FINAL REPORT

Self-organizing peptidic systems-catalyzed reactions in continuous flow reactor and structure characterization thereof

OTKA PD103994

Dr. István Mándity

The research activities provided results of which can be classified mainly into two topics e.g. continuous flow process development and foldamer technology. Nonetheless, these two topics are closely related and supports each other. First various continuous-flow (CF) reactions were developed with the results as follows:

1. A solid-supported peptide-catalyzed (CF) process was developed for asymmetric aldol reactions. The catalyst was readily synthesized and immobilized by solid-phase peptide synthesis (SPPS) on a swellable polymer support in one single step. Ignoring the peptide cleavage from the resin means no work-up, no purification, and no product loss. The structure of the prepared catalysts have been verified after the SPPS. This have been performed either by suspension phase ^{13}C-NMR measurements or by HPLC and MS investigations after the cleavage of a small portion of the peptide. After thorough optimization of the reaction conditions, synthetically useful beta-hydroxyketone products were obtained in high yields and stereoselectivities with short reaction times. It was found that the heterogeneous catalytic reaction is diffusion-controlled under the present conditions; thus, elevation of the pressure is necessary to maximize conversion of the flow process. Moreover, it has been shown, that the properties of the solid support determines the reaction result. Besides being simple and efficient, this method is also rapid and promisingly productive, with short residence times on the catalyst bed. The immobilized peptidic catalyst is highly recyclable, while further advantageous features are the ease of product isolation and the possibility of facile scale-up, furnishing sustainable catalytic methodology. The gained substances can be transferred by simple chemical steps to foldamer building blocks.
2. A simple and inexpensive CF method has been developed for the 1,3-dipolar cycloaddition of organic azides and acetylenes, which utilized Cu powder as the cheapest available Cu(I) source. The need for costly apparatus and special catalyst species is eliminated and the high pressure / high temperature conditions were successfully moderated through the joint use of basic and acidic additives to maximize

the reaction rates. The benefits of flow processing meant that the risks that were associated with the handling of dangerous azides were significantly reduced and gram-scale syntheses were successfully achieved in a safe and simple manner. This CF technique has a wide scope for azides and alkynes and selectively results in synthetically useful 1,4-disubstituted 1,2,3-triazole compounds in excellent yields within short process times, some of which have notable biological activity. The utilization of additives in flow processes not only improved the safety but also efficacy of these reactions and typically afforded higher yields than under the high-temperature conditions. Some highly functionalized derivatives of the antifungal cispentacin were also effectively synthetized with this CF methodology and also with conventional batch procedures, thus demonstrating the outstanding synthetic capabilities of flow processing. The cispentacin derivatives are interesting precursors for new triazole carbanucleosides with possible biological activities. The gained substances are foldameric building blocks.

3. The asymmetric α-amination of aldehydes with azodicarboxylate esters as electrophilic nitrogen sources is a key step in the synthesis of various natural products and bioactive compounds including amino acid derivatives. A CF process to conduct such reactions was developed. We investigated heterogeneous prolyl-peptides as chiral catalyst for asymmetric α-amination reactions. Tripeptides of H-L/D-Pro-Pro-Xaa-NH-TentaGel from previous studies offered only low or moderate enantioselectivities, however, after the omission of the central proline residue, the resulting novel dipeptides (H-L/D-Pro-Xaa-NH-TentaGel) exhibited improved enantioselectivities. H-L-Pro-Asp-NH-TentaGel was found most promising, yielding quantitative conversion and an *ee* of 75% in the batch model reaction of propanal with dibenzyl azodicarboxylate. α-Hydrazino aldehydes are configurationally labile products and racemization can occur. However, employing CF conditions and strategic residence time control, we were able to obtain *ee*s ≥90% and high conversions simultaneously. In contrast, batch-based operations provided much lower *ee* of 75%. Unlike in most of the earlier examples, the heterogeneous catalyst proved highly robust, as no decrease in catalyst activity or selectivity was detected during a continuous scale-up experiment of 20 h. The gained substances can be transferred by simple chemical steps to foldamer building blocks.
4. A simple methodology has been developed for the efficient and stereoselective syntheses of trimethyl 3-aminocyclopentane-1,2,4-tricarboxylates from readily

available starting materials. The stereocontrolled oxidative cleavage of norbornene beta-amino acid derivatives yielded a library of functionalized cispentacin analogues and molecular scaffolds of potential interest for the biomedical and pharmaceutical fields. Because of the nature of the cyclopentane ring, in which the equatorial and axial substituents cannot be unambiguously defined, NOE cross-peaks cannot be used simply for structure determination. However, the NOE cross-peak integrals can be used for the determination of proton distances, which can be correlated with the 3D structure. These 3-aminocyclopentane-1,2,4-tricarboxylates will compose the basis of the planned molecular basket and will yield to a highly functionalized heterogenized organocatalyst and foldamer template.

5. A highly efficient continuous flow-solid phase peptide synthesis (CF-SPPS) peptide synthesis technique has been developed which allows the application of only 1.5 equivalents of amino acids during coupling, while maintaining quantitative conversions. The CF meso-scale reactor that was used allowed the application of high temperature and pressure during the syntheses. The complete reaction parameter optimization that was carried out resulted in quantitative amide bond formation when 1.5 equivalents of the amino acid were applied, with low coupling and deprotection times and low solvent consumption. Under the optimized conditions the couplings of all 20 proteinogenic amino acids with 1.5 amino acid equivalents proceeded with excellent conversions. To demonstrate the efficiency of the CF-SPPS methodology, known difficult sequences were synthetized. The purities of the resulting crude peptides were comparable with literature result, but the CF-SPPS methodology requires much less amino acid and solvent. As further evidence of the effectiveness, β-peptide foldamers with alicyclic side-chains were synthetized in excellent yields. Exceptionally difficult structures containing *trans*-ACHC residues were also obtained. Importantly, exotic and expensive artificial amino acids were incorporated into sequences through the use of exceptionally low numbers of amino acid equivalents at low costs. The synthetic strategy outlined here can be utilized for the economical construction of challenging peptides. Small amounts of peptides can be produced with the high purity required for biological assays. Exceptionally among automated peptide synthesizers, on the basis of HPLC knowledge the methodology can be automated in a reliable manner. This technique may also be used for the synthesis of other biopolymers, such as oligosaccharides and oligonucleotides, and opens up wide ranges of future perspectives.

6. A patent application has been filled concerning the CF peptide synthesis.
7. A book chapter has been written with the title “Experimental procedures for conducting organic reactions in CF” by Pieter Nieuwland. Kaspar Koch. Rene Becker. Sandor B. Otvos. Istvan M. Mandity. and Ferenc Fulop. The chapter describes basic organic transformation mainly for students with the aim of providing an introduction for CF reactions.
8. A review was written concerning the strategic application of residence time control as an efficient tool with which to guide CF transformations and that the residence time can be fine-tuned and readily maintained in CF devices. It can be concluded that the residence time offers an extra advantage with which reactions can be directed. Representative examples have been provided, where the residence time governs the chemoselectivity and the diastereoselectivity. The residence time generally influences the conversion, e.g. the longer the residence time, the higher the conversion. Such obvious examples have not been discussed in this mini-review. Flash chemical transformations, where appropriate residence time control is crucial, have been discussed. The strategic application of residence time control allows adequate and easy handling of very labile and reactive reagents. Consequently, merely by means of the fine-tuning of the residence time under general reaction conditions, higher conversions and yields can be achieved than with conventional batch technologies. Novel chemical windows have been opened in the field of organocatalytic and photochemical transformations too by strategic residence time control. The neat, catalyst-free CF synthesis of Rufinamide, an anticonvulsant drug, has been described. Selected examples have been described where metal catalysts play a vital role, e.g. the Heck cross-coupling reaction, selective hydrogenation on Pd surfaces, gold catalysis and metathesis reaction. Examples are shown for CF oxidation, reduction and cyclopropanation reaction, where strategic residence time control allows the formation of unprecedented reaction products not observed in batch reactions. Through fine-tuning of the residence time for CF reaction optimization, harsh reaction conditions or aggressive reagents can be avoided. Importantly, the fine-tuning of the residence time can open up significant new fields. CF transformation may have the potential to revolutionize the fine-chemical and pharmaceutical industries. The search for such applications of CF systems started shortly after their discovery, and increasing

numbers of studies are currently supporting their utility in drug development. Moreover, efforts in this area are expected to intensify.

9. The deuteration of chalcones was performed too in a CF hydrogenation reactor. The major concern by the optimization of the reaction was the reduction of the ketone functional groups as side reaction parallel with the desired deuteration of the C-C double bond. Application of 5% $Pd/BaSO_4$ or 5% $Pt/A1_2O_3$ as catalyst under various conditions showed low selectivity toward the deuterated ketone derivative. Thus, the lead poisoned Lindlar catalyst was used, of which resulted the deuterated ketone derivative as major product. At 80 bar pressure and 20 °C temperature 98% conversion was gained, while the product ratio was 92:8. One recycling step of the reaction mixture on the catalyst bed resulted complete conversion, but the product ratio went wrong, 83:17 was observed.
10. Patent application has been filled claiming deuterated morphine derivatives.

The results gained in the foldamer technology are as follows:

11. Tetrameric H10/12 helix stabilization was achieved by the application of aromatic side-chains in β-peptide oligomers by intramolecular backbone–side-chain CH–π interactions. Because of the enlarged hydrophobic surface of the oligomers, a further aim was the investigation of the self-assembly in a polar medium for the β-peptide H10/12 helices. NMR, ECD and molecular modeling results indicated that the oligomers formed by *cis*-[1*S*,2*S*]- or *cis*-[1*R*,2*R*]-1-amino-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (ATENAC) and *cis*-[1*R*,2*S*]- or *cis*-[1*S*,2*R*]-2-aminocyclohex-3-enecarboxylic acid (ACHEC) residues promote stable H10/12 helix formation with an alternating backbone configuration even at the tetrameric chain-length. These results support the view that aromatic side-chains can be applied for helical structure stabilization. Importantly, this is the first observation of a stable H10/12 helix with tetrameric chain-length. The hydrophobically driven self-assembly was achieved for the helix-forming oligomers, seen as vesicles in transmission electron microscopy images. The self-association phenomenon, which supports the helical secondary structure of these oligomers, depends on the hydrophobic surface area, because a higher number of aromatic side-chains yielded larger vesicles. These results serve as an essential element for the design of helices relating to the H10/12 helix. Moreover, they open up a novel area for bioactive foldamer construction, while

the hydrophobic area gained through the aromatic side-chains may yield important receptor–ligand interaction surfaces which can provide amplified binding strength.

12. A book was written concerning the secondary structure design of β-peptides. Novel β-peptidic secondary structures have been gained with five and six membered alicyclic side-chains. We analyzed various secondary structures of different foldameric and α-peptidic structures as a function of the back-bone stereochemical pattern. The results showed that the homochiral and the alternating heterochiral systems do not cover all the possibilities to create periodic secondary structures. The absolute configurations can be regarded as the basic instruction set in the assembly language of the peptidic foldamer sequences, and an stereochemical patterning approach was established. We tested the stereochemical patterning approach (SPA) experimentally in terms of sequences with a novel backbone stereochemical pattern, giving an H14/16 helix and an H9-12 helix. These are de novo designed helices. Further evidence in connection with the relevance of the SPA is the two distorted structures with the swapped backbone pattern, because they do not form helical structures. Their secondary structure is loop-like. Heterochiral β-peptides were created with cis- and trans-β-amino acids containing various five or six membered alicyclic side-chains.
13. A review was written concerning the biomedical applications of foldamers. Foldamers are artificial self-organizing systems with various critical properties: (i) a stable and designable secondary structure, (ii) a larger molecular surface as compared with ordinary organic drug molecules, (iii) appropriate control of the orientation of the side-chain functional groups, (iv) resistance against proteolytic degradation, which leads to potentially increased oral bioavailability and a longer serum half-life relative to ordinary α-peptides, and (v) the lower conformational freedom may result in increased receptor binding in comparison with the natural analogs. The general properties and types of the foldamers are covered, with highlighted examples of medicinal chemical applications, including antibacterial and cargo molecules, anti-Alzheimer compounds and protein–protein interaction modifiers. Expert opinion: Various new foldamers have been created with a range of structures and biological applications. Membrane-acting antibacterial foldamers have been introduced. A general property of these structures is their amphiphilic nature. The amphiphilicity can be stationary or induced by the membrane binding. Cell-penetrating foldamers have been described which serve as cargo molecules, and foldamers have been used as autophagy inducers. Anti-Alzheimer compounds too have been created and the greatest breakthrough was

attained via the modification of protein–protein interactions which can serve as the chemical and pharmaceutical basis of the relevance of foldamers in the future.