Enantioselective Sulfoxidation of Thioanisole by Cascading a Choline Oxidase and a Peroxygenase in the Presence of Natural Deep Eutectic Solvents

Li, Yongru; Ma, Yunjian; Li, Peilin; Zhang, Xizhen; Ribitsch, Doris; Alcalde, Miguel; Hollmann, Frank; Wang, Yonghua

DOI
10.1002/cplu.201900751

Publication date
2020

Document Version
Final published version

Published in
ChemPlusChem

ChemPubSoc Europe

DOI: 10.1002/cplu.201900751

CHEMPLUSCHEM Communications

Enantioselective Sulfoxidation of Thioanisole by Cascading a Choline Oxidase and a Peroxygenase in the Presence of Natural Deep Eutectic Solvents

Yongru Li,[a] Yunjian Ma,[a] Peilin Li,[a] Xizhen Zhang,[b] Doris Ribitsch,[c] Miguel Alcalde,[d] Frank Hollmann,*[e] and Yonghua Wang*[a]

A bienzymatic cascade for selective sulfoxidation is presented. The evolved recombinant peroxygenase from *Agrocybe aegerita* catalyses the enantioselective sulfoxidation of thioanisole whereas the choline oxidase from *Arthrobacter nicotianae* provides the H_2O_2 necessary via reductive activation of ambient oxygen. The reactions are performed in choline chloride-based deep eutectic solvents serving as co-solvent and stoichiometric reductant at the same time. Very promising product concentrations (up to 15 mM enantiopure sulfoxide) and catalyst performances (turnover numbers of 150,000 and 2100 for the peroxygenase and oxidase, respectively) have been achieved.

Chiral sulfoxides are widely found in drugs, chiral intermediates as well as chiral ligands in catalysis.[1] Since the first report by Märcker in 1865 on the HNO_3-promoted sulfoxidation aromatic sulfides,[2] many further synthetic methodologies have been added to the portfolio including transition metal catalysis, peracids and hydrogen peroxide or hypohalites.[3] Nevertheless, the issue of overoxidation to the (mostly undesired) sulfone remains to be solved satisfactorily. More importantly, enantioselective methods, highly desired especially for products in the pharmaceutical and agrochemical sectors are still scarce.[3c,4] To address the latter challenge, biocatalysis is of particular interest due to the often inherent enantioselectivity of enzymes.[5] Therefore, it is not very astonishing that chiral sulfoxidation (of benzyl methyl sulfide, thioanisole) is a common model reaction for monooxygenases and peroxidases.[6]

One issue that needs to be addressed in biocatalytic sulfoxidation reactions is the poor solubility of most thiols in aqueous media, preferred in biocatalysis. Thioanisole, for example, is soluble in to approximately 4 mM. As a consequence, the majority of biocatalytic reports on thioanisole sulfoxidation use such low reagent concentrations, which is not attractive neither from an economic nor an environmental point-of-view.[7]

In recent years, (natural) deep eutectic solvents ((NA)DES) have enjoyed an increasing popularity in biocatalysis.[8] Particularly, their often stabilizing effect on enzymes but also their solubilisation of less hydrophilic reagents are of interest. Especially choline chloride (ChCl) is the basis of many (NA)DES. Recently, we have demonstrated that ChCl can be used not only as solvent component but also as sacrificial electron donor for the reductive activation of molecular oxygen by the enzyme choline oxidase from *Arthrobacter nicotianae* (*An*ChOx) to promote perhydrolase-[9] and peroxygenase-catalysed oxyfunctionalisation reactions.[10] Inspired by the recent contribution by Brenna and co-workers on the peroxygenase-catalysed sulfoxidation of thiols[6] we became interested in expanding this reaction concept to ChCl-based DES exploiting the dual functionality of these DES as co-solvent and as co-substrate (Figure 1 and Figure 2).

In a first set of experiments we used *An*ChOx in various DES to generate H_2O_2, which then spontaneously oxidised thioanisole (Figure 1).

Especially urea- and propanediol-containing DES gave the highest conversions. Interestingly, these were not necessarily those solvents in which *An*ChOx exhibits its highest activity.[10] In carbohydrate-based DES, the overall sulfoxidation activity was the lowest. Possibly, carboxylate groups activate the *in situ* formed H_2O_2 for the spontaneous sulfoxidation reaction.[11] Overall, this procedure appeared to be a viable method for the preparation of racemic sulfoxides. However, especially if biological activity of the sulfoxide products is desired, enantioselective sulfoxidation is preferred. Therefore, we also evaluated peroxygenase-catalysed, enantioselective oxygenation of thioanisole (Figure 2).[6]

Pleasingly, in the bienzymatic system, enantiomerically pure (*R*)-sulfoxide was formed in all experiments (ee > 99%, Ta-

[a] Y. Li, Dr. Y. Ma, P. Li, Prof. Dr. Y. Wang
School of Food Science and Engineering
South China University of Technology
Guangzhou 510640 (P. R. China)
E-mail: yonghw@scut.edu.cn
[b] X. Zhang
School of Biology and Biological Engineering
South China University of Technology
Guangzhou 510640 (P. R. China)
[c] Dr. D. Ribitsch
Austrian Centre for Industrial Biotechnology (ACIB)
Konrad Lorenz Straße 22, 3430 Tulln (Austria)
[d] Prof. Dr. M. Alcalde
Department of Biocatalysis
Institute of Catalysis, CSIC
28049 Madrid (Spain)
[e] Prof. Dr. F. Hollmann
Department of Biotechnology
Delft University of Technology
Van der Maasweg 9, 2629HZ, Delft (The Netherlands)
E-mail: F.Hollmann@TUDelft.nl

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cplu.201900751

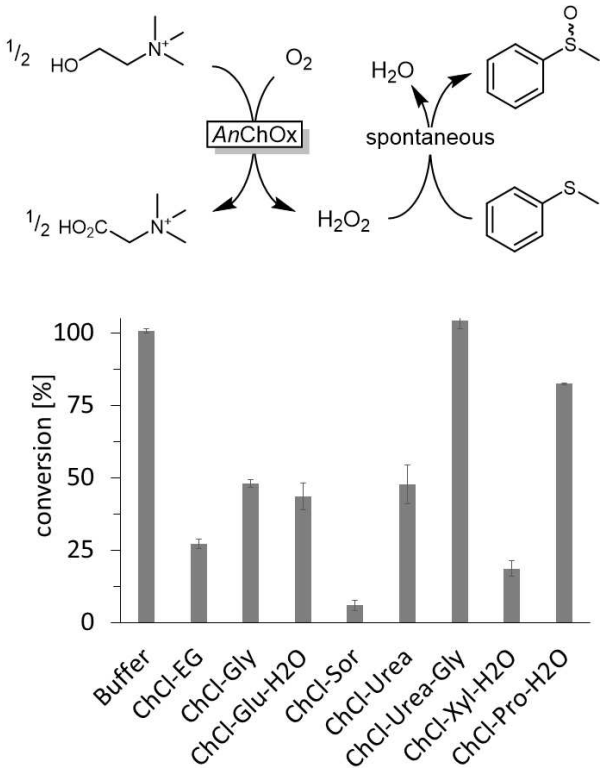

Figure 1. Chemoenzymatic sulfoxidation of thioanisole using *An*ChOx as *in situ* H_2O_2 generation catalyst. Conditions: 1 mL reaction mixture containing [*An*ChOx] = 5 μM, [Methyl phenyl sulfide] = 35 mM, [ChCl] = 100 mM in buffer system and 50% v/v NADES in NADES system. NaPi buffer (pH 7.0, 50 mM) was used in buffer system. 500 rpm, 24 h, 30 °C.

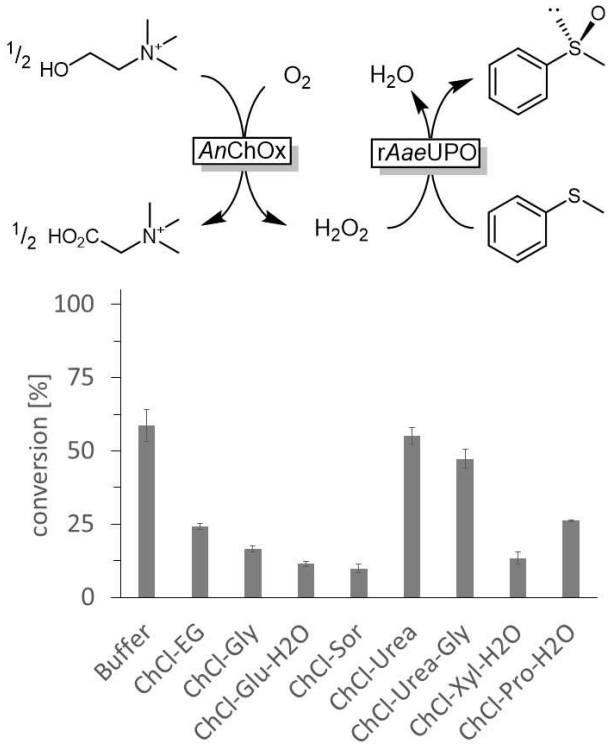

Figure 2. Bienzymatic sulfoxidation of thioanisole using *An*ChOx as *in situ* H_2O_2 generation catalyst and *rAae*UPO as selective sulfoxidation catalyst. Conditions: 1 mL reaction mixture containing [*An*ChOx] = 5 μM, [*rAae*UPO] = 100 nM, [Methyl phenyl sulfide] = 35 mM, [ChCl] = 100 mM in buffer system and 50% v/v NADES in NADES system. NaPi buffer (pH 7.0, 50 mM) was used in buffer system. 500 rpm, 24 h, 30 °C.

ble S4). Nevertheless, conversions in this system never exceeded approx. 50–60%.

As the best results were obtained with Urea-containing DES, we further characterised the influence of several relevant reaction parameters of the efficiency of the bienzymatic sulfoxidation reaction.

First, we evaluated the influence of the water content in the DES reaction mixture (Figure 3). In accordance to our previous findings with bienzymatic hydroxylation and epoxidation reactions,[10] no sulfoxidation activity was found in neat DES and at least 25% (v/v) of water (buffer) were necessary. This is, however, in stark contrast to previous findings on the perhydrolase-catalysed reactions in DES.[9,12] In the latter case, significant enzyme activity was found also in neat DES. For all further experiments, a 1:1 volumetric ratio of buffer and DES was used.

Increasing the concentration of the H_2O_2-generation catalyst (*An*ChOx) influenced the overall product formation in a saturation-type manner (Figure 4).

Interestingly, in buffer, the highest product formation was observed already at *An*ChOx concentrations as low as 3 μM whereas in the presence of 50% (v/v) of ChCl-Urea the overall productivity steadily increased over the entire *An*ChOx concentration range investigated. This may be attributed to the reduced activity of *An*ChOx in the presence of DES.[10] In any case, it can be asserted that the H_2O_2 generation rate (under these conditions) was overall rate limiting (*vide infra*).

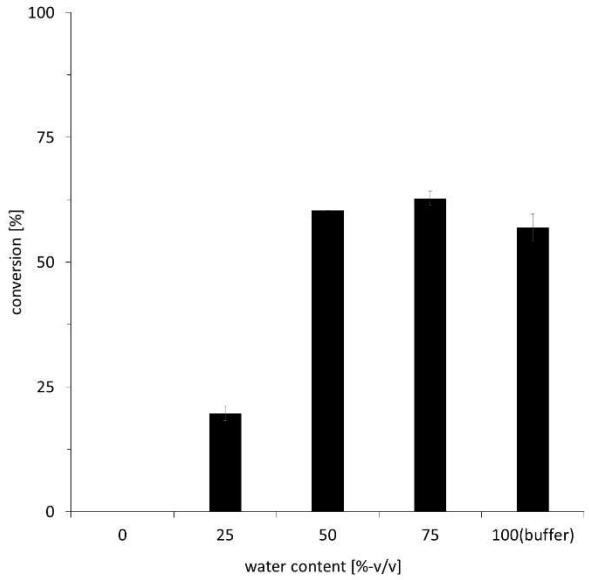

Figure 3. Influence of the water content in the reaction medium on the product formation of the bienzymatic sulfoxidation reaction. Conditions: [*An*ChOx] = 7 μM, [*rAae*UPO] = 100 nM, [Methyl phenyl sulfide] = 25 mM. 0–75% (v/v %) NADES was added in NaPi buffer (pH 7.0, 50 mM). 500 rpm, 24 h, 30 °C.

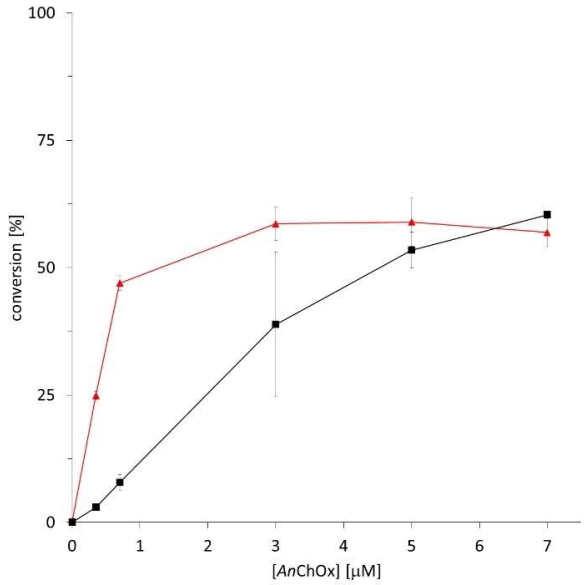

Figure 4. Influence of the H_2O_2-generation rate (of the oxidase concentration) on the performance of the bienzymatic sulfoxidation reaction in aqueous buffer (▲) and in DES (▪). General conditions: [*An*ChOx] = 0.35–7 μM, [r*Aae*UPO] = 100 nM, [Methyl phenyl sulfide] = 25 mM. In NaPi buffer (pH 7.0, 50 mM) system, [ChCl] = 100 mM, and in NADES system, 50% (v/v %) NADES was added in NaPi buffer (pH 7.0, 50 mM), 500 rpm, 24 h, 30 °C.

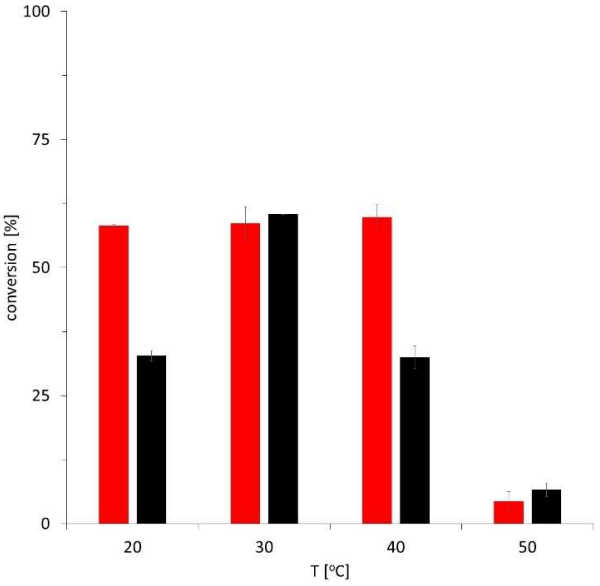

Figure 5. Comparison of the influence of the reaction temperature on the product formation of the bienzymatic sulfoxidation reaction performed in buffer (▪) and in DES (▪). General conditions: [*An*ChOx] = 7 μM in NADES system, [*An*ChOx] = 3 μM in phosphate buffer system, [r*Aae*UPO] = 100 nM, [Methyl phenyl sulfide] = 25 mM. In NaPi buffer (pH 7.0, 50 mM) system, [ChCl] = 100 mM, and in NADES system, 50% (v/v %) NADES was added in NaPi buffer (pH 7.0, 50 mM). 500 rpm, 24 h, 20–50 °C.

Previous studies using hydrolases in DES had established a stabilising effect of DES on the biocatalyst.[12] We therefore also compared the influence of the reaction temperature on the bienzymatic sulfoxidation reaction in buffer and DES-media (Figure 5). Very much to our surprise, no stabilising effect of the DES was observed.

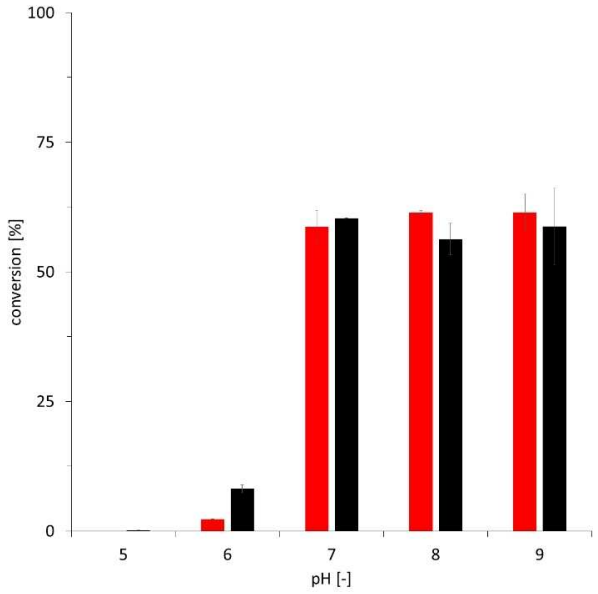

Figure 6. Comparison of the influence of the reaction pH on the product formation of the bienzymatic sulfoxidation reaction performed in buffer (▪) and in DES (▪). General conditions: [*An*ChOx] = 7 μM in NADES system, [*An*ChOx] = 3 μM in phosphate buffer system, [r*Aae*UPO] = 100 nM, [Methyl phenyl sulfide] = 25 mM. In NaPi buffer (pH 7.0, 50 mM) system, [ChCl] = 100 mM, and in NADES system, 50% (v/v %) NADES was added in NaPi buffer (pH 5.0–9.0, 50 mM). 500 rpm, 24 h, 30 °C.

Investigating the pH dependency of both reaction systems (Figure 6) we found no sharp optimum; the highest reaction rates were observed in alkaline media.

Finally, we also varied the r*Aae*UPO concentration to investigate its effect on the initial rate and the final product concentration (Figure 7). Interestingly, varying the peroxygenase concentration between 50 nM and 200 nM had no significant effect in both reaction media, confirming our previous assumption that the r*Aae*UPO-catalysed sulfoxidation is not the overall rate-limiting step of the bienzymatic cascade. Interestingly, while the rate of the overall reaction in the DES-system was approximately half of the buffer system (in accordance with the lower *An*ChOx activity under these conditions Figure 4) the final product concentration in all experiments was essentially identical (13.9 mM ±1.5 mM corresponding to 55% conversion, Table S5–6).

Overall, in the current contribution we have demonstrated that sulfoxidation reactions are feasible in ChCl-based DES. By using *An*ChOx as *in situ* H_2O_2 generation catalyst, we could use the DES not only as a (co-)solvent but also as a reagent for the reaction. Recently, there is an increasing interest in this dual use of DES.[9–10,13]

Using *An*ChOx alone, full conversion of thioanisole was achieved albeit producing racemic products. If combined with r*Aae*UPO, enantiomerically pure sulfoxide was obtained exclusively, albeit at the expense of significantly reduced yields. Today, we are lacking a plausible explanation for this observation. One possible reason may be inhibition of *An*ChOx by the

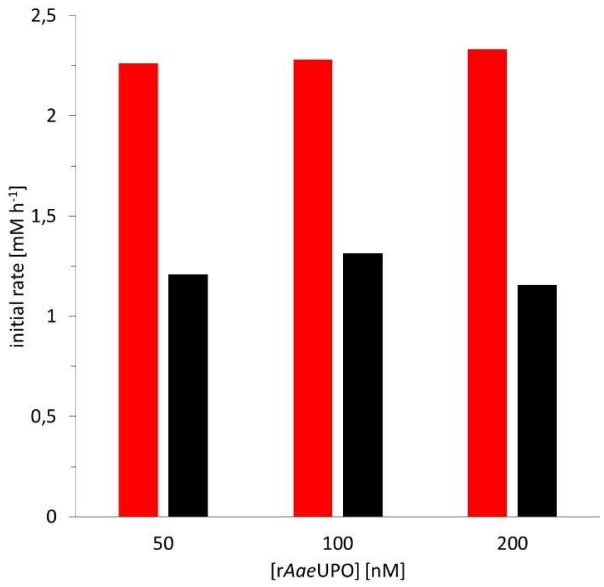

Figure 7. Influence of the r*Aae*UPO concentration on the rate of the bienzymatic sulfoxidation reaction in buffer (▪) and in DES (▪). General conditions: The total reaction volume is 1 mL and the final conditions of this reaction were: [*An*ChOx] = 7 μM in NADES system, [*An*ChOx] = 3 μM in phosphate buffer system, [r*Aae*UPO] = 50–200 nM, [Methyl phenyl sulfide] = 25 mM. In NaPi buffer (pH 7.0, 50 mM) system, [ChCl] = 100 mM, and in NADES system, 50% (v/v %) NADES was added in NaPi buffer (pH 7.0, 50 mM). 500 rpm, 24 h, 30 °C.

(*R*)-sulfoxide, but further experiments will be necessary to identify the molecular reason, alleviate it and bring the production system to preparative applicability. It is worth mentioning that in none of our experiments the corresponding overoxidation product (sulfone) was observed.

Another interesting outcome of this study is that in case of the proposed bienzymatic sulfoxidation reaction, no specific benefit of DES was found. The presence of ChCl-based DES did not improve the stability or the activity of the biocatalysts. This is in stark contrast to previous findings on hydrolases in DES[9,12,14] or even the *An*ChOx-r*Aae*UPO-system if used for hydroxylation and epoxidation reactions.[10] This discrepancy calls for further studies aiming at more systematic investigation and understanding of the effects of DES on biocatalysts!

Acknowledgements

This work was supported by the National Key R&D Program of China (2018YFC0311104), Key Program of Natural Science Foundation of China (31930084), National Outstanding Youth Science Foundation of China (31725022). F.H. gratefully acknowledges funding by the European Research Commission (ERC consolidator grant, No. 648026) and the Netherlands Organization for Scientific Research (VICI grant, No. 724.014.003).

Keywords: biocatalysis · enantioselectivity · natural deep eutectic solvents · peroxygenases · sulfoxidation

[1] J. Han, V. A. Soloshonok, K. D. Klika, J. Drabowicz, A. Wzorek, *Chem. Soc. Rev.* **2018**, *47*, 1307–1350.
[2] C. Märcker, *Justus Liebigs Ann. Chem.* **1865**, *136*, 75–95.
[3] a) T. Okada, H. Matsumuro, S. Kitagawa, T. Iwai, K. Yamazaki, Y. Kinoshita, Y. Kimura, M. Kirihara, *Synlett* **2015**, *26*, 2547–2552; b) M. M. Khodaei, K. Bahrami, A. Karimi, *Synthesis* **2008**, *2008*, 1682–1684; c) S. Liao, I. Čorić, Q. Wang, B. List, *J. Am. Chem. Soc.* **2012**, *134*, 10765–10768.
[4] a) J. Sun, C. Zhu, Z. Dai, M. Yang, Y. Pan, H. Hu, *J. Org. Chem.* **2004**, *69*, 8500–8503; b) H. Egami, T. Katsuki, *J. Am. Chem. Soc.* **2007**, *129*, 8940–8941; c) C. Drago, L. Caggiano, R. F. W. Jackson, *Angew. Chem. Int. Ed.* **2005**, *44*, 7221–7223; d) W. Dai, J. Li, B. Chen, G. Li, Y. Lv, L. Wang, S. Gao, *Org. Lett.* **2013**, *15*, 5658–5661.
[5] G. Torrelo, U. Hanefeld, F. Hollmann, *Catal. Lett.* **2015**, *145*, 309–345.
[6] I. Bassanini, E. E. Ferrandi, M. Vanoni, G. Ottolina, S. Riva, M. Crotti, E. Brenna, D. Monti, *Eur. J. Org. Chem.* **2017**, *2017*, 7186–7189.
[7] Y. Ni, D. Holtmann, F. Hollmann, *ChemCatChem* **2014**, *6*, 930–943.
[8] a) M. Pätzold, S. Siebenhaller, S. Kara, A. Liese, C. Syldatk, D. Holtmann, *Trends Biotechnol.* **2019**, *37*, 943–959; b) I. Juneidi, M. Hayyan, M. A. Hashim, *Proc. Biochem.* **2018**, *66*, 33–60; c) F. Z. Ibn Majdoub Hassani, S. Amzazi, I. Lavandera, *Molecules* **2019**, *24*, 2190; d) V. Gotor-Fernández, C. E. Paul, *J. Biotechnol.* **2019**, *293*, 24–35.
[9] Y. Ma, P. Li, Y. Li, S. J.-P. Willot, W. Zhang, D. Ribitsch, Y. H. Choi, T. Zhang, R. Verpoort, F. Hollmann, Y. Wang, *ChemSusChem* **2019**, *12*, 1310–1315.
[10] Y. Ma, Y. Li, S. Ali, P. Li, W. Zhang, M. Rauch, S. Willot, D. Ribitsch, Y. H. Choi, M. Alcalde, F. Hollmann, Y. Wang, *ChemCatChem* **2020**, DOI: 10.1002/cctc.201901842.
[11] A. J. Kotlewska, F. van Rantwijk, R. A. Sheldon, I. Arends, *Green Chem.* **2011**, *13*, 2154–2160.
[12] a) P. Zhou, X. Wang, C. Zeng, W. Wang, B. Yang, F. Hollmann, Y. Wang, *ChemCatChem* **2017**, *9*, 934–936; b) P. Zhou, X. Wang, B. Yang, F. Hollmann, Y. Wang, *RSC Adv.* **2017**, *7*, 12518–12523.
[13] Á. Mourelle-Insua, I. Lavandera, V. Gotor-Fernández, *Green Chem.* **2019**, *21*, 2946–2951.
[14] a) T. Zhang, Y. Ma, C. P. Tan, F. Hollmann, J. Wang, B. Yang, Y. Wang, *J. Am. Oil Chem. Soc.* **2019**, *96*, 671–679; b) D. Lan, X. Wang, P. Zhou, F. Hollmann, Y. Wang, *RSC Adv.* **2017**, *7*, 40367–40370.

Manuscript received: December 23, 2019
Revised manuscript received: January 17, 2020
Accepted manuscript online: January 17, 2020