CHEMOENZYMATIC MICROFLUIDIC CASCADE REACTION: COUPLING OF A DIELS-ALDER REACTION WITH A TRANSKETOLASE-CATALYZED REACTION

Mariana Santos [1], Brian O'Sullivan[1], Sarah Müller[2], Alina Bunescu[2], Frank Baganz[1], Marco P. C. Marques [1], Helen Hailes [1], Nicolas Szita [1] and Roland Wohlgemuth[3]

[1]University College London, Gordon Street, London, WC1E 6BT, United Kingdom

[2]Sigma-Aldrich, Member of Merck Group, Riedstrasse 2, D-89555 Steinheim, Germany

[3] Institute of Technical Biochemistry, Technical University Lodz, 90-924 Lodz, Poland

ABSTRACT

A chemoenzymatic microfluidic cascade reaction is demonstrated for the first time, where a Diels-Alder reaction is followed by a transketolase reaction, for the synthesis of 3,4-dimethylcyclohex-3-ene-2'-keto-1',3'-propanediols, which are used as scaffolds for a number of interesting pharmaceutical compounds. For an efficient organic synthesis, an enzymatic reaction would be advantageous, as it would minimize the number of process steps by eliminating the need for protective chemistry [1]. However, most catalysts and reactions conditions used with DA reactions are not compatible with a subsequent enzymatic reaction (issues revolve e.g. around solvent compatibility, differing reaction rates, and mis-match of pH). We used the spatial confinement of reactions afforded by cascaded microreactors, which has been well established for enzyme-enzyme reactions [2], to overcome these challenges and to achieve a chemoenzymatic reaction in continuous flow. Each reaction was optimized individually or in a step-wise synthesis, considering solvents and catalyst combinations, before being coupled in continuous flow.

KEYWORDS: Chemoenzymatic reaction, Diels-Alder, Transketolase, Microfluidic cascade.

INTRODUCTION

We present a chemoenzymatic microfluidic cascade system for the coupling of a Diels-Alder reaction with a tranketolase reaction for the novel synthesis of dihydroxyketone 1-(3,4-dimethyl-3-cyclohexen-1-yl)-1,3-dihydroxypropan-2-one (DCDHP), which is used as a building block in the production of several antibiotics such as thiamphenicol [3] (Figure 1).

The Diels-Alder reaction is a classic reaction in organic synthesis, and a powerful method for forming new carbon-carbon bonds simultaneously and stereospecifically, facilitating the construction of numerous complex molecules. However, there is still a vast potential to be realized in the use of aqueous solvent environments, which are usually a prerequisite for subsequent transformations with biocatalysts, since traditional organic solvents may affect enzymatic activity. To address these challenges a microfluidic cascade approach was adopted. In our configuration, spatial compartmentalization of each reaction step was achieved, allowing reaction conditions to be individually optimized. Influence of the organic solvent in the enzymatic activity was minimized by controlling the flow rates. This integration of a Diels-Alder with a transketolase reaction resulted in a novel more efficient synthesis of DCDHP.

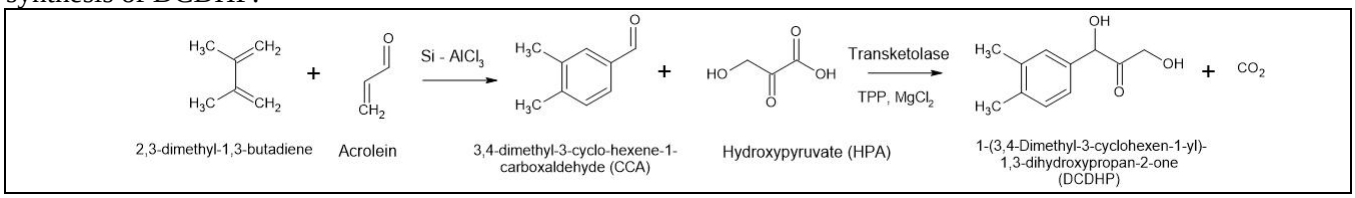

Figure 1: Reaction scheme for the chemoenzymatic Diels-Alder – Transketolase reaction.

EXPERIMENTAL

To achieve a fast reaction and a high throughput, we tested multiple catalysts and solvents for the Diels Alder reaction. Aluminium chloride and acetonitrile showed the highest potential for a full conversion. A blank HPLC column kit was converted into a packed-bed reactor packed with aluminium chloride immobilized on silica gel. In order to assemble the chemoenzymatic microfluidic cascade system the packed bed microreactor was connected in

sequence with a coil microreactor (Figure 2). The chemoenzymatic reaction was initiated by pumping an equimolar mixture of 100 mM of 2,3-dimethyl 1,3-butadiene and acrolein in acetonitrile through the packed bed reactor, at a flow rate of 4 $\mu L.min^{-1}$. The resulting output was diluted in the four-way connector with 5 mM HPA in 25 mM Tris-HCl buffer pH 7 at 4 $\mu L.min^{-1}$ and a transketolase solution, previously incubated for 30 minutes with co-factors, at a flow rate of 32 $\mu L.min^{-1}$.The total flow rate entering the coil reactor was 40 $\mu L.min^{-1}$. Samples collected from the outlet of the coil microreactor were analysed by HPLC for HPA depletion and by colorimetric assay for DCDHP formation.

RESULTS AND DISCUSSION

The chemoenzymatic reaction was successfully assembled by connecting the output of the Diels-Alder packed-bed reactor with a coil reactor, using a four-way connector that allowed the supply of co-substrate hydroxypyruvate and transketolase. Full substrate conversion was achieved in the assembled chemoenzymatic cascade system, for a continuous output of 5 mM of DCDHP (Figure 2). By immobilizing the Diels-Alder catalyst and performing the reaction in continuous flow the production rate improved 5 times, in comparison with batch results.

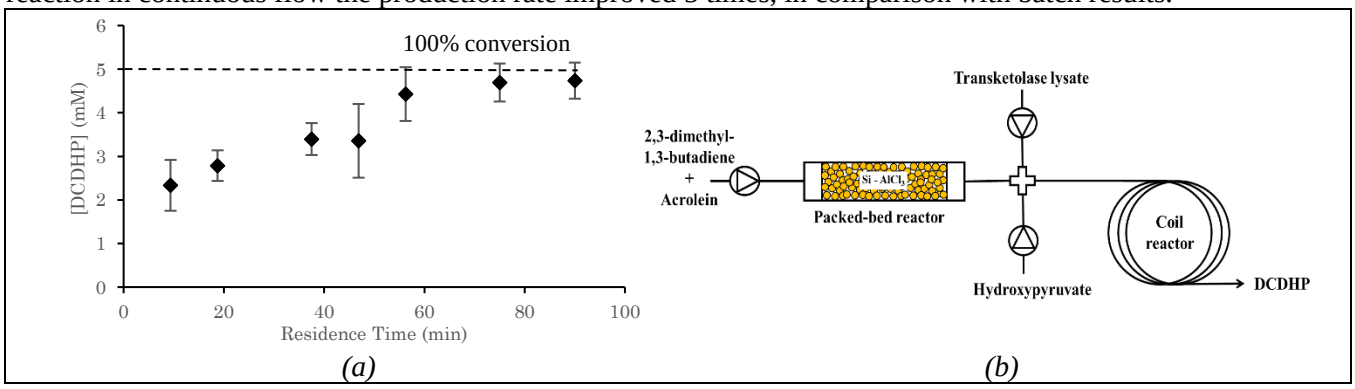

Figure 2: (a) Synthesis of DCDHP in the assembled Diels-Alder – transketolase microfluidic cascade system. Error bars refer to standard deviation from three experiments (N = 3). (b) Scheme of the microfluidic setup for the chemoenzymatic cascade reaction.

CONCLUSION

For the first time, DCDHP was synthetized by a chemoenzymatic reaction in a microfluidic cascade system without the need for protection chemistry. The use of a microreactors to establish this chemoenzymatic cascade synthesis allow the de-convolution of operation windows, preventing enzyme inhibition. Furthermore, full conversion was achieved in the Diels-Alder reaction in the micro packed-bed reactor, whereas in batch the reaction yield was 5 times lower, around only 20%. The results obtained showcase the advantages of microreactor technology to conduct chemoenzymatic cascade reactions.

ACKNOWLEDGEMENTS

This work was supported by the Engineering and Physical Sciences Research Council (EPSRC) and it is dedicated to the memory of Dr. Alexandra Romek.

REFERENCES

[1] H. C. Hailes, P. A. Dalby, and J. M. Woodley, "Integration of biocatalytic conversions into chemical syntheses," *J. Chem. Technol. Biotechnol.*, 2007.

[2] P. Gruber, M. P. C. Marques, B. O'Sullivan, F. Baganz, R. Wohlgemuth, and N. Szita, "Conscious coupling: The challenges and opportunities of cascading enzymatic microreactors," *Biotechnol. J.*, vol. 12, no. 7, pp. 1–13, 2017.

[3] S. Hajra, A. Karmakar, T. Maji, and A. K. Medda, "Stereoselective syntheses of (-)-chloramphenicol and (+)-thiamphenicol," *Tetrahedron*, 2006.

CONTACT

*email: mariana.santos.16@ucl.ac.uk; n.szita@ucl.ac.uk