Proceedings of the 35th European Peptide Symposium
Patrick B. Timmons, Chandralal M. Hewage, Michal Lebl (Editors)
European Peptide Society & PSP, 2018

Peptide couplings by reactive extrusion: Efficient, continuous and green

Thomas-Xavier Métro[1], Yves Yeboue[1], Benjamin Gallard[2], Nicolas Le Moigne[2], Jean Martinez[1], Frédéric Lamaty[1]

[1]Institut des Biomolecules Max Mousseron, France
[2]IMT Mines Ales, France

https://doi.org/10.17952/35EPS.2018.087

Therapeutic peptides exhibit a wide array of advantageous characteristics that place them among the most promising active pharmaceutical ingredients.[1] Yet, industrial production of peptides is hampered by the large amounts of toxic organic solvents that are required during the synthesis and purification steps.[2-3] Although presenting highly concerning environmental issues, polar aprotic solvents such as DMF and NMP are regularly used.[4] These problematic solvents could be avoided since it is known that solvent-less/solvent-free synthesis of small peptides is possible by using ball-milling.[5-8] Enabling to produce 4g of a dipeptide for the best case,[9] this approach was not demonstrated to be further scalable. In addition, the ball-milling process was limited to discontinuous batch production, thereby hampering a wide utilisation by the peptide industry.

In this work, we envisioned overcoming these hurdles by using reactive extrusion. Extruders are composed of a barrel containing one or two rotating screws enabling the efficient transport and mixing along the barrel. Of note, the presence of a recirculation pipe can be utilised to increase the residence time and hence the mixing time of the material to be extruded (Figure 1).

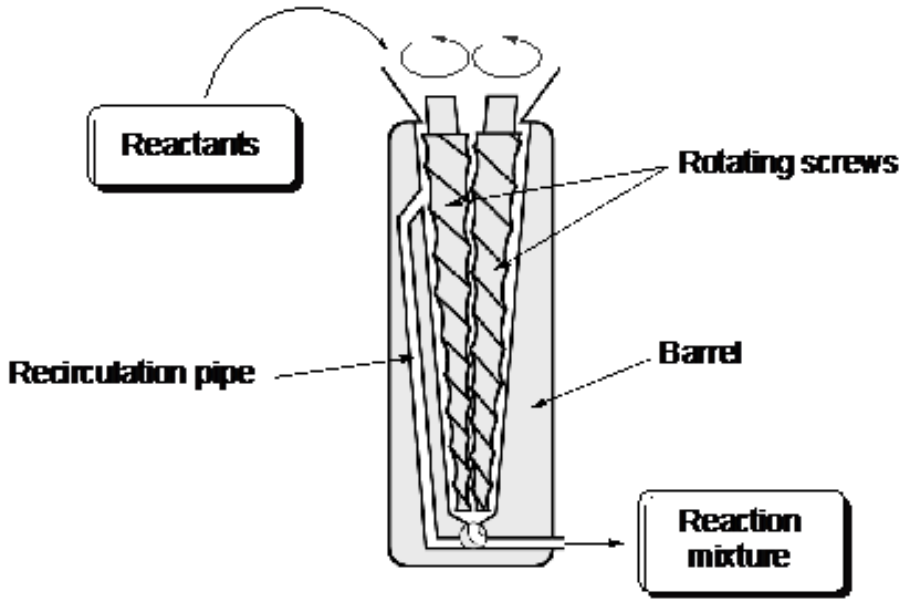
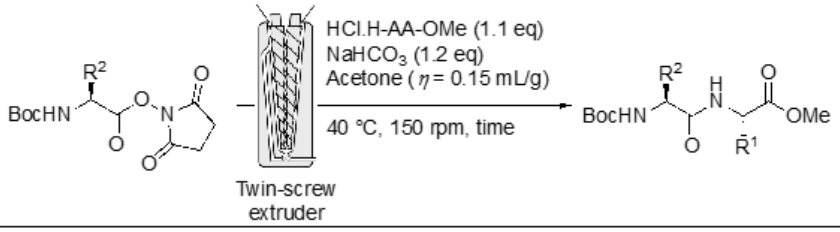


Figure 1: Schematic representation of a twin-screw extruder.

This equipment enables to work under continuous flow conditions while drastically reducing the amount of solvent required for contacting the reactants (if not completely discarding). On the contrary to classical solution-based flow chemistry, extruders allows for the efficient mixing of highly concentrated mixtures that contain a high proportion of solids. Although widely used in the food and plastic industry and identified as a key research area by the pharmaceutical industry,[10] production of high added-value chemicals by reactive extrusion has been scarce. For our part, we first studied the capacity of a co-rotating twin-screw extruder to produce the dipeptide Boc-Trp-Gly-OMe. After a thorough screening of reaction conditions, it appeared that the peptidic bond of Boc-Trp-Gly-OMe could be formed by recirculating inside a twin-screw extruder a mixture composed of Boc-Trp-OSu (1.0 eq.), HCl.H-Gly-OMe (1.1 eq.) and NaHCO3 (1.2 eq.) along with a small amount of acetone (1.5 mL for a total mass of 10g of reactants) while operating the extruder at 40 °C with a screw speed set 150 rpm.

Table 1: Synthesis of dipeptides and tripeptides by using a twin-screw extruder.



Entry	Boc-AA-OSu	HCl·H-AA-OMe	Mixing time (min)	Yield (%)	Purity (%)	Product
1	Trp	Gly	10	85	>99[a]	Boc-Trp-Gly-OMe
2	Trp	Phe	10	61	>99[a]	Boc-Trp-Phe-OMe
3	Asp(OBzl)	Phe	1.5[b]	92	>99[a]	Boc-Asp(OBzl)-Phe-OMe
4	Asp(OBzl)	Trp-Gly	10	86	96[c]	Boc-Asp(OBzl)-Trp-Gly-OMe
5	Asp(OBzl)	Trp-Phe	10	89	94[c]	Boc-Asp(OBzl)-Trp-Phe-OMe

[a] >99% ee or de determined by chiral HPLC. [b] Reaction mixture was extruded without recirculation. Residence time of 1.5 min. [c] Purity determined by HPLC.

As such an amount of acetone is not sufficient to completely solubilize the reactants, acetone would be more appropriately described here as a liquid additive than a solvent. After EtOAc solubilisation and aqueous washings of the extrudate, Boc-Trp-Gly-OMe was isolated in 85% yield and >99% enantiomeric excess (Table 1, entry 1). When HCl.H-Gly-OMe was replaced with HCl.H-Phe-OMe, Boc-Trp-Phe-OMe could be produced in 61% yield and >99% diastereomeric excess (Table 2, entry 2). To our delight, Boc-Asp(OBzl)-Phe-OMe could be formed without recirculating the reaction mixture in the extruder: Boc-Asp(OBzl)-OSu was fully converted after the 1.5 min residence time inside the barrel, leading to the production of Boc-Asp(OBzl)-Phe-OMe in 92% yield and >99% de (Table 2, entry 3). Tripeptides could also be formed by using this approach. Dipeptides hydrochlorides HCl.H-Trp-Gly-OMe and HCl.H-Trp-Phe-OMe were first synthesized by solvent-free gaseous HCl treatment. After being reacted with both NaHCO3 and Boc-Asp(OBzl)-OSu in the extruder, Boc-Asp(OBzl)-Trp-Gly-OMe and Boc-Asp(OBzl)-Trp-Phe-OMe were isolated in 86% and 89% yield and excellent purity (96% and 94% respectively; Table 1, entries 4 & 5). As an illustration of the potential application of this strategy to the production of industrially-relevant peptides, Boc-Asp(OBzl)-Phe-OMe was transformed into the renowned sweetener Aspartame by hydrogenation, Boc removal under solvent-free acidic conditions before final precipitation at the isoelectric point. By doing so, Aspartame was produced in 81% yield in three steps starting from Boc-Asp(OBzl)-OSu.

In conclusion, dipeptides and tri-peptides can be produced in high yields, high stereoisomeric excesses and very short reaction times by using reactive extrusion.[11] The capacity to implement synthesis under continuous conditions is clearly setting the path to intensified industrial peptide production. On the contrary to synthesis in solvent-based continuous flow and in SPPS, the presence of solids could be very easily handled by the mechanical forces occurring in the extruder. Thus, this strategy enabled to work under highly concentrated reaction conditions, while avoiding the use of highly problematic solvents and bases (such as DMF and Et_3N).

Acknowledgements

Université de Montpellier, Centre National de la Recherche Scientifique (CNRS), and LabEx CheMISyst (through ANR programme ANR-10-LABX-05-01) are acknowledged for financial support.

References

(1) Lau, J. L.; Dunn, M. K. Therapeutic peptides: Historical perspectives, current development trends, and future directions. Biorg. Med. Chem. 2017, 26, 2700-2707, DOI: 10.1016/j.bmc.2017.06.052.

(2) Thayer, A. M. Making Peptides At Large Scale. Chem. Eng. News 2011, 89, 21-25.

(3) Patel, P. Executive reveals how Amgen shifted to greener chemistry. Chem. Eng. News 2017, 95, 27-28.

(4) According to Regulation (EC) No 1272/2008, DMF is classified as presenting reproductive toxicity and DCM , THF and 1,4-dioxane as toxic and/or carcinogenic (Category 2).

(5) Declerck, V.; Nun, P.; Martinez, J.; Lamaty, F. Solvent-Free Synthesis of Peptides. Angew. Chem. Int. Ed. 2009, 48, 9318-9321, DOI 10.1002/anie.200903510.

(6) Maurin, O.; Verdié, P.; Subra, G.; Lamaty, F.; Martinez, J.; Métro, T.-X. Peptide synthesis: ball-milling, in solution, or on solid support, what is the best strategy? Beilstein J. Org. Chem. 2017, 13, 2087-2093, DOI 10.3762/bjoc.13.206.

(7) Porte, V.; Thioloy, M.; Pigoux, T.; Métro, T.-X.; Martinez, J.; Lamaty, F. Peptide Mechanosynthesis by Direct Coupling of N-Protected α-Amino Acids with Amino Esters. Eur. J. Org. Chem. 2016, 3505-3508, DOI 10.1002/ejoc.201600617.

(8) Bonnamour, J.; Métro, T.-X.; Martinez, J.; Lamaty, F. Environmentally benign peptide synthesis using liquid-assisted ball-milling: application to the synthesis of Leu-enkephalin. Green Chem. 2013, 15, 1116-1120, DOI 10.1039/c3gc40302e.

(9) Métro, T.-X.; Colacino, E.; Martinez, J.; Lamaty, F. In Ball Milling Towards Green Synthesis: Applications, Projects, Challenges, The Royal Society of Chemistry: 2015, 114-150, DOI 10.1039/9781782621980-00114.

(10) Jiménez-González, C.; Poechlauer, P.; Broxterman, Q. B.; Yang, B.-S.; am Ende, D.; Baird, J.; Bertsch, C.; Hannah, R. E.; Dell'Orco, P.; Noorman, H.; Yee, S.; Reintjens, R.; Wells, A.; Massonneau, V.; Manley, J. Key Green Engineering Research Areas for Sustainable Manufacturing: A Perspective from Pharmaceutical and Fine Chemicals Manufacturers. Org. Process Res. Dev. 2011, 15, 900-911, DOI 10.1021/op100327d.

(11) Yeboue, Y.; Gallard, B.; Le Moigne, N.; Jean, M.; Lamaty, F.; Martinez, J.; Métro, T.-X. Peptide couplings by reactive extrusion: solid-tolerant and free from carcinogenic, mutagenic and reprotoxic chemicals. ACS Sustainable Chem. Eng. 2018, in press, DOI: 10.1021/acssuschemeng.8b04509.