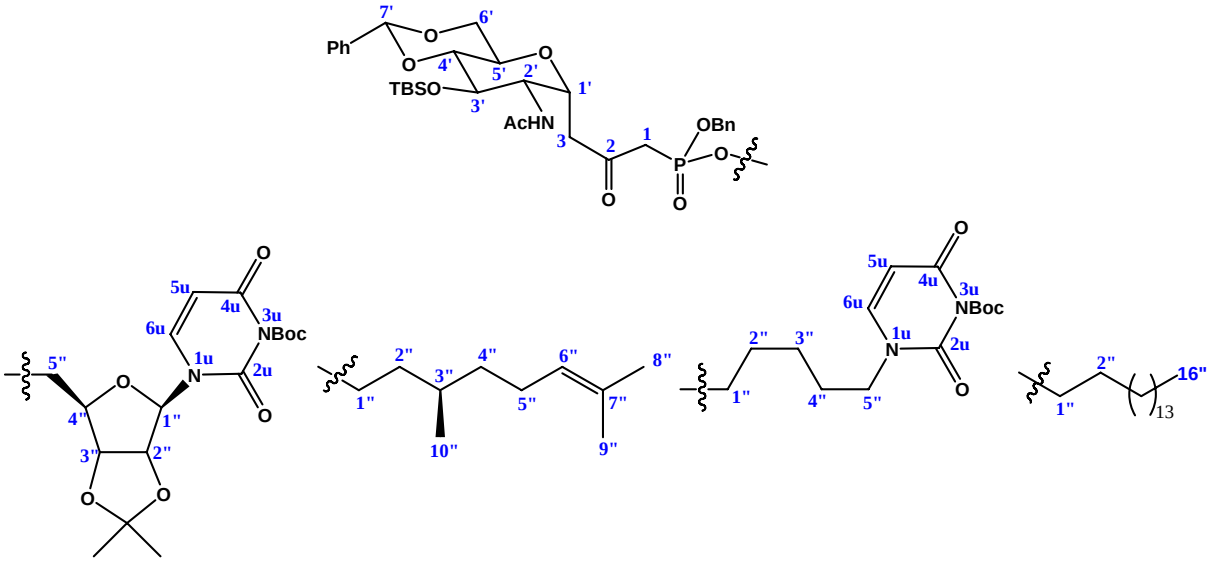
Electronic Supplementary information for:

Synthesis and biological evaluation of potential new inhibitors of the bacterial transferase MraY with a β-ketophosphonate structure†

Nicolas Auberger,[a] Rok Frlan,[a,b]
Bayan Al-Dabbagh,[c,d] Ahmed Bouhss,[c] Muriel Crouvoisier,[c]
Christine Gravier-Pelletier [a,] * and Yves Le Merrer [a]

1. Numbering system



2. Comprehensive experimental section

When a compound is obtained as a mixture of epimers, two situations may happen:
1) One of both epimers is largely predominant: in this case, only one is described.
2) The epimers are both present in substantial quantities: if the distinction between a major epimer and a minor one is possible, they are marked by symbols : * (major) and ° (minor).

"eq" and "ax" are used for description of respectively equatorial and axial protons.

3-(2-Acetamido-4,6-*O*-(*R*)-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl)-prop-1-ene 2α and 3-(2-acetamido-4,6-*O*-(*R*)-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranosyl)-prop-1-ene 2β

To a solution of **1** (6.00 g, 18 mmol) in DMF (180 mL) were added *tert*-butyldimethylsilyl chloride (6.78 g, 45 mmol, 2.5 eq.) and imidazole (4.90 g, 72 mmol, 4 eq.). The reaction mixture was stirred at r.t. for 16 h and concentrated to dryness. The residue was then dissolved in CH_2Cl_2 and washed with saturated aqueous NH_4Cl solution, dried ($MgSO_4$) and concentrated. Purification by flash chromatography (CH_2Cl_2/acetone, 9:1) afforded **2β** (480 mg, 6%, white solid): R_f 0.72 (CH_2Cl_2/acetone = 9:1); mp 159 °C; $[\alpha]_D^{20}$ - 58 (*c* 1.0, CH_2Cl_2);

^{1}H NMR δ 7.49-7.45 (m, 2H, H_{ar}), 7.37-7.33 (m, 3H, H_{ar}), 5.86 (ddt, 1H, $J_{H2\text{-}H1trans}$ = 17.1 Hz, $J_{H2\text{-}H1cis}$ = 10.3 Hz, $J_{H2\text{-}H3}$ = 6.8 Hz, H_2), 5.61 (d, 1H, $J_{NH\text{-}H2'}$ = 9.0 Hz, NH), 5.49 (s, 1H, $H_{7'}$), 5.08 (dm, 1H, $J_{H1trans\text{-}H2}$ = 17.1 Hz, H_{1trans}), 5.05 (dm, 1H, $J_{H1cis\text{-}H2}$ = 10.3 Hz, H_{1cis}), 4.29 (dd, 1H, $J_{H6'eq\text{-}H6'ax}$ = 10.4 Hz, $J_{H6'eq\text{-}H5'}$ = 4.8 Hz, $H_{6'eq}$), 3.83 (dd, 1H, $J_{H3'\text{-}H2'}$ = 9.4 Hz, $J_{H3'\text{-}H4'}$ = 8.3 Hz, $H_{3'}$), 3.75 (ddd, 1H, $J_{H2'\text{-}H1'}$ = 10.2 Hz, $J_{H2'\text{-}H3'}$ = 9.4 Hz, $J_{H2'\text{-}NH}$ = 9.0 Hz, $H_{2'}$), 3.69 (dd, 1H, $J_{H6'ax\text{-}H6'eq}$ = 10.4 Hz, $J_{H6'ax\text{-}H5'}$ = 9.8 Hz, $H_{6'ax}$), 3.52 (ddd, 1H, $J_{H1'\text{-}H2'}$ = 10.2 Hz, $J_{H1'\text{-}H3a}$ = 7.7 Hz, $J_{H1'\text{-}H3b}$ = 3.2 Hz, $H_{1'}$), 3.45 (dd, 1H, $J_{H4'\text{-}H5'}$ = 9.3 Hz, $J_{H4'\text{-}H3'}$ = 8.3 Hz, $H_{4'}$), 3.39 (ddd, 1H, $J_{H5'\text{-}H6'ax}$ = 9.8 Hz, $J_{H5'\text{-}H4'}$ = 9.3 Hz, $J_{H5'\text{-}H6'eq}$ = 4.8 Hz, $H_{5'}$), 2.42-2.35, 2.30-2.23 (2m, 2H, H_3), 1.99 (s, 3H, CH_3CO), 0.82 (s, 9H, Si*t*Bu), 0.02, -0.04 (2s, 6H, SiMe); ^{13}C NMR δ 170.0 ($CH_3\underline{C}O$), 137.4, 129.1, 128.2, 126.5 (C_{ar}), 134.6 (C_2), 117.1 (C_1), 102.0 ($C_{7'}$), 82.7 ($C_{4'}$), 79.0 ($C_{1'}$), 73.8 ($C_{3'}$), 70.5 ($C_{5'}$), 69.0 ($C_{6'}$), 57.1 ($C_{2'}$), 36.7 (C_3), 25.8 (Si*t*Bu), 23.9 ($\underline{C}H_3CO$), 18.2 (Si*t*Bu), -3.8, -4.8 (SiMe).

Further elution afforded **2α** (7.19 g, 89%, white solid): R_f 0.62 (CH_2Cl_2/acetone = 9:1); mp 210 °C; $[\alpha]_D^{20}$ + 18 (*c* 1.0, CH_2Cl_2); ^{1}H NMR δ 7.50-7.45 (m, 2H, H_{ar}), 7.38-7.33 (m, 3H, H_{ar}), 5.77 (dddd, 1H, $J_{H2\text{-}H1trans}$ = 17.1 Hz, $J_{H2\text{-}H1cis}$ = 10.3 Hz, $J_{H2\text{-}H3a}$ = 7.6 Hz, $J_{H2\text{-}H3b}$ = 6.0 Hz, H_2), 5.50 (s, 1H, $H_{7'}$), 5.49 (d, 1H, $J_{NH\text{-}H2'}$ = 7.1 Hz, NH), 5.14 (dm, 1H, $J_{H1trans\text{-}H2}$ = 17.1 Hz, H_{1trans}), 5.11 (dm, 1H, $J_{H1cis\text{-}H2}$ = 10.3 Hz, H_{1cis}), 4.35 (ddd, 1H, $J_{H1'\text{-}H3a}$ = 10.7 Hz, $J_{H1'\text{-}H2'}$ = 5.6 Hz, $J_{H1'\text{-}H3b}$ = 4.8 Hz, $H_{1'}$), 4.24-4.18 (m, 2H, $H_{2'}$, $H_{6'eq}$), 3.84 (dd, 1H, $J_{H3'\text{-}H4'}$ = 10.0 Hz, $J_{H3'\text{-}H2'}$ = 8.3 Hz, $H_{3'}$), 3.69 (dd, 1H, $J_{H4'\text{-}H3'}$ = 10.0 Hz, $J_{H4'\text{-}H5'}$ = 9.8 Hz, $H_{4'}$), 3.59 (ddd, 1H, $J_{H5'\text{-}H4'}$ = 9.8 Hz, $J_{H5'\text{-}H6'ax}$ = 9.4 Hz, $J_{H5'\text{-}H6'eq}$ = 4.5 Hz, $H_{5'}$), 3.53 (dd, 1H, $J_{H6'ax\text{-}H5'}$ = 9.4 Hz, $J_{H6'ax\text{-}H6'eq}$ = 8.6 Hz, $H_{6'ax}$), 2.49 (ddd, 1H, $J_{H3a\text{-}H3b}$ = 14.8 Hz, $J_{H3a\text{-}H1'}$ = 10.7 Hz, $J_{H3a\text{-}H2}$ = 7.6 Hz, H_{3a}), 2.32 (ddd, 1H, $J_{H3b\text{-}H3a}$ = 14.8 Hz, $J_{H3b\text{-}H2}$ = 6.0 Hz, $J_{H3b\text{-}H1'}$ = 4.8 Hz, H_{3b}), 1.99 (s, 3H, CH_3CO), 0.84 (s, 9H, Si*t*Bu), 0.07, 0.00 (2s, 6H, SiMe); ^{13}C NMR δ 170.2 ($CH_3\underline{C}O$), 137.3, 129.2, 128.3, 126.4 (C_{ar}), 134.1 (C_2), 117.4 (C_1), 102.0 ($C_{7'}$), 83.4 ($C_{4'}$), 74.0 ($C_{1'}$), 70.6 ($C_{3'}$), 69.5 ($C_{6'}$), 64.0 ($C_{5'}$), 55.3 ($C_{2'}$), 31.3 (C_3), 25.8 (Si*t*Bu), 23.5 ($\underline{C}H_3CO$), 18.3 (Si*t*Bu), -3.7, -4.7 (SiMe); MS (ESI): m/z = 917 $[2M+Na]^+$ 100%; HRMS calcd for $C_{24}H_{37}NNaO_5Si^+$ 470.2339, found 470.2336.

Methyl 2-(2-acetamido-4,6-*O*-(*R*)-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl)acetate 3

To a solution of **2α** (2.00 g, 4.47 mmol) in CH_2Cl_2 (55 mL) was added a solution of sodium hydroxide (1.79 g, 44.7 mmol, 10 eq.) in methanol (25 mL). Ozone was bubbled through the solution at - 78 °C for 3 h until it became blue. The solution was then purged with argon, warmed to r.t., diluted with CH_2Cl_2 and washed with saturated aqueous NH_4Cl solution. Aqueous phase was back-extracted with CH_2Cl_2. Combined organic extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography (CH_2Cl_2/acetone, 9:1) afforded ester **3** (1.44 g, 67%, white solid): R_f 0.37 (CH_2Cl_2/acetone = 9:1); mp 198 °C; $[\alpha]_D^{20}$ + 21 (*c* 1.0, CH_2Cl_2); IR 1743 cm^{-1}; ^{1}H NMR δ 7.49-7.44 (m, 2H, H_{ar}), 7.38-7.33 (m, 3H, H_{ar}), 5.52 (d, 1H, $J_{NH\text{-}H2'}$ = 7.0 Hz, NH), 5.50 (s, 1H, $H_{7'}$), 4.78 (ddd, 1H, $J_{H1'\text{-}H2a}$ = 9.4 Hz, $J_{H1'\text{-}H2'}$ = 5.7 Hz, $J_{H1'\text{-}H2b}$ = 5.5 Hz, $H_{1'}$), 4.22 (ddd, 1H, $J_{H2'\text{-}H3'}$ = 9.7 Hz, $J_{H2'\text{-}NH}$ = 7.0 Hz, $J_{H2'\text{-}H1'}$ = 5.7 Hz, $H_{2'}$), 4.20 (dd, 1H, $J_{H6'eq\text{-}H6'ax}$ = 9.4 Hz, $J_{H6'eq\text{-}H5'}$ = 3.5 Hz, $H_{6'eq}$), 3.81 (dd, 1H, $J_{H3'\text{-}H2'}$ = 9.7 Hz, $J_{H3'\text{-}H4'}$ = 8.8 Hz, $H_{3'}$), 3.70 (s, 3H, OMe), 3.69-3.62 (m, 2H, $H_{5'}$, $H_{6'ax}$), 3.53 (dd, 1H, $J_{H4'\text{-}H5'}$ = 9.2 Hz, $J_{H4'\text{-}H3'}$ = 8.8 Hz, $H_{4'}$), 2.73 (dd, 1H, $J_{H2a\text{-}H2b}$ = 14.3 Hz, $J_{H2a\text{-}H1'}$ = 9.4 Hz, H_{2a}), 2.62 (dd, 1H, $J_{H2b\text{-}H2a}$ = 14.3 Hz, $J_{H2b\text{-}H1'}$ = 5.5 Hz, H_{2b}), 1.97 (s, 3H, CH_3CO), 0.83 (s, 9H, Si*t*Bu), 0.05, -0.02 (2s, 6H, SiMe); ^{13}C NMR δ 171.2 (C_1), 170.3 ($CH_3\underline{C}O$), 137.3, 129.2, 128.3, 126.4 (C_{ar}), 102.1 ($C_{7'}$), 83.1 ($C_{4'}$), 72.3 ($C_{1'}$), 70.4 ($C_{3'}$), 69.3 ($C_{6'}$), 64.9

($C_{5'}$), 54.9 ($C_{2'}$), 52.2 (OMe), 34.0 (C_2), 25.8 (Si*t*Bu), 23.4 ($\underline{C}H_3CO$), 18.3 (Si*t*Bu), -3.7, -4.8 (SiMe); MS (ESI): m/z = 480 $[M+H]^+$ 100% ; HRMS calcd for $C_{24}H_{37}NNaO_7Si^+$ 502.2237, found 502.2226.

(3-*N*-*tert*-Butyloxycarbonyl-2',3'-*O*-isopropylidene)-uridine 6

To a solution of 2',3'-*O*-isopropylidene-uridine (1.50 g, 5.3 mmol) in THF (30 mL) were added at 0 °C trimethylsilyl chloride (1.35 mL, 10.6 mmol, 2 eq.) and DIEA (1.85 mL, 10.6 mmol, 2 eq.). The reaction mixture was stirred at r.t. for 1 h and hydrolyzed with saturated aqueous NH_4Cl solution. Aqueous phase was extracted with EtOAc and organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was taken up in THF (30 mL). Di-*tert*-butyl dicarbonate (1.27 g, 5.8 mmol, 1.1 eq.), Et_3N (820 µL, 5.8 mmol, 1.1 eq.) and DMAP cat. were successively added. The mixture was stirred at r.t. for 3 h, then cooled to 0 °C and stirred with 1 M aqueous HCl solution for 5 min. After dilution with EtOAc and decantation, organic phase was washed with saturated aqueous NaCl solution, dried ($MgSO_4$) and concentrated. Purification by flash chromatography (cyclohexane/acetone, 2:1) afforded **6** (1.67 g, 82%, white solid): R_f 0.26 (cyclohexane/acetone = 2:1); $[\alpha]_D^{20}$ - 23 (*c* 1.0, CH_2Cl_2); ^{1}H NMR δ 7.48 (d, 1H, $J_{H6\text{-}H5}$ = 8.2 Hz, H_6), 5.71 (d, 1H, $J_{H5\text{-}H6}$ = 8.2 Hz, H_5), 5.66 (d, 1H, $J_{H1'\text{-}H2'}$ = 2.9 Hz, $H_{1'}$), 4.94 (dd, 1H, $J_{H2'\text{-}H3'}$ = 6.4 Hz, $J_{H2'\text{-}H1'}$ = 2.9 Hz, $H_{2'}$), 4.89 (dd, 1H, $J_{H3'\text{-}H2'}$ = 6.4 Hz, $J_{H3'\text{-}H4'}$ = 3.2 Hz, $H_{3'}$), 4.29 (ddd, 1H, $J_{H4'\text{-}H5'b}$ = 3.5 Hz, $J_{H4'\text{-}H3'}$ = 3.2 Hz, $J_{H4'\text{-}H5'a}$ = 2.4 Hz, $H_{4'}$), 3.90 (ddd, 1H, $J_{H5'a\text{-}H5'b}$ = 12.0 Hz, $J_{H5'a\text{-}OH}$ = 3.8 Hz, $J_{H5'a\text{-}H4'}$ = 2.4 Hz, $H_{5'a}$), 3.78 (ddd, 1H, $J_{H5'b\text{-}H5'a}$ = 12.0 Hz, $J_{H5'b\text{-}OH}$ = 6.4 Hz, $J_{H5'b\text{-}H4'}$ = 3.5 Hz, $H_{5'b}$), 2.81 (dd, 1H, $J_{OH\text{-}H5'b}$ = 6.4 Hz, $J_{OH\text{-}H5'a}$ = 3.8 Hz, OH), 1.58 (s, 9H, CMe_3), 1.55, 1.34 (2s, 6H, CMe_2); ^{13}C NMR δ 160.5 (C_4), 148.7 (C_2), 147.5 (CO_{Boc}), 141.7 (C_6), 114.5 ($\underline{C}Me_2$), 102.5 (C_5), 95.7 ($C_{1'}$), 87.4 ($\underline{C}Me_3$), 87.1 ($C_{4'}$), 84.1 ($C_{2'}$), 80.4 ($C_{3'}$), 62.7 ($C_{5'}$), 27.6 ($C\underline{Me}_3$), 27.4, 25.4 ($C\underline{Me}_2$); MS (ESI): m/z = 791 $[2M+Na]^+$ 100%; HRMS calcd for $C_{17}H_{24}N_2NaO_8^+$ 407.1430, found 407.1431.

Benzyl (3-*N*-*tert*-butyloxycarbonyl-2',3'-*O*-isopropylidene)-uridin-5'-yl methylphosphonate 7

At 0 °C, diisopropyl azodicarboxylate (DIAD) (320 µL, 1.6 mmol, 1.5 eq.) was added dropwise to a solution of **5** (200 mg, 1.1 mmol, 1 eq.), **6** (413 mg, 1.1 mmol, 1 eq.) and PPh_3 (423 mg, 1.6 mmol, 1.5 eq.) in THF (10 mL). The reaction mixture was stirred at r.t. for 3 h then concentrated. Purification by flash chromatography (EtOAc/cyclohexane, 2:1) afforded **7** (400 mg, 67%, white solid) as a mixture of epimers (d.r. = 54/46): R_f 0.13 (EtOAc/cyclohexane = 2:1); ^{1}H NMR δ 7.39-7.33 (m, 5H, H_{ar}), 7.32, 7.30 (2d, 1H, $J_{H6\text{-}H5}$ = 8.1 Hz, H_6*, H_6°), 5.70, 5.66 (2d, 1H, $J_{H5\text{-}H6}$ = 8.1 Hz, H_5*, H_5°), 5.70-5.68 (m, 1H, $H_{1'}$), 5.12-5.04 (m, 2H, CH_2Ph), 4.87, 4.80 (2dd, 1H, $J_{H2'\text{-}H3'}$ = 6.4 Hz, $J_{H2'\text{-}H1'}$ = 2.2 Hz, $H_{2'}$°, $H_{2'}$*), 4.77, 4.71 (2dd, 1H, $J_{H3'\text{-}H2'}$ = 6.4 Hz, $J_{H3'\text{-}H4'}$ = 3.7 Hz, $H_{3'}$°, $H_{3'}$*), 4.32-4.28 (m, 1H, $H_{4'}$), 4.25-4.15, 4.15-4.07 (2m, 2H, $H_{5'}$), 1.59 (2s, 9H, *t*Bu°, *t*Bu*), 1.54 (bs, 3H, CMe_2), 1.48, 1.48 (2d, 3H, $J_{CH3\text{-}P}$ = 17.6 Hz, CH_3*, CH_3°), 1.33, 1.33 (2s, 3H, CMe_2°, CMe_2*); ^{13}C NMR δ 160.3 (C_4), 148.3 (C_2), 147.5 (CO_{Boc}), 140.8, 140.7 (C_6*, C_6°), 136.2, 136.2 (2d, $J_{Cqar\text{-}P}$ = 5.0 Hz, Cq_{ar}*, Cq_{ar}°), 128.9, 128.8, 128.1 (CH_{ar}), 114.8 ($\underline{C}Me_2$), 102.3 (C_5), 94.5, 94.4 ($C_{1'}$°, $C_{1'}$*), 87.2 ($\underline{C}Me_3$), 85.8, 85.7 (2d, $J_{C4'\text{-}P}$ = 7.0 Hz, $C_{4'}$°, $C_{4'}$*), 84.7, 84.6 ($C_{2'}$°, $C_{2'}$*), 80.6, 80.5 ($C_{3'}$°, $C_{3'}$*), 67.8, 67.7 (2d, $J_{CH2Ph\text{-}P}$ = 6.0 Hz, CH_2Ph°, CH_2Ph*), 64.8, 64.7 (2d, $J_{C5'\text{-}P}$ = 6.0 Hz, $C_{5'}$*, $C_{5'}$°), 27.6 ($C\underline{Me}_3$), 27.3, 25.5, 25.4 ($C\underline{Me}_2$), 11.5, 11.5 (2d, $J_{CH3\text{-}P}$ = 144.0 Hz, CH_3*, CH_3°); ^{31}P NMR (202 MHz, $CDCl_3$) δ 32.6 (s, 0.46P, P°), 32.4 (s, 0.54P, P*); MS

(ESI): m/z = 1127 $[2M+Na]^+$ 100%; HRMS calcd for $C_{25}H_{33}N_2NaO_{10}P^+$ 575.1771, found 575.1777.

Benzyl (*R*)-citronellyl methylphosphonate 8

At 0 °C, DIAD (880 µL, 4.4 mmol, 1.5 eq.) was added dropwise to a solution of **5** (550 mg, 3 mmol, 1 eq.), (*R*)-citronellol (540 µL, 3 mmol, 1 eq.) and PPh_3 (1.16 g, 4.4 mmol, 1.5 eq.) in THF (25 mL). The reaction mixture was stirred at r.t. for 1 h 30 then concentrated. Purification by flash chromatography (EtOAc/cyclohexane, 2:1) afforded **8** (920 mg, 95%, colorless oil) as a mixture of epimers (d.r. = 1/1): R_f 0.44, 0.31 (EtOAc/cyclohexane, 2:1); ^{1}H NMR δ 7.41-7.28 (m, 5H, H_{ar}), 5.12-5.02 (m, 3H, CH_2Ph, H_6), 4.10-3.90 (m, 2H, H_1), 2.04-1.87 (m, 2H, H_5), 1.72-1.64 (m, 1H, H_{2a}), 1.67 (s, 3H, H_8), 1.59 (s, 3H, H_9), 1.58-1.51 (m, 1H, H_3), 1.48-1.39 (m, 1H, H_{2b}), 1.46 (d, 3H, J_{CH3-P} = 17.5 Hz, CH_3P), 1.36-1.26, 1.20-1.11 (2m, 2H, H_4), 0.88 (2d, 3H, J_{H10-H3} = 6.6 Hz, H_{10}); ^{13}C NMR δ 136.7 (d, J_{Cq-P} = 5.7 Hz, Cq), 131.5 (C_7), 128.7, 128.5, 128.0 (CH_{ar}), 124.7 (C_6), 67.2 (d, $J_{CH2Ph-P}$ = 5.7 Hz, CH_2Ph), 64.2 (d, J_{C1-P} = 6.2 Hz, C_1), 37.5 (d, J_{C2-P} = 6.3 Hz, C_2), 37.1 (C_4), 29.2 (C_3), 25.8 (C_8), 25.5 (C_5), 19.4 (C_{10}), 17.8 (C_9), 11.5, 11.5 (2d, J_{CH3-P} = 144.5 Hz, CH_3P); ^{31}P NMR δ 31.5, 31.5 (2s); MS (ESI): m/z = 671 $[2M+Na]^+$ 100%; HRMS calcd for $C_{18}H_{29}NaO_3P^+$ 347.1752, found 347.1746.

Dibenzyl 3-(2-acetamido-4,6-*O*-(*R*)-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl)-2-oxopropylphosphonate 11

To a solution of **17** (890 mg, 3.2 mmol, 3.25 eq.) in THF (15 mL) was added dropwise *n*BuLi (1.5 mL, 3.43 mmol, 3.5 eq.) at - 78 °C. After 1 h stirring at - 78 °C, the mixture was added to a cold solution of **3** (475 mg, 0.99 mmol, 1 eq.) in THF (3 mL). The reaction mixture was slowly warmed to r.t. overnight and quenched with saturated aqueous NH_4Cl solution. Aqueous phase was extracted with EtOAc and combined organic layers were dried ($MgSO_4$) and concentrated. Purification by flash chromatography (CH_2Cl_2/acetone, 8:2) afforded **11** (600 mg, 83%, white solid): R_f 0.47 (CH_2Cl_2/acetone = 8:2); $[\alpha]_D^{20}$ + 26 (*c* 1.0, CH_2Cl_2); IR (cm^{-1}) 1718 (CO), 1250 (PO); ^{1}H NMR δ 7.51-7.47 (m, 2H, H_{ar}), 7.42-7.30 (m, 13H, H_{ar}), 6.62 (d, 1H, $J_{NH-H2'}$ = 8.7 Hz, NH), 5.50 (s, 1H, $H_{7'}$), 5.12, 5.02 (AB from ABX, 2H, J_{AB} = 11.6 Hz, J_{A-P} = 9.5 Hz, J_{B-P} = 10.0 Hz, CH_2Ph), 5.01 (d, 2H, J_{H-P} = 8.4 Hz, CH_2Ph), 4.78 (ddd, 1H, $J_{H1'-H3a}$ = 8.0 Hz, $J_{H1'-H2'}$ = 5.7 Hz, $J_{H1'-H3b}$ = 5.1 Hz, $H_{1'}$), 4.31 (ddd, 1H, $J_{H2'-H3'}$ = 9.8 Hz, $J_{H2'-NH}$ = 8.7 Hz, $J_{H2'-H1'}$ = 5.7 Hz, $H_{2'}$), 4.15 (dd, 1H, $J_{H6'eq-H6'ax}$ = 10.3 Hz, $J_{H6'eq-H5'}$ = 4.3 Hz, $H_{6'eq}$), 3.83 (dd, 1H, $J_{H3'-H2'}$ = 9.8 Hz, $J_{H3'-H4'}$ = 8.3 Hz, $H_{3'}$), 3.65 (dd, 1H, $J_{H6'ax-H6'eq}$ = 10.3 Hz, $J_{H6'ax-H5'}$ = 9.2 Hz, $H_{6'ax}$), 3.51 (dd, 1H, $J_{H4'-H5'}$ = 9.6 Hz, $J_{H4'-H3'}$ = 8.3 Hz, $H_{4'}$), 3.47 (ddd, 1H, $J_{H5'-H4'}$ = 9.6 Hz, $J_{H5'-H6'ax}$ = 9.2 Hz, $J_{H5'-H6'eq}$ = 4.3 Hz, $H_{5'}$), 3.19 (dd, 1H, $J_{H3a-H3b}$ = 17.1 Hz, $J_{H3a-H1'}$ = 8.0 Hz, H_{3a}), 3.10, 3.05 (AB from ABX, 2H, J_{AB} = 13.5 Hz, J_{A-P} = 23.2 Hz, J_{B-P} = 21.9 Hz, H_{1a}, H_{1b}), 2.63 (dd, 1H, $J_{H3b-H3a}$ = 17.1 Hz, $J_{H3b-H1'}$ = 5.1 Hz, H_{3b}), 1.85 (s, 3H, CH_3CO), 0.83 (s, 9H, Si*t*Bu), 0.10, 0.01 (2s, 6H, SiMe); ^{13}C NMR δ 197.4 (d, J_{C2-P} = 4.7 Hz, C_2), 170.7 ($CH_3\underline{C}O$), 137.4 (Cq_{ar}), 135.4, 135.3 (2d, J_{Cq-P} = 6.0 Hz, Cq_{ar}), 129.2, 129.1, 129.0, 129.0, 128.9, 128.5, 128.2, 128.2, 126.4 (CH_{ar}), 102.0 ($C_{7'}$), 83.4 ($C_{4'}$), 70.5 ($C_{1'}$), 70.1 ($C_{3'}$), 69.3 ($C_{6'}$), 68.6, 68.5 (2d, $J_{CH2Ph-P}$ = 6.5 Hz, CH_2Ph), 65.7 ($C_{5'}$), 54.0 ($C_{2'}$), 43.9 (C_3), 42.9 (d, J_{C1-P} = 124.8 Hz, C_1), 25.8 (Si*t*Bu), 23.0 ($\underline{C}H_3CO$), 18.2 (Si*t*Bu), -3.9, -4.7 (SiMe); ^{31}P NMR δ 21.4 (s); MS (ESI): m/z = 1469 $[2M+Na]^+$ 100%; HRMS calcd for $C_{38}H_{50}NNaO_9PSi^+$ 746.2890, found 746.2898.

Benzyl 3-(2-acetamido-4,6-*O*-(*R*)-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl)-2-oxopropylphosphonate 12

To a solution of **11** (600 mg, 0.83 mmol) in toluene (8 mL) was added DABCO (110 mg, 0.99 mmol, 1.2 eq.). The reaction mixture was then refluxed for 7 h and concentrated. The residue was taken up in methanol, acidified with DOWEX H^+ (50WX8-100) ion exchange resin. Methanol was removed in vacuo to afford **12** (475 mg, 90%, white solid): $[\alpha]_D^{20}$ + 18 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (acetone-d_6) δ 7.54-7.32 (m, 11H, NH, H_{ar}), 5.62 (s, 1H, $H_{7'}$), 5.18-5.08 (m, 2H, CH_2Ph), 4.66 (ddd, 1H, $J_{H1'-H3a}$ = 6.8 Hz, $J_{H1'-H3b}$ = 6.3 Hz, $J_{H1'-H2'}$ = 5.9 Hz, $H_{1'}$), 4.28 (ddd, 1H, $J_{H2'-H3'}$ = 10.0 Hz, $J_{H2'-NH}$ = 9.4 Hz, $J_{H2'-H1'}$ = 5.9 Hz, $H_{2'}$), 4.09 (dd, 1H, $J_{H6'eq-H6'ax}$ = 9.7 Hz, $J_{H6'eq-H5'}$ = 4.5 Hz, $H_{6'eq}$), 3.99 (dd, 1H, $J_{H3'-H2'}$ = 10.0 Hz, $J_{H3'-H4'}$ = 8.9 Hz, $H_{3'}$), 3.68 (dd, 1H, $J_{H6'ax-H5'}$ = $J_{H6'ax-H6'eq}$ = 9.7 Hz, $H_{6'ax}$), 3.62 (ddd, 1H, $J_{H5'-H6'ax}$ = 9.7 Hz, $J_{H5'-H4'}$ = 9.2 Hz, $J_{H5'-H6'eq}$ = 4.5 Hz, $H_{5'}$), 3.52 (dd, 1H, $J_{H4'-H5'}$ = 9.2 Hz, $J_{H4'-H3'}$ = 8.9 Hz, $H_{4'}$), 3.37 (dd, 1H, J_{H1a-P} = 22.8 Hz, $J_{H1a-H1b}$ = 13.4 Hz, H_{1a}), 3.35 (dd, 1H, $J_{H3a-H3b}$ = 17.1 Hz, $J_{H3a-H1'}$ = 6.8 Hz, H_{3a}), 3.24 (dd, 1H, J_{H1b-P} = 21.9 Hz, $J_{H1b-H1a}$ = 13.4 Hz, H_{1b}), 3.00 (dd, 1H, $J_{H3b-H3a}$ = 17.1 Hz, $J_{H3b-H1'}$ = 6.3 Hz, H_{3b}), 1.84 (s, 3H, CH_3CO), 0.82 (s, 9H, Si*t*Bu), 0.08, 0.00 (2s, 6H, SiMe); ^{13}C NMR (acetone-d_6) δ 199.9 (d, J_{C2-P} = 5.0 Hz, C_2), 170.5 ($CH_3\underline{C}O$), 139.0 (Cq_{ar}), 137.6 (d, J_{Cq-P} = 6.0 Hz, Cq_{ar}), 129.6, 129.4, 129.3, 129.1, 128.7, 128.7, 127.3 (CH_{ar}), 102.5 ($C_{7'}$), 84.4 ($C_{4'}$), 72.0 ($C_{1'}$), 71.0 ($C_{3'}$), 69.7 ($C_{6'}$), 68.0 (d, $J_{CH2Ph-P}$ = 5.0 Hz, CH_2Ph), 65.9 ($C_{5'}$), 54.6 ($C_{2'}$), 43.8 (d, J_{C1-P} = 124.0 Hz, C_1), 43.5 (C_3), 26.3 (Si*t*Bu), 23.1 ($\underline{C}H_3CO$), 18.8 (Si*t*Bu), -3.8, -4.5 (SiMe); ^{31}P NMR (acetone-d_6) δ 18.8 (s); MS (ESI): m/z = 632 $[M-H]^-$ 100%; HRMS calcd for $C_{31}H_{43}NO_9PSi^-$ 632.2445, found 632.2460.

Dibenzyl 3-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-2-oxopropylphosphonate 13

To a suspension of **11** (100 mg, 0.14 mmol) in water (5 mL) was added trifluoroacetic acid (5 mL) at 0 °C. The reaction mixture was stirred at r.t. for 1 h and concentrated. Purification by flash chromatography (CH_2Cl_2/MeOH, 85:15) and lyophilization afforded **13** (70 mg, 97%, white solid): R_f 0.48 (CH_2Cl_2/MeOH = 85:15); $[\alpha]_D^{20}$ + 38 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 7.67 (d, 1H, $J_{NH-H2'}$ = 7.8 Hz, NH), 7.41-7.31 (m, 10H, H_{ar}), 5.07-4.98 (m, 5H, $OH_{4'}$, CH_2Ph), 4.88 (d, 1H, $J_{OH3'-H3'}$ = 5.3 Hz, $OH_{3'}$), 4.44 (dd, 1H, $J_{OH6'-H6'b}$ = 6.0 Hz, $J_{OH6'-H6'a}$ = 5.8 Hz, $OH_{6'}$), 4.40 (ddd, 1H, $J_{H1'-H3a}$ = 9.1 Hz, $J_{H1'-H2'}$ = 5.6 Hz, $J_{H1'-H3b}$ = 4.6 Hz, $H_{1'}$), 3.71 (ddd, 1H, $J_{H2'-H3'}$ = 10.0 Hz, $J_{H2'-NH}$ = 7.8 Hz, $J_{H2'-H1'}$ = 5.6 Hz, $H_{2'}$), 3.56 (ddd, 1H, $J_{H6'a-H6'b}$ = 11.6 Hz, $J_{H6'a-OH6'}$ = 5.8 Hz, $J_{H6'a-H5'}$ = 2.5 Hz, $H_{6'a}$), 3.50 (dd, 1H, J_{H1a-P} = 21.8 Hz, $J_{H1a-H1b}$ = 14.4 Hz, H_{1a}), 3.47 (dd, 1H, (ddd, 1H, $J_{H6'b-H6'a}$ = 11.6 Hz, $J_{H6'b-OH6'}$ = 6.0 Hz, $J_{H6'b-H5'}$ = 5.7 Hz, $H_{6'b}$), 3.41 (dd, 1H, J_{H1b-P} = 21.5 Hz, $J_{H1b-H1a}$ = 14.4 Hz, H_{1b}), 3.40 (ddd, 1H, $J_{H3'-H2'}$ = 10.0 Hz, $J_{H3'-H4'}$ = 8.0 Hz, $J_{H3'-OH3'}$ = 5.3 Hz, $H_{3'}$), 3.35 (ddd, 1H, $J_{H5'-H4'}$ = 8.8 Hz, $J_{H5'-H6'b}$ = 5.7 Hz, $J_{H5'-H6'a}$ = 2.5 Hz, $H_{5'}$), 3.15 (ddd, 1H, $J_{H4'-H5'}$ = 8.8 Hz, $J_{H4'-H3'}$ = 8.0 Hz, $J_{H4'-OH4'}$ = 5.3 Hz, $H_{4'}$), 2.90 (dd, 1H, $J_{H3a-H3b}$ = 16.1 Hz, $J_{H3a-H1'}$ = 9.1 Hz, H_{3a}), 2.66 (dd, 1H, $J_{H3b-H3a}$ = 16.1 Hz, $J_{H3b-H1'}$ = 4.6 Hz, H_{3b}), 1.77 (s, 3H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ 200.5 (d, J_{C2-P} = 6.0 Hz, C_2), 169.3 ($CH_3\underline{C}O$), 136.2 (d, J_{Cq-P} = 6.0 Hz, Cq_{ar}), 128.4, 128.2, 127.7 (CH_{ar}), 74.9 ($C_{5'}$), 70.6 ($C_{4'}$), 70.3 ($C_{3'}$), 69.3 ($C_{1'}$), 67.0, 66.9 (2d, $J_{CH2Ph-P}$ = 6.0 Hz, CH_2Ph), 60.9 ($C_{6'}$), 52.6 ($C_{2'}$), 41.8 (d, J_{C1-P} = 126.0 Hz, C_1), 41.4 (C_3), 22.6 ($\underline{C}H_3CO$); ^{31}P NMR (DMSO-d_6) δ 21.8 (s); MS (ESI): m/z = 544 $[M+Na]^+$ 100%; HRMS calcd for $C_{25}H_{32}NNaO_9P^+$ 544.1712, found 544.1707.

Benzyl 3-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-2-oxopropylphosphonate 14

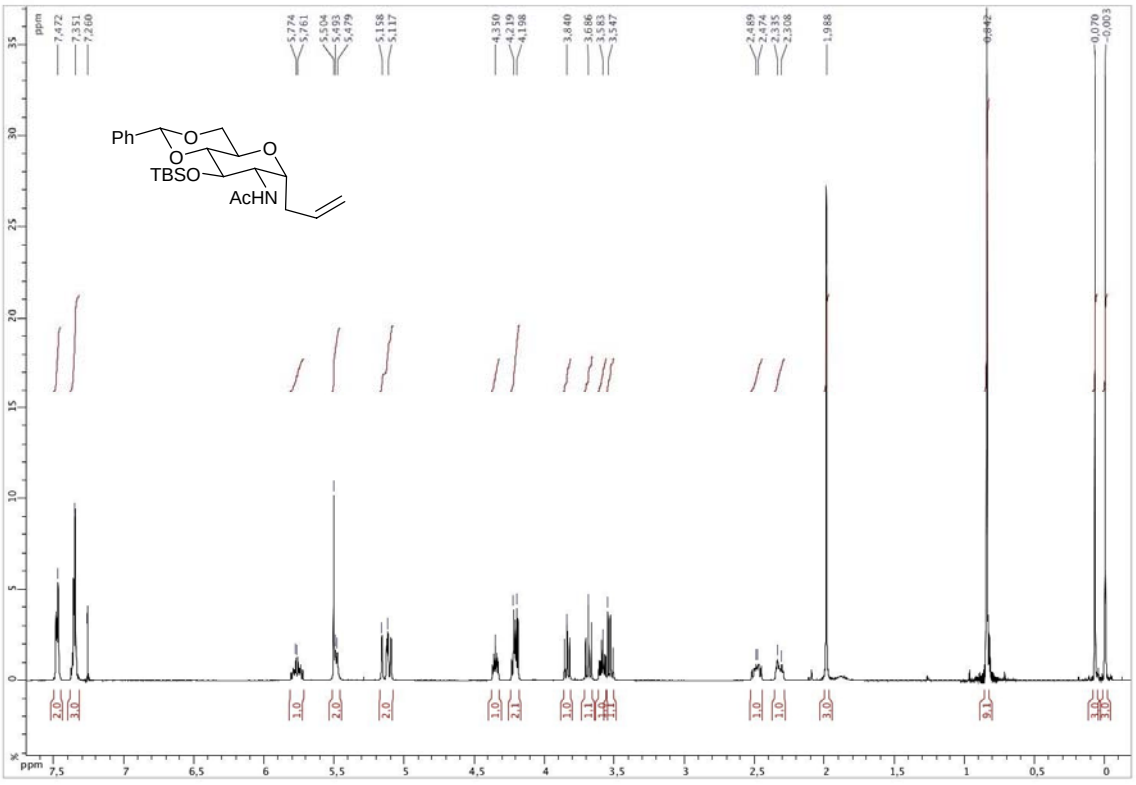
To a solution of **13** (34 mg, 65 µmol) in toluene (1 mL) was added DABCO (9 mg, 78 µmol, 1.2 eq.). The reaction mixture was then refluxed for 4 h and concentrated. The residue was taken up in water, acidified with DOWEX H^+ (50WX8-100) ion exchange resin. Water was removed in vacuo to afford **14** (25 mg, 88%, white solid): ^{1}H NMR (DMSO-d_6) δ 7.73 (d, 1H, $J_{NH\text{-}H2'}$ = 8.0 Hz, NH), 7.42-7.28 (m, 5H, H_{ar}), 4.94 (d, 2H, $J_{CH2Ph\text{-}P}$ = 7.5 Hz, CH_2Ph), 4.66 (ddd, 1H, $J_{H1'\text{-}H3a}$ = 8.3 Hz, $J_{H1'\text{-}H3b}$ = $J_{H1'\text{-}H2'}$ = 5.2 Hz, $H_{1'}$), 3.72 (ddd, 1H, $J_{H2'\text{-}H3'}$ = 10.3 Hz, $J_{H2'\text{-}NH}$ = 8.0 Hz, $J_{H2'\text{-}H1'}$ = 5.2 Hz, $H_{2'}$), 3.56 (dd, 1H, $J_{H6'a\text{-}H6'b}$ = 11.5 Hz, $J_{H6'a\text{-}H5'}$ = 2.2 Hz, $H_{6'a}$), 3.46 (dd, 1H, $J_{H6'b\text{-}H6'a}$ = 11.5 Hz, $J_{H6'b\text{-}H5'}$ = 5.7 Hz, $H_{6'b}$), 3.41 (dd, 1H, $J_{H3'\text{-}H2'}$ = 10.3 Hz, $J_{H3'\text{-}H4'}$ = 8.1 Hz, $H_{3'}$), 3.35 (ddd, 1H, $J_{H5'\text{-}H4'}$ = 8.8 Hz, $J_{H5'\text{-}H6'b}$ = 5.7 Hz, $J_{H5'\text{-}H6'a}$ = 2.2 Hz, $H_{5'}$), 3.17, 3.12 (AB from ABX, 2H, J_{AB} = 13.3 Hz, $J_{A\text{-}P}$ = 21.7 Hz, $J_{B\text{-}P}$ = 22.0 Hz, H_1), 3.12 (dd, 1H, $J_{H4'\text{-}H5'}$ = 8.8 Hz, $J_{H4'\text{-}H3'}$ = 8.1 Hz, $H_{4'}$), 2.88 (dd, 1H, $J_{H3a\text{-}H3b}$ = 16.2 Hz, $J_{H3a\text{-}H1'}$ = 8.3 Hz, H_{3a}), 2.75 (dd, 1H, $J_{H3b\text{-}H3a}$ = 16.2 Hz, $J_{H3b\text{-}H1'}$ = 5.2 Hz, H_{3b}), 1.76 (s, 3H, CH_3CO); ^{13}C NMR (DMSO-d_6) δ 201.5 (d, $J_{C2\text{-}P}$ = 5.5 Hz, C_2), 169.3 ($CH_3\underline{C}O$), 137.2 (d, $J_{Cq\text{-}P}$ = 7.0 Hz, Cq_{ar}), 128.3, 127.8, 127.4 (CH_{ar}), 74.8 ($C_{5'}$), 70.8 ($C_{4'}$), 70.4 ($C_{3'}$), 69.5 ($C_{1'}$), 66.1 (d, $J_{CH2Ph\text{-}P}$ = 5.0 Hz, CH_2Ph), 61.0 ($C_{6'}$), 52.7 ($C_{2'}$), 43.9 (d, $J_{C1\text{-}P}$ = 120.0 Hz, C_1), 41.1 (C_3), 22.6 ($\underline{C}H_3CO$); ^{31}P NMR (DMSO-d_6) δ 16.2 (s); MS (ESI): m/z = 430 $[M\text{-}H]^-$ 100%;.

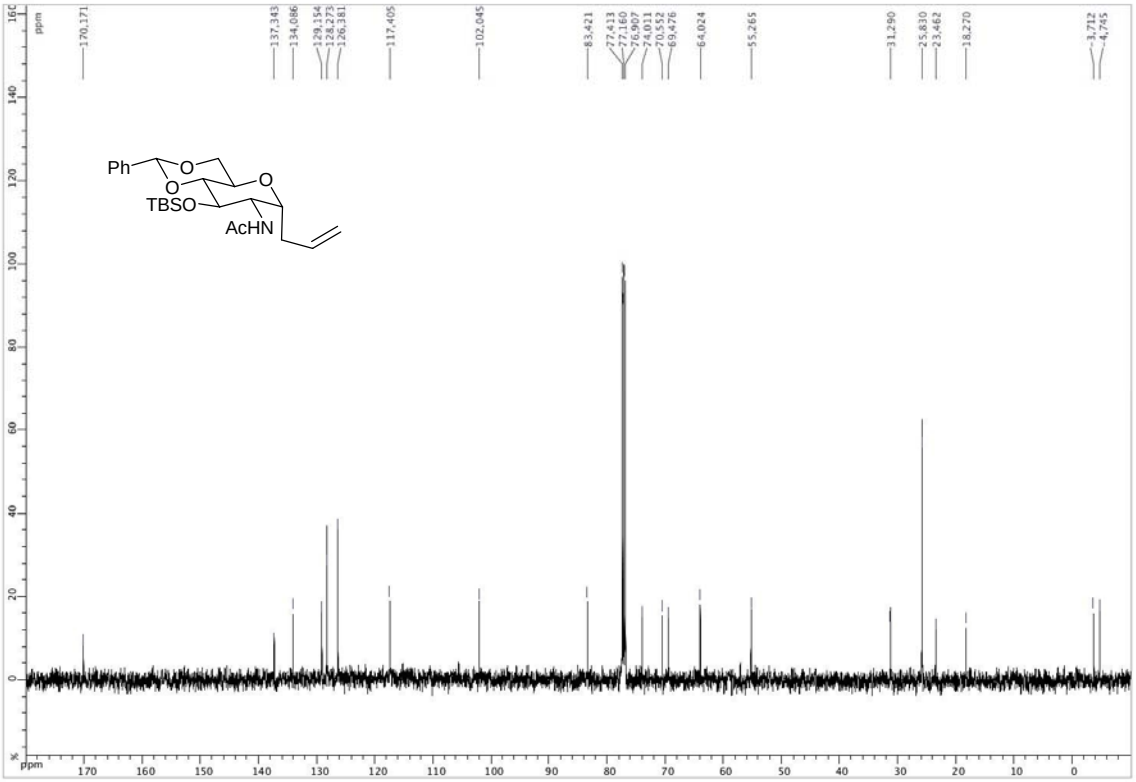
3-*N*-*tert*-Butyloxycarbonyl-1-(5'-hydroxypentyl)-uracil 17

To a solution of 1-(5'-hydroxypentyl)uracil (500 mg, 2.5 mmol) in THF (10 mL) were added at 0 °C trimethylsilyl chloride (650 µL, 5.0 mmol, 2 eq.) and DIEA (880 µL, 5.0 mmol, 2 eq.). The reaction mixture was stirred at r.t. for 1 h 30 and hydrolyzed with saturated aqueous NH_4Cl solution. Aqueous phase was extracted with EtOAc and the organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was taken up in THF (10 mL). Boc_2O (606 mg, 2.8 mmol, 1.1 eq.), Et_3N (390 µL, 2.8 mmol, 1.1 eq.) and DMAP cat. were successively added. The mixture was stirred at r.t. for 16 h, then cooled to 0 °C and stirred with 1 M aqueous HCl solution for 2 min. After dilution with EtOAc and decantation, organic phase was washed with saturated aqueous NaCl solution, dried ($MgSO_4$) and concentrated. Purification by flash chromatography (CH_2Cl_2/MeOH, 9:1) afforded **17** (498 mg, 66%, yellow oil): R_f 0.54 (CH_2Cl_2/MeOH, 9:1); ^{1}H NMR δ 7.12 (d, 1H, $J_{H6\text{-}H5}$ = 8.0 Hz, H_6), 5.70 (d, 1H, $J_{H5\text{-}H6}$ = 8.0 Hz, H_5), 3.73 (t, 2H, $J_{H1'\text{-}H2'}$ = 7.4 Hz, $H_{1'}$), 3.68-3.61 (m, 2H, $H_{5'}$), 1.74 (tt, 2H, $J_{H2'\text{-}H3'}$ = 7.7 Hz, $J_{H2'\text{-}H1'}$ = 7.4 Hz, $H_{2'}$), 1.65-1.56 (m, 2H, $H_{4'}$), 1.60 (s, 9H, CMe_3), 1.47-1.39 (m, 2H, $H_{3'}$); ^{13}C NMR δ 160.9 (C_4), 149.2 (C_2), 148.0 (CO_{Boc}), 143.7 (C_6), 102.0 (C_5), 86.9 ($\underline{C}Me_3$), 62.5 ($C_{5'}$), 49.4 ($C_{1'}$), 32.1 ($C_{4'}$), 28.8 ($C_{2'}$), 27.6 ($C\underline{Me}_3$), 22.9 ($C_{3'}$); MS (ESI): m/z = 321 $[M+Na]^+$ 100%; HRMS calcd for $C_{14}H_{22}N_2NaO_5^+$ 321.1426, found 321.1425.

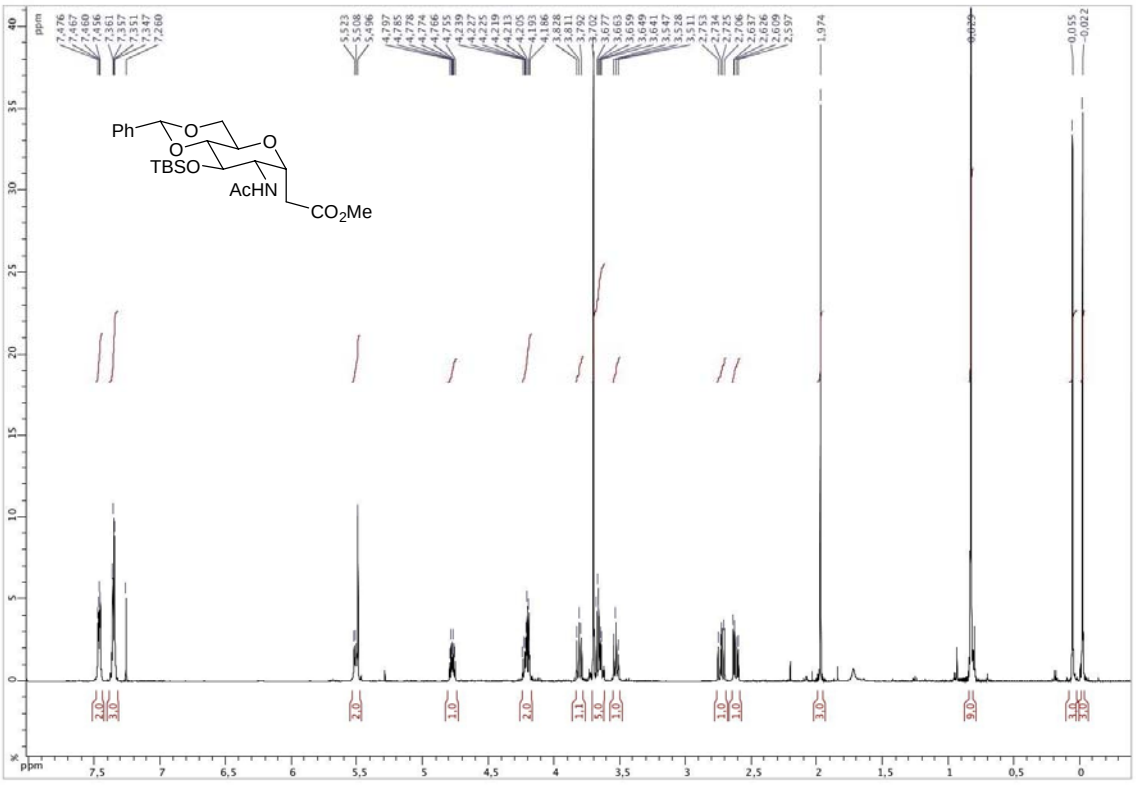
3. ^{1}H , ^{13}C and ^{31}P NMR Spectra

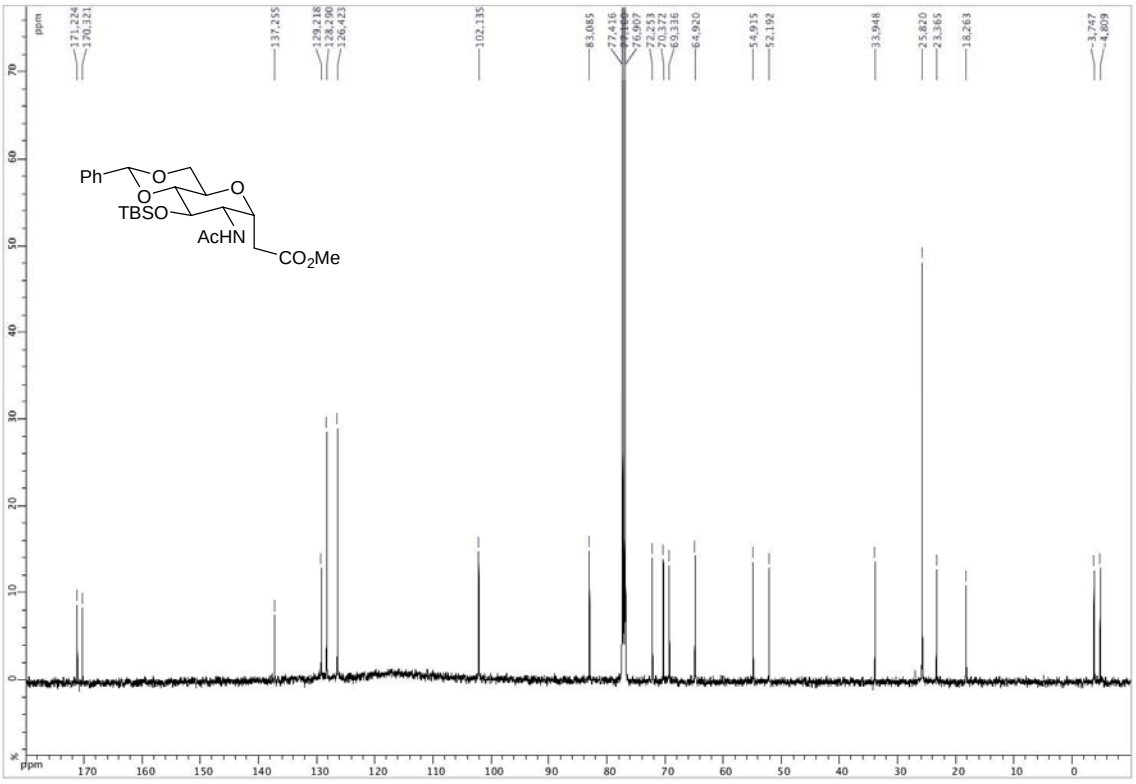
Compound **2α** (^{1}H, ^{13}C)



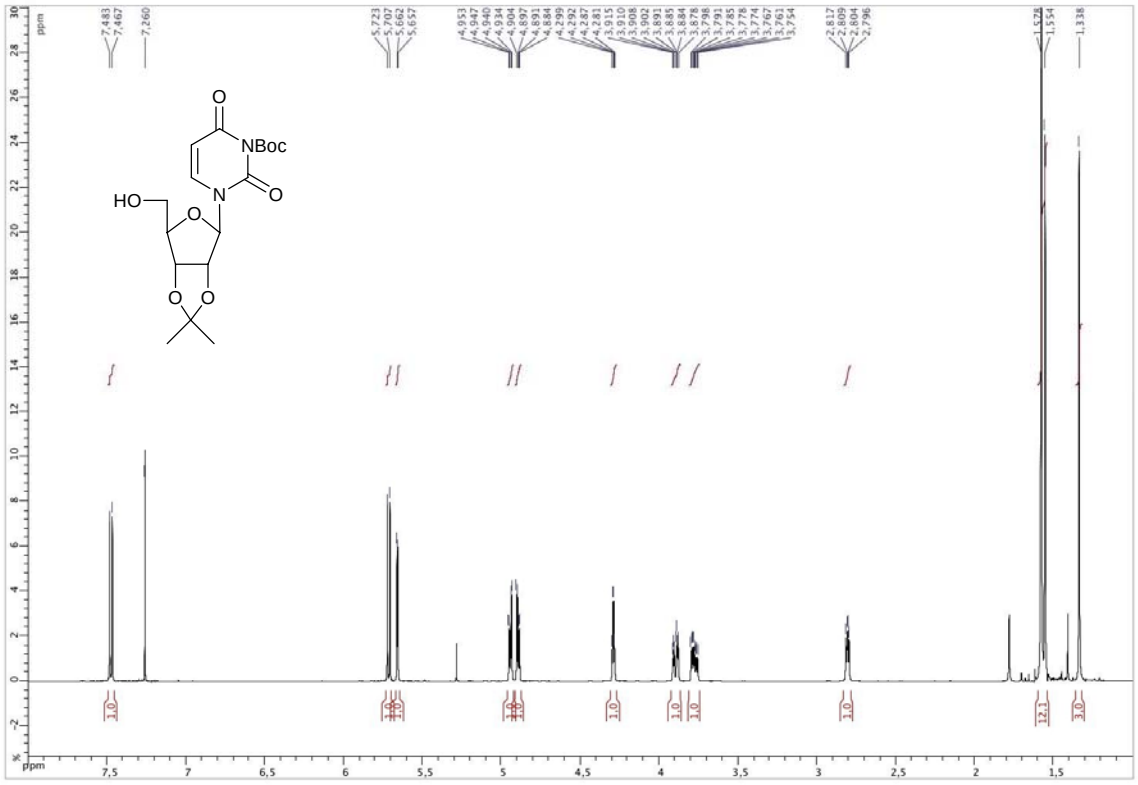


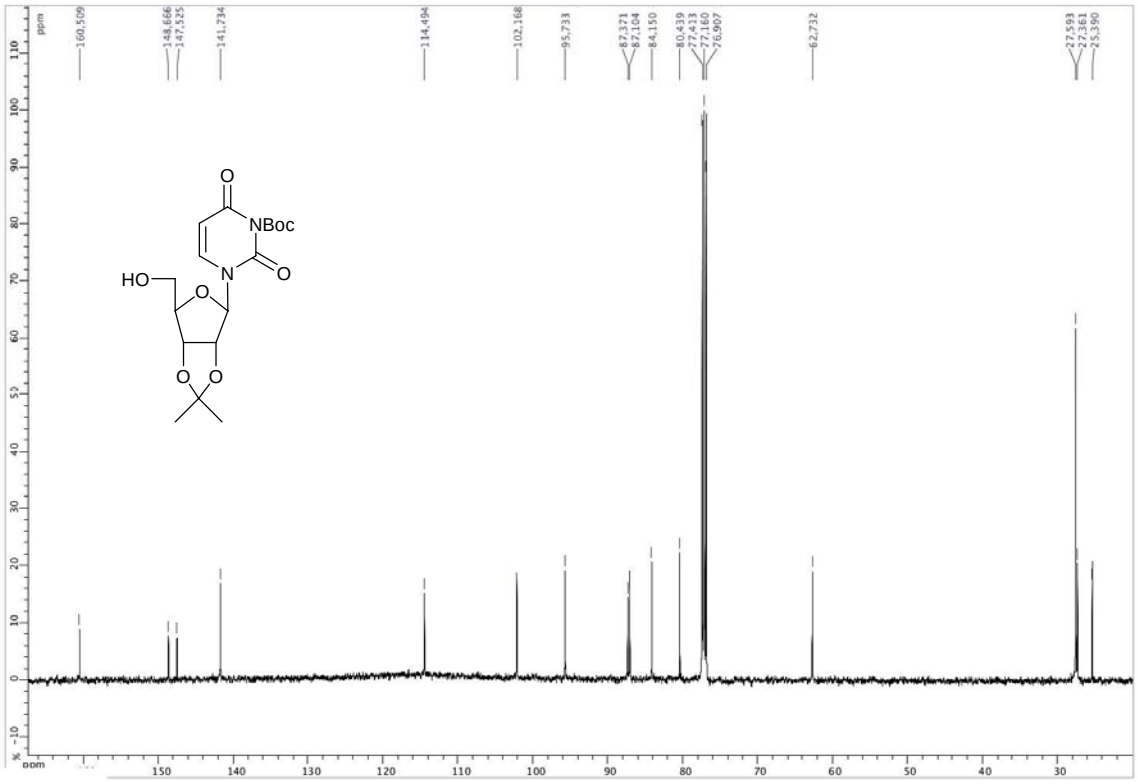
Compound **3** (^{1}H, ^{13}C)



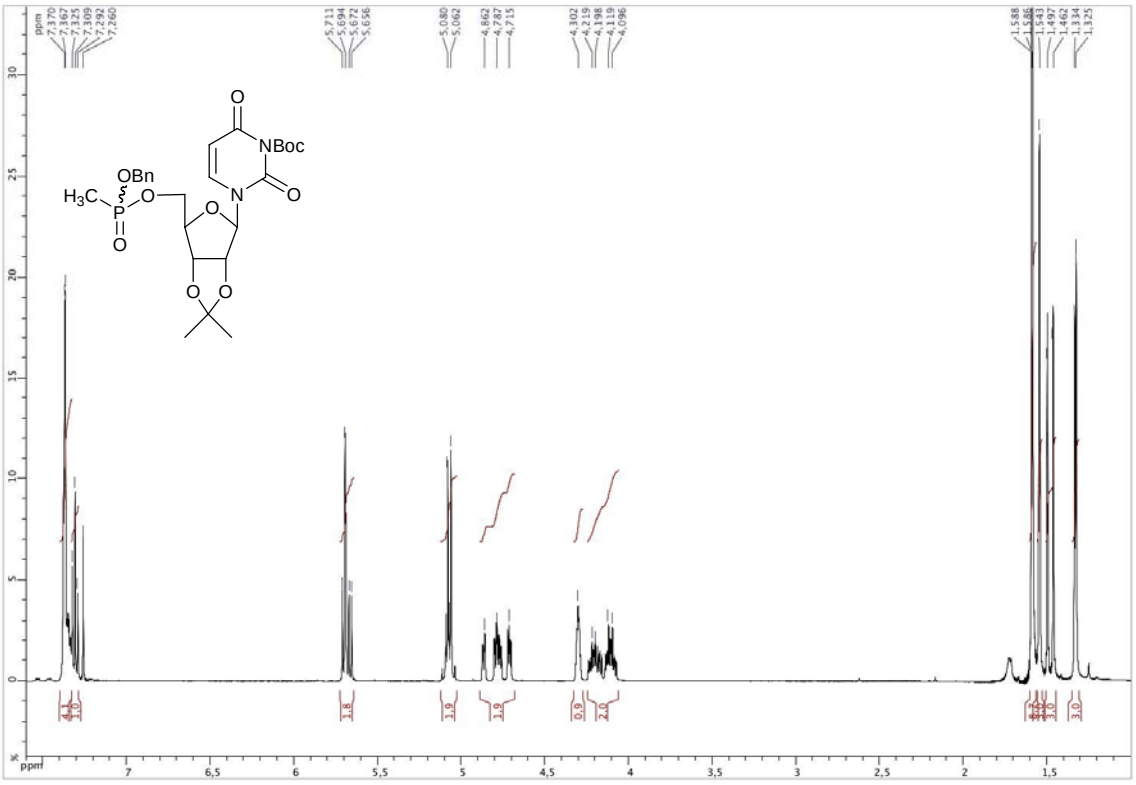


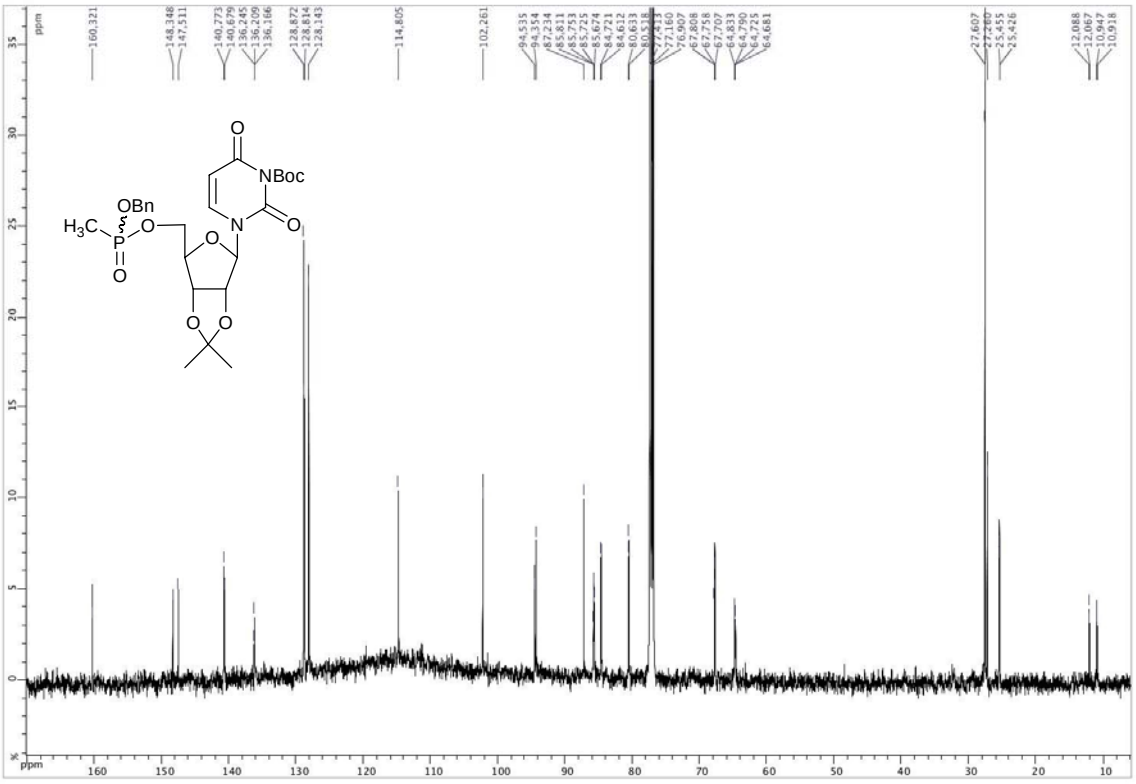
Compound **6** (^{1}H, ^{13}C)

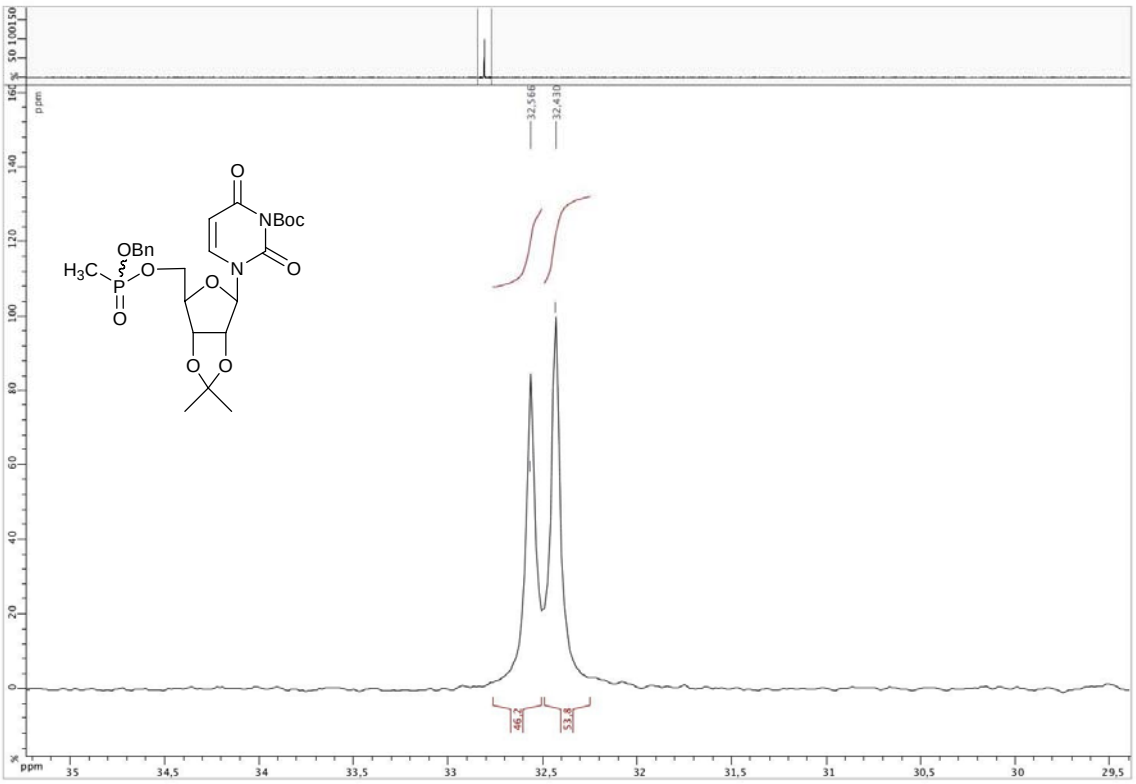




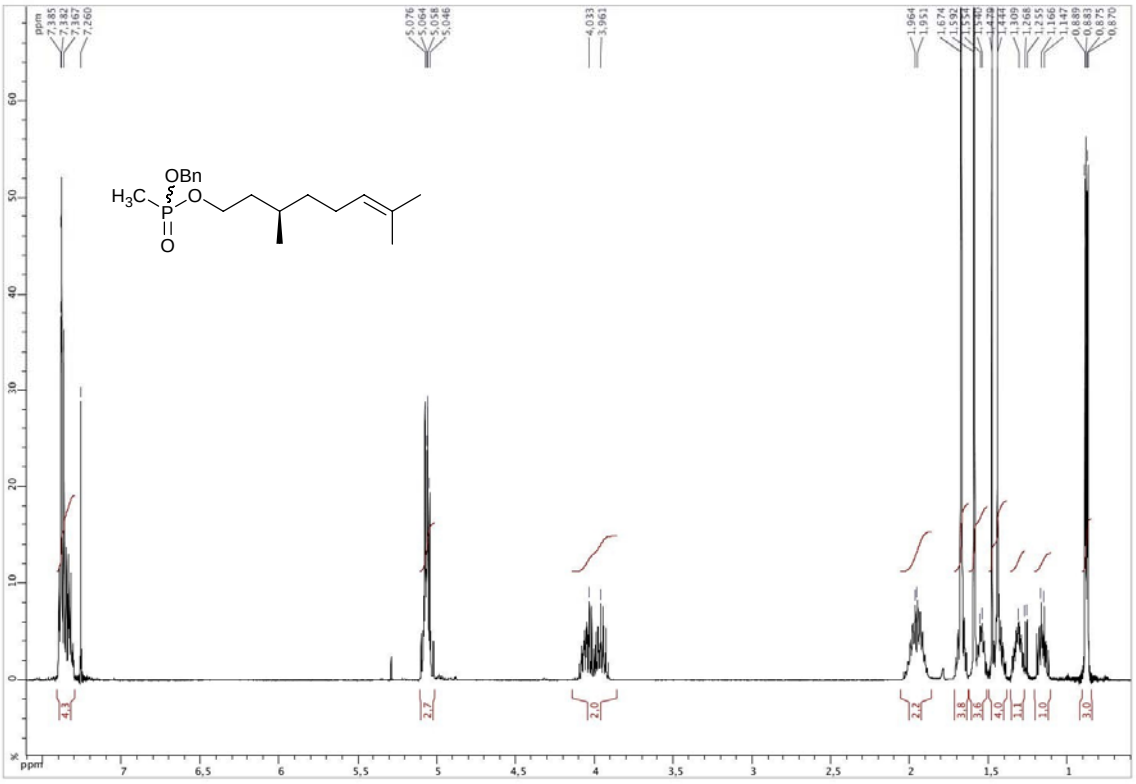
Compound **7** (^{1}H, ^{13}C, ^{31}P)

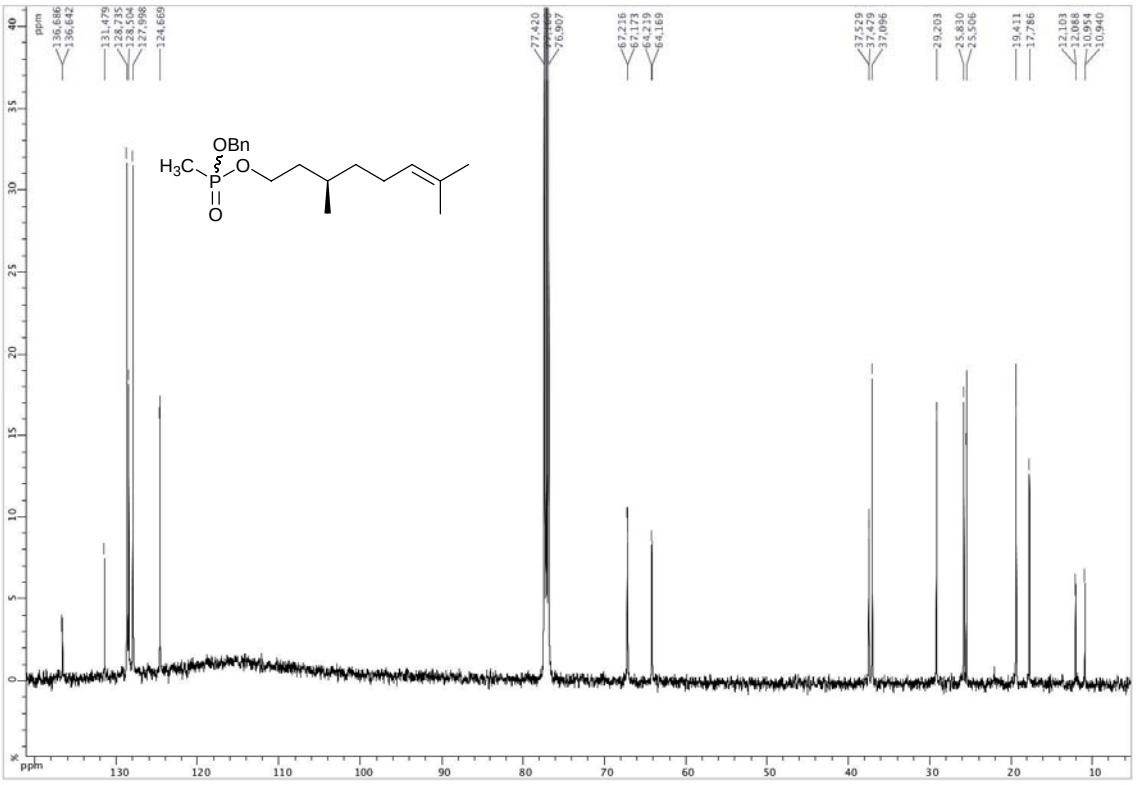


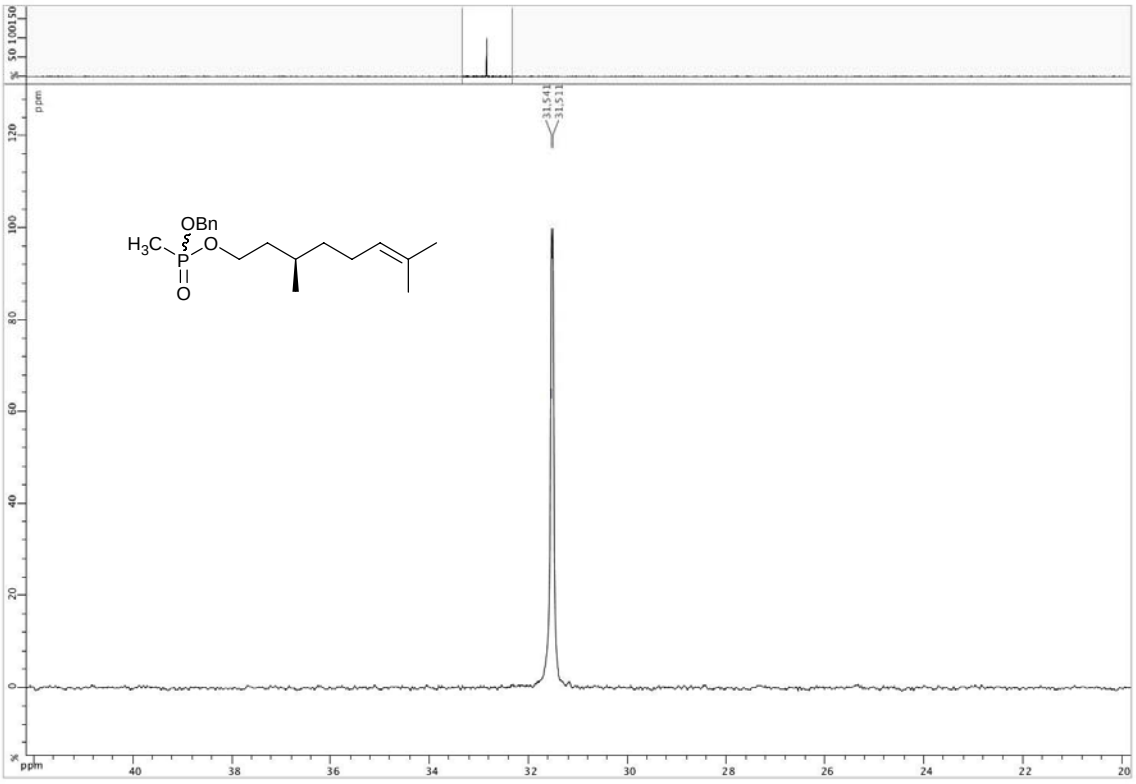




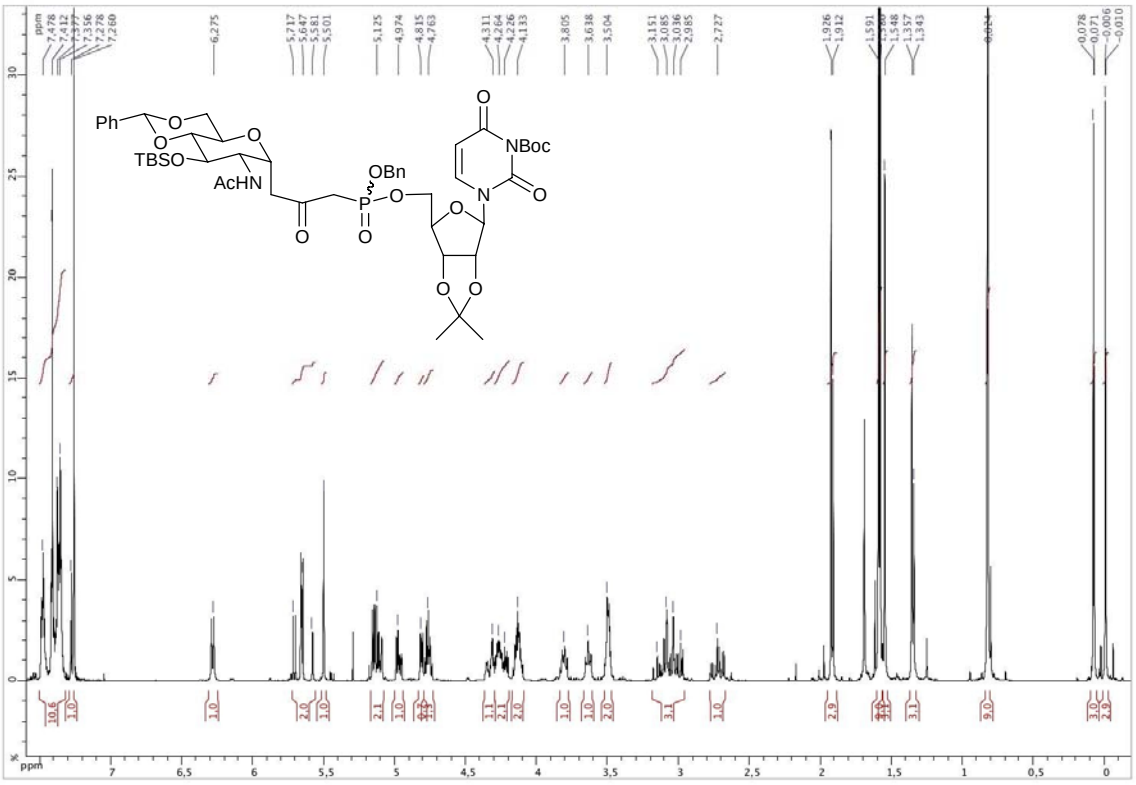
Compound **8** (^{1}H, ^{13}C, ^{31}P)

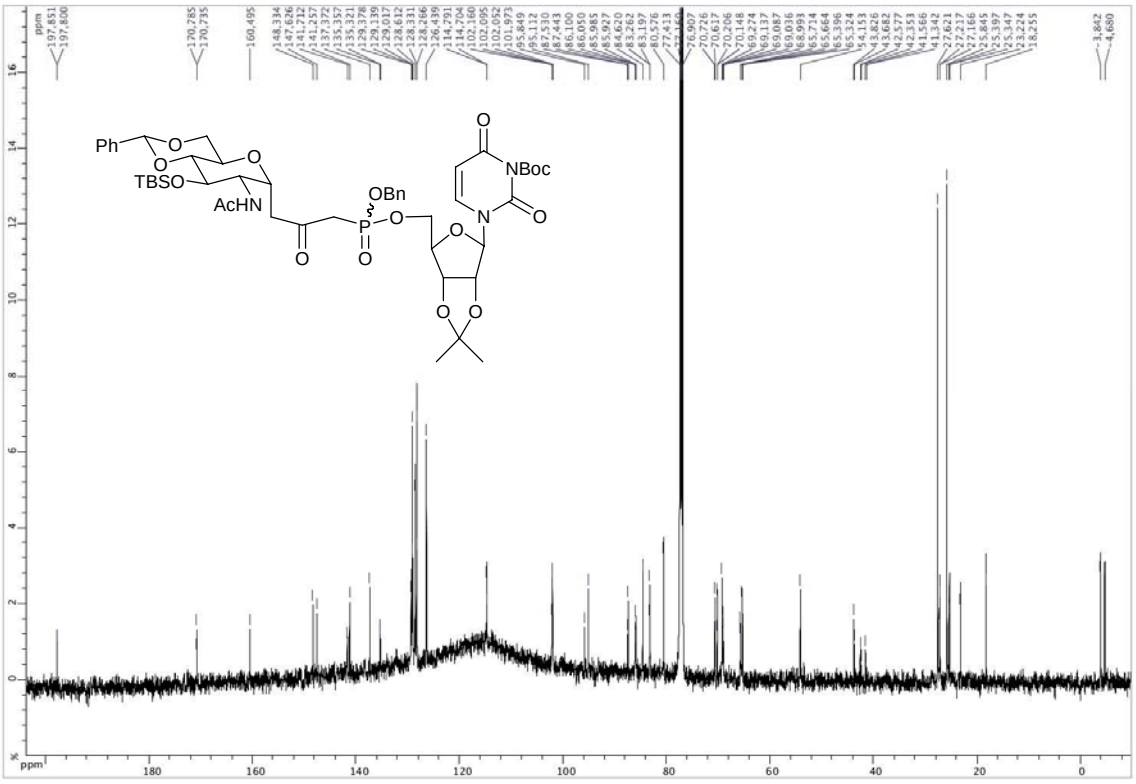


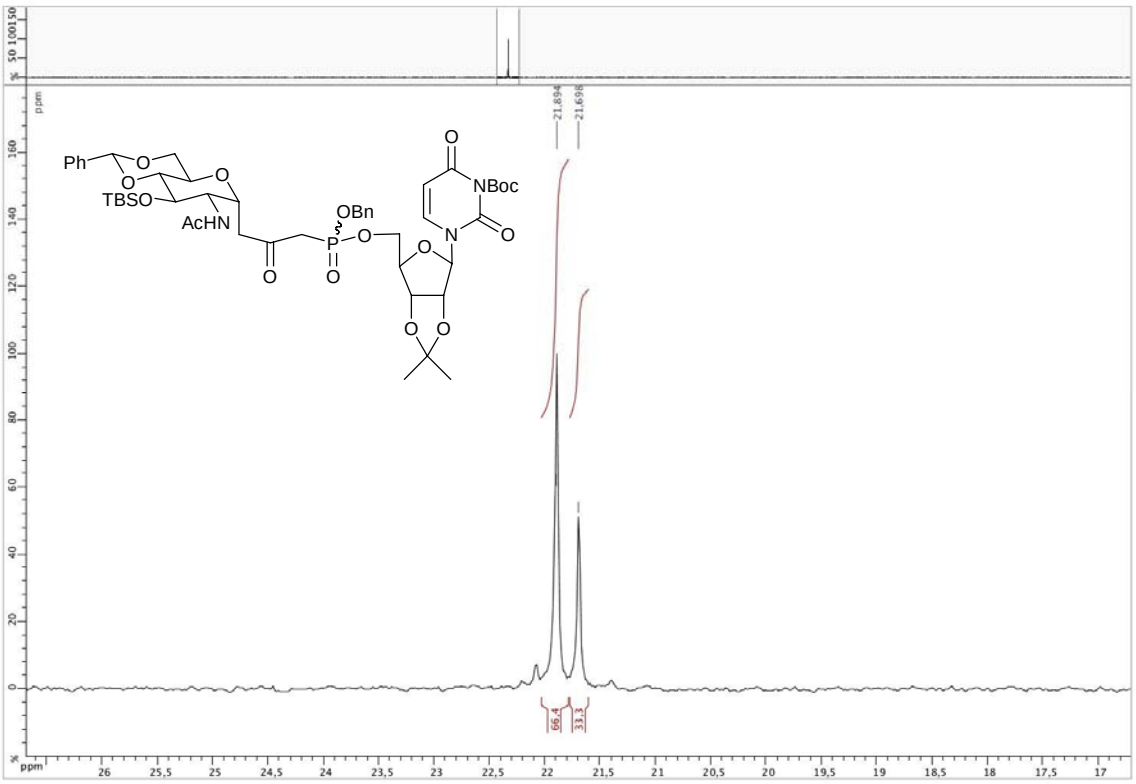




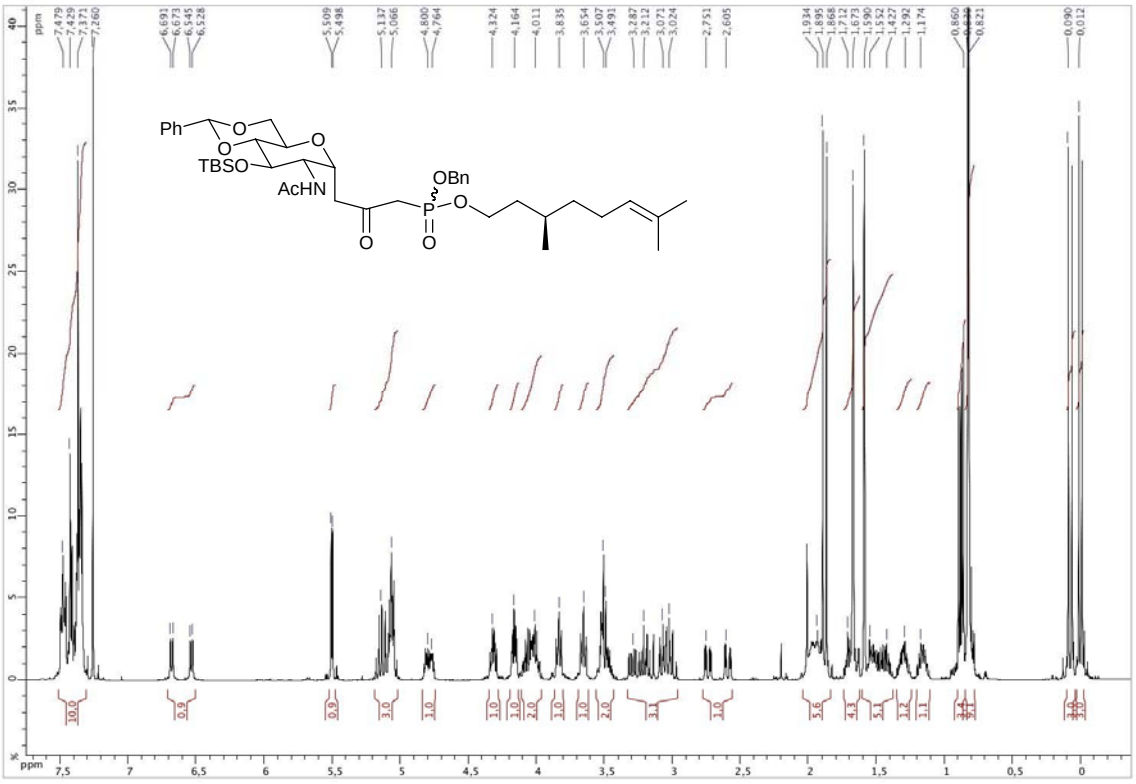
Compound **9** (^{1}H, ^{13}C, ^{31}P)

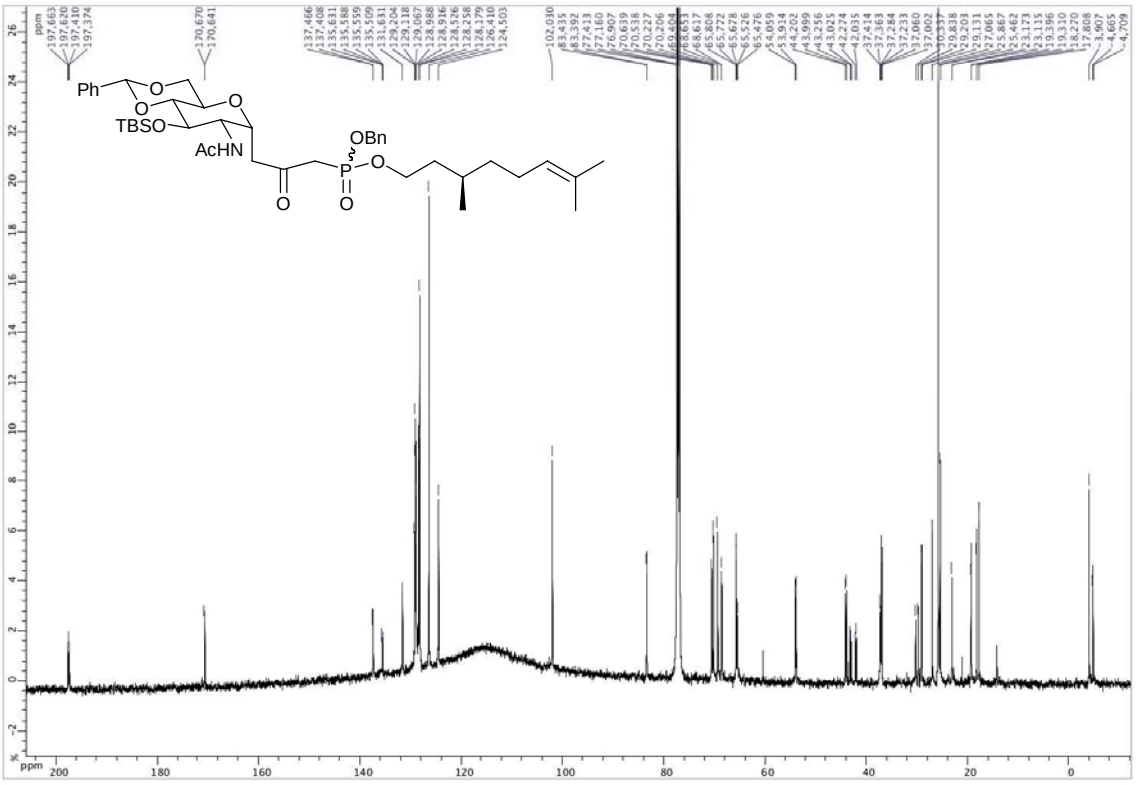


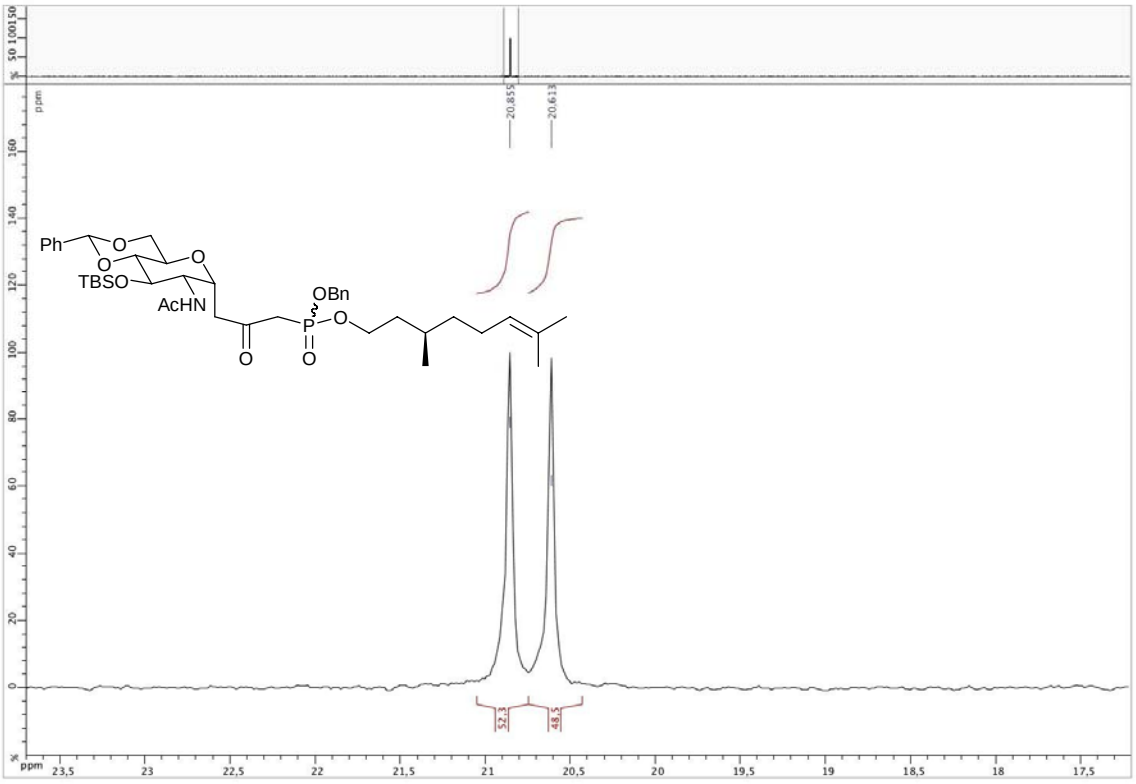




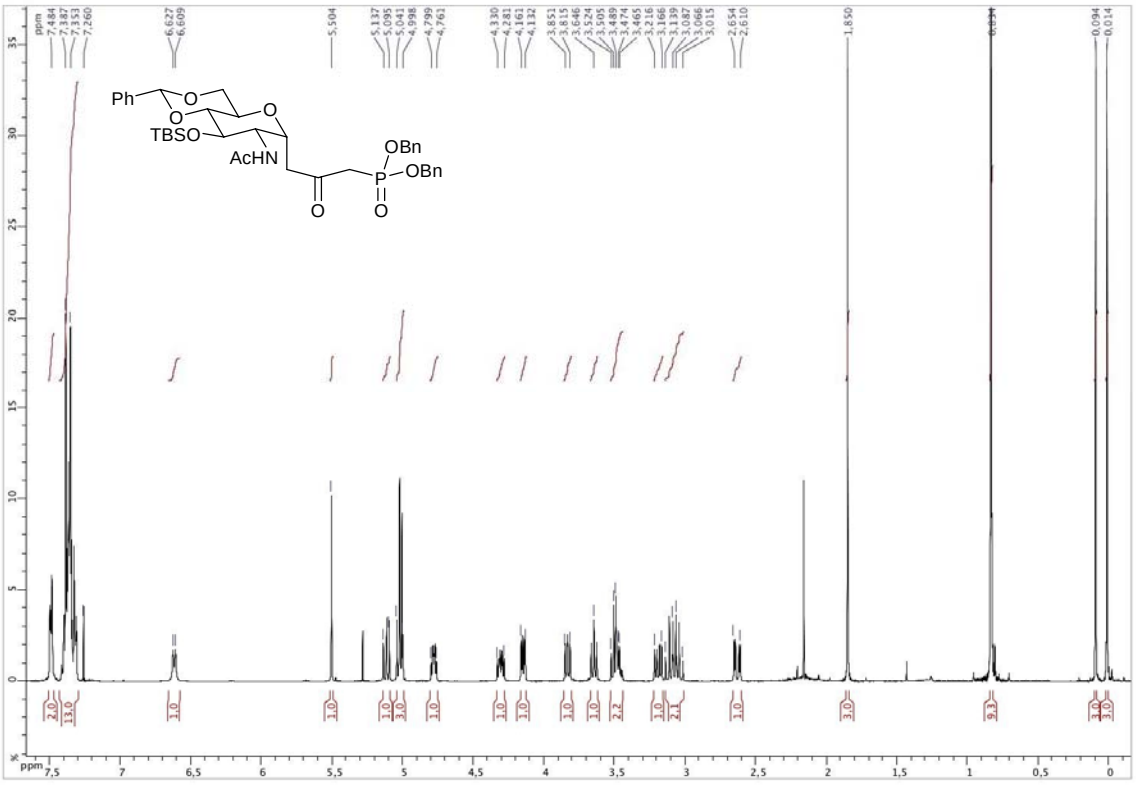
Compound **10** (^{1}H, ^{13}C, ^{31}P)

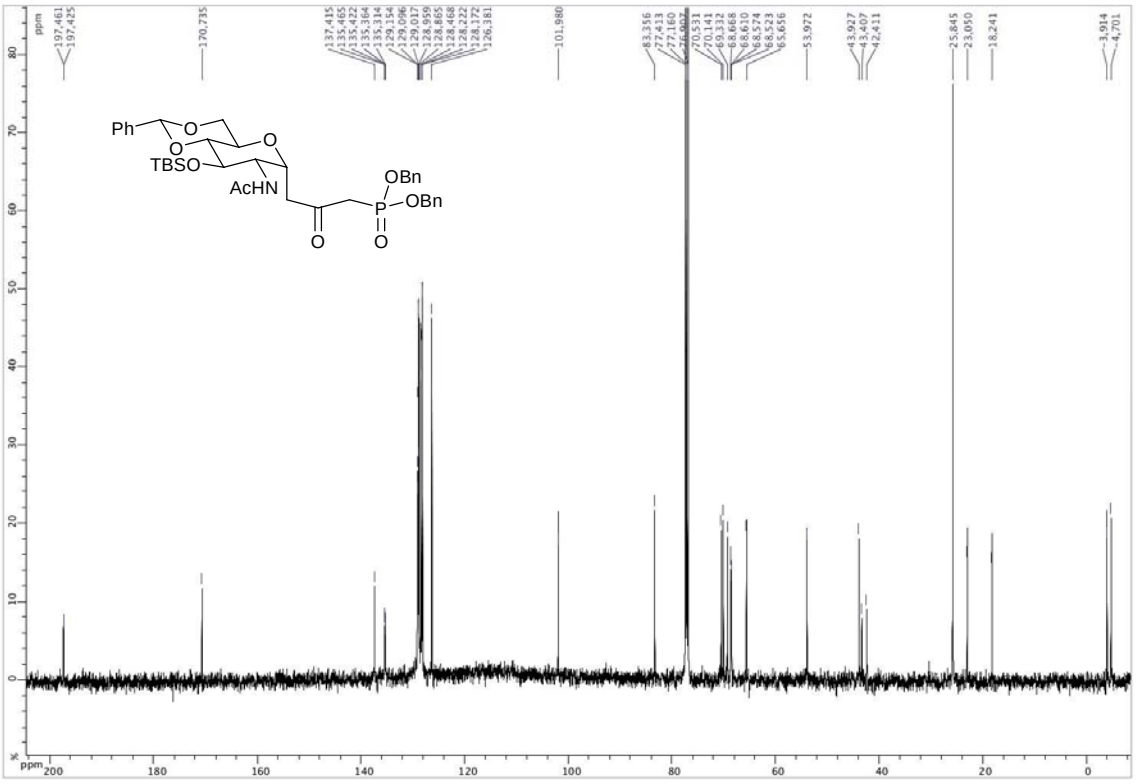


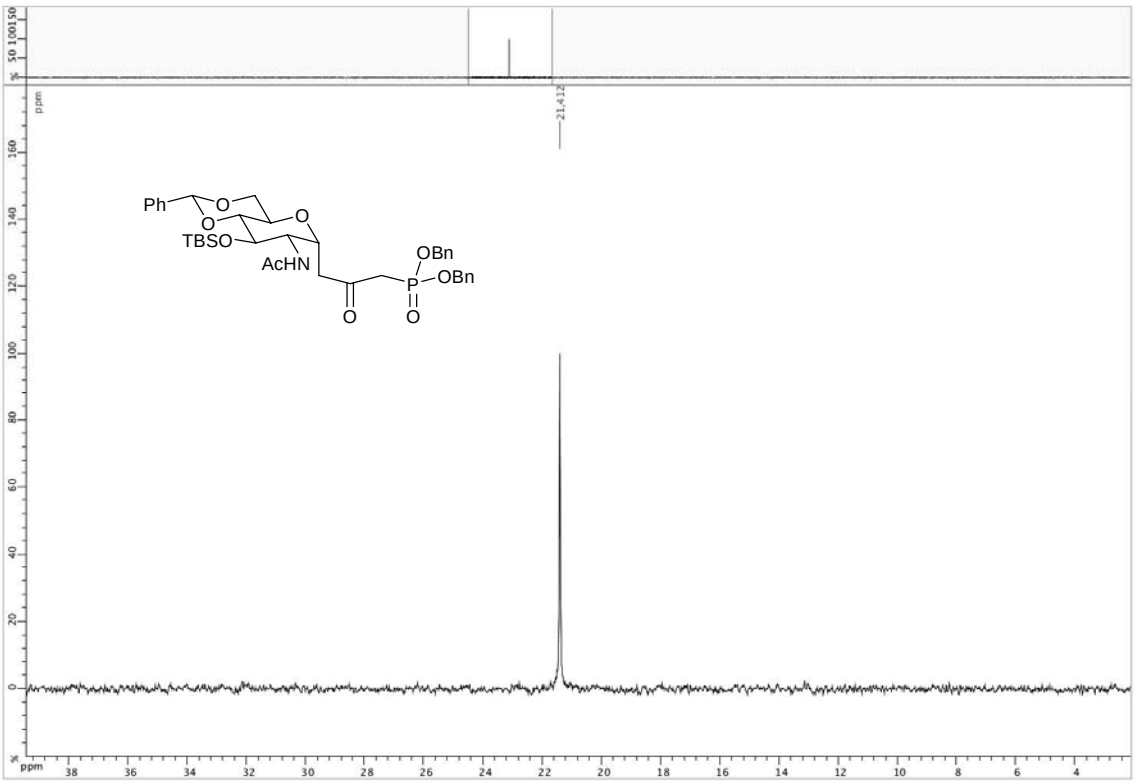




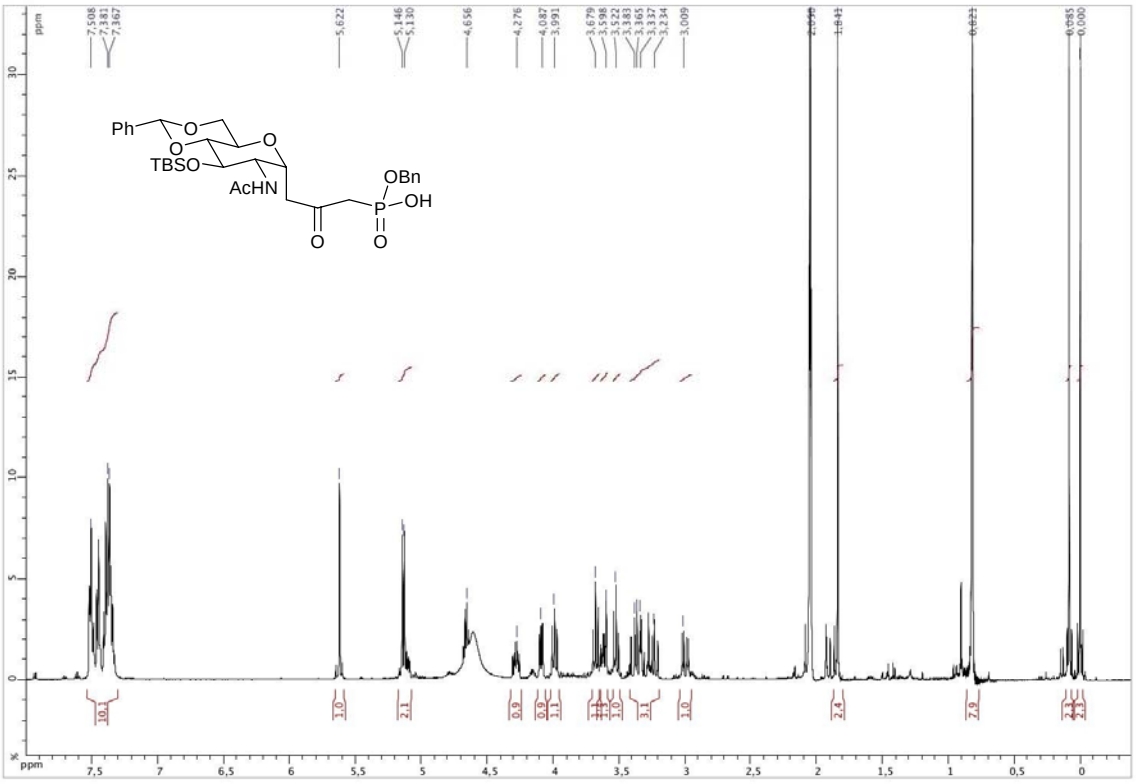
Compound **11** (^{1}H, ^{13}C, ^{31}P)

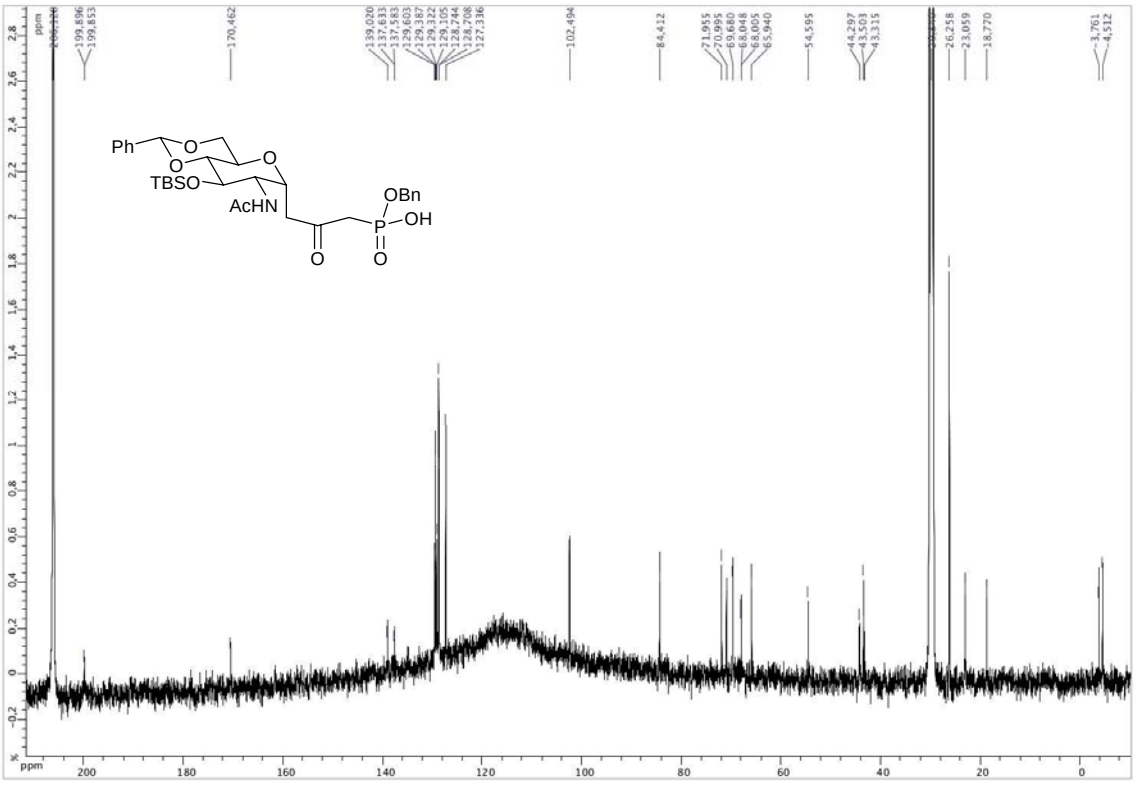


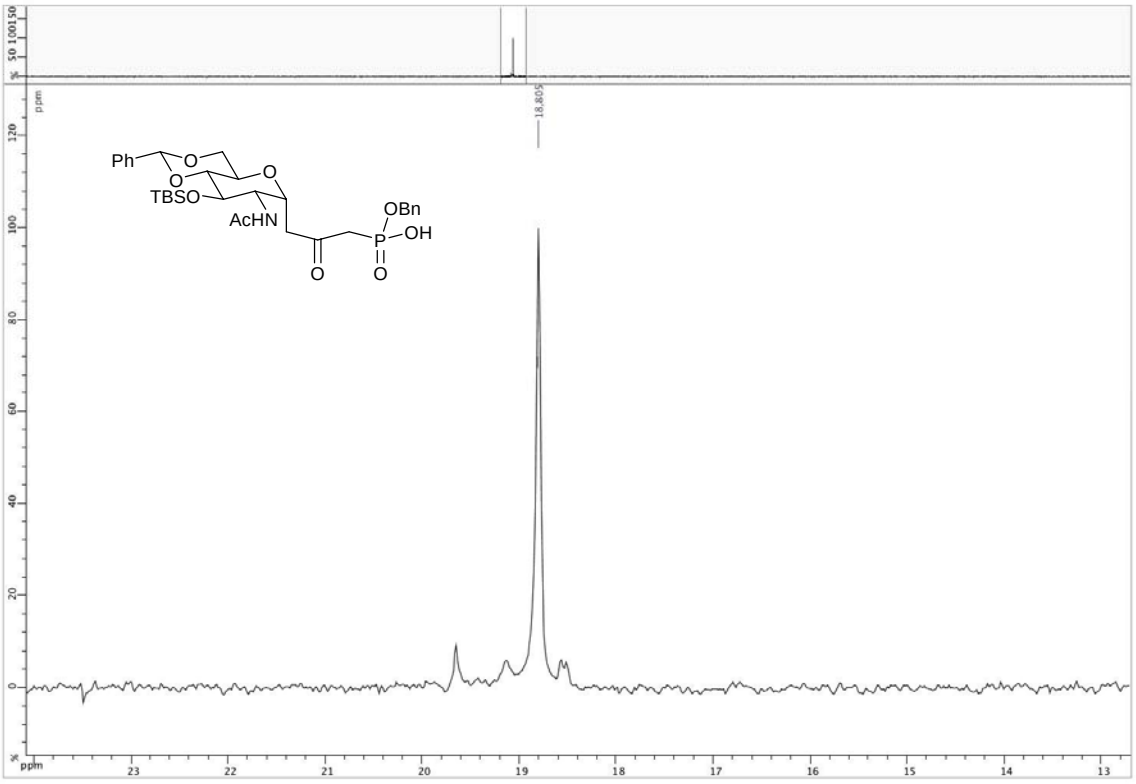




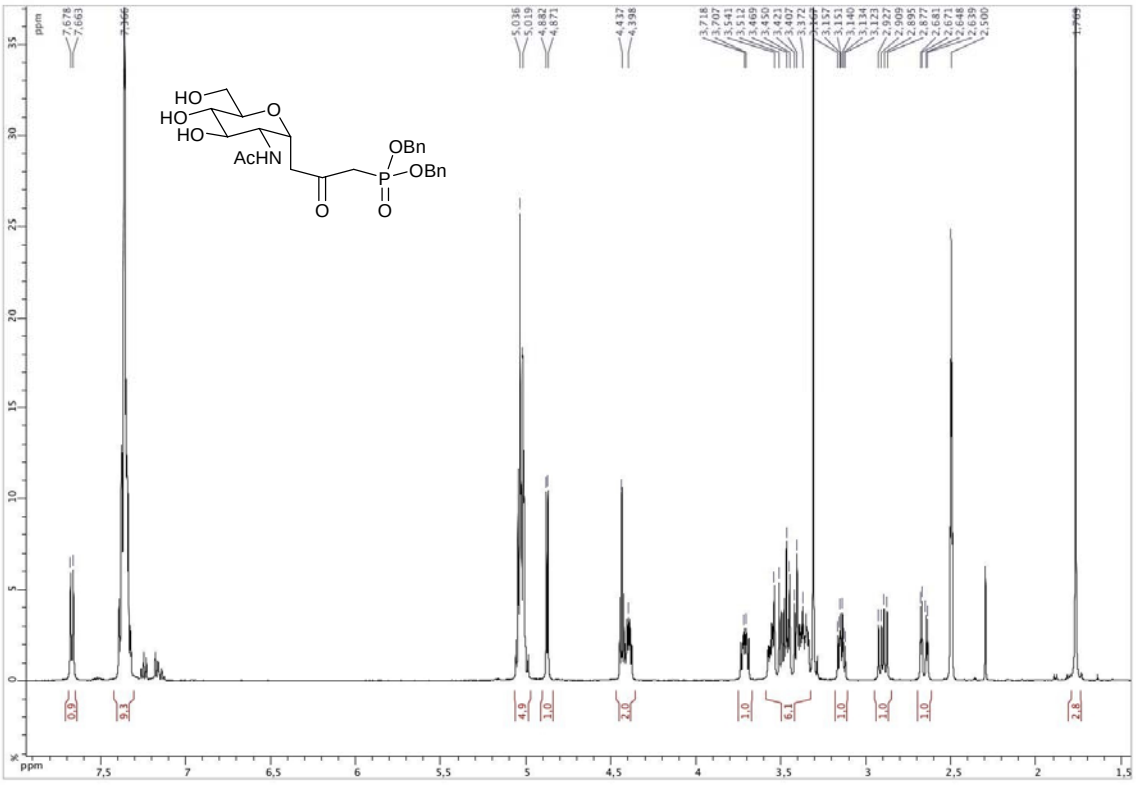
Compound **12** (^{1}H, ^{13}C, ^{31}P)

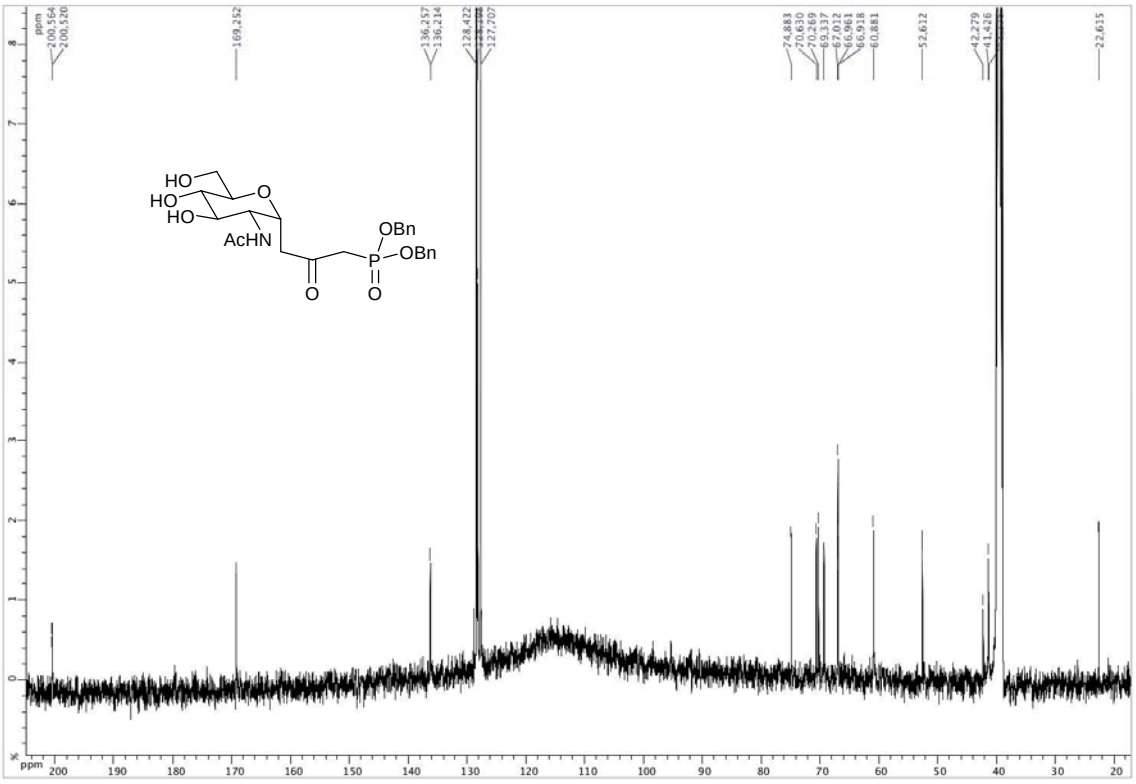


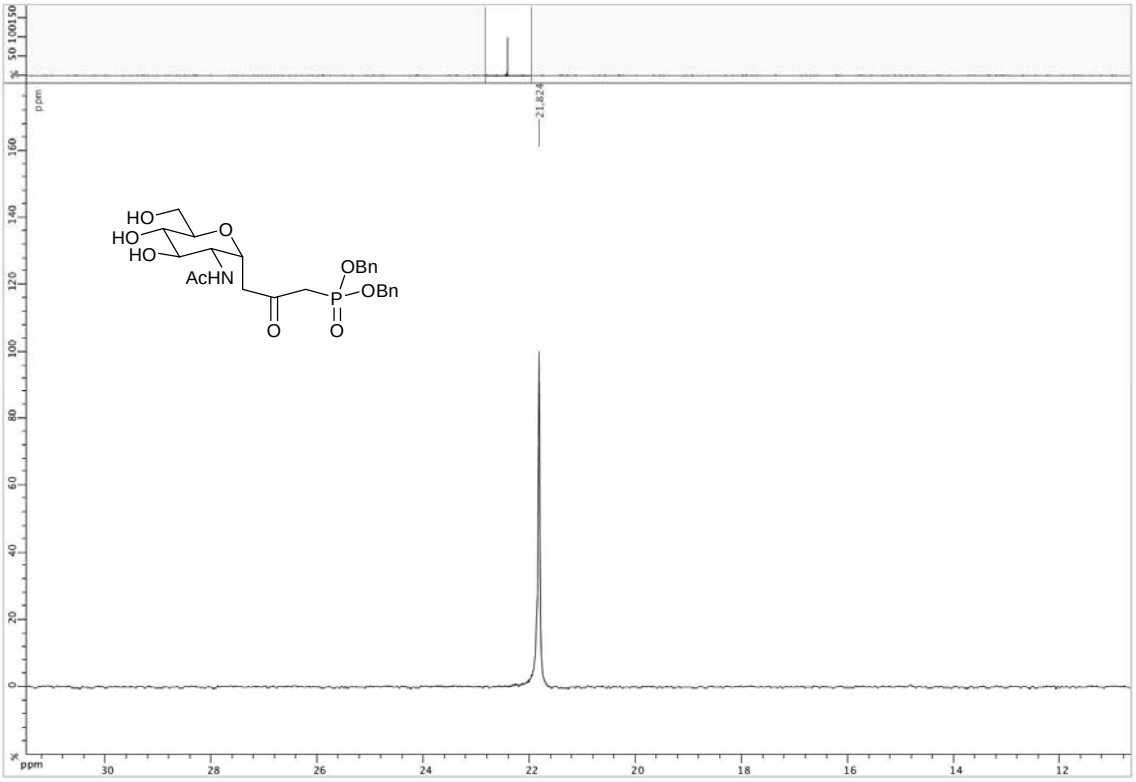




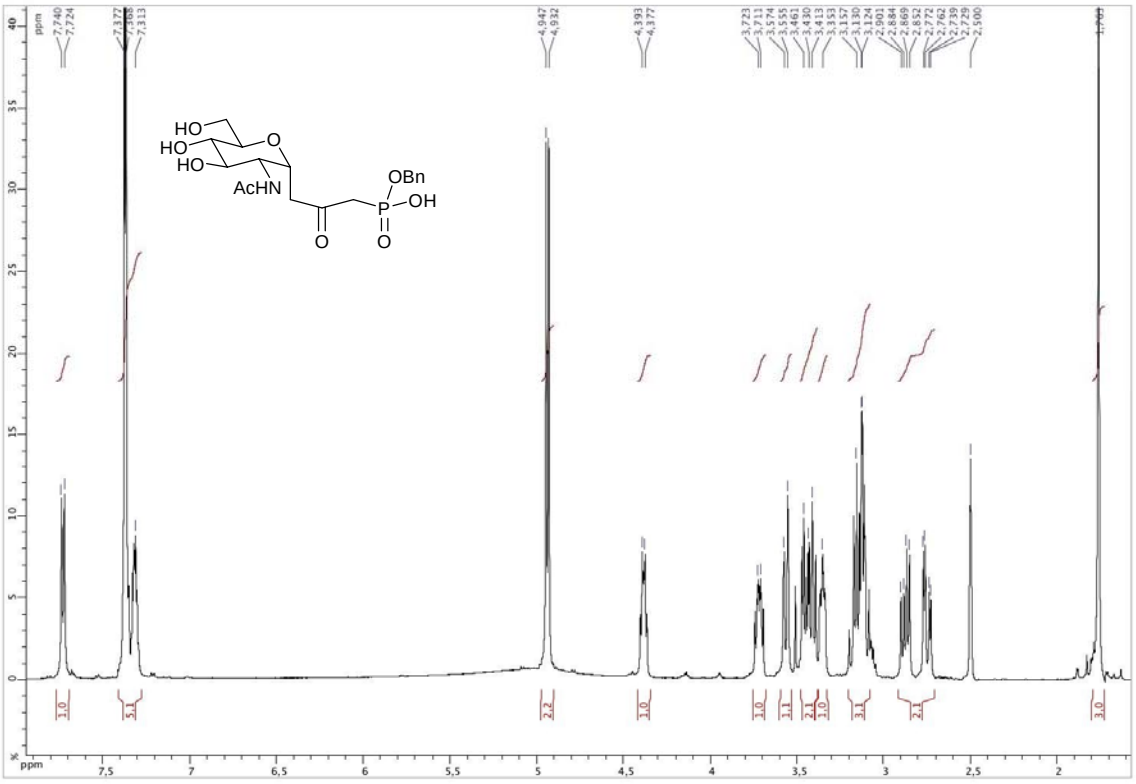
Compound **13** (^{1}H, ^{13}C, ^{31}P)

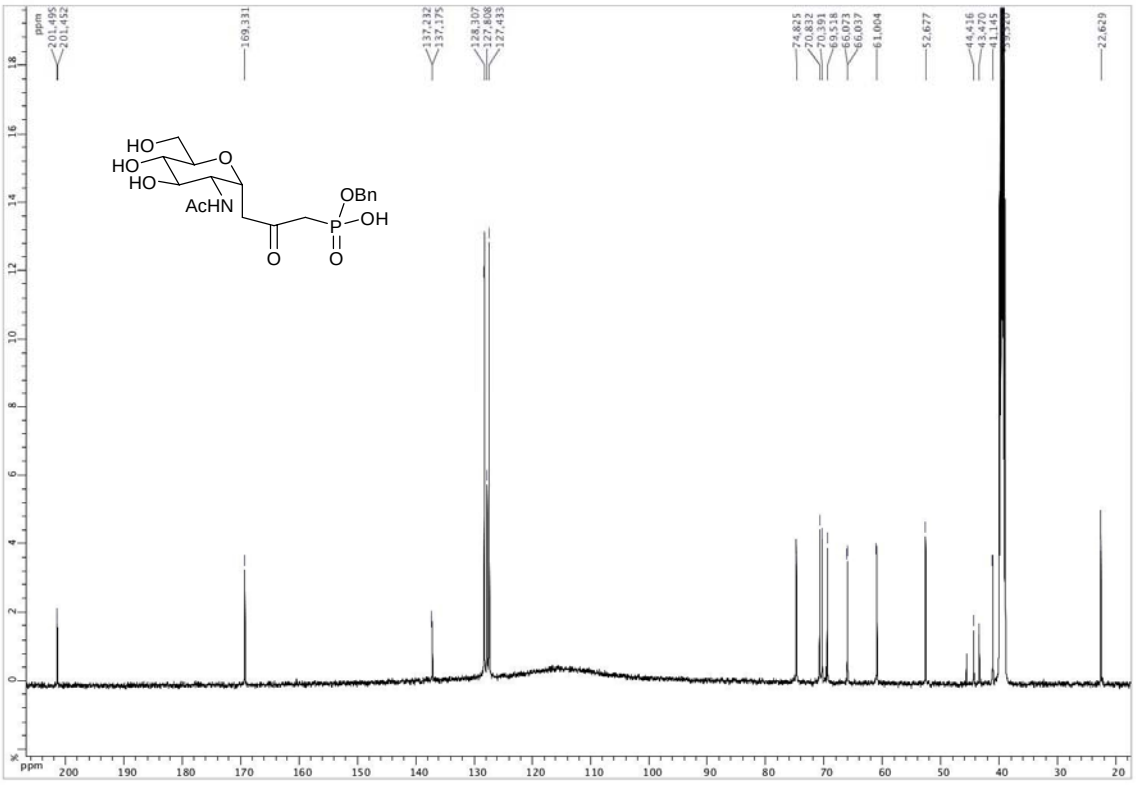


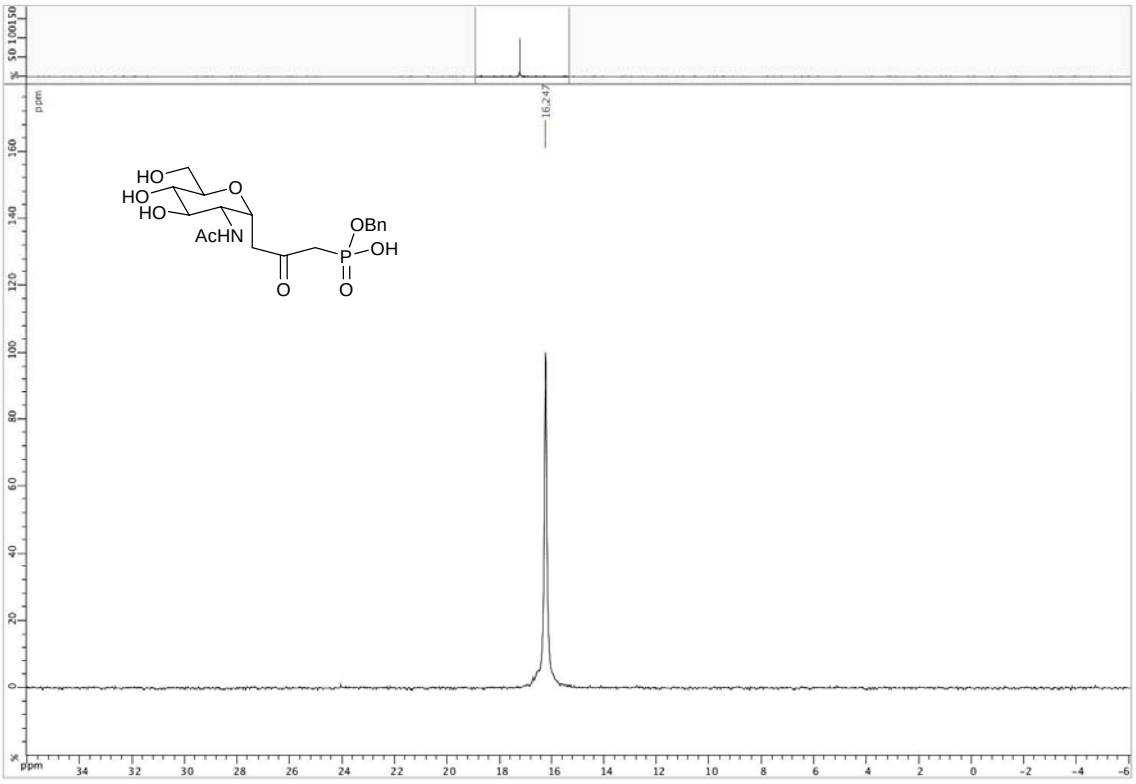




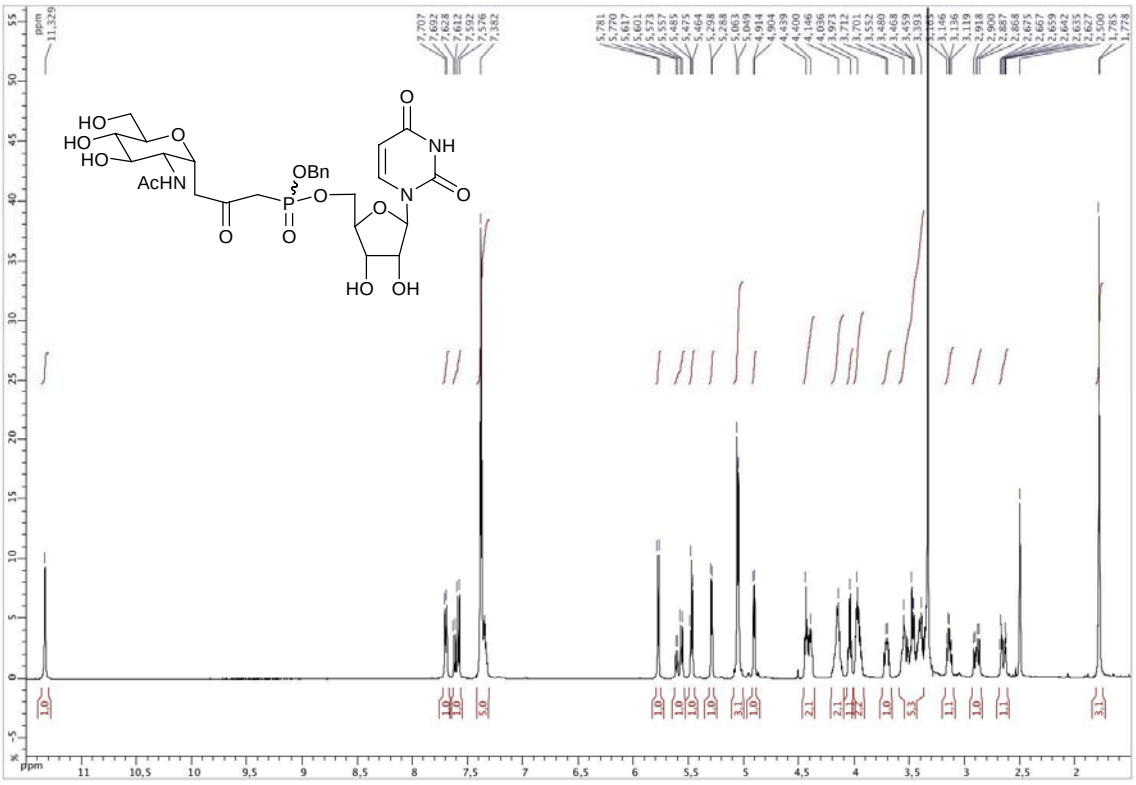
Compound **14** (^{1}H, ^{13}C, ^{31}P)

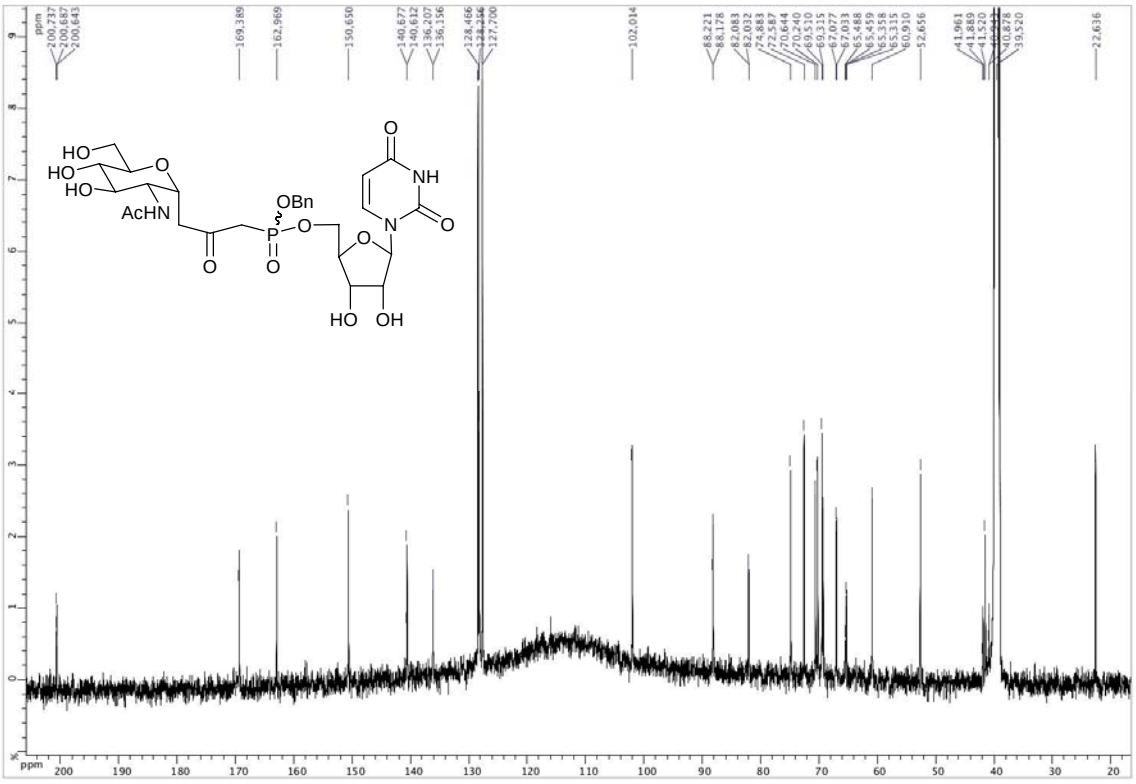


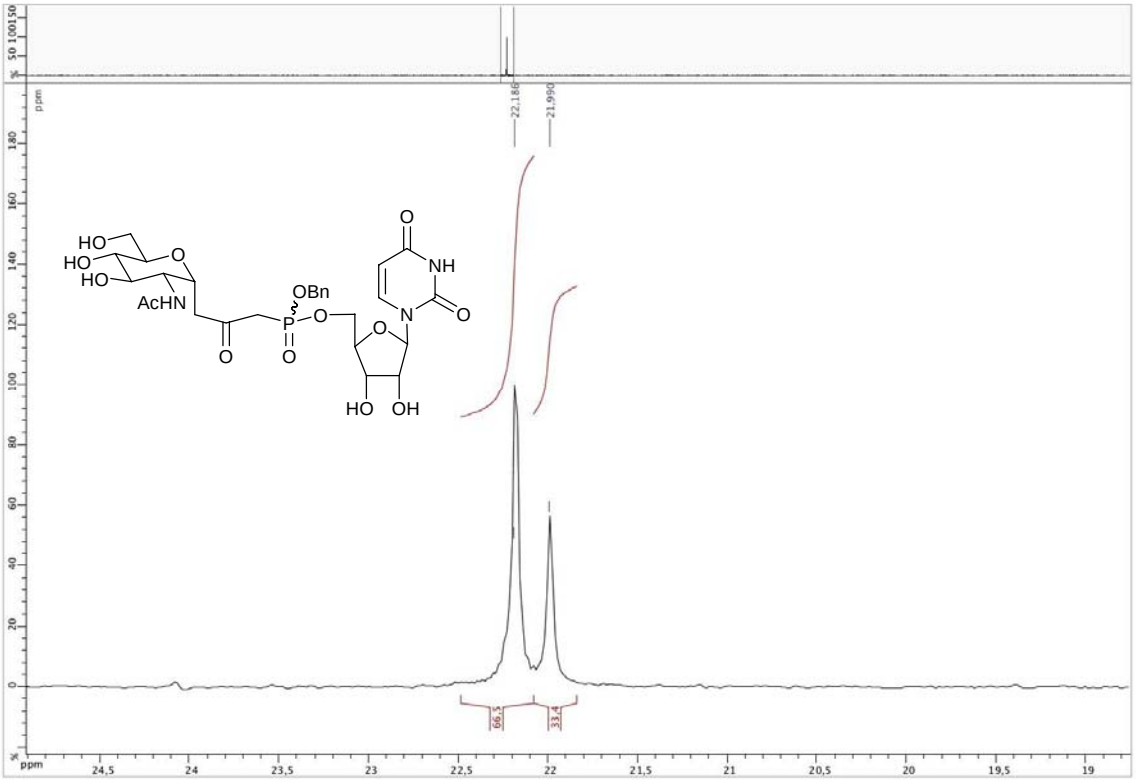




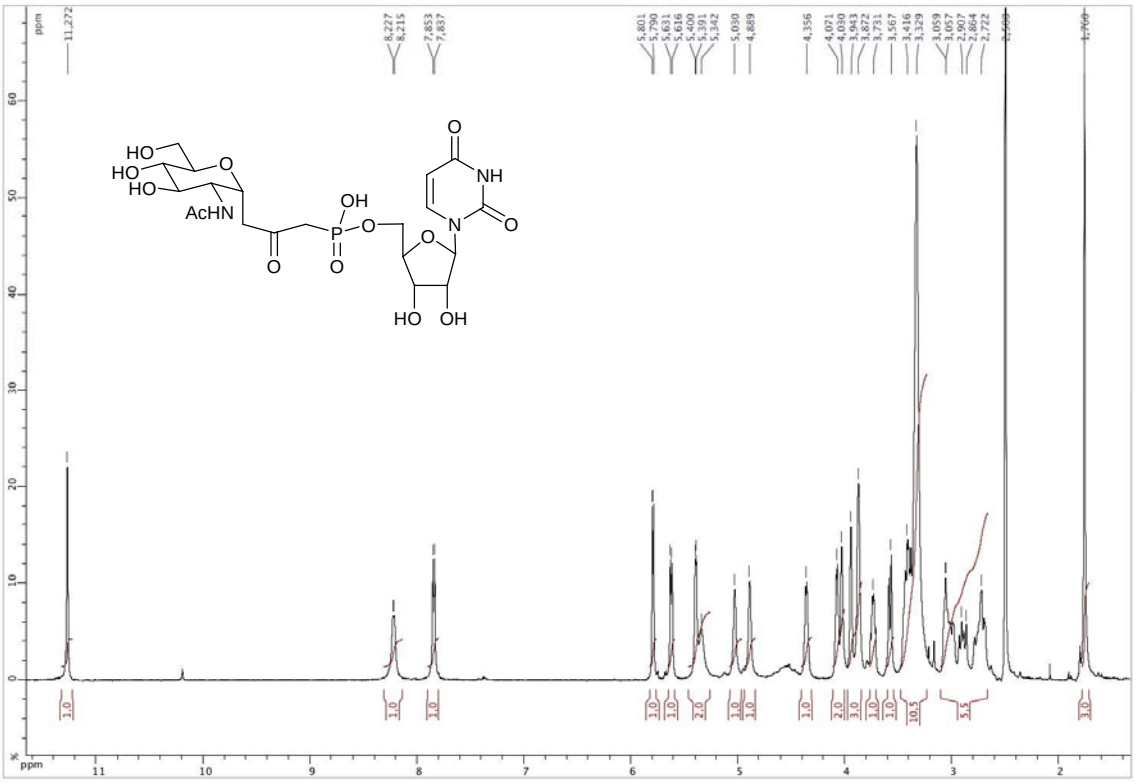
Compound **15** (^{1}H, ^{13}C, ^{31}P)

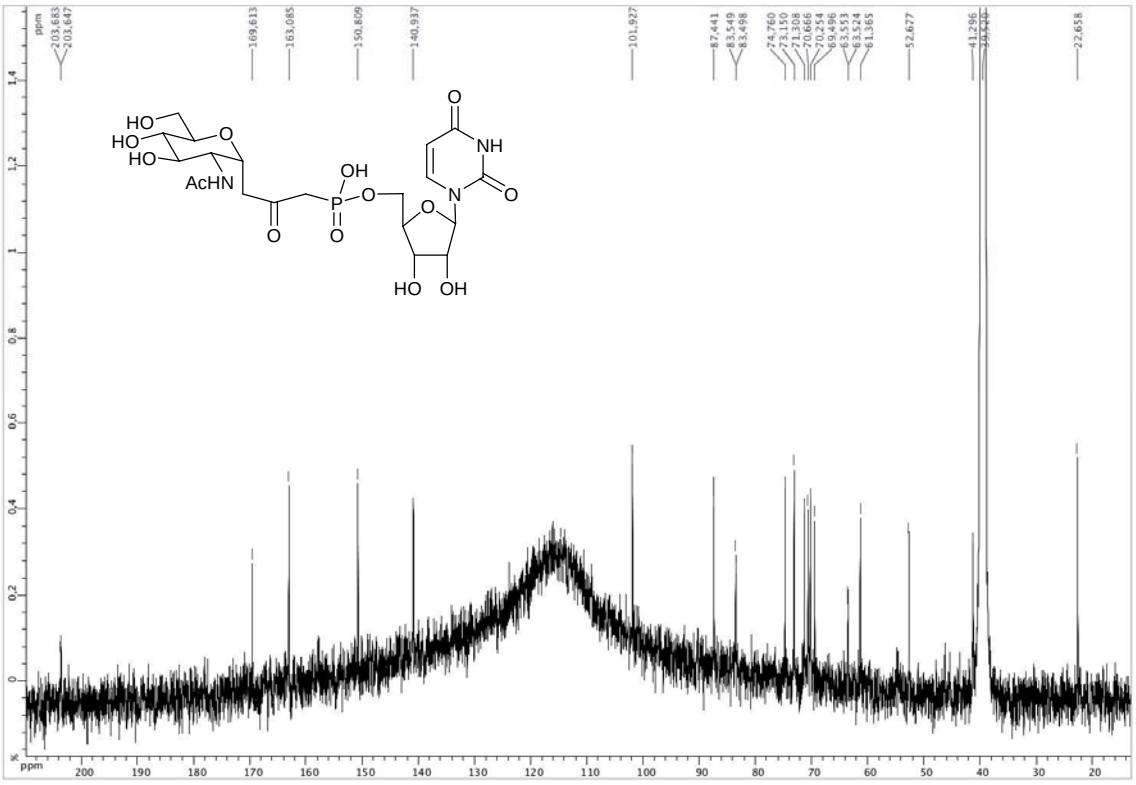


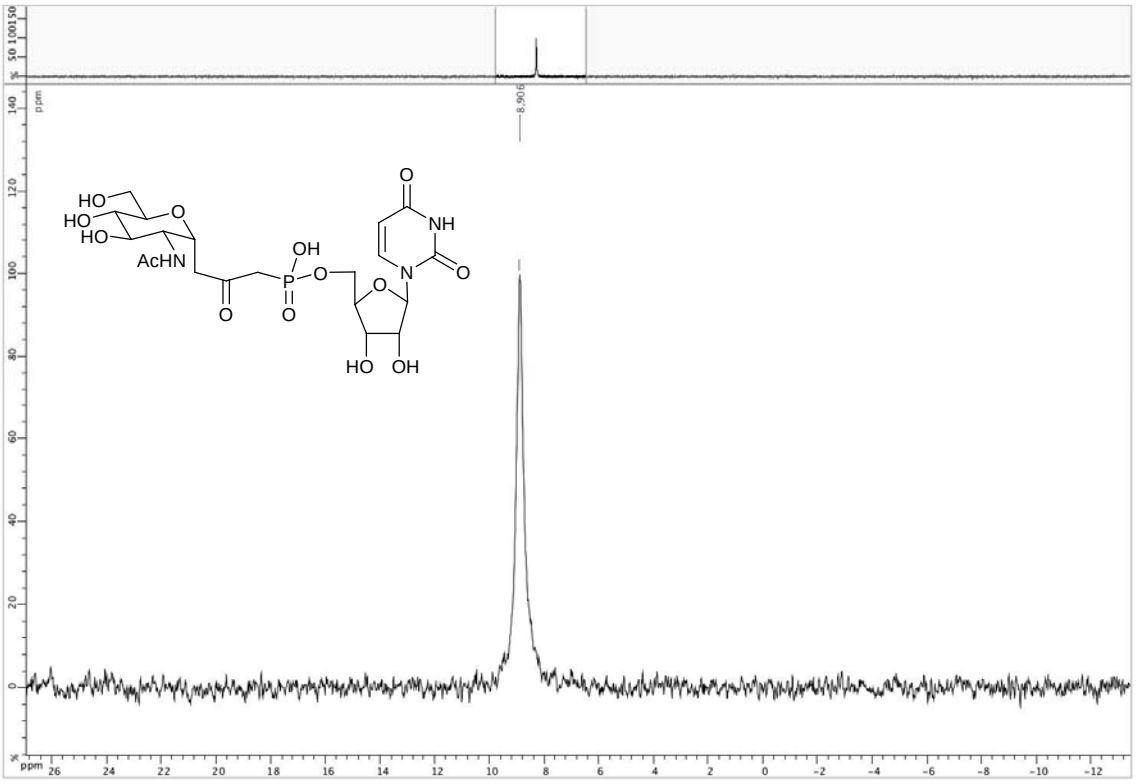




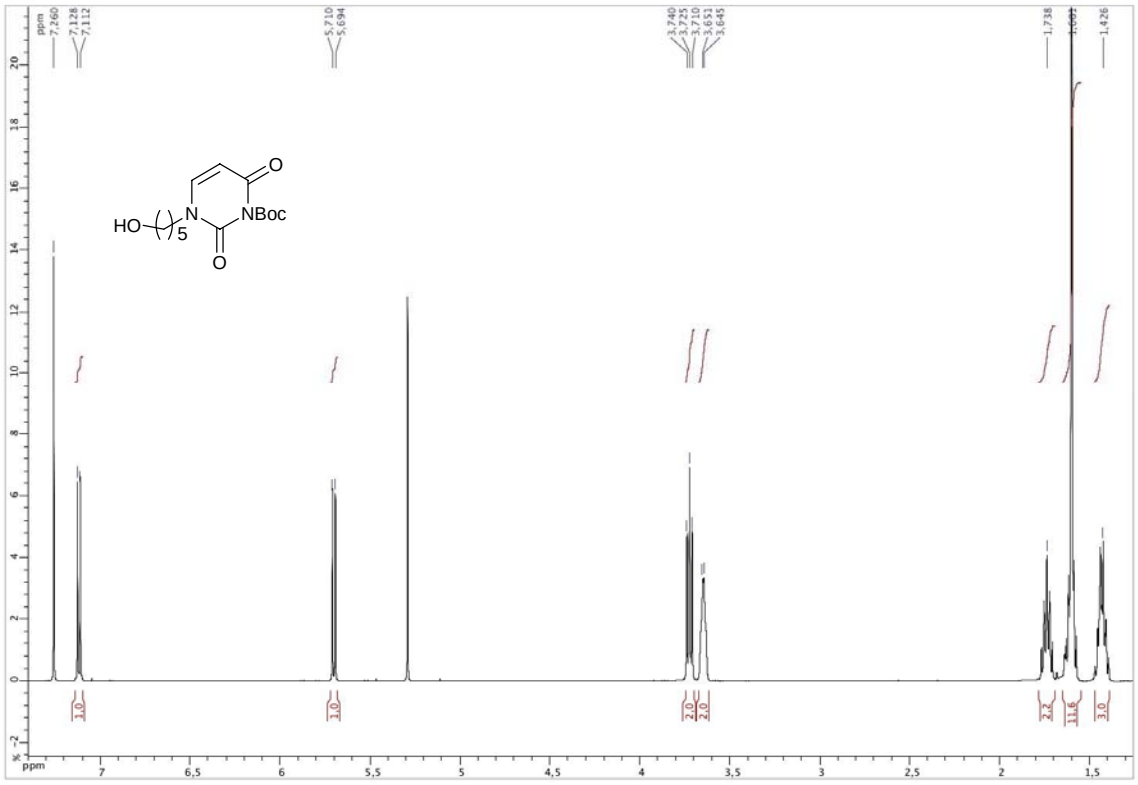
Compound **16** (^{1}H, ^{13}C, ^{31}P)

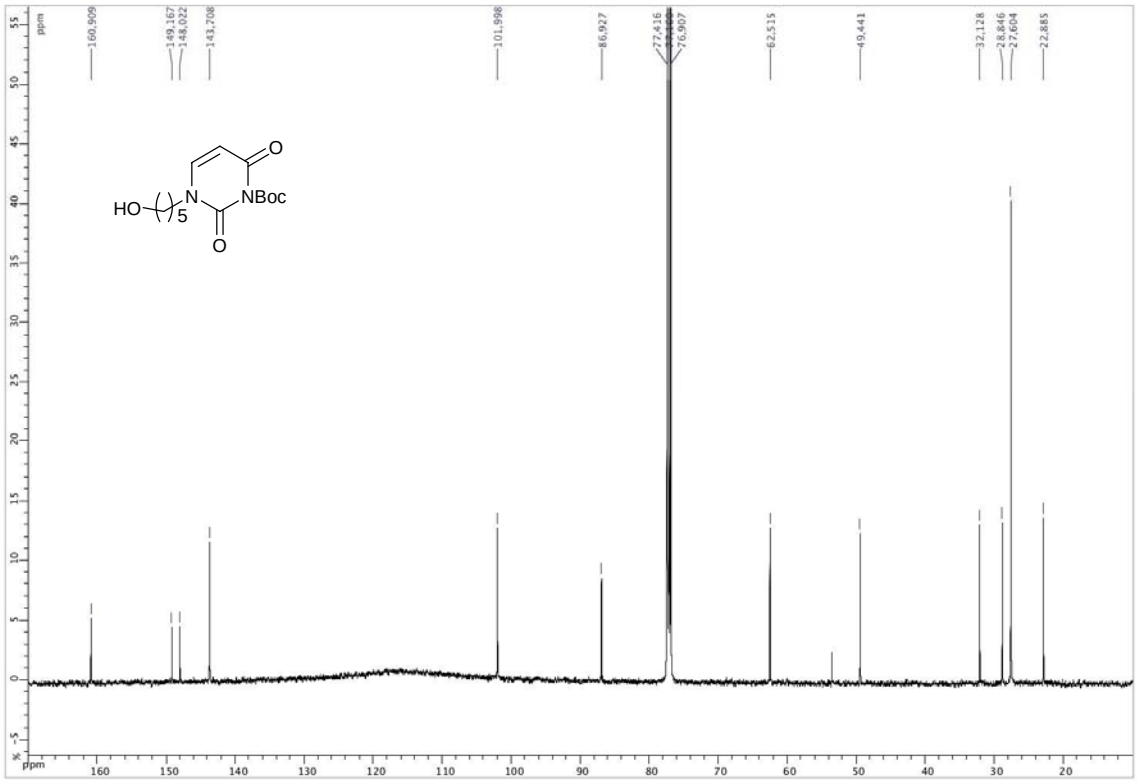




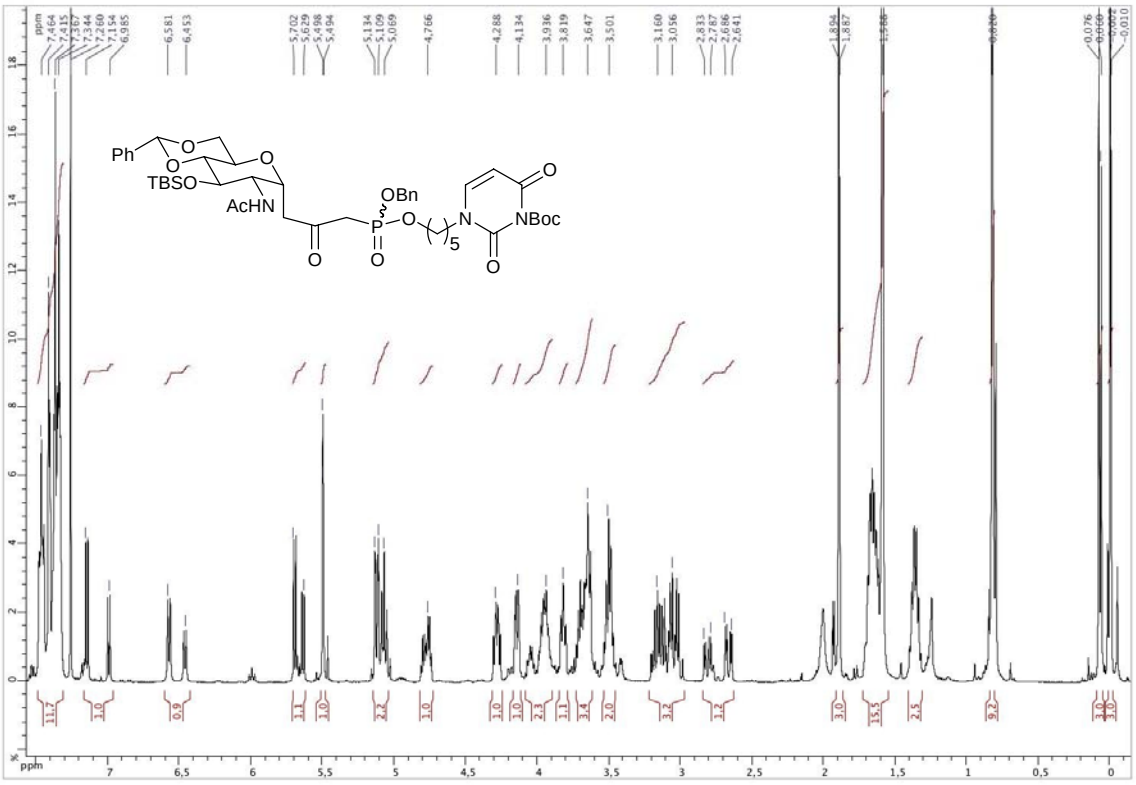


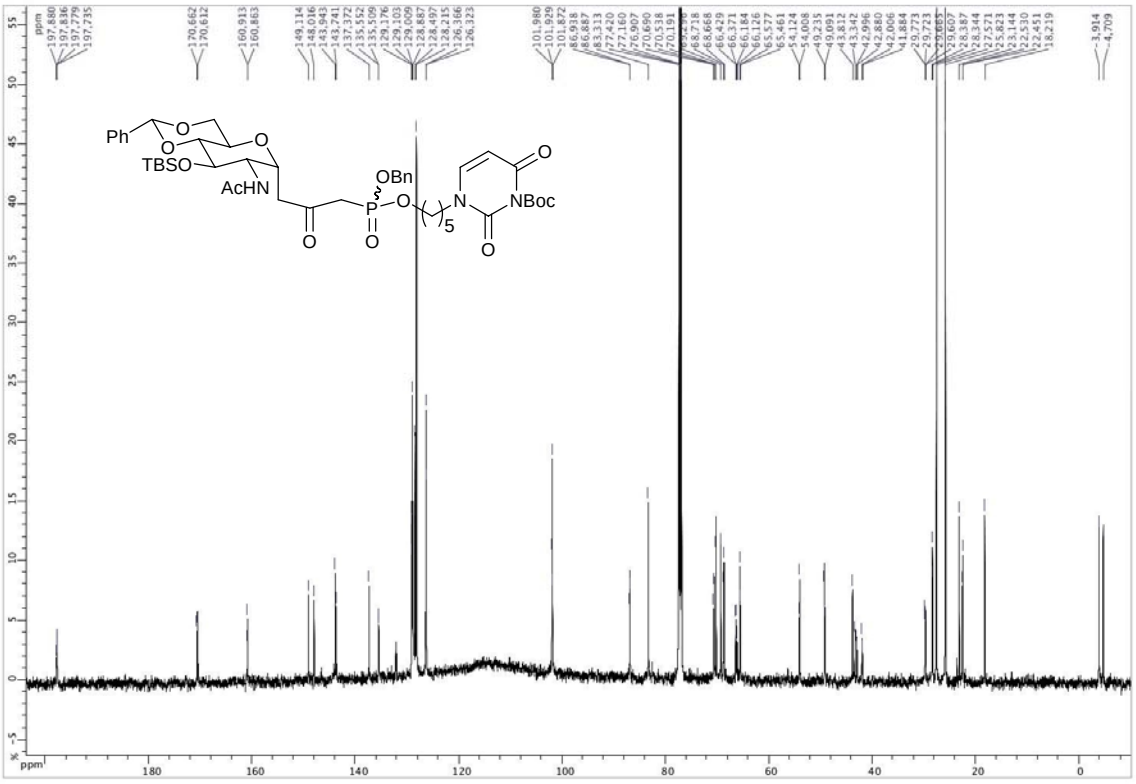
Compound **17** (^{1}H, ^{13}C)

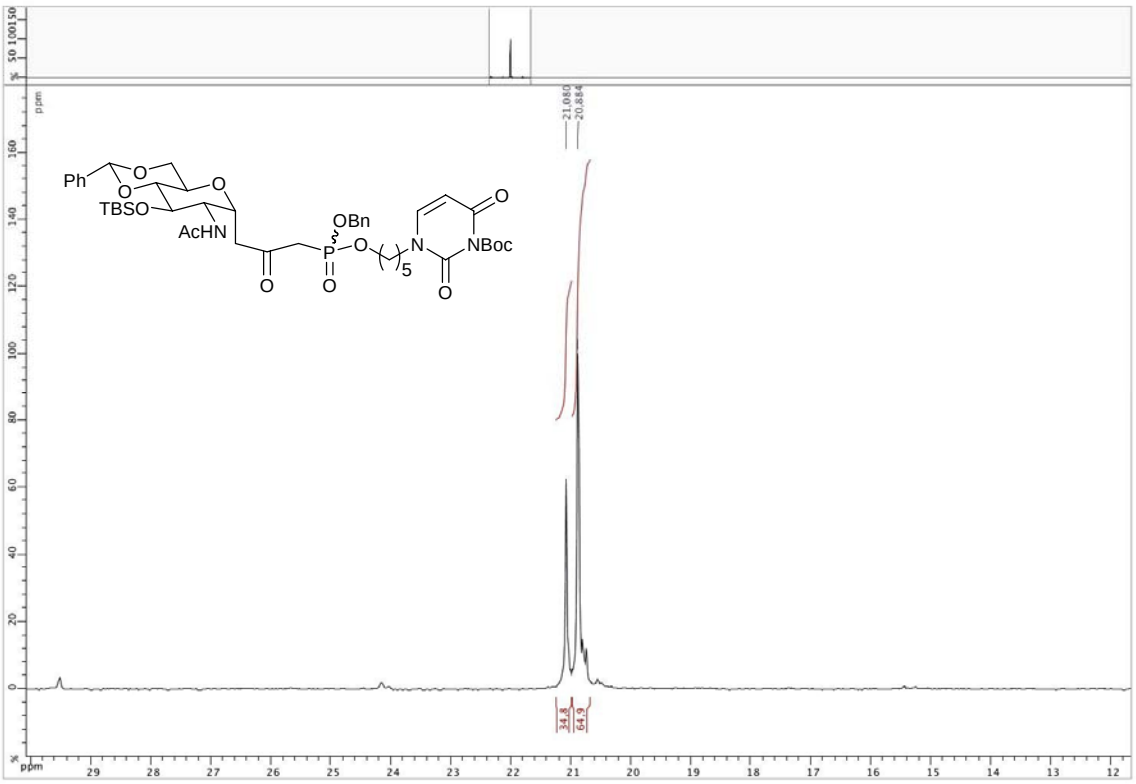




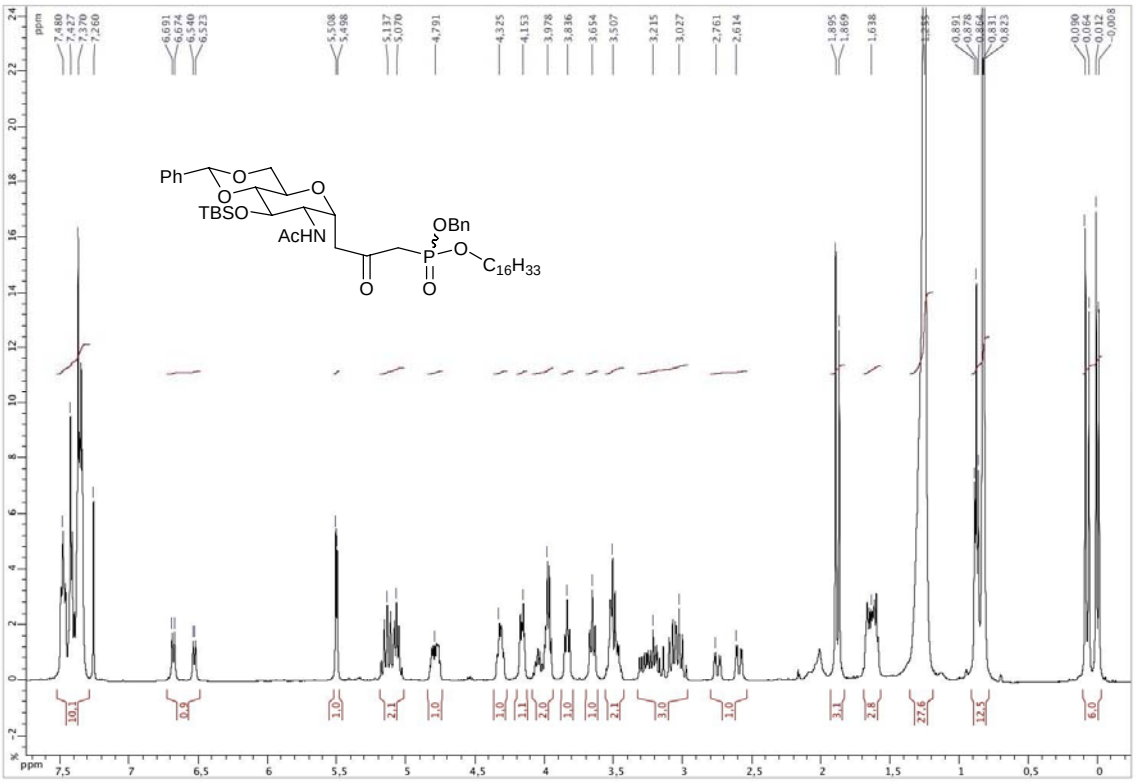
Compound **18** (^{1}H, ^{13}C, ^{31}P)

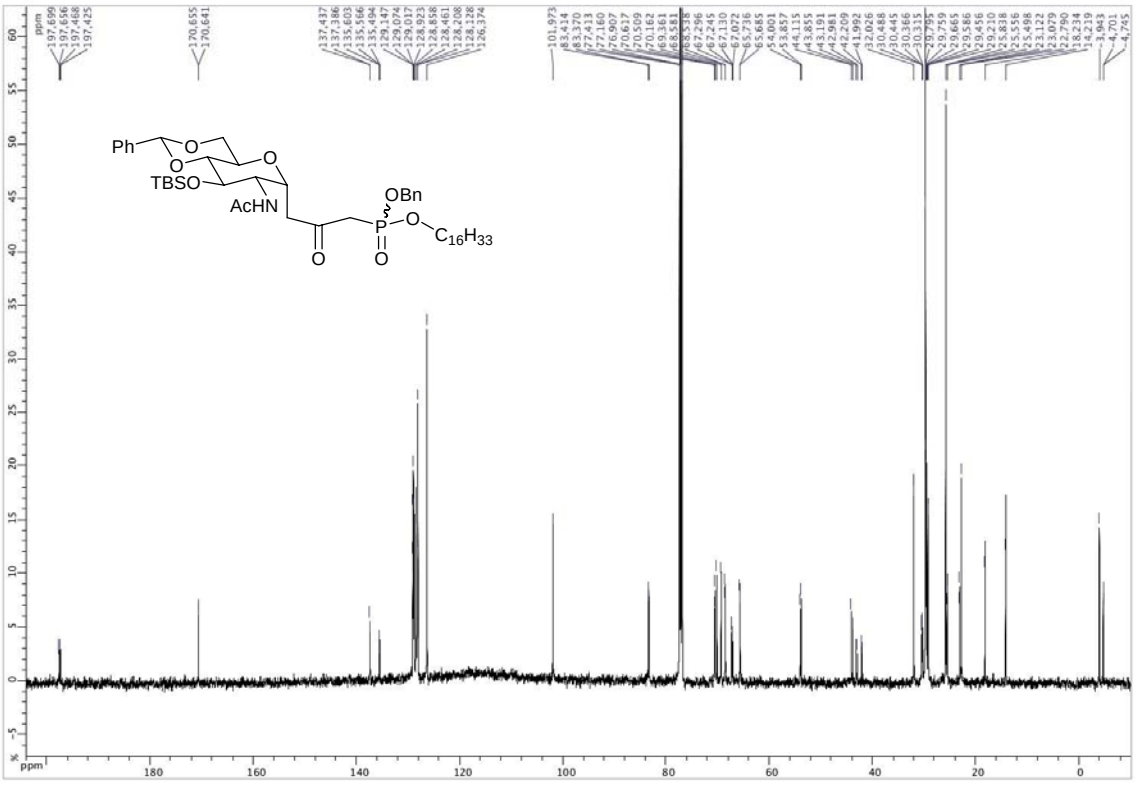


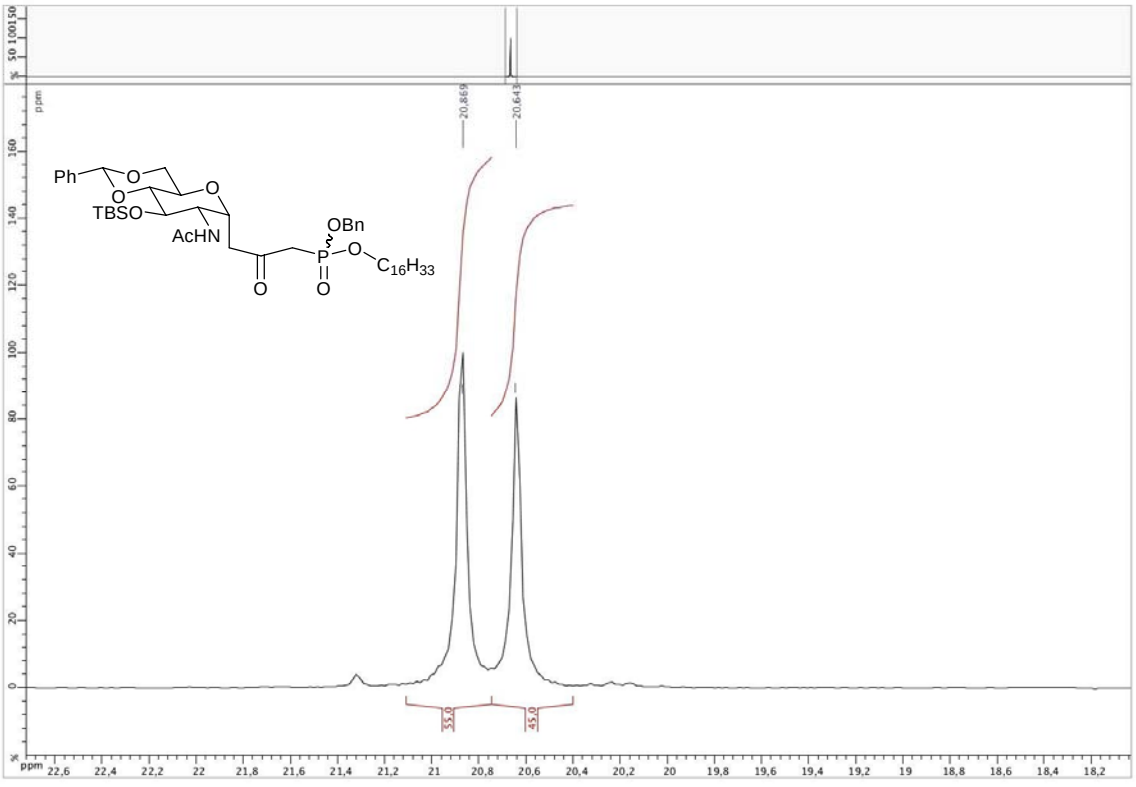




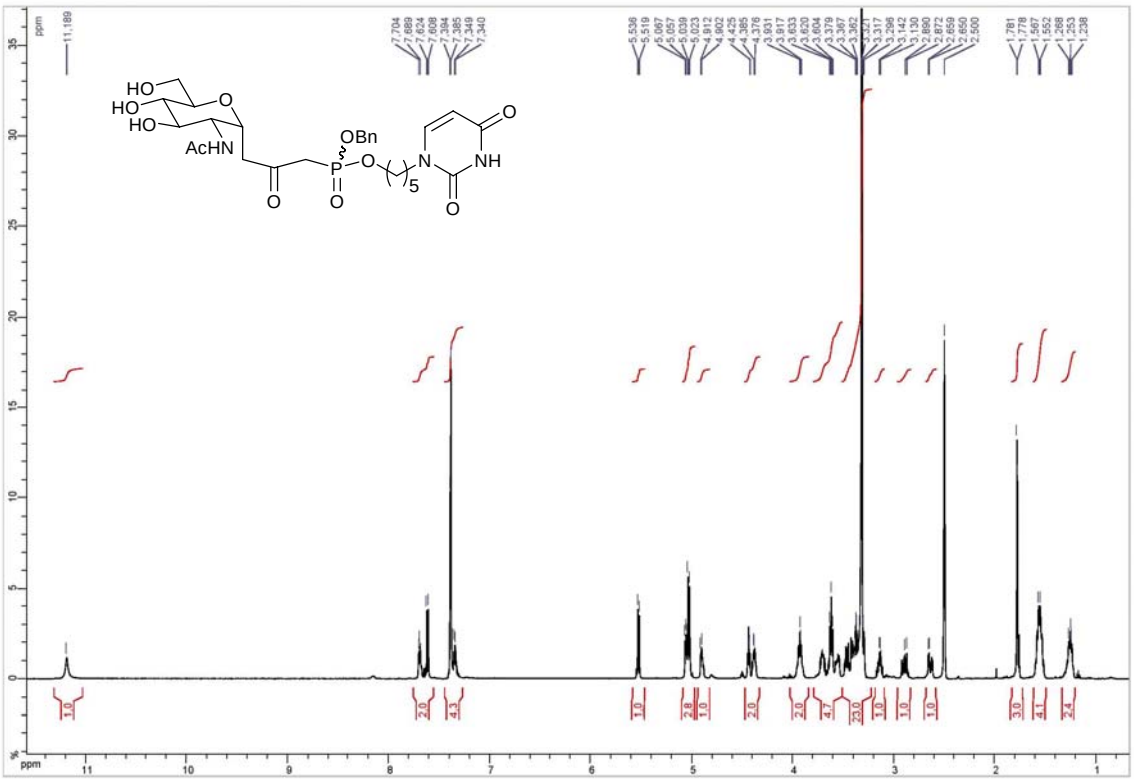
Compound **19** (^{1}H, ^{13}C, ^{31}P)

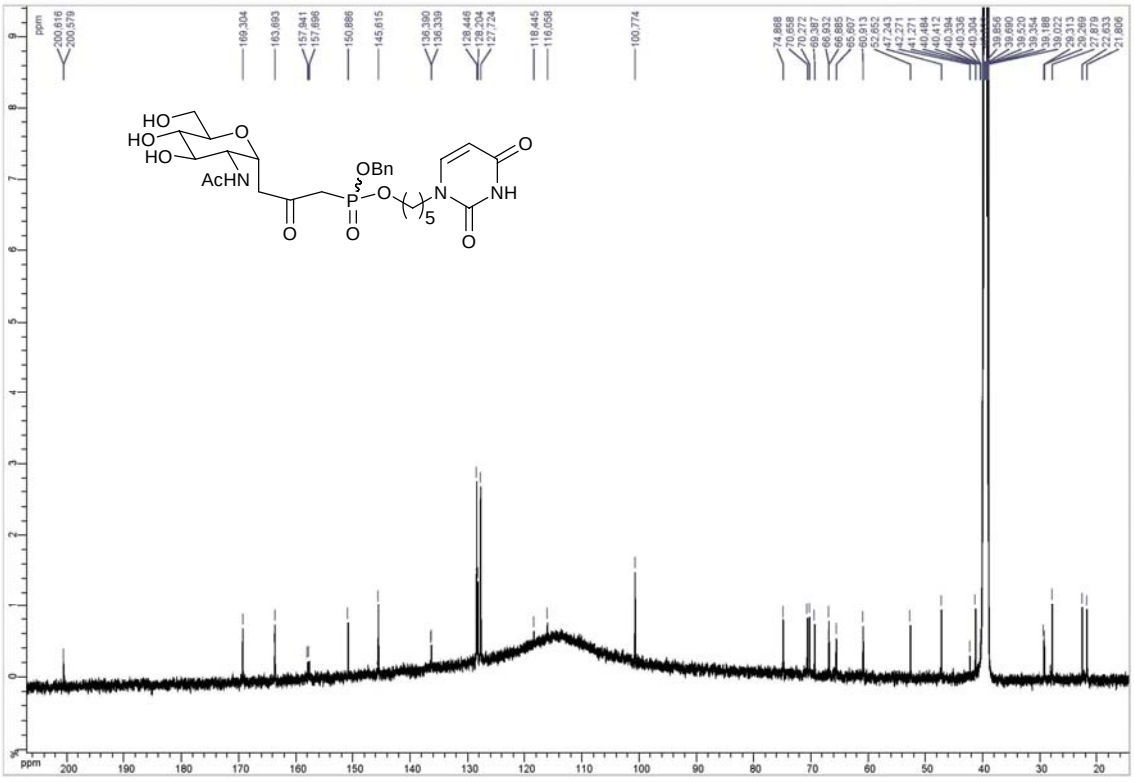


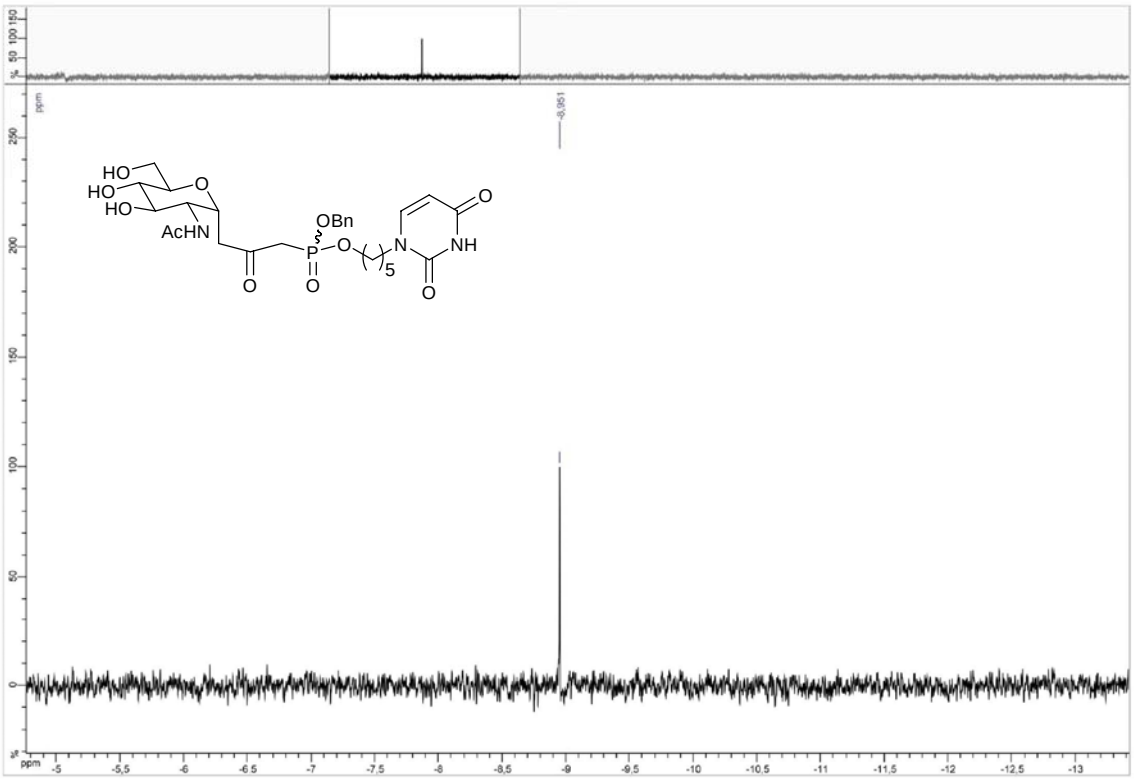




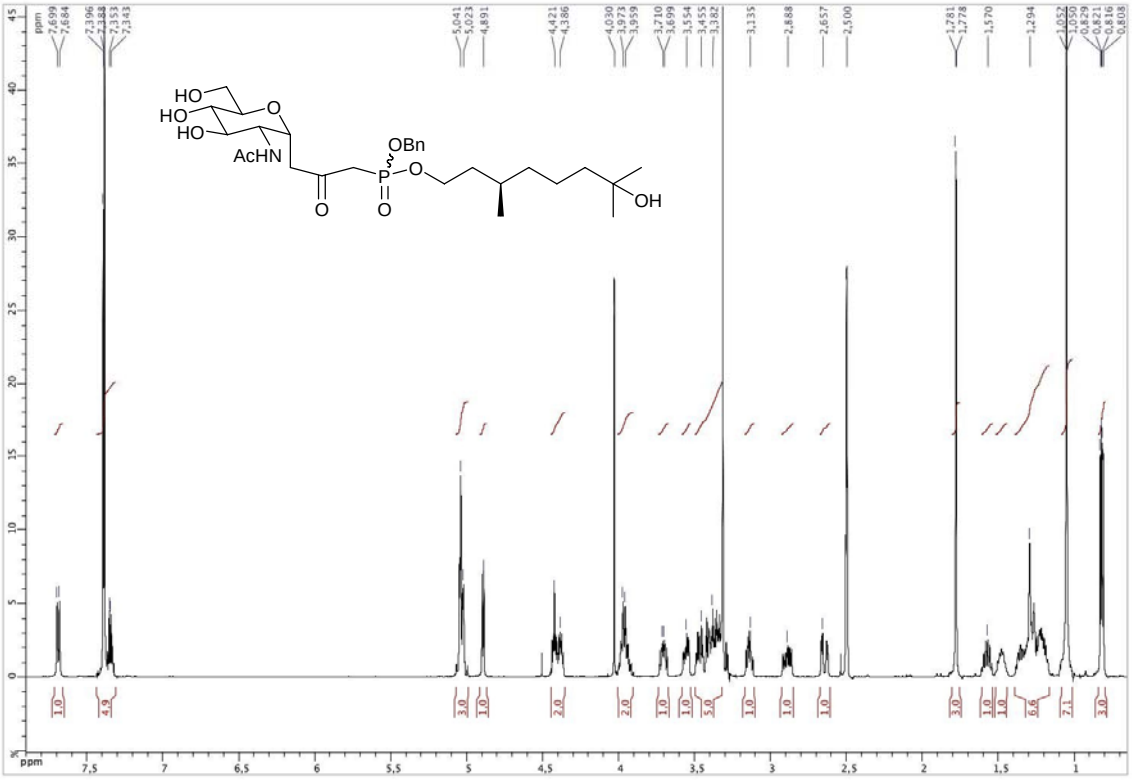
Compound **20** (^{1}H, ^{13}C, ^{31}P)

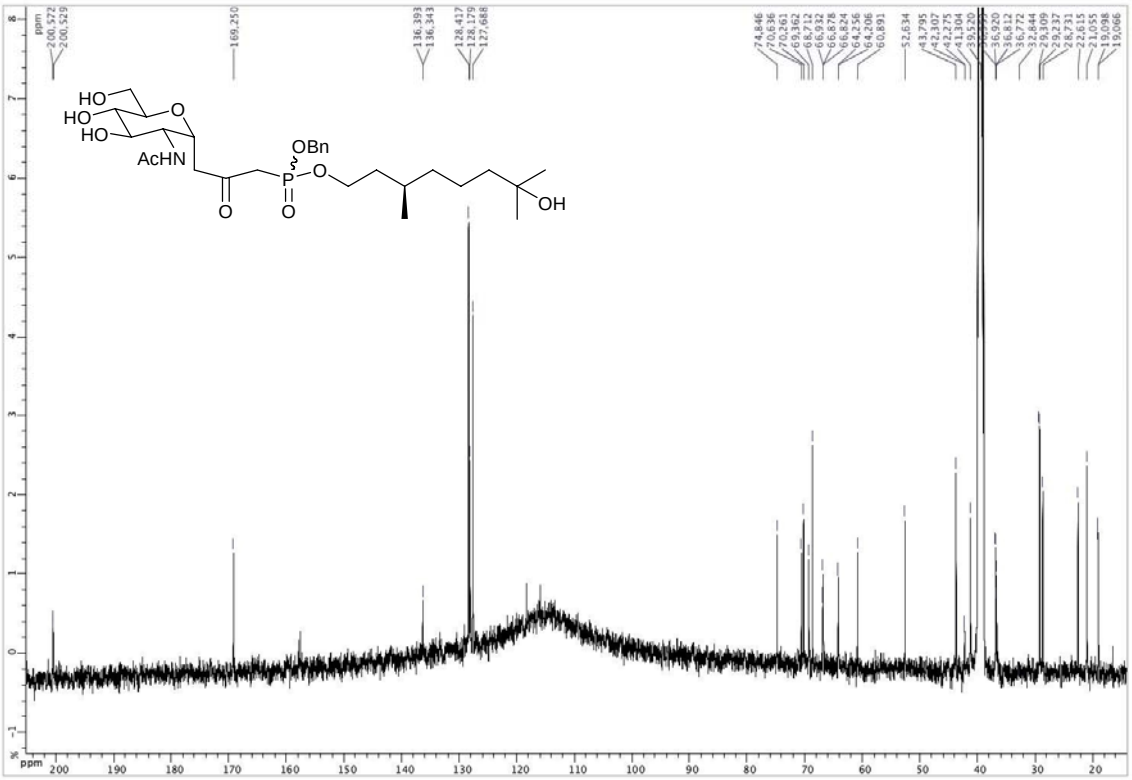


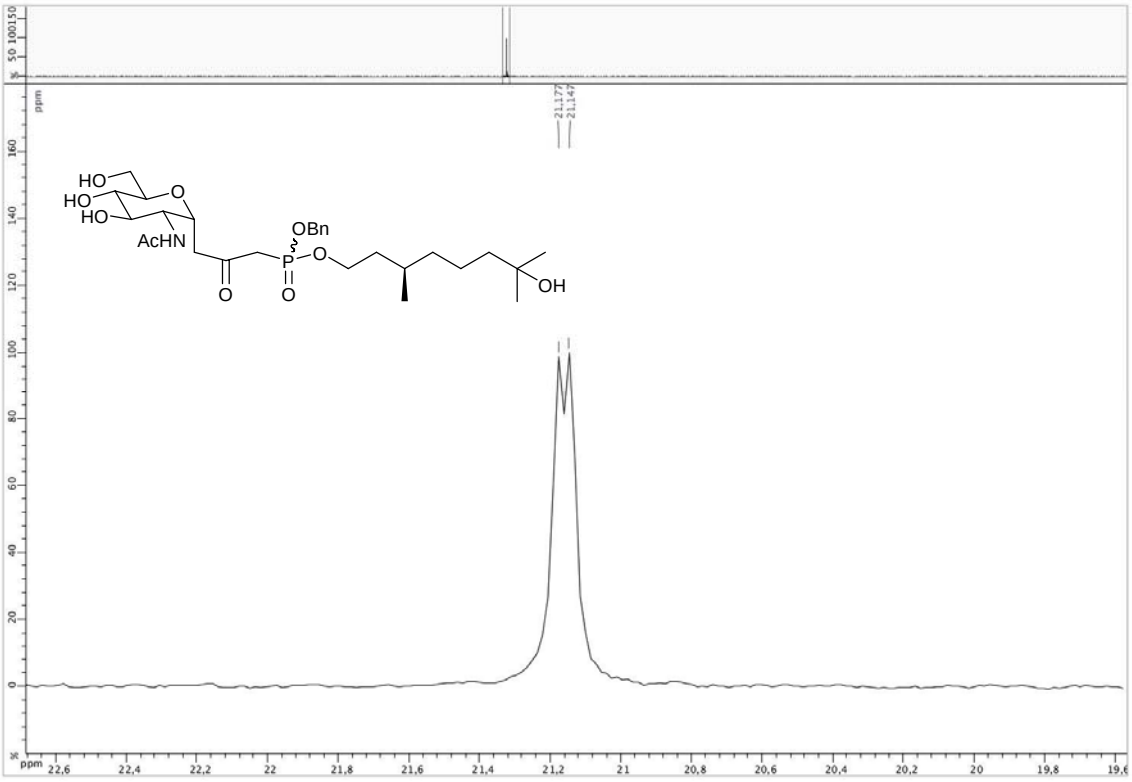




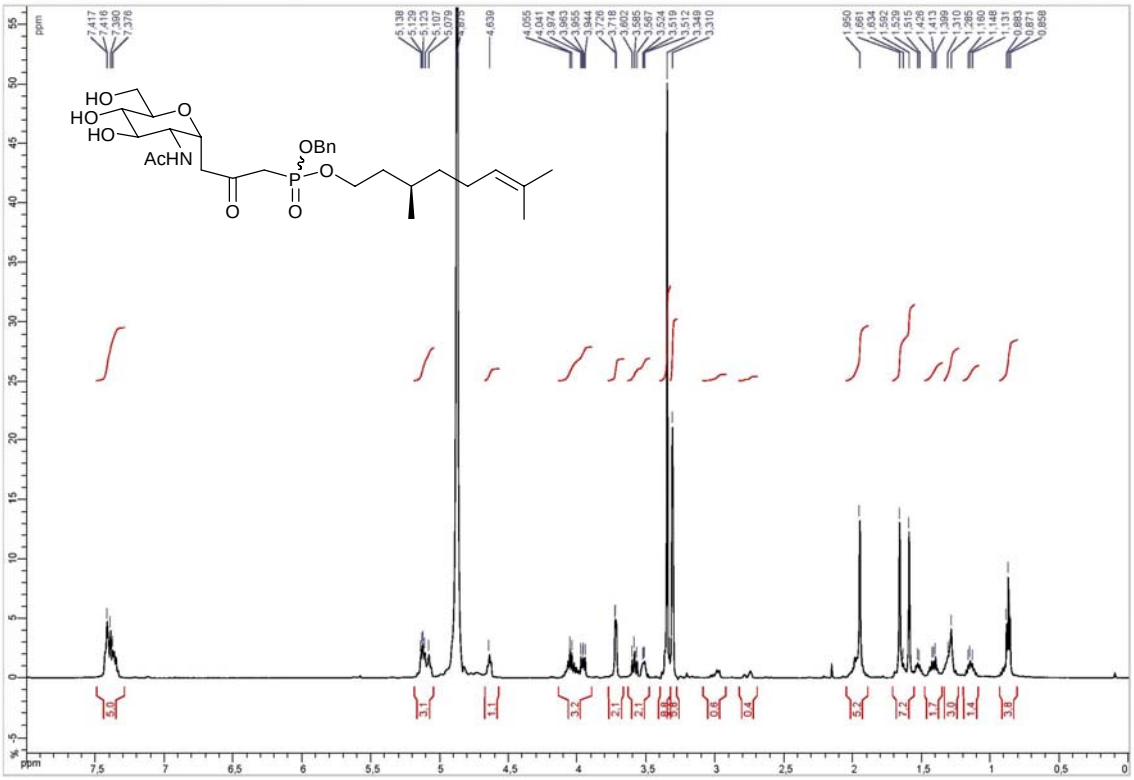
Compound **21** (^{1}H, ^{13}C, ^{31}P)

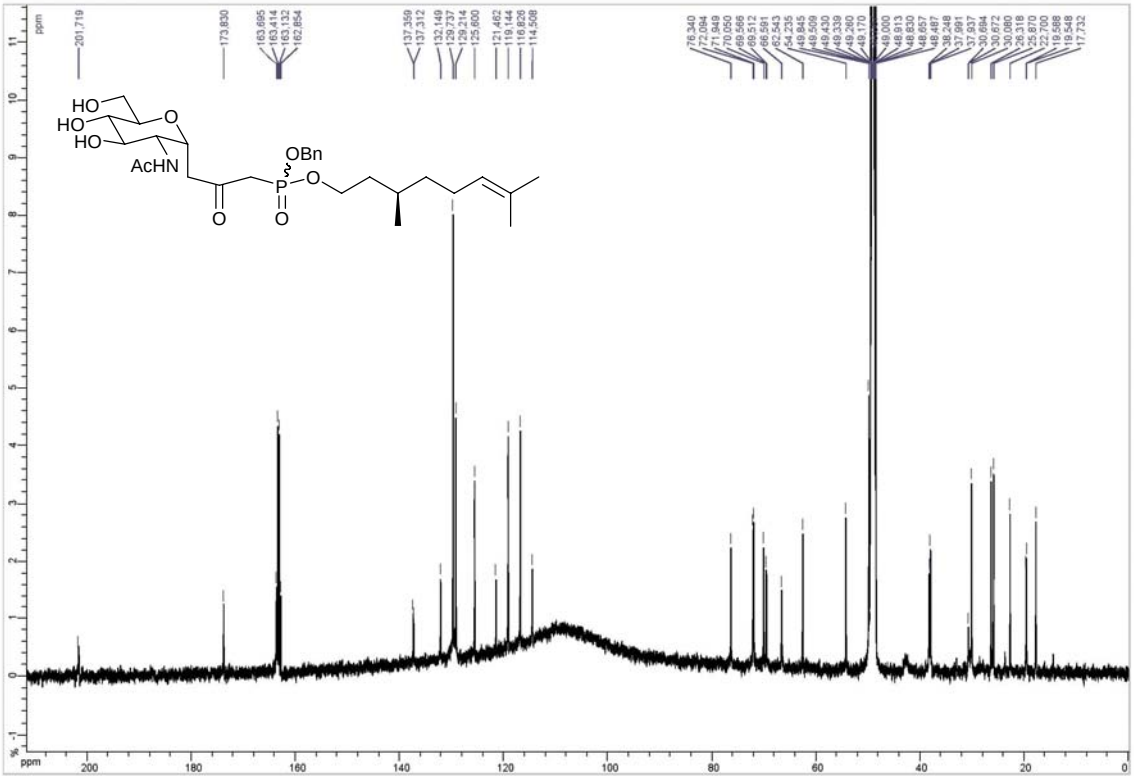


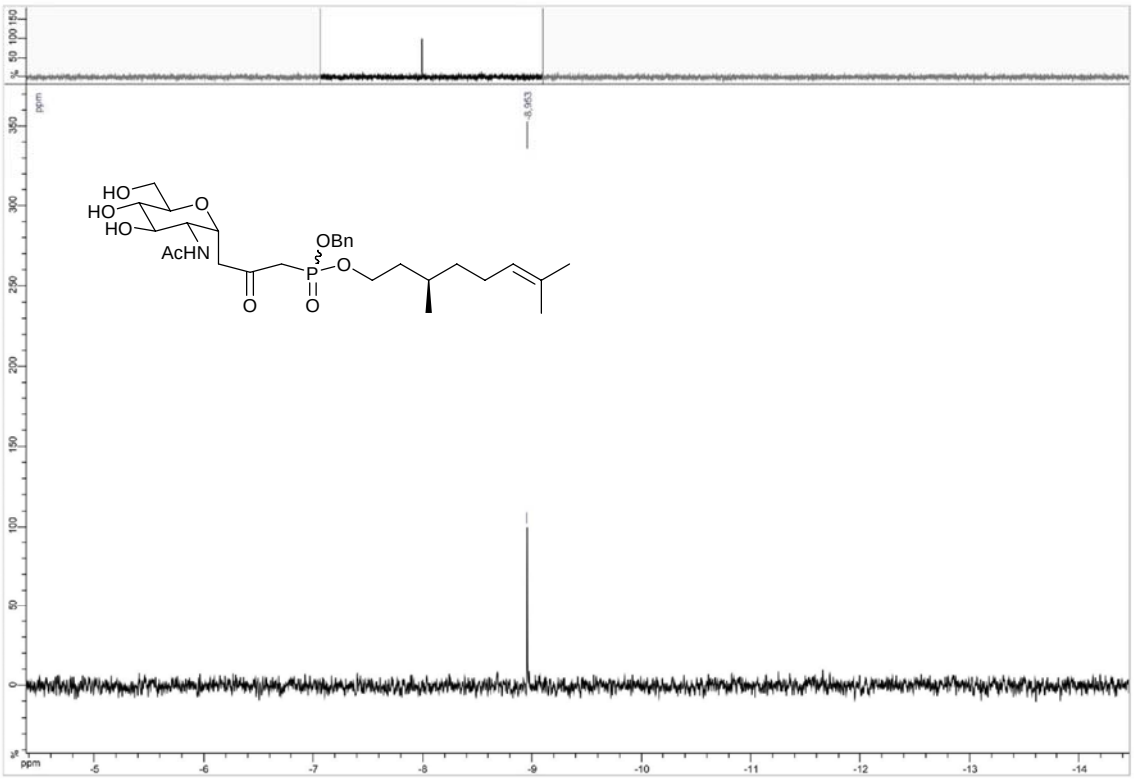




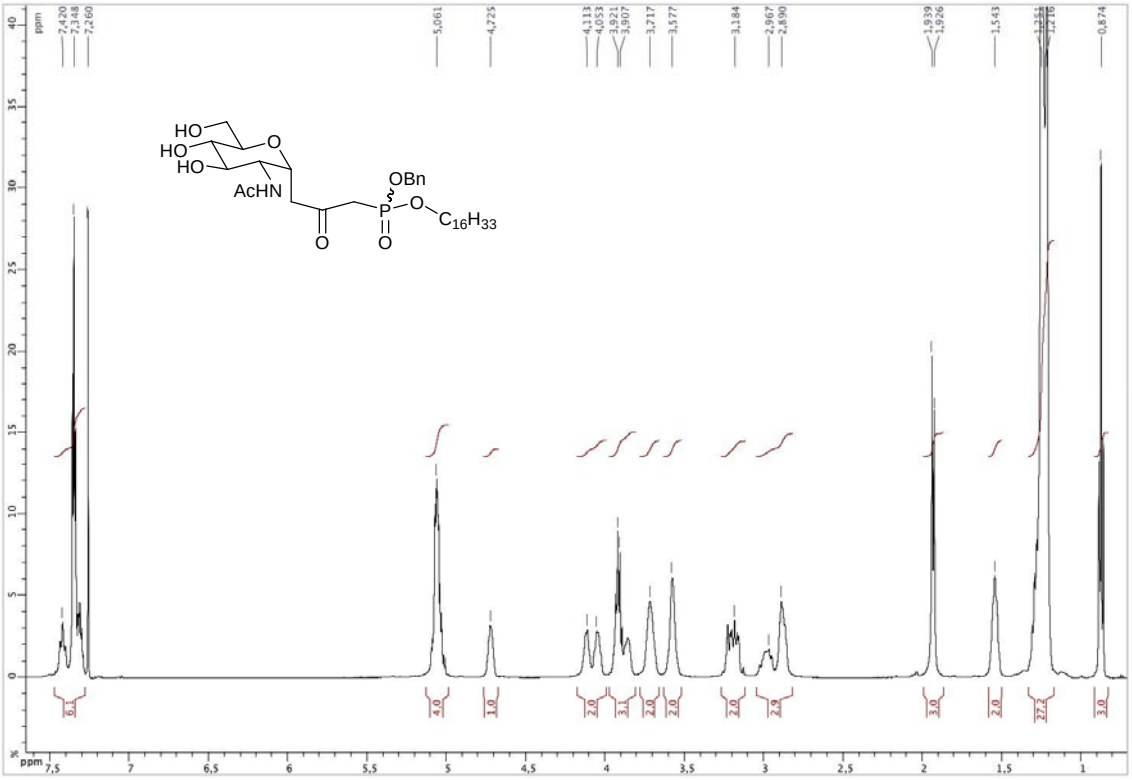
Compound **22** (^{1}H, ^{13}C, ^{31}P)

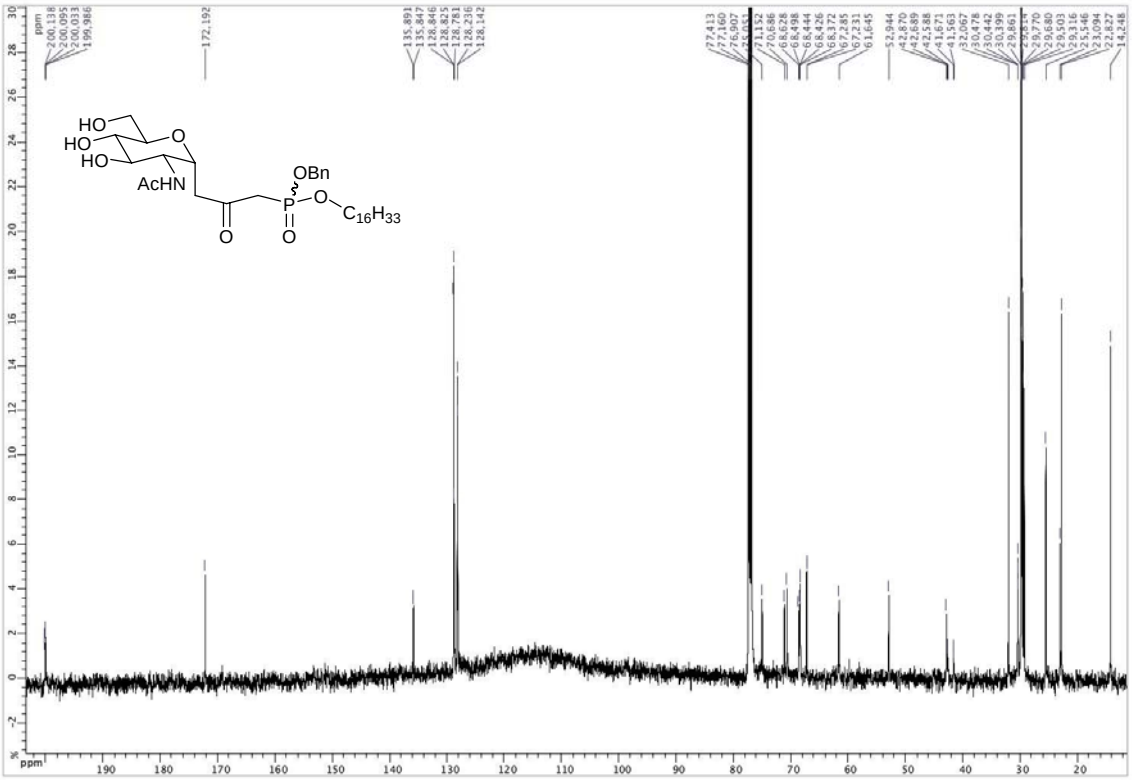


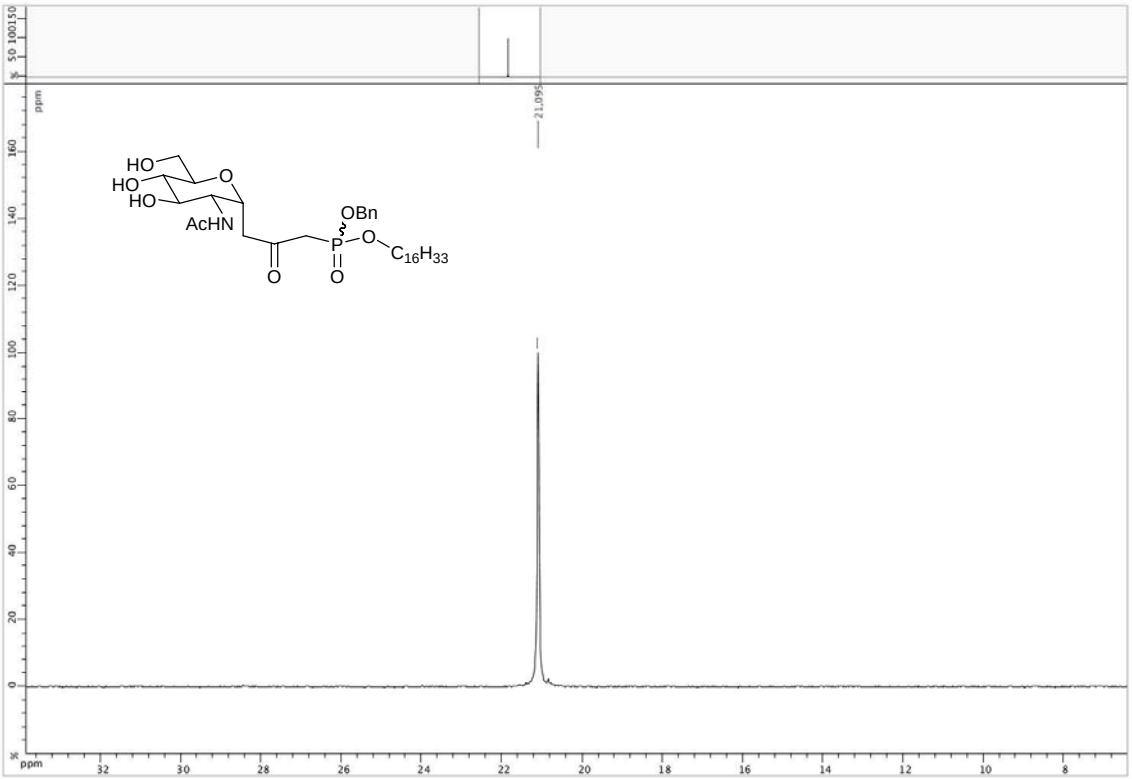




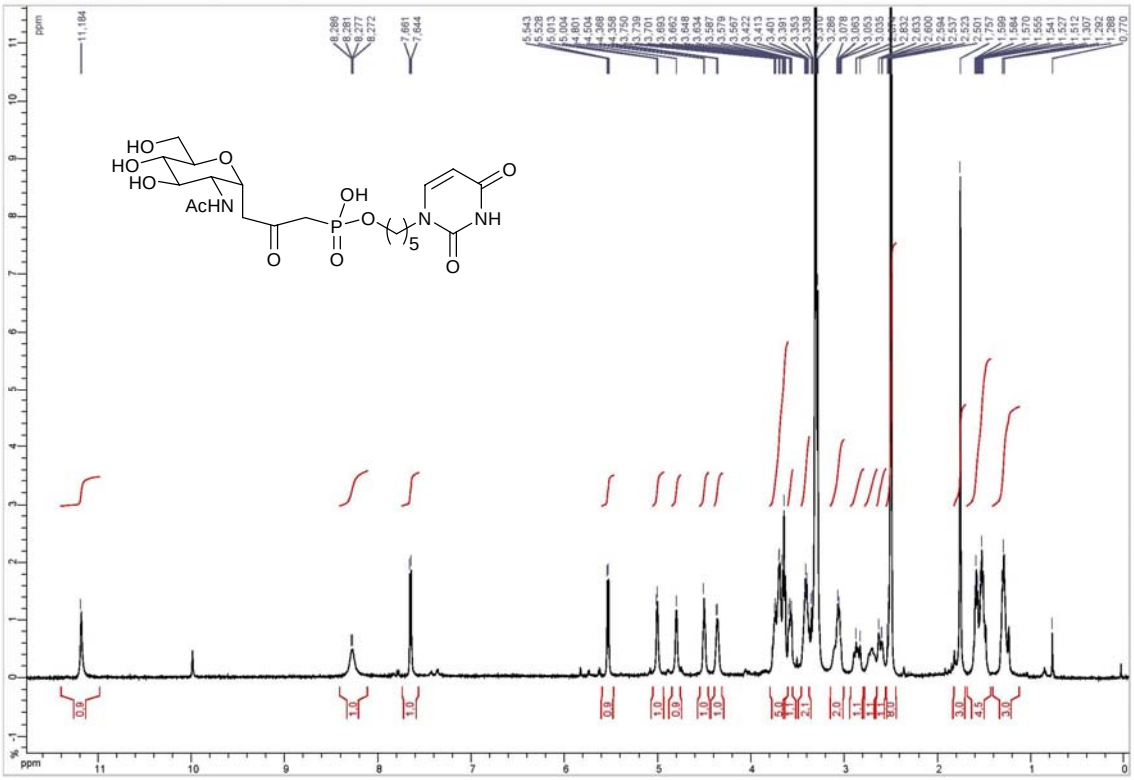
Compound **23** (^{1}H, ^{13}C, ^{31}P)

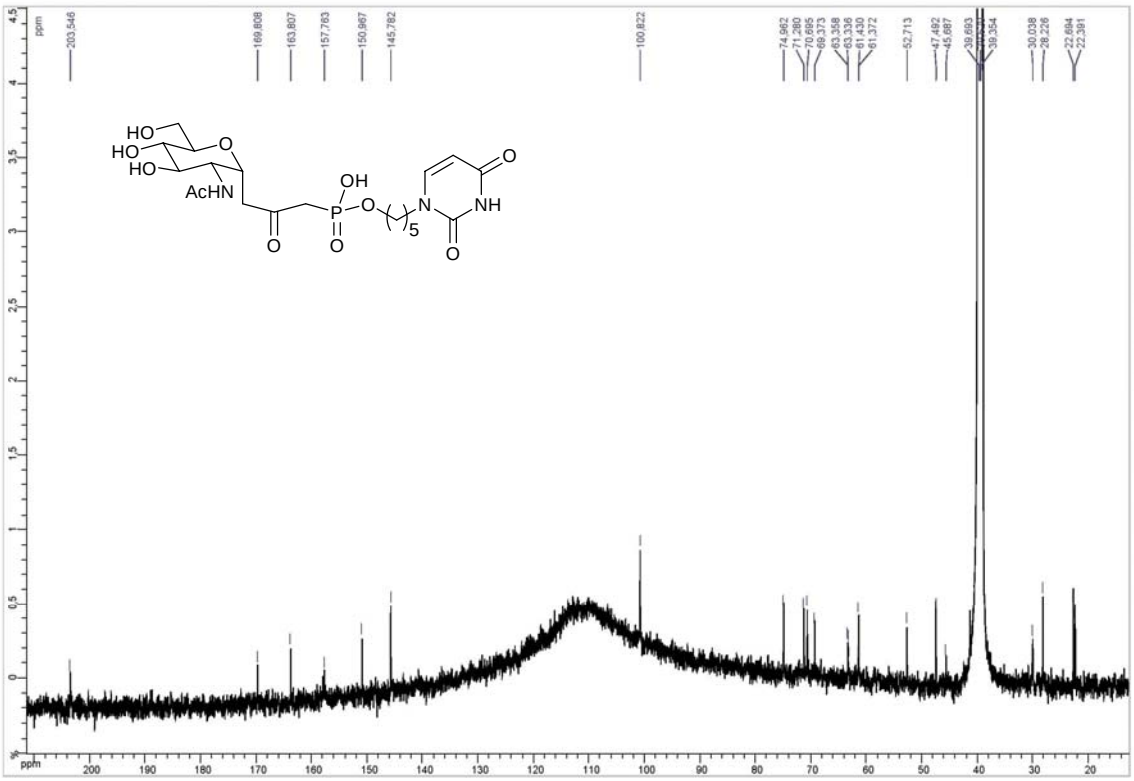


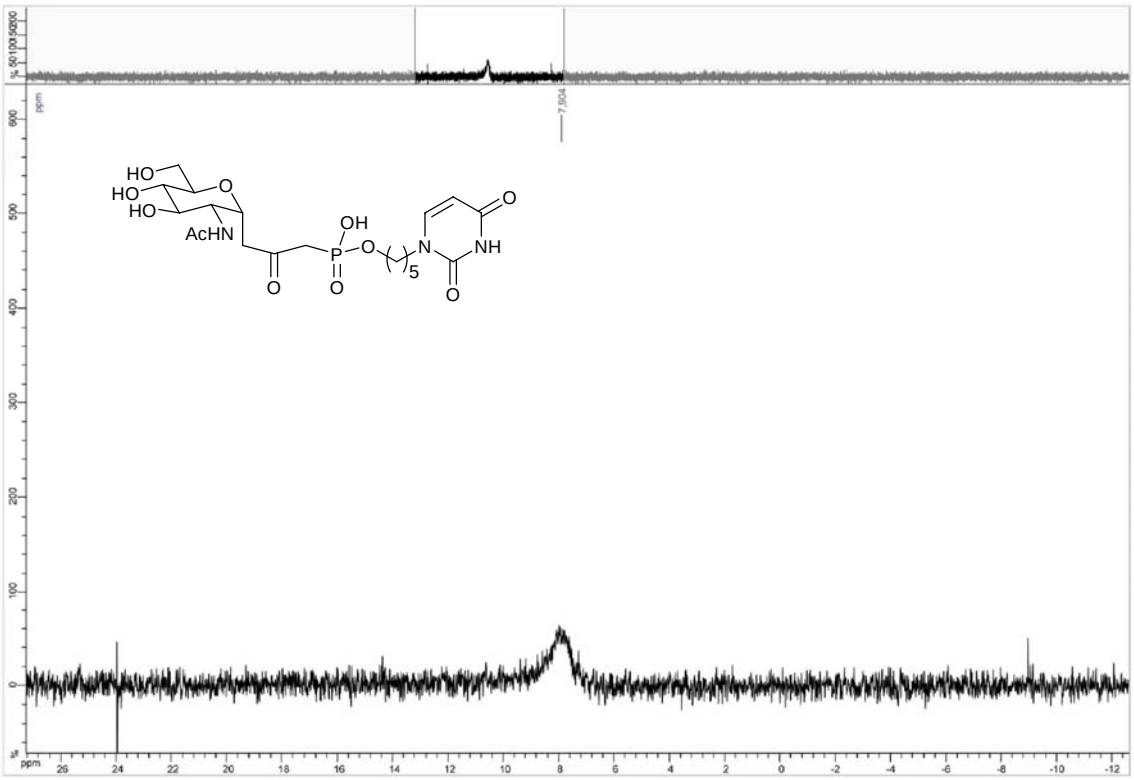




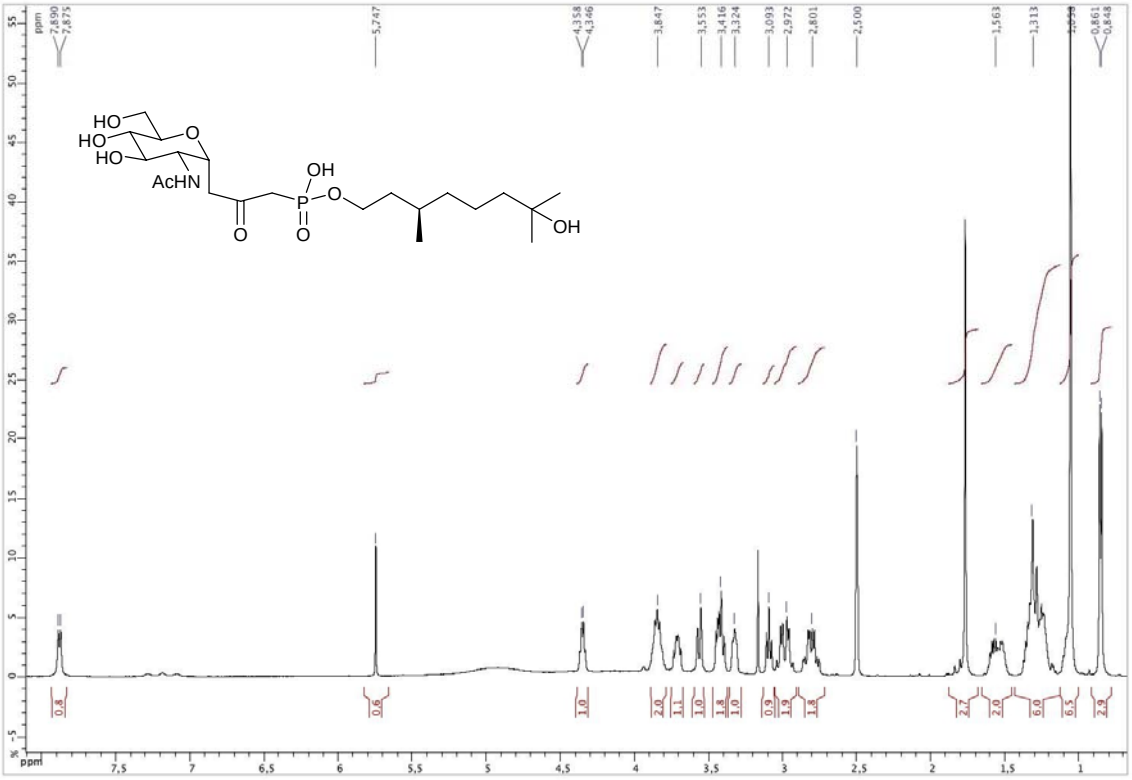
Compound **24** (^{1}H, ^{13}C, ^{31}P)

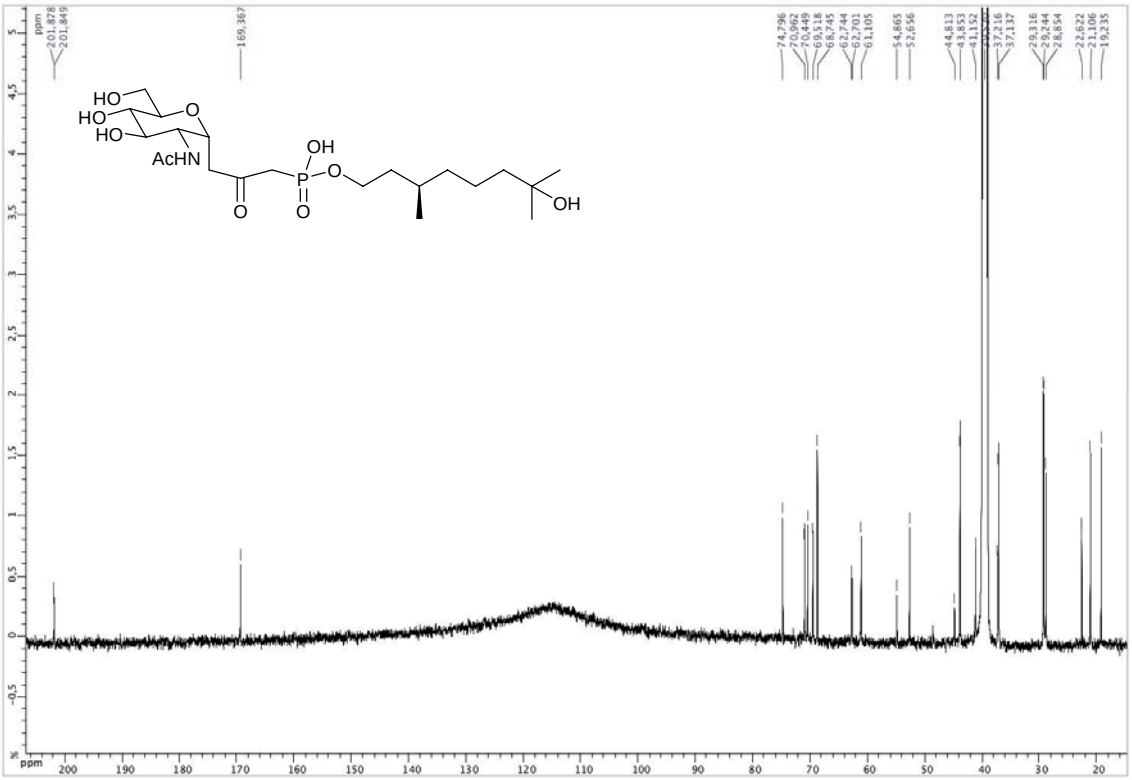


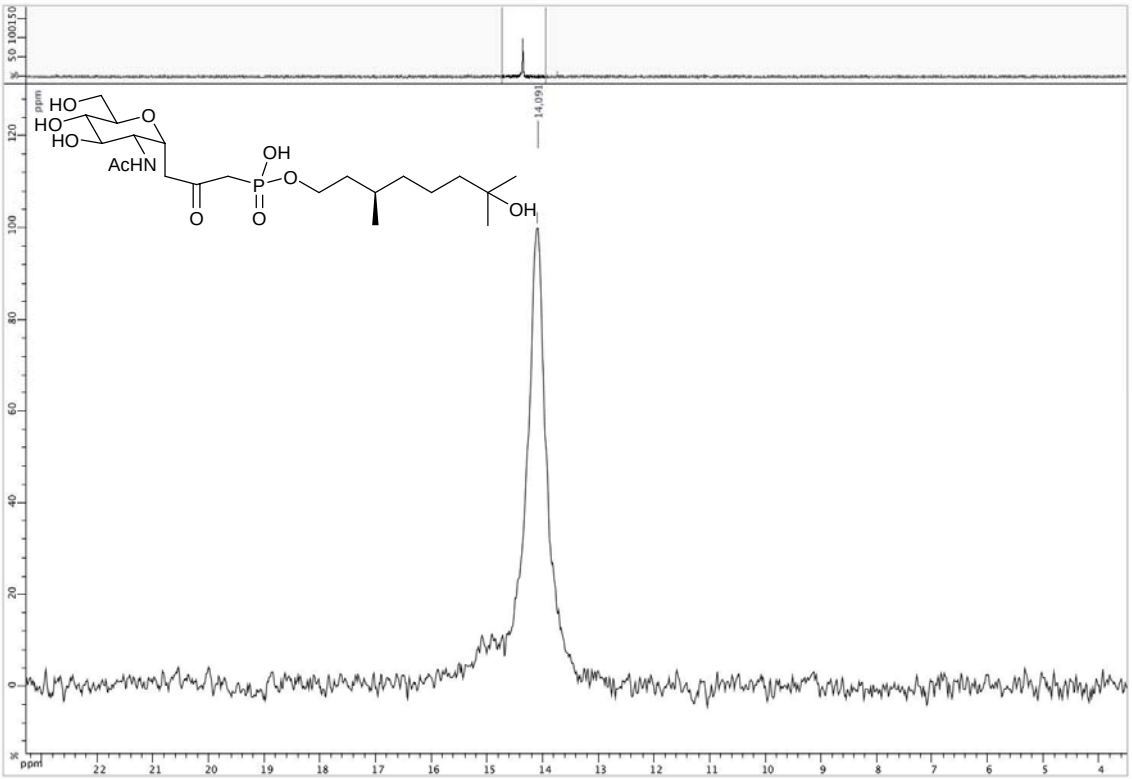




Compound **25** (^{1}H, ^{13}C, ^{31}P)







Compound **26** (^{1}H, ^{13}C, ^{31}P)