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Total synthesis of antiviral drug, nirmatrelvir (PF-07321332)

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ABSTRACT

The emergence and rapid spread of coronavirus disease 2019 (COVID-19), a potentially fatal disease, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has swiftly led to public health crisis worldwide. Hence vaccines and antiviral therapeutics are an important part of the healthcare response to combat the ongoing threat by COVID-19. Here, we report an efficient synthesis of nirmatrelvir (PF-07321332), an orally active SARS-CoV-2 main protease inhibitor.

1. Introduction

The present pandemic has challenged scientists to quickly identify molecules/scaffolds that have shown antiviral activity and can help in mitigation of the disease caused by SARS-CoV-2. Researchers from academic institutions and industries explored molecules which had earlier progressed in the drug discovery pipeline and verified their activity against COVID-19. In addition to early molecules such as remdesivir, favipiravir, umifenovir, *etc.* PF-00835231 [1], which had shown potential activity against SARS-CoV-1 was also considered. To build structure activity relationship (SAR) around PF-00835231, analogues were synthesized by modifying the constituent amino acids and six important analogues were considered for further development. The most promising analogue, PF-07321332 (nirmatrelvir), has been approved by FDA in December 2021 as an antiviral combination pill that can be taken at home to prevent people infected with COVID-19 from becoming severely ill. Nirmatrelvir (1) is a SARS-CoV-2 main protease (Mpro) inhibitor and sold in combination with ritonavir, a CYP3A inhibitor, as Paxlovid™ (brand name) [2].

Nirmatrelvir (1) is a tripeptide protein mimetic and was identified by carrying out SAR studies on some of the clinical candidates/approved molecules. Lufotrelvir (2) and dipeptide of boceprevir (3) formed the basis of constitution of nirmatrelvir. The pyrrolidine amino acid fragment

of lufotrelvir [3] and the dipeptide fragment of boceprevir [4] gave rise to the basic skeleton of nirmatrelvir. The phosphate prodrug functionality of 2 was replaced with a –CN group in 1. During the studies carried out for identification of boceprevir, the potency of the molecule increased due to the incorporation of the *gem*-dimethyl cyclopropyl proline moiety. Using this clue, the same was introduced in analogues for identification of potent molecules for treating SARS-CoV-2. Thus, the bicyclic amino acid in boceprevir 3 and nirmatrelvir 1 has helped in increasing the potency of the molecules (Fig. 1).

2. Results and discussion

Our research group has focused on the synthesis of peptides and peptidomimetics [5]. The consideration of nirmatrelvir, for clinical trials, as an oral antiviral drug, enthused us to take up the synthesis of 1. The molecule is comprised of three amino acids (*L*-*tert*-leucine, bicyclic proline and cyano lactam residue), all unnatural and thus, developing scalable and stereoselective synthetic strategies to achieve the target molecule are imperative. Based on the literature, it was noticed that the bicyclic *gem*-dimethyl cyclopropyl proline amino acid is the key fragment, which increased the potency of the analogue. We thus set out to first synthesize bicyclic amino acid (4) and then followed by the synthesis of nirmatrelvir (1). The retrosynthetic strategy is based on the conversion

Abbreviations: SARS-CoV, severe acute respiratory syndrome coronavirus; SAR, structure activity relationship; FDA, Food and Drug Administration; MsCl, methanesulfonyl chloride; Boc, *tert*-butyloxycarbonyl; HATU, 1-[*bis*(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; NMM, *N*-methylmorpholine; DMAP, 4-dimethylaminopyridine; TMSCl, trimethylsilyl chloride; LiHMDS, lithium *bis*(trimethylsilyl)amide; DMP, Dess–Martin periodinane.

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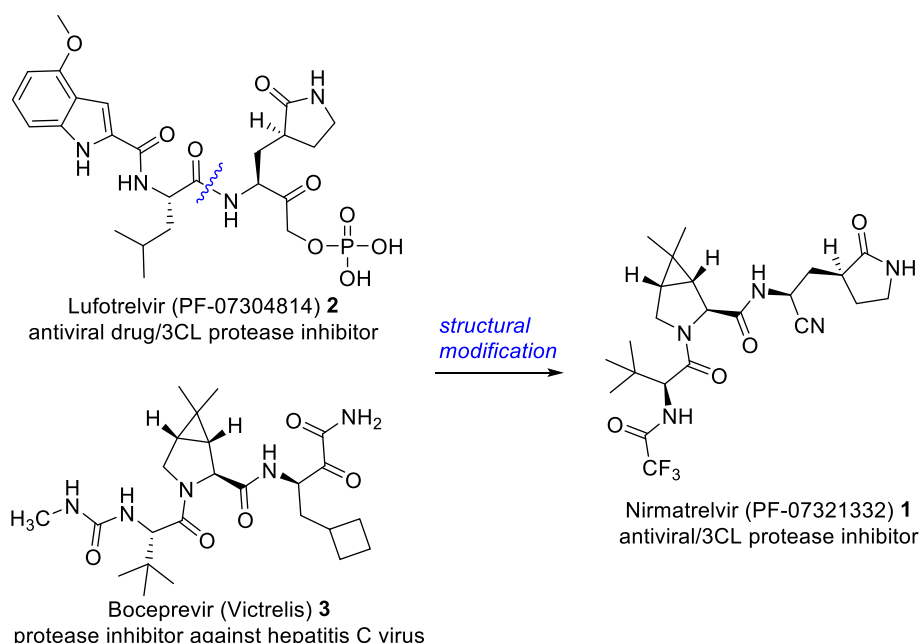


Fig. 1. Structure of antivirals.

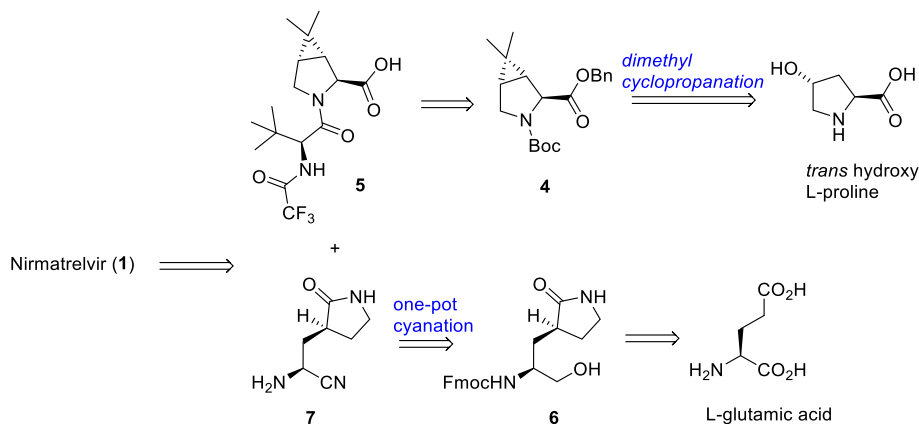
of *trans*-4-hydroxy proline to alkene *via* elimination of hydroxy group which would undergo cyclopropanation to yield **4** and further its conversion to dimer acid **5**. On the other hand, L-glutamic acid could be converted to alcohol **6** which can be oxidized to aldehyde followed by its conversion to nitrile **7**. The coupling of fragments **5** and **7** will lead to nirmatrelvir **1** (Scheme 1).

In literature, the synthesis of bicyclic amino acid [6–8] is reported from pyroglutamic acid [6] (involving nine steps) or (+)-3-carene7 (involving eleven steps). The proposed synthesis of key bicyclic fragment **4** commenced from Boc-*trans*-4-hydroxy L-proline benzyl ester **8** which was obtained from commercially available *trans* 4-hydroxy L-proline, using literature protocol [9]. The hydroxy group was mesylated using methanesulfonyl chloride, triethylamine and DMAP to get **9** in quantitative yield. Compound **9** was treated with diphenyldiselenide [10] and sodium borohydride to afford phenylselenenyl derivative **10** in 78% yield. The oxidation of intermediate selenide **10**, with hydrogen peroxide and subsequent elimination with pyridine smoothly gave the alkene **11** in 75% yield. This compound upon cobalt(II)-catalyzed dimethylcyclopropanation [11,12] using 2,2-dichloropropane, zinc metal, zinc bromide and Co(II)-complex gave target bicyclic dimethylcyclopropyl amino acid fragment **4** in 68% yield. Thus, the synthesis of bicyclic amino acid **4** was achieved from Boc *trans*-4-hydroxy L-proline benzylester **8** in four steps

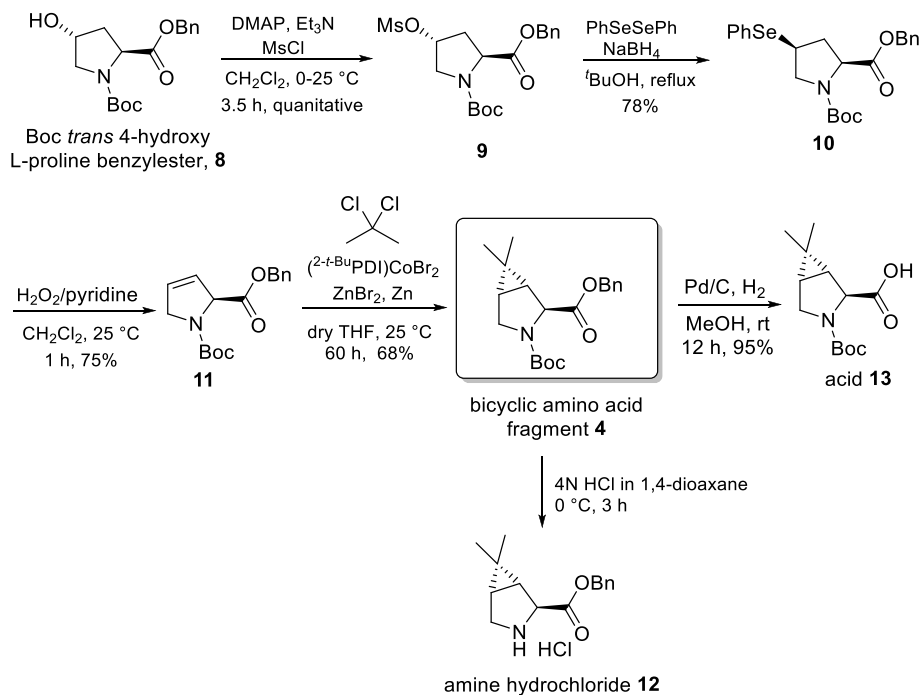
in 40% overall yield. To facilitate peptide coupling on either side, the amino acid residue **4** was treated with either 4 N hydrochloric acid to give free amine benzyl ester as HCl salt **12** or Pd/C–H₂ to give Boc-protected free acid **13** (Scheme 2).

The amine hydrochloride of bicyclic fragment **12** was coupled with *N*-trifluoroacetyl L-*tert*-leucine **14** (obtained from *N*-trifluoro acetylation on L-*tert*-leucine) [13] using HATU, in presence of NMM, DMAP to give dipeptide **15** in 71% yield (Scheme 3).

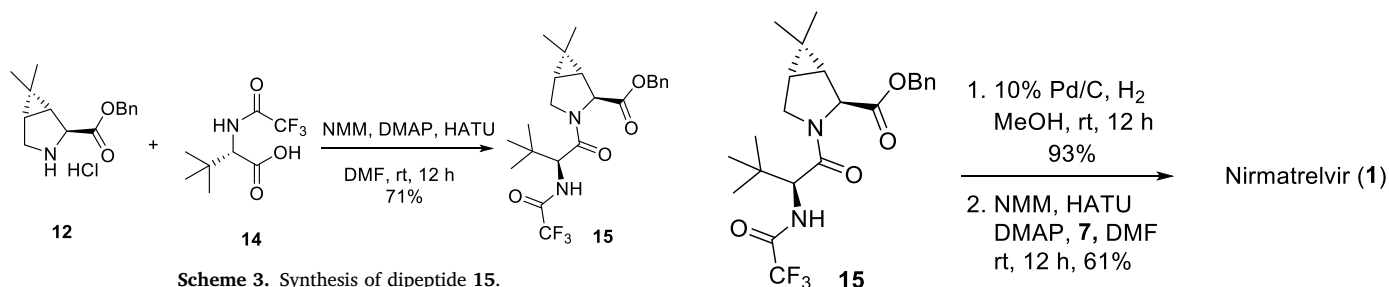
The synthesis of cyano amine **7** was achieved from protected L-glutamic acid **16** (Scheme 4). L-Glutamic acid was silylated with TMSCl, esterified using methanol [14] and the amine group was protected with FmocCl in presence of sodium carbonate as a base to yield protected glutamic acid **16**. The diester **16** was subjected to mono-alkylation with bromoacetonitrile using LiHMDS in THF to give cyano compound **17**. The cyano diester **17** was cyclized to lactam **18** using CoCl₂·6H₂O and sodium borohydride [15]. The ester functionality in lactam **18** was reduced to alcohol **6** using sodium borohydride in THF:MeOH (2:1). Alcohol **6**, upon oxidation with Dess-Martin periodinane (DMP), afforded aldehyde **19**. A one-pot conversion of aldehyde **19** to nitrile **20** was achieved by the treatment of aldehyde with iodine and ammonia solution [16]. Finally, the Fmoc-deprotection of compound **20** using diethylamine resulted in the formation of cyano amine fragment **7**.



Scheme 1. Retrosynthesis of bicyclic amino acid (**4**) and nirmatrelvir (**1**).



Scheme 2. Synthesis of cyclopropyl fragment 4 and its coupling partners (12, 13).



Scheme 3. Synthesis of dipeptide 15.

Scheme 5. Synthesis of Nirmatrelvir 1.

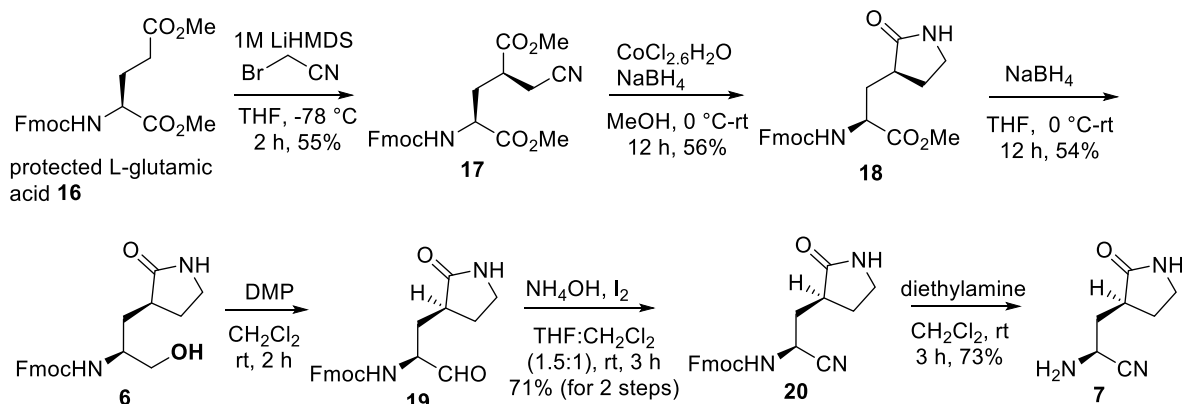
Dipeptide 15 on debenzoylation using Pd/C, H₂ in methanol gave dimer acid 5 in 93% yield. The dimer acid (5) is ready to be coupled with amine 7 to give Nirmatrelvir [17]. The amine 7 was coupled with dimer acid 5, using HATU in presence of NMM and DMAP, to afford the target molecule nirmatrelvir 1 (Scheme 5). The spectral data of compound 1 was found to be in agreement with the reported literature values [18].

The present work provides nirmatrelvir 1 in three steps from amine hydrochloride 12, whereas the earlier report [18] utilises six steps. The use of Burgess reagent is avoided in our protocol, thereby reducing the

cost of synthesizing the product. The protocol uses appropriately substituted amino acids to give better yields with lesser number of steps.

3. Conclusions

In conclusion, synthesis of nirmatrelvir (PF-07321332) has been achieved in fewer steps than the reported protocols, using chemicals which are easily accessible. The key steps of the synthesis involve an



Scheme 4. Synthesis of cyano amine fragment 7.

asymmetric Co(II)-catalyzed dimethyl cyclopropanation to provide bicyclic amino acid and one-pot conversion of aldehyde group to nitrile for other amino acid. The manuscript provides an alternate route for the synthesis of the approved molecule which has the potential to be scaled up.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tchem.2022.100033>.

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