Supporting Information

A Facile Chemoenzymatic Synthesis of SARS-CoV-2 Glycopeptides for Probing Glycosylation Functions

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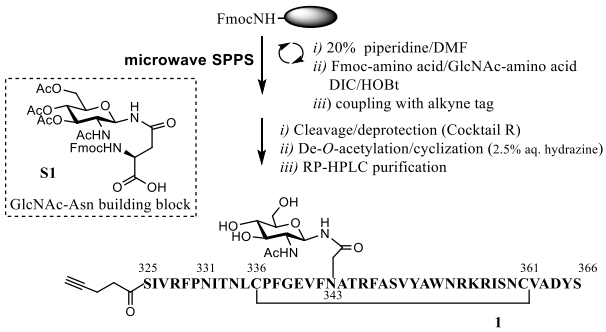
General Procedures.

Analytical reverse-phase HPLC was performed on a Waters Alliance® e2695 HPLC system equipped with a dual absorbance 2489 UV/Vis detector. Separations were performed using a C18 column (YMC-Triart C18, 4.6 × 250 mm, 5 μm) at a flow rate of 1 mL/min using a linear gradient of 10-50% MeCN containing 0.1% FA over 30 min. ESI-MS spectra were obtained using a Waters SQ Detector 2 single quadrupole mass spectrometer. MALDI-TOF analysis was performed using a Bruker UltrafleXtreme (UTX) mass spectrometer with TOF/TOF detection. Preparative RP-HPLC was performed on a Waters 600 HPLC system equipped with a dual absorbance UV detector using a C18 column (Waters-Symmetry Prep C18, 19 × 300 mm, 7 μm) at a flow-rate of 10 mL/min, or a C18 column (Waters XBridge, Prep Shield, 10 × 250 mm, 5 μm) at a flow-rate of 4 mL/min. The column was eluted using a linear gradient of 20-50% MeCN containing 0.1% FA over 30 min.

General Procedure for SPPS of SARS-CoV-2 GlcNAc-peptides

Peptide synthesis was performed under microwave synthesis conditions using a CEM Liberty Blue microwave peptide synthesizer. Synthesis was based on Fmoc chemistry using Rink Amide resin (0.66 mmol/g) on a 0.1 mmol scale. Couplings were performed using 5 equiv of Fmoc-protected amino acids (or 3 equiv of glycosylamino acids), 5 equiv of DIC and 5 equiv of HOBt in DMF. Double couplings were performed at 45°C for 20 min (2×) for Fmoc-Cys(Trt)-OH, Fmoc-His(Trt)-OH and Fmoc-Arg(Pbf)-OH. The selectively protected glycosylamino acid building block Fmoc-Asn(Ac_3GlcNAc)-OH (**S1**) was introduced at pre-determined sites and the coupling was performed at 45°C for 40 min. All other amino acids were coupled at 90 °C for 2 min. Fmoc deblocking was performed in 20% piperidine in DMF containing 0.1 M HOBt. The N-terminus was capped with an alkyne tag by treatment with 4-pentynoic acid following the same method as introduction of glycosylamino acid. After synthesis, the resin was washed with DMF (3×) and DCM (3×), dried. Resin cleavage and global peptide deprotection were achieved using freshly prepared cocktail R (TFA/Thioanisole/Ethanedithiol/Anisole [90/5/3/2]) and shaking for 2 h. The peptide was separated from the resin by filtration and the peptide was precipitated onto cold (-20°C) diethyl ether. A solution of the crude peptide in water (1 mg/mL) with 2.5% hydrazine was shaken at rt for 3 h to cyclize the peptide and/or remove the acetyl protecting group simultaneously. The crude peptides were purified by RP-HPLC and the purity and identity were confirmed by analytical HPLC and LC−MS analysis.



Scheme S1: SPPS of GlcNAc-peptide **1**

*GlcNAc-peptide **1** (42-mer-N343-GN).* Analytical RP-HPLC, t_R = 18.43 min. ESI-MS: calcd., M = 5108.80 (average isotopes); found, 858.91 $[M+K+5H]^{6+}$, 1022.90 $[M+5H]^{5+}$, 1278.31 $[M+4H]^{4+}$, 1703.88 $[M+3H]^{3+}$. Deconvolution mass, 5109.0.

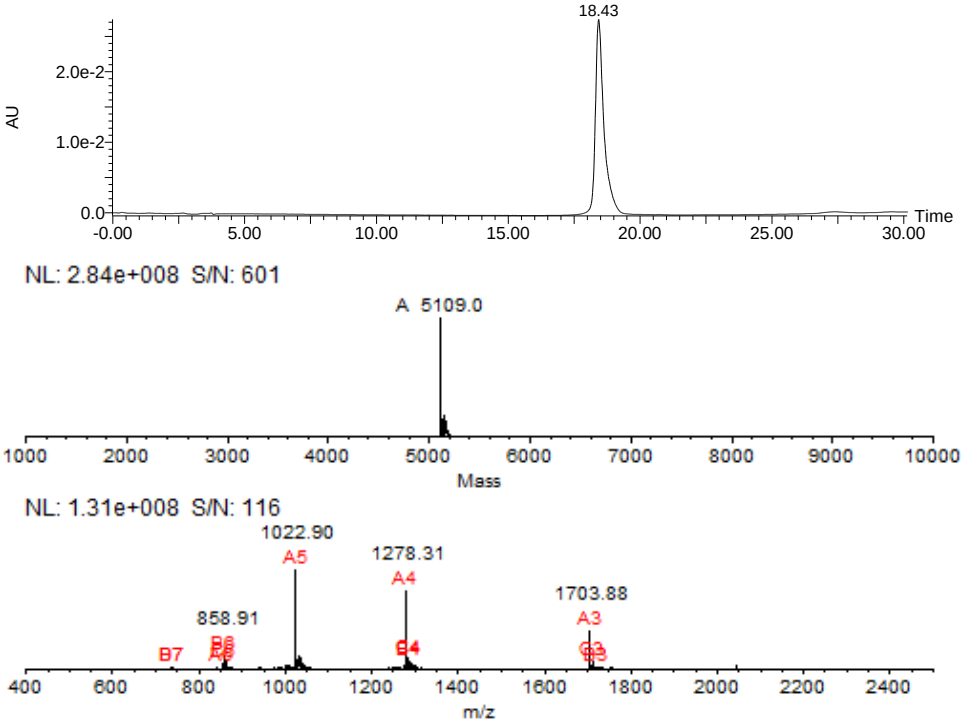
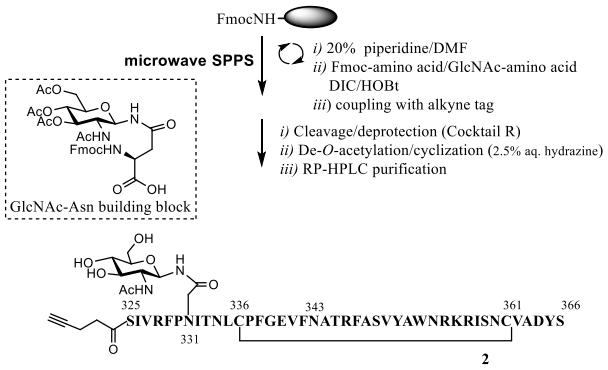


Figure S1. HPLC and ESI-MS profiles of GlcNAc-peptide **1**



Scheme S2: SPPS of GlcNAc-peptide **2**

*GlcNAc-peptide **2** (42-mer-N331-GN)*. Analytical RP-HPLC, t_R = 18.63 min. ESI-MS: calcd., M = 5108.80 (average isotopes); found, 858.97 $[M+K+5H]^{6+}$, 1022.65 $[M+5H]^{5+}$, 1278.24 $[M+4H]^{4+}$, 1704.00 $[M+3H]^{3+}$. Deconvolution mass, 5109.0.

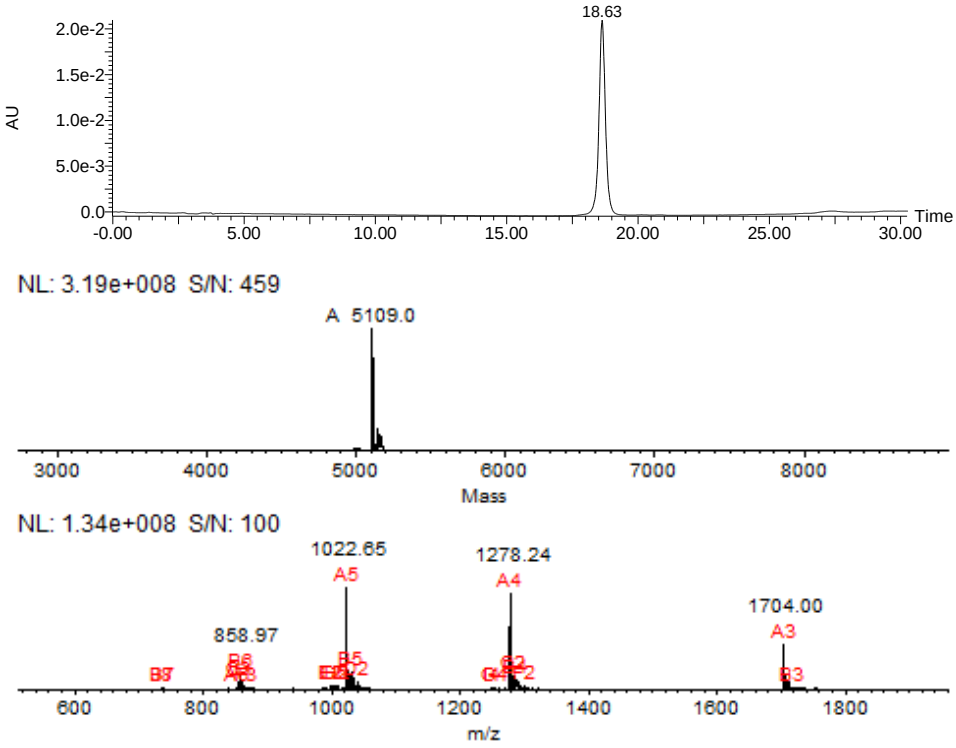
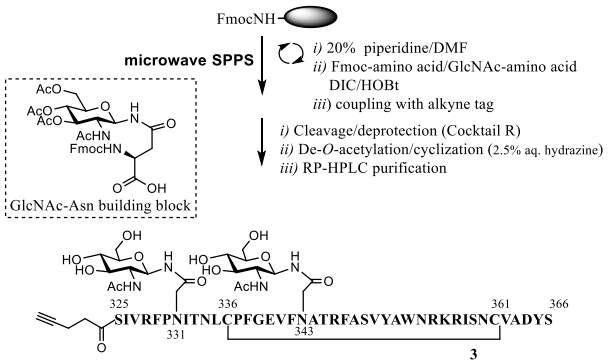


Figure S2. HPLC and ESI-MS profiles of GlcNAc-peptide **2**



Scheme S3: SPPS of GlcNAc-peptide **3**

*GlcNAc-peptide **3** (42-mer-N331-GN, N343-GN)*. Analytical RP-HPLC, t_R = 18.12 min. ESI-MS: calcd., M = 5311.99 (average isotopes); found, 892.74 $[M+K+5H]^{6+}$, 1063.41 $[M+5H]^{5+}$, 1329.08 $[M+4H]^{4+}$, 1771.67 $[M+3H]^{3+}$. Deconvolution mass, 5311.8.

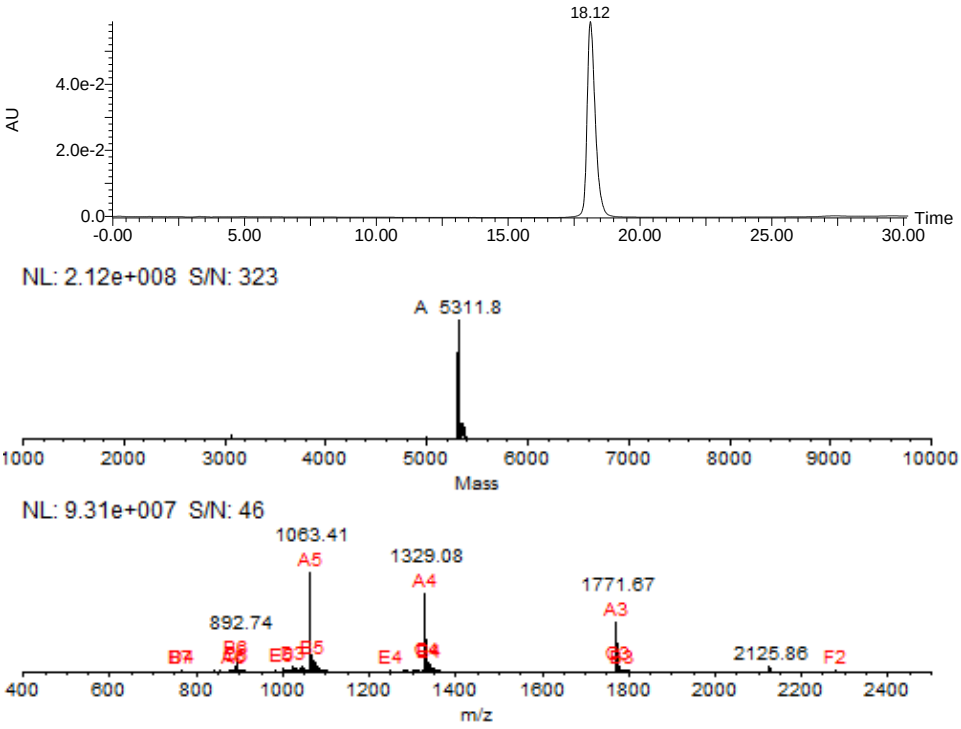
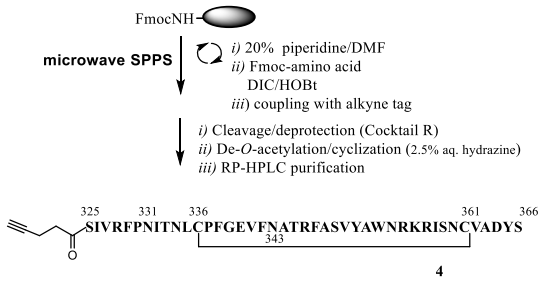


Figure S3. HPLC and ESI-MS profiles of GlcNAc-peptide **3**



Scheme S4: SPPS of GlcNAc-peptide **4**

*Peptide **4** (42-mer)*. Analytical RP-HPLC, t_R = 18.93 min. ESI-MS: calcd., M = 4905.60 (average isotopes); found, 825.20 $[M+K+5H]^{6+}$, 982.01 $[M+5H]^{5+}$, 1227.21 $[M+4H]^{4+}$, 1636.34 $[M+3H]^{3+}$. Deconvolution mass, 4905.2.

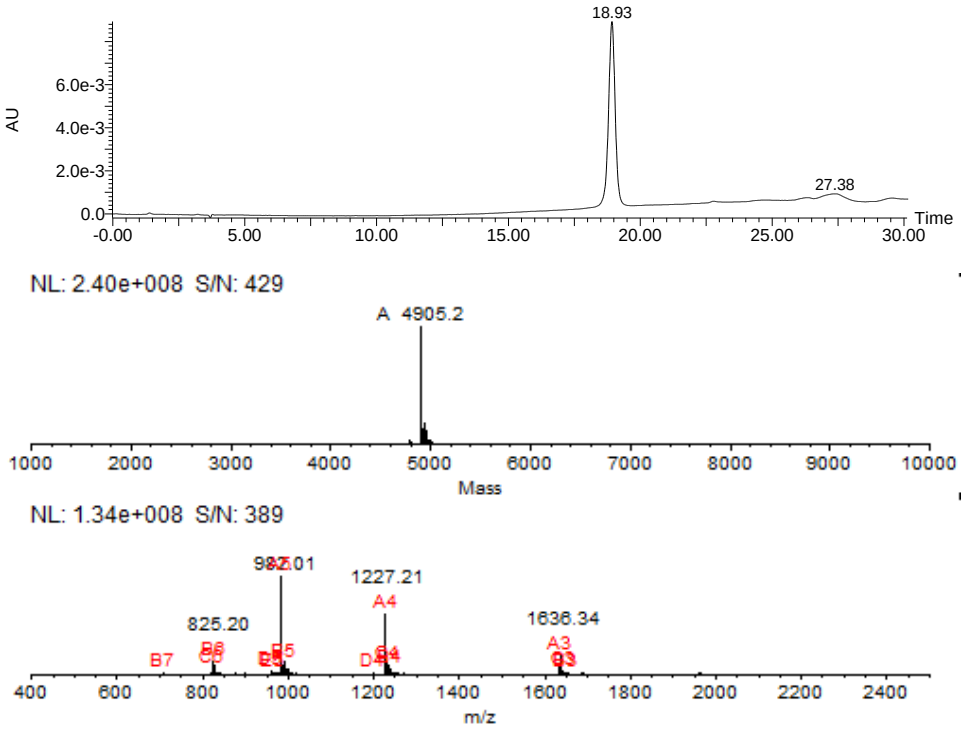


Figure S4. HPLC and ESI-MS profiles of peptide **4**

Preparation of Glycan Donors for Chemoenzymatic Reactions.

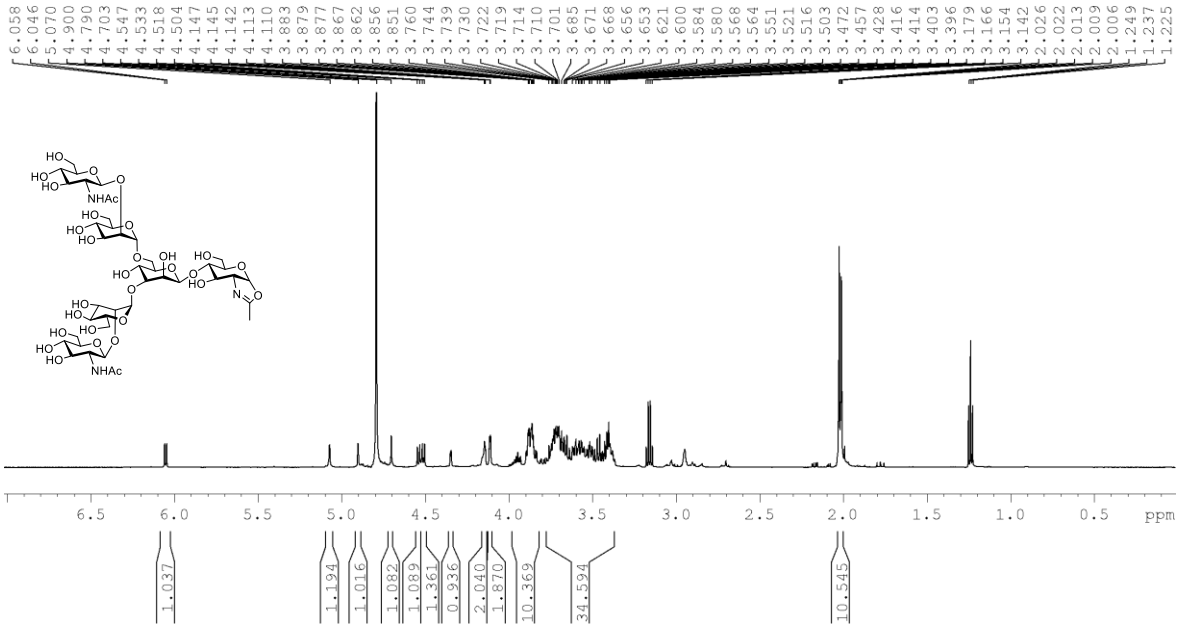
α-L-Fucosyl fluoride (**S2**) was synthesized over a three-step conversion from α-L-fucose following the literature reported procedure.[1]

High-mannose-type N-glycan (Man5GlcNAc) was prepared by Endo-A-catalyzed hydrolysis of soybean agglutinin (SBA) isolated from soybean flour followed by α1,2-mannosidase digestion.[2] The obtained glycan were converted into Man5-oxazoline following the previous method using DMC in the presence of triethylamine.[3]

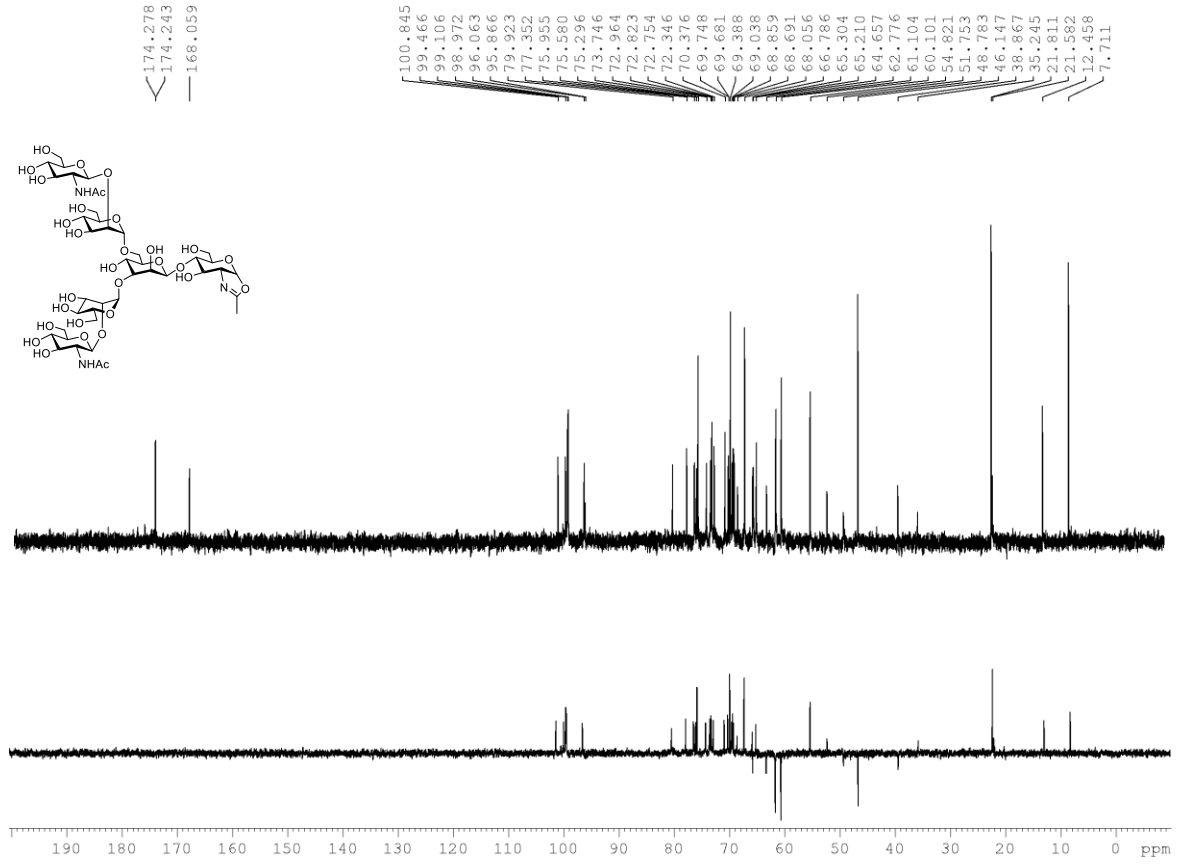
Galactosylated biantennary complex-type N-glycan (G2GlcNAc) prepared by Endo-S2-catalyzed hydrolysis of sialylglycopeptides (SGP) isolated from egg yolk powder[4] followed by neuraminidase digestion. The obtained glycan was converted into G2-oxazoline following the previous method using DMC in the presence of triethylamine.[3]

For the synthesis of degalactosylated biantennary complex-type N-glycan (G0GlcNAc)-oxazoline (**8**), a solution of SGP (200 mg) was dissolved in 4 mL PBS buffer (100 mM, pH = 7.4). Endo-S2 (final concentration of 0.1 mg/mL), neuraminidase (final concentration of 0.05 mg/mL) and *β*1,4-galactosidase (final concentration of 0.05 mg/mL) were added sequentially to the solution. The solution was incubated at 30 °C until LC-MS showed each step was complete. The enzymes were then deactivated at 95 °C for 3 min. The crude G0 glycan was purified by gel filtration on a Sephadex G-10 (GE Healthcare) column to remove hydrolyzed monosaccharides followed by cation exchange chromatography (HiTrap SP HP) to remove the cleaved peptides to give pure G0GlcNAc (66 mg, 85% over all steps). The G0-oxazoline (**8**) was prepared by treatment of a solution of G0GlcNAc (50 mg, 45 μmol) in water (1 mL) with triethylamine (2.2 mmol) and 2-chloro-1,3- dimethylimidazolinium chloride (DMC) (1.1 mmol) on ice for 30 min. The glycan oxazoline product was purified by gel filtration on a Sephadex G-10 column eluting with 0.1% triethylamine. The carbohydrate containing fractions were pooled and lyophilized to give **8** (45.8 mg, 93%). ^{1}H NMR (600 MHz, D_2O) δ 6.05 (d, *J* = 7.2 Hz, H-1-GlcNAc-Ox), 5.07 (s, 1H), 4.90 (s, 1H), 4.70 (s, 1H), 4.54 (d, *J* = 8.4 Hz), 4.51 (d, *J* = 8.4 Hz), 4.36 –4.35 (m, 1H), 4.18 – 4.10 (m, 4H), 3.95 – 3.82 (m, 10H), 3.77 – 3.35 (m, 34H), 2.05 – 1.98 (m, 9H). ^{13}C NMR (150 MHz, D_2O) δ 174.3, 174.2, 168.1, 100.9, 99.5, 99.1, 99.0, 96.1, 95.9, 79.9, 77.4, 76.0, 75.6, 75.3, 73.8, 73.0, 72.8, 72.7, 72.4, 70.4, 69.8, 69.7, 69.39, 69.0, 68.9, 68.7, 68.1, 66.8, 65.3, 65.2, 64.7, 62.8, 61.1, 60.1, 54.8, 51.8, 48.8, 38.9, 35.3, 21.8, 21.6, 12.5. HRMS (ESI-TOF) *m*/*z* $[M+H]^+$ Calcd for $C_{42}H_{70}N_3O_{30}^+$: 1096.4039, found: 1096.4025.

1H NMR spectrum of G0-Oxazoline **8**

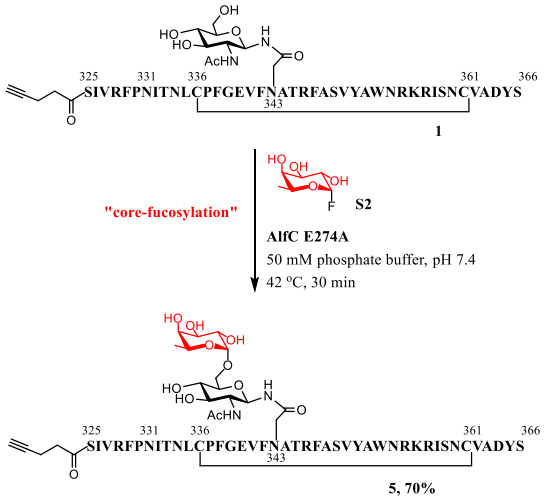


13C and 135DEPT NMR spectrum of G0-Oxazoline **8**



General Procedure for AlfC E274A Catalyzed Core Fucosylation of SARS-CoV-2 GlcNAc-peptides.

A solution of GlcNAc-peptide (1 μmol) and α-fucosyl fluoride (5 μmol) in phosphate buffer (50 mM, pH 7.4, 1 mL) was incubated with AlfC mutant E274A (final concentration, 0.2 mg/mL) at 42 °C. The reaction was monitored by RP-HPLC-MS. After 30 min, the reaction was quenched with 0.1% aq. TFA. The mixture was centrifuged and filtered through a 0.45 μm syringe filter. The filtrate was purified by RP-HPLC to give fucosylated GlcNAc-peptide.



Scheme S5: AlfC E274A Catalyzed Core Fucosylation of GlcNAc-peptide **5**

*Fucosylated GlcNAc-peptide **5** (42-mer-N343-GN-F)*. GlcNAc-peptide **1** (10 mg, 2.0 μmol) was converted to 7.2 mg fucosylated GlcNAc-peptide **5** (yield, 70%). Analytical RP-HPLC, t_R = 18.35 min. ESI-MS: calcd., M = 5254.94 (average isotopes); found, 1052.26 $[M+5H]^{5+}$, 1314.91 $[M+4H]^{4+}$, 1752.83 $[M+3H]^{3+}$. Deconvolution mass, 5255.4.

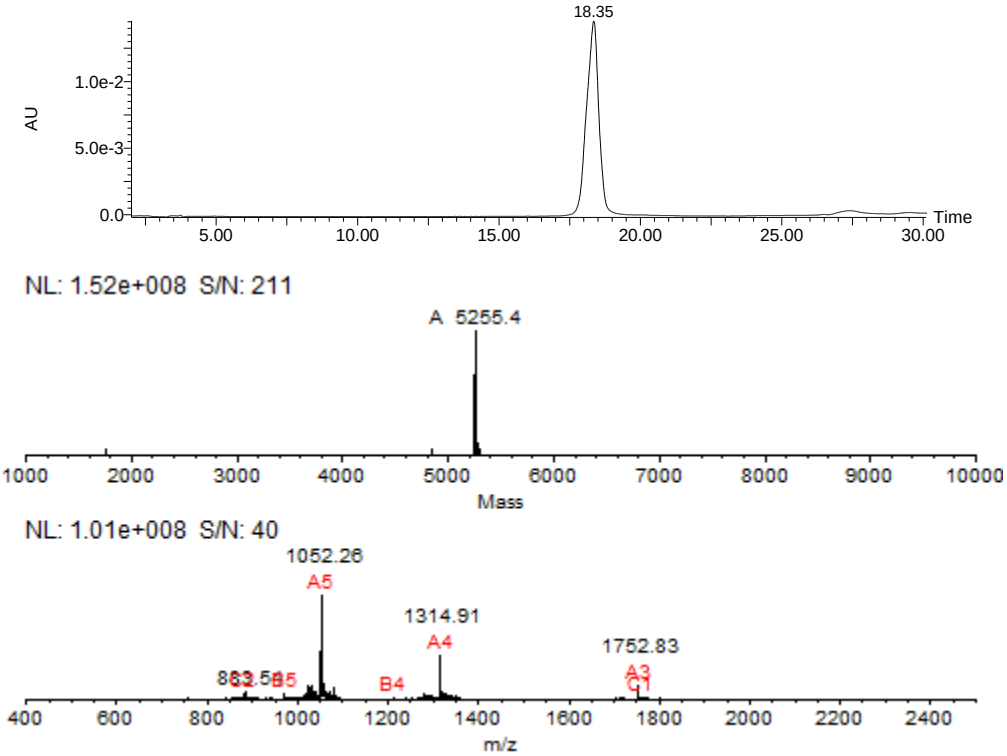
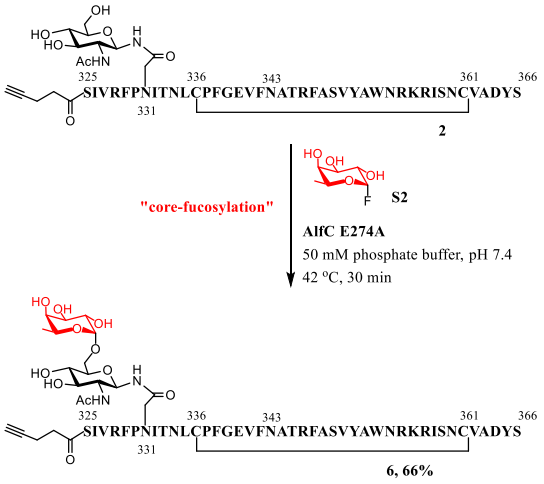


Figure S5. HPLC and ESI-MS profiles of peptide **5**



Scheme S6. AlfC E274A Catalyzed Core Fucosylation of GlcNAc-peptide **6**

*Fucosylated GlcNAc-peptide **6** (42-mer-N331-GN-F)*. GlcNAc-peptide **2** (2.5 mg, 0.5 μmol) was converted to 1.7 mg fucosylated GlcNAc-peptide **6** (yield, 66%). Analytical RP-HPLC, t_R = 18.6 min. ESI-MS: calcd., M = 5254.94 (average isotopes); found, 1052.13 $[M+5H]^{5+}$, 1314.91 $[M+4H]^{4+}$, 1752.39 $[M+3H]^{3+}$. Deconvolution mass, 5255.4.

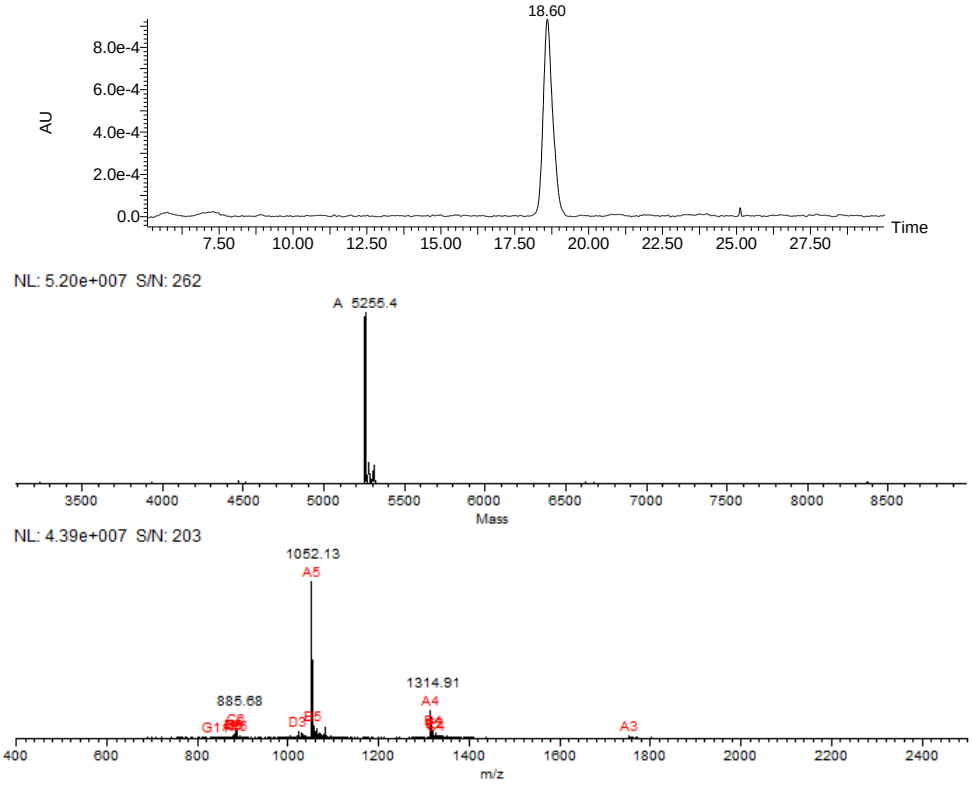
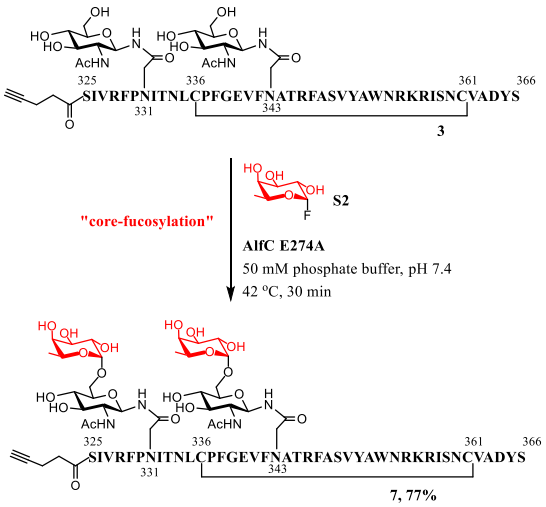


Figure S6. HPLC and ESI-MS profiles of core fucosylated GlcNAc-peptide **6**



Scheme S7. AlfC E274A Catalyzed Core Fucosylation of GlcNAc-peptide **7**

Fucosylated GlcNAc-peptide ***7*** *(42-mer-N331-GN-F, N343-GN-F).* GlcNAc-peptide **3** (5.4 mg, 1.0 μmol) was converted to 4.4 mg fucosylated GlcNAc-peptide **7** (yield, 77%). Analytical RP-HPLC, t_R = 17.92 min. ESI-MS: calcd., M = 5604.28 (average isotopes); found, 941.76 $[M+K+5H]^{6+}$, 1121.94 $[M+5H]^{5+}$, 1402.10 $[M+4H]^{4+}$, 1869.00 $[M+3H]^{3+}$. Deconvolution mass, 5604.4.

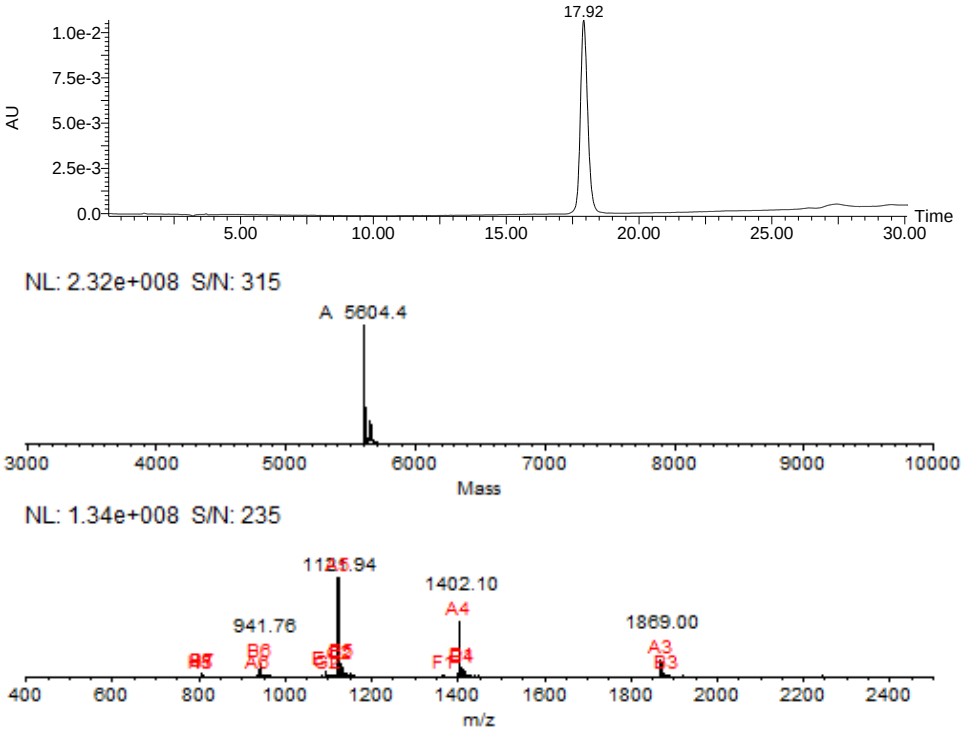
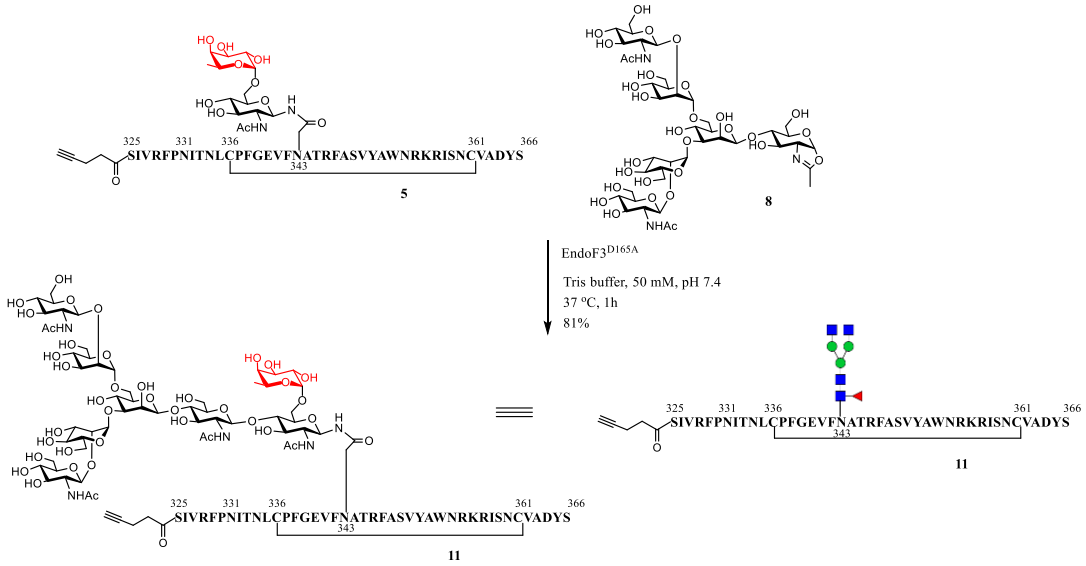


Figure S7. HPLC and ESI-MS profiles of core fucosylated GlcNAc-peptide **7**

General Procedure for Endo-F3 D165A Catalyzed transglycosylation of Fucosylated SARS-CoV-2 GlcNAc-peptides.

A solution of fucosylated GlcNAc-peptide (1 μmol) and G0-oxazoline (**8**, 3 μmol) or G2-oxazoline (**9**, 3 μmol) in Tris buffer (50 mM, pH 7.4, 0.5 mL) was incubated with Endo-F3 mutant D165A (final concentration, 0.2 mg/mL) at 37 °C. The reaction was monitored by RP-HPLC-MS. After 1 h, the reaction was quenched with 0.1% aq. TFA. The mixture was centrifuged and filtered through a 0.45 μm syringe filter. The filtrate was purified by RP-HPLC to give fucosylated glycopeptide.



Scheme S8. Chemoenzymatic synthesis of glycopeptide **11**

*Fucosylated Glycopeptide **11** (42-mer-N343-GN-F-G0).* Fucosylated GlcNAc-peptide **5** (7.0 mg, 1.3 μmol) was converted to 6.9 mg fucosylated glycopeptide **11** (yield, 81%). Analytical RP-HPLC, t_R = 18.15 min. ESI-MS: calcd., M = 6350.94 (average isotopes); found, 1271.31 $[M+5H]^{5+}$, 1588.77 $[M+4H]^{4+}$, 2117.99 $[M+3H]^{3+}$. Deconvolution mass, 6351.4.

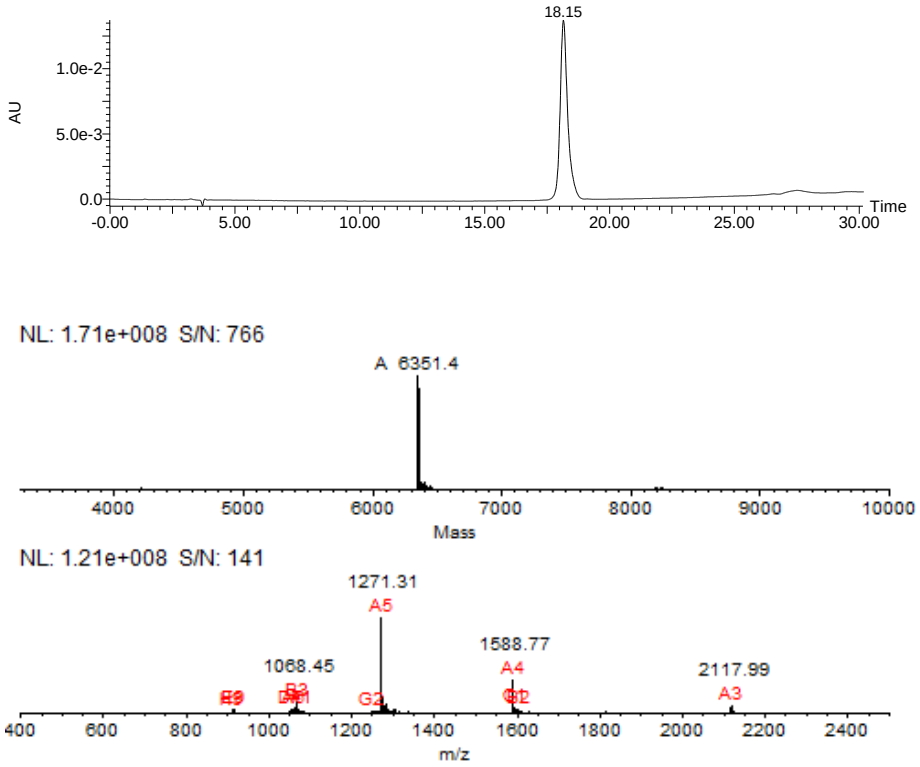
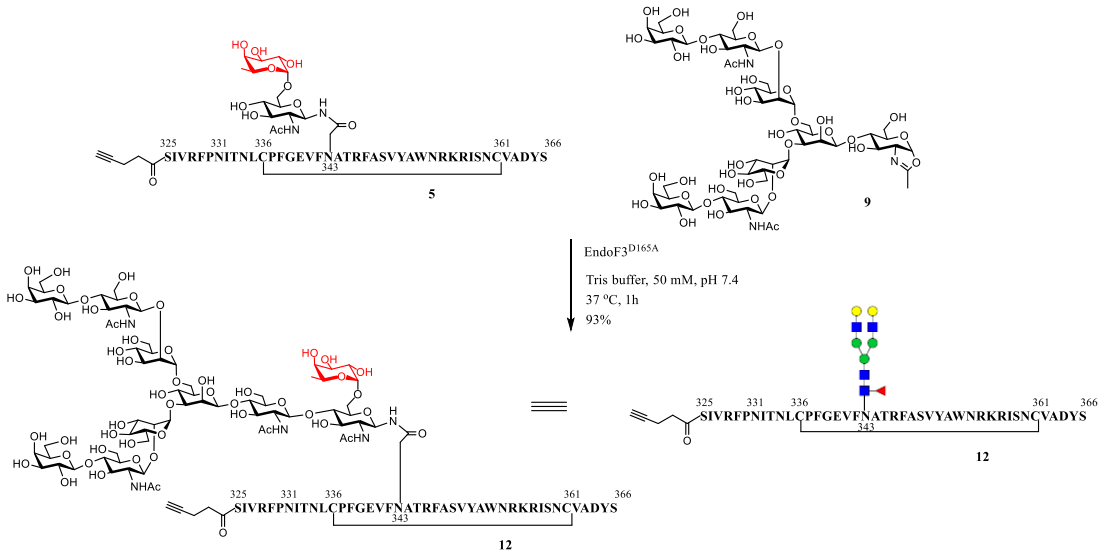


Figure S8. HPLC and ESI-MS profiles of core fucosylated glycopeptide **11**



Scheme S9. Chemoenzymatic synthesis of glycopeptide **12**

*Fucosylated Glycopeptide **12** (42-mer-N343-GN-F-G2)*. Fucosylated GlcNAc-peptide **5** (1.7 mg, 0.32 μmol) was converted to 2.0 mg fucosylated glycopeptide **12** (yield, 93%). Analytical RP-HPLC, t_R = 17.97 min. ESI-MS: calcd., M = 6675.23 (average isotopes); found, 1122.51 $[M+Na+K+4H]^{6+}$, 1335.89 $[M+5H]^{5+}$, 1669.98 $[M+4H]^{4+}$, 2225.97 $[M+3H]^{3+}$. Deconvolution mass, 6674.9.

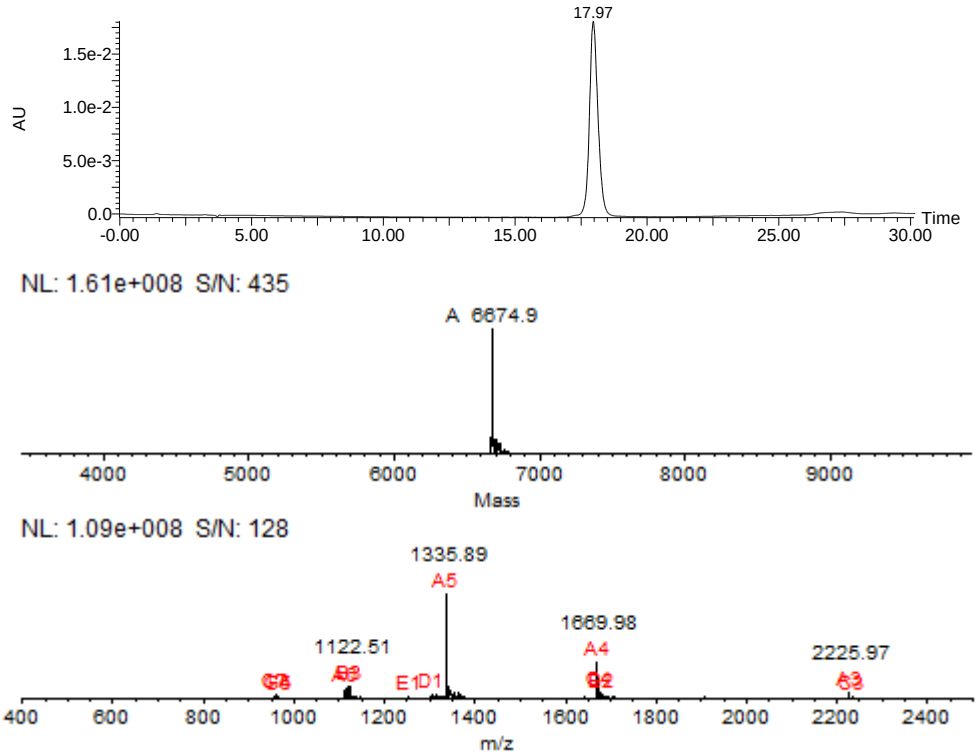
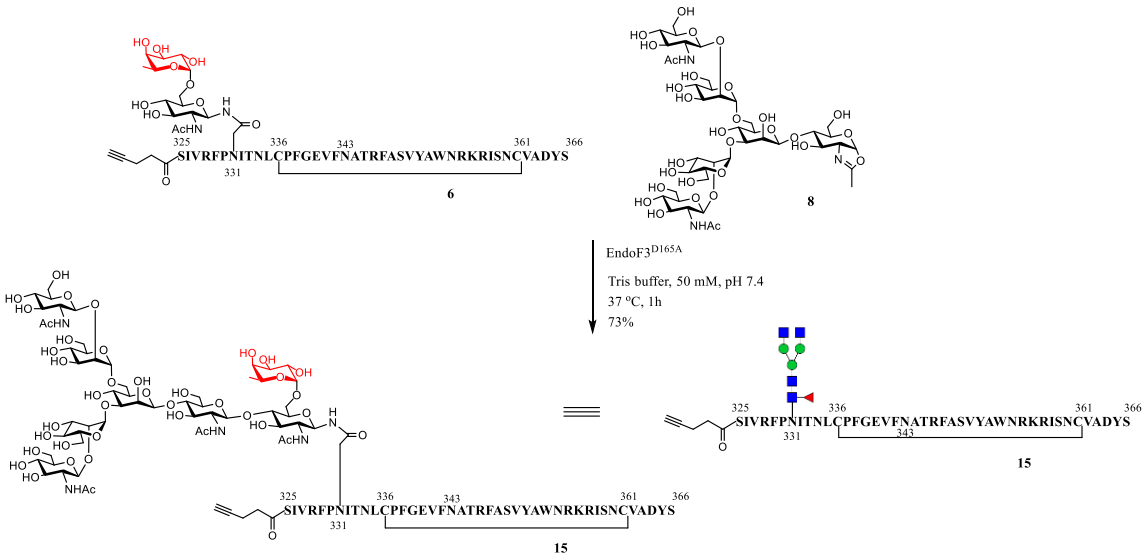


Figure S9. HPLC and ESI-MS profiles of core fucosylated glycopeptide **12**



Scheme S10. Chemoenzymatic synthesis of glycopeptide **15**

*Fucosylated Glycopeptide **15** (42-mer-N331-GN-F-G0)*. Fucosylated GlcNAc-peptide **6** (1.5 mg, 0.29 μmol) was converted to 1.4 mg fucosylated glycopeptide **15** (yield, 77%). Analytical RP-HPLC, t_R = 18.17 min. ESI-MS: calcd., M = 6350.94 (average isotopes); found, 1066.18 $[M+K+5H]^{6+}$, 1271.00 $[M+5H]^{5+}$, 1588.65 $[M+4H]^{4+}$, 2117.86 $[M+3H]^{3+}$. Deconvolution mass, 6350.9.

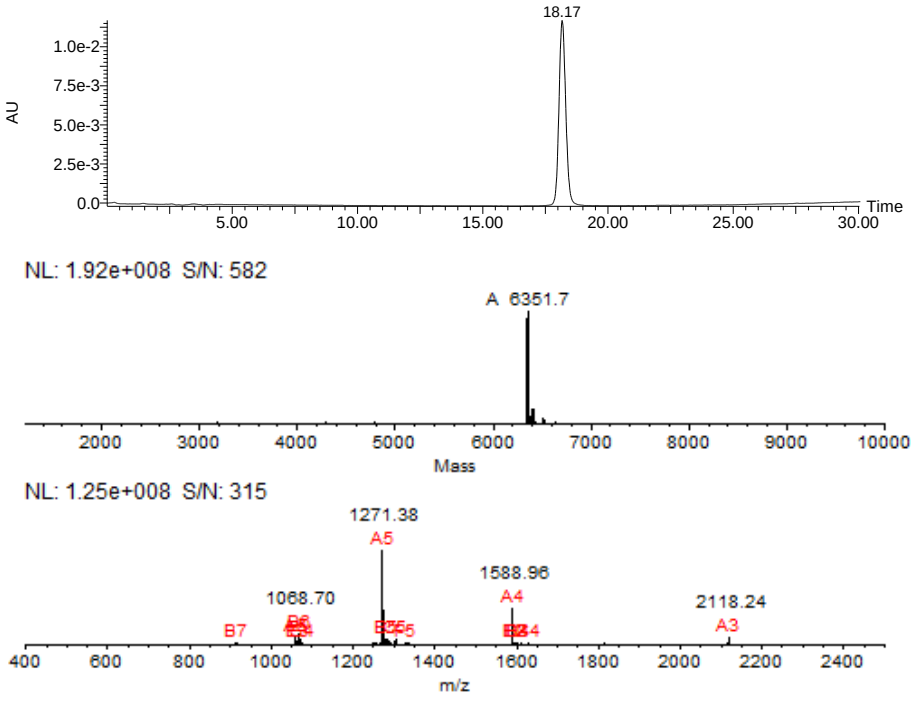
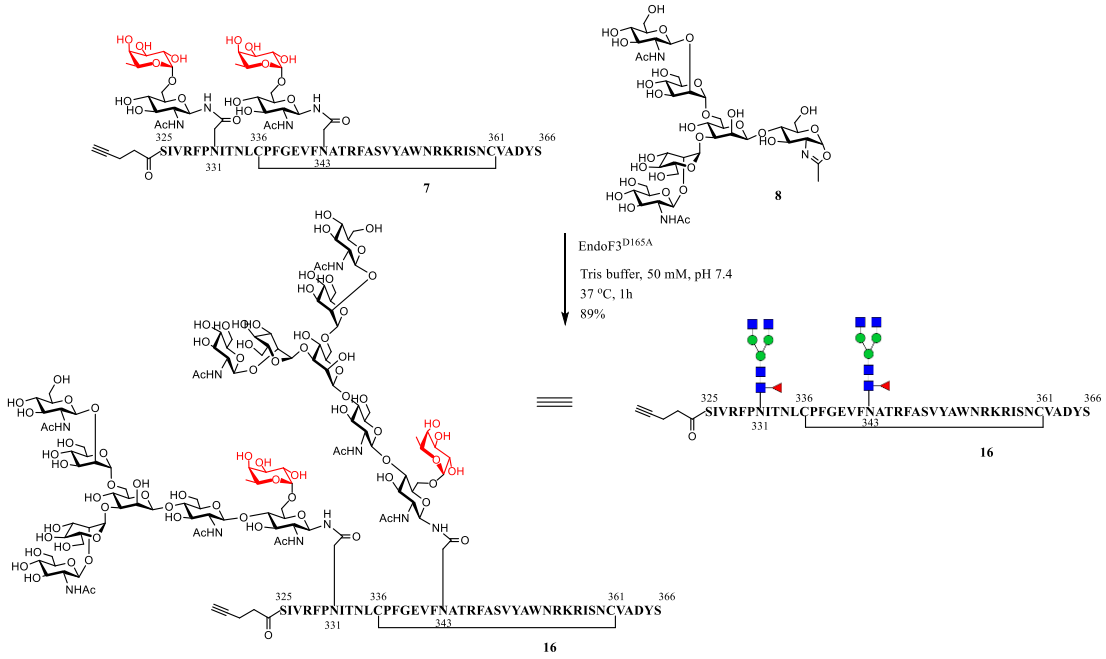


Figure S10. HPLC and ESI-MS profiles of core fucosylated glycopeptide **15**



Scheme S11. Chemoenzymatic synthesis of glycopeptide **16**

*Fucosylated Glycopeptide **16** (42-mer-N331-GN-F-G0, N343-GN-F-G0)*. Fucosylated GlcNAc-peptide **7** (3.0 mg, 0.54 μmol) was converted to 3.3 mg fucosylated glycopeptide **7** (yield, 79%). Analytical RP-HPLC, t_R = 17.37 min. ESI-MS: calcd., M = 7796.29 (average isotopes); found, 1120.36 $[M+K+6H]^{7+}$, 1300.55 $[M+6H]^{6+}$, 1560.11 $[M+5H]^{5+}$, 1949.96 $[M+4H]^{4+}$. Deconvolution mass, 7796.1.

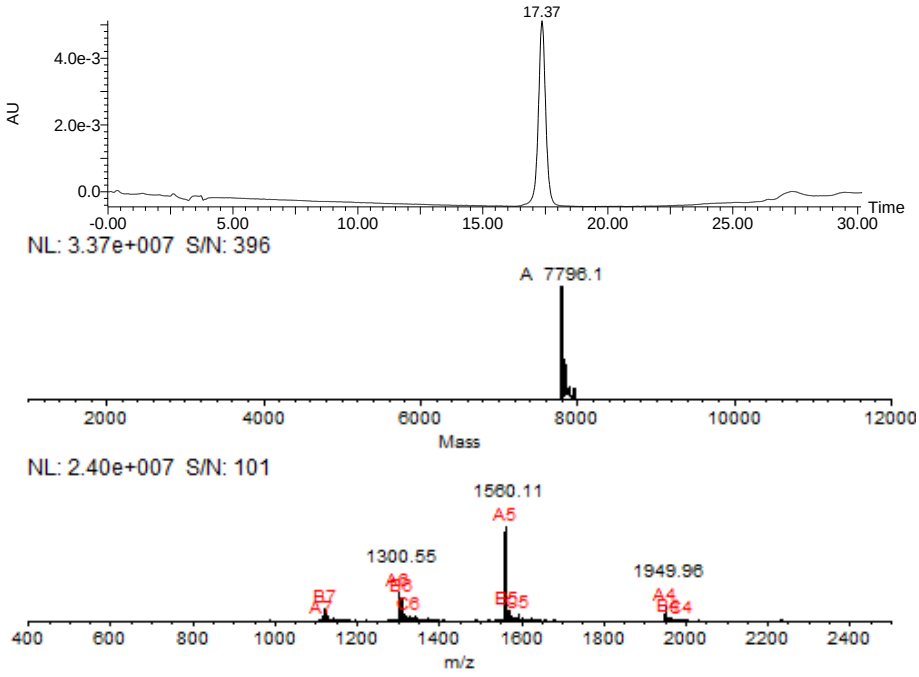
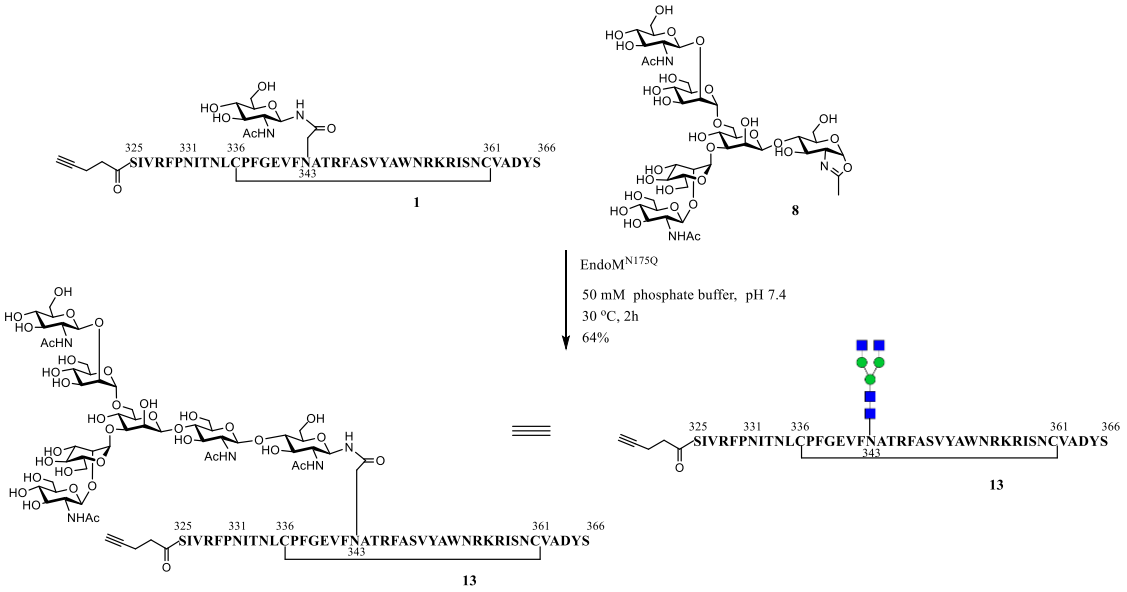


Figure S11. HPLC and ESI-MS profiles of core fucosylated glycopeptide **16**

General Procedure for EndoM-N175Q Catalyzed transglycosylation of SARS-CoV-2 GlcNAc-peptides.

A solution of GlcNAc-peptide (1 μmol) and glycan oxazoline (3 μmol) in a phosphate buffer (50 mM, pH 7.4, 0.5 mL) was incubated with EndoM mutant N175Q (final concentration, 0.1 mg/mL) at 30 °C. The reaction was monitored by RP-HPLC-MS. After 2 h, the reaction was quenched with 0.1% aq. TFA. The mixture was centrifuged and filtered through a 0.45 μm syringe filter. The filtrate was purified by RP-HPLC to give glycosylated peptide.



Scheme S12. Chemoenzymatic synthesis of glycopeptide **13**

*Fucosylated Glycopeptide **13** (42-mer-N343-GN-G0)*. GlcNAc-peptide **1** (4.0 mg, 0.78 μmol) was converted to 3.1 mg glycopeptide **13** (yield, 64%). Analytical RP-HPLC, t_R = 18.10 min. ESI-MS: calcd., M = 6204.80 (average isotopes); found, 1242.02 $[M+5H]^{5+}$, 1552.11 $[M+4H]^{4+}$, 2068.91 $[M+3H]^{3+}$. Deconvolution mass, 6204.3.

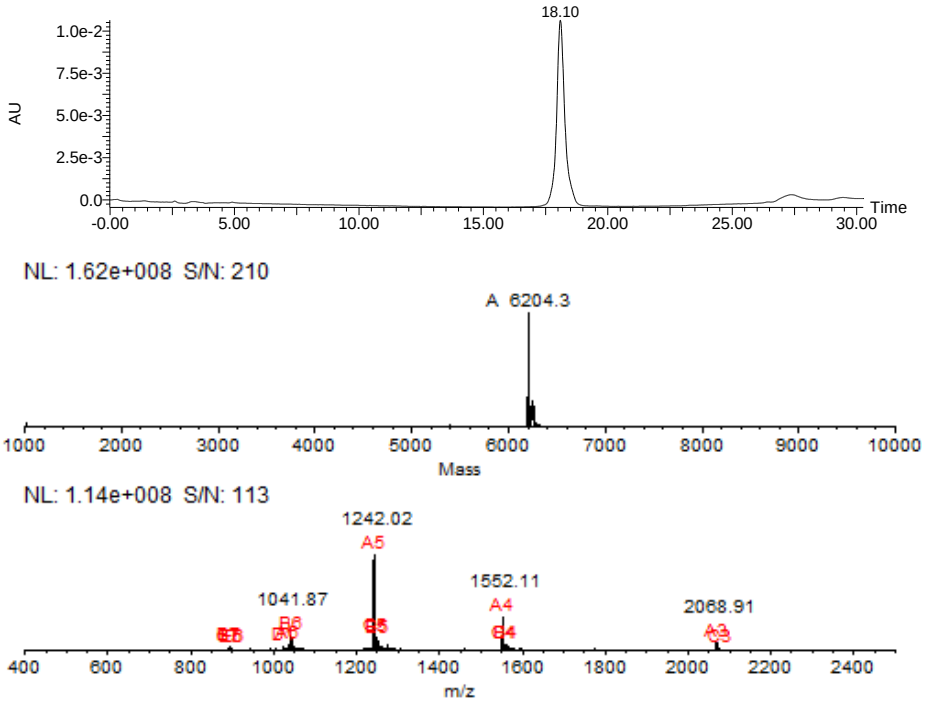
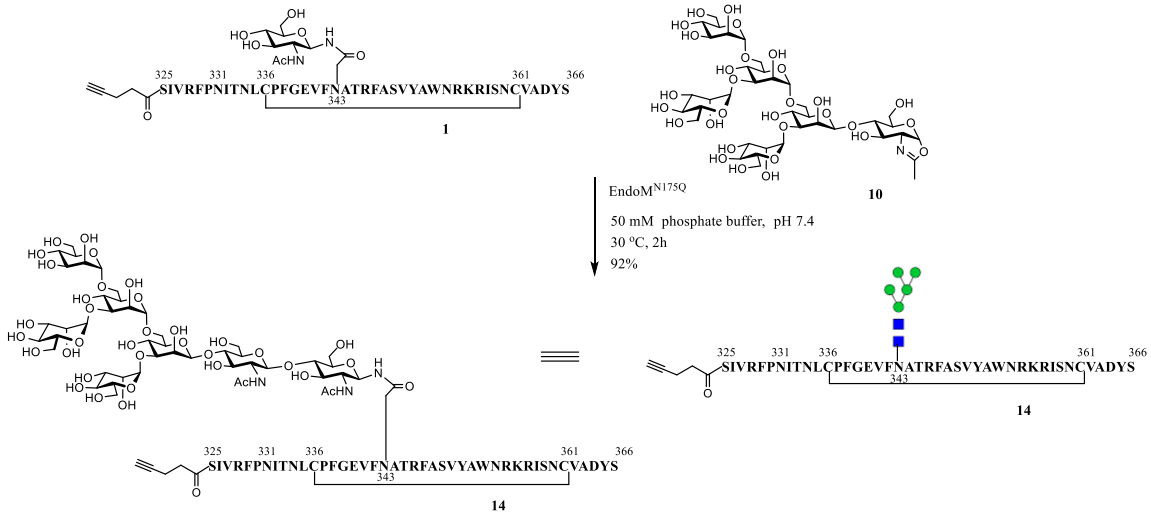


Figure S12. HPLC and ESI-MS profiles of core fucosylated glycopeptide **13**



Scheme S13. Chemoenzymatic synthesis of glycopeptide **14**

*Fucosylated Glycopeptide **14** (42-mer-N343-GN-Man5)*. GlcNAc-peptide **1** (4.0 mg, 0.78 μmol) was converted to 4.4 mg glycopeptide **14** (yield, 92%). Analytical RP-HPLC, t_R = 18.07 min. ESI-MS: calcd., M = 6122.70 (average isotopes); found, 1027.69 $[M+K+5H]^{6+}$, 1225.51 $[M+5H]^{5+}$, 1531.69 $[M+4H]^{4+}$, 2041.88 $[M+3H]^{3+}$. Deconvolution mass, 6122.8.

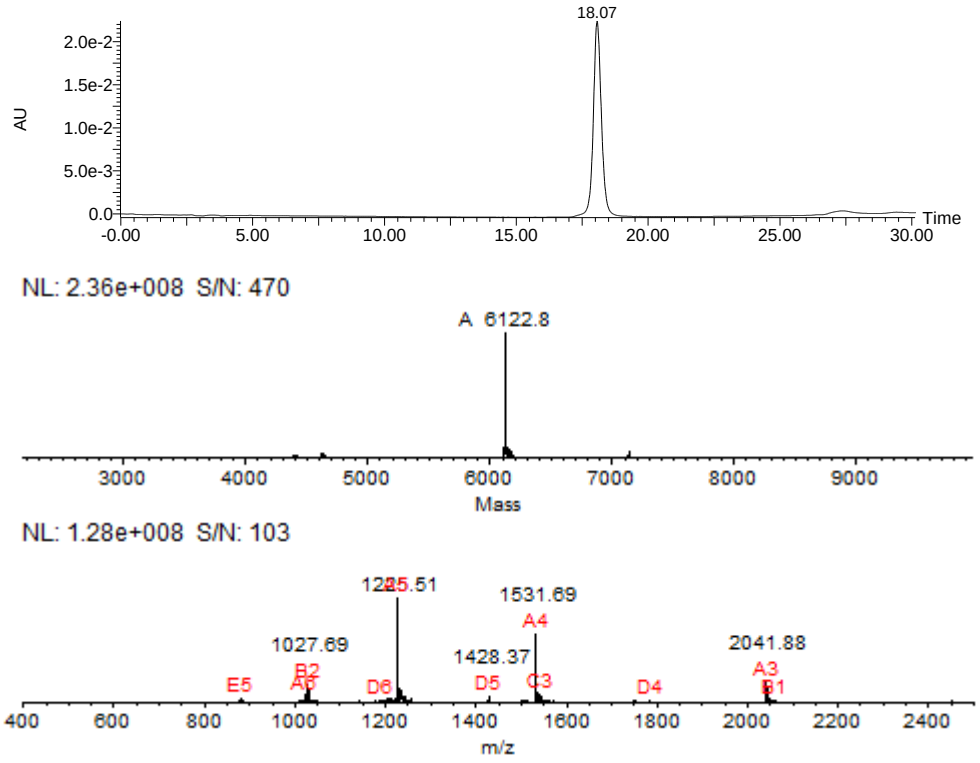
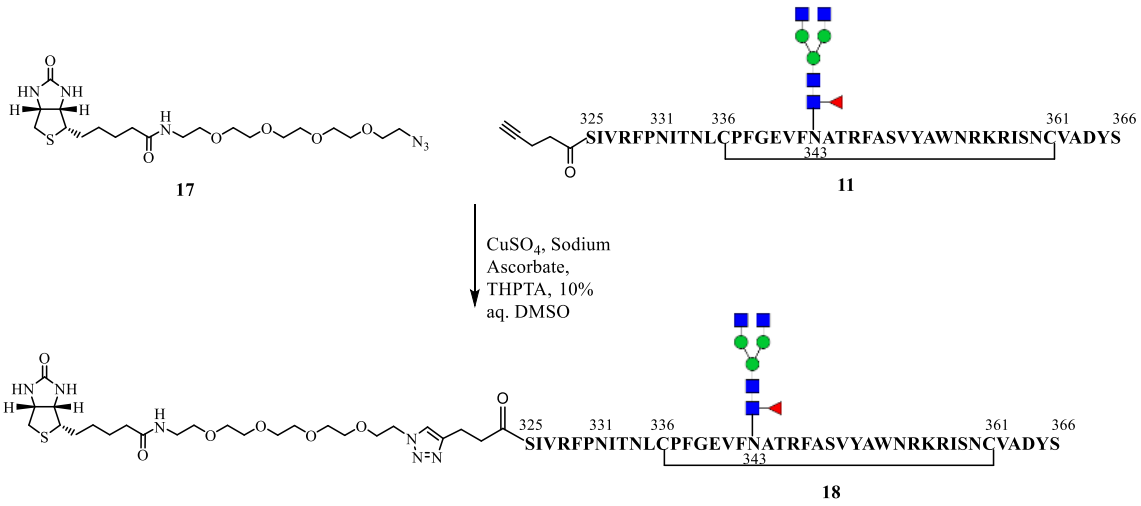


Figure S13. HPLC and ESI-MS profiles of core fucosylated glycopeptide **14**

General procedure for preparation of biotinylated glycopeptides:

To a solution of (glyco)peptide **4**, **11-16** (0.2 mg) and biotin-$(PEG)_4$-N_3 (**17**, 2 equiv) in a mixture of water/DMSO (9:1, v/v, 0.1 mL) was added an aqueous solution of $CuSO_4$ (0.2 equiv), THPTA (0.2 equiv) and sodium ascorbate (2 equiv) and the mixture was incubated at 37 ºC in dark. The reaction was monitored by RP-HPLC-MS. After 6 h, the reaction was diluted with water (2 mL) and filtered through a 0.45 μm syringe filter. The filtrate was purified by RP-HPLC to give biotinylated glycopeptide.



Scheme S14. Synthesis of biotinylated glycopeptide **18**

*Biotinylated glycopeptide **18** (Biotin-42-mer-N343-GN-F-G0)*. Glycopeptide **11** (0.20 mg, 0.031 μmol) was converted to 0.18 mg biotinylated glycopeptide **18** (yield, 84%). Analytical RP-HPLC, t_R = 18.13 min. ESI-MS: calcd., M = 6839.55 (average isotopes); found, 1141.22 $[M+6H]^{6+}$, 1369.22 $[M+5H]^{5+}$, 1711.37 $[M+4H]^{4+}$. Deconvolution mass, 6841.3.

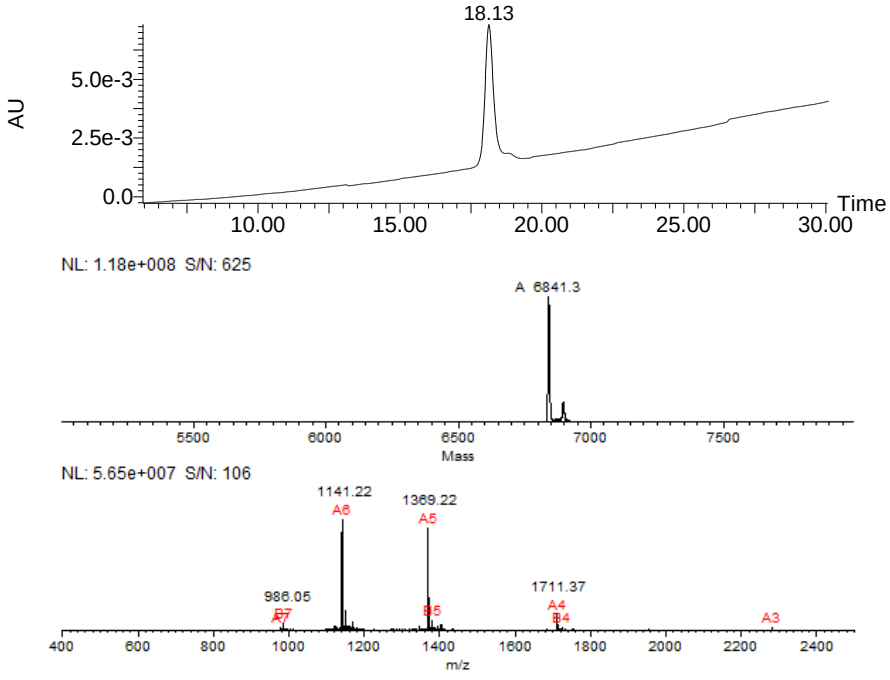
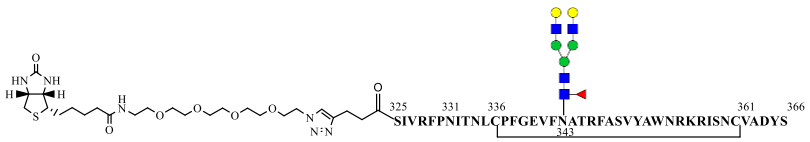


Figure S14. HPLC and ESI-MS profiles of biotinylated glycopeptide **18**



Biotinylated glycopeptide **19**

*Biotinylated glycopeptide **19** (Biotin-42-mer-N343-GN-F-G2)*. Glycopeptide **12** (0.20 mg, 0.030 μmol) was converted to 0.18 mg biotinylated glycopeptide **19** (yield, 84%). Analytical RP-HPLC, t_R = 18.05 min. ESI-MS: calcd., M = 7163.83 (average isotopes); found, 1195.27 $[M+6H]^{6+}$, 1434.23 $[M+5H]^{5+}$, 1792.27 $[M+4H]^{4+}$. Deconvolution mass, 7165.9.

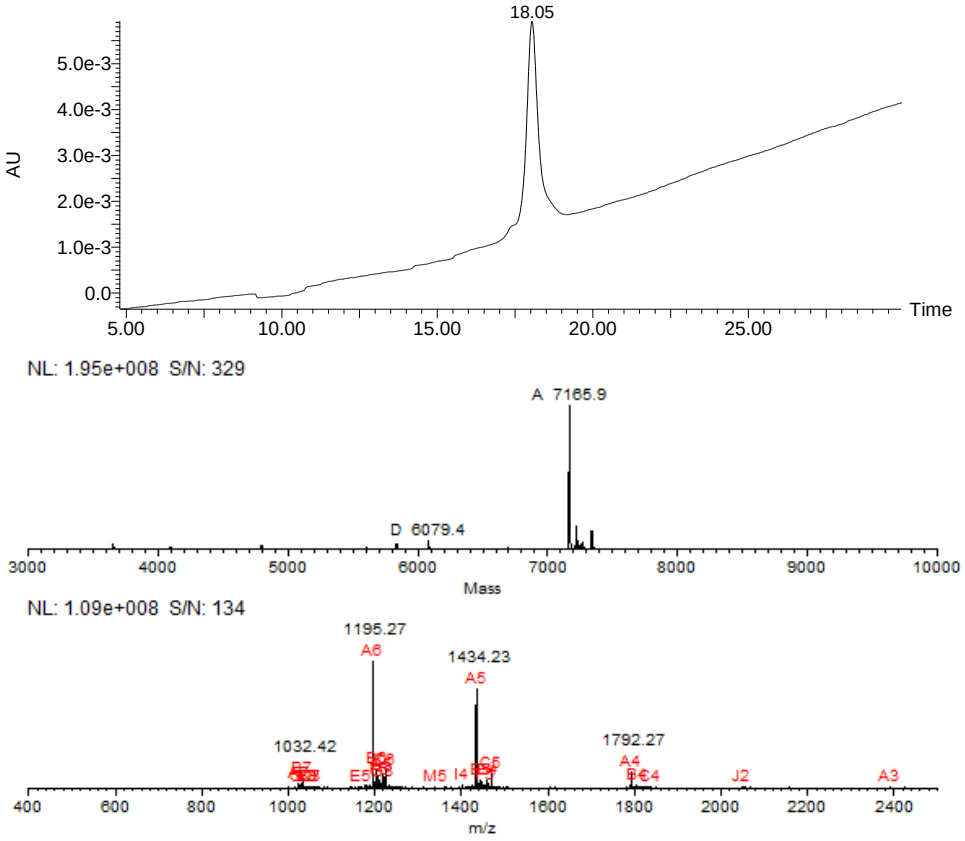
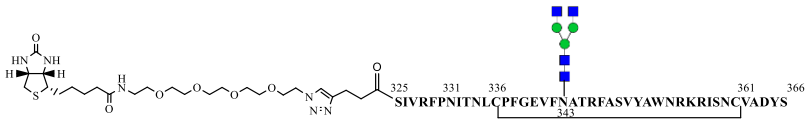


Figure S15. HPLC and ESI-MS profiles of biotinylated glycopeptide **19**



Biotinylated glycopeptide **20**

*Biotinylated glycopeptide **20** (Biotin-42-mer-N343-GN-G0)*. Glycopeptide **13** (0.20 mg, 0.032 μmol) was converted to 0.18 mg biotinylated glycopeptide **20** (yield, 83%). Analytical RP-HPLC, t_R = 18.20 min. ESI-MS: calcd., M = 6693.41 (average isotopes); found, 1116.73 $[M+6H]^{6+}$, 1339.80 $[M+5H]^{5+}$, 1674.49 $[M+4H]^{4+}$, 2232.00 $[M+3H]^{3+}$. Deconvolution mass, 6694.2.

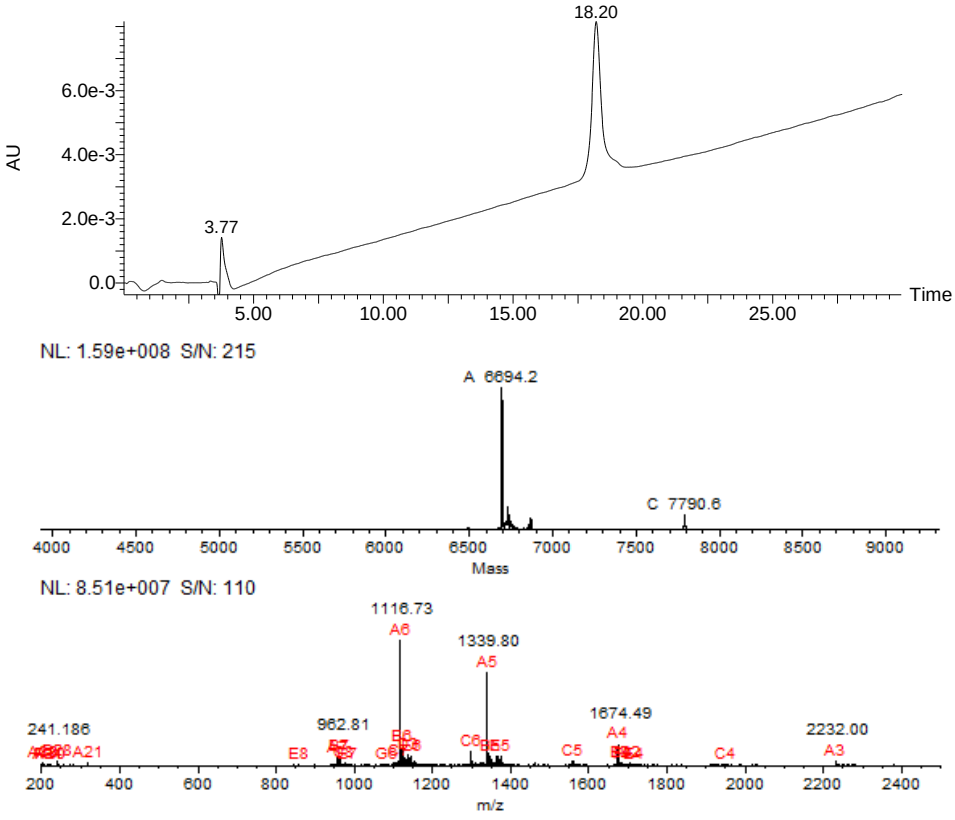
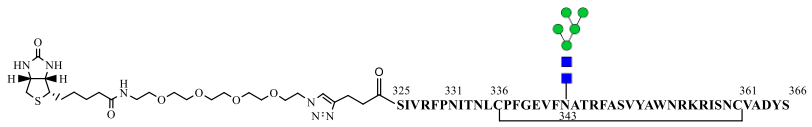


Figure S16. HPLC and ESI-MS profiles of biotinylated glycopeptide **20**



Biotinylated glycopeptide **21**

*Biotinylated glycopeptide **21** (Biotin-42-mer-N343-GN-Man5)*. Glycopeptide **14** (0.20 mg, 0.033 μmol) was converted to 0.17 mg biotinylated glycopeptide **21** (yield, 79%). Analytical RP-HPLC, t_R = 18.15 min. ESI-MS: calcd., M = 6611.30 (average isotopes); found, 1103.05 $[M+6H]^{6+}$, 1323.44 $[M+5H]^{5+}$, 1653.83 $[M+4H]^{4+}$, 2205.08 $[M+3H]^{3+}$. Deconvolution mass, 6612.3.

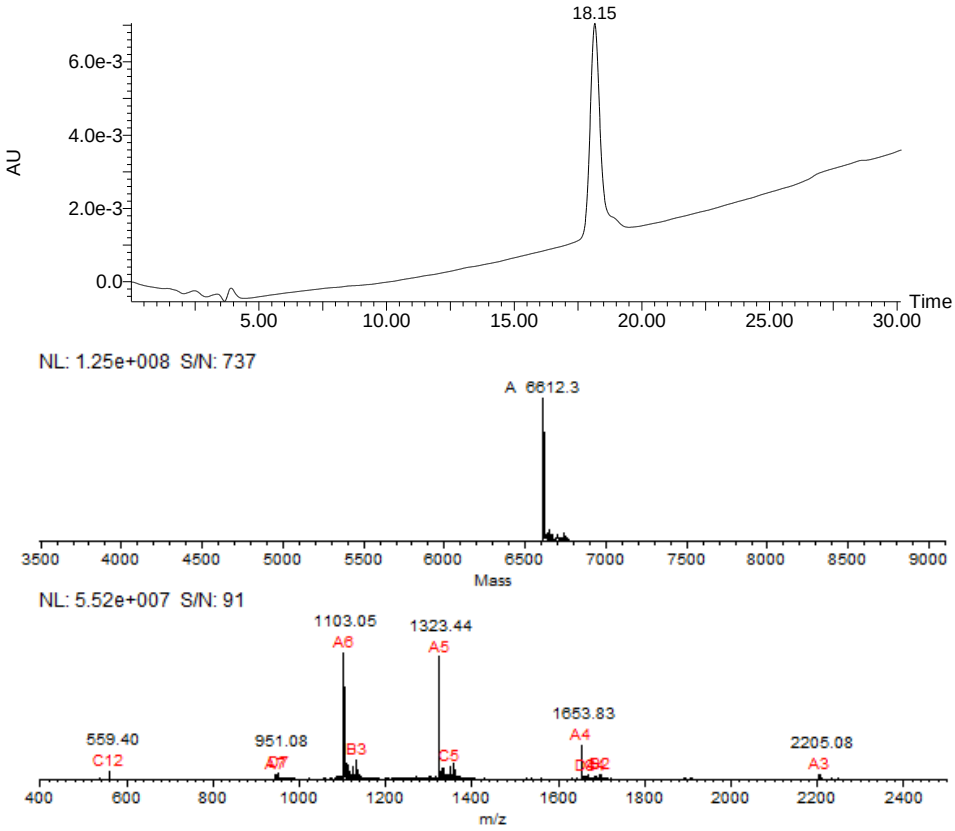
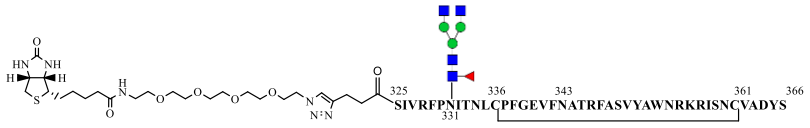


Figure S17. HPLC and ESI-MS profiles of biotinylated glycopeptide **21**



Biotinylated glycopeptide **22**

*Biotinylated glycopeptide **22** (Biotin-42-mer-N331-GN-F-G0)*. Glycopeptide **15** (0.20 mg, 0.031 μmol) was converted to 0.17 mg biotinylated glycopeptide **22** (yield, 79%). Analytical RP-HPLC, t_R = 18.15 min. ESI-MS: calcd., M = 6839.55 (average isotopes); found, 1141.24 $[M+6H]^{6+}$, 1369.12 $[M+5H]^{5+}$, 1711.24 $[M+4H]^{4+}$. Deconvolution mass, 6840.6.

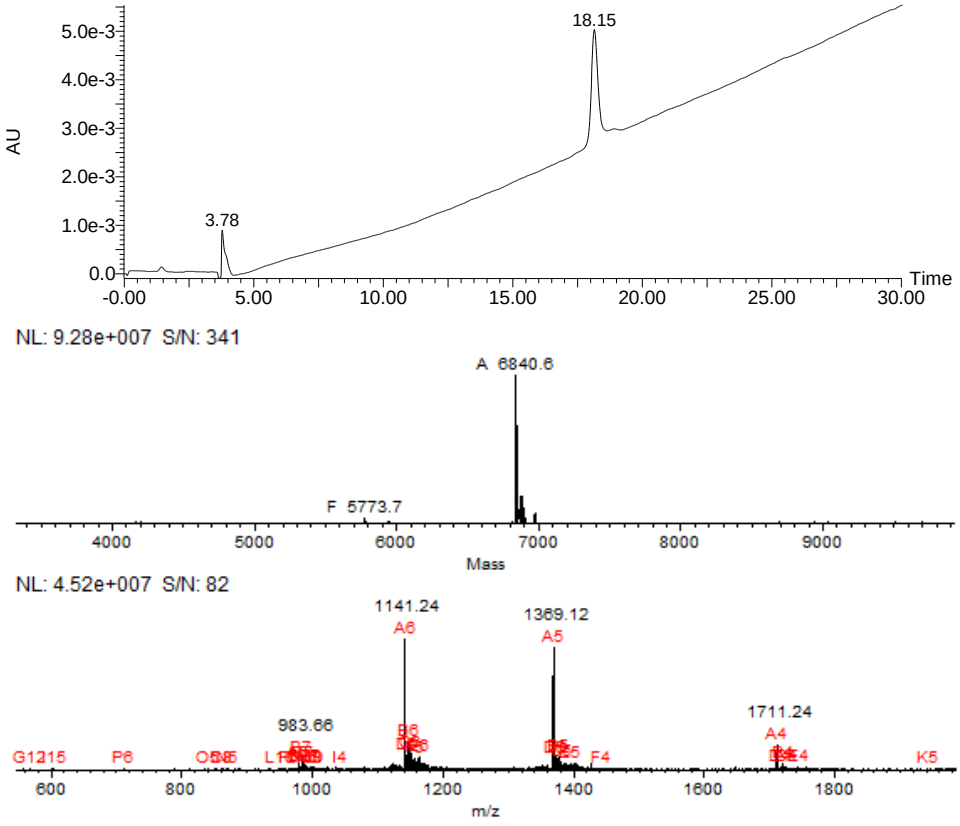
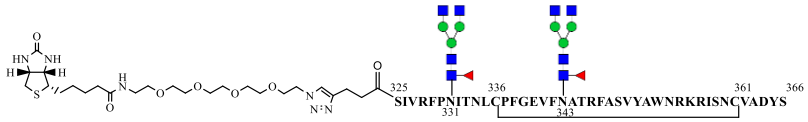


Figure S18. HPLC and ESI-MS profiles of biotinylated glycopeptide **22**



Biotinylated glycopeptide **23**

*Biotinylated glycopeptide **23** (Biotin-42-mer-N331-GNF-G0, N343-GNF-G0)*. Glycopeptide **16** (0.20 mg, 0.026 μmol) was converted to 0.20 mg biotinylated glycopeptide **23** (yield, 94%). Analytical RP-HPLC, t_R = 17.52 min. ESI-MS: calcd., M = 8284.89 (average isotopes); found, 1184.90 $[M+7H]^{7+}$, 1382.09 $[M+5H]^{5+}$, 1658.20 $[M+5H]^{5+}$. Deconvolution mass, 8286.5.

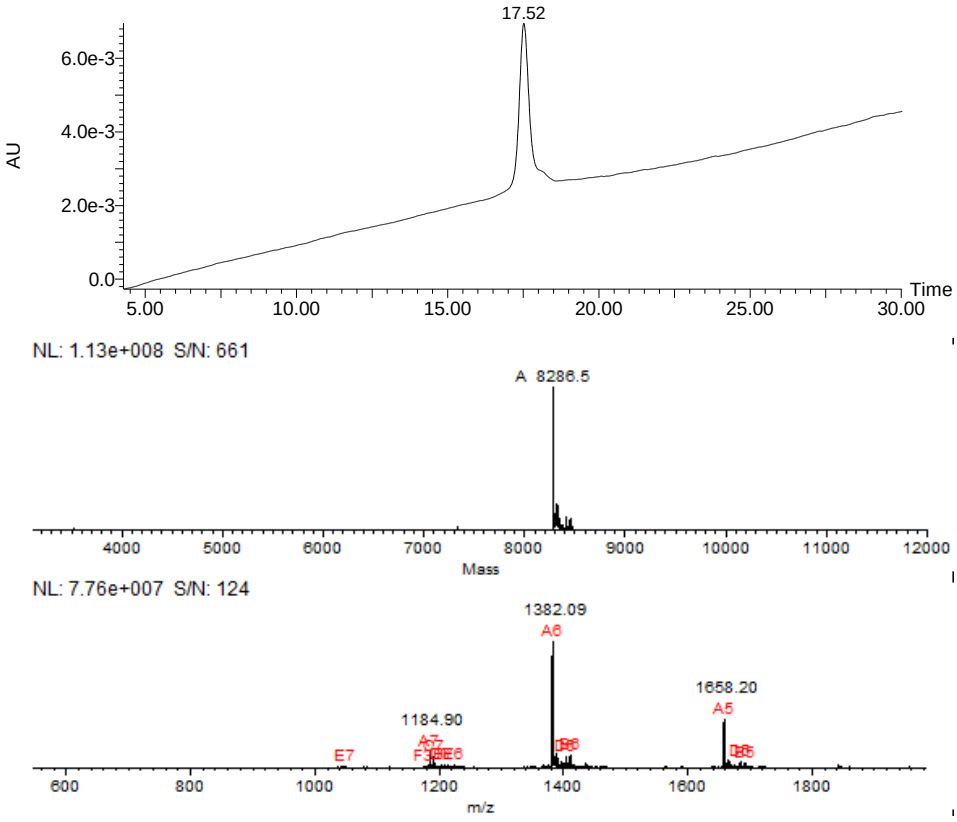
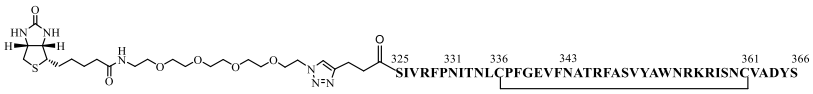


Figure S19. HPLC and ESI-MS profiles of biotinylated glycopeptide **23**



Biotinylated glycopeptide **24**

*Biotinylated glycopeptide **24** (Biotin-42-mer-standard)*. Peptide **4** (0.20 mg, 0.041 μmol) was converted to 0.16 mg biotinylated peptide **24** (yield, 73%). Analytical RP-HPLC, t_R = 18.88 min. ESI-MS: calcd., M = 5394.21 (average isotopes); found, 900.18 $[M+6H]^{6+}$, 1080.04 $[M+5H]^{5+}$, 1349.57 $[M+4H]^{4+}$, 1799.15 $[M+3H]^{3+}$. Deconvolution mass, 5395.1.

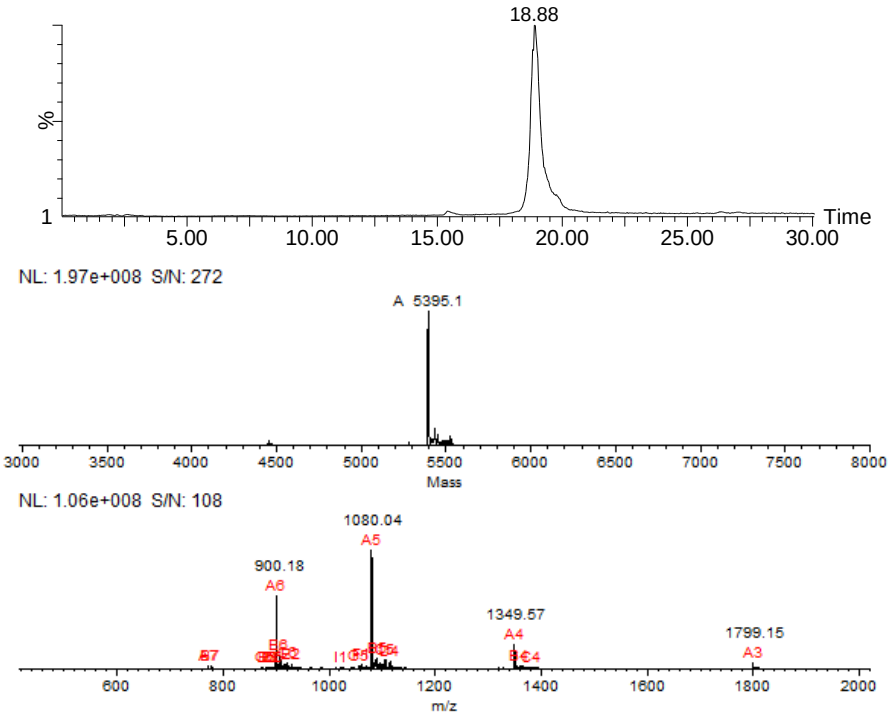


Figure S20. HPLC and ESI-MS profiles of biotinylated glycopeptide **24**

Expression and Characterization of Monoclonal Antibody S309

Gene construct – DNA construct of monoclonal antibody S309 was designed using the Fab sequence of S309[5] (Protein Data Bank accession code – 6WS6) and the heavy chain constant domains of human IgG1 (Uniprot: P01857) with a M428L/N434S double mutation[5]. Synthetic genes of the antibody heavy chain and light chain, codon-optimized and individually cloned into pcDNA3.1 vector using NheI/XhoI sites, were procured from GenScript Biotech Corporation.

Antibody expression and characterization – Recombinant S309 antibody was transiently expressed in HEK293T cells using the outlined procedure. Briefly, HEK293T cells were seeded at a density of 1 x 10^6/mL in serum-free FreeStyle™ F17 Expression Medium (Thermo Fisher Scientific) and cultured at 37°C, 7% CO_2, 150 rpm. After 20 – 24 h, co-transfection was performed with 1.5 µg/mL each of the heavy chain and light chain plasmids and 9 µg/mL of the transfection reagent, polyethylenimine (PEI). 2.2 mM of valproic acid was supplemented a day post-transfection. The culture was harvested three days post-transfection and the supernatant was purified using Protein A chromatography. The purified antibody was characterized using non-reduced and reduced gel electrophoresis (Figure S21) and mass spectrometric (MS) analysis (Figure S22). The antibody was treated with IdeS protease (0.02 mg/ml) in PBS at 37°C for 15 min to generate Fab dimer and Fc monomer fragments. Liquid chromatography (LC)-MS analysis of reduced antibodies was performed on a Thermo Scientific Exactive™ Plus Orbitrap mass spectrometer on a Waters XBridge™ BEH300 C4 column (3.5 µm, 2.1 x 50 mm) using a 6-min linear gradient of 25 - 35% acetonitrile containing 0.1% formic acid at a flow rate of 0.4 mL/min. MS raw data was deconvoluted using MagTran (Amgen).

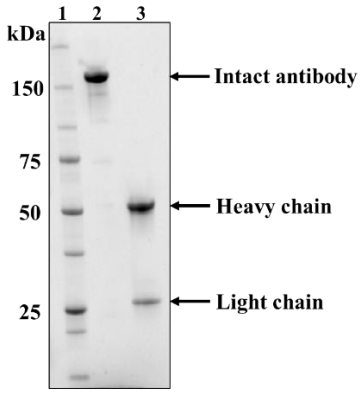
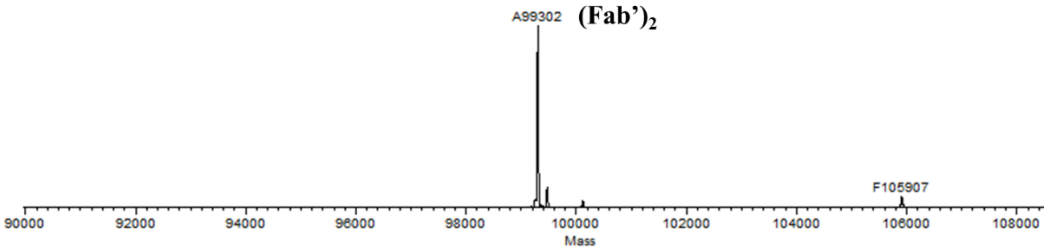


Figure S21. SDS-PAGE analysis of recombinant S309 antibody. Lane 1 – protein ladder showing protein standards of specified sizes, Lane 2 – purified intact S309 antibody run under non-reducing conditions, Lane 3 – reduced S309 antibody showing antibody heavy chain and light chain.

A)



B)

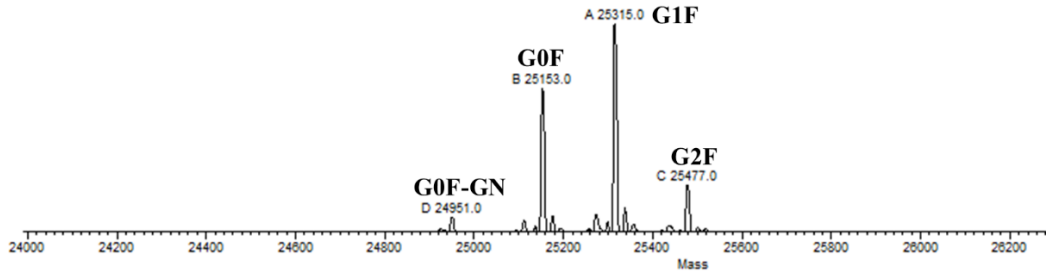


Figure S22. LC-MS analysis of IdeS-treated S309 antibody showing A) Fab dimer and B) Fc monomer

Enzyme-linked immunosorbant assay (ELISA)

The high-binding Ultra Cruz 96-well plates (Santa Cruz Biotech) were firstly coated with 100 ng of the SARS-CoV-2 RBD derived (glyco)peptides or SARS-CoV-2 spike protein trimer antigen in 100 μL of PBS buffer at 4 °C overnight. Afterwards, the plates were washed with PBS/0.05% Tween-20 (PBS-T) three times and then saturated with 2% (w/v%) sodium casein under incubation at 37 °C for 1 h. The plates were again washed with PBS-T for three times. Next, the S309 mAb with various concentrations (1 μg/mL to 0.5 ng/mL with a serial of 2-fold dilutions) was applied to the assay plate. The binding of the mAb was proceeded at room temperature for 1 h and then washed by the PBS-T solution for 4 times. Subsequently, 100 μL of the 1:10,000 diluted HRP-labeled goat anti-human IgG antibody (KPL) was applied to the well plate. The binding occurred at room temperature for 30 min, followed by extensive washes with PBS-T solution for 5 times. Finally, 100 μL of the TMB 2-component microwell peroxidase substrates (SeraCare) was added to each well. The color was developed in dark at room temperature for 30 min, and then quenched with 100 μL of 1 M H_3PO_4 solution. Absorbance values were read by subtracting the optical density (OD) at 450 nm by the OD at 550 nm.

Surface Plasmon Resonance (SPR) Measurements.

SPR measurements were performed on a Biacore T200 instrument (GE Healthcare) at 25 ^{o}C. Approximately 7000 resonance units (RU) of neutravidin was immobilized on a CM5 sensor chip in a sodium acetate buffer (50 μg/mL, pH 4.0), using the amine coupling kit provided by the manufacturer. Biotinylated SARS-CoV-2 spike protein (glyco)peptides were captured on neutravidin-coated CM5 sensor chips in HBS-P buffer (10mM HEPES, 150 mM NaCl, P20 surfactant 0.05% v/v, pH 7.4) until approximately 2000 response unit (RU) was achieved. Recombinant human CLEC4G (Fc tag, Acro Biosystems) were prepared at determined concentrations (2× serial dilutions from 7.8 nM to 2 μM) in HBS-P running buffer (10 mM HEPES, 150 mM NaCl, 500 μM $CaCl_2$, P20 surfactant 0.05% v/v, pH 7.4) while a 10 mM EDTA-NaOH was used as regeneration buffer. A typical binding curve was obtained by flowing *h*CLEC4G sample at 10 μL/s for 180 s association and then flowing running buffer for 240 s dissociation, followed by 60 s regeneration buffer at 10 μL/s. The kinetic constants are obtained by fitting the steady-state RU using Biacore T200 evaluation software.

References

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