Supplementary Information

Rapid Phenolic *O*-Glycosylation of Small Molecules and Complex Unprotected Peptides in Aqueous Solvent

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1. General Information and Reagents

Routine ^{1}H NMR spectra were recorded on 400, 500, or 600 MHz spectrometers at ambient temperature. NMR solvents *d*-chloroform ($CDCl_3$), d_4–methanol (CD_3OD), deuterium oxide (D_2O), and anhydrous d_6-dimethylsulfoxide (DMSO-d_6) were purchased from commercial suppliers and used without further purification. Ampules of d_4-methanol and d_6-dimethylsulfoxide (anhydrous) were used immediately after opening. Spectra were processed using the automatic phasing and polynomial baseline correction features of MestReNova software. Spectral data are reported as follows: chemical shift (multiplicity [singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), doublet of doublet of doublets (ddd), doublet of triplets (dt)], coupling constant, integration). Chemical shifts are reported in ppm (δ), and coupling constants are reported in Hz. ^{1}H resonances are referenced to solvent residual peaks for $CDCl_3$ (7.26 ppm), CD_3OD (3.31 ppm), D_2O (4.79 ppm), or DMSO-d_6 (2.50 ppm). Routine ^{13}C NMR spectra were recorded on Agilent 500 or 600 MHz spectrometers with protons fully decoupled unless otherwise noted. ^{13}C resonances are reported in ppm relative to solvent residual peaks for $CDCl_3$ (77.2 ppm), CD_3OD (49.0 ppm), or DMSO-d_6 (39.52 ppm). Routine ^{19}F NMR spectra were recorded on an Agilent 400 MHz spectrometer. ^{19}F resonances are reported in ppm relative to 1.0% 1,1,1-trifluorotoluene added as the internal standard, with literature resonance peak at –63.72 ppm. For one-dimensional NOESY experiments, 64 scans were acquired with a mixing time of 500 ms. Wilmad 5 mm Thin Wall 7" Class B Glass 100 MHz (WG-5MM-ECONOMY-7) NMR tubes were employed for analysis.

Optical rotations were recorded at the sodium D-line (589 nm) with a 50 mm path length TempTrol cell at 20 °C on a Rudolph Research Analytical Autopol VI Automatic Polarimeter. Data are reported as follows: $[\alpha]_{\lambda}^{temp}$, concentration (*c* in g/100 mL), and solvent. Infrared spectra were recorded on an ATR/FT-IR spectrometer, and ν_{max} are partially reported in cm^{-1}. Reversed-phase flash chromatography was performed using a Biotage Isolera automated flash purification system (CV = column volumes). Analytical HPLC spectra were obtained using an Agilent 1200 Series chromatograph equipped with a photodiode array detector (254 nm); column temperature was unregulated. Analytical UPLC spectra were obtained using a Shimadzu Analytical UPLC system; column temperature was regulated at 40 °C. Semiprep HPLC isolation was achieved using an Agilent 1260 Infinity HPLC system; column temperature was unregulated. Preparative HLPC isolation was achieved using a Shimadzu Prominence HPLC system equipped with a photodiode array detector (254 nm); column temperature was unregulated.

For small molecule and dipeptide substrates, high-resolution liquid chromatography-mass spectrometry (HRMS) was performed by the Mass Spectroscopy Laboratory (MSL) of the School of Chemical Science (SCS) at University of Illinois Urbana Champaign. For polypeptide substrates, the molecular mass was determined by ultra-high-performance liquid chromatography-mass spectrometry (UPLC/MS) performed on a Waters Acquity UPLC/MS instrument equipped with a reversed-phase BEH C18 column (1.7 μm particle size, 2.1 x 50

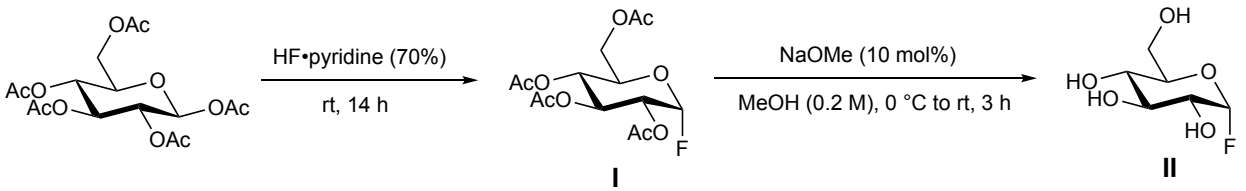
mm), a dual atmospheric pressure chemical ionization (API)/electrospray ionization (ESI) mass spectrometry detector, and a photodiode array detector.

All reagents were purchased from commercial suppliers and used without further purification unless noted otherwise. Methylene chloride (CH_2Cl_2) and tetrahydrofuran (THF) were obtained from a PPT/Glass Contour solvent purification system, in which the solvent was dried over alumina and dispensed under an atmosphere of argon (Ar). Methanol (MeOH), *N,N*-dimethylformamide (DMF), and phenol (liquefied) were purchased from Avantor Performance Materials (Center Valley, PA). Dimethyl sulfoxide (DMSO) was purchased from Biotium Inc. (Fremont, CA). H-PAL (amide) ChemMatrix® resin, 1-hydroxybenzotriazole hydrate (HOBt), SigmaCote®, trifluoroacetic acid (TFA), and trypsin (sequencing grade) were purchased from Sigma-Aldrich (St. Louis, MO). Standard fluorenylmethyloxycarbonyl (Fmoc)-protected amino acid monomers were purchased from Novabiochem (San Diego, CA). *N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU), *N*-methyl pyrolidinone (NMP), piperidine, and DIEA (2 M in NMP) were purchased from AmericanBio, Inc. (Natick, MA). Triisopropylsilane (TIPS) was purchased from Acros Organics (Fair Lawn, NJ). Calcitonin-salmon was purchased from Bachem Americas Inc. (Torrance, CA). Endomorphin-1, endomorphin-2, LH-RH-salmon, Leu-enkephalin, oxytocin, and vasopressin were purchased from Alfa Aesar (Haverhill, MA). For reactions run in water (H_2O), deionized water was used unless stated otherwise.

Room temperature (rt) is defined as 20–24 °C.

2. Synthesis of α-D-Fluoroglycosides

2.1. Synthesis of α-D-Fluoroglucose



Note: Extreme caution should be taken when manipulating HF•pyridine and/or aqueous HF solutions. All HF solutions should be manipulated under a well-ventilated space (>100 L air/min flow rate), and appropriate PPE should be used at all times. Please refer to the MSDS data sheet before any reaction with HF•pyridine.

Note: Prepare an aqueous 1.0 M solution of NaOH (~20 mL) in order to quench any residual HF•pyridine in the syringe.

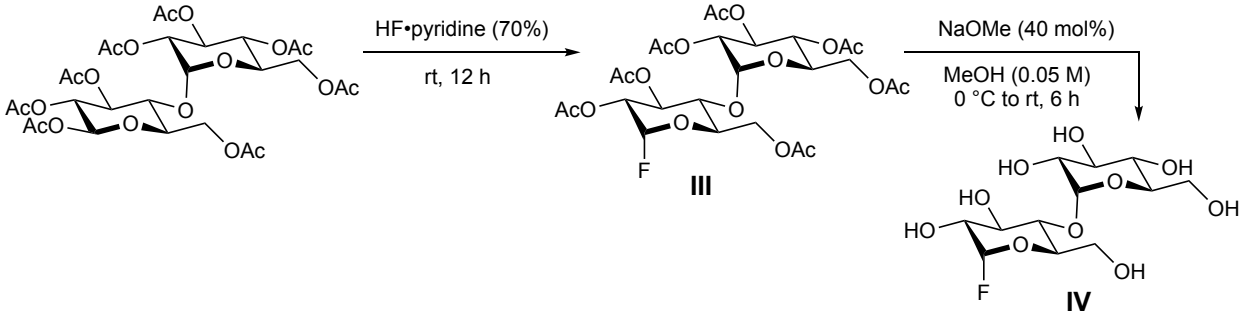
α-D-Fluoroglucose tetraacetate (I). To a 500 mL plastic bottle were added β-D-glucose pentaacetate (10.0 g, 25.7 mmol, 1.0 equiv.) and a magnetic stir bar. The bottle was then placed in an ice-water bath, and a cold (freshly removed from a –20 °C freezer) solution of 70% HF•pyridine (17 mL, 1.5 M) was added to the solid via plastic syringe. The bottle was capped, and the suspension was stirred for 14 h. Over the course of the reaction, the ice bath was allowed to melt and warm to rt. The reaction mixture was then diluted with ice-cold H_2O (40 mL) followed by CH_2Cl_2 (40 mL), and the resulting biphasic mixture was stirred for 5 min. The mixture was transferred to a 250 mL separatory funnel, rinsing with CH_2Cl_2, and the layers were separated. The organic layer was collected, and the aqueous layer was extracted with CH_2Cl_2 (2 x 40 mL). The combined organic layers were then washed with a saturated aqueous $NaHCO_3$ solution (1 x 100 mL) followed by a saturated aqueous NaCl solution (1 x 100 mL), dried over Na_2SO_4, filtered, and evaporated to dryness in a 500 mL round bottom flask. The resulting colorless oil was further dried on high-vacuum for 3 h before carrying on to the next step without purification (yield calculated over the next step).[1]

α-D-Fluoroglucose (II). To a 500 mL round bottom flask containing α-D-fluoroglucose tetraacetate (**I**; 9.0 g, 25.7 mmol, 1.0 equiv.) were added absolute MeOH (130 mL, 0.2 M) and a magnetic stir bar. The resulting clear, colorless solution was stirred for 10 min to fully dissolve the substrate, and the solution was cooled to 0 °C in an ice-water bath. After stirring at 0 °C for 10 min, NaOMe (139 mg, 2.57 mmol, 0.1 equiv.) was added in one portion. The resulting mixture was stirred for 10 min. The ice-water bath was then removed and the mixture was allowed to warm to rt with stirring over 3 h. The reaction flask was cooled in an ice-water bath, and silica gel (~9 g) was added. The resulting suspension was stirred for 10 min, then

[1] For literature characterization data, see: Pelletier, G.; Zwicker, A.; Allen, C. L.; Schepartz, A.; Miller, S. J. *J. Am. Chem. Soc.* **2016**, *138*, 3175.

concentrated to a thick paste *in vacuo*. To the reaction flask was added 7:3 EtOAc:MeOH (50 mL), and the resulting suspension was stirred for 5 min. The mixture was filtered through a fritted funnel and rinsed with 7:3 EtOAc:MeOH (50 mL). The filtrate was transferred to a 250 mL round bottom flask and evaporated to dryness, resulting in a colorless foam which solidified over extensive evaporation on high vacuum (4.59 g, 98% over 2 steps). The product was used without further purification and stored at –20 °C. $[\alpha]_D^{20}$ = +84 (*c* 1.6, H_2O), lit[2] $[\alpha]_D^{20}$ = +84.1 (*c* 1.0, H_2O); **^{1}H NMR** (400 MHz, D_2O) δ 5.68 (dd, *J* = 53.5, 2.8 Hz, 1H), 3.92 – 3.69 (m, 4H), 3.60 (ddd, *J* = 26.3, 9.9, 2.8 Hz, 1H), 3.50 (t, *J* = 9.4 Hz, 1H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 107.9 (d, *J* = 223.0 Hz), 75.4 (d, *J* = 3.0 Hz), 72.7, 71.4 (d, *J* = 24.8 Hz), 69.1, 60.5 ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ –151.1 (dd, *J* = 53.9, 25.9 Hz) ppm; **IR** (cm^{-1}, neat): 3367, 3218, 2932, 2882, 1459, 1405, 1372, 1341, 1310, 1233, 1213, 1165, 1139, 1107, 1089, 1052, 1040, 1019, 995, 919, 894, 856, 774, 707, 583, 553, 520, 507, 429, 407; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_6H_{10}FO_5$ requires *m/z* 181.0512, found 181.0514.

2.2. Synthesis of α-D-Fluoromaltose



Note: Extreme caution should be taken when manipulating HF•pyridine and/or aqueous HF solutions. All HF solutions should be manipulated under a well-ventilated space (>100 L air/min flow rate), and appropriate PPE should be used at all times. Please refer to the MSDS data sheet before any reaction with HF•pyridine.

Note: Prepare an aqueous 1.0 M solution of NaOH (~20 mL) in order to quench any residual HF•pyridine in the syringe.

α-D-Fluoromaltose heptaacetate (III). To a 250 mL plastic bottle were added β-D-maltose octaacetate (10.0 g, 14.7 mmol, 1.0 equiv.) and a magnetic stir bar. The bottle was then placed in an ice-water bath, and a cold (freshly removed from a –20 °C freezer) solution of 70% HF•pyridine (14.7 mL, 1.0 M) was added to the solid via plastic syringe. The bottle was capped, and the suspension was stirred for 12 h. Over the course of the reaction, the ice bath was allowed to melt and warm to rt. The reaction mixture was then diluted with ice-cold H_2O (40 mL)

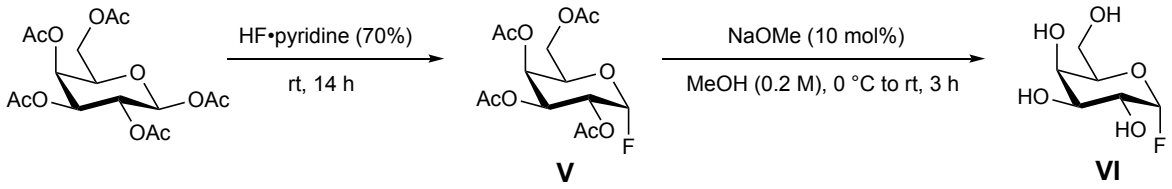
[2] Wilkinson, S. M.; Liew, C. W.; Mackay, J. P.; Salleh, H. M.; Withers, S. G.; McLeod, M. D. *Org. Lett.* **2008**, *10*, 1585.

followed by CH_2Cl_2 (40 mL), and the resulting biphasic mixture was stirred for 5 min. The mixture was transferred to a 250 mL separatory funnel, rinsing with CH_2Cl_2, and the layers were separated. The organic layer was collected, and the aqueous layer was extracted with CH_2Cl_2 (2 x 40 mL). The combined organic layers were then washed with a saturated aqueous $NaHCO_3$ solution (1 x 100 mL) followed by a saturated aqueous NaCl solution (1 x 100 mL), dried over Na_2SO_4, filtered, and evaporated to dryness in a 500 mL round bottom flask. The flask was charged with ~100 mL CH_2Cl_2 followed by ~20 g silica gel, and the resulting suspension was evaporated to dryness. The resulting solid was dry-loaded in an empty Biotage samplet, and the product was purified using a Biotage flash chromatography system (340 g Ultra column; 200 mL/min flow rate; gradient = ramped from 20% EtOAc/80% hexanes to 80% EtOAc/20% hexanes over 17 CV). Fractions containing the product (visualized by TLC stained with ceric ammonium molybdate (CAM)) were evaporated to dryness, resulting in a colorless foam (yield calculated over the next step).[3]

α-D-Fluoromaltose (IV). To a 500 mL round bottom flask containing α-D-fluoromaltose heptaacetate (**III**; 5.6 g, 8.8 mmol, 1.0 equiv.) were added absolute MeOH (176 mL, 0.05 M) and a magnetic stir bar. The resulting suspension was stirred for 10 min to ensure maximum dissolution of substrate, then the mixture was cooled to 0 °C in an ice-water bath. After stirring at 0 °C for 10 min, NaOMe (190 mg, 3.5 mmol, 0.4 equiv.) was added in one portion. The resulting mixture was stirred for 15 min. The ice-water bath was then removed and the mixture was allowed to warm to rt with stirring over 6 h. The reaction flask was cooled in an ice-water bath, and silica gel (~10 g) was added. The resulting suspension was stirred for 15 min, then concentrated to a thick paste *in vacuo*. To the reaction flask was added 7:3 EtOAc:MeOH (50 mL), and the resulting suspension was stirred for 5 min. The mixture was filtered through a fritted funnel and rinsed with 7:3 EtOAc:MeOH (50 mL). The filtrate was transferred to a 250 mL round bottom flask and evaporated to dryness, resulting in a colorless foam which solidified over extensive evaporation on high vacuum (2.60 g, 54% over 2 steps). The product was used without further purification and stored at –20 °C. $\mathbf{[\alpha]_D^{22}}$ = +137 (*c* 0.6, MeOH), lit[4] $\mathbf{[\alpha]_D^{20}}$ = +145.0 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 5.71 (dd, *J* = 53.6, 2.9 Hz, 1H), 5.44 (d, *J* = 3.9 Hz, 1H), 4.03 (t, *J* = 9.5 Hz, 1H), 4.00 – 3.57 (m, 10H), 3.44 (t, *J* = 9.4 Hz, 1H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 107.7 (d, *J* = 224.0 Hz), 101.0, 78.7, 73.6, 73.5, 73.2, 72.6 (d, *J* = 7.6 Hz), 70.8 (d, *J* = 24.5 Hz), 69.9, 60.8, 60.0 ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ –151.3 (dd, *J* = 53.7, 25.8 Hz) ppm; **IR** (cm^{-1}, neat): 3282, 2935, 1590, 1355, 1145, 1076, 1018, 919, 880, 815, 756, 514, 419; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{12}H_{20}FO_{10}$ requires *m/z* 343.1041, found 343.1039.

[3] For literature characterization data, see: Pelletier, G.; Zwicker, A.; Allen, C. L.; Schepartz, A.; Miller, S. J. *J. Am. Chem. Soc.* **2016**, *138*, 3175.
[4] Pelletier, G.; Zwicker, A.; Allen, C. L.; Schepartz, A.; Miller, S. J. *J. Am. Chem. Soc.* **2016**, *138*, 3175.

2.3. Synthesis of α-D-Fluorogalactose



Note: Extreme caution should be taken when manipulating HF•pyridine and/or aqueous HF solutions. All HF solutions should be manipulated under a well-ventilated space (>100 L air/min flow rate), and appropriate PPE should be used at all times. Please refer to the MSDS data sheet before any reaction with HF•pyridine.

Note: Prepare an aqueous 1.0 M solution of NaOH (~20 mL) in order to quench any residual HF•pyridine in the syringe.

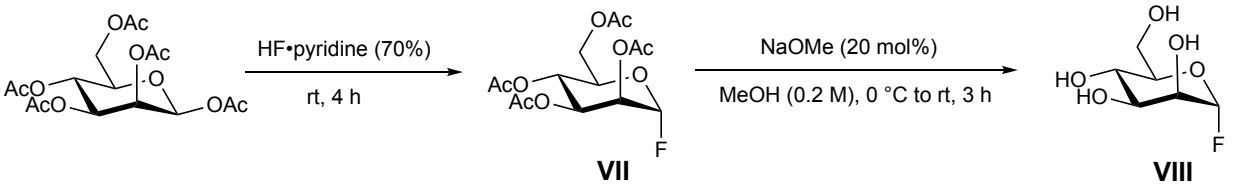
α-D-Fluorogalactose tetraacetate (V). To a 500 mL plastic bottle were added β-D-galactose pentaacetate (10.0 g, 25.7 mmol, 1.0 equiv.) and a magnetic stir bar. The bottle was then placed in an ice-water bath, and a cold (freshly removed from a –20 °C freezer) solution of 70% HF•pyridine (17 mL, 1.5 M) was added to the solid via plastic syringe. The bottle was capped, and the suspension was stirred for 14 h. Over the course of the reaction, the ice bath was allowed to melt and warm to rt. The reaction mixture was then diluted with ice-cold H_2O (40 mL) followed by CH_2Cl_2 (40 mL), and the resulting biphasic mixture was stirred for 5 min. The mixture was transferred to a 250 mL separatory funnel, rinsing with CH_2Cl_2, and the layers were separated. The organic layer was collected, and the aqueous layer was extracted with CH_2Cl_2 (2 x 40 mL). The combined organic layers were then washed with a saturated aqueous $NaHCO_3$ solution (1 x 100 mL) followed by a saturated aqueous NaCl solution (1 x 100 mL), dried over Na_2SO_4, filtered, and evaporated to dryness in a 500 mL round bottom flask. The resulting colorless oil was further dried on high vacuum for 3 h before carrying on to the next step without purification (yield calculated over the next step).[5]

α-D-Fluorogalactose (VI). To a 500 mL round bottom flask containing α-D-fluorogalactose tetraacetate (**V**; 9.0 g, 25.7 mmol, 1.0 equiv.) were added absolute MeOH (130 mL, 0.2 M) and a magnetic stir bar. The resulting clear, colorless solution was stirred for 10 min to fully dissolve the substrate, and the solution was cooled to 0 °C in an ice-water bath. After stirring at 0 °C for 10 min, NaOMe (139 mg, 2.57 mmol, 0.1 equiv.) was added in one portion. The resulting mixture was stirred for 10 min. The ice-water bath was then removed and the mixture was allowed to warm to rt with stirring over 3 h. The reaction flask was cooled in an ice-water bath, and silica gel (~9 g) was added. The resulting suspension was stirred for 10 min, then concentrated to a thick paste *in vacuo*. To the reaction flask was added 7:3 EtOAc:MeOH (50

[5] For literature characterization data, see: Steinmann, A.; Thimm, J.; Matwiejuk, M.; Thiem, J. *Macromolecules* **2010**, *43*, 3606.

mL), and the resulting suspension was stirred for 5 min. The mixture was filtered through a fritted funnel and rinsed with 7:3 EtOAc:MeOH (50 mL). The filtrate was transferred to a 250 mL round bottom flask and evaporated to dryness, resulting in a colorless foam which solidified over extensive evaporation on high vacuum (4.50 g, 96% over 2 steps). The product was used without further purification and stored at –20 °C. **$[\alpha]_D^{20}$** = +116.3 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 5.74 (dd, *J* = 53.8, 2.6 Hz, 1H), 4.12 (dd, *J* = 7.0, 5.3 Hz, 1H), 4.07 (d, *J* = 3.2 Hz, 1H), 3.96 – 3.74 (m, 4H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 108.4 (d, *J* = 222.7 Hz), 74.0, 69.2, 68.4, 67.8 (d, *J* = 24.4 Hz), 60.5 ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ –153.2 (dd, *J* = 54.4, 26.6 Hz) ppm; **IR** (cm^{-1}, neat): 3301, 2897, 1340, 1237, 1160, 1144, 1125, 1064, 1022, 1004, 971, 956, 928, 906, 867, 815, 791, 769, 687, 518, 422; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_6H_{10}FO_5$ requires *m/z* 181.0512, found 181.0519.

2.4. Synthesis of α-D-Fluoromannose



VII **VIII**

Note: Extreme caution should be taken when manipulating HF•pyridine and/or aqueous HF solutions. All HF solutions should be manipulated under a well-ventilated space (>100 L air/min flow rate), and appropriate PPE should be used at all times. Please refer to the MSDS data sheet before any reaction with HF•pyridine.

Note: Prepare an aqueous 1.0 M solution of NaOH (~20 mL) in order to quench any residual HF•pyridine in the syringe.

α-D-Fluoromannose tetraacetate (VII). To a 250 mL plastic bottle were added β-D-mannose pentaacetate (3.9 g, 10.0 mmol, 1.0 equiv.) and a magnetic stir bar. The bottle was then placed in an ice-water bath, and a cold (freshly removed from a –20 °C freezer) solution of 70% HF•pyridine (10 mL, 1.0 M) was added to the solid via plastic syringe. The bottle was capped, and the suspension was stirred for 10 min. The bottle was then removed from the ice-water bath, and the reaction mixture was allowed to gradually warm to rt with stirring over 4 h. The reaction mixture was then diluted with ice-cold H_2O (40 mL) followed by CH_2Cl_2 (40 mL), and the resulting biphasic mixture was stirred for 5 min. The mixture was transferred to a 250 mL separatory funnel, rinsing with CH_2Cl_2, and the layers were separated. The organic layer was collected, and the aqueous layer was extracted with CH_2Cl_2 (2 x 40 mL). The combined organic layers were then washed with a saturated aqueous $NaHCO_3$ solution (1 x 100 mL) followed by a saturated aqueous NaCl solution (1 x 100 mL), dried over Na_2SO_4, filtered, and evaporated to dryness in a 250 mL round bottom flask. The flask was charged with ~50 mL CH_2Cl_2 followed by ~10 g silica gel, and the resulting suspension was evaporated to dryness. The resulting solid

was dry-loaded in an empty Biotage samplet, and the product was purified using a Biotage flash chromatography system (100 g KP-Sil column; 100 mL/min flow rate; gradient = ramped from 10% EtOAc/90% hexanes to 50% EtOAc/50% hexanes over 16 CV). Fractions containing the product (visualized by TLC stained with CAM) were evaporated to dryness, resulting in a colorless oil (yield calculated over the next step).[6]

α-D-Fluoromannose (VIII). To a 250 mL round bottom flask containing α-D-fluoromannose tetraacetate (**VII**; 2.56 g, 7.32 mmol, 1.0 equiv.) were added absolute MeOH (36.6 mL, 0.2 M) and a magnetic stir bar. The resulting clear, colorless solution was stirred for 5 min to fully dissolve the substrate, and the solution was cooled to 0 °C in an ice-water bath. After stirring at 0 °C for 10 min, NaOMe (79.0 mg, 1.46 mmol, 0.2 equiv.) was added in one portion. The resulting mixture was stirred for 5 min. The ice-water bath was then removed and the mixture was allowed to warm to rt while stirring over 3 h. The reaction flask was cooled in an ice-water bath, and silica gel (~2 g) was added. The resulting suspension was stirred for 30 min, then concentrated to a thick paste *in vacuo*. To the reaction flask was added 7:3 EtOAc:MeOH (25 mL), and the resulting suspension was stirred for 5 min. The mixture was filtered through a fritted funnel and rinsed with 7:3 EtOAc:MeOH (25 mL). The filtrate was transferred to a 100 mL round bottom flask and evaporated to dryness, resulting in a colorless oil which was further dried on high vacuum for 48 h (1.32 g, 73% over 2 steps). The product, which was found to be extremely hygroscopic, was used without further purification, and was stored at –20 °C. $\mathbf{[\alpha]_D^{20}}$ = +16 (*c* 1.0, H_2O), lit[7] $\mathbf{[\alpha]_D^{20}}$ = +16.8 (*c* 0.8, MeOH); $^{\mathbf{1}}$**H NMR** (400 MHz, D_2O) δ 5.66 (dd, *J* = 49.3, 1.9 Hz, 1H), 4.16 – 4.09 (m, 1H), 3.97 – 3.84 (m, 2H), 3.84 – 3.70 (m, 3H) ppm; $^{\mathbf{13}}$**C NMR** (126 MHz, DMSO-d_6) δ 108.7 (d, *J* = 217.0 Hz), 76.6, 70.2, 68.8 (d, *J* = 35.6 Hz), 66.0, 60.9 ppm; $^{\mathbf{19}}$**F NMR** (376 MHz, CD_3OD) δ –138.8 (d, *J* = 49.9 Hz) ppm; **IR** (cm^{-1}, neat): 3318, 2923, 1381, 1171, 1095, 1064, 1020, 959, 880, 811, 674, 591, 564, 502; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_6H_{10}FO_5$ requires *m/z* 181.0512, found 181.0517.

[6] For literature characterization data, see: Wen, P.; Crich, D. *Org. Lett.* **2017**, *19*, 2402.
[7] Micheel, F.; Borrmann, D. *Chem. Ber.* **1960**, *93*, 1143.

3. Reaction Optimization Tables

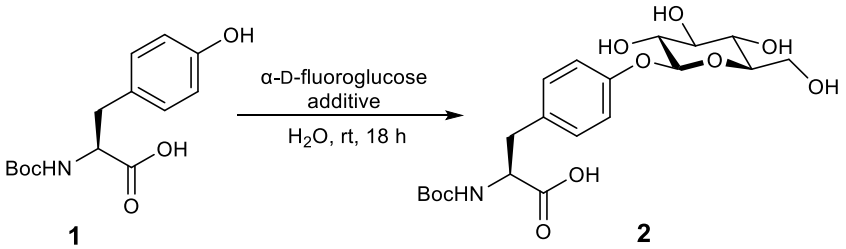
3.1. Optimization of Reagent Loading, Additive, Concentration, Reaction Time

A 1-dram vial containing Boc-L-Tyr-OH (**1**; 1.0 equiv.), α-D-fluoroglucose (**II**), additive, and a magnetic stir bar was charged with solvent (45% NMe_3/H_2O for entry 1, H_2O for all other entries). The vial was sealed with a cap and stirred vigorously at rt (18 h for Table 1 entries 1-15, 1 h for entry 16, 10 min for entry 17) and worked-up as described below:

Work-up for Supplementary Table 1, entries 1–15: The reaction vial was charged with 500 µL of a 0.05 M solution of 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS sodium salt) in D_2O, and the resulting suspension was stirred at rt for 5 min. A 100 µL aliquot of the reaction mixture was transferred to an NMR tube, diluted with ~500 µL D_2O, and analyzed by ^1H NMR.

Work-up for Supplementary Table 1, entries 16 and 17: The reaction vial was charged with 500 µL of a 0.05 M solution of 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS sodium salt) internal standard in D_2O, 300 µL D_2O, and EDTA disodium salt dihydrate (223 mg, 0.6 mmol, 3.0 equiv.). The resulting suspension was stirred at rt for 10 min, then filtered through celite. The eluent was transferred to an NMR tube and analyzed by ^1H NMR.

Supplementary Table 1:
Optimization of Reagent Loading, Additive, Concentration, Reaction Time



1 **2**

Entry	Boc-Tyr-OH (**1**)	α-D-Fluoroglucose		Additive			H_2O		NMR Yield of **2** (%)[b]
	Mass (mg)	Equiv.	Mass (mg)	Identity	Equiv.	Mass (mg)	Conc.[a] (M)	Vol. (µL)	
1[c]	14.1	6	54.6	$Ca(OTf)_2$	6	101.5	0.2	250	100
2	14.1	6	54.6	$Ca(OTf)_2$	6	101.5	0.2	250	0
3	14.1	6	54.6	$Ca(OH)_2$	6	22.2	0.2	250	95
4	14.1	6	54.6	$LiOH•H_2O$	6	12.6	0.2	250	8
5	14.1	6	54.6	NaOH	6	12.0	0.2	250	6
6	14.1	6	54.6	KOH	6	16.8	0.2	250	6
7	14.1	6	54.6	$CsOH•H_2O$	6	50.4	0.2	250	5
8	14.1	6	54.6	$Mg(OH)_2$	6	17.5	0.2	250	0
9	14.1	6	54.6	$Ba(OH)_2•8H_2O$	6	94.7	0.2	250	4
10	14.1	6	54.6	none	-	-	0.2	250	0
11	14.1	3	27.3	$Ca(OH)_2$	3	11.1	**0.2**	250	73
12	56.3	3	109.3	$Ca(OH)_2$	3	44.5	**0.5**	400	92
13	56.3	3	109.3	$Ca(OH)_2$	**3**	44.5	**1.0**	200	97
14	56.3	2	72.8	$Ca(OH)_2$	**2**	29.6	1.0	200	93
15	56.3	1	36.4	$Ca(OH)_2$	**1**	14.8	1.0	200	16
16[d]	56.3	3	109.3	$Ca(OH)_2$	3	44.5	1.0	200	95
17[e]	56.3	3	109.3	$Ca(OH)_2$	3	44.5	1.0	200	94

[a.] Concentration of Boc-L-Tyr-OH (**1**).

[b.] NMR yield of each reaction was determined by comparison of ^{1}H NMR aromatic peaks of starting material (**1**) and product (**2**) with 3-(Trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS sodium salt) internal standard.

[c.] Solvent = 45% NMe_3/H_2O.

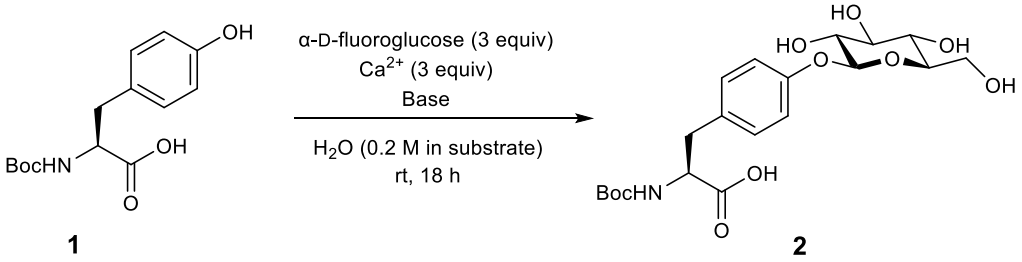
[d.] Reaction time = 1 h.

e. Reaction time = 10 min.

3.2. Evaluation of Base Loading

A 1-dram vial containing Boc-L-Tyr-OH (**1**; 14.1 mg, 0.05 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 27.3 mg, 0.15 mmol, 3.0 equiv.), Ca^{2+} source (0.15 mmol, 3.0 equiv.), and a magnetic stir bar was charged with the following:
Supplementary Table 2, entry 1: A solution of 45% NMe_3/H_2O (250 µL).
Supplementary Table 2, entry 2: A solution of 45% NMe_3/H_2O (25 µL) and H_2O (225 µL).
Supplementary Table 2, entries 3 and 4: H_2O (250 µL).
Supplementary Table 2, entries 5–12: H_2O (250 µL) and the indicated quantity of base.
The vial was sealed with a cap and the reaction mixture was stirred vigorously at rt for 18 h. The mixture was then diluted with D_2O (500 µL), and a 100 µL aliquot of the resulting suspension was transferred to an NMR tube, diluted with ~500 µL D_2O, and analyzed by ^1H NMR.

Supplementary Table 2: Evaluation of Base Loading



1 **2**

Entry	Ca^{2+} Source		Base			NMR Conv. to **2** (%)[a]
	Identity	Mass (mg)	Identity	Equiv.	Amount	
1	$Ca(OH)_2$	11.1	Me_3N	32.8	250 µL of 45% NMe_3/H_2O soln.	100
2	$Ca(OH)_2$	11.1	Me_3N	3.28	25 µL of 45% NMe_3/H_2O soln.	82
3	$Ca(OH)_2$	11.1	none	-	-	73
4	$Ca(OTf)_2$	50.8	none	-	-	0
5	$Ca(OTf)_2$	50.8	Me_3N	3	23 µL of 45% NMe_3/H_2O soln.	0
6	$Ca(OTf)_2$	50.8	Me_3N	6	46 µL of 45% NMe_3/H_2O soln.	8
7	$Ca(OTf)_2$	50.8	$LiOH•H_2O$	3	6.3 mg	38
8	$Ca(OTf)_2$	50.8	$LiOH•H_2O$	6	12.6 mg	74
9	$Ca(OH)_2$	11.1	$LiOH•H_2O$	1	2.1 mg	61
10	$Ca(OH)_2$	11.1	$LiOH•H_2O$	2	4.2 mg	46
11	$Ca(OH)_2$	11.1	$LiOH•H_2O$	4	8.4 mg	17
12	$Ca(OH)_2$	11.1	$LiOH•H_2O$	8	16.8 mg	8

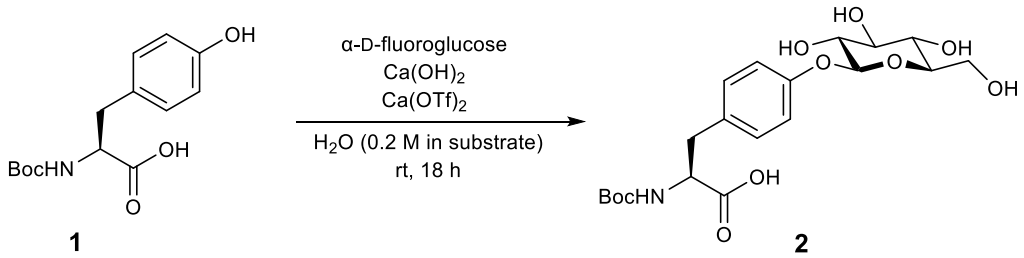
[a] NMR conversion of each reaction was determined by comparison of ^1H NMR aromatic peaks of starting material (**1**) and product (**2**). No significant side-products were observed.

3.3. Evaluation of Reagent Loading

A 1-dram vial containing Boc-L-Tyr-OH (**1**; 14.1 mg, 0.05 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**), $Ca(OH)_2$, $Ca(OTf)_2$, and a magnetic stir bar was charged with H_2O (250 µL). The vial was

sealed with a cap, and the reaction mixture was stirred vigorously at rt for 18 h. The mixture was then diluted with D_2O (500 μL), and a 100 μL aliquot of the resulting suspension was transferred to an NMR tube, diluted with ~500 μL D_2O, and analyzed by ^1H NMR.

Supplementary Table 3: Evaluation of Reagent Loading



1 **2**

Entry	α-D-Fluoroglucose		$Ca(OH)_2$		$Ca(OTf)_2$		NMR Conv. to **2** (%)[a]
	Equiv.	Mass (mg)	Equiv.	Mass (mg)	Equiv.	Mass (mg)	
1	1	9.1	1	3.7	-	-	trace
2	2	18.2	2	7.4	-	-	32
3	3	27.3	3	11.1	-	-	73
4	4	36.4	4	14.8	-	-	83
5	6	54.6	6	22.2	-	-	91
6	10	91.1	10	37.1	-	-	95
7	2	18.2	3	11.1	-	-	53
8	4	36.4	3	11.1	-	-	79
9	6	54.6	3	11.1	-	-	85
10	12	109.3	3	11.1	-	-	88
11	3	27.3	2	7.4	-	-	65
12	3	27.3	4	14.8	-	-	69
13	3	27.3	6	22.2	-	-	71
14	3	27.3	12	44.5	-	-	68
15	3	27.3	3	11.1	1	16.9	79
16	3	27.3	3	11.1	2	33.8	80
17	3	27.3	3	11.1	3	50.7	81
18	3	27.3	3	11.1	6	101.5	87

[a] NMR conversion of each reaction was determined by comparison of ^1H NMR aromatic peaks of starting material (**1**) and product (**2**). No significant side-products were observed.

3.4 Optimization of Substrate/Reagent Concentrations

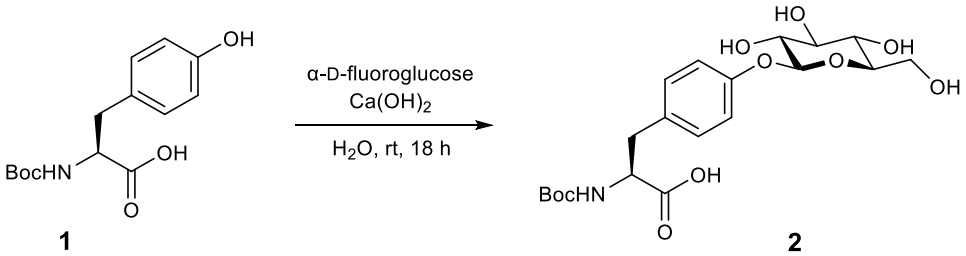
A 1-dram vial or 4-dram vial containing Boc-L-Tyr-OH (**1**), α-D-fluoroglucose (**II**), $Ca(OH)_2$, and a magnetic stir bar was charged with H_2O or D_2O. The vial was sealed with a cap, and the reaction mixture was stirred vigorously at rt for 18 h and worked-up as described below:

Supplementary Table 4, entries 1–5: The mixture was diluted with D_2O (500 µL), and a 100 µL aliquot of the resulting suspension was transferred to an NMR tube and diluted with ~500 µL D_2O.

Supplementary Table 4, entries 6–16: The mixture was filtered through celite, and the eluent was transferred to an NMR tube.

Samples were analyzed by ^{1}H NMR.

Supplementary Table 4: Optimization of Substrate/Reagent Concentrations



1 **2**

Entry	Boc-Tyr-OH (**1**)		α-D-Fluoroglucose			$Ca(OH)_2$			H_2O[a]	NMR conv. to **2** (%)[b]
	Mass (mg)	Conc. (M)	Equiv.	Mass (mg)	Conc. (M)	Equiv.	Mass (mg)	Conc. (M)	Vol. (µL)	
1	56.3	1.0	2	72.8	2.0	2	29.6	2.0	200	93
2	56.3	1.0	3	109.3	3.0	3	44.5	3.0	200	97
3	56.3	0.5	3	109.3	1.5	3	44.5	1.5	400	92
4	14.1	0.2	3	27.3	0.6	3	11.1	0.6	250	73
5	14.1	0.2	6	54.6	1.2	6	22.2	1.2	250	95
6	14.1	0.1	3	27.3	0.3	3	11.1	0.3	500	28
7	42.2	0.1	10	273.5	1.0	10	111.2	1.0	1500	100
8	14.1	0.01	3	27.3	0.03	3	11.1	0.03	5000	0
9	7.0	0.01	20	91.1	0.2	20	37.1	0.2	2500	45
10	4.2	0.01	30	81.9	0.3	30	33.3	0.3	1500	68
11	4.2	0.01	60	163.9	0.6	60	66.7	0.6	1500	93
12	4.2	0.01	100	273.5	1.0	100	111.2	1.0	1500	100
13	1.4	0.001	100	91.1	0.1	100	37.1	0.1	5000	21
14	0.4	0.001	300	81.9	0.3	300	33.3	0.3	1500	68
15	0.4	0.001	600	163.9	0.6	600	66.7	0.6	1500	100
16	0.4	0.001	1000	273.5	1.0	1000	111.2	1.0	1500	100

[a] D_2O used instead of H_2O for entries 6–16.

[b] NMR conversion of each reaction was determined by comparison of ^{1}H NMR aromatic peaks of starting material (**1**) and product (**2**). No significant side-products were observed.

3.5. Reaction Time-Course Study

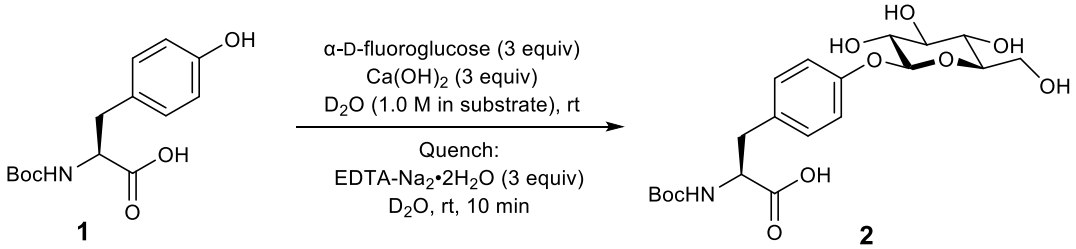
Procedure for Supplementary Table 5, entry 1: A 1-dram vial containing Boc-L-Tyr-OH (**1**; 56.3 mg, 0.2 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 109.3 mg, 0.6 mmol, 3.0 equiv.), $Ca(OH)_2$ (44.5 mg, 0.6 mmol, 3.0 equiv.), EDTA-Na_2•$2H_2O$ (223.3 mg, 0.6 mmol, 3.0 equiv.), and a

magnetic stir bar was charged with D_2O (1000 μL). The vial was sealed with a cap, and the reaction mixture was stirred vigorously at rt for 10 min. The mixture was then filtered through celite, and the eluent was analyzed by ^1H NMR.

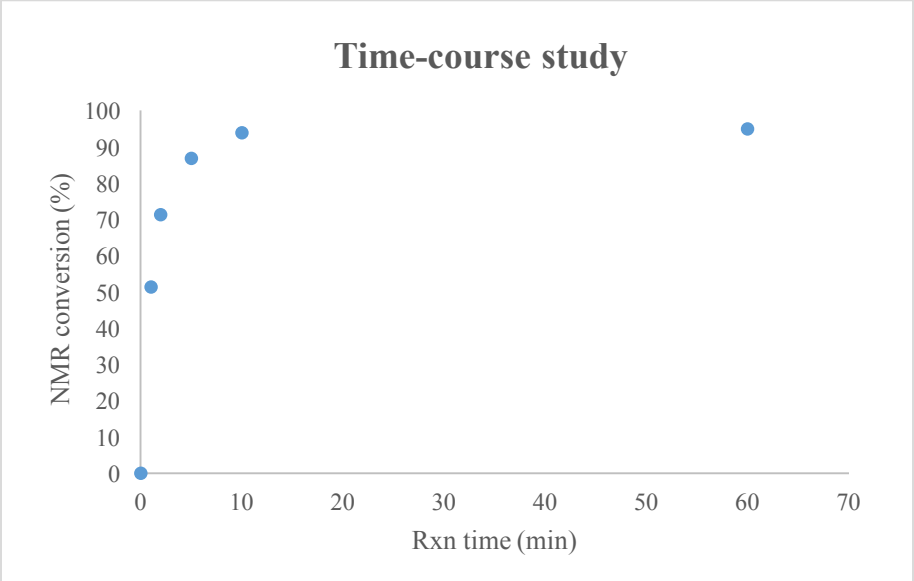
Procedure for Supplementary Table 5, entries 2–6: A 1-dram vial containing Boc-L-Tyr-OH (**1**; 56.3 mg, 0.2 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 109.3 mg, 0.6 mmol, 3.0 equiv.), $Ca(OH)_2$ (44.5 mg, 0.6 mmol, 3.0 equiv.), and a magnetic stir bar was charged with D_2O (200 μL, 1.0 M in substrate). The vial was sealed with a cap, and the reaction mixture was stirred vigorously at rt for the indicated time. The mixture was then charged with EDTA-Na_2•$2H_2O$ (223.3 mg, 0.6 mmol, 3.0 equiv.) followed by D_2O (800 μL) and stirred at rt for 10 min. The mixture was then filtered through celite, and the eluent was analyzed by ^1H NMR.

NMR conversion of each reaction was determined by comparison of ^1H NMR aromatic peaks of starting material (**1**) and product (**2**). No significant side-products were observed.

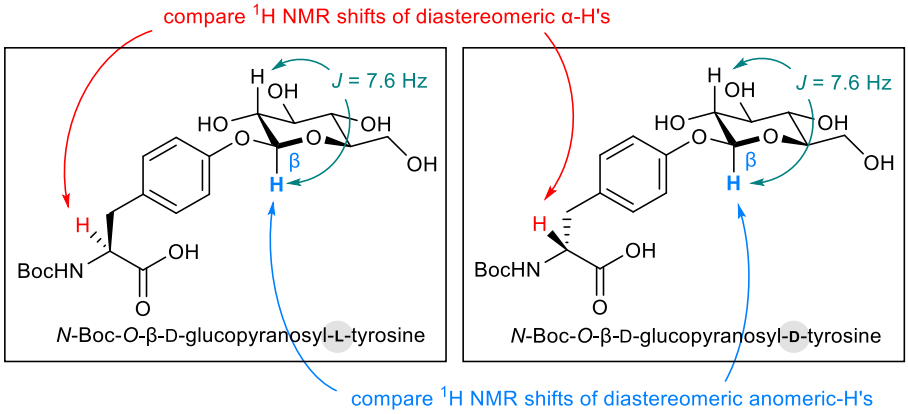
Supplementary Table 5: Reaction Time-Course



Entry	Rxn time (min)	NMR conv. (%)
1	0	0
2	1	52
3	2	71
4	5	87
5	10	94
6	60	95


Time-course study
NMR conversion (%)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
Rxn time (min)

4. Assessing Product Stereochemistry in the Glycosylation of Boc-L-Tyr-OH

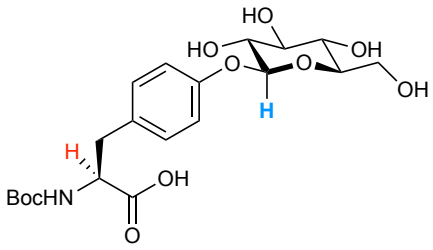


^{1}H NMR analysis and measured optical rotation of the product of Boc-Tyr-OH glucosylation over 10 min reaction time matched that of the product of Boc-Tyr-OH glycosylation over 24 h reaction time. These measurements suggest the following:

- the glycosylation proceeds to the β-glucosyl product within 10 min (anomeric-H J = 7.6 Hz),
- no measurable α/β interconversion occurs in the product of Boc-L-Tyr-OH glucosylation over 24 h reaction time, and
- no measurable amino acid epimerization occurs in the product of Boc-L-Tyr-OH glycosylation over 24 h reaction time.

For this study, the following samples were prepared and purified by reversed-phase flash chromatography:

Sample IX: Reaction product(s) from **10 min** glycosylation of **Boc-L-Tyr-OH**

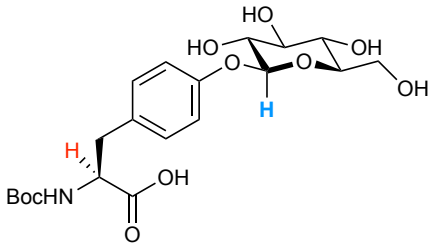


N-Boc-*O*-β-D-glucopyranosyl-L-tyrosine (**IX**)

A 1-dram vial containing Boc-L-Tyr-OH (**1**; 56.3 mg, 0.2 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 109.3 mg, 0.6 mmol, 3.0 equiv.), $Ca(OH)_2$ (44.5 mg, 0.6 mmol, 3.0 equiv.), and a magnetic stir bar was charged with H_2O (200 µL, 1.0 M in substrate). The vial was sealed with a cap, and the mixture was stirred vigorously at rt for 10 min. The resulting bright yellow mixture was then loaded onto a Biotage samplet, rinsing the reaction vial with H_2O, and purified via reversed-phase flash chromatography (60 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 50% MeOH/50% H_2O over

12 CV). Fractions containing the product were concentrated *in vacuo*, frozen, and lyophilized to dryness, resulting in a white powder (81 mg, 92% yield). Characterization matched that of *N*-Boc-*O*-β-D-glucopyranosyl-L-tyrosine (**2**) obtained by General Procedure 1; see Page 23. $[\alpha]_D^{20}$ = –7.2 (*c* 1.0, MeOH).

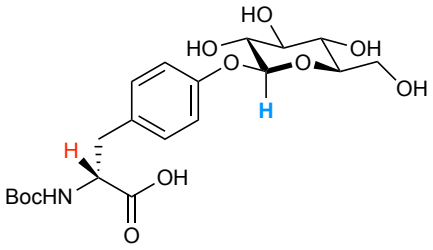
Sample X: Reaction product from **24 h** glycosylation of **Boc-L-Tyr-OH**



N-Boc-*O*-β-D-glucopyranosyl-L-tyrosine (**X**)

A 1-dram vial containing Boc-L-Tyr-OH (**1**; 56.3 mg, 0.2 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 109.3 mg, 0.6 mmol, 3.0 equiv.), $Ca(OH)_2$ (44.5 mg, 0.6 mmol, 3.0 equiv.), and a magnetic stir bar was charged with H_2O (200 µL, 1.0 M in substrate). The vial was sealed with a cap, and the mixture was stirred vigorously at rt for 24 h. The resulting bright yellow mixture was then loaded onto a Biotage samplet, rinsing the reaction vial with H_2O, and purified via reversed-phase flash chromatography (60 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 50% MeOH/50% H_2O over 12 CV). All fractions containing the product mass (determined by LCMS) were concentrated *in vacuo*, frozen, and lyophilized to dryness, resulting in a white powder (81 mg, 92% yield). Full characterization matched that of *N*-Boc-*O*-β-D-glucopyranosyl-L-tyrosine (**2**) obtained by General Procedure 1; see page 23. $[\alpha]_D^{20}$ = –7.3 (*c* 1.0, MeOH).

Sample XI: Reaction product from **10 min** glycosylation of **Boc-D-Tyr-OH** (the expected epimerization product)

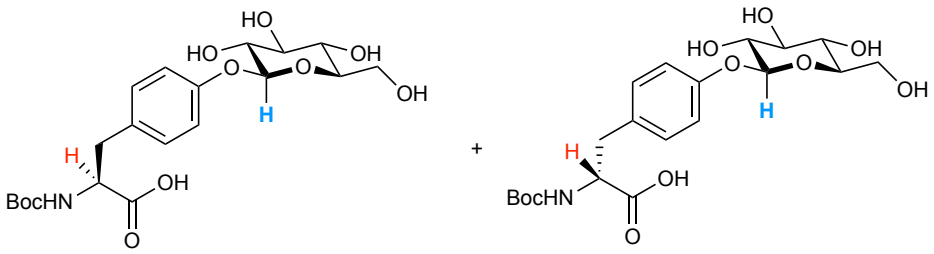


N-Boc-*O*-β-D-glucopyranosyl-D-tyrosine (**XI**)

***N*-Boc-*O*-β-D-glucopyranosyl-D-tyrosine (XI).** A 1-dram vial containing Boc-D-Tyr-OH (56.3 mg, 0.2 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 109.3 mg, 0.6 mmol, 3.0 equiv.), $Ca(OH)_2$ (44.5 mg, 0.6 mmol, 3.0 equiv.), and a magnetic stir bar was charged with H_2O (200 µL, 1.0 M in substrate). The vial was sealed with a cap, and the mixture was stirred vigorously at rt for 10 min. The resulting bright yellow mixture was then loaded onto a Biotage samplet, rinsing the

reaction vial with H_2O, and purified via reversed-phase flash chromatography (60 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 50% MeOH/50% H_2O over 12 CV). Fractions containing the product were concentrated *in vacuo*, frozen, and lyophilized to dryness, resulting in a white powder (82 mg, 92% yield). **$[\alpha]_D^{20}$** = –57.2 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.25 (d, *J* = 8.2 Hz, 2H), 7.09 (d, *J* = 8.2 Hz, 2H), 5.10 (d, *J* = 7.5 Hz, 1H), 4.16 (dd, *J* = 9.1, 4.7 Hz, 1H), 3.94 (dd, *J* = 12.5, 2.2 Hz, 1H), 3.77 (dd, *J* = 12.5, 5.5 Hz, 1H), 3.66 – 3.46 (m, 4H), 3.14 (dd, *J* = 13.9, 4.8 Hz, 1H), 2.90 – 2.68 (m, 1H), 1.40 – 1.23 (m, 9H) ppm; **^{13}C NMR** (151 MHz, DMSO-d_6) δ 175.5, 155.8, 154.7, 132.2, 130.3, 115.6, 100.5, 77.5, 77.0, 76.7, 73.3, 69.7, 60.7, 56.3, 36.7, 28.3 ppm; **IR** (cm^{-1}, neat): 3342, 2977, 2931, 1677, 1580, 1510, 1394, 1367, 1230, 1164, 1071, 1044, 1018, 893, 813, 624, 542, 435; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{20}H_{28}NO_{10}$ requires *m/z* 442.1713, found 442.1718.

Sample XII: Reaction products from glycosylation of **1:1 mixture of Boc-L-Tyr-OH and Boc-D-Tyr-OH**

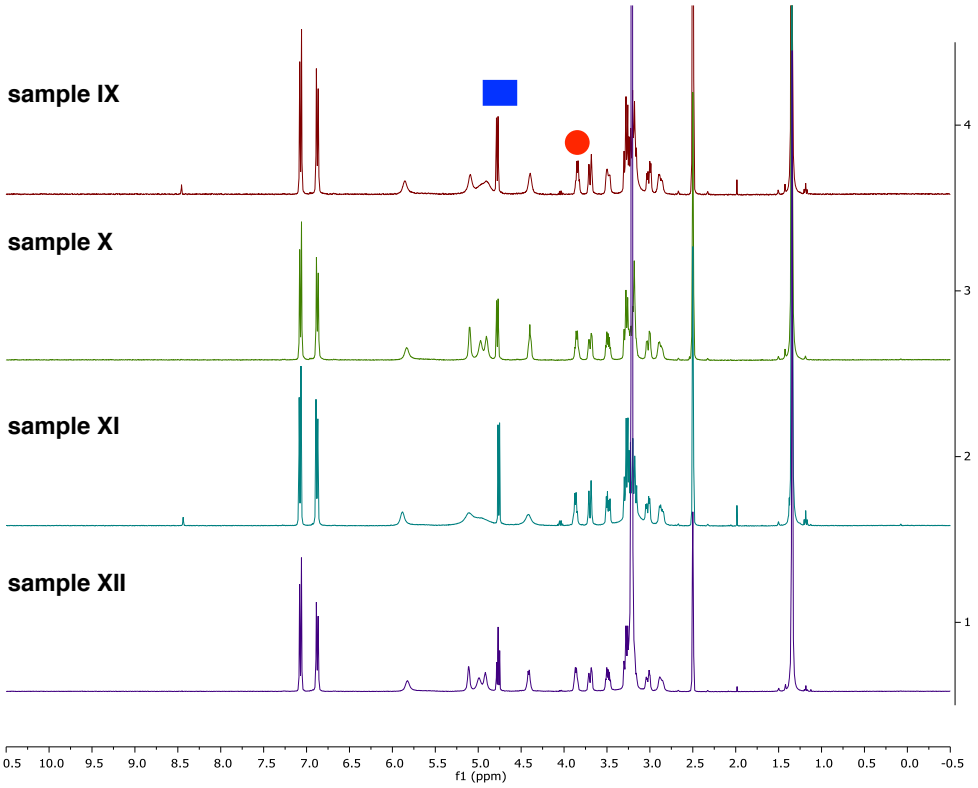


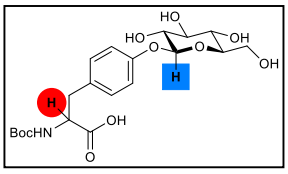
N-Boc-*O*-β-D-glucopyranosyl-L-tyrosine + *N*-Boc-*O*-β-D-glucopyranosyl-D-tyrosine (**XII**)

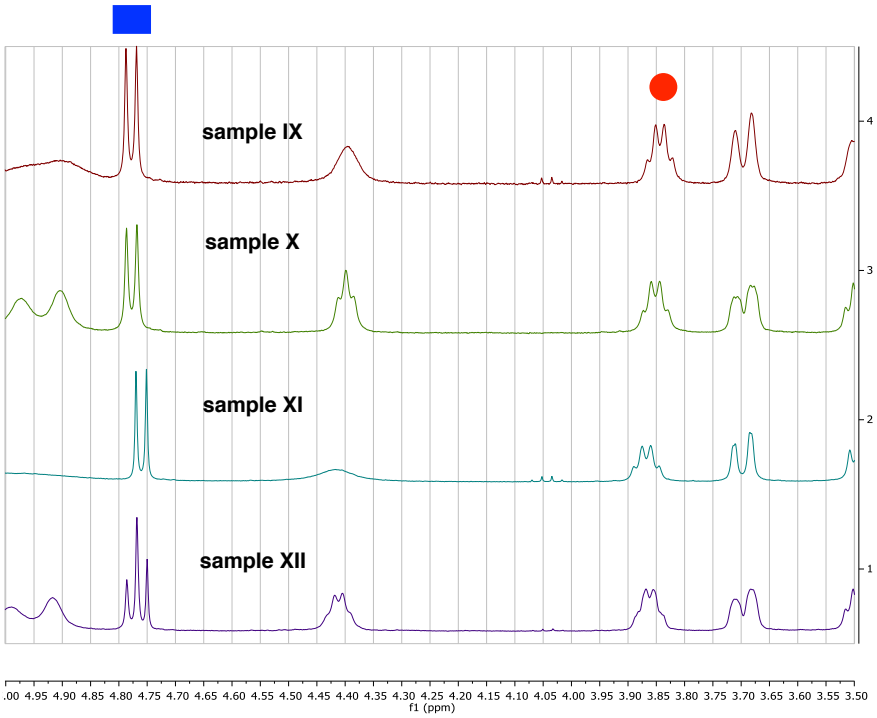
A 1-dram vial containing Boc-L-Tyr-OH (**1**; 70.4 mg, 0.25 mmol, 0.5 equiv.), Boc-D-Tyr-OH (70.4 mg, 0.25 mmol, 0.5 equiv.), α-D-fluoroglucose (**II**; 273.5 mg, 1.5 mmol, 3.0 equiv.), $Ca(OH)_2$ (111.2 mg, 1.5 mmol, 3.0 equiv.), and a magnetic stir bar was charged with H_2O (500 µL). The vial was sealed with a cap, and the mixture was stirred vigorously at rt for 16 h. The resulting bright yellow mixture was then loaded onto a Biotage samplet, rinsing the reaction vial with H_2O, and purified via reversed-phase flash chromatography (30 g C18 column; 25 mL/min flow rate; gradient = 5% MeOH/95% H_2O for 6 CV, ramped from 5% MeOH/95% H_2O to 80% MeOH/20% H_2O over 12 CV). All fractions containing the product mass (determined by LCMS) were concentrated *in vacuo*, frozen, and lyophilized to dryness, resulting in a white powder (206 mg, 93% yield). For the ^{1}H NMR spectrum of the mixture, see Section 4.2.1.

4.2.1. Comparison of Glycosylation Product ^{1}H NMR Spectra

^{1}H NMR analysis of samples **IX**, **X**, **XI**, and **XII** (DMSO, 55 °C) revealed no detectable epimerization or α/β interconversion up to 24 h reaction time. See data below:


sample IX
sample X
sample XI
sample XII
4
3
2
1
0.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


HO
HO
O
O
OH
OH
H
H
BocHN
OH
O

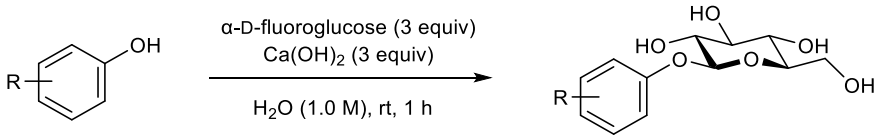

sample IX
sample X
sample XI
sample XII
4
3
2
1
.00 4.95 4.90 4.85 4.80 4.75 4.70 4.65 4.60 4.55 4.50 4.45 4.40 4.35 4.30 4.25 4.20 4.15 4.10 4.05 4.00 3.95 3.90 3.85 3.80 3.75 3.70 3.65 3.60 3.55 3.50
f1 (ppm)

4.2.2. Comparison of Glycosylation Product Optical Rotations

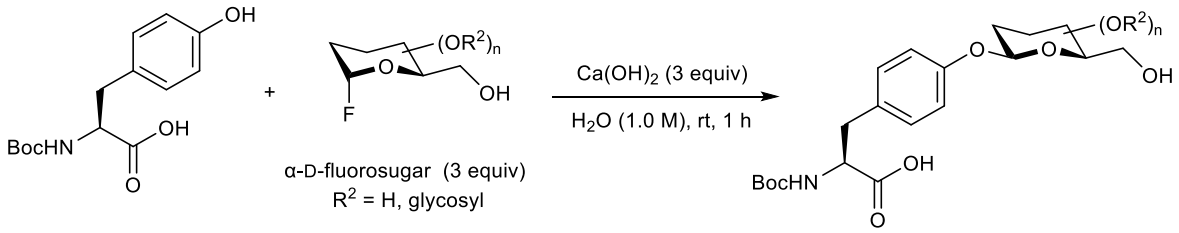
- **Sample IX** (10 min glycosylation of Boc-L-Tyr-OH) $[\alpha]_D^{20} = -7.2$ (*c* 1.0, MeOH)
- **Sample X** (24 h glycosylation of Boc-L-Tyr-OH) $[\alpha]_D^{20} = -7.3$ (*c* 1.0, MeOH)
- **Sample XI** (10 min glycosylation of Boc-D-Tyr-OH) $[\alpha]_D^{20} = -57.2$ (*c* 1.0, MeOH)

5. Exploration of Small Molecule Glycosylation Scope

5.1 General Procedures

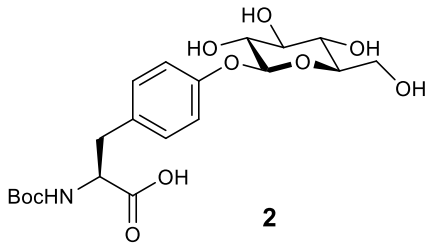


General Procedure 1: A 10 mL round bottom flask containing substrate (0.5 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 273.5 mg, 1.5 mmol, 3.0 equiv.), $Ca(OH)_2$ (111.2 mg, 1.5 mmol, 3.0 equiv.), and a magnetic stir bar was charged with H_2O (500 μL). The flask was sealed with a septum, and the mixture was stirred vigorously at rt for 1 h. The mixture was then loaded onto a Biotage samplet, rinsing the reaction flask with H_2O, and purified via reversed-phase flash chromatography. Fractions containing the product were concentrated *in vacuo*, frozen, and lyophilized to dryness.



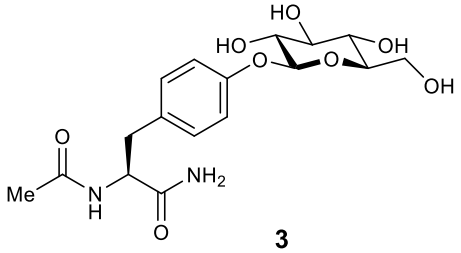
General Procedure 2: A 10 mL round bottom flask containing Boc-L-Tyr-OH (**1**; 140.7 mg, 0.5 mmol, 1.0 equiv.), α-D-fluorosugar (1.5 mmol, 3.0 equiv.), $Ca(OH)_2$ (111.2 mg, 1.5 mmol, 3.0 equiv.), and a magnetic stir bar was charged with H_2O (500 μL). The flask was sealed with a septum, and the mixture was stirred vigorously at rt for 1 h. The mixture was then loaded onto a Biotage samplet, rinsing the reaction flask with H_2O, and purified via reversed-phase flash chromatography. Fractions containing the product were concentrated *in vacuo*, frozen, and lyophilized to dryness.

5.2 Syntheses and Characterization of Small Molecule Glycosylation Products



2

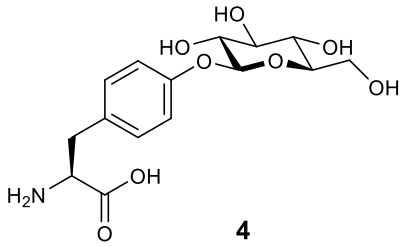
***N*-Boc-*O*-β-D-glucopyranosyl-L-tyrosine (2).** The product was synthesized from Boc-L-Tyr-OH (140.7 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 50% MeOH/50% H_2O over 12 CV) followed by lyophilization resulted in a white solid (201 mg, 91% yield). $[\alpha]_D^{20}$ = –7.2 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.24 (d, *J* = 8.3 Hz, 2H), 7.09 (d, *J* = 8.1 Hz, 2H), 5.09 (d, *J* = 7.6 Hz, 1H), 4.15 (dd, *J* = 8.9, 4.6 Hz, 1H), 3.93 (dd, *J* = 12.5, 2.2 Hz, 1H), 3.77 (dd, *J* = 12.4, 5.4 Hz, 1H), 3.67 – 3.45 (m, 4H), 3.13 (dd, *J* = 14.0, 4.8 Hz, 1H), 2.89 – 2.71 (m, 1H), 1.34 (s, 6H), 1.28 (s, 3H) ppm; **^{13}C NMR** (151 MHz, DMSO-d_6) δ 175.2, 155.7, 154.7, 132.3, 130.3, 115.5, 100.4, 77.4, 77.0, 76.7, 73.3, 69.7, 60.6, 56.3, 36.7, 28.3 ppm; **IR** (cm^{-1}, neat): 3349, 2980, 2932, 1676, 1579, 1510, 1394, 1367, 1229, 1163, 1071, 1043, 1017, 892, 813, 542, 443, 428; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{20}H_{28}NO_{10}$ requires *m/z* 442.1713, found 442.1707.



3

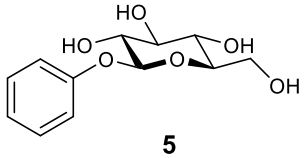
***N*-Acetyl-*O*-β-D-glucopyranosyl-L-tyrosinamide (3).** The product was synthesized from Ac-Tyr-NH_2 (111.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. The product was purified by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV). Mixed product fractions were combined, concentrated *in vacuo*, and subjected to a second column (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV). Combining and lyophilizing pure product fractions from both columns resulted in a white solid (174 mg, 91% yield). $[\alpha]_D^{20}$ = –18.0 (*c* 0.5, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.27 (d, *J* = 8.6 Hz, 2H), 7.10 (d, *J* = 8.6 Hz, 2H), 5.12 (d, *J* = 7.4 Hz, 1H), 4.54 (dd, *J* = 8.9, 6.0 Hz, 1H), 3.94 (dd, *J* = 12.4, 2.2 Hz, 1H), 3.76 (dd, *J* = 12.4, 5.7 Hz, 1H), 3.69 – 3.43 (m, 4H), 3.14 (dd, *J* = 14.0, 6.1 Hz, 1H), 2.95 (dd, *J* = 14.1, 8.9 Hz, 1H), 1.95 (s, 3H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 173.3, 169.0, 156.0, 131.4, 129.9, 115.8, 100.4, 77.0, 76.6, 73.3, 69.7, 60.7, 54.0, 36.9, 22.5

ppm; **IR** (cm^{-1}, neat): 3356, 3301, 1663, 1650, 1533, 1512, 1423, 1374, 1226, 1120, 1074, 1034, 1006, 896, 838, 818, 726, 659, 629, 585, 529, 512, 464, 429; **HRMS** (ESI) *m/z* for $[M+H]^+$ $C_{17}H_{25}N_2O_8$ requires *m/z* 385.1611, found 385.1604.



4

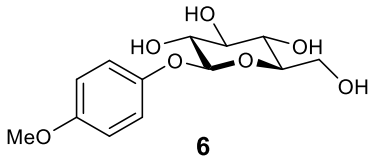
***O*-β-D-Glucopyranosyl-L-tyrosine (4).** The product was synthesized from H-L-Tyr-OH (90.6 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV) followed by lyophilization resulted in a white solid (158 mg, 92% yield). $[\alpha]_D^{20}$ = –61.3 (*c* 0.2, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.29 (d, *J* = 8.7 Hz, 2H), 7.13 (d, *J* = 8.7 Hz, 2H), 5.14 (d, *J* = 7.4 Hz, 1H), 4.00 – 3.90 (m, 2H), 3.76 (dd, *J* = 12.4, 5.6 Hz, 1H), 3.70 – 3.46 (m, 4H), 3.25 (dd, *J* = 14.6, 5.2 Hz, 1H), 3.10 (dd, *J* = 14.6, 7.8 Hz, 1H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 169.8, 156.2, 130.6, 130.3, 116.1, 100.5, 77.0, 76.6, 73.2, 69.7, 60.7, 55.7, 36.1 ppm; **IR** (cm^{-1}, neat): 3366, 3130, 2921, 1640, 1580, 1511, 1433, 1406, 1371, 1348, 1301, 1239, 1228, 1189, 1096, 1073, 1047, 1023, 1007, 894, 881, 842, 812, 795, 745, 656, 634, 604, 536, 499, 475, 452, 434; **HRMS** (ESI) *m/z* for $[M+H]^+$ $C_{15}H_{22}NO_8$ requires *m/z* 344.1345, found 344.1342.



5

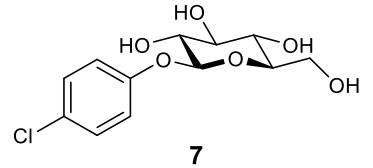
Phenyl-β-D-glucopyranoside (5). The product was synthesized from phenol (47.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 50% MeOH/50% H_2O over 12 CV) followed by lyophilization resulted in a white solid (118 mg, 92% yield). $[\alpha]_D^{18}$ = –62.0 (*c* 1.0, MeOH), lit[8] $[\alpha]_D^{20}$ = –56.2 (*c* 0.2, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.45 – 7.39 (m, 2H), 7.21 – 7.12 (m, 3H), 5.15 (d, *J* = 7.4 Hz, 1H), 3.94 (dd, *J* = 12.5, 2.3 Hz, 1H), 3.76 (dd, *J* = 12.4, 5.7 Hz, 1H), 3.70 – 3.46 (m, 4H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 157.5, 129.3, 121.7, 116.2, 100.4, 77.0, 76.6, 73.2, 69.7, 60.7 ppm; **IR** (cm^{-1}, neat): 3255, 2942, 1621, 1590, 1496, 1397, 1368, 1223, 1112, 1074, 1041, 1014, 927, 890, 817, 754, 692, 530, 505, 472, 422; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{12}H_{15}O_6$ requires *m/z* 255.0869, found 255.0864.

[8] Cocinero, E.J.; Gamblin, D.P.; Davis, B.G.; Simons, J.P. *J. Am. Chem. Soc.* **2009**, *131*, 11117.



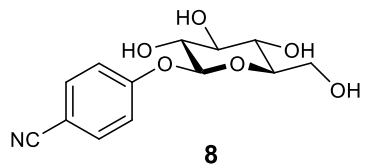
6

4-Methoxyphenyl-β-D-glucopyranoside (6). The product was synthesized from 4-methoxyphenol (62.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 50% MeOH/50% H_2O over 12 CV) followed by lyophilization resulted in a white solid (131 mg, 92% yield). $[\alpha]_D^{20}$ = –64.1 (*c* 0.2, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.16 – 7.09 (m, 2H), 7.03 – 6.95 (m, 2H), 5.02 (d, *J* = 7.6 Hz, 1H), 3.93 (dd, *J* = 12.5, 2.2 Hz, 1H), 3.82 (s, 3H), 3.76 (dd, *J* = 12.4, 5.7 Hz, 1H), 3.64 – 3.45 (m, 4H) ppm; **^{13}C NMR** (151 MHz, DMSO-d_6) δ 154.2, 151.5, 117.6, 114.4, 101.5, 77.0, 76.6, 73.3, 69.8, 60.8, 55.3 ppm; **IR** (cm^{-1}, neat): 3297, 2893, 1615, 1508, 1441, 1398, 1378, 1291, 1224, 1105, 1076, 1046, 1017, 896, 825, 753, 658, 607, 528, 465, 422; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{13}H_{17}O_7$ requires *m/z* 285.0974, found 285.0976.



7

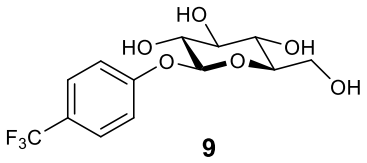
4-Chlorophenyl-β-D-glucopyranoside (7). The product was synthesized from 4-chlorophenol (64.3 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV) followed by lyophilization resulted in a white solid (135 mg, 93% yield). $[\alpha]_D^{20}$ = –62.6 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.45 – 7.34 (m, 2H), 7.17 – 7.07 (m, 2H), 5.11 (d, *J* = 7.4 Hz, 1H), 3.94 (dd, *J* = 12.5, 2.3 Hz, 1H), 3.76 (dd, *J* = 12.4, 5.7 Hz, 1H), 3.68 – 3.46 (m, 4H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 156.2, 129.2, 125.5, 118.0, 100.5, 77.1, 76.5, 73.2, 69.6, 60.7 ppm; **IR** (cm^{-1}, neat): 3304, 2930, 1595, 1490, 1402, 1236, 1070, 1046, 1027, 893, 824, 680, 654, 617, 576, 507, 473; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{12}H_{14}ClO_6$ requires *m/z* 289.0479, found 289.0473.



8

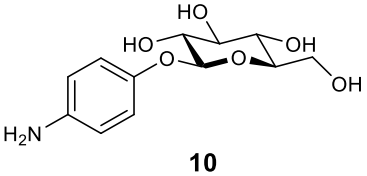
4-(β-D-Glucopyranosyloxy)benzonitrile (8). The product was synthesized from 4-cyanophenol (59.5 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for

3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV) followed by lyophilization resulted in a white solid (113 mg, 80% yield). $[\alpha]_D^{20}$ = –91.1 (*c* 1.5, H_2O), lit[9] $[\alpha]_D^{20}$ = –75.9 (*c* 0.5, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.83 – 7.74 (m, 2H), 7.27 – 7.21 (m, 2H), 5.24 (d, *J* = 7.7 Hz, 1H), 3.94 (dd, *J* = 12.3, 2.2 Hz, 1H), 3.76 (dd, *J* = 12.4, 5.7 Hz, 1H), 3.69 (ddd, *J* = 9.8, 5.7, 2.2 Hz, 1H), 3.65 – 3.61 (m, 2H), 3.57 – 3.48 (m, 1H) ppm; **^{13}C NMR** (151 MHz, DMSO-d_6) δ 160.7, 134.1, 119.0, 117.0, 104.0, 99.7, 77.1, 76.4, 73.1, 69.5, 60.6 ppm; **IR** (cm^{-1}, neat): 3295, 2891, 2230, 1787, 1658, 1607, 1549, 1511, 1379, 1297, 1250, 1177, 1084, 1042, 990, 893, 837, 725, 663, 639, 610, 548, 506; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{13}H_{14}NO_6$ requires *m/z* 280.0821, found 280.0816.



9

4-Trifluoromethylphenyl-β-D-glucopyranoside (9). The product was synthesized from 4-(trifluoromethyl)phenol (81.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 50% MeOH/50% H_2O over 12 CV) followed by lyophilization resulted in a white solid (98 mg, 60% yield). $[\alpha]_D^{20}$ = –64.3 (*c* 0.2, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.72 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 8.6 Hz, 2H), 5.22 (d, *J* = 7.4 Hz, 1H), 3.95 (dd, *J* = 12.4, 2.3 Hz, 1H), 3.77 (dd, *J* = 12.4, 5.7 Hz, 1H), 3.74 – 3.57 (m, 3H), 3.57 – 3.48 (m, 1H) ppm; **^{13}C NMR** (151 MHz, DMSO-d_6) δ 160.1, 126.8, 124.5, 122.2, 116.6, 99.9, 77.1, 76.5, 73.1, 69.6, 60.6 ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ –62.7 (s) ppm; **IR** (cm^{-1}, neat): 3301, 2887, 1617, 1595, 1518, 1423, 1330, 1248, 1162, 1105, 1060, 1047, 1012, 929, 896, 833, 652, 619, 590, 530, 508, 476; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{13}H_{14}F_3O_6$ requires *m/z* 323.0742, found 323.0744.

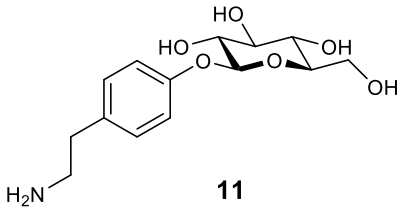


10

4-Aminophenyl-β-D-glucopyranoside (10). The product was synthesized from 4-aminophenol (81.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. The product was purified by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV). Mixed product fractions were combined, concentrated *in vacuo*, and subjected to a second column (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV). Combining and

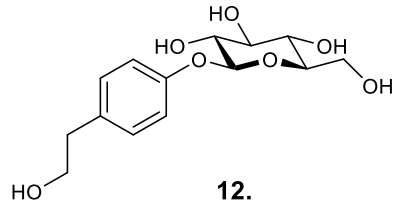
[9] Marton, Z.; Tran, V.; Tellier, C.; Dion, M.; Drone, J.; Rabiller, C. *Carbohydrate Res.* **2008**, *343*, 2939.

lyophilizing pure product fractions from both columns resulted in a white solid (97 mg, 72% yield). **$[\alpha]_D^{20}$** = –55.8 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.05 – 6.97 (m, 2H), 6.86 – 6.80 (m, 2H), 4.98 (d, *J* = 7.7 Hz, 1H), 3.93 (dd, *J* = 12.4, 2.2 Hz, 1H), 3.75 (dd, *J* = 12.4, 5.6 Hz, 1H), 3.66 – 3.43 (m, 4H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 148.8, 143.6, 117.7, 114.5, 102.1, 76.9, 76.7, 73.3, 69.9, 60.8 ppm; **IR** (cm^{-1}, neat): 3310, 2897, 1628, 1508, 1397, 1217, 1067, 1034, 893, 830, 765; **HRMS** (ESI) *m/z* for $[M+H]^+$ $C_{12}H_{18}NO_6$ requires *m/z* 272.1134, found 272.1129.



11

4-(2-Aminoethyl)phenyl-β-D-glucopyranoside (11). The product was synthesized from tyramine (68.6 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV) followed by lyophilization resulted in a white solid (124 mg, 83% yield). **$[\alpha]_D^{20}$** = –36.5 (*c* 0.2, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.19 – 7.11 (m, 2H), 7.03 – 6.93 (m, 2H), 4.97 (d, *J* = 7.3 Hz, 1H), 3.78 (dd, *J* = 12.4, 2.3 Hz, 1H), 3.60 (dd, *J* = 12.4, 5.7 Hz, 1H), 3.53 – 3.29 (m, 4H), 3.11 (t, *J* = 7.2 Hz, 2H), 2.82 (t, *J* = 7.2 Hz, 2H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 155.9, 132.5, 129.5, 116.2, 100.6, 77.0, 76.6, 73.3, 69.8, 60.7, 42.5, 36.3 ppm; **IR** (cm^{-1}, neat): 3261, 2916, 1611, 1510, 1382, 1226, 1067, 1013, 897, 824, 723, 547, 436; **HRMS** (ESI) *m/z* for $[M+H]^+$ $C_{14}H_{22}NO_6$ requires *m/z* 300.1447, found 300.1443.

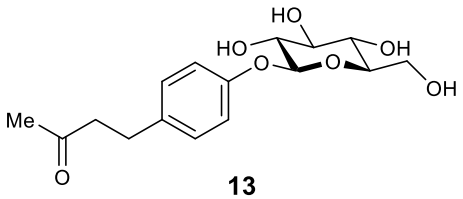


12.

4-(2-Hydroxyethyl)phenyl-β-D-glucopyranoside (12). The product was synthesized from 2-(4-hydroxyphenyl)ethanol (69.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV) followed by lyophilization resulted in a white solid (123 mg, 82% yield). **$[\alpha]_D^{23}$** = –61.9 (*c* 0.11, MeOH), lit[10] **$[\alpha]_D^{20}$** = –57.6 (*c* 0.5, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.34 – 7.23 (m, 2H), 7.15 – 7.06 (m, 2H), 5.11 (d, *J* = 7.4 Hz, 1H), 3.93 (dd, *J* = 12.4, 2.3 Hz, 1H), 3.81 (t, *J* = 6.6 Hz, 2H), 3.76 (dd, *J* = 12.4, 5.7 Hz, 1H), 3.65 – 3.44 (m, 4H), 2.83 (t, *J* = 6.6

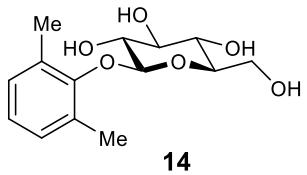
[10] Miyase, T.; Ueno, A.; Takizawa, N.; Kobayashi, H.; Oguchi, H. *Phytochemistry* **1989**, *28*, 3483.

Hz, 2H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 155.7, 132.7, 129.6, 116.1, 100.6, 77.0, 76.6, 73.2, 69.7, 62.4, 60.7, 38.2 ppm; **IR** (cm^{-1}, neat): 3329, 2886, 1612, 1513, 1402, 1312, 1228, 1188, 1083, 1036, 991, 896, 848, 826, 625, 588, 540, 518; **HRMS** (ESI) *m/z* for $[M+H]^+$ $C_{14}H_{21}O_7$ requires *m/z* 301.1287, found 301.1284.



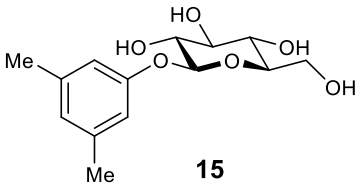
13

4-[4-(β-D-Glucopyranosyloxy)phenyl]-2-butanone (13). The product was synthesized from 4-(4-hydroxyphenyl)-2-butanone (82.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV) followed by lyophilization resulted in a white solid (154 mg, 94% yield). **$[\alpha]_D^{20}$** = –50.9 (*c* 0.5, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.29 – 7.17 (m, 2H), 7.15 – 7.03 (m, 2H), 5.09 (d, *J* = 7.5 Hz, 1H), 3.93 (dd, *J* = 12.4, 2.3 Hz, 1H), 3.75 (dd, *J* = 12.4, 5.7 Hz, 1H), 3.66 – 3.44 (m, 4H), 2.95 – 2.78 (m, 4H), 2.19 (s, 3H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 207.8, 155.7, 134.4, 129.0, 116.2, 100.6, 77.0, 76.6, 73.3, 69.8, 60.7, 44.4, 29.8, 28.3 ppm; **IR** (cm^{-1}, neat): 3282, 2921, 1703, 1612, 1511, 1402, 1364, 1227, 1164, 1099, 1068, 1040, 1010, 897, 812, 657, 571, 535, 413; **HRMS** (ESI) *m/z* for $[M+H]^+$ $C_{16}H_{23}O_7$ requires *m/z* 327.1444, found 327.1440.



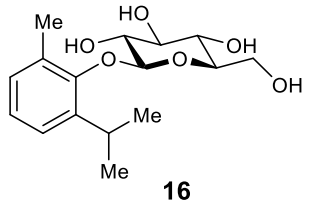
14

2,6-Dimethylphenyl-β-D-glucopyranoside (14). The product was synthesized from 2,6-dimethylphenol (61.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (30 g C18 column; 25 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 6 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 24 CV) followed by lyophilization resulted in a white solid (60 mg, 42% yield). **$[\alpha]_D^{20}$** = –1.8 (*c* 0.5, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.15 (d, *J* = 7.3 Hz, 2H), 7.11 – 7.06 (m, 1H), 4.85 (d, *J* = 7.7 Hz, 1H), 3.79 (dd, *J* = 12.4, 2.4 Hz, 1H), 3.73 (dd, *J* = 12.3, 4.9 Hz, 1H), 3.65 (t, *J* = 8.4 Hz, 1H), 3.58 (t, *J* = 9.0 Hz, 1H), 3.51 (t, *J* = 9.2 Hz, 1H), 3.30 (ddd, *J* = 9.4, 4.8, 2.3 Hz, 1H), 2.33 (s, 6H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 153.6, 131.4, 128.5, 124.0, 104.2, 76.8, 76.4, 74.1, 70.0, 61.2, 17.0 ppm; **IR** (cm^{-1}, neat): 3317, 2922, 1475, 1389, 1264, 1190, 1074, 1036, 896, 834, 765, 593, 540, 514; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{14}H_{19}O_6$ requires *m/z* 283.1182, found 283.1180.



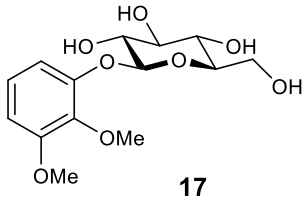
15

3,5-Dimethylphenyl-β-D-glucopyranoside (15). The product was synthesized from 3,5-dimethylphenol (61.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. The product was purified by reversed-phase flash chromatography (30 g C18 column; 25 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 6 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 24 CV). Mixed product fractions were combined, concentrated *in vacuo*, and subjected to a second column (30 g C18 column; 25 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 6 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 24 CV). Combining and lyophilizing pure product fractions from both columns resulted in a white solid (109 mg, 77% yield). $[\alpha]_D^{20}$ = –62.4 (*c* 0.8, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 6.71 (s, 1H), 6.65 (s, 2H), 4.95 (d, *J* = 7.6 Hz, 1H), 3.78 (dd, *J* = 12.5, 2.2 Hz, 1H), 3.60 (dd, *J* = 12.4, 5.8 Hz, 1H), 3.50 – 3.30 (m, 4H), 2.14 (s, 6H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 157.6, 138.4, 123.3, 114.1, 100.6, 77.0, 76.6, 73.3, 69.7, 60.7, 21.1 ppm; **IR** (cm^{-1}, neat): 3266, 2919, 1596, 1474, 1407, 1373, 1318, 1297, 1177, 1161, 1103, 1072, 1055, 1029, 987, 893, 853, 829, 688, 670, 644, 539, 412; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{14}H_{19}O_6$ requires *m/z* 283.1182, found 283.1180.



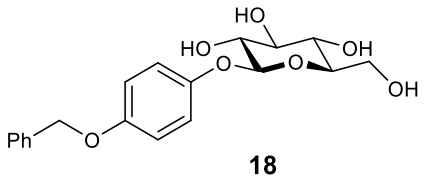
16

5-Methyl-2-(1-methylethyl)phenyl-β-D-glucopyranoside (16). The product was synthesized from thymol (75.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (30 g C18 column; 25 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 6 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 24 CV) followed by lyophilization resulted in a white solid (36 mg, 23% yield). $[\alpha]_D^{20}$ = –57.9 (*c* 0.2, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.29 (d, *J* = 7.8 Hz, 1H), 7.03 (d, *J* = 1.7 Hz, 1H), 7.00 (d, *J* = 7.6 Hz, 1H), 5.06 (d, *J* = 7.7 Hz, 1H), 3.94 (dd, *J* = 12.5, 2.2 Hz, 1H), 3.76 (dd, *J* = 12.4, 5.8 Hz, 1H), 3.68 – 3.55 (m, 3H), 3.57 – 3.48 (m, 1H), 3.38 (hept, *J* = 7.0 Hz, 1H), 2.31 (s, 3H), 1.19 (d, *J* = 6.9 Hz, 6H) ppm; **^{13}C NMR** (151 MHz, DMSO-d_6) δ 154.6, 135.7, 134.1, 125.4, 122.4, 115.8, 101.3, 77.1, 76.9, 73.5, 69.9, 60.8, 25.6, 23.0, 22.7, 21.0 ppm; **IR** (cm^{-1}, neat): 3339, 2966, 2927, 2868, 1616, 1503, 1451, 1391, 1369, 1281, 1249, 1159, 1121, 1092, 1069, 1045, 1014, 940, 900, 813, 768, 634, 583, 573, 498, 447, 430, 420, 406; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{16}H_{23}O_6$ requires *m/z* 311.1495, found 311.1499.



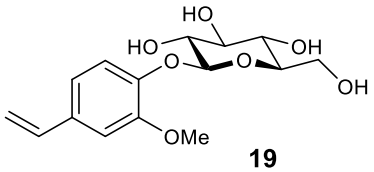
17

2,3-Dimethoxyphenyl-β-D-glucopyranoside (17). The product was synthesized from 2,3-dimethoxyphenol (77.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV) followed by lyophilization resulted in a white solid (127 mg, 80% yield). $[\alpha]_D^{20}$ = –47.1 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.15 (t, *J* = 8.5 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 2H), 5.14 (d, *J* = 7.5 Hz, 1H), 3.92 (dd, *J* = 12.5, 2.2 Hz, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 3.76 (dd, *J* = 12.5, 5.5 Hz, 1H), 3.66 – 3.59 (m, 3H), 3.55 – 3.49 (m, 1H) ppm; **^{13}C NMR** (151 MHz, DMSO-*d*$_6$) δ 153.2, 151.3, 138.3, 123.4, 109.1, 106.4, 101.0, 77.1, 76.8, 73.3, 69.7, 60.7, 60.2, 55.8 ppm; **IR** (cm^{-1}, neat): 3336, 2938, 1602, 1498, 1477, 1369, 1295, 1258, 1178, 1080, 1041, 1018, 992, 896, 774, 732, 561, 486; **HRMS** (ESI) *m/z* for $[M+Na]^+$ $C_{14}H_{20}O_8Na$ requires *m/z* 339.1056, found 339.1053.



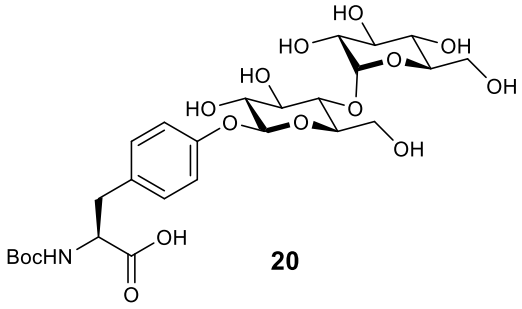
18

4-(Phenylmethoxy)phenyl-β-D-glucopyranoside (18). The product was synthesized from 4-(benzyloxy)phenol (100.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV) followed by lyophilization resulted in a white solid (18 mg, 10% yield). $[\alpha]_D^{20}$ = –29.0 (*c* 0.1, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.54 – 7.38 (m, 5H), 7.16 – 7.09 (m, 2H), 7.09 – 7.03 (m, 2H), 5.16 (s, 2H), 5.03 (d, *J* = 7.6 Hz, 1H), 3.93 (dd, *J* = 12.5, 2.3 Hz, 1H), 3.76 (dd, *J* = 12.5, 5.7 Hz, 1H), 3.65 – 3.45 (m, 5H) ppm; **^{13}C NMR** (151 MHz, DMSO-*d*$_6$) δ 153.3, 151.6, 137.3, 128.4, 127.7, 127.6, 117.5, 115.5, 101.4, 77.0, 76.6, 73.3, 69.8, 69.6, 60.7 ppm; **IR** (cm^{-1}, neat): 3303, 2859, 1620, 1508, 1461, 1379, 1311, 1233, 1105, 1078, 1048, 1020, 897, 823, 783, 735, 695, 659, 623, 536, 465, 422; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{19}H_{21}O_7$ requires *m/z* 361.1287, found 361.1281.



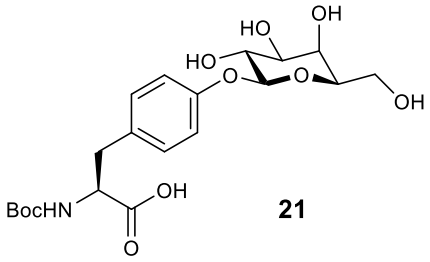
19

4-Ethenyl-2-methoxyphenyl-β-D-glucopyranoside (19). The product was synthesized from 2-methoxy-4-vinylphenol (75.1 mg, 0.5 mmol, 1.0 equiv.) using General Procedure 1. Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV) followed by lyophilization resulted in a white solid (16 mg, 10% yield). $[\alpha]_D^{20}$ = –46.9 (*c* 0.1, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.25 (d, *J* = 1.9 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 7.11 (dd, *J* = 8.5, 1.9 Hz, 1H), 6.76 (dd, *J* = 17.7, 11.0 Hz, 1H), 5.80 (dd, *J* = 17.7, 0.9 Hz, 1H), 5.29 (dd, *J* = 10.9, 0.8 Hz, 1H), 5.15 (d, *J* = 7.7 Hz, 1H), 3.96 – 3.89 (m, 4H), 3.76 (dd, *J* = 12.5, 5.5 Hz, 1H), 3.66 – 3.59 (m, 3H), 3.52 (ddd, *J* = 9.4, 6.2, 3.1 Hz, 1H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 149.0, 146.5, 136.4, 131.2, 119.1, 115.1, 112.4, 109.8, 99.9, 77.0, 76.9, 73.2, 69.6, 60.6, 55.6 ppm; **IR** (cm^{-1}, neat): 3292, 1585, 1513, 1417, 1324, 1265, 1225, 1131, 1071, 1023, 914, 858, 803; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{15}H_{19}O_7$ requires *m/z* 311.1131, found 311.1124.



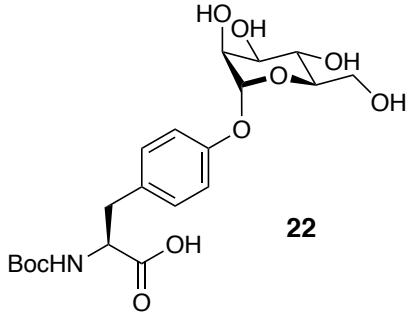
20

***N*-Boc-*O*-β-D-maltopyranosyl-L-tyrosine (20).** The product was synthesized by General Procedure 2 using α-D-fluoromaltose (**S4**; 516.2 mg, 1.5 mmol, 3.0 equiv.). Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 50% MeOH/50% H_2O over 12 CV) followed by lyophilization resulted in a white solid (223 mg, 74% yield). $[\alpha]_D^{20}$ = +41.0 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.25 (d, *J* = 8.2 Hz, 2H), 7.09 (d, *J* = 8.2 Hz, 2H), 5.46 (d, *J* = 3.8 Hz, 1H), 5.11 (d, *J* = 7.9 Hz, 1H), 4.16 (dd, *J* = 9.1, 4.6 Hz, 1H), 4.01 – 3.66 (m, 9H), 3.65 – 3.54 (m, 2H), 3.44 (t, *J* = 9.5 Hz, 1H), 3.14 (dd, *J* = 14.0, 4.8 Hz, 1H), 2.88 – 2.73 (m, 1H), 1.41 – 1.22 (m, 9H) ppm; **^{13}C NMR** (151 MHz, DMSO-d_6) δ 175.5, 155.6, 154.7, 132.4, 130.3, 115.6, 100.9, 100.1, 79.3, 77.5, 76.3, 75.2, 73.6, 73.3, 72.9, 72.5, 69.9, 60.9, 60.3, 56.3, 36.8, 28.3 ppm; **IR** (cm^{-1}, neat): 3342, 2928, 1676, 1583, 1509, 1394, 1367, 1230, 1155, 1019, 894, 534, 435; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{26}H_{38}NO_{15}$ requires *m/z* 604.2241, found 604.2250.



21

***N*-Boc-*O*-β-D-galactopyranosyl-L-tyrosine (21).** The product was synthesized by General Procedure 2 using α-D-fluoroglactose (**S6**; 273.5 mg, 1.5 mmol, 3.0 equiv.). Purification by reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 50% MeOH/50% H_2O over 12 CV) followed by lyophilization resulted in a white solid (103 mg, 46% yield). $[\alpha]_D^{20}$ = +3.3 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.25 (d, *J* = 8.2 Hz, 2H), 7.10 (d, *J* = 8.2 Hz, 2H), 5.03 (d, *J* = 7.0 Hz, 1H), 4.16 (dd, *J* = 8.9, 4.7 Hz, 1H), 4.02 (dd, *J* = 3.1, 1.0 Hz, 1H), 3.91 – 3.73 (m, 5H), 3.13 (dd, *J* = 13.8, 4.9 Hz, 1H), 2.88 – 2.70 (m, 1H), 1.41 – 1.24 (m, 9H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 175.3, 155.8, 154.6, 132.2, 130.2, 115.6, 101.1, 77.4, 75.4, 73.4, 70.3, 68.0, 60.3, 56.3, 36.8, 28.3 ppm; **IR** (cm^{-1}, neat): 3328, 2975, 1677, 1581, 1509, 1394, 1367, 1230, 1163, 1052, 558, 530, 435; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{20}H_{28}NO_{10}$ requires *m/z* 442.1713, found 442.1718.



22

***N*-Boc-*O*-α-D-mannopyranosyl-L-tyrosine (22).** A 10 mL round bottom flask was charged with a solution of α-D-fluoromannose (**S8**; 285.0 mg, 1.57 mmol, 3.0 equiv.) in ~2 mL MeOH and evaporated to dryness (solid α-D-fluoromannose was too hygroscopic to weigh accurately). To the reaction flask were then added Boc-Tyr-OH (146.3 mg, 0.52 mmol, 1.0 equiv.), $Ca(OH)_2$ (116.3 mg, 1.57 mmol, 3.0 equiv.), a magnetic stir bar, and H_2O (520 μL). The flask was sealed with a septum, and the mixture was stirred vigorously at rt for 1 h. The mixture was then loaded onto a Biotage samplet, rinsing the reaction flask with H_2O, and purified via reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% CH_3CN/100% H_2O for 2 CV, ramped from 0% CH_3CN/100% H_2O to 50% CH_3CN/50% H_2O over 18 CV). Mixed product fractions were combined, concentrated *in vacuo*, and subjected to a second column (120 g C18 column; 50 mL/min flow rate; gradient = 0% CH_3CN/100% H_2O for 2 CV, ramped from 0% CH_3CN/100% H_2O to 30% CH_3CN/70% H_2O over 18 CV). Combining and lyophilizing pure product fractions from both columns resulted in a white solid (75 mg, approx. 25% yield; see

NMR spectra for relative level of purity). $[\alpha]_D^{20}$ = +104.5 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.25 (d, *J* = 8.3 Hz, 2H), 7.12 (d, *J* = 8.3 Hz, 2H), 5.60 (d, *J* = 1.9 Hz, 1H), 4.22 – 4.11 (m, 2H), 4.05 (dd, *J* = 8.9, 3.4 Hz, 1H), 3.86 – 3.69 (m, 4H), 3.13 (dd, *J* = 13.8, 4.8 Hz, 1H), 2.89 – 2.65 (m, 1H), 1.42 – 1.23 (m, 9H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 175.4, 154.9, 154.7, 132.5, 130.4, 116.2, 99.2, 77.4, 74.8, 70.7, 70.2, 66.7, 61.0, 56.2, 36.7, 28.3 ppm; **IR** (cm^{-1}, neat): 3312, 2934, 1677, 1582, 1509, 1392, 1367, 1226, 1164, 1120, 1049, 1014, 979, 887, 823, 555, 505; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{20}H_{28}NO_{10}$ requires *m/z* 442.1713, found 442.1715.

To assign the stereochemistry of **22** at the anomeric position, 1D NOESY and coupled ^{13}C NMR experiments were performed (Pages 114–115). Strong NOE[11] signals from the anomeric proton to H2 and H8, as well as a $^1J_{CH}$ coupling constant[12] of 173 Hz confirmed the presence of the α-anomer.

6. Syntheses of GLP-1 and Y19F GLP-1

GLP-1 (**23**): HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG-$CONH_2$

Y19F GLP-1 (**25**) : HAEGTFTSDVSSFLEGQAAKEFIAWLVKGRG-$CONH_2$

Peptide Synthesis (23 and 25). Peptides were synthesized on 100 μmol scale using standard solid-phase peptide synthesis. Peptides were synthesized on H-PAL ChemMatrix® resin (to generate products carrying *C*-terminal amides) on an Initiator+ Alstra synthesizer from Biotage (Charlotte, NC) using microwave acceleration. Prior to the first amino acid coupling, the resin was swelled in DMF (4.5 mL) at 70 °C for 20 min. For standard amino acid couplings, DMF (4.5 mL), HBTU (5 equiv.), HOBt (5 equiv.), DIEA (10 equiv.), and Fmoc-protected amino acid (5 equiv.) were subjected to microwave-assisted standard amino acid couplings (75 °C for 5 min). All arginine residues were coupled at 50 °C. In addition, all arginine residues were coupled twice. Fmoc deprotections were performed using 20% piperidine in DMF (4.5 mL) with microwave assistance (70 °C for 3 min, twice). To prevent aspartimide formation, 0.1 M HOBt was added to the deprotection solution. The resin was washed thoroughly with DMF (4 x 4.5 mL) between each coupling and deprotection step. Following synthesis, the resin was transferred to a custom glass reaction vessel containing a stir bar. Both the vessel and the stir bar were coated with SigmaCote before the resin was transferred. Once the peptide synthesis was complete, the resin was washed thoroughly with alternating DMF (10 mL) and DCM (10 mL) three times, washed with MeOH (5 mL), and dried overnight under nitrogen. The peptide was cleaved from the resin using a cocktail (4 mL per 50 μmol of resin) containing TFA (88%),

[11] Cosgrove, K. L., Bernhardt, P. V., Ross, B. P., McGeary, R. P. Determination of the Anomeric Configuration of 2,3,4,6-Tetra-*O*-Acetyl-D-Mannopyranosyl Azide. *Aust. J. Chem.* **2006**, *59*, 473–476.
[12] Bock, K., Pedersen, C. A Study of 13CH Coupling Constants in Hexopyranoses. *J. Chem. Soc., Perkin Trans. 2* **3**, 293–297 (1974).

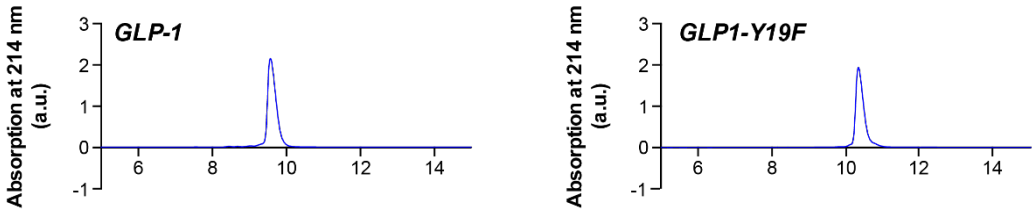
phenol (5%), water (5%), and TIPS (2%) for 2 h at rt. Cleaved peptides were precipitated in diethyl ether (40 mL, chilled to -80 °C), pelleted by centrifugation, dissolved in a mixture of acetonitrile (15%) in water, lyophilized to dryness, and reconstituted in 1–2 mL DMSO prior to purification by HPLC.

Peptide purification (23 and 25). Peptide solutions in DMSO were filtered through nylon syringe filters (0.45um, 4mm from Thermo Fisher, Waltham, MA) prior to HPLC analysis. All peptides were purified using an Agilent 1260 Infinity HPLC system, a reverse phase Triaryl-C18 (YMC-Triaryl-C18, 150 mm x 10 mm, 5 µm, 12 nm) column (YMC America, Inc., Allentown, PA), and eluent gradients of water in acetonitrile containing 0.1% TFA. Peptides were detected at 214 and 280 nm. Peptide purity was verified using a Shimadzu Analytical UPLC system (ES Industries, West Berlin, NJ; Shimadzu Corporation, Kyoto, Japan) and a C8 reverse phase (Sonoma C8(2), 3 µm, 100 Å, 10 cm x 2.1 nm) analytical column. Analytical samples were eluted using eluent gradients of water in acetonitrile containing 0.1% TFA over 20–25 min and were detected at 214 and 280 nm.

UPLC/MS for GLP-1 (**23**): Exact mass calculated for $[C_{151}H_{229}N_{41}O_{46} + H]^+$ requires m/z = 3353.69. Found 3353.44.

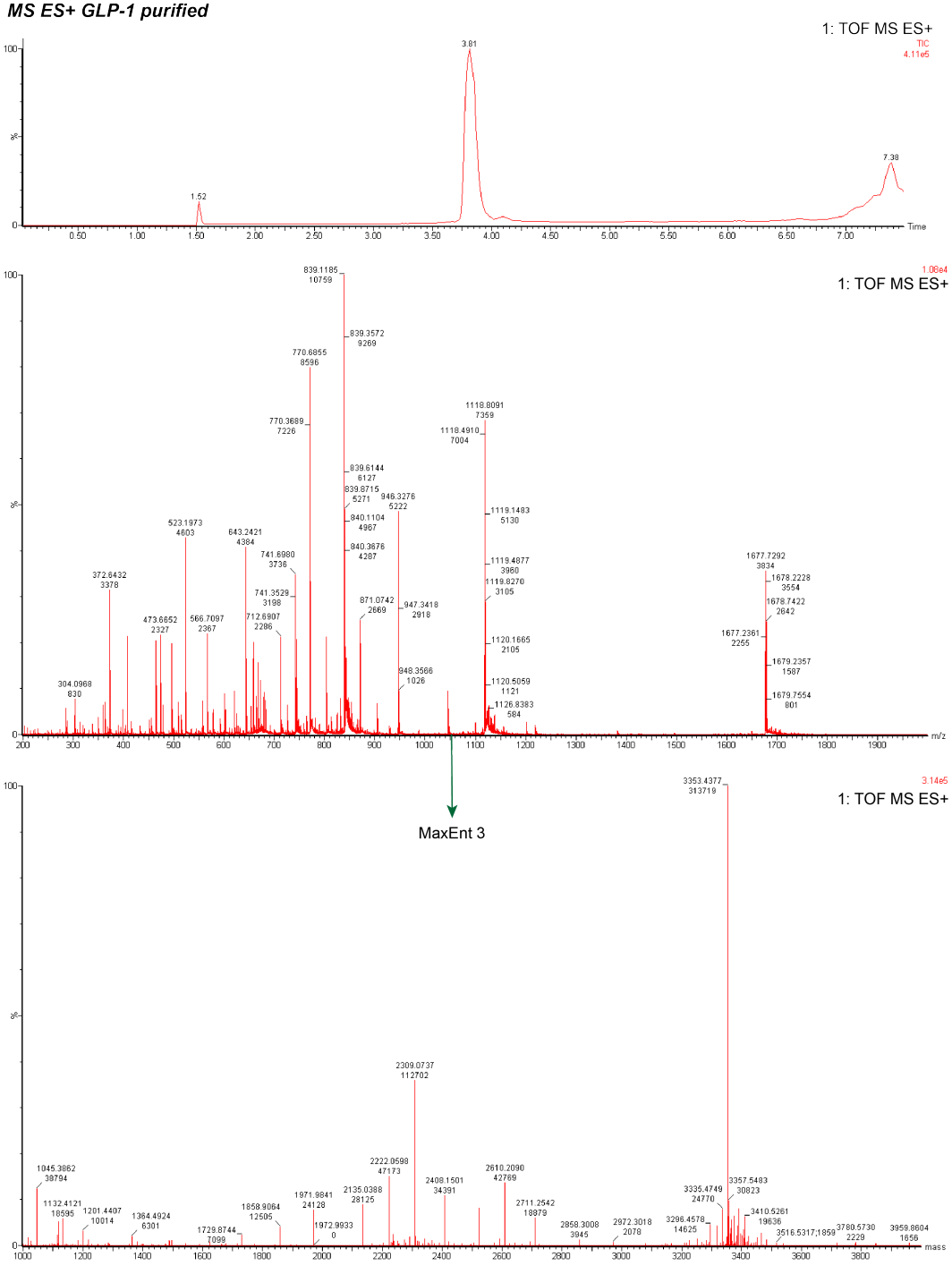
UPLC/MS for Y19F GLP-1 (**25**): Exact mass calculated for $[C_{151}H_{229}N_{41}O_{45} + H]^+$ requires m/z = 3337.70. Found 3337.43.

UPLC Spectra for purified GLP-1 (23) and Y19F GLP-1 (25)



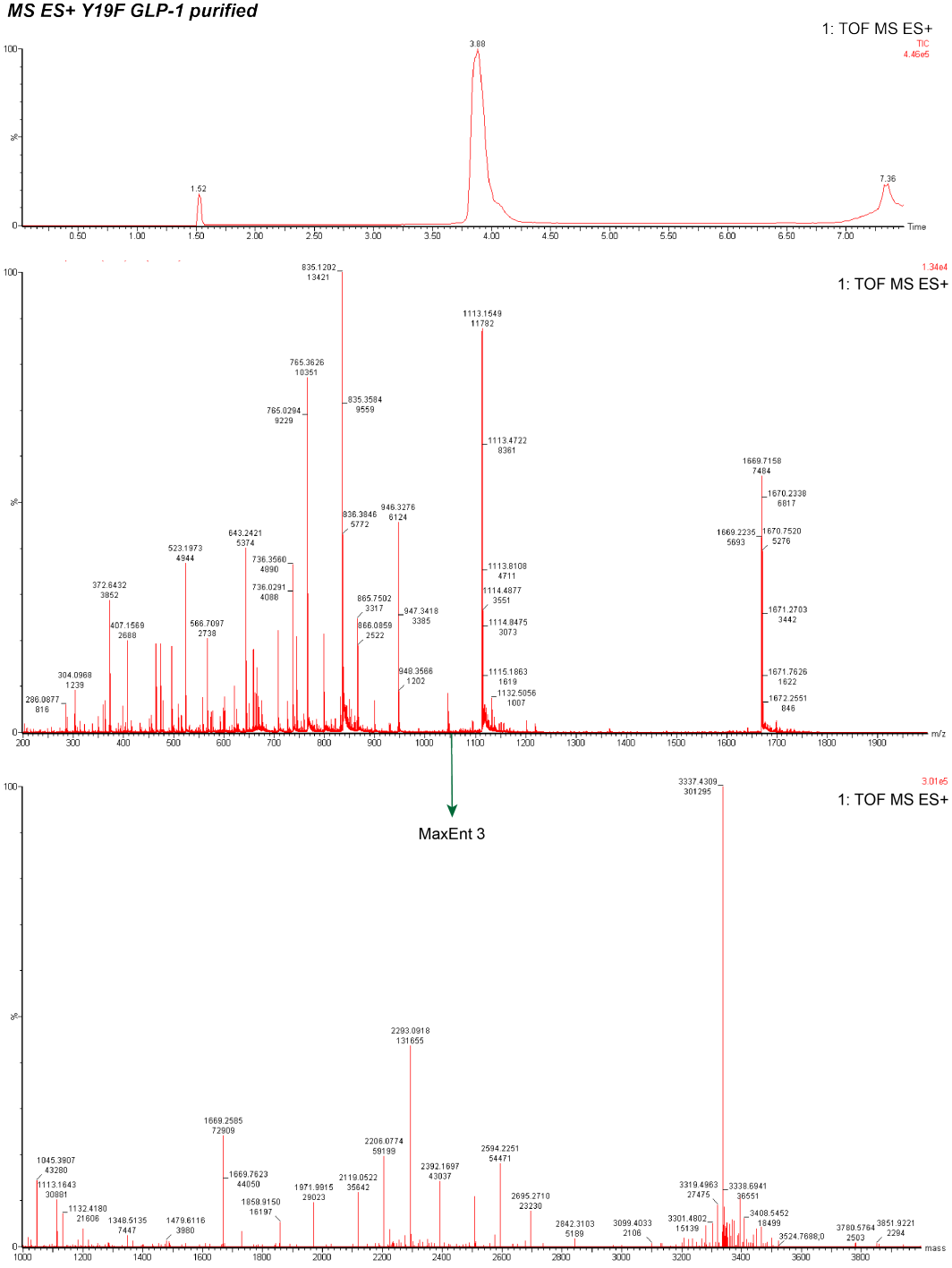
MS ES+ Spectra for purified GLP-1 (23)

MS ES+ GLP-1 purified



MS ES+ Spectra for purified Y19F GLP-1 (25)

MS ES+ Y19F GLP-1 purified



7. Glycosylation of GLP-1 and Y19F GLP-1

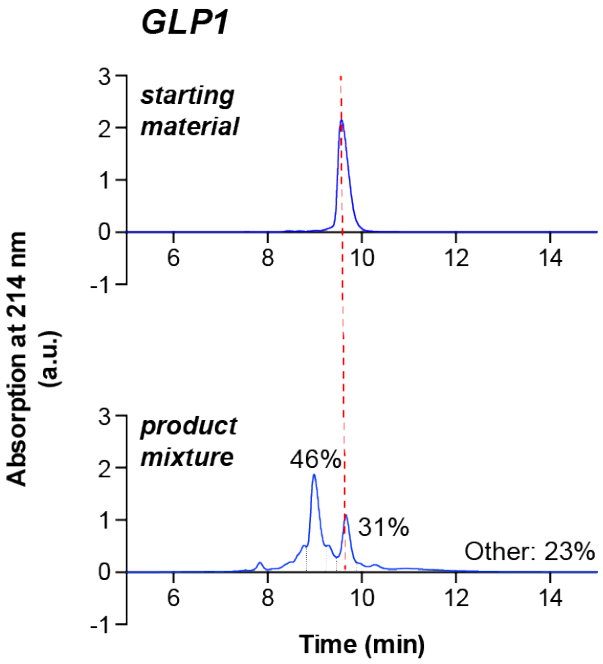
7.1 Reaction Procedure

***O*-Tyr-glucopyranosyl-GLP-1 (24) and glucopyranosyl-Y19F GLP-1**. A 1-dram vial containing GLP-1 or Y19F GLP-1 (**23** or **25**; 1.0 mg, 0.30 μmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 55 mg, 0.30 mmol, 1000 equiv.), $Ca(OH)_2$ (22 mg, 0.30 mmol, 1000 equiv.), and a magnetic stir bar was charged with H_2O (to reach 1.0 mM in substrate, 1.0 M in reagents, 300 μL). The vial was sealed with a cap, and the reaction mixture was stirred vigorously at rt for 10 min. The suspension was then quenched with EDTA solution (0.5 M, pH = 8.0, 900 μL, 1500 equiv.), and a 0.9 mL aliquot of the resulting solution was transferred to a SpinOUT™ column (GT-100) to remove salts and insoluble particles. The SpinOUT™ column manufacturer's protocol was followed, and the peptides were buffer-exchanged into water. That solution was frozen, lyophilized to dryness, and re-dissolved in water (500 μL) to enable UPLC and LCMS analysis.

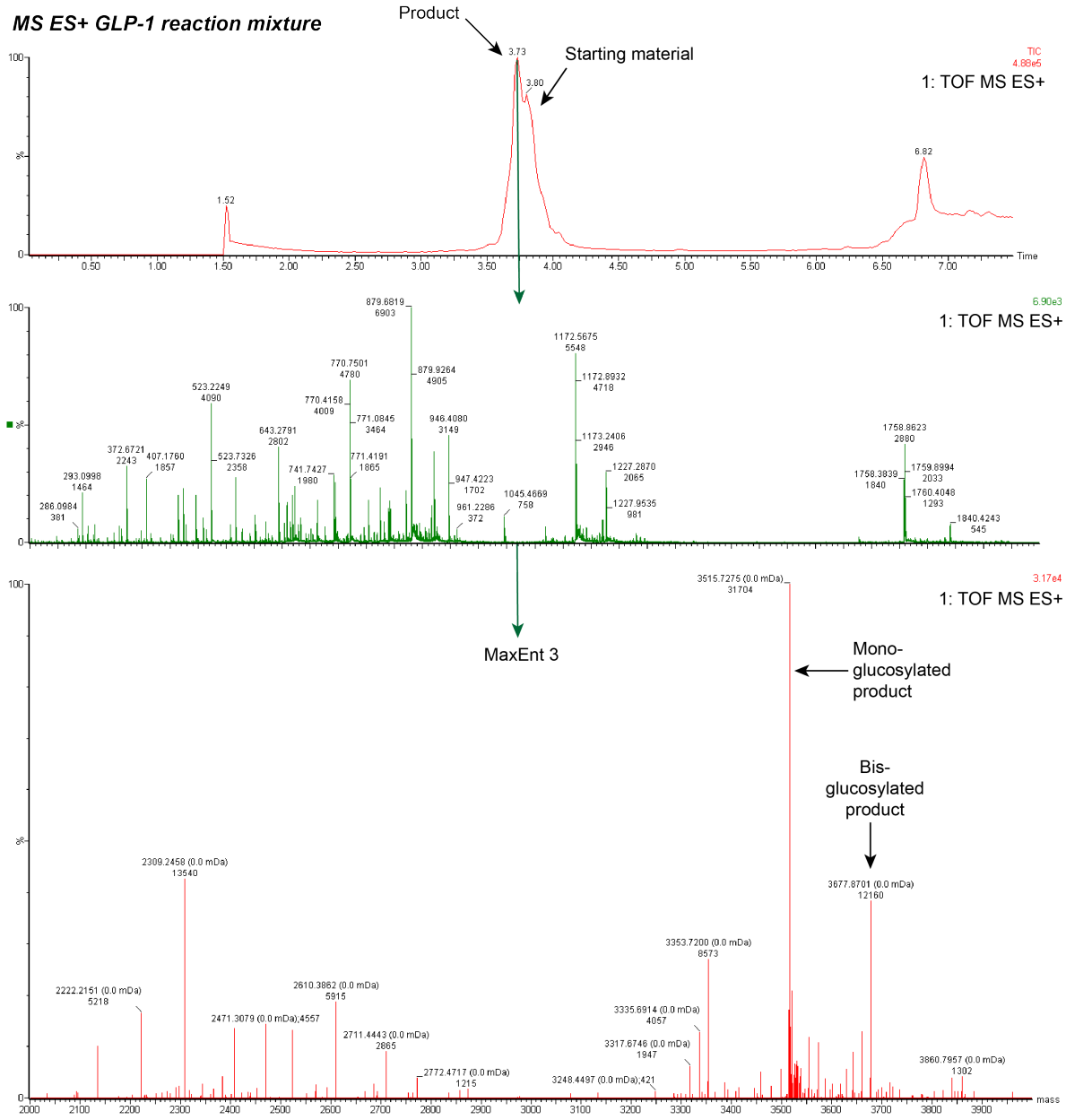
HPLC yields were calculated by integrating the peak corresponding to the monosubstituted peptide at 214 nm.

UPLC/MS for *O*-Tyr-glucopyranosyl-GLP-1 (**24**): Exact mass calculated for $[C_{157}H_{239}N_{41}O_{51} + H]^+$ requires m/z = 3515.74. Found 3515.73.

UPLC spectra of product mixture from glycosylation of GLP-1 (23)

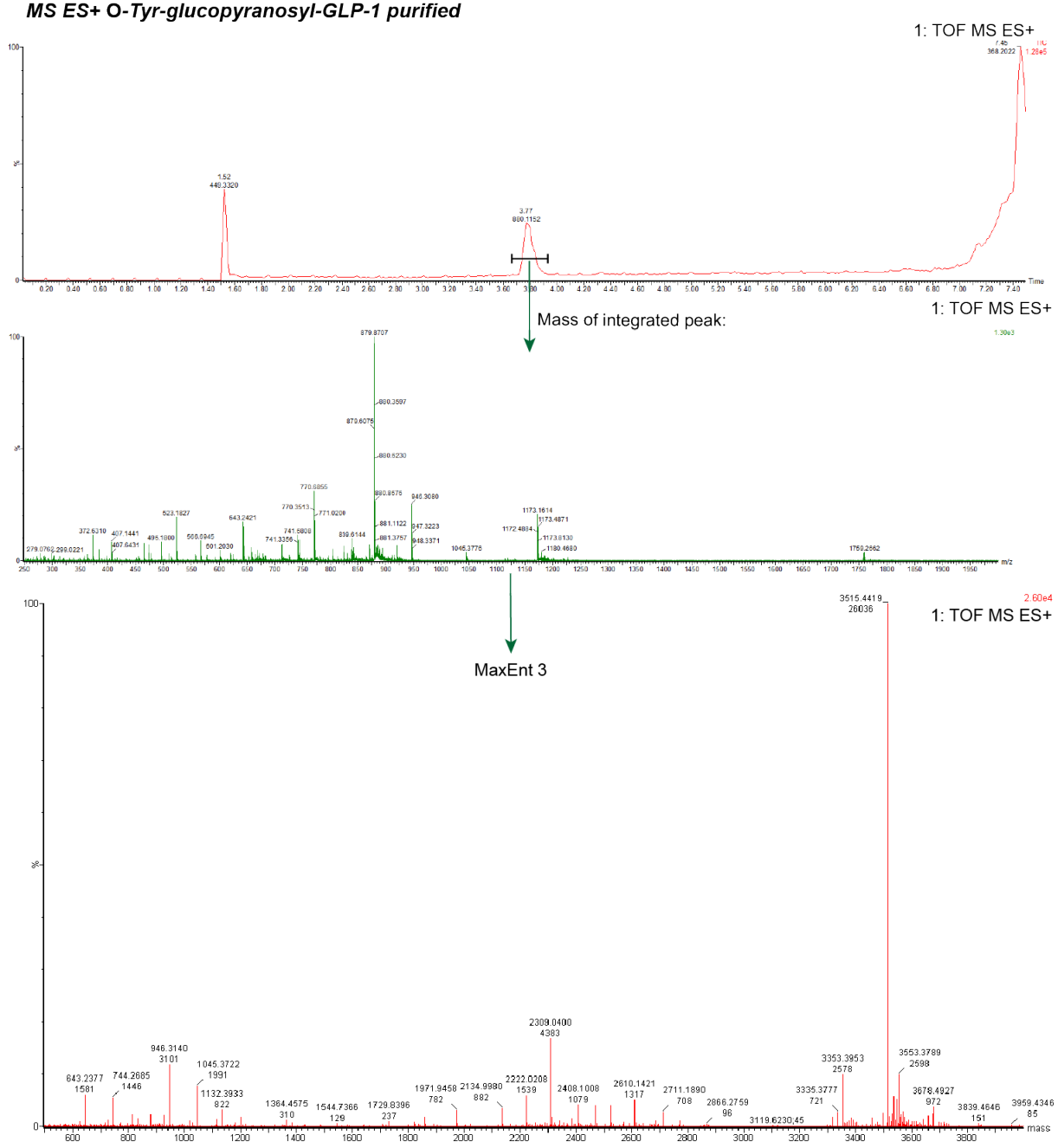


MS ES+ Spectra for GLP-1 glycosylation reaction mixture

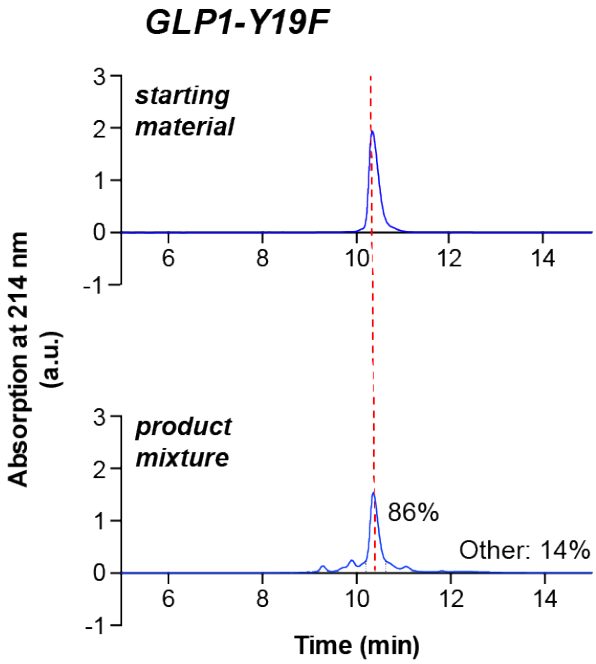


MS ES+ Spectra for purified *O*-Tyr-glucopyranosyl-GLP-1 (24)

MS ES+* O-*Tyr-glucopyranosyl-GLP-1 purified



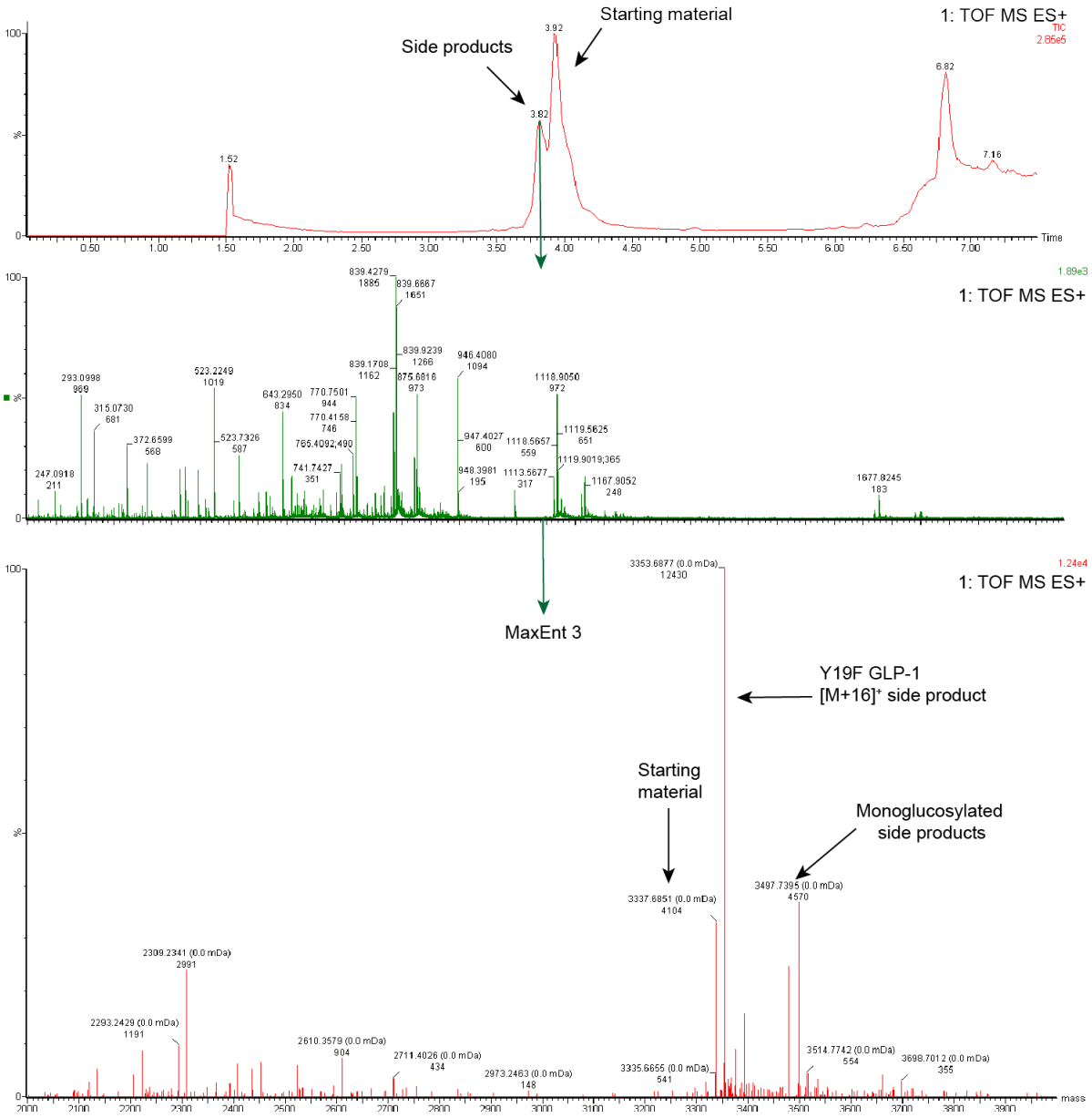
UPLC spectra of product mixture from glycosylation of Y19F GLP-1 (25)



Note: "Other 14%" includes peak shoulders and was identified as mono-glucosyl product(s) and $[M + 16]^+$ side product by LCMS.

MS ES+ Spectra for Y19F GLP-1 glycosylation reaction mixture

MS ES+ Y19F GLP-1 reaction mixture



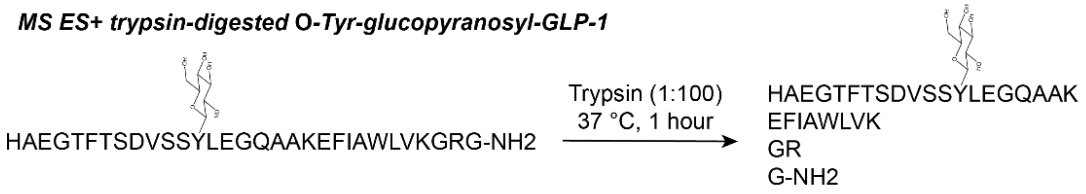
7.2 LC-MS/MS Characterization

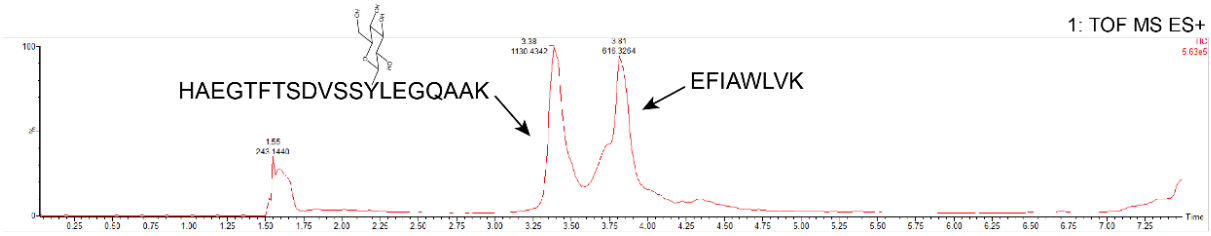
Trypsin digestion: *O*-Tyr-glucopyranosyl GLP-1 (**24**; purified by HPLC and lyophilized to dryness) was dissolved in 100 mM Tris-HCl, pH 8.5 for trypsinization (1:100 trypsin to protein weight ratio) at 37 °C for 1 hour. Trypsinized peptides were concentrated, filtered with a C18 ZipTip (Millipore), and analyzed by LC-MS to ensure complete trypsinization.

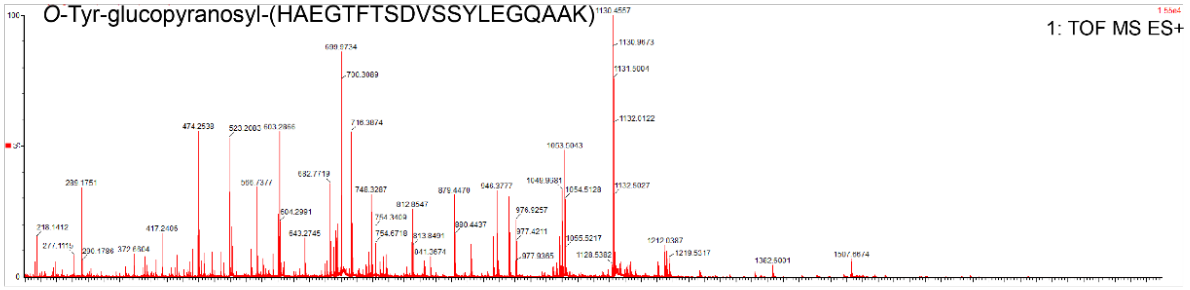
UPLC/MS for the two major trypsin fragments of *O*-Tyr-glucopyranosyl-GLP-1 (*O*-Tyr-glucopyranosyl-HAEGTFTSDVSSYLEGQAAK $[M + 2H]^{2+}$ and EFIAWLVK $[M + H]^{+}$): Exact masses require m/z = 1130.52 and m/z = 1005.58. Found 1130.46 and 1005.51.

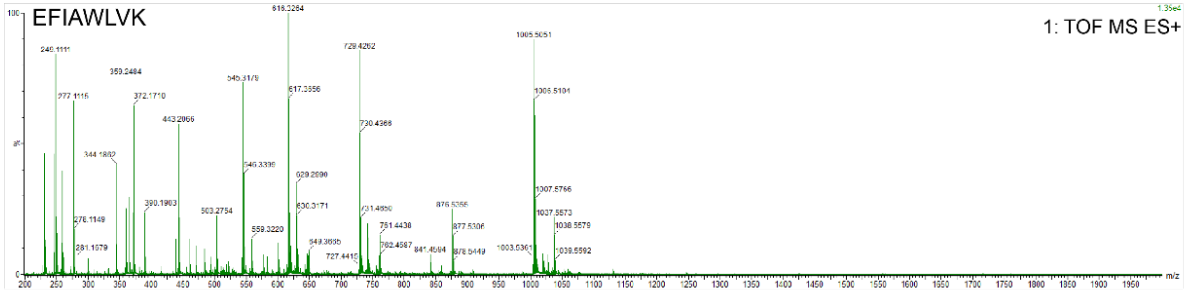
MS/MS procedure: After desalting with a ZipTip, the eluted sample was dried using a SpeedVac then dissolved in MS loading buffer (0.2% Trifluoroacetic Acid, 2% acetonitrile in water). An estimated 125 ng was injected for LC-MS/MS analysis on a Thermo Scientific Q Exactive Plus mass spectrometer equipped with a Waters nanoAcquity UPLC system utilizing a binary solvent system (Buffer A: 100% water, 0.1% formic acid; Buffer B: 100% acetonitrile, 0.1% formic acid). Trapping was performed at 5µl/min, 97% Buffer A for 3 min using a Waters Symmetry® C18 180µm x 20mm trap column. Peptides were separated using an ACQUITY UPLC PST (BEH) C18 nanoACQUITY Column 1.7 µm, 75 µm x 250 mm (37ºC) and eluted at 300 nl/min with the following gradient: 3% Buffer B at initial conditions; 10% B at 1 minute; 35% B at 38 minutes; 90% B at 43-48 min; return to initial conditions at 50 minutes. MS was acquired in profile mode over the 300-1,700 m/z range using 1 microscan, 70,000 resolution, AGC target of 3E6, and a full max ion time of 100 ms. Data dependent MS/MS were acquired in centroid mode using 1 microscan, 17,500 resolution, AGC target of 1E5, full max IT of 100 ms, 1.7 m/z isolation window, and a normalized collision energy of 28. Up to 20 MS/MS were collected per MS scan on species with an intensity threshold of 1E4, charge states 1-5, peptide match off, and dynamic exclusion set to 15 seconds.

MS ES+ trypsin-digested O-Tyr-glucopyranosyl-GLP-1

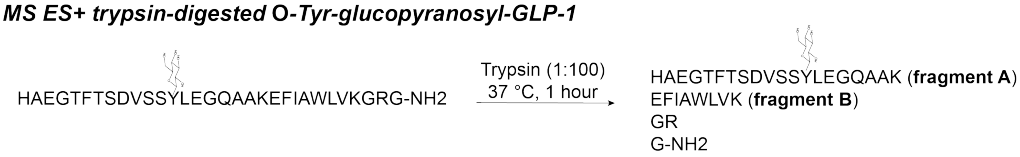


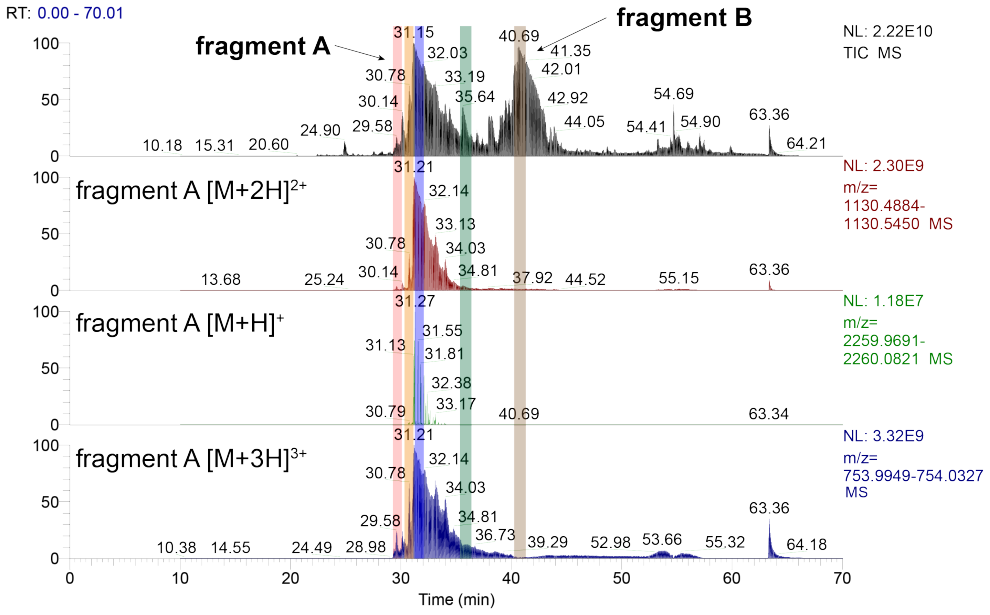


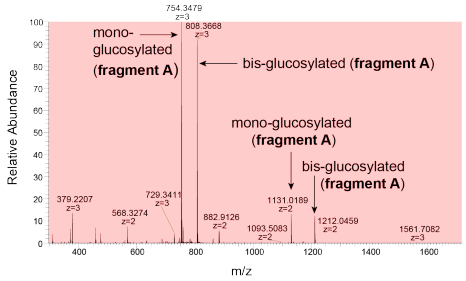


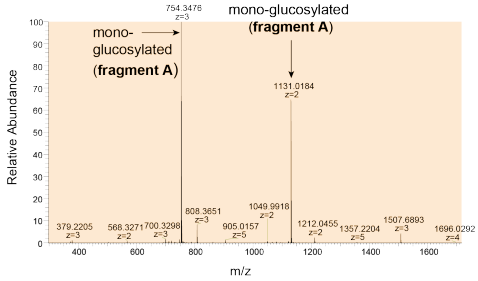


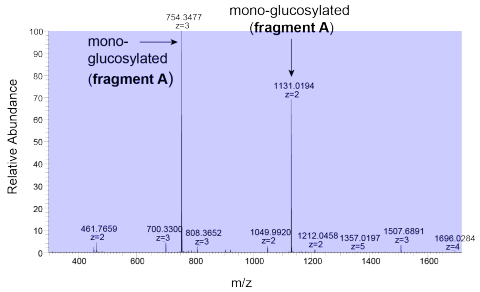
MS ES+ trypsin-digested O-Tyr-glucopyranosyl-GLP-1

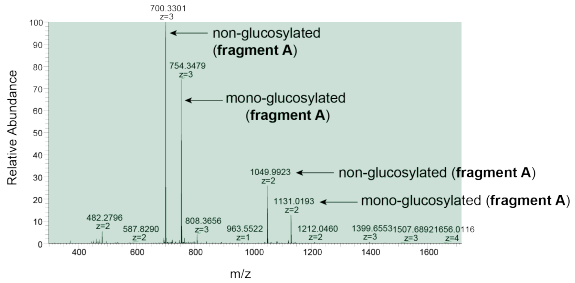


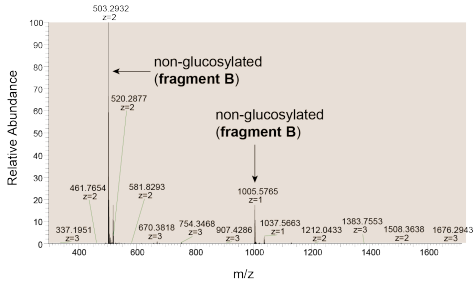




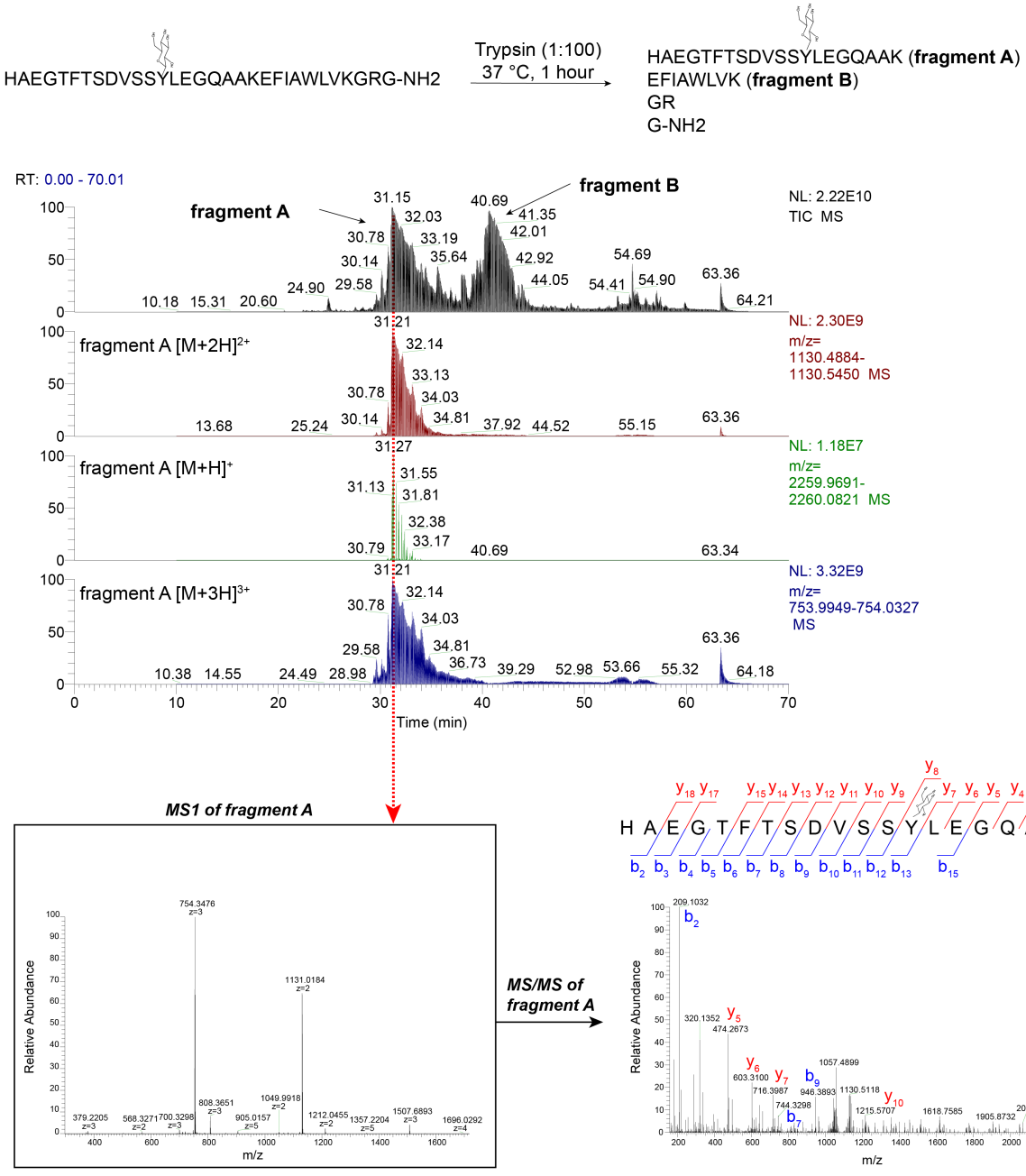


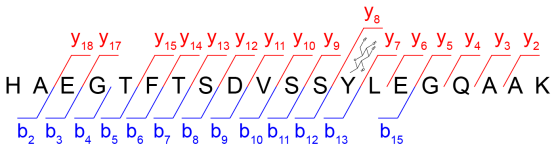


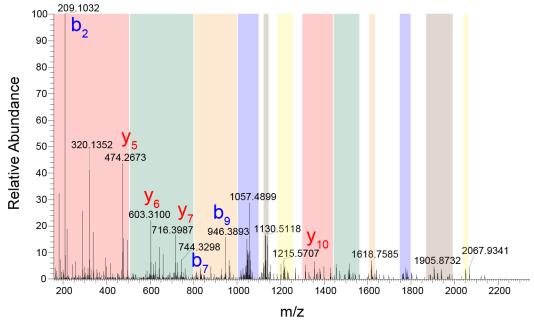


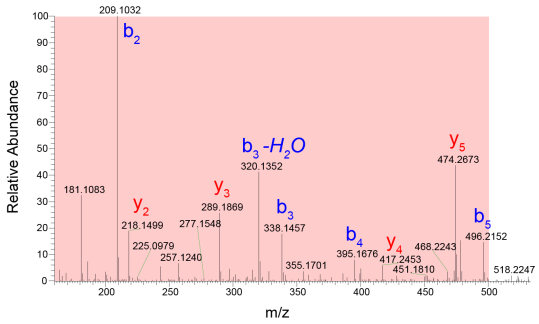


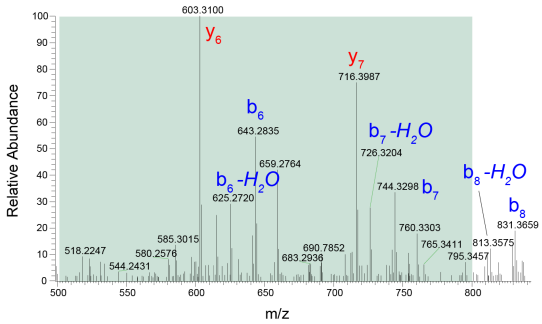
MS ES+ trypsin-digested O-Tyr-glucopyranosyl-GLP-1

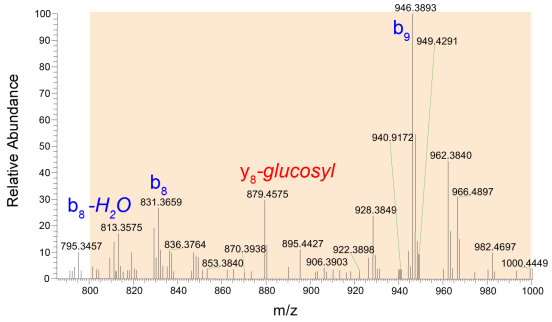


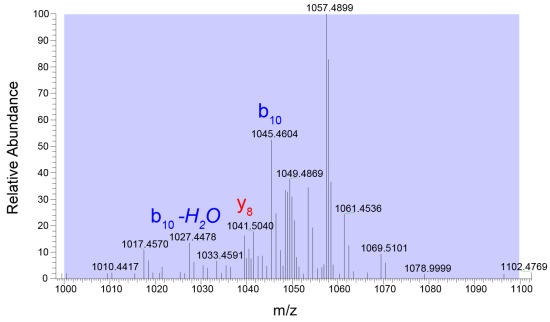


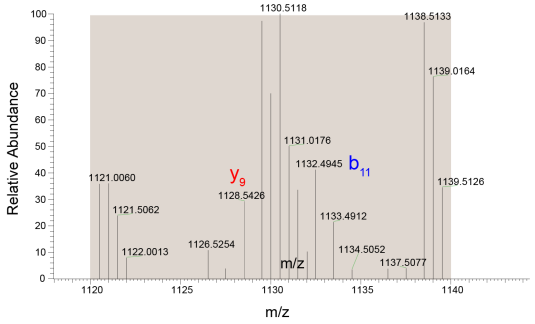


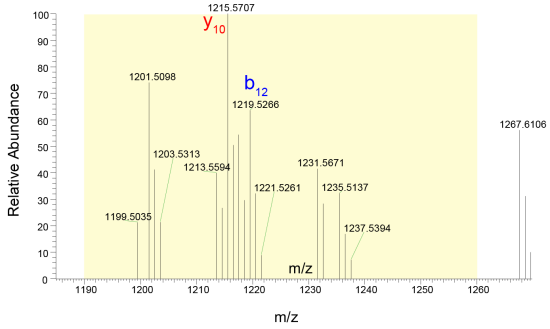




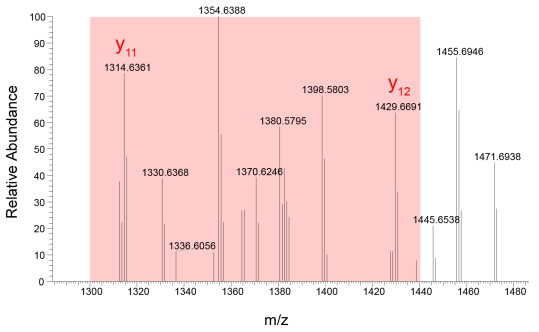








(Figure continues on next page)


Relative Abundance
m/z
y11
1314.6361
1354.6388
y12
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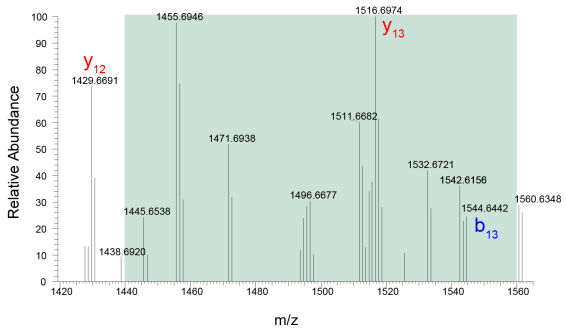
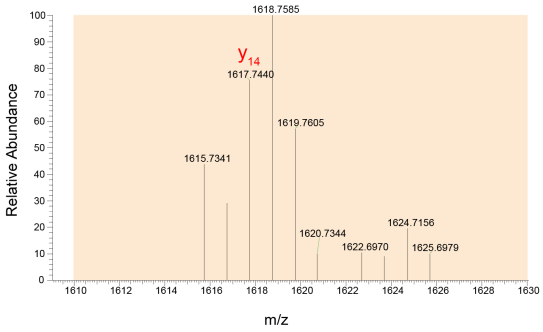
1398.5803
1380.5795
1455.6946
1471.6938
1330.6368
1370.6246
1445.6538
1336.6056


Relative Abundance
m/z
y12
1429.6691
1455.6946
1516.6974
y13
1511.6682
1471.6938
1532.6721
1542.6156
1496.6677
1560.6348
1445.6538
1544.6442
b13
1438.6920


Relative Abundance
m/z
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1618.7585
1619.7605
1615.7341
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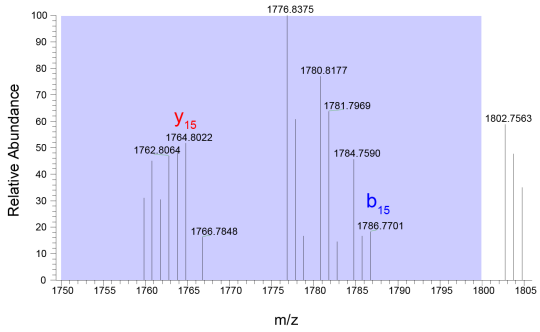
1620.7344
1622.6970
1625.6979


Relative Abundance
m/z
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1780.8177
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y15
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1802.7563
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b15
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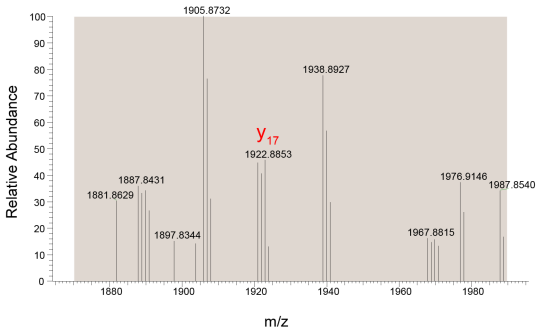
1766.7848


Relative Abundance
m/z
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1938.8927
y17
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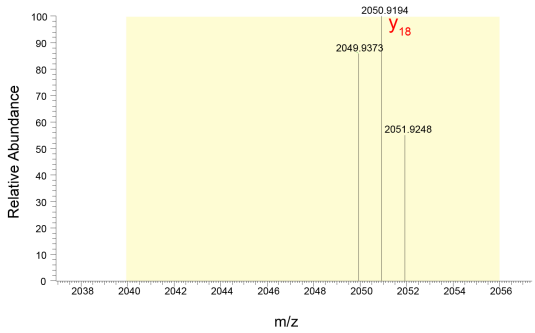
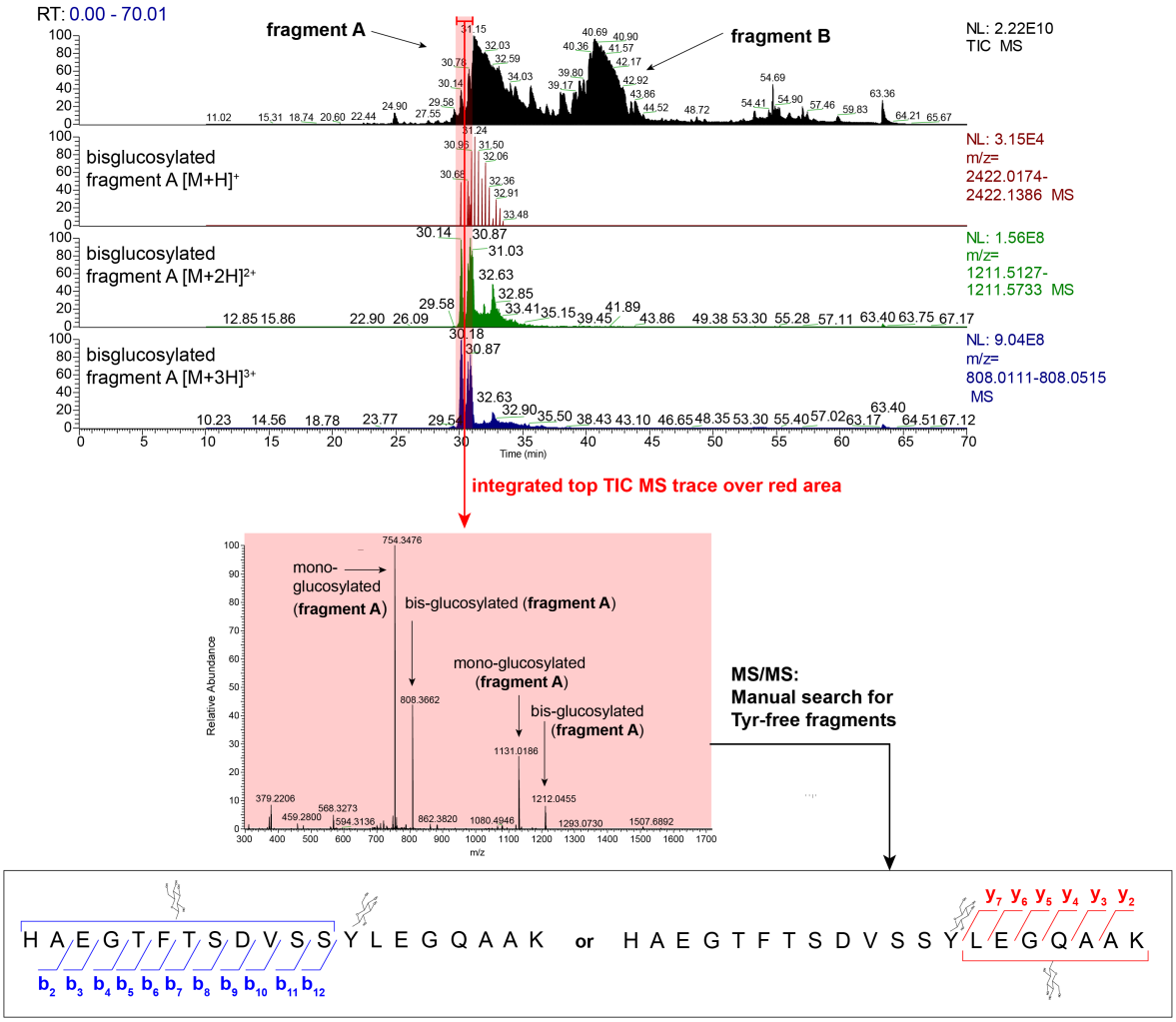
1887.8431
1881.8629
1976.9146
1987.8540
1897.8344
1967.8815


Relative Abundance
m/z
2050.9194
y18
2049.9373
2051.9248

MS ES+ trypsin-digested O-Tyr-glucopyranosyl-GLP-1: Off-target glycosylation



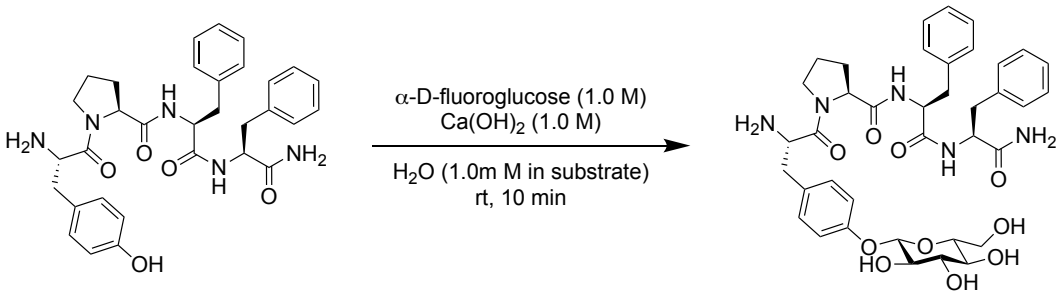
Ion	Expected MH^{+1} (mono)	Found MH^{+1} (mono)	Ion	Expected MH^{+1} (mono)	Found MH^{+1} (mono)
b2	371.1561	371.1571	y2	309.1656	not found
b3	500.1987	500.1996	y3	380.2027	not found
b4	557.2202	not found	y4	451.2399	not found
b5	658.2679	658.2676	y5	579.2984	not found
b6	805.3363	805.3373	y6	765.3625	not found
b7	906.3840	906.3852	y7	878.4466	not found
b8	993.4160	993.4196			
b9	1108.4429	1108.4464			
b10	1207.5113	1207.5127			
b11	1294.5434	1294.5470			
b12	1381.5754	1381.5753			

8. Exploration of Peptide Glycosylation Scope

8.1. General Procedure 3

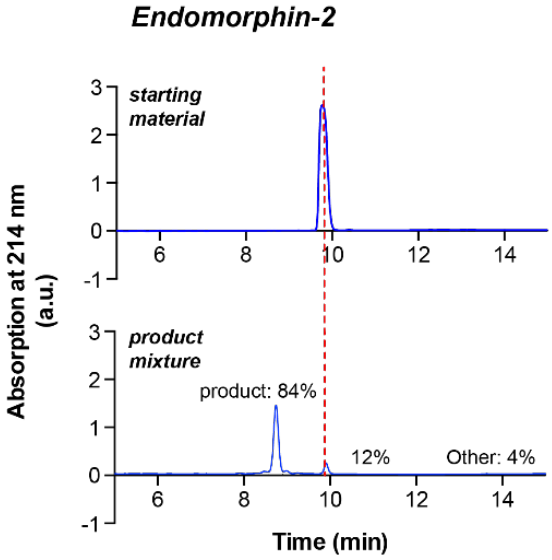
A 1-dram vial containing peptide (0.5 mg or 1 mg, 1.0 equiv.), α-D-fluoroglucose (**II**; 1000 equiv.), $Ca(OH)_2$ (1000 equiv.), and a magnetic stir bar was charged with H_2O (1.0 mM in substrate, 1.0 M in reagents). The vial was sealed with a cap, and the reaction mixture was stirred vigorously at rt for 10 min. The suspension was then quenched with 0.5 M EDTA solution, pH 8.0 (1500 equiv.), and a 0.9 mL aliquot of the resulting solution was transferred to a SpinOUT™ column (GT-100) to remove salts and insoluble particles. The SpinOUT™ column manufacturer's protocol was followed, and the product mixture was buffer-exchanged into water. The resulting solution was frozen, lyophilized to dryness, and re-dissolved in water (500 μL) to enable UPLC and LCMS analysis. UPLC yields were calculated using a C8 reversed-phase (Sonoma C8(2), 3 μm, 100 Å, 10 cm x 2.1 nm) analytical column. Analytical samples were eluted using eluent gradients of water in acetonitrile containing 0.1% TFA over 20–25 min and were detected at 214 and 280 nm.

8.2. Syntheses and Characterization of Peptide Glycosylation Products

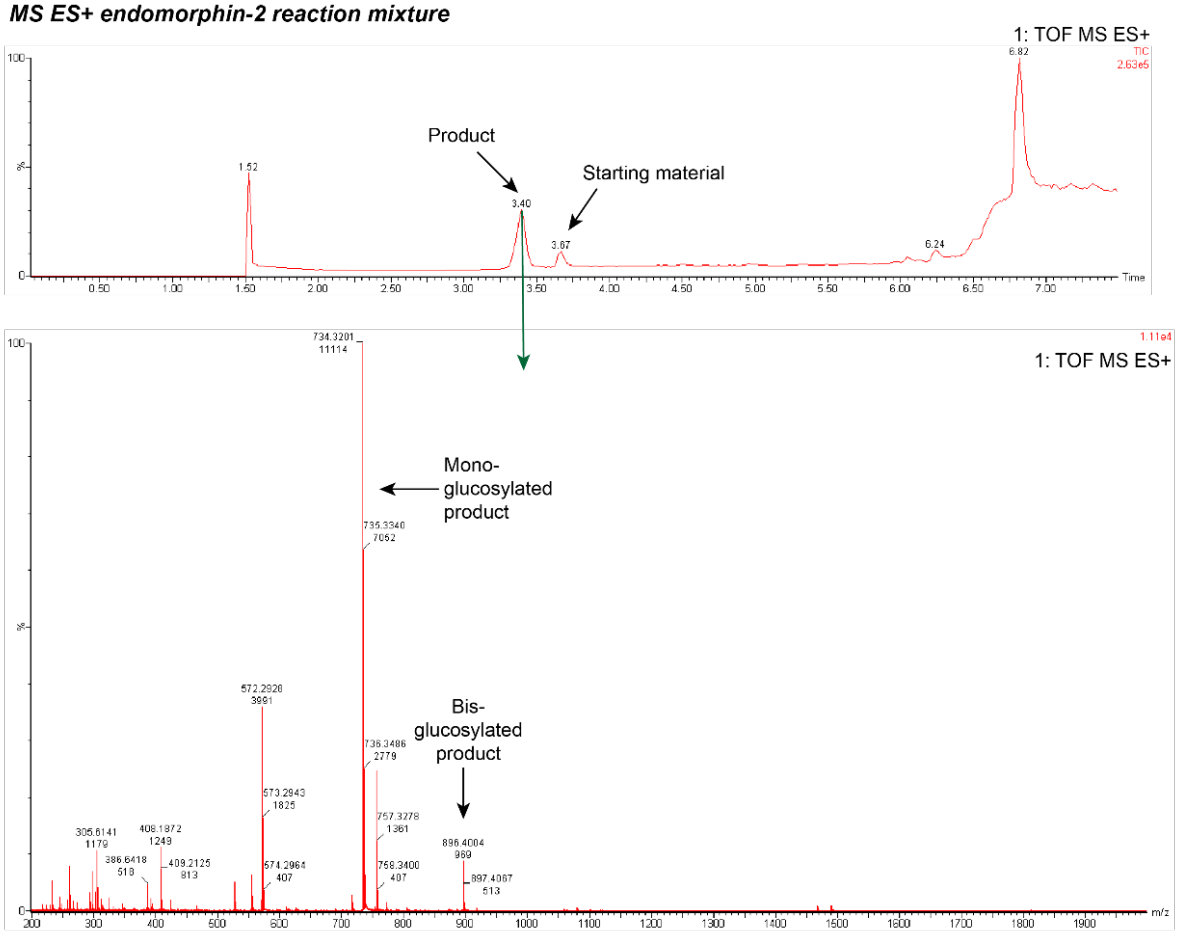


endomorphin-2 **26**

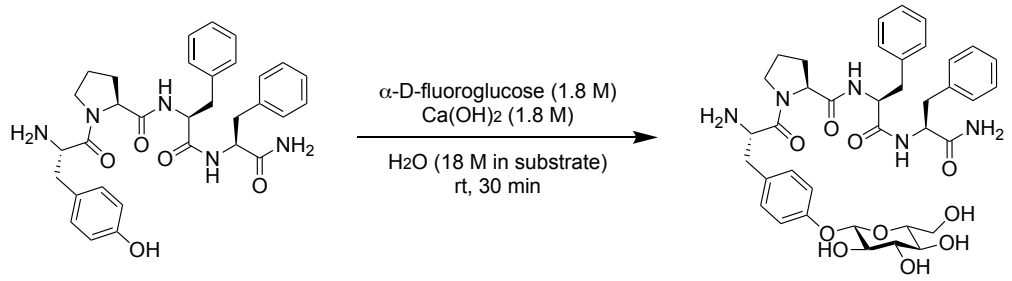
***O*-Tyr-glucopyranosyl endomorphin-2 (26).** The product was synthesized from endomorphin-2 (0.5 mg, 0.87 µmol, 1.0 equiv.) using General Procedure 3. UPLC analysis indicated 84% product present in the reaction mixture. **UPLC/MS** for *O*-Tyr-glucopyranosyl endomorphin-2: Exact mass calculated for $[C_{38}H_{47}N_5O_{10} + H]^+$ requires m/z = 734.34. Found 734.32.



MS ES+ endomorphin-2 reaction mixture



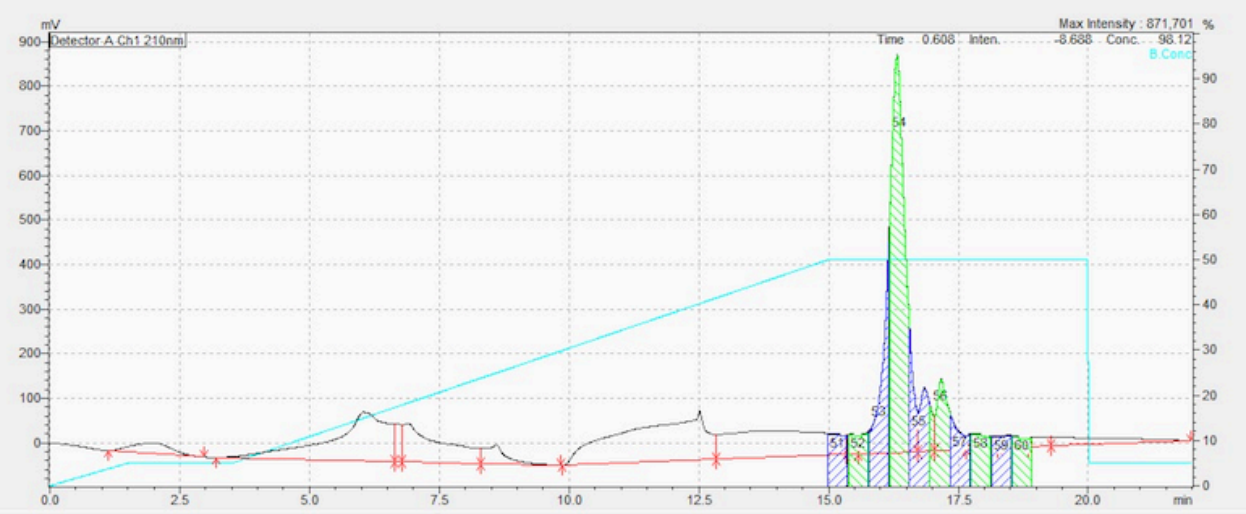
Characterization of Endomorphin-2 Glycosylation Product



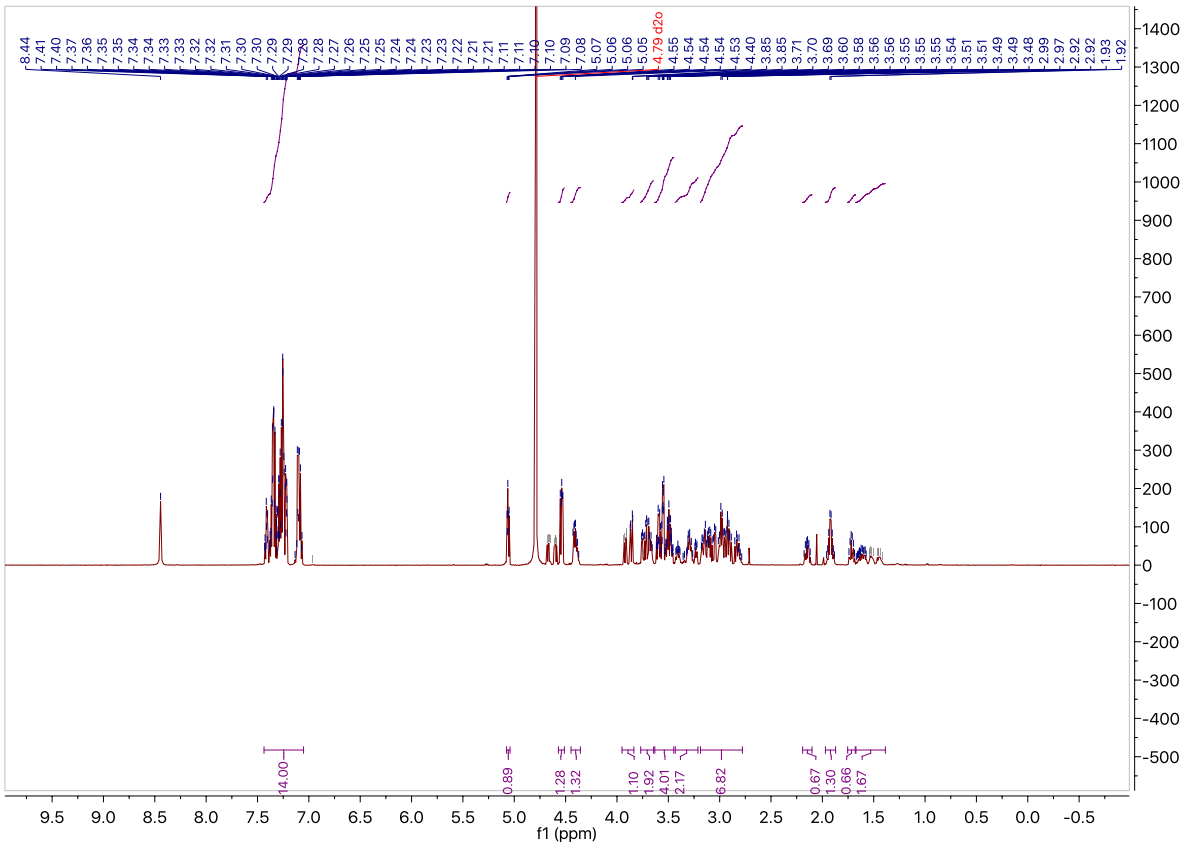
endomorphin-2 **26**

***O*-Tyr-glucopyranosyl endomorphin-2 (26).** A 1-dram vial containing endomorphin 2 (10.0 mg, 0.0175 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 319.0 mg, 1.75 mmol, 100.0 equiv.), $Ca(OH)_2$ (130.0 mg, 1.75 mmol, 100.0 equiv.), and a magnetic stir bar was charged with H_2O (970 μL, 0.018 M in substrate). The vial was sealed with a cap, and the mixture was stirred vigorously at rt for 30 min. The resulting bright yellow mixture was filtered over celite. The pad was eluted with H_2O (15 mL). The collected filtrate was transferred into 15 HPLC vials (~1 mL per vial) for purification via reverse phase prep HPLC (Symmetry prep C8 7μm 7.8 19 X 300 mm column. Injection volume = 1000 μL per run. Solvent A = 0.1% formic acid in water, solvent B = 0.1% formic acid in acetonitrile, flow = 10 mL/min, λ = 210 nm; started at 5% B and held for 3.5 min, ramped to 50% B over 11.5 min, held at 50% B for 5 min, product at 16 min). Fractions containing the product were frozen and lyophilized to dryness, resulting in a white solid (**26**, 5.1 mg, 40% yield); **^{1}H NMR** (600 MHz, D_2O): δ 7.44–7.05 (m, 14H), 5.07-5.04 (m, 1H), 4.54 (dd, *J* = 8.6, 6.4 Hz, 1H), 4.41 (td, *J* = 10.9, 9.7, 6.5 Hz, 1H), 3.86 (dd, *J* = 12.5, 2.2 Hz, 1H), 3.71 (tdd, *J* = 20.1, 11.6, 6.4 Hz, 2H), 3.63–3.45 (m, 4H), 3.43–3.21 (m, 2H), 3.19–2.78 (m, 7H), 2.15 (dq, *J* = 13.0, 7.4 Hz, 1H), 1.97–1.87 (m, *J* = 6.4 Hz, 1H), 1.71 (dq, *J* = 12.9, 6.6 Hz, 1H), 1.67–1.39 (m, 2H); **^{13}C NMR** (151 MHz, D_2O): δ 175.09, 174.74 (d, *J* = 3 Hz), 172.26, 170.92, 156.01, 136.41, 136.31, 136.28, 135.85, 131.04, 130.44, 129.10, 129.08, 129.01, 128.80, 128.72, 128.59, 128.53, 127.25, 126.97, 126.92, 117.00, 116.77, 100.28, 100.01, 76.05, 75.98, 75.41, 75.39, 72.76, 69.29, 69.21, 60.43, 60.35, 60.21, 59.42, 55.10, 54.27, 54.24, 52.97, 47.70, 37.07, 36.96, 31.45, 28.94, 24.50, 21.67; **IR** (cm^{-1}, neat): 1646, 1590, 1511, 1429, 1348, 1231, 1071; **UPLC/MS**: Exact mass calculated for $[C_{38}H_{47}N_5O_{10} + H]^+$ requires m/z = 734.34. Found 734.15; $[\alpha]_D^{20}$ = –62.0 (*c* 0.001, MeOH).

Purification of **26** via reverse phase prep HPLC (product at 16 min)

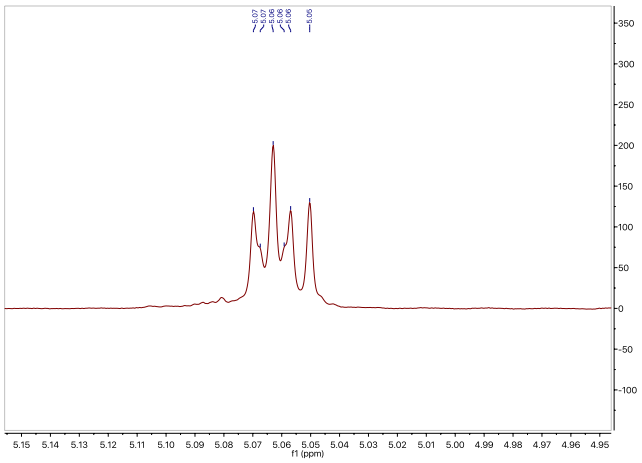


^{1}H NMR spectrum of **26** (600 MHz, D_2O, 25 °C)



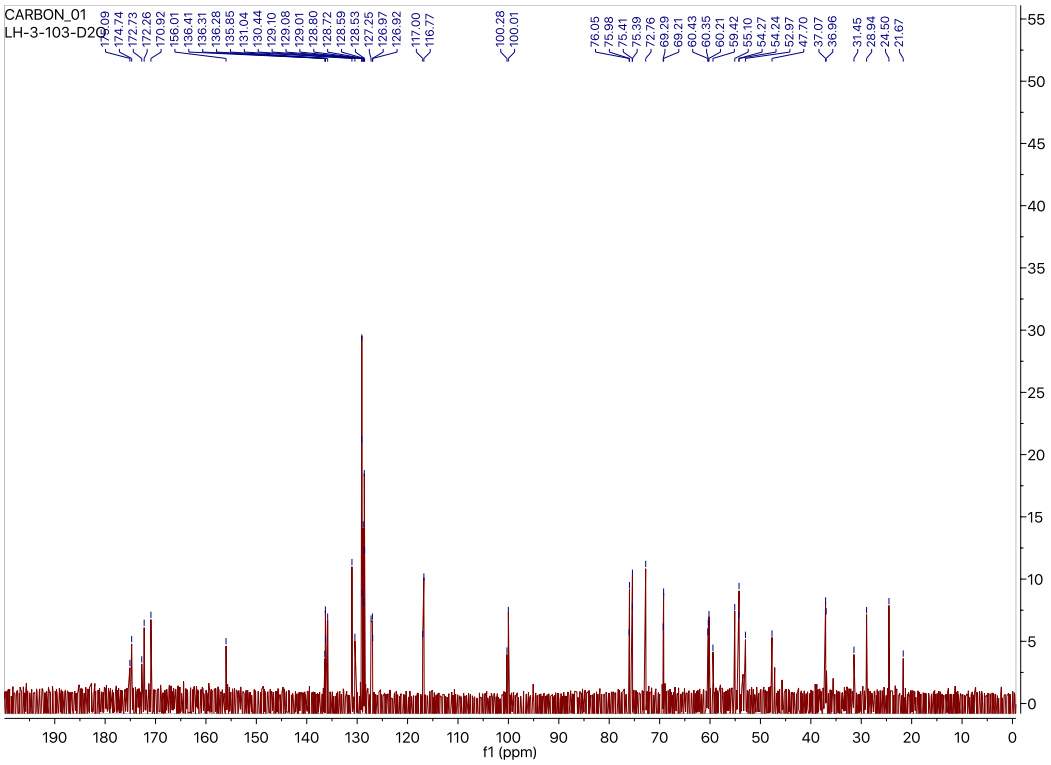
Note: peak at 8.44 ppm correspond to the proton of formate ($HCOO^-$).

^{1}H NMR spectrum of **26**
(600 MHz, D_2O, 25 °C, δ 5.07–5.04 ppm)

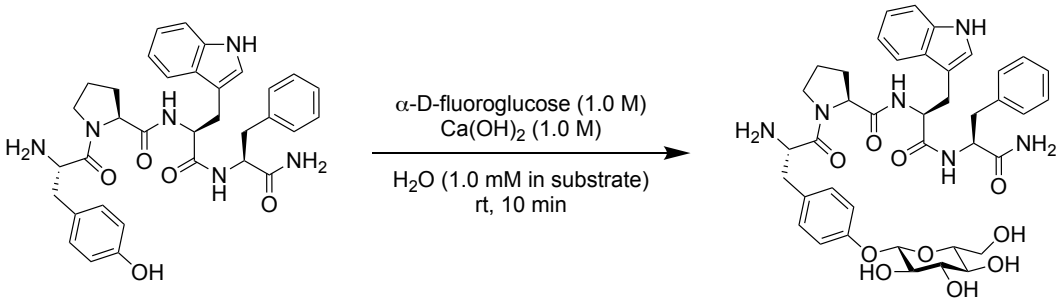


Note: the anomeric proton is splitted into a multiplet due the virtual long-range coupling. When the chemical shifts of the H-2 and H-3 axial protons are within 0.05 ppm, extra splitting may be observed for the anomeric beta H-1 proton.[13]

^{13}C NMR spectrum of **26** (151 MHz, D_2O, 25 °C)

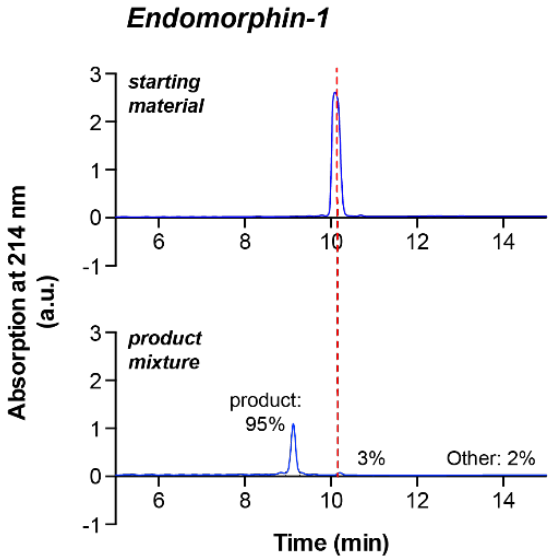


[13] Dahmén, J.; Frejd, T.; Gronberg, G.; Magnusson, G.; Noori, G. *Carbohydr. Res*. **1984**, *125*, 161–164.

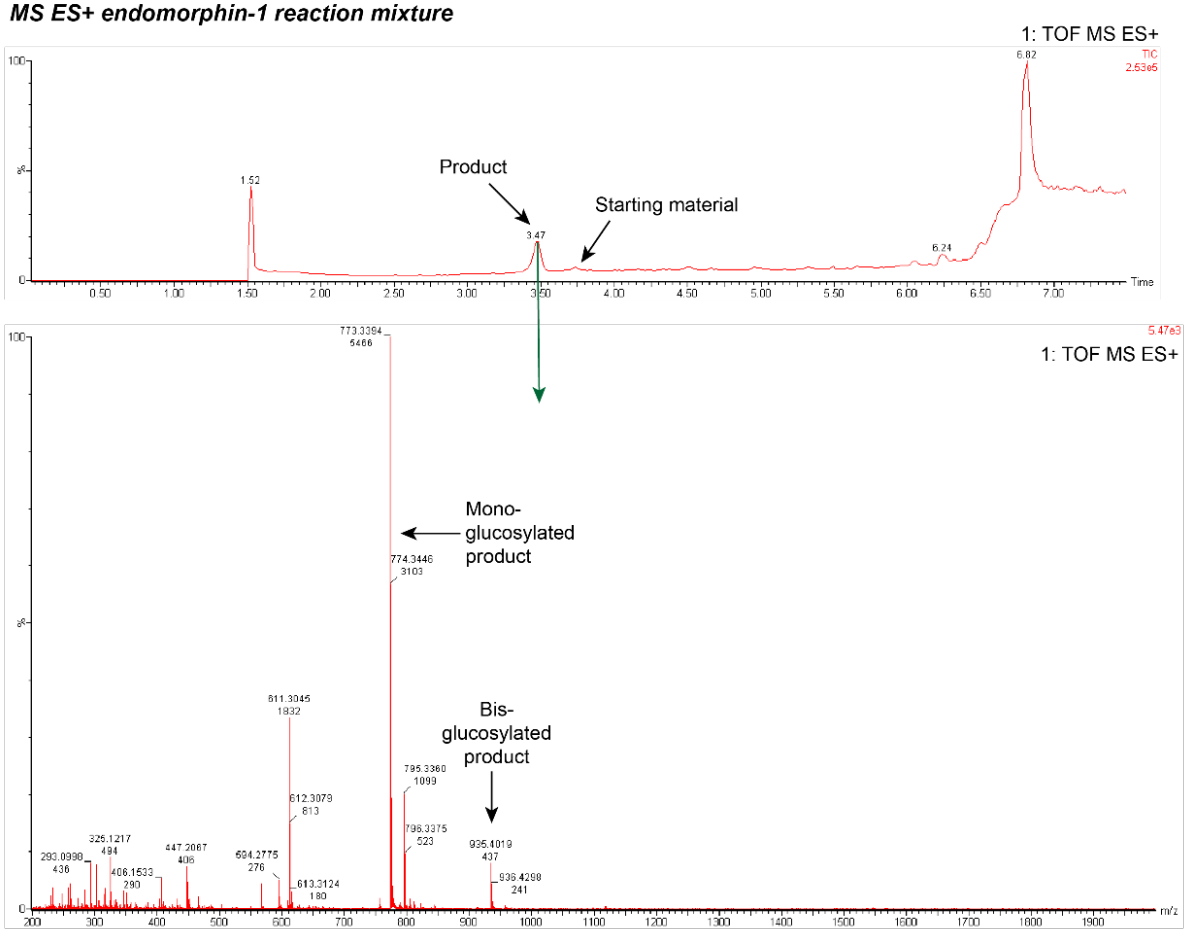


endomorphin-1 **27**

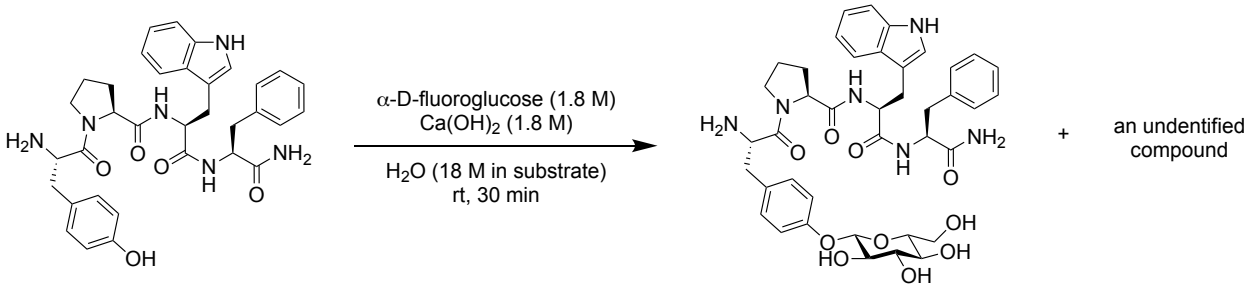
***O*-Tyr-glucopyranosyl endomorphin-1 (27).** The product was synthesized from endomorphin-1 (0.5 mg, 0.82 μmol, 1.0 equiv.) using General Procedure 3. UPLC analysis indicated 95% product present in the reaction mixture. **UPLC/MS** for *O*-Tyr-glucopyranosyl endomorphin-1: Exact mass calculated for $[C_{40}H_{48}N_6O_{10} + H]^+$ requires m/z = 773.35. Found 773.34.



MS ES+ endomorphin-1 reaction mixture



Characterization of Endomorphin-1 Glycosylation Product

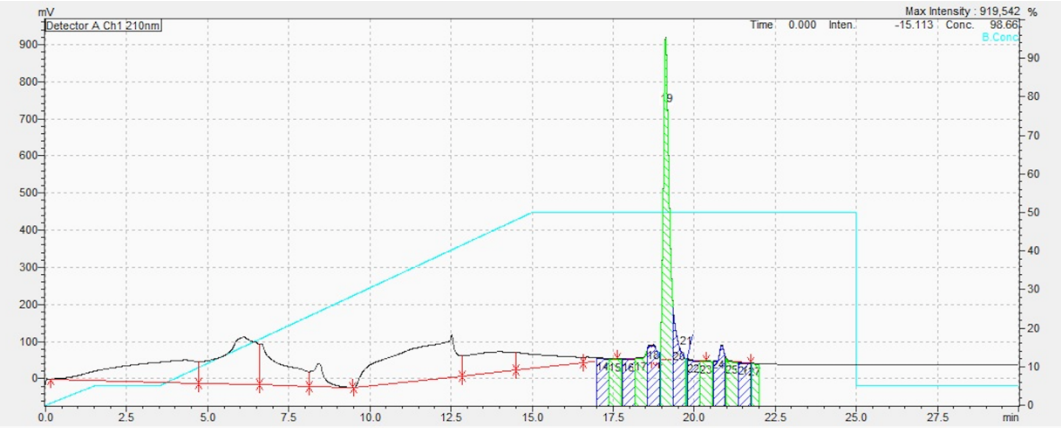


endomorphin-1 **27**

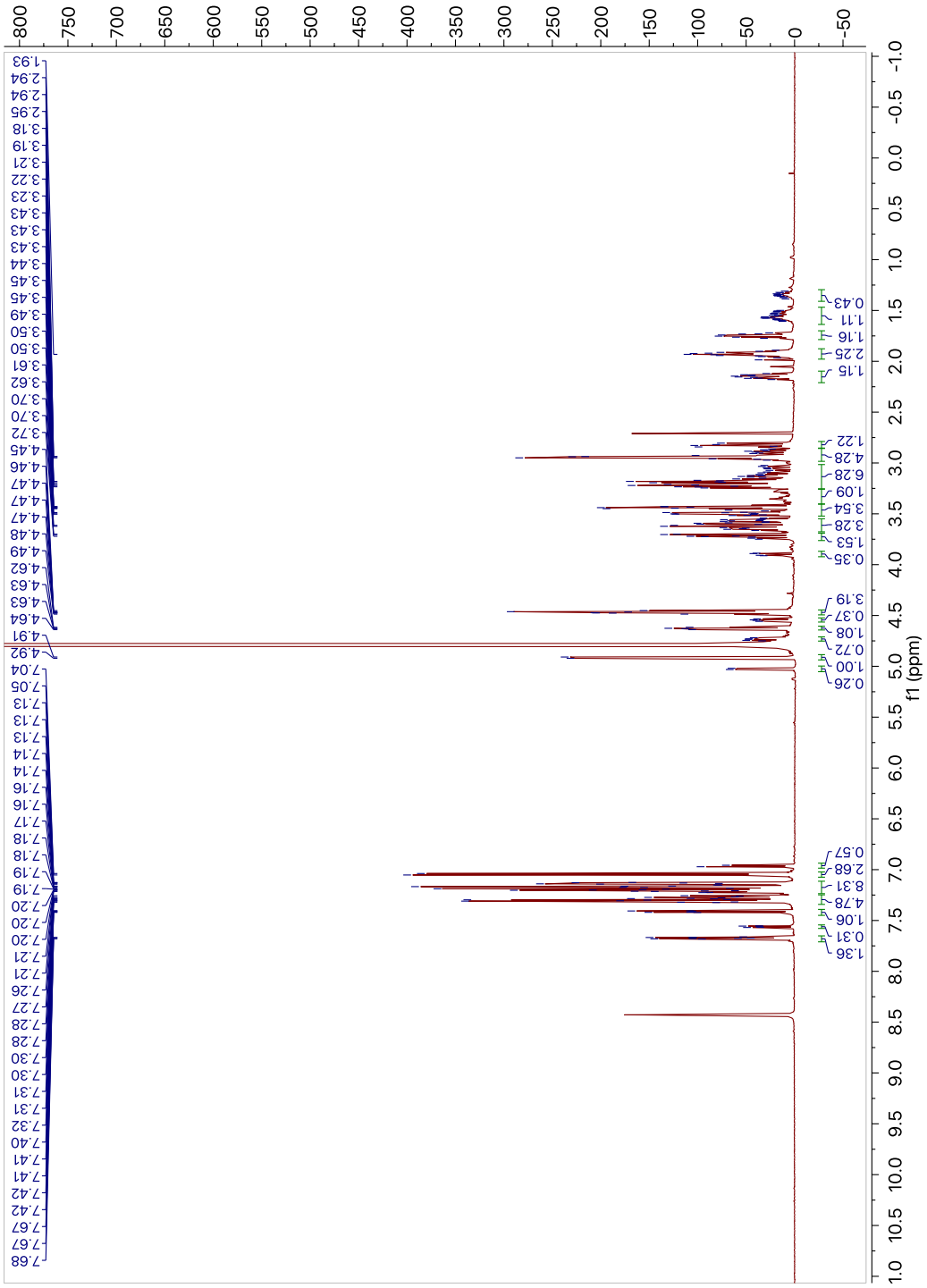
***O*-Tyr-glucopyranosyl endomorphin-1 (27).** A 1-dram vial containing endomorphin 1 (9.0 mg, 0.015 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 273.0 mg, 1.5 mmol, 100.0 equiv.), $Ca(OH)_2$ (111.0 mg, 1. 5 mmol, 100.0 equiv.), and a magnetic stir bar was charged with H_2O (833 µL, 0.018 M in substrate). The vial was sealed with a cap, and the mixture was stirred vigorously at rt for 30 min. The resulting bright yellow mixture was filtered over celite. The pad was eluted with H_2O (15 mL). The collected filtrate was transferred into 15 HPLC vials (~1 mL per vial) for purification via reverse phase prep HPLC (Symmetry prep C8 7µm 7.8 19 X 300 mm column. Injection volume = 1000 µL per run. Solvent A = 0.1% formic acid in water, solvent B = 0.1% formic acid in acetonitrile, flow = 10 mL/min, λ = 210 nm; started at 5% B and held for 3.5 min, ramped to 50% B over 11.5 min, held at 50% B for 5 min, product at 19 min). Fractions containing the product were frozen and lyophilized to dryness, resulting in a white solid.

*Compound **27** was isolated along with an unidentified compound (combined isolated weight = 5.8 mg). The mixture was not separable by reverse phase prep-HPLC equipped with C8, C18, and HYDRO columns. Ratio of **27** and the unknown compound was estimated to be (3.8:1.0), as determined by ^{1}H NMR. Approximate yield of **27** is 40% (4.6 mg)*

Purification of **27** via reverse phase prep HPLC (product at 19 min)

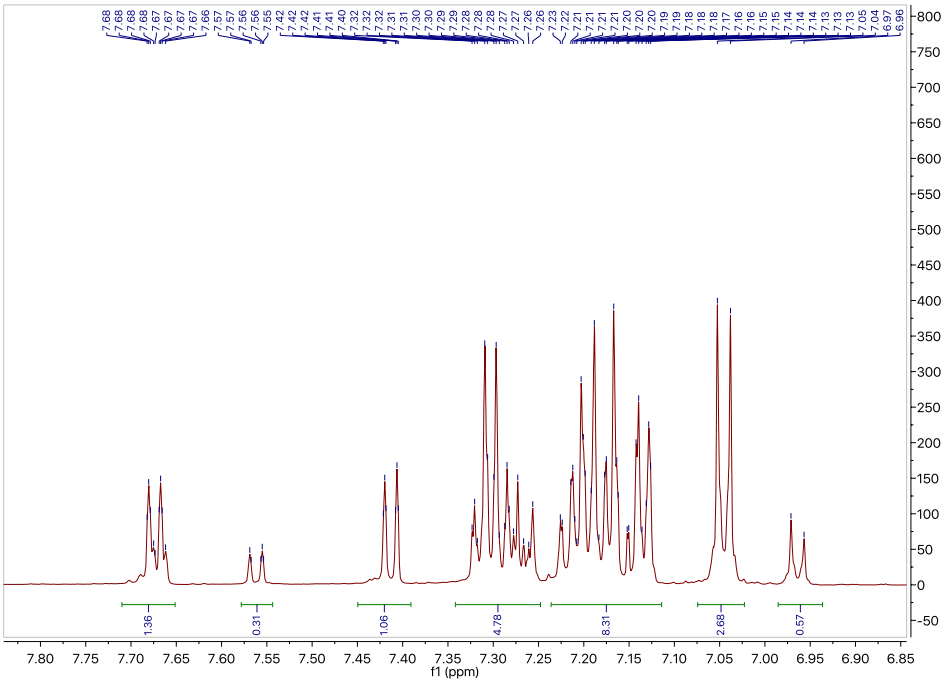


^{1}H NMR spectrum of **27** and an unidentified compound (600 MHz, D_2O, 25 °C)

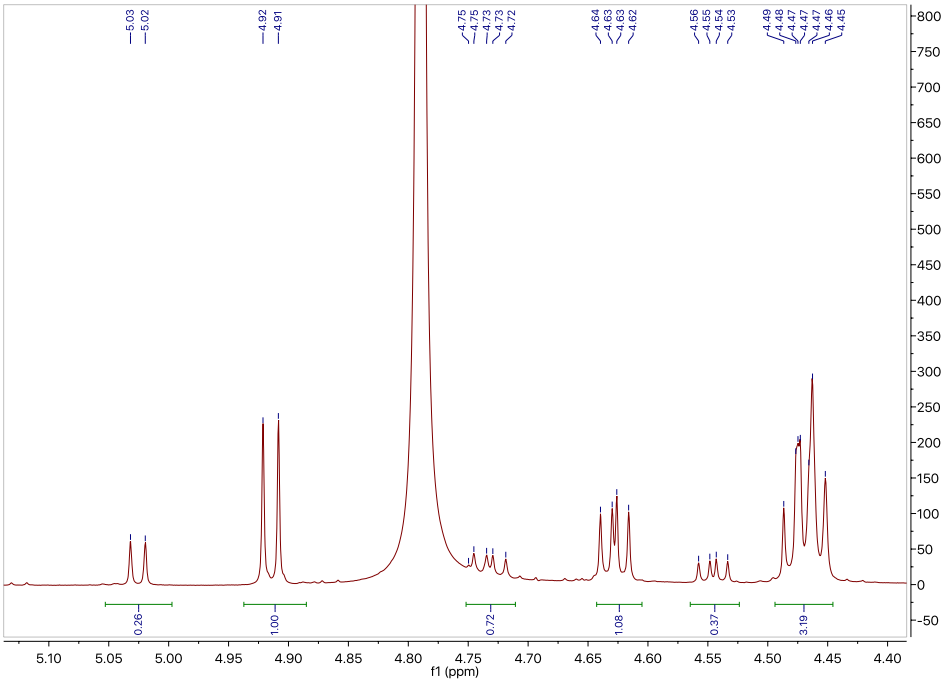


Note: peak at 8.41 ppm correspond to the proton of formate ($HCOO^-$).

^{1}H NMR spectrum of **27** and an unidentified compound
(600 MHz, D_2O, 25 °C, δ 7.80–6.85 ppm)

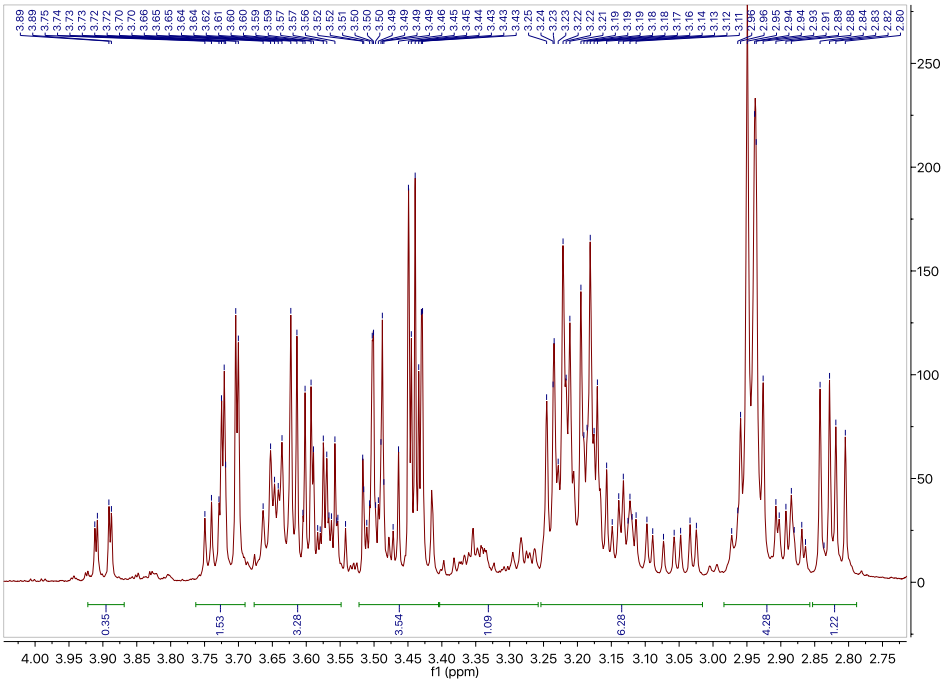


^{1}H NMR spectrum of **27** and an unidentified compound
(600 MHz, D_2O, 25 °C, δ 5.10–4.40 ppm)

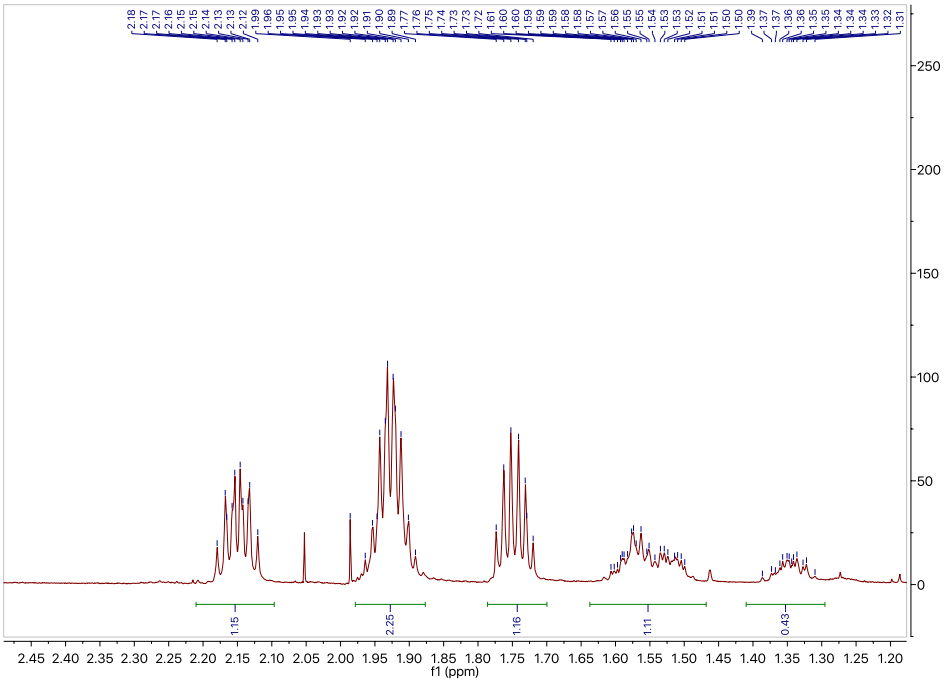


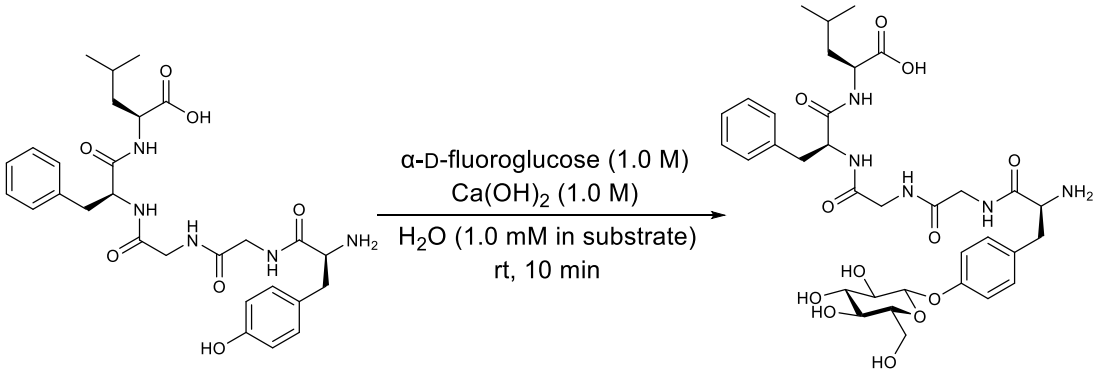
Note: δ 5.03 ppm (d, J = 7.4 Hz), 4.91 ppm (d, J = 7.7 Hz).

^{1}H NMR spectrum of **27** and an unidentified compound
(600 MHz, D_2O, 25 °C, δ 4.00–2.75 ppm)



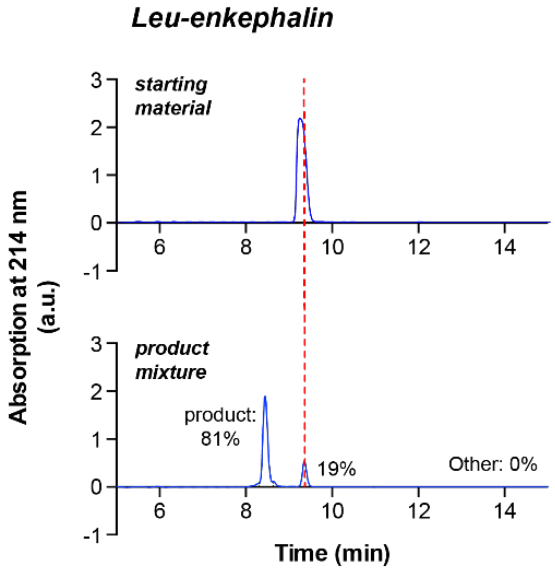
^{1}H NMR spectrum of **27** and an unidentified compound
(600 MHz, D_2O, 25 °C, δ 2.45–1.20 ppm)



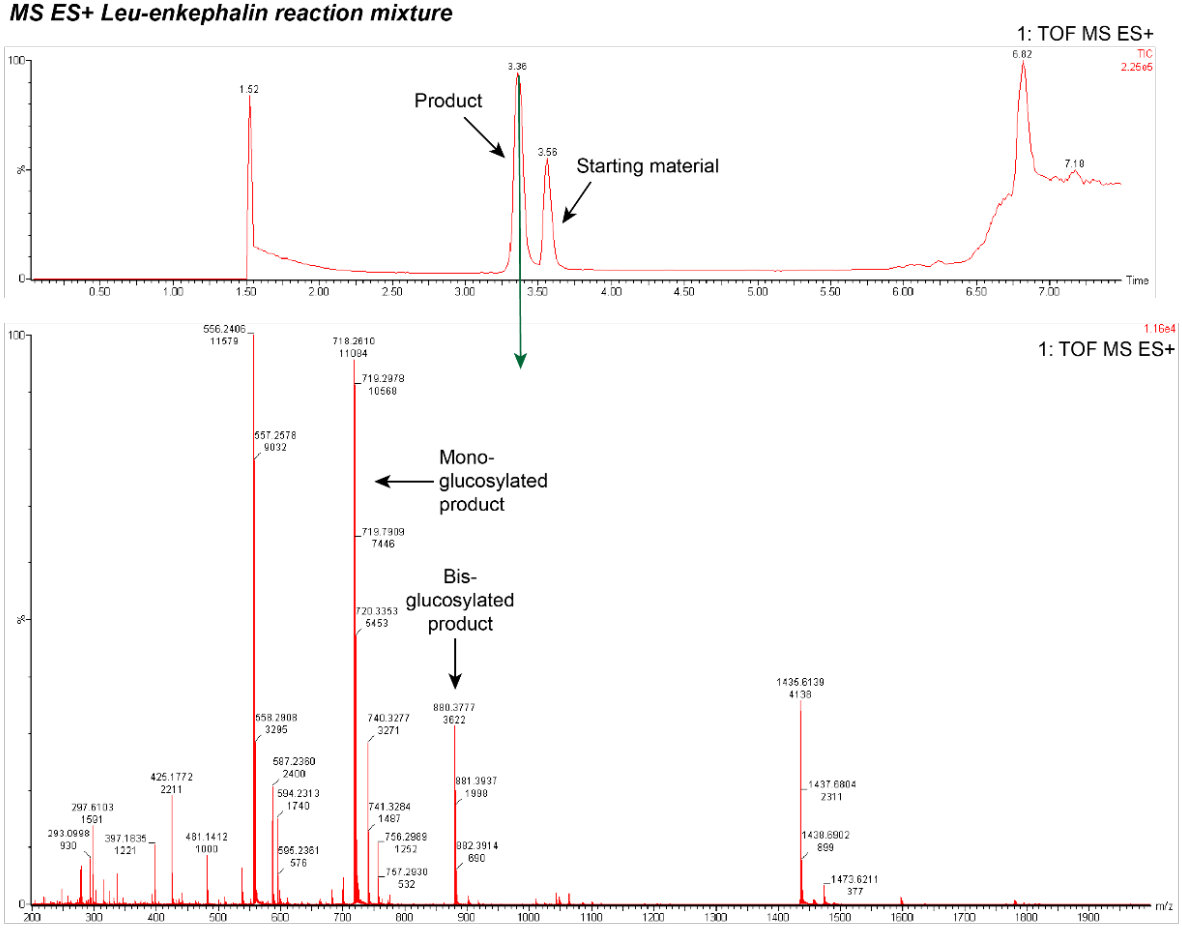


leu-enkephalin **28**

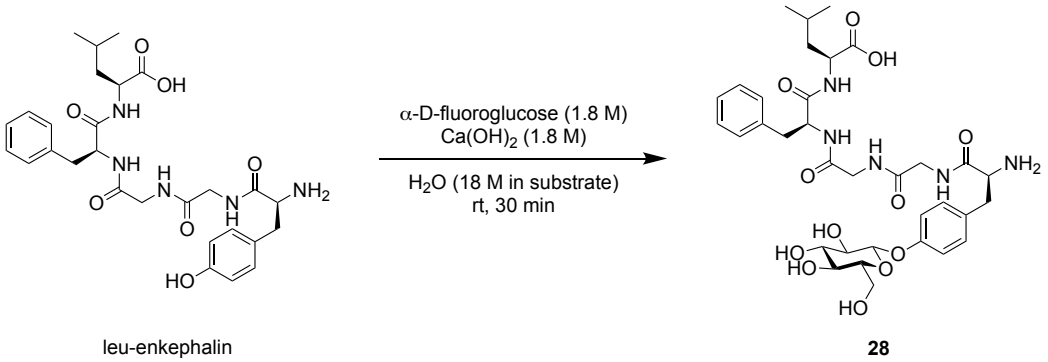
***O*-Tyr-glucopyranosyl leu-enkephalin (28).** The product was synthesized from leu-enkephalin (0.5 mg, 0.90 μmol, 1.0 equiv.) using General Procedure 3. UPLC analysis indicated 81% product present in the reaction mixture. **UPLC/MS** for *O*-Tyr-glucopyranosyl leu-enkephalin: Exact mass calculated for $[C_{34}H_{47}N_5O_{12} + H]^+$ requires m/z = 718.33. Found 718.26.



MS ES+ Leu-enkephalin reaction mixture



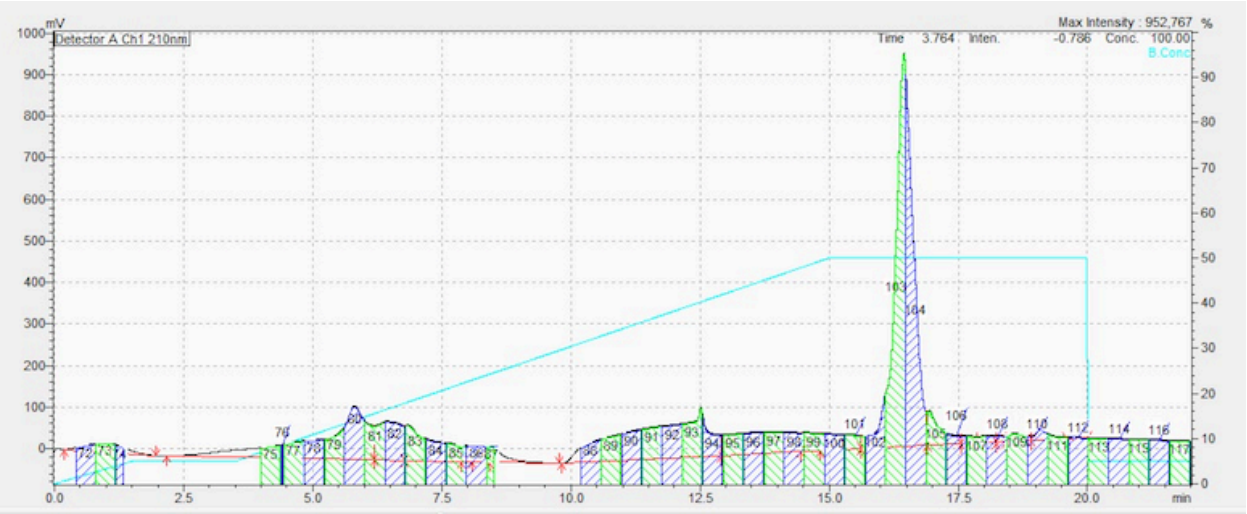
Characterization of Leu-Enkephalin Glycosylation Product



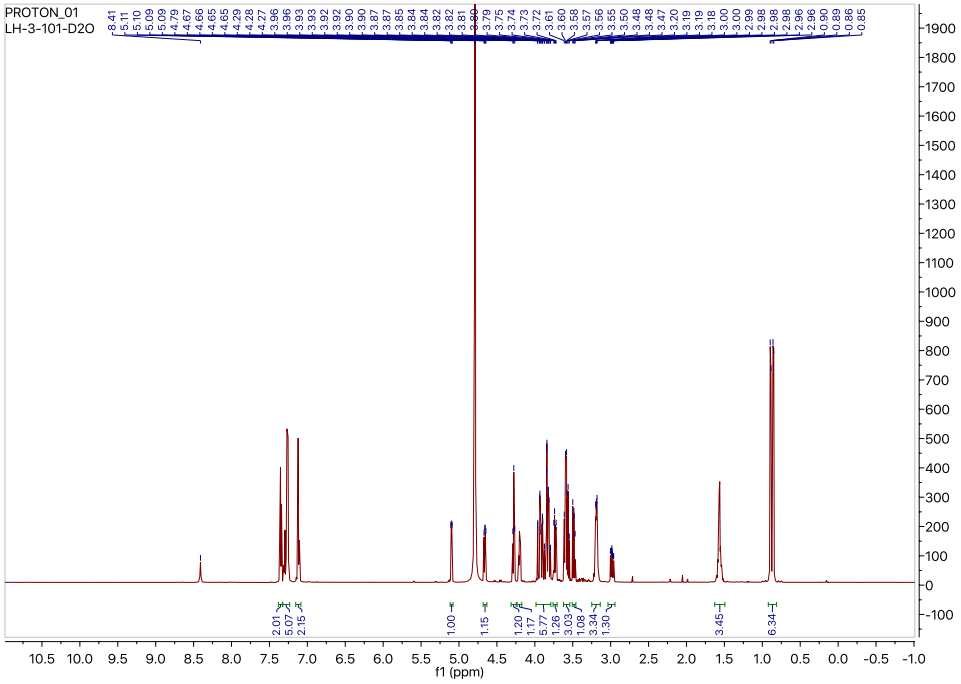
leu-enkephalin **28**

***O*-Tyr-glucopyranosyl leu-enkephalin (28).** A 1-dram vial containing leu-enkephalin (10.0 mg, 0.018 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 328.0 mg, 1.8 mmol, 100.0 equiv.), $Ca(OH)_2$ (133.0 mg, 1.8 mmol, 100.0 equiv.), and a magnetic stir bar was charged with H_2O (1000 μL, 0.018 M in substrate). The vial was sealed with a cap, and the mixture was stirred vigorously at rt for 30 min. The resulting bright yellow mixture was filtered over celite. The pad was eluted with H_2O (15 mL). The collected filtrate was transferred into 15 HPLC vials (1 mL per vial) for purification via reverse phase prep HPLC (Symmetry prep C8 7μm 7.8 19 X 300 mm column. Injection volume = 1000 μL per run. Solvent A = 0.1% formic acid in water, solvent B = 0.1% formic acid in acetonitrile, flow = 10 mL/min, λ = 210 nm; started at 5% B and held for 3.5 min, ramped to 50% B over 11.5 min, held at 50% B for 5 min, product at 16 min). Fractions containing the product were frozen and lyophilized to dryness, resulting in a white solid (**28**, 4.6 mg, 36% yield); **^{1}H NMR** (600 MHz, D_2O): δ 7.37–7.25 (m, 7H), 7.12–7.11 (m, 2H), 5.10 (dd, *J* = 7.5, 2.6 Hz, 1H), 4.66 (ddd, *J* = 9.2, 5.5 Hz, 1H), 4.28 (t, *J* = 7.2 Hz, 1H), 4.21–4.19 (m, 1H), 3.98–3.79 (m, 6H), 3.73 (dd, *J* = 12.5, 5.7 Hz, 1H), 3.61–3.55 (m, 3H), 3.50–3.47 (m, 1H), 3.22–3.15 (m, 3H), 2.98 (ddd, *J* = 14.1, 9.2, 2.2 Hz, 1H), 1.60–1.52 (m, 3H), 0.87 (dd, *J* = 22.1, 5.9 Hz, 6H); **^{13}C NMR** (150 MHz, D_2O): δ 172.07, 172.05, 171.01, 170.65, 169.81, 136.36, 130.72, 129.13, 128.59, 128.17, 126.99, 116.87, 99.92, 76.00, 75.41, 72.77, 69.28, 60.41, 54.64, 54.35, 53.62, 42.37, 42.02, 40.50, 36.86, 35.84, 24.38, 22.25, 22.24, 20.80, 20.78; **IR** (cm^{-1}, neat): 1707, 1563, 1488, 1450, 1375, 1253, 1131, 1073, 1022, 921; **UPLC/MS**: Exact mass calculated for $[C_{34}H_{47}N_5O_{12} + H]^+$ requires m/z = 718.22. Found 718.33; **$[\alpha]_D^{20}$** = –5.33 (*c* 0.003, MeOH).

Purification of **28** via reverse phase prep HPLC (product at 16 min)

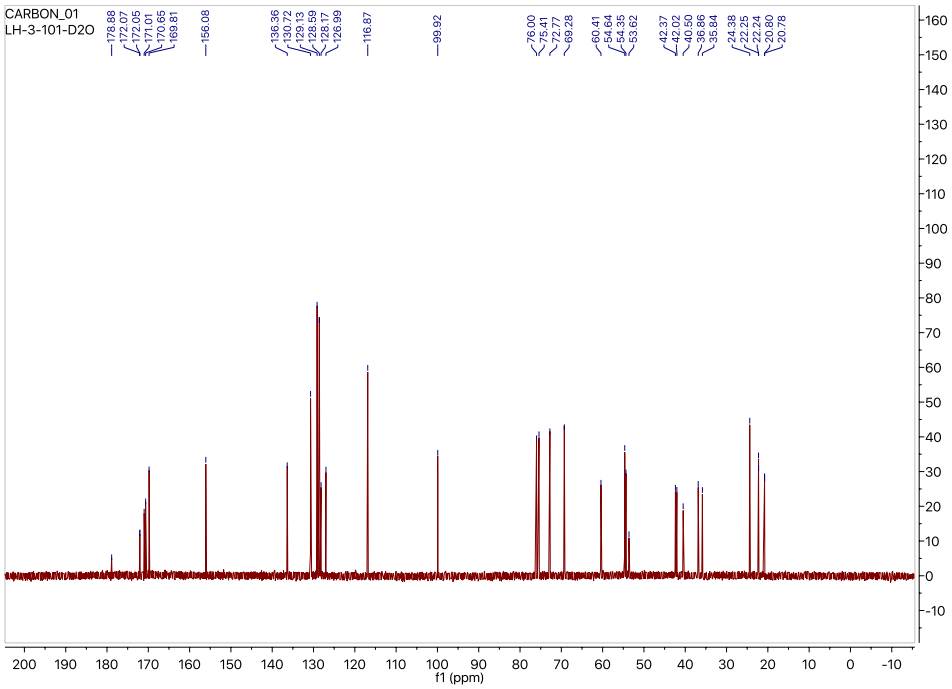


^{1}H NMR spectrum of **28** (600 MHz, D_2O, 25 °C)

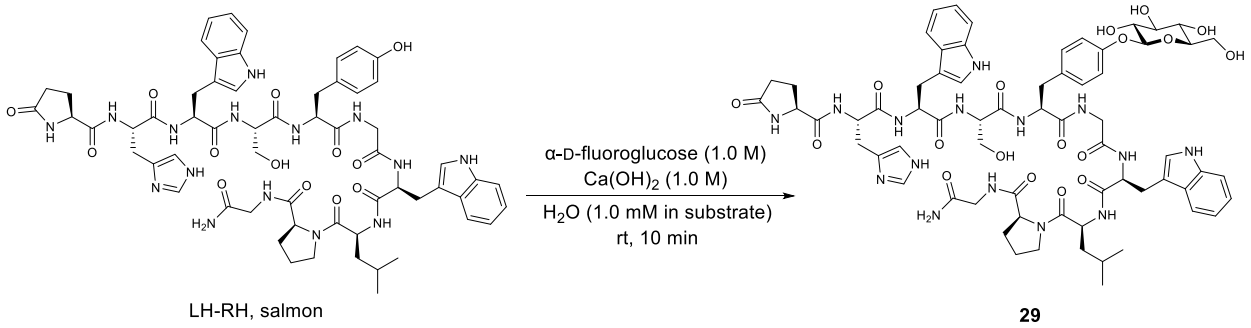


Note: the peak at 8.41 ppm corresponds to the proton of formate ($HCOO^-$).

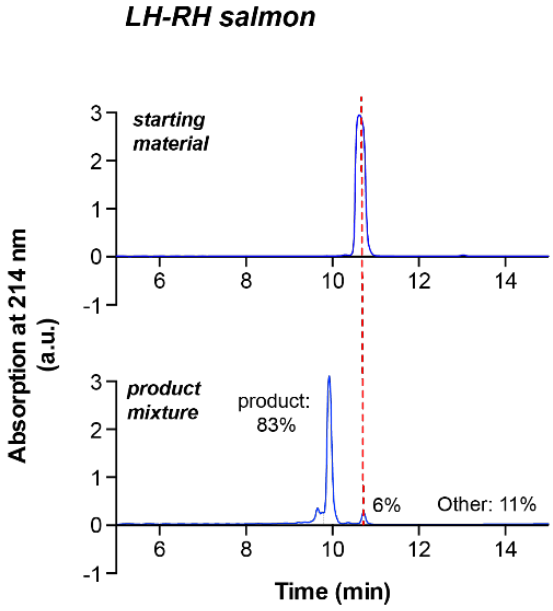
^{13}C NMR spectrum of **28** (150 MHz, D_2O, 25 °C)



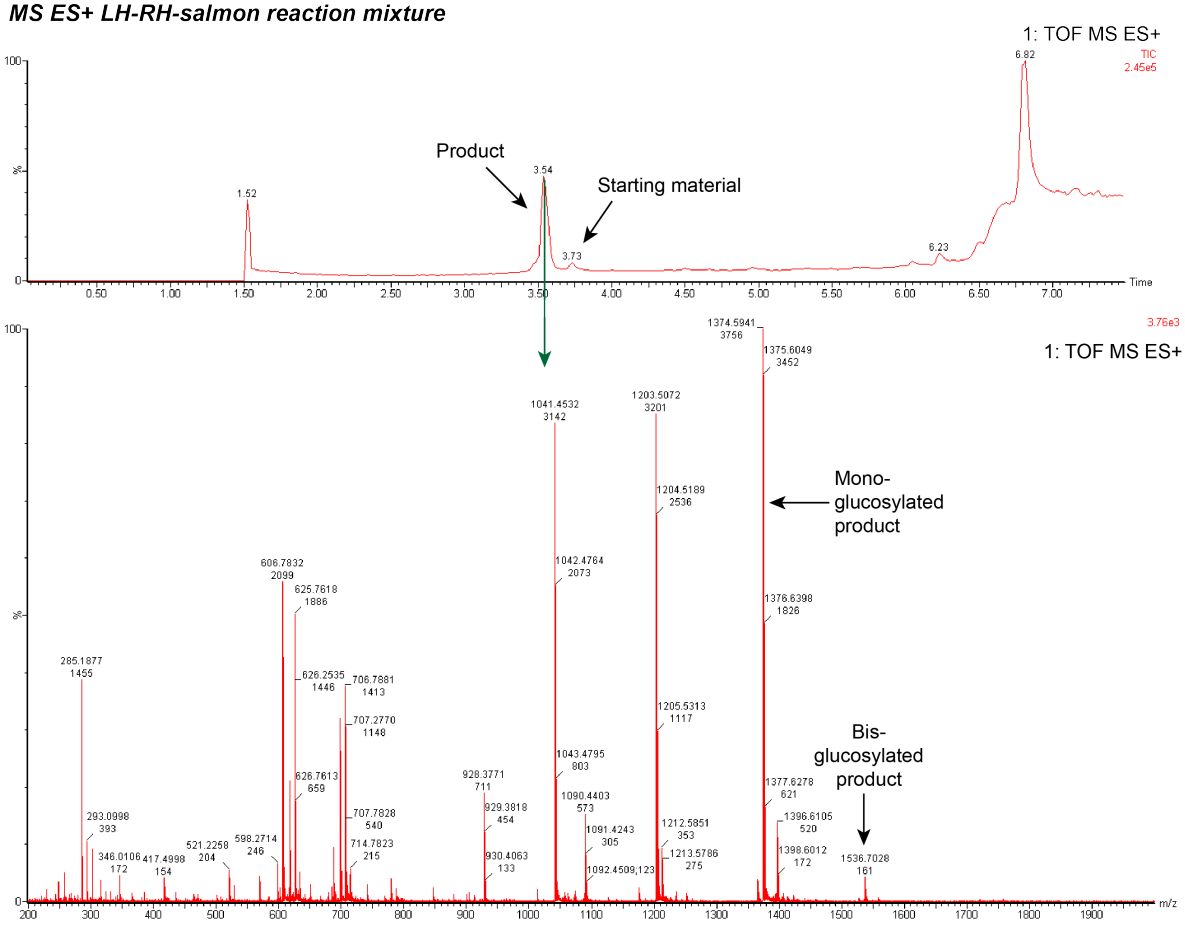
Note: peak at 178.88 ppm correspond to the carbonyl of formate ($HCOO^-$).

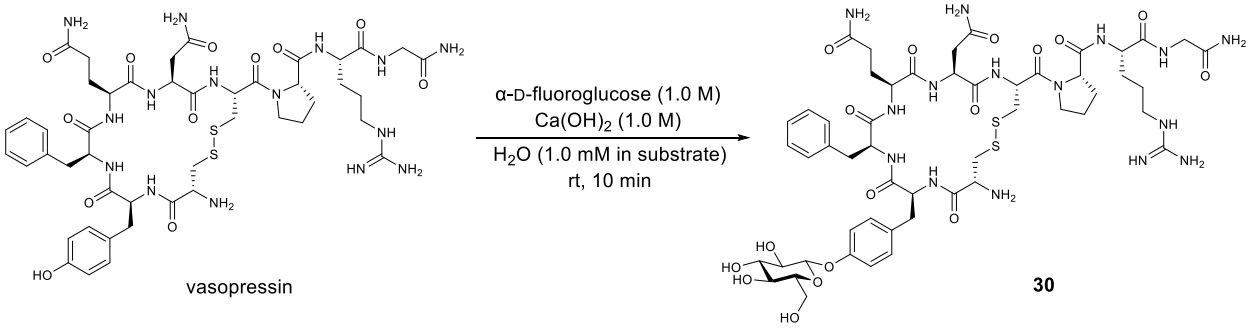


***O*-Tyr-glucopyranosyl LH-RH (29).** The product was synthesized from LH-RH-salmon (1 mg, 0.82 µmol, 1.0 equiv.) using General Procedure 3. UPLC analysis indicated 83% product present in the reaction mixture. **UPLC/MS** for *O*-Tyr-glucopyranosyl LH-RH: Exact mass calculated for $[C_{66}H_{83}N_{15}O_{18} + H]^+$ requires m/z = 1374.61. Found 1374.59.

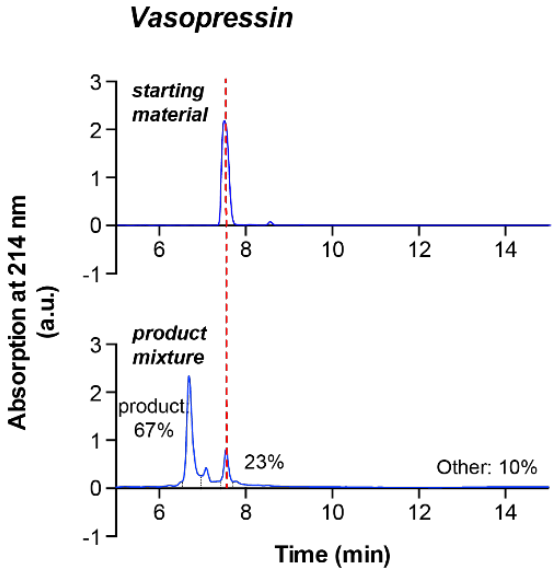


MS ES+ LH-RH-salmon reaction mixture

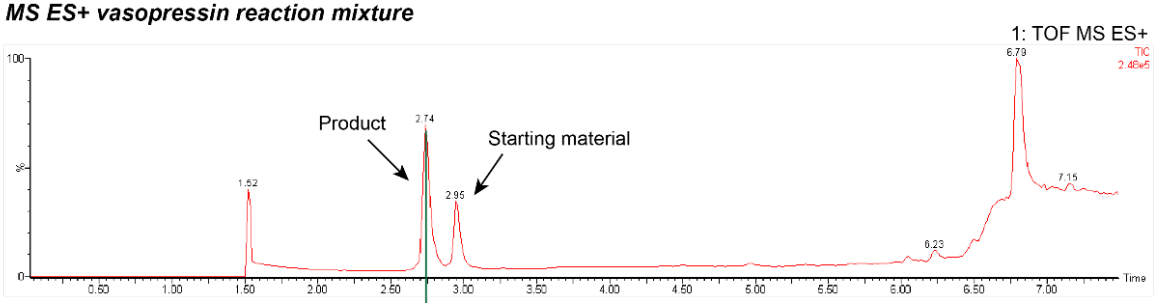


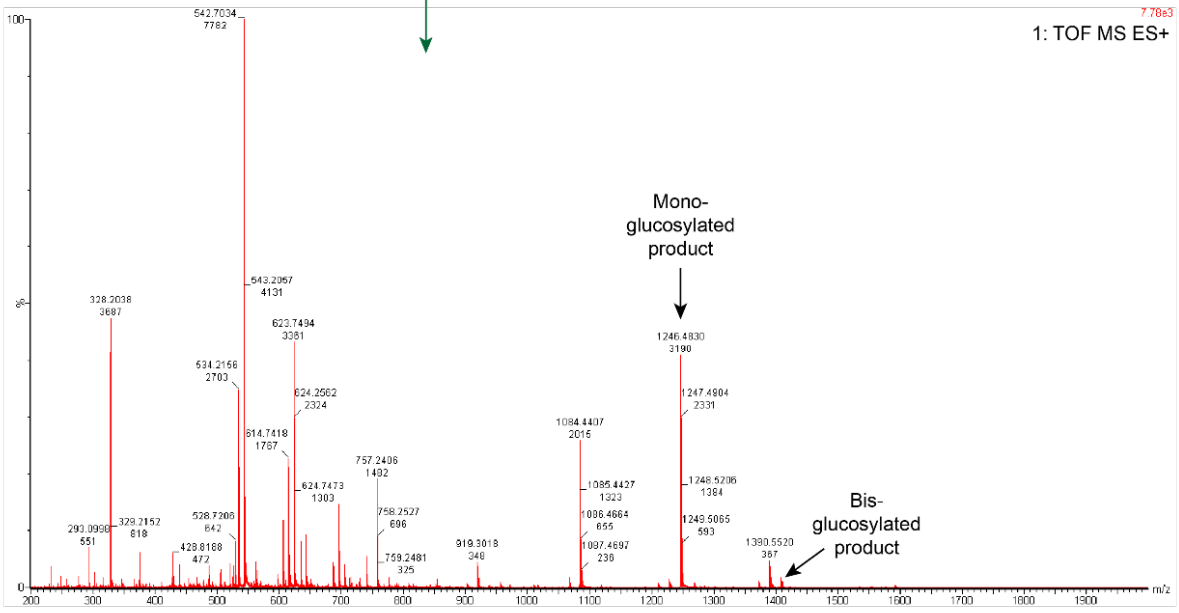


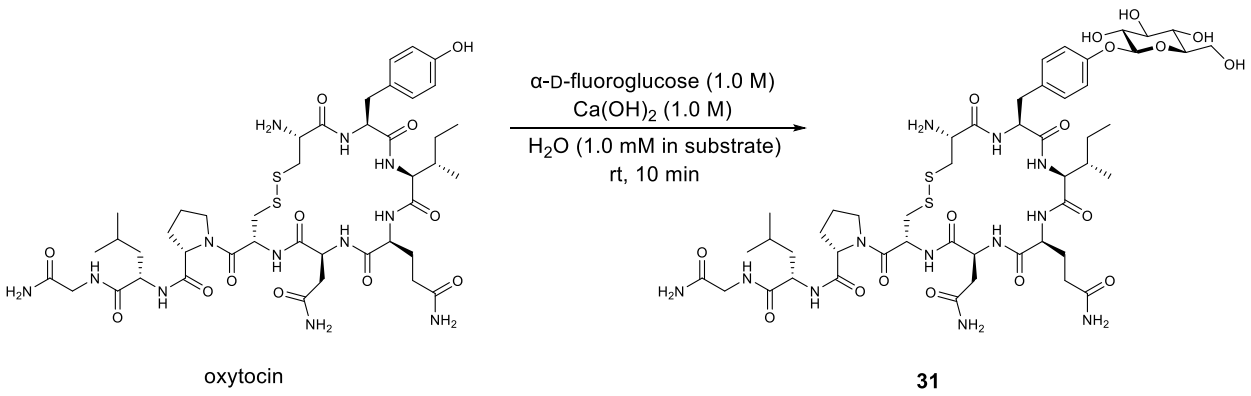
***O*-Tyr-glucopyranosyl vasopressin (30).** The product was synthesized from vasopressin (1 mg, 0.92 µmol, 1.0 equiv.) using General Procedure 3. UPLC analysis indicated 67% product present in the reaction mixture. **UPLC/MS** for *O*-Tyr-glucopyranosyl vasopressin: Exact mass calculated for $[C_{52}H_{75}N_{15}O_{17}S_2 + H]^+$ requires m/z = 1246.50. Found 1246.48.



MS ES+ vasopressin reaction mixture

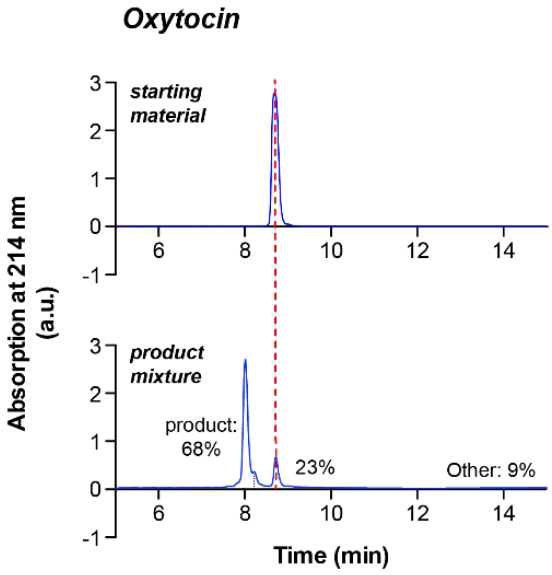




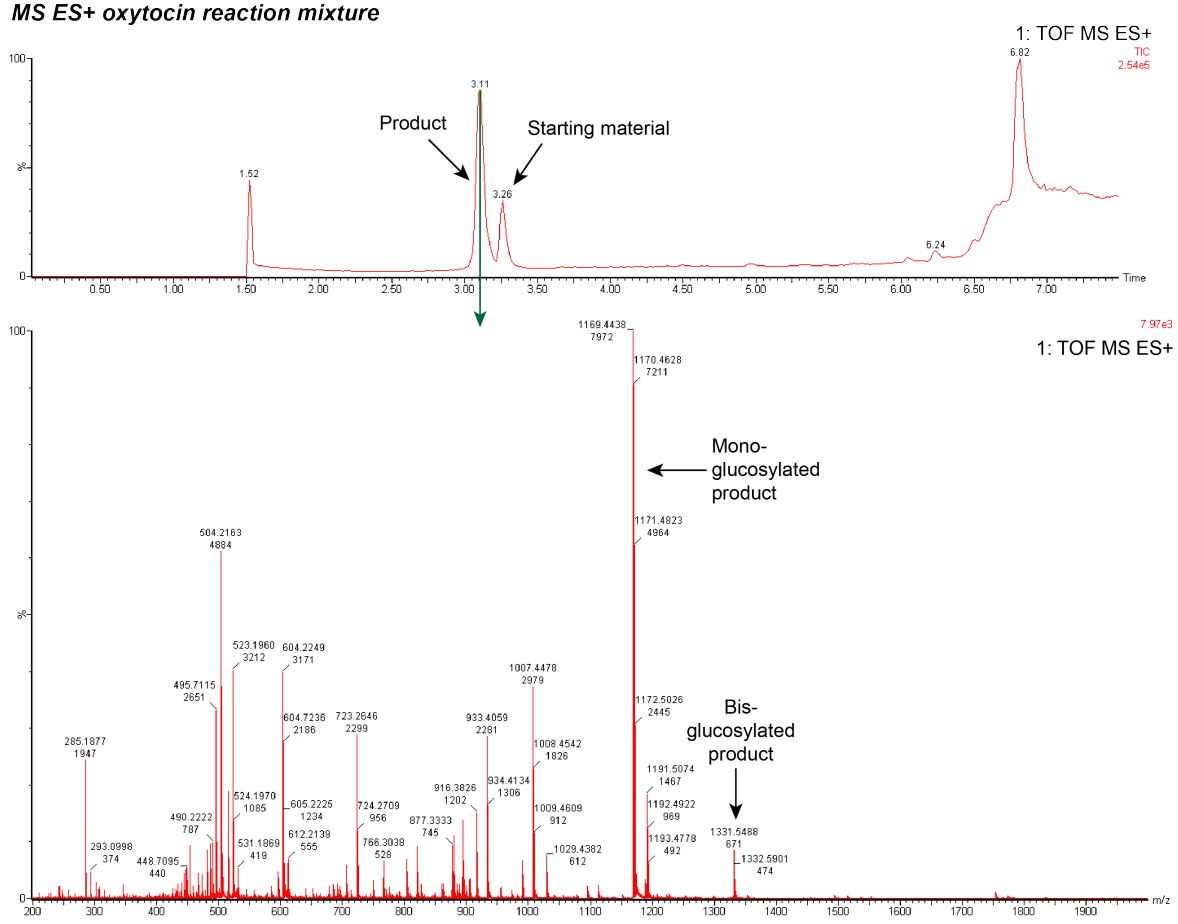


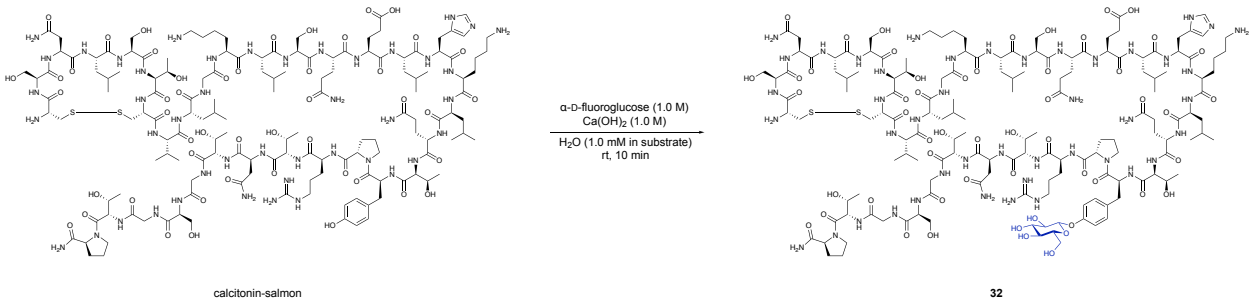
oxytocin **31**

***O*-Tyr-glucopyranosyl oxytocin (31).** The product was synthesized from oxytocin (1 mg, 0.99 µmol, 1.0 equiv.) using General Procedure 3. UPLC analysis indicated 68% product present in the reaction mixture. **UPLC/MS** for *O*-Tyr-glucopyranosyl oxytocin: Exact mass calculated for $[C_{49}H_{76}N_{12}O_{17}S_2 + H]^+$ requires m/z = 1169.50. Found 1169.44.

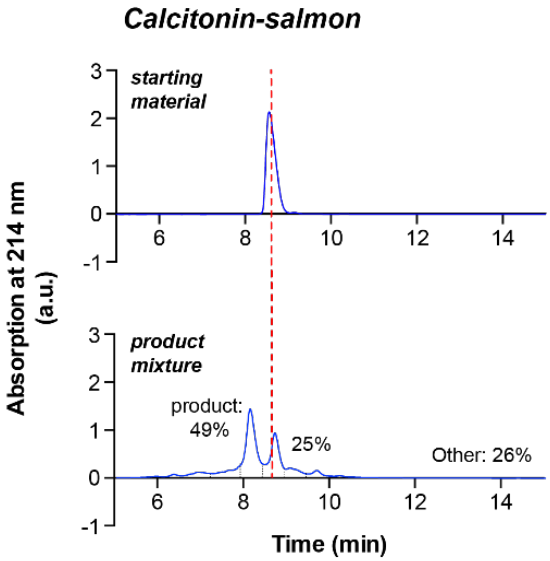


MS ES+ oxytocin reaction mixture

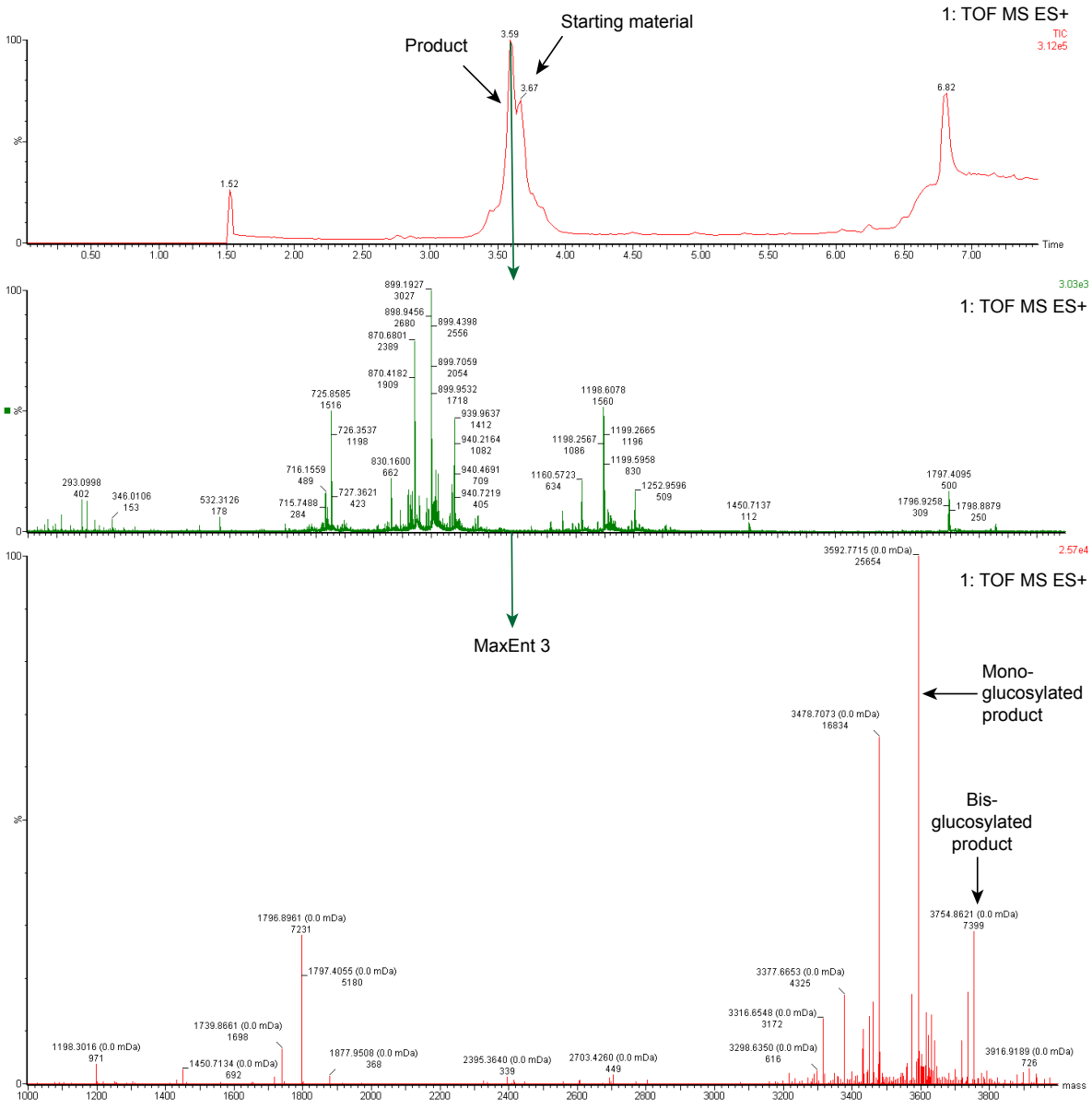




***O*-Tyr-glucopyranosyl calcitonin (32).** The product was synthesized from calcitonin-salmon (1 mg, 0.29 μmol, 1.0 equiv.) using General Procedure 3. UPLC analysis indicated 49% product present in the reaction mixture. **UPLC/MS** for *O*-Tyr-glucopyranosyl calcitonin: exact mass calculated for $[C_{151}H_{250}N_{44}O_{53}S_2 + H]^+$ requires m/z = 3592.77. Found: 3592.77.

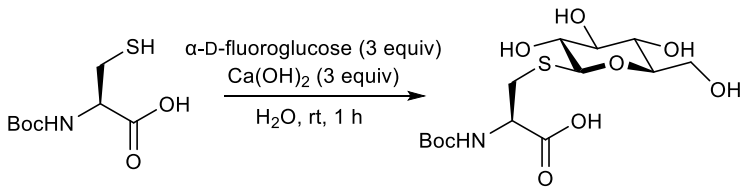


MS ES+ calcitonin-salmon reaction mixture



9. Investigation of Cysteine Reactivity

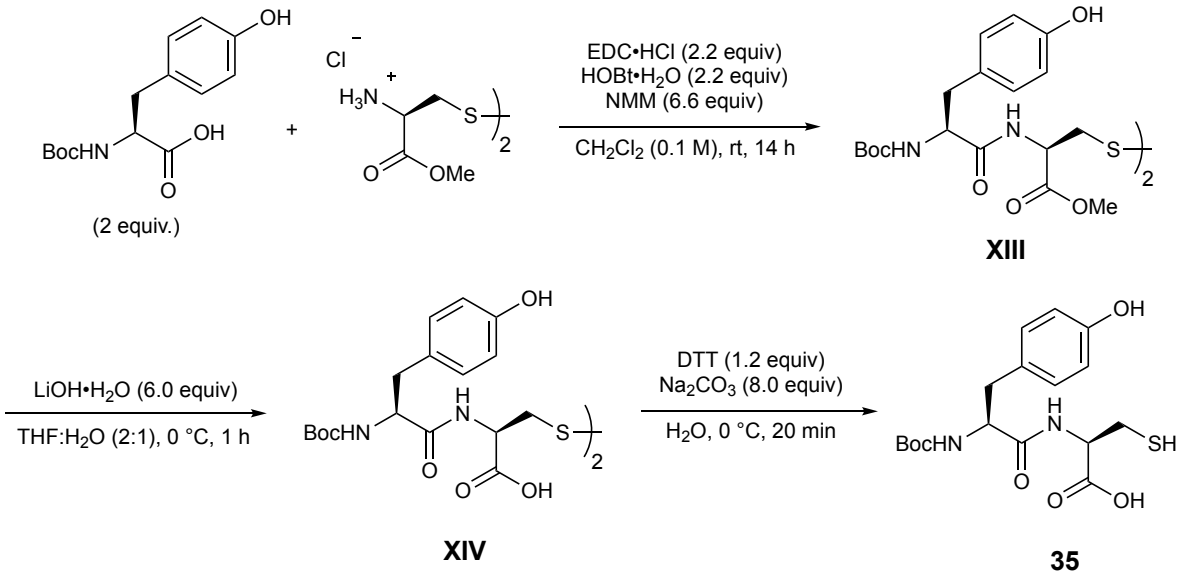
9.1. Glycosylation of Boc-Cys-OH



33 **34**

***N*-Boc-*S*-β-D-glucopyranosyl-L-cysteine (34).** A 10 mL round bottom flask containing Boc-Cys-OH (**33**; 110.7 mg, 0.5 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 273.5 mg, 1.5 mmol, 3.0 equiv.), $Ca(OH)_2$ (111.2 mg, 1.5 mmol, 3.0 equiv.), and a magnetic stir bar was charged with H_2O (500 μL). The flask was sealed with a septum, and the mixture was stirred vigorously at rt for 1 h. The mixture was then loaded onto a Biotage samplet, rinsing the reaction flask with H_2O, and purified via reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 90% MeOH/10% H_2O over 12 CV). Fractions containing the product were concentrated *in vacuo*, frozen, and lyophilized to dryness to yield a white solid (188 mg, 98% yield). $[\alpha]_D^{20}$ = –31.9 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 4.55 (d, *J* = 9.8 Hz, 1H), 4.14 (m, 1H), 3.94 (dd, *J* = 12.8, 2.0 Hz, 1H), 3.76 (dd, *J* = 12.6, 5.6 Hz, 1H), 3.56 – 3.41 (m, 3H), 3.37 (t, *J* = 9.2 Hz, 1H), 3.24 (dd, *J* = 14.2, 4.2 Hz, 1H), 3.01 (dd, *J* = 14.2, 7.4 Hz, 1H), 1.47 (s, 9H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 173.9, 154.9, 85.2, 81.2, 78.1, 77.7, 72.9, 70.1, 61.4, 55.2, 32.2, 28.3 ppm; **IR** (cm^{-1}, neat): 3315, 2975, 1679, 1587, 1519, 1393, 1367, 1249, 1163, 1050, 1025, 867, 577, 409; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{14}H_{24}NO_9S$ requires *m/z* 382.1172, found 382.1170.

9.2. Synthesis of Boc-Tyr-Cys-OH



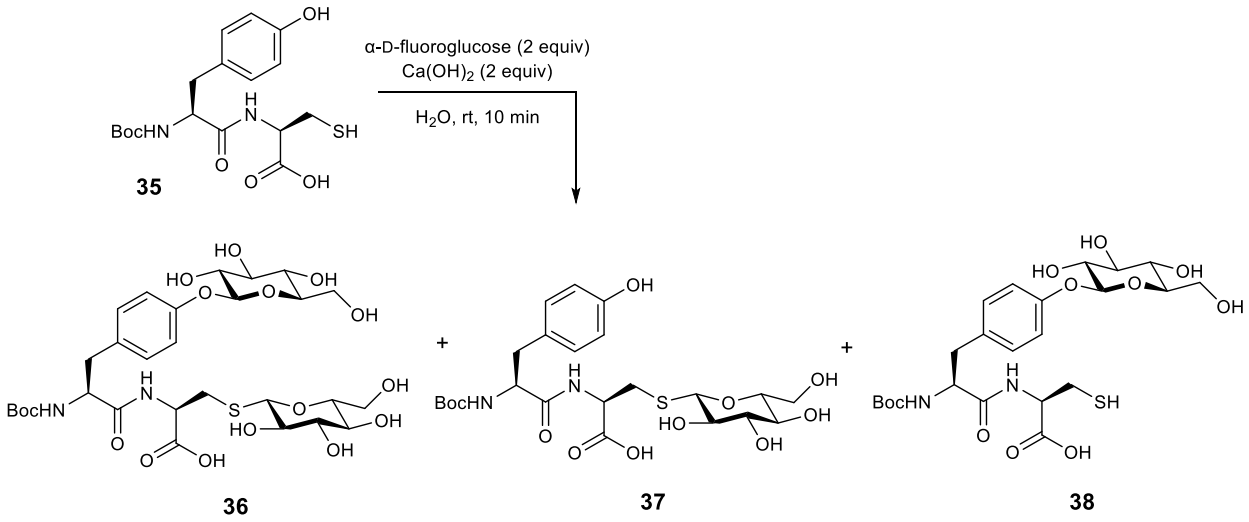
(Boc-Tyr-Cys-OMe)$_2$ (XIII). To a 50 mL round bottom flask were added Boc-Tyr-OH (843.9 mg, 3.0 mmol, 2.0 equiv.), (H-Cys-OMe•HCl)$_2$ (511.9 mg, 1.5 mmol, 1.0 equiv.), EDC•HCl (632.6 mg, 3.3 mmol, 2.2 equiv.), HOBt•H_2O (503.2 mg, 3.3 mmol, 2.2 equiv.), and a magnetic stir bar. The flask was sealed with a rubber septum then evacuated and back-filled with N_2 three times. CH_2Cl_2 (15 mL, 0.1 M) was added via syringe, and the resulting suspension was stirred at rt for 5 min. *N*-methylmorpholine (1.09 mL, 9.9 mmol, 6.6 equiv.) was added slowly via syringe, and the resulting yellow solution was stirred at rt for 14 h. The solution was then transferred to a 125 mL separatory funnel, diluting to a total volume of 50 mL with CH_2Cl_2. The solution was washed sequentially with a 10% aqueous citric acid solution (1 x 50 mL), water (1 x 50 mL), a saturated $NaHCO_3$ solution (1 x 50 mL), and a saturated NaCl solution (1 x 50 mL). The organic layer was then dried over Na_2SO_4, filtered, and dried *in vacuo* to generate an off-white foam. The product was purified using a Biotage reversed-phase flash chromatography system (120 g C18 column; 50 mL/min flow rate; mobile phase buffered with 0.1% formic acid; gradient = 20% CH_3CN/80% H_2O for 2 CV, ramped from 20% CH_3CN/80% H_2O to 95% CH_3CN/5% H_2O over 12 CV). Fractions containing the product were evaporated to dryness, resulting in a white foam (829 mg, 69.5% yield). **$[\alpha]_D^{20}$** = –65.7 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, CD_3OD) δ 7.05 (d, *J* = 8.1 Hz, 4H), 6.69 (d, *J* = 8.2 Hz, 4H), 6.61 (d, *J* = 8.4 Hz, 1H), 4.75 (dd, *J* = 7.8, 5.2 Hz, 2H), 4.37 – 4.20 (m, 2H), 3.72 (s, 6H), 3.19 (dd, *J* = 14.0, 5.2 Hz, 2H), 3.07 – 2.96 (m, 4H), 2.75 (dd, *J* = 13.9, 8.9 Hz, 2H), 1.37 (s, 18H) ppm; **^{13}C NMR** (126 MHz, CD_3OD) δ 174.5, 172.0, 157.6, 157.2, 131.4, 129.1, 116.2, 80.7, 57.5, 53.1, 49.9, 40.5, 38.5, 28.7 ppm; **IR** (cm^{-1}, neat): 3310, 2979, 1658, 1614, 1514, 1437, 1366, 1224, 1159, 1049, 1019, 889, 828, 808, 779, 540, 490, 424; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{36}H_{49}N_4O_{12}S_2$ requires *m/z* 793.2788, found 793.2797.

(Boc-Tyr-Cys-OH)$_2$ (XIV). A 100 mL round bottom flask containing (Boc-Tyr-Cys-OMe)$_2$ (**XIII**; 600 mg, 0.75 mmol, 1.0 equiv.) and a magnetic stir bar was charged with THF (10 mL).

The resulting clear colorless solution was cooled in an ice-water bath, then a solution of $LiOH•H_2O$ (190 mg, 4.53 mmol, 6.0 equiv.) in H_2O (5 mL) was added dropwise. The resulting yellow solution was stirred at 0 °C for 1 h, monitoring reaction progress by TLC. The solution was then charged with 1 M HCl (4.5 mL) and stirred for 2 min. The solution was transferred to a 125 mL separatory funnel and extracted with a 75% $CHCl_3$/25% iPrOH solution (3 x 30 mL). The combined organic layers were evaporated to dryness and purified using a Biotage reversed-phase flash chromatography system (120 g C18 column; 50 mL/min flow rate; mobile phase buffered with 0.1% formic acid; gradient = 20% CH_3CN/80% H_2O for 2 CV, ramped from 20% CH_3CN/80% H_2O to 50% CH_3CN/50% H_2O over 12 CV). Fractions containing the product were combined with ~50 mL 1-methoxy-2-propanol (to prevent foaming) and evaporated to dryness, resulting in a white foam (336 mg, 58% yield). Extensive drying on high vacuum (3 d) did not result in complete removal of 1-methoxy-2-propanol, as indicated by NMR analysis. $\mathbf{[\alpha]_D^{20}}$ = –81.0 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, CD_3OD) δ 7.07 (d, *J* = 8.0 Hz, 4H), 6.70 (d, *J* = 8.2 Hz, 4H), 4.75 (dd, *J* = 8.1, 4.6 Hz, 2H), 4.43 – 4.20 (m, 2H), 3.31 – 3.24 (m, 2H), 3.15 – 2.98 (m, 4H), 2.75 (dd, *J* = 14.1, 9.4 Hz, 2H), 1.38 (s, 18H) ppm; **^{13}C NMR** (151 MHz, CD_3OD) δ 174.7, 173.2, 157.7, 157.2, 131.4, 129.3, 116.2, 80.7, 57.5, 53.3, 40.8, 38.5, 28.7 ppm; **IR** (cm^{-1}, neat): 3312, 2978, 1658, 1615, 1514, 1449, 1392, 1367, 1234, 1159, 1105, 1051, 1021, 961, 889, 827, 777, 543, 489; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{34}H_{45}N_4O_{12}S_2$ requires *m/z* 765.2475, found 765.2491.

Boc-Tyr-Cys-OH (35). To a 50 mL round bottom flask equipped with a magnetic stir bar was added $(Boc\text{-}Tyr\text{-}Cys\text{-}OH)_2$ (**XIV**; 200 mg, 0.26 mmol, 1.0 equiv.) followed by H_2O (5 mL). To the resulting suspension was added Na_2CO_3 (221 mg, 2.08 mmol, 8.0 equiv.), generating a clear, colorless solution. The solution was cooled in an ice-water bath with stirring, and dithiothreitol (DTT; 47.8 mg, 0.31 mmol, 1.2 equiv.) was added in one portion. After stirring 20 min, formic acid (78 μL, 2.08 mmol, 8.0 equiv.) was added via pipet, and the solution was stirred for 1 min. The solution was then loaded onto a Biotage samplet and purified via reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; mobile phase buffered with 0.1% formic acid; gradient = 20% CH_3CN/80% H_2O for 2 CV, ramped from 20% CH_3CN/80% H_2O to 50% CH_3CN/50% H_2O over 12 CV). Fractions containing the product were concentrated *in vacuo*, frozen, and lyophilized to dryness, resulting in a white solid (155 mg, 78% yield). $\mathbf{[\alpha]_D^{20}}$ = +2.0 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.18 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 4.55 (s, 1H), 4.34 (dd, *J* = 8.8, 5.9 Hz, 1H), 3.13 – 3.01 (m, 1H), 2.95 (d, *J* = 5.6 Hz, 2H), 2.89 (dd, *J* = 13.9, 8.9 Hz, 1H), 1.36 (s, 9H) ppm; **^{13}C NMR** (126 MHz, DMSO-*d_6*) δ 171.9, 171.4, 155.7, 155.2, 130.1, 128.0, 114.8, 78.1, 56.0, 54.2, 36.4, 28.1, 25.6 ppm; **IR** (cm^{-1}, neat): 3304, 2978, 1653, 1614, 1514, 1443, 1393, 1367, 1303, 1228, 1157, 1051, 1021, 827, 805, 778, 543; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{17}H_{23}N_2O_6S$ requires *m/z* 383.1277, found 383.1280.

9.3. Glycosylation of Boc-Tyr-Cys-OH



A 1-dram vial containing Boc-Tyr-Cys-OH (**35**; 38.4 mg, 0.1 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 36.4 mg, 0.2 mmol, 2.0 equiv.), $Ca(OH)_2$ (14.8 mg, 0.2 mmol, 2.0 equiv.), and a magnetic stir bar was charged H_2O (100 µL). The vial was sealed with a cap, and the reaction mixture was stirred vigorously at rt for 10 min. The mixture was then diluted with H_2O (500 µL), and EDTA-Na_2•$2H_2O$ (74.4 mg, 0.2 mmol, 2.0 equiv.) was added. The resulting suspension was stirred at rt for 5 min and then diluted with 2.4 mL of a 4:1 H_2O:CH_3CN solution containing 5 mM sodium benzoate. The mixture was then filtered through a 0.22 µm PVDF syringe filter, and the eluent was analyzed by reversed-phase HPLC (Phenomenex Luna 5 µm C8(2) 250 x 4.6 mm column; λ = 254 nm; flow rate = 1 mL/min; solvent A = H_2O buffered with 0.1% formic acid; solvent B = CH_3CN buffered wth 0.1% formic acid; gradient = ramped from 95% solvent A/5% solvent B to 85% solvent A/15% solvent B over 2 min, ramped from 85% solvent A/15% solvent B to 60% solvent A/40% solvent B over 18 min, ramped from 60% solvent A/40% solvent B to 5% solvent A/95% solvent B over 1 min, held at 5% solvent A/95% solvent B for 4 min).

HPLC Trace for Glycosylation of Boc-Tyr-Cys-OH:

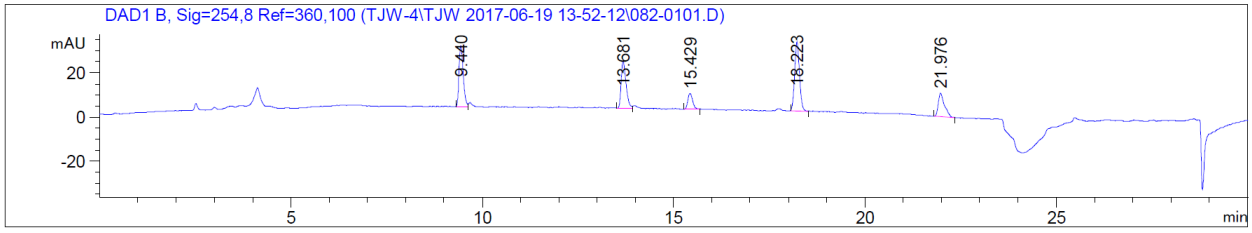
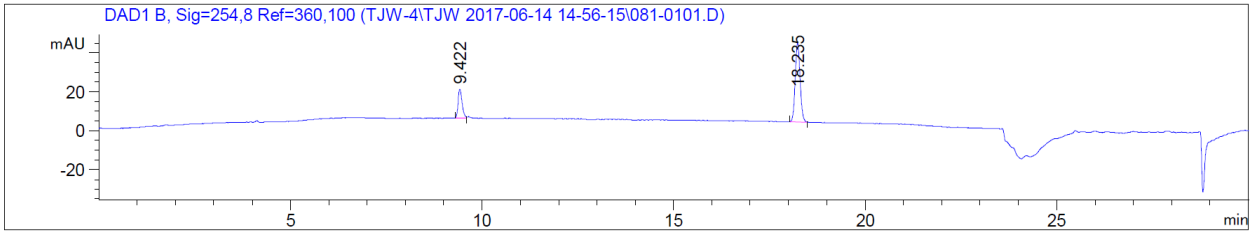


Table S6: Peak List for the Glycosylation of Boc-Tyr-Cys-OH

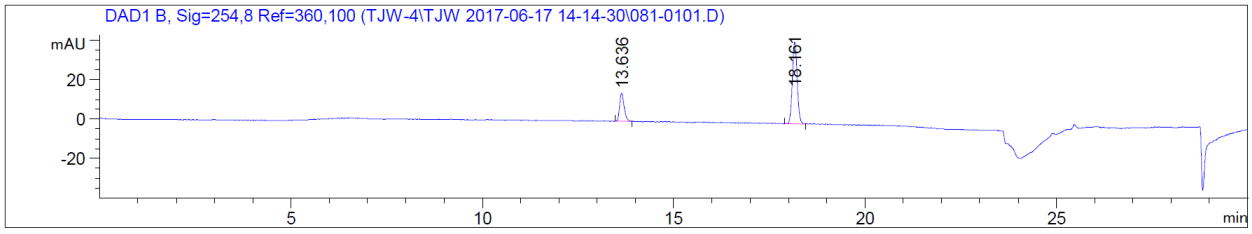
Entry	Ret. Time (min)	Identity	Peak Area (mAU*s)	Peak Area (%)
1	9.4	Boc-Tyr(*O*-β-D-glu)-Cys(*S*-β-D-glu)-OH (**36**)	210.298	36
2	13.6	Boc-Tyr-Cys(*S*-β-D-Glc)-OH (**37**)	194.186	33
3	15.4	Boc-Tyr(*O*-β-D-Glc)-Cys-OH (**38**)	60.702	10
4	18.2	Sodium benzoate (standard)	-	-
5	22.0	Boc-Tyr-Cys-OH (**35**)	120.288	21

Peak assignments were determined by isolation of each product (see below for isolation details):

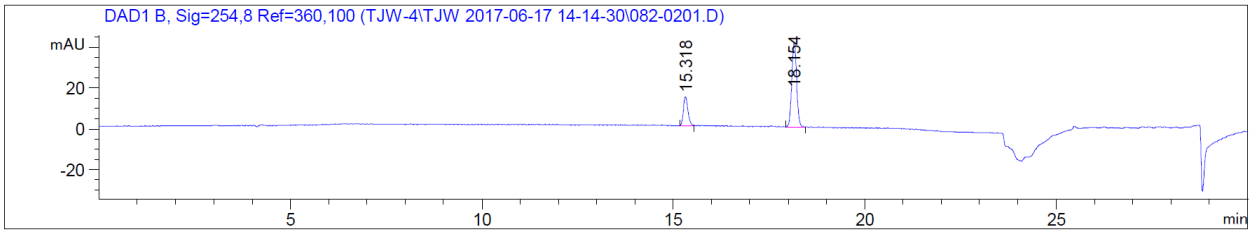
HPLC Trace for Boc-Tyr(*O*-β-D-glu)-Cys(*S*-β-D-glu)-OH (36):



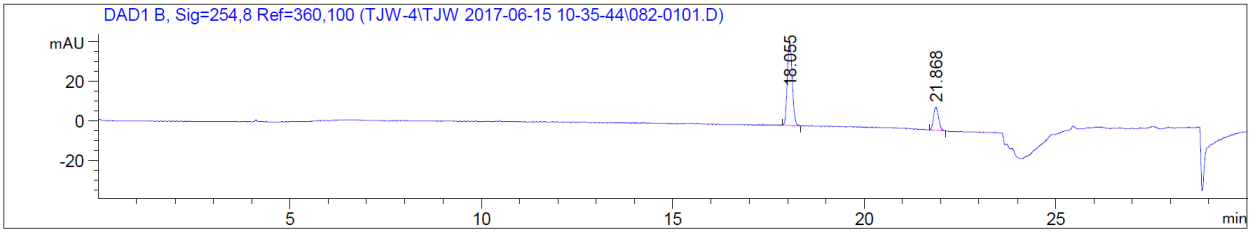
HPLC Trace for Boc-Tyr-Cys(*S*-β-D-Glc)-OH (37):



HPLC Trace for Boc-Tyr(*O*-β-D-Glc)-Cys-OH (37):



HPLC Trace for Boc-Tyr-Cys-OH (35):

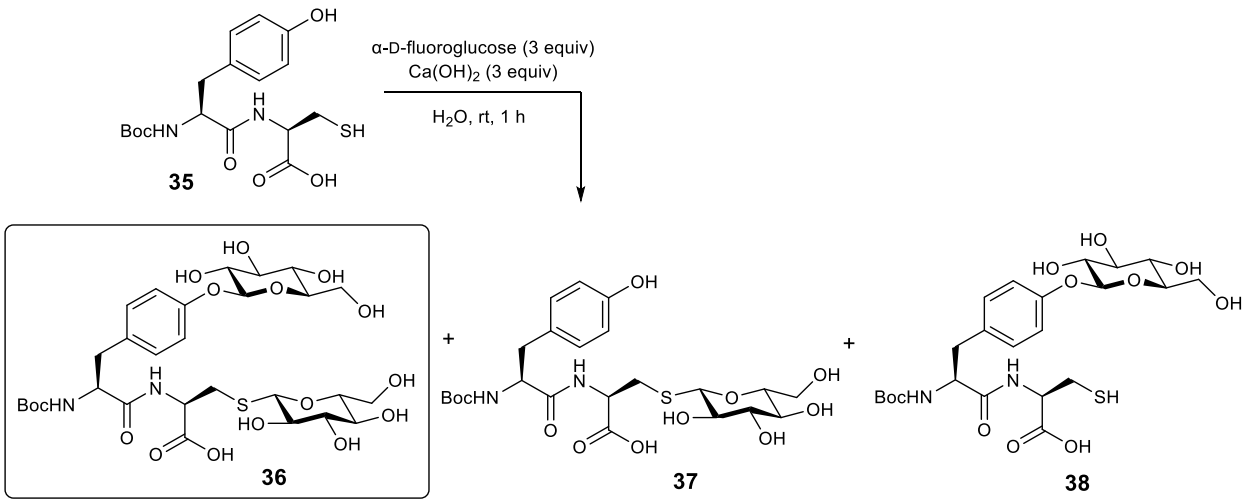


9.4 Syntheses of Pure Boc-Tyr-Cys-OH Glycosylation Products

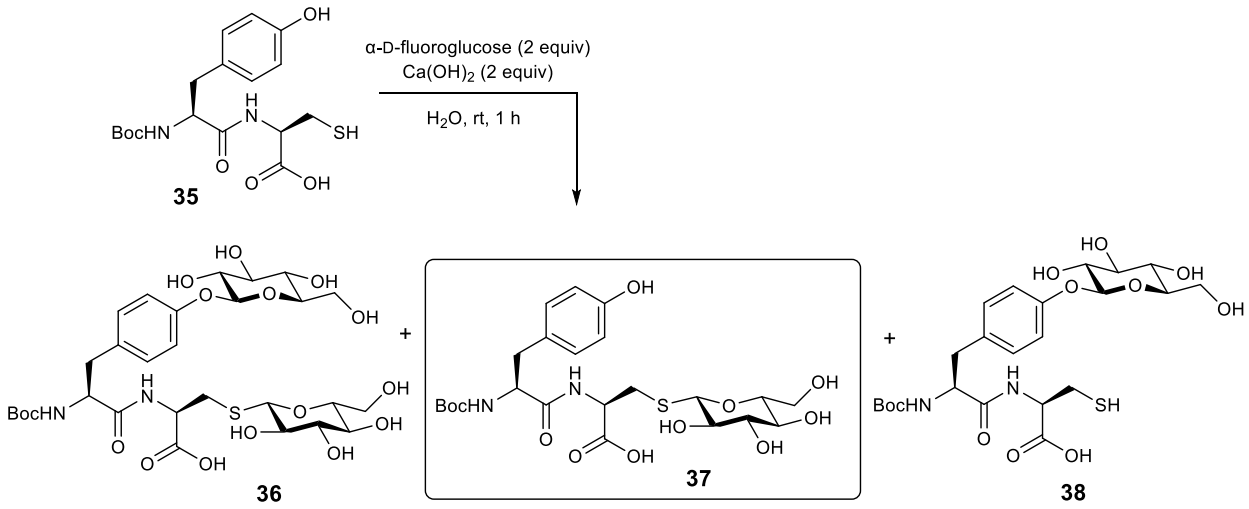
Pure samples for product identification were obtained via

- **Boc-Tyr(*O*-β-D-glu)-Cys(*S*-β-D-glu)-OH (36):** glycosylation of Boc-Tyr-Cys-OH (**35**) followed by reversed-phase flash chromatography,
- **Boc-Tyr-Cys(*S*-β-D-Glc)-OH (37):** glycosylation of Boc-Tyr-Cys-OH (**35**) followed by reversed-phase flash chromatography and reversed-phase preparative HPLC, and
- **Boc-Tyr(*O*-β-D-Glc)-Cys-OH (38):** glycosylation of the Cys-protected substrate (Boc-Tyr-Cys-OH)$_2$ (**S14**) followed by cystine reduction.

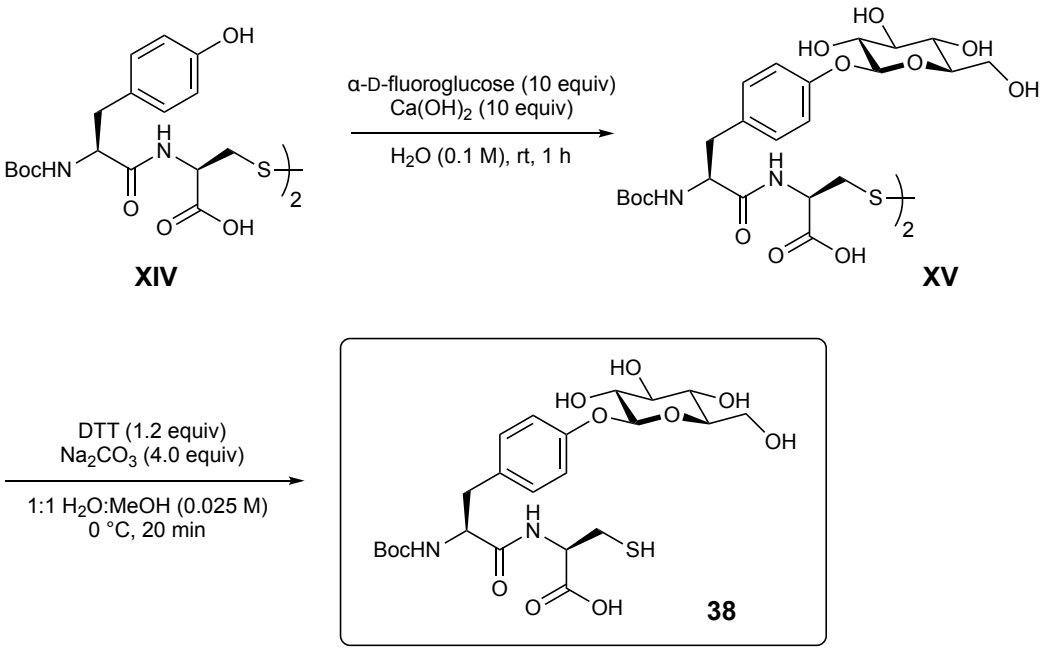
Experimental procedures and characterization for each product are listed below:



Boc-Tyr(*O*-β-D-glu)-Cys(*S*-β-D-glu)-OH (36). A 1-dram vial containing Boc-Tyr-Cys-OH (**35**; 76.9 mg, 0.2 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 109.3 mg, 0.6 mmol, 3.0 equiv.), $Ca(OH)_2$ (44.5 mg, 0.6 mmol, 3.0 equiv.), and a magnetic stir bar was charged H_2O (200 μL). The vial was sealed with a cap, and the reaction mixture was stirred vigorously at rt for 1 h. The mixture was then loaded onto a Biotage samplet, rinsing the reaction vial with H_2O, and purified via reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 25% MeOH/75% H_2O over 16 CV). Fractions containing the product were combined, concentrated *in vacuo*, frozen, and lyophilized to dryness, resulting in a white powder (89 mg, 63% yield). $[\alpha]_D^{20}$ = –37.3 (*c* 1.0, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.28 (d, *J* = 8.1 Hz, 2H), 7.11 (d, *J* = 8.2 Hz, 2H), 5.10 (d, *J* = 7.6 Hz, 1H), 4.54 (d, *J* = 9.8 Hz, 1H), 4.43 (dd, *J* = 6.9, 4.9 Hz, 1H), 4.39 (dd, *J* = 9.8, 5.0 Hz, 1H), 3.93 (td, *J* = 12.4, 2.1 Hz, 2H), 3.75 (ddd, *J* = 21.9, 12.5, 5.3 Hz, 2H), 3.68 – 3.13 (m, 10H), 3.08 (dd, *J* = 13.9, 6.9 Hz, 1H), 2.86 (dd, *J* = 13.9, 9.9 Hz, 1H), 1.34 (s, 9H) ppm; **^{13}C NMR** (126 MHz, DMSO-*d*$_6$) δ 173.3, 170.9, 155.9, 155.2, 131.7, 130.0, 115.9, 100.5, 85.6, 81.1, 78.2, 78.1, 77.0, 76.7, 73.3, 70.1, 69.7, 61.4, 60.7, 56.3, 54.2, 36.7, 32.4, 28.2, 27.9 ppm; **IR** (cm^{-1}, neat): 3304, 1649, 1592, 1510, 1393, 1368, 1230, 1161, 1070, 1041, 893, 420; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{29}H_{43}N_2O_{16}S$ requires *m/z* 707.2333, found 707.2347.



Boc-Tyr-Cys(*S*-β-D-Glc)-OH (37). A 1-dram vial containing Boc-Tyr-Cys-OH (**35**; 76.9 mg, 0.2 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 72.8 mg, 0.4 mmol, 2.0 equiv.), $Ca(OH)_2$ (29.6 mg, 0.4 mmol, 2.0 equiv.), and a magnetic stir bar was charged H_2O (200 µL). The vial was sealed with a cap, and the reaction mixture was stirred vigorously at rt for 1 h. The mixture was then loaded onto a Biotage samplet, rinsing the reaction vial with H_2O, and purified via reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 25% MeOH/75% H_2O over 16 CV). Fractions containing a mixture of mono-glucosyl products (**37** and **38**) were combined, concentrated *in vacuo*, frozen, and lyophilized to dryness, resulting in a white powder. The product was further purified by reversed-phase preparative HPLC (Phenomenex Luna 5 µm C18(2) 250 x 21.2 mm column; λ = 254 nm; flow rate = 20 mL/min; solvent A = H_2O buffered with 0.1% formic acid; solvent B = CH_3CN buffered wth 0.1% formic acid; gradient = held at 95% solvent A/5% solvent B for 5 min, ramped from 95% solvent A/5% solvent B to 70% solvent A/30% solvent B over 40 min, ramped from 70% solvent A/30% solvent B to 5% solvent A/95% solvent B over 5 min, held at 5% solvent A/95% solvent B for 5 min). Fractions containing the product were combined, concentrated *in vacuo*, frozen, and lyophilized to dryness, resulting in a white powder (19 mg, 17% yield). $\mathbf{[\alpha]_D^{20}}$ = –21.6 (*c* 0.2, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.18 (d, *J* = 8.1 Hz, 2H), 6.87 (d, *J* = 8.1 Hz, 2H), 4.58 (t, *J* = 6.0 Hz, 1H), 4.54 (d, *J* = 9.8 Hz, 1H), 4.38 – 4.30 (m, 1H), 3.95 – 3.87 (m, 1H), 3.73 (dd, *J* = 12.5, 4.8 Hz, 1H), 3.56 – 3.41 (m, 3H), 3.37 – 3.23 (m, 2H), 3.16 – 2.99 (m, 2H), 2.86 (dd, *J* = 13.9, 9.2 Hz, 1H), 1.36 (s, 9H) ppm; **^{13}C NMR** (151 MHz, DMSO-d_6) δ 171.9, 171.8, 155.7, 155.1, 130.1, 128.2, 114.8, 85.1, 81.0, 78.0 (2), 73.1, 69.9, 61.1, 56.0, 52.9, 36.7, 30.8, 28.2 ppm; **IR** (cm^{-1}, neat): 3305, 2931, 1657, 1515, 1393, 1367, 1245, 1161, 1104, 1022, 806, 540, 420; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{23}H_{33}N_2O_{11}S$ requires *m/z* 545.1805, found 545.1811.

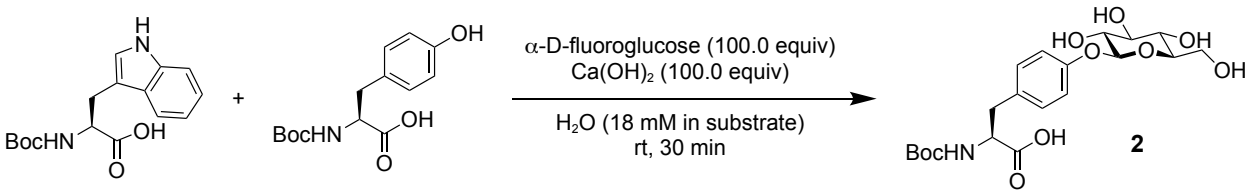


(Boc-Tyr(*O*-β-D-Glc)-Cys-OH)$_2$ (XV). A 10 mL round bottom flask containing (Boc-Tyr-Cys-OH)$_2$ (**XIV**; 100.0 mg, 0.13 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 237.4 mg, 1.3 mmol, 10.0 equiv.), $Ca(OH)_2$ (96.3 mg, 1.3 mmol, 10.0 equiv.), and a magnetic stir bar was charged with H_2O (1.3 mL, 0.1 M in substrate). The reaction flask was sealed with a septum, and the mixture was stirred vigorously at rt for 1 h. The resulting bright yellow mixture was then loaded onto a Biotage samplet, rinsing the reaction flask with H_2O, and purified via reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 40% MeOH/60% H_2O over 20 CV). Although separation of the product and the mono-glucosyl product was incomplete, fractions containing the pure product were combined, concentrated *in vacuo*, frozen, and lyophilized to dryness, resulting in a white powder (80 mg, 56% yield). Product present in mixed fractions was discarded in lieu of additional purification. $[\alpha]_D^{20}$ = –77.7 (*c* 0.5, 1:1 H_2O:MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.26 (d, *J* = 8.0 Hz, 4H), 7.08 (d, *J* = 8.1 Hz, 4H), 5.06 (d, *J* = 7.6 Hz, 2H), 4.54 (dd, *J* = 8.1, 4.2 Hz, 2H), 4.38 (bs, 2H), 3.92 (dd, *J* = 12.4, 2.2 Hz, 2H), 3.77 (dd, *J* = 12.5, 5.4 Hz, 2H), 3.67 – 3.44 (m, 8H), 3.34 – 3.10 (m, 4H), 3.04 (dd, *J* = 14.1, 8.4 Hz, 2H), 2.83 (dd, *J* = 14.0, 10.1 Hz, 2H), 1.31 (s, 18H) ppm; **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.9, 170.7, 155.9, 155.3, 131.9, 130.1, 115.8, 100.5, 78.0, 76.9, 76.6, 73.2, 69.7, 60.6, 56.5, 53.6, 44.1, 36.6, 28.2 ppm; **IR** (cm^{-1}, neat): 3329, 1681, 1633, 1589, 1511, 1390, 1367, 1317, 1229, 1164, 1044, 893, 832, 807, 452; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{46}H_{65}N_4O_{22}S_2$ requires *m/z* 1089.3532, found 1089.3523.

Boc-Tyr(*O*-β-D-Glc)-Cys-OH (38). A 20 mL vial containing (Boc-Tyr(*O*-β-D-glu)-Cys-OH)$_2$ (**XV**; 50.0 mg, 0.046 mmol, 1.0 equiv.) and a magnetic stir bar was charged with 1:1 H_2O:MeOH

(1.84 mL, 0.025 M). To the resulting suspension was added Na_2CO_3 (19.5 mg, 0.18 mmol, 4.0 equiv.), and the mixture was cooled in an ice-water bath with stirring. Dithiothreitol (DTT; 8.5 mg, 0.055 mmol, 1.2 equiv.) was added in one portion, and the mixture was stirred for 20 min. Formic acid (6.9 μL, 0.18 mmol, 4.0 equiv.) was added via pipet, and the mixture was stirred for 1 min. The mixture was then loaded onto a Biotage samplet, rinsing the reaction vial with H_2O, and purified via reversed-phase flash chromatography (60 g Ultra C18 column; 50 mL/min flow rate; mobile phase buffered with 0.1% formic acid; gradient = 5% CH_3CN/95% H_2O for 2 CV, ramped from 5% CH_3CN/95% H_2O to 50% CH_3CN/50% H_2O over 16 CV). Fractions containing the product were concentrated *in vacuo*, frozen, and lyophilized to dryness, resulting in a white powder (32 mg, 63% yield). $\mathbf{[\alpha]_D^{20}}$ = –29.3 (*c* 0.5, MeOH); **^{1}H NMR** (400 MHz, D_2O) δ 7.27 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.6 Hz, 2H), 5.10 (d, *J* = 7.5 Hz, 1H), 4.55 (s, 1H), 4.37 (dd, *J* = 8.8, 6.0 Hz, 1H), 3.94 (dd, *J* = 12.5, 2.2 Hz, 1H), 3.77 (dd, *J* = 12.5, 5.6 Hz, 1H), 3.67 – 3.47 (m, 4H), 3.22 – 3.03 (m, 1H), 3.02 – 2.88 (m, 3H), 1.36 (s, 9H) ppm; **^{13}C NMR** (126 MHz, DMSO-*d*$_6$) δ 171.9, 171.4, 156.0, 155.3, 131.3, 130.0, 115.9, 100.5, 78.2, 76.9, 76.6, 73.2, 69.7, 60.7, 55.9, 40.0, 36.4, 28.2, 25.6 ppm; **IR** (cm^{-1}, neat): 3304, 2930, 1658, 1510, 1392, 1367, 1303, 1229, 1162, 1072, 1041, 1016, 894, 830, 779, 506, 436; **HRMS** (ESI) *m/z* for $[M–H]^-$ $C_{23}H_{33}N_2O_{11}S$ requires *m/z* 545.1805, found 585.1812.

10. Competition Experiment between Boc-Trp-OH and Boc-Tyr-OH

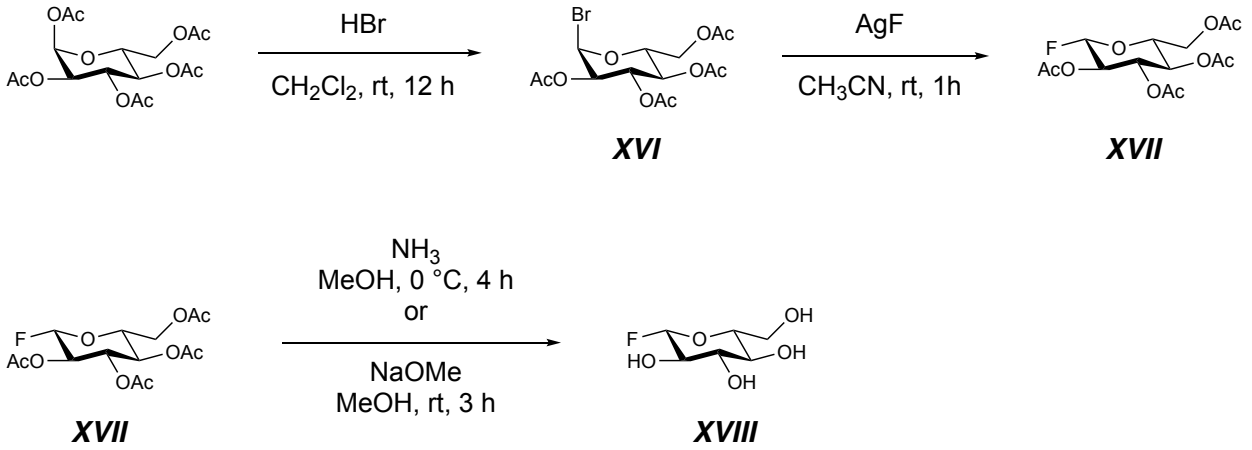


***N*-Boc-*O*-β-D-glucopyranosyl-L-tyrosine (2)**. A 4 mL vial containing Boc-Trp-OH (10.0 mg, 0.033 mmol, 1.0 equiv.), Boc-Tyr-OH (9.3 mg, 0.033 mmol, 1.0 equiv.), α-D-fluoroglucose (**II**; 601.1 mg, 3.3 mmol, 100.0 equiv.), $Ca(OH)_2$ (244.5 mg, 3.3 mmol, 100.0 equiv.), and a magnetic stir bar was charged with H_2O (1.83 mL). The vial was sealed with a septum, and the mixture was stirred vigorously at rt for 30. The mixture was then loaded onto a Biotage samplet, rinsing the reaction flask with H_2O, and purified via reversed-phase flash chromatography (60 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H_2O to 50% MeOH/50% H_2O over 20 CV, ramped from 50% MeOH/50% H_2O to 100% MeOH/0% H_2O over 10 CV). Fractions containing the product were concentrated *in vacuo*, frozen, and lyophilized to dryness to yield a white solid (13.0 mg, 89% yield). Spectral data match those previously reported in page 23.

Note that Boc-Tyr-OH was exclusively glycosylated under the calcium-mediated glycosylation conditions in the presence of Boc-Trp-OH.

11. Investigation of Stereospecificity and Reactivity of Glycosylation Using β-D-Fluoroglucose

11.1. Synthesis of β-D-Fluoroglucose



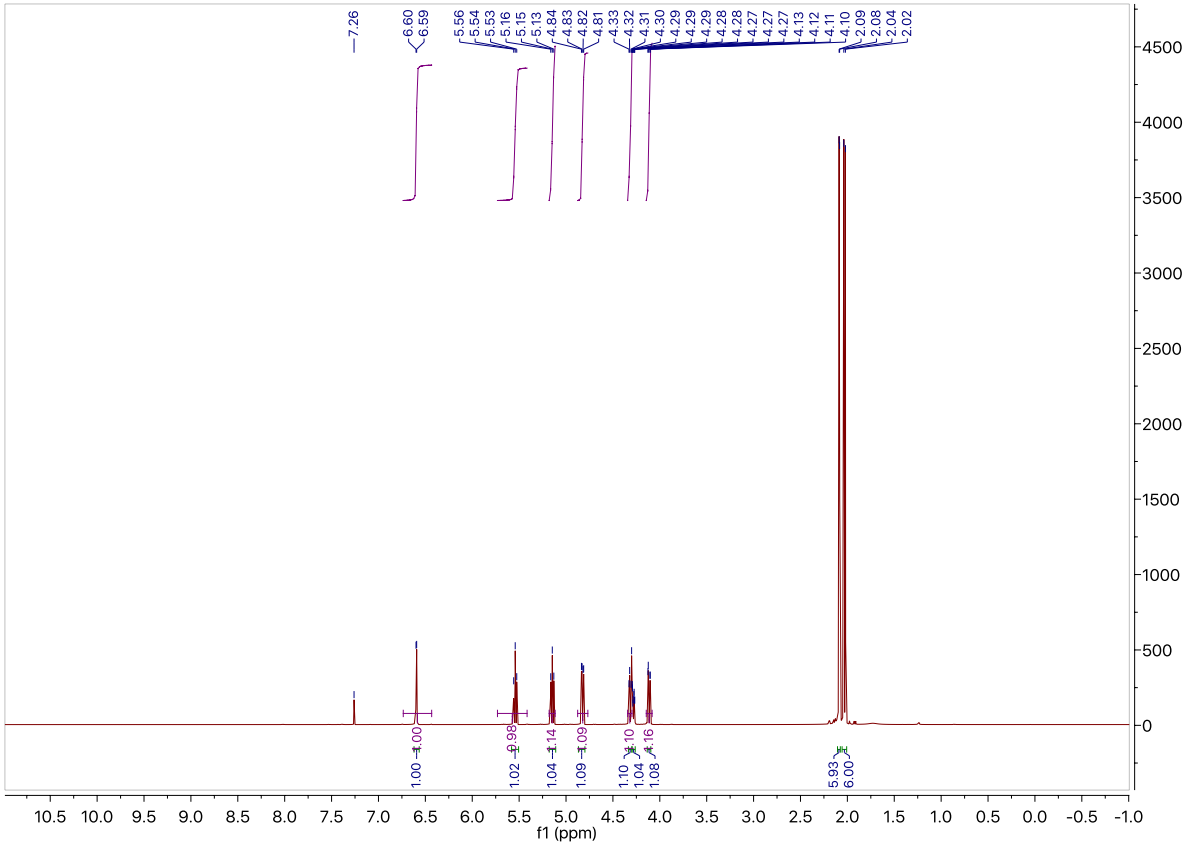
XVI ***XVII*** ***XVII*** ***XVIII***

α-D-Bromoglucose tetraacetate (XVI). To a solution of peracylated D-glucose (780.0 mg, 2.0 mmol) in anhydrous CH_2Cl_2 (7.8 mL), hydrobromic acid (33% w/w in AcOH, 4 mL) was added dropwise at 0 °C. The reaction mixture was gradually warmed to rt and stirred for 2 h. The reaction mixture was diluted with H_2O (10 mL) and CH_2Cl_2 (10 mL). The layers were separated. The organic layer was washed with saturated $NaHCO_3$ solution (2 X 10 mL) and brine (10 mL). The organics were then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude solid was purified via silica gel chromatography (eluted with EtOAc:Hexanes = 1:2) to obtain glucosyl bromide as the alpha isomer (699.0 mg, 85% yield). Spectral data match those previously reported.[14]

α-D-Bromoglucose tetraacetate (XVI); ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.60 (d, *J* = 4.0 Hz, 1H), 5.54 (t, *J* = 9.7 Hz, 1H), 5.15 (t, *J* = 9.8 Hz, 1H), 4.82 (dd, *J* = 10.0, 4.1 Hz, 1H), 4.31 (dd, *J* = 12.5, 4.1 Hz, 1H), 4.11 (dd, *J* = 12.5, 1.9 Hz, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H).

[14] Machida, T.; Novoa, A.; Gillon, E.; Zheng, S.; Claudinon, J.; Eierhoff, T.; Imberty, A.; Romer, W.; Winssinger, N. *Angew. Chem. Int. Ed*. **2017**, *56*, 6762–6766

^{1}H NMR spectrum of α-D-bromoglucose tetraacetate (**XVI**, 400 MHz, $CDCl_3$, 25 °C)

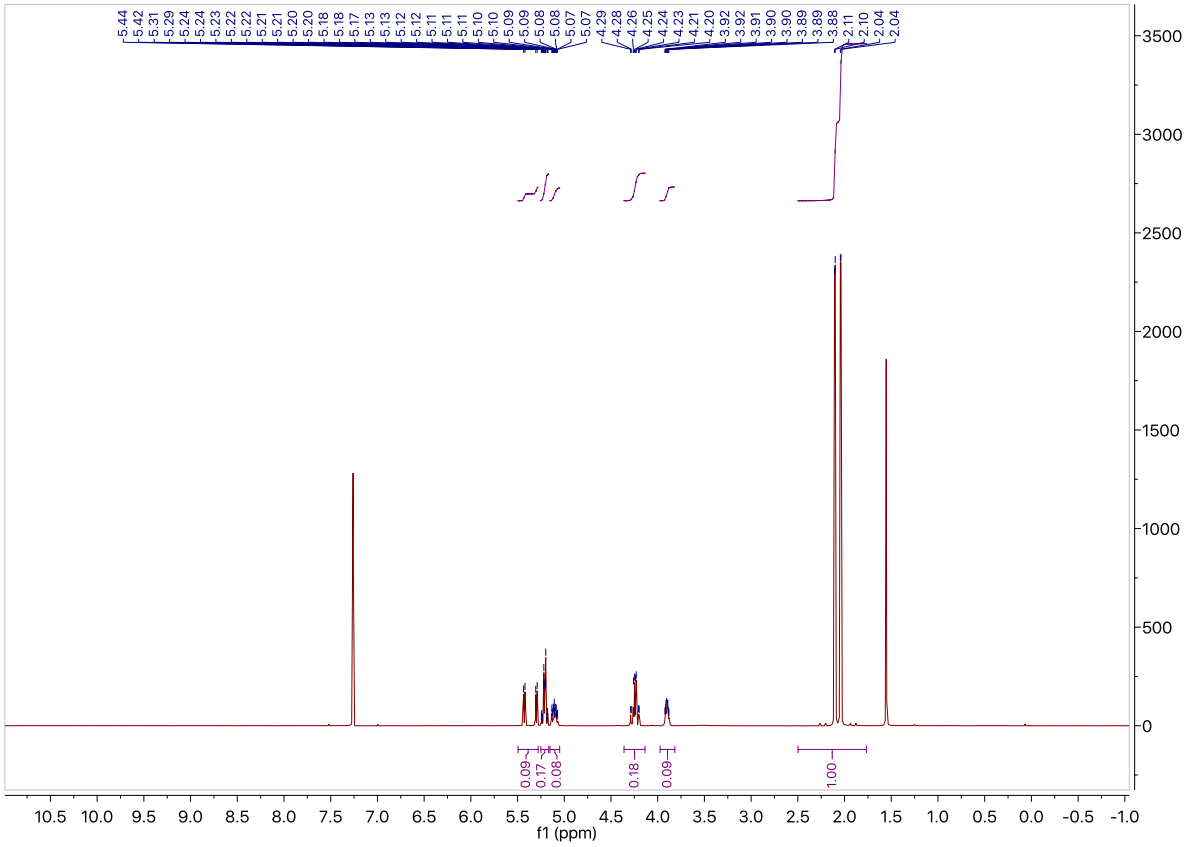


β-D-Fluoroglucose tetraacetate (XVII). To a solution of α-D-bromoglucose tetraacetate (300.0 mg, 0.73 mmol, 1.0 equiv.) in anhydrous acetonitrile (1.0 mL) was added AgF (300.0 mg, 2.36 mmol, 6.4 equiv.). The resulting heterogenous mixture was stirred at rt for 1 h under N_2. The mixture was then filtered over celite to remove the insoluble solids. The pad was eluted with acetonitrile (10 mL). The solvent was concentrated under reduced pressure to give a white solid and further dried on high-vacuum for 3 h before carrying on to the next step without purification (yield calculated over the next step). Spectral data match those previously reported.[15]

β-D-Fluoroglucose tetraacetate (XVII): ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.36 (dd, J = 52.0, 6.0 Hz, 1H), 5.25–5.17 (m, 2H), 5.15–5.05 (m, 1H), 4.36–4.14 (m, 2H), 3.90 (dp, J = 7.4, 2.6 Hz, 1H), 2.07 (dd, J = 24.8, 2.7 Hz, 12H); ^{19}F NMR (376 MHz, $CDCl_3$): δ –137.85 (dd, J = 51.8, 10.8 Hz).

[15] Møller, B. L.; Olsen, C. E.; Motawia, M. S. *J. Nat. Prod*. **2016**, *79*, 1198–1202.

^{1}H NMR spectrum of β-D-Fluoroglucose tetraacetate (**XVII**, 400 MHz, $CDCl_3$, 25 °C)



β-D-Fluoroglucose tetraacetate ***(XVII)*** *may be deacetylated to give β-D-fluoroglucose* ***(XVIII)*** *either with alcoholic ammonia (procedure A) or with a catalytic amount of sodium methoxide in alcohol (procedure B).* ***β-D-fluoroglucose was prepared just before use****.*

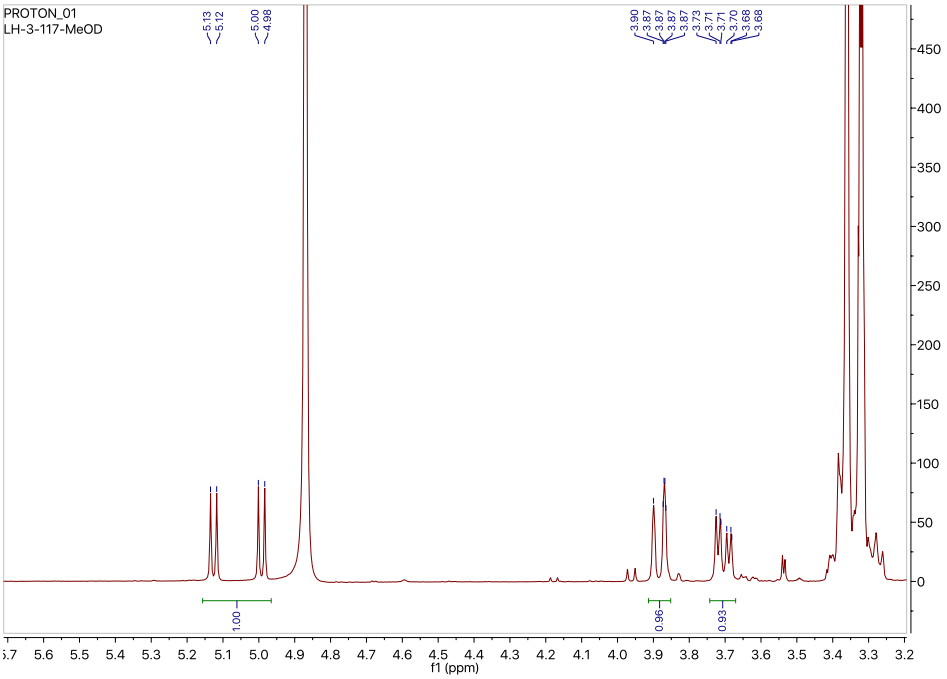
Procedure A: To a flask containing β-D-fluoroglucose tetraacetate (**XVII**, 300.0 mg, 0.86 mmol, 1.0 equiv.) and absolute methanol (6.1 mL) was added 7 M ammonia-methanol (2.5 mL, 20 equiv. ammonia) at 0 °C. The resulting mixture was stirred at 0 °C for 4 h. The solvent was removed by rigorous N_2 flow and dried in vacuo on ice. The resulting clear oil was dissolved in 7:3 EtOAc:MeOH (5 mL) and then silica gel (~5 g) was added. The resulting suspension was stirred for 10 min, then concentrated to a thick paste in vacuo. To the reaction flask was added 7:3 EtOAc:MeOH (30 mL), and the resulting suspension was stirred for 5 min. The mixture was filtered through a fritted funnel and rinsed with 7:3 EtOAc:MeOH (30 mL). The filtrate was concentrated to dryness in vacuo, giving β-D-fluoroglucose **(XVIII)** as a clear oil (131.0 mg, 84% yield). *β-D-fluoroglucose was prepared just before use*.[16]

Procedure B: To a flask containing β-D-fluoroglucose tetraacetate (**XVII**, 255.7 mg, 0.73 mmol, 1.0 equiv.) was added absolute MeOH (3.7 mL) and a magnetic stir bar. The resulting colorless solution was stirred at 0 °C until the substrate was fully dissolved. NaOMe (3.9 mg, 0.073 mmol, 0.1 equiv.) was added in one portion. The resulting mixture was stirred at for 3 h. The reaction flask was cooled in an ice-water bath, and silica gel (~5 g) was added. The resulting suspension was stirred for 10 min, then concentrated to a thick paste in vacuo. To the reaction flask was added 7:3 EtOAc:MeOH (30 mL), and the resulting suspension was stirred for 5 min. The mixture was filtered through a fritted funnel and rinsed with 7:3 EtOAc:MeOH (30 mL). The filtrate was concentrated to dryness in vacuo, giving β-D-fluoroglucose **(XVIII)** as a clear oil (100.0 mg, 75% yield). *β-D-fluoroglucose was prepared just before use*.[16]

β-D-fluoroglucose (XVIII); ^{1}H NMR (400 MHz, D_2O): δ 5.09 (dd, *J* = 53.1, 7.1 Hz, 1H), 3.77 (ddd, *J* = 12.4, 2.3, 1.0 Hz, 1H), 3.62 (ddd, *J* = 12.5, 5.5, 1.0 Hz, 1H). 3.44–3.26 (m, 4H); ^{19}F NMR (376 MHz, D_2O): δ –143.73 (dd, *J* = 53.2, 13.6 Hz).

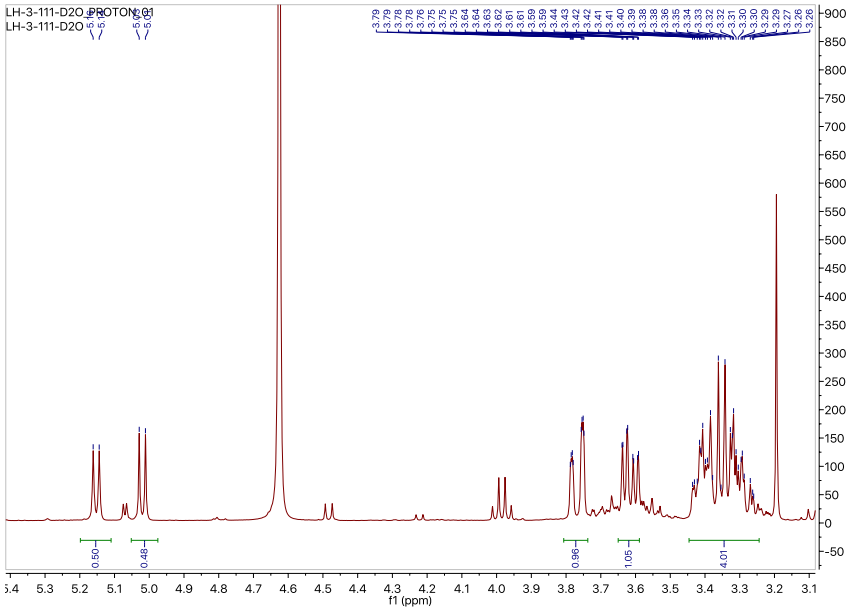
[16] Yamamoto, K.; Davis, B. G. *Angew. Chem. Int. Ed*. **2012**, *51*, 7449–7453.

^{1}H NMR spectrum of the crude reaction mixture of β-D-fluoroglucose **(XVIII)** prepared using Procedure A (**XVIII**, 400 MHz, MeOD, 25 °C)

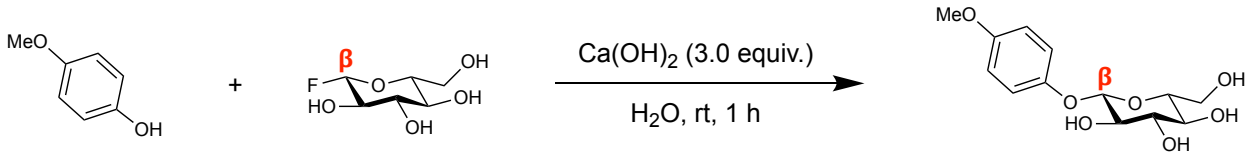


Note that the peaks at 3.44–3.26 ppm overlap with MeOD and MeOH signals.

^{1}H NMR spectrum of the crude reaction mixture of β-D-fluoroglucose (**XVIII**) prepared using Procedure B (**XVIII**, 400 MHz, D_2O, 25 °C)



11.2 Glycosylation of 4-Methoxyphenol with β-D-Fluoroglucose



XVIII ***6***

4-Methoxyphenyl-β-D-glucopyranoside (6). A dram vial containing 4-methoxyphenol (25.0 mg, 0.20 mmol, 1.0 equiv.), β-D-fluoroglucose (**XVIII**, 109.29 mg, 0.60 mmol, 3.0 equiv.), $Ca(OH)_2$ (44.45 mg, 0.60 mmol, 3.0 equiv.), and a magnetic stir bar was charged with H_2O (200 µL, 1.0 M). The vial was sealed with a septum, and the mixture was stirred vigorously at rt for 1 h. The mixture was then loaded onto a Biotage samplet, rinsing the reaction flask with H_2O, and purified via reversed-phase flash chromatography (120 g C18 column; 50 mL/min flow rate; gradient = 0% MeOH/100% H_2O for 3 CV, ramped from 0% MeOH/100% H2O to 50% MeOH/50% H2O over 12 CV). Fractions containing the product were concentrated in vacuo, frozen, and lyophilized to dryness to give a white solid (14.7 mg, 26% yield). Spectral data match those previously reported in Page 25.

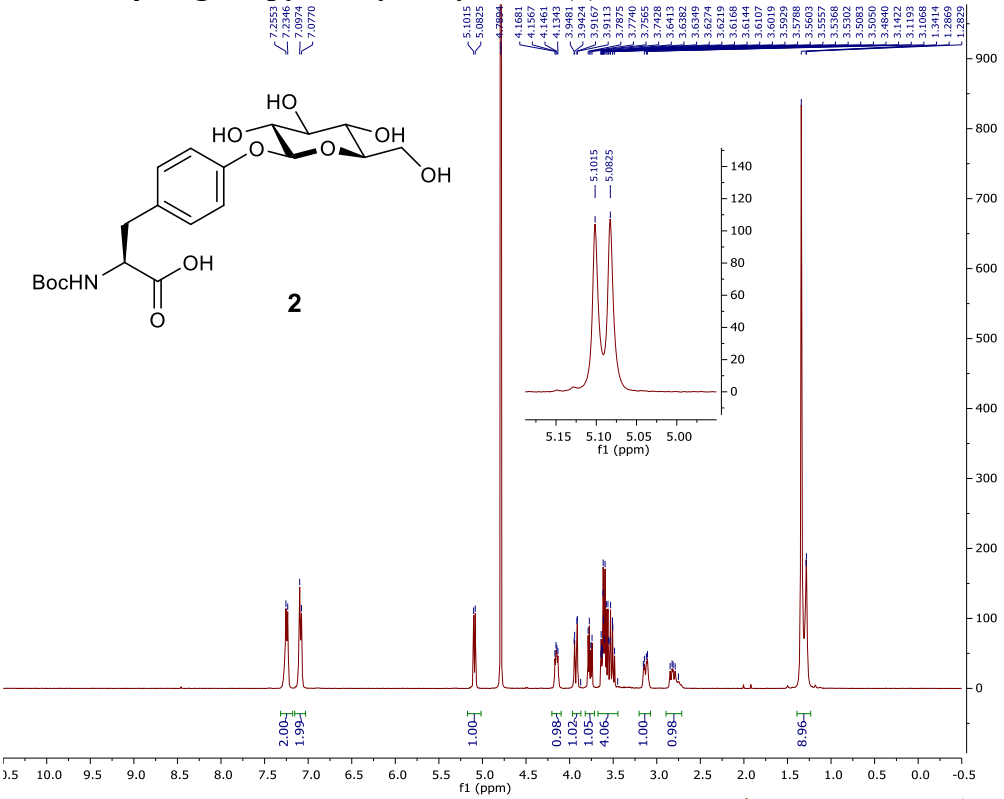
Note that upon mixing β-D-fluoroglucose (1.0 equiv.) and $Ca(OH)_2$ (1.0 equiv) in D_2O (0.7 mL), the fluoroglucose underwent hydrolysis to D-glucose, as demonstrated by ^1H NMR analysis.

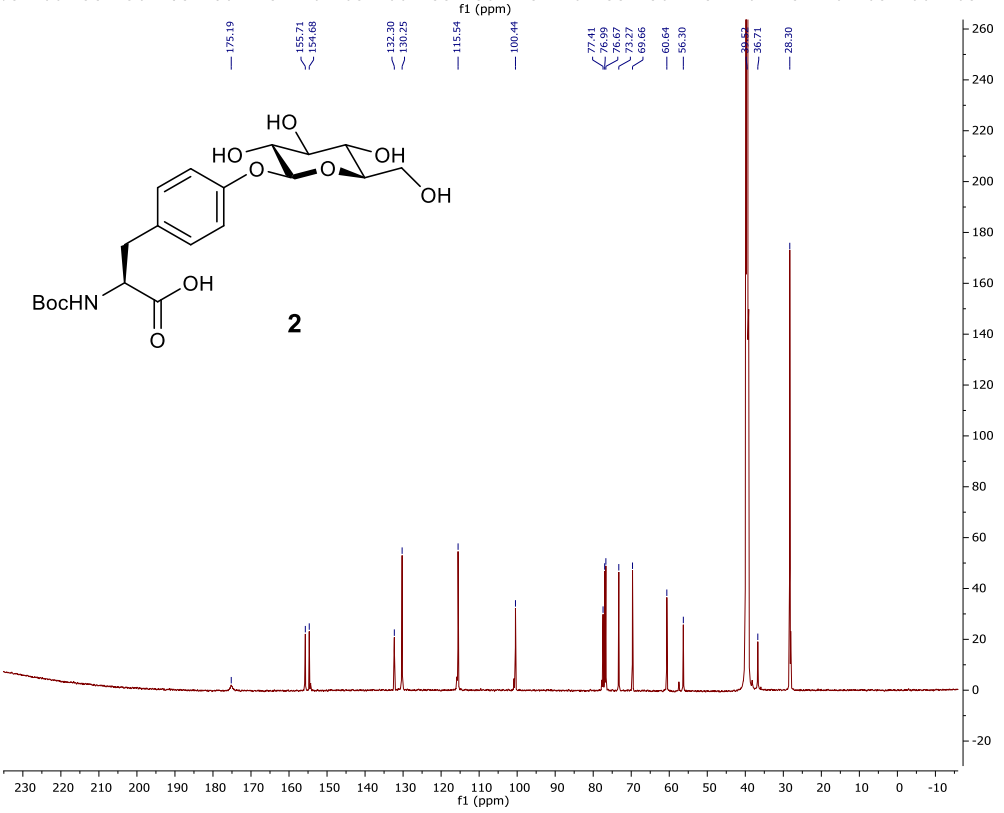
*Using β-D-fluoroglucose, retention in the stereochemistry at the anomeric center of **6** was observed. This observation may be explained by double displacement from C2 of β-D-fluoroglucose via the formation of epoxide intermediate.*[17]

[17] *Micheel, F.; Klemer, A. Eur. J. Inorg. Chem. **1958**, 91, 194–197.*

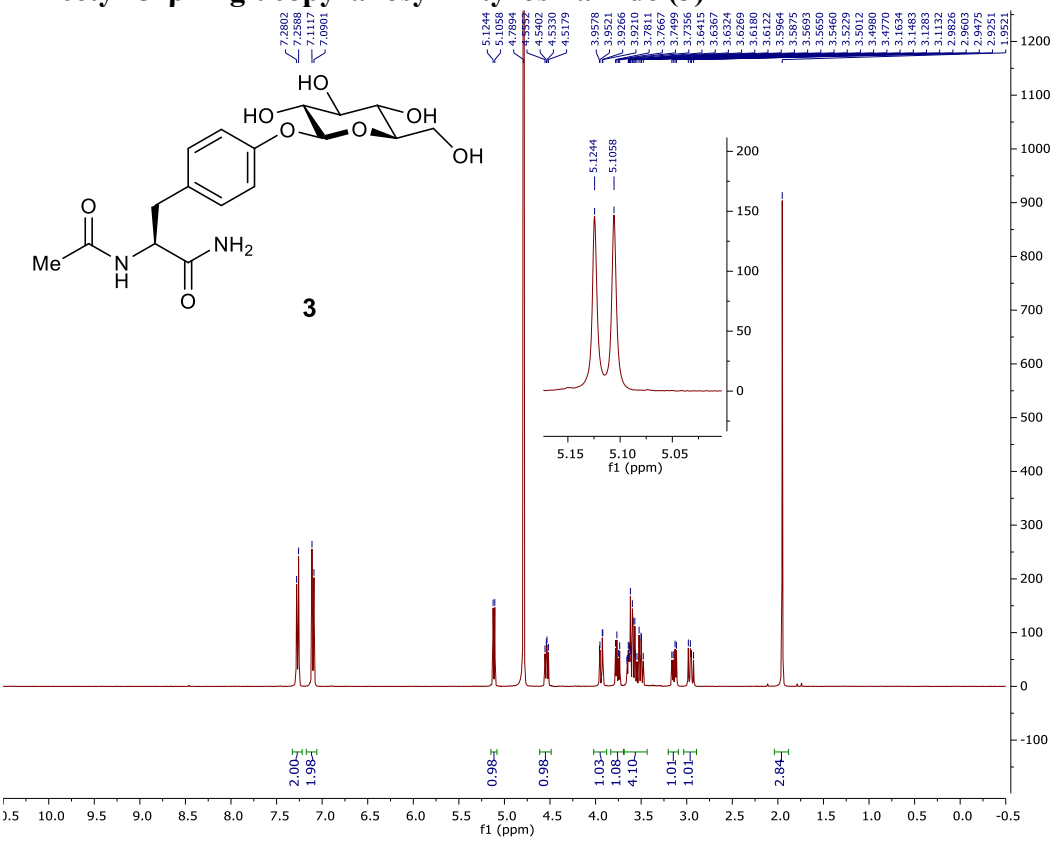
12. NMR Spectra of Synthesized Compounds

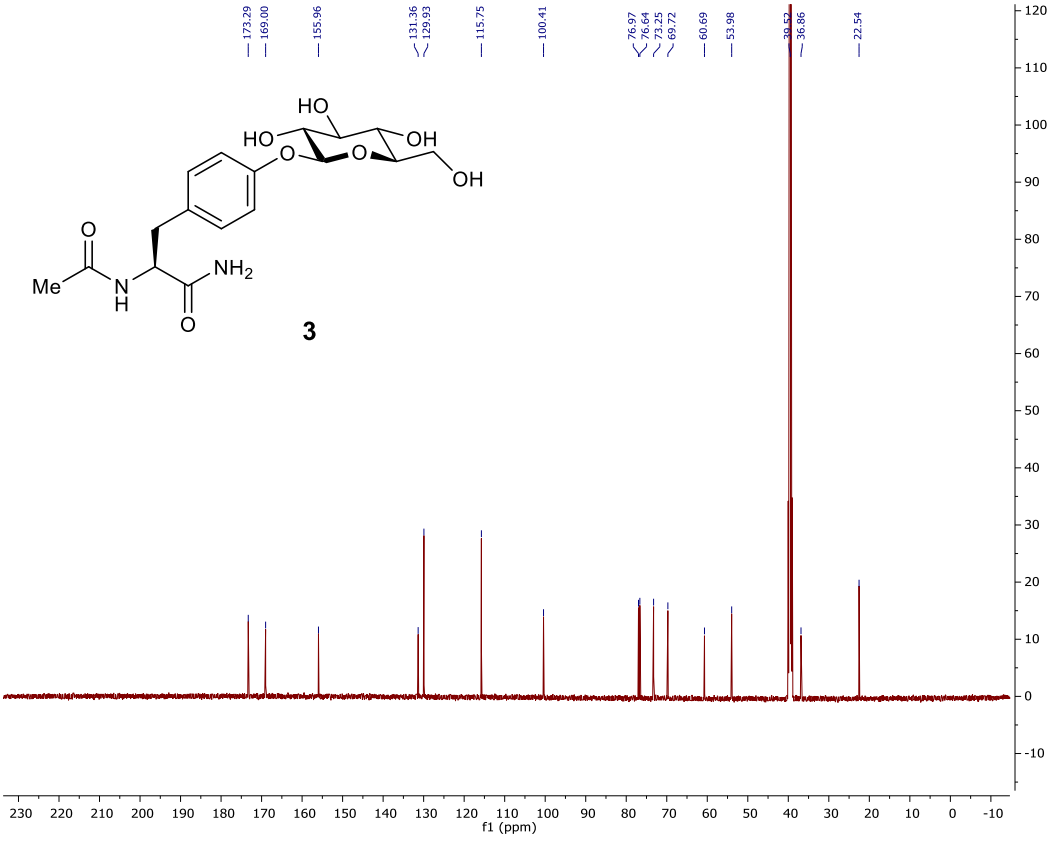
N-Boc-*O*-β-D-glucopyranosyl-L-tyrosine (2)



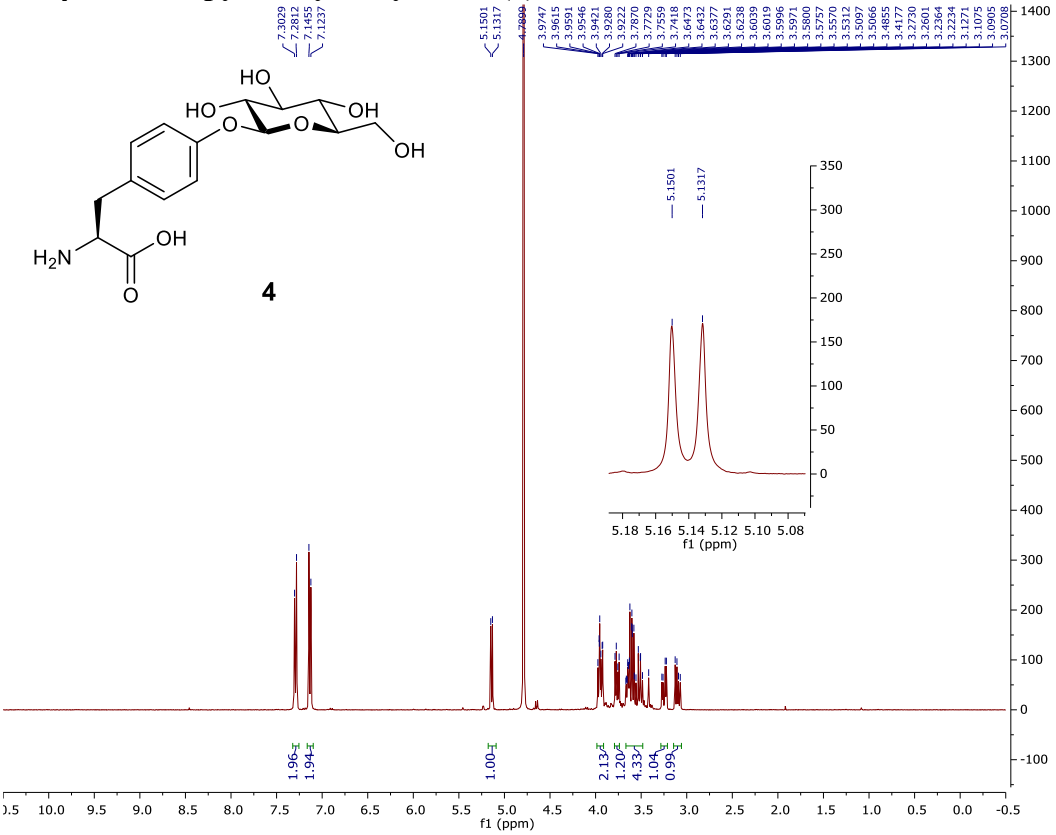


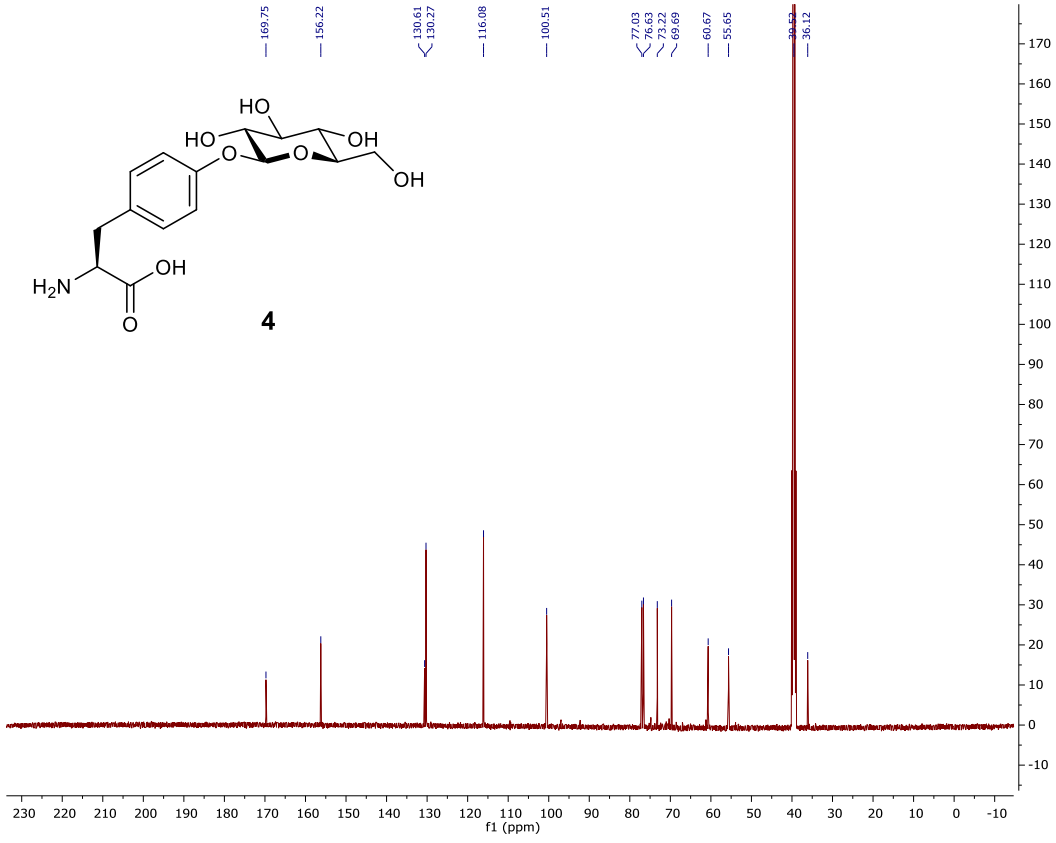
N-Acetyl-*O*-β-D-glucopyranosyl-L-tyrosinamide (3)



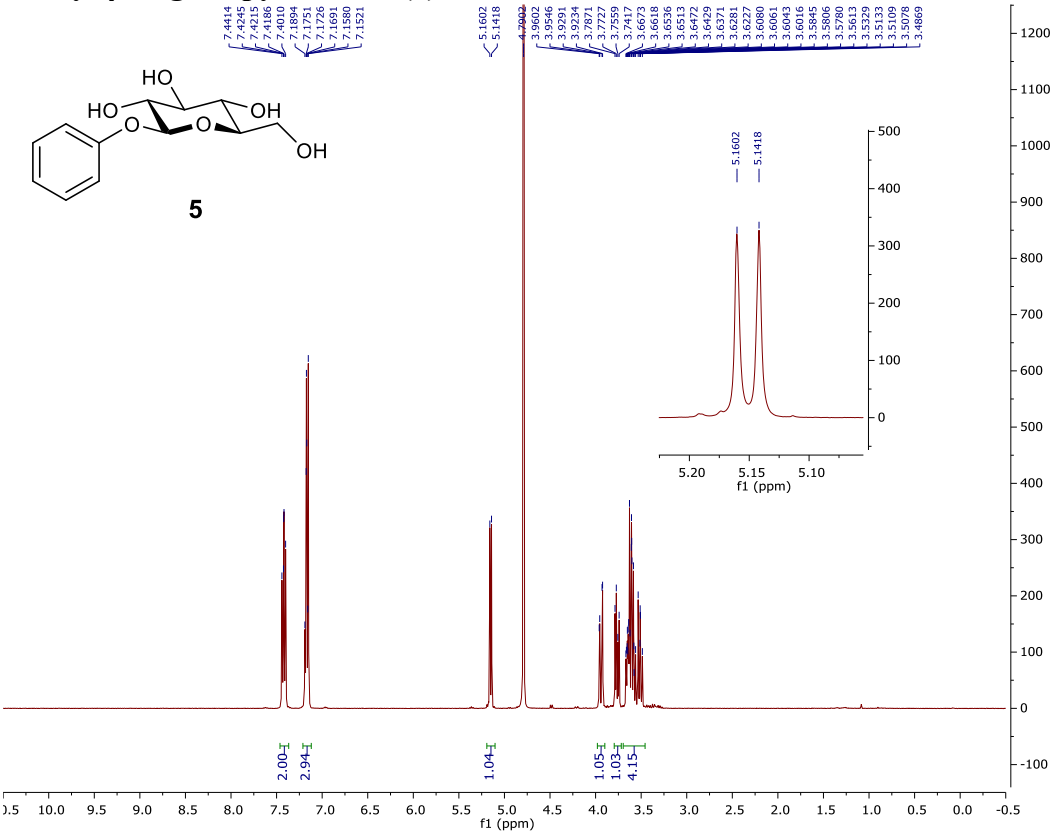


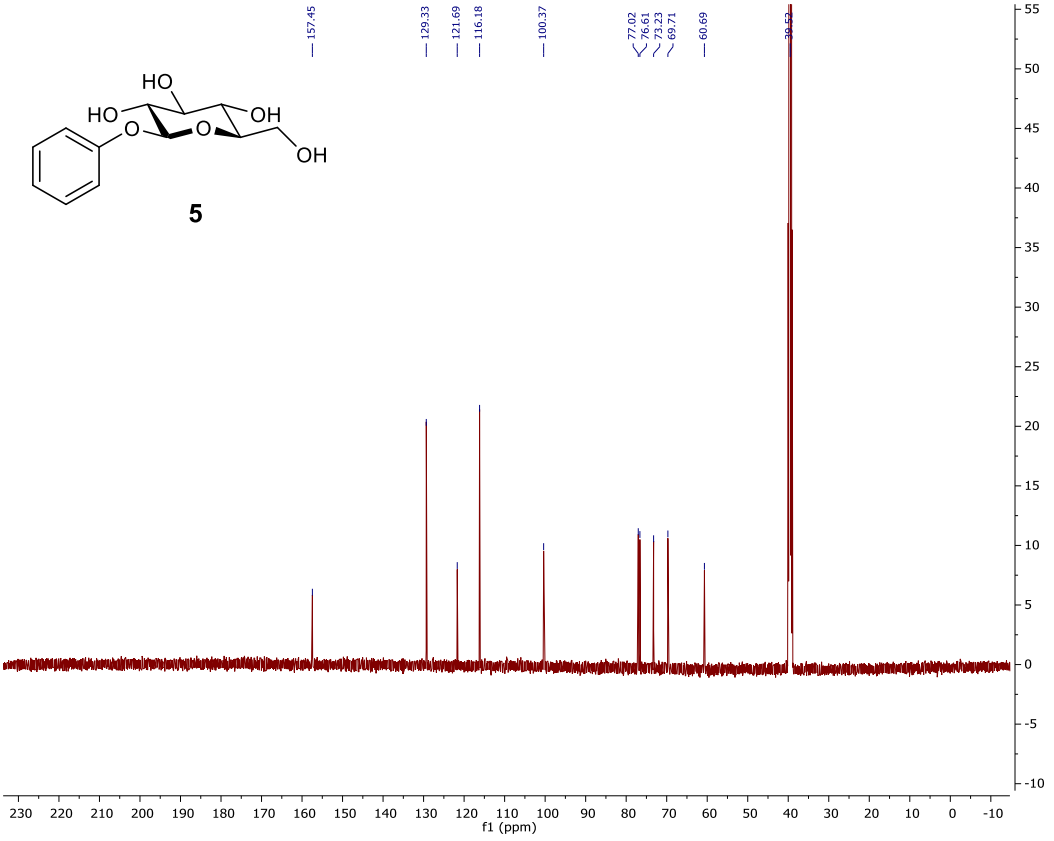
O-β-D-Glucopyranosyl-L-tyrosine (4)



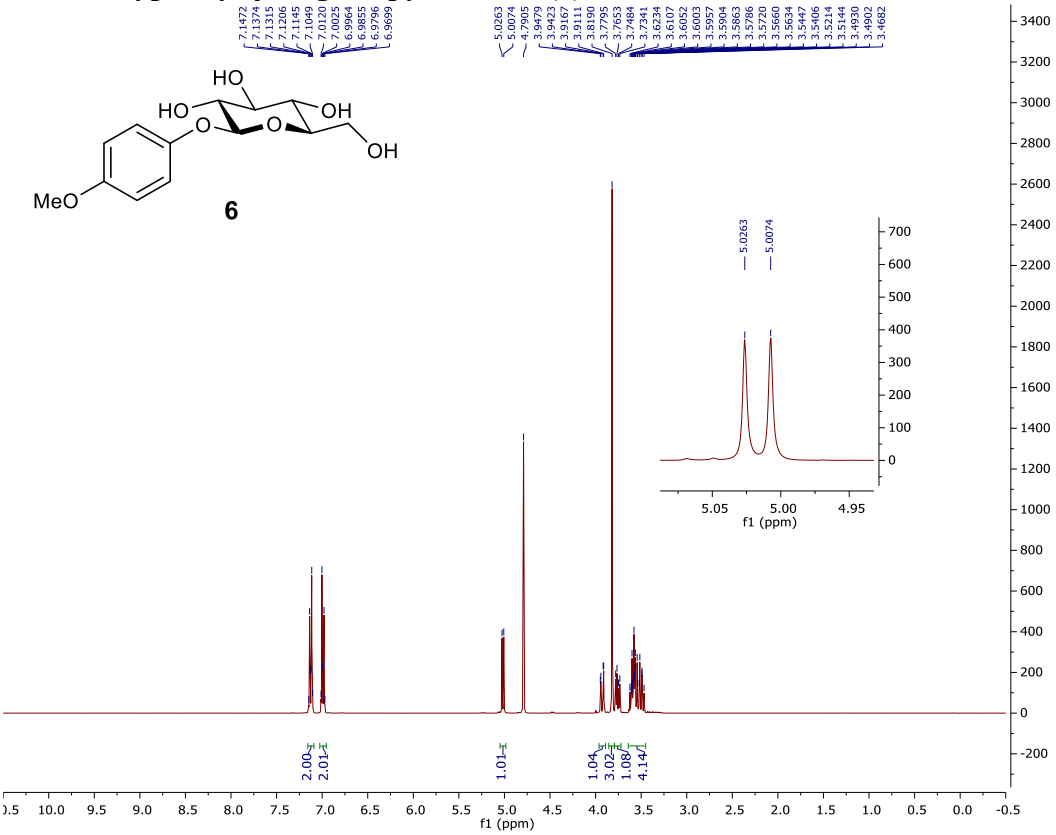


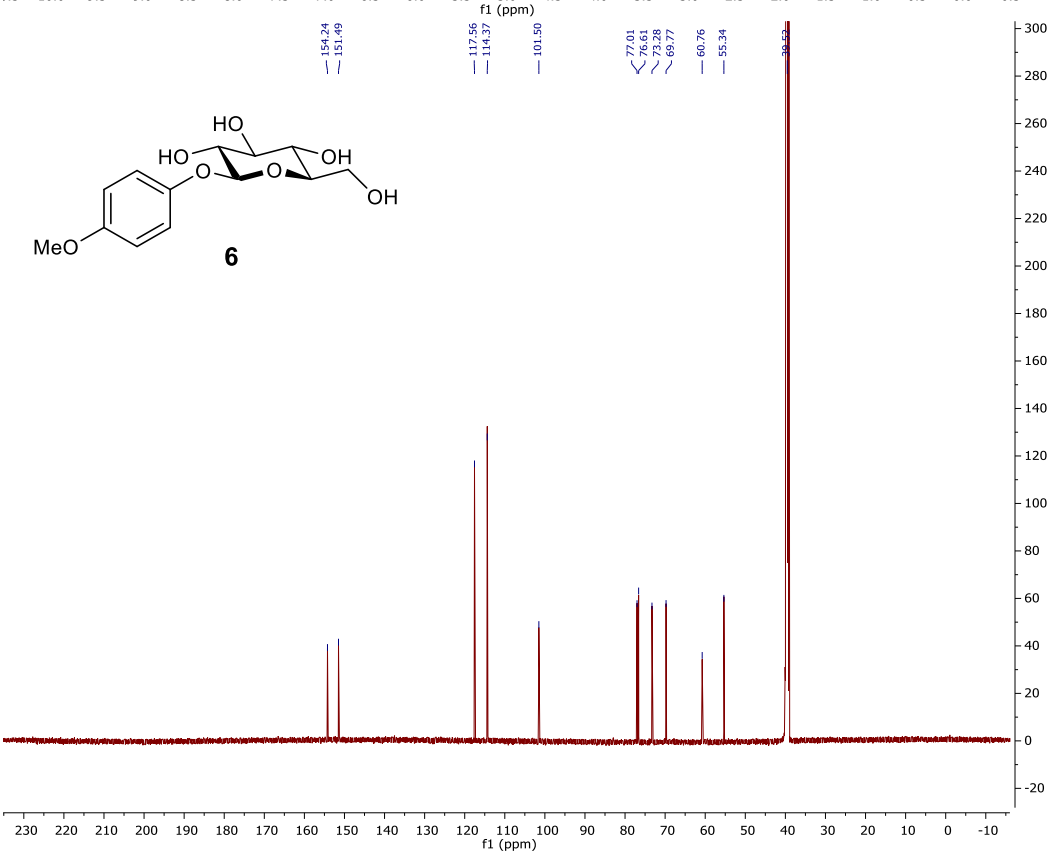
Phenyl-β-D-glucopyranoside (5)



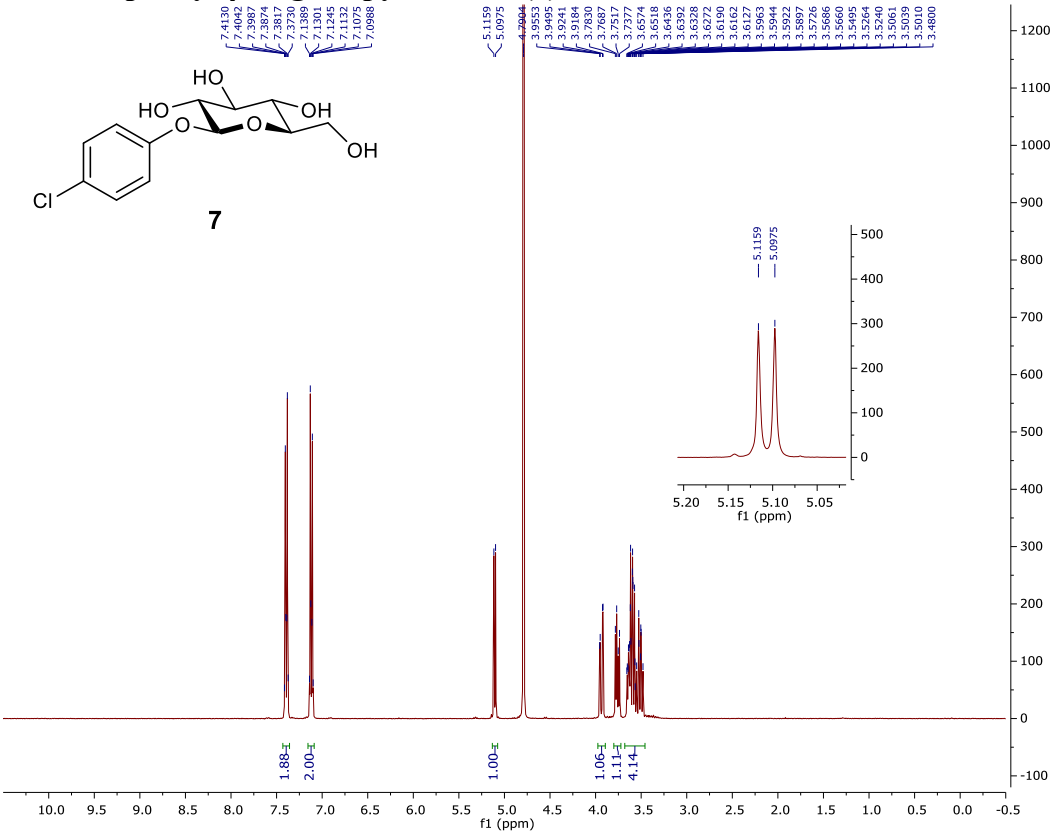


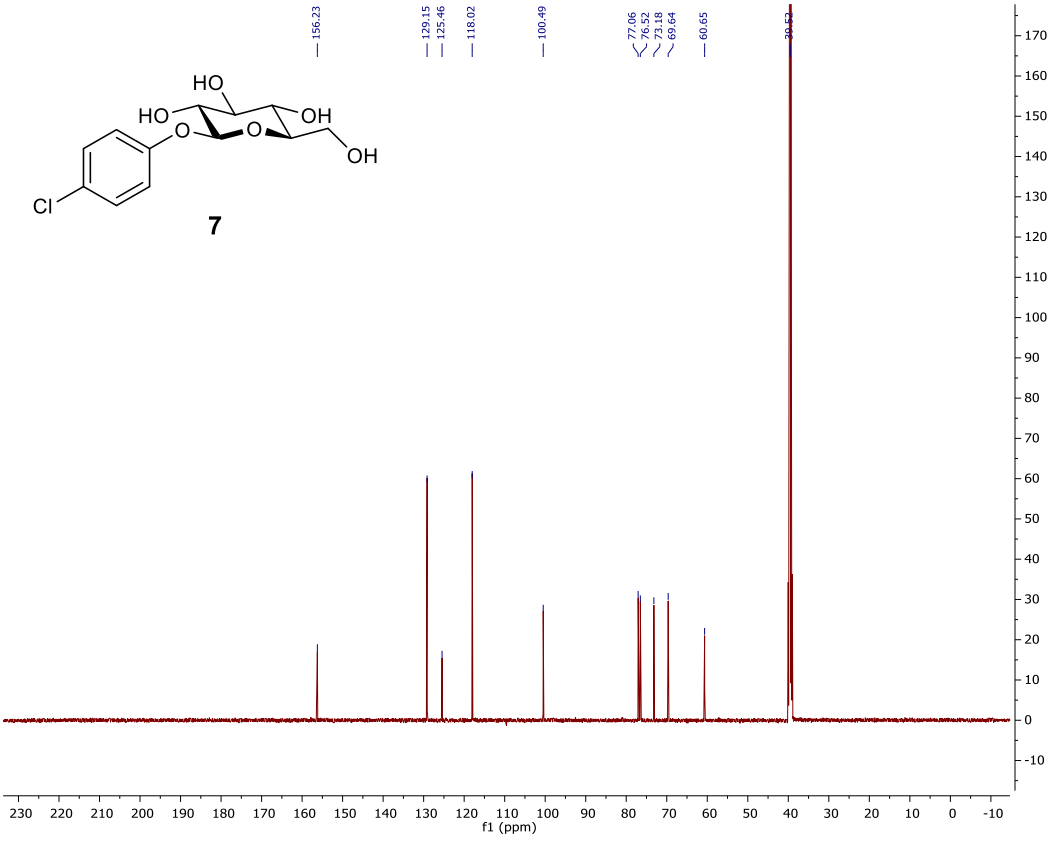
4-Methoxyphenyl-β-D-glucopyranoside (6)



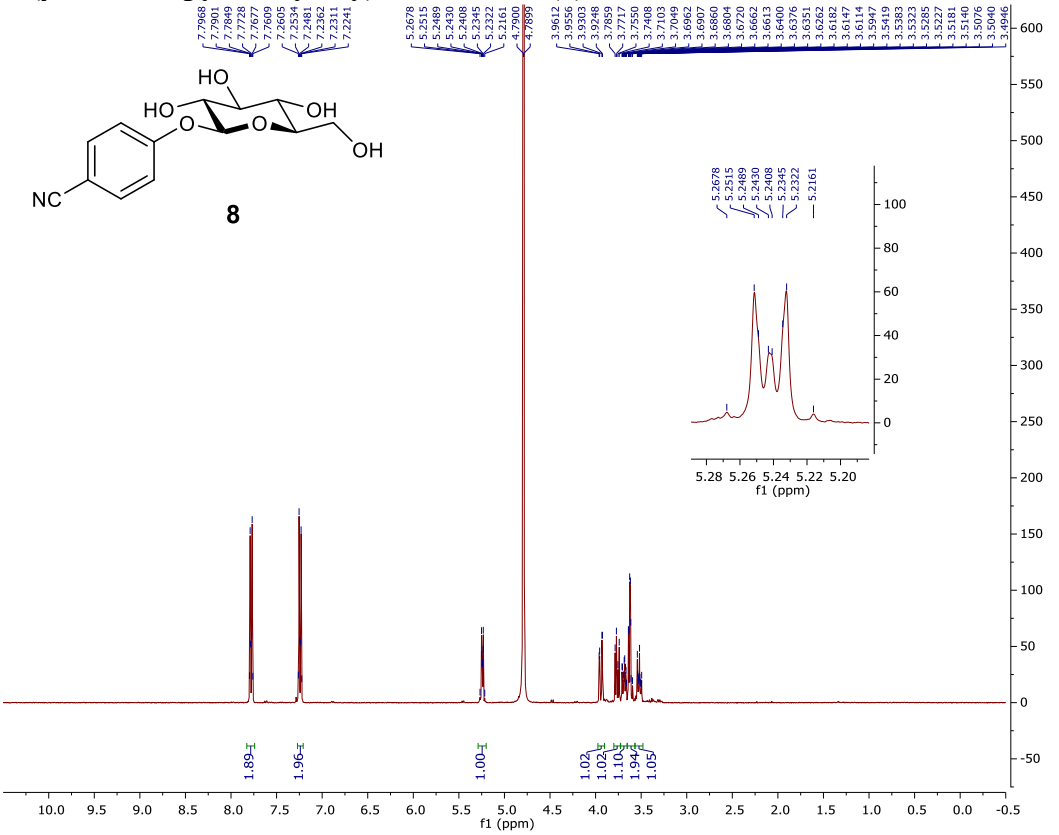


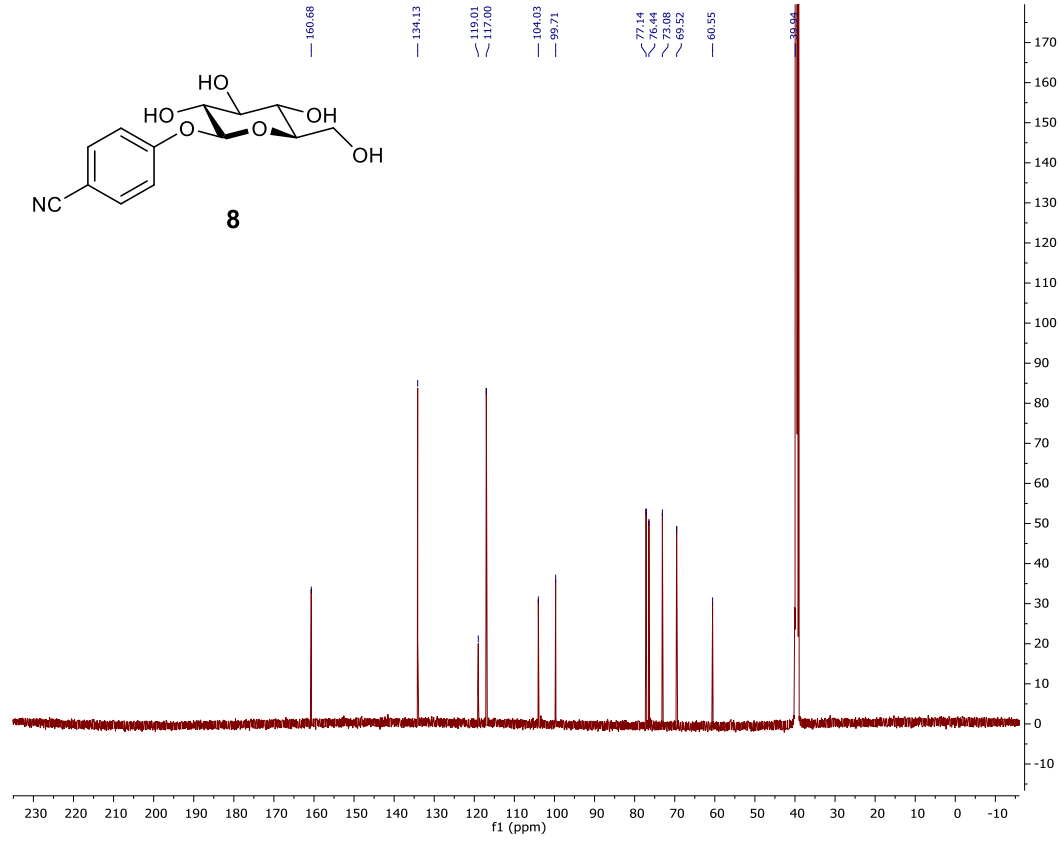
4-Chlorophenyl-β-D-glucopyranoside (7)





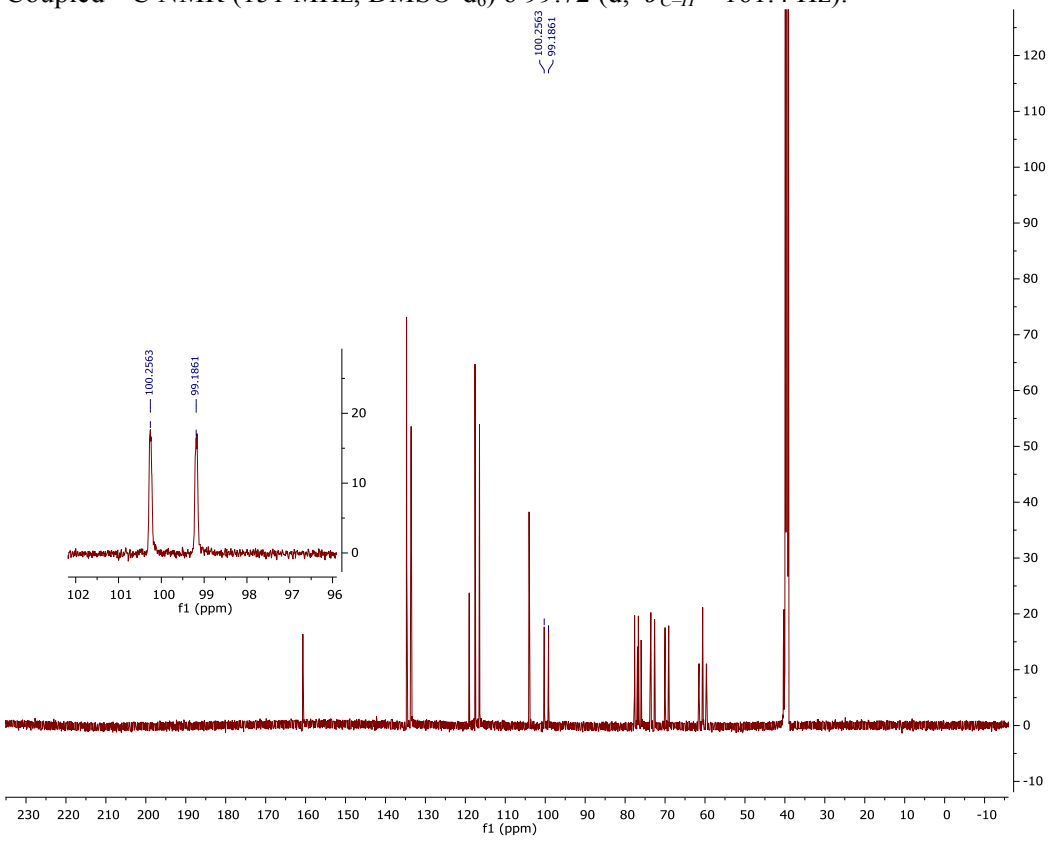
4-(β-D-Glucopyranosyloxy)benzonitrile (8)



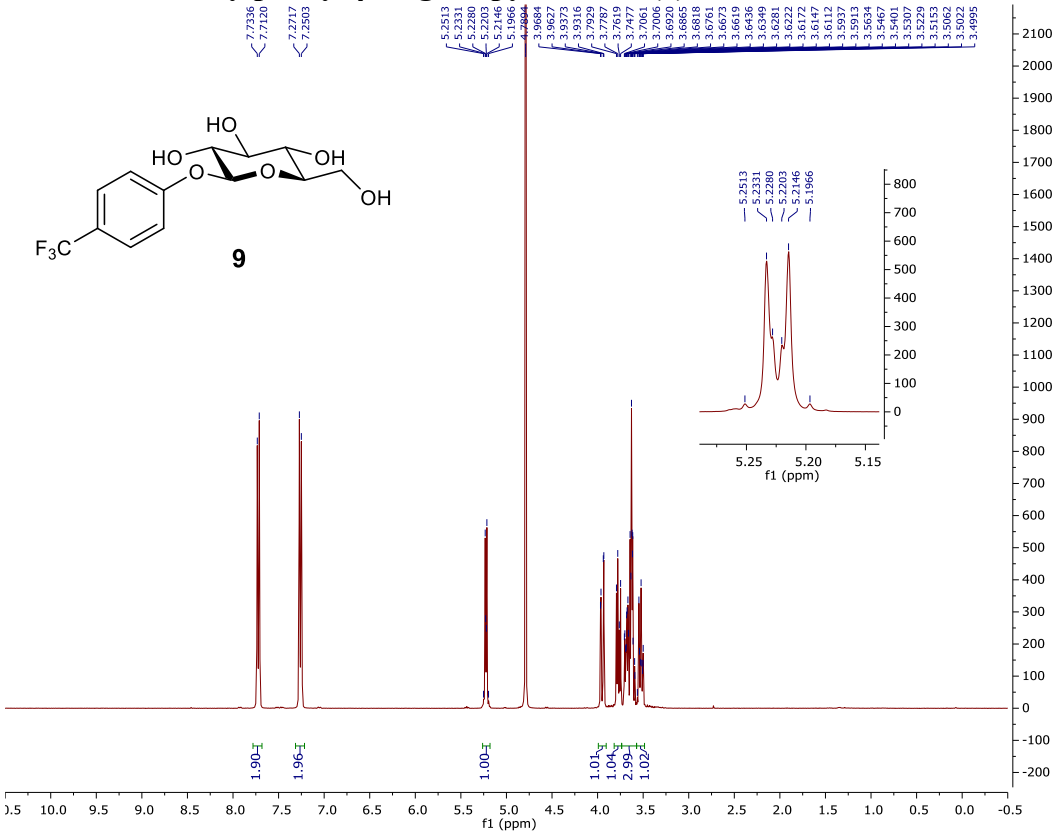


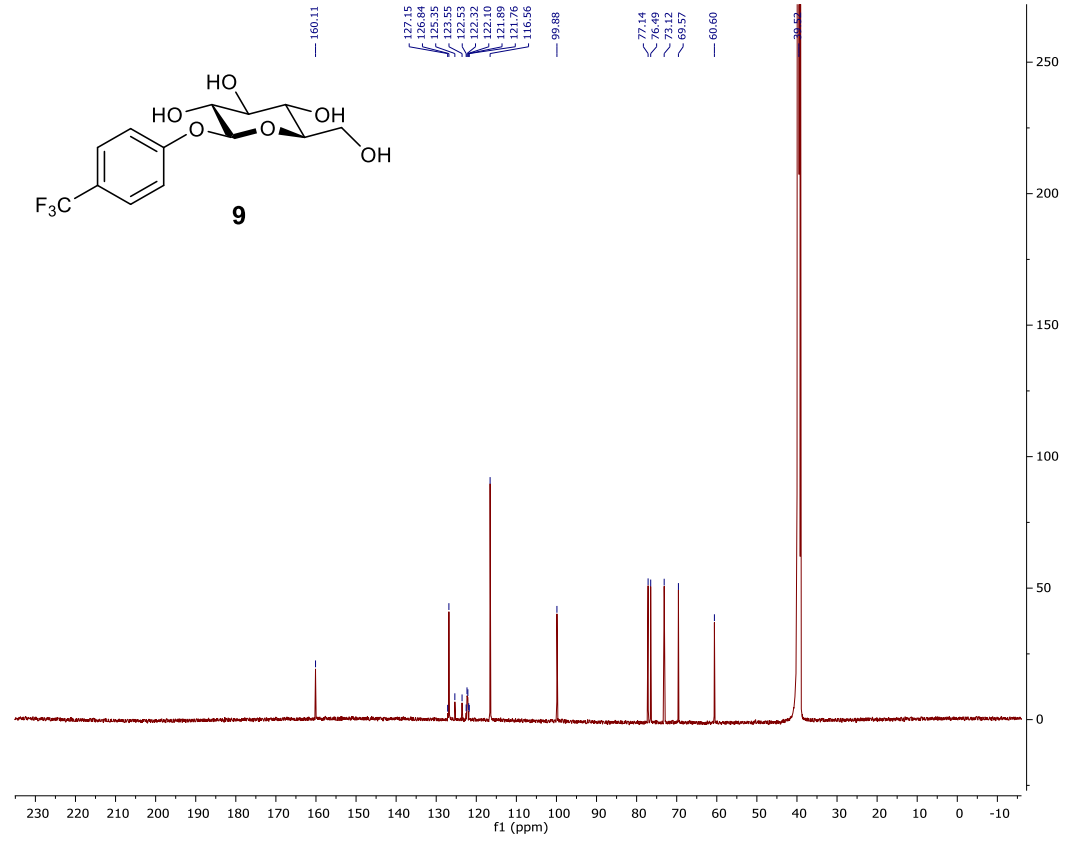
4-(β-D-Glucopyranosyloxy)benzonitrile (8)

Coupled ^{13}C NMR (151 MHz, DMSO-d_6) δ 99.72 (d, $^{1}J_{C-H}$ = 161.4 Hz).



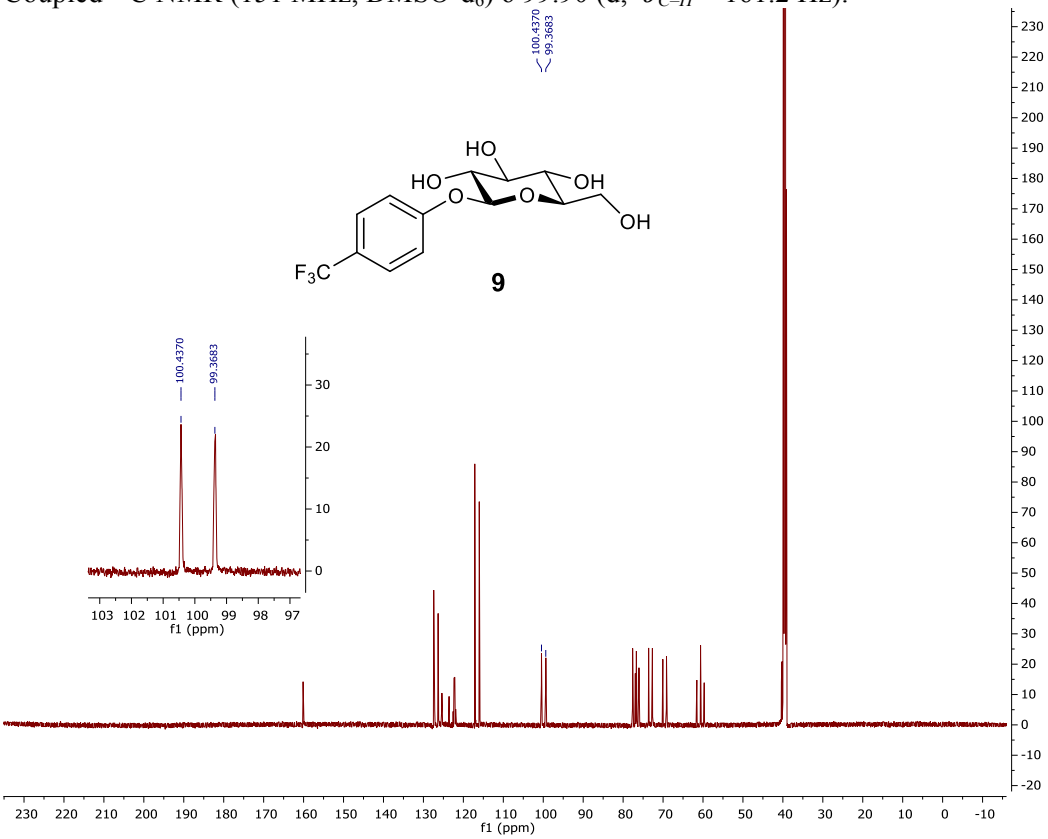
4-Trifluoromethylphenyl-β-D-glucopyranoside (9)

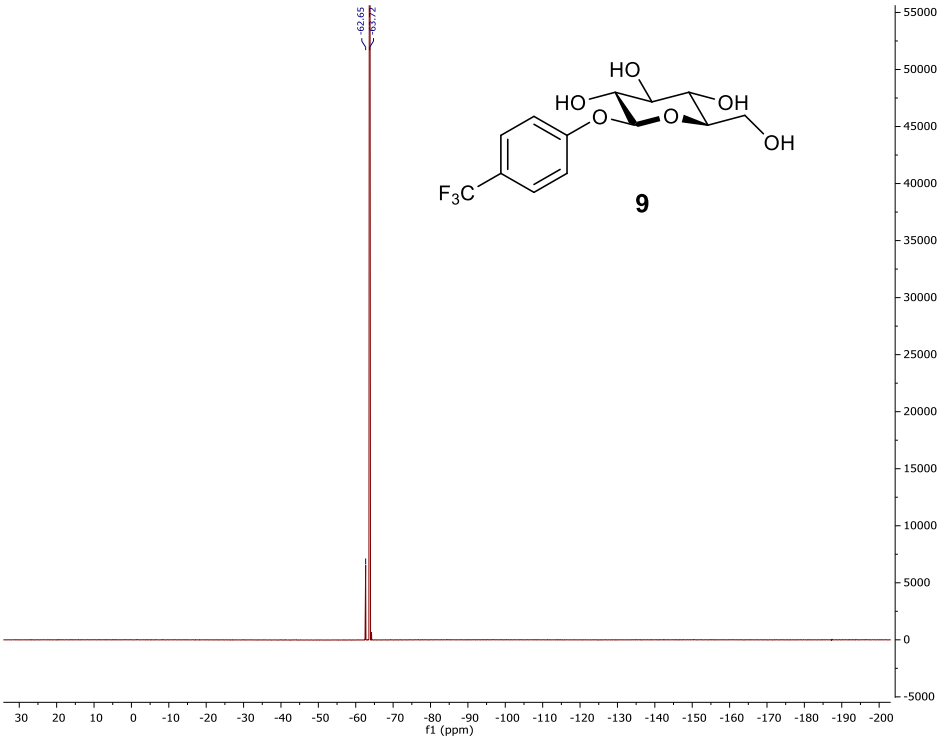




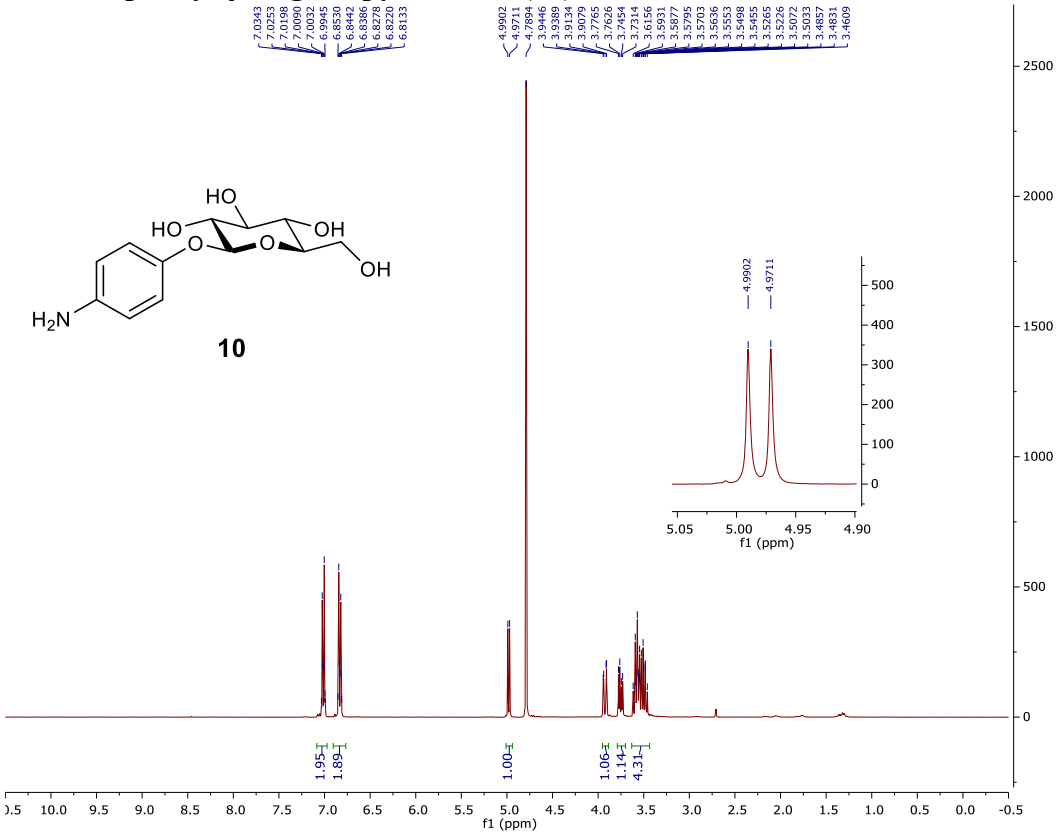
4-Trifluoromethylphenyl-β-D-glucopyranoside (9)

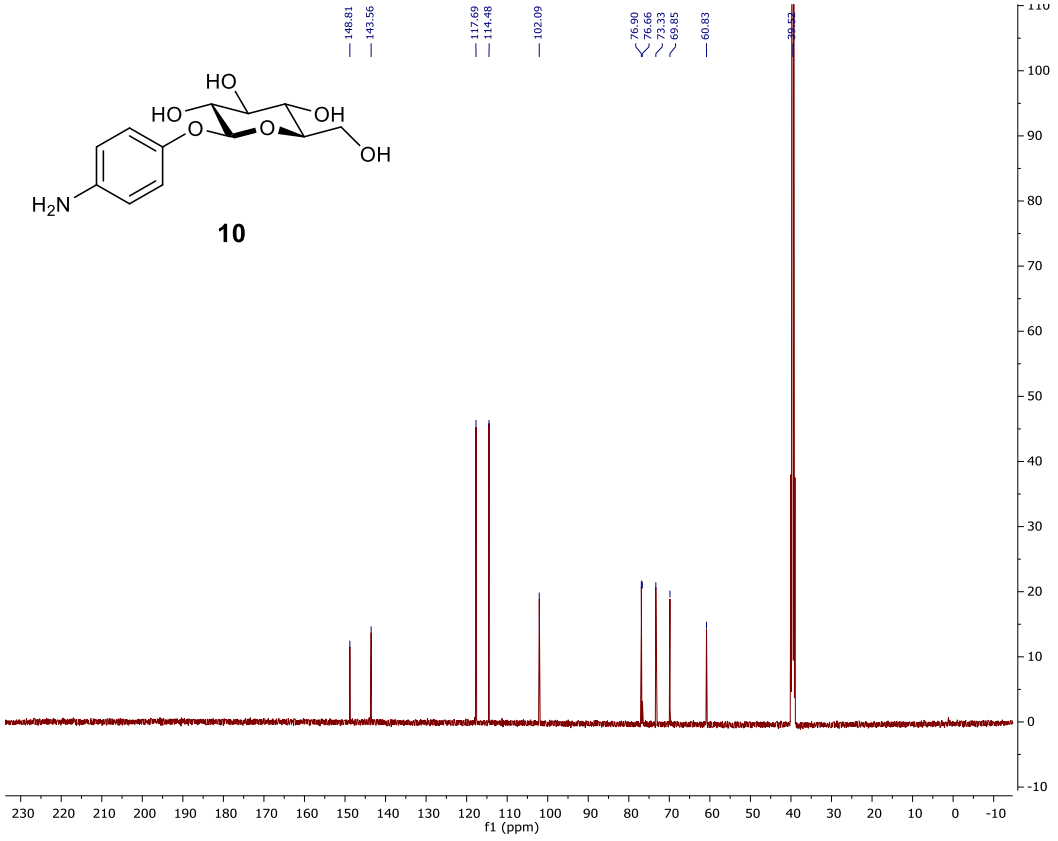
Coupled ^{13}C NMR (151 MHz, DMSO-d_6) δ 99.90 (d, $^{1}J_{C-H}$ = 161.2 Hz).



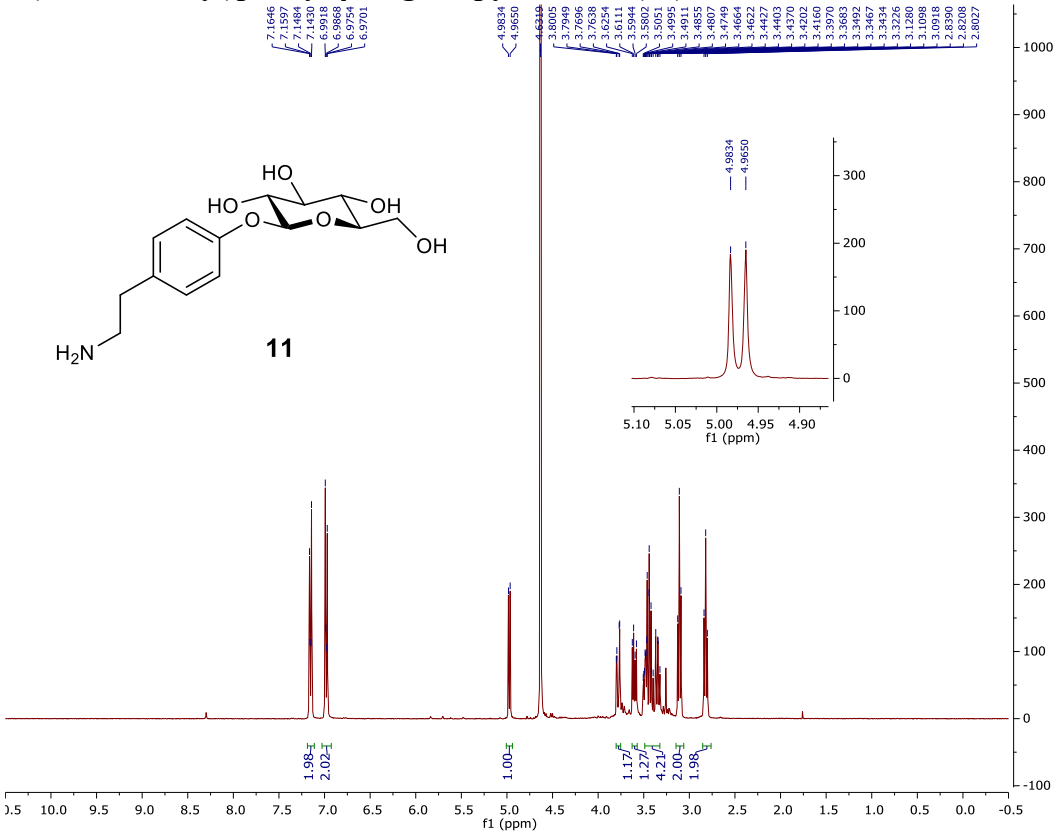


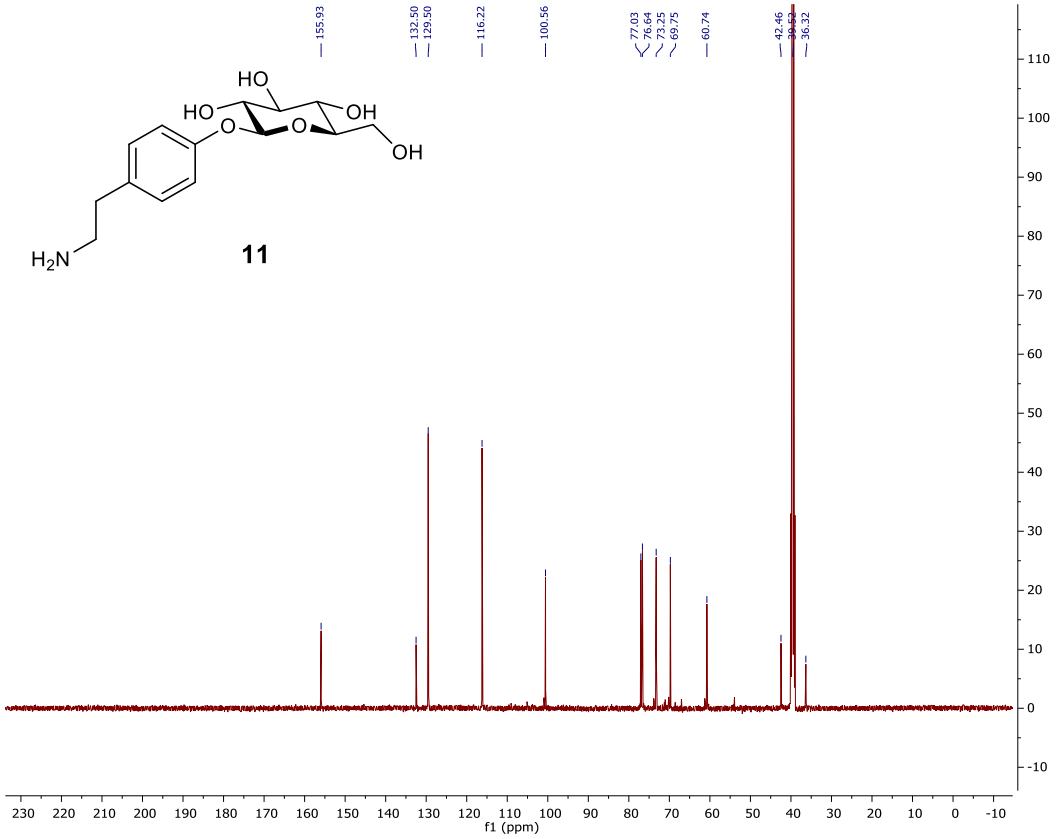
4-Aminophenyl-β-D-glucopyranoside (10)



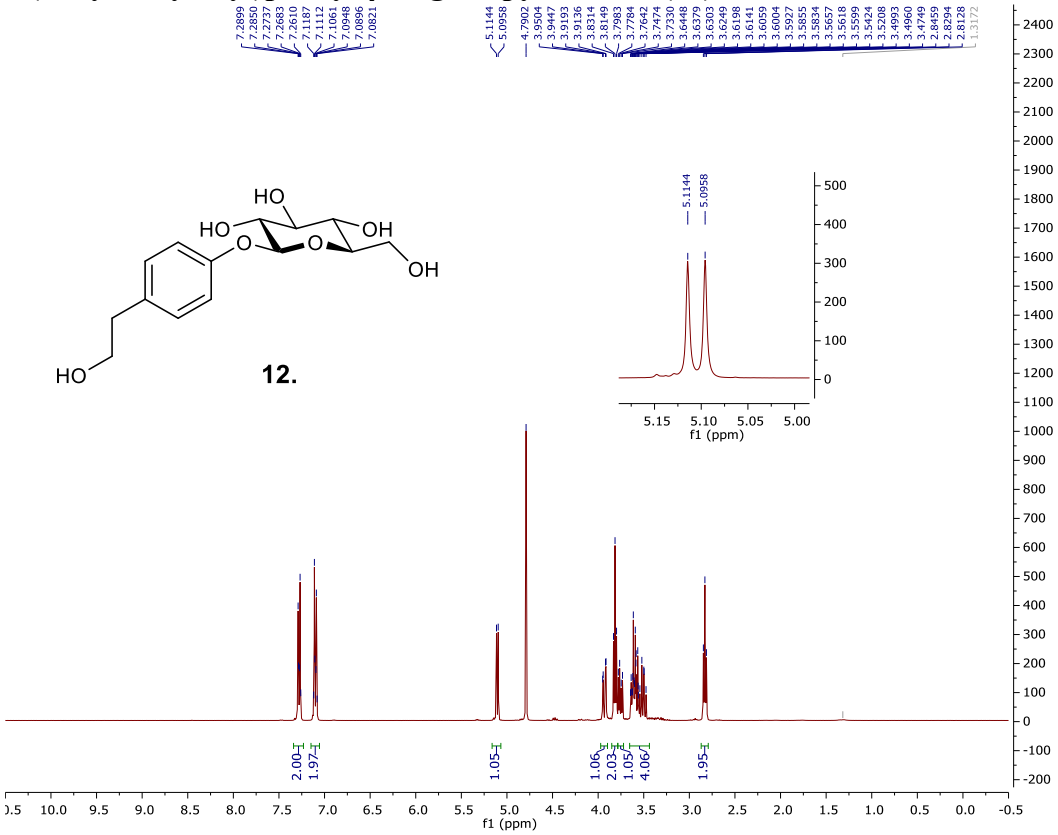


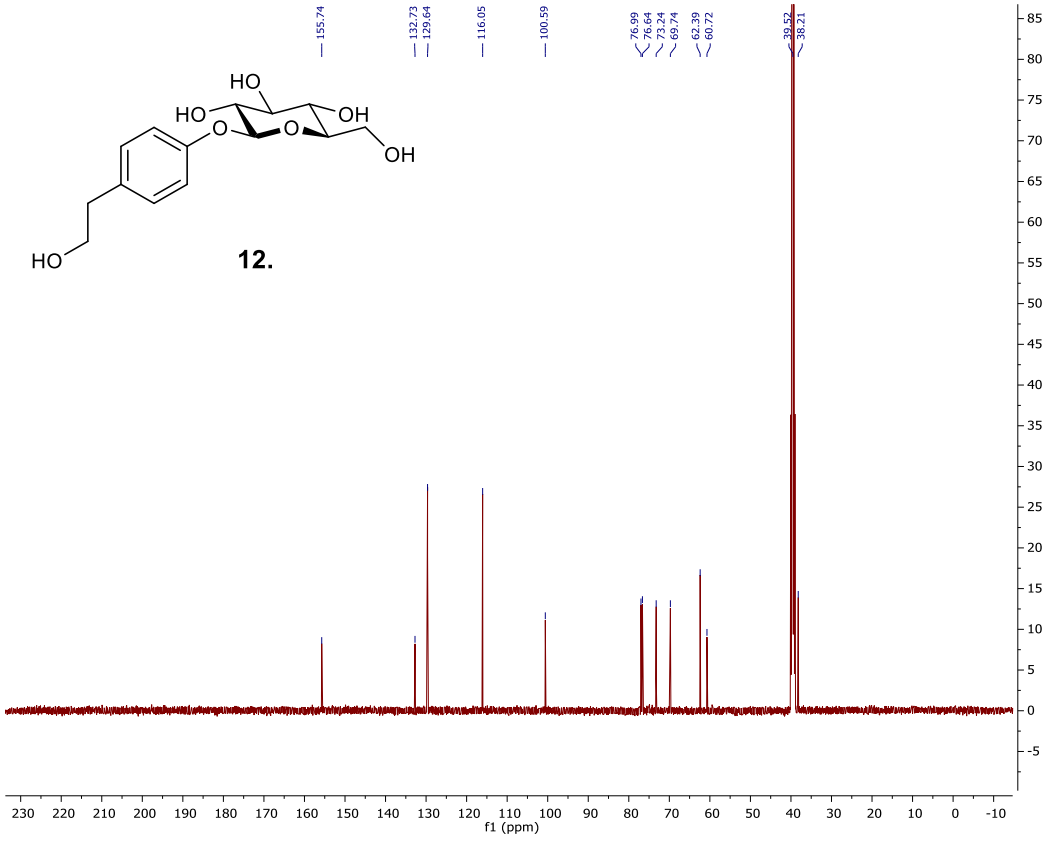
4-(2-Aminoethyl)phenyl-β-D-glucopyranoside (11)



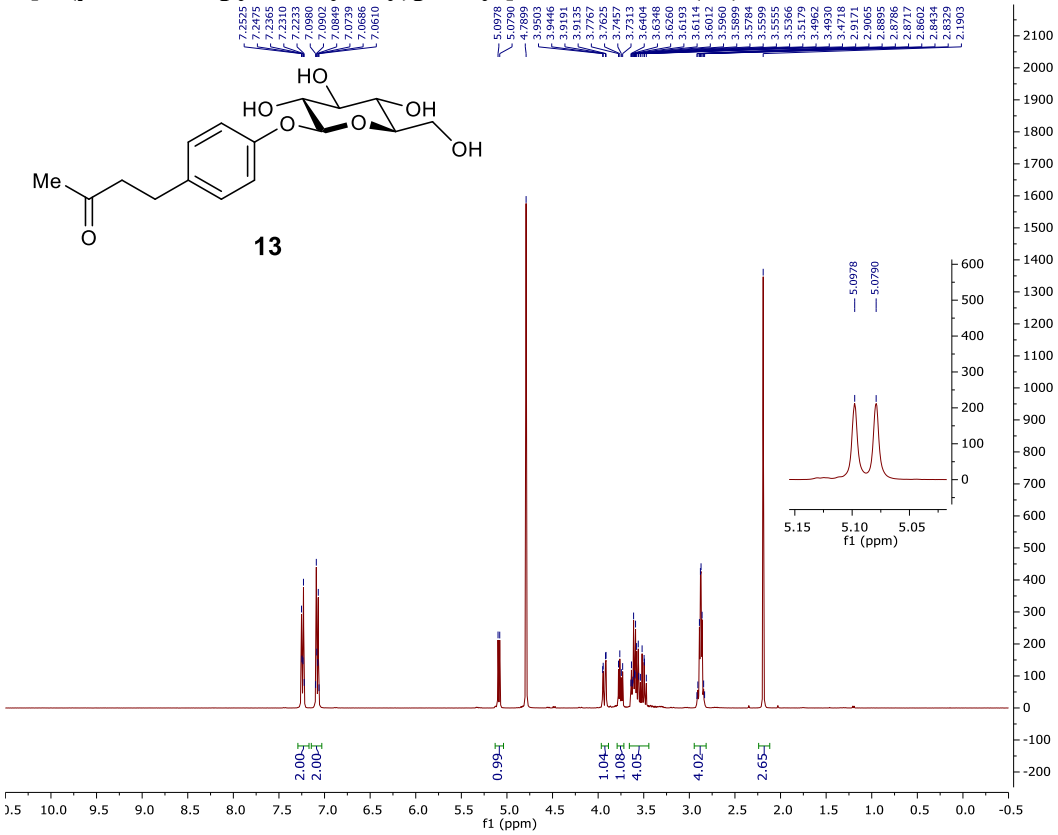


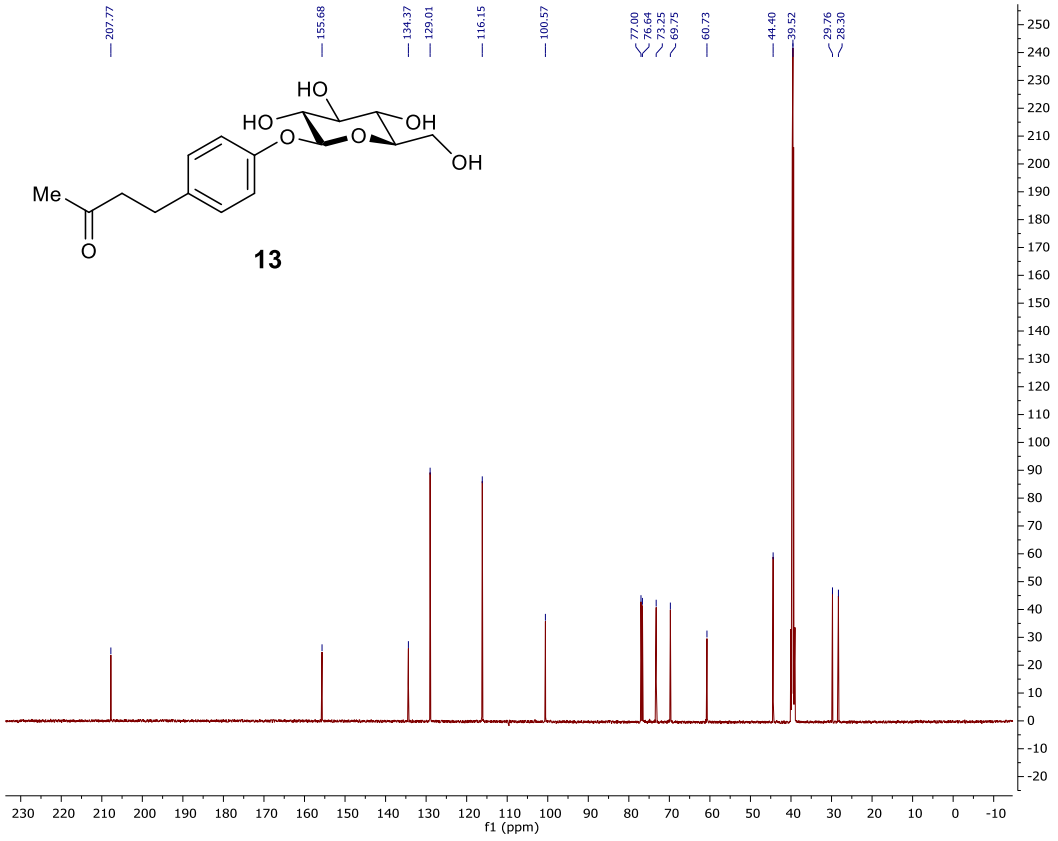
4-(2-Hydroxyethyl)phenyl-β-D-glucopyranoside (12)



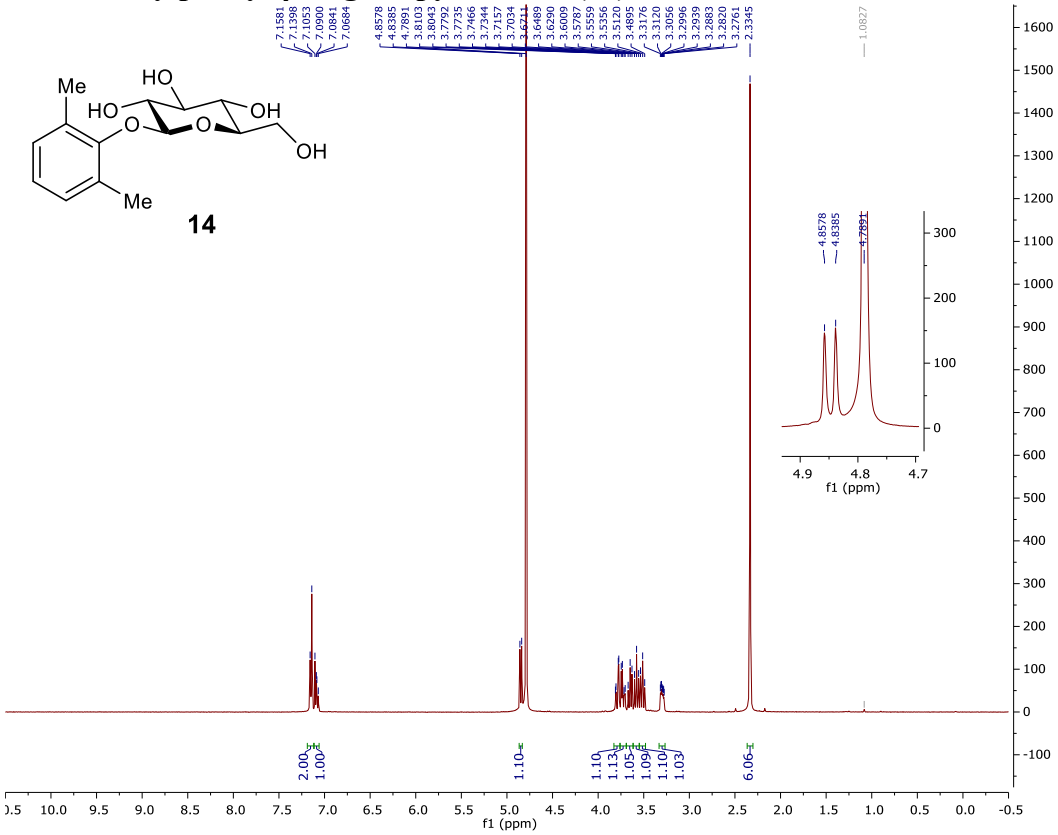


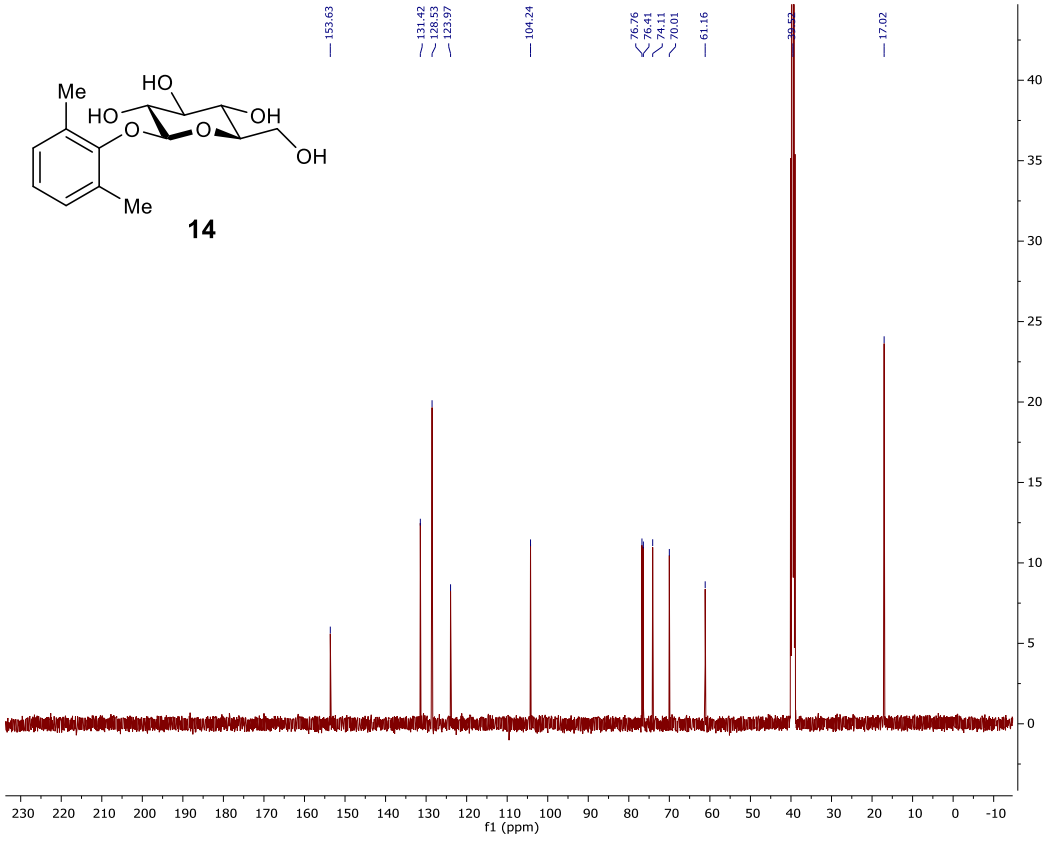
4-[4-(β-D-Glucopyranosyloxy)phenyl]-2-butanone (13)



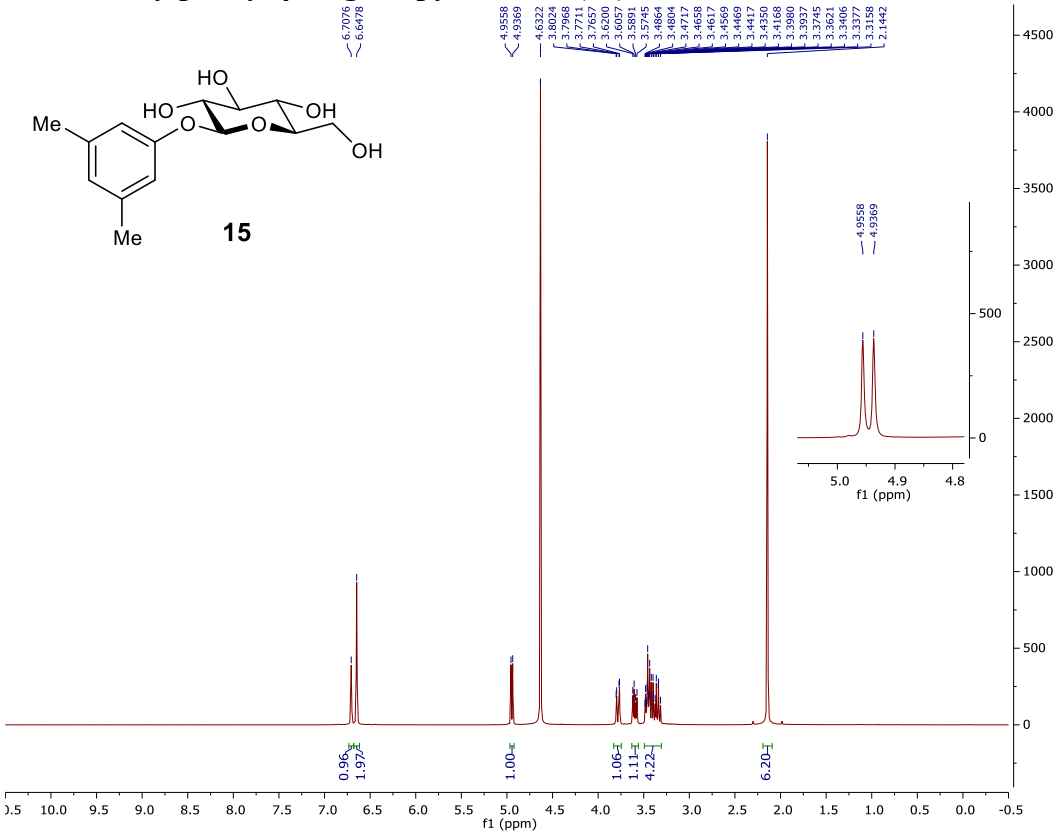


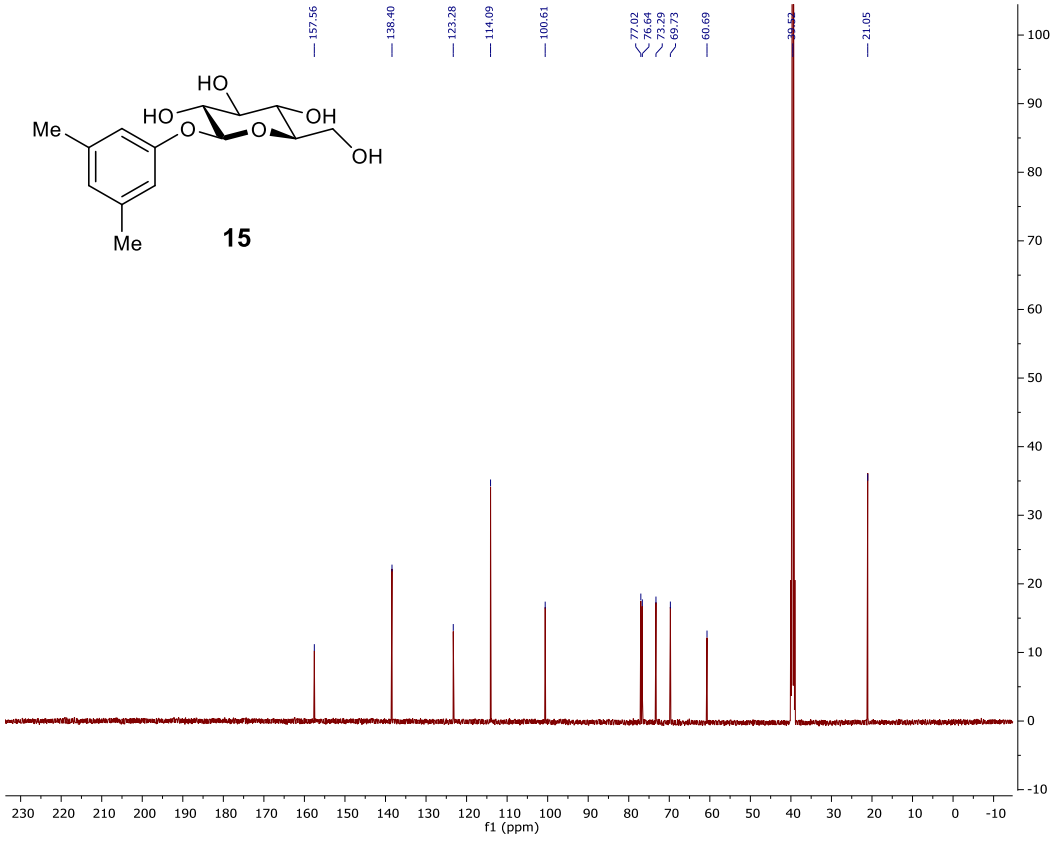
2,6-Dimethylphenyl-β-D-glucopyranoside (14)



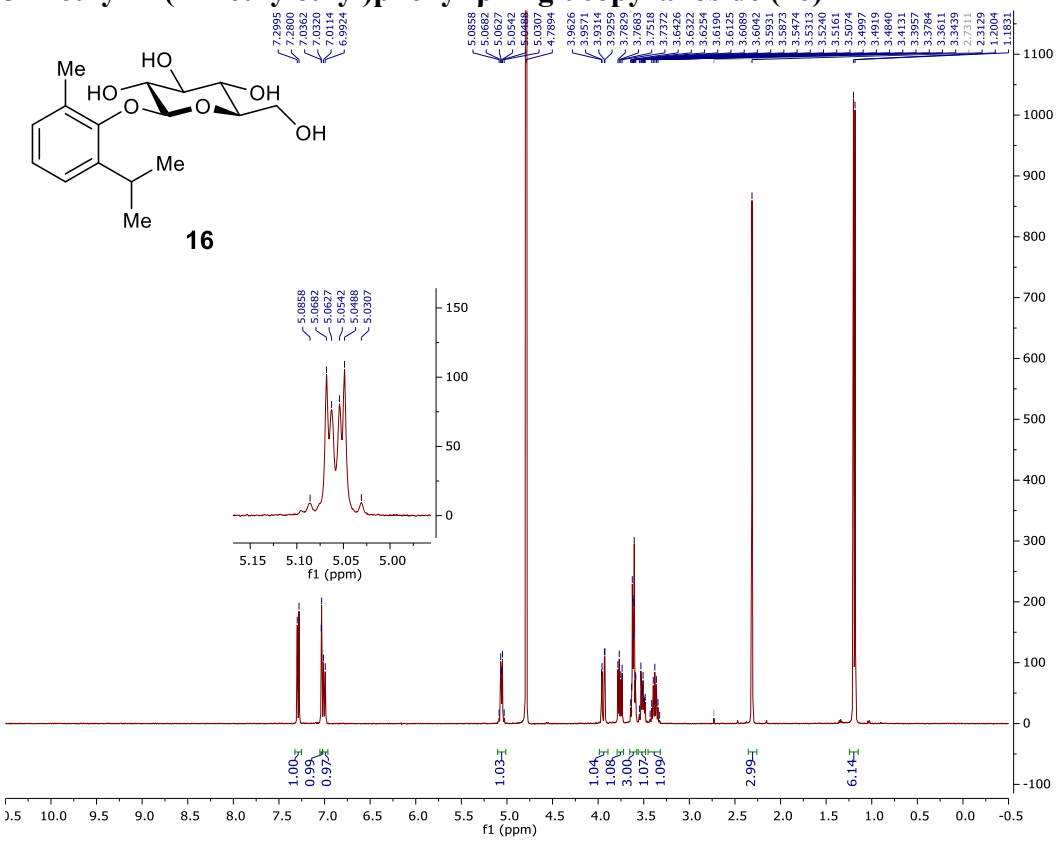


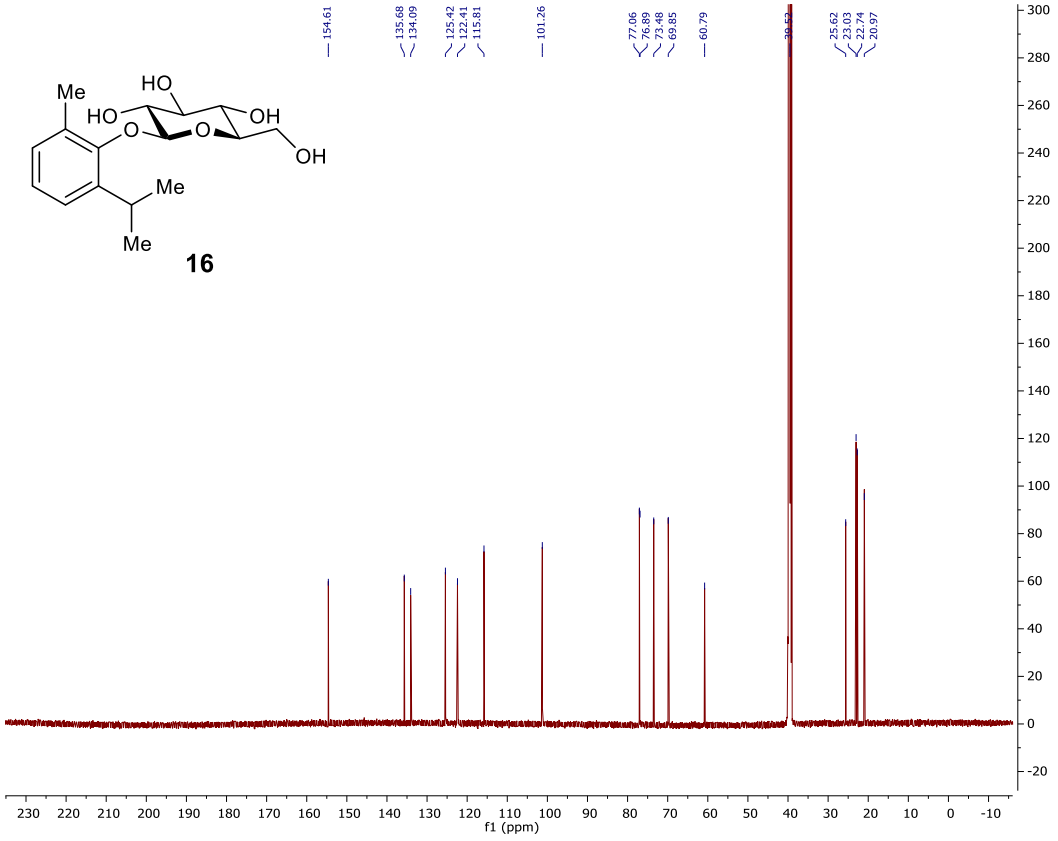
3,5-Dimethylphenyl-β-D-glucopyranoside (15)



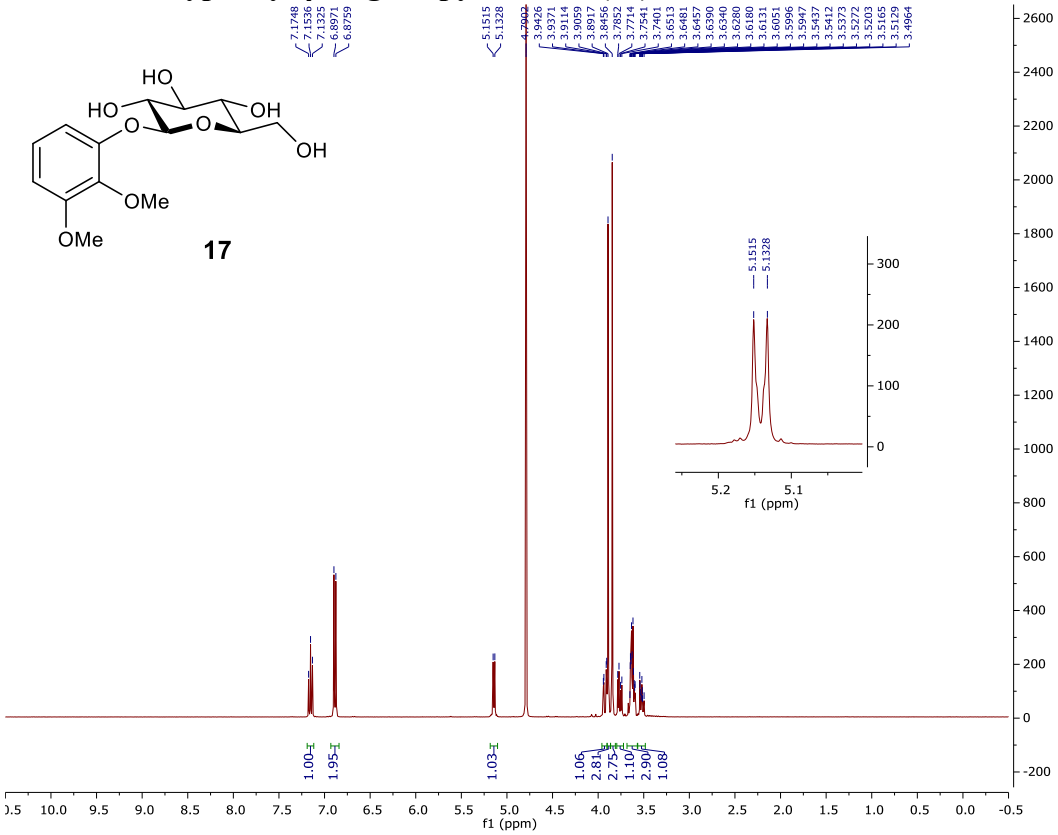


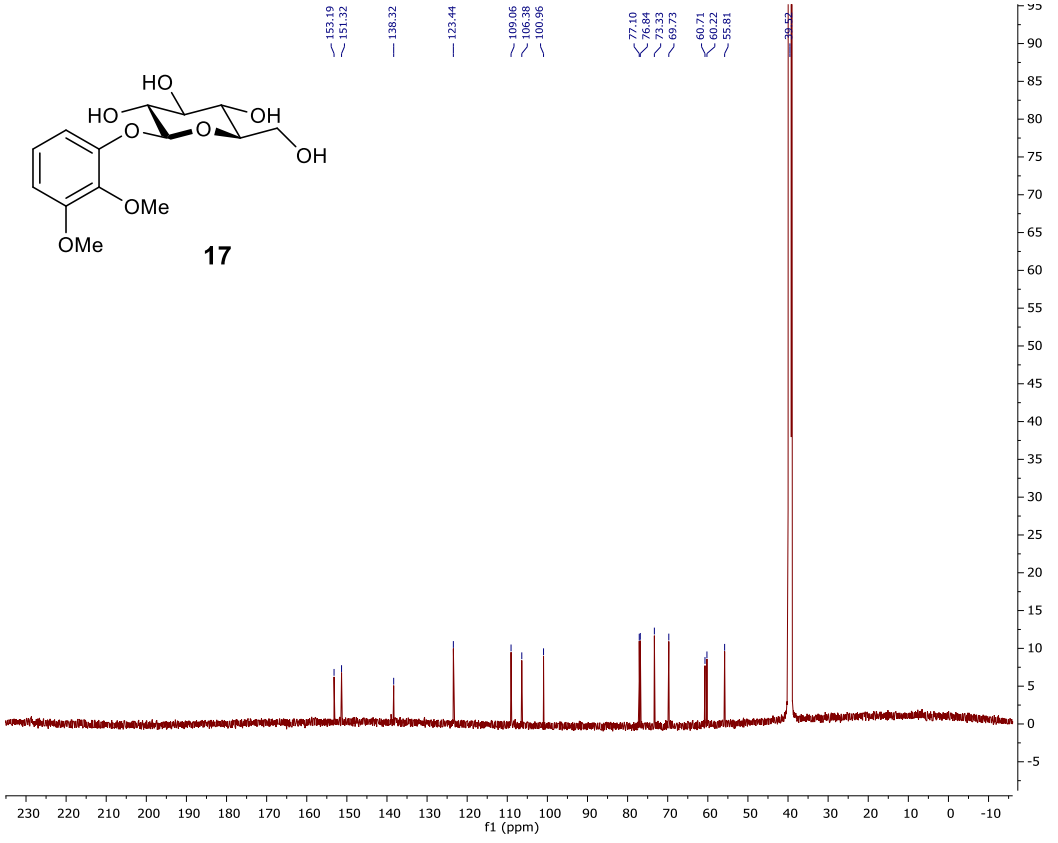
5-Methyl-2-(1-methylethyl)phenyl-β-D-glucopyranoside (16)



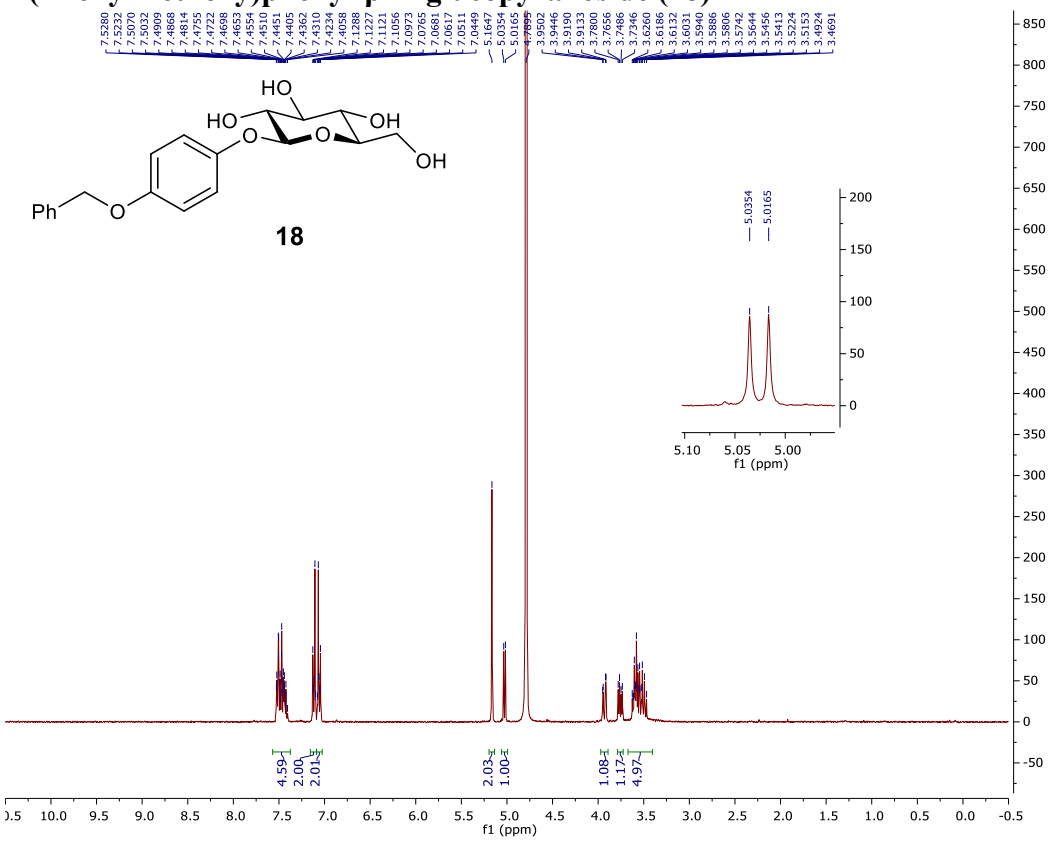


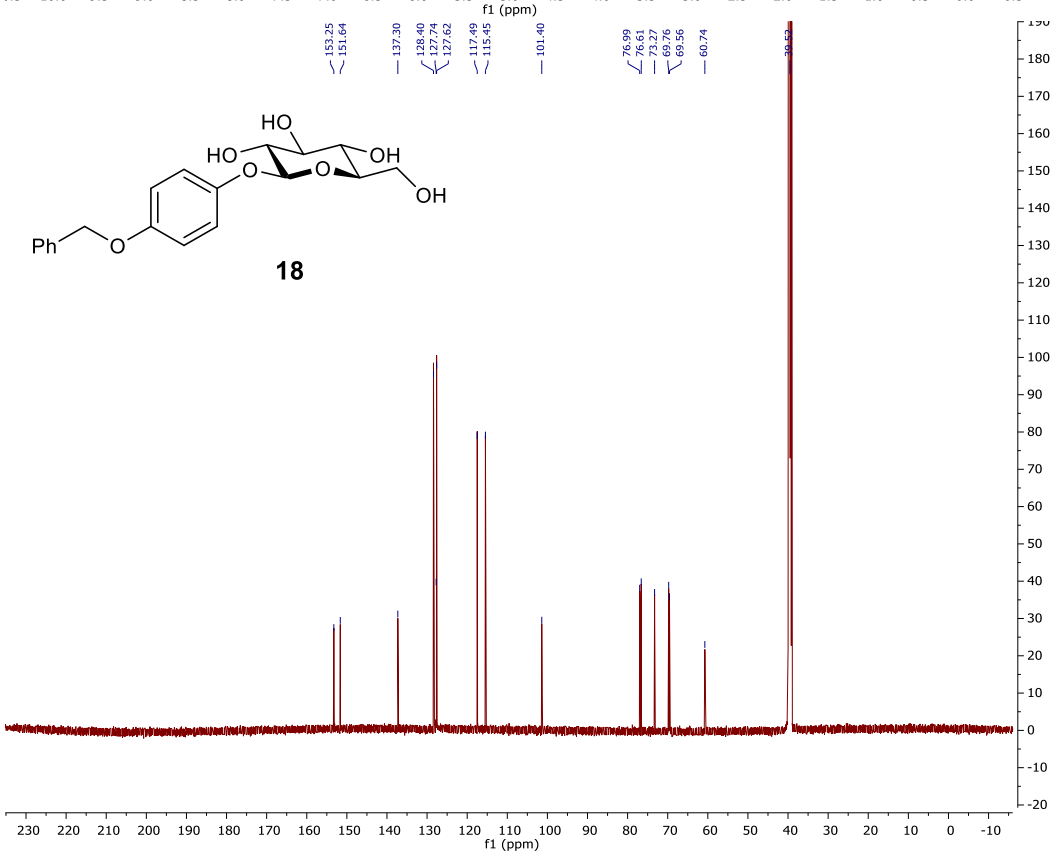
2,3-Dimethoxyphenyl-β-D-glucopyranoside (17)



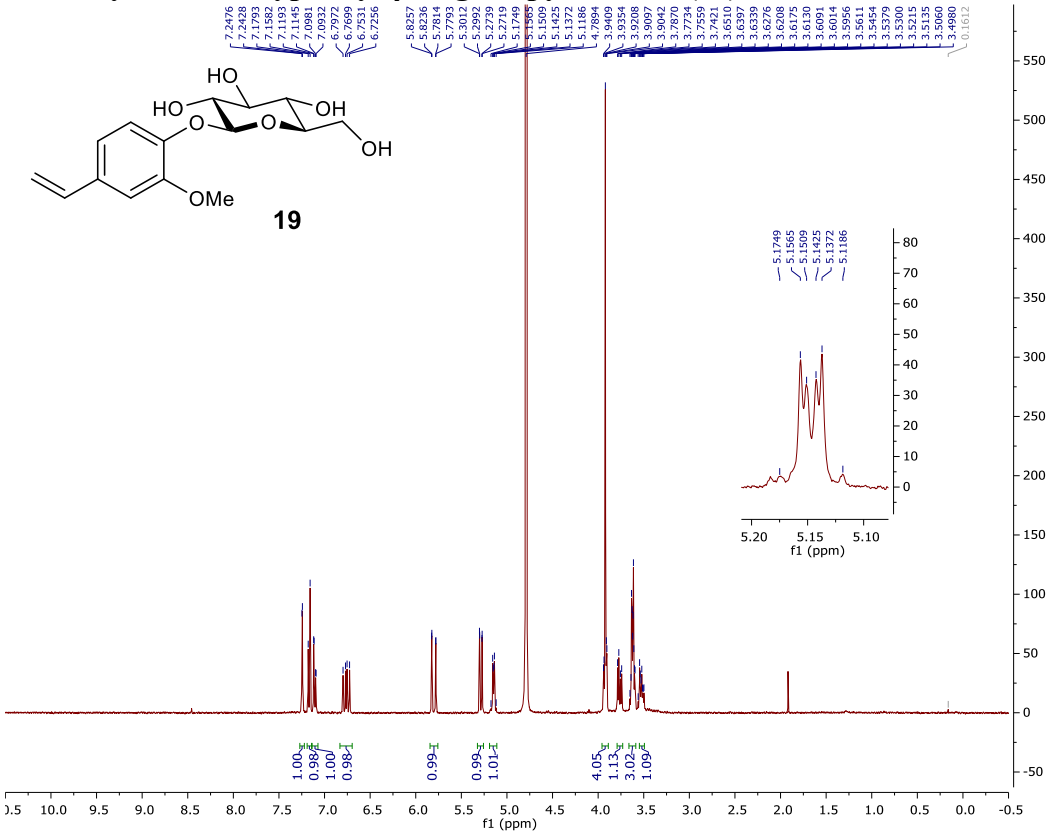


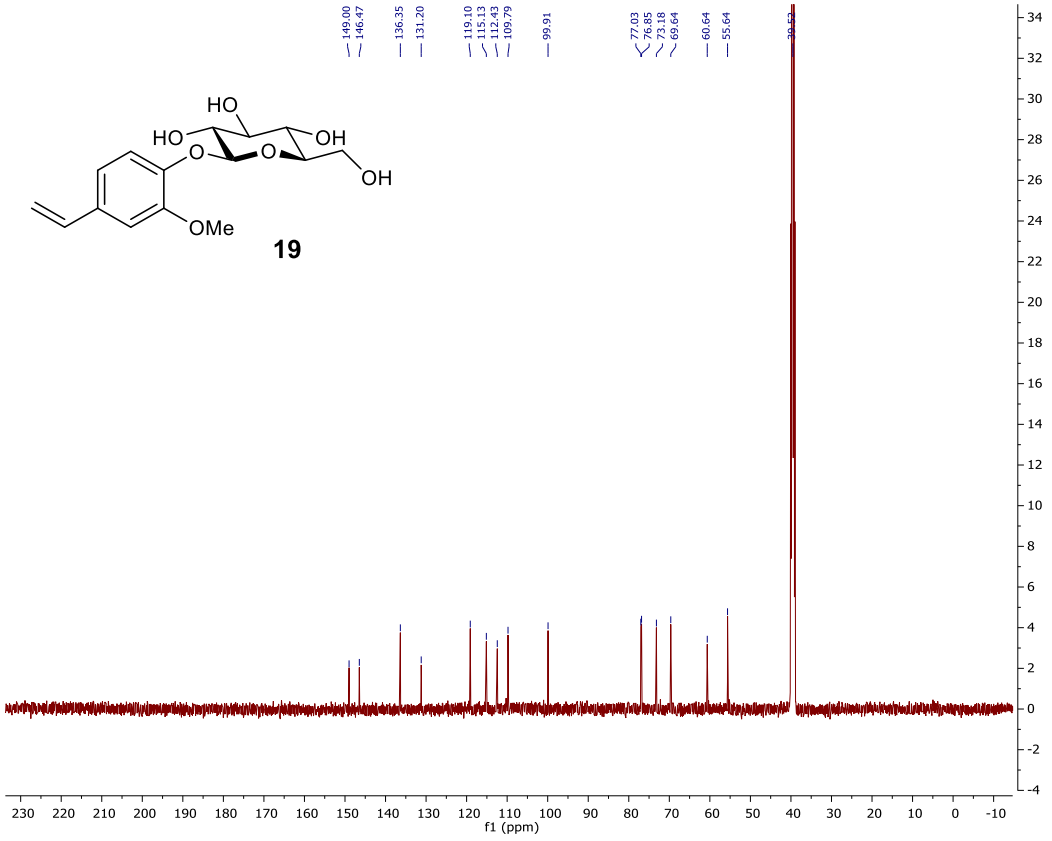
4-(Phenylmethoxy)phenyl-β-D-glucopyranoside (18)



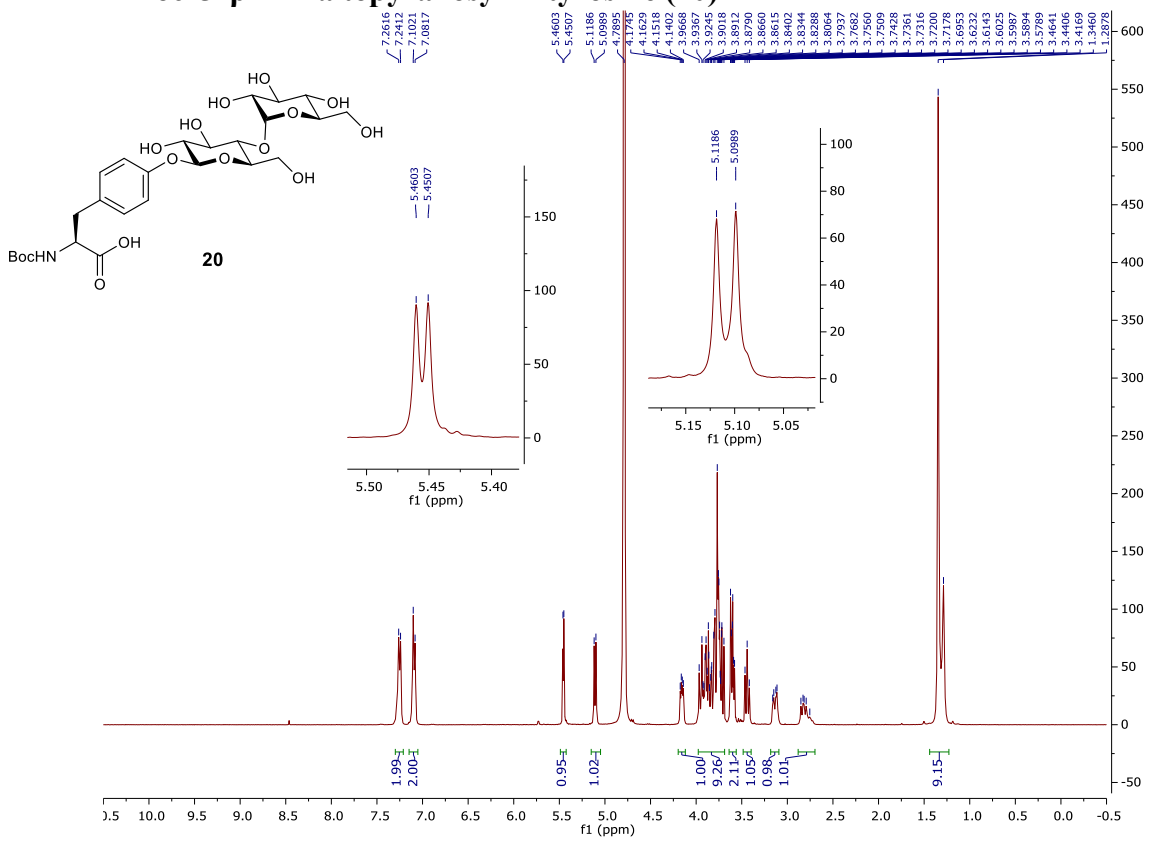


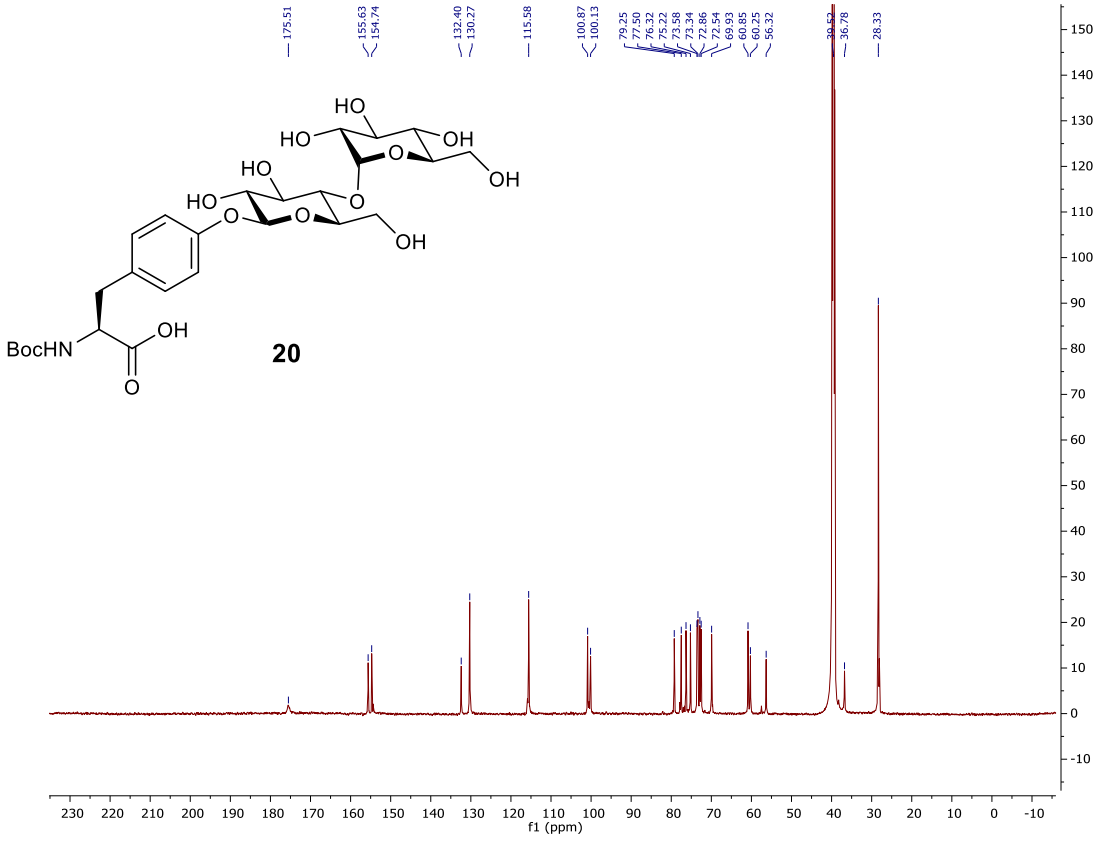
4-Ethenyl-2-methoxyphenyl-β-D-glucopyranoside (19)



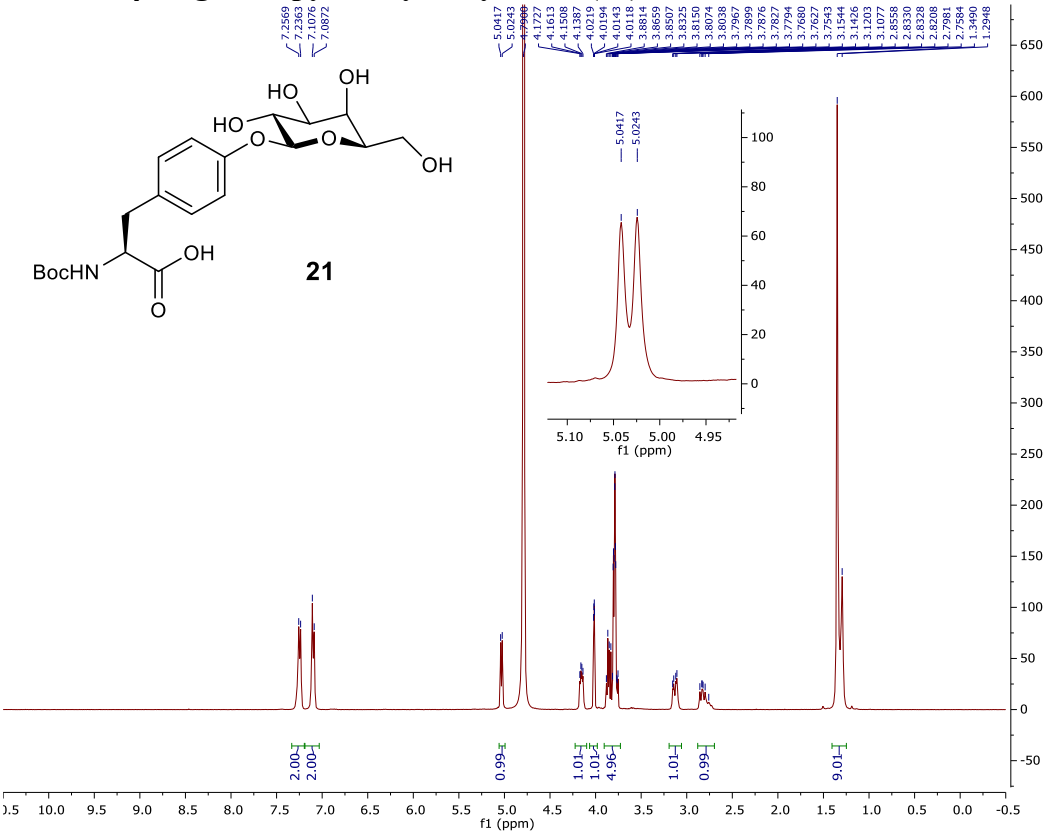


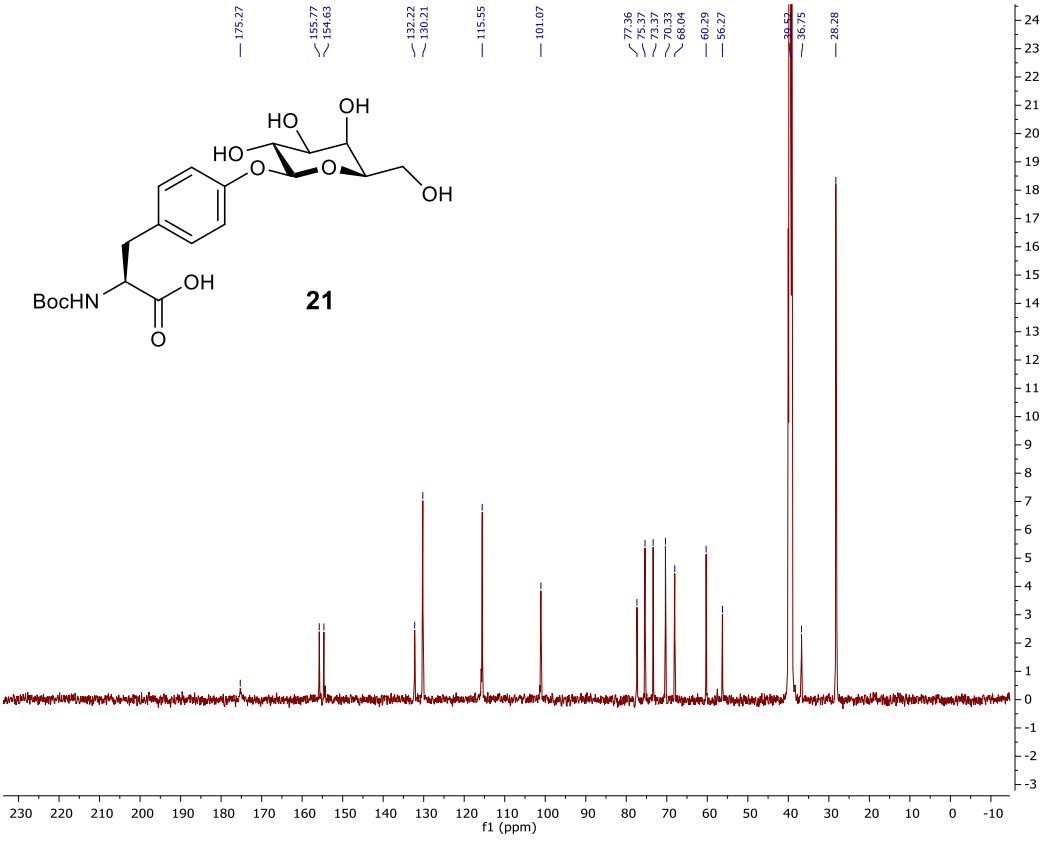
N-Boc-*O*-β-D-maltopyranosyl-L-tyrosine (20)



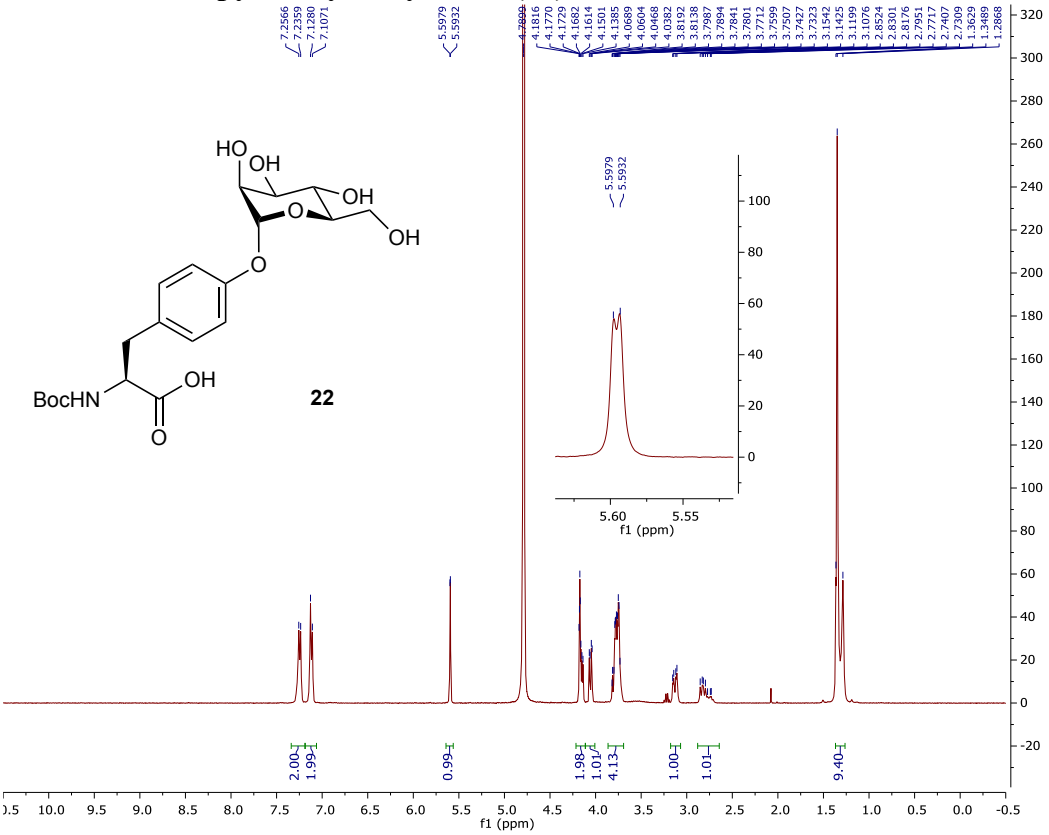


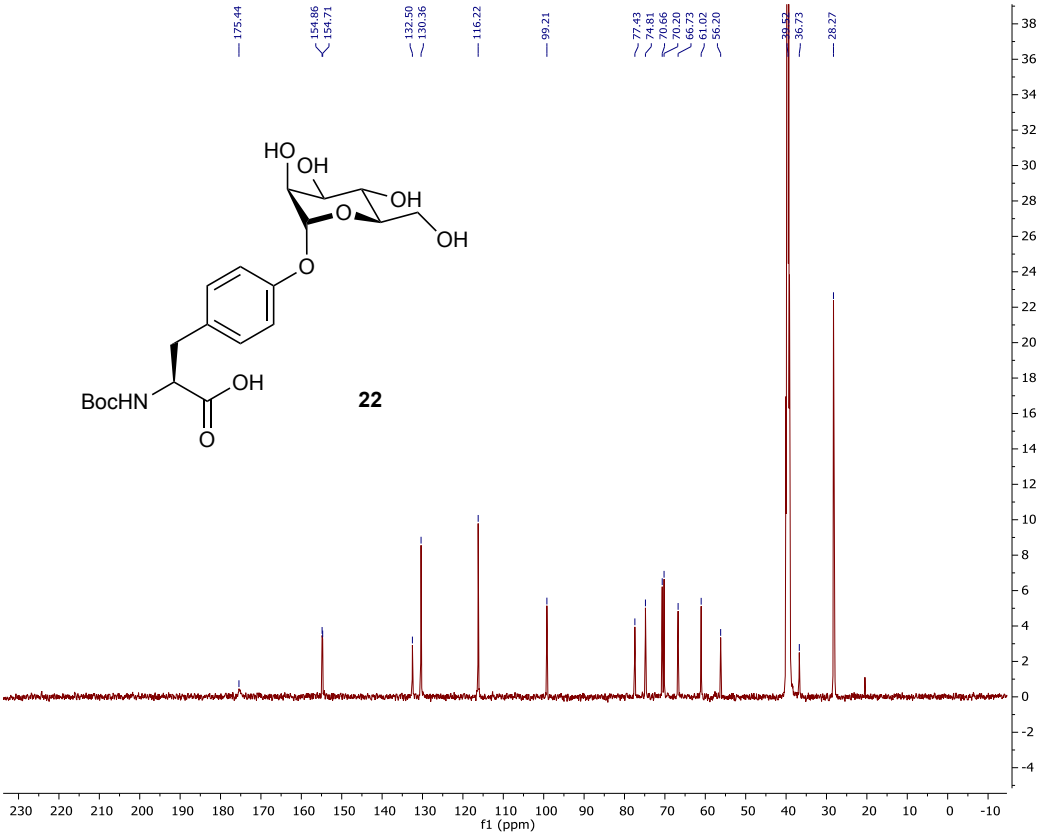
N-Boc-*O*-β-D-galactopyranosyl-L-tyrosine (21)

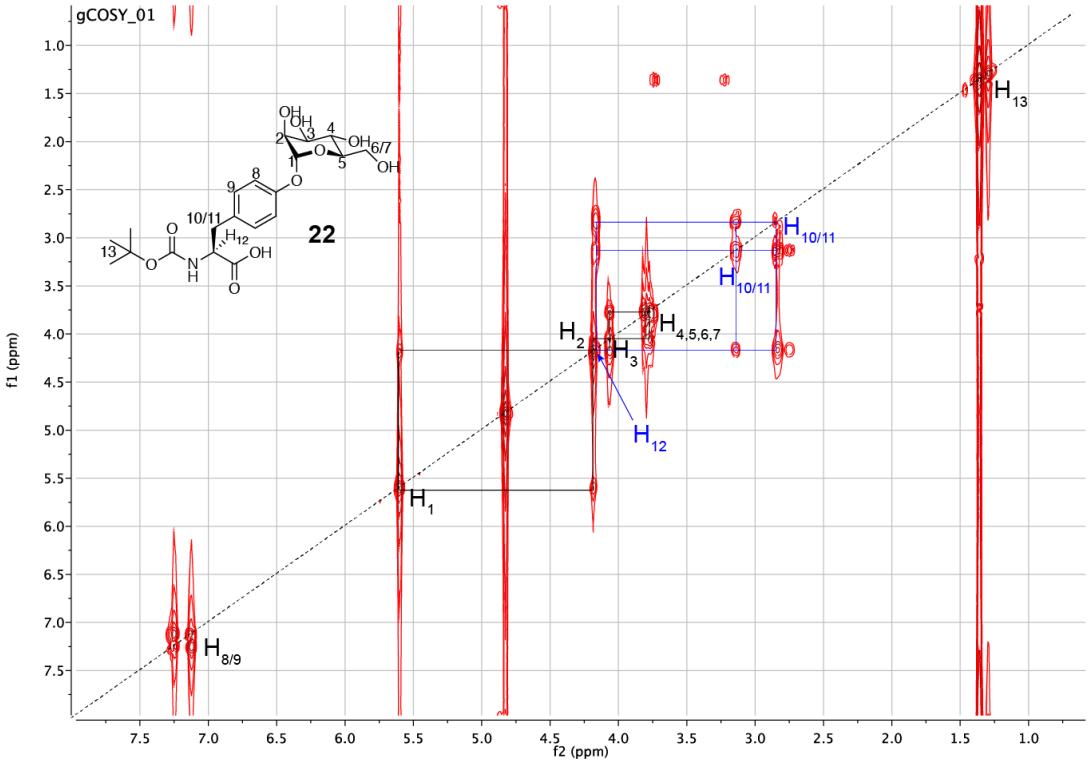


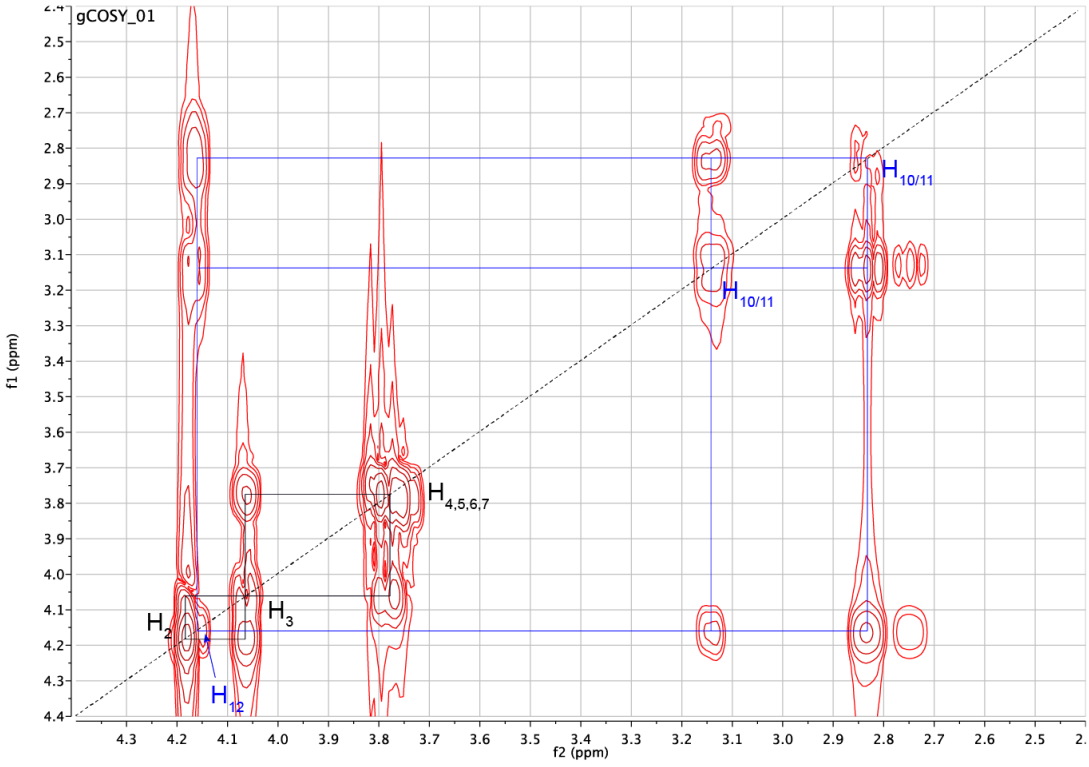


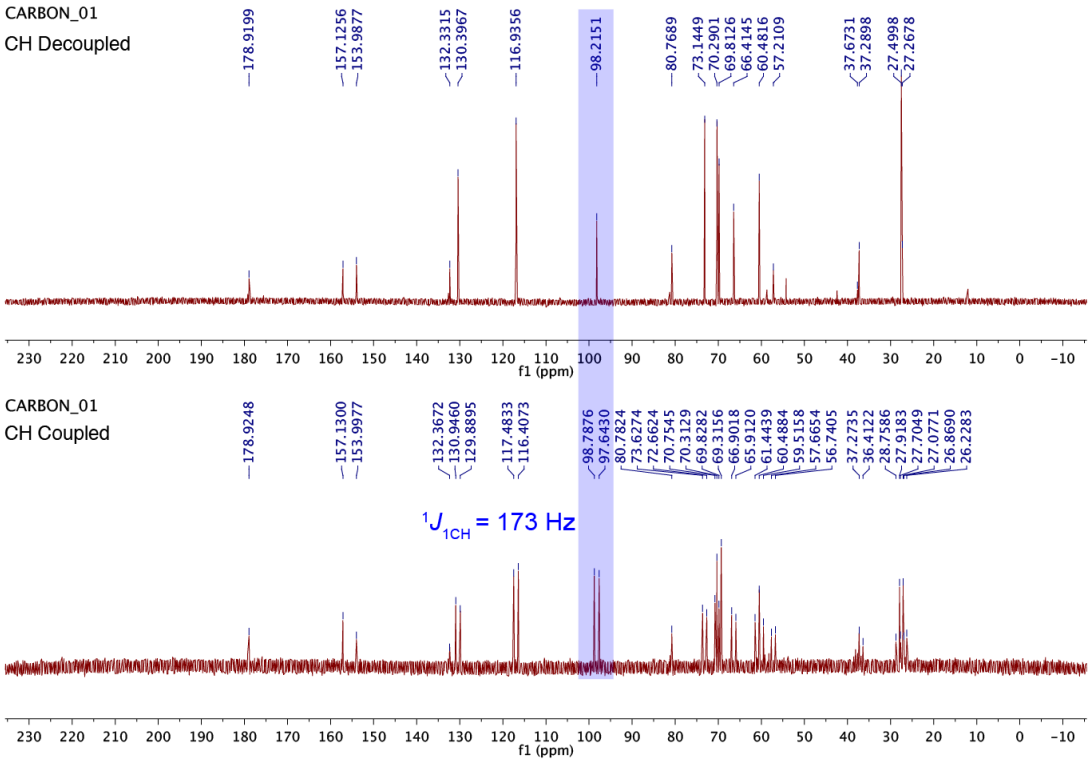
_N_-Boc-_O_-α-D-mannopyranosyl-L-tyrosine (22)

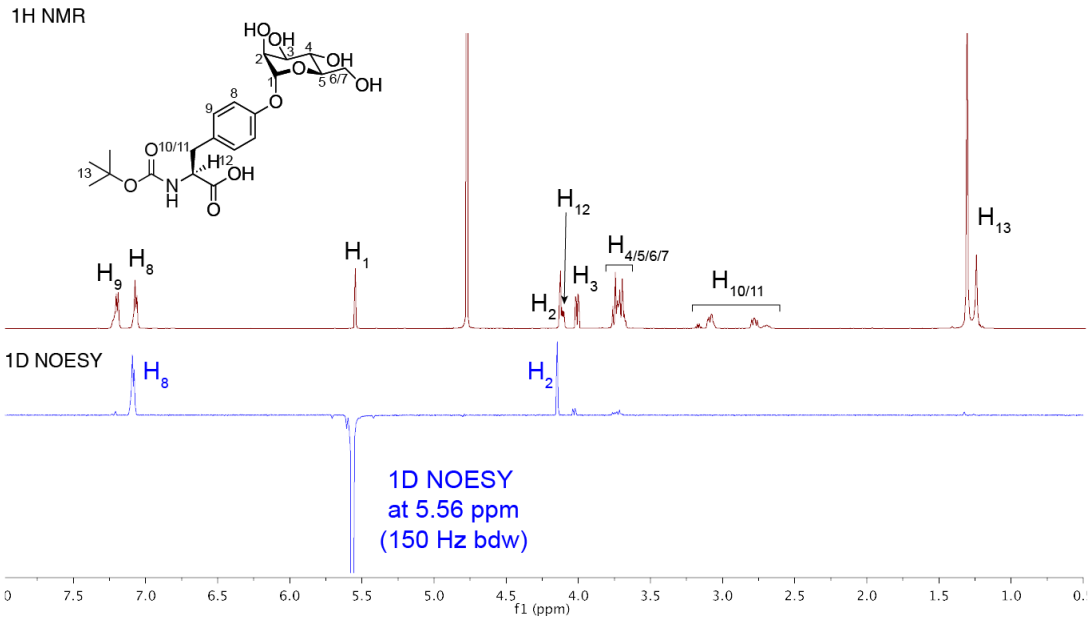




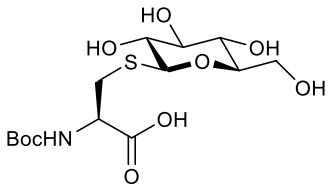

gCOSY_01
22
H13
H10/11
H10/11
H2
H4,5,6,7
H3
H12
H1
H8/9
f1 (ppm)
f2 (ppm)


gCOSY_01
H10/11
H10/11
H4,5,6,7
H2
H3
H12
f1 (ppm)
f2 (ppm)

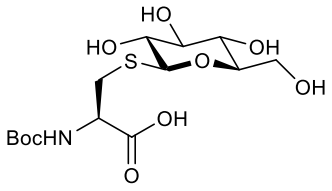

CARBON_01
CH Decoupled
178.9199
157.1256
153.9877
132.3315
130.3967
116.9356
98.2151
80.7689
73.1449
70.2901
69.8126
66.4145
60.4816
57.2109
37.6731
37.2898
27.4998
27.2678
f1 (ppm)
CARBON_01
CH Coupled
178.9248
157.1300
153.9977
132.3672
130.9460
129.8895
117.4833
116.4073
98.7876
97.6430
80.7824
73.6274
72.6624
70.7545
70.3129
69.8282
69.3156
66.9018
65.9120
61.4439
60.4884
59.5158
57.6654
56.7405
37.2735
36.4122
28.7586
27.9183
27.7049
27.0771
26.8690
26.2283
$^1J_{1CH}$ = 173 Hz
f1 (ppm)


1H NMR
H9
H8
H1
H12
H3
H2
H4/5/6/7
H10/11
H13
1D NOESY
H8
H2
1D NOESY
at 5.56 ppm
(150 Hz bdw)
f1 (ppm)

N-Boc-*S*-β-D-glucopyranosyl-L-cysteine (34)

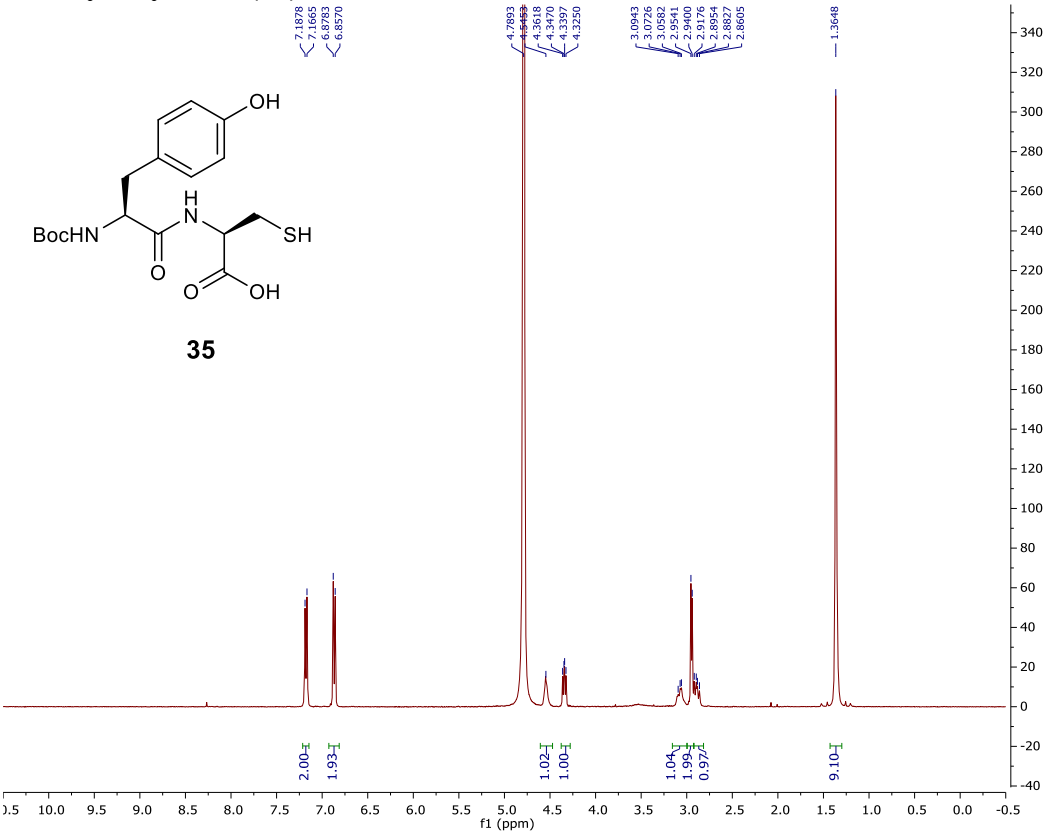


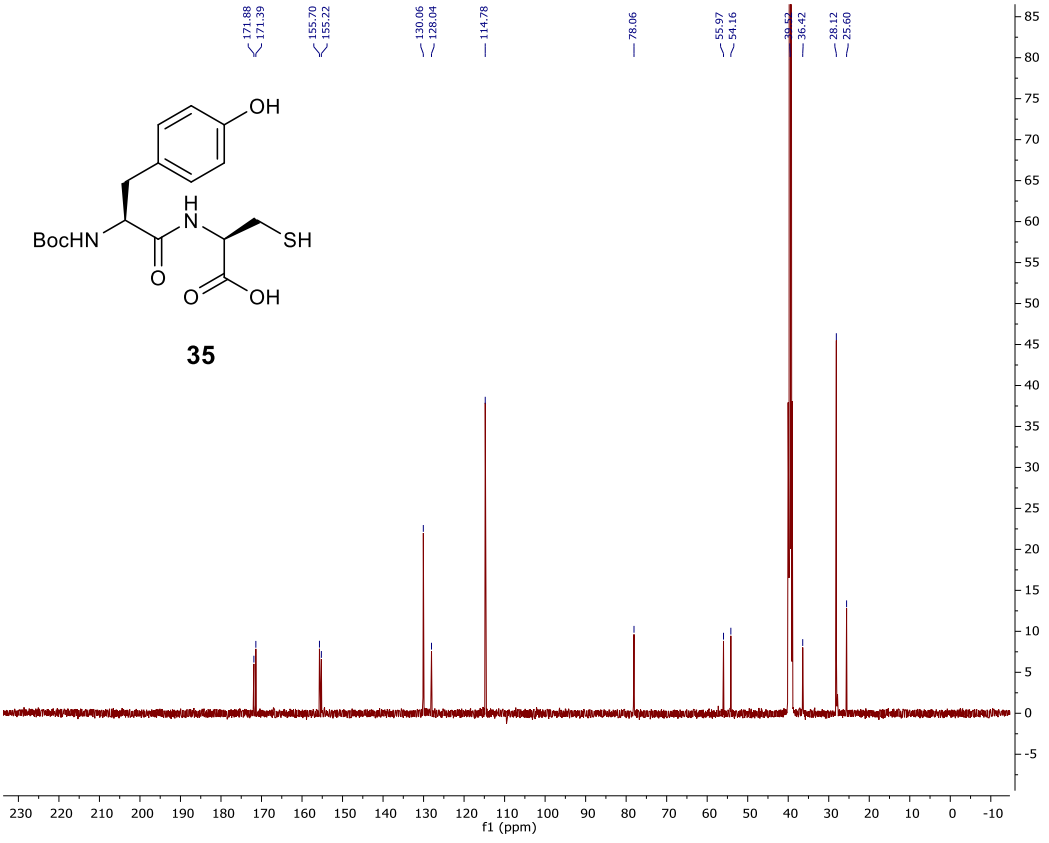
34



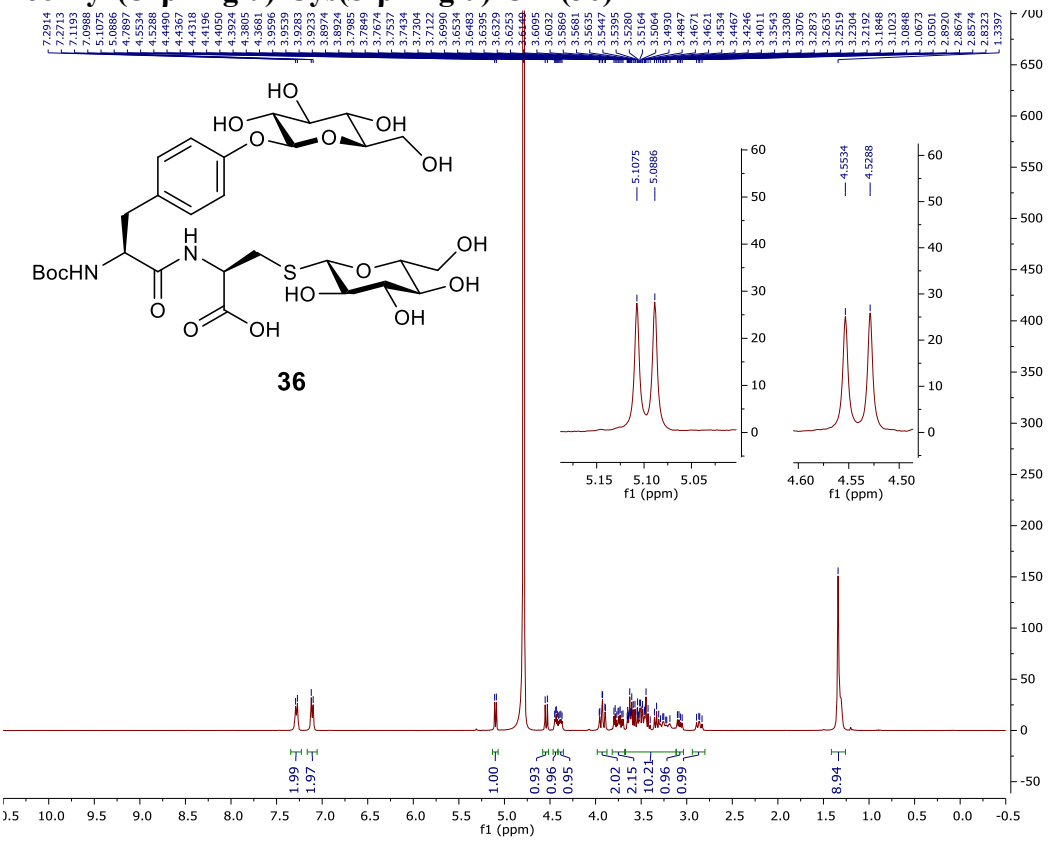
34

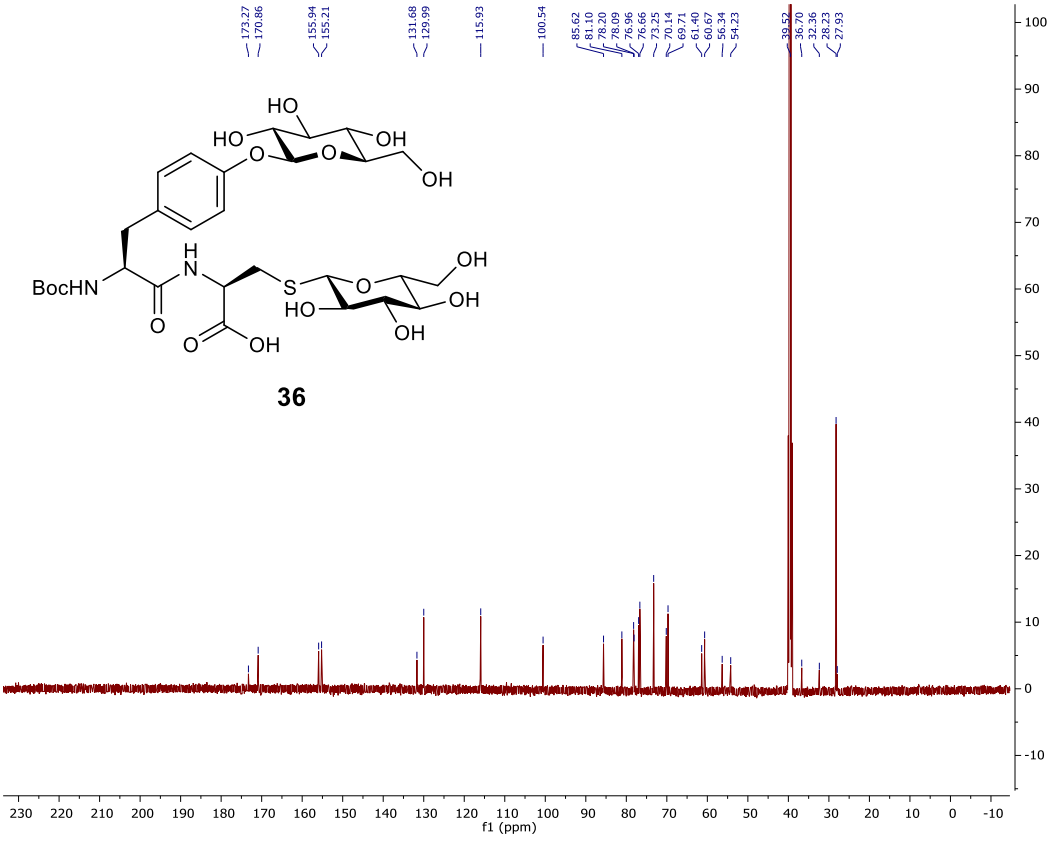
Boc-Tyr-Cys-OH (35)



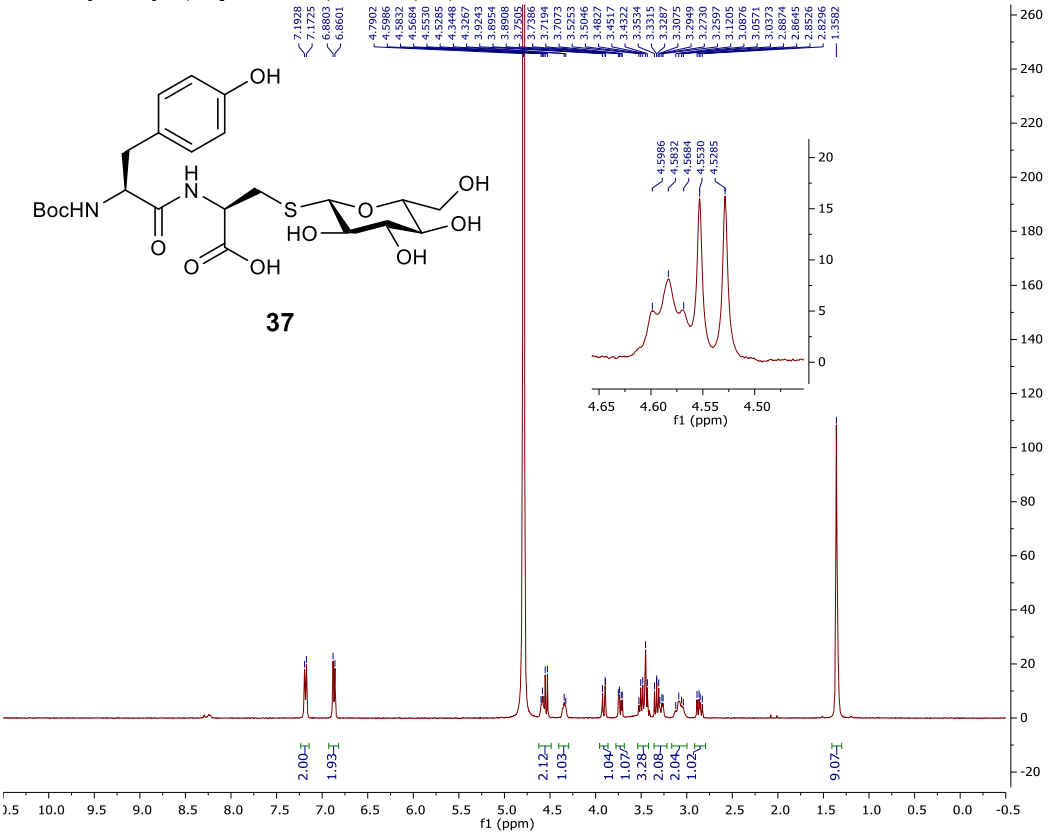


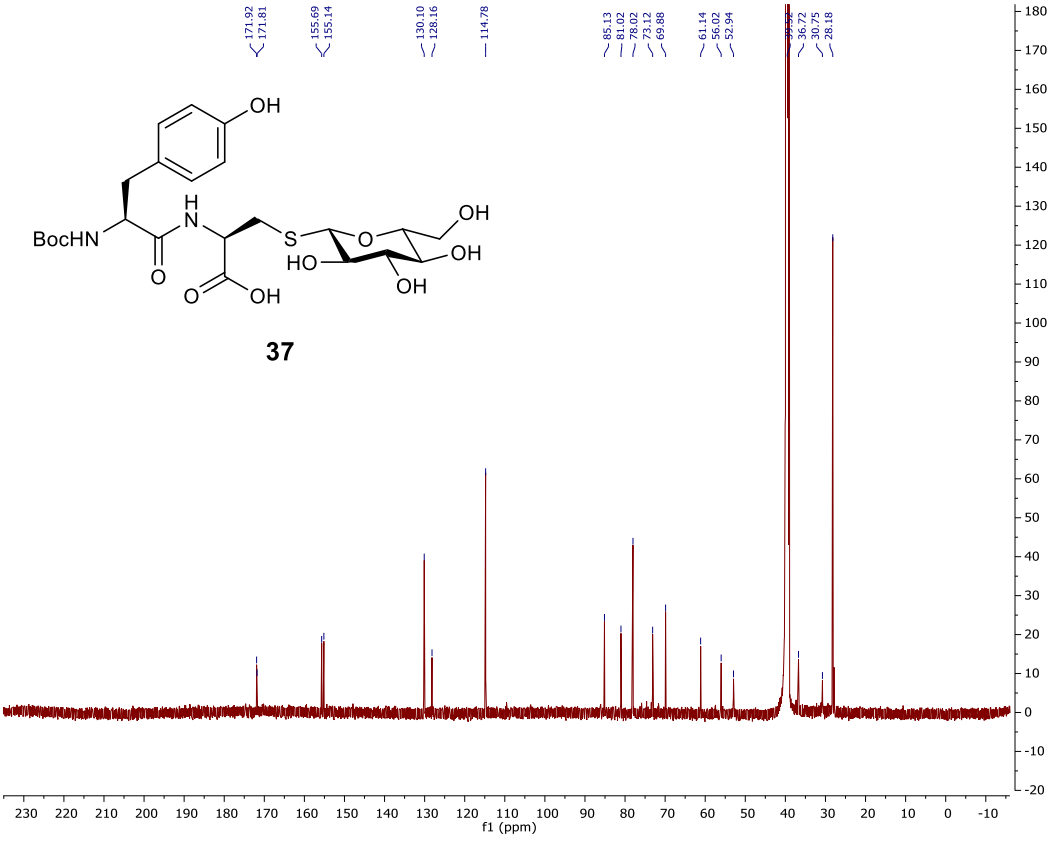
Boc-Tyr(*O*-β-D-glu)-Cys(*S*-β-D-glu)-OH (36)



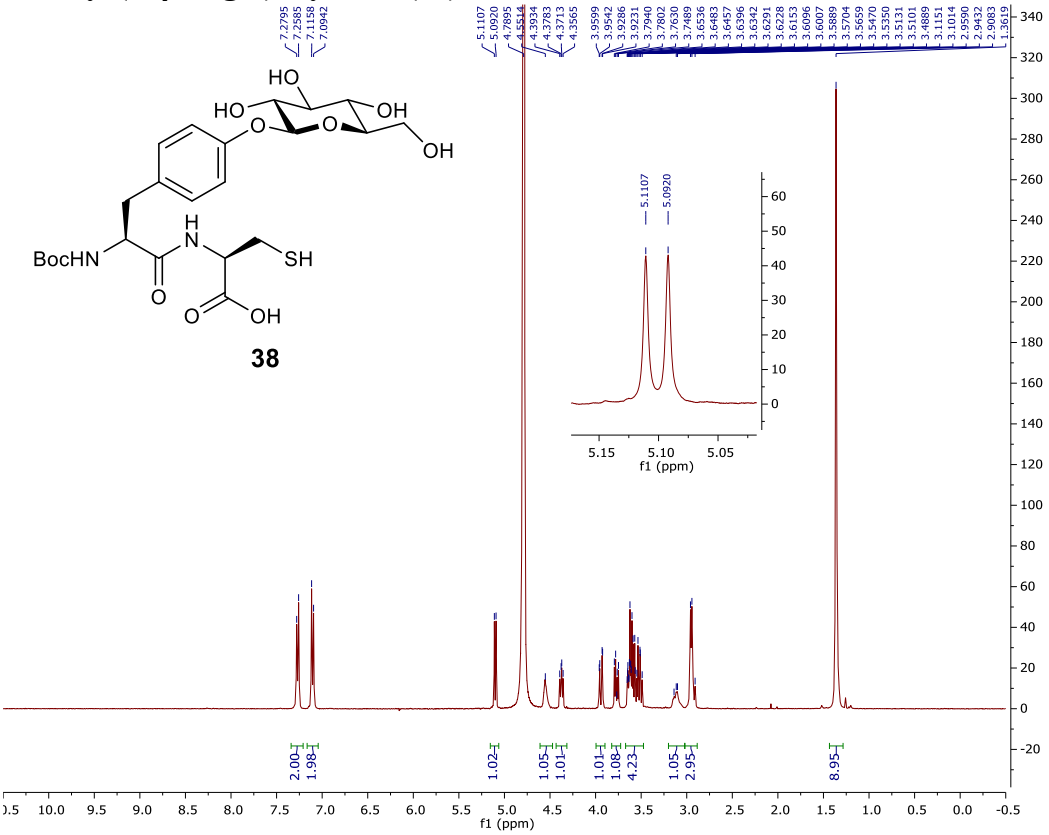


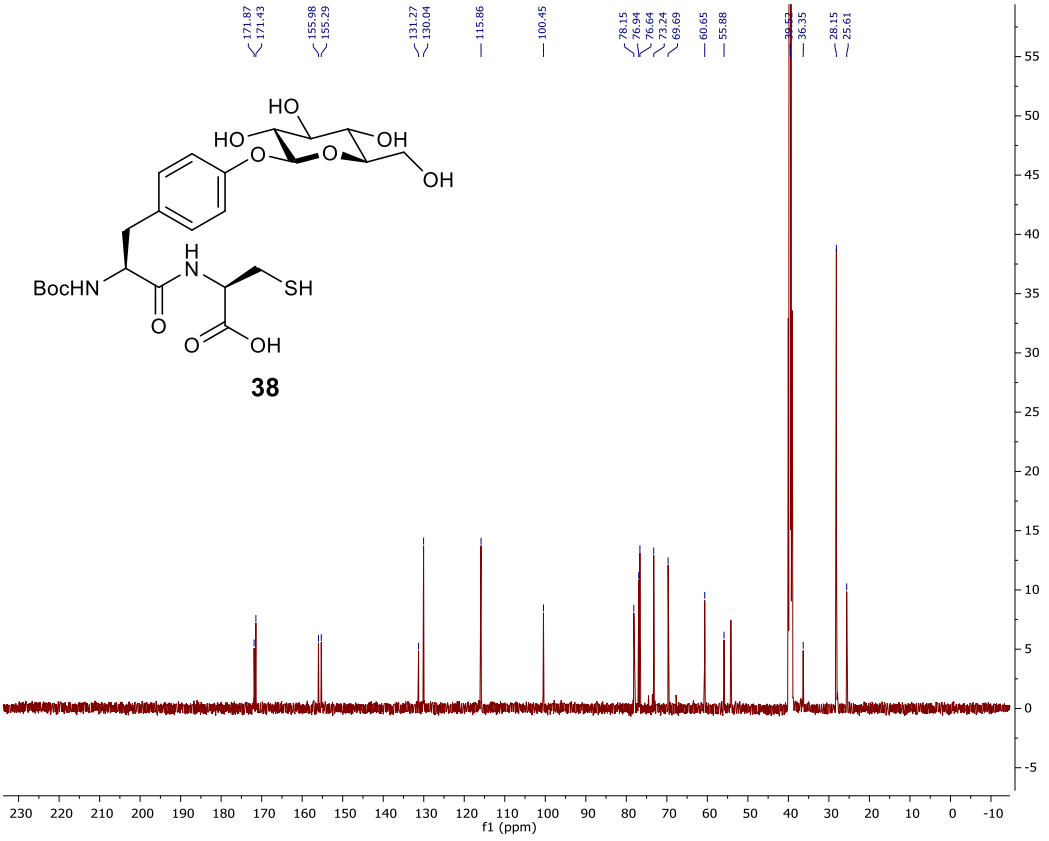
Boc-Tyr-Cys(*S*-β-D-Glc)-OH (37)



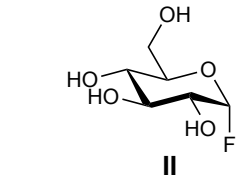


Boc-Tyr(*O*-β-D-glu)-Cys-OH (38)

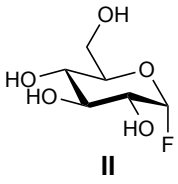




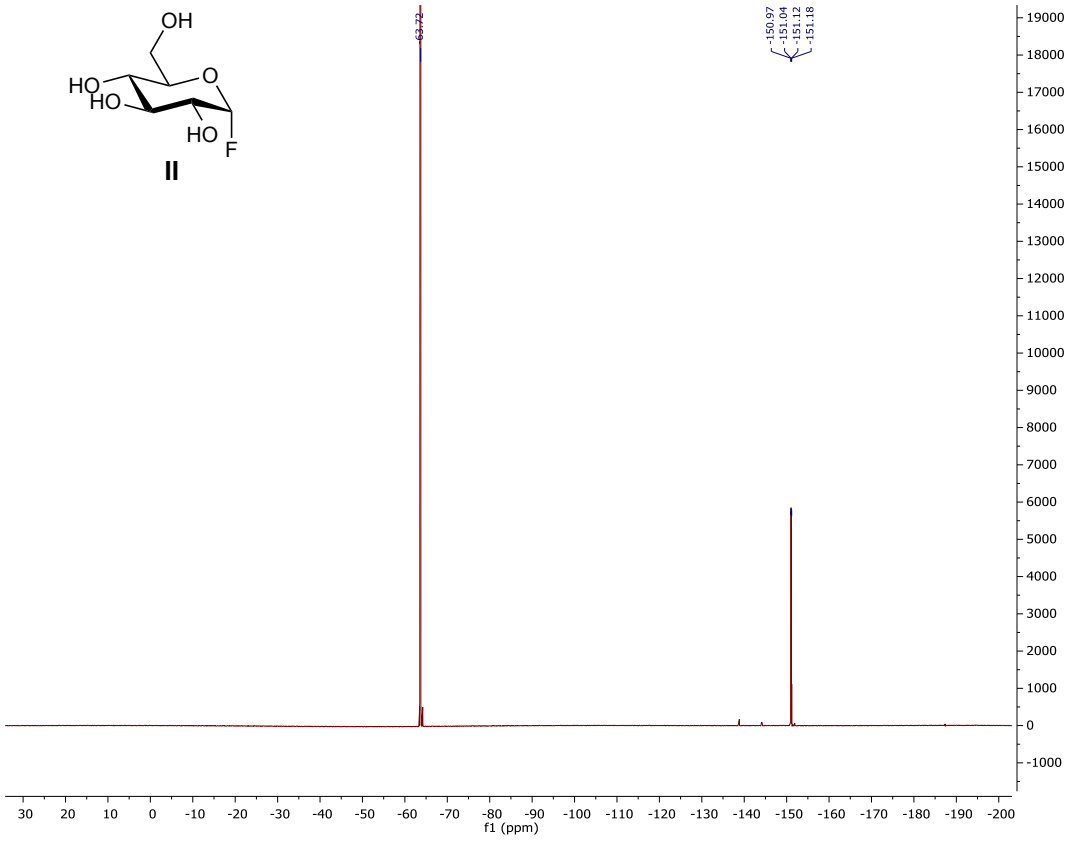
α-D-Fluoroglucose (II)



II

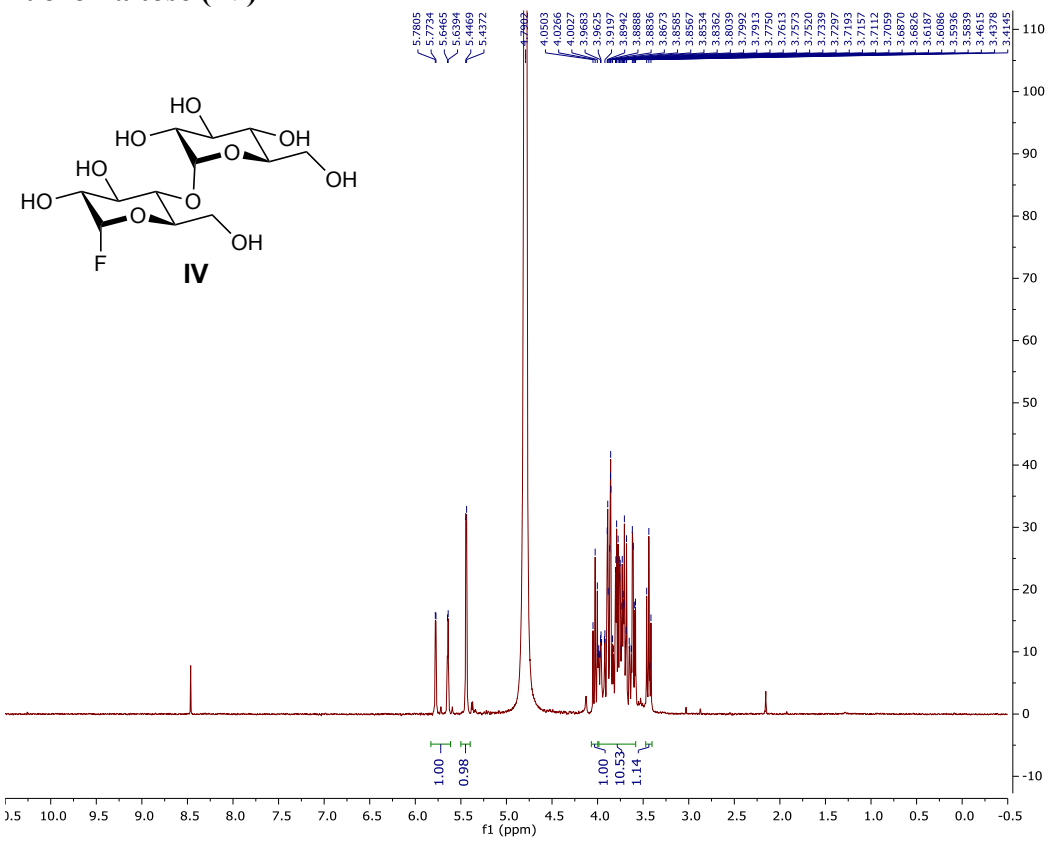


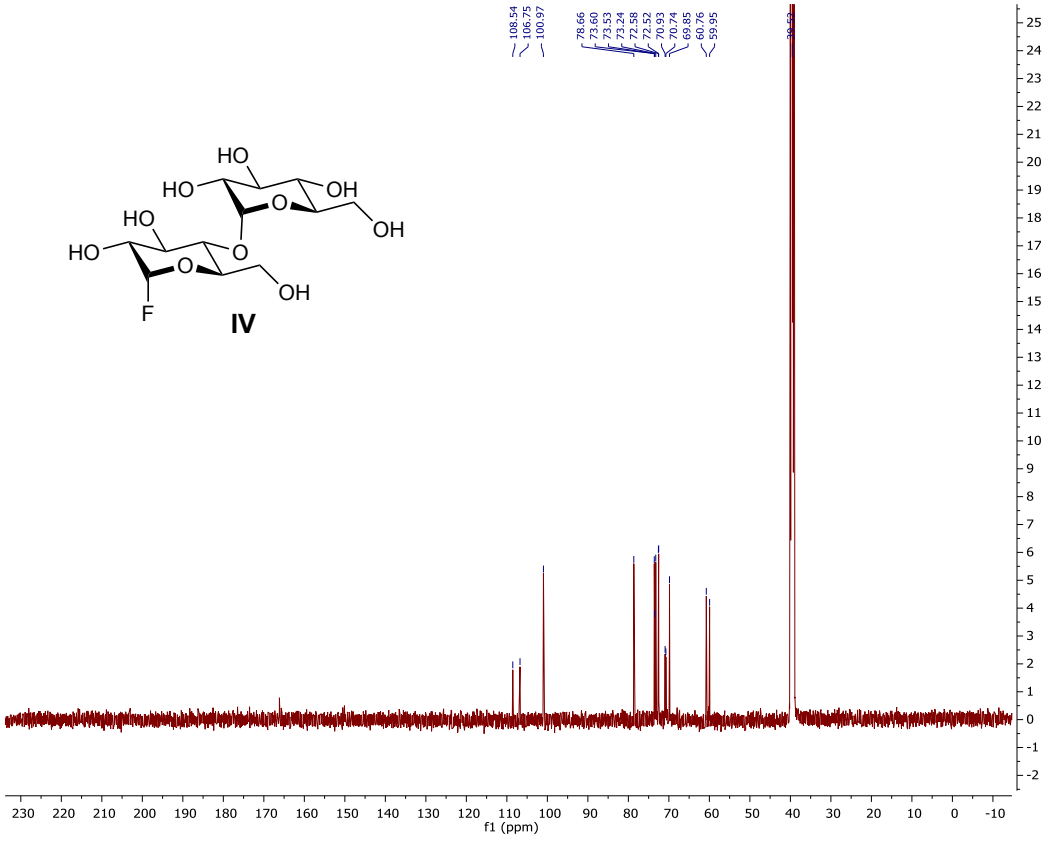
II

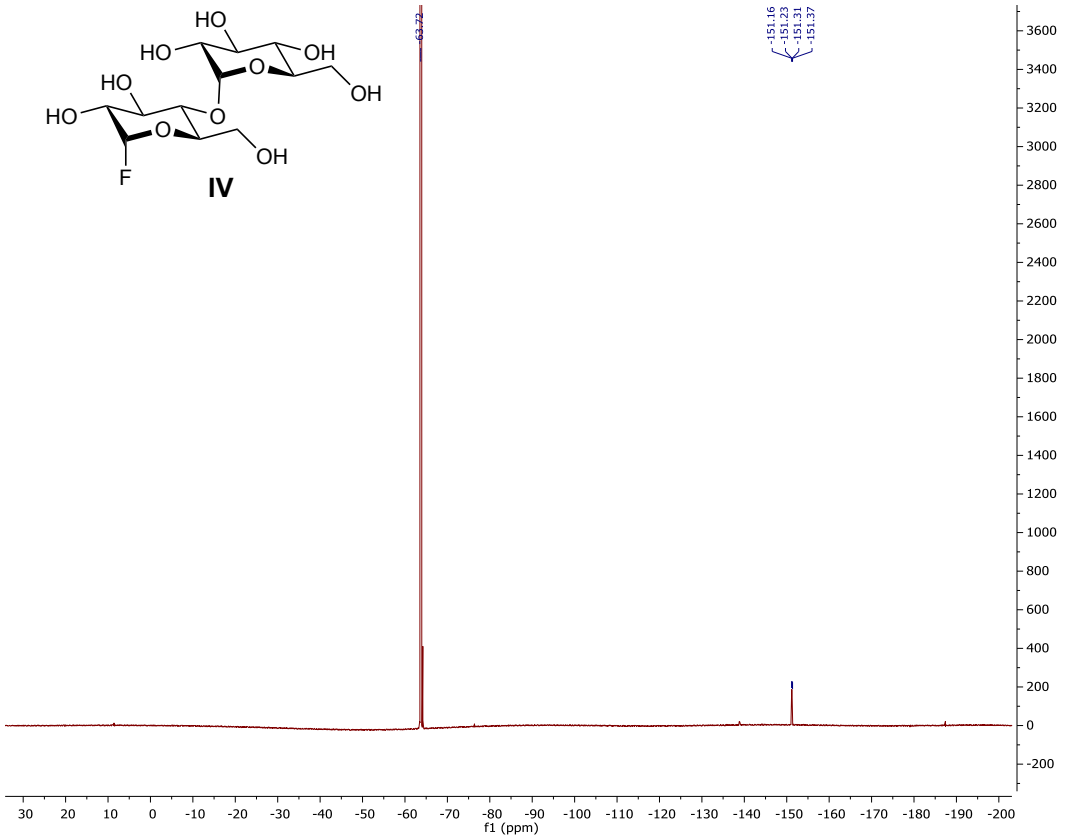

OH
HO
HO
O
HO
F
63.72
-150.97
-151.04
-151.12
-151.18
19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

II

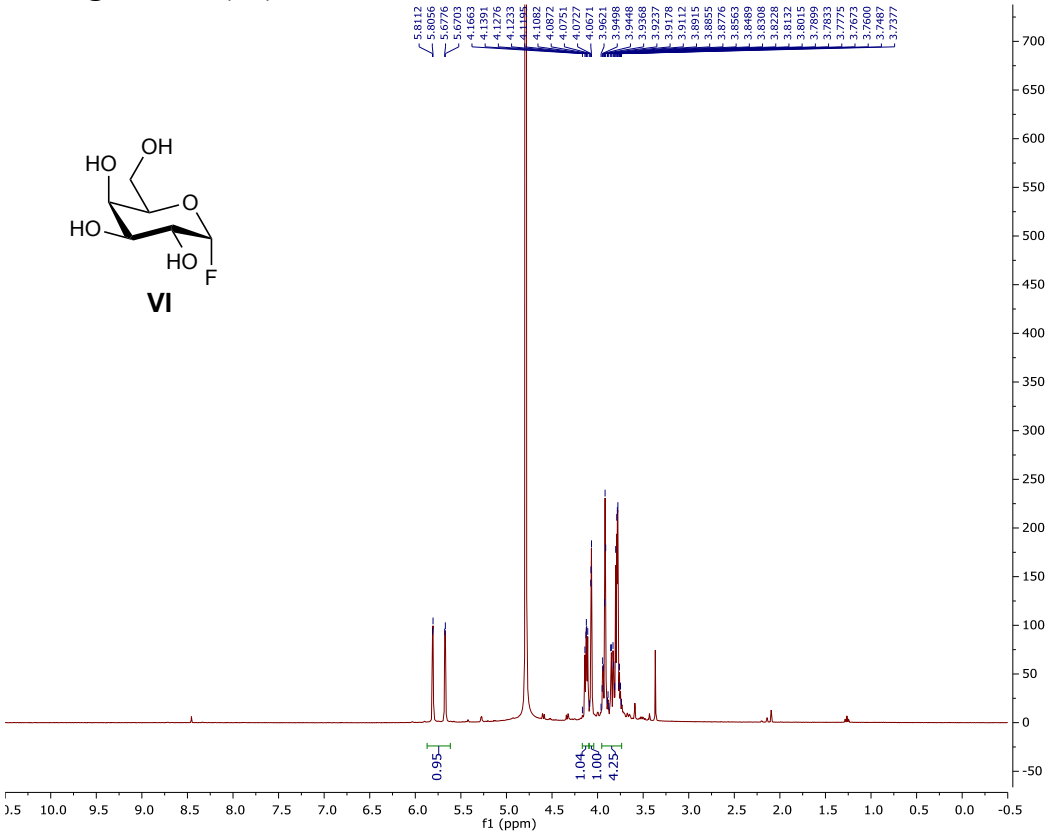
α-D-Fluoromaltose (IV)



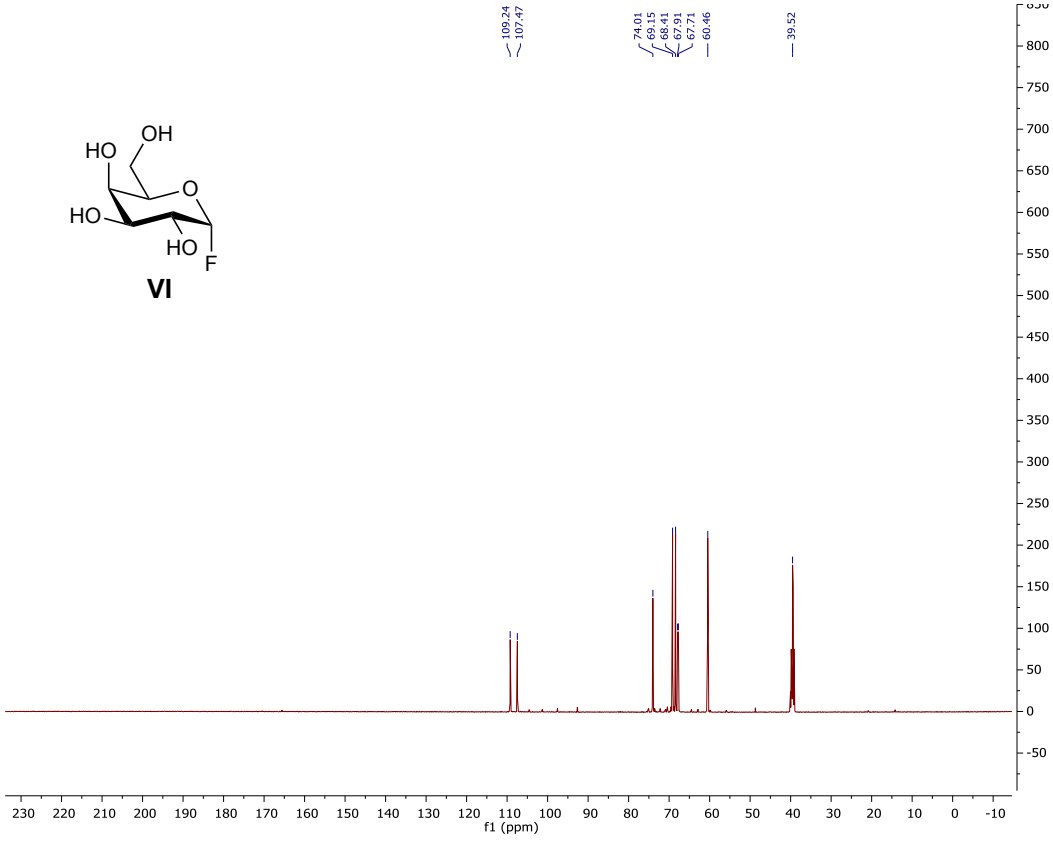



HO
HO
OH
OH
HO
HO
O
O
O
OH
F
IV
-63.72
-151.16
-151.23
-151.31
-151.37
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

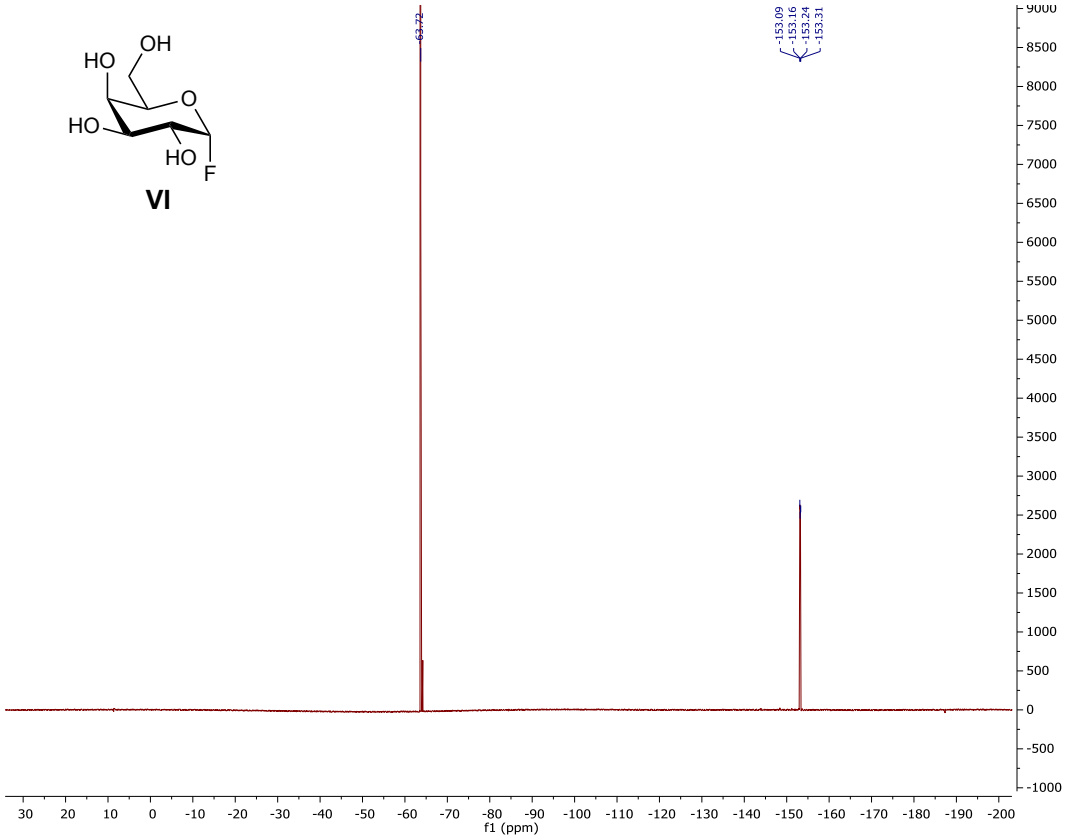
α-D-Fluorogalactose (VI)



VI

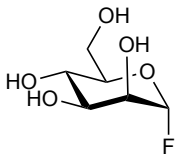


VI

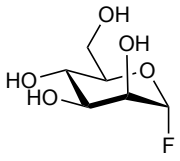


VI

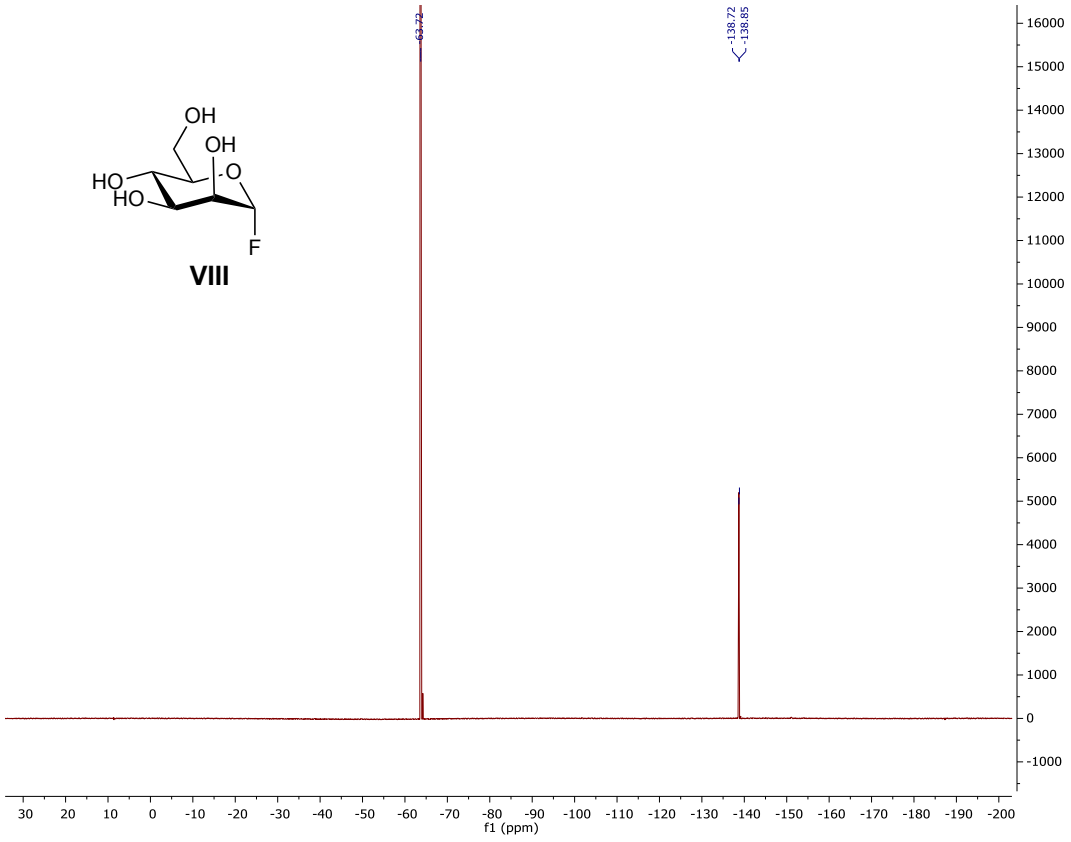
α-D-Fluoromannose (VIII)



VIII

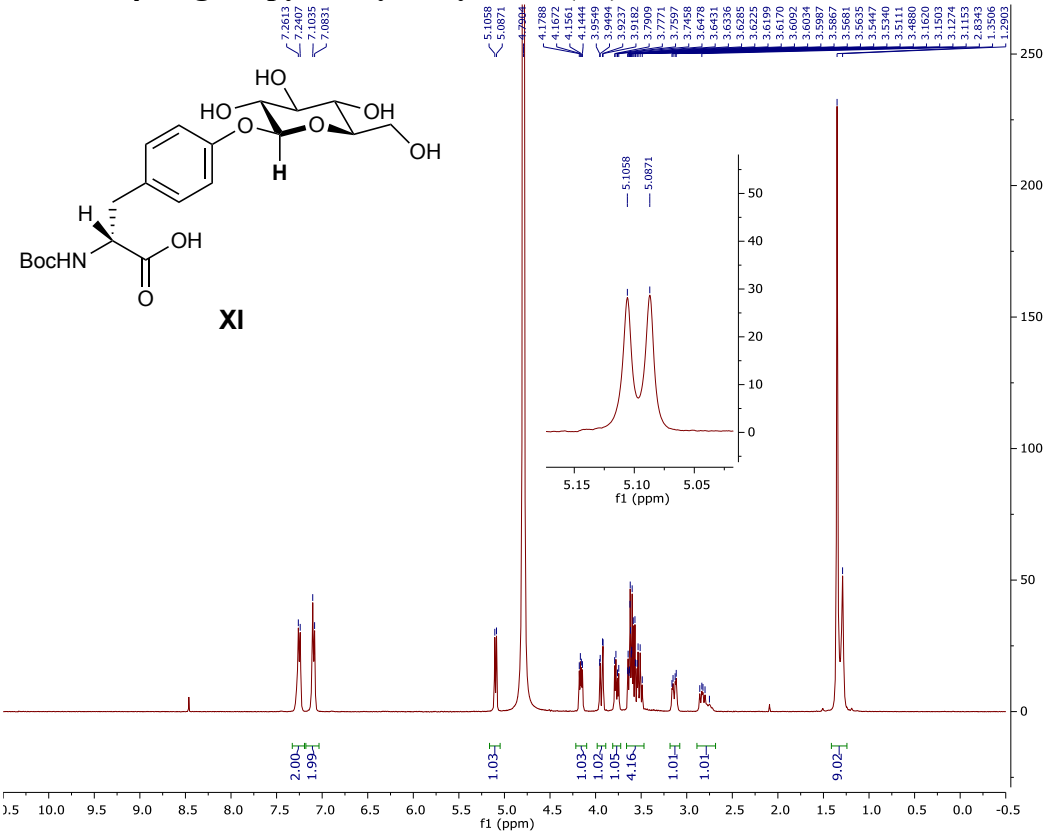


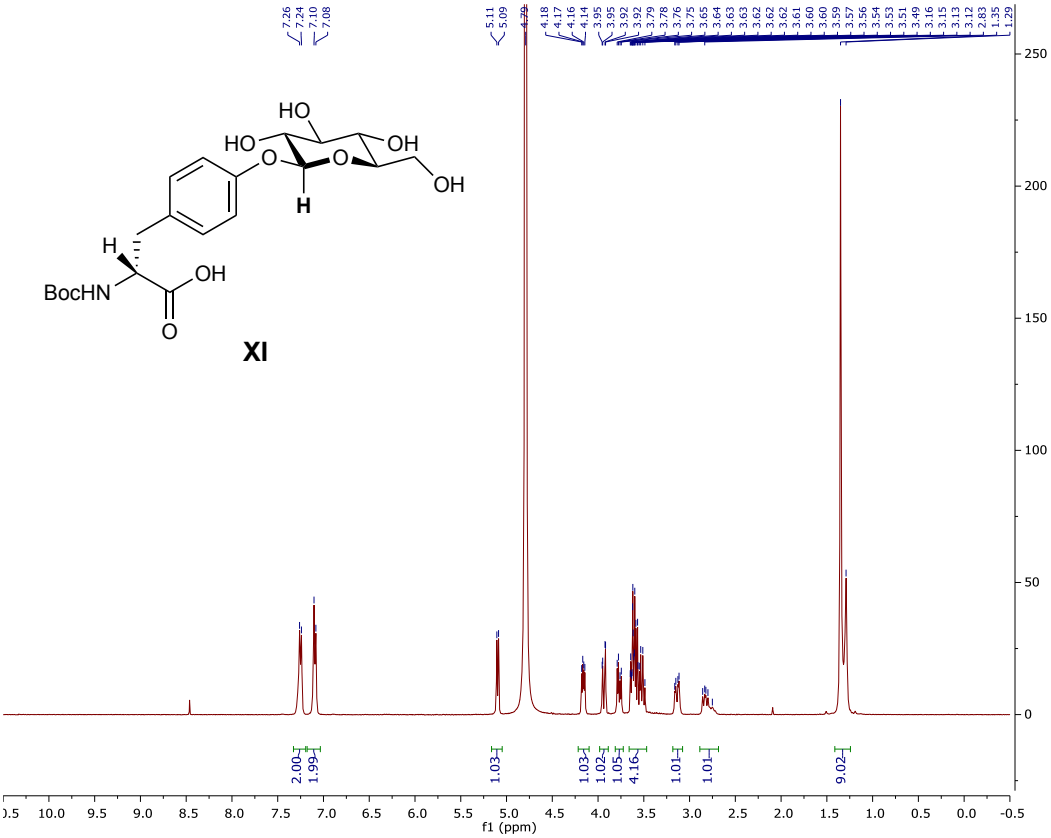
VIII


OH
OH
HO
HO
O
F
63.72
-138.72
-138.85
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

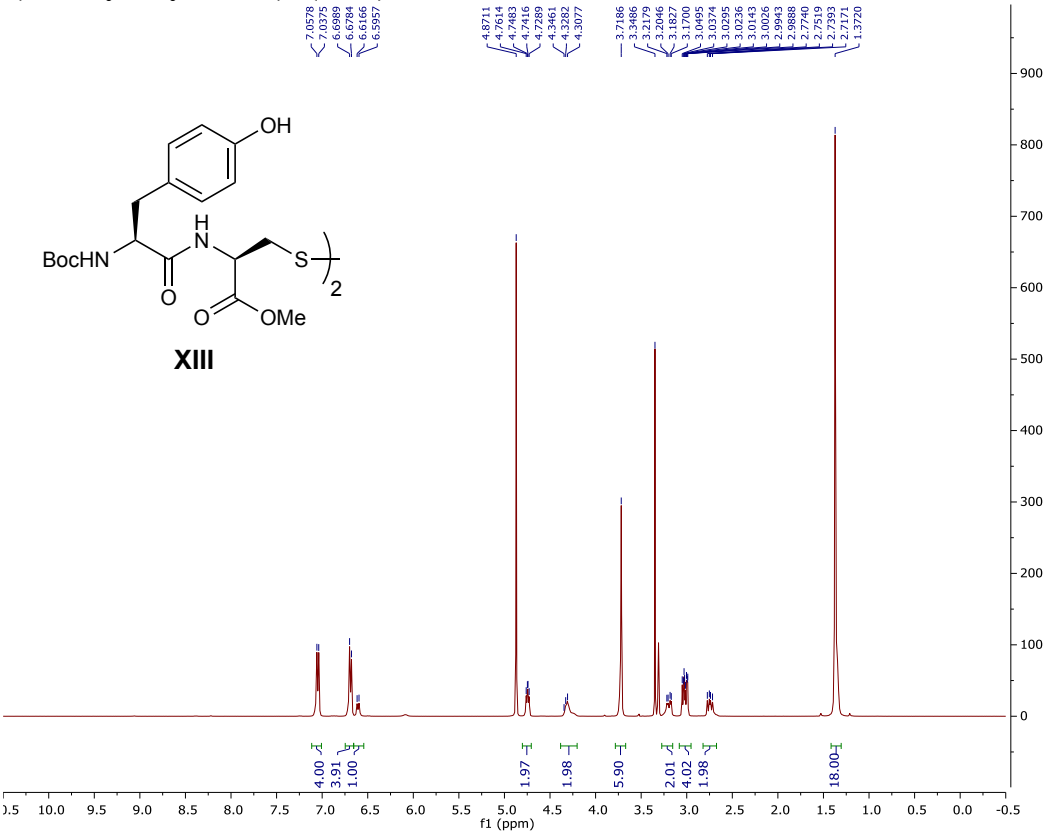
VIII

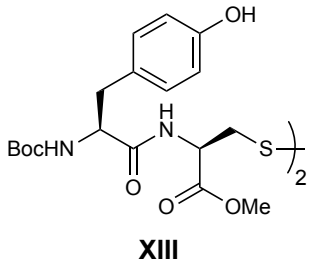
N-Boc-*O*-β-D-glucopyranosyl-D-tyrosine (XI)





(Boc-Tyr-Cys-OMe)$_2$ (XIII)

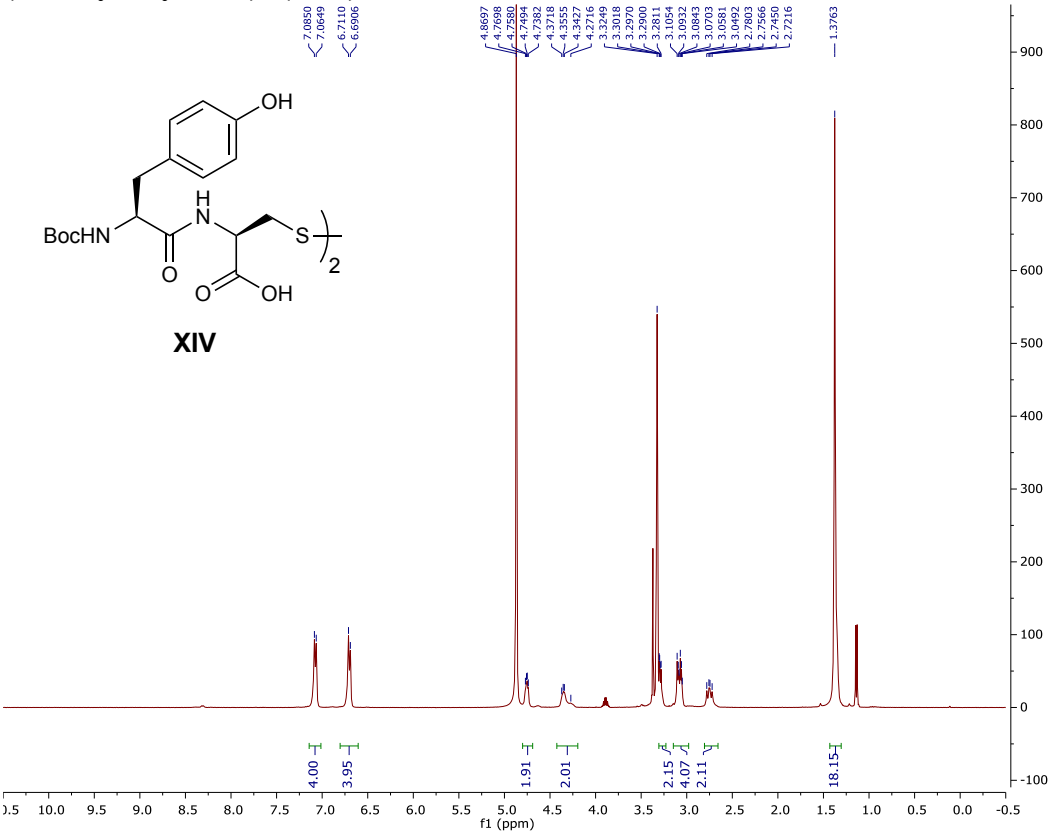


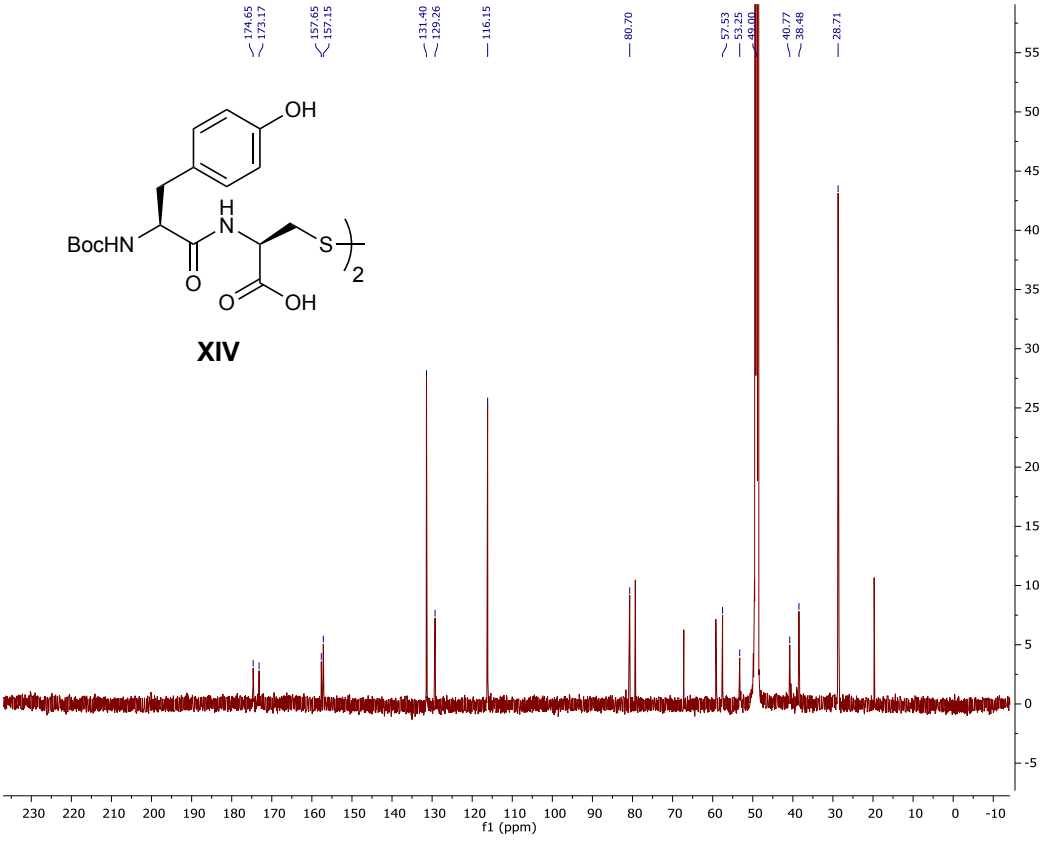


XIII

XIII

(Boc-Tyr-Cys-OH)$_2$ (XIV)





(Boc-Tyr(*O*-β-D-Glc)-Cys-OH)$_2$ (XV)

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