Supporting Information.

"Biologic" level structures through chemistry: A total synthesis of a unimolecular pentavalent MUCI glycopeptide construct.

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Experimental Procedures

I. Materials and Methods:

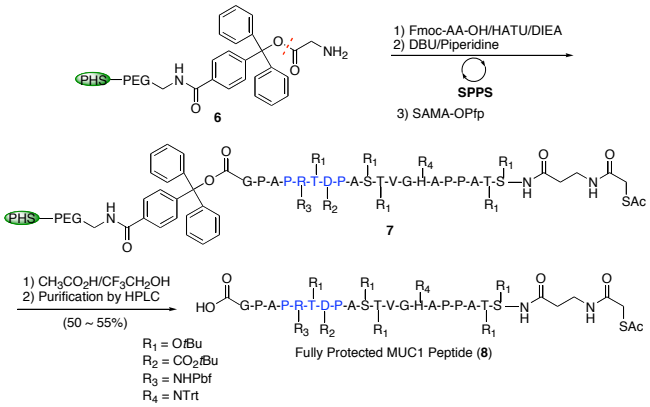
Reagents: All commercial materials were used as received unless otherwise noted. Trifluoroethanol (TFE), trifluoroacetic acid (TFA), acetic acid (CH_3CO_2H), *N*,*N*-diisopropylethyl amine (DIEA), diazabicycloundecene (DBU), piperidine, *N*-hydroxybenzotriazole (HOBT), triethyl silane (Et_3SiH), phenol (PhOH), anhydrous methanol (MeOH), anhydrous methyl sulfoxide (DMSO), and anhydrous *N*,*N*-dimethyl formamide (DMF) were purchased from Aldrich. [*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*', *N*'-tetramethyluronium hexafluorophosphate] (HATU) was purchased from GenScript and used without further purification. All amino acids and resins for solid phase peptide synthesis were purchased from NovaBiochem; All other solvents from Fisher Scientific (HPLC grade).

HPLC: All separation involved a mobile phase of 0.05% TFA (v/v) in water (solvent A)/0.0425% TFA in acetonitrile (solvent B). Preparative and analytical HPLC separation were performed using a Rainin HXPL solvent delivery system equipped with a Rainin UV-1 detector and Microsorb Dynamax-100Å C18 axial compression columns. LC-MS chromatographic separations were performed using a Waters 2695 Separations Module and a Waters 996 Photodiode Array Detector equipped with Varian Microsorb C18 2 X 150 mm, and C4 2 X 250 mm columns at a flow rate of 0.2 mL/min.

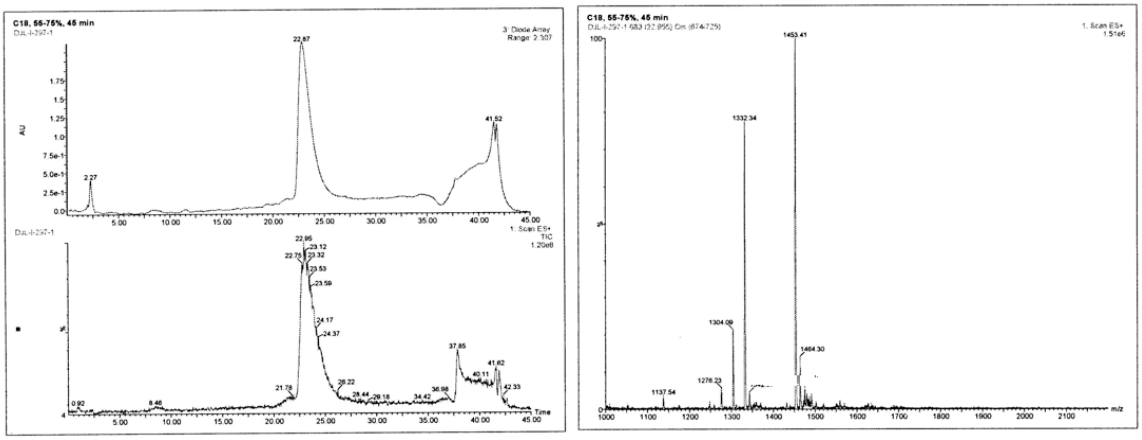
ESMS and LC-MS: Electrospray mass spectroscopy and LCMS analyses were obtained on a Waters Micromass ZQ mass spectrometer in conjugation with the Waters HPLC apparatus described above.

II. Detailed Experimental Procedures

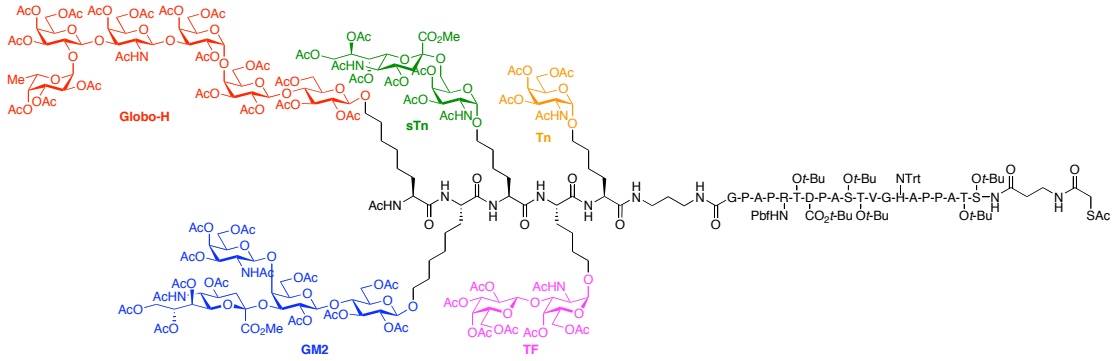
1. Preparation of the Peptide (**8**)



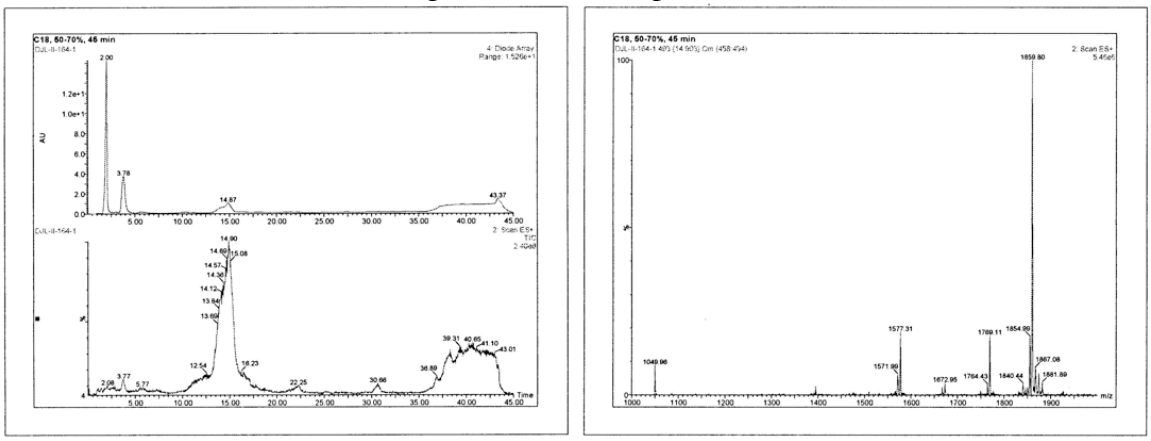
The Fully Protected MUC1 Peptide (8): Fmoc-Gly-NovaSyn® TGT resin (purchased from NovaBiochem) was used. Fmoc quantitation of the resin prior to deprotection indicated a loading of 0.23 mmol/g. 217.4 mg of this resin (**6**) was subjected continuous flow automated peptide synthesis. For coupling steps, resin was treated with a 4-fold excess of HATU and Fmoc amino acids in DIEA/DMF, and for deblocking, a solution of 2% piperidine/2% DBU in DMF was used. The amino acids used were, in order of synthesis, Fmoc-Pro-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Thr(O-*t*Bu)-OH, Fmoc-Asp(O-*t*Bu)-OH, Fmoc-Pro-OH, Fmoc-Ala-OH, Fmoc-Ser(O-*t*Bu)-OH, Fmoc-Thr(O-*t*Bu)-OH, Fmoc-Val-OH, Fmoc-Gly-OH, Fmoc-His(N-Trt)-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Pro-OH, Fmoc-Ala-OH, Fmoc-Thr(O-*t*Bu)-OH, Fmoc-Ser(O-*t*Bu)-OH, Fmoc-β-Ala-OH, SAMA-Opfp (*S*-acetylthioglycolic acid pentafluorophenyl ester). The resin was then transferred to a manual peptide synthesis vessel and treated with a cleavage solution (10 mL, CH_3CO_2H/TFE/CH_2Cl_2 = 1:1:8) for 1 hour. The beads were filtered, rinsed with another 10 mL cleavage solution. This 1-hour cleavage cycle process was repeated for two times, and the combined filtrate was concentrated by nitrogen (N_2) flow and lyophilized to afford 73 mg (51%) as the crude peptide. This crude peptide (8.0 mg) was purified by preparative reverse-phase HPLC using a gradient of 60-80% B buffer over 30 minutes, flow rate 16 mL/min, 265 nm UV detection. The peak with retention time of 22.13 minutes was collected and lyophilized to afford 5.90 mg of **8** as a white solid (74% yield based on the loaded crude material). Post-purification analytical LC-MS analysis showed a clean product spectrum with a base peak of 2905.09 $[M+H]^+$ and 1453.41 $[M+2H]^+$.



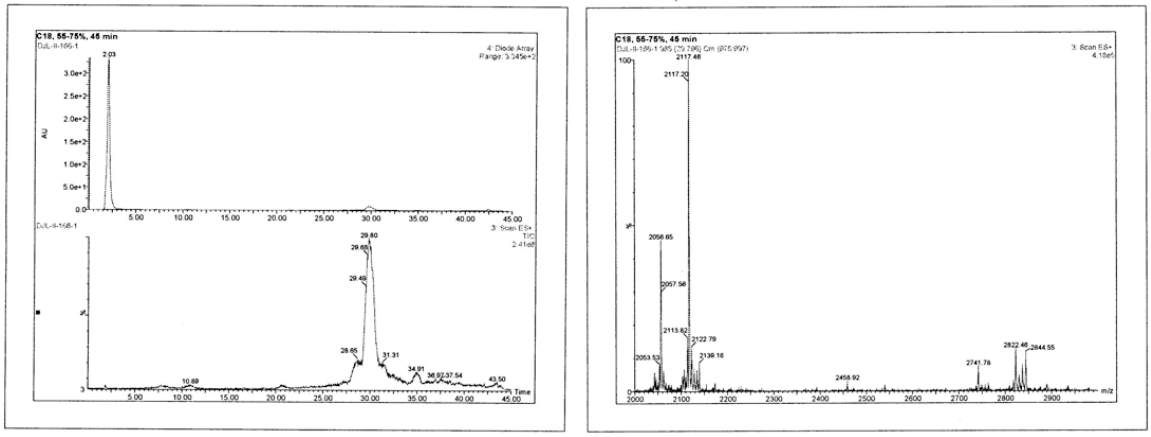
2. Preparation of the Fully Protected Unimolecular Pentavalent-MUC1 Glycopeptide



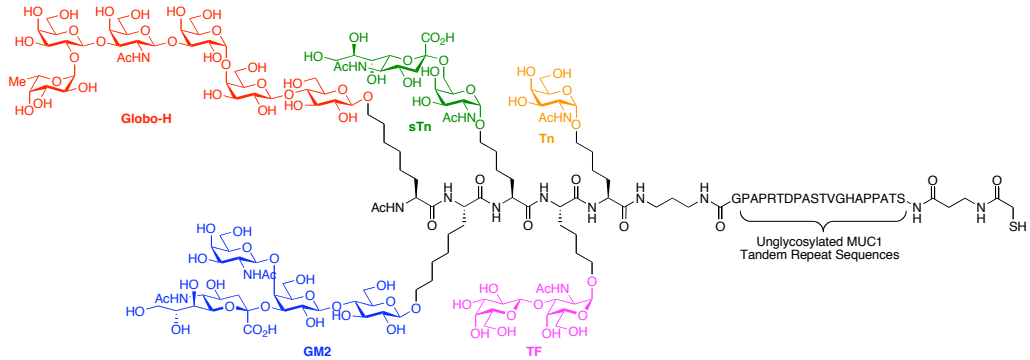
The Fully Protected Unimolecular Pentavalent-MUC1 Glycopeptide: To a stirred solution of **5** (2.70 mg, 0.48 μmol, 1 equiv) in CH_2Cl_2 (300 μL) was added trifluoroacetic acid (TFA, 30 μL) at room temperature. The resulting reaction mixture was stirred for 3 hours. 3 μL of aliquot of the reaction mixture was taken out and diluted with 30 μL of CH_3CN for LC-MS analysis; C18 column, B: 50-70% over 30 minutes, retention time: 14.87 minutes, MS(ESI): $C_{239}H_{346}N_{14}O_{135}$, Calc. 5572.06, Observed 2788.94 $[M+2H]^{2+}$, 1859.80 $[M+3H]^{3+}$. The solvent was removed by nitrogen flow (N_2), and the crude material was used for the next step without further purification.



To a stirred solution of amine prepared above (theoretically 2.65 mg, 0.48 μmol, 1 equiv) and peptide (**8**) (1.66 mg, 0.57 μmol, 1.2 equiv) in DMSO (250 μL), a solution of HATU (0.90 mg, 2.38 μmol, 5 equiv) and HOBT (0.32 mg, 2.38 μmol, 5 equiv) in DMSO, and DIEA (0.83 μL, 4.76 μmol, 10 equiv) were added at room temperature. The resulting reaction mixture was stirred for 24 hours. 3 μL of aliquot of the reaction mixture was taken out and diluted with 30 μL of CH_3CN for LC-MS analysis; C18 column, B: 55-75% over 30 minutes, retention time: 29.80 minutes, MS(ESI): $C_{382}H_{558}N_{40}O_{168}S_2$, Calc. 8457.5792, Observed 2117.46 $[M+4H]^{4+}$, 1694.00 $[M+5H]^{5+}$. The final reaction mixture was diluted with 1 mL of CH_3CN/H_2O (1:1), and this crude mixture was purified by preparative reverse-phase HPLC using a gradient of 60-80% B buffer over 30 minutes, flow rate 16 mL/min, 264 nm UV detection. The peak with retention time of 26.50 minutes was collected and lyophilized to afford 2.90 mg as a white solid (72% yield over 2 steps). Post-purification analytical LC/ESI MS analysis showed a clean product spectrum with a base peak of 2117.46 $[M+4H]^{4+}$, 1693.87 $[M+5H]^{5+}$.

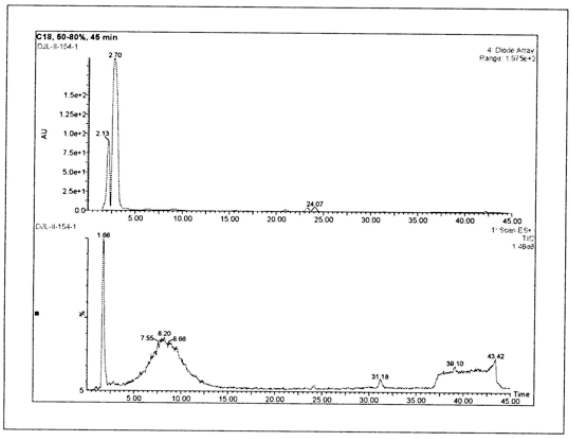


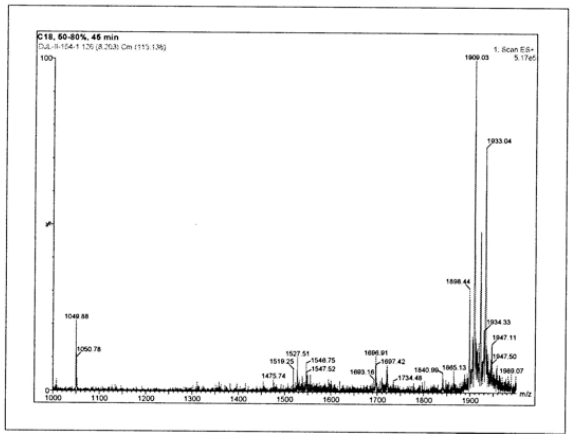
3. Preparation of the Unimolecular Pentavalent-MUC1 Glycopeptide (**9**)



The Unimolecular Pentavalent-MUC1 Glycopeptide (9): The fully protected unimolecular pentavalent-MUC1 glycopeptide (1.1 mg, 0.14 μmol) was treated with a cleavage solution (150 μL, TFA/H_2O/PhOH/Et_3SiH = 8.75:0.5:0.5:0.25). The resulting mixture was stirred for 3.5 hours at room temperature. 3 μL of aliquot of the reaction mixture was taken out and diluted with 30 μL of CH_3CN for LC-MS analysis; C18 column, B: 50-80% over 30 minutes, retention time: 9.13 minutes, MS(ESI):

$C_{326}H_{480}N_{40}O_{168}S$, Calc. 7627.0120, Observed 1909.03 $[M+4H]^{4+}$. The reaction mixture was dried by nitrogen flow (N_2), and rinsed with Et_2O, and dried in vacuo. The crude material was used for the next step without further purification.





To a stirred solution of the glycopeptide prepared above in MeOH (120 μL), 0.3M aqueous NaOH (60 μL) solution at room temperature. The resulting reaction mixture was stirred for 24 hours, which was then acidified with 1M aqueous HCl solution until the pH of the reaction mixture reached 4-5. 3 μL of aliquot of the reaction mixture was taken out and diluted with 30 μL of CH_3CN for LC-MS analysis; C18 column, B: 01-10% over 30 minutes, retention time: 35.85 minutes, MS(ESI): $C_{234}H_{386}N_{40}O_{120}S$, Calc. 5708.5053, Observed 1905.54 $[M+3H]^{3+}$, 1429.18 $[M+4H]^{4+}$. The final reaction mixture was diluted with 1 mL of CH_3CN/H_2O (1:1), and this crude mixture was purified by preparative reverse-phase HPLC using a gradient of 1-10% (5 minutes), 20-50% (30 minutes) B buffer, flow rate 16 mL/min, 220 nm UV detection. The peak with retention time of 12.10 minutes was collected and lyophilized to afford 0.5 mg of **9** as a white solid (60% yield over 2 steps). Post-purification analytical LC/ESI MS analysis showed a clean product spectrum with a base peak of 1905.02 $[M+3H]^{3+}$.