# Immobilised *Burkholderia cepacia* lipase in dry organic solvents and ionic liquids: A comparison

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Lipase PS from *Burkholderia cepacia* in its free, commercial form (BCL-PS), immobilised in a sol–gel (BCLxero) and as a CLEA (BCL-CLEA) was tested in dry organic solvents, ionic liquids and their mixtures. Utilising the acylations of secondary alcohols **1–3** the influence of the enzyme preparation on its activity and enantioselectivity was studied. BCL-CLEA displays higher activity (initial rates) than BCLxero for all substrates in the ILs but loses its activity rapidly. Thus, BCLxero is suitable for kinetic resolution in ILs and in their mixtures with organic solvents. It is not possible to label one IL better than the other without taking the nature of the substrate into account. In neat solvents, the nature of the solvent affects enantioselectivity (*E*) only when furyl-substituted alcohol **2** serves as a substrate while variation in *E* is more evident for reactions in solvent mixtures.



## Introduction

Ionic liquids (IL) and enzymes, as well as their application, were both described long ago but it has taken until recently before the potential of combining them was recognised. They have been thoroughly reviewed as separate subjects and in combination.[1–10] Using enzymes in ionic liquids many new applications of biocatalysis have came about. However, to fully appreciate the possibilities of applying enzymes in ionic liquids, the parameters influencing the behaviour of enzymes in ILs need to be understood. One of the parameters which has, to date, virtually not been systematically investigated, is the influence of the enzyme preparation on its stability in different dry ILs or IL/organic solvent mixtures.[5]

One of the enzymes best studied in ILs is *Burkholderia cepacia* lipase (BCL), also known as *Pseudomonas cepacia* lipase and lipase PS or lipase PS "Amano" SD.[11–20] Here we report on the application of three different BCL forms, the commercial free enzyme powder from Amano which is sold diluted with dextrin, a cryoprotector,[21] BCL encapsulated inside a sol–gel[22] and BCL cross-linked as a CLEA.[23] These forms of BCL are utilised in different, often used ILs and in IL/organic solvent mixtures for the enantioselective acylation of diverse racemic alcohols. A water miscible ionic liquid (IL) with a hydrophilic anion [EMIM][$BF_4$] and water immiscible ionic liquids with hydrophobic anions [EMIM][$Tf_2N$] and [BMIM][$PF_6$] were chosen. These ILs are thoroughly characterized (Table 1), and display hydrogen bond donor abilities similar to those of alcohols while their nucleophilicities are much lower and entirely anion dependent, *i.e.*, the ILs are unique as solvents showing high polarity and low nucleophilicity.[24–26] The cations are both modestly hydrophilic. Although it is well-known that [$BF_4$] and [$PF_6$] can release HF this is not the case when they are used under dry conditions, as described here.[9,16,25] Both [EMIM] and [BMIM] were recently assessed for their ecotoxicity and fall into a similar range as most organic solvents.[27,28] The advantage of using these ILs lies therefore mainly in the fact that they are non-volatile and that a lot of previous work has been performed in them. This allows a systematic comparison of the different immobilisation techniques.

As test reactions, the kinetic resolution of aromatic alcohol **1**, heteroaromatic alcohol **2** and *N*-acylated amino alcohol **3** were investigated (Scheme 1). In addition, the potential side reaction, hydrolysis of the acetate products **4–6** due to residual water in the enzymatic reaction mixture, was explored. This side reaction causes the release of acetic acid, reduces the yield, affects enantiopurities and is normally neglected. The significance of this side reaction is often ignored although ample evidence of its importance is available.[29–34] Esterification of the acid was previously proposed as the continuous source of water.[35]

In an earlier study we could already demonstrate that there is a significant difference in behaviour between the BCL preparations, and that depending on the substrate (**1–3**), different organic solvents should be employed.[36] The commercial BCL (BCL-PS) is in essence dextrin, containing 3% free enzyme. The encapsulated enzyme is prepared with the sol–gel technique, utilising both MTMS (methyltrimethoxysilane) and TMOS (tetramethoxysilane) as precursors for the sol–gel.[36,37] The initially obtained aquagel is lyophilised to obtain the xerogel which is then ready to be used as a catalyst. The final preparation (BCLxero) is the enzyme encapsulated in reverse phase silica in which part of the capsules will be broken due to the drying process. The cross linked BCL (BCL-CLEA) is prepared

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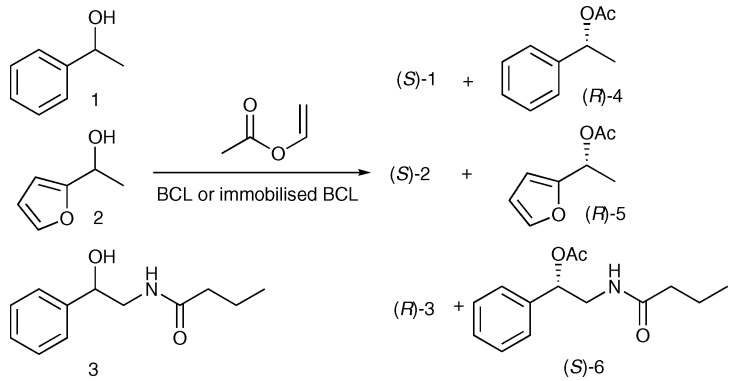
**Table 1** Properties of dry ILs: log *P* (logarithm for the partition coefficient of an IL between 1-octanol and water), $E^N_T$ (Reichardt's normalized polarity scale) and viscosity (25 °C)

| Ionic liquid | log *P*[24] | $E^N_T$[24] | Viscosity [cP][25] | Water content [ppm][a] | Water solubility[26] |
|---|---|---|---|---|---|
| [EMIM][$BF_4$] | –3.53 | 0.697 | 43 | 986 | yes |
| [EMIM][$Tf_2N$] | –1.18 | 0.661 | 28 | 45 | no |
| [BMIM][$PF_6$] | –2.06 | 0.670 | 450 | 486 | no |
| Toluene | 2.8 | — | — | 77 | no |
| DIPE | 1.9 | 0.546 | — | 321 | no |
| TBME | 1.35 | — | — | 622 | no |

[a] Determined by Karl Fisher titration.




**Scheme 1** Kinetic resolution of three different alcohols catalysed by different BCL preparations, performed in organic solvents, ILs and IL/organic solvent mixtures.



according to standard methodology.[36,38] While the BCLxero had the same or lower activity than BCL-PS in organic solvents the BCL-CLEA had higher activity (Tables 2 and 3).[36] On the other hand BCLxero proved more stable in organic solvents than BCL-CLEA. We here describe the behaviour of the BCL preparations in three different ILs and in their mixtures with selected organic cosolvents with three different substrates (**1–3**). To make direct comparison of the results possible, the protein content was kept as 100 mg $ml^{-1}$ BCLxero and BCL-PS, on one hand, and as 50 mg $ml^{-1}$ for BCL-CLEA and BCL-PS, on the other hand.

## Results and discussion

Racemates **1–3** were first subjected to acylation with vinyl acetate in toluene, DIPE and TBME as well as in [EMIM][$Tf_2N$], [EMIM][$BF_4$] and [BMIM][$PF_6$] in the presence of the BCLxero and BCL-CLEA (Table 2). Toluene for **1**, DIPE for **2** and TBME for **3** were previously best detected for the acylation in the presence of BCLxero and BCL-CLEA.[36] On the other hand, the ILs were chosen as best for the previous acylation of **3** with lipase PS-C II (BCL preparation on Toyonite from Amano[16]).[39] Activities in the table are given as initial rates measured by

**Table 2** Acylation of **1–3** (0.1 M) with vinyl acetate (0.2 M) in organic solvents and in ionic liquids in the presence of BCLxero and BCL-CLEA at room temperature

| | | | BCLxero[a] | | BCL-CLEA[b] | |
|---|---|---|---|---|---|---|
| Entry | Substrate | Solvent | $v_0$[c] | Conv. [%][d]/*E* | $v_0$[c] | Conv. [%][d]/*E* |
| 1 | **1** | Toluene | 1.42 ± 0.02 | 50/>200 | 7.0 ± 0.9 | 50/>200 |
| 2 | **1** | Toluene | — | — | 3.86 ± 0.25[e] | 46/>200 |
| 3 | **1** | [EMIM][$Tf_2N$] | 0.31 ± 0.01 | 30/>200 | 0.32 ± 0.01 | 5/>200 |
| 4 | **1** | [EMIM][$BF_4$] | 0.19 ± 0.01 | 23/>200 | 1.65 ± 0.07 | 24/>200 |
| 5 | **1** | [BMIM][$PF_6$] | 0.41 ± 0.01 | 34/>200 | 1.1 ± 0.1 | 18/>200 |
| 6 | **2** | DIPE | 1.47 ± 0.02 | 51/131 | 30.1 ± 0.4 | 53/58 |
| 7 | **2** | [EMIM][$Tf_2N$] | 0.90 ± 0.01 | 28/38 | 0.17 ± 0.01 | 3/— |
| 8 | **2** | [EMIM][$BF_4$] | 0.15 ± 0.02 | 23/46 | 2.28 ± 0.25 | 34/40 |
| 9 | **2** | [BMIM][$PF_6$] | 0.27 ± 0.04 | 41/78 | 1.34 ± 0.38 | 21/49 |
| 10 | **3** | TBME | 1.54 ± 0.03 | 49/>200 | 6.7 ± 0.3 | 49/>200 |
| 11 | **3** | [EMIM][$Tf_2N$] | 0.03 ± 0.01 | 5/>200 | 0.06 ± 0.01 | 1/>200 |
| 12 | **3** | [EMIM][$BF_4$] | 0.04 ± 0.01 | 5/>200 | 1.91 ± 0.01 | 17/— |
| 13 | **3** | [BMIM][$PF_6$] | 0.08 ± 0.01 | 13/>200 | 0.67 ± 0.07 | 10/>200 |

[a] Based on 100 mg of BCL-PS powder. [b] Based on 50 mg of BCL-PS powder. [c] μmol $min^{-1}$ $g^{-1}$. [d] Conversion after 24 h/*E*. [e] CLEA prepared by adding bovine serum albumin.

**Table 3** Acylation of **1–3** (0.1 M) with vinyl acetate in organic solvents and the mixtures of IL:organic solvent (1:2) in the presence of BCL-PS at room temperature

| Entry | Substrate | Solvent | Phases | BCL-PS | *t* [h] | *c* [%] | *E* | $v_0$ [μmol min$^{-1}$ g$^{-1}$] |
|---|---|---|---|---|---|---|---|---|
| 1 | **1** | Toluene | 1 | 50 mg [a] | 24 | 49 | >200 | 3.4 ± 0.2 |
| 2 | **1** | [EMIM][$NTf_2$]:toluene | 2 | 50 mg [a] | 48 | 17 | >200 | 0.13 ± 0.01 |
| 3 | **1** | [EMIM][$BF_4$]:toluene | 2 | 50 mg [a] | 24 | 47 | >200 | 1.15 ± 0.01 |
| 4 | **1** | [BMIM][$PF_6$]:toluene | 2 | 50 mg [a] | 48 | 49 | >200 | 0.79 ± 0.01 |
| 5 | **2** | DIPE | 1 | 50 mg [a] | 24 | 51 | 131 | 3.8 ± 0.2 |
| 6 | **2** | [EMIM][$NTf_2$]:DIPE | 2 | 50 mg [a] | 48 | 30 | 51 | 0.26 ± 0.004 |
| 7 | **2** | [EMIM][$BF_4$]:DIPE | 2 | 50 mg [a] | 24 | 51 | 134 | 1.27 ± 0.01 |
| 8 | **2** | [BMIM][$PF_6$]:DIPE | 2 | 50 mg [a] | 24 | 50 | 77 | 0.79 ± 0.01 |
| 9 | **3** | TBME | 1 | 50 mg [a] | 48 | 33 | 14 | 2.38 ± 0.03 |
| 10 | **3** | [EMIM][$BF_4$]:TBME | 2 | 50 mg [a] | 48 | 23 | 50 | 0.84 ± 0.001 |
| 11 | **3** | [BMIM][$PF_6$]:TBME | 2 | 50 mg [a] | 48 | 48 | 70 | 0.79 ± 0.001 |
| 12 | **3** | [EMIM][$NTf_2$]:TBME | 2 | 50 mg [a] | 48 | 24 | >200 | 0.16 ± 0.004 |
| 13 | **1** | Toluene | 1 | 100 mg [b] | 24 | 50 | >200 | 2.7 ± 0.3 |
| 14 | **1** | [EMIM][$NTf_2$]:toluene | 2 | 100 mg [b] | 48 | 17 | 51 | 0.084 ± 0.002 |
| 15 | **1** | [EMIM][$BF_4$]:toluene | 2 | 100 mg [b] | 24 | 50 | >200 | 0.57 ± 0.001 |
| 16 | **1** | [BMIM][$PF_6$]:toluene | 2 | 100 mg [b] | 48 | 50 | >200 | 0.46 ± 0.01 |
| 17 | **2** | DIPE | 1 | 100 mg [b] | 24 | 50 | 115 | 3.5 ± 0.2 |
| 18 | **2** | [EMIM][$NTf_2$]:DIPE | 2 | 100 mg [b] | 48 | 45 | 88 | 0.21 ± 0.001 |
| 19 | **2** | [EMIM][$BF_4$]:DIPE | 2 | 100 mg [b] | 6 | 50 | 115 | 1.54 ± 0.01 |
| 20 | **2** | [BMIM][$PF_6$]:DIPE | 2 | 100 mg [b] | 24 | 47 | 196 | 0.43 ± 0.001 |
| 21 | **3** | TBME | 1 | 100 mg [b] | 24 | 25 | 30 | 1.69 ± 0.05 |
| 22 | **3** | [EMIM][$BF_4$]:TBME | 2 | 100 mg [b] | 24 | 33 | 50 | 0.41 ± 0.01 |
| 23 | **3** | [BMIM][$PF_6$]:TBME | 2 | 100 mg [b] | 48 | 16 | 15 | 0.067 ± 0.001 |
| 24 | **3** | [EMIM][$NTf_2$]:TBME | 2 | 100 mg [b] | 48 | 30 | 30 | 0.12 ± 0.003 |

[a] Corresponds to protein in BCL-CLEA. [b] Corresponds to protein in BCLxero.



μmol min$^{-1}$ g$^{-1}$ of the original BCL-PS used to prepare the xero gel or the CLEA, thus allowing direct comparison. The results clearly indicate that initial rates in the organic solvents (entries 1, 6 and 10) are always higher than those in the studied ILs. The acylation in organic solvents proceeds smoothly close to 50% conversion in a highly enantioselective manner while in the ILs there is a tendency for the progress of the acylation to cease. Finally, the acylation of **3** in the ILs with both BCL preparations practically stops at early stages (entries 11–13). The BCL-CLEA displays higher initial rates than the BCLxero for all substrates in [EMIM][$BF_4$] and in [BMIM][$PF_6$] (entries 4, 5, 8, 9, 12 and 13). Diffusion limitations can be proposed as a reason for apparent low activities of the BCLxero. However, diffusion limitation should be most evident in the viscose [EMIM][$Tf_2N$] rather than in [EMIM][$BF_4$] or [BMIM][$PF_6$] (Table 1). This is not the case ruling out the influence of diffusion on the reaction rates of BCLxero (Table 2, compare entries 3, 4, 5 and 7, 8, 9). It can therefore be concluded that all three ILs have a negative effect on the initial activity of BCLxero. For the more active BCL-CLEA the diffusion limitations do become evident in the same examples explaining the relatively low initial rate of BCL-CLEA in [EMIM][$Tf_2N$]. But as with BCLxero all ILs seem to have a negative influence on the initial rates of BCL-CLEA. Poor reactivity was reported earlier for the BCL-PS preparation in ILs.[12]

While the initial rates of BCLxero are significantly lower than those of BCL-CLEA the overall conversion in the IL with BCLxero is much better for the acylation of **1** and **2**. Indeed, with BCLxero conversions continue to increase toward 50% conversion with time. In contrast BCL-CLEA seems to loose its activity rather rapidly and even with high initial rates the reactions tend to stop at low conversions (entries 3, 4, 8, 9, 12, 13, Table 2). This loss of activity might be due to the earlier described acetaldehyde-oligomer formation induced by ILs when vinyl acetate is used as an acyl donor.[17,40] Free BCL-PS was also previously shown to loose its activity in [BMIM][$PF_6$] and other *N*,*N*-dialkyl imidazolium ion based ILs.[12] The stabilisation of BCL *via* immobilisation on the ceramic Toyonite carriers for the use in ILs has earlier been described.[16,17,39] The results described here indicate that the xerogel can also protect BCL against deactivation, while cross-linking does not provide this stabilisation. Consequently BCLxero is the BCL preparation of choice in IL.

No clear trend as to which IL is the best can be deduced from Table 2. It seems that, much like the organic solvents, there is a "best IL" for each of the three substrates. Thus it can be said that with the organic solvent of choice for each substrate the enzyme preparations always perform better than with the IL of choice.

For kinetic resolutions of racemates, enantioselectivity (measured by the enantiomer ratio *E*) is often a more important parameter than reactivity. In the case of **2**, considerable solvent effects on enantioselectivity are evident when either BCLxero or BCL-CLEA was used (entries 6–9 Table 2). As for the activity, the enzyme enantioselectivity is highly dependent on the substrate. For this substrate [BMIM][$PF_6$] is the IL of choice. The excellent enantioselectivity for the acylations of **1** and **3** was not affected by the solvents used (entries 1–5 and 10–13, Table 2).

In our previous work for the acylation of **3** with lipase PS–C II, activities and enantioselectivities were enhanced when TBME was added to [EMIM][$Tf_2N$] and [EMIM][$BF_4$].[39] Accordingly, toluene, DIPE and TBME were mixed with the ILs studied here, and the acylation of **1–3** in the respective mixtures was investigated using all three BCL preparations, BCL-PS, BCLxero and

**Table 4** Acylation of **1–3** (0.1 M) with vinyl acetate (0.2M) in the mixture of IL:organic solvent in the presence of BCLxero (based on 100 mg of the original BCL-PS powder) at room temperature

| Entry | Substrate | Solvent system | Phases | Conv.[a] [%] | ee[b] [%]/$E$ | $v_0$ [μmol min$^{-1}$ g$^{-1}$] |
|---|---|---|---|---|---|---|
| 1 | **1** | [EMIM][$Tf_2N$]:toluene (2:1) | 1 | 40/49 | 98/>200 | 0.24 ± 0.05 |
| 2 | **1** | [EMIM][$Tf_2N$]:toluene (1:1) | 2 | 22/34 | >99/>200 | 0.14 ± 0.001 |
| 3 | **1** | [EMIM][$Tf_2N$]:toluene (1:2) | 2 | 45/49 | >99/>200 | 0.98 ± 0.08 |
| 4 | **1** | [EMIM][$Tf_2N$]:toluene (1:3) | 2 | —/30 | >99/>200 | 0.25 ± 0.001 |
| 5 | **1** | [EMIM][$BF_4$]:toluene (2:1) | 1 | 33/45 | 99/>200 | 0.40 ± 0.02 |
| 6 | **1** | [EMIM][$BF_4$]:toluene (1:1) | 2 | 43/48 | 99/>200 | 0.60 ± 0.12 |
| 7 | **1** | [EMIM][$BF_4$]:toluene (1:2) | 2 | 39/47 | 99/>200 | 0.35 ± 0.03 |
| 8 | **1** | [EMIM][$BF_4$]:toluene (1:3) | 2 | 29/40 | >99/>200 | 0.28 ± 0.001 |
| 9 | **1** | [BMIM][$PF_6$]:toluene (2:1) | 1 | 37/47 | 99/>200 | 0.84 ± 0.01 |
| 10 | **1** | [BMIM][$PF_6$]:toluene (1:2) | 2 | 46/50 | 99/>200 | 0.72 ± 0.05 |
| 11 | **2** | [EMIM][$Tf_2N$]:DIPE (1:2) | 2 | 51/54 | 85/66 | 0.71 ± 0.04 |
| 12 | **2** | [EMIM][$BF_4$]:DIPE (2:1) | 2 | 51/— | 91/66 | 1.19 ± 0.14 |
| 13 | **2** | [EMIM][$BF_4$]:DIPE (1:2) | 2 | 54/— | 87/61 | 2.19 ± 0.09 |
| 14 | **2** | [BMIM][$PF_6$]:DIPE (2:1) | 2 | 51/— | 87/42 | 1.48 ± 0.16 |
| 15 | **2** | [BMIM][$PF_6$]:DIPE (1:2) | 2 | 34/33 | 96/60 | 0.68 ± 0.04 |
| 16 | **3**[c] | [EMIM][$Tf_2N$]:TBME (2:1) | 1 | 10/18 | >99/>200 | 0.12 ± 0.01 |
| 17 | **3**[c] | [EMIM][$Tf_2N$]:TBME (1:2) | 2 | 26/37 | 97/126 | 0.31 ± 0.01 |
| 18 | **3**[c] | [EMIM][$BF_4$]:TBME (2:1) | 1 | 32/42 | 96/71 | 0.46 ± 0.01 |
| 19 | **3**[c] | [EMIM][$BF_4$]:TBME (1:2) | 2 | 28/37 | 96/89 | 0.30 ± 0.02 |
| 20 | **3**[c] | [BMIM][$PF_6$]:TBME (2:1) | 1 | 5/5 | >99/— | 0.15 ± 0.04 |
| 21 | **3**[c] | [BMIM][$PF_6$]:TBME (1:2) | 2 | 41/44 | 97/102 | 1.06 ± 0.11 |

[a] Conversion after 24 h/48 h. [b] ee for the formed ester product at the conversion after 48 h. [c] Reaction temperature 48 °C.

**Table 5** Acylation of **1–3** (0.1 M) with vinyl acetate (0.2 M) in the mixture of an IL and an organic solvent in the presence of BCL-CLEA (based on 50 mg of the original BCL-PS powder) at room temperature

| Entry | Substrate | Solvent system | Phases | Conv.[a] [%] | ee[b] [%]/$E$ | $v_0$ [μmol min$^{-1}$ g$^{-1}$] |
|---|---|---|---|---|---|---|
| 1 | **1** | [EMIM][$Tf_2N$]:toluene (2:1) | 1 | 11/19 | >99/>200 | 1.21 ± 0.62 |
| 2 | **1** | [EMIM][$Tf_2N$]:toluene (1:2) | 2 | 10/18 | >99/>200 | 1.57 ± 0.47 |
| 3 | **1** | [EMIM][$BF_4$]:toluene (2:1) | 1 | 41/49 | 98/>200 | 5.83 ± 1.44 |
| 4 | **1** | [EMIM][$BF_4$]:toluene (1:2) | 2 | 41/48 | >99/>200 | 6.60 ± 0.71 |
| 5 | **1**[c] | [EMIM][$BF_4$]:toluene (1:2) | 2 | 32/43 | >99/>200 | 1.98 ± 0.36 |
| 6 | **1** | [BMIM][$PF_6$]:toluene (2:1) | 1 | 15/28 | >99/>200 | 1.80 ± 0.36 |
| 7 | **1** | [BMIM][$PF_6$]:toluene (1:2) | 2 | 20/32 | >99/>200 | 1.90 ± 0.41 |
| 8 | **2** | [EMIM][$Tf_2N$]:DIPE (1:2) | 2 | 19/31 | 95/49 | 1.80 ± 0.18 |
| 9 | **2** | [EMIM][$BF_4$]:DIPE (2:1) | 2 | 34/46 | 92/57 | 3.57 ± 0.26 |
| 10 | **2** | [EMIM][$BF_4$]:DIPE (1:2) | 2 | 40/50 | 93/79 | 4.82 ± 0.15 |
| 11 | **2** | [BMIM][$PF_6$]:DIPE (2:1) | 2 | 18/36 | 96/79 | 0.54 ± 0.13 |
| 12 | **2** | [BMIM][$PF_6$]:DIPE (1:2) | 2 | 33/47 | 94/78 | 2.57 ± 0.42 |
| 13 | **3**[d] | [EMIM][$Tf_2N$]:TBME (1:2) | 2 | 4/7 | >99/— | 0.35 ± 0.04 |
| 14 | **3**[d] | [EMIM][$BF_4$]:TBME (2:1) | 1 | 19/29 | 95/43 | 0.54 ± 0.13 |
| 15 | **3**[d] | [EMIM][$BF_4$]:TBME (1:2) | 2 | 22/33 | 95/55 | 1.69 ± 0.40 |
| 16 | **3**[d] | [BMIM][$PF_6$]:TBME (2:1) | 1 | 29/40 | 95/73 | 3.14 ± 0.38 |
| 17 | **3**[d] | [BMIM][$PF_6$]:TBME (1:2) | 2 | 31/41 | 96/105 | 3.29 ± 0.38 |

[a] Conversion after 24 h/48 h. [b] ee for the formed ester product at the conversion after 48 h. [c] CLEA prepared by adding bovine serum albumin. [d] Reaction temperature 48 °C.

BCL-CLEA (Tables 3–5). The addition of an organic solvent lowers the viscosity of an IL.[41] Another important factor is that the organic solvents used are not in all proportions soluble in the ILs and separation into two phases may take place as given in the tables.

All BCL preparations display the highest initial rates in the pure organic solvent. Mixtures of ILs with organic solvents slow down the reaction in most cases, independent of the fact whether it is a mono- or biphasic mixture.

For substrate **1** BCL-PS clearly performs best in toluene, and only in [EMIM][$BF_4$]:toluene 1:2 mixture equal enantioselectivity and close to 50% conversions after 24 h were observed (Table 3, entries 1, 3, 13, 15). In IL organic solvent mixtures BCLxero always displays lower activities for this substrate than in toluene, but keeps excellent enantioselectivity (Table 2 entry 1 and Table 4 entries 1–10). For BCL-CLEA the activity towards **1** also uniformly drops in IL organic solvent mixtures compared to the pure organic solvent, while excellent enantioselectivities are maintained (Table 2 entry 1 and Table 5 entries 1–7).

For substrate **2** BCL-PS again performs best in the organic solvent and in [EMIM][$BF_4$]:DIPE 1:2 mixture while in the other IL:DIPE mixtures lower activity and enantioselectivity are observed (Table 3). In contrast BCLxero displays practically the same (51–54%) conversion toward **2**, independent of the solvent system (Table 4, entries 11–14), the mixture [BMIM][$PF_6$]:DIPE (1:2) being an exception (entry 15). However, the

enantioselectivity in IL:DIPE mixtures is always lower than in pure DIPE (Table 2 entry 6 and Table 4 entries 11–15). BCL-CLEA is less active and less enantioselective toward **2** when used in IL:DIPE mixtures compared to its use in pure DIPE (Table 2 entry 6 and Table 5 entries 8–12).

In the case of substrate **3**, BCLxero and BCL-CLEA have the highest activity and enantioselectivity in TBME (compare Table 2 entry 10 with Tables 4 and 5). This is not the case for BCL-PS. Here improved enantioselectivity can be observed in some cases in IL:TBME mixtures (Table 3, entries 9–12 and 21–24). In this case hydrolysis by the water in the enzyme may complicate the interpretation of the observed results as will be discussed below. However, the initial rates are always highest in the pure organic solvent.

Thus, no clear trend-line can be deduced as to which reaction medium should be used, mono- or biphasic. Instead it seems that for each substrate the best solvent (mixture) has to be found separately.

We then studied the hydrolysis of the produced enantiopure esters (*R*)-**4**, (*R*)-**5** and (*S*)-**6** with the residual water in the system with the two most successful BCL preparations, BCL-PS and BCLxero. The potential of hydrolysis for the produced ester in the acylation of **1–3** can be seen in such experiments while the real proportion of the hydrolysis during the acylation cannot be determined. Moreover, the proportion in the acylation is certainly much less that the data in Table 6 suggest because the concentration of the hydrolysable ester is initially zero and the substrate alcohol (**1–3**) then competes with water as a nucleophile. In the case of all the substrates, the presence of BCLxero causes considerably less hydrolysis than BCL-PS (Table 6). The hydrolysis of (*S*)-**6** in the solvent mixture is an exception (entry 6). In this case, the hydrolysable ester is butanoate rather than acetate and the reaction takes place at an elevated temperature. Another observation is that hydrolysis is always more significant in the solvent mixture than in a neat organic solvent. This might be explained by the relatively high water content (986 ppm) of [EMIM][$BF_4$] and by its water miscibility. However, the same amount of water from the IL is present in all the solvent mixtures (entries 2, 4 and 6), and accordingly the water contents of the organic solvents (77 ppm for toluene, 321 ppm for DIPE and 622 ppm for TBME) make the difference. BCLxero in toluene and in the solvent mixture caused practically no hydrolysis in the case of (*R*)-**4** (entries 1 and 2) while considerable hydrolysis by the residue water was observed in the case of (*S*)-**6** in the solvent mixture (entry 6). While the acylation of **3** in TBME with vinyl acetate and BCLxero proceeded smoothly without clear indications about hydrolysis of the produced (*S*)-**6** (Table 2, entry 10), the hydrolysis in [EMIM][$BF_4$] (1:2) (Table 4, entry 19) evidently caused the observed difficulties in leading the acylation reaction to 50% conversion and variations in *E* values with time.

## Conclusions

The present work indicates that the nature of the BCL preparation (native BCL-PS, BCLxero and BCL-CLEA in the present work) on its activity is one of the crucial variables when enzymes are used to catalyse organic reactions in the presence of ILs. When these enzyme preparations were applied to the acylation of three different aromatic secondary alcohols (**1–3**) in the most commonly used ionic liquids ([EMIM][$Tf_2N$], [EMIM][$BF_4$] and [BMIM][$PF_6$]) in biocatalysis it was shown that the ILs have a negative influence on the initial rates (activities) of the enzyme preparations compared to the reactions in selected organic solvents or in their mixtures with an IL. While BCL-CLEA displays higher activity (initial rates) than BCLxero for all substrates in the ILs it loses its activity rapidly. In organic solvents and in the ILs, the nature of the solvent affects *E* only with **2** serving as the substrate while $E > 200$ is evident with **1** and **3**. This work reveals that it is not enough to consider activities of the BCL preparations to find the one with highest stability against an IL. Rather the overall conversion and possible side reactions, the tendency for hydrolysis of the ester produced in particular, needs to be taken into consideration. Contrary to earlier results,[42] it is not possible to label one IL better than the other without taking the nature of the substrate and the ester produced into account. Overall, the results show that BCLxero is a good choice, especially in cases where the produced ester is an activated ester and thus susceptible to hydrolysis as a side reaction.

## Experimental

### Materials

Lipase PS "Amano" SD (BCL-PS, from *Burkholderia cepacia*) was purchased from Amano Pharmaceuticals Co., Ltd (Nagoya, Japan). 1-Phenylethanol (98%) and 2-amino-1-phenylethanol (98%) were products of Aldrich and 1-(2-furyl)ethanol (>97%) was from Fluka. Amide **3** was prepared by the reaction of 2-amino-1-phenylethanol with butanoic anhydride (0.95 eqv.). (*R*)-**4**, (*R*)- **5** and (*S*)-**6** were available from our

**Table 6** Conversion (%) after 24 h in the hydrolysis of enantiopure esters **4–6** (0.05 M) by the residual water in the enzyme preparation and the solvent system at room temperature

| Entry | Substrate | Solvent | log *P* | Conv. [%] by BCL-PS[a] | Conv. [%] by BCLxero[b] |
|---|---|---|---|---|---|
| 1 | (*S*)-**4** | Toluene | 2.8 | 4 | 1 |
| 2 | (*S*)-**4** | [EMIM][$BF_4$]:toluene (1:2) | | 27 | 2 |
| 3 | (*S*)-**5** | DIPE | 1.9 | 20 | 10 |
| 4 | (*S*)-**5** | [EMIM][$BF_4$]:DIPE (1:2) | | 22 | 26 |
| 5 | (*R*)-**6**[c] | TBME | 1.35 | 86 | 16 |
| 6 | (*R*)-**6**[c] | [EMIM][$BF_4$]:TBME (1:2) | | 35 | 45 |

[a] 100 mg $mL^{-1}$ of BCL-PS. [b] BCLxero based on 100 mg $mL^{-1}$ of BCL-PS. [c] Butanoate instead of acetate; reaction temperature 48 °C.

previous work.[36] Methyltrimethoxysilane (MTMS, Aldrich, >99%), tetramethoxysilane (TMOS, Fluka, >99%) and glutaraldehyde (Fluka, 25% in water) were used as supplied. [EMIM][$Tf_2N$], [EMIM][$BF_4$] and [BMIM][$PF_6$] were prepared by the methods described in the literature.[36,39] Ethyl bromide (>99%), 1-methylimidazole (>99%), 1-chlorobutane (>99.5%), $LiNTf_2$ (>99%) and sodium fluoroborate (>98%) were products of Fluka. $HPF_6$ (60% in $H_2O$) was from Aldrich. Vinyl acetate and the solvents were of the highest grade from Aldrich, J.T. Baker and Lab-Scan Ltd. Water contents of the neat solvents were measured by Karl Fischer titration and were: toluene 77 ppm, TBME 622 ppm, DIPE 321 ppm, [EMIM][$Tf_2N$] 45 ppm, [EMIM][$BF_4$] 986 ppm and [BMIM][$PF_6$] 486 ppm.

### Analysis

The progress of the reactions was followed by taking samples (50 μL) at intervals and extracting the products into toluene, TBME or DIPE accordingly to organic solvent in the reaction. The samples were derivatized with propionic anhydride in the presence of 4,4-dimethylaminopyridine (DMAP, 1% in pyridine) to achieve a good baseline separation and analyzed by GC equipped with Chrompack CP-Chirasil-DEX CB column (25 m × 0.25 mm) and Chrompack CP-Chirasil-L-valine column. The determination of $E$ was based on equation $E = \ln[(1 - c)(1 - ee_S)]/\ln[(1 - c)(1 + ee_S)]$ with $c = ee_S/(ee_S + ee_P)$ using linear regression ($E$ as the slope of the line $\ln[(1 - c)(1 - ee_S)]$ *versus* $\ln[(1 - c)(1 + ee_S)]$. The protein content of lipase PS-SD powder was determined using bicinchoninic acid assay using bovine serum albumin as the standard protein.

### Encapsulation of BCL in a sol–gel matrix

The sol–gel precursor was prepared according to a literature method.[36,37] Acidic water (1.38 mL, pH= 2.85 by HCl) was added to a mixture of MTMS (2.1 g, 15.4 mmol), TMOS (9.08 g, 58.5 mmol) and distilled water (10.4 mL) and the mixture was stirred until it was homogenous. The formed methanol was removed by evaporizing in a rotary evaporator until the odours of MTMS, TMOS and MeOH were not detectable any longer. The mixture was cooled to 0 °C and water was added until the total volume corresponded to the initial MTMS/TMOS volume. The sol precursor was used immediately for the encapsulation of BCL. BCL-PS powder (containing 3% protein, 100 mg) was dissolved in $KH_2PO_4$-buffer (200 μL, 0.1 M, pH = 7.0). The sol precursor mixture (200 μL) was added and the mixture was stirred magnetically until homogenous, followed by the removal of the stirring bar. The mixture gelled (1–2 min) and the gel was aged at 4 °C for 24 h followed by lyophilization at 0.2 atm for 4 h. The formed xerogel was stored at 4 °C and used as a pellet.

### Preparation of BCL-CLEA

The preparation of CLEA was based on a literature method.[36,38] BCL-PS powder (containing 3% protein, 50 mg) in $KH_2PO_4$ buffer (1 mL, 0.1 M, pH = 7) was added dropwise to saturated $(NH_4)_2SO_4$ (9 mL) at 4 °C. Glutaraldehyde (377 μL, 100 mM, 25% in water) was added and the mixture was stirred at 4 °C for 5 h. The suspension was diluted with 3 mL of the buffer and centrifugated. The pellet was washed two times with the buffer (3 mL) and once with acetonitrile (3 mL). After centrifugation, the obtained CLEA was dried in vacuum and stored at 4 °C. When BSA was used, it was added in $KH_2PO_4$ buffer together with BCL-PS.

### Enzymatic acylation

For enzymatic acylation, an organic solvent (1 mL) or the mixture of ionic liquid, solvent (1 mL) and vinyl acetate (0.2 M) were added to one of the lipase preparations (for BCLxero corresbonds to 100 mg and for BCL-CLEA 50 mg of original BCL-PS powder; with BCL-PS both 50 and 100 mg were used) and the addition of a substrate (0.1 M) started the reaction. The reactions were shaken at room temperature (23 °C) for the reactions of **1** and **2** and at 48 °C for the reaction of **3**.

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