Introduction

Amidoximes, having both hydroxamino and imino groups at the same carbon atom, and their O-substituted derivatives form a unique class of pharmaceutical products with varied activities such as anti-bacterial, anti-tuberculosis, insecticidal, antiviral etc. A number of amidoximes are currently in use as drugs, or are under clinical trials. Molnupiravir, also known as EIDD-2801 and MK-4482, is one such compound being studied in late-stage clinical trials for combating infections caused by COVID-19, and acts by disrupting RNA synthesis. Its advantages over remdesivir, the other drug in use, are oral bioavailability and structural simplicity allowing its easier manufacture.

The reported synthetic route for molnupiravir disclosed by the original developers consists of a five-step synthesis starting from uridine. The method suffers from low yield (ca. 17%) and the use of an inefficient (yield 29%) triazole insertion step (Scheme 1A). In a revised route with an improved yield reported by Kappe, Dallinger, and co-workers, the triazole insertion was introduced in the first step of a modified procedure. Sned and co-workers published another route to molnupiravir with 60% yield starting with cytidine and using acetonide protection, esterification, hydroxymamination and acetonide deprotection. The same group also published two different two-step routes from cytidine, where selective enzymatic esterification of the primary alcohol replaced the diol protection/deprotection sequence. However, the drawbacks include use of expensive oxime ester, need for immobilized enzyme in large amounts, formation of diester (both at O- and NHO-), along with the desired monoester, and the necessity for removal of O-ester during transamination (Scheme 1B).

Merck & Co (USA) carried out an elegant enzymatic synthesis starting from ribose and uracil. However it entails the use of cocktails of costly enzymes and buffers used in the glycosylation step, while the high water solubility of the final product makes its isolation tricky. Our interest in nucleoside synthesis led us to devise an alternative to overcome the shortcomings. We envisaged that the amino group at C-4 might get substituted by hydroxylamine to form 4-hydroxylamino derivative (Scheme 1C). In the case of cytidine, this exchange may be facilitated by the presence of an electron withdrawing group (perhaps an acyl) on the nitrogen at C-4 and thereby N-acyl cytidine can be converted directly to amidoximes under mild conditions by the removal of corresponding amides. For this, our initial task was to synthesize N-acyl protected cytidine from the free base and thereafter attempt to selectively convert it to the 4-hydroxylamino derivative under controlled conditions to avoid deprotection of the ester at the primary hydroxyl group (Scheme 1C).

The advantages of the envisaged process will include (a) avoiding chlorinating solvents like POCl₃, and (b) better yield in the coupling step as compared to uracil. This would also present an alternative strategy to EIDD-2801, employing ribose and cytosine rather than cytidine. Thus, we focused on the development of an efficient route to access N-acyl cytidine derivative for a regio and stereo selective glycosylation.

Results and discussion

We initiated the process by synthetising the key intermediate 2 from commercially available 3-(-)-ribose. It could be locked in its furanoid form employing an HCl mediated acetonide protection procedure, followed by benzoylation of the free hydroxyl group of 1 with benzoyl chloride in pyridine at ambient temperature (Scheme 2). Hydrolysis of 2 with trifluoroacetic acid and water (9 : 1) resulted in the removal of the acetonide protection.
group at 2,3-position and the methyl group from anomeric hydroxy in a single step. Subsequent acetylation with acetic anhydride in pyridine furnished 1,2,3-tri-O-acetyl-5-benzoyl-β-D-ribofuranose in 90% overall yield (Scheme 2). The key step involved in the synthesis of molnupiravir is a stereo and regioselective glycosylation, where coupling of nucleobases 4a–c with 3 is desired. Silylation of nucleobases with hexamethyldisilazane (HMDS) in catalytic amount of TMSOTf prior to coupling with sugar donors is well known Silyl Hilbert-Johnson reaction which is reported mostly for the synthesis of uridine derivatives. But, mostly cytidine is obtained from uridine after a series of steps which involves chlorination with POCl3 and substituent installation of 1,2,4-triazole to get amine with the treatment of hydroxylamine (Scheme 3A). For direct access of cytidine Raffaele et al. reported microwave accelerated glycosylation approach from N-benzoyl cytosine with 50% yield (Scheme 3B). So in order to improve the yield further optimization of reaction was needed. Therefore we firstly treated N-protected cytosine 4a with HMDS and TMSOTf (catalytic amount) in acetonitrile at 80 °C for 1 h, then added 3 and SnCl4 and stirred at the same temperature only to end up with a mixture of regioisomers of cytidine derivatives in low yield (ESI, † Table 1, entry 1).

To increase the yield and regioselectivity we screened different solvents like DCM, DCE, DMF, and 1,4-dioxane. This showed that DCE was the best solvent to obtain a high yield of the product but regioselectivity remained a concern (ESI, † Table 1, entries 2–5). In order to improve this, we planned to perform the glycosylation with N-masked cytosine derivatives. To our delight, replacing cytosine with N-acetyl cytosine 4b and N-benzoyl cytosine 4c ensured the desired regioselectivity (ESI, † Table 1, entries 6–7). Decreasing the temperature from 80 °C to 60 °C resulted in still more increase in the selectivity and yield of the product, but further lowering to 40 °C was not helpful (ESI, † Table 1, entries 8–9). Also, using promoters other than SnCl4 gave unsatisfactory results (ESI, † Table 1, entries 10–12). Although decreased proportion of promoter affects the yield of the product, but increase beyond the stoichiometric amount did not improve the yield (ESI, † Table 1, entry 13–14). Consolidating the outcome of our optimization study, it appeared that using 1 equiv. of N-acetyl cytosine 4b with HMDS (1.5 equiv.) and TMSOTf (0.1 equiv.) at 80 °C for 1 h followed by cooling the reaction mixture down to 60 °C before the addition of 0.5 equiv. of 3 and 1 equiv. SnCl4 and allowing the reaction mixture to stir for 3 h at the same temperature delivers the desired nucleoside in good yield (Scheme 3).
In order to remove all the protecting groups, 5 was exposed to ammonia (20%) in methanol at 50 °C which resulted in deprotection of N-acetyl, O-acetyl and O-benzoyl groups within 5 h to deliver cytidine 6 (Scheme 3). To selectively introduce the isobutyl ester at the 5'-hydroxy and 4-amino positions of 6, the other hydroxy groups at 2' and 3' needed to be protected first. Using the reported conditions for the acetonide protection (5 mol% H2SO4 in acetone),21 different hydroxylamine was employed acetonide diester (Table 2, entry 3). Yield of the desired product was found better at the boiling point of water instead of 50 °C (Table 2, entry 4). Also time played a critical role on the outcome of the reaction. For instance, lowering of the time period of the reaction from 12 h to 40 min increased the yield of the desired product (Table 2, entry 5). To check the role of NaOAc, a reaction in the absence of it was performed only to get 9b in 20% yield (Table 2, entry 6). Replacement of NaOAc with other bases failed to improve the yield (Table 2, entries 7–11). On decreasing the proportion of hydroxylamine hydrochloride, the yield of the product was found to be decreased whereas an increase had little influence (Table 2, entries 12–13). Consolidating these outcomes, employing acetamide diester 8 (1 equiv.), hydroxyl amine hydrochloride (4.0 equiv.), and sodium acetate (2.5 equiv.) in water at 100 °C for 40 min proved to be the best condition for obtaining the desired product 9a (Scheme 3). Deprotection of the acetamide group of 8 to EIDD-2801 has been described to proceed in H2SO4 in combination with iPrOH as solvent. However, we failed to achieve satisfactory conversion under the reported conditions. But screening of different acids like formic acid, acetic acid, and trifluoroacetic acid (TFA) for different time periods revealed that an aqueous solution of TFA worked best,

| Entry | Cytosine | Promotor (equiv.) | Solvent | Temp. (°C) | Time (h) | Yieldb (%) |
|-------|----------|-------------------|---------|------------|----------|------------|
| 1     | 4a       | SnCl4 (1.0)       | ACN     | 80         | 12       | 20         |
| 2     | 4a       | SnCl4 (1.0)       | DCM     | 80         | 5        | 28         |
| 3     | 4a       | SnCl4 (1.0)       | DCE     | 80         | 3        | 54         |
| 4     | 4a       | SnCl4 (1.0)       | DMF     | 80         | 3        | 5          |
| 5     | 4a       | SnCl4 (1.0)       | 1,4 dioxane | 80     | 3        | 15         |
| 6     | 4b       | SnCl4 (1.0)       | DCE     | 80         | 3        | 64         |
| 7     | 4c       | SnCl4 (1.0)       | DCE     | 80         | 3        | 62         |
| 8     | 4b       | SnCl4 (1.0)       | DCE     | 60         | 3        | 70         |
| 9     | 4b       | SnCl4 (1.0)       | DCE     | 40         | 5        | 68         |
| 10    | 4b       | TMSOTf (1.0)      | DCE     | 60         | 3        | 48         |
| 11    | 4b       | ZnCl2 (1.0)       | DCE     | 40         | 3        | 18         |
| 12    | 4b       | TiCl4 (1.0)       | DCE     | 40         | 3        | 11         |
| 13    | 4b       | SnCl4 (0.5)       | DCE     | 60         | 3        | 52         |
| 14    | 4b       | SnCl4 (2.0)       | DCE     | 60         | 3        | 70         |

*Reaction conditions: 1 equiv. of N-acetyl cytosine 4b, HMDS (1.5 equiv.) and TMSOTf (0.1 equiv.) at 80 °C for 1 h followed by cooling the reaction mixture down to 60 °C before the addition of 0.5 equiv. of 3 and 1 equiv. SnCl4 and allowing the reaction mixture to stir for 3 h. b Isolated yield.

Table 1: Optimization of the reaction conditions

© 2021 The Author(s). Published by the Royal Society of Chemistry RSC Adv., 2021, 11, 36143–36147 | 36145
effecting the highest conversion (90%) to EIDD-2801 10 in 1 h at room temperature (Scheme 3). Finally, keeping in mind the requirement of large amount of drug and the availability of cytidine as a cheap raw material, we have carried out the 2-step synthesis of EIDD-2801 from cytidine 6, one of the intermediates in our synthetic route, in 62% overall yield (Scheme 4).

### Table 2  Standarization of reaction conditions

| Entry | Hydroxyl-amine deriv. (4 equiv.) | Base (2.5 equiv.) | Solvent | Time (h) | Yield † 9a : 9b |
|-------|---------------------------------|-------------------|---------|----------|-----------------|
| 1     | NH₂OH (2 M)                     | NaOAc             | iPrOH   | 12       | 0 : 50          |
| 2     | NH₂OH - HCl                     | NaOAc             | iPrOH   | 12       | 10 : 30         |
| 3     | NH₂OH - HCl                     | NaOAc             | H₂O     | 12       | 30 : 20         |
| 4     | NH₂OH - HCl                     | NaOAc             | H₂O     | 2        | 50 : 15         |
| 5     | NH₂OH - HCl                     | —                 | H₂O     | 40 min   | 88 : 0          |
| 6     | NH₂OH - HCl                     | Na₂CO₃            | H₂O     | 40 min   | 0 : 20          |
| 7     | NH₂OH - HCl                     | Na₂CO₃            | H₂O     | 1        | 00 : 15         |
| 8     | NH₂OH - HCl                     | NaHCO₃            | H₂O     | 1        | 00 : 23         |
| 9     | NH₂OH - HCl                     | Cs₂CO₃            | H₂O     | 1        | 00 : 18         |
| 10    | NH₂OH - HCl                     | KOAc              | H₂O     | 1        | 40 : 10         |
| 11    | NH₂OH - HCl                     | Ca(OAc)₂          | H₂O     | 1        | 23 : 14         |
| 12    | NH₂OH - HCl (2.0 equiv.)        | NaOAc             | H₂O     | 1        | 57 : 12         |
| 13    | NH₂OH - HCl (6.0 equiv.)        | NaOAc             | H₂O     | 1        | 75 : 0          |

† Reaction conditions: 1 equiv. acetonide diester 8, 4.0 equiv. (except entry 12, 13) hydroxyl amine hydrochloride (2 M NH₂OH for entry 1), and 2.5 equiv. base in water (iPrOH for entry 1,2) at 100 °C (50 °C for entry 1–3) for specified time. †† Isolated yield.

### Conclusions

In conclusion, we have developed an efficient process for the synthesis of molnupiravir starting with cheaper commercially available ribose and N₄-acetyl cytosine, which ensures stereo and regioselective glycosylation and regioselective conversion of N-acyl to hydroxylamino derivative in good to excellent yields. Other N-hydroxy cytidine derivatives could also be synthesized by employing this method.

### Conflicts of interest

There are no conflicts to declare.

### Acknowledgements

The authors are thankful to CSIR-India (HCP-29), SERB-India (EMR/2016/004710) for funding. A. A. is thankful to CSIR New Delhi for SRF. IIIM Publication No. CSIR-IIIM/IPR/00311.

### Notes and references

1. K. Fylaktakidou, D. Hadjipavlou-Litina, K. Litinas, E. Varella and D. Nicolaides, *Curr. Pharm. Des.*, 2008, 14, 1001–1047.
2. T. P. Sheahan, A. C. Sims, S. Zhou, R. L. Graham, A. J. Pruijssers, M. L. Agostini, S. R. Leist, A. Schäfer,
K. H. Dinnon 3rd, L. J. Stevens, J. D. Chappell, X. Lu, T. M. Hughes, A. S. George, C. S. Hill, S. A. Montgomery, A. J. Brown, G. R. Bluemling, M. G. Natchus, M. Saindane, A. A. Kolkyhalov, G. Painter, J. Harcourt, A. Tamin, N. J. Thornburg, R. Swanstrom, M. R. Denison and R. S. Baric, Sc. Trans. Med., 2020, 12(541), eabb5883.

3 (a) M. L. Agostini, A. J. Pruijssers, J. D. Chappell, J. Gribble, X. Lu, E. L. Andres, G. R. Bluemling, M. A. Lockwood, T. P. Sheahan and A. C. Sims, J. virol., 2019, 93(24), e01348-19; (b) N. Urakova, V. Kuznetsova, D. K. Crossman, A. Sokratian, D. B. Guthrie, A. A. Kolykhalov, M. A. Lockwood, M. G. Natchus, M. R. Crowley, G. R. Painter, E. I. Frolova and I. Frolov, J. Virol., 2018, 92(3), e01965-17.

4 Ridgeback Biotherapeutics and Merck Announce Preliminary Findings from a Phase 2a Trial of Investigational COVID-19 Therapeutic Molnupiravir. Merck, March 6, 2021, https://www.merck.com/news/ridgeback-biotherapeutics-and-merck-announce-preliminary-findingsfrom-a-phase-2a-trial-of-investigational-covid-19-therapeuticmolnupiravir/, accessed 2021-04-14.

5 (a) Merck and Ridgeback Bio Announce Closing of Collaboration and Licensing Transaction, July 1, 2020, Merck, https://www.merck.com/news/merck-and-ridgeback-bio-announce-closing-of-collaboration-and-licensing-transaction/, accessed 2021-04-14; (b) Efficacy and Safety of Molnupiravir (MK-4482) in Hospitalized Adult Participants with COVID-19 (MK-4482-001), https://clinicaltrials.gov/ct2/show/NCT04575584, accessed 2021-04-14.

6 D. J. Paymode, N. Vasudevan, S. Ahmad, A. L. Kadam, F. S. P. Cardoso, J. Burns, D. W. Cook, R. W. Stringham and D. R. Sneed, Org. Process Res. Dev., 2021, 25, 1822.

7 G. R. Painter, G. R. Bluemling, M. G. Natchus and D. Guthrie, N2-Hydroxycytidine and Derivatives and Anti-viral Uses Related Thereto, US 2016/106050 Al, June 13, 2019.

8 A. Steiner, D. Znidar, S. B. ¨O. tv¨os, D. R. Sneed, D. Dallinger and C. O. Kappe, Eur. J. Org. Chem., 2020, 2020, 6736.

9 V. Gopalsamuthiram, C. Williams, J. Noble, T. F. Jamison, B. F. Gupton and D. R. Sneed, Synlett, 2021, 32, 326.

10 N. Vasudevan, G. P. Ahlqvist, C. P. McGeough, D. J. Paymode, F. S. Cardoso, T. Lucas, J.-P. Dietz, T. Opatz, T. F. Jamison, F. B. Gupton and D. R. Sneed, Chem. Commun., 2020, 56, 13363–13364.

11 G. P. Ahlqvist, C. P. McGeough, C. Senanayake, J. D. Armstrong, A. Yadaw, S. Roy, S. Ahmad, D. R. Sneed and T. F. Jamison, ACS Omega, 2021, 6, 10396–10402.

12 T. Benkovics, J. A. McIntosh, S. M. Silverman, J. Kong, P. Maliges, T. Itoh, H. Yang, M. A. Huffman, D. Verma, W. Pan, H.-I. Ho, J. Vroom, A. Knight, J. Hurtak, W. Morris, N. A. Strotman, G. Murphy, K. M. Maloney and P. S. Fier, Evolving to an Ideal Synthesis of Molnupiravir, an Investigational Treatment for COVID-19, ChemRxiv, 2020, DOI: 10.26434/chemrxiv.13472373.v1.

13 (a) F. Rasool and D. Mukherjee, Org. Lett., 2017, 19, 4936; (b) A. Ahmed, F. Rasool, G. Singh, M. Katoch and D. Mukherjee, Eur. J. Org. Chem., 2020, 2020, 4408.

14 A. B. Smith, Q. H. Paul, A. S. Breslin and G. K. Beauchamp, Org. Lett., 2005, 7, 5075.

15 U. Niedballa and H. Vorbr¨uggen, J. Org. Chem., 1976, 41, 2084–2086.

16 A. Amanteal, M. Henz and P. Strazewski, Helv. Chim. Acta, 1996, 79, 244.

17 B. C. Bookser and N. B. Raffaele, J. Org. Chem., 2007, 72, 173–179.

18 A. Ahmed, J. S. Banday, Q. N. Ahmed, D. Mukherjee, Non infringing process for the synthesis of N4-hydroxycytidine and its derivatives, IN202111020678, May, 6, 2021.