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Aldehyde-based racemization in the dynamic kinetic resolution of *N*-heterocyclic α-amino esters using *Candida antarctica* lipase A

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Abstract—The present research introduces approaches for the dynamic kinetic resolution of the methyl esters of proline and pipecolic acid. As the result, a method was developed which is based on the acylation of the secondary amino group of the amino esters with vinyl butanoate by *Candida antarctica* lipase A. In the optimized method, acetaldehyde as a racemizing agent is released in situ from vinyl butanoate in the presence of triethylamine, allowing ca. 90% of the racemic proline and 70% of the pipecolic acid methyl esters to be acylated in the forms of highly enantiopure (ee=97%) butanamides with the *S*-absolute configurations.

1. Introduction

N-Heterocyclic amino acids, such as proline and pipecolic acid, are valuable building blocks of many pharmaceuticals.[1] In our previous paper the enzymatic kinetic resolution of methyl pipecolinate (*rac*-**2**; Scheme 1) as a non-proteinogenic α-amino ester was studied.[2] As the most important result, *Candida antarctica* lipase A (CAL-A) was reported to catalyze the acylation of the secondary ring nitrogen with 2,2,2-trifluoroethyl esters in a highly enantioselective manner in organic solvents (enantioselectivity ratio $E>100$).

Dynamic kinetic resolution enables the transformation of a racemate to a new product as one enantiomer. This becomes possible through the racemization of one of the enantiomers whereas the other reacts to a stable product. Most racemizing methods of α-amino acids are based on the acidic α-hydrogen at an amino acid or its derivative. This allows enol formation under acidic and enolate formation under basic conditions. However, free amino acids are generally difficult to racemize. Derivatization of an amino acid as a Schiff base, hydantoin or oxazolone results in a more acidic α-proton, and accordingly promotes racemization.[3–5] Acidic and basic conditions are known to aid aldehyde-promoted racemization through a Schiff base.[6–8] The previously published racemizations of amino acids or esters including the racemization of (*S*)-proline were effectively performed in acetic acid or in the mixture of acetone–acetic acid using various aldehydes or ketones at elevated temperatures.[6,9,10] Traditional racemization by aldehydes is assisted by metal catalysts.[11] Metal catalysts also alone are able to provoke racemization particularly in alkaline solutions.[12]

Several biocatalytic dynamic kinetic resolutions of amino acids have been published including both enzymatic and chemical racemization steps. Especially oxazolones, thiazolones and hydantoins have been used as a starting material.[13] Among amino acid racemases, hydantoin and *N*-acylamino acid racemases have previously gained significance in industrial dynamic kinetic resolution processes.[14] Regarding aldehyde-based methods, salicylaldehyde and pyridoxal have been utilized for the racemization of phenylglycine methyl ester during the lipase-catalyzed ammoniolysis in organic solvents.[15,16] The pyridoxal 5-phosphate-catalyzed racemization of amino esters together with alcalase-catalyzed resolution is also known.[7] Moreover, Schiff bases of α-amino esters have served as starting materials for enzyme-catalyzed ester hydrolysis.[8,17]

The above results prompted us to start work in order to develop an aldehyde-based dynamic kinetic resolution method for *N*-heterocyclic amino esters *rac*-**1** and *rac*-**2** in the presence of CAL-A and an acyl donor (Scheme 1). In order to study the general usability of CAL-A, the normal kinetic resolution of *rac*-**1** and *rac*-**2** as secondary amines and that of some common α-amino esters as primary amines with 2,2,2-trifluoroethyl butanoate have been investigated. Racemization of (*S*)-**1** and (*S*)-**2** in the presence of additives (acids or bases) and aldehydes has been studied. Finally, the dynamic kinetic resolution method was optimized and the preparative scale experiment performed using racemic proline methyl ester as a substrate.

Keywords: Dynamic kinetic resolution; Proline methyl ester; Pipecolic acid methyl ester; *Candida antarctica* lipase A; Aldehyde-based racemization.
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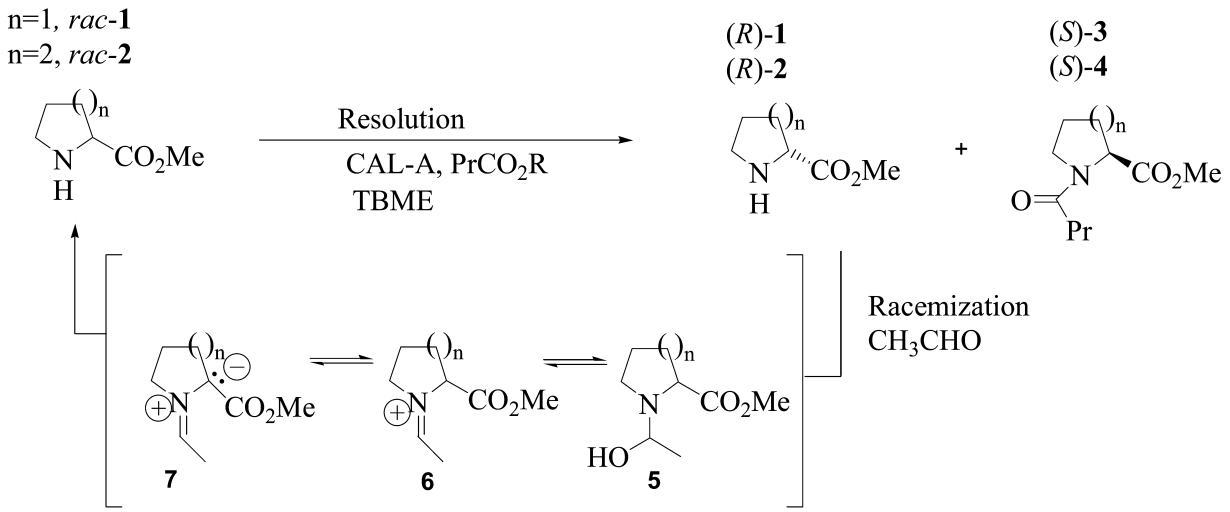

Scheme 1.

2. Results and discussion

In order to develop a satisfactory method for dynamic kinetic resolution where the product enantiomer is formed with high yield and enantiomeric excess, the resolution reaction should be highly enantioselective and the less reactive enantiomer rapidly racemized under the conditions where the product of an enzyme-catalyzed reaction remains stable and the enzyme catalytically active. Under such conditions, the starting material is always practically racemic, allowing the maximal enantiopurity (as determined by the *E* value of the corresponding kinetic resolution) to be reached at the theoretical zero conversion for the more reactive enantiomer throughout the reaction. Thus, it can be calculated that in order to achieve the product with ee>95%, the *E* value for kinetic resolution should be 40 or higher.[18]

2.1. Kinetic resolution

We have shown earlier that CAL-A catalyzes highly enantioselective *N*-acylations between β-amino esters and achiral esters with absolute chemoselectivity while some other common lipases tend to lead interesterification products.[19–22] We have also shown that CAL-A is exceptional among lipases by catalyzing acylations of the secondary amino group of (*S*)-**2**.[2] In order to find the general usability of CAL-A for the resolution of α-amino esters, various methyl esters (Table 1) were subjected to enzymatic resolution under the best conditions of the previous works. According to the results in Table 1, enantioselectivity requirements for a successful dynamic kinetic resolution are properly fulfilled only for the acylation of sterically hindered *N*-heterocyclic amino esters **1** and **2** with 2,2,2-trifluoroethyl esters (entries 16–21). Aspartic acid dimethyl ester as a primary amine is also acceptable with *E*=33 and with a calculated theoretical ee=94% at zero conversion for the (*S*)-amide product (entry 1). However, the observed high stability against racemization did not persuade us to continue studies for its dynamic kinetic resolution. Thus, there was only a small drop in ee from 99 to 73% when the (*S*)-amino ester (0.1 M) was incubated in TBME (*tert*-butyl methyl ether) for 24 h while the enzymatic acylation of the racemic compound with 2,2,2-trifluoroethyl butanoate in TBME smoothly proceeded to 53% conversion in 3 h. On the above basis and because 2,2,2-trifluoroethyl butanoate as an acyl donor (entries 16 and 19) was more effective than the corresponding acetate (entries 18 and 21) the studies were continued using butanoates as achiral acyl donors and *rac*-**1** and *rac*-**2** as substrates.

2.2. Racemization experiments

Racemization of amino esters with aldehydes is a process where an aldehyde reacts with the starting material in the formation of carbinolamine **5** which in the presence of an acid forms a Schiff base **6** (Scheme 1). The subsequent base-catalyzed release of a proton is responsible for the deterioration of enantiopurity in the formation of the carbanion intermediate **7**.[3,7,8] When racemization is planned to proceed in situ at the same time with the enzymatic acylation mild reaction conditions are necessary for the enzyme to stay active. In the present work, methyl ester (*S*)-**1** (0,1 M, ee=99%) and acetaldehyde (0.1 M) were chosen for racemization in TBME with and without additives (Table 2). Carboxylic acids and ammonium acetate (entries 2–4) clearly favour racemization while bases such as triethylamine (entry 6) do not have an impact over the effect of acetaldehyde alone (entry 1). As another observation, the amount of **1** clearly decreases in the presence of carboxylic acids (entries 2 and 3). Salt formation between **1** and the acid is one explanation for the decrease. Indeed, some precipitation was observed in the mixture of **1** or **2** (0.1 M) and acetic or butanoic acid (0.1 M) in TBME at room temperature while the precipitate disappeared at elevated temperature (48 °C). Another possible explanation for the loss of an amino ester is the stabilization of a species such as **6** through salt formation. The loss of **1** when no additives were added shows that part of the starting material is in the form of a Schiff base (entry 1). The formation of various condensation products (although not detected by the present GC method) cannot be totally ruled out either.

Different aldehydes were next tested for the acetic acid-catalyzed racemization of (*S*)-**1** in TBME (Table 3).

Table 1. CAL-A-catalyzed acylation at room temperature of the methyl esters of α-amino acids (0.1 M) with trifluoroethyl butanoate (0.2 M) in TBME (A) and in CH_3CN (B) and with butyl butanoate as a solvent and an acyl donor (C)

Entry	Methyl ester of	Conditions	*E*	*N*-Acylated product formed (%)	Time (h)
1	aspartic acid	A	33±2	16[a]	0.5
2	aspartic acid	B	20±1	19[a]	0.5
3	aspartic acid	C	17±1	9[a]	0.5
4	glutamic acid	A	12±1	37[b]	0.5
5	glutamic acid	B	12±1	33[b]	0.5
6	glutamic acid	C	13	53[c]	0.5
7	methionine	A	10±1	49[c]	0.5
8	methionine	B	7±1	100[c]	0.5
9	methionine	C	8±0	90[c]	0.5
10	phenylglycine	A	7±0	77[c]	0.5
11	phenylglycine	B	1.5±0.1	54[c]	0.5
12	phenylglycine	C	6±0	35[c]	0.5
13	valine	A	15±1	38[c]	0.5
14	valine	B	9±0	44[c]	0.5
15	valine	C	18±0	24[c]	0.5
16	proline (**1**)	A	>100	50[c]	0.5
17	proline (**1**)	C	>100	44[c]	0.5
18	proline[d] (**1**)	A	>100	2	0.5
19	pipecolic acid[e] (**2**)	A	>100	49[c]	9
20	pipecolic acid[e] (**2**)	B	>100	39[c]	22
21	pipecolic acid[d,e] (**2**)	A	>100	29[c]	24

[a] 5 mg/mL of the enzyme preparation.
[b] 10 mg/mL of the enzyme preparation.
[c] 75 mg/mL of the enzyme preparation.
[d] $CH_3CO_2CH_2CF_3$ as an acyl donor.
[e] Ref. 2.

Acetaldehyde (entry 2) is clearly most favourable for the purpose. Increasing acetic acid (entries 1–4, Table 4) and acetaldehyde (entries 5–9) concentrations have a positive effect on racemization although acetic acid (0.1 M, entry 5) alone is not effective. Increasing acetaldehyde concentrations also decrease the amount of **1** in the presence of acetic acid (0.1 M; entries 5–9). This indicates improved possibilities for the adducts like **6** and/or improved possibility for side reactions. As the best compromise, 1 equiv. of acetaldehyde and acetic acid with respect to the starting material were chosen for the subsequent dynamic kinetic resolution experiments.

Contrary to the above results for proline methyl ester, the racemization of (*S*)-**2** proceeds slowly in the presence of acetaldehyde (0.1 M) and various acetic acid concentrations in TBME (Table 4, entries 10–12). Elevated temperature (48 °C) favours racemization and also leads to the enhanced disappearance of the starting material (entries 13–15).

2.3. Dynamic kinetic resolution

As shown in Table 1, the traditional kinetic resolution of *rac*-**1** and *rac*-**2** with 2,2,2-trifluoroethyl butanoate and CAL-A smoothly proceeds in a highly enantioselective manner with *E*>100 in TBME (entries 16 and 19). High enantiopurity is also observed for the formed (*S*)-**3** when vinyl butanoate is used in the place of 2,2,2-trifluoroethyl butanoate although the acylation of *rac*-**1** then is approaching toward the conditions of dynamic kinetic resolution due to the acetaldehyde produced from vinyl alcohol (Fig. 1; Table 5, entry 2). Thus, after an hour 75% of the racemate (●) was transformed to the product (■) with $ee^{(S)\text{-}\mathbf{3}}$=98% and at this point the reaction mixture contained only 4% of the unreacted starting material. The enantiopurity of (*S*)-**4** is also excellent (entry 8) for the acylation of *rac*-**2** with vinyl butanoate, but the yield of the product only slightly increases after 40% is reached (Fig. 2). It is clear according to Figures 1 and 2 that a considerable amount of the starting material has disappeared somewhere.

Three different strategies were now chosen for improving the yield of the dynamic kinetic resolution of *rac*-**1** and *rac*-**2**. In method I, acetic acid and acetaldehyde (both 0.1 M) were added to the reaction mixture where 2,2,2-trifluoroethyl butanoate served as an acyl donor. In methods II and III vinyl butanoate served as an acyl donor and as the in situ

Table 2. Effect of additives on the racemization of (*S*)-**1**[a] (0.1 M) with acetaldehyde (0.1 M) in TBME after 24 h at room temperature

Entry	Additive (0.1 M)	ee (%)	**1** left (%)
1	No additive	72	92
2	Acetic acid	0	86
3	Butanoic acid	0	85
4	$CH_3CO_2^-NH_4^+$	3	96
5	DMAP[b]	72	98
6	Triethylamine	76	93

[a] Original $ee^{(S)\text{-}\mathbf{1}}$=99%.
[b] *N*,*N*-Dimethylaminopyridine.

Table 3. Effect of different aldehydes on the racemization of (*S*)-**1**[a] (0.1 M) in the presence of acetic acid (0.1 M) in TBME after 2 h at room temperature

Entry	Aldehyde (0.1 M)	ee (%)	**1** left (%)
1	Pivalaldehyde	96	100
2	Acetaldehyde	0	95
3	Pyridoxal	97	29
4	Benzaldehyde	10	93
5	4-Methylbenzaldehyde	4	89

[a] Original $ee^{(S)\text{-}\mathbf{1}}$=99%.

Table 4. Effects of acetaldehyde and acetic acid concentrations on the racemization of (*S*)-**1**[a] (0.1 M) and (*S*)-**2**[b] (0.1 M) in TBME after 2 h at room temperature

Entry	Acetic acid (M)	Acetaldehyde (M)	$ee^{(S)\text{-}\mathbf{1}}$ or $^{(S)\text{-}\mathbf{2}}$ (%)	**1** or **2** left (%)
1	0	0.1	**1**; 86	95
2	0.01	0.1	**1**; 79	96
3	0.05	0.1	**1**; 27	96
4	0.1	0.1	**1**; 0	95
5	0.1	0	**1**; 99	97
6	0.1	0.05	**1**; 66	100
7	0.1	0.1	**1**; 0	95
8	0.1	0.25	**1**; 1	86
9	0.1	0.5	**1**; 2	46
10	0	0.1	**2**; 95	100
11	0.1	0.1	**2**; 93	98
12	0.5	0.1	**2**; 83	93
13	0.1[c]	0.1	**2**; 90	100
14	0.25[c]	0.1	**2**; 82	97
15	0.5[c]	0.1	**2**; 76	74

[a] Original $ee^{(S)\text{-}\mathbf{1}}$=99%.
[b] Original $^{(S)\text{-}\mathbf{2}}$=95%.
[c] Temperature 48 °C.

source of acetaldehyde. In method II, the reaction proceeded in the presence of acetic acid (0.1 M) with the purpose to enhance racemization. In method III, triethylamine (or ammonium acetate) was added to the mixture of a substrate (0.1 M) and vinyl butanoate (0.2 or 0.4 M) in TBME in order to enhance racemization and to bind the liberated butanoic acid. It is worth mentioning that 2,2,2-trifluoroethyl and vinyl butanoates as activated esters are partly hydrolyzed by water in the seemingly dry enzyme preparation, always giving some butanoic acid in the reaction mixture independent of which method is considered.

In accordance with the fast racemization in the case of (*S*)-**1** with acetic acid (Table 4, entry 4), *rac*-**1** (0.1 M) was effectively transformed to (*S*)-**3** in TBME using either method I or II (Table 5, entries 1, 3, and 4). The same methods were useless for the dynamic kinetic resolution of *rac*-**2** (entries 7 and 9). Accordingly, the reaction by method I almost stopped at ca. 30% yield for (*S*)-**4** after 16 h and thereafter was slowly approaching to 43% yield at 75% total conversion in 3–4 days. The reaction closely followed the progression curves shown in Figure 2 where no acid was added. There was no increase in the amount of (*S*)-**4** either when normal kinetic resolution was allowed to proceed first to 48% conversion ($ee^{(R)\text{-}\mathbf{2}}$=91% and $ee^{(S)\text{-}\mathbf{4}}$=99%) before acetic acid (0.1 M) and acetaldehyde (0.1 M) were added. Instead and as expected, the less reactive enantiomer was subject to slow racemization ($ee^{(R)\text{-}\mathbf{2}}$=73%, $ee^{(S)\text{-}\mathbf{4}}$=99% after 70 h). Under the conditions of method II, the enzymatic acylation of *rac*-**2** stopped when only 25% of (*S*)-**4** was obtained ($ee^{(S)\text{-}\mathbf{4}}$=96%) at the point where 33% ($ee^{(R)\text{-}\mathbf{2}}$=3) of the starting material was left unreacted (entry 9). Replacing acetaldehyde with benzaldehyde did not alleviate the problem and the acylation of *rac*-**2** in the presence of acetic acid (0.1 M) stopped at 28% yield for the product. The above-suggested improved existence of stable adducts like **6** with an added acid (or that formed from an acyl donor) and the precipitation of amino esters as acetate (or butanoate) salts can explain where at least part of the original substrate disappeared.

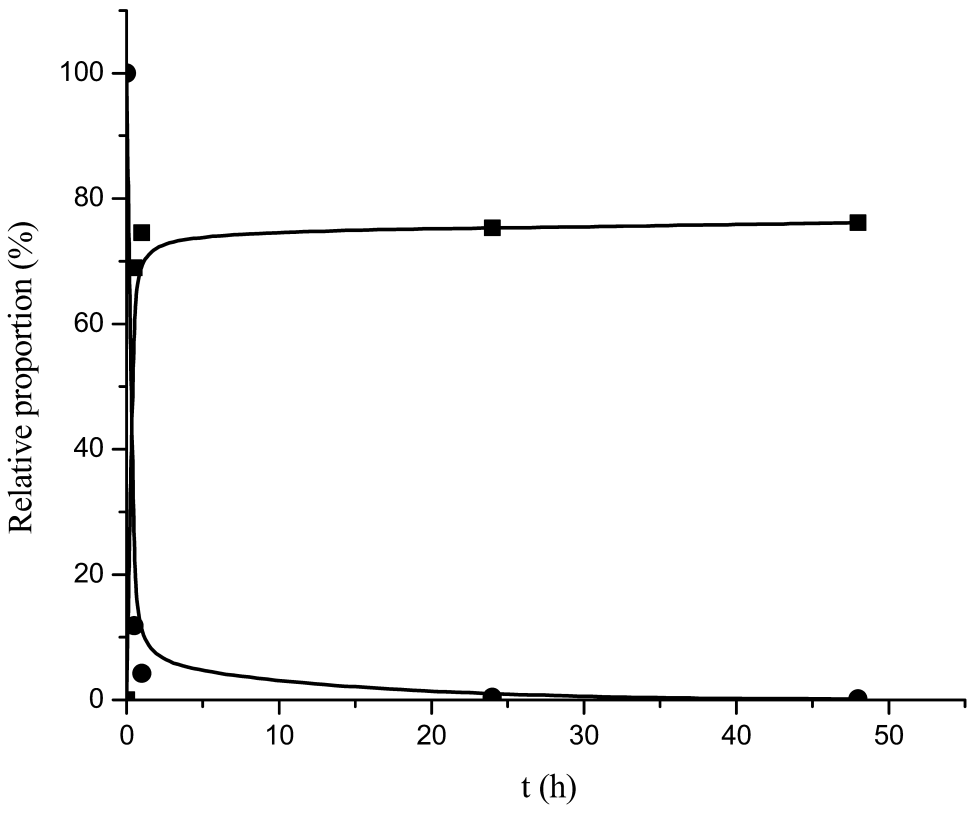

Figure 1. Progression curves for the formation of (*S*)-**3** (■) and disappearance of *rac*-**1** (●) when *rac*-**1** (0.1 M) reacts with vinyl butanoate (0.2 M) in TBME in the presence of CAL-A preparation (75 mg/mL).

Table 5. Dynamic kinetic resolution of *rac*-**1** and *rac*-**2** (both 0.1 M) with $PrCO_2R$ in TBME in the presence of CAL-A-preparation (75 mg/mL) and additives (0.1 M)

Entry	Substrate	R	ee$^{(S)\text{-}\mathbf{1}\text{ or }(S)\text{-}\mathbf{2}}$	(*S*)-**3** or (*S*)-**4** formed[a] (%)	Time (h)	Temperature (°C)	Additive
1	*rac*-**1**	CH_2CF_3[b]	99	97	25	25	CH_3CO_2H
2	*rac*-**1**	$CH{=}CH_2$[b]	98	75	1	25	None
3	*rac*-**1**	$CH{=}CH_2$[b]	96	82	3	25	CH_3CO_2H
4	*rac*-**1**	$CH{=}CH_2$[b]	97	88	3	48	CH_3CO_2H
5	*rac*-**1**	$CH{=}CH_2$[c]	97	86	1	25	$CH_3CO_2^-NH_4^+$
6	*rac*-**1**	$CH{=}CH_2$[c]	97	86	1	25	TEA
7	*rac*-**2**	CH_2CF_3[b]	97	43	187	48	CH_3CO_2H
8	*rac*-**2**	$CH{=}CH_2$[b]	97	39	24	25	None
9	*rac*-**2**	$CH{=}CH_2$[b]	96	25	45	48	CH_3CO_2H
10	*rac*-**2**	$CH{=}CH_2$[c]	97	50	24	25	TEA
11	*rac*-**2**	$CH{=}CH_2$[c]	97	61	24	48	TEA
12	*rac*-**2**	$CH{=}CH_2$[c]	97	69	24	56	TEA

[a] Values indicate maximum yields obtained in the reaction.
[b] 0.2 M.
[c] 0.4 M.

Finally in method III we decided to use base-induced racemization of the Schiff base (although the racemization tests of Table 2 were not promising) and to neutralize the formed butanoic acid by adding triethyl amine (0.1 M) in the enzymatic reaction mixture of *rac*-**1** and *rac*-**2** with vinyl butanoate in TBME. At this stage the concentration of vinyl butanoate was also doubled to 0.4 M. Surprisingly, the addition of triethyl amine caused ca. 10% enhancements in the product yield of (*S*)-**3** (Table 5, entry 6) compared to the case where no additives were present (entry 2). The same result was obtained when 0.05 and 0.1 M triethylamine was used. The presence of ammonium acetate affected in the same way (entry 5). For the acylation of *rac*-**2**, the positive effect of triethyl amine was even more pronounced and still increased at elevated temperature. Finally, ca. 70% of *rac*-**2** was transformed to (*S*)-**4** by refluxing the mixture of *rac*-**2** (0.1 M), vinyl butanoate (0.4 M) and CAL-A preparation (75 mg/mL) in TBME in the presence of triethyl amine (0.1 M) (entry 12).

From an economical point of view, vinyl acetate should be preferred over vinyl butanoate as an achiral acyl donor. When vinyl butanoate was replaced with vinyl acetate in the presence of triethyl amine 84% of initially *rac*-**1** was transformed to (*S*)-**3** with only 92% ee in 24 h at 48 °C. The reaction at room temperature stopped when 77% product was yielded. Due to higher enantiopurity, the dynamic kinetic resolution of *rac*-**1** in gram-scale was finally performed with vinyl butanoate as described in Section 4 using method III.

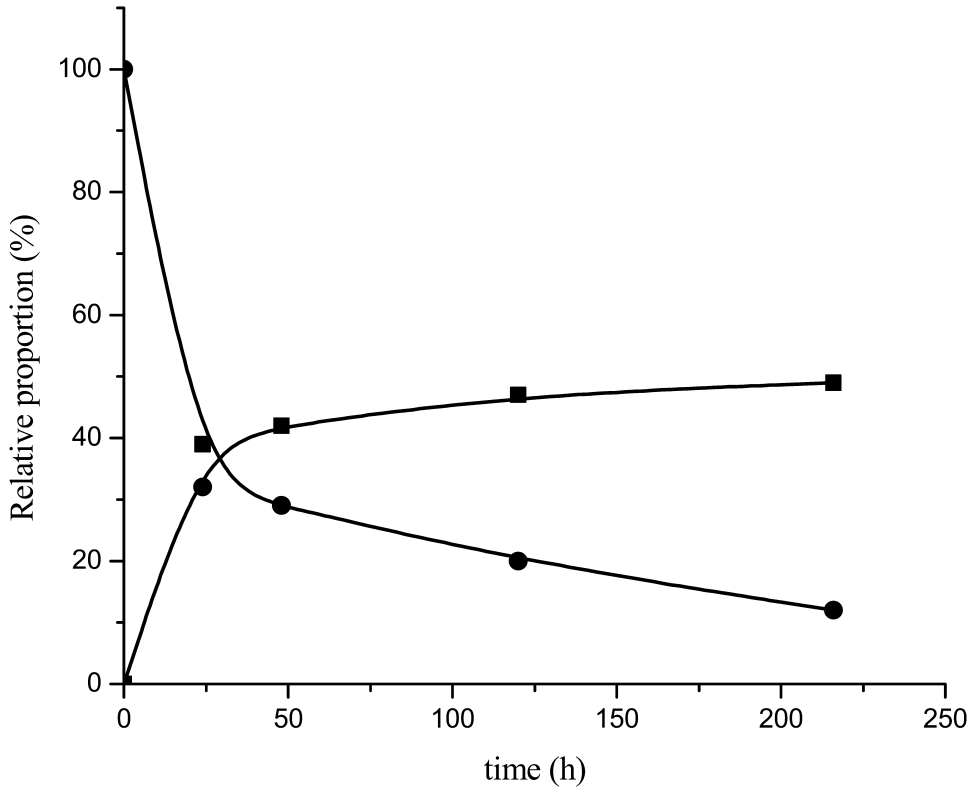

Figure 2. Progression curves for the formation of (*S*)-**4** (■) and disappearance of *rac*-**2** (●) when *rac*-**2** (0.1 M) reacts with vinyl butanoate (0.2 M) in TBME in the presence of CAL-A preparation (75 mg/mL).

3. Conclusions

The previously reported[2] exceptional property of lipase A from *Candida antarctica* to catalyze highly enantioselective *N*-acylations of *N*-heterocyclic amino esters as secondary amines has been confirmed in the present work. This property was used for dynamic kinetic resolution studies where the methyl esters of proline (*rac*-**1**) and pipecolic acid (*rac*-**2**) have served as racemic substrates and vinyl butanoate and 2,2,2-trifluoroethyl butanoate as achiral acyl donors in TBME (Tables 1 and 5). Negligible to moderate enantioselectivity with *E* values from 1 to 33 did not give reasons to use CAL-A for the dynamic kinetic resolution of various natural α-amino esters through the acylation of primary amino groups (Table 1).

For the enzymatic acylation of *rac*-**1** with vinyl esters the reaction was clearly approaching the conditions of dynamic kinetic resolution with 75% yield for (*S*)-**3**, showing the aldehyde-based racemization ability of the less reactive (*R*)-**1** under the acylation conditions (Scheme 1; Fig. 1; Table 5, entry 2). For the acylation of *rac*-**2** the reaction tended to stop when only 40% of the starting material was transformed to (*S*)-**4**. Three different strategies were chosen for improving the yields. In methods I and II, acetic acid served to catalyze aldehyde-based racemization. The methods failed badly with *rac*-**2** as the substrate. Salt formation between the acid and amino esters and/or intermediates such as **6** was suggested to explain the observed loss of the substrate and accordingly the low yield for the produced amide (*S*)-**4**. In method III, triethyl amine was added to the acylation mixture of *rac*-**1** and *rac*-**2** with vinyl butanoate in order to bind the butanoic acid which is liberated when the acyl donor is enzymatically hydrolyzed by the water in the enzyme preparation. In methods II and III the use of vinyl butanoate allows the releasing acetaldehyde to racemize the amino ester in situ. Under optimized conditions, ca. 90% of the racemic proline and 70% of the pipecolic acid methyl esters were acylated to highly enantiopure (ee=97%) butanamides with the *S*-absolute configurations.

4. Experimental

4.1. Materials and methods

Racemic proline, butyl butanoate and (*S*)-glutamic acid were obtained from Acros, methyl pipecolinate hydrochloride, valine, (*S*)-valine methyl ester hydrochloride, racemic and (*R*)-phenylglycine, racemic and (*R*)-methionine, *tert*-butyl methyl ether and dihexyl ether from Aldrich, methanol and petroleum ether from J. T. Baker and thionyl chloride from Riedel De-Haën. Vinyl butanoate, hexadecane, ethyl acetate, glutamic acid and (*S*)-proline were the products of Fluka. Dimethyl aspartate was a product of Sigma. All solvents were of the best analytical grade. (*S*)-methyl pipecolinate (ee=95%) was the product of the enzymatic kinetic resolution.[2] Amino acid methyl esters were synthesized by esterifying the acid with thionyl chloride in methanol followed by bubbling with ammonia in chloroform. Trifluoroethyl esters were synthesized from trifluoroethanol and an acid chloride. Absolute configurations were determined by synthesizing an enantiopure amino ester from a commercially available amino acid and by comparing the peak of the enantiomer with the peaks of a racemic compound in a chromatogram.

CAL-A was the product of Roche (Chirazyme L5, lyo.). Before use, CAL-A (20% (w/w)) was adsorbed on Celite by dissolving the enzyme (5 g) and sucrose (3 g) in Tris–HCl buffer (250 mL, 20 mM, pH 7.8) followed by the addition of celite (17 g). The mixture was dried by letting water evaporate. The enzyme preparation gave the initial velocity of 0.028 mmol/min/g for the acylation of racemic valine methyl ester (0.1 M) with trifluoroethyl butanoate (0.2 M) in TBME ($E=15.5\pm0.2$).

^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ on a Jeol Lambda 400 Spectrometer tetramethylsilane being as an internal standard. MS-spectrum was recorded on a VG Analytical 7070E instrument equipped with a VAXstation 3100 M76 computer. Optical rotation was measured using a Jasco DIP-360 polarimeter. The determination of *E* was based on equation $E=\ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$.[18] Using linear regression *E* was achieved as the slope of a line.

Typical reaction volume for enzymatic reactions was 1–3 mL. Substrate (0.1 M) and an acyl donor (0.2 M) were dissolved in TBME and an acid or base and acetaldehyde were added. CAL-A preparation (5–75 mg/mL) started the reaction. The progress of the reactions was followed by GC on Chrompack CP-Chirasil-DEX CB or Chrompack CP-Chirasil-L-Valine column by taking samples (0.1 mL) at intervals and derivatizing them with acetic anhydride (butanoate as an acyl donor) or butanoic anhydride (acetate as an acyl donor) and *N*,*N*-dimethylaminopyridine in pyridine (1% solution). *N*,*N*-dimethylaminopyridine solution was not used for those samples where aldehyde-induced racemization was present. Quantitative analysis of the reactions was performed by using dihexyl ether or hexadecane as internal standards (0.1 or 0.2 M).

4.1.1. Enzymatic gram-scale resolution. Before performing a gram scale reaction the amount of the enzyme preparation was optimized and as the best compromise 25 mg/mL was chosen. Vinyl butanoate (5.9 mL, 46.5 mmol) and *rac*-**1** (1.50 g, 11.6 mmol) were dissolved in TBME (116 mL). Addition of triethylamine (1.6 mL, 11.6 mmol) and CAL-A on Celite (2.904 g, 25 mg/mL) started the reaction. The reaction was stopped when 90% of *rac*-**1** was transformed to (*S*)-**3** after 5 h by filtering off the enzyme. The crude product was purified by column chromatography on Silicagel. After first purification (EtOAc–petroleum ether (3/7)) the product still contained butanoic acid. It was removed by dissolving the crude product in methanol and by adding thionyl chloride (200 μL) in an ice bath. After evaporation the product was repeatedly purified by column chromatography yielding (*S*)-**3** (viscous liquid, 1.79 g, 78%, 9.0 mmol, ee=97%, $[\alpha]_D^{20}$ −96.4 (*c*=1.04, MeOH)).

(*S*)-**3**. ^{1}H NMR: δ (ppm) 0.99 (t, *J*=7.4 Hz, 3H, CH_3CH_2), 1.69 (m, 7.6 Hz, 2H, CH_3CH_2), 1.90 (m, 2H, CH_2CON), 2.0–2.4 (m, 4H, CH_2CH_2CHN), 3.53 (m, 2H, CH_2N), 3.72

(s, 3H, CO_2CH_3), 4.49 (m, J=4.0, 8.5 Hz, 1H, C*H*), ^{13}C NMR: δ (ppm) 13.7 (CH_3CH_2), 17.9 (CH_3CH_2), 24.7 (CH_2CH_2CH), 29.1 (CH_2CH), 36.2 (CH_2CO), 46.9 (CH_2N), 52.0 (CO_2CH_3), 58.4 (*C*H), 171.8 (*C*O), 172.9 (*C*O). HRMS calculated for $C_{10}H_{17}NO_3$ M^+=199.1208. Found M^+=199.1216.

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References and notes

1. Petersen, M.; Sauter, M. *Chimia* **1999**, *53*, 608–612.
2. Liljeblad, A.; Lindborg, J.; Kanerva, A.; Katajisto, J.; Kanerva, L. T. *Tetrahedron Lett.* **2002**, *43*, 2471–2474.
3. Rios, A.; Crugeiras, J.; Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **2001**, *123*, 7949–7950.
4. Pietzsch, M.; Syldatk, C. In *Hydrolysis and Formation of Hydantoins*. *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 2, pp 761–799.
5. De Jersey, J.; Zerner, B. *Biochemistry* **1969**, *8*, 1967–1974.
6. Yamada, S.; Hongo, C.; Yoshioka, R.; Chibata, I. *J. Org. Chem.* **1983**, *48*, 843–846.
7. Chen, S.-T.; Huang, W.-H.; Wang, K.-T. *J. Org. Chem.* **1994**, *59*, 7580–7581.
8. Parmar, V. S.; Singh, A.; Bisht, K. S.; Kumar, N.; Belokon, Y. N.; Kochetkov, K. A.; Ikonnikov, N. S.; Orlova, S. A.; Tararov, V. I.; Saveleva, T. F. *J. Org. Chem.* **1996**, *61*, 1223–1227.
9. Barry, L. G.; Pugnière, M.; Castro, B. *Int. J. Pept. Protein Res.* **1993**, *41*, 323–325.
10. Grigg, R.; Gunaratne, H. Q. N. *Tetrahedron Lett.* **1983**, *24*, 4457–4460.
11. Gillard, R. D.; O'Brien, P. *J. Chem. Soc. Dalton Trans.* **1978**, 1444–1447.
12. Buckingham, D. A.; Marzilli, L. G.; Sargeson, A. M. *J. Am. Chem. Soc.* **1967**, *89*, 5133–5138.
13. Williams, J. M. J.; Parker, R. J.; Neri, C. In *Enzymatic Kinetic Resolution*. *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 1, pp 287–312.
14. May, O.; Verseck, S.; Bommarius, A.; Drauz, K. *Org. Process Res. Dev.* **2002**, *6*, 452–457.
15. Hacking, M. A. P. J.; Wegman, M. A.; Rops, J.; van Rantwijk, F.; Sheldon, R. A. *J. Mol. Catal. B: Enzym.* **1998**, *5*, 155–157.
16. Wegman, M. A.; Hacking, M. A. P. J.; Rops, J.; Pereira, P.; van Rantwijk, F.; Sheldon, R. A. *Tetrahedron: Asymmetry* **1999**, *10*, 1739–1750.
17. Belokon, Y. N.; Kochetkov, K. A.; Plieva, F. M.; Ikonnikov, N. S.; Maleev, V. I.; Parmar, V. S.; Kumar, R.; Lozinsky, V. I. *Appl. Biochem. Biotechnol.* **2000**, *84*, 97–106.
18. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
19. Liljeblad, A.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1999**, *10*, 4405–4415.
20. Gedey, S.; Liljeblad, A.; Lázár, L.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2001**, *12*, 105–110.
21. Kanerva, L. T.; Csomós, P.; Sundholm, O.; Bernáth, G.; Fülöp, F. *Tetrahedron: Asymmetry* **1996**, *7*, 1705–1716.
22. Solymár, M.; Liljeblad, A.; Lázár, L.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2002**, *13*, 1923–1928.