Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d0ob01561j

Fatty alcohol synthesis from fatty acids at mild temperature by subsequent enzymatic esterification and metal-catalyzed hydrogenation

Kim E. Schlipköter,[a] Tobias Betke,[b] Joscha Kleber,[a] Harald Gröger *[b] and Andreas Liese *[a]

Fatty alcohols are important products in chemical industry to be used in the formulation of surfactants and lubricants. This work describes a two step approach for the production of myristyl alcohol under neat conditions by combining a lipase catalyzed esterification of myristic acid and myristyl alcohol with a ruthenium catalyzed hydrogenation of the intermediate myristyl myristate. The esterification was carried out in a bubble column reactor with the commercial immobilized lipase B from *Candida antarctica* as a biocatalyst, while the hydrogenation was conducted under pressurized conditions being catalyzed by the homogeneous chemocatalyst Ru-Macho-BH. By investigating the reaction steps separately, comparable reaction rates were found for the esterification of short chain and long chain alcohols. Additionally, the hydrogen pressure could be reduced to 35 bar compared to the current industrial Lurgi process. Characterization of cross interactions by the reactants myristic acid and sodium myristate in the hydrogenation demonstrates that the metal catalyst was completely deactivated, even at a low amount of 0.5 mol% of myristic acid. Complete conversion of myristic acid in the esterification with equal amounts of myristic acid and myristyl alcohol was obtained, overcoming any limitation in the hydrogenation. In comparison to the Lurgi process starting also from fatty acid and fatty alcohols, the chemoenzymatic two step reaction sequence could be realized at lower reaction temperatures of 60 and 100 °C as well as lower hydrogen pressures of 35 bar.

Received 29th July 2020,
Accepted 19th September 2020

DOI: 10.1039/d0ob01561j

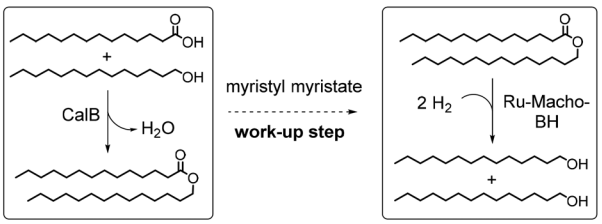
rsc.li/obc

Introduction

The hydrogenation of fatty acids to their corresponding alcohols is an important industrial task due to the broad use of fatty alcohols in various fields of specialty chemicals such as surfactants and lubricants.[1] However, direct hydrogenation of fatty acids requires harsh reaction conditions such as, *e.g.*, temperatures of >200 °C.[2] In contrast, hydrogenation of fatty acid esters is being carried out at lower temperature, and a range of efficient homogeneous metal-catalyzed ester hydrogenations under mild conditions were recently reported.[2,3] On the other hand, esters need to be prepared from a fatty acid and in general short chain alcohols like methanol that, however, is wasted afterwards. The ester formation typically requires harsh conditions when using a chemocatalyst or consumption of additional reagents leading to an increased formation of waste.[4] In industry, the so-called Lurgi-process gained particular importance and therein a reduction of the intermittently formed wax esters to fatty alcohols is realized by a more sustainable process compared to other processes. Harsh temperatures (230–270 °C) are required for the previous solvent free esterification of the fatty acid with a fatty alcohol. The intermediate, the wax ester, is hydrogenated by means of a heterogeneous catalyst. In total, side products or waste as well as energy consuming downstream processes are circumvented.[5] Alternatively, esters can be prepared under mild, solvent-free conditions utilizing a biocatalyst.[6–8] The production of emollient esters by a biocatalytic route offers advantages over conventional processes in terms of waste reduction and energy consumption.[4,9]

In this contribution a conceptually waste-free process sequence is reported which combines the advantages of starting directly from fatty acids and same chain length fatty alcohols with the advantage of applying smooth hydrogenation conditions being known for esters (Scheme 1). The process concept is based on an initial, solvent-free esterification of a fatty acid (exemplified for myristic acid as a C14-fatty acid) with its corresponding fatty alcohol in the first reactor. The

[a]*Institute of Technical Biocatalysis, Hamburg University of Technology, Denickestr. 15, 21073 Hamburg, Germany. E-mail: liese@tuhh.de*
[b]*Chair of Industrial Organic Chemistry and Biotechnology, Department Chemistry, University of Bielefeld, post office box 100131, 33501 Bielefeld, Germany. E-mail: harald.groeger@uni-bielefeld.de*



Scheme 1 Process concept of a less energy-intensive fatty alcohol production.

reaction is catalyzed at mild temperature by a heterogenized lipase following previously developed protocols.[10,11] Subsequently, separation of the formed ester from the immobilized lipase by simple filtration and subsequent transfer of the formed ester into a second reaction vessel for direct hydrogenation under pressure conditions. In consequence, at temperatures of less than 120 °C two molecules of the same desired alcohol (here: C14-alcohol) are delivered within this second reaction. This ester hydrogenation is catalyzed by a pincer-type transition metal catalyst.[12,13] By means of such a chemoenzymatic approach, a sustainable, "zero-waste" (besides waste from catalysts) and economically attractive conversion of myristic acid to myristyl alcohol could be realized.

Results and discussion

Initially, we focused on the biocatalytic esterification of myristic acid as first step of this cascade. We started with investigation of the influence of several alcohols on the reaction rate in order to gain an insight into the impact of a longer chain (C14-)alkyl alcohol compared to the short-chain alcohols such as ethanol and propanol, which have been reported in literature.[10,14] It could be demonstrated that not only full conversions were obtained in all cases by integrating water removal *via* molecular sieves, but also comparable reaction courses under neat conditions (Fig. 1). Hence, the chain length seemed to be of lower impact for the reaction rate of the esterification.

For the second step, the Takasago-catalyst Ru-MACHO-BH was found to reach full conversion of myristyl myristate hydrogenation. This catalyst tolerates the long alcohol moiety of the fatty ester and proceeds very efficiently at 100 °C, which is necessary for catalyst activation.[12,13] The conversion dependency on hydrogen pressure was investigated in replicate experiments of 5 hours by application of hydrogen pressures between 10 and 50 bar (Fig. 2). It could be shown, that even with a comparable low hydrogen pressure of 10 bar a conversion of 10% could still be reached. Overall, the conversion increases progressively from 10% to 40% by a reaction time of 5 h when raising the hydrogen pressure from 10 to 50 bar. The difference in the final conversion between 25 and 50 bar is just 7%, which indicates a feasibility of the reaction already at a low pressure of 25 bar. Compared to the Lurgi process, which

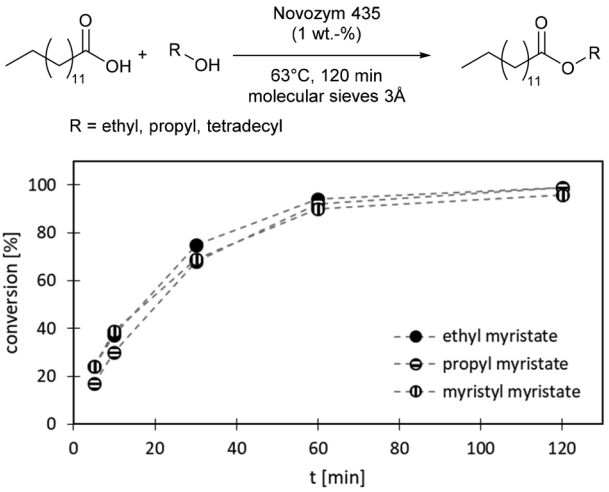


Fig. 1 Esterification of myristic acid with short chain alcohols and myristyl alcohol. Conditions: T = 63 °C, myristic acid (5 g, 21.9 mmol), Novozym 435 (1 wt% regarding myristic acid), 1 eq. alcohol, 2.6 g molecular sieves 3 Å, t = 120 min.

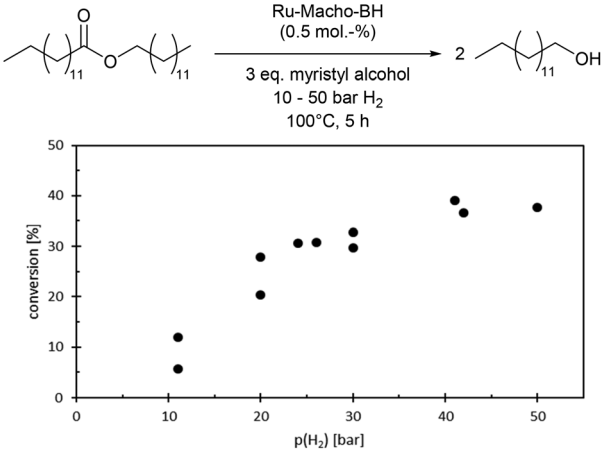


Fig. 2 Hydrogenation of myristyl myristate to myristyl alcohol under hydrogen pressures between 10 and 50 bar. Conditions: $p(H_2)$ = 10–50 bar, T = 100 °C, myristyl myristate (2.1 g, 5 mmol), c(Ru-Macho-BH) = 0.5 mol%, 3 eq. myristyl alcohol, U = 250 rpm, t = 5 h, double measurements.

requires 70 to 100 bar hydrogen pressure, this new approach enables a possible hydrogen pressure reduction of 50 bar.[5,15] Considering Henry's law, the distribution of hydrogen between the gas and the liquid phase is determined by the partial pressure and gives an explanation for the increase of the conversion with increasing hydrogen pressure, a higher concentration of hydrogen becomes available for the reaction. To realize a two step reaction sequence, a filtration step needed to be integrated to intermittantly separate the immobilized biocatalyst to prevent interactions with the Takasago-catalyst. Since myristyl alcohol was used as solvent in the pressure screening of the hydrogenation catalyst Ru-Macho-BH, the impact of alcohol residues from the prior esterification step as critical component in the hydrogenation can be neglected. To investi-

gate, if residual non-converted myristic acid from the esterification step could influence the activity of the Ru-Macho-BH catalyst, conversion studies were carried out with myristic acid as additive. In detail, amounts of 0.5 and 1 mol% of myristic acid as well as (for comparison) sodium myristate were added to the hydrogenation reaction with a concentration of Ru-Macho-BH in the same range of 0.5 and 1 mol% (Fig. 3). In the presence of myristic acid no conversion could be observed in contrast to the addition of sodium myristate, where 16 and 3% conversion were reached, respectively. However, all conversion points were lower than in the experiments without its addition. In conclusion, the chemocatalyst Ru-Macho-BH seemed to be significantly inhibited by the acid function.

Because of the acid sensitivity of the hydrogenation catalyst, full conversion of myristic acid is required in the preceding esterification to prevent an additional downstream step besides filtration of the biocatalyst. Thermodynamically driven, to enable full conversion of the acid, a high alcohol to acid ratio is required in the esterification. To determine the optimal ratio of myristic acid and myristyl alcohol, also enabling high reaction rates, different amounts of myristyl alcohol between 35 and 82 mol% of the total mass of 120 g reaction mixture were applied in the esterification (Fig. 4). To simplify the experimental setup, the reactor was changed to a bubble column reactor, because it allows by aeration with dry air simultaneous efficient mixing of the reaction mixture and separation of the byproduct water, which is formed during the esterification, enabling full conversion of myristic acid.[7,10,11]

Fig. 4 demonstrates, that the reaction rate increased with decreasing myristyl alcohol amount. Conversions are calculated based on the concentration of the key component, which was added in lower amounts, in most cases myristic acid. Full conversion was achieved with 35 mol% myristyl alcohol at 120 min, with equal amounts of the acid and the alcohol after

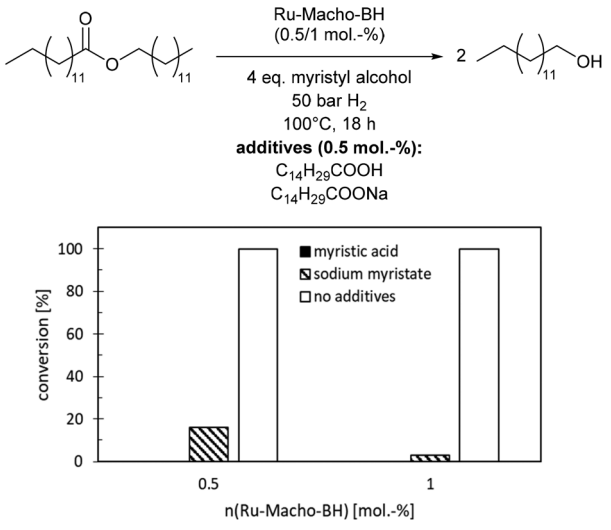


Fig. 3 Influence of additives from the esterification step on the conversion of the hydrogenation of myristyl myristate. Conditions: $p(H_2)$ = 50 bar, T = 100 °C, c(Ru-Macho-BH) = 0.5/1 mol%, 4 eq. myristyl alcohol, t = 18 h, myristic acid/sodium myristate = 0.5/1 mol%.

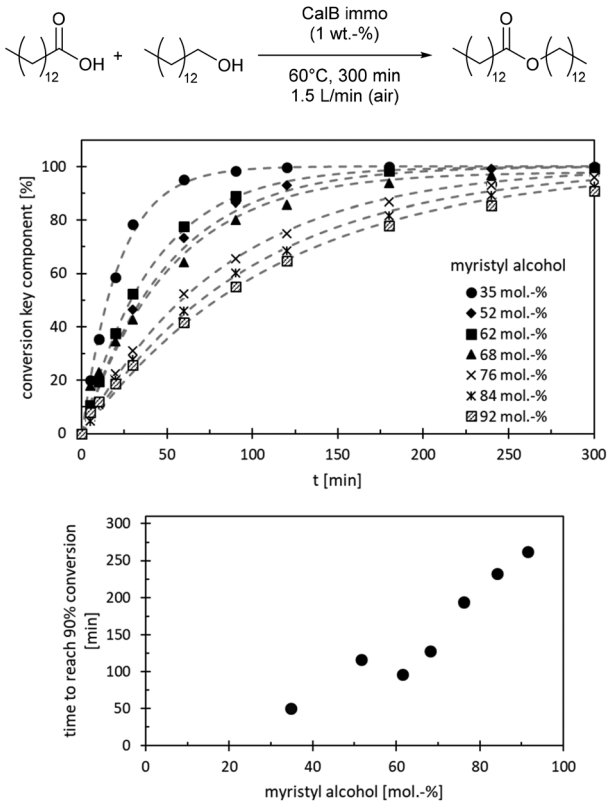


Fig. 4 Progress curves of the esterification in a bubble column with different substrate ratios of myristyl alcohol and myristic acid to form myristyl myristate as well as the time to reach 90% conversion in the esterification depending on the myristyl alcohol amount. Conditions: T = 60 °C, m(total) = 120 g, c(CalB immo) = 1 wt%, V(air) = 1.5 L min^{-1}, t = 300 min.

180 min and with an excess of alcohol of 68 mol% after 5 hours. Exceeding 68 mol% of alcohol, full conversion could not be reached during the monitored time range of 5 hours. This substrate dependency of the activity was already reported by previous investigations of this lipases. In this case, an optimum of 0.6 mol mol^{-1} of myristic acid was found.[16] To efficiently integrate the esterification as first step in the two step reaction sequence a compromise between highest biocatalyst activity and short reaction time to reach maximum conversion, resulting in minimum residual acid, needs to be met. A fast reaction requires an implementation of an acid excess in the esterification combined with an additional downstream process next to the filtration to separate the acid prior to charging the reaction mixture to the hydrogenation. Otherwise, an application with equal or lower amounts of myristic acid neglected the second downstream process, but this implies a longer reaction time to reach full conversion. As one aim of this approach is to lower process costs by minimizing the number of downstream processing steps subsequent to the esterification, equal amounts of both substrates were chosen for further investigations of the two step process.

To prove the feasibility of this chemoenzymatic reaction sequence a two step process was carried out (Fig. 5). The con-

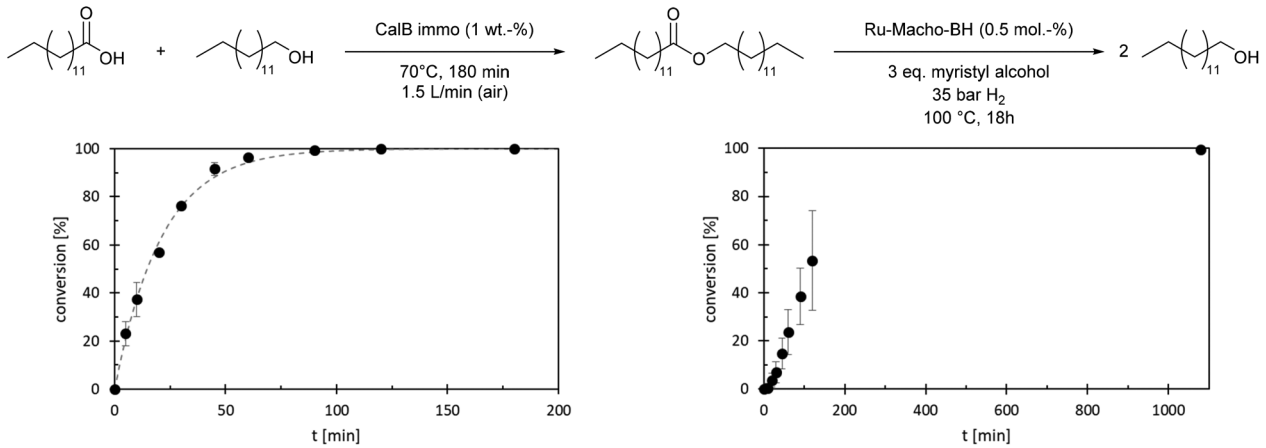


Fig. 5 Conversion of the complete reaction sequence to produce myristyl alcohols consisting of esterification in a bubble column and hydrogenation in an autoclave. Conditions: esterification: T = 70 °C, myristyl acid (50 g, 220 mmol), m(CalB immo) = 1 wt%, 1 eq. myristyl alcohol, $\dot{V}$ (air) = 1.5 L min^{-1}, t = 180 min, hydrogenation: T = 100 °C, p(H2) = 35 bar, myristyl myristate (60 g, 142 mmol), c(Ru-Macho-BH) = 0.5 mol%, 3 eq. myristyl alcohol, U = 600 rpm, t = 18 h, for both reactions 3 determinations.

version in the solvent-free esterification increased constantly and reached full conversion after 120 min. In the subsequent hydrogenation complete conversion could also be reached. As space–time yield (STY) for the esterification 590 g (L h)$^{-1}$ was determined. Overall, a two step process for synthesis of myristyl alcohol was realized, at a relative short reaction time.

Conclusions

A two step chemoenzymatic approach to synthesize myristyl alcohol could be realized successfully in a 100 g scale. In the first reaction step an enzyme catalyzed neat esterification of myristyl alcohol with myristic acid was carried out in a bubble column reactor, reaching a space–time yield of 590 g (L h)$^{-1}$. In a subsequent hydrogenation of the esterification product myristyl myristate catalyzed by Ru-Macho-BH myristyl alcohol was yield. Complete conversion of myristic acid in the first reaction step was achieved and the immobilized lipase was be removed by filtration to apply the obtained solution in the second chemocatalyzed reaction step. It was possible to reduce the overall reaction time significantly compared to the actual industrial process, by application of the lipase CalB immo to catalyze the esterification and the homogeneous Ru-Macho-BH in the hydrogenation in contrast to the state of the art of non-catalyzed esterification followed by a heterogeneously catalyzed hydrogenation. This goes along with reduction to mild reaction conditions of 60 and 100 °C reaction temperature, respectively, and 35 bar hydrogen pressure. However, an effective separation of the homogeneous ruthenium catalyst, Ru-Macho-BH, needs to be identified to enable recyclization.

Experimental

Myristic acid (≥99%), myristyl alcohol (≥98%) were ordered by Merck KGgA (Darmstadt, Deutschland). Lipase B from *Candida antarctica* (CalB immo, 9.100 LU g^{-1}) was purchased by c-LEcta GmbH (Leipzig, Deutschland). Phenolphthalein (1% in ethanol), *n*-hexane (99%) as well as potassiumhydroxide solution in ethanol (0.1 N) were used from Carl Roth GmbH & Co. KG (Karlsruhe, Deutschland). 2-Methyltetrahydrofuran (98%) as well as Ru-Macho-BH were ordered by Tokyo Chemical Industry (TCI, Zwijndrecht, Belgium). Because of no commercial availability, myristyl myristate was synthesised starting from myristic acid and myristyl alcohol by enzyme catalyzed esterification following the procedure below.

Analysis

Esterification with different alcohol chain length and the influences of myristic acid on the hydrogenation were recorded at 500 MHz by a DRX 500 NMR (Bruker Cooperation). The chemical shift δ is given in ppm and referenced to the corresponding solvent signal ($CDCl_3$). Coupling constants are given in Hz.

The samples of esterification in the bubble column had been solved in 10 mL of a 2-methyltetrahydrofuran–water-mixture (5 : 1, volumetric) and been analysed by titration with ethanoic KOH-solution and phenolphthalein (20 µL) until a colour change to pink was visible.

All other samples were analyzed isothermic at 230 °C column temperature *via* GC-FID (HP 5890 Series II) with an Optima FFAP Plus column (30 m × 0.25 mm × 0.25 µm, Macherey-Nagel) and hydrogen as a carrier gas. Typical retention times were: myristyl alcohol 1.10 min, myristic acid 2.26 min, myristyl myristate 7.85 min.

Procedure of esterification with different alcohols

For esterification with different alcohols, myristic acid (5 g, 21.9 mmol), ethanol, propanol or tetradecanol (1 eq., 21.9 mmol) and molecular sieves (3 Å, 2.6 g) were given into a heat dried Schlenk flask, equipped with a thermometer on the inside of the flask. The reaction mixture was heated up and

stirred, upon which the acid started melting. After reaching a temperature of 60–70 °C, CalB (Novozyme 435, 1 wt% regarding myristic acid) was added and the reaction progress was monitored by ^{1}H-NMR-analysis of collected samples (50 μL). The progress calculation took place by analysis of the α-methylgroups of the alcohols and esters.

^{1}H-NMR (500 MHz, $CDCl_3$). 1-Ethanol: δ[ppm] = 0.87 (t, J = 6.9 Hz, 3H), 1.20–1.36 (m, 24H), 1.62 (tt, J = 14.4, 7.2 Hz, 2H), 2.33 (t, J = 7.5 Hz, 2H), 3.72 (q, J = 7.0 Hz, 1H), ester: δ[ppm] = 0.90 (t, J = 6.8 Hz, 3H), 1.28 (m, 23H), 1.63 (tt, J = 14.4, 7.2 Hz, 2H), 2.30 (t, J = 7.6 Hz, 2H), 4.14 (q, J = 7.1 Hz, 2H).

1-Propanol: δ[ppm] = 0.88 (t, J = 6.9 Hz, 3H), 0.94 (t, J = 7.4 Hz, 1H), 1.17–1.40 (m, 24H), 1.53–1.71 (m, 3H), 2.34 (t, J = 7.5 Hz, 2H), 3.61 (t, J = 6.6 Hz, 1H), ester: δ[ppm] = 0.88 (t, J = 6.9 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H), 1.25–1.33 (m, 20H), 1.58–1.70 (m, 4H), 2.29 (t, J = 7.6 Hz, 2H), 4.02 (t, J = 6.7 Hz, 2H).

1-Tetradecanol: δ[ppm] = 0.88 (t, J = 6.9 Hz, 6H), 1.27 (m, 42H), 1.59 (m, 4H), 2.29 (t, J = 7.5 Hz, 2H), 3.64 (t, J = 6.6 Hz, 2H), ester: δ[ppm] = 0.88 (t, J = 6.9 Hz, 6H), 1.17–1.42 (m, 42H), 1.61 (m, 4H), 2.28 (t, J = 7.5 Hz, 2H), 4.05 (t, J = 6.7 Hz, 2H)

Procedure of pressure screening for hydrogenation

For the pressure screening 20 mL autoclaves equipped with magnetic stirrers were applied. In a standard procedure myristyl myristate (2 g, 4.7 mmol) and myristyl alcohol (3 g, 14.0 mol) were melted in the reactors at 60 °C. A time point zero sample was taken when the mixture was liquified and Ru-Macho-BH (0.5 mol%, 13.8 mg, 24 μmol) added. The reactors were closed, pressurized by hydrogen (10–50 bar) and heated to 100 °C. After 5 h the reaction was stopped by depressurizing the reactors and a GC sample (20 μL) was taken, while heating to 80 °C.

Procedure for the hydrogenation with additives

Myristyl myristate (3.40 g, 8.00 mmol), 1-tetradecanol (6.43 g, 30.0 mmol) and additive (40–80 μmol, 0.5–1.0 mol%) were charged into a stainless steel autoclave under continuous flow of argon gas. Ru-Macho-BH (40 μmol, 0.5 mol%) was weighted under inert gas and added to the reaction mixture. The autoclave was sealed and heated to 60 °C to melt the compounds. After purging with nitrogen gas three times, the reactor was pressurized with hydrogen gas (50 bar) and heated to 100 °C for 18 h. At the end of the reaction the pressure was released and the autoclave opened. The crude product was transferred to a flask in its liquid state. Absolute conversion was determined *via* ^{1}H-NMR ($CDCl_3$).

^{1}H-NMR (500 MHz, $CDCl_3$): myristyl myristate: δ[ppm] = 0.88 (t, J = 6.9 Hz, 6H), 1.17–1.42 (m, 42H), 1.61 (m, 4H), 2.28 (t, J = 7.5 Hz, 2H), 4.05 (t, J = 6.7 Hz, 2H), 1-tetradecanol: δ[ppm] = 0.88 (t, J = 6.8 Hz, 3H), 1.17–1.44 (m, 23H), 1.50–1.61 (tt, J = 14.6, 6.6 Hz, 2H), 3.64 (t, J = 6.6 Hz, 2H)

Procedure for all esterification in the bubble column

All esterifications of myristic acid with myristyl alcohol were carried out in a 100 mL glas bubble column following this standard procedure. Myristic acid (between 80 g, 350.3 mmol and 11 g, 48.2 mmol) and myristyl alcohol (between 40 g, 186.9 mmol and 110 g, 514.0 mmol) were liquefied separately in a water bath (60 °C) and then filled in the bubble column with an air flow of 1.5 L min^{-1}. After sampling, CalB immo (1 wt% regarding the total mass) was added. Sampling occurred at defined time points. At the end of the reaction, CalB immo was separated by filtration through a filter paper (270 mm, 65 g m^{-3}) at 70 °C in a heating chamber.

Procedure of hydrogenation in two step process

For hydrogenation a 450 mL bench top autoclave from Parr Instruments Company was applied. In a typical procedure myristyl myristate (60 g, 141.5 mmol) and myristyl alcohol (3 eq., 90 g, 420.5 mmol) were weighted in and melted. The reactands and Ru-Macho-BH (0.5 mol%, 707 μmol) were charged to the reactor, which was flushed three times with argon after closing. The mixture was heated up to 100 °C, hydrogen (35 bar) was added as soon as 100 °C was reached and samples were withdrawn *via* a sampling port over 18 h. After decrease of the hydrogen pressure of 5 bar pressure, hydrogen was added again up to 35 bar The withdrawn samples were analysed by GC-analysis.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Harald Gröger and Tobias Betke gratefully acknowledge generous support from the German Federal Ministry of Education and Research (BMBF) within the funding programme "Biotechnologie 2020 +, Nächste Generation biotechnologischer Verfahren" (grant number: 031A184A).

References

1 (*a*) H. J. Richtler and J. Knaut, *J. Am. Oil Chem. Soc.*, 1984, **61**, 160–175; (*b*) J. Knaut and H. J. Richtler, *J. Am. Oil Chem. Soc.*, 1985, **62**, 317–327.
2 S. Werkmeister, K. Junge and M. Beller, *Org. Process Res. Dev.*, 2014, **18**, 289–302.
3 (*a*) J. Zhang, G. Leitus, Y. Ben-David and D. Milstein, *Angew. Chem., Int. Ed.*, 2006, **45**, 1113–1115; (*b*) N. T. Fairweather, M. S. Gibson and H. Guan, *Organometallics*, 2015, **34**, 335–339; (*c*) M. L. Clarke, *Catal. Sci. Technol.*, 2012, **2**, 2418; (*d*) D. Spasyuk, S. Smith and D. G. Gusev, *Angew. Chem.*, 2012, **124**, 2826–2829.
4 O. Thum and K. Oxenbøll, *SOFW J.*, 2008, **134**, 44–47.
5 Rudolf Boensch and Klaus Noweck, Lurgi GmbH, *United States Pat*, 8426654B2, 2009.
6 T. Garcia, M. Martinez and J. Aracil, *Biocatal. Biotransform.*, 1996, **14**, 67–85.
7 O. Thum, L. Hilterhaus and A. Liese, EP2080807A2, 2009.
8 G. Hills, *Eur. J. Lipid Sci. Technol.*, 2003, **105**, 601–607.
9 O. Thum, *Tenside Surfactants Deterg.*, 2004, **41**, 287–290.

10 L. Hilterhaus, O. Thum and A. Liese, *Org. Process Res. Dev.*, 2008, **12**, 618–625.
11 J. J. Müller, M. Neumann, P. Scholl, L. Hilterhaus, M. Eckstein, O. Thum and A. Liese, *Anal. Chem.*, 2010, **82**, 6008–6014.
12 W. Kuriyama, T. Matsumoto, O. Ogata, Y. Ino, K. Aoki, S. Tanaka, K. Ishida, T. Kobayashi, N. Sayo and T. Saito, *Org. Process Res. Dev.*, 2012, **16**, 166–171.
13 T. Touge, K. Aoki, H. Nara and W. Kuriyama, WO2012/144650A1, 2012.
14 (*a*) A. J. Kruis, A. C. Bohnenkamp, C. Patinios, Y. M. van Nuland, M. Levisson, A. E. Mars, C. van den Berg, S. W. M. Kengen and R. A. Weusthuis, *Biotechnol. Adv.*, 2019, **37**, 107407; (*b*) A. Larios, H. S. García, R. M. Oliart and G. Valerio-Alfaro, *Appl. Microbiol. Biotechnol.*, 2004, **65**, 373–376.
15 M. A. Sánchez, G. C. Torres, V. A. Mazzieri and C. L. Pieck, *J. Chem. Technol. Biotechnol.*, 2017, **92**, 27–42.
16 J. J. Müller, *Enzymatische Veresterungen in Blasensäulenreaktoren: Kinetik, Stofftransport und Online Analytik*, PhD thesis, Hamburg University of Technology, 2012.