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Optimization of 2-alkoxyacetates as acylating agent for enzymatic kinetic resolution of chiral amines

Márk Oláh [a], Dániel Kovács [a], Gabriel Katona [b], Gábor Hornyánszky [a, c], László Poppe [a, b, c, *]

[a] Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Műegyetem rkp. 3, Budapest H-1111, Hungary
[b] Biocatalysis and Biotransformation Research Centre, Babeș-Bolyai University of Cluj-Napoca, Arany János Str. 11, Cluj-Napoca RO-400028, Romania
[c] SynBiocat Ltd., Szilasliget u. 3, Budapest H-1172, Hungary

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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%).

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

* Corresponding author. Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Műegyetem rkp. 3, Budapest H-1111, Hungary.
E-mail address: poppe@mail.bme.hu (L. Poppe).

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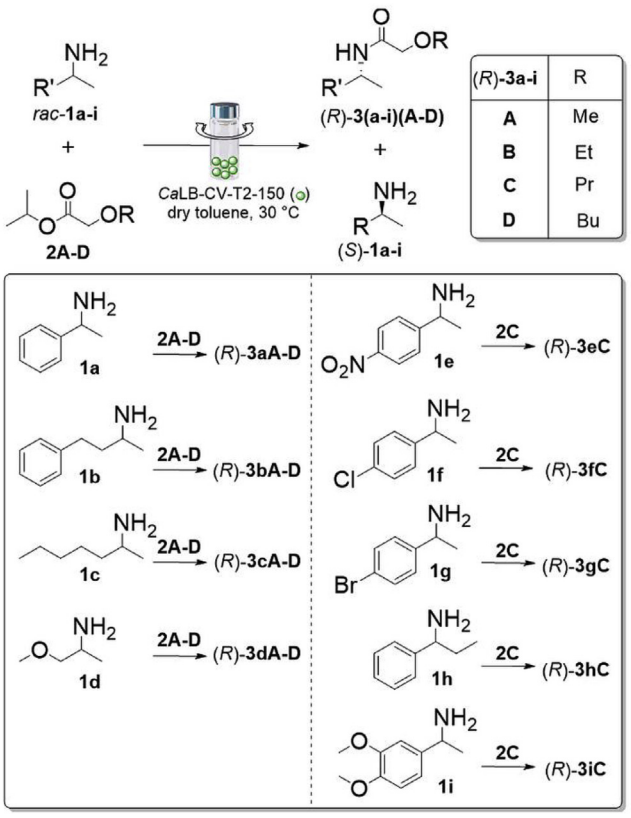
provide enhanced catalytic activity in enzymatic KRs. It is important to note that esters of strong acids (pK_a<2.0) as acylating agents in KR could facilitate not just the enzymatic but the chemical acylation of substrate during enzymic resolution thereby lowering the enantiomeric purity of product 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).

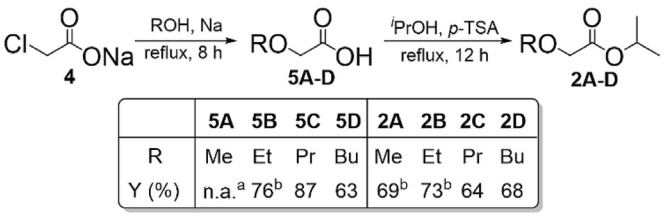
2. Results and discussion

2.1. Synthesis of 2-alkoxyacetic 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).



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.



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

The corresponding 2-alkoxyacetic 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).

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 ($ee_{(R)\text{-}\mathbf{3(a\text{-}d)\ (A\text{-}D)}}$) (Scheme 1).

Analysis of the conversion values (Fig. 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 ($ee_{(R)\text{-}\mathbf{3(a\text{-}d)(A\text{-}D)}}$) 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 (Fig. 1, panels A–C). In contrast, KR of 1-methoxypropan-2-amine *rac*-**1d** showed slightly lower but acceptable enantiomer selectivity (*ee* >95%, Fig. 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).