Molnupiravir (MK-4482)

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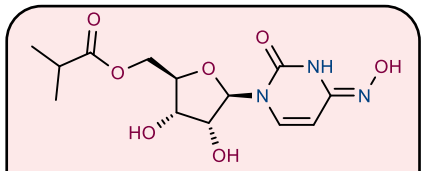
MERCK

Projected Sales By 2022:
3 billion USD

Molnupiravir was initially discovered as an anti-vrial at Emory University in 2018. The drug would laterbe acquired by Ridgeback Therapeutics, who would then partner with Merck.

The drug has shown significant promise as a COVID-19 thereapeutic and as of June 2021, the US Department of Health and Human Services has agreed to purchase 1.2 billion USD worth of courses (approximately 1.7 million).



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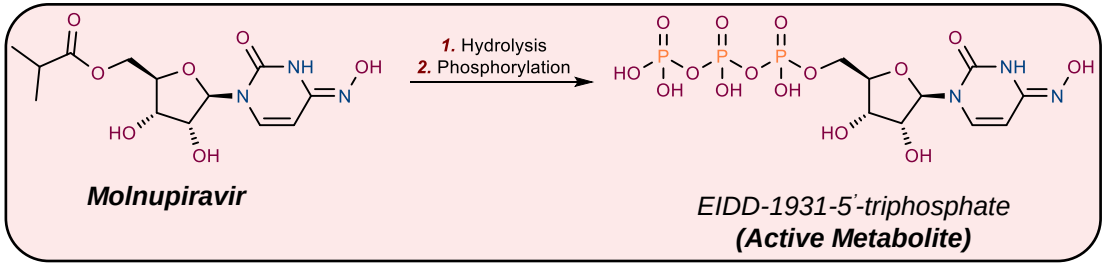
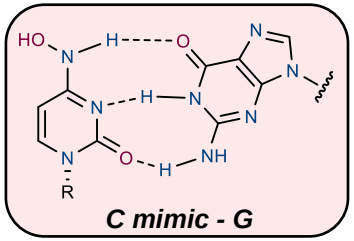
Mechanism of Action and Pre-Clinical Data:

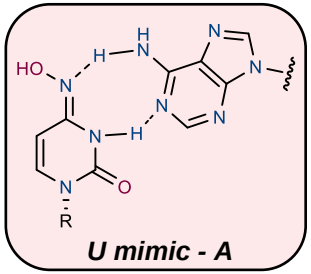


Molnupiravir

EIDD-1931-5'-triphosphate
(Active Metabolite)



C mimic - G

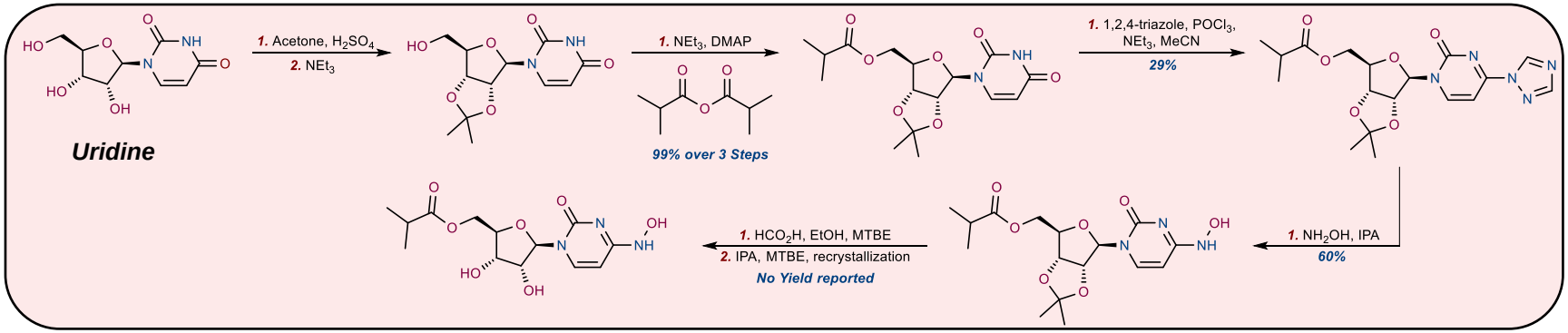


U mimic - A

Molnupiravir is metablized into a ribonucleoside analogue, resembling cytidine. Viral RNA polymerase incorporates this active metabolite into newly synthesized RNA, rather than cytidine. Depending on tautomeric form, the metabolite can interestingly mimic cytidine or uridine, thus being incorporated into a number of mutations, inducing lethal mutagenesis.

Molnupiravir went into Phase II Clinical Trials in October 2020, as of October 2021, preclinical data has demonstrated that treatment with molnupiravir reduced risk of hospitalization and death from COVID-19 by about 50% for newly diagnosed, high-risk patients.

Reported Discovery Route:



Uridine

Benkovics, T. *Chem Rxiv.* **2020.** 10.26434/chemrxiv.13472373.v1

Jamison, T. *ACS Omega* 2021, 6, 15, 10396–1040. https://doi.org/10.1021/acsomega.1c00772

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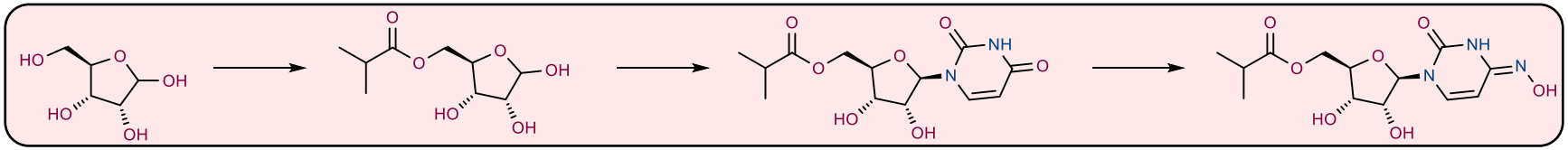
Inspiration for the Merck Process Route:

Merck highlights a few problems with the existing synthetic routes and discusses their vision for an "ideal" synthesis:

1. No existing synthesis utilizes true commodity raw materials available on multi-kilogram scales without a need for building blocks.
2. No existing synthesis is green on the levels Merck envisions.
3. No scalable routes have been free of purification steps and met the above criteria.

"…neither cytidine nor uridine should be considered as true commodity raw materials, as both are typically prepared in at least four steps from ribose, their syntheses are not inherently green, and each has supply chain risks on the scales needed". (Ac protection, Bn protection, chlorination then addition of uracil, deprotection.)

The Envisioned Optimum Route:



Esterification: While this step seems trivial, there are numerous issues highlighted:

1. Ribose adopts its pyranose form in many solvents, where 1º alcohol exists as hemi-acetal
2. Ribose has poor solubility profile in many intert process solvents
3. The esterified ribose is more soluble than ribose, which leads to over-reaction.
4. Product is highly water soluble and hard to crystallize.

Anomeric Incorporation of Uracil: While this step seems trivial, there are numerous issues highlighted:

1. Direct addition of Uracil to this anomeric position is unknown.
2. Biosynthetically, this O-PO_3 bond is normally forged by phosphorylation of the free 5' OH which is then enzymatically isomerized to the anomeric OH (which can then go on to incorporate the nucleobase).

Conversion of amidic carbonyl to oxime: Other methods have explored this transformation on very similar systems; however, optimization was nonetheless desired.

While this route seems straightforward in principle, it would require the development of two novel reactions.

Benkovics, T. *Chem Rxiv.* **2020.** 10.26434/chemrxiv.13472373.v1

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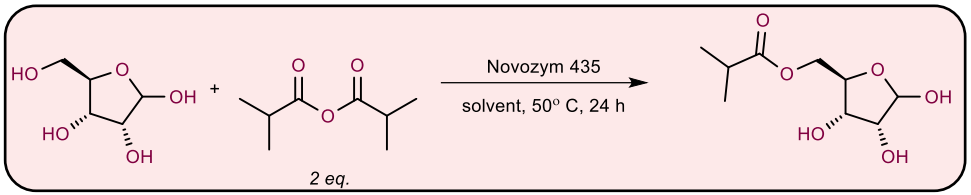
The Developed Process Route – A Testament to the Power of Biocatalysis:

Biocatalytic Esterification:



Lipase enzymes are well-precedented to aid in selective esterification, thus the known system (available on multi-metric ton quantities from Novozymes was explored. Novozym 435 is an immobilized Ca1B lipase..

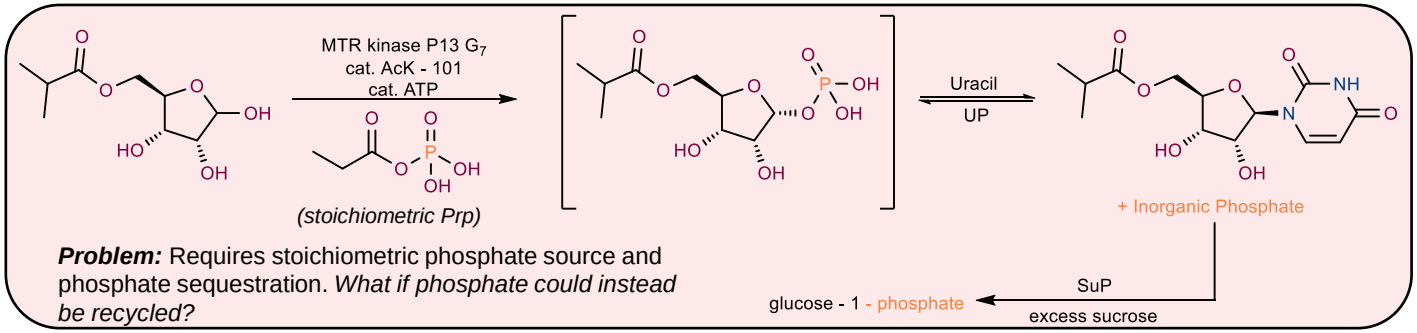
Product was able to be filtered and extracted in water to be used directly in the next step with few remaining impurities.

Entry	Solvent	Novozym 435 (wt%)	Conversion (%)	Yield (%)
1	EtOH	50	0	N/A
2	iPrOH	50	8	N/A
3	tBuOH	50	98	N/A
4	THF	50	47	N/A
5	Acetone	50	99	N/A
6	tBuOH	20	86	79
7	Acetone	20	99	94
8	Acetone	10	99	94

Biocatalytic Addition of Uracil (Gen 1): A Novel Direct Disconnection

Nature utilizes MTR kinases to phosphorylate the 1 position of 5-S-methylthioribose. However, these MTR kinases do not participate in nucleoside biosynthesis in nature. Could these enzymes be leveraged or modified to do this transformation?

Numerous MTR kinases were screened, and the best lead underwent rounds of directed evolution which would eventually result in MTR kinase P13 G_7, capable of undergoing this transformation with 99% yield with lower loadings.



Problem: Requires stoichiometric phosphate source and phosphate sequestration. *What if phosphate could instead be recycled?*

MTR – 5-S-methylthioribose *– Phosphorylation of anomeric OH*
AcK – Acetate Kinase *– Aids in Regeneration of ATP*
UP – Uridine Phosphorylase *– Adds Uridine to O-PO_3* and helps drive equilibrium to the right
Prp *– propionyl phosphate (stoichiometric phosphate source)*

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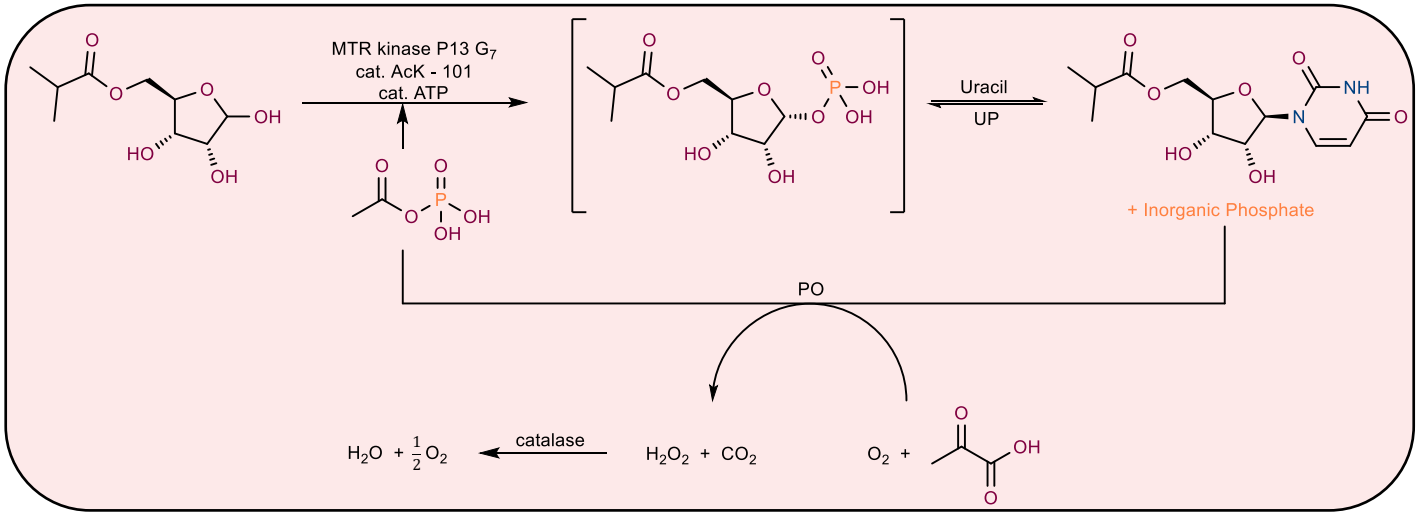
Biocatalytic Addition of Uracil (Gen 2): A Novel Catalytic Cycle



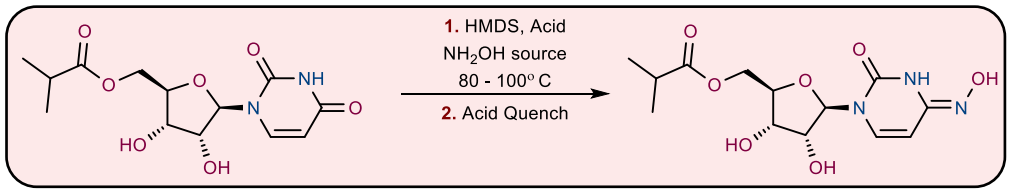
MTR – 5-S-methylthioribose *– Phosphorylation of anomeric OH*
AcK – Acetate Kinase *– Aids in Regeneration of ATP*
UP – Uridine Phosphorylase *– Adds Uridine to O-PO_3*
PO – Pyruvate Oxidase *– Generates Acetyl Phosphate from Pyruvic Acid and Inorganic Phosphate*
Catalase *– Breaks down H2O2 generated in this process into water and oxygen*

**Biocatalytic cycle can be run at >80g/L concentration.*

**No hazardous side products, only H_2O and O_2.*

Oxime Formation:

Early screens revealed phosphate and silyl activation worked to add the oxime, however, they wanted to avoid the use of TMSCl and phosphates as they are either corrosive or toxic. Thus, HMDS was selected as the silyl activator to optimize with.



Entry	NH_2OH Reagent	Solvent	Acid	Conversion
1	NH_2OH * HCl	sulfolane	none	0
2	NH_2OH * HCl	sulfolane	TfOH	27
3	NH_2OH * HCl	sulfolane	H_2SO_4	85
4	$(NH_2OH)_2$ * H_2SO_4	sulfolane	H_2SO_4	89
5	$(NH_2OH)_2$ * H_2SO_4	2-MeTHF	H_2SO_4	79
6	$(NH_2OH)_2$ * H_2SO_4	DME	H_2SO_4	89
7	$(NH_2OH)_2$ * H_2SO_4	none	H_2SO_4	89
8	$(NH_2OH)_2$ * H_2SO_4	none	$(NH_4)HSO_4$	69
9	$(NH_2OH)_2$ * H_2SO_4	none	$(NH_4)HSO_4$ + 0.5 eq. imid.	99

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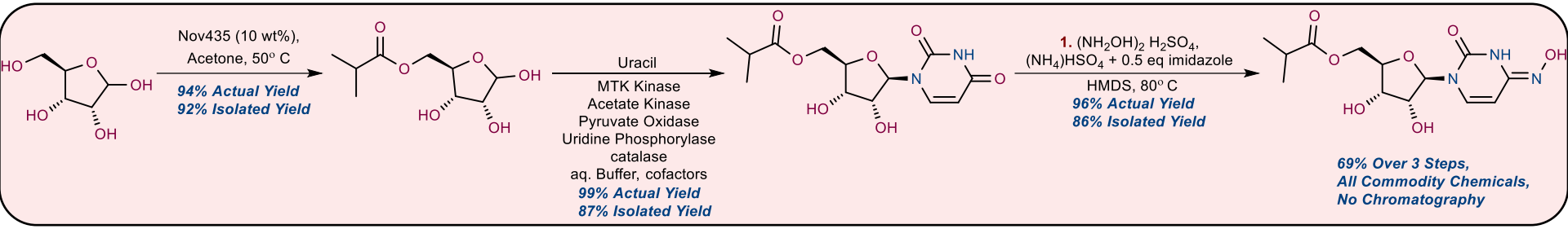
The Final Synthesis:



After final product is synthesized, the following protocol is followed to purify:
-pH adjusted and inorganic impurities washed away
-pH adjusted again and recrystallization

Previous discovery routes: approximately 10% over 10 steps