A concise route to MK-4482 (EIDD-2801) from cytidine†

N. Vasudevan, Grace P. Ahlqvist, Catherine P. McGeough, Dinesh J. Paymode, Flavio S. P. Cardoso, Tobias Lucas, Jule-Phillip Dietz, Till Opatz, Timothy F. Jamison, Frank B. Gupton and David R. Snead*

A two-step route to MK-4482 (EIDD-2801, 1) was developed consisting of an esterification and hydroamination of cytidine. The selective acylation and direct amination eliminate the need for protecting and activating groups and proceed in overall yield of 75%, a significant advancement over the reported yield of 17%. The step count is reduced from five transformations to two, and expensive uridine is replaced with the more available cytidine.

Remdesivir has presented itself as an option for COVID-19 treatment; however, improvements upon this initial solution are still desired. Fulfilling demand is complicated by issues related to raw material supply, price, and synthetic route length. MK-4482 (EIDD-2801) presents an interesting complement to remdesivir for COVID-19 treatment. It is structurally simple in comparison, and it can likely be made from abundant raw materials. These factors would be expected to alleviate supply chain difficulties and reduce costs. Encouragingly, MK-4482 shows potential to treat mice with remdesivir resistant strains of COVID-19, and the active pharmaceutical ingredient (API) is orally bioavailable. These advantages prompted Merck to license the drug candidate from Ridgeback Biotherapeutics.

The initial disclosure of MK-4482 is the only synthesis which appears in the open literature (Fig. 1), and not unexpectedly there are significant opportunities for improvement over this early route:

- The API is constructed over five chemical transformations.
- The route suffers from low yield (17% maximum, yield of diol deprotection not disclosed).
- The step count is lengthened by derivatizations and protections.

- Uridine, an expensive material of limited availability, is the synthetic starting point.

Building the API from cytidine instead of uridine presents several advantages. First, raw material costs can be decreased because cytidine is ~40% of the price of uridine. Secondly, there is potential to reduce the synthesis to a two-step sequence comprising esterification and transamination (Fig. 2).

We began our exploration by examining direct transamination of cytidine with hydroxylamine. Older literature studies suggested that mono-hydroxamination can be achieved under the right concentration, temperature and pH, while minimizing over-reaction of the substrate. More recently this finding was repeated by Purohit with preparative HPLC separation while Painter claimed difficulties with the procedure leading to 20% yield. In our hands, with slight adjustment of the reaction conditions, N(4)-hydroxycytidine (NHC, 3) was synthesized in 70% assay yield (AY). Importantly, upon concentration, pure NHC was obtained by simple crystallization directly from the reaction mixture in 50% isolated yield.

We also explored transamination of cytidine isobutyryl ester 4, and surprisingly, with the use of NH₂OH·H₂SO₄ in iPrOH,
approach would work to form the desired but also for cytidine. Isobutyric oxime ester agents was of particular interest due to the structural similarity and anhydride acyl donors. The use of oxime ester transfer of cytidine has achieved this goal by making use of vinyl esters over-acylation of the product, resulting in an isolated yield of the intermediate at scale. Conversion was stopped at 90% to halt byproducts are formed which might hamper efforts to purify might not be preferable to the enzymatic route. More reagents suggests that the selective for one of four hydroxyl groups, and the literature obtained from MK-4482. The esterification of NHC to MK-4482. The esterification of NHC-branched esters.

Surprisingly immobilized CALB (Candida Antarctica Lipase B) provided the desired selectivity not only for N-hydroxycytidine but also for cytidine. Isobutyric oxime ester 5 was used as the acyl transfer agent with solid supported enzyme (200 wt%, 1.5 mol%). A sufficient excess of the oxime ester was necessary to drive the reaction to completion, and early results have been best with 1,4-dioxane. MK-4482 was isolated in 74% yield from 3, and 4 was isolated in 78% yield from cytidine. A traditional chemically catalyzed acylation was developed to provide a non-enzymatic option to reach 4. Though inexpensive this option might not be preferable to the enzymatic route. More reagents are added to the reaction system and a greater number of byproducts are formed which might hamper efforts to purify the intermediate at scale. Conversion was stopped at 90% to halt over-acylation of the product, resulting in an isolated yield of 76% of 4. Similarly, an alternative route to 3 was developed from uridine.

This completes two concise routes to MK-4482, which differ in the order of synthetic transformations. When conducting esterification first, MK-4482 is obtained in 75% yield, and it is made in 37% yield when hydroxamination is conducted first. The step cost is reduced from five transformations to two, and the more expensive uridine is replaced with cytidine. The use of protecting groups and derivatization is eliminated. We plan to further report on the optimization of this preliminary result to refine catalyst loadings, solvent selection, and yield while developing process-amenable isolation sequences.

We thank the Bill and Melinda Gates Foundation for their longstanding support of our research. Grace Ahlgqvist would also like to acknowledge support by the National Science Foundation Graduate Research Fellowship under Grant No. 1745302.

Conflicts of interest

There are no conflicts to declare.

Notes and references


6. Prices obtained through analysis of Indian import/export records.


11. See ESI for details.