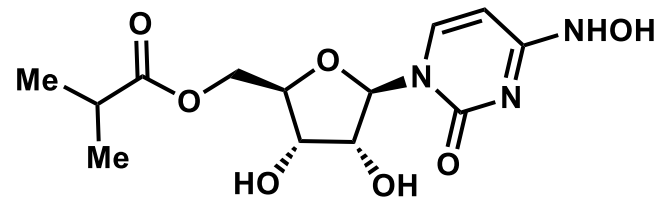


# Molnupiravir: Enzyme Recycling Information for M4ALL's Two-step Enzymatic Process

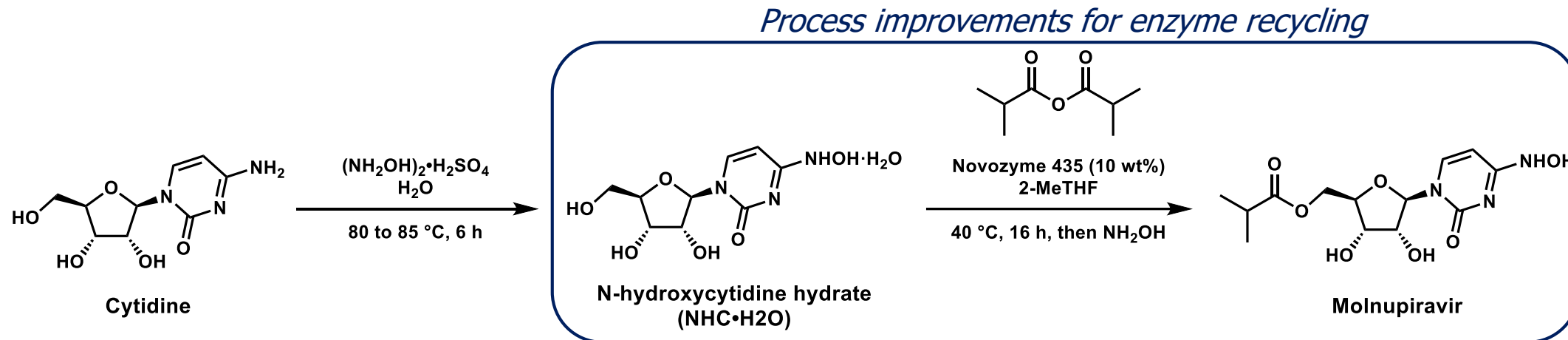
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January 2022



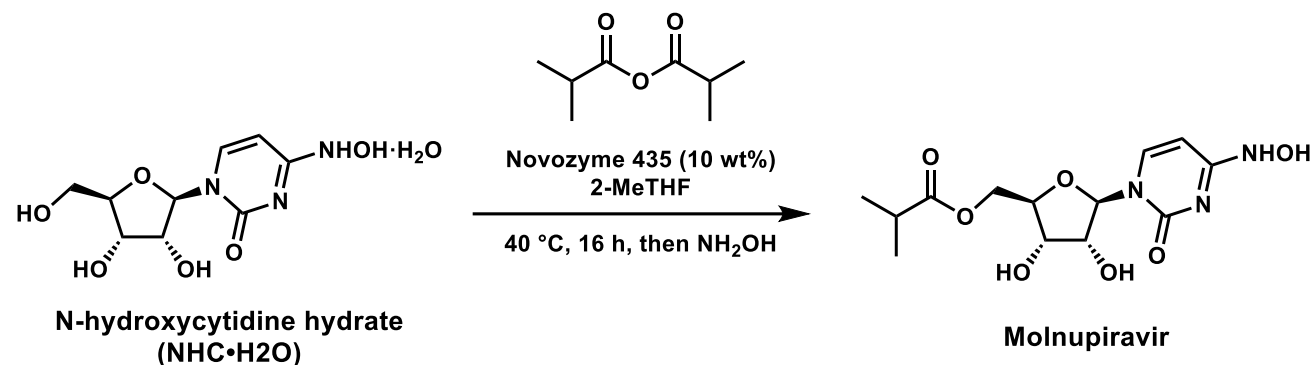
# Enzyme Recycling Lowers the Overall Raw Material Cost in M4ALL's Enzymatic Route to Molnupiravir



- M4ALL recently disclosed a low-cost, two-step process to molnupiravir starting from cytidine
  - The total raw material costs for this process are estimated to be \$260/kg
  - A significant cost driver is the polymer-supported lipase enzyme Novozyme 435 (50% of the total RM cost)
- To further lower raw material costs, we sought to identify the critical process parameters that were necessary to recycle the enzyme
- *The following modifications to our original procedure should make it possible to use a single charge of Novozyme for a total of 4 batches of molnupiravir*
  - A rotating bed reactor (RBR) reduces mechanical breakdown of the catalyst caused by a conventional stir blade
  - Low-cost, commercially-available isobutyric anhydride generates product of identical quality without catalyst deactivation
- Combined, *these modifications provide the following benefits for manufacturers* who take up this process
  - Total raw material costs could potentially be reduced to \$160/kg, assuming 3 reusages of the enzyme
  - An additional synthetic step is removed from the process (preparation of the oxime ester)

# Acylation Reaction Details for Enzyme Recycling

To a 200 mL baffled glass vessel<sup>1</sup> equipped with an internal temperature probe, N-hydroxycytidine hydrate<sup>2</sup> (NHC·H<sub>2</sub>O; 20.0 g, 72.14 mmol, 1.0 equiv.), 2-methyltetrahydrofuran (2-MeTHF, 100 mL, 5V), and isobutyric anhydride (23.93 mL, 144.3 mmol, 2.0 equiv.) were added. Novozyme 435 (2.0 g, 10 wt.% to NHC) was equally divided into the four compartments between the stir blades of the rotating bed reactor (RBR) basket.<sup>3</sup> This basket was then lowered into the reaction mixture and fixed into vessel on the shaft to the stirrer motor. The atmosphere was purged with N<sub>2</sub> gas for several minutes, before being sealed for the duration of the reaction. The mixture was then stirred at 840 RPM at 40 °C (internal temperature) for 16 h. At this point, an IPC (HPLC, 260 nm) of the reaction mixture should show an LCAP for molnupiravir products of >=91.<sup>4</sup> Then the reaction mass was drained from the glass vessel into a secondary reactor. The enzyme (still in the RBR basket) was washed by addition of 2-MeTHF (100 mL) to the original reactor. This wash was stirred at 840 RPM for 15 min at 40 °C before being drained into the secondary reactor with the original reaction mass. This process was repeated a second time, at which point the enzyme was ready for reuse.<sup>3</sup> Treatment of the final reaction mass with hydroxylamine and isolation of the crude molnupiravir were conducted in the same manner as described in our campaign report.<sup>5</sup>



Enzyme Recycle	Enzyme Activity (PLU)	Product LCAP		
		IPC 16 h <sup>4</sup>	Isolated Crude	Assay Yield (% , qNMR) <sup>6</sup>
Initial	11900	93.5	92.2	79
1	ND	93.9	93.8	ND
2	ND	92.7	91.6	ND
3	2800	91.0	91.0	80

- <https://www.spinchem.com/product/9200-001/>
- Purified by a single recrystallization from water (2.5 V). 100 wt% by HPLC.
- If enzyme is being recycled, it is left in the RBR basket after the 2-MeTHF washes from the previous batch, described at the end of the experimental procedure, and reused directly in the subsequent reaction.
- There are two peaks in the chromatogram that correspond to molnupiravir after treatment with hydroxylamine. These details are described in the campaign report (link below). The IPC data given in the table are the sum of the two LCAP values.
- [https://medicines4all.vcu.edu/media/medicines4all/assets/documents/TCG-M4ALL%20Final%20Campaign%20report\\_Molnupiravir.pdf](https://medicines4all.vcu.edu/media/medicines4all/assets/documents/TCG-M4ALL%20Final%20Campaign%20report_Molnupiravir.pdf)
- Determined from isolated crude product before crystallization.

# Enzyme Recycling Experiments: Key Findings

## Reactor Design

- In our original report, extremely slow stirring speeds were necessary to avoid mechanical decomposition of the supported enzyme by the stir blade
- A rotating bed reactor (RBR, purchased from SpinChem, Sweden) was explored to mitigate this issue
- *With the RBR, stir rates of up to 1000 rpm were tested without significant physical degradation of the catalyst*

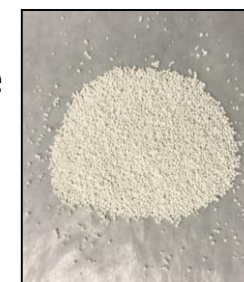
## Oxime Ester

- In our initial report, an isobutyryl oxime ester was used as the acylating reagent
  - This compound requires at least one additional synthetic step for preparation
- The nature and purity of this compound was found to have an impact on the reaction product profile and enzyme recyclability
  - Purification of the ester was demonstrated via distillation, but this is not recommended for manufacturing because of a relatively low thermal decomposition temperature
  - With recycled enzyme and purified ester ( $\geq 99\%$  purity), the reaction profile could be further improved by addition of substoichiometric triethylamine to stabilize pH
- *Isobutyric anhydride was found to be a superior acylating reagent if enzyme recyclability is required*
  - This low-cost, commercially-available reagent provides similar reaction profiles with recycled enzyme and without the addition of triethylamine for pH adjustment
  - In addition, lower reagent utilization (2 eq vs 3 eq) and a reduction in step count (no oxime ester synthesis) further improve manufacturing costs for this process

SpinChem Rotating Bed Reactor



Fresh Enzyme



After 3 usages in RBR

