Biocatalysis

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One-Pot Enzymatic Resolution and Separation of *sec*-Alcohols Based on Ionic Acylating Agents**

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Enantiomerically pure alcohols are important intermediates in the pharmaceutical industry for the production of active pharmaceutical ingredients. On a large scale, and asides from chiral natural resources, they can be obtained by classical chemical asymmetric or biotechnological processes. The approaches used most often are the asymmetric hydrogenation of ketones,[1] Corey–Bakshi–Shibata borane reduction,[2] the kinetic resolution of epoxides,[3] enzymatic resolution,[4] ketone reduction,[5] and fermentation.[6]

Enzymatic kinetic resolution (EKR) of racemic alcohols is a well-established method and often the unique and/or most practical route for the preparation of enantiomeric alcohols, especially when both enantiomers are needed.[7,8] However, in this method, some limitations need to be circumvented to improve economical and environmental aspects. One problem is generally the need of a large excess of acylating agent to achieve the desired conversions, with consequent generation of reactive side products that can change the reaction course. Another major drawback in this transformation is the product separation step. The enantiomers, one as an alcohol and the other as an ester, formed during the acylation are usually separated by chromatographic techniques. On the laboratory scale this is not a problem, but on a large scale this approach presents serious limitations.[9,10] To circumvent this disadvantage, several new approaches have been reported; for example, Theil and co-workers described the efficient enzymatic resolution of *sec*-alcohols using fluorous acylating agents.[11,12] To isolate the products, namely the *R*-fluorous ester (98% *ee*) and the unreacted *S*-alcohol (99% *ee*), from the reaction, repeated extractions with a fluorous solvent were needed. In 2001, Curran, Theil, and co-workers described another attractive kinetic resolution process involving fluorous triphasic reactions[12] whereby a simple U-tube holding a lower fluorous phase that serves a barrier to separate two organic phases was used. One side contained the source phase MeOH/CH_3Cl, and the other side contained a receiving phase containing MeOH/MeO^-. A mixture of *S*-fluorous ester (99% *ee*) and the unreacted *R*-alcohol (91% *ee*) obtained from EKR was added to the source side of the U-tube, and it was possible to separate each enantiomer of alcohol with only a slight loss in *ee* (about 2–4%). In 2006, Matsumoto and co-workers described an EKR approach based on monomethoxy-poly(ethylene glycol)-supported carbonates as anchoring agents.[13] Such a suitable water-soluble supported carbonate containing one of the enantiomers could be separated from the *R*-alcohol by hexane extraction. By repeating the extraction with EtOAc, it was then possible to remove from the water layer the supported carbonate containing one of the enantiomers.

During the course of our recent investigations, Salunkhe and co-workers have reported another similar EKR involving anchored ionic liquids containing a carboxylate group in DMSO.[14] Ibuprofen-anchored ionic liquids were resolved, and (*S*)-ibuprofen was isolated in 87% yield (86% *ee*).

Over the last few years, the use of ionic liquids (ILs) to replace organic solvents for EKR has gained much attention and appears to be a good alternative.[15–22] The high stability of ionic liquid media, the ease of product recovery combined with enzyme stability,[23] and the possibility to reuse the reaction media make ILs a powerful tool for biocatalysis. Additionally, task-specific ionic liquids (TSILs) have shown a considerable advantage in several reactions.[24]

Herein, we report a practical, reusable, and efficient process for the resolution and separation of both enantiomers of *sec*-alcohols, without the need for laborious chromatography separation, based on the combination of TSILs as acylating and anchoring ionic liquids and an enzyme working sequentially as a kinetic resolution agent and to detach one enantiomer of the alcohol at the end (Scheme 1).

A central feature of this EKR and separation of alcohols is the combination of an ionic liquid and an ionic acylating agent. An acylating agent was envisaged that contained two distinct parts, namely, an ionic moiety and an ester moiety recognized by an enzyme, to allow the selective resolution and separation of an ionically anchored ester from the unreacted alcohol. The major advantage of this process compared with other procedures described before is the possibility to separate both free enantiomers of a racemic mixture only by enzymatic resolution in a one-pot reaction using one equivalent of acylating agent. This approach takes advantage of the unique properties of ILs, that they are formed only by ions, which provides an ionic pool crucial for entrapping one of the enantiomers as an ionic ester moiety.

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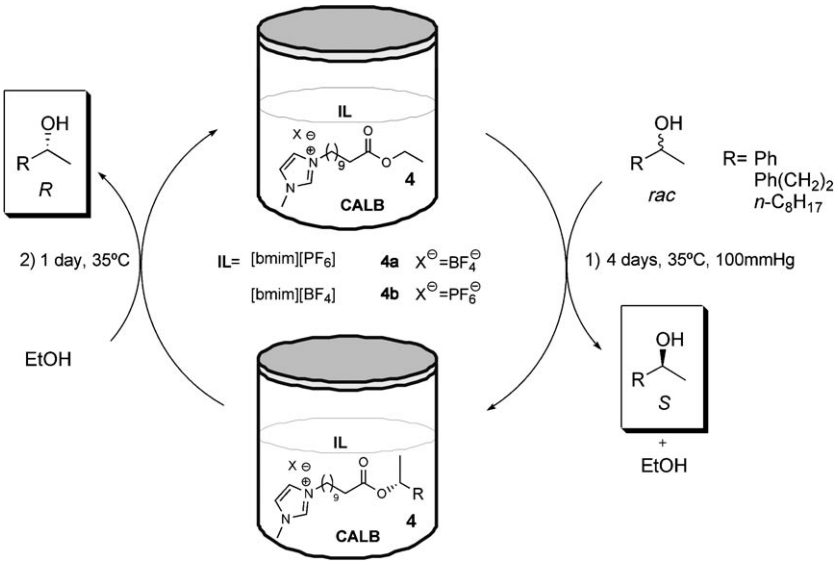


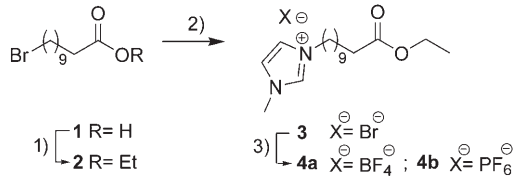
Scheme 1. Methodology for the enzymatic resolution and separation of *sec*-alcohols. CALB = lipase B from *Candida antarctica*.

Additionally, as a result of ILs being immiscible with several organic solvents, the unreacted alcohol can be removed by repeated extraction with a common organic solvent. The fact that ILs are almost non-volatile[25] allows the ethanol formed during transesterification to be evaporated, thus moving the equilibrium toward the formation of products. It is also known that ILs provide a stable and friendly environment for enzymes, which thereby retain their catalytic activity. The anchored enantiomer that results as the product of step 1 (Scheme 1) can be removed in step 2 by a second enzymatic transesterification (reversible reaction) using a primary alcohol, such as EtOH.

After screening several potential acylating agent candidates containing different cations and alkyl chains, the imidazolium cation appeared the most appropriate for this transformation. The acylating agent **4** can be obtained simply by nucleophilic substitution of ethyl 11-bromoundeconate **2** (see Scheme 2). The effect of anion type in the acylating agent and solvent was disclosed by using enzymatic resolution of 1-phenylethanol with CALB as a model transformation, which allowed the identification of **4a** and **4b**, and the ionic liquids [bmim][PF_6] and [bmim][BF_4] (bmim = 1-*n*-butyl-3-methylimidazolium) as the best candidates (Table 1).

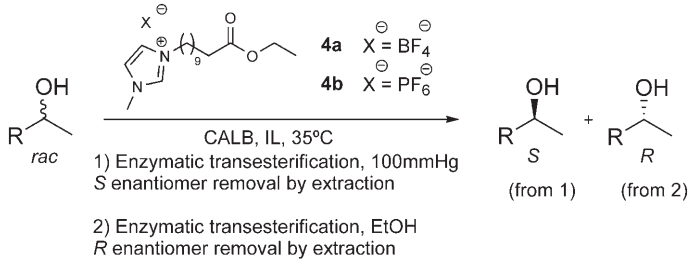
The use of different combinations of acylating agent **4a**/**4b** with [bmim][PF_6]/[bmim][BF_4] and CALB allowed us to obtain *S* and *R* enantiomers in good yields and with good enantiomeric excesses (Table 1). The best result was generally obtained by combination of the acylating agent **4b** and the ionic liquid [bmim][PF_6] containing the same PF_6^- anion. Better yields and enantioselectivities were obtained when longer reaction times were applied for the first enzymatic transformation (96 h). (*S*)-1-Phenylethanol was isolated in 51% yield (80.9% *ee*), and its *R* enantiomer was isolated in 41.3% yield (99.3% *ee*). The first enzymatic transesterification (step 1) turned out to be slower than the second (step 2).

The resolution can also be performed using the acylating agent **4a** without solvent, as **4a** is liquid at room temperature. However, in this case, it was observed that the viscosity of the reaction mixture increases with reaction time, forming a slurry after 48 h and providing both enantiomers in moderate yield and *ee* (step 1:



Scheme 2. Synthesis of an acylating agent based on the imidazolium cation. 1) EtOH, H_2SO_4, toluene, reflux, Dean–Stark apparatus, 15 h, 90%; 2) methyl imidazole, *i*Pr_2O, 70°C, 48 h, 98%; 3) $NaBF_4$ (**4a**), KPF_6 (**4b**), CH_2Cl_2, RT, 48 h, **4a** 87%; **4b** 74%.

Table 1: Enzymatic resolution and separation of racemic *sec*-alcohols using CALB as a biocatalyst.[a]



Racemic alcohol	Acylating agent	IL[b]	Step 1: *S* enantiomer			Step 2: *R* enantiomer		
			t [h]	yield [%]	*ee* [%][c]	*t* [h]	yield [%]	*ee* [%][c]
R = Ph	**4a**	[bmim][PF_6]	48	67.0	54.5	24	25.9	88.9
			96[d]	68.8	62.2	24	22.5	98.5
	4b	[bmim[PF_6]	96[d]	51.0	80.9	24	41.3	99.3
	4a	[bmim][BF_4]	96[d]	74.3	56.2	24	19.2	98.9
	4a	–	48	64.5	39.4	24	19.4	99.0
R = $Ph(CH_2)_2$	**4a**	[bmim][PF_6]	96	50.5	62.4	24	33.2	96.4
	4b	[bmim][PF_6]	96	51.6	60.0	24	30.6	91.9
R = *n*-C_8H_{17}	**4b**	[bmim][PF_6]	96	51.5	38.5[e]	24	25.0	96.5[e]

[a] All reactions were carried out in IL (0.8 mL) with alcohol (0.41 mmol), acylating agent (0.41 mmol), and CALB (20 mg) at 35°C. [b] Initial water content: 1.9 μg H_2O per μL [bmim][PF_6]; 3.8 μg H_2O per μL [bmim][BF_4]. [c] Determined by HPLC. [d] Result corresponds to the first cycle of reuse of the reaction medium (enzyme, acylating agent, IL). [e] Determined by GC of the corresponding butyrate ester.

Table 2: Reuse of the reaction medium for the enzymatic resolution and separation of 1-phenylethanol (yields and *ee* values are given as percentages).[a]

Acylating agent	IL[b]	Step 1: (*S*)-1-phenylethanol[c]								Step 2: (*R*)-1-phenylethanol[c]							
		1st cycle		2nd cycle		3rd cycle		4th cycle		1st cycle		2nd cycle		3rd cycle		4th cycle	
		yield	*ee*	yield	*ee*	yield	*ee*	yield	*ee*	yield	*ee*	yield	*ee*	yield	*ee*	yield	*ee*
4b	[bmim][PF_6]	51.0	80.9	56.9	75.2	62.3	71.1	73.2	51.0	41.3	99.3	43.1	99.2	44.9	98.7	38.4	97.9
4a	[bmim][PF_6]	68.8	62.2	58.3	63.0	62.5	57.2	63.3[d]	64.3	22.5	98.5	36.4	97.0	40.0	99.2	42.5[e]	97.6
4a	[bmim][BF_4]	74.3	56.2	64.5	58.7	76.7	50.4	64.2[f]	58.3	19.2	98.9	38.8	92.6	35.6	98.6	34.2[g]	85.3

[a] All reactions were carried out in IL (0.8 mL) with 1-phenylethanol (0.41 mmol), acylating agent (**4a**, **4b**; 0.41 mmol), and CALB (20 mg) at 35 °C; step 1: 96 h, 100 mmHg, *S* enantiomer removed by extraction with Et_2O; step 2: EtOH (2.5 equiv), 24 h, *R* enantiomer removed by extraction with Et_2O. [b] Initial water content: 1.9 μg H_2O per μL [bmim][PF_6]; 3.8 μg H_2O per μL [bmim][BF_4]. [c] Enantiomeric excess (*ee*) determined by HPLC. [d] 5th cycle: 62.9% yield, 59.6% *ee*; 6th cycle: 79.7% yield, 45.0% *ee*. [e] 5th cycle: 39.3% yield, 99.1% *ee*; 6th cycle: 32% yield, 85.2% *ee*. [f] 5th cycle: 76.1% yield, 43.1% *ee*. [g] 5th cycle: 23.5% yield, 76.8% *ee*.

64.5% yield, 39.4% *ee*; step 2: 19.4% yield, 99.0% *ee*). Comparing these results with the ones in the presence of the IL demonstrates that the IL solvent plays an important role in that a better homogenization of the reaction medium is achieved.

This methodology was applied to different substrates. When 4-phenyl-2-butanol was used as substrate, both free enantiomers were obtained: the *S* enantiomer (step 1: 51.6% yield, 60% *ee*) and the *R* enantiomer (step 2: 30.6% yield, 91.9% *ee*). 2-*n*-Decanol provided (*S*)-2-decanol in 51.5% yield and 38.5% *ee* (step 1) and (*R*)-2-decanol in 25% yield and 96.5 *ee* (step 2). These results demonstrate the excellent ability of this enzymatic resolution in the separation of different substrates.

The possibility of reusing the reaction medium (the enzyme, the ionic acylating agent, and the ionic liquid) was studied for the best observed acylating agents, **4a** and **4b**, and ionic liquids, [bmim][PF_6] and [bmim][BF_4], using 1-phenylethanol as a model substrate (Table 2). Under these conditions, it was possible to perform up to four (**4b**/[bmim][PF_6]), five (**4a**/[bmim][BF_4]), and six (**4a**/[bmim][PF_6]) consecutive enzymatic resolution–separation steps using the same catalytic reaction medium, without considerable decrease in the resolution efficiency, which corresponds respectively to eight, ten, and twelve overall enzymatic reactions. Further optimization of each cycle with respect to a specific substrate such as an enzyme, the reaction time, and the temperature should allow each enantiomer to be obtained in high yield and with high enantiomeric excess.

In conclusion, we have demonstrated a new, simple, reliable, reusable, and efficient preparative methodology for the one-pot resolution–separation of secondary alcohols based on the enzymatic resolution and temporal attachment of one enantiomer to an ionic acylating reagent in an ionic liquid environment, both of which are non-volatile thus allowing non-reversible transformation by removal of ethanol under vacuum. This attachment allows a selective extraction of the free enantiomer by solvent extraction owing to the ionic liquid environment. Subsequently, the attached enantiomer is released by a second enzymatic transesterification with ethanol, which regenerates the reaction mixture of active enzyme, ionic acylating agent, and ionic liquid for another reaction cycle. This method could be adapted to other target substrates and extended to other functional groups by choosing an appropriate combination of enzyme and ionic anchoring agent.

Experimental Section

General procedure for enzymatic kinetic resolution and separation of *sec*-alcohols: CALB (Novozym 435; 20 mg) and racemic *sec*-alcohol (0.41 mmol) were added to a stirred solution of acylating agent (**4a**–**b**; 0.41 mmol) in ionic liquid (0.8 mL). The reaction mixture was stirred for 4 days under reduced pressure (100 mmHg) at 35 °C in a thermostatic bath. After this time, the reaction mixture was extracted with Et_2O (3 × 7 mL) and the organic phases were collected and passed through a pipette-sized column packed with silica gel. The solvent was then evaporated under reduced pressure to give the *S*-configured *sec*-alcohol.

The reaction mixture was dried under reduced pressure (20 mmHg) for 2 h. After this time, EtOH (2.5 equiv) was added and the mixture was stirred for 1 day at 35 °C in a thermostatic bath. Then, the reaction mixture was extracted again with Et_2O (3 × 7 mL), and the organic phase was collected and passed through a pipette-sized column packed with silica gel. The solvent was then evaporated under reduced pressure to give the *R*-configured *sec*-alcohol.

Reuse experiment: The reaction mixture was dried under reduced pressure (20 mmHg) for 2 h, then more racemic *sec*-alcohol (0.41 mmol) was added to the mixture, and the reaction was performed as described above.

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