5'-Morpholino modification of the sense strand of an siRNA makes it a more effective passenger

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Electronic Supplementary Information

Contents

1. Experimental Details:

1.1 General synthetic details

Commercially available starting materials, reagents, and solvents were used as received. All moisture-sensitive reactions were carried under anhydrous conditions under argon atmosphere. Flash chromatography was performed on a Teledyne ISCO Combi Flash system using pre-packed ReadySep Teledyne ISCO silica gel columns. TLC was performed on Merck silica-coated plates 60 F_{254}. Compounds were visualized under UV light (254 nm) or after spraying with the *p*-anisaldehyde staining solution followed by heating. ESI-HRMS spectra were recorded on Waters QTof API US spectrometer using the direct flow injection in the positive mode (capillary = 3000 kV, cone = 35, source temperature = 120 °C, and desolvation temperature = 350 °C). ^{1}H and ^{13}C NMR spectra were recorded at room temperature on Varian spectrometers, and chemical shifts in ppm are referenced to the residual solvent peaks. Coupling constants are given in Hertz. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), broad signal (br), or multiplet (m). ^{31}P NMR spectra were recorded under proton-decoupled mode; chemical shifts are referenced to external H_3PO_4 (80%).

1.2 Synthesis of nucleosides and phosphoramidites

Synthesis of nucleoside 1a-1d

Nucleoside **1a**-**1d** were prepared following literature procedure.[1]

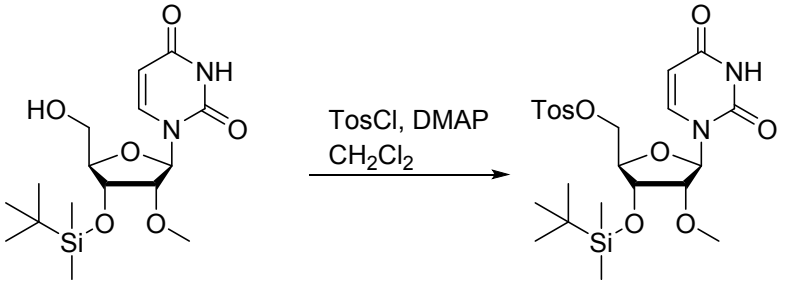
1a: ^{1}H (400 MHz, d6-DMSO) δ 11.35 (s, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 5.82 (d, *J* = 4.8 Hz, 1H), 5.65 (d, *J* = 8.1 Hz, 1H), 5.17 (t, *J* = 4.9 Hz, 1H), 4.28 (t, J = 4.7 Hz, 1H), 3.83 (t, *J* = 4.9 Hz, 2H), 3.65 (dd, *J* = 15.5, 4.9 Hz, 1H), 3.58 – 3.46 (m, 1H), 3.33 (s, 3H), 0.87 (s, 9H), 0.08 (s, 6H). ^{13}C (126 MHz, d6-DMSO) δ 163.03, 150.47, 140.30, 101.86, 86.13, 85.00, 82.17, 69.68, 60.00, 57.58, 25.62, 17.78, -4.85, -4.96.

1b: ^{1}H (400 MHz, d6-DMSO) δ 11.22 (s, 1H), 8.76 (s, 2H), 8.04 (d, *J* = 7.3 Hz, 2H), 7.64 (t, *J* = 7.5 Hz, 1H), 7.54 (t, *J* = 7.6 Hz, 2H), 6.14 (d, *J* = 5.9 Hz, 1H), 5.20 (t, *J* = 5.5 Hz, 1H), 4.59 – 4.57 (m, 1H), 4.53 (t, *J* = 5.3 Hz, 1H), 3.98 (q, *J* = 4.0 Hz, 1H), 3.76 – 3.66 (m, 1H), 3.63 – 3.53 (m, 1H), 3.32 (s, 3H), 0.92 (s, 9H), 0.13 (s, 6H). ^{13}C (126 MHz, d6-DMSO) δ 165.58, 152.03, 151.70, 150.48, 143.05, 133.26, 132.43, 128.46, 128.43, 125.84, 86.37, 85.48, 81.91, 70.22, 60.76, 57.64, 25.65, 17.82, -4.84, -4.85.

1c: ^{1}H (400 MHz, d6-DMSO) δ 10.91 (s, 1H), 8.44 (d, *J* = 7.5 Hz, 1H), 7.19 (d, *J* = 7.5 Hz, 1H), 5.82 (d, *J* = 2.4 Hz, 1H), 5.23 (t, *J* = 4.7 Hz, 1H), 4.23 (dd, *J* = 7.0, 4.8 Hz, 1H), 3.92 – 3.85 (m, 1H), 3.81 – 3.68 (m, 1H), 3.60 – 3.50 (m, 1H), 3.43 (s, 3H), 2.09 (s, 3H), 0.85 (s, 9H), 0.05 (s, 6H). ^{13}C (126 MHz, d6-DMSO) 170.98, 162.39, 154.42, 145.02, 95.25, 88.05, 84.06, 83.11, 68.68, 59.00, 57.77, 25.59, 24.32, 17.76, -4.86, -5.09.

1d: ^{1}H (400 MHz, d6-DMSO) δ 12.10 (s, 1H), 11.60 (s, 1H), 8.32 (s, 1H), 5.86 (d, *J* = 6.7 Hz, 1H), 5.16 (t, *J* = 5.3 Hz, 1H), 4.46 (dd, *J* = 4.6, 2.4 Hz, 1H), 4.32 (dd, *J* = 6.7, 4.6 Hz, 1H), 3.93 – 3.90 (m, 1H), 3.64 – 3.58 (m, 1H), 3.57 – 3.50 (m, 1H), 3.29 (s, 3H), 2.76 (p, *J* = 6.8 Hz, 1H), 1.11 (d, *J* = 6.8 Hz, 6H), 0.89 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H). ^{13}C (126 MHz, d6-DMSO) 180.12, 154.74, 148.82, 148.26, 137.33, 120.10, 86.65, 84.29, 82.36, 70.39, 60.79, 57.72, 34.70, 25.64, 18.84, 17.82, -4.88, -4.87.

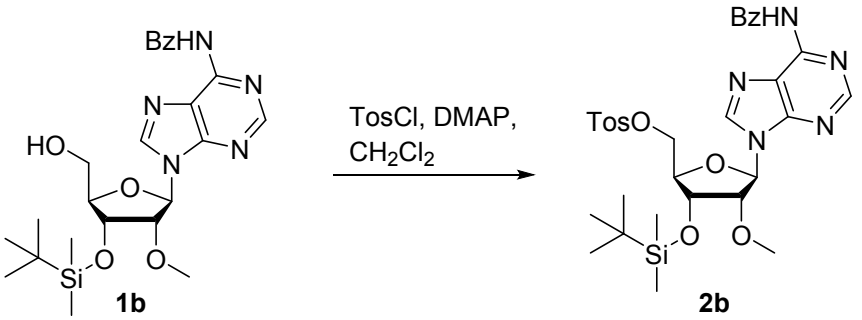
Synthesis of nucleoside 2a



1a **2a**

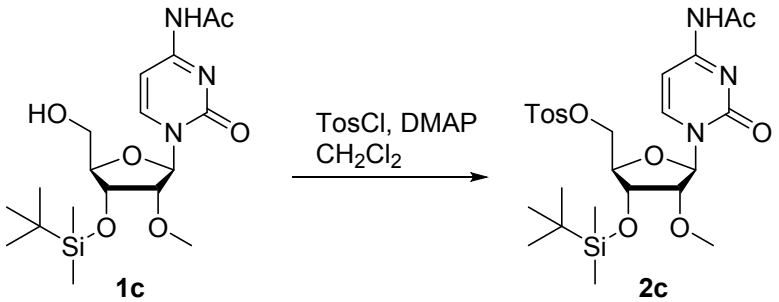
Alcohol **1a** (7.45 g, 20 mmol) and 4-dimethyaminopyridine (DMAP, 4.89 g, 40 mmol) were dissolved in dry CH_2Cl_2. 4-Methylbenzenesufonyl chloride (TosCl, 5.72 g, 30 mmol) was added at 0 °C (ice bath), and the reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h. The reaction was quenched by adding saturated aqueous $NaHCO_3$ (150 mL) and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were dried ($MgSO_4$), and the crude product was purified by flash chromatography using a gradient of 0 – 70 % EtOAc in hexanes to afford nucleoside **2a** (8.40 g, 79%) as a white form. MS (ESI$^+$) m/z calcd for $C_{23}H_{35}N_2O_8SSi$ $[M + H]^+$ 527.1878, found 527.1883. ^{1}H (400 MHz, d6-DMSO) δ 11.40 (d, *J* = 2.2 Hz, 1H), 7.88 – 7.73 (m, 2H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 2H), 5.73 (d, *J* = 4.3 Hz, 1H), 5.60 (dd, *J* = 8.1, 2.2 Hz, 1H), 4.21 (dd, *J* = 8.9, 4.5 Hz, 2H), 4.15 (d, *J* = 5.4 Hz, 1H), 3.92 – 3.90 (m, *J* = 5.5, 3.5 Hz, 1H), 3.86 (t, *J* = 4.8 Hz, 1H), 3.30 (s, 3H), 2.40 (s, 3H), 0.81 (s, 9H), 0.03 (s, 3H), 0.01 (s, 3H). ^{13}C (126 MHz, d6-DMSO) δ 162.97, 150.27, 145.31, 140.47, 131.82, 130.24, 127.69, 102.04, 87.49, 80.90, 80.77, 69.49, 68.88, 57.63, 25.51, 21.09, 17.66, -4.89, -5.25.

Synthesis of nucleoside 2b



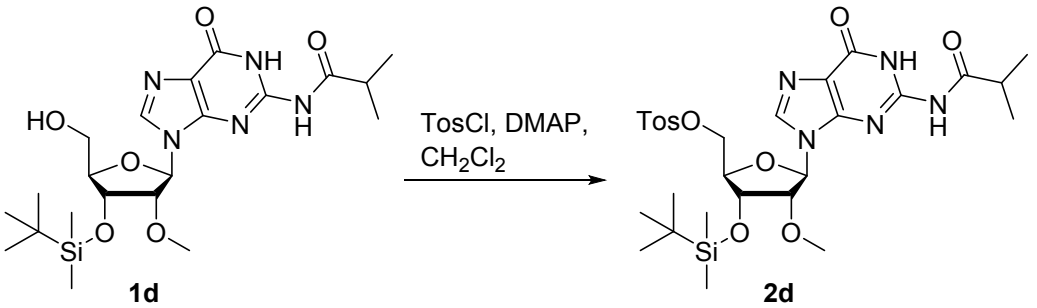
Alcohol **1b** (1.00 g, 2.00 mmol) and 4-dimethylaminopyridine (DMAP, 0.49 g, 3.00 mmol) were dissolved in dry CH_2Cl_2 (20 mL), and the reaction mixture was cooled to 0-4 °C (ice bath). 4-Toluenesulfonyl chloride (TosCl, 0.48 g, 2.50 mmol) was added, and reaction mixture was stirred at 0 °C (ice bath) for 1 h and then at room temperature for 3 h. The reaction was diluted with CH_2Cl_2 (100 mL), washed with saturated aqueous $NaHCO_3$ (100 mL), dried, and concentrated. The residue was purified by column chromatography using a gradient of 0 – 4% MeOH in CH_2Cl_2 to obtain **2b** (1.10 g, 84%) as a white foam. MS (ESI^+) m/z calcd for $C_{31}H_{40}N_5O_7SSi$ $[M + H]^+$ 654.2412, found 654.2423. ^1H (400 MHz, d6-DMSO) δ 11.24 (s, 1H), 8.69 (s, 1H), 8.61 (s, 1H), 8.11 – 7.99 (m, 2H), 7.73 – 7.68 (m, 2H), 7.68 – 7.62 (m, 1H), 7.56 (dd, *J* = 8.3, 6.8 Hz, 2H), 7.42 – 7.29 (m, 2H), 6.10 (d, *J* = 5.2 Hz, 1H), 4.61 (t, *J* = 4.4 Hz, 1H), 4.55 (t, *J* = 5.0 Hz, 1H), 4.37 – 4.28 (m, 2H), 4.09 – 4.06 (m, 1H), 3.29 (s, 3H), 2.35 (s, 3H), 0.87 (s, 9H), 0.10 (s, 3H), 0.08 (s, 3H). ^{13}C (126 MHz, d6-DMSO) δ 165.59, 151.73, 151.60, 150.53, 145.09, 143.34, 133.25, 132.46, 131.85, 129.99, 128.47, 128.44, 127.49, 125.90, 85.93, 81.98, 80.90, 69.93, 69.23, 57.71, 25.54, 20.99, 17.70, -4.92, -5.12.

Synthesis of nucleoside 2c



Alcohol **1c** (2.50 g, 6.04 mmol) and 4-dimethylaminopyridine (DMAP, 1.47 g, 12.03 mmol) were taken in dry CH_2Cl_2 (50 mL) and reaction mixture was cooled to 0-4^oC (ice bath). 4-Toluenesulfonyl chloride (TosCl, 1.44 g, 7.55 mmol) was added and reaction mixture was stirred in ice bath for 3h. The reaction was diluted with CH_2Cl_2 (100 mL), washed with saturated aqueous $NaHCO_3$ (2 × 30 mL), dried, and concentrated. The residue was purified by column chromatography using a gradient of 0 – 5% MeOH in CH_2Cl_2 to obtain **2c** (2.71 g, 79%) as a white foam. MS (ESI$^+$) m/z calcd for $C_{25}H_{38}N_3O_8SSi$ $[M + H]^+$ 568.2143, found 568.2144. ^1H (400 MHz, d6-DMSO) δ 10.94 (s, 1H), 7.85 (d, *J* = 7.5 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 2H), 7.49 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 7.5 Hz, 1H), 5.78 (d, *J* = 2.2 Hz, 1H), 4.30 (dd, *J* = 11.4, 2.8 Hz, 1H), 4.24 (dd, *J* = 11.4, 4.9 Hz, 1H), 4.09 (dd, *J* = 7.6, 4.9 Hz, 1H), 4.05 – 3.98 (m, 1H), 3.80 (dd, *J* = 4.9, 2.3 Hz, 1H), 3.40 (s, 3H), 2.41 (s, 3H), 2.10 (s, 3H), 0.79 (s, 9H), 0.02 (s, 3H), -0.01 (s, 3H). ^{13}C (101 MHz, d6-DMSO) δ 171.04, 162.45, 154.18, 145.40, 144.50, 131.73, 130.28, 127.66, 95.43, 89.09, 81.95, 80.29, 69.12, 68.37, 57.78, 25.46, 24.34, 21.09, 17.59, -4.88, -5.39.

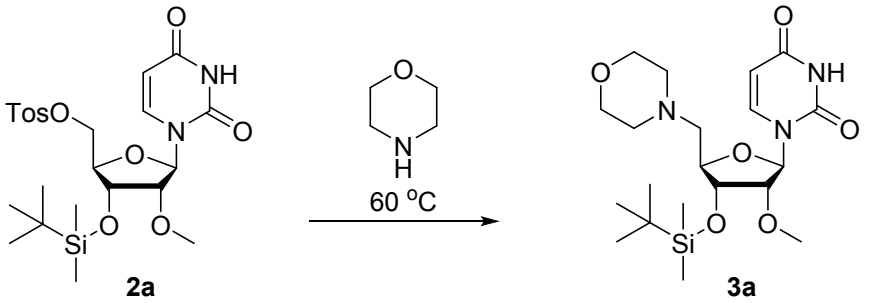
Synthesis of nucleoside 2d



Alcohol **1d** (4.81 g, 10 mmol) and 4-dimethylaminopyridine (DMAP, 2.45 g, 20 mmol) were dissolved in dry CH_2Cl_2 (40 mL). 4-Toluenesulfonyl chloride (TosCl, 2.86 g, 15 mmol) was added at 0^oC (ice bath) and the reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h. The reaction was quenched by adding saturated aqueous $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (2 × 60 mL). The combined organic phases were dried ($MgSO_4$), and the crude product was purified by column chromatography using a gradient of 0 – 4% MeOH in CH_2Cl_2 to afford nucleoside **2d** (3.60 g, 56%) as a white form. MS (ESI$^+$) m/z calcd for $C_{28}H_{42}N_5O_8SSi$ $[M + H]^+$ 636.2518, found 636.2521. ^1H (400 MHz, d6-DMSO) δ 12.08 (s, 1H), 11.53 (s, 1H), 8.17 (s, 1H), 7.81 – 7.70 (m, 2H), 7.40 (d, *J* = 8.1 Hz, 2H), 5.83 (d, *J* = 5.3 Hz, 1H), 4.37 – 4.30 (m, 2H), 4.27 – 4.22 (m, 2H), 4.03 – 4.00 (m, 1H), 3.27 (s, 3H), 2.77 – 2.73 (m, 1H), 2.38 (s, 3H), 1.11 (d, *J* = 6.8 Hz, 6H), 0.85 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H). ^{13}C (126 MHz, d6-DMSO) δ

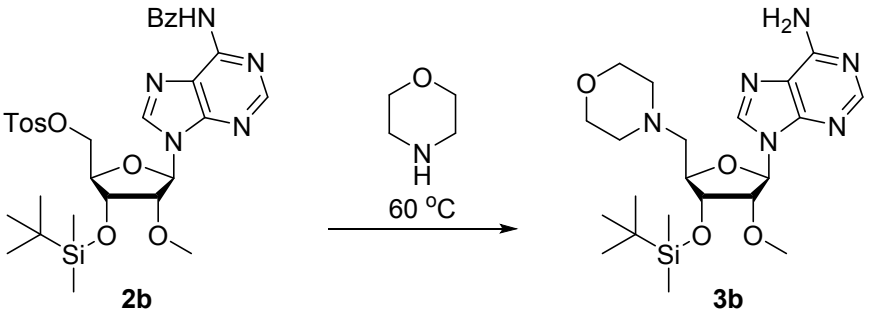
180.04, 154.75, 148.71, 148.24, 145.19, 137.33, 131.93, 130.10, 127.57, 120.19, 84.52, 82.49, 81.26, 69.92, 69.49, 57.83, 34.77, 25.56, 21.06, 18.85, 18.83, 17.72, -4.96, -5.12.

Synthesis of nucleoside 3a



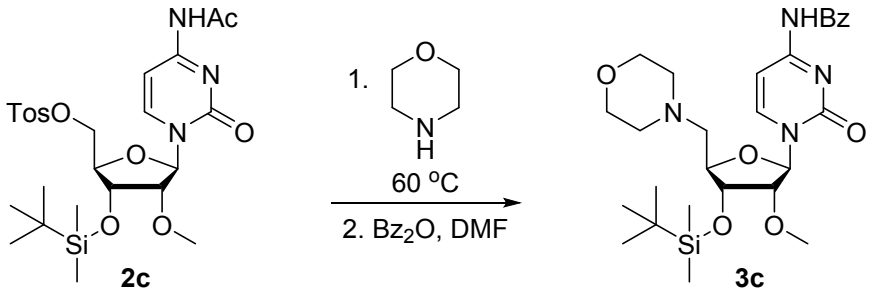
Nucleoside **2a** (2.0 g, 3.79 mmol) was dissolved in morpholine (25 mL), and the reaction mixture was stirred at 60 °C for 18 h. Solvent was removed at reduced pressure, and the residue was dissolved in CH_2Cl_2 (100 mL) and washed with H_2O (50 ml). The aqueous phase was back extracted with CH_2Cl_2 (2 × 30 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated, and the crude was purified by column chromatography using a gradient of 0 – 5 % MeOH in CH_2Cl_2 to afford nucleoside **3a** (1.1 g, 66%) as white powder. MS (ESI^+) m/z calcd for $C_{20}H_{36}N_3O_6Si$ $[M + H]^+$ 442.2368, found 442.2378. ^1H (400 MHz, d6-DMSO) δ 11.37 (s, 1H), 7.69 (d, J = 8.1 Hz, 1H), 5.78 (d, J = 4.5 Hz, 1H), 5.67 (d, J = 8.0 Hz, 1H), 4.15 (t, J = 5.2 Hz, 1H), 3.90 – 3.85 (m, 2H), 3.55 (t, J = 4.6 Hz, 4H), 3.32 (s, 3H), 2.59 (dd, J = 13.5, 5.1 Hz, 1H), 2.47 – 2.42 (m, 5H), 0.87 (s, 9H), 0.09 (s, 6H). ^{13}C (101 MHz, d6-DMSO) δ 162.96, 150.35, 140.90, 102.11, 87.07, 81.46, 80.92, 71.66, 66.14, 59.73, 57.42, 54.01, 25.61, 17.76, -4.70, -4.99.

Synthesis of nucleoside 3b



Nucleoside **2b** (2.4 g, 3.6 mmol) was dissolved in morpholine (20 mL), and the reaction mixture was stirred at 60 °C for 16 h. Solvents were removed at reduced pressure, and the residue was dissolved in CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (50 mL). The aqueous phase was back extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were concentrated, and the residue was purified by column chromatography using a gradient of 0 – 7% MeOH in CH_2Cl_2 to yield nucleoside **3b** (1.35 g, 78%) as a white foam. MS (ESI^+) m/z calcd for $C_{21}H_{37}N_6O_4Si$ $[M + H]^+$ 465.2640, found 465.2630. ^1H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.94 (s, 1H), 5.99 (d, *J* = 3.4 Hz, 1H), 5.79 (s, 2H), 4.50 (dd, *J* = 6.2, 5.0 Hz, 1H), 4.37 (dd, *J* = 5.1, 3.4 Hz, 1H), 4.24 – 4.15 (m, 1H), 3.70 (t, *J* = 4.7 Hz, 4H), 3.46 (s, 3H), 2.69 (dd, *J* = 5.5, 3.6 Hz, 2H), 2.63 – 2.45 (m, 4H); 0.94 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H). δ_C (101 MHz, $CDCl_3$) 155.74, 153.18, 149.69, 140.18, 120.75, 87.98, 82.58, 81.99, 72.77, 67.05, 60.89, 58.67, 54.75, 25.97, 18.38, -4.28, -4.56.

Synthesis of nucleoside 3c



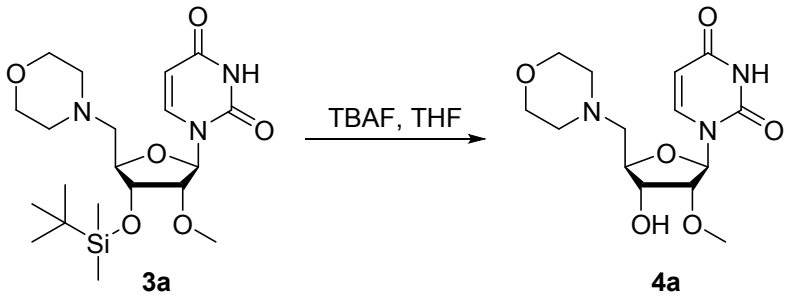
Nucleoside **2c** (3.7 g, 6.51 mmol) was dissolved in morpholine (20 mL). The resulting solution was heated at 60 °C for 24 h. Solvents were removed, and the crude was purified by column chromatography using a gradient of 0-12% MeOH in CH_2Cl_2. The isolated product (3.5 g) was dissolved DMF (15 mL). To this solution was added benzoic anhydride (1.61 g, 7.11 mmol), and the reaction mixture was stirred at room temperature for 16 h. Solvent was removed, and the residue was dissolved in EtOAc (100 mL) and washed with H_2O (2 × 50 mL). The combined aqueous phases were back extracted with EtOAc (50 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The residue was purified by column chromatography using a gradient of 0 – 5% MeOH in CH_2Cl_2 to afford nucleoside **3c** (2.35 g, 66% over 2 steps) as white foam. MS (ESI^+) m/z calcd for $C_{27}H_{41}N_4O_6Si$ $[M + H]^+$ 545.2790, found 545.2791. ^1H (400 MHz, d6-DMSO) δ 11.33 (s, 1H), 8.24 (d, *J* = 7.5 Hz, 1H), 8.03 – 7.97 (m, 2H), 7.65 – 7.58 (m, 1H), 7.55 – 7.47 (m, 2H), 7.40 (d, *J* = 7.5 Hz, 1H), 5.88 (d, *J* = 2.8 Hz, 1H), 4.08 (dd, *J* = 7.1, 4.9 Hz, 1H), 3.98 (dt, *J* = 6.9, 5.1 Hz, 1H), 3.83 (dd, *J* = 5.0, 2.8 Hz, 1H), 3.58 (t, *J* = 4.6 Hz, 4H), 3.42 (s, 3H), 2.63 (d, *J* = 5.1 Hz, 2H), 2.52 – 2.43 (m, 5H), 0.87 (s, 9H), 0.08 (s, 6H). ^{13}C (101 MHz, d6-DMSO) δ 167.55, 163.09,

154.18, 145.29, 133.11, 132.71, 129.20, 128.43, 96.54, 88.72, 82.17, 81.29, 71.47, 66.17, 59.46, 57.57, 54.07, 25.59, 17.72, -4.65, -5.08.

Synthesis of nucleoside 3d

Nucleoside **2d** (1.4 g, 2.20 mmol) was dissolved in morpholine (20 mL). The resulting solution was heated at 60 °C for 40 h. Solvent was removed, and the crude was dissolved in $CHCl_3$ (100 mL) and washed with H_2O (50 mL). The aqueous phase was back extracted with $CHCl_3$ (50 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated at reduced pressure. The residue was dissolved in MeOH (15 mL) and to this was added dimethylformamide dimethyl acetal (DMF-DMA, 380 mg, 3.2 mmol). The resulting reaction mixture was stirred at room temperature for 18 h. The solvents were removed, and the crude was purified by column chromatography using a gradient of 0 – 6% MeOH in CH_2Cl_2 to afford nucleoside **3d** (0.78 g, 66%) as a white foam. MS (ESI^+) m/z calcd for $C_{24}H_{42}N_7O_5Si$ $[M + H]^+$ 536.3011, found 536.2994. ^1H (400 MHz, d6-DMSO) δ 11.37 (s, 1H), 8.51 (s, 1H), 8.06 (s, 1H), 5.87 – 5.86 (m, 1H), 4.41 (d, *J* = 3.6 Hz, 2H), 3.96 (td, *J* = 6.1, 2.6 Hz, 1H), 3.53 (t, *J* = 4.6 Hz, 4H), 3.28 (s, 3H), 3.14 (s, 3H), 3.03 (s, 3H), 2.64 (dd, *J* = 13.3, 5.9 Hz, 1H), 2.54 – 2.49 (m, 1H), 2.46 – 2.34 (m, 4H), 0.90 (s, 9H), 0.12 (s, 6H). ^{13}C (101 MHz, d6-DMSO) δ 157.76, 157.50, 157.23, 149.72, 137.28, 120.02, 85.23, 82.41, 81.05, 72.18, 66.15, 60.14, 57.54, 53.91, 40.69, 34.63, 25.66, 17.84, -4.68, -4.86.

Synthesis of nucleoside 4a

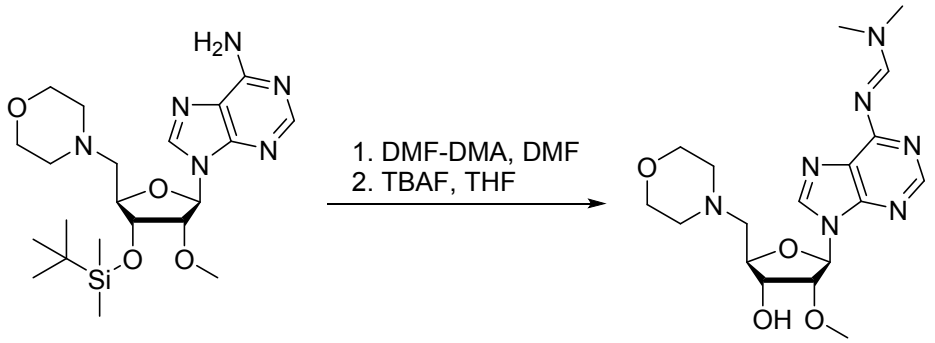


3a **4a**

Nucleoside **3a** (500 mg, 1.13 mmol) was dissolved in THF (5 mL). To this was added tetra-n-butylammonium fluoride (TBAF, 1 M in THF, 1.5 mL, 1.5 mmol). The reaction mixture was stirred at room temperature for 1 h. Solvents were removed, and the residue was purified by column chromatography using a gradient of 0-5% MeOH in EtOAc to obtain alcohol **4a** (320 mg, 86%) as white amorphous powder. MS (ESI^+) m/z calcd for $C_{14}H_{22}N_3O_6$ $[M + H]^+$ 328.1503, found 328.1501. ^1H NMR (500 MHz, d6-DMSO) δ

11.35 (d, *J* = 2.2 Hz, 1H), 7.71 (d, *J* = 8.1 Hz, 1H), 5.76 (d, *J* = 4.0 Hz, 1H), 5.65 (dd, *J* = 8.0, 2.1 Hz, 1H), 5.18 (d, *J* = 6.5 Hz, 1H), 3.94 (q, *J* = 6.0 Hz, 1H), 3.88 (td, *J* = 6.4, 3.9 Hz, 1H), 3.79 (dd, *J* = 5.2, 4.1 Hz, 1H), 3.55 (t, *J* = 4.7 Hz, 4H), 3.36 (s, 3H), 2.63 (dd, *J* = 13.6, 3.9 Hz, 1H), 2.56 – 2.36 (m, 5H). δ_C (101 MHz, d6-DMSO) 163.00, 150.31, 140.79, 101.96, 87.07, 81.63, 81.48, 70.23, 66.14, 59.88, 57.63, 53.99.

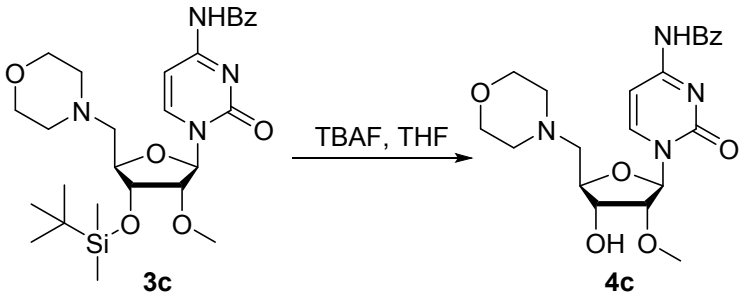
Synthesis of nucleoside 4b



3b **4b**

To a solution of nucleoside **3b** (1.3 g, 2.79 mmol) in DMF (10 mL) was added dimethylformamide dimethyl acetal (DMF-DMA, 0.75 mL, 5.60 mmol). The reaction mixture was stirred at 50 °C for 5 h. The solvent was removed at reduced pressure, and the crude was dissolved in THF (6 mL). TBAF (1M in THF, 3.3 mL, 3.3 mmol) was added, and the reaction mixture was stirred at room temperature for 2 h. The solvent was removed at reduced pressure, and the residue was purified by column chromatography using a gradient of 0 – 7% MeOH in CH_2Cl_2 to yield **4b** (0.81 g, 69%) as a white foam. MS (ESI$^+$) m/z calcd for $C_{18}H_{28}N_7O_4$ [M + H]$^+$ 406.2197, found 406.2209. ^{1}H NMR (400 MHz, d6-DMSO) δ 8.90 (s, 1H), 8.47 (s, 1H), 8.42 (s, 1H), 6.02 (d, *J* = 5.0 Hz, 1H), 5.29 (d, *J* = 6.0 Hz, 1H), 4.41 (t, , *J* = 5.0 Hz, 1H), 4.31 (q, , *J* = 5.2 Hz, 1H), 4.04 – 4.00 (m, 1H), 3.52 (t, *J* = 4.4 Hz, 4H), 3.34 (s, 3H), 3.19 (s, 3H), 3.12 (s, 3H), 2.67 (dd, *J* = 13.4, 4.3 Hz, 1H), 2.54 (dd, *J* = 13.4, 7.0 Hz, 1H), 2.44 – 2.33 (m, 4H). ^{13}C (126 MHz, d6-DMSO) δ 159.27, 157.97, 152.00, 151.19, 141.46, 125.68, 85.60, 82.28, 81.50, 70.51, 66.11, 60.39, 57.62, 53.85, 40.63, 34.53.

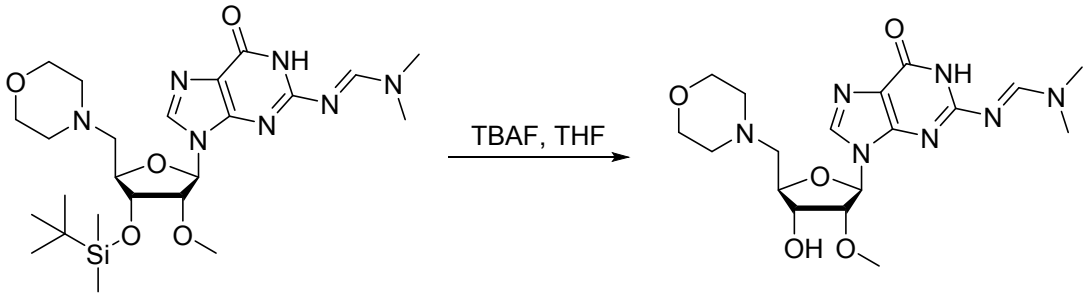
Synthesis of nucleoside 4c



3c **4c**

Protected nucleoside **3c** (2.35 g, 1.13 mmol) was dissolved in THF (10 mL). To this was added tetra-n-butylammonium fluoride (TBAF, 1M in THF, 6.5 mL, 6.5 mmol). The reaction mixture was stirred at room temperature for 1 h. Solvents were removed, and the residue was purified by column chromatography using a gradient of 0-8% MeOH in EtOAc to obtain alcohol **4c** (1.48 g, 80%) as a white foam. MS (ESI$^+$) m/z calcd for $C_{21}H_{27}N_4O_6$ $[M + H]^+$ 431.1925, found 431.1936. ^{1}H NMR (500 MHz, d6-DMSO) δ 11.30 (s, 1H), 8.30 (d, *J* = 7.6 Hz, 1H), 8.04 – 7.94 (m, 2H), 7.62 (t, *J* = 7.4 Hz, 1H), 7.51 (t, *J* = 7.7 Hz, 2H), 7.39 (d, *J* = 7.5 Hz, 1H), 5.84 (d, *J* = 2.0 Hz, 1H), 5.18 (d, *J* = 7.0 Hz, 1H), 3.99 – 3.95 (m, 1H), 3.90 – 3.86 (m, 1H), 3.73 (dd, *J* = 5.0, 2.1 Hz, 1H), 3.58 (t, *J* = 4.7 Hz, 4H), 3.47 (s, 3H), 2.71 (dd, *J* = 13.8, 3.1 Hz, 1H), 2.63 (dd, *J* = 14.0, 6.5 Hz, 1H), 2.56 – 2.41 (m, 4H), δ_C (101 MHz, d6-DMSO) 167.50, 163.07, 154.13, 145.19, 133.13, 132.70, 129.21, 128.43, 128.40, 96.36, 88.74, 82.81, 81.31, 70.09, 66.24, 59.36, 57.86, 54.12.

Synthesis of nucleoside 4d

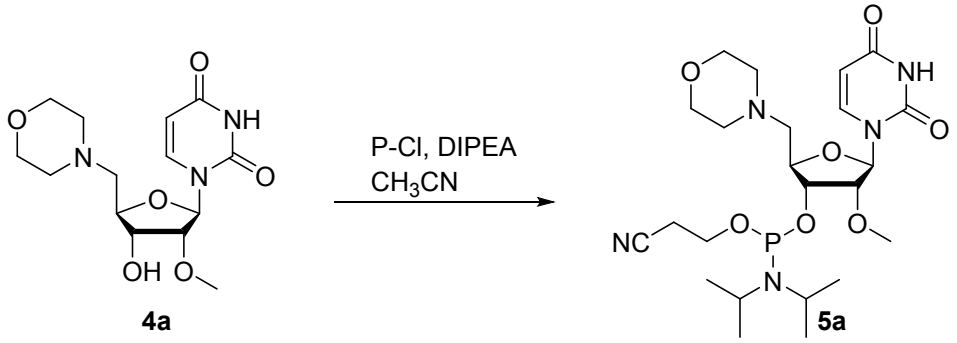


3d **4d**

To a solution of **3** (0.63 g, 1.25 mmol) in THF (5 mL) was added tetra-n-butylammonium fluoride (TBAF, 1M in THF, 1.5 mL, 1.5 mmol). The reaction mixture was stirred at room temperature for 2 h. The solvent was removed at reduced pressure, and the residue was purified by column chromatography using a gradient of 0 – 8% MeOH in CH_2Cl_2 to yield **4d** (0.46 g, 88%) as a white foam. MS (ESI$^+$) m/z calcd for $C_{18}H_{28}N_7O_5$ $[M + H]^+$ 422.2146, found 422.2154. ^{1}H NMR (400 MHz, d6-DMSO) δ 11.35 (s, 1H), 8.54 (s, 1H), 8.03 (s, 1H), 5.87 (d, *J* = 5.1 Hz, 1H), 5.28 (d, *J* = 5.4 Hz, 1H), 4.24 (dt, *J* = 12.5, 5.0 Hz, 2H), 4.00 – 3.96

(m, 1H), 3.53 (t, *J* = 4.6 Hz, 4H), 3.34 (s, 3H), 3.15 (s, 3H), 3.03 (s, 3H), 2.65 (dd, *J* = 13.3, 4.7 Hz, 1H), 2.55 – 2.50 (m, 1H), 2.40 (dt, *J* = 9.2, 4.9 Hz, 4H). ^{13}C NMR (126 MHz, d6-DMSO) δ 157.87, 157.52, 157.28, 149.79, 136.79, 119.76, 84.84, 82.34, 81.87, 70.52, 66.12, 60.54, 57.55, 53.85, 40.70, 34.67.

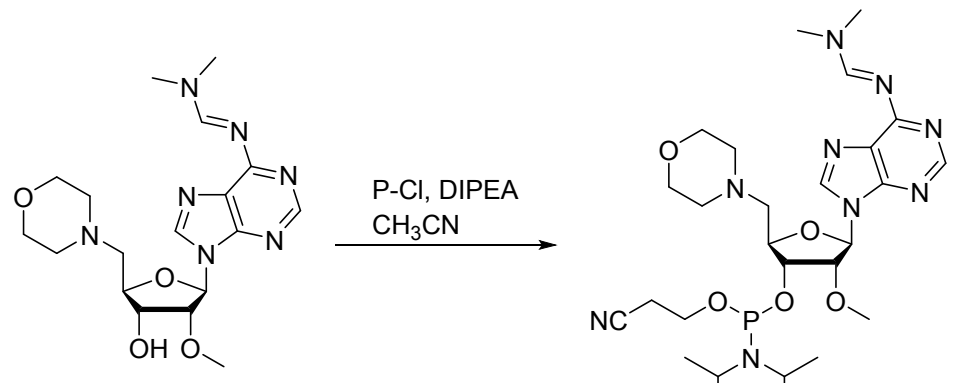
Synthesis of nucleoside 5a



4a **5a**

To a solution of **4a** (6.2 gm, 18 mmol) in dry CH_3CN (60 mL) was added ethyl thiotetrazole (2.4 g, 18 mmol). 2-Cyanoethyl N, N, N', N'-tetraisopropylphosphordiamidite (6.8 gm, 22 mmol,) was added slowly to the reaction mixture and stirred at room temperature for 3 h. The reaction mixture was filtered and purified by column chromatography using a gradient of EtOAc in hexanes containing 0.2% triethylamine to yield **5a** (5.0 g, 50%). MS (ESI$^+$) m/z calcd for $C_{23}H_{39}N_5O_7P$ $[M + H]^+$ 528.2582, found 528.2592. ^{31}P NMR (202 MHz, CD_3CN) δ 151.04, 150.76.

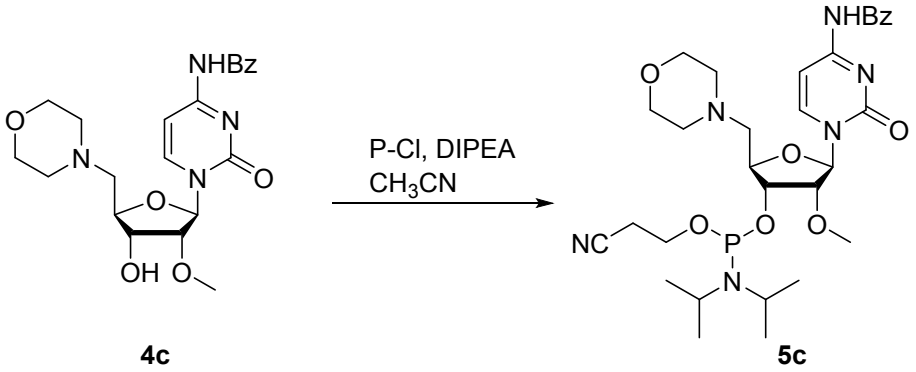
Synthesis of nucleoside 5b



4b **5b**

Nucleoside **4b** (0.82 g, 1.35 mmol) was co-evaporated with dry CH_3CN and re-dissolved in dry CH_3CN (6 mL). N, N-diisopropylethylamine (DIPEA, 1.0 g, 7.73 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (P-Cl, 0.95 g, 4.03 mmol) were added, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL) followed by brine (20 mL). The combined aqueous phase was back extracted with CH_2Cl_2 (20 mL), and the combined organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography using a gradient of 0 – 3% MeOH in CH_2Cl_2 (containing 0.2% Et_3N) to obtain phosphoramidite **5b** (0.90 g, 73%) as a white foam. MS (ESI^+) m/z calcd for $C_{27}H_{45}N_9O_5P$ $[M + H]^+$ 606.3276, found 606.3286. ^{31}P (202 MHz, CD_3CN) δ 151.04, 150.70

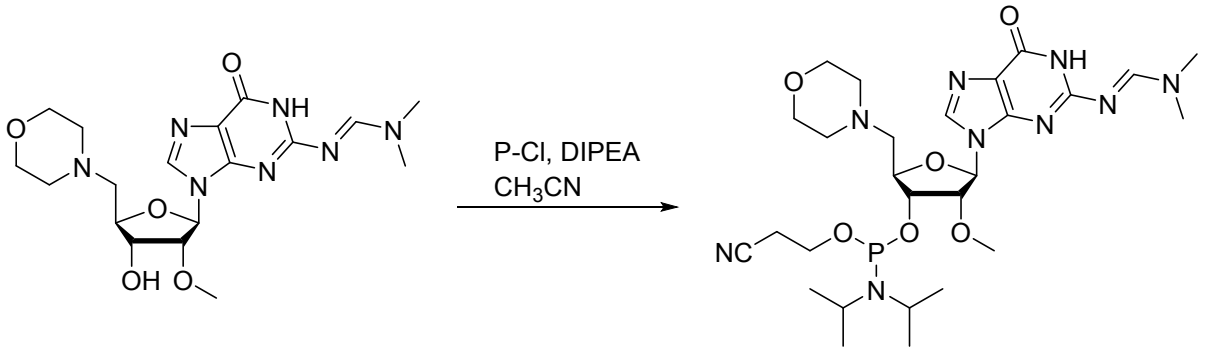
Synthesis of nucleoside 5c



4c **5c**

Nucleoside **4c** (0.50 g, 1.16 mmol) was co-evaporated with dry CH_3CN (5mL) and re-dissolved in dry CH_3CN (5mL). N, N -diisopropylethylamine (DIPEA, 0.60 g, 4.64 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (P-Cl, 0.55 g, 2.32 mmol) were added, and the reaction mixture was stirred at room temperature for 2 h. The solvents were evaporated under reduced pressure, and the crude was purified by column chromatography using a gradient of 0 – 2% MeOH in CH_2Cl_2 (containing 0.2% Et_3N) to obtain phosphoramidite **5c** (0.40 g, 57%) as a white foam. MS (ESI^+) m/z calcd for $C_{30}H_{44}N_6O_7P$ $[M + H]^+$ 631.3004, found 631.3034. ^{31}P (202 MHz, CD_3CN) δ 151.25, 150.89.

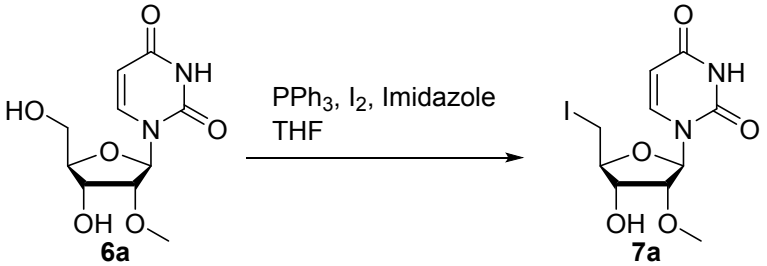
Synthesis of nucleoside 5d



4d **5d**

Nucleoside **4d** (0.40 g, 0.95 mmol) was co-evaporated with dry CH_3CN and re-dissolved in dry CH_3CN (5mL). N, N -diisopropylethylamine (DIPEA, 0.60 g, 4.64 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (P-Cl, 0.66 g, 2.78 mmol) were added, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL) followed by brine (20 mL). The combined aqueous phase was back extracted with CH_2Cl_2 (20 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography using a gradient of 0 – 3% MeOH in CH_2Cl_2 (containing 0.2% Et_3N) to obtain phosphoramidite **5d** (460 mg, 77%). MS (ESI^+) m/z calcd for $C_{27}H_{45}N_9O_6P$ $[M + H]^+$ 622.3225, found 622.3240. ^{31}P (202 MHz, CD_3CN) δ 151.24, 150.89.

Synthesis of nucleoside 7a

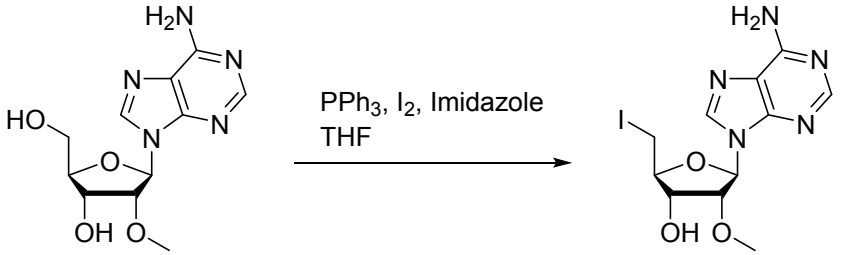


6a **7a**

2'-*O*-Methyluridine (**6a**, 2.58 g, 10 mmol), imidazole (1.36 g, 20 mmol), and triphenylphosphine (3.93 g, 15 mmol) were suspended in dry THF (50 mL). To this was added a solution of iodine (3.16 g, 12.5 mmol)

in THF (20 mL) dropwise at 0 °C. The reaction mixture was slowly warmed to room temperature and stirred for another 3 h. The solvent was removed at reduced pressure, and the residue was purified by column chromatography using a gradient of 0 – 7% MeOH in CH_2Cl_2 to afford the desired nucleoside **7a** (3.26 g, 86%) as a pale-yellow foam. MS (ESI$^+$) m/z calcd for $C_{10}H_{14}IN_2O_5$ $[M + H]^+$ 368.9942, found 368.9951. ^{1}H NMR (400 MHz, d6-DMSO) δ 11.40 (d, *J* = 2.1 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 1H), 5.85 (d, *J* = 5.4 Hz, 1H), 5.68 (dd, *J* = 8.1 Hz, 2.2 Hz, 1H), 5.44 (d, *J* = 6.0 Hz, 1H), 4.04 – 4.00 (m, 1H), 3.97 (t, *J* = 5.4 Hz, 1H), 3.88 – 3.79 (m, 1H), 3.54 (dd, *J* = 10.6 Hz, 5.4 Hz, 1H), 3.39 (dd, *J* = 10.6 Hz, 6.8 Hz, 1H), 3.33 (s, 3H). ^{13}C NMR (101 MHz, d6-DMSO) δ 162.87, 150.41, 140.86, 102.31, 86.70, 83.31, 81.22, 71.44, 57.60, 7.23.

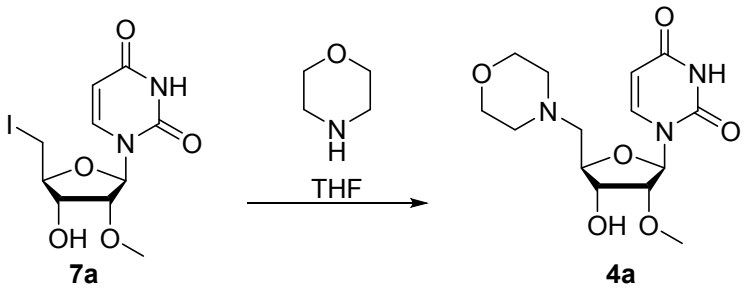
Synthesis of nucleoside 7b



6b **7b**

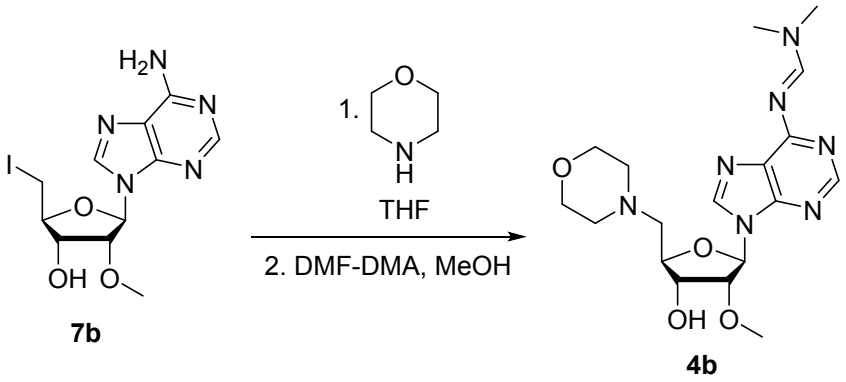
Nucleoside **6b** (1.44 g, 5.0 mmol), imidazole (0.70 g, 10.0 mmol) and triphenylphosphine (2.00 g, 7.5 mmol) were suspended in dry THF (30 mL). To this was added a solution of iodine (1.60 g, 6.3 mmol) in THF (10 mL) dropwise at 0 °C. The reaction mixture was slowly warmed to room temperature and stirred for another 3 h. The solvent was removed at reduced pressure, and the residue was purified by column chromatography using a gradient of 0 – 8% MeOH in CH_2Cl_2 to afford the nucleoside **7b** (1.60 g, 82%) as an amorphous solid. MS (ESI$^+$) m/z calcd for $C_{11}H_{15}IN_5O_3$ $[M + H]^+$ 392.0214, found 392.0222. ^{1}H NMR (400 MHz, d6-DMSO) δ 8.37 (s, 1H), 8.15 (s, 1H), 7.32 (s, 2H), 6.02 (d, *J* = 5.9 Hz, 1H), 5.54 (d, *J* = 5.5 Hz, 1H), 4.59 (t, *J* = 5.4 Hz, 1H), 4.38 – 4.34 (m, 1H), 4.02 – 3.98 (m, 1H), 3.60 (dd, *J* = 10.5, 5.9 Hz, 1H), 3.47 (dd, *J* = 10.4, 7.0 Hz, 1H), 3.31 (s, 3H, OCH_3). ^{13}C NMR (126 MHz, d6-DMSO) 156.12, 152.78, 149.27, 139.80, 119.11, 85.58, 84.42, 81.45, 71.45, 57.63, 7.52. Note: The compound was found to be unstable in solution. NMR samples were prepared at the time of the experiment.

Alternative synthesis of nucleoside 4a



5′-Iodo-2′-*O*-methyluridine **7a** (1.0 g, 2.71 mmol) was dissolved in THF (5 mL). Morpholine (5 mL) was added, and the reaction mixture was stirred at room temperature for 16 h. A white solid precipitated during this period. The solid was removed by filtration and washed with CH_2Cl_2 (50 mL). The filtrate was purified by column chromatography using a gradient of 0 – 8% MeOH in CH_2Cl_2 to afford the nucleoside **4a** (0.55 g, 62%) as a white powder.

Alternative synthesis of nucleoside 4b



Nucleoside **7b** (1.0 g, 2.55 mmol) was dissolved in THF (5 mL) and morpholine (5 mL). The reaction mixture was then stirred for 2 days at room temperature. A white solid precipitated. The solid was removed by filtration, washed with CH_2Cl_2 (50 mL), and purified by column chromatography using a gradient of 0 – 8% MeOH in CH_2Cl_2 to obtain white solid (0.7 g). The solid was dissolved in dry MeOH (6 mL) and to this solution was added dimethylformamide dimethyl acetal (DMF-DMA, 0.80 g, 6.7 mmol). The resulting

solution was stirred for 16 h at room temperature. The solvents were removed, and the crude was purified by column chromatography using a gradient of 0 – 7% MeOH in CH_2Cl_2 to afford nucleoside **4b** (550 mg, 48% over 2 steps) as a white solid material.

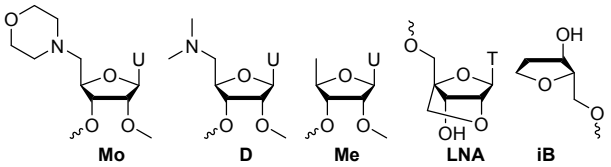
1.3 Oligonucleotide synthesis

Oligonucleotides were synthesized on a Mermade 192 synthesizer using universal or custom supports following protocol described by Schlegel et al.[2] A solution of 0.25 M 5-(*S*-ethylthio)-1*H*tetrazole in acetonitrile was used as the activator. The phosphoramidite solutions were 0.10 M in anhydrous acetonitrile or 9:1 acetonitrile:DMF (2′-OMe-C, 2′-OMe-A and 2′-OMe-G) . The oxidizing reagent was 0.02 M I_2 in THF/pyridine/H_2O. *N,N*-Dimethyl-*N*′-(3-thioxo-3*H*-1,2,4-dithiazol-5-yl)methanimidamide (DDTT), 0.1 M in pyridine, was used as the sulfurizing reagent. The detritylation reagent was 3% dichloroacetic acid (DCA) in CH_2Cl_2. After trityl-off synthesis, oligonucleotides were removed from solid support by incubating the columns with 40% aqueous methylamine (150 µL for 30 min). A very light vacuum was applied to drain the solution into a plate, and procedure was repeated. The plate containing combined solution was shaken for 1h at room temperature whereupon a cold mixture of acetonitrile:EtOH (1.2 mL) was added, and the plate was left at −20 °C for 16h. This resulted in precipitation of oligonucleotides which were then recovered by centrifuging the plate at 3000 rpm at 4 °C for 45 minutes. The supernatant was removed and the pellets were dissolved in 20 mM aqueous NaOAc. The oligonucleotides were then desalted using GE Hi-trap desalting column using water as eluent. The oligonucleotides were identified by using HPLC-MS and were obtained in purity of ≥80%. Hybridization to generate siRNA duplexes was performed by mixing equimolar amounts of complementary strands in 1×PBS buffer, pH 7.4, and by heating in an over at 100 °C for 45 min followed by slow cooling to room temperature.

Table S1: Mass spec analysis of modified oligonucleotides

Entry	siRNA	siRNA duplex	Mass	
			calculated	found
1	Parent	5′-U•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-u•U•gAuGcCcAuauUuGuCaCa•a•a	8715.3 7531.9	8714.0 7530.7
2	S5′-**Mo**	5′-**Mo**•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-u•U•gAuGcCcAuauUuGuCaCa•a•a	8797.5 7531.9	8794.9 7530.6

3	S5′-**D**	5′- **D**•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-u•U•gAuGcCcAuauUuGuCaCa•a•a	8755.4 7531.9	8752.6 7531.0
4	S5′-**Me**	5′-**Me**•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-u•U•gAuGcCcAuauUuGuCaCa•a•a	8712.4 7531.9	8709.8 7530.5
5	S5′-**LNA**	5′-**LNA**•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-u•U•gAuGcCcAuauUuGuCaCa•a•a	8739.4 7531.9	8737.9 7530.9
6	S5′-**iB**	5′-**iB**U•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-u•U•gAuGcCcAuauUuGuCaCa•a•a	8895.4 7531.9	8893.6 7530.7
7	AS5′-**Mo**	5′-U•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-**Mo**•U•gAuGcCcAuauUuGuCaCa•a•a	8715.3 7602.0	8713.9 7599.2
8	AS5′-**D**	5′-U•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-**D**•U•gAuGcCcAuauUuGuCaCa•a•a	8715.3 7560.0	8714.0 7557.9
9	AS5′-**Me**	5′-U•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-**Me**•U•gAuGcCcAuauUuGuCaCa•a•a	8715.3 7516.9	8713.9 7514.3
10	AS5′-**LNA**	5′-U•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-**LNA**•U•gAuGcCcAuauUuGuCaCa•a•a	8715.3 7543.9	8713.8 7542.6
11	AS5′-**iB**	5′-U•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-**iB**u•U•gAuGcCcAuauUuGuCaCa•a•a	8715.3 7711.9	8713.8 7709.8
12	S/As-5′-**Mo**	5′-**Mo**•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$ 5′-**Mo**•U•gAuGcCcAuauUuGuCaCa•a•a	8795.5 7602.0	8794.9 7599.2



Structure of modifications: 5′-deoxy-5′-morpholino-2′-*O*-methyl uridine (**Mo**), 5′-deoxy-5′-dimethylamino-2′-*O*-methyl uridine (**D**), 5′-deoxy-2′-*O*-methyl uridine (**Me**), locked nucleic acid (**LNA**), and inverted abasic site (**iB**).

1.4 *In vitro* RNAi activity

To 5 µL of siRNA in each well of a 384-well collagen-coated plate were added 4.9 µL of Opti-MEM and 0.1 µL of Lipofectamine RNAiMax (Invitrogen). The final siRNA concentrations were 0.1 or 10 nM. Plates were incubated at room temperature for 15 min, and 40 µL of William's E Medium (Life Technologies) containing ~5 x10^3 primary mouse hepatocytes cells were added. Each condition was assessed in quadruplicate. Cells were incubated for 24 h prior to RNA purification. RNA was isolated with DynaBeads (ThermoFisher), and reverse transcribed into cDNA according to manufacturer's protocol (Applied Biosystems). Multiplex qPCR reactions were performed in duplicate using a gene-specific TaqMan assay for *apob* (ThermoFisher Scientific, #Mm01545156_m1) and mouse *Gapdh* (#4352339E) as an endogenous

control. Real-Time PCR was performed on a Roche LightCycler 480 using LightCycler 480 Probes Master Mix (Roche). Data were analyzed using the ΔΔCt method, normalizing *apob* expression to *Gapdh*, followed by normalization to the average of the control siRNA-transfected wells. Data were expressed as mean log difference from cells treated with the parent siRNA. Various designs were compared to parent design by multiple linear regression using the software package R.

1.5 Quantification of whole liver and Ago2-associated siRNA levels

Mice (n=3 per group) were sacrificed on days 3, 7, and 15 post-dose, and livers were snap-frozen in liquid nitrogen and ground into powder for downstream analysis. Total siRNA liver levels and Ago2-bound siRNA were measured by SL-qPCR based on previously published methods.[1] For SL-qPCR of *apob*-targeting siRNAs, the following probes and primers were used: sense stem loop primer 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGATGCCCAT-3', sense forward primer 5-gccgcgcTGTGACAAATATG-3', sense probe 5'-CTGGATACGACTGATGCCC-3', antisense stem loop primer 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTTTGTGACAA-3', antisense forward primer 5'-gccgcgcTTGATGCCCATA-3', antisense probe 5'-CTGGATACGACTTTGTGAC-3', and universal reverse primer 5'-GTGCAGGGTCCGAGGT-3'.

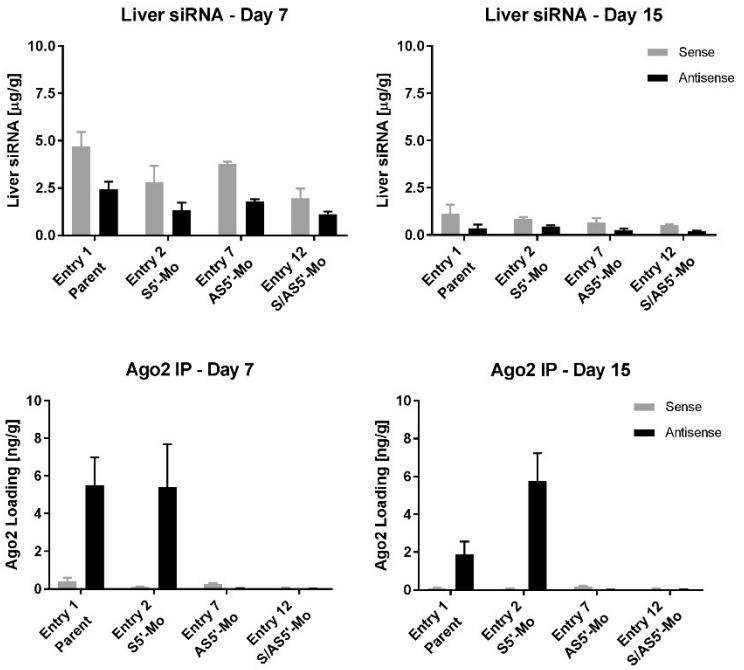


Figure S1. Mice (n=3 per group) were treated with a single dose of 3 mg/kg parent, AS5′-Mo, S5′-Mo, or S/AS5′-Mo siRNAs targeting *apob*. Cohorts of mice were sacrificed at 3, 7, and 15 days post-dose, and liver lysates were generated. (A) Liver levels of the antisense and passenger strand of siRNAs were quantified by RT-qPCR. (B) Ago2 was immunoprecipitated, and Ago2-bound antisense (antisense) and passenger (sense) strand levels were quantified by RT-qPCR.

1.6 *In vivo* RNAi activity targeting *FIX* gene

Table S2. siRNAs targeting *FIX* gene

Entry	siRNA	siRNA duplex
1	Parent	5′-u•g•gaagCfaGfUfAfuguugaugga-GalNAc$_3$ 5′- u•Cf•cauCfaAfCfauacUfgCfuucca•a•a
2	S5′-**Mo**	5′-**Mo**u•g•gaagCfaGfUfAfuguugaugga-GalNAc$_3$ 5′- u•Cf•cauCfaAfCfauacUfgCfuucca•a•a
3	AS5′-**Mo**	5′-u•g•gaagCfaGfUfAfuguugaugga-GalNAc$_3$ 5′-**Mo**u•Cf•cauCfaAfCfauacUfgCfuucca•a•a

Abbreviations and symbols: S, sense strand; AS, antisense strand; Mo, morpholino; uppercase, 2′-F; lowercase, 2′-*O*Me; •, phosphorothioate; GalNAc$_3$, hydroxyprolynyl trivalent N-acetyl-galactosamine ligand.

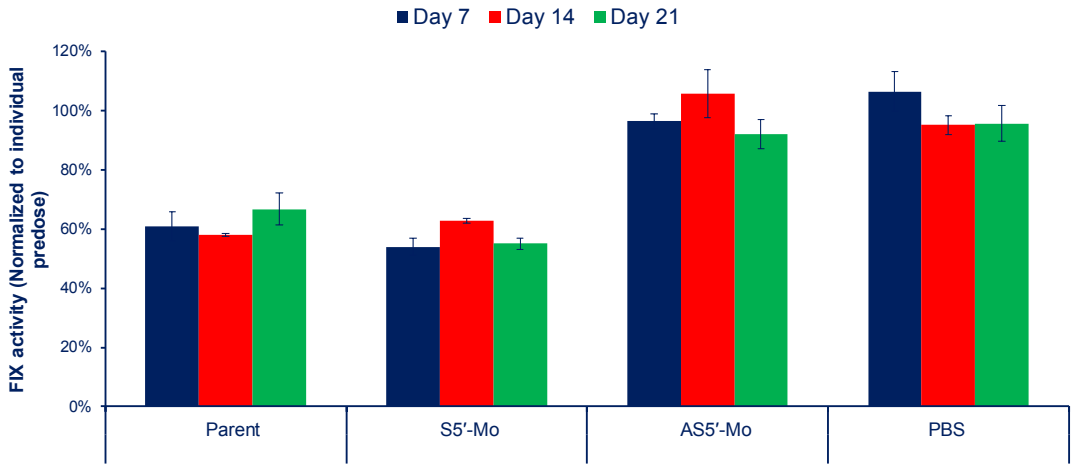
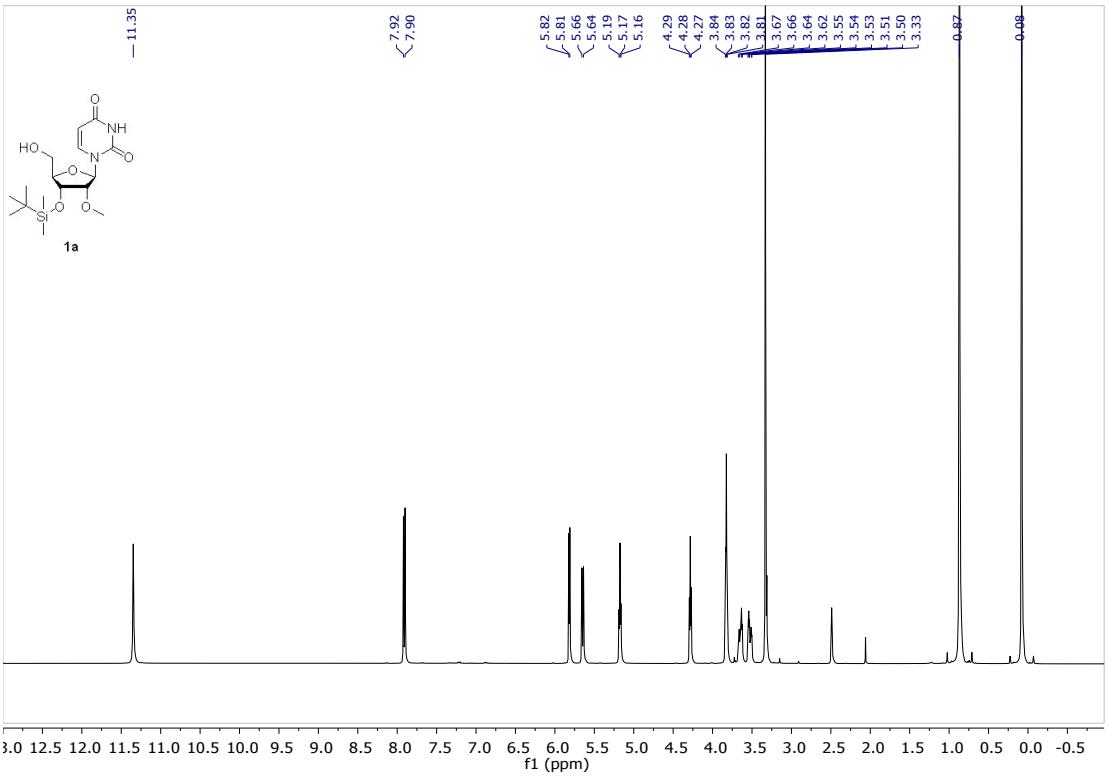
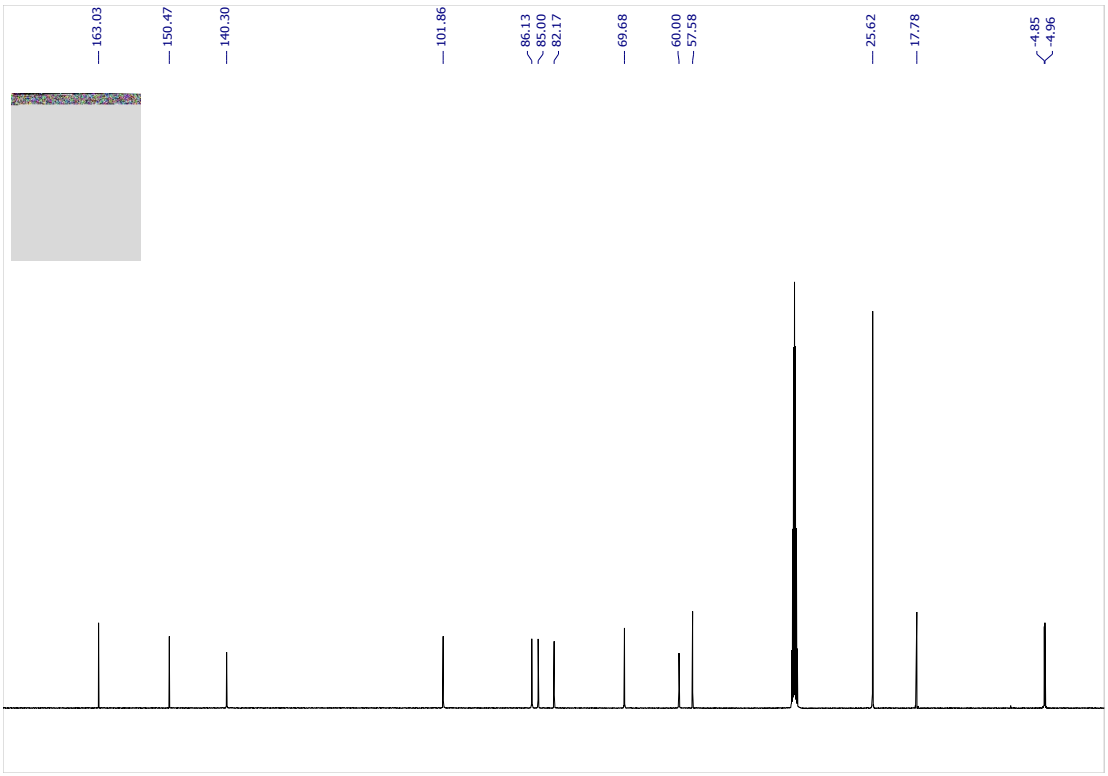


Figure S2: Serum FIX levels in mice on days 7 (blue), 14 (red), and 21 (green) following a single subcutaneous 1 mg/kg dose of indicated siRNAs (Table S2). FIX levels were normalized to the individual pre-dose values. The experiments were conducted as described in literature.[3]

1.7 Representative NMR spectra

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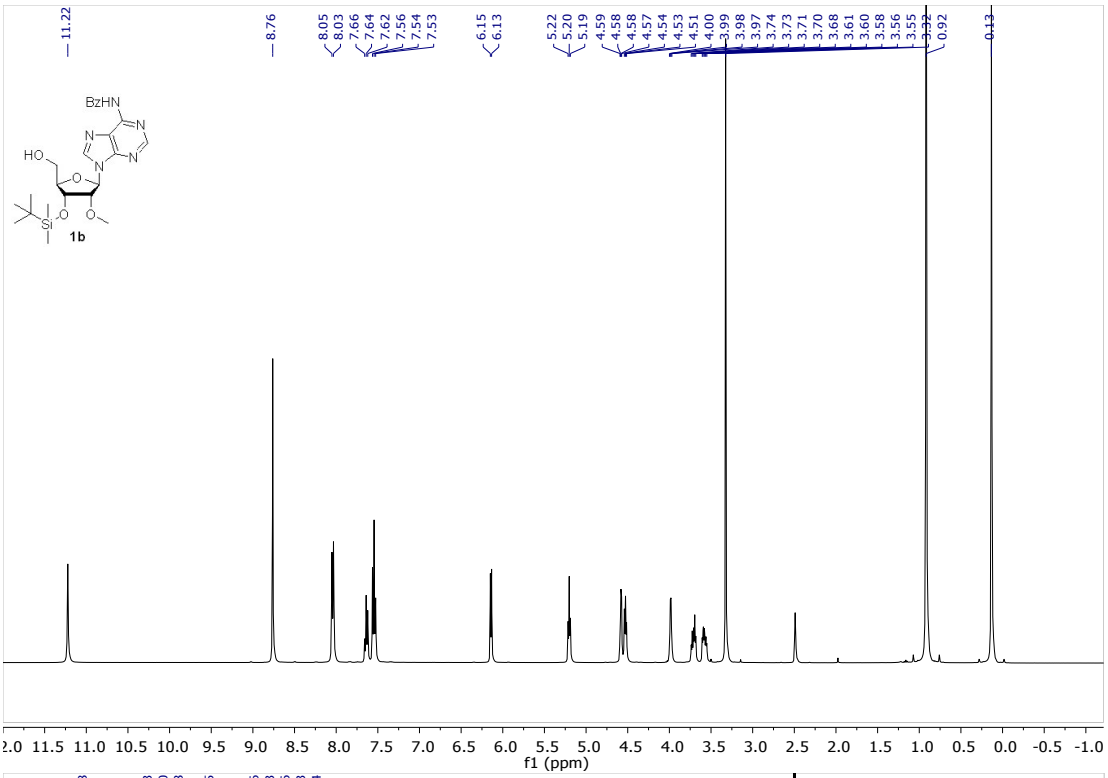
82.17
69.68
60.00
57.58
25.62
17.78
-4.85
-4.96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7.56
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7.53
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6.13
5.22
5.20
5.19
4.59
4.58
4.58
4.57
4.54
4.53
4.51
4.00
3.99
3.98
3.97
3.74
3.73
3.71
3.70
3.68
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3.58
3.56
3.55
0.92
0.13
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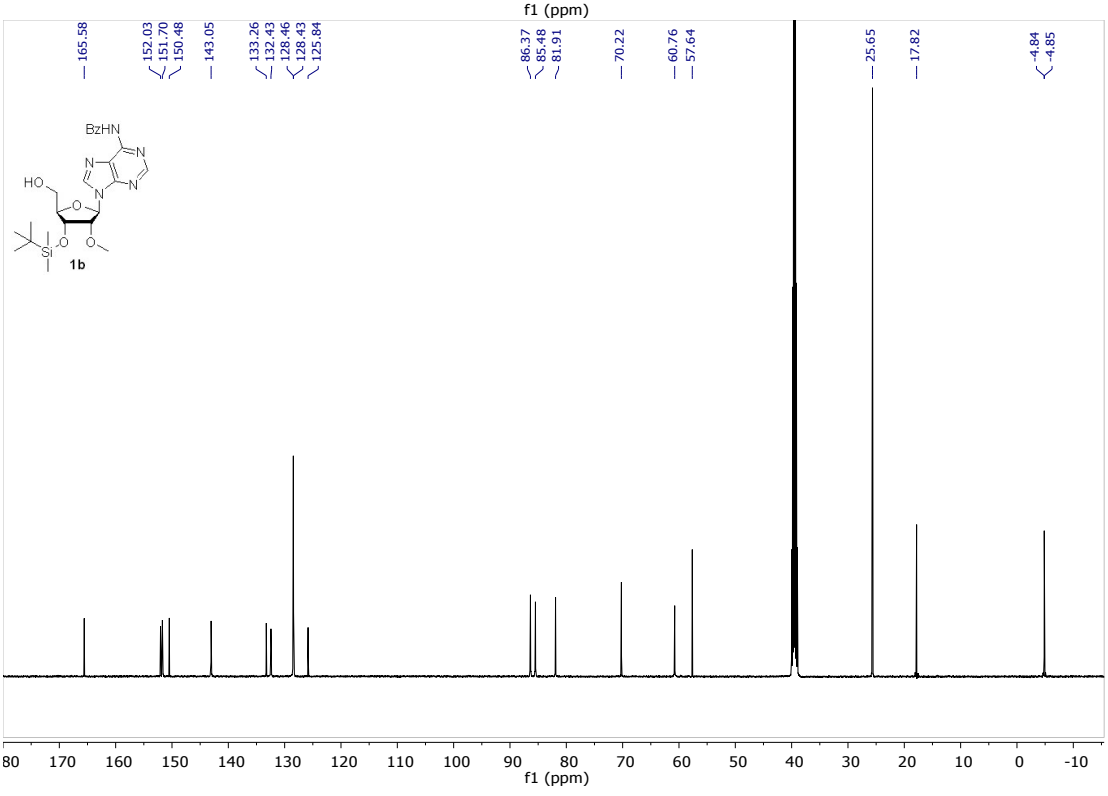
HO
Si
1b
f1 (ppm)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128.46
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81.91
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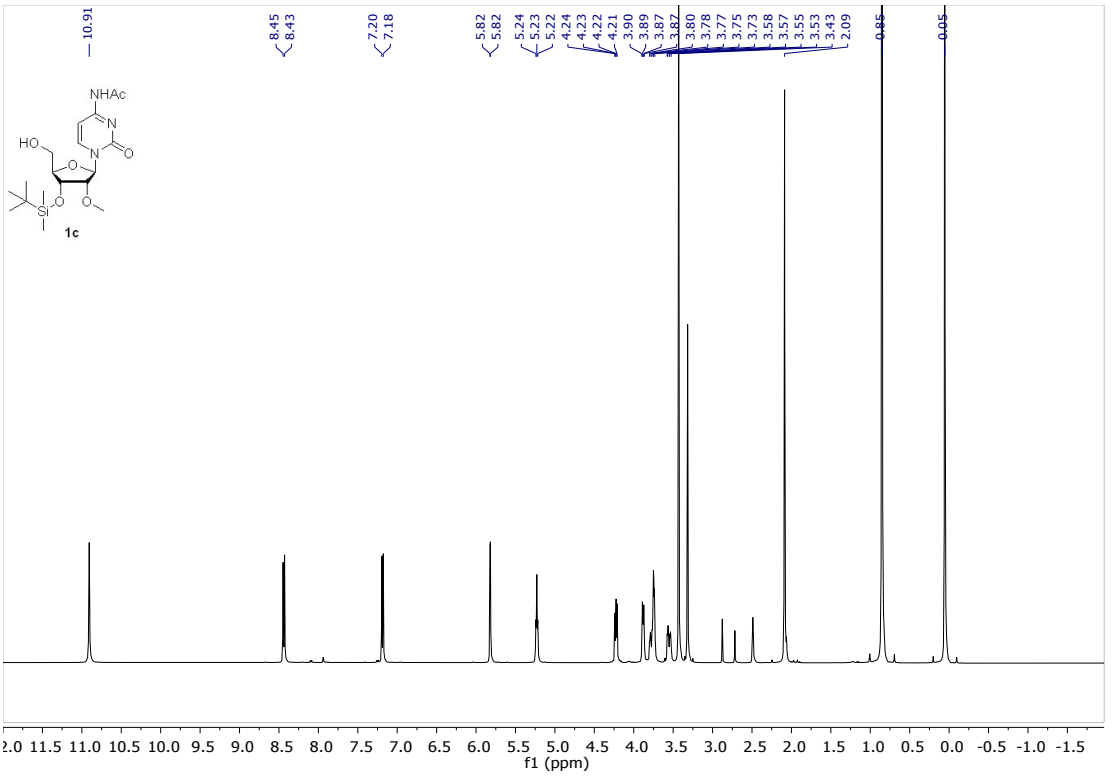
25.65
17.82
-4.84
-4.85
BzHN
HO
Si
1b
f1 (ppm)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5.82
5.82
5.24
5.23
5.22
4.24
4.23
4.22
4.21
3.90
3.89
3.87
3.87
3.80
3.78
3.77
3.75
3.73
3.58
3.57
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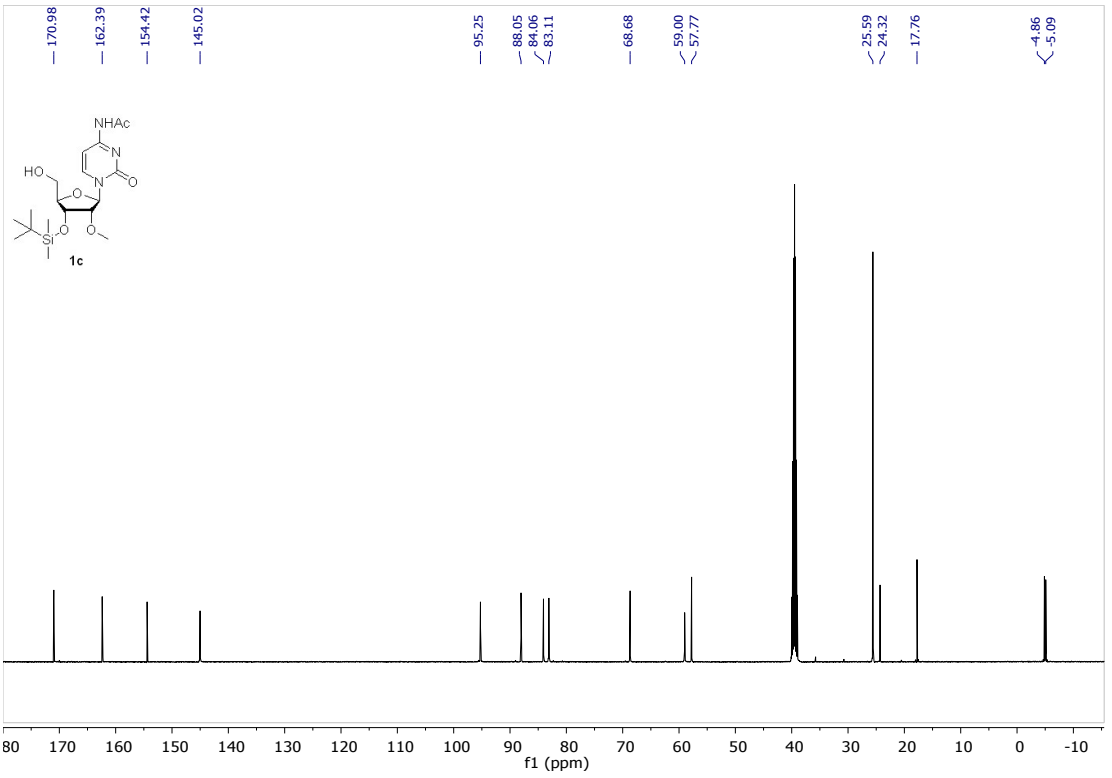
O
N
O
Si
1c
f1 (ppm)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83.11
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HO
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N
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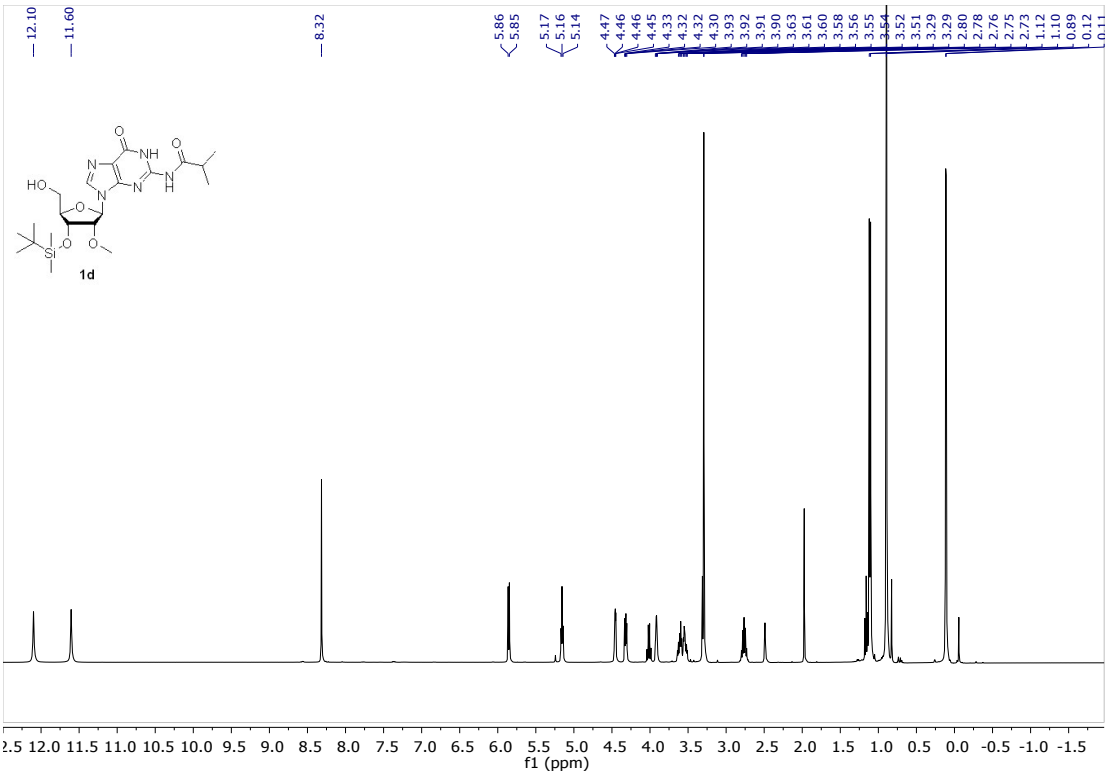
Si
1c
f1 (ppm)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4.32
4.30
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3.92
3.91
3.90
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3.54
3.52
3.51
3.29
3.29
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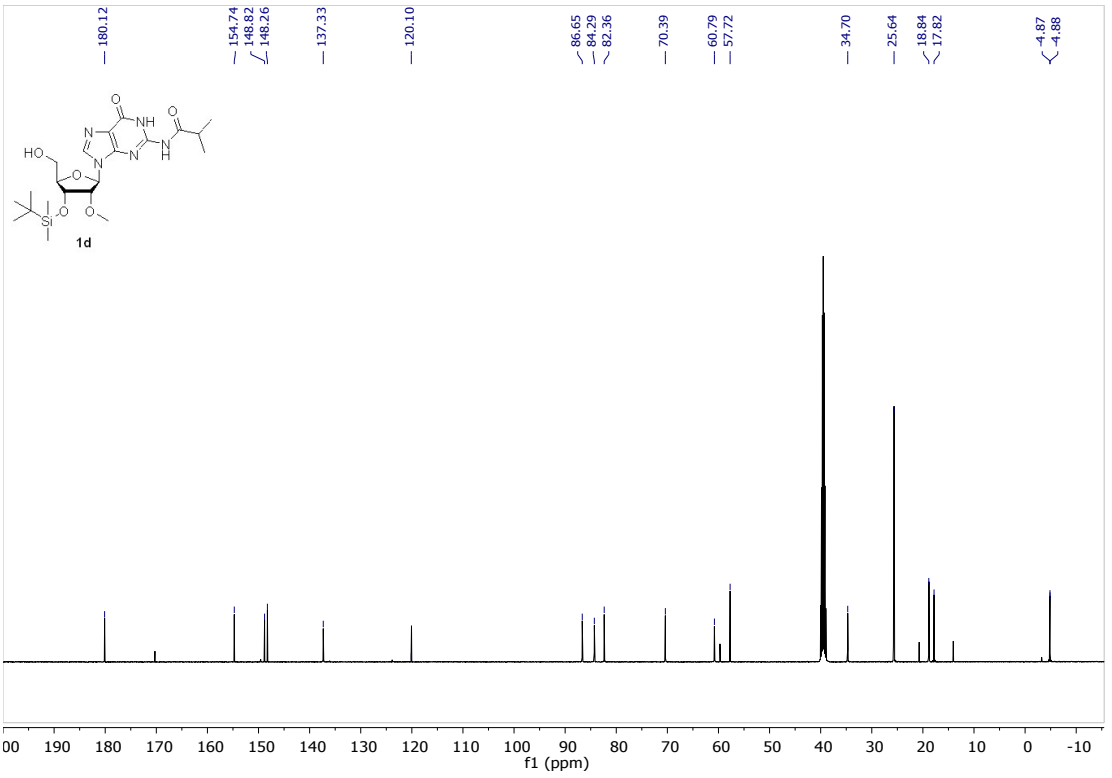
f1 (ppm)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17.82
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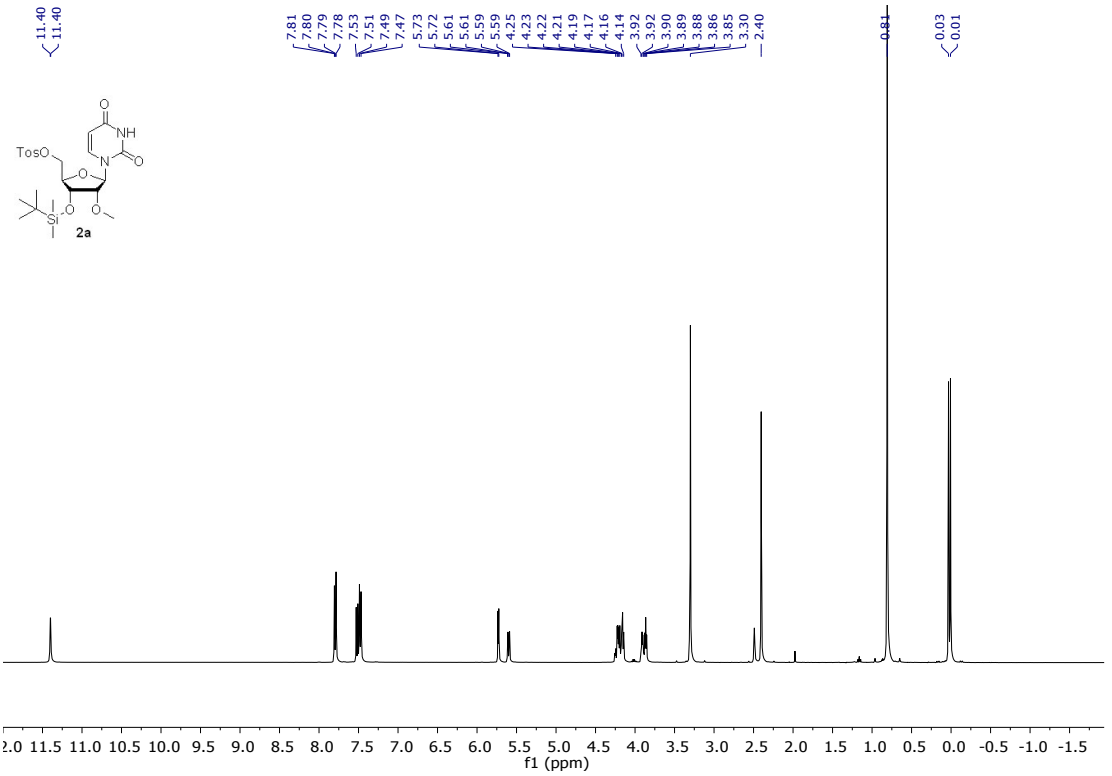
4.88
1d
f1 (ppm)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5.61
5.61
5.59
5.59
4.25
4.23
4.22
4.21
4.19
4.17
4.16
4.14
3.92
3.92
3.90
3.89
3.88
3.86
3.85
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0.03
0.01
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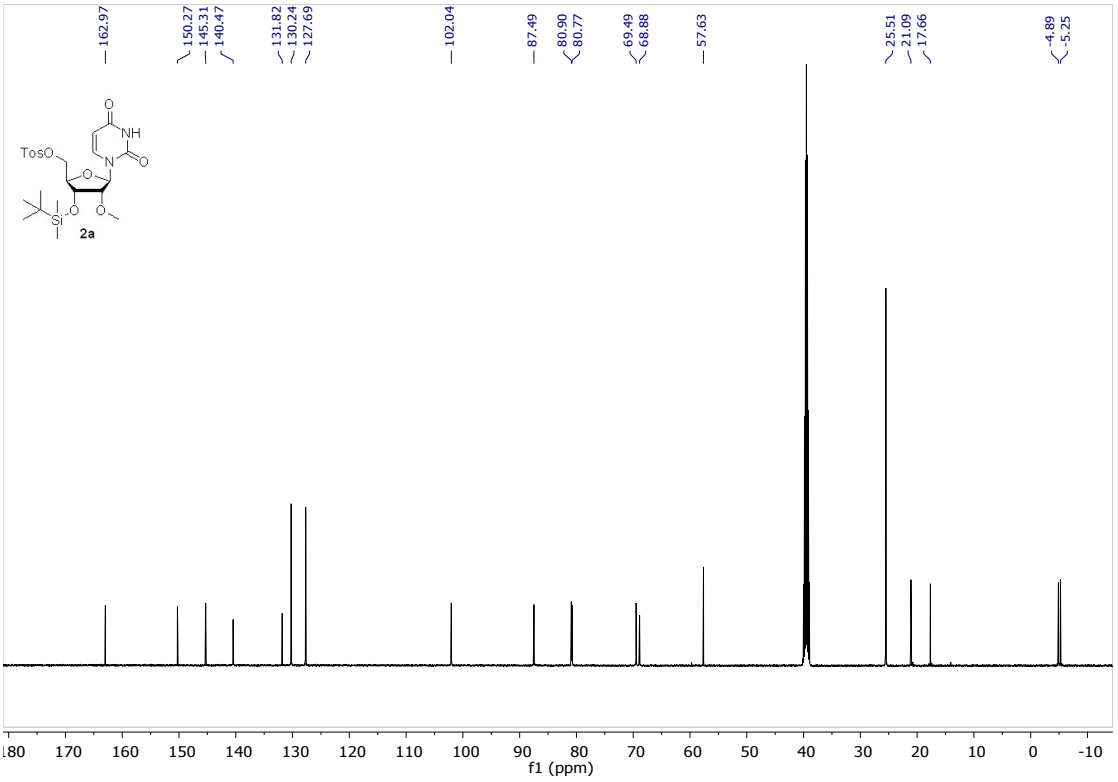
N
O
O
O
Si
2a
f1 (ppm)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80.77
69.49
68.88
57.63
25.51
21.09
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-4.89
-5.25
TosO
NH
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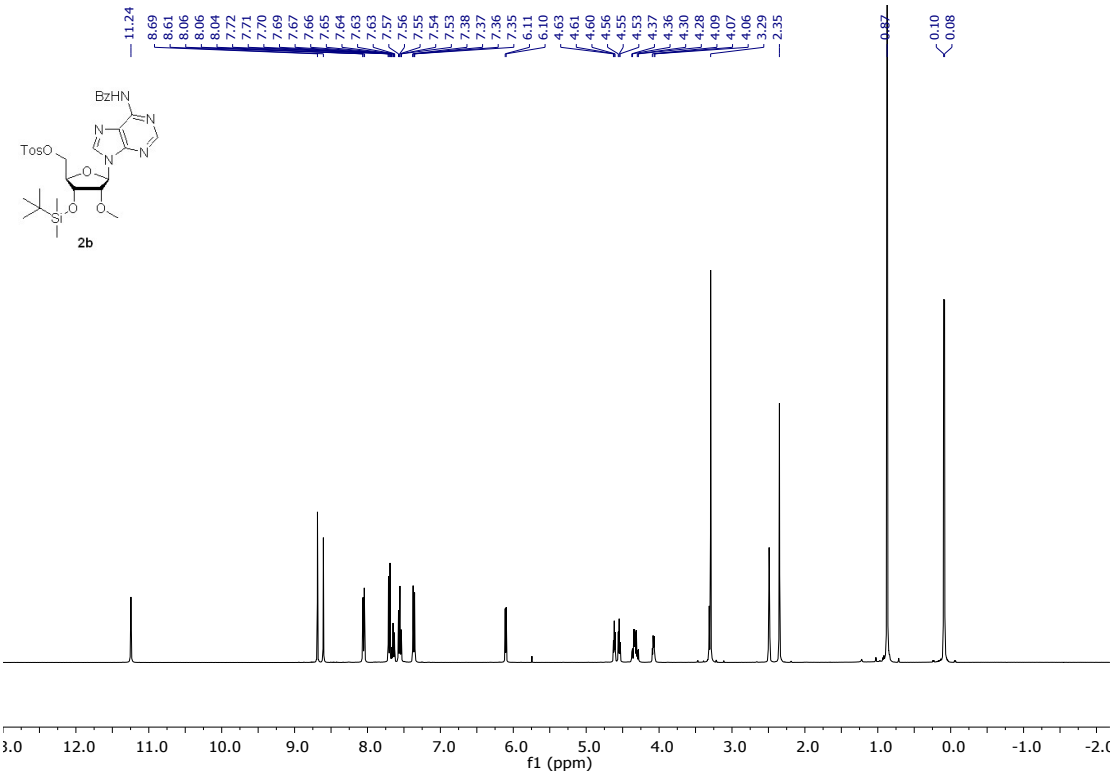
N
O
O
O
Si
2a
f1 (ppm)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7.63
7.57
7.56
7.55
7.54
7.53
7.38
7.37
7.36
7.35
6.11
6.10
4.63
4.61
4.60
4.56
4.55
4.53
4.37
4.36
4.30
4.28
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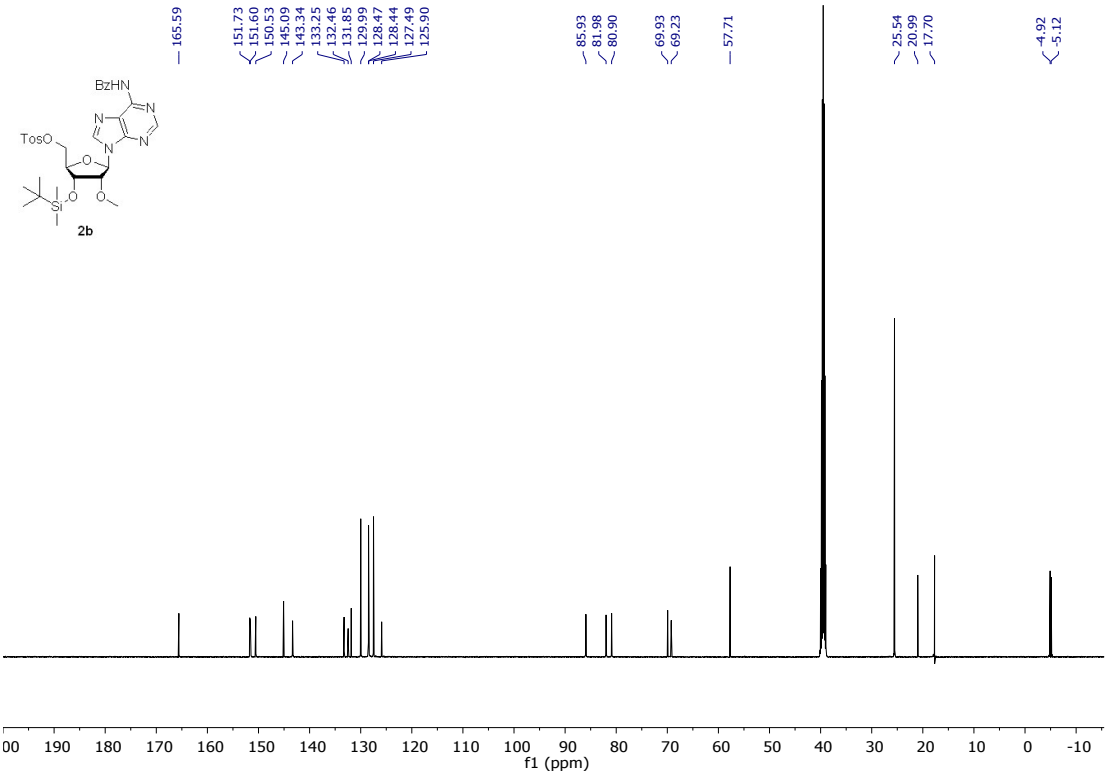
0.10
0.08
BzHN
TosO
2b
f1 (ppm)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85.93
81.98
80.90
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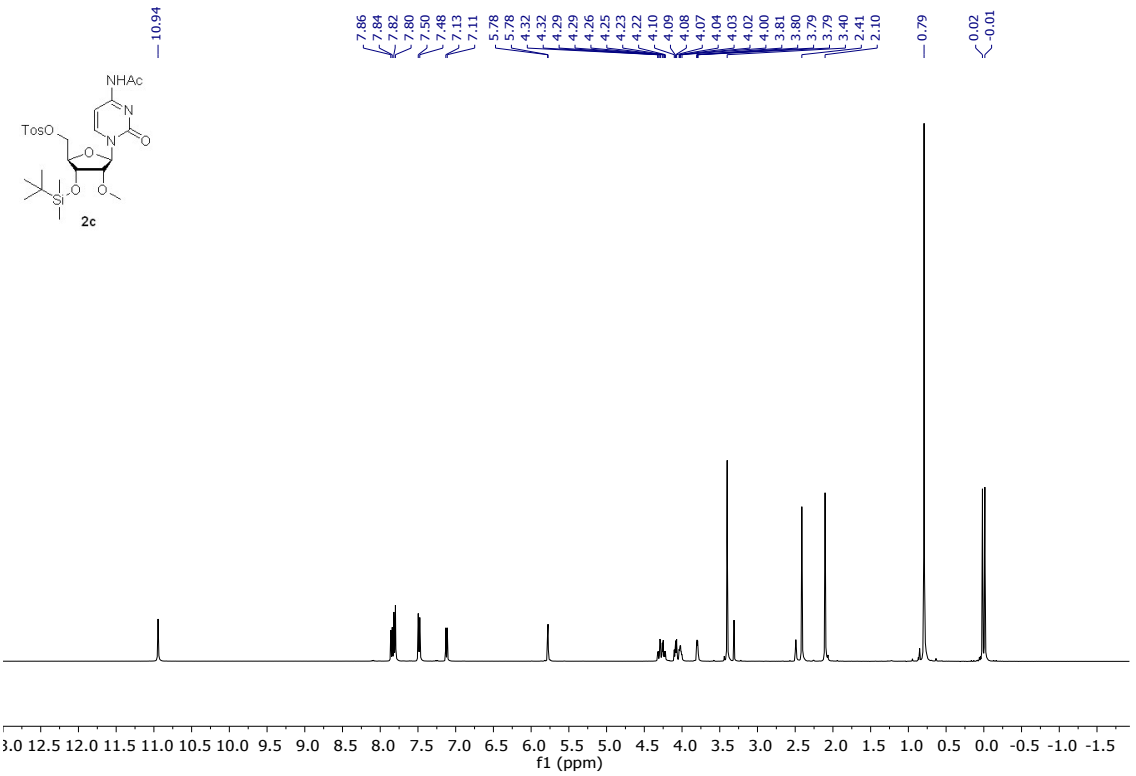
BzHN
TosO
2b
f1 (ppm)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4.26
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4.23
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4.09
4.08
4.07
4.04
4.03
4.02
4.00
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3.80
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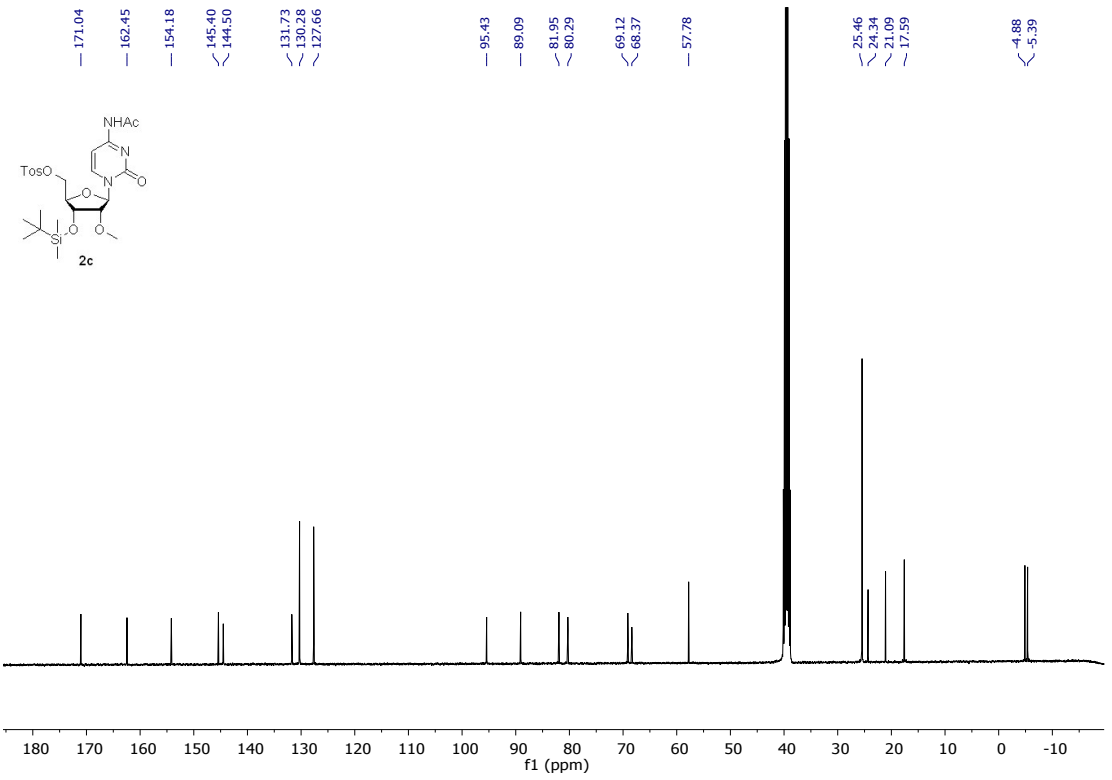
2c
f1 (ppm)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69.12
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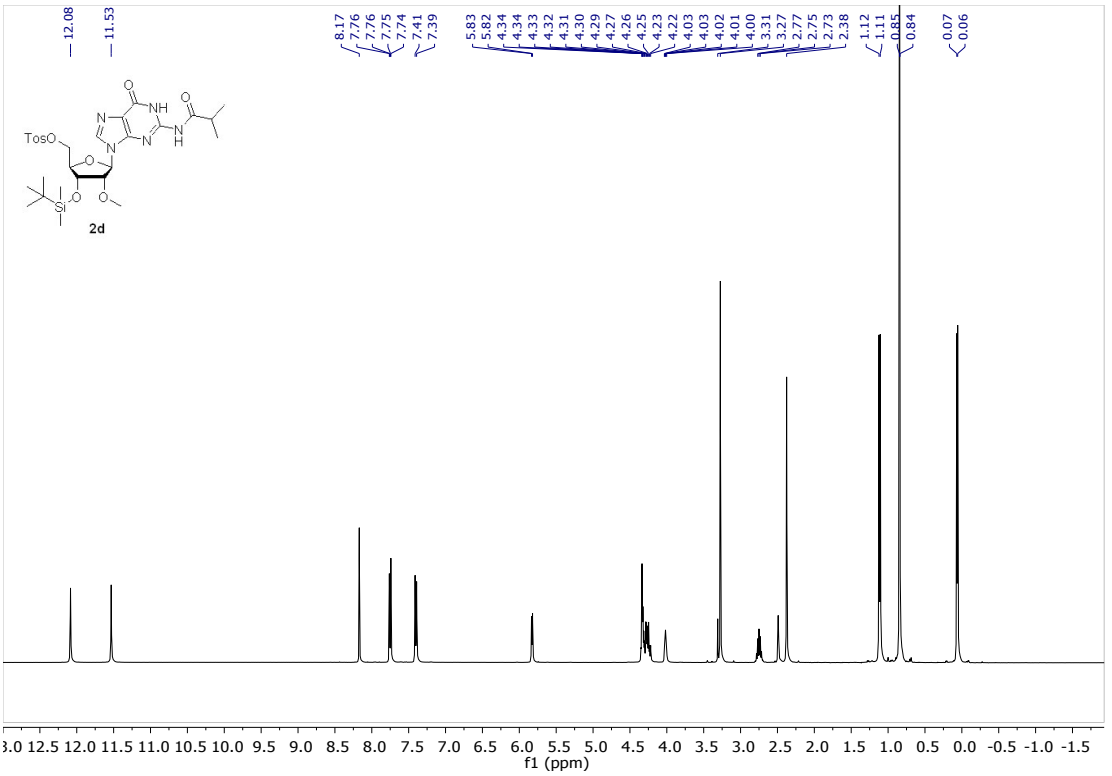
2c
f1 (ppm)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4.34
4.33
4.32
4.31
4.30
4.29
4.27
4.26
4.25
4.23
4.22
4.03
4.03
4.02
4.01
4.00
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3.27
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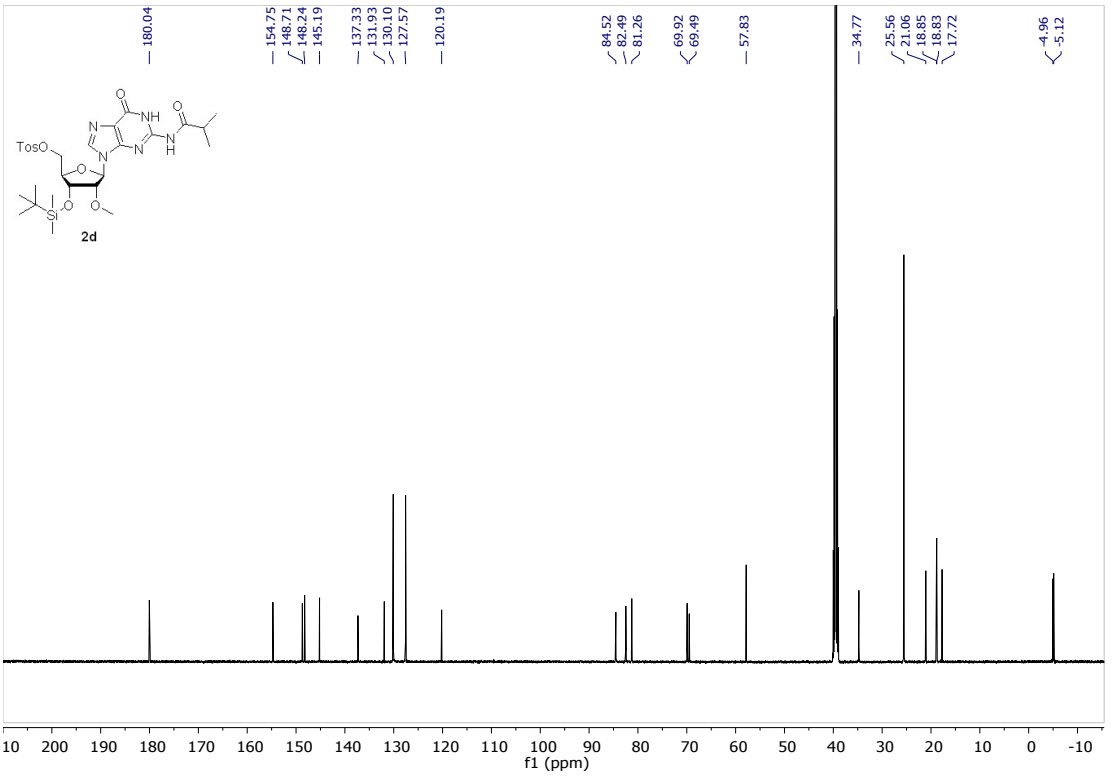
1.12
1.11
0.85
0.84
0.07
0.06
TosO
2d
f1 (ppm)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69.92
69.49
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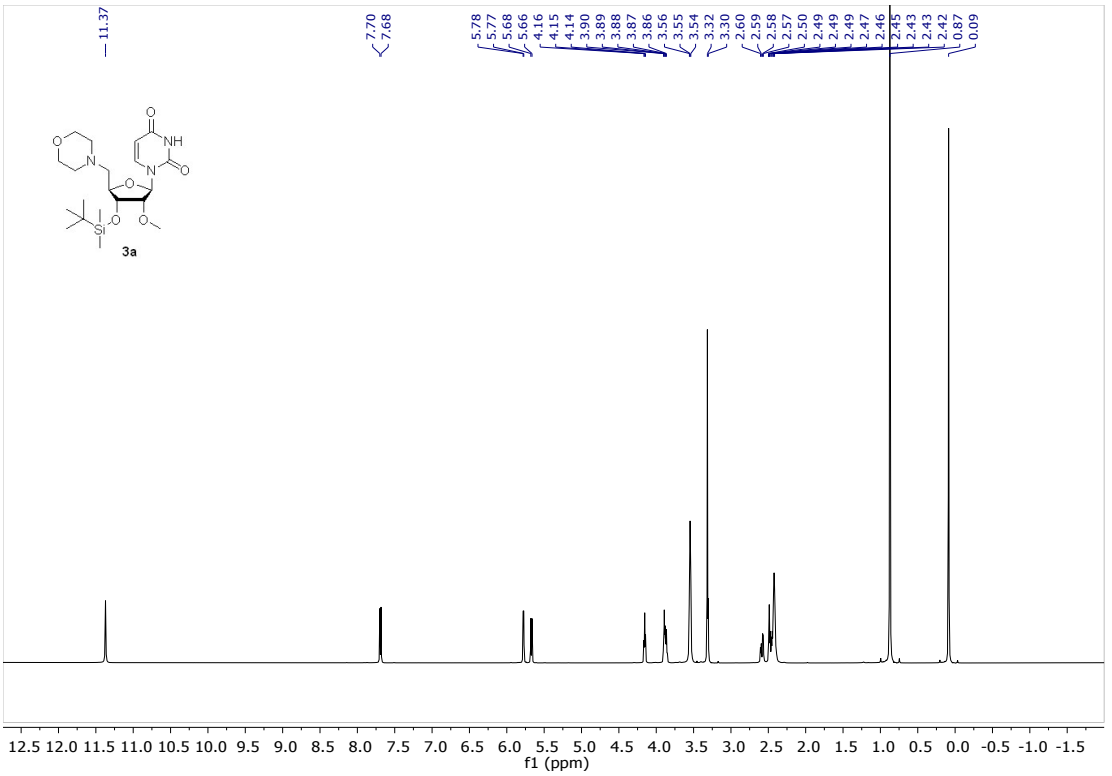
18.85
18.83
17.72
-4.96
-5.12
TosO
2d
f1 (ppm)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2.58
2.57
2.50
2.49
2.49
2.49
2.47
2.46
2.45
2.43
2.43
2.42
0.87
0.09
3a
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5
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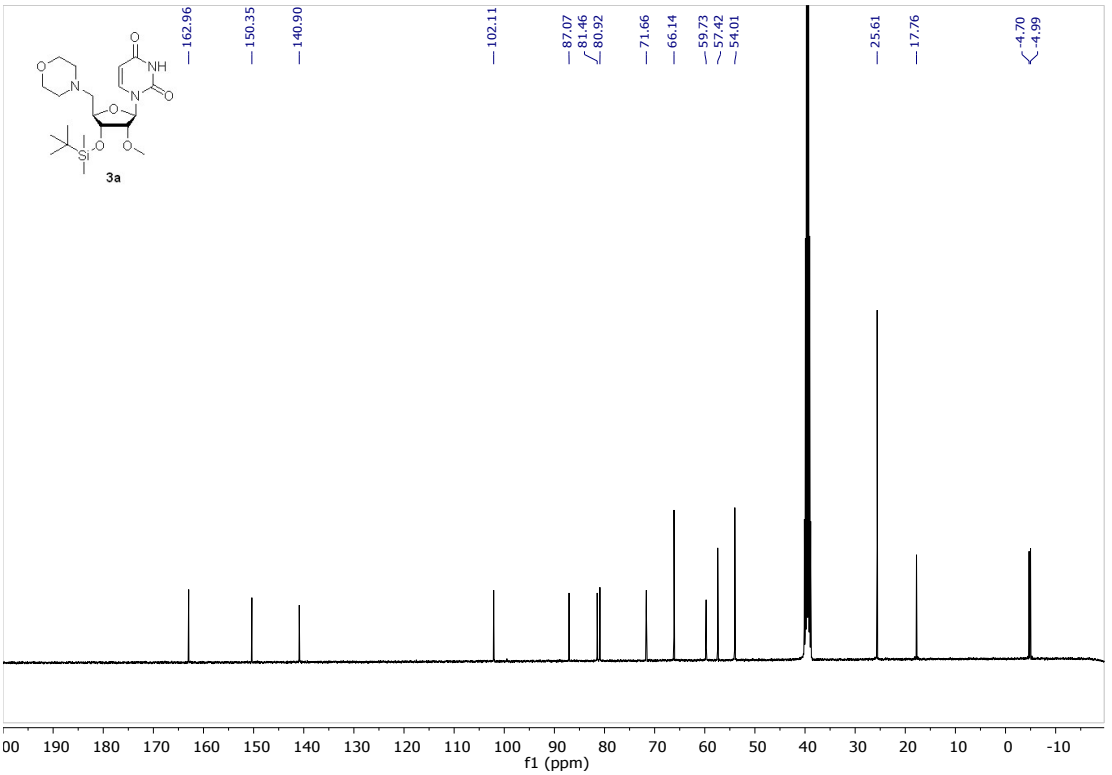
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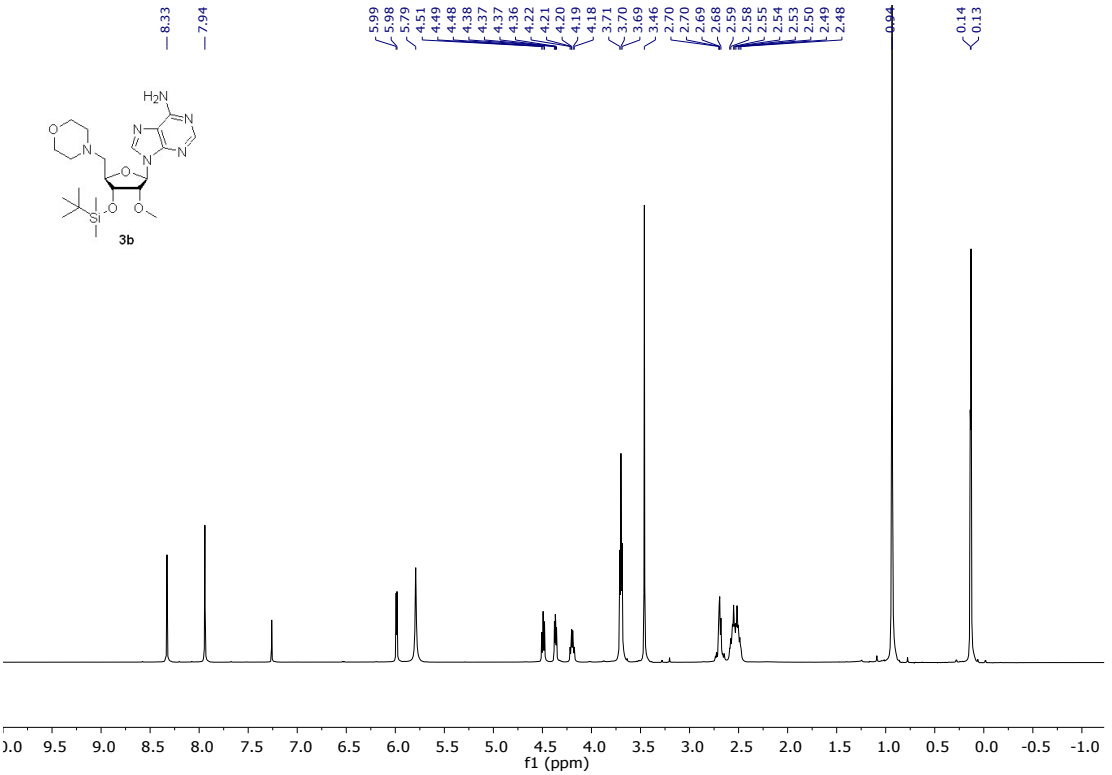
f1 (ppm)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4.18
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3.46
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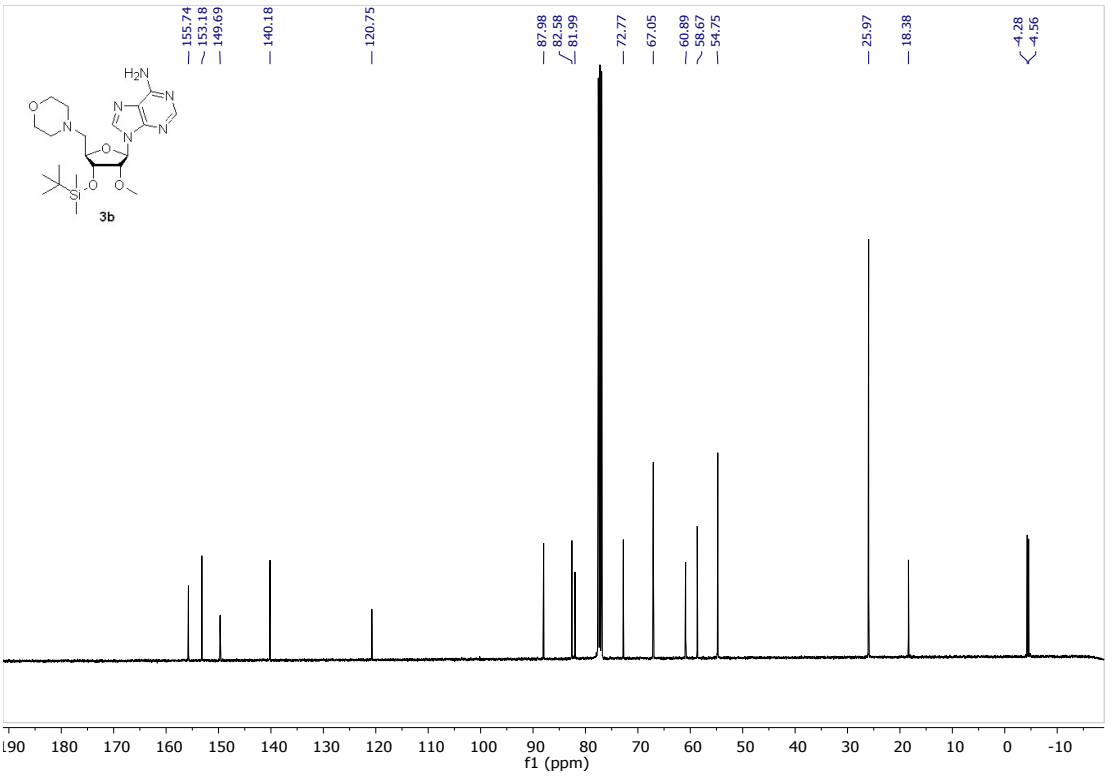
f1 (ppm)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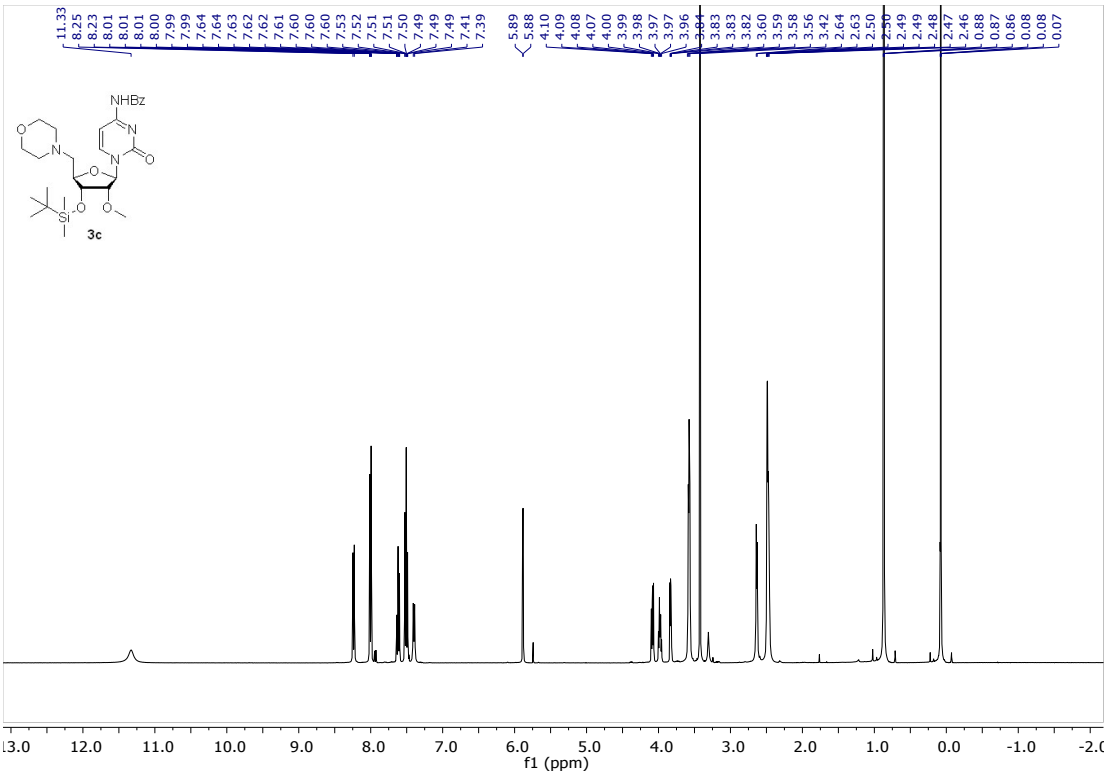
f1 (ppm)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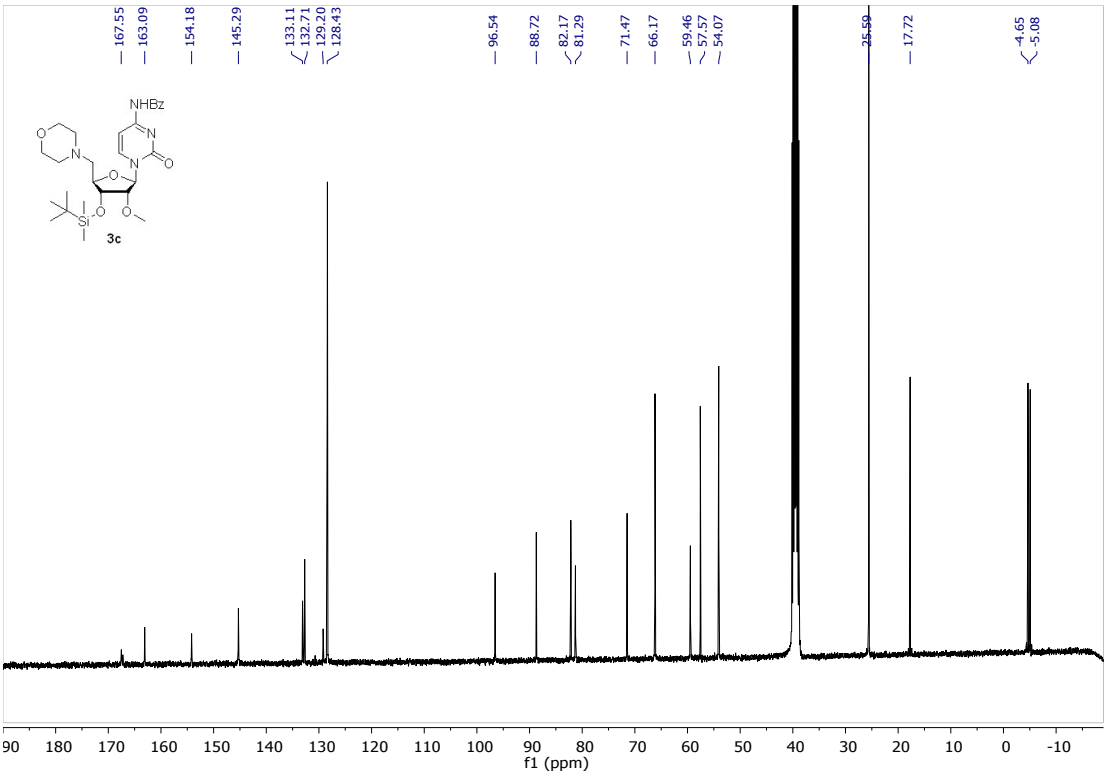
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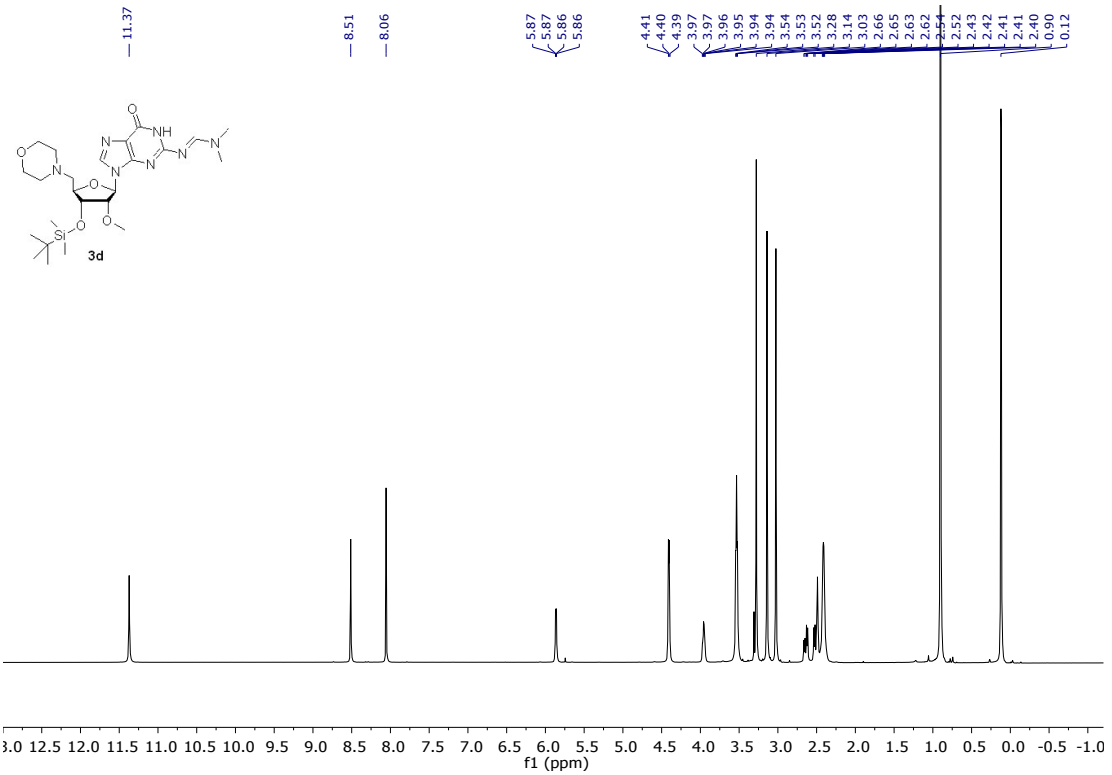
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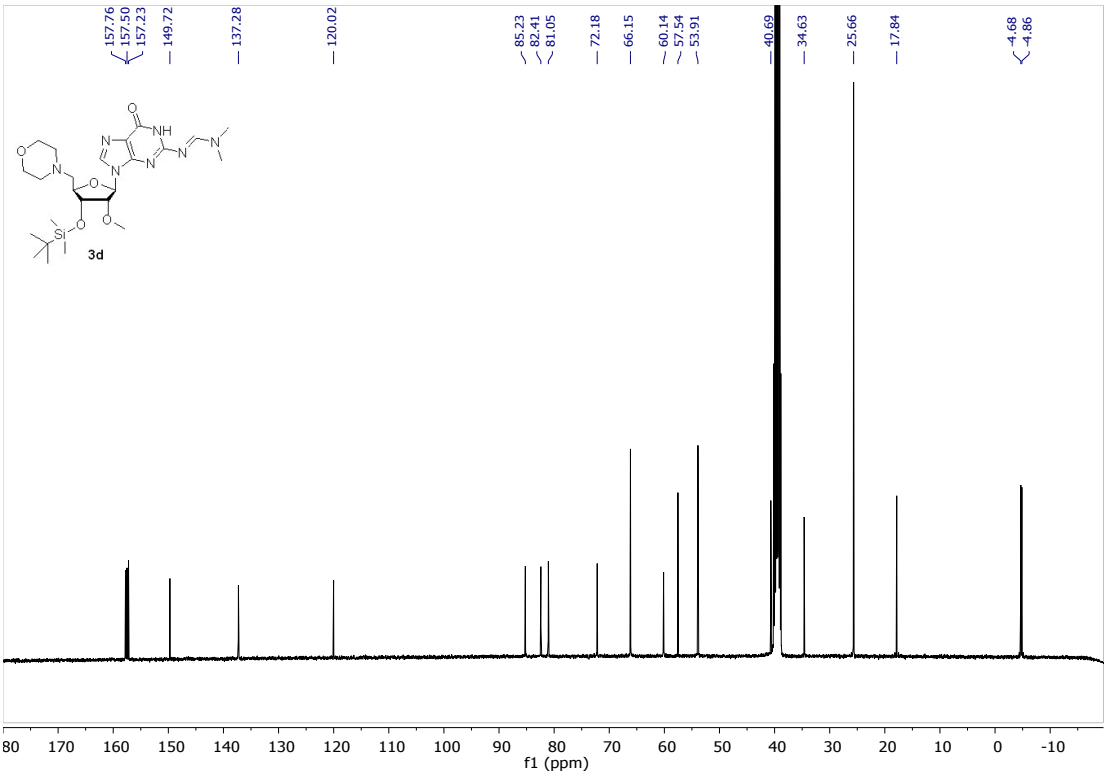
f1 (ppm)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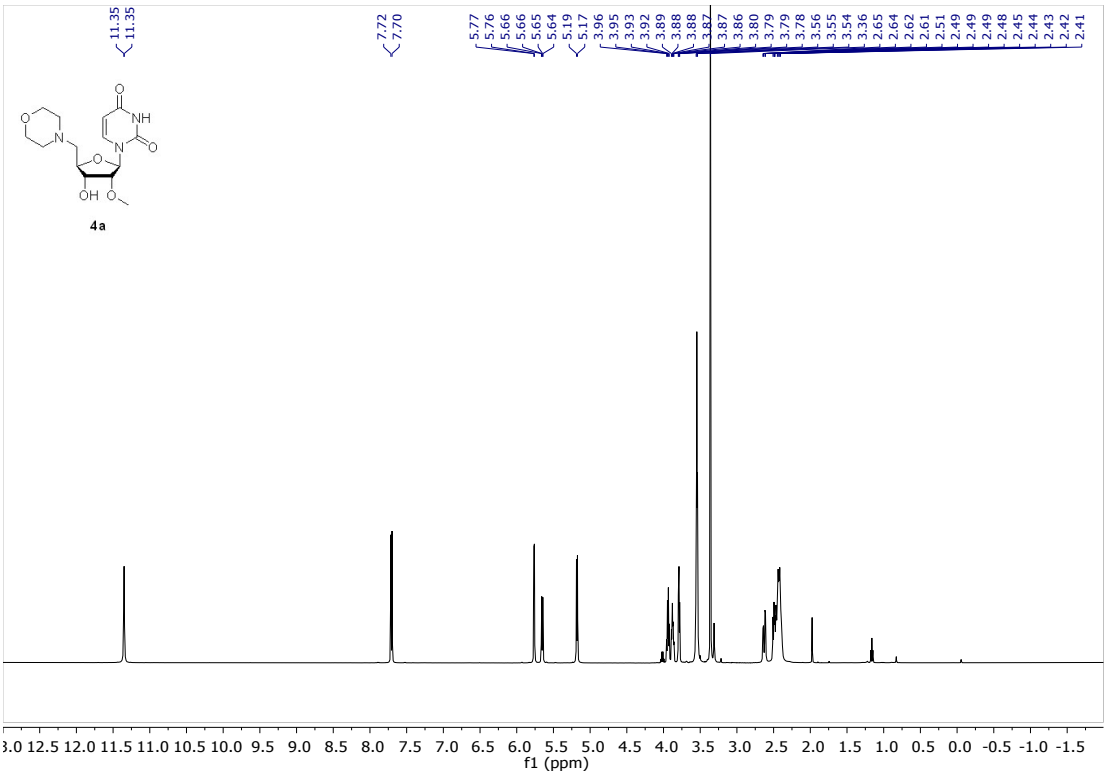
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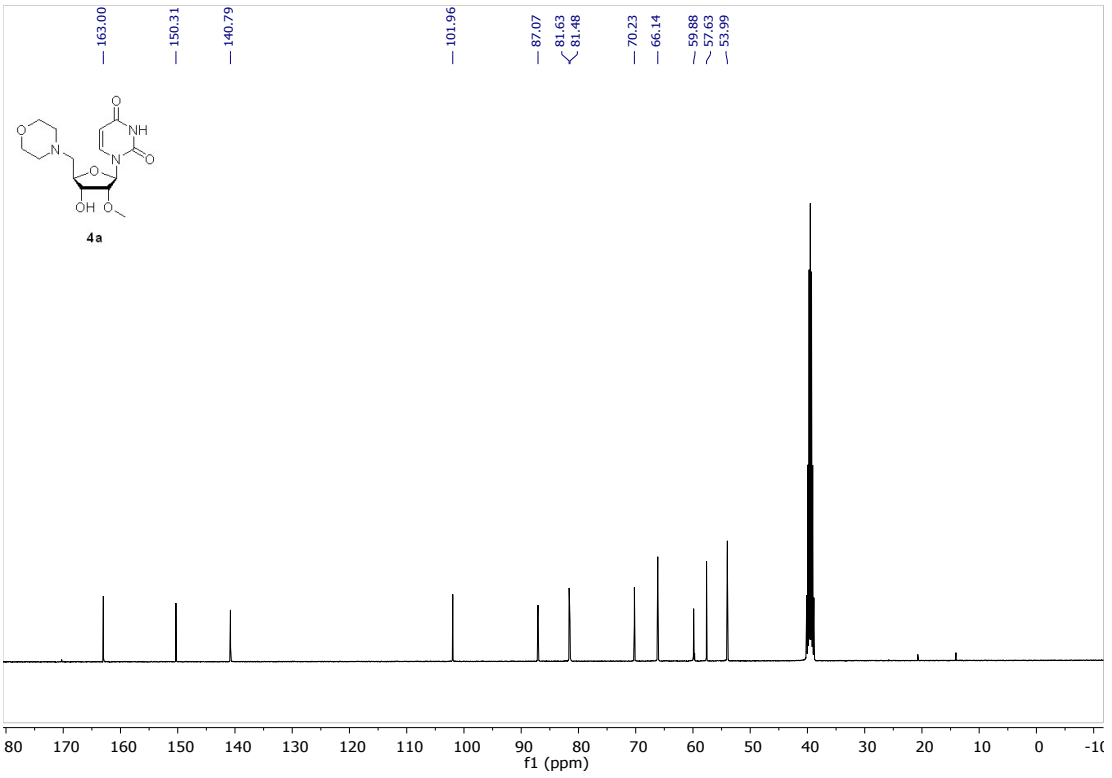
O
4a
f1 (ppm)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NH
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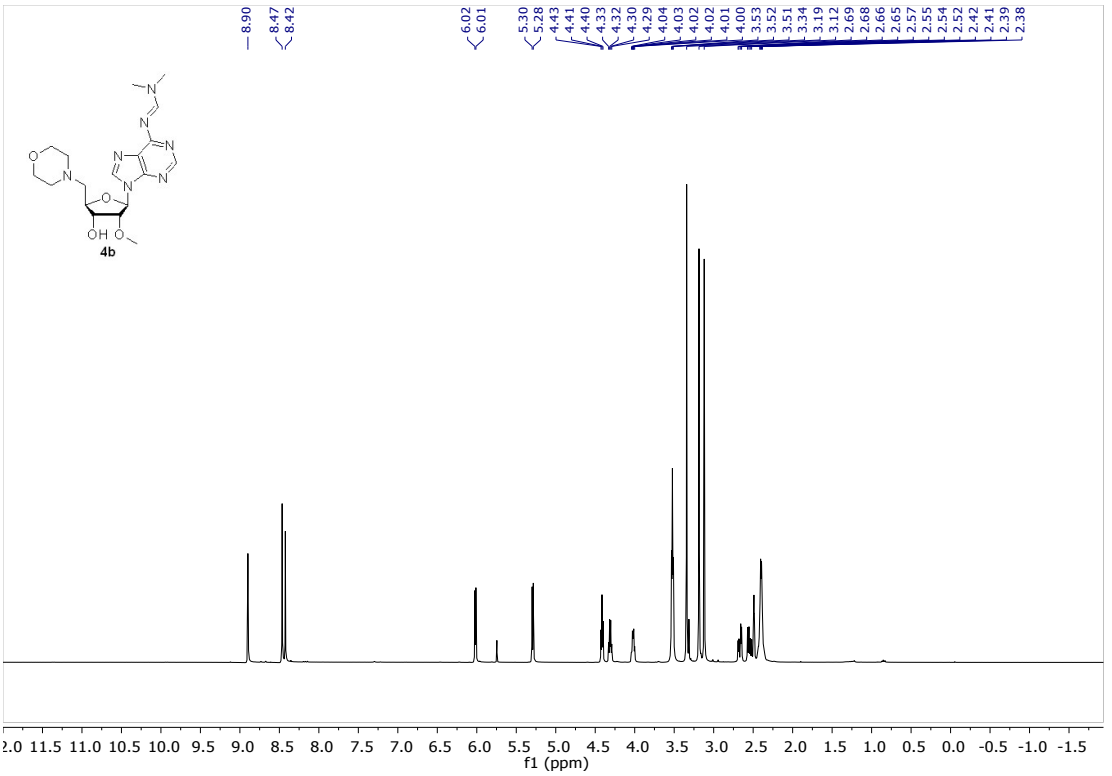
OH
O
4a
f1 (ppm)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4.02
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4.01
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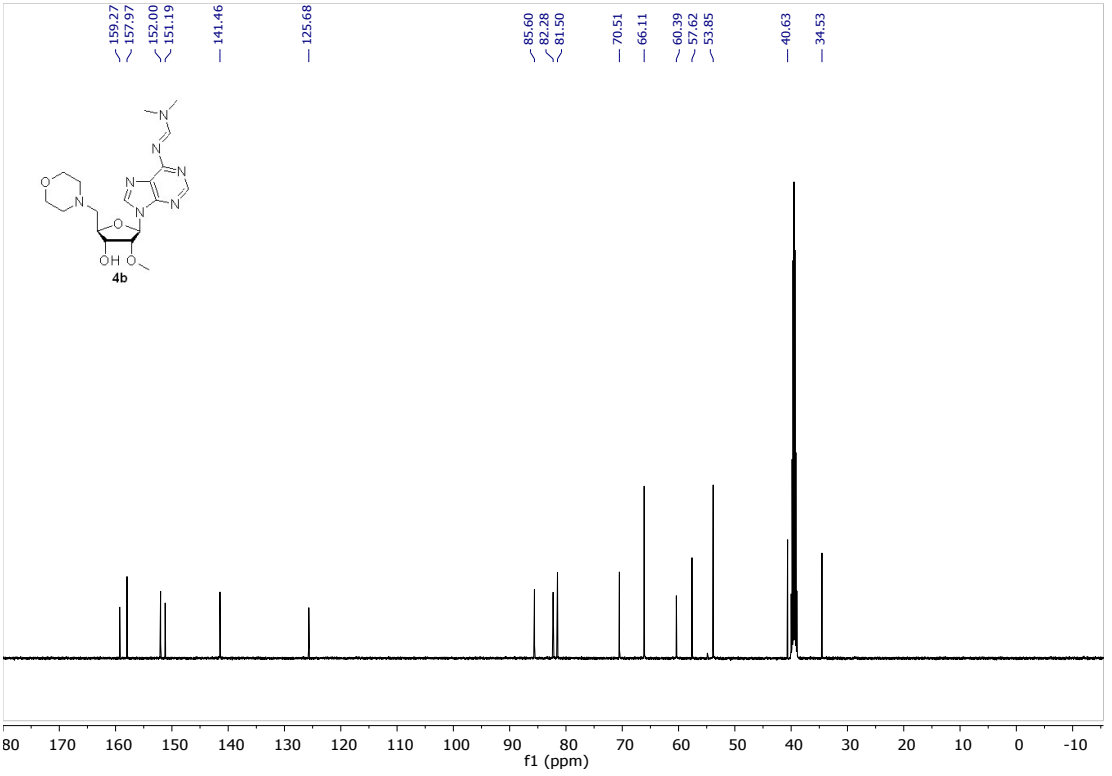
f1 (ppm)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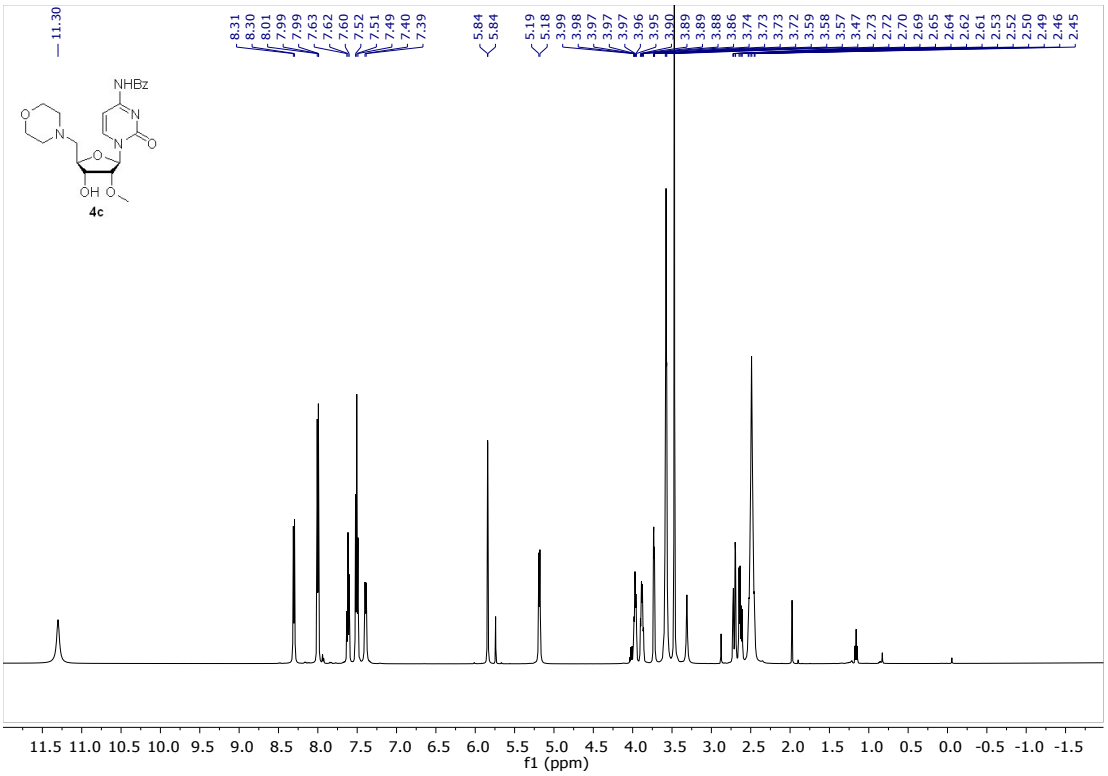
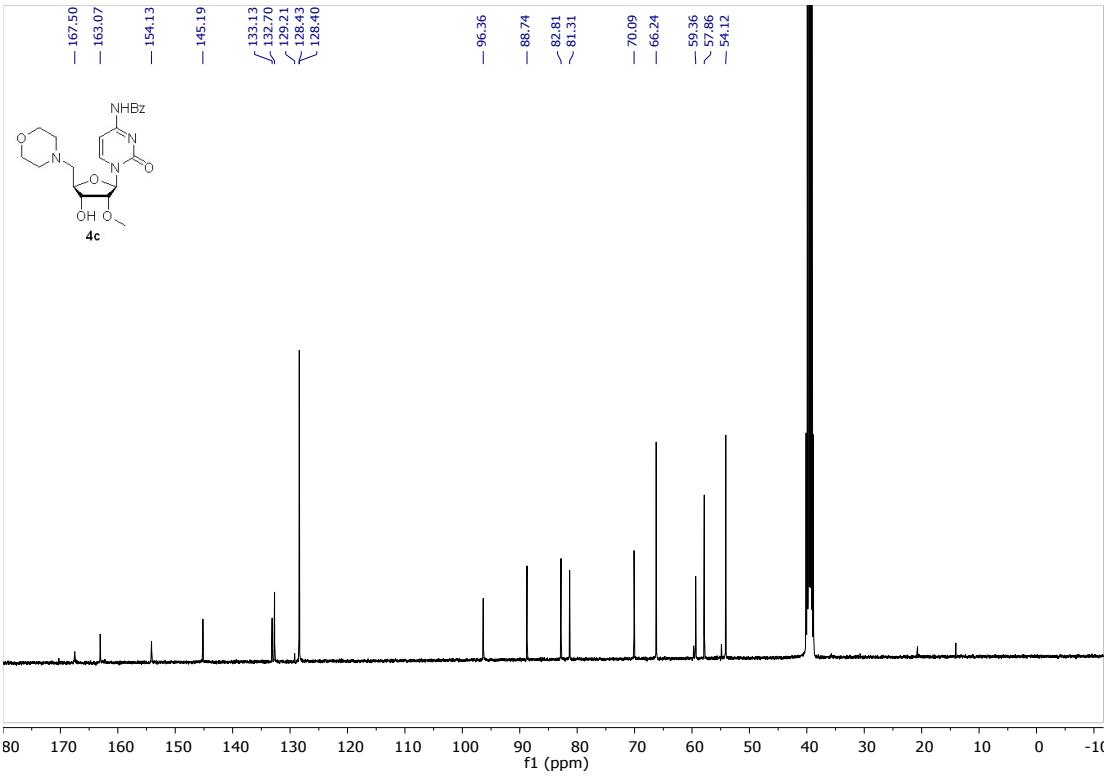
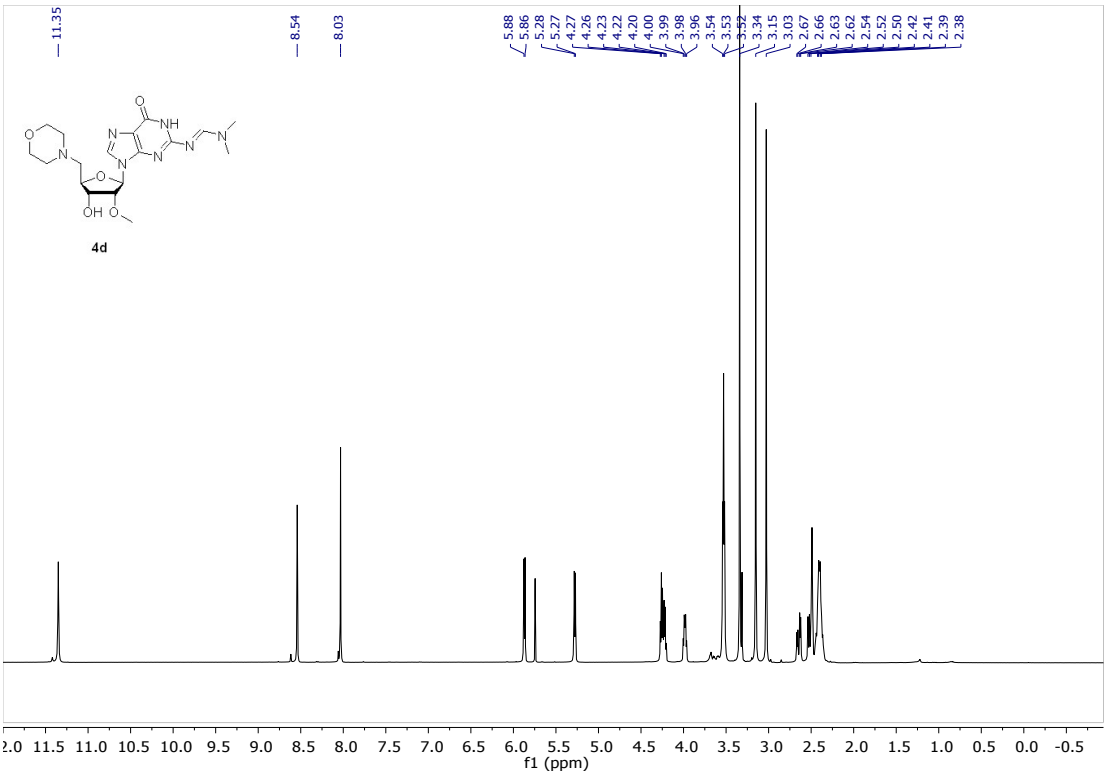
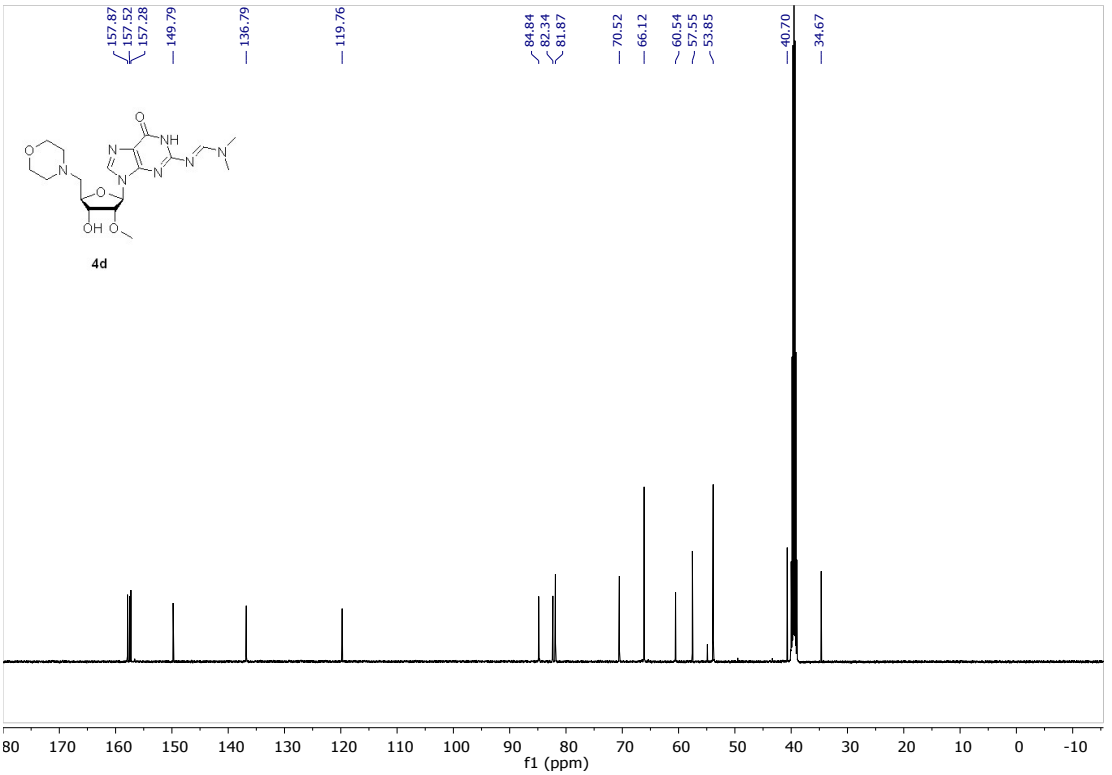
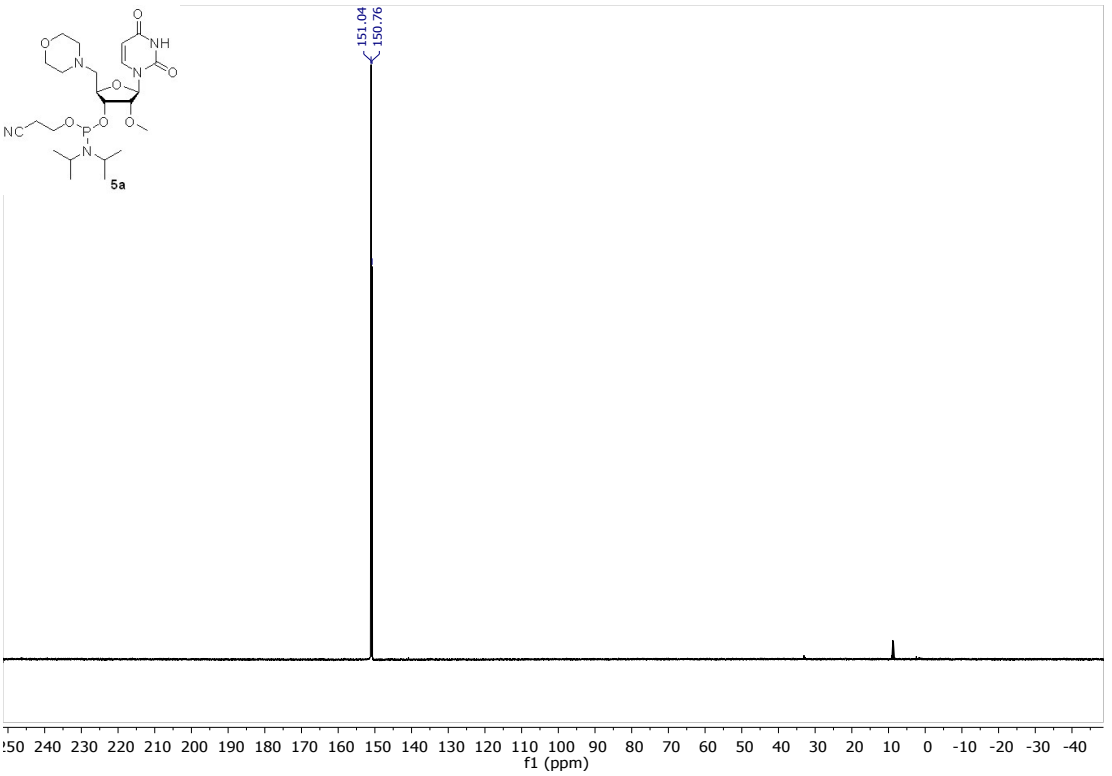
81.50
70.51
66.11
60.39
57.62
53.85
40.63
34.53
4b
f1 (ppm)

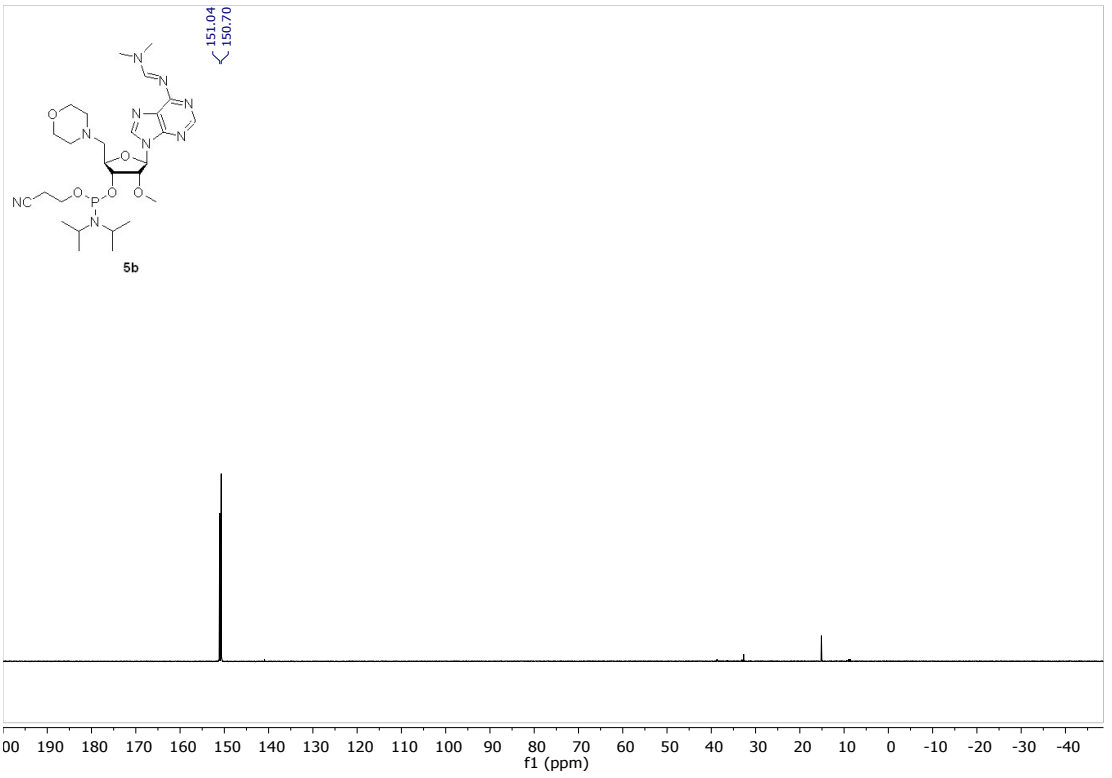

11.30
8.31
8.30
8.01
7.99
7.99
7.63
7.62
7.60
7.52
7.51
7.49
7.40
7.39
5.84
5.84
5.19
5.18
3.99
3.98
3.97
3.97
3.97
3.96
3.95
3.90
3.89
3.89
3.88
3.86
3.74
3.73
3.73
3.72
3.59
3.58
3.57
3.47
2.73
2.72
2.70
2.69
2.65
2.64
2.62
2.61
2.53
2.52
2.50
2.49
2.46
2.45
NHBz
OH
O
4c
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5
f1 (ppm)

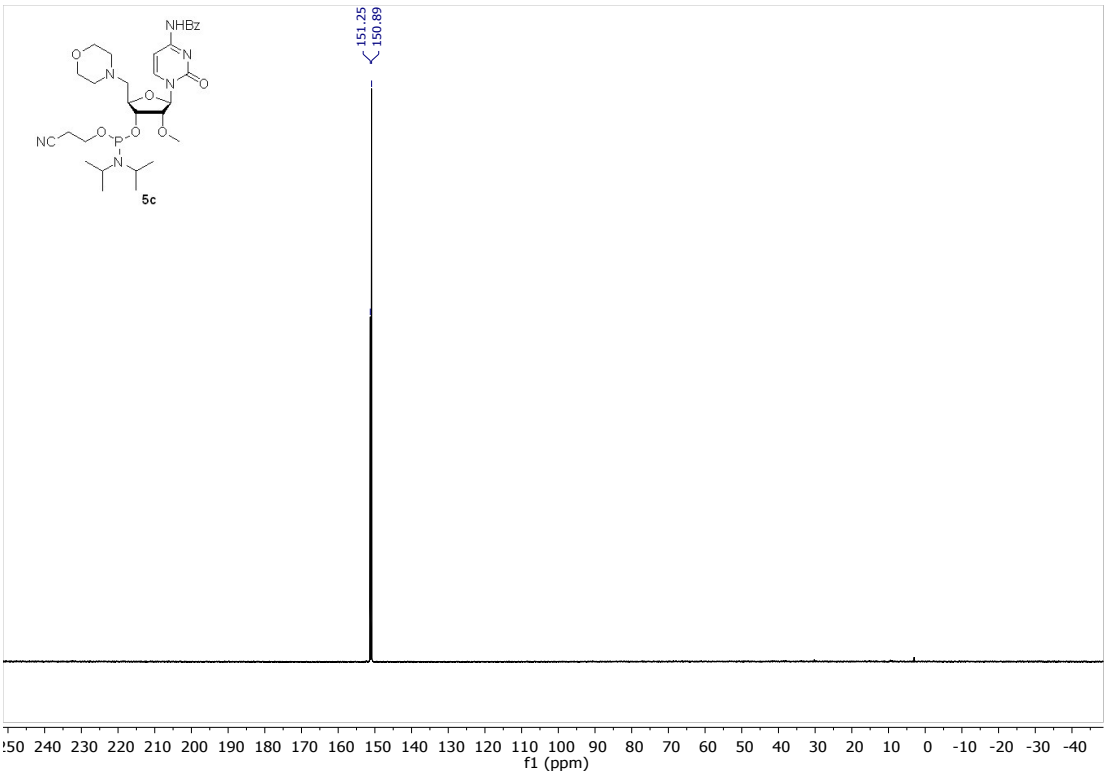

167.50
163.07
154.13
145.19
133.13
132.70
129.21
128.43
128.40
96.36
88.74
82.81
81.31
70.09
66.24
59.36
57.86
54.12
NHBz
OH
O
4c
80 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1(
f1 (ppm)

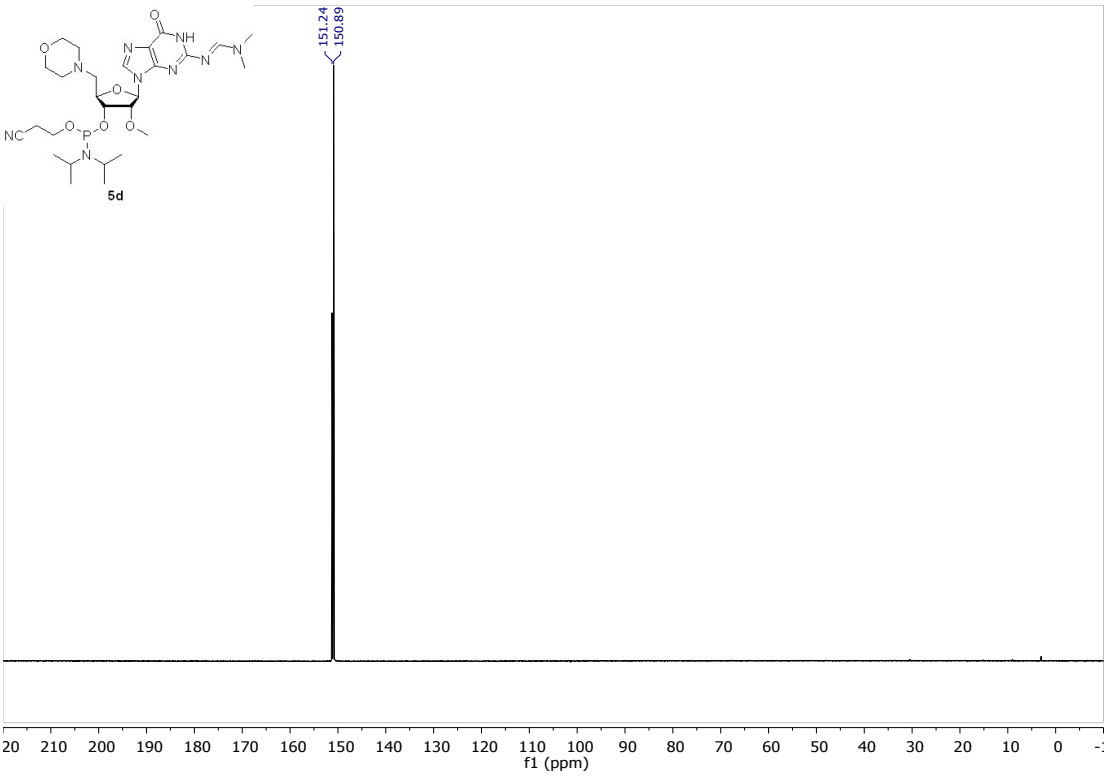

11.35
8.54
8.03
5.88
5.86
5.28
5.27
4.27
4.26
4.23
4.22
4.20
4.00
3.99
3.98
3.96
3.54
3.53
3.52
3.34
3.15
3.03
2.67
2.66
2.63
2.62
2.54
2.52
2.50
2.42
2.41
2.39
2.38
4d
f1 (ppm)

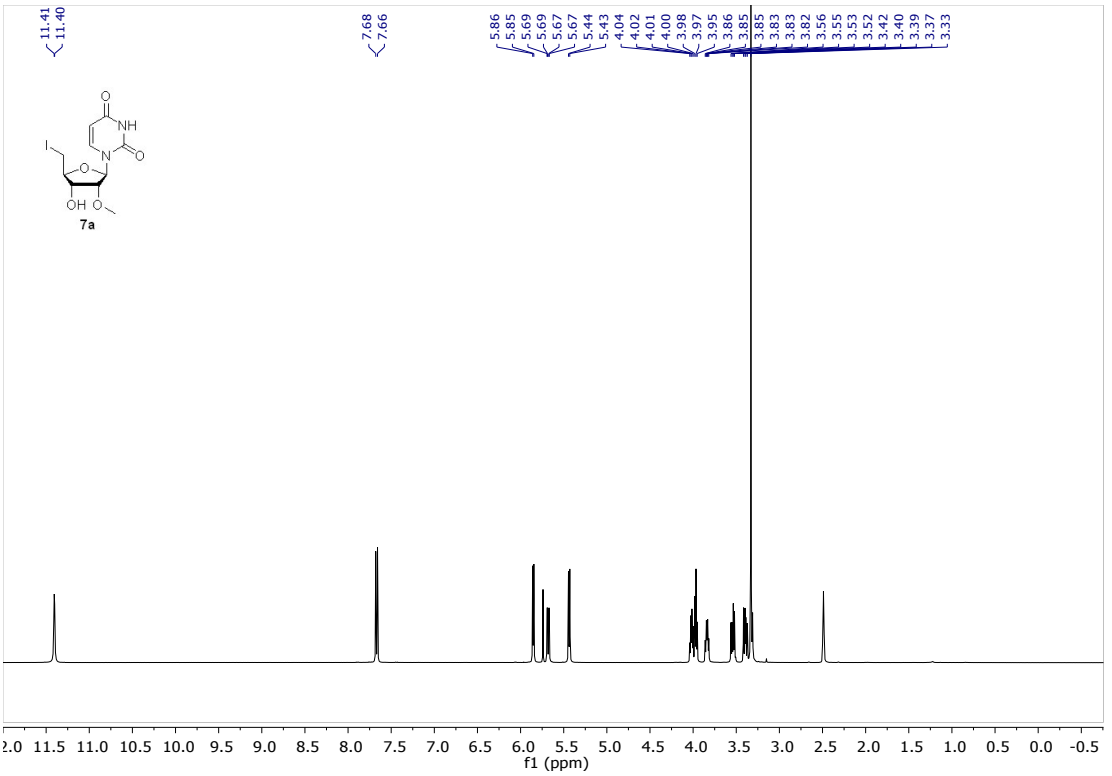

157.87
157.52
157.28
149.79
136.79
119.76
84.84
82.34
81.87
70.52
66.12
60.54
57.55
53.85
40.70
34.67
4d
f1 (ppm)

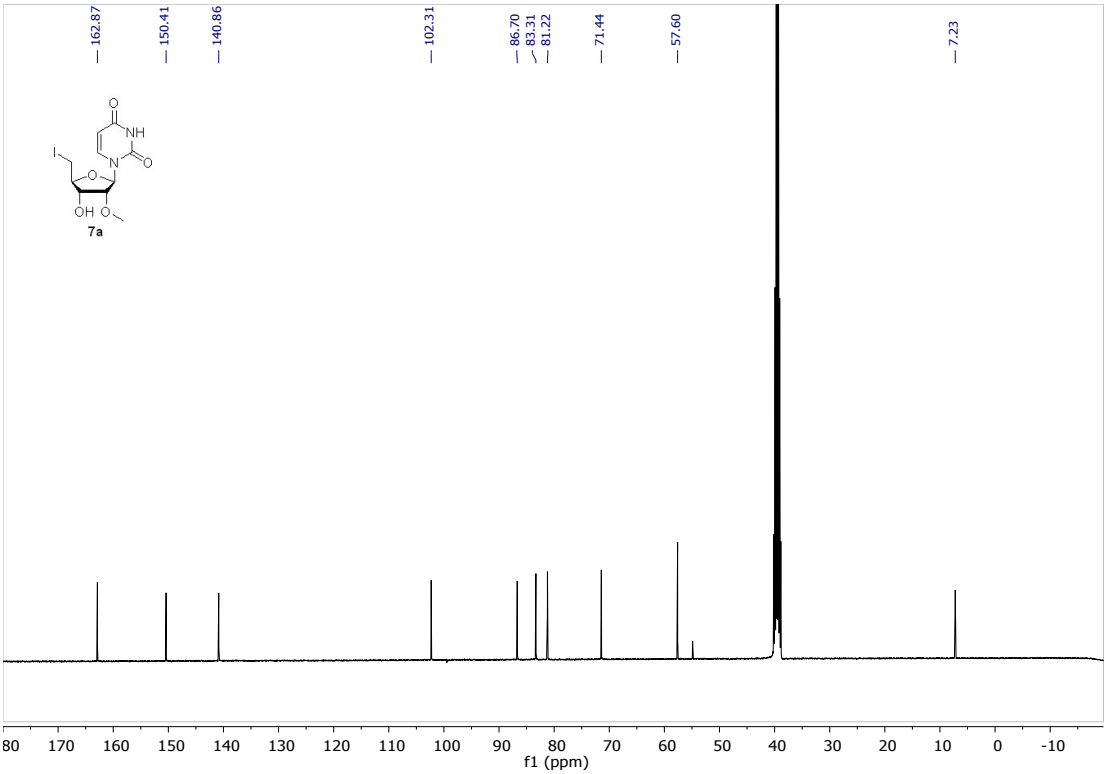

151.04
150.76
5a
250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40
f1 (ppm)

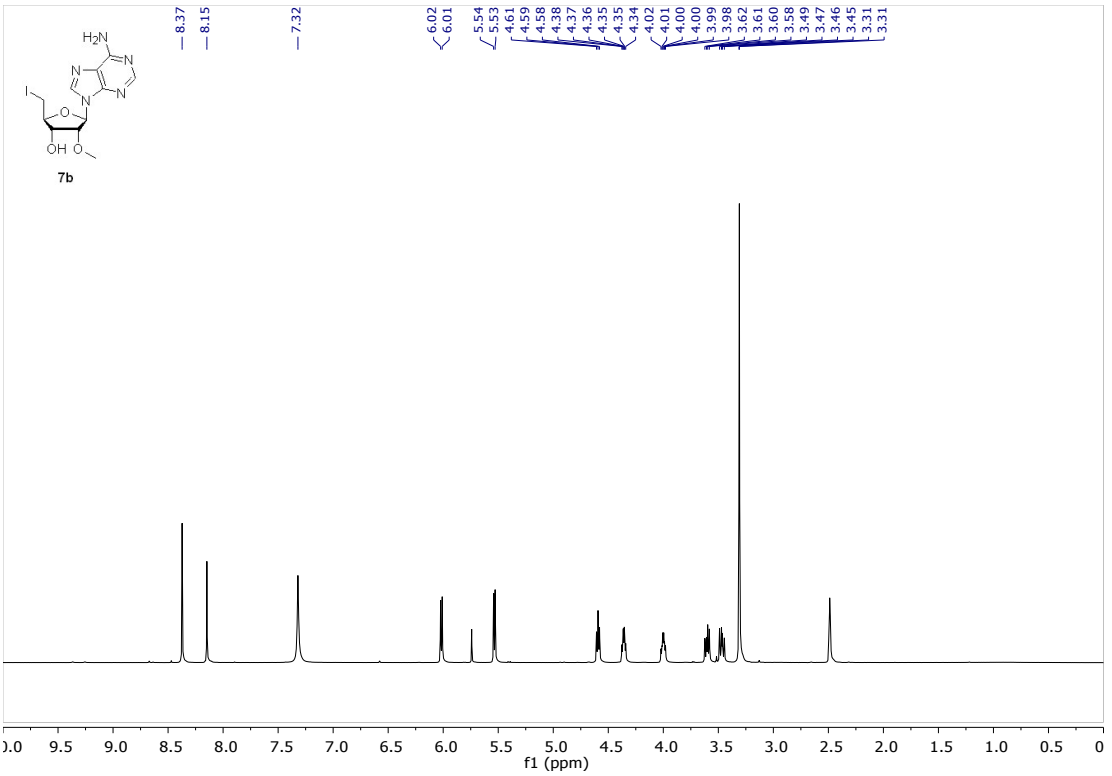

151.04
150.70
5b
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40
f1 (ppm)

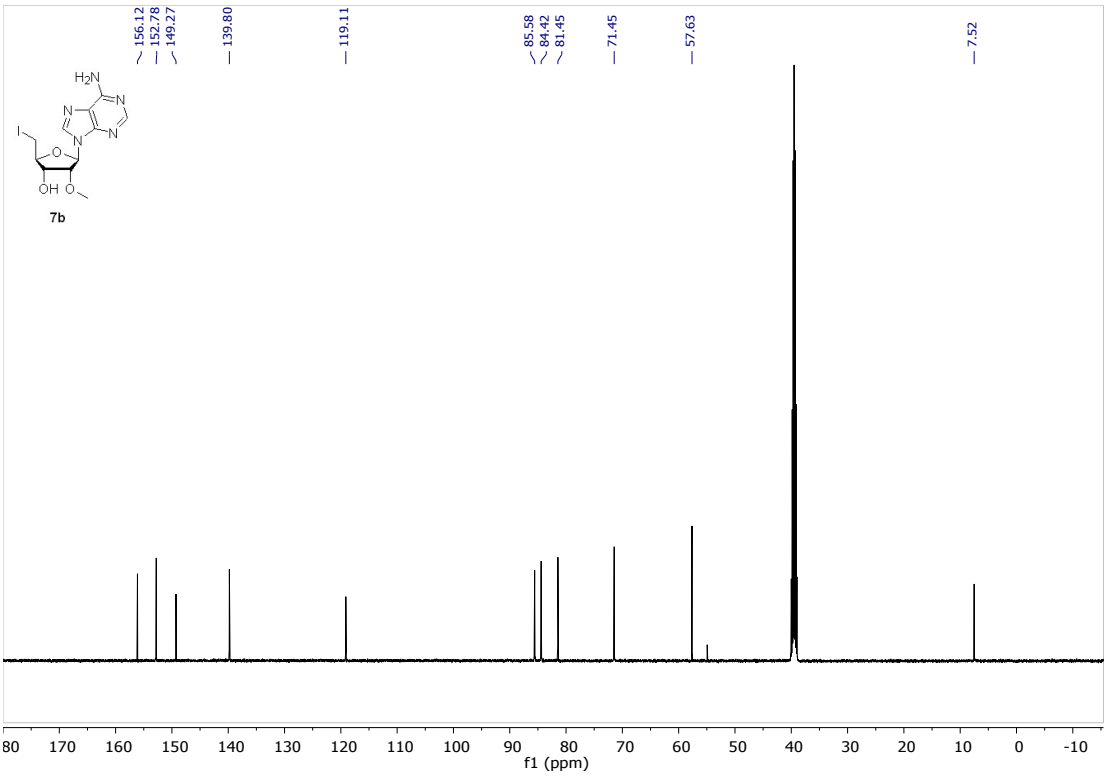

NHBz
NC
5c
151.25
150.89
f1 (ppm)


NC
5d
151.24
150.89
f1 (ppm)

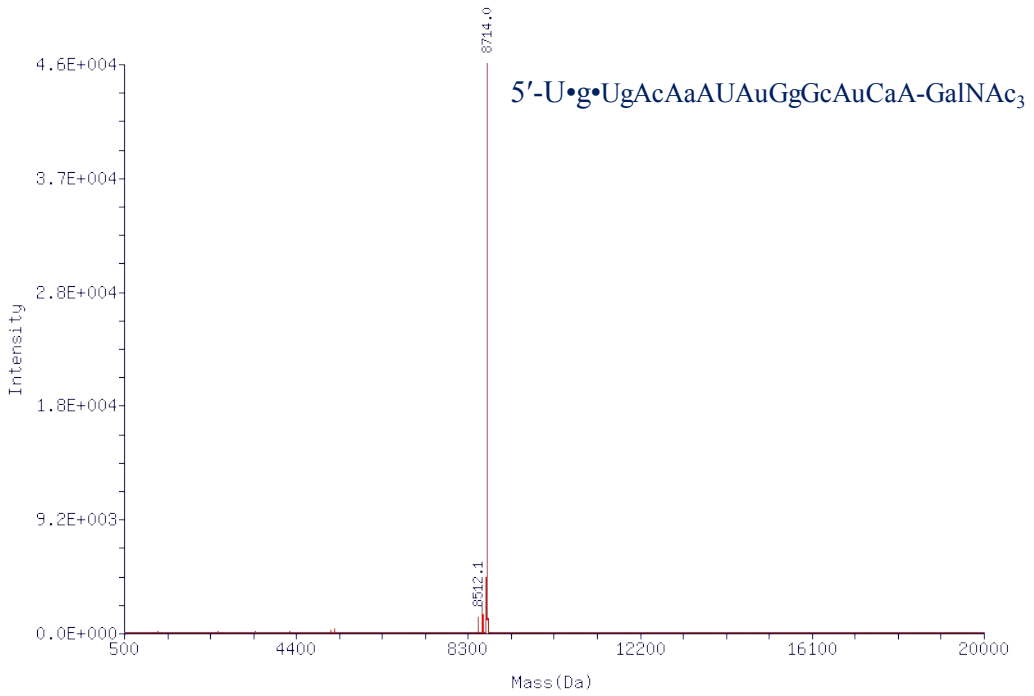

11.41
11.40
7.68
7.66
5.86
5.85
5.69
5.69
5.67
5.67
5.44
5.43
4.04
4.02
4.01
4.00
3.98
3.97
3.95
3.86
3.85
3.85
3.83
3.83
3.82
3.56
3.55
3.53
3.52
3.42
3.40
3.39
3.37
3.33
NH
O
N
O
I
O
OH O
7a
f1 (ppm)

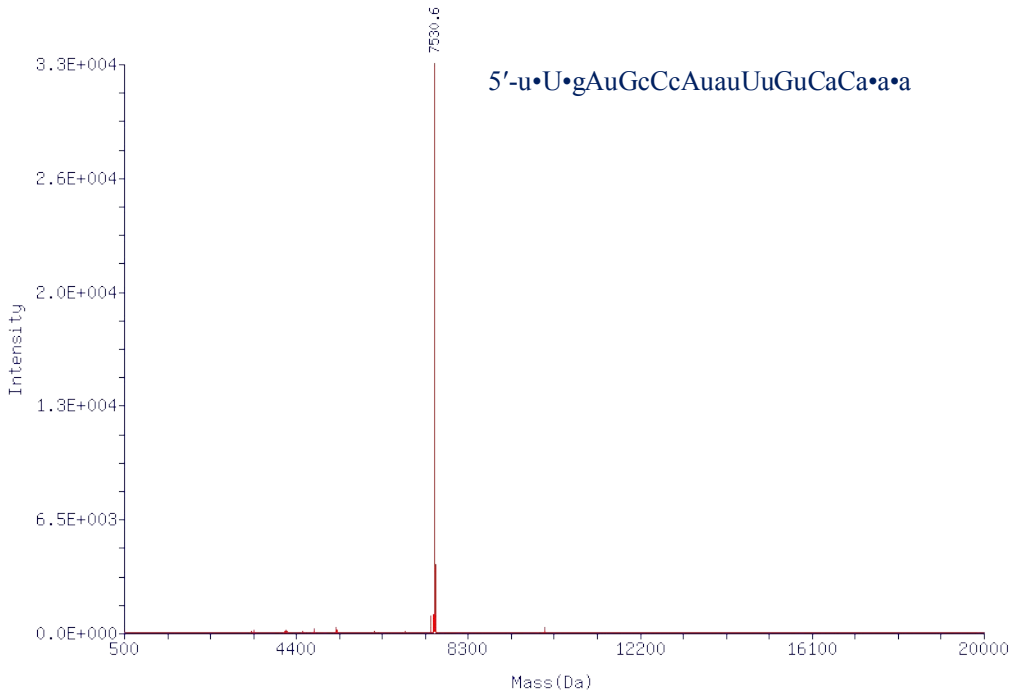

162.87
150.41
140.86
102.31
86.70
83.31
81.22
71.44
57.60
7.23
NH
O
N
O
I
O
OH O
7a
f1 (ppm)

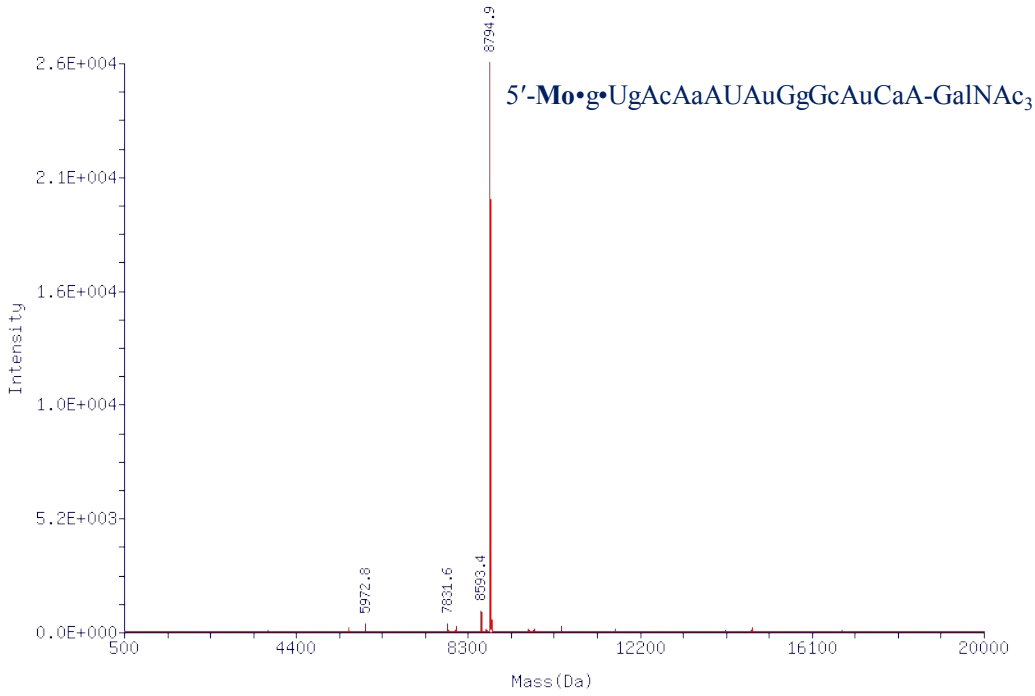

8.37
8.15
7.32
6.02
6.01
5.54
5.53
4.61
4.59
4.58
4.38
4.37
4.36
4.35
4.35
4.34
4.02
4.01
4.00
4.00
3.99
3.98
3.62
3.61
3.60
3.58
3.49
3.47
3.46
3.45
3.31
3.31
7b
f1 (ppm)

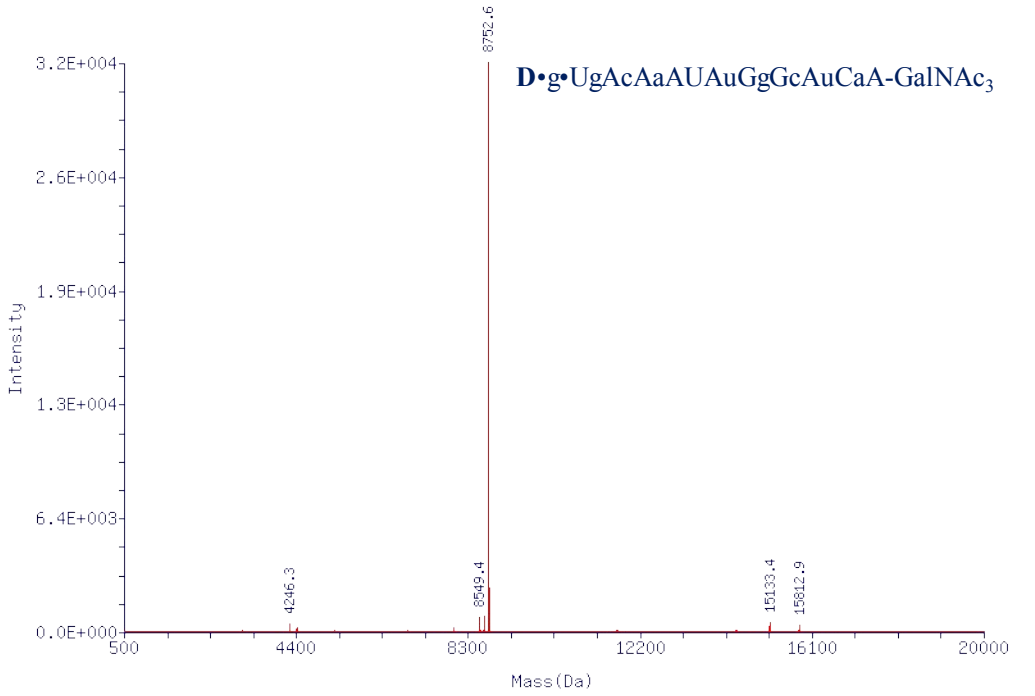

156.12
152.78
149.27
139.80
119.11
85.58
84.42
81.45
71.45
57.63
7.52
7b
f1 (ppm)

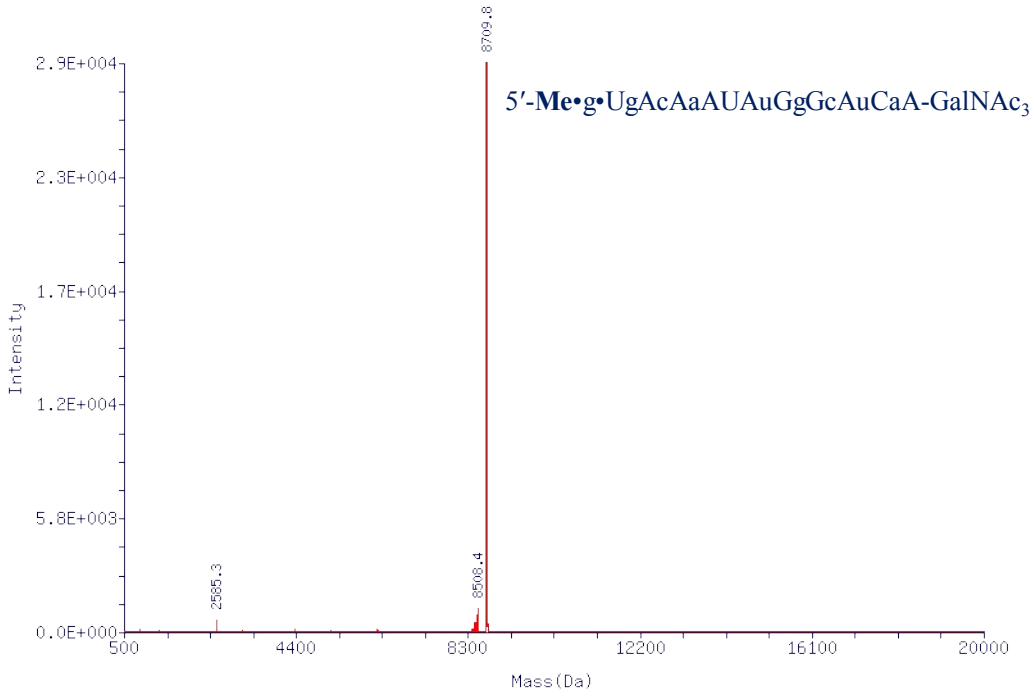
1.8 Representative MS spec of Oligonucleotides



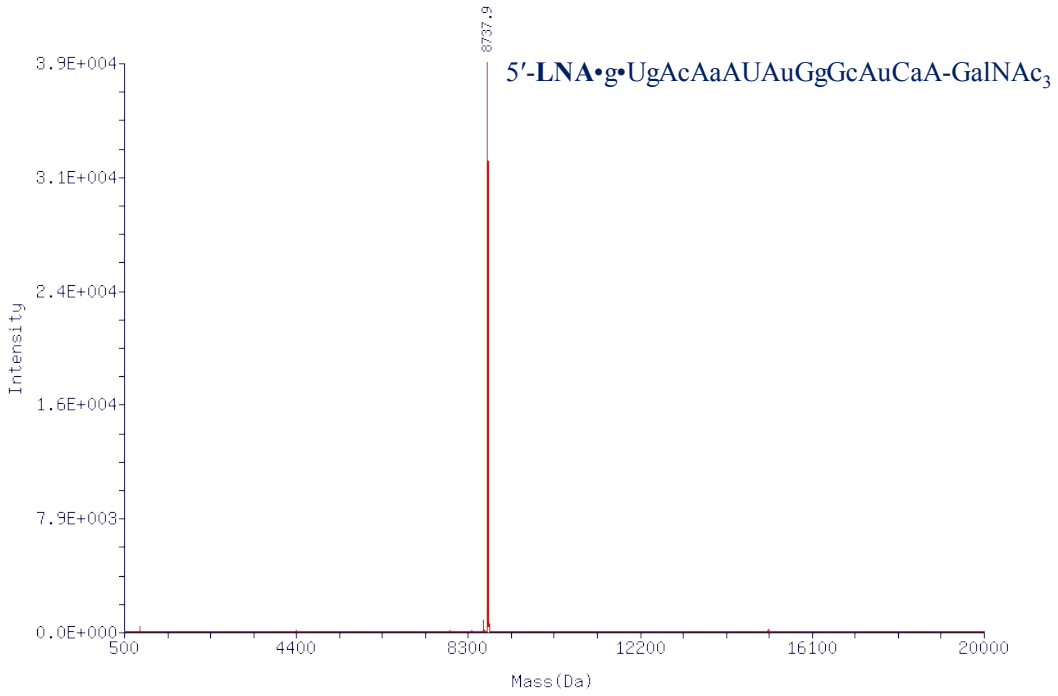



8794.9
5′-Mo•g•UgAcAaAUAuGgGcAuCaA-GalNAc3
2.6E+004
2.1E+004
1.6E+004
1.0E+004
5.2E+003
0.0E+000
Intensity
5972.8
7831.6
8593.4
500
4400
8300
12200
16100
20000
Mass(Da)

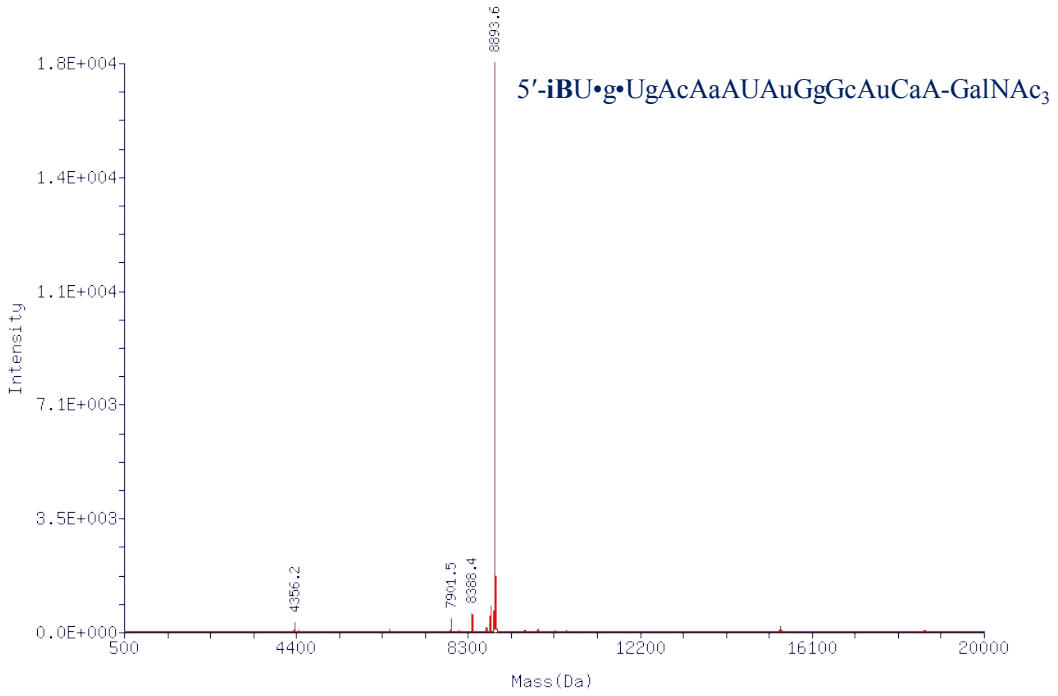

8752.6
D•g•UgAcAaAUAuGgGcAuCaA-GalNAc3
3.2E+004
2.6E+004
1.9E+004
1.3E+004
6.4E+003
0.0E+000
Intensity
4246.3
8549.4
15133.4
15812.9
500
4400
8300
12200
16100
20000
Mass(Da)

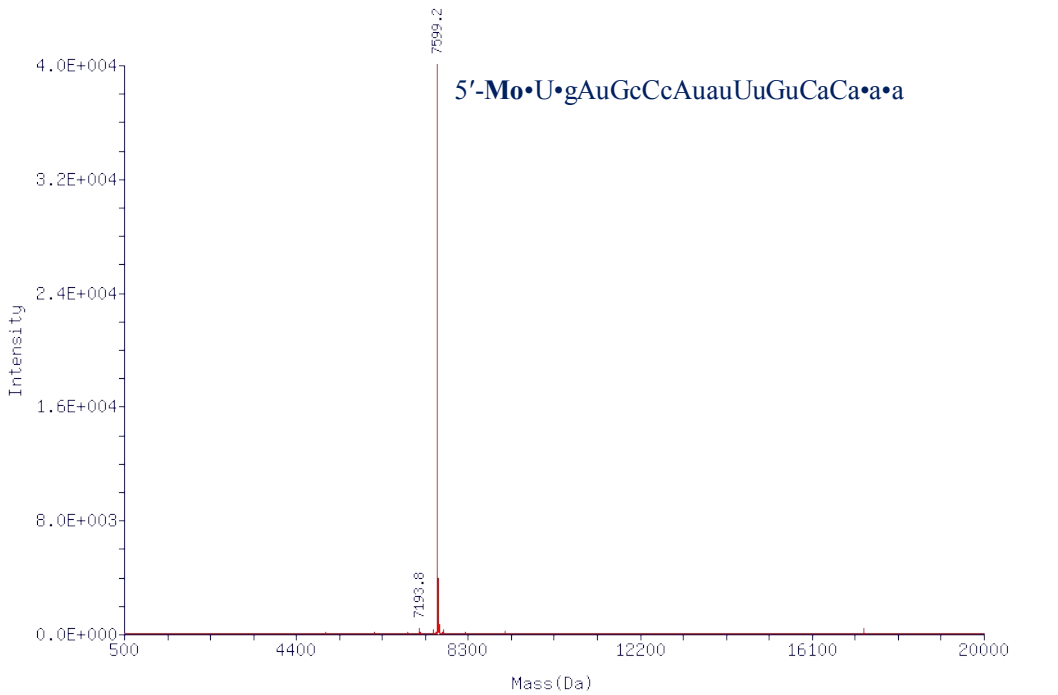


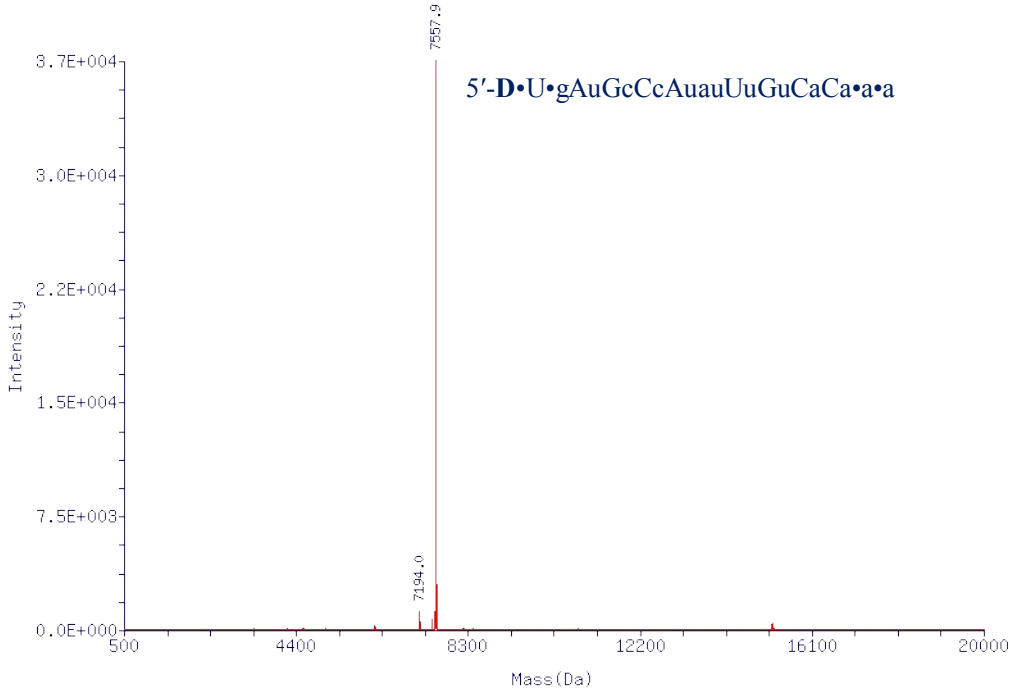
5′-**Me**•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$

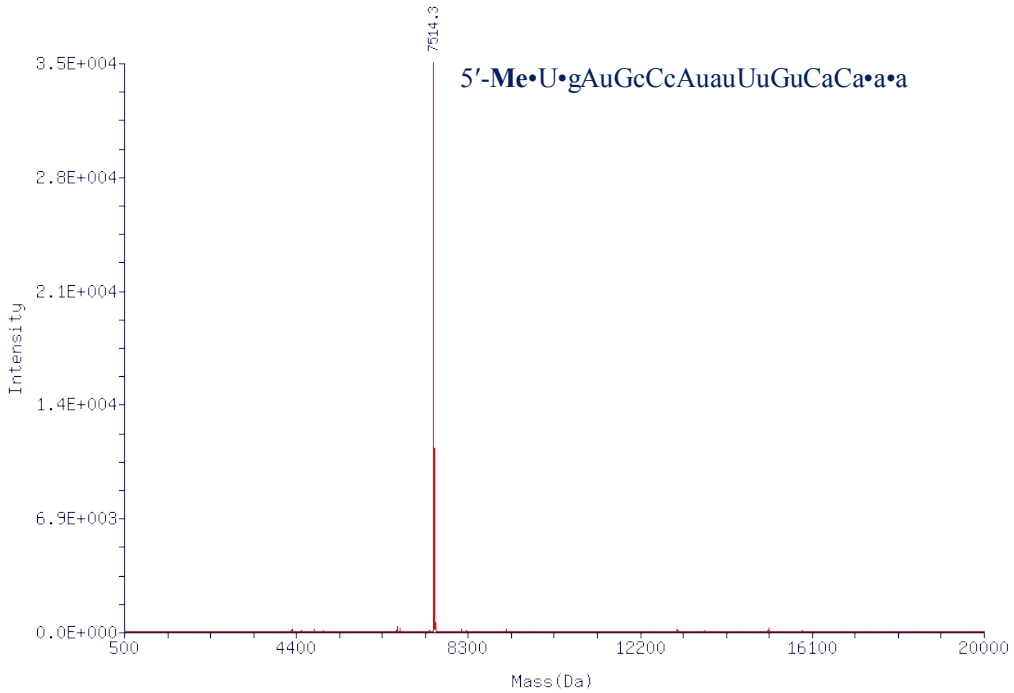


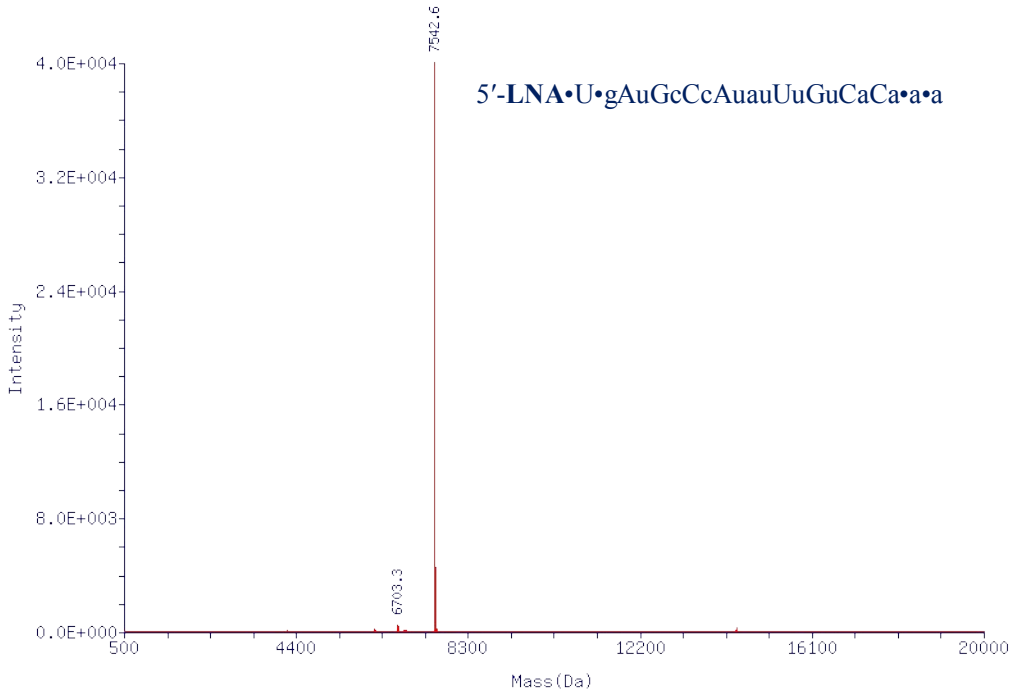
5′-**LNA**•g•UgAcAaAUAuGgGcAuCaA-GalNAc$_3$

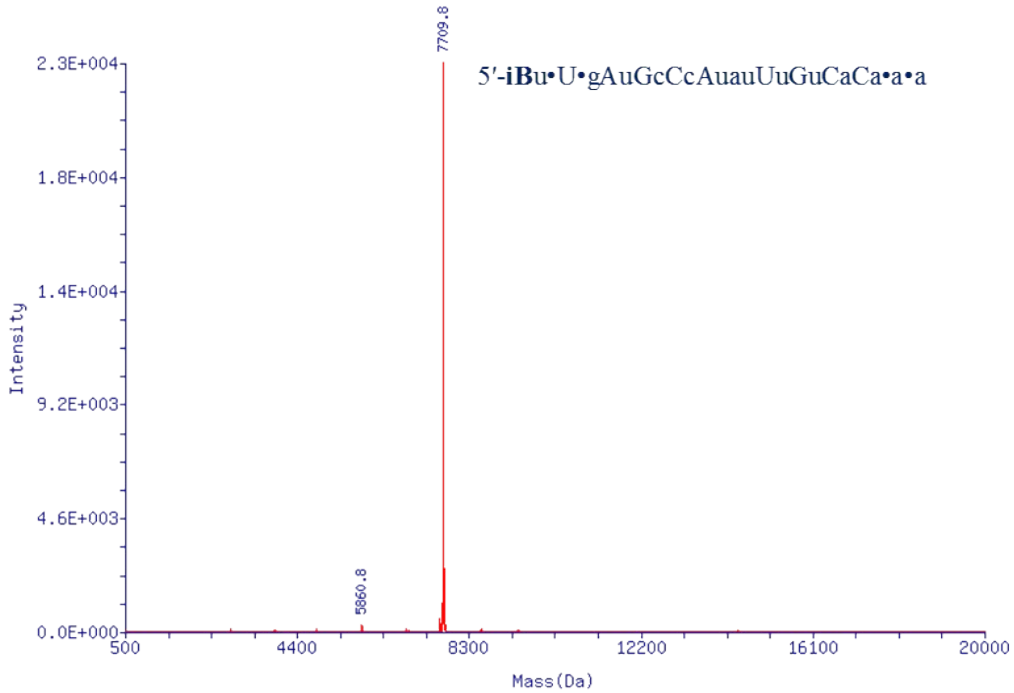

8893.6
5′-iBU•g•UgAcAaAUAuGgGcAuCaA-GalNAc3
1.8E+004
1.4E+004
1.1E+004
7.1E+003
3.5E+003
0.0E+000
Intensity
4356.2
7901.5
8388.4
500
4400
8300
12200
16100
20000
Mass(Da)

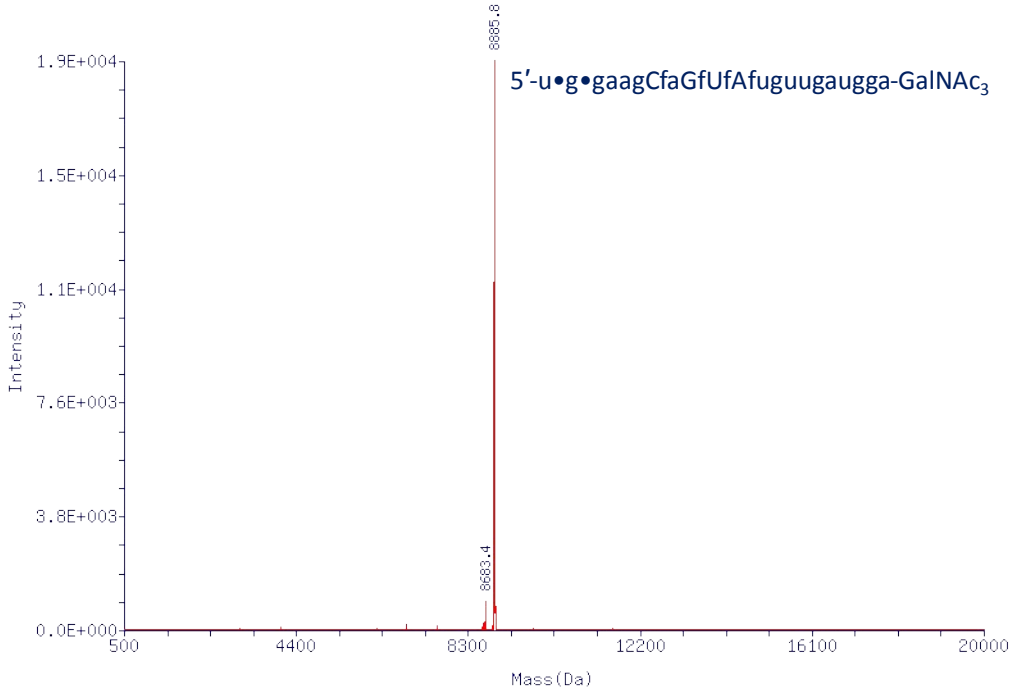

7599.2
5′-Mo•U•gAuGcCcAuauUuGuCaCa•a•a
4.0E+004
3.2E+004
2.4E+004
1.6E+004
8.0E+003
0.0E+000
Intensity
7193.8
500
4400
8300
12200
16100
20000
Mass(Da)

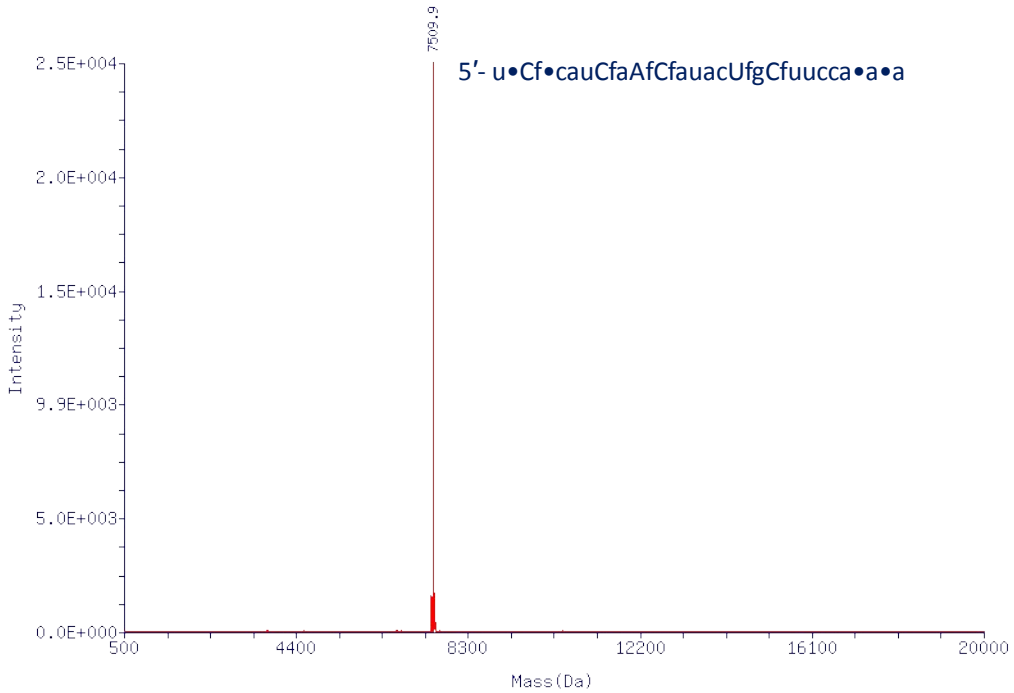

7557.9
7194.0
5′-D•U•gAuGcCcAuauUuGuCaCa•a•a
3.7E+004
3.0E+004
2.2E+004
1.5E+004
7.5E+003
0.0E+000
Intensity
500
4400
8300
12200
16100
20000
Mass(Da)

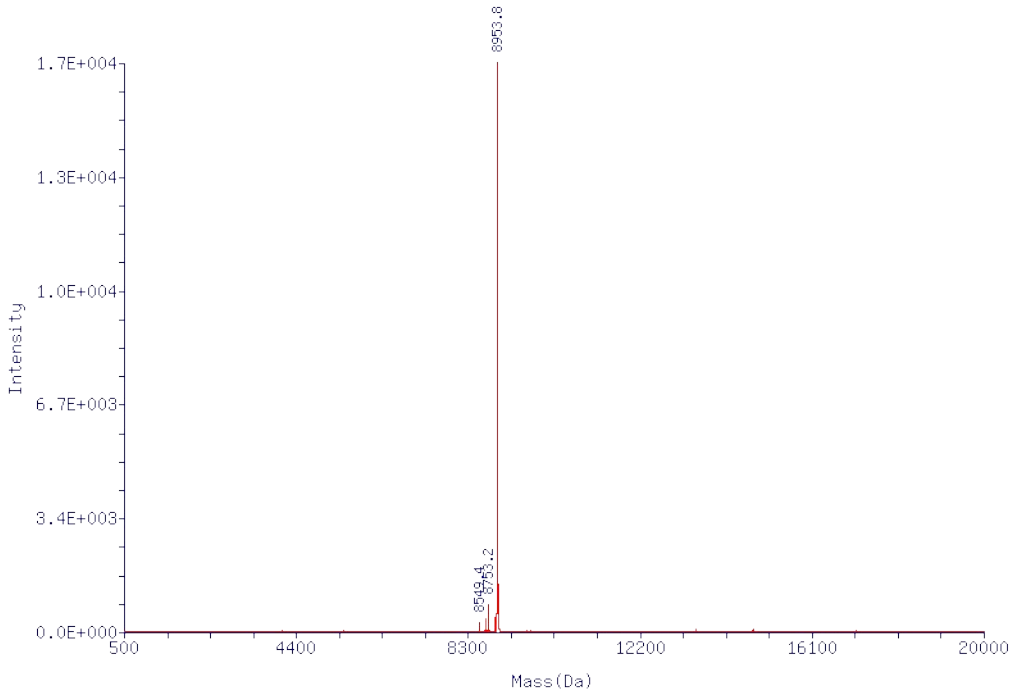

7514.3
5′-Me•U•gAuGcCcAuauUuGuCaCa•a•a
3.5E+004
2.8E+004
2.1E+004
1.4E+004
6.9E+003
0.0E+000
Intensity
500
4400
8300
12200
16100
20000
Mass(Da)

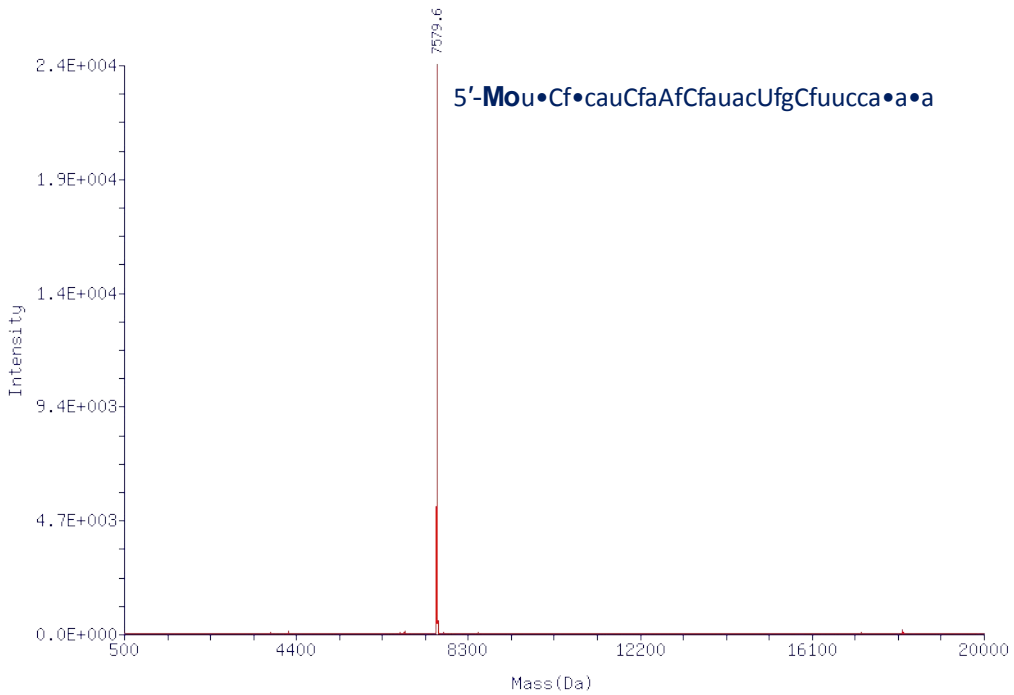

7542.6
5′-LNA•U•gAuGcCcAuauUuGuCaCa•a•a
4.0E+004
3.2E+004
2.4E+004
1.6E+004
8.0E+003
0.0E+000
Intensity
6703.3
500
4400
8300
12200
16100
20000
Mass(Da)


7709.8
5′-iBu•U•gAuGcCcAuauUuGuCaCa•a•a
2.3E+004
1.8E+004
1.4E+004
9.2E+003
4.6E+003
0.0E+000
Intensity
5860.8
500
4400
8300
12200
16100
20000
Mass(Da)


8885.8
8683.4
5′-u•g•gaagCfaGfUfAfuguugaugga-GalNAc3
1.9E+004
1.5E+004
1.1E+004
7.6E+003
3.8E+003
0.0E+000
Intensity
500
4400
8300
12200
16100
20000
Mass(Da)


7509.9
5′- u•Cf•cauCfaAfCfauacUfgCfuucca•a•a
2.5E+004
2.0E+004
1.5E+004
9.9E+003
5.0E+003
0.0E+000
Intensity
500
4400
8300
12200
16100
20000
Mass(Da)





5'-**Mo**u•Cf•cauCfaAfCfauacUfgCfuucca•a•a

1. R. G. Parmar, C. R. Brown, S. Matsuda, J. L. S. Willoughby, C. S. Theile, K. Charisse, D. J. Foster, I. Zlatev, V. Jadhav, M. A. Maier, M. Egli, M. Manoharan and K. G. Rajeev, *J Med Chem*, 2018, **61**, 734-744.
2. M. K. Schlegel, D. J. Foster, A. V. Kel'in, I. Zlatev, A. Bisbe, M. Jayaraman, J. G. Lackey, K. G. Rajeev, K. Charissé, J. Harp, P. S. Pallan, M. A. Maier, M. Egli and M. Manoharan, *Journal of the American Chemical Society*, 2017, **139**, 8537-8546.
3. R. Parmar, J. L. S. Willoughby, J. Liu, D. J. Foster, B. Brigham, C. S. Theile, K. Charisse, A. Akinc, E. Guidry, Y. Pei, W. Strapps, M. Cancilla, M. G. Stanton, K. G. Rajeev, L. Sepp-Lorenzino, M. Manoharan, R. Meyers, M. A. Maier and V. Jadhav, *ChemBioChem*, 2016, **17**, 985-989.