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Article

Toward a Practical, Nonenzymatic Process for Investigational COVID-19 Antiviral Molnupiravir from Cytidine: Supply-Centered Synthesis

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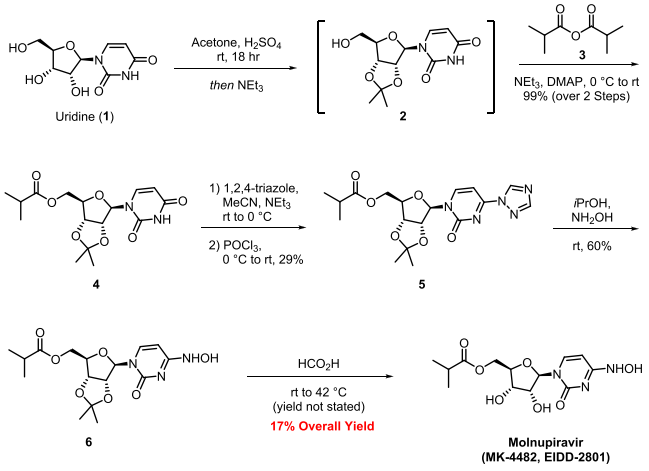
ABSTRACT: A scalable four-step synthesis of molnupiravir from cytidine is described herein. The attractiveness of this approach is its fully chemical nature involving inexpensive reagents and more environmentally friendly solvents such as water, isopropanol, acetonitrile, and acetone. Isolation and purification procedures are improved in comparison to our earlier study as all intermediates can be isolated via recrystallization. The key steps in the synthesis, namely, ester formation, hydroxyamination, and deprotection were carried out on a multigram scale to afford molnupiravir in 36−41% yield with an average purity of 98 wt % by qNMR and 99 area% by HPLC.

KEYWORDS: *COVID-19, antivirals, molnupiravir, EIDD-2801, MK-4482*

INTRODUCTION

Molnupiravir (also known as EIDD-2801 and MK-4482) is a promising drug candidate for treating COVID-19. Merck licensed the compound from Ridgeback Biotherapeutics in 2020, and clinical results with outpatients advanced molnupiravir to Phase 3 clinical trials.[1] Molnupiravir offers complementary advantages over remdesivir such as oral bioavailability and structural simplicity, thus reducing the manufacturing complexity.[2,3] The original synthesis of molnupiravir developed by Painter utilizes uridine (**1**) and proceeds in five steps with a low overall yield (17% overall; two steps have assumed yields) with the aid of an acetonide-protecting group strategy (Scheme 1).[4]

Scheme 1. Discovery Route to Molnupiravir



As the above synthesis had a high step count accompanied by a low yield, an alternative synthesis of molnupiravir was necessary. In our original synthetic route, we found that Novozyme 435, a common lipase enzyme, could selectively esterify the primary hydroxy groups of cytosine derivatives without the need for acetonide protection, thus reducing the overall step count to two (Route I, Scheme 2).[5a,5] The order of this two-step sequence can be varied either by carrying out the hydroxyamination of cytidine (**7**) first followed by regioselective acylation or vice versa. Also, Kappe and co-workers in the year 2020 reported a high-yield synthesis of molnupiravir from uridine.[6] In a recent preprint disclosure, Merck demonstrated a similar enzymatic strategy for molnupiravir using ribose **9** and uracil proceeding in 69% yield over three steps.[7] Although the enzymatic routes are indeed attractive because of the low step count, we felt that demonstrating a scalable, nonenzymatic reaction sequence would still be valuable to ensure maximum global access to this important drug candidate.

Our preliminary results on a fully chemical route to molnupiravir (Route II, Scheme 2) were disclosed recently.[5b] The details of our acetonide approach to molnupiravir are shown in Scheme 3. The synthesis began with protection of **7** as its acetonide **10** in 94% yield using 2,2-dimethoxypropane in conjunction with acetone and sulfuric acid. Chemoselective

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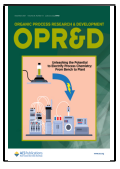
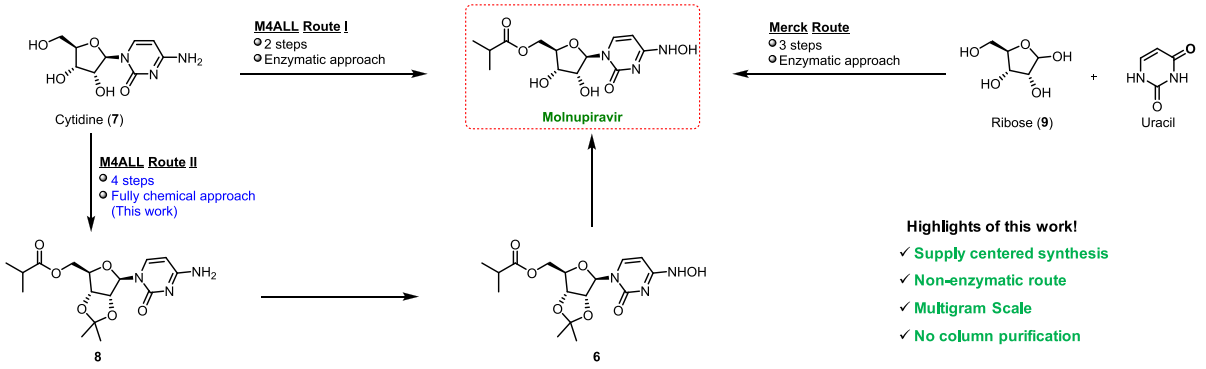
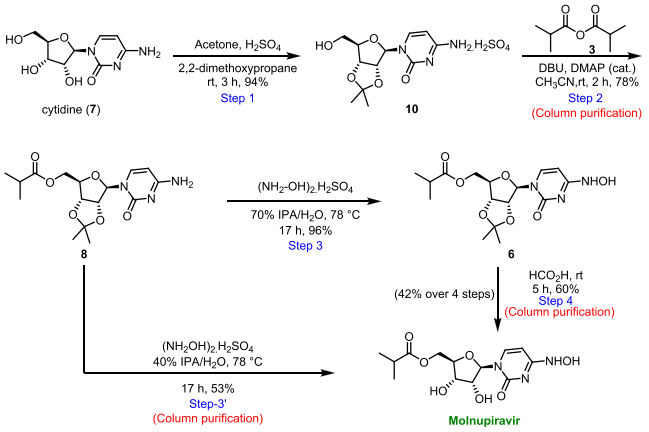


Scheme 2. Development Routes toward Molnupiravir



Scheme 3. Our Initial Acetonide Approach to Molnupiravir (M4ALL Route II)



esterification was then accomplished using isobutyric anhydride and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetonitrile to afford the ester **8** in 78% isolated yield. Hydroxyamination gave the penultimate intermediate **6** in excellent yield (96%), and upon deprotection with formic acid, molnupiravir was isolated in 42% overall yield in four steps. Alternatively, we can reduce one step in this sequence by using a solution of water/IPA (60/40), whereby molnupiravir is obtained in a single step from **8** in an overall 39% yield (Step 3′).

While this three-step approach appears attractive, challenges foreseen to control the impurity profile of molnupiravir due to telescoping the entire operation made it less favorable for further development. Also, there is some safety and environmental concerns related to hydroxylamine. It was reported that hydroxylamine may explode on heating.[8] It is also an irritant to respiratory tract, eyes, skin, and other mucous membranes making it a possible mutagen.[9] However, hydroxylamine and its derivatives are more safely handled in the form of salts. The products from Steps 1 and 3 were isolated by crystallization; however, column chromatography was used to isolate the products of Step 2 and molnupiravir.[5b] This prompted us to explore further optimization to avoid some of these drawbacks.

We now report herein an optimized, operationally simpler process featuring chromatography-free isolation. The scalability of the entire process has been demonstrated by synthesizing multigram quantities of molnupiravir.

RESULTS AND DISCUSSION

Impurity Profiling in Four-Step Synthesis of Molnupiravir. Prior to further developmental work, we identified the impurities in each step of our route either by direct isolation from the reaction mixtures or by synthesis of authentic materials for comparison with reaction in-process control data (Figure 1). Step 1 is very clean and no impurities were

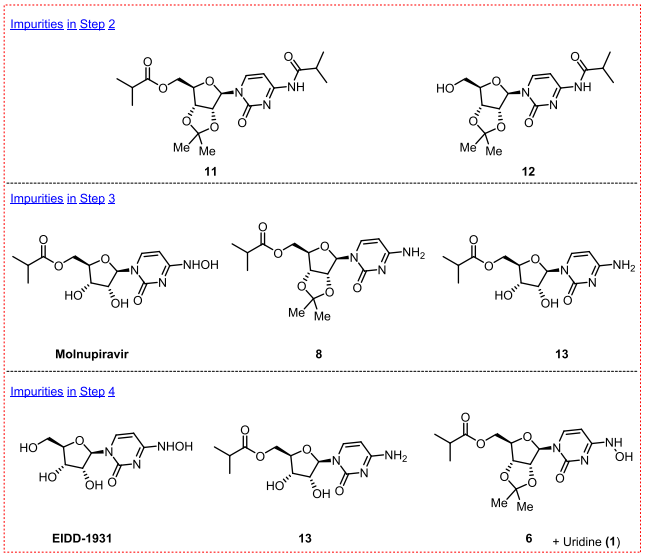


Figure 1. Side products in Step 2, Step 3, and Step 4.

observed either during the reaction or after the isolation of the product, whereas the key impurities generated in Step 2 were the diacylated side compound **11** and the *N*-acylated compound **12**. For Step 3, the key impurities in the crude reaction mixture were the starting material amine **8**, molnupiravir, and the acetonide-deprotected compound **13** of the starting material. For Step 4, the key impurities were **6**, **8**, EIDD-1931, **13**, and uridine (**1**).

Step 1: Acetonide Protection—Impurity Profile and Reaction Optimization. The acetonide protection of the vicinal diol of cytidine was performed using 2,2-dimethoxypropane (5 equiv) in acetone mediated by sulfuric acid (2.3 equiv) to afford **10**, which crystallized from the reaction mixture as its sulfate salt and was isolated in 94% yield with 95 wt % purity based on quantitative nuclear magnetic resonance (qNMR) spectroscopy. Purity by high-performance liquid chromatography (HPLC) was 100%.

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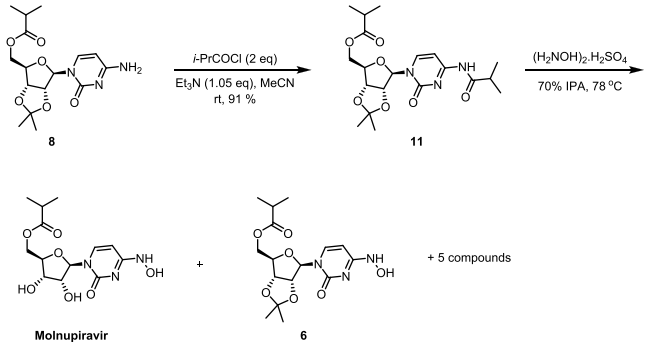
Step 2: Esterification—Impurity Profile and Reaction Optimization. A time profile for the acylation of **10** was recorded to determine the formation of product and impurities in this reaction (see Figure S1, Supporting Information). When the reaction was performed using isobutyric anhydride (1.1 equiv) and triethylamine (2.5 equiv) as the base in the presence of catalytic *N*,*N*-dimethylamino pyridine (DMAP, 20 mol %) in acetonitrile as a solvent at room temperature for 18 h, it was observed that within first 30 min, the reaction proceeded to more than 85% conversion and also that conversion quickly tapered off over the course of additional reaction time. When the reaction was stirred for additional 17 h of reaction period, only a marginal increase in product formation was observed along with the two side products, namely, over-acylated compound **11** and the *N*-acylated compound **12** (Figure 1) in 6.3 and 0.2 area % by HPLC (LCAP), respectively.

We were curious to know if **11** could be transformed to the desired product **6** of the subsequent step by transamination with hydroxylamine since this would eliminate the need for purging while providing a yield boost for the subsequent step. To probe this possibility, **11** was prepared from *O*-monoacylated **8** by reaction with *iso*-butyryl chloride (Scheme 4). Isolated **11** was treated with 3.2 equiv of hydroxylamine

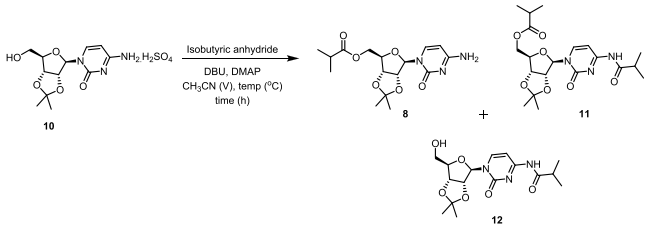
Scheme 4. Synthesis of Diacylated Side Product 11 and Its Subsequent Hydroxyamination Products



sulfate in 70% IPA-H_2O at 78 °C for 24 h. This reaction led to the formation of at least seven compounds by HPLC, of which the major constituents were **6** and molnupiravir in LCAP 21 and 31%, respectively. Although we were disappointed that this reaction did not lead to a higher level of the desired compound **6**, it did provide some useful insights for Step 3. In particular, this reaction profile matches that seen for the hydroxyamination step (Step 3), which suggests that the source of impurities in that reaction stems from side reactions arising from **11** (Scheme 4). This experiment clearly demonstrates that minimization of side product **11** in Step 2 is critical.

A systematic optimization of key variables in Step 2 was conducted to maximize the formation of the monoacylated product **8** and minimize the diacylation side reaction leading to compound **11**. The results of this optimization are provided in Table 1. The optimization was performed using the base (2.1 equiv) in the presence of catalytic DMAP (20 mol %) in acetonitrile as a solvent over the period of 8 h reaction time. However, after screening some of the bases as shown in Table 1 (entries 1−5), it was observed that the reaction performed well with DBU as the base and gives a good yield of the desired product within 3 h of reaction time (entries 5−11).

Table 1. Optimization of Regioselective Acylation of 10



entry[a]	base	temp (°C)	solvent (V)	LCAP[b,c] 8	10	11	12
1	Et_3N	19	10	59	0	17	0.5
2	Bu_3N	19	10	52.6	0	21.6	1.1
3	DIPEA	19	10	60.4	0	17.5	0
4	lutidine	19	10	34.5	0	30.2	6.5
5	DBU	19	10	93.5	0.2	6.1	0.1
6	DBU	19	2.5	89.3	7.1	3.6	0.3
7	DBU	19	5	89.4	5.3	4.3	0.3
8	DBU	19	7	92.5	0.4	6.1	0.1
9	**DBU**	**0**	**10**	**94.7**	**2.2**	**2.1**	**0.6**
10	DBU	40	10	88.7	6	4.6	0.4
11	DBU	56	10	85.5	8.5	5.4	0.6

[a]Reaction conditions: isobutyric anhydride (1.1 equiv), base (2.1 equiv), DMAP (20 mol %), and CH_3CN. [b]Reaction time: 8 h for entries 1−4 and 3 h for entries 5−11. [c]LCAP at 260 nm.

Furthermore, the temperature and reaction concentration were studied (entries 5−11) using DBU as the base and it was observed that the most impactful result from this parameter screening is the role of the reaction concentration on the conversion of the starting material **10** to the product **8**. Increasing the amount of the solvent from 2.5 to 10 V (entries 5−8) increased the LCAP of **8** from 89 to 94% and reduced the overall contribution from the impurities **10**, **11**, and **12** from 11 to 4.3%. Also, reaction temperature has a major effect on the formation of byproduct **11** and it was observed that decreasing the reaction temperature from 19 to 0 °C decreases the formation of byproduct **11** from 6.2 to 2.2% (entry 5 vs entry 9). Because the formation of byproduct **11** (major byproduct) requires two consecutive second-order reactions, here we reasoned that decreasing the reaction temperature would lead to a dramatic decrease in the rate of the second acylation reaction.

After optimization of the key reaction variables for the regioselective acylation of **10**, we found the optimal conditions for the formation of **8** in a high yield to be isobutyric anhydride (1.1 equiv) and DBU (2.1 equiv) in CH_3CN (10 V) at 0 °C for a reaction time of 3 h (entry 9). Although DBU was found to be the optimal base for this reaction, its removal from the reaction mixture is challenging. In our first study, chromatographic separation was used to isolate the pure compound, but this will be costly on scale. Given the large pK_a difference between DBU (∼12.5) and the cytidine amine functionality (∼4.2), we reasoned that a mild acidic workup could potentially remove DBU from the organic layer without extraction of the product **8**. The reaction mixture was concentrated to remove acetonitrile and then dissolved in dichloromethane. Several aqueous acid washes were performed for DBU removal, including 10% ammonium chloride, 10% acetic acid, 10% sulfuric acid, and 85% H_3PO_4. It was observed that using 10% acetic acid resulted in the maximum recovery of the ester **8** and extraction of DBU into the aqueous layer. The

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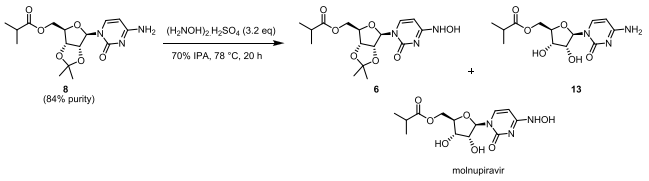
acid wash was followed with a saturated sodium bicarbonate wash to remove the residual acetic acid. The product was then isolated by concentrating the organic layer to dryness. The purity of the final product was found to be 84 wt % by qNMR in chloroform-d, and LCAP was 94.7% for **8**, 2.1% for **11**, and 0.6% for **12**.

A comparison of HPLC data for an in-process control versus the organic layer post 10% acetic acid and bicarbonate workup shows that the relative ratios of cytosine-containing materials were relatively unaffected by the extraction conditions. A trace amount of DBU was detected (0.5 area%) in the final product **8** (Figure S2, Supporting Information).

Step 3: Hydroxyamination—Impurity Profile and Reaction Optimization. In our preliminary study, the transamination of **8** using hydroxylamine sulfate resulted in 96% yield (94 wt % purity) of **6**. Unreacted hydroxylamine sulfate was removed by filtration after dissolution of **6** in a minimal amount of acetonitrile. However, in our previous study,[5b] the starting material for this reaction had been purified by column chromatography. Conducting the same transamination reaction with ester **8** (84 wt % purity) that was obtained using our optimized acetic acid workup gives a mixture of the product **6**, molnupiravir, and some unreacted starting material (Scheme 5). A typical distribution of products at the end of 20 h of the

Scheme 5. Hydroxyamination and Side Products Formed



reaction period with an internal reaction temperature of 73 °C is 86% product **6**, 6% of starting material **8**, 3% molnupiravir, and ∼1% of the acetonide-deprotected ester **13** by HPLC. After isolation, this translated to 89% average-adjusted isolated yield with 73 wt % purity from duplicate runs before removal of unreacted hydroxylamine sulfate.

Qualitative solubility studies were carried out using different solvents (Figure S3, Supporting Information) for purification of the product **6** (Table 2) and two methods were identified. The first method (Method A) involves recrystallization of crude **6** (73 wt % purity) from 2.5 V of isopropyl acetate. The mass recovery using this method is 66% and the purity is 98 wt % by qNMR in acetone-d_6 and more **6** was present in the mother liquor from Method A. In the second purification

Table 2. Recrystallization of Crude Product 6 in Different Solvents

entry	solvent	solvent (V)	% mass recovery[a]	wt % purity[b]
1	chlorobenzene	10	53	98
2	CPME	10	23	97
3	anisole	10	30	69
4	toluene/chlorobenzene (1:1)	5	61	96
5	toluene	10	84	82
6	***i*-PrOAc**	**2.5**	**66**	**98**
7	CH_3CN	2.5	64	96

[a]Adjusted to purity. [b]By qNMR.

method (Method B), recrystallization of the crude **6** (73% purity) from 2.5 V of acetonitrile provided 64% adjusted isolated yield of **6** with a purity of 96 wt % by qNMR. Additional product **6** (second crop) from Method B was isolated by concentrating the mother liquor and recrystallizing the remaining solid in 2.5 V of acetonitrile. This secondary isolation affords an additional 11% mass yield of material with a purity of 90 wt % by qNMR.

The two methods described above afford the Step 3 product **6** of >96% purity. More notably, the amount of **13** was reduced postpurification by either of these methods from 1.7 area% to 0.1 area% (see the HPLC trace in Figure S4, Supporting Information, a peak at a retention time of 4.998 min). The key impurities in **6** obtained by either of these methods were molnupiravir and **8** (2.6 area% and 0.3 area% by Method A and 2.1 area% and 0.5 area% by Method B). Method A was preferred for scale up as it provided **6** with a higher purity.

Step 4: Acetonide Deprotection—Impurity Profile and Optimization. With access to a relatively pure **6** from Step 3, screening of conditions for deprotection of the acetonide group in **6** was next performed (see Figure S5, Supporting Information). Most protic acids such as sulfuric acid, phosphoric acid, hydrochloric acid, or Lewis acids such as zirconium tetrachloride provided molnupiravir in low to modest yields (<50%) (as estimated by HPLC area% of the crude reaction mixtures) along with a variety of side products. For example, increased formation of **8** and **13** under some reaction conditions (TFA in EtOAc, TFA in DCM or HCl in DCM, and H_2SO_4 in DCM) possibly indicates a self-oxidation reduction, previously reported with aryl hydroxylamines.[10] Of all the acids screened, only neat formic acid and trifluoroacetic acid provide conversions to molnupiravir in >90% yield. Finally, formic acid was selected for further development as it gave consistent results over different scales during the development of molnupiravir.

A range of recrystallization conditions was evaluated to purify molnupiravir (Table 3). Using 10 volumes of 1:1

Table 3. Recrystallization of Molnupiravir from Various Solvents

entry	solvent	solvent (V)	% mass recovery[a]	wt % purity[b]
1	IPA	10	91	89
2	IPA−EtOAc (1:1)	10	18	
3	**H_2O**	**2**	**69**	**98.5**
4	*n*-BuOH−H_2O (1:1)	5	59	99.7
5	CH_3CN	10	95	95.7
6	EtOAc−CH_3CN (1:1)	10	82[c]	97

[a]Adjusted to purity. [b]By qNMR. [c]Combined from three crops.

EtOAc/acetonitrile, the condition used for purification via the enzymatic route,[5d] an 82% mass recovery of molnupiravir was obtained in three crops with 97 wt % purity (HPLC area % purity of the product was 97.8% and area% purity of known impurities **6** and **8** was 0.4 and 0.3%, respectively). Improved purity could be obtained by crystallization from five volumes of 1:1 *n*-BuOH/water with 99.7 wt % purity (the HPLC area % purity of the product was 99.7% and area% purity of the known impurity **8** was 0.2%, entry 4) or two volumes of water with 98.5 wt % purity (the HPLC area% purity of the product was 98.6% and area% purity of known impurities **6** and **8** were 0.3 and 0.8%, respectively, entry 3) although with reduced mass

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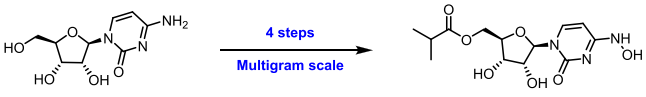
recovery. With two volumes of *n*-BuOH/water (1:1), precipitation of the product was observed with a lower purity. Based on the balance of purity of molnupiravir and its recovery, water was selected as the final crystallization solvent.

Scalability. A preliminary evaluation of the scalability of the entire sequence was conducted at 100 g scale. Acetonide protection proceeded smoothly to afford the product of Step 1 in an excellent yield and purity (>95%) on a 100 g scale (Table 4). The optimized Step 2 conditions resulted in >90% isolated

Table 4. Scale Up of the Four-Step Sequence to Molnupiravir



Cytidine Molnupiravir

step	scale (g)	LCAP of product[a]	wt % purity[b]	yield (%)[c]
1	100	99.1	95.0	94
	300	99.2	95.5	92
2	150	98.6	89.0	92
	140	94.0	85.8	95
3	130	97.3	94.0	70
	120	98.7	95.0	76
4	80	98.6	98.5	59
	100	99.6	97.6	61

[a]LCAP at 260 nm. [b]By qNMR. [c]Yield adjusted to purity.

yield with good purity on a 150 g scale. At a 130 g scale, the hydroxyamination (Step 3) showed modest yields of the product (70−76%) postrecrystallization with isopropyl acetate with good purity (∼95%) The final deprotection on a 80 g scale afforded molnupiravir in 59−61% yield with 98.5% wt % purity, which represents an overall yield of 36−41% over the four steps. Consistent results were observed in repetition batches on varying scales for each step as can be seen below in Table 4. The filtrate from the final recyrstallization contained additional molnupiravir (14%). The impurity profile of the molnupiravir obtained from this route indicates the presence of **6** (0.3 area%) and **8** (0.8 area%) as the two main impurities (Figure S6, Supporting Information).

CONCLUSIONS

In summary, improvements and reaction details for our four-step route to molnupiravir from highly available cytidine are disclosed. Compound **10** from Step 1 is isolated directly from the reaction by filtration and washing, whereas compound **8** from Step 2 is obtained directly by washing the organic layer with 10% acetic acid. The pure compound **6** from Step 3 is obtained by recrystallization of the crude from isopropyl acetate, and molnupiravir from Step 4 is obtained by recrystallization from water. We have demonstrated comparable overall yields to our previous study and we have also been able to substitute column chromatographic purification with simple purification procedures that can be performed at a large scale.

EXPERIMENTAL SECTION

General. For all compounds, ^{1}H and ^{13}C NMR spectra were recorded using 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 = 3.31 ppm for ^{1}H and 49.0 ppm for ^{13}C, and D_2O = 4.79 ppm for ^{1}H). Coupling constants *J* are reported in hertz (Hz). The following abbreviations were used to denote 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; and br, broad. Reactions were monitored by HPLC using the methods indicated. Quantitative NMR measurements were done using either mesitylene or 1,3,5-trimethoxybenzene as the internal standard. Glassware was oven-dried at 120 °C, assembled while hot, and cooled to an ambient temperature under an inert atmosphere. Unless otherwise noted, reactions involving air-sensitive reagents and/or requiring anhydrous conditions were performed under a nitrogen atmosphere.

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-dinethylaminopyridine), LCAP (Area % by HPLC), qNMR (Quantitative NMR in the presence of an internal standard), DCM (methylene chloride), and IPA (isopropanol).

*4-Amino-1-((3aR,4R,6R,6aR)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)pyrimidin-2(1H)-one (**1**) Sulfuric Acid Salt.* 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) were added followed by 2,2-dimethoxypropane (251.9 mL, 2.055 mol, 5 equiv) under a nitrogen atmosphere. Neat sulfuric acid (50.7 mL, 0.94 mol, 2.3 equiv) was added to the above suspension and left for 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 for 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.[5b]

*((3aR,4R,6R,6aR)-6-(4-Amino-2-oxopyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl isobutyrate (**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) were added at room temperature. The reaction mixture was stirred for 10 min and isobutyric anhydride (65.11 mL, 0.39 mol, 1.05 equiv) was added dropwise at 0 °C in two equal portions at half hour intervals each and the reaction mixture was maintained for 2 h at the same temperature. The reaction mixture was then directly concentrated under a reduced pressure to afford a waxy solid. The resultant material was redissolved 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 a 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

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anhydrous sodium sulfate and concentrated under a 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.[5b]

*((3aR,4R,6R,6aR)-6-(4-(Hydroxyamino)-2-oxopyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-methyl isobutyrate (**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) were added and the resultant solution was heated to an internal temperature of 72−73 °C for 19 h at which time HPLC showed the formation of a product in addition to the 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 a cosolvent 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 dried under a 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.[5b]

((2R,3S,4R,5R)-3,4-Dihydroxy-5-(4-(hydroxyamino)-2-oxopyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl isobutyrate (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. The solvent was removed under a 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. For 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. The solid was filtered and the wet solid was 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 and 98.3% purity by HPLC at 260 nm) of 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.[5b]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.1c00219.

Reaction optimization, experimental details, copies of ^{1}H and ^{13}C NMR spectra of all new compounds, and HPLC method (PDF)

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Notes

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