DOI: 10.1002/adsc.201300236

Development of a Continuously Operating Process for the Enantioselective Synthesis of a β-Amino Acid Ester *via* a Solvent-Free Chemoenzymatic Reaction Sequence

Simon Strompen,[a] Markus Weiß,[b] Harald Gröger,[b,c] Lutz Hilterhaus,[a] and Andreas Liese[a,*]

[a] Hamburg University of Technology, Institute of Technical Biocatalysis, Denickestr. 15, 21073 Hamburg, Germany
Fax: (+49)-40-42878-2127; phone: (+49)-40-42878-3018; e-mail: liese@tuhh.de
[b] University of Erlangen-Nürnberg, Department of Chemistry and Pharmacy, 91052 Erlangen, Germany
[c] Present address: Bielefeld University, Faculty of Chemistry, Universitätsstr. 25, 33615 Bielefeld, Germany
harald.groeger@uni bielefeld.de

Received: April 9, 2013; Published online: August 9, 2013

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.201300236.

Abstract: A sequential, chemoenzymatic process for a continuously operating production of the chiral β-amino acid ester ethyl (*S*)-3-(benzylamino)-butanoate was developed. The reactor set-up combined a plug-flow reactor for the thermal aza-Michael addition of benzylamine to *trans*-ethyl crotonate coupled to a subsequent packed-bed reactor for the lipase (Novozym 435)-catalyzed kinetic resolution of the racemic intermediate product, which was formed in the initial step. The coupled reactors were operated continuously for a time period of 4 days without significant loss of enzyme activity. The target β-amino acid ester was obtained with 92% conversion in the plug-flow reactor and 59% conversion in the packed bed reactor at high enantiomeric excess of >98%. A space-time yield of 0.4 $kg\,L^{-1}\,d^{-1}$ was calculated for the total reactor system and 1.8 $kg\,L^{-1}\,d^{-1}$ based solely on the volume of the packed bed reactor. A total turnover number of 158,000 was calculated for the biocatalyst assuming the same deactivation rate as observed in batch experiments. The continuously operating, solvent-free process thus represents an efficient method for the enantioselective production of a value added (*S*)-β-amino acid ester starting from cheap substrates.

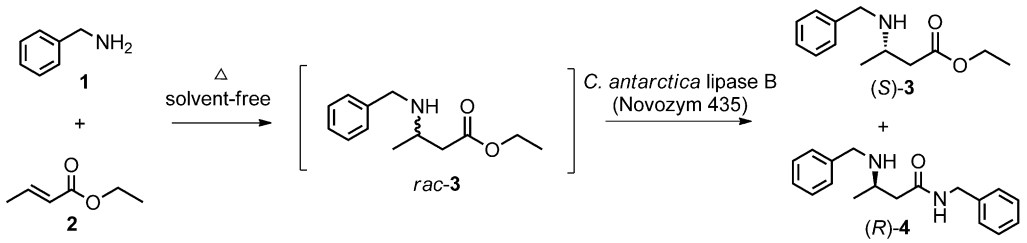
Abbreviations: CALB = *Candida antarctica* lipase B; CSTR = continuously stirred tank reactor; d_i = inner diameter [mm]; *ee* = enantiomeric excess; ε = porosity; $k_{cat,obs}$ = apparent turnover number [h^{-1}]; k_{deact} = enzyme deactivation constant [h^{-1}]; L = length of reactor [m]; $\bar{m}_p$ = average mass of a single particle [g]; N435 = Novozym 435 (*Candida antarctica* lipase B immobilized on acrylic resin); PBR = packed-bed reactor; PFR = plug-flow reactor; PTFE = polytetrafluoroethylene; ρ_{bp} = bulk particle density [$kg\,m^{-3}$]; ρ_p = density of carrier material [$kg\,m^{-3}$]; Q = productivity [$kg\,kg_{N435}^{-1}\,d^{-1}$]; R = channel radius of curvature [cm]; *ttn* = total turnover number [$mol\,mol^{-1}$]; T = temperature [°C]; τ = residence time [h]; u_f = fluid velocity [$cm\,min^{-1}$]; $\dot{v}$ = volumetric flow rate [$mL\,min^{-1}$]; v_t = flow rate of substance [$mmol\,min^{-1}$]; V = volume [mL]; χ_i = mole fraction of compound *i*; X_i = conversion of compound *i*; STY = space-time yield [$kg\,L^{-1}\,d^{-1}$].

Keywords: β-amino acids; biocatalysis; chemoenzymatic reaction sequence; continuously operating process; kinetic resolution; solvent-free conditions

Introduction

In order to improve the industrial applicability of (bio)catalytic processes it is generally desirable to operate at high substrate concentrations. The use of solvent-free reactions as the extreme is therefore especially interesting. Higher yields and reaction rates can often be achieved, while energy costs and waste formation are reduced.[1] Additionally, the necessity for smaller reactor sizes leads to decreased capital investments. A number of solvent-free biocatalytic reactions including, e.g., polymerizations and esterifications have been described in the literature up to date.[2] However, only rather few examples in solvent-free asymmetric catalysis exist using either chemo- or biocatalysts.[3]

With regard to reactor design, continuously operating processes represent an attractive approach applied in the chemical and pharmaceutical industry. As



Scheme 1. Solvent-free chemoenzymatic reaction sequence for the synthesis of β-amino acid ester (*S*)-**3**.

major advantages one can see an increased level of control that facilitates scale-up, the possibility to achieve high space-time yields by making efficient use of substrates and catalysts, improved safety aspects and a significantly reduced physical space requirement as opposed to conventional batch reactors.[4] Additionally, a decreased labor input of skilled workers is required in case of stable processes with long operation times. The stability of the process, however, is often limited by catalyst deactivation or wash-out, contamination in the case of bioprocesses, or mechanical failure of pumps, valves, membranes or pipes (depending on the specific process, e.g., caused by precipitate formation, membrane fouling etc.).

In an attempt to classify (continuously operating) catalytic reactions in the field of applied biocatalysis, the consideration of the type of biological principles of cell metabolism has been suggested as a concept.[5] Hereafter, coupled reactions carried out in continuous flow are termed fourth generation processes. Such processes mimic the concept of cell metabolism in which a constant flux of nutrients in a continuous multireaction network is required for the cell to stay alive. The compatibility of all (chemo)enzymatic steps involved is the major challenge in the development of new cascade processes, which is most probably why relatively few examples of fourth generation processes can be found in the literature.

This article describes the development of such a multistep process for the production of ethyl (*S*)-3-(benzylamino)butanoate [(*S*)-**3**] that combines the above-mentioned advantages of a continuously operating reactor set-up and solvent-free reactions. The reaction sequence presented initially by Weiß and Gröger[6] comprises a thermal aza-Michael addition of benzylamine (**1**) and *trans*-ethyl crotonate (**2**), and a subsequent *Candida antarctica* lipase B (CALB)-catalyzed kinetic resolution of the racemic intermediate β-amino acid ester *via* aminolysis (Scheme 1). A kinetic investigation of both individual steps has been published previously.[7] An environmental assessment of the batch process including downstream processing to produce the free β-amino acid revealed the efficiency of the process with regard to raw material consumption, as well as environmental and safety issues.[8]

Results and Discussion

Theoretical Aspects

The aza-Michael addition of **1** and **2** has been found previously to yield *rac*-**3** as the main product (Scheme 1).[7] However, in a slow successive side reaction, the aminolysis of *rac*-**3** to form *rac*-**4** was observed. While a continuous stirred tank reactor (CSTR) would favor the formation of the final product in racemic form (*rac*-**4**) and thus lead to decreased yields of *rac*-**3**, maximal formation of the intermediate product *rac*-**3** can be achieved using a plug-flow reactor (PFR). Similarly, the lipase (Novozym 435)-catalyzed aminolysis of *rac*-**3** may be carried out continuously in a CSTR or a PFR in the form of a packed-bed reactor (PBR). For kinetic reasons, the latter reactor type is favored in order to achieve maximal yields of the desired chiral product (*S*)-**3** with high

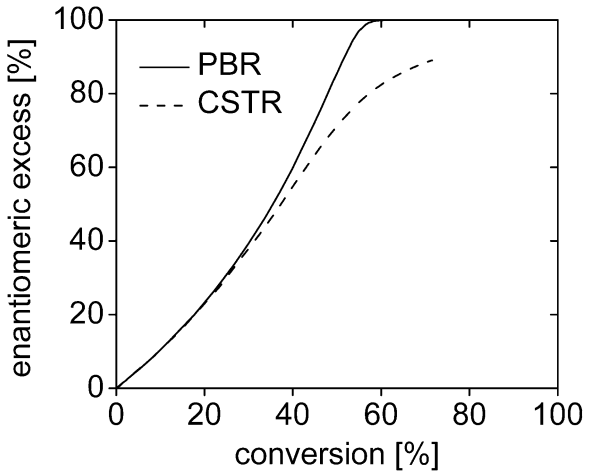
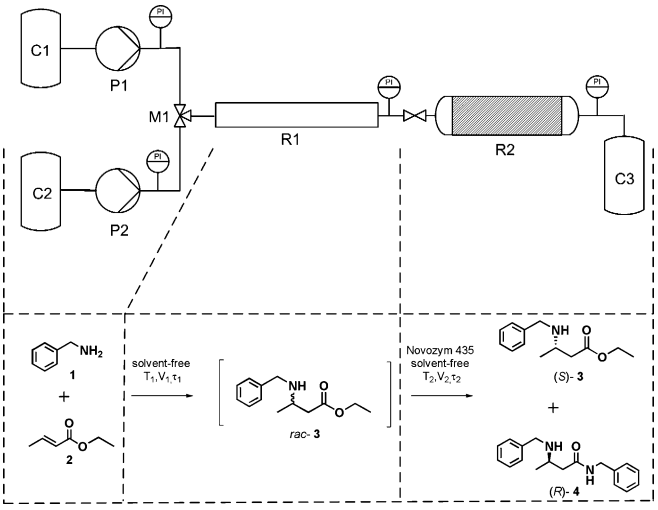


Figure 1. Simulation of enantiomeric excess as a function of conversion in the CALB (Novozym 435)-catalyzed kinetic resolution of *rac*-**3** with **1** in a PBR and CSTR reactor. The simulation was based on the mass balance describing each reactor[10] and the kinetic model.[7] Initial concentrations for each compound were defined as obtained from an aza-Michael addition carried out without prior purification of intermediates. $[\mathbf{1}]_0 = 2.15\ \text{mmol g}^{-1}$, $[(R)\text{-}\mathbf{3}]_0 = 1.50\ \text{mmol g}^{-1}$, $[(S)\text{-}\mathbf{3}]_0 = 1.50\ \text{mmol g}^{-1}$, $[(R)\text{-}\mathbf{4}]_0 = 0.69\ \text{mmol g}^{-1}$, $[(S)\text{-}\mathbf{4}]_0 = 0.069\ \text{mmol g}^{-1}$.



Scheme 2. Coupled reactor set-up for the continuously operating chemoenzymatic production of ethyl (*S*)-3-(benzylamino)butanoate [(*S*)-**3**]. C1, C2: substrate containers. C3: product container. M1: mixing T-piece. R1: tube reactor. R2: packed-bed reactor. Optimized parameters for temperature T, reactor volume V and residence time τ of the coupled reactor are specified in Table 2.

enantiomeric excess.[9] In a CSTR, the continuous operation at high conversion of the favored enantiomer (*R*)-**3** necessarily leads to operation at a high resulting concentration of the unfavored enantiomer (*S*)-**3**, thus kinetically favoring its conversion and causing decreased yields of (*S*)-**3** as remaining substrate (Scheme 1). The effect becomes obvious when plotting the enantiomeric excess as a function of conversion for both PBR and CSTR as shown in Figure 1 using the respective equations describing the mass balance for each reactor type[10] and the kinetic parameters determined previously (see the Supporting Information and Ref.[7]).

Taking these aspects into account, a coupled reactor design comprising a tube reactor for the aza-Michael addition and a packed bed for the subsequent lipase (Novozym 435)-catalyzed aminolysis was devised as shown in Scheme 2.

Continuously Operating Process in a Tube Reactor for Aza-Michael Addition

With the kinetic and thermodynamic data of the aza-Michael addition from batch experiments in hand, a transfer of the system to a continuously operated plug-flow reactor can be realized. In an ideal PFR back-mixing or turbulent flow profiles do not occur. The reaction time in a batch process and the residence time τ in a PFR are used analogously. In an otherwise identical mathematical description of the two reactors, these two dimensions are exchanged.[10] Consequently, in a PFR the concentration changes with the length of the reactor instead of time as in a batch reactor. In order to verify if the assumption of an ideal reactor is true for envisioned reactor geometries, the conversion in an ideally mixed batch reactor was compared to the conversion obtained experimentally in a tube reactor (Figure 2a). A significantly decreased conversion was observed for the tube reactor as compared to the batch reactor indicating non-ideal flow characteristics.

This effect may be attributed to the very low flow velocities used here in order to allow for long reaction times and thereby sufficiently high degrees of conver-

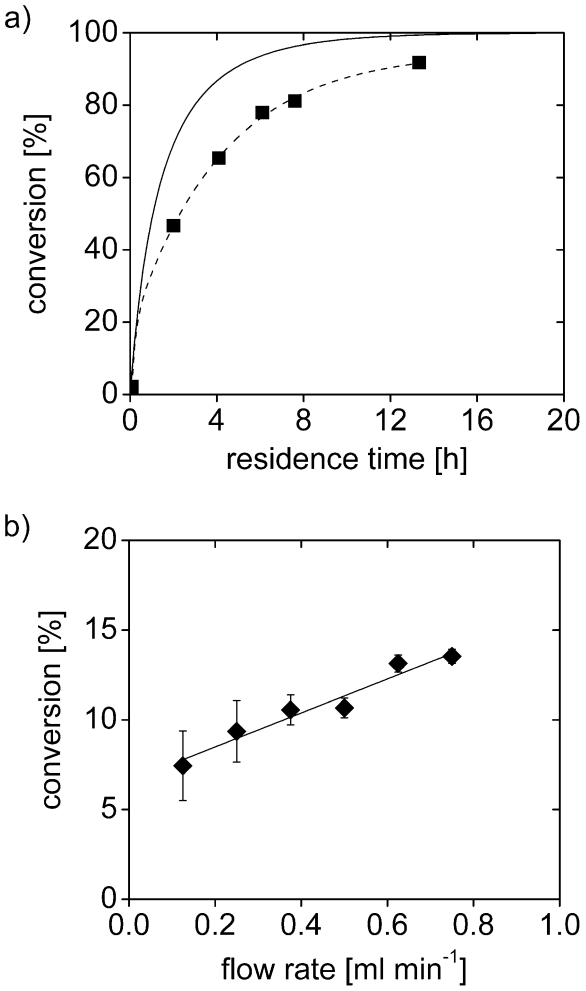


Figure 2. Aza-Michael addition in a plug flow reactor carried out with a 1.7:1 initial molar ratio of **1** and **2**. (a) Prediction of conversion in ideal reactor based on kinetic model[7] (–) with $k_{1,80\,°C} = 14.1 \pm 0.6 \cdot 10^{-2}$ g mmol^{-1} h^{-1} and $k_{2,80\,°C} = 2.1 \pm 0.3 \cdot 10^{-3}$ g mmol^{-1} h^{-1} and experimental results (▲). Visual aid (–). Tube reactor (PTFE), $V = 41.6$ mL, L = 82.7 m, $d_i = 0.8$ mm, $T = 80\,°C$. (b) Conversion as a function of flow rate at constant residence time $\tau = 4$ min using plug flow reactors of constant diameter but increasing length. Tube reactor (stainless steel), $V = 0.5$–3 mL, L = 1–6 m, $d_i = 0.75$ mm, $R = 2.5$ cm, $T = 140\,°C$.

sion. The Reynolds number (Re) is commonly used to characterize the flow regime in continuous reactors and can be calculated for tube reactors according to Eq. (1):

$$Re = \frac{\rho_1 \cdot \mu_f \cdot d_i}{\mu_i} \quad (1)$$

While no data on the dynamic viscosity of *rac*-**3** or reaction mixtures of varying substrate/product composition are available, Reynolds numbers for the pure substrates were calculated for the reactor with an inner tube diameter $d_i = 0.8$ mm and a flow velocity of $u_f = 6.3\ m\,h^{-1}$. Thermophysical properties of the compounds benzylamine ($\mu_{1,80\,°C} = 0.715$ MPa s; $\rho_{1,80\,°C} = 0.933\ g\,mL^{-1}$) and *trans*-ethyl crotonate ($\mu_{2,70\,°C} = 0.439$ MPa s; $\rho_{2,70\,°C} = 0.870\ g\,mL^{-1}$) were taken from the Infotherm database.[11] Accordingly, Reynolds numbers of Re = 1.8 for benzylamine (**1**) and Re = 2.8 for *trans*-ethyl crotonate (**2**) were calculated. Given the very low Reynolds numbers, the flow regime can be considered laminar also for substrate/product mixtures. However, for tube or pipe reactors small vortices causing a local, lateral mixing of the liquid phase are usually desired. In the absence of vortices in an entirely laminar flow regime, a non-ideal flow profile within the tube is caused by friction of the fluid and the tube wall. Consequently, the fluid velocity is slowed down towards the tube wall while higher velocities are found in the center of the tube. Effects such as channeling or stagnant regions, however, reduce the performance of the reactor and should be avoided.[12] In fact, this is a common problem encountered for slow reactions in continuous flow necessitating several hours reaction time. To circumvent such a problem, a nested-pipe reactor has been presented by Minnich and co-workers with reactor volumes ranging from 0.25–8 L.[13]

Dean vortices can be used to circumvent the problem of channel wall effects.[13] Such vortices are generated in a curved pipe by a pressure driven flow of the fluid when the higher velocity stream in the center experiences a greater centripetal force and is hence directed outward.[14] In Figure 2b, conversion of the aza-Michael addition at 140 °C in a coiled steel capillary reactor of varying length is plotted as a function of the flow rate at a constant residence time of $\tau = 4$ min. With increasing flow rate an increased conversion is observed. The improved mixing resulting from Dean vortices is likely to be responsible for this effect. In a microreactor setup, Howell et al. observed Dean vortex formation starting at Reynolds numbers between 1–10 that became stronger with increasing flow velocity.[15] Unfortunately, the necessary high flow velocities to improve conversion cannot be realized easily in a tube reactor set-up without at the same time drastically reducing the reaction time or increasing the length of the tube. It was therefore decided to elongate the reaction time for the tube reactor compared to batch experiments in order to partly compensate for the decreased reaction rate.

Packed-Bed Reactor for Biocatalytic Aminolysis

In order to transfer previous results from batch experiments to a continuous packed-bed reactor set-up, geometrical parameters of reactor and catalyst preparation must be considered. A borosilicate glass reactor of $d_i = 1$ cm was chosen as a suitable PBR due to its chemical resistance and flexibility with regard to adjustment of reactor length. The heterogeneous biocatalyst Novozym 435, according to the manufacturer, has a bulk particle density, i.e., an average density of a large volume of particles, of

$$\rho_{bp} = \frac{m_{p,tot}}{V_{PBR}} = 430\ \frac{kg}{m^3} \quad (2)$$

With a Sauter mean diameter of the particles of 498 μm and an average mass of a single particle of $\bar{m}_p = 6.095 \cdot 10^{-9}$ g, the density of the carrier material is calculated to

$$\rho_p = \frac{\bar{m}_p}{\frac{\pi}{6} \cdot d_p^3} = 946\ \frac{kg}{m^3} \quad (3)$$

The porosity ε describing the void volume in the reactor may be calculated as

$$\varepsilon = 1 - \frac{\rho_{bp}}{\rho_p} = 0.543 \quad (4)$$

The data were used to estimate residence times depending on the amount of catalyst and the volumetric flow rate. However, deviations were observed on comparing calculated and experimentally determined bulk particle density and porosity which, in turn, impact on parameters such as void and total volume and the residence time (see Table 1 for observed parameters of PBR used in coupled reactor set-up). All calculations and simulations were based on experimentally determined parameters.

Long reaction times of the aza-Michael addition described in the previous section necessitate low flow rates. However, in reactions carried out with enzymes immobilized on porous carriers, external mass transfer

Table 1. Data characterizing two packed-bed reactors in series containing immobilized *C. antarctica* lipase B (Novozym 435) used in a coupled, continuous flow reactor set-up.

PBR	d_i [cm]	m_{N435} [g]	V_{total} [mL]	V_{void} [mL]	L [cm]	$\dot{v}$ [mL min^{-1}]	u_f [cm min^{-1}]	τ [h]	ρ_{bp} [g mL^{-1}]	ε [−]
1	1	4.3	11.9	7.8	15.2	0.052	0.10	2.5	0.36	0.65
2	1	2.0	5.1	3.4	6.5	0.049	0.09	1.2	0.39	0.67

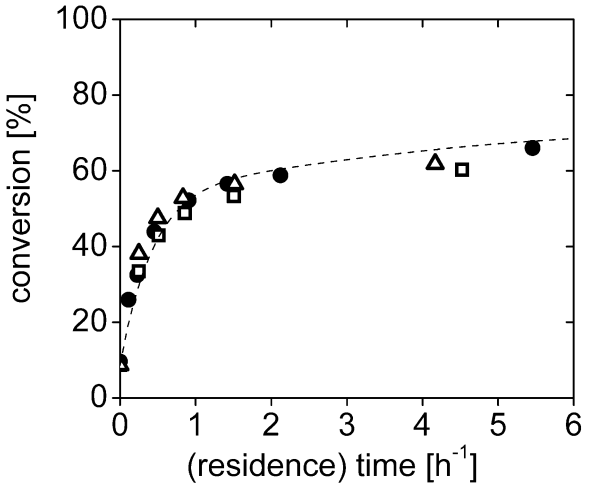


Figure 3. Diffusion limitation in packed bed reactor for CALB (Novozym 435)-catalyzed aminolysis. Comparison of conversion of benzylamine **1** as a function of residence time in batch reactor and two packed bed reactors. $T = 60\,°C$, $\chi_{0,rac\text{-}3} = 62\%$. Batch reactor (●); PBR_1, $V_1 = 3.5$ mL void volume, $m_1 = 2$ g Novozym 435 (□); PBR_2, $V_2 = 7$ mL void volume, $m_1 = 4$ g Novozym 435 (△); simulation based on kinetic model (- -).

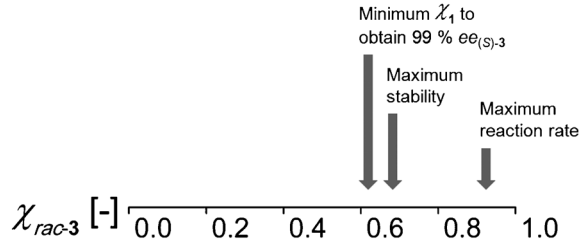
or internal diffusion limitations are a common problem. For batch reactions, such limitations are not significant.[7] The potential presence of diffusion limitation can be determined experimentally for packed-bed reactors by comparing the conversion of two PBRs with different amounts of catalyst. Equal residence times in both reactors of different catalyst loading can be achieved by varying the flow rate. Similar conversion levels at equal residence times are expected in case no diffusion limitation is present. A decreased degree of conversion in the reactor that is operated at lower flow rates (lower catalyst load) on the other hand indicates the presence of diffusional limitations. Figure 3 shows the conversion as a function of residence time for two PBRs of 3.5 mL (PBR_1) and 7 mL (PBR_2) void volume. Additionally, the conversion for a batch reaction in terms of reaction time known to be not limited by mass transfer phenomena is shown. Since equally high catalyst concentrations cannot be realized in batch experiments, corresponding values were calculated from reactions carried out with a lower catalyst concentration as compared to PBR experiments. Negligible differences are visible comparing batch reactor and PBRs.

Coupled Reactor Set-Up for Continuous Production of (*S*)-3

Optimization of the overall reactor performance mainly depends on the enzymatic reaction carried out in the PBR. A high operational stability of the biocatalyst and a good turnover frequency are crucial in order to develop any economically attractive process. Important aspects to consider with respect to the substrate ratio effects in the biocatalytic aminolysis are therefore summarized graphically in Scheme 3.

A high enantiomeric excess (*ee*) of ideally >99% of the product *rac*-**3** is a prerequisite in order to achieve a value-added product, for which approximately 60% conversion of *rac*-**3** are necessary given the intrinsic enantioselectivity of CALB (Novozym 435) for this substrate. Consequently, benzylamine **1** as a remaining component from stage 1 must be available as a substrate in a sufficient amount in stage 2. At the same time, the excess of **1** should be kept as low as possible for kinetic, stability and atom efficiency reasons. The requirements can be fulfilled best using an initial molar ratio of approximately 1.7:1 of substrates **1** and **2**. Despite non-ideal flow conditions in the PFR (stage 1), 92% conversion can be achieved in a reasonable time frame ($\tau = 13.3$ h, $T = 80\,°C$) when applying this starting molar ratio.

The kinetic model (see the Supporting Information and ref.[7]) was used to simulate $ee_{(S)\text{-}3}$ and conversion as a function of residence time with a fixed amount of 6.3 g CALB (Novozym 435) assuming no impact of diffusional limitation. As starting conditions, the con-



Scheme 3. Summary of substrate ratio effects as obtained from batch experiments carried out at 60 °C.[7]

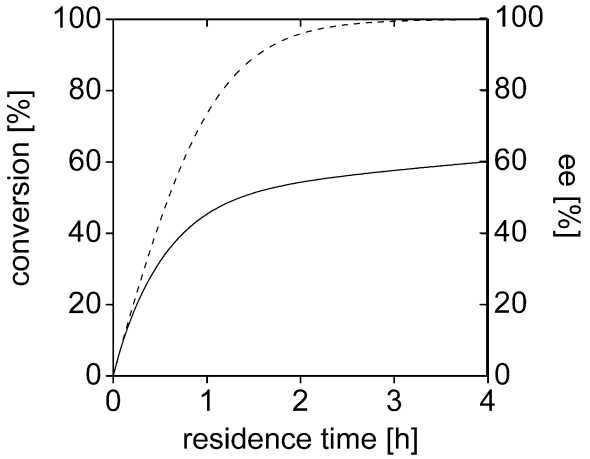


Figure 4. Simulation of conversion (–) and enantiomeric excess (- -) as a function of residence time in a packed bed reactor ($m_{N435}=6.3$ g, 60 °C) based on kinetic model.[7] Initial substrate concentrations used for simulation were those obtained in previous experiments from aza-Michael addition carried out in a PFR with an initial substrate ratio of 1.7:1 for **1** and **2** at $\tau=13.3$ h, $T=80$ °C and 92% conversion of **2**.

centrations of the substrates **1**, *rac*-**3** and product *rac*-**4** were used as observed at the tube reactor outlet. Figure 4 shows that a residence time τ of approximately 2.7 h is necessary to obtain 99% *ee* at 58% conversion.

Experiments employing the coupled reactor set-up (Scheme 2) were carried out starting from the substrates benzylamine **1** and *trans*-ethyl crotonate **2**. Table 1 summarizes the geometric reactor data and operational parameters characterizing the PBR used in this continuous reactor set-up. In order to be able to collect conversion/*ee* data at two different degrees of conversion within a single experiment, two PBRs were coupled in series. Slightly lower flow rates were observed for the second reactor due to a density increase from approximately 1.00 g mL^{-1} at ambient temperature at the inlet of the first PBR to about 1.06 g mL^{-1} at ambient temperature at the inlet of the second PBR caused by ongoing conversion in the solvent-free system.

The reactor was continuously operated for 88 h without significant loss of activity. With a residence time $\tau=2.5$ h a conversion of 55% at 97% $ee_{(S)\text{-}\mathbf{3}}$ was achieved (Figure 5a). A further elongation of the residence time to $\tau=3.7$ h led to an increased conversion of 59% and an average $ee_{(S)\text{-}\mathbf{3}}$ of 98% (Figure 5b). With regard to conversion, the results for both residence times are in good agreement with the simulated data based on the kinetic model. The enantioselectivity is slightly lower as expected. Enzyme deactivation was not observed to a significant degree in the time frame of operation. Diffusional limitations were not regarded in the kinetic model due to observations indicating the presence of no or only weak limitations

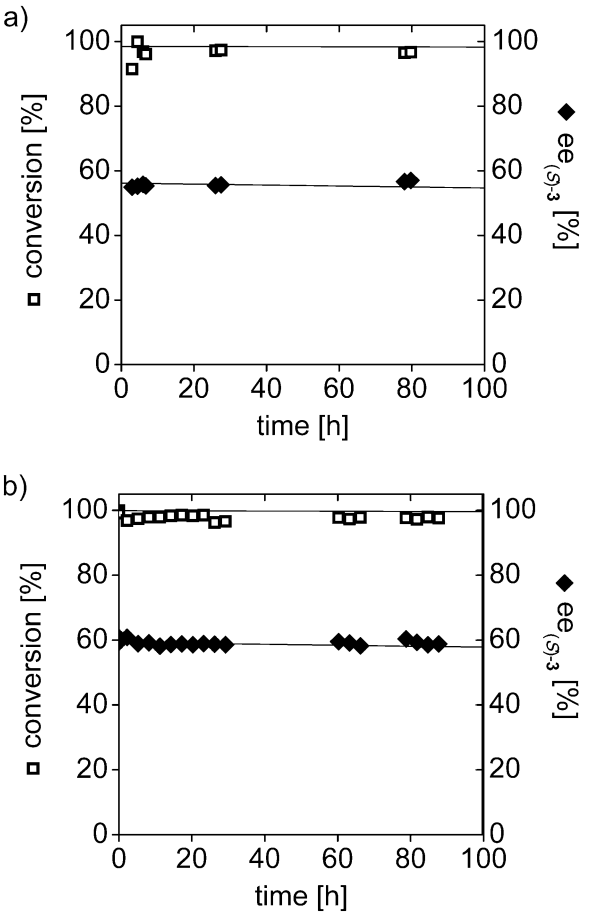


Figure 5. Conversion and *ee* as a function of reaction time in coupled reactor for the continuous aza-Michael addition of **1** and **2** and subsequent CALB (Novozym 435)-catalyzed aminolysis. Samples were taken using sampling valves at (a) residence time $\tau_{PBR}=2.5$ h and (b) $\tau_{PBR}=3.5$ h at the outlet of the packed bed reactor. Symbols represent experimental data: $X_{rac\text{-}\mathbf{3}}$ (◆); $ee_{(S)\text{-}\mathbf{3}}$ (□). Lines represent predicted data as obtained from a simulation based on the kinetic model) see Supporting Infoirmation and ref.[7] PTFE tube reactor: 80 °C, $V=41.6$ mL, L = 82.7 m, $d_i=0.8$ mm. A constant conversion of 92% was measured at the outlet of the tube reactor at $\tau=13.3$ h (see Figure 2a).Packed-bed reactor: 60 °C, $V_{total}=17$ mL, $L_{total}=21.7$ cm, $d_i=1$ cm, 6.3 g Novozym 435. Flow rate for substrates **1** and **2**, respectively: $\nu_1=0.303$ mmol min^{-1}, $\nu_2=0.180$ mmol min^{-1}.

(Figure 3). The operation of the process was aborted after 88 h due to blocked capillaries and sampling valves caused by a colorless precipitate. The precipitate may have been formed from benzylamine, which upon exposure to carbon dioxide forms the corresponding carbamic acid salt or the corresponding acid of *rac*-**3** after enzyme-catalyzed hydrolysis with residual water contained in the substrate solutions.[16]

Parameters characterizing the performance of the sequential process are summarized in Table 2 for the aza-Michael addition and for the CALB (Novozym 435)-catalyzed aminolysis. For the PBR, a space-time

Table 2. Basic reaction engineering parameters characterizing the performance of the coupled-reactor (Scheme 2) for the continuous production of (*S*)-**3**.

	χ_0 [−]	T [°C]	τ [h]	*X* [%]	STY[e] [kg L^{-1} d^{-1}]	Q [kg^{-1} kg$^{-1}_{N435}$ d^{-1}]	*ttn*[f] [−]	spec. *ttn* [mol$^{-1}_{N435}$]	$ee_{(S)\text{-}3}$ [%]
TR	0.63[a]	80	13.3	92[c]	1.2				
PBR	0.53[b]	60	3.67	59[d]	1.8	4.9	158.000	239	98

[a] Initial molar ratio of benzylamine (**1**).
[b] Initial molar ratio of *rac*-**3**.
[c] Conversion of *trans*-ethyl crotonate (**2**).
[d] Conversion of *rac*-**3**.
[e] Based solely on the volume of the respective reactor unit.
[f] Expected according to stability determined in batch experiments [see Eq. (5)].

yield (STY) of 1.8 kg L^{-1} d^{-1} was obtained at about 59% conversion, 98% *ee* and a catalyst productivity Q of 4.9 kg kg$^{-1}_{N435}$h^{-1}. The total turnover number *ttn* was calculated according to the method proposed by Rogers and Bommarius[17] from $k_{cat,obs}$ and the deactivation constant k_{deact} Eq. (5):

$$ttn = \frac{k_{cat,obs}}{k_{deact}} \quad (5)$$

With a production rate of 10.4 U g^{-1} Novozym 435 under process conditions, a molecular mass of CALB of 33000 g mol^{-1} and an estimated protein load of 5% (w/w) CALB on the carrier material, an apparent $k_{cat,obs} = 414$ h^{-1} is calculated. A deactivation constant of $k_{deact} = 0.0026$ h^{-1} had been determined in batch experiments and was used for an estimation of the ttn in a continuous process. Even though the process mode in general may influence enzyme stability, experimental results indicate a high lipase stability for both batch reactor and continuously operated PBR.

Conclusions

The chemoenzymatic reaction sequence towards the synthesis of short-chain aliphatic β-amino acids developed initially by Weiß and Gröger[6] has been transferred successfully to a continuously operated, coupled reactor set-up. Suitable conditions for an increased reaction rate and stability of the catalyst had been identified previously in a kinetic investigation.[7] The developed kinetic model proved useful for the simulation and optimization of the continuous process. The coupled chemo-enzymatic process could be operated for more than 80 h without significant loss of activity. An STY of 1.8 kg L^{-1} d^{-1} (128 g L^{-1} h^{-1}) for the desired β-amino acid ester product (*S*)-**3** was achieved. According to Straathof et al.,[18], industrial processes for the production of fine chemicals range in between 0.1 and 130 g L^{-1} h^{-1}. For comparison, α-amino acids are produced at 30–130 g L^{-1} h^{-1}.[18] The productivity of the process presented here is thus comparable to established industrial processes and shows the high potential of solvent-free processes even in the case of only moderate reaction rates.

Experimental Section

General Remarks

Benzylamine (99.5%, ≤0.3% water K.F.) and *trans*-ethyl crotonate (96%) were obtained from Acros Organics (Belgium). Commercial Novozym 435 (*Candida antarctica* lipase B immobilized on acrylic resin) was obtained from Novozymes (Denmark). Analytical grade solvents used for HPLC analysis were purchased from Carl Roth (Germany). Reference compounds *rac*-**3** and *rac*-**4** were synthesized as described previously[6,7] and in the Supporting Information supplied with this article. A commercial PTFE capillary (Bohlender, Germany) was used as a tube reactor and a Superformance® borosilicate glass column (Merck, Germany) filled with Novozym 435 as the packed-bed reactor.

Continuously Operating Process in a Tube Reactor for Aza-Michael Addition

The substrates **1** and **2** were mixed prior to the reaction in order to avoid changes in substrate composition due to back-pressure changes and pump inaccuracies upon change of flow rates. The premixed substrates were stored on ice/NaCl (−15 °C) in order to largely prevent an ongoing reaction. Substrates were subsequently pumped through a temperature controlled tube for the continuous reaction using a Pharmacia 2248 HPLC pump (Sweden). Flow rates were adjusted in the range from 0.05 mL min^{-1} to 0.8 mL min^{-1}. A polytetrafluoroethylene (PTFE) tube (V = 41.6 mL, L = 82.7 m, $d_i = 0.8$ mm) coiled around a custom-made steel spindle with a diameter of 10.8 cm was inserted into a thermostatted cylinder was applied at temperatures up to 80 °C. Coiled stainless steel capillaries (V = 0.5–3 mL, L = 1–6 m, $d_i = 0.075$ mm, $R = 2.5$ cm) were applied for experiments on flow velocity effects at 140 °C. Temperature control was achieved by immersing the steel capillary reactor into a ther-

mostat (Ecoline Star Edition E200, Lauda, Germany) filled with silicon oil. Samples for quantification were collected at the outlet of the reactor and quenched by dilution in 1,3-dioxolane prior to HPLC analysis.

Continuously Operating Process in a Packed-Bed Reactor for Biocatalytic Aminolysis

In analogy to continuous aza-Michael addition experiments, substrate mixtures consisting predominantly of benzylamine (**1**) and *rac*-**3** were prepared and used as a substrate feed for the Novozym 435-catalyzed aminolysis in order to avoid fluctuation of the substrate composition. The mixtures were obtained as (crude) product resulting from the solvent-free aza-Michael addition of benzylamine (**1**) to *trans*-ethyl crotonate (**2**). A representative batch reaction for preparative substrate synthesis was carried out using 0.98 mol of benzylamine and 0.58 mol of *trans*-ethyl crotonate at 60 °C for 26 h in a round-bottom glass flask. At 95% conversion the composition of the product representing the substrate feed solution for the biocatalytic aminolysis was 2.3 mol g^{-1} benzylamine (**1**), 0.152 mol g^{-1} *trans*-ethyl crotonate (**2**), 3.031 mol g^{-1} *rac*-**3** and 0.117 mol g^{-1} *rac*-**4**. A borosilicate glass reactor (Superformance R, Merck, Germany) of d_i = 1 cm was filled with Novozym 435 and thermostatted at 60 °C using an Ecoline Star Edition E100 thermostat (Lauda, Germany). Substrates were pumped through the packed-bed reactor using a Pharmacia 2248 HPLC pump at flow rates of 0.02–0.4 mL min^{-1}. Pressure was monitored at the reactor inlet using an analogous pressure gauge. Samples were collected at the outlet of the reactor and quenched in 1,3-dioxolane or *iso*-hexane:2-propanol 90:10 (v/v) for quantification and chiral analysis *via* HPLC.

Coupled Reactor Set-Up for Continuous Production of (*S*)-3

The PTFE tube reactor (V = 41.6 mL, L = 82.7 m, d_i = 0.8 mm) and packed-bed reactor (V_{total} = 17 mL, L_{total} = 21.7 cm, d_i = 1 cm, 6.3 g Novozym 435) were coupled for the continuous production of (*S*)-ethyl 3-(benzylamino)butanoate [(*S*)-**3**]. Two Pharmacia 2248 HPLC pumps were used for the continuous supply of substrates **1** (0.303 mmol min^{-1}) and **2** (0.180 mmol min^{-1}). PTFE pressure retention valves (Bohlender, Germany) set to 2 bar were installed at the pump outlet in order to improve the performance of the pumps and maintain constant flow velocities. Mixing was achieved using a simple mixing T-piece prior to the reactor inlet. The tube reactor for the aza-Michael addition was thermostatted at 80 °C using a water bath. The temperature of the packed bed reactor was maintained at 60 °C. Pressure gauges and sampling valves were installed before and after both reactor units.

Determination of Conversion

Conversions of all reactions were analyzed by HPLC on an Agilent system equipped with a diode array detector (215 nm) and a Nucleodur C8 ec column (5 μm, 25 cm, 0.46 cm; Macherey–Nagel, Germany). A mixture consisting of 47.5/52.5 (v/v) MeOH/sodium phosphate buffer (50 mM, pH 6.5) was used as the eluent at a flow rate of 1 mL min^{-1}. Retention times: **1**: 3.9 min, **2**: 8.5 min, *rac*-**3**: 15.5 min, *rac*-**4**: 18 min.

Determination of Enantiomeric Excess

The enantiomeric excess of **3** was determined using a Chiralcel OD-H column (5 μm, 25 cm, 0.46 cm; Daicel, Japan) cooled down to 10 °C and UV detection at 223 nm. *iso*-Hexane/2-propanol 95/5 (v/v), 0.2% (v/v) DEA was used as the eluent at a flow rate of 0.75 mL min^{-1}. Retention times: (*R*)-**3**: 10 min, (*S*)-**3**: 12.5 min. The *ee* of the product **4** was measured on a Nucleocel α S column (5 μm, 25 cm, 0.46 cm; Macherey–Nagel, Germany) at 20 °C and UV detection at 258 nm. A mixture consisting of *n*-hexane/2-propanol 95/5 (v/v), 0.2 % (v/v) DEA was used as the eluent at a flow rate of 0.8 mL min^{-1}. Retention times: (*S*)-**4**: 29 min, (*R*)-**4**: 32 min.

Computational Methods

Simulation of reactor performances based on kinetic parameters published previously was performed using the inherent ode45 algorithm of MATLAB (MathWorks, Natick, USA).[7]

References

[1] Selected examples on the concept, benefits and challenges of solvent-free approaches a) P. T. Anastas, J. Warner, in: *Green Chemistry: Theory and Practice*; Oxford University Press, New York, **1998**; b) H. R. Hobbs, N. R. Thomas, *Chem. Rev.* **2007**, *107*, 2786–2820; c) P. J. Walsh, H. Li, C. A. de Parrodi, *Chem. Rev.* **2007**, *107*, 2503–2545; d) J. M. DeSimone, *Science* **2002**, *297*, 799.

[2] For efficient examples see a) L. Hilterhaus, O. Thum, A. Liese, *Org. Process Res. Dev.* **2008**, *12*, 618–625; b) C. Korupp, R. Weberskirch, J. J. Müller, A. Liese, L. Hilterhaus, *Org. Process Res. Dev.* **2010**, *14*, 1118–1124; c) A. Mahapatro, A. Kumar, R. A. Gross, *Biomacromolecules* **2004**, *5*, 62–68.

[3] See for example a) J. von Langermann, A. Mell, E. Paetzold, T. Daußmann, U. Kragl, *Adv. Synth. Catal.* **2007**, *349*, 1418–1424; b) K.-N. Uhm, S.-J. Lee, H.-k. Kim, H.-Y. Kang, Y. Lee, *J. Mol. Catal. B: Enzym.* **2007**, *45*, 34–38; c) J. Xiong, J. Wu, G. Xu, L. Yang, *Chem. Eng J.* **2008**, *138*, 258–263; d) H.-H. Li, Y.-H. He, Y. Yuan, Z. Guan, *Green Chem.* **2011**, *13*, 185–189.

[4] Selected reviews on continuous (bio)catalytic processes; a) C. Wiles, P. Watts, *Eur. J. Org. Chem.* **2008**, 1655–1671; b) N. N. Rao, S. Lütz, K. Würges, D. Minör, *Org. Process Res. Dev.* **2009**, *13*, 607–616; c) P. Lozano, E. Garcia-Verdugo, S. V. Luis, M. Pucheault, M. Vaultier, *Curr. Org. Synth.* **2011**, *8*, 810–823.

[5] R. Yuryev, S. Strompen, A. Liese, *Beilstein J. Org. Chem.* **2011**, *7*, 1449–1467.

[6] M. Weiß, H. Gröger, *Synlett* **2009**, 1251–1254.

[7] S. Strompen, M. Weiß, T. Ingram, I. Smirnova, H. Gröger, L. Hilterhaus, A. Liese, *Biotechnol. Bioeng.* **2012**, *109*, 1479–1489.

[8] a) M. Weiß, T. Brinkmann, H. Gröger, *Green Chem.* **2010**, *12*, 1580–1588; b) M. Eissen, M. Weiß, T. Brink-

mann, S. Steinigeweg, *Chem. Eng. Technol.* **2010**, *33*, 629–637.
[9] J. L. L. Rakels, H. T. Paffen, A. J. J. Straathof, J. J. Heijnen, *Enz. Microb. Technol.* **1994**, *16*, 791–794.
[10] H. Chmiel, *Bioprozesstechnik*, Spektrum, Heidelberg, **2006**.
[11] Website: www.fiz-chemie.de/infotherm/.
[12] O. Levenspiel, *Chemical Reaction Engineering*, John Wiley & Sons, New York, **1999**.
[13] C. B. Minnich, L. Greiner, C. Reimers, M. Uerdingen, M. A. Liauw, *Chem. Eng. J.* **2011**, *168*, 759–764.
[14] D. Kaufhold, F. Kopf, C. Wolff, S. Beutel, L. Hilterhaus, M. Hoffmann, T. Scheper, M. Schlüter, A. Liese, *J. Membrane Sci.* **2012**, *423–424*, 342–347.
[15] P. B. Howell Jr, D. R. Mott, J. P. Golden, F. S. Ligler, *Lab Chip* **2004**, *4*, 663–669.
[16] L. Heuer, *Benzylamine*, in: *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH, Weinheim, **2006**.
[17] T. A. Rogers, A. S. Bommarius, *Chem. Eng. Sci.* **2010**, *65*, 2118–2124.
[18] A. J. Straathof, S. Panke, A. Schmid, *Curr. Opin. Biotechnol.* **2002**, *13*, 548–556.

