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Enzymatic kinetic resolution of *sec*-alcohols using an ionic liquid anhydride as acylating agent

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ABSTRACT

A task-specific ionic liquid bearing an anhydride moiety was synthesized for the first time in good yield (83%) through a carbodiimide-mediated coupling reaction. The enantiomeric separation of a series of *sec*-alcohols was performed via enzymatic kinetic resolution, employing an ionic anhydride as acylating agent and *Candida antarctica* Lipase B as a biocatalyst. A fast and efficient recovery of both enantiomers was achieved separately due to the ionic nature of the acyl donor, combined with the possibility of carrying out the enzymatic step in an organic solvent.

1. Introduction

In addition to ionic liquids being recognized as good solvents or co-solvents in biocatalysis,[1] they are also used for other purposes such as enzyme coatings in ionic-liquid-coated enzymes endowed with improved catalytic performances, or as phase transfer agents in supported-ionic-liquid membranes.[2] Moreover, the structure of an ionic liquid can be modified in order to include a group of atoms that confer an additional and specific function to the ionic liquid itself. These are known as task-specific ionic liquids.[3,4] Two examples of its application in biocatalysis include the work of Salunkhe et al.[5] who proposed that an ibuprofen-anchored ionic liquid can be used as a substrate in enzymatic kinetic resolution to isolate (*S*)-ibuprofen, and our own work on the use of ester ionic liquids as promising acylating agents with which to perform lipase-catalyzed kinetic resolutions in ionic liquid solvents.[6,7]

Lipases are ubiquitous in nature. They are versatile biocatalysts used extensively in organic synthesis and in many industries (e.g., for foods, cosmetics and biofuel production).[8–13] The enzymatic kinetic resolution of racemic compounds is among the principal transformations promoted by lipases. The success of the method relies on the fact that enantiomers react at different rates with the chiral active site of the biocatalyst. Furthermore, it allows us to recover both enantiomers of a racemic mixture. The interest in obtaining enantiomerically pure compounds is clear since they are key intermediates in the production of fine chemicals. Many chiral intermediates used in the pharmaceutical industry are obtained via enzymatic kinetic resolution.[14] The lipase-catalyzed acylation of *sec*-alcohols is widely employed to obtain enantiomerically pure or enriched forms of different compounds.[13,15]

Some lipase-catalyzed reactions have to be carried out in non-aqueous media, either to prevent reverse hydrolysis of the products or to bypass solubility issues. Lipases have shown good activity and stability in organic solvents and in ionic liquids. Even so, thermodynamic aspects can present a problem to enzymatic reactions carried out in non-aqueous solvents (e.g. if nucleophiles are released as reaction by-products).

Herein we report the synthesis of a new task-specific ionic liquid bearing an anhydride moiety for enzyme recognition and its successful application as an acyl donor in the enzymatic resolution of *sec*-alcohols. Acyl-transfer reactions become irreversible when anhydrides are employed as acylating agents because no nucleophiles are released throughout the reaction. It also avoids the use of another ionic liquid as a solvent to undergo vacuum extraction of the by-products, which is mandatory with acylating ionic esters.[6]

2. Results and discussion

Ionic anhydride **3** was obtained from ester **1** (Scheme 1). Ester 1 was first hydrolyzed to yield the ionic acid **2** (Step a), which was further converted into one-half equivalent of **3** by means of a carbodiimide-mediated coupling (Step b). The possibility of undertaking the synthesis of the anhydride under mild conditions, such as those afforded by the carbodiimide-coupling, is fundamental because it allows us to introduce the anhydride functionality in the last step of the synthesis. That, in turn, can assure a straightforward regeneration of the acylating agent.

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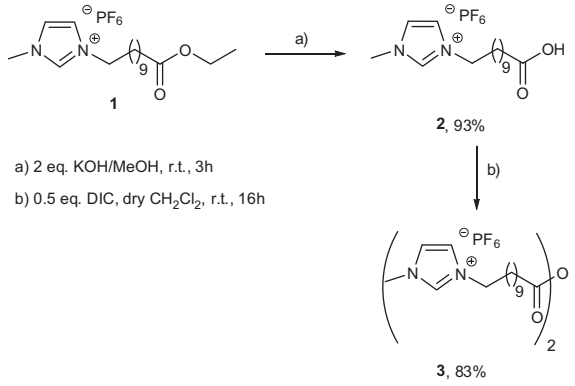
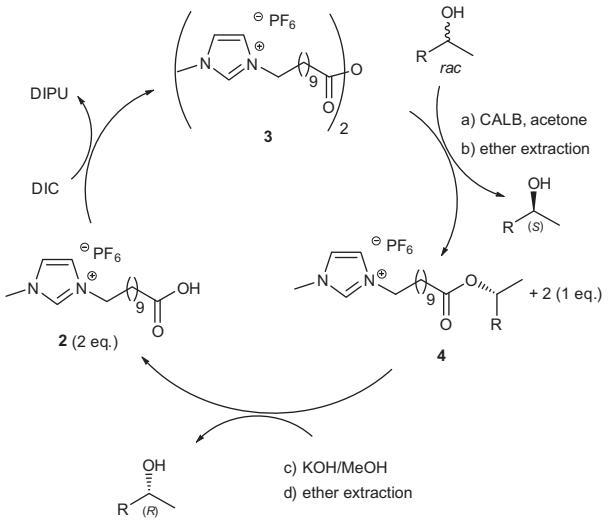


Scheme 1. Synthesis of the ionic liquid anhydride **3**, used as acyl donor in the enzymatic kinetic resolution of *sec*-alcohols (DIC = *N*,*N*′-diisopropylcarbodiimide).

Previous to the synthesis of anhydride **3** according to Scheme 1, we failed in several attempts to prepare an ionic anhydride from the bromide analogue of **2**, which was likely due to the very low solubility of that compound in suitable organic solvents. Thus, we decided to replace the bromide with another counter-anion, in order to reduce the polarity of the ionic acid. Among the anions that reportedly do not affect lipase activity (BF_4^-, PF_6^-, and NTF_2^-),[16,17] we chose PF_6^- based on our previous experience in the enzymatic kinetic resolution of *sec*-alcohols with CALB and imidazolium cation-based ionic liquids.[6] By replacing the bromide with PF_6^-, we solved our solubility issues and managed to obtain the anhydride under mild conditions and in good yield.

The length of the alkyl chain linking the anhydride group to both ionic moieties (C11) was also chosen based on the previously observed effects of the ionic group on the active center of the enzyme.[7] Therein we showed that long alkyl-chain acyl donors give better results in the resolution of 1-phenylethanol, our model molecule. We also attempted the enzymatic kinetic resolution of substrate **5** with a shorter-chain anhydride (C7, data not shown), but we observed no catalytic activity. Ionic liquids are favorable solvents with regard to both medium recycling possibility and the catalytic activity of lipases. Nonetheless, enzymatic kinetic resolution reactions are often very slow when ionic liquids are used as reaction media (taking from hours to days), which might be due to mass transfer limitations caused by their high viscosity.[18] By using an anhydride-functionalized ionic acylating agent to carry out the enzymatic kinetic resolution of *sec*-alcohols, it is possible to obtain both enantiomers separately by means of two simple ether extractions and by using an 'enzyme-friendly' organic solvent as the reaction medium (Scheme 2). One extraction is performed after the enantioselective lipase-catalyzed esterification to recover the (*S*)-enriched form of the *sec*-alcohol while the other one affords the remaining (*R*)-enriched *sec*-alcohol; the latter is performed after chemical hydrolysis of the chiral ester produced in the enzymatic reaction. The recovery of enantiomerically enriched mixtures or pure enantiomers via solvent extraction is only possible due to the different physico-chemical properties of the reaction components; again, we are able to take advantage of the unique properties of task-specific ionic liquids, providing an ionic phase where the fast-reacting enantiomers can be trapped as ionic esters. Separation of the enantiomers via extraction has the advantage of being more expeditious and energy effective than distillation, as well as requiring lesser amounts of organic solvents than chromatographic separations.

All of the lipase-catalyzed acylations were performed in distilled acetone. It is known that solvent effects play a determinant role in CALB catalytic performance and that, in general, polar solvents may displace the essential water causing enzyme inactivation,



Scheme 2. Methodology of the enzymatic kinetic resolution and separation of the enantiomers of *sec*-alcohols. CALB was used as the biocatalyst and ionic anhydride **3** as the acylating agent (DIPU = *N*,*N*′-diisopropylurea).

whereas hydrophobic solvents are favorable to enzymatic reactions.[19] Acetone, a mildly polar solvent, has previously been shown to provide good results in CALB-catalyzed reactions.[20,21]

The main resolution step in our enzymatic kinetic resolutions is the enzymatic reaction (Step a, Scheme 2), wherein the biocatalyst selectively transforms one of the enantiomers into a product, which is a new chemical entity with distinct physico-chemical properties from the initial substrate, leaving the other enantiomer unchanged. In this step, we used one equivalent of acylating agent to react with the racemic alcohol, which represents a 2:1 ratio of acylating agent to the fast-reacting enantiomer. Ionic anhydride 3 was then employed in the enzymatic kinetic resolution of the aromatic, aliphatic, and allylic *sec*-alcohols presented in Figure 1.

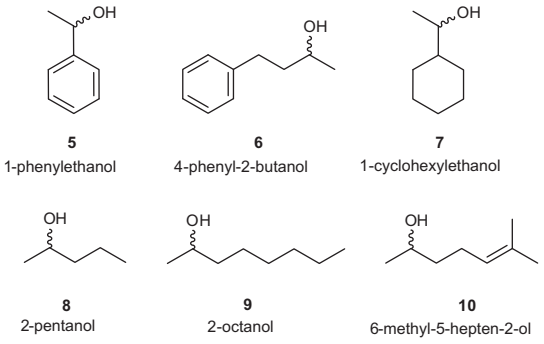


Figure 1. Secondary alcohols employed in the enzymatic kinetic resolutions catalyzed by CALB, using **3** as the acyl donor.

After Step a, the immobilized enzyme was removed by filtration and the solvent was evaporated under reduced pressure, to give in solution a mixture of ionic compounds (carboxylic acid, ester, and non-reacted anhydride) and the unreacted alcohol. CALB displayed high selectivity toward the (*R*)-enantiomers of the *sec*-alcohols. Therefore, the non-ionic phase was mainly composed of the (*S*)-enantiomer, which could be extracted with diethyl ether (Step b, Scheme 2). The product of the enzymatic esterification was the (*R*)-enriched ionic ester 4, which had to be chemically hydrolyzed (Step c, Scheme 2) to give the (*R*)-*sec*-alcohol, after ether extraction (Step d, Scheme 2). Finally, the two equivalents of ionic acid