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New Enzymatic Two-Step Cascade Reaction for the Preparation of a Key Intermediate for the Taxol Side-Chain

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Enzymatic strategies are reported for the synthesis of (2*R*,3*S*)-3-amino-2-hydroxy-3-phenylpropionic acid (*ee* > 98 %), a key intermediate of the side-chain of Taxol®, by enzymatic hydrolysis in organic media. The new enzymatic cascade reaction, which took place through *Candida antarctica* lipase B-catalysed deacylation followed by lactam ring-opening of racemic *cis*-3-acetoxy-4-phenylazetidin-2-one with H_2O in iPr_2O at 60 °C, resulted in two different enantiopure products (*ee* ≥ 98 %), one of them being the desired key intermediate for the side-chain of Taxol®.

Introduction

Chiral β-amino acids can exhibit biological activity (e.g., cispentacin[1] and Icofungipen[2]), but they can also serve as building blocks for the synthesis of β-peptides[3] that have increased activity and stability, similar to those of natural peptides, and peptides with well-defined three-dimensional structures.[4] β-Amino acids are likewise used in heterocyclic[5] and combinatorial[6] chemistry and in drug research.[7] In recent years, acyclic β-amino acids have attracted much attention especially following their recognition as an important class of compounds in the design and synthesis of potential pharmaceutical drugs such as Taxol®. (paclitaxel) and its analogue Taxotère® (docetaxel), currently considered to be among the most important drugs in cancer chemotherapy.[8]

Initially, the only source of Taxol was the bark of the pacific yew tree, *Taxus brevifolia*, but its Taxol content was found to be relatively low. In efforts to overcome this supply problem, chemists have been working on semi-syntheses. These methods involve synthetic side-chain coupling to C13–O of the more readily available baccatin III derivatives, which can be isolated in higher yield from the needles of various Taxus species (e.g., *Taxus baccata*). Because it is now evident that a 3-phenylisoserine-derived side-chain is essential for the antitumour activity of Taxol, there is a need to develop efficient processes for the preparation of (2*R*,3*S*)-3-amino-2-hydroxy-3-phenylpropionic acid [(2*R*,3*S*)-**4**] or its direct enantiopure sources such as (3*R*,4*S*)-3-acetoxy-4-phenylazetidin-2-one [(3*R*,4*S*)-**2**]. Barua and co-workers have given a comprehensive overview of the approaches to the synthesis of the C13 side-chain of Taxol.[9] Although methods (e.g., asymmetric epoxidation routes, routes involving asymmetric dihydroxylation, strategies using a chiral auxiliary, inverse electron demand Diels–Alder reaction, enol–imine condensation, strategies using asymmetric catalysts) have been developed for the synthesis of the Taxol side-chain, most approaches are based on enzymatic routes (microbial reduction, asymmetric acylation, transesterification and hydrolysis). As an example, Taneja and co-workers recently reported the enzymatic synthesis of several 4-substituted (phenyl, 2-furyl or 2-thienyl) *N*-protected (4-methoxyphenyl)azetidin-2-one derivatives (*ee* ≥ 99 %) by lipase (*Arthrobacter* sp.)-catalysed enantioselective cleavage of the ester at C3 ($E > 200$) in phosphate buffer (pH 7.0).[10] As another example, Sih and co-workers described the preparation of optically active 3-hydroxy-4-phenyl-β-lactam derivatives by lipase-catalysed asymmetric acylation reactions (vinyl acetate, $E > 100$) of the secondary OH group and hydrolysis (H_2O, $E > 100$) of the ester function at C3 in aqueous medium.[11] In addition to OAc hydrolysis, they observed the ring-opening of the *N*-protected [C(O)Ph] β-lactam as a competitive reaction when lipase P-30 was used as catalyst with MeOH as the nucleophile and the reaction was performed in *t*BuOMe. The authors mentioned that two penicillinases, from *Escherichia coli* 205 and *Enterobacter cloacae*, catalysed the ring-opening reactions less enantioselectively ($E \leq 16$).

Our aim was to develop direct enzymatic strategies for the synthesis of enantiopure 3-amino-2-hydroxy-3-phenylpropionic acid with the appropriate absolute configuration, essential for the biological activity of Taxol. We first planned to carry out the hydrolysis of racemic *cis*-3-acetoxy-4-phenylazetidin-2-one [(3*R**,4*S**)-(±)-**2**] in an organic medium. Previous extensive investigations of the lipase-catalysed ring-cleavage of β-lactams[12] suggested the possibility of lipase-catalysed enantioselective ring-opening of racemic *cis*-3-hydroxy-4-phenylazetidin-2-one [(3*S**,4*R**)-(±)-**3**].

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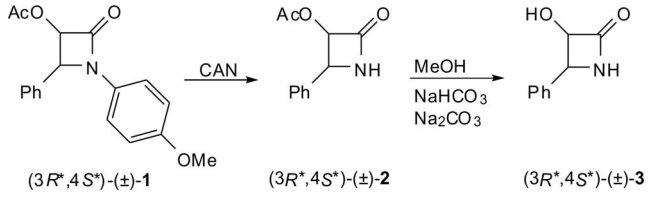
Results and Discussion

Racemic *cis*-3-acetoxy-1-(4-methoxyphenyl)azetidin-2-one [(3*R**,4*S**)-(±)-**1**] was prepared by Staudinger reaction of acetoxyacetyl chloride with the Schiff's base derived from benzaldehyde and *p*-anisidine in the presence of TEA according to a literature procedure.[10] The treatment of (3*R**,4*S**)-(±)-**1** with CAN followed by neutralization with a 5% $NaHCO_3$ solution resulted in the desired lactam *cis*-3-acetoxy-4-phenylazetidin-2-one [(3*R**,4*S**)-(±)-**2**] (Scheme 1). The hydrolysis of (3*R**,4*S**)-(±)-**2** in MeOH with saturated aqueous $NaHCO_3$ and Na_2CO_3 furnished racemic *cis*-3-hydroxy-4-phenylazetidin-2-one [(3*R**,4*S**)-(±)-**3**].[13]



(3*R**,4*S**)-(±)-**1** (3*R**,4*S**)-(±)-**2** (3*R**,4*S**)-(±)-**3**

Scheme 1. Synthesis of (*R**,4*S**)-(±)-**2** and (3*R**,4*S**)-(±)-**3**.

Lipase-Catalysed Hydrolysis of Racemic *cis*-3-Acetoxy-4-phenylazetidin-2-one [(3*R**,4*S**)-(±)-2]

Expecting that the enzymatic reaction might possibly lead to both cleavage of the ester at C3 and to N1–C2 cleavage of the lactam ring, we conducted preliminary screening experiments in iPr_2O with the enzymes (30 mg mL^{-1}) lipase PS-SD and lipase PS-IM (*Burkholderia cepacia*), lipase AK (*Pseudomonas fluorescens*), lipase AY (*Candida rugosa*), CAL-A (*Candida antarctica* lipase A) and PPL (porcine pancreatic lipase). The reactions of (3*R**,4*S**)-(±)-**2** (0.05 M) were performed with H_2O (0.5 equiv.) at 45 °C. Virtually no cleavage of the ester at C3 nor ring-opening reactions were observed for the majority of the lipases tested (conv. < 2% after 4 d); the only exceptions were with lipase AK and lipase PS-IM, which catalysed the hydrolysis of the ester at C3 (Scheme 2) with very different reaction rates and with moderate-to-good enantioselectivities[14] (conv. = 9%, *E* = 10 for lipase AK after 4 d and conv. = 29%, *E* = 34 for lipase PS-IM after 20 h). Interestingly, the same type of *Pseudomonas* lipases (P-30, AK and K-10) were successfully used by Sih and co-workers for the enantioselective (*E* > 100) *O*-deacylation of (3*R**,4*S**)-(±)-**2** in phosphate buffer at pH = 6.8.[11]

To increase the enantioselectivity we tested several solvents, such as Me_2CO, 1,4-dioxane, *t*BuOMe, *n*-hexane and toluene (Table 1), for the lipase PS-IM (30 mg mL^{-1})-catalysed hydrolysis of (3*R**,4*S**)-(±)-**2** at 50 °C. The best combination of enantioselectivity and reaction rate was observed for iPr_2O (entry 3), which was therefore chosen as the reaction medium for further optimization and gram-scale reaction.

Table 1. Conversion and enantioselectivity of the hydrolysis of (3*R**,4*S**)-(±)-**2**.[a]

Entry	Solvent	Conv. [%]	ee_S [%][b]	ee_p [%][b]	*E*
1	Me_2CO	24	21	67	6.2
2	1,4-dioxane	22	23	82	12
3	iPr_2O	48	81	89	42
4	*t*BuOMe	44	68	88	31
5	*n*-hexane	49	77	79	19
6	toluene	30	26	60	5.1

[a] 0.05 M substrate, 30 mg mL^{-1} lipase PS-IM, 0.5 equiv. of H_2O in the solvent tested at 50 °C after 23 h. [b] According to GC.

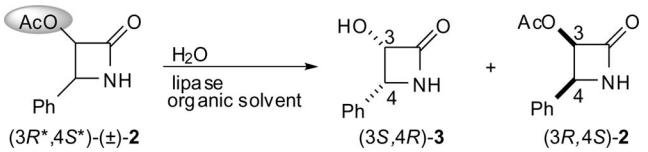
On the basis of our earlier results on secondary OAc hydrolysis in organic solvents,[15] we analysed the lipase PS-IM-catalysed reactions in iPr_2O in the presence of EtOH (3–10%), but no improvement in *E* (which apparently remained unchanged) was observed. Further, the reaction rate progressively decreased with increasing EtOH (data not shown).

When the reaction temperature for lipase PS-IM (30 mg mL^{-1})-catalysed OAc hydrolysis in iPr_2O in the presence of H_2O (0.5 equiv.) was lowered, surprisingly, *E* dropped dramatically (after 23 h: conv. = 48%, *E* = 42 at 50 °C; conv. = 32%, *E* = 5.9 at 25 °C; conv. = 7%, *E* = 5.5 at 3 °C).

Next, the reactions were performed with 50 or 70 mg mL^{-1} instead of 30 mg mL^{-1} of lipase PS-IM in iPr_2O in the presence of H_2O (0.5 equiv.) at 50 °C; significant increases in the reaction rate were observed (after 6 h, $E \approx 40$: conv. = 17% with 30 mg mL^{-1}; conv. = 51% with 50 mg mL^{-1}; conv. = 53% with 70 mg mL^{-1}).

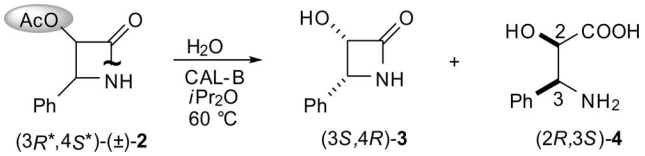
Note that not even traces of ring-opened β-amino acid could be detected by GC.

Surprisingly, analysis of the CAL-B (*Candida antarctica* lipase B, 30 mg mL^{-1})-catalysed hydrolysis of (3*R**,4*S**)-(±)-**2** (0.05 M) with H_2O (0.5 equiv.) in iPr_2O at 60 °C revealed two consecutive enzymatic reactions: cleavage of the ester at C3 with relatively low *E* (*ee* < 20% for **2** throughout the whole reaction) and a highly selective N1–C2 cleavage of the lactam ring (*ee* ≥ 98% for **4**; Scheme 3). The overall reaction rate was governed by the reaction rate for the first step. The *ee* for the intermediate lactam was de-



(3*R**,4*S**)-(±)-**2** (3*S*,4*R*)-**3** (3*R*,4*S*)-**2**

Scheme 2. Lipase-catalysed hydrolysis of (3*R**,4*S**)-(±)-**2**.

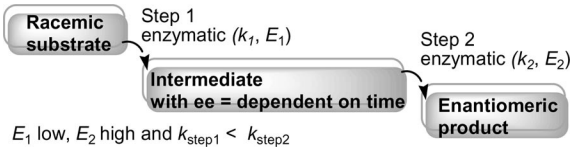


(3*R**,4*S**)-(±)-**2** (3*S*,4*R*)-**3** (2*R*,3*S*)-**4**

Scheme 3. CAL-B-catalysed two-step cascade reaction.

pendent on time and increased progressively up to 99%. At 50% overall conversion (after 87 h), therefore, this new type of two-step cascade reaction of racemic substrate (3*R**,4*S**)-(±)-**2** resulted in two different enantiopure (*ee* ≥ 98%) products: β-lactam (3*S*,4*R*)-**3** and β-amino acid (2*R*,3*S*)-**4**.

Domino or cascade reactions, in which the reaction sequence is triggered by a biocatalyst, are known in the literature but in the majority of cases the biotransformation is combined with a chemical reaction to furnish a domino sequence.[16] Two-step resolution processes catalysed by the same biocatalyst have also been described, but in most cases the enzymatic mixture requires work-up after the first step.[17] Examples without work-up after the first step can also be found in the literature, for example, the enzymatic preparation of enantiomers of 1-phenylethane-1,2-diol through the sequential one-pot methanolysis of the corresponding diacetate or acylation of the racemic diol.[18] The present enzymatic two-step cascade reaction of (3*R**,4*S**)-(±)-**2** (Scheme 4) consists of two consecutive enzymatic transformations without any "intervention" and requires a fast first enzymatic step (ester hydrolysis at C3 with relatively low *E*) and a highly enantioselective second enzymatic step (cleavage of the lactam ring). Note that, although both reactions are hydrolytic processes, in contrast with the earlier example, two totally different moieties of the substrate take part in the reactions: hydrolysis of the ester at C3 and hydrolytic cleavage of the amide bond.



Scheme 4. Enzymatic two-step cascade reaction.

Several solvents, such as Me_2CO, *t*BuOMe, *n*-hexane, 1,4-dioxane, Et_2O, toluene and CH_2Cl_2, and different amounts of CAL-B were tested for the cascade reaction. As the usual calculations for determining *ee* and the conversions of the first- and second-step enzymatic reactions could not be applied, to gain information concerning the course of the transformations with respect to time, samples taken at intervals were analysed by GC with a chiral column (see Exp. Sect.) and the corresponding mmol fractions of **2**, **3** and **4** were determined from the corresponding areas.

As an example, Figure 1 illustrates the rates of the reactions observed during the CAL-B-catalysed (80 mg mL^{-1}) hydrolysis of (3*R**,4*S**)-(±)-**2** (0.05 M substrate in *i*Pr_2O) with H_2O (0.5 equiv.) at 60 °C under which conditions the transformation was complete within 48 h to give (3*S*,4*R*)-**3** and (2*R*3*S*)-**4** with high *ee* (>98%). Under the same conditions, none of the tested solvents ensured a faster first-step reaction than *i*Pr_2O (data not shown), but a considerable increase in reaction rate was observed with higher amounts of enzyme (Table 2, entries 1, 2 and 6).

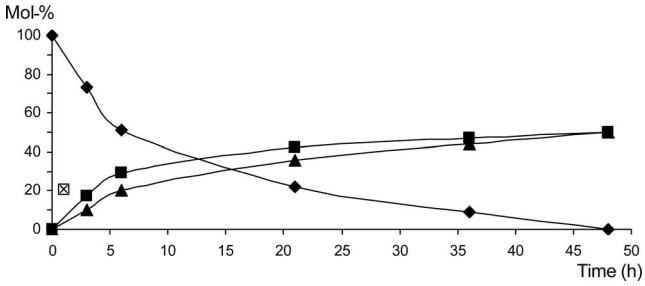


Figure 1. Mol fractions of **2** (♦), **3** (■) and **4** (▲) present at various times (3 h: 73% **2**, 17% **3**, 10% **4**; 6 h: 51% **2**, 29% **3**, 20% **4**; 21 h: 22% **2**, 42% **3**, 36% **4**; 36 h: 9% **2**, 47% **3**, 44% **4**; 48 h: 0% **2**, 50% **3**, 50% **4**) in the CAL-B-catalysed two-step cascade reaction of (3*R**,4*S**)-(±)-**2**.

Table 2. Effects of quantity of CAL-B on the hydrolysis of (3*R**,4*S**)-(±)-**2**.[a]

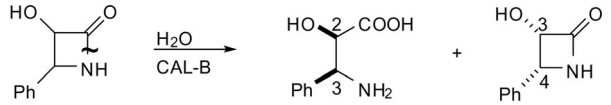
Entry	CAL-B [mg mL^{-1}]	Time [h]	Mol fraction [%]		
			2	**3**	**4**
1	30	72	18	45	37
2	50	54	10	47	43
3	50[b]	54	17	44	39
4	50[c]	54	28	38	34
5	50[d]	54	51	28	21
6	80	44	3	49	48

[a] 0.05 M substrate in *i*Pr_2O with 0.5 equiv. of H_2O at 60 °C. [b] Already used once. [c] Already used twice. [d] Already used three times.

Because a relatively high quantity of enzyme ensured the fastest reaction (Table 2, entry 6), we next analysed the reusability of the enzyme. The hydrolysis of (3*R**,4*S**)-(±)-**2** was tested with CAL-B that had already been used in one, two or three cycles (Table 2, entries 3–5). The catalytic activity of the reused CAL-B progressively declined although the overall enantioselectivity was apparently not affected.

Lipase-Catalysed Ring-Cleavage of Racemic *cis*-3-Hydroxy-4-phenylazetidin-2-one [(3*R**,4*S**)-(±)-3]

In view of our results on the lipase-catalysed enantioselective ring-cleavage of unprotected carbocyclic and aryl-substituted β-lactams in organic solvent,[12] preliminary experiments involving the CAL-B-catalysed ring-cleavage of (3*R**,4*S**)-(±)-**3** (Scheme 5) were limited to solvent testing (Table 3). The reactions were performed with 0.5 equiv. of H_2O at 60 °C. The ring-cleavage was slowest in Me_2CO and THF (entries 1 and 3), somewhat faster in 1,4-dioxane and CH_2Cl_2 (entries 2 and 7), even faster in *i*Pr_2O (entry 4) and fastest in *t*BuOMe and toluene (entries 5 and 6), but all these solvents afforded high enantioselectivities ($E > 200$). On the principle that "the best solvent is no solvent",[19] the hydrolysis of (3*R**,4*S**)-(±)-**3** was also performed in a solvent-free system: a mixture of thoroughly powdered (3*R**,4*S**)-(±)-**3** (9 mg, 0.05 mmol), CAL-B (50 mg) and H_2O (0.025 mmol) was shaken intensely at 60 °C. Unfortunately, the reaction was slow, leading to only 7% conversion in 26 h (ee_S = 8%, $ee_P > 98\%$; $E > 200$).



(3*R**,4*S**)-(±)-**3** (2*R*,3*S*)-**4** (3*S*,4*R*)-**3**

Scheme 5. Lipase-catalysed hydrolysis of (3*R**,4*S**)-(±)-**3**.

Table 3. Conversion and enantioselectivity of the hydrolysis of (3*R**,4*S**)-(±)-**3**.[a]

Entry	Solvent	Conv. [%]	ee_S[b] [%]	ee_P[c] [%]	*E*
1	Me_2CO	5	5	>98	>200
2	1,4-dioxane	28	38	>98	>200
3	THF	7	8	>98	>200
4	iPr_2O	44	77	>98	>200
5	*t*BuOMe	49	95	>98	>200
6	toluene	48	91	>98	>200
7	CH_2Cl_2	29	41	>98	>200

[a] 0.05 M substrate in the solvent tested, 50 mg mL^{-1} CAL-B and 0.5 equiv. of H_2O at 60 °C after 19 h. [b] Determined by GC. [c] Determined by GC after double derivatization.

On the basis of the preliminary results, the gram-scale resolutions of (3*R**,4*S**)-(±)-**2** and (3*R**,4*S**)-(±)-**3** were performed under the optimized conditions and the results are reported in Table 4 and the Exp. Sect. The absolute configurations were proved by comparing the [*α*] values with literature data[20] (see the Exp. Sect.).

Conclusions

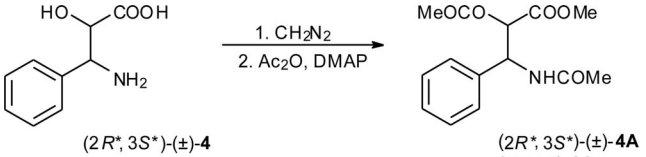
Lipase PS-IM catalysed the cleavage of ester (3*R**,4*S**)-(±)-**2** at C3 with moderate enantioselectivity (*E* = 24) when H_2O (0.5 equiv.) was used as the nucleophile and the reaction was performed in iPr_2O at 50 °C. The CAL-B-catalysed enantioselective ring-cleavage of β-lactam (3*R**,4*S**)-(±)-**3** (*E* > 200) with added H_2O (0.5 equiv.) in *t*BuOMe at 60 °C furnished the desired β-amino acid (2*R*,3*S*)-**4** with high *ee* (>98%) and in good yield (45%). A new type of enzymatic two-step cascade reaction has been used successfully for the synthesis of (2*R*,3*S*)-**4**. When the hydrolysis of (3*R**,4*S**)-(±)-**2** was performed in the presence of CAL-B with H_2O (1 equiv.) in iPr_2O at 60 °C, a 100% overall conversion of (3*R**,4*S**)-(±)-**2** resulted in two different enantiopure products, the lactam (3*S*,4*R*)-**3** and amino acid (2*R*3*S*)-**4** with *ee* ≥ 98%.

One advantage of this procedure is the use of lipases, which are commercially available, stable, have high enantioselectivity and can be applied on an industrial scale.[21] In parallel, a new GC method has been developed for the enantioseparation of racemic 3-amino-2-hydroxy-3-phenylpropionic acid on a CP-Chirasil-Dex CB column after a simple and rapid double derivatization (esterification followed by *N*-acylation).

Experimental Section

Materials and Methods: CAL-B (lipase B from *Candida antarctica*), produced by the submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin (Catalogue no. L4777), and porcine pancreatic lipase (PPL) were from Sigma–Aldrich. CAL-A (lipase A from *Candida antarctica*) was purchased from Roche Diagnostics Corporation. Lipase PS-IM (immobilized on diatomaceous earth) and PS-SD (*Burkholderia cepacia*) were kind gifts from Amano Enzyme Europe Ltd. Lipase AK (*Pseudomonas fluorescens*) was from Amano Pharmaceuticals and lipase AY (*Candida rugosa*) was from Fluka. The solvents were of the highest analytical grade.

Typical Small-Scale Experiment: Racemic substrate (0.05 M solution) in an organic solvent (1 mL) was added to the lipase to be tested (30, 50, 70 or 80 mg mL^{-1}). H_2O (0.5 equiv.) was next added. The mixture was shaken at 3, 50 or 60 °C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analysing them by GC. The *ee* values for the unreacted substrate enantiomers were determined by GC on a Chromopack Chiralsil-Dex CB column (25 m) [190 °C, 140 kPa, retention times [min] (3*R*,4*S*)-**2**: 9.48 (antipode: 10.76); (3*S*,4*R*)-**3**: 18.09 (antipode: 16.41)]. On the basis of a recently developed enantioseparation of β-amino acids by GC,[22] the *ee* values for the β-amino acids produced were determined by a novel GC method using a Chromopack Chirasil-Dex CB column after a simple and rapid double derivatization with CH_2N_2[23] (**CAUTION!** derivatization with diazomethane should be performed under a well-functioning hood) and then Ac_2O in the presence of 4-(dimethylamino)pyridine and pyridine (Scheme 6).



(2*R**, 3*S**)-(±)-**4** (2*R*,3*S*)-**4** (2*S*,3*R*)-**4**

(2*R**, 3*S**)-(±)-**4A** (2*R*,3*S*)-**4A** (2*S*,3*R*)-**4A**

Scheme 6. Derivatization process.

Table 4. Lipase-catalysed preparative-scale hydrolysis of (3*R**,4*S**)-(±)-**2** and (3*R**,4*S**)-(±)-**3**.[a]

	Lipase	*T* [h]	Conv. [%]	*E*	Product enantiomer [s]				Unreacted enantiomer			
					Yield [%]	Isomer	*ee*[b] [%]	$[\alpha]_D^{25}$	Yield [%]	Isomer	*ee*[c] [%]	$[\alpha]_D^{25}$ ($CHCl_3$)
(3*R**,4*S**)-(±)-**2**	PS-IM	31	56	24	48	(3*S*,4*R*)-**3**	74	–131 ($CHCl_3$)[d]	33	(3*R*,4*S*)-**2**	95	–49[e]
(3*R**,4*S**)-(±)-**2**	CAL-B	58	50[f]	>200[f]	43	(2*R*,3*S*)-**4**	>98	–7.2 (H_2O)[g]	no unreacted enantiomer			
					49	(3*S*,4*R*)-**3**	>98	–171 ($CHCl_3$)[d]				
(3*R**,4*S**)-(±)-**3**	CAL-B	18	49	>200	48	(2*R*,3*S*)-**4**	98	–6.9 (H_2O)[g]	46	(3*S*,4*R*)-**3**	>99	–169[h]

[a] 0.5 equiv. of H_2O, 50 mg mL^{-1} lipase PS-IM in iPr_2O at 50 °C for the hydrolysis of (3*R**,4*S**)-(±)-**2**; 80 mg mL^{-1} CAL-B in iPr_2O for the cascade reaction of (3*R**,4*S**)-(±)-**2**; 50 mg mL^{-1} CAL-B in *t*BuOMe for the hydrolysis of (3*R**,4*S**)-(±)-**3** at 60 °C. [b] Determined by GC after double derivatization (see the Exp. Sect., Scheme 6, Figure 2). [c] Determined by GC (see the Exp. Sect.). [d] *c* = 0.35. [e] *c* = 0.45. [f] Overall values. [g] *c* = 0.34. [h] *c* = 0.26.

Derivatization Process: To prepare the corresponding (2*R**,3*S**)-(±)-**4A**, (2*R*,3*S*)-**4A** and (2*S*,3*R*)-**4A**, β-amino acids (2*R**,3*S**)-(±)-**4**, (2*R*,3*S*)-**4** and (2*S*,3*R*)-**4**·HCl [obtained by hydrolysis of (3*S*,4*R*)-**3** with 18% HCl[12c]] were double-derivatized. A saturated solution of CH_2N_2 in Et_2O was added dropwise to a 0.05 M MeOH solution (0.5 mL) of the β-amino acid until a yellow colour persisted. In the next acylation step, Ac_2O (15 μL) and a mixture of DMAP and pyridine (5:95 w/w; 15 μL) were added to the same test-tube. After shaking for 2–3 s, the double-derivatized samples [(2*R**,3*S**)-(±)-**4A**, (2*R*,3*S*)-**4A** and (2*S*,3*R*)-**4A**] were analysed on GC chiral columns [140 °C for 7 min→190 °C (temperature rise: 10 °C min^{-1}, 100 kPa)] (Figure 2).

Absolute Configuration: The literature[20] [*α*] value for (2*S*,3*R*)-3-amino-2-hydroxy-3-phenylpropionic acid {$[\alpha]_D^{23}$ = +14.4 (*c* = 0.5, 6 N HCl), *ee* = 100%} is in good accordance with the value measured for (2*S*,3*R*)-**4** under the same conditions {$[\alpha]_D^{25}$ = +15 (*c* = 0.25, 6 N HCl), *ee* > 98%} and the [*α*] value measured for the antipode (2*R*,3*S*)-**4** {$[\alpha]_D^{25}$ = –15 (*c* = 0.25, 6 N HCl), *ee* > 98%}. Optical rotations [*α*] were measured with a Perkin–Elmer 341 polarimeter.

Melting Points: (3*R*,4*S*)-**2**: 187–188 °C (from *i*Pr_2O/EtOAc) (ref.[11] 151–153 °C); (3*S*,4*R*)-**3**: 186–188 °C (from *i*Pr_2O) (ref.[11] 187–188 °C); (2*R*,3*S*)-**4**: 252–255 °C with decomposition (from H_2O/Me_2CO). (2*S*,3*R*)-**4**: 251–256 °C with decomposition (from H_2O/Me_2CO) (ref.[20] 255–256 °C with decomposition). Melting points were determined with a Kofler apparatus.

Gram-Scale Resolution of Racemic *cis*-3-Acetoxy-4-phenylazetidin-2-one [(3*R,4*S**)-(±)-2]**

Lipase PS-IM-Catalysed Cleavage at C3: (3*R**,4*S**)-(±)-**2** (1.00 g, 4.88 mmol) was dissolved in *i*Pr_2O (40 mL). Lipase PS-IM (2 g, 50 mg mL^{-1}) and H_2O (44 μL, 2.44 mmol) were added and the mixture was shaken in an incubator shaker at 50 °C for 31 h. The reaction was stopped by filtering off the enzyme at 56% conversion. The solvent was evaporated and the residue of (3*R*,4*S*)-**2** and (3*S*,4*R*)-**3** was separated by column chromatography (EtOAc/hexane, 3:1). (3*R*,4*S*)-**2**: yield 330 mg, 33%. R_f = 0.62. $[\alpha]_D^{25}$ = –49 (*c* = 0.45, $CHCl_3$), *ee* = 95% {ref.[11] $[\alpha]_D^{27}$ = –51 (*c* = 1, $CHCl_3$), *ee* = 96%}. (3*S*,4*R*)-**3**: yield 381 mg, 48%. R_f = 0.25. $[\alpha]_D^{25}$ = –131 (*c* = 0.35, $CHCl_3$), *ee* = 74% {ref.[20] $[\alpha]_D^{27}$ = –193 (*c* = 1, $CHCl_3$), *ee* = 100%}.

CAL-B-Catalysed Two-Step Cascade Reaction: (3*R**,4*S**)-(±)-**2** (1.00 g, 4.88 mmol) was dissolved in *i*Pr_2O (50 mL). CAL-B (4 g, 80 mg mL^{-1}) and water (88 μL, 4.88 mmol) were added and the mixture was shaken in an incubator shaker at 60 °C for 58 h. The reaction was stopped by filtering off the enzyme at 49% conversion. The solvent was evaporated and the residue (3*S*,4*R*)-**3** crystallized { 390 mg, 49%, $[\alpha]_D^{25}$ = –171 (*c* = 0.35, $CHCl_3$), *ee* > 99%}. The filtered-off enzyme was washed with distilled water (3 × 20 mL) and the water was evaporated to yield the crystalline β-amino acid (2*R*,3*S*)-**4** {379 mg, 43%, $[\alpha]_D^{25}$ = –7.2 (*c* = 0.34, H_2O), $[\alpha]_D^{25}$ = –15 (*c* = 0.25, 6 N HCl), *ee* > 98%}.

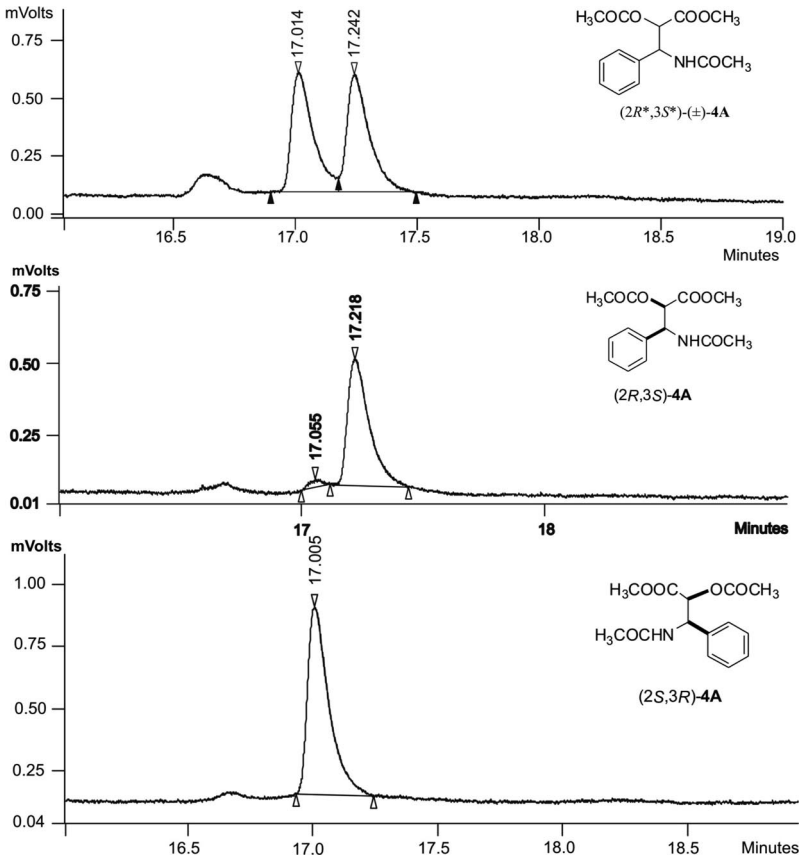


Figure 2. Enantioseparation of (2*R**,3*S**)-(±)-**4A** (dead time t_0 = 1.28 min, retention factor k' = 12.32, selectivity α = 1.01 and resolution R_S = 1.33).

Gram-Scale Resolution of Racemic *cis*-3-Hydroxy-4-phenylazetidin-2-one [(3*R,4*S**)-(±)-3]:** (3*R**,4*S**)-(±)-**3** (1.00 g, 6.13 mmol) was dissolved in *t*BuOMe (40 mL). CAL-B (2 g, 50 mg mL^{-1}) and water (55 μL, 3.07 mmol) were added and the mixture was shaken in an incubator shaker at 60 °C for 18 h. The reaction was stopped by filtering off the enzyme at 50% overall conversion. The solvent was evaporated and the residue (3*S*,4*R*)-**3** crystallized {460 mg, 46%, $[a]_D^{25}$ = –169 (*c* = 0.26, $CHCl_3$), *ee* > 99%}. The filtered-off enzyme was washed with distilled water (3 × 20 mL) and the water was evaporated to give the crystalline β-amino acid (2*R*,3*S*)-**4** {533 mg, 48%, $[a]_D^{25}$ = –6.9 (*c* = 0.34, H_2O), *ee* = 98%}.

^{1}H NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer and were as follows. The data for (3*R*,4*S*)-**2** were similar to those for (3*R**,4*S**)-(±)-**2**: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.71 (s, 3 H, C*H*$_3$), 5.07–5.08 (d, *J* = 4.6 Hz, 1 H, C*H*CO), 5.91–5.93 (dd, *J* = 4.6, *J* = 2.6 Hz, 1 H, C*H*NH), 6.36 (br. s, 1 H, N*H*), 7.30–7.42 (m, 5 H, C_6H_5) ppm. $C_{11}H_{11}NO_3$ (205.07): calcd. C 64.38, H 5.40, N 6.83; found for (3*S*,4*R*)-**2**: C 64.11, H 5.22, N 6.80.

The data for (3*S*,4*R*)-**3** were similar to those for (3*R**,4*S**)-(±)-**3**: ^{1}H NMR (400 MHz, DMSO, 25 °C): δ = 4.69–4.70 (d, *J* = 4.8 Hz, 1 H, C*H*CO), 4.92–4.95 (m, 1 H, O*H*), 5.79–5.81 (d, *J* = 7.2 Hz, 1 H, C*H*NH), 7.32–7.36 (m, 5 H, C_6H_5), 8.44 (br. s, 1 H, N*H*) ppm. $C_9H_9NO_2$ (163.06): calcd. C 66.25, H 5.56, N 8.58; found for (3*S*,4*R*)-**3**: C 66.22, H 5.44, N 8.65.

(2*R*,3*S*)-**4**: ^{1}H NMR (400 MHz, D_2O): δ = 4.36–4.37 [d, *J* = 5.9 Hz, 1 H, C*H*(OH)(COOH)], 4.59–4.61 (d, *J* = 5.9 Hz, 1 H, C*H*NH_2), 7.43–7.58 (m, 5 H, C_6H_5) ppm. $C_9H_{11}NO_3$ (181.07): calcd. C 59.66, H 6.12, N 7.73; found C 59.43, H 6.10, N 7.81.

(2*S*,3*R*)-**4**·HCl: ^{1}H NMR (400 MHz, D_2O): δ = 4.57–4.58 [d, *J* = 6.4 Hz, 1 H, C*H*(OH)(COOH)], 4.64–4.66 (d, *J* = 6.4 Hz, 1 H, C*H*NH_2), 7.44–7.50 (m, 5 H, C_6H_5) ppm. $C_9H_{11}NO_3$·HCl (217.05): calcd. C 49.67, H 5.56, N 6.44; found C 49.49, H 5.41, N 6.60.

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