Summary of PhD Thesis

THE PHARMACEUTICAL CHEMICAL TENDENCY TOWARDS CONTINUOUS-FLOW PROCESSING: NOVEL CHEMICAL REACTIVITIES AND ENTITIES

Imane Nekkaa

Supervisors:

Prof. Dr. Ferenc Fülöp
Dr. István Mándity

University of Szeged
Institute of Pharmaceutical Chemistry

Szeged

2018

University of Szeged

Doctoral School of Pharmaceutical Sciences

Educational Programme: Pharmaceutical Chemistry and Drug Research

Programme director: Prof. Dr. Ferenc Fülöp

Institute: Institute of Pharmaceutical Chemistry

Supervisors: Prof. Dr. Ferenc Fülöp

Dr. István Mándity

Imane Nekkaa

The pharmaceutical chemical tendency towards continuous-flow processing: novel chemical reactivities and entities

Final examination committee:

Head: Prof. Dr. György Dombi

Members: Prof. Dr. István Pálinkó

Dr. Pál Szabó

Reviewer committee:

Head: Prof. Dr. Piroska Révész

Reviewers: Prof. Dr. Pál Perjési

Dr. Éva Frank

Members: Dr. Géza Tóth

Dr. István Zupkó

A. INTRODUCTION AND AIMS

Synthetic chemistry plays a key part in the drug discovery process. New reactivity patterns are discovered every day, along with new reactions and applications of established reactions. Besides the conventional laboratory-based techniques, continuous-flow (CF) processing is emerging as one of the techniques that can significantly impact the synthetic process.

This interest can be explained, at least in part, by the number of potential advantages that CF processes have over traditional batch chemistry. Namely, the well-regulated flow reactor concept provides an increased parameter space for chemical synthesis and enables reactions to be performed with an unprecedented level of control. It is due to the greatly enhanced heat and mass transfer and improved mixing properties, which can translate into higher reaction rates, outstanding selectivity, and safer and greener chemistry. Thus, flow chemistry has long been selected to provide a simple means to use more rigorous reaction conditions.

Focusing on the latter understanding, our major aim was to probe the versatility of the CF technology by developing novel sustainable synthetic methodologies with possible usefulness for the pharmaceutical industry. For this purpose, a study was conducted where the following areas were reinvestigated comparing classical batch methods to CF-based techniques: *i*) retro-Diels–Alder (rDA) reaction, *ii*) cyclisation reactions, *i.e*. a three-step domino ring-closure reaction and spirocyclisation (cyclocondensation), *iii*) synthesis of β-peptide foldamers *via* CF solid-phase peptide synthesis (SPPS) followed by rDA reaction.

The rDA reaction represents a straightforward and an efficient approach for the synthesis and design of novel heterocyclic scaffolds with diverse pharmacological potentials. However, it has been less explored due to the harsh reaction conditions involved. The endothermic requirements of rDA make it an ideal reaction to be performed under CF processes, where high heat and mass transfer are operative. Fused pyrimidinone derivatives are well known for their pharmacological properties. Herein, we have designed a novel synthetic process for the preparation and transformation of distinct pyrimidinone-fused moieties such as; pyrrolopyrimidinones, pyrimidoisoindoles, and spiropyrimidinones, by means of the highly controlled CF rDA reaction.

β-Peptide foldamers play the role as a novel class of drug scaffolds with tailored molecular shape and surface. These unnatural oligomers have strong tendency to adopt specific and predictable conformations in solution. By application of the CF rDA reaction on homooligomer peptides containing an enantiomeric bicyclic residue in the middle of the chain, the effect of new structural elements on conformation was studied. Moreover, we wanted to gain an insight into the origin of biological homochirality, through the investigation of the stereochemical discrimination in the synthesis of β-peptides towards the homochiral oligomers.

B. EXPERIMENTAL SECTION

CF experiments were carried out in:

Continuous-flow retro-Diels–Alder (CF rDA) reactor: CF rDA reactions were performed on a modular flow system equipped with heated 304 stainless steel tubing coil and an adjustable back-pressure regulator. The tube reactor was heated in an oven (Figure 1). Solutions of the starting materials were loaded into the reactor via an HPLC pump. The most important reaction parameters such as temperature, pressure, flow rate and substrate concentration were systematically fine-tuned to determine optimal conditions. The CF rDA products were characterised by means of (^{1}H, ^{13}C) NMR, HPLC-MS and FT-IR spectroscopy. In the cases of chiral compounds, *ee* was assigned with a Phenomenex IA column.

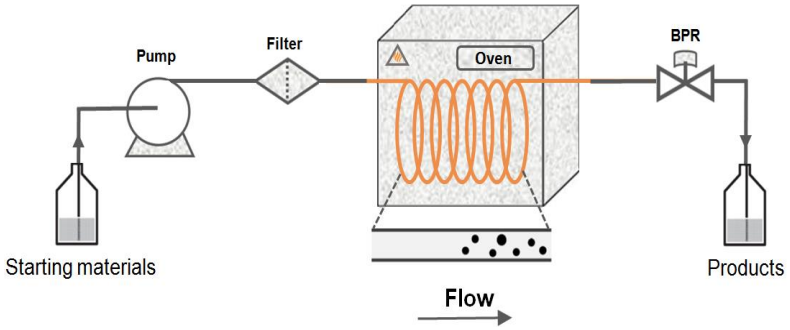


Figure 1. Setup of the CF rDA reactor.

***Continuous-flow solid-phase peptide synthesizer (CF-SPPS)*:** Peptides were synthesized by using the CF-SPPS reactor constructed previously (Figure 2), involving Fmoc chemistry. The peptides were purified by RP-HPLC and characterized by HPLC-MS. Structures were determined by the use of molecular modelling, NMR (TOCSY, ROESY) techniques and ECD.

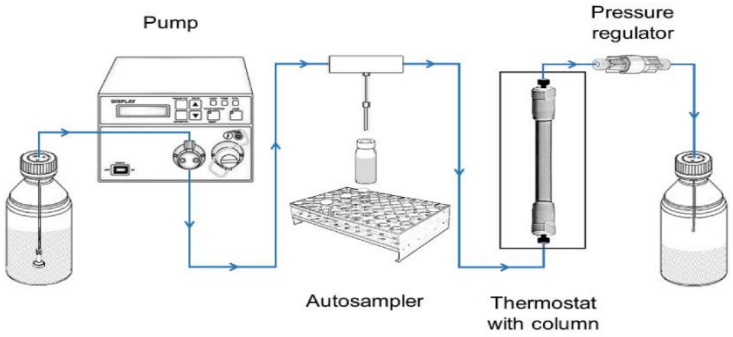


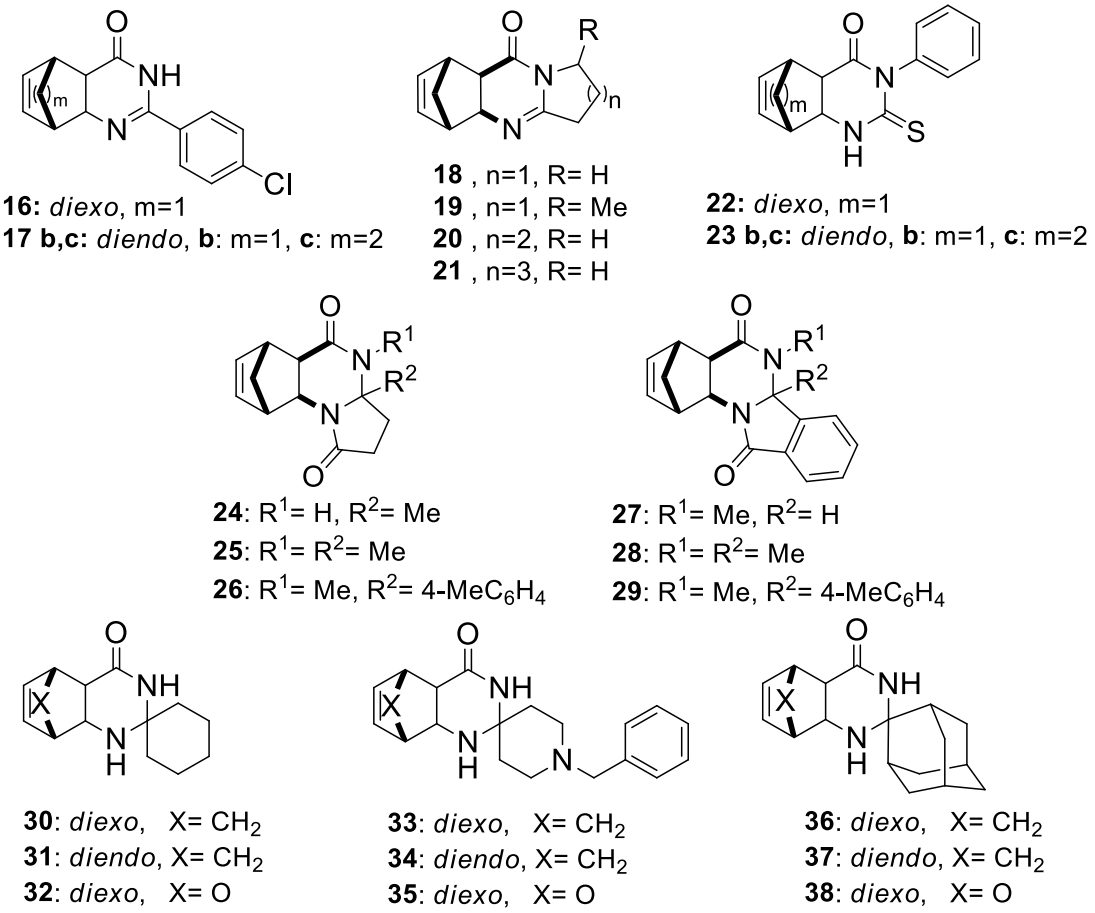
Figure 2. Schematic representation of the CF-SPPS reactor.

CF hydrogenation reactor: Reactions were performed in an H-Cube® flow reactor apparatus equipped with a gas-generation unit consisting of a reservoir for deionised water and a built-in electrolysis cell for the generation of H_2. The gas generated *in situ* is combined via a gas–liquid mixer with the solution of the substrate, and then the mixture is then transported to the catalyst (10% Pd/C) bed, where the reactions take place.

C. RESULTS AND DISCUSSION*

Novel, sustainable CF synthetic methodologies were developed for the synthesis of pharmaceutically relevant intermediates and potentially bioactive compounds.

1. The starting materials **16**–**38** (Scheme 1) were selected to comprise; *i*) molecules where good, medium and no conversion was observed under batch rDA conditions, *ii*) more complex racemic or enantiomeric fused pyrimidinone moieties, *iii*) molecules which have never been subjected to rDA reactions under batch conditions.

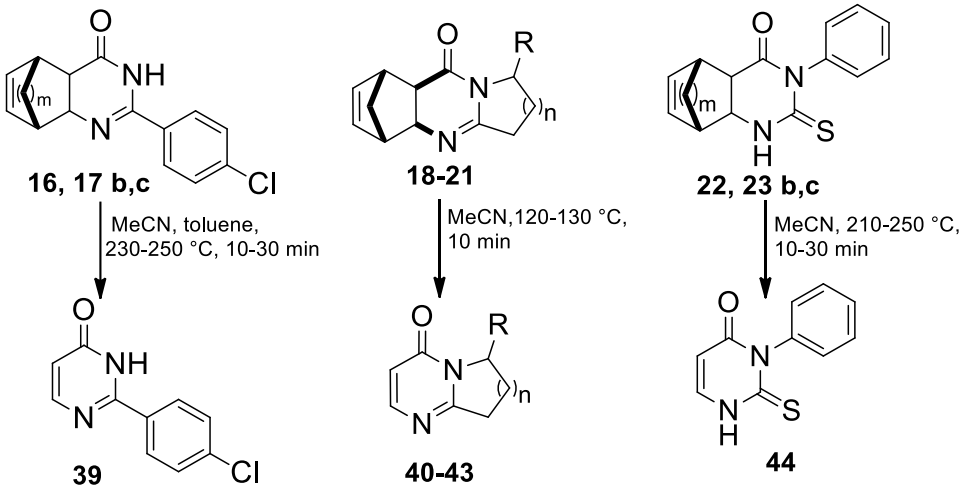


Scheme 1. The selected starting materials for the CF rDA investigation.

2. Pyrimidinones **16**–**38** have been previously prepared by literature methods. In addition, we developed a time-efficient CF-based cyclisation method for the synthesis of **24**–**38**. To this end, solutions of β-amino amides were mixed with γ-keto acids or cyclic ketones and loaded into the CF reactor (Figure 1). Using the same operating conditions and solvents as used before in batch syntheses. The intermediate **24**–**38** were obtained in slightly higher or similar yields (79–95%) than found previously in batch method. Note, however, that shorter reactions were needed for cyclisation in the CF reactor.

***** Compound numbering is identical to that applied in the thesis.

3. To validate our protocol, we initiated the CF rDA investigation of tri- and tetracyclic derivatives **16–23**. After optimising the reaction conditions, compounds **16–23** were introduced into the CF rDA reactor (Figure 1, Scheme 2). HPLC–MS measurements revealed full conversions to the desired pyrimidinones **39–44**, whereas only a moderate conversion of **23c** to **44** was observed. The stereochemistry (*diendo vs*. *diexo*) of the starting pyrimidinones and elimination of cyclopentadiene or cyclohexadiene has no significant effect on the reaction yields.



Scheme 2. The CF rDA synthesis of pyrimidinones **39–44**.

On the basis of the results previously reported for the preparation pyrimidinones **39–44** under batch conditions. The rDA were performed by: *i*) heating under neat conditions or *ii*) heating under reflux in CB or DCB, and under microwave conditions in DCB. A comparison between the literature results and CF rDA results is presented in Figure 3. These findings validate that the proposed CF rDA protocol is superior to the existing conventional batch technologies, especially in case of compound **23c** which did not undergo decomposition under batch conditions.

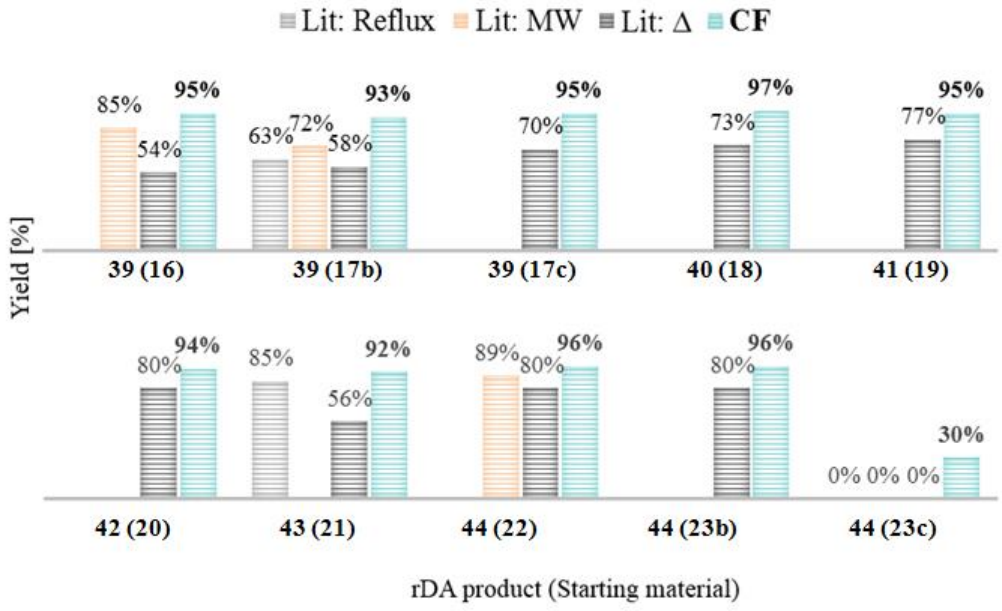
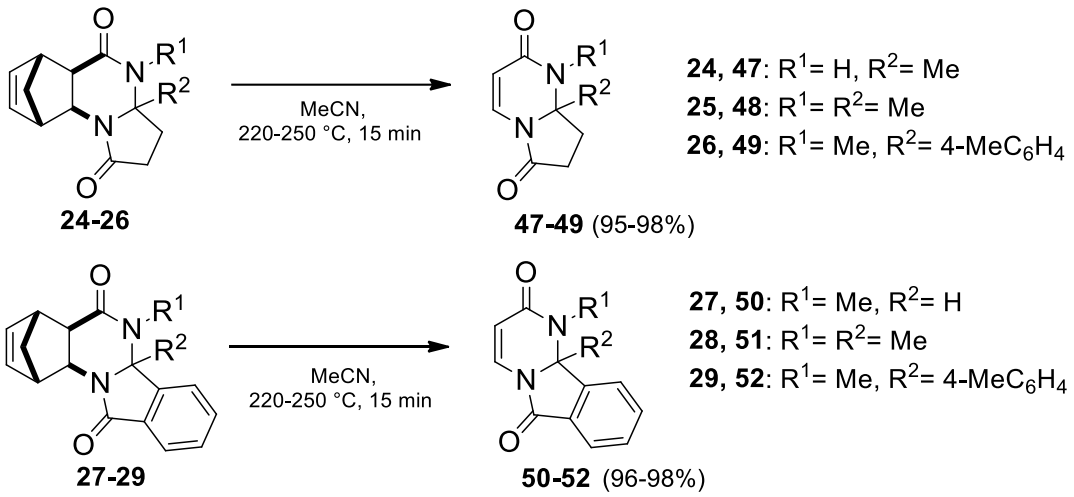


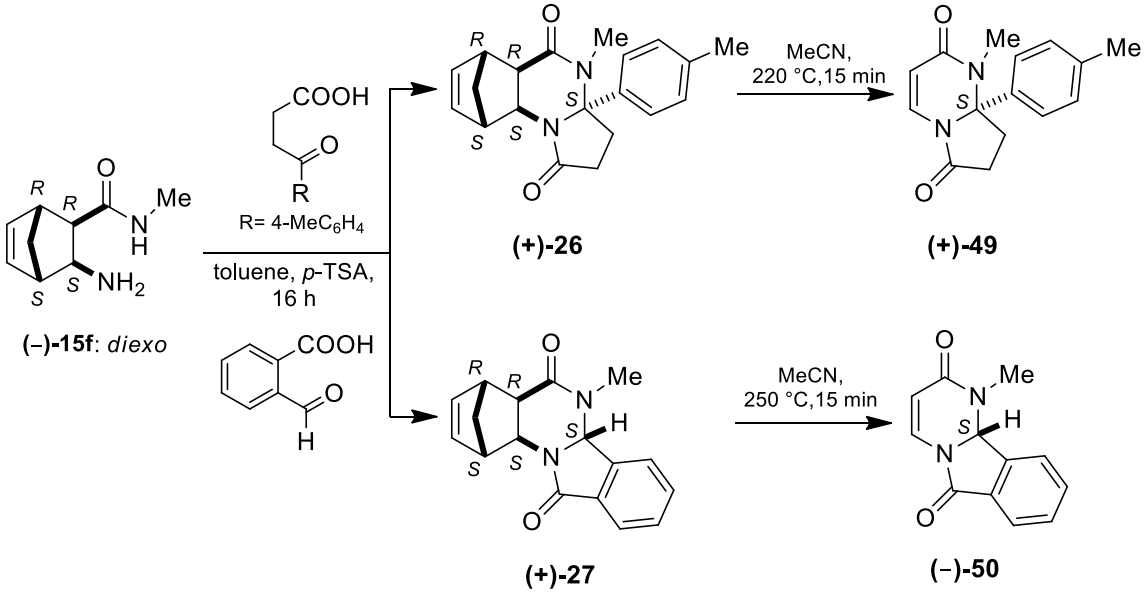
Figure 3. Comparison in terms of isolated yields between CF rDA and different batch methods for the synthesis of pyrimidinone derivatives **39–44**.

4. As a further investigation on the ability of the CF rDA approach; the synthesis of more complex racemic and enantiomeric fused-pyrimidinone moieties; pyrrolopyrimidinone (**47**–**49**), pyrimidoisoindole (**50**–**52**) derivatives was tested. Accordingly, upon subjecting compounds **24**–**29** to CF rDA conditions. The rDA products **47**–**52** were afforded in good yields (Scheme 3).



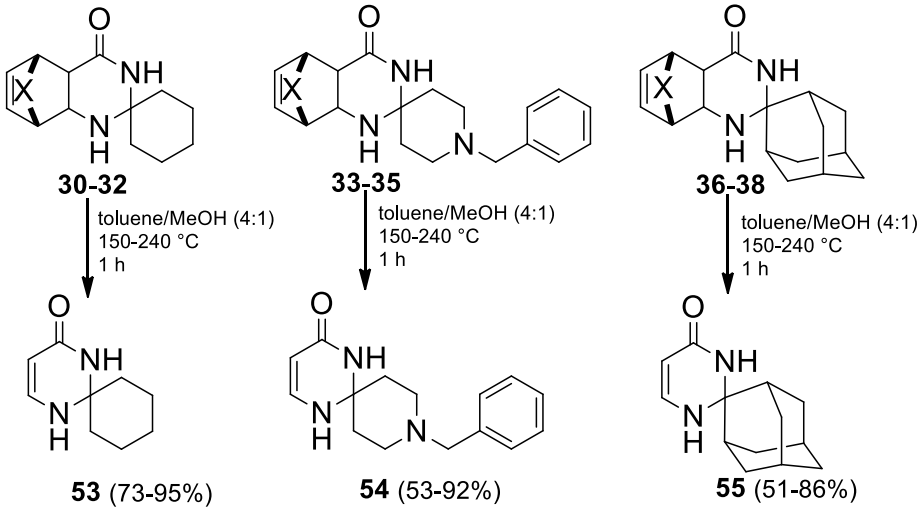
Scheme 3. The CF rDA synthesis of more complex fused pyrimidinones **47**–**52**.

To establish the range of applicability of our CF rDA process, the syntheses of enantiomerically pure pyrrolo[1,2-*a*]pyrimidine **49** and pyrimido[2,1-*a*]isoindole **50** through rDA reaction under CF conditions were undertaken (Scheme 4). In a stereocontrolled ring-closing reaction, (–)-**15f** was reacted with γ-keto acids to afford (+)-**26** and (+)-**27** as single diastereoisomers in good yields. The ready loss of cyclopentadiene through the CF rDA protocol at 220 °C resulted the enantiomer (+)-**49** in high yield (97%) with an *ee* value of 97%. While, the CF-induced thermolysis of pentacyclic (+)-**27**, at 250 °C, gave the expected (–)-**50** within a residence time of 15 min at full conversion, in yield of 97% and with an *ee* value of 98%.



Scheme 4. The synthesis of enantiomeric pyrimidinones through CF rDA reaction.

5. The capacity of the CF rDA protocol for providing new chemical entities was tested on spiroquinazolinones **30–38** (Scheme 5), which have not been subjected to rDA reactions previously through conventional batch approaches. Spiro-compounds **30–38** were introduced into the flow reactor (Figure 1) under the optimized reaction conditions**.** The results revealed only moderate conversions to new spiropyrimidinones **53–55** were observed for the methylene-bridged **30**, **31**, **33**, **34**, **36**, and **37**. It is interesting that epoxy-bridged **32**, **35**, and **38**, in which an oxygen atom has been introduced at position C-7 of the β-aminonorbornene carboxamide skeleton, gave retrodiene products **53–55** in almost quantitative yields.



Scheme 5. The CF rDA synthesis of spiropyrimidinones **53–55** as new chemical entities.

In line with our previous findings, we wanted to explore whether spiroquinazolinone **30–38** undergo thermal decomposition under microwave conditions. To this end, microwave irradiation was applied on **30–38**. Slightly higher than medium or no conversions to the retrodiene products **53–55** were found. The best MW-promoted cycloreversion for the synthesis of compounds **53–55** was achieved with epoxy-spiroquinazolinones **32**, **35** and **38** irradiated in DCB at 180 °C for 30 min, these conditions afforded yields of 78%, 89%, and 78%, respectively (Figure 4). These data clearly demonstrate the superiority of CF technology compared to the microwave method.

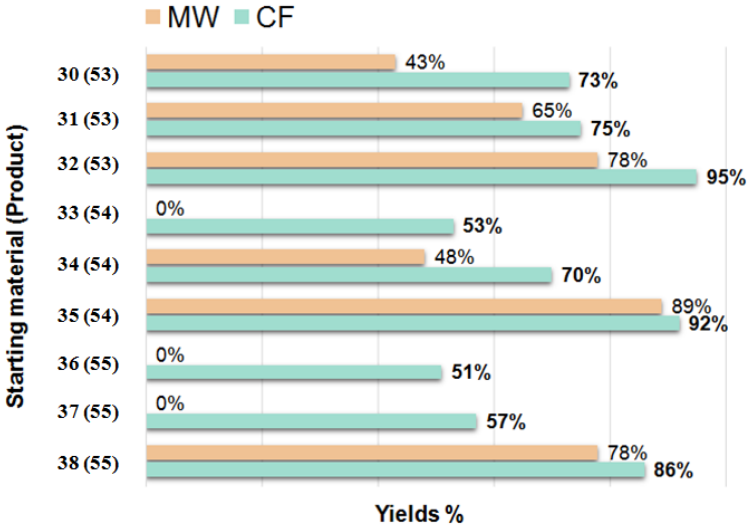
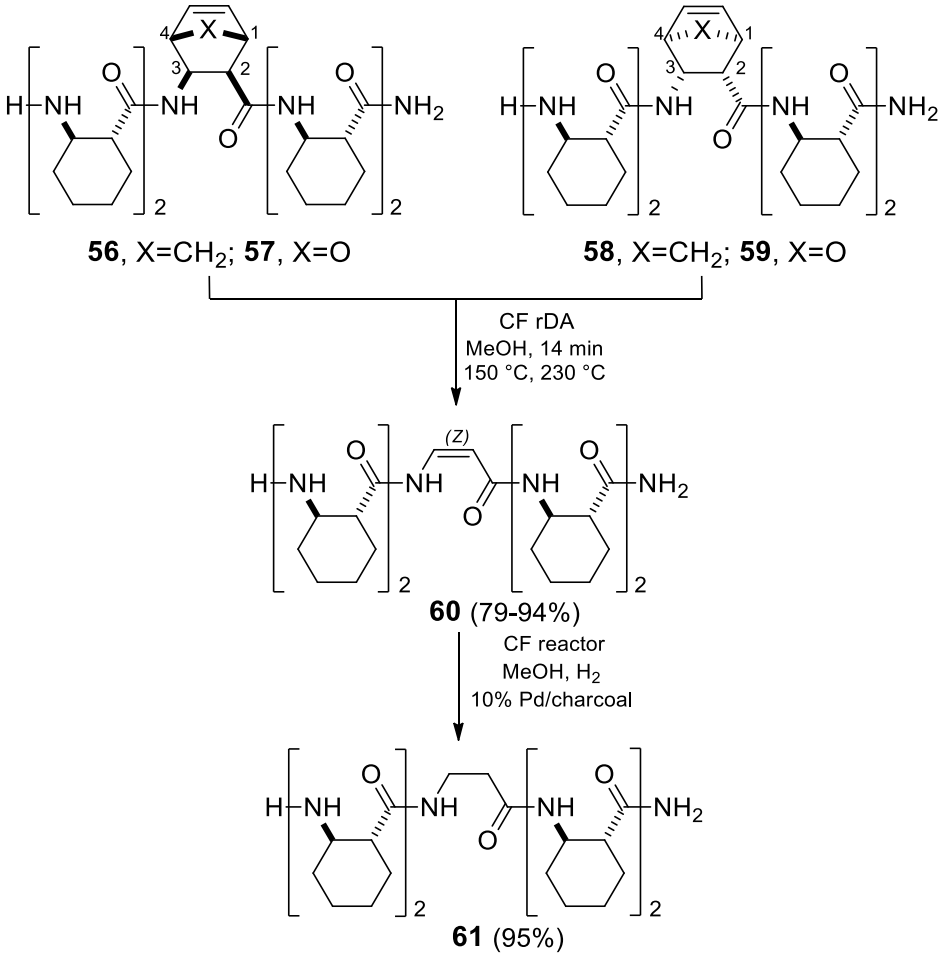


Figure 4. Comparison in terms of isolated yields between CF rDA and microwave processes for the synthesis of new spiropyrimidinones **53–55**.

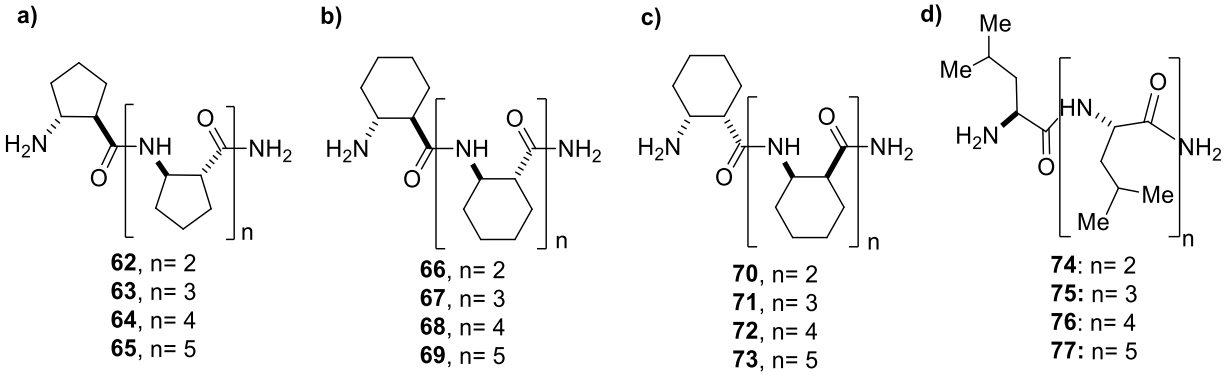
- MW-induced rDA (orange)
- CF rDA (blue).

6. Furthermore, we examined the helix forming property of oligomers containing enantiomeric bicyclic β-amino acid residues and their chemical derivatives. To this end, pentamers made of [1*R*,2*R*]-2-aminocyclohexanecarboxylic acid ([1*R*,2*R*]-ACHC) building blocks possessing an enantiomer of bicyclic residue in the middle of the peptide chain were assembled by means of CF-SPPS (Figure 2). As bridged residues, enantiomers of *diexo*-3-aminobicyclo[2.2.1]hept-5-ene-2-carboxylic acid (*diexo*-ABHEC) and *diexo*-3-amino-7-oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid (*diexo*-AOBHEC) were used to compare the ability of folding regarding the configuration and to study the effect of an oxygen-bridged residue on self-organisation. We proved the versatility of the CF rDA protocol by providing distinct peptidic structures. Namely, oligomer **60** was obtained, in a high yields, when **56**–**59** were treated under CF rDA conditions. β-Alanine-containing peptide **61** was afforded upon subjecting **60** to the CF hydrogenation reactor (Scheme 6).



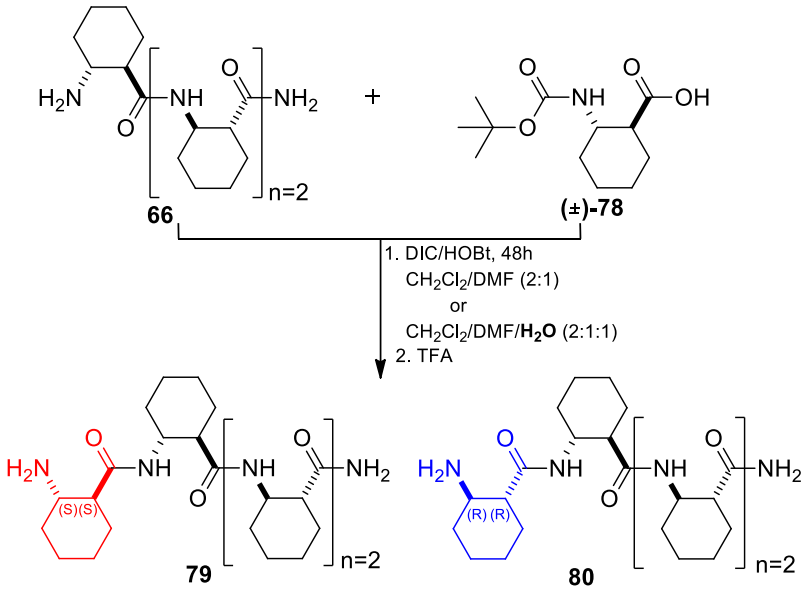
Scheme 6. Investigated foldamer structures composed of [1*R*,2*R*]-ACHC units incorporating [1*R*,2*R*,3*S*,4*S*]-ABHEC (**56**), [1*R*,2*S*,3*R*,4*S*]-AOBHEC (**57**), [1*S*,2*S*,3*R*,4*R*]-ABHEC (**58**), [1*S*,2*R*,3*S*,4*R*]-AOBHEC (**59**), *Z*-dehydro-β-alanine (**60**) and β-alanine (**61**) as third β-amino acid units.

7. We further proved that biological homochirality as an inherent property is also occurring with the unnatural β-peptide foldamers. The phenomenon was investigated by means of chain-length-dependent stereochemical discrimination in the synthesis of β-peptides with various side chains and secondary structures (Scheme 7). As reference, the natural α-L-leucine (L-Leu) oligopeptides were selected. The peptides **62-77** were assembled by CF-SPPS (Figure 2)



Scheme 7. The investigated β-peptide structures.

The stereochemical discrimination property of these peptides **62-77** was tested by means of the solution-phase peptide coupling technique as demonstrated in Scheme 8 with the example of oligomers **66**. Importantly, the effect of water on the stereochemical discrimination was investigated too, since it is known to be a crucial factor.



Scheme 8. Chain elongation of **66** with racemic amino-acid **(±)-78** yielding diastereomers **79** (heterochiral) and **80** (homochiral).

β-Peptide oligomers composed of either *cis* or *trans* alicyclic β-amino acids showed a tendency towards the homochiral constructs. The size of the side chain drastically influenced the selectivity of the stereodiscriminative chain-elongation reaction. It is noteworthy that water as a co-solvent increases the selectivity. Theoretical calculations indicated that water plays a crucial role in this phenomenon through the induction of self-association.

D. LIST OF PUBLICATIONS AND LECTURES

Papers related to the thesis:

I. István M. Mándity, **Imane Nekkaa**, Gábor Paragi, Ferenc Fülöp:
Homochirality of β-Peptides: A Significant Biomimetic Property of Unnatural Systems.
ChemistryOpen. **2017**, *6*, 492–496. IF: 2.801*

II. **Imane Nekkaa**, Márta Palkó, István M. Mándity, Ferenc Fülöp:
Continuous-flow retro-Diels–Alder Reaction: An Efficient Method for the Preparation of Pyrimidinone Derivatives.
Beilstein J. Org. Chem. **2018**, *14*, 318–324. IF: 2.340*

III. **Imane Nekkaa**, Márta Palkó, István M. Mándity, Ferenc Miklós, Ferenc Fülöp:
Continuous-flow retro-Diels–Alder Reaction: A Novel Process Window for Designing New Heterocyclic Scaffolds.
Eur. J. Org. Chem. **2018**, 4456–4464. IF: 2.882*

IV. **Imane Nekkaa**, Dóra Bogdán, Tamás Gáti, Szabolcs Béni, Tünde Juhász, Márta Palkó, Gábor Paragi, Gábor K. Tóth, Ferenc Fülöp, István M. Mándity:
Flow-chemistry Enabled Efficient Patterning of β-Peptides: Backbone Topology *vs*. Helix Formation.
Submitted for publication

* 2017 Impact Factors

Scientific lectures related to the thesis:

V. **Imane Nekkaa**, István M. Mándity, Ferenc Fülöp:
Stereochemical Discrimination in the Synthesis of β-Peptide Oligomers: Origin of Homochirality.
XXXVII. Kémiai Előadói Napok, Szeged, 2015. Október 26–28.

VI. **Imane Nekkaa**, István M. Mándity, Ferenc Fülöp:
Stereochemical Discrimination in the Synthesis of β-Peptide Oligomers: Origin of Homochirality.
8th International Conference Chemistry towards Biology. 28th August–1st September 2016. Brno, Czech Republic.

VII. **Imane Nekkaa**, István M. Mándity, Ferenc Fülöp:
Homochirality in the Unnatural Peptide World: A Significant Biomimetic Property.
A Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány, Szeged 2017, May 26.

VIII. **Imane Nekkaa**, István M. Mándity, Ferenc Fülöp:
Homochirality of β-Peptides: A Significant Biomimetic Property of Unnatural Systems.
7th BBBB International Conference on Pharmaceutical Sciences. New Trends and Achievements in Pharmaceutical Sciences and Pharmacy Practice, 5–7 October 2017, Balatonfüred, Hungary.

IX. **Imane Nekkaa**, Márta Palkó, István M. Mándity, Ferenc Fülöp:
Continuous-flow retro-Diels–Alder Reaction: An Efficient Method for the Preparation of Pyrimidinone Derivatives.
Flow Chemistry Europe 2018, 6–7 February 2018, Cripps Court, Magdalene College, Cambridge, UK.

X. **Imane Nekkaa**, Márta Palkó, István M. Mándity, Ferenc Miklós, Ferenc Fülöp:
Continuous-flow retro-Diels–Alder Reaction: A Novel Process Window for Designing New Heterocyclic Scaffolds.
Heterociklusos és Elemorganikus Kémiai Munkabizottság ülése. Balatonszemes, 2018, June 6–8.