Nature, accepted
DOI 10.1038/nature14343

Multistep Continuous Flow Synthesis of (*R*)- and (*S*)-Rolipram Using Heterogeneous Catalysts

Tetsu Tsubogo,[1] Hidekazu Oyamada[1] & Shū Kobayashi[1]

[1] Department of Chemistry, School of Science and Green & Sustainable Chemistry Social Cooperation Laboratory, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

Chemical manufacturing is conducted using two systems: batch systems or continuous flow systems. Flow systems have several advantages over batch systems, particularly in terms of productivity, heat and mixing efficiency, safety, and reproducibility[1-4]. However, for over 50 years, pharmaceutical manufacturing has been conducted using batch systems, because the synthesis of complex molecules, like drugs, has been difficult to achieve with continuous flow systems[5, 6]. Here, we describe for the first time, the continuous flow synthesis of drugs using only columns packed with heterogeneous catalysts. Commercially available starting materials were successively passed through four columns containing achiral and chiral heterogeneous catalysts to afford (*R*)-rolipram[7], an anti-inflammatory drug and one of the family of γ-aminobutyric acid (GABA) derivatives[8], directly. In addition, by simply replacing a column packed with a chiral heterogeneous catalyst with another column bearing the opposing enantiomer, antipole (*S*)-rolipram was obtained. Similarly, (*R*)-phenibut, another drug belonging to the GABA family, was synthesized. The flow systems described in this report are simple and stable, and no leaching of metal catalysts occurred. Our results demonstrate multistep (eight-step here) chemical transformations for the syntheses of drugs can proceed smoothly under flow conditions using only heterogeneous catalysts, without the isolation of any intermediates and without the separation of any catalysts, co-products, byproducts, and excess reagents. We anticipate our synthesis to be an ideal model for future pharmaceutical manufacturing.

While the chemical and biotechnology industries have preferred the use of continuous flow systems because of their advantages such as high productivity and efficiency, fine chemical production has been conducted using batch systems because the synthesis of more complex molecules has been difficult to achieve with continuous flow systems. However, recent pharmaceutical manufacturing requires high quality of synthesis, environmentally benign methods and reproducibility of manufacturing. To meet these demands, it is believed that continuous flow systems are superior to batch systems.

Methods for continuous flow systems have been developed more recently than methods for batch systems. We divided the continuous flow systems into four types (I–IV; see Fig. 1). Type I: substrates (A and B) are passed through a column or hollow loop, during which reactions occur. Unreacted A or B or any by-products are not separated. Type II: one of the substrates (B) is supported in a column. If an excess amount of B is used, one substrate (A) is consumed. However, once supported B is consumed, the column must be changed. Type III: A reacts with B in the presence of a homogeneous catalyst. While catalysis proceeds smoothly, the catalyst cannot be separated. Type IV: A reacts with B in the presence of a heterogeneous catalyst. If catalysis proceeds smoothly, no separation is required.

Based on the recent regulations of green sustainable chemistry[9], synthesis with catalysts is preferred to synthesis without catalysts because of energy saving and waste reduction. Consequently, types III and IV are recommended in continuous flow systems. Furthermore, while catalysts are contaminated with products in type III, no contamination of catalysts is expected under ideal conditions in type IV. Therefore, as type IV is regarded as the best method for continuous flow synthesis[10-13], we elected to use type IV for drug synthesis. Although recent technological improvements have made it possible to synthesize relatively complex molecules including drugs using continuous flow systems[14-17], there have been no examples of drug synthesis using only Type IV continuous flow systems.

(Figure 1)

γ-Aminobutyric acid (GABA) and derivatives are an important class of compounds in neuroscience[8]. Rolipram is one of the GABA family. It is an anti-inflammatory drug[7, 18], which is a selective phosphodiesterase 4 (PDE4) inhibitor and particularly effective for the PDE4B subtype of PDE4[19]. Moreover, rolipram is known as a possible antidepressant and has been reported to have anti-inflammatory, immunosuppressive, and antitumor effects[7, 18]. Rolipram has also been proposed as a treatment for multiple sclerosis, and has been suggested to have antipsychotic effects[20]. Furthermore, it has been reported that (*R*)-rolipram has anti-inflammatory activity, whereas (*S*)-rolipram does not[19]. There are many GABA derivatives that are drugs or have potential biological activities in the area of neurotransmitter and brain science[21] (Fig. 2).

(Figure 2)

We selected (*R*)- and (*S*)-rolipram for the target of our continuous flow synthesis because as mentioned, rolipram itself is a very interesting and promising drug for several targets and because the completed flow synthesis may be applicable to the synthesis of other GABA derivatives. We planned to synthesize (*R*)- and (*S*)-rolipram from commercially available starting materials using continuous flow systems, using only type IV columns (Fig. 1). Our synthetic strategy is shown in Fig. 3. Commercially available aldehyde **2** and nitromethane **3** could be converted to nitroalkene **4**. Catalytic asymmetric 1,4-addition of malonate **5** to **4** could afford enantiomerically enriched γ-nitro ester **6**. The nitro group of **6** could be reduced selectively to afford γ-lactam **7** after cyclization. Finally, the ester group of **7** could be removed to afford **1**.

(Figure 3)

First, we examined the flow synthesis of **4** from **2** and **3**, using a heterogeneous catalyst (Fig. 4, stage 1)[22, 23]. The formation of nitroalkenes from aldehydes and nitroalkanes is known to proceed in the presence of a base[24]. We selected toluene as a solvent because the following step, the asymmetric 1,4-addition, proceeded smoothly in toluene. We examined several heterogeneous amines, and finally found that a silica-supported amine with anhydrous calcium chloride showed a high yield of **4** when using almost equimolar amounts of **2** and **3** at 50-75 °C. Under the optimized conditions, a silica-supported amine (Chromatorex DM1020; Fuji Silysia, Kasugai, Japan; 4.5 g, 0.73 mmol/g) and finely crushed anhydrous

calcium chloride (13.5 g) were introduced into a SUS column (ϕ 10 mm x 300 mm, column I). The toluene solution of **2** and **3** was introduced from the bottom of the column, and the desired product **4** was obtained in >90% yield. The system was found to be stable at 75 °C for at least one week (>90% yield). It was further confirmed that this flow system was applicable to other aldehydes; several nitroalkenes were obtained in high yields (Supplementary Information). It was noted that, while excess amounts of aldehydes (1.5–1.7 equiv) were required to obtain high yields of nitroalkenes in batch systems, equimolar amounts of aldehydes afforded the desired nitroalkenes in high yields under continuous flow conditions.

We next examined the asymmetric 1,4-addition of malonate **5** to **4** using a chiral heterogeneous catalyst (Fig. 4, stage 2). Catalytic asymmetric reactions provide one of the most efficient routes to enantiomerically enriched products[25]. Recently, we developed a polymer-supported chiral calcium catalyst, which was successfully used for the asymmetric 1,4-addition of malonates to nitroalkenes under continuous flow conditions[26]. We set column II, which was filled with this supported calcium catalyst (PS-(*S*)-Pybox-calcium chloride), and connected it with column I. We also included a valve (switching paths A and B) to drain the synthesized nitroalkene solution (receiver 1) and an MS 4A column (**X**, ϕ 5□□□□□□50□□□) to stabilize the system. A solution of nitroalkene **4** synthesized in column I and a toluene solution of malonate **5** and triethylamine were mixed and introduced into column II. After optimization of the reaction conditions, it was found that when the reaction was conducted at 0 °C, using slightly excess amounts of nitromethane **3** and malonate **5** (**4** was formed), the desired γ-nitro ester **6** was obtained in high yield with high enantioselectivity. Under the optimized conditions, the mixture of **2**, **3**, and **5** was precooled at 0 °C using a loop, and column II was separated into two columns (column II-1 and column II-2, each ϕ□□□□□□□□□1□□□□□, PS-(*S*)-Pybox, 750 mg, 0.85 mmol/g; $CaCl_2$•$2H_2O$, 375 mg; Celite®, 1.4 g. This was required due to the size of the cooling bath). We collected the crude product solution in receiver 2. It was confirmed to contain mainly **6**, with small amounts of **3** and **5**, and triethylamine. The crude product was quenched with an aqueous ammonium chloride solution, and after a usual work-up, the desired γ-nitro ester **6** was obtained in 84% yield with 94% ee. At this stage, we also tested several aldehydes in this continuous flow system. It was found that, in all cases, the desired γ-nitro esters were obtained in high yields with high enantioselectivities (Supplementary Information).

The next step involved the reduction of the nitro group to the corresponding amino group (Fig. 4, stage 3). Experimental conditions required the flow of the toluene solution obtained from column II to be under atmospheric pressure. We selected a continuous flow hydrogenation[27, 28] and examined several commercially available supported Ni and Pd catalysts[18, 29]; however, the desired reduction did not proceed at all. Having recently developed a polysilane-supported palladium/alumina (Pd/PSi-Al_2O_3) catalyst, which worked well for the hydrogenation of alkenes, alkynes, and also nitrobenzene derivatives under flow conditions[30], we then tested Pd/PSi-Al_2O_3 for the hydrogenation of **6**. Unfortunately, the reaction did not proceed.

At this stage, therefore, we decided to develop a new heterogeneous catalyst for our purpose. After several trials, we developed a polysilane-supported palladium/carbon (Pd/PMPSi-C) catalyst, which worked well for the reduction. We then connected column III (column III-1 and column III-2, both ø□□□□□□□□□1□□□□□; Pd/PMPSi-C, 4.8 g, 0.29 mmol/g [dry], 34% wet; Celite®, 1.2 g) with the already constructed flow system (columns I and II). The mixed solution (crude **6** in toluene) and hydrogen gas (3 mL/min) were introduced into column III (filled with Pd/PMPSi-C and Celite®) at 100 °C by downflow.

Under the conditions, the desired reduction proceeded smoothly to afford γ-lactam **7** in 74% yield with 94% ee. It is noted that the reduction of the nitro group proceeded smoothly under atmospheric pressure of hydrogen, and that no epimerization occurred under the conditions. We also tested other substrates and in all cases the reduction proceeded well to afford the desired γ-lactams in high yields with high enantioselectivities (Supplementary Information).

The final stage in the synthesis of rolipram (**1**) involved the hydrolysis and decarboxylation of the ester part of **7** (Fig. 4, stage 4). It was found that the desired transformations proceeded in the presence of a silica-supported carboxylic acid (Si-COOH; Chromatorex ACD, Fuji Silysia). We then connected column IV (ϕ□10□□□□□□□300□□□), which was filled with Si-COOH (13.5 g, 0.38 mmol/g) and Celite® (0.5 g), with columns I–III and examined the continuous flow starting from **2** and **3**. We then added small columns of Amberlyst 15Dry® (**Y**) and Celite® (**Z**), and *o*-xylene was introduced. The main flow from column III was combined with *o*-xylene and water, and the total flow was passed through column IV from the top down at 120 °C. Finally, we obtained (*S*)-rolipram ((*S*)-**1**, 50% yield from **2** after preparative TLC, 997.8 mg/24 h, 96% ee). The flow system was found to be stable for at least one week (Supplementary Information). Recrystallization from water/methyl alcohol gave optically pure (*S*)-rolipram (>99% ee). Direct recrystallization of the crude product afforded chemically and enantiomerically pure (*S*)-rolipram without chromatography.

(Figure 4)

Thus, the synthesis of (*S*)-rolipram was completed. Commercially available starting materials were successively passed through the columns containing heterogeneous achiral and chiral catalysts to directly afford the drug with high enantioselectivity. Eight-step chemical transformations were conducted smoothly during the flow without isolation of any intermediates and without the separation of any catalysts, co-products, byproducts, and excess reagents. In the flow system, each step can be monitored by using receivers (real-time analysis is possible). It is noteworthy that all four columns employed are the desirable type IV flow system (Fig. 1), and that the product does not contain any metal (palladium, <0.01 ppm), as confirmed by inductively coupled plasma analysis. Moreover, this is the first example that a chiral catalyst has been successfully used in multistep continuous flow synthesis of drugs or biologically important compounds.

This flow system could also be applicable to the synthesis of other GABA derivatives (Fig. 2). Antipole (*R*)-Rolipram was also synthesized by continuous flow by simply replacing column II packed with PS-(*S*)-Pybox-calcium chloride with column II′ bearing PS-(*R*)-Pybox-calcium chloride (the opposing enantiomer). The procedure remained the same and similar productivity was obtained ((*R*)-**1**, 50% yield from **2**, 96% ee). We also synthesized (*R*)-phenibut[21] from benzaldehyde by slightly modifying the flow system. We believe that all the compounds shown in Fig. 2 could be synthesized by using continuous flow systems.

The present multistep continuous flow synthesis is for the laboratory scale, and the drugs were obtained on a gram-scale. On the other hand, we have confirmed that the system is stable and the flow is at steady state during the synthesis. Indeed, the system is stable for at least one week, and the same yields and the enantioselectivities were obtained for the syntheses of (*R*)- and (*S*)-rolipram. Furthermore, it has also been confirmed that heterogeneous catalysts used in this flow system are robust, air-stable, and have a long lifetime. For example, the chiral calcium catalyst can be used for more than several months

without loosing any catalytic activity and selectivity (enanitioselectivity). We are now scaling up the system toward multi-kilogram syntheses of drugs.

1. Wiles, C. & Watts, P. Continuous flow reactors: a perspective. *Green Chem*. **14**, 38-54 (2012).
2. Ley, S. V. & Baxendale, I. R. New tools and concepts for modern organic synthesis. *Nat*. *Rev*. *Drug Disc*. **1**, 573–586 (2002).
3. Geyer, K., Codée, J. D. C. & Seeberger, P. H. Microreactors as tools for synthetic chemists–the chemists' round-bottomed flask of the 21st century? *Chem*. *Eur*. *J*. **12**, 8434–8442 (2006).
4. Hartman, R. L., McMullen, J. P. & Jensen, K. F. Deciding whether to go with the flow: Evaluating the merits of flow reactors for synthesis. *Angew. Chem. Int. Ed*. **50**, 7502-7519 (2011).
5. Van Arnum, P. Advancing flow chemistry in API manufacturing. *Pharm. Technol.* **37**, 78–82 (2013).
6. Poechlauer, P. *et al*. Continuous processing in the manufacture of active pharmaceutical ingredients and finished dosage forms: An industry perspective. *Org. Process Res. Dev.* **16**, 1586–1590 (2012).
7. Sommer, N. *et al*. The antidepressant rolipram suppresses cytokine production and prevents autoimmune encephalomyelitis. *Nature Med.* **1**, 244–248 (1995).
8. Macdonald, R. L. & Olsen, R. W. GABAA receptor channels. *Annu*. *Rev*. *Neurosci*. **17**, 569–602 (1994).
9. Anastas, P. T. & Warner, J. C. Ed. *Green Chemistry Theory and Practice* (Oxford University Press, Oxford, 1998).
10. Kirschning, A., Solodenko, W. & Mennecke, K. Combining enabling techniques in organic synthesis: Continuous flow processes with heterogenized catalysts. *Chem. Eur. J*. **12**, 5972-5990 (2006).
11. Frost, C. G. & Mutton, L. Heterogeneous catalytic synthesis using microreactor technology. *Green Chem*. **12**, 1687-1703 (2010).
12. Tsubogo, T., Ishiwata, T. & Kobayashi, S. Asymmetric carbon–carbon bond formation under continuous-flow conditions with chiral heterogeneous catalysts. *Angew*. *Chem., Int*. *Ed*. **52**, 6590–6604 (2013).
13. Battilocchio, C.; Hawkins, J. M. & Ley, S. V. A mild and efficient flow procedure for the transfer hydrogenation of ketones and aldehydes using hydrous zirconia. *Org. Lett.* **15**, 2278-2281 (2013).
14. Pastre, J. C., Browne, D. L. & Ley, S. V. Flow chemistry syntheses of natural products. *Chem. Soc. Rev.* **42**, 8849–8869 (2013).
15. Hartwig, J. *et al*. Heating under high-frequency inductive conditions: Application to the continuous synthesis of the neurolepticum olanzapine (Zyprexa). *Angew. Chem., Int. Ed.* **52**, 9813–9817 (2013).
16. Webb, D & Jamison, T. F. Continuous flow multi-step organic synthesis. *Chem. Sci.* **1**, 675-680 (2010).
17. Hopkin, M. D., Baxendale, I. R. & Ley, S. V. An expeditiouus synthesis of imatinib and analogues utilising flow chemistry methods. *Org. Biomol. Chem.* **11**, 1822-1839 (2013).
18. Barnes, D. M. *et al*. Development of a catalytic enantioselective conjugate addition of 1,3-dicarbonyl compounds to nitroalkenes for the synthesis of endothelin-A antagonist ABT-546. Scope, mechanism, and further application to the synthesis of the antidepressant rolipram. *J*. *Am*. *Chem*. *Soc*. **124**, 13097–13105 (2002).

19. Day, J. P. *et al.* Elucidation of a structural basis for the inhibitor-driven, p62 (SQSTM1)-dependent intracellular redistribution of cAMP phosphodiesterase-4A4 (PDE4A4). *J. Med. Chem.* **54**, 3331–3347 (2011).
20. Maxwell, C. R., Kanes, S. J., Abel, T. & Siegel, S. J. Phosphodiesterase inhibitors: A novel mechanism for receptor-independent antipsychotic medications. *Neuroscience* **129**, 101–107 (2004).
21. Malykh, A. G. & Sadaie, M. R. Piracentam and piracetam-like drugs. *Drugs* **70**, 287–312 (2010).
22. Motokura, K., Tada, M. & Iwasawa, Y. Layered materials with coexisting acidic and basic sites for catalytic one-pot reaction sequences. *J. Am. Chem. Soc.* **131**, 7944–7945 (2009).
23. Soldi, L. *et al.* Use of immobilized organic base catalysts for continuous-flow fine chemical synthesis. *J. Catal.* **258**, 289–295 (2007).
24. Worrall, D. E. Nitrostyrene. *Org. Synth.* **9**, 66–69 (1929).
25. Ojima, I. Ed. Catalytic *asymmetric synthesis, 3rd Edition* (Wiley, Hoboken, New Jersey, 2010).
26. Tsubogo, T., Yamashita, Y. & Kobayashi, S. Toward efficient asymmetric carbon–carbon bond formation: Continuous flow with chiral heterogeneous catalysts. *Chem. Eur. J.* **18**, 13624–13628 (2012).
27. Kobayashi, J. *et al.* A microfluidic device for conducting gas–liquid–solid hydrogenation reactions. *Science* **304**, 1305–1308 (2004).
28. O'Brien, M. *et al.* Hydrogenation in flow: Homogeneous and heterogeneous catalysis using Teflon AF-2400 to effect gas-liquid contact at elevated pressure. *Chem. Sci.* **2**, 1250-1257 (2011).
29. Hynes, P. S., Stupple, P. A. & Dixon, D. J. Organocatalytic asymmetric total synthesis of (*R*)-rolipram and formal synthesis of (3*S*,4*R*)-paroxetine. *Org. Lett.* **10**, 1389–1391 (2008).
30. Oyamada, H., Naito, T. & Kobayashi, S. Continuous flow hydrogenation using polysilane-supported palladium/alumina hybrid catalysts. *Beilstein J. Org. Chem.* **7**, 735–739 (2011).

Supplementary Information is available in the online version of the paper.

Acknowledgments This work was partially supported by a Grant-in-Aid for Science Research from the Japan Society for the Promotion of Science (JSPS), Global COE Program, The University of Tokyo, MEXT, Japan, and ACT-C and Center of Innovation (COI) Program, the Japan Science and Technology Agency (JST).

Author Contributions T.T. and H.O. designed and performed the experiments. S.K. conceived, designed and directed the investigations and wrote the manuscript with revisions provided by T.T.

Author Information The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to S.K. (shu_kobayshi@chem.s.u-tokyo.ac.jp).

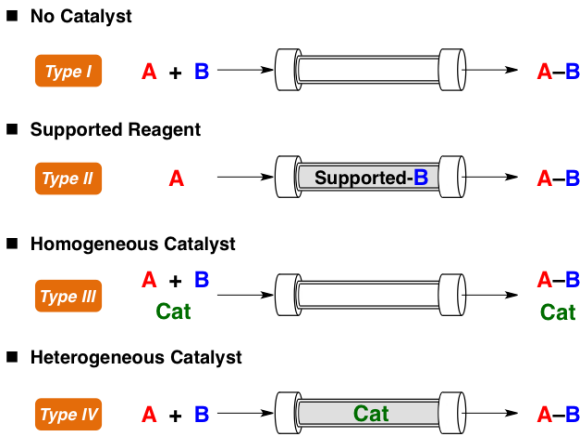


Fig. 1 | The four types of continuous flow systems. The continuous flow systems so far reported can be divided into four types I–IV. **Type I:** Substrates (A and B) are passed through a column or hollow loop, during which reactions occur. Unreacted A or B or any by-products are not separated. **Type II:** One of the substrates (B) is supported in a column. If an excess amount of B is used, one substrate (A) is consumed. However, once supported B is consumed, the column must be changed. **Type III:** A reacts with B in the presence of a homogeneous catalyst. While catalysis proceeds smoothly, the catalyst cannot be separated. **Type IV:** A reacts with B in the presence of a heterogeneous catalyst. If catalysis proceeds smoothly, no separation is required. Therefore, Type IV is regarded as the best method for continuous flow synthesis.

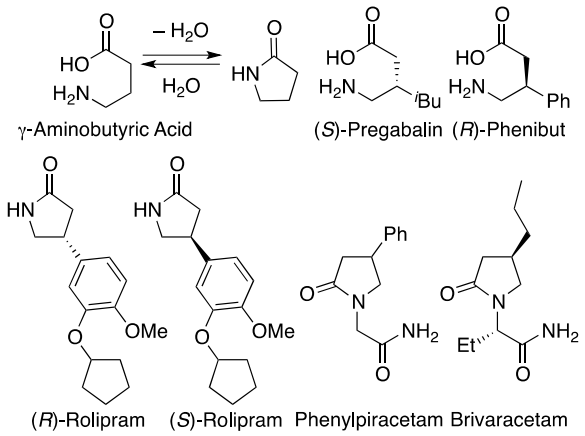


Fig. 2 | GABA derivatives. γ-Aminobutyric acid (GABA) and derivatives are drugs or have potential biological activities in the area of neurotransmitter and brain science. Rolipram, one of the GABA family, is an anti-inflammatory drug, which is a selective phosphodiesterase 4 (PDE4) inhibitor and particularly effective for the PDE4B subtype of PDE4. Moreover, rolipram is known as a possible antidepressant and has been reported to have anti-inflammatory, immunosuppressive, and antitumor effects. Rolipram has also been proposed as a treatment for multiple sclerosis, and has been suggested to have antipsychotic effects. It has been reported that (*R*)-rolipram has anti-inflammatory activity, whereas (*S*)-rolipram does not.

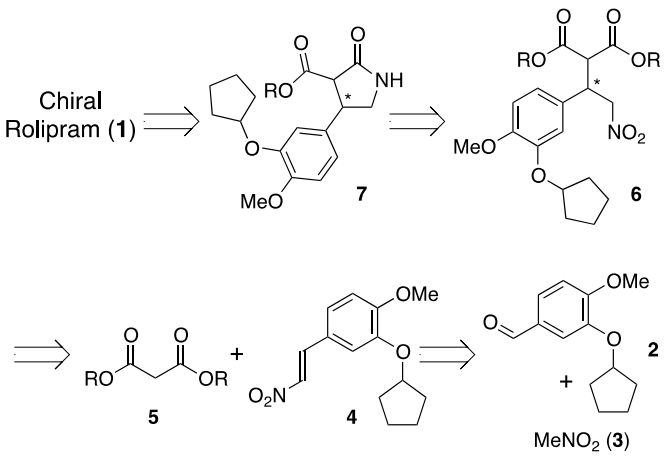
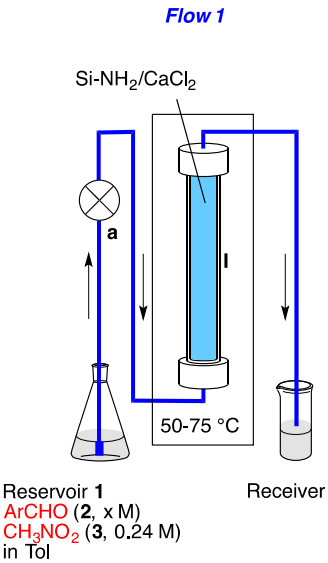


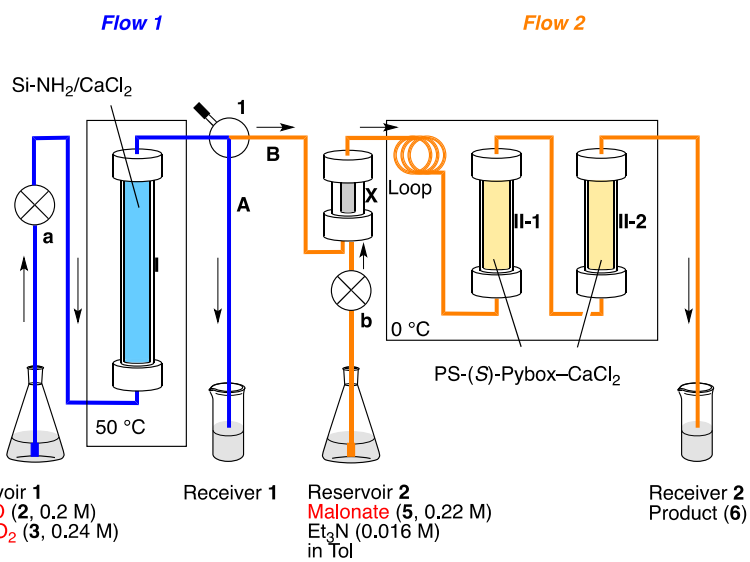
Fig. 3 | Retrosynthetic analysis. Commercially available aldehyde **2** could react with nitromethane **3** to afford nitroalkene **4**. Asymmetric 1,4-addition of malonate **5** to **4** with a chiral catalyst could give enantiomerically enriched γ-nitro ester **6**, whose nitro group could be selectively reduced to afford γ-lactam **7** after cyclization. Finally, the ester group of **7** could be removed to afford optically active rolipram **1**. (*R*)- and (*S*)-rolipram could be synthesized by using column II packed with PS-(*S*)-Pybox-calcium chloride and column II′ bearing PS-(*R*)-Pybox-calcium chloride (the opposing enantiomer), respectively.



Reservoir **1**
ArCHO (**2**, x M)
CH_3NO_2 (**3**, 0.24 M)
in Tol

Receiver **1**

Stage 1: Synthesis of nitroalkenes.



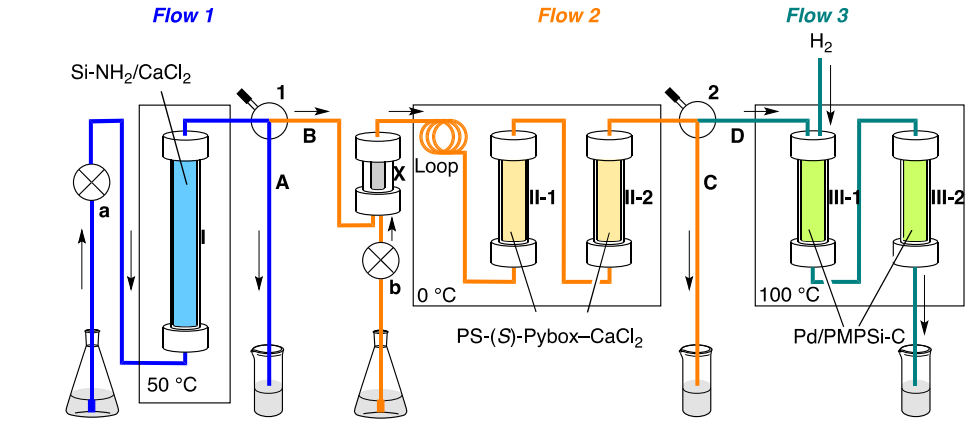
Reservoir **1**
ArCHO (**2**, 0.2 M)
CH_3NO_2 (**3**, 0.24 M)
in Tol

Receiver **1**

Reservoir **2**
Malonate (**5**, 0.22 M)
Et_3N (0.016 M)
in Tol

Receiver **2**
Product (**6**)

Stage 2: Synthesis of γ-nitro esters.



Reservoir **1**
ArCHO (**2**, 0.2 M)
CH_3NO_2 (**3**, 0.24 M)
in Tol

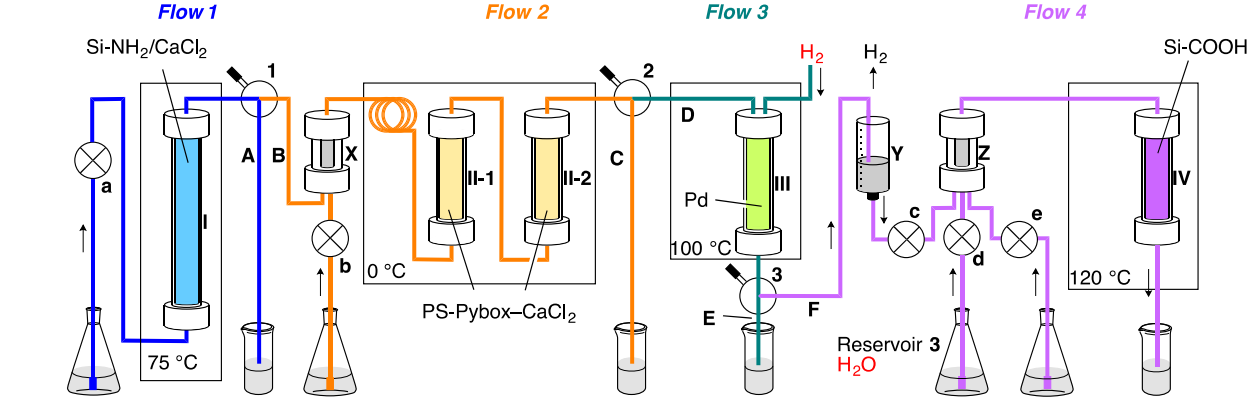
Receiver **1**

Reservoir **2**
Malonate (**5**, 0.22 M)
Et_3N (0.016 M)
in Tol

Receiver **2**

Receiver **3**
Product (**7**)

Stage 3: Synthesis of γ-lactams.



Reservoir **1**
ArCHO (**2**, 0.1 M)
CH_3NO_2 (**3**, 0.12 M)
in Tol

Receiver **1**

Reservoir **2**
Malonate (**5**, 0.11 M)
Et_3N (0.016 M)
in Tol

Receiver **2**

Receiver **3**

Reservoir **4** Rolipram (**1**)
o-Xylene

Stage 4: Synthesis of (*R*)- and (*S*)-rolipram.

Fig. 4 | Diagram of the series of flow reactors. Stage 1: Synthesis of nitroalkene **4** from aldehyde **2** and nitromethane **3**. **Stage 2:** Synthesis of γ-nitro ester **6** from aldehyde **2** and nitromethane **3**. **Stage 3:** Synthesis of γ-lactam **7** from aldehyde **2** and nitromethane **3**. **Stage 4:** Synthesis of (*R*)- and (*S*)-rolipram **1** from aldehyde **2** and nitromethane **3**. Totally, commercially available starting materials **2**, **3**, **5**, H_2, and H_2O were successively passed through the columns **I**, **II**, **III**, and **IV** containing heterogeneous achiral and chiral catalysts to directly afford **1** with high enantioselectivity. Eight-step chemical transformations were conducted smoothly during the flow without isolation of any intermediates and without the separation of any catalysts, co-products, byproducts, and excess reagents. It is noteworthy that all four columns employed are the desirable type IV flow system (Fig. 1).