

# Towards a greener synthesis of (*S*)-3-aminobutanoic acid: process development and environmental assessment†

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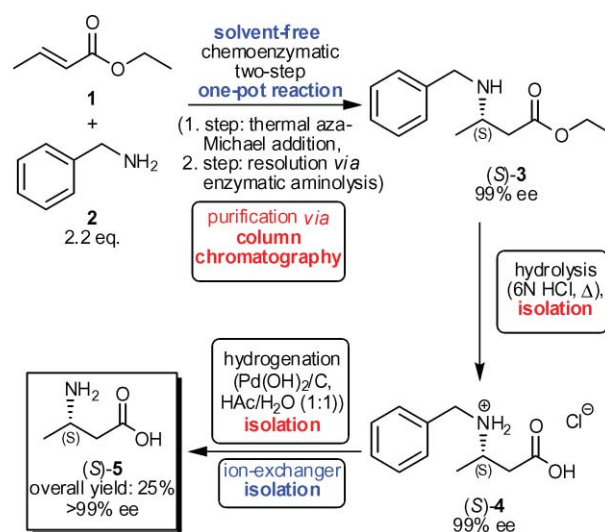
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An improved, greener process for the enantioselective chemoenzymatic synthesis of (*S*)-3-aminobutanoic acid has been developed. Reaction steps comprise an initial aza-Michael addition starting from cheap prochiral compounds, subsequent enzymatic resolution *via* aminolysis using commercially available *Candida antarctica* lipase B in a solvent-free one-pot process, hydrolysis of the resulting ester and removal of the *N*-benzyl moiety *via* hydrogenation. After isolation, the desired (*S*)-3-aminobutanoic acid was obtained in an overall yield of 28% and with an excellent enantiomeric excess of 99% ee. Notably, this reaction sequence does not require column chromatography with organic solvents and only one purification step of an intermediate is needed. The environmental impact of this optimized process has been evaluated and an E-factor of 41 has been calculated for the overall process. A comparative assessment with the previous process was done *via* mass balancing using the E-factor, the selectivity index  $S^{-1}$  as well as an SHE assessment.

## Introduction

Enantiomerically pure  $\beta$ -amino acids are attractive key building blocks for the synthesis of pharmaceuticals.<sup>1</sup> The tendency to use chiral  $\beta$ -amino acids in drug synthesis has increased over recent years as well as the search for new synthetic methods for their enantioselective preparation.<sup>2</sup> For the stereoselective synthesis of aromatic  $\beta$ -amino acids a range of efficient methods exist,<sup>3,4</sup> which in part are already applied on industrial scale. In contrast, efficient stereoselective methodologies for aliphatic, in particular short-chain  $\beta$ -amino acids, are known to a lesser extent.<sup>5</sup> In addition, numerous synthetic steps and tedious work-up operations (in particular column chromatographical separation) often limits the applicability and scalability of several routes. In order to make those multi-step syntheses more attractive for large-scale applications it would be desirable to: (i) have a limited number of synthetic steps, (ii) use cheap and easily available starting materials, (iii) avoid isolation and (complex) purification of intermediates, and (iv) replace column chromatography (requiring large amount of organic solvent) by greener work-up operations. Recently we developed a chemoenzymatic approach towards short-chain  $\beta$ -amino acids exemplified for (*S*)-3-aminobutanoic acid ((*S*)-**5**, Scheme 1),<sup>6</sup> which already fulfils the criteria (i) and (ii) for a sustainable and potentially technical application.

However, this process still requires isolation and purification of intermediates (in contrast to criteria (iii)) and a column



**Scheme 1** Original lab synthesis of (*S*)-**1** according to ref. 6: scope and limitations.

chromatographical purification step leading to a high demand of organic solvent (in contrast to criteria (iv)). As a consequence, this results in a high E-factor of 359 for the overall process.

In the following, we report the process development towards a greener synthesis of (*S*)-3-aminobutanoic acid (as hydrochloride salt), which overcomes the former limitations. The product (*S*)-3-aminobutanoic acid hydrochloride, (*S*)-**9** (Scheme 2), has been chosen as a representative of chiral short-chain  $\beta$ -amino acids. The process development has been influenced by environmental considerations according to the twelve green chemistry principles by Anastas and Warner,<sup>7</sup> addressing issues such as the use of non-toxic chemicals, minimization of organic solvents and reduction of isolation steps. In addition we carried out an environmental assessment of the optimized process, in which critical

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† Electronic supplementary information (ESI) available: Material and ecotoxicity data used for the environmental assessment, and data resulting from the environmental assessment for the optimized and original process. See DOI: 10.1039/c002721a

process steps of the original process have been replaced. Thus, this work also contributes to increase the (still) limited number of examples in the field of small chiral molecules describing a process development in combination with an environmental assessment of the process. For the assessment of the optimized process (and the original process for comparison) we used the software programmes EATOS<sup>8</sup> and Umberto/Sabento.<sup>9</sup> Both software programmes EATOS (primarily developed for chemical syntheses) and Umberto/Sabento (Sabento: primarily developed for biotechnological processes) rely on mass balancing, and allow the calculation of, e.g., the E-factor according to a concept by Sheldon.<sup>10,11</sup> We used these software programmes, which are based on slightly different assessment algorithms, since they complement each other. EATOS allows an easy and fast calculation of the E-factor and environmental impact of the separate reaction steps within a sequence. Umberto/Sabento allows a graphical breakdown of the whole process by the illustration of all mass flows in a Sankey diagram.<sup>12</sup>

## Results and discussion

### Process development towards a sustainable synthesis

Our recently developed lab scale process for the preparation of the enantiomerically pure (*S*)- $\beta$ -amino acid (*S*)-**5** (Scheme 1), represented the starting point for the process development work. This process is a multi-step synthesis with a solvent-free, chemoenzymatic one-pot reaction as the initial step. This key step led to the desired intermediate (*S*)-**3** in high enantiomeric excess of 99% ee after separation of the immobilized enzyme through filtration, washing with methyl *tert*-butyl ether (MTBE), and purification *via* column chromatography (Scheme 1). The ester moiety in (*S*)-**3** was subsequently hydrolysed under harsh acidic conditions under formation of the hydrochloride salt of the acid (*S*)-**4**. After deprotection of the *N*-benzyl protecting group *via* hydrogenation and subsequent ion-exchange chromatography, the desired (*S*)-3-aminobutanoic acid, (*S*)-**5**, was obtained in 25% overall yield and enantiomerically pure form (> 99% ee).

The use of cheap and readily available (prochiral) substrates (**1** and **2**) is advantageous, as is using a commercially available biocatalyst, limiting the number of synthetic steps, using solvent-free and highly enantioselective initial chemoenzymatic synthesis of (*S*)-**3**, and the satisfactory overall yield of 25% (taking into account the presence of a resolution step within a four-step synthesis). Since organic solvents, which are used as reaction media and for product isolation and purification (extraction, column chromatography), typically are the main contributors of “auxiliary waste” produced during chemical processes,<sup>13</sup> an improved multi-step process should exclude organic solvents.<sup>14,15</sup> The avoidance of organic solvents in reaction steps has been addressed in our original work by the development of a solvent-free protocol for the initial key step.

However, critical steps of this lab process with respect to sustainability are in particular (i) the large amounts of waste, which result from organic solvents used for the column chromatographic step (as a typical lab purification procedure), (ii) single use of the biocatalyst, and (iii) isolation of the resulting intermediates and product after each reaction stage during the

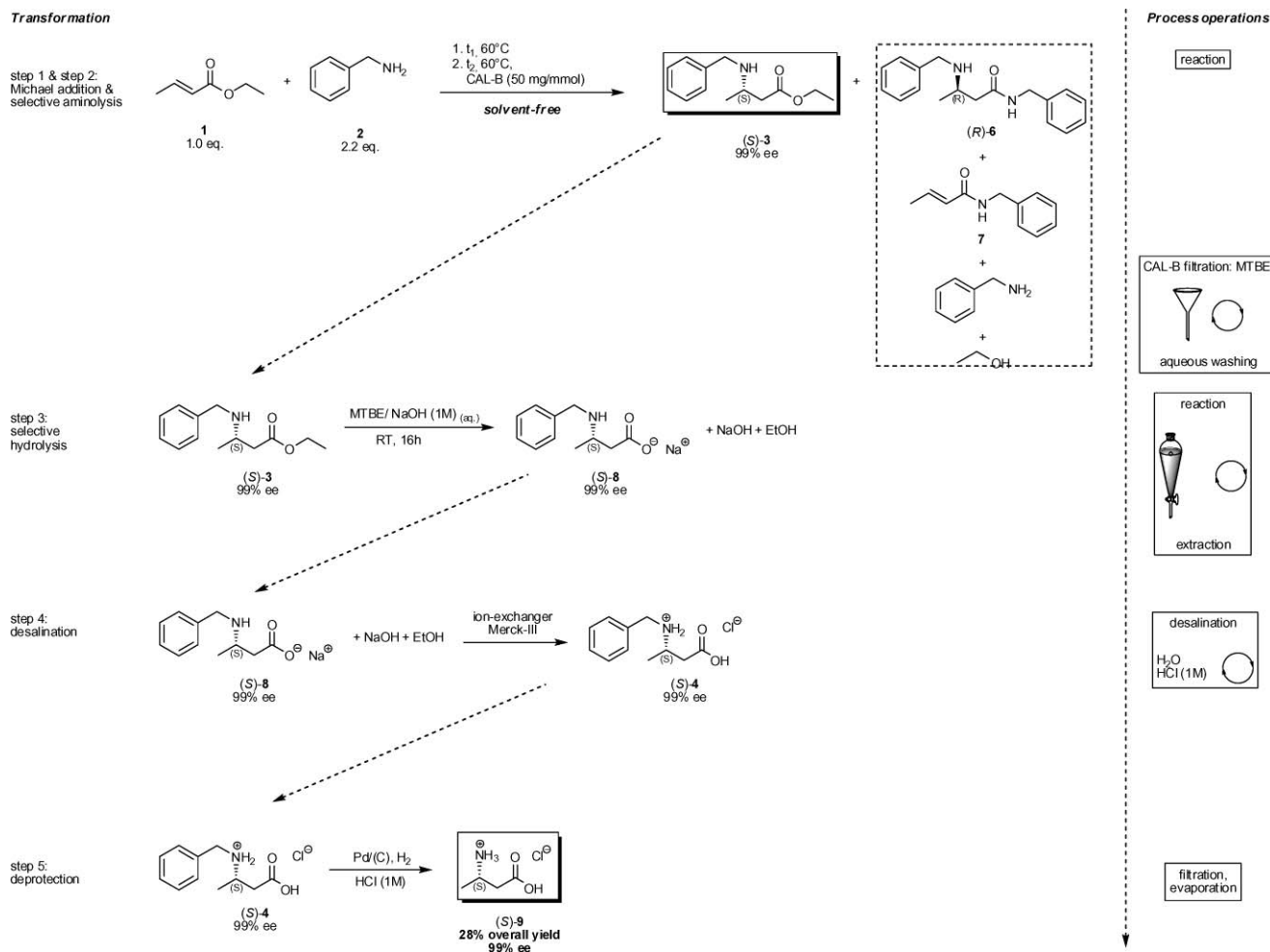
whole process. In addition, the conditions of each reaction step also provide potential for optimization. Thus, in spite of general advantages this original process results in a disadvantageous mass balance and a non-satisfactory high E-factor of 359, which makes a scale up of this process less attractive (for details of the mass balancing, see Electronic Supporting Information (ESI)<sup>†</sup> and Table 4). This high E-factor is mainly due to the use of large quantities of organic solvents, which were necessary for the chromatographic purification step.

To overcome these limitations we envisioned a process with improved sustainability, which fulfils the following criteria: (i) multiple usage (recycling) of the environmentally friendly biocatalyst, (ii) reduced number of purification steps (ideally only isolation of the final product (*S*)-**5** in this multi-step synthesis), and (iii) prevention of waste organic solvents by avoiding the use of column chromatography. If possible, organic solvents in reactions should also be substituted with water as an environmentally friendly solvent with exceptional properties (safe, non-flammable, non-toxic *etc.*).<sup>16</sup>

The concept and realization of such a new, greener process is depicted in Scheme 2 as a flow sheet, and discussed in the following. At the beginning of the process the previously developed solvent-free one-pot reaction is carried out (steps 1 and 2). After thermal aza-Michael addition of benzylamine **2** to (*E*)-ethyl but-2-enoate, **1**, a biocatalytic resolution through aminolysis is initiated after addition of *Candida antarctica* lipase B (CAL-B). This one-pot process resulted in the formation of the desired  $\beta$ -amino ester (*S*)-**3** with 99% ee besides *N*-benzyl (*R*)-3-(benzylamino)butanamide, (*R*)-**6**, (*E*)-*N*-benzyl but-2-enamide, **7**, and ethanol as further products. Subsequently, the immobilized enzyme CAL-B was simply filtered and washed with MTBE, followed by an additional washing step with saturated sodium hydrogen carbonate solution.<sup>17</sup> Since (*S*)-**3** and (*R*)-**6** (in the MTBE filtrate) are difficult to separate from each other by simple, non-chromatographical procedures, we decided to selectively hydrolyse the ester moiety of (*S*)-**3** in the presence of the  $\beta$ -amino amide (*R*)-**6** and enamide **7**. This hydrolysis of (*S*)-**3** (step 3), leading to the desired water-soluble sodium salt of (*S*)-3-(benzylamino)butanoic acid, (*S*)-**8**, had to be fully chemoselective for the ester moiety, since a partial cleavage of amide (*R*)-**6** would lead to the opposite enantiomer (*R*)-**8**, thus decreasing the optical purity of the resulting sodium (*S*)-3-(benzylamino)butanoate, (*S*)-**8**.

Thus, a screening of different reaction conditions for such a chemoselective hydrolysis was conducted to explore the optimum conditions. As a substrate, *rac*-**3** was used for this purpose (instead of (*S*)-**3** for practical reasons). Besides a chemoselective course of the reactions we focused on a minimization of the required amount of sodium hydroxide (used under mild reaction conditions) and the feasibility of conducting the hydrolysis in MTBE, since this solvent was used in the previous unit operation step (filtration of the immobilized enzyme CAL-B).

The results are summarized in Table 1. It turned out that both the concentration of the NaOH solution and the equivalents of NaOH (referring to the amount of *rac*-**3**) are crucial for the outcome of the hydrolysis. In terms of minimized inorganic waste and amount of water as well as high conversion entry 3 showed the optimal reaction conditions. Next, we studied the chemoselective course of the reaction when using a mixture



**Scheme 2** Improved, greener synthesis of (*S*)-3-aminobutanoic acid hydrochloride, (*S*)-9.

**Table 1** Screening of conditions for hydrolysis of *rac*-3

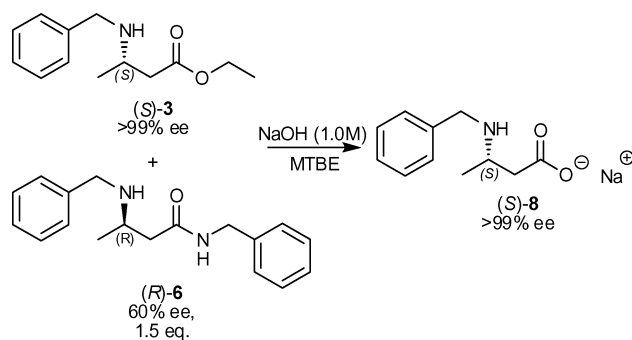
Entry <sup>a</sup>	Base	Solvent ratio <sup>b</sup>	Eq. <sup>c</sup>	pH	Cleavage <sup>d</sup>	E-Factor <sup>e</sup>
1	NaOH (1.0M)	1.9/1	5.0	14	+	3.9
2	NaOH (2.0M)	0.4/1	2.2	14	~	6.0
3	NaOH (1.0M)	0.8/1	2.0	14	+	2.0
4	NaOH (0.5M)	1.5/1	2.0	14	+	2.9
5	NaOH (1.0M)	0.4/1	1.1	14	~	1.4
6	KCl/NaOH (0.2M)	10/1	5.2	12.6	+	14.3

<sup>a</sup> Typical reaction conditions: *rac*-3 (42.0 mg, 0.19 mmol), 0.5 mL MTBE, 18 h stirring at room temperature. <sup>b</sup> Solvent ratio: ratio of the volume of aqueous solution of the base to the volume of MTBE. <sup>c</sup> Equivalents = eq. refers to the amount of *rac*-3. <sup>d</sup> Denotation of symbols: "+" complete cleavage, "~" partial cleavage, the reaction was monitored by TLC (ethylacetate:2-PrOH), 95:5 (v/v), 0.2% diethylamine. <sup>e</sup> E = waste (kg)/product (kg); the E-factor was calculated based on the assumption of complete hydrolysis of *rac*-3 and a recycling rate of the aqueous phase of 90%.

of ester (*S*)-3 (>99% ee) and amide (*R*)-6 (60% ee). We were pleased to find that stirring a mixture of isolated ester

(*S*)-3 (>99% ee) and 1.5 equivalents of amide (*R*)-6 (60% ee) as starting material under basic conditions (1 M NaOH) led to a highly chemoselective formation of the sodium salt (*S*)-8 with >99% ee (Scheme 3), thus indicating - as desired - hydrolysis of only the ester (*S*)-3 (and not amide (*R*)-4). It should also be noted that in contrast acidic hydrolysis (1.0 M HCl) of such a mixture consisting of ester (*S*)-3 (>99% ee) and amide (*R*)-6 (60% ee) led to a dramatic decrease in the enantiomeric excess of the resulting acid compared with the enantiomeric excess of the starting material (*S*)-3 (data not shown).

With such a chemoselective hydrolysis in hand, next we applied these conditions for the hydrolysis of product (*S*)-3 (99% ee), which results from step 2 of the chemoenzymatic one-pot process as a solution in MTBE (Scheme 2). After treatment of this organic layer containing (*S*)-3 with a solution of NaOH (1.0 M), the resulting hydrolytic reaction led to a highly chemoselective formation of the desired intermediate (*S*)-8 with unchanged 99% ee (Scheme 2, step 3). After completion of hydrolysis (indicated by TLC) the sodium salt of (*S*)-3-(benzylamino)-butanoate, (*S*)-8, was obtained *via* simple separation of the aqueous layer from the hydrophobic components. By means of an anion-exchanger *N*-benzylated (*S*)-β-amino acid hydrochloride (*S*)-4 was isolated from the aqueous layer from step 3 in pure form (Scheme 2, step 4). Subsequent

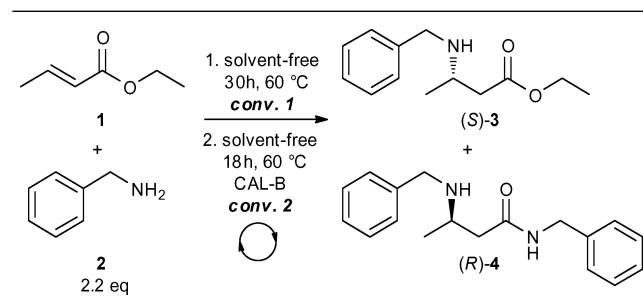


**Scheme 3** Chemoselective hydrolysis of (*S*)-3 (control experiment).

hydrogenation of (*S*)-4 over palladium in 1 M HCl then gave the desired (*S*)-3-aminobutanoic acid hydrochloride, (*S*)-9, with excellent enantiomeric excess of 99% ee (Scheme 2, step 5), and a good overall yield of 28% (after five process steps starting from inexpensive prochiral compounds and including one resolution step). Notably, all the synthetic steps can be combined in an elegant manner without the need for a complex purification unit operation for either intermediates or product. The selected downstream-processing steps are based on simple unit operation steps such as extraction and ion-exchange chromatography, thus avoiding the solvent-consuming (and waste-producing) column chromatography as “standard work up protocol” for lab procedures.

In order to further improve the overall process economy we studied the re-usage of the immobilized enzyme CAL-B for the one-pot reaction. The conditions of the recycling experiments and the results are shown in Table 2. After each one-pot reaction under formation of (*S*)-3 the immobilized CAL-B was filtered, washed with MTBE, and dried prior to re-use. The one-pot reaction starting from prochiral compounds **1** and **2** has been repeated four times with the recycled enzyme immobilisate. The results shown in Table 2 indicate that enzyme recycling is possible in principle, however at the expense of a continuous loss

**Table 2** Recycling of CAL-B in the one-pot reaction



Entry <sup>a</sup>	Reaction	Conv. Step 1 (%) <sup>b</sup>	Conv. Step 2 (%) <sup>b</sup>	ee ( <i>S</i> )-3 <sup>c</sup>
1	1	95.5	60.6	99
2	2	93.0	56.0	96
3	3	94.0	55.0	93
4	4	93.5	55.0	91
5	5	93.0	52.0	88

<sup>a</sup> Typical reaction conditions (*E*)-ethyl but-2-enoate, **1** (114.2 mg, 1.0 mmol), benzylamine, **2** (235.5 mg, 2.2 mmol), lipase CAL-B (50 mg mmol<sup>-1</sup>). <sup>b</sup> Conversions were determined by means of <sup>1</sup>H-NMR spectroscopy. <sup>c</sup> Determination of enantiomeric excess *via* chiral HPLC.

of reactivity. After 5 reaction cycles, conversion drops to 52% (Table 2, entry 5) compared with 60.6% after the same reaction time when using fresh enzyme (entry 1).

As a main reason for the decreased enzyme activity we assume the loss of CAL-B due to mechanical abrasion during the reaction (caused by the magnetic stirrer used in our experiments) and subsequent filtration. Potential process options for the future in order to circumvent this critical issue are the use of stirrers other than magnetic stirrers for such batch-type operations.

### Environmental assessment of the optimized process

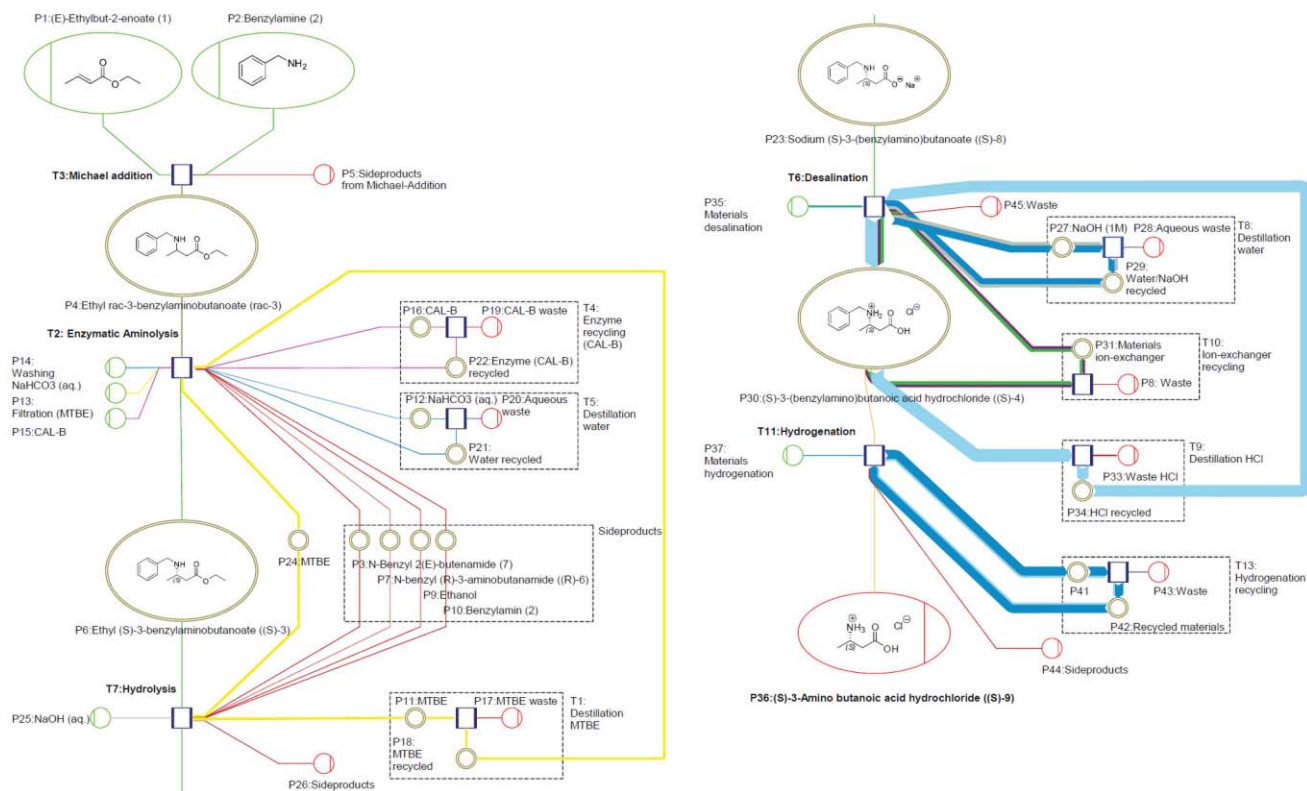
After the successful preparation of (*S*)-3-amino butanoic acid hydrochloride, (*S*)-9 (28% overall yield, 99% ee), we started the environmental assessment of the developed process with the software programmes EATOS and Umberto/Sabento. Since not all material data was provided by the material databases of both programmes, a collection of such ecological information was done first (for material data, see ESI†). For this purpose we used the free-access databases of BGIA gestis, BGIA report,<sup>18</sup> eChemportal,<sup>19</sup> ecotox data<sup>20</sup> and the material data safety sheets (MSDS) of the commercial chemicals suppliers Acros,<sup>21</sup> Sigma-Aldrich<sup>22</sup> and Merck.<sup>23</sup>

Based on the collection of the material data, the results of the chemical reactions of the overall process (shown in Scheme 2) and the process assumptions given below, a material flow network was modelled. This fully specified material flow model is shown in Scheme 4 as a Sankey diagram. In the Sankey diagram the intermediates are shown after each reaction step for clarity. In general there are three types of places “P”, which are mapped by colored circles: green input places (materials enter the material network here), yellow connection places (materials are passed from one transition to another) and red output places (materials leave the system boundary here). The blue boxes denote “transition sites”, which are sites where changes in the mass composition occur through chemical reactions, work up procedures and recycling steps. This enables an overview of the mass flow in the process, and an analysis of what happens to each material. In contrast to the reaction equations of Scheme 2 one can immediately recognize mass intensive parts of a reaction sequence, since the line width of every material arrow is proportional to the mass of each material used. The dashed lined boxes show areas of special interest, *i.e.* side-products and recycling areas.

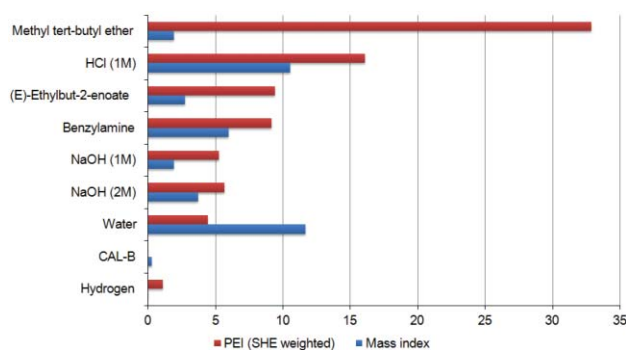
For the mass balancing the following assumptions have been used as boundary conditions: (i) 90% recycling rate for pure organic solvents,<sup>24</sup> aqueous solutions (HCl (1 M), NaOH (1 M)) and water,<sup>25</sup> (ii) 85% recycling rate for CAL-B,<sup>26</sup> (iii) full reusability for palladium catalyst and ion exchanger,<sup>27</sup> and (iv) 85% recycling rate for mixture of organic solvents.

The results of the mass balancing visualized in the Sankey diagram in Scheme 4 are discussed in the following in more detail. An overview about two types of indices for the input materials is shown in Fig. 1. Therein, the blue bar is related to the used amount (mass) of each material (in kg) theoretically required for the hypothetical preparation of one kg of final product (*S*)-9, whereas the red bar shows the fraction of the (substance-specific) potential environmental impact for the





**Scheme 4** Sankey diagram (material network) of the Umberto/Sabento modelling according to Scheme 2; mass flows are indicated as follows: light blue = HCl (1 M), dark blue = water, grey = NaOH (1 M), purple = auxiliary materials, yellow = organic solvents, pink = enzyme, green = (organic) material transfer, red = (organic) waste transfer.



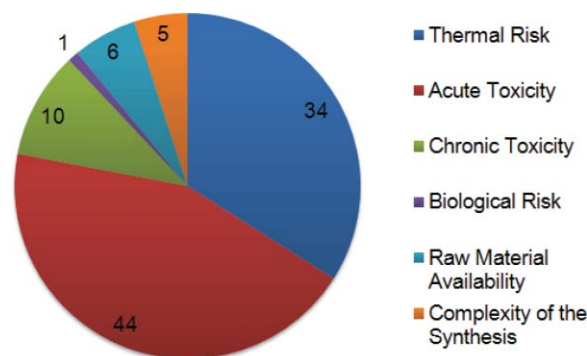
**Fig. 1** Selected mass indices (in kg) and substance-specific PEI indices (in PEI/kg) of input materials according to the Sankey diagram in Scheme 4 (in the text section the resulting percentage mass indices (in %), calculated by dividing the substance specific mass index by the total sum of mass indices, are discussed).

input side (PEI(input)) of each material after safety health environment weighing (SHE). Accordingly, water (28.2%), 1 M HCl (HCl used for work-up, 25.4%) and benzylamine, **2** (14.4%), determine the three main parts of the total input material mass flow. In addition it should be noted that only 68% of the total material demand is needed for product isolation.

Since from a sustainability perspective the impact of a material, in particular waste material, is not only related to its required mass but also to its hazard properties, it is important to examine the PEI of each material besides its amount. According to Fig. 1 methyl *tert*-butyl ether is identified to have the highest

PEI, whereas its mass proportion (4.5%) is relatively small compared to the other materials. In contrast 1 M HCl (HCl used for work up, 25.4%) represents the second largest mass proportion, together with the second largest substance-specific PEI. This underlines the importance of the reduction of organic solvents from syntheses and work up procedures. The substance-specific PEI of the substrates benzylamine, **2** (14.4% of total mass input) and (*E*)-ethyl but-2-enoate, **1** (6.9% of total mass input), was calculated to be nearly equal within this process, although the required mass of benzylamine is significantly higher.

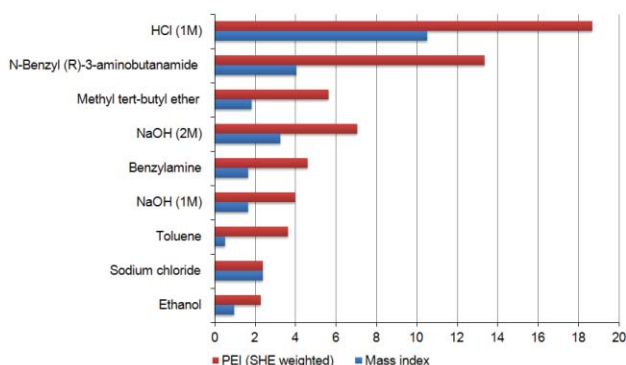
In addition, the different impact categories of the process have been studied and evaluated. The results are visualized in Fig. 2. Therein the different impact categories that arise from



**Fig. 2** Impact categories of the input materials according to the Sankey diagram in Scheme 4.

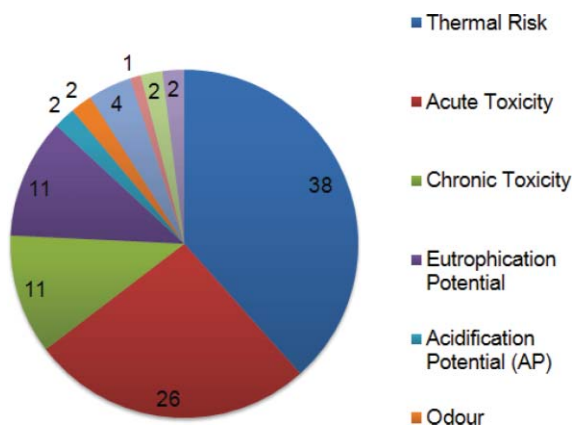
the substances used within the process (input materials) are displayed. Accordingly, the major impact potentials with respect to the input materials are assigned to thermal risks (34%) and acute toxicity (44%).

Furthermore, an environmental assessment of the output materials (which represents waste material) was carried out in analogous manner. The resulting mass and PEI indices of the output materials are displayed in Fig. 3. As can be seen in Fig. 3, a 1 M solution of HCl (25.5%), (*R*)-3-(benzylamino)-butanamide (*R*)-4 (9.8%), a 2M solution of NaOH (7.3%) and methyl *tert*-butyl ether (4.5%) represent the main proportion of the total mass output (waste). The substance-specific PEI values follow the same order. It is also noteworthy that small quantities of organic solvents like toluene (2% of total mass output) and methyl *tert*-butyl ether (4.5%) show a relatively high substance-specific PEI value compared to the aqueous mass flows (1 M HCl and 1 M NaOH), which would require much more mass to cause a similar substance-specific PEI value. In addition, 66% of the total output waste volume is of aqueous origin, whereas only 4.5% are caused by the only organic solvent MTBE.



**Fig. 3** Selected mass indices (in kg) and substance-specific PEI indices (in PEI/kg) of output materials according to the Sankey diagram in Scheme 4 (in the text section the resulting percentage mass indices (in %), calculated by dividing the substance specific mass index by the total sum of mass indices, are discussed).

The overall risk of the process with respect to the output materials (waste materials) has also been classified into the different impact categories. The results are displayed in Fig. 4.



**Fig. 4** Impact categories of the output materials according to the Sankey diagram in Scheme 4.

**Table 3** Mass balance<sup>a</sup> of the process according to the Sankey diagram in Scheme 4

Input	Quantity/kg	Output	Quantity/kg
Hydrogen	0.0708	Hydrogen	0.0569
NaOH (0.1 M)	0.2531	NaOH (0.1 M)	0.2531
NaOH (1 M)	2.0632	NaOH (1 M)	1.7341
NaOH (2 M)	3.9236	NaOH (2 M)	3.0506
MTBE	1.8734	MTBE	1.8734
CAL-B	0.1898	CAL-B	0.1898
Benzylamine, 2	5.9620	Benzylamine, 2	1.8101
( <i>E</i> )-Ethyl but-2-enoate, 1	2.8860	Coupling-/Side products	6.3807
NaHCO <sub>3</sub>	0.3645	Inorganic salts	2.8328
HCl (1 M)	12.1645	HCl (1 M)	10.5696
Water	11.7721	Water	11.7721
		( <i>S</i> )-3-Aminobutanoic acid hydrochloride, ( <i>S</i> )-9	1.0
Total	41.5232	Total	41.5232
		<b>E-factor<sup>b</sup></b>	<b>41</b>

<sup>a</sup> Mass balance calculated for the hypothetical production of 1 kg (*S*)-3-aminobutanoic acid hydrochloride, (*S*)-9. For a more detailed mass balance, see ESI†. <sup>b</sup> E-factor = (amount of total waste)[kg]/(amount of product)[kg]; the E-factor is given above without decimal places (E = 41); the calculated E-factor with decimal places is 40.5232.

Accordingly, about two thirds of the total risks of the produced waste output can be assigned to thermal risks (38%) and acute toxicity (26%). Among further risks there are also risks for eutrophication (11%) and chronic toxicity (11%).

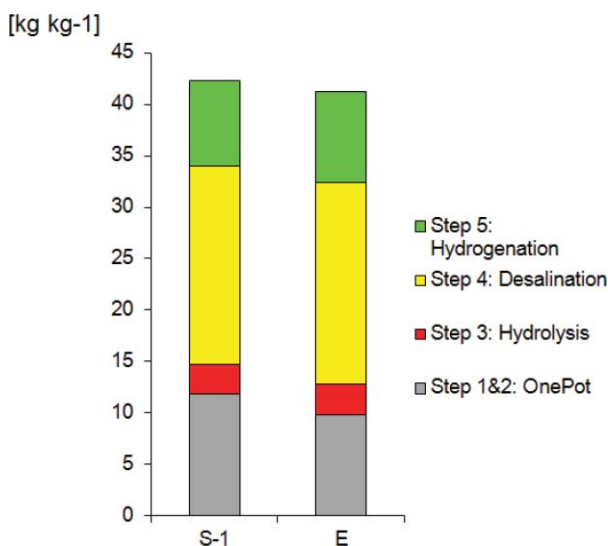
In addition to this type of EHS assessment, which considers the properties of the substances, a comparative assessment only based on mass balances can also be done. Here an important indication for the sustainability of a chemical process is the so-called E-factor which describes the amount of waste (in kg) produced per kg of product. Thus, the E-factor, which has been introduced by Sheldon,<sup>10,11</sup> reflects the performance of a preparative process considering, *e.g.*, stoichiometry of reagents, product yield, amount of catalyst and solvents. Thus, for the calculation of the E-value a detailed mass balance of the process is required. Such a mass balance of the overall process for the synthesis of (*S*)-9 (according to Scheme 4) is given in Table 3. Based on this mass balance an E-factor of 41 was calculated. For comparison, typical E-factors in the industrial synthesis of pharmaceuticals are in the range of 25–100. Thus, the achieved E-factor of 41 can be considered to be in a satisfying range, especially when taking into account the relatively low molar mass of the target molecule (*S*)-3-aminobutanoic acid hydrochloride, (*S*)-9, and the presence of a resolution within the five-step synthesis.

In multi-step syntheses a further interesting aspect is the evaluation of which steps contribute to the consumption of substances and formation of waste, and to what extent. Thus, for our process we calculated the distribution of the mass volume of the used resources (represented by selectivity  $S^{-1}$ ;  $S^{-1}$  = (amount of resources [kg])/(amount of product) [kg]) and the waste (represented by the E-factor) on the individual process steps 1 to 5 (according to Schemes 2 and 4). The results of this evaluation ( $S^{-1}$ , E-factor referring to each step) are shown in Fig. 5. The separate steps contribute to the overall amount of

**Table 4** Comparison of key data of the optimized process according to Schemes 2, 4, and the original process according to Scheme 1 and ref. 6

Entry	New optimized process (this work)	Original process <sup>d</sup> (according to ref. 6)
E-factor <sup>a</sup> (reaction)	13	45
E-factor <sup>a</sup> (incl. isolation)	41	359
Selectivity <sup>b</sup> S <sup>-1</sup> (incl. isolation)	36	366
PEI <sup>c</sup> (input)	86	6580
PEI <sup>c</sup> (output)	121	6666
PEI <sup>c</sup> (total)	107	6632

<sup>a</sup> E-factor = (amount of total waste[kg])/(amount of product[kg]).  
<sup>b</sup> Selectivity S<sup>-1</sup> = (amount of resources[kg])/(amount of product[kg]).  
<sup>c</sup> PEI = potential environmental impact = PEI/(kg product), calculated with Umberto/Sabento. <sup>d</sup> For details of the calculation for the original process according to ref. 6, see ESI†.



**Fig. 5** Distribution of the mass volume of resources (S<sup>-1</sup>) and waste (E) on the different process steps 1–5 according to the Sankey diagram in Scheme 4 (calculated with EATOS).

used substances (resources) and produced waste as follows: The chemoenzymatic one-pot synthesis of (*S*)-3 (steps 1 and 2) as a key step only demands 28.1% of used substances and only causes 23.9% of formed waste. Whereas subsequent hydrolysis (step 3) also contributes to a minor extent to the demand of resources (6.7%) and formation of waste (7.0%), desalination (step 4) plays a major role in this field with 45.6% of demand of resources and 47.5% of formed waste. The hydrogenation (step 5) as a final step contributes to 19.6% of demand of resources and 21.6% of formed waste. The improvements from the process development becomes evident when comparing the optimized process (according to Scheme 2 and 4) with the original process (shown in Scheme 1). In Table 4, the key data for both the optimized process (described in this work) and our previously published original process<sup>6</sup> are shown (for details see ESI†). Comparing the calculated E-factors (including isolation) of both processes a reduction of more than eight times (41 versus 359) could be achieved with the new optimized process.

The same tendency has been found for the resource demand (represented by S<sup>-1</sup>). The decrease of isolation process unit

operations for intermediates in the optimized process also turned out to be beneficial and contributed to a reduction of waste. This is underlined by the significantly improved E-factor (reaction) of the new, optimized process.

The advantages of the optimized process (Schemes 2, 4) in comparison with the original process (Scheme 1) in terms of sustainability is also underlined by the analysis of the potential environmental impact PEI. The PEI (total) summarizes all substance-specific PEIs (for selected data, see Fig. 1 and 3), and is therefore a key indicator for the SHE assessment of a process. The PEI (input) and PEI (output) are referring to all substance-specific PEIs from the input and output materials, respectively. In comparison to the original process,<sup>6</sup> the developed optimized process is about 62 times more benign in terms of environmental, health and safety aspects (Table 4).

## Conclusion

In conclusion, we reported a successful process development towards an efficient approach with improved sustainability to the hydrochloride of (*S*)-3-aminobutanoic acid, (*S*)-9. This compound is a representative of the pharmaceutically interesting product class of short-chain aliphatic β-amino acids and corresponding salts. The target product (*S*)-9 has been obtained within a five-step chemoenzymatic synthesis in an overall yield of 28% and with excellent enantiomeric excess of 99% ee. Notably, column chromatographical work-up steps with organic solvents were not required, and isolations and purifications are based on simple unit operations such as extraction or ion-exchange chromatography. In addition, the developed synthesis of (*S*)-9 was analysed *via* mass balancing and SHE assessment with the software programmes EATOS and Umberto/Sabento. The analysis of key environmental indicators such as, *e.g.*, S<sup>-1</sup>, E-factor, PEI, revealed a significantly improved sustainability of the new optimized process in comparison to the original process. A reduction of the E-factor by more than eight times was achieved in addition to a reduction of the PEI (total) by more than 60 times. This fact also indicates a significant diminishment of hazardous substances for the optimized process.

## Experimental

### General

<sup>1</sup>H NMR (<sup>13</sup>C NMR) spectra were recorded at room temperature on a Bruker Avance 300 or JEOL JNM GX 400 spectrometer operating at 300 MHz or 400 MHz. Chemical shifts (δ) are reported in parts per million relative to tetramethylsilane (TMS) (δ0.00). NMR raw data was analyzed with the program MestreC 4.9.9.9. HPLC spectra were recorded on a Jasco HPLC system with a PU-1587 Intelligent Prep. Pump and a LC-Net II and ADC device at room temperature. As stationary phase the following Daicel® columns were used: OD, OJ-H, AD-H, IA and IB. Mass spectra (MALDI) were recorded on a Shimadzu Biotech Axima Confidence spectrometer. Mass spectra (EI) were recorded on a Micromass Zabspec spectrometer with electron ionization. Elemental analysis were measured on a EA 1119 CHNS, CE instrument. IR spectra were recorded on a Thermo scientific Nicolet IR-100 FT-IR spectrometer. The absorption



is given in wavelength  $\tilde{\nu}$ ; [cm<sup>-1</sup>]. The optical rotation of chiral compounds was measured on a Perkin Elmer instruments polarimeter (model 341) with a Na-lamp (wavelength  $\lambda = 589$  nm). Preparative (flash) column chromatography was performed over Merck Silica gel 60 (230–400 mesh) as stationary phase. Thin layer chromatography (TLC) was performed on silica gel TLC cards (Alugramm® SIL G, layer thickness 0.20 mm, Fluka) with fluorescens indicator. Chemicals and materials were purchased from commercial suppliers Acros and Sigma-Aldrich unless otherwise stated. The used enzyme *Candida antarctica* lipase B in immobilized form is a commercial product from Novozymes A/S (product name: Novozym®435).

#### Procedure for hydrolysis reactions of *rac*-3 according to Table 1.

In a 5 mL round bottom flask *rac*-3 (0.19 mmol, 42 mg) was diluted in 0.5 mL MTBE. After addition of 1.1–5.0 eq of a base the reaction mixture was stirred for 18 h at room temperature. Reaction progress was monitored by TLC control (ethyl acetate/2-PrOH, 95 : 5 (v/v), 0.2% diethylamine).

**Procedure for the control experiment for chemoselective hydrolysis of (*S*)-3 according to Scheme 3.** In a 5 mL round bottom flask (*S*)-3 (99% ee) (0.19 mmol, 42 mg) and (*R*)-4 (60% ee) (0.28 mmol, 80 mg) was diluted in 0.5 mL MTBE. After addition of 0.76 mL NaOH (2 M) the reaction mixture was stirred for 18 h. The completion of the reaction was indicated by TLC control (ethyl acetate/2-PrOH, 95 : 5 (v/v), 0.2% diethylamine). The aqueous phase was extracted four times with 1 mL ethyl acetate. The remaining water was removed at 60 °C under reduced pressure. After cooling to room temperature thionyl chloride (0.38 mmol, 45 mg) was added. The reaction mixture was cooled to –10 °C (ice/NaCl-bath) and ethanol was added dropwise. After stirring for 30 min at –10 °C, the reaction mixture was stirred for 1 h at room temperature. After heating to 60 °C the reaction mixture was stirred again for 1 h, diluted in 5 mL NaHCO<sub>3</sub> and extracted four times with 2 mL dichloromethane. After drying over MgSO<sub>4</sub> the solvent was evaporated under reduced pressure to yield ethyl (*S*)-(3-benzylamino) butanoate, (*S*)-3, as a yellowish oil. Yield: 18 mg (43%). Enantiomeric excess: >99% ee. Retention time (HPLC): 11.80 min ((*S*)-3), 13.38 min ((*R*)-3) (Daicel column IB, hexanes:2-PrOH, 99 : 1 (v/v), 0.1% diethylamine, flow 1.0 ml min<sup>-1</sup>, 254 nm).

**Procedure for recycling experiments with CAL-B in the synthesis of (*S*)-3 according to Table 2.** In a 5 mL round bottom flask with glass stopper (*E*)-ethyl but-2-enoate (**1**, 2 mmol, 228 mg), and benzylamine (**2**, 4.4 mmol, 480  $\mu$ l) was heated at 60 °C and stirred for 30 h. After completion of the thermal Michael addition the conversion (step 1) was determined by means of <sup>1</sup>H-NMR spectroscopy (5  $\mu$ l sample). Then, lipase from *Candida antarctica* B (CAL-B) was added (50 mg mmol<sup>-1</sup>, 100 mg) and the reaction was stirred for another 18 h at 60 °C. The conversion (step 2) was determined by means of <sup>1</sup>H-NMR spectroscopy, the enantiomeric excess was determined by chiral HPLC (IB column, hexanes:2-PrOH, 99 : 1 (v/v), 0.1% diethylamine) from a 5  $\mu$ l sample of the reaction mixture. The enzyme was washed eight times with 1 mL MTBE. After evaporation of the solvent, the enzyme was re-used again.

#### Preparation of (*S*)-3-(benzylamino)butanoic acid hydrochloride, (*S*)-4, and (*S*)-3-aminobutanoic acid hydrochloride, (*S*)-9, according to Schemes 2 and 4 (optimized process)

**Steps 1 and 2.** In a 10 mL round bottom flask with glass stopper (*E*)-ethyl but-2-enoate (**1**, 20 mmol, 2.28 g) and benzylamine (**2**, 44 mmol, 4.81 mL) was heated at 60 °C and stirred for 30 h. After completion of the thermal Michael addition the conversion (step 1) was determined (step 1: 93.0% total conversion, 91.0% product related conversion) by means of <sup>1</sup>H-NMR spectroscopy (5  $\mu$ l sample). Then, lipase *Candida antarctica* B (CAL-B) was added (50 mg mmol<sup>-1</sup>, 1.0 g) and the reaction was stirred for another 16 h at 60 °C. The conversion (step 2) was determined (step 2: 61.2% conversion) by means of <sup>1</sup>H-NMR spectroscopy (5  $\mu$ l sample). The enantiomeric excess of ethyl (*S*)-3-(benzylamino) butanoate, (*S*)-3, was determined by chiral HPLC chromatography. Enantiomeric excess: 99% ee. Retention time (HPLC): 11.80 min ((*S*)-3), 13.38 min ((*R*)-3) (Daicel column IB, hexanes:2-PrOH, 99 : 1 (v/v), 0.1% diethylamine, flow 1.0 ml min<sup>-1</sup>, 254 nm). The reaction mixture was separated from the immobilized enzyme by filtration, and subsequently washed with methyl *tert*-butyl ether (MTBE, 20 mL). The resulting organic solution was washed three times with a solution of aqueous saturated NaHCO<sub>3</sub> (1 mL). The organic layer containing the product (*S*)-3 was used directly for the next step. The assignment of the absolute configuration was done by comparison with data in ref. 6.

**Step 3.** An aqueous solution of NaOH (1 M, 14.4 mL) was added to the organic layer resulting from step 2 in a 10 mL round bottom flask. The resulting reaction mixture was stirred at room temperature for 16 h. The reaction progress was monitored according to the consumption of (*S*)-3 by TLC (ethyl acetate:2-PrOH, 95 : 5 (v/v), 0.1% diethylamine, *R<sub>f</sub>* 0.60). After completion of the reaction, the aqueous layer was separated in a separatory funnel. Finally the organic phase was extracted two times with an aqueous solution of NaOH (0.1 M, 1 mL).

**Step 4, (*S*)-3-(benzylamino)butanoic acid hydrochloride (*S*)-4.** To the aqueous layer resulting from step 3 was added ion exchanger Merck-III (14.4 g). The resulting mixture was stirred for one hour. After washing with water (40 mL) the (*S*)-3-(benzylamino)butanoic acid hydrochloride, (*S*)-4, was eluted with an aqueous solution of HCl (1 M, 70 mL). Pure (*S*)-3-(benzylamino)butanoic acid hydrochloride, (*S*)-4, was yielded as a white solid after evaporation of the aqueous hydrochloric acid under reduced pressure at 60 °C. Yield: 1.30 g (29%, related to the amount of (*E*)-ethyl but-2-enoate, **1**). The enantiomeric excess of (*S*)-4 was determined by chiral HPLC chromatography of its ethyl ester, (*S*)-3 (after derivatization of (*S*)-4 with thionylchloride according to the procedure for the control experiment for chemoselective hydrolysis of (*s*)-3 according to Scheme 3). Enantiomeric excess: 99% ee. Optical rotation [ $\alpha$ ]<sub>D</sub><sup>25</sup> +15.7 (*c* 1.0 in H<sub>2</sub>O). Elemental analysis: Found: C, 57.09; H, 7.03; N, 6.13%. C<sub>11</sub>H<sub>16</sub>ClNO<sub>2</sub> requires C, 57.52; H, 7.02; N, 6.10%. IR  $\nu_{\max}$ /cm<sup>-1</sup>: 3061 (CH<sub>arom.</sub>), 2911 (CH<sub>aliph.</sub>), 2735 (CH<sub>aliph.</sub>), 1723 (C=O), 1456 (C=C), 1173 (C–C). <sup>1</sup>H-NMR (400 MHz, D<sub>2</sub>O): 1.42 (3H, d, *J*<sub>H,H</sub> = 6.7 Hz, Me), 2.78 (1H, dd, *J* = 6.6 Hz, *J* = 17.4 Hz, CHCH<sub>2</sub>CO), 2.85 (1H, dd, *J* = 17.4 Hz, *J* = 6.2 Hz, CHCH<sub>2</sub>CO), 3.70 (1H, m, 1H, CH), 4.24,



4.31 (2H, 2d,  $J_{\text{H,H}} = 13.2$  Hz,  $\text{CH}_2$ ), 7.47–7.50 (5H, m, CH).  $^{13}\text{C}$ -NMR (100 MHz,  $\text{D}_2\text{O}$ ): 16.08 ( $\text{CH}_3$ ), 36.87 ( $\text{CHCH}_2\text{COOH}$ ), 48.89 ( $\text{CHCH}_2\text{COOH}$ ), 50.78 ( $\text{NH}_2\text{-CH}_2\text{-C6}$ ), 129.62 ( $\text{C}=\text{C}$ ), 129.98 ( $\text{C}=\text{C}$ ), 130.02 ( $\text{C}=\text{C}$ ), 130.89 ( $\text{C}=\text{C}$ ), 171.60 ( $-\text{COOH}$ ). MS (MALDI):  $m/z = 194$  ( $\text{M}^+$ ).

**Step 5, (S)-3-aminobutanoic acid hydrochloride, (S)-9.** The product resulting from step 4, (S)-4 (1.5 mmol, 290 mg), was dissolved in 3 mL HCl (1M) in a Fischer–Porter bottle, followed by the addition of Pd(C) (–10%, 87 mg). The bottle is evacuated and flushed with inert gas for three times. After final evacuation the bottle was filled with (65 psi corresponding to 0.44 MPa) of hydrogen. The reaction mixture was heated to 65–70 °C and stirred for 22 h. Then, the reaction mixture was separated from the Pd(C) catalyst *via* filtration and eluted with water (15 mL). The pure product (S)-3-aminobutanoic acid hydrochloride, (S)-9, was yielded as a white solid after evaporation of the solvent at 60 °C under reduced pressure. Yield: 209 mg (99% related to the amount of (S)-3-(benzylamino)butanoic acid hydrochloride, (S)-4, 28% related to the amount of (E)-ethyl but-2-enoate, 1,  $^1\text{H}$ -NMR (400 MHz,  $\text{D}_2\text{O}$ ): 1.34 (d,  $^3J = 6.7$  Hz, 3H, Me), 2.73 (dd,  $J = 7.4$  Hz,  $J = 17.5$  Hz, 1H,  $\text{CHCH}_2\text{CO}$ ), 2.78 (dd,  $J = 5.7$  Hz,  $J = 17.5$  Hz, 1H,  $\text{CHCH}_2\text{CO}$ ), 3.71–3.78 (m, 1H,  $\text{CH}_3\text{CHCH}_2$ ).  $^{13}\text{C}$ -NMR (100 MHz,  $\text{CDCl}_3$ ): 17.91 (C-4), 37.95 (C-3), 44.61 (C-2), 174.38 (C-1). The spectroscopic data are in accordance with those reported in the literature.<sup>28</sup> Enantiomeric excess: 99% ee (determined by optical rotation of the free (S)-3-amino butanoic acid and comparison with literature data).<sup>29</sup>

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