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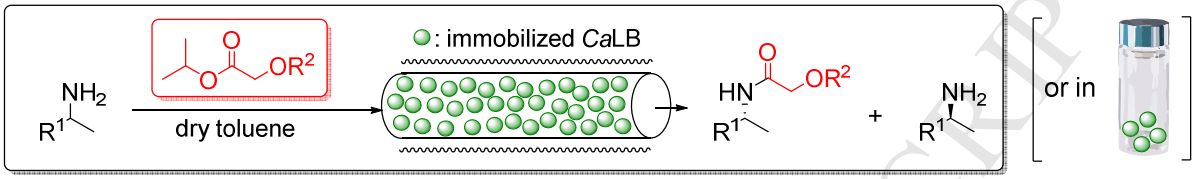

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ABSTRACT

In this study, the activity of acetic acid esters modified with electron withdrawing 2-alkoxy-groups was investigated as acylating agent in kinetic resolution (KR) of racemic amines. A homologous series of the isopropyl esters of four 2-alkoxyacetic acids (2-methoxy-, 2-ethoxy-, 2-propoxy- and 2-butoxyacetic acids) were prepared and investigated for enantiomer selective *N*-acylation catalyzed by lipase B from *Candida antarctica* under batch and continuous-flow conditions. In the first set of experiments, isopropyl 2-propoxyacetate showed the highest effectivity with all of the four racemic amines [(±)-1-phenylethylamine, (±)-4-phenylbutan-2-amine, (±)-heptan-2-amine and (±)-1-methoxypropane-2-amine] in the set enabling excellent conversions (≥46%) and enantiomeric excess values (*ee* ≥99%) with each amines in continuous-flow mode KRs under the optimized reaction conditions. In a second set of experiments, KRs of five additional amines – being substituted derivatives of (±)-1-phenylethylamine – further demonstrated the usefulness of isopropyl 2-propoxyacetate – being the best acylating agent in the first set of KRs – in KRs leading to (*R*)-*N*-propoxyacetamides with high *ee* values (≥99.8%).

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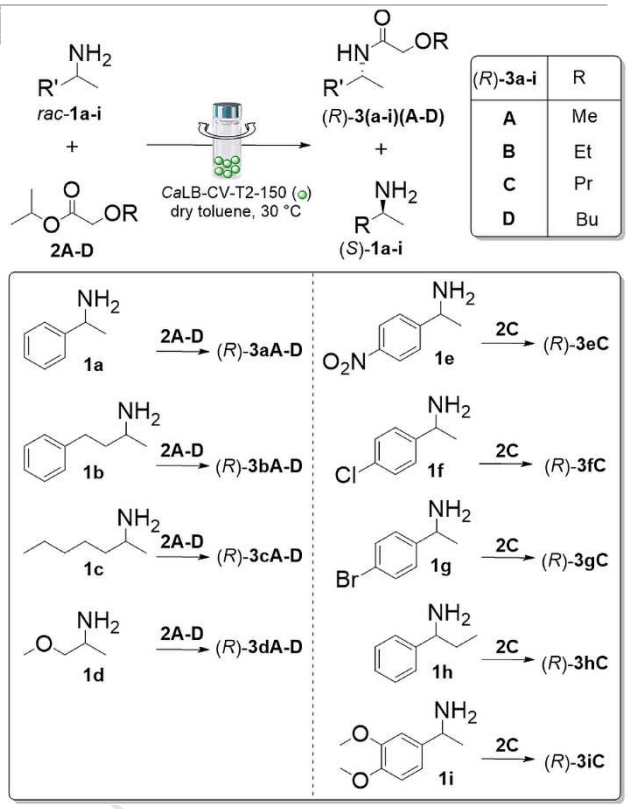
1. Introduction

The production of chiral organic molecules[1] in enantiopure form is an important objective from physiological,[2] economic, and environmental[3] aspects as well. Among the possible technological solutions, kinetic resolution (KR)[4,5] is a widely used method which can afford almost enantiopure compounds with a theoretical yield of 50% starting from racemates.[6] To distinguish between the two enantiomers of a racemate by this method, use of an adequate chiral auxiliary namely a chiral reagent,[7] a chiral metal-ligand complex[8] or an enzyme[9] is fundamental. Moreover, in many cases an effective KR could be extended to a dynamic kinetic resolution (DKR) method by an *in situ* combination with racemization of the residual enantiomer to raise the theoretical yield up to 100%.[10]

To ensure the required enantiomer selectivity, enzymes as biocatalysts offer the necessary chiral environment due to their inherent chirality deriving from their building blocks, the chiral amino acids.[11] This initiated numerous studies in the past few decades to apply many classes of enzymes (such as hydrolases,[12] transferases,[13] oxidoreductases[14] or lyases[15]) to perform synthetic organic reactions.[16] Since cofactor-free enzymatic methods are preferred for the large-scale production of chemicals, lipase-catalyzed KRs gained popularity in the enantiomer separation of alcohols, amines and their derivatives.[17,18] Besides the advantageous catalytic properties of lipases (substrate specificity, activity, selectivity), their enhanced stability and easy recovery is an indispensable issue in an effective and industrially applicable synthetic process. Thus, different immobilization methods were developed to keep these enzymes in active and stable form over numerous recycling steps.[19-21] After the proper technique is chosen to immobilize an enzyme influencing activity of the enzyme, there are further parameters which influence the productivity of lipase-catalyzed KRs of amines, e.g. the type of solvent or the water content of reaction components.[22]

Many investigations emphasized the key role of the acyl donor in KR of amines. In the first DKR of (±)-1-phenylethylamine, ethyl acetate was applied as acylating agent by Reetz and Schimossek in 1996.[23] Later, it was found that the modification of acylating agent's carboxylic acid moiety with electron withdrawing groups could provide enhanced catalytic activity in enzymatic KRs. It is important to note that esters of strong acids (pK_a<2.0) as acylating agents in KR could facilitate not just the enzymatic but the chemical acylation of substrate during enzymic resolution thereby lowering the enantiomeric purity of product acetamide even under ambient conditions.[24-27]

In order to further increase the productivity of enzymatic KR processes for amines by enzymatic acylation, we decided to extend our previous study on the esters of 2-ethoxyacetic acid as enhanced acylating agents for lipase-catalyzed KR of (±)-1-phenylethylamine *rac*-**1a**.[28] In this study enzymatic KRs of *rac*-**1a** and eight other racemic amines [(±)-4-phenylbutan-2-amine *rac*-**1b**, (±)-heptan-2-amine *rac*-**1c**, (±)-1-methoxypropane-2-amine *rac*-**1d,** (±)-1-(4-nitrophenyl)ethan-1-amine *rac*-**1e**, (±)-1-(4-chlorophenyl)ethan-1-amine *rac*-**1f**, (±)-1-(4-bromophenyl)ethan-1-amine *rac*-**1g**, (±)-1-phenylpropan-1-amine *rac*-**1h**, (±)-1-(3,4-dimethoxyphenyl)ethan-1-amine *rac*-**1i**] catalyzed by a covalently immobilized lipase B from *Candida antarctica* (*Ca*LB-CV-T2-150) were performed using as acylating agents an extended set of isopropyl alkoxyacetates **2A-D** [isopropyl 2-propoxy- (**2C**) and 2-butoxyacetate (**2D**), in addition to the already known isopropyl 2-methoxy- (**2A**) and 2-ethoxyacetate (**2B**)] under batch and continuous-flow conditions (Scheme 1).

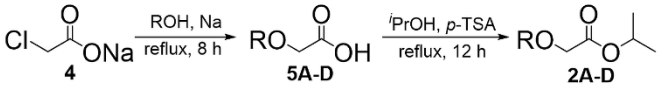


Scheme 1. KR of racemic amines *rac*-**1a-i** using isopropyl esters of 2-alkoxyacetic acids **2A-D** as acylating agent catalyzed by *Ca*LB-CV-T2-150 in batch mode

2. Results and Discussion

2.1. Synthesis of 2-alkyloxy acid isopropyl esters

First, the desired homologous series of alkoxyacetic acid isopropyl esters **2A-D** being potential acylating agents for kinetic resolution (KR) of racemic amines were synthesized (Scheme 2). The corresponding 2-alkyloxyacetic acids **5A,B** were already available, while **5C,D** were prepared by reacting sodium 2-chloroacetate **4** with the corresponding alcohols (R-OH) in the presence of sodium. Esterification of the acids **5A-D** with 2-propanol using *p*-toluenesulfonic acid catalysis, followed by vacuum distillation led to isopropyl esters **2A-D** (Scheme 2).



	5A	5B	5C	5D	2A	2B	2C	2D
R	Me	Et	Pr	Bu	Me	Et	Pr	Bu
Y (%)	n.a.[a]	76[b]	87	63	69[b]	73[b]	64	68

Scheme 2. Synthesis of 2-alkyloxyacetic acids **5A-D** and their isopropyl esters **2A-D** ([a] obtained commercially, [b] synthesized as described earlier[27])

*2.2. Comparison the activity of 2-alkoxyacetic acid esters **2A-D** in kinetic resolution of chiral amines **1a-d** in batch mode*

The biocatalytic applicability of four 2-alkoxyacetic acid esters **2A-D** as acylating agents in the *Ca*LB-CV-T2-150-catalyzed KRs of the selected four racemic amines **1a-d** was studied in batch mode using shake flasks by comparison of the achievable conversion and enantiomeric excess (*ee*) values of formed (*R*)-2-alkoxyacetamides ($ee_{(R)\text{-}\mathbf{3(a-d)(A-D)}}$) (Scheme 1).

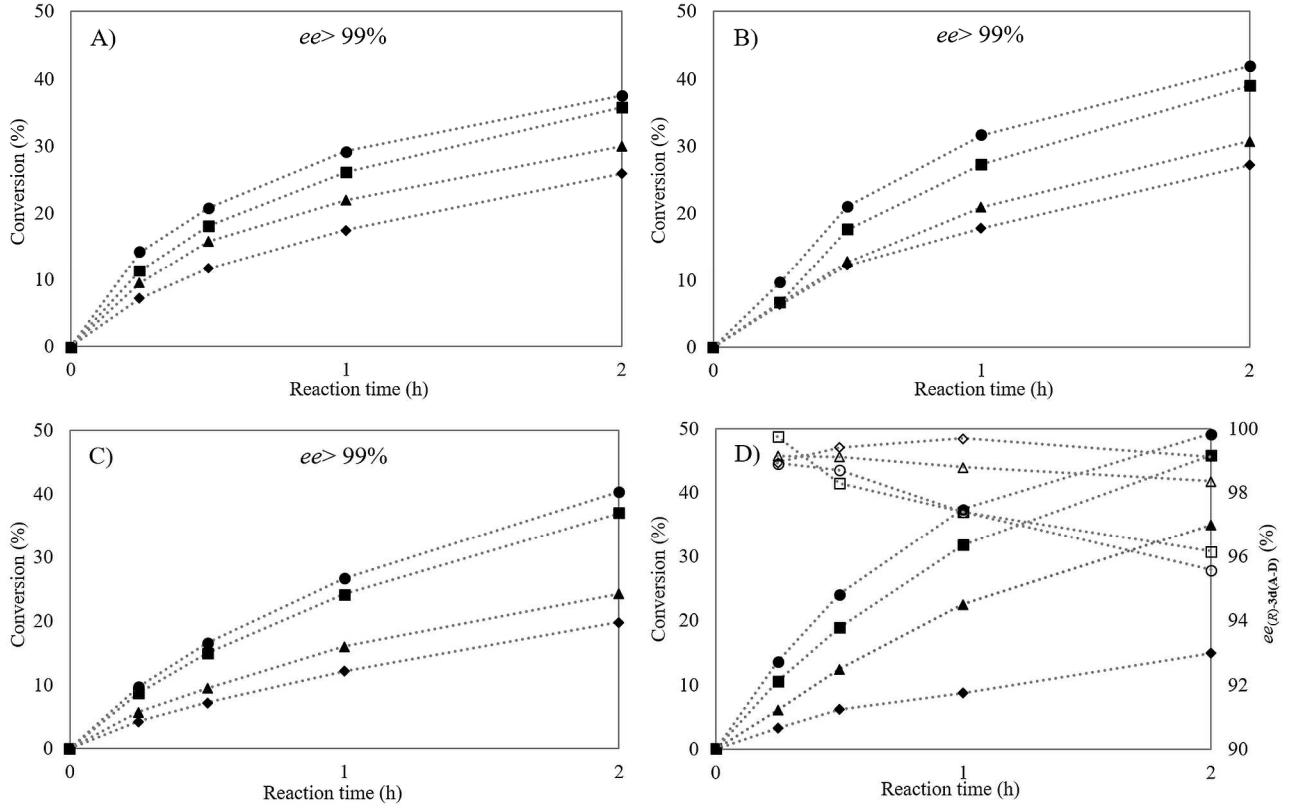
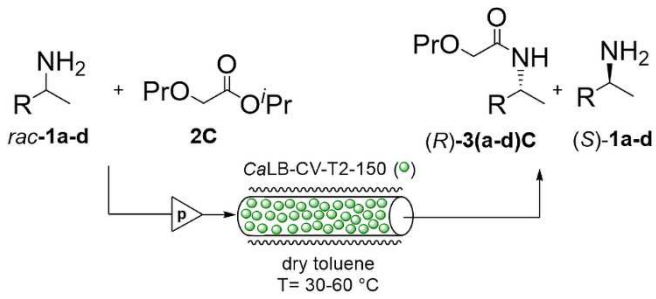


Figure 1. *Ca*LB-CV-T2-150-catalyzed batch mode KR of *rac*-**1a-d** (0.778 M) with various acylating agents **2A-D** (1.0 equiv., 0.778 M) using shake flasks [Panel A: KR of *rac*-**1a**, Panel B: KR of *rac*-**1b**, Panel C: KR of *rac*-**1c**, Panel D: KR of *rac*-**1d**; Conditions – *Ca*LB-CV-T2-150: 15.0 mg, dry toluene: 1.0 mL, reaction temperature: 30 °C, shaking: 750 rpm, conversion and *ee* determined by GC after sampling directly from reaction mixture and treated by acetic anhydride; Markers: conversions with **2A** (◆), **2B** (■), **2C** (●), **2D** (▲), *ee* values for (*R*)-**3dA** (◊), (*R*)-**3dB** (□), (*R*)-**3dC** (○), (*R*)-**3dD** (Δ)]

Analysis of the conversion values (Figure 1, sections A-D) indicated similar tendency with all the four esters **2A-D** for each of the amines *rac*-**1a-d**. The 2-methoxyacetate **2A** showed the lowest activity while increased number of carbon atoms in the alkoxy moiety of esters **2B-D** led to enhanced acylating activity. Among these four isopropyl esters, the one with 2-propoxy moiety **2C** proved to be optimal in terms of reaction rate with amines *rac*-**1a-d**. Isopropyl 2-propoxyacetate **2C** overcome the performance of the already applied isopropyl 2-methoxyacetate **2A**[29] or isopropyl 2-ethoxyacetate **2B**[28] as acylating agent in *Ca*LB-catalyzed KRs of these amines. Even isopropyl 2-buthoxyacetate **2D** enabled higher efficiency in enzymatic *N*-acylation of amines rac-**1a-d** than isopropyl 2-methoxyacetate **2A**.

Besides activity, enantiomer selectivity of the enzyme in KRs is another important parameter to be compared with the different acylating agents **2A-D**. Thus, *ee* values of formed (*R*)-2-alkoxyacetamides ($ee_{(R)\text{-}3(a\text{-}d)(A\text{-}D)}$) were determined to characterize the influence of acylating agents **2A-D** on the enantiomer selectivity. In case of the two aromatic ring-containing amines *rac*-**1a,b** and the aliphatic heptan-2-amine *rac*-**1c** of comparable size, the enantiomer selectivity was remarkable with *ee* values over 99% even at the highest conversions (Figure 1, panels A-C). In contrast, KR of 1-methoxypropan-2-amine *rac*-**1d** showed slightly lower but acceptable enantiomer selectivity (*ee* >95%, Figure 1, panel D), even if the conversion with isopropyl 2-propoxyacetate **2C** (being the most active acylating agent for *rac*-**1d**) reached 49.6% (the theoretical limit in a fully selective KR is 50%).

After the first series of experiments in shake flasks, the effect of temperature on the activity and the selectivity of KRs of the amines *rac*-**1a-d** was investigated in continuous-flow mode using packed-bed columns filled with *Ca*LB-CV-T2-150 as biocatalyst and applying isopropyl 2-propoxyacetate **2C** as the optimal acylating agent in the shake flask KRs. Thus, a solution of racemic amine (*rac*-**1a-d**) and ester (**2C**) in dry toluene was fed to *Ca*LB-CV-T2-150-filled column by a syringe pump at different flow rates (50, 100, 200 μL min^{-1}) and the column was thermostated to 30, 40, 50 and 60 °C in an HPLC column thermostat (Scheme 3).



Scheme 3. Kinetic resolution of racemic primary amines *rac*-**1a-d** using isopropyl 2-propoxyacetate **2C** as acylating agent catalyzed by *Ca*LB-CV-T2-150 in continuous-flow mode Kinetic resolution of amines rac-**1a-d** in continuous-flow mode using isopropyl 2-propoxyacetate **2C** as acylating agent

Data for the continuous-flow mode KRs of amines *rac*-**1a-d** with ester **2C** at 200 µL min $^{-1}$ flow rate are presented in Figure 2. In the whole temperature range, conversions of the 1-methoxypropan-2-amine *rac*-**1d** were the highest and at 60 °C, the conversion from *rac*-**1d** with **2C** ($c_{rac\text{-}1d}$= 47%) approached the theoretical limit of a fully selective KR process (c= 50%).

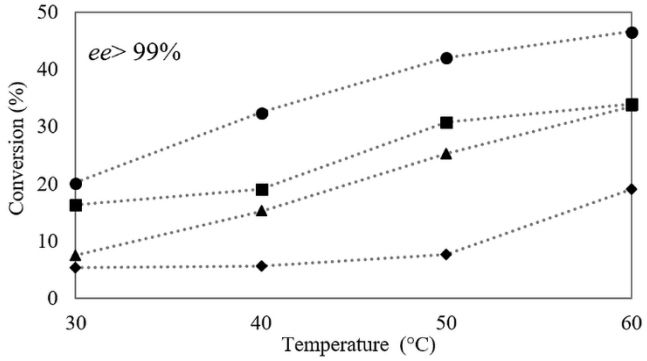


Figure 2. Continuous-flow mode KR of racemic amines *rac*-**1a-d** at different temperatures using isopropyl 2-propoxyacetate **2C** as acylating agent [conditions: *rac*-**1a-d** 0.63 M, **2C** 0.38 M (0.6 equiv.), flow rate 200 µL min^{-1}, column filling: *Ca*LB-CV-T2-150 216 mg, markers: **1a** (◆), **1b** (■), **1c** (▲), **1d** (●)].

The KRs of amines *rac*-**1b** and *rac*-**1c** in continuous-flow mode behaved quite similar, as indicated by the progress of their temperature-conversion curves. This can be explained by considering that *rac*-**1b** and *rac*-**1c** are flexible amines of comparable size. In this series of experiments, KR of amine *rac*-**1a** being a relatively rigid molecule with the direct binding of the rigid aromatic ring to the center of asymmetry proved to be the less active substrate for the covalently immobilized *Ca*LB-CV-T2-150 biocatalyst.

After finding that in the 30 – 60 °C temperature range the highest conversions from amines *rac*-**1a-d** with acylating agent **2C** could be achieved at 60 °C, the flow rate was fine-tuned in the range of 50–200 µL min^{-1} to reach nearly the theoretical conversions for all substrates (c ≥46%) with high enantiomeric purities ($ee_{(R)\text{-}3(a\text{-}d)C}$ ≥99.1%) (Table 1).

Table 1. Optimized reaction conditions for continuous-flow mode KR of racemic amines *rac*-**1a-d** with isopropyl 2-propxyacetate **2C** as acylating agent

Subst.[a]	T [°C]	v [µL min^{-1}]	c [%]	$Y_{(R)\text{-}3(a\text{-}d)C}$ [%]	$ee_{(R)\text{-}3(a\text{-}d)C}$ [%]	E [-]
rac-**1a**	60	50	46	45	99.7	»200
rac-**1b**	30	100	49	49	99.2	»200
rac-**1c**	40	50	48	46	99.1	»200
rac-**1d**	60	200	47	43	99.9	»200

[a] Conditions: *rac*-**1a-d** 0.63 M, **2C** 0.38 M (0.6 equiv.), column filled with *Ca*LB-CV-T2-150 (216 mg).

In Table 2, a thorough comparison is listed for various esters used as acylating agents in amine KRs involving ethyl acetate being the most often used acylating agent in such processes. It is clearly apparent that presence of electron withdrawing 2-alkyloxy groups in the acetates significantly enhance their acylating ability in enzymatic KR for *rac*-**1a,b** (ca. 7-18 times) as compared to the non-activated ethyl acetate. Use of isopropyl instead of ethyl esters as acylating agent, led to the products (*R*)-**3a,b** with higher *ee* values due to lowering the degree of non-selective chemical acylation as undesired side-reaction.[28]

To broaden the substrate scope, KRs of further five racemic amines [(±)-1-(4-nitrophenyl)ethan-1-amine *rac*-**1e**, (±)-1-(4-chlorophenyl)ethan-1-amine *rac*-**1f**, (±)-1-(4-bromophenyl)ethan-1-amine *rac*-**1g**, (±)-1-phenylpropan-1-amine *rac*-**1h**, (±)-1-(3,4-dimethoxyphenyl)ethan-1-amine *rac*-**1i**] were investigated in batch mode using isopropyl 2-propoxyacetate (**2C**) as acylating agent (Scheme 1). The additional set of substrates were variously substituted derivatives of (±)-1-phenylethylamine *rac*-**1a**. The conversions from *rac*-**1e-i** and *rac*-**1a** with the best acylating agent **2C** reflecting to the relative activities are directly compared in PanelA of Figure 3. The enantiomeric excess values of (*R*)-**3e-iC** and (*R*)-**3aC** reflecting to the relative selectivities of the KRs from the additional five amines *rac*-**1e-i** are shown in Pane B of Figure 3. Only the *p*-chlorophenyl derivative *rac*-**1f** showed higher activity than the *rac*-**1a** while the conversions of the four other substrates (*rac*-**1e,g-i**) were lower than that of *rac*-**1a**. The enantiomer selectivity of CaLB-CV-T2-150 for the derivatives containing a halogen (chlorine or bromine) *rac*-**1f,g** was a bit lower than that for *rac*-**1a** as indicated by the *ee* values of their products (*R*)-**3fC** and (*R*)-**3gC**. After 8 h of reaction time, the (*R*)-propoxyacetamides (*R*)-**3e-iC** were isolated and characterized with high *ee* values (≥99.8%) (Table 3).

Table 2. Comparison of various acylating agents in KRs from racemic amines *rac*-**1a,b** using *Ca*LB-catalyzed reactions in batch mode (30 °C, shaken flask)[18,28]

Subst.	Acylating agent[a]	Concentration [M]		Time [h]	Biocatalyst		c [%]	$ee_{(R)\text{-}3a,b}$ [%]	E [-]
		Substrate	Acylating agent (equiv.)		*Ca*LB-type	Amount [mg]			
rac-**1a**[b]	ethyl acetate	0.385	0.770 (2.0)	1	CV-T2-150	50.0	2.0	99.6	>200
rac-**1a**[b]	isopropyl acetate	0.385	0.770 (2.0)	1	CV-T2-150	50.0	4.1	99.8	»200
rac-**1a**[b]	isopropyl acetate	0.385	0.231 (0.6)	1	CV-T2-150	50.0	0.9	99.7	»200
rac-**1a**[b]	ethyl 2-methoxyacetate	0.385	0.231 (0.6)	1	CV-T2-150	50.0	6.5	99.8	»200
rac-**1a**[b]	ethyl 2-ethoxyacetate	0.385	0.770 (2.0)	1	CV-T2-150	50.0	14.4	99.0	>200
rac-**1a**[b]	**2A**	0.385	0.231 (0.6)	1	CV-T2-150	50.0	7.6	>99.9	»200
rac-**1a**[b]	**2A**	0.778	0.778 (1.0)	1	CV-T2-150	15.0	17.5	99.4	>200
rac-**1a**[b]	**2B**	0.385	0.231 (0.6)	1	CV-T2-150	50.0	16.0	>99.9	»200
rac-**1a**[b]	**2B**	0.385	0.770 (2.0)	1	CV-T2-150	50.0	36.1	>99.9	»200
rac-**1a**[b]	**2B**	0.778	0.778 (1.0)	1	CV-T2-150	15.0	26.1	99.6	»200
rac-**1a**[b]	**2C**	0.778	0.778 (1.0)	1	CV-T2-150	15.0	29.2	99.7	»200
rac-**1a**[b]	**2D**	0.778	0.778 (1.0)	1	CV-T2-150	15.0	21.9	99.6	»200
rac-**1b**[c]	ethyl acetate	0.200	10.2[d]	4	Novozym™ 435	300.0	51	86	41
rac-**1b**[b]	**2C**	0.778	0.778 (1.0)	4	CV-T2-150	15.0	46.8	99.9	»200

[a] **2A**: isopropyl 2-methoxyacetate, **2B**: isopropyl 2-ethoxyacetate, **2C**: isopropyl 2-propoxyacetate, **2D**: isopropyl 2-butoxyacetate, [b] Data obtained by Oláh et al.[28]
[c] Data obtained by González-Sabín et al.[19] [d] Used as solvent (15 mL)

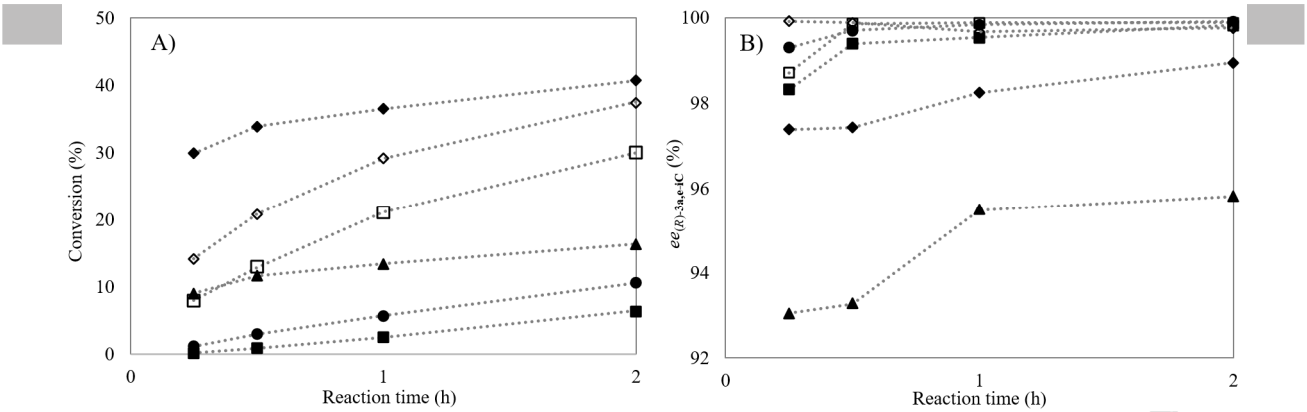


Figure 3. *Ca*LB-CV-T2-150-catalyzed KR of *rac*-**1a,e-i** (0.778 M) with isopropyl 2-propoxyacetate **2C** (1.0 equiv., 0.778 M) in shaken flasks in batch mode [Panel A: conversion values of KRs from *rac*-**1a,e-i**; Panel B: *ee* values of formed (*R*)-*N*-propoxyacetamides (*R*)-**3aC** and (*R*)-**3(e-i)C** (results for KR of *rac*-**1a** were also shown in Figure 1, Panel A); conditions – *Ca*LB-CV-T2-150: 15.0 mg, dry toluene: 1.0 mL, reaction temperature: 30 °C, shaking: 750 rpm, conversion and *ee* were determined by GC after sampling directly from reaction mixture and derivatized with by acetic anhydride; markers: **1a** (◊), **1e** (□), **1f** (◆), **1g** (▲), **1h** (●) and **1i** (■).]

Table 3. KR of racemic amines *rac*-**1e-i** with isopropyl 2-propoxyacetate **2C** as acylating agent in batch mode[a]

Subst. [a]	*c* [%]	$Y_{(R)\text{-}3(e\text{-}i)C}$ [%]	$ee_{(R)\text{-}3(e\text{-}i)C}$ [%]	*E* [-]
rac-**1e**	42.3	40	99.9	»200
rac-**1f**	47.8	46	99.9	»200
rac-**1g**	22.2	19	99.8	»200
rac-**1h**	22.6	19	99.8	»200
rac-**1i**	24.9	24	99.8	»200

[a] Conditions: *rac*-**1e-i** (0.778 M), **2C** (0.778 M, 1.0 equiv.), *Ca*LB-CV-T2 (15.0 mg), dry toluene (1.0 mL), reaction time: 8 h, reaction temperature: 30 °C, shaking: 750 rpm.

3. Conclusions

Our study focused on the optimization of isopropyl 2-alkoxyacetates as acylating agent in enzymatic kinetic resolution of chiral amines with the robust lipase B from *Candida antarctica*. The study with a homologous series of isopropyl 2-alkoxyacetates **2A-D** – ranging from 2-methoxy to 2-butoxy derivatives – in *Ca*LB-catalyzed KRs of racemic aromatic and aliphatic primary amines in batch and continuous-flow modes revealed isopropyl 2-propoxyacetate **2C** as the most efficient acylating agent providing higher activity than the previously applied 2-methoxy- or 2-ethoxyacetic acid esters. The most efficient ester **2C** was applied and optimized as acylating agent in KRs of four racemic amines *rac*-**1a-d** under continuous-flow conditions leading to the enantioenriched forms of the four valuable amines of industrial interest in high enantiomeric purity ($99.1\% \leq ee \leq 99.9\%$) at nearly 50% conversions ($46\% \leq c \leq 49\%$). The KRs of amines with **2C** were extended to five further racemic amines *rac*-**1e-i** in batch mode to yield enantioriched (*R*)-2-propoxy-N-acetmides (*R*)-**3e-iC** with high *ee* (≥99.8%). The results indicated the increased potential and applicability of the most efficient isopropyl 2-propoxyacetate **2C** in lipase-catalyzed KRs.

4. Experimental Section

4.1. Materials

*Ca*LB-CV-T2-150 (lipase B from *Candida antarctica*, covalently attached to dry acrylic beads of 150-300 µm particle size) was the product of ChiralVision BV (Leiden, The Netherlands). All other reagents and solvents were purchased from Sigma Aldrich (Saint Louis, MO, USA), Alfa Aesar Europe (Karlsruhe, Germany), Merck (Darmstadt, Germany) and used as received.

4.2. Methods

TLC was carried out using Kieselgel 60 F254 (Merck) sheets. Spots were visualized under UV light (Vilber Lourmat VL-6.LC, 254 nm) or after treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates. The NMR spectra were recorded in $CDCl_3$ on a Bruker Avance 500 spectrometer operating at 300 MHz or 500 MHz for ^1H and 125 MHz for ^{13}C, and signals are given in ppm on the δ scale. Infrared spectra were recorded on a Bruker ALPHA FT-IR spectrometer and wavenumbers of bands are listed in cm^{-1}. Optical rotation was measured on Perkin–Elmer 241 polarimeter at the D-line of sodium. The polarimeter was calibrated with measurements of both enantiomers of menthol. Samples (20 µL) from kinetic resolution reactions were diluted with ethanol (1000 µL) treated with acetic anhydride (30 µL, at 60 °C at 750 rpm in a thermostatted shaker, for derivatization of unreacted amines into *N*-acetamides), dried over Na_2SO_4 and analyzed in parallel by two GC equipment: an Agilent 5890 equipped with a Hydrodex β-TBDAc column (Macherey-Nagel; 25 m×0.25 mm×0.25 µm, film of heptakis-(2,3-di-*O*-acetyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrin and an Agilent 4890 equipped with column Hydrodex β-TBDM column (Macherey-Nagel; 25 m × 0.25 mm × 0.25 µm, film of heptakis-(2,3-di-*O*-methyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrin) [FID (250 °C), injector (250 °C), H_2 (12 psi, split ratio: 1:50].

Conversion (*c*) and enantiomeric excess (*ee*) were determined by GC. Conversion was calculated using the equation $c = ee_S \times (ee_S + ee_P)^{-1}$ (where ee_S is the *ee* of the substrate and ee_P is the *ee* of the product).[30] The specific reaction rates in continuous-flow systems (r_{flow}) were calculated using the equation $r_{flow} = [P] \times v/m_B$ (where $[P]$ [mol mL^{-1}] is the molar concentration of the product, v [mL min^{-1}] is the flow rate and m_B [g] is the mass of the applied biocatalyst). The yields of the isolated (*R*)-amides were related to the corresponding racemates of substrates.[31] Enantiomeric ratio (*E*) was calculated from *c* and enantiomeric excess of the product (ee_P) using the equation $E = \ln[1-c(1+ee_P)]/\ln[1-c(1-ee_P)]$.[32] Due to sensitivity of *E* value above 100 to small deviations of experimental errors, *E* values calculated in the range of 100–200 were given as >100, those in the range of 200–500 as >200 and above 500 as »200.

*4.3. Synthesis of 2-alkoxy acids **5A-D** and their isopropyl esters **2A-D***

2-Methoxy- and 2-ethoxyacetic acids **5A**,**B** and their isopropyl esters **2A**,**B** were synthesized as described earlier.[27]

Sodium (7.00 g, 304 mmol) was dissolved in the corresponding dry alcohol (n-propanol or n-butanol, 300 mL) at 60 °C. To the forming solution of sodium alcoholate in the alcohol were added triethylbenzylammonium chloride (0.50 g, 18 mmol) and sodium chloroacetate **4** and the resulted mixture was refluxed for 12 h. After the alcohol was evaporated *in vacuum*, the residue was dissolved in water (200 mL) and 5N HCl was added (to pH= 2). The solution was extracted with ethyl acetate (3×100 mL). The organic phases were unified, washed with brine (50 mL) and dried over Na_2SO_4. After removal of the solvent by vacuum rotary evaporation, the residue was purified with vacuum distillation to yield 2-propoxyacetic **2C** or 2-butoxyacetic acid **2D** as colorless oil.

2-Propoxyacetic acid **5C** (15.40 g, 130 mmol, 76%): Bp 103 °C (9 torr); n_D^{20} 1.4231; d_H (300 MHz, $CDCl_3$) 10.05 (1H, s, COOH), 4.13 (2H, s, CH_2COOH), 3.51 (2H, t, *J*=6.6 Hz, CH_2O), 1.75–1.54 (2H, m, CH_3CH_2), 0.94 (3H, t, *J*=7.4 Hz, CH_3); d_C (75 MHz, $CDCl_3$) 175.54, 73.67, 67.70, 22.66, 10.33; n_{max} (liquid film): 2965, 2939, 2879, 1734, 1430, 1203, 1121, 962, 675 cm^{-1}. Elemental analysis for $C_5H_{10}O_3$: required: C 50.84, H 8.53, found C 50.73, H 8.56.

2-Butoxyacetic acid **5D** (14.27 g, 108 mmol, 63%): Bp 111 °C (7 torr); n_D^{20} 1.4258; d_H (500 MHz, $CDCl_3$) 7.29 (1H, s, COOH), 4.13 (2H, s, CH_2COOH), 3.59 (2H, t, *J*=6.2 Hz, CH_2O), 1.68–1.58 (2H, m, J_1=14.0 J_2=6.8 Hz, CH_2Et), 1.47–1.36 (2H, m, J_1=14.4 J_2=7.2 Hz, CH_3CH_2), 0.96 (3H, t, *J*=7.0 Hz, CH_3); d_C (126 MHz, $CDCl_3$) 174.41, 71.86, 67.73, 31.45, 19.09, 13.78; n_{max} (liquid film): 2959, 2935, 2873, 1725, 1430, 1242, 1201, 1121, 933, 673 cm^{-1}. Elemental analysis for $C_6H_{12}O_3$: required: C 54.53, H 9.15, found C 54.61, H 9.08.

The corresponding 2-alkoxyacetic acid [2-propoxy- **5C** (2.00 g, 16.9 mmol), 2-butoxyacetic acid **5D** (2.24 g, 16.9 mmol)] and *p*-toluenesulfonic acid (0.05 equiv., 0.155 g, 0.845 mmol) were dissolved in 2-propanol (30 mL) and refluxed for 12 h. After evaporation of 2-propanol from the reaction mixture, the desired isopropyl ester (**2C** or **2D)** was obtained by vacuum distillation.

Isopropyl 2-propoxyacetate **2C** (1.73 g, 10.8 mmol, 64%): Bp 60 °C (8 torr); n_D^{20} 1.4068; d_H (300 MHz, $CDCl_3$) 5.09 (1H, hept, *J*=6.2 Hz, MeCHMe), 4.03 (2H, s, CH_2COO), 3.48 (2H, t, *J*=6.7 Hz, CH_2Et), 1.73–1.56 (2H, m, CH_2Me), 1.26 (6H, d, *J*=6.3 Hz, MeCHMe), 0.94 (3H, t, *J*=7.4 Hz, CH_2CH_3); d_C (75 MHz, $CDCl_3$) 170.38, 73.68, 68.69, 68.53, 22.97, 22.00, 10.61; n_{max} (liquid film): 2979, 2938, 2878, 1749, 1730, 1466, 1375, 1277, 1205, 1127, 1104, 957, 931, 726, 584, 411 cm^{-1}. Elemental analysis for $C_8H_{16}O_3$: required: C 59.98, H 10.07, found C 60.03, H 9.98.

Isopropyl 2-butoxyacetate **2D** (2.04 g, 11.7 mmol, 68%): Bp 76 °C (8 torr); n_D^{20} 1.4012; d_H (300 MHz, $CDCl_3$) 5.18–5.01 (1H, m, MeCHMe), 4.02 (2H, s, CH_2COO), 3.52 (2H, t, *J*=6.6 Hz, CH_2Pr), 1.67–1.53 (2H, m, CH_2Et), 1.49–1.31 (2H, m, CH_2Me), 1.26 (6H, d, *J*=6.3 Hz, MeCHMe), 0.92 (3H, t, *J*=7.3 Hz, CH_2CH_3); d_C (75 MHz, $CDCl_3$) 170.23, 71.63, 68.54, 68.36, 31.64, 21.83, 19.20, 13.87; n_{max} (liquid film): 2960, 2935, 2874, 1750, 1730, 1467, 1375, 1278, 1204, 1139, 1104, 956, 932, 725, 584, 410 cm^{-1}. Elemental analysis for $C_9H_{18}O_3$: required: C 62.04, H 10.41, found C 61.97, H 10.52.

*4.4. Kinetic resolution of racemic amines rac-**1a-i** in shake flask*

Into a screw cap reaction vial were added a mixture of dry toluene (1.0 mL), immobilized *Ca*LB enzyme (15.0 mg, *Ca*LB-CV-T2-150), the corresponding racemic amine *rac*-**1a-d** (0.778 mmol) and the corresponding isopropyl 2-alkoxyacetate **2A-D** (1.0 equiv., 0.778 mmol). The reaction mixture was shaken (750 rpm) at 30 °C and monitored by taking samples (20 µL) after different reaction times (0.25, 0.5, 1, 2, 3, 4, 6, 8 h). After 8 h, the reactions were worked up.

The raw reaction mixtures of *rac*-**1a-c** and *rac*-**1e-i** were filtered on glass filter, evaporated in vacuum, picked up in dichloromethane (20 mL) and extracted with HCl (2×10 mL, 5N). The aqueous phase was extracted with dichloromethane (10 mL) and the combined organic phases were dried on Na_2SO_4 and after rotary evaporation the corresponding (*R*)-2-alkoxyacetamide [(*R*)-**3(a-c)**(**A-D**), (*R*)-**3(e-i)C**] was obtained as light yellow crystal or oil.

From the raw KR reaction mixtures of *rac*-**1d** the *Ca*LB-CV-T2-150 was filtered off on a glass filter, the volatiles were evaporated in vacuum. The formed amide [(*R*)-**3d**(**A-D**)] was afforded as light yellow oil after preparative TLC.

For (*R*)-2-methoxy-*N*-(1-phenylethyl)acetamide (*R*)-**3aA** and (*R*)-2-ethoxy-*N*-(1-phenylethyl)acetamide (*R*)-**3aB** the physical properties agreed with the published data.[28]

(*R*)-2-Propoxy-*N*-(1-phenylethyl)acetamide (*R*)-**3aC** (47 mg, 0.212 mmol, 27%): *ee*= 99.8%, $[\alpha]_D^{20}$= +70.6 (*c*=10 mg mL^{-1}, CH_2Cl_2); d_H (500 MHz, $CDCl_3$) 7.41–7.25 (5H, m, Ar), 6.83 (1H, s, NH), 5.21 (1H, p, *J*=7.0 Hz, NHCH), 3.96 (2H, q, *J*=15.2 Hz, CH_2CONH), 3.50 (2H, dt, *J*=12.9, 7.4 Hz, CH_2Et), 1.71–1.59 (2H, m, *J*=14.0, 7.3 Hz, CH_2Me), 1.55 (3H, d, *J*=6.8 Hz, $CHCH_3$), 0.95 (3H, t, *J*=7.4 Hz, CH_2CH_3). Elemental analysis for $C_{13}H_{19}NO_2$: required: C 70.56, H 8.65, N 6.33, found C 70.62, H 8.59, N 6.28.

(*R*)-2-Butoxy-*N*-(1-phenylethyl)acetamide (*R*)-**3aD** (42 mg, 0.178 mmol, 23%): *ee*= 99.8%, $[\alpha]_D^{20}$= +72.9 (*c*= 10 mg mL^{-1}, CH_2Cl_2); d_H (500 MHz, $CDCl_3$) 7.42–7.25 (5H, m, Ar), 6.82 (1H, s, NH), 5.21 (1H, p, *J*= 6.9 Hz, NHCH), 3.96 (2H, q, *J*= 15.2 Hz, CH_2CONH), 3.52 (2H, t, *J*= 6.6 Hz, CH_2Pr), 1.70–1.57 (2H, m, CH_2Et), 1.54 (3H, d, *J*= 6.8 Hz, $CHCH_3$), 1.48–1.33 (2H, m, J_1= 14.9 J_2= 7.9 Hz, CH_2CH_3), 0.95 (3H, t, *J*= 7.1 Hz, CH_2CH_3). Elemental analysis for $C_{14}H_{21}NO_2$: required: C 71.46, H 9.00, N 5.95, found C 71.55, H 8.97, N 5.89.

(*R*)-2-Methoxy-*N*-(4-phenylbutan-2-yl)acetamide (*R*)-**3bA** (69 mg, 0.312 mmol, 40%): *ee*= 97.5%, $[\alpha]_D^{20}$= +27.3 (*c*= 10 mg mL^{-1}, CH_2Cl_2); d_H (500 MHz, $CDCl_3$) 7.26 (5H, dt, J_1= 41.1, J_2= 6.9 Hz, Ar), 6.37 (1H, d, *J*= 6.3 Hz, NH), 4.19–4.07 (1H, m, NHCH), 3.90 (2H, s, CH_2CONH), 3.42 (3H, s, OCH_3), 2.71–2.63 (2H, m, $ArCH_2$), 1.82 (2H, dd, J_1= 15.8, J_2= 6.9 Hz, Ar CH_2CH_2), 1.22 (3H, d, *J*= 6.6 Hz, $CHCH_3$). Elemental analysis for $C_{13}H_{19}NO_2$: required: C 70.56, H 8.65, N 6.33, found C 70.61, H 8.59, N 6.28.

(*R*)-2-Ethoxy-*N*-(4-phenylbutan-2-yl)acetamide (*R*)-**3bB** (72 mg, 0.306 mmol, 40%): *ee*= 96.4%, $[\alpha]_D^{20}$= +18.2 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 7.36–7.13 (5H, m, Ar), 6.39 (1H, s, NH), 4.17–4.06 (1H, m, NHCH), 3.98–3.89 (2H, m, CH_2CONH), 3.58 (2H, q, *J*= 7.0 Hz, CH_2CH_3), 2.71–2.63 (2H, m, $ArCH_2$), 1.86–1.76 (2H, m, $ArCH_2CH_2$), 1.26 (3H, t, *J*= 7.0 Hz, $CHCH_3$), 1.23 (3H, d, *J*= 6.6 Hz, CH_2CH_3). Elemental analysis for $C_{14}H_{21}NO_2$: required: C 71.46, H 9.00, N 5.95, found C 71.40, H 9.01, N 5.96.

(*R*)-2-Propoxy-*N*-(4-phenylbutan-2-yl)acetamide (*R*)-**3bC** (107 mg, 0.429 mmol, 55%): *ee*= 92.2%, $[\alpha]_D^{20}$= +13.2 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 7.34–7.15 (5H, m, J_1=

37.0, J_2= 10.3, J_3= 5.9 Hz, Ar), 6.47 (1H, d, J= 26.4 Hz, NH), 4.18–4.07 (1H, m, CHNH), 3.95 (2H, s, CH_2CONH), 3.48 (2H, t, J= 6.6 Hz, CH_2Et); 2.70–2.63 (2H, m, $ArCH_2$), 1.87–1.76 (2H, m, $ArCH_2CH_2$), 1.72–1.60 (2H, m, CH_2CH_3), 1.23 (3H, d, J= 6.6 Hz, $CHCH_3$), 0.98–0.93 (3H, m, CH_2CH_3). Elemental analysis for $C_{15}H_{23}NO_2$: required: C 72.25, H 9.30, N 5.62, found C 72.35, H 9.31, N 5.61.

(*R*)-2-Butoxy-*N*-(4-phenylbutan-2-yl)acetamide (*R*)-**3bD** (112 mg, 0.426 mmol, 61%): *ee*= 92.6%, $[\alpha]_D^{20}$= +11.1 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 7.33–7.16 (5H, m, J_1= 11.0, J_2= 10.3, J_3= 6.3 Hz, Ar), 6.40 (1H, d, J= 22.6 Hz, NH), 4.16–4.07 (1H, m, CHNH), 3.94 (2H, s, CH_2CONH), 3.52 (2H, t, J= 6.6 Hz, CH_2Pr), 2.71–2.63 (2H, m, $ArCH_2$), 1.86–1.77 (2H, m, $ArCH_2CH_2$), 1.68–1.57 (2H, m, CH_2Et), 1.48–1.36 (2H, m, CH_2Me), 1.23 (3H, d, J= 6.6 Hz, $CHCH_3$), 0.97 (3H, t, J= 7.4 Hz, CH_2CH_3). Elemental analysis for $C_{16}H_{25}NO_2$: required: C 72.97, H 9.57, N 5.32, found C 72.95, H 9.58, N 5.33.

(*R*)-2-Methoxy-*N*-(heptan-2-yl)acetamide (*R*)-**3cA** (58 mg, 0.311 mmol, 40%): *ee*= 97.9%, $[\alpha]_D^{20}$= +0.03 (*c*= 10 mg mL^{-1}, CH_2Cl_2), $[\alpha]_D^{20}$= +0.43 (*c*= 39 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 6.28 (1H, s, NH), 4.04–3.95 (1H, m, CHNH), 3.85 (2H, s, CH_2CONH), 3.40 (3H, s, OCH_3), 1.48–1.37 (2H, m, CH_2Bu), 1.26 (6H, t, J= 13.9 Hz, 3×CH_2), 1.13 (3H, d, J= 6.5 Hz, $CHCH_3$), 0.86 (3H, t, J= 6. Hz, CH_3CH_2). Elemental analysis for $C_{10}H_{21}NO_2$: required: C 64.13, H 11.30, N 7.48, found C 64.20, H 11.35, N 7.46.

(*R*)-2-Ethoxy-*N*-(heptan-2-yl)acetamide (*R*)-**3cB** (66 mg, 0.327 mmol, 42%): *ee*= 96.5%, $[\alpha]_D^{20}$= -3.29 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz $CDCl_3$) 6.33 (1H, s, NH), 4.04–3.94 (1H, m, CHNH), 3.89 (2H, s, CH_2CONH), 3.54 (2H, q, J= 7.0 Hz, OCH_2), 1.49–1.38 (2H, m, CH_2Bu), 1.28 (6H, d, J= 6.4 Hz, 3×CH_2), 1.22 (3H, t, J= 7.0 Hz, CH_3CH_2O), 1.13 (3H, d, J= 6.6 Hz, $CHCH_3$), 0.86 (3H, t, J= 6.7 Hz, CH_3CH_2). Elemental analysis for $C_{11}H_{23}NO_2$: required: C 65.63, H 11.52, N 6.96, found C 65.57, H 11.48, N 6.95.

(*R*)-2-Propoxy-*N*-(heptan-2-yl)acetamide (*R*)-**3cC** (80 g, 0.373 mmol, 48%): *ee*= 94.2%, $[\alpha]_D^{20}$= -3.02 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 6.34 (1H, d, J= 6.3 Hz, NH), 4.03–3.94 (1H, m, NHCH), 3.88 (2H, s, CH_2CONH), 3.43 (2H, t, J= 6.6 Hz, OCH_2Et), 1.66–1.56 (2H, m, OCH_2CH_2), 1.40 (2H, dd, J_1= 17.0 J_2= 10.1 Hz, $CHCH_2$), 1.26 (6H, t, J= 11.2 Hz, 3×CH_2), 1.12 (3H, d, J= 6.6 Hz, $CHCH_3$), 0.91 (3H, t, J= 6.1 Hz, $OCH_2CH_2CH_3$), 0.85 (3H, t, J= 6.5 Hz, CH_3). Elemental analysis for $C_{12}H_{25}NO_2$: required: C 66.93, H 11.70, N 6.50, found C 66.89, H 11.64, N 6.44.

(*R*)-2-Butoxy-*N*-(heptan-2-yl)acetamide (*R*)-**3cD** (95 mg, 0.412 mmol, 53%): *ee*= 98.1%, $[\alpha]_D^{20}$= -3.76 (*c*= 10 mg mL^{-1}, CH_2Cl_2); d_H (500 MHz, $CDCl_3$) 6.36 (1H, d, J= 7.0 Hz, NH), 4.03–3.96 (1H, m, NHCH), 3.90 (2H, s, CH_2CONH), 3.49 (2H, t, J= 6.5 Hz, OCH_2Pr), 1.65–1.54 (2H, m, OCH_2CH_2Et), 1.42 (2H, dd, J_1= 13.1 J_2= 5.9 Hz, $OCH_2Ch_2CH_2Me$), 1.38 (2H, dd, J_1= 14.9 J_2= 7.4 Hz, CH_2CH), 1.33–1.23 (6H, m, 3×CH_2), 1.14 (3H, d, J= 6.6 Hz, $CHCH_3$), 0.93 (3H, t, J= 7.4 Hz, $OCH_2Ch_2CH_2CH_3$), 0.87 (3H, t, J= 6.6 Hz, CH_3). Elemental analysis for $C_{13}H_{27}NO_2$: required: C 68.08, H 11.87, N 6.11, found C 69.98, H 11.93, N 6.06.

(*R*)-2-Methoxy-*N*-(1-methoxypropan-2-yl)acetamide (*R*)-**3dA** (48 mg, 0.298 mmol, 30%): R_f= 0.40 (EtOAc) *ee*= 99.7%, $[\alpha]_D^{20}$= +15.5 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 6.65 (1H, s, NH), 4.30–4.12 (1H, m, NHCH), 3.88 (2H, s, CH_2CONH), 3.42 (3H, s, $COCH_2OCH_3$), 3.38 (5H, d, J= 4.5 Hz, CH_3OCH_2), 1.21 (3H, d, J= 6,8 Hz, $CHCH_3$). Elemental analysis for $C_7H_{15}NO_3$: required: C 52.16, H 9.38, N 8.69, found C 52.35, H 9.44, N 8.61.

(*R*)-2-Ethoxy-*N*-(1-methoxypropan-2-yl)acetamide (*R*)-**3dB** (33 mg, 0.187 mmol 24%): R_f= 0.45 (EtOAc), *ee*= 98.3%, $[\alpha]_D^{20}$= +9.7 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500, MHz $CDCl_3$) 6.71 (1H, s, NH), 4,20 (1H, dt, J_1= 11.5, J_2= 6.3 Hz, NHCH), 3.92 (2H, s, CH_2CONH), 3.57 (2H, q, J= 7.0 Hz, CH_2CH_3), 3.42–3.35 (5H, m, CH_3OCH_2), 1.25 (3H, t, J= 7.0 Hz, CH_2CH_3), 1.21 (3H, d, J= 6.8 Hz, $CHCH_3$). Elemental analysis for $C_8H_{17}NO_3$: required: C 54.84, H 9.78, N 7.99, found C 55.04, H 9.82, N 8.01.

(*R*)-2-Propoxy-*N*-(1-methoxypropan-2-yl)acetamide (*R*)-**3dC** (19 mg, 0.101 mmol, 13%): R_f= 0.50 (EtOAc), *ee*= 97.2%, $[\alpha]_D^{20}$= +5.2 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 6.74 (1H, s, NH), 4.25–4.18 (1H, m, J_1= 8.8 Hz, J_2= 6.7 Hz, J_3= 4.2 Hz, J_4= 2.3 Hz, NHCH), 3.93 (2H, s, CH_2CONH), 3.47 (2H, t, J= 6.6 Hz, CH_2Et), 3.41–3.36 (5H, m, CH_3OCH_2), 1.69–1.61 (2H, m, CH_2CH_3), 1.22 (3H, d, J= 6.8 Hz, $CHCH_3$), 0.97 (3H, t, J= 7.4 Hz, CH_2CH_3). Elemental analysis for $C_9H_{19}NO_3$: required: C 57.12, H 10.12, N 7.40, found C 57.25, H 10.03, N 7.34.

(*R*)-2-Butoxy-*N*-(1-methoxypropan-2-yl)acetamide (*R*)-**3dD** (40 mg, 0.195 mmol, 25%): R_f= 0.60 (EtOAc), *ee*= 95.4%, $[\alpha]_D^{20}$= +9.3 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 6.72 (1h, s, NH), 4.26–4.16 (1H, m, CHNH), 3.92 (2H, s, CH_2CONH), 3.51 (2H, t, J= 6.5 Hz, CH_2Pr), 3.38 (5H, d, J= 7.2 Hz, CH_3OCH_2), 1.64–1.56 (2H, m, CH_2Et), 1.45–1.36 (2H, m, CH_2CH_3), 1.22 (3H, d, J= 6.8 Hz, $CHCH_3$), 0.95 (3H, t, J= 7.4 Hz, CH_2CH_3). Elemental analysis for $C_{10}H_{21}NO_3$: required: C 59.09, H 10.41, N 6.89, found C 58.93, H 10.54, N 6.79.

(*R*)-2-Propoxy-*N*-(1-(4-nitrophenyl)ethyl)acetamide (*R*)-**3eC** (83 mg, 0.101 mmol, 40%): R_f= 0.71 (5% $MeOH/CH_2Cl_2$), *ee*= 99.9%, $[\alpha]_D^{20}$= +50.8 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, CDCl3) 8.19–8.13 (2H, m, 2×CH), 7.46 (2H, d, J= 8.6 Hz, 2×CH), 6.87 (1H, d, J= 6.9 Hz, NH), 5.19 (1H, p, J= 7.2 Hz, CHNH), 3.97–3.87 (2H, m, CH_2CONH), 3.50–3.42 (2H, m, OCH_2Et), 1.68–1.56 (2H, m, CH_3CH_2), 1.52 (3H, d, J= 7.0 Hz, CH_3CH), 0.97–0.89 (3H, m, CH_3CH_2). Elemental analysis for $C_{13}H_{18}N_2O_4$: required: C 58.63, H 6.81, N 10.52, found C 58.49, H 6.75, N 10.63.

(*R*)-2-Propoxy-*N*-(1-(4-chlorophenyl)ethyl)acetamide (*R*)-**3fC** (92 mg, 0.109 mmol, 46%): R_f= 0.71 (5% $MeOH/CH_2Cl_2$), *ee*= 99.9%, $[\alpha]_D^{20}$= +56.5 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 7.33–7.19 (4H, m, 4×CH), 6.77 (1H, d, J= 6.7 Hz, NH), 5.12 (1H, p, J= 7.1 Hz, CHNH), 3.92 (2H, q, J= 15.3 Hz, CH_2CONH), 3.44 (2H, td, J_1= 6.6 J_2= 1.2 Hz, OCH_2Et), 1.61 (2H, dt, J_1= 14.1 J_2= 7.1 Hz, CH_3CH_2), 1.48 (3H, d, J= 7.0 Hz, CH_3CH), 0.96–0.88 (3H, m, CH_3CH_2). Elemental analysis for $C_{13}H_{18}ClNO_2$: required: C 61.05, H 7.09, N 5.48, found C 60.91, H 7.11, N 5.41.

(*R*)-2-Propoxy-*N*-(1-(4-bromophenyl)ethyl)acetamide (*R*)-**3gC** (44 mg, 0.062 mmol, 19%): R_f= 0.74 (5% $MeOH/CH_2Cl_2$), *ee*= 99.8%, $[\alpha]_D^{20}$= +50.0 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 7.45 (2H, d, J= 8.4 Hz, 2×CH), 7.18 (2H, d, J= 8.3 Hz, 2×CH), 6.78 (1H, s, NH), 5.16–5.06 (1H, m, CHNH), 3.93 (2H, q, J= 15.3 Hz, CH_2CONH), 3.45 (2H, t, J= 6.2 Hz, OCH_2Et), 1.61 (2H, td, J_1= 14.2 J_2= 7.2 Hz, CH_3CH_2), 1.48 (3H, d, J= 6.9 Hz, CH_3CH), 0.92 (3H, t, J= 7.3 Hz, CH_3CH_2). Elemental analysis for $C_{13}H_{18}BrNO_2$: required: C 52.01, H 6.04, N 4.67, found C 51.92, H 6.07, N 4.58.

(*R*)-2-Propoxy-*N*-(1-phenylpropyl)acetamide (*R*)-**3hC** (34 mg, 0.053 mmol, 14%): R_f= 0.69 (5% $MeOH/CH_2Cl_2$), *ee*= 99.8%, $[\alpha]_D^{20}$= +53.4 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$)

7.37–7.21 (m, Ar), 6.84 (1H, d, *J*= 7.4 Hz, NH), 4.92 (1H, dd, J_1= 15.9 J_2= 7.4 Hz, CHNH), 3.93 (2H, dd, J_1= 36.1 J_2= 15.3 Hz, CH_2CONH), 3.45 (2H, qd, J_1= 6.8 J_2= 2.8 Hz, OCH_2Et), 1.88–1.80 (2H, m, CH_3CH_2CH), 1.68–1.57 (2H, m, $CH_3CH_2CH_2$), 1.48 (3H, d, *J*= 7.0 Hz, CH_3CH), 0.96–0.90 (3H, m, CH_3CH_2CH), 0.89 (3H, t, *J*= 7.4 Hz, $CH_3CH_2CH_2$). Elemental analysis for $C_{14}H_{21}NO_2$: required: C 71.46, H 9.00, N 5.95, found C 71.39, H 9.06, N 5.89.

(*R*)-2-Propoxy-*N*-(1-(3,4-dimethoxyphenyl)ethyl)acetamide (*R*)-**3iC** (53 mg, 0.071 mmol, 24%): R_f= 0.63 (5% MeOH/CH_2Cl_2), *ee*= 99.8%, $[\alpha]_D^{20}$= +70.9 (*c*= 10 mg mL^{-1}, CH_2Cl_2), d_H (500 MHz, $CDCl_3$) 6.94–6.81 (3H, m, 3×CH), 6.76 (1H, d, *J*= 7.4 Hz, NH), 5.13 (1H, p, *J*= 7.0 Hz, CHNH), 3.91 (8H, dd, J_1= 17.0 J_2= 4.5 Hz, CH_2CONH, 2×OCH_3), 3.45 (2H, t, *J*= 6.5 Hz, CH_2Pr), 1.61 (2H, dq, J_1= 14.2 J_2= 7.1 Hz, CH_3CH_2), 1.51 (3H, d, *J*= 6.9 Hz, CH_2Et), 1.45–1.36 (2H, m, CH_2CH_3), 1.22 (3H, d, *J*= 6.8 Hz, $CHCH_3$), 0.92 (3H, t, *J*= 7.4 Hz, CH_2CH_3). Elemental analysis for $C_{15}H_{23}NO_4$: required: C 64.04, H 8.24, N 4.98, found C 64.11, H 8.19, N 5.01.

*4.5. General methods for kinetic resolution of racemic amines rac-**1a-d** with ester **2C** in continuous-flow mode*

Packed-bed columns for continuous-flow biotransformation were made by filling *Ca*LB-CV-T2-150 into stainless steel columns (PTFE layer inside; inner diameter: 4 mm; total length: 70 mm; packed length: 65 mm; inner volume: 0.816 mL) according to the filling process of ThalesNano Inc. The columns were settled by silver metal [Sterlitech Silver Membrane from Sigma-Aldrich, Z623237, pore size 0.45 µm; pure metallic silver, 99.97% with no extractable or detectable contaminants] and PTFE [Whatman® Sigma-Aldrich, WHA10411311, pore size 0.45 µm] filter membranes. The sealings were made of PTFE. The reaction mixtures were pumped through the *Ca*LB-filled columns by syringe pumps (Chemyx) and heated by an HPLC column thermostat.

Before use, the *Ca*LB-CV-T2-150-filled columns were washed with toluene (200 µL min^{-1}, 30 min). After setting a new reaction parameter (temperature, substrate concentration, flow rate), samples were analyzed by GC every 10 min up to 60 min from the start of the actual experiment. After the stationary operation has been established (40 min after the start of the experiment), samples were collected (20 µL sample was diluted with ethanol to 1 mL) and analyzed by TLC and GC. When a series of experiments was finished, the actual *Ca*LB-CV-T2-150-filled column was washed with the toluene (200 µL min^{-1}, 30 min) and stored in refrigerator (4 °C).

*4.6. Continuous-flow kinetic resolution of racemic amines rac-**1a-d** with ester **2C** at different reaction temperatures*

The solution of racemic amine (*rac*-**1a-d**, 0.65 mmol mL^{-1}) and isopropyl 2-propoxyacetate (**2C**, 0.6 equiv., 0.39 mmol mL^{-1}) in dry toluene was pumped through the *Ca*LB-CV-T2-150-filled column thermostated to different temperatures (30, 40, 50 and 60 °C) at different flow rates (50, 100 and 200 µL min^{-1}). Sampling of the reactions was performed as described in section 4.5. After the stationary operation has been established, 10 mL of reaction mixture was collected and worked up according to the description in Section 4.4 to obtain the corresponding amides (*R*)-**3(a-d)C**:

(*R*)-2-Propoxy-*N*-(1-phenylethyl)acetamide (*R*)-**3aC**, 45%, $ee_{(R)\text{-}\mathbf{3aC}}$= 99.7%.

(*R*)-2-Propoxy-*N*-(4-phenylbutan-2-yl)acetamide (*R*)-**3bC**, 49%, $ee_{(R)\text{-}\mathbf{3bC}}$= 99.2%.

(*R*)-2-Propoxy-*N*-(heptan-2-yl)acetamide (*R*)-**3cC**, 46%, $ee_{(R)\text{-}\mathbf{3cC}}$= 99.1%.

(*R*)-2-Propoxy-*N*-(1-methoxypropan-2-yl)acetamide (*R*)-**3dC**, 43%, $ee_{(R)\text{-}\mathbf{3dC}}$= 99.9%.

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Supplementary Material

Please see the Supplementary material to find details of the preparation of the racemic 2-alkoxyacetamides for GC analysis, GC methods, representative chromatograms and spectra (^1H, ^{13}C NMR, FT-IR).

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