Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

An Identification Method to Distinguish Sugar Isomers on Glycopeptides

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1. Materials and Methods

Continuous flow equipment was assembled from commercially available components supplied from IDEX Health & Science Technologies and Cole Parmer. Reactors were constructed from high-purity perfluoroalkoxy (PFA) tubing. For all reactions, PFA tubing with a 0.02" inner diameter (Part #1512L) and 0.020" low-pressure tee assembly (Part #P-712), with complementary PEEK fittings, were used. Static mixers were purchased from StaMixCo (Part # HT-40-1.70-10-PTFE). Varian Prostar 210 HPLC pumps with 10 mL/min pump heads were used to uptake and deliver reagents.

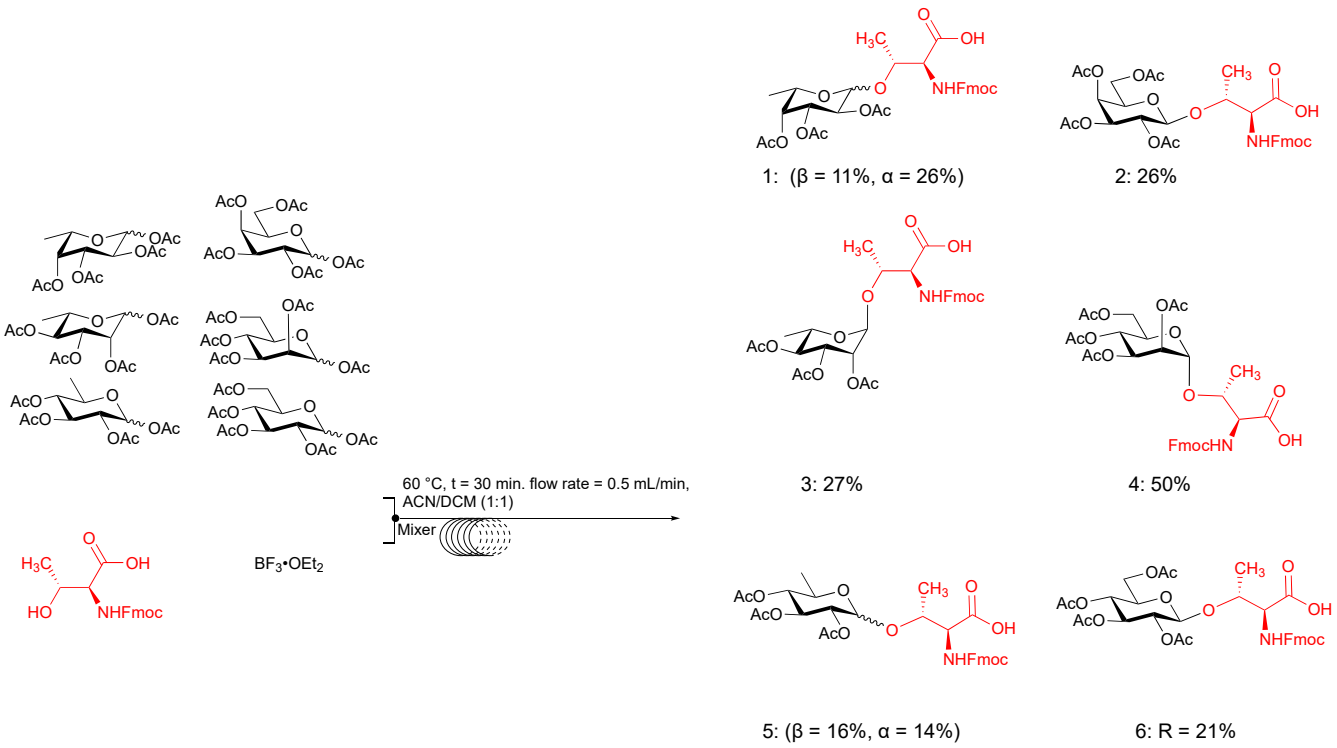
Air- and moisture sensitive reactions were performed in oven-dried glassware and were kept under an argon atmosphere. Commercially available reagents were used without further purification unless otherwise noted. Reactions were monitored using thin-layer chromatography (TLC). Characterization of synthesized molecules was completed using nuclear magnetic resonance and mass spectrometry present at Indiana University. ^{1}H NMRs were obtained on a Varian Inova 500 (500 MHz), Varian Inova 600 (600 MHz), or Bruker (500 MHz) instrument. ^{13}C NMR, 2D COSY, and 2D HSQC data was obtained using a Varian Inova 500 (500 MHz), Varian Inova 600 (600 MHz), or Bruker (500 MHz) instrument. All NMR spectra were recorded in $CDCl_3$ and referenced to 7.26 ppm or 77.2 ppm. All NMR spectra were processed using Mestrenova software. NMR abbreviations used to express multiplicity in spectra data follows established nomenclature rules. High-resolution mass spectra (HRMS) were obtained using electrospray Ionization (ESI) on an LTQ-Orbitrap XL.

2. Experimental Procedures

2.1 General Experimental

Prior to running each reaction, the reactor was equilibrated with identical volumes of anhydrous solvent. For instance, anhydrous 1:1 CH_2Cl_2/ACN were used to equilibrate the reactor prior to running the reactions.

2.2 Synthesis of Glycosylated Amino Acid Library



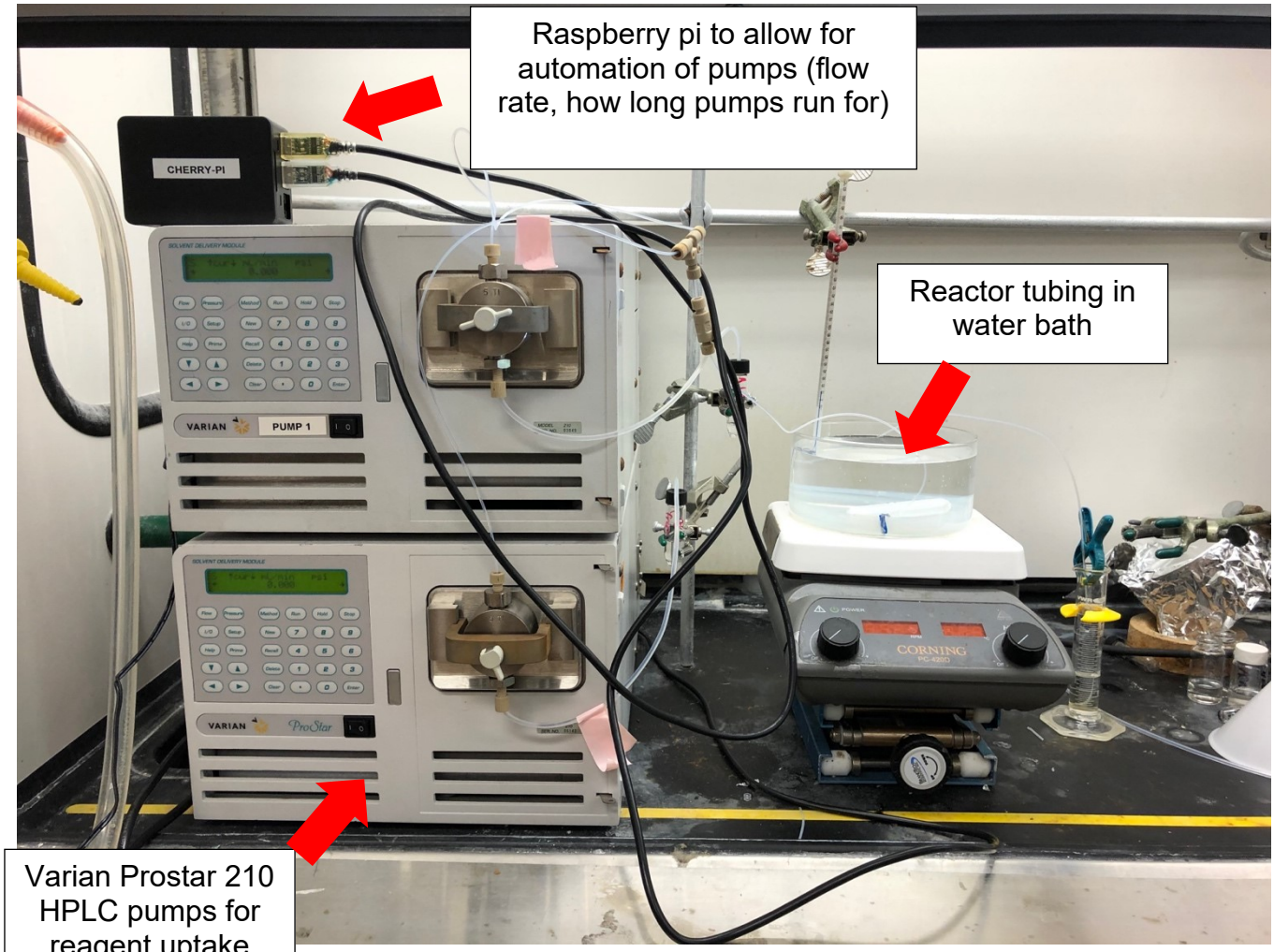
Tubing from reactor vessel to pump heads 1 ft in length, tubing to tee mixers 1 ft in length, reactor tubing 250 ft in length

Solution 1: Peracetylated sugar was dissolved in 1:1 CH_2Cl_2/ACN in a reactor vessel.

Solution 2: Fmoc amino acid was dissolved in 1:1 CH_2Cl_2/ACN and promoter was added to the reactor vessel.

The PFA tubing (I.D. 1/8 in.) was placed through the septa of two separate conical vials with solutions 1 and 2 while under inert atmosphere. A reactor coil made from high purity PFA tubing (0.020 in I. D., 250 ft, 15 mL) was placed in a hot bath at 60 °C. Two Varian Prostar 210 HPLC pumps were used to deliver the reagents and were connected to a Raspberry pi via USB connection. The flow rate for the pumps was set to 0.25 mL/min, giving an overall reaction flow rate of 0.5 mL/min. The donor solution was flowed with the acceptor and BF_3•OEt_2 solution at a flow rate of 0.5 mL/min through PFA tubing with a residence time of 30 min. The reaction solution was collected at steady state for two minutes in a

vial, and the volume of the collected reaction solution was determined. The steady state fraction was then concentrated in vacuo, and the anomers were then separated using semipreparative-HPLC on a Phenomenex C-18 column (100 Å, 5 µm, 250 x 10 mm) under isocratic conditions with 0.1% aqueous trifluoroacetic acid (TFA)/ 0.1% TFA in acetonitrile (1:1) to give the desired glycosylated amino acids.

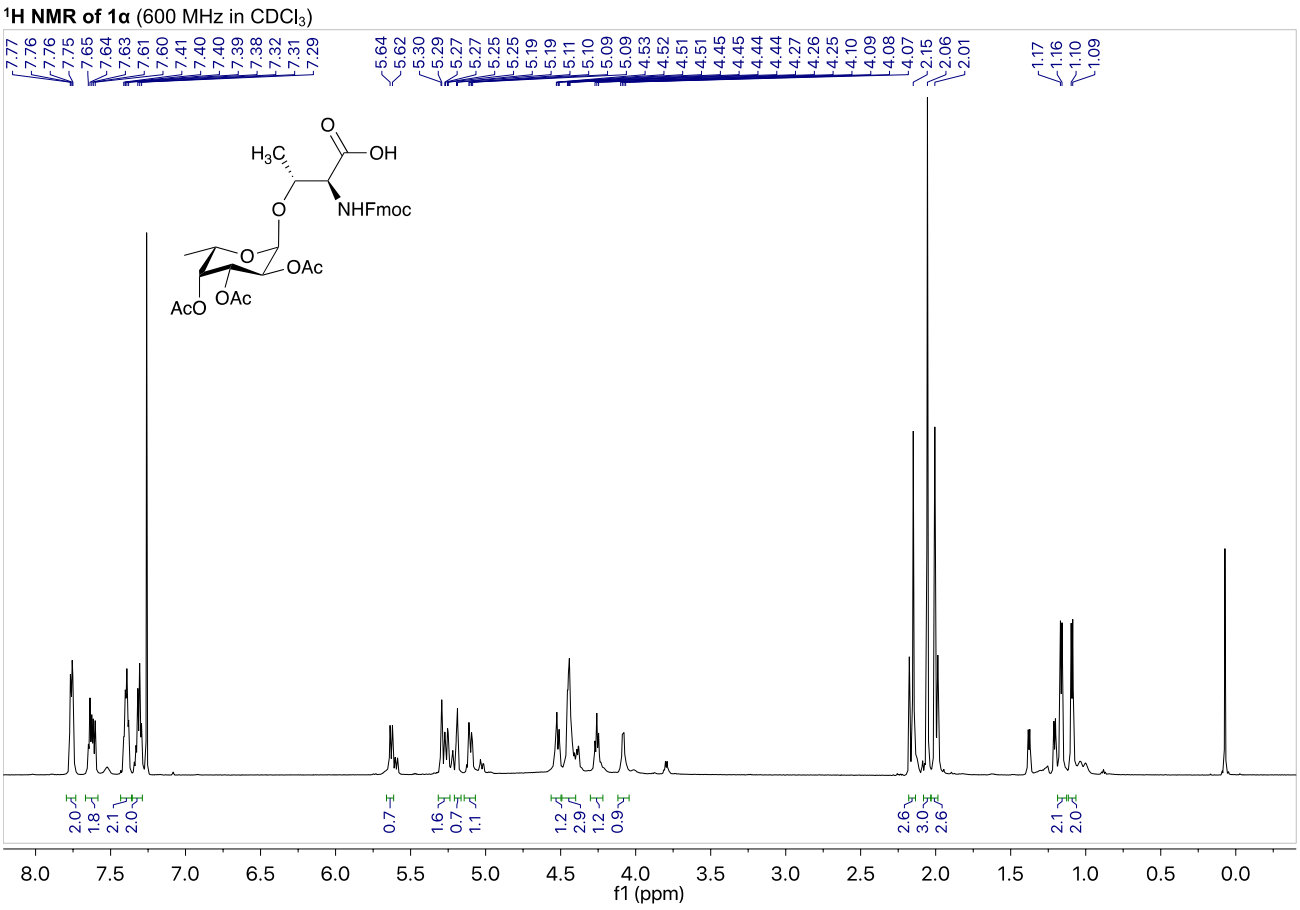


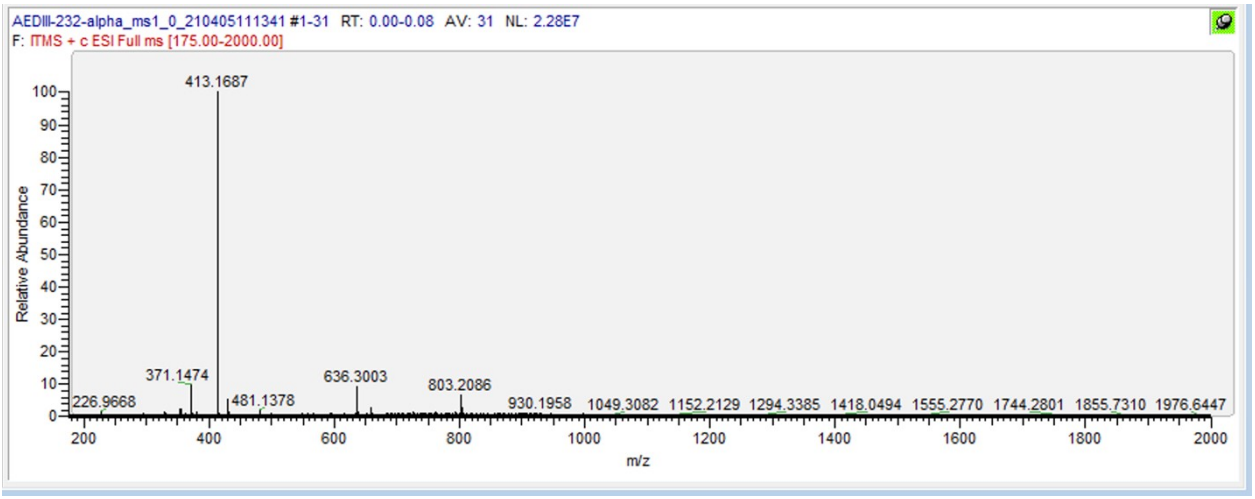
Fmoc-Thr(α-L-Fuc(Ac)$_3$)-OH. Tetraacetylated fucose (250.6 mg, 0.7541 mmol) was dissolved in anhydrous ACN (3.8 mL) and anhydrous dichloromethane (3.8 mL) and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into the solution. Fmoc-Thr-OH (309.2 mg, 0.9058 mmol) was diluted in anhydrous ACN (3.6 mL) and dichloromethane (3.6 mL) and boron trifluoride diethyletherate was added (0.560 mL, 4.51 mmol), and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into

the solution. A reactor coil high purity PFA tubing (0.020 in I.D., 250 ft, 15 mL) was placed in a hot bath at 60 °C. Two Varian Prostar 210 HPLC pumps were used to deliver the reagents and were connected to a Raspberry pi via USB connection. Desired flow rate, run time, and reaction parameters and components were entered into Jupyter notebook, and this information was used to start the pumps at a given flow rate. The donor solution was flowed with the acceptor and BF_3•OEt_2 solution at a flow rate of 0.5 mL/min through PFA tubing with a residence time of 30 min. The reaction solution was collected at steady state for two minutes in a vial, and the volume of the collected reaction solution was determined. The steady state fraction was then concentrated in vacuo, and the anomers were then separated using semipreparative-HPLC on a Phenomenex C-18 column (100 Å, 5 μm, 250 x 10 mm) under isocratic conditions for 30 minutes with 0.1% aqueous TFA/ 0.1% TFA in acetonitrile (1:1) to give **1α** (8.2 mg, 26%) and **1β** (3.5 mg, 11%). The spectroscopic data was in good agreement with previously reported values.[1,2]

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.76 (dd, *J* = 7.7, 2.9 Hz, 2H), 7.63 (dt, *J* = 13.6, 7.2 Hz, 2H), 7.43 – 7.37 (m, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), 5.63 (d, *J* = 9.3 Hz, 1H), 5.35 – 5.25 (m, 2H), 5.19 (d, *J* = 3.9 Hz, 1H), 5.10 (dd, *J* = 10.7, 3.9 Hz, 1H), 4.52 (dd, *J* = 9.2, 2.4 Hz, 1H), 4.45 (dd, *J* = 7.0, 3.3 Hz, 3H), 4.26 (t, *J* = 7.3 Hz, 1H), 4.08 (q, *J* = 6.6 Hz, 1H), 2.15 (s, 2H), 2.06 (s, 3H), 2.01 (s, 3H), 1.16 (d, *J* = 6.2 Hz, 2H), 1.09 (d, *J* = 6.5 Hz, 2H).

LRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{35}NO_{12}Na$ 636.2; Found 636.2

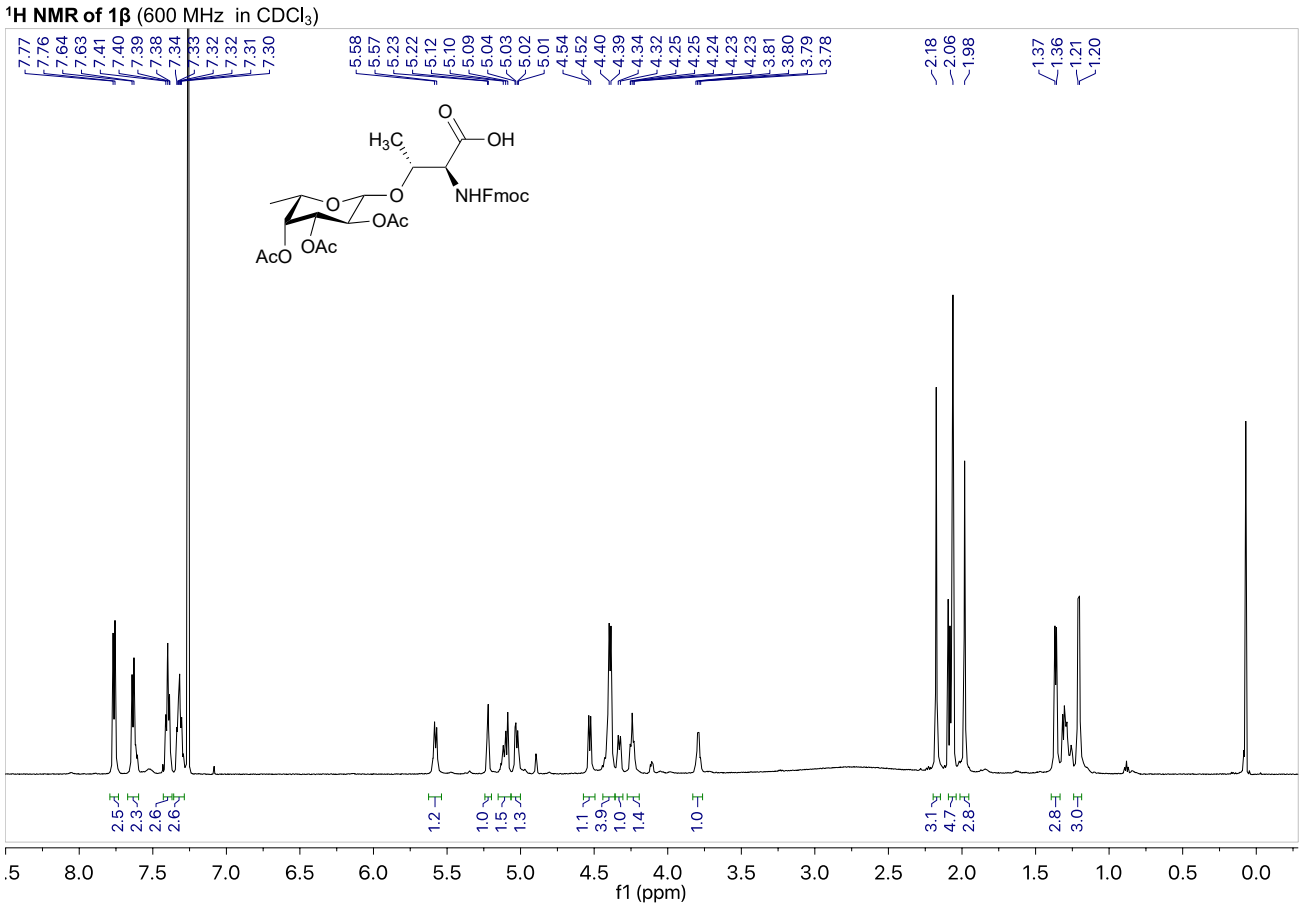


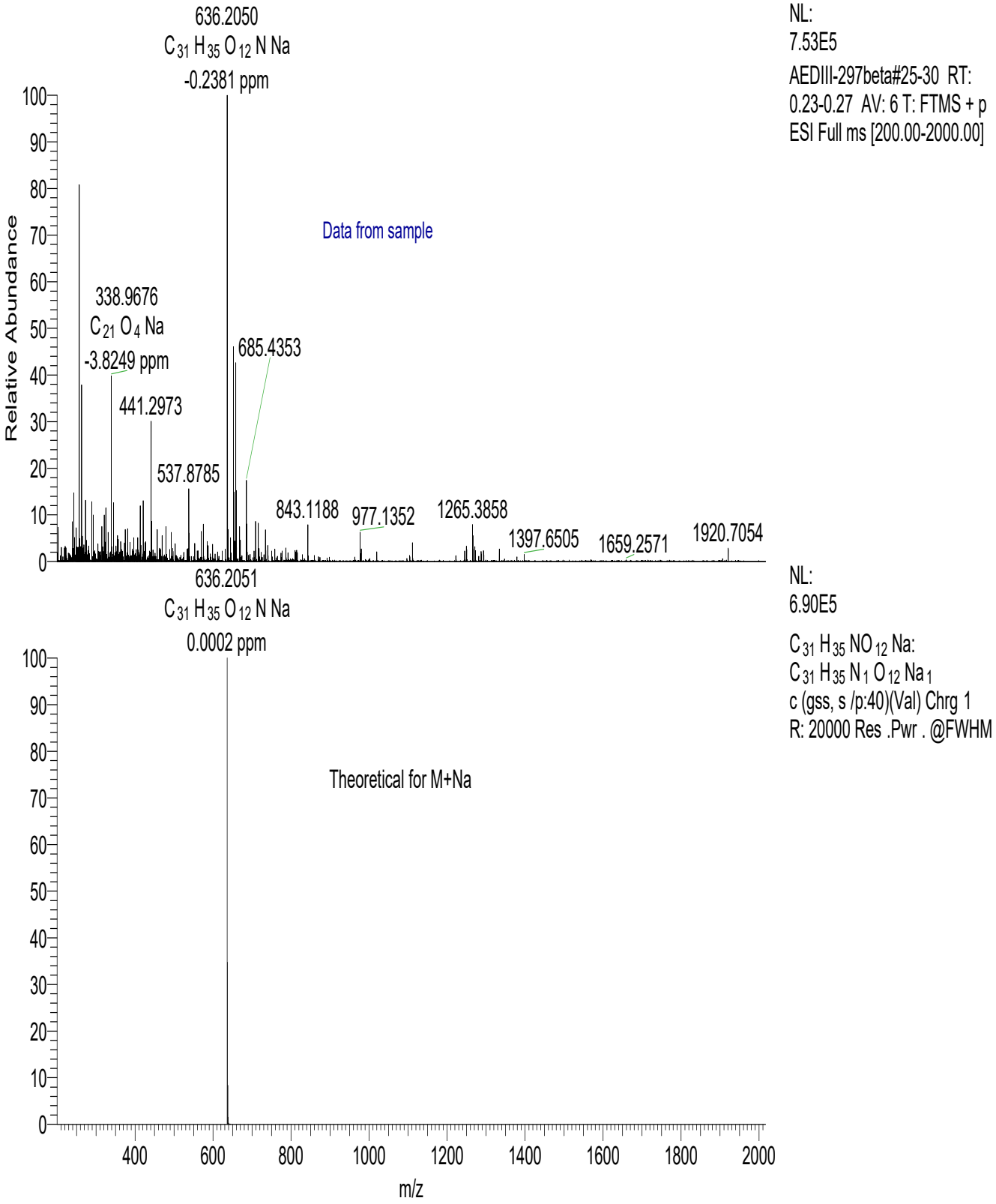


Fmoc-Thr(β-L-Fuc(Ac)$_3$)-OH. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.76 (d, *J* = 7.6 Hz, 3H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 3H), 7.32 (td, *J* = 7.5, 4.0 Hz, 3H), 5.58 (d, *J* = 8.8 Hz, 1H), 5.22 (d, *J* = 3.4 Hz, 1H), 5.16 – 5.07 (m, 1H), 5.03 (dd, *J* = 10.4, 3.4 Hz, 1H), 4.53 (d, *J* = 7.8 Hz, 1H), 4.39 (d, *J*

= 7.5 Hz, 4H), 4.33 (d, *J* = 9.1 Hz, 1H), 4.24 (t, *J* = 7.1 Hz, 1H), 3.79 (q, *J* = 6.4 Hz, 1H), 2.18 (s, 4H), 2.06 (s, 6H), 1.98 (s, 3H), 1.36 (d, *J* = 6.4 Hz, 3H), 1.21 (d, *J* = 6.4 Hz, 4H).

HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{35}NO_{12}Na$ 636.2051; Found 636.2050




636.2050
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NL:
7.53E5
AEDIII-297beta#25-30 RT:
0.23-0.27 AV: 6 T: FTMS + p
ESI Full ms [200.00-2000.00]
Data from sample
Relative Abundance
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537.8785
843.1188
977.1352
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1397.6505
1659.2571
1920.7054
636.2051
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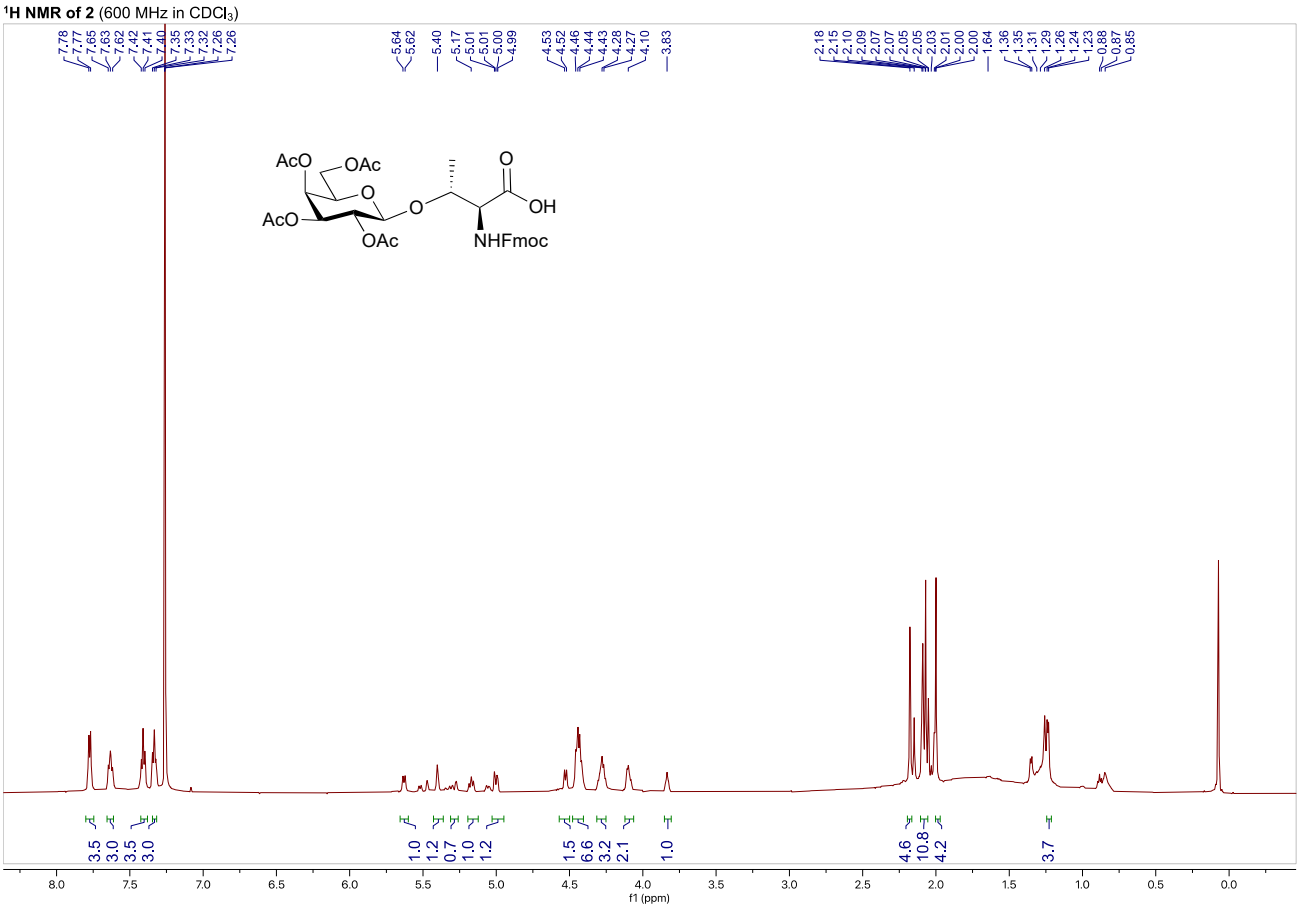
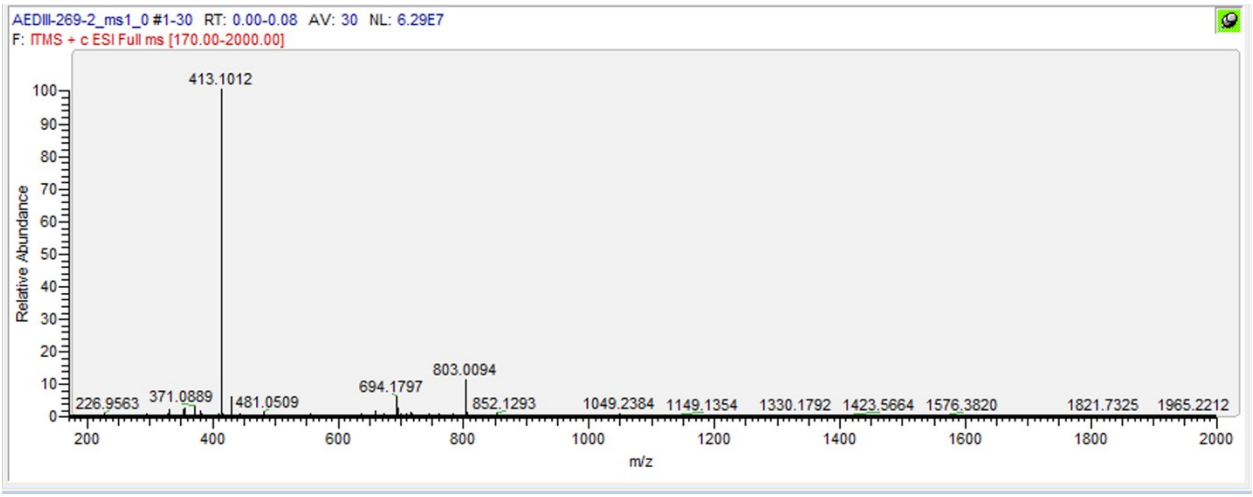
0.0002 ppm
NL:
6.90E5
C31 H35 NO12 Na:
C31 H35 N1 O12 Na1
c (gss, s /p:40)(Val) Chrg 1
R: 20000 Res .Pwr . @FWHM
Theoretical for M+Na
m/z

Fmoc-Thr(β-D-Gal(Ac)$_4$)-OH. Peracetylated galactose (250.4 mg, 0.6405 mmol) was dissolved in anhydrous ACN (3.8 mL) and anhydrous dichloromethane (3.8 mL) and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into the solution. Fmoc-Thr-OH (263.6 mg, 0.7722 mmol) was diluted in anhydrous ACN (3.6 mL) and dichloromethane (3.6 mL) and boron trifluoride diethyletherate was added (0.470 mL, 3.84 mmol), and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into the solution. A reactor coil high purity PFA tubing (0.020 in I.D., 250 ft, 15 mL) was placed in a hot bath at 60 °C. Two Varian Prostar 210 HPLC pumps were used to deliver the reagents and were connected to a Raspberry pi via USB connection. Desired flow rate, run time, and reaction parameters and components were entered into Jupyter notebook, and this information was used to start the pumps at a given flow rate. The donor solution was flowed with the acceptor and BF_3•OEt_2 solution at a flow rate of 0.5 mL/min through PFA tubing with a residence time of 30 min. The reaction solution was collected at steady state for two minutes in a vial, and the volume of the collected reaction solution was determined. The steady state fraction was then concentrated in vacuo, and the anomers were then separated using semipreparative-HPLC on a Phenomenex C-18 column (100 Å, 5 μm, 250 x 10 mm) under isocratic conditions for 30 minutes with 0.1% aqueous TFA/ 0.1% TFA in acetonitrile (1:1) to give **2** (7.0 mg, 26%). The spectroscopic data was in good agreement with previously reported values.[3]

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 7.6, 3.4 Hz, 3H), 7.63 (dd, *J* = 11.2, 6.1 Hz, 3H), 7.41 (t, *J* = 7.5 Hz, 3H), 7.36 – 7.30 (m, 3H), 5.66 (d, *J* = 8.9 Hz, 1H), 5.39 (d, *J* = 3.4 Hz, 1H), 5.16 (dd, *J* = 10.4, 8.0 Hz, 1H), 5.07 – 4.98 (m, 1H), 4.52 (d, *J* = 8.0 Hz, 1H), 4.48 – 4.43 (m, 5H), 4.27 (dt, *J* = 11.2, 5.3 Hz, 3H), 4.09 (ddd, *J* = 17.9, 9.1, 5.1 Hz, 1H), 3.85 (t, *J* = 6.3 Hz, 1H), 2.16 (d, *J* = 13.7 Hz, 5H), 2.11 – 2.04 (m, 9H), 2.01 (d, *J* = 7.1 Hz, 4H), 1.24 (d, *J* = 6.3 Hz, 3H).

LRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{33}H_{37}NO_{14}Na$ 694.2; Found 694.2



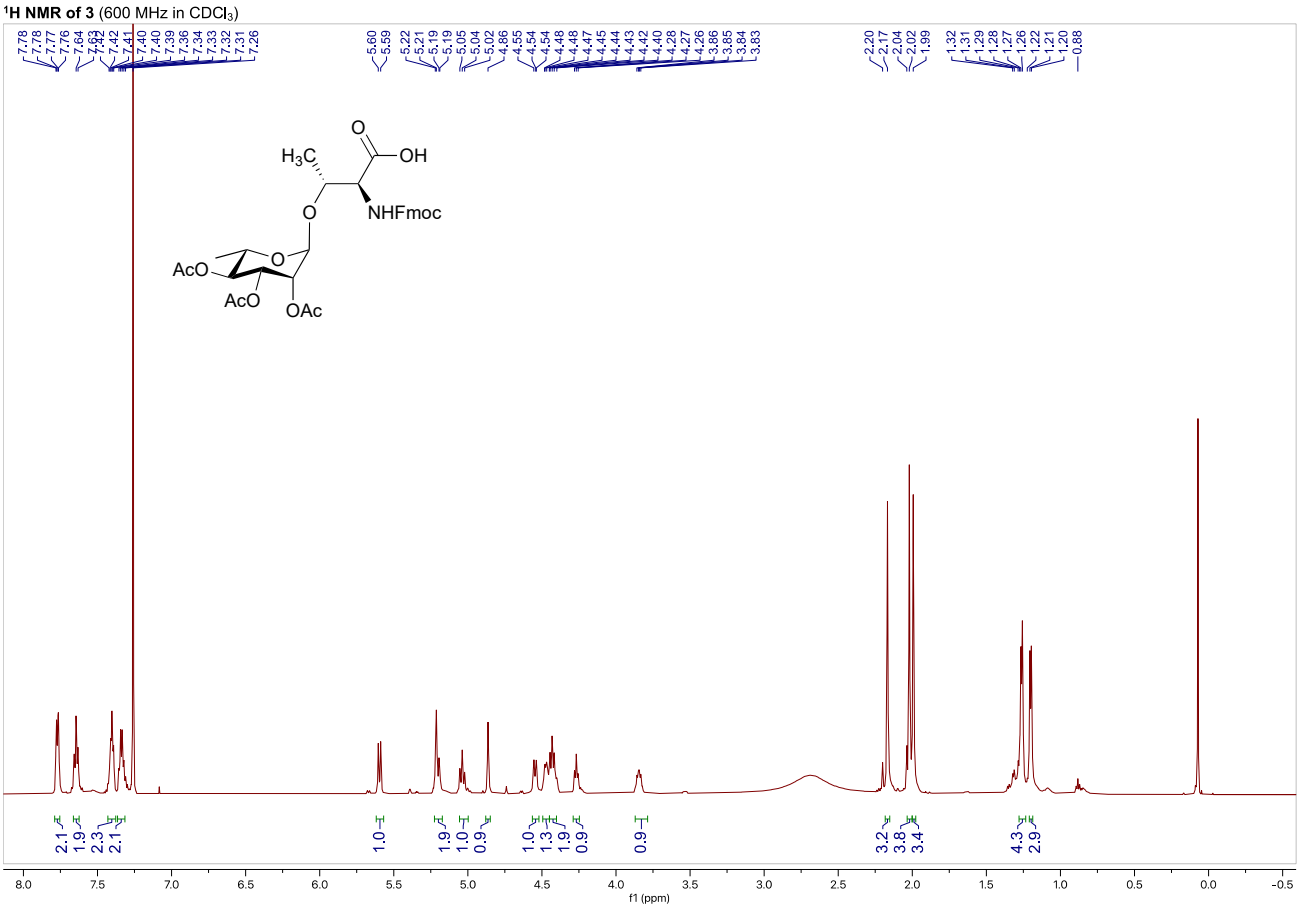


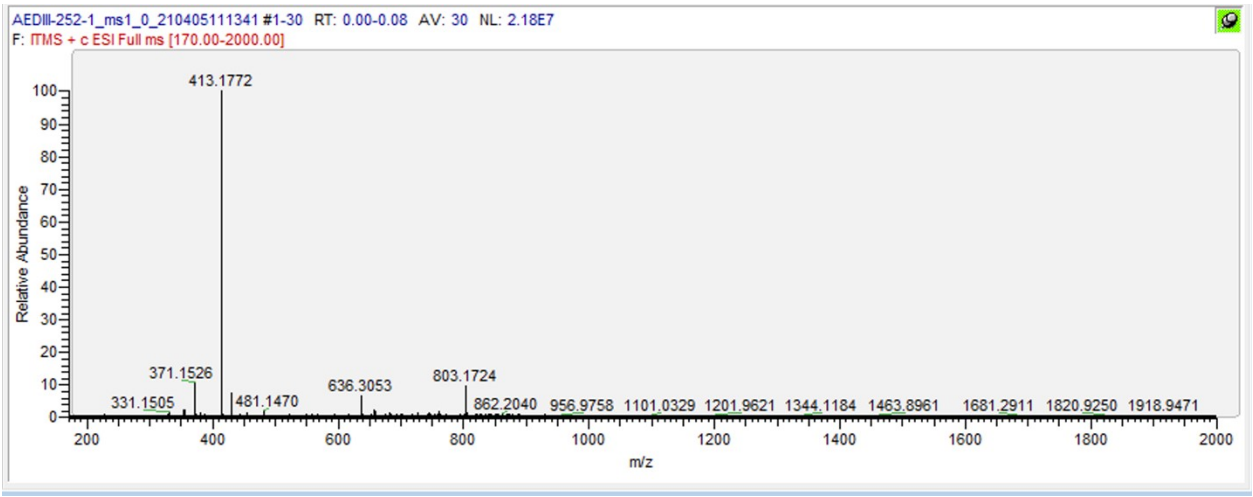
Fmoc-Thr(α-L-Rha(Ac)$_3$)-OH. Tetraacetylated rhamnose (252.2 mg, 0.7589 mmol) was dissolved in anhydrous ACN (3.8 mL) and anhydrous dichloromethane (3.8 mL) and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into the solution. Fmoc-Thr-OH (307.8 mg, 0.9017 mmol) was diluted in anhydrous ACN (3.6 mL) and dichloromethane (3.6 mL) and boron trifluoride diethyletherate was added (0.560 mL, 4.51 mmol), and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into

the solution. A reactor coil high purity PFA tubing (0.020 in I.D., 250 ft, 15 mL) was placed in a hot bath at 60 °C. Two Varian Prostar 210 HPLC pumps were used to deliver the reagents and were connected to a Raspberry pi via USB connection. Desired flow rate, run time, and reaction parameters and components were entered into Jupyter notebook, and this information was used to start the pumps at a given flow rate. The donor solution was flowed with the acceptor and BF_3•OEt_2 solution at a flow rate of 0.5 mL/min through PFA tubing with a residence time of 30 min. The reaction solution was collected at steady state for two minutes in a vial, and the volume of the collected reaction solution was determined. The steady state fraction was then concentrated in vacuo, and the anomers were then separated using semipreparative-HPLC on a Phenomenex C-18 column (100 Å, 5 μm, 250 x 10 mm) under isocratic conditions for 30 minutes with 0.1% aqueous TFA/ 0.1% TFA in acetonitrile (1:1) to give **3** (8.1 mg, 27%). The spectroscopic data was in good agreement with previously reported values.[4]

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.64 (t, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.36 – 7.30 (m, 2H), 5.60 (d, *J* = 9.5 Hz, 1H), 5.20 (d, *J* = 11.1 Hz, 2H), 5.04 (t, *J* = 9.5 Hz, 1H), 4.86 (s, 1H), 4.55 (d, *J* = 9.6 Hz, 1H), 4.44 (dd, *J* = 14.9, 8.1 Hz, 3H), 4.27 (t, *J* = 7.2 Hz, 1H), 3.85 (d, *J* = 8.9 Hz, 1H), 2.17 (s, 3H), 2.01 (d, *J* = 13.8 Hz, 7H), 1.26 (d, *J* = 6.3 Hz, 4H), 1.20 (d, *J* = 6.3 Hz, 3H).

LRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{35}NO_{12}Na$ 636.2; Found 636.2



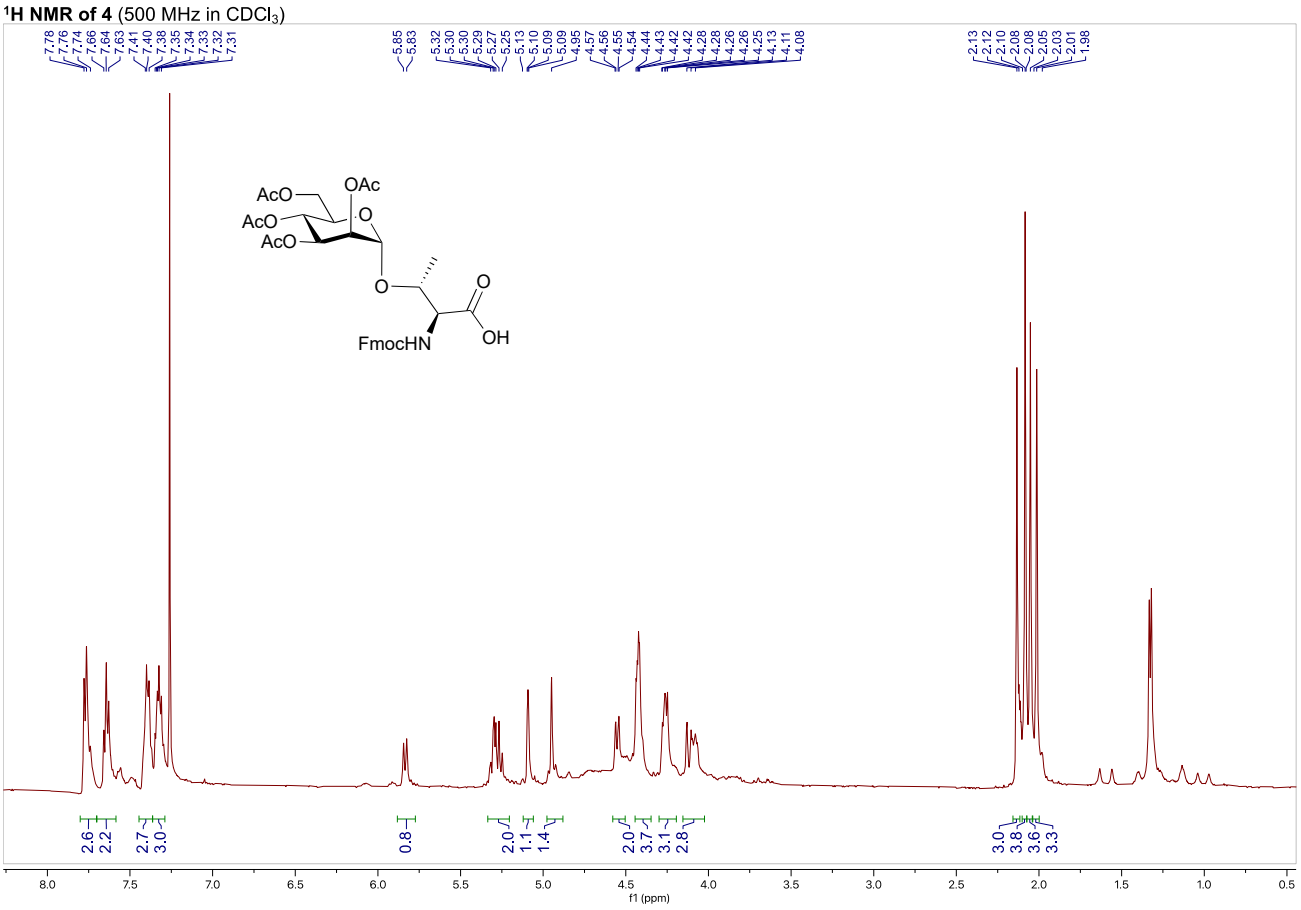


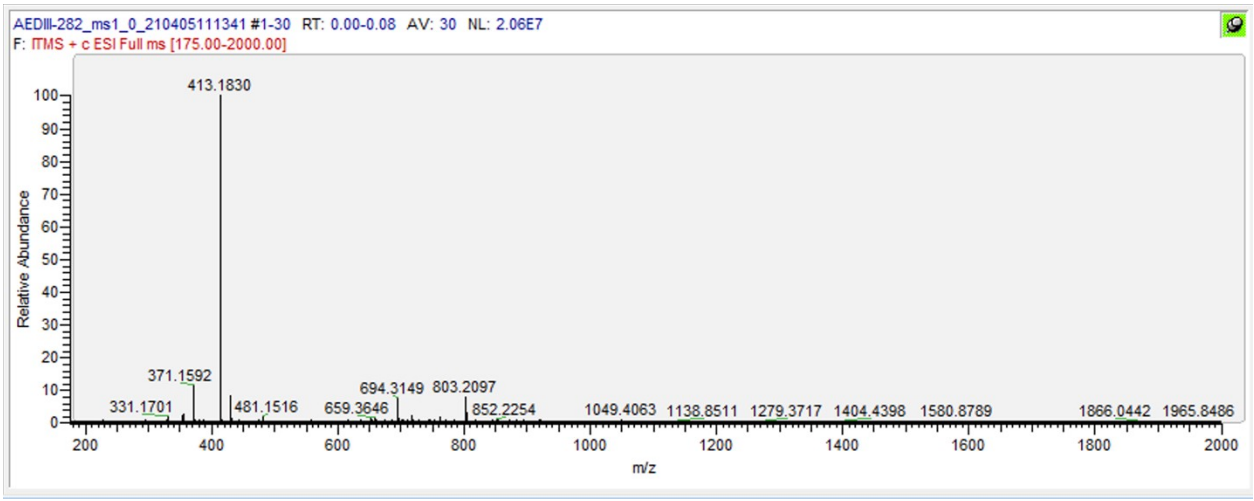
Fmoc-Thr(α-D-Man(Ac)$_4$)-OH. Peracetylated mannose (249.0 mg, 0.6379 mmol) was dissolved in anhydrous ACN (3.2 mL) and anhydrous dichloromethane (3.2 mL) and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into the solution. Fmoc-Thr-OH (264.8 mg, 0.7757 mmol) was diluted in anhydrous ACN (3.2 mL) and dichloromethane (3.2 mL) and boron trifluoride diethyletherate was added (0.470 mL, 3.84 mmol), and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into

the solution. A reactor coil high purity PFA tubing (0.020 in I.D., 250 ft, 15 mL) was placed in a hot bath at 60 °C. Two Varian Prostar 210 HPLC pumps were used to deliver the reagents and were connected to a Raspberry pi via USB connection. Desired flow rate, run time, and reaction parameters and components were entered into Jupyter notebook, and this information was used to start the pumps at a given flow rate. The donor solution was flowed with the acceptor and BF_3•OEt_2 solution at a flow rate of 0.5 mL/min through PFA tubing with a residence time of 30 min. The reaction solution was collected at steady state for two minutes in a vial, and the volume of the collected reaction solution was determined. The steady state fraction was then concentrated in vacuo, and the anomers were then separated using semipreparative-HPLC on a Phenomenex C-18 column (100 Å, 5 μm, 250 x 10 mm) under isocratic conditions for 30 minutes with 0.1% aqueous TFA/ 0.1% TFA in acetonitrile (1:1) to give **4** (10.5 mg, 39%). The spectroscopic data was in good agreement with previously reported values.[3]

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.7 Hz, 4H), 7.64 (t, *J* = 7.4 Hz, 3H), 7.44 – 7.28 (m, 6H), 5.84 (d, *J* = 9.7 Hz, 1H), 5.34 – 5.23 (m, 2H), 5.09 (t, *J* = 2.5 Hz, 1H), 4.95 (s, 1H), 4.55 (dd, *J* = 9.5, 2.6 Hz, 1H), 4.43 (dd, *J* = 7.3, 3.5 Hz, 3H), 4.26 (dd, *J* = 9.3, 6.3 Hz, 3H), 4.15 – 4.06 (m, 2H), 2.13 (s, 3H), 2.08 (s, 4H), 2.05 (s, 3H), 2.01 (s, 3H), 1.33 (d, *J* = 6.5 Hz, 4H)

LRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{33}H_{37}NO_{14}Na$ 694.2; Found 694.2



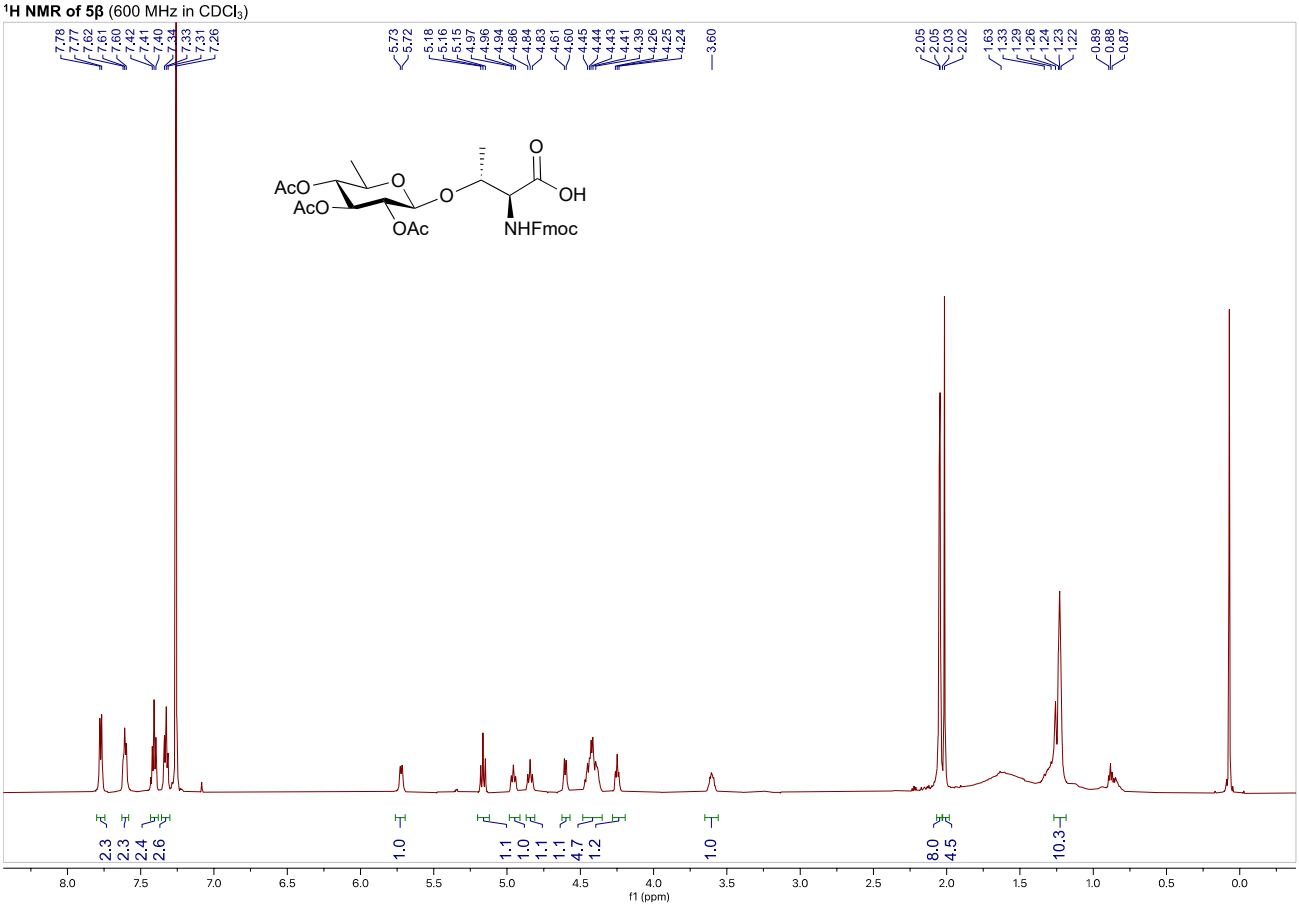


Fmoc-Thr(β-D-Quin(Ac)$_3$)-OH. Tetraacetylated quinovose (98.9 mg, 0.2976 mmol) was dissolved in anhydrous ACN (1.5 mL) and anhydrous dichloromethane (1.5 mL) and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into the solution. Fmoc-Thr-OH (128.6 mg, 0.3767 mmol) was diluted in anhydrous ACN (1.5 mL) and dichloromethane (1.5 mL) and boron trifluoride diethyletherate was added (0.223 mL, 1.80 mmol), and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into

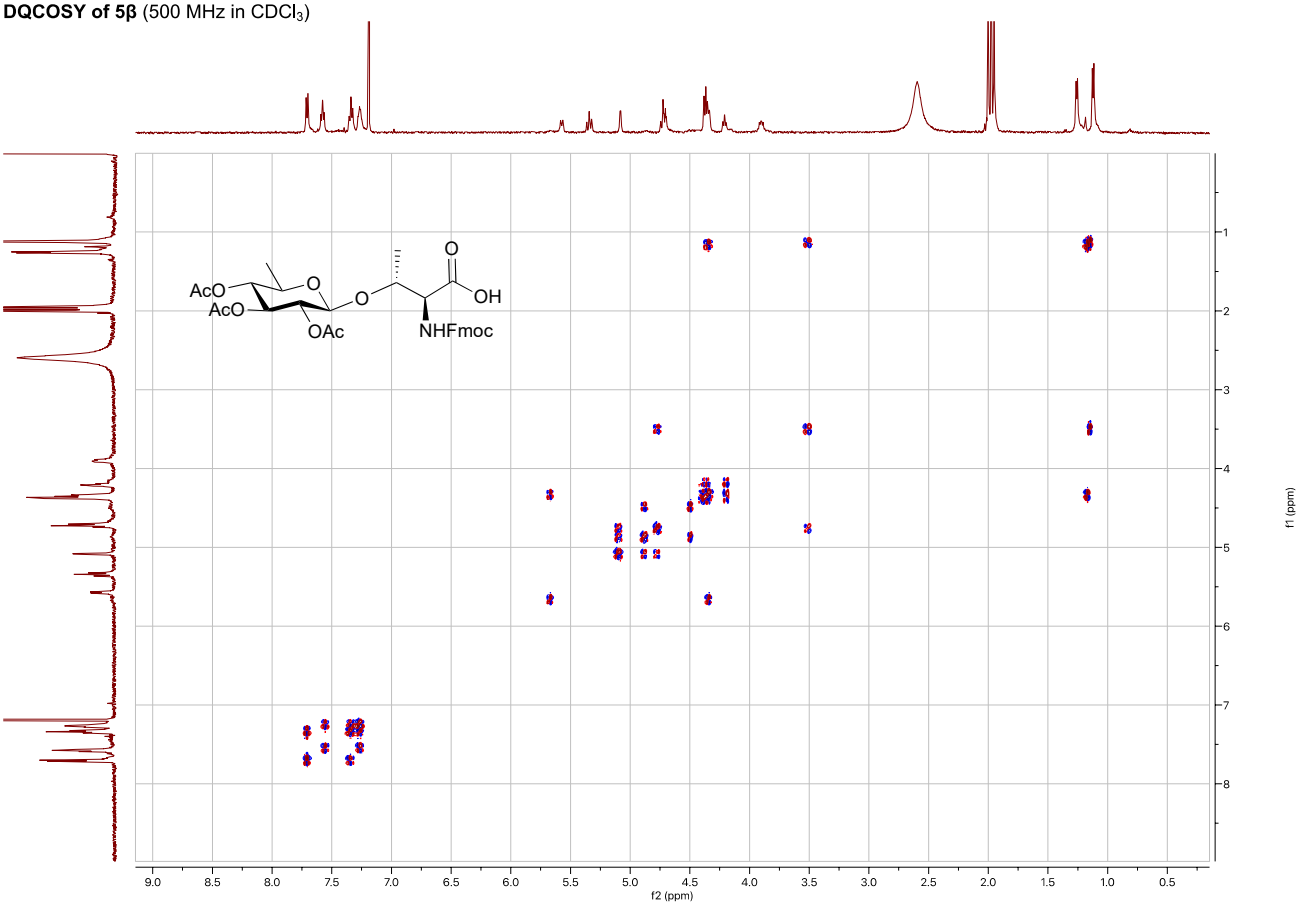
the solution. A reactor coil high purity PFA tubing (0.020 in I.D., 250 ft, 15 mL) was placed in a hot bath at 60 °C. Two Varian Prostar 210 HPLC pumps were used to deliver the reagents and were connected to a Raspberry pi via USB connection. Desired flow rate, run time, and reaction parameters and components were entered into Jupyter notebook, and this information was used to start the pumps at a given flow rate. The donor solution was flowed with the acceptor and BF_3•OEt_2 solution at a flow rate of 0.5 mL/min through PFA tubing with a residence time of 30 min. The reaction solution was collected at steady state for two minutes in a vial, and the volume of the collected reaction solution was determined. The steady state fraction was then concentrated in vacuo, and the anomers were then separated using semipreparative-HPLC on a Phenomenex C-18 column (100 Å, 5 μm, 250 x 10 mm) under isocratic conditions for 30 minutes with 0.1% aqueous TFA/ 0.1% TFA in acetonitrile (1:1) to give **5α** (2.4 mg, 14%) and **5β** (2.8 mg, 16%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.78 (d, *J* = 7.6 Hz, 2H), 7.65 (t, *J* = 6.9 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.33 (d, *J* = 3.4 Hz, 2H), 5.64 (d, *J* = 9.1 Hz, 1H), 5.41 (t, *J* = 10.0 Hz, 1H), 5.15 (d, *J* = 3.9 Hz, 1H), 4.79 (t, *J* = 9.9 Hz, 2H), 4.43 (dd, *J* = 14.9, 7.8 Hz, 4H), 4.28 (t, *J* = 7.2 Hz, 1H), 3.96 (d, *J* = 8.4 Hz, 1H), 2.13 – 1.95 (m, 11H), 1.33 (d, *J* = 6.3 Hz, 3H), 1.19 (d, *J* = 6.3 Hz, 4H).
HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{35}NO_{12}Na$ a 636.2051; Found 636.2050

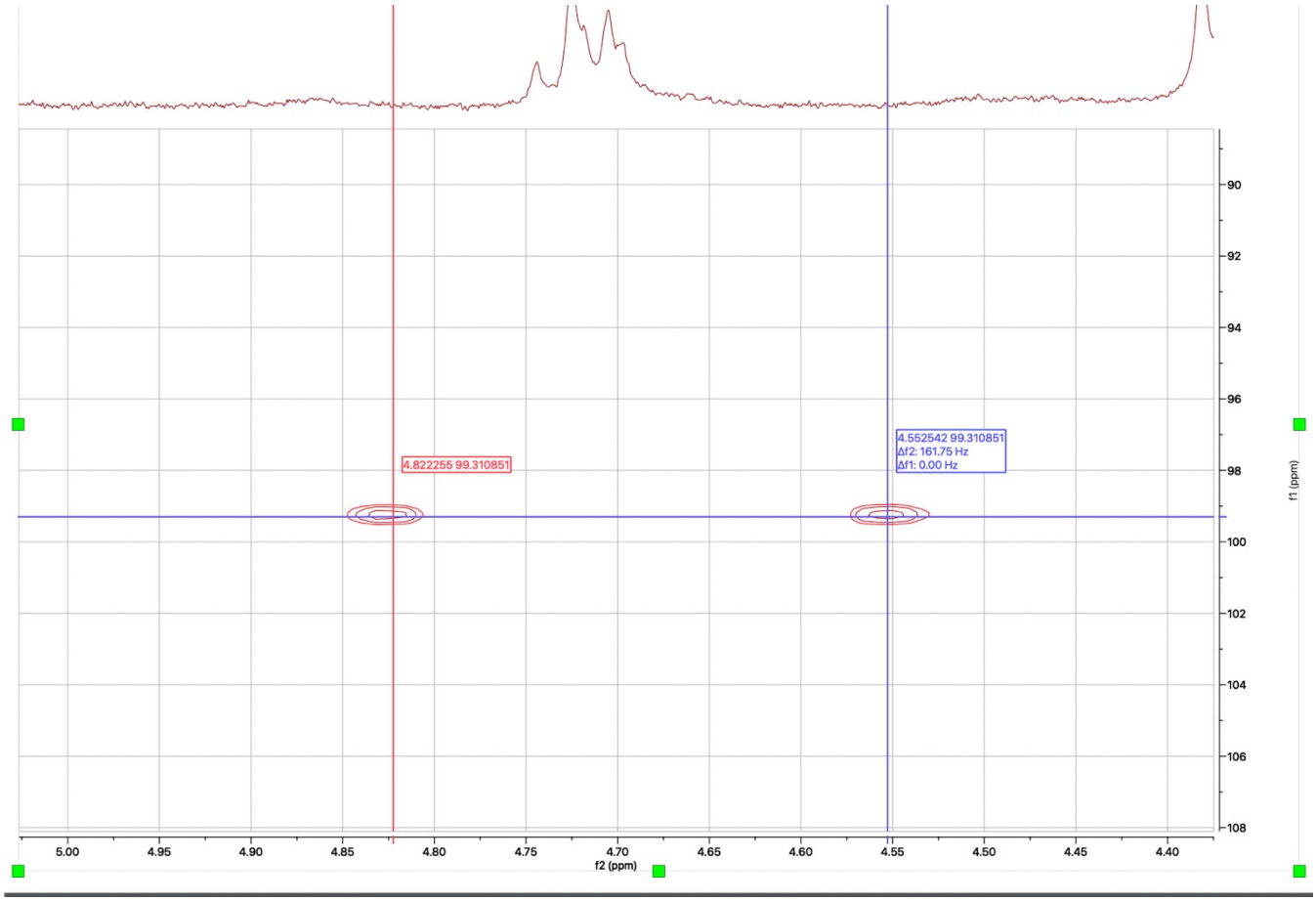
^{1}H NMR of 5β (600 MHz in $CDCl_3$)



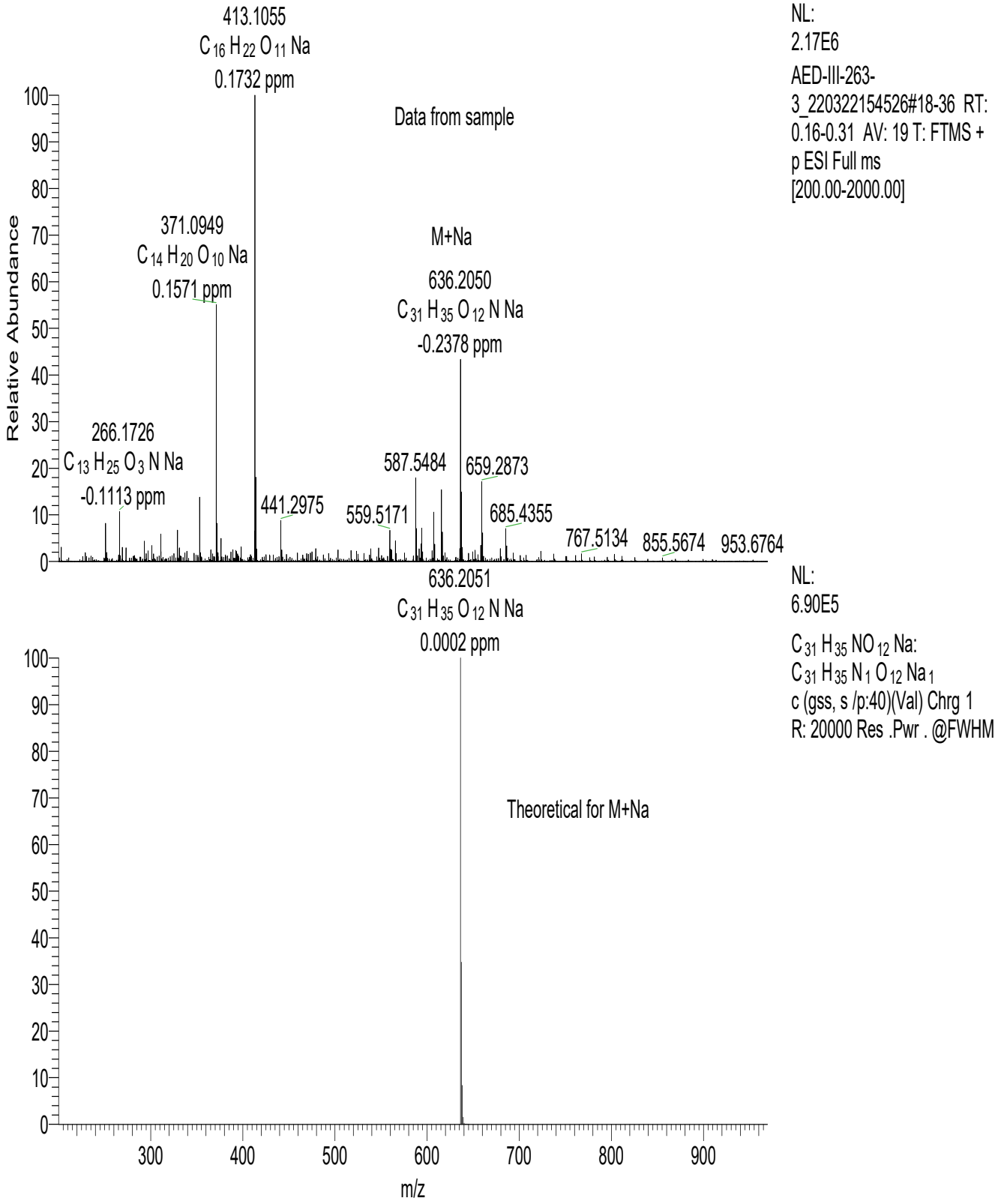
DQCOSY of 5β (500 MHz in $CDCl_3$)



Coupled HSQC of 5β (500 MHz, 125 MHz in $CDCl_3$)



160 Hz indicates an axial proton at C1, therefore confirms β-linkage.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C16 H22 O11 Na
0.1732 ppm
Data from sample
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2.17E6
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C14 H20 O10 Na
0.1571 ppm
M+Na
636.2050
C31 H35 O12 N Na
-0.2378 ppm
266.1726
C13 H25 O3 N Na
-0.1113 ppm
587.5484
659.2873
441.2975
559.5171
685.4355
767.5134
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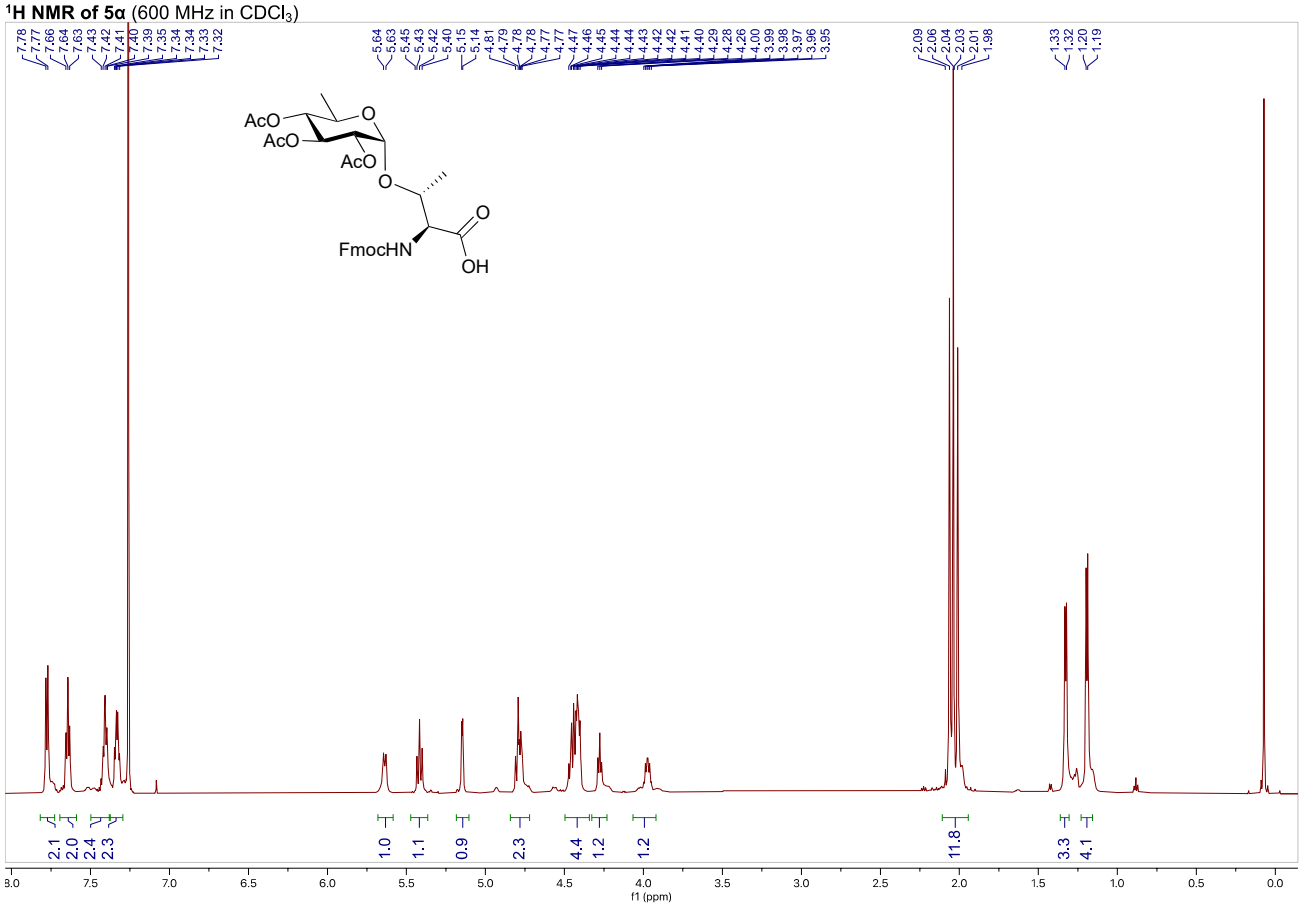
953.6764
Relative Abundance
636.2051
C31 H35 O12 N Na
0.0002 ppm
NL:
6.90E5
C31 H35 NO12 Na:
C31 H35 N1 O12 Na1
c (gss, s /p:40)(Val) Chrg 1
R: 20000 Res .Pwr . @FWHM
Theoretical for M+Na
m/z

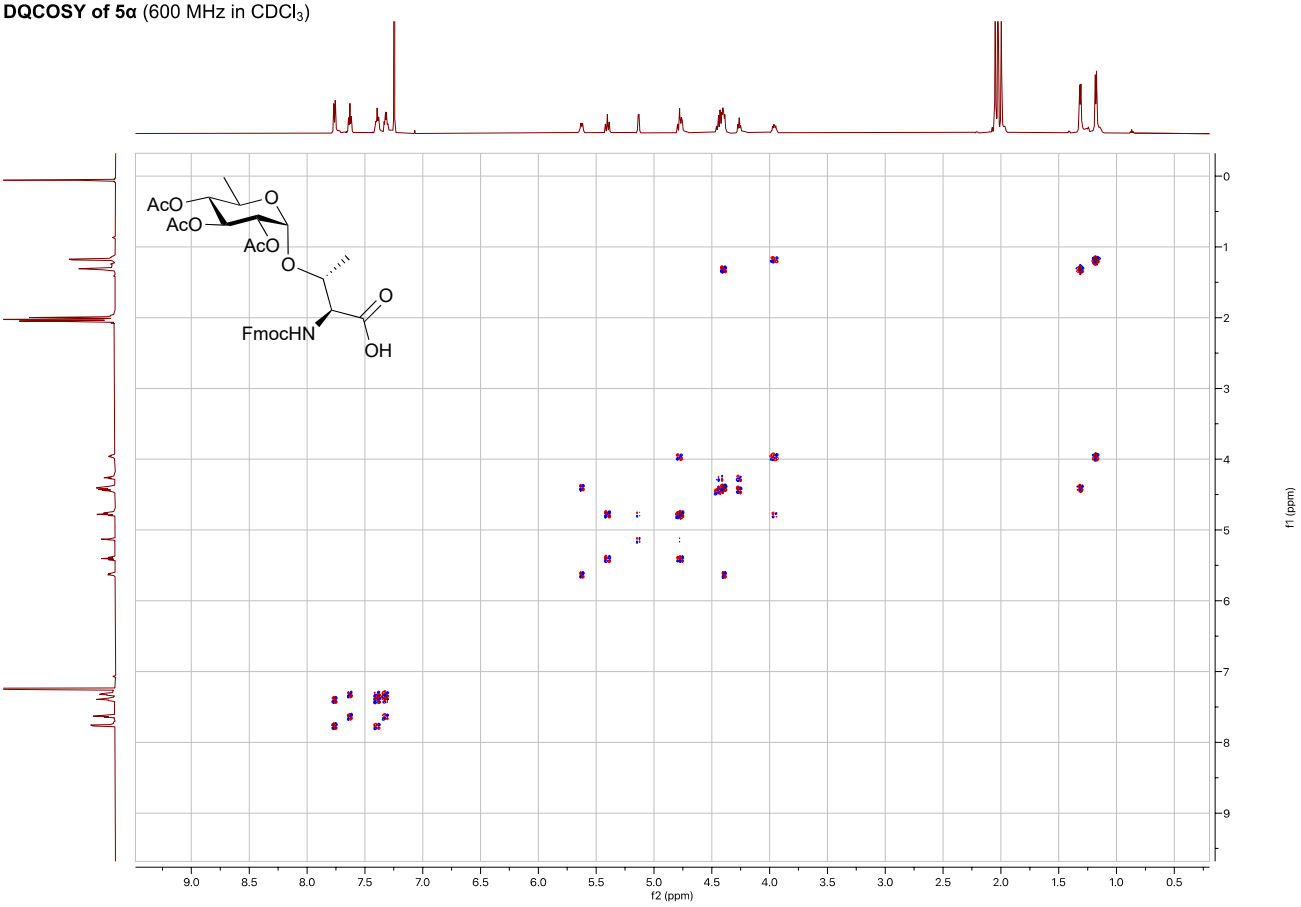
Fmoc-Thr(α-D-Quin(Ac)$_3$)-OH.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.64 (t, *J* = 7.1 Hz, 2H), 7.43 – 7.38 (m, 2H), 7.36 – 7.30 (m, 2H), 5.64 (d, *J* = 9.1 Hz, 1H), 5.42 (t, *J* = 9.9 Hz, 1H), 5.15 (d, *J* = 3.9 Hz, 1H), 4.82 – 4.75 (m, 2H), 4.49 – 4.39 (m, 4H), 4.28 (t, *J* = 7.2 Hz, 1H), 3.97 (dq, *J* = 12.3, 6.3 Hz, 1H), 2.04 (p, *J* = 15.5 Hz, 9H), 1.33 (d, *J* = 6.3 Hz, 3H), 1.19 (d, *J* = 6.3 Hz, 3H).

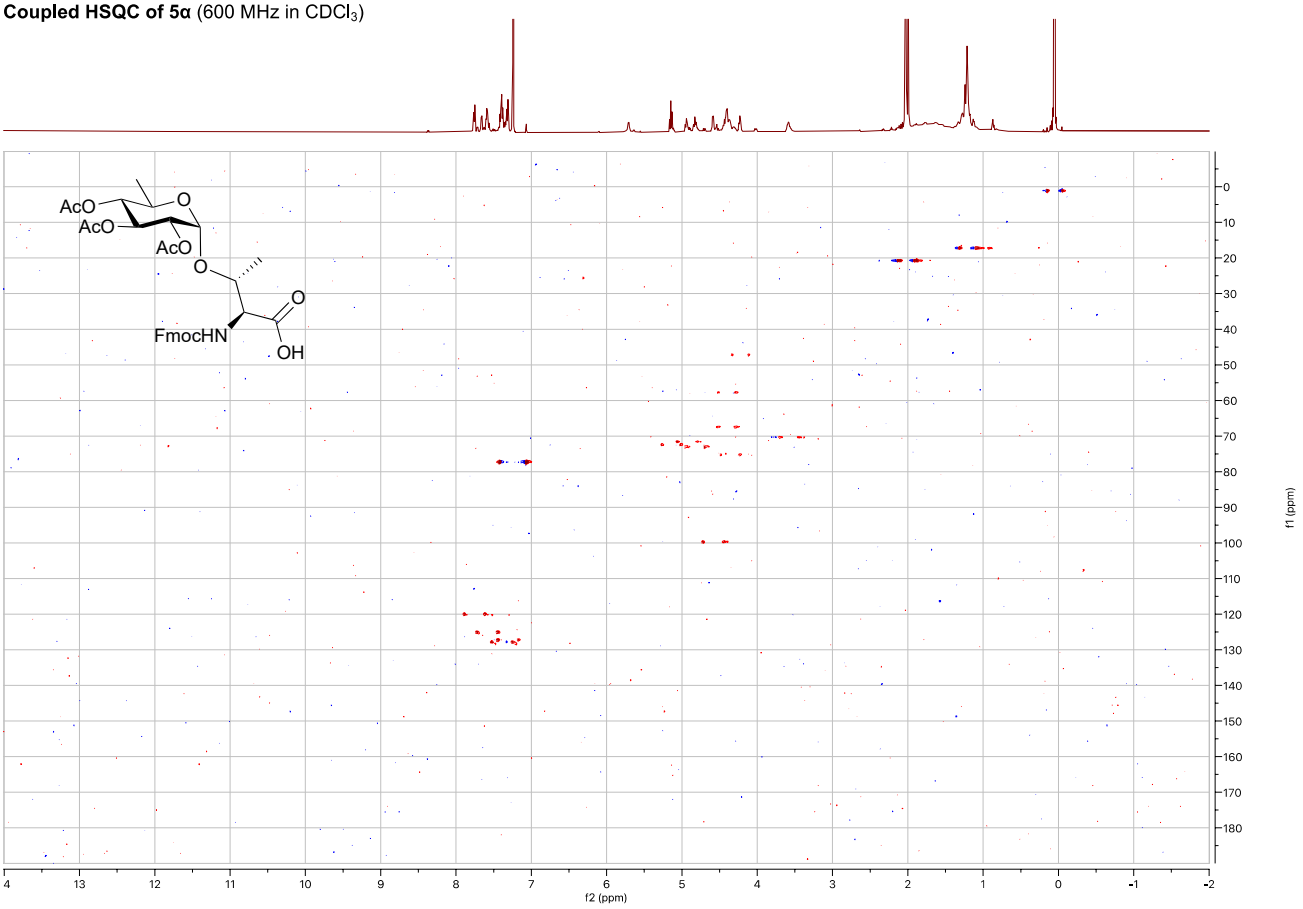
HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{35}NO_{12}Na$ 636.2051; Found 636.2051, $[2M+Na]^+$ Calcd for $C_{31}H_{35}NO_{12}Na$ 1249.4211; Found 1249.4213.

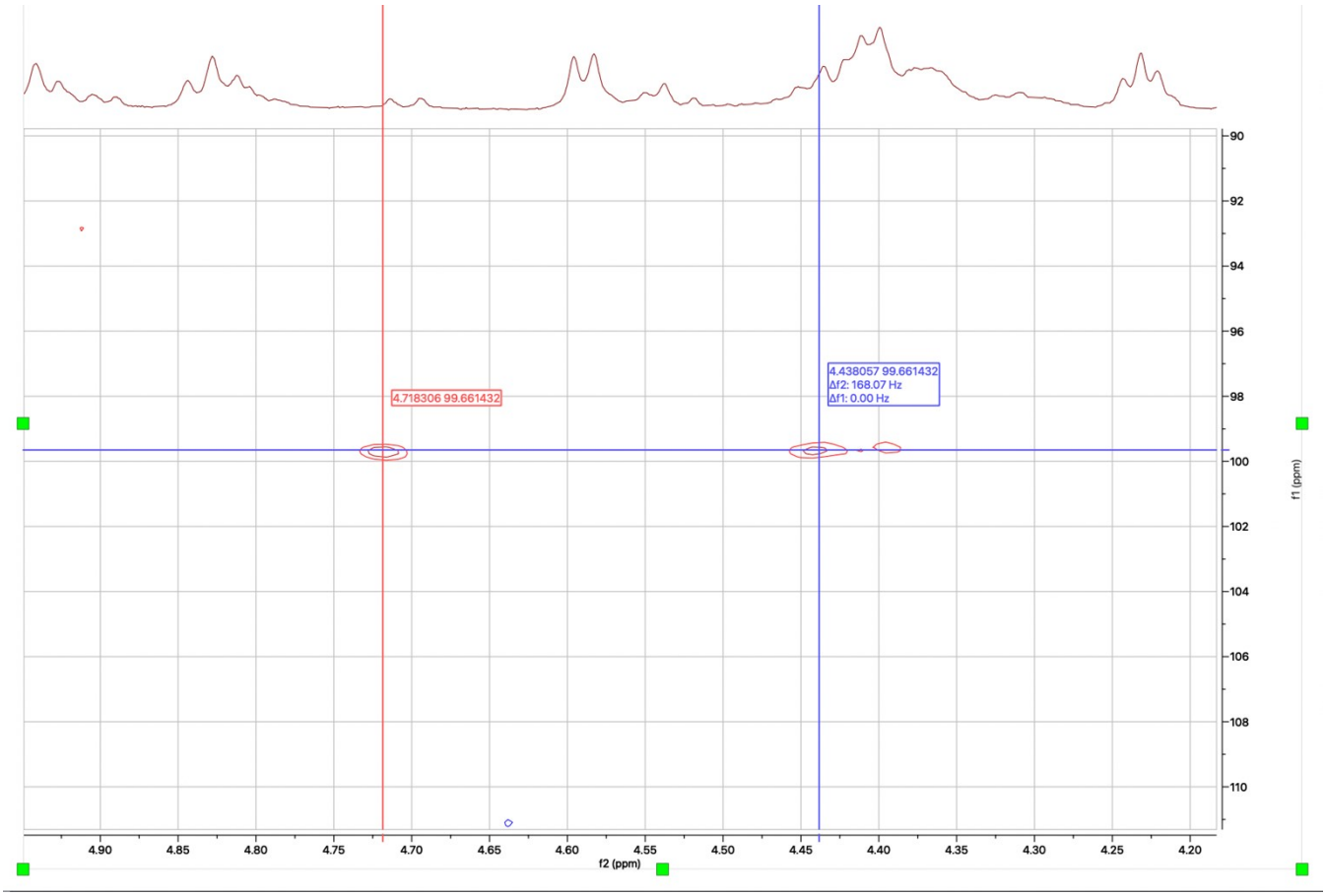


DQCOSY of 5α (600 MHz in $CDCl_3$)



Coupled HSQC of 5α (600 MHz in $CDCl_3$)





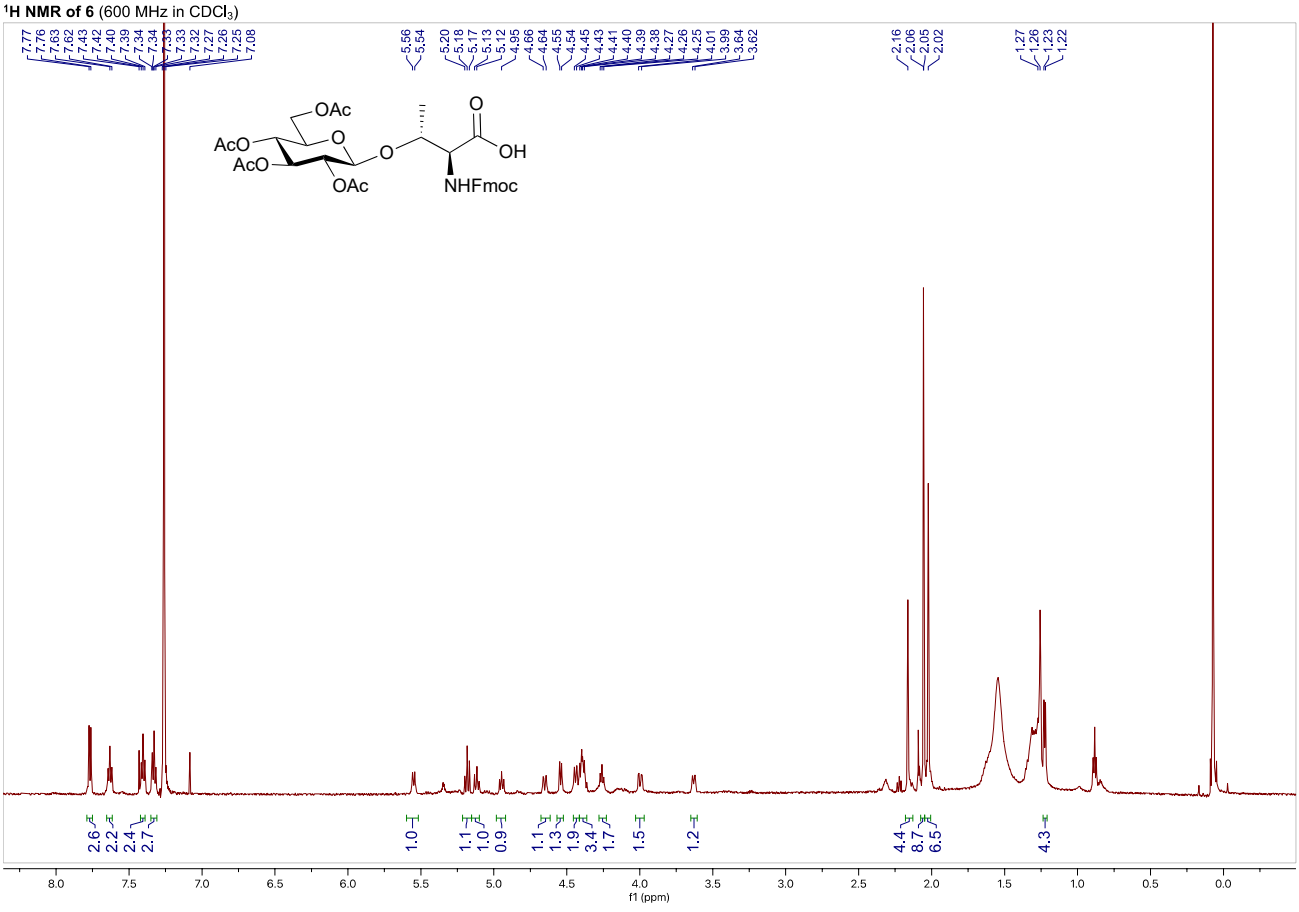
~170 Hz indicates an equatorial proton at C1, therefore confirms α-linkage.

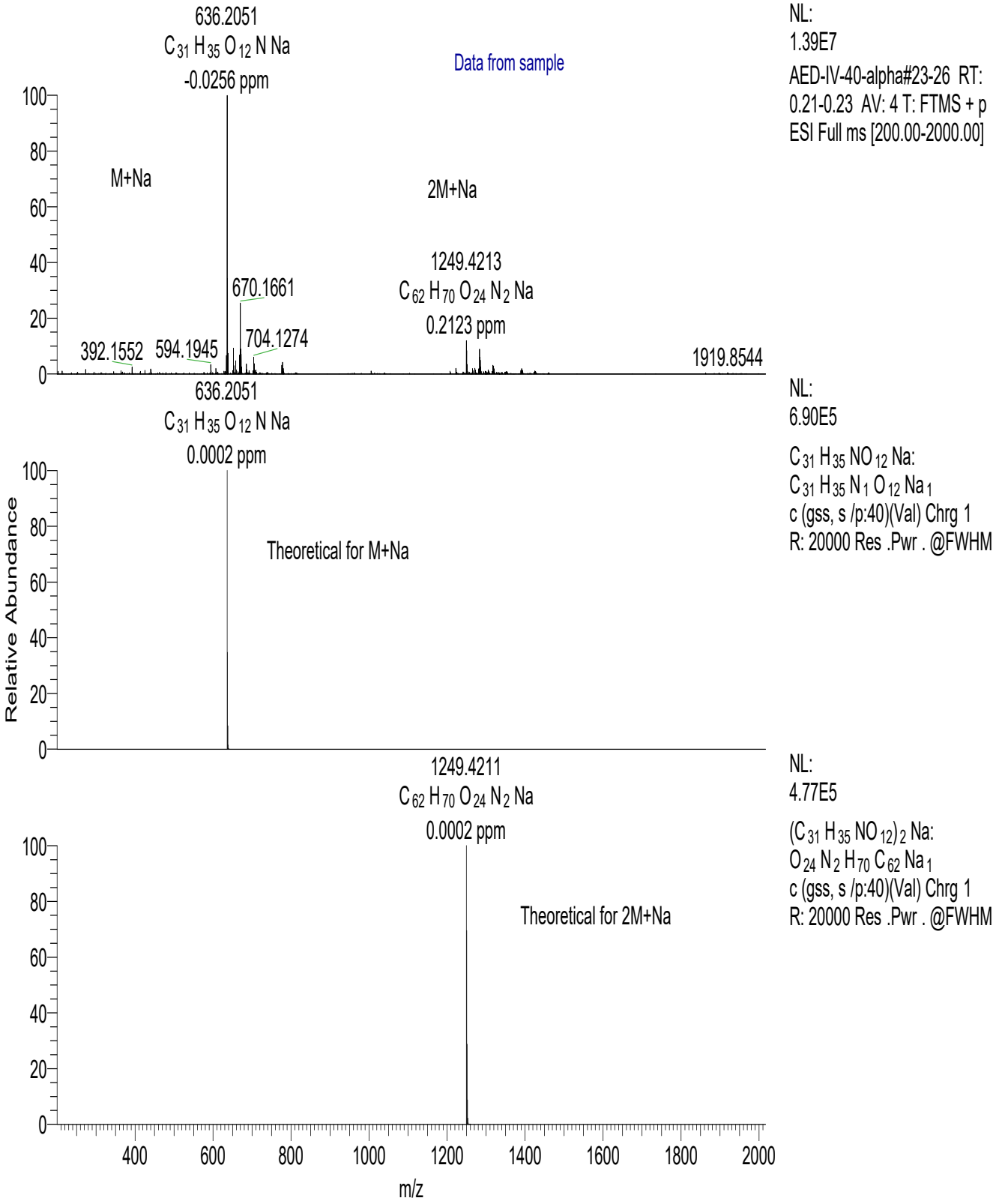
Fmoc-Thr(β-D-Glc(Ac)$_4$)-OH. Peracetylated glucose (250.3 mg, 0.6412 mmol) was dissolved in anhydrous ACN (3.2 mL) and anhydrous dichloromethane (3.2 mL) and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into the solution. Fmoc-Thr-OH (265.3 mg, 0.7772 mmol) was diluted in anhydrous ACN (3.2 mL) and dichloromethane (3.2 mL) and boron trifluoride diethyletherate was added (0.470 mL, 3.84 mmol), and PFA tubing (I.D. 1/8 in, 12 in) was inserted through the septa into the solution. A reactor coil high purity PFA tubing (0.020 in I.D., 250 ft, 15 mL) was placed in a hot bath at 60 °C. Two Varian Prostar 210 HPLC pumps were used to deliver the reagents and were connected to a Raspberry pi via USB connection. Desired flow rate, run time, and reaction parameters and components were entered into Jupyter notebook, and this information was used to start the pumps at a given flow rate. The donor solution was flowed with the acceptor and BF_3•OEt_2 solution at a flow rate of 0.5 mL/min through PFA tubing with a residence time of 30 min. The reaction solution was

collected at steady state for two minutes in a vial, and the volume of the collected reaction solution was determined. The steady state fraction was then concentrated in vacuo, and the anomers were then separated using semipreparative-HPLC on a Phenomenex C-18 column (100 Å, 5 µm, 250 x 10 mm) under isocratic conditions for 30 minutes with 0.1% aqueous TFA/ 0.1% TFA in acetonitrile (1:1) to give **12** (6.6 mg, 21%). The spectroscopic data was in good agreement with previously reported values.[3]

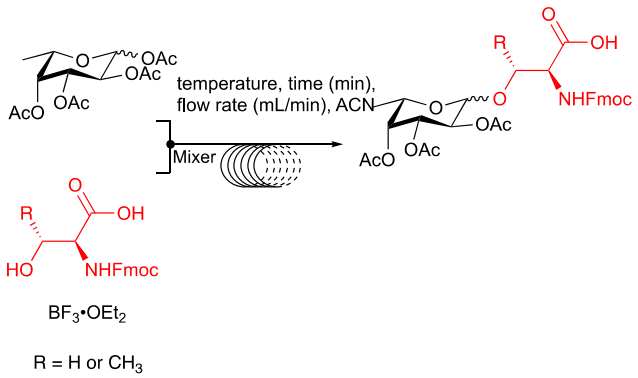
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.63 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.33 (td, *J* = 7.5, 1.2 Hz, 2H), 5.62 (d, *J* = 9.3 Hz, 1H), 5.24 – 5.16 (m, 1H), 5.11 (t, *J* = 9.7 Hz, 1H), 4.95 (dd, *J* = 9.6, 7.9 Hz, 1H), 4.59 – 4.52 (m, 2H), 4.48 – 4.35 (m, 4H), 4.26 (q, *J* = 7.9 Hz, 1H), 4.03 (dd, *J* = 12.4, 3.6 Hz, 1H), 3.64 (dt, *J* = 10.1, 3.2 Hz, 1H), 2.15 (s, 3H), 2.11 – 2.01 (m, 10H), 1.23 (d, *J* = 6.4 Hz, 3H).

LRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{33}H_{37}NO_{14}Na$ 694.2; Found 694.2



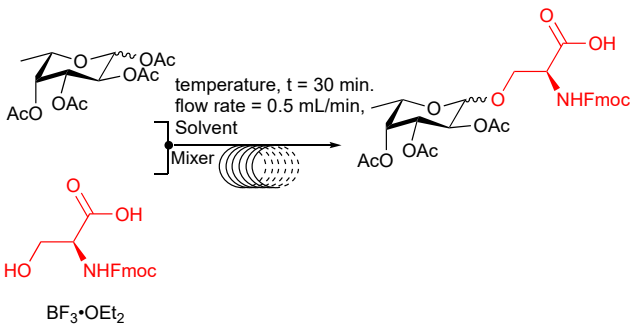

636.2051
C31 H35 O12 N Na
-0.0256 ppm
Data from sample
NL:
1.39E7
AED-IV-40-alpha#23-26 RT:
0.21-0.23 AV: 4 T: FTMS + p
ESI Full ms [200.00-2000.00]
M+Na
2M+Na
1249.4213
C62 H70 O24 N2 Na
0.2123 ppm
670.1661
704.1274
392.1552
594.1945
1919.8544
636.2051
C31 H35 O12 N Na
0.0002 ppm
NL:
6.90E5
C31 H35 NO12 Na:
C31 H35 N1 O12 Na1
c (gss, s /p:40)(Val) Chrg 1
R: 20000 Res .Pwr . @FWHM
Theoretical for M+Na
Relative Abundance
1249.4211
C62 H70 O24 N2 Na
0.0002 ppm
NL:
4.77E5
(C31 H35 NO12)2 Na:
O24 N2 H70 C62 Na1
c (gss, s /p:40)(Val) Chrg 1
R: 20000 Res .Pwr . @FWHM
Theoretical for 2M+Na
m/z

Optimization of protected glycosylated amino acid building block synthesis



	temp. (°C)	residence time (min.)	α yield	α % yield	β yield	β % yield	ratio (α/β)
Fuc-Thr	0 °C	6	0.805 mg	1 %	20.2 mg	22 %	1: 25
Fuc-Thr	80 °C	6	4.21 mg	5 %	5.62 mg	6 %	1: 1.3
Fuc-Thr	0 °C	7.5	1.35 mg	1.5 %	27.2 mg	29 %	1: 20
Fuc-Ser	0 °C	6	1.18 mg	1.3 %	3.26 mg	3.6 %	1: 2.8
Fuc-Ser	80 °C	6	3.21 mg	3.6 %	0.63 mg	0.7 %	5.1: 1
Fuc-Ser	0 °C	7.5	0.07 mg	0.08 %	3.82 mg	4.2 %	1: 55

Initial optimization efforts investigated the effect of temperature on α/β selectivity. Fuc-Ser refers to R=H and Fuc-Thr refers to R=CH_3. These efforts showed that at higher temperatures, the alpha anomer became more prevalent, and at lower temperatures, the beta anomer was favored. Yields were based off of HPLC calibration curves, and unfortunately, did not reflect the isolated yields that were later collected, showing a need to optimize for yields further.



Temp (°C)	res time (min)	solvent	α-mass (mg)	β-mass (mg)	α/β
40	30	ACN	2.5	1.1	2.3:1
60	30	ACN	5.0	1.1	4.5:1

Temp (°C)	res time (min)	solvent	α-mass (mg)	β-mass (mg)	α/β
60	30	ACN	5.0	1.1	4.5:1
60	30	ACN/DCM (1:1)	6.1	1.6	3.8:1

Temp. (°C)	res. time (min)	Solvent	Promoter eq.	α-mass (mg)	β-mass (mg)	α/β
60	30	ACN/DCM (1:1)	6	6.1	1.6	3.8:1
60	50	ACN/DCM (1:1)	0.2	3.7	3.4	1.1:1

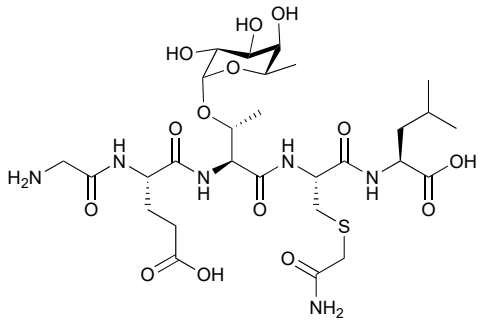
The next optimization efforts were focused on optimization of isolated yields by investigating reaction temperature, ratio of acetonitrile to dichloromethane, and promoter equivalents. These efforts showed that a higher temperature of 60 °C was ideal for amount of material isolated, and using DCM as a solvent alongside acetonitrile was beneficial for limiting by-product formation. Stoichiometric promoter equivalents, allowed for more selectivity towards the alpha anomer.

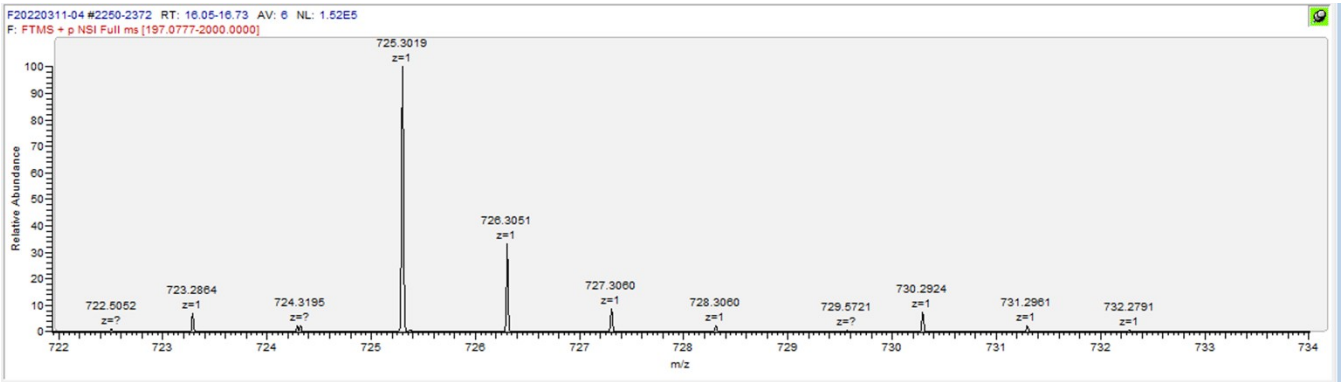
General solid phase peptide synthesis protocol:

Wang resin (0.9 mmol/ g) was swelled with dichloromethane for 30 min. The resin was washed with dichloromethane and DMF, and the resin was dried under vacuum. A solution of Fmoc-Leu-OH (5.0 equiv.), HOBt (5.0 equiv.), N,N'-diisopropylcarbodiimide (5.0 equiv.), and DMAP (0.2 equiv.) in DMF was prepared and added to the resin. The resin loading step was agitated overnight. Following resin loading, the resin was washed with DMF and dichloromethane and vacuum dried and then was subjected to capping conditions (500 µL of 8:1:1 DMF/Ac_2O/pyridine) for 30 min. The resin was washed with DMF and dichloromethane and vacuum dried and then was subjected to Fmoc deprotection conditions (20% piperidine in DMF) for 21 min, and this sequence of coupling, capping, and Fmoc deprotection was repeated for all necessary amino acids. Amino acid coupling conditions involved making a solution of Fmoc amino acid (5.0 equiv.), HOBt (5.0 equiv.), DIC (5.0 equiv.) in DMF and agitation times ranged from 3 h to overnight. Coupling of the glycosylated amino acid involved making a solution of PyBOP (5.0 equiv), HOBt (4.0 equiv) in DMF and then adding Hünigs

base (3.0 equiv.) and agitating for 4 h. Upon completion of the final coupling, the last Fmoc was deprotected (20% piperidine in DMF) for 21 min. The glycopeptide was then cleaved from the resin (9:0.5:0.5 TFA/TIPS/H_2O) for 1 h. This material was purified via a C18 Sep Pak, and the organic elution was collected and lyophilized. To the lyophilized material, 1:1 MeOH/H_2O (500 µL) was added followed by hydrazine monohydrate (28 µL), and this was agitated for 1 h. Then more hydrazine monohydrate (10 µL) was added, and this was agitated for 3 h. Following deacetylation, iodoacetamide (100 µL, 20 mM in water) was added to alkylate the cysteine residue, and this reaction was allowed to proceed in a dark drawer for 45 min. Samples were then submitted for LCMS data analysis without further purification.

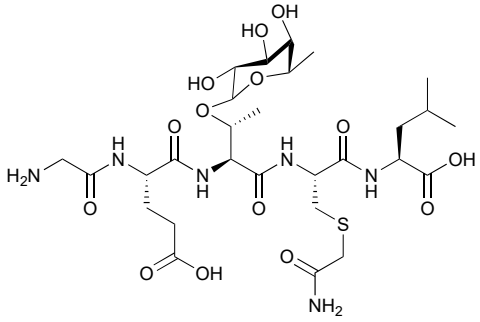
GET(Fuc-α)CL

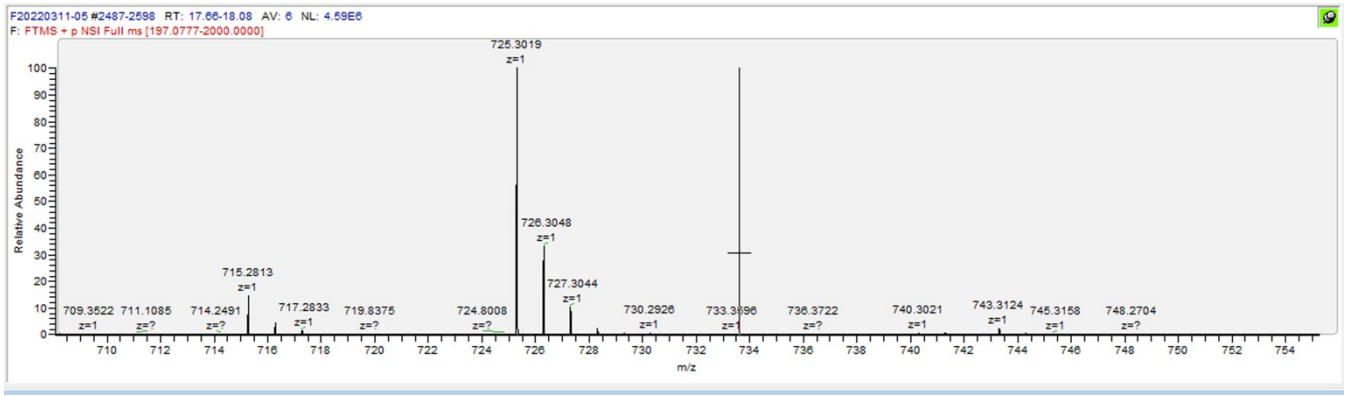




HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{28}H_{49}N_6O_{14}S$, 724.2949; Found 725.3019

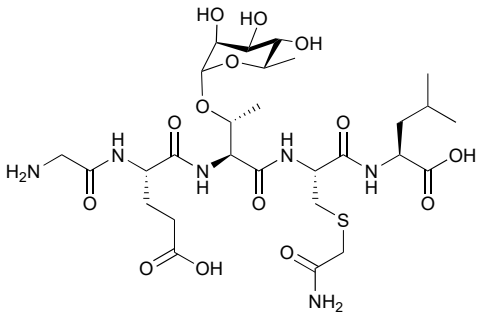
GET(Fuc-β)CL

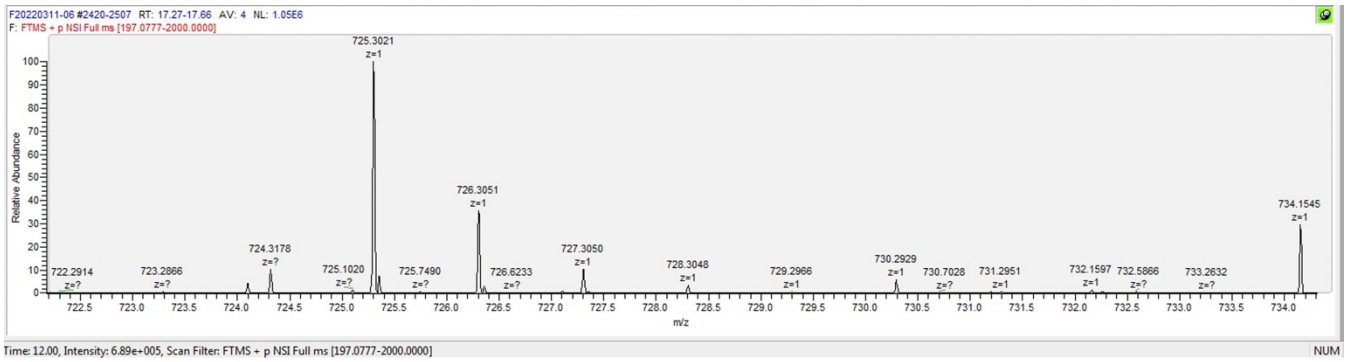




HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{28}H_{49}N_6O_{14}S$, 724.2949; Found 725.3019

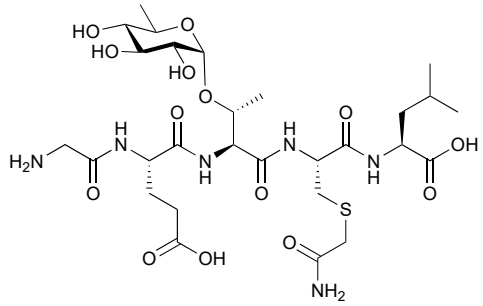
GET(Rha-α)CL

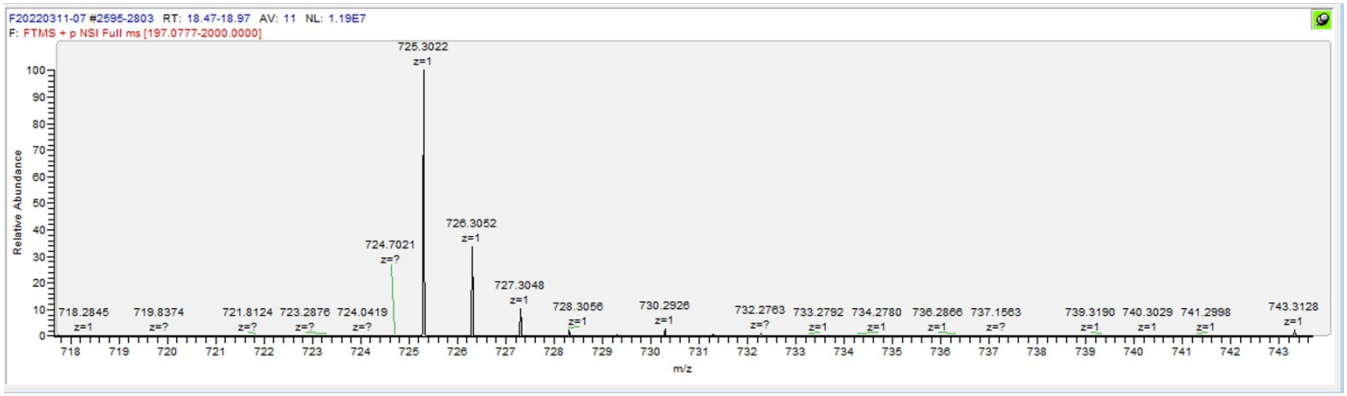




HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{28}H_{49}N_6O_{14}S$, 724.2949; Found 725.3021

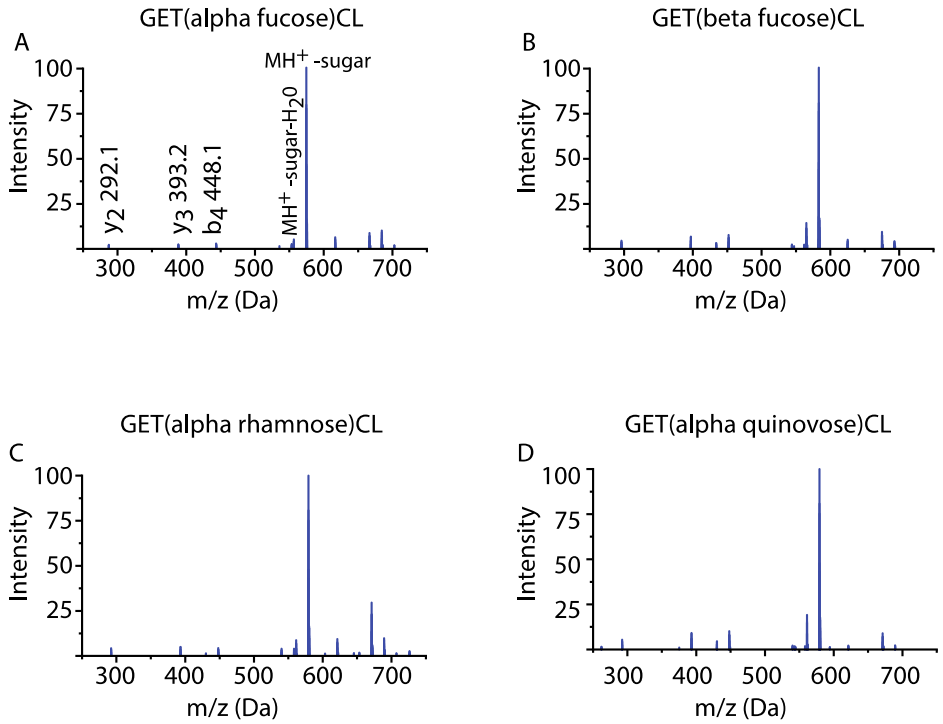
GET(Quin-α)CL



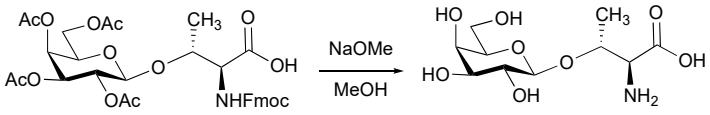


HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{28}H_{49}N_6O_{14}S$, 724.2949; Found 725.3022

MS2 spectra for synthesized glycopeptides

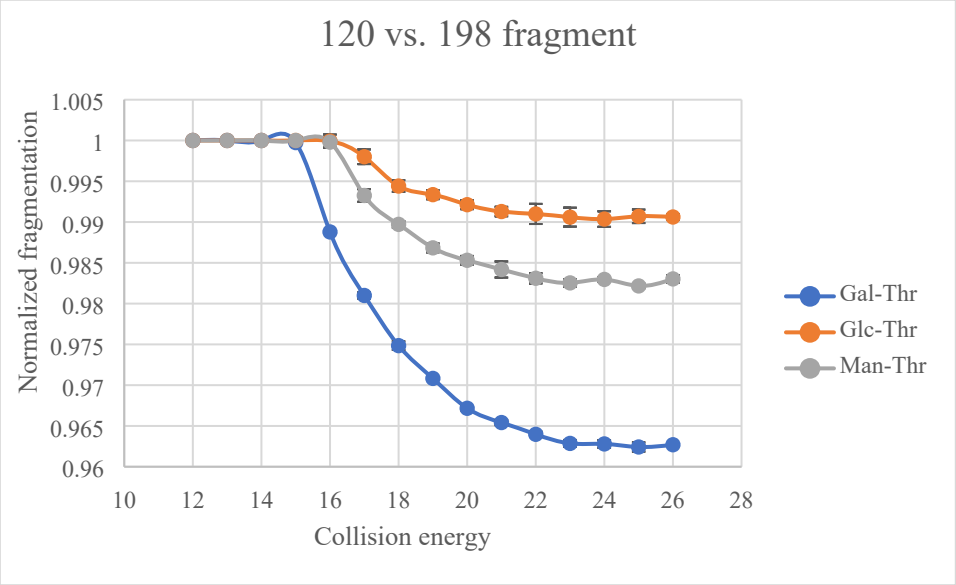


2.3 Deprotected Glycosylated amino acid mass spectrometry protocol



A protected glycosylated amino acid (1.0 mg, 1.49 μmol) was dissolved in methanol (200 μL), and sodium methoxide solution (22 μL, 25 wt. %) was added. This was stirred at 23 °C for 3 h and then quenched with 1 M HCl, and solvent was removed under reduced pressure. The material left after evaporation was diluted in DI water, washed with ether, and the aqueous layer was lyophilized to give the fully deprotected glycosylated amino acid. Solutions of the fully deprotected glycosylated amino acids were then prepared in 0.1% formic acid water buffer (0.1 μg/μL). Samples were then subjected to LCMS collision induced dissociation experiments, where the precursor mass at 282.1 Daltons, was isolated and CID spectra were taken over increasing collision energies. Collision energies ranged from 12.00% to 26.00% relative energy. For each collision energy, all the MS2 spectra during the elution window were summed. Exact mass data was then exported to Microsoft Excel where the areas of the

precursor and various fragments were integrated. Integrations were normalized, and then plotted with respect collision energy. Triplicate data was collected for each glycosylated amino acid, and standard deviations were reported for each collision energy.

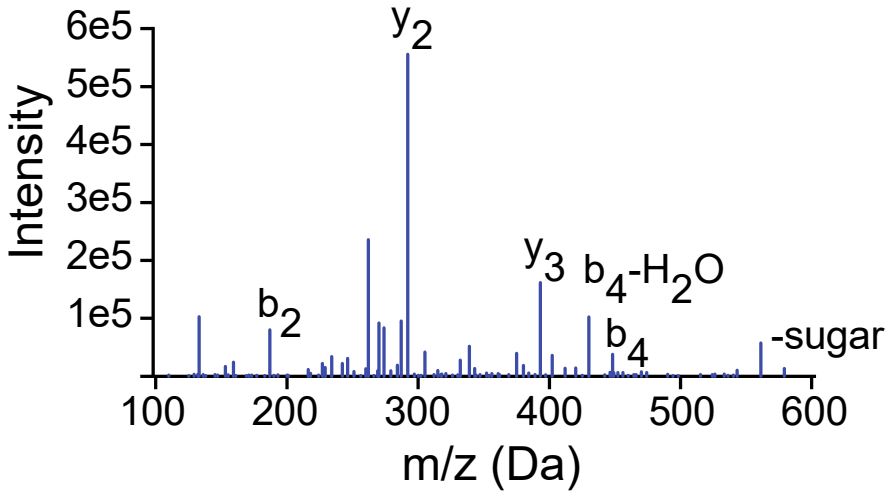


2.4 Notch digestion protocol

Tris(2-carboxyethyl)phospine hydrochloride (TCEP) and iodoacetamide were removed from the refrigerator to allow for them to warm up to room temperature prior to use. A solution of urea (8 M, 1 mL) in ammonium bicarbonate (100 mM) was prepared. A solution of TCEP (100 mM, 1 mL) in ammonium bicarbonate (25 mM) was prepared, and this was a stock solution, so the final concentration of TCEP was 10 mM. A solution of iodoacetamide (200 mM, 1 mL) in ammonium bicarbonate (25 mM) was prepared. This was a stock solution, and the final concentration of iodoacetamide was 20 mM. Urea (8 M, 10 µL) was added to the protein sample to dissolve it. TCEP (100 mM, 1 µL) was added and this was then vortexed and spun briefly. The reaction was allowed to proceed at 56 °C for 1 h. Iodoacetamide (200 mM, 1 µL) was then added and this was then vortexed and spun briefly. The reaction was allowed to proceed in a dark drawer for 45 min at ambient temperature. Ammonium

bicarbonate solution (100 mM, 68 µL) was then added to bring the final volume up to 80 µL and the final urea concentration to 1 M. The pronase solution (0.4 µg/µL in 0.1 % acetic acid) was prepared, and 0.5 µL was added to the sample. This was then digested for 16 hours at 37 °C.

Below is the MS2 data that was used to identify the Notch glycopeptide fragment, GET(dHex)CL, from the proteolytic digest,:



2.5 Orbitrap Fusion Lumos GET(dHex)CL Protocol

Samples were analyzed by LC-MS on an Orbitrap Fusion Lumos (ThermoFisher) equipped with an Easy NanoLC1200 HPLC (ThermoFisher) operated in positive ion mode. Peptides were separated on a 75 µm × 15 cm Acclaim PepMap100 separating column (Thermo Scientific) downstream of a 2 cm guard column (Thermo Scientific). Buffer A was 0.1% formic acid in water. Buffer B was 0.1% formic acid in 80% acetonitrile. Peptides were separated on a 30 minute gradient from 0% B to 28% B over 22 minutes, then to 100% B over 30 seconds. The spray voltage was set at 1.9 kV. Precursor ions were measured in the Orbitrap with a resolution of 60,000 and an AGC target of 4.0e5 ions with a maximum injection time of 50 ms. A tMSn experiment was also conducted to fragment the GET(dHex)CL peptide

at 725.3 Da the entire time. Precursor masses were isolated in the quadrupole with a 2 m/z window. Peptides were fragmented using CID with a range of collision energies from 11 to 36. Fragment ions were measured in the Orbitrap at a resolution of 7500 with an AGC target of 2.0e5 and a maximum injection time of 400 ms. Glycopeptides were identified in their protonated state, and no evidence of sodiated adducts was observed following LC. Deoxyhexose units such as fucose in the case on N-glycans have been demonstrated to undergo transfer reactions during MS2 fragmentation. Mass spectrometry of proton adducts of fucosylated *N*-glycans, which involves fucose transfer between antennae can give rise to misleading fragments; however, we did not notice such an effect in our data. To the extent that it occurred, it did not prevent differentiation of isomers using the collision energy scan approach.

2.6 Processing of Orbitrap Fusion Lumos Data

Samples were subjected to LCMS collision induced dissociation experiments, where the precursor mass at 725.3 Daltons, was isolated and CID spectra were taken over increasing collision energies. Collision energies ranged from 11.00% to 36.00% relative energy. For each collision energy, all the MS2 spectra during the elution window were summed. Exact mass data was then exported to Microsoft Excel where the areas of the precursor and various fragments were integrated. Integrations were normalized, and then plotted with respect collision energy. Triplicate data was collected for each glycosylated amino acid and standard deviations were reported for each collision energy. Data was then processed in Origin and fitted to a Boltzmann curve to extract x_o and dx.

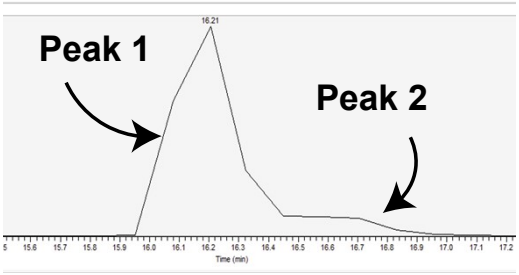
2.7 Information on instrument variation and sample stability

The GET(Fuc-α)CL standard varied by approximately 1.00% relative collision energy over the course of four days, whereas the variation of the other synthetic standards was minimal. Fucose's ability to migrate during collision induced dissociation has been described in the context of fucosylated glycans,

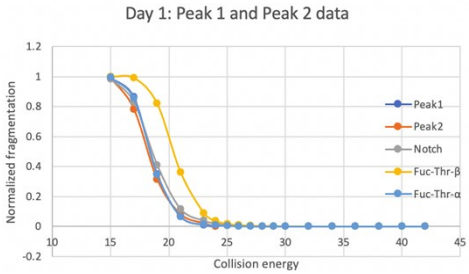
such as the Lewis blood group antigens.[5,6] Given that the variation that occurred in our data occurred over a period of several days, rather than consistently occurring for every experiment, it seemed likely that this is a chemical transformation that was occurring in solution, rather than as a result of collision energy, thus we believe that is unlikely that this phenomena is occurring. To the extent that it did occur, it did not prevent differentiation of the isomers using the collision energy scan approach.

For the GET(Fuc-α)CL , two separate LC elution curves were observed. The collision energy scan data was extracted for each elution peak. For the data that was collected on day 4, the less abundant LC peak's curve overlayed with the Notch curve, while the more intense peak was between the Notch curve and the curve for GET(Fuc-β)CL. The more intense peak likely represented the transformation that was occurring over the course of four days. The experiment was repeated with freshly synthesized GET(Fuc)CL, and the alpha- and beta- anomer standards showed the same respective difference in collision energy (as measured by X_0) compared to the initial day 1 data; however, they both fragmented at a higher collision energy. This shift to higher collision energies is likely due to routine cleaning and tuning of the instrument and highlights the importance of measuring the standards and sample on the same day.

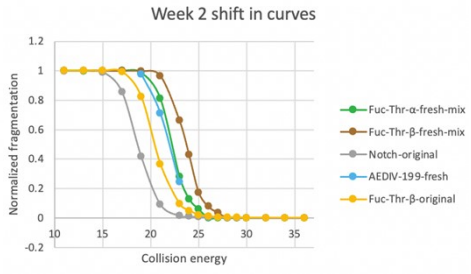
Two separate elution peaks were observed by LC for GET(Fuc-α)CL



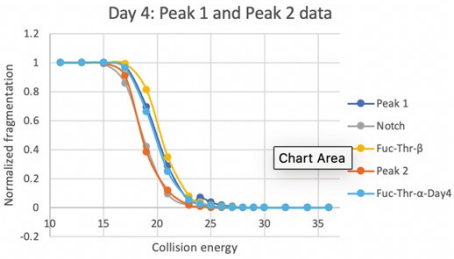
Initial data for two peaks shows same curve for all GET(Fuc-α)CL compounds

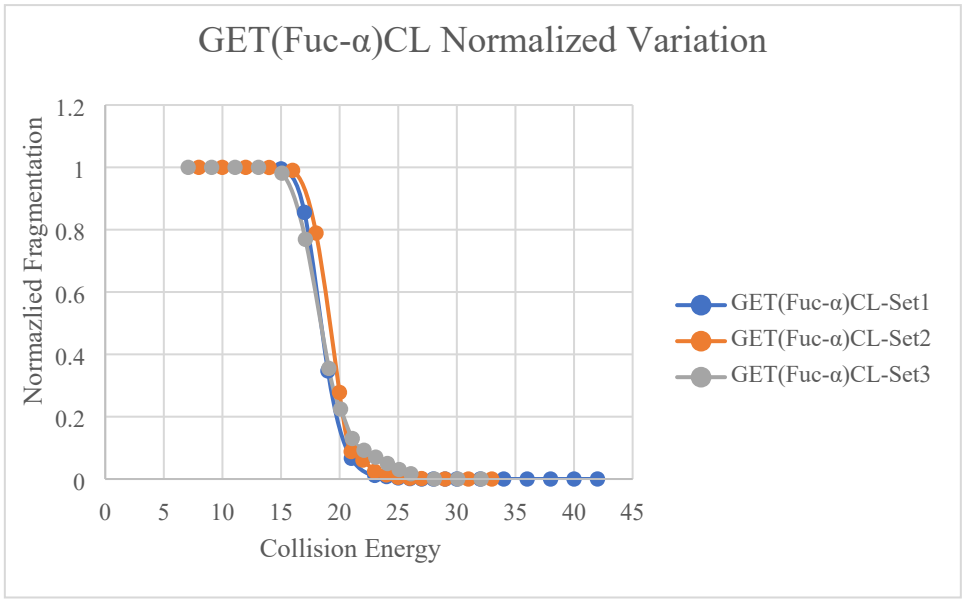


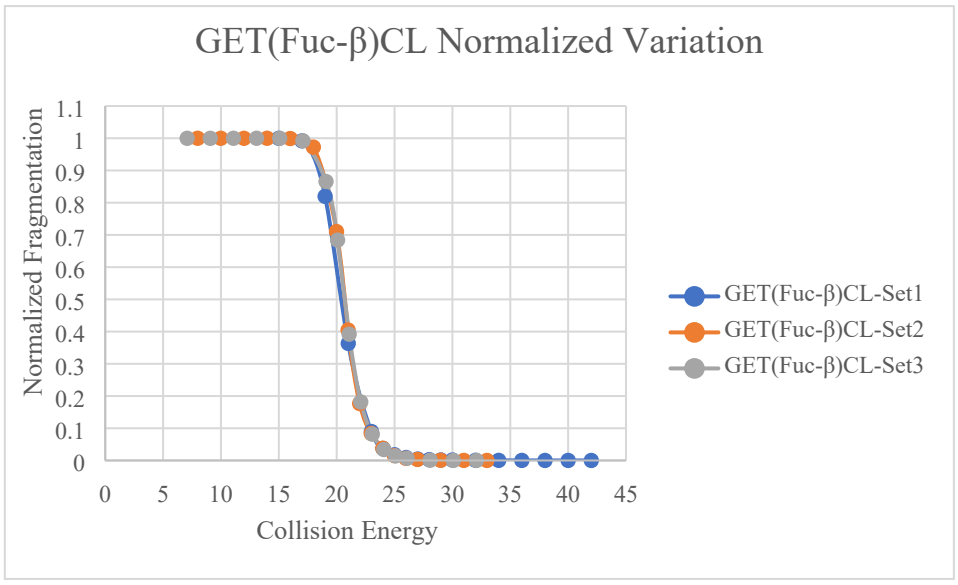
Variation in collision energy at which fragmention occurs due to routine cleaning and tuning of instrument

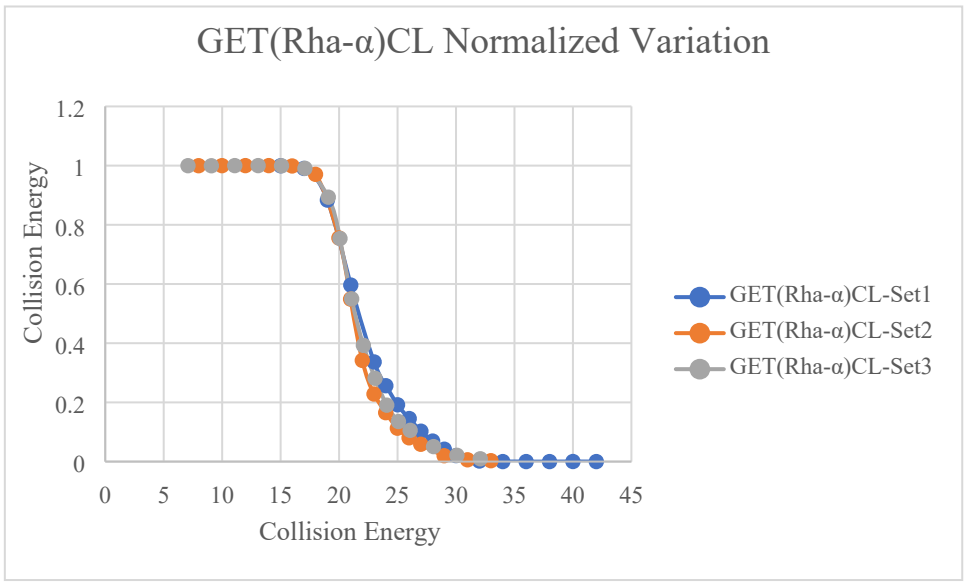


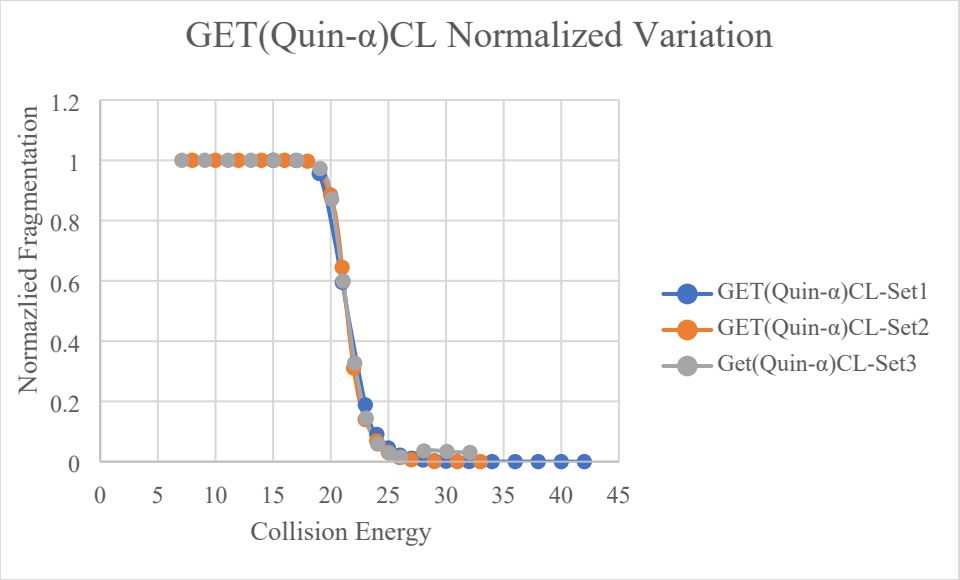
Data collected four days after samples were prepared to demonstrate the need for collecting data when samples are prepared




GET(Fuc-α)CL Normalized Variation
Normazlied Fragmentation
1.2
1
0.8
0.6
0.4
0.2
0
0 5 10 15 20 25 30 35 40 45
Collision Energy
GET(Fuc-α)CL-Set1
GET(Fuc-α)CL-Set2
GET(Fuc-α)CL-Set3


GET(Fuc-β)CL Normalized Variation
Normalized Fragmentation
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 5 10 15 20 25 30 35 40 45
Collision Energy
GET(Fuc-β)CL-Set1
GET(Fuc-β)CL-Set2
GET(Fuc-β)CL-Set3


GET(Rha-α)CL Normalized Variation
Collision Energy
1.2
1
0.8
0.6
0.4
0.2
0
0 5 10 15 20 25 30 35 40 45
Collision Energy
GET(Rha-α)CL-Set1
GET(Rha-α)CL-Set2
GET(Rha-α)CL-Set3



	Average x_0	Standard deviation x_0		Average dx	Standard deviation dx
GET(Fuc-α)CL	18.651	0.405	GET(Fuc-α)CL	0.985	0.194
GET(Fuc-β)CL	20.611	0.157	GET(Fuc-β)CL	0.912	0.097
GET(Rha-α)CL	21.417	0.211	GET(Rha-α)CL	1.583	0.423
GET(Quin-α)CL	21.417	0	GET(Quin-α)CL	0.860	0.156

To demonstrate consistency and reproducibility of the data, we collected data for the standards on three separate days over a period of two weeks, and the order of fragmentation for the standards remained consistent. Additionally, the ability to normalize the data with respect to one of the standards was demonstrated. Normalization was done by extracting X_0 values using a Boltzmann curve fit in Origin for each curve and normalizing the data with respect to the GET(Quin-α)CL standard. After normalization, standard deviations ranged from 0.157 to 0.405 for X_0 for the synthesized standards, which show that GET(Fuc-α)CL and GET(Fuc-β)CL can be separated from GET(Rha-α)CL and GET(Quin-α)CL. While GET(Rha-α)CL and GET(Quin-α)CL have the same X_0 value of 21.417, the

slopes of these curves at X_0 are consistently different across all sets of data, where the slope of the GET(Rha-α)CL standard curve is 1.583, and the slope of the GET(Quin-α)CL curve is 0.860.

3. Automation Platform

Automation of the continuous flow setup was enabled through Mechwolf, a Python-controlled, open-source program.

Code and Jupyter notebook pages are uploaded for every reaction. These can then be downloaded and stoichiometry can be adjusted as need be.

Example of Jupyter notebook page for Synthesis of Rhamnosylated serine 5:

In [4]:
```
import mechwolf as mw
import graphviz
import math
from datetime import timedelta
from astropy.table import QTable, Table, Column
from sigfig import round
import json
```

In [5]:
```
with open('AEDIV-23') as f:
    data = json.load(f)
```

Note: To make the notebook page more readable, each reaction includes a separate .JSON files. This type of file, which stores the information about reagents, is referenced in the notebook page in order to fill out the stoichiometry table and solvent volume tables. .JSON files can be updated to change the scale of reactions or adjust which reagents are used in a reaction, and this will be reflected in the Jupyter Notebook page.

Reagents

In [6]:
```
print(f"The limiting reagent is {data['limiting reagent']}")
mw_limiting = 332.31

try:
    mass_scale = float(data['mass scale'])
except:
    print('Invalid input: numerical values only')

moles = mass_scale / mw_limiting

print(f'\nMoles: {moles}')
```

```
The limiting reagent is acetylated rhamnose

Moles: 0.0007496012759170654
```

In [7]:
```
try:
    concentration = float(data['concentration'])
except:
    print('Invalid input: numerical values only')

volume = (moles * 1000) / concentration

print(f'\nVolume: {volume} mL')
```

```
Volume: 14.992025518341308 mL
```

In [8]:
```
class Reagent:

    def __init__(self, reagent):
        assert 'name' in reagent, 'ERROR: one of the reagents does not have a name field'
        assert 'inChi' in reagent, f'Error: {reagent["name"]} does not have an inChi field'
        assert 'inChi Key' in reagent, f'Error: {reagent["name"]} does not have an inChi key field'
        assert 'molecular weight' in reagent, f'Error: {reagent["name"]} does not have a molecular weight field'
        assert 'eq' in reagent, f'Error: {reagent["name"]} does not have an eq field'
        assert 'vessel' in reagent, f'Error: {reagent["name"]} does not have an vessel # field'

        self.name = reagent['name']
        self.inChi = reagent['inChi']
        self.mol_weight = reagent['molecular weight']
        self.eq = reagent['eq']
        self.vessel = reagent['vessel']
        self.moles = round(moles * self.eq, decimals = 4)
        self.mass = round(self.moles * self.mol_weight, decimals = 4)

    def __str__(self):
        return f"{self.name}"

class Solid(Reagent):

    def __init__(self, reagent):
        super().__init__(reagent)

class Liquid(Reagent):

    def __init__(self, reagent):
        super().__init__(reagent)

        assert 'density' in reagent, f'Error: {reagent["name"]} does not have a density field'
        self.density = reagent['density']
        self.volume = round(self.eq * moles * (self.mol_weight / self.density), decimals = 4)

reagent_list = []
for solid in data['solid reagents']:
    reagent_list.append(Solid(solid))

for liquid in data['liquid reagents']:
    reagent_list.append(Liquid(liquid))
```

Stoichiometry Table

Reagent Table

In [9]:
```
reagent_table = QTable()
reagent_table['Reagent'] = [reagent.name for reagent in reagent_list]
reagent_table['Molecular Weight (g/mol)'] = [reagent.mol_weight for reagent in reagent_list]

if moles < 0.0005:
    reagent_table['mmol'] = [reagent.moles * 1000 for reagent in reagent_list]
    reagent_table['Mass (mg)'] = [round(reagent.mass * 1000, decimals = 4) for reagent in reagent_list]
else:
    reagent_table['mol'] = [reagent.moles for reagent in reagent_list]
    reagent_table['Mass (g)'] = [round(reagent.mass, decimals = 4) for reagent in reagent_list]

reagent_table['Volume (mL)'] = [reagent.volume if hasattr(reagent, 'volume') else "N/A" for reagent in reagent_list]
reagent_table['Density (g/mL)'] = [reagent.density if hasattr(reagent, 'density') else "N/A" for reagent in reagent_
reagent_table['eq'] = [reagent.eq for reagent in reagent_list]

reagent_table.pprint(max_lines=-1, max_width=-1)

print(f'\nSolvent: {data["solvent"]}')
```

```
      Reagent       Molecular Weight (g/mol)  mol   Mass (g) Volume (mL) Density (g/mL)  eq
------------------ ------------------------ ------ -------- ---------- -------------- ----
acetylated rhamnose                   332.31 0.0007   0.2326        N/A            N/A  1.0
        Fmoc-Ser-OH                   327.34 0.0009   0.2946        N/A            N/A  1.2
            BF3OEt2                   141.93 0.0045   0.6387     0.5569           1.15 6.02

Solvent: 1:1 ACN/DCM
```

Reaction vessel table

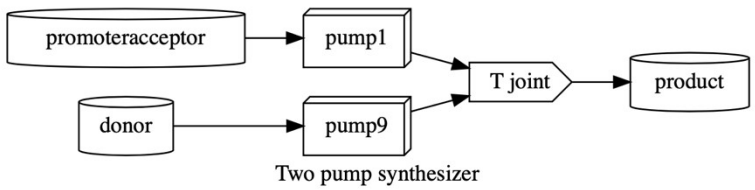
In [10]:
```
def get_vessel_reagents(reagents, ves_max):

    def get_reagents_for_vessel_number(ves_num):
        reagent_for_vessel_num = ""
        for reagent in reagents:
            if reagent.vessel == ves_num:
                reagent_for_vessel_num += f'{reagent.name}, '

        return reagent_for_vessel_num[:-2]
    return list(map(get_reagents_for_vessel_number, range(1, ves_max)))

vessel_table = QTable()
vessel_max = max([reagent.vessel for reagent in reagent_list]) + 1
vessel_table['vessel'] = list(range(1, vessel_max))
vessel_table['Reagent'] = get_vessel_reagents(reagent_list, vessel_max)
vessel_table['Solvent Volume'] = data['solvent volume']

vessel_table.pprint(max_lines = -1, max_width = -1)
```

```
vessel       Reagent        Solvent Volume
------ -------------------- --------------
     1  acetylated rhamnose            7.6
     2 Fmoc-Ser-OH, BF3OEt2            7.6
```

Define Apparatus

In [14]:
```
A = mw.Apparatus("Two pump synthesizer")

A.add(donor, pump9, fat_short_tube)
A.add(pump9, mixer, thin_short_tube)

A.add(promoteracceptor, pump1, fat_short_tube)
A.add(pump1, mixer, thin_short_tube)

A.add(mixer,product,thin_rxn_tube)


A.visualize()
```

Out[14]:



Two pump synthesizer

Define Passive Components

Reaction Vessels

In [11]:
```
donor = mw.Vessel("donor", name = "donor")
promoteracceptor = mw.Vessel("promoteracceptor", name = "promoteracceptor")
product = mw.Vessel("product", name = "product")
```

Tubes and Mixer

In [12]:
```
fat_short_tube = mw.Tube(length="12 in", ID="1/16 in", OD="1/8 in", material="PFA")
thin_short_tube = mw.Tube(length="12 in", ID="0.04 in", OD="1/16 in", material="PFA")
thin_rxn_tube = mw.Tube(length="3000 in", ID="0.04 in", OD="1/16 in", material="PFA")

mixer = mw.TMixer(name="T joint")

#static mixer
```

Define Active Components

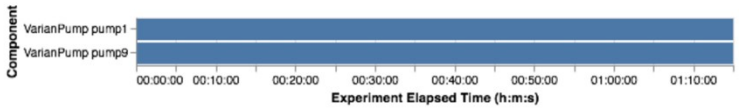
In [13]:
```
pump9 = mw.VarianPump(serial_port='/dev/serial/by-id/usb-FTDI_USB-RS422_Cable_FT1UI0ZM-if00-port0',
                      max_rate='10 ml/min', name ='pump9')

pump1 = mw.VarianPump(serial_port='/dev/serial/by-id/usb-FTDI_USB-RS422_Cable_FT1WLW8P-if00-port0',
                      max_rate='10 ml/min', name='pump1')
```

Define protocol

In [8]:
```
P=mw.Protocol(A)

start = timedelta(seconds=0)

P.add(pump9, start='0 s', duration='4500 s', rate='0.25 ml/min')
P.add(pump1, start='0 s', duration='4500 s', rate='0.25 ml/min')


#P.add(sensor, start='0sec', duration='30 secs', rate='1 Hz')
P.visualize()
```

```
<vega.vegalite.VegaLite at 0x6a689ef0>
```

Out[8]:



In [9]:
```
exp=P.execute(dry_run=100)
```

```
VBox(children=(HTML(value='<h3>Experiment 2020_12_15_12_15_11_dd722e61</h3>'), HBox(children=(Button(button_st…
```

In [10]:
```
exp=P.execute()
```

```
Execute? [y/N]: y
```

```
VBox(children=(HTML(value='<h3>Experiment 2020_12_15_12_17_37_dd722e61</h3>'), HBox(children=(Button(descripti…
```

References

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