Glycosyl modified nucleoside motifs as a synthetic route to Glycoconjugation of oligonucleotides

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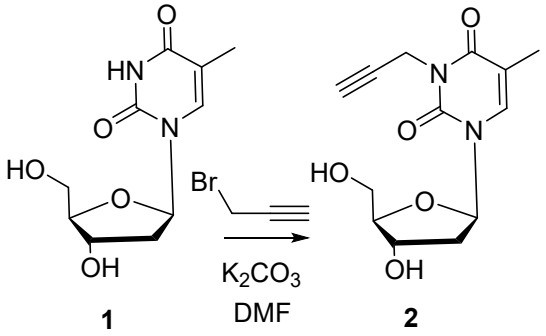
A/ Materials and Methods for the Chemical Synthesis

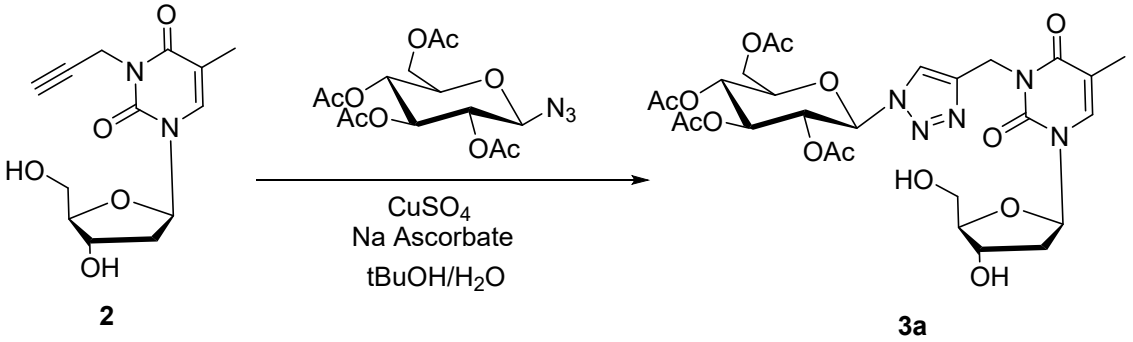
All commercial reagents and solvents (Fluka, Sigma-Aldrich, Alfa-Aesar) were used without further purification. For reactions requiring anhydrous conditions, dry solvents were used under inert atmosphere (nitrogen or argon). Analytical thin layer chromatography (TLC) was performed on precoated silica gel F254 plates with a fluorescent indicator (Merck). The detection of compounds was accomplished with UV light (254 nm). All compounds were characterized using ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy (Bruker Avance DPX-300 spectrometer, ^{1}H at 300.13 MHz and ^{13}C at 75.46 MHz). Assignments were made by ^{1}H-^{1}H COSY and HSQC experiments. Chemical shifts (δ) are given in parts per million (ppm) relative to tetramethylsilane or residual solvent peaks ($CHCl_3$: ^{1}H: 7.26, ^{13}C: 77.0). Coupling constants *J* are given in Hertz (Hz); peak multiplicity is reported as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, m = multiplet. High-resolution mass spectra (HRMS).

I. **Synthesis of propargyl thymidine 2**

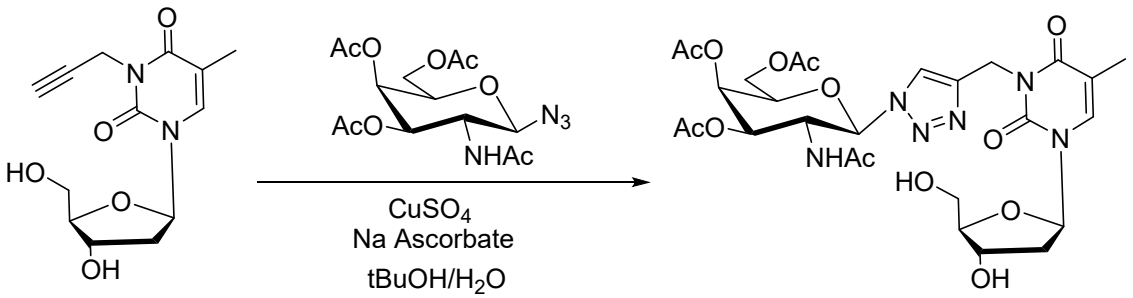


***N*²-propargyl-thymidine (2):** Thymidine **1** (3 g; 12.4 mmol; 1.0 equiv.), DMF (30 mL), propargyl bromide (2.07 mL, 18.6 mmol; 1.5 equiv.), and K_2CO_3 (2.57 g; 18.6 mmol; 1.5 equiv.) were added to a 250 mL RB flask, which was then capped and reaction mixture stirred at 25°C for 18 h. After completion, the mixture was washed with water and organic mixture extracted three times with EtOAc (50 mL x 3) and dried over anhydrous Na_2SO_4 and solvent evaporated using rotary evaporator. The crude mixture was then purified by column chromatography using silica gel as the stationary phase (24 g silica; 95-05% DCM/MeOH), giving *N*-propargylated product **2** (1.60 g; 62%). **TLC** R_f 0.41 (DCM/MeOH 8:2; UV). **^{1}H NMR (300 MHz, MeOD)** δ 7.86 (s, 1H), 6.32-6.28 (t, *J* = 6 Hz, 1H), 4.65-4.64 (d, *J* = 3 Hz, 2H), 4.43-4.38 (m, 1H), 3.92 (m, 1H), 3.84-3.71 (qd, *J*= 12 Hz, *J*= 3 Hz, 2H), 2,54 (t, *J*=3 Hz, 1H), 2.34-2.17 (m, 2H), 1.91 (s, 3H).

II. **Synthesis of carbohydrate modified thymidines 3a to 3f**

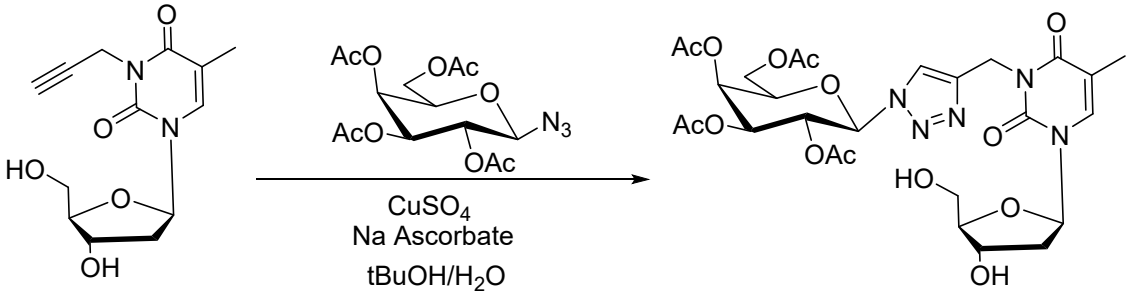


5-[1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside)-1H-1,2,3-triazol-4-yl]-2'-deoxyThymidine (3a): To a solution of **2** (0.20 g, 0.71 mmol, 1.0 equiv) and 1-azido-2,3,4,6-tetra-O-acetyl-β-Dglucopyranoside (0.21 g, 0.77 mmol, 1.1 equiv) in 6 mL of *tert*-BuOH/H_2O (50/50) was added copper sulfate pentahydrate (27.0 mg, 0.10 mmol, 0.1 equiv) followed by sodium ascorbate (42.0 mg, 0.20 mmol, 0.2 equiv). The mixture was stirred at 75 °C for 20 hours. After cooling to room temperature, solvents were removed under reduced pressure. The crude was extracted three times with EtOAc/EDTA. The organic phase was concentrated under reduced pressure and purified by column chromatography (12 g silica, 80/20 EtOAc/MeOH), giving the trizole-glucose-thymidine product **3a** (410 mg, 0.62 mmol, 87 %). **TLC:** R_f 0.27 (EtOAc/MeOH; 8:2 UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.85 (s, 1H), 7.44 (s, 1H), 6.24–6.19 (t, *J* = 6 Hz, 1H), 5.84–5.82 (d, *J* = 6 Hz, 1H), 5.44–5.35 (Quin, *J* = 9 Hz, 2H), 5.27-5.15 (m, 3H), 4.59 (s, 1H), 4.30-4.25 (m, 1H), 4.16–4.12 (m, 1H), 4.00-3.82 (m, 4H), 2.64 (m, 1H), 2.39–2.34 (m, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.93 (s, 3H), 1.85 (s, 3H), 1.62 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.78, 170.12, 169.49, 169.13, 169.06, 163.12, 150.79, 143.97, 135.25, 122.40, 110.40, 87.36, 86.98, 85.80, 75.26, 72.88, 71.40, 70.34, 67.81, 62.42, 61.66, 40.41, 36.04, 20.87, 20.66, 20.34, 13.39. Mass 653.2181, HRMS (ESI) calcd for [$C_{27}H_{35}N_5O_{14}$ + Na^+] 676.2072, found 676.2046.



2 **3b**

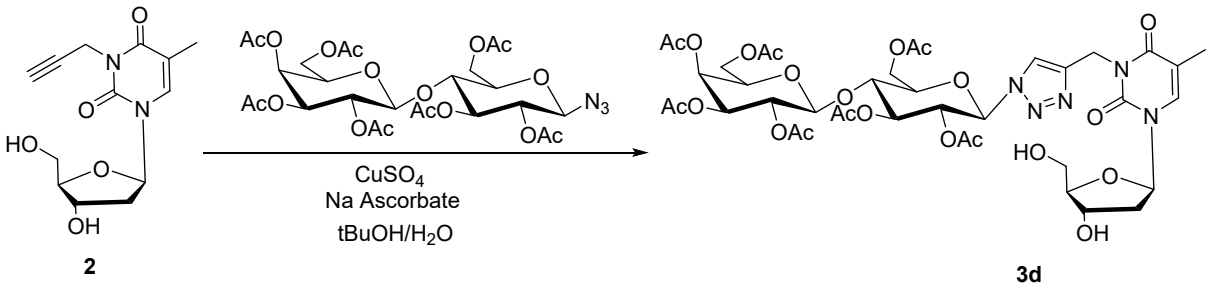
5-[1-(2,3,4,6-tetra-O-acetyl-β-D-galactosaminepyranoside)-1H-1,2,3-triazol-4-yl]-2'-deoxyThymidine (3b): To a solution of **2** (0.15 g, 0.53 mmol, 1.0 equiv) and Galnac-Azide (0.219 g, 0.59 mmol, 1.1 equiv) in 4 mL of *tert*-BuOH/H_2O (50/50) was added copper sulfate pentahydrate (13.5 mg, 0.05 mmol, 0.1 equiv) followed by sodium ascorbate (21.0 mg, 0.10 mmol, 0.2 equiv). The mixture was stirred at 75 °C for 20 hours. After cooling to room temperature, solvents were removed under reduced pressure. The resulting solid was washed with deionized water (5.0 mL), DCM (5.0 ml) and absolute methanol (5.0 mL). After drying, the resulting white solid was used in the next step without further purification. Yield: 79 % (0.275 g). **TLC:** R_f 0.19 (EtOAc/MeOH; 80:20 UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.09-8.04 (m, 2H), 7.83 (s, 1H), 6.23–6.19 (t, *J* = 6 Hz, 1H), 6.10–6.07 (d, *J* = 9 Hz, 1H), 5.36–5.26 (m, 2H), 5.11-4.96 (m, 4H), 4.60-4.53 (q, *J* = 9 Hz 1H), 4.24-4.01 (m, 4H), 3.78 (s, 1H), 3.58 (m, 1H), 2.12 (m, 2H), 2.00 (s, 6H), 1.94 (s, 3H), 1.84 (s, 3H); **^{13}C NMR (75 MHz, DMSO)** δ 170.19, 169.73, 169.61, 169.46, 162.41, 160.01, 150.30, 143.00, 135.18, 122.38, 108.63, 87.46, 84.95, 84.61, 73.49, 72.41, 70.33, 68.05, 61.88, 61.27, 52.00, 35.93, 22.36, 20.62, 20.49, 20.36, 12.98. Mass 652.2340, HRMS (ESI) calcd for [$C_{27}H_{36}N_6O_{13}$ + Na^+] 675.2232, found 675.2205.



2 **3c**

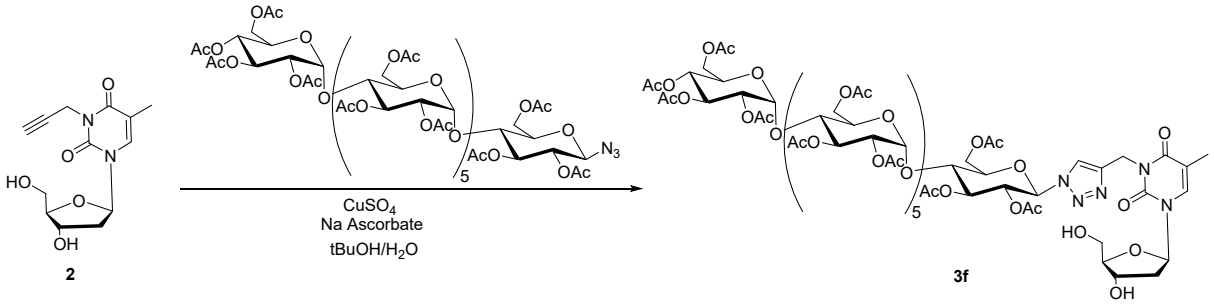
5-[1-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside)-1H-1,2,3-triazol-4-yl]-2'-deoxyThymidine (3c): To a solution of **2** (0.20 g, 0.71 mmol, 1.0 equiv) and 1-azido-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (0.290 g, 0.78 mmol, 1.1 equiv) in 6 mL of *tert*-BuOH/H_2O (50/50) was added copper sulfate pentahydrate (17.4 mg, 0.07 mmol, 0.1 equiv) followed by sodium ascorbate (28.0 mg, 0.14 mmol, 0.2 equiv). The mixture was stirred at 75 °C for 20 hours. After cooling to room temperature, solvents were removed under reduced pressure. The crude was extracted three times with EtOAc/EDTA. The organic phase was concentrated under reduced pressure and purified by column chromatography (12 g silica, 80/20 EtOAc/MeOH), giving the trizole-galactose-thymidine product **3c** (422 mg, 0.64 mmol, 93 %). **TLC:** R_f 0.25 (EtOAc/MeOH 80/20; UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.91 (s, 1H), 7.62 (s, 1H), 6.24 (m, 1H), 5.82–5.79 (d, *J* = 9 Hz, 1H), 5.52–5.46 (m, 2H), 5.21 (m, 2H), 5.12-5.07 (m, 1H), 4.59-4.52 (m, 1H), 4.16–4.12 (m, 1H), 4.00-3.82 (m, 8H), 2.64 (m, 1H), 2.28–2.23 (m, 2H), 2.12 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.85 (s, 3H), 1.80 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.47, 170.17, 169.88, 169.06, 163.23, 150.68, 143.59, 135.24, 122.76, 109.95, 88.22, 87.25, 86.33, 86.10, 73.97,

72.82, 70.79, 67.85, 66.91, 61.93, 61.25, (53.52 CH2 DCM), 40.28, 35.83, 20.70, 20.63, 20.48, 20.22, 13.16. Mass 653.2181, HRMS (ESI) calcd for [$C_{27}H_{35}N_5O_{14}$ + Na^+] 676.2072, found 676.2055.



2 **3d**

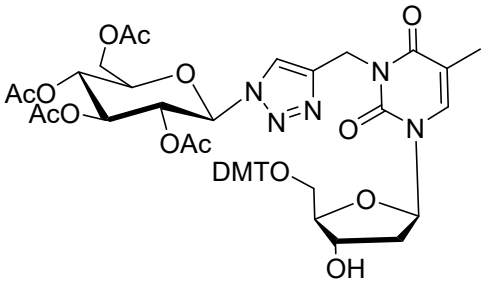
5-**[1-(2,3,4,6-tetra-O-acetyl-β-D-lactopyranoside)-1H-1,2,3-triazol-4-yl]-2'-deoxyThymidine (3d):** To a solution of **2** (0.20 g, 0.71 mmol, 1.0 equiv) and 1-azido-2,3,4,6-tetra-O-acetyl-β-D-lactopyranoside (0.510 g, 0.78 mmol, 1.1 equiv) in 6 mL of *tert*-BuOH/H_2O (50/50) was added copper sulfate pentahydrate (17.4 mg, 0.07 mmol, 0.1 equiv) followed by sodium ascorbate (28.0 mg, 0.14 mmol, 0.2 equiv). The mixture was stirred at 75 °C for 20 hours. After cooling to room temperature, solvents were removed under reduced pressure. The crude was extracted three times with EtOAc/EDTA. The organic phase was concentrated under reduced pressure and purified by column chromatography (12 g silica, 80/20 EtOAc/MeOH), giving the trizole-lactose-thymidine product **3d** (480 mg, 0.53 mmol, 76 %). **TLC:** R_f 0.20 (EtOAc/MeOH; 80/20 UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.82 (s, 1H), 7.48 (s, 1H), 6.24–6.20 (t, *J* = 6 Hz, 1H), 5.80–5.77 (d, *J* = 9 Hz, 1H), 5.41–5.37 (m, 3H), 5.27-5.08 (m, 3H), 4.99-4.95 (dd, *J* = 9Hz, *J* = 3Hz, 1H), 4.55-4.52 (d, *J* = 9Hz, 2H), 4.48-4.44 (d, *J* = 9Hz, 1Hz), 4.16-4.04 (m, 3H), 3.98-3.79 (m,6H), 2.31 (m, 2H), 2.15 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.04 (s, 6H), 1.96 (s, 3H), 1.89 (s, 3H), 1.82 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.57, 170.52, 170.27, 170.21, 169.75, 169.30, 163.24, 150.80, 143.67, 135.32, 122.89, 110.18, 101.19, 87.23, 86.86, 85.53, 75.95, 75.65, 72.75, 71.10, 71.05, 70.92, 70.58, 69.19, 66.77, 62.22, 61.90, 60.98, 40.38, 35.95, 20.95, 20.83, 20.79, 20.75, 20.62, 20.35, 13.31. Mass 941.3026, HRMS (ESI) calcd for [$C_{39}H_{51}N_5O_{22}$ + Na^+] 964.2923, found 964.2887.



2 **3f**

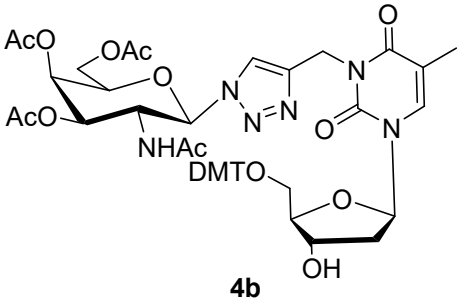
5-[(O-acetyl-β-D-heptamaltoside)-1H-1,2,3-triazol-4-yl]-2'-deoxyThymidine (3f): To a solution of **2** (0.100 g, 0.35 mmol) and 1-azido-O-acetyl-β-D-heptamaltoside (0.133 g, 0.063 mmol) in 6 mL of *tert*-BuOH/H_2O (50/50) was added copper sulfate pentahydrate (40 mg, 0.16 mmol) followed by sodium ascorbate (54.0 mg, 0.27 mmol). The mixture was stirred at 115 °C for 20 hours. After cooling to room temperature, solvents were removed under reduced pressure and purified by silica gel column chromatography using 5-10% MeOH/DCM containing 0.5% TEA giving the trizole-heptamaltose-thymidine product **3f** (80 mg, 0.033 mmol, 53 %). **TLC:** R_f 0.20 (MeOH/DCM; 10/20 UV). **^{1}H NMR (300 MHz, $CDCl_3$ & CD_3OD)** δ 8.44 (s, 1H), 7.59 (s. 1H), 7.56 (m, 5H), 7.29 (m, 1H), 6.15-5.71 (m, 7H), 4.71-4.64 (m 7H), 4.41-4.29 (m, 6H), 3.79-3.64 (m, 18H), 2.76-2.73 (m, 14H), 2.69 (s, 3H), 1.83-1.89 (m, 20H), 1.08-1.03 (m, 46H); **^{13}C NMR (75 MHz, CD_3OD)** δ 162.26, 162.04, 161.61, 161.53, 149.29, 149.13, 148.84, 148.81, 135.82, 135.69, 133.93, 133.89, 133.84, 133.77, 110.49, 108.51, 107.89, 107.82, 88.40, 86.04, 85.99, 84.38, 84.29, 83.03, 77.78, 75.93, 69.26, 69.22, 61.20, 59.96, 59.91, 53.08, 50.82, 48.79, 47.01, 46.72, 46.44, 46.15, 45.87, 45.59, 45.30, 44.99, 40.35, 39.05, 38.46, 38.39, 35.96, 29.27, 29.09, 11.88, 10.37, 10.34, 10.30.

III. **Synthesis of DMT protected carbohydrate modified thymidines 4a to 4f :**



4a

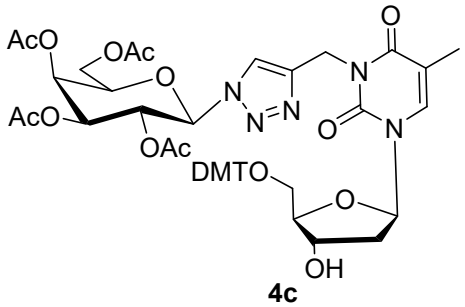
(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(4-((3-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl) methoxy) methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (4a): To a solution of **3a** (0.35 g, 0.53 mmol, 1.0 equiv) in 8 mL of pyridine was added DMT-Cl (0.260 g, 0.68 mmol, 1.3 equiv) followed by DMAP (6.5 mg, 0.053 mmol, 0.1 equiv). The mixture was stirred at room temperature for overnight. After 20 hours, toluene was added as co-solvent, solvents were removed under reduced pressure. The crude was purified by column chromatography (12 g silica, 98/02/0.1 EtOAc/MeOH/TEA), giving the DMT-trizol-glucose-thymidine protected product **4a** (410 mg, 0.42 mmol, 81 %). **TLC:** R_f 0.65 (EtOAc/MeOH/TEA : 98/02/0.2; UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.85 (s, 1H), 7.44 (s, 1H), 7.28 (m,1H), 7.18 (m, 3H), 6.84–6.82 (d, J = 6 Hz, 4H), 6.24-6.19 (t, J = 6Hz, 1H), 5.85–5.82 (d, J = 9Hz, 1H), 5.47–5.35 (Quin, J = 9Hz, 2H), 5.28-5.15 (m, 3H), 4.58 (m, 1H), 4.31-4.26 (dd, *J* = 12Hz, *J* = 3Hz, 1H), 4.00-3.86 (m, 4H), 3.80 (s, 3H), 3.78 (s, 1H), 2.74 (s, 1H), 2.67-2.55 (m, 1H), 2.40-2.33 (m, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.93 (s, 3H), 1.85 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.77, 170.12, 169.48, 169.04, 159.83, 159.43, 159.38, 158.88, 158.79, 150.78, 147.47, 143.98, 139.60, 135.23, 130.22, 129.49, 129.38, 129.28, 128.26, 128.21, 128.17, 128.00, 127.91, 127.24, 122.36, 113.44, 113.32, 110.40, 87.36, 86.95, 85.79, 77.36, 75.26, 72.88, 71.40, 70.33, 67.81, 62.42, 61.66, 55.40, 40.41, 36.05, 20.87, 20.67, 20.34, 19.99, 13.40. Mass 955.3487, HRMS (ESI) calcd for [$C_{48}H_{53}N_5O_{16}$ + Na^+] 978.3379, found 978.3348.



4b

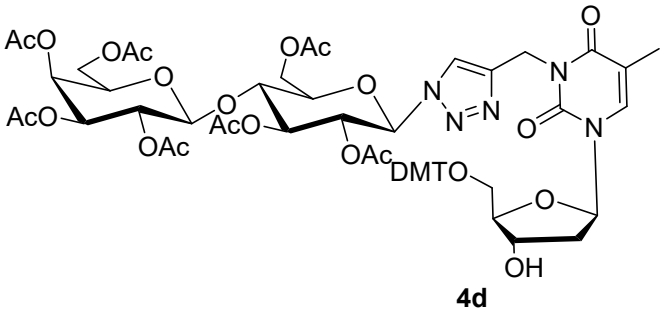
(2R,3R,4R,5R,6R)-5-acetamido-2-(acetoxymethyl)-6-(4-((3-((2R,4S,5R)-5-((bis(4-methoxyphenyl) (phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin -1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4-diyl diacetate (4b): To a solution of **3b** (0.380 g, 0.58 mmol, 1.0 equiv) in 8 mL of pyridine was added DMT-Cl (0.260 g, 0.68 mmol, 1.3 equiv) followed by DMAP (7.0 mg, 0.058 mmol, 0.1 equiv). The mixture was stirred at room temperature for overnight. After 20 hours, toluene was added as co-solvent, solvents were removed under reduced pressure. The crude was purified by column chromatography (12 g silica, 98/02/0.1 EtOAc/MeOH/TEA), giving the DMT-trizol-galnac-thymidine protected product **4b** (506 mg, 0.52 mmol, 91 %). **TLC:** R_f 0.69 (EtOAc/MeOH/TEA : 98/02/0.1; UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.92 (s, 1H), 7.65-7.61 (d, 1H, *J* = 12Hz), 7.30 (s,2H), 7.30-7.25 (m, 4H), 6.81–6.79 (m, 4H), 6.45 (m, 1H), 5.87–5.84 (d, *J* = 9 Hz, 1H), 5.58–5.52 (m, 2H), 5.37-5.32 (dd, *J* = 15 Hz, *J* = 6 Hz, 1H), 5.26-5.22 (dd, *J* = 9 Hz, *J* = 3 Hz, 1H), 5.15-5.11 (d, J = 12Hz, 1H), 4.64 (s, 1H), 4.27-4.09 (m, 4H), 3.75-3.74 (d, *J* = 3Hz, 6H), 3.57-3.43 (m, 5H), 3.31 (m, 1H), 2.78-2.70 (q, *J* = 15Hz, *J* = 9Hz, 3H), 2.61 (m, 2H), 2.45-2.29 (m, 2H), 2.23-2.21 (d, J= 6Hz, 3H), 2.01-1.69 (m, 6H), 1.83-1.82 (d, *J*= 3Hz, 3H), 1.44-1.43 (d, *J* = 3Hz, 3H). ^{13}C NMR

(75 MHz, $CDCl_3$) δ 170.19, 169.99, 169.67, 168.79, 162.91, 158.56, 150.49, 144.11, 143.64, 135.20, 133.87, 129.98, 128.08, 128.02, 127.83, 126.99, 122.21, 113.10, 110.29, 86.73, 85.95, 85.24, 73.82, 70.75, 67.64, 66.84, 63.14, 62.94, 62.88, 61.20, 58.32, 58.07, 57.85, 55.09, 45.81, 43.03, 40.04, 35.83, 24.46, 24.37, 24.28, 20.60, 20.51, 20.35, 20.20, 20.10, 20.00, 12.31. Mass 954.3647, HRMS (ESI) calcd for [$C_{48}H_{54}N_6O_{15}$ + Na^+] 977.3545, found 977.3238.



4c

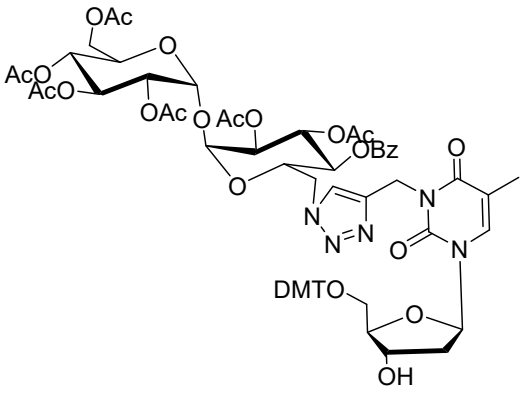
(2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-(4-((3-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy) methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (4c): To a solution of **3c** (0.30 g, 0.3 mmol, 1.0 equiv) in 8 mL of pyridine was added DMT-Cl (0.155 g, 0.4 mmol, 1.3 equiv) followed by DMAP (3.6 mg, 0.03 mmol, 0.1 equiv). The mixture was stirred at room temperature for overnight. After 20 hour,s toluene added as co-solvent, solvents were removed under reduced pressure. The crude was purified by column chromatography (12 g silica, 98/02/0.1 EtOAc/MeOH/TEA), giving the DMT-trizol-galactose-thymidine protected product **4c** (506 mg, 0.52 mmol, 91 %). **TLC:** R_f 0.69 (EtOAc/MeOH/TEA : 98/02/0.1; UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.85 (s, 1H), 7.59 (s, 1H), 7.40-7.37 (d, J = 9HZ, 2H), 7.30-7.28 (m, 6H), 6.84–6.82 (d, J = 9 Hz, 4H), 6.48-6.44 (t, J = 6Hz, 1H), 5.82–5.79 (d, J = 9Hz, 1H), 5.57–5.51 (m, 2H), 5.37-5.32 (d, J = 15Hz, 1H), 5.23-5.12 (m, 2H), 4.58 (s, 1H), 4.20-4.04 (m, 5H), 3.78 (s, 6H), 3.48-3.34 (m, 2H), 2.42-2.26 (m, 3H), 2.24 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.84 (s, 3H), 1.49 (s, 3H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.48, 170.25, 169.97, 169.09, 163.20, 158.86, 150.74, 144.46, 143.91, 135.55, 135.48, 134.13, 130.22, 129.27, 128.26, 128.13, 127.98, 127.90, 127.27, 127.20, 122.47, 113.42, 113.30, 110.48, 87.07, 86.36, 86.10, 85.50, 77.36, 74.19, 72.42, 71.04, 67.87, 67.01, 63.61, 61.39, 60.53, 55.38, 41.25, 36.04, 21.18, 20.87, 20.78, 20.62, 20.39, 14.33, 12.69. Mass 955.3487, HRMS (ESI) calcd for [$C_{48}H_{53}N_5O_{16}$ + Na^+] 978.3379, found 978.3374.



4d

(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(((2R,3R,4S,5R,6R)-4,5-diacetoxy-2-(acetoxymethyl)-6-(4-((3-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (4d): To a solution of **3d** (0.30 g, 0.22 mmol, 1.0 equiv),) and in 8 mL of Pyridine was added DMT-Cl (0.112 g, 0.3 mmol, 1.3 equiv) followed by DMAP (3.0 mg, 0.03 mmol, 0.1 equiv). The mixture was stirred at room temperature for night. After 20 hours Toluene added as co-solvent, solvents were removed under reduced pressure. The crude was purified by column chromatography (12 g silica, 98/02/0.1 EtOAc/MeOH/TEA), giving the DMT-trizol-lactose-

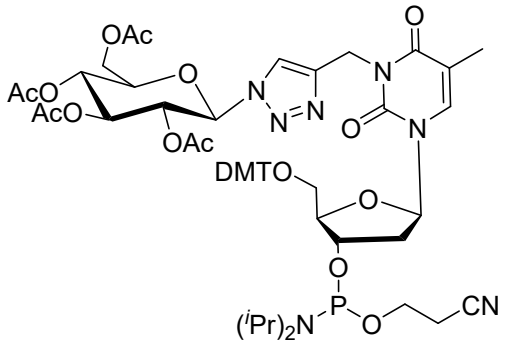
thymidine protected product **4d** (506 mg, 0.52 mmol, 91 %). **TLC:** R_f 0.37 (EtOAc/MeOH/TEA : 98/02/0.1; UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.80 (s, 1H), 7.58 (s, 1H), 7.42-7.28 (m, 5H), 7.18-7.15 (d, J= 9Hz, 3H), 6.84–6.82 (d, J = 9 Hz, 4H), 6.23-6.18 (t, J = 6Hz, 1H), 5.80–5.77 (d, J = 9Hz, 1H), 5.40–5.37 (m, 4H), 5.19-5.10 (m, 2H), 4.99-4.93 (dd, *J* = 9Hz, *J* = 3Hz, 1H), 4.57-4.45 (m, 3H), 4.14-4.10 (m, 3H), 3.91-3.86 (m, 5H), 3.80 (s, 6H), 3.78 (s, 1H), 2.39-2.33 (m, 2H), 2.16 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.06 (s, 6H), 1.97 (s, 3H), 1.93 (s, 3H), 1.84 (s, 3H), 1.50-147 (d, J = 6Hz, 2H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.79, 170.41, 170.18, 169.69, 169.62, 169.55, 169.21, 169.12, 167.39, 167.14, 158.61, 142.71, 139.46, 130.08, 129.14, 128.26, 128.01, 127.86, 127.77, 127.09, 113.30, 113.17, 110.26, 101.12, 95.08, 87.66, 87.31, 86.80, 85.45, 77.21, 76.20, 76.13, 76.00, 75.84, 75.58, 72.64, 71.79, 71.38, 71.31, 70.94, 70.86, 70.45, 69.04, 68.24, 68.10, 67.32, 66.60, 62.30, 55.32, 55.25, 40.25, 35.87, 20.83, 20.67, 20.62, 20.38, 20.29, 20.24, 19.33, 19.16, 13.25. Mass 1243.43, HRMS (ESI) calcd for [$C_{60}H_{69}N_5O_{24}$ + Na^+] 1266.4224, found 1266.4182.



4e

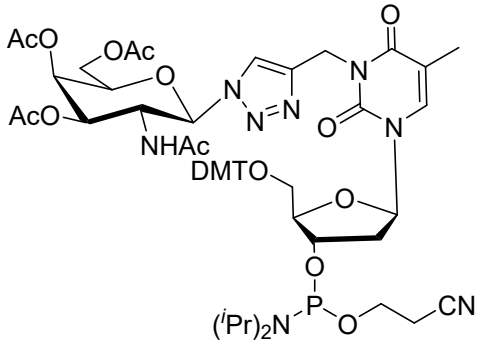
(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(((2R,3R,4S,5R,6R)-3,4-diacetoxy-5-(benzoyloxy)-6-((4-((3-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-2,3-dihydropyrimidin-1(6H)-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (4e): $CuSO_4.5H_2O$ (17 mg, 0.11 mmol) and sodium-L-ascorbate (42 mg, 0.21 mmol) were added to a solution of compounds DMT protected ***N*^{2}-propargyl-thymidine** (621 mg, 1.07 mmol) and corresponding sugar (848 mg, 1.17 mmol) in a mixture of THF/H_2O 1/1 (8.4 mL) at room temperature under an argon atmosphere. Then the reaction mixture was stirred at 77 °C overnight. After completion of the reaction, the solution was cooled down to room temperature and water (15 mL) was added. The aqueous layer was extracted three times with DCM (3 x 20 mL), and the combined organic layer was washed twice with water (2 x 20 mL), dried over Na_2SO_4, filtered and concentrated under reduce pressure. The crude compound was purified by flash chromatography over irregular silica (0 to 5% MeOH in DCM/MeOH over 20 CV) to afford compound **4e** as white crystals (1.07 g, 77%). **TLC:** Rf = 0.38 (DCM/MeOH 95/5). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.08 – 8.01 (m, 2H), 7.65 (dd, *J* = 14.0, 6.5 Hz, 2H), 7.51 (dd, *J* = 14.6, 6.7 Hz, 3H), 7.40 (d, *J* = 6.9 Hz, 2H), 7.34 – 7.27 (m, 5H), 6.87 – 6.78 (m, 4H), 6.46 (dd, *J* = 7.8, 5.4 Hz, 1H), 5.73 (t, *J* = 9.9 Hz, 1H), 5.43 – 5.31 (m, 3H), 5.26 – 5.17 (m, 1H), 5.11 (dd, *J* = 10.3, 3.9 Hz, 1H), 5.07 – 4.90 (m, 3H), 4.84 (d, *J* = 3.9 Hz, 1H), 4.76 – 4.64 (m, 1H), 4.61 – 4.55 (m, 1H), 4.32 – 4.21 (m, 3H), 4.10 – 3.95 (m, 3H), 3.78 (s, 6H), 3.42 (dd, *J* = 10.6, 3.1 Hz, 1H), 3.34 (dd, *J* = 10.1, 2.8 Hz, 1H), 2.73 (d, *J* = 3.5 Hz, 1H), 2.55 – 2.43 (m, 1H), 2.40 – 2.26 (m, 1H), 2.12 – 2.00 (m, 9H), 2.00 – 1.87 (m, 9H), 1.67 (s, 2H), 1.55 (s, 3H).

IV. **Synthesis of phosphoramidites 5a to 5f:**



5a

(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(4-((3-((2R,4S,5R)-5-((bis(4methoxyphenyl)(phenyl)methoxy) methyl)-4-(((2-cyanoethoxy)(diisopropylamino)phosphaneyl)oxy)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (5a): Triazol-DMT **4a** (240 mg; 0.25 mmol; 1 equiv.) was dissolved in anhydrous DCM (6 mL) at room temperature, DIPEA (0.090 mL; 0.50 mmol; 2 equiv.) and CEP-Cl (0.071 mL; 0.32 mmol; 1.27 equiv.) were added and the mixture was stirred for 40 minutes. TLC analysis showed full conversion. Anhydrous MeOH (0.1 ml) added to stop the reaction. The crude was evaporated and the product was purified using silica column chromatography (4 g; DCM/TEA 2% or 3%) to give the product **5a** as a mixture of diastereomers (180 mg; 0.155 mmol; 62%). **TLC:** R_f 0.56 (2.5% TEA/DCM; UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.87-7.84 (d, *J* = 9 Hz, 1H), 7.64-7.59 (d, *J* = 15 Hz, 1H), 7.39-7.37 (m, 2H), 7.29-7.24 (m, 5H), 6.83–6.79 (dd, *J* = 9 Hz, *J* = 3 Hz, 4H), 6.47-6.41 (m, 1H), 5.85–5.82 (d, *J* = 9 Hz, 1H), 5.48–5.24 (m, 3H), 5.21-5.13 (t, *J*= 12 Hz, 2H), 4.63-4.60 (m, 1H), 4.304.25 (dd, *J* = 12 Hz, *J* = 3 Hz, 1H), 4.14-4.10 (m, 2H), 3.99 -3.94 (m, 1H), 3.91-3.79 (m, 2H), 3.77-3;76 (d, *J*= 3 Hz, 1H), 3.73-3.49 (m, 5H), 2.65-2.59 (q, *J* = 12 Hz, *J* = 6 Hz, 2H), 2.41-2.37 (t, *J* = 12 Hz, 1H), 2.07 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.82 (s, 3H), 1.43 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.62, 170.02, 169.38, 168.85, 163.10, 158.79, 150.73, 144.33, 144.03, 135.46, 135.39, 135.34, 134.04, 130.25, 130.20, 128.32, 128.24, 128.04, 127.21, 122.28, 122.20, 117.66, 117.44, 113.32, 110.53, 86.97, 85.68, 85.47, 75.15, 74.11, 73.89, 73.65, 73.43, 72.87, 70.24, 67.75, 63.38, 63.11, 61.60, 58.64, 58.49, 58.38, 58.29, 58.24, 58.04, 55.32, 50.83, 50.60, 46.16, 43.44, 43.35, 43.27, 43.19, 43.11, 42.95, 40.27, 36.05, 24.78, 24.68, 24.58, 24.50, 20.79, 20.60, 20.25, 12.52. **^{31}P NMR (121 MHz, $CDCl_3$)** 152.66-152.13. Mass 1155.4566, HRMS (ESI) calcd for $[C_{57}H_{70}N_7O_{17}P + Na^+]$ 1178.4458, found 1178.4420.

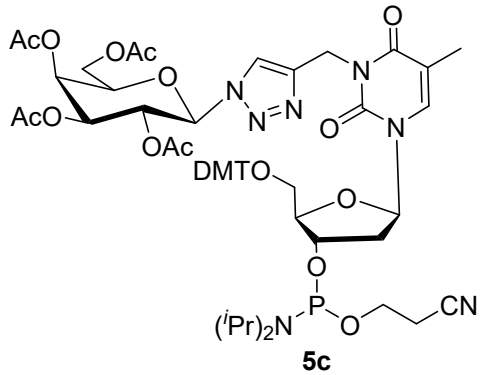


5b

(2R,3R,4R,5R,6R)-5-acetamido-2-(acetoxymethyl)-6-(4-((3-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl) methoxy)methyl)-4-(((2-cyanoethoxy)(diisopropylamino)phosphaneyl)oxy)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4-diyl diacetate (5b): Triazol-DMT **4b** (150 mg; 0.157 mmol; 1 equiv.) was dissolved in anhydrous DCM (6 mL) at room temperature, DIPEA (0.078 mL; 0.45 mmol; 2.85 equiv.) and CEP-Cl (0.045 mL; 0.2 mmol; 1.29 equiv.) were added and the mixture was stirred for 40 minutes. TLC analysis showed full conversion. Anhydrous MeOH (0.1 ml) added to stop the reaction. The crude was evaporated and the product was purified using silica column chromatography (4 g; DCM/TEA 2% or 3%) to give the product **5b** as a mixture of diastereomers (50 mg; 0.045 mmol; 28.5%). **TLC:** R_f 0.53 (EtOAc/MeOH/TEA: 6:4:0.2;

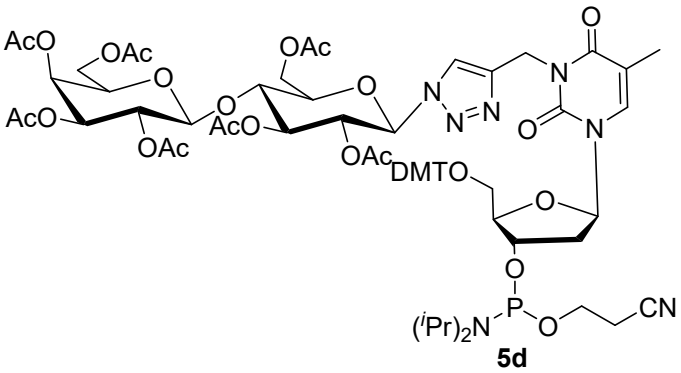
UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.00 (s, 1H), 7.87 (s, 1H), 7.64-7.60 (s, J= 12Hz, 1H), 6.82-6.80 (m, 5H), 6.43 (m, 1H), 6.16-6.13 (d, J= 9Hz, 1H), 5.87 (s, 1H), 5.61–5.55 (t, J= 9Hz, 1H), 5.61-5.19 (m, 3H), 4.62 (m, 1H), 4.28-4.04 (m, 8H), 3.76 (s, 6H), 3.62-3.43 (m, 9H), 3.31 (m, 1H), 2.77-2.73 (t, *J* = 6Hz, 4H), 2.61 (m, 1H), 2.41-2.37 (t, *J* = 6 Hz, 1H), 2.06-2.03 (s, 9H), 1.73 (s, 3H), 1.42 (m, 4H), 1.27-1.24 (m, 23H), 1.15-1.13 (d, J = 6Hz, 10H), 1.04-1.02 (d, J = 6Hz, 3H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.82, 170.80, 170.76, 170.62, 169.48, 163.13, 158.81, 150.75, 144.36, 143.58, 135.50, 130.28, 130.23, 128.34, 128.27, 128.07, 123.05, 117.04, 113.35, 110.54, 86.98, 86.96, 85.48, 85.40,

77.58, 77.36, 77.16, 76.74, 74.82, 72.28, 68.30, 61.88, 58.32, 58.25, 55.36, 55.34, 53.64, 45.46, 45.37, 43.46, 43.37, 43.29, 43.21, 24.74, 24.70, 24.65, 24.60, 23.09, 23.06, 23.01, 22.97, 20.84, 20.75, 20.71, 20.24, 20.15, 12.57. **^{31}P NMR (121 MHz, $CDCl_3$)** 152.08-151.65. Mass 1154.4726, HRMS (ESI) calcd for [$C_{57}H_{71}N_8O_{16}P$ + H^+] 1155.4798, found 1155.4763.



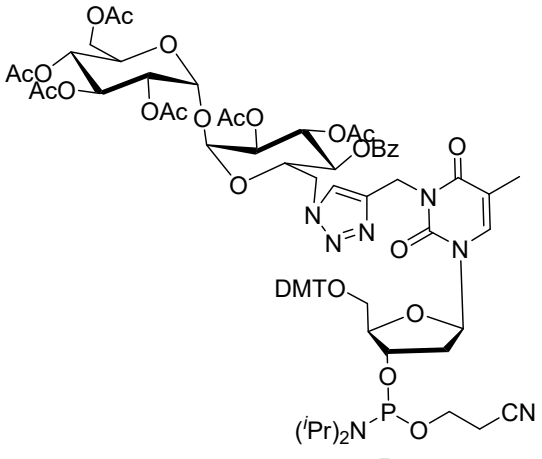
5c

(2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-(4-((3-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl) methoxy) methyl)-4-(((2-cyanoethoxy)(diisopropylamino)phosphaneyl)oxy)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (5c): Triazol-DMT **4c** (150 mg; 0.157 mmol; 1 equiv.) was dissolved in anhydrous DCM (6 mL) at room temperature, DIPEA (0.040 mL; 0.23 mmol; 1.46 equiv.) and CEP-Cl (0.060 mL; 0.269 mmol; 1.71 equiv.) were added and the mixture was stirred for 40 minutes. TLC analysis showed full conversion. Anhydrous MeOH (0.1 ml) added to stop the reaction. The crude was evaporated and the product was purified using silica column chromatography (4 g; DCM/TEA 2% or 3%) to give the product **5c** as a mixture of diastereomers (30 mg; 0.025 mmol; 16.5%). **TLC:** R_f 0.54 (2.5% TEA/DCM; UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.91 (s, 1H), 7.65-7.63 (d, J = 6Hz, 1H), 7.43-7.37 (m, 2H), 7.30-7.26 (m, 5H), 6.84–6.80 (dd, J = 9 Hz, J = 3Hz, 4H), 6.50-6.43 (m, 1H), 5.82–5.79 (d, J = 9Hz, 1H), 5.53–5.52 (m, 2H), 5.40-5.35 (d, J= 18Hz, 1H), 5.22-5.18 (dd, J = 9Hz, J = 3Hz, 1H), 5.165.11 (d, *J* = 15Hz, 1H), 4.63 (m, 1H), 4.22-4.10 (m, 5H), 3.78 (s, 3H), 3.63-3.60 (m, 6H), 3.32-3.28 (m, 1H), 2.78-2.73 (t, *J* = 9Hz, 1H), 2.63-2.59 (t, *J* = 6Hz, 1H), 2.41-2.37 (t, *J* = 6 Hz, 3H), 2.24 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.85 (s, 3H), 1.44 (s, 3H), 1.28 (s, 3H), 1.27 (s, 3H), 1.25 (s, 3H), 1.16 (s, 9H), 1.14 (s, 3H), 1.05-1.03 (d, 3H). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.33, 170.11, 169.83, 168.94, 163.09, 158.72, 150.66, 144.25, 143.82, 135.38, 135.31, 135.26, 130.18, 130.13, 128.24, 128.17, 127.97, 127.15, 122.28, 113.25, 110.47, 86.89, 86.23, 85.39, 74.05, 70.93, 67.71, 66.86, 63.32, 63.04, 61.24, 58.17, 58.10, 57.95, 55.25, 45.35, 45.27, 43.37, 43.28, 43.20, 43.11, 40.14, 35.96, 24.60, 24.51, 24.42, 23.08, 22.96, 22.91, 22.88, 20.74, 20.65, 20.49, 20.26, 20.15, 20.06, 12.45. **^{31}P NMR (121 MHz, $CDCl_3$)** 152.19-151.62. Mass 1155.4566, HRMS (ESI) calcd for [$C_{57}H_{70}N_7O_{17}P$ + Na^+] 1178.4458, found 1178.4420.



5d

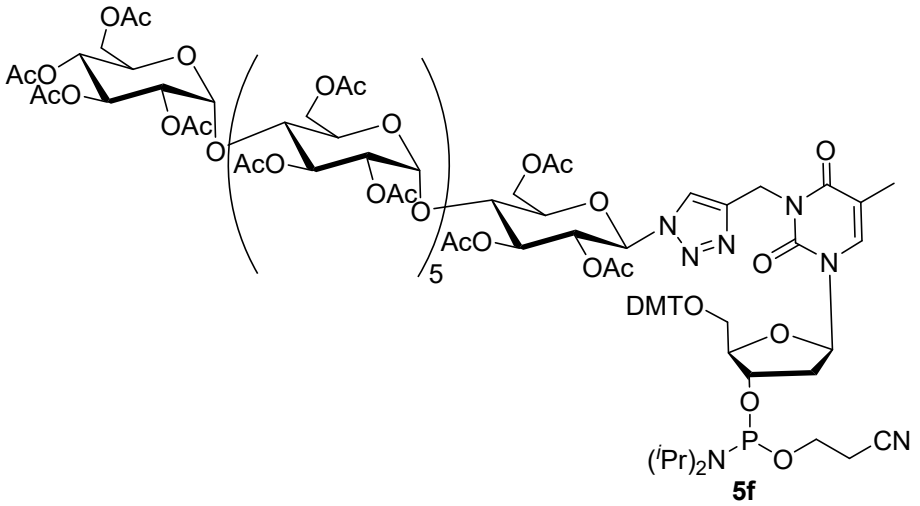
(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(((2R,3R,4S,5R,6R)-4,5-diacetoxy-2-(acetoxymethyl)-6-(4-((3-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(((2-cyanoethoxy)(diisopropyl amino)phosphaneyl)oxy)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl) methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (5d): Triazol-DMT **4d** (100 mg; 0.080 mmol; 1 equiv.) was dissolved in anhydrous DCM (6 mL) at room temperature, DIPEA (0.090 mL; 0.50 mmol; 6.25 equiv.) and CEP-Cl (0.071 mL; 0.32 mmol; 4.0 equiv.) were added and the mixture was stirred for 40 minutes. TLC analysis showed full conversion. Anhydrous MeOH (0.1 ml) added to stop the reaction. The crude was evaporated and the product was purified using silica column chromatography (4 g; DCM/TEA 2% or 3%) to give the product **5d** as a mixture of diastereomers (68 mg; 0.046 mmol; 57%). **TLC:** R_f 0.60 (2.5% TEA/DCM; UV). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.80 (s, 1H), 7.63-7.59 (d, *J* = 12 Hz, 1H), 7.38-7.33 (t, *J* = 6 Hz, 2H), 7.28-7.15 (m, 5H), 6.83–6.79 (m, 4H), 6.44 (m, 1H), 5.82–5.79 (d, *J* = 9 Hz, 1H), 5.76–5.73 (d, *J* = 9 Hz, 1H), 5.43-5.25 (m, 4H), 5.17-5.08 (m, 2H), 4.98-4.93 (dd, *J* = 12 Hz *J* = 3 Hz, 1H), 4.61 (m, 1H), 4.52-4.43 (dd, *J* = 15 Hz, *J* = 6 Hz, 2H), 4.14-4.07 (m, 4H), 3.93-3.87 (m, 3H), 3.86-3.81 (m, 2H), 3.76-3.73 (d, *J* = 9 Hz, 6H), 3.55-3.51 (d, *J* = 12 Hz, 5H , aliphatic imp), 3.31-3.22 (q, *J* = 12 Hz, *J* = 6Hz , 4H, Aliphatic imp), 2.70-2.56 (m, 5H), 2.15 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 6H), 1.95 (s, 3H), 1.82 (s, 3H), 1.42-1.02(m, 30H, impurity). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.39, 170.30, 170.13, 170.06, 169.57, 169.12, 169.06, 163.08, 158.81, 158.70, 150.65, 150.62, 143.71, 143.58, 135.36, 135.30, 135.26, 134.98, 130.10, 128.23, 128.15, 128.06, 127.96, 127.29, 127.12, 122.44, 118.35, 113.34, 113.24, 110.45, 101.06, 86.88, 85.40, 77.47, 77.04, 76.62, 75.81, 75.55, 72.66, 70.93, 70.84, 70.41, 69.04, 66.63, 61.78, 60.87, 58.57, 58.52, 58.20, 58.15, 57.63, 55.24, 50.71, 50.65, 46.38, 35.96, 24.58, 24.53, 24.49, 24.41, 21.47, 20.81, 20.66, 20.61, 20.48, 20.23, 20.15, 20.07, 19.06, 12.41. **^{31}P NMR (121 MHz, $CDCl_3$)** 152.62-152.09.



5e

(2R,3R,4S,5R)-2-(acetoxymethyl)-6-(((2R,3R,4S,5R,6R)-3,4-diacetoxy-5-(benzoyloxy)-6-((4-((3-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(((2-cyanoethoxy)(diisopropyl amino)phosphino)oxy)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-2,3-dihydropyrimidin-1(6H)-yl)

methyl)-1H-1,2,3-triazol-1-yl)methyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (5e): Compound **4e** (501 mg, 0.38 mmol) was dried under vacuum, put under an Ar atmosphere and dissolved in dry DCM (6.5 mL). Then DIEA (0.13 mL, 077 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.1 mL, 0.58 mmol) were added to the reaction mixture and the solution was stirred for 2.5 h at rt. After reaction completion, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$. The resulting mixture was extracted three times with DCM, dried over $MgSO_4$, filtered off and concentrated under reduce pressure. The crude product was purified by column chromatography over irregular silica (pentane/EtOAc 35/65 with 1% TEA) to afford compound **5e** (426 mg, 74%) as a white solid. **TLC:** Rf = 0.5 (Pent/EtOAC 35/65 + 1% TEA). **^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.03 (d, *J* = 7.6 Hz, 2H), 7.71 – 7.56 (m, 3H), 7.49 (t, *J* = 7.6 Hz, 2H), 7.43 – 7.35 (m, 2H), 7.32 – 7.27 (m, 4H), 6.81 (dd, *J* = 8.8, 3.3 Hz, 4H), 6.47 – 6.37 (m, 1H), 5.72 (t, *J* = 9.8 Hz, 1H), 5.43 (t, *J* = 9.7 Hz, 1H), 5.37 (d, *J* = 3.8 Hz, 1H), 5.31 – 5.15 (m, 2H), 5.15 – 5.04 (m, 2H), 5.03 – 4.94 (m, 2H), 4.88 (d, *J* = 3.8 Hz, 1H), 4.68 – 4.55 (m, 2H), 4.39 – 4.23 (m, 2H), 4.23 – 4.13 (m, 2H), 4.07 – 3.95 (m, 2H), 3.87 – 3.69 (m, 7H), 3.66 – 3.41 (m, 4H), 3.35 – 3.24 (m, 1H), 2.61 (t, *J* = 6.2 Hz, 1H), 2.57 – 2.43 (m, 1H), 2.40 (t, *J* = 6.5 Hz, 1H), 2.36 – 2.23 (m, 1H), 2.13 – 2.03 (m, 9H), 2.00 – 1.89 (m, 9H), 1.72 (s, 3H), 1.46 (s, 3H), 1.15 (d, *J* = 6.7 Hz, 9H), 1.04 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.62, 170.06, 169.62, 169.51, 169.25, 165.70, 163.22, 158.75, 150.71, 144.34, 143.67, 135.49, 135.44, 134.07, 130.13, 128.78, 128.37, 128.28, 128.21, 128.00, 127.17, 125.03, 117.65, 117.43, 113.27, 110.28, 110.24, 92.33, 91.61, 86.89, 86.87, 85.71, 85.67, 85.61, 85.41, 85.33, 73.97, 73.74, 73.50, 73.28, 70.60, 70.34, 69.94, 69.45, 69.20, 68.80, 68.66, 68.29, 63.31, 63.06, 61.99, 58.46, 58.30, 58.22, 58.04, 55.30, 55.28, 50.79, 43.42, 43.33, 43.25, 43.16, 40.22, 36.14, 29.73, 24.69, 24.64, 24.54, 24.48, 20.70, 20.62, 20.51, 20.39, 20.27, 20.18, 12.51; **^{31}P NMR (121 MHz, $CDCl_3$)** δ 152.08, 151.55.



(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-(((2R,3R,4S,5R,6R)-4,5-diacetoxy-2-(acetoxymethyl)-6-(((2R,3R,4S,5R,6R)-4,5-diacetoxy-2-(acetoxymethyl)-6-(4-((3-((2R,4S,5R)-5-((bis(4-methoxy phenyl) (phenyl)methoxy)methyl)-4-(((2-cyanoethoxy)(diisopropylamino) phosphaneyl) oxy)tetrahydro-furan-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (5f): Compound **4f** (30 mg, 0.010 mmol) was dried under vacuum, put under an Ar atmosphere and dissolved in dry acetonitrile (5 mL). Then diisopropylammoniumtetrazolide (14 mg, 0.081 mmol, 8.0 equiv) and 2-cyanoethyl *N*,*N*,*N*,*N*-tetraisopropylphosphorodiamidite (0.060 mL, 0.188 mmol, 18.0 equiv) were added to the reaction mixture and the solution was stirred for overnight at rt. After reaction completion, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$. The resulting mixture was extracted three times with DCM, dried over $MgSO_4$, filtered off and

concentrated under reduce pressure. The crude product was purified by column chromatography over silica to afford compound **5f** (15 mg, 47%) as a white solid. **TLC:** Rf = 0.7 (5% MeOH/DCM + 1% TEA, UV). **^{1}H NMR (300 MHz, Acetone-d6)** δ 7.75 (s, 1H), 7.66 (m, 1H), 7.50 (d, *J* = 7.5 Hz, 2H), 7.39-7.24 (m, 7H), 6.92 (d, *J* = 8.5 Hz, 4H), 6.40 (m, 2H), 5.92-5.51 (m, 3H), 5.40 (m, 2H), 4.85-4.77 (m, 1H), 4.73 (d, *J* = 7.0 Hz, 2H), 4.53-4.35 (m, 14H), 4.03-3.95 (m, 2H), 3.80 (s, 6H), 3.77 – 3.66 (m, 2H), 3.60-3.34 (m, 7H), 3.00-2.77 (m, 33H), 2.69-2.38 (m, 7H), 1.97-1.87 (m, 5H), 1.52-1.48 (m, 6H), 1.38 (d, *J* = 6.5 Hz, 14H), 1.31-1.07 (m, 38H), 0.89 (m, 7H); **^{31}P NMR (121 MHz, CD_3CN)** δ 152.92, 152.73.

B/ **Synthesis and characterization of Oligonucleotides**

Synthesis. Phosphorothioate DNA and DNA/2'MOE gapmer sequence were synthesized using the standard β-phosphoramidite cycle of detritylation, coupling, sulfurization and capping on a 1.0/2.0 µmole scale. Dichloroacetic acid (DCA) (3% in CH_2Cl_2) was used for detritylation, 5-Ethylthio-1H-tetrazole (ETT) (0.25 M in MeCN) was used as an activator, and sulfurization was achieved using sulfurizing reagent II, 3-((Dimethylaminomethylidene)amino)-3H-1,2,4-dithiazole-3-thione, DDTT (2 g, 60% pyridine/40% MeCN). Cap A (N-methylimidazole in MeCN) and Cap B (B1: acetic anhydride in MeCN and B2: 2,6-lutidine in MeCN) was used for the capping step. Standard DNA phosphoramidites and the modified phosphoramidites (dT Glucose and dT Trehalose phosphoramidites) were dissolved in ACN at 0.08M with 18 equivalents used for coupling at 1µmolar or 2µmolar scale. Cleavage and deprotection were done in ammonia for 8h at 55°C since universal support (UnySupport, dA or dT support, Glen Research) was used. The concentrated oligos were resuspended in distilled water for dialysis (spectra por 6, MWCO 2kD, Sartorius) against NaCl 9%. The obtained sequences were characterized by RP-HPLC and ESI or LC mass spectrometry.

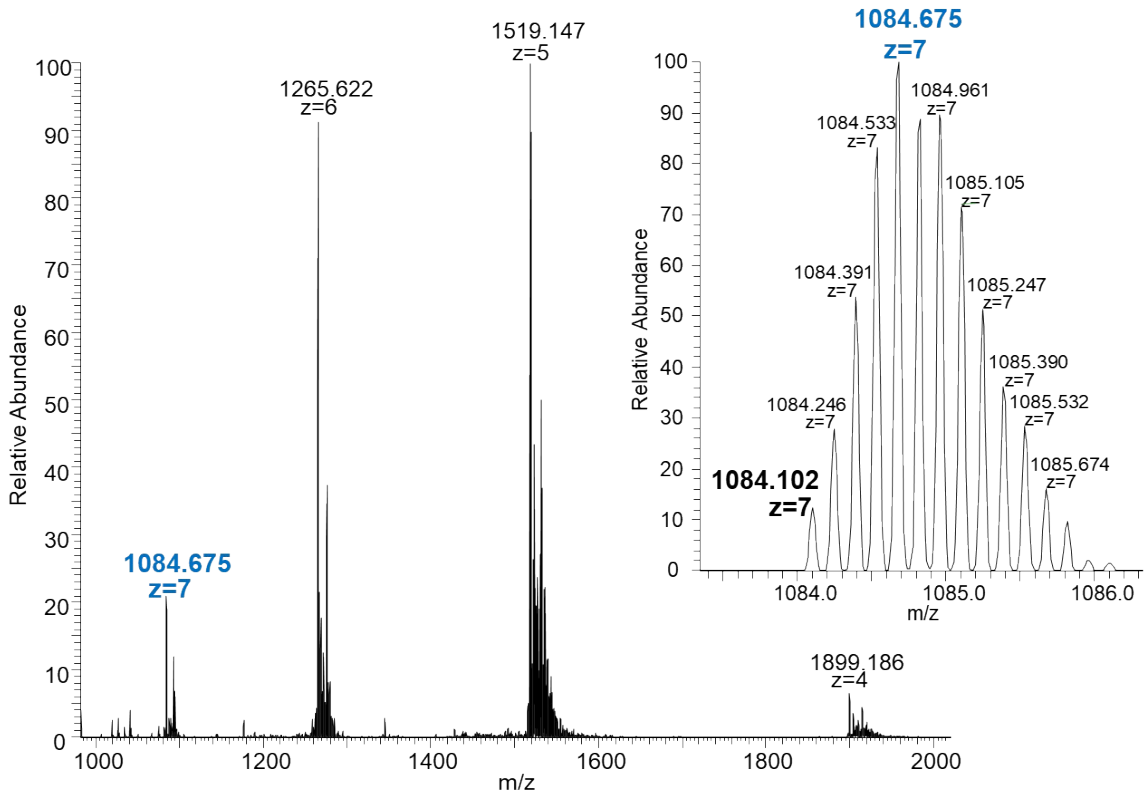
Mass analysis. ESI mass spectrometry analysis were carried out on a Thermo Fisher Exactive (IECB, Pessac) and a Q-Exactive (Cesamo, Talence). Oligonucleotide samples were prepared using 3.5 kD vivacon membrane (Sartorius) for dialysis against 50 mM ammonium acetate, lyophilized and resuspended in a methanol, water mixture (1:1). Spectra show multi-charged ions (M-z)/z obtained in the negative mode. Measured experimental monoisotopic masses were determined using the value of the first peak of the isotope distribution. LC-MS analysis were carried out on ISQ-EM spectrometer from Thermo Fischer (Simple Quadrupole - Extended Mass range of 10 - 2000 m/z, HESI - source of ionization and full scan negative mode. IP-RP-HPLC analysis were carried out using a colonne acquity BEH C18 column (50 x 2,1 mm, 1,7 µm) with the following conditions: Mobile phase (A) 8,5 mM TEA/100 mM HFIP (pH 8,2) and (B) 50%(A)/50%ACN (v/v) increasing from 5% to 18% B in 20 min. Flow: 0,30 mL/min. Column Temperature : 65°C. Sample : ONs at 10 µM in ultrapure water. Volume injected : 2 µL. Detection UV at 260 nm. Detection by Mass Spectrometry in mode Full Scan, Centroïd, negative polarity : -3000V, Vaporizer and Ion transfer tube temperature : 350°C, Gas pressure : 75 psig (Sheath for vaporization), 7,5 psig (auxiliary for drying), 0 psig (counter-current sweep).

Table S1: Mass calculations, mono-isotopic theoretical mass and experimental mass values.:[a]LC-MS, [b]High resolution ESI-MS, [c]crude product. ON1, ON4 and ON7 were synthesized in 2µmolar scale whereas ON2-ON3 and ON5-ON6 were synthesized on 1µmolar scale.

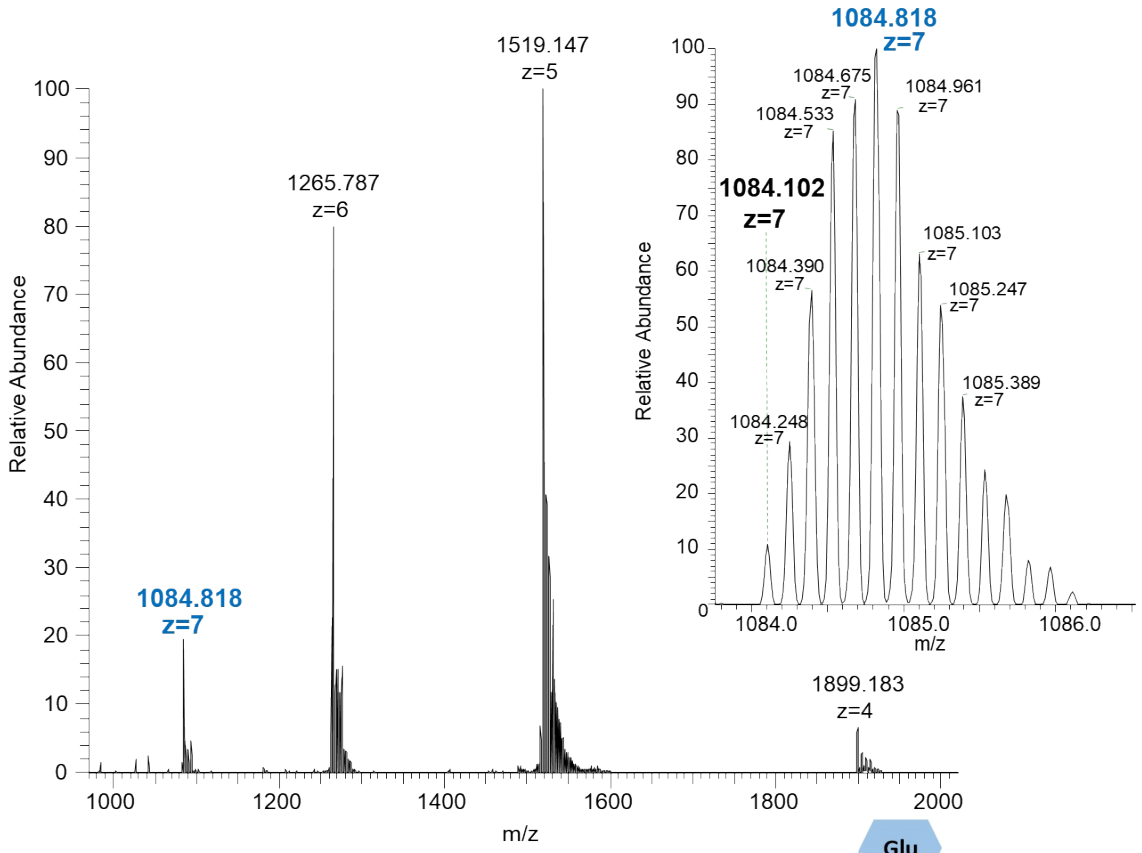
Name	Product (nmoles)	Yield (%)	M calc	M exp	ΔM (ppm)

ON1	568	28.4	7037.7	7036.11[a]	159
ON2	372	37.2	7595.763	7595.769[b]	-0.7
ON3	342	34.2	7595.763	7595.783[b]	-2.6
ON4	403	20.1[c]	7280.9	7282.89[a]	-199
ON5	37.65	3.76	8608.193	8608.213[b]	-2.3
ON6	40	4	8769.274	8769.132[b]	16.2
ON7	540	27	7104.7	7103.7[a]	100

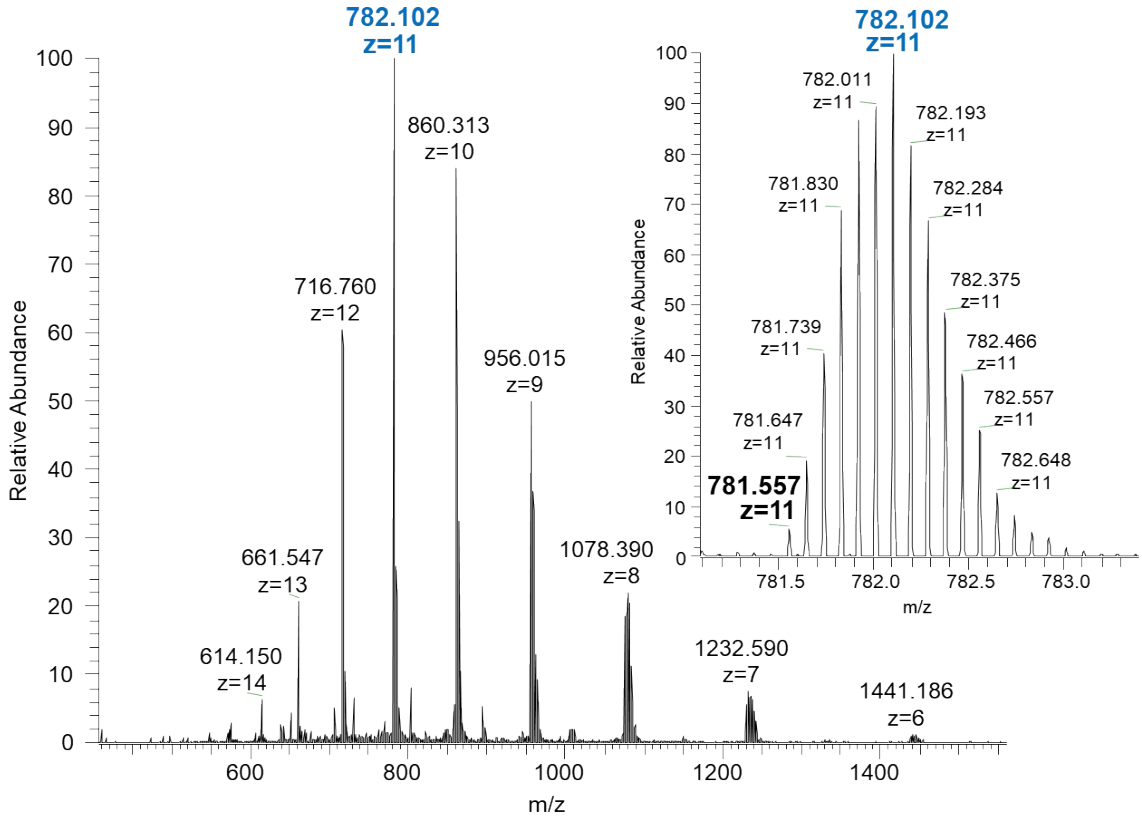
Mass spectrum of ON2: 5' Glu **T**TG TTG GAT CAT ATT CGT CCA CA 3'



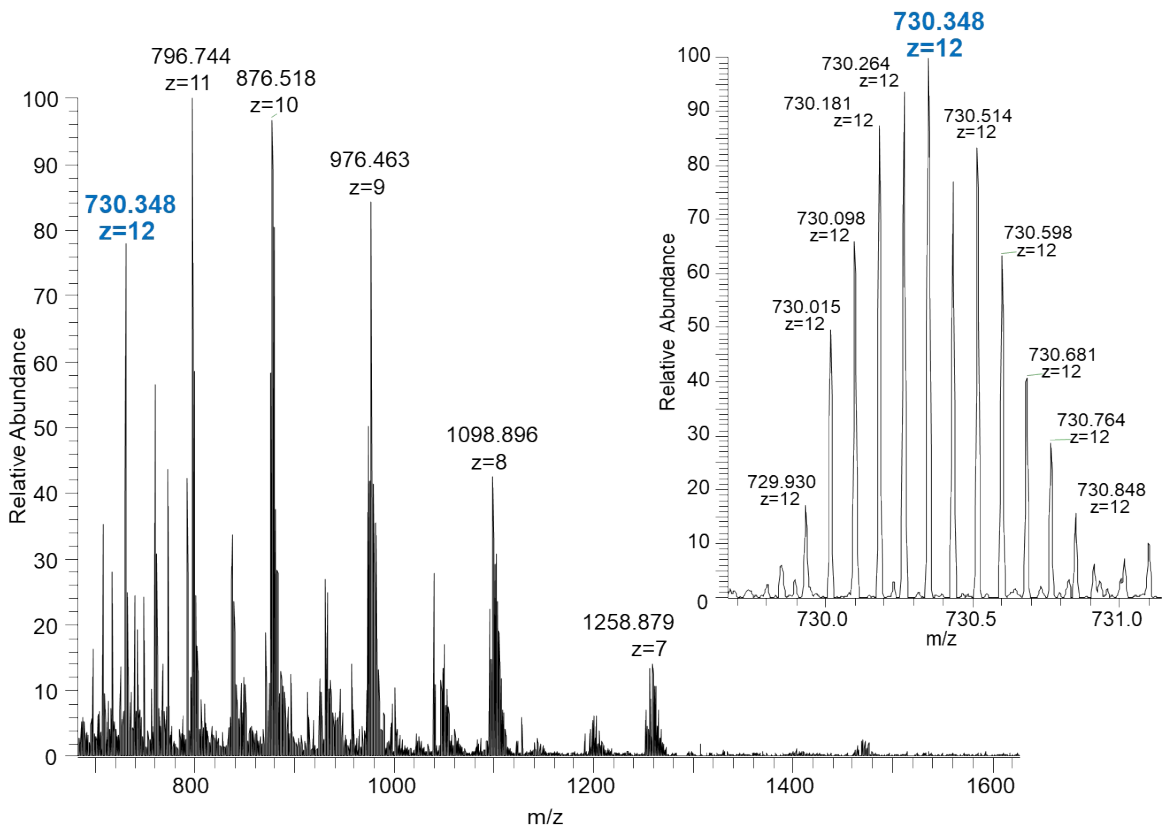
Mass spectrum of ON3: 5' TGT TGG ATC ATA TTC GTC CAC AT Glu 3'



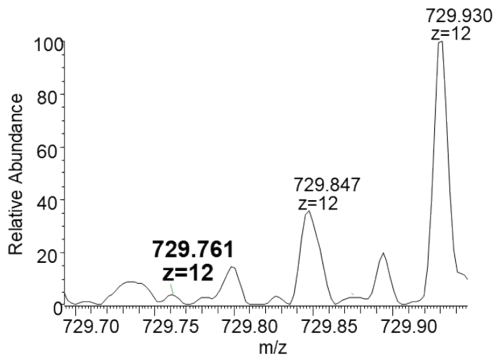
Mass spectrum of ON5: 5' ^{m}U^{m}C^{m}C^{m}U^{m}CC AAC ATT TGT CAC ^{m}U^{m}UG^{m}C^{m}UT Glu 3'



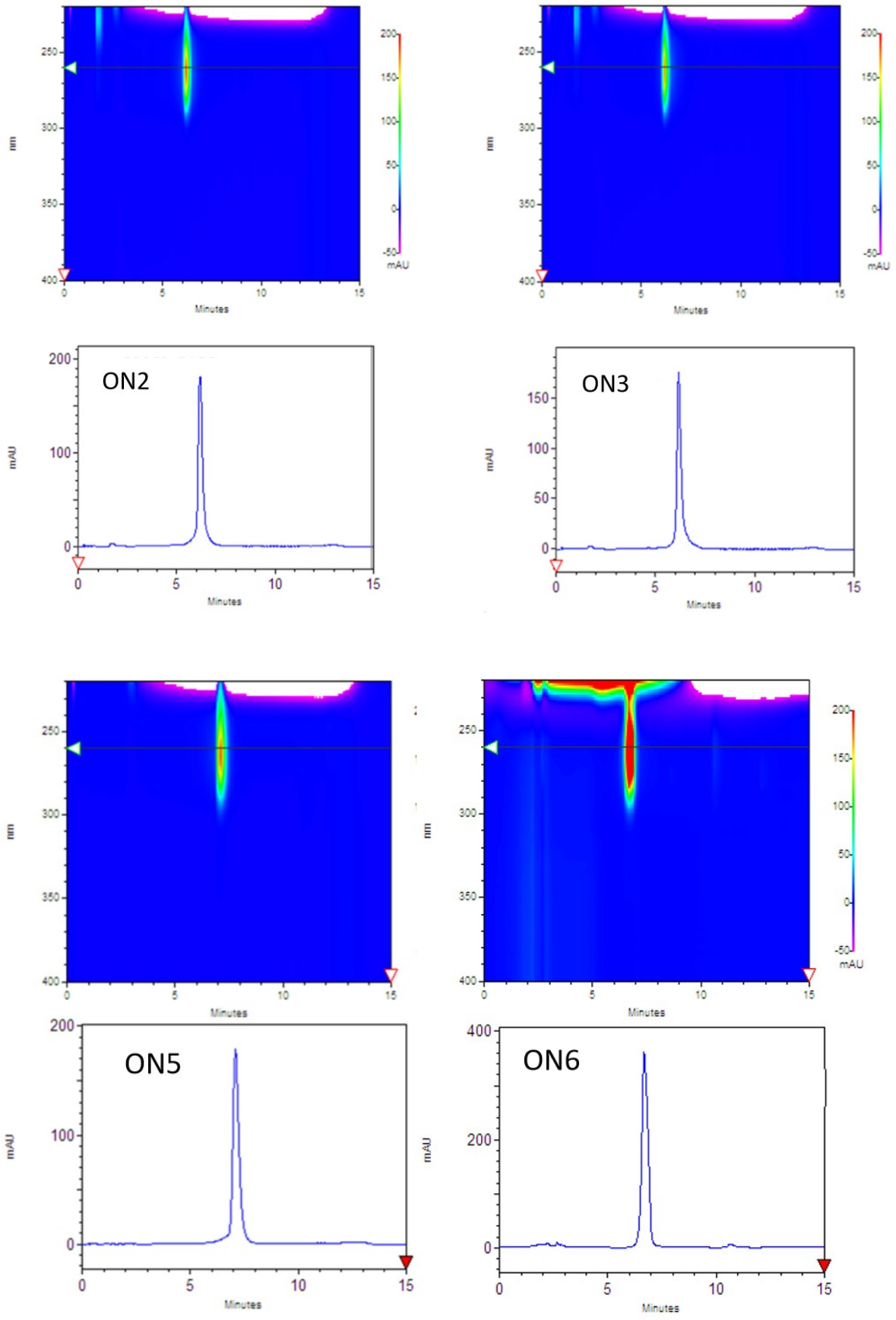
Mass spectrum of ON6: 5' Glu Glu $T^mU^mC^mC^mU^mC$ CAA CAT TTG TCA $C^mU^mUG^mC^mU$ 3'



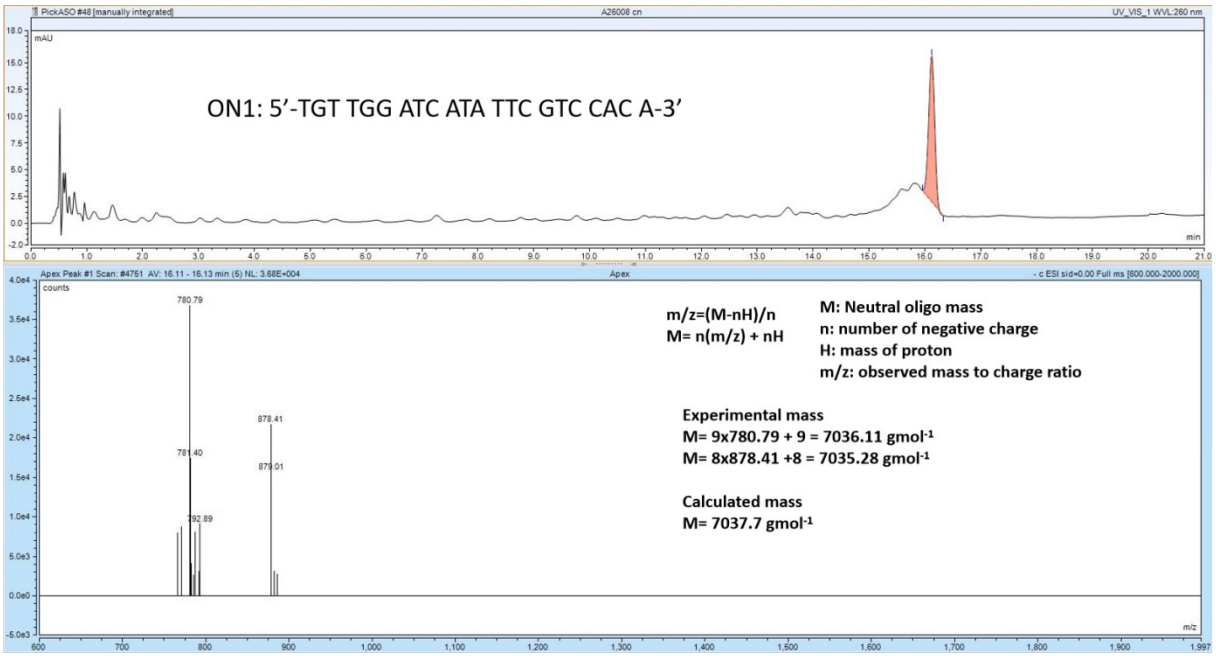
Zoom of isotope distribution z=12 of ON6 spectra:



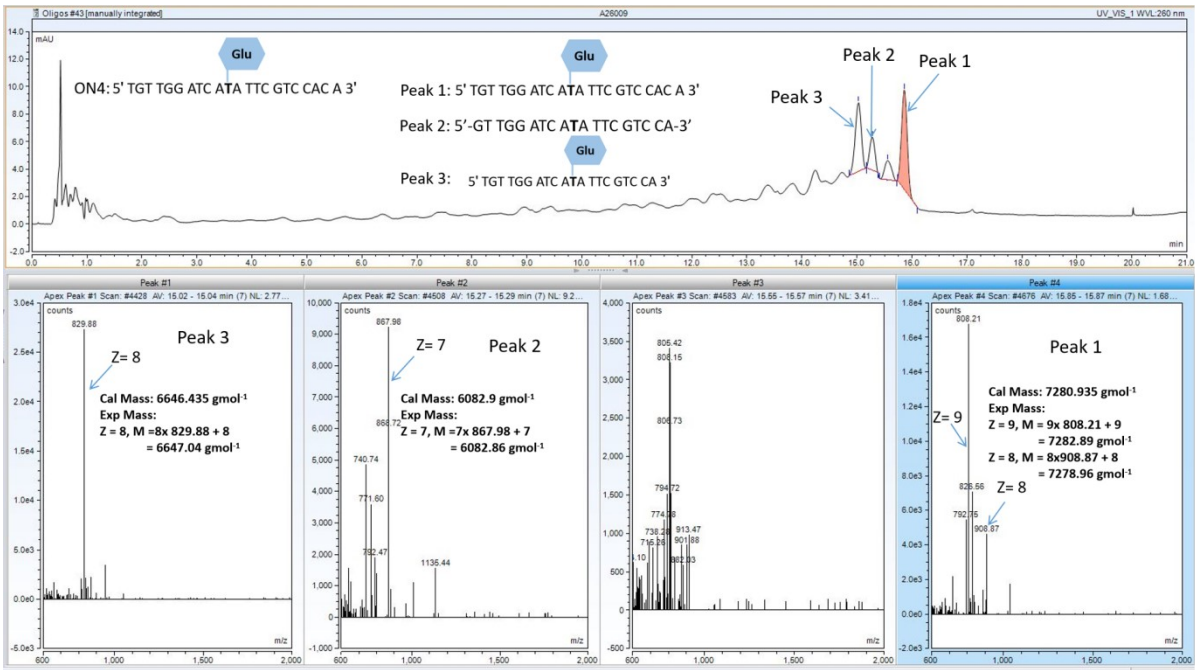
HPLC chromatograms of ON2-ON3 and ON5-ON6:



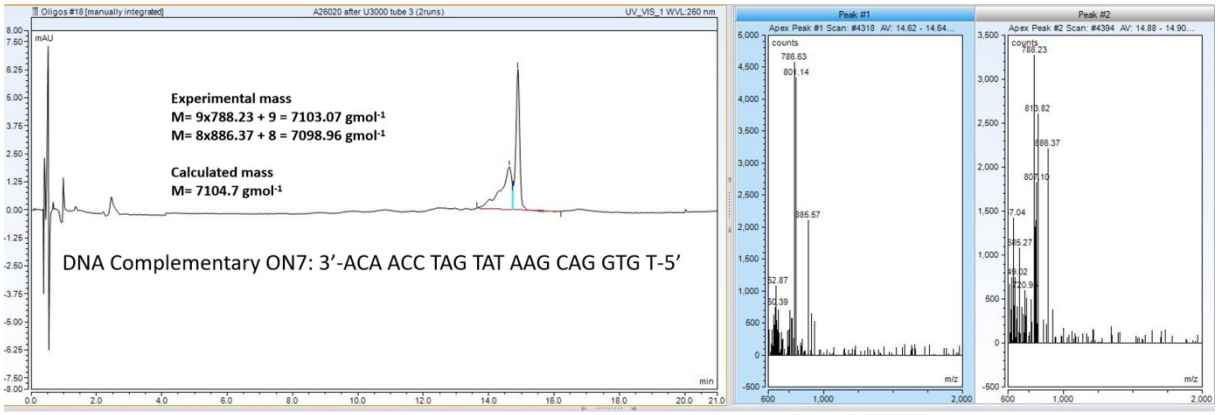
HPLC chromatogram and mass spectrum of ON1: 5’-TGT TGG ATC ATA TTC GTC CAC A-3’



HPLC chromatogram and mass spectrum of ON4: 5' TGT TGG ATC ATA TTC GTC CAC A 3'



HPLC chromatogram and mass spectrum of ON7: 3'-ACA ACC TAG TAT AAG CAG GTG T-5'



UV-melting studies:

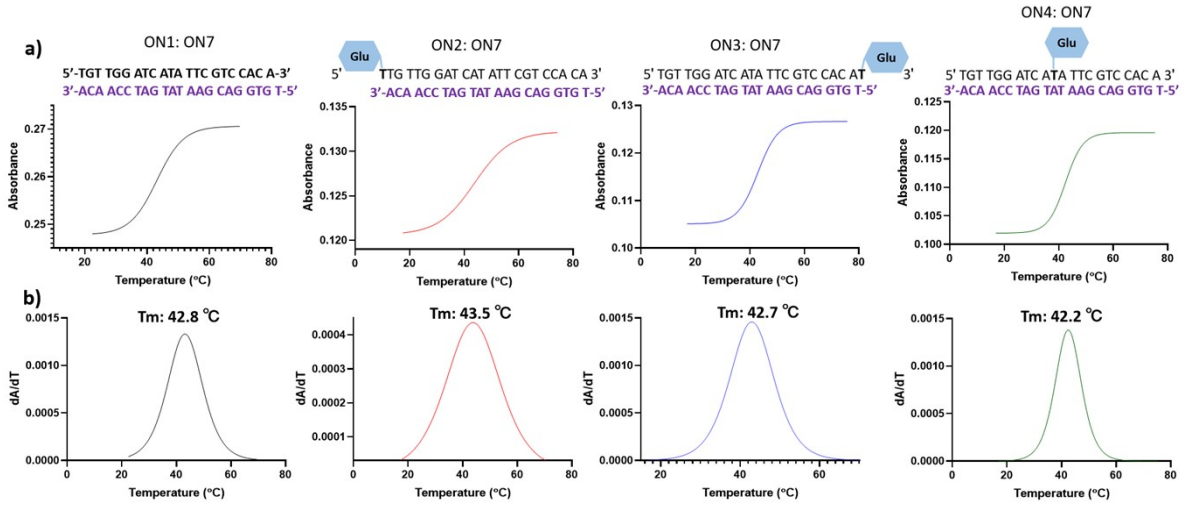
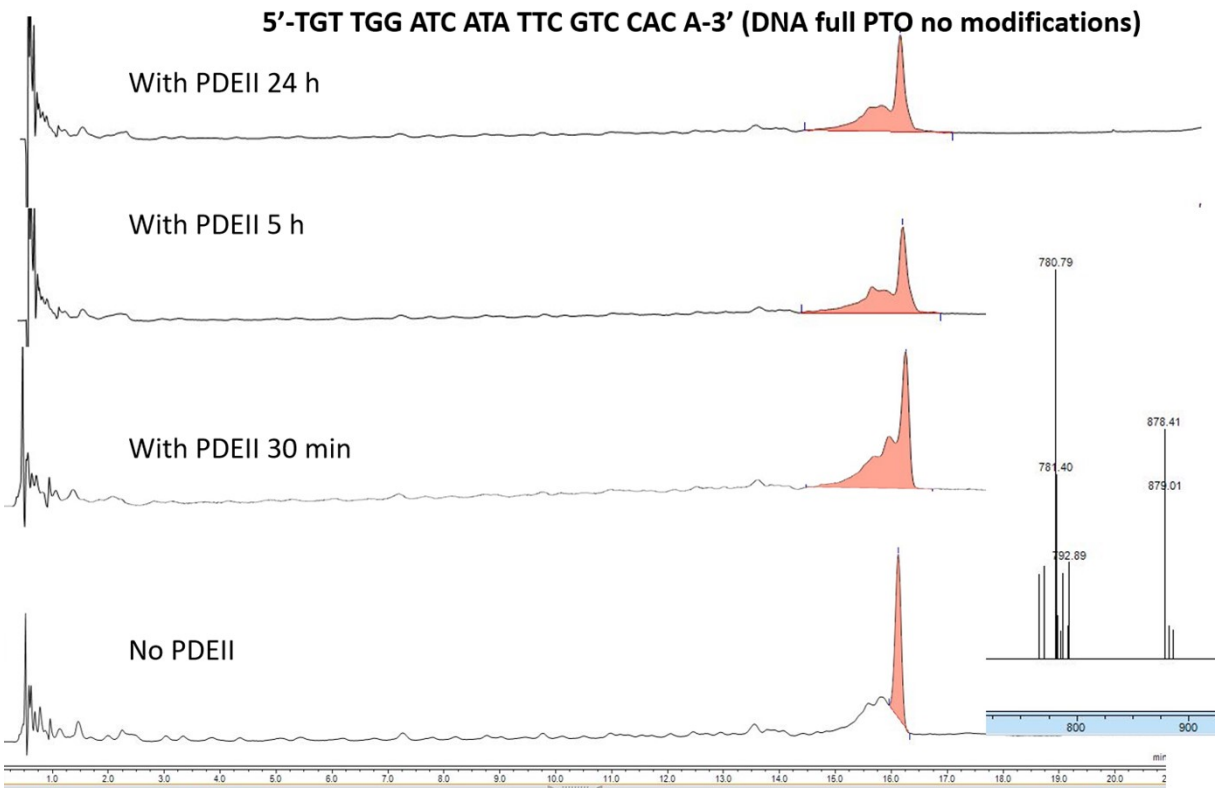


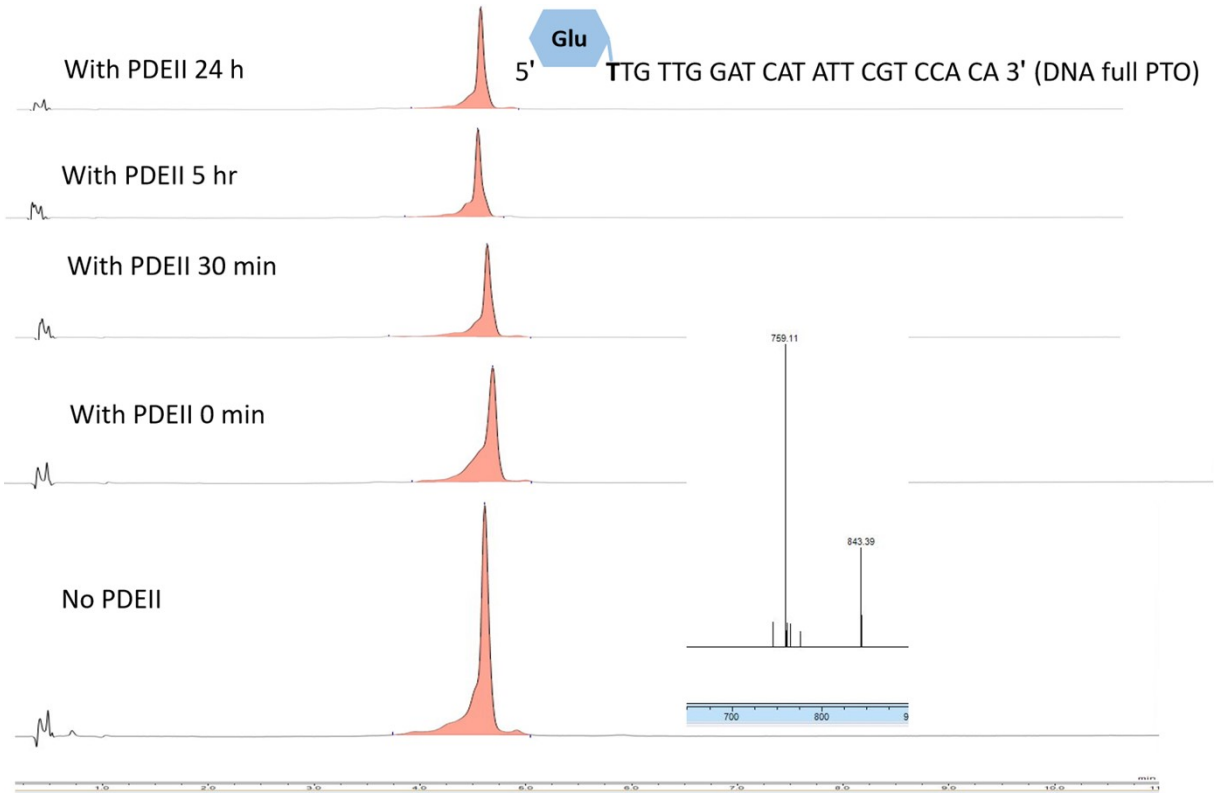
Figure S1: UV melting studies for modified/unmodified ONs (ON1-ON4) against complementary DNA (ON7). a) Representative UV melting curves measured using 0.5 µM of each ON in 10 mM Na-phosphate buffer, pH = 7.24 and 100 mM KCl; b) 1st derivative of melting curves.

Nuclease stability assays of oligonucleotide sequences: HPLC analysis

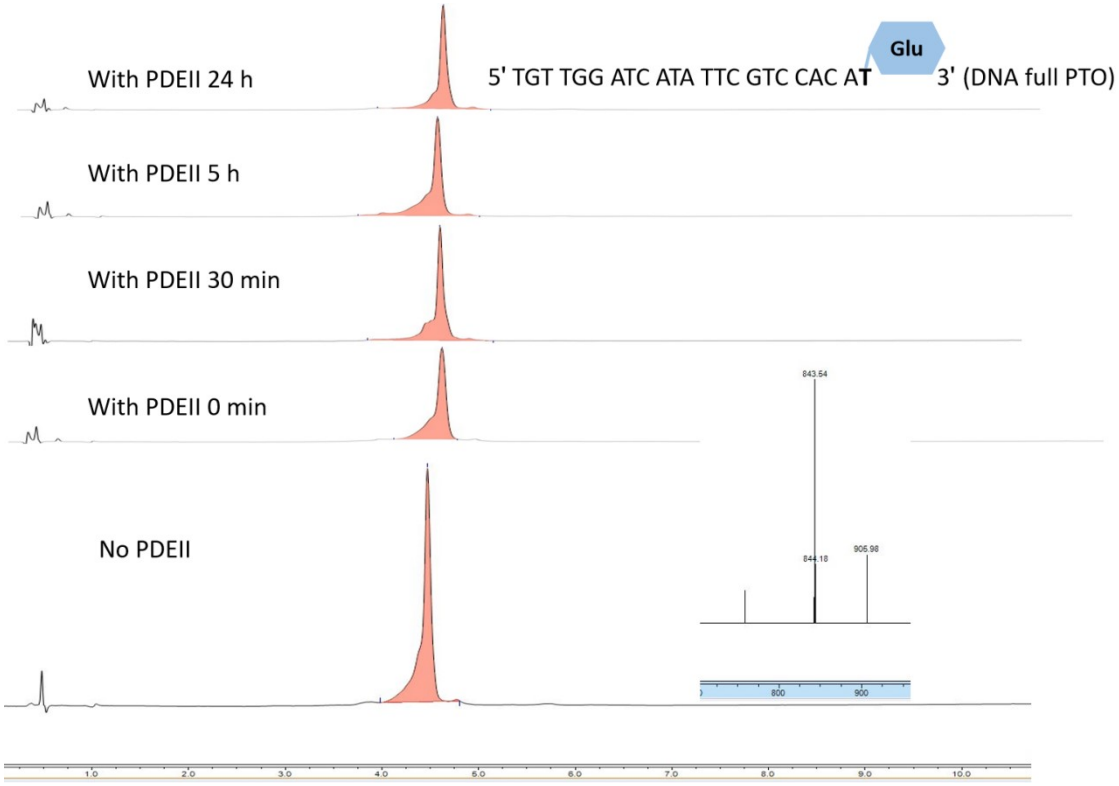
A)



B)



C)



D)

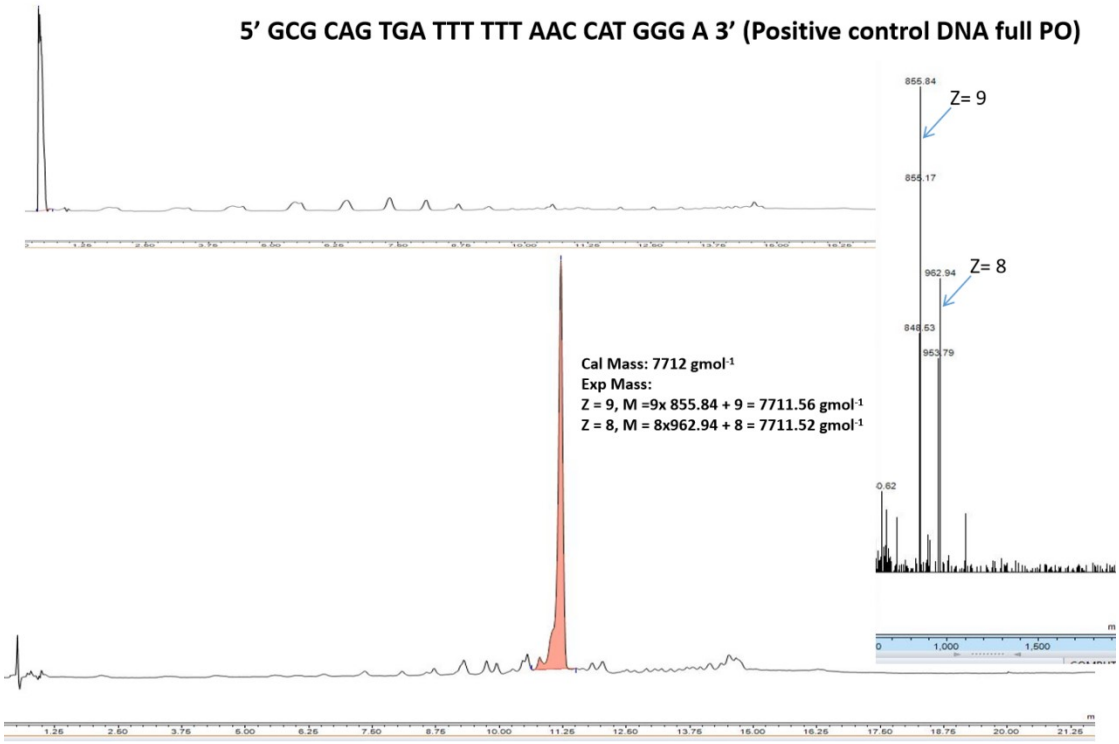
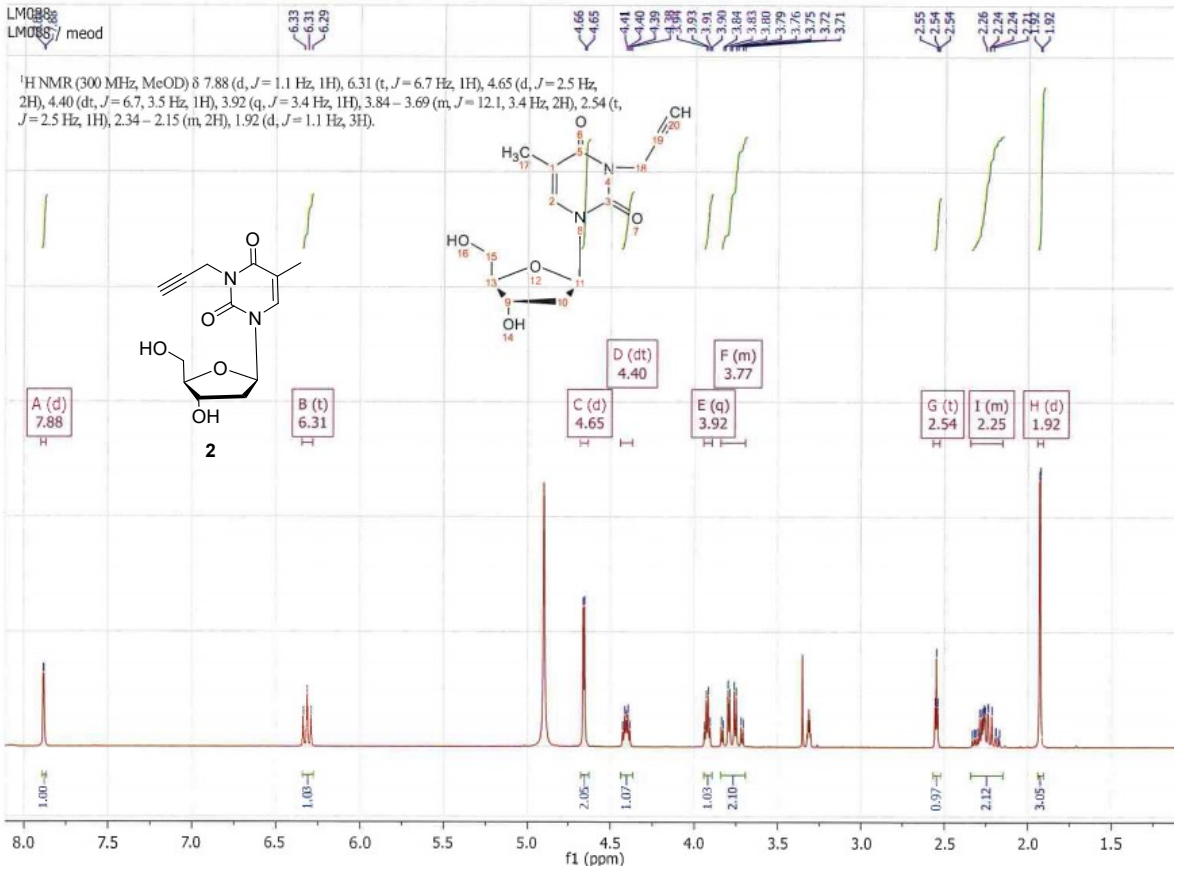
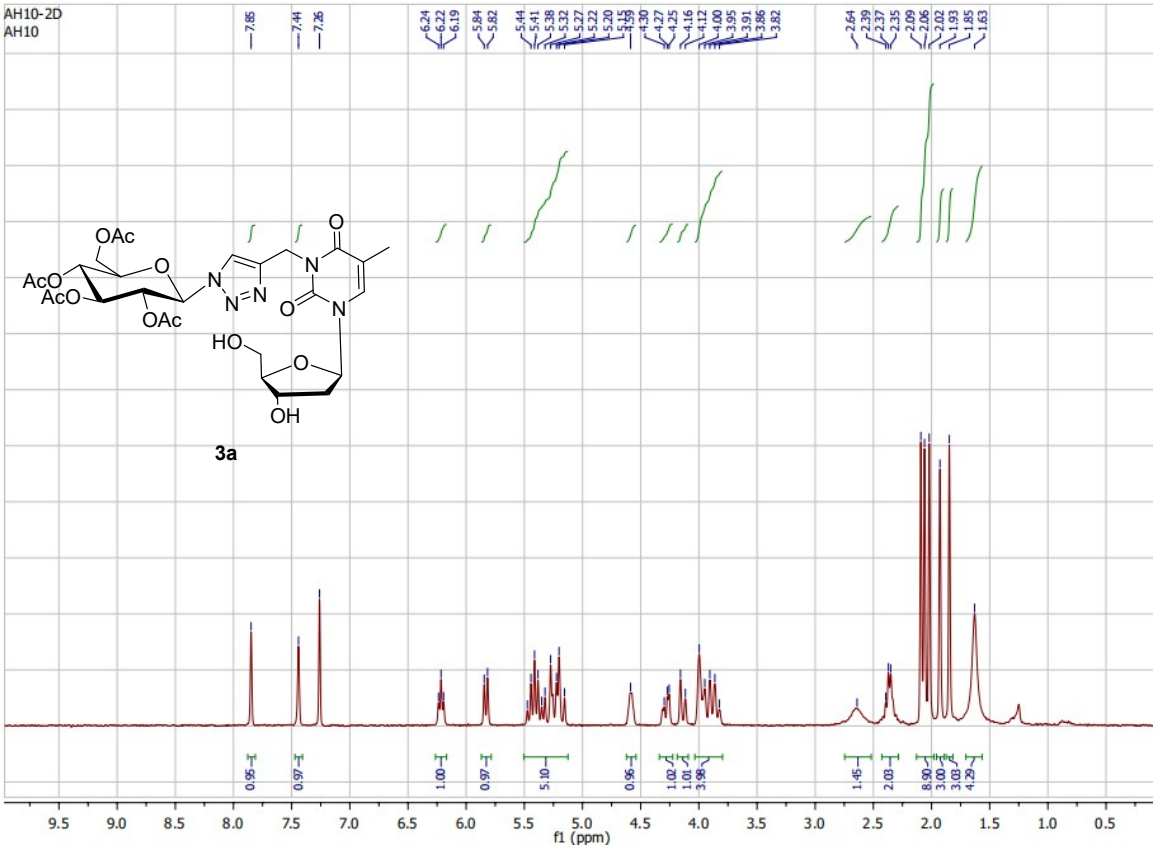


Figure S2: Comparative nuclease stability assays of oligonucleotide sequences (10 µM) performed in the presence of phosphodiesterase II from bovine spleen, a 5' exonuclease (0.6mU/µL), 50 mM Na-acetate buffer and 10 mM $MgCl_2$ incubated at 37 ℃ . (A-C) ON1-ON3 with full phosphorothioate linkages irrespective of modifications remain undigested even after 24 hours of incubation. (D) a positive control with full phosphodiester linkages underwent rapid degradation within 30 minutes of incubation.

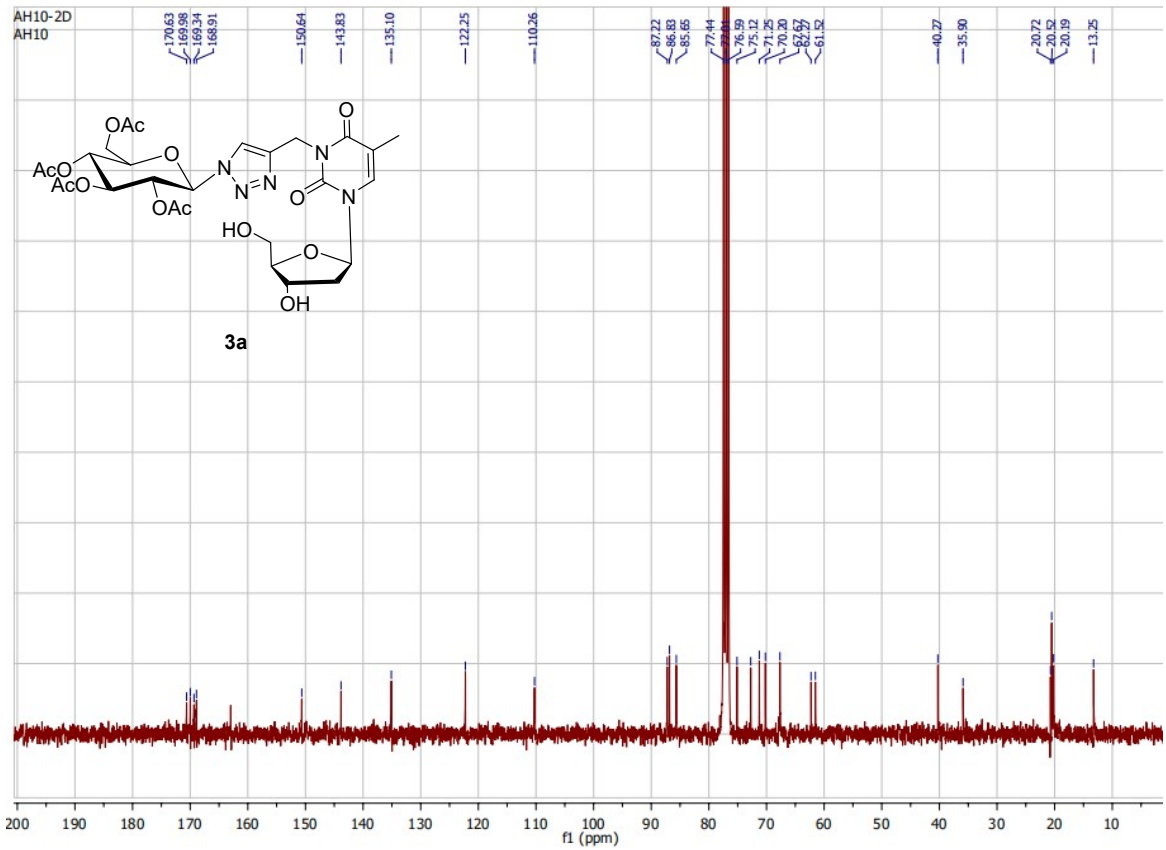
C/ NMR Spectra



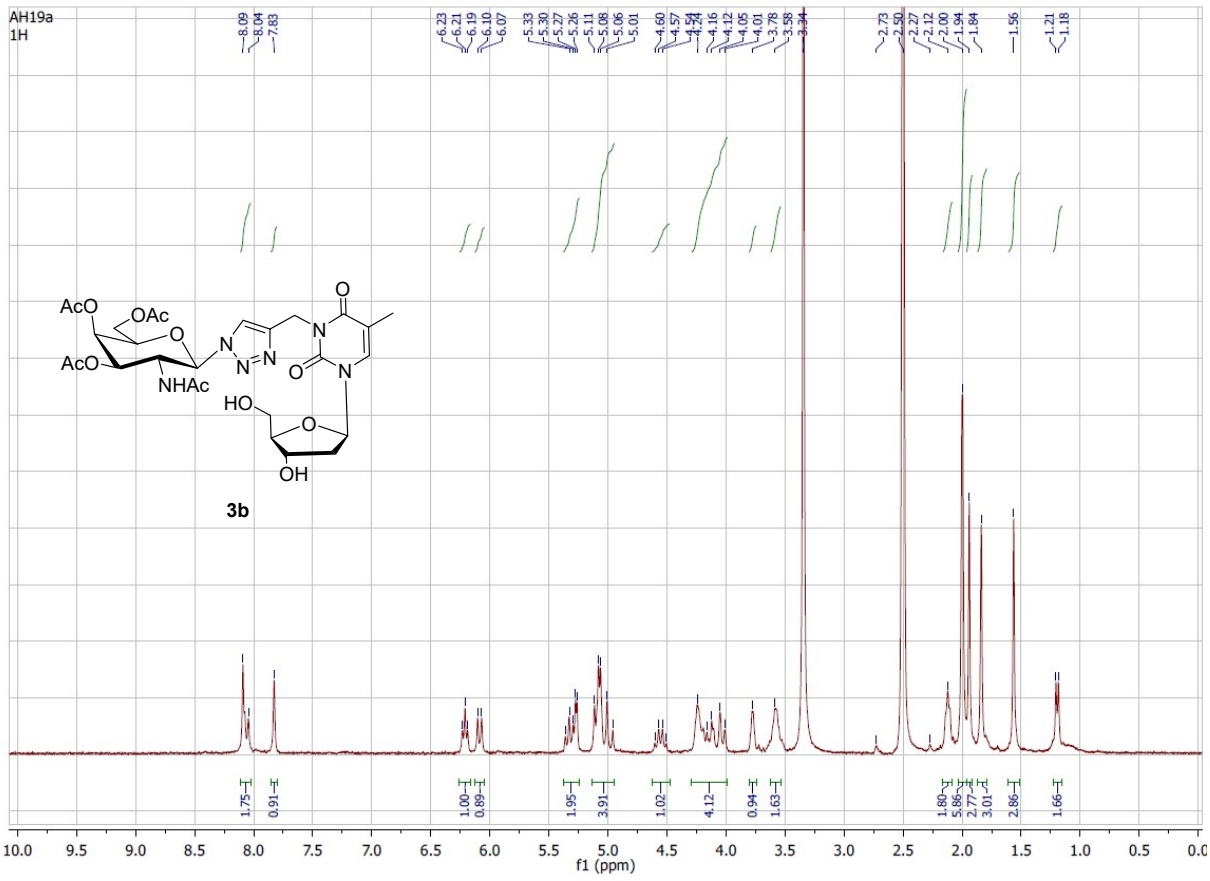
^{1}H NMR of compound 2 (300 MHz, CD_3OD)



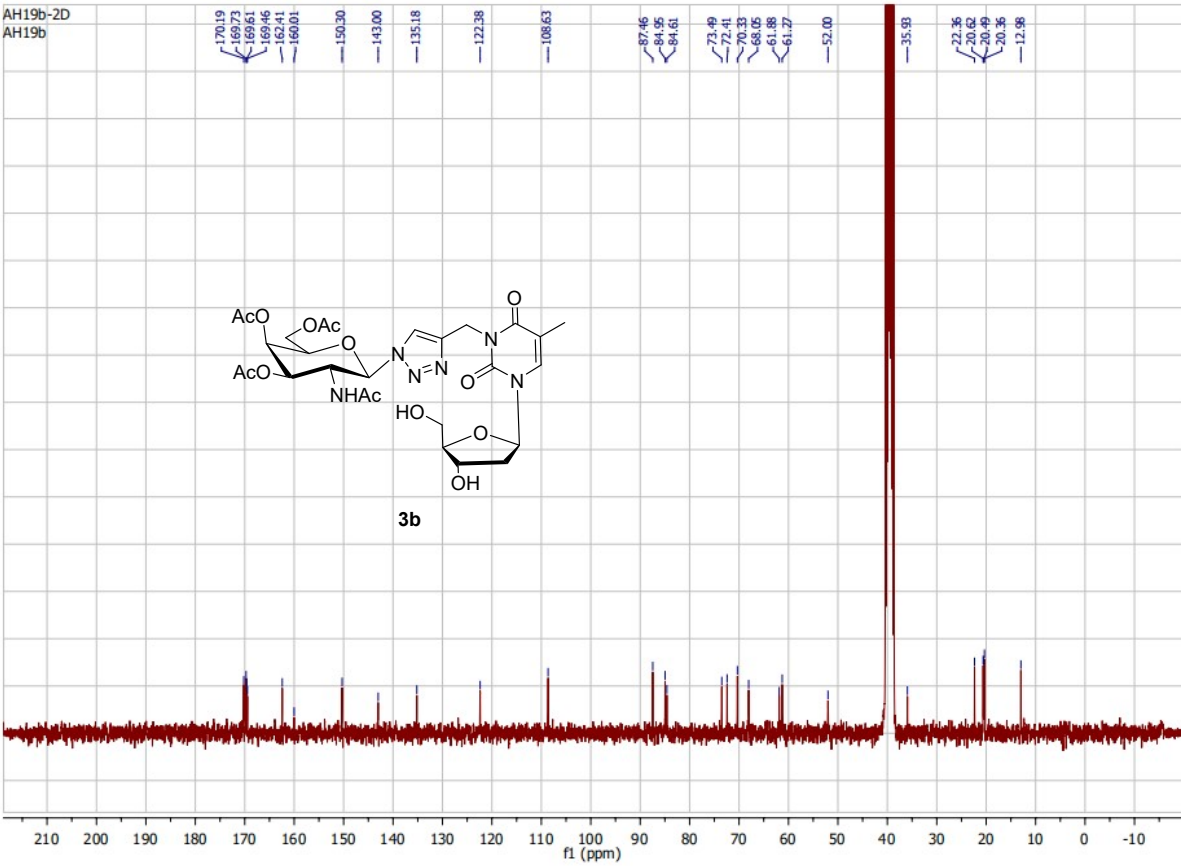
^{1}H NMR of compound 3a (300 MHz, $CDCl_3$)



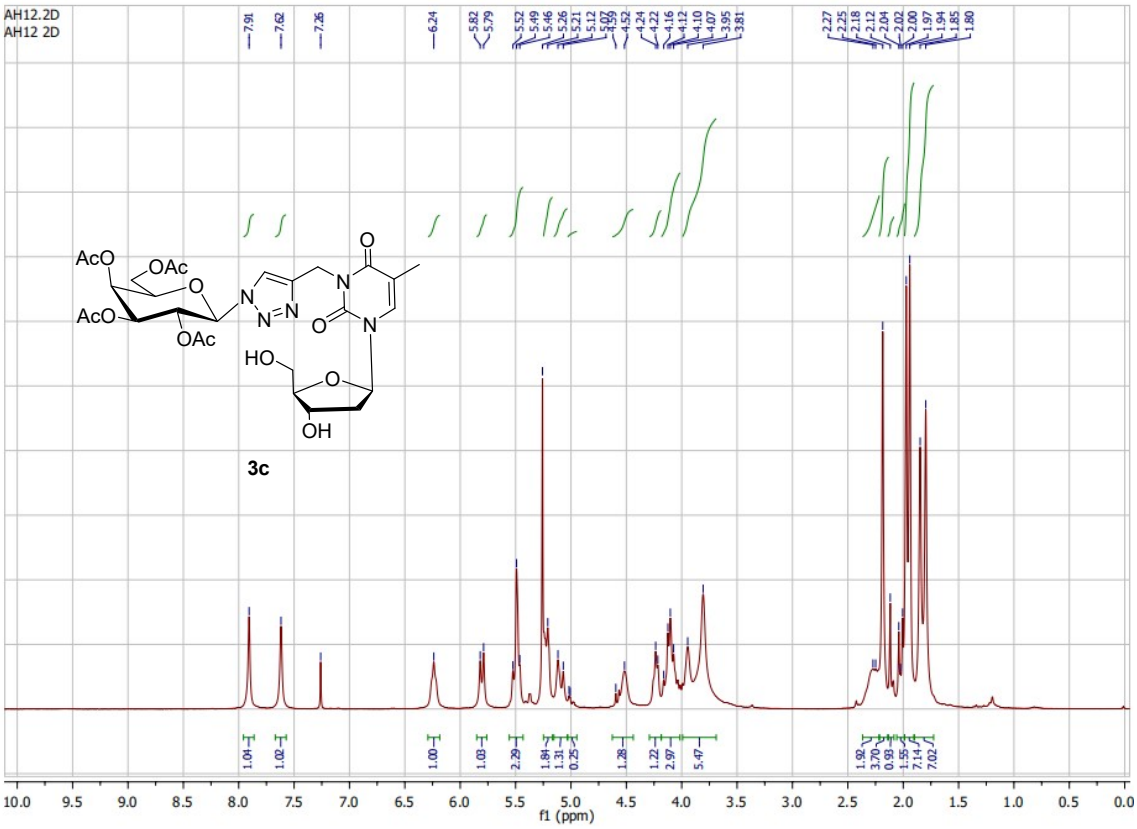
^{13}C NMR of compound 3a (75 MHz, $CDCl_3$)



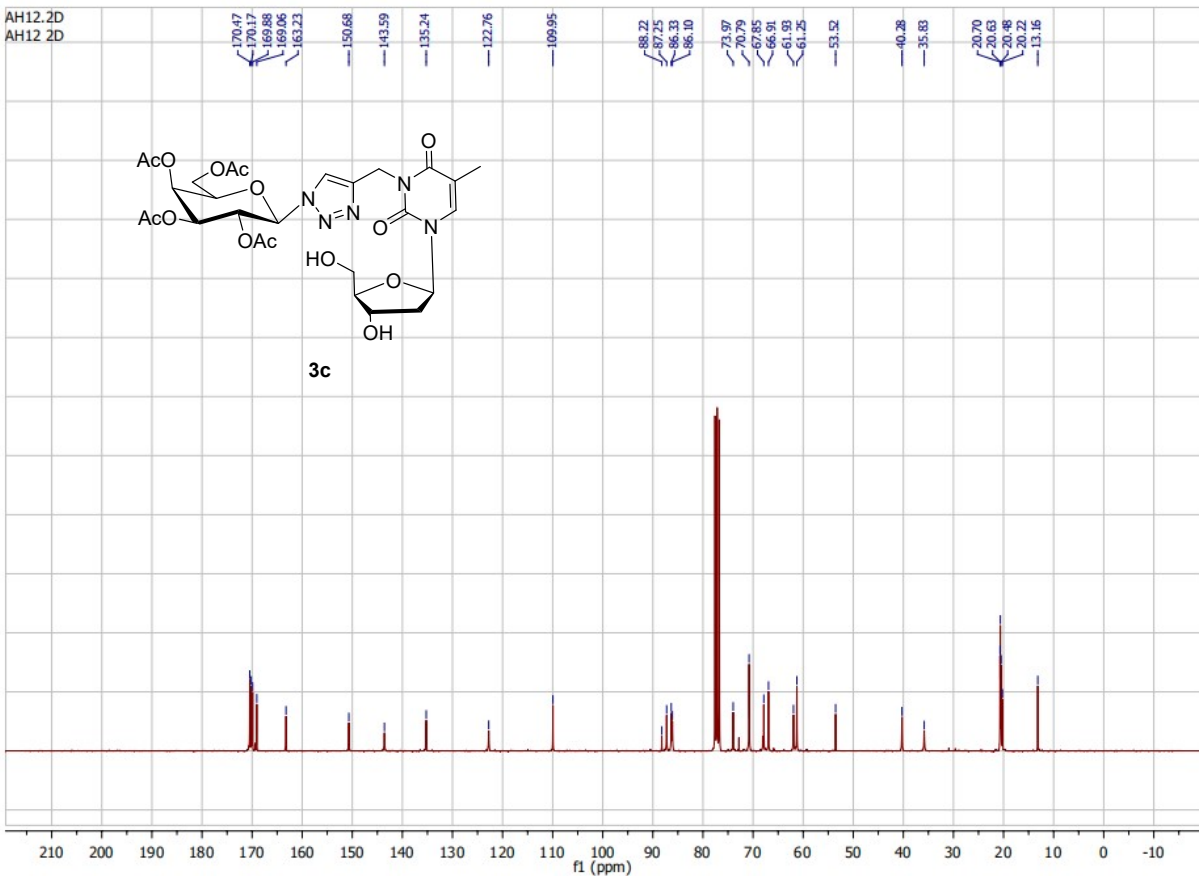
^{1}H NMR of compound 3b (300 MHz, $CDCl_3$)



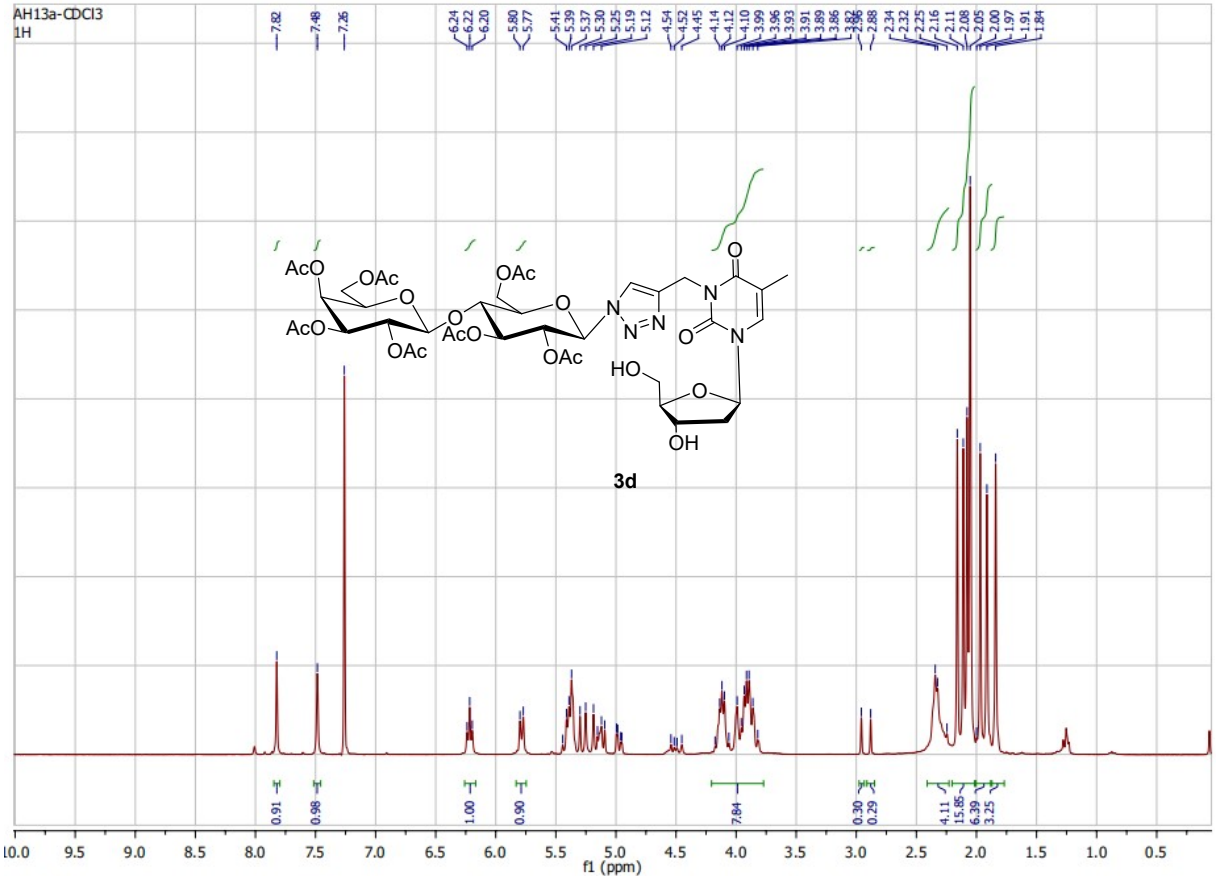
^{13}C NMR of compound 3b (75 MHz, $CDCl_3$)



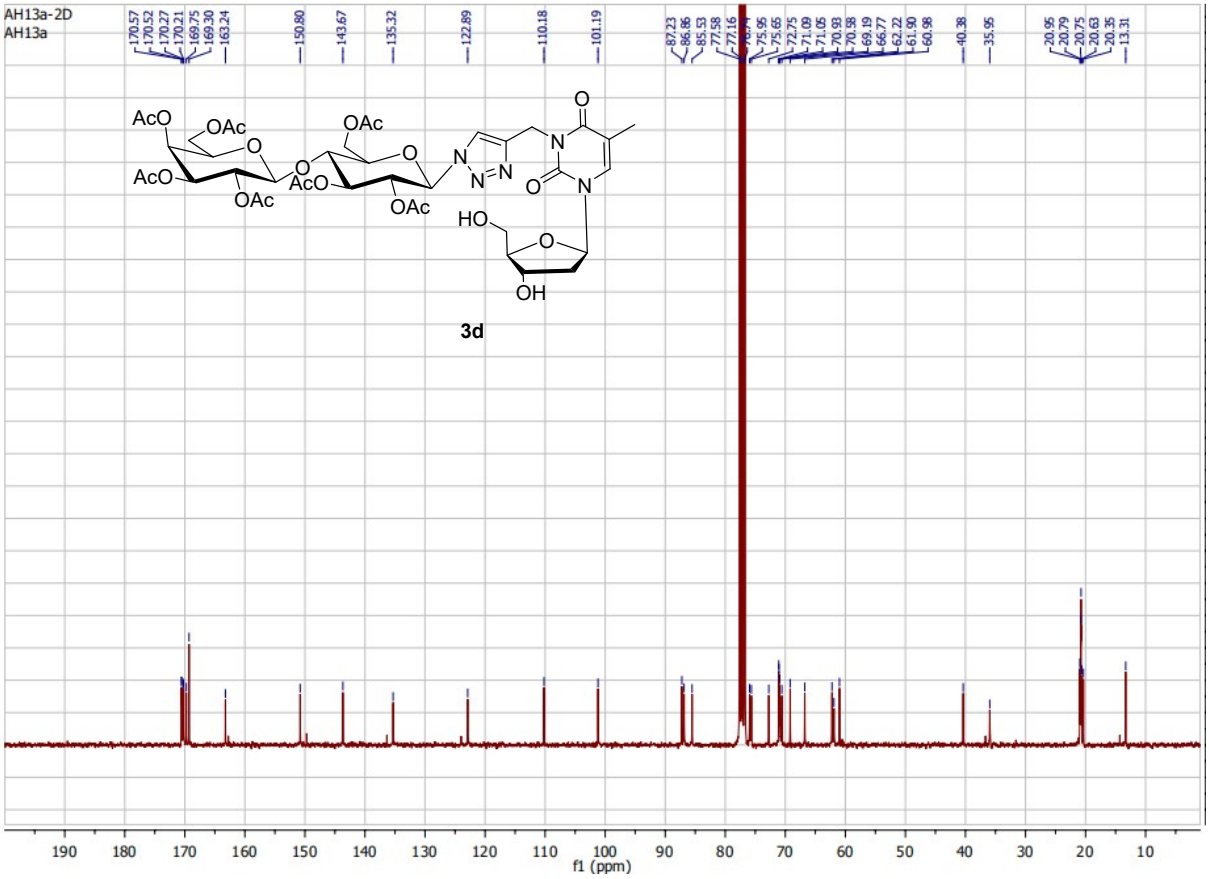
^{1}H NMR of compound 3c (300 MHz, $CDCl_3$)



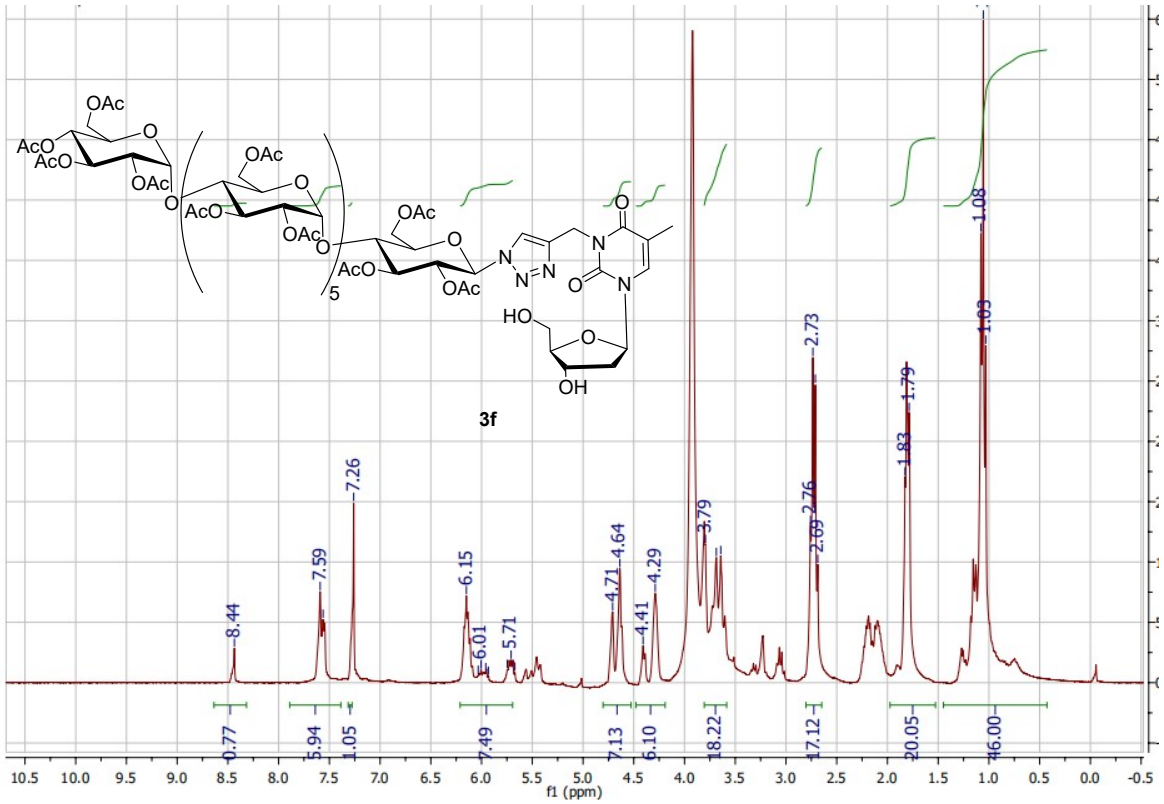
^{13}C NMR of compound 3c (75 MHz, $CDCl_3$)



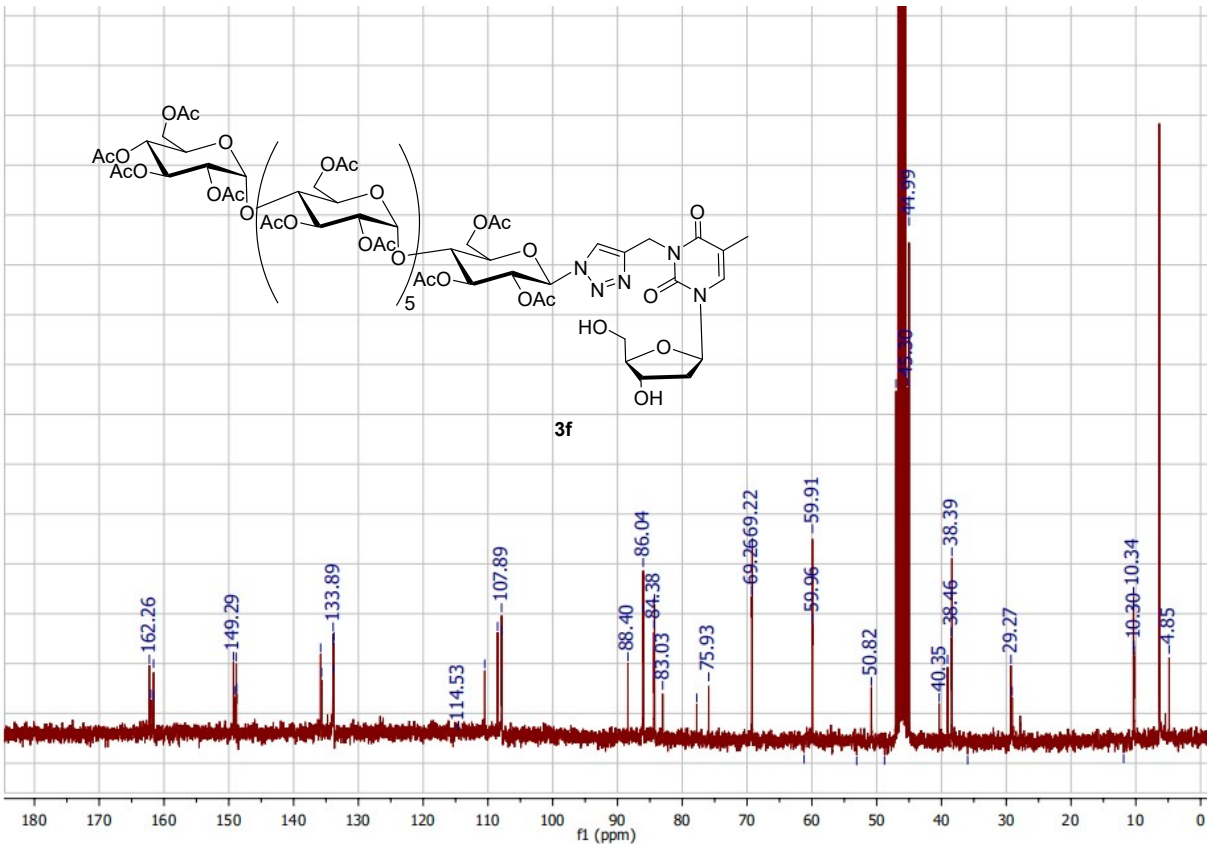
^{1}H NMR of compound 3d (300 MHz, $CDCl_3$)



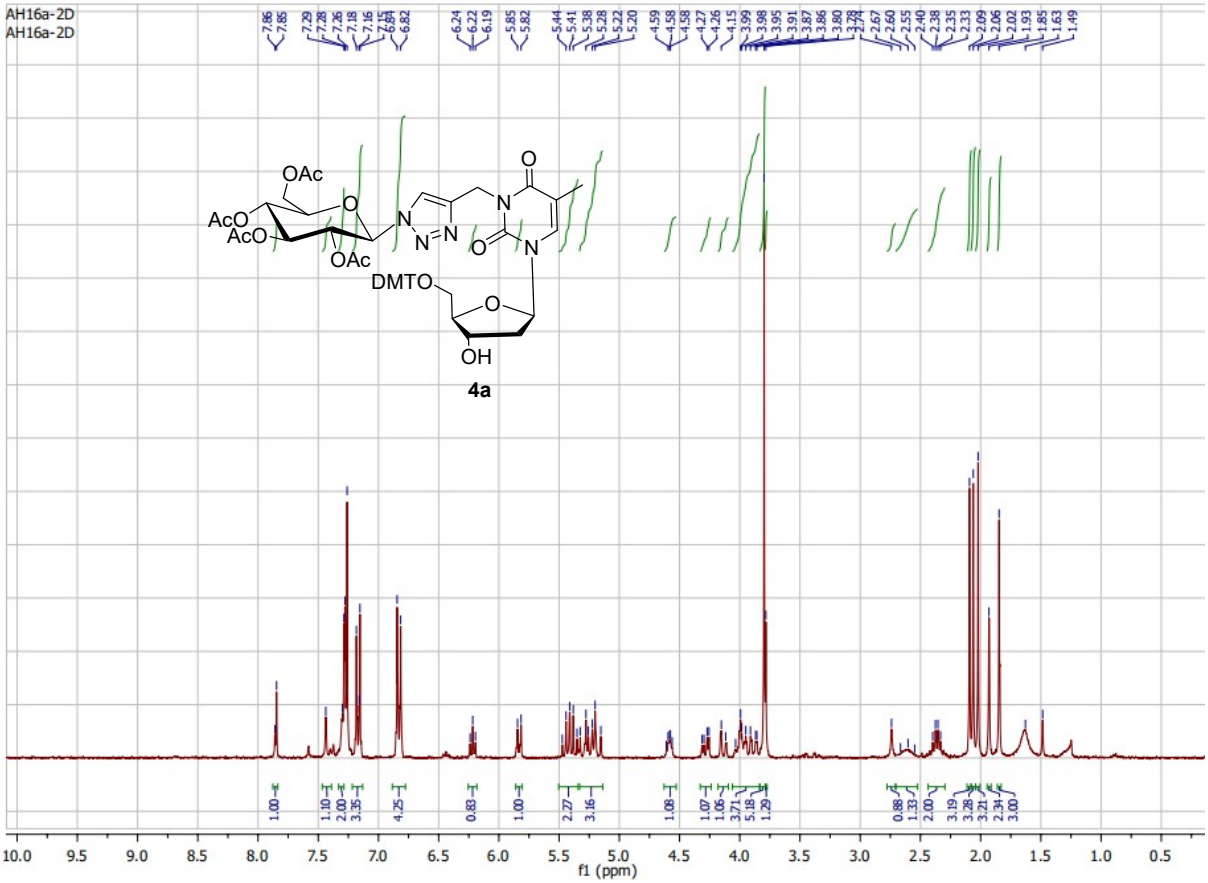
^{13}C NMR of compound 3d (75 MHz, $CDCl_3$)



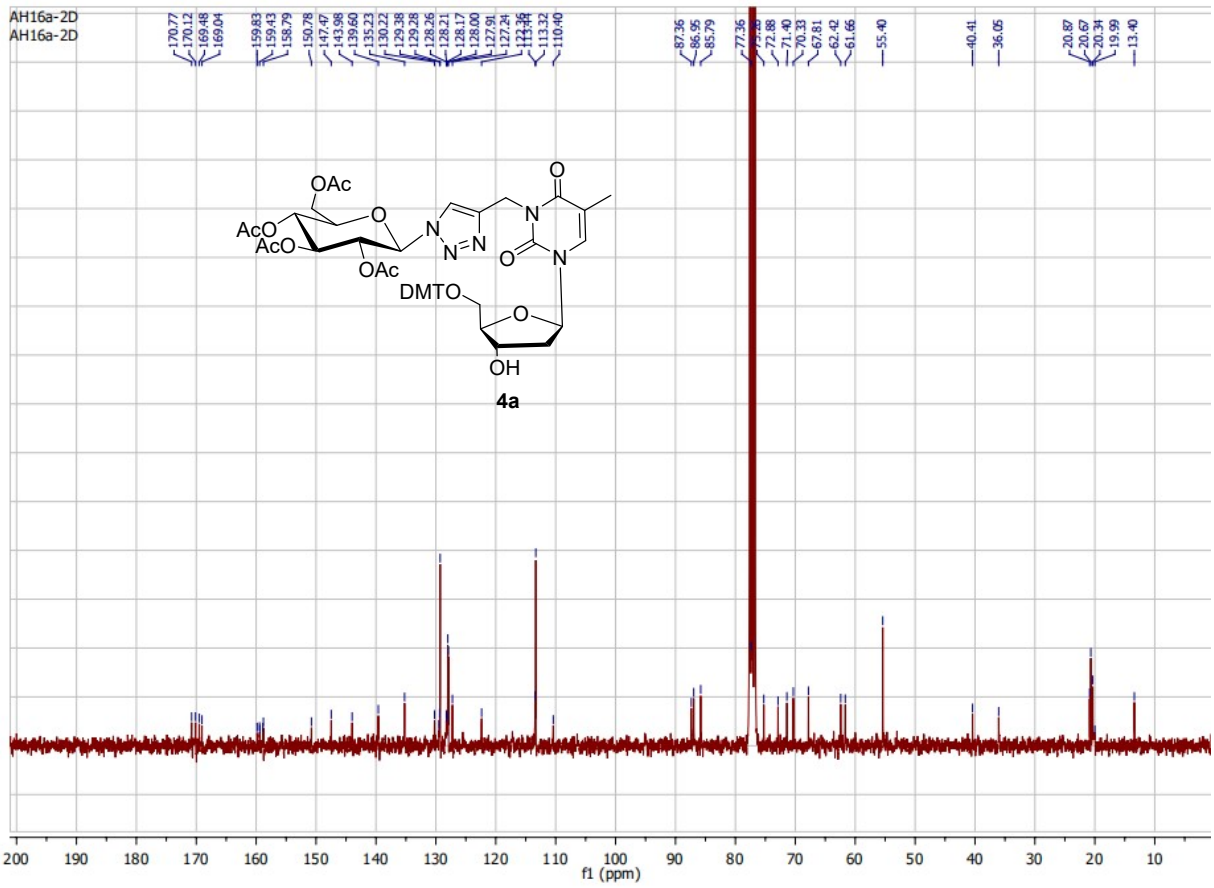
^{1}H NMR of compound 3f (300 MHz, $CDCl_3$ & CD_3OD)



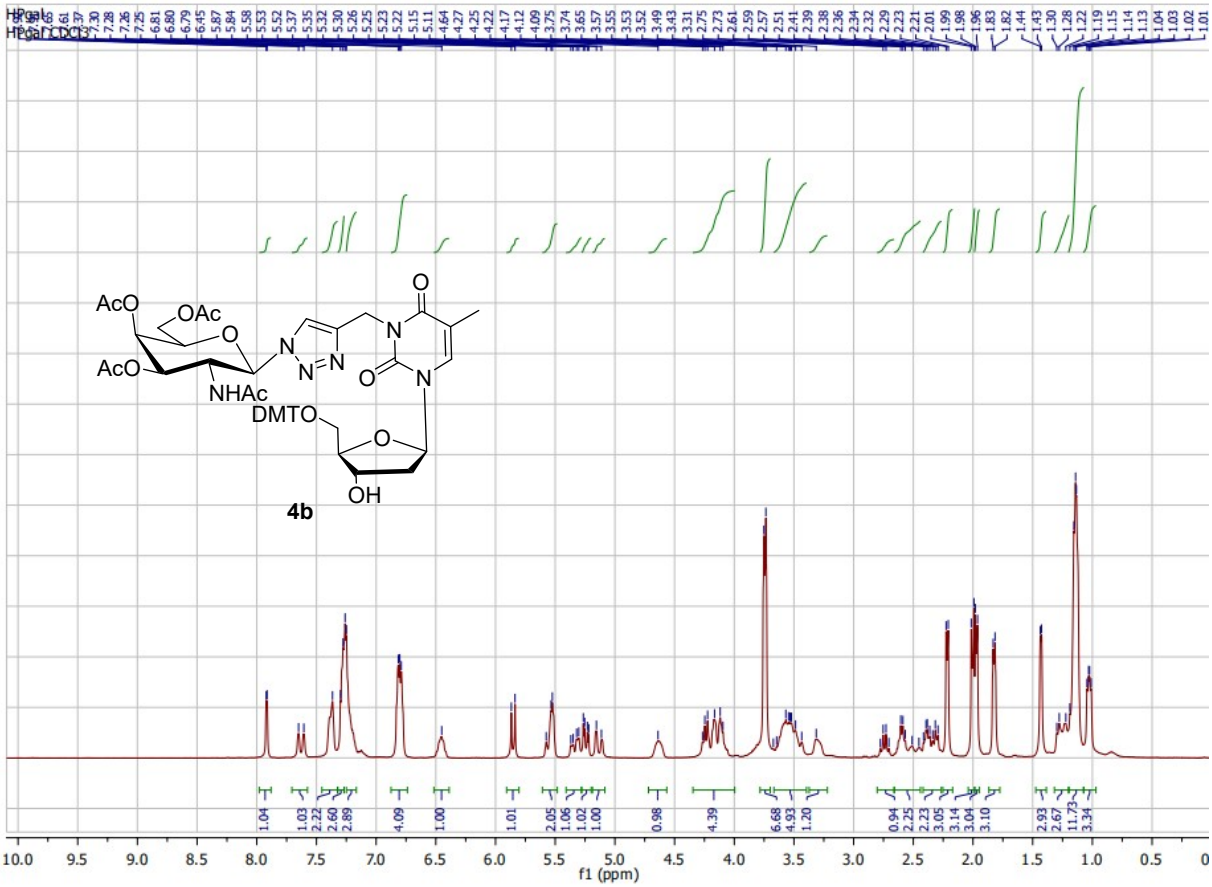
^{13}C NMR of compound 3f (75 MHz, CD_3OD)



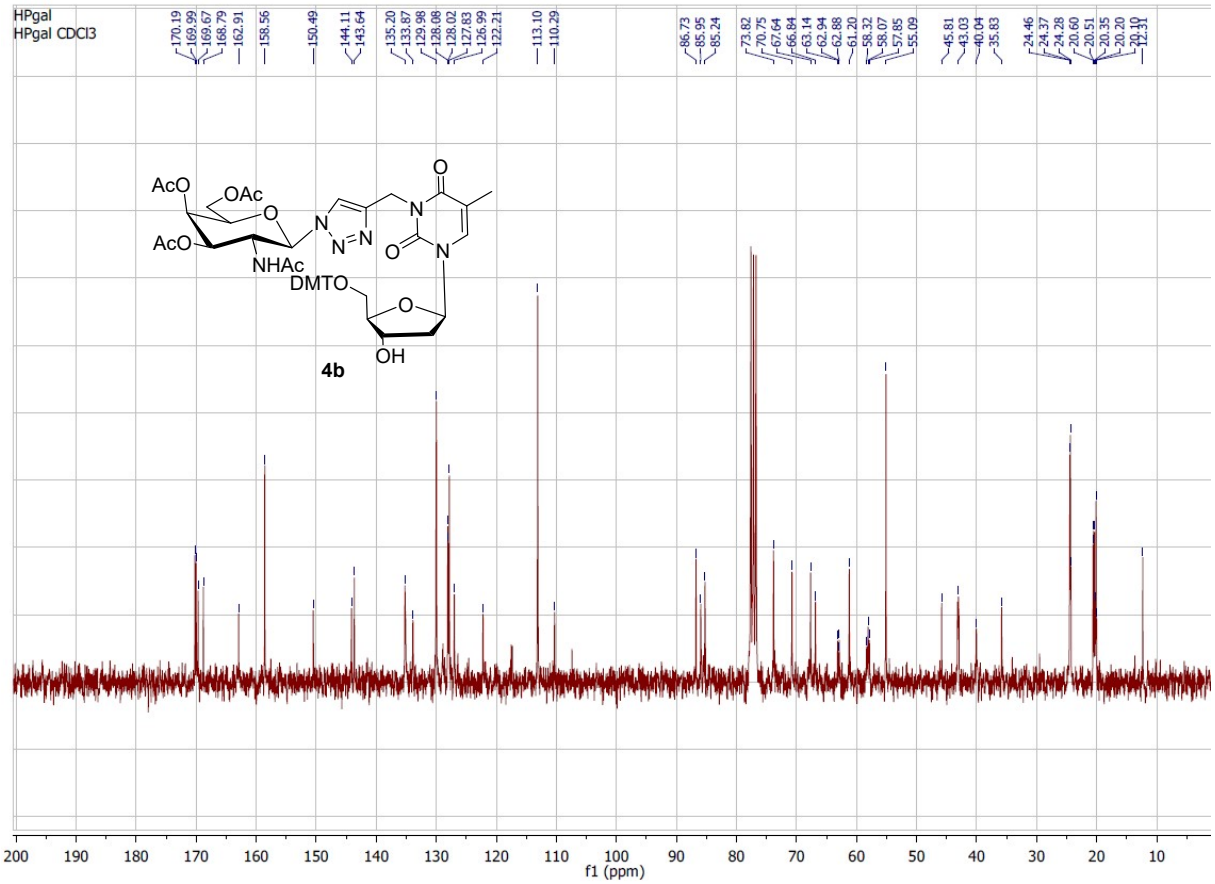
^{1}H NMR of compound 4a (300 MHz, $CDCl_3$)



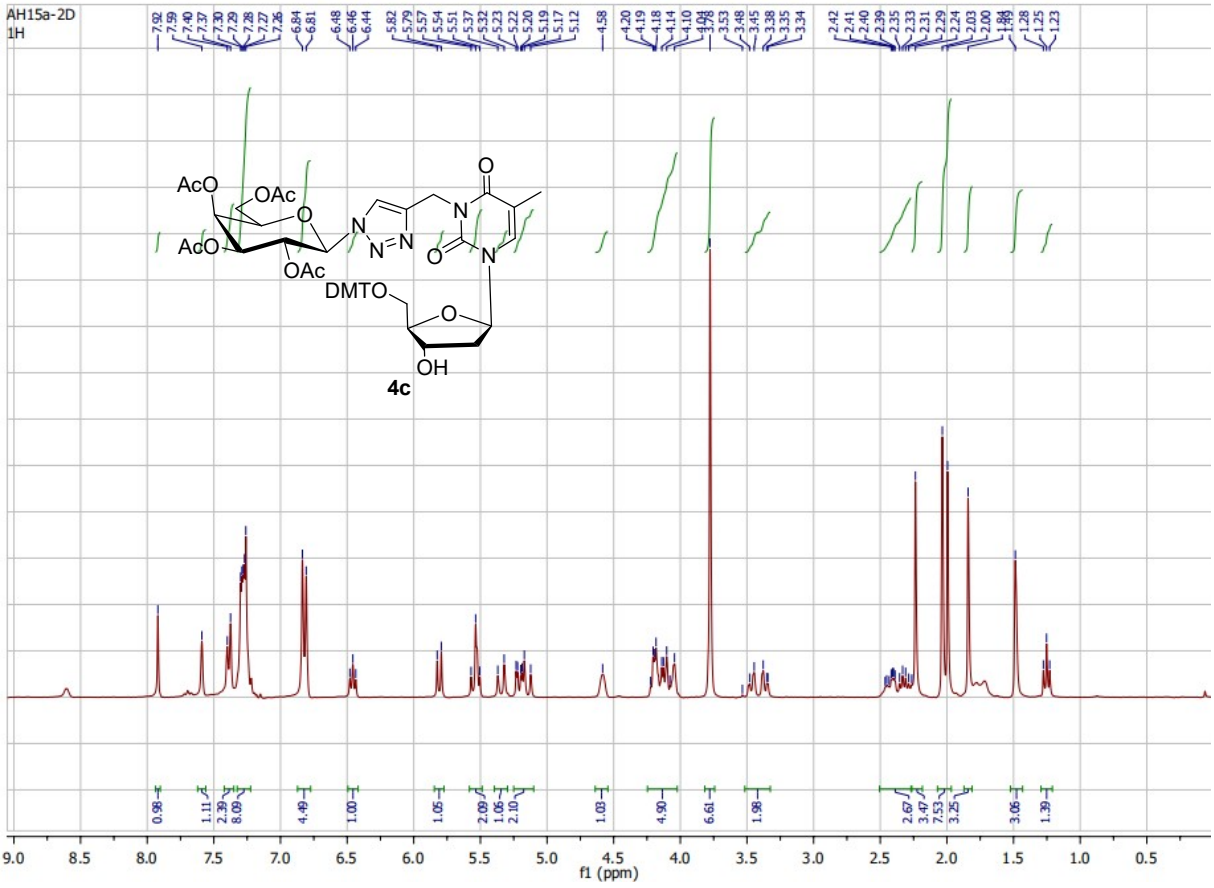
^{13}C NMR of compound 4a (75 MHz, $CDCl_3$)



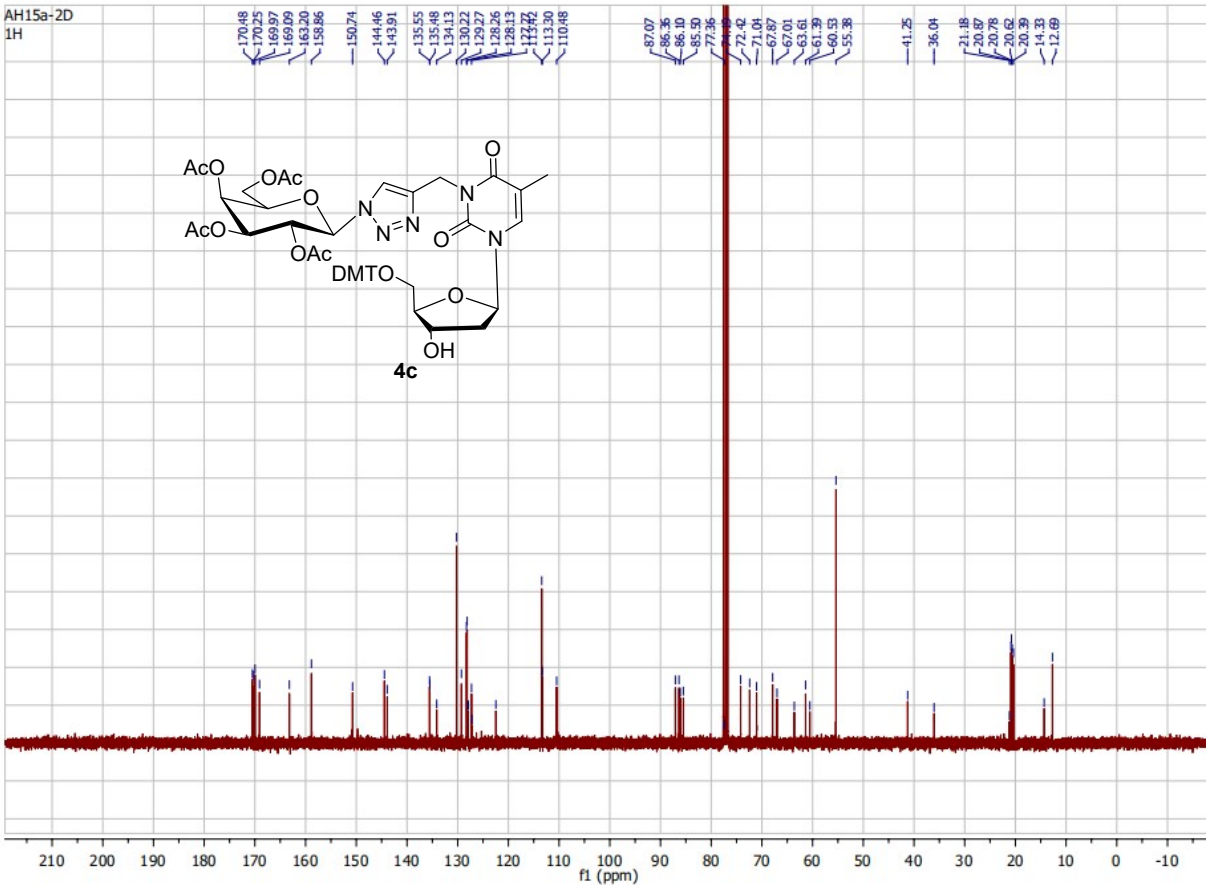
^{1}H NMR of compound 4b (300 MHz, $CDCl_3$)



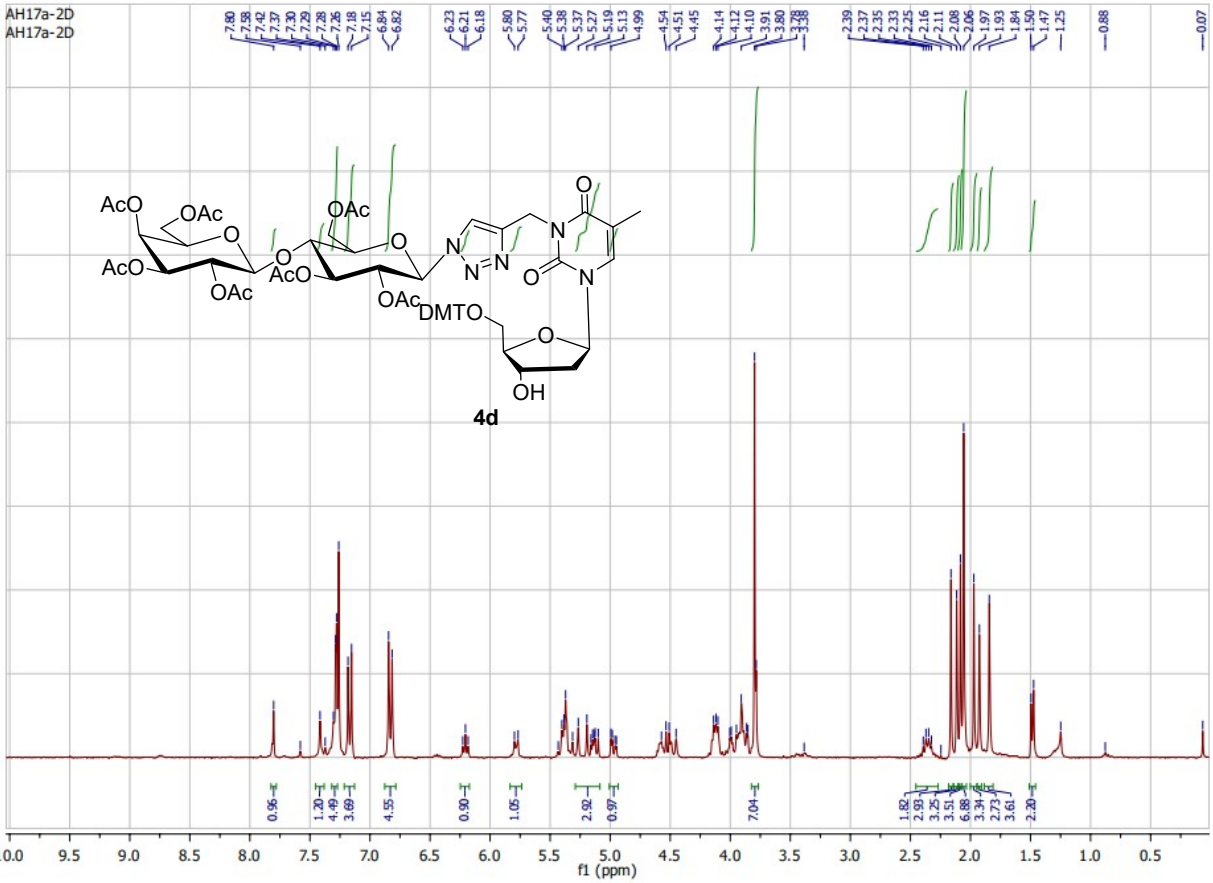
^{13}C NMR of compound 4b (75 MHz, $CDCl_3$)



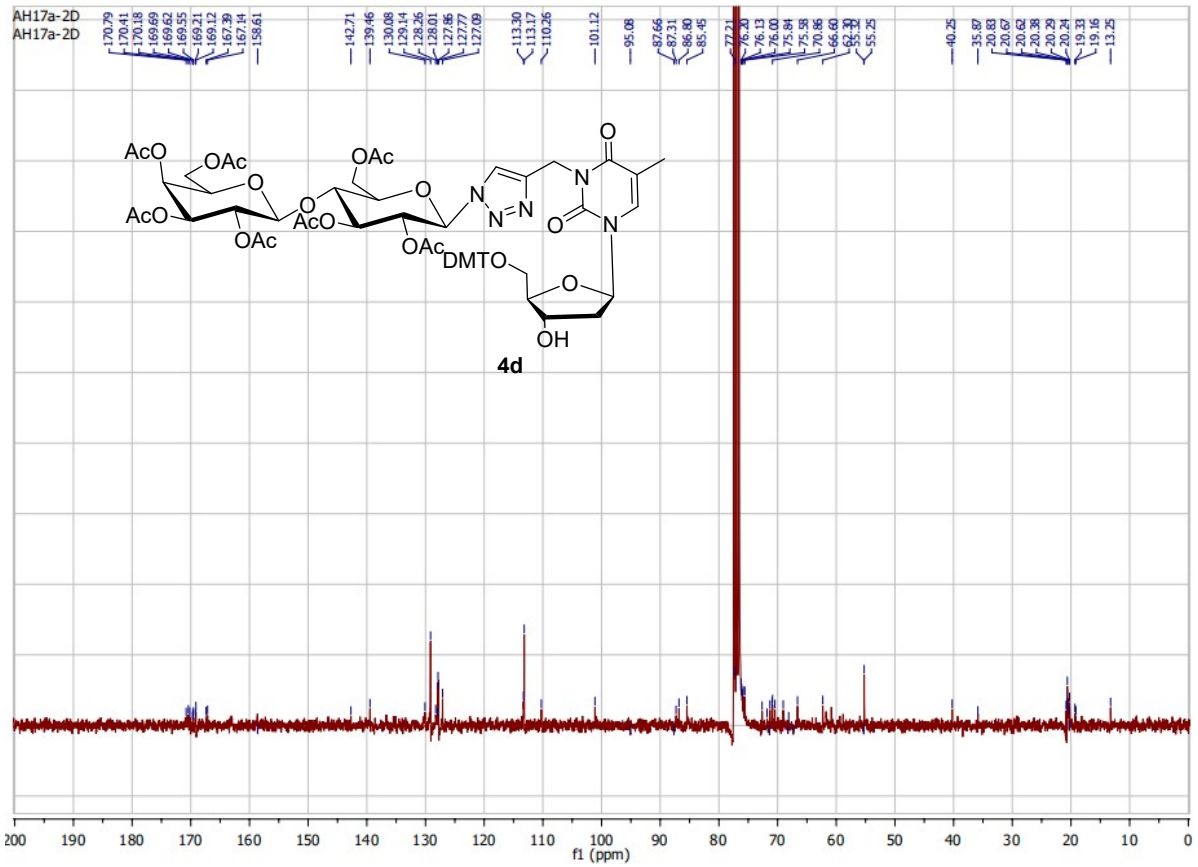
^{1}H NMR of compound 4c (300 MHz, $CDCl_3$)



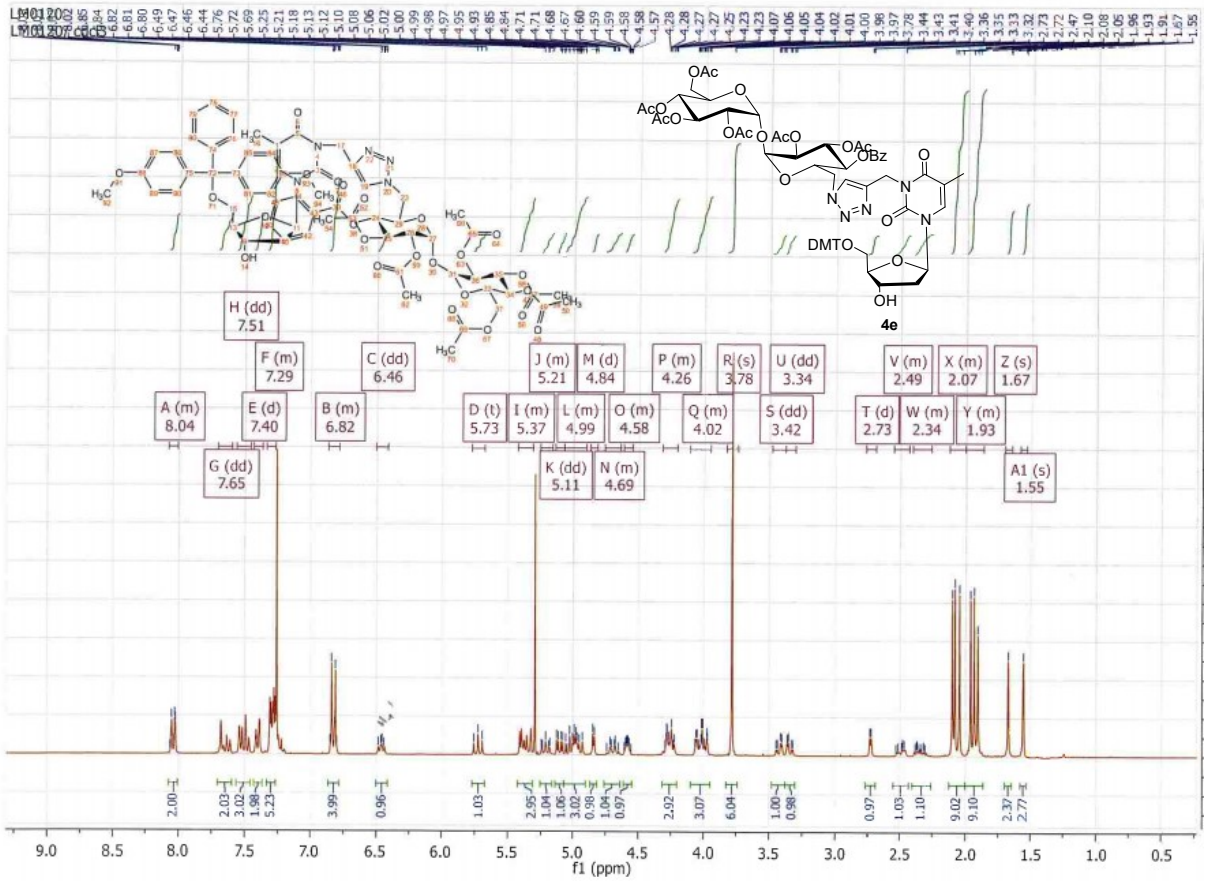
^{13}C NMR of compound 4c (75 MHz, $CDCl_3$)



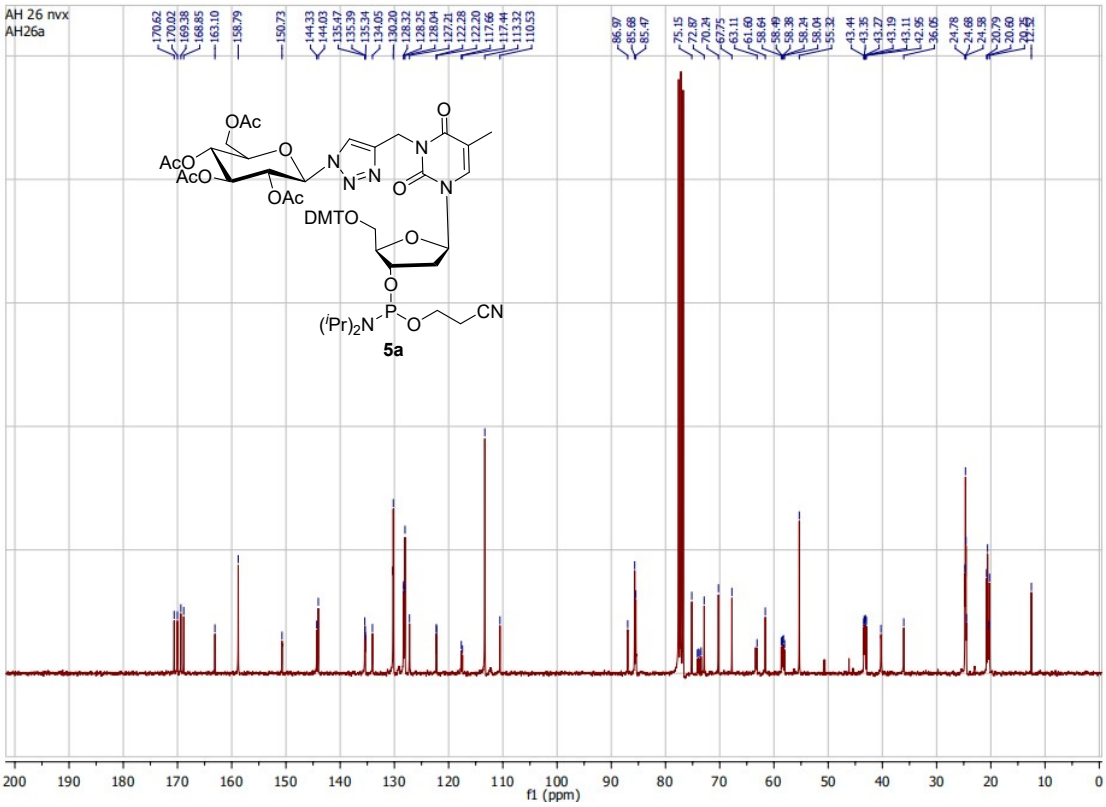
^{1}H NMR of compound 4d (300 MHz, $CDCl_3$)



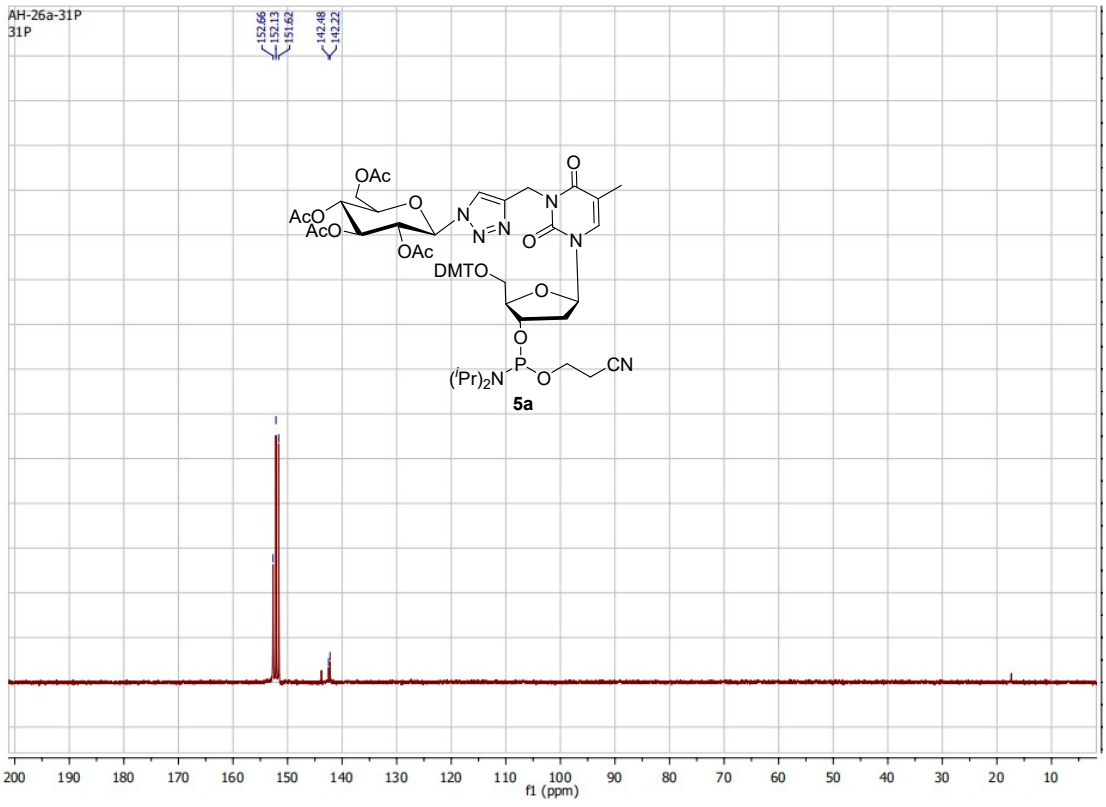
^{13}C NMR of compound 4d (75 MHz, $CDCl_3$)



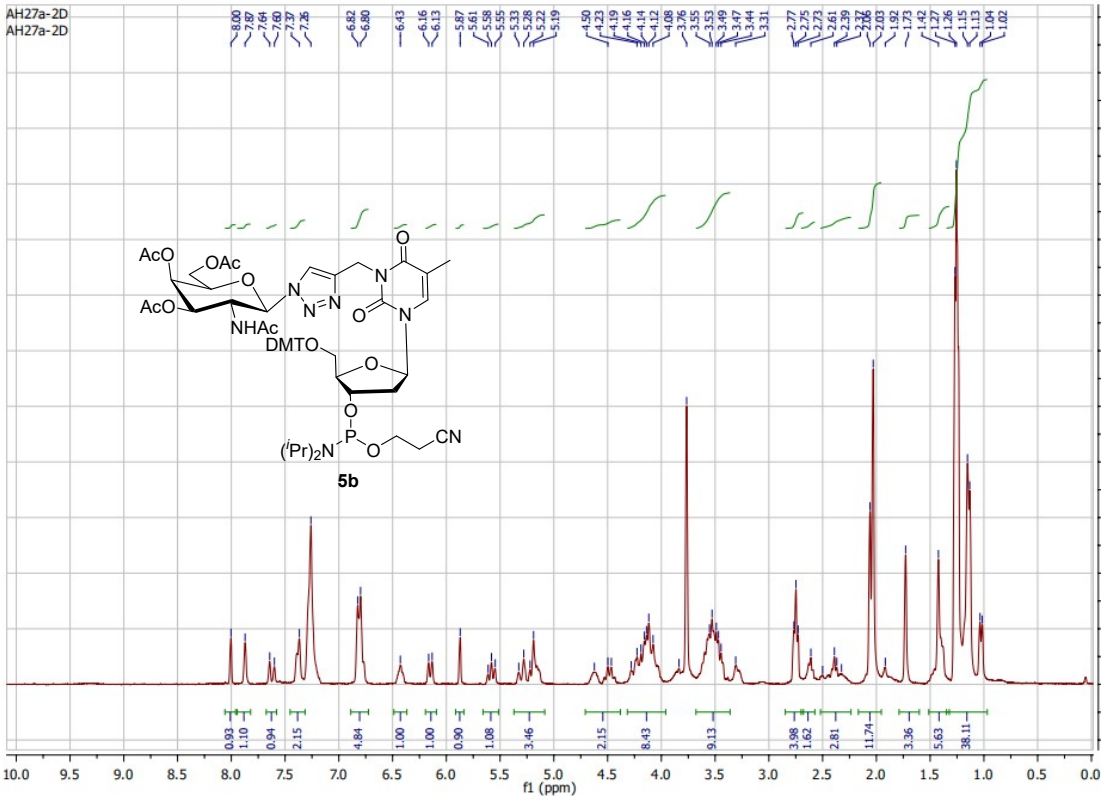
^{1}H NMR of compound 4e (300 MHz, $CDCl_3$)



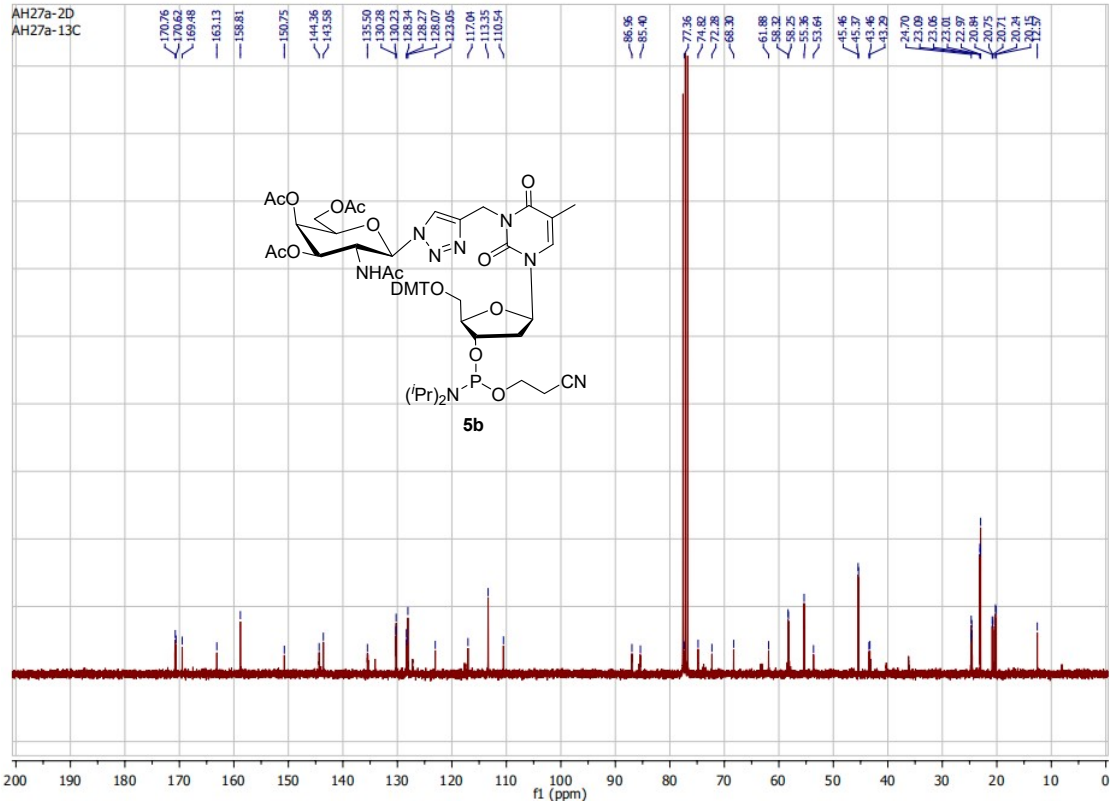
^{13}C NMR of compound 5a (75 MHz, $CDCl_3$)



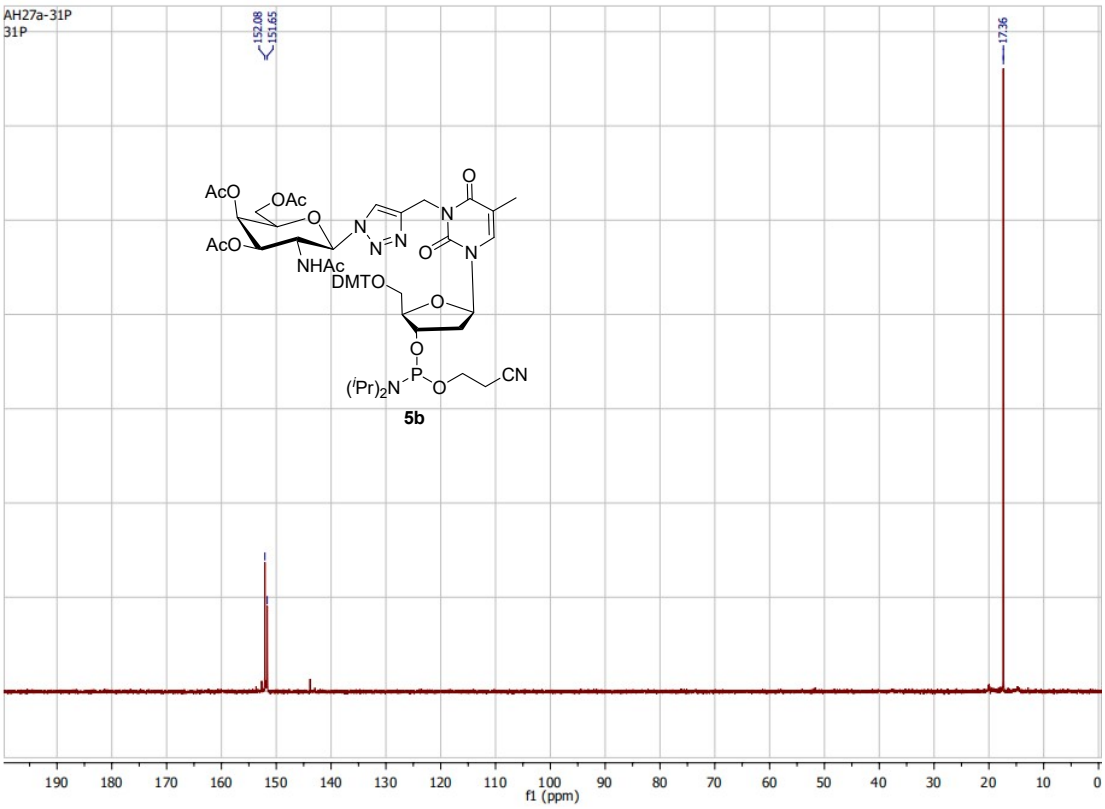
^{31}P NMR of compound 5a (121.5 MHz, $CDCl_3$)



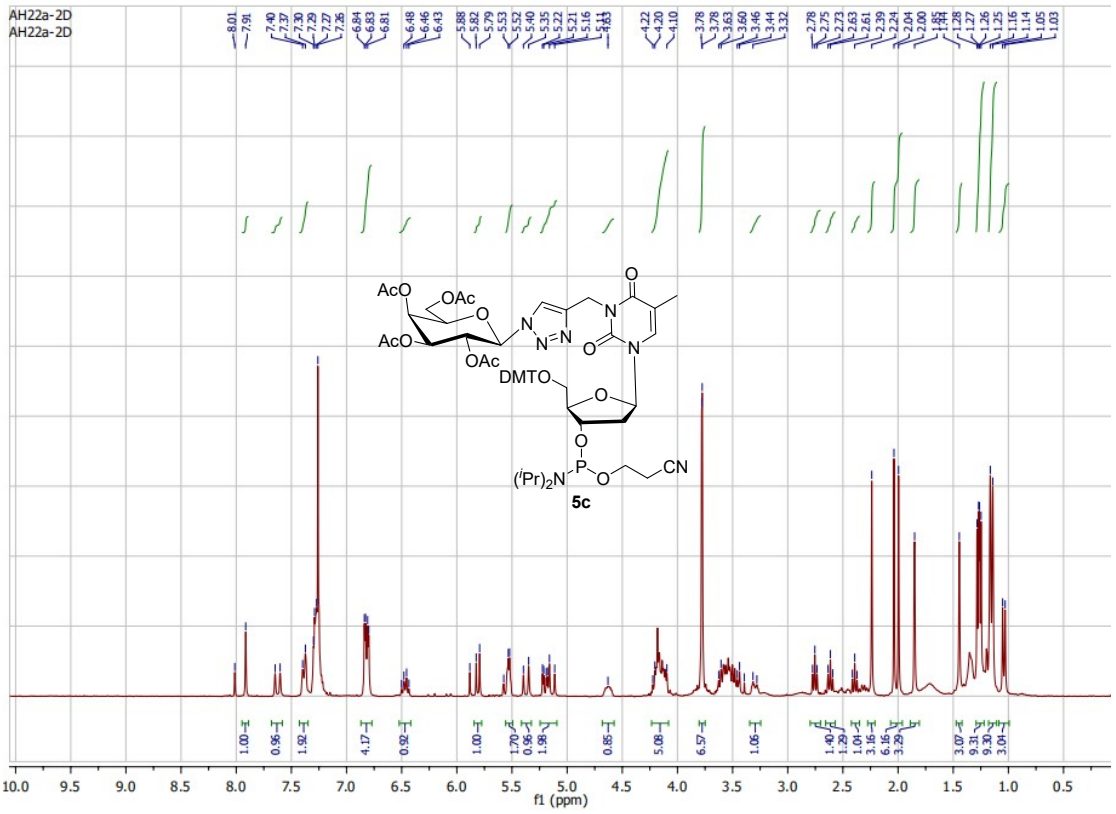
^{1}H NMR of compound 5b (300 MHz, $CDCl_3$)



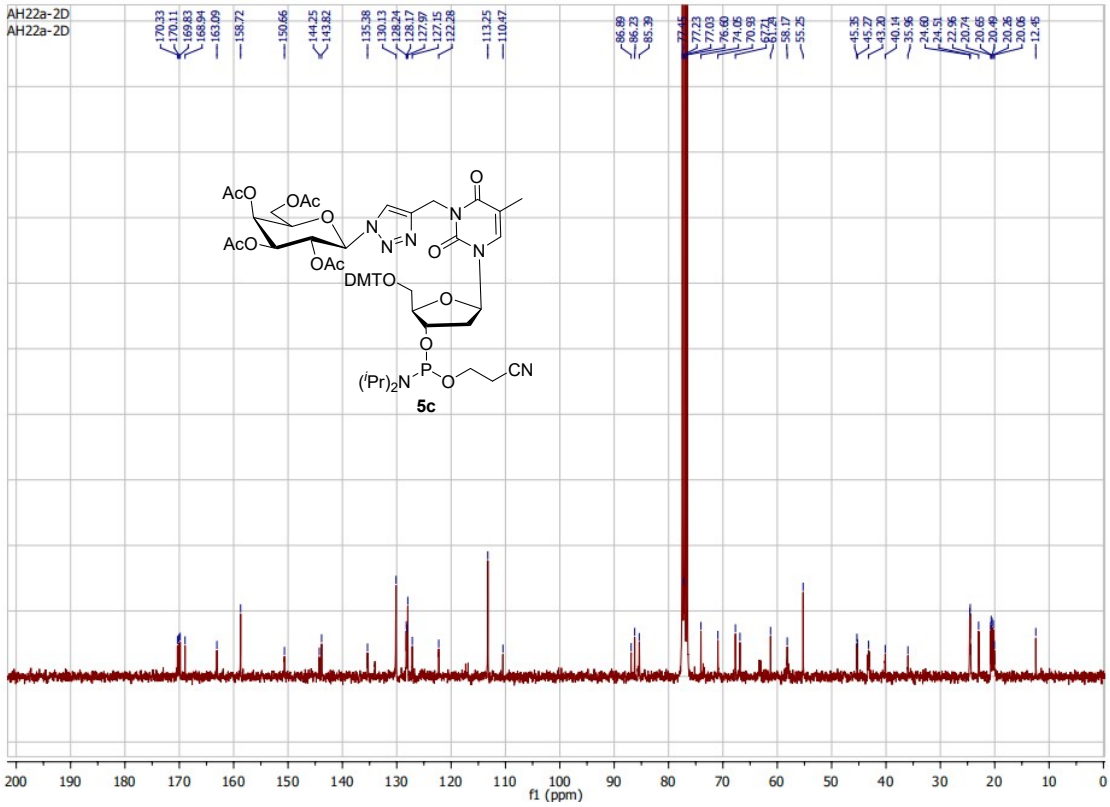
^{13}C NMR of compound 5b (75 MHz, $CDCl_3$)



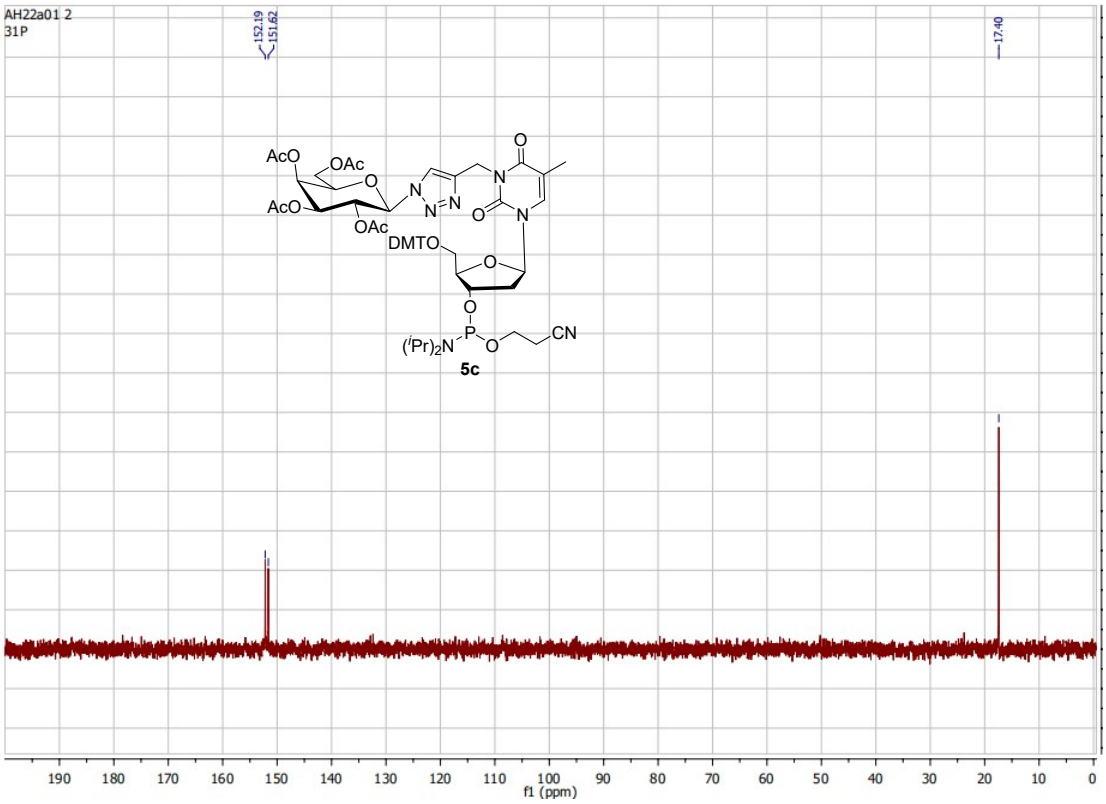
^{31}P NMR of compound 5b (121.5 MHz, $CDCl_3$)



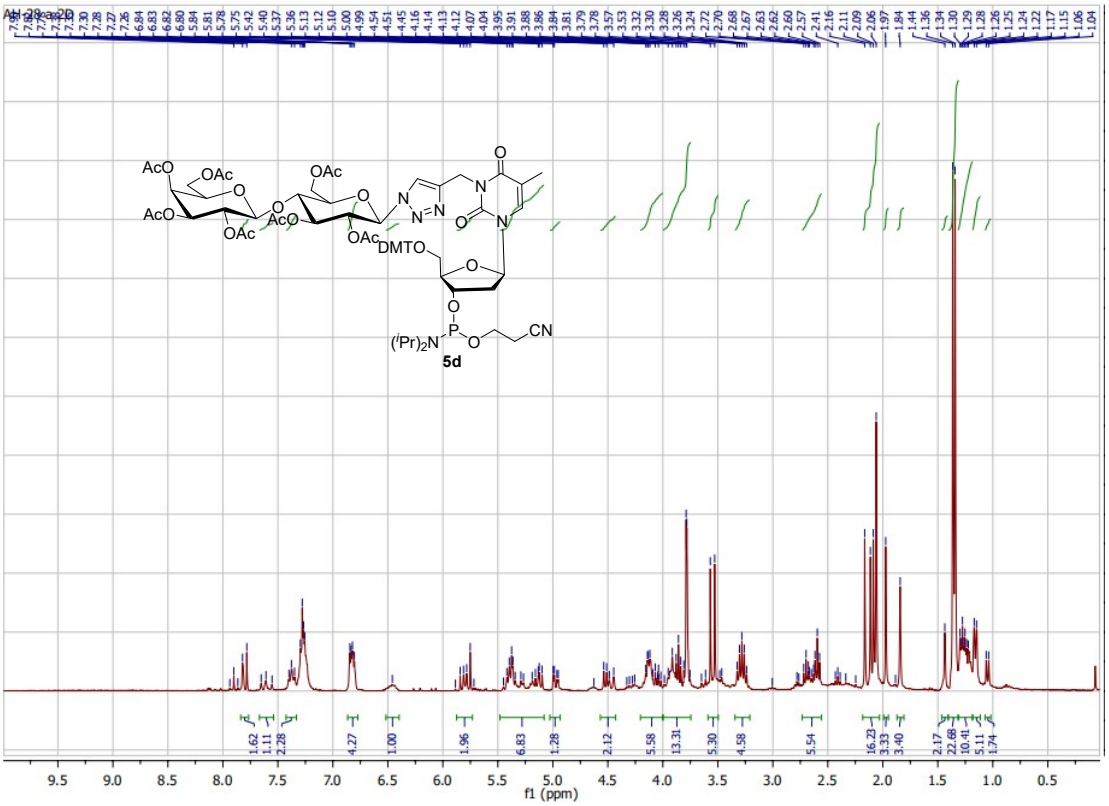
^{1}H NMR of compound 5c (300 MHz, $CDCl_3$)



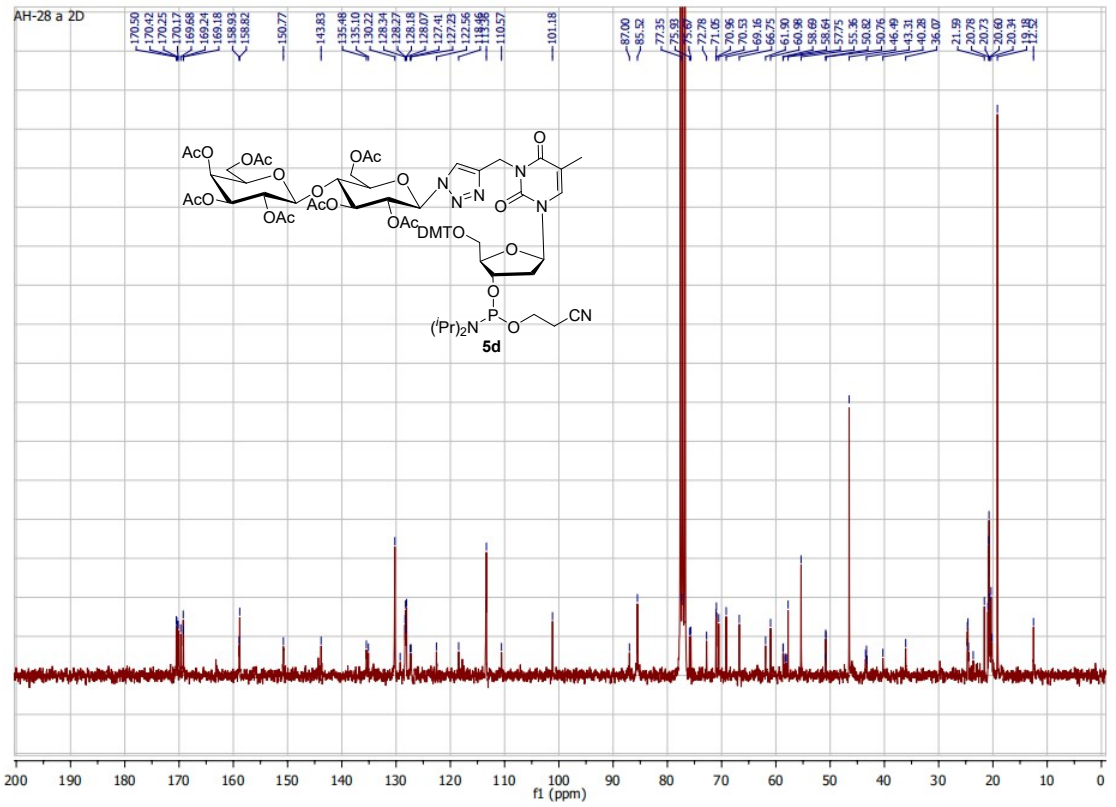
^{13}C NMR of compound 5c (75 MHz, $CDCl_3$)



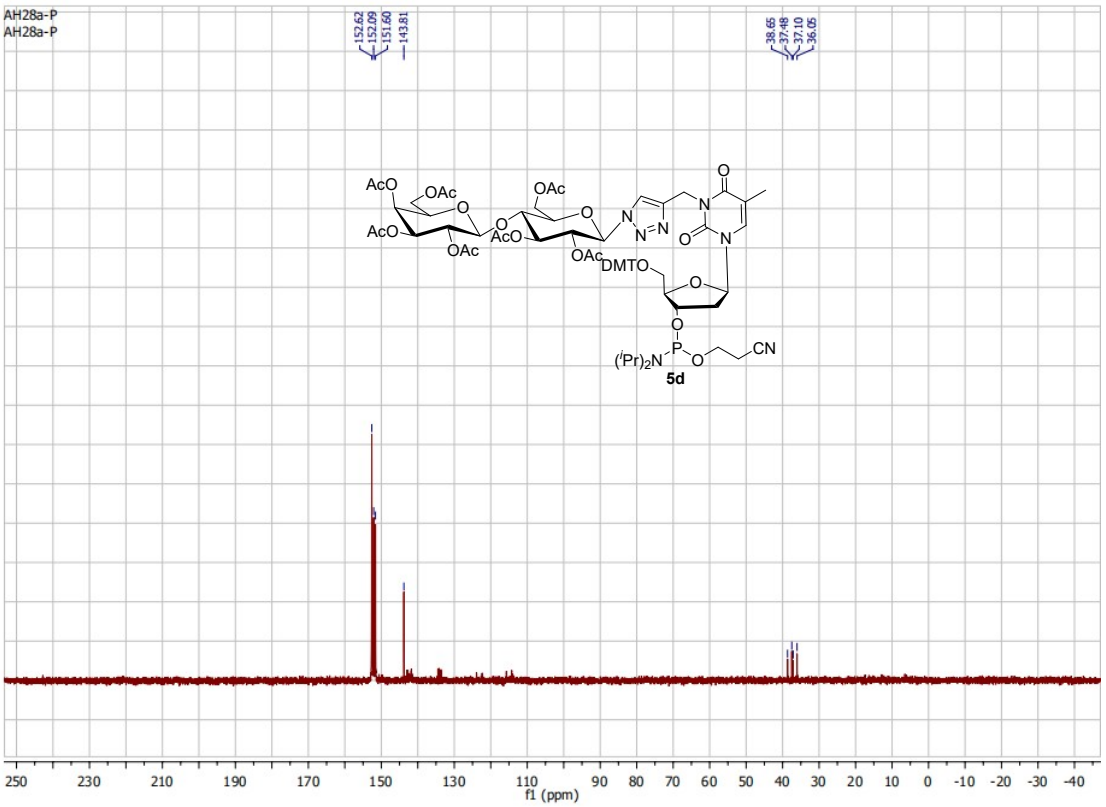
^{31}P NMR of compound 5c (121.5 MHz, $CDCl_3$)



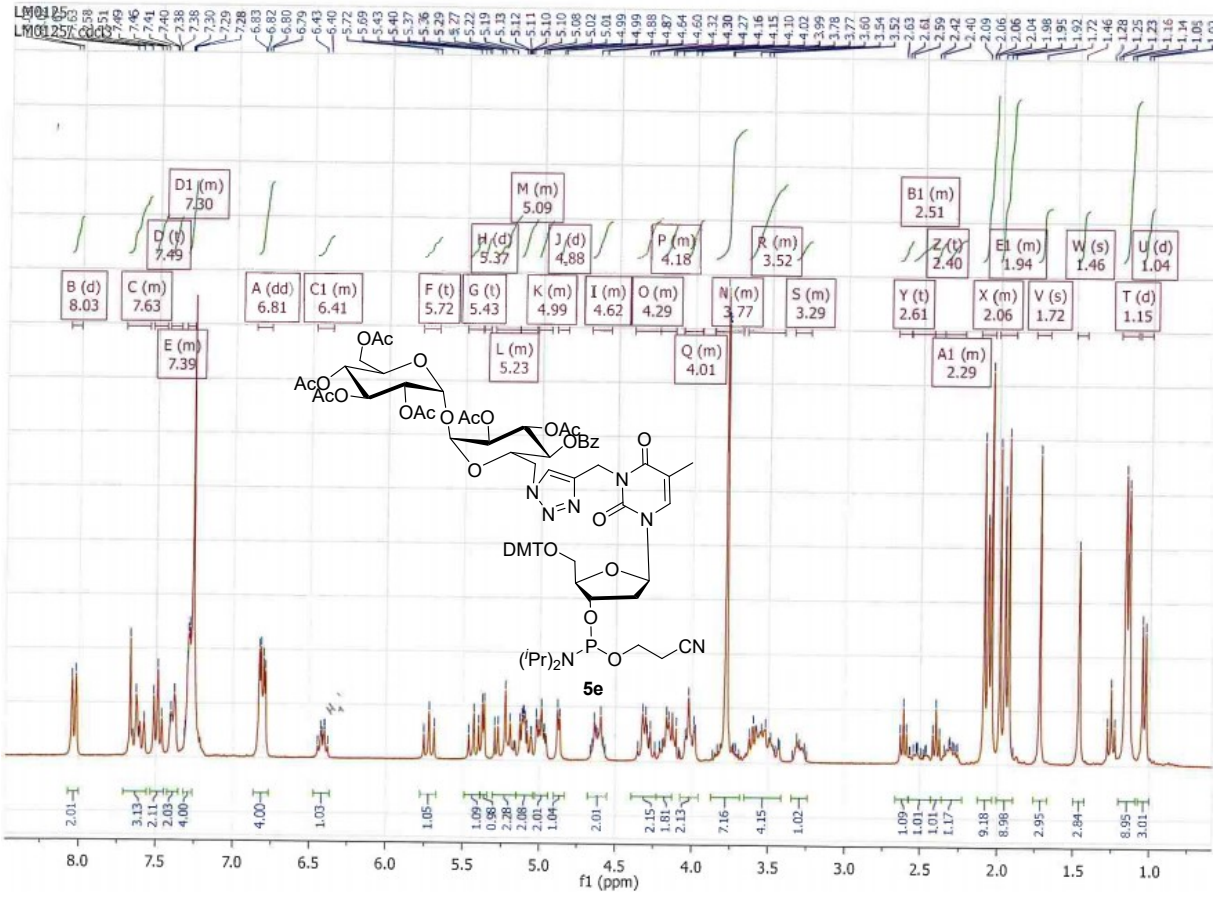
^{1}H NMR of compound 5d (300 MHz, $CDCl_3$)



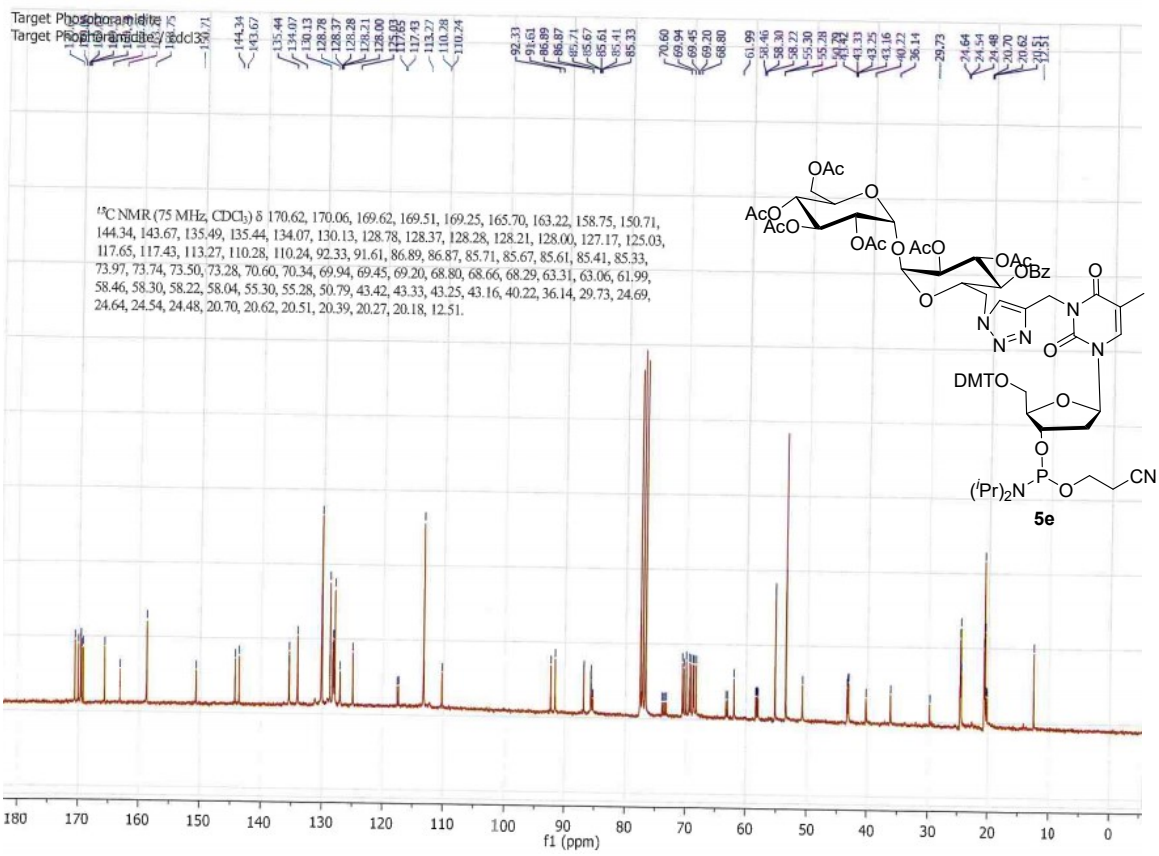
^{13}C NMR of compound 5d (75 MHz, $CDCl_3$)



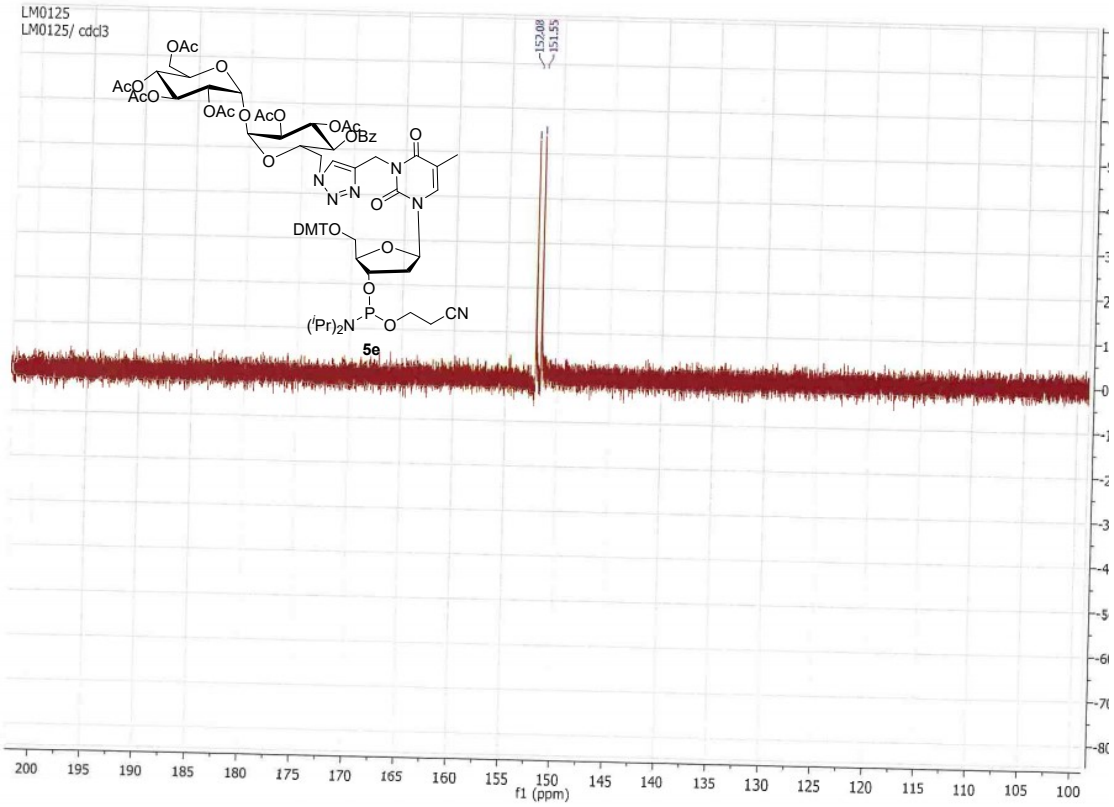
^{31}P NMR of compound 5d (121.5 MHz, $CDCl_3$)



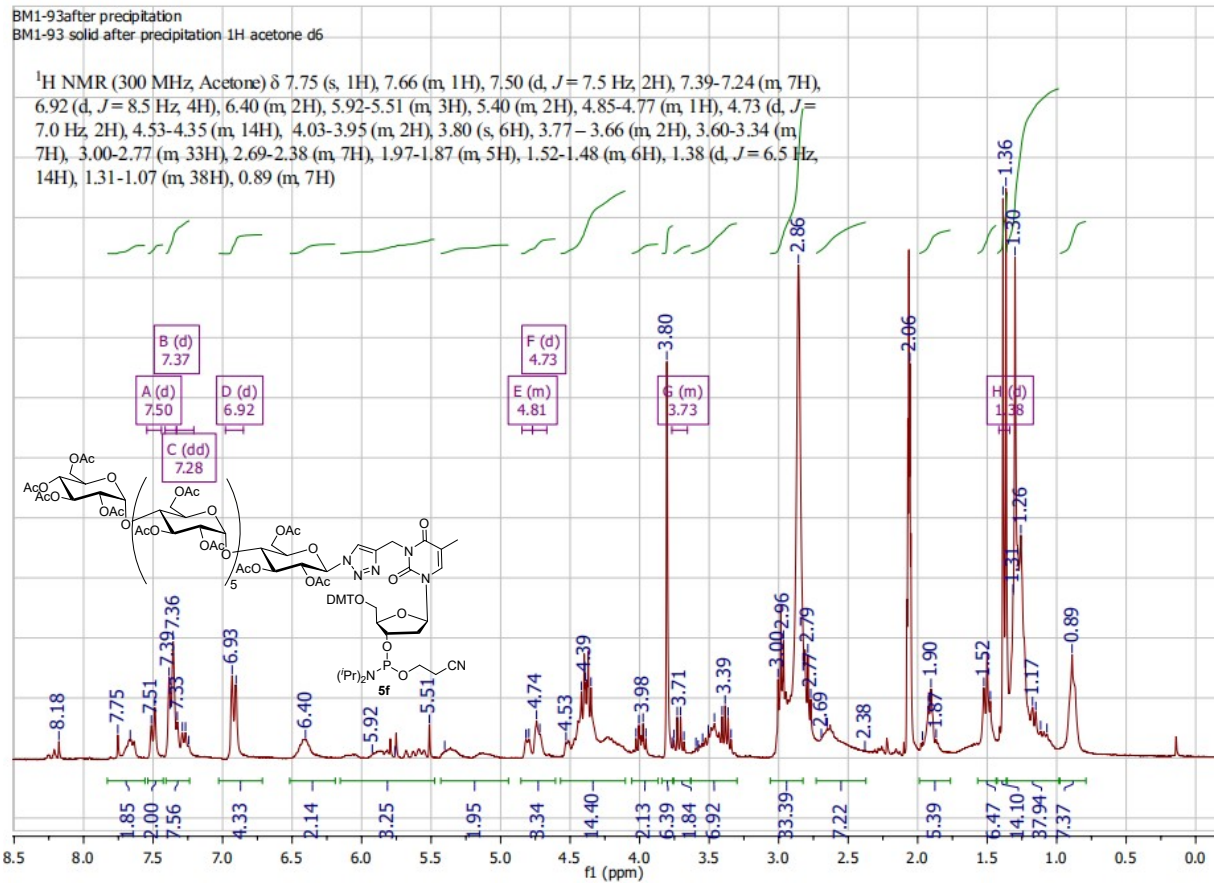
^{1}H NMR of compound 5e (300 MHz, $CDCl_3$)



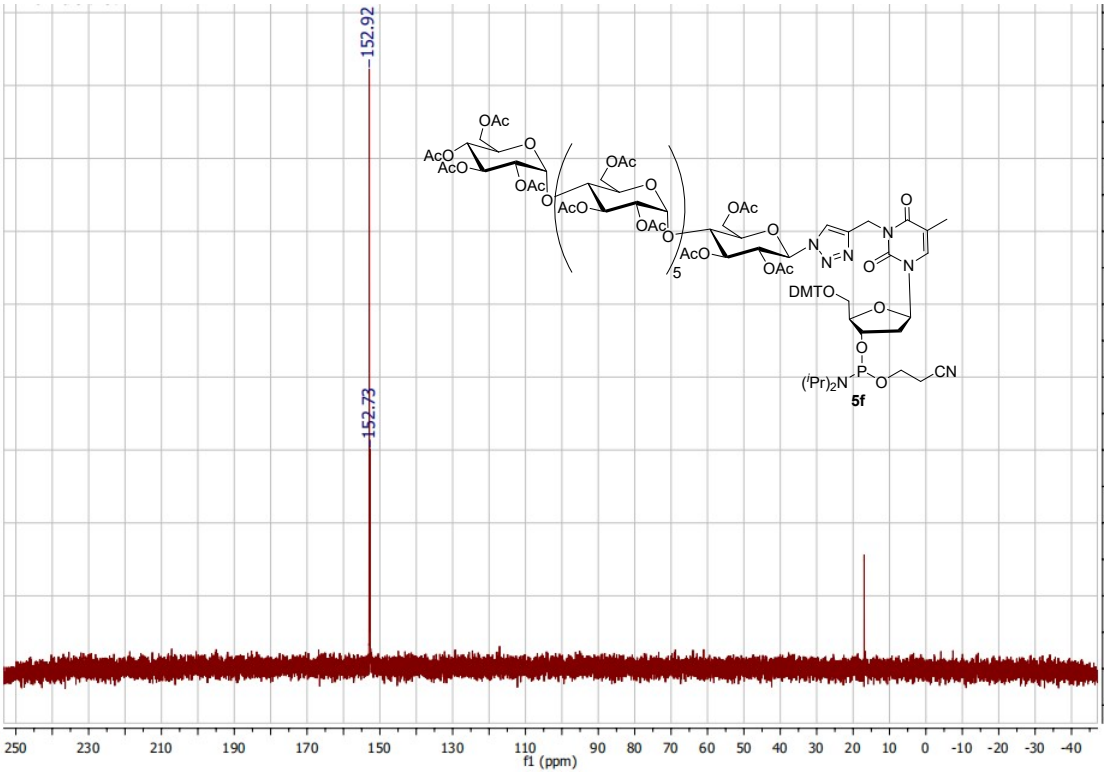
^{13}C NMR of compound 5e (75 MHz, $CDCl_3$)



^{31}P NMR of compound 5e (121.5 MHz, $CDCl_3$)



^{1}H NMR of compound 5f (300 MHz, Acetone-d6)



^{31}P NMR of compound 5f (121.5 MHz, CD_3CN)