Efforts to Develop a Cost-Effective and Scalable Synthetic Process for Nirmatrelvir

Appasaheb L. Kadam, Barreddi Chiranjeevi, Aline A. Nunes, Aravindan Jayaraman, Saeed Ahmad, Sarah L. Aleshire, Kai O. Donsbach, B. Frank Gupton, Michel C. Nuckols, Charles S. Shanahan**

Medicines for All Institute, Virgina Commonwealth University, Richmond, Virginia 23298, United States

*Co-Corresponding Authors can be reached at Michel C. Nuckols: mnuckols2@vcu.edu or Charles S. Shanahan: shanahanc@vcu.edu

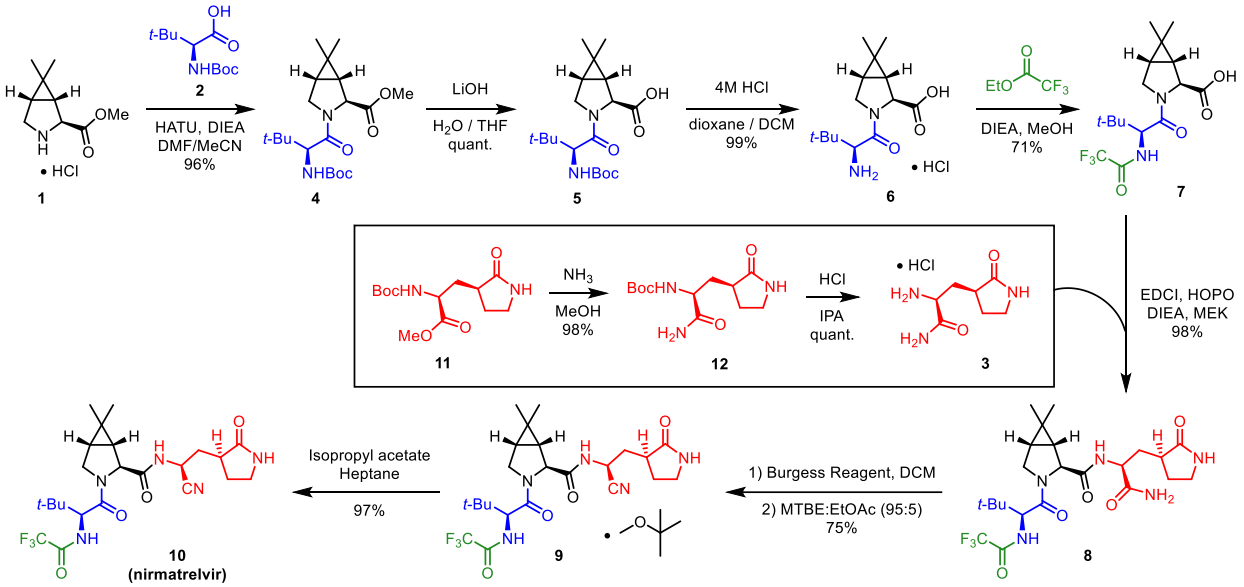
ABSTRACT:

Nirmatrelvir, the novel antiviral component of Pfizer's orally available combination therapy Paxlovid, used to treat COVID-19, presents a significant synthetic challenge. Herein, we report process optimization insights that could enable a scalable and cost-effective manufacturing process to make nirmatrelvir. The disclosed development opens up a path to three new complete routes, offering options to eliminate some of the major cost-drivers for nirmatrelvir and deliver the final API in higher yield and lower overall cost while maintaining quality requirements.

INTRODUCTION:

Paxlovid, an orally bioavailable drug for the treatment of COVID-19, was given emergency use authorization by US FDA in December 2021, thus spurring significant development efforts around the world to make this drug widely available. While numerous coronavirus outbreaks have caused significant global health concerns,[1] the early investigations (especially into the 2003 SARS outbreak) allow for rapid development of the novel antiviral component of Paxlovid, nirmatrelvir, is a 3CL protease inhibitor and was developed by Pfizer.[2] It has shown potent antiviral activity in clinical studies,[3] is safe for use, has a robust oral bioavailability and presents the world with an effective tool at battling the global pandemics and saving lives. The chemical complexity and high costs of known starting materials to make nirmatrelvir will likely limit the global availability of this critical drug, making synthetic innovations at all stages of nirmatrelvir production valuable in improving patient access.[i] Pfizer's originally disclosed synthesis of nirmatrelvir (Scheme 1),[1a] offers numerous opportunities for cost improvements, and herein we disclose some practical opportunities to that end. Recent reports from the Lipshutz[4] and Ruijter & Turner[5] research groups offer alternative routes to assemble nirmatrelvir.

[i] Based on analysis of the overall synthesis of nirmatrelvir and its starting materials, publicly available costs of the known, commercially available starting materials (i.e. from Zauba and Datamyne), and published costs of required reagents to manufacture nirmatrelvir.



Scheme 1 - Pfizer's Originally Disclosed[1a] Route to Nirmatrelvir

Pfizer's synthesis is 8 operations (6 synthetic steps) from the key starting materials proline derivative **1**, *N*-Boc-*tert*-leucine **2**, and lactam **3** (derived in two steps from **11**) and provides a reported 48% overall yield of nirmatrelvir. We estimate the major cost-drivers of this route in Scheme 1 to be the three key starting materials **1**, **2**, and **3,** the HATU coupling reagent used in the first step, Burgess reagent used in the final synthetic step, and the moderate yield of the trifluoracetamide installation in step 4. Furthermore, the disclosed Pfizer synthesis utilized column chromatography in the first coupling step, which would also require a practical work-around to be developed. We describe herein our progress to address these issues to encourage continued action to help drive down the cost of nirmatrelvir and improve patient access, however, work is still ongoing in our laboratories to further optimize and scale-up our process. We are also investigating new disconnection strategies that would lead to more efficient syntheses of nirmatrelvir and further development will be reported.

RESULTS AND DISCUSSION:

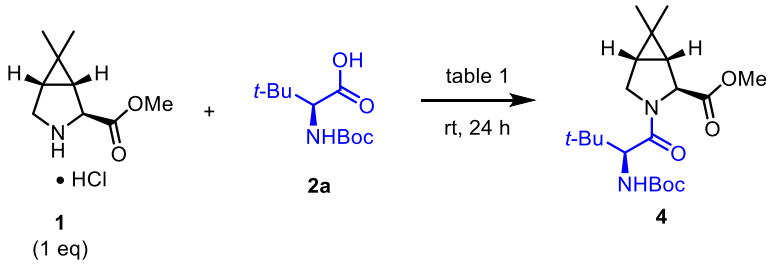
The synthesis starts with an amide coupling reaction between proline derivative **1** and *N*-Boc-*tert*-leucine **2** using HATU as the coupling reagent to obtain amide **4** in a reported 96% yield. Although high yields have been reported for this step, HATU is a significant cost-driver for the API, and it also carries potential safety risk in the form of shock sensitivity and explosiveness posing potential scale-up risks.[6]

In our efforts to replace HATU in the synthesis of **4,** we screened numerous coupling reagents under a variety of reaction conditions (bases, additives, solvents, etc). Our efforts revealed that DCC and HBTU provided the most competitive activity compared to HATU but, given that HBTU has a similar safety concern relative to HATU, we opted to focus our efforts on DCC (Table 1). A subsequent DOE study revealed that DCC, sub-stoichiometric HOAt and *N*-methylmorpholine (NMM) in DCM provided yields of approximately 88-90% (Table 1, Entries 11-13).[ii] The DOE tested four factors (molar equivalents of **2a**, molar equivalents of DCC, molar equivalents of HOAt as an additive and the ratio of DMF:DCM in the solvent system) and required 27 unique experiments to complete. We fixed the primary cost driver proline derivative **1** as the limiting reagent, the temperature to room temperature, the reaction time to 24 h, and the base to NMM (3 eq) as we found in early experiments NMM to be highly beneficial and less impactful to cost at higher equivalents. From this study we found that increasing the molar equivalents of the less expensive *tert*-leucine **2** provided **4** in better yields, but that moving much beyond 1.5-1.6 eq was unnecessary as yields did not improve further. Given the cost of **2** relative to the much higher priced **1**, excess **2** could be tolerated without greatly affecting the cost of **4**. Furthermore, we

[ii] While Pfizer's Science paper describes a yield of 96% with HATU in the first step, in our hands the yields were in the range of 85-90% over numerous replicate experiments, thus we feel the DCC result is competitive with HATU.

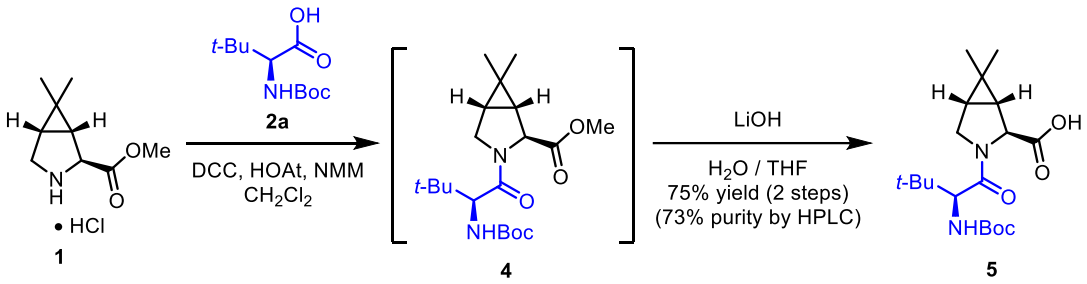
determined that the use of DMF as a co-solvent did not appear necessary, which results in a more process-friendly solvent system as DCM could be used as the sole solvent. At this point, the preferred condition lacking DMF as a co-solvent (Table 1, Entry 13) has been successfully repeated multiple times on multigram scale and optimization of these conditions will continue as we scale-up our process.

Table 1 - Optimization of DCC-Mediated Coupling of 1 & 2a



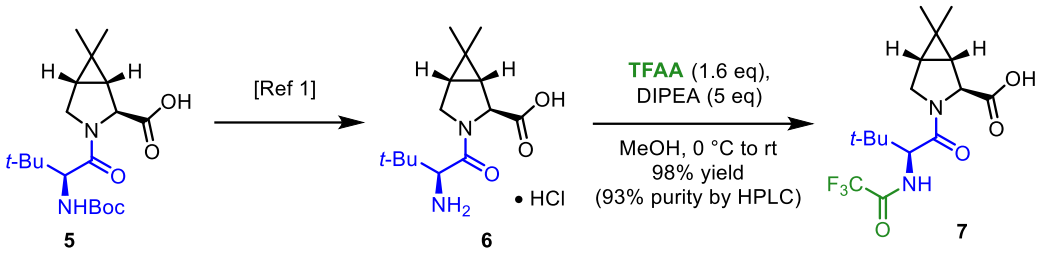
Entry	Batch Size (g)	2a (eq)	Coupling Reagent (eq)	Additive (eq)	Base (eq)	Solvent System	qNMR Assay Yield (%)
1	0.2	1.1	T3P (1.5)	-	DIPEA (4.0)	EtOAc (20.0 vol.)	20
2	0.2	1.1	CDI (1.3)	-	Triethylamine (2.0)	DCM (15.0 vol.)	Trace
3	0.2	1.1	Pivaloyl chloride (1.1)	-	NMM (2.5)	THF (10.0 vol.)	55
4	0.2	1.1	Isobutyl chloroformate (1.1)	-	Triethylamine (2.5)	THF (10.0 vol.)	21
6	0.2	1.1	DMTMM (1.1)	-	NMM (2.5)	DCM: DMF (10.0 Vol, 80:20)	22
7	0.2	1.1	EDCI (1.2)	HOPO (0.5)	NMM (2.5)	DCM: DMF (10.0 Vol, 80:20)	68
8	0.2	1.1	DIC (1.1)	HOPO (0.5)	NMM (2.5)	DCM: DMF (10.0 Vol, 80:20)	51
9	0.2	1.1	HBTU (1.1)	-	DIPEA (3.0)	ACN: DMF (20.0 Vol, 90:10)	86
10	0.2	1.1	DCC (1.1)	HOAt (0.5)	NMM (2.5)	DCM: DMF (10.0 Vol, 80:20)	75
11	0.2	1.8	DCC (1.6 eq.)	HOAt (0.7)	NMM (3.0)	DCM: DMF (10.0 Vol, 75:25)	90
12	**0.2**	**1.8**	**DCC (1.6 eq.)**	**HOAt (0.3)**	**NMM (3.0)**	**DCM: DMF (10.0 Vol, 25:75)**	**90**
13	**0.2**	**1.6**	**DCC (2.1eq.)**	**HOAt (0.5)**	**NMM (3.0)**	**DCM (10.0 Vol)**	**88**

We next turned our attention of the purification of **4** (or proceeding to intermediate **5**) while avoiding column chromatography. We first devised a strategy to avoid chromatographic purification using the HATU coupling method as a first case study (following the process in Scheme 1) by carrying **4** on crude to the subsequent saponification step. Using the Pfizer HATU procedure, we were able to obtain **5** in 73% overall yield (99 wt% purity by HPLC) after conducting a simple aqueous workup followed by precipitation from a solution of acetonitrile by addition of water. This purification technique is now being optimized for DCC to remove the less polar urea by-products. To this point, we have been able to effectively recapture DCU in sufficient purity to effectively be recycled,[7] and our current best result on this telescoped procedure with DCC as the coupling reagent is providing a 75% yield (corrected for purity, over 2 steps), providing **5** in ~73 wt% purity (by HPLC). We are currently optimizing this process to improve throughput and purity of **5** and will report these results in future communications.



Scheme 2 - Telescoped Procedure to Provide Pure 5 without Column Chromatography

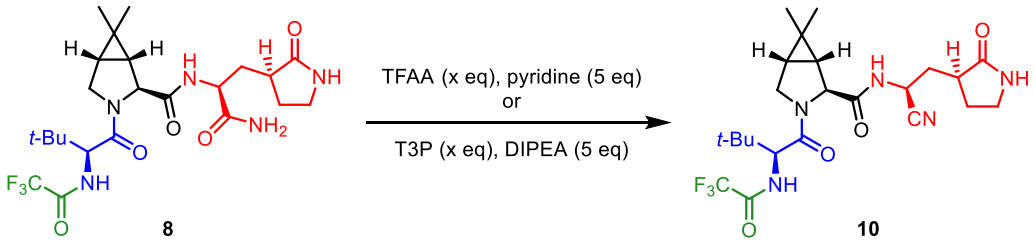
The next opportunity for cost improvement was to improve the yield of the trifluoracetamide installation in step 4, where Pfizer reported using ethyl trifluoroacetate to provide **7** in 71% yield (refer to Scheme 1). We found that a 98% isolated yield of **7** (93% purity by HPLC) could be obtained by simply switching to trifluoroacetic anhydride (TFAA) along with excess DIPEA in methanol (Scheme 3).

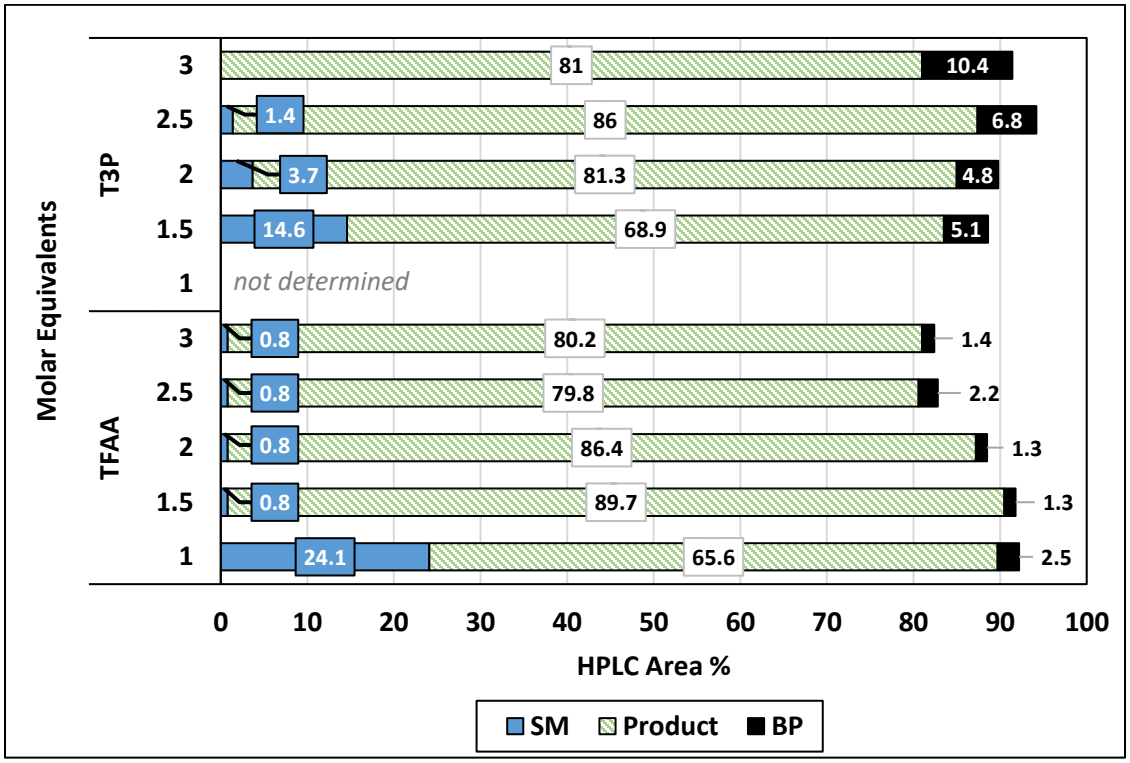


Scheme 3 - Optimization of Trifluoroacetamide Group of Acid 7

With an optimized route to carboxylic acid **7** in hand, we turned our attention to discovering methods to replace the expensive Burgess reagent, used in the last synthetic step to convert the primary amide to the key cyano group. We calculated Burgess reagent to be one of the biggest raw material cost-contributors to the Pfizer strategy, making its replacement with a low-cost alternative paramount. To accomplish this goal, we screened various dehydrating reagents and found both TFAA and T3P to be similarly effective (Graph 1),[8] but increasing amounts of T3P produced more of a single major side product than TFAA. In all cases the side product appears to be the same by HPLC and NMR analysis of the crude product, but so far has not been positively identified.[iii] Under all conditions, we also observed numerous minor impurities along the baseline in the HPLC, however, these minor impurities and the major side product can be removed by the subsequent recrystallization from MTBE:EtOAc, per Pfizer's procedure.[1a] From this study we found that TFAA at 1.5 – 2 eq was most effective in converting the amide to cyano without forming significant side product and furnished nirmatrelvir in nearly 90% assay yield (HPLC).

[iii] We are still investigating the nature of this by-product but have found the same by-product to be produced by the Pfizer Burgess reagent process at about 4% (HPLC area %). This by-product is removed during the recrystallization step.

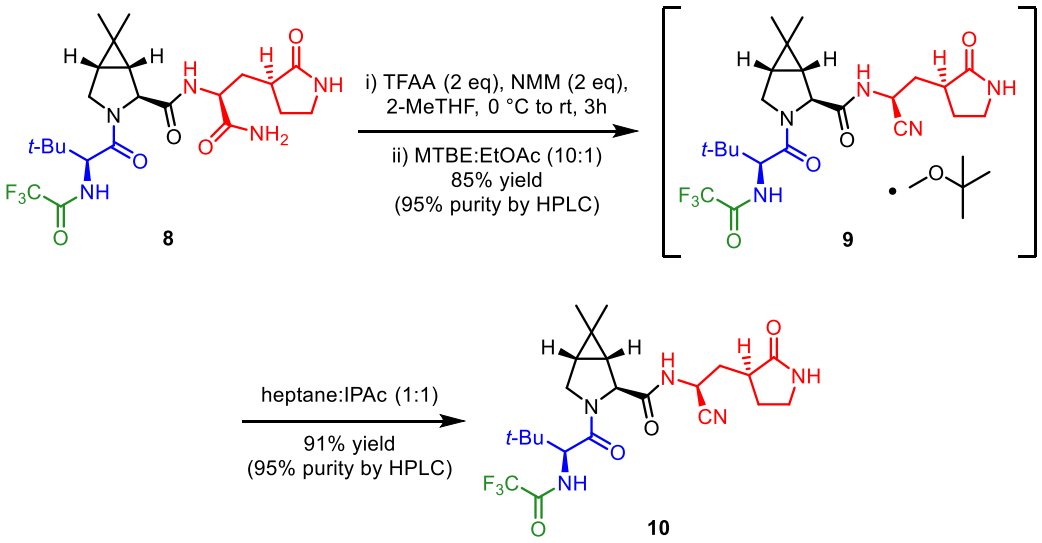




Graph 1 - Results of Amide Dehydration Conditions Screening

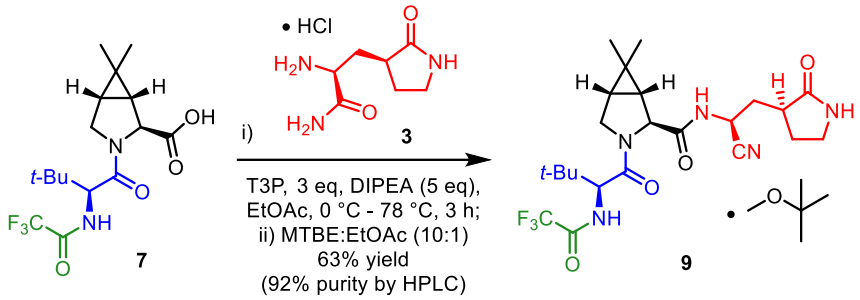
With alternative dehydration conditions in hand, we optimized the TFAA method by screening several different bases and reaction conditions and found that pyridine and/or NMM as the base could both offer high yields and purity. When executing the NMM conditions on gram scale, the dehydration reaction, followed by recrystallization from MTBE:EtOAc, gave the MTBE-solvate of nirmatrelvir (**9**) in 85% yield (95 wt% purity by HPLC) (Scheme 4). The same process with Burgess reagent was reported to give 75% yield and, given that TFAA is considerably less expensive than Burgess reagent, this development amounts to a significant overall cost reduction

in nirmatrelvir. We also tested the final recrystallization procedure that Pfizer reported to deliver the MTBE-free final crystals of nirmatrelvir to establish that TFAA could successfully replace Burgess reagent in the last step. In the event, we found that the purity was largely unchanged, but some material loss was observed, likely exacerbated by operating here at sub-gram scale. We are further optimizing this process as well and report developments in subsequent communications.



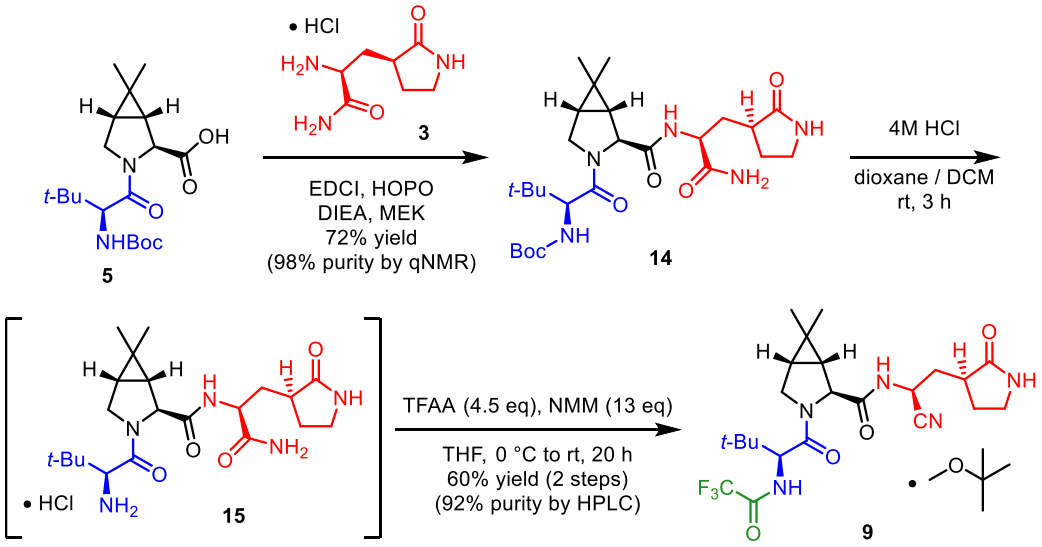
Scheme 4 - Replacement of Burgess Reagent with Low-Cost TFAA as the Dehydrating Reagent

While T3P was not found to be as effective a dehydrating agent compared to TFAA, we were intrigued to explore its ability to mediate both the coupling of **3** and **7** and effect the amide dehydration in one-pot as had similarly been demonstrated by GlaxoSmithKline in their denagliptin synthesis.[9] In our case, this two-step/one-pot sequence from **7** to nirmatrelvir proceeded quite effectively to deliver nirmatrelvir (Scheme 5). After the initial recrystallization procedure, we were able to isolate the nirmatrelvir-MTBE solvate in 63% overall yield (92 wt% purity by HPLC). We are continuing to optimize this telescoped process and believe this demonstrates clearly that a viable one-pot process has the potential lead to further improvements in cost by reducing step count and replacing ECDI with T3P in the overall sequence.



Scheme 5 - Telescoped Coupling/Amide Dehydration to Synthesize Nirmatrelvir

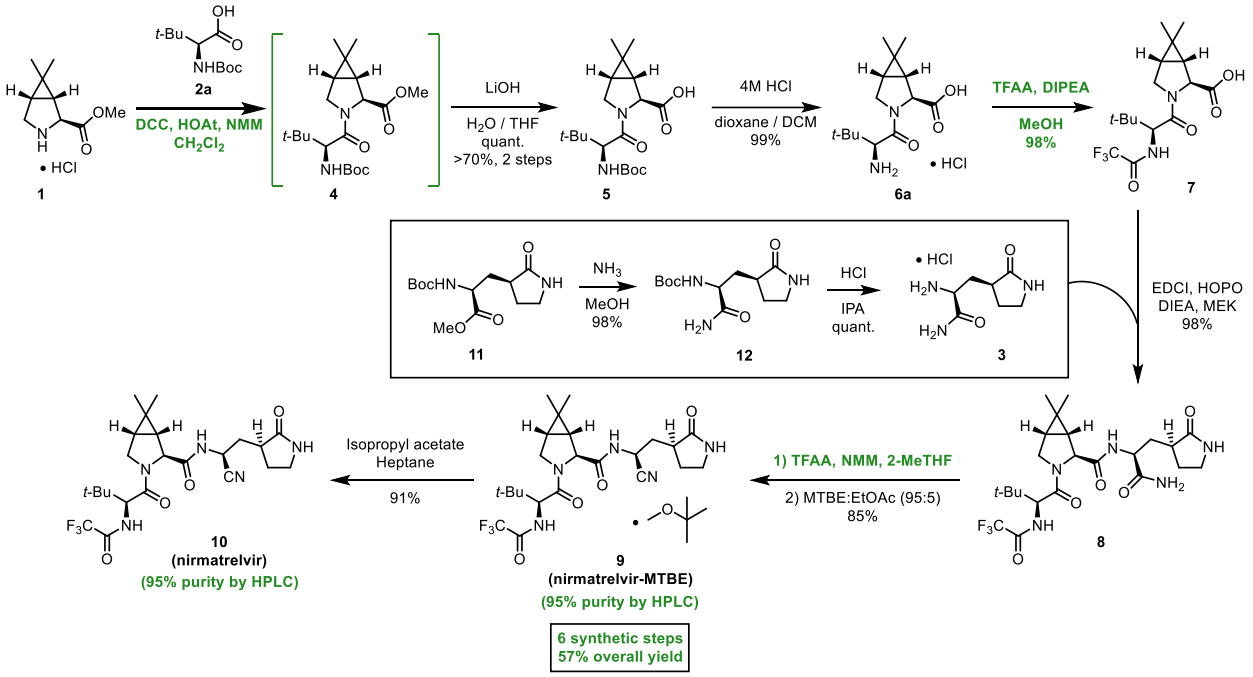
Another option to cut the number of overall steps, is the direct coupling of **3** with *N*-Boc protected **5** prior to TFA installation (Scheme 6). We utilized the coupling conditions reported by Pfizer for the coupling of **7** and **3** (refer to Scheme 1, step 5) and we were able to obtain *N*-Boc protected **14** in 72% yield (98% purity by qNMR) after column chromatography. We next removed the Boc-protecting group with HCl (4M in dioxane) to furnish the amino acid salt **15** quantitatively. This material was taken on without purification to the one-pot trifluoroacetylation/amide-dehydration reaction. This strategy is ultimately made possible by the dual action of the TFAA reagent that can both trifluoroacetylate the free *tert*-leucine residue and dehydrate the primary amide in one single operation. In this streamlined endgame, we were able to deliver nirmatrelvir (**10**) in an unoptimized 60% yield over the last two telescoped steps (92 wt% purity by HPLC) and mitigate the impact of utilizing the Boc-protecting group as part of the overall strategy. We are currently working to optimize this process with respect to the coupling step to improve yield along with an upgraded purification strategy that avoids column chromatography. Progress will be reported in due course as this process option is fully optimized and scaled-up.



Scheme 6 – Telescoped Utilization of N-Boc-tert-Leucine as Starting Material Leveraging a One-Pot Trifluoroacetylation/Amide-Dehydration Reaction

CONCLUSIONS:

We have thus far developed several scalable options to prepare nirmatrelvir to improve upon the process safety and provide nirmatrelvir at lower cost over previously published routes. In our hands, the Pfizer disclosed strategy in Scheme 1 was able to provide the nirmatrelvir-MTBE solvate (**9**) in approximately 48% overall yield and in 6 synthetic steps from starting materials **1**, **2**, and **3**. Incorporating the improvements that we have disclosed, we have further improved the utilization of these starting materials by increasing the overall yield by ~10%. Removing the major cost drivers HATU and Burgess Reagent and improving the Step 4 yield (Scheme 7) provides further cost savings potential.



Scheme 7 - Streamlined and Scale-Up Ready Process to Make Nirmatrelvir from Starting Materials 1, 2 and 3

The key insights we have disclosed here can be combined to produce three complete routes to nirmatrelvir, and we are currently working to optimize and verify these routes at kilogram scale to determine the most cost-effective and environmentally friendly overall process. Future disclosures will reflect our progress to develop an end-to-end manufacturing process for nirmatrelvir and we will make these process updates available as they are finalized.

ACKNOWLEDGMENTS:

This work was supported by funding from the Bill & Melinda Gates Foundation. We would like to express gratitude to Trevor Laird and John Dillon for insightful discussions and suggestions. We also thank Silpa Sundaram (BMGF) and Dr. Susan Hershenson (BMGF) for fostering an ecosystem where rapid decisions on project direction can be made.

ABBREVIATIONS:

DCC, dicyclohexylcarbodiimide; COVID-19, coronavirus disease 2019; USFDA, United States Food and Drug Administration; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; HBTU, hexafluorophosphate benzotriazole tetramethyl uronium; DOE, design of experiment; DIC, N,N'-diisopropylcarbodiimide; HOAt, 1-Hydroxy-7-azabenzotriazole; NMM, N-methylmorpholine; PMI, process mass intensity; DMF, N,N-dimethylformamide; DCM, dichloromethane; API, active pharmaceutical ingredient; TFAA, trifluoroacetic anhydride; DIPEA, diisopropyl ethylamine; T3P, propylphosphonic anhydride; MTBE, methyl *tert*-butyl ether; ECDI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; TFA, trifluoroacetate or trifluoroacetamide (depending on structure context); THF, tetrahydrofuran; CDI, carbonyl diimidazole; DMTMM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride; HOPO, 2-hydroxypyridine-N-oxide.

REFERENCES:

1 Guarner, J. *American J. Clinical Pathology* **2020**, *153*, 420–421.

2. a) Owen et al. *Science* **2021**, *374*, 1586–1593; b) Owen et al. "Nitrile-Containing Antiviral Compounds" US20220062232A1, 2022.

3. Hammond J, Leister-Tebbe H, Gardner A, Abreu P, Bao W, Wisemandle W, Baniecki M, Hendrick VM, Damle B, Simón-Campos A, Pypstra R, Rusnak JM; EPIC-HR Investigators *New England Journal of Medicine* **2022**, *15*, 1397-1408.

4. Kincaid, J. R. A.; Caravez, J. C.; Iyer, K. S.; Kavthe, R. D.; Fleck, N.; Aue, D. H.; Lipshutz, B. H. *ChemRxiv* **2022**. *This content is a preprint and has not been peer-reviewed*.

5. Preschel, H. D.; Otte, R. T.; Zhuo, Y.; Ruscoe, R. E.; Burke, A. J.; Cowan, R.; Horst, B.; Hennig, S.; Janssen, E.; Green, A.; Turner, N. J.; Ruijter, E. *ChemRxiv* **2022**. *This content is a preprint and has not been peer-reviewed.*

6. a) Sperry, J.B.; Minteer, C.J.; Tao, J.; Johnson, R.; Duzguner, R.; Hawksworth, M.; Oke, S.; Richardson, P.F.; Barnhart, R.; Bill, D.R.; Giusto, R.A.; Weaver, J.D. *Org. Process Res. Dev.* **2018**, *22*, 1262–1275; b) Magano, J. *Org. Process Res. Dev.* **2022**, *26* 1562–1689; c) Dunetz, J.R., Magano, J.; Weisenburger, G.A. *Org. Process Res. Dev.* **2016**, 20, 140-177.

7. a) Stevens, C.L; Singhal, G.H.; Ash, A.B. *J. Org. Chem.* **1967**, *32,* 2895; b) "Method for recycling recycled N,N'-dicyclohexylurea" CN105439870A, 2015.

8. a) Villhauer, E.B N-substituted 2-cyanopyrrolidines US74629596A, 1996; b) Vishwanatha, T.M; Panguluri, N.R.; Sureshbabu, V.V. *Synthesis* **2013**; *45*, 1569-1601.

9. Patterson, D.E.; Powers, J.D., LeBlanc, M.; Sharkey, T.; Boehler, E.; Irdam, E.; Osterhout, M.H. *Org. Process Res. Dev.* **2009**, *13*, 900-906.