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Solvent-free kinetic resolution of primary amines catalyzed by *Candida antarctica* lipase B: effect of immobilization and recycling stability

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ABSTRACT

Highly enantioselective (E >200) N-acylation of nine racemic primary amines with isopropyl methoxyacetate in the presence of *Candida antarctica* lipase B (Novozym 435) has been reported to yield the unreacted (*S*)-amines (ee ⩾98%) and produced the (*R*)-amides (ee ⩾95%) at 50% conversion under solvent-free conditions. One of the amines and the acyl donor have been used in an equimolar ratio at room temperature (23 °C). Under the reaction conditions, the reuse stability of Novozym 435 with 1-phenylethylamine (as a model compound) has been shown to be poor while somewhat improved stability has been observed with an in-house prepared sol–gel CAL-B catalyst.

1. Introduction

Nitrogen containing enantiopure compounds, such as amines, amino acids, and their derivatives, are important chiral building blocks and final products especially in the pharmaceutical and fine chemical industries. With increasing interest in environmental aspects, research on the preparation of enantiomers containing amino groups is increasingly focused on the methods that fulfill the principles of green chemistry. In this respect, biocatalytic approaches especially in organic solvents, are extensively applied and have also been reviewed.[1–7] Reported strategies cover enzymatic kinetic and dynamic kinetic resolution methods and various methods that exploit enzymatic asymmetric synthesis. There are several factors (enzyme availability, expense, ease of use, reuse possibility, solubility and stability of the substrates, and products etc.) in addition to enzymatic stereo-, regio-, and chemo-selectivities which need to be taken into account when the viability of a suitable biocatalytic method is considered.

Lipases (E.C. 3.1.1.3) are useful catalysts for the kinetic resolution of primary[1–7] and less common secondary[8–10] amino group containing enantiomers through enantioselective N-acylation in dry organic solvents since lipases lead to irreversible N-acylation due to the fact that amide bonds, other than those in β-lactams,[11–13] are unreactive toward lipases. However, caution should be taken to prevent the partial hydrolysis of an acyl donor by the so-called residual water in the lipase preparation or by water otherwise present in the reaction mixture as the acid liberated by this side-reaction may cause, for instance, amine precipitation through ion-pair formation. Lipase-catalyzed ester hydrolysis is possible, especially when *Candida antarctica* lipase B as a Novozym 435 preparation (CAL-B adsorbed on a divinylbenzene-crosslinked, hydrophobic macroporous polymer based on methyl and butyl methacrylic esters) is involved. Firstly, the hydrophobic immobilization material will readily release any adsorbed water into the reaction mixture.[14,15] Accordingly, a catalyst obtained by other immobilization methods could be of interest. Secondly, a water tunnel, existing in some enzymes such as CAL-B, allows easy access for water from the medium to the active site.[16]

The aim herein is to investigate the CAL-B-catalyzed solvent-free kinetic resolution of amines *rac*-**1a**–**i** under ambient conditions (Scheme 1). Enantioselective N-acylations under solvent-free conditions are rare, and those reported have been performed under harsh reaction conditions with elevated temperatures and reduced pressures.[17–19] The use of solvent-free reaction systems (systems where an acyl donor is not in the role of a solvent) is a step toward green chemistry as solvents represent a large part of the waste formed in a conventional kinetic resolution using enzymatic reactions in organic solvents. As limitations for solvent-free reactions, substrates need to be liquids or one of them should dissolve the other. Other bottlenecks are enzyme tolerance and selectivities together with slow reactions in approximately equimolar mixtures of an amine and acyl donor. Reuse stability, rather than initial rate-based entities is the best measure for enzyme tolerance in synthetic applications. In addition to the classical Novozym 435, an in-house prepared sol–gel CAL-B has been investigated as a reusable catalyst for the N-acylation of *rac*-**1a**. Sol–gel entrapment was selected as an immobilization method since the entrapment is easy to perform by the acid- or base-catalyzed hydrolysis of silanes.[20–22] Sol–gel lipase catalysts have also given promising

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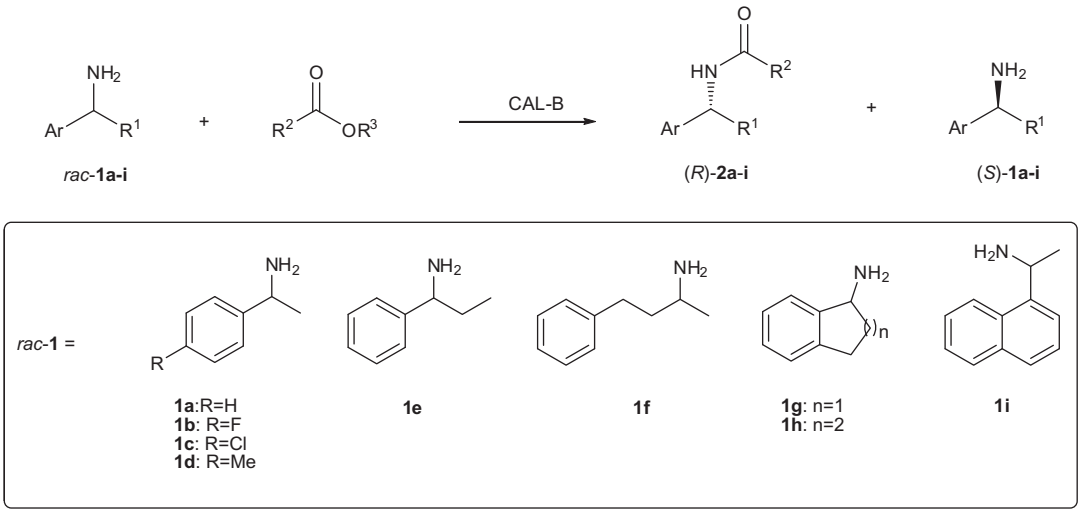


Scheme 1. N-acylation of primary amines **1** with CAL-B.

results as to enzymatic reactivity, enantioselectivity, and reuse stability.

2. Results and discussion

2.1. Optimization of solvent-free reaction conditions

Isopropyl, ethyl, and 2,2,2-trifluoroethyl esters together with acyl activated methoxyacetates are often used as acyl donors for the lipase-catalyzed N-acylation of amino group containing compounds in organic solvents.[1–10] The high enantioselectivity of CAL-B, previously observed for the N-acylation of 1-phenylethylamine *rac*-**1a** with ethyl acetate[23] and later with ethyl and isopropyl methoxyacetates[21,24] in organic solvents, forms the basis for the present investigation under solvent-free conditions. The kinetic resolution of *rac*-**1a** (0.1 M, a model substrate) with potential acyl donors (0.2 M) was first surveyed in toluene (Table 1). To minimize the risk of enzymatic hydrolysis of the acyl donor the reactions were performed in the presence of molecular sieves (4 Å). Acylation with isopropyl acetate (entry 3) gave moderate enantioselectivity (measured as the enantiomer ratio value, $E = 49$) while both butanoate and methoxyacetate esters proceeded with excellent enantioselectivity (E >200, entries 1, 2, and 4). The same behavior was seen under solvent-free conditions (entries 5–15). The acyl activated methoxyacetates both gave excellent reactivities (entries 5–8), the activation being mainly connected to the methoxy oxygen of the acyl donor and to the two-step mechanism of serine hydrolases, proceeding through the so-called acyl-enzyme intermediate. Thus, the existence of a weak hydrogen bond between the β-oxygen atom of the methoxyacetate moiety in the intermediate and the amine hydrogen of the reactive amine counterpart has been proven.[25] The effect of the enzyme content on the acylation of *rac*-**1a** with equimolar amounts of isopropyl methoxyacetate revealed that 25 mg of the enzyme was optimal, giving 50% conversion in 6 h. The reaction with 10 mg of the catalyst was not yet complete in the same time and there was no improvement on the reaction with 50 mg of the enzyme (entries 5–7). The benefit of a solvent-free reaction is clear when the enzyme efficiencies (mg of the enzyme)/(mmol of the substrate), 100 mg mmol^{-1} for the reaction of *rac*-**1a** (0.1 M) in toluene and 12.5 mg mmol^{-1} for that of *rac*-**1a** (2 mmol) reacting with an equimolar amount of methoxyacetate, are considered. On the other hand, acylations with unactivated esters were too slow to be of any practical use (conversions of the order of 10% after 6 h, entries 9–15). An increase in the temperature or in the amount of the acyl donor did not help the situation.

The kinetic resolution of *rac*-**1a** (2 mmol) with ethyl and isopropyl methoxyacetates (2 mmol) in the presence of Novozym 435

Table 1
Novozym 435-catalyzed kinetic resolution of *rac*-**1a** (0.1 M in toluene or 2 mmol) with acyl donor (0.2 M or 1–10 equiv) in the presence of molecular sieves (4 Å, 50 mg) after 6 h

Entry	Solvent	Acyl donor (equiv)	T (°C)	Enzyme (mg)	Conv. (%)	ee$^{(S)\text{-}\mathbf{1a}}$ (%)	ee$^{(R)\text{-}\mathbf{2a}}$ (%)	E
1	Toluene[a]	Isopropyl methoxyacetate (0.2 M)	23	10	50	96	98	>200
2	Toluene[a]	Ethyl methoxyacetate (0.2 M)	23	10	49	94	99	>200
3	Toluene[a]	Isopropyl acetate (0.2 M)	23	10	25	32	95	49
4	Toluene[a]	Isopropyl butanoate (0.2 M)	23	10	20	25	>99	>200
5	—	Isopropyl methoxyacetate (1)	23	10	47	87	99	>200
6	—	Isopropyl methoxyacetate (1)	23	25	50	98	99	>200
7	—	Isopropyl methoxyacetate (1)	23	50	50	98	>99	>200
8	—	Ethyl methoxyacetate (1)	23	25	45	81	99	>200
9	—	Isopropyl acetate (1)	23	25	7	7	95	72
10	—	Isopropyl acetate (1)	47	25	10	10	95	16
11	—	Isopropyl butanoate (1)	23	25	6	6	>99	>200
12	—	Isopropyl butanoate (1)	47	25	8	9	>99	>200
13	—	Isopropyl butanoate (1)	70	25	10	11	>99	>200
14[b]	—	Isopropyl butanoate (5)	23	25	13	15	>99	>200
15[b]	—	Isopropyl butanoate (10)	23	25	18	22	>99	>200

[a] Reaction volume 1 mL.
[b] 0.5 mmol substrate.

Table 2
Novozym 435-catalyzed kinetic resolution of *rac*-**1a** (2 mmol) with acyl donor (0.6–1.25 equiv) in the presence of molecular sieves (4 Å, 50 mg)

Entry	Alkyl methoxyacetate (equiv)	*T* (°C)	CAL-B (mg)	Time[a] (h)	Conv. (%)	ee$^{(S)\text{-}\mathbf{1a}}$ (%)	ee$^{(S)\text{-}\mathbf{2a}}$ (%)	*E*
1	Isopropyl (1)	rt	25	6	50	98	99	>200
2	Isopropyl (1)	47	25	6	50	97	98	>200
3	Isopropyl (1)	70	25	6[b]	34	71	96	—[c]
4	Isopropyl (1.25)	rt	25	4	50	99	99	>200
5	Isopropyl (0.75)	rt	25	6 (24)	47	87	>99	>200
6	Isopropyl (0.6)	rt	25	6 (56)	43	75	>99	>200
7	Ethyl (1)	rt	25	6 (24)	45	81	99	>200
8	Ethyl (1)	47	25	6[b]	40	65	97	—[c]
9	Ethyl (1)	70	25	6[b]	30	40	86	—[c]

[a] Reaction time required to reach 50% conversion in parentheses.
[b] Reaction halted before 50% conversion.
[c] *E* value could not be reliably determined.

(25 mg) was optimized further with respect to the temperature and the amount of acyl donor under the solvent-free conditions (Table 2). A gradual increase in temperature from 23 to 70 °C caused the enzyme to lose its stability with time (entries 1–3 and 7–9) and the reactions to halt before the full 50% conversion was reached. Moreover, the enantioselectivity dropped at elevated temperatures as seen on the basis of the ee$^{(R)\text{-}\mathbf{2a}}$ values, the effect being more pronounced with ethyl than with isopropyl methoxyacetate. On the other hand, an increase in isopropyl methoxyacetate content from 0.6 to 1.25 equiv decreased the time to the full 50% conversion by 52 h (entries 1 and 4–6). From an economical point of view, the optimal conditions for the kinetic resolution of *rac*-**1a** with isopropyl methoxyacetate (1 equiv) and Novozym 435 (25 mg mL^{-1}) at 23 °C were settled.

For substrate scope studies, the Novozym 435-catalyzed solvent-free kinetic resolution under the optimized conditions was extended to encompass nine structurally different primary amines (*rac*-**1a**–**i**, Scheme 1). The results are presented in Table 3. With *rac*-**1i** (entry 5), an elevated reaction temperature (47 °C) was needed to increase the solubility of the amine and the amide product formed. The time needed to reach 50% conversion was naturally dependent on the structure of the racemate. All of the kinetic resolution products (the unreacted (*S*)-amine and the product (*R*)-amide, Scheme 1) were obtained in highly enantiomerically enriched forms (ee ⩾95%, mostly 99% or higher) at 50% conversions. The products were separated from the kinetic resolution mixtures with high isolated yields by column chromatography. It is worth noting that separation by distillation should be possible after scale-up as described for reactions in solvents in a BASF patent.[24] The results indicate that the general nature of the solvent-free method is comparable to reactions[1–7] previously described in organic solvents. Actually, the difference between the solvent (reaction times given in Table 3 in the parentheses) and solvent-free reactions is in times needed to reach 50% conversion and as already stated, in enzyme efficiency (100 mg enzyme/1 mmol substrate and only 12.5 mg enzyme/1 mmol substrate). Specific rotations, mostly in accordance with the corresponding literature values when available,[26–33] were determined in dichloromethane apart from (*S*)-**1i** and (*R*)-**2i** where methanol was the solvent. With (*R*)-**2a**, (*R*)-**2d**, (*R*)-**2e,** and (*R*)-**2g** (entries 1, 4, 6, and 7), the specific rotations obtained in chloroform differ considerably from the literature values determined in methanol[26] (see Section 4). The values determined in methanol herein are in accordance with the literature values indicating the considerable solvent dependence of $[\alpha]_D^{25}$.

2.2. Immobilization of CAL-B

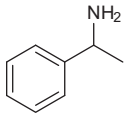
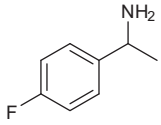
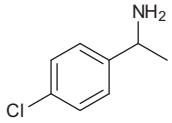
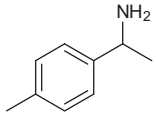
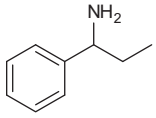
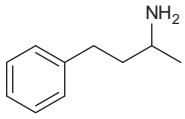
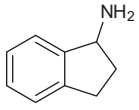
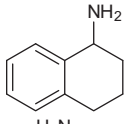
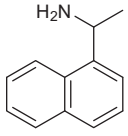
In enzymatic applications, enzyme immobilization plays an important role in stabilizing the catalyst and allowing its reuse in successive reactions. Immobilization material and the method used for immobilization considerably affect the outcome of a catalyst. As CAL-B encapsulated in sol–gel matrices was previously shown to have excellent stability in solvent reactions,[21] attention was now directed to the preparation of sol–gel CAL-B catalysts. A previously described sol–gel encapsulation method was utilized.[21,22] The method uses the fluoride ion-catalyzed hydrolysis of the mixture of tetramethylorthosilicate (TMOS) and methyltrimethoxysilane (MTMS) followed by condensation and gelation. The [TMOS]/[MTMS] ratio 1/5 and gel/water ratio 1/10 were shown to be optimal in accordance with previous observations with lipases.[21,22] Additives in sol–gel CAL-B, such as isopropyl alcohol (IPA), polyvinyl alcohol (PVA), and 18-crown-6, were previously reported to result in a dramatic increase in CAL-B activity.[21] For that reason, Celite, Celite together with sucrose, 18-crown-6, 18-crown-6 together with Celite and PVA together with IPA were used as additives in sol–gel CAL-B preparations. Celite itself is a generally used immobilization material for adsorption-based immobilization, and the presence of sucrose together with Celite has been shown to enhance the activity and stability of *Pseudomonas fluorescens* lipase when encapsulated by the sol–gel method.[22] The enzyme loading in each catalyst batch was kept constant (75 mg) against 3 mmol of a mixture of TMOS and MTMS. After maturation, the obtained sol–gel CAL-B preparation was dried by lyophilization.

The sol–gel CAL-B catalysts were characterized using the N-acylation of *rac*-**1a** (0.1 M) with isopropyl methoxyacetate (0.2 M) in toluene (Fig. 1). A solvent reaction rather than a solvent-free one was chosen for catalyst optimization in order to minimize the effect of possible diffusional limitations. The protein content of the catalysts was set to 2.6 mg mL^{-1} in each reaction. Independent of the nature of additives, the reactions all reached 50% conversion in 24 h allowing the kinetic resolution of *rac*-**1a**. However, the presence of different additives in the catalysts caused clear differences in reactivity over the first hours of the N-acylation, the reaction being slowest with Celite and most effective with the mixture of PVA and IPA. The additive-free sol–gel preparation was of the similar activity as the preparation obtained in the presence of PVA and IPA. The additive-free sol–gel CAL-B catalyst was chosen for next reuse studies under solvent-free conditions in order to keep the encapsulation as simple as possible. Interestingly, the enantioselectivity of the sol–gel CAL-B catalyst was excellent (*E* >200) independent of the hydrophobic character (the ratio of TMOS and MTMS) and the preparation method of the sol–gel.

2.3. Reuse of the CAL-B catalysts under solvent-free conditions

With Novozym 435 and the optimized sol–gel CAL-B (without additives) in hand, the studies were turned to reuse experiments for the acylation of *rac*-**1a** (2 mmol) with isopropyl methoxyacetate

Table 3
Preparative kinetic resolution of *rac*-**1a-i** (2 mmol) with isopropyl methoxyacetate (2 mmol) and Novozym 435 (25 mg) under solvent-free conditions in the presence of molecular sieves (4 Å, 50 mg) at 23 °C

Entry	Substrate	Time[a] (h)	(*S*)-**1**			(*R*)-**2**		
			Isolated yield (%)	ee (%)	$[\alpha]_D^{25}$[b]	Isolated yield (%)	ee (%)	$[\alpha]_D^{25}$[b]
1		6 (6)	35	98	−30.6	48	97	+101
2		3 (6)	37	>99	−24.4	39	97	+84.7
3		2 (6)	46	>99	−28.4	47	98	+102
4		3 (2)	45	>99	−30.5	45	98	+105/+120[c]
5		24 (48)	41	99	−9.4	45	99	+93.5/+103[c]
6		1 (1)	45	>99	+4.1	48	98	+34.1
7		2 (1)	39	>99	+20.8	44	95	+62.2/+105[c]
8		2 (24)	34	98	+34.7	45	99	+75.7
9[d]		6 (48)	35	>99	−59.2[d]	37	99	+30.0[c]

[a] Time to reach the 50 ± 0.5% conversion; in parentheses the time needed to reach 50% conversion in toluene [amine (0.1 M), isopropyl methoxyacetate (0.2 M), Novozym 435 (10 mg mL^{-1})].
[b] (*c* 1, $CHCl_3$).
[c] (*c* 2, MeOH).
[d] Reaction temperature 47 °C.

(2 mmol) under solvent-free conditions. Since the protein content of Novozym 435 is not known, the specific activities (initial rates divided by the mass of the CAL-B preparation) of the catalysts were determined as 0.25 μmol min^{-1} mg^{-1} for Novozym 435 and 0.41 μmol min^{-1} mg^{-1} for sol–gel CAL-B. On this basis, 25 mg of Novozym 435 and 16 mg of sol–gel CAL-B (without additives) were used in order to make the N-acylations comparable (Fig. 2).

For these reused experiments, every cycle was left to proceed to 50% conversion (or to the conversion reached in 24 h when the enzymatic activity had considerably decreased) before the enzyme preparation was separated by gentle centrifugation and the new substrates were added. The high stability of CAL-B as a Novozym 435 preparation is well known. Thus, it was not surprising that both Novozym 435 and sol–gel CAL-B gave reproducible results in terms of fully retained reactivity and enantioselectivity (*E* >200) for the kinetic resolution of *rac*-**1a** (0.1 M) with isopropyl methoxyacetate (0.2 M) in toluene over the five reuse cycles investigated (results not shown). However, for the kinetic resolution of *rac*-**1a** (2 mmol) with isopropyl methoxyacetate (2 mmol) under solvent-free conditions the reactivity of Novozym 435 significantly decreased with every reuse, the second cycle already requiring two days in order to reach the full 50% conversion instead of 6 h in the first cycle. In the following reaction cycles, the trend in the loss of reactivity increased, destroying the reuse possibility of the catalyst (Fig. 2A). The sol–gel CAL-B catalyst (16 mg) also lost part of its activity in every reuse although much less than Novozym 435 (Fig. 2B). Although the 50% conversion was reached in 16 h (compared to 6 h with Novozym 435) in the first cycle, the next cycles still were feasible. In spite of the loss in activity during reuse, both Novozym 435 and sol–gel CAL-B retained the same excellent

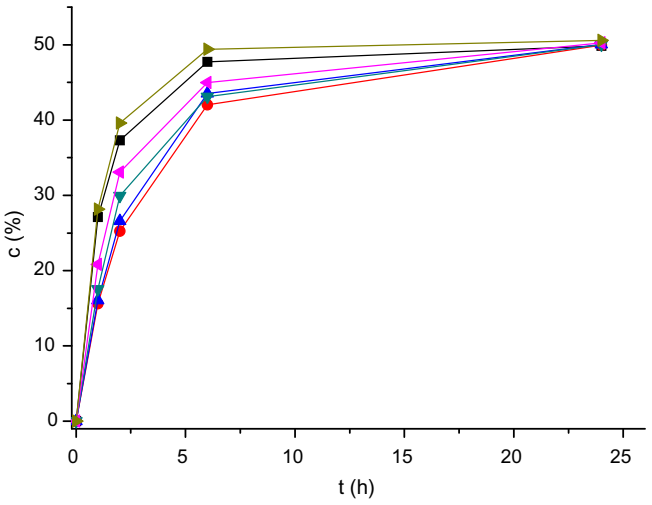


Figure 1. Effect of additives on the sol–gel CAL-B catalysts. Progression curves for the N-acylation of *rac*-**1a** (0.1 M) with isopropyl methoxyacetate (0.2 M) in toluene at 23 °C: Additive-free (■), Celite (●), Celite and sucrose (▲), 18C6 (▼), 18C6 and Celite (◀) and PVA and IPA (▶).

enantioselectivity (E >200) over the five sequentially performed reactions.

3. Conclusion

A solvent free kinetic resolution method has been developed, utilizing *Candida antarctica* lipase B as Novozym 435 and sol–gel CAL-B catalysts and equimolar amounts of racemic amines and isopropyl methoxyacetate under ambient reaction conditions. Optimization of the reaction conditions was performed with 1-phenylethylamine *rac*-**1a** as a model substrate. The substrate scope of the method with Novozym 435 was extended to 9 structurally different primary amines *rac*-**1a**–**i**, which all were resolved with excellent enantioselectivity (E >200), and the resolution products (*S*)-**1** and (*R*)-**2** were separated with good isolated yields and ee ⩾95%. We have shown that the reusability of CAL-B under the demanding solvent-free conditions is dependent on the immobilizate, Novozym 435 losing considerable activity in every reuse while sol–gel CAL-B, being somewhat more stable, allows a couple of cycles with reasonable time extensions. We have shown that only loss of reactivity restricts the reuse possibilities as the enantioselectivity (E >200) is excellent with both catalyst preparations from cycle to another as it is also for the traditional solvent reaction in toluene.

4. Experimental

4.1. Materials and methods

Solvents were purchased from standard suppliers and were dried prior to use. Amines were purchased from Fluka and Sigma–Aldrich and were used as received. Isopropyl methoxyacetate was prepared from the corresponding acid chloride and isopropyl alcohol. Poly(vinyl alcohol) (M_w 31,000–50,000) and Celite (Filter Agent Celite, high-purity analytical grade) were purchased from Sigma–Aldrich. Lipase B from *Candida antarctica* (CAL-B, Novozym 435) was purchased from Novozymes and free CAL-B-powder from Biocatalytics. The protein content of the free CAL-B-powder was determined using the bicinchonic acid assay with bovine serum albumin as the standard protein.[34,35] NMR spectra were recorded with a Bruker Avance 500 spectrometer using tetramethylsilane (TMS) as an internal standard. The NMR spectra of the separated amines (*S*)-**1a**–**i** are identical to those of the commercial amines *rac*-**1a**–**i**. The NMR spectra of the amides (*R*)-**2a**, (*R*)-**2d**, (*R*)-**2e**, (*R*)-**2g,** and (*R*)-**2i** formed are identical with those reported in the literature.[26] HRMS were measured in ESI$^+$ mode with Bruker micOTOF-Q quadrupole-TOF spectrometer. HRMS were not obtained for (*S*)-**1a**–**i** because of low intensity. Chiral GC analyses **1a**–**h** were performed with a gas chromatograph equipped with a Chrompack CP-Chirasil-DEX CB column and chiral HPLC analysis **1i** with an instrument equipped with a Chiralcel OD-H column using a mixture of *n*-hexane and 2-propanol as eluent. Optical rotations were determined with the aid of a Perkin-Elmer 341 polarimeter using sodium D line, and the $[\alpha]_D^{25}$-values are given in units of 10^{-1} deg cm^2 g^{-1}. Melting points were recorded with a Gallenkamp apparatus and are uncorrected. The values of enantiomer ratio (E) and conversions (c) were obtained using the equation $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)$ with $c = ee_S/(ee_S+ee_P)$.[36]

4.2. Kinetic resolution of *rac*-1a in toluene and under solvent-free conditions

One of the acyl donors (0.2 M; isopropyl acetate, isopropyl butanoate. ethyl or isopropyl methoxyacetate) was added into a reaction vessel containing molecular sieves (4 Å, 50 mg) and the suspension of Novozym 435 (10 mg mL^{-1}), toluene (1 mL) and *rac*-**1a** (0.1 M), or *rac*-**1a** (2 mmol) and an acyl donor (2 mmol) were added into a reaction vessel containing Novozym 435 (10–50 mg) and molecular sieves (4 Å, 50 mg). The reaction mixture was shaken (170 rpm) at room temperature (23 °C) or at 47 °C. The reactions were monitored by taking samples which were derivatized with acetic or propionic anhydride and analyzed by GC or HPLC.

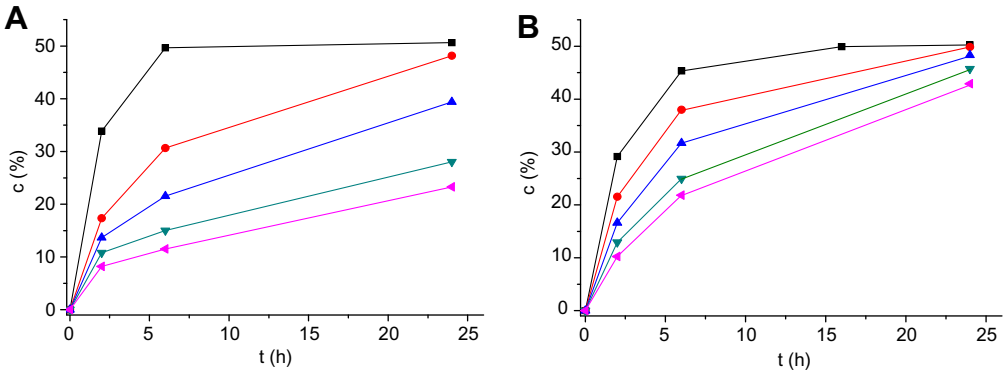


Figure 2. Progression curves for the solvent-free kinetic resolution of *rac*-**1a** (2 mmol) with isopropyl methoxyacetate (2 mmol) at 23 °C in reuse with (A) Novozym 435 (25 mg) and (B) sol–gel CAL-B (16 mg): cycle 1 (■), cycle 2 (●), cycle 3 (▲), cycle 4 (▼) and cycle 5 (◀).

4.3. Preparative-scale solvent-free kinetic resolution of *rac*-1a–i

One of the amines *rac*-**1a–i** (2 mmol) and isopropyl methoxyacetate (2 mmol) were added into a reaction vessel containing Novozym 435 (25 mg) and molecular sieves (4 Å, 50 mg). The reaction mixture was shaken (170 rpm) at room temperature (23 °C) if not otherwise stated. The reaction was stopped by filtering off the enzyme at (50 ± 0.5)% conversion. Isolation of the products was performed by silica gel chromatography using a mixture of hexane and ethylacetate and/or mixture of dichloromethane and methanol as eluent.

4.3.1. Compound (*S*)-1a

Clear oil in 35% yield {ee = 98%, $[\alpha]_D^{25} = -30.6$ (*c* 1, $CHCl_3$); lit.[27] $[\alpha]_D^{25} = -32.7$, ee >99% (*c* 1.04, $CHCl_3$)}.

4.3.2. Compound (*R*)-2a

White solid in 48% yield {ee = 97%, $[\alpha]_D^{25} = +101$ (*c* 1, $CHCl_3$); lit.[26] $[\alpha]_D^{25} = +110$, ee = 99% (*c* 2, MeOH)}. HRMS $[M+Na]^+$ found ($[M+Na]^+$ calculated for $C_{11}H_{15}NO_2Na$): 216.1028 (216.0995). The NMR data identical with those reported in the literature.[26]

4.3.3. Compound (*S*)-1b

Light yellow oil in 37% yield {ee >99%, $[\alpha]_D^{25} = -24.4$ (*c* 1, $CHCl_3$)}.

4.3.4. Compound (*R*)-2b

White solid in 39% yield {mp 68–69 °C, ee = 97%, $[\alpha]_D^{25} = +84.7$ (*c* 1, $CHCl_3$)}. HRMS $[M+Na]^+$ found ($[M+Na]^+$ calculated for $C_{11}H_{14}FNO_2Na$): 234.0921 (234.0901); ^1H NMR (DMSO, 500 MHz), δ (ppm): 8.20 (d, 1H, N*H*), 7.37 (2H, Ar*H*), 7.13 (2H, Ar*H*), 4.99 (quint., *J* = 7.0 Hz, 1H, C*H*), 3, 1 (2H, CH_2), 3.30 (s, 3H, OCH_3), 1.38 (d, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (DMSO, 126 MHz), δ (ppm): 167.97 (*C*O), 161.87, 159. 94 (d, arom *C*), 140.64 (arom *C*), 127.99, 127.92 (arom *C*H), 114.85, 114.68 (arom *C*H), 71.38 (CH_2), 58.42 (OCH_3), 46.73 (*C*H), 21.98 (CH_3).

4.3.5. Compound (*S*)-1c

Pale yellow oil in 46% yield {ee >99%, $[\alpha]_D^{25} = -28.4$ (*c* 1, $CHCl_3$); lit.[28] $[\alpha]_D^{25} = -23.7$, ee >99% (*c* 2, MeOH)}.

4.3.6. Compound (*R*)-2c

White solid in 47% yield {mp 79–80 °C, ee = 98%, $[\alpha]_D^{25} = +102$ (*c* 1, $CHCl_3$)}. HRMS $[M+Na]^+$ found ($[M+Na]^+$ calculated for $C_{11}H_{14}ClNO_2Na$): 250.0597 (250.0605); ^1H NMR (DMSO, 500 MHz), δ (ppm): 8.23 (d, *J* = 8.0 Hz, 1H, N*H*), 7.36 (4H, Ar*H*), 4.98 (quint., *J* = 7.0 Hz, 1H, C*H*), 3.81 (2H, CH_2), 3.30 (s, 3H, OCH_3), 1.37 (d, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (DMSO, 126 MHz), δ (ppm): 168.06 (*C*O), 143.51 (arom *C*), 131.05 (arom *C*), 128.04 (arom *C*H), 127.94 (arom *C*H), 71.37 (CH_2), 58.43 (OCH_3), 46.83 (*C*H), 21.79 (CH_3).

4.3.7. Compound (*S*)-1d

Light yellow oil in 45% yield {ee >99%, $[\alpha]_D^{25} = -30.5$ (*c* 1, $CHCl_3$)}.

4.3.8. Compound (*R*)-2d

White solid in 45% yield {ee = 98%, $[\alpha]_D^{25} = +105$ (*c* 1, CHCl3), $[\alpha]_D^{25} = +119.7$ (*c* 2, MeOH); lit.[26] $[\alpha]_D^{25} = +124$ (*c* 2, MeOH), ee = 99.1%}. HRMS $[M+Na]^+$ found ($[M+Na]^+$ calculated for $C_{11}H_{14}ClNO_2Na$): 230.1182 (230.1152). The NMR data identical with those reported in the literature.[26]

4.3.9. Compound (*S*)-1e

Yellow oil in 35% yield {ee >99%, $[\alpha]_D^{25} = -59.2$ (*c* 2, MeOH); lit.[29] $[\alpha]_D^{25} = -18.0$, ee = 90% (*c* 1.0, EtOH)}.

4.3.10. Compound (*R*)-2e

White solid in 37% yield {ee = 99%, $[\alpha]_D^{25} = -30.0$ (*c* 2, MeOH); lit.[26] $[\alpha]_D^{25} = +30.8$, ee = 99.1% (*c* 2, MeOH)}. HRMS $[M+Na]^+$ found ($[M+Na]^+$ calculated for $C_{15}H_{17}NO_2Na$): 266.1161 (266.1152). The NMR data identical with those reported in the literature.[26]

4.3.11. Compound (*S*)-1f

Light yellow oil in 41% yield {ee = 99%, $[\alpha]_D^{25} = -9.4$ (*c* 1, $CHCl_3$); lit.[30] $[\alpha]_D = -6.7$, ee >99% (c 0.5, $CHCl_3$)}.

4.3.12. Compound (*R*)-2f

White solid in 45% yield {mp 69–70 °C, ee = 99%, $[\alpha]_D^{25} = +93.5$ (*c* 1, $CHCl_3$); $[\alpha]_D^{25} = +103$ (*c* 2, MeOH); lit.[26] $[\alpha]_D^{25} = +107.4$ (*c* 2, MeOH)}. HRMS $[M+Na]^+$ found ($[M+Na]^+$ calculated for $C_{12}H_{17}NO_2Na$): 230.1177 (230.1152); ^1H NMR (DMSO, 500 MHz), δ (ppm): 8.12 (d, *J* = 8.5 Hz, 1H, N*H*), 7.31 (m, 4H, Ar*H*), 7.21 (tt, *J* = 7.0 Hz, *J* = 2.0 Hz, 1H, Ar*H*), 4.73 (q, *J* = 8.5 Hz, *J* = 6.5 Hz, 1H, C*H*), 3.82 (q, *J* = 4.5 Hz, 2H, OCH_2), 3.30 (s, 3H, OCH_3), 1.74 (m, *J* = 6.5 Hz, *J* = 7.0 Hz, 2H, CH_2), 0.83 (t, *J* = 7.0 Hz, 3H, CH_3); ^{13}C NMR (DMSO, 126 MHz), δ (ppm): 168.29 (*C*O), 143.52 (C(1′)), 128.07, 126.57, 126.49 (C(2′), C(3′), C(4′), C(5′), C(6′)), 71.41 (OCH_2), 58.41 (OCH_3), 53.70 (CH), 28.65 (CH_2), 11.06 (CH_3).

4.3.13. Compound (*S*)-1g

Pale yellow semisolid in 39% yield {ee >99%, $[\alpha]_D^{25} = +20.8$ (*c* 1, $CHCl_3$) lit.[31] $[\alpha]_D^{25} = +20.0$, ee = 96% (*c* 1.0, $CHCl_3$)}.

4.3.14. Compound (*R*)-2g

White solid in 44% yield {mp 63 °C, ee = 95%, $[\alpha]_D^{25} = +62.2$ (*c* 1, $CHCl_3$), $[\alpha]_D^{25} = +105$ (*c* 2, MeOH); lit.[26] $[\alpha]_D^{25} = +112.4$ (*c* 2, MeOH), ee = 98.2%}. HRMS $[M+Na]^+$ found ($[M+Na]^+$ calculated for $C_{12}H_{15}NO_2Na$): 228.1002 (228.0995). The NMR data identical with those reported in the literature.[26]

4.3.15. Compound (*S*)-1h

Pale yellow semisolid in 34% yield {ee = 98%, $[\alpha]_D^{25} = +34.7$ (*c* 1, $CHCl_3$); lit.[33] $[\alpha]_D^{25} = +27.9$ (*c* 0.51, MeOH), ee>99.5%}.

4.3.16. Compound (*R*)-2h

Solid in 45% yield (mp 87 °C, ee = 99%, $[\alpha]_D^{25} = +75.7$ (*c* 1, $CHCl_3$)}. HRMS $[M+Na]^+$ found ($[M+Na]^+$ calculated for $C_{12}H_{15}NO_2Na$): 242.1179 (242.1152); ^1H NMR (DMSO, 500 MHz), δ (ppm): 8.01 (d, *J* = 9.0 Hz, 1H, N*H*), 7.14 (arom., m, 3H), 7.08 (arom., m, 1H), 5.03 (m, 1H, C*H*NH_2), 3.87 (s, 2H, CH_2), 3.30 (s, 3H, OCH_3), 2.67–79 (m, 2H), 2.87 (m, 2H), 1.67–1.78 (m, 2H); ^{13}C NMR (126 MHz, DMSO): δ 168.35 (*C*O), 137.33, 137.02 (arom *C*), 128.62, 127.67, 126.55, 125.72 (arom *C*H), 71.33 (CH_2), 58.41 (OCH_3), 46.05 (*C*HNH_2), 29.57 (C(2)), 28.67 (C(2)), 20.20 (C(3)).

4.3.17. Compound (*S*)-1i

Pale yellow oil in 45% yield {ee >99%, $[\alpha]_D^{25} = +4.1$ (*c* 1, $CHCl_3$); lit.[32] $[\alpha]_D^{25} = +6.4$ (*c* 0.47, $CHCl_3$), ee = 98%}.

4.3.18. Compound (*R*)-2i

White solid in 48% yield (ee = 98%, $[\alpha]_D^{25} = +34.1$ (*c* 1, $CHCl_3$)}. HRMS $[M+Na]^+$ found ($[M+Na]^+$ calculated for $C_{12}H_{15}NO_2Na$): 244.1328 (244.1308). The NMR data were identical with those reported in the literature.[26]

4.4. Preparation of a sol–gel CAL-B catalyst

Phosphate buffer (0.1 M, pH 7.5, 390 μL producing gel/water with a ratio of 1:10) and possible additives (Celite 50 mg, Celite 50 mg together with sucrose 5 mg or 18-crown-6 (0.5 mmol, 132 mg), 18-crown-6 (0.5 mmol, 132 mg) together with Celite 50 mg) were added to a vial containing the CAL-B powder

(75 mg) and mixed on a Vortex-mixer at room temperature while IPA (isopropyl alcohol, 100 μL) and PVA (polyvinyl alcohol, 4% w/v) were introduced to the enzyme solution. After addition of the possible additives the aqueous NaF (1 M, 50 μL) was added and the mixture was shaken on a Vortex-mixer before the addition of TMOS/MTMS (1:5, 3 mmol). The mixture was stirred effectively until heat-formation was observed. The vial was cooled on an ice bath and the mixture was gently shaken until gelation was complete. Loosely closed vials were stored in a desiccator overnight for maturation. Sol–gel CAL-B preparations were dried in a lyophilizator and gently crushed with a spatula before use.

4.5. Reuse of the CAL-B preparations

1-Phenylethylamine (12.7 μL, 0.1 M in toluene or 2 mmol), isopropylmethoxyacetate (27.2 μL, 0.2 M in toluene or 2 mmol) were added to a vial containing Novozym 435 or sol–gel CAL-B preparation, and the reaction mixture was shaken at 23 °C. The reactions were stopped at 50% conversion by centrifuging gently the catalyst and pipetting off the liquid phase. New reagents (and toluene for solvent reactions) were added to start the next reaction cycle. Reuse was repeated five times under identical conditions.

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