Expedient Synthesis of Antiviral Drug Molnupiravir from D-Ribose and Cytosine

Saptashwa Chakraborty[1], Yogesh Sutar[1], Kameshwar Prasad[1], Jayashree Rajput[1], and Srinivas Hotha[1]*

[1] *Department of Chemistry, Indian Institute of Science Education and Research Pune, Pune – 411 008, India*

s.hotha@iiserpune.ac.in

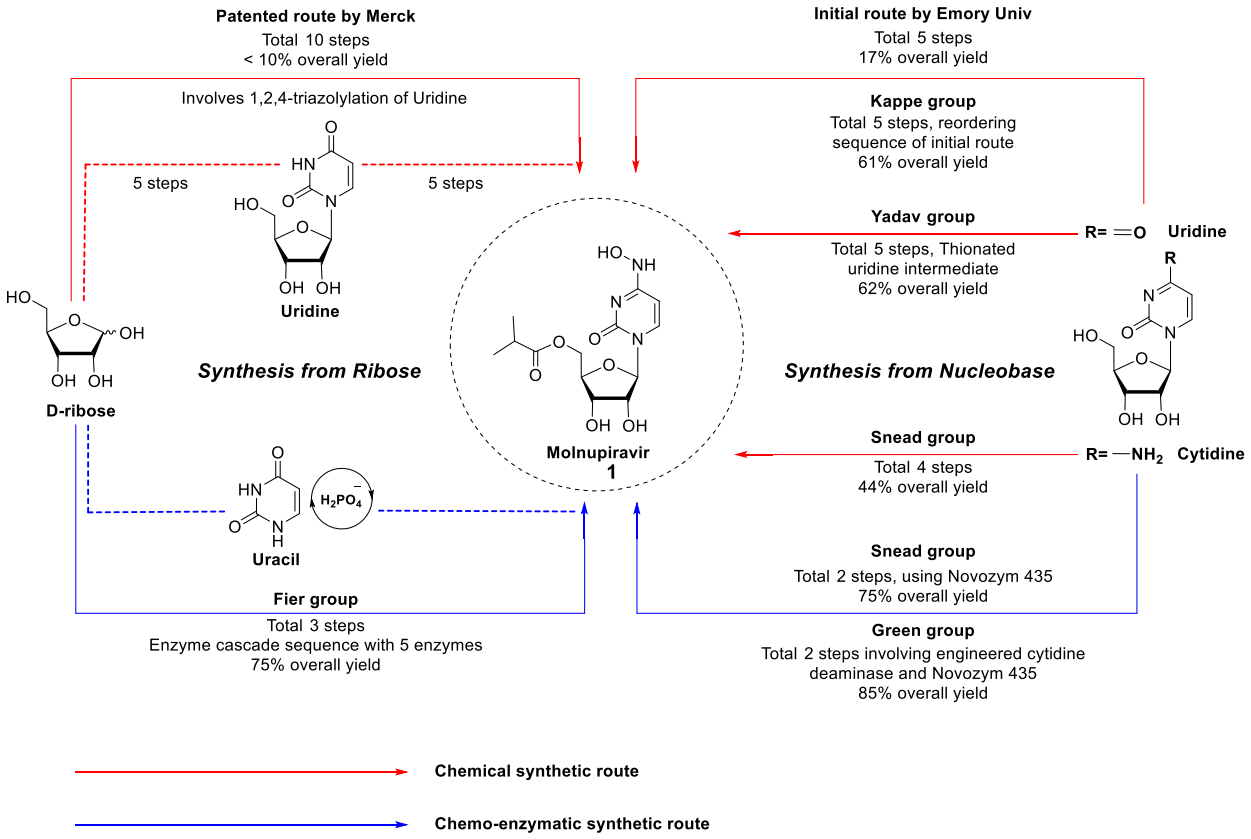
Keywords: Glycosidation, Glycosyl Donor, Molnupiravir, *N*-Glycosides, COVID-19

Abstract:

Molnupiravir is an antiviral drug that is approved to treat Sars-Cov2-infected patients. Determining a short and sustainable synthesis from commodity raw materials is essential given the compound's potential for high demand. Herein, we report a cost-effective scalable synthetic route for the API by exploiting mild *N*-glycosylation conditions utilizing metal-free activation of an alkynyl carbonate donor. The overall yield is increased up to 48% using minimum purification and sequential one-pot reaction.

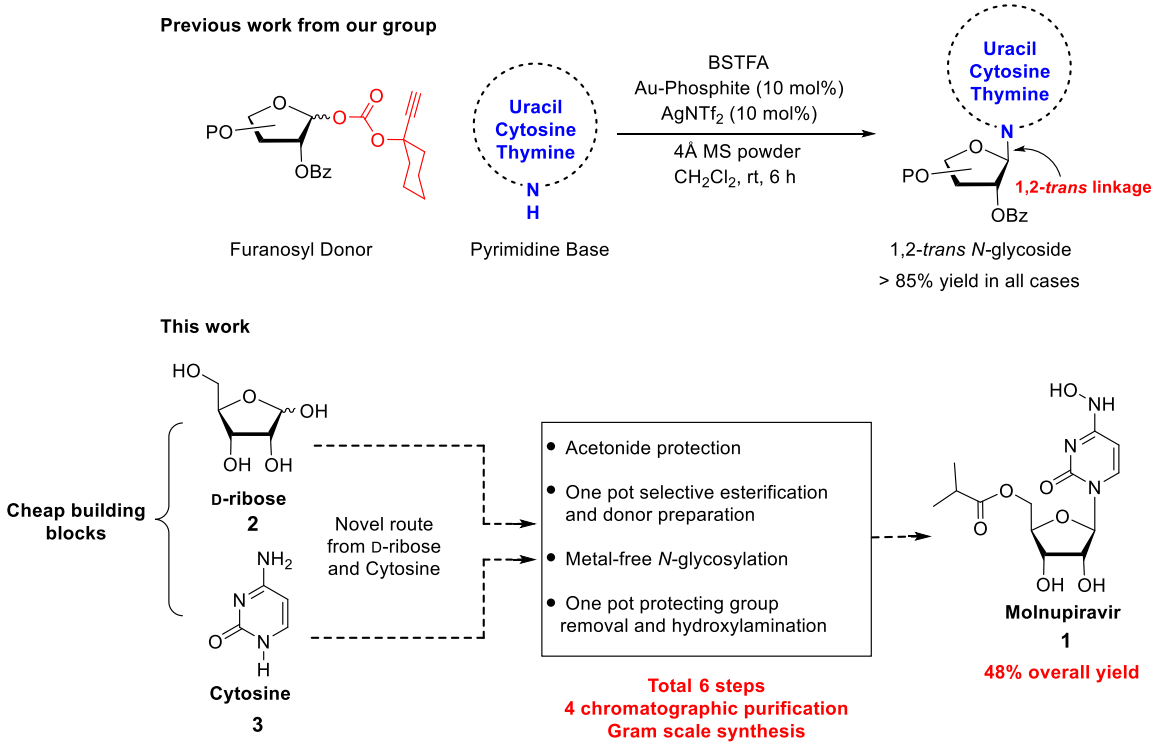
Introduction:

The COVID-19 pandemic has highlighted the lack of readily available antiviral agents and the dire need for developing economic processes to manufacture antiviral drugs on a large scale efficiently. Although the development of vaccines against this lethal virus promises a long-term solution, there are concerns regarding the mutation of the virus to escape acquired immunity.[3,4] Hence, it is equally essential to identify novel antiviral agents to treat viral infection and develop rapid and efficient synthetic routes to produce medicines from readily available commercial reagents. Molnupiravir or EIDD-2801 is a ribonucleoside prodrug that was first developed as an anti-influenza drug at Emory University in 2018.[5] This N^4-D-β-hydroxycytidine (NHC) analog has been approved as an antiviral drug for treating COVID-19 infected adult patients who are at risk of progressing to hospitalization.[6] Molnupiravir (**1**) is an attractive candidate to study for the global treatment of viral infection because of its structural simplicities, wide range of antiviral activities, and oral availability. Since the clinical trial and especially after the approval of this drug for COVID-19 treatment, there has been significant interest in developing cost-effective routes for bulk production of Molnupiravir (**1**). The first synthetic route reported by Painter *et al.*, is comprised of five



Scheme 1: Synthesis of Molnupiravir by chemical and chemo-enzymatic methods

steps starting from Uridine with unclear yields, unoptimized reaction conditions, and 17% overall yield[5]. Merck Co. in collaboration with Ridgeback Biotherapeutics has patented a 10-step route starting from ribose and uracil, although the overall yield of the process is less than 10%.[10] Later on, many groups reported chemical synthetic routes either starting from uridine or cytidine. The main disadvantages of the aforementioned synthetic routes are either low yielding triazolation step or the usage of nucleosides as starting material that is prepared in multiple steps from commercially available building blocks. The first chemo-enzymatic synthesis was reported from Snead group where Novozyme 435 was used for selective esterification. An engineered ribosyl-1-kinase and uridine phosphorylase enzymes cascade arrayed with a pyruvate-oxidase-enabled phosphate recycling strategy was used by Fier group to streamline the synthesis of the API starting from ribose and uracil[16]. Very recently, a biocatalytic process to produce Molnupiravir (**1**) using an engineered ribosyl kinase has been reported. Though advancement has taken place with enzymatic synthesis of this anti-viral drug, high cost and sometimes poor expression and scalability of enzymes, catalyst recycling, enzyme leaching, and purification are some of the major hurdles in developing

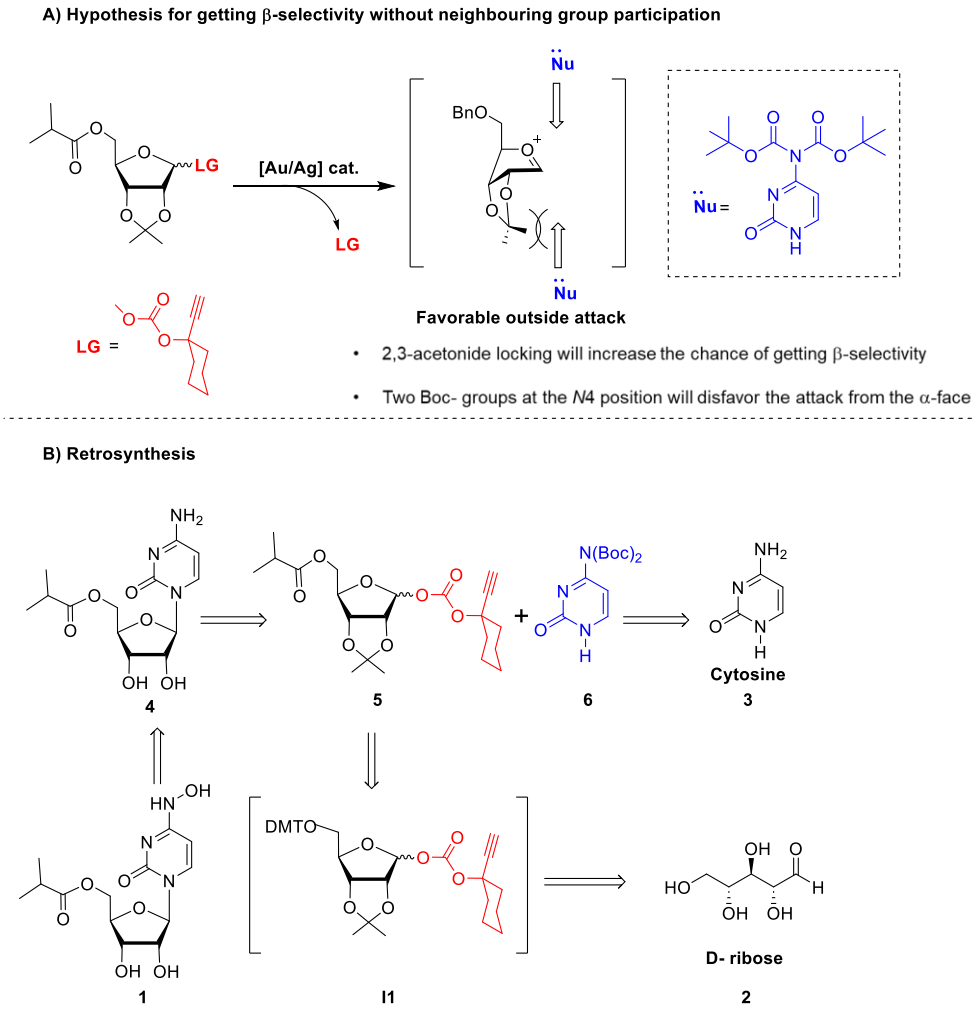


Scheme 2: Novel route for the synthesis of Molnupiravir

cost-effective production. Although scientists are currently working on standardizing robust large-scale manufacturing routes[18–20], there is scope for chemists to develop novel methods for synthesizing this antiviral drug from simple building blocks in minimum steps. Herein, we reported a convergent six-step gram-scale preparation of Molnupiravir with an overall yield of 48% from D-ribose and cytosine involving metal-free activation of glycosyl carbonate donor. The process involves a strategic sequential array of reactions to minimize purification.

Results and Discussion: A novel method from our group for synthesizing various *N*-glycosides in an efficient and high-yielding fashion inspired us to synthesize Molnupiravir in minimum steps. The glycosylation condition is mild, hence it has its advantages over conventional methods for coupling *N*-acceptors equipped with various protecting groups. The newly developed mild glycosylation method enables strategic implementation of protecting groups that help in increasing the solubility of the sparingly soluble nucleobases as well as improving regioselectivity. A ribofuranosyl donor and cytosine acceptor was chosen over conventionally used uracil to avoid the complex triazolation step. Instead of using commercially available N^4-Bz cytosine, we decided to protect the N^4 amine group of cytosine with bis-Boc group to increase the solubility of the nucleobase[21] as well as bypass the deacylation of N^4-Bz group as the primary hydroxyl of the prodrug is equipped with an isopropyl ester group. To minimize the overall number of steps, the implementation of an ester group at the *C*2 position of the ribofuranosyl donor was avoided.

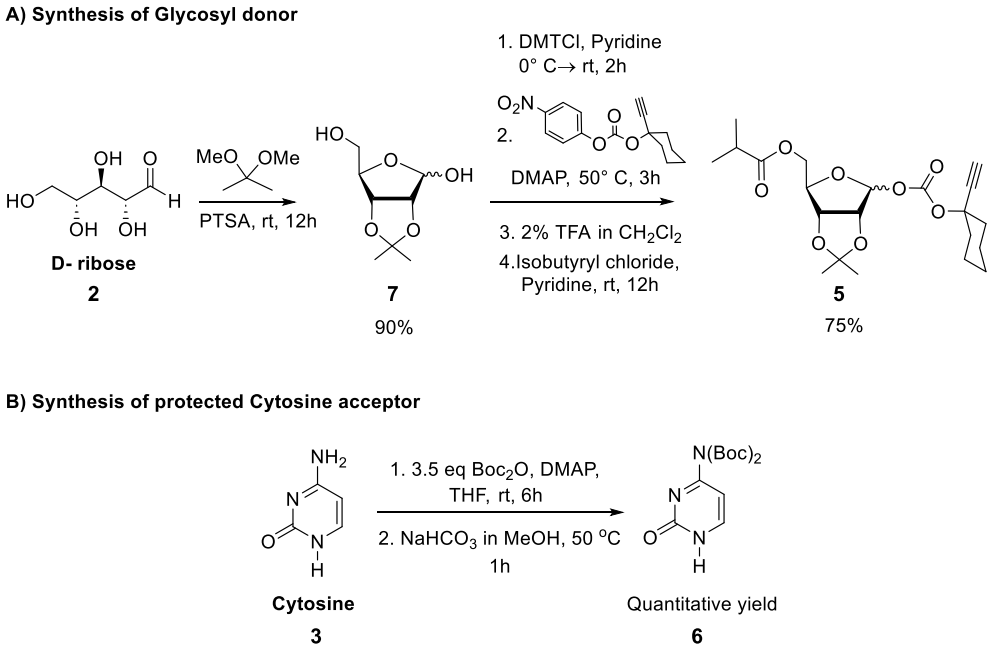
Thus, to attain the desired β-stereoselectivity at the anomeric center without neighboring group participation, we envisioned the installation of an acetonide group at the C2 and C3 positions. Although ribofuranosyl cation prefers 1,3-*cis* or inside attack of a nucleophile, here, in this case, the locked conformation of the oxacarbenium ion at the transition state would favor an unnatural outside attack of the nucleophile as the inside face is sterically hindered, therefore increasing the chance to get higher β-selectivity.[22] Two bulky Boc groups at the N^4 position will also favor attack from the α-side.



Scheme 3: A) Hypothesis of getting β-selectivity and B) Retrosynthesis of Molnupiravir

Retrosynthesis of the antiviral drug Molnupiravir **1** was exercised to accomplish the synthesis in a convergent fashion. Target molecule **1** could be obtained by hydroxylamination at the N^4 position of compound **4.** Compound **4** is a partially deprotected cytidine analogue that can be synthesized by coupling glycosyl donor **5** and acceptor **6**. Glycosyl donor **5** could be derived by deprotection of dimethoxy trityl

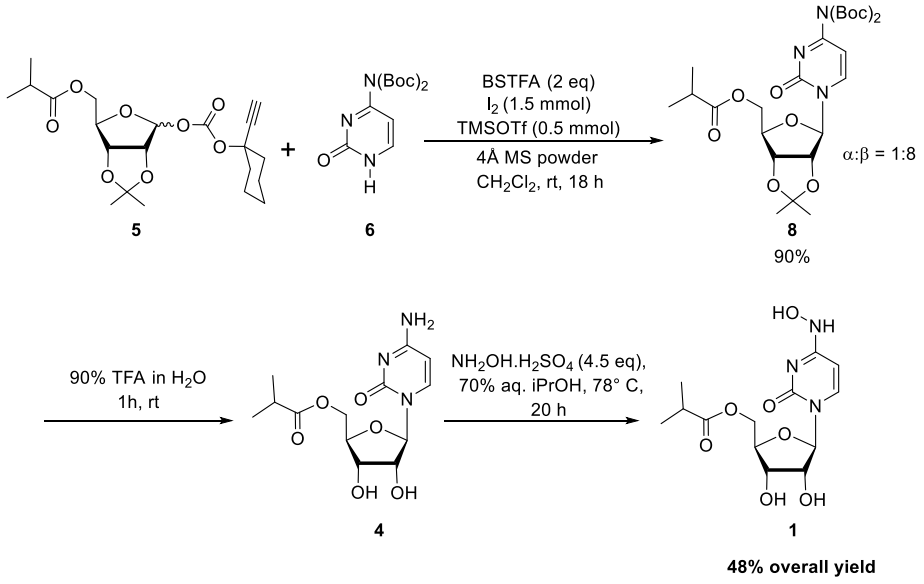
(DMT) group of intermediate compound **I1** that can be produced by multiple sequential reactions starting from cheap commodity chemical D-ribose **2**. On the other hand, glycosyl acceptor **6** can be prepared from cytosine in a sequential two-step reaction. Synthesis of glycosyl donor **5** started from D-ribose. Initially, anomeric allyl protection to lock the sugar ring in its furanoside form was unsuccessful as a significant amount of ribopyranoside was forming along with the furanoside, which will eventually lead to overall yield loss. So, we decided to protect 2,3 hydroxyls of D-ribose with acetonide group with 2,2-dimethoxy propane and PTSA. This way, ribofuranoside **7** was formed in 90% yield. Next, compound **7** was subjected to temporary selective primary hydroxyl group protection with dimethyl trityl group. The free anomeric hydroxyl group was then converted to ethynyl cyclohexyl propargyl donor in the same pot. Later, the acid labile DMT group was cleaved by using 3% trifluoroacetic acid, and esterification with isobutyryl chloride was performed. After three sequential reactions, donor **5** was isolated in 75% overall yield after one purification. For glycosyl acceptor **6**, commercially available cytosine **3** was protected with bis-Boc group in high yields. After the successful synthesis of donor **5** and acceptor **6**, these two compounds were



Scheme 4: Syntheses of glycosyl donor and acceptor in minimum steps

subjected to *N*-glycosylation reaction by following previously optimized conditions[23] with Au phosphite and $AgNTf_2$ co-catalysts. Surprisingly, we found that *O*-glycosidic product formed majorly, and less than 20% *N*-glycosidic product was detected. In our previous work, no such observation was found when the corresponding glycosyl donors are of armed, disarmed, and superarmed nature and $AgNTf_2$ and AgOTf as silver co-catalysts[23]. We hypothesized that both the stability of the oxacarbenium ion and the counter anion

present in the reaction mixture have an important role in the outcome of the glycosylation. A set of armed, disarmed, and superarmed ribofuranosyl donors were synthesized and subjected to glycosylation reaction with **6**, Au-phosphite, and $AgNTf_2$ or AgOTf as silver co-catalysts. In all the cases exclusive *N*-glycosidic product was observed. Next, we changed our attention to screen the role of counter anions that essentially come from the silver catalyst. A panel of Ag(I) catalysts with different counter anions were screened for the *N*-glycosylation reaction and an exclusive *N*-glycosidic product **8** was identified with AgOTf as the co-catalyst (see supporting information for details). The glycosylation yield was successfully increased up to 90% when TMSOTf was used as an additive. The best-optimized condition for the *N*-glycosylation reaction was 12 mol% of each Au-phosphite, AgOTf and TMSOTf. A recent finding from our group[24] describes pyrimidine *N*-glycosylation using a metal-free condition. Hence, to increase the cost-effectiveness of the overall procedure, glycosylation was performed using I_2 and TMSOTf. Mild glycosylation condition afforded nucleosidation with acetonide-protected ribofuranosyl donor and bis-Boc protected adenine to furnish the desired β isomer **8** in 90% yield along with 8:1 ratio of β:α isomer. The glycosylation reaction is compatible with gram-scale preparation. After the successful synthesis of the protected cytidine analog **8**, both Boc and acetonide groups were successfully removed in a single step by using trifluoro acetic acid to get compound **4** in quantitative yield. Next hydroxylamination at the N4 position was performed to get Molnupiravir **1**. The NMR and mass spectroscopy data were in accordance with previously reported values. H1 was identified at δ 6.02 ppm with a doublet (*J*=3.9 Hz*)* and anomeric C1 was identified at δ 85.5 ppm.



Scheme 4: Completion of synthesis

Conclusion:

We have successfully synthesized Molnupiravir (**1**) in just six steps and using four chromatographic purifications from commodity chemical D-ribose and Cytosine. The β-selectivity was achieved without anchimeric assistance and by the strategic implementation of acetonide group on the glycosyl donor and bis-Boc group on N^4 position of Cytosine. A stoichiometric amount of I_2 and TMSOTf could replace the costly metal catalysts for the *N*-glycosylation step effectively. The overall yield of the newly discovered synthetic route is 48%.

References:

(1) Coronavirus disease (COVID-19) – World Health Organization https://www.who.int/emergencies/diseases/novel-coronavirus-2019.

(2) Wang, Z.; Yang, L. Turning the Tide: Natural Products and Natural-Product-Inspired Chemicals as Potential Counters to SARS-CoV-2 Infection. *Front. Pharmacol.* **2020**, *11*, 1013.

(3) Harvey, W. T.; Carabelli, A. M.; Jackson, B.; Gupta, R. K.; Thomson, E. C.; Harrison, E. M.; Ludden, C.; Reeve, R.; Rambaut, A.; Peacock, S. J.; Robertson, D. L. SARS-CoV-2 Variants, Spike Mutations and Immune Escape. *Nat Rev Microbiol* **2021**, *19*, 409–424.

(4) Wibmer, C. K.; Ayres, F.; Hermanus, T.; Madzivhandila, M.; Kgagudi, P.; Oosthuysen, B.; Lambson, B. E.; de Oliveira, T.; Vermeulen, M.; van der Berg, K.; Rossouw, T.; Boswell, M.; Ueckermann, V.; Meiring, S.; von Gottberg, A.; Cohen, C.; Morris, L.; Bhiman, J. N.; Moore, P. L. SARS-CoV-2 501Y.V2 Escapes Neutralization by South African COVID-19 Donor Plasma. *Nat Med* **2021**, *27*, 622–625.

(5) Painter, G. R.; Guthrie, D. B.; BLUEMLING, G. R.; Natchus, M. G. N4-Hydroxycytidine and Derivatives and Anti-Viral Uses Related Thereto. WO2016106050A1, June 30, 2016.

(6) Merck and Ridgeback's Investigational Oral Antiviral Molnupiravir Reduced the Risk of Hospitalization or Death by Approximately 50 Percent Compared to Placebo for Patients with Mild or Moderate COVID-19 in Positive Interim Analysis of Phase 3 Study - Merck.com https://www.merck.com/news.

(7) Vieira, T.; Stevens, A. C.; Chtchemelinine, A.; Gao, D.; Badalov, P.; Heumann, L. Development of a Large-Scale Cyanation Process Using Continuous Flow Chemistry En Route to the Synthesis of Remdesivir. *Org. Process Res. Dev.* **2020**, *24*, 2113–2121.

(8) Kabinger, F.; Stiller, C.; Schmitzová, J.; Dienemann, C.; Kokic, G.; Hillen, H. S.; Höbartner, C.; Cramer, P. Mechanism of Molnupiravir-Induced SARS-CoV-2 Mutagenesis. *Nat Struct Mol Biol* **2021**, *28*, 740–746.

(9) Cox, R. M.; Wolf, J. D.; Plemper, R. K. Therapeutically Administered Ribonucleoside Analogue MK-4482/EIDD-2801 Blocks SARS-CoV-2 Transmission in Ferrets. *Nat Microbiol* **2021**, *6*, 11–18.

(10) Merck & Co. joins race for COVID-19 vaccines and therapies https://cen.acs.org/pharmaceuticals/drug-development/Merck.

(11) Steiner, A.; Znidar, D.; Ötvös, S. B.; Snead, D. R.; Dallinger, D.; Kappe, C. O. A High-Yielding Synthesis of EIDD-2801 from Uridine**. *Eur. J. Org. Chem.* **2020**, *2020*, 6736–6739.

(12) 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. *Syn lett* **2020**, 3.

(13) Dey, R.; Nayak, S.; Das, P.; Yadav, S. Short Synthesis of Molnupiravir (EIDD-2801) *via* a Thionated Uridine Intermediate. *ACS Omega* **2021**, *6*, 28366–28372.

(14) Hu, T.; Xie, Y.; Liu, Y.; Xue, H.; Zhu, F.; Aisa, H. A. A Convenient and Cost Efficient Route Suitable for "One-Pot" Synthesis of Molnupiravir. 5.

(15) 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. A Concise Route to MK-4482 (EIDD-2801) from Cytidine. *Chem. Commun.* **2020**, *56*, 13363–13364.

(16) 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**, *7*, 1980–1985.

(17) Burke, A. J.; Birmingham, W. R.; Zhuo, Y.; Thorpe, T. W.; Zucoloto da Costa, B.; Crawshaw, R.; Rowles, I.; Finnigan, J. D.; Young, C.; Holgate, G. M.; Muldowney, M. P.; Charnock, S. J.; Lovelock, S. L.; Turner, N. J.; Green, A. P. An Engineered Cytidine Deaminase for Biocatalytic Production of a Key Intermediate of the Covid-19 Antiviral Molnupiravir. *J. Am. Chem. Soc.* **2022**, *144*, 3761–3765.

(18) Paymode, D. J.; Vasudevan, N.; Ahmad, S.; Kadam, A. L.; Cardoso, F. S. P.; Burns, J. M.; Cook, D. W.; Stringham, R. W.; Snead, D. R. Toward a Practical, Two-Step Process for Molnupiravir: Direct

Hydroxamination of Cytidine Followed by Selective Esterification. *Org. Process Res. Dev*. **2021**, *25*, 1822–1830.

(19) Ahlqvist, G. P.; McGeough, C. P.; Senanayake, C.; Armstrong, J. D.; Yadaw, A.; Roy, S.; Ahmad, S.; Snead, D. R.; Jamison, T. F. Progress Toward a Large-Scale Synthesis of Molnupiravir (MK-4482, EIDD-2801) from Cytidine. *ACS Omega* **2021**, *6*, 10396–10402.

(20) Fier, P. S.; Xu, Y.; Poirier, M.; Brito, G.; Zheng, M.; Bade, R.; Sirota, E.; Stone, K.; Tan, L.; Humphrey, G. R.; Chang, D.; Bothe, J.; Zhang, Y.; Bernardoni, F.; Castro, S.; Zompa, M. A.; Taylor, J.; Sirk, K. M.; Diaz-Santana, A.; Diribe, I.; Emerson, K. M.; Krishnamurthi, B.; Zhao, R.; Ward, M.; Xiao, C.; Ouyand, H.; Zhan, J.; Morris, W. J. Development of a Robust Manufacturing Route for Molnupiravir, an Antiviral for the Treatment of COVID-19. *Org. Process Res. Dev.* **2021**, *25*, 2806–2815.

(21) Porcheddu, A.; Giacomelli, G.; Piredda, I.; Carta, M.; Nieddu, G. A Practical and Efficient Approach to PNA Monomers Compatible with Fmoc-Mediated Solid-Phase Synthesis Protocols. *Eur. J. Org. Chem.* **2008**, *2008*, 5786–5797.

(22) Ichikawa, S.; Hayashi, R.; Hirano, S.; Matsuda, A. Highly β-Selective C-Allylation of a Ribofuranoside Controlling Steric Hindrance in the Transition State. *Org. Lett.* **2008**, *10*, 5107–5110.

(23) Chakraborty, S.; Mishra, B.; Das, K,P.; Pasari, S.; Hotha, S. Synthesis of N-glycosides by Silver Assisted Gold Catalysis, *Angew. Chem. Int. Ed.* **2023**, 62, e2022141

(24) Prasad, K., Sharma, A., Pati, S., Taillefer, M., Jaroschik, F. and Hotha, S. Metal Free Activation of Alkynyl Glycosyl Carbonate Donors. *Advanced Synthesis & Catalysis*, **2023**, *365*(14), 2344-2349.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.