Fluorosugar Chain Termination Agents as Probes of the Sequence Specificity of a Carbohydrate Polymerase

Christopher D. Brown[†,§], Max S. Rusek[†] and Laura L. Kiessling[†,‡].

[†]*Department of Chemistry, University of Wisconsin--Madison, 1101 University Ave., Madison, WI 53706*

[‡]*Department of Biochemistry, University of Wisconsin--Madison, 433 Babcock Dr., Madison, WI 53706*

[§]*University of Wisconsin School of Medicine and Public Health,750 Highland Ave., Madison, WI 53705*

CORRESPONDING AUTHOR kiessling@chem.wisc.edu

Supporting Information:

I. General Procedures and Materials

All compounds were purchased from Sigma Aldrich (Milwaukee, WI) or Fischer Scientific (Pittsburgh, PA). Tetrahydrofuran (THF) and toluene were distilled from sodium/benzophenone ketyl. Diisopropylethylamine (DIEA), triethylamine (TEA) and dichloromethane (CH_2Cl_2) were distilled from calcium hydride. All reactions were run under nitrogen atmosphere unless otherwise stated. Analytical thin layer chromatography (TLC) was carried out on E. Merck (Darmstadt) TLC plates pre-coated with silica gel 60 F254 (250 μm layer thickness). Analyte visualization was accomplished using a UV lamp and charring with p-anisaldehyde solution. Flash chromatography was performed on Scientific Adsorbents Incorporated silica gel (32-63 μm, 60 Å pore size) using distilled reagent grade hexanes and ACS grade ethyl acetate (EtOAc) or methanol, CH_2Cl_2 and acetic acid. UDP sugars were purified by semi-preparative HPLC using a Dionex carbopak column eluting in a 300mM triethylammonium acetate buffer at neutral pH.^{1}H. ^{19}F, ^{31}P and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC-300 or Varian Inova-500 spectrometers, and chemical shifts are reported relative to tetramethylsilane or residual solvent peaks in parts per million ($CHCl_3$: ^{1}H: 7.26, ^{13}C: 77.0; MeOH: ^{1}H: 3.31, ^{13}C: 49.15; D_2O: ^{1}H: 4.79, ^{13}C: referenced to ^{1}H. ^{19}F and ^{31}P referenced to ^{1}H) Peak multiplicity is reported as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), doublet of triplets (dt), etc. High resolution electrospray ionization mass spectra (HRESI-MS) were obtained on a Micromass LCT. Polymerization reactions consisted of 10 μL total volume containing final concentrations of 0.2 μM His$_6$-GlfT2, approximately 200 μM acceptor, 1.0 mM UDP donor sugar in 50 mM Hepes, pH 7.0, 25 mM $MgCl_2$, and 100 mM NaCl. Reactions were incubated at room temperature for 18 h, then quenched with 30 μL of a 1:1 mixture of

$CHCl_3$/MeOH. Quenched reaction mixtures were evaporated to dryness under vacuum in a SpeedVac SC100 (Varian) then resuspended in 10 μL of 50% MeCN in MQ water for MALDI MS analysis. Samples for MALDI MS analysis were spotted as a 1:3 mixture with α-cyano-4-hydroxycinnamic acid matrix and spectra were recorded in positive linear mode using a Bruker Ultraflex III mass spectrometer.

II. Experimental Procedures

2,3-di-*O*-benzyl-6-deoxy-6-iodo-β-D-methylgalactopyranose (**5**): 2,3-di-*O*-benzyl-β-D-methylgalactopyranose (4.4 g, 11.8 mmol) was dissolved in dry THF (100 mL) under N_2 atmosphere. Triphenylphosphine (4.6 g, 17.6 mmol) and imidazole (2.5 g, 36.6 mmol). The flask was fitted with a flame dried condenser and the contents warmed to 50 ˚C. A solution of iodine (4.5 g, 17.6 mmol) in dry THF (20 mL) was added dropwise over 2h. Once the color of the reaction was a persistent yellow the contents were cooled to RT, quenched with saturated $Na_2S_2O_3$ solution until the yellow color dissipated. THF was removed *in vacuo* then the contents were diluted with water, extracted 2x into DCM, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica chromatography (gradient 10% → 40% EtOAc in Hex) gave 4.61 g of a colorless oil (81%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 7.42 – 7.22 (m, 10H, Ar-H), 4.89 (d, *J*= 11.1, 1H, OCH_2Ph), 4.73 (m, 3H, OCH_2Ph, OCH_2Ph), 4.26 (d, *J*= 7.7, 1H, H-1), 4.11 (dd, *J*= 3.3, 0.9, 1H, H-4), 3.59 (s, 3H, OCH_3), 3.61 – 3.46 (m, 2H, H-2, H-5), 3.50 (dd, *J*= 9.4, 3.5, 1H, H-3), 3.41 (m, 2H, H-6a, H-6b); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 138.75, 137.88, 128.73, 128.53, 128.24, 128.07, 127.85, 124.71, 80.59, 78.70, 75.24, 74.87, 72.96, 67.41, 57.28.

2,3-di-*O*-benzyl-5-eno-β-D-methyl galactofuranose (**6**): Iodogalactopyanose **5** (4.61 g, 9.52 mmol) was dissolved in anhydrous THF (25 mL) under N_2 atmosphere and chilled to -78 ˚C. A 2.5M solution of n-butyllithium in hexanes (8.4 mL, 20.9 mmol) was added and the reaction was warmed to -40 ˚C over 1hr and then stirred for another 2h. The remaining n-butyllithium was quenched with a 10% w/v solution of NH_4Cl, the product was extracted 3x into ether, the organics dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting oil was dissolved in DCM (16mL) and treated with 12M NaOH (2 mL). A large stir bar was used to vigorously mix the biphasic solution. Methyl iodide (1.5 mL, 24.1 mmol) and tetrabutylammonium iodide (358 mg, .97 mmol) were added and the reaction stirred at RT overnight. The reaction was diluted with DCM, washed 1x with 5% citric acid, 1x with H_2O, and 1x with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (gradient 5% → 20% EtOAc) allowed isolation of the β anomer (1.54 g, 47%). Characterization of β anomer: ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 7.41 – 7.21 (m, 10H, Ar-H), 5.92 (ddd, *J*= 17.2, 10.3, 7.1, 1H, H-5), 5.41 (dt, *J*= 17.2, 1.0, 1H, H-6 *trans*), 5.24 (dt, *J*= 10.3, 1.0, 1H, H-6 *cis*), 4.92 (s, 1H, H-1), 4.56 (s, 2H, OCH_2Ph), 4.53 (ABq, *J*=11.7, 2H, OCH_2Ph), 4.43 (t, *J*= 6.9, 1H. H-4), 4.00 (dd, *J*=3.4, 1.1, 1H, H-2), 3.77 (dd, *J*= 7.0, 3.4, 1H, H-3), 3.39 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 138.05, 137.81, 136.23, 128.69, 128.62, 128.13, 128.03, 118.32, 107.27, 88.88, 87.67, 82.36, 72.56, 72.32, 55.13

5,6-anhydro-2,3-di-*O*-benzyl-β-D-methyl galactofuranose and **5,6-anhydro-2,3-di-*O*-benzyl-β-L-methyl altrofuranose** (**7**): 5-eno-β-D-methyl galactofuranoside **6** (1.1 g, 3.23 mmol) was dissolved in reagent grade DCM (32 mL) and chilled to 0 ˚C. mCPBA (2.17 g, 9.69 mmol) was added and the contents stirred at RT for 2 days. The reaction was quenched with saturated

$Na_2S_2O_3$ and $NaHCO_3$ solution and extracted 1x into DCM. The organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (gradient 5% → 20% EtOAc in hexane) gave 947 mg of colorless oil as a 1:1 diastereomeric mixture of epoxides (97%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 7.41 – 7.21 (m, 20H, Ar-H), 4.95 (d, *J*= 3.7, 2H, H-1g, H-1a), 4.63 – 4.44 (m, 8H, 2x OCH_2Phg, 2x OCH_2Pha), 4.03 – 3.97 (m, 2H, H-2g, H-2a), 3.96 – 3.88 (m, 2H,H-3g, H-3a), 3.80 (t, *J*= 6.6, 2H, H-4g, H-4a), 3.39 (s, 3H, OCH_3), 3.38 (s, 3H, OCH_3), 3.11 (m, 2H, H-5g, H-5a), 2.75 (m, 4H, H-6g, H-6a); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 137.79, 137.64, 128.70, 128.69, 128.50, 128.26, 128.15, 128.10, 128.06, 107.69, 107.57, 101.31, 88.09, 87.91, 84.62, 83.82, 82.14, 82.02, 72.65, 72.34, 72.22, 72.15, 55.28, 55.21, 52.17, 51.65, 45.65, 44.03

5,6-anhydro-2,3-di-*O*-benzyl-beta-D-methyl galactofuranose (**8**) and **2,3-di-*O*-benzyl-beta-L-methylaltrofuranose** (**9**)**:** (*S*,*S*)-*N*,*N*'-Bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediaminocobalt (II) (334mg, .5528 mmol) was dissolved in 200 µL of reagent grade DCM and treated with glacial acetic acid (25 µL, .442 mmol). The solution stirred open to air for 30 minutes. DCM was removed *in vacuo*. A solution of epoxide **7** (1.97g, 5.528 mmol) in THF (8mL, 0.7M) and water (50 µL, 2.763 mmol) was added to the brown residue and the reaction was capped and stirred overnight. The reaction was stopped when TLC indicated 50% conversion to diol. Quenched with 5 mL of 2M NH_3 in MeOH and stirred for 30 minutes. Concentrated *in vacuo*. Purified by silica gel chromatography (DCM → 60% EtOAc in hexane, 10% stepwise gradient). Isolated 1.083 g (55%) of epoxide and 958mg (46%) of diol. Average isolated yields for three separate experiments were 48% for epoxide, 45% for diol. Characterization of epoxide **8**: ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 7.4-7.2 (m, 10H, Ar-H), 4.93 (s, 1H, H-1), 4.54 (ABq, 2H, J = 12.1, $-OCH_2Ph$), 4.53 (ABq, 2H, J = 11.6 Hz, $-OCH_2Ph$), 3.99 (dd, J = 3.1, 1.1, 1H, H-2), 3.93 (dd, J = 6.8, 2.9, 1H, H-3), 3.79 (dd, J = 6.7, 6.0, 1H, H-4), 3.37 (s, 3H, OCH_3), 3.09 (ddd, J = 5.9, 4.1, 2.7, 1H, H-5), 2.74 (AB part of an ABX, J_{AX} = 4.1, J_{BX} = 2.7, 2H, H-6a, H-6b); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 137.80, 137.64, 128.70, 128.69, 128.15, 128.05, 107.57, 88.11, 84.63, 82.02, 72.64, 72.33, 55.21, 52.16, 44.02; MS (EMM)$^+$: *m/z*: 379.1503 $(M+Na)^+$ ($M+Na^+$ calcd 379.1516) Characterization of diol (#): ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 7.4 – 7.2 (m, 10H, Ar-H), 4.93 (s, 1H, H-1), 4.53 (ABq, J = 11.9, 2H, OCH_2Ph) 4.50 (ABq, J = 11.8, 2H, OCH_2Ph), 4.07 (m, 2H, H-2, H-3), 3.99 (broad s, 1H, H-4), 3.92 (m, 1H, H-5), 3.64 (m, 2H, H-6a, H-6b), 3.37 (2, 3H, OCH_3), 2.56 (d, J = 3.9, 1H, 5-OH), 2.20 (t, J = 6.0, 1H, 6-OH); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 137.56, 137.38, 128.75, 128.73, 128.36, 128.28, 128.26, 128.22, 107.39, 87.56, 82.76, 82.51, 72.49, 72.18, 71.51, 63.57, 55.15; MS (EMM)$^+$: *m/z*: 397.1634 $(M+Na)^+$ ($M+Na^+$ calcd. 397.1622)

2,3-di-*O*-benzyl-6-deoxy-6-fluoro-β-D-methyl galactofuranose: Epoxide **8** (45 mg, .126 mmol) was placed in a 3mL Eppendorf tube and dissolved in HF-triethylamine (180 µL, .7M). The tube was capped, warmed to 70 °C using a sand bath and incubated for 4 days. The contents were cooled to room temperature, diluted with acetone and carefully filtered through a silica plug. Concentrated *in vacuo*. Purified by silica gel chromatography (5% → 20% EtOAc in hexane gradient). Isolated 44 mg of fluoroalcohol as a pale yellow oil (94%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 7.4 – 7.2 (m, 10H, Ar-H), 4.54 (m, 5H, 2x OCH_2Ph, H-6a), 4.34 (1/2 of the AB part of an ABMX, 1H, H-6b), 4.14 (dd, J = 5.8, 2.9, 1H, H-4), 4.05 (dd, J = 5.7, 2.0, 1H, H-3), 3.98 (d, J = 1.5, 1H, H-2) 3.90 (dtd, J = 14.3, 5.9, 3.1, 1H, H-5), 3.37 (s, 3H, OCH_3), 2.23 (broad s, 1H, 5-OH); ^{19}F NMR (280 MHz, $CDCl_3$, δ (ppm)) -233.7 (td, 48.7, 14.1); ^{13}C NMR (75

MHz, $CDCl_3$, δ (ppm)) 137.71, 137.33, 128.74, 128.29, 128.23, 128.19, 128.16, 107.72, 87.11, 85.25, 83.17, 82.99, 81.31, 81.24, 72.62, 72.16, 70.01, 69.74, 55.18; MS (EMM)$^+$: *m/z*: 399.1579 (M+Na)$^+$ (M+Na$^+$ calcd. 399.1579)

6-deoxy-6-fluoro-beta-D-methyl galactofuranose (**10**)*:* 2,3-di-*O*-benzyl-6-deoxy-6-fluoro-β-D-methyl galactofuranose (44 mg, .117 mmol) was dissolved in reagent grade methanol (4.5 mL, .025M) and treated with 20 wt% palladium hydroxide on carbon (44mg). The flask was evacuated and backfilled with H_2 from a balloon three times. Stirred at STP overnight. Filtered through celite, washed with methanol and concentrated in vacuo. Isolated 22 mg of a colorless oil (96%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 4.91 (s, 1H, H-1), 4.55 (AB part of an ABMX, J = , 2H, H-6a, H-6b), 4.5 (m, 3H, H-2, H-3, H-5), 4.1 (s, 1H, H-4), 3.40 (s, 3H, OCH_3); ^{19}F NMR (280 MHz, $CDCl_3$, δ (ppm)) -233.5 (td, 48.1, 14.0) ^{13}C NMR (75 MHz, CD_3OD, δ (ppm)) 110.72, 86.51, 84.27, 83.84 (d, J = 27), 83.30, 78.68, 70.48 (d, J = 89), 55.45; MS (EMM)$^+$: *m/z*: 219.0630 (M+Na)$^+$ (M+Na$^+$ calcd. 219.0640)

2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-β-D-methyl galactofuranose: Fluoro Gal*f* **10** (250mg, 1.274 mmol) was dissolved in dry pyridine (12 mL) under N_2 atmosphere and chilled to 0 °C. Benzoyl chloride (565 µL) was added dropwise over 5 minutes. The contents were warmed to RT and stirred for 3 hours. The reaction was diluted with toluene and azeotroped 3x. Placed on high vacuum line to dry. Purified by silica chromatography (5% → 20% EtOAc in hexane). Isolated 560 mg of white crystalline powder (86%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 8.1 (dd, J = 9.1, 7.2, 4H, Ar-H), 7.90 (d, J = 7.2, 2H, Ar-H), 7.58 (m, 3H, Ar-H), 7.42 (t, J = 7.8, 2H, Ar-H), 7.30 (t, J = 7.5, 4H, Ar-H), 5.85 (dq, J = 16.0, 5.2, 1H, H-5), 5.59 (d, J = 5.4, 1H, H-3), 5.45 (d, J = 0.9, 1H, H-2), 5.20 (s, 1H, H-1), 4.80 (ddq, J = 46.7, 9.8, 5.9, 2H, H-6a, H-6b), 4.63 (dd, J = 5.2, 3.9, 1H, H-4), 3.47 (s, 3H, OCH_3); ^{19}F NMR (280 MHz, $CDCl_3$, δ (ppm)) -235.5 (td, 46.6, 16.0) ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 165.92, 165.87, 165.71, 133.72, 133.61, 133.56, 130.22, 130.18, 130.05, 129.54, 129.20, 128.64, 107.01, 82.50, 82.38, 80.51, 80.44, 80.20, 77.75, 70.99, 70.71, 55.18; MS (EMM)$^+$: *m/z*: 531.1450 (M+Na$^+$ calcd. 531.1426)

1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-D-galactofuranose: 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-β-D-methyl galactofuranose (100 mg, .197 mmol) was placed in a flame-dried round bottom flask and treated with a 1.4% v/v solution of concentrated H_2SO_4 in acetic anhydride (2 mL, .1M). The reaction stirred at RT for 2 hours. Quenched with saturated $NaHCO_3$ solution, diluted with brine and extracted into DCM 3x. Dried organic layer over $MgSO_4$, filtered and concentrated *in vacuo*. Azeotroped remaining acetic acid with toluene 2x. Purified by silica gel chromatography (5% → 20% EtOAc in hexane) to give 97 mg of white crystalline solid (92%, 5:1 mix of β:α). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 8.10 (m, 6.5H, Ar-H), 7.90 (d, J = 7.5, 2H, Ar-H), 7.6 – 7.2 (m, 13H, Ar-H), 6.61 (d, J = 4.8, .2H, H-1α), 6.50 (s, 1H, H-1β), 6.17 (t, J = 6.6, .2H, H-2α), 5.89 (dq, J = 16.2, 5.0, 1H, H-5β), 5.75 (m, .4H, H-3α, H-5α), 5.63 (d, J = 4.7, 1H, H-3β), 5.59 (s, 1H, H-2β), 5.0 – 4.8 (m, ~4H, H-4α, H-4-β, H-6aα, H-6aβ, H-6bα, H-6bβ), 2.20 (s, 3H, C(O)CH_3 β), 2.13 (s, .6H, C(O)CH_3 α); ^{19}F NMR (280 MHz, $CDCl_3$, δ (ppm)) -235.5 (td, J = 46.3, 15.7, β anomer), -237.5 (td, J = 46.1, 19.3, α anomer); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 169.35, 165.84, 165.72, 165.50, 133.92, 133.80, 133.65, 130.24, 130.10, 129.34, 129.07, 128.89, 128.77, 128.68, 128.65, 99.55, 93.65, 82.85, 82.79, 82.27, 81.64, 79.97, 77.68, 76.49, 70.74, 70.46, 21.29, 21.23; MS (EMM)$^+$: *m/z*: 559.1380 (M+Na$^+$ calcd. 559.1375)

Dibenzyl 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-D-galactofuranose (100 mg, .186 mmol) was placed in a flame dried round bottom flask and dissolved in dry DCM (2.0 mL) under N_2 atmosphere. The solution was chilled to 0 °C and treated with a 33 wt% HBr in AcOH solution (120 μL). After stirring for 20 minutes the reaction was warmed to RT and stirred for 4 hours. Azeotroped using freshly distilled toluene 3x to isolate the crude glycosyl bromide as an orange oil. The oil was dissolved in dry toluene (1.0 mL) under N_2. The solution was added dropwise over 30 minutes to a stirring solution of dibenzyl phosphate (208 mg) in dry toluene (1.0 mL). The contents stirred at RT for 2.5 hours. Concentration *in vacuo* followed by silica gel chromatography (12% EtOAc in toluene) allowed isolation of 97 mg of the α-anomer as a slightly cloudy viscous oil (69%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 8.14 (d, J = 7.0, 2H, Ar-H), 8.0 (t, J = 7.1, 4H, Ar-H), 7.60 – 7.00 (m, 19, Ar-H), 6.32 (dd, J = 5.6, 4.7, 1H, H-1), 6.13 (t, J = 7.2, 1H, H-3), 5.70 (m, 2H, H-2, H-5), 5.05 – 4.55 (m, 7H, 2x OCH_2Ph, H-6a, H-6b, H-4); ^{19}F NMR (280 MHz, $CDCl_3$, δ (ppm)) -237.2 (td, J = 46.8, 17.3); ^{31}P NMR (122 MHz, $CDCl_3$, δ (ppm)) -1.88 (m); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 165.80, 165.76, 165.72, 135.65, 135.54, 133.92, 133.84, 133.58, 130.33, 130.24, 130.15, 130.04, 129.34, 128.87, 128.73, 128.65, 128.00, 127.89, 97.85, 97.79, 81.69, 79.37, 79.29, 76.68, 73.43, 71.53, 71.25, 69.62, 69.55, 69.45; MS (EMM)$^+$: *m/z*: 777.1890 (M+Na$^+$ calcd. 777.1872)

Triethylammonium 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: Dibenzyl 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate (40 mg, .053 mmol) was dissolved in ethyl acetate (500 μL, .1M) and triethylamine (50 μL). 10% Pd on carbon (20 mg) was added. The stoppered flask was evacuated and back filled with H_2 from a balloon. The contents stirred at STP for 90 minutes. The reaction was filtered through celite, washed with ethyl acetate and concentrated *in vacuo*. Isolated 33 mg of cloudy, viscous oil (90%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 8.10 (t, J = 7.2, 4H, Ar-H), 7.90 (d, J = 7.2, 2H, Ar-H), 7.5 – 7.2 (m, 9H, Ar-H), 6,21 (t, J = 7.2, 1H, H-1), 6.13 (dd, J = 7.4, 4.5, 1H, H-3), 5.69 (dq, J = 18.7, 4.8, 1H, H-5), 5.60 (ddd, J = 7.7, 4.5, 1.4), 5.00 (m, 2H, H-6a, H-6b), 4.54 (t, J = 5.4, 1H, H-4), 2.85 (q, J = 7.2, $NC\underline{H_2}CH_3$), 1.2 (t, J = 7.3, $NCH_2\underline{CH_3}$); ^{19}F NMR (280 MHz, $CDCl_3$, δ (ppm)) -238.1 (td, J = 48.7, 18.9); ^{31}P NMR (122 MHz, $CDCl_3$, δ (ppm)) -1.88 (d, J = 7.9); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 174.93, 66.03, 165.70, 133.43, 133.23, 133.16, 130.41, 130.03, 129.83, 129.54, 129.40, 128.50, 128.44, 128.26, 95.98, 82.61, 80.34, 74.16, 72.44, 72.19, 45.46, 8.57; MS (EMM)$^-$: *m/z*: 573.0941 ([M-H]$^-$, calcd. 573.0967)

Tributylammonium 6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: Benzoylated Gal*f* monophosphate (#) (42 mg, .06 mmol) was dissolved in a 5:2:1 mixture of methanol:water:triethylamine (2.3 mL) and warmed to 30 °C. The reaction stirred for 4 days. Concentrated *in vacuo* then azeotroped 3x with toluene. Purified by anion exchange chromatography (Biorad AG1-X8 resin, gradient elution 250 mM → 500 mM NH_4HCO_3 solution) using a peristaltic pump. Column fractions were combined, frozen on dry ice and lyophilized. The residue was treated with ~200 μL Dowex-50WX8-200 resin (pH 7, pretreated with 2.0M $HNBu_3Cl$) for 4 hours. The resin was removed by filtration through a cotton plug and the filtrate was frozen on dry ice and lyophilized. Isolated 18 mg of fluffy white solid (56%, 1.1 equivalents of tributylammonium). ^{1}H NMR (300 MHz, D_2O, δ (ppm)) 5.45 (t, J = 4.8, 1H, H-1), 4.65 – 4.40 (AB part of an ABMX, J = 47.0, 5.7, 3.7, 2H, H-6a, H-6b), 4.21 (dd, J = 8.6, 7.2, 1H, H-3), 4.06 (ddd, J = 8.4, 4.3, 2.3, 1H, H-2), 3.88 (dtd, 20.8, 5.4, 3.9, 1H, H-5), 3.83 (dd, J = 7.2, 5.6, 1H, H-4), 3.10 (m, 7H), 1.65 (m, 7H), 1.38 (sextet, J = 7.3, 7H), 0.9 (t, J = 7.3, 10H); ^{19}F NMR (280 MHz, D_2O, δ (ppm)) -235.6 (td, J = 46.9, 21.1); ^{31}P NMR (122 MHz, D_2O, δ (ppm)) -0.3 (d, J = 3.5); ^{13}C NMR (75 MHz, D_2O, δ (ppm)) 97.14 (d, J = 4.9), 85.20, 82.98, 81.07 (d, J = 7.0), 76.63 (d, J = 7.5), 73.59, 70.76 (d, J = 19), 52.85, 25.36, 19.43, 12.92; MS (EMM)$^-$: *m/z*: 261.0176 ([M-H]$^-$, calcd. 261.0181)

Uridine diphosphate 6-deoxy-6-fluoro-α-D-galactofuranose (**2**): A solution of tributylammonium UMP (16mg, .03 mmol) in anhydrous DMF (470 μL) was stirred with activated 4 Å molecular sieves under N_2 for 1 hour. The molecular sieves were removed and the solution was treated with diisopropylethylamine (17 μL, .1 mmol) and 1-methyl-3-benzenesulfonylimidazolium triflate (12.4 mg, 0.4 mmol) and stirred for 1 minute. The solution was added dropwise over 30 seconds to a solution of tributylammonium 6-deoxy-6-fluoro-α-D-galactofuranose monophosphate (10mg, .02 mmol) and $MgCl_2$ (3mg, .03 mmol) in anhydrous DMF (220 μL) at 0 °C. The contents were raised to room temperature and stirred for 2 hours. The reaction was quenched with 1.0 mL of 100 mM ammonium acetate and washed 1x with 1.0 mL of DCM. The DCM was extracted with 1.0 mL of 100 mM ammonium acetate, the aqueous layers were combined, frozen on dry ice and stored at -20 °C until purification by HPLC. Purified by HPLC using a semiprep carbopak dionex column, 300mM tirthylammonium acetate, pH = 6.0-7.0, flow rate = 1mL/min. Collected peak eluting at 40 – 45 minutes, froze on dry ice and lyophilized to give a light brown solid. ^{1}H NMR (500 MHz, D_2O, δ (ppm)) 7.98 (d, J = 8.3, 1H, H-5u), 5.99 (m, 2H, H-1r, H-6u) 5.67 (t, J = 4.2, 1H, H-1g) 4.62 (m, 2H, H-6ag, H-6bg) 4.15 – 4.42 (m, 7H, H-2g, H-2r, H-3g, H-3r, H-4r, H-5ar, H-5br) 4.01 (dq, J = 20.5, 5.0, 1H, H-5g) 3.91 (t, J = 7.5, 1H, H-4g); ^{19}F NMR (280 MHz, D_2O, δ (ppm)) -234.8; ^{13}C NMR (125 MHz, D_2O, δ (ppm)) 183.52, 168.86, 154.46, 144.27, 105.28, 100.30, 90.95, 87.08, 85.93, 85.85, 85.75, 83.82, 83.77, 79.33, 79.27, 76.38, 76.19, 73.52, 73.38, 72.28, 67.50, 49.28, 10.81; MS (EMM)$^+$: *m/z*: 591.0406 ([M+Na]$^+$, calcd. 591.0400)

2,3,6-tri-*O*-benzyl-β-L-methyl altrofuranose: Dibutyltin oxide (244 mg, .979 mmol) was placed in a flame-dried round bottom flask flushed with N_2. A solution of dibenzyl Alt*f* **9** (282 mg, .753 mmol) in hot toluene (2.5 mL) was added and the flask was equipped with a claisen adapter, thermometer and reflux condenser. The ensemble was tilted 45° such that the bend in the claisen adapter could collect refluxing solvent. A spatula tip of $MgSO_4$ was placed in the adapter and the reaction was heated to reflux. After 5 hours, the claisen adapter was removed and the condenser was fitted directly to the reaction flask. Benzyl bromide (116 uL, .979 mmol) and tetrabutylammonium bromide (24 mg, .075 mmol) were added the reflux continued overnight. The contents were cooled to RT and quenched with aqueous KF. Toluene was isolated, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (5% → 20% EtOAc in hexane) gave 237 mg of colorless oil (70%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 7.4 – 7.2 (m, 15H, Ar-H), 4.94 (s, 1H, H-1), 4.50 (m, 6H, 3x OCH_2Ph), 4.22 (t, J = 4.9, 1H, H-4), 4.15 (dd, J = 4.8, 1.1, 1H, H-3), 3.99 (m, 1H, H-5), 3.95 (d, J = 1.2, 1H, H-2), 3.55 (AB part of an ABX, J_{AX} = 3.9, J_{BX} = 6.7, J_{AB} = 9.9, 2H, H-6a, H-6b), 3.37 (s, 3H, OCH_3), 2.50 (d, J = 3.5, 1H, 5-OH);); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 138.02, 137.55, 128.69, 128.64, 128.61, 128.30, 128.16, 128.10, 128.02, 127.96, 107.46, 87.32, 83.31, 82.65, 73.68, 72.22, 71.90, 71.13, 70.82, 55.07; MS (EMM)$^+$: *m/z*: 487.2116 ([M+Na]$^+$, calcd. 487.2092)

2,3,6-tri-*O*-benzyl-5-deoxy-5-fluoro-β-D-methyl galactofuranose: 2,3,6-tri-*O*-benzyl-β-L-methyl altrofuranose (26 mg, .056 mmol) was dissolved in dry DCM (500 μL) under N_2 and chilled to -10 °C. DAST (11 μL, .084 mmol) was added dropwise. After stirring for 30 minutes the reaction was warmed to RT and stirred for an additional hour. The contents were chilled to 0 °C and stirred with aqueous $NaHCO_3$ (2.0 mL) for 30 minutes. Extracted into chloroform, washed with water, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (5% → 10% EtOAc in hexane) gave 12 mg of a colorless oil (46%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 7.4 – 7.2 (m, 15H, Ar-H) 4.93 (s, 1H, H-1), 4.75 (ddt, J = 48.4, 6.7, 3.7, 1H, H-5), 4.51 (m, 6H, 3x OCH_2Ph), 4.12 (ddd, J = 22.6, 6.9, 3.5, 1H, H-4), 4.05 (m, 1H, H-3), 3.98 (m, 1H, H-2), 3.7 (m, 2H, H-6a, H-6b), 3.37 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 138.03, 137.74, 137.63, 128.67, 128.14, 127.96, 127.91, 107.51, 92.03, 89.65, 87.91, 82.78 (d, J = 6.5), 80.21 (d, J = 17.9), 73.74, 72.62, 72.25, 69.69, 69.37, 55.20, 29.92; ^{19}F NMR (280 MHz, CD_3OD, δ (ppm)) -204.9 (dq, J = 44.9, 23.7); MS $(EMM)^+$: *m/z*: 489.2036 ($[M+Na]^+$, calcd. 489.2048)

5-deoxy-5-fluoro-beta-D-methyl galactofuranose (**11**)*:* 2,3,6-tri-*O*-benzyl-5-deoxy-5-fluoro-β-D-methyl galactofuranose (51 mg, .109 mmol) was dissolved in reagent grade methanol (4.5 mL, .025M) and treated with 10 wt% palladium hydroxide on carbon (25mg). The flask was evacuated and backfilled with H_2 from a balloon three times. Stirred at STP overnight. Filtered through celite, washed with methanol and concentrated in vacuo. Isolated 22 mg of a colorless oil (100%). ^{1}H NMR (300 MHz, CD_3OD, δ (ppm)) 4.71 (d, J = 1.6, 1H, H-1), 4.58 (ddt, J = 48.4, 6.9, 3.8, 1H, H-5), 4.0 – 3.6 (m, 5H, H-2, H-3, H-4, H-6a, H-6b), 3.33 (s, 3H, OCH_3); ^{19}F NMR (280 MHz, CD_3OD, δ (ppm)) -209.3 (dq, J = 48.6, 20.1); ^{13}C NMR (75 MHz, CD_3OD, δ (ppm)) 109.25, 93.37, 91.01, 82.35, 81.30, 81.05, 76.92, 76.85, 61.49, 61.17, 54.22; MS $(EMM)^+$: *m/z*: 219.0638 ($[M+Na]^+$, calcd. 219.0640)

2,3,6-tri-O-benzoyl-5-deoxy-5-fluoro-β-D-methyl galactofuranose: Fluoro Gal*f* **11** (20mg, .099 mmol) was dissolved in dry pyridine (1.0 mL) under N_2 atmosphere and chilled to 0 °C. Two crystals of dimethylamino pyridine were added. Benzoyl chloride (60 μL) was added dropwise over 5 minutes. The contents were warmed to RT and stirred for 1 hour. The reaction was diluted with toluene and azeotroped 3x . Placed on high vacuum line to dry. Purified by silica chromatography (5% → 10% EtOAc in hexane). Isolated 35 mg of colorless oil (70%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 8.08 (m, 6H, Ar-H), 7.58 (m, 3H, Ar-H), 7.45 (m, 6H, Ar-H), 5.63 (d, J = 4.5, 1H, H-3), 5.54 (s, 1H, H-2), 5.38 (m, 1H, H-5), 5.20 (s, 1H, H-1), 4.70 (m, 2H, H-6a, H-6b), 4.43 (ddd, J = 27.0, 4.7, 2.1), 3.48 (s, 3H, OCH_3); ^{19}F NMR (280 MHz, $CDCl_3$, δ (ppm)) -208.0 (dq, J = 48.6, 27.0); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm))166.42, 166.22, 165.70, 139.90, 133.83, 133.77, 133.48, 130.40, 130.19, 130.01, 129.79, 129.31, 129.17, 128.73, 128.64, 107.40, 90.59, 88.17, 82.65, 82.41, 81.34, 77.89, 77.81, 64.30, 63.98, 55.35; MS $(EMM)^+$: *m/z*: 531.1399 ($[M+Na]^+$, calcd. 531.1426)

1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-D-galactofuranose: 2,3,6-tri-O-benzoyl-5-deoxy-5-fluoro-β-D-methyl galactofuranose (84 mg, .165 mmol) was placed in a flame-dried round bottom flask and treated with a 1.4% v/v solution of concentrated H_2SO_4 in acetic anhydride (2.0 mL). The reaction stirred at RT for 1.5 hours. Quenched with 2.0 mL of saturated $NaHCO_3$ solution, diluted with brine and extracted into DCM 3x. Dried organic layer over $MgSO_4$, filtered and concentrated *in vacuo*. Azeotroped remaining acetic acid with toluene 2x.

Purified by silica gel chromatography (5% → 20% EtOAc in hexane) to give 81 mg of white crystalline solid (91%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 8.08 (m, 6H, Ar-H), 7.58 (m, 3H, Ar-H), 7.45 (m, 6H, Ar-H), 6.60 (d, J = 4.5, .2H, H-1α), 6.52 (s, 1H, H-1β), 6.14 (t, J = 6.9, .2H, H-3α), 5.77 (dd, J = 7.4, 4.6, .2H, H-2α), 5.69 (m, 2H, H-2β, H-3β), 5.36 (m, 1H, H-5β), 5.20 (m, .2H, H-5α), 4.65 (m, 3.4H, H-6aα, H-6-aβ, H-6bα, H-6bβ, H-4β), 4.43 (ddd, J = 26.2, 6.4, 2.8, 1H, H-4α), 2.20 (s, 3H, C(O)CH_3 β), 2.09 (s, .6H, C(O)CH_3 α); ^{19}F NMR (280 MHz, $CDCl_3$, δ (ppm)) -206.1 (dq, J = 51.6, 21.2, α) -207.8 (dq, J = 48.7, 24.5, β); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 169.69, 169.20, 166.34, 166.28, 166.15, 165.99, 165.44, 134.05, 133.96, 133.49, 130.26, 130.16, 130.09, 130.01, 129.81, 129.74, 129.04, 128.96, 128.86, 128.80, 128.64, 99.80, 93.14, 90.49, 90.40, 88.07, 87.96, 85.02, 84.77, 80.73, 80.46, 77.71, 77.63, 75.70, 74.29, 74.22, 64.16, 63.83, 63.58, 21.27, 21.17; MS (EMM)$^+$: *m/z*: 559.1364 ([M+Na]$^+$, calcd. 559.1375)

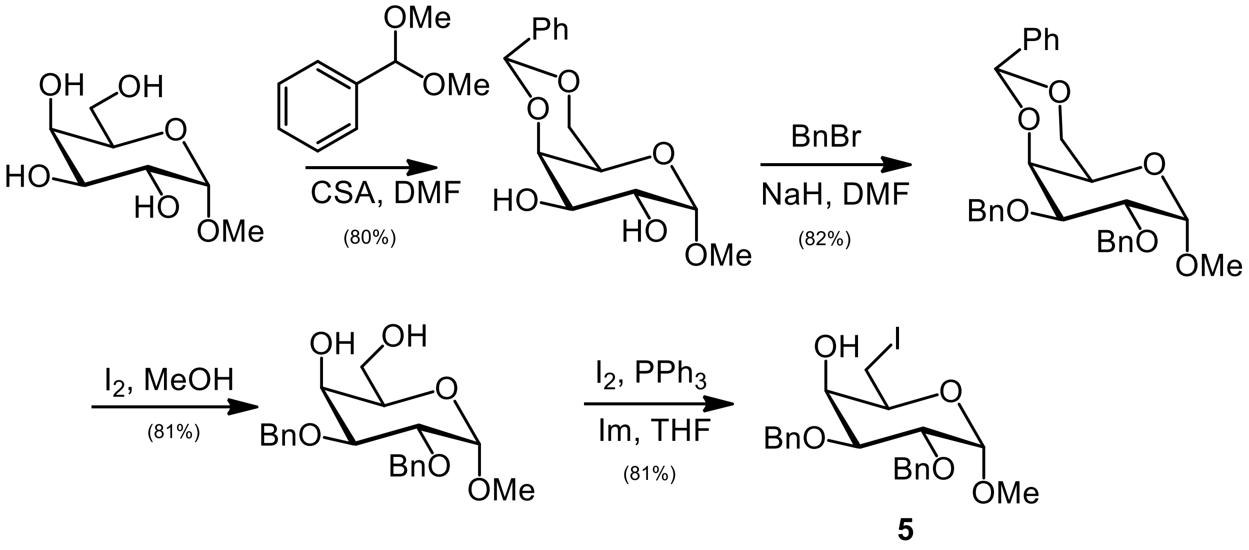
Dibenzyl 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: 1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-D-galactofuranose (22 mg, .041 mmol) was placed in a flame dried round bottom flask and dissolved in dry DCM (450 µL) under N_2 atmosphere. The solution was chilled to 0 °C and treated with a 33 wt% HBr in AcOH solution (28 µL). After stirring for 20 minutes the reaction was warmed to RT and stirred for 4 hours. Azeotroped using freshly distilled toluene 3x to isolate the crude glycosyl bromide as an orange oil. The oil was dissolved in dry toluene (450 µL) under N_2. The solution was added dropwise over 30 minutes to a stirring solution of dibenzyl phosphate (49 mg, .176 mmol) in dry toluene (500 µL). The contents stirred at RT for 2.5 hours. Concentration *in vacuo* followed by silica gel chromatography (12% EtOAc in toluene) allowed isolation of 28 mg of the α-anomer as a slightly cloudy viscous oil (90%). ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 8.00 (m, 6H, Ar-H), 7.55 (m, 3H, Ar-H), 7.4 – 7.2 (m, 16H, Ar-H), 6.31 (t, J = 4.7, 1H, H-1), 6.14 (t, J = 6.9, 1H, H-3), 5.70 (ddd, J = 7.1, 4.3, 2.2, 1H, H-2), 5.17 (dtd, J = 47.1, 6.4, 3.2, 1H, H-5), 5.07 (AB part of an ABX, J = 6.5, 3.5, 2H, $POCH_2Ph$), 4.90 (AB part of an ABX, J = 11.6, 6.9, 2H, $POCH_2Ph$), 4.61 (m, 2H, H-6a, H-6b), 4.50 (ddd, J = 24.4, 6.4, 3.5, 1H , H-4); ^{19}F NMR (280 MHz, $CDCl_3$, δ (ppm)) -203.8 (dq, J = 56.9, 28.9); ^{31}P NMR (122 MHz, $CDCl_3$, δ (ppm)) -2.4 (m); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 166.13, 165.96, 165.76, 134.00, 133.89, 133.46, 130.26, 130.15, 129.96, 129.60, 128.76, 128.70, 128.63, 128.58, 128.05, 127.85, 101.02, 97.61, 97.54, 90.59, 88.15, 80.86, 80.60, 76.54, 76.44, 73.56, 73.47, 69.56, 69.47, 63.32; MS (EMM)$^+$: *m/z*: 777.1898 ([M+Na]$^+$, calcd. 777.1872)

Triethylammonium 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: Dibenzyl 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate (29 mg, .038 mmol) was dissolved in ethyl acetate (1.0 mL, .05M) and triethylamine (38 µL). 10% Pd on carbon (15 mg) was added. The stoppered flask was evacuated and back filled with H_2 from a balloon. The contents stirred at STP overnight. The reaction was filtered through celite, washed with ethyl acetate and concentrated *in vacuo*. Isolated 14 mg of cloudy, viscous oil (54%).^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) 8.1 (d, J = 7.5, 2H, Ar-H), 7.9 (m, 3H, Ar-H), 7.5 – 7.2 (m, 9H, Ar-H), 6.2 (m, 2H, H-1, H-3), 5.62 (m, 1H, H-2), 5.30 (m, 1H, H-5), 4.7 (m, 2H, H-6a, H-6b), 4.5 (dt, J = 19.3, 6.2, 1H, H-4), 3.00 (q, J = 7.3, 3.8H, $N\underline{CH_2}CH_3$), 1.2 (t, J = 7.5, 10.7H, $NCH_2\underline{CH_3}$); ^{19}F NMR (280 MHz, $CDCl_3$, δ (ppm)) -199.7 (dq, J = 47.1, 25.1); ^{31}P NMR (122 MHz, $CDCl_3$, δ (ppm)) 0.0 (d, J = 3.5); ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)) 164.83, 164.74, 164.58, 132.26, 132.06, 131.90, 129.14, 128.93, 128.81, 128.58, 128.47, 128.44, 127.92, 127.25, 127.1795.17, 95.11, 91.02, 78.54, 78.24, 75.80, 75.70, 73.09, 72.98, 62.71, 62.43, 44.38, 7.37; MS (EMM)$^-$: *m/z*: 573.0966 ([M]$^-$, calcd. 573.0967)

Tributylammonium 5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: Triethylammonium 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate (14 mg, .021 mmol) was dissolved in a 5:2:1 mixture of methanol:water:triethylamine (1.0 mL) and warmed to 30 °C. The reaction stirred for 4 days. Concentrated *in vacuo* then azeotroped 3x with toluene. Purified by anion exchange chromatography (Biorad AG1-X8 resin, gradient elution 250 mM → 500 mM NH_4HCO_3 solution) using a peristaltic pump. Column fractions were combined, frozen on dry ice and lyophilized. The residue was treated with ~200 µL Dowex-50WX8-200 resin (pH 7, pretreated with 2.0M $HNBu_3Cl$) for 4 hours. The resin was removed by filtration through a cotton plug and the filtrate was frozen on dry ice and lyophilized. Isolated 10 mg of fluffy white solid (82%, 1.5eq. of $HNBu_3$); ^{1}H NMR (300 MHz, D_2O, δ (ppm)) 5.51 (dd, J = 6.2, 4.2, 1H, H-1), 4.69 (dtd, J = ~50, 5.8, 3.9, 1H, H-5), 4.19 (t, J = 7.8, 1H , H-3), 4.15 (ddd, J = 8.0, 4.4, 2.0, 1H, H-2), 3.95 (dt, J = 19.5, 6.3, 1H, H-4), 3.84 (m, 2H, H-6a, H-6b), 3.10 (m, 9H, NBu), 1.62 (m, 9H, NBu), 1.33 (sextet, J = 7.3, 10H, NBu), 0.9 (t, J = 7.2, 13H); ^{19}F NMR (280 MHz, D_2O, δ (ppm)) -199.7 (dq, J = 46.4, 23.4); ^{31}P NMR (122 MHz, D_2O, δ (ppm)) -0.4 (broad s); ^{13}C NMR (75 MHz, D_2O, δ (ppm)) 96.91, 96.85, 95.41, 93.10, 79.82, 79.57, 76.74, 76.69, 73.59, 73.50, 60.71, 60.43, 52.85, 25.36, 19.44, 12.93; MS (EMM)$^-$: *m/z*: 261.0182 ([M]$^-$, calcd. 261.0181)

5-deoxy-5-fluoro-α-D-galactofuranose uridine diphosphate (**3**): A solution of tributylammonium UMP (5mg, .01 mmol) in anhydrous DMF (150 µL) was stirred with activated 4 Å molecular sieves under N_2 for 1 hour. The molecular sieves were removed and the solution was treated with diisopropylethylamine (5 µL, .03 mmol) and 1-methyl-3-benzenesulfonylimidazolium triflate (4 mg, 0.01 mmol) and stirred for 1 minute. The solution was added dropwise over 30 seconds to a solution of tributylammonium 5-deoxy-5-fluoro-α-D-galactofuranose monophosphate (3mg, .007 mmol) and $MgCl_2$ (1 3mg, .01 mmol) in anhydrous DMF (75 µL) at 0 °C. The contents were raised to room temperature and stirred for 2 hours. The reaction was quenched with 1.0 mL of 100 mM ammonium acetate and washed 1x with 1.0 mL of DCM. The DCM was extracted with 1.0 mL of 100 mM ammonium acetate, the aqueous layers were combined, frozen on dry ice and stored at -20 °C until purification by HPLC. Purified by HPLC using a semiprep carbopak dionex column, 300mM tirthylammonium acetate, pH = 6.0-7.0, flow rate = 1mL/min. Collected peak eluting at 40 – 45 minutes, froze on dry ice and lyophilized to give a light brown solid. ^{1}H NMR (500 MHz, D_2O, δ (ppm)) 7.83 (d, J = 8.0, 1H, H-5u), 5.86 (m, 2H, H-1r, H-6u) 5.55 (t, J = 4.8, 1H, H-1g) 4.56 (m, 1H, H-5g) 4.00 – 4.25 (m, 7H, H-2g, H-2r, H-3g, H-3r, H-4r, H-5ar, H-5br) 3.89 (dt, J = 18.9, 6.5, 1H, H-4g) 3.71- 3.80 (m, 2H, H-6ag, H-6bg); ^{19}F NMR (280 MHz, D_2O, δ (ppm)) -232.0; ^{13}C NMR (125 MHz, D_2O, δ (ppm)) 144.25, 105.25, 102.84, 100.32, 90.97, 85.90, 85.83, 79.02, 76.41, 75.63, 72.20, 67.42, 49.28, 10.81; MS (EMM)$^+$: *m/z*: 591.0406 ([M+Na]$^+$, calcd. 591.0400).

III. Supplemental Figures



Scheme S1: Synthesis of iodosugar **5** from methyl-D-galactopyranose.

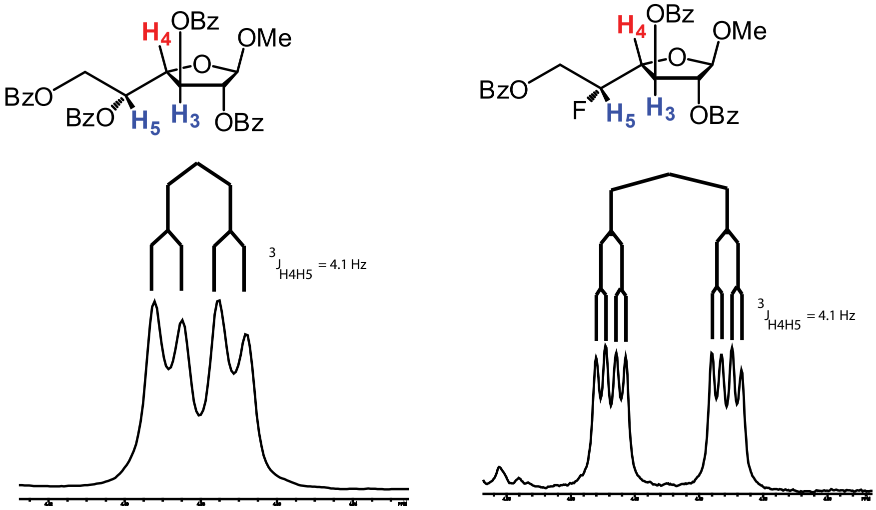
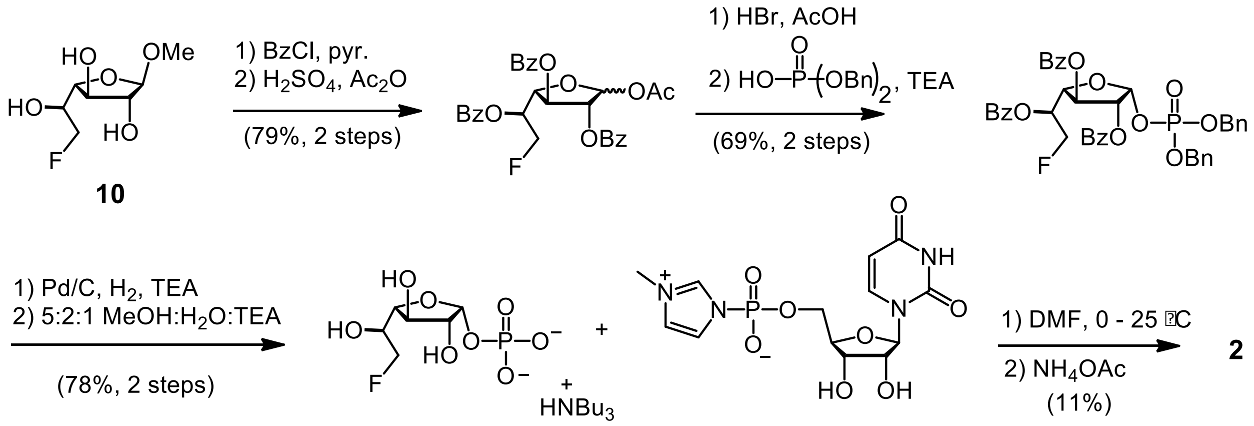
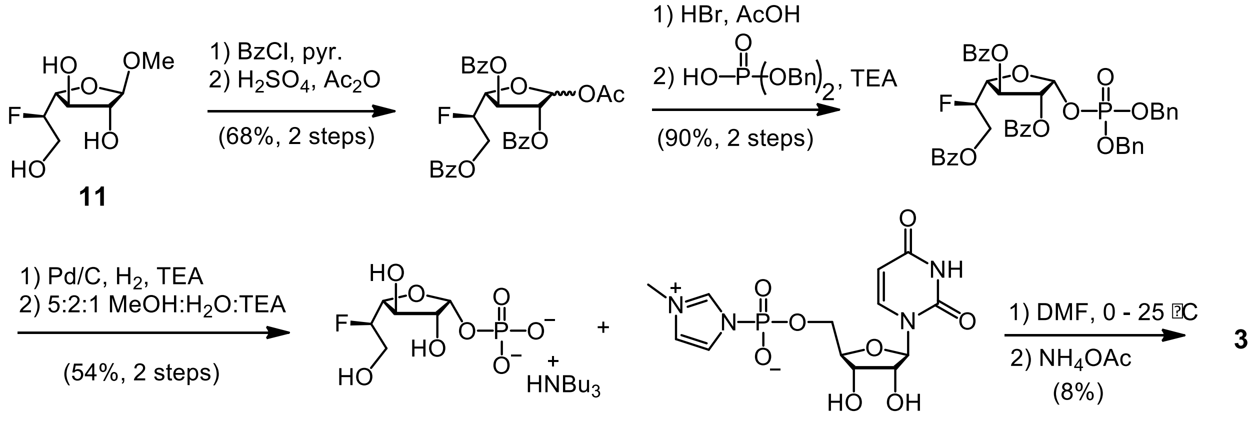


Figure S2: ^{1}H NMR $^3J_{H4H5}$ coupling constants of benzoylated 5-fluorofuranose and 2,3,6-tri-*O*-benzoyl-β-Gal*f* are identical. Thus, 5-fluorofuranose is assumed to have D-Gal*f* stereochemistry.



Scheme S3: Synthesis of **2** from 6F-Gal*f* intermediate **10**.



Scheme S4: Synthesis of **3** from 5F-Gal*f* intermediate **11**.

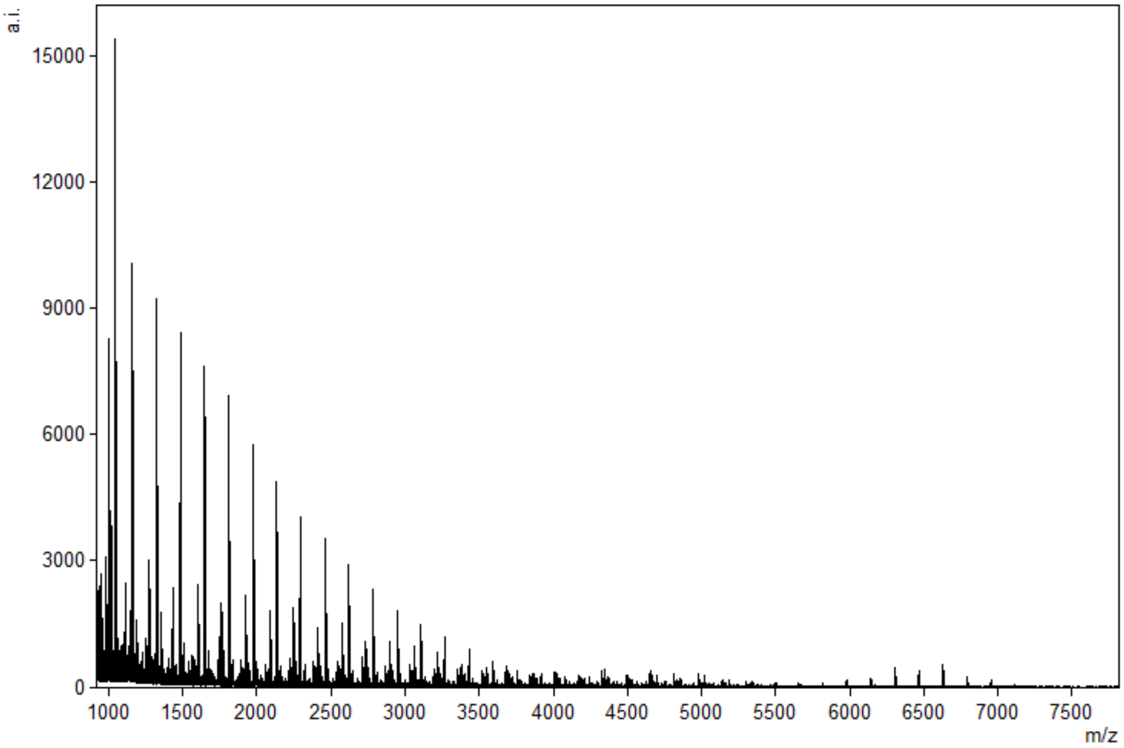
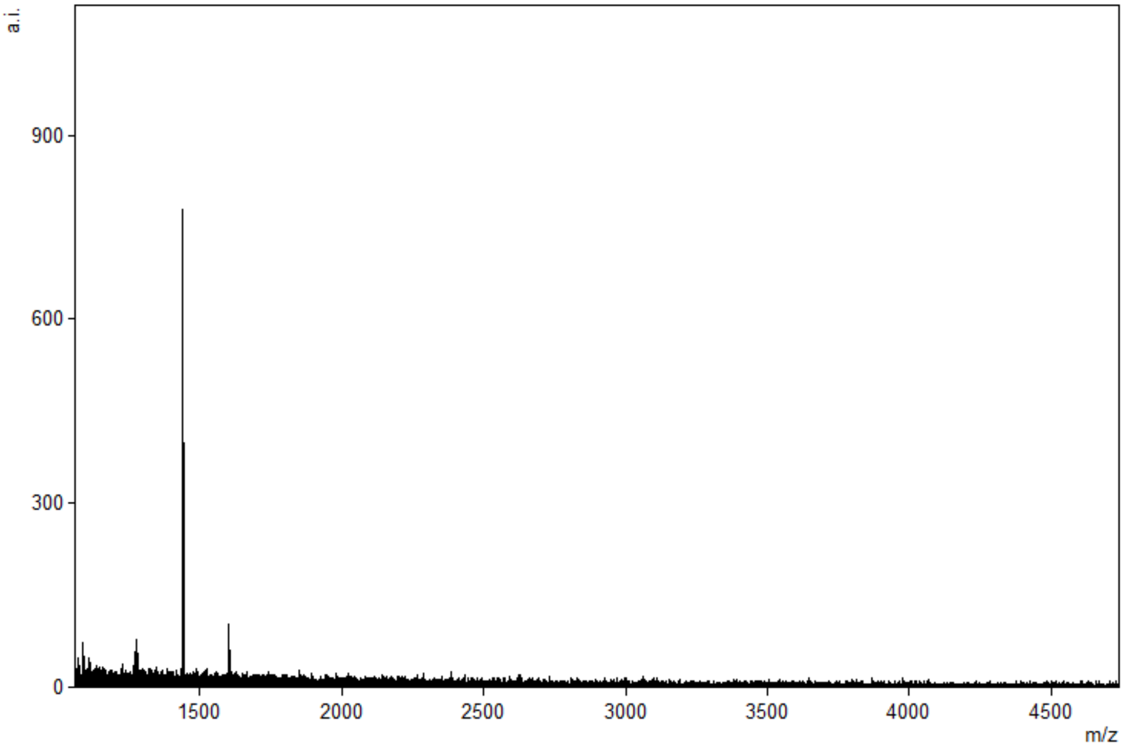


Figure S5: MALDI of products obtained by incubating GlfT2 with hexasaccharide acceptor **12** and UDP-Gal*f*.

A



B

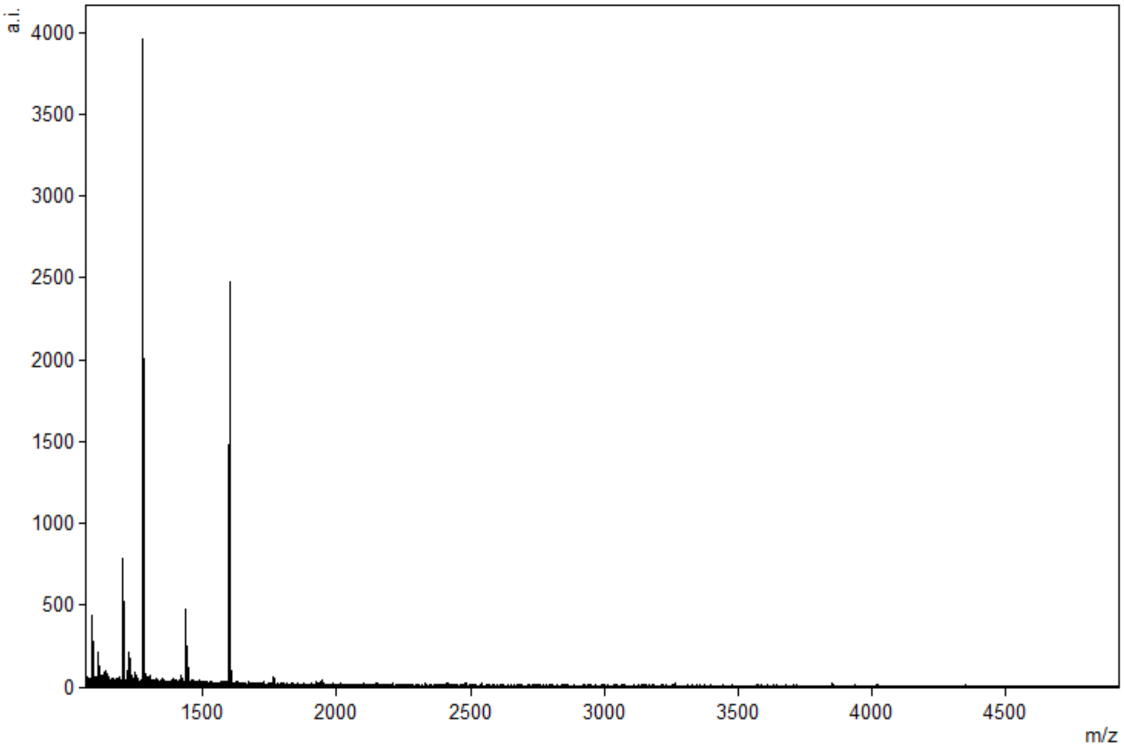
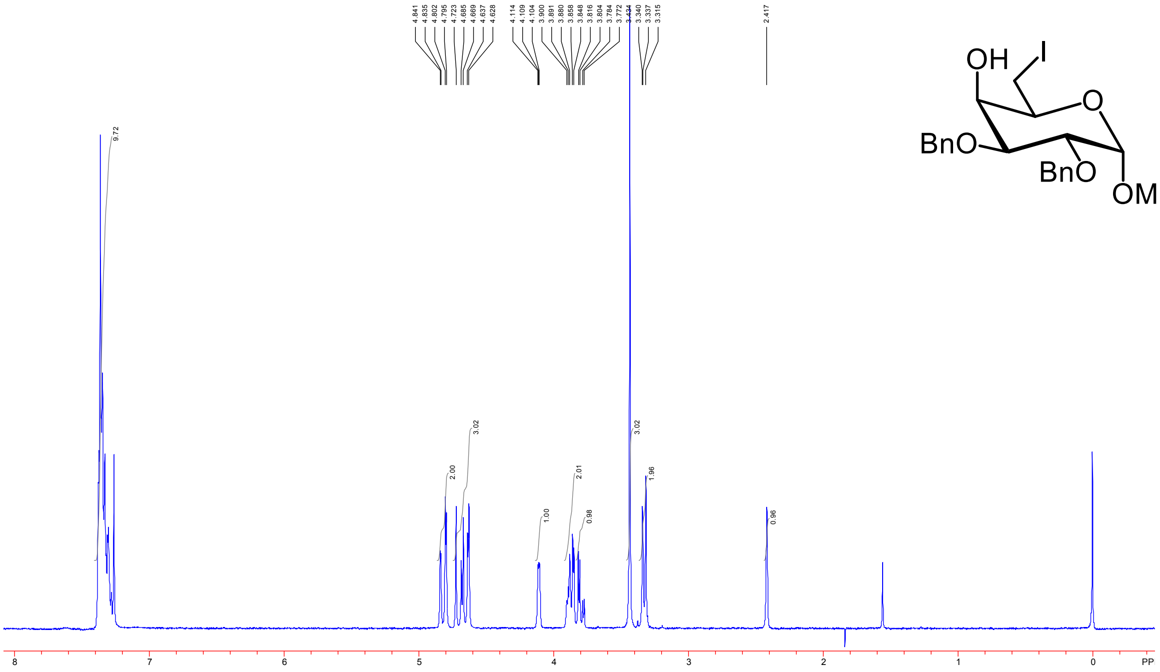


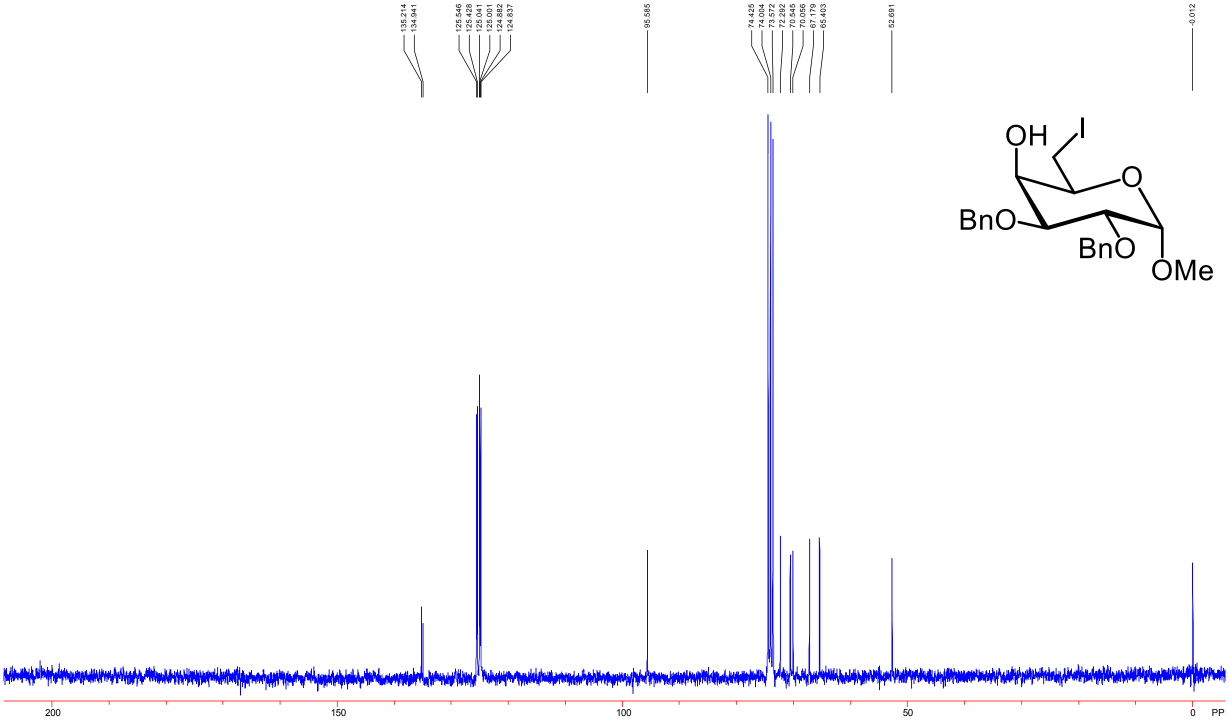
Figure S6: Full spectra for polymerization of hexasaccharide **12** with **2** (A) and **3** (B).

IV. Spectral Data

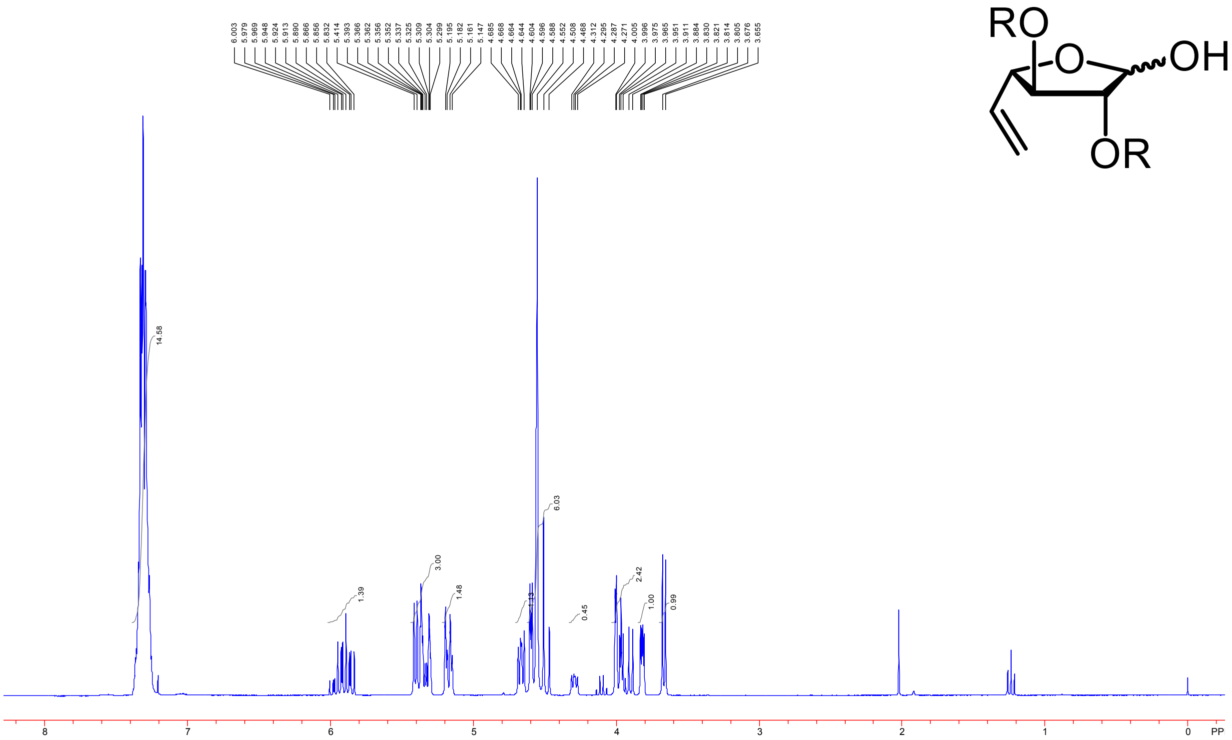
2,3-di-*O*-benzyl-6-deoxy-6-iodo-β-D-methylgalactopyranose (**5**): ^{1}H NMR



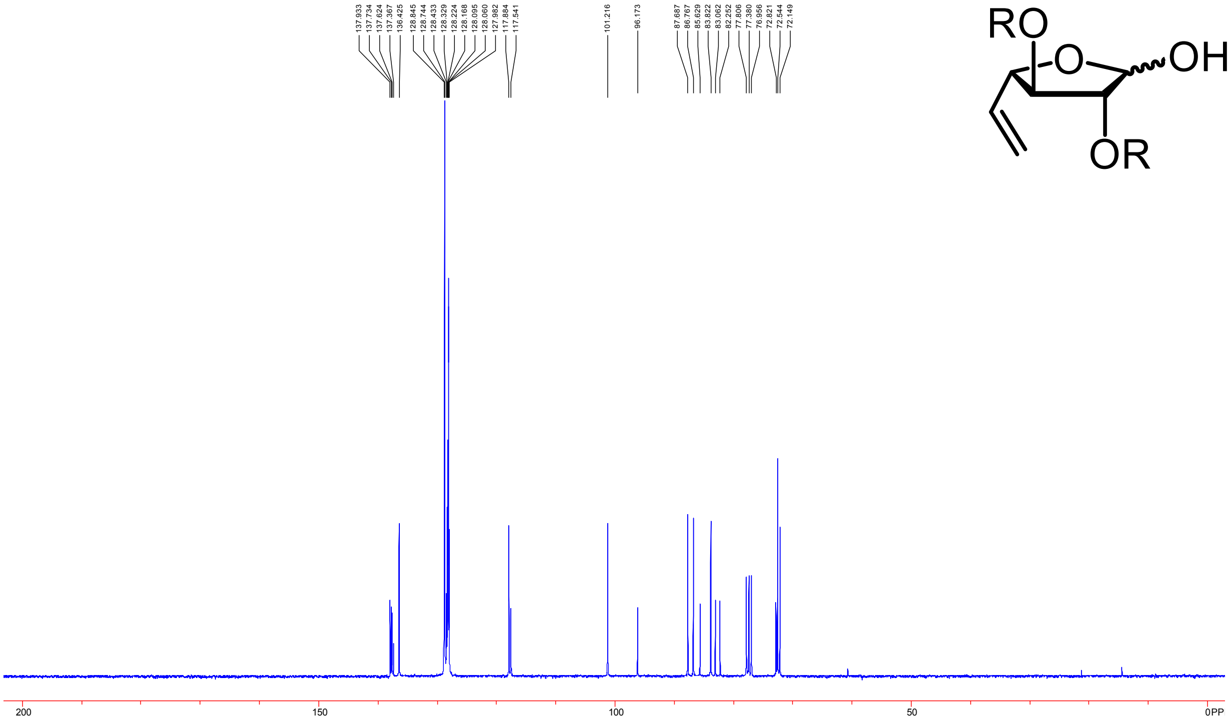
2,3-di-*O*-benzyl-6-deoxy-6-iodo-β-D-methylgalactopyranose (**5**): ^{13}C NMR



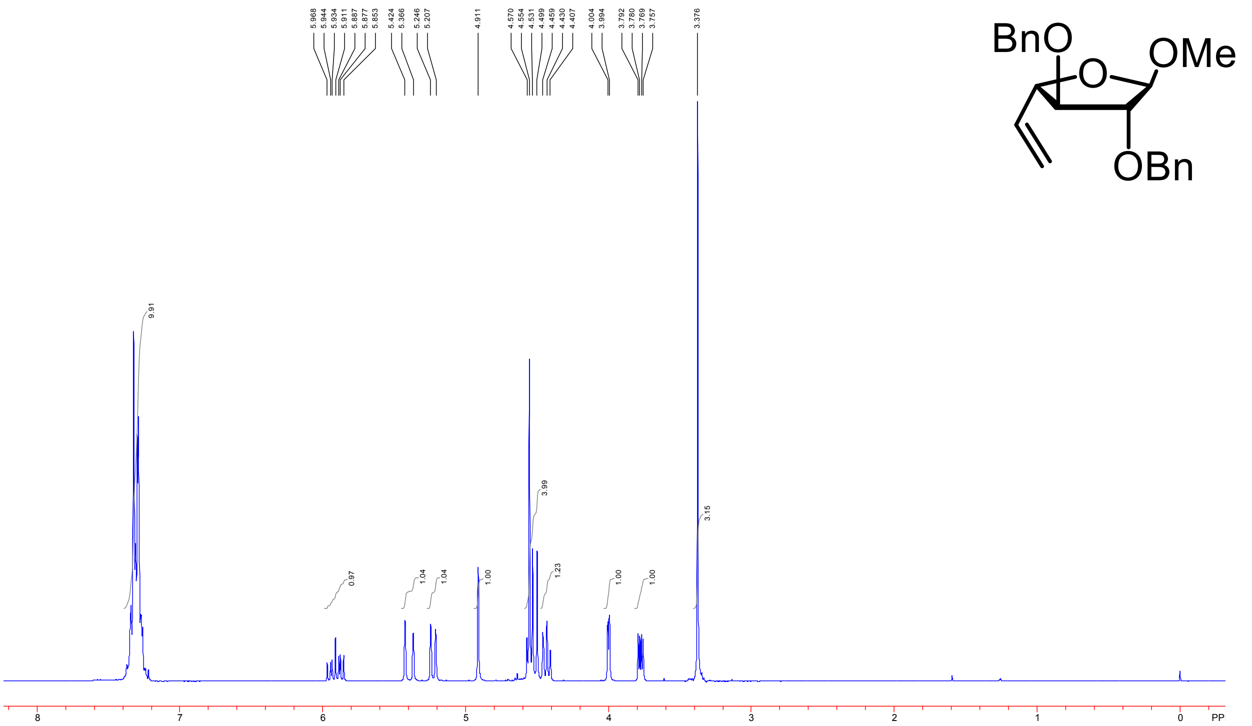
2,3-di-*O*-benzyl-5-eno-β-D-methyl galactofuranose*:* ^{1}H NMR



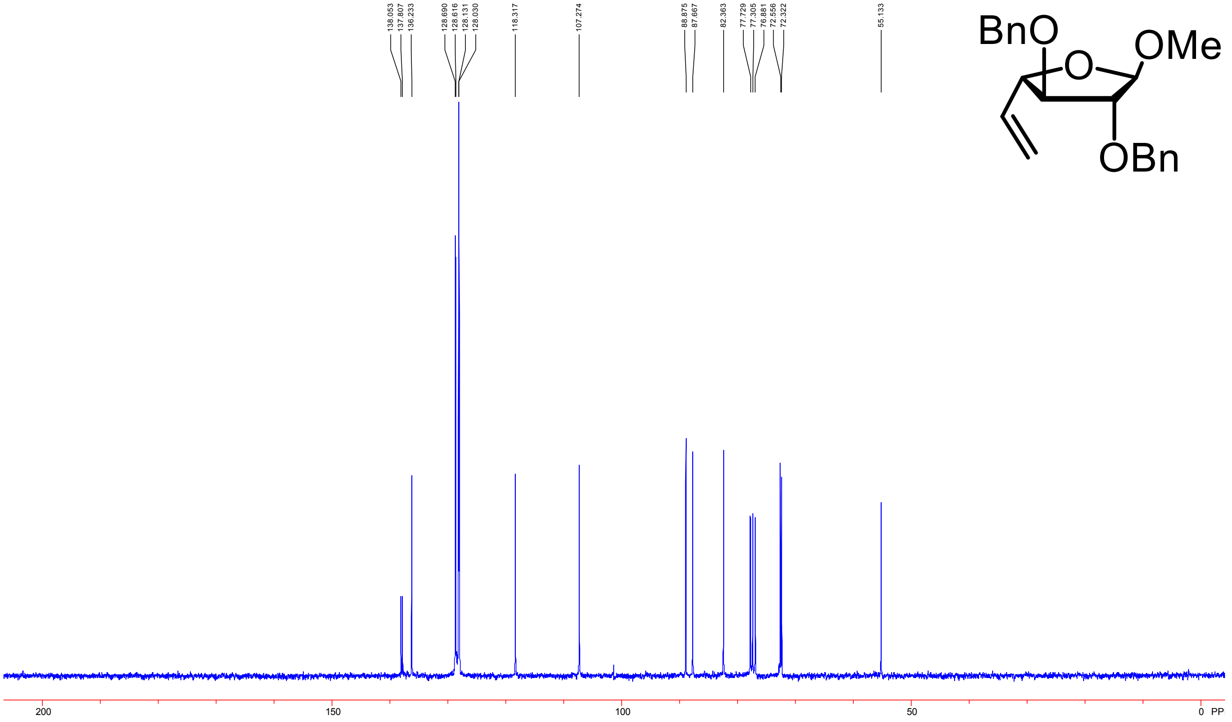
2,3-di-*O*-benzyl-5-eno-β-D-methyl galactofuranose: ^{13}C NMR



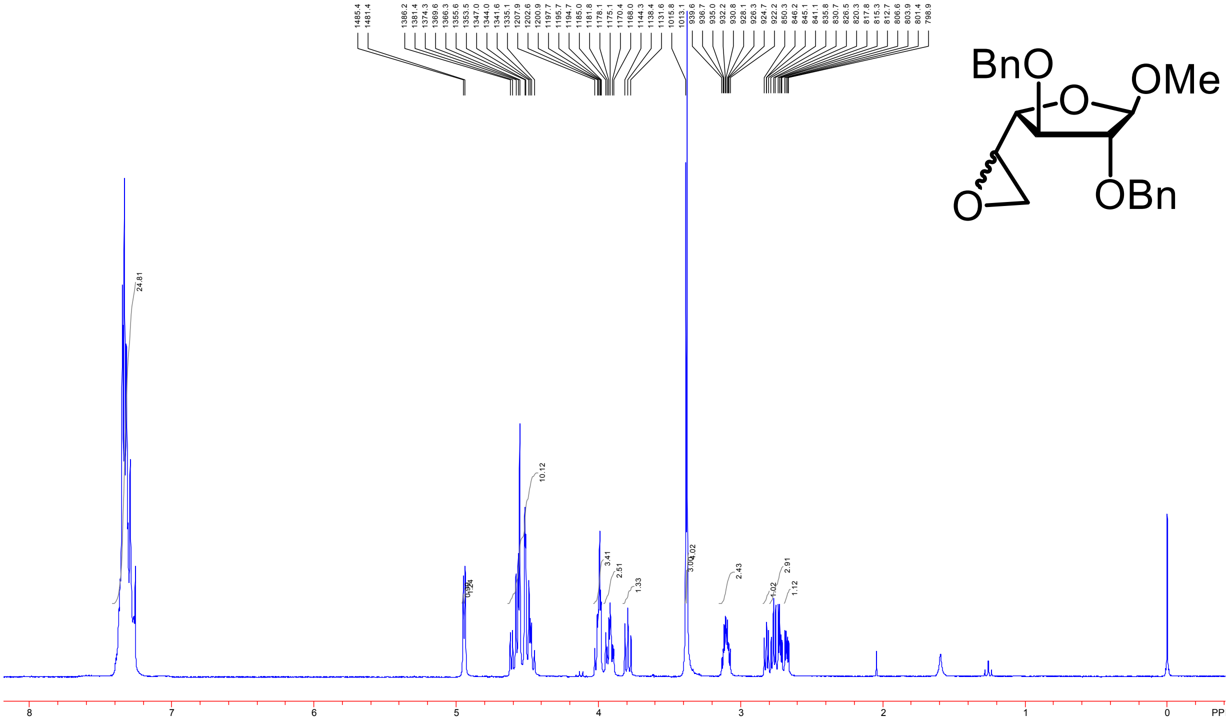
2,3-di-*O*-benzyl-5-eno-β-D-methyl galactofuranose (**6**): ^{1}H NMR



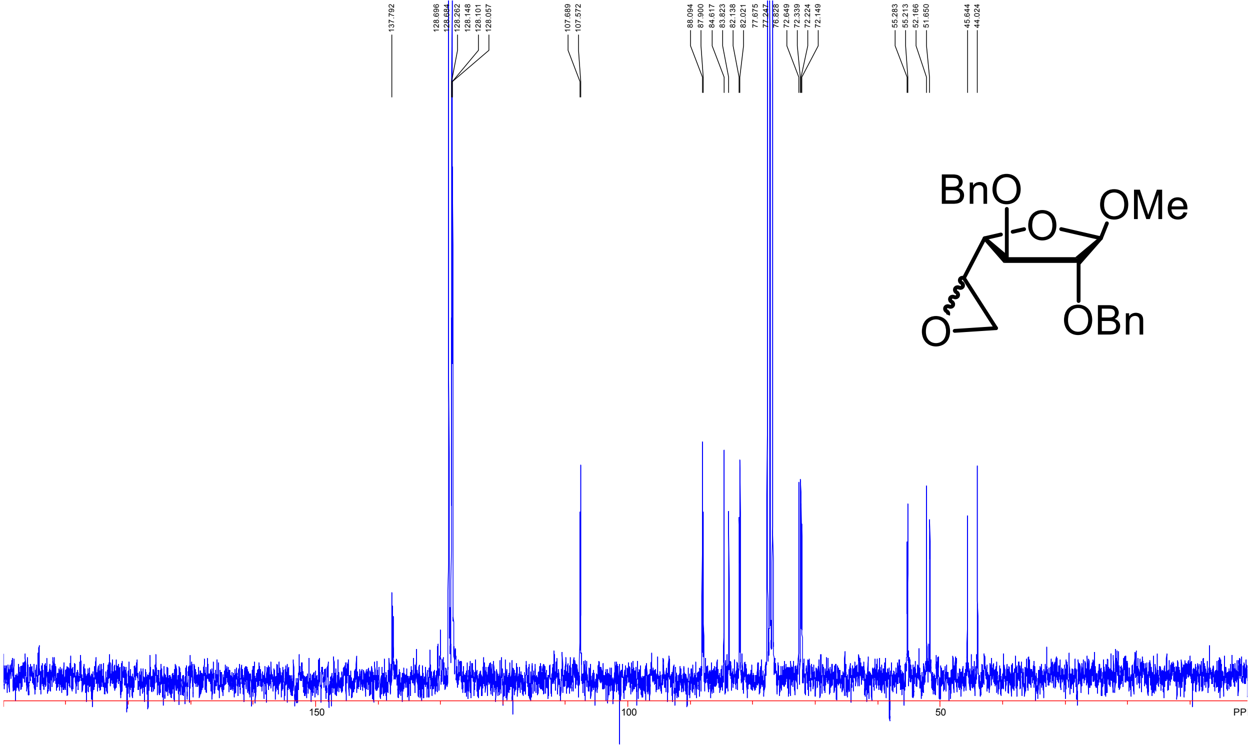
2,3-di-*O*-benzyl-5-eno-β-D-methyl galactofuranose (**6**): ^{13}C NMR



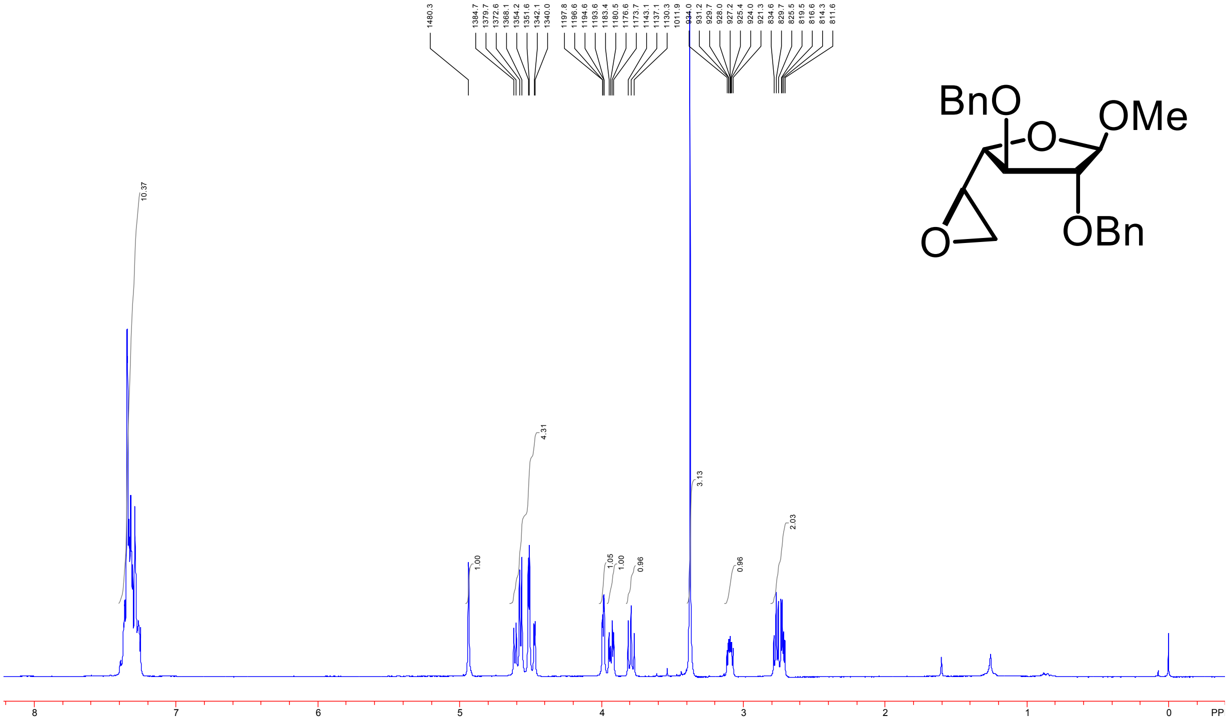
5,6-anhydro-2,3-di-O-benzyl-β-D-methyl galactofuranose and 5,6-anhydro-2,3-di-O-benzyl-β-L-methylaltrofuranose (**7**): ^{1}H NMR



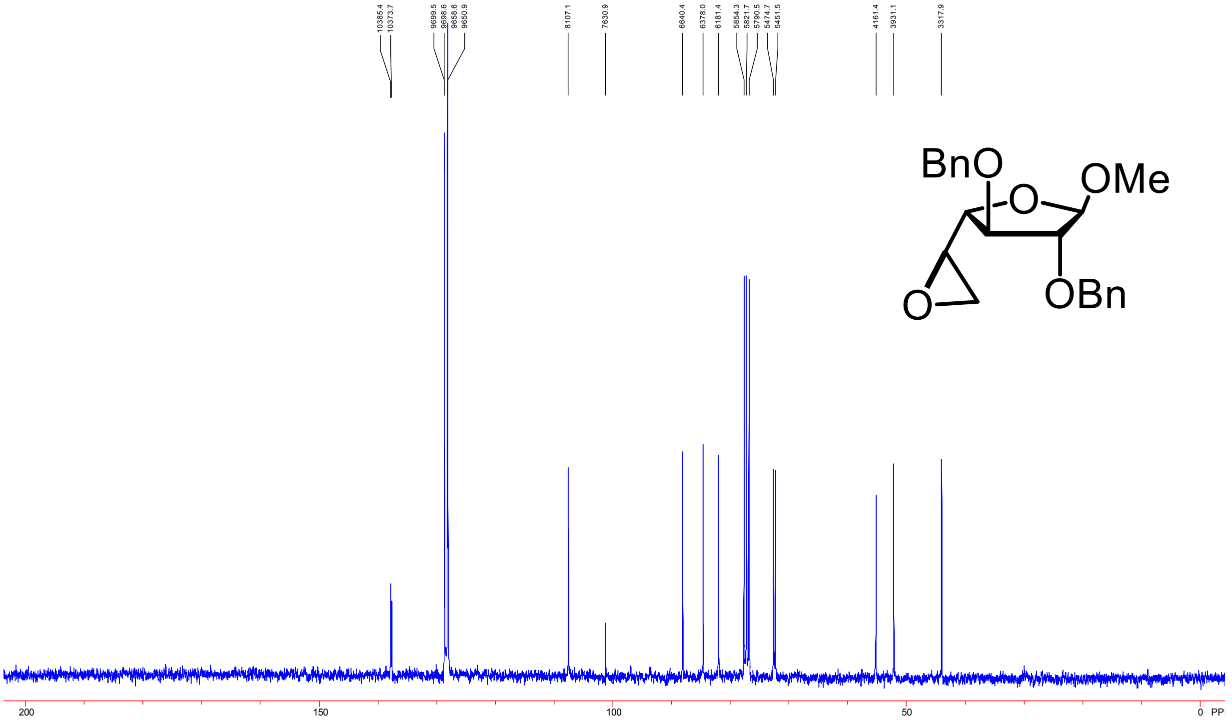
5,6-anhydro-2,3-di-O-benzyl-β-D-methyl galactofuranose and 5,6-anhydro-2,3-di-O-benzyl-β-L-methylaltrofuranose (**7**): ^{13}C NMR



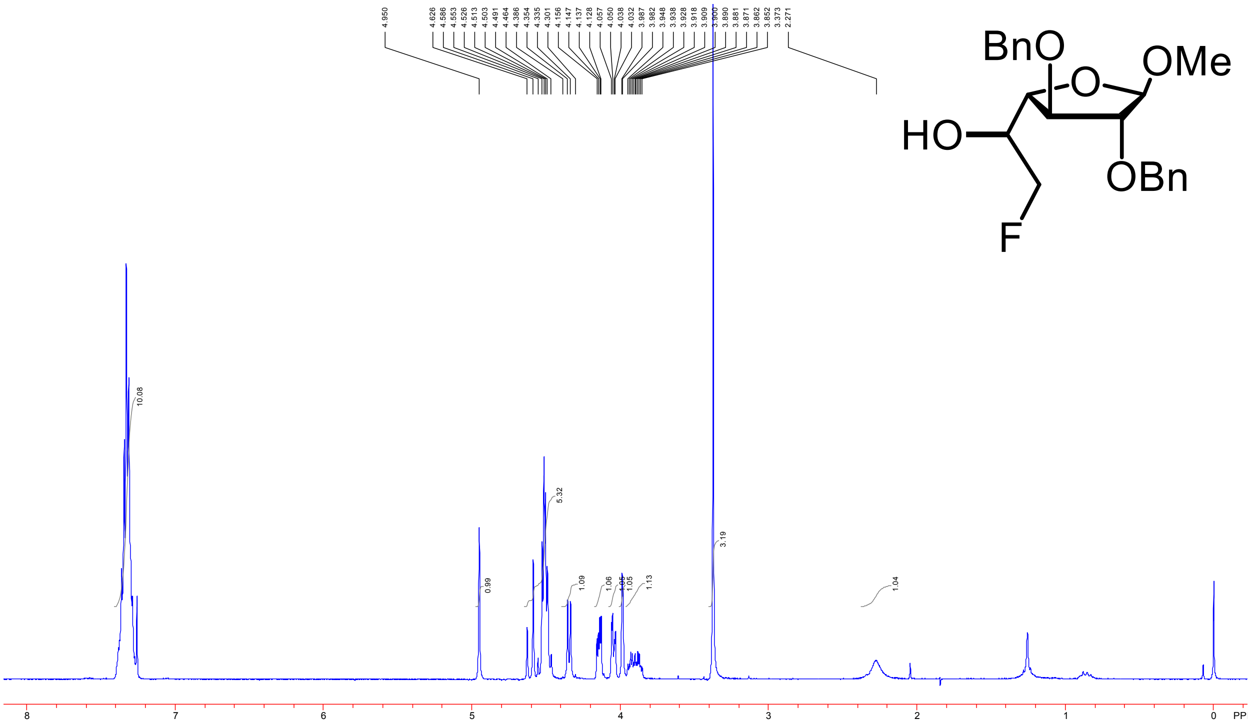
5,6-anhydro-2,3-di-*O*-benzyl-beta-D-methyl galactofuranose (**8**): ^{1}H NMR



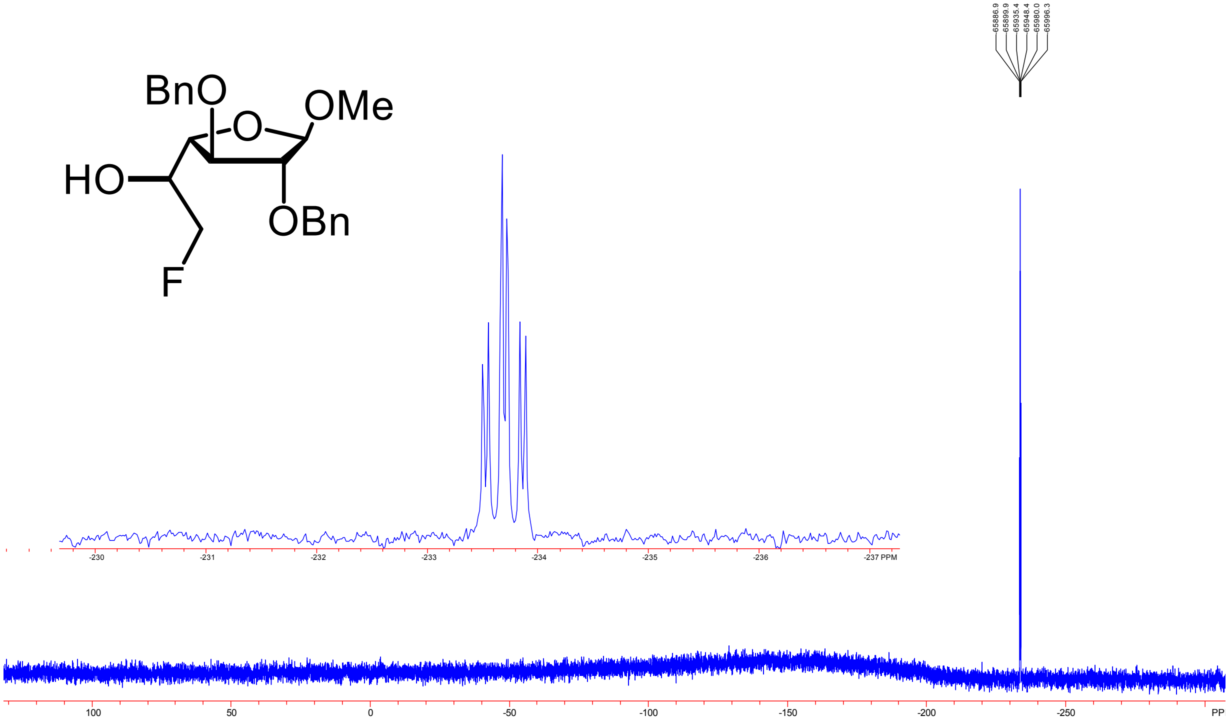
5,6-anhydro-2,3-di-*O*-benzyl-beta-D-methyl galactofuranose (**8**): ^{13}C NMR



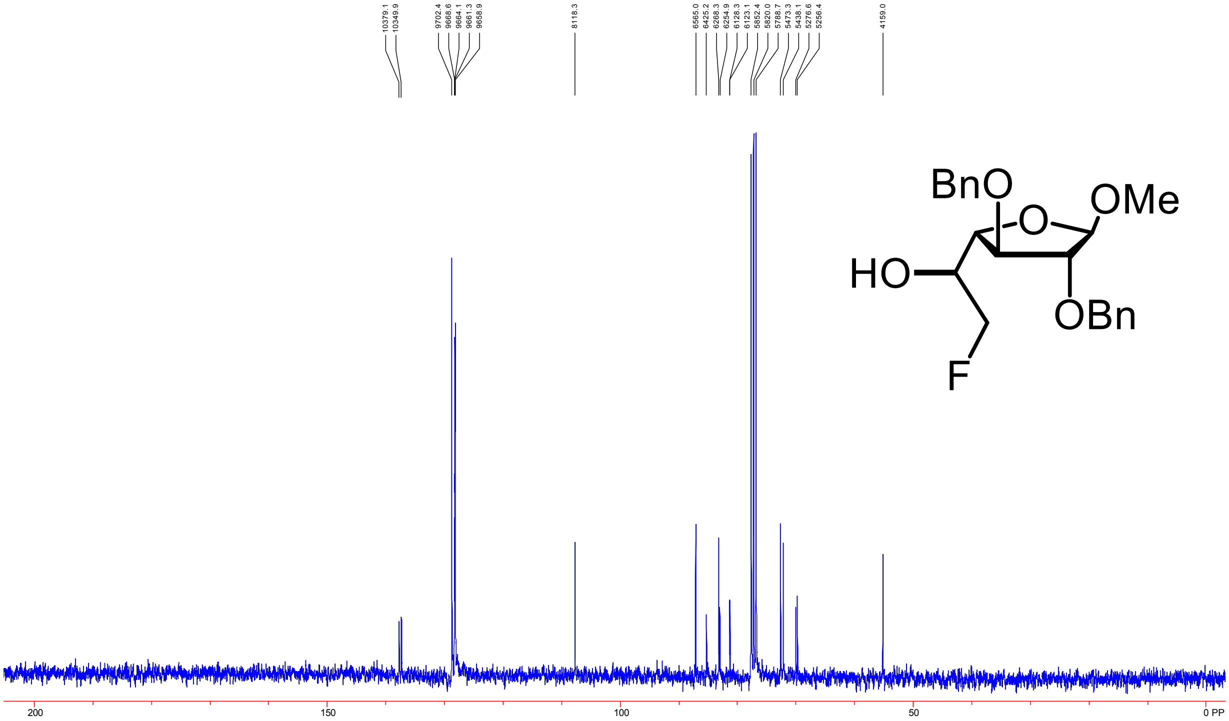
2,3-di-*O*-benzyl-6-deoxy-6-fluoro-β-D-methyl galactofuranose: ^{1}H NMR



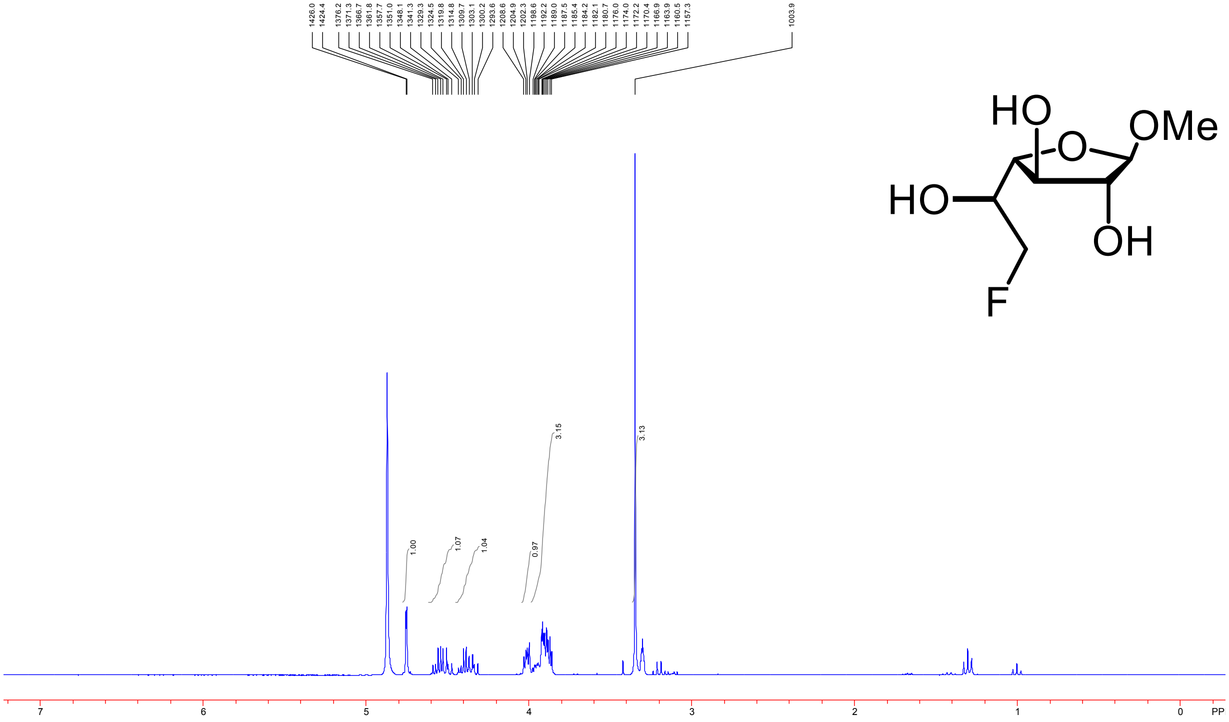
2,3-di-*O*-benzyl-6-deoxy-6-fluoro-β-D-methyl galactofuranose: ^{19}F NMR



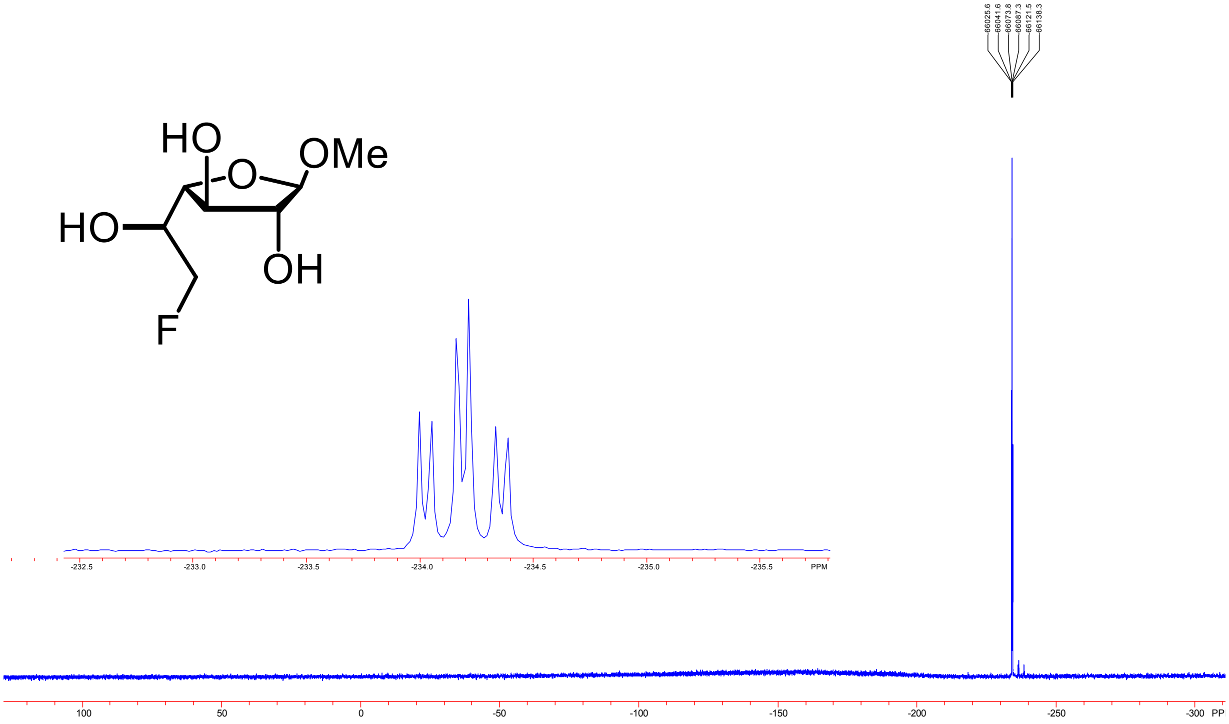
2,3-di-*O*-benzyl-6-deoxy-6-fluoro-β-D-methyl galactofuranose: ^{13}C NMR



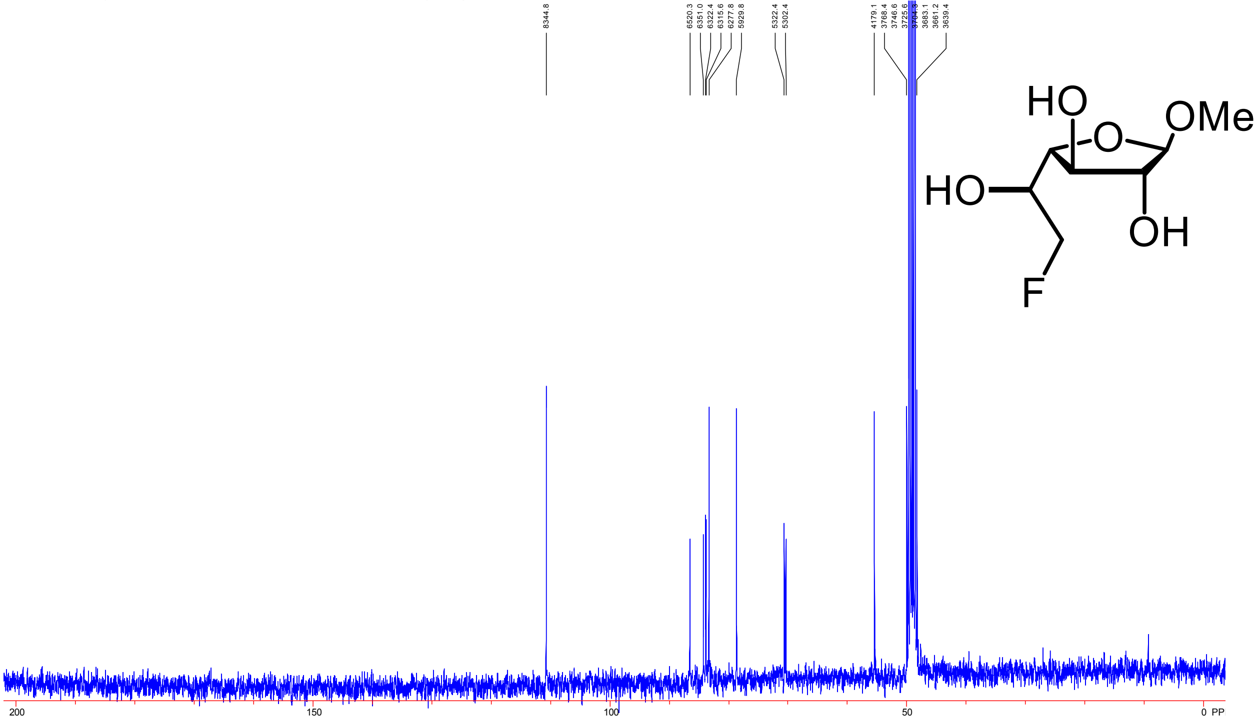
6-deoxy-6-fluoro-beta-D-methyl galactofuranose (**10**): ^{1}H NMR



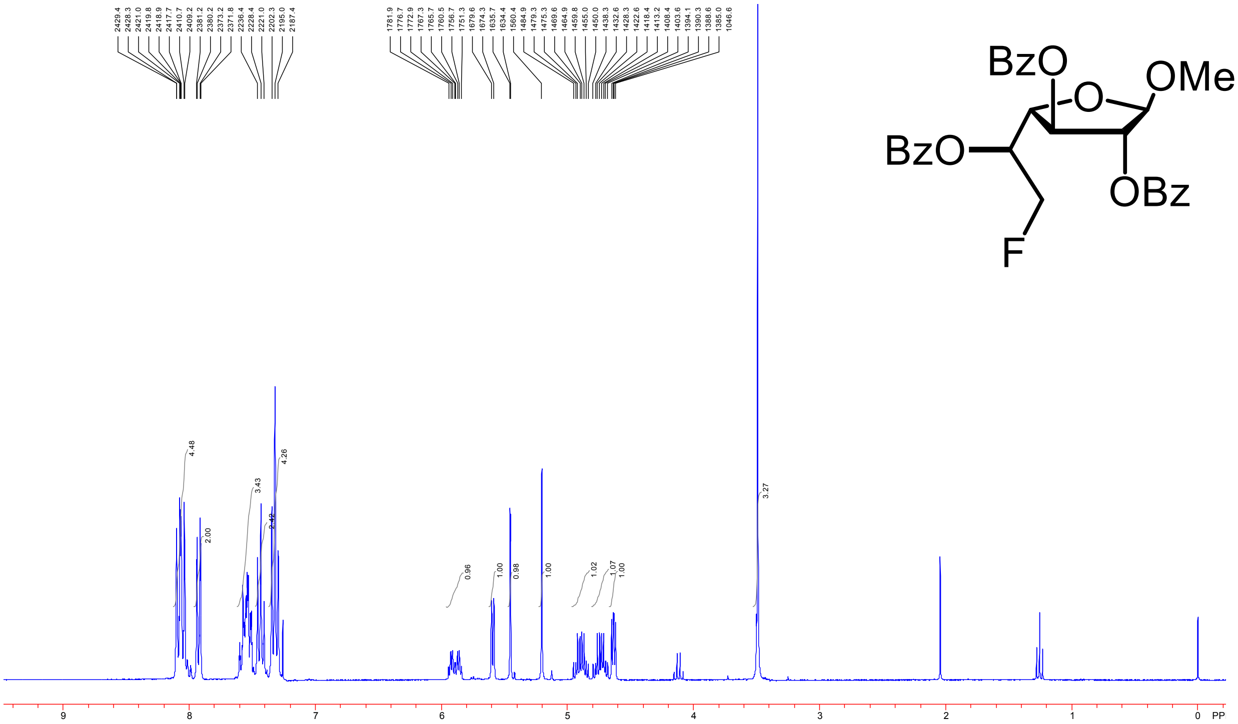
6-deoxy-6-fluoro-beta-D-methyl galactofuranose (**10**): ^{19}F NMR



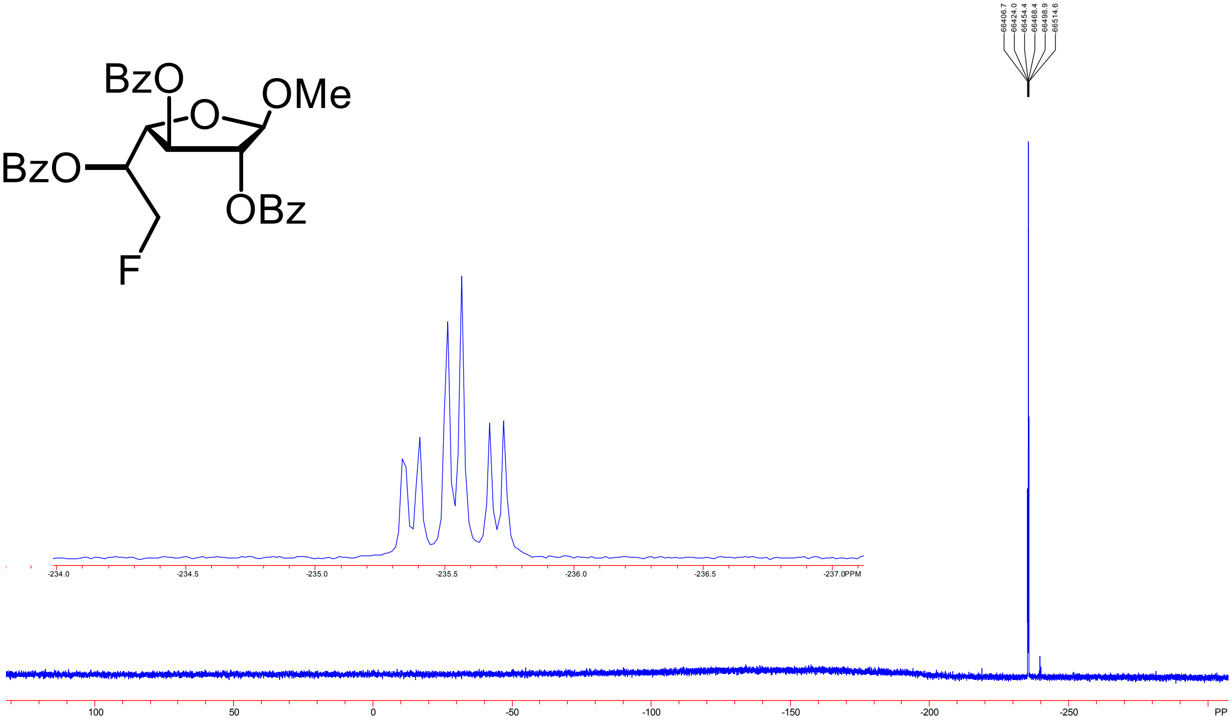
6-deoxy-6-fluoro-beta-D-methyl galactofuranose (**10**): ^{13}C NMR



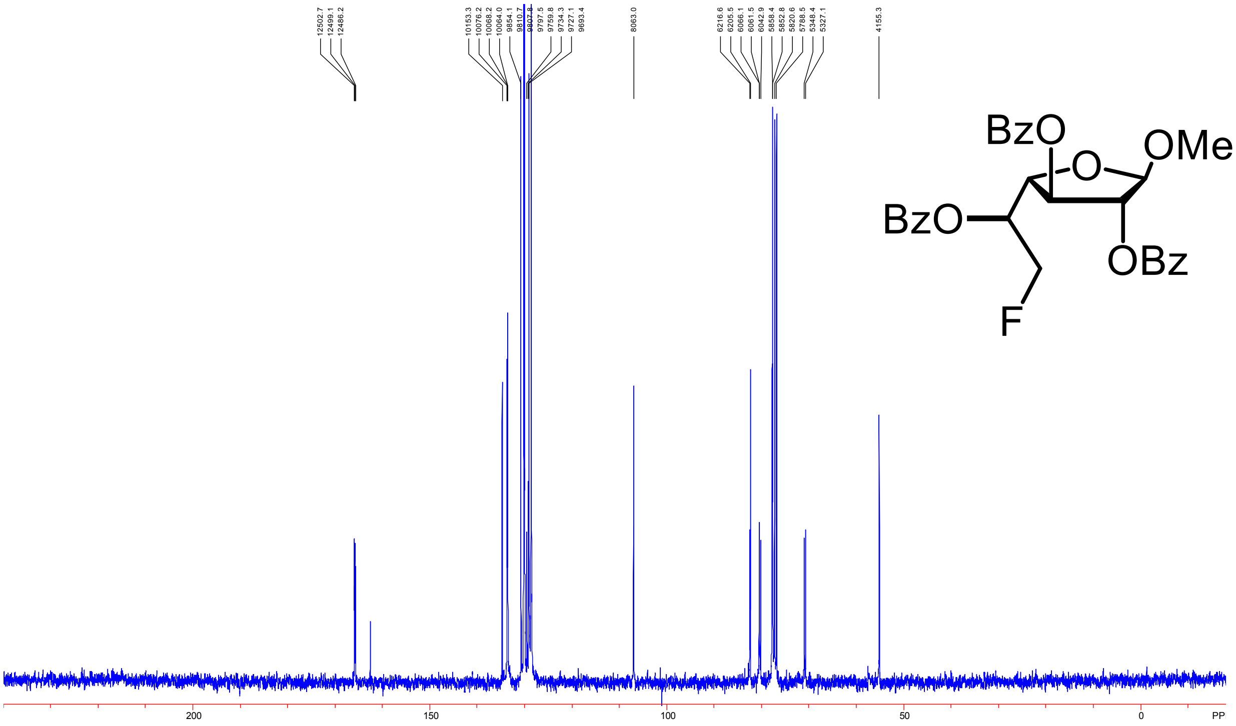
2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-β-D-methyl galactofuranose: ^{1}H NMR



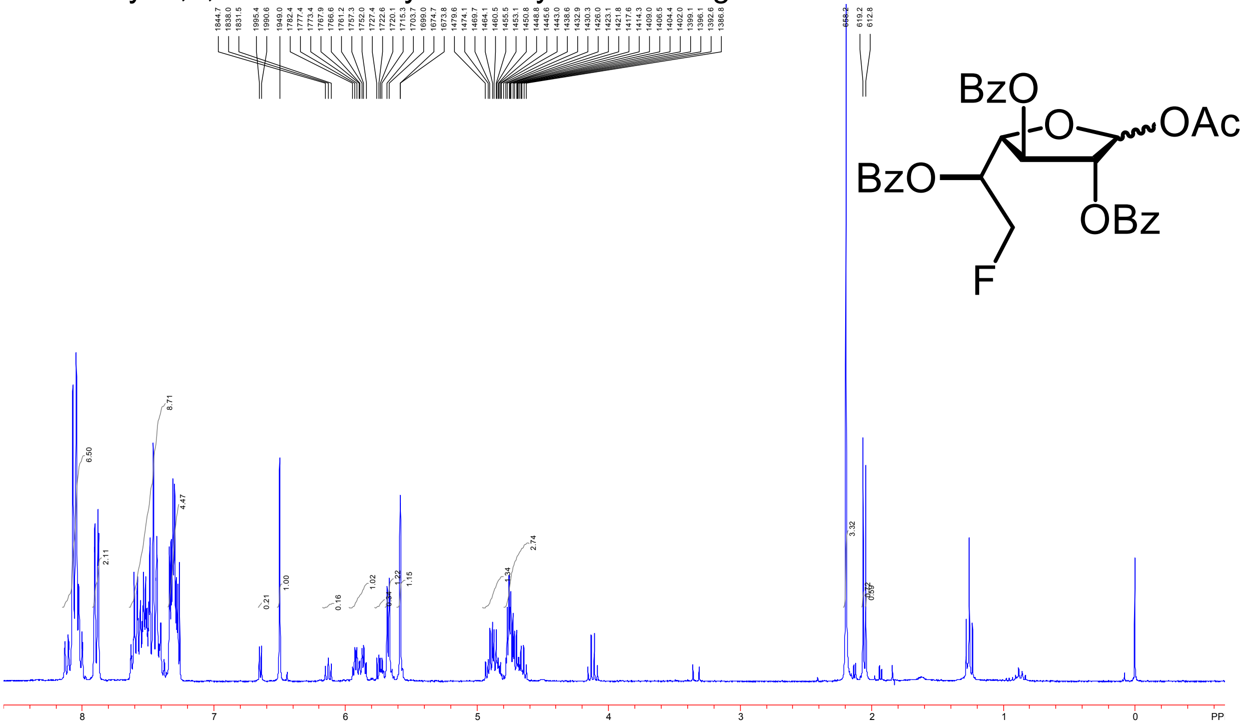
2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-β-D-methyl galactofuranose: ^{19}F NMR



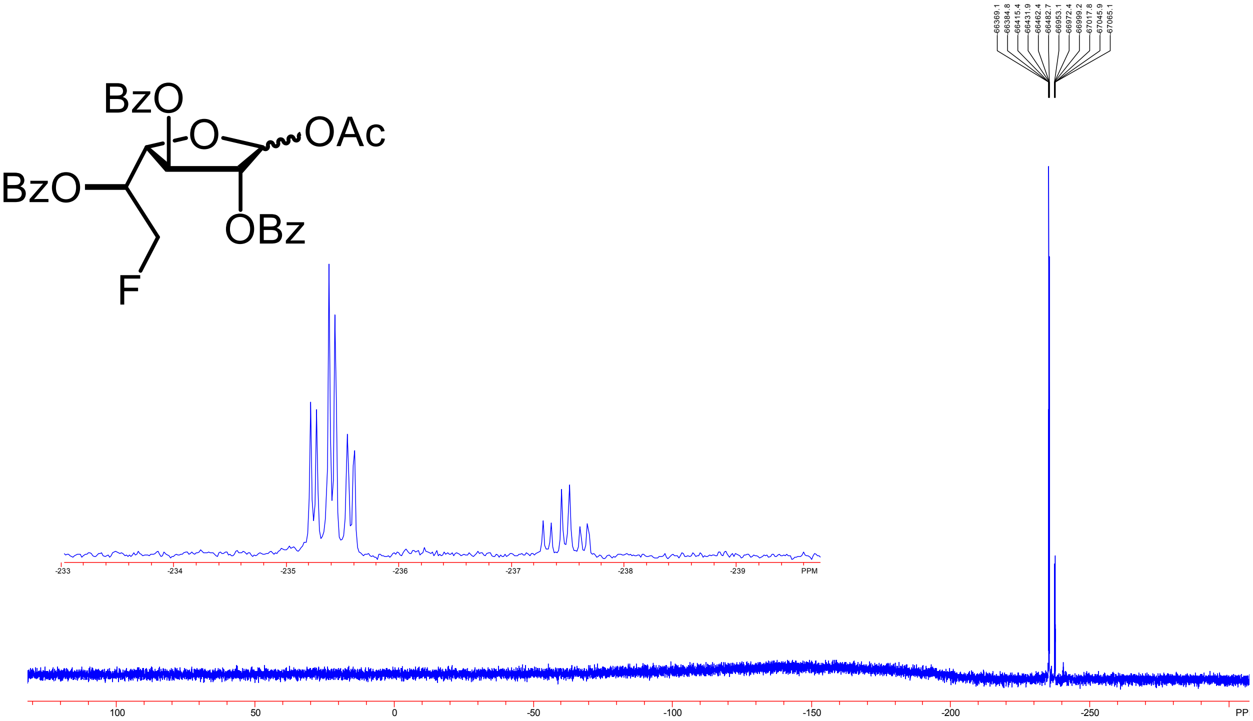
2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-β-D-methyl galactofuranose: ^{13}C NMR



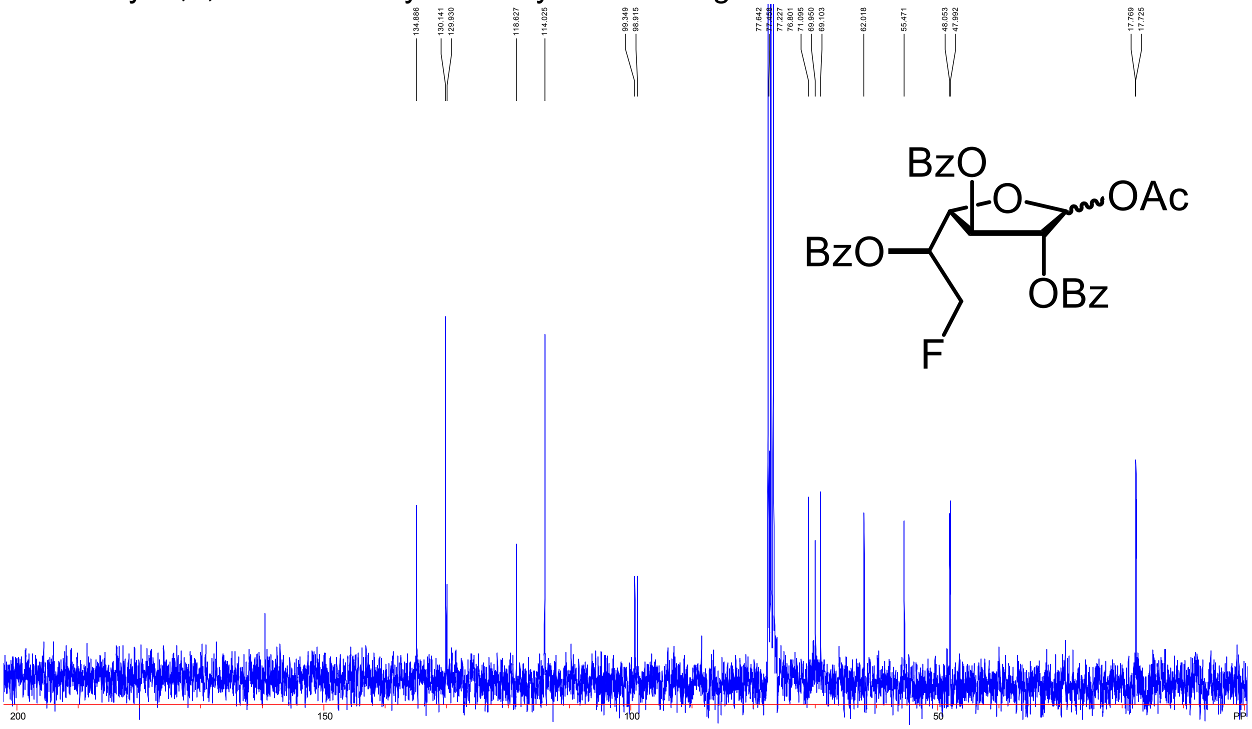
1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-D-galactofuranose: ^{1}H NMR



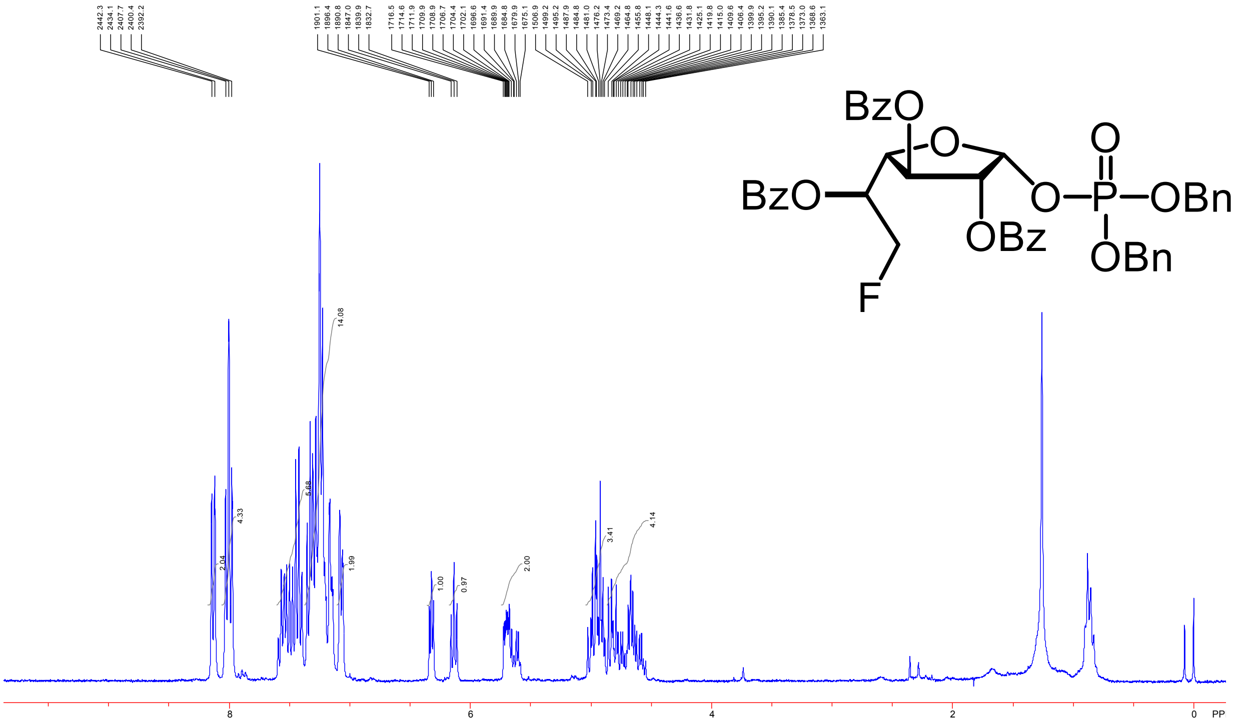
1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-D-galactofuranose: ^{19}F NMR



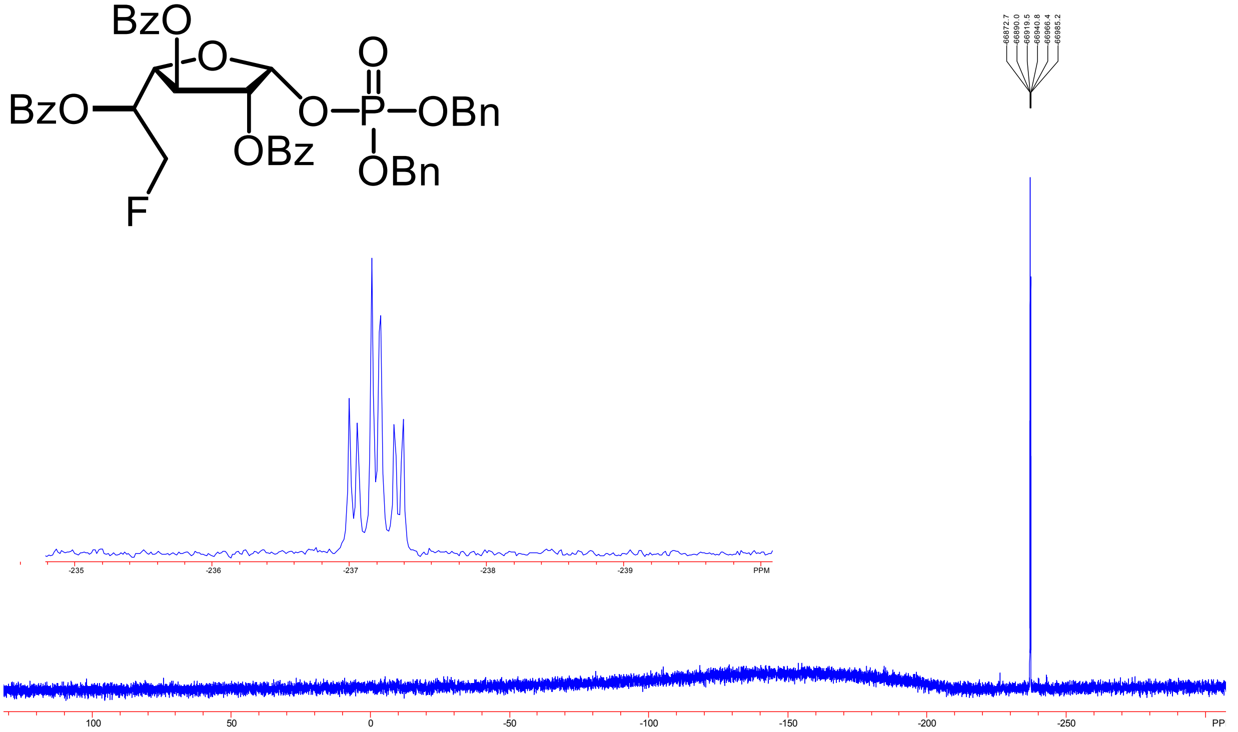
1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-D-galactofuranose: ^{13}C NMR



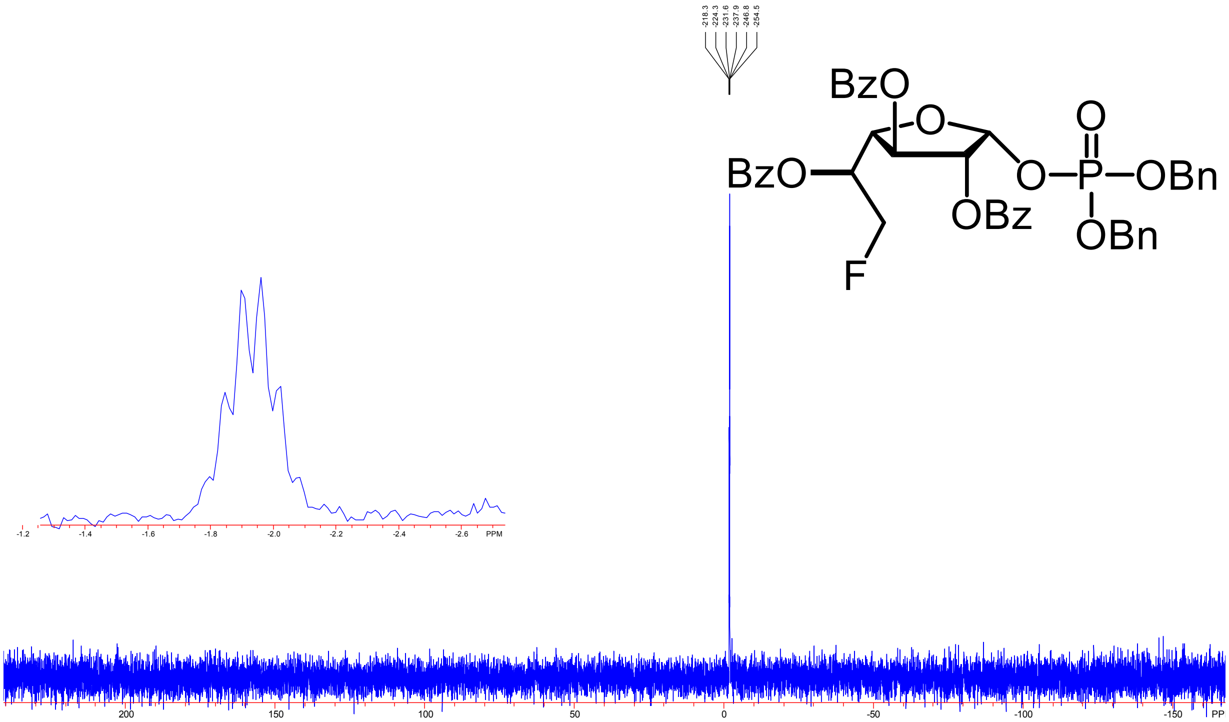
Dibenzyl 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: ^{1}H NMR



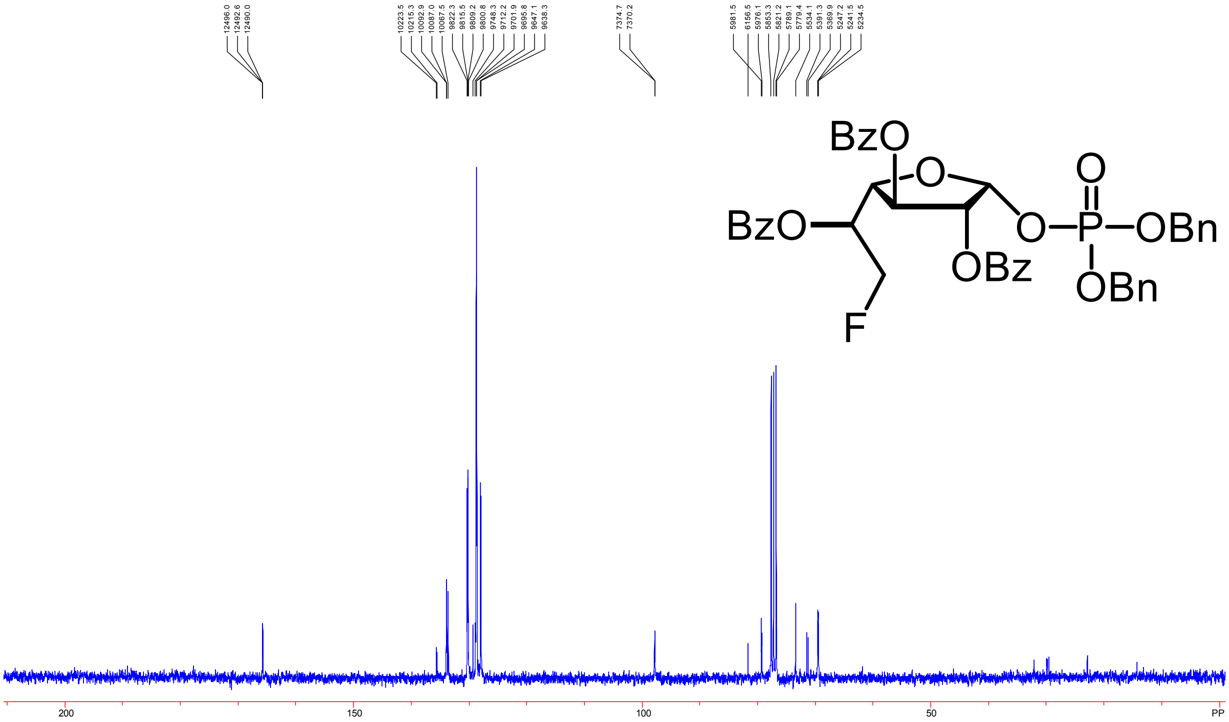
Dibenzyl 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: ^{19}F NMR



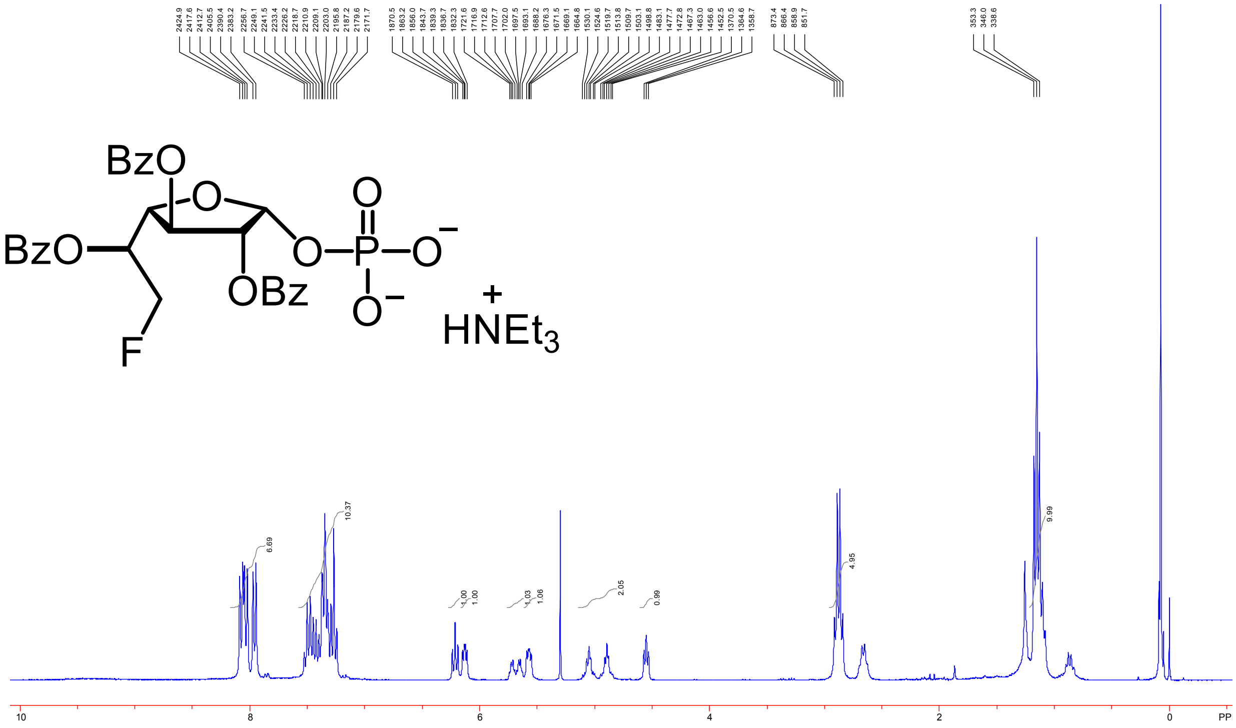
Dibenzyl 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: ^{31}P NMR



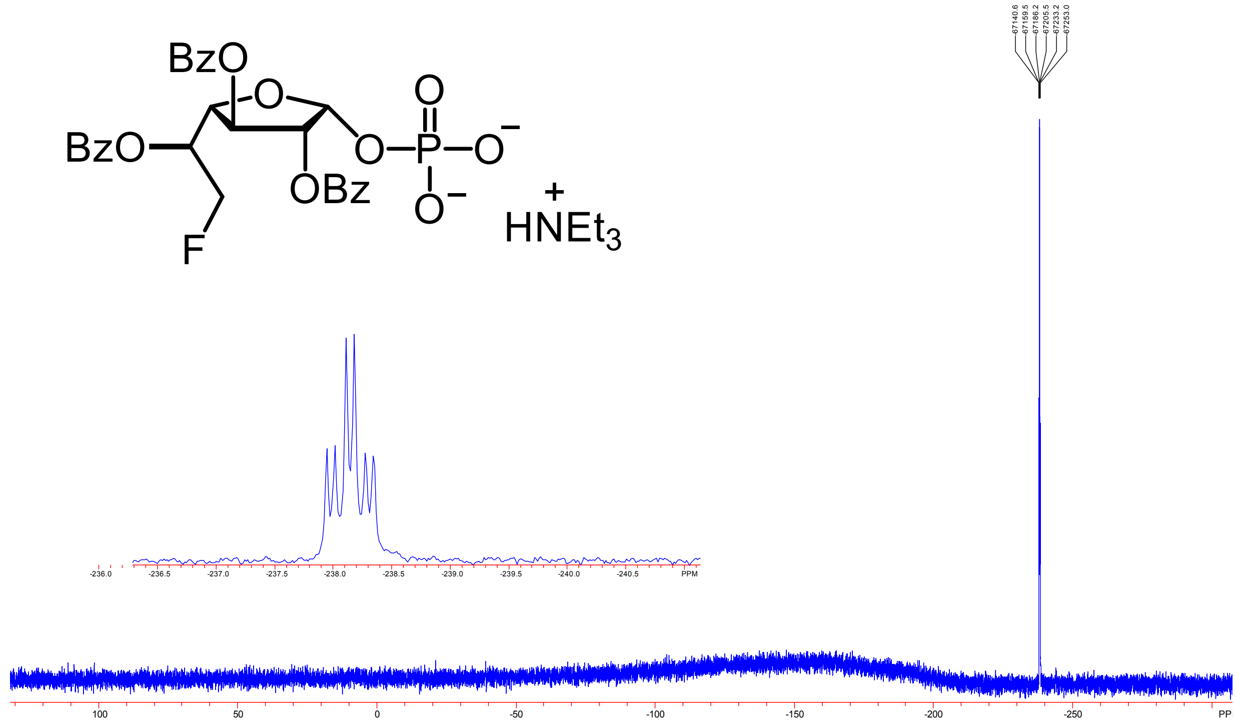
Dibenzyl 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: ^{13}C NMR



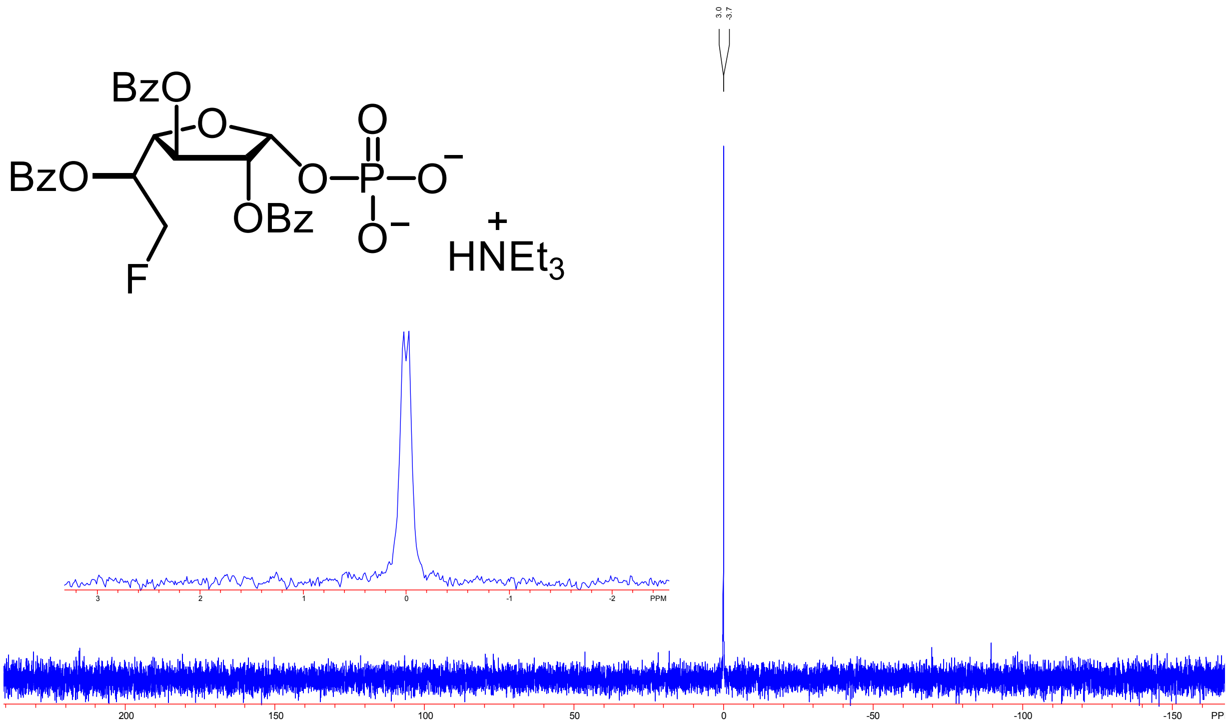
Triethylammonium 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: 1H NMR



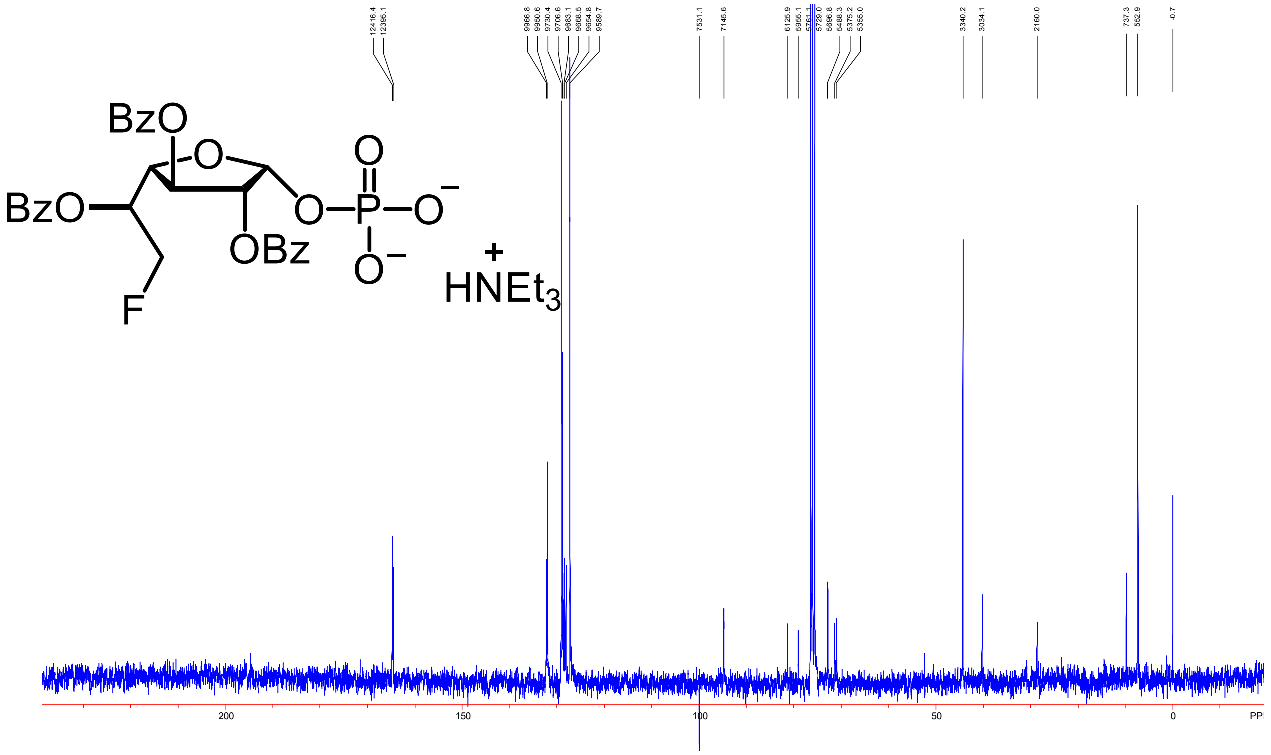
Triethylammonium 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: ^{19}F NMR



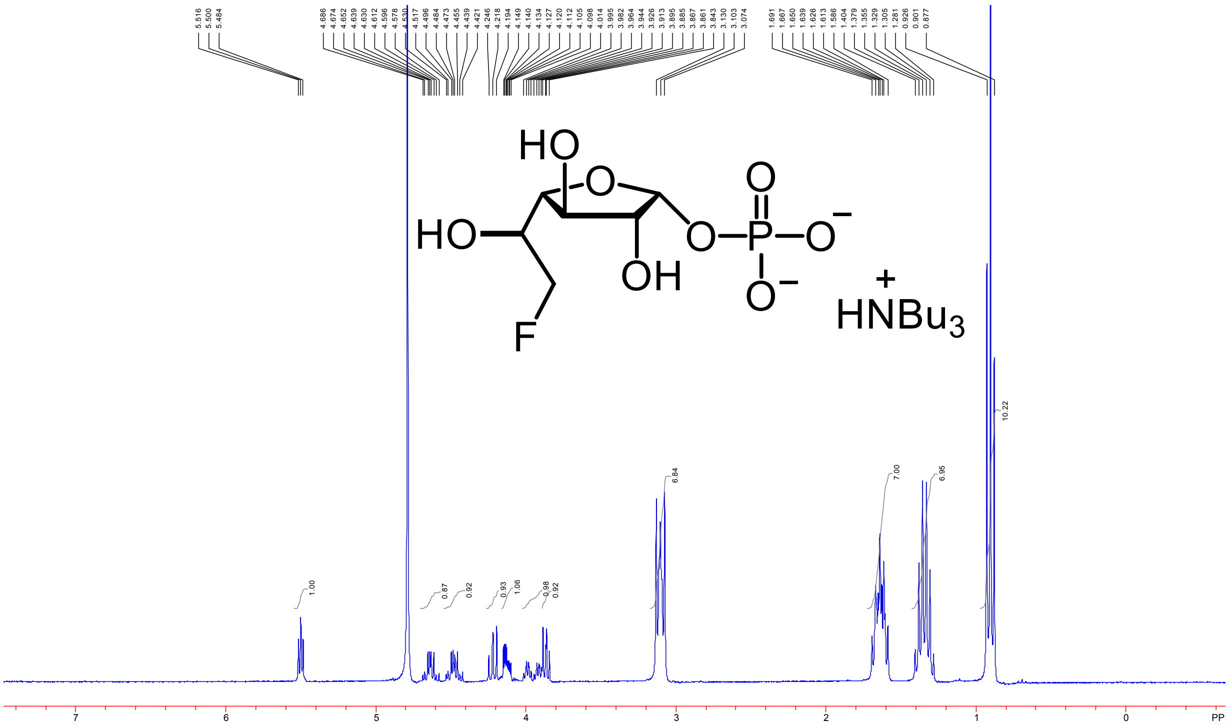
Triethylammonium 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate:
31P NMR



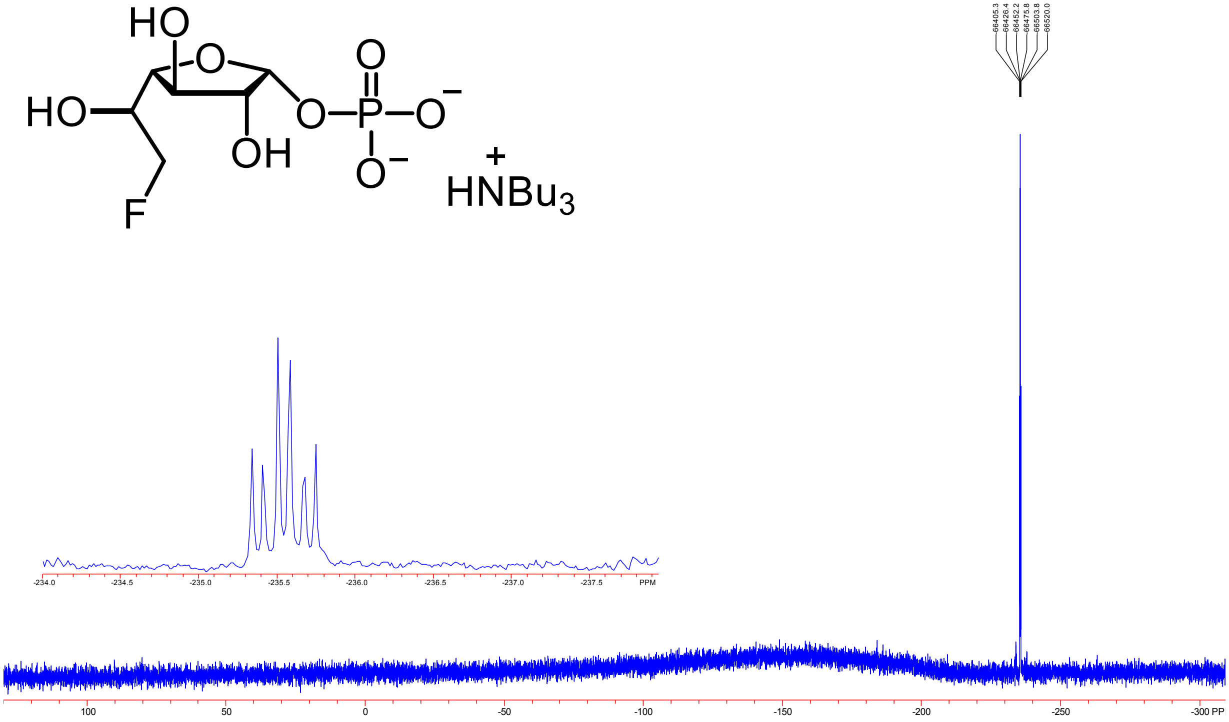
Triethylammonium 2,3,5-tri-*O*-benzoyl-6-deoxy-6-fluoro-α-D-galactofuranose monophosphate:
^{13}C NMR



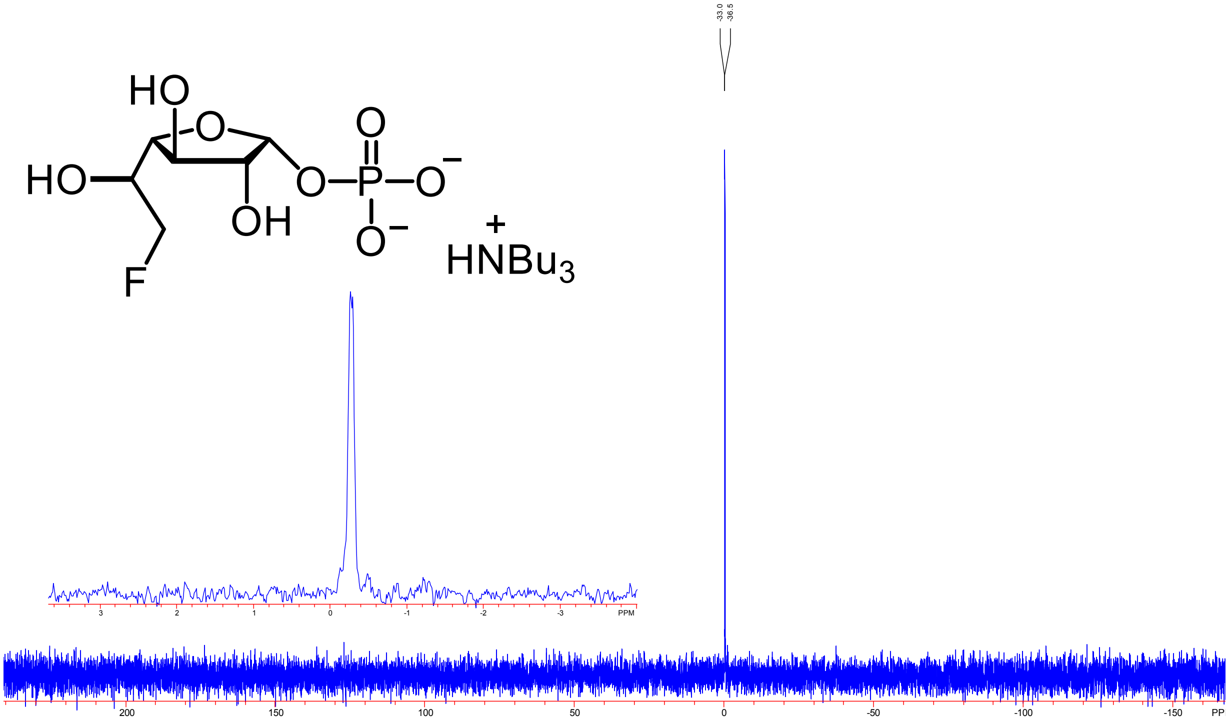
Tributylammonium 6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: ^{1}H NMR



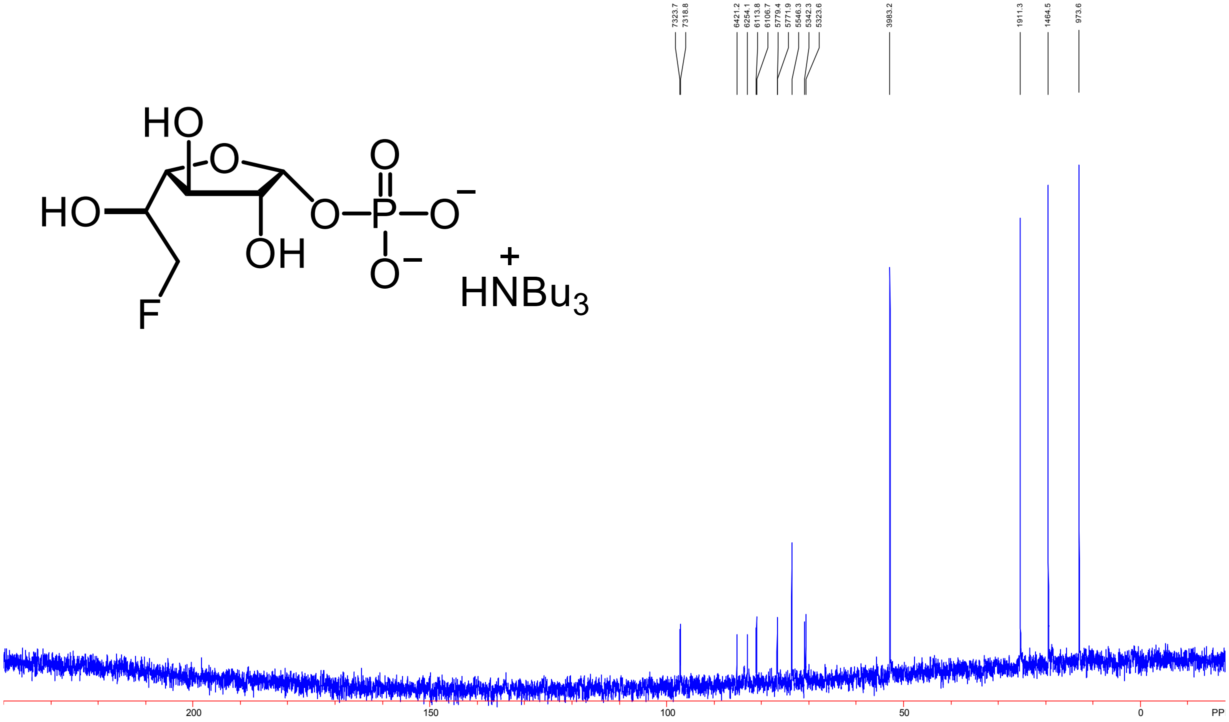
Tributylammonium 6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: ^{19}F NMR



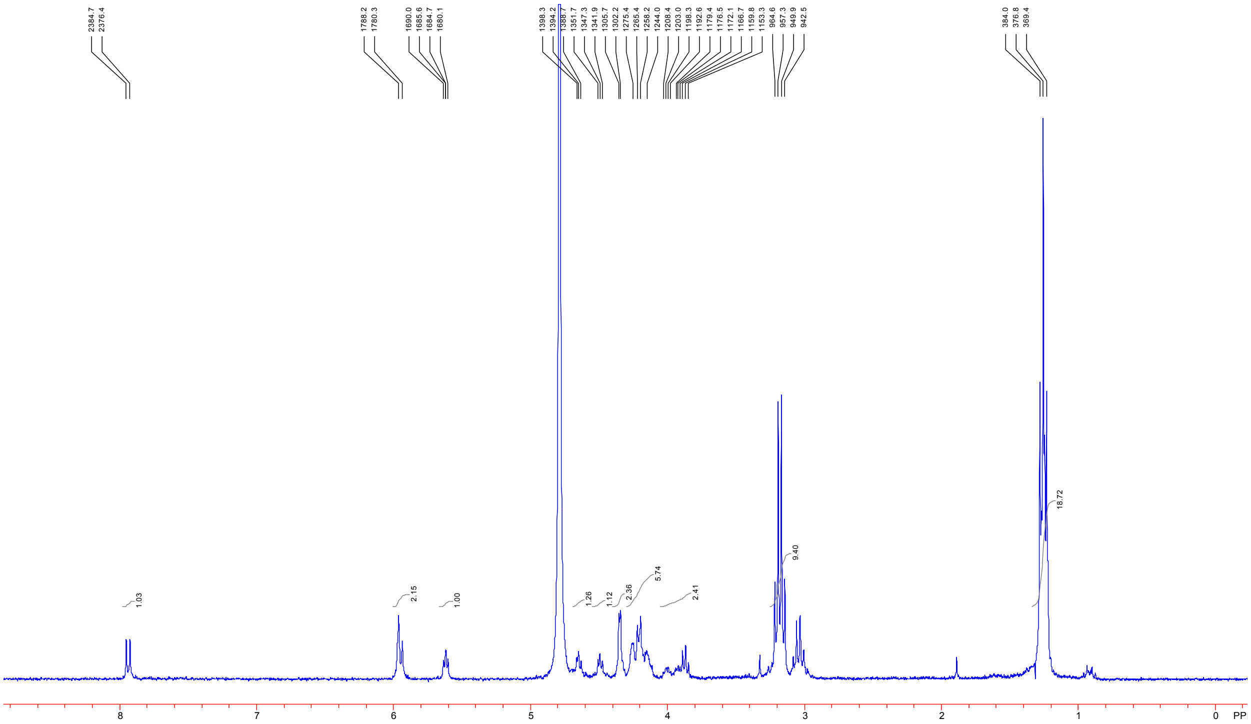
Tributylammonium 6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: ^{31}P NMR



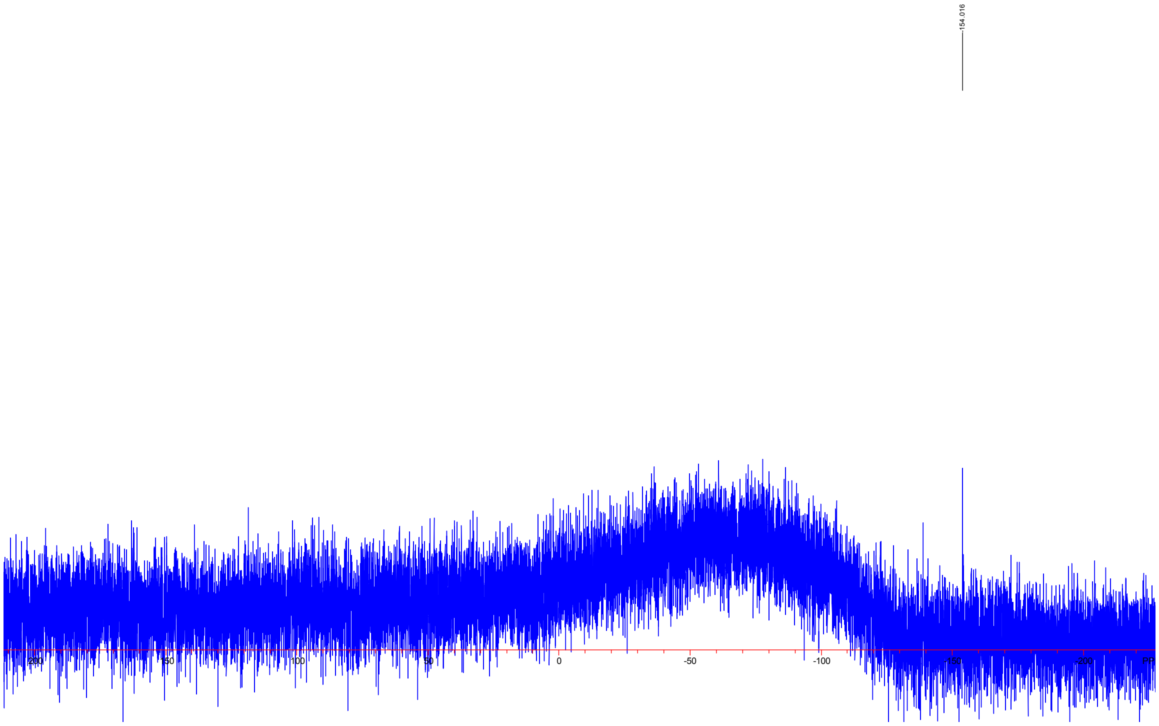
Tributylammonium 6-deoxy-6-fluoro-α-D-galactofuranose monophosphate: ^{13}C NMR



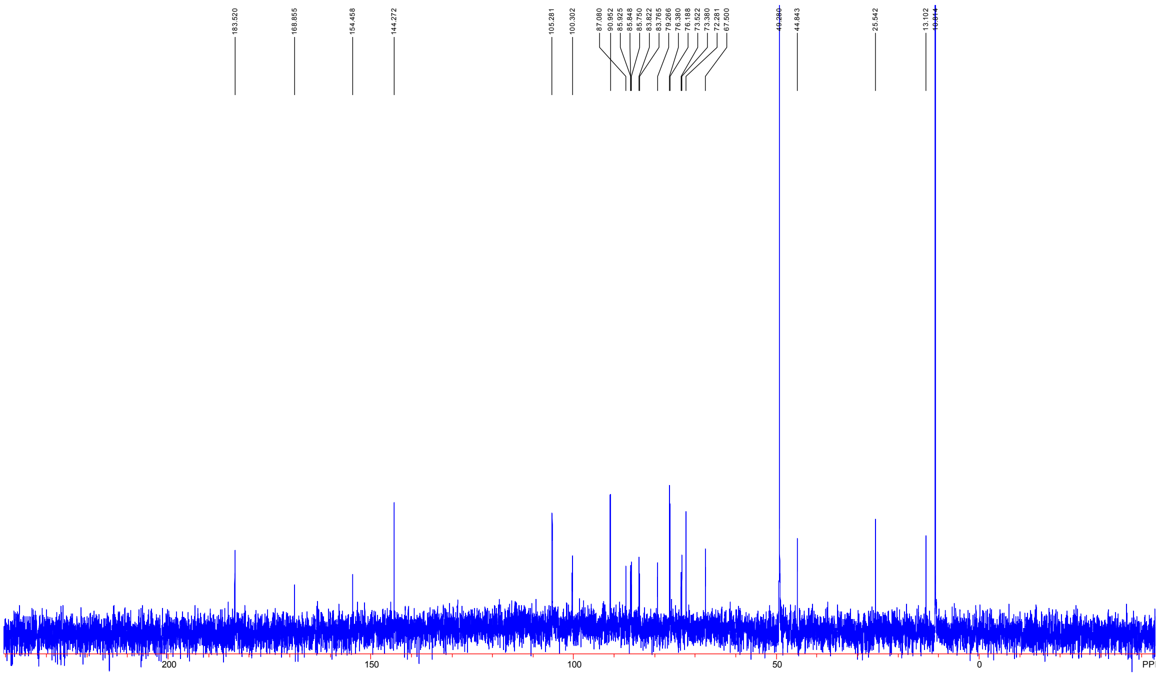
UDP-6F-Galf (**2**): ^{1}H NMR



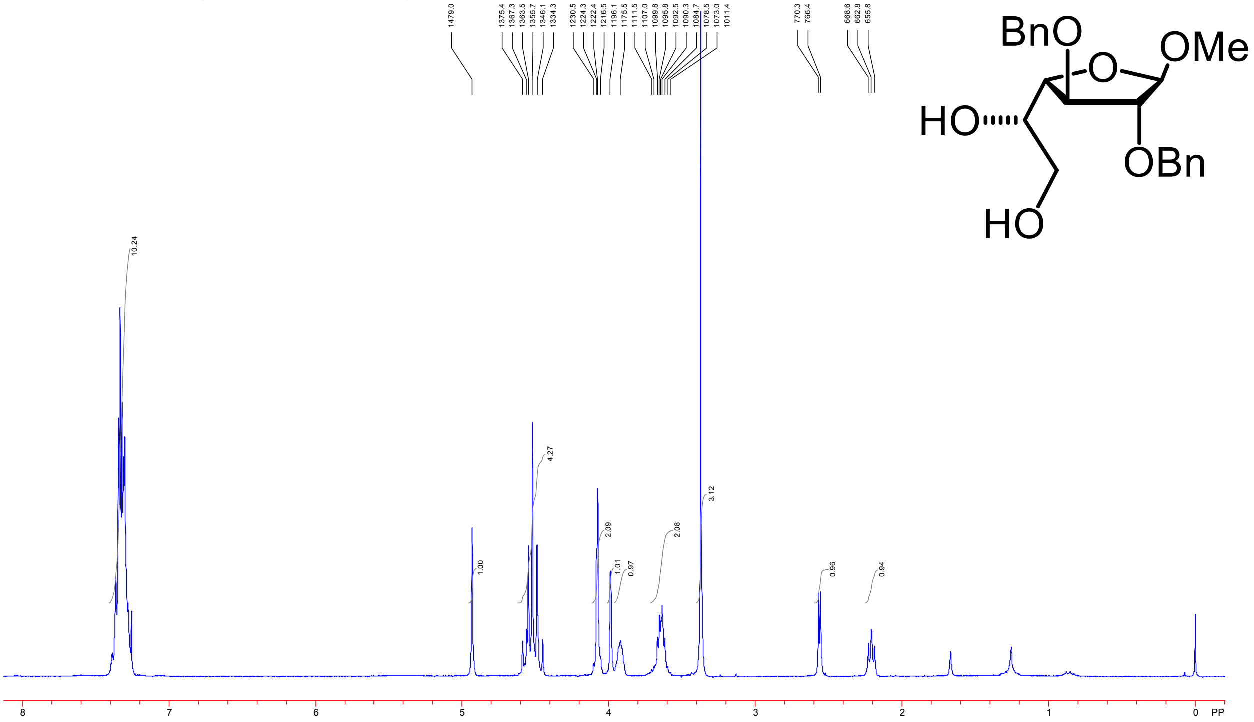
UDP-6F-Galf (**2**): ^{19}F NMR



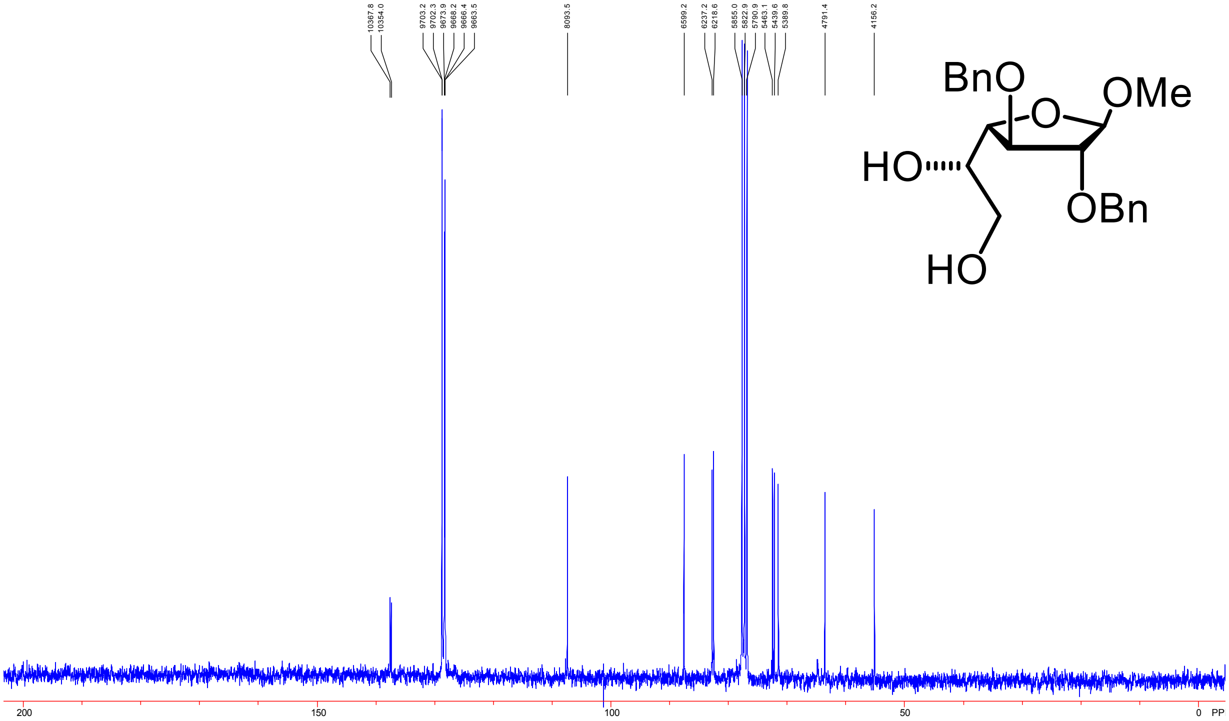
UDP-6F-Galf (**2**): ^{13}C NMR



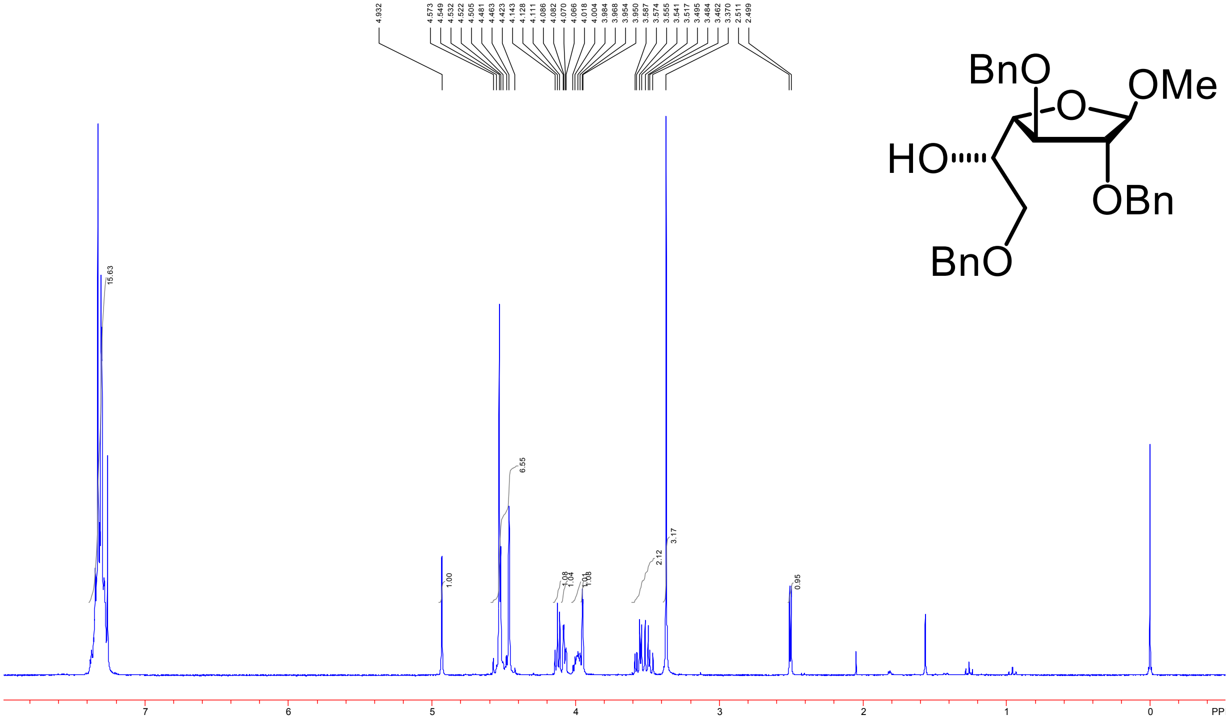
2,3-di-*O*-benzyl-beta-L-methylaltrofuranose (**9**): ^{1}H NMR



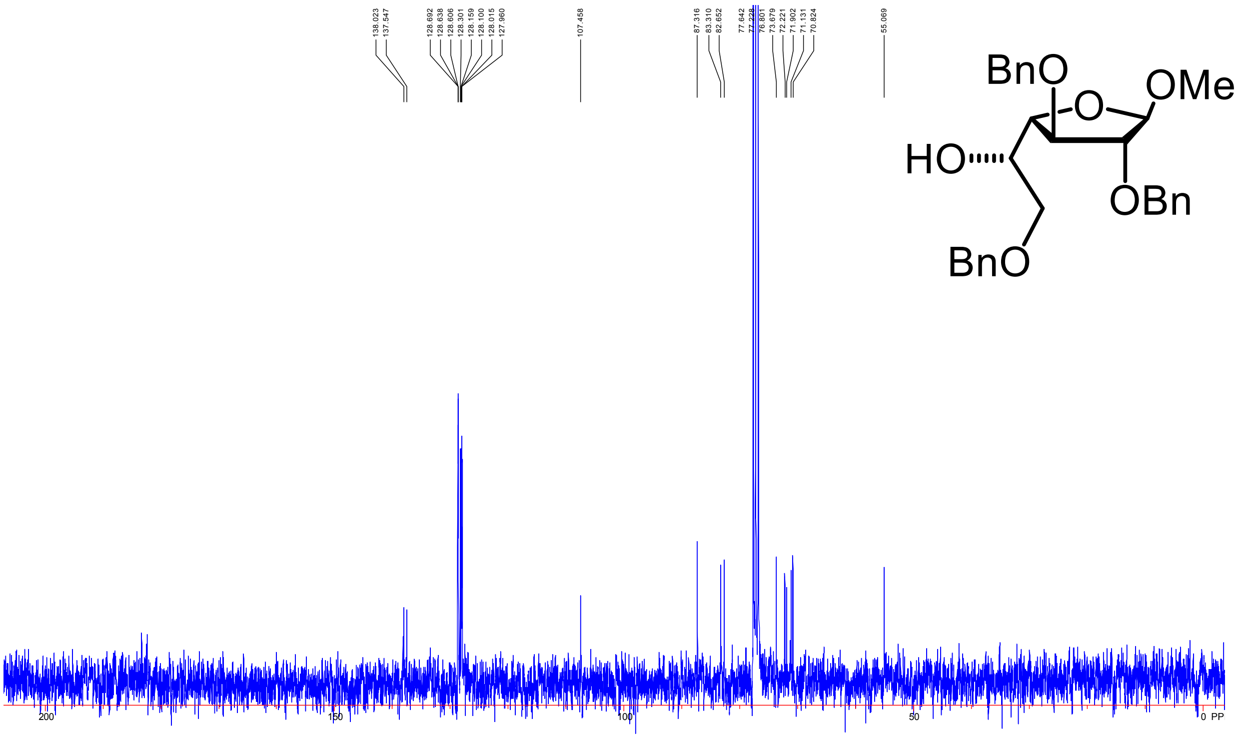
2,3-di-*O*-benzyl-beta-L-methylaltrofuranose (**9**): ^{1}H NMR



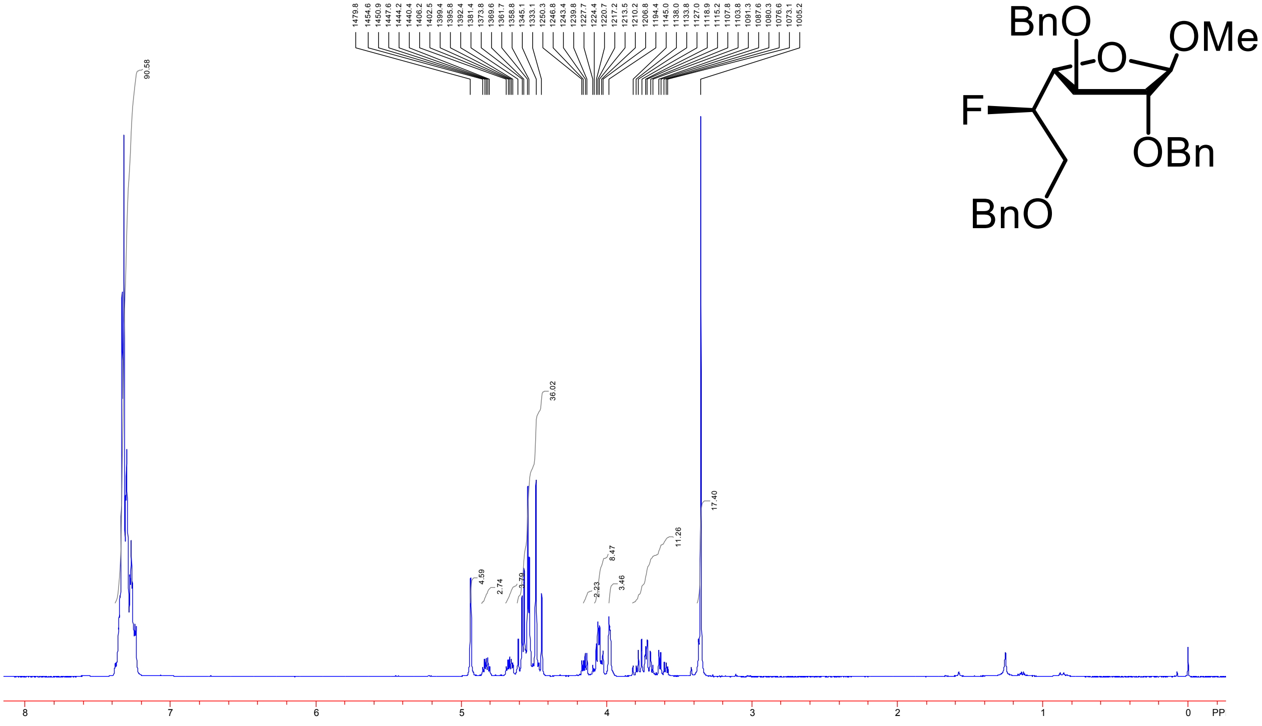
2,3,6-tri-*O*-benzyl-β-L-methylaltrofuranose: ^{1}H NMR



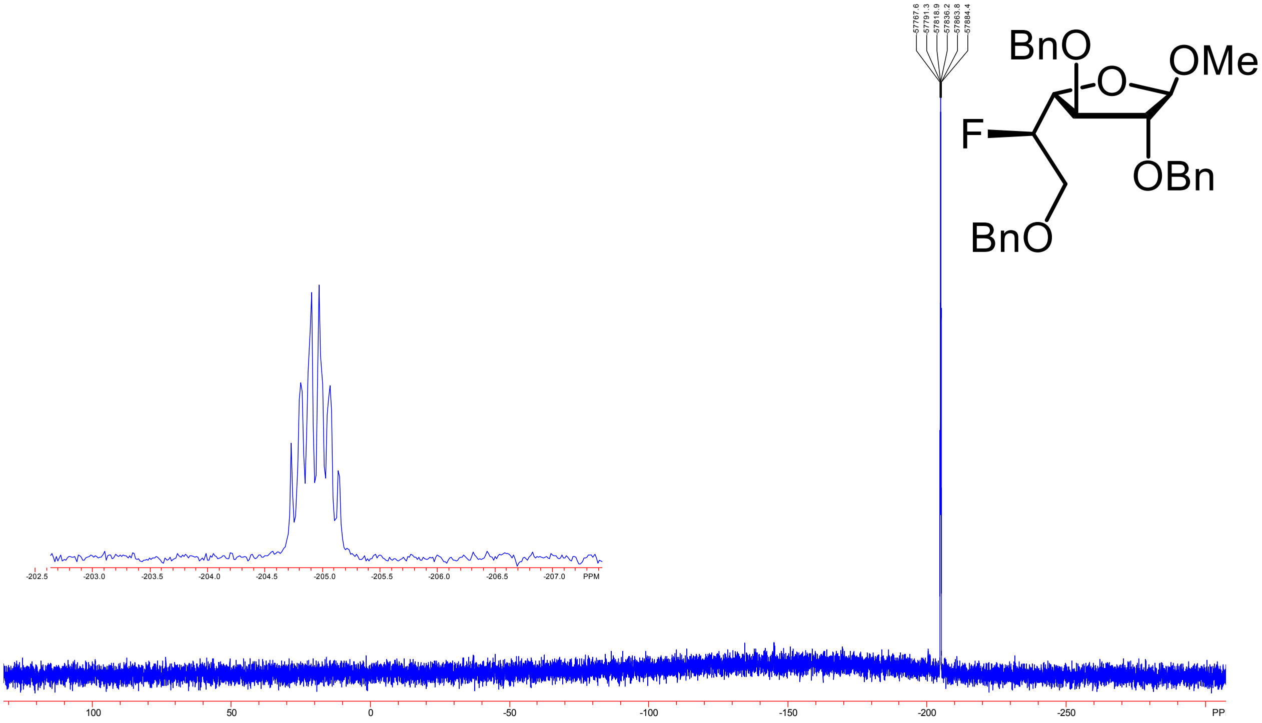
2,3,6-tri-*O*-benzyl-β-L-methylaltrofuranose: ^{13}C NMR



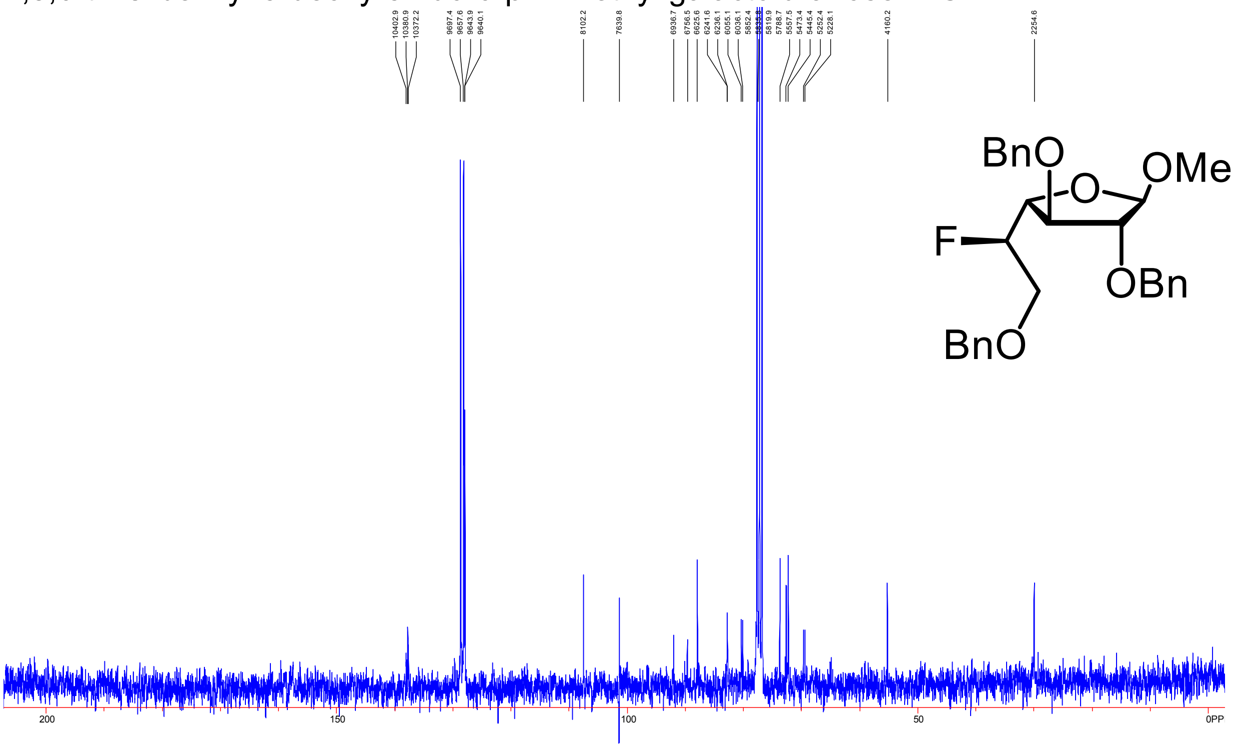
2,3,6-tri-*O*-benzyl-5-deoxy-5-fluoro-β-D-methyl galactofuranose: ^{1}H NMR



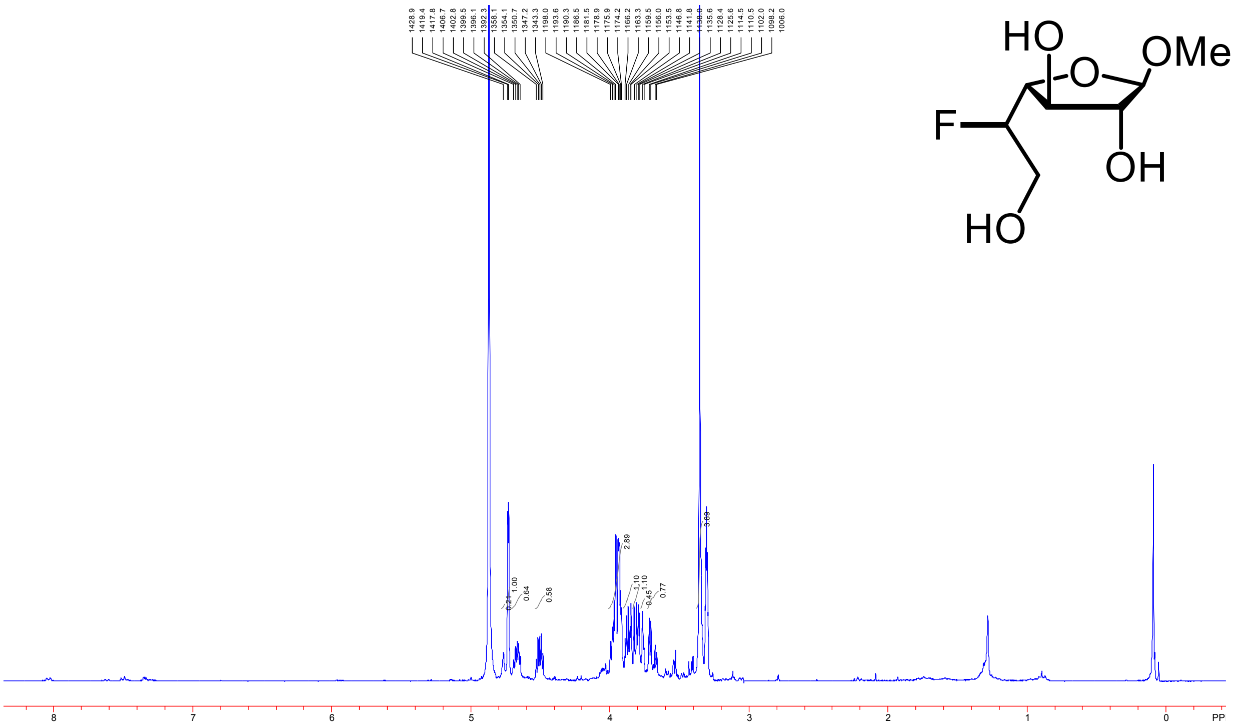
2,3,6-tri-*O*-benzyl-5-deoxy-5-fluoro-β-D-methyl galactofuranose: ^{19}F NMR



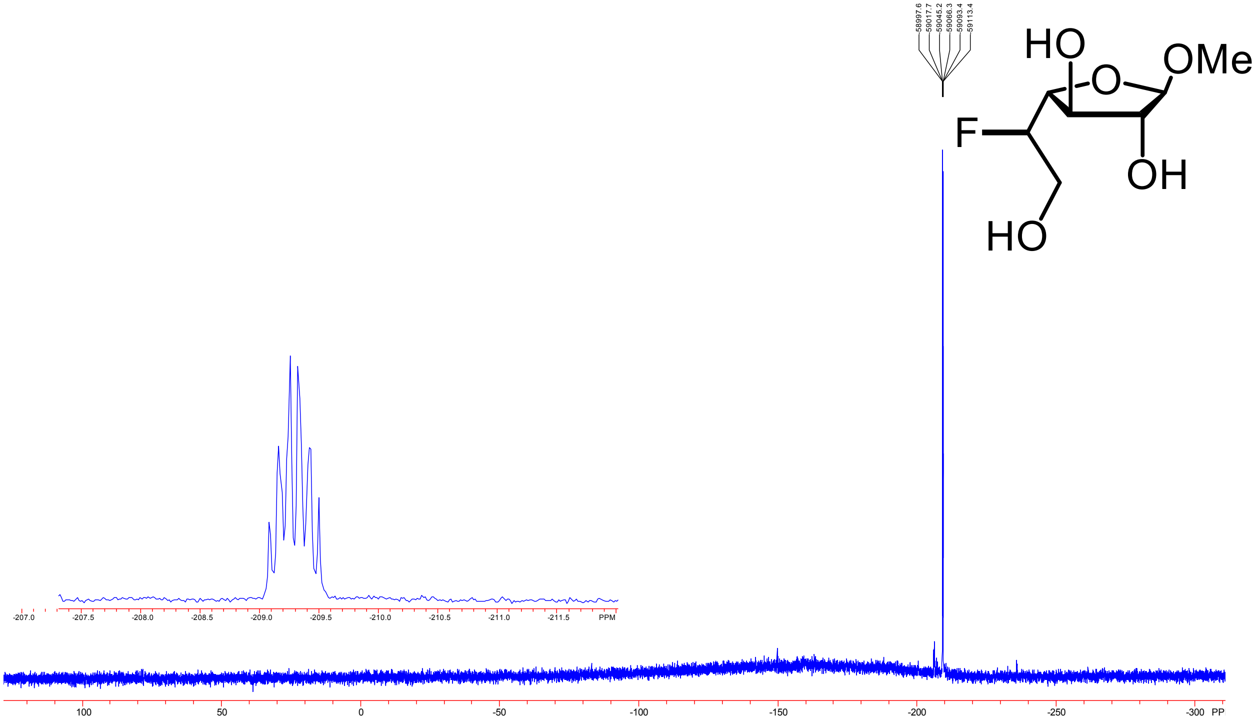
2,3,6-tri-*O*-benzyl-5-deoxy-5-fluoro-β-D-methyl galactofuranose: ^{13}C NMR



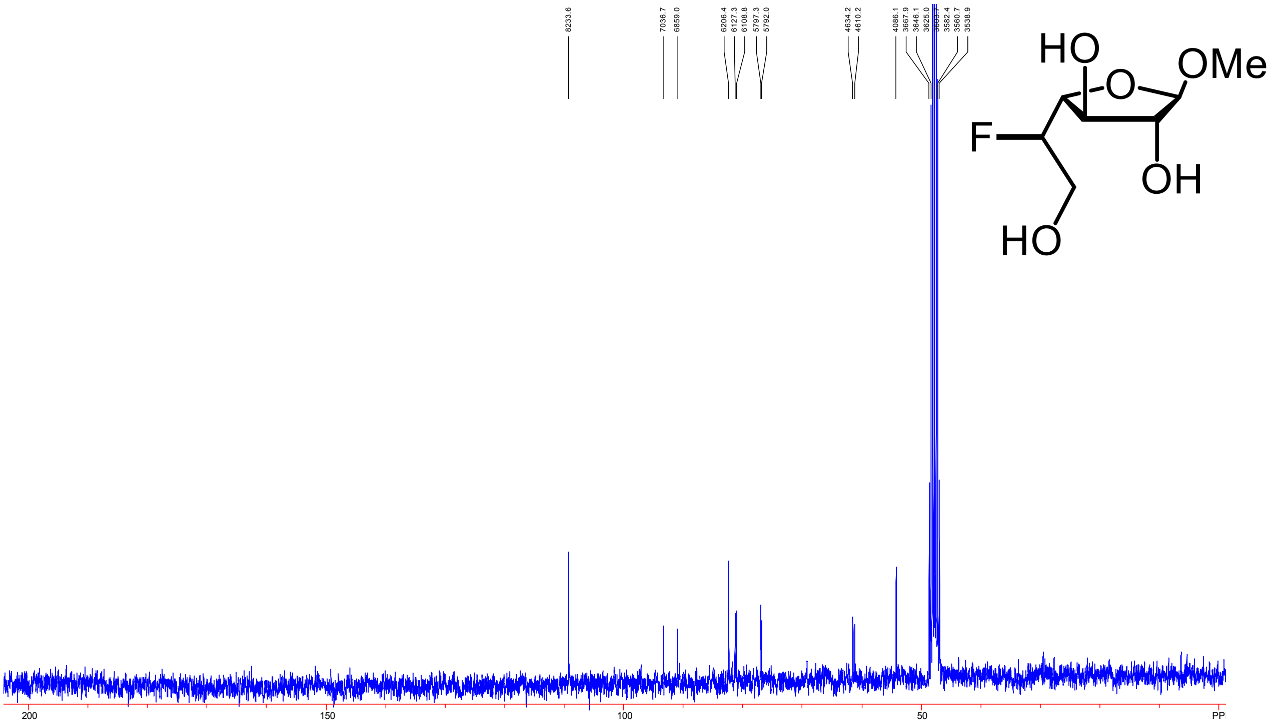
5-deoxy-5-fluoro-beta-D-methyl galactofuranose (**11**): ^{1}H NMR



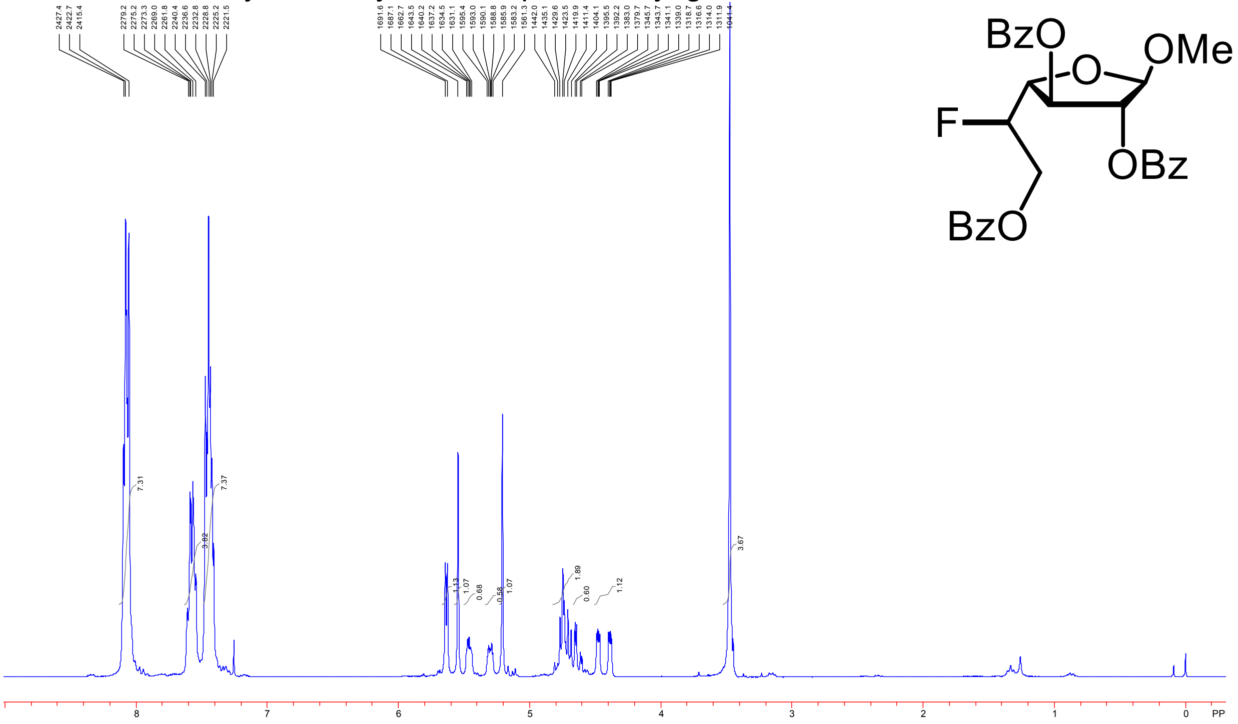
5-deoxy-5-fluoro-beta-D-methyl galactofuranose (**11**): ^{19}F NMR



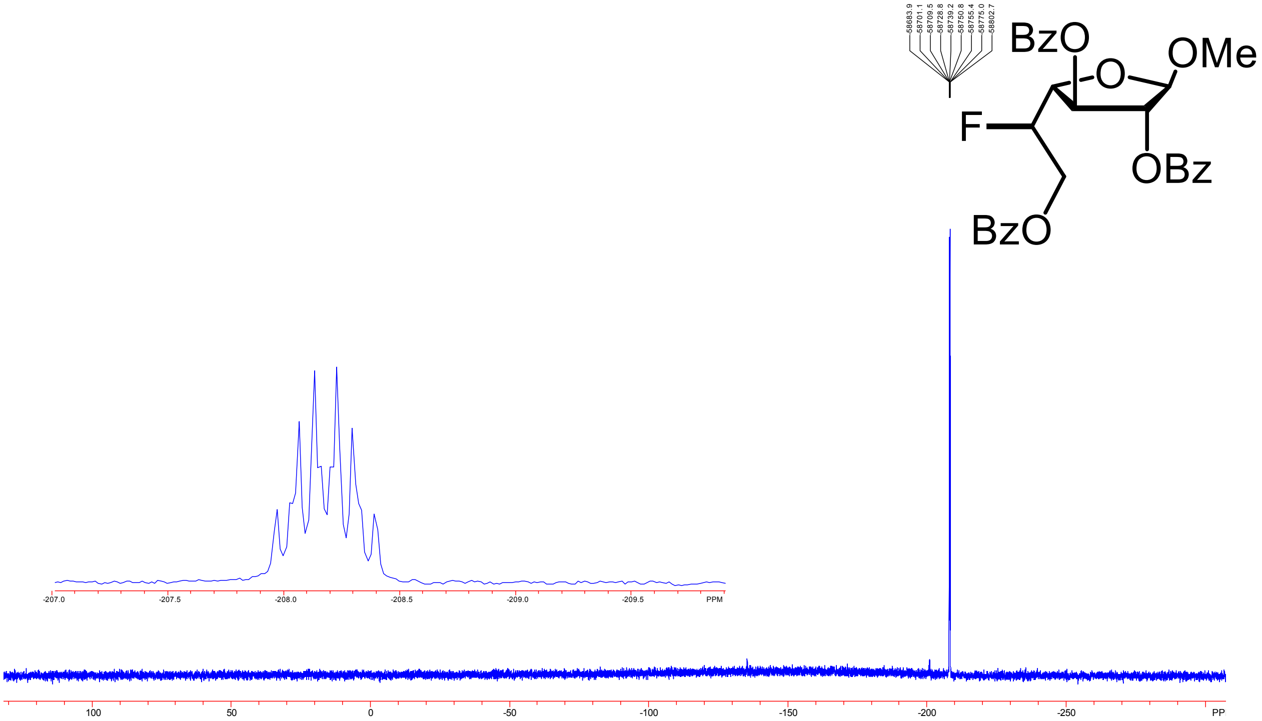
5-deoxy-5-fluoro-beta-D-methyl galactofuranose (**11**): ^{13}C NMR



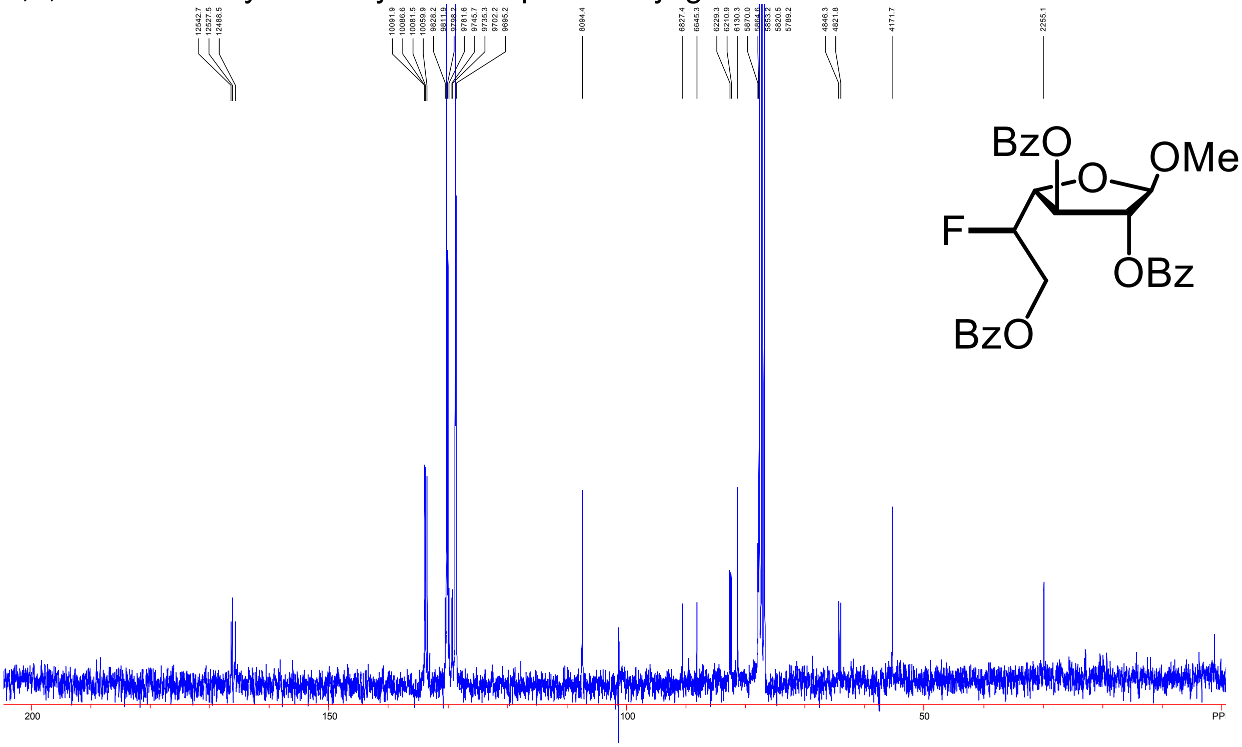
2,3,6-tri-O-benzoyl-5-deoxy-5-fluoro-β-D-methyl galactofuranose: ^{1}H NMR



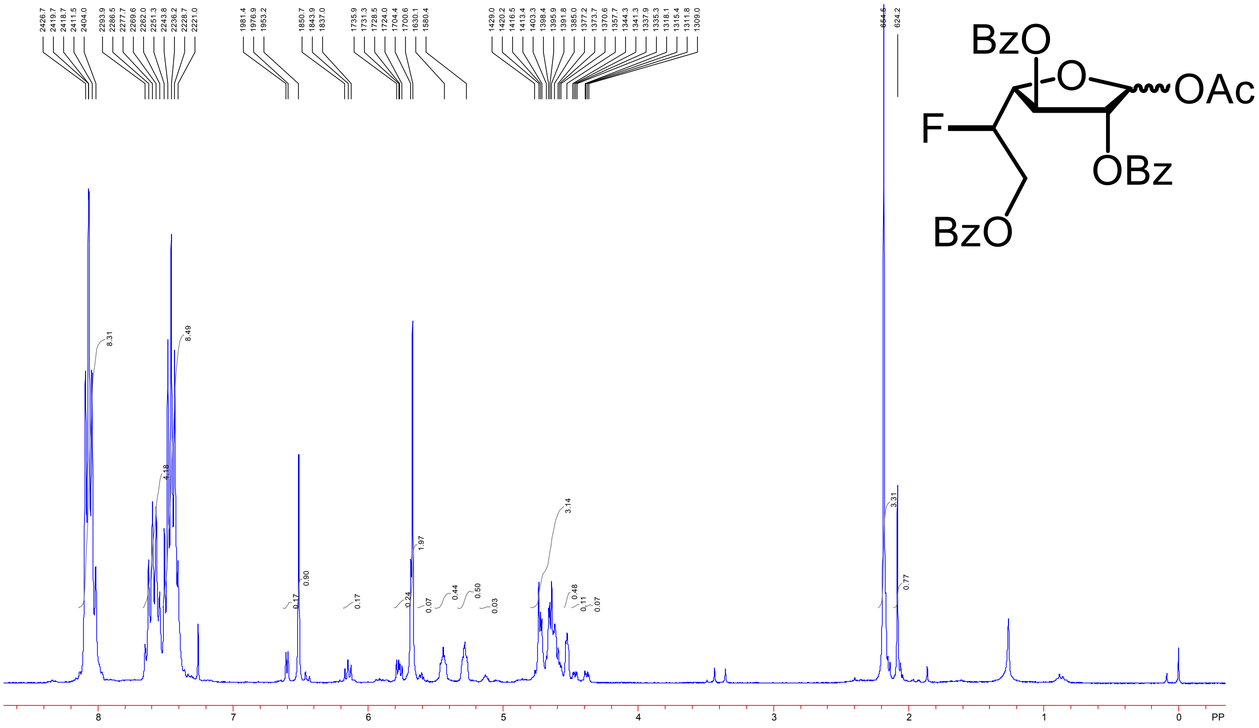
2,3,6-tri-O-benzoyl-5-deoxy-5-fluoro-β-D-methyl galactofuranose: ^{19}F NMR



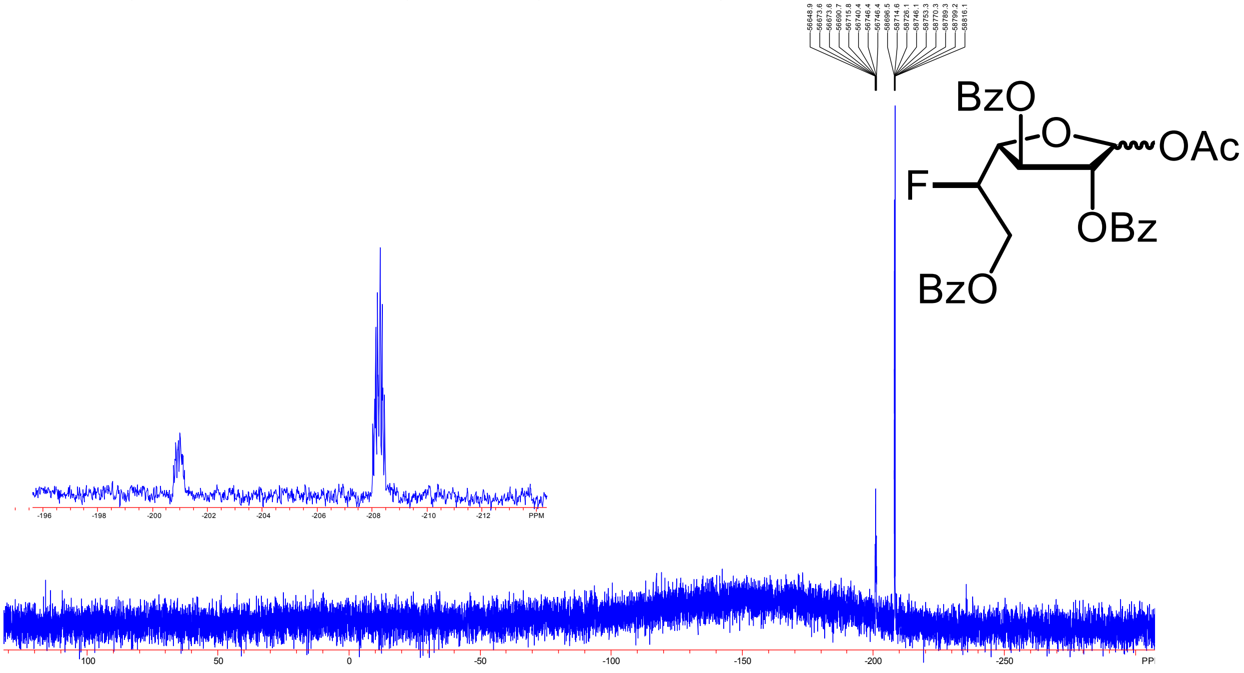
2,3,6-tri-O-benzoyl-5-deoxy-5-fluoro-β-D-methyl galactofuranose: ^{13}C NMR



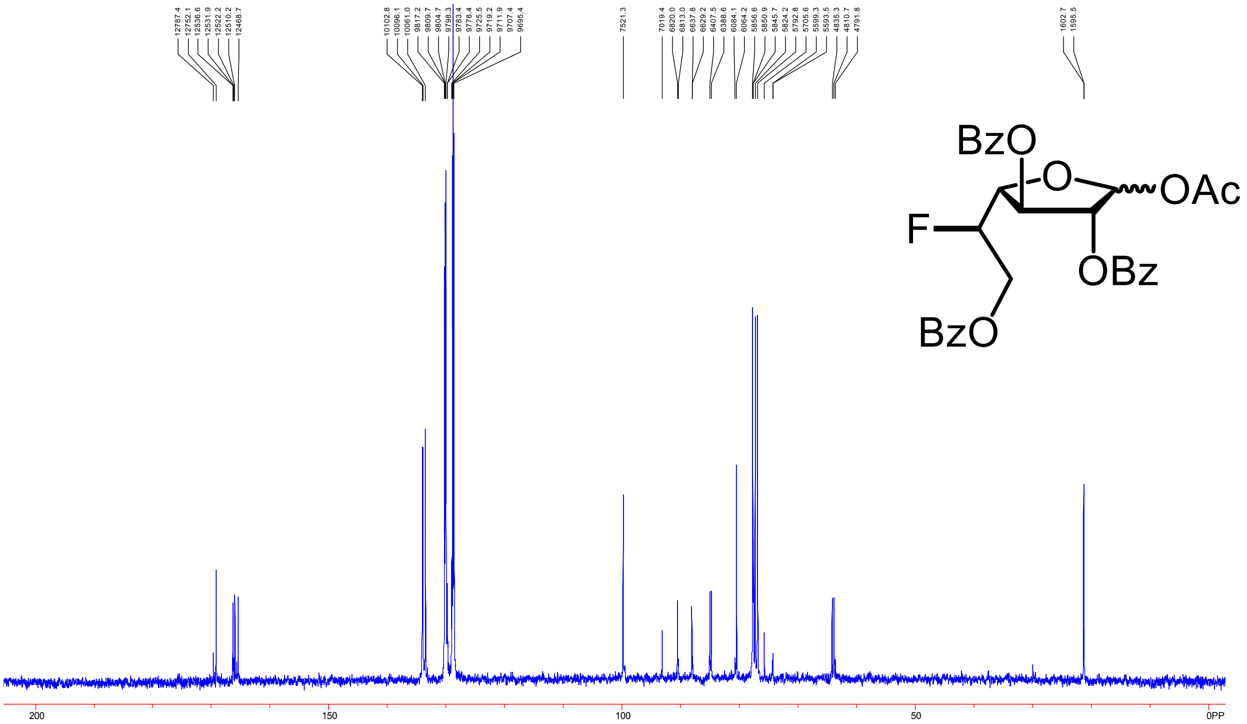
1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-D-galactofuranose: 1H NMR



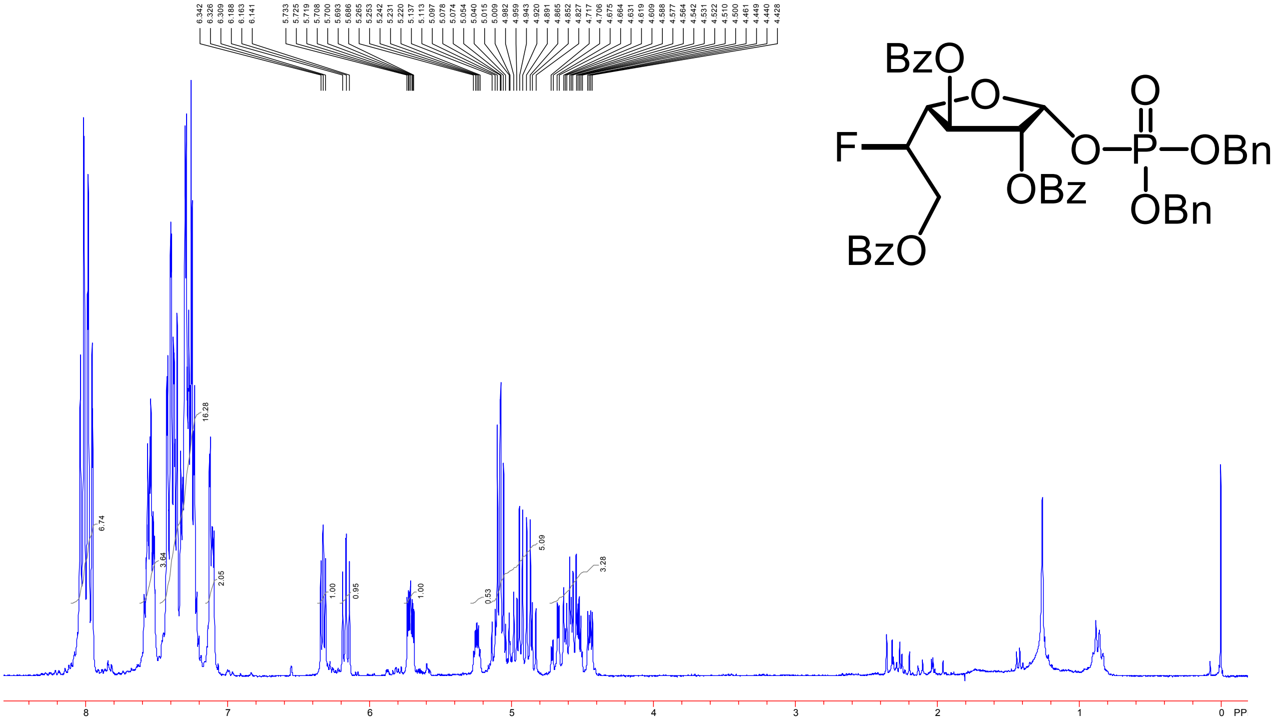
1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-D-galactofuranose: ^{19}F NMR



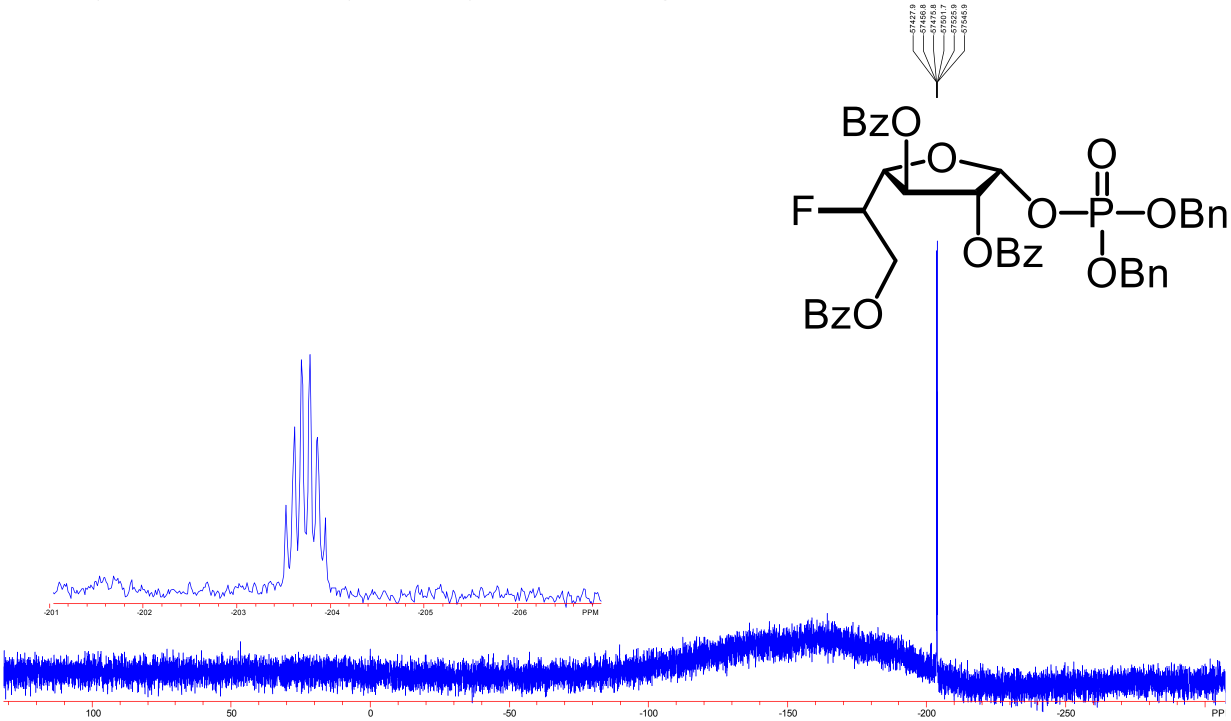
1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-D-galactofuranose: ^{13}C NMR



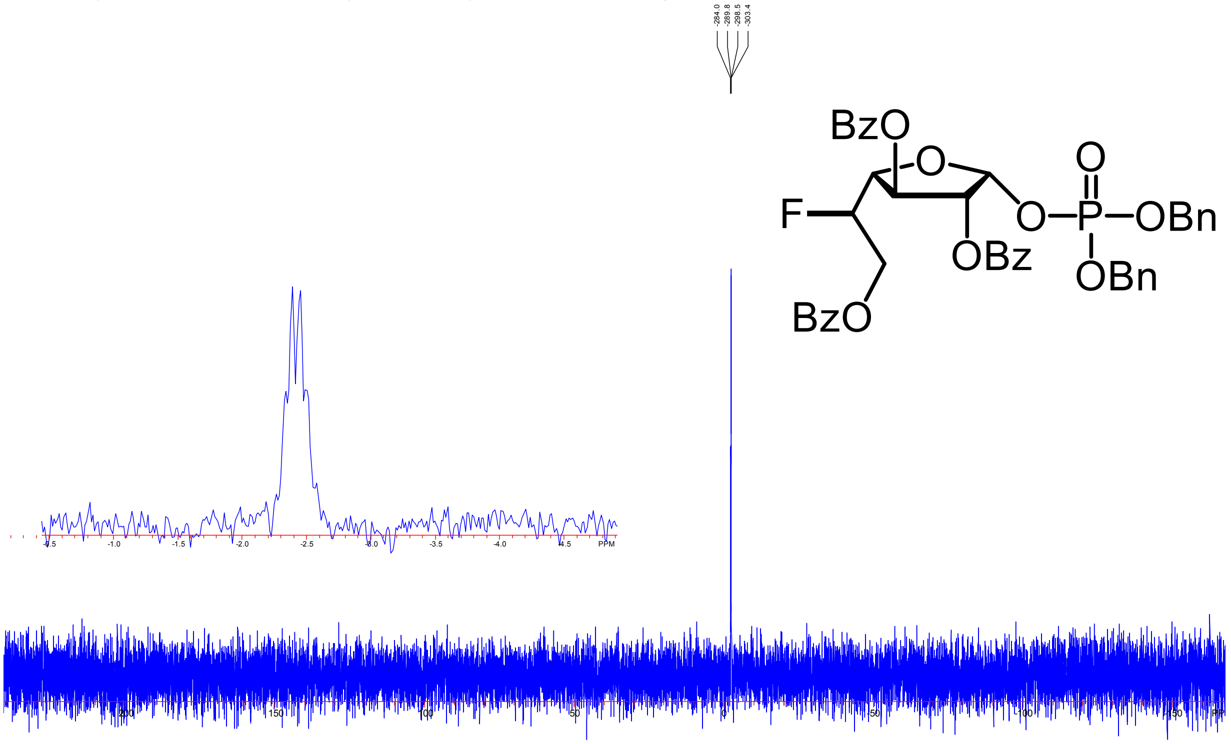
Dibenzyl 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: ^{1}H NMR



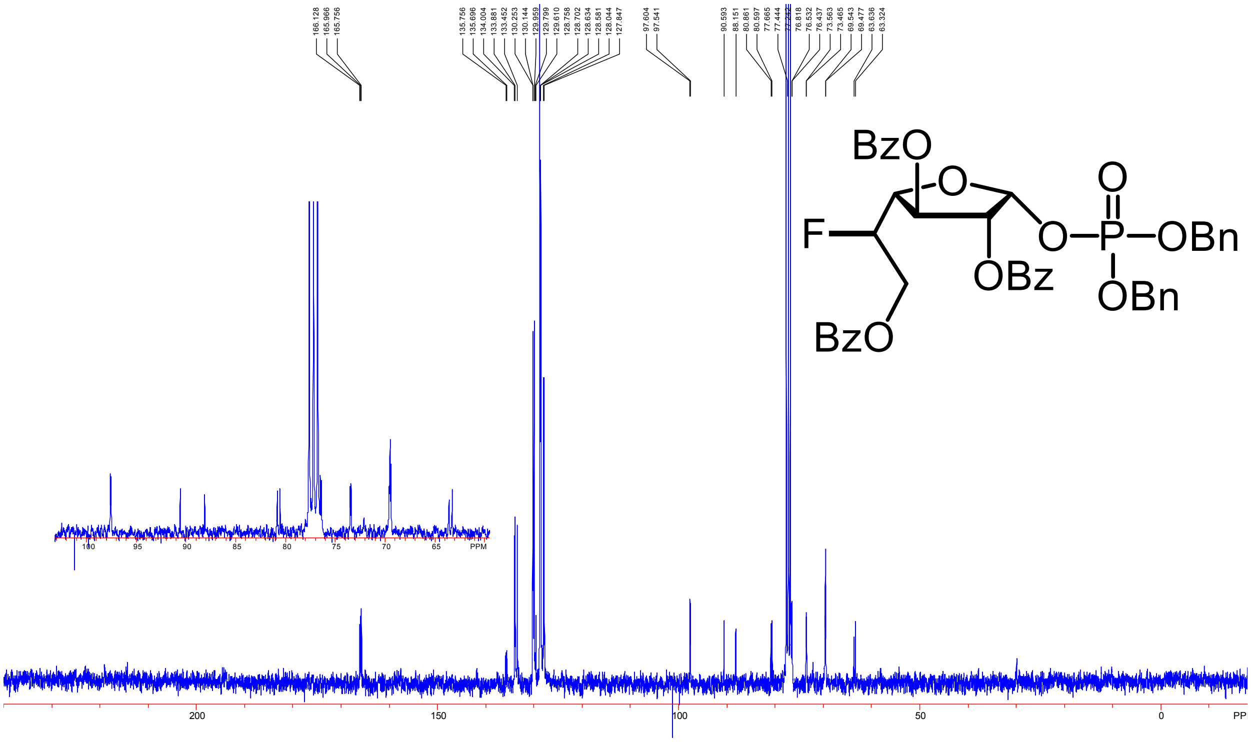
Dibenzyl 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: ^{19}F NMR



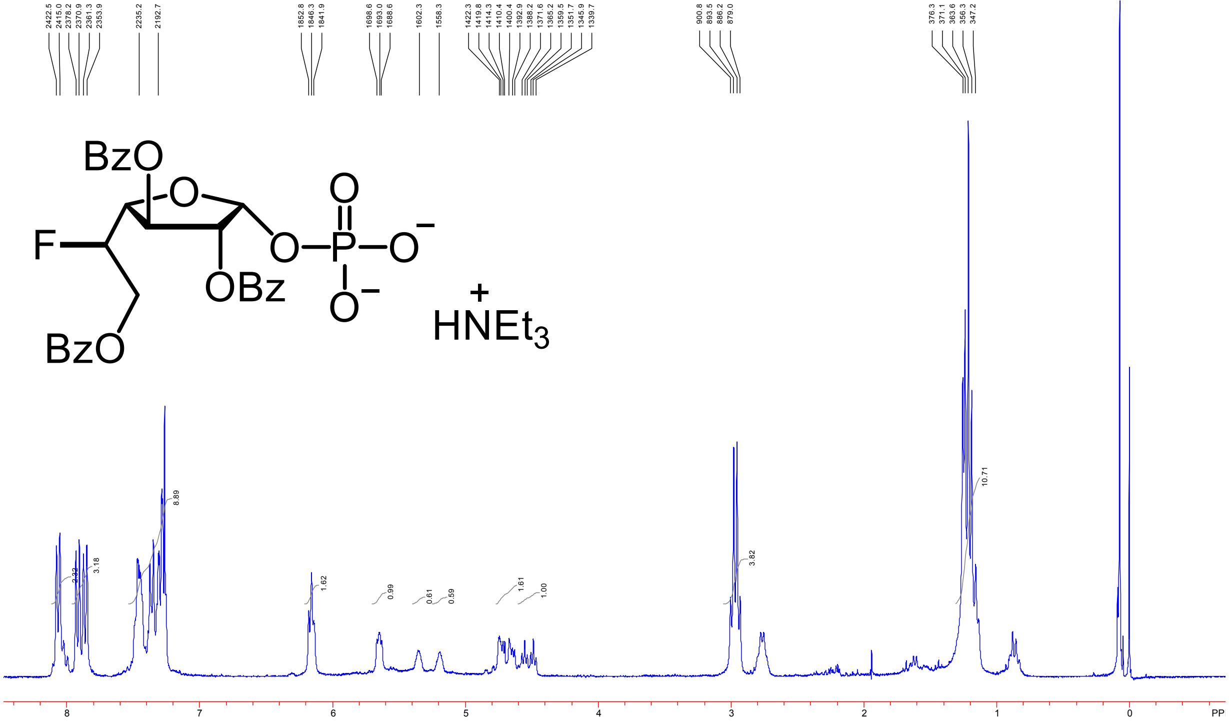
Dibenzyl 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: ^{31}P NMR



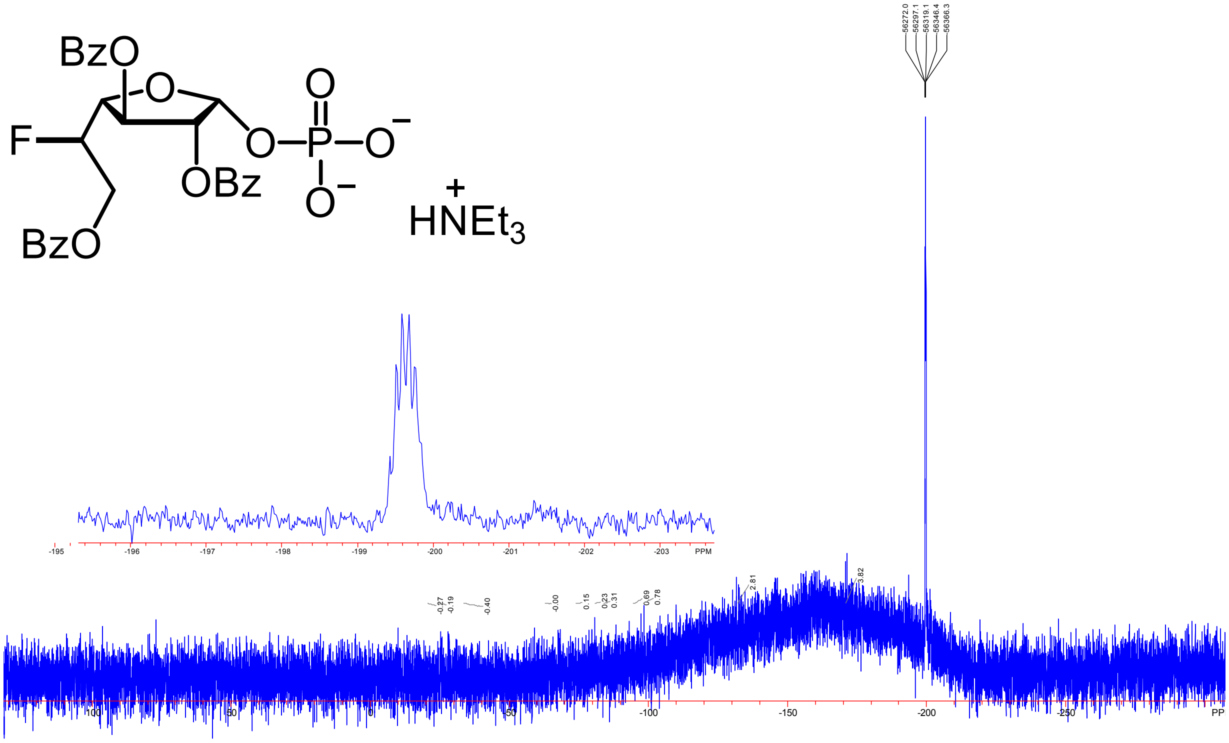
Dibenzyl 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: ^{13}C NMR



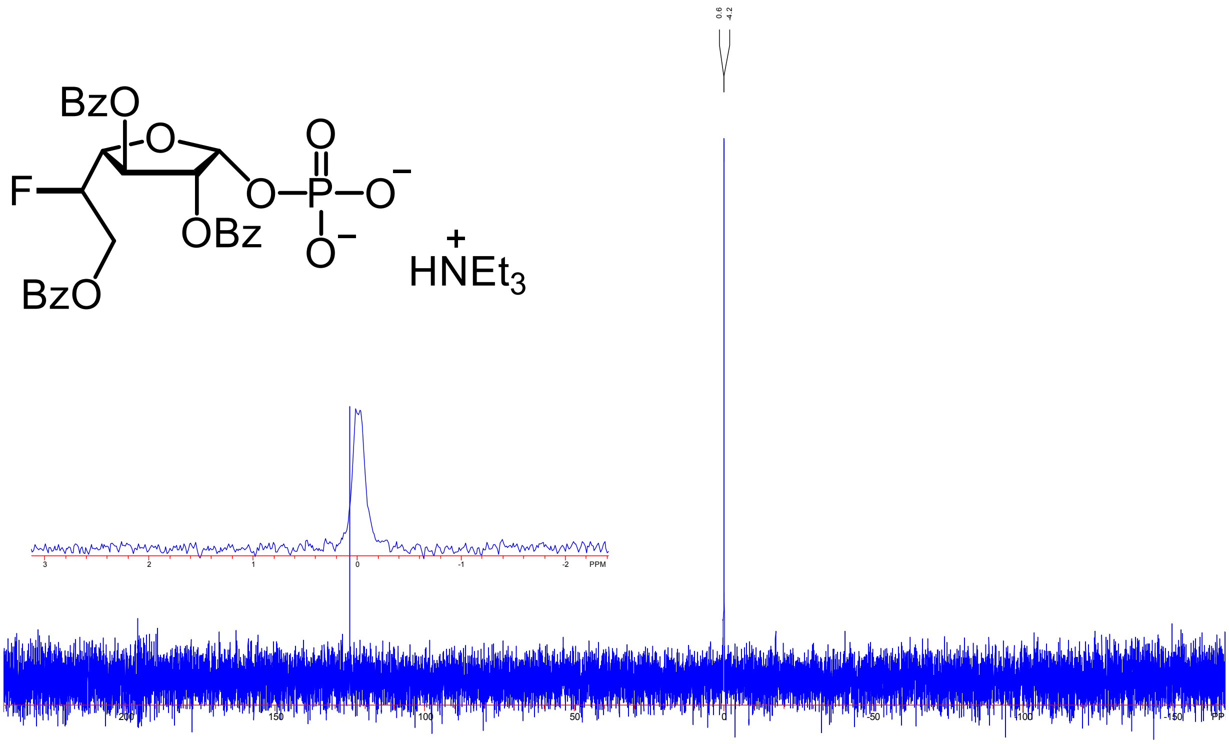
Triethylammonium 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: ^{1}H NMR



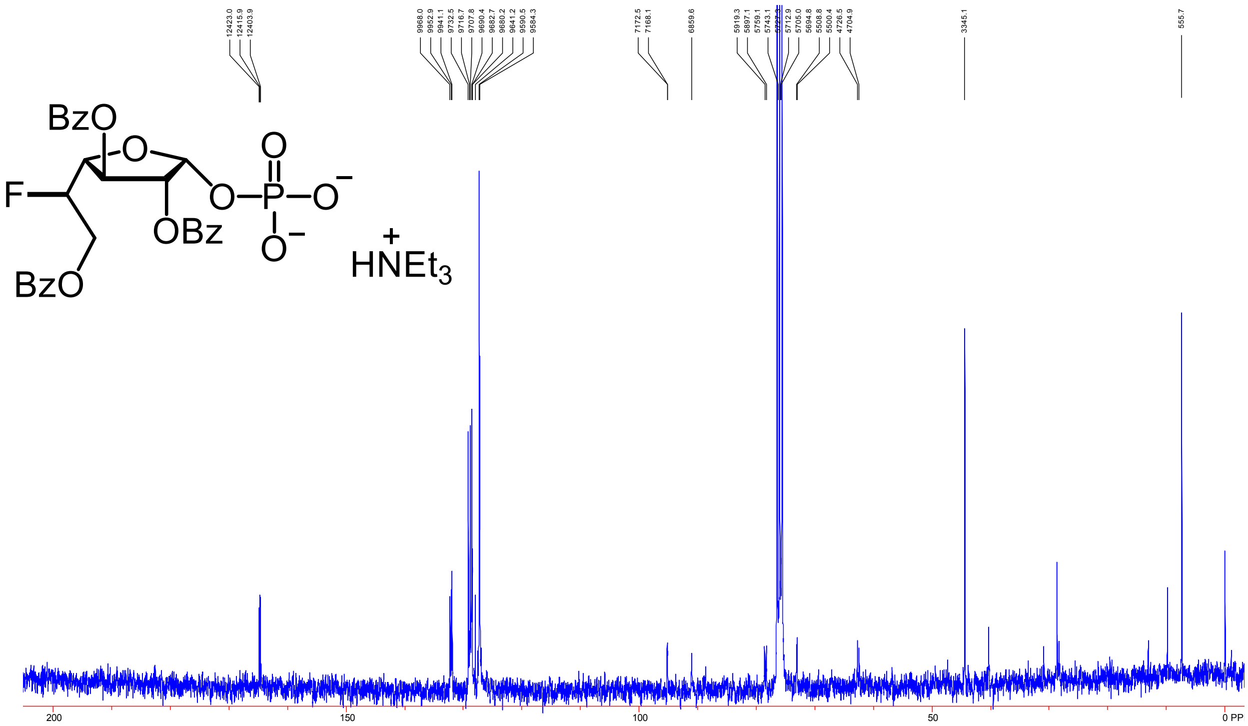
Triethylammonium 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: ^{19}F NMR



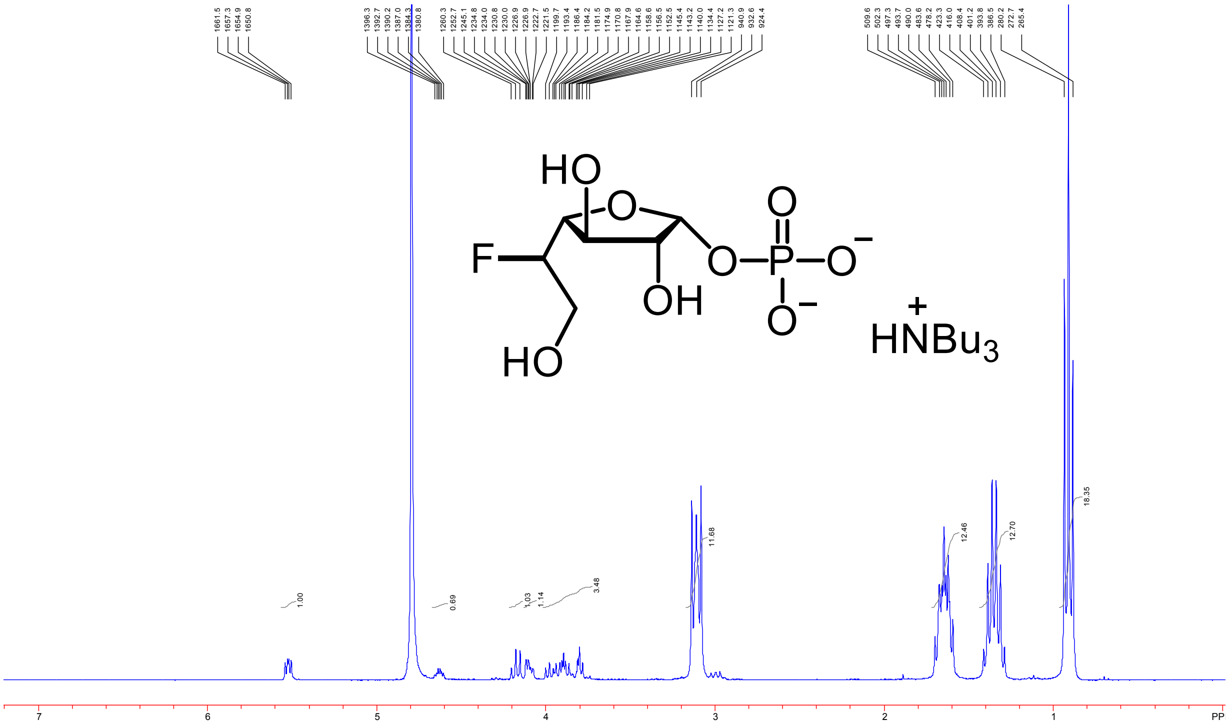
Triethylammonium 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: 31P NMR



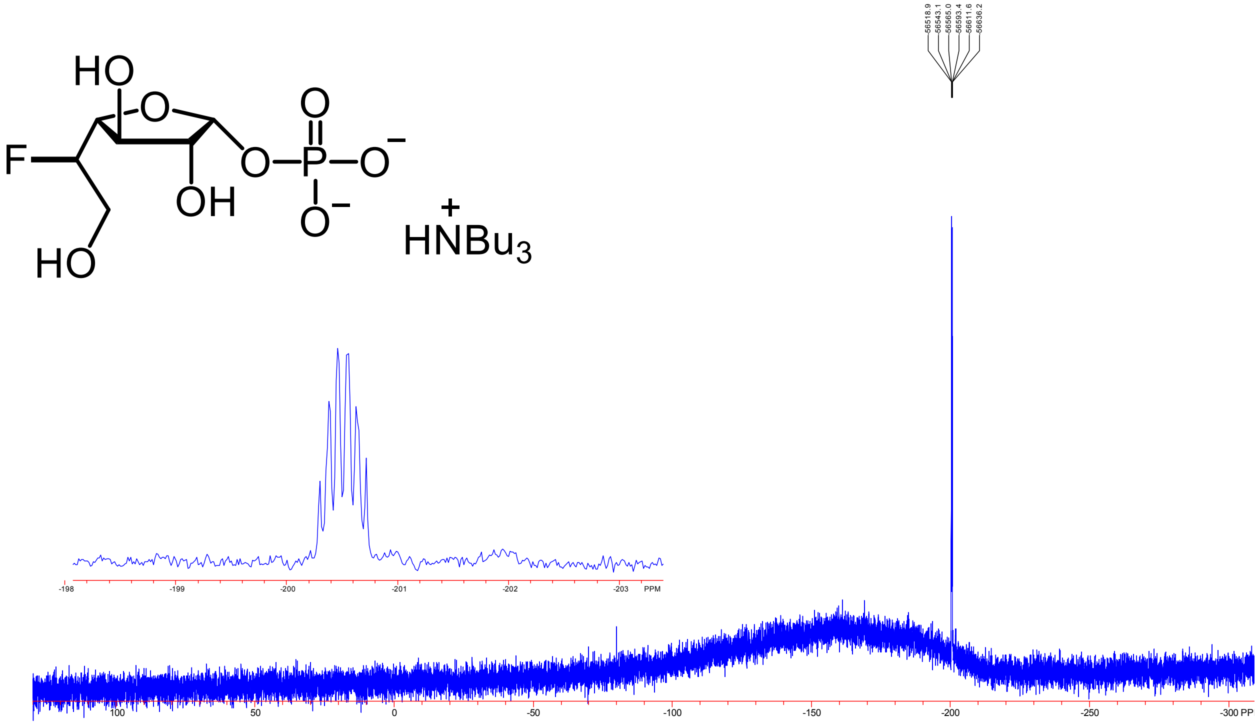
Triethylammonium 2,3,6-tri-*O*-benzoyl-5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: 13C NMR



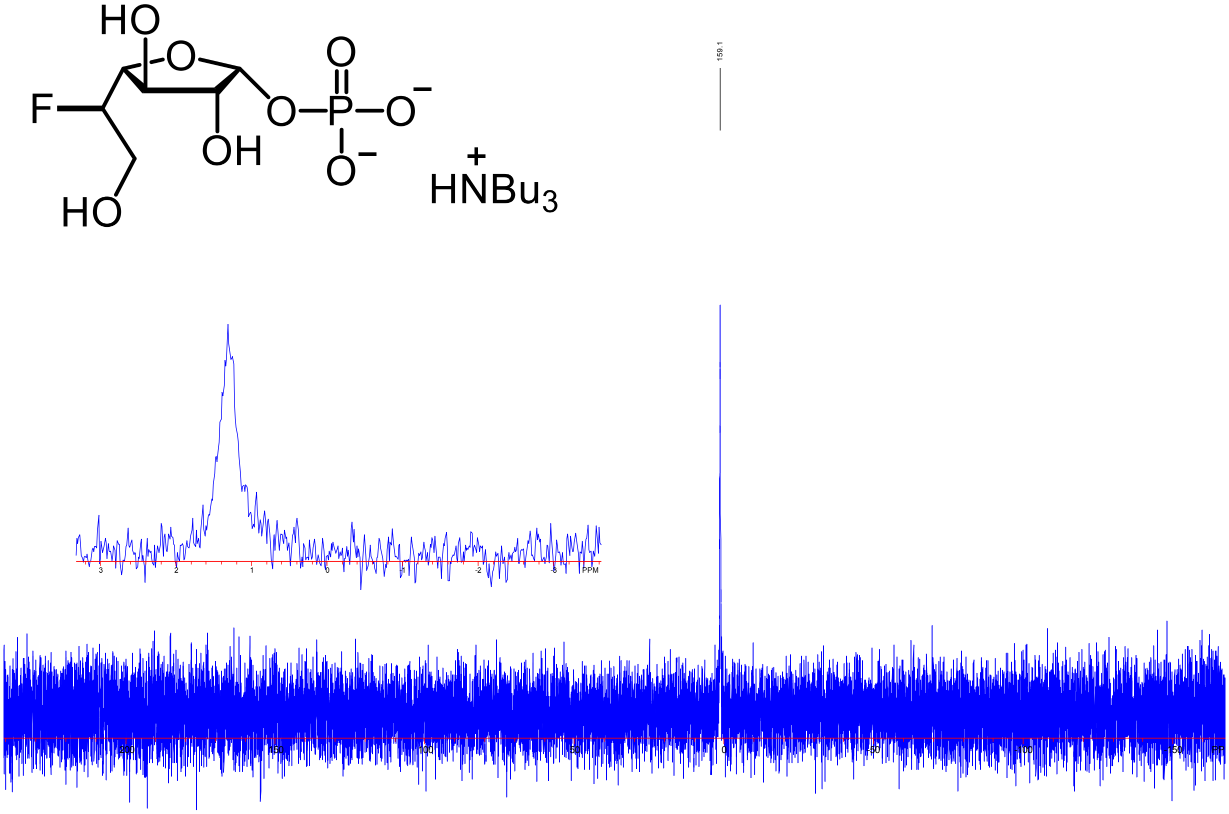
Tributylammonium 5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: 1H NMR



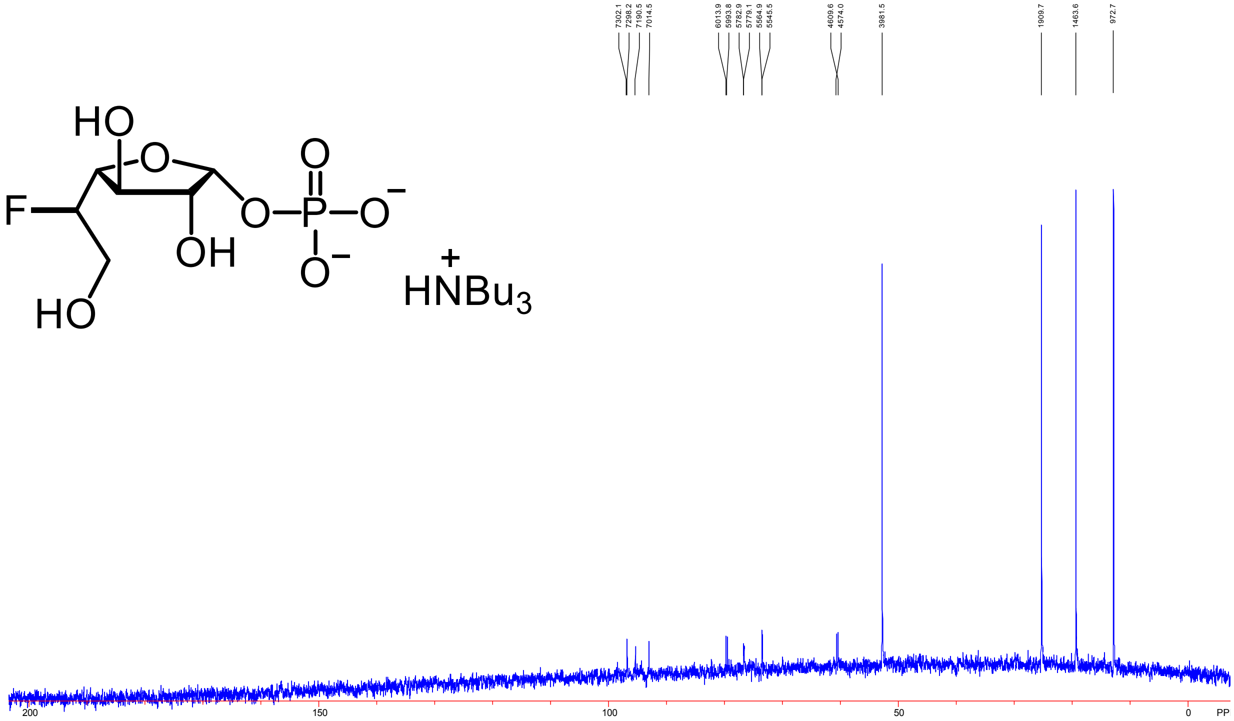
Tributylammonium 5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: ^{19}F NMR



Tributylammonium 5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: ^{31}P NMR

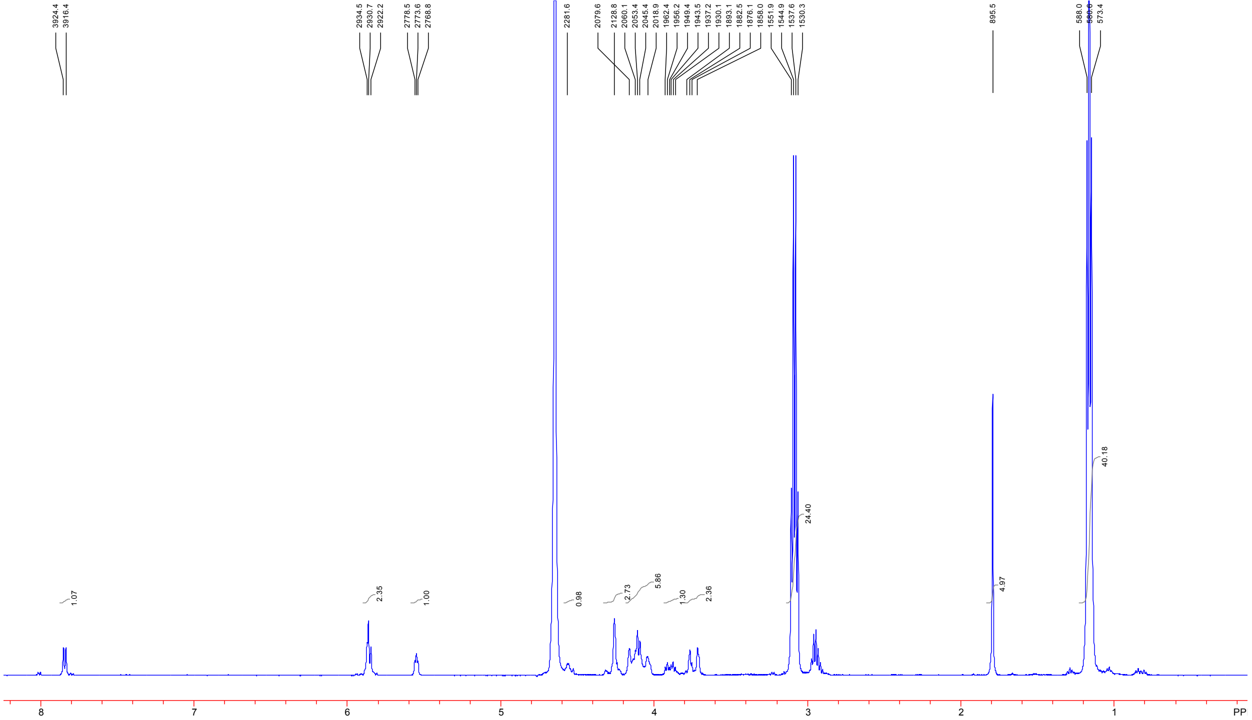


Tributylammonium 5-deoxy-5-fluoro-α-D-galactofuranose monophosphate: ^{13}C NMR

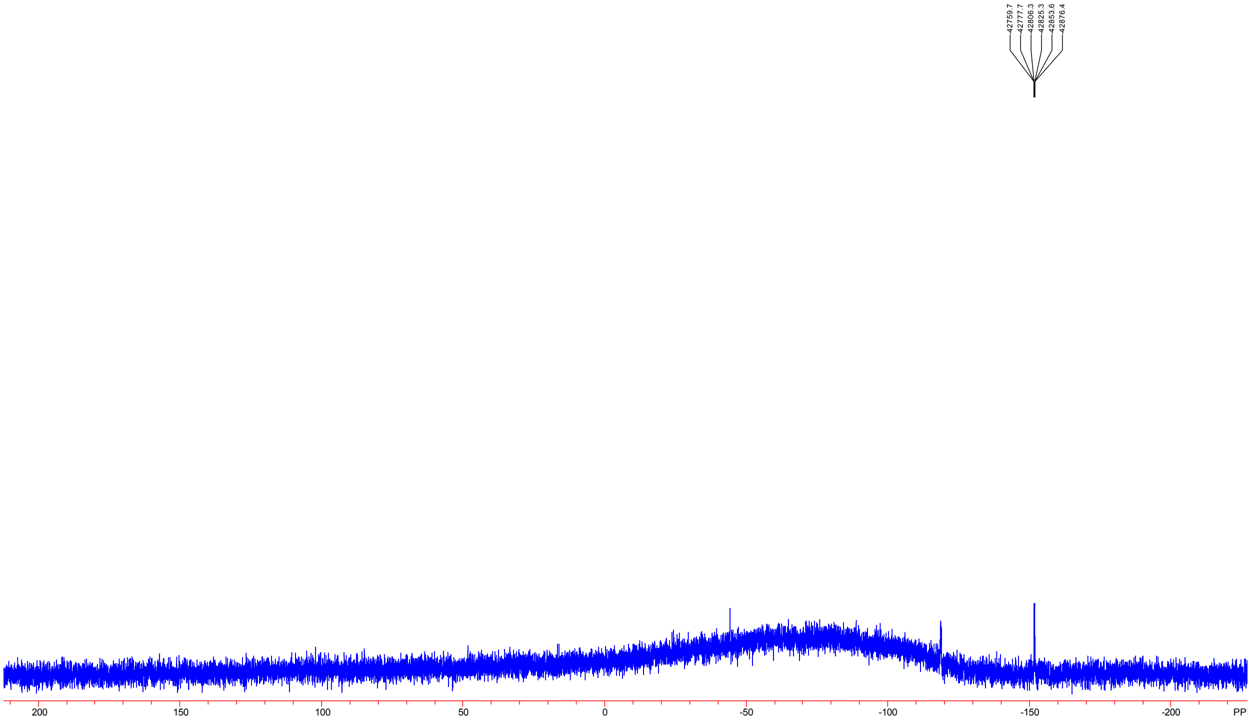


CDB-2-224C.001 (**25-C**)

UDP 5F-Galf (**3**): ^{1}H NMR



UDP 5F-Galf (**3**): ^{19}F NMR



UDP 5F-Gal*f* (**3**): ^{13}C NMR