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Alternate end-game strategies towards Nirmatrelvir synthesis: Defining a continuous flow process for the preparation of an anti-COVID drug

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ABSTRACT

Scalable alternate end-game strategies for the synthesis of the anti-COVID drug molecule Nirmatrelvir (**1**, PF-07321332) have been described. The first involves a direct synthesis of **1** via amidation of the carboxylic acid **7** (suitably activated as a mixed anhydride with either pivaloyl chloride or T3P) with the amino-nitrile **10·HCl**. T3P was found to be a more practical choice since the reagent promoted efficient and concomitant dehydration of the amide impurity **9** (derived from the amino-amide contaminant **8·HCl** invariably present in **10·HCl**) to **1**. This observation allowed for the development of the second strategy, namely a continuous flow synthesis of **1** from **9** mediated by T3P. Under optimized conditions, this conversion could be achieved within 30 min in flow as opposed to 12–16 h in a traditional batch process. The final API had quality attributes comparable to those obtained in conventional flask processes.

As of August 2022, the on-going COVID-19 pandemic has claimed 6.5 million lives worldwide [1]. While the vaccine program continues to be rolled out globally, the incessant emergence of new variants (such as B.1.1.529 or Omicron) of the causative SARS-CoV-2 virus has made identification of newer antivirals for effective clinical management of COVID-19 a necessity [2]. A very recent therapeutic option in this regard is Paxlovid, which has been authorized by USFDA in July 2022 for conditional use in treating mild-to-moderate COVID-19 [3]. Paxlovid is an oral combination therapy of the direct-acting antiviral Nirmatrelvir (**1**, PF-07321332) and Ritonavir which acts as a pharmacokinetic booster [4]. Nirmatrelvir is an irreversible inhibitor of SARS-CoV-2 viral protease M^{pro} and has even shown promising *in-vitro* activity against the SARS-CoV-2 variant Omicron [5–7].

From a structural and manufacturing standpoint, Nirmatrelvir (Fig. 1), with its six chiral centres (some of which are highly prone towards epimerization) and the highly orchestrated assembly of three fragments that its preparation entails, is undoubtedly-one of the more complex and synthetically challenging anti-COVID drugs known [8–15]. While acquisition of all the three Nirmatrelvir fragments involves multi-step syntheses, the known process for the endgame is particularly challenging. It employs expensive and difficult-to-handle reagents such as the Burgess reagent or involves prolonged reaction times (*vide infra*) which increases the possibility of impurity formation owing to epimerization. Given the topical importance of **1** and against the background of our own research into one of its key synthetic fragments [16,17], our interest was drawn into delineating a robust and cost-effective endgame strategy for the synthesis of the API. The present manuscript documents our efforts in this regard.

The best known and widely employed route (owing largely to the ease of accessing the intermediate **8** in pure form) for the synthesis of **1** has been illustrated in Scheme 1 [8,9]. As already alluded to, the endgame in this route consists of: (a) amidation of the carboxylic acid **7** with the amino-amide **8** at 25 °C for 16 h in presence of HOPO (2-hydroxypyridine 1-oxide), DIPEA and EDC·HCl to furnish the amide **9**, and (b) Burgess reagent mediated dehydration of the carboxamide moiety in **9** at 25 °C for 4 h to obtain **1**.

An alternate and a more convergent route to **1** is illustrated in Scheme 2. This affords a direct access to the API via amidation of

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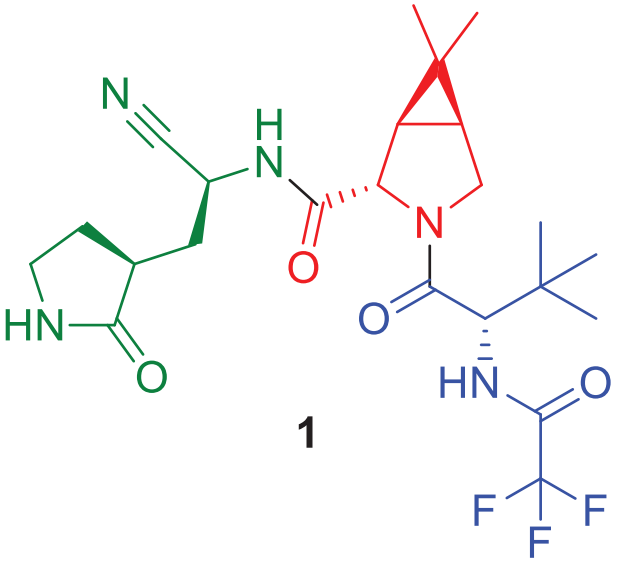
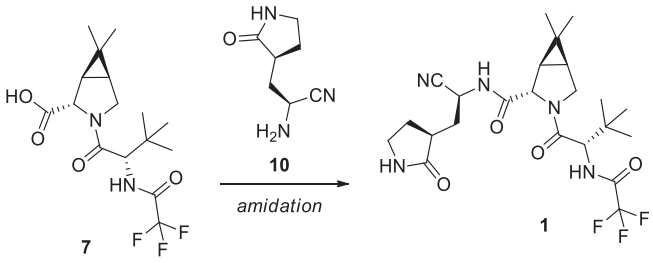


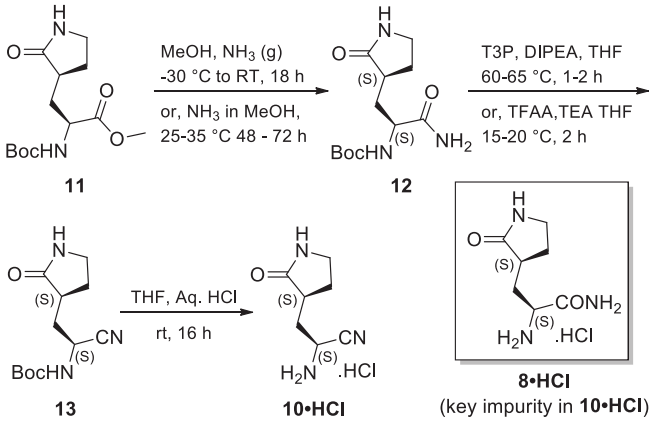
Fig. 1. Chemical structure of Nirmatrelvir **1** with the three key synthetic fragments highlighted.

7 with the amino-nitrile **10**, thus bypassing the need to employ amide dehydration at the final stage of an API synthesis [9]. Needless to say, the difficulty in accessing **10** in sufficient purity has been a major bottleneck in the adoption of this route for manufacturing **1**. In general, the purity requirements for advanced intermediates that are taken forward to the final active pharmaceutical ingredient are stringently defined to ensure the desired pharmaceutical quality and regulatory aspects of the pharmaceutical industry.

Against this background, we aimed to develop a process that can afford **10** (preferably as a solid crystalline salt) with enough purity to be deemed as a regulatory starting material (RSM). As illustrated in Scheme 3, our synthesis commenced with converting the ester **11** to the carboxamide **12**. When carried out in commercially available methanolic ammonia at RT, this reaction took 2–3 days to complete. Use of a fortified solution of ammonia in methanol (obtained by passing ammonia gas through a pre-cooled solution of **11** in methanol) allowed us to reduce the reaction time to 18 h. Dehydration of the carboxamide **12** to the nitrile **13** could be achieved with both trifluoroacetic anhydride (TFAA) and



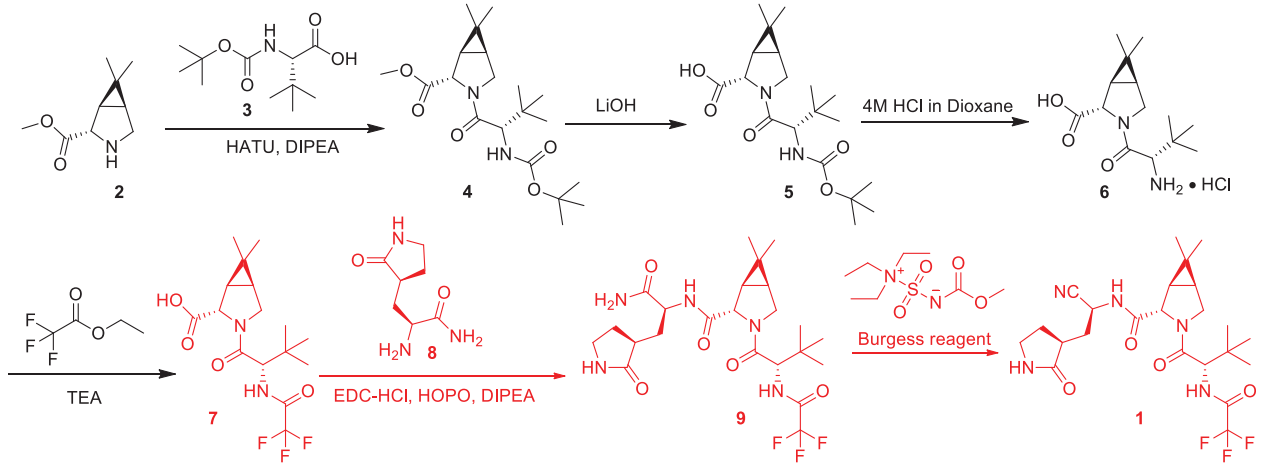
Scheme 2. Direct synthesis of Nirmatrelvir **1** via amidation of **7** with nitrile **10**.



Scheme 3. Synthesis of **10·HCl** from **11**.

1-propanephosphonic acid anhydride (T3P). However, it was observed that addition of TFAA caused the reaction mixture to form an extremely heavy suspension which was quite difficult to stir. This led us to prefer using T3P as the dehydrating agent during the scale-up batches.

Initial attempts towards Boc removal in **13** using TFA led to a highly contaminated product even in the presence of carbocation scavengers such as thioanisole. Minimizing hydrolysis of the nitrile **10** to the amide **8** during the course of the reaction and isolation posed as one of the two key challenges; the other being the ability to define a consistent range of yield and content of the impurity **8** in the isolated **10**. Screening of various acids (both organic and inorganic) and reaction conditions eventually led us to identify



Scheme 1. Known route of synthesis for Nirmatrelvir **1**, starting from the chiral bicyclic proline **2** – a fragment that is also employed in the construction of the anti-HCV drug Boceprevir.[16] The endgame steps have been indicated in red.

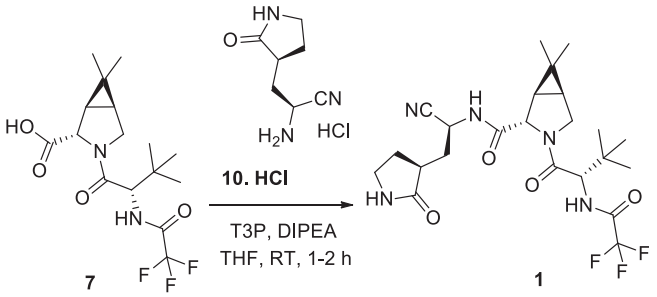
Boc removal in **13** with aqueous HCl in THF as the best way forward. Under optimized reaction conditions, the desired amino-nitrile could be easily precipitated out directly from the reaction milieu as a crystalline hydrochloride salt (**10·HCl**) consistently in 70 % yield and with >95 % purity (1–2 % of the amino-amide **8** still persisted as an impurity).

With the amino-nitrile **10·HCl** in hand, we shifted our attention to identifying a cost-effective and scalable strategy for carrying out the final step in the synthesis of **1** (Scheme 4). Both **7** and **10** are stereochemically embellished and contain functional groups sensitive towards acids and bases. Hence, as the first approach, we chose to activate the carboxylic acid **7** as a pivaloyl mixed anhydride **14** which conveniently reacted with **10·HCl** to furnish the final API **1**. Needless to say, the amino-amide impurity **8**, present in **10**, also reacted with **14** under the reaction conditions employed to give **9** (this was confirmed in a separate study shown in Scheme 4) as the major contaminant in **1**.

During our efforts described so far, we were cognizant of the fact that not being able to obtain the nitrile **10** with very high purity can potentially compromise its use as a regulatory precursor to the final API **1**. Hence, we decided to address this inherent shortcoming and envisaged the use of T3P as a common reagent to carry out three transformations in the same pot, namely: (a) amidation of **7** with **10** to furnish **1**, (b) amidation of **7** with the impurity **8** to furnish **9**, and (c) dehydration of **9**, thus formed, to furnish **1**. To our delight, even a sample of the amino-nitrile **10·HCl**, containing 5 % of the amino-amide **8**, afforded Nirmatrelvir **1** with ~97 % purity when reacted with **7** in presence of T3P (Scheme 5).

The foregoing observation indicated that T3P can be successfully employed as a reagent to obtain a direct access to the final API **1** from the carboxamide **9**. Additionally, unlike the Burgess reagent, T3P is well-known for its low toxicity, long shelf-life stability and easy handling [18]. Initial development studies through conventional batch chemistry revealed that the dehydration step was rather slow and required almost 12–16 h to complete even upon refluxing the reaction mixture.

It was at this juncture that we contemplated the use of continuous flow chemistry as a handy tool for facilitating conventional reactions under unconventional operating windows [19,20]. A technique that enables a chemical transformation to be carried

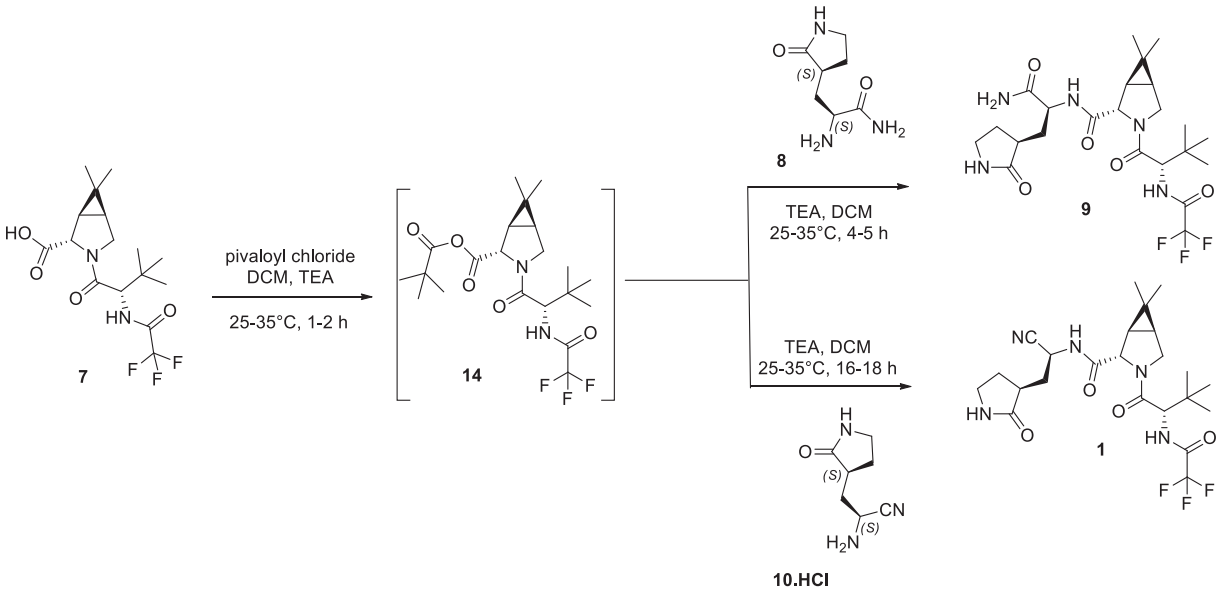


Scheme 5. Synthesis of Nirmatrelvir **1** from **7** and **10·HCl** using T3P.

out in tubes or pipes as opposed to a conventional round-bottom flask, flow has carved for itself a tremendously attractive niche in the domain of organic synthesis [21–24]. It presents several advantages such as (a) high surface-to-volume ratio that enhances heat and mass transfer ultimately resulting in better selectivity of products formed [25], (b) absence of a headspace that promotes sensitive reactions to be easily carried out [26], (c) excellent handling of exotherms thus resulting in enhancing reaction profile and controlling runaway reactions [27–29], (d) enhanced scale-up technologies available for large-scale production post-optimization [30].

For the chemical transformation **9** → **1**, application of continuous flow techniques was expected to bring about an improvement owing to the following reasons: (a) application of a back-pressure could prevent the liquid stream from evaporating, thus allowing exploration of temperatures higher than the boiling points of the solvents present; (b) an increase in the operating temperatures of the process could bring down the overall time needed for the conversion, thus assuring a time-efficient mode of synthesis; and (c) adapting a lab-based flow process into the industrial regime is easily possible due to various commercially available flow systems capable of large-scale manufacturing.

Fig. 2 shows the schematic representation of the continuous flow experimental setup that was employed. A photograph of the actual experimental setup is included in Fig. S1 of the Supplemen-



Scheme 4. Synthesis of **1** and **9** from **7** using the mixed-anhydride approach.

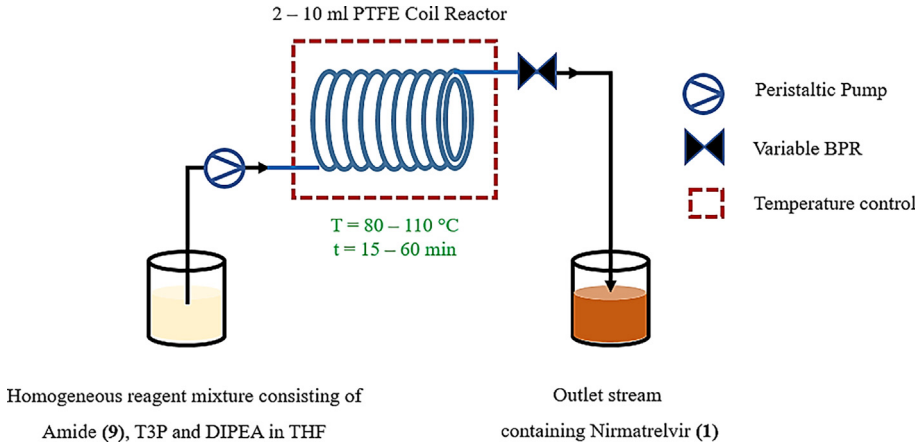


Fig. 2. Schematic representation of the continuous flow setup used for synthesis of **1** via T3P-mediated dehydration of the amide **9**.

Table 1
Summary of the continuous flow experimental trials carried out for the T3P-mediated dehydration of **9** to **1**.[a]

Sr. No.	Molar equivalents			Temp. (°C)	ResidenceTime (min)	HPLC (% Area under the curve)		
	Amide (9)	DIPEA	T3P			Amide (9)	Nitrile (1)	Σ (Others)
1	1	2.5	2	100	30	1.4	85.69	12.91
2	1	2.5	2	110	15	24.61	65.18	10.21
3[b]	1	2.5	2	100	30	0	89.44	10.56
4	1	2.5	1.5	100	30	13.1	72.42	14.48
5	1	2.5	2	80	30	17.11	69.71	13.18
6	1	2.5	2	110	30	0	87.38	12.62

[a] All experiments were performed with 10 volumes of THF. All runs were performed in either 2 mL or 10 mL PTFE coils with Vapourtec [31] V-3 peristaltic pumps and variable BPR. Purity of the amide employed as input was typically >85 % by HPLC. Yield and purity of crude **1** isolated from the output stream were 60–75 % and >90 % respectively [typical results from batch experiments, yield: 65–68 %, purity: >90 %].
[b] Performed to check process reproducibility.

tary Information file. A summary of the experimental runs conducted through flow have been compiled in the Table 1. As clearly indicated in Table 1, a complete conversion of the carboxamide **9** to Nirmatrelvir **1** could be achieved in flow with a reaction temperature of 100 °C and a residence time of 30 min (as compared to 12–16 h in batch) using 2.5 mol equiv. of DIPEA and 2.0 mol equiv. of T3P. The yield and purity of the crude API isolated from the output stream were comparable to those obtained by conventional batch process.

In summary, we have demonstrated a viable strategy for commercial adoption of an alternate and a more convergent synthetic route to Nirmatrelvir **1** – an important antiviral drug needed in countering the COVID-19 pandemic. During the course of our efforts, we have delineated a scalable synthesis and practical means of isolating the amino-nitrile **10** in a pure form as a crystalline hydrochloride salt. This salt **10·HCl** afforded a direct access to Nirmatrelvir **1** via amidation with the carboxylic acid **7** activated as a pivaloyl mixed anhydride. Using T3P to couple **7** and **10·HCl** had the added benefit of being able to concomitantly convert the amino-amide **8·HCl** (invariably present as an impurity in **10·HCl**) to the carboxamide **9** which was then dehydrated by T3P to **1**. This allowed us to obtain the final API in ~97 % purity even with a sample of the amino-nitrile **10·HCl**, containing 5 % of the amino-amide **8**. Subsequently, a continuous flow process was developed for achieving an efficacious and scalable T3P mediated conversion of **9** to **1**. Unlike a batch process that took 12–16 h to complete even at the reflux temperature of the reaction mixture, the same chemical transformation **9** → **1** could be achieved within just 30 min in flow without the need to operate under extreme temperature and pressure regimes. Efforts to obtain a direct access to **1** in flow from the carboxylic acid **7** and the amino-amide **8** via T3P-mediated tandem amidation-dehydration are currently underway, and the results will be communicated soon.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data (Experimental procedures; characterization data of compounds **1**, **9**, **10·HCl**, **12** and **13**; and details of the experimental setup employed in the continuous flow T3P-mediated dehydration of **9** to **1**) to this article can be found online at https://doi.org/10.1016/j.tetlet.2023.154344.

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