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A multi-step continuous flow synthesis of pomalidomide

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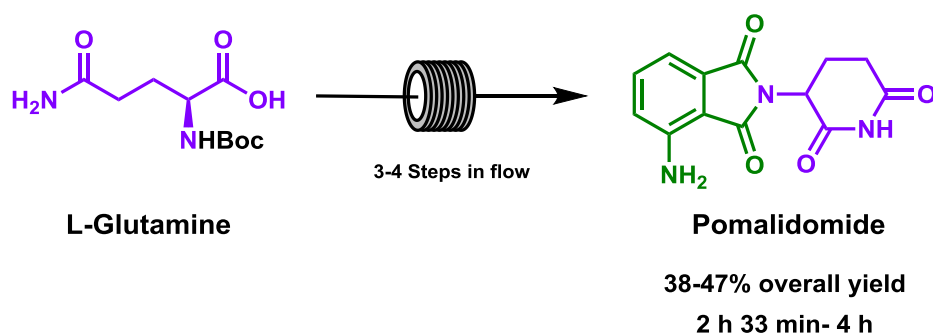
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Graphical abstract



Abstract : The immunomodulatory drugs (IMiDs) thalidomide, pomalidomide, and lenalidomide are widely used to treat multiple myeloma. The permanent need of IMiDs analogues in clinical screening and practice has created the demand to find a reliable and robust method for their preparation. To reach this goal, the use of flow chemistry is quite appealing, allowing a safe operation, an excellent reproducibility and an efficient process.

Herein, we describe a continuous 3-4 step flow approach for the synthesis of the pomalidomide (38-47% overall yield) and a few analogues.

Keywords: active pharmaceutical ingredients, IMiDs, thalidomide, glutamine, reduction,

Thalidomide (2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione) was developed by Chemie Grunenthal in 1956 (Figure 1).^{1,2} Initially, it was put in application as a sedative and had much softer side effects than barbiturates; it was therefore prescribed to pregnant women for the treatment of morning nausea. A few years later, the raise of the cases of phocomelia and malformations of the newborns were detected, caused by the use of thalidomide during pregnancy.^{3,4} As a result, the drug was withdrawn from the marketplace. After further study, it was discovered that thalidomide is efficient against various diseases, namely, a number of cancers, graft-versus-host disease, and a number of skin conditions. For that reason, it was reapproved to be marketed.

The sedative effect is linked to (*R*)-isomer, whereas the teratogenic effects are associated with the (*S*)-isomer. At physiological conditions the asymmetric tertiary carbon-center of thalidomide is epimerized, hence the clinical trials of a single isomer are not possible and the drug is marketed as a racemic mixture.^{5,6} The immunomodulatory thalidomide analogues (IMiDs) are constantly being searched for and tested, to boost some properties for the treatment of autoimmune and inflammatory diseases and also reduce the harmfulness of side effects. One of the best thalidomide analogues with direct antitumor effect, ability to inhibit angiogenesis, and immunomodulatory role is pomalidomide, the 4-amino substituted analog of thalidomide (Figure 1). It was proved to be a potent inhibitor of tumor necrosis factor (TNF- α) release as assessed in cultured human peripheral blood mononuclear cells challenged to lipopolysaccharide (LPS), a major component of the outer membrane of gram-negative bacteria that is used as a pharmacological tool to induce TNF- α synthesis and release.⁷

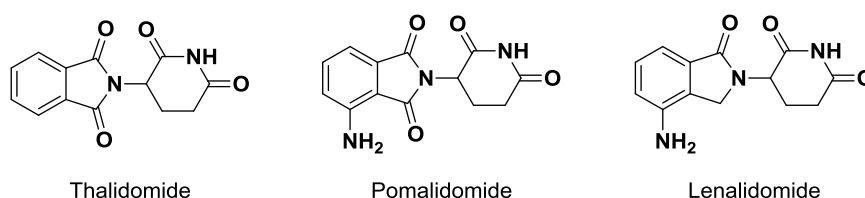


Figure 1. Chemical structures of thalidomide, pomalidomide, lenalidomide.

Besides, continuous flow chemistry has been gaining interest in recent years because of improvements in efficiency and safety as the scale of production increases.^{8,9} Therefore, we conjectured that the development of a continuous flow process to access pomalidomide would be beneficial to the subsequent development of an industrially viable flow route. Moreover, such a process would offer an interesting synthetic manifold to pave the way of the development of libraries in the context of a drug discovery program.^{10–14} Hence, we report herein a continuous flow synthesis of pomalidomide and analogues, as a proof of concept. Most of the synthetic pathways to build up pomalidomide are based on the cyclisation of glutamine and the subsequent condensation with phthalic anhydride. Previous reports described the stepwise deprotection and condensation reactions,¹⁵ as an alternative, the condensation could be followed by cyclisation.⁷ Here, we chose a recent method, which combined the thermal Boc deprotection with the condensation step.¹⁶ Our synthesis of pomalidomide **11** begins by the treatment of the commercially available Boc-L-glutamine **1** with *N*-hydroxysuccinimide **2** and *N,N'*-Diisopropylcarbodiimide **3** in DMF,¹⁷ in which racemization of the product occurs. The resultant *N*-Boc glutarimide ring **4** is either deprotected by under acidic conditions to afford to the glutarimide moiety **5** as hydrochloride salt, or directly involved in the step of the condensation with 4-nitrophthalic anhydride **6**, affording the 4-nitro-substituted thalidomide derivative **7**. A final reduction of the nitro group proceeded smoothly to yield the 4-amino-substituted analogue **11** (Figure 2).

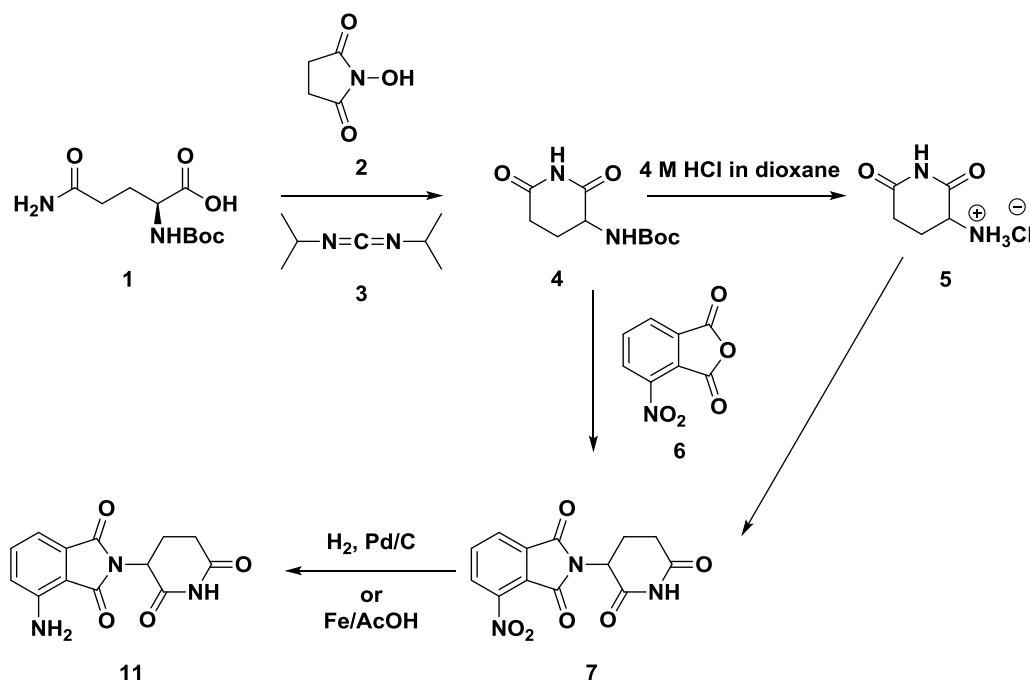


Figure 2. Synthesis of pomalidomide **11**

The mixture of Boc-L-Glutamine **1** and *N*-hydroxysuccinimide **2** was dissolved in DMF (0.25 M), this solution was then combined at 0.1 mL/min flow rate with the solution of DIC **3** in DMF (0.25 M) within a T-mixer at the same flow rate. Then, the blended solution passed through a 22 mL PTFE reactor (1.6 mm ID) at 80 °C placed into a water bath, the residence time amounted to 1 h 50 min, whereas in batch this reaction takes 6 hours. All attempts to downsize the reactor, or an increase the flow rate, or the concentration, led to inferior conversions. We checked as well several carbodiimides and solvents (namely DCC in DCM, DCC in DMF, EDC in DMF, DIC in DMF) and in terms of the best solubility, DIC in DMF was chosen (For more details see SI, Table SI1). The product **4** was then obtained in 71% yield on 1 mmol scale (Figure 3), (productivity: 0.011 kg/h.L).

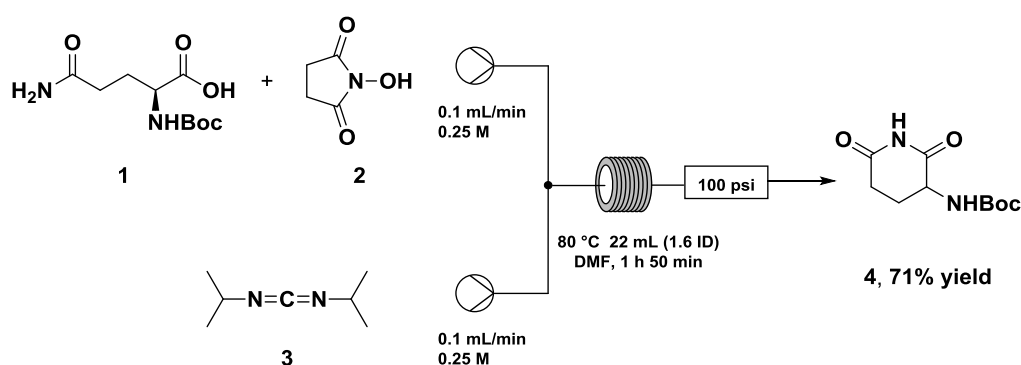


Figure 3. Continuous flow cyclisation of Boc-L-Glutamine **1**

Then, the deprotection of the amino group on the obtained cyclic amide **4** was achieved. To do so, it should be diluted in dioxane. A 0.2 M solution of **4** was the most convenient to handle, a more concentrated solution tended to precipitate. The solution was then combined with the second one 4 M HCl in dioxane, flowed through a 10 mL tubular reactor at 50°C at 1.0 mL/min total flow rate. The residence time of this transformation was 10 min. After the solvent was evaporated, the pure product **5** was delivered in quantitative yield (productivity: 0.097 kg/h.L) (Figure 4)

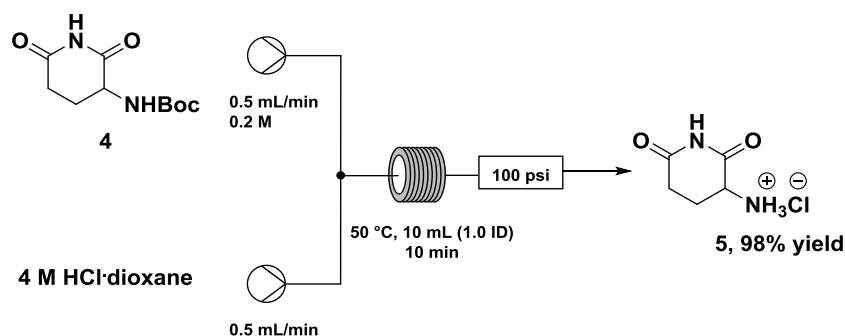


Figure 4. Continuous flow deprotection of cyclic amide **4**

With regard to the next step, we tested various reaction conditions in order to carry out the condensation step between the commercially available phthalic anhydride **6** and the *N*-Boc-deprotected cyclic L-Glutamine **5**, namely: AcOH/Ac₂O, MeOH/H₂O, 1 M NaOH, propionic acid/butyric acid, Et₃N/ACN, AcOH/H₂O/dioxane, etc (For more details see SI). We found that the best conditions to perform this reaction in flow made use of the following solvent mixture: AcOH/H₂O/ 1,4-dioxane (2 :1 :1), 0.1 M at 130 °C at a flow rate of 0.1 mL/min in a 10 mL PTFE coil reactor. The residence time amounted to 1.66 h. The yield of this transformation was 71% calculated on 1 mmol scale (productivity: 0.013 kg/h·L), (Figure 5).

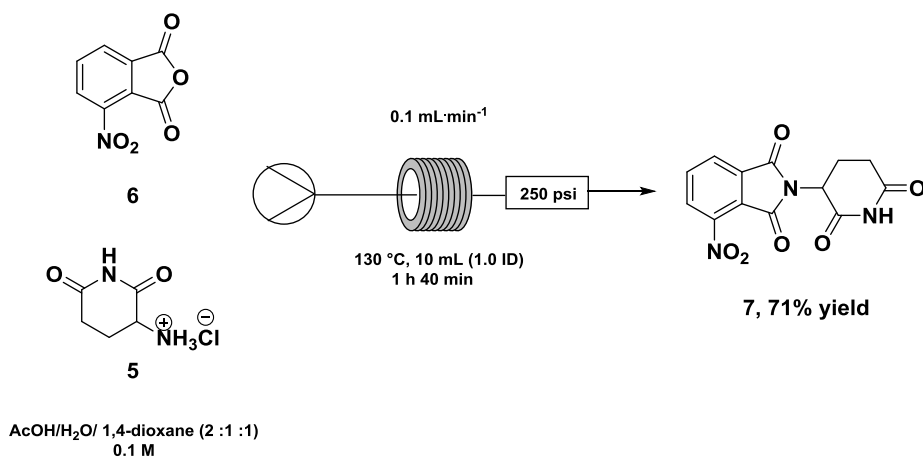


Figure 5. Continuous flow condensation reaction of the salt **5** with 4-nitrophthalic anhydride **6**

Nevertheless, the purity of the product was not fully satisfactory and it was decided to move on with the direct use of *N*-Boc-protected cyclic L-Glutamine, allowing us to skip the deprotection step and to shorten the synthetic route. With this in mind, we evaluated a few conditions and the following turned out to be the best : the commercially available phthalic anhydride **6** and the *N*-Boc-protected cyclic L-Glutamine **4** were mixed in a trifluoroethanol/ toluene mixture (2:1) at the concentration of 0.1 M and then passed through a 10 mL high temperature tube reactor made of stainless steel, at 150 °C, at 0.1 mL/min. The flow system

was equipped with a 250 psi BPR at the outlet of the reactor. Regarding the purity and conversion, these conditions were the most favorable. Indeed, attempts with HFIP/toluene, trifluoroethanol/1,4-dioxane mixtures at various proportions and temperatures were less efficient in terms of yields and purity. By these means, we produced a greyish solid **7** in 68% yield, based on a 0.9 mmol scale (productivity: 124 mg/h). Besides, it was recently reported by Hashimoto and co-workers that thalidomide tetrafluorophthalimides and tetrachlorophthalimides are possible therapeutic agents, which enhance the TNF-R production in PMA-treated HL-60 cells.^{18,19} In view of this, we applied the above-mentioned conditions to the synthesis of these promising bioactive molecules (Figure 6). Using our developed conditions, the chloro, perchloro and perbromo analogues were synthesized in moderate to good yield under the developed standard conditions.

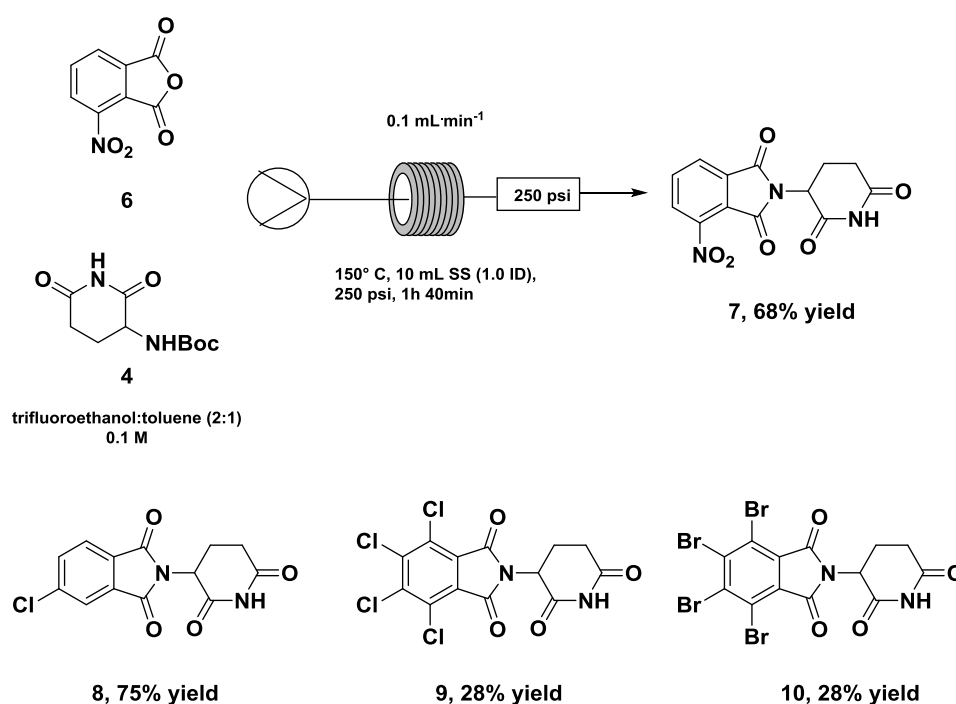


Figure 6. Continuous flow condensation reaction of **4** with 4-nitrophthalic anhydride **6** and IMiDs prepared by the same method

To complete the synthesis of the pomalidomide a final reduction of the nitro function is needed. We used the H-cubeTM with 30 mm CatCart® and tested various catalysts: Raney nickel, platinum (IV) oxide, Pd/C (various %). We discovered that a single cycle of 3 min (1 mL/min) with CatCart® 10% Pd/C at 45 °C and 0.01 M EtOH with full mode of hydrogenation was sufficient to reduce the nitro group. We obtained a bright yellow oil in 78% yield, starting from 0.5 mmol of **7** (productivity: 0.043 kg/h·L) (Figure 7).

The reduction could also be carried out inside of a Vapourtec column reactor heated at 80 °C. In order to do so, the Omnifit column should be packed with a mixture of 20 equiv of Fe

and silica gel, and the solution of the nitro-compound **7** in AcOH (0.02 M) passed through it at a flow rate of 0.2 mL/min. The reduced product **11** was thus obtained in 95% yield (productivity: 0.062 g/h) (For more details see SI).

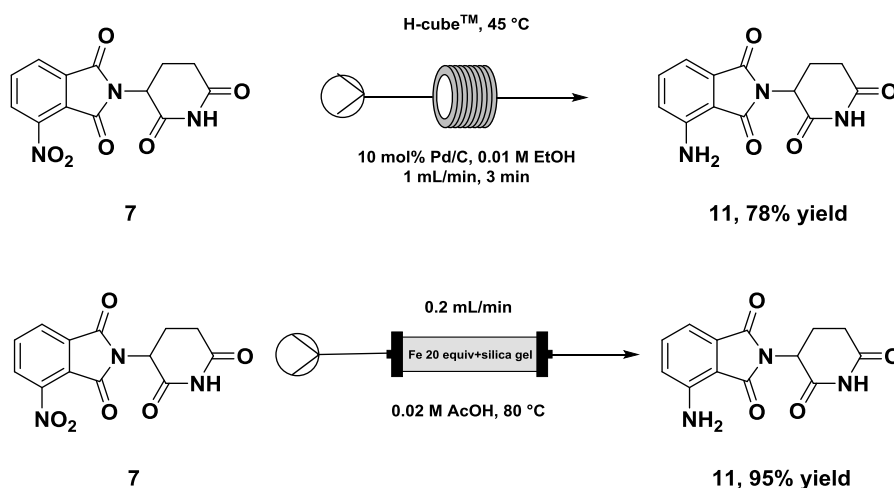


Figure 7. Continuous flow reduction of nitro group of **7** using H-cube™ or Fe + silica gel filled column reactor

In summary, we have demonstrated the 3-/4-steps flow-based synthetic approaches to pomalidomide, which was applied to the synthesis of other immunomodulatory thalidomide analogues (IMiDs). Starting from the commercially available *N*-Boc-L-Glutamine, after the cyclisation, the *N*-Boc glutarimide ring is either deprotected or directly involved in the step of the condensation with 4-nitrophthalic anhydride. The final step of the smooth reduction of the nitro group delivered pomalidomide. We believe that this first synthesis in flow of such important scaffolds would pave the way to further development toward the implementation of continuous flow for the synthesis of libraries of bioactive molecules or towards the development of industrial continuous flow processes with higher efficiency.

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest

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