Supporting Information

Towards a Practical, Non-enzymatic Process for Investigational COVID-19 Antiviral Molnupiravir from Cytidine: Supply Centered Synthesis

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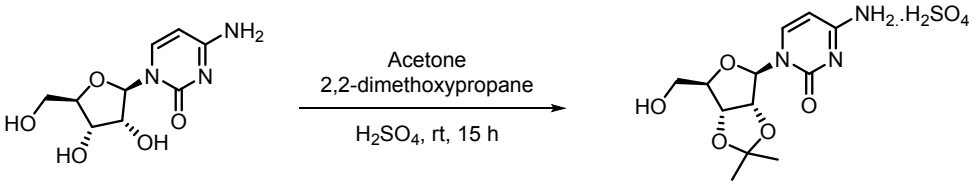
General Remarks:

Instrumentation: For all compounds, ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer. Chemical shifts were measured relative to the residual solvent resonance for ^{1}H and ^{13}C NMR ($CDCl_3$= 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C, DMSO-d_6= 2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C, CD_3OD= 3.31ppm for ^{1}H and 49.0 ppm for ^{13}C, D_2O= 4.79 ppm for ^{1}H). Coupling constants J are reported in hertz (Hz). The following abbreviations were used to designate signal multiplicity: s, singlet; d, doublet; t, triplet; q, quartet, p, pentet; dd, doublet of doublet; ddd, doublet of doublet of doublet; dt, double of triplet; ddt, doublet of doublet of triplet; m, multiplet; br, broad. Reactions were monitored by HPLC using the methods indicated. Glassware was oven-dried at 120 °C, assembled while hot, and cooled to ambient temperature under an inert atmosphere. Unless noted otherwise, reactions involving air sensitive reagents and/or requiring anhydrous conditions were performed under a nitrogen atmosphere.

Reagents and solvents: Cytidine **7** was purchased from Chem Impex. All other reagents and solvents were purchased from Aldrich Chemical Company, Fisher Scientific, Alfa Aesar, Acros Organics, Oakwood, or TCI. Liquid reagents were purified by distillation when necessary. Unless otherwise noted, solid reagents were used without further purification. Acronyms: DBU (1,8-Diazabicylo[5.4.0]undec-7-ene), MTBE (Methyl *tert*-butyl ether), CPME (Cyclopentyl methyl ether), DMAP (N,N-Dimethylamino pyridine), DCM (Methylene chloride), IPA (Isopropanol).

Representative Procedures:

4-Amino-1-((3a*R*,4*R*,6*R*,6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)pyrimidin-2(1*H*)-one (1) sulfuric acid salt.

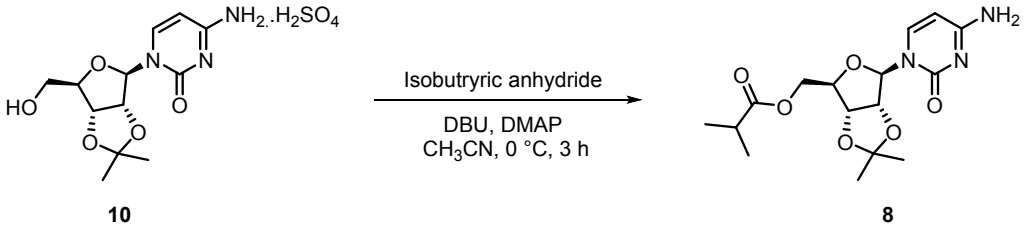


Cytidine 7 **10**

To the mechanically stirred 2000 mL three neck round bottom flask, cytidine **1** (100 g, 0.41 mol, 1 equiv) and anhydrous acetone (1300 mL) was added, followed by 2,2-dimethoxypropane (251.9 mL, 2.055 mol, 5 equiv) under nitrogen atmosphere. Neat sulfuric acid (50.7 mL, 0.94 mol, 2.3 equiv) was added to the above suspension and left stirring for 15 h. The insoluble residue was filtered and the solid precipitate was washed multiple times with acetone (1000 mL) followed by MTBE (400 mL). The solid was left drying under vacuum for a day to obtain 155 g (94% corrected yield, 95 wt% Purity by NMR in DMSO-d_6) of compound **10** as an off-white solid.

Data matched with those previously reported.[1]

((3a*R*,4*R*,6*R*,6a*R*)-6-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl isobutyrate (8).



10 **8**

To the mechanically stirred 2000 mL three neck round bottom flask, compound **10** (150 g, 95 wt% Purity, 0.37 mol, 1 equiv) and dry acetonitrile (1500 mL) followed by DMAP (9.13 g, 0.075

mol, 0.2 equiv) and DBU (117.4 mL, 0.78 mol, 2.1 equiv) was added at room temperature. Reaction mixture was stirred for 10 min and added Isobutyric anhydride (65.11 mL, 0.39 mol, 1.05 equiv) at 0 °C dropwise in two equal portions at half hour intervals each and the reaction mixture was maintained for 2 h at the same temp. The reaction was then directly concentrated under reduced pressure to afford a waxy solid. The resultant material was re-dissolved in dichloromethane (600 mL) and washed with 10 % acetic acid (1000 mL) once. To the organic layer in a 2000 mL three neck round bottom flask was then added clear solution of saturated sodium bicarbonate (1000 mL) dropwise with stirring until effervescence ceased. The layers were then separated and the dichloromethane layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain 136.5 g (91.9% corrected yield, 89.3 wt% Purity by NMR in DMSO-d_6) of compound **8** as a white foamy solid.

^{1}H NMR (600 MHz, CD_3OD) δ 7.90 (s, 1H), 7.63 (d, J = 7.4 Hz, 1H), 5.86 (d, J = 7.4 Hz, 1H), 5.71 (d, J = 6.9 Hz, 1H), 5.03 (dd, J = 4.7, 1.6 Hz, 1H), 4.84 (dd, J = 6.3, 3.4 Hz, 1H), 4.31 (m,1H), 2.55 (septet, J = 7 Hz, 1H), 1.53 (s, 3H), 1.34 (s, 3H), 1.13 (ddd, *J* = 3.2, 1.8, 1.3 Hz, 6H) ppm.

^{13}C NMR (151 MHz, CD_3OD) δ 178.49, 168.31, 158.14, 145.15, 115.34, 97.21, 96.25, 87.04, 86.71, 83.36, 65.86, 35.35, 27.74, 25.75, 19.75, 19.60, 19.52 ppm.

Data matched with those previously reported.[1]

Reaction profile of the esterification step over time was done and the result is as shown below in Fig S1.

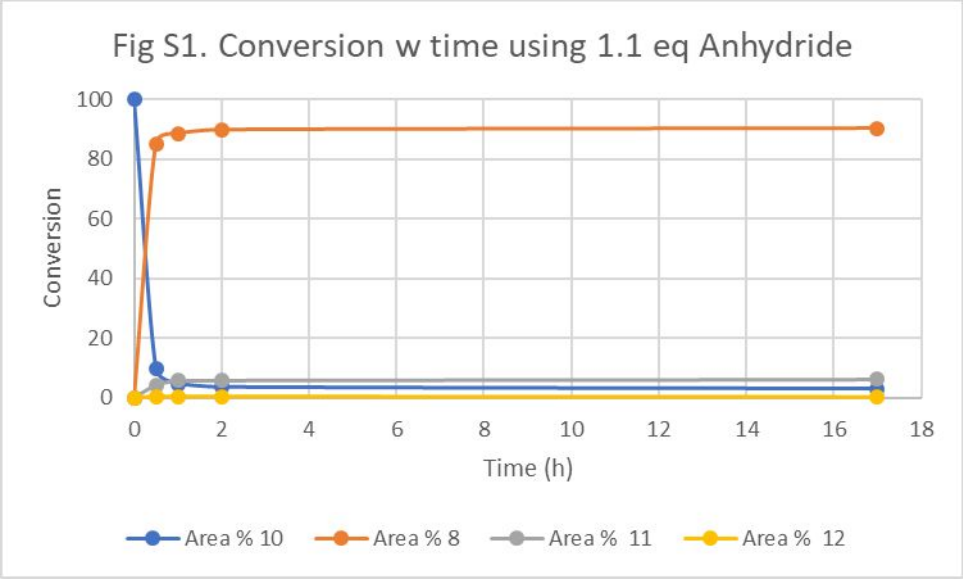
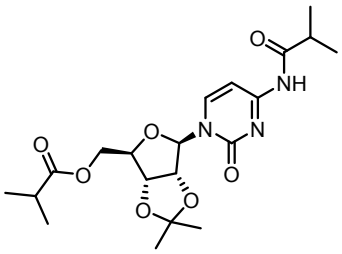


Figure S1: Reaction profile of the esterification step (step 2) over time.

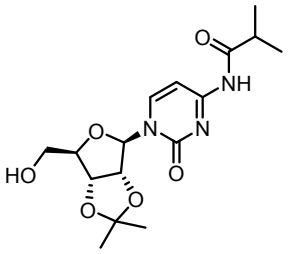
Analytical data for diacylated side product **11** obtained by preparative HPLC.



11

^{1}H NMR (CDCl$_3$, 600 MHz) δ 7.76 (d, 1H, J = 7.4 Hz), 7.41 (d, 1H, J = 7.4 Hz), 5.77 (s, 1H), 5.01 (d, 1H, J = 6.4 Hz), 4.82 (dd, 1H, J = 4.1, 1.7 Hz), 4.47-4.44 (m, 1H), 4.37 (dd, 1H, J = 8.6, 3.5 Hz), 4.32 (dd, 1H, J = 6.1, 5.9 Hz), 2.64-2.56 (m, 1H), 2.51 (septet, J = 6.9 Hz, 1H), 1.58 (s,3H), 1.35 (s, 3H), 1.23 (d, 6H, J = 6.8 Hz), 1.14 (s, 6H, J = 7.1 Hz) **^{13}C NMR (151 MHz, CDCl$_3$)** δ 176.48, 145.97, 114.25, 96.32, 86.21, 85.28, 81.14, 64.07, 33.82, 27.09, 25.24, 19.01, 18.96, 18.95, 18.86 **HRMS** (m/z, M+Na) calc 446.1903 m/z obtained 446.1880

Analytical data for *N*-Acylated side product **12** below.



12

^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.89 (s, 1H), 8.22 (d, 1H, J = 7.5 Hz), 7.22 (d, 1H, J = 7.4 Hz), 5.82 (s, 1H), 4.86 (dd, 1H, J = 4.4, 1.4 Hz), 4.74 (dd, 1H, J = 3.2, 2.9 Hz), 4.19 (q, 1H, J = 4.1 Hz), 3.64 (dd, 1H, J = 3.2, 7.6 Hz), 3.55 (dd, 1H, J = 4.9, 6.9 Hz), 2.71 (septet, 1H, J = 6.8 Hz), 1.48 (s, 3H), 1.29 (s, 3H), 1.06 (d, 6H, J = 6.8 Hz) **^{13}C NMR (151 MHz, DMSO-d_6)** δ 177.87, 162.95, 154.43, 146.53, 112.51, 95.22, 93.52, 87.73, 84.81, 80.58, 61.25, 34.92, 26.98, 25.11, 19.01, 18.93 **HRMS** (m/z, M+Na) calc 376.1485 m/z obtained M+Na 376.1467

Typical HPLC profile for esterification under the above conditions is as shown below under optimized workup conditions using 10 % acetic acid (Fig 3) at 210 nm to visualize the disappearance of DBU.

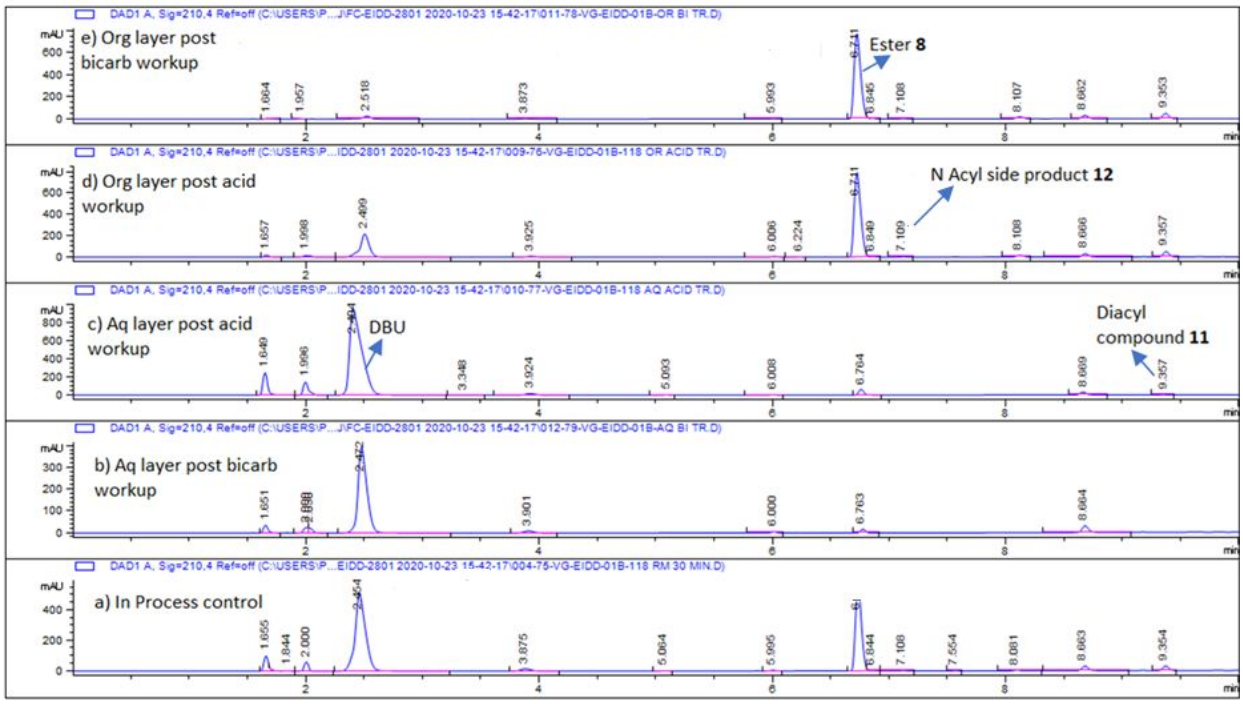
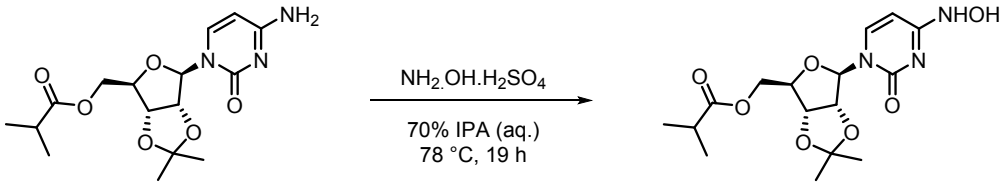


Figure S2. HPLC profile of the esterification reaction at different stages at 210 nm

((3a*R*,4*R*,6*R*,6a*R*)-6-(4-(Hydroxyamino)-2-oxopyrimidin-1(2*H*)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl isobutyrate (6).



8 **6**

To the mechanically stirred 2000 mL three neck round bottom flask, compound **8** (130 g, 89.3 wt% Purity, 0.33 mol, 1 equiv) and hydroxylamine sulfate (172.7 g, 1.05 mol, 3.2 equiv) followed by 70 % IPA (1300 mL) was added and the resultant solution was heated to an internal temperature 72-73 ˚C for 19 h upon which time HPLC (For HPLC method, refer Page 20) showed the formation of product in addition to starting material and molnupiravir. At this juncture, the two layers were separated. The top layer (IPA) was concentrated to afford a thick residue. To this, acetonitrile (200 mL) was added as co-solvent and concentrated to dryness. To the crude product, Isopropyl acetate (375 mL) was added and heated to 80-85°C for 30 min while stirring. It was then cooled to room temperature under slow stirring for 12 h and the solid which formed was filtered and washed with isopropyl acetate (150 mL) and sucked dry under vacuum pressure to obtain 84.8 g (70.1% corrected yield, 97 % purity by HPLC, 94 wt% Purity by NMR in acetone-d_6) of compound **6** as a white powdery solid.

^{1}H NMR (600 MHz, CD_3OD): δ 6.85 (d, J = 8.2 Hz, 1H), 5.69 (d, J = 2.2 Hz, 1H), 5.57 (d, J = 8.2 Hz, 1H), 4.97-4.99 (dd, J = 6.4, 2.2 Hz, 1H), 4.79-4.81 (dd, J = 6.3, 4.8 Hz, 1H), 4.26 (d, J = 5.3 Hz, 2H), 4.21 (q, J = 4.9 Hz, 1H), 2.60 (septet, J = 7 Hz, 1H), 1.53 (s, 3H), 1.34 (s, 3H), 1.15- 1.17 (dd, J = 7, 1.8 Hz, 6H) ppm.

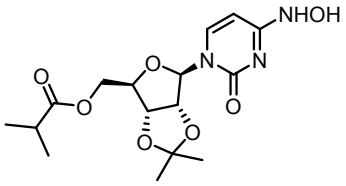
^{13}C NMR (151 MHz, CD_3OD): δ 178.61, 151.42, 146.49, 134.21, 115.73, 99.73, 94.53, 85.62, 85.58, 82.87, 65.54, 35.36, 30.97, 27.79, 25.82, 19.61, 19.58 ppm.

Data matched with those previously reported.[1]

Solubility study of hydroxaminated product 6

Solubility check of the hydroxaminated product **6** in different solvents is shown below (Figure S4).

At the beginning, different solvents were screened for recrystallization of compound **6** (above table). However, out of all solvents screened above, acetonitrile and isopropyl acetate looks promising. The nice crystalline product was obtained from isopropyl acetate with good recovery. On the other hand, acetonitrile provided low recovery of the crystallized product.



6

Entry	Solvent	Solubility at		
		rt	80 °C	Return to rt
1	Hexane			
2	TBME			
3	MeOH			
4	H_2O			
5	Toluene			
6	CH_3CN			
7	Ethyl acetate			
8	DCM			
9	THF			
10	Acetone			
11	Isopropyl acetate			
12	Chlorobenzene			
13	CPME			
14	Anisole			
15	Toluene/chlorobenzene (1:1)			
	PPT			
	Solution			

	Recrystallized			

Figure S3. Solvent screening for recrystallization of hydroxaminated product **6**.

HPLC traces of compound 6 after/before recrystallization from Isopropyl acetate

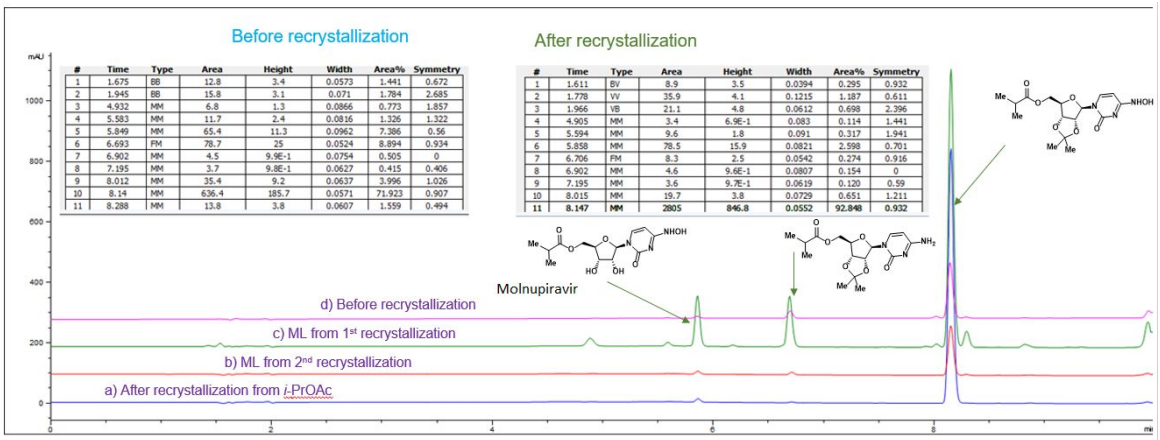
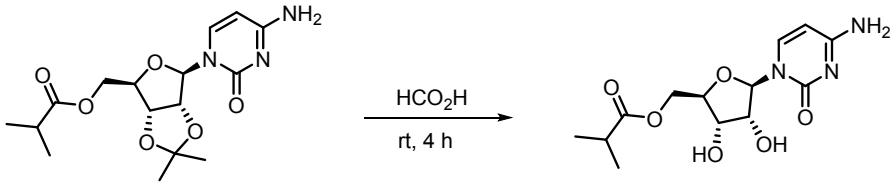


Figure S4. Recrystallization from Isopropyl acetate HPLC trace (before and after) at 260 nm **((2R,3S,4R,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl isobutyrate (13).**

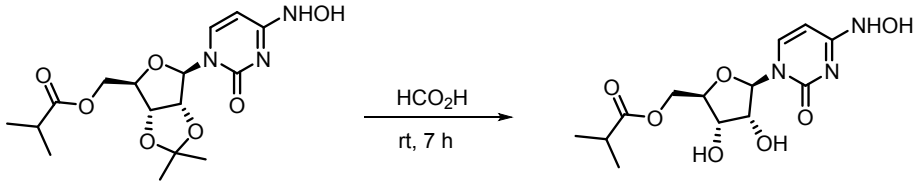


8 **13**

The acetonide ester **8** (0.503 g, 83 wt% purity) was taken in a 20 mL vial and formic acid (8 mL) was then added. The reaction mixture was stirred at room temperature for 4 h. The solvent was then removed on a rotary evaporator and under high vacuum. Ethanol (5 mL) was then added. The resultant solution was concentrated. MTBE (5 mL) was then added to the residue upon which addition, solid formation observed was observed on the sides of the vial. Concentration was done again followed by addition of isopropanol (5 mL) again to the residue. The resultant solution was concentrated to give the crude product (0.47 g, 52 wt% Purity, 70% Assay yield). Purification was done by flash chromatography on silica gel (Biotage Isolera) and the product eluted in 9% MeOH/dichloromethane to yield 0.24 g of **13** (98 wt% Purity in DMSO-d_6, 68 % isolated yield corrected for purity) as a white crystalline solid.

The analytical data (^{1}H NMR and ^{13}C NMR of the pure material shown below) matched the reported values for the two-step synthesis recently communicated.[2]

((2*R*,3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-(4-(hydroxyamino)-2-oxopyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl isobutyrate (Molnupiravir).



6 **Molnupiravir**

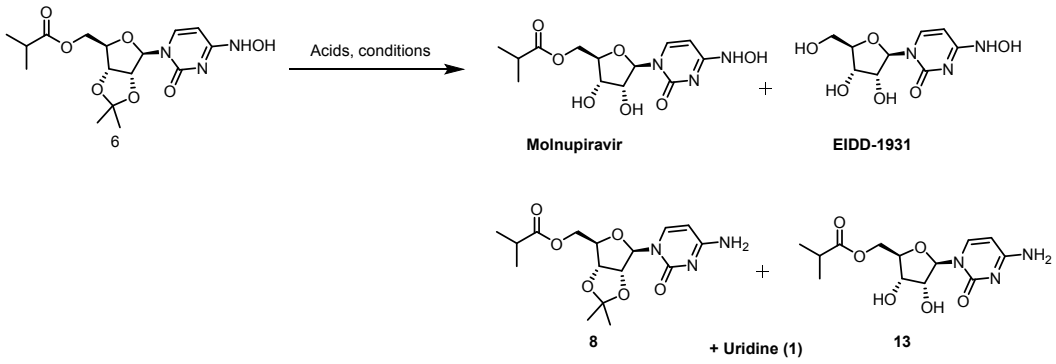
To the mechanically stirred 2000 mL three neck round bottom flask, compound **6** (80 g, 94 wt% purity, 0.20 mol) followed by formic acid (1300 mL) was added. The resultant solution was stirred at room temperature for 7 h. Solvent was removed under reduced pressure and fresh EtOH (500 mL) was added. The resultant solution was again concentrated under vacuum to afford a waxy solid as a crude product. *Recrystallization from water*: To the crude (70 g), water (140 mL) was charged and heated to 60-65 °C for 10 min. The reaction mixture was cooled to 25-30 °C and slowly stirred for 12 h. solid was filtered and wet solid washed with MTBE (210 mL) and dried under vacuum to get 40.0 g (58.7% corrected yield, 98.5 wt% purity by NMR in methanol-d_4, 98.3 % Purity by HPLC at 260 nm) pure molnupiravir as an off-white solid.

^{1}H NMR (600 MHz, CD_3OD): δ 6.91 (d, J = 8.2 Hz, 1H), 5.82 (d, J = 4.8 Hz, 1H), 5.61 (d, J = 8.2 Hz, 1H), 4.29 (d, J = 3.6 Hz, 2H), 4.14 (t, J = 4.9 Hz, 1H), 4.08 (p, J = 4.9 Hz, 2H), 2.62 (septet, J = 7.0 Hz, 1H), 1.19 (d, J = 7.0 Hz, 6H) ppm.

^{13}C NMR (151 MHz, CD_3OD): δ 178.6, 151.81, 146.44, 132.04, 99.84, 90.74, 82.88, 74.67, 71.80, 65.23, 35.45, 27.49, 19.65, 19.61 ppm.

Data matched with those previously reported.[1]

Acid screening for the deprotection of acetonide to molnupiravir



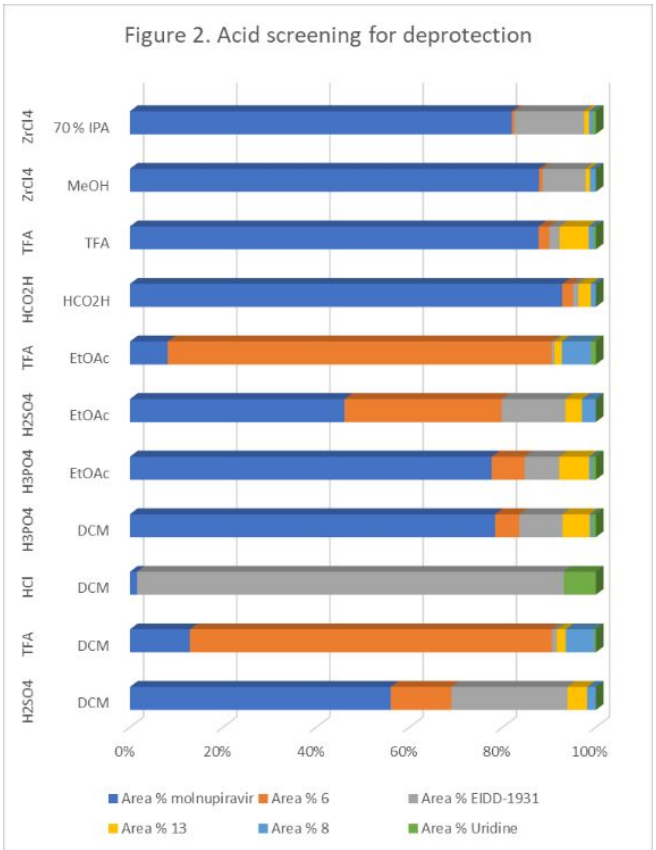


Figure S5. Acid screening for deprotection to molnupiravir

HPLC trace of recrystallized Molnupiravir from water

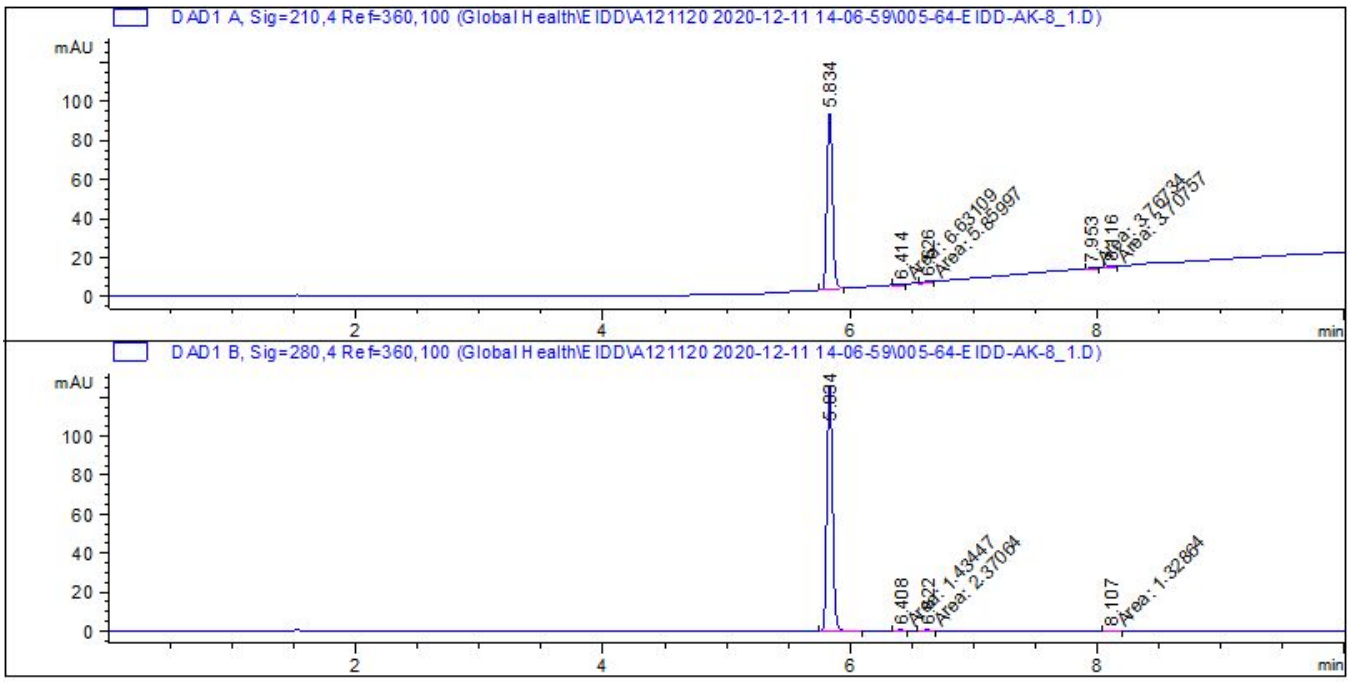


Figure S6. HPLC trace of recrystallized Molnupiravir from water

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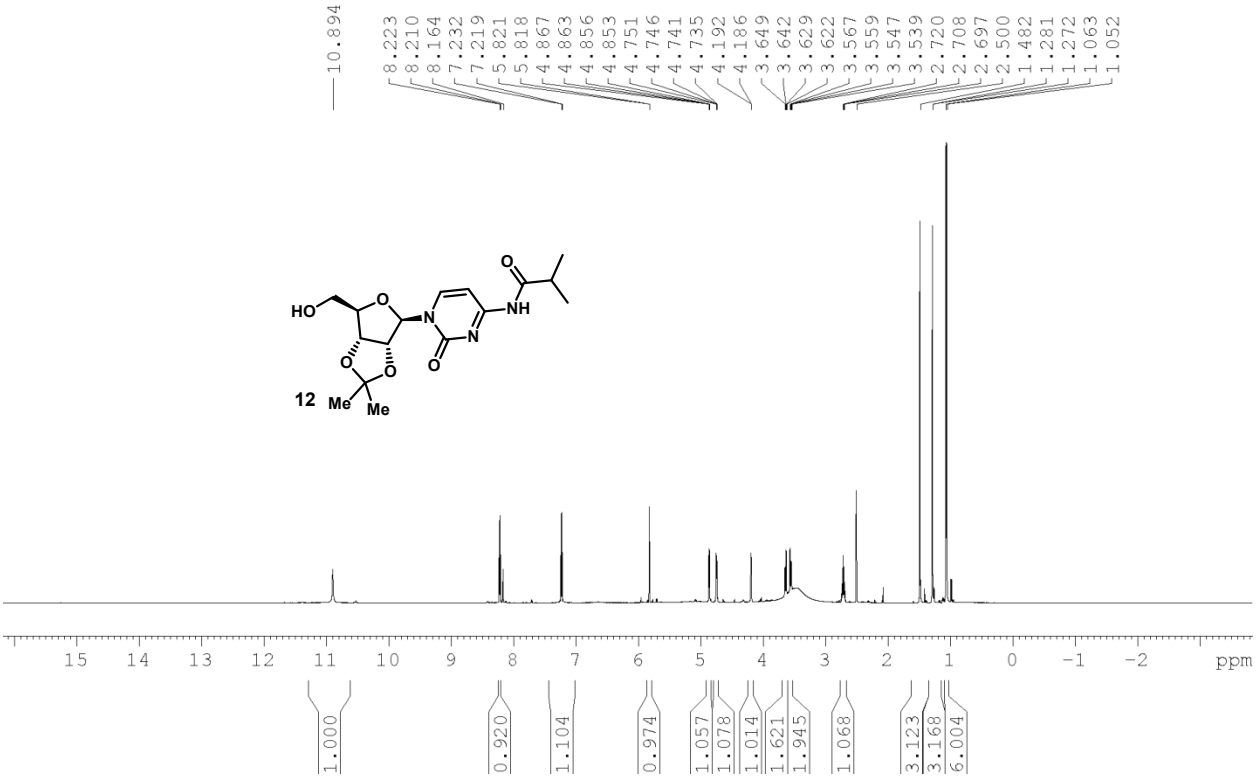
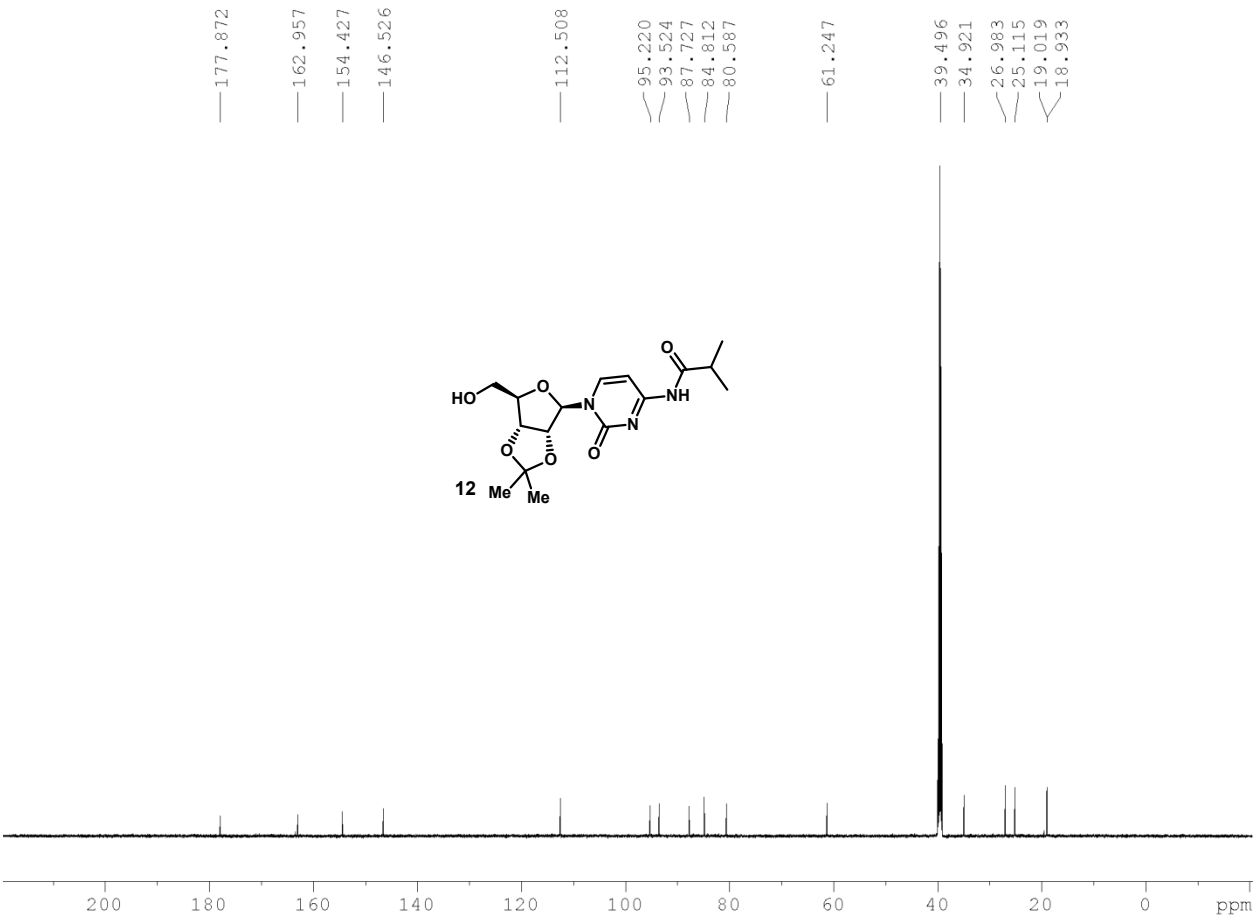
1. Gopalsamuthiram, V.; Williams, C.; Noble, J.; Jamison, T. F.; Gupton, B. F.; Snead, D. R. A Concise Route to MK-4482 (EIDD-2801) from Cytidine: Part 2. *Synlett* **2021**, *32*, 326–328.

2. a) Vasudevan, N.; Ahlqvist, G.P.; McGeough, C.P.; Paymode, D.J.; Cardoso, F.S.P.; Lucas, T.; Dietz, J-P.; Opatz, T.; Jamison, T.F.; Gupton, F.B.; Snead, D.R. *Chem.Comm*.**2020**, *56*, 13363-13364 b) Ahlqvist, G.P.; McGeough, C.P.; Senanayake, C.; Armstrong, J.D.; Yadaw, A.; Roy, S.; Ahmed, S.; Snead, D.R.; Jamison, T.F. *ACS Omega* **2021**, *6*, 10396–10402.

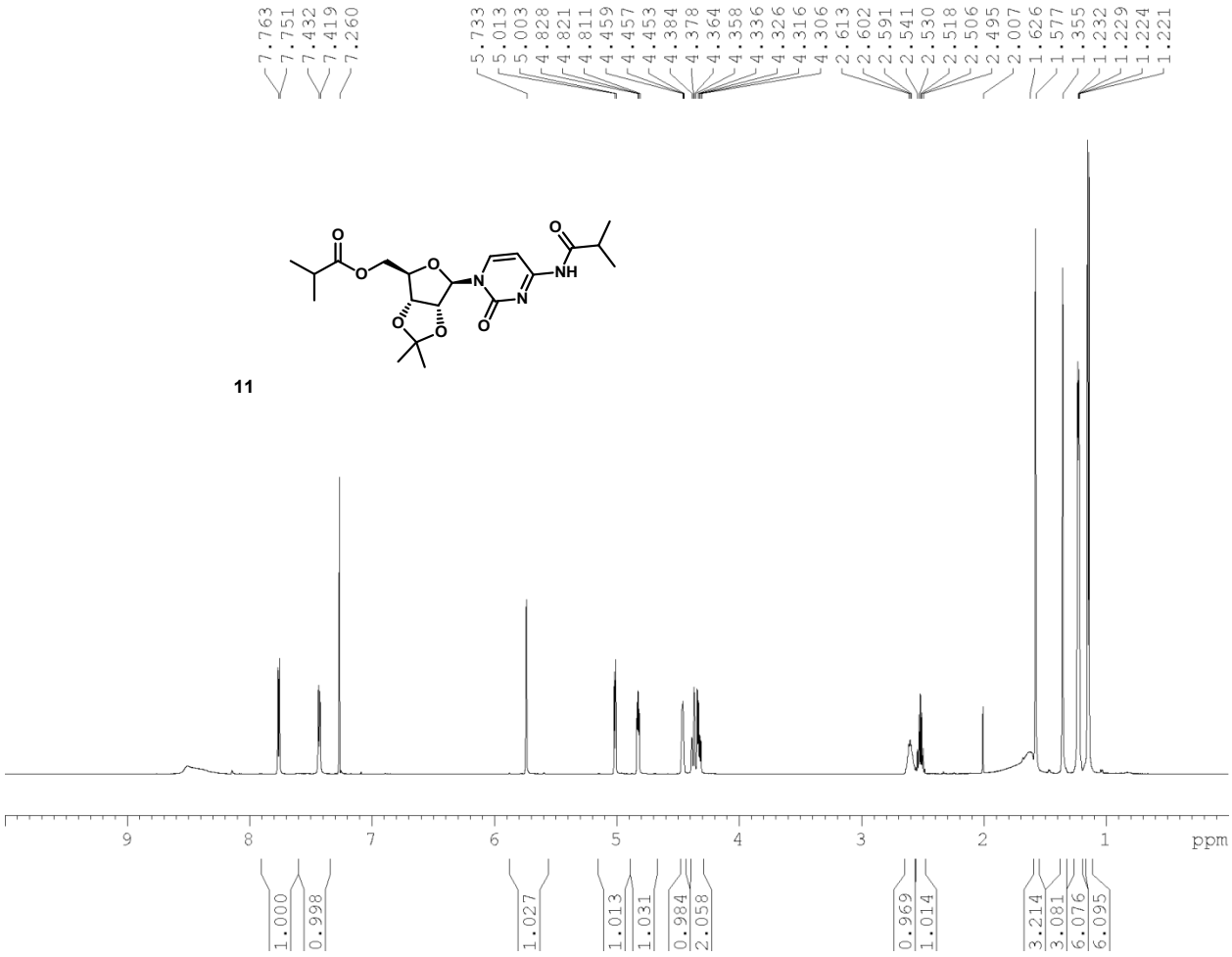
Copies of ^{1}H and ^{13}C NMR data of all new compounds



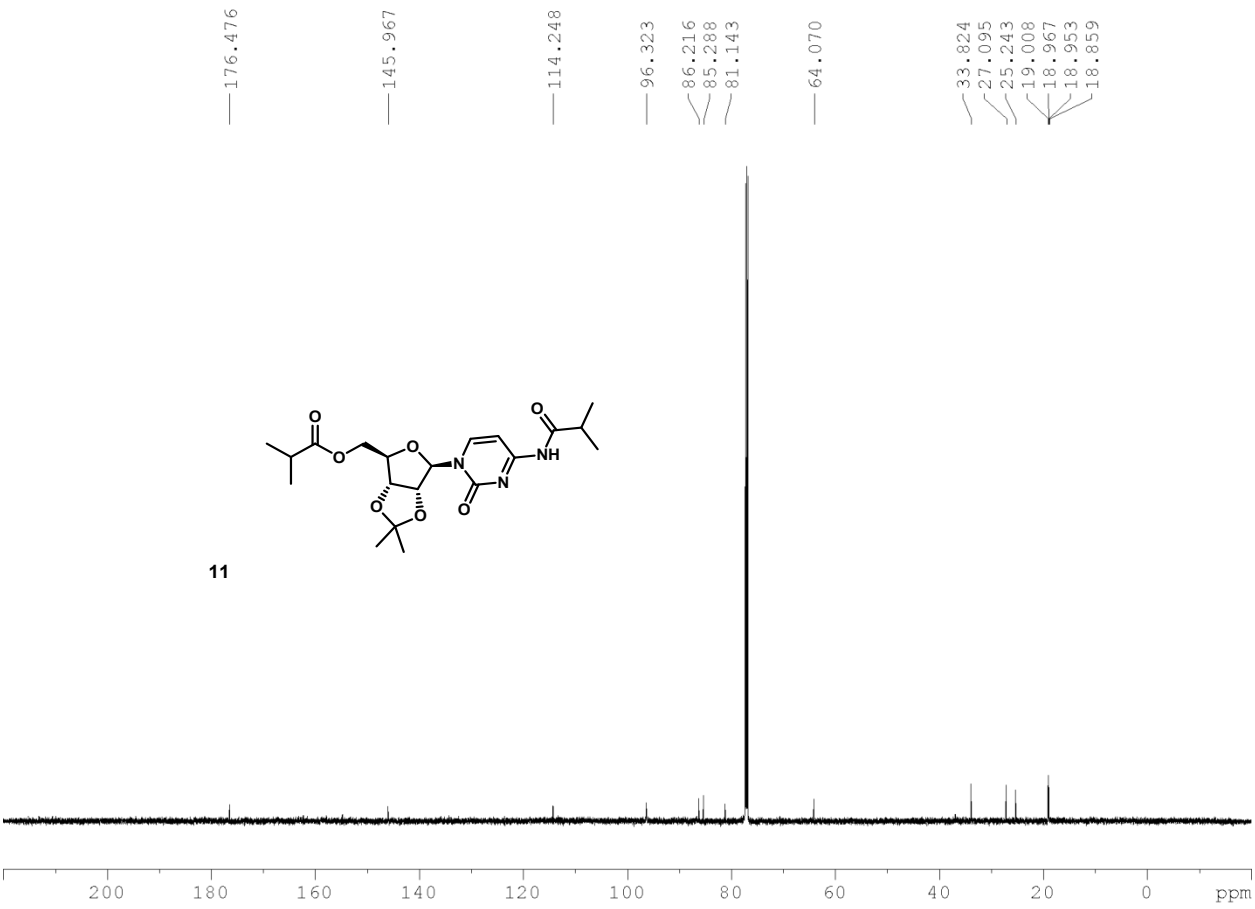
^{1}H NMR Spectrum of **12**



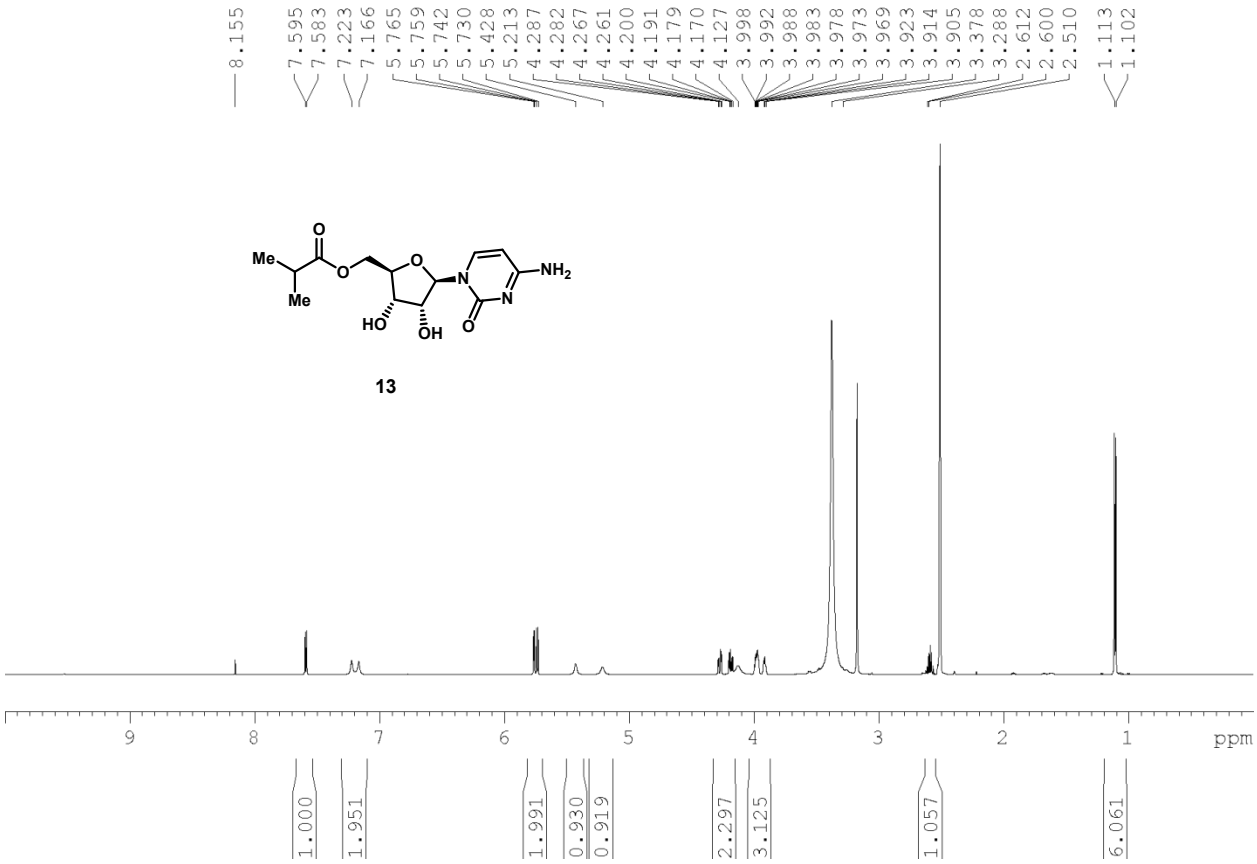
^{13}C NMR Spectrum of **12**



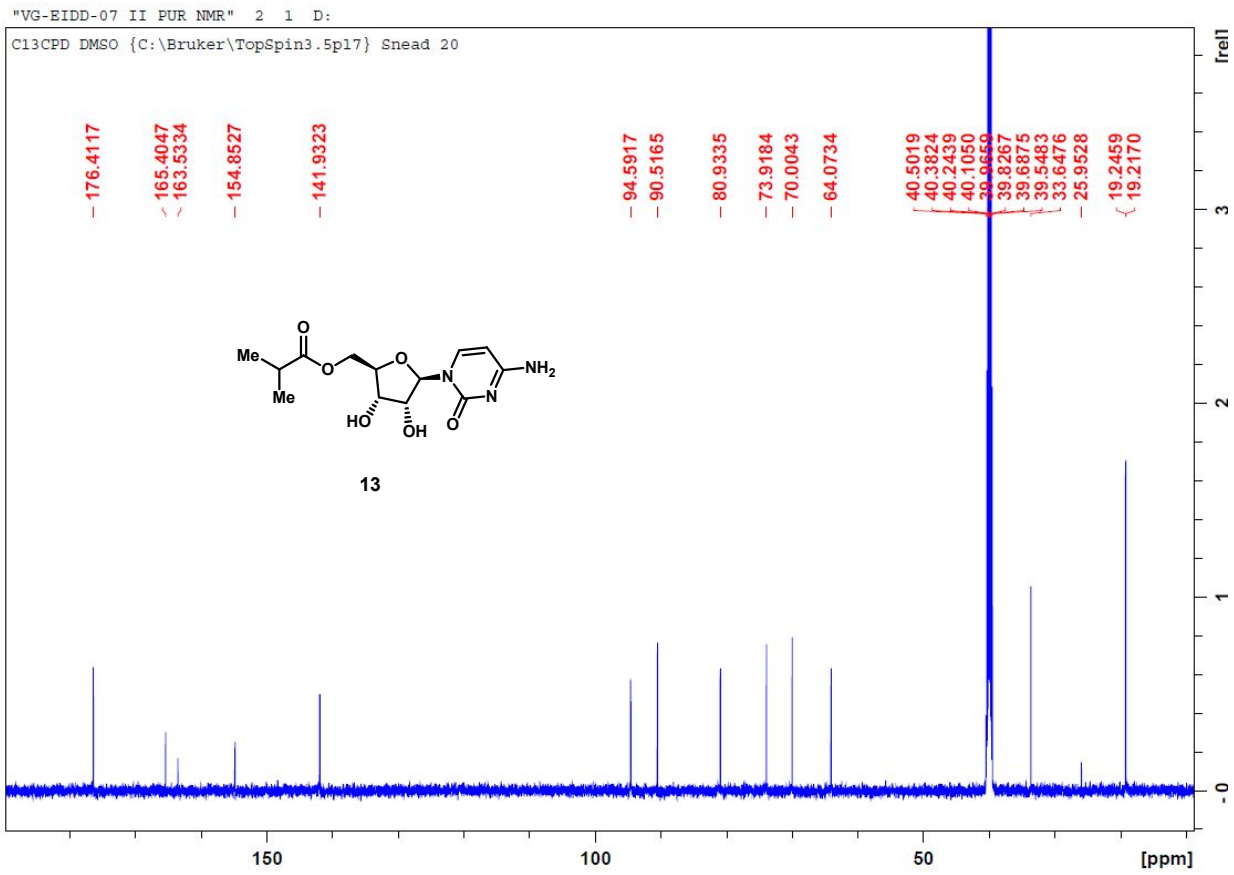
^{1}H NMR Spectrum of **11** in $CDCl_3$



^{13}C NMR Spectrum of **11** in $CDCl_3$



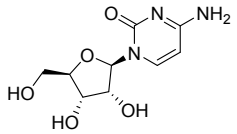
^{1}H NMR spectrum of **13** in DMSO-d_6



^{13}C NMR spectrum of **13** in DMSO-d_6

HPLC Method

Structures & IDs :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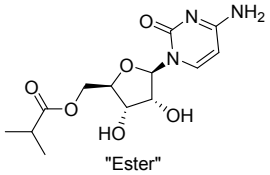
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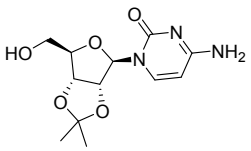
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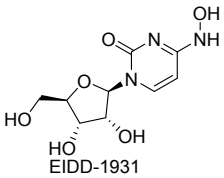
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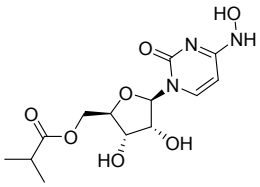
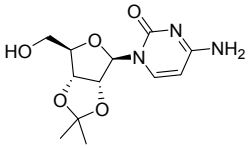
N-hydroxycytidine


Molnupiravir


Acetonide Ester

Conditions

Mobile Phase A: 0.1% Phosphoric acid in Water
(1 mL HPLC grade H_3PO_4 in 1000 mL HPLC grade water)
Mobile Phase B: Acetonitrile
Flow rate: 1.5 mL/min
Injection volume: 1 µL
Column: Agilent Zorbax Eclipse C18 (4.6 x 250 mm; 5 µL)
Column temp: 30 °C
Detector wavelength: 260 nm (primary)
210 nm (for information only)
Sample preparation: Pipet 20 µL of reaction solution and add 1 mL water to dilute

Gradient Table

Time (min)	% A	% B
0.0	90%	10%
2.0	90%	10%
8.0	70%	70%
10.0	70%	70%
Post Time: 4 min		

Retention Times

***Approximate values. Run standards before assigning peaks based on RT*

Compound	**Time (min)**
Cytidine	1.73
Acetonide	3.91
Acetanide ester	6.77
EIDD-1931 (NHC)	1.52
Ester	4.97
Molnupiravir	5.83