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Candida antarctica lipase catalyzed resolution of ethyl (±)-3-aminobutyrate

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Abstract: *Candida antarctica* lipase efficiently catalyzes acetylation and hydrolysis of ethyl (±)-3-aminobutyrate. © 1997, Elsevier Science Ltd. All rights reserved.

The occurrence of β-amino acids in natural products such as alkaloids and antibiotics,[1] and their synthetic utility as precursors of β-lactams[2] and therapeutically enhanced peptides,[3] has always attracted the attention of organic chemists. This is reflected by the ever increasing research activity in the field of the stereoselective synthesis of β-amino acids or their ester derivatives.[4]

Despite the well-documented properties of hydrolytic enzymes to produce optically active compounds by means of kinetic resolutions of racemates, their application for the preparation of β-amino acids remains only slightly explored.[5] The enantioselective hydrolysis of *N*-phenylacetyl derivatives of β-amino acids with penicillin acylase[5a] is one of the limited examples.

For some time we have been studying the lipase-catalyzed aminolysis reaction of either racemic esters or amines, and this methodology has provided us an easy access to optically active amines, esters and amides.[6] Continuing with this study, and taking into account the scarcity of enzymatic routes in the field of β-amino acids, we now wish to report the lipase-catalyzed resolution of ethyl β-aminobutyrate **1** through aminolysis. β-Amino esters are attractive and versatile substrates because they are able to behave as nucleophiles (amino group) or acyl donors (ester function) in aminolysis processes.

In the course of our investigations, we have found that *Candida antarctica* lipase (CAL) shows a high activity in aminolysis reactions. Therefore, the enzymatic transformations of (±)-**1** are carried out with CAL as catalyst. First, we investigated the acetylation of (±)-**1** using ethyl acetate as acyl donor and solvent (Scheme 1). The reaction is monitored by chiral-GC and stopped when both a high conversion and a high enantiomeric purity (95%) of the amidoester (*R*)-**3** are achieved. Unfortunately, the ee of the remaining (*S*)-**1** can not be determined by this technique. To facilitate the isolation of the unreacted β-amino ester **1**, the reaction mixture[7] is treated with benzyloxycarbonylchloride to transform (*S*)-**1** into its Cbz-derivative (*S*)-**4**. After the usual work-up, compounds (*R*)-**3** and (*S*)-**4** are easily separated by flash-chromatography. Furthermore, the enantiomeric excess of (*S*)-**1** can be determined by HPLC analysis of its Cbz-derivative (*S*)-**4** using a Chiralcel-OD column. Inspection of the results obtained in this reaction (Table 1) reveals that CAL is an effective catalyst (E=74)[8] in the enantioselective acetylation of the β-amino ester **1**. If the reaction is allowed to reach a higher conversion (53% after 12 h) compound (*S*)-**1** is obtained with 99% ee (measured in the Cbz-derivative **4**, Table 1, entry 2). A similar enantiomeric ratio and rate of reaction are achieved when the acetylation is carried out in 1,4-dioxane (entry 3). However, using hexane as solvent only a 15% conversion of (*R*)-**3** is attained after 4 days of reaction, although the enantiomeric purity of **3** (95% ee) is also very high. It is noteworthy that in no case is dimerization of the substrate observed, that is, in these conditions CAL only catalyzes the aminolysis of ethyl acetate.

To assign the absolute configuration to compounds **3** and **4**, the latter is hydrolyzed with NaOH in ethanol (RT, 30 min) to the acid **5**[9] and the Cbz-group is subsequently removed by hydrogenolysis[10]

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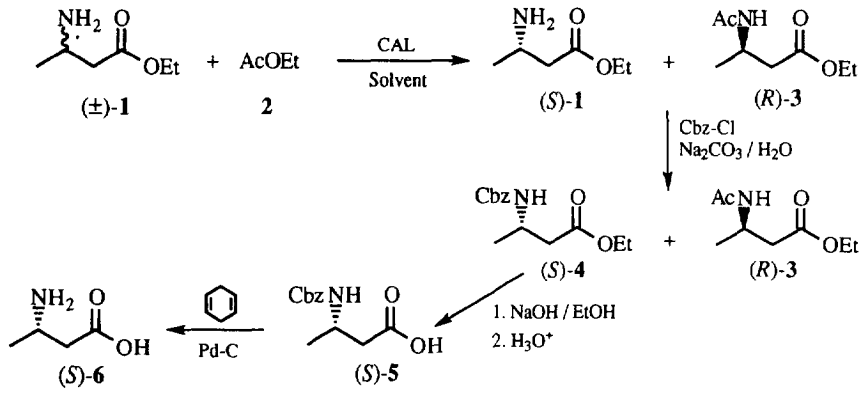
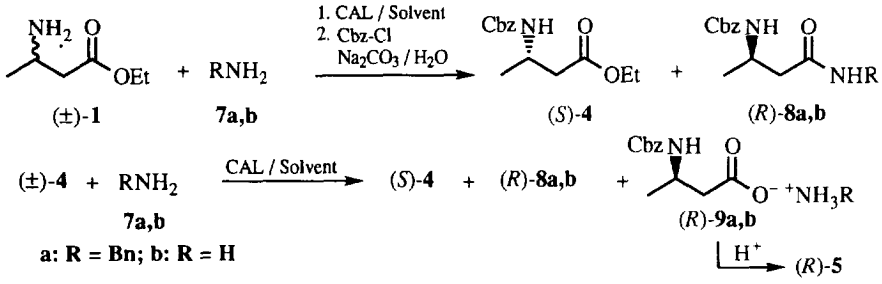


Scheme 1.

Table 1. Acetylation of (±)-1 catalyzed by CA lipase[a]

			remaining ester (*S*)-4		product (*R*)-3			
Entry	Solvent	t, h	Yield,[b] %	ee, %	Yield,[b] %	ee, %	conv.,[c] %	E^c
1	AcOEt	6	52	62	35	95	40	74
2	AcOEt	12	45	99	47	88	53	82
3	1,4-dioxane[d]	8	49	75	38	95	44	88

[a] Typical scale: (±)-1 (5 mmol), CAL-SP 435 (300 mg), solvent (15 mL). [b] After flash chromatography. [c] See ref. 8. [d] AcOEt (1.5 mL)



Scheme 2.

to yield free β-amino acid **6**. Comparison of the specific rotation of **6** ($[\alpha]_D^{25}$+38.6, c=0.48, H_2O; 99% ee) with reported data for (*R*)-(−)-**6**[11] establishes the *S*-configuration for **4** and, therefore, for the ester **1**. This means that the CAL preferentially catalyzes the acetylation of the *R* enantiomer of (±)-**1**.

Next we examined the enantioselective properties of CAL in the aminolysis of **1**, with it acting as acyl-donor, with benzylamine **7a** and 1,4-dioxane and toluene as solvents. After filtration of the enzyme, the reaction mixture formed by the remaining ester **1** and the aminolysis product is transformed into the corresponding mixture of the Cbz-derivatives (*S*)-**4** and (*R*)-**8a** (Scheme 2). This makes the isolation of both compounds easier and affords suitable derivatives for analysis by chiral-HPLC. As shown in Table 2 (entries 1 and 2), the corresponding amide (*R*)-**8a** is obtained with moderate ee and the enantiomeric ratio (*E*) is low in both cases. However, at >50% conversion (entry 2), the remaining (*S*)-**1** [isolated as (*S*)-**4**] can be obtained with high ee (93%). Also unsuccessful is the ammonolysis of (±)-**1**; CAL catalyzes the formation of (*R*)-**8b** in 1,4-dioxane but with very low *E* (entry 3).

Table 2. Amidation of (±)-**1** and (±)-**4** with RNH_2 catalyzed by CA lipase[a]

Entry	Substrate	R	Solvent	t, h	ee, %			Yield,[b] %			conv,[c] %	E[c]
					(*S*)-**4**	(*R*)-**8**	(*R*)-**5**	(*S*)-**4**	(*R*)-**8**	(*R*)-**5**		
1	(±)-**1**	Bn	1,4-dioxane	3	55	77	–	33	22	–	42	13
2	(±)-**1**	Bn	toluene	8	93	66	–	34	15	–	58	16
3	(±)-**1**	H	1,4-dioxane	8	71	36	–	28	33	–	66	4
4	(±)-**4**	Bn	toluene	13	62	69	–	42	40		47	10
5	(±)-**4**	H	TBA	8	98	75	75	38	27	27		
6	(±)-**4**	H	1,4-dioxane	8	87	84	94	38	3	39		

[a] Typical scale: 0.5 mmol of (±)-**1** or (±)-**4**, CAL (100 mg), $BnNH_2$ (0.5 mmol), solvent (2 mL); for ammonolysis processes, NH_3 (g) is bubbled through the solvent at 0 °C for 10 min, under nitrogen atmosphere. [b] After flash chromatography. [c] See ref. 8.

Table 3. Hydrolysis of (±)-**4** catalyzed by CA lipase

Entry	Solvent	t, h	ee, %		c,[a] %	E[a]
			(*S*)-**4**	(*R*)-**5**		
1	1,4-dioxane[b]	8	21	93	18	33
2	1,4-dioxane[c]	8	67	98	41	>100
3	Buffer, pH = 7	1	77	83	48	25

[a] See ref. 8. [b] With H_2O (1 eq). [c] With H_2O (1 eq) + Et_3N (1 eq).

Since the derivatization of the reaction mixture with Cbz-Cl is neccessary, and the introduction of slight modifications in the substrates can modify the biocatalytic activity of the enzymes, we decided to try the aminolysis and ammonolysis of (±)-**4** in different solvents. The most significative results are collected in Table 2. Only when aminolysis of (±)-**4** is carried out in toluene, is amide (*R*)-**8a** the sole reaction product (entry 4). With the more hydrophilic 1,4-dioxane and *tert*-butyl alcohol (TBA), besides the aminolysis or ammonolysis product **8a** or **8b**, a high percentage of hydrolysis product **9**, which is isolated as the acid (*R*)-**5**, is obtained. Thus, in the reaction of (±)-**4** with ammonia in TBA (see entry 5), (*R*)-**8b** and (*R*)-**5** are obtained in a 1:1 ratio showing the same ee (75%). More surprising is the ammonolysis reaction in 1,4-dioxane (entry 6) where only a small amount of amide (*R*)-**8b** is isolated (3%) and the major product is the acid (*R*)-**5**, which is obtained with very high ee (94%).

The above results mean that the hydrolysis of (±)-**4** is more favoured than its aminolysis or ammonolysis, even with conventionally dried organic solvents. Moreover, the high conversion and ee obtained for (*R*)-**5** in the latter reaction indicate that CAL catalyzed hydrolysis of (±)-**4** in the presence of ammonia is very enantioselective. These facts prompted us to investigate the hydrolysis of (±)-**4** under different conditions. As shown in Table 3, hydrolysis of (±)-**4** in 1,4-dioxane with an equimolecular amount of water yields (*S*)-**4** and (*R*)-**5**, but the reaction is slower and less enantioselective than that in the presence of ammonia (compare entry 1 in Table 3 with entry 6 in Table 2). This could be due to the fact that ammonia precludes the reversibility of the enzymatic hydrolysis in organic solvent, because it removes the product (acid **5**) by formation of its ammonium salt **9b**. Similarly, if the base is Et_3N instead of NH_3, thus avoiding amide formation, the reaction is also very enantioselective; (*R*)-**5** is obtained with very high ee at a high extent of conversion (entry 2). Finally, for the purpose of comparing the hydrolysis of (±)-**4** in organic and aqueous media, hydrolysis of (±)-**4** is conducted in phosphate buffer (entry 3). The reaction is faster than in organic solvents but less enantioselective. These results again reveal the advantages of carrying out enzymatic hydrolyses in organic solvents in the presence of an amine.[12]

The stereochemical preference of CAL in all the described processes[13] is always the same. CAL transforms the (*R*)-enantiomer of (±)-**1** when it acts either as a nucleophile or as an acyl donor;

furthermore, the presence of a Cbz-group does not modify the enantiopreference of the CAL, because the (*R*)-enantiomer of (±)-**4** is transformed too.

In conclusion, ethyl (±)-3-aminobutyrate and its Cbz-derivative have been successfully resolved by CAL catalyzed acetylation and hydrolysis processes. The high enantioselectivity achieved in these reactions as well as the simplicity of the procedure make this strategy a useful alternative for the resolution of β-amino acids. The scope and limitations of this methodology are currently under study.

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9. M.p. 108–110°C; $[\alpha]_D^{20}$ −17.9, c=0.99, $CHCl_3$; 99ee (determined from its methyl ester derivative by chiral-HPLC, hexane:propan-2-ol 95:5 as eluent, 0.6 mL/min; t_R: 35.7 min. For *rac*-methyl ester derivative, two peaks t_R 31.1 and 35.5 min; Rs, 2.8). Reported data: (Drey, C. N. C., Mtetwa, E. *J. Chem. Soc., Perkin Trans. 1*, **1982**, 1587) m.p. 108–110°C; $[\alpha]_D^{20}$ −26, c=1, $CHCl_3$.
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13. Satisfactory spectral data are obatined for all new compounds. Conditions for chiral-GC: for (*R*)-**3**, Cydex-B column; P, 180 KPa; T, 110°C; t_R, 32.7 min; for (±)-**3**, two peaks t_R 31.8 and 32.7 min; Rs, 1.0. Conditions for chiral-HPLC (Chiralcel-OD column): for (*S*)-**4**, t_R, 32.7 min; for (±)-**4**, two peaks t_R 31.1 and 33.2 min; Rs, 1.3 (hexane:propan-2-ol 95:5, 0.5 mL/min). For (*R*)-**8a**, t_R, 26.8 min; for (±)-**8a**, two peaks t_R 23.9 and 26.8 min; Rs, 1.3 (hexane:ethanol 95:5, 1 mL/min). For (*R*)-**8b**, t_R, 12.4 min; for (±)-**8b**, two peaks t_R 12.4 and 16.4 min; Rs, 3.3 (hexane:propan-2-ol 85:15, 1 mL/min).

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