Supporting Information for

Phosphodiesters serve as potentially tunable aglycones for 2-deoxy-2-fluoro sugar inactivators of retaining β-glycosidases

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Contents

General Experimental Procedures

General synthetic procedures: Solvents and reagents used were either reagent or spectral grade. Anhydrous solvents were prepared as follows under a nitrogen atmosphere: methanol was distilled over magnesium turnings; acetonitrile, pyridine, toluene and dichloromethane were individually distilled over calcium hydride. All solvents were used immediately after cooling to room temperature following distillation.

Synthetic reactions were monitored by TLC using Merck Kieselgel 60 F_{254} aluminum-backed sheet (thickness 0.2 mm). Compounds were detected by illumination using ultraviolet light (λ=254 mm) followed by charring with 10% ammonium molybdate in 2 M H_2SO_4 and heating. Flash chromatography was performed under positive pressure using the in-house air system on Silicycle SilicaFlash F60 (230-400 mesh) silica gel using the specified eluants. ^{1}H NMR spectra were recorded on a Bruker AV300 spectrometer at 300 MHz or a Bruker AV400 spectrometer at 400 MHz, equipped with either indirect or direct proton detection. Proton chemical shifts are reported in δ units (ppm), and are referenced to $CDCl_3$ at 7.27 ppm. ^{19}F NMR spectra were recorded on a Bruker AV300 spectrometer at 282 MHz. Fluorine chemical shifts are reported in δ units (ppm), and are referenced to $CFCl_3$ at 0 ppm. ^{31}P NMR spectra were recorded on a Bruker AV300 spectrometer at 121 MHz. Phosphorus chemical shifts are reported in δ units (ppm), and are referenced to 85% H_3PO_4 at 0 ppm. ^{13}C NMR spectra were recorded on a Bruker AV300 spectrometer at 75 MHz or a Bruker AV400 spectrometer at 100 MHz, equipped with either indirect or direct carbon detection. Carbon chemical shifts are reported in δ units (ppm), and are referenced to $CDCl_3$ at 77.23 ppm. All NMR data was processed using ACD/NMR Processor Academic Edition. Low resolution ESI mass spectrometry was performed on a Waters LC-MS equipped with an autosampler. High resolution ESI mass spectrometry was performed by the University of British Columbia Department of Chemistry Mass Spectrometry Laboratory using a Waters/Micromass LCT mass spectrometer. Scans of ^{13}C NMR spectra are not available owing to data losses from a computer crash.

<u>General procedure A for bromination</u>

The compound of interest was dissolved in an appropriate volume of 33% (by weight) HBr in acetic acid (in a ratio of 500 mg of starting material to 1 mL of HBr solution) in an open flask equipped with a drying tube on top, and allowed to stir until the reaction was complete as

determined by TLC. The solution was diluted in an appropriate volume of ethyl acetate, then washed successively with ice-cold H_2O (2x), sat. $NaHCO_3$ (1x) and sat. NaCl. The organic layer was then dried over $MgSO_4$ for 10 minutes, filtered, and concentrated under reduced pressure.

General procedure B for deprotection of esters

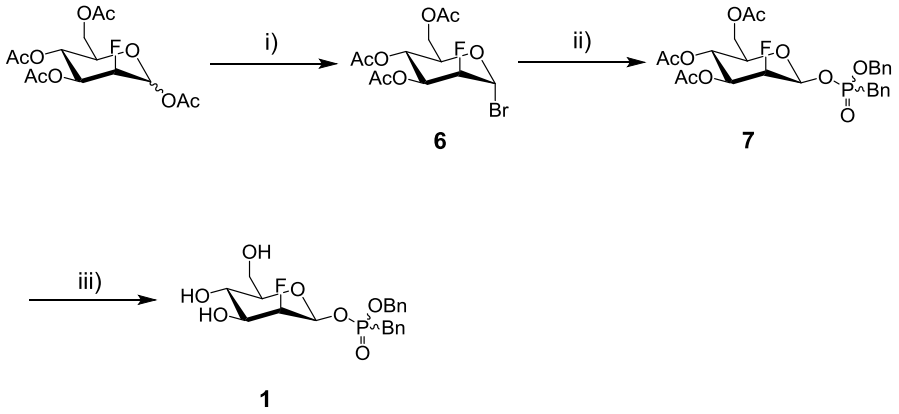
The compound of interest was dissolved in an appropriate volume of dry methanol (in a ratio of 50 mg starting material to 1 mL of methanol), and a small chunk of sodium was added. The reaction was stirred under $N_{2(g)}$ for 30 minutes. The solution was neutralized by addition of a small scoop of silica gel, and evaporated under reduced pressure to leave the crude product adsorbed onto silica gel.

General Enzymatic Procedures

All buffer chemicals and other reagents were obtained from Sigma-Aldrich Chemical Company, unless otherwise noted. 2',4'-dinitrophenyl α-D-mannopyranoside was kindly provided by Dr. Hongming Chen. β-Glucosidase from *Agrobacterium* sp (Abg) was cloned and expressed by Ms. Karen Rupitz. β-Mannosidase from *Cellulomonas fimi* (Man2A) was expressed and purified by Dr. Dominik Stoll. *E. coli* β-galactosidase (Lac-Z), Jack Bean α-mannosidase (JBAM) and Yeast α-glucosidase (Yag) were all purchased from Sigma.

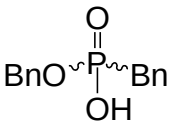
All enzymatic data analyses were performed using GraFit.[1] For all data analyses, the error bars have been omitted for clarity, although all calculated errors were ≤10% of the calculated value. All enzyme reactions were performed at 37 °C unless otherwise specified. All enzymatic buffers were made up using water from a Millipore Direct-Q™ 5 Ultrapure Water System.

Synthesis of benzyl benzyl-(2-deoxy-2-fluoro-β-D-mannopyranosyl) phosphonate (1)



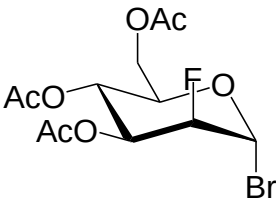
Scheme S1. Synthesis of benzyl benzyl-(2-deoxy-2-fluoro-β-D-mannopyranosyl) phosphonate (**1**). i) 33% (w/v) HBr/AcOH, 90%; ii) Benzyl benzylphosphonic acid, Ag_2CO_3, MeCN, 63%; iii) NaOMe, MeOH, 77%.

Benzyl benzylphosphonic acid



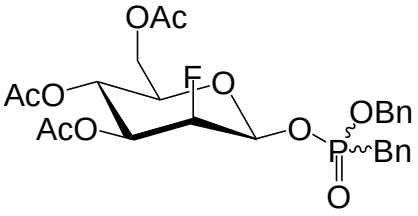
Benzyl dibenzylphosphonate (0.765 g, 2.17 mmol, 1 eq)[2] and lithium bromide (0.377 g, 4.34 mmol, 2 eq) were dissolved in 40 mL dry acetonitrile, and refluxed overnight under $N_{2(g)}$. The resulting white powder was filtered and washed with a small amount of acetonitrile. The powder was dissolved in 15 mL methanol, and acidified by stirring with Amberlite IR-120 (H+) resin for 10 minutes. The resin was filtered off and the filtrate collected. The solvent was evaporated under reduced pressure to yield benzyl benzylphosphonic acid as a colourless oil (0.541 g, 2.06 mmol, 95%). The compound was used without further characterization or purification.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl bromide **(6)**



3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-mannopyranose (0.518 g, 1.48 mmol)[3] was brominated according to General Procedure A overnight. The crude product (0.494 g, 1.33 mmol, 90%) was used without further purification or characterization.

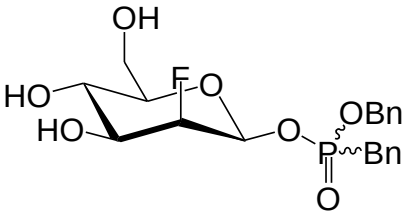
Benzyl benzyl-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-β-D-mannopyranosyl) phosphonate **(7)**



6 (0.125 g, 0.337 mmol) was dissolved in 5 mL dry acetonitrile. The crude benzyl benzylphosphonic acid (0.139 g, 0.518 mmol, 1.5 eq) was dissolved in 1 mL dry acetonitrile and added, along with silver carbonate (0.325 g, 1.18 mmol, 3.5 eq) to the solution **6**. The resulting slurry was stirred for three days under $N_{2(g)}$, in the dark. The solution was filtered through a short plug of silica using ethyl acetate as the eluent to remove the silver salts. The filtrate was collected and the solvent evaporated under reduced pressure. The product was purified by flash chromatography (1:1 petroleum ether : ethyl acetate) to yield **7**, as a mixture of diastereomers, as a colourless oil (0.118 g, 0.214 mmol, 63%). **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 7.33-7.22 (20 H, m, Ar-*H*) 5.45-5.25 (4 H, m, $H1_a$, $H1_b$, $H3_a$, $H3_b$), 5.10-4.94 (6 H, m, $H4_a$, $H4_b$, OCH_2Ph_a, OCH_2Ph_b), 4.50-4.05 (6 H, m, $H2_a$, $H2_b$, $H6_a$, $H6_b$, $H6'_a$, $H6'_b$), 3.86-3.74 (2 H, m, $H5_a$, $H5_b$), 3.35-3.21 (4 H, m, PCH_2Ph_a, PCH_2Ph_b), 2.08 (3 H, s, Ac), 2.07 (6 H, s, 2 x Ac), 2.03 (3 H, s, Ac), 2.02 (3 H, s, Ac), 1.97 (3 H, s, Ac); **^{19}F NMR:** (282 MHz, $CDCl_3$): δ -199.97-200.30 (2 F,

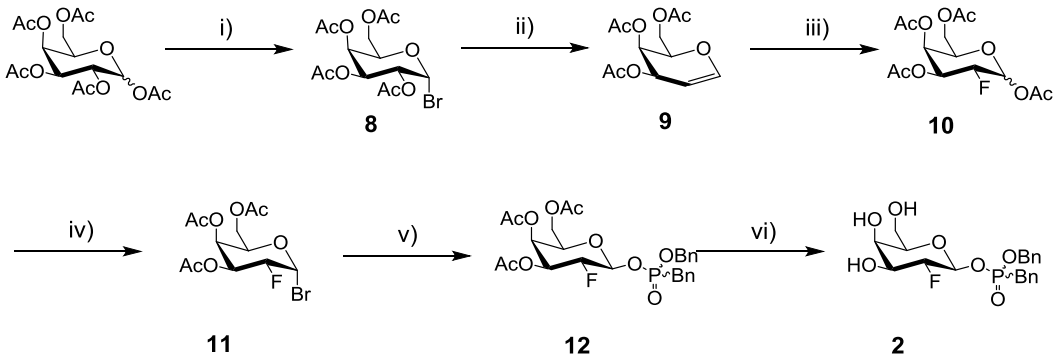
m, $F2_a$, $F2_b$); **^{31}P (^{1}H decoupled) NMR:** (121 MHz, $CDCl_3$): δ 28.10, 27.98; **ESI-MS (high res):** m/z calc.: 575.1453; **Found:** 575.1456 $[M + Na]^+$.

Benzyl benzyl-(2-deoxy-2-fluoro-β-D-mannopyranosyl) phosphonate **(1)**



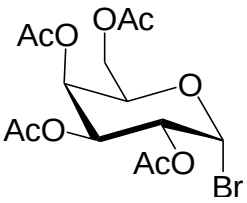
7 (35.1 mg, 0.0635 mmol) was dissolved in 5 mL dry methanol, and deacetylated according to General Procedure B. The crude product was purified by flash chromatography (19:1 ethyl acetate : methanol) to yield **1**, as a mixture of diastereomers, as a colourless oil (20.9 mg, 0.0490 mmol, 77%). **^{1}H NMR:** (300 MHz, CD_3OD) δ 7.30-7.27 (20 H, m, Ar-H), 5.31-5.21 (2 H, m, $H1_a$, $H1_b$), 5.07 (2 H, d, J_{H-P} 7.7 Hz, OC$\underline{H_2}$Ph$_a$), 5.03 (2 H, d, J_{H-P} 8.0 Hz, OC$\underline{H_2}$Ph$_b$) 4.23-3.99 (2 H, m, $H2_a$, $H2_b$), 3.88-3.84 (2 H, m, $H3_a$, $H3_{b)}$), 3.72-3.58 (4 H, m, H_{4a}, H_{4b}, H_{5a}, H_{5b}) 3.43- 3.30 (8 H, m, $H6_a$, $H6_b$, $H6'_a$, $H6'_b$, PC$\underline{H_2}$Ph$_a$, PC$\underline{H_2}$Ph$_b$); **^{19}F NMR:** (282 MHz, CD_3OD): δ -(201.00-201.34) (2F, m, F_{2a}, F_{2b}); **^{31}P (^{1}H decoupled) NMR:** (121 MHz, CD_3OD): δ 28.93, 28.52; **ESI-MS (high res):** m/z calc.: 449.1136; **Found:** 449.1134 $[M + Na]^+$; **Anal. calc. for** $C_{20}H_{24}FO_7P$: C, 56.34, H, 5.67; **Found:** C, 56.12, H, 5.78.

Synthesis of benzyl benzyl-(2-deoxy-2-fluoro-β-D-galactopyranosyl) phosphonate (2)



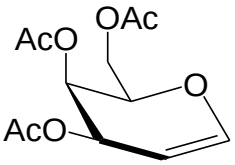
Scheme S2. Synthesis of benzyl benzyl-(2-deoxy-2-fluoro-β-D-galactopyranosyl) phosphonate (**2**). i) 33% (w/v) HBr/AcOH; ii) Zn, AcOH, H_2O, 48% over two steps; iii) Selectfluor^{TM}, MeCN, AcOH, 21%; iv) 33% (w/v) HBr/AcOH; v) Benzyl benzylphosphonic acid, Ag_2CO_3, MeCN, 80% from **10**; vi) NaOMe, MeOH, 98%.

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide **(8)**



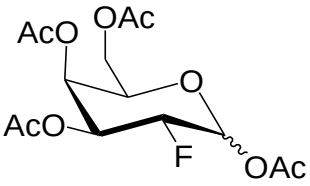
1,2,3,4,6-Penta-*O*-acetyl-D-galactopyranoside (7.68 g, 19.7 mmol) was treated according to General Procedure A (15 mL, 2 hours) to yield, after work-up, crude **8** as a colourless gum. The product was used without further characterization or purification.

3,4,6-Tri-*O*-acetyl-D-galactal **(9)**



8 was dissolved in 300 mL of 1:1 acetic acid : water. Zinc (14.5 g, 0.222 mmol, 11 eq) was added, and the resulting slurry mechanically stirred at 0 °C overnight. The solution was filtered through Celite to remove the solids, and the solvent removed under reduced pressure. The product was purified by flash chromatography (4:1 petroleum ether : ethyl acetate) to yield **9** as a white solid (2.54 g, 9.33 mmol, 48% from1,2,3,4,6-penta-*O*-acetyl-D-galactopyranoside). **^{1}H NMR:** (400 MHz, $CDCl_3$) δ 6.39 (1 H, d, $J_{H1\text{-}H2}$ 6.6 Hz, H1), 5.48 (1 H, m, H3), 5.35 (1 H, m, H4), 4.66 (1 H, ddd, $J_{H5\text{-}H4}$ 6.3 Hz, $J_{H5\text{-}H6}$ 2.6 Hz, $J_{H5\text{-}H6'}$ 1.5 Hz, H5), 4.26 (1 H, t, $J_{H2\text{-}H1} = J_{H2\text{-}H3}$ 6.6 Hz, H2), 4.22-4.12 (2 H, m, H6, H6'), 2.05 (3 H, s, Ac), 2.01 (3 H, s, Ac), 1.95 (3 H, s, Ac).

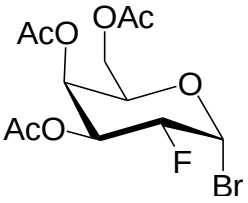
1,3,4,6-Tetra-*O*-acetyl -2-deoxy-2-fluoro-D-galactopyranose **(10)**



9 (0.845 g, 3.10 mmol) was dissolved in 60 mL of 2:1 acetonitrile : acetic acid and stirred at 70 °C under $N_{2(g)}$. To this solution was added Selectfluor (1.39 g, 3.93 mmol, 1.25 eq) and the mixture allowed to stir for 2.5 hours before being cooled to room temperature. Following solvent evaporation under reduced pressure, the residue was dissolved in ethyl acetate and washed with 2 x H_2O, 1 x saturated $NaHCO_3$, 1 x brine and dried over $MgSO_4$. After 10 minutes, the $MgSO_4$ was filtered off, and the solvent evaporated under reduced pressure. The product was purified by column chromatography (4:1 hexane : ethyl acetate) to yield **10** (3:2 β:α) as a colourless oil (0.224 g, 0.639 mmol, 21%). **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 6.39 (1 H, dd, $J_{H1\alpha\text{-}H2\alpha}$ 48.5 Hz, $J_{H1\alpha\text{-}H2\alpha}$ 3.9 Hz, H1α), 5.74 (1 H, dd, $J_{H1\beta\text{-}F2\beta}$ 8.0 Hz, $J_{H1\beta\text{-}H2\beta}$ 4.1 Hz, H2β), 5.45-5.09 (4 H, m, H3α, H3β, H4α, H4β), 4.85 (1 H, ddd, $J_{H2\alpha\text{-}F2\alpha}$ 49.1 Hz, $J_{H2\alpha\text{-}H3\alpha}$ 10.2 Hz, $J_{H2\alpha\text{-}H1\alpha}$ 4.0 Hz, H2α), 4.57 (1 H, ddd, $J_{H2\beta\text{-}F2\beta}$ 51.6 Hz, $J_{H2\beta\text{-}H3\beta}$ 9.6 Hz, $J_{H2\beta\text{-}H1\beta}$ 4.1 Hz, H2β), 4.27-4.00 (6 H, m, H5α, H5β, H6α, H6β, H6'α, H6'β), 2.12 (6 H, s, 2 x Ac), 2.11 (6 H, s, 2 x Ac), 2.08 (3 H, s, Ac), 1.99

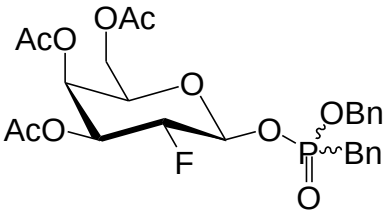
(3 H, s, Ac), 1.97 (3 H, s, Ac), 1.96 (3 H, s, Ac) **^{19}F NMR:** (282 MHz, $CDCl_3$): δ -208.5 (1 F, ddd, $J_{F2\beta\text{-}H2\beta}$ 51.6 Hz, $J_{F2\beta\text{-}H3\beta}$ 14.4 Hz, $J_{F2\beta\text{-}H1\beta}$ 8.0 Hz, F2β), -202.63 (1 F, ddd, $J_{F2\alpha\text{-}H1\alpha}$ 48.5 Hz, $J_{F2\alpha\text{-}H3\alpha}$ 49.1 Hz, $J_{F2\alpha\text{-}H3\alpha}$ 12.4 Hz, $J_{F2\alpha\text{-}H1\alpha}$ 3.9 Hz, F2α).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl bromide **(11)**



10 (0.104 g, 0.297 mmol) was treated according to General Procedure A (3 mL,4 hours) to yield, after work-up, crude **11** as a colourless gum. The product was used without further characterization or purification.

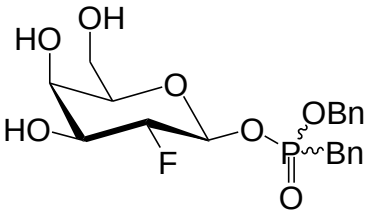
Benzyl benzyl-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-β-D-galactopyranosyl) phosphonate **(12)**



11 was dissolved in 5 mL dry acetonitrile. Benzyl benzylphosphonic acid (0.180 g, 0.686 mmol, 2.3 eq) was dissolved in 2 mL dry acetonitrile and added, along with silver carbonate (0.351 g, 1.27 mmol, 4.3 eq) to the solution of **11**. The resulting slurry was stirred overnight under $N_{2(g)}$, in the dark. The solution was filtered through a short plug of silica using ethyl acetate as the eluent to remove the silver salts. The filtrate was collected and the solvent evaporated under reduced pressure. The product was purified by flash chromatography (1:1 → 1:2 petroleum ether : ethyl acetate) to yield **12**, as a mixture of diastereomers, as a colourless oil (0.130 g, 0.235 mmol, 80% from **10**). **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 5.45-5.25 (4 H, m, $H1_a$, $H1_b$, $H3_a$, $H3_b$), 5.09-4.94 (6 H, m, $H4_a$, $H4_b$, $OC\underline{H_2}Ph_a$, $OC\underline{H_2}Ph_b$), 4.48-4.05 (6 H, m, $H2_a$, $H2_b$, $H6_a$, $H6_b$, $H6'_a$, $H6'_b$), 3.84-

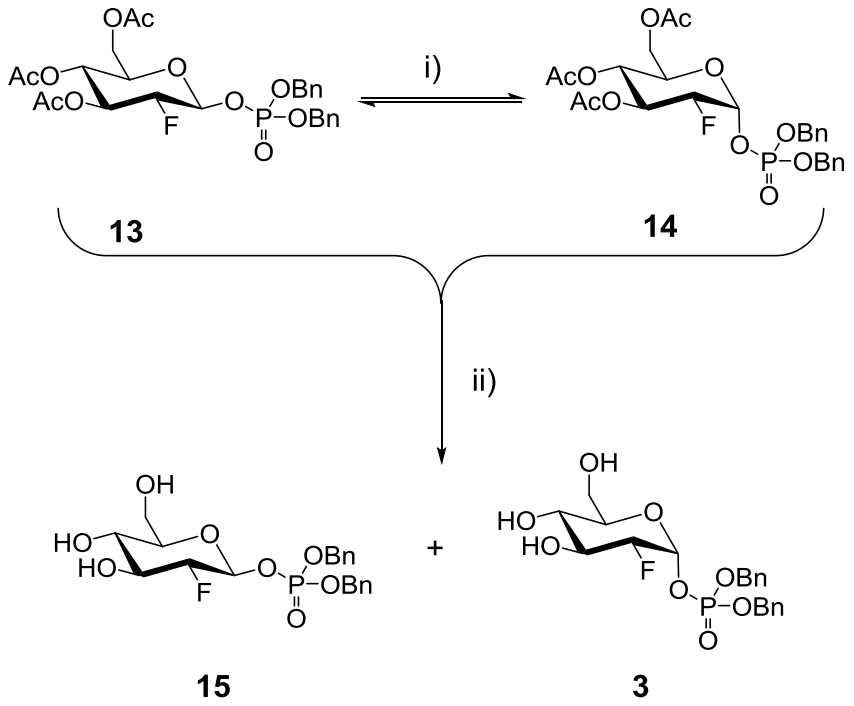
3.75 (2 H, m, H5$_a$, H5$_{b)}$), 3.32-3.22 (4 H, m, PC$\underline{H_2}$Ph$_a$, PC$\underline{H_2}$Ph$_b$) 2.08 (9 H, s, 3 x Ac), 2.07 (6 H, s, 2 x Ac), 2.03 (3 H, s, Ac); **^{19}F NMR:** (282 MHz, $CDCl_3$): δ -199.98-200.30 (2 F, m, F2$_a$, F2$_b$); **^{31}P (^{1}H decoupled) NMR:** (121 MHz, $CDCl_3$): δ 28.08, 27.96; **ESI-MS (high res):** m/z calc.: 575.1458; **Found:** 575.1453 $[M + Na]^+$.

Benzyl benzyl-(2-deoxy-2-fluoro-β-D-galactopyranosyl) phosphonate **(2)**



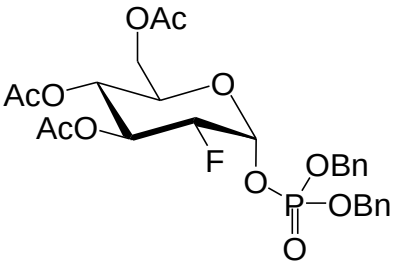
12 (21.9 mg, 0.0396 mmol) was dissolved in 5 mL dry methanol, and deacetylated according to General Procedure B. The crude product was purified by flash chromatography (9:1 ethyl acetate : methanol) to yield pure **2**, as a mixture of diastereomers at phosphorus, as a colourless oil (16.6 mg, 0.0389 mmol, 98%). **^{1}H NMR:** (300 MHz, CD_3OD) δ 7.30-7.25 (20 H, m, Ar-H), 5.30-5.21 (2 H, m, H1$_a$, H1$_b$), 5.08 (2 H, d, J_{H-P} 7.7 Hz, OC$\underline{H_2}$Ph$_a$), 5.04 (2 H, d, J_{H-P} 8.0 Hz, OC$\underline{H_2}$Ph$_b$) 4.23-3.99 (2 H, m, H2$_a$, H2$_b$), 3.88-3.84 (2 H, m, H3$_a$, H3$_{b)}$), 3.70-3.54 (4 H, m, H$_{4a}$, H$_{4b}$, H$_{5a}$, H$_{5b}$) 3.43- 3.32 (8 H, m, H6$_a$, H6$_b$, H6'$_a$, H6'$_b$, PC$\underline{H_2}$Ph$_a$, PC$\underline{H_2}$Ph$_b$); **^{19}F NMR:** (282 MHz, CD_3OD): δ -(209.60-209.95) (2 F, m, F$_{2a}$, F$_{2b}$); **^{31}P (^{1}H decoupled) NMR:** (121 MHz, CD_3OD): δ 28.87, 28.45; **ESI-MS (high res):** m/z calc.: 449.1136; **Found:** 465.1133 $[M + Na]^+$; **Anal. calc. for** $C_{20}H_{24}FO_7P$: C, 56.34, H, 5.67; **Found:** C, 56.26, H, 5.72.

Synthesis of benzyl benzyl-(2-deoxy-2-fluoro-α-D-glucopyranosyl) phosphate (3)



Scheme S3. Synthesis of dibenzyl (2-deoxy-2-fluoro-α-D-glucopyranosyl) phosphate (**3**). i) Dibenzyl phosphoric acid, toluene, 22%; ii) NaOMe, MeOH, 77%.

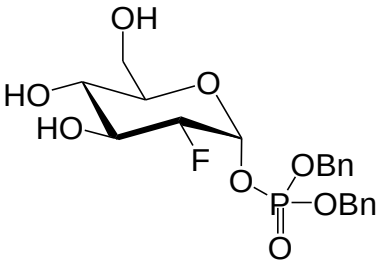
Dibenzyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl) phosphate (**14**)



Dibenzyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl) phosphate (**13**) (0.463 g, 0.814 mmol)[4] was dissolved in 10 mL dry toluene. Dibenzyl phosphoric acid (0.450 g, 1.62 mmol, 2.0 eq) was added and the solution stirred under $N_{2(g)}$ at 110 °C for 2 days. The solvent was then evaporated under reduced pressure, and the product was partially purified by flash

chromatography (3:1 → 2:1 → 1:1 hexanes : ethyl acetate) to yield an inseparable mixture of 1:4 **13** to **14** as a colourless oil (0.149 g, 0.262 mmol, 22%). **13**: Characterization previously described.[4] **14**: **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 7.34-7.32 (10 H, m, Ar-H), 5.95 (1 H, dd, J_{H1-P} 6.7 Hz, J_{H1-H2} 3.6 Hz, H1), 5.51 (1 H, dt, J_{H3-F2} 11.9 Hz, $J_{H3-H2} = J_{H3-H4}$ 9.6 Hz, H3), 5.09-4.99 (5 H, m, H4, 2 x OC$\underline{H_2}$Ph), 4.56 (1 H, ddt, J_{H2-F2} 48.6 Hz, J_{H2-H3} 9.6 Hz, $J_{H2-H1} = J_{H2-P}$ 3.6 Hz, H2), 4.14 (1 H, dd, $J_{H6-H6'}$ 12.6 Hz, J_{H6-H5} 4.0 Hz, H6), 4.01 (1 H, ddd, J_{H5-H4} 9.4 Hz, J_{H5-H6} 4.0 Hz, $J_{H5-H6'}$ 2.0 Hz, H5), 3.85 (1 H, dd, $J_{H6'-H6}$ 12.6 Hz, $J_{H6'-H5}$ 2.0 Hz, H6'), 2.06 (3 H, s, Ac), 2.01 (3 H, s, Ac), 1.97 (3 H, s, Ac); **^{19}F NMR:** (282 MHz, $CDCl_3$): δ -200.81 (1 F, dd, J_{F2-H2} 48.6 Hz, J_{F2-H3} 11.9 Hz, F2); **^{31}P (^{1}H decoupled) NMR:** (121 MHz, $CDCl_3$): δ -2.10; **^{13}C NMR:** (75 MHz, $CDCl_3$) δ 170.54, 169.92, 169.60 (3 x C=O), 135.53-135.30 (2 C, m), 128.78 (2 C), 128.73 (2 C), 128.70 (2 C), 128.69 (2 C), 128.07 (2 C) (12 x Ar), 95.99 (dd, J_{C1-F2} 24 Hz, J_{C1-P} 5 Hz, C1), 89.37 (dd, J_{C2-F2} 192 Hz, J_{C2-P} 9 Hz, C2), 72.78 (C5), 72.42 (d, J_{C3-F2} 19 Hz, C3), 69.87 (d, J_{C-P} 8 Hz, O$\underline{C}$H$_2$Ph), 69.80 (d, J_{C-P} 8 Hz, O$\underline{C}$H$_2$Ph), 67.71 (d, J_{C4-F2} 7 Hz, C4), 61.50 (C6), 20.72, 20.65, 20.31 (3 x Ac); **ESI-MS (high res):** m/z calc.: 591.4712; **Found:** 591.4718 $[M + Na]^+$.

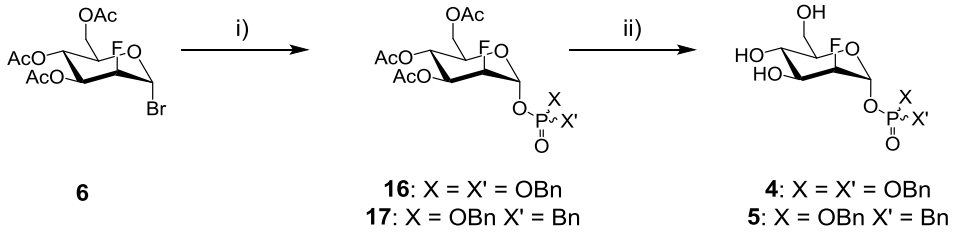
Dibenzyl (2-deoxy-2-fluoro-α-D-glucopyranosyl) phosphate (**3**)



The impure **14** (51.9 mg, 0.0913 mmol) was dissolved in 10 mL dry methanol, and deacetylated according to General Procedure B. The crude product was purified by flash chromatography (50:1 ethyl acetate : methanol) to yield a 1:19 mixture of **15** to **3** as a colourless oil (31.0 mg, 0.0701 mmol, 77%). **15**: Characterization previously described.[4] **^{1}H NMR:** (300 MHz, CD_3OD) δ 7.36-7.33 (10 H, m, Ar-H), 5.92 (1 H, dd, J_{H1-P} 6.1 Hz, J_{H1-H2} 3.7 Hz, H1), 5.11-5.08 (2 H, m, 2 x OC$\underline{H_2}$Ph), 4.38 (1 H, ddd, J_{H2-F2} 48.5 Hz, J_{H2-H3} 9.5 Hz, J_{H2-H1} 3.7 Hz, H2), 3.85 (1 H, dt, J_{H3-F2} 12.9 Hz, $J_{H3-H2} = J_{H3-H4}$ 9.5 Hz, H3), 3.73-3.63 (2 H, m, H4, H5), 3.53-3.41 (2 H, m, H6, H6');

^{19}F NMR: (282 MHz, CD_3OD): δ -202.20 (1 F, dd, J_{F2-H2} 48.5 Hz, J_{F2-H3} 12.9 Hz, F2); **^{31}P (^{1}H decoupled) NMR:** (121 MHz, CD_3OD): δ -2.35; **^{13}C NMR:** (100 MHz, CD_3OD) δ 134.64 (2 C, d, J_{C-P} 7 Hz), 128.82, 128.75, 128.71 (2 C), 128.61 (2 C), 128.11 (4 C) (12 x Ar), 96.41 (dd, J_{C1-F2} 24 Hz, J_{C1-P} 7 Hz, C1), 88.92 (dd, J_{C2-F2} 194 Hz, J_{C2-P} 8 Hz, C2), 77.54 (d, J_{C3-F2} 18 Hz, C3), 74.62 (d, J_{C4-F2} 7 Hz, C4), 72.58 (C5), 70.29 (d, J_{C-P} 10 Hz, O<u>C</u>H_2Ph), 70.26 (d, J_{C-P} 8 Hz, O<u>C</u>H_2Ph), 60.73 (C6); **ESI-MS (high res):** m/z calc.: 465.1085; **Found:** 465.1078 $[M + Na]^+$; **Anal. calc. for** $C_{20}H_{24}FO_8P$: C, 54.30, H, 5.47; **Found:** C, 54.22, H, 5.56.

Synthesis of dibenzyl (2-deoxy-2-fluoro-α-D-mannopyranosyl) phosphate (4) and benzyl benzyl-(2-deoxy-2-fluoro-α-D-mannopyranosyl) phosphonate (5)



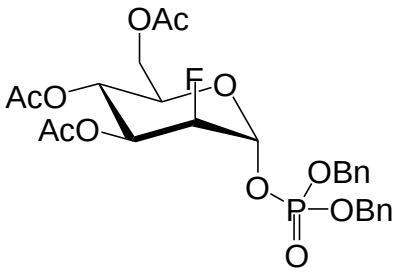
6

16: X = X' = OBn
17: X = OBn X' = Bn

4: X = X' = OBn
5: X = OBn X' = Bn

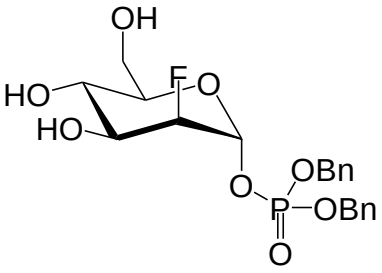
Scheme S4. Synthesis of dibenzyl (2-deoxy-2-fluoro-α-D-mannopyranosyl) phosphate (**4**) and benzyl benzyl-(2-deoxy-2-fluoro-α-D-mannopyranosyl) phosphonate (**5**). i) Dibenzyl phosphoric acid, toluene (for **16**) or benzyl benzylphosphonic acid, MeCN (for **17**), Ag_2CO_3, **16**: 7%, **17**: 37%; ii) NaOMe, MeOH, **4**: 76%, **5**: 83%.

Dibenzyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl) phosphate **(16)**



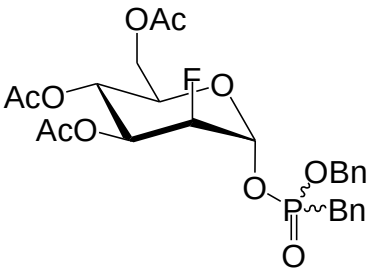
6 (0.494 g, 1.33 mmol) was dissolved in 10 mL dry toluene. Dibenzyl phosphoric acid (0.617 g, 2.22 mmol, 1.7 eq) and silver carbonate (0.769 g, 2.79 mmol, 1.9 eq) were added to the toluene solution. The resulting slurry was stirred overnight under $N_{2(g)}$, in the dark. The solution was filtered through a short plug of silica using ethyl acetate as the eluent to remove the silver salts. The filtrate was collected and the solvent evaporated under reduced pressure. The product was purified by flash chromatography (9:1 toluene : acetone) to yield **16** as a colourless oil (0.0547 g, 0.0962 mmol, 7%) along with substantial amounts of hydrolysis product, 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranose (0.324 g, 1.05 mmol, 79%) . **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 7.36-7.35 (10 H, m, Ar-H), 5.73 (1 H, br td, J_{H1-P} 6.4 Hz, J_{H1-F2} 5.5 Hz, J_{H1-H2} 1.9 Hz, H1), 5.32 (1 H, t, $J_{H4-H3} = J_{H4-H5}$ 10.0 Hz, H4), 5.17 (1 H, ddd, J_{H3-F2} 27.6 Hz, J_{H3-H4} 10.0 Hz, J_{H3-H2} 2.5 Hz, H3), 5.11-5.07 (4 H, m, 2 x OC*H*$_2$Ph), 4.54 (1 H, br dt, J_{H2-F2} 48.9 Hz, J_{H2-H3} 2.5 Hz, J_{H2-H1} 1.9 Hz, H2), 4.16 (1 H, dd, $J_{H6-H6'}$ 12.3 Hz, J_{H6-H5} 4.1 Hz, H6), 3.99 (1 H, ddd, J_{H5-H4} 10.0 Hz, J_{H5-H6} 4.1 Hz, $J_{H5-H6'}$ 2.1 Hz, H5), 3.93 (1 H, dd, $J_{H6'-H6}$ 12.3 Hz, $J_{H6'-H5}$ 2.1 Hz, H6'), 2.09 (3 H, s, Ac), 2.03 (3 H, s, Ac), 2.00 (3 H, s, Ac); **^{19}F NMR:** (282 MHz, $CDCl_3$): δ -203.69 (1 F, ddd, J_{F2-H2} 48.9 Hz, J_{F2-H3} 27.6 Hz, J_{F2-H1} 5.5 Hz, F2); **^{31}P (^{1}H decoupled) NMR:** (121 MHz, $CDCl_3$): δ -2.38; **^{13}C NMR:** (75 MHz, $CDCl_3$) δ 170.71, 170.02, 169.43 (3 x C=O), 135.38, 135.31, 128.04 (3 C), 128.90 (3 C), 128.37 (2 C), 128.23 (2 C), (12 x Ar), 94.46 (dd, J_{C1-F2} 32 Hz, J_{C1-P} 5 Hz, C1), 88.78 (dd, J_{C2-F2} 196 Hz, J_{C2-P} 8 Hz, C2), 70.40 (C5), 70.19 (d, J_{C-P} 10 Hz, O*C*H$_2$Ph), 70.09 (d, J_{C-P} 6 Hz, O*C*H$_2$Ph), 69.00 (d, J_{C3-F2} 17 Hz, C3), 65.08 (C4), 61.55 (C6), 20.71, 20.70 (2 C), (3 x Ac); **ESI-MS (high res):** m/z calc.: 591.4712; **Found:** 591.4710 $[M + Na]^+$.

Dibenzyl (2-deoxy-2-fluoro-α-D-mannopyranosyl) phosphate **(4)**



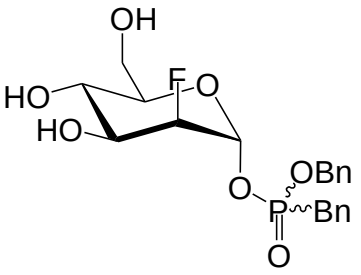
16 (14.9 mg, 0.0262 mmol) was dissolved in 2 mL dry methanol, and deacetylated according to General Procedure B. The crude product was purified by flash chromatography (50:1 ethyl acetate : methanol) to yield pure **4** as a colourless oil (8.8 mg, 0.020 mmol, 76%). **^{1}H NMR:** (300 MHz, CD_3OD) δ 7.37 (10 H, m, Ar-H), 5.71 (1 H, td, $J_{H1-F2} = J_{H1-P}$ 6.2 Hz, J_{H1-H2} 1.9 Hz, H1), 5.11 (2 H, d, J_{H-P} 8.6 Hz, OC*H*$_2$Ph), 5.10 (d, J_{H-P} 9.6 Hz, OC*H*$_2$Ph), 4.46 (1 H, dt, J_{H2-F2} 48.9 Hz, $J_{H2-H3} = J_{H2-H1}$ 1.9 Hz, H2), 3.71-3.60 (5 H, m, H3, H4, H5, H6, H6'); **^{19}F NMR:** (282 MHz, CD_3OD): δ -205.90 (1 F, ddd, J_{F2-H2} 48.9 Hz, J_{F2-H3} 22.6 Hz, J_{F2-H1} 6.2 Hz, F2); **^{31}P (^{1}H decoupled) NMR:** (121 MHz, CD_3OD): δ -2.54; **^{13}C NMR:** (75 MHz, CD_3OD) δ 134.34 (2 C), 128.43 (2 C), 128.41 (2 C), 128.39 (2 C), 128.24 (2 C), 128.11 (2 C) (12 x Ar), 94.21 (dd, J_{C1-F2} 30 Hz, J_{C1-P} 6 Hz, C1), 88.94 (dd, J_{C2-F2} 198 Hz, J_{C2-P} 8 Hz, C2), 77.65 (d, J_{C3-F2} 19 Hz, C3), 75.15 (d, J_{C4-F2} 5 Hz, C4), 72.48 (C5), 70.43 (d, J_{C-P} 9 Hz, O*C*H$_2$Ph), 70.39 (d, J_{C-P} 7 Hz, O*C*H$_2$Ph), 60.73 (C6); **ESI-MS (high res):** m/z calc.: 465.1085; **Found:** 465.1082 $[M + Na]^+$; **Anal. calc. for** $C_{20}H_{24}FO_8P$: C, 54.30, H, 5.47; **Found:** C, 54.14, H, 5.59.

Benzyl benzyl-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl) phosphonate **(17)**



6 (0.479 g, 1.29 mmol) was dissolved in 10 mL dry acetonitrile. Benzyl benzylphosphonic acid (0.307 g, 1.83 mmol, 1.4 eq) was dissolved in 2 mL dry acetonitrile and added, along with silver carbonate (0.452 g, 1.64 mmol, 1.3 eq) to the solution of **6**. The resulting slurry was stirred overnight under $N_{2(g)}$, in the dark. The solution was filtered through a short plug of silica using ethyl acetate as the eluent to remove the silver salts. The filtrate was collected and the solvent evaporated under reduced pressure. The product was purified by flash chromatography (1:1 petroleum ether : ethyl acetate) to yield **17**, as a mixture of diastereomers at the phosphorus, as a colourless oil (0.267 g, 0.483 mmol, 37%). **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 7.31-7.26 (20 H, m, Ar-*H*) 5.79-5.63 (2 H, m, $H1_a$, $H1_b$), 5.31-4.97 (8 H, m, $H3_a$, $H3_b$, $H4_a$, $H4_b$, OCH_2Ph_a, OCH_2Ph_b), 4.52-3.83 (8 H, m, $H2_a$, $H2_b$, $H5_a$, $H5_b$, $H6_a$, $H6_b$, $H6'_a$, $H6'_b$), 3.31-3.13 (4 H, m, PCH_2Ph_a, PCH_2Ph_b), 2.08 (3 H, s, Ac), 2.07 (6 H, s, 2 x Ac), 2.01 (3 H, s, Ac), 1.97 (6 H, s, 2 x Ac); **^{19}F NMR:** (282 MHz, $CDCl_3$): δ -(203.25-203.92) (2 F, m, $F2_a$, $F2_b$); **^{31}P (^{1}H decoupled) NMR:** (121 MHz, $CDCl_3$): δ 27.71, 27.25; **ESI-MS (high res):** m/z calc.: 575.1453; **Found:** 575.1448 $[M + Na]^+$.

Benzyl benzyl-(2-deoxy-2-fluoro-α-D-mannopyranosyl) phosphonate **(5)**

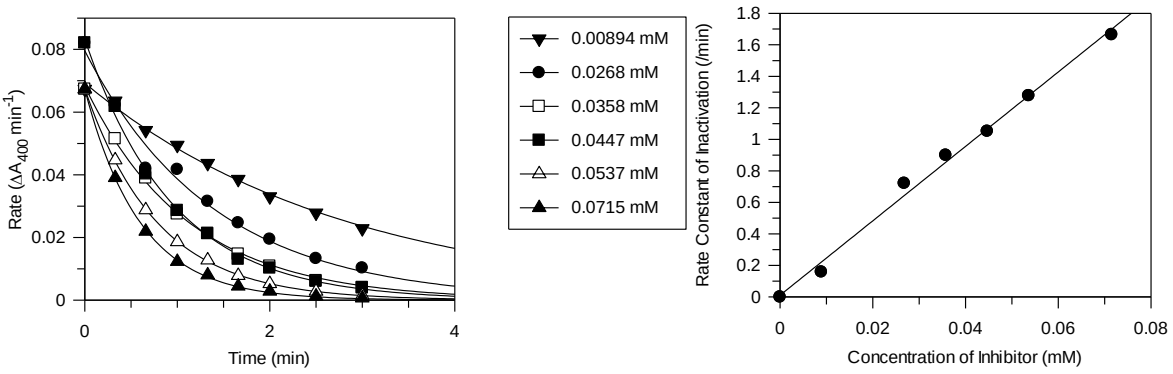


17 (56.5 mg, 0.133 mmol) was dissolved in 5 mL dry methanol, and deacetylated according to General Procedure B. The crude product was purified by flash chromatography (9:1 ethyl acetate : methanol) to yield pure **5**, as a mixture of diastereomers at the phosphorus, as a colourless oil (36.2 mg, 0.851 mmol, 83%). **^{1}H NMR:** (300 MHz, $CDCl_3$) δ 7.33-7.29 (20 H, m, Ar-*H*) 5.70-5.67 (2 H, m, $H1_a$, $H1_b$), 5.10-5.04 (2 H, m, $H4_a$, $H4_b$), 4.86-4.81 (4 H, m, OCH_2Ph_a, OCH_2Ph_b) 4.48-4.25 (2 H, m, $H2_a$, $H2_b$), 3.81-3.30 (12 H, m, $H3_a$, $H3_b$, $H5_a$, $H5_b$, $H6_a$, $H6_b$, $H6'_a$, $H6'_b$, PCH_2Ph_a, PCH_2Ph_b); **^{19}F NMR:** (282 MHz, $CDCl_3$): δ -(205.30-205.90) (2 F, m, $F2_a$, $F2_b$); **^{31}P (^{1}H decoupled) NMR:** (121 MHz, $CDCl_3$): δ 28.55, 28.37; **ESI-MS (high res):** m/z calc.: 449.1136; **Found:** 449.1126 $[M + Na]^+$; **Anal. calc. for** $C_{20}H_{24}FO_7P$: C, 56.34, H, 5.67; **Found:** C, 56.23, H, 5.78.

Kinetic Characterization of 1-5 with Retaining Glycosidases

Kinetics using Abg

Abg is known to hydrolyze both mannosides and galactosides.[5] Therefore, a diastereomeric mixture (at phosphorus) of benzyl benzyl-(2-deoxy-2-fluoro-β-D-mannopyranosyl) phosphonate (**1**) was evaluated as an inactivator of Abg (0.3 μg/mL , 50 mM NaH_2PO_4/Na_2HPO_4 pH = 6.8). 4'-Nitrophenyl-β-D-fucopyranoside was used as the substrate for this assay[5] since the 2-fluoro-mannosyl covalent intermediate formed can be observed over the course of the assay through transglycosylation onto the O4 hydroxyl of the typical substrate, 4'-nitrophenyl β-D-glucopyranoside.[6] The resulting activity versus time curve for various concentrations of inactivator and a re-plot of the individual k_{obs} values as a function of inactivator concentration are shown in Figure S1.

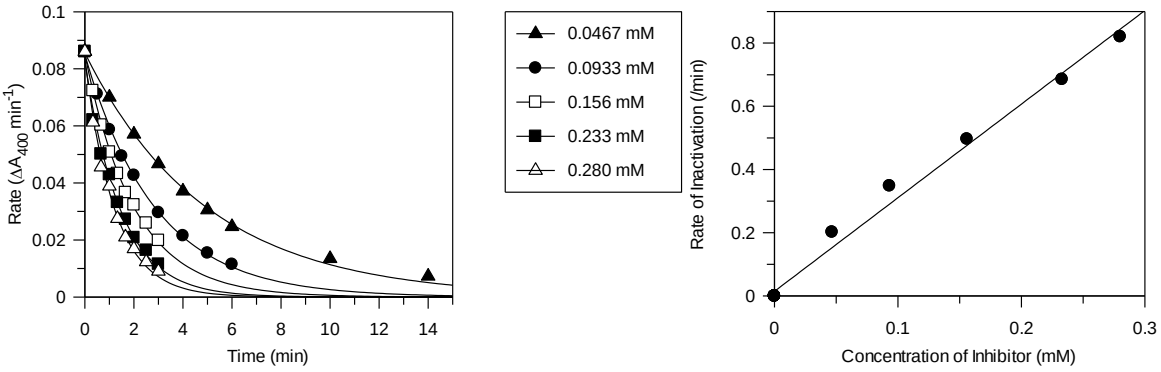


a) b)

Figure S1. Inactivation of Abg with **1**. a) Non-linear plot of residual enzyme activity versus time at the indicated inactivator concentrations fitted to an exponential decay equation. b) Plot of the observed rate constants of inactivation versus concentration of inactivator.

In this instance, it was not possible to calculate the individual k_i and K_i values for **1**, since inactivation was too rapid at concentrations approaching saturation to allow sampling. However, a second order rate constant of inactivation (k_i/K_i = 23.9 $min^{-1}mM^{-1}$) was obtained from linear fitting of the re-plot of the rate constants of inactivation.

A diastereomeric mixture (at the phosphorus atom) of benzyl benzyl-(2-deoxy-2-fluoro-β-D-galactopyranosyl) phosphonate (**2**) was also tested as an inactivator of Abg (Figure S2).



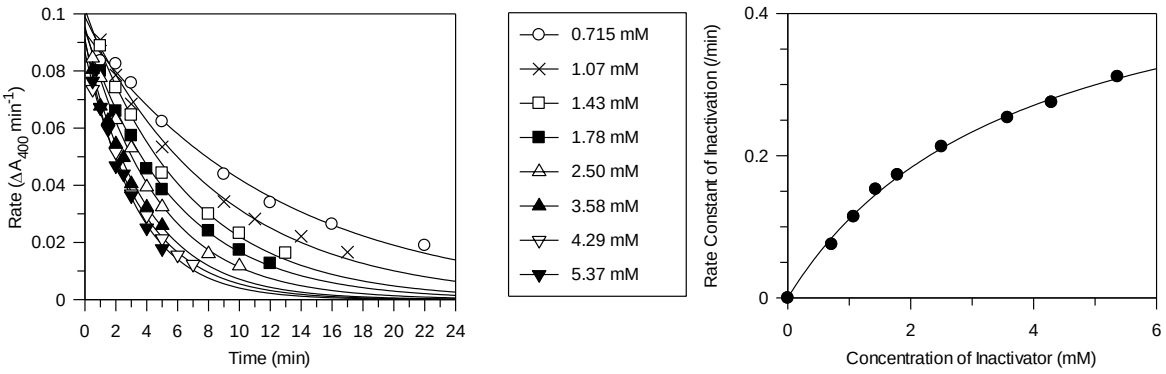
a) b)

Figure S2. Inactivation of Abg with **2**. a) Non-linear plot of residual enzyme activity versus time at the indicated inactivator concentrations fitted to an exponential decay equation. b) Plot of the observed rate constants of inactivation versus concentration of inactivator.

As described for **1**, only a second-order rate constant of inactivation (k_i/K_i = 3.0 $min^{-1}mM^{-1}$) was obtained for **2** as an inactivator of Abg.

Kinetics using Man2A

Benzyl benzyl-(2-deoxy-2-fluoro-β-D-mannopyranosyl) phosphonate (**1**) was tested as an inactivator of Man2A (0.4 μg/mL, 50 mM NaH_2PO_4/Na_2HPO_4, pH = 6.8). The substrate used was 4'-nitrophenyl-β-D-mannopyranoside. The resulting activity versus time plot for various concentrations of inactivator and a re-plot of the individual k_{obs} values plotted as a function of inactivator concentration are shown for **1** in Figure S3.



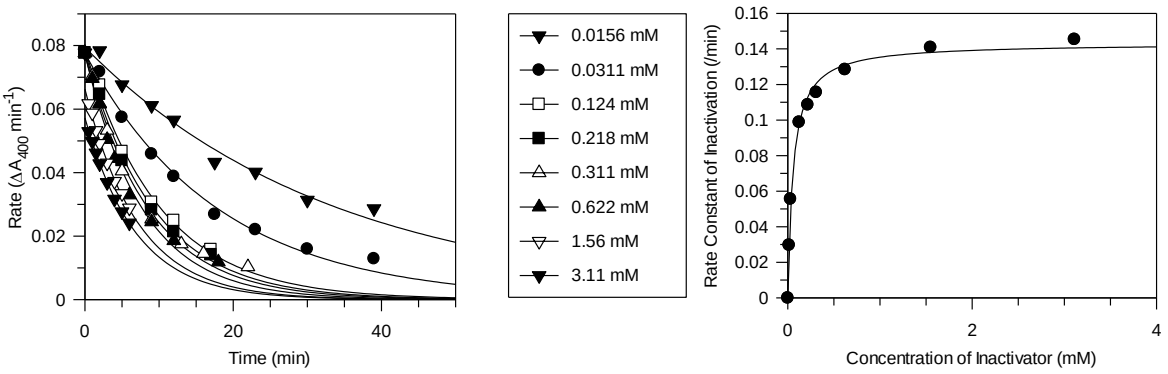
a) b)

Figure S3. Inactivation of Man2A by **1**. a) Non-linear plot of residual enzyme activity versus time at the indicated inactivator concentrations fitted to an exponential decay equation. b) Plot of the observed rate constants of inactivation versus concentration of inactivator.

Compound **1** behaves as a covalent inactivator of Man2A, and individual values for k_i = 0.52 min^{-1}, K_i = 3.7 mM, and k_i/K_i = 0.14 $min^{-1}mM^{-1}$ were obtained.

Kinetics using Lac-Z

Benzyl benzyl-(2-deoxy-2-fluoro-β-D-galactopyranosyl) phosphonate (**2**) was tested as a covalent inactivator of Lac-Z (1.28 μg/mL, 50 mM NaH_2PO_4/Na_2HPO_4,1 mM $MgCl_2$, pH = 7.0). 4'-Nitrophenyl-β-D-galactopyranoside was used as the substrate for the assay. The resulting activity versus time plot for various concentrations of inactivator and a re-plot of the individual k_{obs} values plotted as a function of inactivator concentration are shown in Figure S4.



a) b)

Figure S4. Inactivation of Lac-Z with **2**. a) Non-linear plot of residual enzyme activity versus time at the indicated inactivator concentrations fitted to an exponential decay equation. b) Plot of the observed rate constants of inactivation versus concentration of inactivator.

Compound **2** was an efficient inactivator of Lac-Z, with kinetic parameters of k_i = 0.14 min^{-1}, K_i = 0.058 mM, and k_i/K_i = 2.5 $min^{-1}mM^{-1}$.

Kinetic evaluation of 3 as a covalent inactivator of Yag

Dibenzyl (2-deoxy-2-fluoro-α-D-glucopyranosyl) phosphate (**3**) was tested as a covalent inactivator of Yag (1.96 μg/mL, 50 mM NaH_2PO_4/Na_2HPO_4, pH = 6.8). The substrate used was 4'-nitrophenyl-α-D-glucopyranoside. No accumulation of the covalent intermediate was observed at 37 °C. To test whether accumulation could be observed kinetically, both the incubation and the assay temperatures were lowered to 4 °C to slow the rate of turnover of any covalent glycosyl-enzyme intermediate formed enough to be observed kinetically. This method has been used to observe the covalent glycosyl-enzyme intermediate formed between 5-fluoro-β-L-idosyl fluoride and Yag.[7, 8] However, even at 4 °C, no time-dependent decrease in enzyme activity was observed.

To test whether the compound was acting as a substrate for Yag, a TLC assay was used to observe the formation of 2-deoxy-2-fluoro-glucose released by the enzyme, as can be seen in Figure S5.

Figure S5. TLC analysis (9:1 ethyl acetate : methanol) of hydrolysis of **3** by Yag, 18 minutes incubation at 37 °C. Lane 1 = positive control (2-deoxy-2-fluoro-glucose). Lane 2 = **3** + Yag. Lane 3 = negative control, **3** only.

The fastest running spot can be identified as intact **3** both by its R_f and by co-spot with an authentic standard (data not shown). The middle spot corresponds to the product of hydrolysis of **3**, 2-deoxy-2-fluoro-glucose. The darker product spot in the middle lane (containing enzyme) relative to the right-hand lane (containing no-enzyme) shows that **3** is hydrolyzed in an enzyme-dependent manner. This suggests that **3** is a substrate for Yag. Therefore, **3** was tested as a competitive substrate for Yag under steady state conditions, as follows. A solution of Yag was individually incubated with the substrate 4'-nitrophenyl-α-D-glucopyranoside and varying amounts of inhibitor, and the rate of enzyme-catalyzed substrate hydrolysis monitored. The resulting inverse rate versus inhibitor concentration plot can be seen in Figure S6.

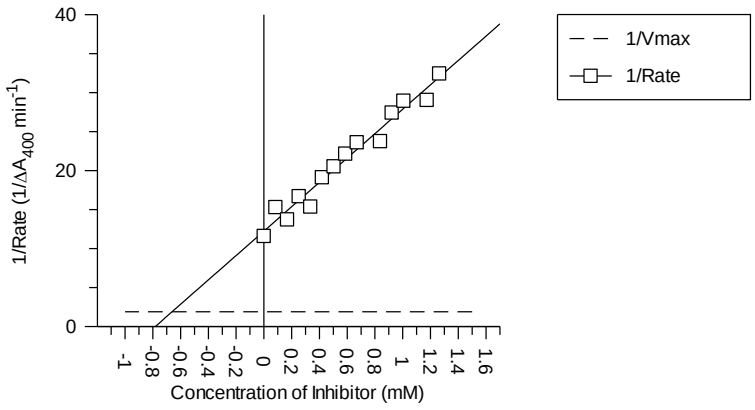


Figure S6. Testing of **3** as a competitive substrate for Yag by plotting 1/rate of pNP-α-Glc hydrolysis catalyzed by Yag vs. concentration of **3**.

An apparent K_i' value for **3** for Yag of approximately 0.7 mM can be obtained from the intersection of the 1/rate and $1/V_{max}$ lines, which should correspond to the K_m value for this compound as a substrate.

Kinetics using JBAM

Dibenzyl (2-deoxy-2-fluoro-α-D-mannopyranosyl) phosphate (**4**) and benzyl benzyl-(2-deoxy-2-fluoro-α-D-mannopyranosyl) phosphonate (**5**) were both evaluated as potential inactivators of JBAM (1.74 μg/mL, 50 mM sodium citrate, pH = 4.5). However, no accumulation of the covalent intermediate was observed at 37 °C. To test whether accumulation could be observed kinetically, both the incubation and the assay temperatures were lowered to 4 °C to slow the rate of turnover of any covalent glycosyl-enzyme intermediate formed enough to be observed kinetically, as described above. However, even at 4 °C, no time-dependent decrease in enzyme activity was observed with either **4** or **5**. To test whether either compound is a substrate for JBAM, a TLC assay was used to test for the presence of the product of hydrolysis, 2-deoxy-2-fluoro-mannose. The image of the TLC plate for **4** in the presence (right lane) and absence (left lane) of JBAM is shown in Figure S7.

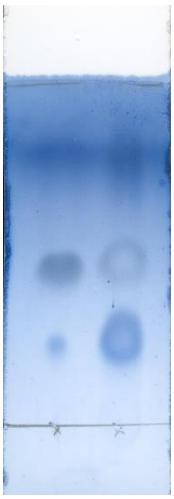


Figure S7. TLC analysis (9:1 ethyl acetate : methanol) of **4** incubated in the presence (right lane) and absence (left lane) of JBAM. The top spot is intact inactivator, and the middle spot is 2-deoxy-2-fluoro-glucose (both confirmed by co-spot analysis, not shown).

This TLC assay shows that **4** is hydrolyzed at an increased rate in the presence of enzyme. Therefore, dibenzyl phosphate derivative **4** was evaluated as a competitive substrate for JBAM under steady-state conditions. The resulting graph of inverse rate versus concentration of **4** is shown in Figure S8.

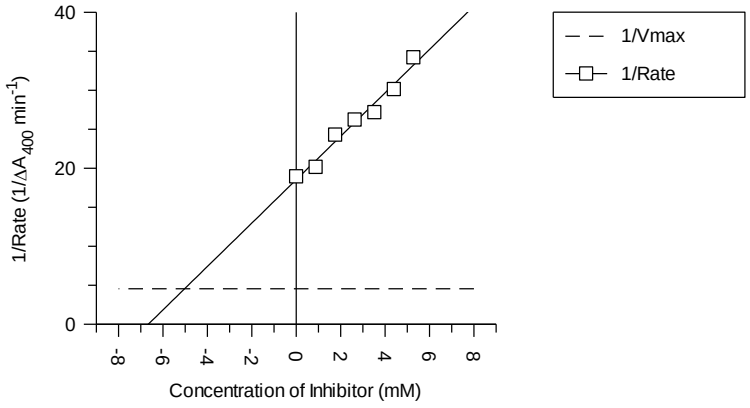


Figure S8. Testing of **4** as a competitive substrate for JBAM by plotting 1/rate of DNP-α-Man hydrolysis catalyzed by JBAM vs. concentration of **4**.

From this graph, a K_i' value (corresponding to the K_m value for **4** as a substrate) of ~5 mM can be obtained. This, coupled with the TLC data, demonstrates that **4** behaves as a substrate for JBAM, rather than a covalent inactivator.

Treatment of the other compound, benzyl benzylphosphonate derivative **5** with JBAM, even for extended periods of time (30 mins), did not increase rates of hydrolysis above that observed for the control reaction. Therefore, it was concluded that this compound does not act as a substrate for JBAM. To test whether **5** was still capable of binding to the active site of the enzyme, it was tested as a competitive inhibitor of the substrate, 2',4'-dinitrophenyl-α-D-mannopyranoside. No reduction in the rate of hydrolysis of substrate was observed at the highest concentration of **5** tested (6 mM).

References

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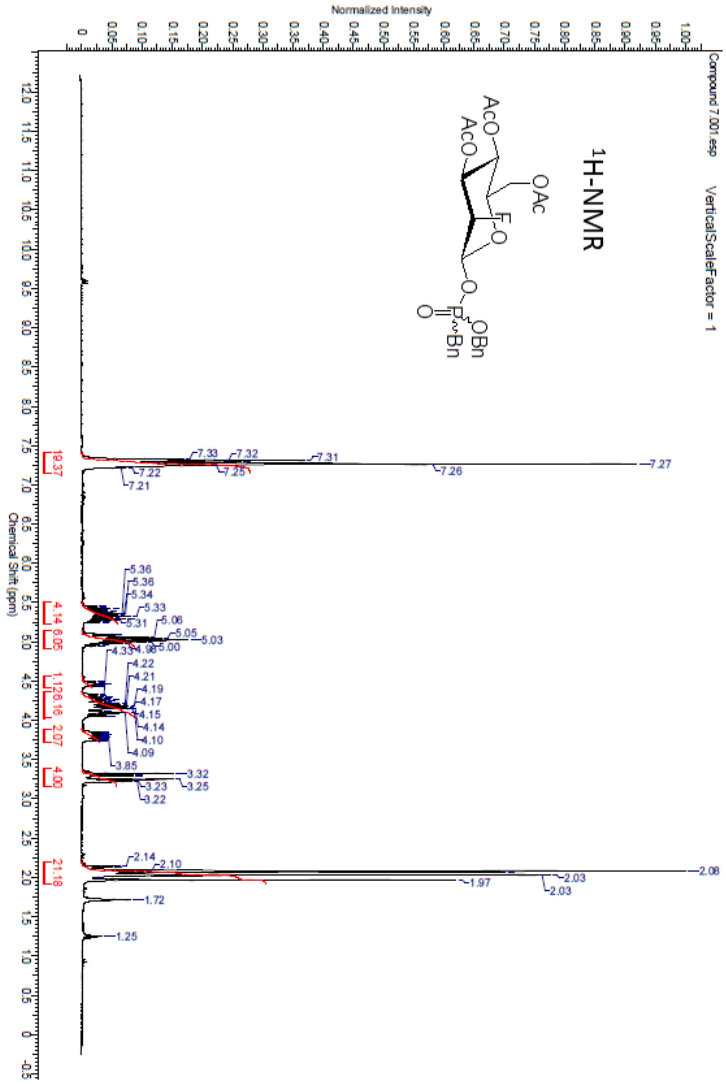
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OAc
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O
O
P
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OBn
Chemical Shift (ppm)
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7.32
7.31
7.27
7.26
7.25
7.22
7.21
5.36
5.36
5.34
5.33
5.31
5.06
5.05
5.03
5.00
4.98
4.33
4.22
4.21
4.19
4.17
4.15
4.14
4.10
4.09
3.85
3.32
3.25
3.23
3.22
2.14
2.10
2.08
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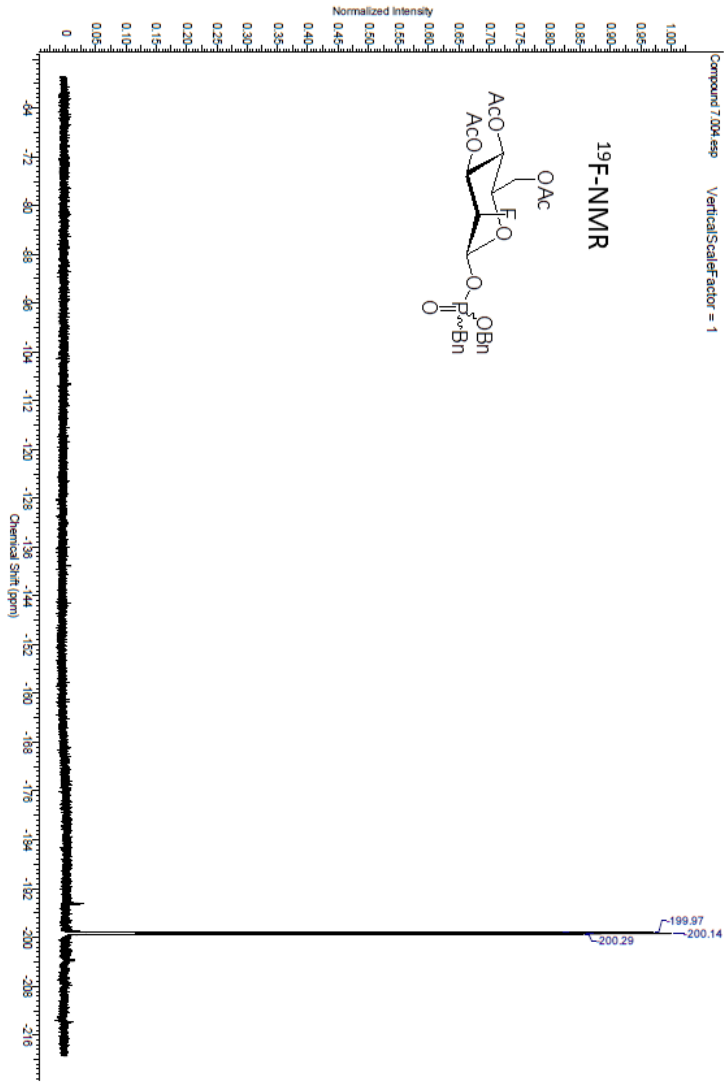
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O
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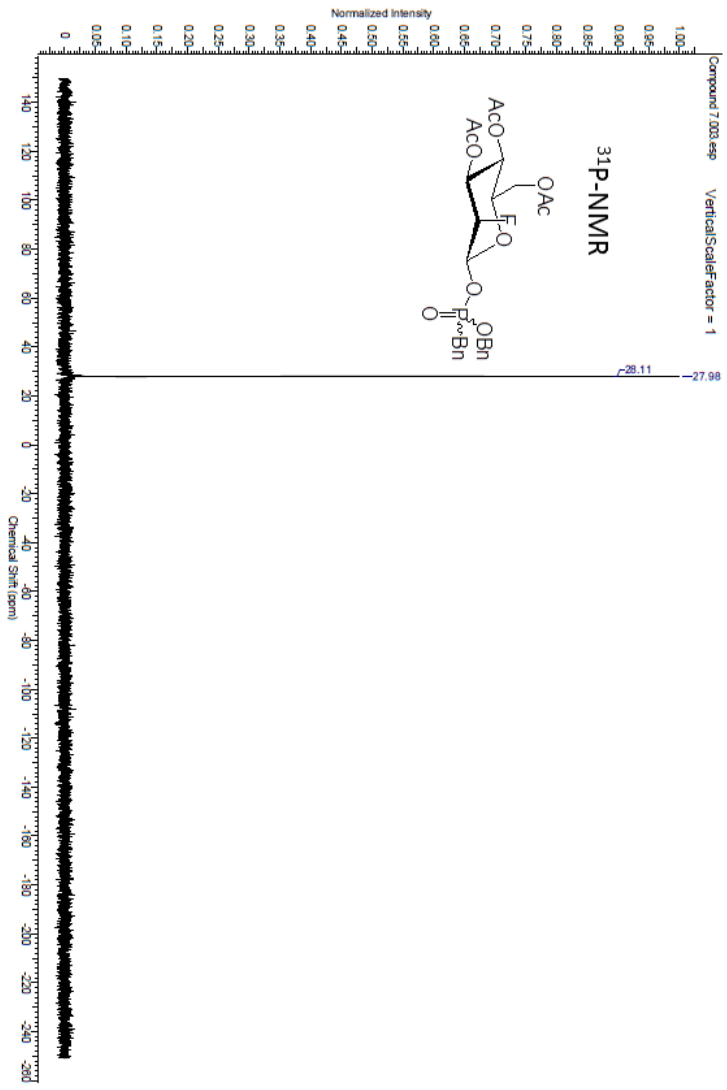
200.29
Chemical Shift (ppm)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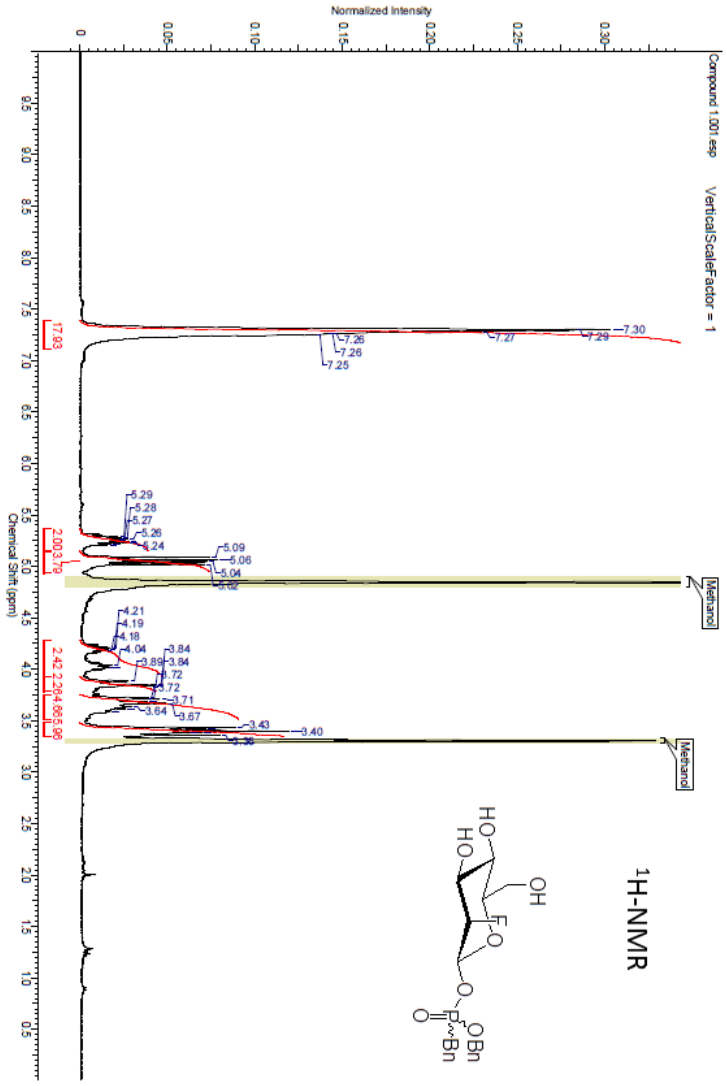
Chemical Shift (ppm)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F
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O
O
P
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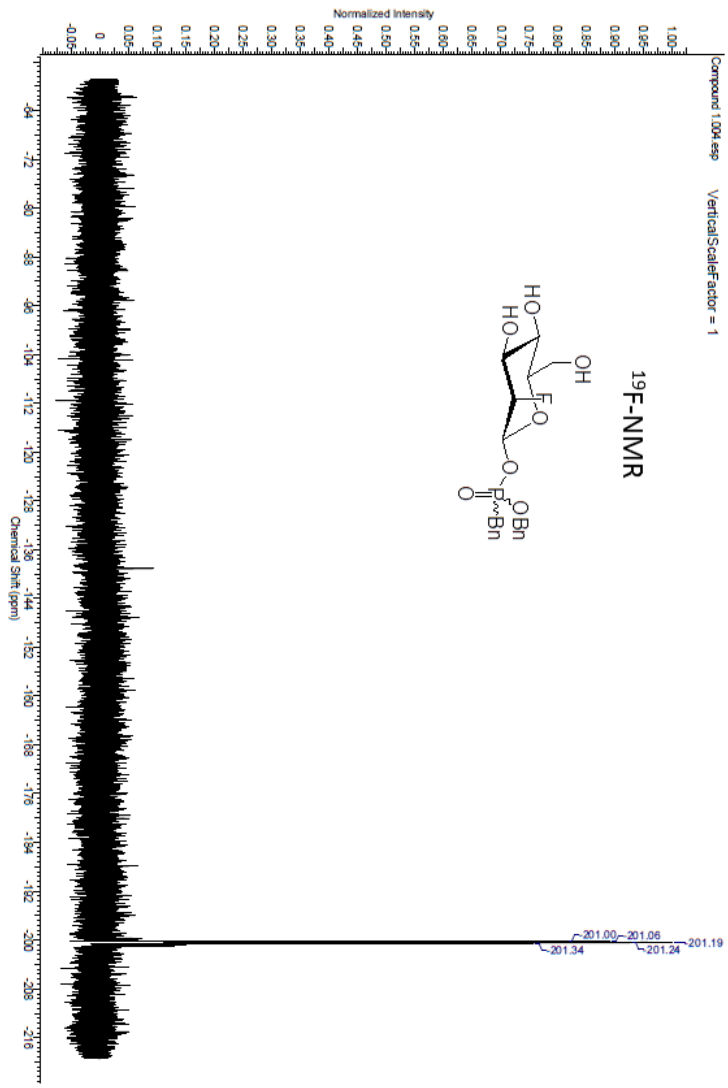
Bn
O
1H-NMR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Compound 1.004.esp
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HO
OH
F
O
O
O
P
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OBn
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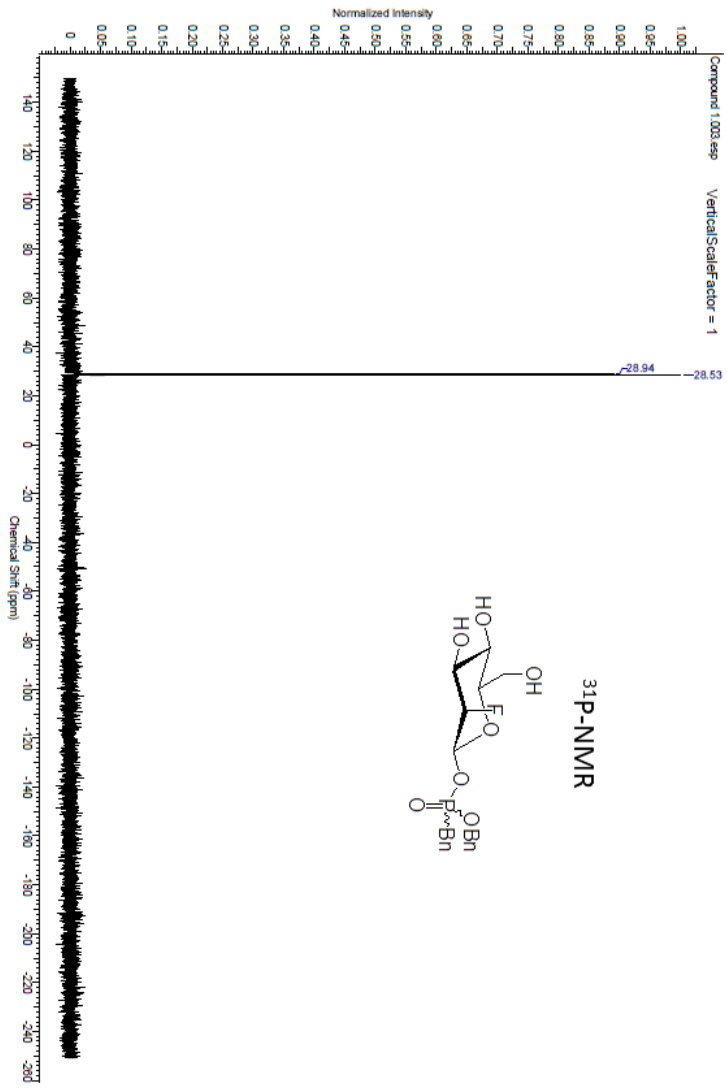
201.06
201.19
201.34
201.24
Chemical Shift (ppm)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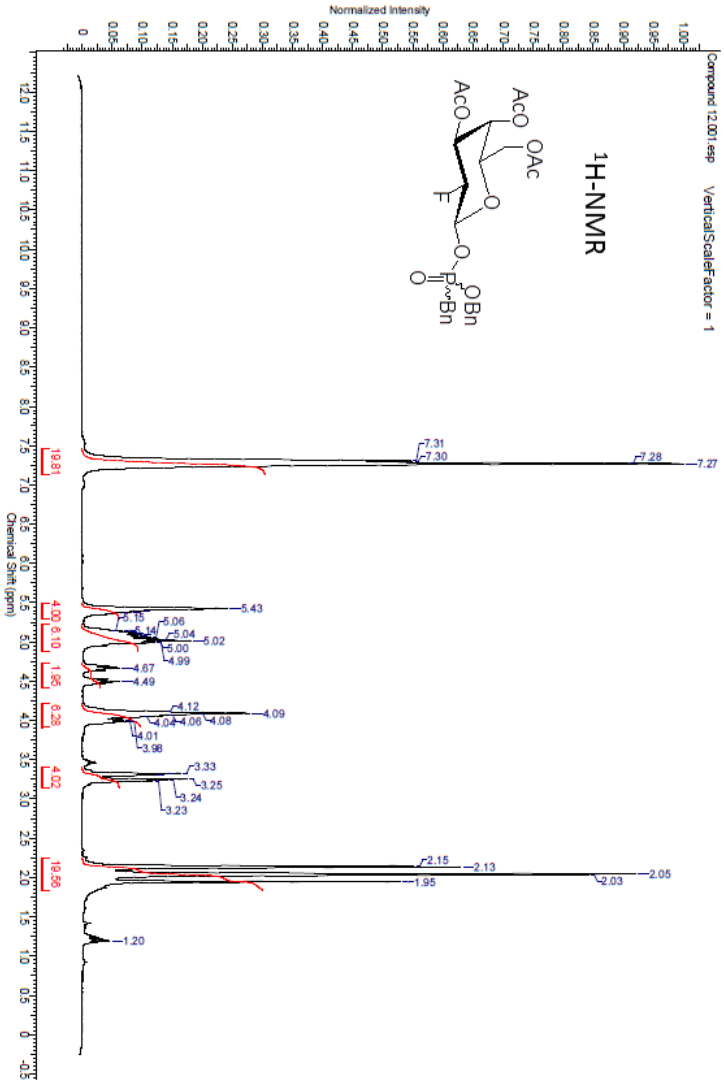
^{31}P-NMR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OAc
F
O
O
P
OBn
OBn
O
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6.10
1.95
6.28
4.02
19.56
7.31
7.30
7.28
7.27
5.43
5.15
5.06
5.04
5.02
5.00
4.99
4.67
4.49
4.12
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4.08
4.06
4.04
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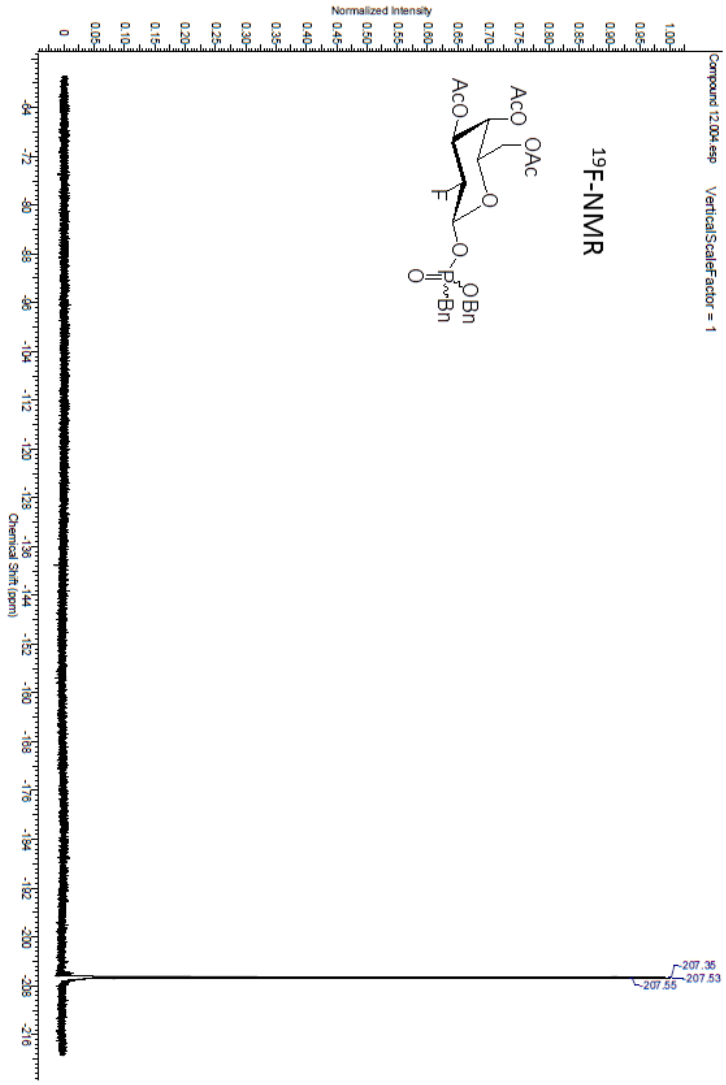
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O
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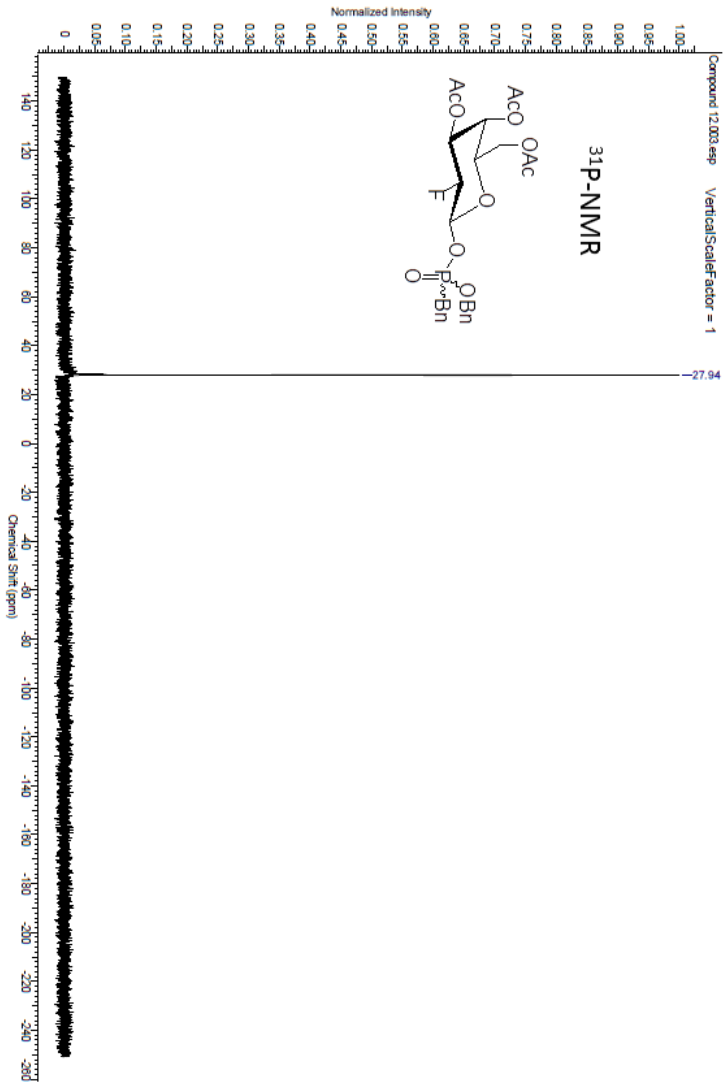
207.55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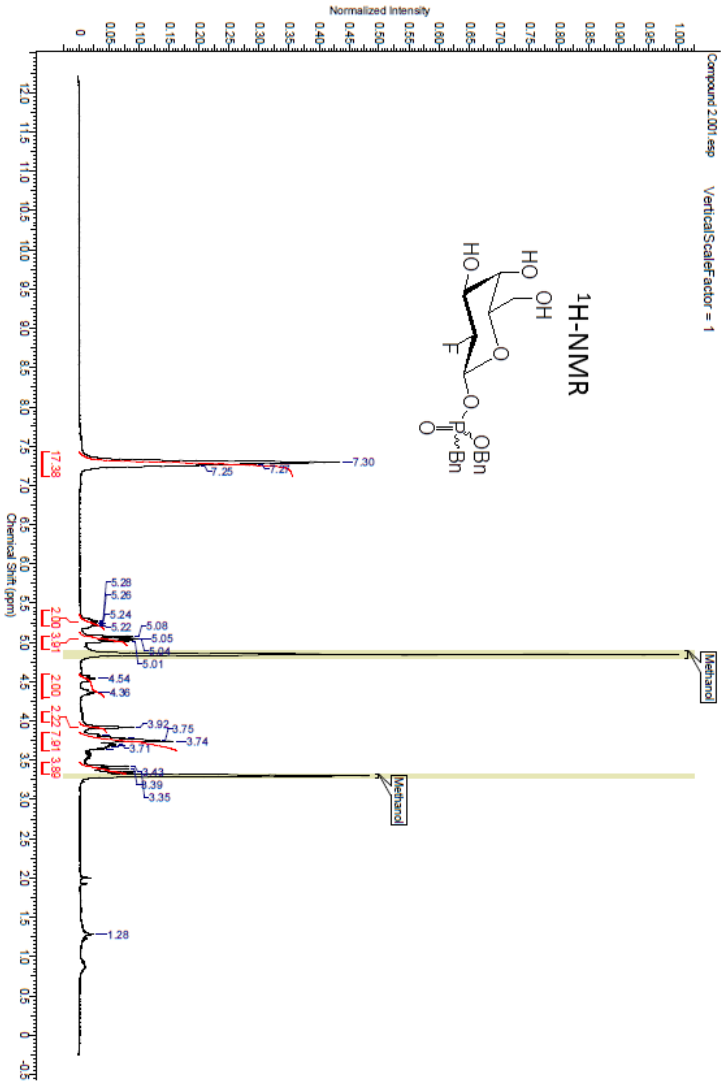
Normalized Intensity
Chemical Shift (ppm)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OH
F
O
O
P
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OBn
O
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2.00
3.81
2.00
2.52
7.91
3.99
7.30
7.25
7.24
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5.04
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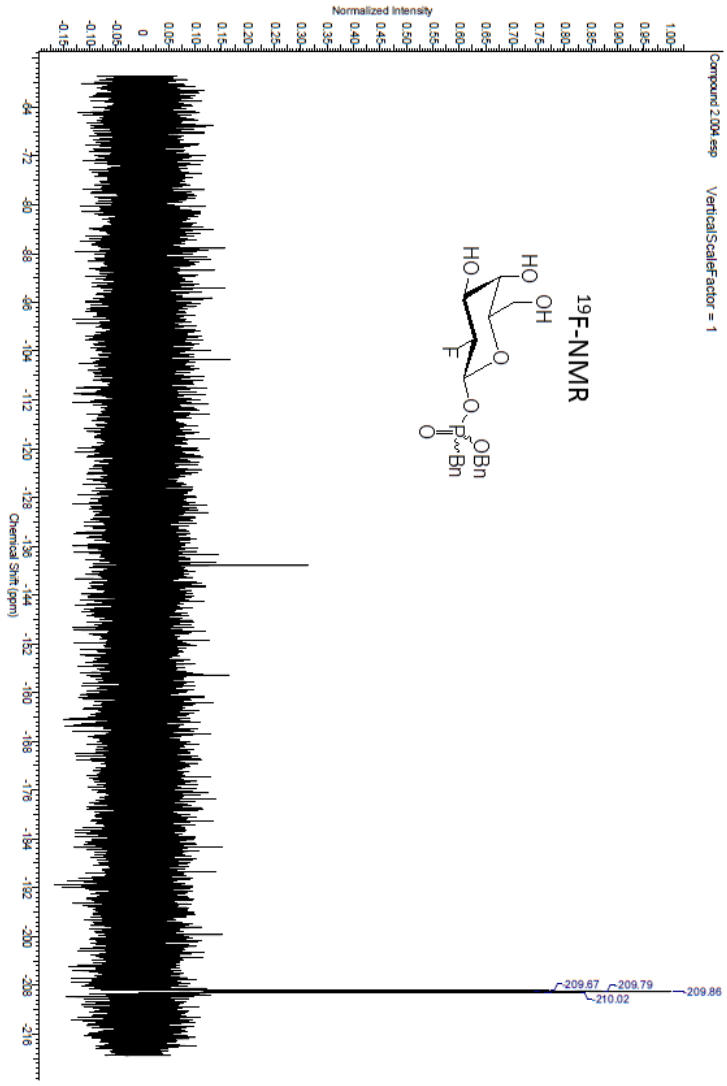
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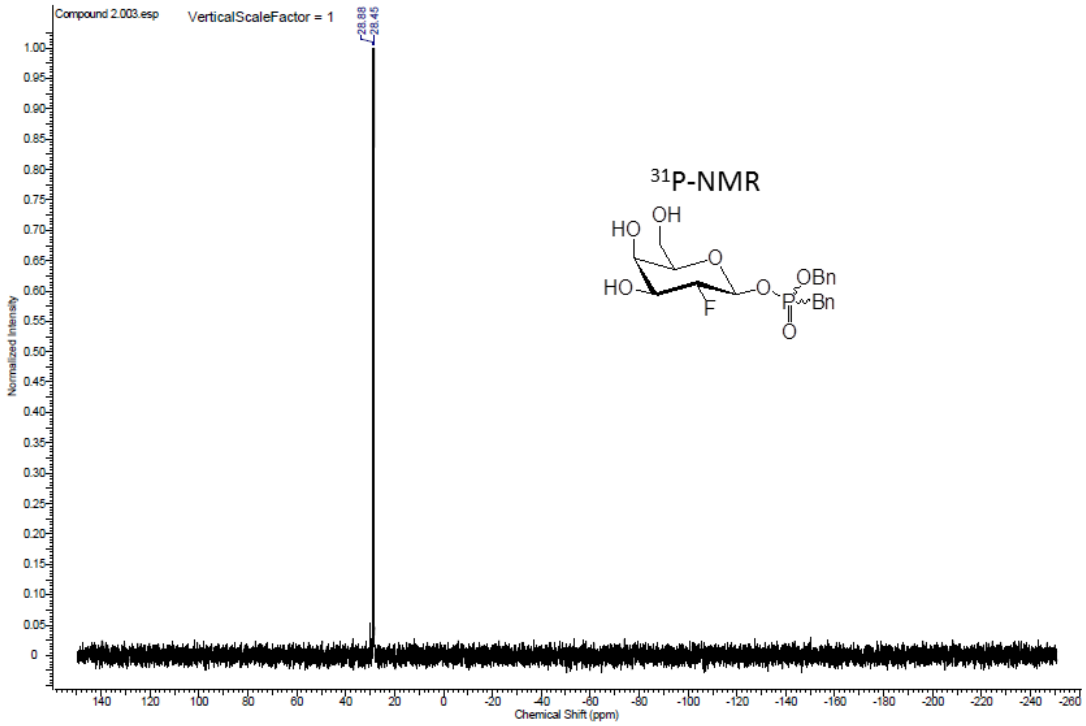
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P
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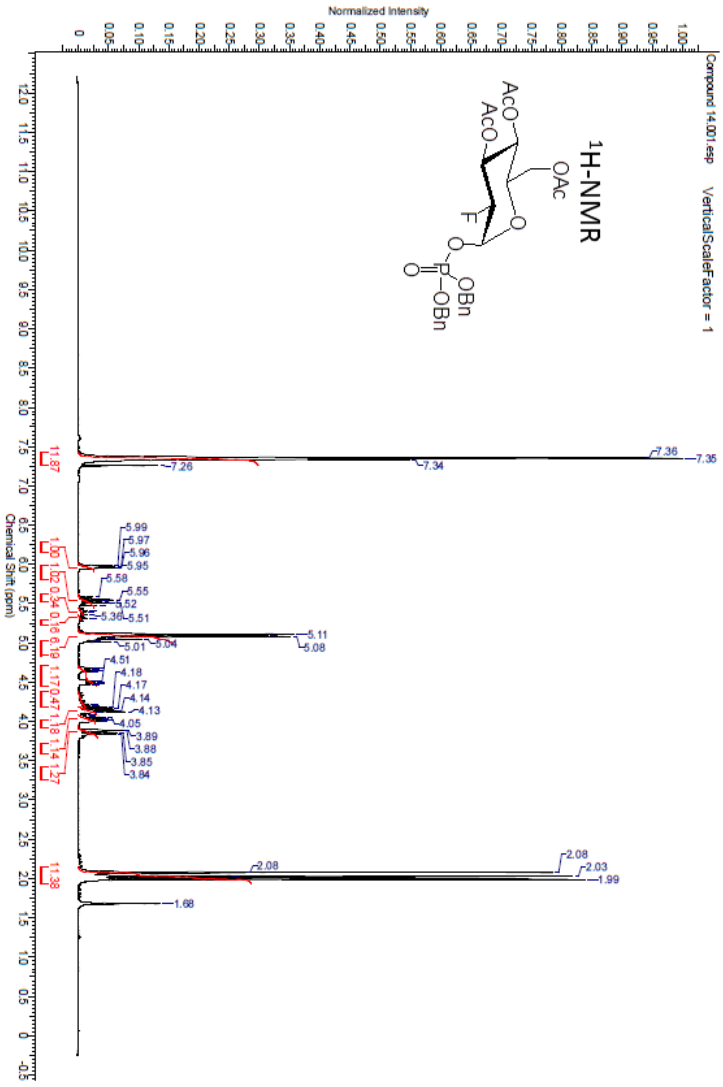
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O
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OBn
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7.26
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5.96
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5.58
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5.52
5.36
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5.11
5.08
5.01
5.04
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4.13
4.05
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1.02
0.34
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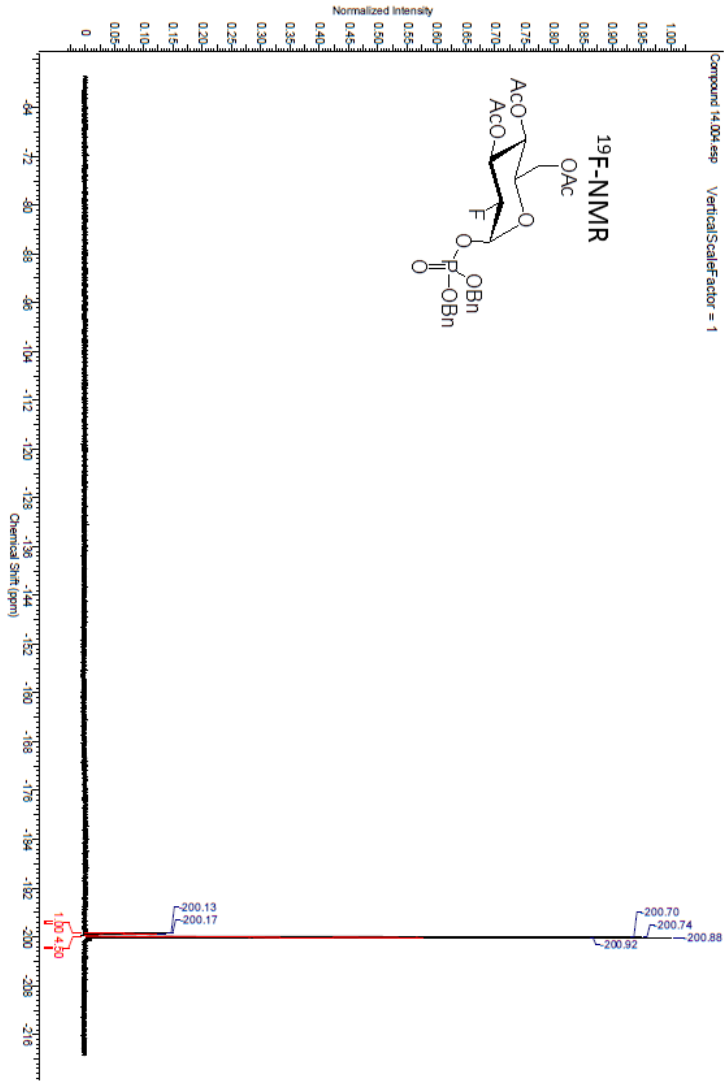
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P
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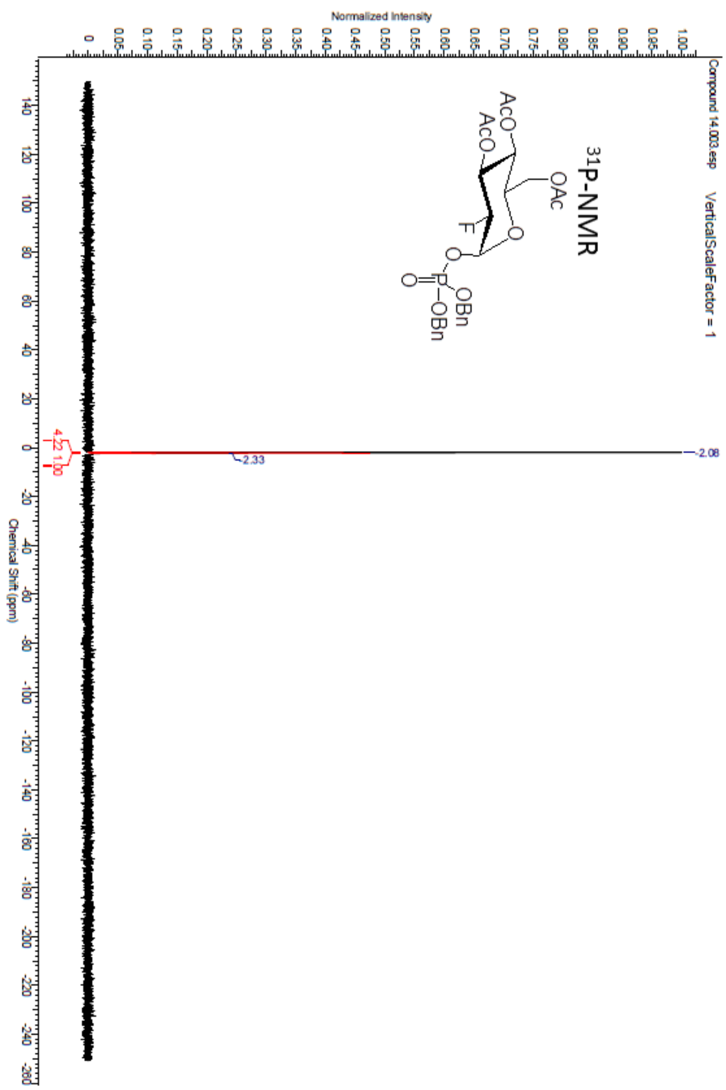
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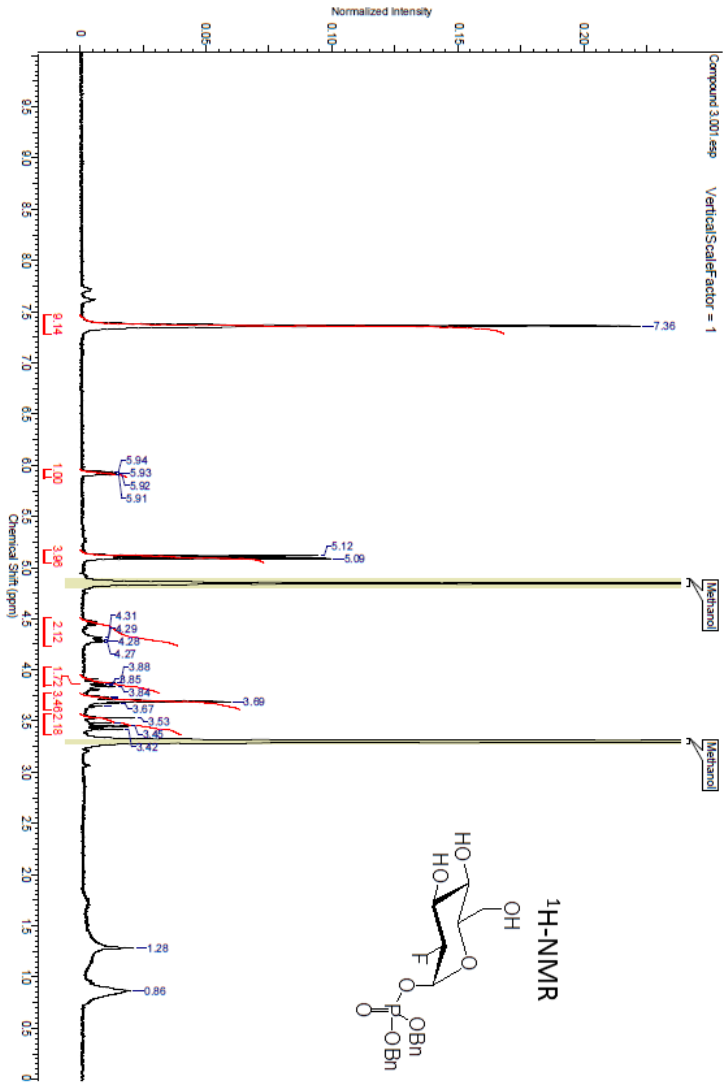
Chemical Shift (ppm)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O
P
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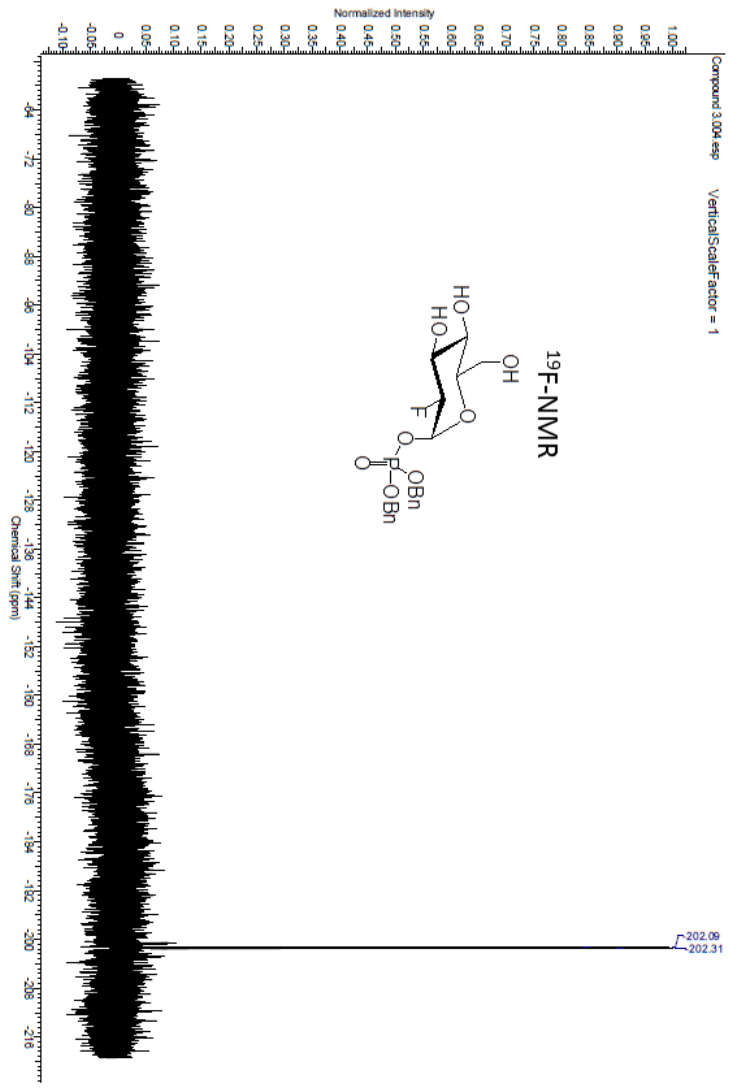
OBn
OBn
^{1}H-NMR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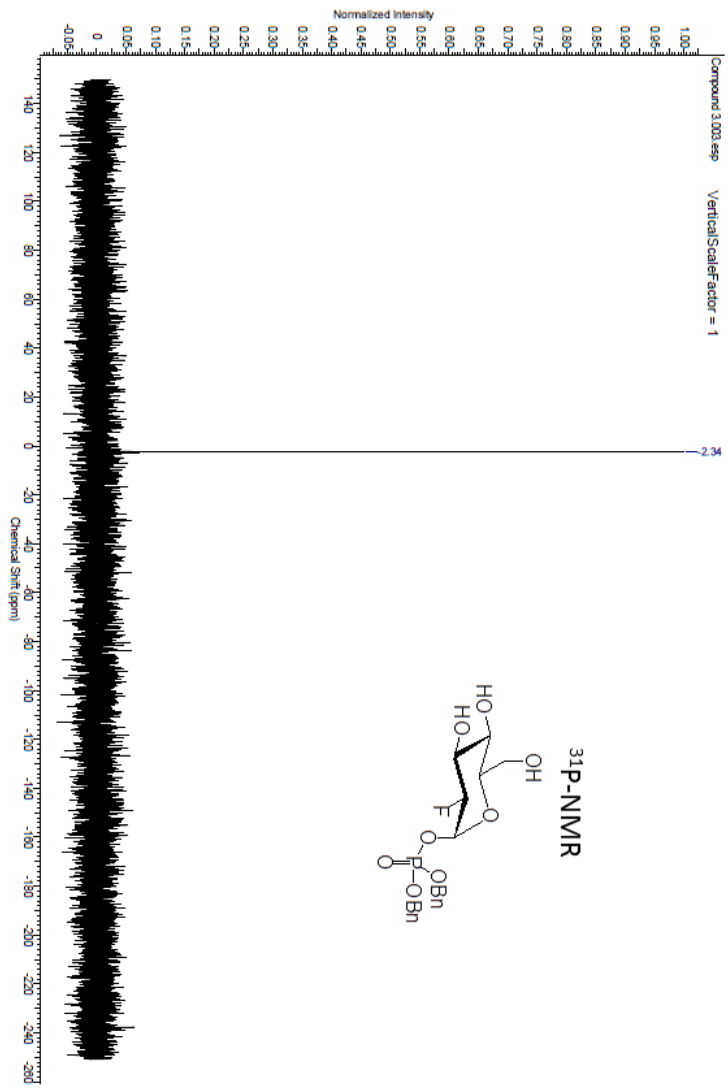
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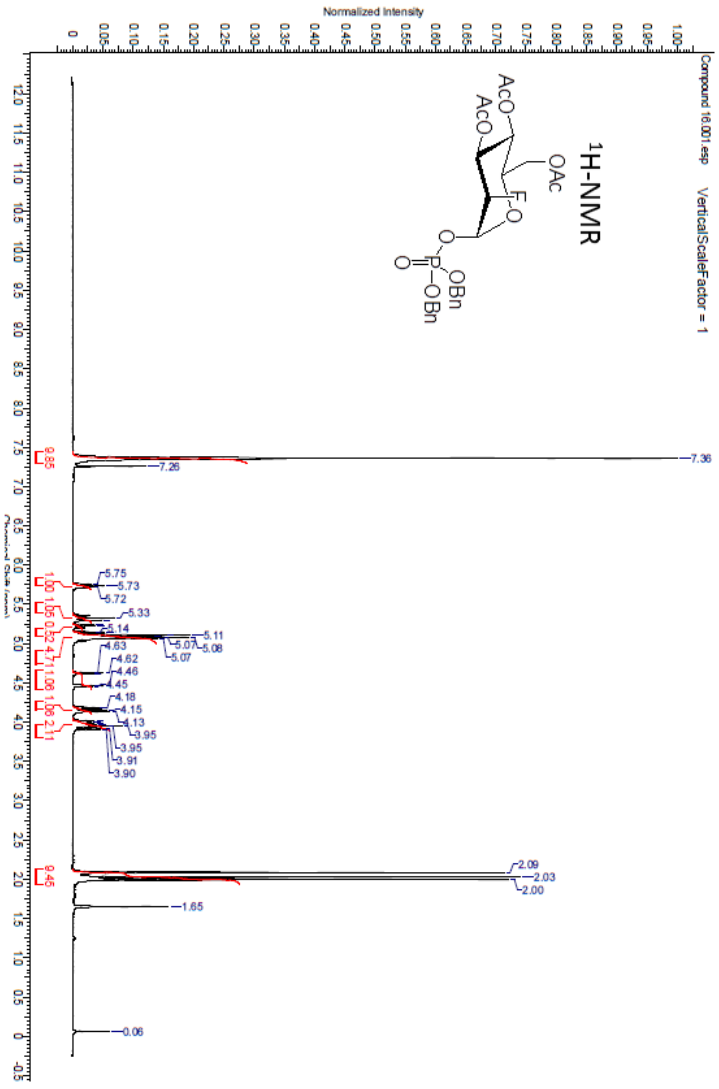
OBn
31P-NMR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O
P
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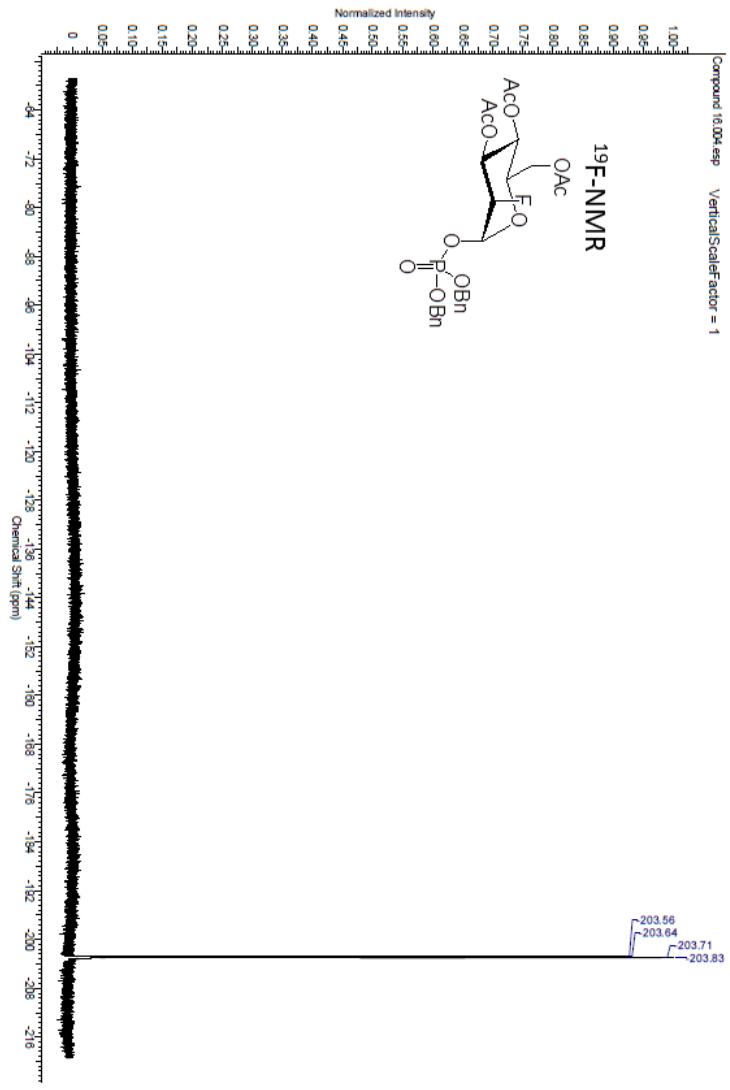
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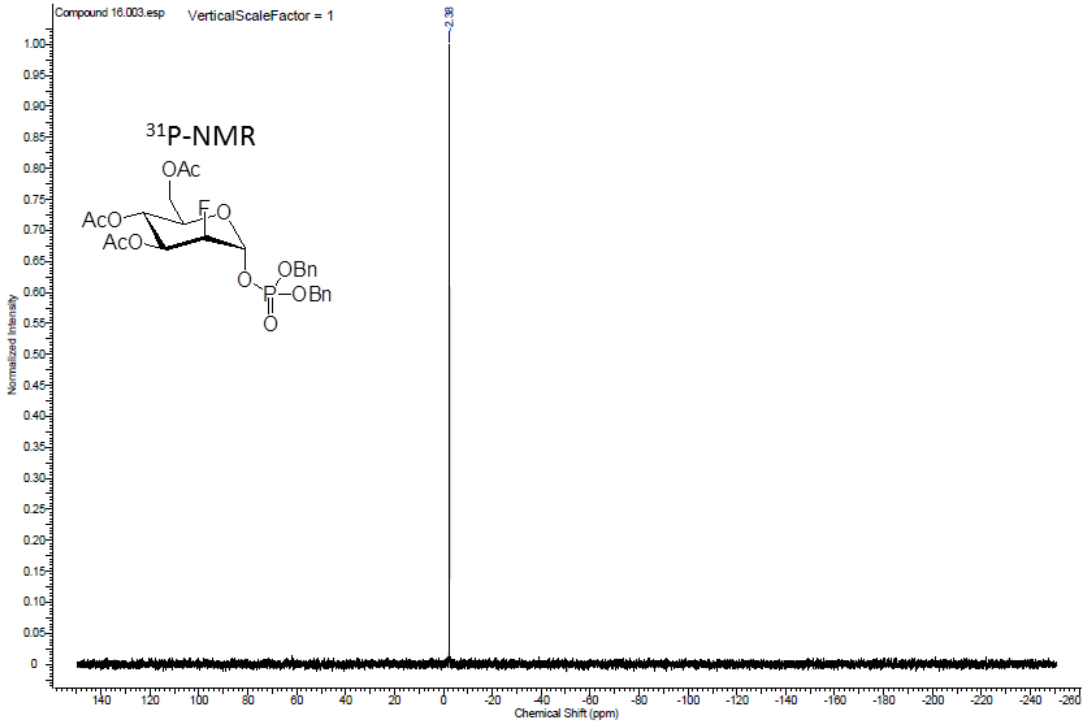
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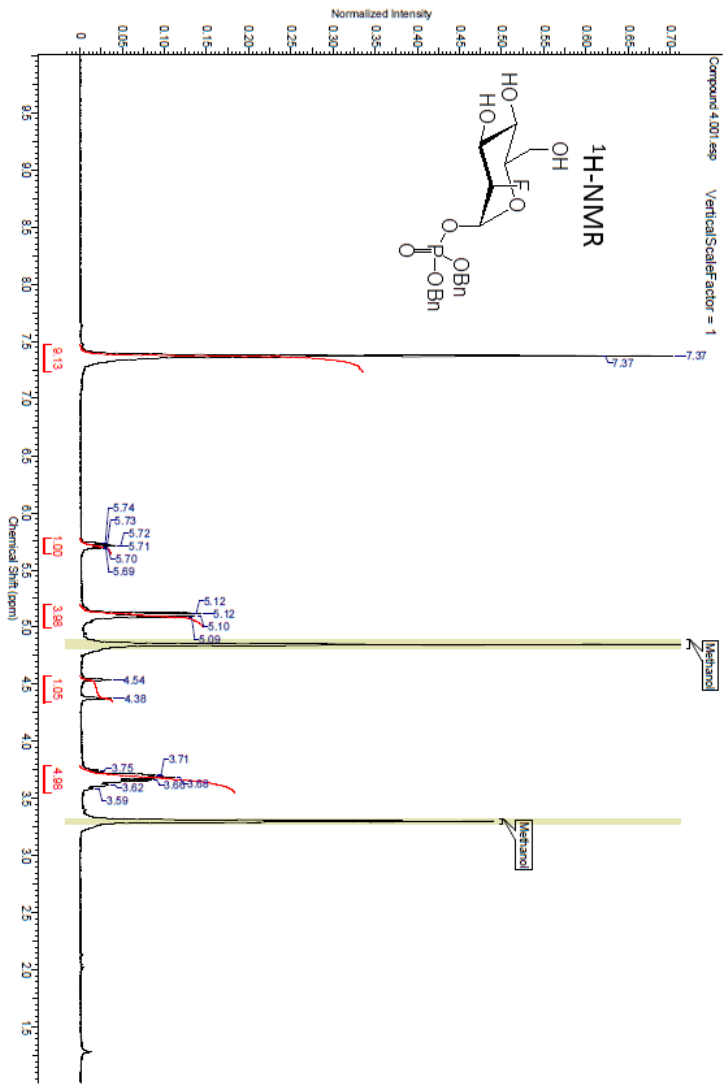
Chemical Shift (ppm)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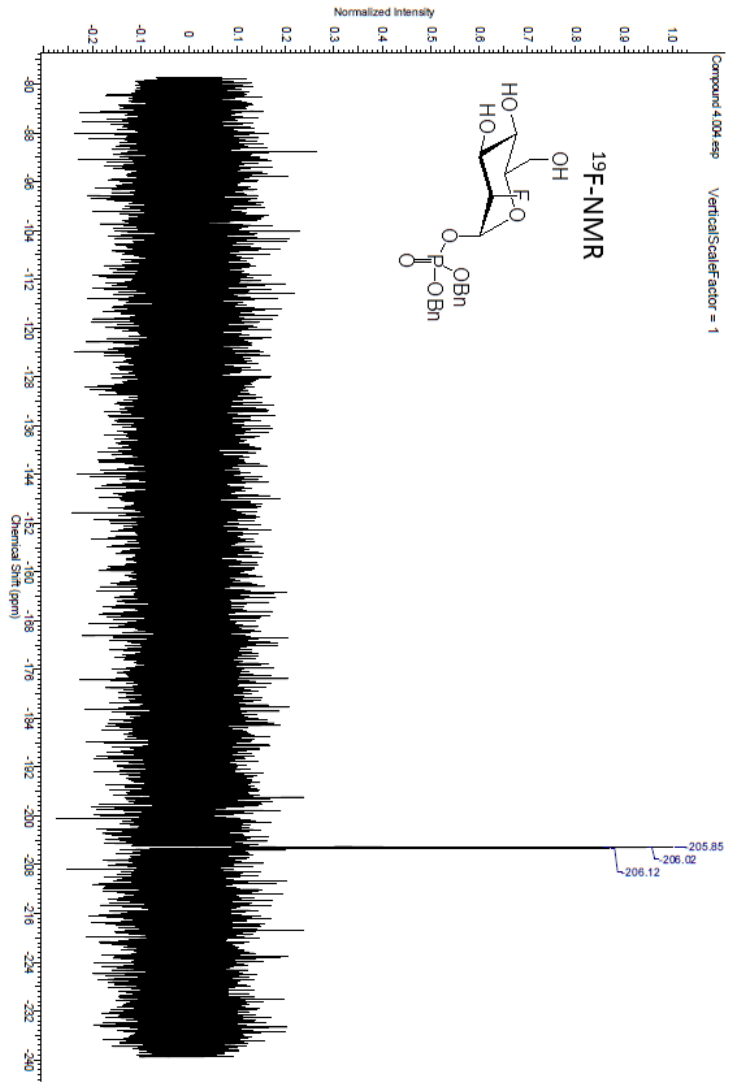
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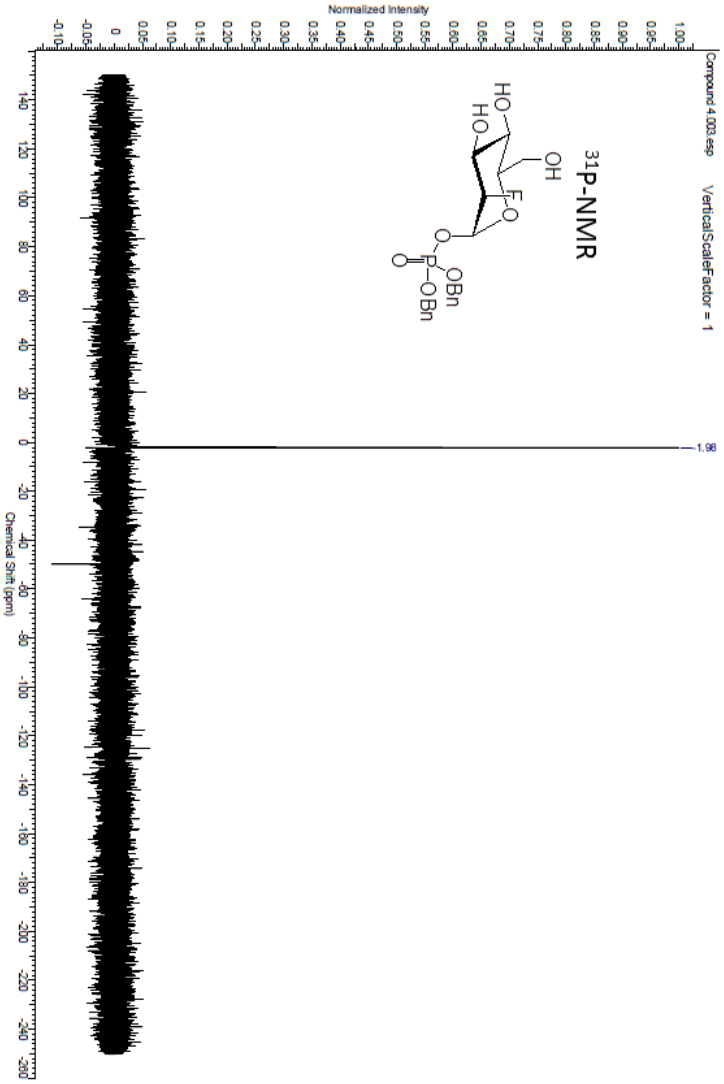
Chemical Shift (ppm)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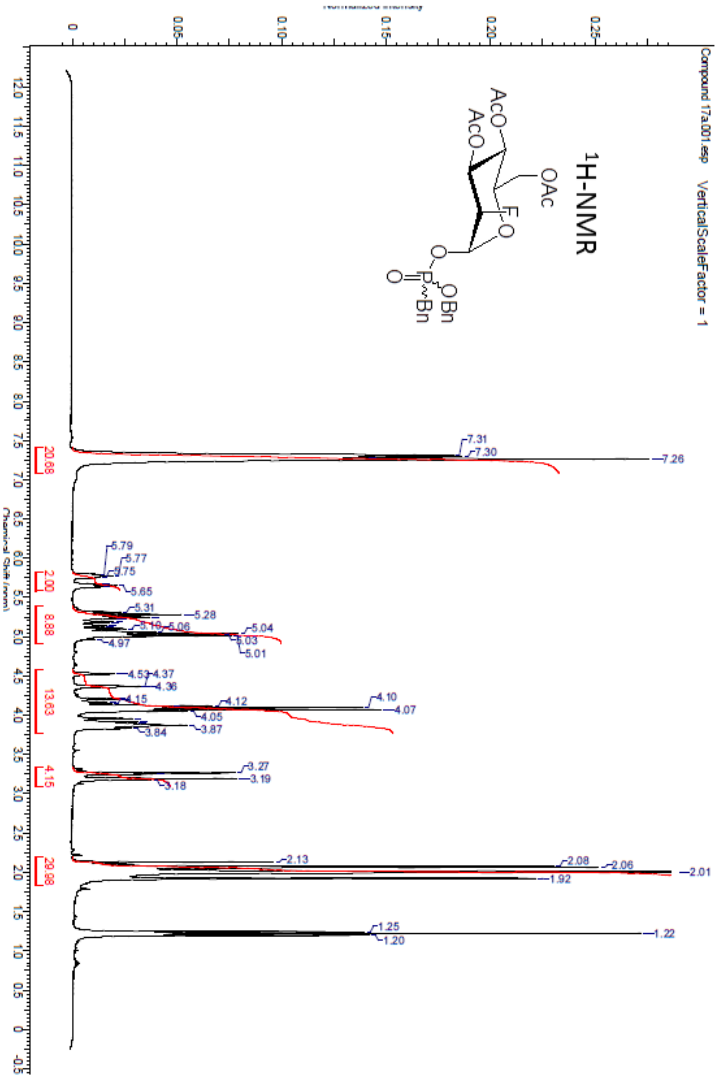
1.98
Chemical Shift (ppm)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P
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Bn
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7.26
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5.06
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3.19
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2.08
2.06
2.01
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1.25
1.20
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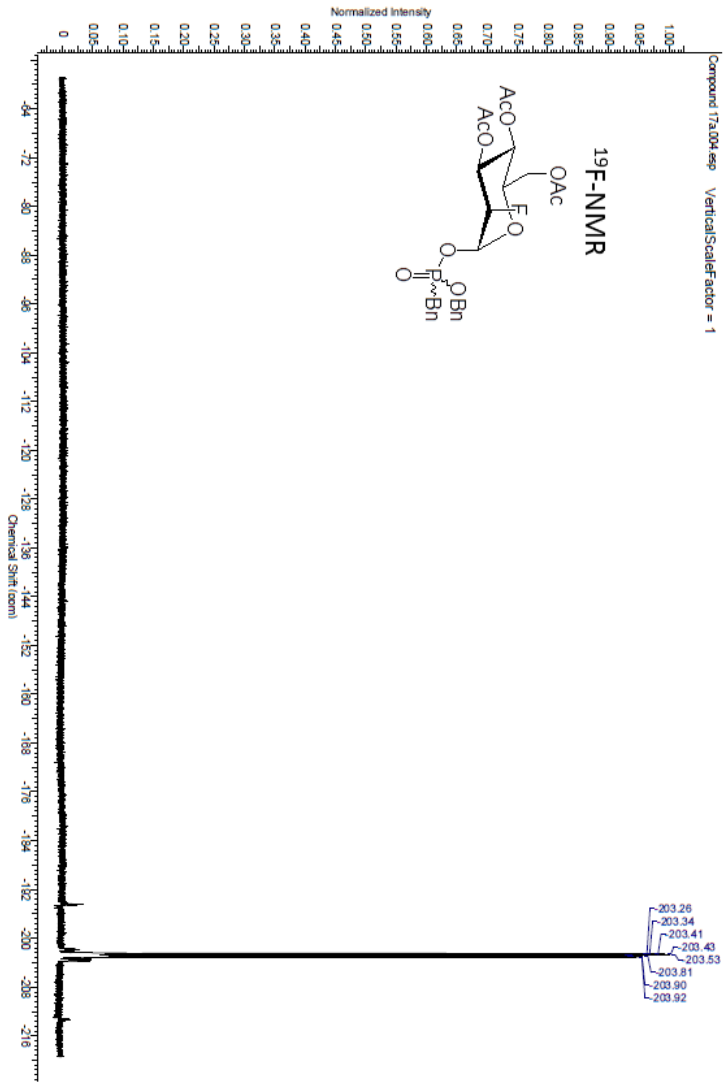
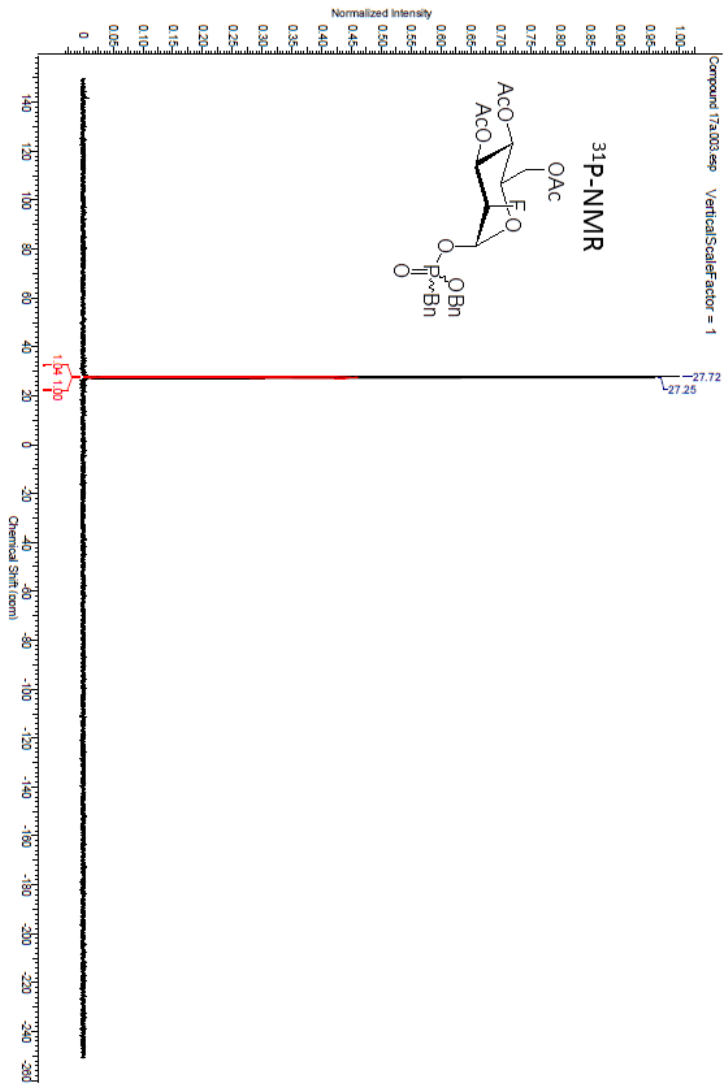
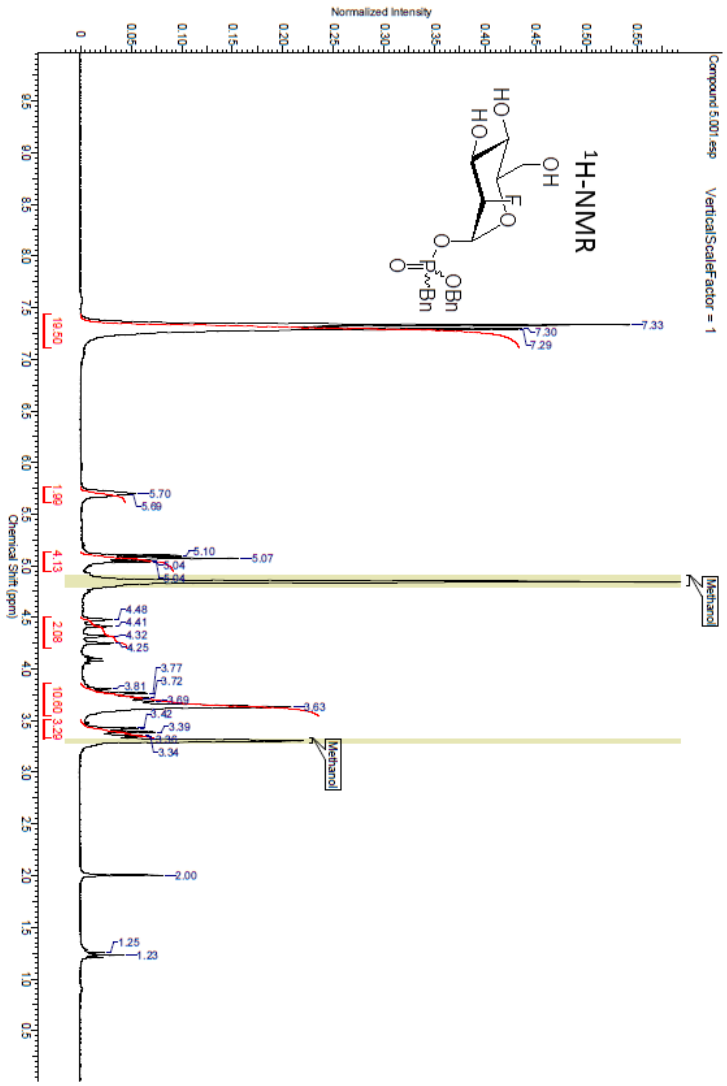
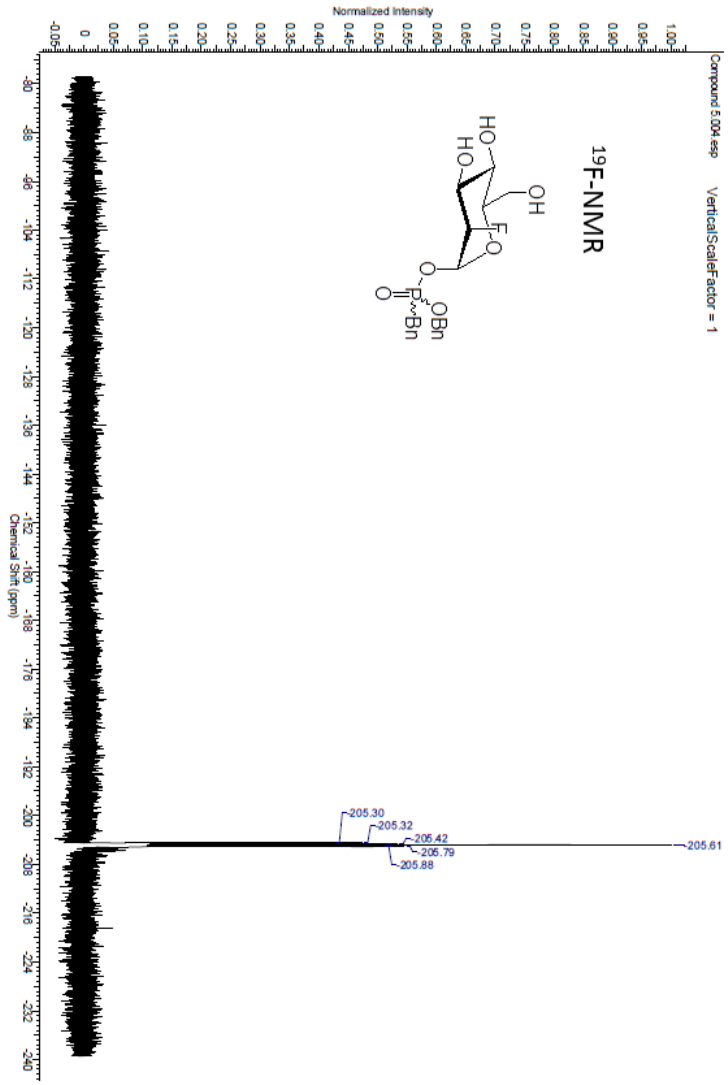
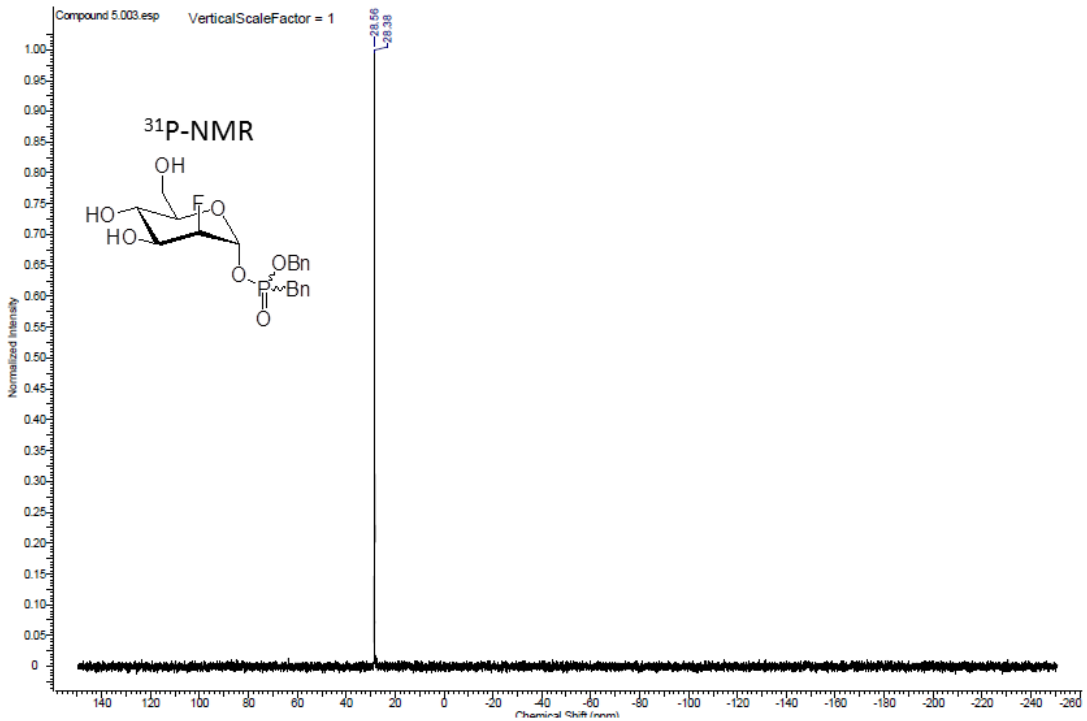
13.63
4.15
20.98


19F-NMR
Compound 17a.004.esp VerticalScaleFactor = 1
Normalized Intensity
Chemical Shift (ppm)
AcO
AcO
OAc
F
O
O
O
P
OBn
Bn
203.26
203.34
203.41
203.43
203.53
203.81
203.90
203.92


Normalized Intensity
Compound 17a.000.esp VerticalScaleFactor = 1
31P-NMR
AcO
AcO
OAc
F
O
O
O
P
OBn
Bn
O
27.72
27.25
1.04
1.00
Chemical Shift (ppm)


Normalized Intensity
Chemical Shift (ppm)
Compound 5.001.esp
VerticalScaleFactor = 1
1H-NMR
HO
HO
OH
F
O
O
P
O
OBn
Bn
19.50
1.99
4.13
2.08
10.60
3.29
7.33
7.30
7.29
5.70
5.09
5.10
5.07
5.04
4.94
4.48
4.41
4.32
4.25
3.77
3.72
3.81
3.69
3.63
3.42
3.39
3.34
2.00
1.25
1.23
Methanol
Methanol


Normalized Intensity
Compound 5.004.esp VerticalScaleFactor = 1
19F-NMR
HO
HO
OH
F
O
O
P
OBn
Bn
O
205.30
205.32
205.42
205.79
205.88
205.61
Chemical Shift (ppm)


Compound 5.003.esp
VerticalScaleFactor = 1
28.56
28.38
31P-NMR
OH
HO
HO
F
O
O
P
OBn
Bn
O
Normalized Intensity
Chemical Shift (ppm)