Room Temperature Ionic Liquids in the Kinetic Resolution of Adrenaline-Type Aminoethanols by *Burkholderia cepacia* Lipase under Normal and Microwave Conditions

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Abstract: The lipase PS-C II (lipase from *Burkholderia cepacia* immobilized on ceramic particles)-catalyzed acylations of *N*-acylated 2-amino-1-phenylethanol (*rac*-**1**) and *N*-acylated norphenylephrine (*rac*-**2**) have been studied in imidazolium- and pyridinium-based ionic liquids, in *tert*-butyl methyl ether (TBME) and in their mixtures. Enzymatic chemo- and enantioselectivities in the presence of hydrophobic EMIM·NTf_2 and hydrophilic EMIM·BF_4 have been studied in more detail using high substrate concentrations. The preparative-scale kinetic resolution of *rac*-**1** and *rac*-**2** (both 0.45 M) with vinyl butanoate in the mixture of EMIM·NTf_2 with TBME (2:1) took 9 and 27 hours, respectively. Microwave-assisted acylation of *rac*-**1** and *rac*-**2** in the presence of EMIM·NTf_2 proceeded similarly to the reactions under normal conditions.

Keywords: 2-amino-1-phenylethanols; enantioselective acylation; ionic liquids; lipase catalysis; microwave synthesis

Introduction

Room temperature nitrogen-based ionic liquids (RTILs) most commonly consist of a 1,3-dialkylimidazolium cation part and a BF_4^-, PF_6^-, $ROSO_3^-$ or $(CF_3SO_2)N^-$ (Tf_2N^-) anion part.[1–6] In addition, pyridinium salts are attracting growing interest, especially as solvents for organic and catalytic reactions.[4,6] RTILs as recyclable and thermally stable organic salts with low melting points are easy to prepare by simple procedures such as those shown in Scheme 1. Many of them are commercially available. Modification of either the anion, the cation or the attached substituents (R^1 and R^2) can be used to tune up their chemical and physical properties such as polarity, solvent miscibility, hydrophobicity and viscosity.

RTILs serve as non-toxic, non-volatile and non-flammable alternatives to classical organic solvents in various applications. Since the group of Russell[7] discovered that thermolysin catalyzes peptide synthesis in 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) containing 5% (v/v) of water, several different RTILs have served as solvents for enzymatic reactions.[6–22] Transformations of secondary alcohols represent the most thoroughly studied enzymatic applications.[6,8,11–19] In these transformations, *Candida antarctica* lipase B (Novozym 435, CAL-B) and *Burkholderia cepacia* lipase (lipase PS) are among the most studied enzymes. Interestingly, RTILs permit enzyme-catalyzed reactions in a solvent polarity range of $ET^N = 0.6–0.9$ (Reichardt's normalized polarity scale) and at $\log P < -2.3$ (the logarithm for the partition coefficient of an RTIL between 1-octanol and water).[6,8,9,23] The excellent dissolution characteristics of RTILs for various organic and inorganic molecules are at least partly explained by these data. On the other hand, lipases are reported to be as fast as or even faster and more stable in RTILs than, for instance, in tetrahydrofuran (THF), toluene and hexane with E_T^N and $\log P$ in the ranges 0.009–0.207 and 0.49–3.5, respectively.[6,8–18] It has been suggested that RTILs stabilize enzymes by protecting the hydration layer surrounding the enzyme[11,12] or by a permanent activating conformational change.[9] The results for lipase-catalyzed reactions in RTILs have been promising not only with respect to enhanced enzymatic activities and stabilities but also with respect to enzymatic regio- and enantioselectivities.[8,12–18]

In our previous work, lipase PS catalyzed the highly (*S*)-selective *O*-acylation of 2-amino-1-phenylethanols with butanoic anhydride in toluene/THF (3:1).[24,25] The addition of THF allowed the use of 0.05 M substrate concentration. Our continuous efforts toward green and sustainable chemistry inspired us to study the possibility to replace conventional organic solvents partly or totally by imidazolium- and pyridinium-based RTILs (Scheme 2) and to use high concentrations of *N*-acylat-

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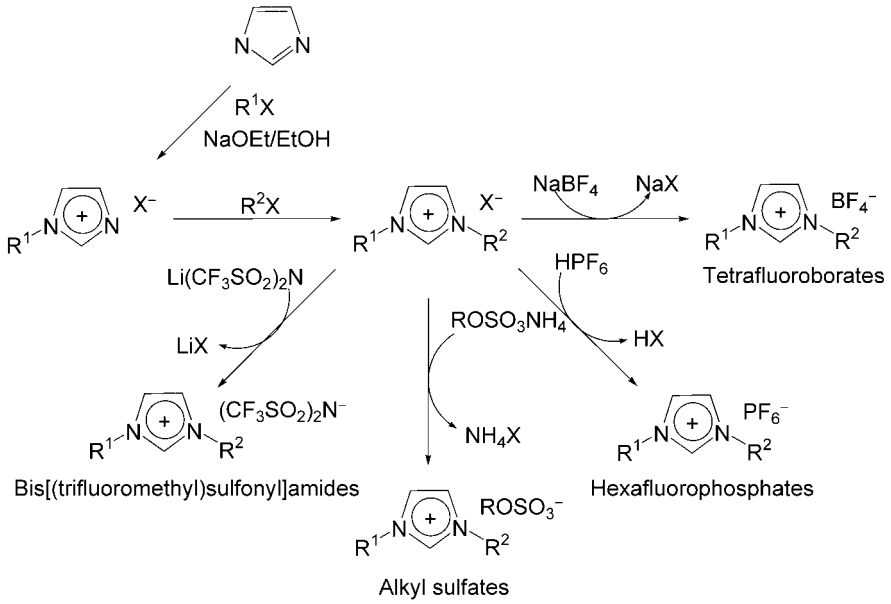
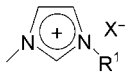
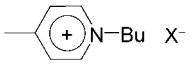


Scheme 1. Synthesis of some imidazolium-based ionic liquids; X = I, Br or Cl; R^1 = Me; R^2 = Et, Bu, hexyl, octyl, $(CH_2)_2OH$; R = Me, Et, $EtOCH_2CH_2$, $PhOCH_2CH_2$.



[EMIM][BF_4]: R^1 = Et, X = BF_4
[BMIM][BF_4]: R^1 = Bu, X = BF_4
[HexMIM][BF_4]: R^1 = Hexyl, X = BF_4
[BMIM][PF_6]: R^1 = Bu, X = PF_6
[EMIM][NTf_2]: R^1 = Et, X = $(CF_3SO_2)_2N$
[BMIM][NTf_2]: R^1 = Bu, X = $(CF_3SO_2)_2N$
[EMIM][TfO]: R^1 = Et, X = CF_3SO_3



[BMPyr][BF_4]: X = BF_4
[BMPyr][PF_6]: X = PF_6

Scheme 2. Imidazolium- and pyridinium-based ionic liquids.

ed aminoethanols as substrates for lipase PS (mainly lipase PS-C II and lipase PS adsorbed on Celite[26]). *N*-Acylated substrates rather than the free amino alcohols were chosen to restrict the number of acylation steps to those shown in Scheme 3. Thus, our focus has been on the enantioselective benzylic acylation of *rac*-**1** and *rac*-**2** and on chemoselectivity between the benzylic and phenolic acylations of *rac*-**2**. The decision to keep to *N*-acylated substrates was further supported by the early observation of the present study where the acylation of free 2-amino-1-phenylethanol with 2,2,2,-trifluoroethyl butanoate in EMIM · BF_4 tended to stop already at 40% conversion. For comparison, toluene/THF (3:1) and *t*-BuOMe (TBME) were used as conventional solvents. The lipase-catalyzed kinetic resolutions of *rac*-**1** and *rac*-**2** were also examined under microwave conditions.

Results and Discussion

Asymmetric Acylation of *N*-Acylated *rac*-1

Many benefits have been addressed to the use of RTILs as media for enzymatic reactions. These benefits include the intrinsic ability to dissolve polar substances. In our efforts to improve the previous enzymatic kinetic resolution of amino ethanols,[24,25] *rac*-**1** was first subjected to acylation with butanoic anhydride and lipase PS-C II by using BMPyr · BF_4 as an alternative solvent for the previous toluene/THF system. Under the reaction conditions, a relatively fast chemical acylation parallel to the enzymatic one was observed, and after the reaction time of 24 h unreacted **1** and produced **3** both were racemic (Scheme 3, Table 1, row 7). Low enantioselectivities were also monitored in some other RTILs in the presence of butanoic anhydride (e.g., "$E = 15$" for the reaction in EMIM · NTf_2 where E includes the contribution from the chemical reaction). It is possible that the equivalent amount of butanoic acid formed when the anhydride reacts contaminated the ionic liquid, retarding enzymatic acylation with respect to chemical acylation. As a support to this, the addition of triethylamine was previously shown to enhance reactivity for the lipase PS-C-catalyzed acylation of secondary alcohols with succinic anhydride in BMIM · PF_6.[18] In toluene/THF (3:1), the chemical *O*-acylation of *rac*-**1** with butanoic anhydride was not detected, and excellent enantioselectivity was observed ($E > 200$, row 2). Accordingly, the idea to use acid anhydrides as acyl donors in ionic liquids was dismissed.

Table 1. Lipase (50 mg/mL)-catalyzed acylation of *rac*-**1** (0.05 M) with vinyl butanoate (0.1 M) at 47 °C.

Row	Solvent	Lipase preparation	Time [h]	Conversion [%]	*E*
1	Toluene/THF (3/1)	PS on Celite	2	49	>200
2	Toluene/THF (3/1)[a]	PS on Celite	2	42	>200
3	Toluene/THF (3/1)	PS-C II	2	50	>200
4	BMPyr·BF_4	PS on Celite	24	4	>200
5	BMPyr·BF_4	PS-C II	24	31	190
6	BMPyr·BF_4[b]	PS-C II	24	20	160
7	BMPyr·BF_4[c]	PS-C II	24	83	--

[a] Butanoic anhydride (0.05 M) as an acyl donor.
[b] 2,2,2-Trifluoroethyl butanoate (0.1 M) as an acyl donor.
[c] *rac*-**1** (0.2 M) and butanoic anhydride (0.4 M); at the end both unreacted **1** and produced **3** were racemic.

When *rac*-**1** was subjected to acylation with vinyl butanoate and lipase PS on Celite in toluene/THF (Table 1, row 1) and in BMPyr·BF_4 (row 4) the former solvent system was clearly favored. We suppose that the adsorption forces are not enough to keep the lipase adsorbed on Celite in polar RTILs. In accordance with this, there are reported signs that an immobilized enzyme gives higher rates than the free enzyme in RTILs just as is observed in conventional organic solvents.[9,11] As further support, in the present work lipase PS on Celite and lipase PS-C II (covalently immobilized on ceramic particles) behaved similarly for the acylation of *rac*-**1** in toluene/THF (rows 1 and 3) while lipase PS-C II was clearly more favorable for the reaction in BMPyr·BF_4 (rows 4 and 5). 2,2,2-Trifluoroethyl butanoate as an acyl donor (row 6) was somewhat less effective than vinyl butanoate (row 5). Accordingly, the focus from here on has been on lipase PS-C II-catalyzed acylations with vinyl butanoate. No reaction was observed without lipase PS-C II in RTILs.

Table 2. Lipase PS-C II (50 mg/mL)-catalyzed acylation of *rac*-**1** (0.2 M) with vinyl butanoate (0.4 M) in RTILs at 47 °C; reaction time 6 h.

Row	Ionic liquid	E_T^{N}[f]	Conversion [%]	*E*
1	EMIM·NTf_2[a]		21	>200
2	EMIM·NTf_2[b]		27	>200
3	EMIM·NTf_2		45	>200
4	BMIM·NTf_2	0.64	39	160
5	BMPyr·BF_4[c]		12	>200
6	BMPyr·BF_4[d]		29	140
7	EMIM·BF_4[c]		–	–
8	EMIM·BF_4[d]	0.71	31	160
9	BMIM·BF_4[d]	0.68	23	80
10	HexMIM·BF_4		19	100
11	BMPyr·PF_6	0.63	19	70
12	BMIM·PF_6[c, e]		28	90
13	BMIM·PF_6[d, e]	0.68	41	120
14	EMIM·TfO		26	190

[a] Novozym 435 (CAL-B) in the place of lipase PS-C II.
[b] 2,2,2-Trifluoroethyl butanoate as an acyl donor.
[c] Commercial RTIL, not purified.
[d] Commercial RTIL, purified.
[e] *Rac*-**1** (0.1 M) and vinyl butanoate (0.2 M).
[f] Solvent polarity according to Reichardt's normalized polarity scale.[8,9,23]

Different RTILs were next screened for the acylation of *rac*-**1** with vinyl butanoate using lipase PS-C II catalysis. Reactivity (conversion obtained at certain time) decreased as the polarity of the RTIL decreased with increasing hydrophobic nature of tetrafluoroborates (X = BF_4, R^1 = Et, Bu or Hex) and bisamides (X = NTf_2, R^1 = Et or Bu) (Scheme 2, Table 2, rows 3 and 4 and 8–10). The same trend was reported also for the lipase PS-catalyzed acylation of 1-phenylethanol in RTILs.[8] The results in Table 2 show highly selective reactions in most RTILs although only in the case of EMIM·NTf_2 was the same order of excellent enantioselectivity ($E > 200$) reached as for the reaction in toluene/THF (Table 1, row 3). EMIM·NTf_2 was also the most effective of the RTILs in terms of reactivity. The results confirm the above observation that 2,2,2-trifluoroethyl butanoate is less effective than vinyl butanoate as an acyl donor (Table 2, row 2 compared to row 3). It is also clear that the same amount (50 mg/mL) of Novozym 435 in EMIM·NTf_2 (row 1) gives a less effective reaction than lipase PS-C II (row 3). Halides and acidic impurities in RTILs were previously reported to slow down lipase-catalyzed reactions.[8] In accordance with this, the results for the lipase PS-C II-catalyzed reactions of *rac*-**1** with vinyl butanoate in purified and unpurified commercial BMPyr·BF_4 and EMIM·BF_4 clearly indicated the importance of purification (rows 5–8). EMIM·NTf_2 (hydrophobic, water-immiscible) and EMIM·BF_4 (hydrophilic, water-miscible) as imidazolium-based RTILs were prepared and carefully purified in our laboratory for the use in the following studies.

As we had wished, EMIM·NTf_2 and EMIM·BF_4 allowed the use of relatively high substrate concentrations (0.45 and 0.35 M, respectively) for the lipase PS-C II-catalyzed acylation of *rac*-**1** (Table 3). Interestingly, the concentration of *rac*-**1** in EMIM·NTf_2 had no clear effect on reactivity or enantioselectivity (rows 1–4). For

Table 3. Effect of substrate concentration on the lipase PS-C II (50 mg/mL)-catalyzed acylation of *rac*-**1** (1 equivalent) with vinyl butanoate (2 equivalents) in RTILs at 47 °C; reaction time 6 h.

Row	Ionic liquid	*rac*-**1** [M]	Conversion [%]	*E*
1	EMIM·NTf_2	0.2	45	>200
2	EMIM·NTf_2	0.25	44	>200
3	EMIM·NTf_2	0.35	39	>200
4	EMIM·NTf_2	0.45	42	>200
5	EMIM·BF_4	0.1	31	65
6	EMIM·BF_4	0.2	31	160
7	EMIM·BF_4	0.25	42	100
8	EMIM·BF_4	0.35	18	35

the reaction in EMIM·BF_4, enantioselectivity had a maximum value in 0.2 M substrate (row 6), and both reactivity and enantioselectivity dramatically dropped when 0.35 M substrate was used (row 8).

According to the above results, the lipase PS-C II-catalyzed acylation of *rac*-**1** in toluene/THF proceeded ideally, except that low substrate concentrations were used (Table 1). The lipase PS-C II-catalyzed acylation of *rac*-**1** in EMIM·NTf_2 proceeded enantioselectively at high substrate concentrations, except that reactivity was slow compared to that in toluene/THF (Table 3). The reaction in mixed solvents was expected to be a reasonable compromise. Because TBME dissolves various substances relatively well and is generally accepted by lipases, the lipase PS-C II-catalyzed acylation of *rac*-**1** with vinyl butanoate was first studied in TBME. The enantioselective reaction proceeded smoothly up to 0.2 M substrate concentration (Table 4, rows 1–3). For reasons which are not yet clear the reaction turned more enantioselective with increasing substrate concentration. The results show that TBME is well suited as a cosolvent for EMIM·NTf_2 and EMIM·BF_4. As shown in Table 4, TBME at high concentrations does not dissolve in the two RTILs.

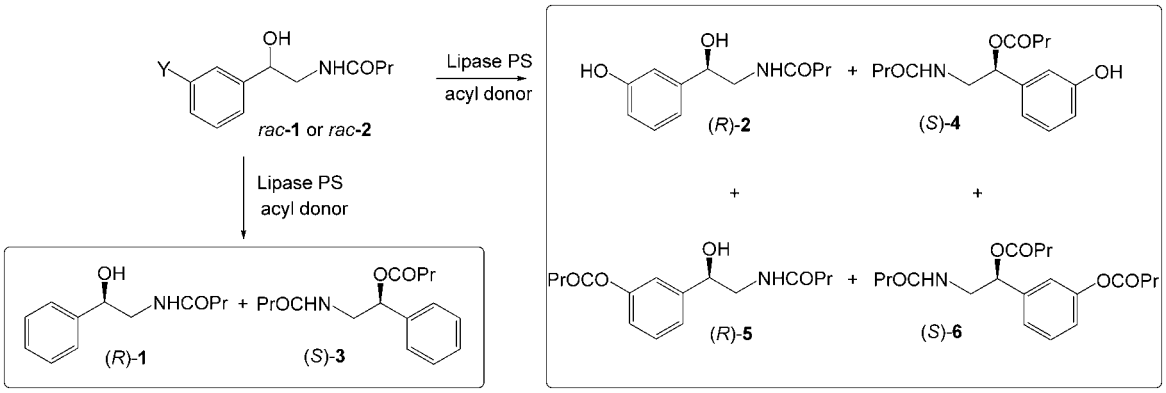
The kinetic resolution of *rac*-**1** with vinyl butanoate and lipase PS-C II was studied in various mixtures of TBME and RTILs (Table 4). As expected on the basis of the above results, the acylation proceeded more effectively with increasing amounts of TBME in EMIM·NTf_2 and EMIM·BF_4 (rows 5, 7 and 10 and 12, 13 and 16). At highest substrate concentrations, reactivity tended to drop (row 5 compared to row 6; rows 7 and 8 compared to row 9; row 13 compared to row 14 and 15). The presence of TBME markedly improved the value of $E=160$ in pure EMIM·BF_4, and excellent enantioselectivity ($E>200$) was obtained independent of the composition of the solvent mixture or the substrate concentration. Although (*R*)-**1** was more soluble in the RTIL phase and (*S*)-**3** more soluble in the TBME phase, the compounds were partly present in both phases. Because one-phasic systems are beneficial for monitoring the reaction the mixture EMIM·NTf_2/TBME (2:1) was chosen for the gram-scale resolution of *rac*-**1** (0.45 M) as described in the Experimental Section. To some extent TBME is soluble in EMIM·NTf_2 but not *vice versa*, allowing the extraction of the products with TBME.

Asymmetric Acylation of *N*-Acylated *rac*-2

Bifunctional *rac*-**2** was subjected to lipase PS-C II-catalyzed acylation with vinyl butanoate in EMIM·NTf_2, in TBME and in their mixtures as was done in the case of *rac*-**1** above (Scheme 3). In all these solvent possibilities, the reaction proceeded with excellent *S*-enantioselectivity at the benzylic HO as can be seen from the ee values 94 –>99% for the produced (*S*)-**4** and (*S*)-**6** (Table 5,

Table 4. Lipase PS-C II (50 mg/mL)-catalyzed acylation of *rac*-**1** (1 equivalent) with vinyl butanoate (2 equivalents) in the mixture of RTIL and TBME at 47 °C; reaction time 2 h.

Row	Solvent	RTIL:TBME	Number of phases	*rac*-**1** [M]	Conversion [%]	*E*
1	TBME	–	1	0.05	44	52
2	TBME	–	1	0.1	38	65
3	TBME	–	1	0.2	53	110
4	EMIM·NTf_2	–	1	0.2	29	>200
5	EMIM·NTf_2/TBME	2:1	1	0.2	33	>200
6	EMIM·NTf_2/TBME	2:1	1	0.45	25	>200
7	EMIM·NTf_2/TBME	1:1	2	0.2	38	>200
8	EMIM·NTf_2/TBME	1:1	2	0.35	37	>200
9	EMIM·NTf_2/TBME	1:1	2	0.5	32	>200
10	EMIM·NTf_2/TBME	1:2	2	0.2	44	>200
11	EMIM·BF_4	–	1	0.2	20	160
12	EMIM·BF_4/TBME	2:1	1	0.2	43	>200
13	EMIM·BF_4/TBME	1:1	2	0.2	47	>200
14	EMIM·BF_4/TBME	1:1	2	0.35	34	190
15	EMIM·BF_4/TBME	1:1	2	0.5	39	>200
16	EMIM·BF_4/TBME	1:2	2	0.2	48	>200



Scheme 3. Lipase PS-C II-catalyzed acylation of *N*-acylated amino alcohols *rac*-**1** and *rac*-**2**; **1**, Y = H and **2**, Y = OH.

rows 1–8). In addition, the acylation at the phenolic HO resulted in the formation of product **5**, the enantiomeric purification of which took place when the *S*-selective benzylic acylation gave (*S*)-**6** (Scheme 3). (*S*)-**6** was also produced through the phenolic acylation of (*S*)-**4**. Evidently, the phenolic acylation does not lead to enantiodiscrimination as indicated by the practically racemic nature of **5** at the early stage of the reaction and also as previously shown for the reaction in toluene/THF.[25] Accordingly, all the four products [(*S*)-**4** and (*S*)-**6** as well as (*R*)-**2** and (*R*)-**5**] were in enantiopure form when the reaction proceeded far enough (Table 5, rows 1–7). Clearly, this conversion was reached at much shorter time in the reaction in TBME than when the reaction proceeded in the presence of the ionic liquid (row 1 compared to rows 2, 4 and 2). It can be expected that finally *rac*-**2** is totally transformed into enantiopure (*R*)-**5** and (*S*)-**6**. However, it is not reasonable to wait that long because the products (*S*)-**4** and (*S*)-**6**, in one hand, and (*R*)-**2** and (*R*)-**5**, in the other hand, can be joined followed by the deprotection to free *S*- and *R*-amino alcohols with HCl/MeOH and NH_3-bubbling as previously done.[25] For preparative-scale resolution a 0.45 M concentration of *rac*-**2** in EMIM·NTf_2/TBME (2:1) was used. The four enantiopure products were all separated in close to quantitative chemical yields and identified as described in the Experimental Section.

The present results show that chemoselectivity for the reaction of *rac*-**2** with vinyl butanoate in the presence of lipase PS-C II was highly dependent on the nature of the medium. This is shown in Figure 1 where the progression curves for the acylation in TBME (**a**), in EMIM·NTf_2 (**b**) and in their (2:1) mixture (**c**) are presented. Thus, high chemoselectivity for benzylic acylation in TBME turned to low chemoselectivity in EMIM·NTf_2. Low chemoselectivity is clearly seen as the high amounts of **5** (▲, being almost racemic in the beginning) and (*S*)-**6** (◆) with time compared to the amount of (*S*)-**4** (■) in EMIM·NTf_2. On the other hand, the reaction in EMIM·BF_4 proceeded slowly at low enantio- and chemoselectivity (Table 5, row 9 compared to row 2).

Microwave-Assisted Lipase-Catalyzed Acylation of *rac*-1 and *rac*-2

Ionic liquids as non-volatile solvents are good candidates for microwave-assisted reactions. Ionic liquids do not absorb microwaves efficiently and water-ionic liquid or organic solvent-ionic liquid mixtures are recommended. In the microwave vessel, the reaction is allowed to proceed at higher temperatures than the boiling point of the solvent. Some reports of the use of microwaves for lipase-catalyzed reactions in organic media exist.[27–29] According to these reports, rate and enantioselectivity enhancements are caused by the irradiation.

In the present work, the preliminary results for the lipase PS-C II-catalyzed acylation of *rac*-**1** and *rac*-**2** with vinyl butanoate in TBME and EMIM·NTf_2 and in their mixtures using the Emrys™Creator EXP microwave synthesizer are given (Table 6). In TBME, a fast reaction of *rac*-**1** under traditional and microwave conditions gave 50% conversion in a highly enantioselective manner (rows 1–4). The enzyme worked excellently even at 80 °C (row 4). For the reaction in EMIM·NTf_2, reactivity was almost the same under the microwave conditions at 60 °C as it was under normal conditions at 47 °C (rows 5 and 6). The same trend was observed in the 2:1 mixture of EMIM·NTf_2/TBME (rows 7 and 8 and 11 and 12). However, a dramatic drop both in reactivity and enantioselectivity was evident at temperatures higher than 60 °C when the microwave conditions were used (rows 8–10). At 110 °C 0.5–1.0 bar and at 60 °C 0.2 bar or lower pressures were observed.

The kinetic resolution of *rac*-**2** in EMIM·NTf_2/TBME (2:1) gave highly enantiopure products in 8 hours (row 16). The chemoselective behavior of *rac*-**2** in EMIM·NTf_2/TBME (2:1) followed closely the curves obtained in the same solvent system under the normal conditions of Figure 1.

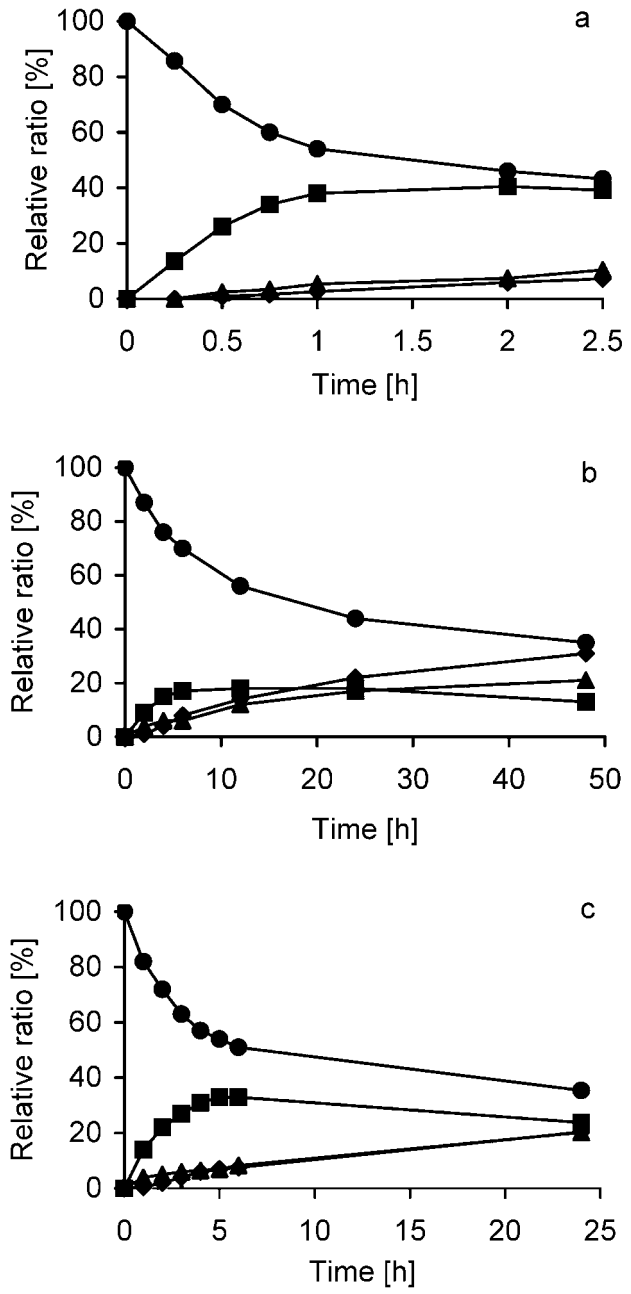


Figure 1. Progression curves for the lipase PS-C II-catalyzed acylation of *rac*-**2** with vinyl butanoate in (**a**) TBME, (**b**) EMIM·NTf_2 and (**c**) EMIM·NTf_2/TBME (2:1); **2** (●), **4** (■), **5** (▲) and **6** (◆).

Conclusion

The lipase-catalyzed acylation of *N*-acylated 2-amino-1-phenylethanol (*rac*-**1**) and *N*-acylated norphenylephrine (*rac*-**2**) with vinyl butanoate were studied in toluene/THF (3:1), in TBME, in various RTILs and in mixtures of TBME with EMIM·NTf_2 and EMIM·BF_4 (Schemes 2 and 3, Tables 1–5). Against expectations, our results for the lipase PS-C II-catalyzed acylation of *rac*-**1** and *rac*-**2** with vinyl butanoate showed lower reactivity in RTILs than in toluene/THF or in TBME. Highly *S*-enantioselective benzylic *O*-acylation allowed the gram-scale resolution of *rac*-**1** and *rac*-**2** (both 0.45 M) in the mixture of EMIM·NTf_2 with TBME (2:1) in 9 and 27 hours, respectively. Accordingly, by turning to RTILs it was possible to improve the previous preparative system [kinetic resolution of the free amino alcohols (0.05 M) in toluene/THF (3:1)][24,25] with respect to considerably enhanced substrate concentrations. As a drawback, *N*-acylated amino alcohols were now used as substrates, needing an extra synthetic step compared to the use of free amino alcohols. Prior *N*-acylation was necessary in order to focus only on the *O*-acylations and because of the fact that the reaction of 2-amino-2-phenylethanol with 2,2,2-trifluoroethyl butanoate in RTILs tended to stop before 50% conversion was reached.

Benzylic and phenolic *O*-acylations compete for the lipase PS-C II-catalyzed acylation of *rac*-**2** in RTILs and in organic solvents. The present results showed low chemoselectivity in the presence of EMIM·NTf_2 compared to the high chemoselectivity in TBME (or toluene/THF) (Figure 1). We also showed that there was no clear improvement with respect to reactivity, enantioselectivity and chemoselectivity under microwave compared to the normal conditions when the lipase PS-C II-catalyzed acylations were performed in the presence of EMIM·NTf_2 (Table 6).

Experimental Section

General Remarks

Lipases from *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) as native (lipase PS) and as immobilized on ceramic particles (lipase PS-C II) were purchased from Amano Pharmaceuticals Co., Ltd (Nagoya, Japan). Lipase PS powder was adsorbed on Celite® in Tris-HCl buffer (pH 7.8) in the presence of sucrose (1:1:0.6) as described earlier.[26] *Candida antarctica* lipase B (Novozym 435, CAL-B) was a kind gift from Novozymes. Activity of the lipase PS-C II was 11.4 μmol min^{-1} mg^{-1} for the disappearance of 1-phenylethanol (0.16 mmol) in the acylation with vinyl butanoate (0.20 mmol) in TBME (1 mL). Activity of lipase PS on Celite was 12.1 μmol min^{-1} mg^{-1}. BMPyr·BF_4, BMIM·BF_4, HexMIM·BF_4, BMPyr·PF_6 and EMIM·TfO were products of Fluka. EMIM·BF_4, BMIM·PF_6, EMIM·NTf_2 and BMIM·NTf_2 were prepared by slightly modifying the methods described in the literature.[2,8,30] RTILs were purified as previously described.[8] 2-Amino-1-phenylethanol and norphenylephrine hydrochloride were products of Aldrich. Norphenylephrine was prepared by bubbling ammonia through its hydrochloride solution in $CHCl_3$/ethanol (3:1). *Rac*-**1** and *rac*-**2** were prepared by the reaction of the corresponding amino alcohol with butanoic anhydride using stoichiometric control in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine. 2,2,2-Trifluoroethyl butanoate was prepared from butanoyl chloride and 2,2,2-trifluoroethanol. Vinyl butanoate and organic solvents

Table 5. Lipase PS-C II (50 mg/mL)-catalyzed acylation of *rac*-**2** (1 equivalent) with vinyl butanoate (2 equivalents) in RTILs and in their mixtures with TBME at 47 °C.

Row	Ionic liquid	**2** [M]	RTIL:TBME	Time [h]	Conv. [%]	Relative ratios [%] (ee [%]) (*R*)-**2**	(*S*)-**4**	(*R*)-**5**	(*S*)-**6**
1	TBME	0.2	–	2	54	46 (99)	40 (>99)	8 (>99)	6 (>99)
2	EMIM·NTf_2	0.2	–	24	55	45 (97)	18 (95)	17 (95)	22 (>99)
3	EMIM·NTf_2	0.35	–	48	70	30 (>99)	24 (94)	23 (94)	23 (>99)
4	EMIM·NTf_2/TBME	0.2	1:1	24	57	43 (96)	21 (>99)	14 (>99)	22 (>99)
5	EMIM·NTf_2/TBME	0.2	2:1	24	64	36 (>99)	24 (>99)	20 (>99)	20 (>99)
6	EMIM·NTf_2/TBME	0.35	2:1	24	58	42 (>99)	36 (98)	10 (95)	12 (>99)
7	EMIM·NTf_2/TBME	0.45	2:1	27	69	31 (>99)	33 (95)	16 (99)	20 (98)
8	EMIM·NTf_2/TBME	0.8	2:1	48	60	40 (98)	37 (96)	11 (88)	12 (>99)
9	EMIM·BF_4	0.2	–	168	85	15 (70)	17 (67)	20 (72)	48 (73)

Table 6. Lipase PS-C II (50 mg/mL)-catalyzed acylation of *rac*-**1** and *rac*-**2** (0.2 M) with vinyl butanoate (0.4 M) under microwave irradiation and classical heating.

Row	Substrate	Solvent	Heating method	*t* [°C]	Conv.[%] (*t* [h])	*E*
1	**1**	TBME[a]	Classical	47	27 (1)	110
2	**1**	TBME [a]	Classical	60	50 (1)	>200
3	**1**	TBME[a]	MW60	60	46 (1)	>200
4	**1**	TBME[a]	MW90	80	51 (1)	>200
5	**1**	EMIM·NTf_2	Classical	47	29 (2)	>200
6	**1**	EMIM·NTf_2	MW90	60	23 (2)	>200
7	**1**	EMIM·NTf_2/TBME 2:1	Classical	47	33 (2)	>200
8	**1**	EMIM·NTf_2/TBME 2:1	MW90	60	30 (2)	>200
9	**1**	EMIM·NTf_2/TBME 2:1	MW90	80	26 (2)	110
10	**1**	EMIM·NTf_2/TBME 2:1	MW90	110	2 (2)	3
11	**1**	EMIM·NTf_2/TBME 2:1[b]	Classical	47	50 (8)	>200
12	**1**	EMIM·NTf_2/TBME 2:1[b]	MW90	60	49.5 (8)	>200
13	**2**	EMIM·NTf_2/TBME 2:1	Classical	47	27 (2)	–
14	**2**	EMIM·NTf_2/TBME 2:1	MW90	60	33 (2)	–
15	**2**	EMIM·NTf_2/TBME 2:1[b]	Classical	47	69 (27)	>99/95/99/98[c]
16	**2**	EMIM·NTf_2/TBME 2:1[b]	MW90	60	51 (8)	94/96/86/>99[c]

[a] 0.1 M substrate.
[b] 0.45 M substrate.
[c] ee values for (*R*)-**2**/(*S*)-**4**/(*R*)-**5**/(*S*)-**6**.

were of the highest grade from Aldrich, Lab Scan Ltd, Riedel-de Haen or J. T. Baker.

The progress of the reactions was followed by taking samples (30–100 μL) at intervals, filtering off the enzyme (reaction in organic solvents) or extracting the products into TBME (reaction in RTILs) and analyzing the samples by GC on a Chrompack CP-Chirasil-L-valine capillary column after derivatization of the free alcohol groups with acetic or propionic anhydride. The determination of E was based on equation $E = \ln[(1 - ee_S)/(1 - ee_S/ee_P)]/\ln[(1+ee_S)/(1+ee_S/ee_P)]$ with $c = ee_S/(ee_S+ee_P)$.[31] E values with these equations can be calculated only when competitive reactions are not observed. Absolute configurations were determined by comparing the specific rotations to those in the literature[25]. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker 400 Spectrometer at room temperature. Microwave reactions were performed in an Emrys™Creator EXP microwave synthesizer with absorption level very high and power ranging from 60 W (MW60) to 90 W (MW90). Optical rotations were measured using a Perkin Elmer 300 polarimeter, the values being in units of 10^{-1} deg cm^{-2} g^{-1}.

Enzymatic Reactions

In a typical lipase-catalyzed acylation, an acyl donor (2 equivalents) was added into one of the amino alcohol derivatives *rac*-**1** or *rac*-2 in an organic solvent, RTIL or in their mixture (0.5–1 mL) and the enzyme preparation (50 mg/mL) was added to start the enzymatic reaction at 47 °C. The reactions under microwave conditions were typically performed as small-scale experiments (0.5 mL) at a power level 60 or 90 W at temperatures ranging from 60 to 110 °C, 60 °C being the lowest temperature allowed by the instrument. The instrument was cooled down at intervals and the samples were taken through the septum of the vial and thereafter the process started again.

Preparative-Scale Resolution of *rac*-1

Lipase PS-C II (0.49 g) and vinyl butanoate (1.4 mL, 10.6 mmol) were added to *rac*-**1** (1.00 g, 4.8 mmol) in EMIM·NTf_2/TBME (2:1; 9.7 mL). After the reaction time of 9 h at 47 °C the enzyme was filtered off at 50% conversion and the products were extracted into TBME. Purification by column chromatography [silica, ethyl acetate/hexane (1:1)] afforded the unreacted (*R*)-**1** [0.44 g, 2.1 mmol, ee 99%, $[\alpha]_D^{25}$: −82,4 (*c* 1, $CHCl_3$)] and the produced (*S*)-**3** [0.61 g, 2.2 mmol, ee 98%, $[\alpha]_D^{25}$: +52.4 (*c* 1, $CHCl_3$)].

(*R*)-**1**: ^{1}H NMR ($CDCl_3$, 25 °C): δ = 0.91(t, 3H, *J* = 7.5 Hz, CH_3CH$_2$), 1.62 (q, 2H, CH$_3$CH_2CH$_2$), 2.13 (t, 2H, *J* = 7.5 Hz, CH$_3$CH$_2$CH_2CO), 3.30 and 3.65 (m, 2H, *J* = 7.4 Hz, CHCH_2-NHCOPr), 3.97 (s, 1H, O*H*), 4.80 (dd, 1H, C*H*OH), 6.14 (d, 1H, CH$_2$N*H*CO), 7.33 (s, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 13.7, 19.1, 38.5, 47.5, 73.7, 125.8, 127.8, 128.5, 141.9, 174.7. MS: *m/z* = 207.1259 (M^+) calculated for $C_{12}H_{17}NO_2$: 207.1261.

(*S*)-**3**: ^{1}H NMR ($CDCl_3$, 25 °C): δ = 0.94 (m, 2 × 3H, CH_3-CH$_2$), 1.65 (m, 2 × 2H, *J* = 7.3 Hz, CH$_3$CH_2CH$_2$), 2.12 and 2.35 (tt, 2 × 2H, *J* = 7.5 Hz, CH$_3$CH$_2$CH_2CO), 3.57–3.72 (m, 2H, CHCH_2NHCOPr), 5.77 (s, 1H, CH$_2$N*H*CO), 5.86 (dd, 1H, *J* = 4.3 Hz, C*H*CH$_2$NHCOPr), 7.34 (s, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 13.6, 13,7, 18.4, 19.1, 36.3, 38.6, 44.3, 74.3, 126.4, 128.4, 128.7, 137.9, 173.0. MS: *m/z* = 277.1672 (M^+); calculated for $C_{16}H_{23}NO_3$: 277.1678.

Preparative-Scale Resolution of *rac*-2

Lipase PS-C II (0.50 g) and vinyl butanoate (1.25 mL, 9.85 mmol) were added to *rac*-**2** (1.00 g, 4.5 mmol) in EMIM·NTf_2/TBME (2:1; 10 mL). After the reaction time of 27 h at 47 °C the enzyme was filtered off at 69% conversion and the products were extracted into TBME. Purification by column chromatography [gradient from ethyl acetate/hexane (1:1) to pure ethyl acetate] afforded unreacted (*R*)-**2** (0.34 g, 1.5 mmol, ee > 99%, $[\alpha]_D^{20}$: +5.0 (*c* 2, MeOH)) and the acylated products (*S*)-**4** [0.34 g, 1.2 mmol, ee 95%, $[\alpha]_D^{20}$: +46.8 (*c* 1, MeOH)], (*R*)-**5** [0.19 g, 0.64 mmol, ee 99%, $[\alpha]_D^{20}$: +8.6 (*c* 2, MeOH)] and (*S*)-**6** [0.34 g, 0.92 mol, ee 98%, $[\alpha]_D^{20}$: +32.6 (c 1, MeOH)].

(R)-**2**: ^{1}H NMR ($CDCl_3$, 25 °C): δ = 0.83 (t, 3H, *J* = 7.4 Hz, CH_3CH$_2$), 1.49 (m, 2H, *J* = 7.4 Hz, CH$_3$CH_2CH$_2$), 2.05 (t, 2H, CH$_3$CH$_2$CH_2CO), 3.05 and 3.27 (m, 2H, CHCH_2NHCOPr), 5.37 (d, 1H, C*H*CH$_2$NHCOPr), 6.63 (dd, 1H, C_6H_4), 6.73 (d, 1H, *J* = 7.8 Hz, C_6H_4), 6.76 (s, 1H, C_6H_4), 7.10 (t, 1H, *J* = 7.8 Hz, C_6H_4), 7.83 (t, 1H, CH$_2$N*H*CO), 9.29 (s, 1H, ArO*H*); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 13.7, 18.7, 37.3, 46.9, 71.5, 112.9, 113.9, 116.6, 128.9, 145.4, 157.2 and 172.3; MS: *m/z* = 223.1212 (M^+); calculated for $C_{12}H_{17}NO_3$: 223.1208.

(*S*)-**4**: ^{1}H NMR ($CDCl_3$, 25 °C): δ = 0.84 (tt, 2 × 3H, *J* = 7.4 Hz, CH_3CH$_2$), 1.43–1.59 (qq, 2 × 2H, *J* = 7.4 Hz, CH$_3$CH_2-CH$_2$), 2.05 and 2.31 (tt, 2 × 2H, *J* = 8.5 Hz, CH$_3$CH$_2$CH_2CO), 3.27–3.41 (m, 2H, CHCH_2NHCOPr), 5.66 (dd, 1H, *J* = 4.2 Hz, C*H*CH$_2$NHCOPr), 6.69 (t, 3H, C_6H_4), 7.15 (t, 1H, C_6H_4), 7.99 (t, 1H, CH$_2$N*H*CO), 9.46 (s, 1H, ArO*H*); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 13.9, 14.0, 18.4, 19.1, 36.0, 37.7, 44.2, 74.0, 113.4, 115.3, 117.1, 129.9, 140.5, 157.8, 172.5, 172.7. MS: *m/z* = 293.1636 (M^+); calculated for $C_{16}H_{23}NO_4$: 293.1627.

(R)-**5**: ^{1}H NMR ($CDCl_3$, 25 °C): δ = 0.90 and 1.00 (tt, 2 × 3H, CH_3CH$_2$), 1.60 and 1.75 (qq, 2 × 2H, *J* = 7.4 Hz, CH$_3$CH_2CH$_2$), 2.11 and 2.52 (tt, 2 × 2H, CH$_3$CH$_2$CH_2CO), 3.25–3.60 (m, 2H, CHCH_2NHCOPr), 4.3 (s, 1H, CHO*H*), 4.75 (d, C*H*CH$_2$-NHCOPr), 6.9 (d, 1H, C_6H_4), 7.1 (s, 1H, C_6H_4), 7.25 (d, 1H, C_6H_4), 7.4 (t, 1H, CH$_2$NHCO); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 13.6, 18.4, 19.0, 33.9, 36.1, 47.3, 73.0, 119.1, 121.9, 124.5, 130.6, 151.0, 154.0, 172.2, 175.0; MS: *m/z* = 293.1634 (M^+); calculated for $C_{16}H_{23}NO_4$: 293.1627.

(*S*)-**6**: ^{1}H NMR ($CDCl_3$, 25 °C): δ = 0.92 (m, 6H, *J* = 7.4 Hz, CH_3CH$_2$), 1.04 (t, 3H,CH_3CH$_2$), 1.56–1.66 (m, 2 × 2H, *J* = 7.4 Hz, CH$_3$CH_2CH$_2$), 1.75 (q, 2H, CH$_3$CH_2CH$_2$), 2.12, 2.36, 2.5 (3 × t, 3 × 2H, *J* = 7.3 Hz, CH$_3$CH$_2$CH_2CO), 3.27–3.41 (m, 2H, CHCH_2NHCOPr), 5.86 (dd, 1H, *J* = 4.6 Hz, C*H*CH$_2$-NHCOPr), 7.03 (d, 1H, *J* = 7.9 Hz, C_6H_4), 7.09 (s, 1H, C_6H_4), 7.24 (d, 1H, C_6H_4), 7.38 (t, 1H, *J* = 7.9 Hz, CH$_2$N*H*CO); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 15.0, 20.4, 21.3, 30.1, 37.9, 38.0, 39.9, 46.2, 61.2, 75.8, 121.9, 123.6, 125.9, 131.7, 142.5, 153.4, 174.7, 175.3, 177.4; MS: *m/z* = 363.2059 (M^+); calculated for $C_{20}H_{29}NO_5$: 363.2046.

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