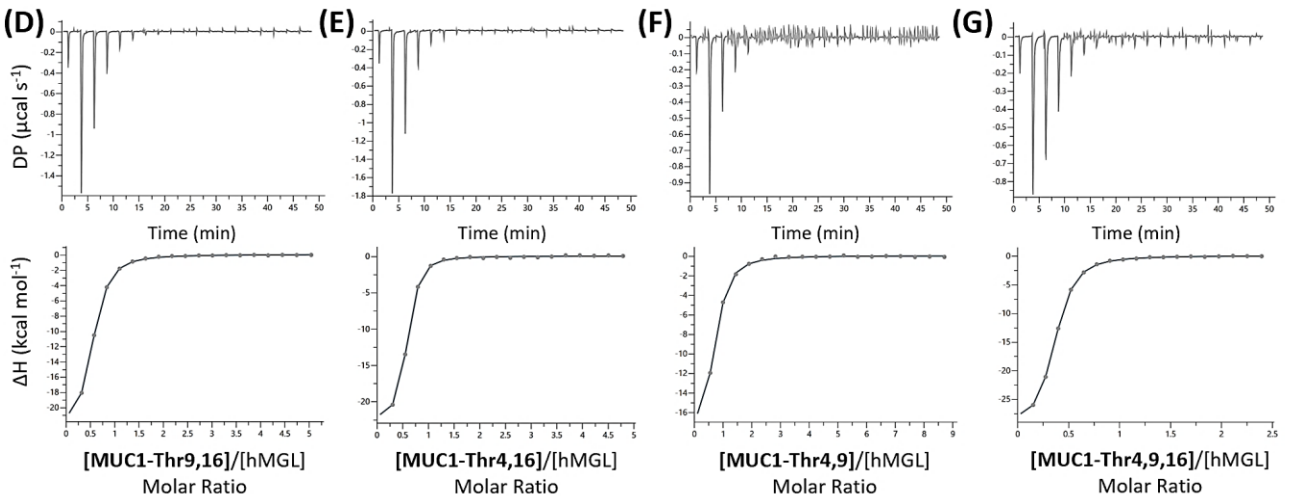
MUC1-Thr4,9,16: **HGVT*SAPDT*RPAPGST*APPA**



n	0.99
K_a (10^4 M^{-1})	14.66
ΔH (kcal/mol)	-11.40 ± 0.35
-TΔS (kcal/mol)	4.38
K_d (μM)	**6.82** ± 0.29

n	1.01
K_a (10^4 M^{-1})	11.87
ΔH (kcal/mol)	-10.50 ± 0.16
-TΔS (kcal/mol)	3.53
K_d (μM)	**8.42** ± 0.25

n	1.03
K_a (10^4 M^{-1})	14.47
ΔH (kcal/mol)	-10.00 ± 0.18
-TΔS (kcal/mol)	2.95
K_d (μM)	**6.91** ± 0.30



n	0.46
K_a (10^4 M^{-1})	81.30
ΔH (kcal/mol)	-24.00±0.16
-TΔS (kcal/mol)	16.00
K_d (µM)	**1.23 ± 0.02**

n	0.48
K_a (10^4 M^{-1})	144.92
ΔH (kcal/mol)	-22.80±0.20
-TΔS (kcal/mol)	14.30
K_d (µM)	**0.69 ± 0.02**

n	0.50
K_a (10^4 M^{-1})	70.92
ΔH (kcal/mol)	-20.70±1.12
-TΔS (kcal/mol)	12.70
K_d (µM)	**1.41 ± 0.13**

n	0.32
K_a (10^4 M^{-1})	166.67
ΔH (kcal/mol)	-30.30±0.11
-TΔS (kcal/mol)	21.80
K_d (µM)	**0.60 ± 0.01**

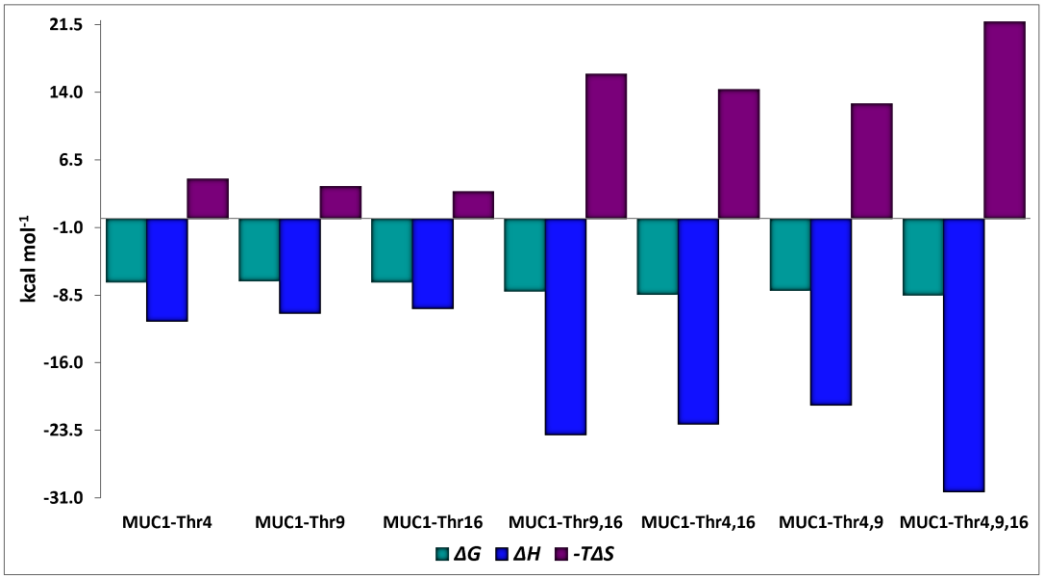
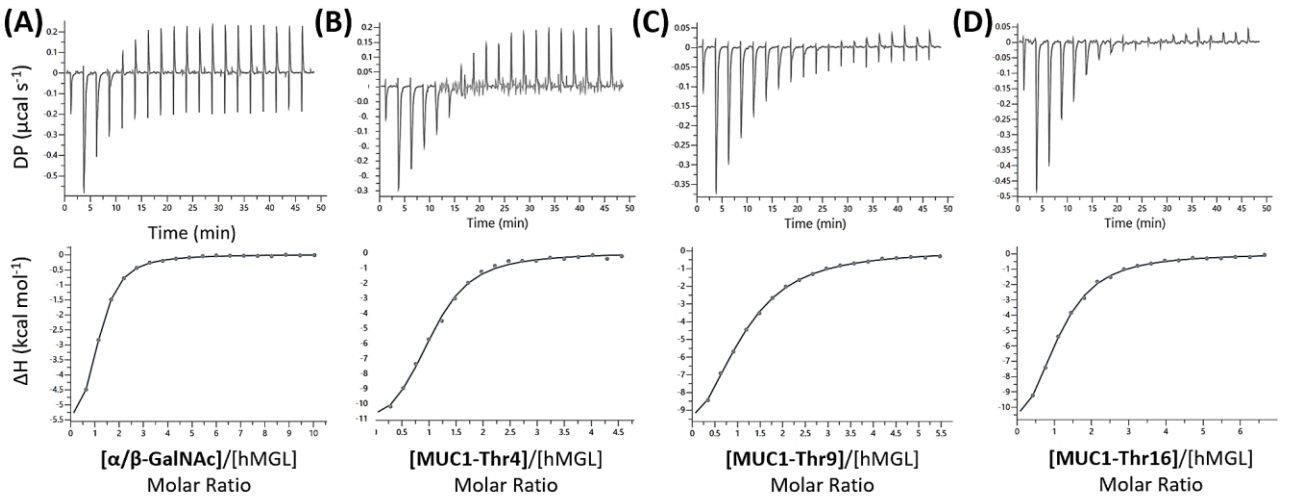


Figure S30. Thermodynamics of free Gal*N*Ac and MUC1 glycopeptide-hMGL interactions. Isotherms corresponding to binding of (**A**) α/β-Gal*N*Ac (1.50 mM) with hMGL (28.6 μM), (**B**) MUC1-Thr4 (0.50 mM) with hMGL (21.0 μM), (**C**) MUC1-Thr9 (0.50 mM) with hMGL (17.5 μM), (**D**) MUC1-Thr16 (0.50 mM) with hMGL (14.4 μM), (**E**) MUC1-Thr9,16 (0.50 mM) with hMGL (20.0 μM), (**F**) MUC1-Thr4,16 (0.50 mM) with hMGL (19.0 μM), (**G**) MUC1-Thr4,9 (0.50 mM) with hMGL (24.5 μM), and (**H**) MUC1-Thr4,9,16 (0.25 mM) with hMGL (21.0 μM). The titrations, integrated data, and signature plots are shown in the upper, middle, and lower panels. The titrations were performed in buffered (10 mM HEPES sodium salt, 50 mM NaCl, and 2 mM $CaCl_2$ at pH 7.4) D_2O at 25˚C**.**

MUC1-Thr4: **HGVT*SAPDTRPAPGSTAPPA**
MUC1-Thr9: **HGVTSAPDT*RPAPGSTAPPA**
MUC1-Thr16: **HGVTSAPDTRPAPGST*APPA**
MUC1-Thr9,16: **HGVTSAPDT*RPAPGST*APPA**
MUC1-Thr4,16: **HGVT*SAPDTRPAPGST*APPA**
MUC1-Thr4,9: **HGVT*SAPDT*RPAPGSTAPPA**
MUC1-Thr4,9,16: **HGVT*SAPDT*RPAPGST*APPA**

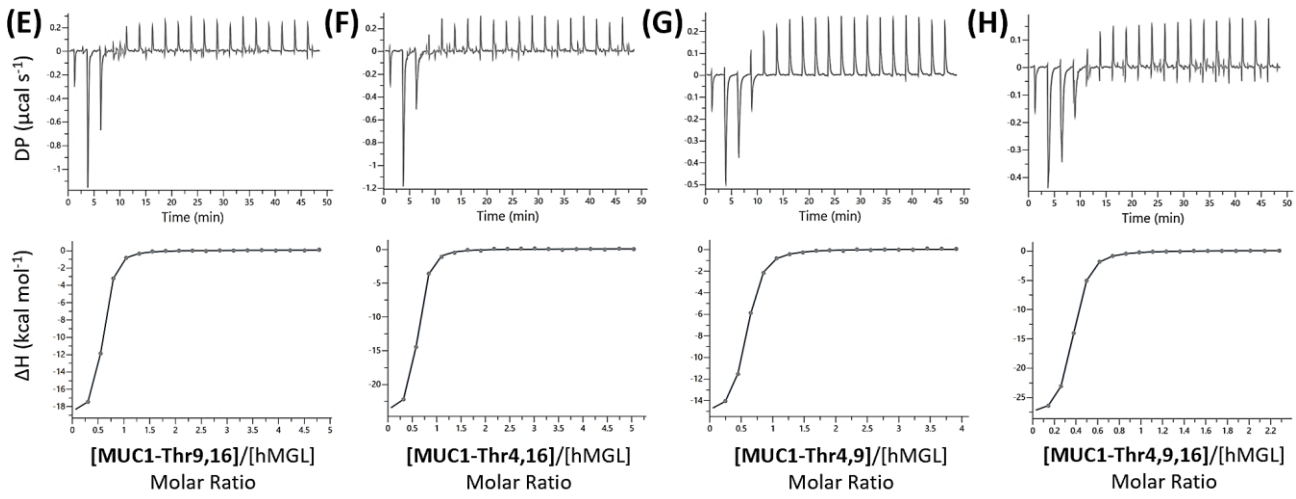


n	0.97
K_a (10^4 M^{-1})	13.79
ΔH (kcal/mol)	-6.81 ± 0.10
-TΔS (kcal/mol)	-0.20
K_d (μM)	**7.25 ± 0.02**

n	1.00
K_a (10^4 M^{-1})	26.88
ΔH (kcal/mol)	-12.50±0.50
-TΔS (kcal/mol)	5.13
K_d (μM)	**3.72 ± 0.47**

n	1.01
K_a (10^4 M^{-1})	10.84
ΔH (kcal/mol)	-14.10±0.55
-TΔS (kcal/mol)	7.22
K_d (μM)	**9.22 ± 0.61**

n	1.01
K_a (10^4 M^{-1})	16.75
ΔH (kcal/mol)	-14.18±0.63
-TΔS (kcal/mol)	7.63
K_d (μM)	**5.97 ± 0.48**

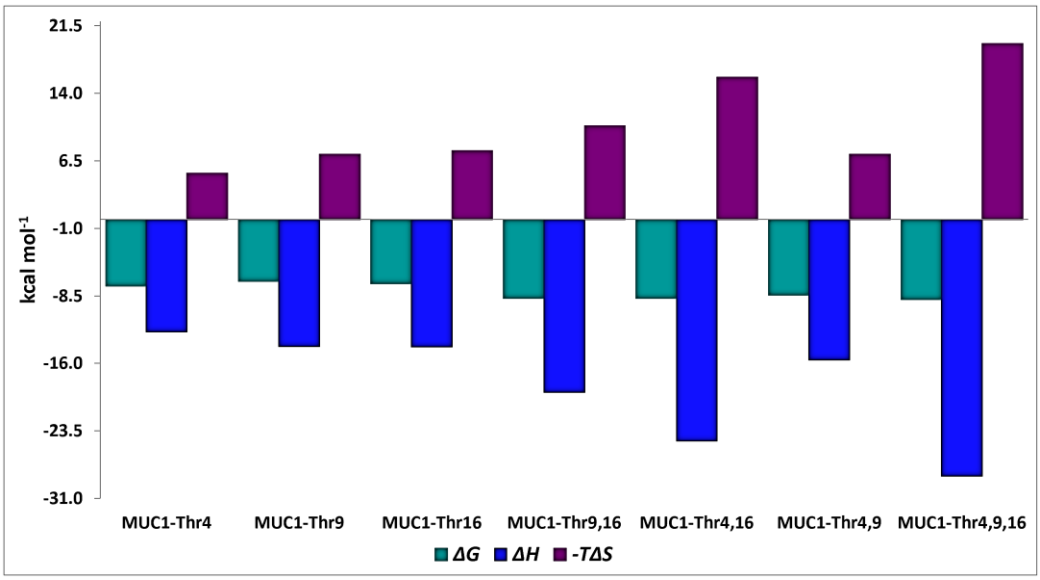


n	0.49
K_a (10^4 M^{-1})	263.15
ΔH (kcal/mol)	-19.20±0.01
-TΔS (kcal/mol)	10.40
K_d (µM)	**0.38 ± 0.01**

n	0.50
K_a (10^4 M^{-1})	263.15
ΔH (kcal/mol)	-24.60±0.17
-TΔS (kcal/mol)	15.80
K_d (µM)	**0.38 ± 0.02**

n	0.50
K_a (10^4 M^{-1})	144.50
ΔH (kcal/mol)	-15.60±0.01
-TΔS (kcal/mol)	7.22
K_d (µM)	**0.69 ± 0.03**

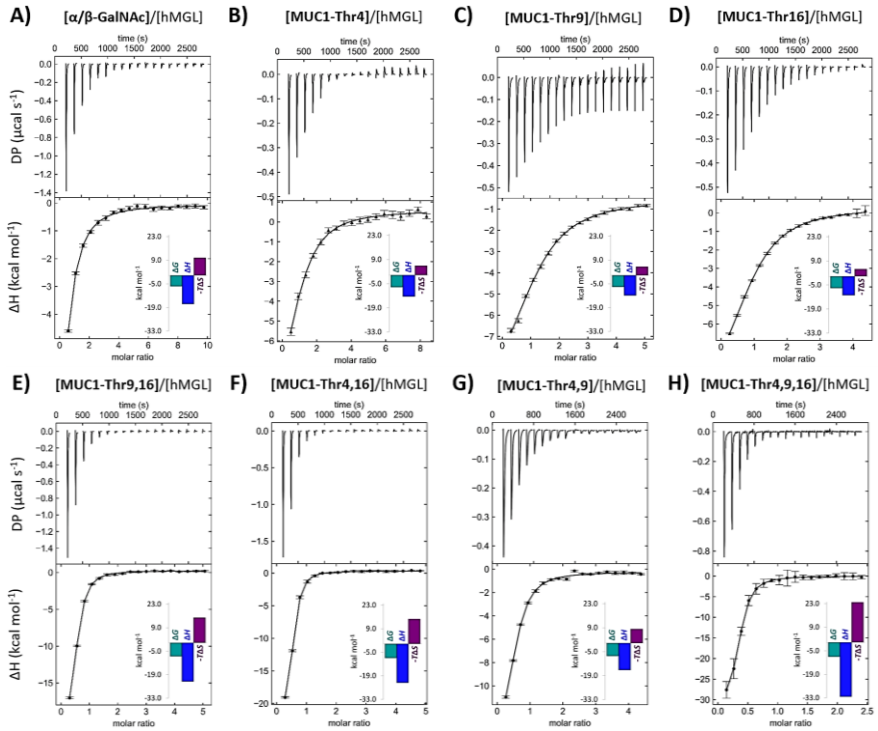
n	0.32
K_a (10^4 M^{-1})	338.98
ΔH (kcal/mol)	-28.50±0.01
-TΔS (kcal/mol)	19.50
K_d (µM)	**0.29 ± 0.01**



NITPIC, SEDPHAT, and GUSSI

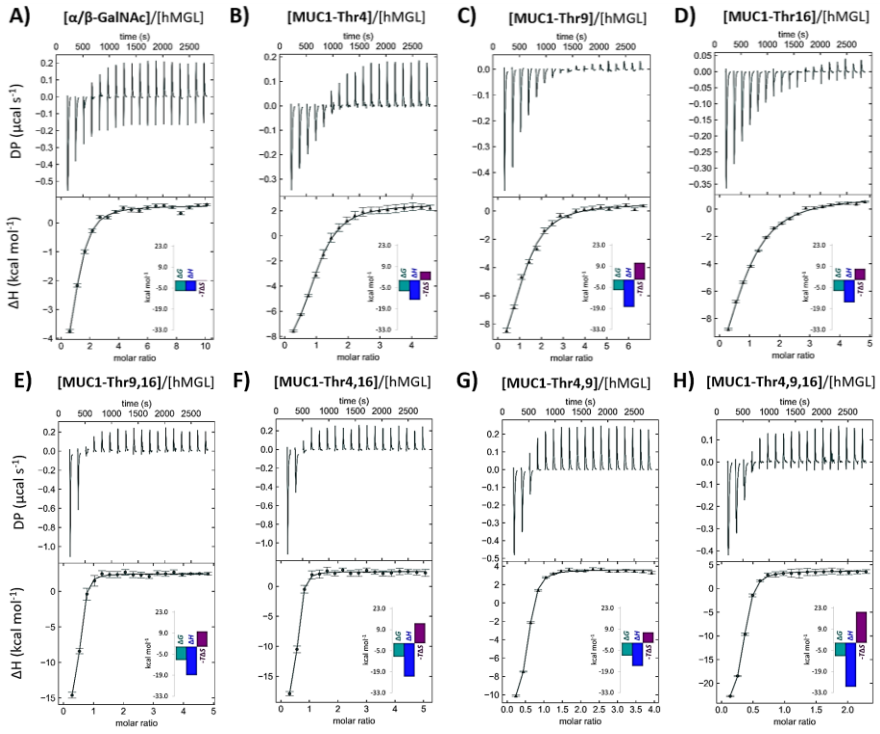
Figure S31. NITPIC/SEDPHAT/GUSSI thermodynamic values in water. Thermodynamics of MUC1 glycopeptide-hMGL interactions. Isotherms corresponding to binding of (**A**) α/β-Gal*N*Ac (1.50 mM) with hMGL (29.6 μM), (**B**) MUC1-Thr4 (0.50 mM) with hMGL (11.5 μM), (**C**) MUC1-Thr9 (0.44 mM) with hMGL (19.0 μM), (**D**) MUC1-Thr16 (0.50 mM) with hMGL (22.0 μM), (**E**) MUC1-Thr9,16 (0.50 mM) with hMGL (19.0 μM), (**F**) MUC1-Thr4,16 (0.50 mM) with hMGL (20.0 μM), (**G**) MUC1-Thr4,9 (0.50 mM) with hMGL (22.0 μM), and (**H**) MUC1-Thr4,9,16 (0.25 mM) with hMGL (20.0 μM). Monte-Carlo error analysis.

Ligand	*Entry*	K_a x10^4 M^{-1}	ΔG kcal mol^{-1}	ΔH kcal mol^{-1}	$-T\Delta S$ kcal mol^{-1}	*n*	K_d μM	$\chi 2$
GalNAc		5.23 ± 1.08e-3	-6.44	-16.88 ± 7.49e-2	10.44	0.96	19.11	0.416
MUC1-Thr4	**7**	12.89 ± 4.61e-4	-6.97	-12.38 ± 1.20e-2	5.41	0.91	8.90	0.321
MUC1-Thr9	**8**	10.25 ± 7.28e-4	-6.83	-11.82 ± 4.79e-3	4.99	1.17	9.75	0.351
MUC1-Thr16	**9**	10.27 ± 7.27e-5	-6.83	-11.01 ± 1.00e-3	4.17	0.91	11.30	0.129
MUC1-Thr9,16	**10**	78.18 ± 1.78e-5	-8.04	-23.06 ± 3.19e-4	15.03	0.46	2.80	0.444
MUC1-Thr4,16	**11**	135.20 ± 1.26e-5	-8.36	-2[illegible] ± 1.[illegible]e-4	14.72	0.47	1.50	0.212
MUC1-Thr4,9	**12**	33.97 ± 2.88e-5	-7.54	-15.77 ± 4.56e-4	8.22	0.49	2.94	1.24
MUC1-Thr4,9,16	**13**	163.80 ± 6.29e-5	-8.47	-32.26 ± 1.11e-3	23.78	0.33	0.61	0.034



NITPIC/SEDPHAT/GUSSI thermodynamic values in deuterium oxide. Thermodynamics of MUC1 glycopeptide-hMGL interactions. Isotherms corresponding to binding of (**A**) α/β-Gal*N*Ac (1.50 mM) with hMGL (28.6 μM), (**B**) MUC1-Thr4 (0.50 mM) with hMGL (21.0 μM), (**C**) MUC1-Thr9 (0.44 mM) with hMGL (17.5 μM), (**D**) MUC1-Thr16 (0.50 mM) with hMGL (14.4 μM), (**E**) MUC1-Thr9,16 (0.50 mM) with hMGL (20.0 μM), (**F**) MUC1-Thr4,16 (0.50 mM) with hMGL (19.0 μM), (**G**) MUC1-Thr4,9 (0.50 mM) with hMGL (24.5 μM), and (**H**) MUC1-Thr4,9,16 (0.25 mM) with hMGL (21.0 μM). Monte-Carlo error analysis.

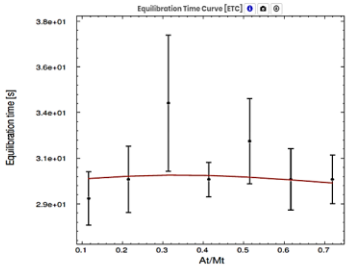
Ligand	*Entry*	K_a x10^4 M^{-1}	ΔG kcal mol^{-1}	ΔH kcal mol^{-1}	$-T\Delta S$ kcal mol^{-1}	*n*	K_d μM	$\chi 2$
GalNAc		11.45 ± 3.36e-4	-6.90	-6.91 ± 3.30e-3	0.01	0.97	8.60	1.11
MUC1-Thr4	**7**	23.59 ± 1.22e-4	-7.33	-13.01 ± 1.00e-3	5.68	0.96	3.70	0.156
MUC1-Thr9	**8**	9.89 ± 1.76e-4	-6.82	-18.02 ± 5.79e-3	11.21	0.82	12.60	0.942
MUC1-Thr16	**9**	19.47 ± 4.67e-4	-7.22	-14.74 ± 2.11e-3	7.33	1.07	5.50	0.646
MUC1-Thr9,16	**10**	265.90 ± 3.60e-5	-8.77	-18.86 ± 1.40e-4	10.10	0.47	0.38	0.126
MUC1-Thr4,16	**11**	325.70 ± 2.52e-5	-8.89	-22.13 ± 1.01e-4	13.25	0.44	0.62	0.185
MUC1-Thr4,9	**12**	136.80 ± 9.53e-6	-8.37	-15.26 ± 5.34e-5	6.88	0.50	1.40	0.996
MUC1-Thr4,9,16	**13**	257.00 ± 2.33e-5	-8.75	-29.11 ± 3.50e-4	20.37	0.32	1.10	0.159



AFFINImeter KinITC

Figure S32. Affinimeter KinITC data in water.

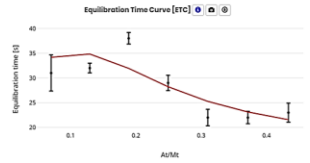
(A) **MUC1-Thr16 (9)**



Kinetic Information [χ^2 = 0.31]

k_{off} [s^{-1}]	k_{on} [$M^{-1}.s^{-1}$]	Instrument respo... time [s]
(0.5994 ± 1.9365)e+00	(0.9647 ± 3.1166)e+05	(6.2865 ± 1.3858)e+00

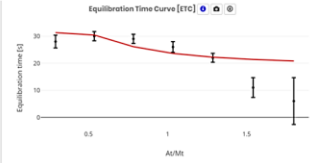
(B) **MUC1-Thr9,16 (10)**



Kinetic information

Param.	Value	± Error
χ^2	5.330e+0	--
k_{on} [$M^{-1}s^{-1}$]	3.200e+4	4.13e+3
k_{off} [s^{-1}]	4.862e-2	6.28e-3
Response time [s]	3.003e+0	3.87e-1

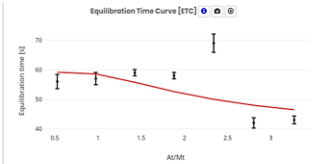
(C) **MUC1-Thr4,16 (11)**



Kinetic information

Param.	Value	± Error
χ^2	2.218e+0	--
k_{on} [$M^{-1}s^{-1}$]	5.866e+4	1.36e+4
k_{off} [s^{-1}]	5.142e-2	1.19e-2
Response time [s]	4.034e+0	4.24e-1

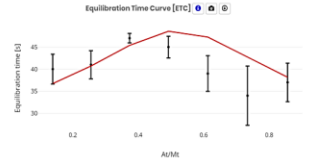
(D) **MUC1-Thr4,9 (12)**



Kinetic information

Param.	Value	± Error
χ^2	1.752e+1	--
k_{on} [$M^{-1}s^{-1}$]	1.641e+4	2.17e+3
k_{off} [s^{-1}]	6.006e-2	7.96e-3
Response time [s]	8.380e+0	4.53e-1

(E) **MUC1-Thr4,9,16 (13)**



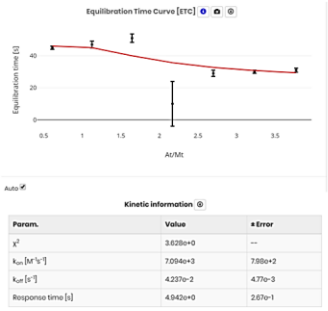
Kinetic information

Param.	Value	± Error
χ^2	1.466e+0	--
k_{on} [$M^{-1}s^{-1}$]	2.578e+4	5.66e+3
k_{off} [s^{-1}]	1.931e-2	4.24e-3
Response time [s]	4.269e+0	6.77e-1

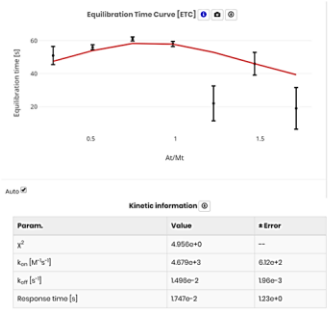
Figure S33. AFFINImeter KinITC data in deuterium.

Ligand	Entry	k_{on} $x10^6$ M^{-1} s^{-1}	k_{off} s^{-1}	τ s
		D_2O		
GalNAc		0.0070	0.0423	23.60
MUC1-Thr4	**7**	0.0046	0.0149	66.84
MUC1-Thr9	**8**	-	-	-
MUC1-Thr16	**9**	-	-	-
MUC1-Thr9,16	**10**	-	-	-
MUC1-Thr4,16	**11**	0.0446	0.0058	172.41
MUC1-Thr4,9	**12**	0.0257	0.0203	49.06
MUC1-Thr4,9,16	**13**	0.0351	0.0162	61.72

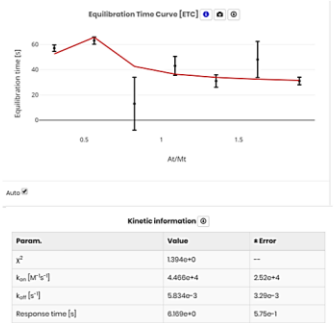
(A) **α/β-GalNAc**



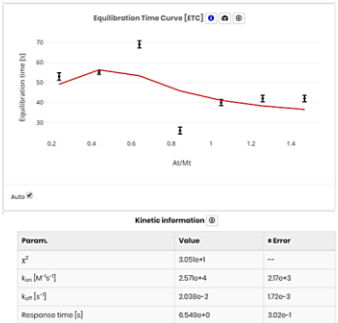
(B) **MUC1-Thr4 (7)**



(C) **MUC1-Thr4,16 (11)**



(D) **MUC1-Thr4,9 (12)**



(E) **MUC1-Thr4,9,16 (13)**