The Development of a Robust Manufacturing Route for Molnupiravir, an Antiviral for the Treatment of COVID-19

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1. General experimental details

Unless otherwise noted, all reactions were performed under ambient atmosphere. Either Chem Glass jacketed vessels or Mettler-Toledo vessels with overhead agitation were used in the lab evaluation.

Reagents. Reagents were purchased in reagent grade from commercial suppliers and used without further purification, unless otherwise described.

Purification. Purification of desired compounds was performed by direct crystallization with or without extractive workup. NMR experiments utilized deuterated solvents purchased from Sigma Aldrich or Cambridge Isotope Laboratories. HPLC eluent mixtures made use of HPLC grade MeCN and H2O.

NMR analysis. Proton nuclear magnetic resonance (\(^1\)H NMR) spectra and carbon nuclear magnetic resonance (\(^{13}\)C NMR) spectra, were recorded at 25 °C unless stated otherwise on a Bruker 500 MHz spectrometer or a Bruker 400 MHz spectrometer. Chemical shifts for protons are reported in parts per million (ppm) are referenced to residual proton signals of the NMR solvent according to values reported in the literature.\(^1\) Chemical shifts for carbon are reported in parts per million (ppm) are referenced to the carbon resonances of the NMR solvent according to values reported in the literature.\(^1\) For samples in CDCl\(_3\), the residual solvent signal was referenced to 7.26 ppm for \(^1\)H NMR and 77.2 ppm for \(^{13}\)C NMR. For samples in CD\(_3\)CN, the residual solvent signal was referenced to 1.94 ppm for \(^1\)H NMR and 132 ppm and 118.26 ppm for \(^{13}\)C NMR. For samples in CD\(_3\)OD, the residual solvent signal was referenced to 3.31 ppm for \(^1\)H NMR and 49.00 ppm for \(^{13}\)C NMR. For samples in DMSO-\(d_6\), the residual solvent signal was referenced to 2.50 ppm for \(^1\)H NMR and 39.5 ppm for \(^{13}\)C NMR. Data are presented as follows: chemical shift, integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, dt = doublet of triplets), coupling constants (J) in Hertz (Hz).

High resolution mass spectrometry (HRMS) analysis. High resolution mass spectrometry data were collected on Agilent G6224A TOF LC/MS. Details of condition can be found in following table.

| HPLC Condition | Column | Two-way connector |
|----------------|--------|-------------------|
| Mobile Phase | 0.1% FA in H\(_2\)O: 0.1% FA in ACN(70/30, V/V,A/B) |
| Wavelength(nm) | 220 |
| Flow Rate (mL/min) | 0.3 |
| Column Temp.(°C) | 30 |
| Injection Volume (µL) | 1 |
| Acquisition Time (min) | 2 |
| Post Time (min) | off |

| MS Condition | Ion Source | Dual ESI |
|--------------|------------|----------|
| Polarity | Positive |
| Nebulization Gas | Nitrogen |
| Drying Gas (l/min) | 10 |
Nebulizer pressure (psi) | 40  
---|---
Gas Temperature (°C) | 350  
Vcap (V) | 4000  
Fragmentor (V) | 35  

**Physical measurements.**

Powder X-Ray Diffraction: X-ray diffraction measurements were acquired on an Empyrean X-ray diffraction instrument (Panalytical Inc., Natick, MA) with a Cu Kα source operating at 45 kV and 40 mA. Samples were prepared on a zero background silicon sample holder and data were collected from 2–40° 2θ range.

Particle Size Analysis: particle size measurements were obtained using a Microtrac S3500 laser diffraction particle size analyzer (Microtrac MRB, Montgomeryville PA, USA). Each sample was dispersed in Isopar G with 0.25% lecithin. The sample was sonicated for 30 seconds prior to data collection. The results reported are in volume distributions.

**HPLC method information for each step**

Chromatographic Conditions and Approximate Relative Retention Times

*Step 1*

| Instrument | Waters H-Class UPLC with UV detector |
|---|---|
| Column | Acquity UPLC HSS T3(100 x 3.0 mm, 1.8 μm) |
| Column Temperature | 35 ºC |
| Sampler Temperature | 10 ºC |
| Wavelength | UV 210 nm |
| Flow Rate | 1.0 mL/min |
| Injector Volume | 2 μL |
| Needle Wash | MeOH:H₂O=1:1 (v/v) |
| Mobile Phase A | 0.1% H₃PO₄ in water For example, transfer 2.0 mL of H₃PO₄ to 2.0 L of water and mix. |
| Mobile Phase B | ACN |
| Gradient Program | Time(min) A% B% |
| Compound ID | Retention Time (RT, min) | Relative Retention Time (RRT) | Relative Response Factor (RRF) |
|-------------|--------------------------|------------------------------|-------------------------------|
| Acetone     | 1.5                      | 0.26                         | N/A                           |
| Uridine     | 1.7                      | 0.29                         | 1.25                          |
| 1           | 5.7                      | 1.00                         | 1.00                          |
| 2,2-DMP     | 7.8                      | 1.36                         | N/A                           |

**Step 2**

| Instrument | Waters H-class UPLC with UV detector or equivalent |
|------------|---------------------------------------------------|
| Column     | Waters Acquity UPLC HSS T3 (100 mm x 3.0 mm, 1.8 µm) PN: 186004680 |
| Wavelength | 210 nm                                           |
| Reference Wavelength | Off                                               |
| Column Temperature | 35°C                                               |
| Sample Tray | 5°C                                                |
| Flow Rate  | 1.0 mL/min                                       |
| Injector Volume | 2 µL                                            |
| Needle Wash | ACN:H₂O=1:1(v/v)                                 |
| Mobile Phase A | 0.1% H₃PO₄ in water, v/v                        |
|             | For example, accurately transfer 1 mL H₃PO₄ to 1000 mL pure water, mix well and degas by ultrasonic. |
Mobile Phase B

Degas by ultrasonic.

| Gradient Program | Time(min) | A% | B% |
|------------------|-----------|----|----|
| Initial          | 100       | 0  |    |
| 0.50             | 100       | 0  |    |
| 8.50             | 80        | 20 |    |
| 20.50            | 20        | 80 |    |
| 21.00            | 20        | 80 |    |
| 22.00            | 100       | 0  |    |
| 25.00            | Stop      |    |    |

Run Time 25 min

Data Acquisition Time 25 min

| Compound ID       | Retention Time (RT, min) | Relative Retention Time (RRT) | Relative Response Factor (RRF) |
|-------------------|--------------------------|-------------------------------|--------------------------------|
| Uridine           | 1.8&2.2                  | 0.15&0.18                     | N/A                            |
| DMAP              | 2.7&2.9                  | 0.22&0.24                     | N/A                            |
| isobutyric acid   | 4.9                      | 0.40                          | N/A                            |
| EtOAc             | 5.4                      | 0.44                          | N/A                            |
| 1                 | 6.5                      | 0.53                          | 1.34                           |
| 2                 | 12.3                     | 1.00                          | 1.00                           |
| isobutyric anhydride | 14.4                  | 1.18                          | N/A                            |

Step 3

| Instrument        | Waters H-Class UHPLC with UV detector |
|-------------------|---------------------------------------|
| Column            | Acquity UPLC HSS T3 (100 x 3.0 mm, 1.8 μm), PN: 186004680 |
| Column Temperature| 35 °C                                  |
| Sampler Temperature| 5 °C                                   |
| Wavelength        | UV 210 nm                              |
| Flow Rate       | 1.0 mL/min         |
|-----------------|-------------------|
| Injector Volume | 1 µL for Waters H-Class and 2µL for Agilent 1290 UHPLC |
| Needle Wash     | ACN:H₂O=1:1 (v/v) |
| Mobile Phase A  | 0.1% H₃PO₄ in water For example, accurately transfer 1.0 mL H₃PO₄ to 1000 mL pure water, mix well and degas by ultrasonic. |
| Mobile Phase B  | ACN               |
| Gradient Program| Time(min) A% B%    |
|                 | 0.01 100 0        |
|                 | 0.50 100 0        |
|                 | 8.50 80 20        |
|                 | 20.50 20 80       |
|                 | 21.00 20 80       |
|                 | 22.00 100 0       |
|                 | 25.00 STOP        |
| Run Time        | 25 min            |
| Data Acquisition Time | 25 min          |
| Compound ID     | Retention Time (RT, min) | Relative Retention Time (RRT) | Relative Response Factor (RRF) |
| 1,2,4-Triazole  | 0.6               | 0.05                          | N/A                           |
| EtOAc           | 5.0               | 0.40                          | N/A                           |
| 3b              | 11.1              | 0.90                          | N/A                           |
| 2               | 11.8              | 0.95                          | 0.58                          |
| 3a              | 12.4              | 1.00                          | 1.00                          |
### Step 4

| Instrument       | Waters H-Class UHPLC with UV detector |
|------------------|--------------------------------------|
| **Column**       | Acquity UPLC HSS T3 (100 x 3.0 mm, 1.8 μm), PN: 186004680 |
| **Column Temperature** | 35 °C |
| **Sampler Temperature** | 5 °C |
| **Wavelength**   | UV 210 nm |
| **Flow Rate**    | 1.0 mL/min |
| **Injector Volume** | 3 μL |
| **Needle Wash**  | MeOH:H_{2}O=8:2 (v/v) |
| **Stock Phase**  | 5 mM Ammonium Phosphate Buffer in water  |
| For example, add 449 mg of (NH₄)₂HPO₄ and 759.2 mg of NH₄H₂PO₄ to 2 L of pure water. Sonicate to ensure complete dissolution, filter prior to use. Labeled it as stock phase. |
| **Mobile Phase A** | 1 mM Ammonium Phosphate Buffer in water  |
| For example, Add 200 mL of stock phase and 800 mL of Water to a 1L bottle, mix well and degas by ultrasonic. |
| **Mobile Phase B** | 1 mM Ammonium Phosphate Buffer in ACN:H_{2}O=8:2 (v/v)  |
| For example, Add 200 mL of stock phase and 800 mL of ACN to a 1L bottle, mix well and degas by ultrasonic. |
| **Gradient Program** | |
| Time(min) | A% | B% |
| 0.01 | 100 | 0 |
| 0.50 | 100 | 0 |
| 8.50 | 80 | 20 |
| 20.50 | 20 | 80 |
| 21.00 | 20 | 80 |
| 22.00 | 100 | 0 |
| 25.00 | Stop |
| **Run Time**    | 25 min |
| **Data Acquisition Time** | 25 min |
| Compound ID       | Retention Time (RT, min) | Relative Retention Time (RRT) | Relative Response Factor (RRF) |
|-------------------|--------------------------|-------------------------------|-------------------------------|
| 1,2,4-Triazole    | 0.7                      | 0.05                          | N/A                           |
| Hydroxylamine     | 0.7                      | 0.05                          | N/A                           |
| 2                 | 14.1                     | 0.98                          | 0.33                          |
| 4                 | 14.3                     | 1.00                          | 1.00                          |
| 3                 | 14.8                     | 1.03                          | 0.46                          |
| Dimer impurity    | 19.3                     | 1.35                          | 0.22                          |

### Step 5

| Instrument        | Thermo U3000 HPLC with UV detector or equivalent |
|-------------------|--------------------------------------------------|
| Column            | Waters Atlantis T3, 4.6 × 150 mm, 3.0 μm, PN: 186003729 |
| Wavelength        | 260 nm                                           |
| Reference Wavelength | Off                              |
| Column Temperature| 40°C                                             |
| Sampler Temperature| 5°C                                              |
| Flow Rate         | 1.0 mL/min                                       |
| Injector Volume   | 5 μL                                             |
| Needle Wash       | MeOH:H₂O=8:2 (v/v)                              |
|                   | For example, accurately transfer 800 mL MeOH to 200 mL pure water, mix well and degas by ultrasonic. |
| Mobile Phase A    | 20 mM NH₄H₂PO₄ in water                         |
|                   | For example, accurately transfer 4.6 g NH₄H₂PO₄ to 2000 mL pure water, mix well and degas by ultrasonic. |
| Mobile Phase B    | MeOH                                              |

| Gradient Program  | Time (min) | A% | B% |
|-------------------|------------|----|----|
|                   | Initial    | 100| 0  |
|                   | 2.00       | 100| 0  |
|                   | 43.00      | 20 | 80 |
|                   | 48.00      | 20 | 80 |
## 2. Synthetic procedures for the manufacturing route

**Step 1, high temperature TEA quench process:**

To a 1000 mL vessel, acetone (275 mL) was charged. A sample was taken from the vessel and checked by Karl Fischer titration to ensure that the its water content was below 0.3 wt%. Uridine (55.18 g, 226 mmol, 1 eq.) was charged into the vessel. The batch was agitated and the batch temperature was adjusted to 21-23°C. 2,2-DMP (24.62 g, 236 mmol, 1.05 eq.) was charged. H₂SO₄ (0.222 g, 2.264 mmol, 0.01 eq.) was charged into the vessel while the batch temperature was maintained at 21–23 °C. The batch was warmed up to 50 – 60 °C within 5 hour and agitated for 1 hour. Triethylamine (0.913 g, 9.02 mmol, 0.04 eq.) was charged while the batch was maintained between 50 – 60 °C. The batch was then cooled to 42 – 44 °C over 20 min and intermediate A (0.066 g, 0.24 mol., 0.1% eq.) was charged. n-Heptane (110 mL) was charged into vessel at 42 – 44 °C over 4 hours. The batch was then cooled to – 5 to 5 °C over 4 hours and further aged for 2 – 6 hours. The resulting slurry was filtered, and the cake was washed with n-heptane (110 mL). Intermediate 1 (54.56 g, 84% isolated yield) was isolated as a white solid after drying under reduced pressure at a maximum batch temperature of 55 °C.
**1H NMR (400 MHz, DMSO-d$_6$) δ ppm 1.29 (s, 3 H), 1.49 (s, 3 H), 3.50 - 3.72 (m, 2 H), 4.07 (q, $J$=4.27 Hz, 1 H), 4.75 (dd, $J$=6.27, 3.51 Hz, 1 H), 4.90 (dd, $J$=6.27, 2.51 Hz, 1 H), 5.09 (t, $J$=5.40 Hz, 1 H), 5.64 (dd, $J$=8.03, 1.25 Hz, 1 H), 5.83 (d, $J$=2.76 Hz, 1 H), 7.79 (d, $J$=8.03 Hz, 1 H), 11.38 (br s, 1 H). 13C NMR (101 MHz, DMSO-d$_6$) δ ppm 25.65, 27.52, 61.73, 80.94, 84.14, 86.98, 91.58, 102.20, 113.44, 142.38, 150.80, 163.64.**

HRMS (m/z): calculated for [C$_{12}$H$_{16}$N$_2$O$_6$]+, 285.1081; observed [C$_{12}$H$_{16}$N$_2$O$_6$+H]$^+$, 285.1079.

**Step 1, low temperature TEA quench process:**

To a jacket vessel with 30 L total capacity, acetone (8 L) was charged and the batch temperature was adjusted to 20-25°C. A sample was taken from the vessel and checked by Karl Fischer titration to ensure that the water content was below 0.3 wt%. The batch was agitated. Uridine (2.0 kg, 8.19 mol, 1 eq.) and a rinse with acetone (2 L) were charged. The batch temperature was adjusted to 20-25°C. 2,2-DMP (0.896 kg, 8.60 mol, 1.05 eq.) was charged and conc. H$_2$SO$_4$ (8.0 g, 0.082 mol, 0.01 eq.) was charged into the vessel while the batch temperature was maintained at 20 – 25 °C. The batch was warmed up to 50 – 55 °C within 1.5 hour and agitated for 1 hour. Intermediate 1 (2.3 g, 8.19 mmol) was charged as seed at 50 – 55 °C. The batch was cooled to between 0 to 5°C within 4 h and stirred for 2 h. Triethylamine (33.20 g, 0.33 mol, 0.04 eq.) was charged while the batch was maintained between 0 to 5°C. n-Heptane (4 L) was charged over 2 h and batch was aged for 10 h. The resulting slurry was filtered, and the cake was washed with n-heptane (4 L). Intermediate 1 (2.07 kg, 89.05% isolated yield) was isolated as a white solid after drying under reduced pressure at a maximum batch temperature of 45 °C.

**Step 2**

To a 500 mL vessel, EtOAc (160 mL) was charged followed by intermediate 1 (40 g, 141 mmol, 1 eq.). TEA (21.36 g, 211 mmol, 1.5 eq.), DMAP (0.34 g, 2.78 mmol, 0.02 eq.) and EtOAc (20 mL) as rinse. The batch was cooled to 5 - 10 °C. Isobutyric anhydride (24.48 g, 155 mmol, 1.1 eq.) was charged followed by a rinse with EtOAc (20 mL). The batch temperature was adjusted to 20-25 °C and the batch was agitated for 2 h. The batch was cooled to 10-15 °C and water (80 mL) was charged over 1 h. The batch temperature was adjusted to 20-25 °C and it was stirred for 2h. The aqueous phase was removed and the organic phase was concentrated to 4 vol. at a maximum batch temperature of 50 °C. Azeotrope distillation with acetonitrile (400 mL) was used to control the KF < 1000 ppm at a maximum jacket temperature of 50 °C. 48.3 g 2 in ACN solution was obtained with 29.1wt% and 97.0% assay yield.

**1H NMR (400 MHz, DMSO-d$_6$) δ ppm 1.08 (d, $J$=6.78 Hz, 6 H), 1.30 (s, 3 H), 1.49 (s, 3 H), 4.13 - 4.28 (m, 3 H), 4.79 (dd, $J$=6.27, 3.26 Hz, 1 H), 5.05 (dd, $J$=6.53, 1.76 Hz, 1 H), 5.64 (d, $J$=8.03 Hz, 1 H), 5.79 (d, $J$=1.76 Hz, 1 H), 7.69 (d, $J$=8.03 Hz, 1 H), 11.43 (s, 1 H).**

**13C NMR (101 MHz, DMSO-d$_6$) δ ppm 19.18, 25.62, 27.42, 33.53, 64.26, 81.21, 84.14, 84.70, 93.04, 102.21, 113.74, 143.27, 150.73, 163.70, 176.34**
HRMS (m/z): calculated for \([\text{C}_{16}\text{H}_{22}\text{N}_2\text{O}_7]+\), 355.1500; observed \([\text{C}_{16}\text{H}_{22}\text{N}_2\text{O}_7+\text{H}]+\), 355.1508

**Step 3, with TEA as base and an extractive workup after filtration to remove waste salts:**

To a 1000 mL vessel, 1,2,4-triazole (39.0 g, 565 mmol, 5 eq.) was charged followed by acetonitrile (280 mL). The batch temperature was adjusted to 10-20 °C. Triethylamine (79.5 g, 786 mmol, 7 eq.) and phosphorous oxychloride (23.0 g, 150 mmol, 1.3 eq.) was added sequentially at 10-20 °C. Intermediate 2 (39.8 g, 112 mmol, 1 eq.) was added as EtOAc solution at 10-20 °C. The batch was warmed up to 40-50 °C over 1 h and aged for 19 h. The batch was cooled down to 10-20 °C and filtered to remove the waste cake. The cake was rinsed with with EtOAc (120 mL) which was combined with rest of the filtrate. The combined filtrate was charged back to the vessel followed by water (12 mL). The batch was concentrated to 2-3 vol.

Additional azeotrope distillation was conducted with EtOAc (80 mL). EtOAc (280 mL) was charged into the batch and followed by water (160 mL). After the water phase was removed, the organic phase was concentrated to 160 mL at maximum 40 °C batch temperature. The batch was charged with IPA (160 mL) and then seeded with intermediate C (0.04 g) at 35-45°C and aged for 1.5 h. The batch was solvent switched with IPA (160 mL) at maximum 40 °C batch temperature and IPA (80 mL) was charged to control residual EtOAc and ACN. The batch temperature was adjusted to 40-50 °C and n-heptane (160 mL) was charged at 40-50°C. After being aged at that temperature for 2 h, the batch was cooled to 0-10 °C over 10 h and further aged for 3 h. The slurry was filtered, and the cake was washed with MTBE (120 mL) to give wet solid. After drying under 45 °C, 35.5 g dry solid was obtained in 78% isolated yield.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\ ppm\): 1.03 (t, \(J=7.40\) Hz, 6 H), 1.32 (s, 3 H), 1.52 (s, 3 H), 4.19 - 4.36 (m, 2 H), 4.43 - 4.50 (m, 1 H), 4.83 (dd, \(J=6.27, 3.26\) Hz, 1 H), 5.07 (dd, \(J=6.27, 1.25\) Hz, 1 H), 5.90 (s, 1 H), 6.99 (d, \(J=7.28\) Hz, 1 H), 8.42 (s, 1 H), 8.48 (d, \(J=7.28\) Hz, 1 H), 9.46 (s, 1 H). \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\ ppm\): 18.98, 19.15, 25.56, 27.36, 33.52, 64.54, 81.42, 85.11, 86.07, 94.36, 96.05, 113.31, 144.27, 150.53, 154.01, 154.69, 159.59, 176.21

HRMS (m/z): calculated for \([\text{C}_{18}\text{H}_{23}\text{N}_5\text{O}_6]+\), 406.1721; observed \([\text{C}_{18}\text{H}_{23}\text{N}_5\text{O}_6+\text{H}]+\), 406.1733

**Step 3, with DIPEA as base and direct isolation process**

To a vessel with 30 L total capacity, ACN (6825 mL) was charged to the vessel and sample was taken from the vessel and checked by Karl Fischer titration to test KF to ensure it was < 0.01 wt%. 1H-1,2,4-Triazole (1.49 kg, 21.6 mol, 5.1 eq.) was charged followed by a rinse with acetonitrile (300 mL). DIPEA (4.1 kg, 31.7 mol, 7.5 eq.) was charged followed by a rinse with acetonitrile (75 mL). The batch was cooled to 0-5 °C, before phosphorous oxychloride (0.91 kg, 5.9 mol, 1.4 eq.) was charged below 20 °C followed by a rinse with acetonitrile (75 mL). The batch temperature was adjusted to 25-35 °C and it was aged for 0.5 h. Compound 2 (1.5 kg, 4.2 mol, 1 eq.) in acetonitrile was charged portionwise over more than 1 h at 25-35 °C, followed by a rinse with acetonitrile (75 mL). The batch was agitated at 25-35 °C for 17 h and water (1500 mL) was charged below 35 °C. The batch was concentrated to 5.0 vol. while batch temperature was controlled ≤ 45 °C and jacket temperature < 50 °C. The batch temperature was adjusted to 35-45 °C and it
was aged for more than 1h. Process water (7500 mL) was charged over more than 2 h at 35-45 °C. The mixture was then cooled to 0-10 °C over more than 3 h. The slurry was aged at 5 °C for more than 2 h before filtration. The wet cake was washed with water(7500 mL). Drying the wet cake at maximum 50 °C gave 1656 g intermediate 3 as an off-white solid in 90.5% isolated yield.

**Step 4**

To a vessel with 30L total capacity, 2360 mL ACN was charged followed by intermediate 3 (1535.8 g, 3788 mmol., 1 eq.) and a rinse with 710 mL ACN. The batch temperature was adjusted to 0-8°C, before 52.5% NH₂OH (311 g, 4943 mmol, 1.3 eq.) was charged. The batch was agitated at 0-8°C for 5 h. The batch was then cooled to 0-10°C and water (15.4 L) was charged. The slurry was aged at 0-10°C for 5 h before filtration. The wet cake was washed with water (1.54 L). Drying the wet cake at 45-55 °C gave 1.24 kg intermediate 4 as a white solid in 88.4% isolated yield.

1H NMR (400 MHz, DMSO-d₆) δ ppm 1.09 (d, J=7.03 Hz, 6 H), 1.29 (s, 3 H), 1.48 (s, 3 H), 4.02 - 4.33 (m, 3 H), 4.76 (dd, J=6.27, 4.02 Hz, 1 H), 4.95 (dd, J=6.53, 2.26 Hz, 1 H), 5.57 (d, J=8.03 Hz, 1 H), 5.72 (d, J=2.26 Hz, 1 H), 6.89 (d, J=8.28 Hz, 1 H), 9.67 (s, 1 H), 10.06 (s, 1 H). 13C NMR (101 MHz, DMSO-d₆) δ ppm 19.20, 25.66, 27.47, 33.54, 64.29, 81.06, 83.58, 91.71, 99.16, 113.87, 132.39, 143.75, 149.54, 176.38. HRMS (m/z): calculated for [C₁₆H₂₃N₃O₇⁺], 370.1609; observed [C₁₆H₂₃N₃O₇+H⁺], 370.1616.

**Step 5**

To a 2000 mL vessel, ACN (800 mL) was charged, followed by intermediate 4 (100 g, 271 mmol., 1 eq.). The batch was then warmed up to 55-65 °C over 3 h. 18.3 wt% HCl (46.9 g, 230 mmol, 0.85 eq.) was charged into the vessel at 55-65 °C. The batch was agitated at 55-65 °C for 10 h and then cooled to 15-25 °C. The reaction mixture was quenched with 13.1 wt% Na₂CO₃ solution (192 g, 150 mmol, 0.43 eq.) at 20-25 °C. The mixture was then concentrated to 4.0-6.0 vol. at maximum 35 °C. Azeotropic distillation with EtOAc (1200 mL) was performed and EtOAc (800 mL) was charged to control residual ACN less than 3wt%. The organic layer was washed with 15wt% Na₂SO₄ solution with 1wt% EDTA (200 mL) and then water (75 mL). The final organic layer was then transferred to another vessel with 2000 mL total capacity via a filter pot (0.22 μm). The batch was concentrated to 6 vol. with inner temperature ≤35 °C. EA (200
mL) was charged and the batch temperature was adjusted to around 45 to 55 °C until solid was dissolved. The batch was cooled at 40 to 50 °C. FP (0.3 g) was charged as seed at 40-50 °C and the resulting slurry was aged for 1 h. Then the batch was cooled to 25 to 35 °C over 1.5 h. EtOAc (400 mL) was charged over 6 h while the batch temperature was maintained at 25 to 35 °C. The mixture was concentrated to 4 vol. with inner temperature < 30 °C. EtOAc (400 mL) was charged and then batch concentrate to 4 vol. while jacket temperature ≤ 30 °C. MTBE (400 mL) was charged and the batch was heated at 55 to 65 °C for 3 h. It was then cooled to 15-25 °C over 3 h and agitated at 15-25 °C for 1 h. The batch was filtered and wet cake washed with EtOAc/MTBE solution (75 mL/225 mL) and then MTBE (200 mL). Final product 65.6 g was isolated after drying under reduced pressure with a N2 sweep at a maximum batch temperature of 45 °C in 74% isolated yield.

\[ ^1 \text{H NMR} \ (500 \text{ MHz, Deuterium Oxide}) \delta \text{ ppm} \ 6.99 \ (d, J = 8.3 \text{ Hz, 1H}), \ 5.89 \ (d, J = 5.1 \text{ Hz, 1H}), \ 5.80 \ (d, J = 8.3 \text{ Hz, 1H}), \ 4.47 - 4.34 \ (m, 3H), \ 4.30 \ (q, J = 4.7 \text{ Hz, 2H}), \ 2.72 \ (\text{hept, } J = 7.0 \text{ Hz, 1H}), \ 1.20 \ (\text{dd, } J = 7.0, \ 2.9 \text{ Hz, 6H}). \]

\[ ^{13} \text{C NMR} \ (126 \text{ MHz, Deuterium Oxide}) \delta \text{ ppm} \ 179.85, \ 151.06, \ 146.49, \ 131.11, \ 98.82, \ 88.57, \ 81.03, \ 72.56, \ 69.64, \ 63.63, \ 33.88, \ 18.20, \ 18.12. \]

3. Additional process information for step 3

Premixing of triazole, base and POCl₃ has been evaluated to understand the potential active intermediate in this reaction. NMR studies was run in CD₃CN, with 0.1 eq. Ph₃PO as an internal reference. Both \(^1\)H NMR and \(^{31}\)P NMR indicated that a new triazole/P related intermediate formed from the beginning which remained unchanged after more triazole (2 eq wrt to 1eq POCl₃) was charged. The additional triazole charged remained as free triazole based on this study.
4. NMR spectra

Major peak at -22.3 ppm with 2 eq. triazole charged – remained as major peak with more triazole charged and further aging.
5. Form and Particle Size Data

Two non-solvated forms, Form 1 and Form 2, were identified during process development for molnupiravir. Below are representative powder diffraction patterns of Form 1 and a sample enriched in Form 2. The diffraction patterns are very similar, but Form 2 can be distinguished by the presence of several unique reflections some of which include 17.8, 19.0, 22.1, 23.9 °2θ. Form 1 is the thermodynamically stable form under the process conditions and at ambient temperature.

![Diffraction patterns of Form 1 and Form 2](image)

Below is a representative particle size distribution generated by the manufacturing route of molnupiravir reported by volum distribution. The distribution is monomodal with the presence of a fines tail.

| D10 (µm) | D50 (µm) | D90 (µm) |
|----------|----------|----------|
| 21       | 81       | 167      |
6. References

1. Fulmer, G. R.; Miller, A. J. M.; Sherden, N. H.; Gottlieb, H. E.; Nudelman, A.; Stoltz, B. M.; Bercaw, J. E.; Goldberg, K. I. *Organometallics*. **2010**, 29, 2176-2179.