Green Chemistry

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c6gc02529c

An alternative approach towards poly-ε-caprolactone through a chemoenzymatic synthesis: combined hydrogenation, bio-oxidations and polymerization without the isolation of intermediates†

Severin Wedde,[a] Philipp Rommelmann,[a] Christian Scherkus,[b] Sandy Schmidt,[c] Uwe T. Bornscheuer,[c] Andreas Liese*[b] and Harald Gröger*[a]

Received 9th September 2016,
Accepted 13th December 2016

DOI: 10.1039/c6gc02529c

www.rsc.org/greenchem

A novel synthetic route towards the polymer poly-ε-caprolactone based on a chemoenzymatic reaction sequence was developed. Initial hydrogenation of phenol to cyclohexanol gave a crude product, which was directly used without work-up for a subsequent biocatalytic double oxidation towards ε-caprolactone by means of an alcohol dehydrogenase and a monooxygenase. In order to overcome product inhibition effects, an *in situ*-product removal strategy *via* extraction of ε-caprolactone from an aqueous reaction medium with an organic solvent in the presence of a permeable polydimethylsiloxane membrane was applied. Furthermore, this *in situ*-product removal was combined with lipase-catalyzed polymerization in the organic phase at 25 °C. The obtained crude product contained a polymer fraction with a degree of polymerization comparable to commercial poly-ε-caprolactone.

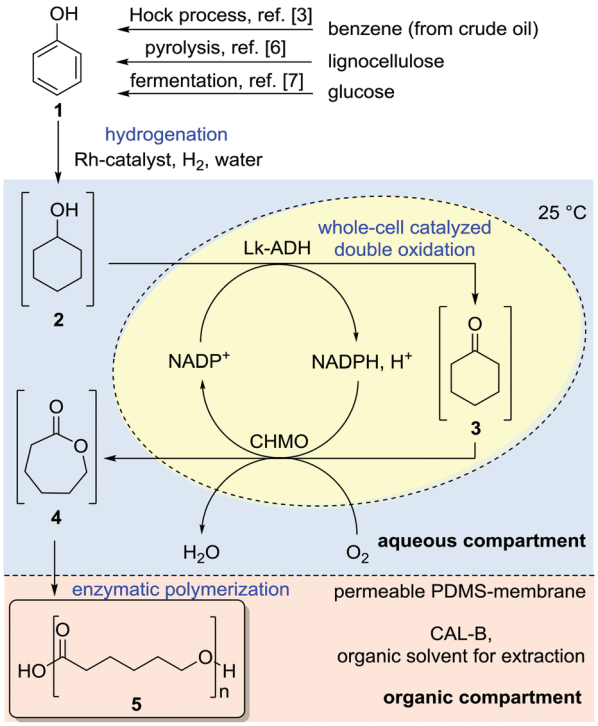
Introduction

As a key challenge for the next few decades in biocatalysis one can regard the integration of biotransformations within chemical multi-step reaction sequences and the application of such chemoenzymatic processes for the production of bulk chemicals. Although biocatalysis has already emerged as an important synthetic tool in the production of chiral fine chemicals and pharmaceuticals, its application in the field of bulk chemicals is still rare.[1] The integration of biocatalytic key steps in chemical multi-step sequences and the design of novel retrosynthetic pathways towards industrial chemicals might also contribute to switch the chemical raw material supply from a petrochemical to a biorenewable basis.[2] Since the largest production volumes of industrial chemicals typically relate to applications in the polymer sector,[3] such an alternative route design for a sustainable production of today's existing petrochemical-based polymers by combining chemo- and biocatalysis would be attractive.[4] Towards this end, many efficient chemo- and bio-catalytic technologies, which are already available, can serve as modules for such new chemoenzymatic reaction sequences.[5]

In the following, we report an alternative synthetic approach towards the petrochemical polymer poly-ε-caprolactone (PCL) starting from phenol and based on the combination of four synthetic key steps comprising metal-catalysed as well as enzymatic reactions. It is noteworthy that besides from crude oil[3] phenol is accessible from lignocellulose *via* pyrolysis[6] or glucose *via* fermentation,[7] thus enabling in principle the synthesis of PCL also from a biorenewable starting material.

In detail, the process concept (which is shown in Scheme 1) is based on an initial access to phenol (*via* the Hock process starting from benzene or by conversion of a biorenewable feedstock),[3,6,7] followed by a metal-catalysed hydrogenation to cyclohexanol and subsequent bi-enzymatic transformation into ε-caprolactone (ε-CL, **4**). In this biotransformation, first cyclohexanol is oxidized by means of a dehydrogenase to cyclohexanone under consumption of $NADP^+$, thus forming NADPH. In the second enzymatic step, namely oxidation of cyclohexanone to ε-CL with a monooxygenase, NADPH is converted back into $NADP^+$. Thus, $NADP^+$ is *in situ* regenerated

[a]*Chair of Organic Chemistry I, Faculty of Chemistry, Bielefeld University, Universitätsstrasse 25, 33615 Bielefeld, Germany. E-mail: harald.groeger@uni-bielefeld.de*
[b]*Institute of Technical Biocatalysis, Hamburg University of Technology, Denickestr. 15, 21073 Hamburg, Germany. E-mail: liese@tuhh.de*
[c]*Institute of Biochemistry, Dept. of Biotechnology & Enzyme Catalysis, University of Greifswald, Felix-Hausdorff-Str. 4, 17487 Greifswald, Germany*
†Electronic supplementary information (ESI) available. See DOI: 10.1039/c6gc02529c



Scheme 1 Concept for a sustainable synthesis route towards the production of PCL (**5**) from phenol (being accessible from biorenewable sources) by combination of metal-catalyzed hydrogenation and multi-enzymatic steps.

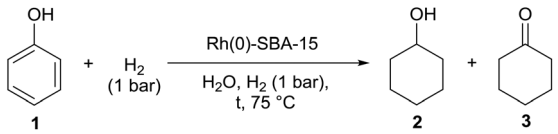
and can be used in catalytic amounts. The final step then consists of an *in situ* removal of ε-CL without work-up and direct polymerization of ε-CL towards PCL. Since for the initial access to phenol established approaches are available,[3,6,7] our major interest was to disclose a proof of concept for a combination of a metal-catalysed hydrogenation and subsequent enzymatic steps without the need for isolation and purification of any of the intermediates.

Results and discussion

To start with the initial hydrogenation step, such reactions with aromatic compounds are extensively described in the literature.[8] Heterogeneous catalysts offer the advantage of excellent product removal and high recycling potential. The catalyst Rh(0)-SBA-15,[8f] consisting of rhodium nanoparticles entrapped in mesoporous silica gel, meets these requirements in general and was used in our study. A screening of the reaction conditions led to an optimized reduction process of phenol (**1**) towards cyclohexanol (**2**; Table 1). Our best result was obtained with a 1 M aqueous phenol solution, using 1 mol% active Rh(0) and 1 bar hydrogen at 75 °C for 24 h, which yielded a conversion of 99% with a selectivity of 99% for cyclohexanol (Table 1, entry 3).

The optimized hydrogenation process was then combined with our developed enzymatic "double oxidation" method[9a,b]

Table 1 Phenol hydrogenation with the heterogeneous catalyst Rh(0)-SBA-15



Entry	Rh cat. [mol%]	t [h]	**1** [c] [%]	**2** [c] [%]	**3** [c] [%]
1[a]	1.9	24	1	99	0
2[a]	1.0	20	1	99	0
3[b]	1.0	24	1	98	1
4[b]	0.5	24	57	16	27

[a] 0.5 M substrate concentration. [b] 1.0 M substrate concentration. [c] The relative amount of each of the compounds **1**, **2** and **3**, respectively, after the reaction is over.

without the isolation of intermediate **2** (according to the reactions shown in Scheme 1). After conducting the hydrogenation and removal of the hydrogen atmosphere as well as subsequent separation of the catalyst by filtration, the aqueous cyclohexanol solution with traces of phenol and cyclohexanone was diluted (for reasons of enzyme inactivation and inhibition at a higher substrate concentration)[9a,b] and then used directly for oxidation to ε-CL (**4**) by means of a cyclohexanone monooxygenase from *Acinetobacter* sp. NCIMB 9871 (CHMO) and an alcohol dehydrogenase from *Lactobacillus kefir* (Lk-ADH) as isolated enzymes just requiring O_2 as the reagent and fully avoiding a co-substrate (Fig. 1). It is noteworthy that the results for ε-CL from this integrated process without cyclohexanol

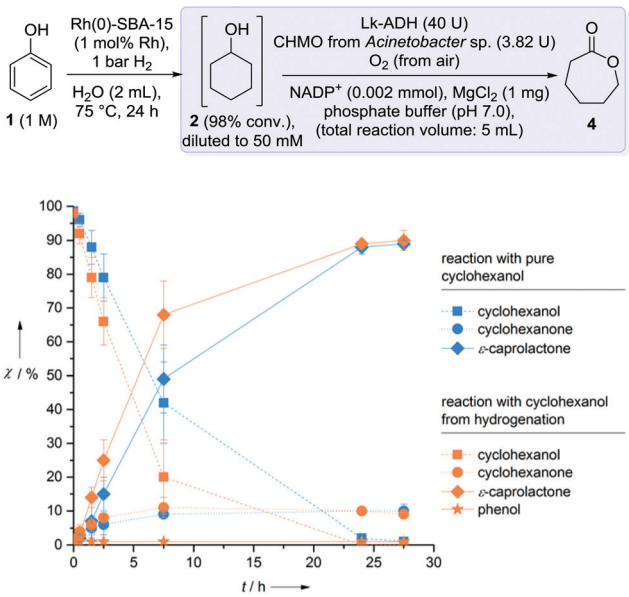


Fig. 1 Biocatalytic oxidation of **2** prepared *in situ via* hydrogenation of phenol (blue-colored symbols: results from the reaction with commercially available cyclohexanol; orange-colored symbols: results from the reaction with cyclohexanol from the hydrogenation process).

isolation are comparable to the one for the biotransformation with commercially available, purified cyclohexanol (**1**) as the substrate (Fig. 1). Almost 90% reaction yield for the desired ε-CL (**4**) could be achieved in both cases after 24 h reaction time. Thus, a residual amount of phenol in the reaction mixture does not influence the enzyme activities negatively. These results show that the "back integration" of ε-CL synthesis with preceded phenol hydrogenation has no negative impact on the enzymatic oxidation cascade.

Next, we focused on the biocatalytic double oxidation step[9–11] as in previous work, it turned out that the process efficiency is hampered by inactivation as well as inhibition especially of the CHMO from *Acinetobacter* sp. by substrate **2**, intermediate **3** and product **4**.[9a,b] Substrate inhibition could be addressed by a fed-batch strategy, and product inhibition was taken into consideration by an *in situ*-product removal approach through oligomerization of ε-CL (**4**) *via* lipase A from *Candida antarctica* in the aqueous reaction medium to its oligomers.[10a] An initial study of water–organic two phase systems for *in situ*-product removal from the aqueous phase revealed that hydrophobic solvents with high log *P*-values were suitable as co-solvents when being used in a low amount,[9c] but more detailed studies showed severe negative effects when increasing the organic solvent amount of such two-phase systems (for details, see the ESI†). The observed decrease in conversion might be attributable to a deactivating effect of the phase boundary on the enzymes.

In order to overcome these limitations when using organic co-solvents, we focused on a new access towards the bulk chemical ε-CL and its polymer product PCL through the combination of the concepts "*in situ*-product removal" and "membrane-based solvent compartmentation". The organic solvents were chosen based on the criterion of environmental sustainability and also based on their classification of being at least "acceptable".[12] For *in situ* extraction we used the following solvents as such with differing log *P* values: methyl-*tert*-butylether (MTBE; log *P* 0.94), cyclohexane (log *P* 3.44) and methylcyclohexane (log *P* 3.88).[12] To avoid the contact of the aqueous with the organic solvent during *in situ* extraction when conducting the biocatalytic double oxidation, we studied the usage of a polydimethylsiloxane (PDMS)-thimble[13] as a selectively permeable membrane (Scheme 1 and Fig. 2A). First we investigated the diffusion of cyclohexanol (**2**), cyclohexanone (**3**) and ε-CL (**4**) from the aqueous reaction compartment (inside the thimble) through the thimble membrane into the organic compartment (outside of the thimble) containing the extracting solvent. Independent of the organic solvent used, the PDMS membrane of 200–250 μm thickness is permeable for all reaction components **2**–**4** (Fig. 2B).

Although ideally it would be preferred to use a membrane, which is exclusively permeable for ε-CL (**4**) alone, diffusion of substrate **2** and intermediate **3** is not critical as the membrane is permeable in both directions, and the substrate concentration can be kept low by applying a fed-batch mode. However, analysis of the aqueous phase revealed that among the organic solvents applied the PDMS-membrane is impermeable only to methylcyclohexane. Thus, methylcyclohexane was selected as the solvent of choice in order to protect the enzymes from the deactivating effect of the extracting solvent (Fig. 2B).

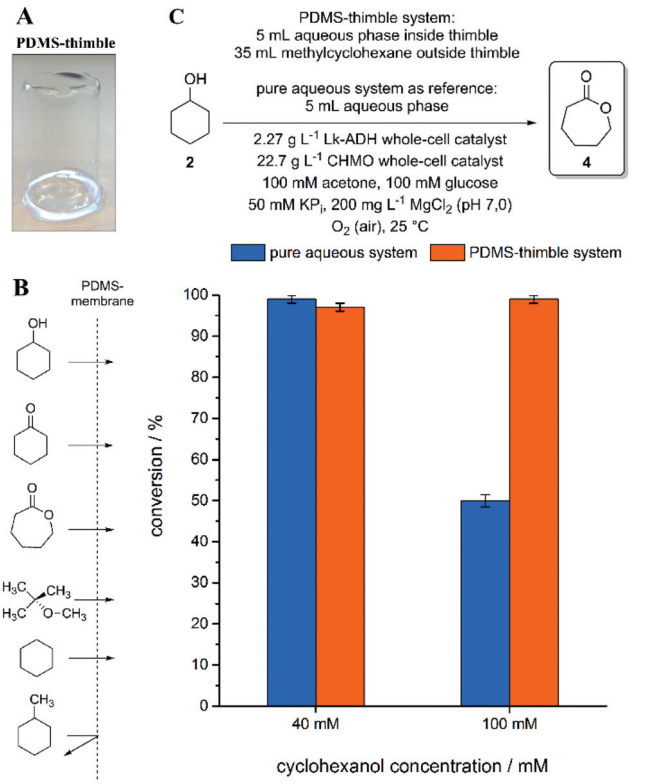


Fig. 2 (A) Photograph of a PDMS thimble. (B) Selectivity of the PDMS-membrane. (C) Whole-cell catalytic biotransformation of cyclohexanol to ε-CL in a pure aqueous buffer (blue) and a PDMS-thimble system (two-compartment one-pot system) with selective component exchange (orange). The compartment inside the PDMS-thimble contains the aqueous solution for the biotransformation. This compartment is selectively separated by the PDMS-membrane from methylcyclohexane (outside the thimble) for *in situ* extraction.

Having a promising membrane-based extraction system without a direct contact of the aqueous and organic phase in hand, we next carried out biotransformations with recombinant whole-cells at 40 and 100 mM substrate concentrations. As at a substrate loading of 40 mM of cyclohexanol in a pure aqueous system the effect of the substrate and product inhibition as well as enzyme inactivation was found not to be critical,[9] in accordance with this previous result[9] nearly quantitative conversion was observed in the case of 40 mM of **2** (Fig. 2C). In contrast, at an increased substrate concentration of 100 mM of **2** these negative effects become evident and conversion dramatically drops to 50%. When using the PDMS-thimble system with separated aqueous and organic phases under the conditions of *in situ*-product removal, at 40 mM substrate loading a similar conversion of 97% was found, thus indicating a proof of concept for the suitability of this methodology. Furthermore, the advantage of the thimble system emerges at 100 mM substrate loading by an increase of conver-

sion from 50% to 99% (Fig. 2C), thus indicating that product inhibition and deactivation as well as the negative impact of the organic solvent on the biocatalyst can be avoided *via* compartmentation of the aqueous phase (for the biotransformation) and the organic phase (for *in situ*-product removal). Since the PDMS-membrane is permeable for substrate **2**, it can diffuse at the beginning of the reaction along the concentration gradient from the aqueous to the organic phase, thus reducing the inhibition of the enzymes in the aqueous compartment. Due to the conversion of cyclohexanol (**2**) to ε-CL in the aqueous compartment, the concentration of **2** decreases and **2** present in the organic compartment diffuses back to the aqueous phase.

In the final step we envisioned an extension of this type of *in situ* extraction towards the integration of a further reactive step, namely a direct polymerization of ε-CL (**4**) to PCL (**5**) catalysed by an immobilized lipase B from *Candida antarctica* (CAL-B). Thus, in a modular fashion this chemoenzymatic synthesis could be applied (dependent on the demand) for the production of ε-CL (**4**, used as a monomer but also needed as a solvent) or alternatively directly the polymer PCL (**5**). The lipase-catalysed transformation of ε-CL (**4**) to PCL (**5**) is a well-known process albeit being reported at higher reaction temperatures and in different solvents.[14] After demonstrating that this enzyme-catalyzed ring-opening polymerization of ε-CL also works at room temperature (25 °C) in methylcyclohexane at 100 mM substrate concentration, we combined the biocatalytic double oxidation of cyclohexanol **2** into ε-CL (**4**) with *in situ* extraction and polymerization of ε-CL to PCL (Fig. 3). We were pleased to find that under these conditions conversion of cyclohexanol (**2**) into ε-CL species (monomer, dimer, oligomer and PCL) is increased to 81% in comparison to the single aqueous system, which only gave 50% conversion for the biocatalytic synthesis of ε-CL (**4**).

In contrast to the PDMS-system without polymerization, however, (Fig. 2) conversion is decreased (81% *versus* 97%), which might be related to the observation of a white thin layer on the PDMS-membrane at the end of the reaction, indicating a blocking or clogging up of the membrane by adsorbed oligomers and polymers of ε-CL as well as the need for further process optimization.

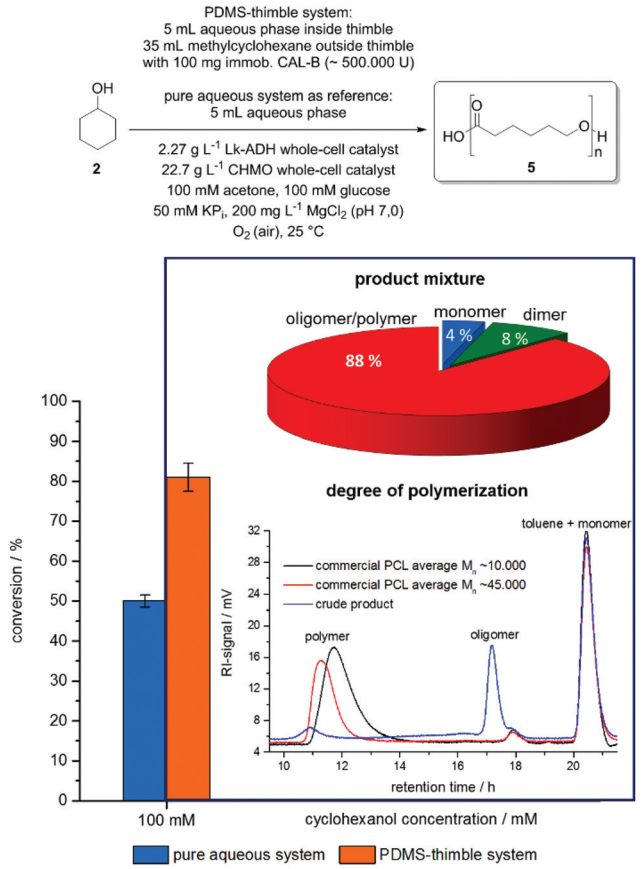


Fig. 3 Whole-cell catalytic biotransformation of cyclohexanol to ε-CL in a pure aqueous buffer system (blue) and to PCL in a PDMS-thimble system (orange). The compartment inside the PDMS-thimble contains the aqueous solution and is selectively separated by the PDMS-membrane from methylcyclohexane (outside the thimble; for *in situ* extraction of ε-CL). *In situ* extraction is coupled with CAL-B-catalyzed polymerization of ε-CL towards PCL in the organic phase. The crude product contains a monomer (ε-CL), a dimer as well as oligomers and polymers. This polymer fraction has a degree of polymerization comparable to commercial PCLs (see gel permeation chromatograms).

Conclusions

In summary, we disclosed a novel synthetic route for the production of the polymer poly-ε-caprolactone based on a chemoenzymatic reaction sequence. Toward this end, an initial hydrogenation of phenol to cyclohexanol was conducted, and the resulting crude product was directly used without work-up in a biocatalytic double oxidation towards ε-caprolactone by means of an alcohol dehydrogenase and a monooxygenase. In order to overcome product inhibition effects, an *in situ*-product removal strategy *via* extraction of ε-caprolactone from an aqueous reaction medium with an organic solvent in the presence of a permeable polydimethylsiloxane-membrane was applied. Furthermore, this *in situ*-product removal by extraction was combined with an enzymatic polymerization in the organic phase at 25 °C utilizing the lipase CAL-B. The obtained crude product contained a polymer fraction with a degree of polymerization comparable to commercial PCL, thus reinforcing the synthetic potential of this process concept towards the petrochemical polymer poly-ε-caprolactone starting from phenol through the combination of a metal-catalysed hydrogenation and three enzymatic steps without the need for isolation and purification of any of the intermediates.

Acknowledgements

The authors thank the German Federal Environmental Foundation (Deutsche Bundesstiftung Umwelt, DBU) for generous support within the DBU-network “ChemBioTec” of the funding priority “Biotechnology” (project AZ 13268-32).

Notes and references

1 Overviews about biocatalysis and industrial applications: (*a*) *Industrial Biotransformations*, ed. A. Liese, K. Seelbach and C. Wandrey, Wiley-VCH, Weinheim, 2nd edn, 2006; (*b*) *Enzyme Catalysis in Organic Synthesis*, ed. K. Drauz, H. Gröger and O. May, Wiley-VCH, Weinheim, 3rd edn, 2012, vol. 1–3.

2 Reviews about chemical processes utilizing biorenewables as raw materials: (*a*) P. Gallezot, *Green Chem.*, 2007, **9**, 295–302; (*b*) A. Corma, S. Iborra and A. Velty, *Chem. Rev.*, 2007, **107**, 2411–2502; (*c*) E. Taarning, I. S. Nielsen, K. Egeblad, R. Madsen and C. H. Christensen, *ChemSusChem*, 2008, **1**, 75–78; (*d*) *Catalytic Process Development for Renewable Materials*, ed. P. Imhof and J. C. van der Waal, Wiley-VCH, Weinheim, 2013; (*e*) J. Zakzeski, P. C. A. Bruijnincx, A. L. Jongerius and B. M. Weckhuysen, *Chem. Rev.*, 2010, **110**, 3552–3599.

3 Overview about the "industrial product tree of organic bulk chemicals": K. Weissermel and H.-J. Arpe, *Industrial Organic Chemistry*, Wiley-VCH, Weinheim, 4th edn, 2003.

4 Review on polymer synthesis through fermentation and biocatalytic cascades: S. Schaffer and T. Haas, *Org. Process Res. Dev.*, 2014, **18**, 752–766.

5 Reviews on the combination of chemo- and bio-catalysis in one-pot processes: (*a*) H. Gröger and W. Hummel, *Curr. Opin. Chem. Biol.*, 2014, **19**, 171–179; (*b*) H. Gröger, in *Cooperative Catalysis*, ed. R. Peters, Wiley-VCH, Weinheim, 2015, ch. 11, pp. 325–349.

6 Q. Bu, H. Lei, S. Ren, L. Wang, J. Holladay, Q. Zhang, J. Tang and R. Ruan, *Bioresour. Technol.*, 2011, **102**, 7004–7007.

7 (*a*) N. J. P. Wierckx, H. Ballerstedt, J. A. M. de Bont and J. Wery, *Appl. Environ. Microbiol.*, 2005, **71**, 8221–8227; (*b*) L. Miao, Q. Li, A. Diao, X. Zhang and Y. Ma, *Appl. Microbiol. Biotechnol.*, 2015, **9**, 5163–5173.

8 Selected examples: (*a*) V. Mevellec, A. Roucoux, E. Ramirez, K. Philippot and B. Chaudret, *Adv. Synth. Catal.*, 2004, **346**, 72–76; (*b*) I. S. Park, M. S. Kwon, K. Y. Kang, J. S. Lee and J. Park, *Adv. Synth. Catal.*, 2007, **349**, 2039–2047; (*c*) K. H. Park, K. Jang, H. J. Kim and S. U. Son, *Angew. Chem., Int. Ed.*, 2007, **46**, 1152–1155; (*d*) V. Molinari, C. Giordano, M. Antonietti and D. Esposito, *J. Am. Chem. Soc.*, 2014, **136**, 1758–1761; (*e*) G. Falini, A. Gualandi and D. Savoia, *Synthesis*, 2009, 2440–2446; (*f*) N. Bhorali and J. N. Ganguli, *Catal. Lett.*, 2013, **143**, 276–281.

9 (*a*) S. Staudt, U. T. Bornscheuer, U. Menyes, W. Hummel and H. Gröger, *Enzyme Microb. Technol.*, 2013, **53**, 288–292; (*b*) H. Wulf, H. Mallin and U. T. Bornscheuer, *Enzyme Microb. Technol.*, 2013, **53**, 283–287; (*c*) A. Reimer, S. Wedde, S. Schmidt, D. Höffer, W. Hummel, U. Kragl, U. T. Bornscheuer and H. Gröger, *J. Heterocycl. Chem.*, 2016, DOI: 10.1002/jhet.2595, online published version.

10 Combination of the biocatalytic double oxidation using CHMO and ADH with further enzymatic reactions(s) in cascade processes: (*a*) S. Schmidt, C. Scherkus, J. Muschiol, U. Menyes, T. Winkler, W. Hummel, H. Gröger, A. Liese, H.-G. Herz and U. T. Bornscheuer, *Angew. Chem., Int. Ed.*, 2015, **54**, 2784–2787; (*b*) J. H. Sattler, M. Fuchs, F. G. Mutti, B. Grischek, P. Engel, J. Pfeffer, J. M. Woodley and W. Kroutil, *Angew. Chem., Int. Ed.*, 2014, **53**, 14153–14157.

11 Alternative biocatalytic approaches towards caprolactone without the need for co-substrate addition: (*a*) A. Bornadel, R. Hatti-Kaul, F. Hollmann and S. Kara, *ChemCatChem*, 2015, **7**, 2442–2445; (*b*) A. Bornadel, R. Hatti-Kaul, F. Hollmann and S. Kara, *Tetrahedron*, 2016, **72**, 7222–7228.

12 (*a*) R. K. Henderson, C. Jiménez-González, D. J. C. Constable, S. R. Alston, G. G. A. Inglis, G. Fisher, J. Sherwood, S. P. Binks and A. D. Curzons, *Green Chem.*, 2011, **13**, 854–862; (*b*) ACS GCI Pharmaceutical Roundtable Solvent Selection Guide Version 2.0 Issued March 21, 2011; http://www.acs.org/gcipharmaraoundtable.

13 For previous applications of this PDMS-thimble membrane technology in compartmentation of non-compatible reactions, see: (*a*) M. B. Runge, M. T. Mwangi, A. L. Miller II, M. Perring and N. B. Bowden, *Angew. Chem., Int. Ed.*, 2008, **47**, 935–939; (*b*) M. T. Mwangi, M. B. Runge, K. M. Hoak, M. D. Schulz and N. D. Bowden, *Chem. – Eur. J.*, 2008, **14**, 6780–6788; (*c*) A. L. Miller II and N. B. Bowden, *J. Org. Chem.*, 2009, **74**, 4834–4840; (*d*) H. Sato, W. Hummel and H. Gröger, *Angew. Chem., Int. Ed.*, 2015, **54**, 4488–4492.

14 M. Labet and W. Thielemans, *Chem. Soc. Rev.*, 2009, **38**, 3484–3504.