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Supporting Information

Conversion of *N*-acyl Amidines to Amidoximes: A Convenient Synthetic Approach to Molnupiravir (EIDD-2801) from Ribose

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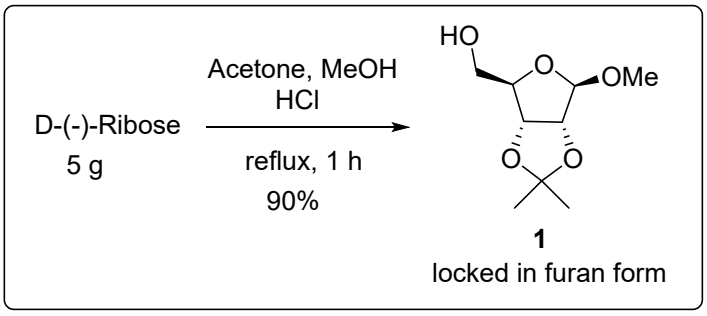
1.General information: ^{1}H and ^{13}C NMR spectra were recorded on 400, 101 and 126 MHz spectrometers with TMS as internal standard. Chemical shifts are expressed in parts per million (δ ppm). Silica gel coated aluminium plates were used for TLC. The products were purified by column chromatography on silica gel (60-120/100-200 mesh) using hexane–ethyl acetate and DCM-MeOH as the eluent to obtain the pure products. Mass spectra were obtained using Q-TOF-LC/MS spectrometer using electron spray ionization. Reagents used were mostly purchased from Sigma Aldrich, TCI and Alfa Aesar.

2. General Procedures:

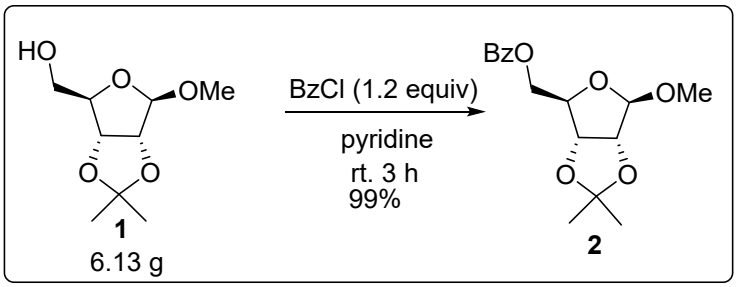
2.1. Preparation of Methyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (1).



Title compound was synthesized according to literature method[1] by dissolving D-ribose (5 g, 1.00 equiv, 0.33 mol) in a mixture of acetone (100 mL) and methanol (26 mL) in an oven dried round bottom flask equipped with a stirring bar magnet then, catalytic amount of conc. HCl (180 µL, 0.2 equiv, 6.66 mmol) was added to it and allowed to stirr on reflux for 1 h. after complete conversion of starting material the mixture was evaporated to remove excess of solvent. The aqueous solution thus obtained was extracted with EtOAc, washed with brine, dried (Na_2SO_4) and evaporated in vacuo to yield **1** (6.13 g, 90% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (s, 1H), 4.47 (d, J = 5.9 Hz, 1H), 4.28 (d, J = 5.9 Hz, 1H), 4.03 (t, J = 4.3 Hz, 1H), 3.30 (d, J = 4.5 Hz, 2H), 3.09 (s, 3H), 1.17 (s, 3H), 1.01 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 112.0, 109.8, 88.1, 85.6, 81.4, 63.8, 55.3, 26.2, 24.6. **HRMS** (ESI+): m/z: $[M+H]^+$ calctd for $C_9H_{17}O_5$, 205.1076; found, 205.1070. $[\alpha]_D$ -19.7 (c 1, $CHCl_3$).

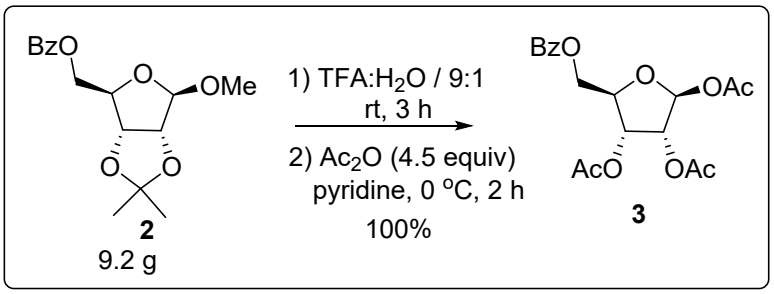
2.2. Preparation of methyl-2,3-*O*-isopropylidene-5-*O*-benzoyl-β-D-ribofuranoside (2).



Compound **1** (6.13 g, 1 euiv., 0.30 mol) as obtained in previous step was dissolved in pyridine (25 mL) at room temperature, benzoyl chloride (4.2 mL, 1.2 equiv, 0.36 mol) was added to it and stirred at same temperature for 3 h. After completion of the reaction, excess of pyridine was quenched with cupric sulfate solution, diluted the reaction mixture with water and extracted with ethyl acetate and washed with brine, dried over Na_2SO_4. The residue was obtained as **2** (9.2 g, 99%yield) was taken to next step without purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 8.03 (m, 2H), 7.64 – 7.54 (m, 1H), 7.48 – 7.42 (m, 2H), 5.04 (s, 1H), 4.83 – 4.76 (m, 1H), 4.67 (d, J = 5.9 Hz, 1H), 4.55 (dt, J = 7.1, 3.7 Hz, 1H), 4.42 – 4.31 (m, 2H), 3.35 (s, 3H), 1.52 (s, 3H), 1.35 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 166.1, 133.2, 129.7, 128.4, 112.6, 109.4, 85.3, 84.3, 81.9, 65.1, 54.9, 26.4, 25.0. **HRMS** (ESI+): m/z calcd. for $C_{16}H_{21}O_6$ $(M+H)^+$ 309.1338, found 309.1346. $[\alpha]_D$ -16.2 (c 1, $CHCl_3$).

2.3. Preparation of 1,2,3-tri-*O*-acetyl-5-*O*-benzoyl-β-D-ribofuranose (3).

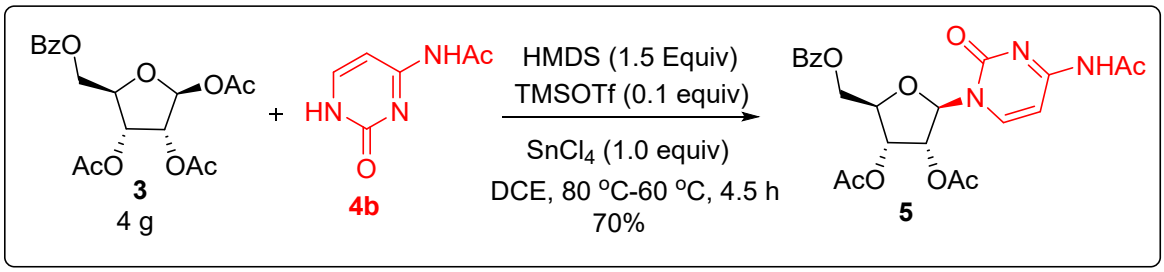


Compound **2** (9.2 g, 1 equiv., 0.30 mol) as crude obtained in previous step was dissolved in a mixture of trifluoroacetic acid (TFA) (22.5 mL) and water (2.5 mL) at room temperature and allowed to stir for 3 hour of time at same temperature. The mixture was evaporated to dryness by repeated concentration of it with toluene. The residue was dissolved in pyridine (50 mL) at 0 °C, treated with Ac_2O (12.7 mL, 4.5 equiv., 1.34 mol), and stirred at rt for 3 h. Excess of pyridine was quenched with cupric sulfate solution, diluted the reaction mixture with water and extracted with ethyl acetate and washed with brine, dried over Na_2SO_4. The residue was purified by

column chromatography by using ethyl acetate and hexane to obtain **3** as a white solid (11.35 g, overall 100%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 7.7 Hz, 2H), 7.49 (t, J = 7.3 Hz, 1H), 7.36 (t, J = 7.6 Hz, 2H), 6.10 (s, 1H), 5.43 (dd, J = 7.0, 5.1 Hz, 1H), 5.31 (d, J = 4.7 Hz, 1H), 4.57 (dd, J = 12.1, 3.5 Hz, 1H), 4.45 – 4.39 (m, 1H), 4.30 (dd, J = 12.1, 4.4 Hz, 1H), 2.05 (s, 3H), 1.96 (s, 3H), 1.85 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 169.6, 169.3, 168.9, 165.9, 133.3, 129.7, 128.4, 98.3, 79.4, 74.3, 70.6, 63.5, 20.8, 20.4, 20.3. **HRMS** (ESI+): m/z calcd. for $C_{18}H_{21}O_9$ $(M+H)^+$ 381.1186, found 381.1181. $[\alpha]_D$ +18.7 (c 1, $CHCl_3$).

2.4. Typical procedure for the synthesis of nucleoside from β-D-ribofuranose 3 and N^4-acetyl-cytosine 4b (5).

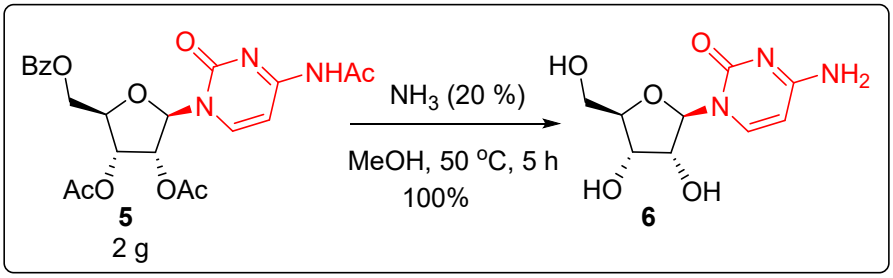


In an atmosphere of nitrogen (3.2 g, 2 equiv., 0.21 mol) of N^4-acetyl-cytosine was dissolved in anhydrous dichloroethane (40 mL) in a round bottom flask, Hexamethyldisilazane (HMDS) (3.3 mL, 1.5 equiv, 0.16 mol) and Trimethylsilyltrifluoromethanesulfonate (TMSOTf) (190 µL, 0.1 equiv., 0.01 mol) were added successively at 80 °C and stirred the mixture for 1 h at the same temperature. The reaction mixture was cooled to 60 °C, then charged with 1,2,3-tri-*O*-acetyl-5-*O*-benzoyl-β-D-ribofuranose **3** (4 g, 1.0 equiv., 0.11 mol) followed by addition of $SnCl_4$ (1.2 mL, 1.0 equiv., 0.11 mol) and allowed the reaction to stir at same temperature for additional 3 hour of time. After completion of the reaction excess $SnCl_4$ was quenched with 1N HCl (10 mL), the reaction mixture was diluted with water and extracted with ethyl acetate and washed with brine, dried over Na_2SO_4. The residue was purified by column chromatography by using dichloromethane and methanol to obtain **5** (3.5 g, 70% yield) as a gummy solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 7.6 Hz, 2H), 7.88 (d, J = 7.6 Hz, 1H), 7.55 (t, J = 7.3 Hz, 1H), 7.42 (t, J = 7.7 Hz, 2H), 7.28 (d, J = 7.6 Hz, 1H), 6.04 (d, J = 3.5 Hz, 1H), 5.55 – 5.49 (m, 1H), 5.42 (t, J = 5.9 Hz, 1H), 4.66 (dd, J = 12.3, 2.3 Hz, 1H), 4.57 – 4.46 (m, 2H), 2.19 (s,

3H), 2.02 (d, J = 10.4 Hz, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.8, 169.1, 169.0, 165.6, 162.8, 154.4, 143.7, 133.2, 129.1, 128.7, 128.3, 96.9, 89.1, 79.3, 73.3, 69.1, 62.5, 24.3, 19.9. **HRMS** (ESI+): m/z calcd. for $C_{22}H_{23}N_3NaO_9$ $(M+Na)^+$ 496.1332, found 496.1323. $[\alpha]_D$ +26.0 (c 1, $CHCl_3$).

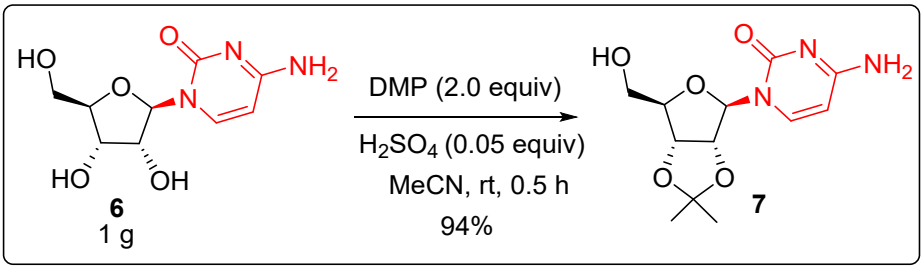
2.5. Global deprotection to obtain Cytidine (6).



Compound **5** (2 g, 1.0 equiv., 4.22 mmol) was dissolved in methanol (20 mL), liquor ammonia (5 mL) was added to it, resulted reaction mixture was stirred at 50 °C for 5 hours of time. After complete consumption of starting material the mixture was concentrated on rota vapour under reduced pressure to dryness by repeated concentration of it with methanol to obtain **6** (1.03 g, 100% yield) as white powder.

^{1}H NMR (400 MHz, MeOD) δ 8.18 (d, J = 7.5 Hz, 1H), 6.07 (d, J = 7.4 Hz, 1H), 5.88 (d, J = 3.0 Hz, 1H), 4.18 (dd, J = 9.6, 4.4 Hz, 2H), 4.11 – 4.01 (m, 1H), 3.91 (d, J = 12.4 Hz, 1H), 3.84 – 3.75 (m, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 164.5, 154.5, 142.5, 95.1, 90.4, 84.7, 74.8, 69.3, 60.5. **HRMS** (ESI+): $[M+H]^+$ calctd for $C_9H_{14}N_3O_5$, 244.0933; found, 244.0931. $[\alpha]_D$ -15.1 (c 1, $CHCl_3$). $[\alpha]_D$ -29.7 (c 1, $CHCl_3$).

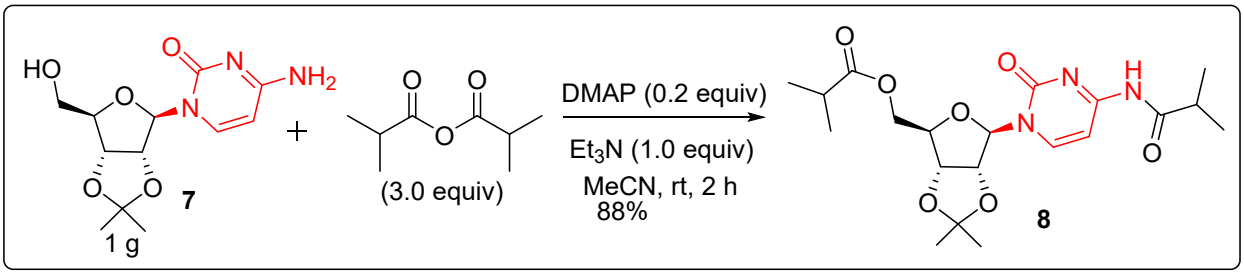
2.6. Preparation of 2,3-acetonide protected cytidine (7).



Acetonide protection of cytidine was done according to a literature report by dissolving **6** (1 g, 1.0 equiv., 4.11 mmol) in 25 mL of anhydrous acetontrile in an oven dried 100 mL 3-necked round bottom flask equipped with a Dean-Stark apparatus flushed with nitrogen. 2,2-Dimethoxypropane (1.0 mL, 2 equiv., 8.23 mmol) and 98% H_2SO_4 (11 µL, 5 mol%) were successively added to it. The reaction mixture was stirred at room temperature for half an hour of time. Then, 10 mL of anhydrous acetonitrle were added and 10 mL of solvent were distilled off. This distillation was performed thrice. After cooling to room temperature, the excess solvent was evaporated under reduced pressure to obtain **7** (1.1 g, 94% yield).

^{1}H NMR (400 MHz, MeOD) δ 7.87 (d, J = 7.6 Hz, 1H), 5.90 (d, J = 7.6 Hz, 1H), 5.77 (d, J = 2.3 Hz, 1H), 4.80 (dd, J = 6.3, 2.4 Hz, 1H), 4.17 (dd, J = 7.4, 3.5 Hz, 1H), 3.70 (dd, J = 12.0, 3.4 Hz, 1H), 3.64 – 3.57 (m, 2H), 1.45 (s, 3H), 1.25 (s, 3H). **^{13}C NMR (101 MHz, MeOD)** δ 164.4, 154.3, 143.7, 113.6, 94.5, 93.9, 87.5, 85.1, 80.8, 61.7, 26.1, 24.2. **HRMS** (ESI+): m/z calcd. for $C_{12}H_{18}N_3O_5$ $(M+H)^+$ 284.1246, found 284.1243. $[\alpha]_D$ +19.7 (c 1, $CHCl_3$).

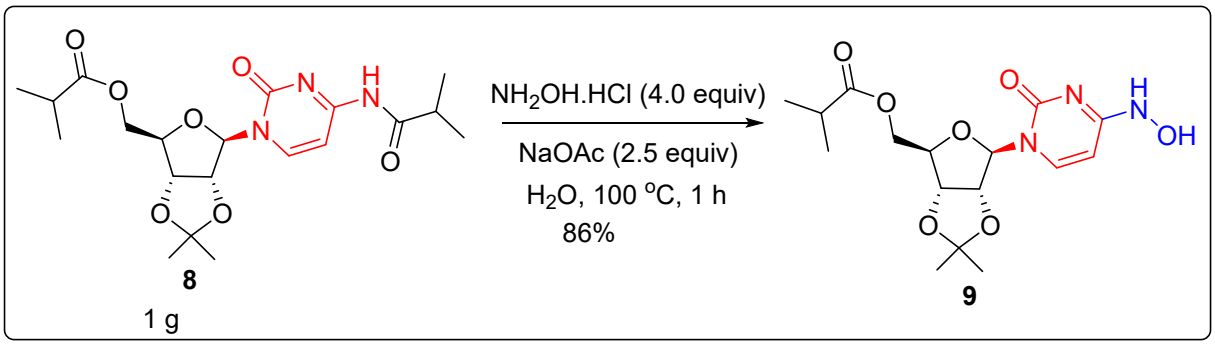
2.7. Esterfication of acetonide protected cytidine (8).



Compound **7** (1 g, 1.00 equiv., 3.53 mmol) was dissolved in acetonitle (25 mL) in an oven dried round bottom flask, Isobutyric anhydride (1.8 mL, 3.0 equiv., 10.6 mmol), triethylamine (491 µL, 1.00 equiv., 3.53 mmol) and 4-Dimethylaminopyridine (87 mg, 0.2 equiv., 0.7 mmol) were added successively. The reaction mixture was stirred at room temperature for 2 h. After completion of reaction, methanol (5 mL) was added to it and stirred the reaction for another additional hour. The solvent was removed under reduced pressure. After addition of 100 mL ethyl acetate, the organic phase was washed with sat. $NaHCO_3$ (2 × 90 mL), water (50 mL) and brine (50 mL) and dried with Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by column chromatography by using ethyl acetate and hexane to obtain **8** (1.32 g, 88% yield) as a whitish solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 7.5 Hz, 1H), 7.50 (d, J = 7.5 Hz, 1H), 5.73 (s, 1H), 5.04 (d, J = 6.4 Hz, 1H), 4.85 (dd, J = 6.2, 3.9 Hz, 1H), 4.52 – 4.43 (m, 1H), 4.39 – 4.30 (m, 2H), 2.77 (dt, J = 13.7, 6.8 Hz, 1H), 2.56 – 2.47 (m, 1H), 1.57 (s, 3H), 1.35 (s, 3H), 1.22 (d, J = 1.1 Hz, 3H), 1.20 (d, J = 1.2 Hz, 3H), 1.16 (s, 3H), 1.14 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 177.9, 176.5, 163.6, 154.6, 146.4, 114.1, 96.6, 96.5, 86.3, 85.2, 81.3, 64.2, 36.4, 33.8, 27.1, 25.2, 19.0, 18.9, 18.9, 18.9. **HRMS** (ESI+): m/z calcd. for $C_{20}H_{30}N_3O_7$ $(M+H)^+$ 424.2084, found 424.2089. $[\alpha]_D$ +13.0 (c 1, $CHCl_3$).

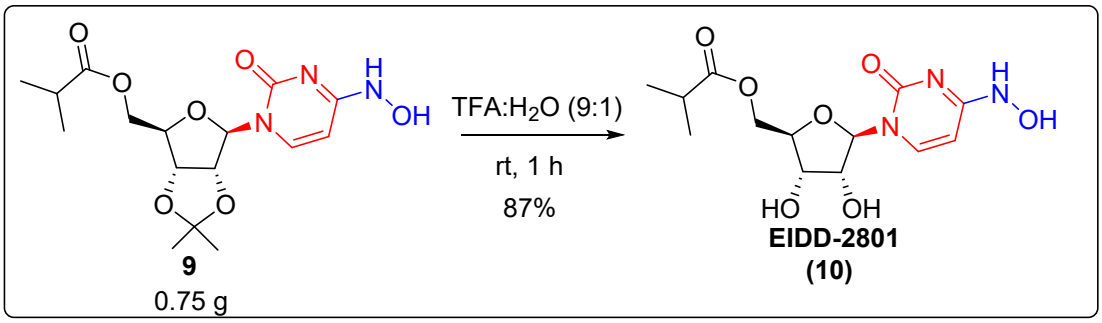
2.8. Synthesis of Acetonide-protected Hydroxylamine (9).



A mixture of **8** (1 g, 1.0 equiv., 2.36 mmol), hydroxylamine hydrochloride (657 mg, 4.0 equiv., 9.46 mmol) and sodium acetate (485 mg, 2.5 equiv., 5.91 mmol) in 50 ml of water was heated at 100 °C for 40 minutes of time. On completely conversion of the starting material the reaction mixture was cooled to room temperature and extracted with ethyl acetate (2 × 50 mL). After removal of the solvent under reduced pressure, the residue was purified by column chromatography by using ethyl acetate and hexane to obtain **9** (0.75 g, 86% yield) as a whitish solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (d, J = 8.1 Hz, 1H), 5.66 (d, J = 8.1 Hz, 1H), 5.60 (d, J = 1.6 Hz, 1H), 4.96 (dd, J = 6.4, 2.0 Hz, 1H), 4.79 (dd, J = 6.3, 3.8 Hz, 1H), 4.35 – 4.24 (m, 3H), 2.59 (dt, J = 13.9, 7.0 Hz, 1H), 1.56 (s, 3H), 1.35 (s, 3H), 1.19 (s, 3H), 1.17 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 176.8, 149.5, 144.7, 132.9, 114.5, 98.6, 93.8, 84.4, 84.2, 81.0, 64.1, 33.8, 33.7, 27.1, 25.3, 18.9, 18.9. **LCMS (ESI-TOF):** m/z: $[M+H]^+$ calctd for $C_9H_{14}N_3O_5$, 244.09; found, 244.15. **HRMS** (ESI+): m/z calcd. for $C_{16}H_{24}N_3O_7$ $(M+H)^+$ 370.1614, found 370.1623. $[\alpha]_D$ +11.3 (c 1, $CHCl_3$).

2.8. Acetonide deprotection to get EIDD-2801 (10).

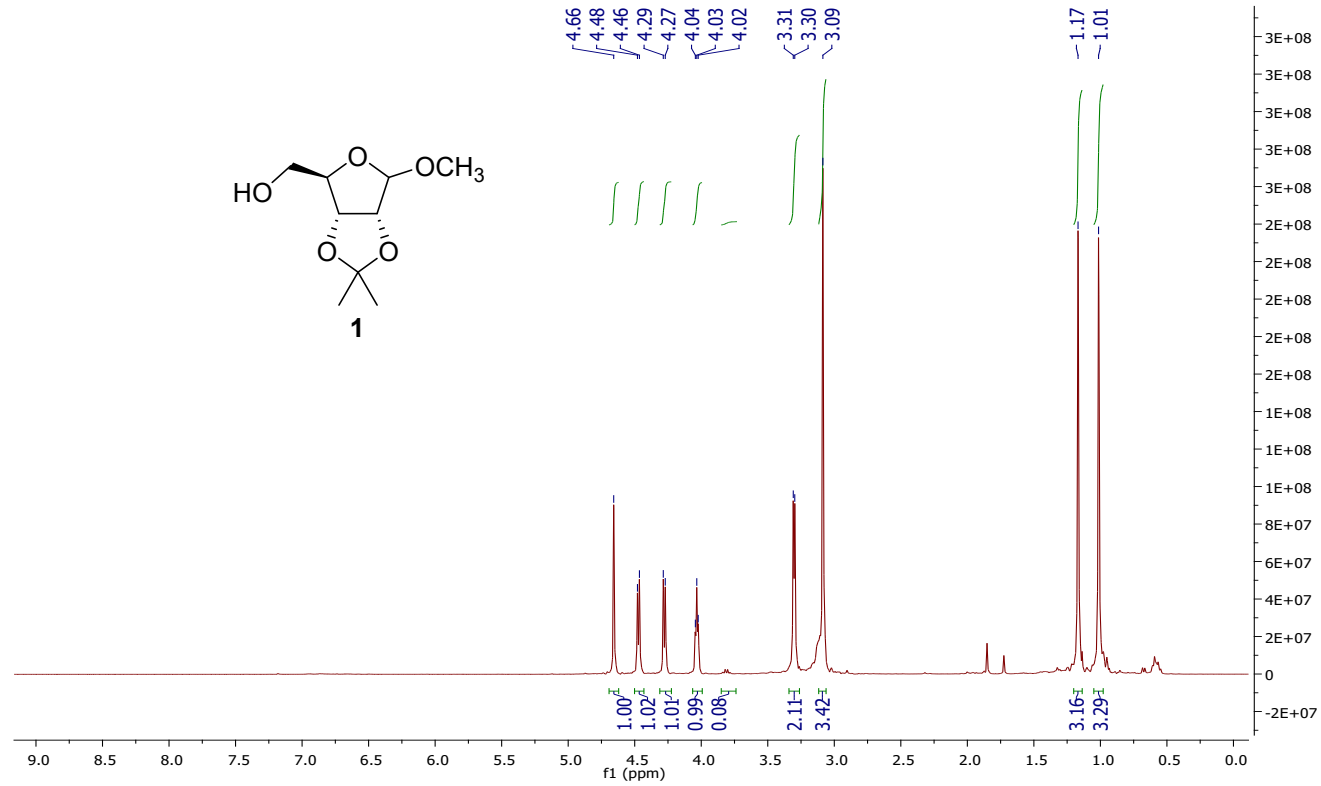


Compound **9** (0.75 g, 1.0 equiv., 2.03 mmol) was treated with trifluoroacetic acid (TFA) (9 mL) and water (1 mL) at room temperature and allowed to stir for 1 hour of time at same temperature. Excess of TFA was quenched with aqueous solution $NaHCO_3$. The reaction mixture was diluted with water and extracted with ethyl acetate and washed with brine, dried over Na_2SO_4. The residue was purified by column chromatography by using dichloromethane and methanol to obtain **10** (0.58 g, 87% yield) as a whitish gummy solid.

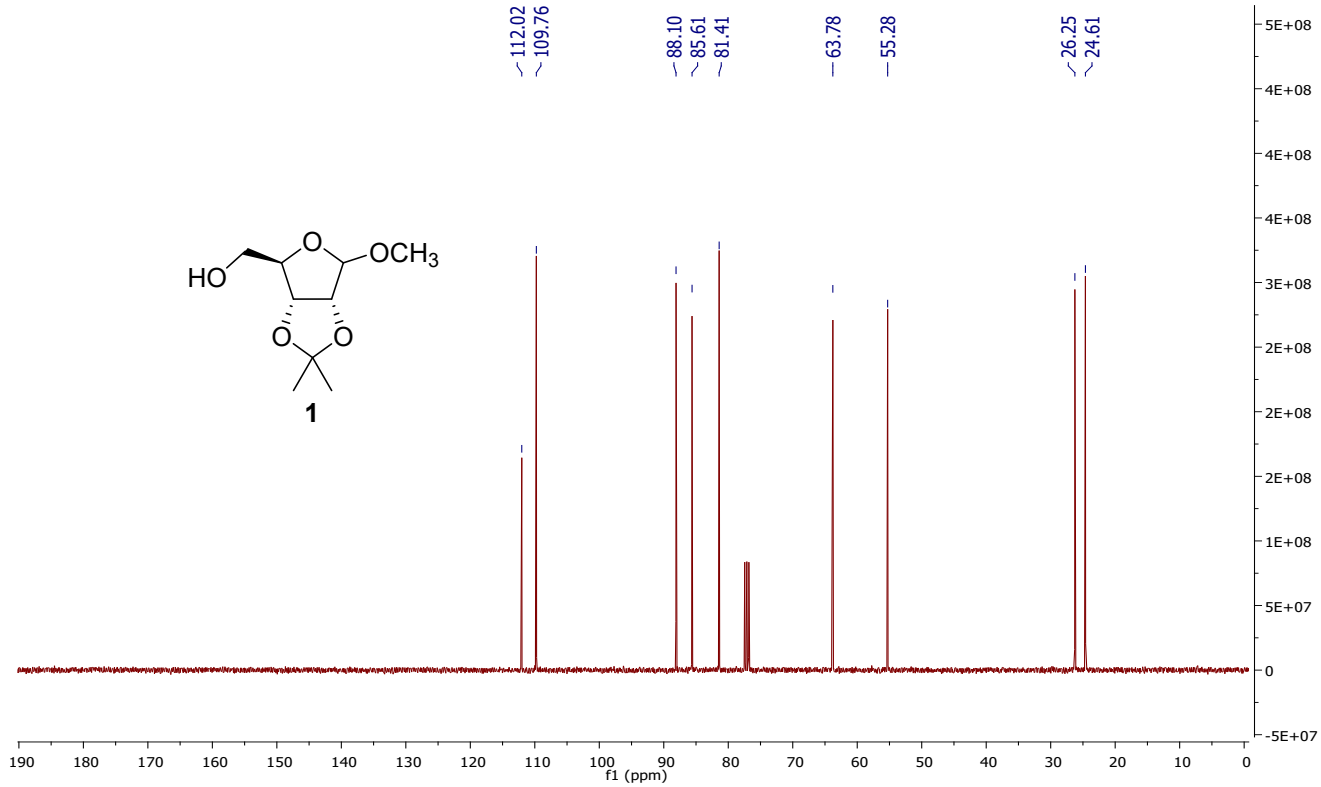
^{1}H NMR (400 MHz, MeOD) δ 6.94 (d, J = 8.2 Hz, 1H), 5.72 (d, J = 4.7 Hz, 1H), 5.56 (d, J = 8.2 Hz, 1H), 4.20 (d, J = 3.9 Hz, 2H), 4.06 – 3.93 (m, 3H), 2.52 (dt, J = 14.0, 7.0 Hz, 1H), 1.09 (s, 3H), 1.07 (s, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 181.2, 153.4, 149.9, 135.7, 101.5, 93.8, 85.1, 77.5, 73.7, 67.6, 37.9, 22.6, 22.6. **HRMS** (ESI+): m/z calcd. for $C_{13}H_{20}N_3O_7$ $(M+H)^+$ 330.1301, found 330.1307. $[\alpha]_D$ -33.2 (c 1, $CHCl_3$).

References:

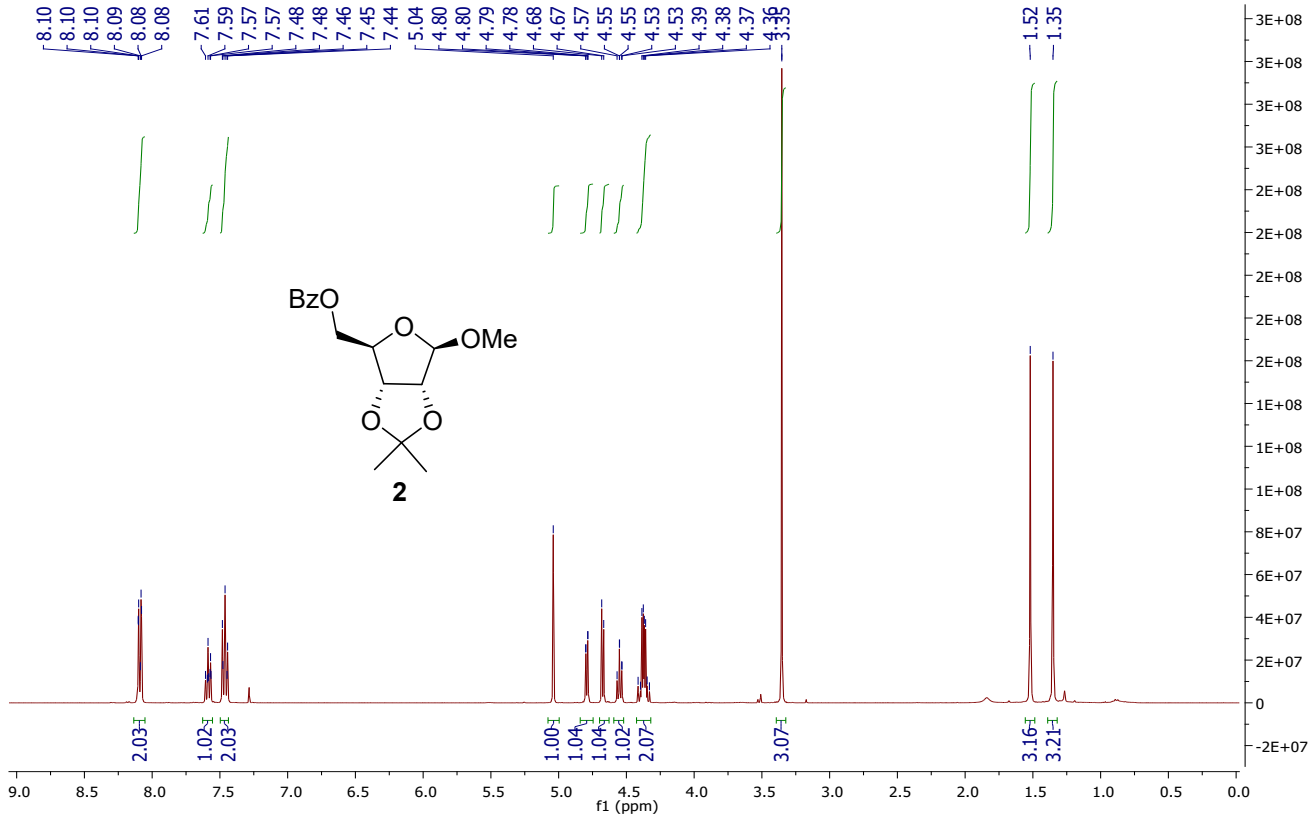
1) Amos B. Smith, III,* Qiang Han, Paul A. S. Breslin, and Gary K. Beauchamp, Org. Lett., Vol. 7, No. 22, 2005



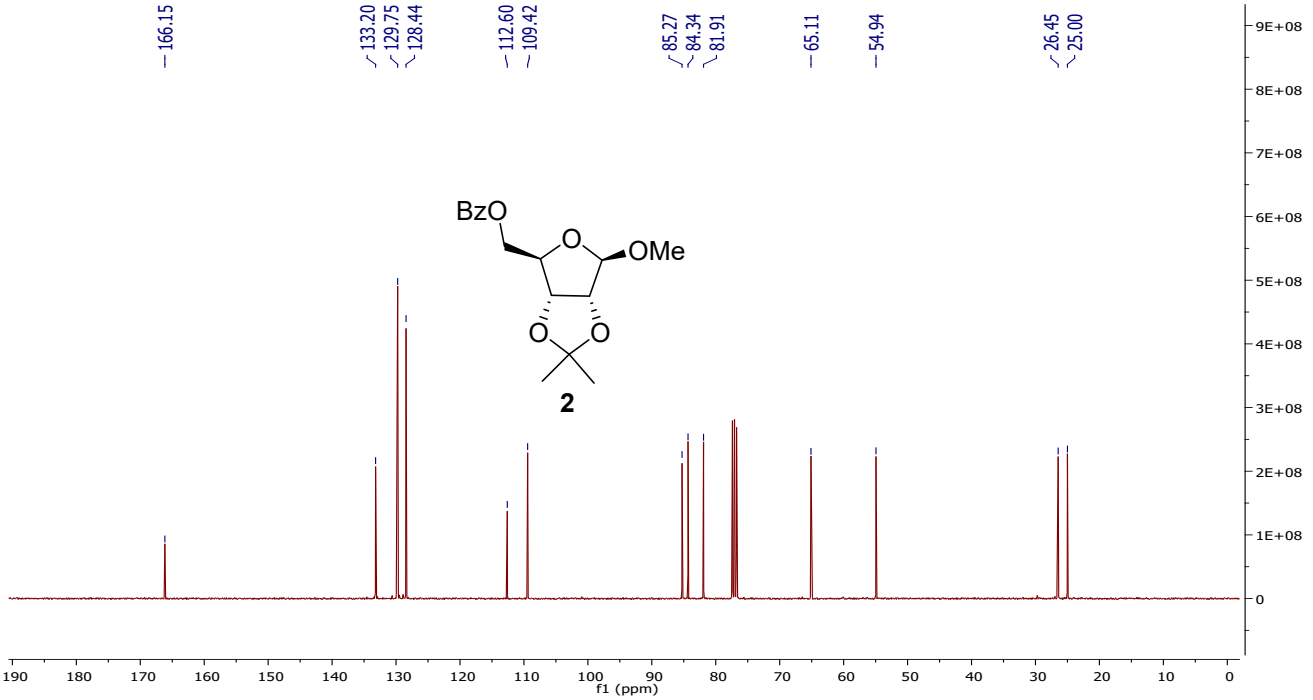
^{1}H NMR (400 MHz, $CDCl_3$) of compound 1



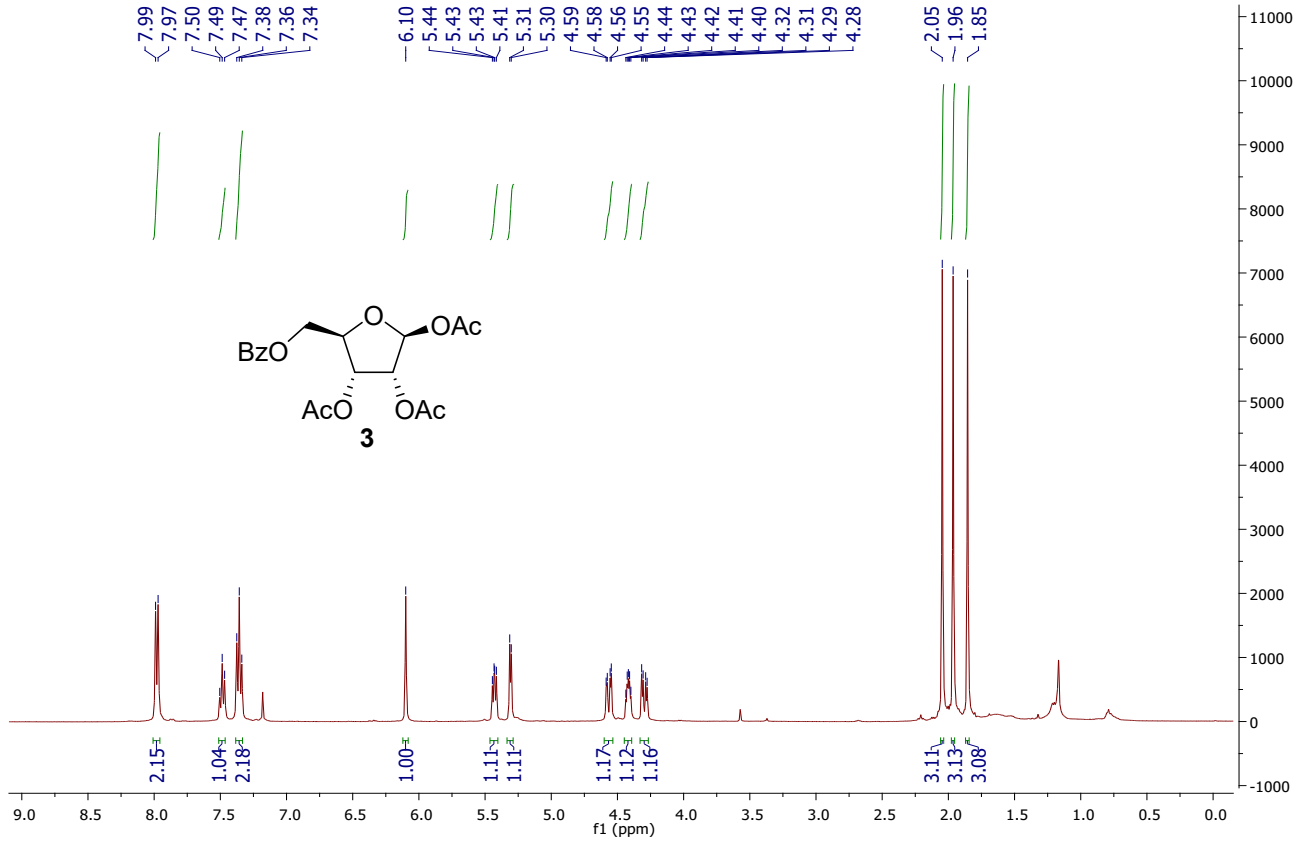
^{13}C NMR (101 MHz, $CDCl_3$) of compound 1



^{1}H NMR (400 MHz, $CDCl_3$) of compound 2



^{13}C NMR (101 MHz, $CDCl_3$) of compound 2



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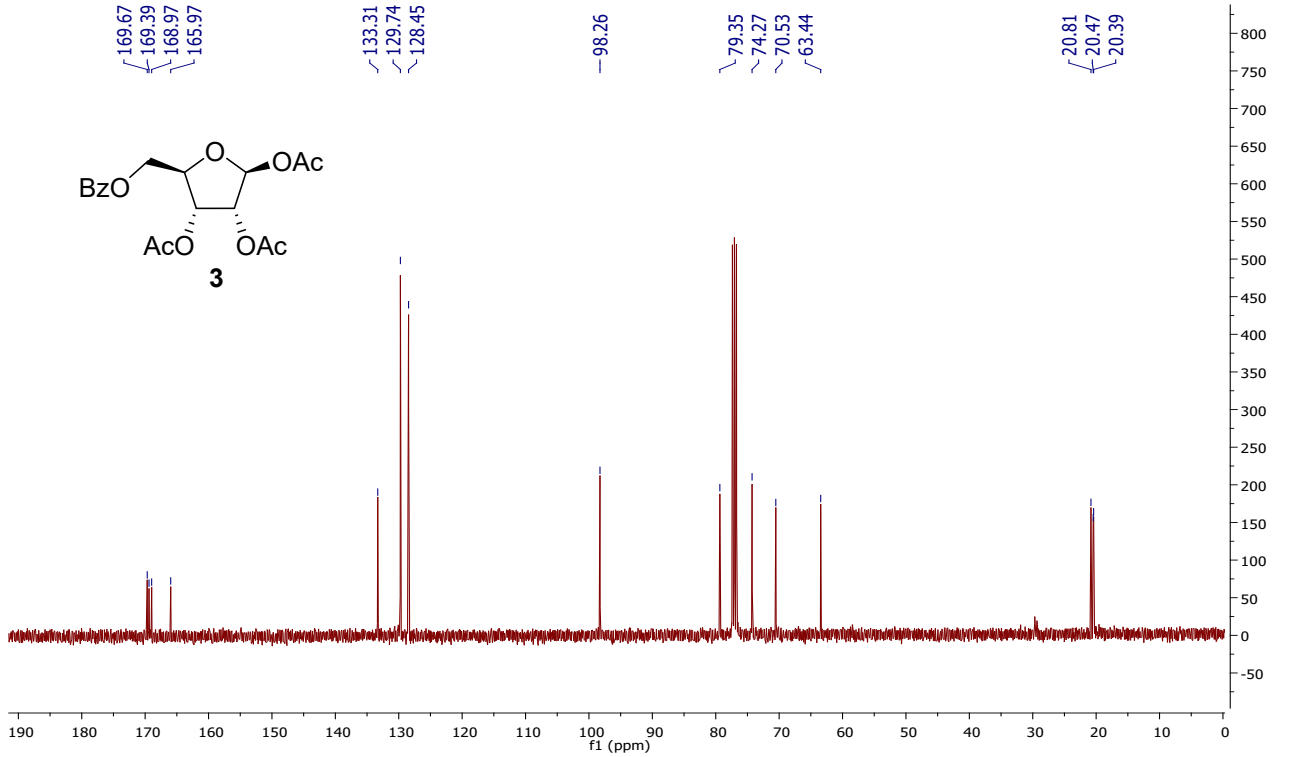
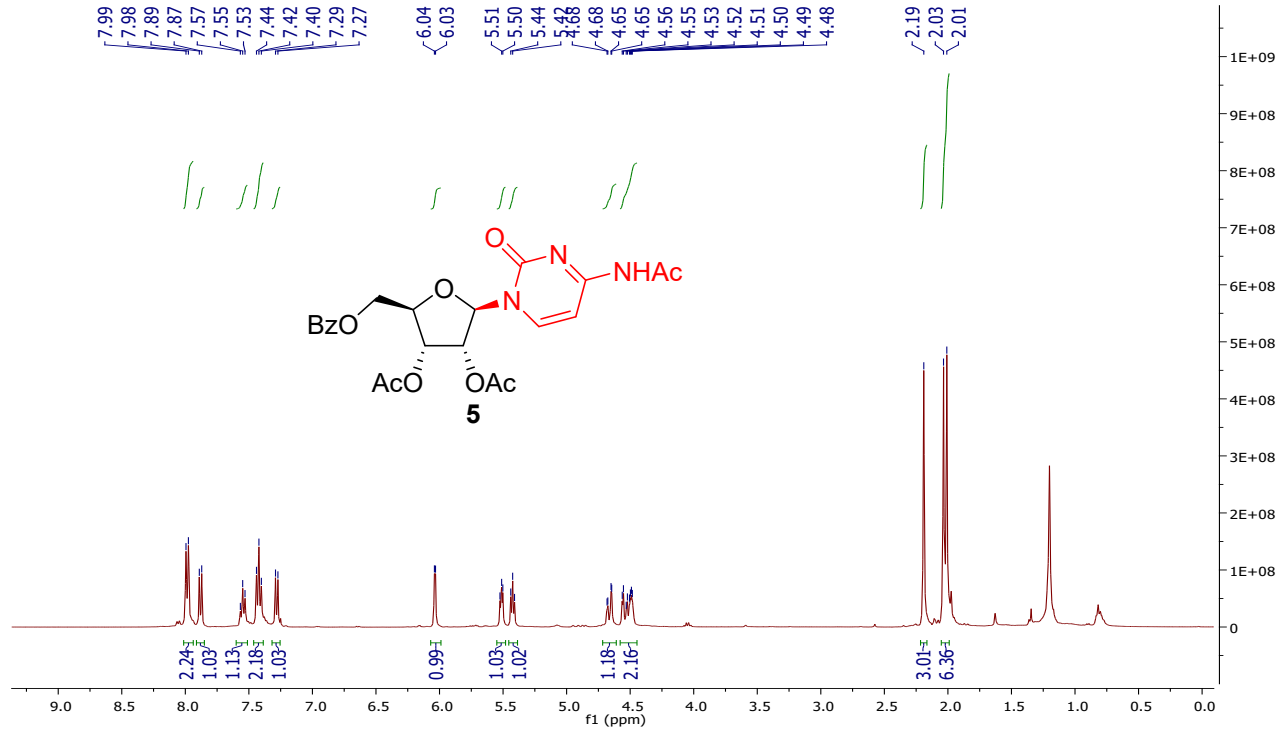
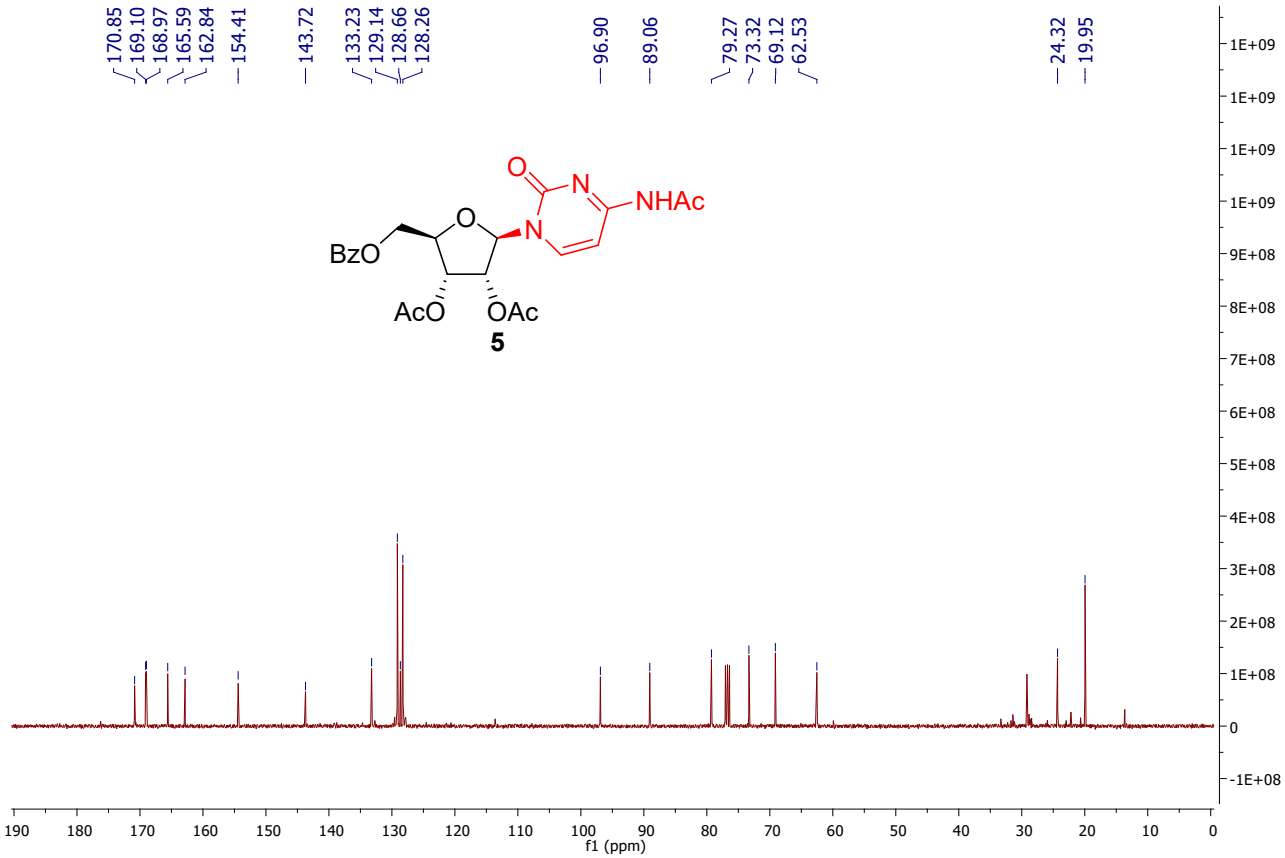


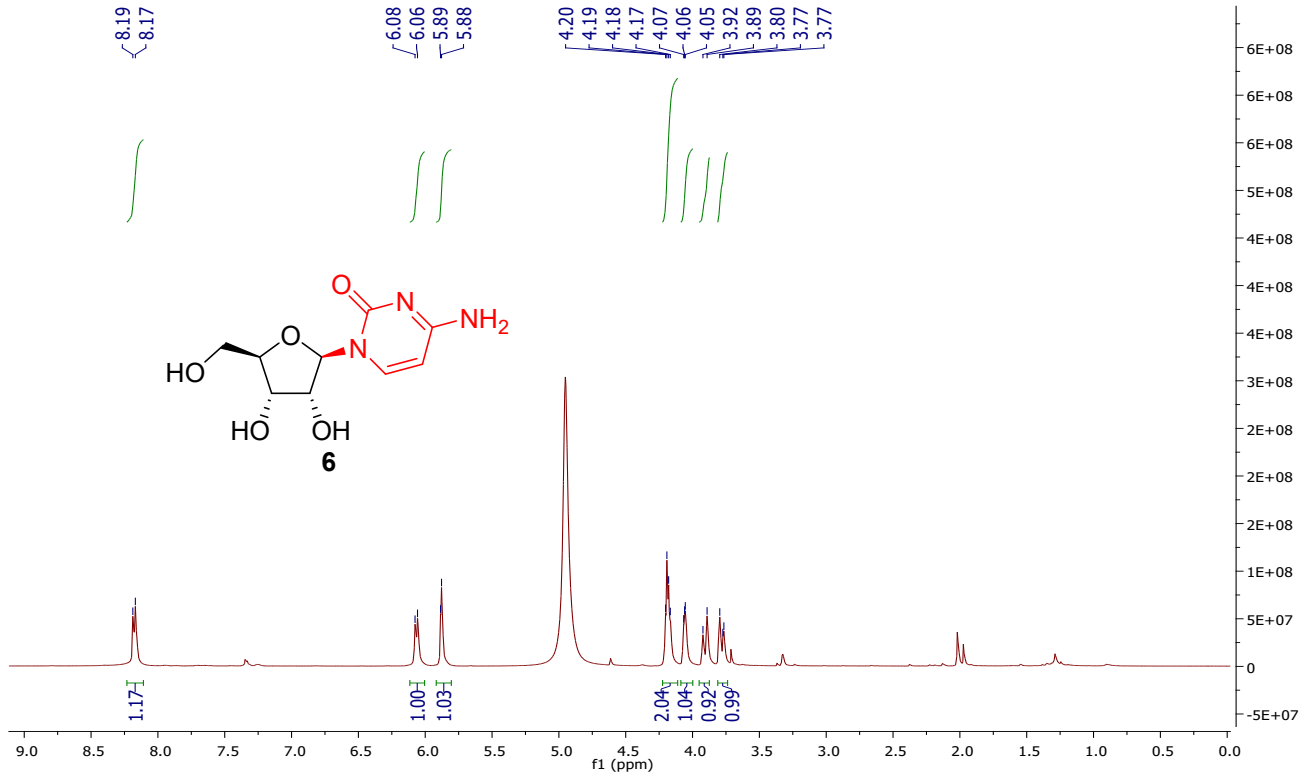
^{13}C NMR (101 MHz, $CDCl_3$) of compound 3



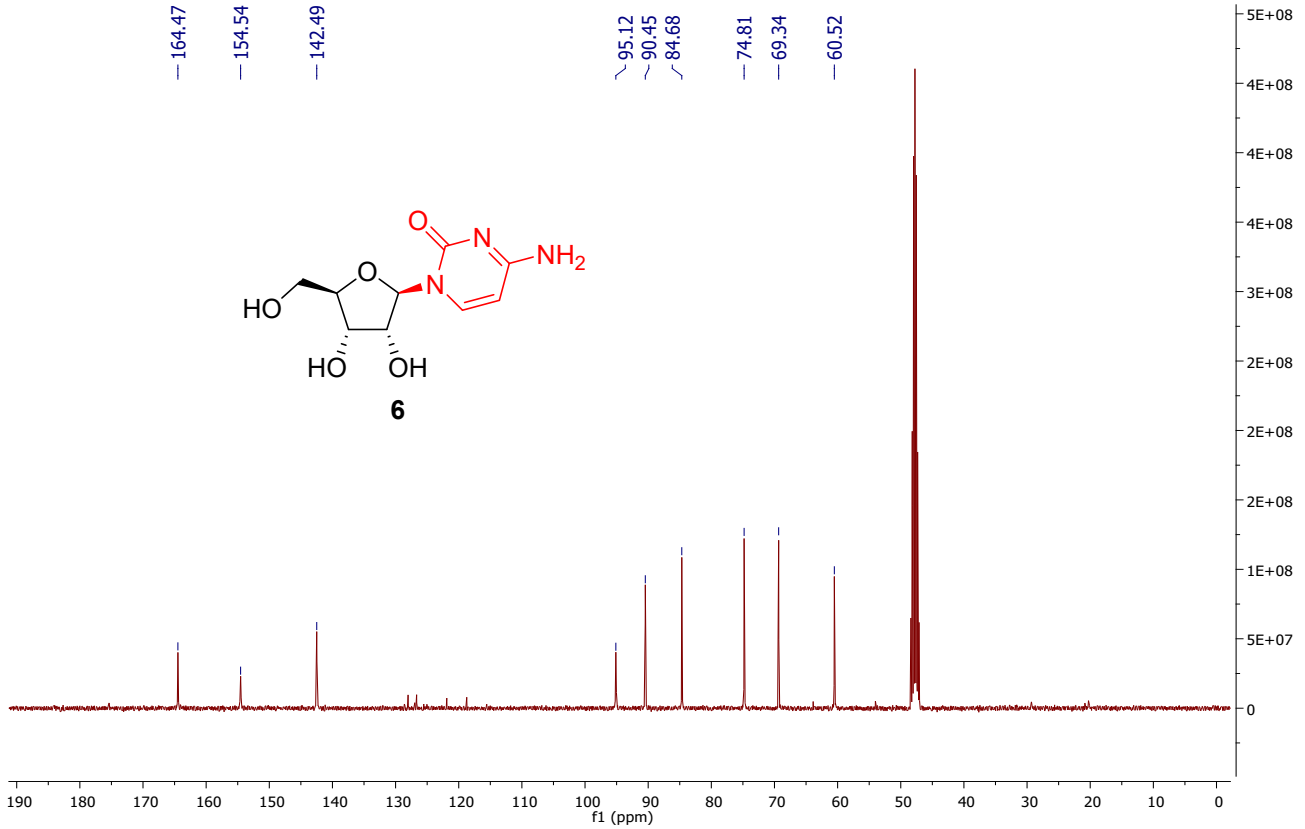
^{1}H NMR (400 MHz, $CDCl_3$) of compound 5



^{13}C NMR (101 MHz, $CDCl_3$) of compound 5



^{1}H NMR (400 MHz, MeOD) of compound 6



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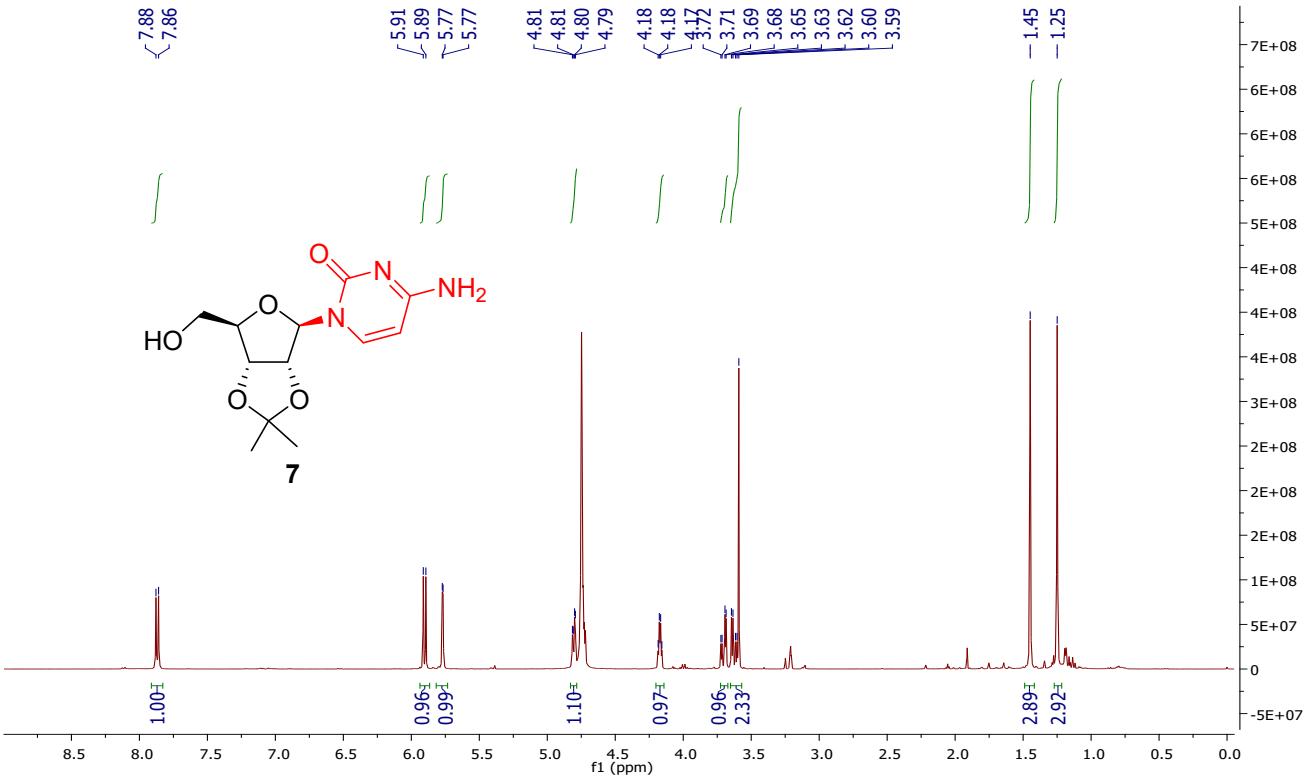
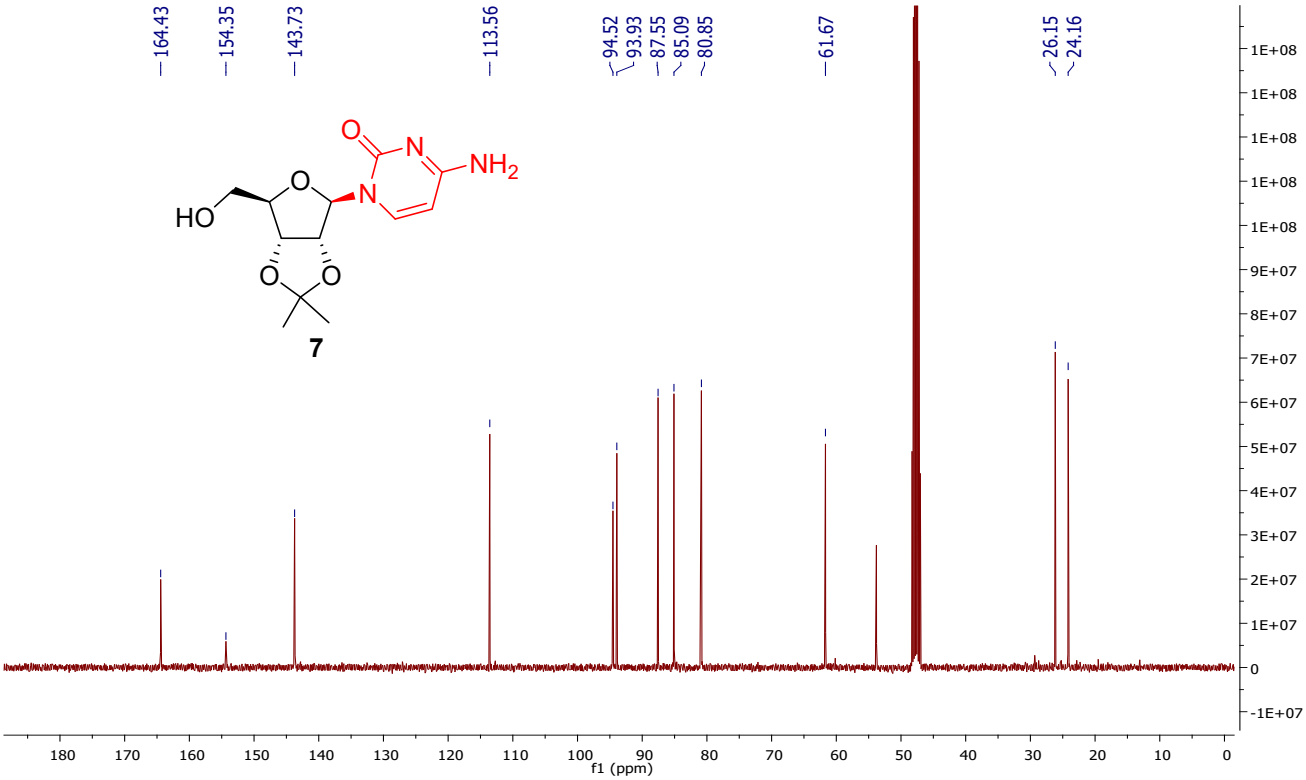
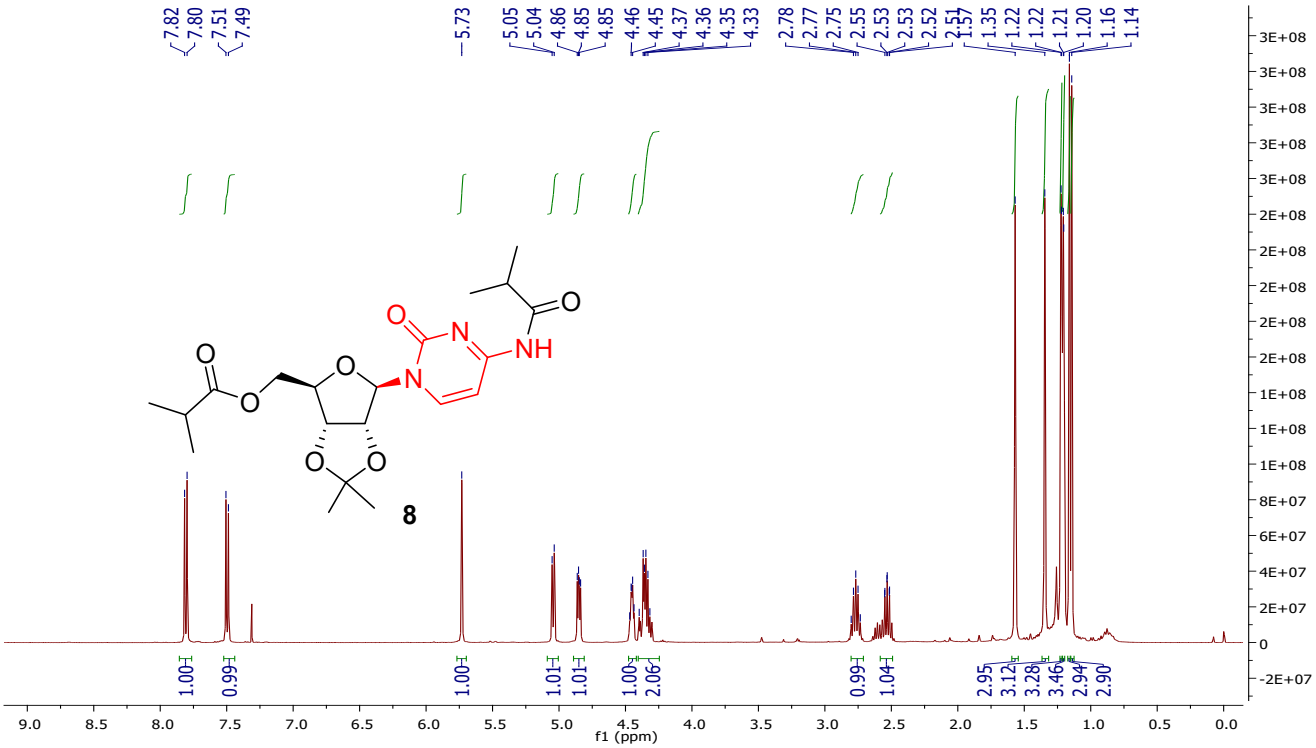


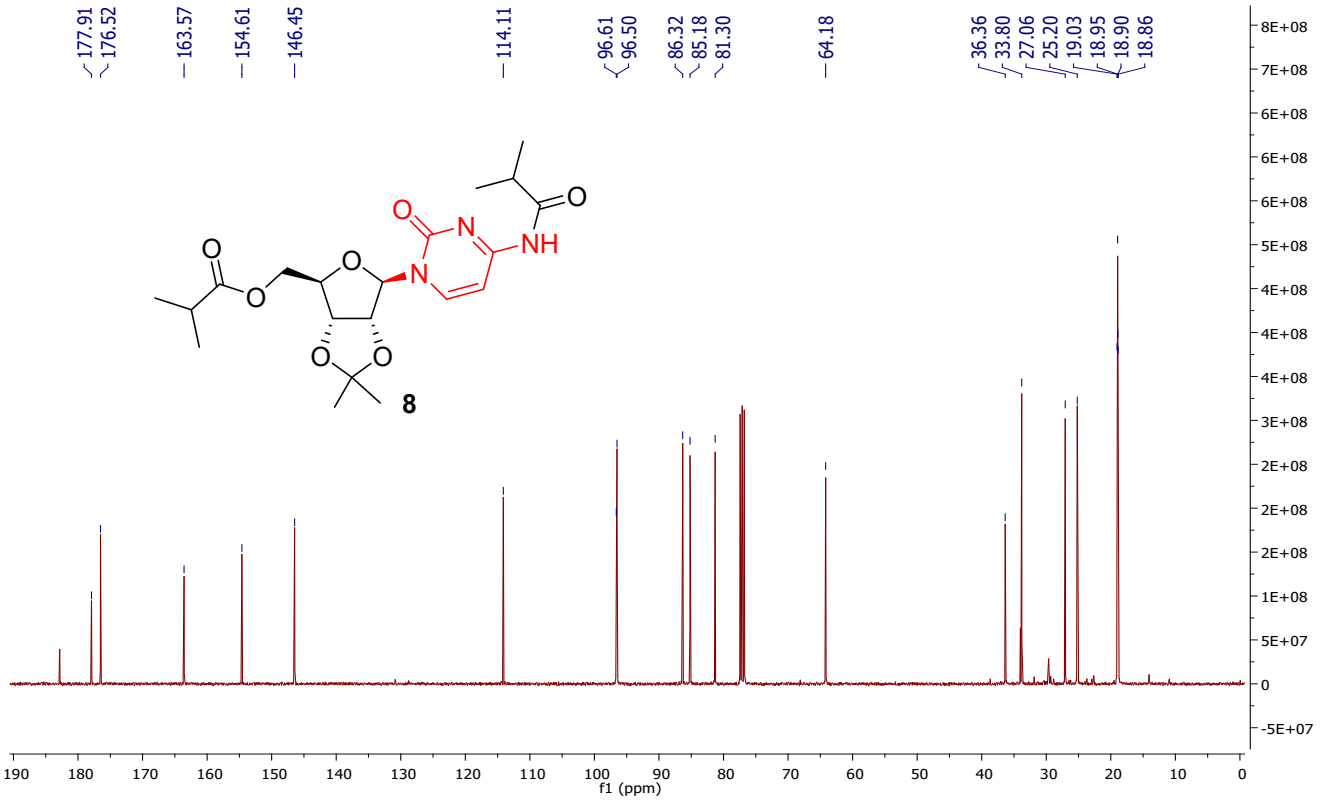
[1]H NMR (400 MHz, MeOD) of compound 7



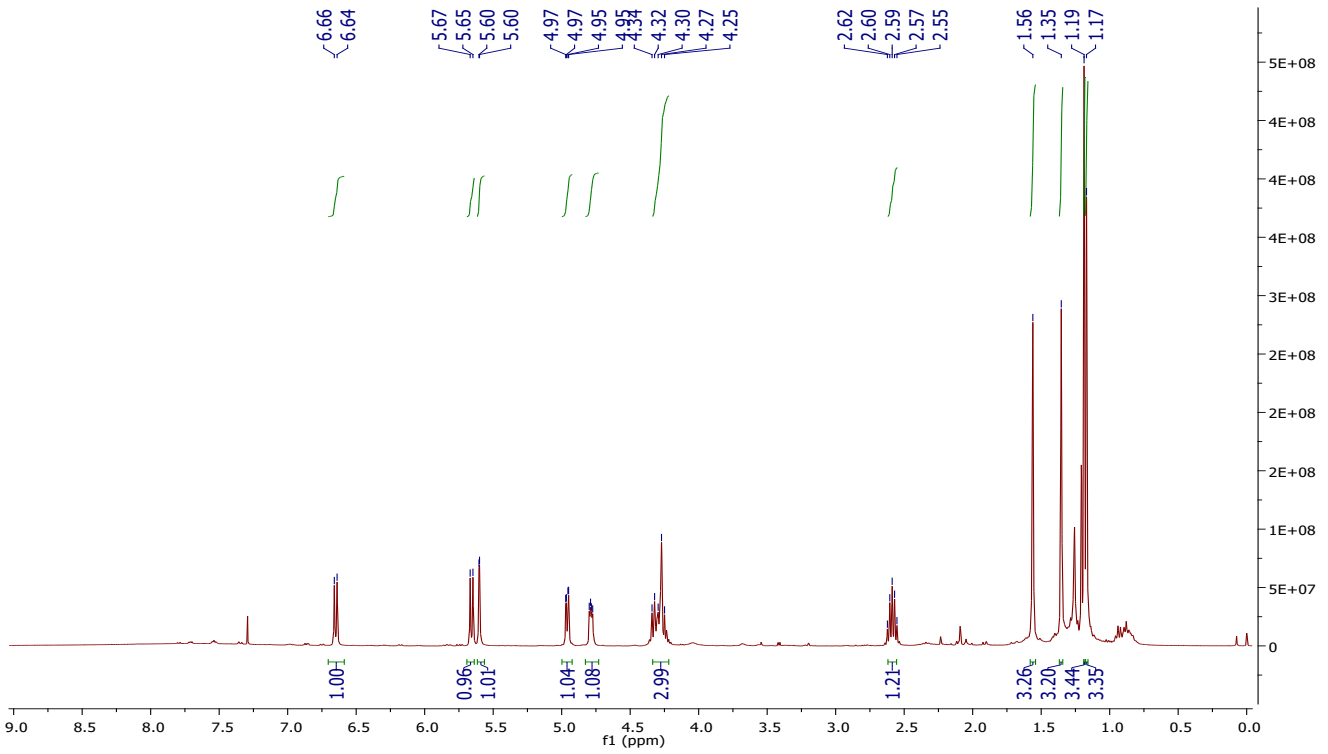
[13]C NMR (101 MHz, MeOD) of compound 7



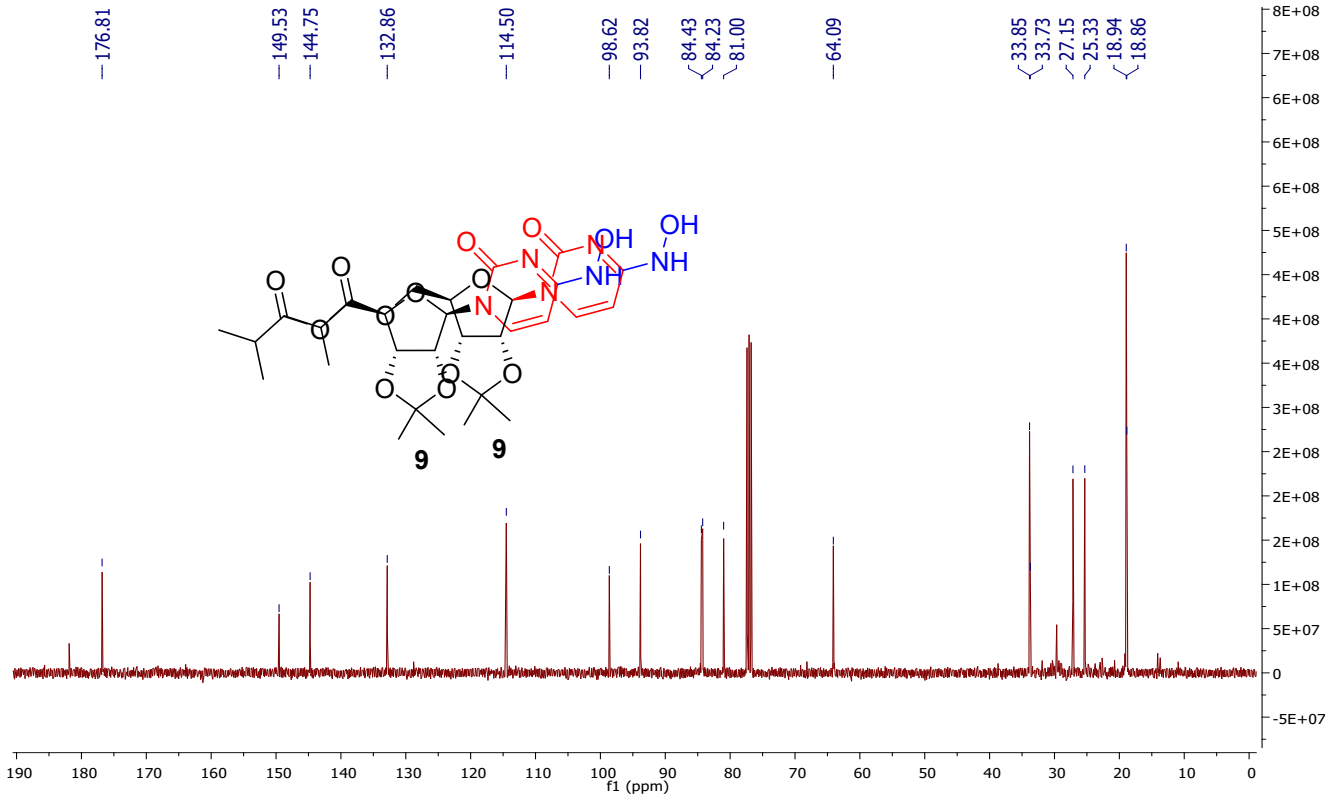
^{1}H NMR (400 MHz, $CDCl_3$) of compound 8



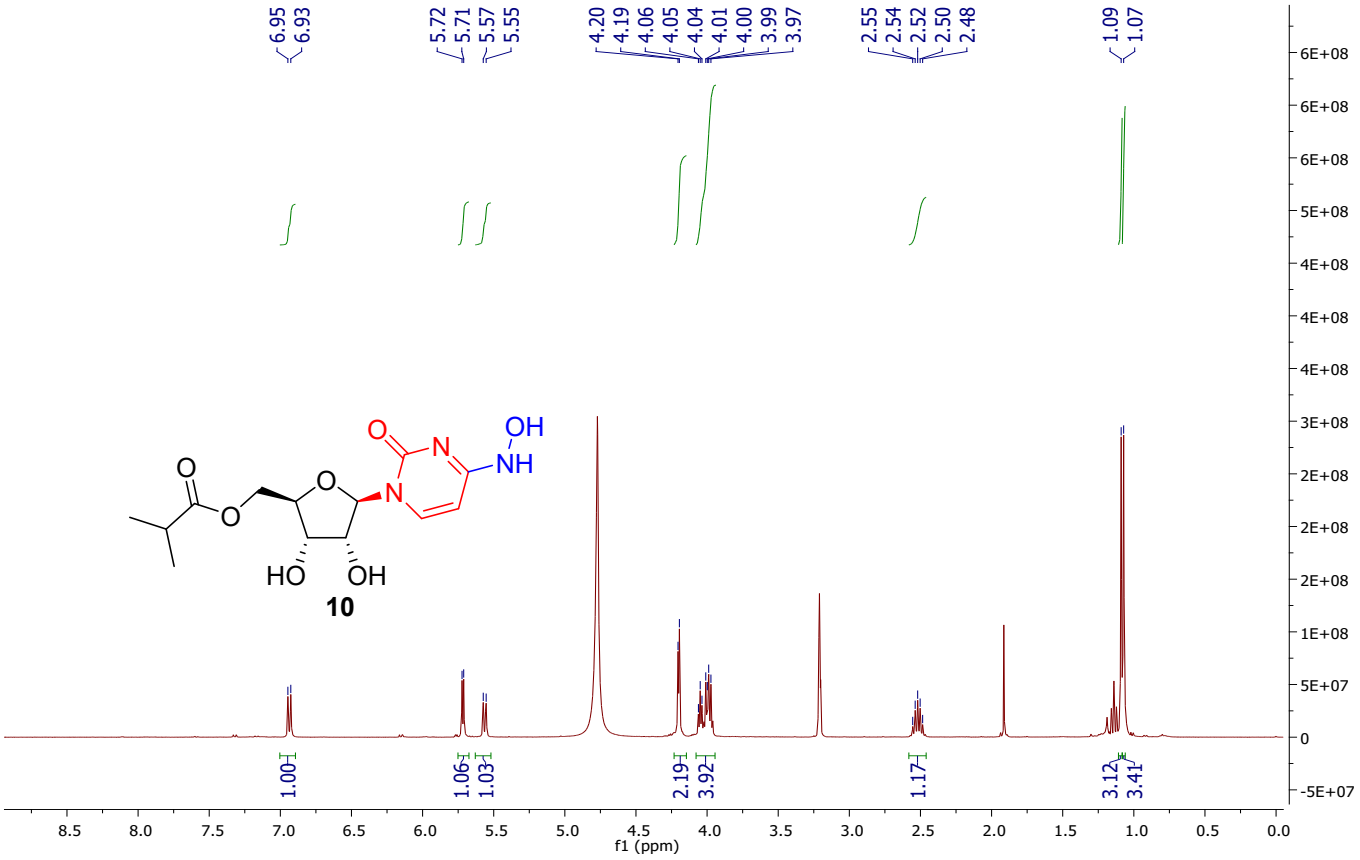
^{13}C NMR (101 MHz, $CDCl_3$) of compound 8



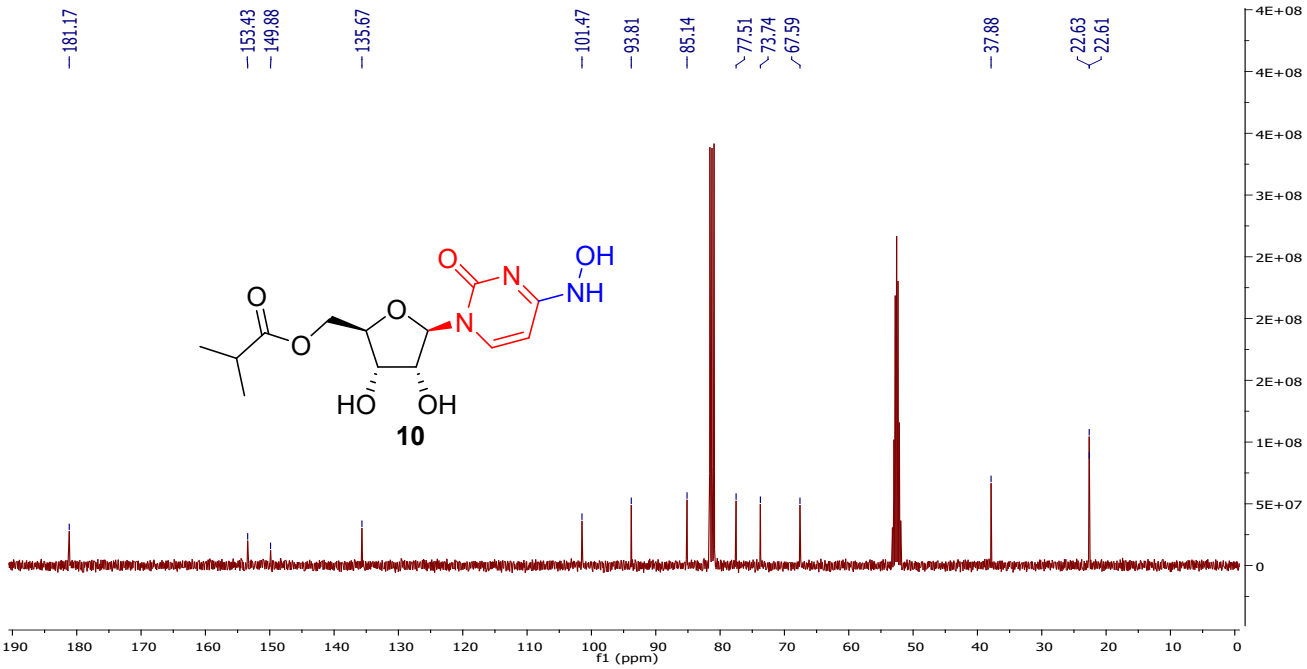
^{1}H NMR (400 MHz, $CDCl_3$) of compound 9



^{13}C NMR (101 MHz, $CDCl_3$) of compound 9



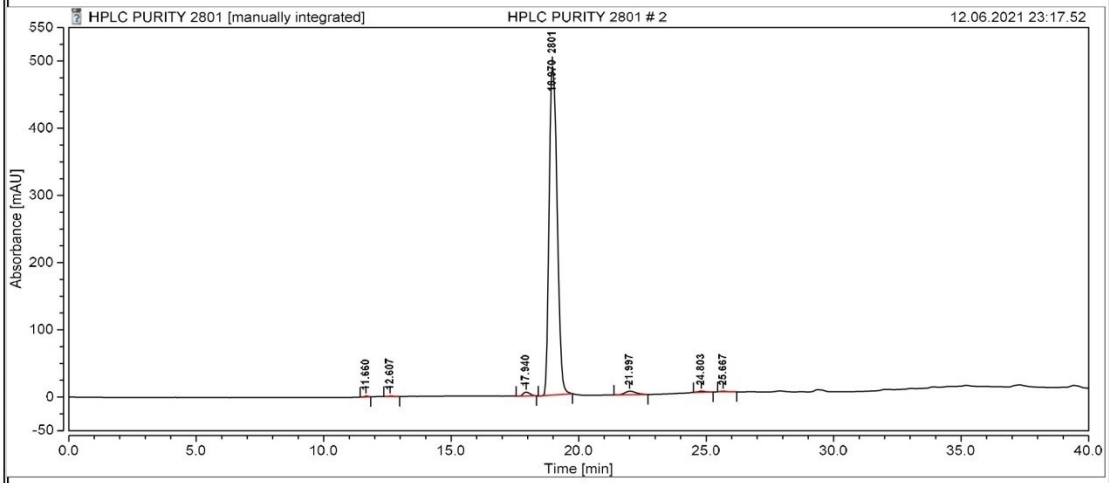
[1]H NMR (400 MHz, MeOD) of **10**



[13]C NMR (101 MHz, MeOD) of compound 10

CSIR-IIIM, DRUG TESTING LABORATORY, JAMMU

Sequence Path :-	Instrument Data\CSIR-IIIM-DTL-03\SEQUENCE\HPLC PURITY 2801.seq		
Sequence Name :-	HPLC PURITY 2801		
Instrument Method :-	HPLC PURITY 2801	**Batch No. :-**	n.a.
Proc. Method :-	HPLC PURITY 2801	**AR No. :-**	n.a.
Injection Name :-	HPLC PURITY 2801	**Test :-**	n.a.
Injection Type :-	Unknown	**Column No. :-**	n.a.
Injection No. :-	2	**Condition :-**	n.a.
Vial Number :-	GE2	**Seq Last Update By :-**	AP_Gupta
Date Acquired :-	12.06.2021 23:17:52	**Proc.Meth.Last Update By :-**	AP_Gupta
Proc.Meth.LastUpdate :-	13.06.2021 11:47:03	**Chromatogram Printed By :-**	AP_Gupta
Instrument ID :-	CSIR-IIIM-DTL-03	**Wavelength :-**	220 nm
Run Time :-	40.0 mins	**Injection Volume :-**	2.0 µL



Peak No.	Ret.Time min	Peak Name	Area mAU*sec	Rel.Area %	Asymmetry	Resolution (USP)	Peak Type
1	11.660		12.683	0.12	1.2	n.a.	BMB*
2	12.607		7.795	0.07	0.9	3.7	BMB*
3	17.940		102.655	0.93	1.3	13.9	BMB*
4	18.970	2801	10683.649	97.06	1.3	2.0	BMB*
5	21.997		155.215	1.41	1.3	4.6	BMB*
6	24.803		28.401	0.26	1.2	4.6	BMB*
7	25.667		17.091	0.16	1.6	1.9	BMB*
Total			**11007.489**	**100.00**			

Analysed By :-

Checked By :-