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Supporting Information

Lipid-Mimicking Phosphorus-Based Glycosidase Inactivators as Pharmacological Chaperones for the Treatment of Gaucher's Disease

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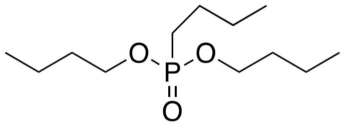
1. Experimental Procedures

Materials and Methods

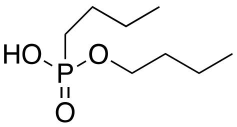
Unless otherwise stated, all reagents were obtained from commercial suppliers and were used without further purification. Flash column chromatography was performed with silica gel (230-400 mesh). TLC was performed on pre-coated silica plates and visualized using UV light and by applying a solution of 10% ammonium molybdate in 2 M H_2SO_4 or an ethanolic solution of ninhydrin 0.1% (w/v), followed by heating. Moisture sensitive reactions were carried out under an atmosphere of dry nitrogen. DCM and CH_3CN were distilled over CaH_2. Methanol was distilled over magnesium and iodine. THF was distilled over sodium/benzophenone. All NMR spectra were acquired on Bruker AV-400 or Bruker AV-600 spectrometers at 25 ºC. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane (^{1}H-NMR) or $CDCl_3$ (^{13}C-NMR). NMR spectra of carbamate-containing compounds were difficult to interpret due to the presence of multiple stable rotamers.

Di-*n*-butyl butylphosphonate (12a)



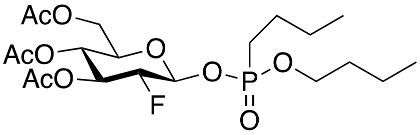
A solution of dibutyl phosphite (271 mg, 1.7 mmol) in dry THF (2.7 mL) was cooled with an ice-bath and subsequently treated with NaH (60%, mineral oil) (112 mg, 2.8 mmol) in one portion. After 30 min, *n*-butyl iodide (0.24 mL, 2.1 mmol) was added dropwise under nitrogen atmosphere and the resulting mixture was allowed to stir overnight while gradually reaching room temperature; then it was quenched by the addition of methanol and evaporated to dryness under vacuum and the residue was partitioned between ethyl acetate and brine. The aqueous phase was extracted once more with ethyl acetate and the combined organic phases were dried over anhydrous sodium sulfate, filtered and evaporated. Purification by flash column chromatography (petroleum ether/EtOAc 4:1 to 7:3) yielded 80 mg (23%) of the desired *n*-butylphosphonate **12a** as a volatile oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ/ppm 4.04 (tdd, J = 9.9, 7.3, $^3J_{H\text{-}P}$ = 6.7 Hz, 2H), 3.98 (ddd, J = 9.9, 7.3, $^3J_{H\text{-}P}$ = 6.7 Hz, 2H), 1.77 – 1.52 (m, 10H), 1.45 – 1.35 (m, 6H), 0.94 (t, J = 7.4 Hz, 6H), 0.91 (t, J = 7.2 Hz, 3H). **^{31}P{^{1}H}** NMR (162 MHz, $CDCl_3$) δ/ppm 32.66. **^{13}C NMR** (101 MHz, $CDCl_3$) δ/ppm 65.2 (d, J = 6.6 Hz, 2C), 32.6 (d, J = 6.6 Hz, 2C), 25.2 (d, J = 140.9 Hz), 24.5 (d, J = 5.1 Hz), 23.7 (d, J = 16.9 Hz), 18.8 (s, 2C), 13.6 (s, 2C), 13.5. **ESI-MS (low res)** *m/z* calc. for $C_{12}H_{27}O_3PNa$ $[M+Na]^+$: 273.2, found: 273.1.

Butyl hydrogen butylphosphonate (13a)



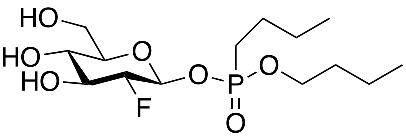
A solution of *n*-butylphosphonate **12a** (80 mg, 0.32 mmol, 1.0 equiv.) in dry DCM (6 mL) was treated with TMSI (1M, DCM) (0.3 mL, 0.32 mmol, 1.0 equiv.) at room temperature under nitrogen atmosphere and stirred for 2 h, after which another aliquot of 0.6 mL of TMSI (1M) was added. The resulting reaction mixture was stirred for another hour and then evaporated under vacuum and azeotropized with toluene until the distillate ran colorless to afford butyl hydrogen butylphosphonate **13a**. This dried crude material was employed in the next step without further purification.

3,4,6-Tri-*O*-acetyl-1-*O*-[*n*-butyl(butylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (16a)



Butyl hydrogen butylphosphonate **13a** (62.0 mg, 0.32 mmol, 1.0 equiv.) was dissolved in a mixture of dry THF (1.5 mL) and dry ACN (1.5 mL), treated with freshly activated 3 Å molecular sieves under inert atmosphere and stirred for 15 min at room temperature before cooling down with an ice-bath. To this mixture, a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide (237 mg, 0.64 mmol, 2.0 equiv.) in dry DCM (2 mL) was added, followed by silver carbonate (353 mg, 1.3 mmol, 4.0 equiv.) in one portion. At this point the flask was purged twice with vacuum/nitrogen, wrapped in foil and the reaction allowed to stir overnight at room temperature. Then, the mixture was filtered through a short Celite pad, washed with acetone and evaporated. The crude residue was purified by flash column chromatography (petroleum ether/EtOAc 3:1 to 1:1) to furnish a 1:1 diastereomeric mixture of peracetylated glycosyl phosphonate **16a** (54 mg, 11.2 mmol, 35%, two steps) as an amorphous solid. **Selected NMR signals:** **^{19}F{^{1}H} NMR** (121 MHz, $CDCl_3$) *δ*/ppm -200.41, -200.43; **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) *δ*/ppm 33.2; **ESI-MS (low res)** *m/z* calc. for $C_{20}H_{34}FO_{10}PNa$ $[M+Na]^+$: 507.2, found: 507.2.

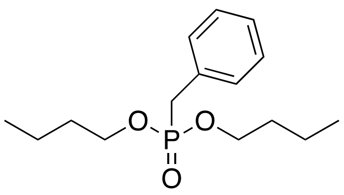
1-*O*-[*n*-butyl(butylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (1)



A solution of peracetylated glycosyl phosphonate **16a** (54 mg, 0.11 mmol, 1.0 equiv.) in dry methanol (2.0 mL) was cooled down with an ice-bath and subsequently treated with a catalytic amount of sodium metal. The reaction was allowed to stir at 0 ºC for 1h, at which point it was quenched by the addition of a scoop of silica gel and then evaporated. The resulting residue was purified by flash column chromatography (dry load) (DCM/MeOH gradient from

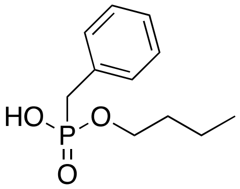
2% to 6%) to yield a 1:1 diastereomeric mixture of glycosyl phosphonate **1** (21 mg, 6.0 mmol, 55%) as a white foam. **^{1}H NMR** (400 MHz, MeOD-d_4) δ/ppm 5.25 (td, J = 7.6, 2.5 Hz, 1H), 5.22 (td, J = 7.8, 2.7 Hz, 1H), 4.06 – 4.99 (m, 6H), 3.86 (d, J = 12.1 Hz, 2H), 3.60 – 3.76 (m, 4H), 3.37 – 3.46 (m, 4H), 1.81 – 1.97 (m, 4H), 1.53 – 1.73 (m, 8H), 1.36 – 1.51 (m, 8H), 0.90 – 1.01 (m, 12H); **^{19}F{^{1}H} NMR** (376 MHz, MeOD-d_4) δ/ppm -200.7; **^{19}F NMR** (376 MHz, MeOD-d_4) δ/ppm -200.7 (ddd, J = 51.8, 15.0, 2.7 Hz); **^{31}P{^{1}H} NMR** (162 MHz, MeOD-d_4) δ/ppm 27.9, 27.4; **^{13}C NMR** (101 MHz, MeOD-d_4) δ/ppm 96.8 (dd, $^{2}J_{C\text{-}F}$ = 24.2, $^{2}J_{C\text{-}P}$ = 5.9 Hz), 96.4 (dd, $^{2}J_{C\text{-}F}$ = 24.2, $^{2}J_{C\text{-}P}$ = 6.6 Hz), 93.9 (d, $^{1}J_{C\text{-}F}$ = 187.1 Hz), 94.7 (d, $^{1}J_{C\text{-}F}$ = 186.33 Hz), 79.11, 79.08, 76.1, 75.9, 70.9, 70.8, 67.0 (d, J = 7.3 Hz), 66.9 (d, J = 7.3 Hz), 62.3, 62.2, 33.6, 33.5, 27.0 (d, $^{1}J_{C\text{-}P}$ = 139.4 Hz), 26.3 (d, $^{1}J_{C\text{-}P}$ = 142.3 Hz), 25.4 (d, J = 5.1 Hz), 25.3 (d, J = 5.1 Hz), 24.75, 24.73, 24.58, 24.55, 19.92, 19.89, 14.1, 14.0. **HRMS ESI** *m/z* calc. for $C_{14}H_{28}FO_7PNa$ [M+Na]$^+$: 381.1449, found: 381.1492.

Di-*n*-butyl benzylphosphonate (12b)



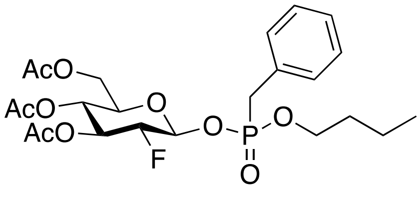
A solution of dibutyl phosphite (252 mg, 1.3 mmol, 1.0 equiv.) in dry THF (2.5 mL) was cooled with an ice-bath and subsequently treated with NaH (60%, mineral oil) (104 mg, 2.6 mmol, 2.0 equiv.) in one portion. After 30 min, benzyl bromide (0.23 mL, 1.95 mmol, 1.5 equiv.) was added dropwise under nitrogen atmosphere and the resulting mixture was allowed to stir overnight while gradually reaching room temperature; then it was quenched by the addition of methanol and evaporated to dryness under vacuum and the residue was partitioned between ethyl acetate and brine. The aqueous phase was extracted with ethyl acetate and the combined organic phases were dried over anhydrous sodium sulfate, filtered and evaporated. Purification by flash column chromatography (petroleum ether/EtOAc 4:1 to 7:3) yielded the desired benzylphosphonate **12b** (133 mg, 0.64 mmol, 49%) as a yellowish oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ/ppm 7.26 – 7.33 (m, 5H), 3.93 (tdd, J = 7.4, 7.4, 5.6, $^{3}J_{H\text{-}P}$ = 1.5 Hz, 4H), 3.15 (d, $^{2}J_{H\text{-}P}$ = 21.6 Hz, 2H), 1.56 (quin, J = 7.4 Hz, 4H), 1.32 (sxt, J = 7.4 Hz, 4H), 0.88 (t, J = 7.4 Hz, 6H). **^{31}P{^{1}H}** NMR (121 MHz, $CDCl_3$) δ/ppm 25.93. **^{13}C NMR** (75 MHz, $CDCl_3$) δ/ppm 131.7 (d, J = 9.2 Hz), 129.8 (d, J = 6.3 Hz, 2C), 128.5 (d, J = 2.9 Hz, 2C), 126.8 (d, J = 3.5 Hz), 65.8 (d, J = 6.9 Hz, 2C), 33.6 (d, J = 137.9 Hz), 32.5 (d, J = 6.3 Hz, 2C), 18.6 (2C), 13.6 (2C). **ESI-MS (low res)** *m/z* calc. for $C_{15}H_{25}O_3PNa$ [M+Na]$^+$: 307.1, found: 307.2.

Benzyl hydrogen butylphosphonate (13b)



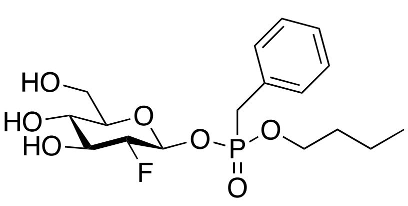
A solution of dibutyl benzylphosphonate **12b** (177 mg, 0.5 mmol) in dry DCM (10 mL) was treated with TMSI (1M, DCM) (0.6 mL, 0.6 mmol, 1.2 equiv.) at room temperature under nitrogen atmosphere and stirred for 2 h, after which another aliquot of 0.6 mL of TMSI (1M) was added. The resulting reaction mixture was stirred for another hour and then evaporated under vacuum and azeotropized with toluene until the distillate ran colorless to afford benzyl hydrogen butylphosphonate **13b**. This dried crude material was employed in the next step without further purification.

3,4,6-Tri-*O*-acetyl-1-*O*-[*n*-butyl(benzylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (16b)



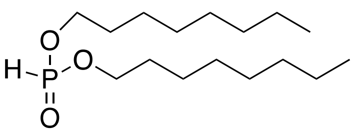
Benzyl hydrogen butylphosphonate **13b** (114 mg, 0.5 mmol, 1.0 equiv.) was dissolved in a mixture of dry THF (3 mL) and dry ACN (3 mL), treated with freshly activated 3 Å molecular sieves under inert atmosphere and stirred for 15 min at room temperature before cooling down with an ice-bath. To this mixture, a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide (278 mg, 0.75 mmol, 1.5 equiv.) in dry DCM (3 mL) was added, followed by silver carbonate (550 mg, 2.0 mmol, 4.0 equiv.) in one portion. At this point the flask was purged twice with vacuum/nitrogen, wrapped in foil and the reaction allowed to stir overnight at room temperature. Then, the mixture was filtered through a short Celite pad, washed with acetone and evaporated. The crude residue was purified by flash column chromatography (petroleum ether/EtOAc 3:1 to 1:1) to furnish a 1:1 diastereomeric mixture of peracetylated glycosyl phosphonate **16b** (132 mg, 0.25 mmol, 51%, two steps) as an amorphous solid. **^{1}H NMR** (400 MHz, $CDCl_3$) *δ*/ppm 7.35 – 7.21 (m, 10H), 5.44 – 5.24 (m, 4H), 5.07 (t, *J* = 9.9 Hz, 1H), 5.03 (t, *J* = 9.7 Hz, 1H), 4.32 – 3.92 (m, 6H), 3.86 – 3.74 (m, 2H), 3.25 (d, *J* = 22.4 Hz, 2H), 3.23 (dd, *J* = 22.2, 3.2 Hz, 2H), 2.09 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.04 (s, 6H), 2.03 (s, 3H), 1.62 – 1.50 (m, 4H), 1.37 – 1.22 (m, 8H), 0.91 – 0.82 (m, 6H); **^{19}F{^{1}H} NMR** (121 MHz, $CDCl_3$) *δ*/ppm -200.1, -200.5; **^{19}F NMR** (121 MHz, $CDCl_3$) *δ*/ppm -200.1 (ddd, *J* = 51.5, 14.8, 2.5 Hz), -200.5 (ddd, *J* = 51.5, 14.8, 2.5 Hz); **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) *δ*/ppm 26.5, 26.2; **ESI-MS (low res)** *m/z* calc. for $C_{23}H_{32}FO_{10}PNa$ $[M+Na]^+$: 541.2, found: 541.1.

1-*O*-[*n*-butyl(benzylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (2)



A solution of the tri-*O*-acetyl derivative **16b** (120 mg, 0.23 mmol, 1.0 equiv.) in dry methanol (3.5 mL) was cooled down with an ice-bath and subsequently treated with a catalytic amount of sodium metal. The reaction was allowed to stir at 0 ºC for 30 min, at which point it was quenched by the addition of a scoop of silica gel and then evaporated. The resulting residue was purified by flash column chromatography (dry load) (DCM/MeOH gradient from 2% to 6%) to yield a 1:1 diastereomeric mixture of glycosyl phosphonate **2 (**71 mg, 0.18 mmol,79%) as a white foam. **^{1}H NMR** (400 MHz, MeOD-d_4) δ/ppm 7.37 – 7.21 (m, 10H), 5.28 (td, J = 7.7, 2.7 Hz, 1H), 5.22 (td, J = 7.7, 2.7 Hz, 1H), 4.16 (dddd, J = 51.8, 8.6, 7.5, 5.6 Hz, 1H), 4.10 – 3.98 (m, 5H), 3.91 – 3.83 (m, 2H), 4.13 – 3.97 (m, 4H), 3.44 – 3.27 (m, 8H), 1.62 – 1.52 (m, 4H), 1.36 – 1.26 (m, 4H), 0.88 (td, J = 7.3, 5.1 Hz, 6H); **^{19}F{^{1}H} NMR** (376 MHz, MeOD-d_4) δ/ppm -200.4, -200.7; **^{19}F NMR** (376 MHz, MeOD-d_4) δ/ppm -200.4 (ddd, J = 51.8, 15.0, 2.7 Hz), -200.7 (ddd, J = 51.8, 15.0, 2.7 Hz); **^{31}P{^{1}H} NMR** (162 MHz, , MeOD-d_4) δ/ppm 27.9, 27.4; **^{13}C NMR** (101 MHz, MeOD-d_4) δ/ppm 132.5 (d, J = 10.3 Hz), 132.4 (d, J = 11.0 Hz), 131.4 (d, J = 6.6 Hz, 2C), 131.3 (d, J = 6.6 Hz, 2C), 129.7 (d, J = 2.9 Hz, 2C), 129.6 (d, J = 2.9 Hz, 2C), 128.3 (d, J = 3.7 Hz), 128.2 (d, J = 3.7 Hz), 97.1 (dd, $^{2}J_{C-F}$ = 24.2, $^{2}J_{C-P}$ = 5.9 Hz), 96.7 (dd, $^{2}J_{C-F}$ = 24.2, $^{2}J_{C-P}$ = 6.6 Hz), 93.8 (d, $^{1}J_{C-F}$ = 187.8 Hz), 93.7 (d, $^{1}J_{C-F}$ = 186.3 Hz), 79.2, 79.1, 76.1, 75.9, 70.9 (d, J = 8.1 Hz), 70.8 (d, J = 8.07 Hz), 67.6 (d, $^{2}J_{C-P}$ = 7.3 Hz), 67.5 (d, $^{2}J_{C-P}$ = 7.3 Hz), 62.2 (2C), 35.2 (d, $^{1}J_{C-P}$ = 137.2 Hz), 34.4 (d, $^{1}J_{C-P}$ = 139.4 Hz), 33.5, 33.4, 19.8, 19.7, 14.0, 13.9; **HRMS ESI** *m/z* calc. for $C_{17}H_{26}FO_7PNa$ [M+Na]$^+$: 415.1292, found: 415.1304.

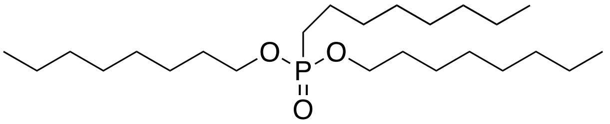
Dioctyl phosphite (11b)



To a solution of diethyl phosphite (2.58 mL, 20.0 mmol, 1.0 equiv.) and 1-octanol (11.1 mL, 70.0 mmol, 3.50 equiv.) was added NaH (80 mg of a 60% dispersion in mineral oil, 2.0 mmol, 0.10 equiv.). The resulting mixture was heated to 160 °C for 3 h in a flask set up for distillation. During this time, EtOH steadily distilled over. The reaction mixture was allowed to reach rt and vacuum was applied to the system to remove the excess of 1-octanol by distillation. The obtained dioctyl phosphite **11b** (5.20 g, 17.0 mmol, 84.9%) was used in the next step without further purification. **^{1}H NMR** (400 MHz, CDCl$_3$) δ/ppm 6.79 (d, J = 691.8 Hz, 1H), 4.22 – 3.82 (m, 4H), 1.81 – 1.60 (m, 4H), 1.44 – 1.17 (m, 20H), 0.96 – 0.79 (m, 6H); **^{31}P{^{1}H} NMR** (122

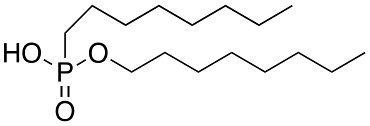
MHz, $CDCl_3$) δ/ppm 7.35; **^{13}C NMR** (101 MHz, $CDCl_3$) δ/ppm 65.9 (d, $^2J_{C-P}$ = 5.9 Hz), 31.9, 30.5 (d, $^3J_{C-P}$ = 6.2 Hz), 29.3, 29.2, 25.6, 22.8, 14.2; **ESI-MS (low res)** *m/z* calc. for $C_{16}H_{35}O_3PNa$ $[M+Na]^+$: 329.2, found: 329.1.

Di-*n*-octyl octylphosphonate (12c)



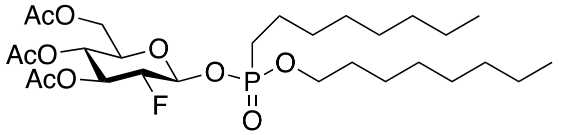
To a suspension of sodium (53.5 mg, 2.33 mmol, 1.25 equiv.) in toluene (1 mL) was slowly added a solution of dioctyl phosphite (0.57 g, 1.86 mmol, 1.0 equiv.) in toluene (0.5 mL). The reaction mixture was stirred for 30 min at rt and further 30 min at 50 °C to bring all of the sodium into solution. 1-Bromooctane (0.35 mL, 2.05 mmol, 1.10 equiv.) was added at rt and the reaction mixture refluxed for 16 h. The reaction was diluted with EtOAc (50 mL) and water (50 mL) and brine (25 mL) were added. The phases were separated and the aq. phase was extracted with EtOAc (25 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. The obtained crude product was purified by flash column chromatography (petroleum ether/EtOAc 4:1) to give dioctyl octylphosphonate **12c** (414 mg, 0.99 mmol, 53.1%) as a colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ/ppm 4.06 – 3.92 (m, 4H), 1.78 – 1.48 (m, 8H), 1.43 – 1.18 (m, 30H), 0.96 – 0.72 (m, 9H); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) δ/ppm 32.16; **^{13}C NMR** (101 MHz, $CDCl_3$) δ/ppm 65.6 (d, $^2J_{C-P}$ = 6.7 Hz, 2C), 32.0, 31.9 (2C), 30.76 (d, $^2J_{C-P}$ = 16.8 Hz), 30.75 (d, $^3J_{C-P}$ = 6.0 Hz, 2C), 29.34 (2C), 29.30 (2C), 29.21, 29.19, 25.7 (2C), 25.7 (d, $^1J_{C-P}$ = 140.4 Hz), 22.8 (3C), 22.6 (d, $^3J_{C-P}$ = 5.3 Hz), 14.2 (3C).

Octyl hydrogen octylphosphonate (13c)



Di-*n*-octyl octylphosphonate **12c** (75.4 mg, 0.18 mmol, 1.0 equiv.) was dissolved in pyridine (1.0 mL). To this solution NaI (135 mg, 0.90 mmol, 5.0 equiv.) was added and the reaction mixture was stirred for 18 h at 115 °C. The pyridine was removed under reduced pressure and the obtained residue suspended in H_2O (15 mL). The aq. phase was washed with Et_2O (2 x 10 mL), acidified to pH 1 using 2M aq. HCl soln. and extracted with CH_2Cl_2 (2 x 10 mL). The combined organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure to give a mixture of the desired octyl hydrogen octylphosphonate **13c** and the side product 1-octylpyridin-1-ium iodide. The two products were separated by extraction using Et_2O (3 x 2 mL) since the side product is insoluble in this solvent to give octyl hydrogen octylphosphonate **13c** (25.0 mg, 81.6 μmol, 45.3%) as a colorless oil. The product was used in the next step without further purification. **^{1}H NMR** (300 MHz, CDCl$_3$) δ/ppm 10.26 (brs, 1H), 3.98 (q, J = 6.8 Hz, 2H), 1.81 – 1.51 (m, 6H), 1.38 – 1.12 (m, 20H), 0.87 (t, J = 6.6 Hz, 6H); **^{31}P{^{1}H} NMR** (122 MHz, CDCl$_3$) δ/ppm 34.77; **^{13}C NMR** (75 MHz, CDCl$_3$) δ/ppm 65.0 (d, $^{2}J_{C\text{-}P}$ = 6.9 Hz), 31.97, 31.94, 30.7 (d, $^{2}J_{C\text{-}P}$ = 16.0 Hz), 30.6 (d, $^{3}J_{C\text{-}P}$ = 5.9 Hz), 29.35, 29.32, 29.26, 29.23, 26.0 (d, $^{1}J_{C\text{-}P}$ = 142.9 Hz), 25.7, 22.8 (2C), 22.4 (d, $^{3}J_{C\text{-}P}$ = 5.0 Hz), 14.2 (2C); **ESI-MS (low res)** *m/z* calc. for $C_{16}H_{34}O_3P$ [M–H]$^{-}$: 305.2, found: 305.1.

3,4,6-Tri-*O*-acetyl-1-*O*-[*n*-octyl(octylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (16c)

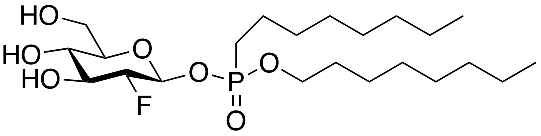


3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide (37.1 mg, 100 μmol, 1.20 equiv.) was dissolved in dry acetonitrile (1.0 mL). To this solution octyl hydrogen octylphosphonate **13c** (25.5 mg, 83.3 μmol, 1.0 equiv.) dissolved in dry acetonitrile (1.0 mL) and silver carbonate (55.2 mg, 200 μmol, 2.40 equiv.) were added at rt. The flask was covered in aluminum foil and the reaction mixture stirred for 16 h at rt. The suspension was diluted with EtOAc (15 mL) and filtered over a small pad of silica. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (petroleum ether/EtOAc 3:2) to give a 1:1 diastereomeric mixture of peracetylated glycosyl phosphonate **16c** (11.0 mg, 18.4 μmol, 22.1%) as an amorphous solid. **^{1}H NMR** (300 MHz, CDCl$_3$) δ/ppm 5.46 – 5.26 (m, 4H), 5.04 (t, J = 9.7 Hz, 2H), 4.37 (dt, J = 50.4, 8.3 Hz, 2H), 4.24 – 4.20 (m, 2H), 4.18 – 3.95 (m, 6H), 3.84[a] (ddd, J = 10.0, 4.9, 2.5 Hz, 1H) 3.83[a] (ddd, J = 9.8, 4.8, 2.4 Hz, 1H), 2.08 (s, 6H), 2.07 (s, 3H), 2.06 (s, 3H), 2.04 (s, 6H), 1.89 – 1.74 (m, 4H), 1.71 – 1.51 (m, 8H), 1.41 – 1.16 (m, 40H), 0.90 – 0.84 (m, 12H); **^{19}F{^{1}H} NMR** (282 MHz, CDCl$_3$) δ/ppm -200.31, -200.34; **^{19}F NMR** (282 MHz, CDCl$_3$) δ/ppm -200.27 (ddd, J = 51.1, 14.0, 2.3 Hz), -200.29 (ddd, J = 51.0, 13.7, 2.3 Hz); **^{31}P{^{1}H} NMR** (122 MHz, CDCl$_3$) δ/ppm 33.2, 32.9; **^{13}C NMR** (75 MHz, CDCl$_3$) δ/ppm 170.6, 170.5, 170.0 (2C), 169.7 (2C), 94.7 (dd, $^{2}J_{C\text{-}F}$ = 25.0, $^{2}J_{C\text{-}P}$ = 4.6 Hz), 94.2 (dd, $^{2}J_{C\text{-}F}$ = 24.0, $^{2}J_{C\text{-}P}$ = 6.1 Hz), 89.6 (dm, $^{1}J_{C\text{-}F}$ = 193.2 Hz, 2C), 72.64 (d, $^{2}J_{C\text{-}F}$ = 19.5 Hz, 2C), 72.61 (2C), 67.9 (d, $^{3}J_{C\text{-}F}$ = 7.2 Hz, 2C), 65.7 (d, $^{2}J_{C\text{-}P}$ = 7.2 Hz), 65.6 (d, $^{2}J_{C\text{-}P}$ = 6.9 Hz), 61.7, 61.6, 31.9 (4C), 30.59 (d, $^{2}J_{C\text{-}P}$ = 17.2 Hz), 30.56 (d, $^{2}J_{C\text{-}P}$ = 17.8 Hz), 30.4 (d, $^{3}J_{C\text{-}P}$ = 6.1 Hz, 2C), 29.32, 29.29, 29.26, 29.18 (2C), 29.16 (2C), 29.13, 26.9 (d, $^{1}J_{C\text{-}P}$ = 139.2 Hz), 26.08 (d, $^{1}J_{C\text{-}P}$ = 142.9 Hz), 25.7, 25.6, 22.8 (4C), 22.1 (d, $^{3}J_{C\text{-}P}$= 4.8 Hz), 22.0 (d, $^{3}J_{C\text{-}P}$ = 4.4 Hz), 20.79 (2C), 20.75 (2C), 20.70 (2C), 14.2 (4C); **ESI-MS (low res)** *m/z* calc. for $C_{28}H_{50}FO_{10}PNa$ $[M+Na]^+$: 619.3, found: 619.3.

^{a}Observed as a d**d**dd (J = 9.8, **7.1**, 4.9, 2.3 Hz, 1H) at 3.83 ppm.

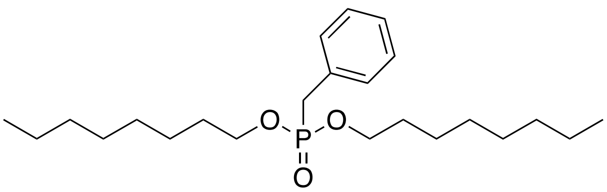
The highlighted doublet arises from a difference in the shift of the two sets of signals obtained from the two isomers. Therefore, it cannot be accounted as a doublet and the respective multiplet hence is reported as two different signals lacking the highlighted doublet.

1-*O*-[*n*-octyl(octylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (3)



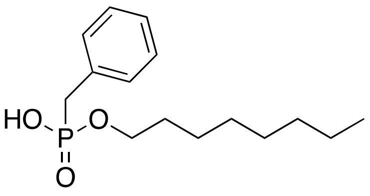
Peracetylated glycosyl phosphonate **16c** (10.1 mg, 17.0 µmol, 1.0 equiv.) was dissolved in dry MeOH (1.0 mL). To this solution a solution of Na (0.17 mg, 7.48 µmol, 0.44 equiv.) dissolved in dry MeOH (0.5 mL) was added. The reaction mixture was stirred for 30 min at rt and the reaction was quenched by the addition of one spatula of silica gel. The solvent was removed under reduced pressure and the impregnated silica gel was directly purified by flash column chromatography (DCM/MeOH 15:1) to give a 1:1 diastereomeric mixture of glycosyl phosphonate **3** (7.50 mg, 15.9 µmol, 93.8%) as an amorphous solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ/ppm 5.29 (td, J = 7.8, 2.9 Hz, 1H), 5.24 (td, J = 8.1, 2.4 Hz, 1H), 4.23 (dt, J = 51.6, 8.4 Hz, 1H), 4.22 (dt, J = 51.0, 8.7 Hz, 1H) 4.13 – 3.97 (m, 4H), 3.94 – 3.47 (m, 16H), 1.89 – 1.74 (m, 4H), 1.70 – 1.53 (m, 8H), 1.38 – 1.21 (m, 40H), 0.88 (t, J = 6.4 Hz, 12H); **^{19}F{^{1}H} NMR** (376 MHz, $CDCl_3$) δ/ppm -199.6, -199.7; **^{19}F NMR** (376 MHz, $CDCl_3$) δ/ppm -199.6 (ddd, J = 51.0, 14.9, 1.8 Hz), -199.7 (ddd, J = 50.8, 14.8, 1.8 Hz); **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) δ/ppm 33.9; **^{13}C NMR** (101 MHz, $CDCl_3$) δ/ppm 95.3 (dd, $^2J_{C\text{-}F}$ = 24.8, $^2J_{C\text{-}P}$ = 5.8 Hz), 95.0 (dd, $^2J_{C\text{-}F}$ = 24.6, $^2J_{C\text{-}P}$ = 6.8 Hz), 92.0 (dd, $^1J_{C\text{-}F}$ = 187.5, $^3J_{C\text{-}P}$ = 7.4 Hz, 2C), 77.0 (2C), 74.84 (d, $^2J_{C\text{-}F}$ = 17.2 Hz, 2C), 70.4 (d, $^3J_{C\text{-}F}$ = 7.7 Hz), 70.1 (d, $^3J_{C\text{-}F}$ = 7.6 Hz), 66.3 (d, $^2J_{C\text{-}P}$ = 7.7 Hz), 66.2 (d, $^2J_{C\text{-}P}$ = 7.9 Hz), 62.2, 61.9, 31.9 (4C), 30.6 (d, $^2J_{C\text{-}P}$ = 17.7 Hz, 2C), 30.4 (d, $^3J_{C\text{-}P}$ = 6.0 Hz), δ 30.3 (d, $^3J_{C\text{-}P}$ = 6.3 Hz), 29.32 (2C), 29.27, 29.25, 29.19, 29.18, 29.15, 29.12, 26.64 (d, $^1J_{C\text{-}P}$ = 140.5 Hz), 25.9 (d, $^1J_{C\text{-}P}$ = 141.7 Hz), 25.7, 25.6, 22.8 (4C), 22.1 (d, $^3J_{C\text{-}P}$ = 5.2 Hz), 22.0 (d, $^3J_{C\text{-}P}$ = 4.4 Hz), 14.2 (4C); **HRMS ESI** *m/z* calc. for $C_{22}H_{44}FO_7PNa$ $[M+Na]^+$: 493.2706, found: 493.2708.

Di-*n*-octyl benzylphosphonate (12d)



Dioctyl phosphite **11b** (3.06 g, 10.0 mmol, 1.0 equiv.) was dissolved in dry DMF (50 mL). To this solution Cs_2CO_3 (9.77 g, 30.0 mmol, 3.0 equiv.) and tetrabutylammonium iodide (11.1 g, 30.0 mmol, 3.0 equiv.) were added at rt and the reaction stirred for 1 h before benzyl bromide (3.56 mL, 30.0 mmol, 3.0 equiv.) was added. The reaction was stirred for 16 h at rt. H_2O (100 mL) and EtOAc (100 mL) were added and the aq. phase extracted with EtOAc (100 mL). The combined organic layers were washed with H_2O (3 x 50 mL) and brine (50 mL), dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography (petroleum ether/EtOAc 3:1) to give dioctyl benzylphosphonate **12d** (1.70 g, 4.29 mmol, 42.9%) as a colorless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ/ppm 7.72 – 6.59 (m, 5H), 4.10 – 3.66 (m, 4H), 3.12 (d, J = 21.6 Hz, 2H), 1.65 – 1.46 (m, 4H), 1.33 – 1.13 (m, 20H), 0.85 (t, J = 6.7 Hz, 6H); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) δ/ppm 25.85; **^{13}C NMR** (75 MHz, $CDCl_3$) δ/ppm 131.9 (d, J = 9.2 Hz), 129.9 (d, J = 6.6 Hz, 2C), 128.6 (d, J = 3.2 Hz, 2C), 126.9 (d, J = 3.7 Hz), 66.3 (d, J = 7.0 Hz, 2C), 33.8 (d, J = 138.2 Hz), 31.9 (2C), 30.7 (d, J = 6.0 Hz, 2C), 29.3 (2C), 29.2 (2C), 25.6 (2C), 22.8 (2C), 14.2 (2C).

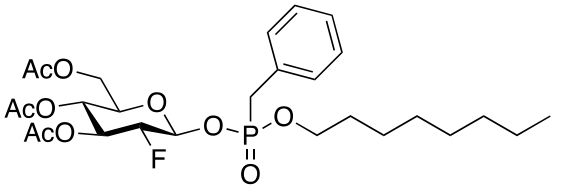
Benzyl hydrogen octylphosphonate (13d)



Dioctyl benzylphosphonate **12d** (301 mg, 0.76 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (10 ml) and cooled to 0 °C. To this solution TMSI (1.14 mL, 1.14 mmol, 1.50 equiv.) was added. The reaction mixture was stirred for 30 min at 0 °C and further for 2 h at rt. The reaction was quenched by the addition of H_2O (5 mL) and the CH_2Cl_2 was removed under reduced pressure. NaOH (1 mL, until pH 10-11) was added, and the aqueous phase was washed with Et_2O (2 x 10 mL). The aqueous phase was acidified with conc. HCl until the pH reached 1. Sat. aq. $Na_2S_2O_3$ (0.5 mL) was added, and the aqueous phase was extracted with CH_2Cl_2 (2 x 20 mL), the combined organic layers were dried over $MgSO_4$ and the solvent removed. The obtained crude product was suspended in Et_2O (5 mL) and filtered over Celite to give benzyl hydrogen octylphosphonate **13d** (125 mg, 440 μmol, 57.8%) as a colorless oil. The product was used in the next step without further purification. **^{1}H NMR** (400 MHz, $CDCl_3$) δ/ppm 11.85 (s, 1H), 7.66 – 7.00 (m, 5H), 3.82 (q, J = 6.7 Hz, 2H), 3.05 (d, J = 22.1 Hz, 2H), 1.66 – 1.49 (m, 2H), 1.39 – 1.17 (m, 10H), 0.90 (t, J = 6.9 Hz, 3H); **^{31}P{^{1}H}**

NMR (122 MHz, CDCl$_3$) δ/ppm 28.58; **^{13}C NMR** (101 MHz, CDCl$_3$) δ/ppm 131.7 (d, J = 9.2 Hz), 130.0 (d, J = 6.6 Hz, 2C), 128.5 (d, J = 3.1 Hz, 2C), 126.9 (d, J = 3.7 Hz), 65.7 (d, J = 7.3 Hz), 33.8 (d, J = 140.6 Hz), 31.9, 30.4 (d, J = 6.6 Hz), 29.3, 29.2, 25.5, 22.8, 14.2.

3,4,6-Tri-*O*-acetyl-1-*O*-[*n*-octyl(benzylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (16d)



3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide (200 mg, 0.54 mmol, 1.20 equiv.) was dissolved in dry acetonitrile (5.50 mL). To this solution benzyl hydrogen octylphosphonate **13d** (128 mg, 0.45 mmol, 1.0 equiv.) dissolved in dry acetonitrile (5.50 mL) and silver carbonate (271 mg, 1.08 mmol, 2.40 equiv.) were added at rt. The flask was covered in aluminum foil and the reaction mixture stirred for 16 h at rt. The suspension was diluted with EtOAc (50 mL) and filtered over a small pad of silica. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (petroleum ether/EtOAc 3:2) to give a 1:1 diastereomeric mixture of peracetylated glycosyl phosphonate **16d** (212 mg, 369 μmol, 82.0%) as an amorphous solid. However, fractions were obtained with just one diastereoisomer during the flash silica gel column chromatography.

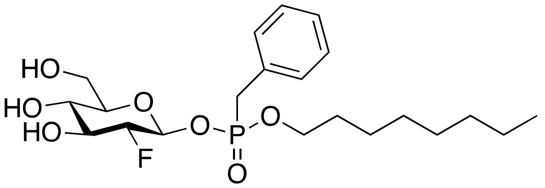
1:1 diastereomeric mixture

^{1}H NMR (300 MHz, CDCl$_3$) δ/ppm 7.35 – 7.15 (m, 10H), 5.45 – 5.19 (m, 4H), 5.06 (t, J = 10.0 Hz, 1H), 5.02 (t, J = 9.7 Hz, 1H), 4.49 – 3.89 (m, 10H), 3.85 – 3.71 (m, 2H), 3.24 (d, J = 22.3 Hz, 2H), 3.21 (dd, J = 22.3, 3.7 Hz, 2H), 2.08 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.03 (s, 6H), 2.02 (s, 3H), 1.63 – 1.46 (m, 4H), 1.34 – 1.15 (m, 20H), 0.92 – 0.79 (m, 6H); **^{19}F{^{1}H} NMR** (282 MHz, CDCl$_3$) δ/ppm -200.12, -200.48; **^{19}F NMR** (282 MHz, CDCl$_3$) δ/ppm -200.16 (ddd, J = 50.6, 13.7, 2.2 Hz), -200.44 (ddd, J = 50.6, 13.7, 2.2 Hz); **^{31}P{^{1}H} NMR** (122 MHz, CDCl$_3$) δ/ppm 26.4, 26.1; **^{13}C NMR** (75 MHz, CDCl$_3$) δ/ppm 170.44, 170.35, 169.81, 169.79, 169.54, 169.52, 130.9 (d, J = 9.9 Hz), 130.6 (d, J = 9.8 Hz), 130.0 (d, J = 6.9 Hz, 2C), 129.9 (d, J = 6.7 Hz, 2C), 128.5 (d, J = 3.3 Hz, 2C), 128.4 (d, J = 3.3 Hz, 2C), 127.1 (d, J = 3.9 Hz), 127.0 (d, J = 3.7 Hz), 95.0 – 94.2 (m, 2C), 90.4 – 88.3 (m, 2C), 72.6 – 72.3 (m, 4C), 67.7 (d, J = 9.2 Hz), 67.6 (d, J = 7.3 Hz), 66.2 (d, J = 7.5 Hz), 66.1 (d, J = 7.2 Hz), 61.5, 61.3, 34.8 (d, J = 137.4 Hz), 33.9 (d, J = 141.1 Hz), 31.8, 31.7, 30.2 (d, J = 6.6 Hz), 30.1 (d, J = 6.4 Hz), 29.15, 29.12, 29.05, 28.98, 25.4, 25.3, 22.6 (2C), 20.71, 20.64 (2C), 20.61, 20.56 (2C), 14.1 (2C); **ESI-MS (low res)** *m/z* calc. for $C_{27}H_{40}FO_{10}PNa$ [M+Na]$^+$: 597.2, found: 597.2.

Single diastereoisomer

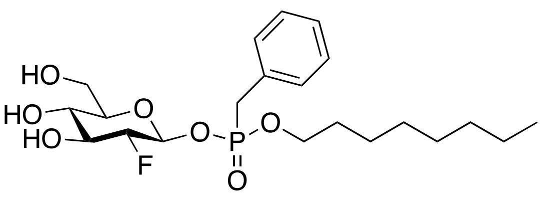
^{1}H NMR (300 MHz, $CDCl_3$) *δ*/ppm 7.50 – 7.10 (m, 5H), 5.40 (td, *J* = 7.8, 2.9 Hz, 1H), 5.31 (dt, *J* = 13.9, 9.4 Hz, 1H), 5.06 (t, *J* = 9.8 Hz, 1H), 4.35 (dt, *J* = 50.6, 8.5 Hz, 1H), 4.16 – 3.88 (m, 4H), 3.77 (ddd, *J* = 10.1, 4.6, 2.3 Hz, 1H), 3.24 (d, *J* = 22.3 Hz, 2H), 2.08 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.63 – 1.46 (m, 2H), 1.34 – 1.15 (m, 10H), 0.87 (t, *J* = 6.7 Hz, 3H); **^{19}F{^{1}H} NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.48; **^{19}F NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.44 (ddd, *J* = 51.1, 13.9, 2.5 Hz); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) *δ*/ppm 26.43; **^{13}C NMR** (75 MHz, $CDCl_3$) *δ*/ppm 170.6, 169.9, 169.7, 131.0 (d, *J* = 9.8 Hz), 130.1 (d, *J* = 6.9 Hz, 2C), 128.5 (d, *J* = 3.2 Hz, 2C), 127.1 (d, *J* = 3.7 Hz), 95.0 (dd, *J* = 23.8, 4.8 Hz), 89.5 (dd, *J* = 192.9, 7.4 Hz), 72.58 (d, *J* = 19.6 Hz), 72.57, 67.8 (d, *J* = 7.3 Hz), 66.2 (d, *J* = 7.7 Hz), 61.4, 34.9 (d, *J* = 137.6 Hz), 31.9, 30.3 (d, *J* = 6.3 Hz), 29.2, 29.1, 25.4, 22.8, 20.83, 20.75, 20.68, 14.2.

1-*O*-[*n*-octyl(benzylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (4) (1:1 mixture of isomers)



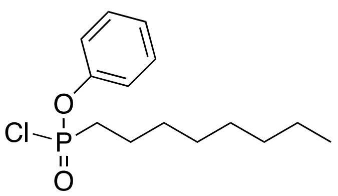
Peracetylated glycosyl phosphonate **16d** (77.0 mg, 133 μmol, 1.0 equiv.) was dissolved in dry MeOH (8.0 mL). To this solution a solution of Na (4.0 mg, 173 μmol, 1.30 equiv.) dissolved in dry MeOH (4.0 mL) was added. The reaction mixture was stirred for 30 min at rt and the reaction was quenched by the addition of one spatula of silica gel. The solvent was removed under reduced pressure and the impregnated silica gel was directly purified by flash column chromatography (DCM/MeOH 15:1) to give a 1:1 diastereomeric mixture of glycosyl phosphonate **4** (50.0 mg, 111 mmol, 83.0%) as an amorphous solid. **^{1}H NMR** (300 MHz, $CDCl_3$) *δ*/ppm 7.35 – 7.16 (m, 10H), 5.30 – 5.16 (m, 2H), 4.24 (dt, *J* = 51.3, 8.5 Hz, 1H), 4.21 (dt, *J* = 51.4, 8.4 Hz, 1H), 4.04 – 3.69 (m, 12H), 3.56 – 3.41 (m, 4H), 3.25 (d, *J* = 22.3 Hz, 2H), 3.22 (dd, *J* = 22.2, 3.3 Hz, 2H), 1.59 – 1.44 (m, 4H), 1.35 – 1.14 (m, 20H), 0.87 (d, *J* = 6.8 Hz, 6H); **^{19}F{^{1}H} NMR** (282 MHz, $CDCl_3$) *δ*/ppm -199.8, -200.1; **^{19}F NMR** (282 MHz, $CDCl_3$) *δ*/ppm -199.7 (dd, *J* = 51.7, 15.1 Hz), -200.1 (dd, *J* = 51.5, 14.8 Hz); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) *δ*/ppm 27.1, 26.8; **^{13}C NMR** (75 MHz, $CDCl_3$) *δ*/ppm 130.5 (d, *J* = 9.8 Hz), 130.3 (d, *J* = 9.9 Hz), 130.0 (d, *J* = 6.6 Hz, 2C), 129.9 (d, *J* = 6.6 Hz, 2C), 128.6 (d, *J* = 3.2 Hz, 2C), 128.5 (d, *J* = 3.1 Hz, 2C), 127.2 (d, *J* = 3.4 Hz, 2C), 95.7 – 95.0 (m, 2C), 92.9 – 90.9 (m, 2C), 77.0 (2C), 74.8 (d, *J* = 17.4 Hz), 74.5 (d, *J* = 17.3 Hz), 70.0 (d, *J* = 7.8 Hz), 69.8 (d, *J* = 7.0 Hz), 66.8 (d, *J* = 7.1 Hz), 66.7 (d, *J* = 7.8 Hz), 61.8, 61.6 34.5 (d, *J* = 139.5 Hz), 33.7 (d, *J* = 142.1 Hz) 31.77, 31.75, 30.2 (d, *J* = 6.4 Hz), 30.1 (d, *J* = 6.6 Hz), 29.16, 29.14, 29.07, 29.05, 25.35, 25.32, 22.7 (2C), 14.1 (2C).

1-*O*-[*n*-octyl(benzylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (4) (single isomer)



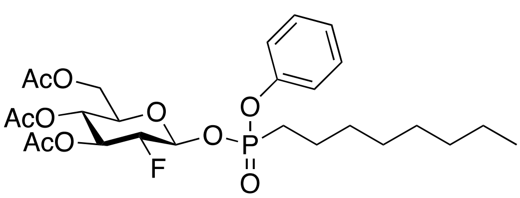
Diastereomerically pure peracetylated glycosyl phosphonate **16d** (14.4 mg, 25.0 μmol, 1.0 equiv.) was dissolved in dry MeOH (1.5 mL). To this solution a solution of Na (0.75 mg, 32.5 μmol, 1.30 equiv.) dissolved in dry MeOH (0.75 mL) was added. The reaction mixture was stirred for 30 min at rt and the reaction was quenched by the addition of one spatula of silica gel. The solvent was removed under reduced pressure and the impregnated silica gel was directly purified by flash column chromatography (DCM/MeOH 15:1) to give a 40:1 diastereomeric mixture of glycosyl phosphonate **4** (8.0 mg, 17.8 μmol, 71.4%) as an amorphous solid. **^{1}H NMR** (300 MHz, $CDCl_3$) *δ*/ppm 7.36 – 7.18 (m, 5H), 5.26 (td, *J* = 7.6, 2.8 Hz, 1H), 4.69 (brs, 2H), 4.21 (dt, *J* = 51.4, 8.3 Hz, 1H), 4.08 – 3.87 (m, 2H), 3.85 – 3.63 (m, 3H), 3.60 – 3.39 (m, 2H), 3.25 (d, *J* = 22.3 Hz, 2H), 2.13 (brs, 1H), 1.61 – 1.46 (m, 2H), 1.37 – 1.16 (m, 10H), 0.88 (d, *J* = 6.7 Hz, 3H); **^{19}F{^{1}H} NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.21 ; **^{19}F NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.16 (ddd, *J* = 51.5, 15.1, 1.7 Hz); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) *δ*/ppm 27.0; **^{13}C NMR** (75 MHz, $CDCl_3$) *δ*/ppm 130.6 (d, *J* = 9.8 Hz), 130.2 (d, *J* = 6.8 Hz, 2C), 128.6 (d, *J* = 3.3 Hz, 2C), 127.3 (d, *J* = 3.4 Hz), 95.5 (dd, *J* = 24.2, 6.1 Hz), 92.0 (dd, *J* = 188.1, 7.1 Hz), 77.0, 74.8 (d, *J* = 17.4 Hz), 70.0 (d, *J* = 7.8 Hz), 66.8 (d, *J* = 7.8 Hz), 61.7, 34.5 (d, *J* = 138.6 Hz), 31.9, 30.3 (d, *J* = 6.6 Hz), 29.3, 29.2, 25.4, 22.8, 14.2; **HRMS ESI** *m/z* calc. for $C_{21}H_{34}FO_7PNa$ $[M+Na]^+$: 471.1924, found: 471.1927.

Phenyl n-octylphosphonochloridate (15a)



A solution of *n*-octylmagensium bromide (2.17 g, 10.0 mmol, 1.0 equiv.) in Et_2O (12 mL) was slowly added to a solution of $POCl_2Ph$ (1.50 mL, 10.0 mmol, 1.0 equiv.) in toluene (50 mL). The reaction mixture was stirred at rt overnight. The solvents were removed under reduced pressure, the obtained crude residue suspended in CH_2Cl_2 and filtered over Celite. The filtrate was concentrated and the crude product purified by flash column chromatography (CH_2Cl_2) to give phenyl n-octylphosphonochloridate **15a** (920 mg, 3.19 mmol, 31.9 %) as a slightly yellow oil. **^{1}H NMR** (300 MHz, $CDCl_3$) *δ*/ppm 7.37 (dd, *J* = 8.7, 7.0 Hz, 2H), 7.32 – 7.02 (m, 3H), 2.46 – 2.20 (m, 2H), 1.94 – 1.76 (m, 2H), 1.54 – 1.42 (m, 2H), 1.41 – 1.13 (m, 8H), 1.01 – 0.68 (m, 3H); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) *δ*/ppm 41.0; **^{13}C NMR** (75 MHz, $CDCl_3$) *δ*/ppm 149.7 (d, *J* = 11.7 Hz), 130.0 (2C), 126.0 (d, *J* = 1.3 Hz), 120.8 (d, *J* = 4.9 Hz, 2C), 34.1 (d, *J* = 121.4 Hz), 31.9, 30.2 (d, *J* = 19.4 Hz), 29.1 (2C), 22.7, 22.6 (d, *J* = 6.0 Hz), 14.2.

3,4,6-Tri-*O*-acetyl-1-*O*-[phenyl (*n*-octylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (16e)

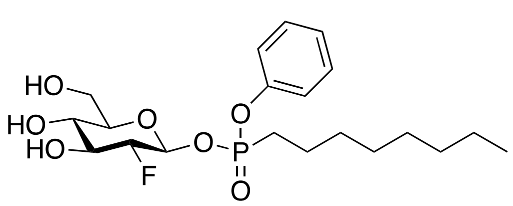


3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-glucopyranose (49.9 mg, 162 µmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (1.5 mL). To this solution 3Å molecular sieves was added and the suspension stirred for 30 min at rt before Et_3N (49.4 µL, 356 µmol, 2.20 equiv.) and phenyl n-octylphosphonochloridate **15a** (93.6 mg, 324 µmol, 2.0 equiv.) dissolved in dry CH_2Cl_2 (0.5 mL) were added. The reaction mixture was stirred over night at rt. The solvent was removed under reduced pressure and the crude product directly purified by flash column chromatography (petroleum ether/EtOAc 3:2) to give a 2:1 diastereomeric mixture of peracetylated glycosyl phosphonate **16e** (80.0 mg, 142 µmol, 88.1%) as an amorphous solid. **^{1}H NMR** (300 MHz, $CDCl_3$) *δ*/ppm 7.35 – 7.26 (m, 4H), 7.24 – 7.12 (m, 6H), 5.44[a] (dt, *J* = 7.9, 2.8 Hz, 1H), 5.41[a] (dt, *J* = 7.8, 2.7 Hz, 1H), 5.32[b] (td, *J* = 9.4, 4.5 Hz, 1H), 5.27[b] (td, *J* = 9.3, 4.5 Hz, 1H), 5.04 (t, *J* = 9.8 Hz, 2H), 4.52 – 4.31 (dm, *J* = 50.7 Hz, 2H), 4.26 (dd, *J* = 12.5, 5.1 Hz, 1H), 4.18 – 4.06 (m, 2H), 3.87 (dd, *J* = 12.5, 2.3 Hz, 1H), 3.80 (ddd, *J* = 10.1, 4.9, 2.3 Hz, 1H), 3.72 (ddd, *J* = 10.1, 4.4, 2.3 Hz, 1H), 2.07 (s, 6H), 2.06 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.82 – 1.62 (m, 4H), 1.49 – 1.36 (m, 4H), 1.34 – 1.18 (m, 20H), 0.96 – 0.77 (m, 6H); **^{19}F{^{1}H} NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.1, -200.4; **^{19}F NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.1 (ddd, *J* = 50.7, 13.9, 2.2 Hz), -200.4 (ddd, *J* = 50.7, 14.0, 2.4 Hz); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) *δ*/ppm 30.2, 29.7; **^{13}C NMR** (75 MHz, $CDCl_3$) *δ*/ppm 170.6, 170.5, 169.91, 169.89, 169.7, 169.6, 150.2 (d, *J* = 9.8 Hz), 149.8 (d, *J* = 9.7 Hz), 129.8 (2C), 129.6 (2C), 125.4, 125.3, 120.9 (d, *J* = 4.4 Hz, 2C), 120.7 (d, *J* = 4.4 Hz, 2C), 94.9 (dd, *J* = 24.1, 5.4 Hz), 94.5 (dd, *J* = 24.7, 6.7 Hz), 89.5 (dd, *J* = 193.1, 8.3 Hz), 89.4 (dd, *J* = 194.3, 7.3 Hz), 72.7 (2C), 72.5 (d, *J* = 7.7 Hz, 2C) (Hz should be around 17), 67.8 (d, *J* = 7.5 Hz), 67.7 (d, *J* = 7.3 Hz), 61.5, 61.4, 31.91, 31.89, 30.6 (d, *J* = 17.8 Hz), 30.5 (d, *J* = 18.0 Hz), 29.2 (2C), 29.1 (2C), 27.2 (d, *J* = 138.5 Hz), 26.3 (d, *J* = 142.2 Hz), 22.8 (2C), 22.1 (d, *J* = 6.5 Hz), 22.0 (d, *J* = 6.4 Hz), 20.8 (2C), 20.73 (2C), 20.67 (2C), 14.2 (2C); **ESI-MS (low res)** *m/z* calc. for $C_{26}H_{39}FO_{10}PNa$ $[M+Na]^+$: 583.2, found: 583.2.

[a] Observed as a tt (*J* = **7.8**, 2.7 Hz) at 5.43 ppm.

[b] Observed as a dtd (*J* = **13.7**, 9.2, 4.3 Hz) at at 5.29 ppm.

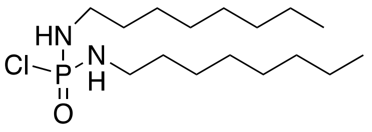
1-*O*-[Phenyl (*n*-octylphosphonyl)]-2-deoxy-2-fluoro-β-D-glucopyranose (5)



Peracetylated glycosyl phosphonate **16e** (16.8 mg, 30.0 µmol, 1.0 equiv.) was dissolved in dry MeOH (2.0 mL). To this solution a solution of Na (1.0 mg, 43.5 µmol, 1.45 equiv.) dissolved in dry MeOH (1.0 mL) was added. The reaction mixture was stirred for 1 h at rt and the reaction was quenched by the addition of one spatula of silica gel. The solvent was removed under reduced pressure and the impregnated silica gel was directly purified by flash column chromatography (DCM/MeOH 15:1) to give a 1:1 diastereomeric mixture of glycosyl phosphonate **5** (9.0 mg, 20.7 µmol, 69.1%) as an amorphous solid. **^{1}H NMR** (300 MHz, CD_2Cl_2) δ/ppm 7.50 – 7.30 (m, 4H), 7.28 – 7.05 (m, 6H), 5.31 – 5.24 (m, 2H), 4.21[a] (dt, J = 51.3, 7.5 Hz, 1H), 4.18[a] (dt, J = 51.4, 7.4 Hz, 1H), 3.85 – 3.51 (m, 6H), 3.49 – 3.31 (m, 4H), 2.13 – 1.91 (m, 4H), 1.82 – 1.62 (m, 4H), 1.50 – 1.38 (m, 4H), 1.36 – 1.21 (m, 16H), 1.02 – 0.65 (m, 6H); **^{19}F NMR{^{1}H}** (282 MHz, CD_2Cl_2) δ/ppm -200.5, -200.8; **^{19}F NMR** (282 MHz, CD_2Cl_2) δ/ppm -200.5 (dd, $^{2}J_{F\text{-}H}$ = 51.4, $^{3}J_{F\text{-}H}$ = 15.0 Hz), -200.8 (dd, $^{2}J_{F\text{-}H}$ = 51.53$^{3}J_{F\text{-}H}$ = 15.0 Hz); **^{31}P{^{1}H} NMR** (122 MHz, CD_2Cl_2) δ/ppm 30.4, 30.1; **^{13}C NMR** (75 MHz, CD_2Cl_2) δ/ppm 150.6 (d, J = 9.5 Hz), 50.5 (d, J = 9.2 Hz), 130.4 (2C), 130.2 (2C), 125.9, 125.9, 121.4 (d, J = 4.3 Hz, 2C), 121.2 (d, J = 4.3 Hz, 2C), 96.0 (dd, J = 24.7, 6.1 Hz), 95.6 (dd, J = 25.2, 7.1 Hz), 92.5 (dd, J = 187.3, 7.8 Hz), 92.4 (dd, J = 187.9, 7.2 Hz), 77.5, 77.3, 75.1 (d, J = 17.3 Hz, 2C), 70.40 (d, J = 7.6 Hz), 70.35 (d, J = 7.3 Hz), 62.1 (2C), 32.4 (2C), 30.9 (d, J = 17.5 Hz, 2C), 29.6 (2C), 29.5 (2C), 27.2 (d, J = 139.1 Hz), 26.5 (d, J = 141.2 Hz), 23.2 (2C), 22.5 (d, J = 5.2 Hz), 22.43 (d, J = 5.2 Hz), 14.4 (2C); **HRMS ESI** *m/z* calc. for $C_{20}H_{33}FO_7PNa$ $[M+Na]^+$: 457.1767, found: 457.1765.

[a] Observed as a dq (J = 51.4, 7.9 Hz, 1H) at 4.20 ppm.

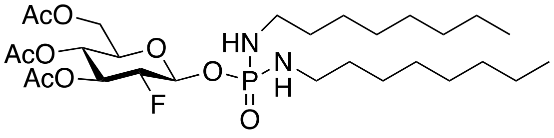
Octylphosphordiamidochloridate (15b)



A solution of *n*-octylamine (3.51 mL, 21.2 mmol, 2.0 equiv.) and Et_3N (3.09 mL, 2.23 mmol, 2.10 equiv.) in toluene (20 mL) was slowly added to a solution of $POCl_3$ (0.98 mL, 10.6 mmol, 1.0 equiv.) in toluene (50 mL) at rt. The reaction mixture was stirred over night at rt. The obtained suspension was filtered over Celite and the filtrate was concentrated under reduced pressure to give octylphosphordiamidochloridate **15b** (3.32 g, 9.80 mmol, 92.4%) as a colorless solid which was used in the next step without further purification. **^{1}H NMR** (300 MHz, $CDCl_3$) δ/ppm 3.14 – 2.91 (m, 1H), 1.67 – 1.47 (m, 4H), 1.36 – 1.15 (m, 20H), 0.95 – 0.77 (m, 6H);

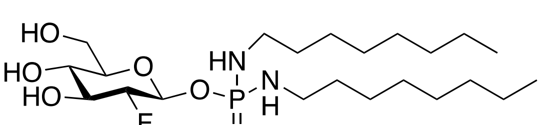
$^{31}P\{^1H\}$ NMR (122 MHz, $CDCl_3$) *δ*/ppm 20.9; **^{13}C NMR** (75 MHz, $CDCl_3$) *δ*/ppm 42.1, 31.9, 31.4 (d, $J = 7.7$ Hz), 29.3 (2C), 26.8, 22.8, 14.2.

3,4,6-Tri-*O*-acetyl-1-*O*-[((di-*n*-octylamino)phosphoryl)oxy]-2-deoxy-2-fluoro-β-D-glucopyranose (16f)



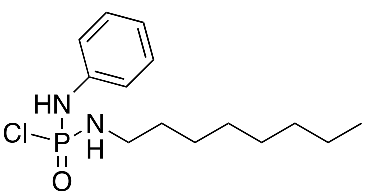
3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-glucopyranose (37.6 mg, 122 μmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (1.0 mL). To this solution 3Å molecular sieves was added and the suspension stirred for 30 min at rt before Et_3N (28.8 μL, 207 μmol, 1.70 equiv.) and phosphordiamidochloridate **15b** (62.0 mg, 183 μmol, 1.50 equiv.) were added at 0 °C. The reaction mixture was stirred for 20 min at 0 °C and overnight at rt. The solvent was removed under reduced pressure and the crude product directly purified by flash column chromatography (petroleum ether/EtOAc 2:1 to 3:2 to pure EtOAc) to give peracetylated glycosyl phosphordiamidate **16f** (19.0 mg, 31.1 μmol, 25.5%) as an amorphous solid. **^1H NMR** (300 MHz, $CDCl_3$) *δ*/ppm 5.37 (dt, $J = 8.1$, 2.7 Hz, 1H), 5.31 (dt, $J = 14.0$, 9.3 Hz, 1H), 5.04 (t, $J = 9.8$ Hz, 1H), 4.32 (dt, $J = 50.5$, 8.4 Hz, 1H), 4.27 – 4.07 (m, 2H), 3.82 (ddd, $J = 10.2$, 4.6, 2.3 Hz, 1H), 2.92 (q, $J = 7.9$ Hz, 4H), 2.51 (brs, 2H), 2.07 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.58 – 1.42 (m, 4H), 1.31 – 1.17 (m, 20H), 0.95 – 0.79 (m, 6H); **$^{19}F\{^1H\}$ NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.40; **^{19}F NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.36 (ddd, $J = 50.4$, 13.9, 2.2 Hz); **$^{31}P\{^1H\}$ NMR** (122 MHz, $CDCl_3$) *δ*/ppm 15.21; **^{13}C NMR** (75 MHz, $CDCl_3$) *δ*/ppm 170.6, 170.0, 169.7, 94.8 (dd, $J = 23.7$, 3.8 Hz), 89.6 (dd, $J = 192.4$, 7.8 Hz), 72.7 (d, $J = 20.3$ Hz), 72.6, 67.9 (d, $J = 7.4$ Hz), 61.6, 41.4, 31.93, 31.92 (d, $J = 5.7$ Hz), 29.4, 29.3, 26.8 (d, $^3J_{C\text{-}P} = 5.7$ Hz), 22.8, 20.84, 20.76, 20.70, 14.2; **ESI-MS (low res)** *m/z* calc. for $C_{25}H_{52}FO_9PNa$ $[M+Na]^+$: 633.3, found: 633.4.

1-*O*-[((Di-*n*-octylamino)phosphoryl)oxy]-2-deoxy-2-fluoro-β-D-glucopyranose (6)



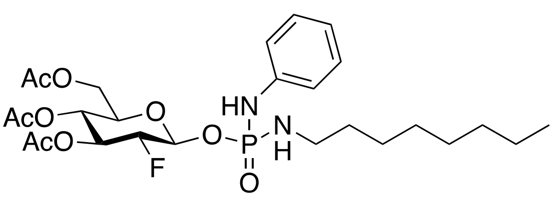
Peracetylated glycosyl phospordiamidate **16f** (15.3 mg, 25.0 μmol, 1.0 equiv.) was dissolved in dry MeOH (1.5 mL). To this solution a solution of Na (0.75 mg, 32.5 μmol, 1.30 equiv.) dissolved in dry MeOH (0.75 mL) was added. The reaction mixture was stirred for 30 min at rt and the reaction was quenched by the addition of one spatula of silica gel. The solvent was removed under reduced pressure and the impregnated silica gel was directly purified by flash column chromatography (DCM/MeOH 10:1) to give a 1:1 diastereomeric mixture of glycosyl phosphordiamidate **6** (12.0 mg, 24.8 μmol, 99.1%) as an amorphous solid. **^{1}H NMR** (300 MHz, CDCl$_3$) *δ*/ppm 5.23 (td, *J* = 8.3, 2.7 Hz, 1H), 4.20 (dt, *J* = 51.4, 8.1 Hz, 1H), 3.97 – 3.69 (m, 3H), 3.59 – 3.38 (m, 2H), 3.20 – 2.97 (m, 2H), 2.95 – 2.80 (m, 4H), 1.56 – 1.42 (m, 4H), 1.36 – 1.19 (m, 20H), 0.96 – 0.79 (m, 6H); **^{19}F{^{1}H} NMR** (282 MHz, CDCl$_3$) *δ*/ppm -199.7; **^{19}F NMR** (282 MHz, CDCl$_3$) *δ*/ppm -199.62 (dd, *J* = 51.6, 15.4 Hz); **^{31}P{^{1}H} NMR** (122 MHz, CDCl$_3$) *δ*/ppm 16.4; **^{13}C NMR** (75 MHz, CDCl$_3$) *δ*/ppm 95.1 (dd, *J* = 23.1, 4.4 Hz), 91.8 – 86.9 (m), 77.3, 74.7 (d, *J* = 16.9 Hz), 70.3 (d, *J* = 7.3 Hz), 61.9, 41.3 (d, *J* = 8.9 Hz), 32.0, 31.9 (d, *J* = 7.8 Hz), 29.5 (2C), 26.9, 22.8, 14.2; **HRMS ESI** *m/z* calc. for $C_{22}H_{46}FN_2O_6PNa$ [M+Na]$^+$: 507.2975, found: 507.2977.

Octyl-phenylphosphordiamidochloridate (15c)



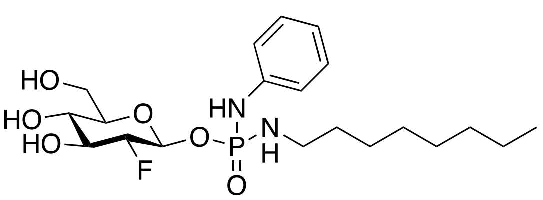
A solution of *n*-octylamine (0.55 mL, 3.30 mmol, 1.0 equiv.) and Et$_3$N (0.48 mL, 3.47 mmol, 1.05 equiv.) in toluene (7 mL) was added dropwise to a solution of POCl$_3$ (0.31 mL, 3.30 mmol, 1.0 equiv.) at 0 °C. The reaction mixture was stirred over night at rt. The obtained suspension was filtered over Celite and the filtrate concentrated under reduced pressure to give octylphosphoramidic dichloride **14b** (0.80 g, 3.25 mmol, 99%) as an intermediate which was used without further purification. Crude octylphosphoramidic dichloride **14b** (0.74 g, 3.0 mmol, 1.0 equiv.) was dissolved in toluene (25 mL). To this solution Et$_3$N (0.44 mL, 3.15 mmol, 1.05 equiv.) and aniline (0.27 mL, 3.0 mmol, 1.0 equiv.) were added dropwise at 0 °C. The reaction mixture was stirred overnight at rt. The obtained suspension was filtered over Celite and the filtrate concentrated under reduced pressure. The crude residue was purified by flash column chromatography (petroleum ether/EtOAc 3:2) to give octyl phenylphosphor-diamidochloridate **15c** (90.0 mg, 0.30 mmol, 10%). **^{1}H NMR** (400 MHz, CDCl$_3$) *δ*/ppm 7.31 (t, *J* = 7.7 Hz, 2H), 7.14 (d, *J* = 7.9 Hz, 2H), 7.08 (t, *J* = 7.4 Hz, 1H), 5.47 (s, 1H), 3.30 (s, 1H), 3.13 – 2.98 (m, 2H), 1.61 – 1.49 (m, 2H), 1.37 – 1.13 (m, 10H), 0.90 (t, *J* = 6.9 Hz, 3H); **^{31}P{^{1}H} NMR** (162 MHz, CDCl$_3$) *δ*/ppm 12.17.

3,4,6-Tri-*O*-acetyl-1-*O*-[((*n*-octylamino)(phenylamino)phosphoryl)oxy]-2-deoxy-2-fluoro-β-D-glucopyranose (16g)



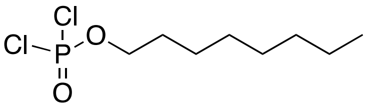
3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-glucopyranose (31.1 mg, 104 µmol, 1.0 equiv.) was dissolved in dry CH$_2$Cl$_2$ (1.0 mL). To this solution 3Å molecular sieves was added and the suspension stirred for 30 min at rt before Et$_3$N (24.5 µL, 177 µmol, 1.70 equiv.) and phosphordiamidochloridate **15c** (47.2 mg, 156 µmol, 1.50 equiv.) dissolved in dry CH_2Cl_2 (1.0 mL) were added at 0 °C. The reaction mixture was stirred for 20 min at 0 °C and overnight at rt. The solvent was removed under reduced pressure and the crude product directly purified by flash column chromatography (petroleum ether/EtOAc 2:3 to 5:8 to 1:3) to give peracetylated glycosyl phosphordiamidate **16g** (6.00 mg, 10.4 µmol, 10.0%) as an amorphous solid. **ESI-MS (low res)** *m/z* calc. for $C_{26}H_{40}FN_2O_9PNa$ $[M+Na]^+$: 597.2, found: 597.2.

1-*O*-[((*n*-octylamino)(phenylamino)phosphoryl)oxy]-2-deoxy-2-fluoro-β-D-glucopyranose (7)



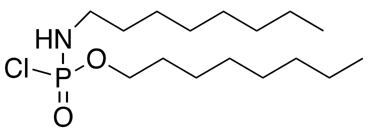
Peracetylated glycosyl phosphordiamidate **16g** (5.75 mg, 10.0 µmol, 1.0 equiv.) was dissolved in dry MeOH (0.8 mL). To this solution a solution of Na (0.40 mg, 17.4 µmol, 1.74 equiv.) dissolved in dry MeOH (0.40 mL) was added. The reaction mixture was stirred for 45 min at rt and the reaction was quenched by the addition of one spatula of silica gel. The solvent was removed under reduced pressure and the impregnated silica gel was directly purified by flash column chromatography (DCM/MeOH 10:1) to give a 1:1 diastereomeric mixture of glycosyl phosphordiamidate **7** (1.40 mg, 3.12 µmol, 31.2%) as an amorphous solid. **^{1}H NMR** (400 MHz, CD_3CN) *δ*/ppm 7.22 (t, *J* = 7.9 Hz, 4H), 7.11 – 7.05 (m, 4H), 6.92 (td, *J* = 7.4, 1.2 Hz, 2H), 5.97 (d, *J* = 8.5 Hz, 2H), 5.27 (td, *J* = 8.5, 8.1, 3.1 Hz, 1H), 5.25 (td, *J* = 7.4, 3.0 Hz, 1H), 4.10 (dt, *J* = 51.4, 8.3 Hz, 1H), 4.09 (dt, *J* = 51.4, 8.3 Hz, 1H), 3.78 (dd, *J* = 12.1, 2.2 Hz, 1H), 3.76 (dd, *J* = 12.1, 2.5 Hz, 1H), 3.69 – 3.55 (m, 6H), 3.44 – 3.32 (m, 4H), 2.94 – 2.81 (m, 4H), 1.47 – 1.36 (m, 4H), 1.33 – 1.19 (m, 20H), 0.88 (t, *J* = 6.9 Hz, 6H); **^{19}F{^{1}H} NMR** (376 MHz, CD_3CN) *δ*/ppm -200.00, -200.09; **^{19}F NMR** (376 MHz, CD_3CN) *δ*/ppm -200.00 (ddd, *J* = 51.4, 15.5, 2.6 Hz), -200.09 (ddd, *J* = 514, 15.5, 2.8 Hz); **^{31}P{^{1}H} NMR** (162 MHz, CD_3CN) *δ*/ppm 7.7, 7.8; **HRMS ESI** *m/z* calc. for $C_{20}H_{34}FN_2O_6PNa$ $[M+Na]^+$: 471.2036, found: 471.2032.

n-Octylphosphorodichloridate (14c)



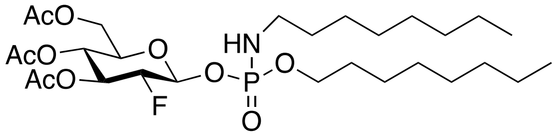
n-Octanol (3.92 mL, 25.0 mmol, 1.0 equiv.) was added dropwise to $POCl_3$ (2.57 mL, 27.5 mmol, 1.10 equiv.) and the reaction mixture stirred for 3 h at rt. The small excess of $POCl_3$ was removed under reduced pressure via distillation to give n-octylphosphorodichloridate **14c** (6.20 g, 25.0 mmol, quant.) as a colorless oil which was used in the next step without further purification. **^{1}H NMR** (300 MHz, $CDCl_3$) δ/ppm 4.33 (dt, J = 9.8, 6.5 Hz, 2H), 1.97 – 1.69 (m, 2H), 1.49 – 1.21 (m, 10H), 0.96 – 0.80 (m, 3H); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) δ/ppm 6.62; **^{13}C NMR** (101 MHz, $CDCl_3$) δ/ppm 72.6 (d, J = 9.8 Hz), 31.8, 29.7 (d, J = 8.8 Hz), 29.2, 29.0, 25.3, 22.7, 14.2.

***n*-Octyl *n*-octylphosphoramidochloridate (15d)**



To a solution of 1-octylamine (1.66 mL, 10.0 mmol, 1.0 equiv.) and NEt_3 (1.46 mL, 10.5 mmol, 1.05 equiv) in toluene (20 mL) was added dropwise a solution of $POCl_2OOct$ (2.47 g, 10.0 mmol, 1.0 equiv.) in toluene (50 mL) at rt. The reaction was stirred for 2 h at rt and was filtered over Celite to remove the obtained $NEt_3 \cdot HCl$ salt. The filtrate was concentrated under reduced pressure and the crude product purified by flash column chromatography (petroleum ether/EtOAc 3:2) to give octyl octylphosphoramidochloridate **15d** (2.62 g, 7.71 mmol, 77.1%) as a colorless oil. **^{1}H NMR** (300 MHz, $CDCl_3$) δ/ppm 4.29 – 3.97 (m, 2H), 3.26 (s, 1H), 3.12 – 2.87 (m, 2H), 1.79 – 1.64 (m, 2H), 1.61 – 1.46 (m, 2H), 1.45 – 1.18 (m, 20H), 0.93 – 0.82 (m, 6H); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) δ/ppm 14.8; **^{13}C NMR** (75 MHz, $CDCl_3$) δ/ppm 68.2 (d, J = 6.4 Hz), 42.1 (d, J = 1.9 Hz), 31.91, 31.88, 31.0 (d, J = 7.7 Hz), 30.0 (d, J = 8.0 Hz), 29.31, 29.27 (2C), 29.17, 26.7, 25.6, 22.8 (2C), 14.2 (2C).

3,4,6-Tri-*O*-acetyl-1-*O*-[(*n*-octyl *n*-octylphosphoramidoyl)oxy]-2-deoxy-2-fluoro-β-D-glucopyranose (16h)



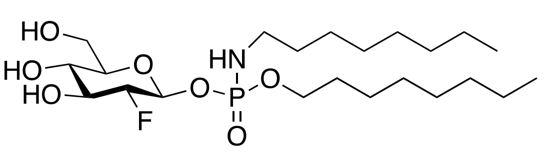
3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-glucopyranose (29.6 mg, 96.0 μmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (1.0 mL). To this solution 3Å molecular sieves was added and the suspension stirred for 30 min at rt before Et_3N (29.3 μL, 211 μmol, 2.20 equiv.) and phosphoramidochloridate **15d** (65.3 mg, 192 μmol, 2.0 equiv.) dissolved in dry CH_2Cl_2 (0.5 mL) were added. The reaction mixture was stirred overnight at rt. The solvent was removed under reduced pressure and the crude product directly purified by flash column chromatography (petroleum ether/EtOAc 1:1) to give a 1:1 diastereomeric mixture of peracetylated glycosyl phosphoramidate **16h** (48.0 mg, 78.5 μmol, 81.7%) as an amorphous solid. **^{1}H NMR** (300 MHz, $CDCl_3$) δ/ppm 5.40 – 5.25 (m, 2H), 5.04^{a} (t, J = 9.8 Hz, 1H), 5.04^{a} (t, J = 9.8 Hz, 0.5H), 4.36 (dt, J = 50.4, 8.4 Hz, 1H), 4.23 – 4.08 (m, 2H), 4.07 – 3.95 (m, 2H), 3.81[b] (ddd, *J* = 10.0, 4.2, 2.3 Hz, 1H), 3.80[b] (ddd, *J* = 10.6, 4.4, 2.3 Hz, 0.5H), 2.98 – 2.85 (m, 2H), 2.71 – 2.58 (m, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.73 – 1.60 (m, 2H), 1.53 – 1.42 (m, 2H), 1.38 – 1.19 (m, 22H), 0.90 – 0.81 (m, 6H); **^{19}F{^{1}H} NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.38, -200.42; **^{19}F NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.34 (ddd, *J* = 50.5, 13.8, 2.3 Hz), -200.38 (ddd, *J* = 50.7, 13.8, 2.9 Hz); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) *δ*/ppm 7.4, 7.8; **^{13}C NMR** (75 MHz, $CDCl_3$) *δ*/ppm 170.6 (2C), 170.0, 169.9, 169.7, 169.6, 95.3 (dd, J = 23.7, 5.1 Hz), 95.2 (dd, J = 24.0, 5.6 Hz), 89.6 (dd, J = 192.4, 8.2 Hz, 2C), 72.63 (2C), 72.61 (d, J = 19.6 Hz, 2C), 67.8 (d, J = 7.3 Hz, 2C), 67.3 (d, J = 5.6 Hz), 67.2 (d, J = 5.6 Hz), 61.6 (2C), 41.5 (2C), 31.9 (4C), 31.7 (d, J = 6.4 Hz), 31.6 (d, J = 6.6 Hz), δ 30.34 (d, J = 7.5 Hz), 30.30 (d, J = 7.4 Hz), 29.50 – 29.10 (8C), 26.7, 26.6, 25.61, 25.6, 22.7 (4C), 20.8 (2C), 20.72 (2C), 20.66 (2C), 14.2 (4C); **ESI-MS (low res)** *m/z* calc. for $C_{28}H_{51}FO_{11}PNa$ $[M+Na]^+$: 634.3, found: 634.4.

[a] Observed as a t**d** (*J* = 9.8, **1.6** Hz, 1H) at 5.04 ppm

[b] Observed as a d**d**dd (*J* = *J* = 10.6, **4.5**, 4.4, 2.3 Hz, 1H) at 3.81 ppm

The highlighted doublets arise from a difference in the shift of the two sets of signals obtained from the two isomers. Therefore, they cannot be accounted as a doublet and the respective multiplet hence is reported as two different signals lacking the highlighted doublet.

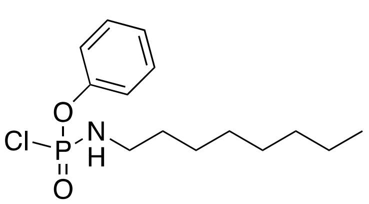
1-*O*-[(*n*-octyl *n*-octyloxyphosphoramidoyl)oxy]-2-deoxy-2-fluoro-β-D-glucopyranose (8)



Peracetylated glycosyl phosphoramidate **16h** (30.6 mg, 50.0 μmol, 1.0 equiv.) was dissolved in dry MeOH (3.0 mL). To this solution a solution of Na (1.49 mg, 65.0 μmol, 1.30 equiv.) dissolved in dry MeOH (1.5 mL) was added. The reaction mixture was stirred for 30 min at rt and the reaction was quenched by the addition of one spatula of silica gel. The solvent was removed under reduced pressure and the impregnated silica gel was directly purified by flash column chromatography (DCM/MeOH 12:1) to give a 1:1 diastereomeric mixture of glycosyl phosphoramidate **8** (19.0 mg, 39.1 μmol, 78.3%) as an amorphous solid. **^{1}H NMR** (300 MHz, CD_2Cl_2) *δ*/ppm 5.23 (td, *J* = 7.3, 2.7 Hz, 1H), 5.18 (td, *J* = 8.3, 3.0 Hz, 1H), 4.24 (dt, *J* = 51.3, 8.4 Hz, 1H), 4.21 (dt, *J* = 51.3, 8.3 Hz, 1H), 4.10 – 3.97 (m, 4H), 3.96 – 3.85 (m, 2H), 3.83 – 3.68 (m, 4H), 3.59 – 3.41 (m, 6H), 3.01 – 2.80 (m, 4H), 1.78 – 1.63 (m, 4H), 1.57 – 1.44 (m, 4H), 1.30 (d, *J* = 6.8 Hz, 40H), 0.97 – 0.82 (m, 12H); **^{19}F NMR{^{1}H}** (282 MHz, CD_2Cl_2) *δ*/ppm -200.2, -200.3; **^{19}F NMR** (282 MHz, CD_2Cl_2) *δ*/ppm -200.2 (ddd, $^{2}J_{F-H}$ = 51.3, $^{3}J_{F-H}$ = 15.2, $^{3}J_{F-H}$ = 2.1 Hz), -200.3 (ddd, $^{2}J_{F-H}$ = 51.53$^{3}J_{F-H}$

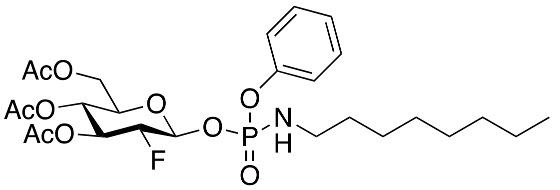
= 15.5, $^{3}J_{F\text{-}H}$ = 2.1 Hz); **^{31}P{^{1}H} NMR** (122 MHz, CD_2Cl_2) δ/ppm 8.1, 7.7; **^{13}C{^{1}H} NMR** (75 MHz, CD_2Cl_2) δ/ppm 96.5 – 95.6 (m, 2C), 92.5 (dd, *J* = 187.2, 8.1 Hz, 2C), 77.8, 77.6, 75.0 (d, *J* = 16.7 Hz, 2C), 70.3 (d, *J* = 6.8 Hz), 70.2 (d, *J* = 6.9 Hz), 67.7 (d, *J* = 5.4 Hz), 67.6 (d, *J* = 5.3 Hz), 62.0, 61.9, 41.8, 41.7, 32.3 (2C), 32.2 (2C), 31.93 (d, *J* = 6.0 Hz), 31.90 (d, *J* = 6.5 Hz), 30.6 (d, *J* = 7.4 Hz, 2C), 29.71 (4C), 29.64 (2C), 29.60 (2C), 27.1 (2C), 26.0 (2C), 23.1 (4C), 14.3 (4C); **HRMS ESI** *m/z* calc. for $C_{22}H_{45}FNO_7PNa$ $[M+Na]^+$: 508.2815, found: 508.2816.

n-Octyl phenylphosphoramidochloridate (15e)



To a solution of 1-octylamine (1.66 mL, 10.0 mmol, 1.0 equiv.) and NEt_3 (1.46 mL, 10.5 mmol, 1.05 equiv) in toluene (20 mL) was added dropwise a solution of $POCl_2OPh$ (1.50 mL, 10.0 mmol, 1.0 equiv.) in toluene (50 mL) at rt. The reaction was stirred overnight at rt and was filtered over Celite to remove the obtained $NEt_3 \cdot HCl$ salt. The filtrate was concentrated under reduced pressure to give octyl pheylphosphoramidochloridate **15e** (3.02 g, 9.94 mmol, 99.4%) as a colorless oil which was sufficiently pure to be used in the next step without further purification. **^{1}H NMR** (300 MHz, $CDCl_3$) δ/ppm 7.41 – 7.33 (m, 2H), 7.30 – 7.19 (m, 3H), 3.60 (brs, 1H), 3.12 (dt, *J* = 13.1, 7.0 Hz, 2H), 1.75 – 1.48 (m, 2H), 1.44 – 1.14 (m, 10H), 0.99 – 0.75 (m, 3H); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) δ/ppm 10.02; **^{13}C NMR** (101 MHz, $CDCl_3$) δ/ppm 150.1 (d, $^{2}J_{C\text{-}P}$ = 8.1 Hz), 130.0, 125.9, 120.7 (d, $^{3}J_{C\text{-}P}$ = 5.4 Hz), 42.3 (d, $^{2}J_{C\text{-}P}$ = 2.1 Hz), 31.9, 30.9 (d, $^{3}J_{C\text{-}P}$ = 7.6 Hz), 29.3, 29.2, 26.6, 22.8, 14.2.

3,4,6-Tri-*O*-acetyl-1-*O*-[(*n*-octyl phenylphosphoramidoyl)oxy]-2-deoxy-2-fluoro-β-D-glucopyranose (16i)



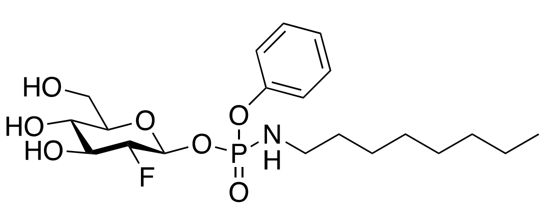
3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-glucopyranose (29.6 mg, 96.0 µmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (0.5 mL). To this solution 3Å molecular sieves was added and the suspension stirred for 30 min at rt before Et_3N (29.3 µL, 211 µmol, 2.20 equiv.) and phosphoramidochloridate **15e** (58.3 mg, 192 µmol, 2.0 equiv.) dissolved in dry CH_2Cl_2 (0.5 mL) were added. The reaction mixture was stirred overnight at rt. The solvent was removed under reduced pressure and the crude product directly purified by flash column chromatography (petroleum ether/EtOAc 2:3) to give a 1:1 diastereomeric mixture of peracetylated glycosyl phosphoramidate **16i** (43.0 mg, 74.7 µmol, 77.8%) as an amorphous solid. **^{1}H NMR** (300 MHz, $CDCl_3$) δ/ppm 7.36 – 7.07 (m, 10H), 5.42 (td, *J* = 7.5, 2.7 Hz, 1H) 5.41 (td, *J* = 7.5, 2.1 Hz, 1H), 5.33[b] (dpseudot, J = 14.1, 8.9 Hz, 1H), 5.31[b] (dpseudot, J = 14.3, 9.1 Hz, 1H), 5.05 (t, *J* = 9.7 Hz, 2H), 4.40 (ddd, *J* = 50.4, 9.3, 7.9, 1H), 4.37 (ddd, *J* = 50.4, 9.0, 7.6, 1H), 4.27 (dd, *J* = 12.4, 4.7 Hz, 1H), 4.22 (dd, *J* = 12.2, 4.7 Hz, 1H), 4.12 (dd, *J* = 12.5, 2.3 Hz, 1H), 4.03 (dd, *J* = 12.4, 2.3 Hz, 1H), 3.82[a] (ddd, *J* = 10.1, 4.6, 2.2 Hz, 1H), 3.79[a] (ddd, *J* = 10.3, 4.6, 2.2 Hz, 1H), 3.13 – 2.81 (m, 4H), 2.07 (s, 3H), 2.05 (s, 6H), 2.02 (s, 6H), 2.01 (s, 3H), 1.47 (q, J = 6.8 Hz, 4H), 1.32 – 1.10 (m, 20H), 0.94 – 0.77 (m, 6H); **^{19}F{^{1}H} NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.1, -200.4; **^{19}F NMR** (282 MHz, $CDCl_3$) *δ*/ppm -200.0 (ddd, *J* = 50.3, 14.3, 2.5 Hz), -200.3 (ddd, *J* = 50.6, 13.9, 2.7 Hz); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) *δ*/ppm 2.81, 2.49; **^{13}C NMR** (75 MHz, $CDCl_3$) *δ*/ppm 170.64, 170.59, 170.0, 169.9, 169.7, 169.6, 150.71 (d, $^{2}J_{C-P}$ = 6.5 Hz), 150.65 (d, $^{2}J_{C-P}$ = 6.6 Hz), 129.8 (2C), 129.7 (2C), 125.15, 125.12, 120.30 (d, $^{3}J_{C-P}$ = 5.3 Hz, 2C), 120.29 (d, $^{3}J_{C-P}$ = 5.3 Hz, 2C), 95.50 (dd, $^{2}J_{C-F}$ = 24.4, $^{2}J_{C-P}$ = 4.4 Hz), 95.47 (dd, $^{2}J_{C-F}$ = 24.4, $^{2}J_{C-P}$ = 4.5 Hz), 89.5 (dd, $^{1}J_{C-F}$ = 193.8, $^{3}J_{C-P}$ = 10.8 Hz), 89.4 (dd, $^{1}J_{C-F}$ = 192.6, $^{3}J_{C-P}$ = 11.1 Hz), 72.7 (2C), 72.54 (d, $^{2}J_{C-F}$ = 20.3 Hz), 72.53 (d, $^{2}J_{C-F}$ = 20.0 Hz), 67.7 (d, $^{3}J_{C-F}$ = 7.2 Hz, 2C), 61.53, 61.50, 41.7 (m, 2C), 31.9 (2C), 31.53 (d, $^{3}J_{C-P}$ = 6.4 Hz), 31.48 (d, $^{3}J_{C-P}$ = 6.7 Hz), 29.32, 29.28 (2C), 29.24, 26.63, 26.57, 22.7 (2C), 20.79 (2C), 20.73 (2C), 20.66 (2C), 14.2 (2C); **ESI-MS (low res)** *m/z* calc. for $C_{26}H_{39}FO_{10}PNa$ [M+Na]$^{+}$: 598.2, found: 598.2.

[a] Observed as a d**d**dd (*J* = 10.3, **8.1**, 4.7, 2.3 Hz) at 3.80 ppm

[b] Observed as a dt**d** (*J* = 14.3, 9.0, **5.0** Hz, 1H) at 5.32 ppm

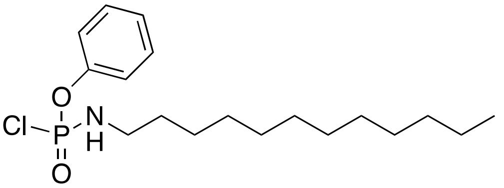
The highlighted doublets arise from a difference in the shift of the two sets of signals obtained from the two isomers. Therefore, they cannot be accounted as a doublet and the respective multiplet hence is reported as two different signals lacking the highlighted doublet.

1-*O*-[(*n*-octyl phenylphosphoramidoyl)oxy]-2-deoxy-2-fluoro-β-D-glucopyranose (9)



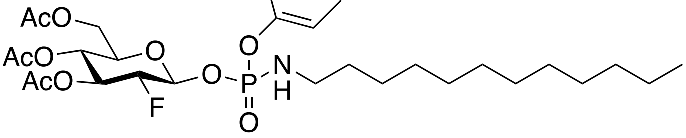
Peracetylated glycosyl phosphoramidate **16i** (14.4 mg, 25.0 µmol, 1.0 equiv.) was dissolved in dry MeOH (1.5 mL). To this solution a solution of Na (0.75 mg, 32.5 µmol, 1.30 equiv.) dissolved in dry MeOH (0.75 mL) was added. The reaction mixture was stirred for 30 min at rt and the reaction was quenched by the addition of one spatula of silica gel. The solvent was removed under reduced pressure and the impregnated silica gel was directly purified by flash column chromatography (DCM/MeOH 10:1) to give a 1:1 diastereomeric mixture of glycosyl phosphoramidate **9** (10.0 mg, 22.2 µmol, 89.0%) as an amorphous solid. **^{1}H NMR** (300 MHz, CD_2Cl_2) *δ*/ppm 7.42 – 7.26 (m, 2H), 7.26 – 7.12 (m, 3H), 5.41 – 5.21 (m, 1H), 4.26 (dpseudoq, *J* = 51.3, 7.8 Hz, 1H), 3.96 – 3.59 (m, 6H), 3.58 – 3.40 (m, 2H), 2.96 (brs, 2H), 1.45 (brs, 1H), 1.35 – 1.21 (m, 10H), 0.88 (t, *J* = 6.7 Hz, 3H); **^{19}F NMR{^{1}H}** (282 MHz, CD_2Cl_2) δ/ppm -200.3, -200.4; **^{19}F NMR** (282 MHz, CD_2Cl_2) δ/ppm -200.2 (ddd, $^2J_{F-H}$ = 51.5, $^3J_{F-H}$ = 15.6, $^3J_{F-H}$ = 2.0 Hz), -200.3 (ddd, $^2J_{F-H}$ = 51.5, $^3J_{F-H}$ = 15.5, $^3J_{F-H}$ = 2.5 Hz); **^{31}P{^{1}H} NMR** (122 MHz, CD_2Cl_2) δ/ppm 3.2, 2.7; **^{13}C NMR** (75 MHz, CD_2Cl_2) δ/ppm 150.7 (d, $^2J_{C-P}$ = 6.6 Hz), 150.6 (d, $^2J_{C-P}$ = 6.4 Hz), 129.8 (4C), 125.2 (2C), 120.4 (d, $^3J_{C-P}$ = 5.0 Hz, 2C), 120.3 (d, $^3J_{C-P}$ = 4.9 Hz, 2C), 96.0 (dd, $^2J_{C-F}$ = 25.0, $^2J_{C-P}$ = 4.8 Hz, 2C), 92.1 (dd, $^1J_{C-F}$ = 186.4, $^3J_{C-P}$ = 10.1 Hz, 2C), 77.3, 77.2, 74.7 (d, $^2J_{C-F}$ = 17.1 Hz, 2C), 69.8 (d, $^3J_{C-F}$ = 7.7 Hz), 69.7 (d, $^3J_{C-F}$ = 7.5 Hz), 61.5, 61.4, 41.7 (d, $^2J_{C-P}$ = 3.6 Hz, 2C), 31.9 (2C), 31.4 (d, $^3J_{C-P}$ = 6.1 Hz), 31.39 (d, $^3J_{C-P}$ = 6.3 Hz), 29.32 (2C), 29.30 (2C), 26.7 (2C), 22.8 (2C), 14.0 (2C); **HRMS ESI** *m/z* calc. for $C_{20}H_{33}FNO_7PNa$ $[M+Na]^+$: 472.1876, found: 472.1880.

n-Dodecyl phenylphosphoramidochloridate (15f)



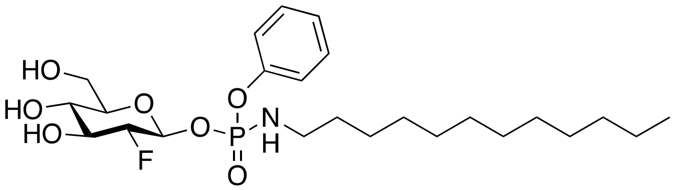
To a solution of 1-dodecylamine (1.85 g, 10.0 mmol, 1.0 equiv.) and NEt_3 (1.46 mL, 10.5 mmol, 1.05 equiv) in toluene (20 mL) was added dropwise a solution of $POCl_2OPh$ (1.50 mL, 10.0 mmol, 1.0 equiv.) in toluene (50 mL) at rt. The reaction was stirred overnight at rt and was filtered over Celite to remove the obtained $NEt_3{\cdot}HCl$ salt. The filtrate was concentrated under reduced pressure to give dodecyl phenylphosphoramidochloridate **15f** (3.00 g, 8.34 mmol, 83.4%) as a slightly yellow oil which was sufficiently pure to be used in the next step without further purification. **^{1}H NMR** (300 MHz, $CDCl_3$) δ/ppm 7.42 – 7.34 (m, 2H), 7.31 – 7.20 (m, 3H), 3.72 – 3.43 (m, 1H), 3.27 – 2.94 (m, 2H), 1.71 – 1.52 (m, 2H), 1.44 – 1.20 (m, 18H), 0.99 – 0.79 (m, 3H); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) δ/ppm 10.00; **^{13}C NMR** (75 MHz, CD_2Cl_2) δ/ppm 150.1 (d, *J* = 8.2 Hz), 130.0 (2C), 125.9 (d, *J* = 1.8 Hz), 120.7 (d, *J* = 5.4 Hz, 2C), 42.3 (d, *J* = 2.1 Hz), 32.1, 30.9 (d, *J* = 7.7 Hz), 29.8 (2C), 29.7, 29.6, 29.5, 29.3, 26.6, 22.8, 14.3.

3,4,6-Tri-*O*-acetyl-1-*O*-[(*n*-dodecyl phenylphosphoramidoyl)oxy]-2-deoxy-2-fluoro-β-D-glucopyranose (16j)



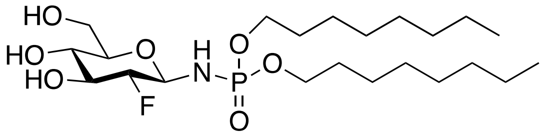
3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-glucopyranose (49.9 mg, 162 µmol, 1.0 equiv.) was dissolved in dry CH_2Cl_2 (1.5 mL). To this solution 3Å molecular sieves was added and the suspension stirred for 30 min at rt before Et_3N (49.4 µL, 356 µmol, 2.20 equiv.) and phosphoramidochloridate **15f** (116 mg, 324 µmol, 2.0 equiv.) dissolved in dry CH_2Cl_2 (0.5 mL) were added. The reaction mixture was stirred overnight at rt. The solvent was removed under reduced pressure and the crude product directly purified by flash column chromatography (petroleum ether/EtOAc 1:1) to give a 3:2 diastereomeric mixture of peracetylated glycosyl phosphoramidate **16j** (94.0 mg, 148 µmol, 91.9%) as an amorphous solid. **^{1}H NMR** (300 MHz, $CDCl_3$) δ/ppm 7.49 – 7.06 (m, 10H), 5.46 – 5.38 (m, 2H), 5.37 – 5.25 (m, 2H), 5.06 (t, J = 9.7 Hz, 2H), 4.53 – 4.28 (m, 2H), 4.27 (dd, J = 12.4, 4.7 Hz, 1H), 4.23 (dd, J = 12.2, 4.7 Hz, 1H), 4.13 (dd, J = 12.5, 2.3 Hz, 1H), 4.04 (dd, J = 12.4, 2.3 Hz, 1H), 3.86 – 3.76 (m, 2H), 3.08 – 2.96 (m, 4H), 2.96 – 2.83 (m, 2H), 2.08 (s, 3H), 2.06 (s, 6H), 2.03 (s, 6H), 2.03 (s, 3H), 1.58 – 1.39 (m, 4H), 1.33 – 1.18 (m, 36H), 0.96 – 0.77 (m, 6H); **^{19}F{^{1}H} NMR** (282 MHz, $CDCl_3$) δ/ppm -200.1, -200.4; **^{19}F NMR** (282 MHz, $CDCl_3$) δ/ppm -200.1 (ddd, J = 50.2, 13.9, 2.1 Hz), -200.4 (ddd, J = 50.4, 14.0, 2.1 Hz); **^{31}P{^{1}H} NMR** (122 MHz, $CDCl_3$) δ/ppm 2.80, 2.49; **^{13}C NMR** (75 MHz, $CDCl_3$) δ/ppm 170.7, 170.6, 170.0, 169.9, 169.7, 169.6, 150.7 (d, J = 6.6 Hz), 150.6 (d, J = 6.6 Hz), 129.8 (2C), 129.7 (2C), 125.2 (2C), 120.34 (d, J = 5.0 Hz, 2C), 120.31 (d, J = 5.1 Hz, 2C), 95.48 (dd, J = 24.2, 4.0 Hz), 95.46 (dd, J = 24.9, 4.2 Hz), 89.4 (dd, J = 192-3, 8.3 Hz), 89.3 (dd, J = 193.7, 9.2 Hz), 72.74, 72.71, 72.5 (d, J = 20.9 Hz, 2C), 67.8 (d, J = 6.8 Hz, 2C), 61.5 (2C), 41.7 (2C), 32.0 (2C), 31.6 (d, J = 6.0 Hz), 31.5 (d, J = 6.5 Hz), 29.9 – 29.6 (m, 8C), 29.5 (2C), 29.4, 29.3, 26.7, 26.6, 22.8 (2C), 20.8 (2C), 20.7 (2C), 20.7 (2C), 14.3 (2C); **ESI-MS (low res)** *m/z* calc. for $C_{30}H_{47}FNO_{10}PNa$ $[M+Na]^+$: 654.3, found: 654.3.

1-*O*-[(*n*-dodecyl phenylphosphoramidoyl)oxy]-2-deoxy-2-fluoro-β-D-glucopyranose (10)



Peracetylated glycosyl phosphoramidate **16j** (75.2 mg, 119.0 µmol, 1.0 equiv.) was dissolved in dry MeOH (6.0 mL). To this solution a solution of Na (2.74 mg, 119.0 µmol, 1.0 equiv.) dissolved in dry MeOH (3 mL) was added. The reaction mixture was stirred for 30 min at rt and the reaction was quenched by the addition of one spatula of silica gel. The solvent was removed under reduced pressure and the impregnated silica gel was directly purified by flash column chromatography (DCM/MeOH 15:1) to give a 1:1 diastereomeric mixture of glycosyl phosphoramidate **10** (51.0 mg, 100.9 µmol, 84.8%) as an amorphous solid. **^{1}H NMR** (300 MHz, DMSO-d_6) δ/ppm 7.40 – 7.32 (m, 4H), 7.25 – 7.12 (m, 6H), 5.58 (d, J = 5.5 Hz, 2H), 5.45 – 5.33 (m, 2H), 5.30 – 5.20 (m, 4H), 4.60 (t, J = 5.6 Hz, 1H), 4.55 (t, J = 5.7 Hz, 1H), 4.03 (dt, J = 51.5, 7.5 Hz, 1H), 3.98 (dt, J = 51.4, 8.0 Hz, 1H), 3.74 – 3.62 (m, 2H), 3.61 – 3.45 (m, 4H), 3.40 – 3.29 (m, 2H), 3.28 – 3.15 (m, 2H), 2.93 – 2.68 (m, 4H), 1.42 – 1.30 (m, 4H), 1.30 – 1.12 (m, 36H), 0.97 – 0.71 (m, 6H). **^{19}F NMR{^{1}H}** (282 MHz, DMSO-d_6) δ/ppm -198.0, -198.1; **^{19}F NMR** (282 MHz, DMSO-d_6) δ/ppm -198.0 (ddd, $^{2}J_{F\text{-}H} = 51.5$, $^{3}J_{F\text{-}H} = 16.0$, $^{3}J_{F\text{-}H} = 2.2$ Hz), -198.1 (ddd, $^{2}J_{F\text{-}H} = 51.6$, $^{3}J_{F\text{-}H} = 15.8$, $^{3}J_{F\text{-}H} = 2.2$ Hz); **^{31}P{^{1}H} NMR** (122 MHz, DMSO-d_6) δ/ppm 3.71, 3.67; **^{13}C NMR** (75 MHz, DMSO-d_6) δ/ppm 150.77 (d, J = 6.0 Hz), 150.72 (d, J = 6.0 Hz), 129.5 (2C), 124.4 (2C), 120.3 (d, J = 5.1 Hz), 120.2 (d, J = 5.0 Hz), 95.3 (dd, J = 24.0, 4.9 Hz), 95.1 (dd, J = 24.3, 4.0 Hz), 92.6 (dd, J = 187.2, 7.0 Hz, 2C), 77.7 (2C), 73.90 (d, J = 17.0 Hz), 73.85 (d, J = 16.5 Hz), 69.3 (d, J = 7.8 Hz), 69.2 (d, J = 7.6 Hz), 60.4, 60.2, 40.8, 40.7, 31.3 (2C), 30.9 (d, J = 6.3 Hz, 2C), 29.1 (2C), 29.0 (6C), 28.7 (4C), 26.0 (2C), 22.1 (2C), 14.0 (2C); **HRMS ESI** *m/z* calc. for $C_{24}H_{41}FNO_7PNa$ [M+Na]$^+$: 528.2502, found: 528.2498.

2-Deoxy-2-fluoro-β-D-glucopyranosyl di-octylphosphoramidate



3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl azide (50 mg, 0.15 mmol) (prepared according to O. Botueira *et al.*, *Org. Lett.* **2015**, *17*, 2836–2839) was dissolved in dry DCM (1.5 mL, 10 mL/mmol) and subsequently treated with trioctyl phosphite (85%) (128 mg, 0.225 mmol) under nitrogen atmosphere. The resulting solution was allowed to stir at room temperature until TLC analysis showed complete consumption of the starting material. The mixture was poured into icy water and extracted twice with DCM. The combined organic phases were washed with brine and dried over anhydrous sodium sulfate, filtered and evaporated under vacuum. This residue was then dissolved in dry methanol (1.5 mL), cooled to 0 ºC and treated with a catalytic amount of sodium metal. After 1 h, the reaction was quenched by the addition of a scoop of silica gel and evaporated under vacuum. Flash column chromatography (DCM/MeOH 5% to 10%) afforded 57 mg of the desired glycosylphosphoramidate as a white foam (79%, two steps). **^{1}H NMR** (400 MHz, MeOD-d_4) δ/ppm 4.59 (q, J = 9.7 Hz, 1H), 4.28 – 3.95 (m, 7H), 3.74 (ddd, J = 10.1, 4.6, 2.2 Hz, 1H), 3.69 (br s, 1H), 3.45 – 3.37 (m, 2H), 1.71 – 1.67 (m, 4H), 1.39 – 1.31 (m, 20H), 0.92 – 0.88 (m, 6H); **^{19}F NMR{^{1}H}** (282 MHz, MeOD-d_4) δ/ppm -195.5; **^{19}F NMR** (282 MHz, MeOD-d_4) δ/ppm -195.5 (dd, $^{2}J_{F\text{-}H} = 50.4$, $^{3}J_{F\text{-}H} = 13.7$ Hz); **^{31}P{^{1}H} NMR** (122 MHz, MeOD-d_4) δ/ppm 4.79; **^{13}C NMR** (101 MHz, MeOD-d_4) δ/ppm 89.5 (dd, J = 192.3, 9.3 Hz, C-2), 82.0 (d, J =

22.3 Hz, C-1), 73.32 (d, *J* = 20.6 Hz), 73.31, 68.0 (d, *J* = 6.7 Hz), 67.1 (2C), 61.9, 31.8 (2C), 29.2 (2C), 29.0 (2C), 28.4 (2C), 24.9 (2C), 23.6 (2C), 14.1 (2C); **HRMS ESI** *m/z* calc. for $C_{22}H_{45}FNO_7PNa$ $[M+Na]^+$: 508.2810, found: 508.2798.

<u>List of Abbreviations employed throught the S.I.:</u>

- ACN: acetonitrile
- DCM: dichloromethane
- DMF: dimethylformamide
- ESI: electrospray ionization
- HRSM: high resolution mass spectrometry
- NMR: nuclear magnetic resonance spectroscopy
- THF: tetrahydrofuran
- TMSI: iodotrimethylsilane

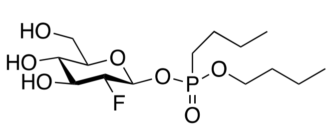
3. Enzyme Kinetics

The kinetic assays were performed using a Varian Cary 300-Bio UV-Visible Spectrophotometer equipped with an externally controlled continuously circulating water bath. All enzymatic data analyses were performed using GraFit Version 4.0.19. GCase was assayed by continuously monitoring (λ = 390 nm) the release of 2,4-dinitrophenol from 2,4-dinitrophenyl-β-D-glucopyranoside. The slope of the resulting absorbance vs. time graph was used to determine the initial rate of enzyme catalyzed hydrolysis. The enzyme reaction was initiated by the addition of 20 μL volume of enzyme solution to a pre-equilibrated assay cuvette containing an appropriate volume (180 μL) of buffer and the chromogenic substrate. All enzyme reactions were performed at 37 °C. All enzymatic buffers were prepared using water from a Millipore Direct-QTM 5 Ultrapure Water System. The conditions used for the analysis of GCase were 50 mM NaOAc, 0.2 % Triton X-100, 0.3% sodium taurocholate pH = 5.5 using 2,4-dinitrophenyl-β-D-glucopyranoside with a final concentration of GCase in the assay cuvette between 0.2 to 1.0 μg/mL.

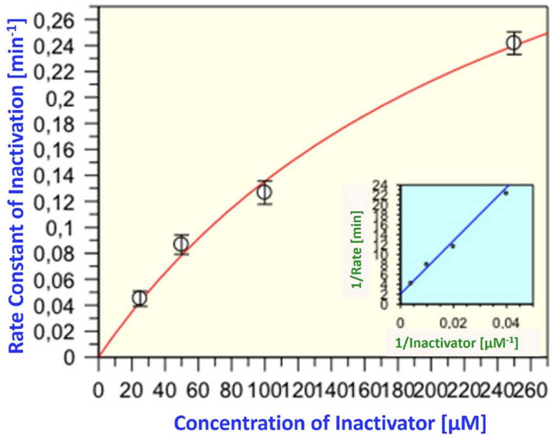
Compounds **1-10** (1:1 diasteromeric mixtures at the phosphorus center) were tested for time-dependent inactivation of GCase using the following general procedure. The enzyme was incubated in a small Eppendorf tube with an appropriate volume of buffer. The inactivation was initiated by the addition of 20 μL of inactivator solution with varying concentrations to the enzyme solution, for a total volume of 200 μL in the Eppendorf tube. At appropriate time points, 20 μL aliquots were withdrawn and added to a solution containing 2-10 x Km of the substrate, along with a large volume of buffer (180 μL). This halts the inactivation reaction both by dilution of the enzyme inactivator and by competition with a large excess of substrate. The residual enzyme activity was then assayed using a continuous enzymatic assay. The rate of release of the chromophore is directly proportional to the concentration of catalytically active enzyme remaining in the solution. Pseudo-first order rate constants for the loss of enzymatic activity for each concentration of inactivator were determined by nonlinear fitting of the decay curve to a single exponential decay equation using GraFit. The individual k_{obs} values were then replotted as a function of inactivator concentration. Individual values for k_i and K_i were obtained by fitting to the equation: $kobs = ki[I]/(Ki + \ [I])$

In many cases though, we found the inactivation was so rapid that it was generally not possible to measure individual values of ki and Ki, but only values of the second-order rate constant ki/Ki estimated from the initial (linear) part of the plot.

a)

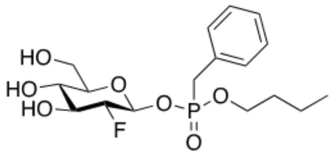


ki/Ki = 1.87 mM^{-1}min^{-1}

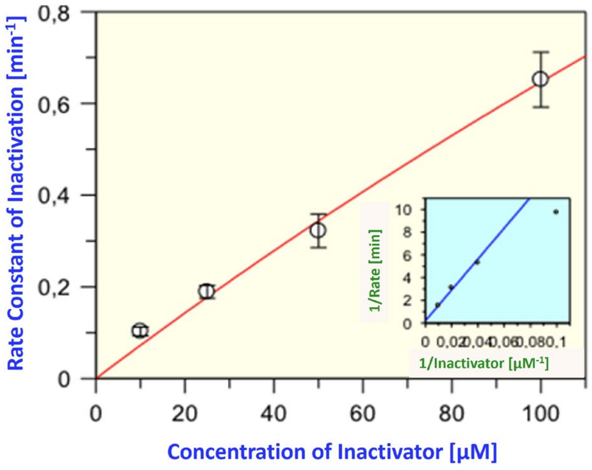


Parameter	Value	Std. Error
ki [min^{-1}]	0.4937	0.0714
Ki [µM]	264.6145	63.0330

b)

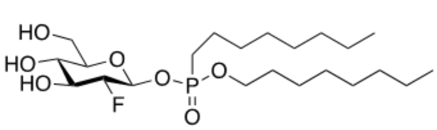


ki/Ki = 7.34 mM^{-1}min^{-1}

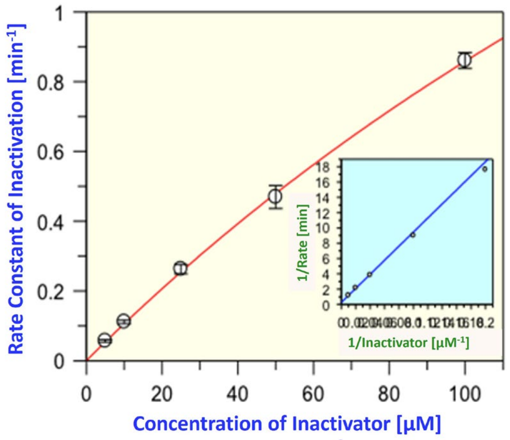


Parameter	Value	Std. Error
ki [min^{-1}]	5.4472	5.0606
Ki [µM]	741.9610	766.5241

c)

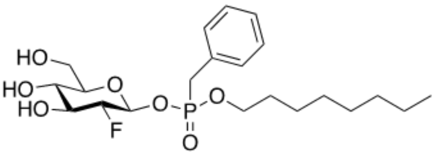


ki/Ki = 10.9 mM^{-1}min^{-1}

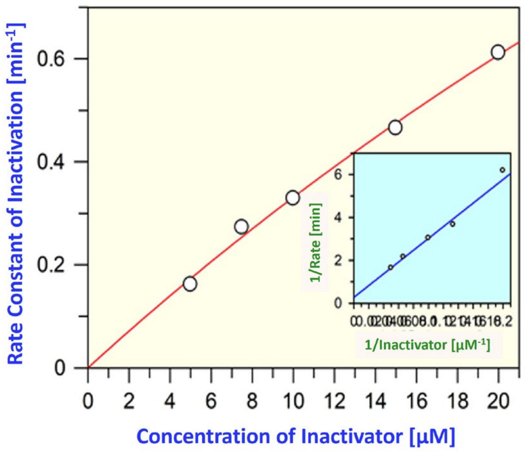


Parameter	Value	Std. Error
ki [min^{-1}]	4.0923	0.5348
Ki [µM]	376.8868	59.7717

d)

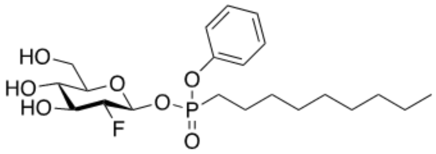


ki/Ki = 36.4 mM^{-1}min^{-1}

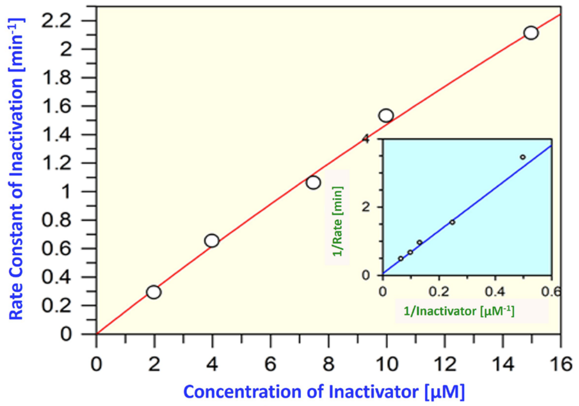


Parameter	Value	Std. Error
ki [min^{-1}]	3.6773	1.3516
Ki [µM]	101.0679	42.7785

e)

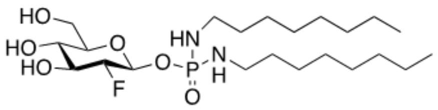


ki/Ki = 160 mM^{-1}min^{-1}

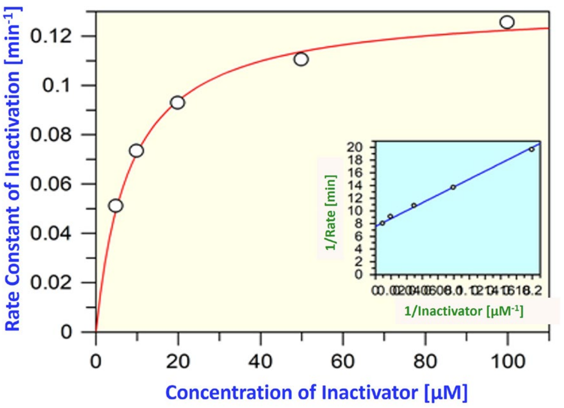


Parameter	Value	Std. Error
ki [min^{-1}]	18.6165	12.5281
Ki [μM]	116.5768	86.3279

f)

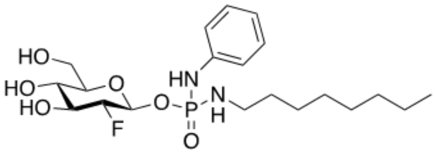


ki/Ki = 16.1 mM^{-1}min^{-1}

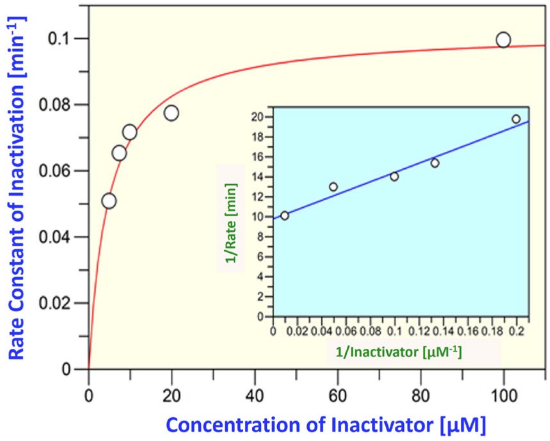


Parameter	Value	Std. Error
ki [min^{-1}]	0.1324	0.0029
Ki [μM]	8.2347	0.6745

g)

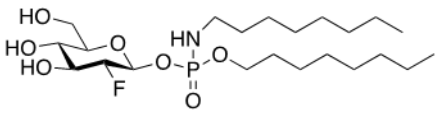


ki/Ki = 21.5 mM^{-1}min^{-1}

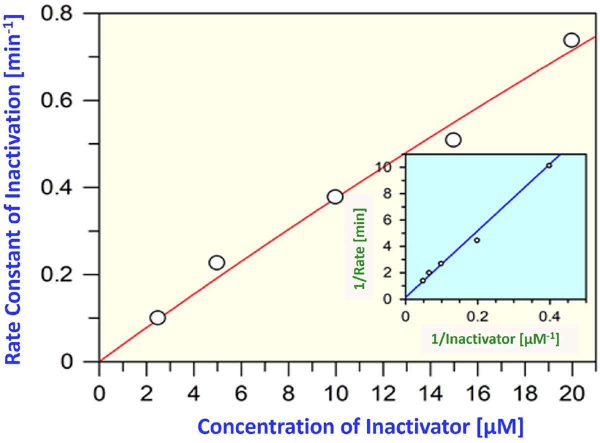


Parameter	Value	Std. Error
ki [min^{-1}]	0.1021	0.0042
Ki [μM]	4.7478	0.6745

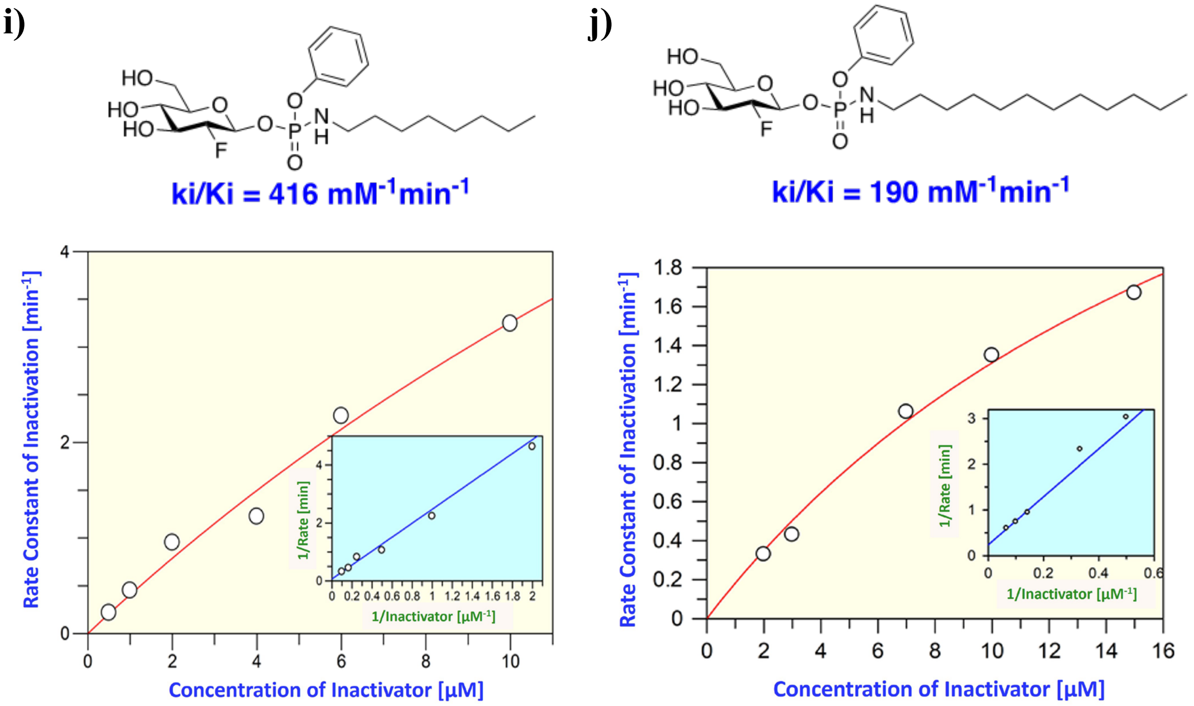
h)



ki/Ki = 39.5 mM^{-1}min^{-1}



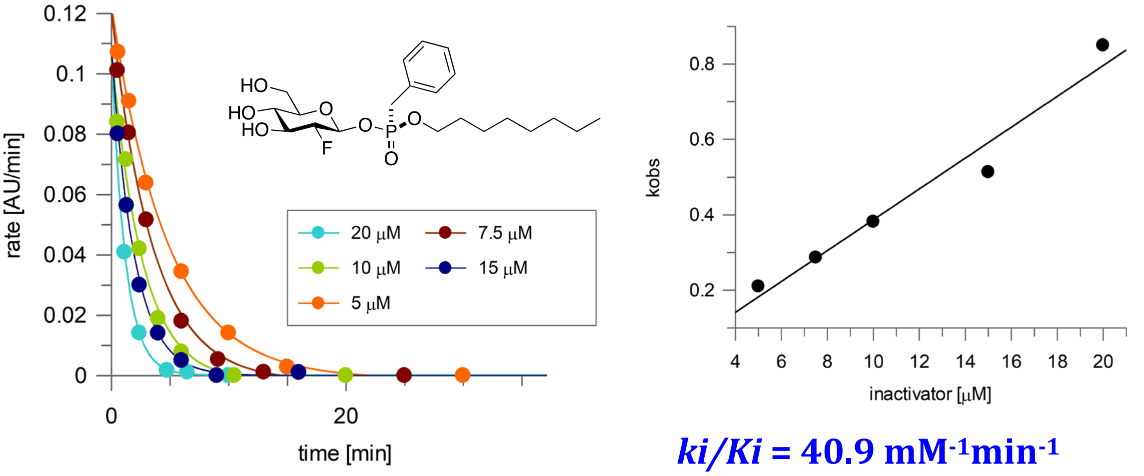
Parameter	Value	Std. Error
ki [min^{-1}]	7.4598	8.3888
Ki [μM]	188.6328	230.2455



Parameter	Value	Std. Error
ki [min^{-1}]	15.0455	7.9077
Ki [µM]	36.1882	22.9617

Parameter	Value	Std. Error
ki [min^{-1}]	4.2185	0.8075
Ki [µM]	22.1668	6.2909

Figure S1. Inactivation of GCase with a) **1**, b) **2**, c) **3**, d) **4**, e) **5**, f) **6**, g) **7**, h) **8**, i) **9** and j) **10**. Replot of the observed rate constants of inactivation versus concentration. Individual rate constants were obtained by non-linear plot of residual enzyme activity versus time at the indicated inactivator concentrations fitted to an exponential decay equation (not shown).

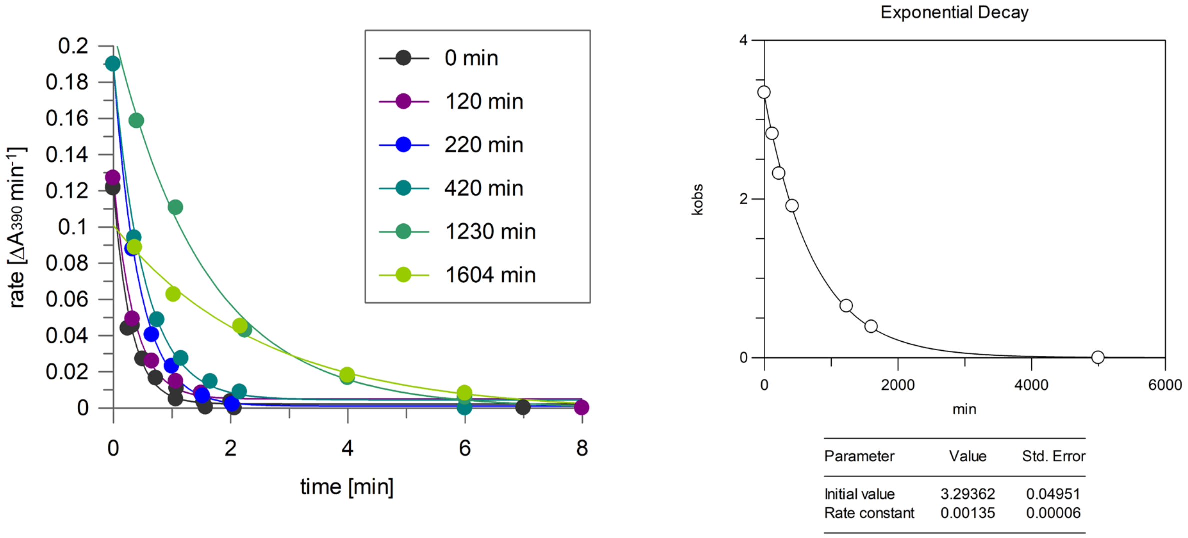


***ki/Ki* = 40.9 mM^{-1}min^{-1}**

Figure S2. Inactivation of GCase with diastereomeric enriched **4**. Individual rate constants were obtained by non-linear plot of residual enzyme activity versus time at the indicated inactivator concentrations fitted to an exponential decay equation (left). Replot of the observed rate constants of inactivation versus concentration (right).

4. Stability Assay

The hydrolysis rate of 2FGlc phosphoramidate **9** was determined by measuring the decay of rate constant of inactivation over time.



Parameter	Value	Std. Error
Initial value	3.29362	0.04951
Rate constant	0.00135	0.00006

Figure S3. A 100 µM solution of inactivator **9** was incubated in an aq. 50 mM NaH_2PO_4/Na_2HPO_4 buffer containing 25% DMSO at pH=6.8 at 37 °C. At appropriate time points the rate constant of inactivation was determined by a non-linear plot of residual enzyme activity versus time fitted to an exponential decay equation (left). Replot of the decay of rate constant over time fitted to an exponential decay equation (right).

Conditions: 100 uM inactivator **9** in buffer/DMSO 4:1, pH = 6.8, incubated at 37 °C

Buffer: 50 mM NaH_2PO_4/Na_2HPO_4

Rate constant: 0.0014 min^{-1}

Half-life: 510 min ± 23 min

5. Cell-based studies

MEF Cells expressing human L444P treated with 4 for three days

Mouse embryonic fibroblast (MEF) cells from transgenic mice expressing human L444P-containing GCase (a common variant underlying neuropathic Gaucher disease) on a murine GCase knockout background were grown on a 24 well plate and continuously treated for three days with daily media changes containing 0 or 100 μM 2FGlc phosphonate inactivator **4**. After 3 days fresh media was added containing no **4**, and after 24 hrs, 48 hrs, 72 hrs, 96 hrs cells were washed then harvested. GBA and HEX activity measured as described [6], Lysates were labelled with MDW933 for 30 minutes and Western Blots (human GBA monoclonal) run. Gel lanes for MDW933 and Westerns were normalized for HEX activity.

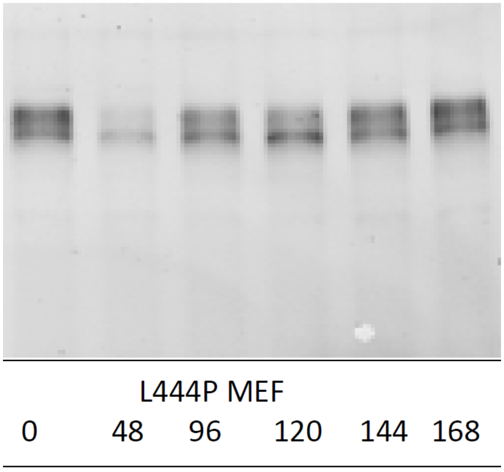
Table S1

Activity Assays:

Cell Type	Days after addition of 4	Relative GBA Activity
L444P MEF	0 (untreated)	1
	24 hrs	0.03
	48 hrs	0.53
	72 hrs	0.95
	96 hrs	1.31

MEF Cells expressing human L444P treated with 4 for seven days

a)



b)

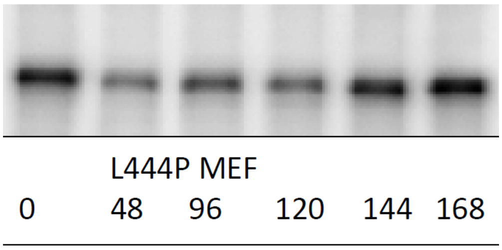


Figure S4. Mouse embryonic fibroblast (MEF) cells from transgenic mice expressing human L444P-containing GCase on a murine GCase knockout background were grown on a 24 well plate and continuously treated for seven days with daily media changes containing 0 or 100 µM 2FGlc phosphonate inactivator **4**. After 7 days, fresh media was added containing no **4**, and after 48 hrs, 96 hrs, 120 hrs, 144 hrs and 168 hrs cells were washed then harvested. Gel lanes were normalized for HEX activity.

- **a)** Lysates were labelled with MDW933 for 30 minutes.
- **b)** Western blots

Human fibroblast cells expressing human L444P treated with 4 for three days

Table S2

Activity Assays:
Human fibroblast cells carrying the L444P mutation were grown on a 24 well plate and continuously treated for three days with daily media changes containing 0 or 100 µM 2FGlc phosphonate inactivator **4**. After 3 days fresh media was added containing no **4**, and after 24 hrs, 48 hrs, 72 hrs, 96 hrs cells were washed then harvested. GBA and HEX activity were measured as described.[6]

Cell Type	Days after addition of 4	Relative GBA Activity
Human L444P Fibro	0 (untreated)	1
	24 hrs	0.29
	48 hrs	0.76
	72 hrs	1.12
	96 hrs	1.29

MEF Cells and Human fibroblasts both expressing human N370S. Treated with 4 for 3 days

MDW 933 labelling

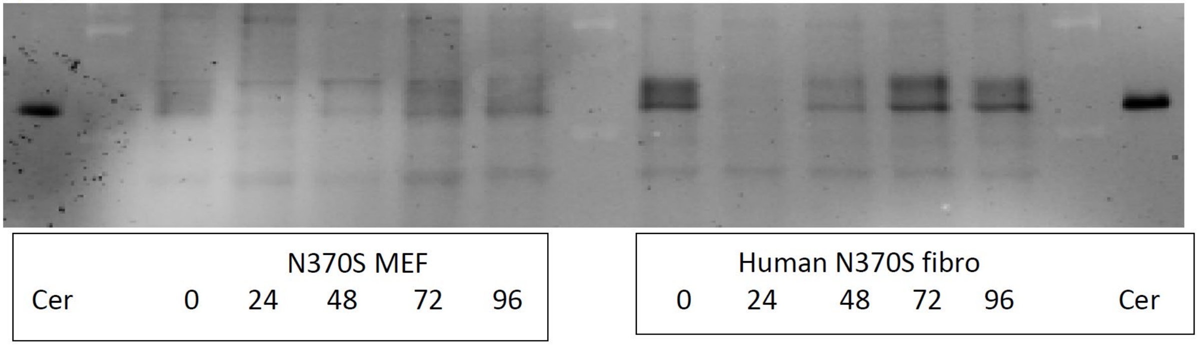
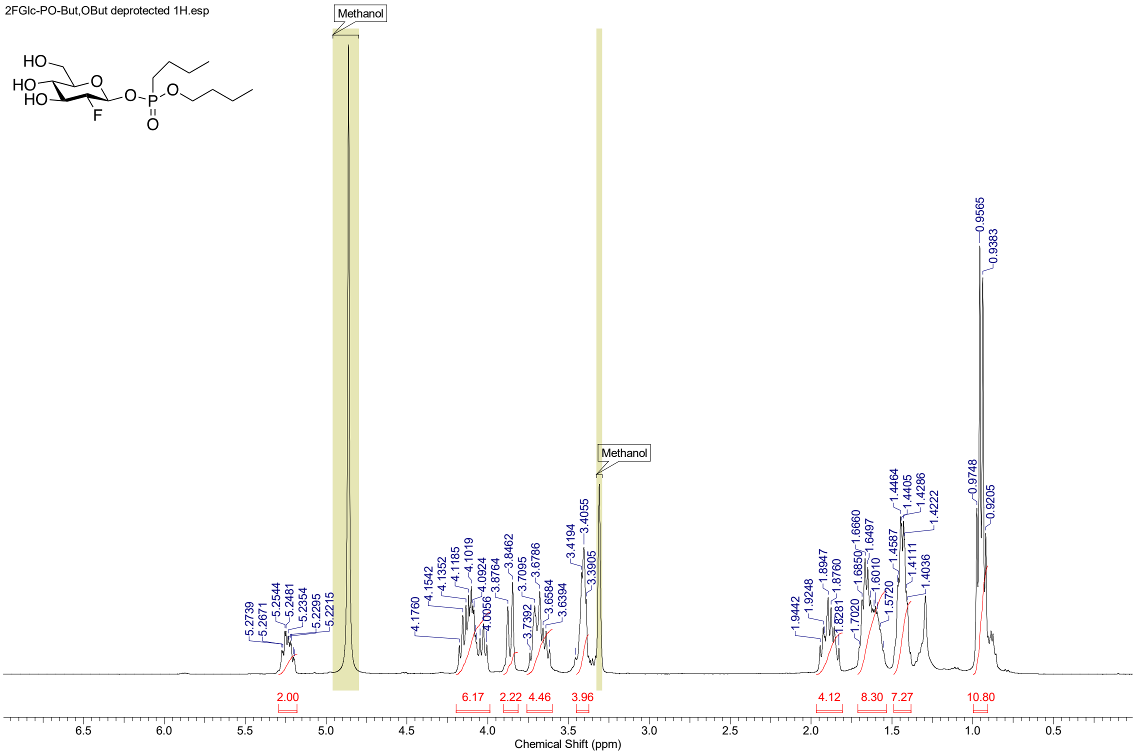


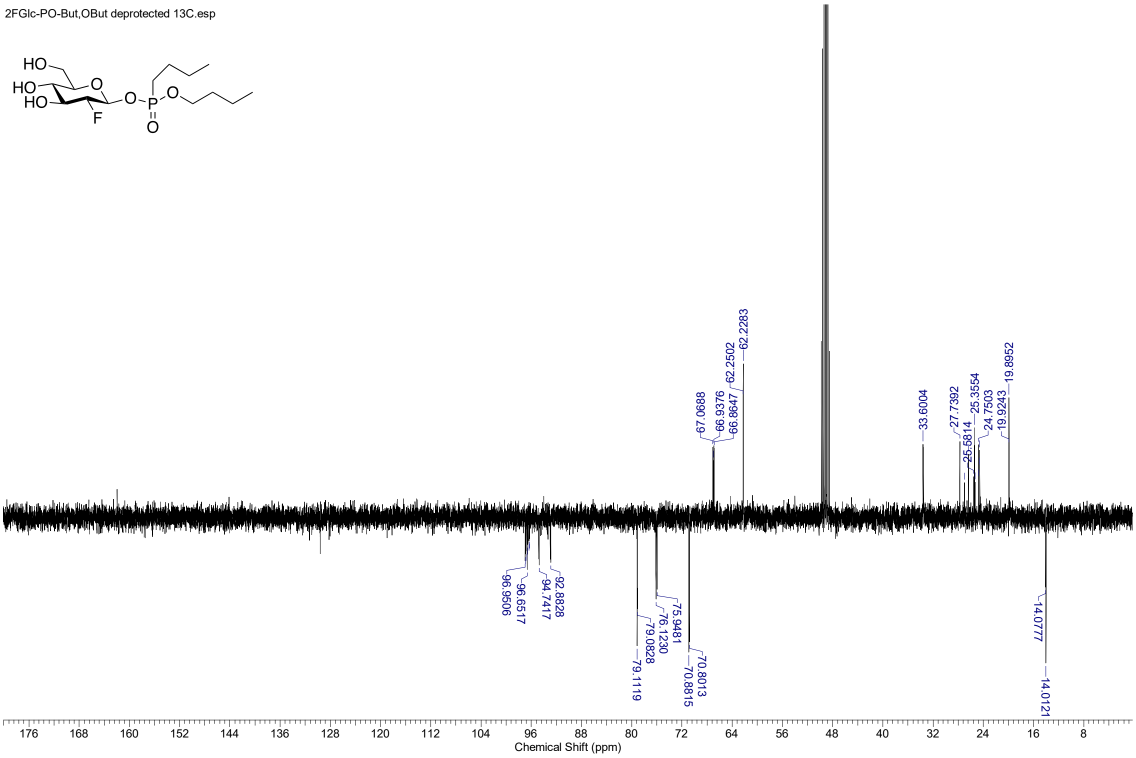
Figure S5. Cells were grown on a 24 well plate and treated with daily media change containing 0 or 100 uM **4**. After 3 days fresh media change with no drug, after 24 hrs, 48 hrs, 72hrs, 96 hrs cells washed then harvested. GBA and HEX activity measured. MDW933 labelling of lysates for 30 minutes Gel lanes normalized for HEX activity.

4. NMR Spectra

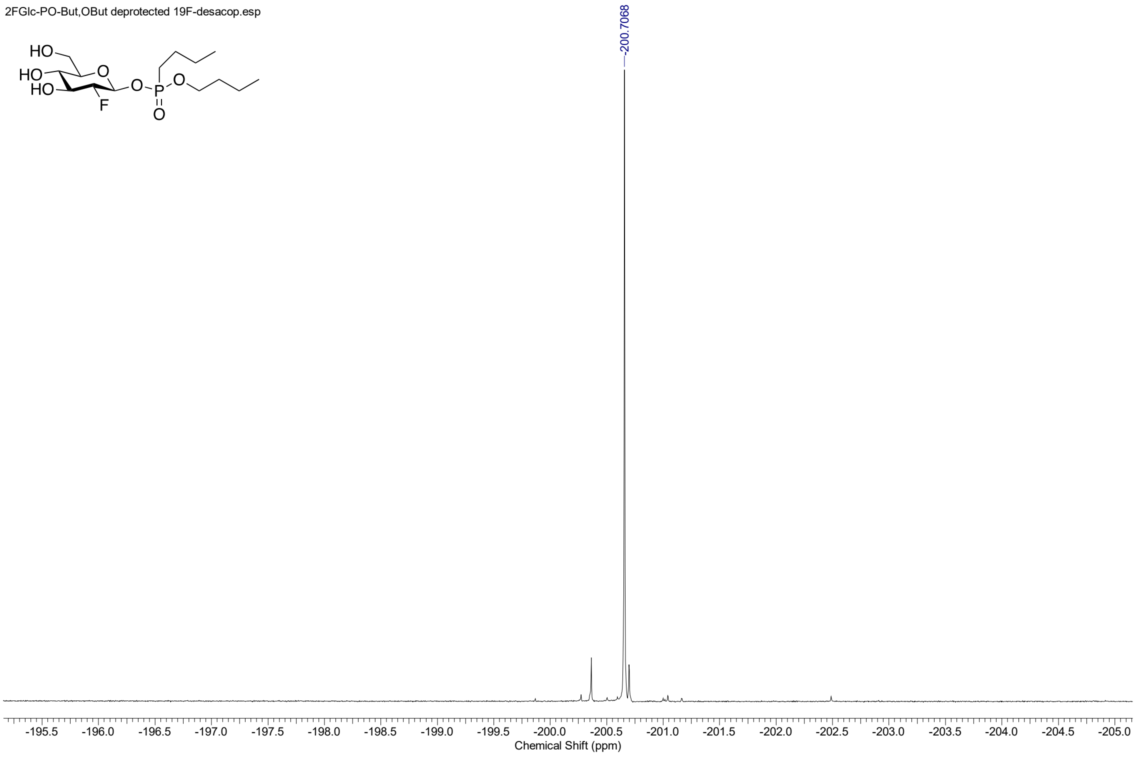
400 MHz ^{1}H NMR spectrum of **1** recorded in MeOD



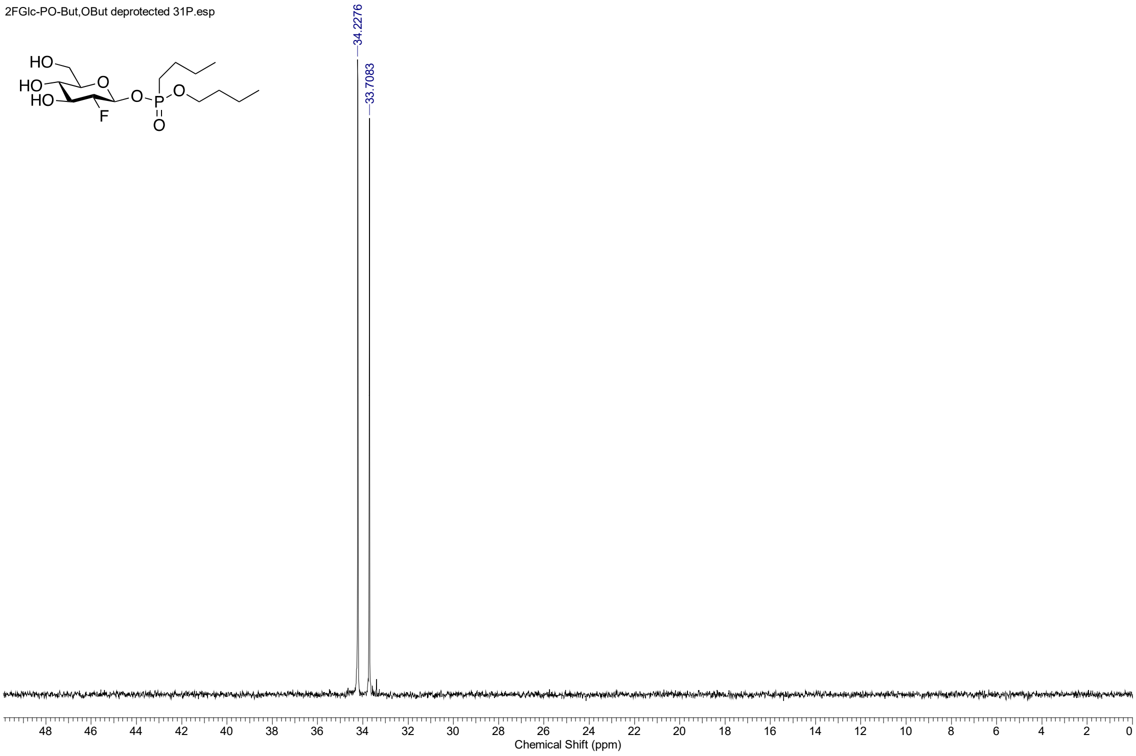
101 MHz ^{13}C NMR spectrum of **1** recorded in MeOD



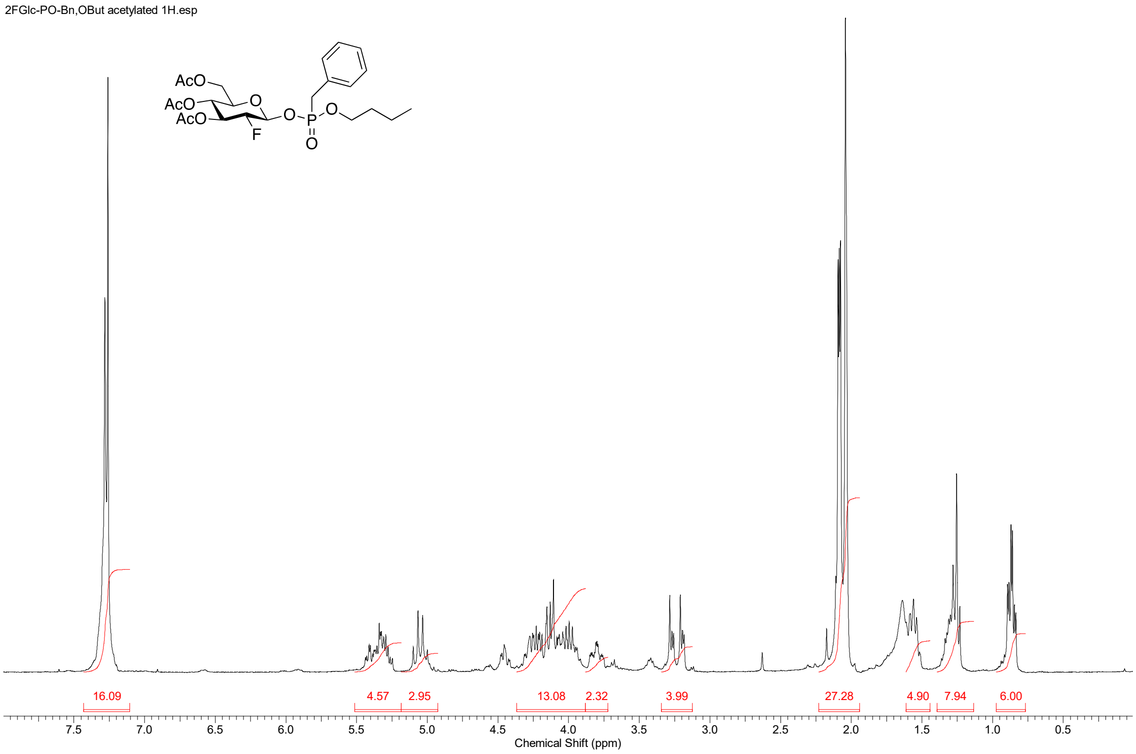
376 MHz ^{19}F NMR spectrum of **1** recorded in MeOD



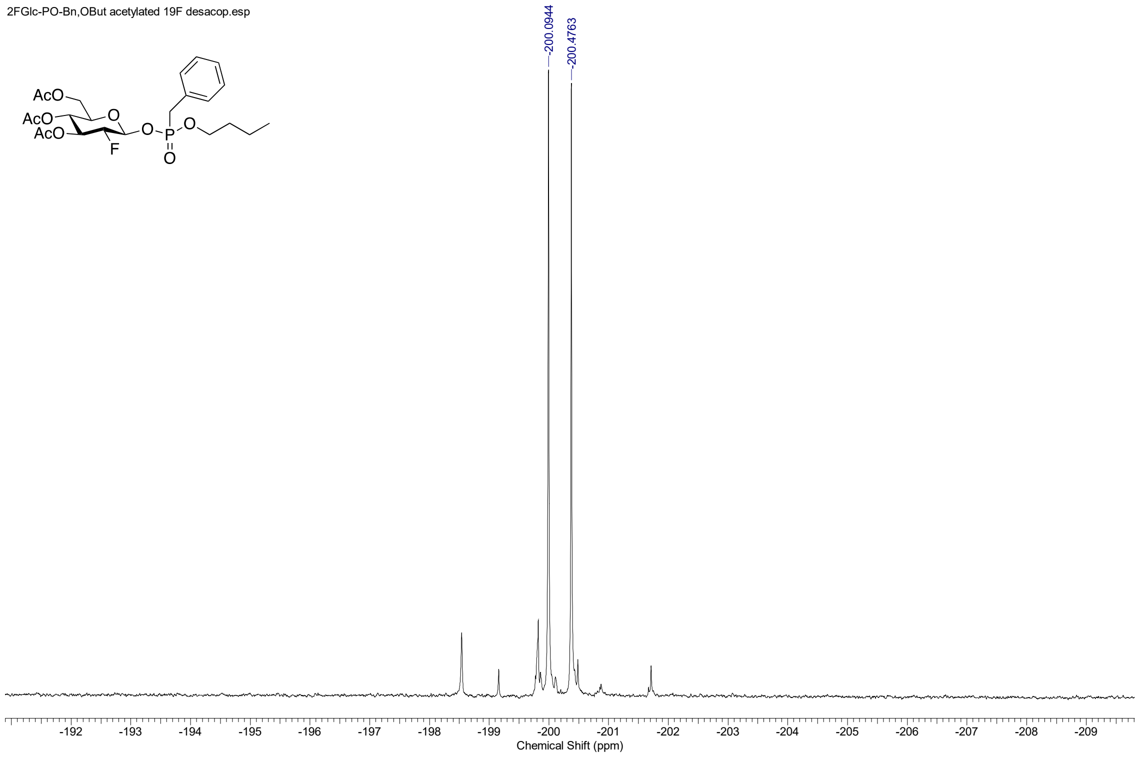
162 MHz ^{31}P NMR spectrum of **1** recorded in MeOD



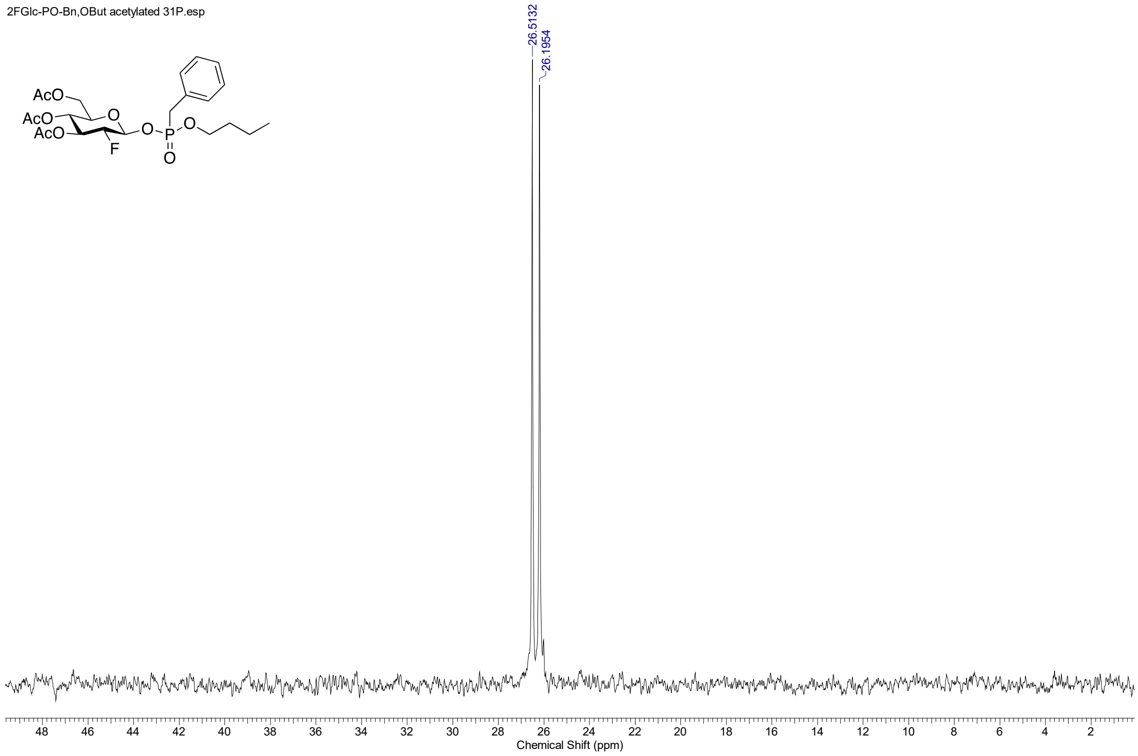
400 MHz ^{1}H NMR spectrum of **16b** recorded in $CDCl_3$



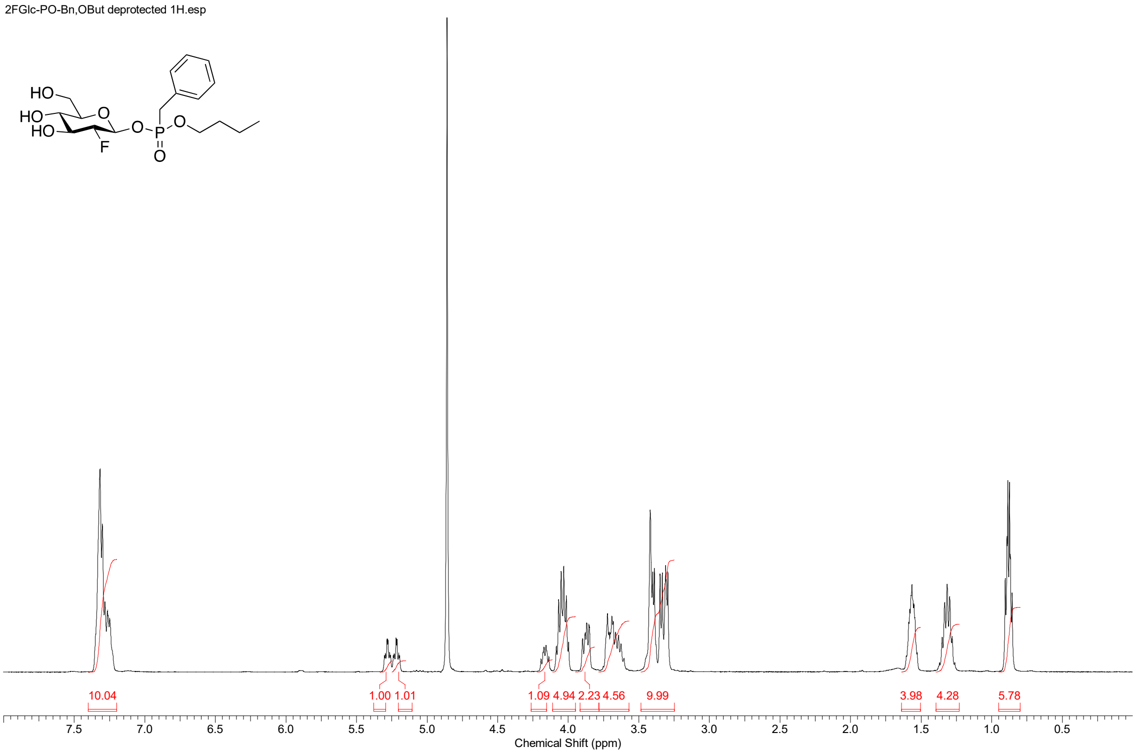
376 MHz ^{19}F NMR spectrum of **16b** recorded in $CDCl_3$



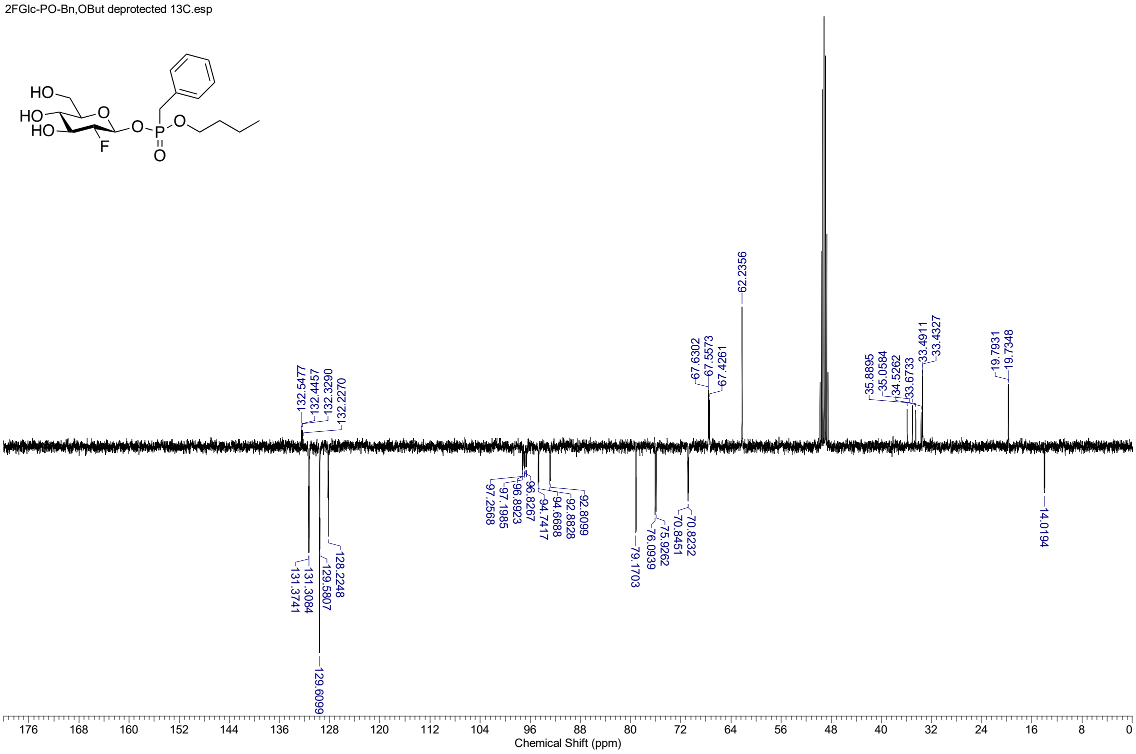
162 MHz ^{31}P NMR spectrum of **16b** recorded in $CDCl_3$



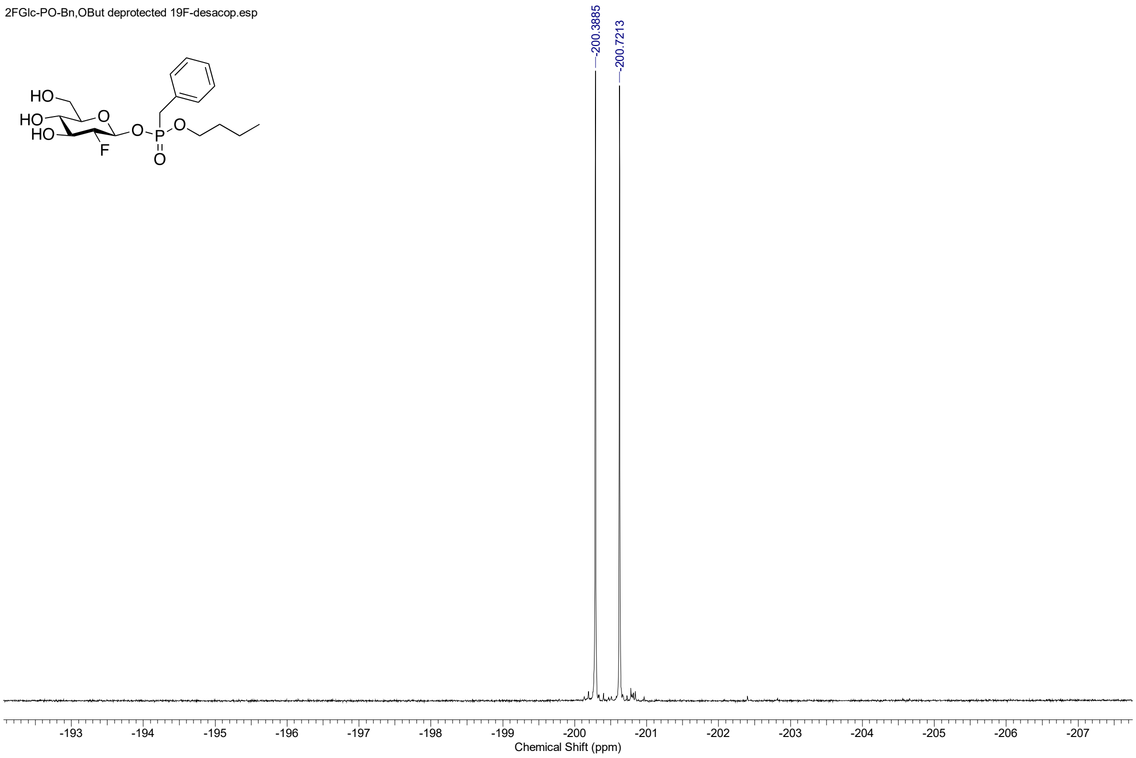
400 MHz ^{1}H NMR spectrum of **2** recorded in MeOD



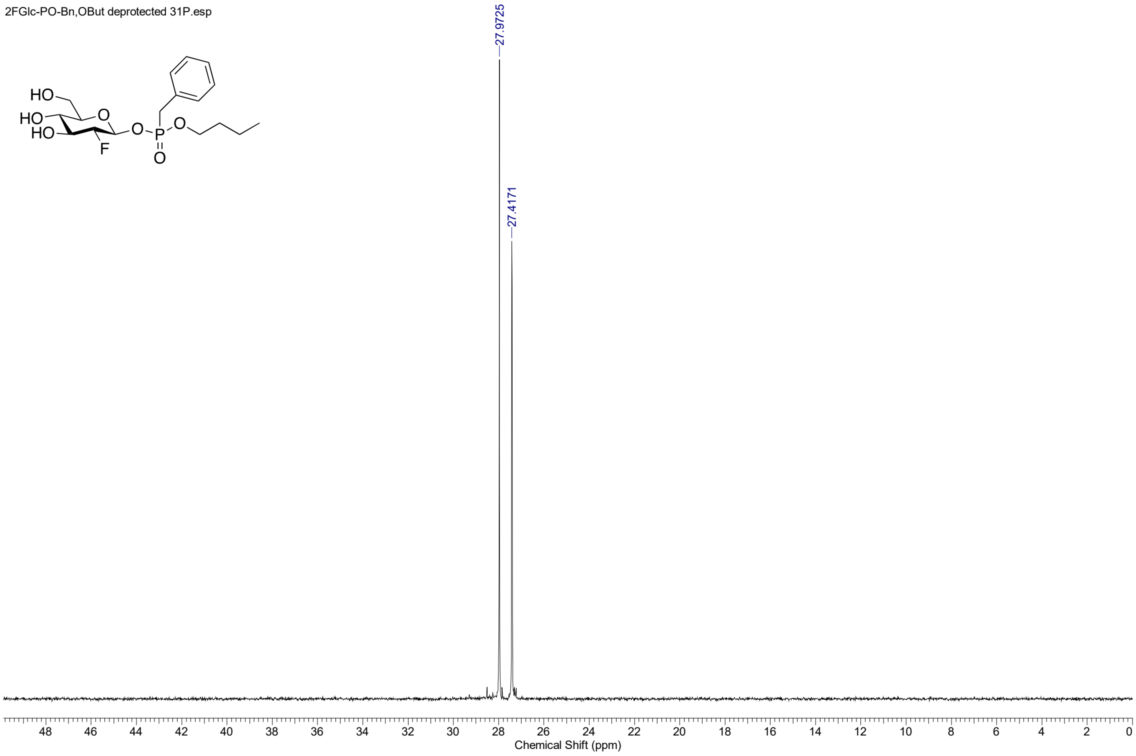
101 MHz ^{13}C NMR spectrum of **2** recorded in MeOD



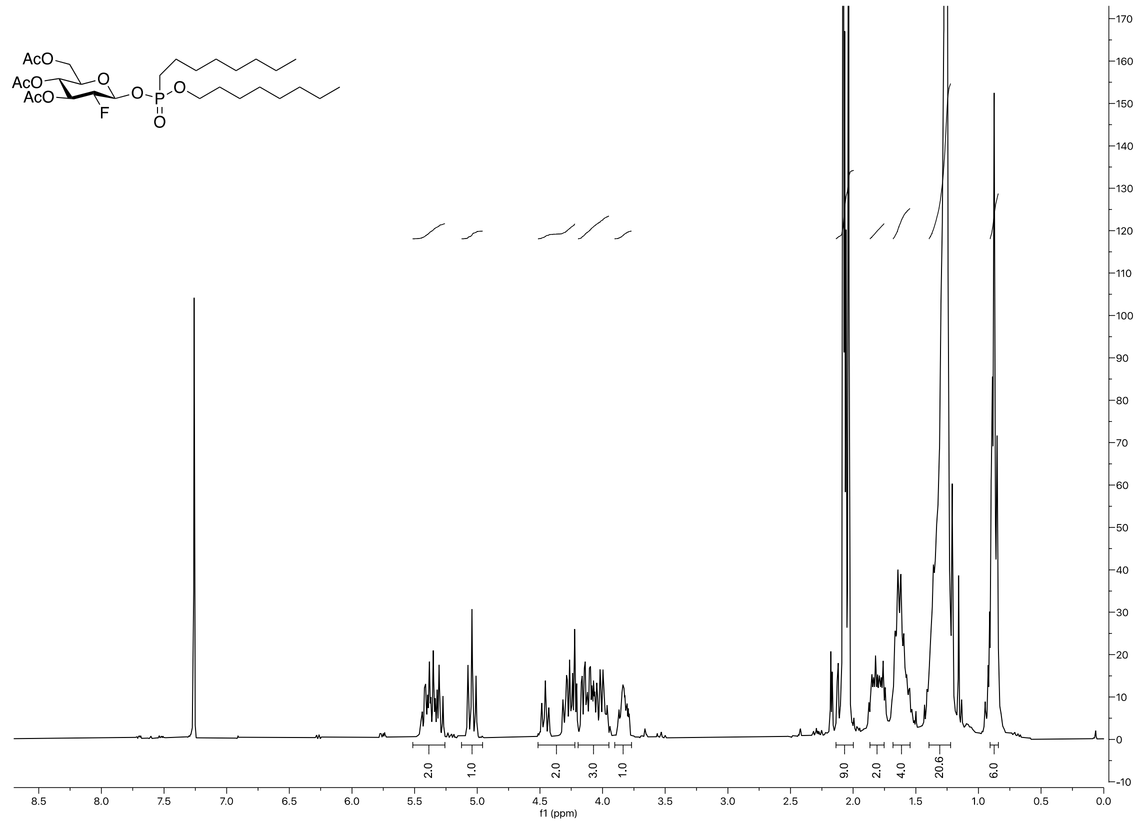
376 MHz ^{19}F NMR spectrum of **2** recorded in MeOD



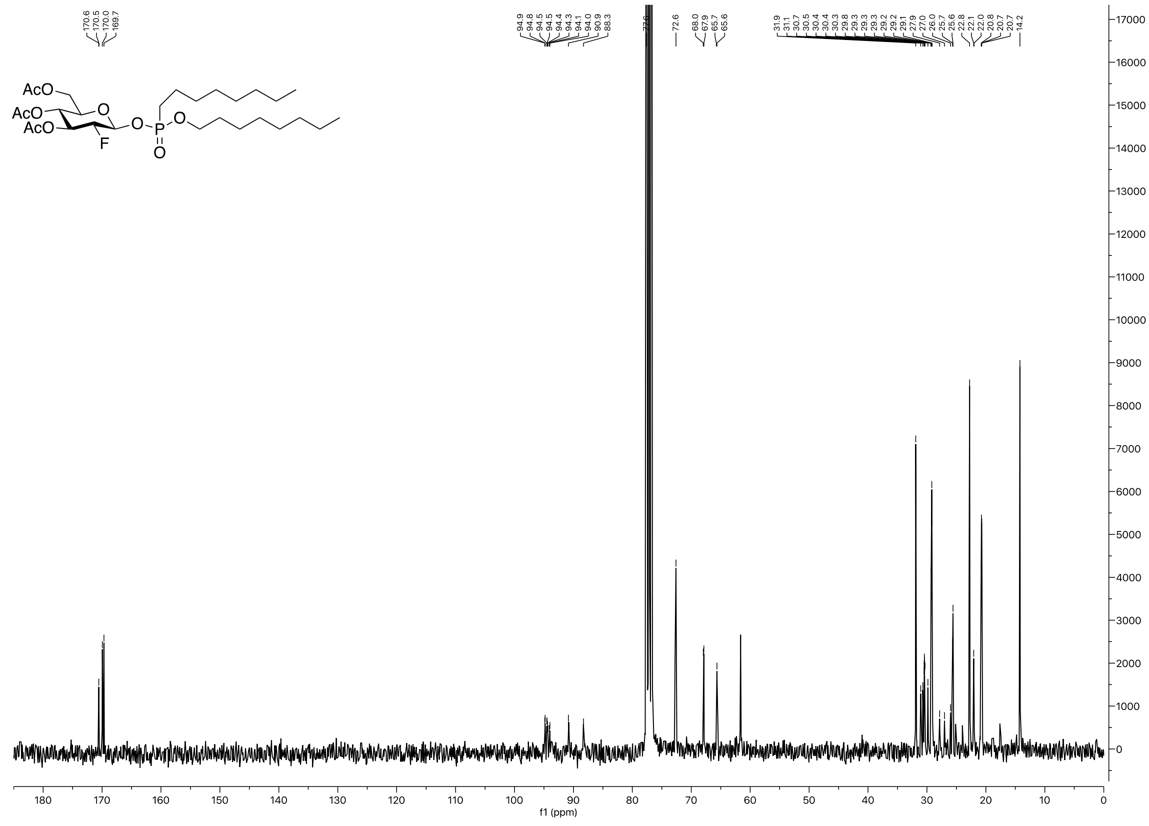
162 MHz ^{31}P NMR spectrum of **2** recorded in MeOD



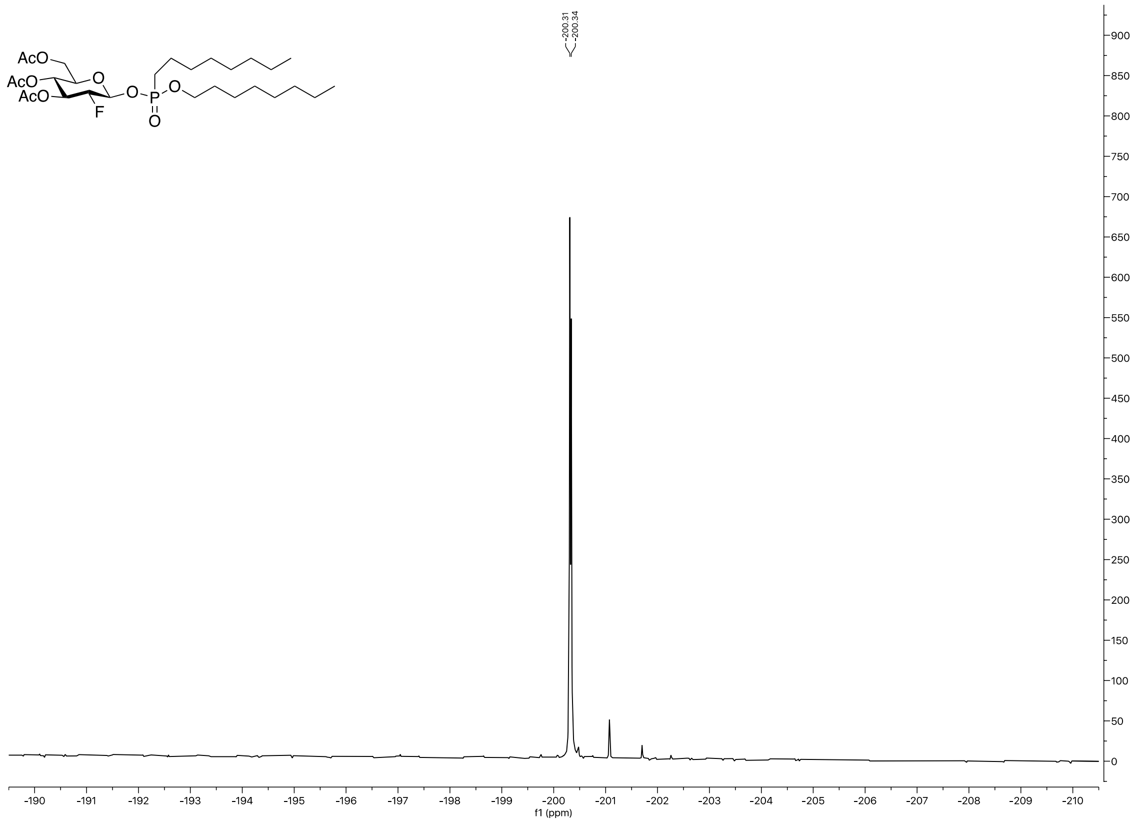
300 MHz ^{1}H NMR spectrum of **16c** recorded in $CDCl_3$



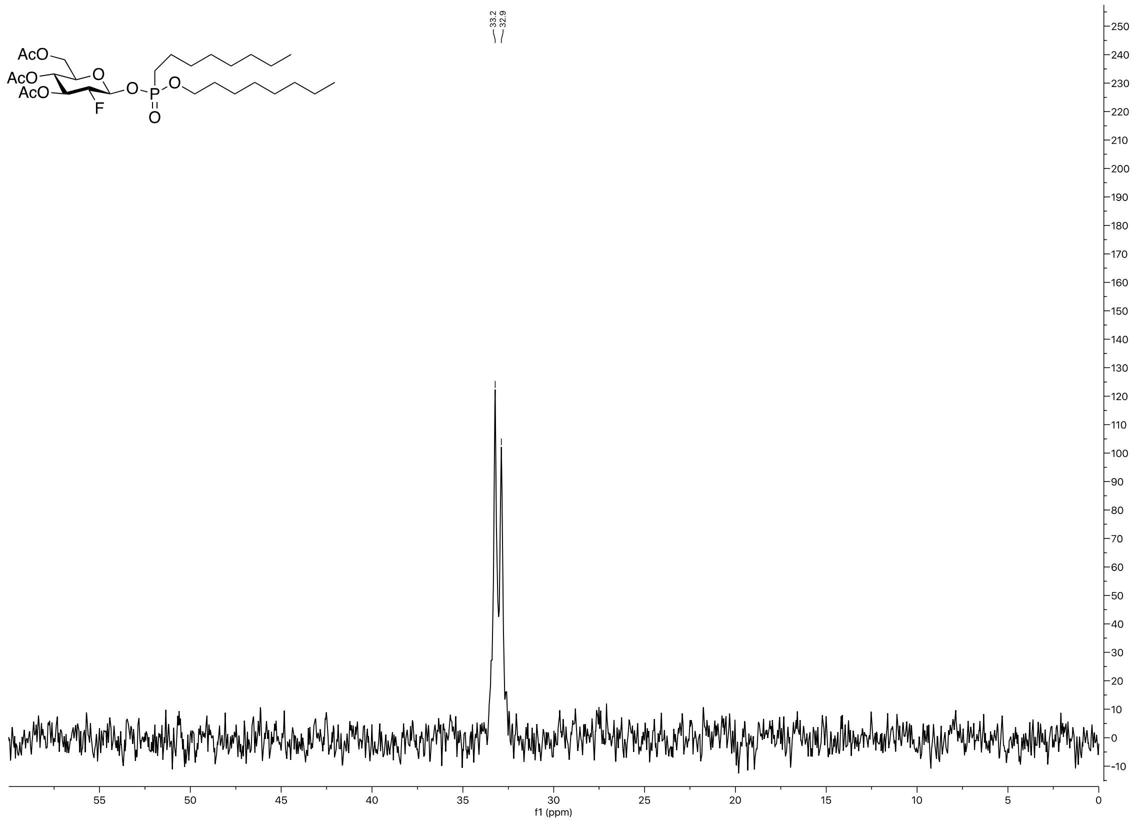
75 MHz ^{13}C NMR spectrum of **16c** recorded in $CDCl_3$



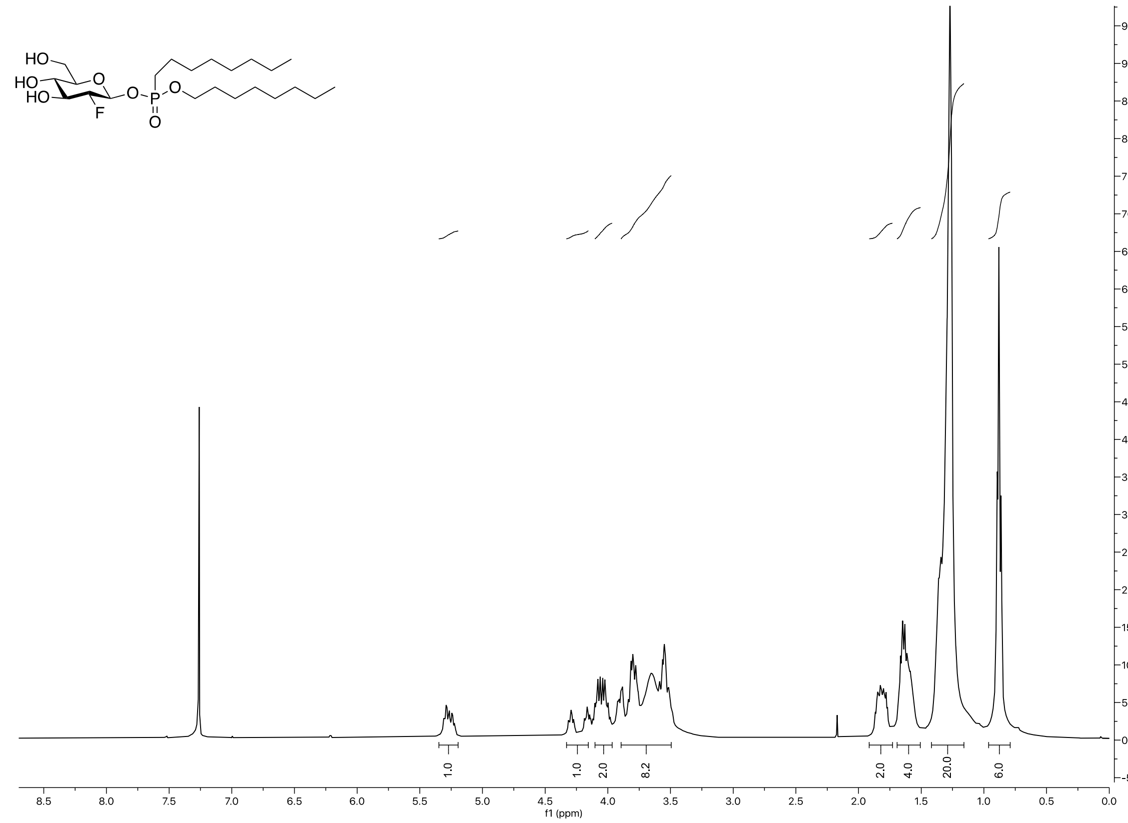
282 MHz ^{19}F NMR spectrum of **16c** recorded in $CDCl_3$



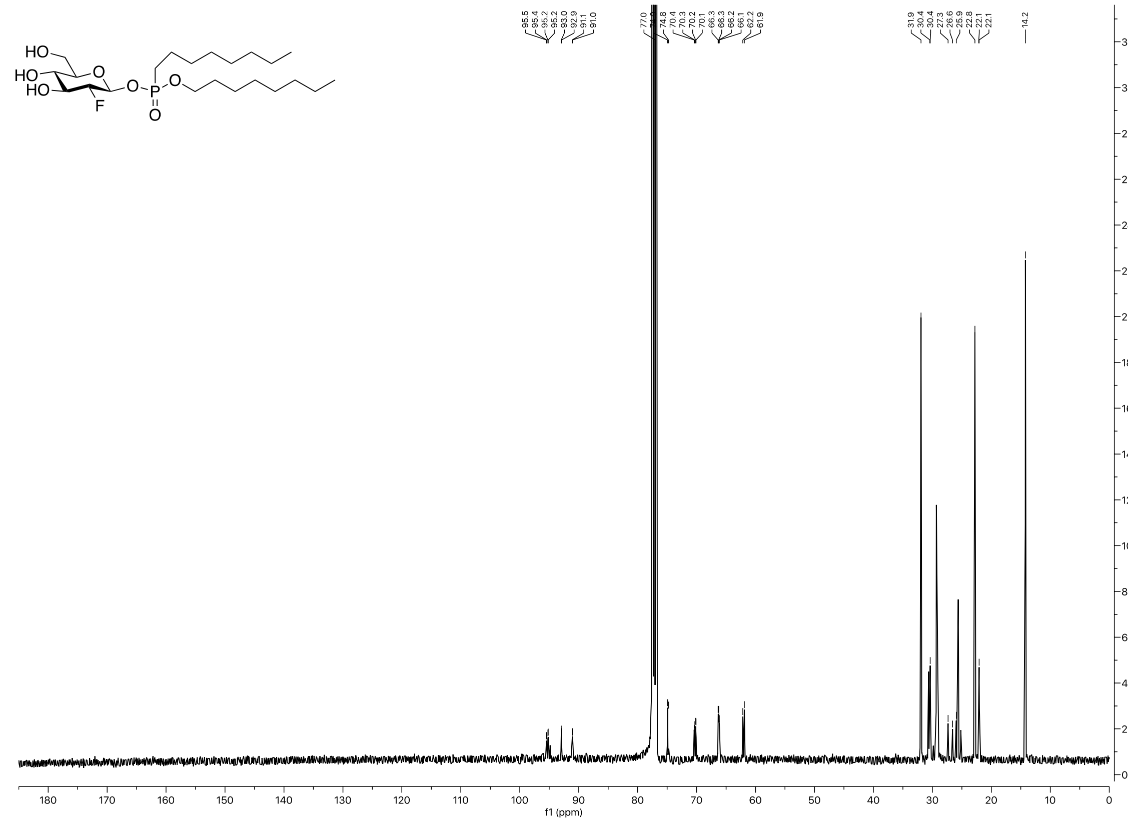
122 MHz ^{31}P NMR spectrum of **16c** recorded in $CDCl_3$



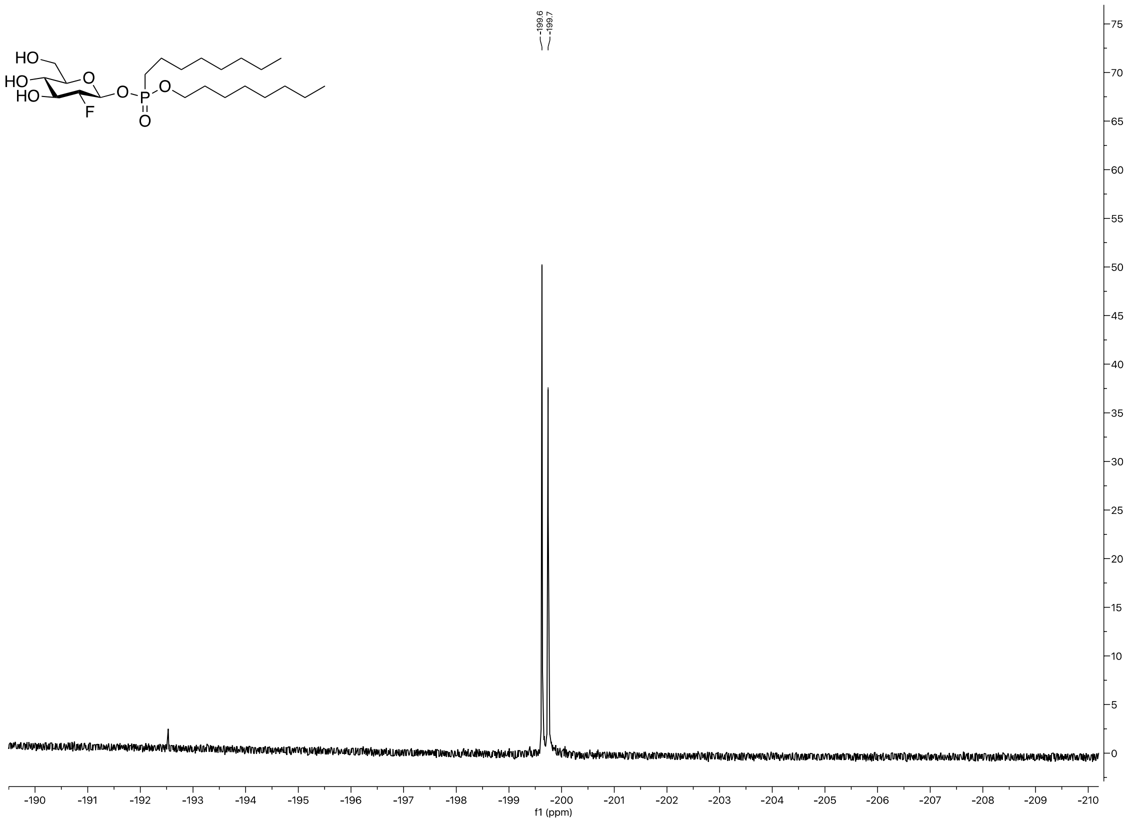
300 MHz ^{1}H NMR spectrum of **3** recorded in CDCl$_3$



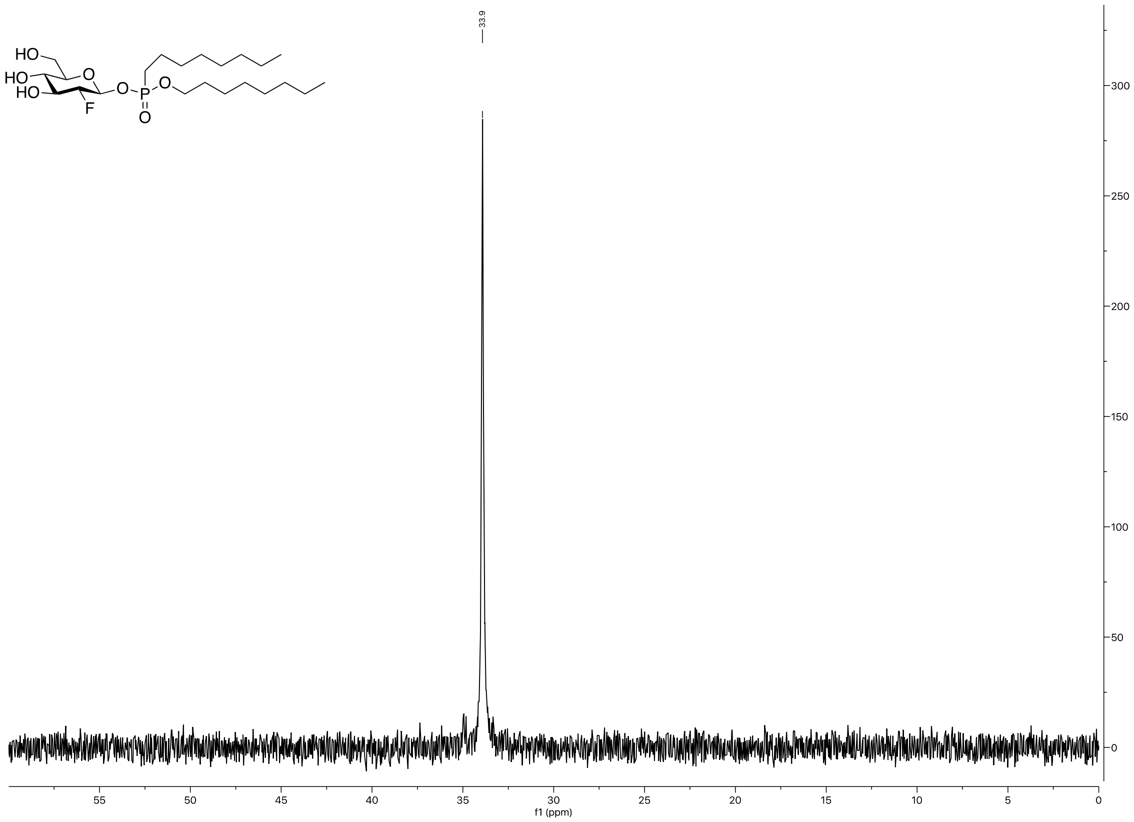
75 MHz ^{13}C NMR spectrum of **3** recorded in CDCl$_3$



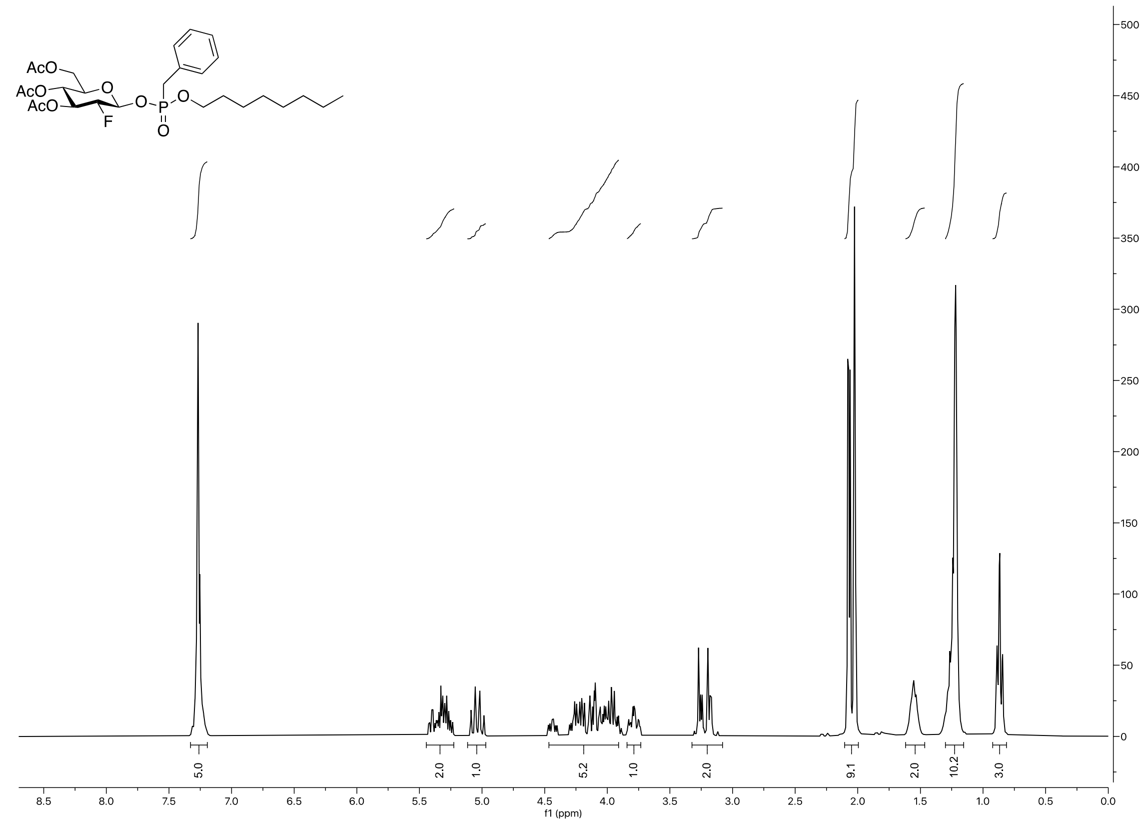
282 MHz ^{19}F NMR spectrum of **3** recorded in CDCl$_3$



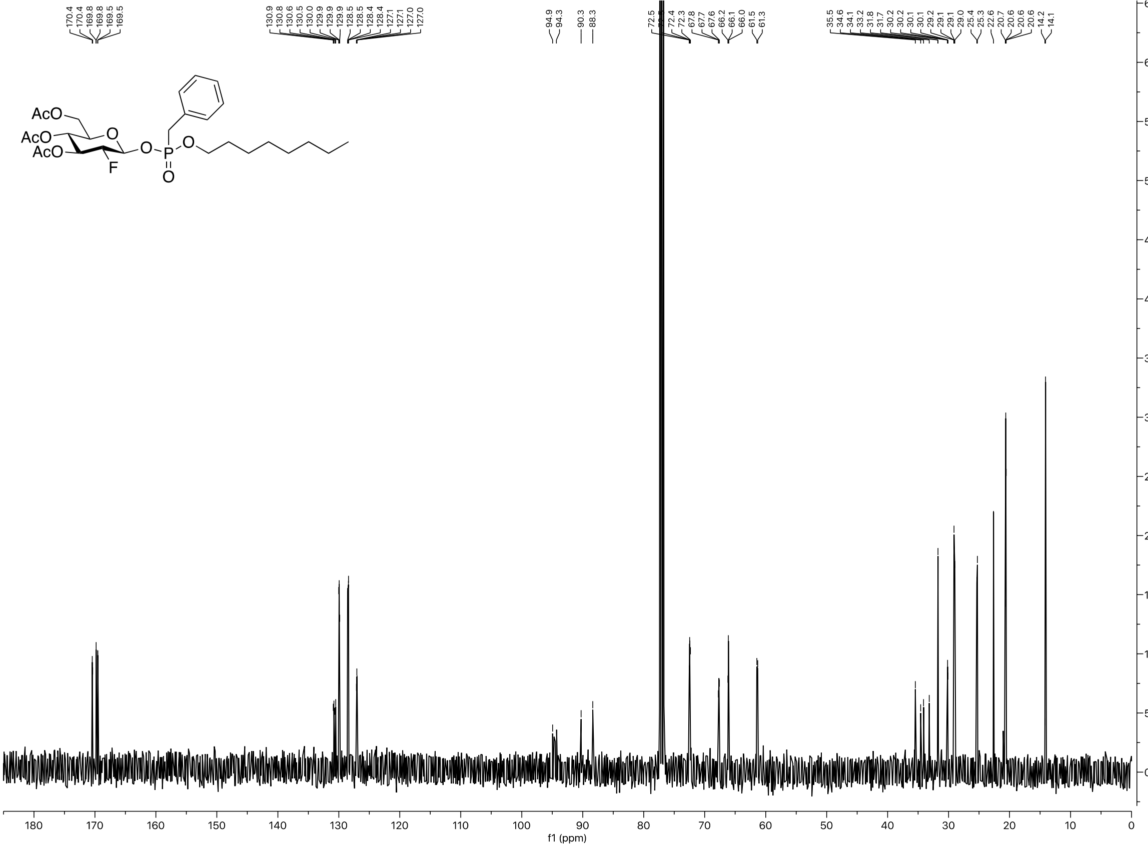
122 MHz ^{31}P NMR spectrum of **3** recorded in CDCl$_3$



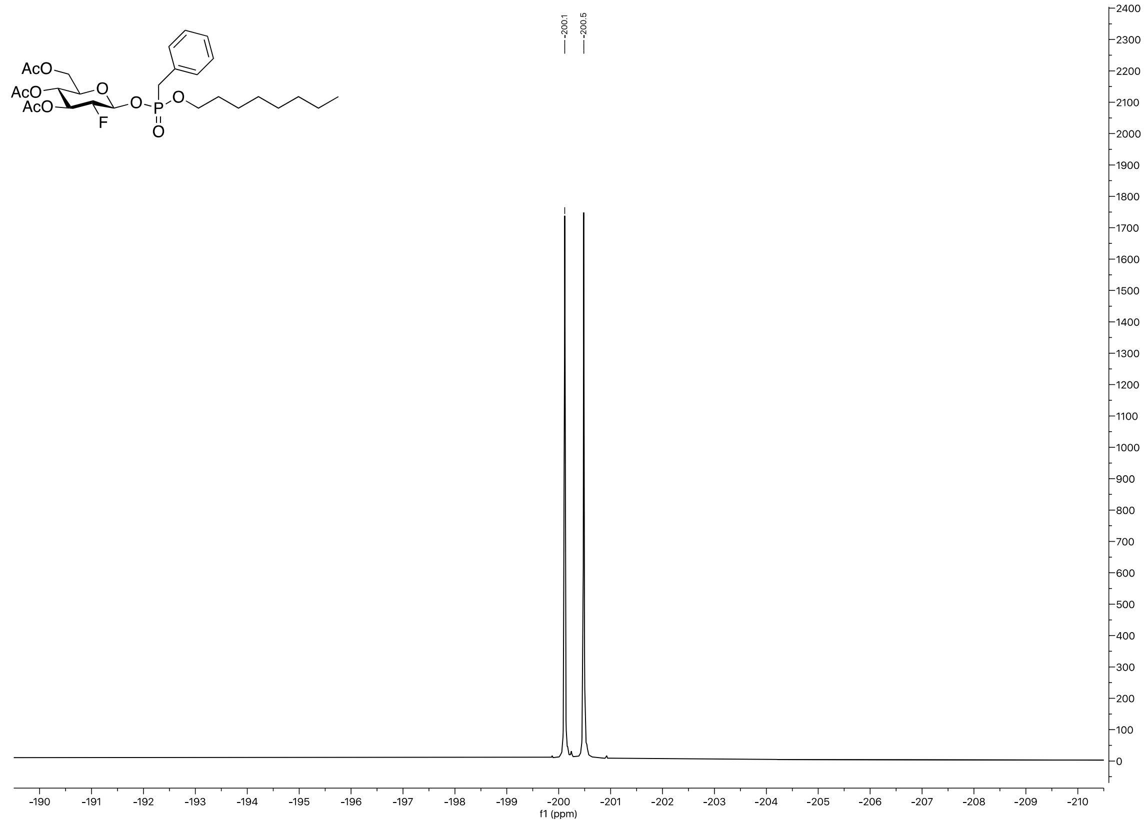
300 MHz ^{1}H NMR spectrum of **16d** recorded in CDCl$_3$



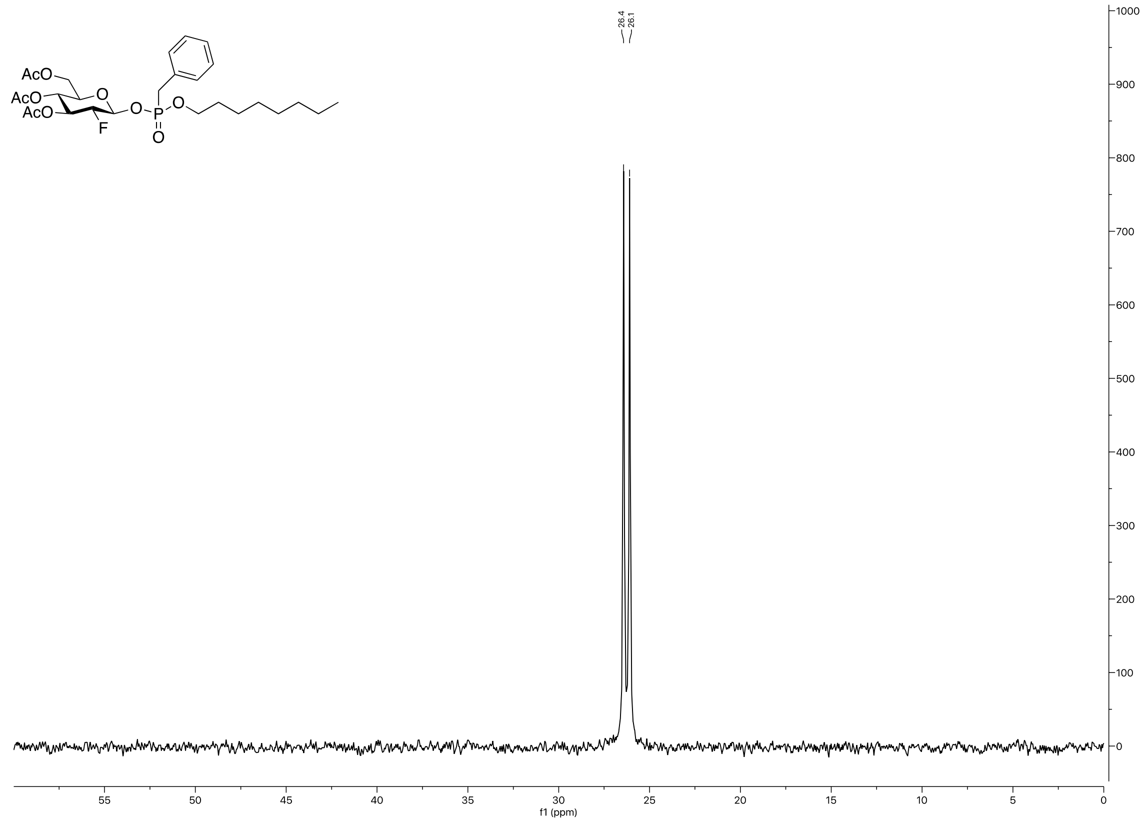
75 MHz ^{13}C NMR spectrum of **16d** recorded in CDCl$_3$



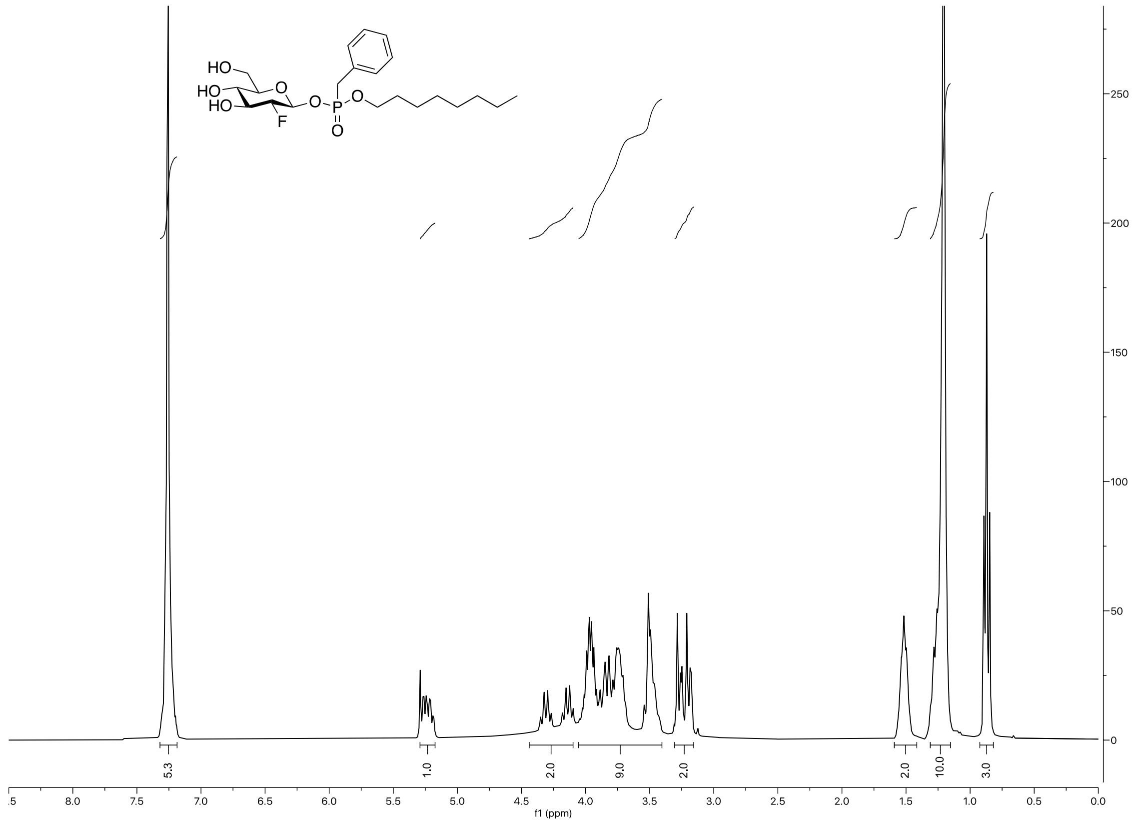
282 MHz ^{19}F NMR spectrum of **16d** recorded in $CDCl_3$



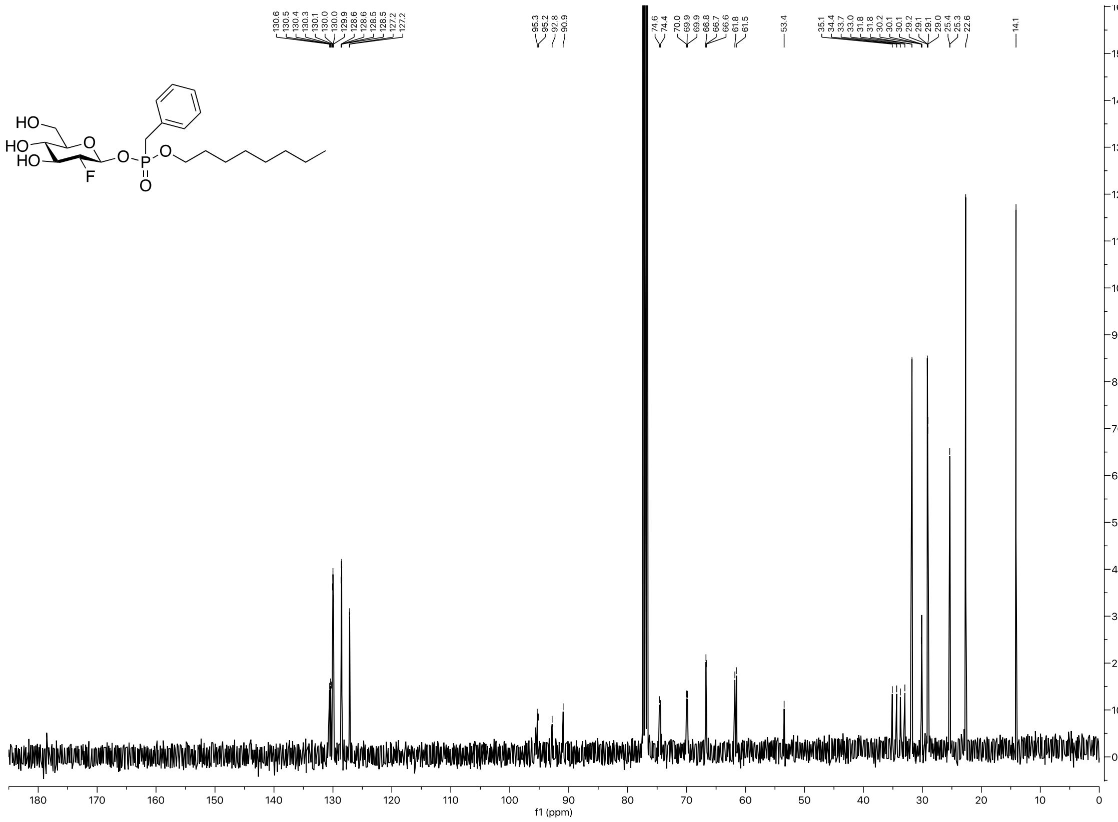
122 MHz ^{31}P NMR spectrum of **16d** recorded in $CDCl_3$



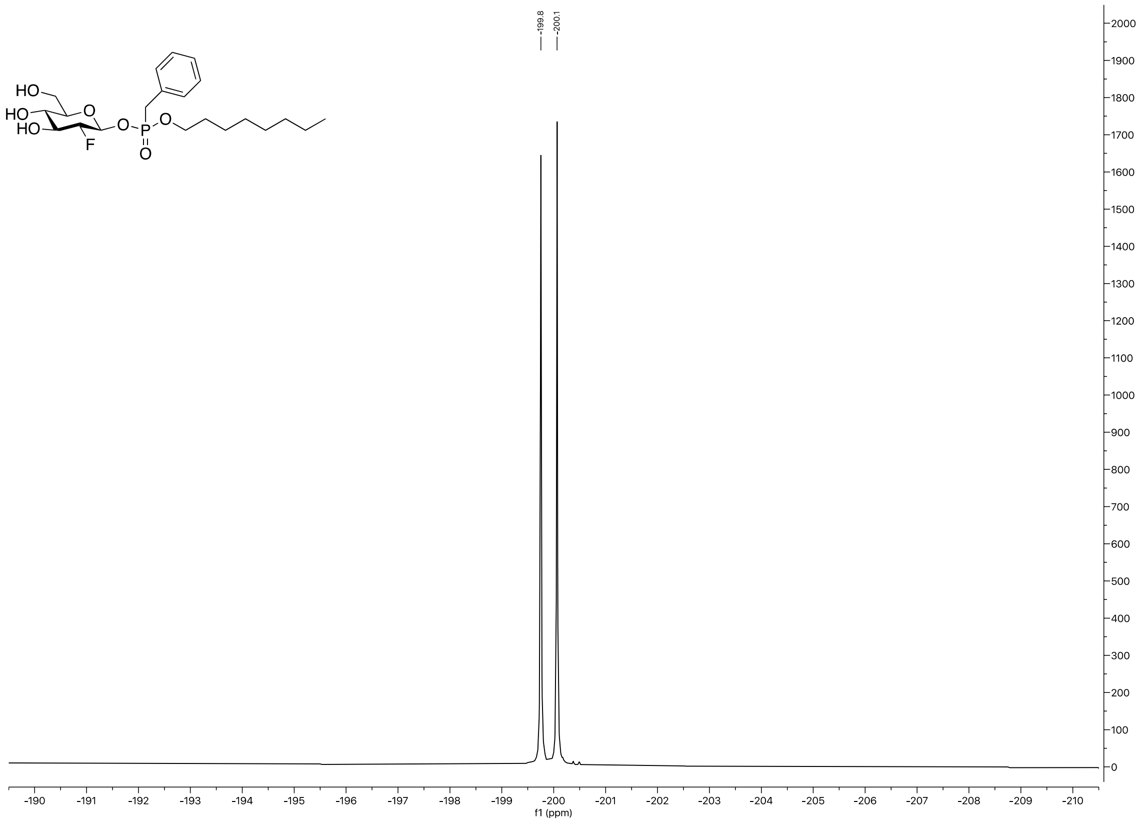
300 MHz ^{1}H NMR spectrum of **4** recorded in $CDCl_3$



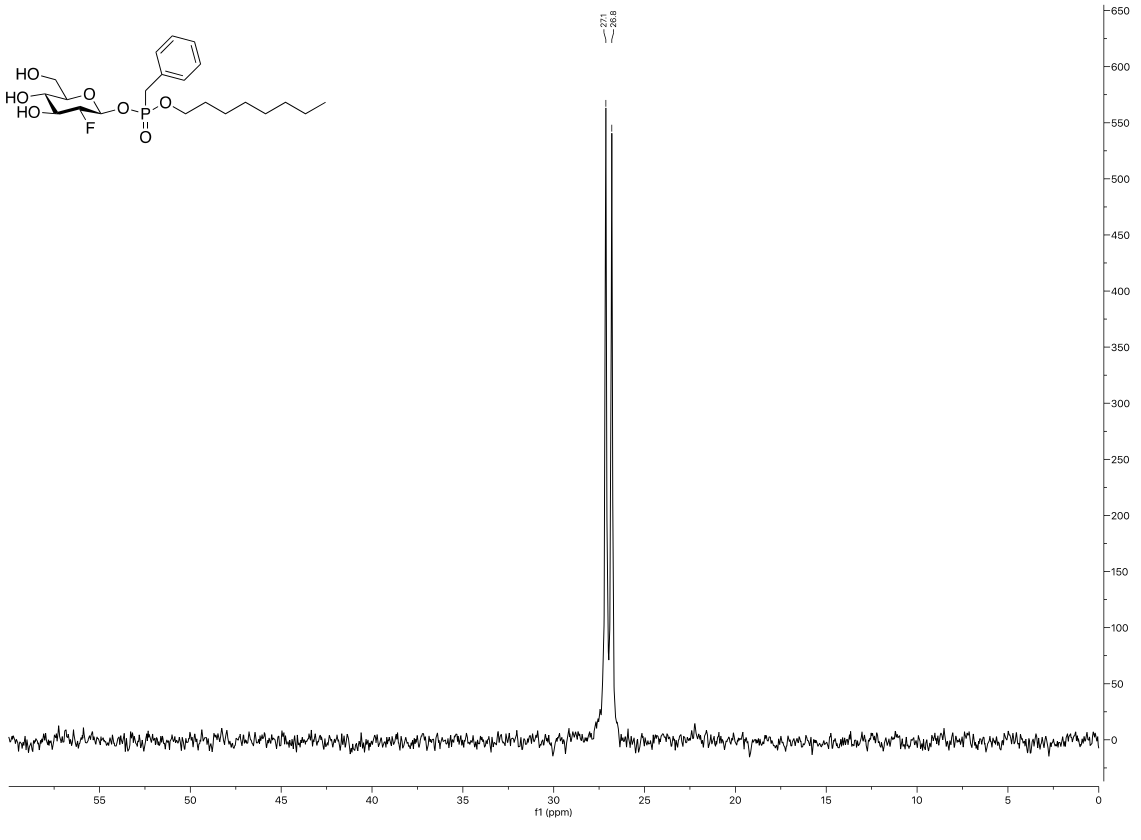
75 MHz ^{13}C NMR spectrum of **4** recorded in $CDCl_3$



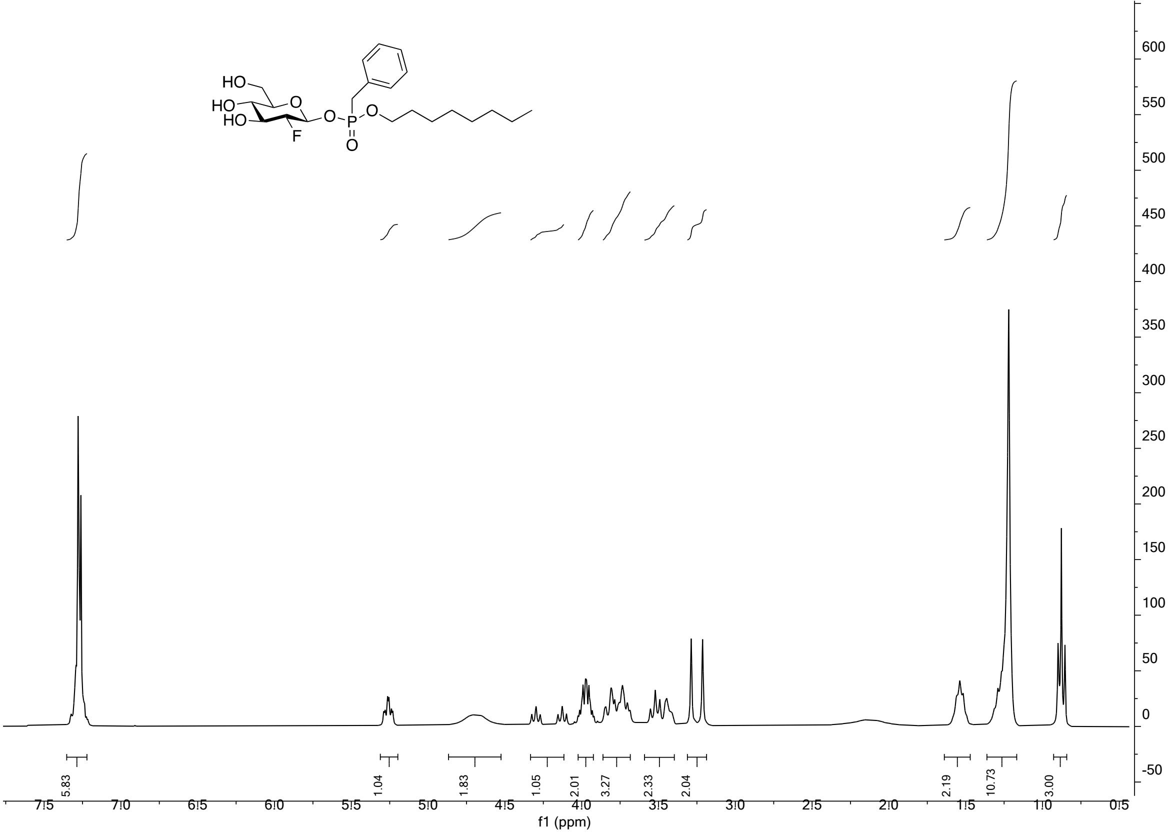
282 MHz ^{19}F NMR spectrum of **4** recorded in $CDCl_3$



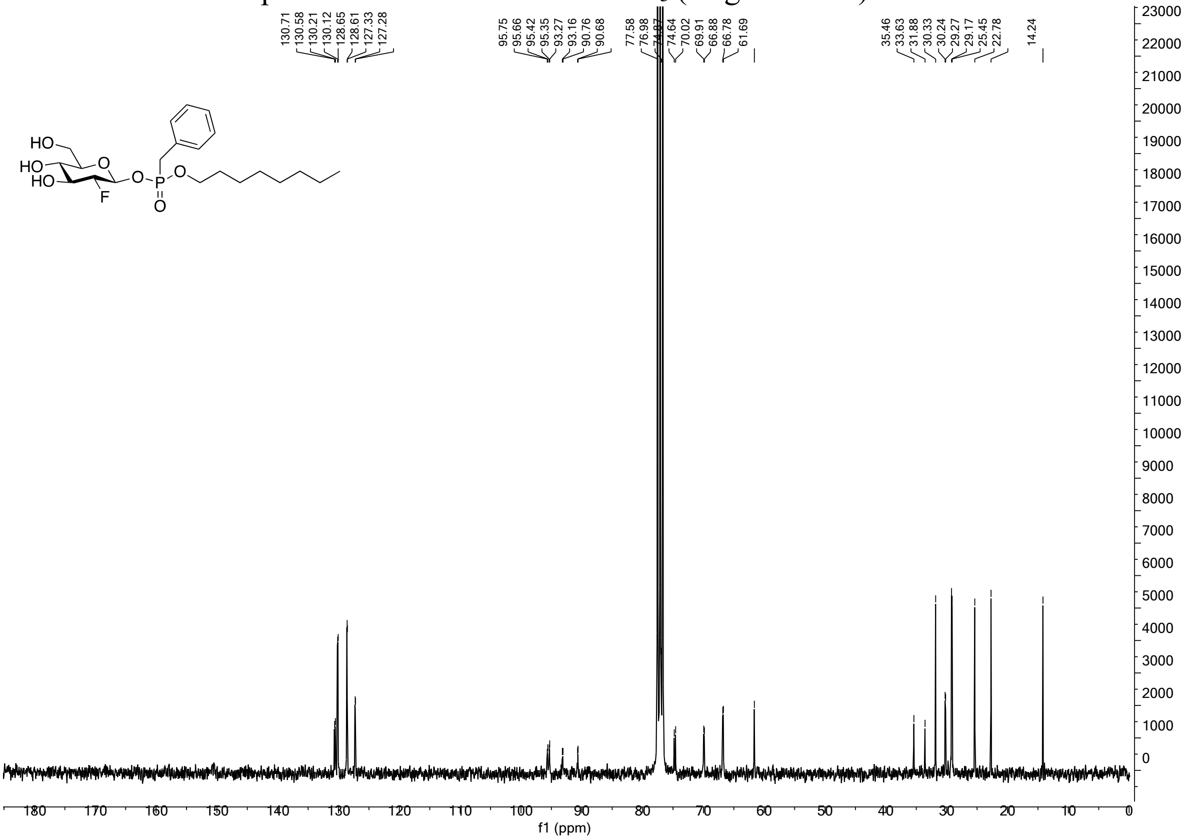
122 MHz ^{31}P NMR spectrum of **4** recorded in $CDCl_3$



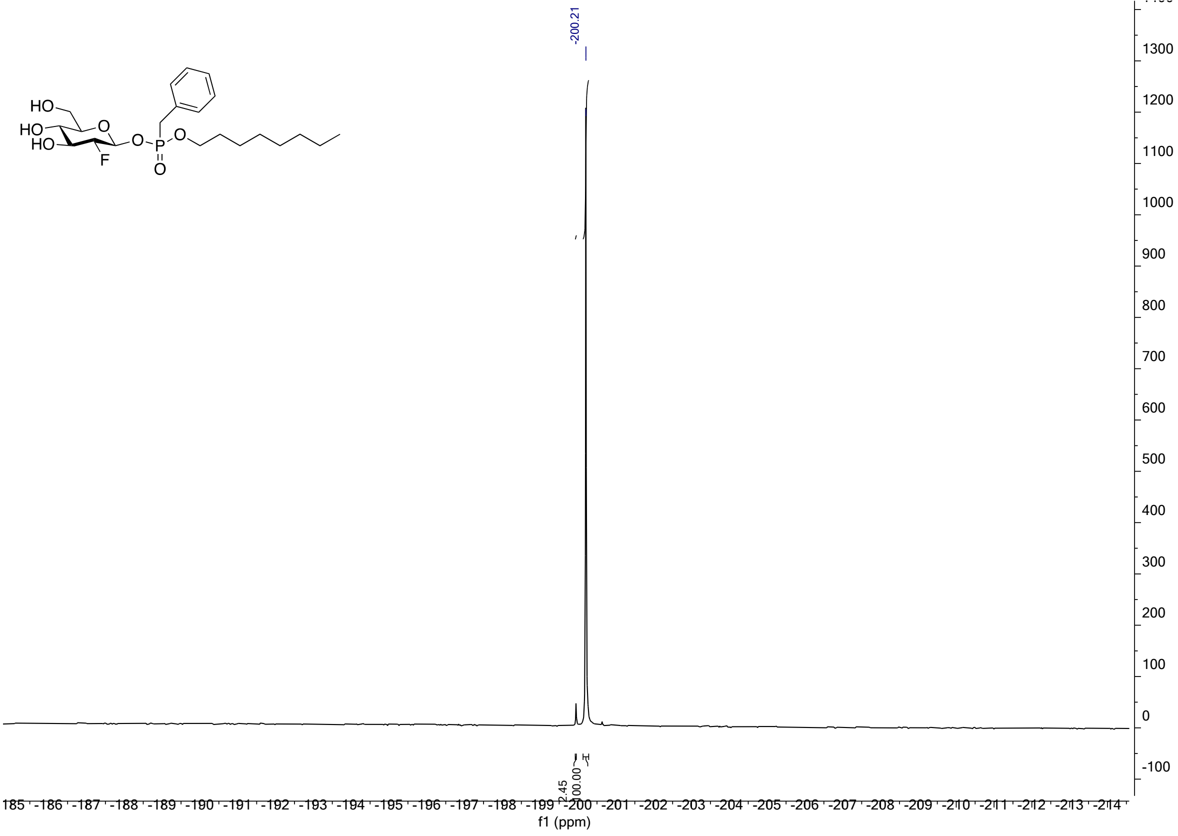
300 MHz ^{1}H NMR spectrum of **4** recorded in CDCl$_3$ (single isomer)



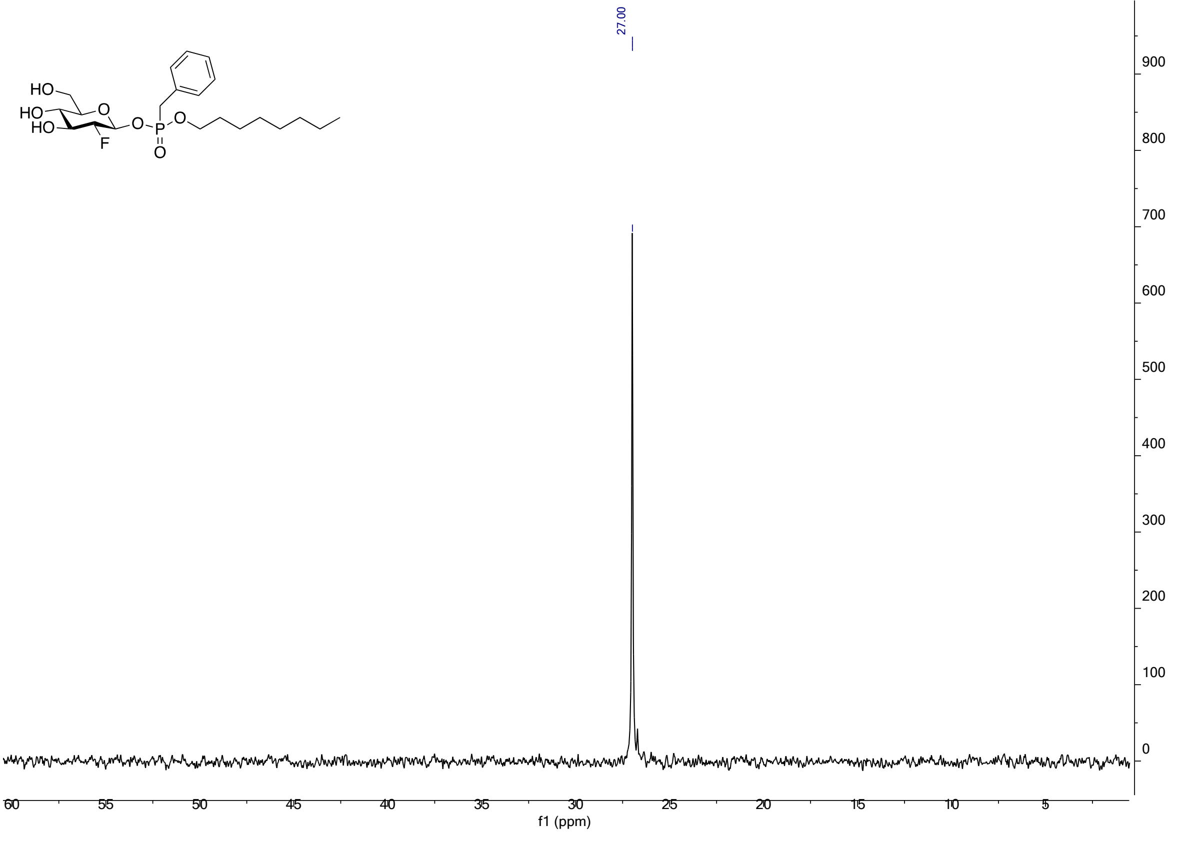
75 MHz ^{13}C NMR spectrum of **4** recorded in CDCl$_3$ (single isomer)



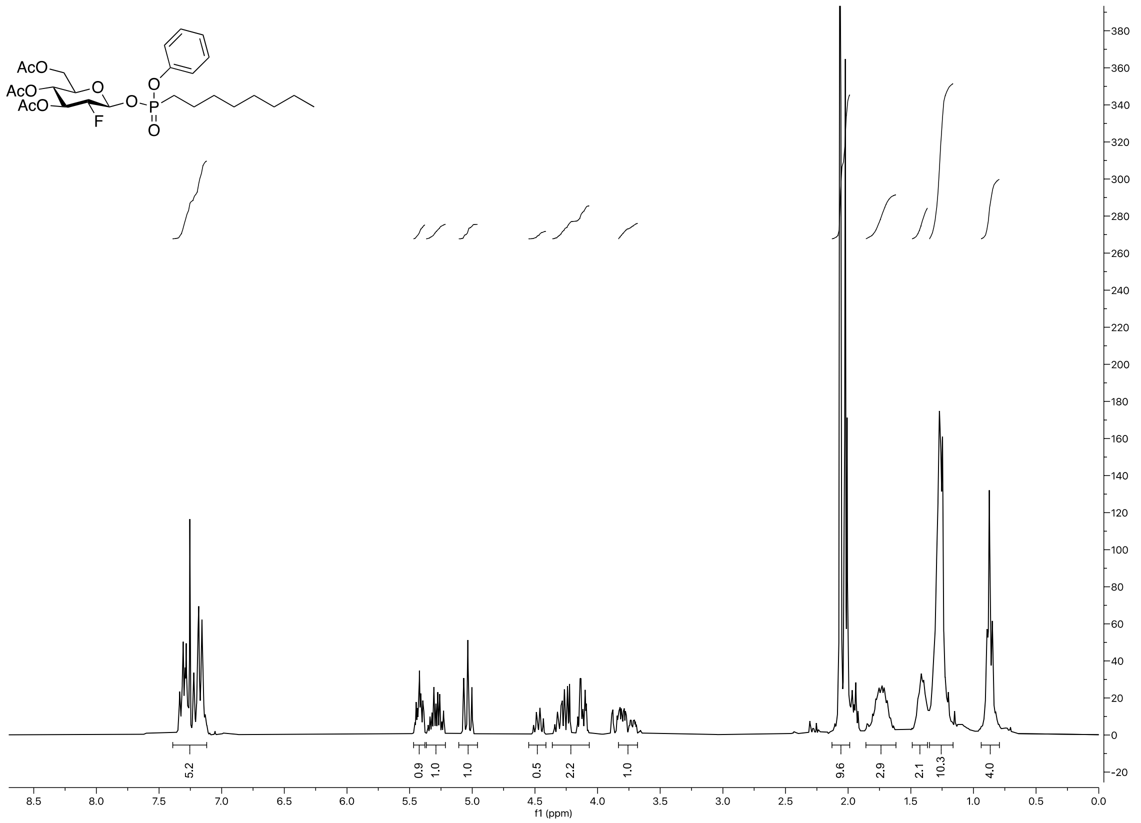
282 MHz ^{19}F NMR spectrum of **4** recorded in $CDCl_3$ (single isomer)



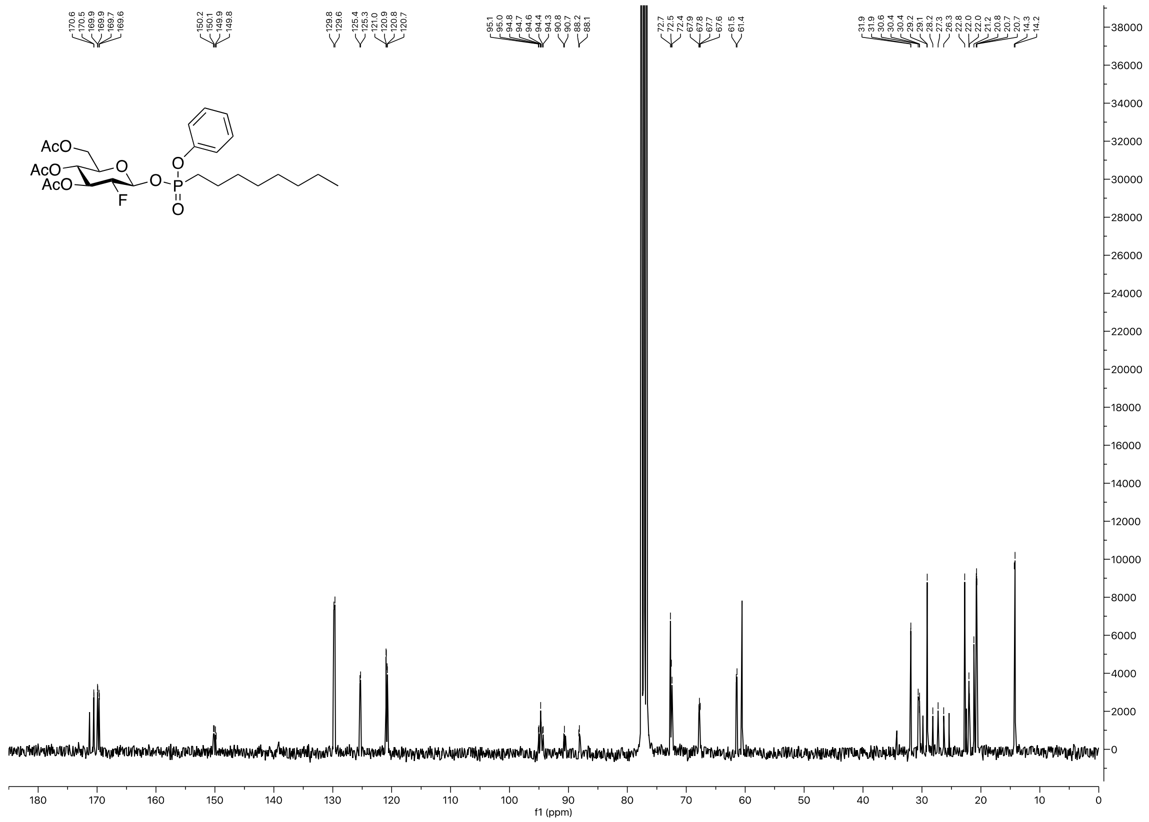
122 MHz ^{31}P NMR spectrum of **4** recorded in $CDCl_3$ (single isomer)



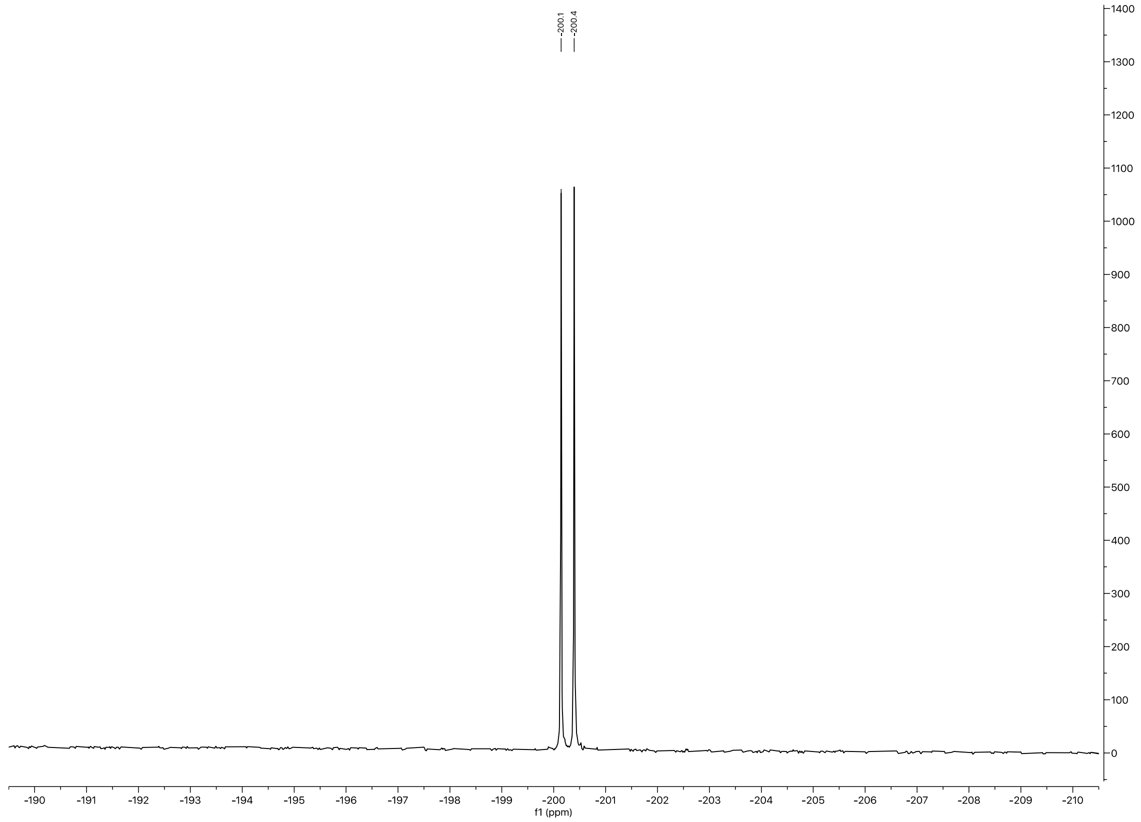
300 MHz ^1H NMR spectrum of **16e** recorded in $CDCl_3$



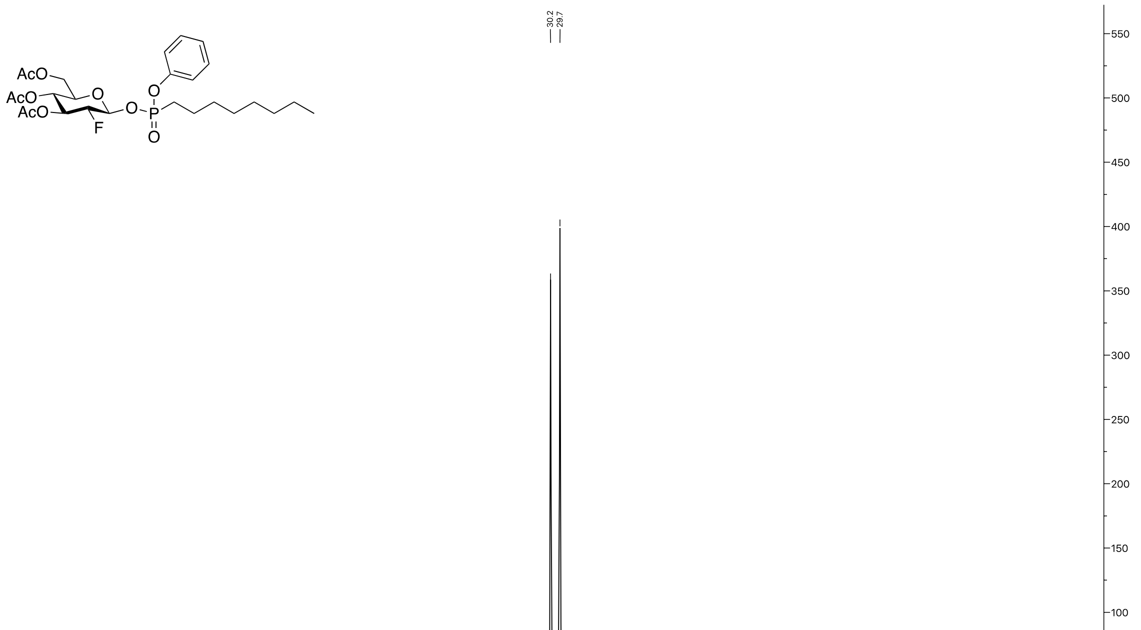
75 MHz ^{13}C NMR spectrum of **16e** recorded in $CDCl_3$



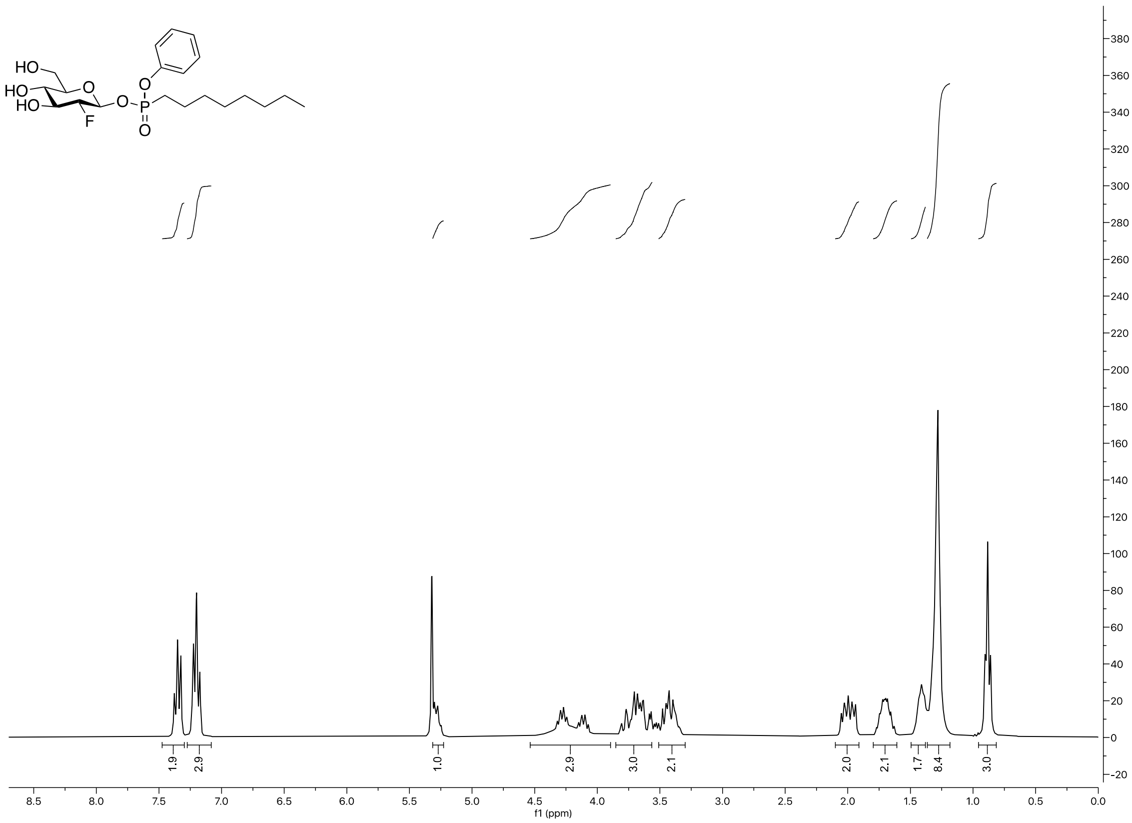
282 MHz ^{19}F NMR spectrum of **16e** recorded in $CDCl_3$



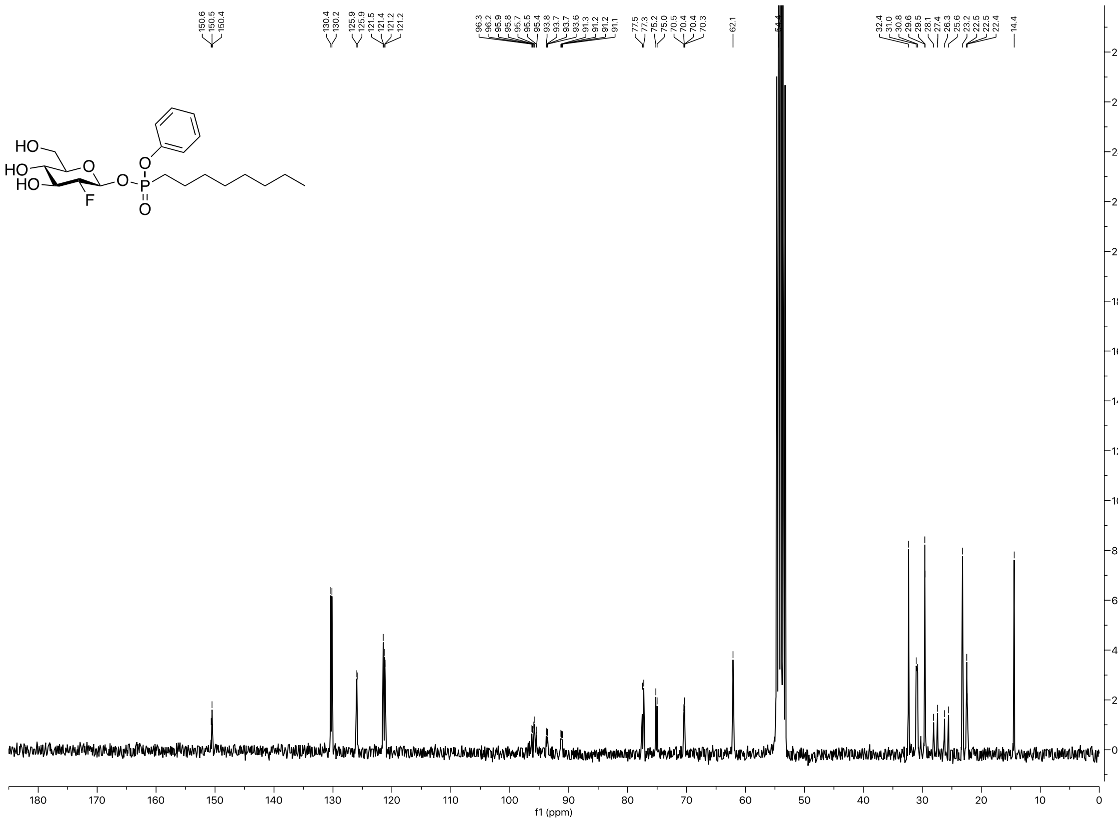
122 MHz ^{31}P NMR spectrum of **16e** recorded in $CDCl_3$



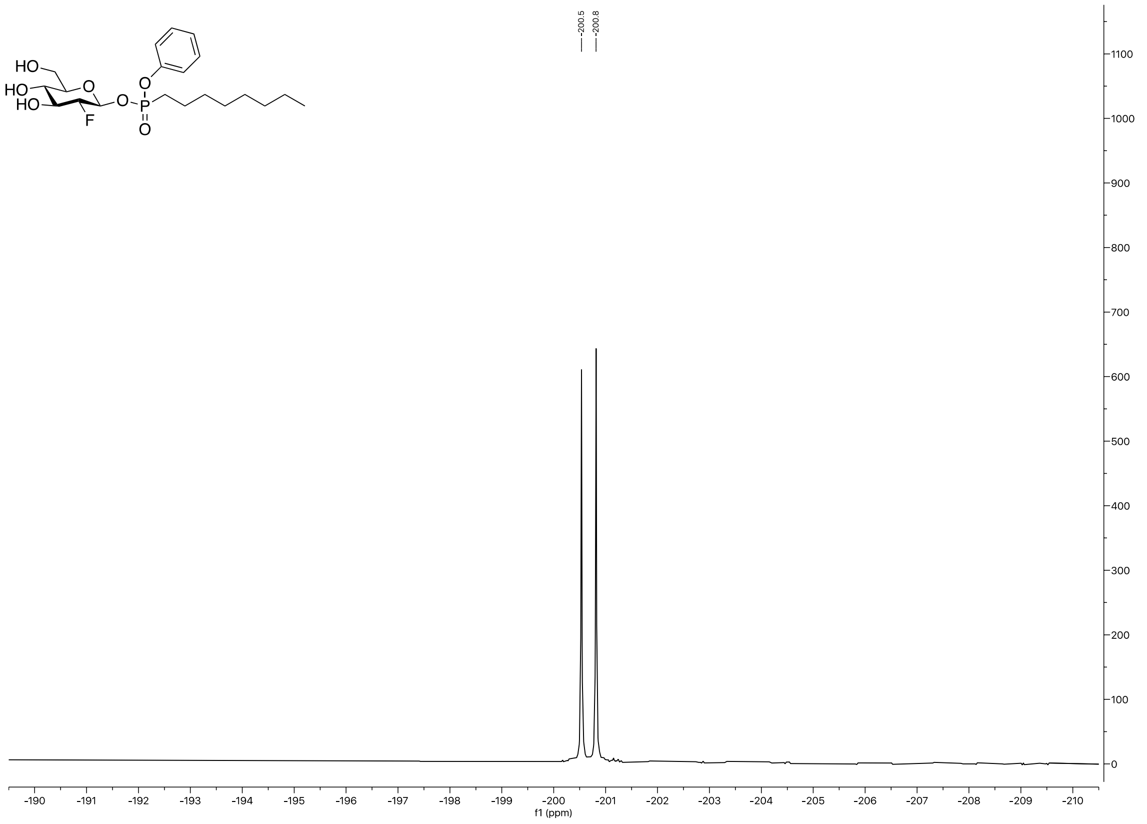
300 MHz ^{1}H NMR spectrum of **5** recorded in CD_2Cl_2



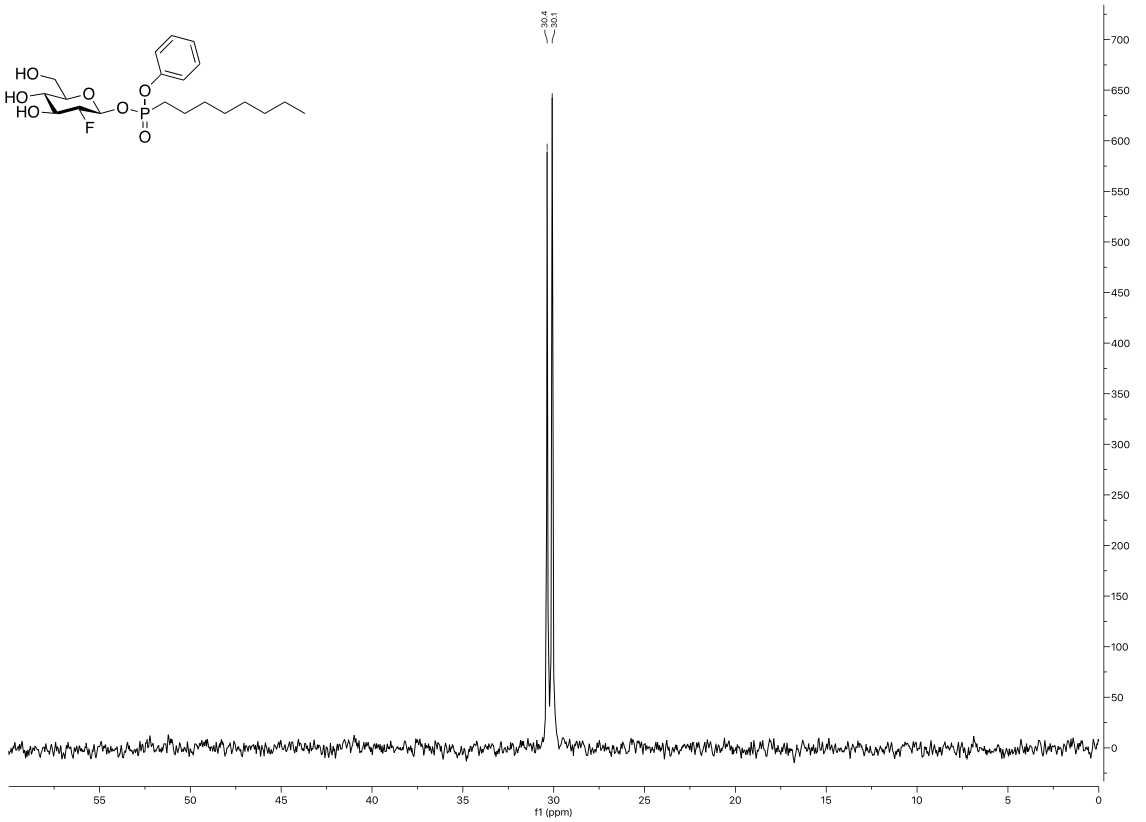
75 MHz ^{13}C NMR spectrum of **5** recorded in CD_2Cl_2



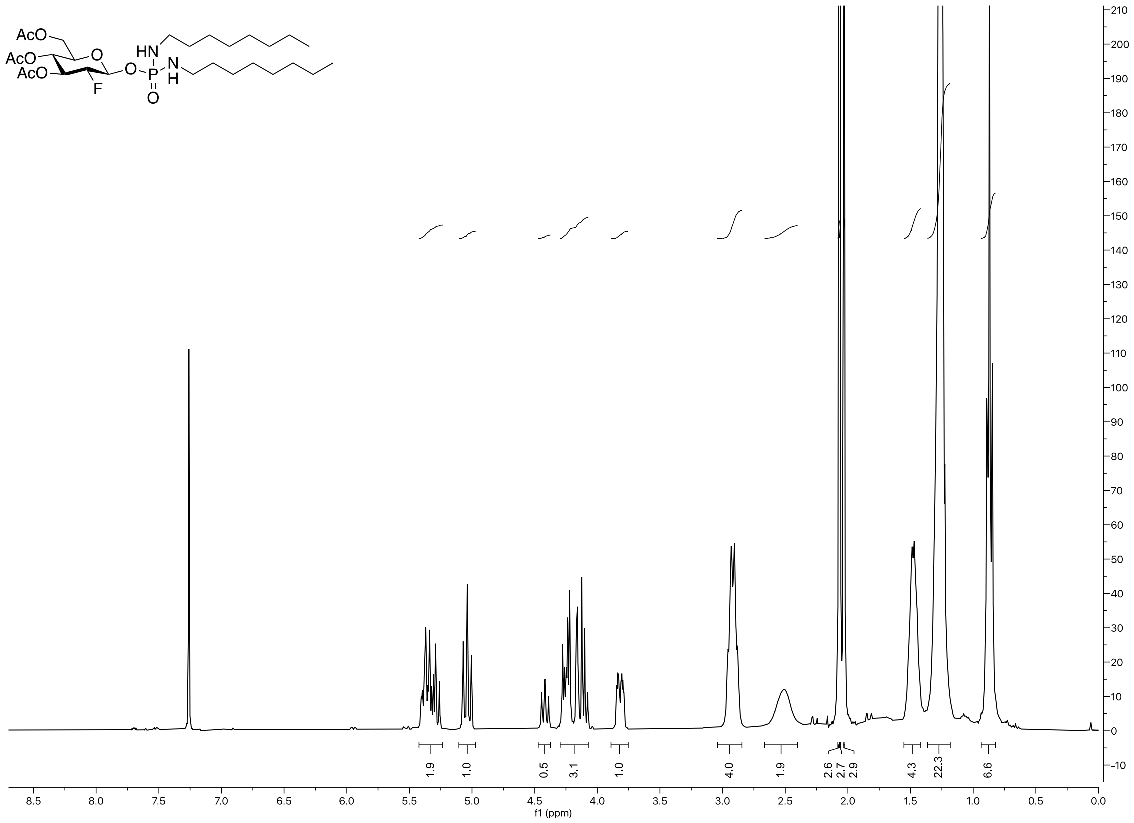
282 MHz ^{19}F NMR spectrum of **5** recorded in CD_2Cl_2



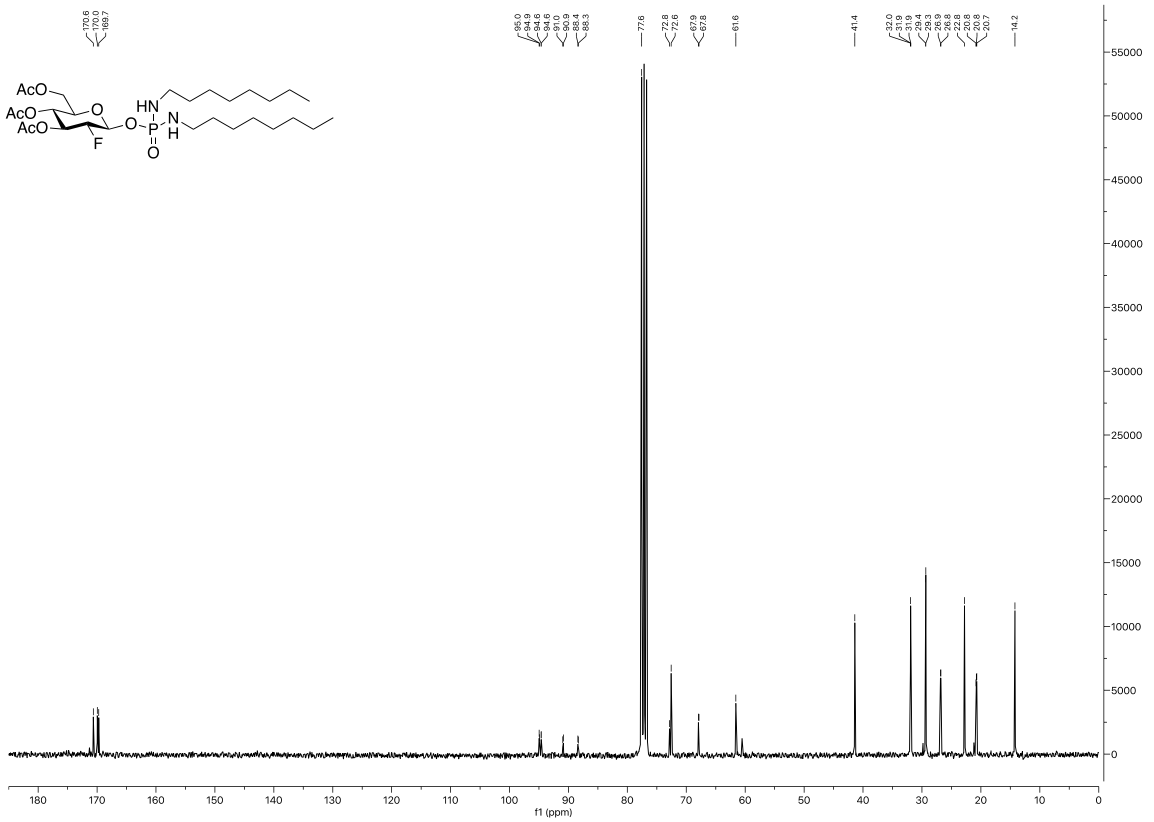
122 MHz ^{31}P NMR spectrum of **5** recorded in CD_2Cl_2



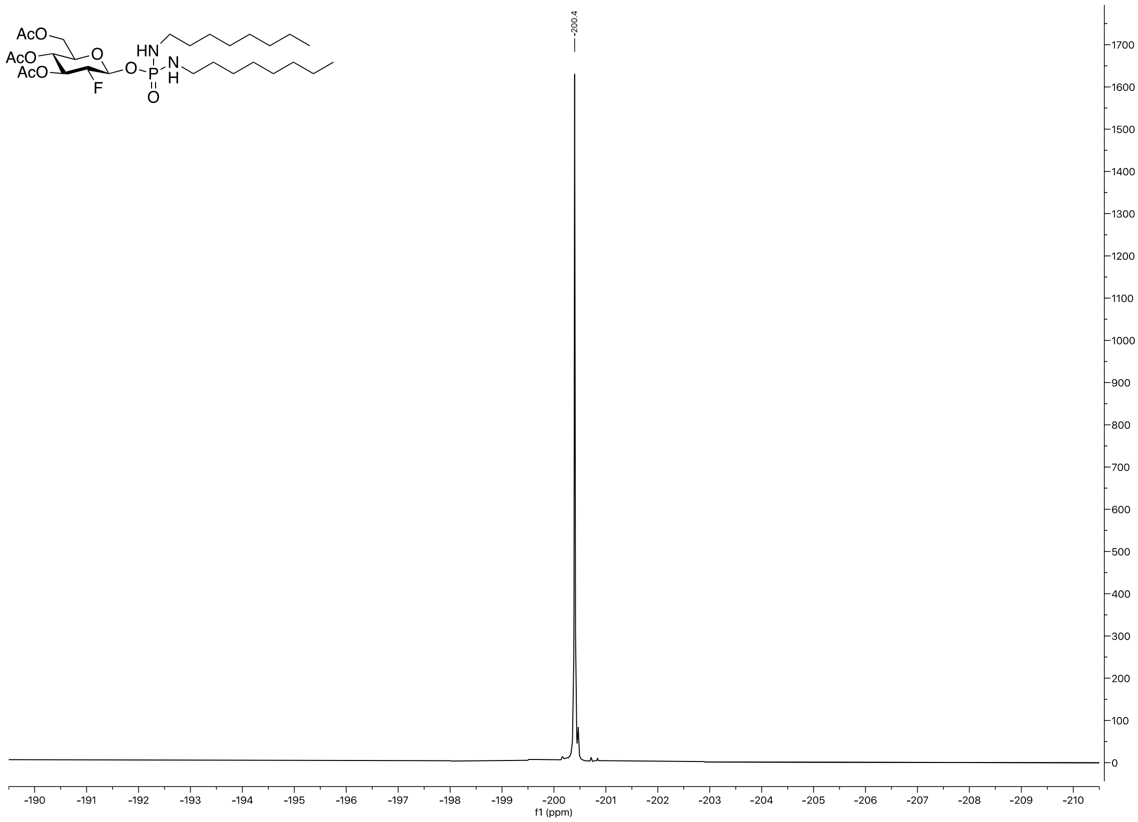
300 MHz ^{1}H NMR spectrum of **16f** recorded in CDCl$_3$



75 MHz ^{13}C NMR spectrum of **16f** recorded in CDCl$_3$



282 MHz ^{19}F NMR spectrum of **16f** recorded in $CDCl_3$



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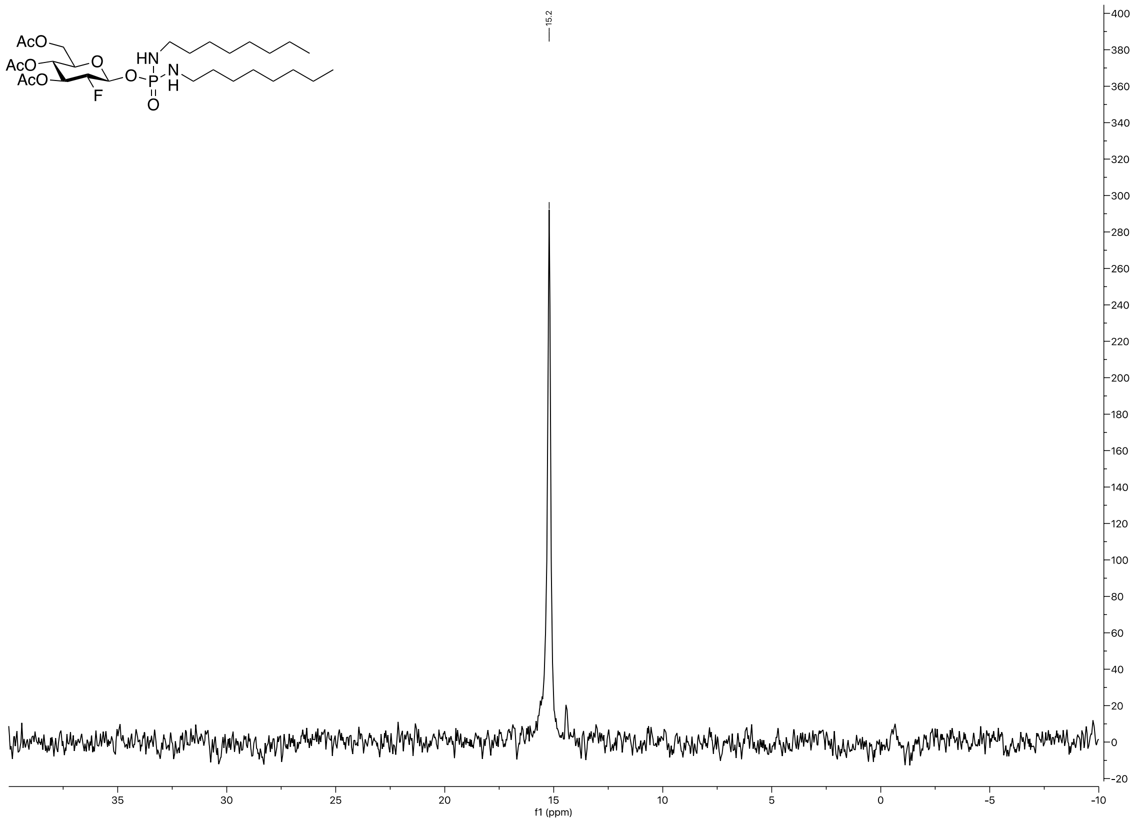
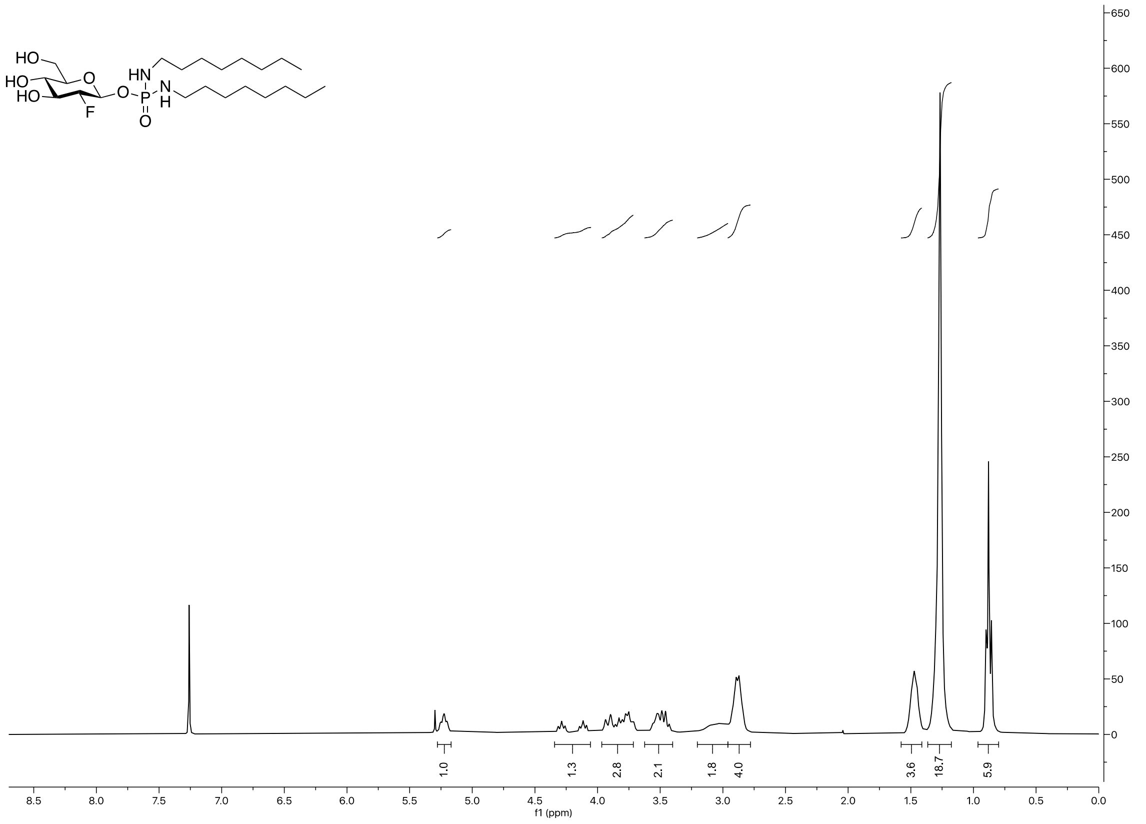
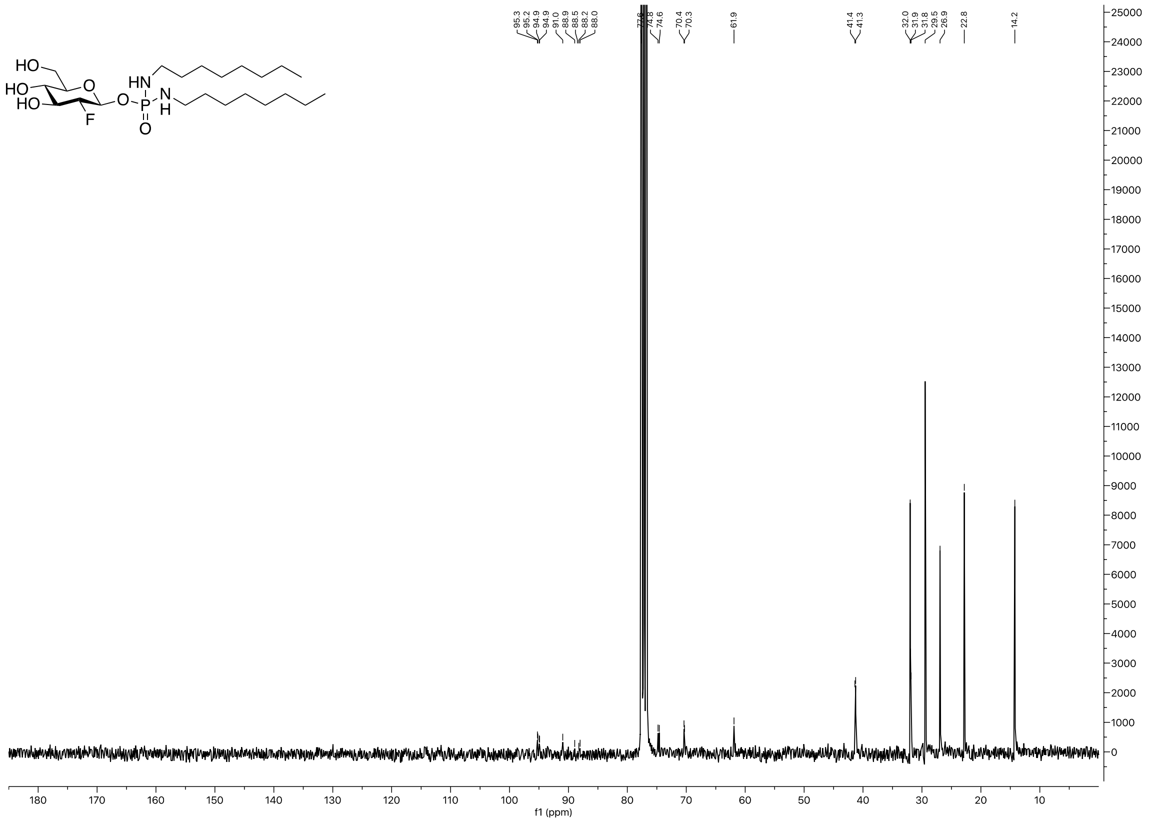


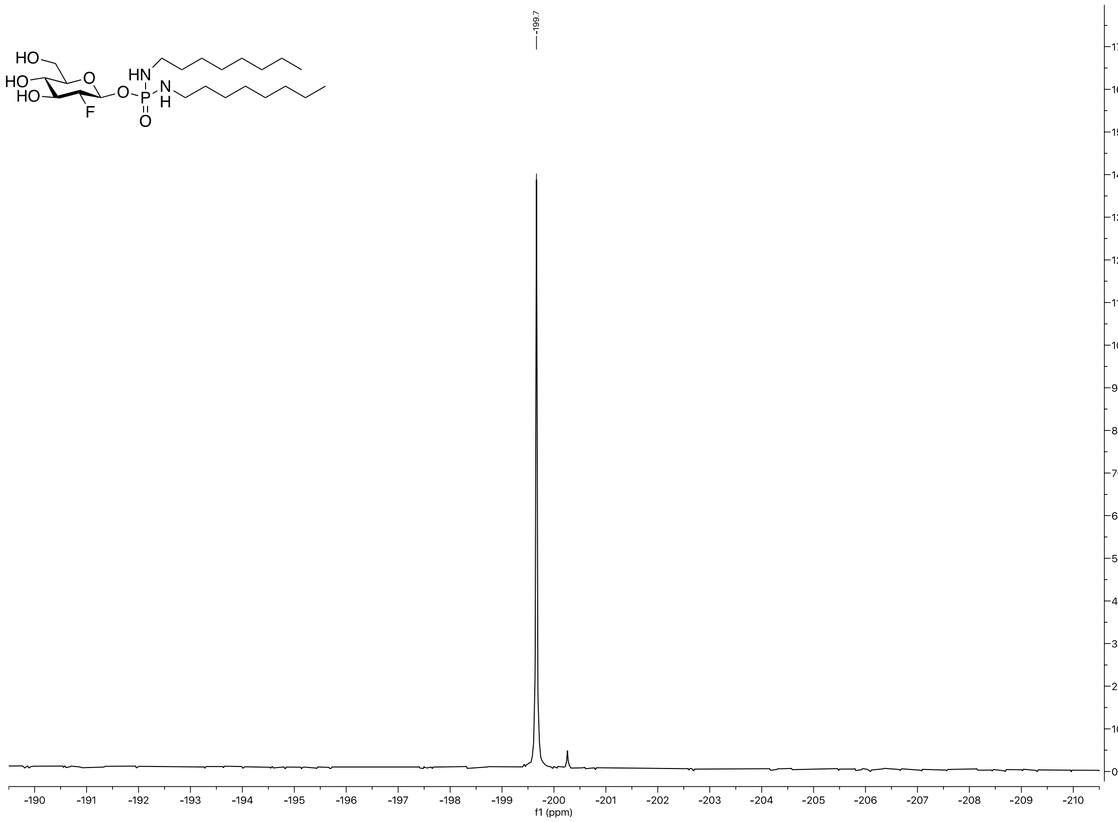
300 MHz ^{1}H NMR spectrum of **6** recorded in $CDCl_3$



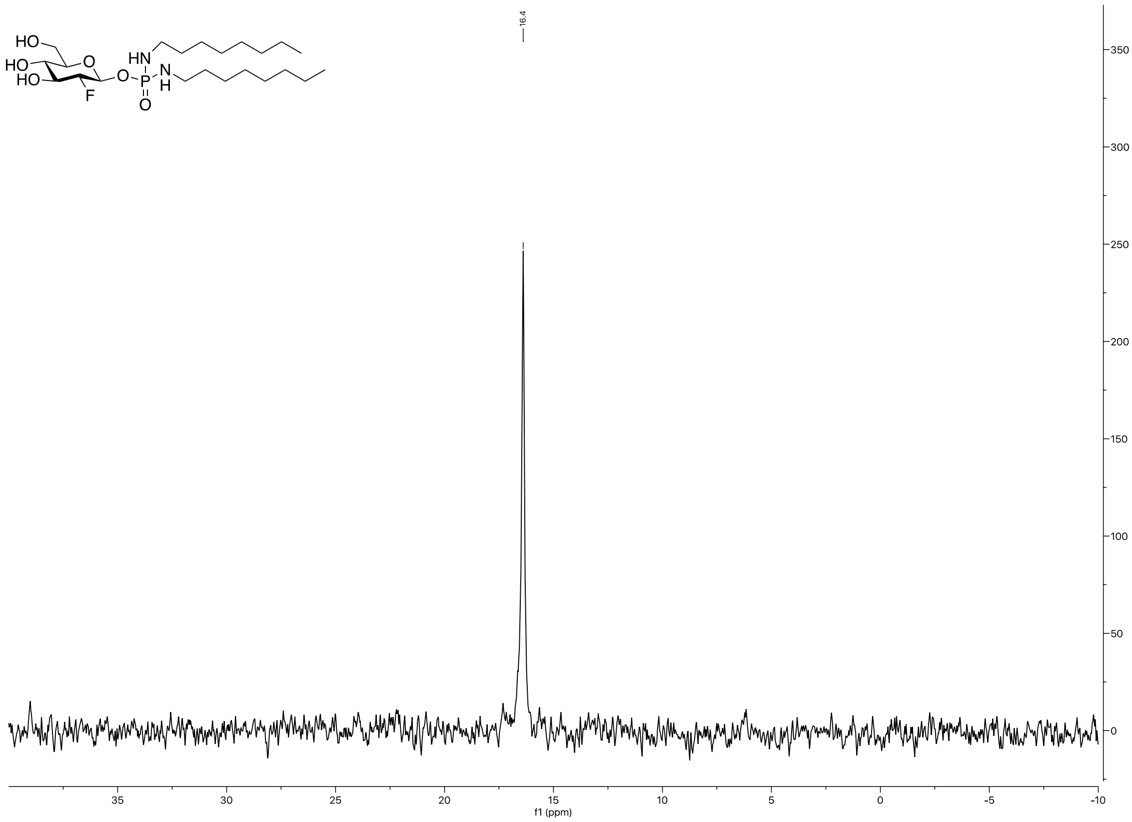
75 MHz ^{13}C NMR spectrum of **6** recorded in $CDCl_3$



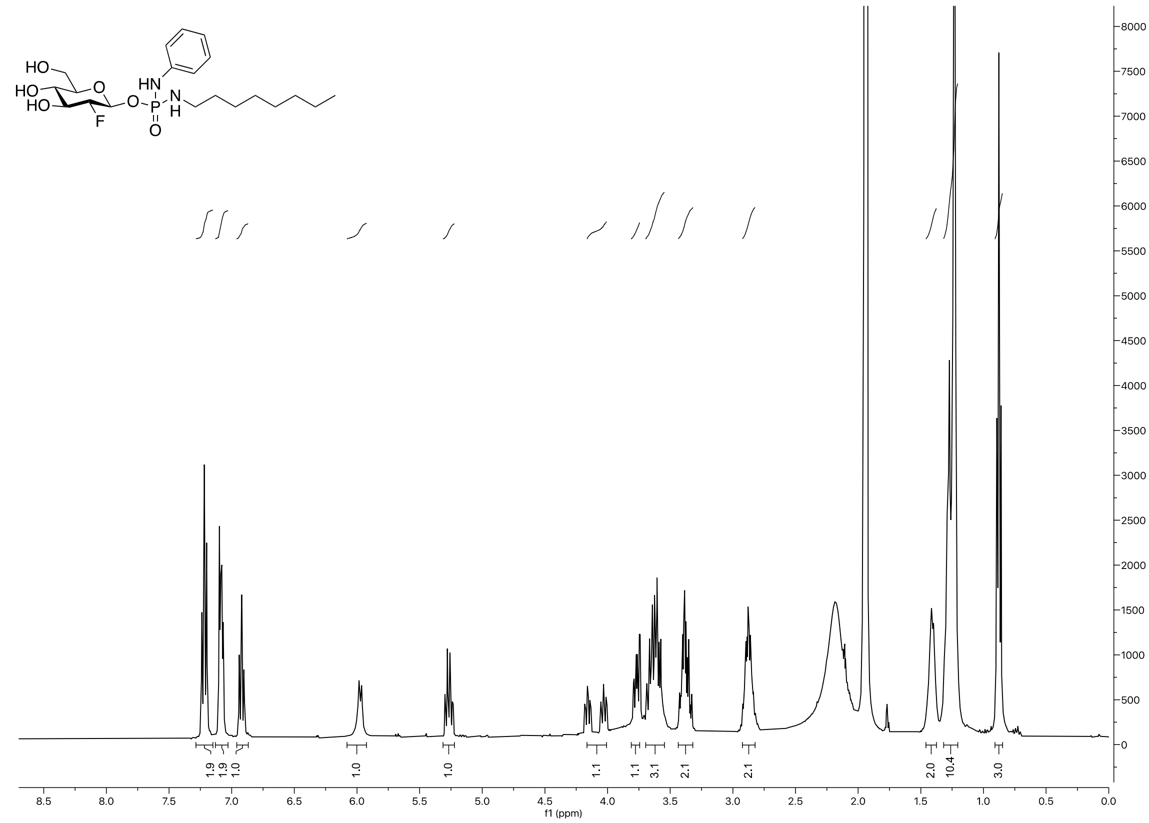
282 MHz ^{19}F NMR spectrum of **6** recorded in CDCl$_3$



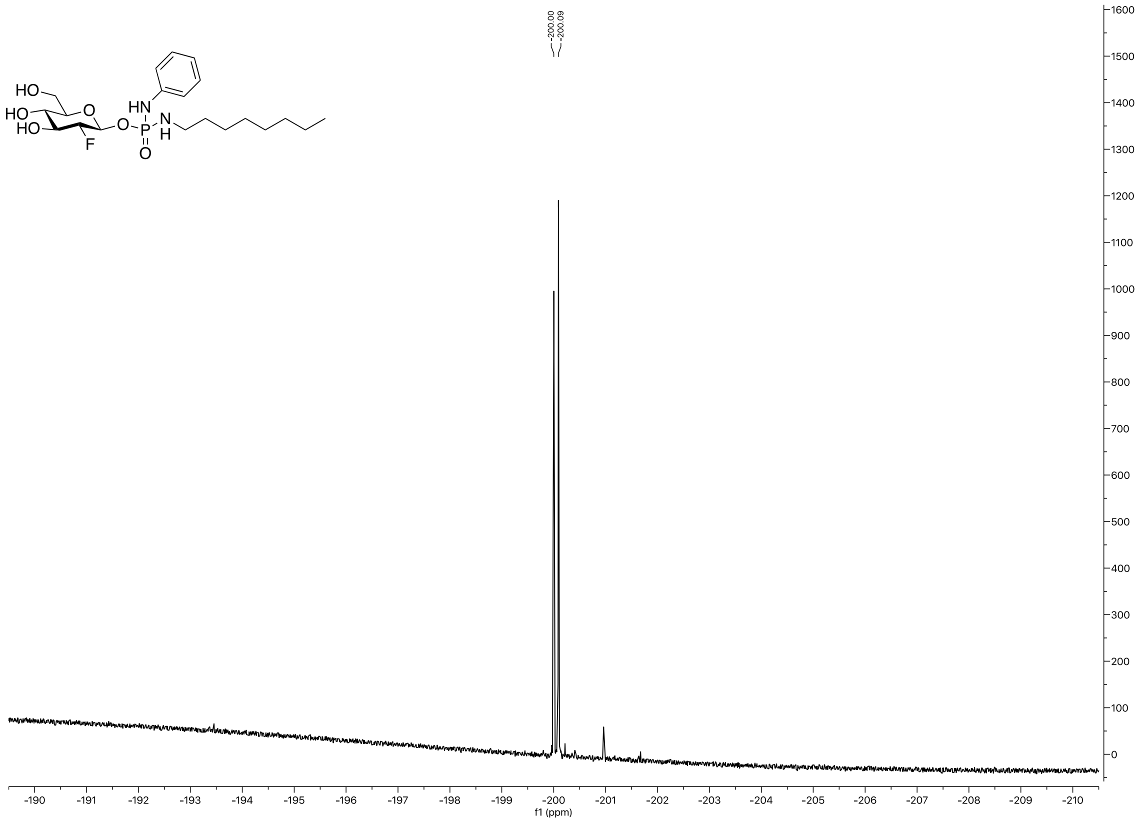
122 MHz ^{31}P NMR spectrum of **6** recorded in CDCl$_3$



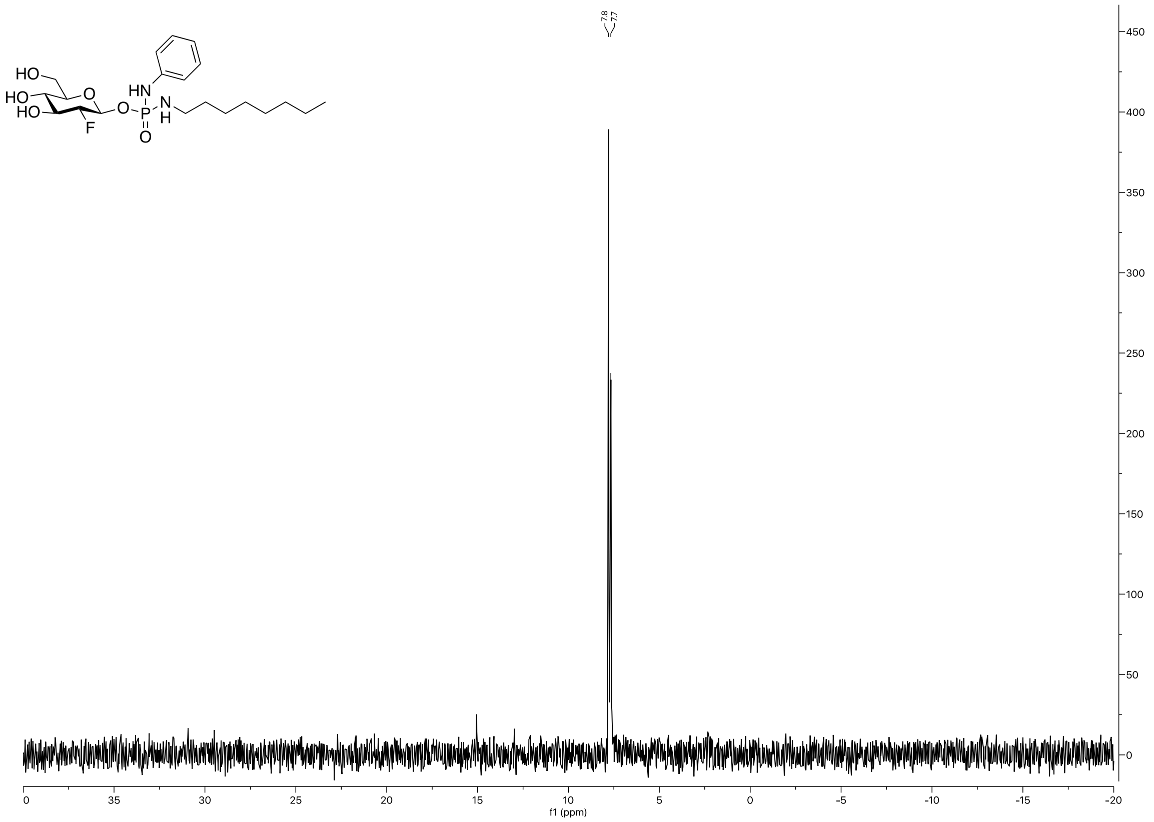
400 MHz ^{1}H NMR spectrum of **7** recorded in CD_3CN



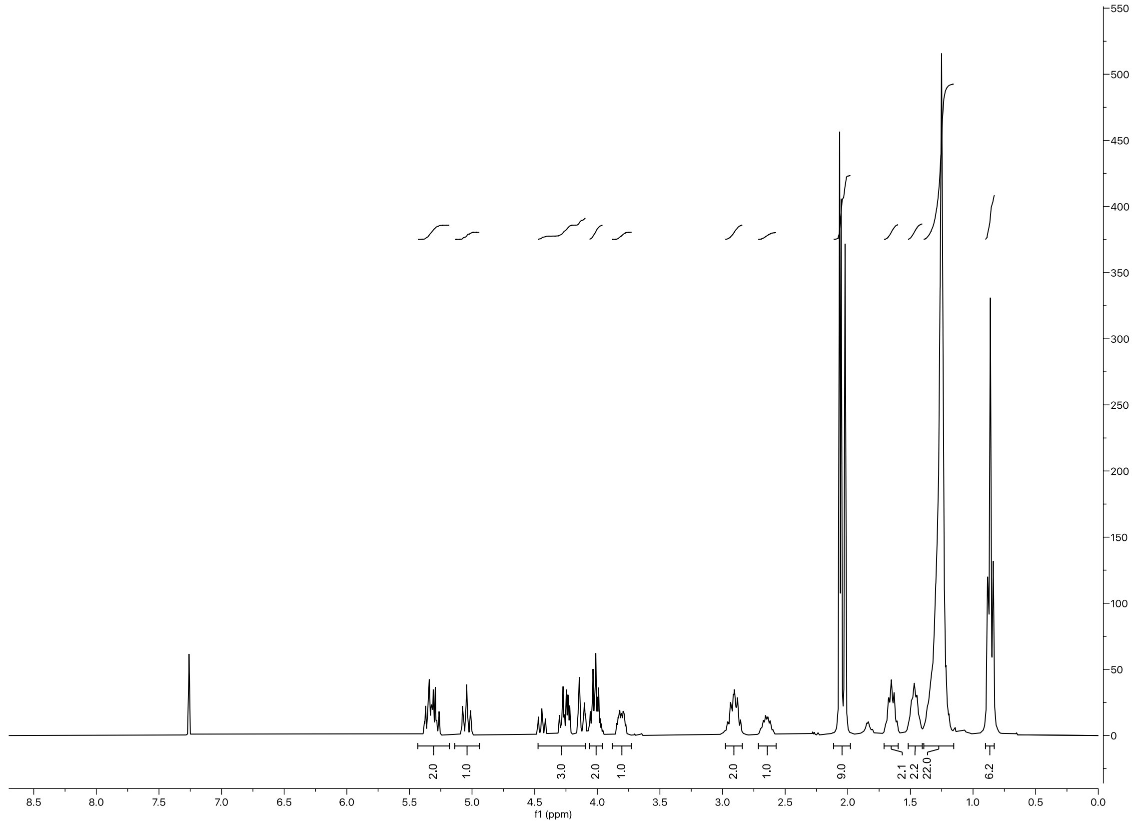
376 MHz ^{19}F NMR spectrum of **7** recorded in CD$_3$CN



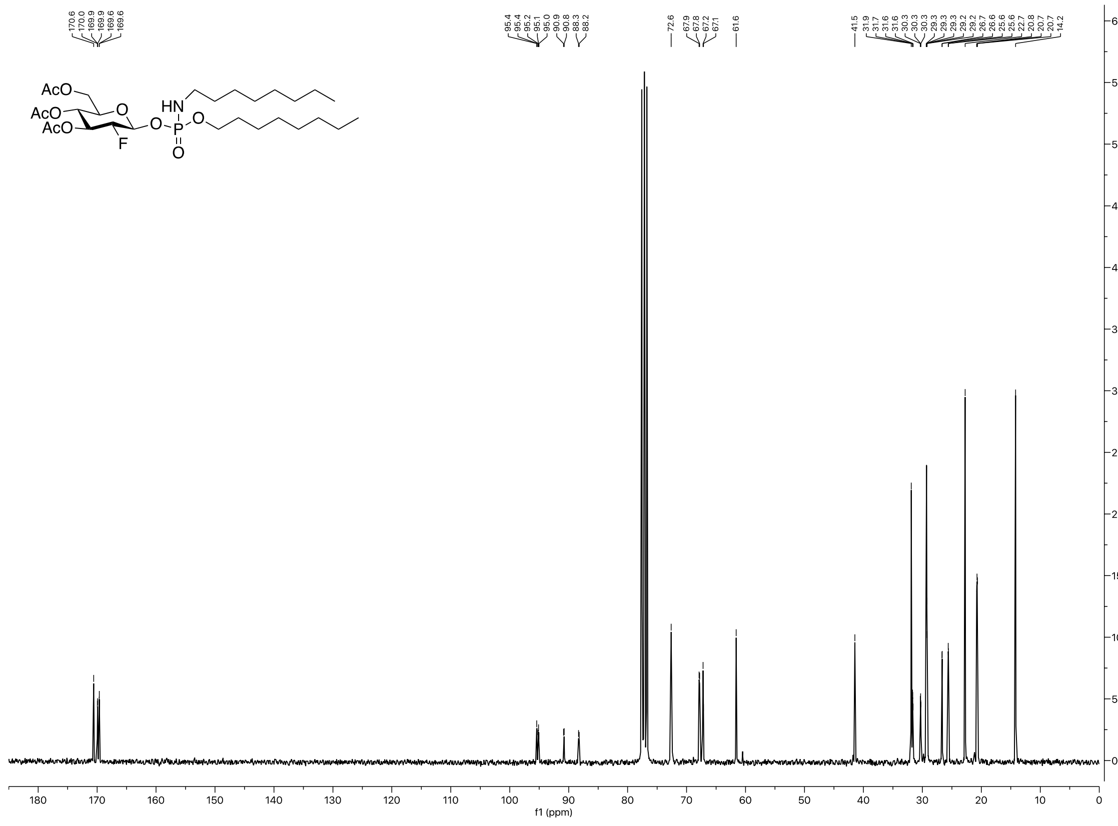
162 MHz ^{31}P NMR spectrum of **7** recorded in CD$_3$CN



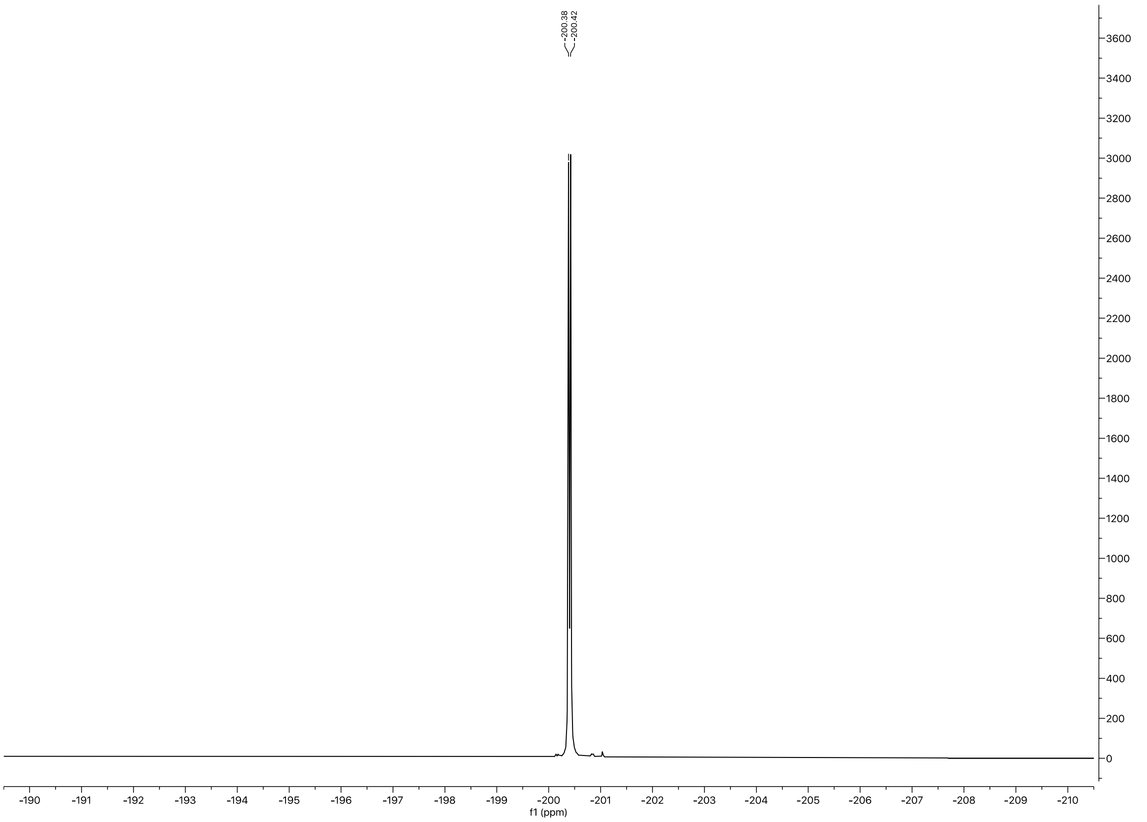
300 MHz ^{1}H NMR spectrum of **16h** recorded in $CDCl_3$



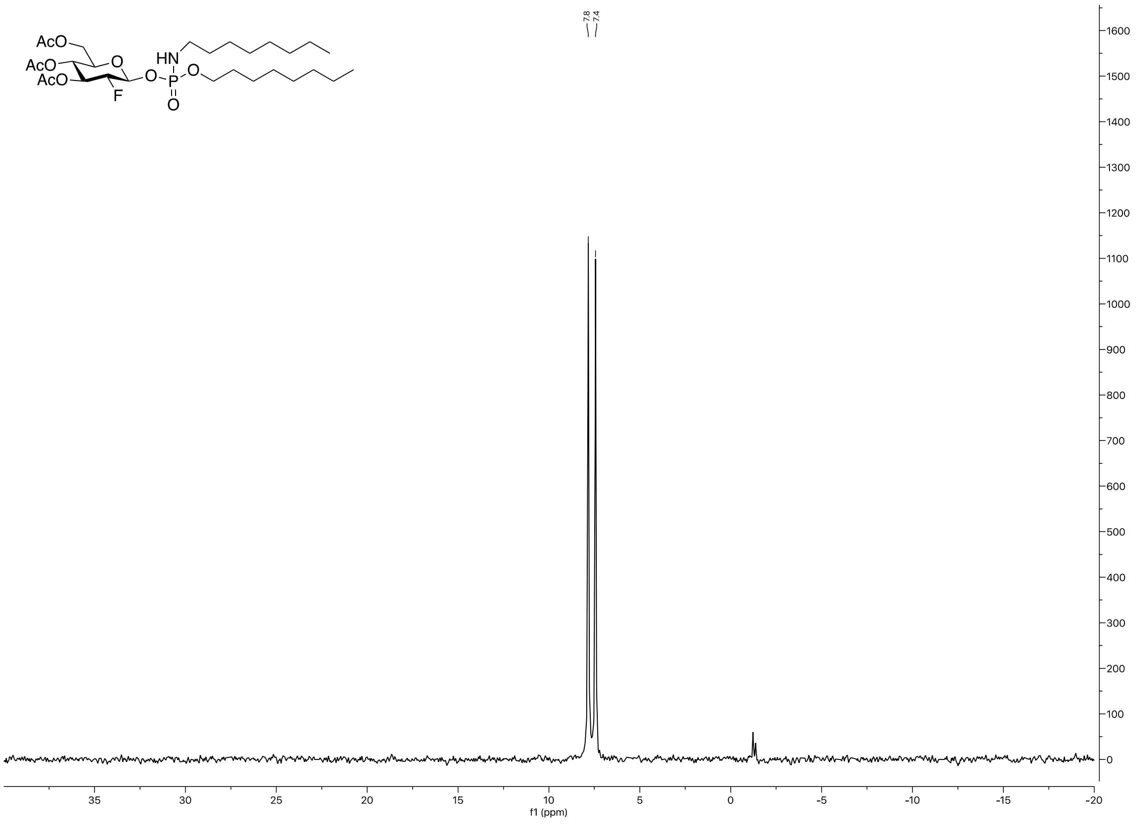
75 MHz ^{13}C NMR spectrum of **16h** recorded in $CDCl_3$



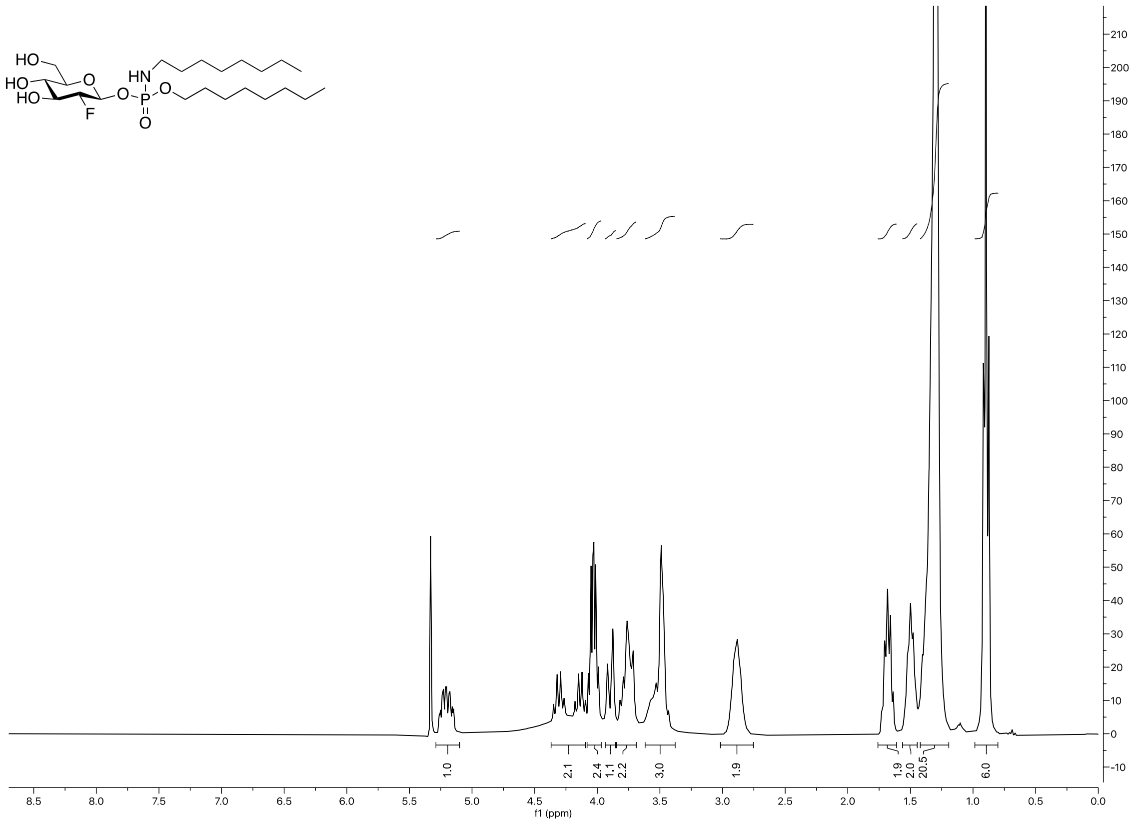
282 MHz ^{19}F NMR spectrum of **16h** recorded in $CDCl_3$



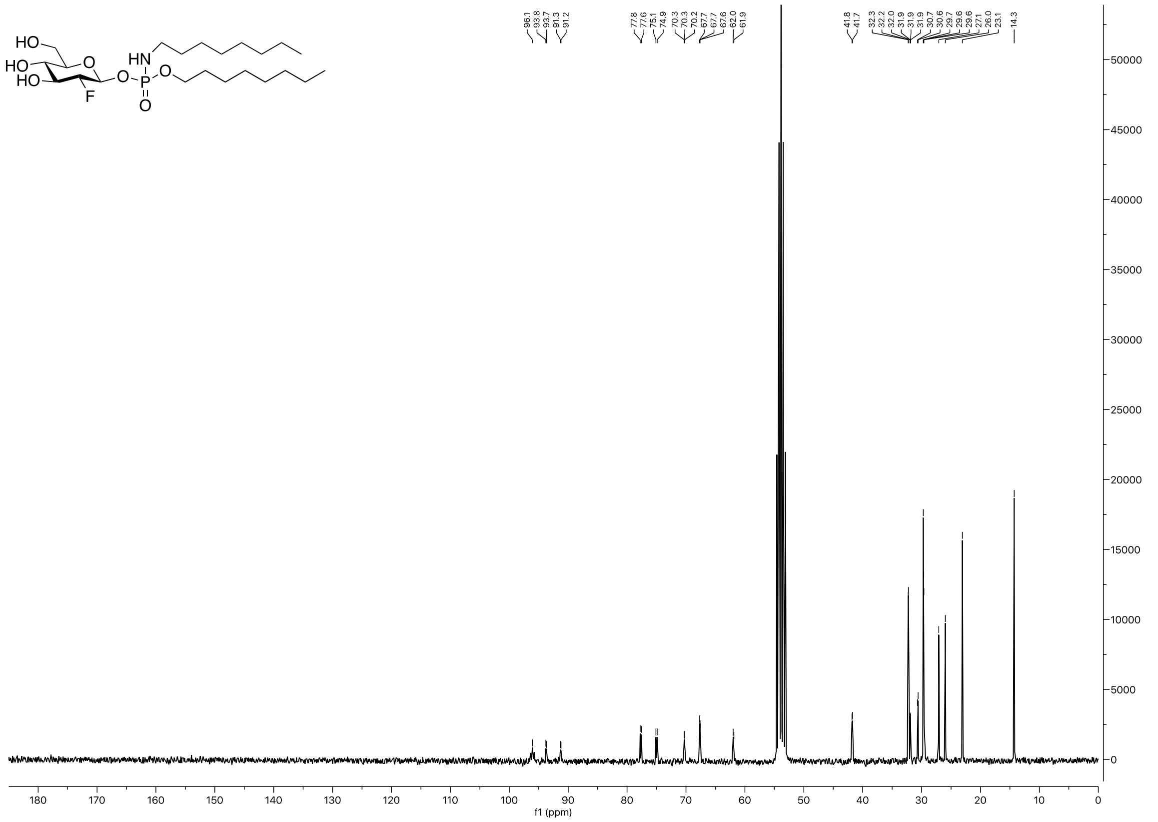
122 MHz ^{31}P NMR spectrum of **16h** recorded in $CDCl_3$



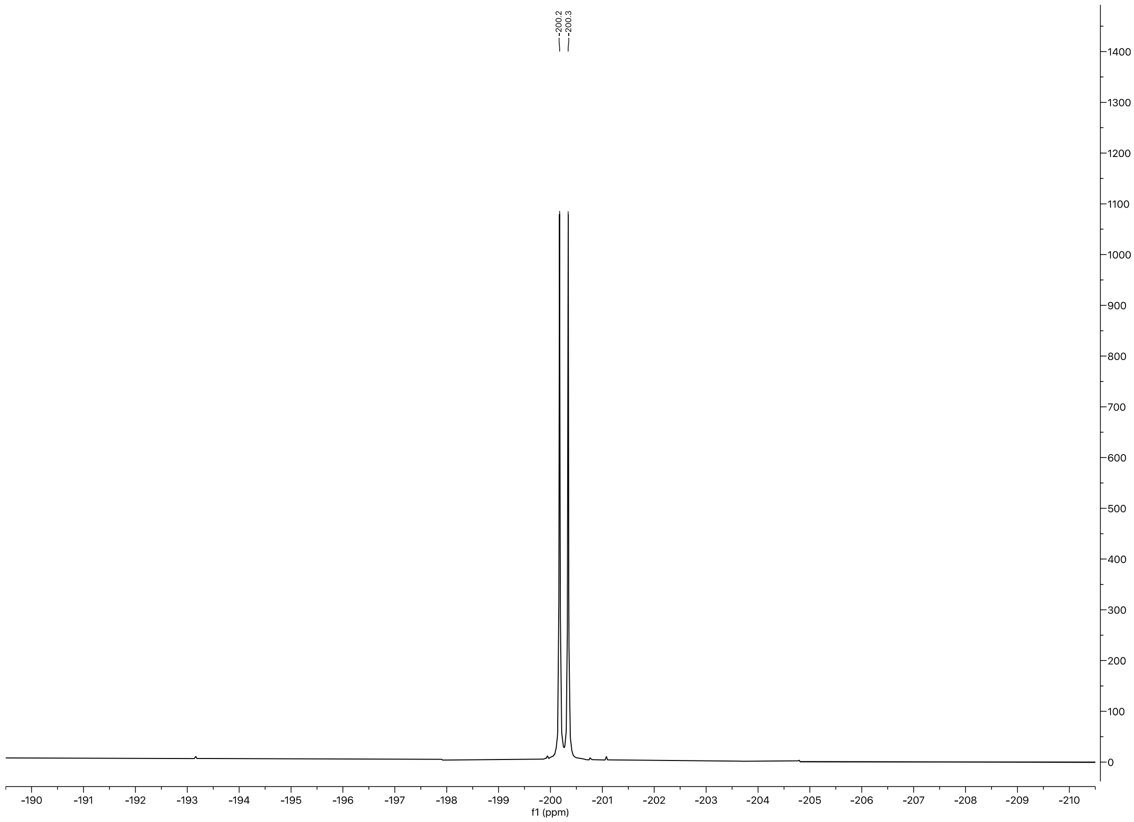
300 MHz ^{1}H NMR spectrum of **8** recorded in CD_2Cl_2



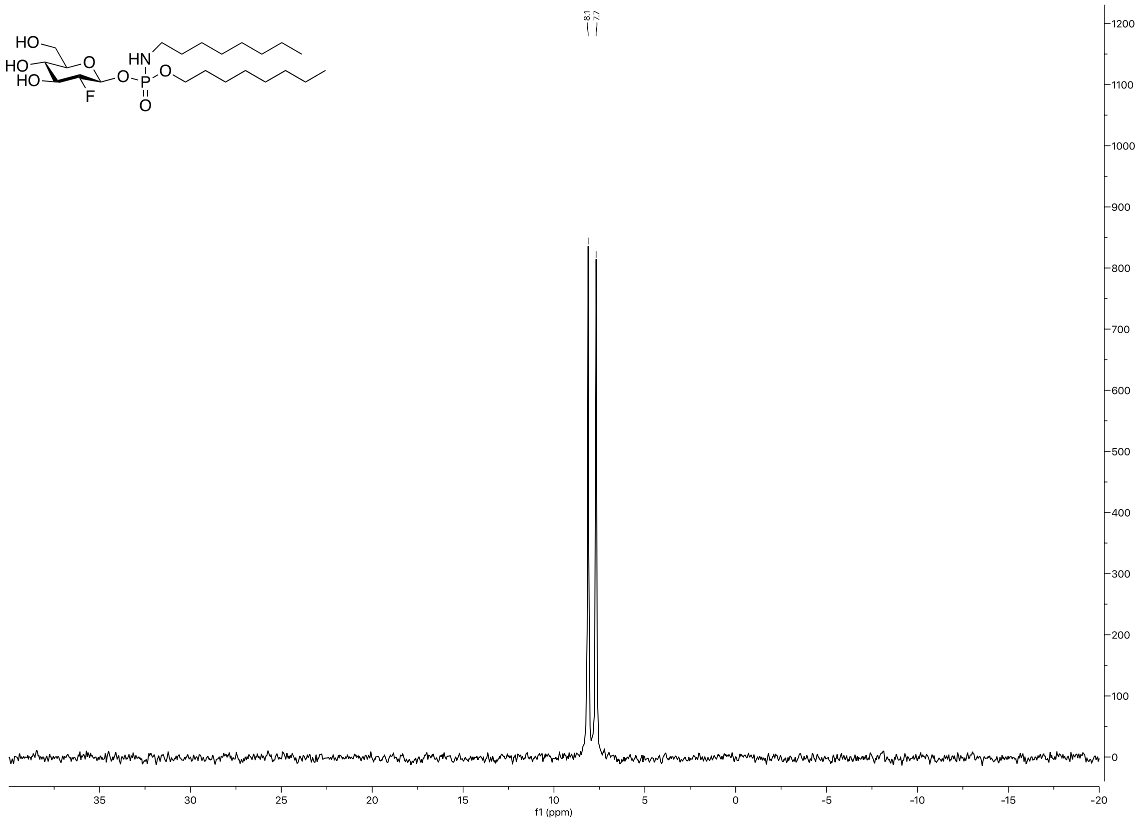
75 MHz ^{13}C NMR spectrum of **8** recorded in CD_2Cl_2



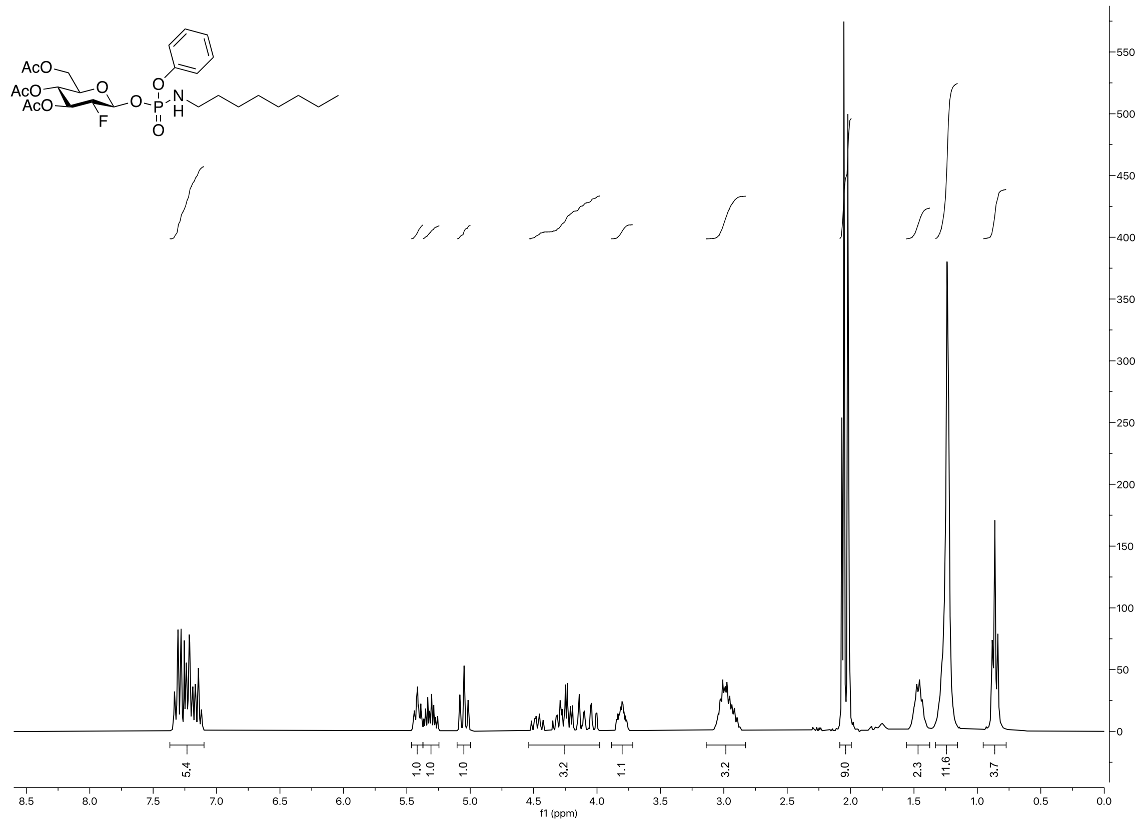
282 MHz ^{19}F NMR spectrum of **8** recorded in CD_2Cl_2



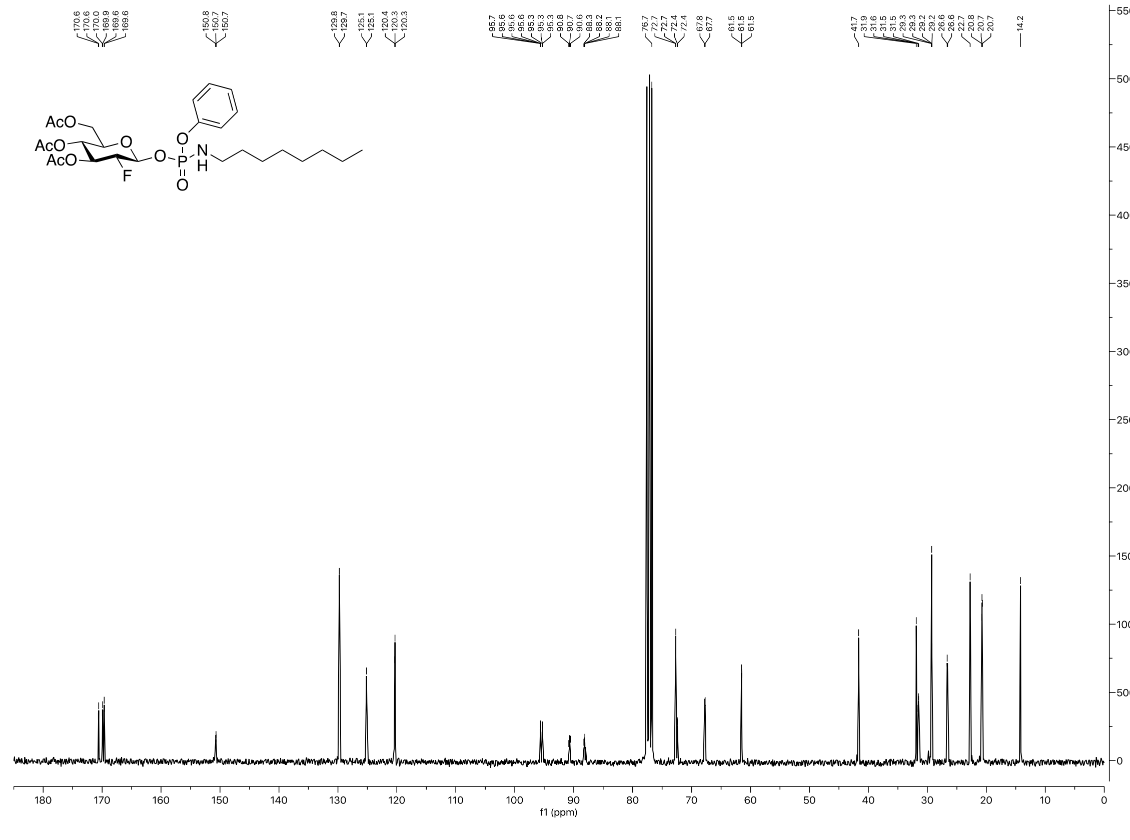
122 MHz ^{31}P NMR spectrum of **8** recorded in CD_2Cl_2



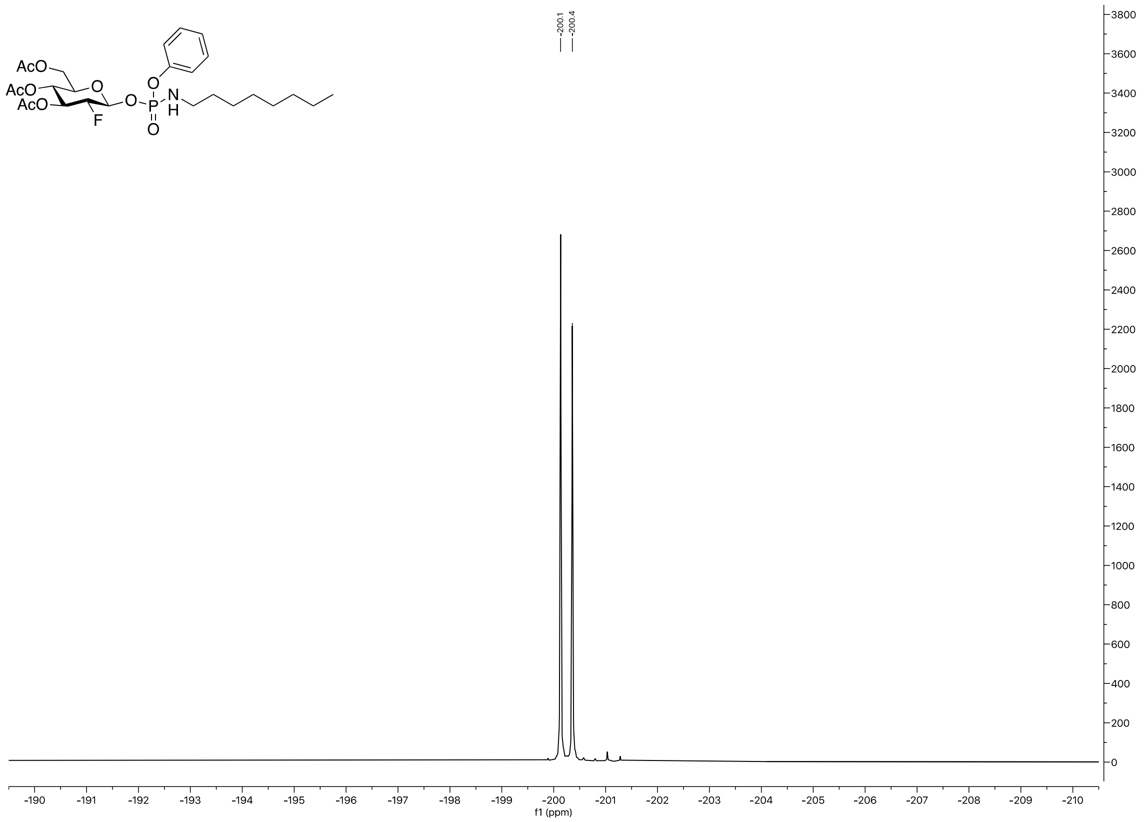
300 MHz ^{1}H NMR spectrum of **16i** recorded in CDCl$_3$



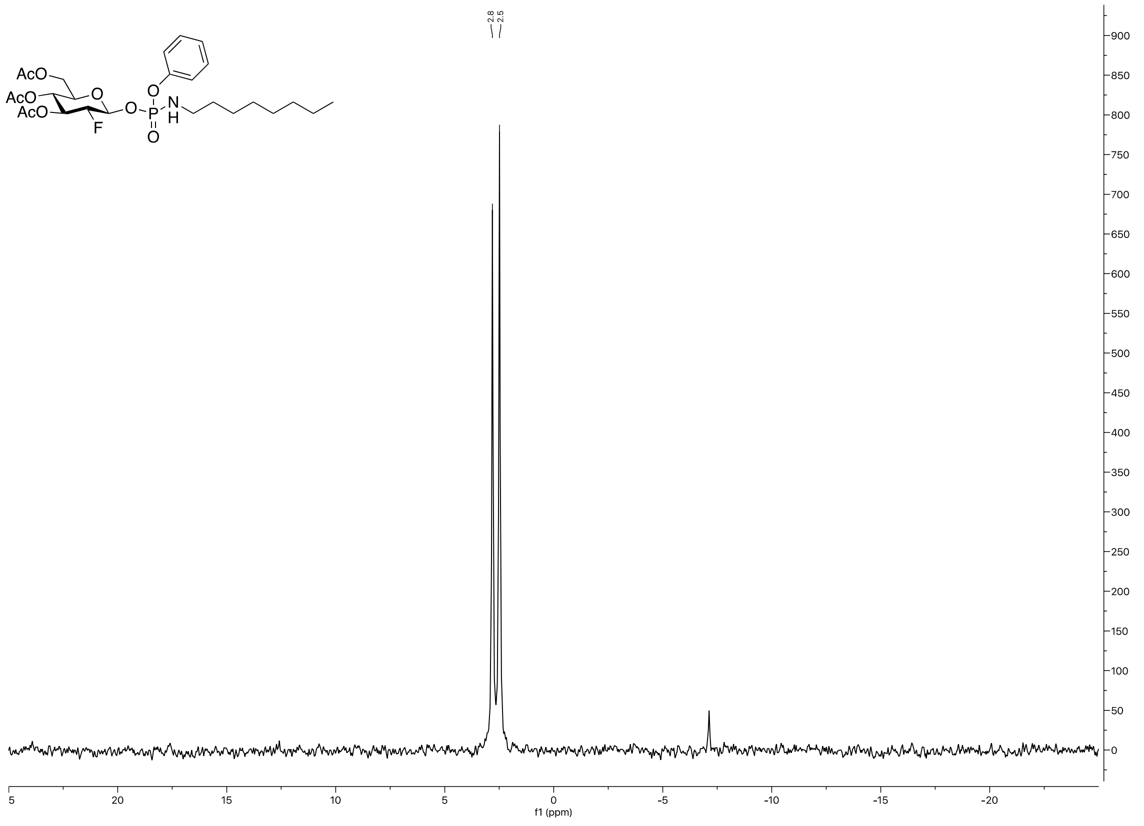
75 MHz ^{13}C NMR spectrum of **16i** recorded in CDCl$_3$



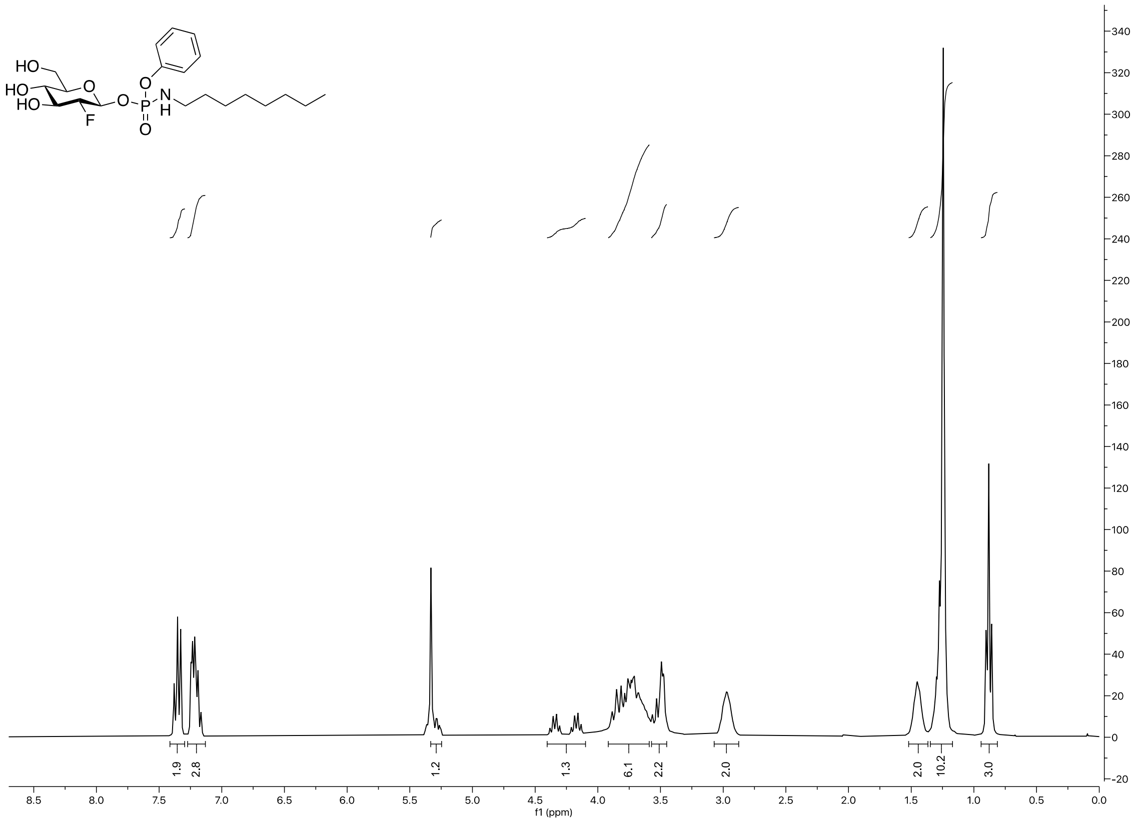
282 MHz ^{19}F NMR spectrum of **16i** recorded in $CDCl_3$



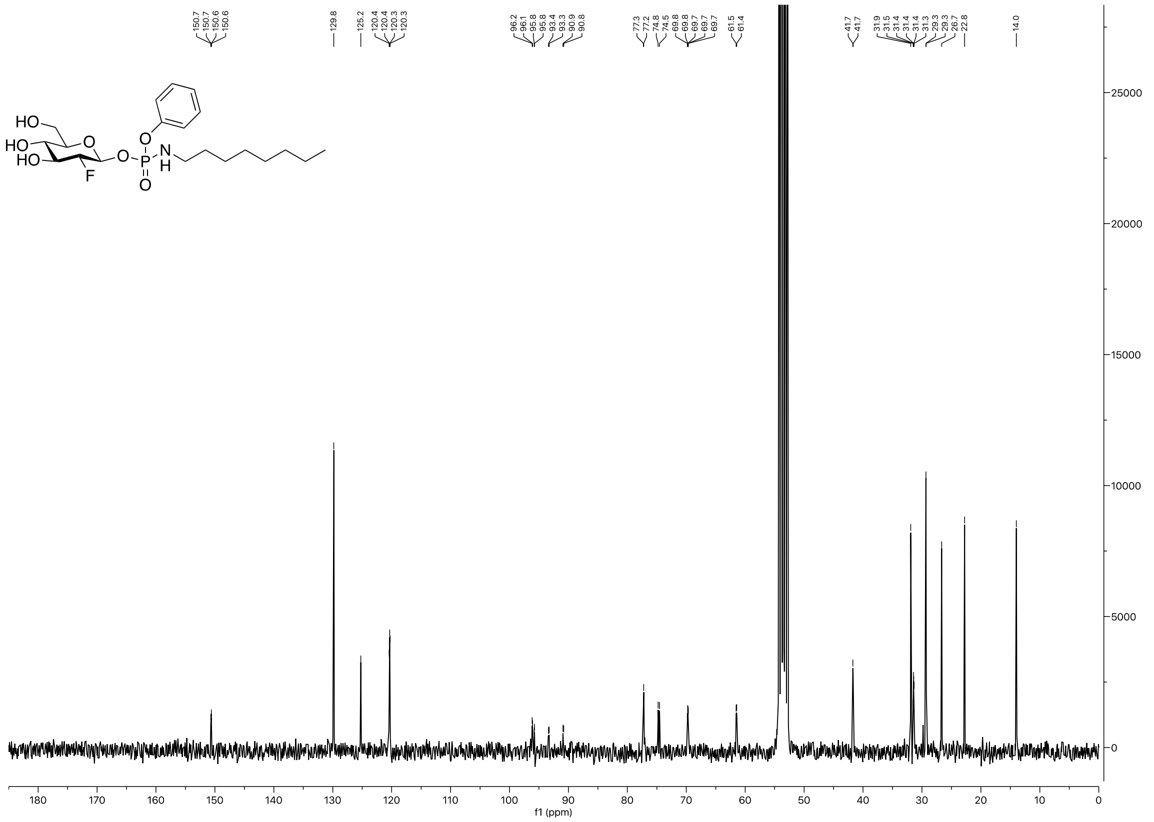
122 MHz ^{31}P NMR spectrum of **16i** recorded in $CDCl_3$



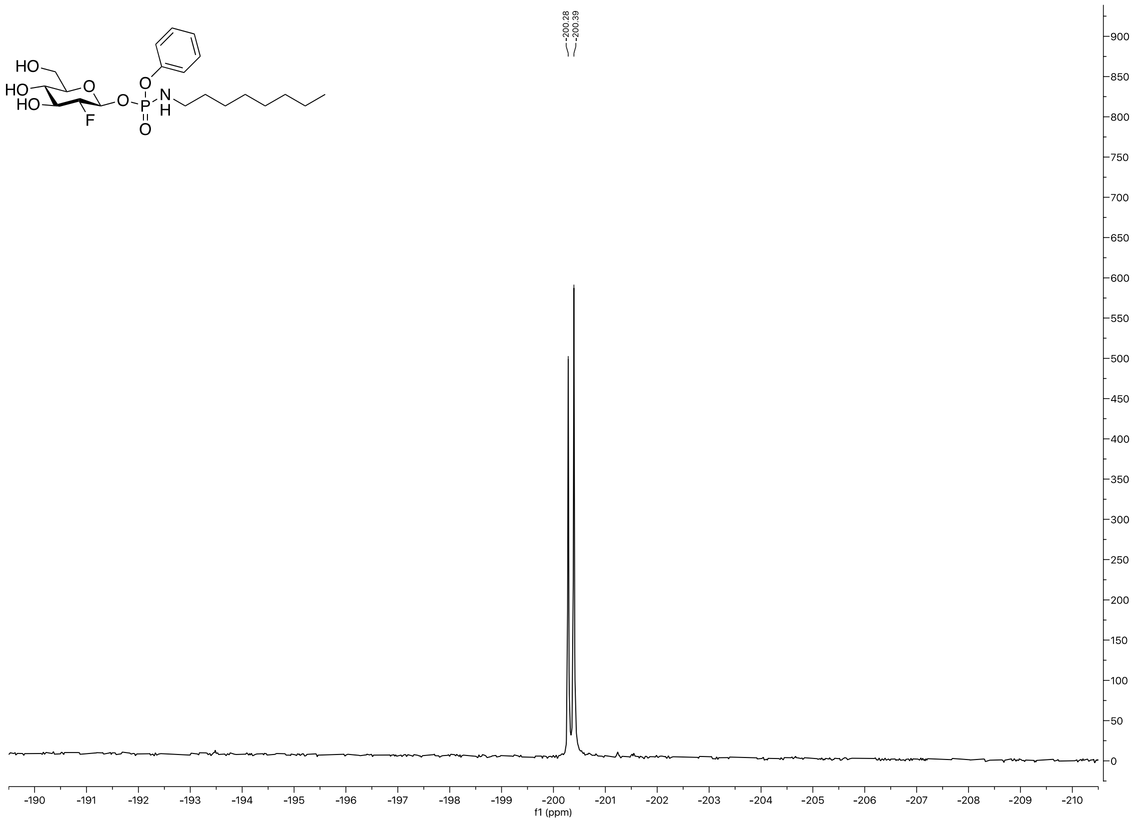
300 MHz ^{1}H NMR spectrum of **9** recorded in CD_2Cl_2



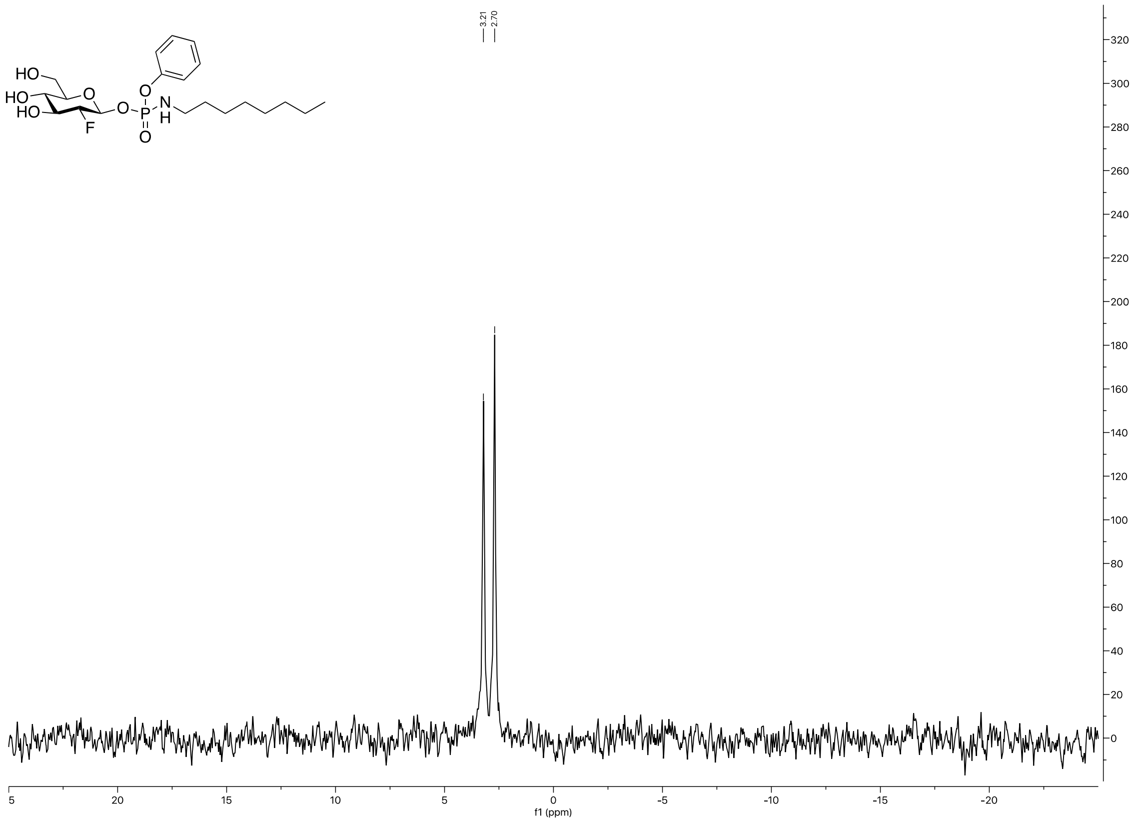
75 MHz ^{13}C NMR spectrum of **9** recorded in CD_2Cl_2



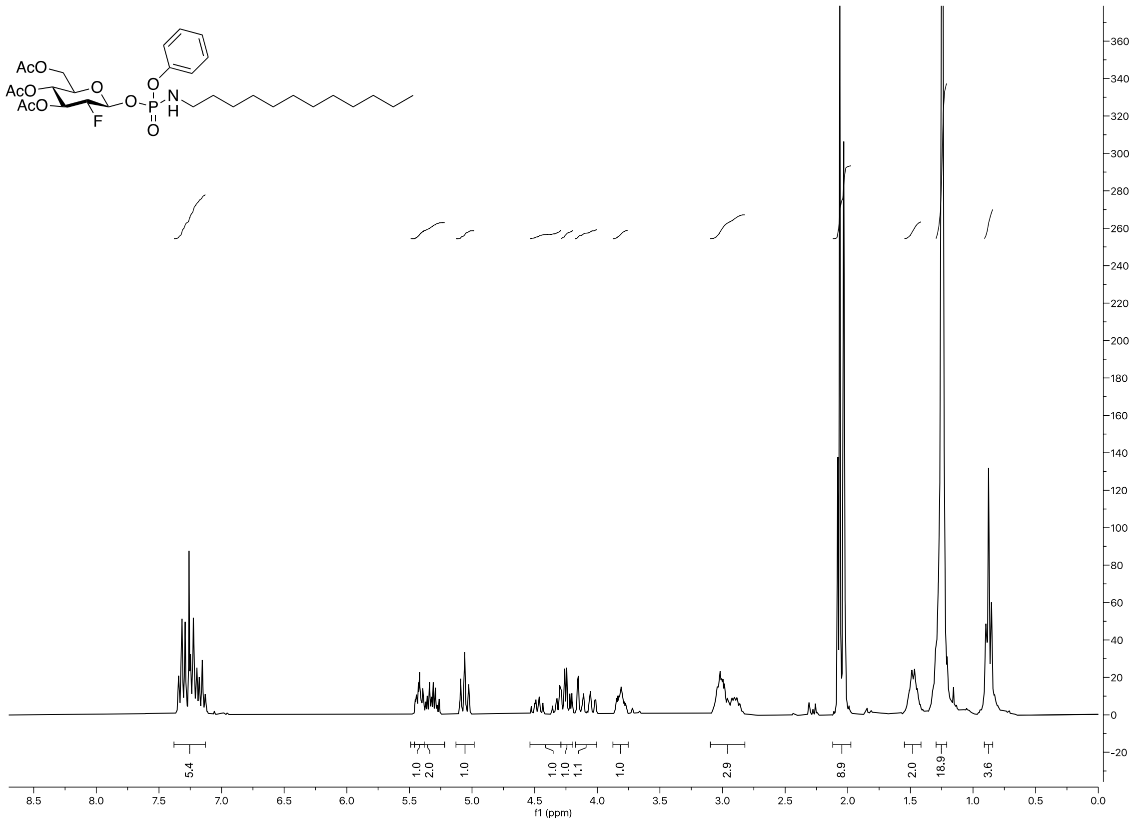
282 MHz ^{19}F NMR spectrum of **9** recorded in CD_2Cl_2



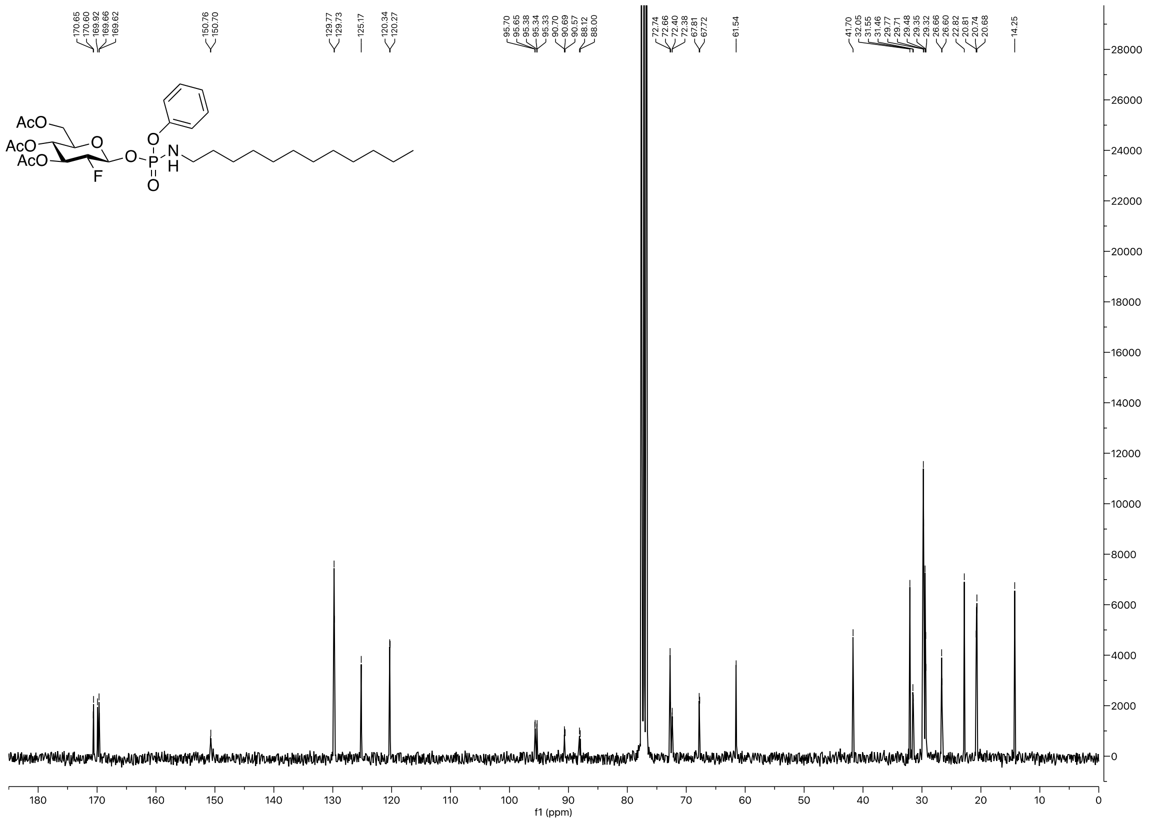
122 MHz ^{31}P NMR spectrum of **9** recorded in CD_2Cl_2



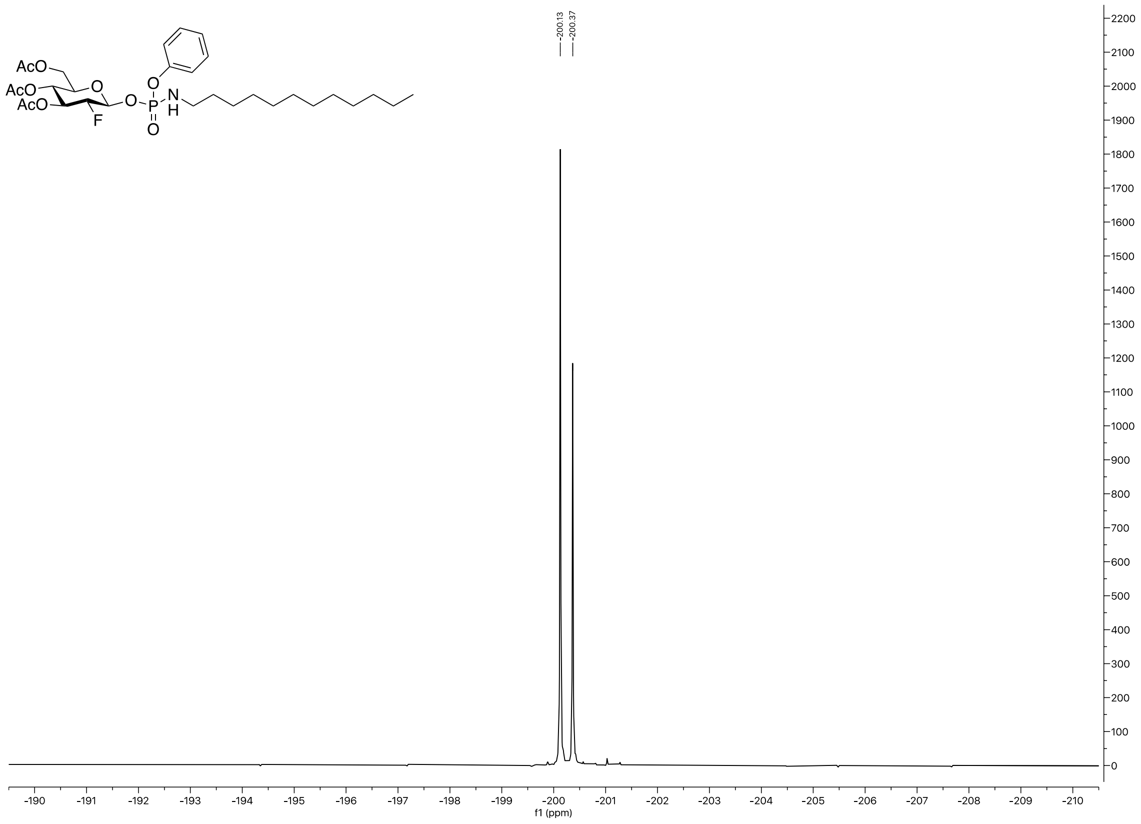
300 MHz ^{1}H NMR spectrum of **16j** recorded in DMSO



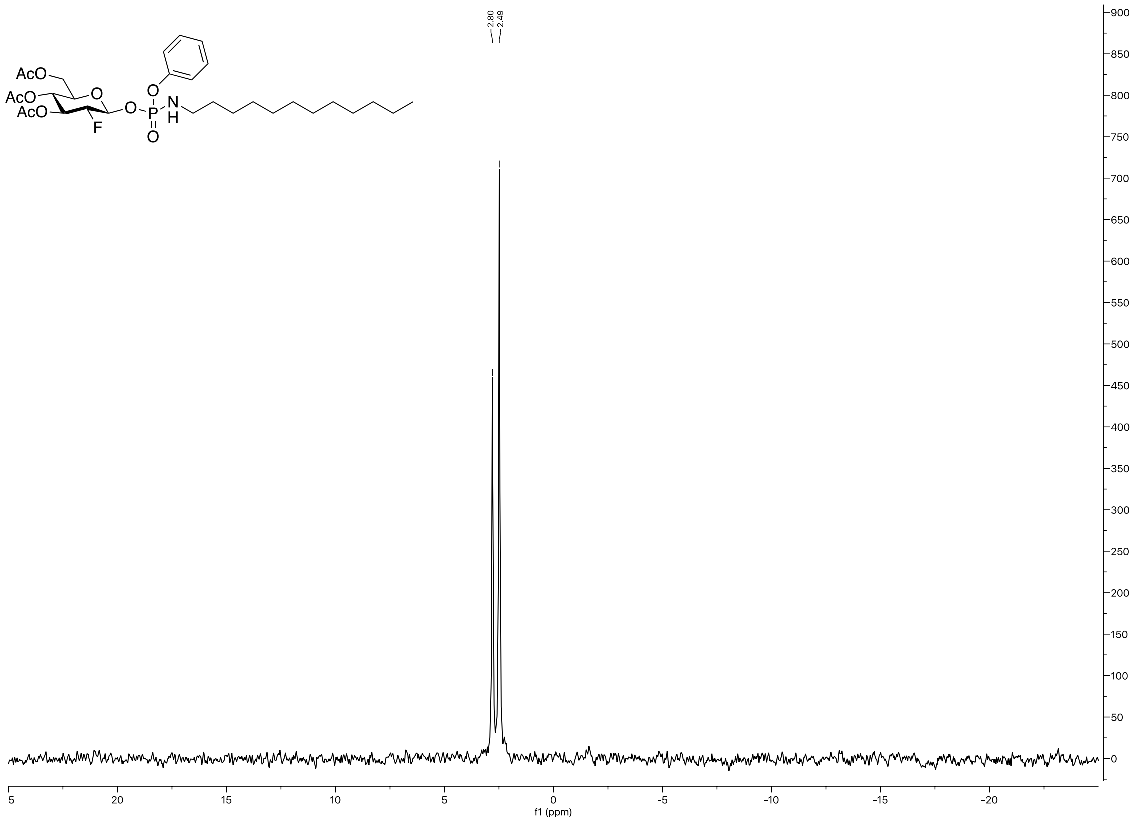
75 MHz ^{13}C NMR spectrum of **16j** recorded in DMSO



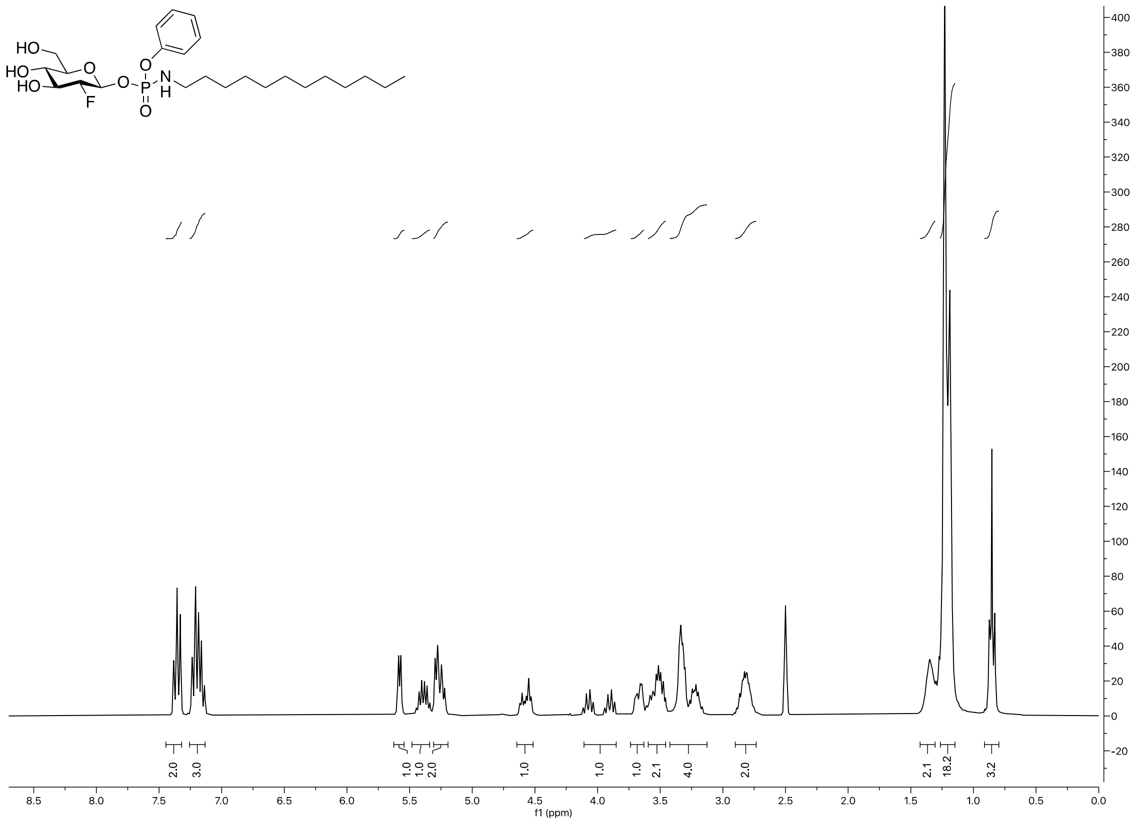
282 MHz ^{19}F NMR spectrum of **16j** recorded in DMSO



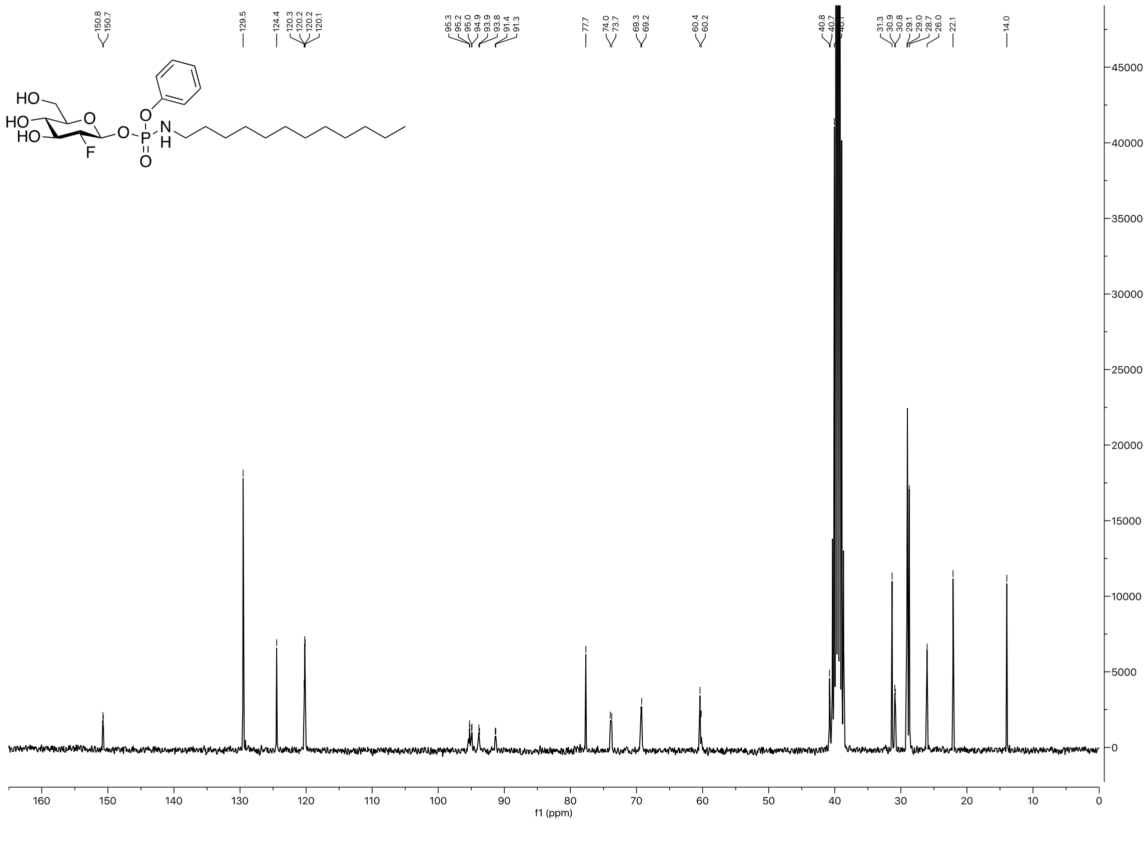
122 MHz ^{31}P NMR spectrum of **16j** recorded in DMSO



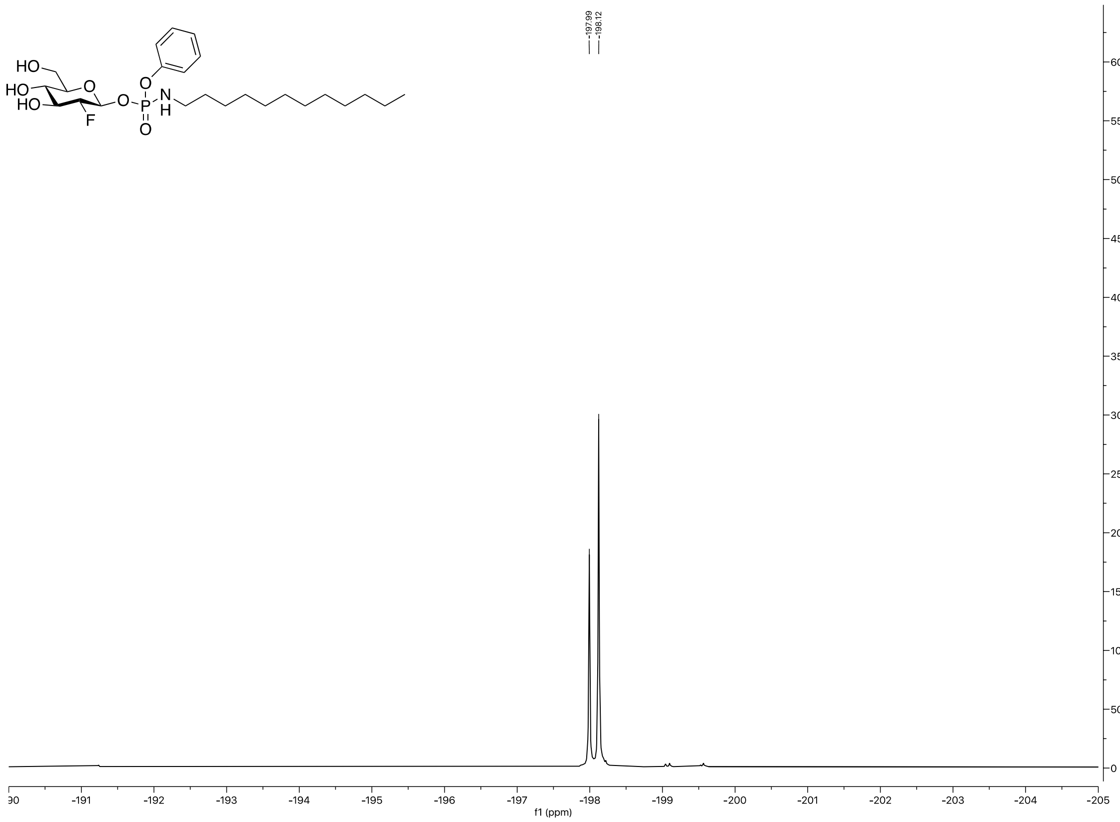
300 MHz ^{1}H NMR spectrum of **10** recorded in DMSO



75 MHz ^{13}C NMR spectrum of **10** recorded in DMSO



282 MHz ^{19}F NMR spectrum of **10** recorded in DMSO



122 MHz ^{31}P NMR spectrum of **10** recorded in DMSO