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Highly enantioselective aminoacylase-catalyzed transesterification of secondary alcohols

M. Bakker, A. S. Spruijt, F. van Rantwijk and R. A. Sheldon*

Laboratory of Organic Chemistry and Catalysis, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands

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Abstract

The aminoacylase (*N*-acyl-L-amino acid amidohydrolase; E.C. 3.5.1.14) from *Aspergillus melleus*, a readily available inexpensive enzyme, catalyzes the transesterification of a wide range of chiral secondary arylalkanols with essentially absolute stereoselectivity ($E > 500$). Moreover, the productivities obtained with 1-phenylethanol, 1-phenylpropanol, 1-(1-naphthyl)ethanol and 1-(2-naphthyl)ethanol were substantially higher than those in the corresponding lipase-catalyzed transesterifications.

1. Introduction

Enantiomerically pure secondary alcohols are important synthetic intermediates and chiral auxiliaries.[1] They are conveniently accessible via kinetic resolution of the racemate, which has mainly involved a lipase-catalyzed transesterification (see Fig. 1). The lipases from the *Pseudomonas* species,[1–3] porcine pancreas,[4,5] *Candida rugosa*[6–8] and *Candida antartica*[8] have been used in such schemes and the steric preferences of the most widely used lipases have been characterized.[9] Because secondary alcohols are sluggish reactants and an irreversible reaction is a prerequisite for efficient kinetic resolution,[10–13] a wide range of activated and/or irreversible acyl donors has been used in combination with lipases, e.g. enol, haloethyl and oxime esters as well as acid anhydrides.[13] Enol esters, such as vinyl and isopropenyl esters, are the most widely used irreversible acyl donors. They shift the reaction equilibrium towards synthesis because the liberated enol tautomerizes to acetaldehyde or acetone, respectively. A disadvantage connected with the use of vinyl esters is that some lipases, notably those from *Candida rugosa* and *Geotrichum candidum*, are deactivated due to the Schiff's base formation of lysine residues with acetaldehyde.[13] Acetone, which is the by-product from isopropenyl esters, does not cause deactivation of the enzyme although in most cases the reaction rate is lower due to steric hindrance.[9–13]

* Corresponding author. Tel: +31 15 278 2683; fax: +31 15 278 1415; e-mail: secretariat-ock@tnw.tudelft.nl

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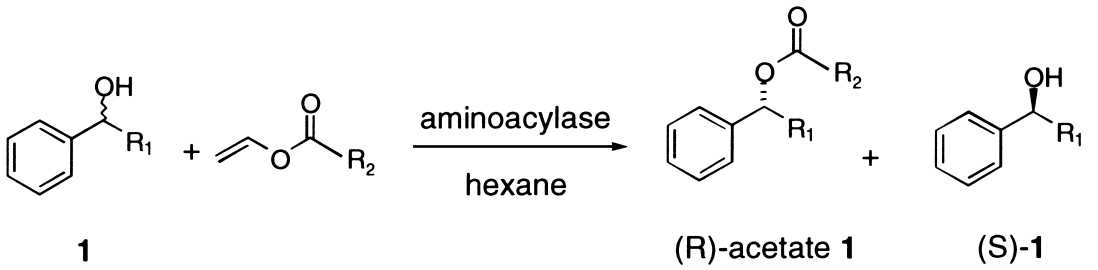


Figure 1. Transesterification of 1-phenylethanol **1** with vinyl acetate

The aminoacylase from *Aspergillus melleus* (*N*-acyl-L-amino acid hydrolase, E.C. 3.5.1.14) is a readily available and versatile catalyst for enantioselective hydrolysis of *N*-acetyl amino acids.[14,15] Although its reaction mechanism is not known in any detail it is generally assumed that it involves coordination of the carbonyl group of the acylating reagent to the Zn^{2+} ion in the active site, similar to the Zn-dependent proteases.[16] Recent work by the group of Herradón et al.[17–19] revealed that the aminoacylase is a transesterification catalyst that combines a highly relaxed substrate specificity with a high selectivity for the (*R*)-enantiomer of the alcohol. Here we present a study of the kinetic resolution of secondary arylalkanols via aminoacylase-mediated transesterification with vinyl acetate. Increased productivities were obtained with a wide scope of substrates and acyl donors.

2. Results and discussion

Initially we investigated the previously reported[17] aminoacylase-mediated acylation of 1-phenylethanol **1** more fully. A range of solvents were compared with regard to the reaction rate in the acylation of (±)-**1** to (*R*)-**1** acetate and (*S*)-**1** by vinyl acetate (see Fig. 1). Hexane, in which solvent the reaction took place at an initial rate of 53 μmol (g protein^{-1}) min^{-1}, was the solvent of choice. The higher rate allowed us to carry out the reaction to 50% conversion in 3 days using only one fifth of the amount of catalyst reported by Herradón et al.[17]

Racemic and (*R*)-**1** were acylated at the same rate in separate experiments, whereas the conversion of (*S*)-**1** was too low to measure, which sets the lower limit of the enantiomeric ratio E[7] at 500. This confirms the absolute stereoselectivity of the aminoacylase and also shows that the catalyst is not inhibited by the unreactive (*S*)-**1**. The linear (zero-order) progress of the reaction (Fig. 2) shows that the presence of acetaldehyde has no deleterious effect on the biocatalyst. The catalyst could easily be recovered by filtration and the products isolated by column chromatography.

We subsequently studied the effect of the acyl donor on the rate and the enantioselectivity of the acyl transfer. A wide range of vinyl carboxylates (Table 1) converted **1** into the corresponding (*R*)-esters and (*S*)-**1** with nearly complete stereoselectivity ($E > 500$). Vinyl butyrate reacted at twice the rate of the acetate, as already reported by Herradón et al.,[17] but hexanoate and higher esters were much less reactive. Analogously to the lipase-catalyzed resolution of 5-phenyl-1-pentan-3-ol,[20] vinyl acrylate and crotonate reacted slower (70%) than vinyl acetate. This is ascribed to the steric hindrance owing to the comparative inflexibility of the α,β-alkenic ester. Moreover, the electron-withdrawing character of the vinyl group is expected to destabilize the coordination of the carbonyl oxygen to the catalytic Zn^{2+} ion of the aminoacylase. Similarly, vinyl benzoate gave a low reaction rate and in this case the enantioselectivity was also mediocre.

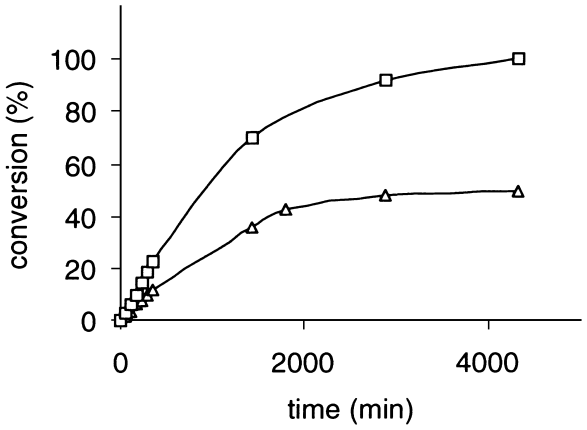


Figure 2. Initial rate and conversions of racemic (△) and (*R*)-1-phenylethanol (□)

Table 1
Effect of the acyl donor on the aminoacylase-mediated acylation of 1-phenylethanol

Substrate	Rate[a] (μmol (g protein)$^{-1}$ min^{-1})	Time (d)	Conv. (%)	ee_{ester} (%)
Vinyl acetate	52.6	3	50	> 99
Vinyl propionate	67.0	2	50	> 99
Vinyl butyrate	109.7	1.3	50	> 99
Vinyl acrylate	26.6	7	37	> 99
Vinyl crotonate	40.2	7	45	> 99
Vinyl hexanoate	15.3	4	50	> 99
Vinyl octanoate	14.6	4	50	> 99
Vinyl decanoate	13.7	6	50	> 99
Vinyl laurate	35.0	6	50	> 99
Vinyl benzoate	1.9	7	14	72
Isopropenyl acetate	1.2	7	8	48
Methyl butyrate	4.3	7	12	> 99

[a] The reaction rate was measured over the first 5 hours.

The effect of the leaving group was briefly investigated. Isopropenyl acetate and methyl butyrate reacted at only a few percent of the rate of vinyl acetate, which renders them unsuitable for this resolution system. We have employed vinyl acetate as the standard acylating agent throughout this work in order to compare our results with related work. In order to assess the scope of the

aminoacylase-catalyzed transesterification, we investigated the acylation of the secondary alcohols **1**–**12** (see Fig. 3).

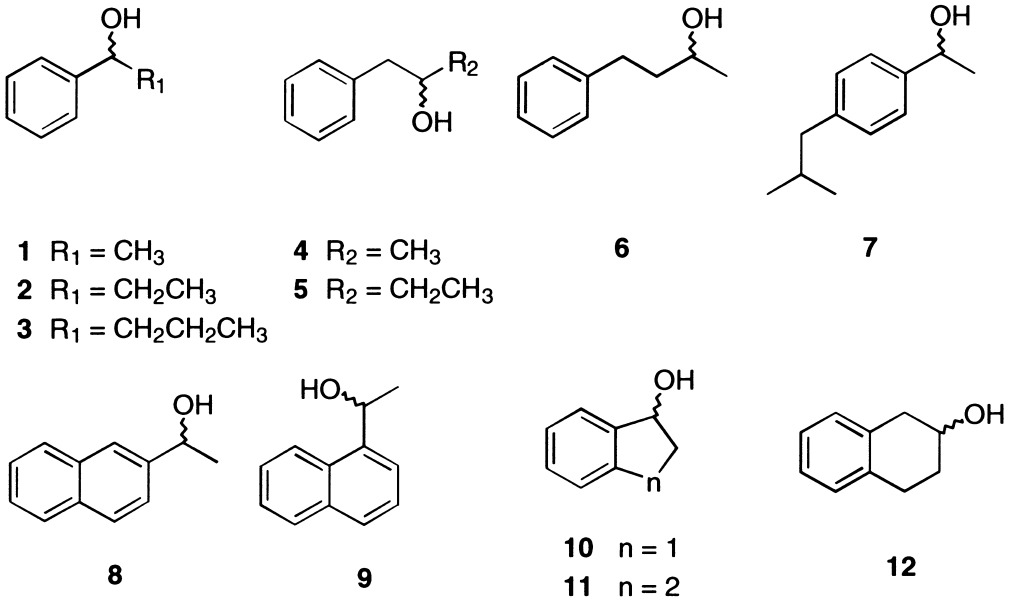


Figure 3. Secondary alcohols used for transesterification reactions

In addition to **1**, its homologues 1-phenyl-propanol **2** and -butanol **3** were likewise converted into the (*R*)-esters with complete enantioselectivity. Alcohol **2** was converted at nearly the same initial rate as **1**, but **3** reacted much slower and complete conversion was not achieved (Table 2). We note that the closely related compound 1-phenylbut-3-enol was also converted quite sluggishly in a comparable reaction.[19] Apparently a C_3 group is not readily accommodated in the part of the nucleophile subsite that in the 'natural' reaction receives the carboxylate group of *N*-acetyl-L-methionine.

When the hydroxyl group was moved to the C-2 positions, **4** and **5**, the acylation became quite slow and the conversion remained low. Moreover, the enantiomeric ratio decreased by a factor of 50. Surprisingly, 4-phenyl-2-butanol **6**, in which the aromatic ring is two carbon atoms removed from the alcohol group, reacted with complete enantioselectivity at a rate comparable with **2**. The opposite enantiomer, i.e. (*S*)-**6**, was preferentially acylated, however. Hence, we conclude that the subsite, which in the acylation of **1** receives the methyl group, preferentially binds the phenylbutyl group in the acylation of **6**.

We next investigated the effect of aromatic substitution. A *p*-isobutyl group **7** had a negligible effect on the reaction, but when the phenyl group in **1** was substituted by naphthyl, the results were more varied. 1-(2-Naphthyl)ethanol **8** was completely converted into the (*R*)-ester but its 1-naphthyl isomer[19] **9** reacted much slower, although the enantioselectivity remained complete.

Finally, we have attempted the resolution of some bicyclic alcohols. 1-Indanol **10** formed only a minute amount of (*R*)-product after 7 days, but 1,2,3,4-tetrahydro-1-naphthol **11** was converted much faster and the acylation of 1,2,3,4-tetrahydro-2-naphthol **12** proceeded at a comparable rate to **11**. We surmise that the half-chair conformation of the six-membered ring in **11** is more

Table 2
Transesterification of secondary aromatic alcohols with vinyl acetate

Substrate (*RS*)	Rate[a]	Time (d)	Conv. (%)	Abs. conf.	Isol. Yield (%)	E[b]	Productivity[c] *acylase* (g (g prot)$^{-1}$h^{-1})	Productivity[c] *lipase* (g (g prot)$^{-1}$h^{-1})
1	52.6	3	50	*R*	49	>500	0.207	0.085[2]
2	44.1	7	48	*R*	44	>500	0.083	0.029[1]
3	10.3	7	22	*R*	19	>500	0.037	n.d.
4	0.3	7	14	*R*	10	12	-	0.033[1]
5	1.2	7	16	*R*	11	10	-	n.d.
6	35.8	7	44	*S*	43	>500	0.074	n.d.
7	50.1	3	50	*R*	47	>500	0.154	n.d.
8	48.0	7	50	*R*	46	>500	0.082	0.058[1]
9	13.8	7	25	*R*	22	>500	0.026	0.001[5]
10	3.3	7	5	*R*	4	>500	0.009	0.054[1]
11	25.9	7	35	*R*	30	>500	0.061	0.060[1]
12	18.7	7	30	*R*	25	>500	0.051	n.d.

[a] Reaction rate measured over the first 5 hours, in μmol product (g protein)$^{-1}$ min^{-1}.

[b] Calculated according to ref.[7]

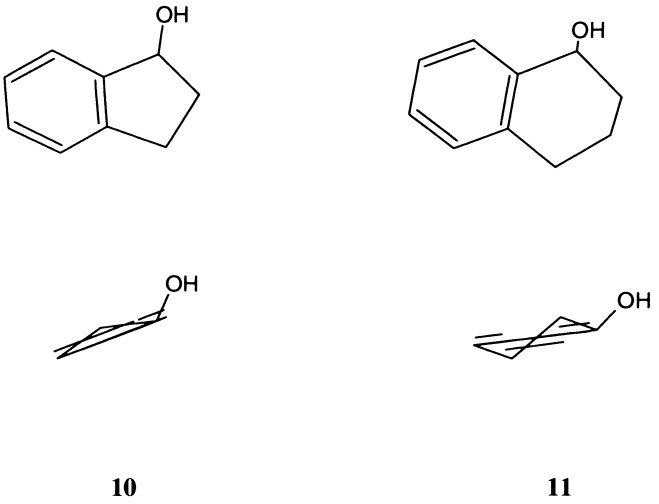
[c] The productivity was only calculated when E > 100.

readily accommodated in the active site than the planar five-membered ring in **10** (Fig. 4). These bicyclic compounds all reacted completely *R*-specifically.

It should be noted that some alcohols, such as **2** and **8**, which reacted at an initial rate that is comparable with **1**, were slow to achieve a complete conversion. Catalyst deactivation apparently plays only a minor role because the enzyme still retained 40% of its original hydrolytic activity after 7 days in hexane. Inhibition by small amounts of acid, originating from donor hydrolysis by traces of water, could very well be involved.

Lipases are also able to catalyze the enantioselective transesterification of secondary alcohols albeit with low productivities, i.e. necessitating the use of relatively large amounts of enzyme to obtain reasonable reaction rates.[1,2,5] For example, *Pseudomonas* lipase mediated the transesterification of **1**[2] with a productivity of 0.09 g (g protein)$^{-1}$ h^{-1}, whereas with aminoacylase 0.2 g (g protein)$^{-1}$ h^{-1} was achieved (Table 2). Moreover, aminoacylase mediated the acylation of **2**, **8** and **9** with substantially higher productivities than those calculated for the lipase-catalyzed reactions.

The main advantage of the aminoacylase is that it readily accepts a wide range of alcohols containing bulky aromatic groups, whereas other catalysts are more restricted in scope and/or exhibit lower *E* values.[9,21,22] This feature, combined with the high volume yields and high productivities that we recently demonstrated[23] makes our procedure very attractive for application in enantioselective transesterifications.



10 **11**

Figure 4. Conformation models of compounds **10** and **11**

In summary, we have shown that the readily available aminoacylase from *Aspergillus melleus* is a very useful catalyst for enantioselective transesterifications. The enzyme is stable, without any modification, in organic solvents and acts highly enantioselectively in the acylation of a wide range of secondary arylalkanols.

3. Experimental

3.1. Materials

Aminoacylase from *Aspergillus melleus* (E.C. 3.5.1.14) and Bradford reagent were purchased from Sigma. *N*-Acetyl-L-methionine, vinyl propionate, vinyl acrylate, vinyl crotonate, vinyl benzoate, (*RS*)-1-phenylethanol, (*RS*)-1-phenyl-1-propanol, (*R*)-1-phenyl-1-butanol, (*RS*)-1-(2-naphthyl)ethanol and (*RS*)-1,2,3,4-tetrahydro-2-naphthol were obtained from Aldrich. Cumene, vinyl acetate, vinyl propionate, (*RS*)-1-phenyl-2-propanol, (*RS*)-1-phenyl-3-butanol and (*RS*)-1-(1-naphthyl)ethanol were purchased from Acros. Vinyl butyrate, methyl butyrate, vinyl laurate, isopropenyl acetate, (*RS*)-1-phenyl-2-propanol, (*S*)-1-phenyl-1-butanol, (*RS*)-1-indanol and (*R*)-1-(1-naphthyl)ethanol were obtained from Fluka, and vinyl hexanoate and vinyl octanoate from Tokyo, Kasei, Kogyo Co. (*RS*)-1,2,3,4-Tetrahydro-1-naphthol was obtained from Lancaster and *p*-isopropyl-1-phenylethanol was kindly donated by the Hoechst Celanese Corporation.

3.2. Analysis

Samples for monitoring the transesterification reactions were quenched by removal of the enzyme by centrifugation. The enantiomeric purity of the alcohols and esters was analyzed by chiral HPLC using a Chiralcel OD column (Daicel Chemical Industries, Ltd., 250×4.6 mm), eluent flow 0.6 ml min^{-1}, and detected on a Waters 486 tunable absorbance detector at 254 nm with Waters Millennium[32] software. A hexane/isopropanol mixture was used as eluent for all alcohols and esters.

The progress of the reactions was monitored by reversed phase HPLC using a custom-packed Symmetry C_{18} cartridge (Waters Radial-pak, 8×100 mm, 7 μm) contained in a Waters RCM 8×10 compression unit, with simultaneous detection on a Waters 486 tunable absorbance detector with Waters Millennium[32] software. The hydrolysis of *N*-acetyl-L-methionine was monitored by reversed phase HPLC on the same symmetry C_{18} column, using acetonitrile:phosphate buffer (50 mM, pH 2.2) 7.5:92.5 (v:v) as eluent (flow 1.5 ml/min for the first 4 min followed by 3 ml/min), with detection at 210 nm. Optical rotations were measured using Perkin–Elmer 241 polarimeter.

3.3. Aminoacylase activity test

The activity of aminoacylase was measured in the hydrolysis of *N*-acetyl-L-methionine. *N*-Acetyl-L-methionine (15.7 mM) was dissolved in a Tris buffer (5 ml, 50 mM, pH 7.5), the pH was adjusted with NaOH (1 M) and enzyme was added. The reaction was quenched after 1 h by adding HCl (1 M, 5 ml) and the conversion was measured by HPLC.

3.4. Enzyme-catalyzed acylation of 1-phenylethanol *1* with enol esters

The experiments were performed at room temperature in a 10 ml reaction vessel containing 50 U aminoacylase from *Aspergillus melleus* (5 mg protein), 100 mg (0.8 mmol) (±)-**1** and 1.2 mmol enol ester dissolved in 5 ml *n*-hexane. The course of the reaction was followed by chiral HPLC (Chiralcel OD column; Diacel Chemical Industries, Ltd.) using cumene or cymene as the internal standard. After 7 days the reaction was stopped and the enzyme was filtered off and the combined filtrates were concentrated in vacuo. Distillation of the residue afforded (*S*)-**1** ($[\alpha]_D^{20} = -45$, $c = 5$, MeOH) and (*R*)-**1** acetate ($[\alpha]_D^{20} = 114$, $c = 2$, MeOH). Similar to the acylation of **1** with vinyl acetate all the other esters were used. Chiral HPLC analysis showed that with all enol esters only (*R*)-**1** was converted to (*R*)-**1** ester.

The racemic esters were synthetically prepared by stirring 1.1 equivalents of anhydride or acid chloride with **1** in pyridine for 4 h. After evaporation, the product was purified by column chromatography or distillation, resulting in a pure racemic compound as reference for HPLC.

3.5. Enzyme-catalyzed acylation of alcohols with vinyl acetate

A mixture of 0.8 mmol (±)-alcohols **1**–**12** and 1.2 mmol vinyl acetate was dissolved in 5 ml *n*-hexane containing 50 U aminoacylase (5 mg protein). The course of the reaction was followed by chiral HPLC (see Table 2) using cumene as the internal standard. When the reaction was complete or after 7 days the enzyme was filtered off and the filtrate was evaporated. The residue was purified for compounds **1**–**12** by silica gel flash chromatography (EtOAc:hexane, 10:90 (v/v)) to afford the unconverted alcohol and the ester. The absolute configurations of the components were assigned on the basis of chiral HPLC by comparison with the literature data.[8,18,19,24] These assignments agreed with the signs of the specific rotation of the unconverted alcohols.

Compounds (*S*)-**1** ($[\alpha]_D^{20} = -45$, $c = 5$, MeOH) and (*R*)-**1** acetate ($[\alpha]_D^{20} = +114$, $c = 2$, MeOH); (*S*)-**2** ($[\alpha]_D^{20} = -28$, $c = 1$, MeOH) and (*R*)-**2** acetate ($[\alpha]_D^{20} = +100$, $c = 1$, $CHCl_3$); (*R*)-**6** ($[\alpha]_D^{20} = +20$, $c = 1$, MeOH) and (*S*)-**6** acetate ($[\alpha]_D^{20} = -10$, $c = 1$, $CHCl_3$); (*S*)-**7** ($[\alpha]_D^{20} = -30$, $c = 1$, MeOH) and (*R*)-**7** acetate ($[\alpha]_D^{20} = +94$, $c = 1$, $CHCl_3$); (*S*)-**8** ($[\alpha]_D^{20} = -25$, $c = 2.2$, MeOH) and (*R*)-**8** acetate ($[\alpha]_D^{20} = +33$, $c = 1$, $CHCl_3$); (*S*)-**9** ($[\alpha]_D^{20} = -76$, $c = 2.2$, MeOH) and (*R*)-**9** acetate ($[\alpha]_D^{20} = +45$, $c = 1$, $CHCl_3$); (*S*)-**10** ($[\alpha]_D^{20} = -34$, $c = 1$, $CHCl_3$) and (*R*)-**10** acetate ($[\alpha]_D^{20} = +45$, $c = 1$, $CHCl_3$); (*S*)-**11**

($[\alpha]_D^{20} = -28$, $c = 1$, $CHCl_3$) and (*R*)-**11** acetate ($[\alpha]_D^{20} = +51$, $c = 1$, $CHCl_3$). The configuration of the unconverted **3** was determined by comparison (chiral HPLC) with the commercially available pure enanatiomers.

The racemic products were synthetically prepared by stirring 1.1 equivalents of acetic anhydride with alcohol in pyridine for 6 h. After removing the pyridine and the excess of acetic anhydride by evaporation the products were purified by column chromatography. The racemic product was used as a reference for the HPLC. The prepared (*S*)-ester was not shown in the HPLC chromatograms of enzymatic-catalyzed reactions, which indicates the chiral discrimination of the catalyst.

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