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Ionic Acylating Agents for the Enzymatic Resolution of *sec*-Alcohols in Ionic Liquids

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Potential acylating agents containing pendant ionic groups have been screened for the enzymatic kinetic resolution of *rac*-secondary alcohols in ionic liquids with CAL-B as biocatalyst. This study has allowed the identification of the 1-methyl-3-alkylimidazolium cation attached to a carboxylate group through a C_{10}-alkyl chain as an efficient acylating agent for this transformation. This strategy was applied to the resolution of 2-hydroxycyclohexanecarbonitrile in which the 1*R*,2*S* enantiomer was isolated in 35 % *ee* (73 % yield) and the 1*S*,2*R* enantiomer in 97 % *ee* (23 % yield).

Introduction

The enzymatic kinetic resolution (EKR) of racemic alcohols, namely by lipases, is a well-established methodology for the preparation of enantiomerically enriched precursors.[1] Lipases also catalyse chemo-, regio- and stereoselective processes such as the EKR of acids, esters or amides,[2] as well as the desymmetrization of prochiral or *meso* compounds.[3] Like other biocatalysts, lipases affect the rates of reversible reactions in which the equilibrium reached is determined by thermodynamics. The equilibrium can be shifted by controlling the concentration of reactants or products, for example, by controlling the concentration of water in the reaction media it is possible to perform ester hydrolysis or synthesis. In cases in which the equilibrium is not controlled, low yields and the erosion of enantioselectivity can be observed. This fact has clearly been illustrated for the kinetic resolution of menthol.[4] Several strategies can be applied to shift the equilibrium. For this, vinyl esters are the most common and efficient acylating agents used to achieve irreversible transesterifications.[5] However, there are some limitations associated with the use of this type of acyl group relating to the formation of acetaldehyde or other side-products (depending of the vinyl ester) that can cause biocatalyst inhibition.[6] The design of new acylating agents for enzymatic resolution has been an important topic in this field of research. To overcome this problem as well as to circumvent difficulties of separation, several different acyl groups have been reported, namely succinic anhydride[7] and carbonates.[8] A different approach involves the resolution by simple esterification using free a carboxylic acid and the continuous removal of water under vacuum[9] or the use of a dehydrating agent such as molecular sieves.[10]

The use of vacuum to remove water is restricted to low volatile substrates and acylating agents such as fatty acids and is not compatible with common volatile organic solvents in which the biocatalyst is very efficient. Another possible approach is to perform the enzymatic reaction in the absence of volatile solvents, which is feasible only for cases in which the alcohol/acid/ester mixture is liquid under the operating conditions.[9]

In recent years the use of ionic liquids (ILs) as solvents for EKR has attracted considerable attention, mainly due to their peculiar properties such as solubility, almost negligible vapour pressure,[11] increase in biocatalyst activity, chemical stability[12] and possible combination with a greener process involving *sc*CO_2 extraction and membrane technology.[13] Many examples of the application of ILs in biocatalysis resolution have been reported,[14] including hydrolysis,[15] esterification[16] and aminolysis.[17]

Task-specific ionic liquids (TSILs) containing a hydroxy group attached to an imidazolium cation have been reported as efficient systems for the resolution of acids by the enzymatic hydrolysis of the presynthesized ester.[18] The use of ILs as reaction media is very convenient for the EKR of free carboxylic acid esterification or transesterification in which water or low-molecular-weight alcohols can be removed under vacuum due to the non-volatility of the IL solvent under the operating conditions.

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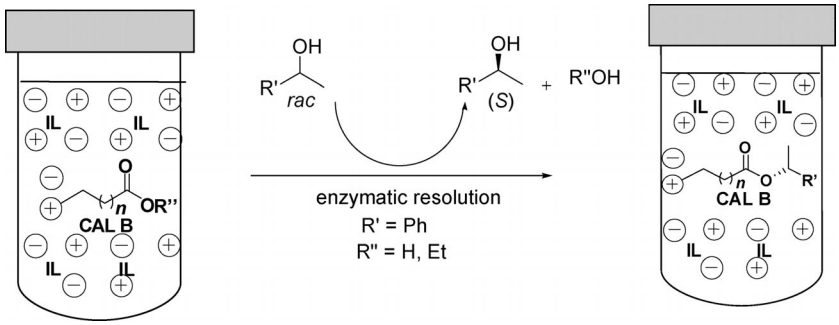


Scheme 1. Enzymatic esterification or transesterification for the resolution of *rac*-*sec*-alcohols by ionic an acylating agent in ionic liquid.

Furthermore, the possibility of identifying a new acylating agent containing one carboxy group (acid or ester) and a pendant stable cation or anion will facilitate product separation due to the preferential partition of the formed optically enriched ester to the ionic liquid phase (Scheme 1). To investigate this possibility, we performed a comprehensive study of EKR in ionic liquids by testing different potential acylating agents.

Results and Discussion

Following the strategy previously reported in the literature of using succinic anhydride as an acylating agent for the resolution of *sec*-alcohols,[7] we envisaged an approach in which both enantiomers could be resolved and separated only by enzymatic resolution. However, when the enzymatic resolution of *rac*-1-phenylethanol was performed in the presence of CAL-B in 1-butyl-3-methylimidazolium hexafluorophosphate [bmim][PF_6] the formation of secondary products was observed, namely succinic diesters.[19] Several different conditions were tested to minimize the formation of secondary products, but with low success. This observation prompted us to perform a more detailed study for other substrates, specifically 2-octanol, for which a remarkable solvent effect on the outcome of the esterification was observed.[20]

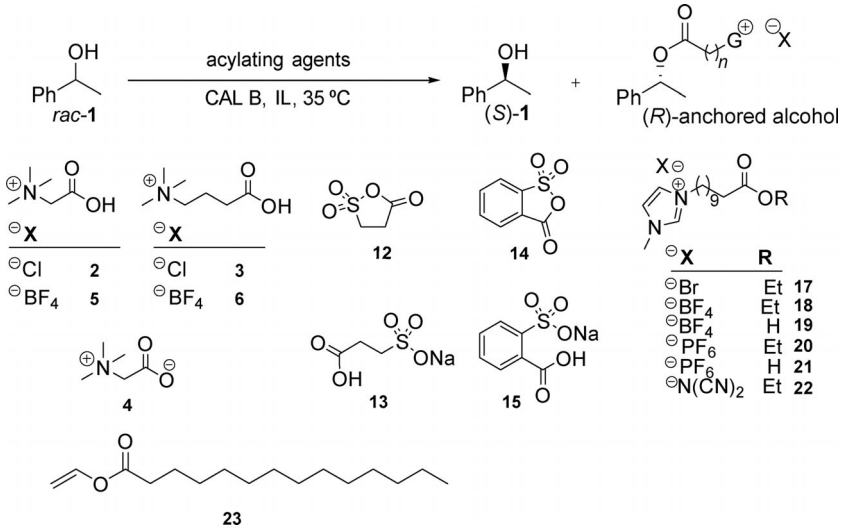
After this first approach, we turned our attention to the design of new efficient acylating agents specifically composed of two different groups, a carboxy group (ester) and a non-labile group at which no secondary reactions could occur.[21] In line with the previous study and to achieve an easy and fast screening of potential acylating agents, the occurrence of any EKR was monitored simply by measuring the enantiomeric excess (*ee*) of the extracted unreacted alcohol (*S*)-**1** (Table 1). Our investigations started with two different acylating agents, carboxyalkyltrimethylammonium chlorides **2** and **3** (entry 1), although in neither case was an *ee* observed. We considered that these poor results could be related to the well-known negative effect of the chlorine atom on enzyme deactivation.[16,22] To circumvent this possibility we decided to use a zwitterionic compound such as **4** and to perform an ionic exchange of the chlorine atom by another enzymatic-friendly ion, namely tetrafluoroborate (compounds **5** and **6**). However, again, no *ee* was observed (entry 1). These results are also in line with those obtained with the ionic acylating agents **8**–**11** with which no reaction occurred with *rac*-1-phenylethanol in acetonitrile in the presence of CAL-B (Scheme 2). In addition, after 24 h of reaction, destruction of the enzyme support was observed. In contrast, no destruction of the enzyme support was observed when the reaction was performed with 2,2,2-trifluoroethyl 4-bromobutanoate (**7**) and high *ee* values of the remaining substrate and product were obtained (Scheme 2).

To overcome the lack of reactivity, which could be attributed to enzyme inhibition of the trimethylalkylammonium group due to its close proximity to the carboxy reaction centre, we turned our attention to the use of a possible acylating agent with a long alkyl chain (C_{11}). Their preparation started with different ammonium salts, however, our attempts to prepare and purify these compounds with acceptable purity and yields failed.

At the same time, a different approach was tested with the aim of replacing succinic anhydride by other cyclic anhydrides, such as compounds **12** and **14**, or sulfonate compounds **13** and **15**. However, when the EKR was performed under the same conditions, no *ee* was observed for the unreacted alcohol and in some cases (**12** and **14**) the alcohol substrate **1** was not even detected. These negative results may be due to the acidity of the intermediate species that is formed when the ring is opened and to the proximity of ionic part of the acylating agent to the reactive carboxy group. After these unsuccessful experiments, we decided to study other possible acylating agents based on the imidazolium cation that contain a longer alkyl chain. This type of acylating agent can be obtained in good yields and purities simply by nucleophilic substitution of ethyl 11-bromoundecanate by methylimidazole (Scheme 3).

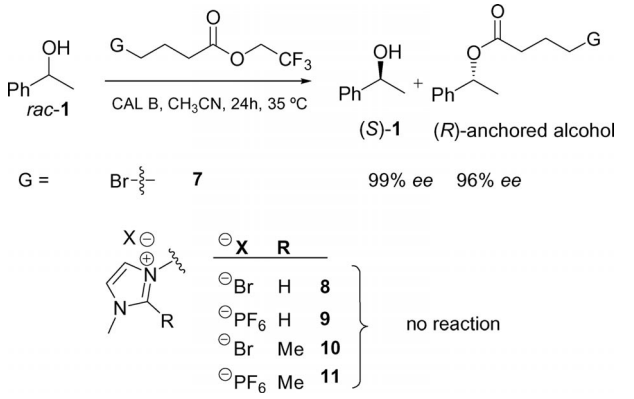
Several imidazolium acylating agents containing an acid or ester group based upon four different anions [Br^-, PF_6^-, BF_4^-, $N(CN)_2^-$] were tested under different conditions. Even with the knowledge that some of these anions form strong hydrogen bonds and present high nucleophilicity consequently reducing the enzyme's half-life[23] probably by changing the enzyme′s conformation by interacting with the positively charged sites in the enzyme structure,[24] we decided to use these anions to prepare acylating agents. This was because we have noticed that the negative effect

Table 1. Screening of different potential ionic acylating agents for the EKR of *rac*-1-phenylethanol with CAL-B in the ionic liquid by measuring the *ee* of the free alcohol.[a]

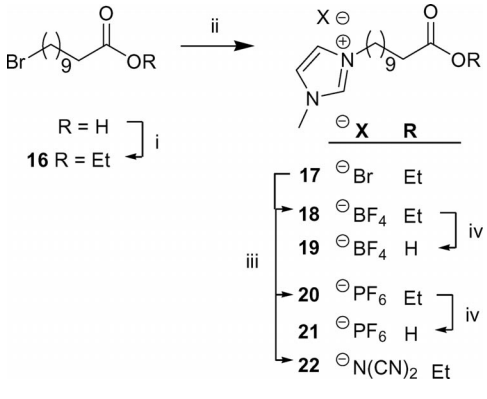


Entry	Acylating agent	Ionic liquid	Vacuum (yes/no)	Time [d]	*ee*[b] (*S*)-**1** [%]	*ee*[c] (*R*)-**1** [%]
1	**2–6**	[bmim][PF_6]	no	7	0	–
2	**12–15**	[bmim][PF_6]	no	7	0	–
3	**17**	[bmim][PF_6]	yes[d]	7	10	97 (24 h)
4	**18**	–	yes[d]	4	5	–
5	**17**	[bmim][BF_4]	yes[d]	7	17	–
6	**18**	[bmim][PF_6]	yes[d]	7	77	91 (48 h)
7	**18**	[bmim][BF_4]	yes[d]	7	37	72 (48 h)
8	**19**	[bmim][BF_4]	no	4	28	–
9	**20**	[bmim][PF_6]	yes[d]	2	77	–
10	**21**	[bmim][PF_6]	no	4	53	–
11	**22**	[bmim][PF_6]	yes[d]	7	32	93 (48 h)
12	**22**	[bmim][BF_4]	yes[d]	7	22	99 (48 h)
13	**23**	–	no	30 min	46	94[e]
14	**23**	[bmim][PF_6]	no	60 min	60	71[f]

[a] All reactions were carried out in 0.25mL of [bmim][PF_6] with 0.20 mmol of alcohol, 0.20 mmol of acylating agent and 10 mg of CAL-B. [b] Determined by HPLC. [c] Determined by HPLC after hydrolysis. [d] 100 Torr. [e] Calculated based on ee_s and a conversion of 33%. [f] Calculated based on *ee* values and a conversion of 46%.



Scheme 2. Enzymatic resolution of *rac*-1-phenylethanol using acylating agents based on 4-substituted 2,2,2-trifluoroethyl butanoates.



Scheme 3. Preparation of acylating agents based on the imidazolium cation. Reagents and conditions: (i) EtOH, H_2SO_4, toluene, reflux, Dean–Stark apparatus, 90%; (ii) methyl imidazole, iPr_2O, 70 °C, 98%; (iii) (MX), CH_2Cl_2 48 h, 87–99%; (iv) NaOH, H_2O, room temp., 83–86%.

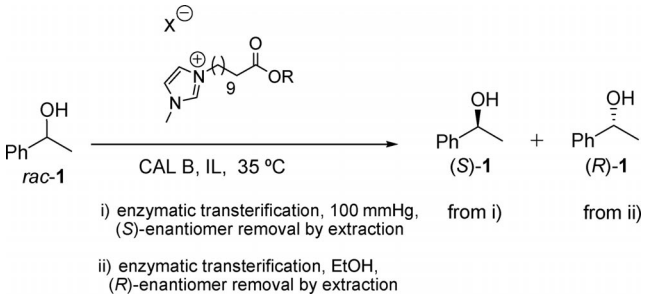
of the more denatured anions is not always observed as in our previous work in which it was possible to resolve the precursor of indinavir in the presence of [Aliquat]-[($N(CN)_2$)] but not in the presence of [bmim][($N(CN)_2$)].[25]

The reaction equilibrium can be controlled and shifted to product formation by performing the reaction under vac-

uum (100 Torr, 35 °C). Under these conditions minimal evaporation of the substrate *rac*-1-phenylethanol occurs. The effect of solvent was studied by performing the reaction in two different ionic liquids ([bmim][PF_6], [bmim][BF_4]) or in the absence of solvent (in the case of the acylating agent **18**, which is a liquid at reaction temperature, entry 4). Some enantioselective enzymatic acylation was observed for these acylating agents under these different conditions (entries 3–12). The best results were obtained for acylating agents containing the ethyl ester group. In contrast, the poorest results were obtained for the bromide salt **17** (entries 3 and 5). Under these conditions, *ee* values of 5–17% were observed for the extracted unreacted alcohol (*S*)-**1**. These data reinforce the negative effect of halogen atoms on this transformation described above.[16,22] The *ee* for the other enantiomer (97%) was determined after hydrolysis (entry 3). Higher *ee* values were obtained for the other acylating agents (entries 6–12). In the case of acylating agent **18**, the reaction was performed under vacuum in two different ILs; the best result was obtained with [bmim][PF_6] as solvent with (*S*)-**1** being obtained with an *ee* of 77%. Isomer (*R*)-**1** was isolated after hydrolysis with 91% *ee* (entry 6). For the acylating agents **19** and **21** no vacuum was applied to the reaction mixture and moderate *ee* values were observed (28–53%). The best result was obtained when the reaction was performed in the presence of compound **20** and [bmim][PF_6] for 2 d. In this case an *ee* of up to 77% was obtained (entry 9). As in previous cases, the best result for acylating agent **22** was obtained with [bmim][PF_6] with an *ee* of 32% observed for (*S*)-**1** (entry 11). The results of these screening tests can be rationalized by the negative effect of the proximity of the permanent ion to the reactive carboxy centre, the alkyl chain length playing an important role in enzyme performance. To the best of our knowledge there is at least one report on the effect of non-ionic acylating alkyl chain lengths on the EKR of 3-methyl-2-butanol with vinyl esters and different alkyl chain lengths using CAL-B. The authors observed that longer alkyl chains gave better *ee* values than shorter alkyl chains.[26] In addition to these encouraging results there are still some reactivity and selectivity differences between vinyl esters and ionic acylating agents. By using vinyl myristate **23** as acylating agent 1-phenylethanol was resolved faster and with higher selectivity than with ionic acylating agents (Table 1, entries 13 and 14 vs. entries 3–12).

The results obtained for the optimized transformation are presented in Table 2. The enzymatic reaction was performed under vacuum using the ionic liquids [bmim][PF_6] and [bmim][BF_4] as the best candidates identified in Table 1. With free carboxylic acids as the acylating agent, the tetrafluoroborate salt **19** and hexafluorophosphate salt **21** gave moderate optical purity of the attached ester (*R*)-**1** (17 and 18%, respectively), allowing the isolation of the remaining unreacted alcohol (*S*)-**1** in higher *ee* (78 and 84%, entries 4 and 6, Table 2). However, the acylating agents **18** and **20** with [bmim][PF_6] or [bmim][BF_4] gave a much better EKR performance than the free carboxylic acid acylating agents **19** and **21**. With the best combination of ester **20** in [bmim][PF_6] (entry 5, Table 2) it was possible to isolate 41% (99% *ee*) of (*R*)-1-phenylethanol from the attached ester and 51% (81% *ee*) of unreacted alcohol (*S*)-1-phenylethanol.

Table 2. EKR of *rac*-1-phenylethanol in IL by acylating agent based on imidazolium cation.



Entry[a]	Acylating agent, X, R	IL	Time [d]	(*S*)-**1** from i Yield [%]	(*S*)-**1** from i *ee*[b] [%]	(*R*)-**1** from ii Yield [%]	(*R*)-**1** from ii *ee* [%]	*E*[c]
1	**18**, BF_4, Et	–	2	65	39	19	99[d]	>200
2	**18**, BF_4, Et	[bmim][PF_6]	2	62	62	30	94[d]	48
			4	69	62	23	99[d]	>200
3	**18**, BF_4, Et	[bmim][BF_4]	4	74	56	19	99[d]	>200
4	**19**, BF_4, H	[bmim][PF_6]	4	40	78	31	17[e]	2
5	**20**, PF_6, Et	[bmim][PF_6]	4	51	81	41	99[d]	>200
6	**21**, PF_6, H	[bmim][PF_6]	4	36	84	35	18[e]	2

[a] All reactions were carried out in 0.8 mL of IL with 0.41 mmol alcohol, 0.41 mmol of acylating agent and 20 mg of CAL-B. [b] Determined by HPLC. [c] *E* value calculated according to Equation (1).[27] [d] Determined by HPLC after enzymatic hydrolysis 1 d, 2.5 equiv. EtOH. [e] Determined by HPLC after enzymatic hydrolysis 1 d, 2.5 equiv. H_2O.

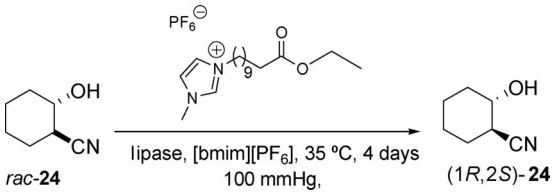
$$E = \frac{\ln\left[1 - c\left(1 + ee_p\right)\right]}{\ln\left[1 - c\left(1 - ee_p\right)\right]} \quad (1)$$

Furthermore, because the ionic acylating agent [ethyl carboxylate $[BF_4]^-$ **18** (entry 1)] is also liquid at room temperature, it is possible to perform the EKR in the absence of solvent. Under this condition, (*S*)-**1** was isolated in 65% yield (39% *ee*) after 2 d and (*R*)-**1** was isolated in 19% yield (99% *ee*) after 1 d. To the best of our knowledge this is the first example of the use of a TSIL at room temperature for the EKR of alcohols.

This strategy was applied to the resolution of a target secondary alcohol with more potential interest, namely 2-hydroxycyclohexanecarbonitrile (**24**), which is a key precursor of an androgen receptor antagonist that is being developed for the treatment of both alopecia and excess sebum (oily skin).[28]

With the purpose of identifying the best biocatalyst for the resolution of this substrate, the enzymatic reaction was performed under the same conditions as previously described using several different enzymes and acylating agent **20** in [bmim][PF_6] (Table 3). From the screening of different enzymes, it was possible to identify CAL-B as the most appropriate catalyst to resolve the substrate with (1*R*,2*S*)-**24** isolated in 70% yield and 29% *ee*.

Table 3. EKR of 2-hydroxycyclohexanecarbonitrile (**24**) in [bmim][PF_6] with an acylating agent **20** based on the imidazolium cation using different enzymes.[a]

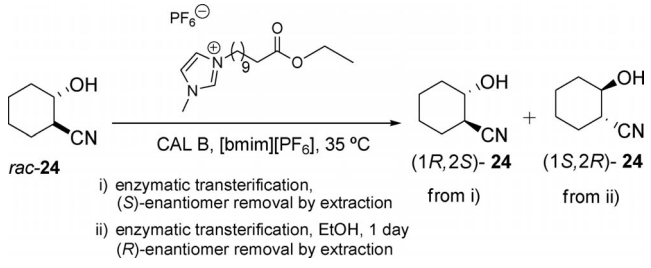


Entry	Lipase[b]	(1*R*,2*S*)-**24**	
		Yield[c] [%]	*ee*[d] [%]
1	CAL-B	70	29
2	Lypozyme	69	31
3	CCL	94	<1
4	PS amano SD	96	5.0
5	Amano AS	99	<1
6	AYS amano	92	<1
7	AK amano	50	26

[a] All reactions were carried out in 0.8 mL of [bmim][PF_6] with 0.41 mmol *rac*-2-hydroxycyclohexanecarbonitrile (**24**), 0.41 mmol of acylating agent **20** and 20 mg of enzyme. [b] Commercial formulations. [c] The yields were calculated on the basis of the initial *rac*-alcohol (0.41 mmol). [d] Determined by GC.

The effects of reaction vacuum, enzyme loading and temperature on the reaction rate were then studied (Table 4). Because this particular substrate **24** is not volatile under the operating conditions, we decided to explore the effect of ethanol evaporation on the course of the reaction by changing the level of vacuum in the reaction. From our results it is possible to see that there is no positive effect of the reduction of pressure on the reaction (entry 4 vs. entry 1). In addition, an increase in enzyme loading had no significant effect on the resolution of **24** (entry 4 vs. entry 5). The effects of temperature and reaction time were explored by performing the reaction for 4 or 5 days and at two different temperatures (35 and 50 °C).

Table 4. EKR of 2-hydroxycyclohexanecarbonitrile (**24**) in [bmim][PF_6] with the acylating agent **20** based on the imidazolium cation.[a]



Entry	Vacuum [Torr]	Time [d]	(1*R*,2*S*)-**24**from i Yield[b] [%]	*ee*[c] [%]	Time [d]	(1*S*,2*R*)-**24**from ii Yield[b] [%]	*ee*[c] [%]
1	100	4	70	29	1	25	96
2[d]	100	4	58 (61)	52 (52)	1	37 (44)	90 (85)
3	100	5	73	35	1	23	97
4	15	4	77	30	1	23	94
5[e]	15	4	80	20	1	20	93

[a] All reactions were carried out in 0.8 mL of [bmim][PF_6] with 0.41 mmol of *rac*-2-hydroxycyclohexanecarbonitrile (**24**), 0.41 mmol of acylating agent **20** and 20 mg of CAL-B. [b] The yields were calculated on basis of the initial *rac*-alcohol (0.41 mmol). [c] Determined by GC. [d] Reaction was carried out at 50 °C. The results obtained for one recycling experiment (ionic liquid, ionic acylating agent and enzyme) are provided in parentheses. [e] Reaction performed with 52 mg of CAL-B.

Under 100 Torr at 35 °C for 4 d, it was possible to isolate (1*R*,2*S*)-**24** from i) in 70% yield and 29% *ee*. Isomer (1*S*,2*R*)-**24** was isolated from ii) in 25% yield and 96% *ee* after 1 d (entry 1). A longer reaction time (5 d) provided (1*R*2*S*)-**24** from i) in 73% yield and 35% *ee*, and (1*S*,2*R*)-**24** from ii) was isolated in 23% yield and 97% *ee* after 1 d (entry 3).

At 50 °C under equivalent conditions, it was possible to isolate (1*R*,2*S*)-**24** from i) in 58% yield and 52% *ee*. Isomer (1*S*,2*R*)-**24** from ii) was isolated in 37% yield and 90% *ee* after 1 d (entry 2). Taking advantage of the nature of the medium this was reused in a second experiment (entry 2, results in parentheses): (1*R*,2*S*)-**24** from i) was isolated in 61% yield and 52% *ee* and (1*S*,2*R*)-**24** from ii) was isolated in 44% yield and 85% *ee*.

Conclusions

This study has allowed the identification of new ionic acylating agents based on the imidazolium cation for EKR in ionic liquids. The strategy opens up new opportunities for efficient resolutions and easy enantiomer separation simply by removal of the unreacted enantiomer alcohol by extraction from the attached reacted opposite enantiomer that remains in the IL medium. The opposite enantiomer can be liberated by a second enzymatic reaction in good yields and *ee* values, as has been demonstrated for 2-hydroxycyclohexanecarbonitrile.

Experimental Section

Procedure for Screening of Task-Specific Ionic Liquids for the Enzymatic Resolution of *rac*-1-Phenylethanol: (Table 1) CAL-B (Novozym 435®; 10 mg) and *rac*-1-phenylethanol (24.4 mg, 0.20 mmol) was added to a stirred solution of the acylating agent (0.20 mmol) in an ionic liquid (0.25 mL) at 35 °C in a thermostatic bath and the mixture was stirred for 7 days. An aliquot of the reaction mixture (50 μL) was treated by passing through a pipette-sized column of SiO_2 with Et_2O (15 mL). The enantiomeric excess of the alcohol was obtained by HPLC analysis [Chiracel OD, hexane/2-propanol (95:5), 1 mL/min, λ = 258 nm].

General Procedure for the Enzymatic Kinetic Resolution of *rac*-1-Phenylethanol: (Table 2) CAL-B (Novozym 435®; 20 mg) and *rac*-1-phenylethanol (50.0 μL, 0.41 mmol) was added to a stirred solution of the acylating agent (0.41 mmol) in an ionic liquid (0.8 mL). The reaction mixture was stirred for 4 d under reduced pressure (100 Torr) 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 filled with silica and the solvent was evaporated under reduced pressure to give (*S*)-1-phenylethanol.

The reaction mixture was dried under reduce pressure (20 Torr) for 2 h and after this time 2.5 equiv. of EtOH was added and mixture

stirred for 1 d at 35 °C in a thermostatic bath. After this time the reaction mixture was extracted again with Et_2O (3 × 7 mL) and the organic phases were collected and passed through a pipette-sized column filled with silica and the solvent was evaporated under reduced pressure to give (*R*)-1-phenylethanol.

Enzymatic Kinetic Resolution of *rac*-2-Hydroxycyclohexanecarbonitrile (24): (Table 3) *rac*-2-Hydroxycyclohexanecarbonitrile (**24**; 51.8 mg, 0.41 mmol) was added to a stirred solution of 1-methyl-3-(11-ethoxycarbonylundecyl)imidazole hexafluorophosphate (**20**; 0.41 mmol) in [bmim][PF_6] (0.8 mL) and CAL-B (Novozym 435®; 20 mg). The reaction mixture was stirred for 5 d under reduced pressure (100 Torr) 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 filled with silica and the solvent was evaporated under reduced pressure to give (1*R*,2*S*)-2-hydroxycyclohexanecarbonitrile (**24**; 38.0 mg, 73% yield, 34.7% *ee*).

The reaction mixture was dried under reduced pressure (20 Torr) for 2 h and after this time 2.5 equiv. of EtOH was added and mixture stirred for 1 d at 35 °C in a thermostatic bath. After this time the reaction mixture was extracted again with Et_2O (3 × 7 mL) and the organic phases were collected and passed through a pipette-sized column filled with silica and the solvent was evaporated under reduced pressure to give (1*S*,2*R*)-2-hydroxycyclohexanecarbonitrile (**24**; 11.9 mg, 23% yield, 97.2% *ee*).

Supporting Information (see also the footnote on the first page of this article): Results of the enzymatic resolution using succinic anhydride, general experimental details, picture of the apparatus used for the enzymatic resolution under vacuum and spectroscopic data.

Acknowledgments

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