Practical, Highly Enantioselective Chemoenzymatic One-Pot Synthesis of Short-Chain Aliphatic β-Amino Acid Esters

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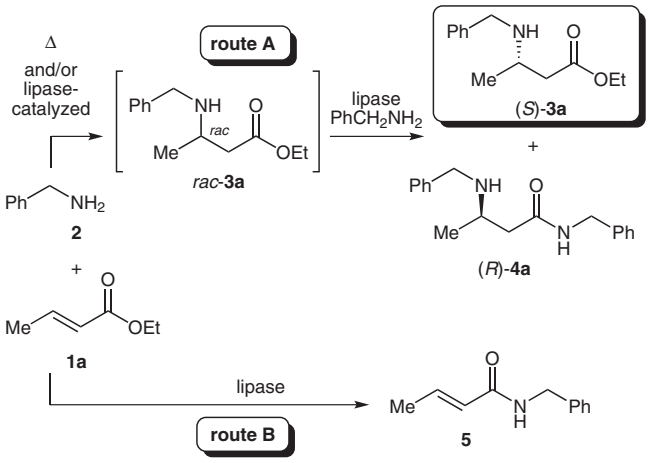
Abstract: A practical, highly enantioselective method for the synthesis of short-chain aliphatic β-amino acid esters was developed starting from prochiral and easily accessible substrates. This chemoenzymatic approach is based on a nonenzymatic aza-Michael addition of benzylamine to enoates and subsequent lipase-catalyzed resolution via enantioselective aminolysis. The two reactions are carried out as a one-pot synthesis under solvent free-conditions affording the β-amino esters in satisfying to good yields and with excellent enantioselectivities of up to 99% ee.

Key words: aminolysis, amino acids, asymmetric catalysis, enzyme catalysis, stereoselective synthesis

Enantiomerically pure β-amino acids are attractive key building blocks for the synthesis of pharmaceuticals.[1] The tendency to use β-amino acids in drug synthesis has increased over recent years as well as the search for new synthetic methods for their enantioselective preparation.[2] For the synthesis of chiral aryl-substituted β-amino acids numerous highly enantioselective approaches have been developed, which are based on the use of chemocatalysts[3] or enzymes.[4] In part, these methods are applied on technical scale. In contrast there is a limited number of scalable and efficient approaches to aliphatic, particularly short-chain β-amino acids and their esters.[5] In general, overall process efficiency and practicability of syntheses for these target molecules is limited by numerous workup steps in multistep syntheses. Furthermore, often enantiomeric excess of the products is below 99% or even 95%, which make additional recrystallization step(s) necessary to obtain the (short-chain) aliphatic β-amino acids in enantiomerically pure form (>99% ee). Thus, among key challenges for a practical route for these types of β-amino acids are (i) minimization of solvent use and isolation steps in multistep approaches and (ii) development of syntheses from readily available substrates leading to products with 95% ee, in particular 99% ee, based on the use of environmental friendly and highly enantioselective catalysts.

In the following we report our preliminary results on a solvent-free, two-step, one-pot synthesis of enantiomerically pure aliphatic β-amino esters through combination of aza-Michael addition and enzymatic aminolysis.[6] As target process we envisioned a one-pot approach for enantiomerically pure β-amino acid esters **3** using only prochiral enoate and benzylamine as raw materials, a lipase as a (bio-)catalyst and no solvent (Scheme 1, route A). The reaction concept is based on an initial Michael addition of benzylamine (**2**) to ethyl crotonate (**1a**) under formation of *rac*-**3a**, followed by subsequent enantioselective enzymatic aminolysis[7] with benzylamine as amine component under formation of (*R*)-**4a** and the (remaining) ester (*S*)-**3a**. The Michael addition might occur noncatalyzed or lipase-catalyzed (which has recently been reported to be non-enantioselective for similar Michael additions).[8] The choice of benzylamine as donor has the advantage that the resulting *N*-benzyl moiety can be easily cleaved from the β-amino acid derivative at a later stage. As an (undesired) side reaction, however, one could expect the direct lipase-catalyzed aminolysis of substrate **1a** under formation of enamide **5** (Scheme 1, route B).



Scheme 1 Proposed reaction course

In our initial enzyme screening we could identify lipase from *Candida antarctica* B (CAL-B) as a biocatalyst and benzylamine as a suitable amine donor, which led to the formation of the desired aza-Michael adduct (*S*)-**3a** in enantiomerically enriched form (Scheme 2).

When carrying out the reaction under neat conditions at 60 °C, the desired β-amino ester (*S*)-**3a** is obtained in 38% yield and with 88% ee.[9] As expected, formation of amide (*R*)-**4a** (48% yield) as well as crotonamide **5** (10% yield) was also observed. The mechanistic picture of this process can be proposed to proceed according to the concept shown in Scheme 1. In accordance with route A, initial aza-Michael addition occurs both noncatalyzed and lipase-catalyzed. Comparison experiments with and without enzyme supported the significant contribution of

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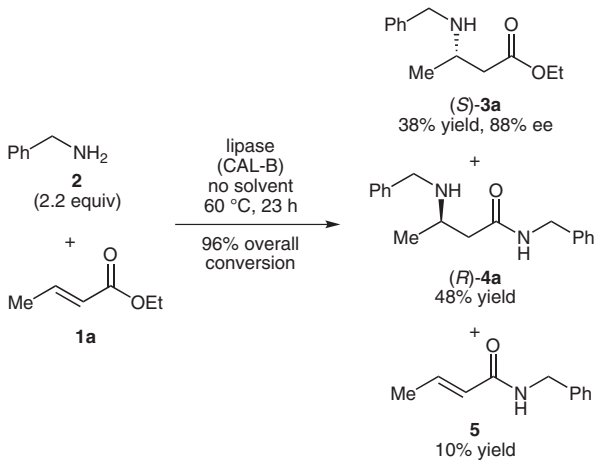


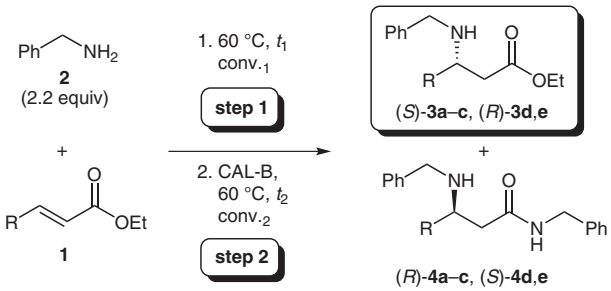
Scheme 2 Initial screening result[9]

lipase-catalyzed Michael addition (e.g., conversion of 31% with enzyme compared to 17% without enzyme after 6 h at r.t.). Taken into consideration yields and enantiomeric excesses of both (*S*)-**3a** (59% yield, 25% ee) and (*R*)-**4a** (18% yield, 77% ee) in a further experiment, it becomes evident that enzymatic Michael addition (and, thus, initial Michael addition in general) proceed without or with negligible enantioselectivity being in the range of the deviation of the analytical methods chosen.[10] Importantly, however, subsequent enzymatic aminolysis of Michael adduct *rac*-**3a** with **2** as amine component proceeds with high enantioselectivity forming the product (*S*)-**3a** with 38% yield and 88% ee (Scheme 2). Notably, highest efficiency of the one-pot synthesis was found under solvent-free conditions. This was underlined by a study of the impact of solvents. The presence of an organic solvent led to less satisfactory results independent of the choice of solvent, in particular with respect to activity (data not shown). As solvents *n*-hexane, toluene, methyl *tert*-butyl ether, and (deuterated) chloroform were used. Besides the desired enzymatic aminolysis resolution, however, the lipase also catalyzes the formation of amide **5** as the expected undesired side product according to Scheme 1, route B.

In order to make this two-step, one-pot process more attractive for preparative use, avoidance of the undesired side product **5** is a prerequisite. Since formation of **5** only occurs via lipase-catalyzed aminolysis of **1a**, the reaction protocol has been modified as follows: In a first step, Michael addition of 2.2 equivalents of **2** to **1a** is carried out in the absence of the enzyme and solvent. After (ideally complete) consumption of **1a** under formation of *rac*-**3a**, the lipase is added catalyzing the aminolysis reaction enantioselectively with the remaining 1.2 equivalents of **2**. Under solvent-free reaction conditions, this one-pot process with enzymatic aminolysis resolution as a key step proceeds with high conversion and delivers ethyl 3-aminobutyrate (*S*)-**3a** in 36% yield and with an excellent enantiomeric excess of 99% (Table 1, entry 1). For the first step a conversion of 95.0% was observed with a high selectivity for *rac*-**3** (93.5%) over *rac*-**4a** (1.5%). The resolution (second step) was stopped at a conversion of 59.8%.

The substrate spectrum turned out to be interesting in particular with respect to the synthesis of aliphatic short-chain β-amino acid esters. When using the homologue enoate **1b** as a Michael acceptor, the two-step, one-pot process under neat conditions furnished the β-amino acid ester (*S*)-**3b** in 30% yield and also with an excellent enantiomeric excess of 98% (Table 1, entry 3). The conversion was 97.5% for the initial aza-Michael addition and 63.1% for the subsequent aminolysis resolution. Extending the chain length of the enoate led to a decreased enantioselectivity, for example, a conversion of 63.7% for the resolution step in the one-pot synthesis was found for the synthesis of 3-aminohexanoate derivative (*S*)-**3c**, which was formed with an enantiomeric excess of 93% (entry 4).

Table 1 One-Pot Synthesis of β-Amino Esters (*S*)-**3**



Entry[a]	R	t_1 (h)	Conv.$_1$ (%)	t_2 (h)	Conv.$_2$ (%)	Product **3**[e,f]	Yield (%)	ee (%)
1	Me	30	95.0[c]	18	59.8	(*S*)-**3a**	36	99
2[b]	Me	30	91.0[c]	18	60.3	(*S*)-**3a**	33	99
3	Et	30	97.5[c]	24	63.1	(*S*)-**3b**	30	98
4	*n*-Pr	48	99.0[c]	48	63.7	(*S*)-**3c**	19	93
5	Ph	96	30.0	23	3.8[d]	(*R*)-**3d**	n.d.[g]	n.d.[g]
6	CF_3	3	99.0[c]	24	60.4	(*R*)-**3e**	36	95

[a] The experimental procedure and analytic data of products (*S*)-**3** are described in the section References and Notes.[11]

[b] This reaction was carried out on a 20 mmol scale.

[c] Selectivities related to conversions: entry 1: 93.5% *rac*-**3a**, 1.5% *rac*-**4a**; entry 2: 89.4% *rac*-**3a**, 1.6% *rac*-**4a**; entry 3: 85.0% *rac*-**3b**, 12.5% *rac*-**4b**; entry 4: 76.0% *rac*-**3c**, 23.0% *rac*-**4c**; entry 6: 99.0% *rac*-**3e**, 0.0% *rac*-**4e**.

[d] Entry 4: Due to low conversion in step 1, the second step was studied using isolated *rac*-**3d** as a substrate.

[e] The corresponding amides (*R*)-**4** were also isolated; yields and ee were as follows: entry 1: (*R*)-**4a**, 55% yield, 60% ee; entry 2: (*R*)-**4a**, 52% yield, 61% ee; entry 3: (*R*)-**4b**, 46% yield, 57% ee; entry 4: (*R*)-**4c**, 65% yield, 36% ee; entry 5: (*R*)-**4d**, yield and ee not determined; entry 6: (*R*)-**4e**, 57% yield, 64% ee.

[f] The different assignment of the absolute configuration in case of **3d** and **3e** [both (*R*)-configuration] compared with the *S*-configuration in the case of **3a–c** is caused by different priorization of the substituents according to the rules of the Cahn–Ingold–Prelog nomenclature.

[g] Not determined.

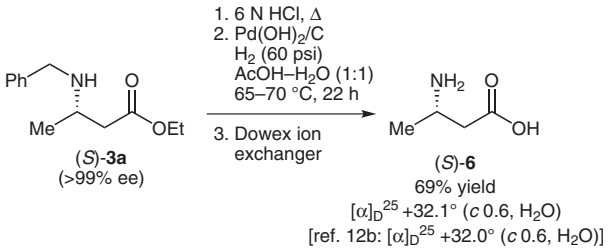
In contrast to the highly enantioselective preparation of short-chain aliphatic β-amino acid esters, this synthetic methodology is not suitable for the preparation of aromatic β-amino acid esters. A very low conversion was observed for the resolution of racemic ethyl 3-benzylamino-3-phenylpropanoate (*rac*-**3d**) with <5% (entry 5). However, fluorosubstituted aliphatic short-chain β-amino acid esters can be also prepared very well using the developed one-pot, two-step synthesis. When starting from 4,4,4-trifluorocrotonate (**1e**), aza-Michael addition and subsequent enzymatic aminolysis gave the fluorosubstituted β-amino acid ester (*R*)-**3e** in 36% yield and with high enantiomeric excess of 95% (entry 6). The conversion was 99.0% for the aza-Michael addition and 60.4% for the subsequent aminolysis resolution step.

In spite of the high enantiomeric excess for the esters **3** in the one-pot process, the corresponding amides **4** were obtained in much less satisfactory enantiomeric excesses in the range of 36–64% ee (although good yields were obtained; see Table 1). In addition, E values calculated for the formation of amides **4** are lower than corresponding E values calculated from the synthesis of the esters **3**. The reason is the formation of racemic amide *rac*-**4** as a byproduct in the initial step of formation of the racemic ester *rac*-**3** (for amount of formed *rac*-**4**, see Table 1), and potentially also during the second reaction step (resolution). The resulting (*R*)-**4a**–**c** and (*S*)-**4d**, **e** from the enzymatic resolution is then isolated jointly with the formed racemic amide *rac*-**4**. Thus, for the isolated products **4** a lower enantiomeric excess is obtained as calculated from the enantioselectivity (E value) of the enzymatic resolution alone.[13]

The efficiency of the enantioselective one-pot process for aliphatic β-amino acid esters has been also demonstrated on a larger lab scale (Table 1, entry 2). On a 20 mmol scale the two-step, one-pot synthesis of (*S*)-**3a** proceeds with nearly unchanged performance, leading to the desired β-amino acid in 33% yield and with excellent enantiomeric excess of 99%.

The prepared (*S*)-amino esters (*S*)-**3** can be easily converted into the corresponding 'free' β-amino acids. This has been successfully demonstrated for the synthesis of enantiomerically pure β-amino butyric acid, (*S*)-**6**, starting from enantiomerically pure (*S*)-**3a** (Scheme 3). After hydrolysis in acidic media and cleavage of the benzyl moiety via Pd-catalyzed hydrogenation subsequent purification with an ion exchanger furnished the desired (*S*)-β-amino butyric acid (*S*)-**6** in 69% yield and with excellent enantiomeric excess (according to the comparison of the measured optical rotation with the literature known value given in the literature[12b,14]).

In conclusion, a practical chemoenzymatic method for the highly enantioselective synthesis of short-chain aliphatic β-amino esters has been developed.[15] Starting from prochiral and easily accessible enoates this approach is based on a nonenzymatic aza-Michael addition to enoates and subsequent lipase-catalyzed resolution via an enantioselective aminolysis. The two reactions are carried out as a one-pot synthesis under solvent free-conditions affording the β-amino esters in satisfying to good yields and with excellent enantioselectivities of up to 99%.



Scheme 3 Synthesis of (*S*)-β-amino butyric acid

Acknowledgment

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(5) For selected efficient enzymatic routes, see: (a) Lipase-catalyzed acylation: Stürmer, R.; Ditrich, K.; Siegel, W. US 6063615, **2000**. (b) PenG acylase-catalyzed hydrolysis, see ref. 4b.

(6) The term 'solvent-free synthesis' refers to the composition of the reaction mixture (excluding workup), thus enabling a

high space-time yield and free choice of solvent at the downstream-processing stage.

(7) For enzymatic aminolysis reactions in general, see: (a) Puertas, S.; Brieva, R.; Rebolledo, F.; Gotor, V. *Tetrahedron* **1993**, *49*, 4007. (b) Sigmund, A. E.; McNulty, K. C.; Nguyen, D.; Silverman, C. E.; Ma, P.; Pesti, J. A.; DiCosimo, R. *Can. J. Chem.* **2002**, *80*, 608. (c) López-Garcia, M.; Alfonso, I.; Gotor, V. *J. Org. Chem.* **2003**, *68*, 648. (d) Review: Gotor, V. *J. Biotechnol.* **2002**, *96*, 35.

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(9) The yields given in Scheme 2 are 'crude yields' since the products have not been isolated.

(10) This result is in accordance with previous reports by Berglund et al. showing a nonenenatioselective course of another type of CAL-B-catalyzed Michael addition, see ref. 8.

(11) **General Procedure for the One-Pot Synthesis of 3 (According to Table 1, Entries 1, 3–6)**
In a 5 mL round-bottom flask a mixture of enoate **1** (1.0 mmol) and benzylamine (**2**, 241.5 μL, 2.2 mmol) was stirred for 3–96 h at 60 °C. After adding 50 mg (entries 1, 3, 5, and 6) or 60 mg (entry 4) of a lipase from *Candida antarctica B* (lipase CAL-B, Novozym 435), the reaction mixture was stirred for further 18–48 h. The crude product was dissolved in MTBE. After filtration from the solid enzyme, the organic phase was concentrated to dryness under vacuo. The resulting oily product was purified by means of column chromatography [entries 1–4: EtOAc–2-PrOH (95:5, v/v), 0.2% Et_2NH; R_f = **3a** (0.62), **4a** (0.22), **3b** (0.66), **4b** (0.22), **3c** (0.50), **4c** (0.16); entry 6: cyclohexane–EtOAc (3:1, v/v); R_f = **3e** (0.60), **4e** (0.13)]. The ee was determined by chiral HPLC chromatography (compounds **3a**,**b**,**e**, and **4c**: Chiracel OJ-H column; compounds **4a**,**e**: Chiracel AD-H column) with hexane–2-PrOH–diethylamine in a ratio of 95:5:0.1 or 99:1:0.1 as eluent or NMR spectroscopy with $Eu(hfc)_3$ (compounds **3c**, **4b**). The absolute configuration of **3a** was assigned according to the direction of optical rotation of the product **6** after derivatization (see Scheme 3) and its comparison with the literature value given in ref. 12. The absolute configuration of the other esters **3b**,**c**,**e** has been assigned in analogy to the result in case of **3a**. The spectral data of the ester products (*S*)-**3a–c**, (*R*)-**3e** were in accordance with literature data. The amides **4a–c**,**e** have been fully characterized (data will be published elsewhere).

(12) (a) Nejman, M.; Sliwinska, A.; Zwierzak, A. *Tetrahedron* **2005**, *61*, 8536. (b) Davies, S. G. *Tetrahedron: Asymmetry* **2007**, *18*, 1554.

(13) For the enzymatic resolution of *rac*-**3a** with benzylamine as amine and lipase CAL-B as biocatalyst under solvent free conditions at 60 °C, an E value of 27 has been obtained for this aminolysis reaction (data not shown).

(14) **Procedure for the Synthesis of (*S*)-β-Amino Butyric Acid, (*S*)-6 (According to Scheme 3)**
In a 10 mL round-bottom flask 4.32 mmol of (*S*)-ethyl 3-(benzylamino)butanoate [(*S*)-**3a**, 955 mg, >99% ee] was dissolved in 25 mL of 6 N HCl and heated to reflux overnight for 18 h. After completion of the reaction remaining solvent was evaporated under reduced pressure at 60 °C. The resulting crude product was dissolved in 15 mL AcOH–H_2O (1:1, v/v) and transferred to a 'Fischer–Porter bottle'. After addition of $Pd(OH)_2$/C (450 mg), the bottle was evacuated and flushed with inert gas for three times. After evacuation of the inert gas, the bottle was filled with hydrogen (60 psi corresponding to 0.41 MPa) and heated to 65–70 °C for 22 h. The reaction mixture was filtered through a thin plug of SiO_2. The resulting hydrochloride of (*S*)-**6** was obtained as a white solid after evaporation of the solvent at reduced pressure at 60 °C. The desired product (*S*)-amino butanoic acid, (*S*)-**6**, was obtained in isolated form after Dowex® ion-exchange chromatography. The spectral data of (*S*)-**6** were in accordance with literature data. Optical rotation data: $[\alpha]_D^{25}$ +32.1 (*c* 0.6, H_2O); for comparison, see ref. 12b: $[\alpha]_D^{25}$ +32.0 (*c* 0.6, H_2O).

(15) During completion of our manuscript we became aware of the following publication, describing processes related to our synthesis shown in Scheme 2: Priego, J.; Ortiz-Nava, C.; Carillo-Morales, M.; Lopez-Munguia, A.; Escalante, J.; Castillo, E. *Tetrahedron* **2009**, *65*, 536.