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Enzymatic Catalysts to Combat COVID-19

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Enzyme engineering provides a scalable route to molnupavir, an orally bioavailable small molecule with therapeutic efficacy against COVID-19.

The COVID-19 pandemic has shined a harsh light on our world's impermanence. Our personal health, the way we interact with loved ones and how we educate our children can turn on a dime due to small changes in the nucleobase sequence of an RNA virus. This raises a philosophical question: do we fight change or embrace it? In this issue of *ACS Central Science*, McIntosh et al. demonstrate the bright side of mutagenesis in developing a scalable, biocatalytic method to synthesize molnupiravir, an orally available antiviral agent with clinical efficacy against COVID-19.[1]

Molnupiravir is a nucleoside analogue whose chemical structure mimics naturally occurring cytidine but with two important changes to enable antiviral function. First, its pyrimidine base is derivatized with a hydroxyl group at the N4 position. The alpha effect pushes the tautomeric equilibrium of the N^4-hydroxylated nucleobase toward the rare imino form (Figure 1). After being taken up by infected cells, the modified nucleoside is converted to a triphosphate by the endogenous cellular biosynthetic machinery, enabling it to function as a substrate for the viral RNA polymerase. Tautomerization to the imino form allows the N^4-hydroxylated CTP to pair with an A rather than a G in the template strand which—together with effects on downstream base incorporation—leads to a deluge of mutations in the virus's RNA.[2] Inefficient use by mammalian RNA polymerases and poor incorporation into DNA nucleotide pools limit the mutagenicity of this agent to human cells, accounting for its therapeutic index. The second change in molnupiravir is an isobutyl ester at the 5′-position of the ribose sugar, which helps the drug permeate the gut so it can liberate the active agent

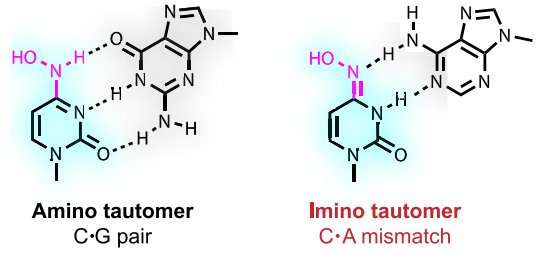

Figure 1. Base-pairing by the amino and imino tautomers of N^4-hydroxycytosine (the pyrimidine base of molnupiravir).

(N^4-hydroxycytidine) in the bloodstream.[3] This property is absolutely critical as it allows the molnupiravir drug to be self-administered in pill form, in contrast to existing treatments. In a planned interim analysis of a phase 3 clinical trial, molnupiravir reduced the risk of hospitalization or death in at-risk patients with COVID-19 by ∼50% relative to the control group.[4] Molnupiravir is currently approved for the treatment of mild-to-moderate COVID-19 in adults by the U.K.'s Medicines and Healthcare Products Regulatory Agency and is still under consideration for Emergency Use Authorization by the United States Food & Drug Administration and other regulatory agencies worldwide.

A stable, orally bioavailable antiviral has the potential to address inequities in vaccine distribution and democratize access to COVID-19 therapeutics worldwide.

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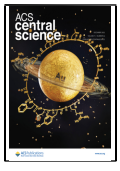

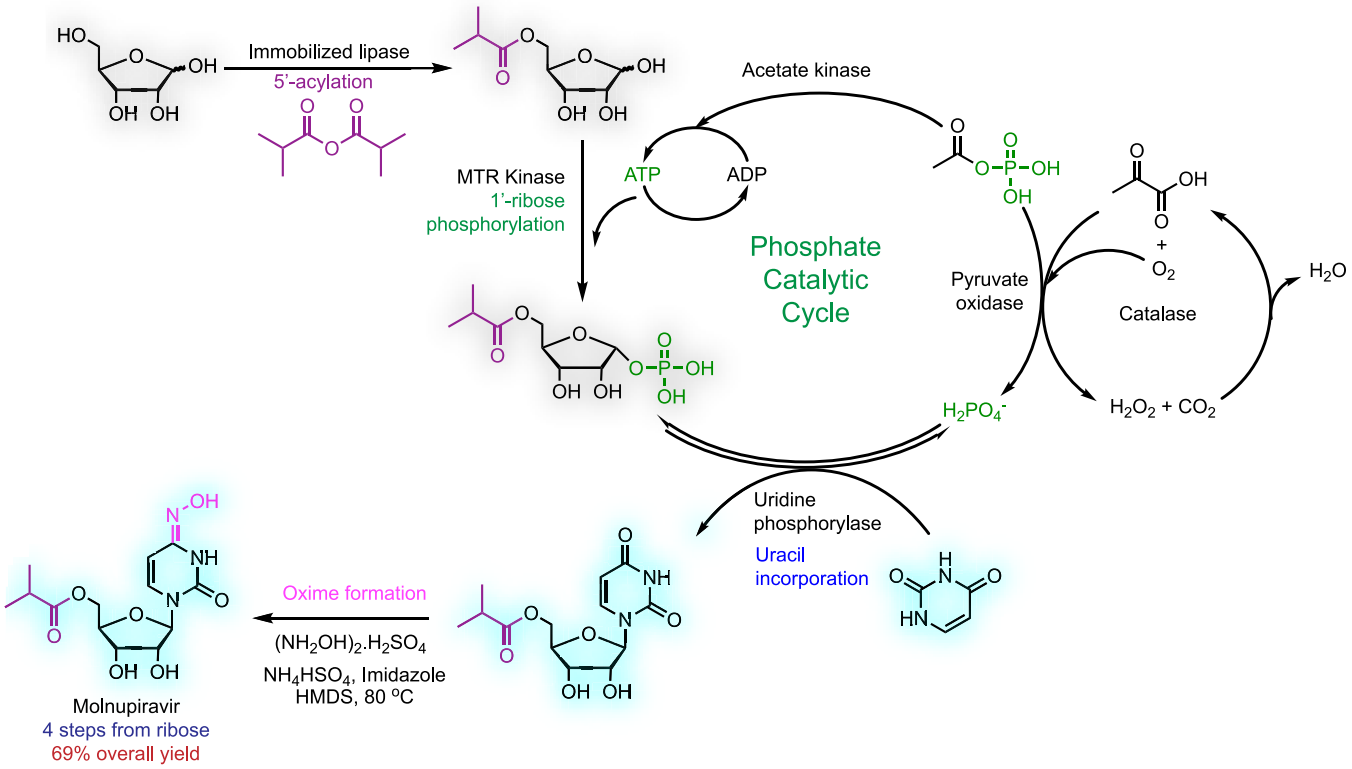

Figure 2. Complete biocatalytic cascade route to molnupavir developed by the Merck group.

Molnupiravir's significance is 2-fold. First, targeting viral replication itself may provide an effective approach against variants. Indeed, no reports of mutations in base excision or replication machinery conferring resistance to molnupiravir have yet been reported. Second, a stable, orally bioavailable antiviral has the potential to address inequities in vaccine distribution and democratize access to COVID-19 therapeutics worldwide. However, achieving this tremendous impact requires meeting an urgent challenge: developing a scalable, cost-effective route to molnupiravir in a time frame that is responsive to an ongoing pandemic.

Biocatalytic processes serve as linchpins in routes to therapeutic agents and have the paired advantages of efficiently mediating complex chemical transformations while yielding byproducts that are environmentally benign. Considering a biocatalytic route to molnupiravir, the Merck team stipulated that any transformation (1) must start from the cost-effective chemical starting material D-ribose and (2) be compatible with 5′-*O*-isobutyrylation, to circumvent the challenges of late-stage acylation.[5] The latter poses a significant technical barrier, as 5′-esterified sugars have never been used in any enzymatic nucleotide synthesis cascade. The Merck team hypothesized this could be solved by identifying two key enzymes: a ribosyl kinase that would phosphorylate a 5′-isobutyrylated sugar at the 1′-position and a nucleoside phosphorylase that would install a uracil base via C−N glycosidic bond formation.[1] To discover catalysts capable of mediating these transformations, the authors first screened a panel of natural enzymes for activity on the esterified sugar. This led to the identification of an *Escherichia coli* uridine phosphorylase and a *Klebsiella spp.* 5S-methylthioribose (MTR) kinase which showed modest activity on the isobutyrylated substrates. They then used site-directed mutagenesis to optimize each enzyme's activity. Iterative cycles of mutagenic optimization led to the identification of ribosyl kinase and uridine phosphorylase variants (containing 6 and 10 mutations, respectively) that displayed a 80−100 fold increased catalytic efficiency on the molnupiravir precursors. These enzymes were then employed in concert with other innovations, including the use of hexamethyldisilazane (HMDS) for parallel transient protection and oxime formation, an industrial-scale enzymatic coupling reaction to regenerate ATP and remove inorganic phosphate, and a chromatography-free strategy product isolation to yield pure molnupiravir in high yield in only four steps.[1]

> Should molnupiravir become a widely used anti-COVID medicine, it is not lost that both its mechanism and production benefit from mutagenesis, the same process currently giving rise to troublesome viral variants.

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The "pandemic preparedness" of the scientific community was fueled by decades of basic research that enabled the development and preclinical validation of novel vaccine platforms.[6,7] This study showcases another powerful example of this principle: harnessing advances in modern enzymology and molecular biology to create an enzymatic cascade that has the potential to accelerate global distribution of a much-needed orally available antiviral therapeutic. This study also raises some new questions. What is the versatility of this cascade for different ester groups? Could ribose esterification serve as a general strategy for optimizing the pharmacology of therapeutic nucleosides? Finally, should molnupiravir become a widely used anti-COVID medicine, it is not lost that both its mechanism and production benefit from mutagenesis, the same process currently giving rise to troublesome viral variants. In science, as in the world, the only thing that is certain is change.

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Notes

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REFERENCES

(1) McIntosh, J. A.; Benkovics, T.; Silverman, S. M.; Huffman, M. A.; Kong, J.; Maligres, P. E.; Itoh, T.; Yang, H.; Verma, D.; Pan, W.; Ho, H.-I.; Vroom, J.; Knight, A. M.; Hurtak, J. A.; Klapars, A.; Fryszkowska, A.; Morris, W. J.; Strotman, N. A.; Murphy, G. S.; Maloney, K. M.; Fier, P. S.; Engineered Ribosyl-1-Kinase Enables Concise Synthesis of Molnupiravir, an Antiviral for COVID-19. *ACS Cent. Sci.* **2021**. DOI: 10.1021/acscentsci.1c00608.

(2) Malone, B.; Campbell, E. A. Molnupiravir: Coding for Catastrophe. *Nat. Struct. Mol. Biol.* **2021**, *28* (9), 706−708.

(3) Toots, M.; Yoon, J.-J.; Cox, R. M.; Hart, M.; Sticher, Z. M.; Makhsous, N.; Plesker, R.; Barrena, A. H.; Reddy, P. G.; Mitchell, D. G.; Shean, R. C.; Bluemling, G. R.; Kolykhalov, A. A.; Greninger, A. L.; Natchus, M. G.; Painter, G. R.; Plemper, R. K. Characterization of Orally Efficacious Influenza Drug with High Resistance Barrier in Ferrets and Human Airway Epithelia. *Sci. Transl. Med.* **2019**, *11* (515). DOI: 10.1126/scitranslmed.aax5866.

(4) Willyard, C. How Antiviral Pill Molnupiravir Shot Ahead in the COVID Drug Hunt. *Nature* **2021**. DOI: 10.1038/d41586-021-02783-1.

(5) Hughes, D. L. Quest for a Cure: Potential Small-Molecule Treatments for COVID-19, Part 2. *Org. Process Res. Dev.* **2021**, *25*, 1089−1111.

(6) Graham, B. S.; Corbett, K. S. Prototype Pathogen Approach for Pandemic Preparedness: World on Fire. *J. Clin. Invest.* **2020**, *130* (7), 3348−3349.

(7) Nance, K. D.; Meier, J. L. Modifications in an Emergency: The Role of N1-Methylpseudouridine in COVID-19 Vaccines. *ACS Cent. Sci.* **2021**, *7* (5), 748−756.