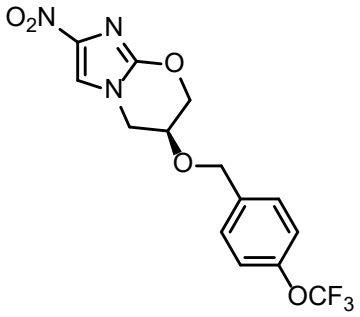
Medicines for All Institute

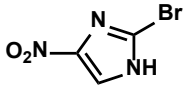
Summary of Process Development Work on the Tuberculosis Drug

Pretomanid



and its key cost-driving Intermediate

2-bromo-4-nitro-1*H*-imidazole ("CBr03")



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Executive Summary

"Tuberculosis (TB) is a global disease, found in every country in the world. It is one of the leading infectious causes of death worldwide. The World Health Organization estimates that 1.8 billion people—close to one quarter of the world's population—are infected with Mycobacterium tuberculosis (M.tb), the bacteria that causes TB," a global pandemic which caused the deaths of about 1.6 million in 2021.[1] "Pretomanid, a novel compound developed by the non-profit organization TB Alliance, was approved by the U.S. Food & Drug Administration (FDA) today for treating some of the most drug-resistant forms of tuberculosis (TB)."[2] A key intermediate in the synthesis of this important TB drug, and other nitroimidazole based APIs, is 2-bromo-4-nitro-1H-imidazole ("CBr03") which we determined to be a key cost-driver in the synthesis of pretomanid. Prior publications that describe the synthesis of CBr03, however, utilized hazardous chemical reagents[4] and intermediates,[3] which could explain the unexpectedly high CBr03 prices relative to the likely low-cost bill of materials for this chemical. To address this limitation Medicines For All (M4ALL) Institute developed a methodology that enabled CBr03 synthesis from commodity and low-hazard reagents, which AAP Pharma Technologies ("AAP") leveraged to develop an end-to-end process to prepare pretomanid. From this insight, AAP developed a scalable process for the synthesis of pretomanid. The current synthetic process to pretomanid is around 10-27% over 4 steps. AAP's route is similar or slightly better at 30% over 4 steps, however, to our current knowledge, a side-by-side, in-depth technoeconomic evaluation of the two routes has yet to be conducted.

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1. Editorial Note:

This report is informed by five sources (reports and publications) that are outlined below. For the most complete understanding of how the final route by AAP was developed, it is recommended that these references are evaluated in chronological order:

1. **Advinus CBr03 publication:**[4]
    1. *Org. Process Res. Dev.* **2013**, *17* (9), 1149–1155
2. **M4ALL analysis report on the Advinus publication**:
    1. See Addendum A in attachments.
3. **M4ALL report on CBr03:**
    1. See Addendum B in attachments.
4. **M4ALL synthesis of (S)-glycidyl pivalate (CISO):**[5]
    1. *SynOpen* **2022**, *06* (4), 258–262.
5. **AAP process report:**
    1. See Addendum C in attachments.

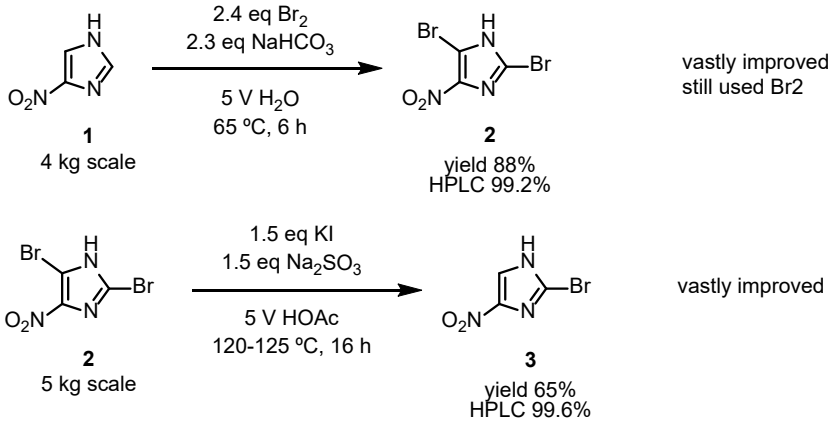
2. Summary of Advinus Work on CBr03:

In 2013 Advinus Therapeutics ("Advinus") published a two-step, kilo-scale synthesis of 2-bromo-4-nitro-1*H*-imidazole (CBr03, **3**) in support of preclinical and clinical development of a nitroimidazo-oxazole-based drug.[4] The first step of the new synthesis avoided shortcomings of previous syntheses of CBr03

including potentially explosive intermediates, low-yielding steps, protection/deprotection steps and hazardous reagent combinations. The second step of the synthesis was also improved over prior art as well as avoiding hazardous reagents, or expensive Pd or Pt metal.

The synthesis starts with the dibromination of 4-nitroimidazole (**1**) using liquid bromine and sodium bicarbonate in water to give 2,5-dibromo-4-nitro-1*H*-imidazole (**2**) in good isolated yield and high purity. The desired product is then obtained by selective debromination of the 5-position with potassium iodide and sodium sulfite in a modest yield with high purity.

Scheme 1 Advinus route to CBr03



3. Summary of M4ALL work on CBr03

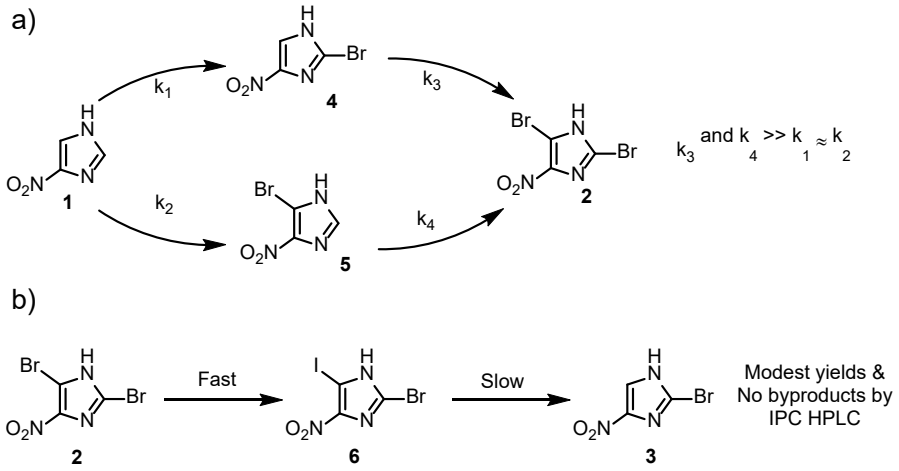
In order to further improve access to pretomanid, M4ALL set out to develop a method to improve safety and expand the number of manufacturers who might practice this chemistry. To that end, M4ALL sought to develop a strategy for the bromination step that would eliminate elemental bromine.

i. MALL Analysis of Advinus Route:

A greater understanding of the Advinus route and procedure was required to be successful in improving the overall process. M4ALL undertook a detailed replication and study of the Advinus route examining the

kinetics of each step, the dibromination and the reduction to CBr03, Scheme 2. This exercise led to the knowledge that dibromination of 4-nitroimidazole (**1**) is fast and that either monobrominated intermediate is rapidly brominated again such that selective bromination to CBr03 is not possible under the Advinus conditions. It was also revealed that the conversion of the dibromo species **2** to the 5-iodo species **6** was rapid and the final conversion of **6** to CBr03 was slower, albeit without impurity formation. This prompted the thought that a higher yield than ~65% could likely be attained and that the culprit could be workup in the Advinus procedure.

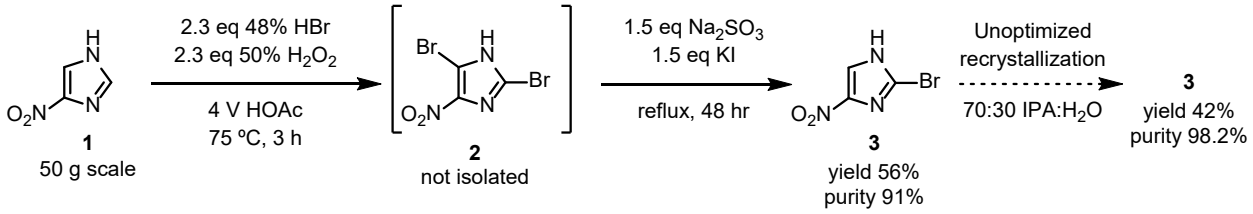
Scheme 2 Kinetics insights from studying Advinus route



ii. MALL Synthesis of CBr03:

A protocol was developed in M4ALL labs that uses hydrobromic acid and hydrogen peroxide to affect the debromination, essentially producing molecular bromine in situ avoiding the need to handle this chemical at scale. The protocol also has modifications that allow for telescoping the two steps of the process into a two-step/one-pot synthesis (e.g., bromination in acetic acid rather than water).

Scheme 3 M4ALL Telescoped route to CBr03



The M4ALL report provides details on the development of this procedure. These details include alternate brominating and oxidizing agents, temperature and solvent screenings, solvent effects in telescoping, mitigation exotherms on scaleup, a 50-gram telescope scaleup demonstration, and a comparison of raw material costs for the two routes (M4ALL and Advinus). In an appendix to this report is found solubility and recrystallization data on CBr03, solvent effects on debromination, reaction mechanism insights, peroxide test protocol, batch sheets for 20- and 50-gram scaleup reactions, and an HPLC analytical method with example chromatogram.

iii. Potential Improvement to the M4ALL CBr03 Synthesis:

While the final report of this work provides considerable detail in how the protocol was developed and some of the thinking behind the approach to developing it, if additional time and scope were to be allowed there are several elements/details which could/should be added to the report:

- An understanding of the solubilities of the inorganic salts in the water/HOAc medium at the end of the synthesis. This may explain the 30wt% inorganic impurities from the telescoped process when AAP attempted to repeat it (see below).
- A larger scaleup reaction, 1 kg, would be advantageous.
- In M4ALL's own analysis of the Advinus route it was pointed out that the dibromo species **2** rapidly converts to the iodo intermediate **6** and slowly *but cleanly* converts to CBr03 without the observation of side products, thus it stands to reason that there is greater product recovery to be had with optimized reaction times. An understanding of this potential *mass balance* issue would be helpful.
- An attempt to recover additional CBr03 from the mother liquor since there is considerable CBr03 in the HPLC trace of the ML from experiment FC-CBr03-075.

Even though AAP's report did encounter issues with the telescoped process, AAP did solidly confirm the efficacy of M4ALL's HBr/H_2O_2 method of dibrominating 4-nitro-1*H*-imidazole on a 1-kilogram scale.

4. AAP Pretomanid Report Summary:

Excerpts from AAP's report below in quotes. Minor edits for clarity indicated by brackets []:

i. Project Background and Key Objectives:

"TB Alliance made a request to develop a process for pretomanid suitable for larger scale synthesis. Overall yield of the current synthetic process is around 10-27% over 4 steps starting from two key starting material[s] called CBr03 and an epoxide called CISO [((*S*)-pivaloyl glycidol)]. CBr03 can be prepared from 4-nitroimidazole (**1**) in two steps with ~50% yield. Selection of a protecting group for (S)-glycidol is critical as it determines the overall yield of this process. The pivaloyl group [of CISO] undergoes a variety of undesired [side] reactions,

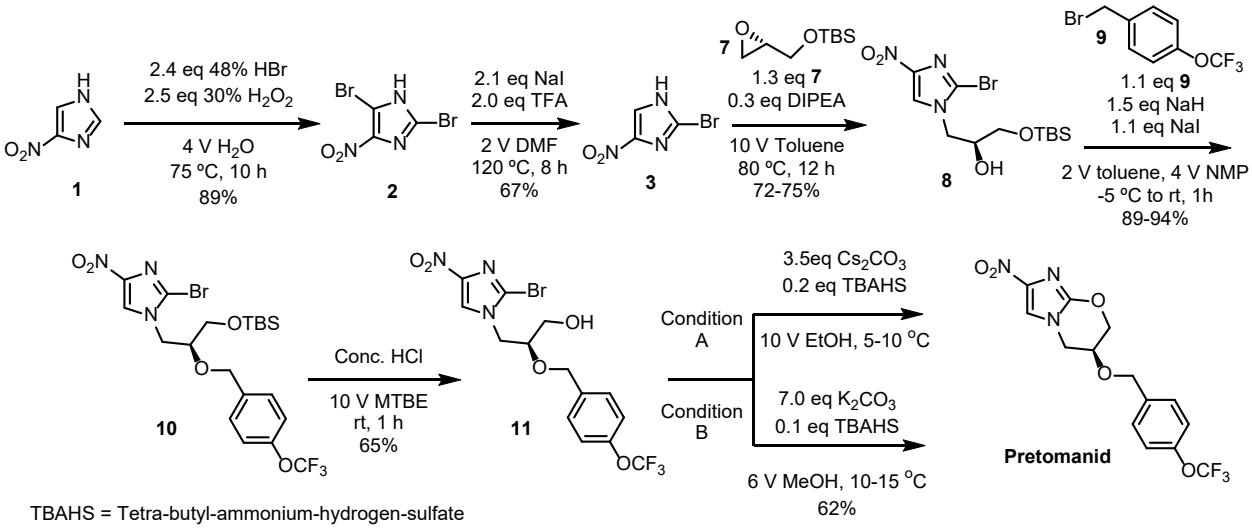
which leads to the low yields in the current pretomanid synthesis. Alternate protecting groups such as *p*-methoxy-benzoyl (PMBz) were explored [by others] and the overall yield improved by 10-20% compared the current baseline. AAP Pharma assessed [these] alternative protecting groups, with the goal of minimizing side reactions thereby increasing yield of this process.

Following the optimized route, a 1 kg scale [4-nitro-1*H*-imidazole] conversion was demonstrated to understand reproducibility of the process. Screening an alternate protecting group of [glycidol] will be the key step in this process. …"

ii. Objectives Achieved:

"A successful process suitable for 1 kg scale synthesis of pretomanid was developed starting from two key intermediates CBr03 and … TBS protected (*S*)-glycidol [(**7**)]. CBr03 was obtained from 4-nitro-imidazole … in two steps with ~60% yield with 99.32% purity. Coupling of TBS protected (*S*)-glycidol and CBr03 afforded [secondary alcohol (**8**)] in ~75% yield and ~95% purity. Reaction of [(**8**)] with 4-trifluoromethoxybenzylbromide [(**9**)] afforded [ether (**10**)], subsequent TBS deprotection afforded the key intermediate [(**11**)] in ~82% purity and 65% yield over two steps. Various solvents and bases were screened for the final cyclization reaction and the results suggested polar protic solvents such as MeOH, EtOH, *n*-propanol and *n*- Butanol are suitable for this cyclization and bases such as Cs_2CO_3 and K_2CO_3 [were] found suitable to achieve pretomanid with ~60% isolated yield and >99% HPLC purity, [Scheme 4].

Scheme 4 Route to pretomanid developed by AAP



> "The chemistry was demonstrated on a 1 kg scale using Cs_2CO_3 in EtOH and on a 300 g scale using K_2CO_3 in MeOH to afford pretomanid with the desired purity and yield. Both [of] the process[es] were compared to understand yield, purity and feasibility of plant scale synthesis."

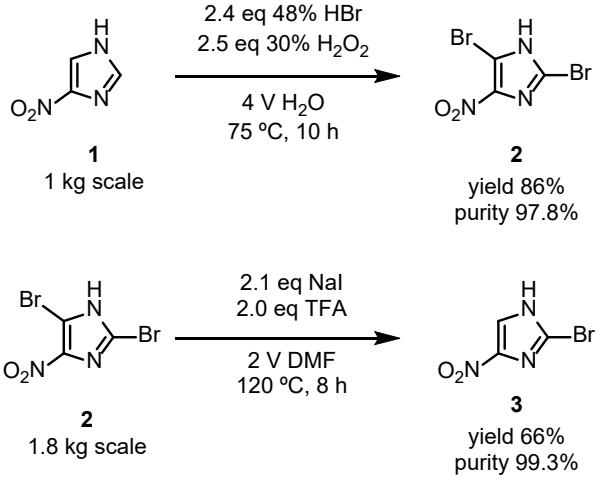
iii. AAP CBr03 Summary:

When repeating the Advinus dibromination method, AAP noted some safety and practical issues with the process, however, they were able to generate the dibromo species **2** in good yield and purity. When attempting to repeat M4ALL's telescoped method to CBr03, they found significant (30wt%) inorganic contamination by ROI (residual on ignition). It is unclear what happened in their hands to have such a large discrepancy from M4ALL's results. As noted above, there could be problems with how much acetic acid is stripped (HOAc-water ratio) causing inorganic salts to precipitate out with the product at the end of the process. This led AAP to pursue developing their own two-step process, which leveraged the HBr/H_2O_2 technique for *in situ* Br_2 generation. For the debromination, AAP opted to keep the M4ALL process but

conduct it in water as the solvent instead of acetic acid which was for the benefit the telescoped process, Scheme 5.

AAP then repeated Advinus' debromination protocol and observed higher amounts of impurities (including the fully-debrominated **1**) and low yields. This led AAP to screen numerous conditions (solvents, catalysts, reducing agents, ratios) and eventually arrived at sodium iodide and trifluoroacetic acid in DMF.

Scheme 5 AAP Two step route to CBr03



iv. Conclusions for AAP CBr03 Synthesis:

Excerpts from AAP's report below in quotes. Minor edits for clarity indicated by brackets []:

> "An alternative synthesis of CBr03 is achieved following [a] two-step process. In the first step di-bromination of [4-nitro-1H-imidazole] was achieved using HBr and H_2O_2 in place of using bromine, which is convenient for plant scale synthesis. Step-1 yield [was] found to be 86-89% and HPLC purity ~98% which is similar [to the] Advinus process and M4ALL process. The yield and HPLC purity of step-2 [was] found [to be] 66% and ≥99% respectively. The overall yield in two steps [was] found [to be] ~57% which is similar [to the] Advinus process. Use of bromine is avoided in step-1, the step-2 reaction and workup

process [are] simple compared to both Advinus and M4ALL process, which are more convenient to operate, therefore, [the] AAP process can be adopted for plant scale manufacturing process.

Cost analysis performed on Advinus and M4ALL process[es] reveals that both alternatives present similar COGs… The reagents used in the first step [are the] same as shown in [the] M4ALL and [the] AAP process, although the 5-6% higher yield observed in [the] AAP process, portion wise addition of H_2O_2 might be the reason behind this result. Therefore step 1 might be cost efficient compared to [the] M4ALL process. The second step appeared [to be] more expensive as 2 equiv. of TFA was used in [the] AAP process whereas less expensive Na_2SO_3 was used in both [the] Advinus and [the] M4ALL process[es]. The AAP process showed slightly higher yield [the] in second step, volume efficiency and workup process … which is convenient for plant scale synthesis. The operation cost might compensate [for] the higher TFA cost therefore, we believe the AAP process will be similar [to the] M4ALL process."

v. AAP Synthesis of Pretomanid:

AAP then goes on to screen reaction conditions for the synthesis of pretomanid, starting with the reaction of CBr03 and protected (*S*)-(-)-glycidols. The protecting groups were pivaloyl (Piv, the protecting group in CISO), *p*-methoxybenzoyl (PMBz), and *t*-butyldimethylsilyl (TBS, or TBDMS). They screened for yield as well as migration of the protecting group, *N*'-alkylation, and closure to the 5 or 6 membered ring. Even though CISO was the chosen route for manufacture of pretomanid, AAP found the TBS protected glycidol to work the best in their hands.

AAP went on to screen numerous conditions for installation of the trifluoromethoxybenzyl ether, deprotection of the TBDMS group, and finally the cyclization to pretomanid. They arrived at two different routes starting from either purified or crude CBr03-glycidol adduct **8,** Scheme 4.

vi. AAP's Conclusion for API Assembly:

Excerpts from AAP's report below in quotes. Minor edits for clarity indicated by brackets []:

> "A TBS group was [chosen] for the protection of (*S*)-(-)-glycidol in the … epoxide opening reaction with CBr03, step 3. This showed ~5% of TBS migration impurity and ~7% of N-regioisomer which is 30-40% lower compared to the process where pivaloyl protected (S)-glycidol [CISO] was used in this step. Significant differences in yield or purity in the benzylation (step-4) and TBS deprotection (step 5) weren't observed compared to the previous reports. [For the cyclization] reaction of **11**, … polar-protic solvents such as MeOH and EtOH were found to be suitable … Furthermore, this reaction was highly sensitive to temperature and bases, rapid cyclization was observed when Cs_2CO_3 was used in EtOH at 5-10 °C, however, product decomposition was a major issue in this condition. Efficient cyclization of **11** was observed in MeOH in the presence of K_2CO_3 at 15-20 °C although 6-7% OMe insertion impurity was found in this process. Demonstration batches of pretomanid were performed following the above processes and HPLC analysis of reaction mixtures showed 80-85% product in both processes. An efficient crystallization process of crude product with 75-80% recovery was found to be MTBE-heptane-water (4: 4: 05 v/v) with 75-80% product recovery and >99% purity. A successful demonstration batch synthesis of showed ~30% yield over four steps (step-3 to step-6) with >99% purity."

[1] https://www.tballiance.org/why-new-tb-drugs/global-pandemic (accessed 2023-02-17).
[2] https://www.tballiance.org/news/fda-approves-new-treatment-highly-drug-resistant-forms-tuberculosis (accessed 2023-02-17).
[3] Wuellner, G.; Herkenrath, F.-W.; Juelich, A.; Yamada, Y.; Kawabe, S. Methods for the Production of 2-Halo-4-Nitroimidazole and Intermediates Thereof. WO2010021409A1, February 25, 2010. https://patents.google.com/patent/WO2010021409A1/en?oq=WO%2f2010%2f021409%2c+2010 (accessed 2023-02-17).
[4] Pedada, S. R.; Satam, V. S.; Tambade, P. J.; Kandadai, S. A.; Hindupur, R. M.; Pati, H. N.; Launay, D.; Martin, D. An Improved Kilogram-Scale Synthesis of 2-Bromo-4-Nitro-1H-Imidazole: A Key Building Block of Nitroimidazole Drugs. *Org. Process Res. Dev.* **2013**, *17* (9), 1149–1155. https://doi.org/10.1021/op400095f.
[5] Noble, J. M.; Chang, L.; Chen, D.; Wang, B.; Dominey, R. N.; Cook, D. W.; Burns, J. M.; Stringham, R. W.; Cardoso, F. S. P.; Snead, D. R. A Practical and Economical Route to (S)-Glycidyl Pivalate. *SynOpen* **2022**, *06* (4), 258–262. https://doi.org/10.1055/s-0042-1751375.