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Isopropyl 2-ethoxyacetate—an efficient acylating agent for lipase-catalyzed kinetic resolution of amines in batch and continuous-flow modes

Márk Oláh [a], Zoltán Boros [a,b], Gábor Hornyánszky [a,b], László Poppe [a,b,*]

[a] Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Műegyetem rkp. 3, H-1111 Budapest, Hungary
[b] Synbiocat Ltd., Lázár deák str. 4/1, H-1173 Budapest, Hungary

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ABSTRACT

Productivity [conversion (c) and specific reaction rate (r_{batch} or r_{flow})] and enantiomer selectivity [enantiomeric ratio (E) and enantiomeric excess (ee) of the products] of ethyl and isopropyl esters of acetic, 2-methoxyacetic and 2-ethoxyacetic acids as acylating agents were compared in the N-acylation of (±)-1-phenylethanamine *rac*-**1** catalyzed by variously immobilized forms of *Candida antarctica* lipase B (*Ca*LB) using shake flasks and continuous-flow reactors. The effect of the temperature in the 0–80 °C range on productivity and enantiomer selectivity in KRs of *rac*-**1** was investigated with the isopropyl esters in continuous-flow mode using *Ca*LB-filled minireactors. Isopropyl 2-ethoxyacetate surpassed the performance of ethyl 2-methoxyacetate in terms of both productivity (1.9–2.9 times higher rate in batch mode) and enantiomeric selectivity ($ee_{(R)\text{-amide}}$>99.9% compared to 99.8%) providing at 40 °C high volumetric productivity (2.22 kg L^{-1} h^{-1}), specific reaction rate and enantiomeric excess (r_{flow}=783 μmol min^{-1} g^{-1}, $ee_{(R)\text{-}\mathbf{2c}}$>99.9%).

1. Introduction

Chiral amines[1–3] are valuable building blocks for synthesis of fine chemicals and final products in pharmaceutical[4] and agrochemical[5] industries. Production of chiral amine derivatives in enantiopure[6,7] form is a desired goal from physiological[8,9] and environmental[10] points of view as well. Chiral amine production relies on differential crystallization of their diastereomeric salts with chiral acids, resolution methods[11] or asymmetric synthesis catalyzed by wide range of chiral metal–ligand complexes or enzymes.[12–15]

As an important tool for enantioselective synthesis, biocatalysis has become a widely used technology due to the increasing availability of different types of enzymes such as hydrolases[16,17] transaminases[18,19] and amine oxidases.[20] In this context, lipases (EC 3.1.1.3)[21] have gained popularity since they can perform kinetic resolution (KR) in aqueous medium[22,23] or in organic solvents[3] as well, do not require any cofactors and can be reused by applying proper immobilization techniques.

Lipases—especially the lipase B from *Candida antarctica*[24]—are suitable catalysts for KRs of primary amines[3] and also of the less common secondary amines[25,26] by enantiomer selective N-acylation of such substrates in organic solvents. A beneficial property of lipases that the amide bond formation is essentially irreversible because lipases are unreactive towards hydrolysis of the amide bond. Many parameters have influence on selectivity and productivity of lipase-catalyzed KRs of amines, such as type of the solvent (organic or ionic liquids),[27] water content of compounds (solvent, enzyme preparation, substrate, acyl donor)[28] and importantly the activity of enzyme preparation. In the past two decades many research have aimed to increase productivity and selectivity of enzymatic KRs of primary amines.

These investigations highlighted the key role of acyl donor to influence productivity and selectivity of the KRs of amines. In early KRs and chemoenzymatic dynamic KRs (DKR) of amines ethyl acetate was applied as acylating agent.[29] Later Bäckvall and coworkers introduced isopropyl acetate as a more suitable acyl donor in stereoselective biotransformations of chiral amines.[13] The comparison of these two acetic acid esters clearly showed as advantages of applying isopropyl group[13,30] higher activity and selectivity in preparation of enantiopure amide products.[31] Besides of ethyl and isopropyl moieties, vinyl and isopropenyl esters[27] were also tested in enzymatic KRs of amines. Later it turned out that the

* Corresponding author. Tel.: +36 1 463 2229; fax: +36 1 463 3297; e-mail address: poppe@mail.bme.hu (L. Poppe).

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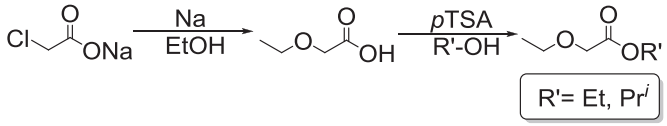
acylating agent could be improved by activating the carboxylic acid moiety with different electron withdrawing groups (e.g., alkoxy, halogen, cyano).[32–34] In an early biocatalytic study in this field, Ladner et al. found that the ethyl 2-methoxyacetate has 100 times higher activity compared to ethyl butyrate of similar size in KR of 1-phenylethylamine catalyzed by *Burkholderia plantarii* lipase.[35] The introduction of methoxy group resulted in many times more active acylating agent as compared to previously used non-activated fatty acid esters, therefore 2-methoxyacetate esters became widely used as enhanced acylating agents in enzymatic *N*-acylation of chiral amines at laboratory as well as at industrial scale.[28,36–42] The excellent reactivity of the activated acetates with lipases could be rationalized by the electronegativity of the methoxy oxygen of the acyl donor and the existence of a weak hydrogen bond to the β-oxygen atom of the 2-methoxyacetate moiety in the so-called acyl-enzyme intermediate.[43] In biocatalytic *N*-acylations with activated acylating agents, however, chemical (non-enzymatic) acylation may generate problems due to lower the stereoselectivity, which call for thorough characterization of the process. The pK_a of an acid[44] correlates with the ability of its esters to the acylation. Thus, esters from strong acids (pK_a<2.0) can react fast not only in enzymic but also in chemical acylation which diminish the selectivity of the enzymic process. Ethyl 2-methoxyacetate proved to be a good balance between enhanced reactivity in enzyme catalyzed reaction but still no chemical acylation ability under ambient conditions of the biotransformations. Therefore it was applied in multiple KRs at industrial scale with excellent yield and selectivity and minimal amounts of enzyme. Such processes have been used by BASF since 1993 to produce a broad spectrum of chiral amines at scale from several hundreds to thousands tons per year. The products, the forming (*R*)-amide and the residual (*S*)-amine can be recovered and separated by distillation in high chemical purities and *ee*.[45]

With the aim to improve the productivity of industrial relevant lipase-catalyzed kinetic resolutions of optically active primary amines, we planned to further investigate the alkoxy-activation of acylating agents using ethyl and isopropyl esters of 2-ethoxyacetic acid compared to the corresponding esters of acetic and 2-methoxyacetic acids in enzymatic *N*-acylation of racemic 1-phenylethylamine *rac*-**1**.

2. Results and discussion

2.1. Preparation of 2-ethoxyacetic acid esters

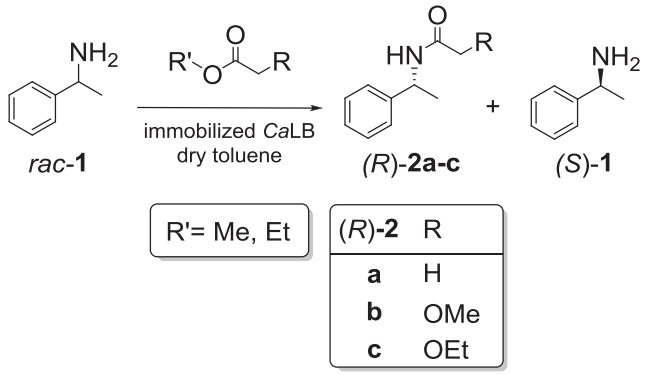
Our studies started with syntheses of ethyl and isopropyl esters of 2-ethoxyacetic acid (Scheme 1.). 2-Ethoxyacetic acid was prepared by reacting sodium 2-chloroacetate with sodium ethoxide.[46] The resulting acid was esterified with ethanol or 2-propanol by acid catalysis and purified with vacuum distillation.



Scheme 1. Synthesis of 2-ethoxyacetic acid esters.

2.2. Influence of the acylating agents and the mode of immobilization of CaLB on kinetic resolution of *rac*-1 in batch mode

Next, the 2-etoxyacetate esters as novel acylating agents were compared to the non-activated ethyl and isopropyl acetate in kinetic resolution of *rac*-**1** catalyzed by six, differently immobilized *Ca*LB biocatalysts (adsorptive: N435, G250O, G250P, PAP31A; covalent: T2-150; sol–gel: SGA10D) (Scheme 2).



Scheme 2. Kinetic resolution of 1-phenylethylamine *rac*-**1** by acylation with different esters by variously immobilized forms of lipase B from *Candida antarctica*.

When applied the acylating agents in 2.0 equiv amounts to *rac*-**1** both the nature of ester and the mode of lipase immobilization affected the conversion and enantiomeric composition of the amides (*R*)-**2a** and (*R*)-**2c** formed (Table 1). It was in accordance with the previous results showing that the mode of immobilization of *Ca*LB could significantly influence its selectivity and productivity in the kinetic resolution of racemic amines with ethyl acetate in batch and continuous-flow modes.[47]

In kinetic resolution of *rac*-**1** catalyzed by the *Ca*LB biocatalysts isopropyl acetate (Pr^{i}OAc) was 1.9–2.8 times more active as ethyl acetate (EtOAc). Moreover, enantiomeric excess of the product (*R*)-**2a** was also higher with Pr^{i}OAc (99.8–>99.9%) than with EtOAc (99.3–99.9%).

In KR of *rac*-**1**, the presence of the electron withdrawing 2-ethoxy group in the acylating agent increased the conversion significantly and rendered ethyl 2-ethoxyacetate as acyl donor more active than Pr^{i}OAc (2–4 times) or EtOAc (6–8 times). Unfortunately, the advantage of using ethyl 2-ethoxyacetate as acylating agent was

Table 1
Immobilized *Ca*LB-catalyzed kinetic resolution of *rac*-**1** with 2.0 equiv amounts of various acylating agents R–CH_2COO-R′

Entry	*Ca*LB[a]	R	R′	Conv.[b][%]	$ee_{(R)\text{-}2a,c}$[b]	E[b] [–]
1	N435	H	Et	9.8	99.9	≫200
2	N435	H	Pr^i	16.5	>99.9	≫200
3	N435	OEt	Et	40.7	99.5	≫200
4	N435	OEt	Pr^i	47.0	99.6	≫200
5	T2-150	H	Et	2.0	99.6	>200
6	T2-150	H	Pr^i	4.1	99.8	≫200
7	T2-150	OEt	Et	14.4	99.0	>200
8	T2-150	OEt	Pr^i	36.1	>99.9	≫200
9	SGA10D	H	Et	0.8	99.1	>200
10	SGA10D	H	Pr^i	1.1	99.4	>200
11	SGA10D	OEt	Et	1.6	90.7	21
12	SGA10D	OEt	Pr^i	7.0	>99.9	≫200
13	G250P	H	Et	4.4	99.8	≫200
14	G250P	H	Pr^i	8.9	>99.9	≫200
15	G250P	OEt	Et	28.6	99.3	>200
16	G250P	OEt	Pr^i	46.2	99.9	≫200
17	G250O	H	Et	1.5	99.5	>200
18	G250O	H	Pr^i	2.9	99.8	≫200
19	G250O	OEt	Et	6.1	97.5	83
20	G250O	OEt	Pr^i	28.6	>99.9	≫200
21	PAP31A	H	Et	5.0	99.8	≫200
22	PAP31A	H	Pr^i	9.7	>99.9	≫200
23	PAP31A	OEt	Et	39.4	99.5	≫200
24	PAP31A	OEt	Pr^i	47.8	99.8	≫200

[a] Conditions: *rac*-**1** (94 mg, 0.385 M), acyl donor (2 equiv, 0.770 M) in dry toluene (2.0 mL), enzyme preparations (50.0 mg). Reaction time 1 h.
[b] Determined by GC.