BIO CATALYSIS IN IONIC LIQUIDS

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Abstract:

This review describes the recent developments of enzymatic catalysis in ionic liquids, reporting the use of different biocatalysts in organic synthesis. Several ionic liquids appear as an alternative to conventional organic solvents, providing comparable or higher rates and, in some cases, improved enantios electivity.

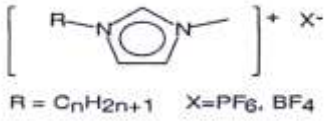
Ionic liquids are receiving increasing attention as solvents for organic synthesis in general and catalytic processes in particular.1,2 This interest stems from their potential as 'green' solvents.† Their non-volatile character and thermal stability makes them potentially attractive alternatives for environment.

† We note, however, that there is very little known with regard to their degradability and aquatic toxicity.

Keywords: Enantios electricity, Green solvents, Hydrophobicity, Transesterification.

Introduction:

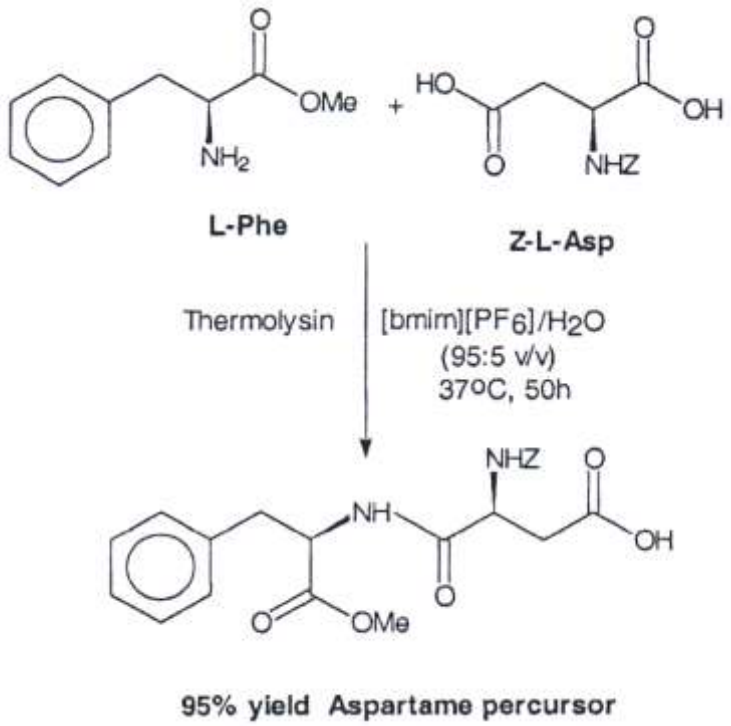
The hydrophobicity /hydrophilicity can be tuned by appropriate modification of the cation or anion, which has earned them the accolade 'designer solvents'.[3] Depending on their structure, they can be immiscible with water or, e.g. alkanes, which renders them useful for performing catalytic reactions in biphasic media, thus facilitating catalyst recovery and recycling. Most studies have involved the use of 1,3-dialkylimidazolium salts, e.g. 1-butyl-3-methylimidazolium tetra fluoroborate, [bmim][BF_4] and hexa fluorophosphate, [bmim][PF_6] which are miscible and immiscible with water, respectively (see Fig. 1).



Recently, the use of ionic liquids as reaction media has been extended to biocatalytic processes. Lye and coworkers[4] reported the use of a biphasic [bmim][PF_6]/H_2O medium for the conversion of 1,3-dicyanobenzene to 3-cyanobenzamide and 3-cyanobenzoic acid catalysed by whole cells of Rhodococcus R312. The ionic liquid acts as a reservoir for the

substrate and product, thereby decreasing substrate and product inhibition observed in water, and, hence, increasing the catalyst productivity. Replacement of toluene by [bmim][PF_6] as the second phase was beneficial as the latter caused less damage to the microbial cells. Similarly, 1-octyl-3-methylimidazolium hexafluorophosphate, [omim][PF6] was used to enhance the recovery of nbutanol from a fermentation broth.[5] In process extraction of nbutanol into the ionic liquid phase followed by recovery from the ionic liquid by pervaporation constitutes an attractive alternative to conventional, energy intensive separation from water by distillation.

The feasibility of using isolated enzymes in ionic liquid media has also been demonstrated. Erbeldinger and coworkers6 reported the thermo lysin-catalysed synthesis of Z-aspartame in [bmim][PF_6]/H_2O (95/5, v/v) as shown in eqn. (1).



(1)

Green Context

The use of enzymes for many reactions is hampered by the need for an aqueous environment, which can lead to side-reactions and other problems. Organic solvents can sometimes be used, but often with significant reduction in efficiency. This short review summarises recent work which indicates that ionic liquids are promising as media for these transformations, allowing high levels of efficiency, good solubility and no volatility.

Reaction rates were comparable to those observed in ethyl acetate/H_2O and the enzyme displayed a higher stability in the ionic liquid medium. It was furthermore shown that the small amount of thermolysin (3.2 mg ml^{-1}) that dissolved in the ionic liquid was not active.

The above mentioned examples all involve bio catalysis in ionic liquid–water mixtures. We were interested to ascertain whether or not an enzyme could function in a water free ionic liquid. I chose lipases for my study since these robust enzymes are known to perform well in essentially anhydrous organic media.

Results and discussion

Lipases in ionic liquids

We showed that Candida antarctica lipase B (CaLB), either as the free enzyme (SP525) or in an immobilised form (Novozym 435), is able to catalyse a variety of transformations in [bmim][BF_4] or [bmim][PF_6] in the absence of added water[7] (the ionic liquid was stored over P_2O_5 and the enzyme was essentially anhydrous). For example, trances terifications (eqn. (2)) proceeded with rates comparable to those observed in tertbutyl alcohol, a commonly employed solvent for such transformations. The immobilised enzyme (Novozym 435) gave higher rates than the free enzyme (SP525) suspended in the ionic liquid. This is consistent with the generally observed higher reactivity of the immobilised lipase, compared to the free enzyme, in organic media.[8]

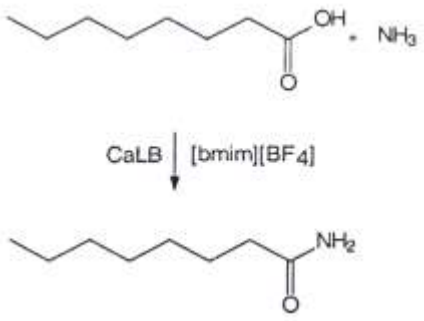
$$CaLB$$

$$R^1CO_2Et + R^2OH \rightleftharpoons R^1CO_2R^2 + EtOH$$

$$[bmin][PF_6]\ or\ [bmin][PF_4]$$
$$40^0C$$

(2)

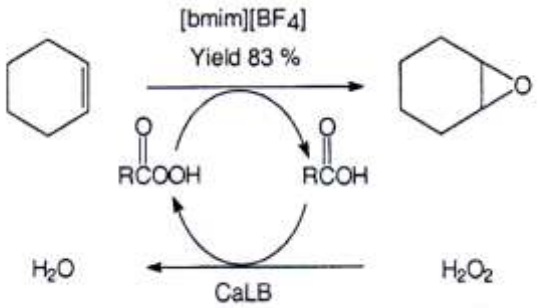
I previously showed[9] that CaLB-catalysed ammoniolysis of carboxylic esters to the corresponding amides occurs smoothly under mild conditions. Primary fatty acid amides, for example, are important commodities that are conventionally prepared by reaction of the ester with ammonia under forcing conditions of temperature and pressure.[10]Heijnen and coworkers[11] subsequently showed that CaLB-catalysed ammoniolysis of carboxylic acids is feasible, albeit with very long reaction times using ammonium carbamate in methyl isobutyl ketone. Hence, we were interested to see if improvements could be achieved in ionic liquid media. Indeed, we found that the reaction of octanoic acid with ammonia (eqn. (3)) in the presence of Novozym 435 at 40 °C in [bmim][BF_4], proceeded to complete conversion in 4 days,[7] compared to 90–100% conversion in 17 days using ammonium carbamate in methylisobutylketone.[11]



(3)

Peroxy carboxylic acids are commonly used oxidants in (industrial) organic synthesis. However, increasing restrictions, with regard to their handling, transport and storage, are making their use prohibitive. Hence, methods for their in situ generation constitute an attractive alternative. It has previously been shown[12] that this is feasible via lipase-catalysed

perhydrolysis of the corresponding carboxylic acid. I have now shown that this reaction can be performed in an ionic liquid.[7] For example, the epoxidation of cyclohexene by peroctanoic acid, generated in situ by Novozym 435-catalysed reaction of octanoic acid with commercially available 60% aqueous hydrogen peroxide in [bmim][BF_4], afforded cyclohexene oxide in 83% yield in 24 h (eqn. (4)). For comparison, a yield of 93% was observed in 24 h in acetonitrile, which was previously shown to be the optimum organic solvent for the reaction.[13]



(4)

Enantioselectivity

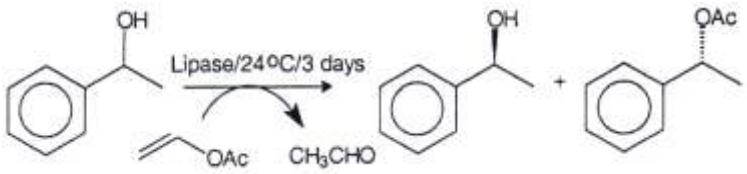
Recently, several groups have investigated lipase-catalysed trances terifications of chiral substrates in ionic liquids. A primary aim of these studies was to ascertain the effect of ionic liquid media on the enantioselectivities of these transformations. For example, Kragl and coworkers[14] investigated the kinetic resolution of 1-phenylethanol (Table 1 eqn. (5)) with nine different lipases in ten different ionic liquids.

Good activities and, in many cases, improved enantioselectivities were observed compared with the same reaction in methyl tert-butyl ether (MTBE). CaLB generally gave the best results and some lipases, e.g. Candida rugosa lipase and Thermomyceslanuginosus lipase, showed almost no activity. The rates and/or enantioselectivities were dependent on both the anion and the alkyl group in the 1-methyl-3-alkylimidazolium cation. In general, the best results were observed in [bmim][CF_3SO_3], [bmim][$(CF_3SO_2)_2N$] and [omim][PF_6]. Surprisingly, virtually no reaction (< 5% conversion) was observed in [bmim][BF_4] and [bmim][PF_6], which is contradictory to what we and others (see later) have observed.

The same resolution of 1-phenylethanol using lipase from Pseudomonas cepacia in several [BF4] ionic liquids was studied by Kazlauskas and coworkers.[15]They observed that the

purification step used in the synthesis of ionic liquid is an important factor in order to obtain higher conversion rates. The use of unpurified ionic liquids resulted in reaction rates at least two to five times slower than in toluene, while after purification of the ionic liquid by method A (addition of silver tetrafluoroborate, removal of silver halide precipitate by filtration, followed by chromatography on silica gel) or B (filtration through silica gel plug, wash with saturated aqueous sodium carbonate) reaction rates increased to a value similar to that in an organic solvent or even higher. The addition of sodium carbonate also gave a dramatic increase in reaction rate. This was attributed to the removal of silver ions and neutralization of acidic impurities in the ionic liquid, which adversely affected enzymatic catalysis.

Table – 1 Lipase medicated enatioselective acylation of 1-phenylethanol.



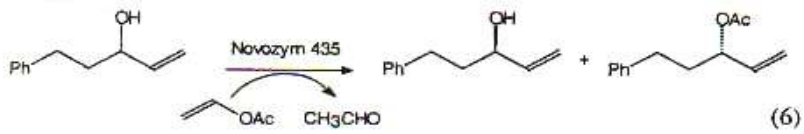
(5)

Comparison of various lipases[14]						
	CaLB		Pseudomonas cepaciasp		Alcaligenes sp.	
Solvent	Conv (%)	ee (%)	Conv (%)	ee (%)	Conv (%)	ee (%)
MTBE	43	> 98	53	84	98	0
[bmim][PF_6]	<5	—	0	—	44	77
[bmim][BF_4]	<5	> 98	7	53	60	81
[hmim][BF_4]	10	> 98	0	—	68	14
[omim][BF_4]	41	> 98	< 5	—	50	> 98
[bmim][CF_3SO_3]	50	> 98	50	> 98	70	82
[bmim][$(CF_3SO_2)_2N$]	50	> 98	47	> 98	89	15

Effects of additives and purification[15]						
Solvent	Purification	Additive	Conv. after 24 h (%)		E	
Toluene	None	None	44		> 200	
DMSO	None	None	0		—	

[bmim][BF_4]	None	None	7.8		> 200	
[bmim][BF_4]	A	None	13		> 200	
[omim][BF_4]	B	None	36		> 200	
[bmim][BF_4]	B	$AgBF_4$	0		—	
[pmim][BF_4]	None	None	0		—	
[pmim][BF_4]	A	None	39		> 200	
[moemim][BF_4]	A	None	0		—	
[moemim][BF_4]	B	None	42		> 200	
[bpyr][BF_4]	A	NaHC O_3	0		—	
[bpyr][BF_4]	A	Na_2CO_3	32		> 200	
bmim = Butylmethylimidazolium; hmim = hexylmethylimidazolium; omim = octylmethylimidazolium; pmim = propylmethylimidazolium; moemim = methoxyethanemethylimidazolium; bpyr = N-butylpyridinium						

Table 2 Lipase mediated enantioselective acylation of 5-phenyl-1-penten-3-ol



(6)

Solvent	Time/h	Yield (%)	(%) Ee	(%) Rate	E
Pr^i_2O	3	45	> 99	17	> 1000
[bmim][PF_6]	5	49	> 99	9.4	> 580
[bmim][PF_4]	3.5	44	> 99	14	> 640
[bmim][TFA]	48	19	91	0.25	227
[bmim][OTf	24	34	> 99	1.8	> 450
[bmim][SbF_6	48	31	> 99	0.8	> 360

Itoh and coworkers[16] similarly observed that the rate of the transesterification of a chiral allylic alcohol (Table 2, eqn. (6)) was strongly dependent on the nature of the anion in [bmim][X]. In contrast, only a minor effect on the enantioselectivity was observed. I note,

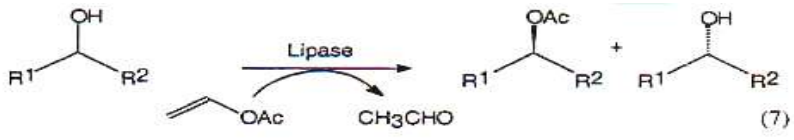
however, that the enantioselectivity wasvery high (E > 200) in all cases which makes it difficult to observe differences. The best results were obtained in [bmim][BF_4] and [bmim][PF_6], in contrast to the results reported (see above) for reaction 5. A comparison of different lipases in [bmim][PF_6] revealed that Novozym 435 gave the highest rate, followed by lipases from Alcaligenes sp. and Pseudomonas cepacia (PCL) while those from Candida rugosa and porcine liver exhibited no activity. It was further shown that the product could be extracted with ether and the ionic liquid, [bmim][PF_6], containing the suspended enzyme, could be recycled (although the activity decreased dramatically after the second recycle).

Kim and coworkers[17] studied the transesterification of several chiral alcohols (Table 3, eqn. (7)) catalysed by CaLB and PCL in [emim][BF_4] and [bmim][PF_6]. Markedly enhanced enantioselectivities were observed compared with the same reactions performed in toluene or THF.

Solubility and stability of enzymes in ionic liquids

The above described results clearly show that enzymes are able to perform in ionic liquid media, even in the absence of water. From both a practical and a theoretical viewpoint it was of interest to ascertain the stabilities of enzymes, in various formulations, in ionic liquids versus (molecular) organic solvents. I was also interested to see if enzymes would dissolve in ionic liquids and retain their activity in dissolved form. To this end I examined the CaLB-catalysed transesterification of racemic phenylglycine methyl ester with ethanol (eqn. (8)).[18]This reaction is not very enantioselective (E = 7 in t-BuOH), which was one of the reasons for choosing it.

Table 3 Lipase mediated enantioselective acylation of secondary alcohols



(7)

R1	R2	Lipase	Solvent	E
CH_3	$PhCH_2CH_2$	CaLB	THF Toluene	141 207

			[emim][BF_4] [bmim]PF_6]	648 > 967
CH_3	$PhCH_2O$- $COCH_2$	CaLB	THF	26
			Toluene [emim][BF_4] [bmim]PF_6]	187 651 155
CH_2Cl	Ph	PCL	THF	56
			Toluene [emim][BF_4] [bmim]PF_6]	158 183 > 450
CH_2Cl	$PhOCH_2$	PCL	THF Toluene [emim][BF_4] [bmim]PF_6]	150 85 172 > 1000

The reaction was studied with four different formulations of CaLB: free enzyme (SP525), immobilised on a support (Nov435), crosslinked enzyme crystals (CLECs)[19] and crosslinked enzyme aggregates (CLEAs).[20] As shown in Table 4 the reaction rates in [bmim][PF_6] and [bmim][CF_3SO_3] were comparable to those observed in tert-butanol. In contrast, no reaction (< 5% conversion) was observed in [bmim][NO_3], [bmim][lactate], [emim][$EtSO_4$] and [$EtNH_3$][NO_3]. I observed similar results in the transesterification of ethyl butyrate with n-butanol in the same solvents. Interestingly, the free enzyme (SP525) dissolved in the ionic liquids in which no activity was observed. However, dissolution as such does not explain the lack of activity since the immobilised preparations were also inactive in these solvents. The ionic liquids in which low activities are observed contain more strongly coordinating anions: lactate, nitrate and ethylsulfate. I suggest that a plausible explanation for this is that coordination of these anions to the enzyme surface, be it in the free or immobilised form, causes conformational changes in the enzyme, leading to a loss of activity.

I studied the effect of dissolving enzymes in ionic liquids on their subsequent hydrolytic activity. CaLB lipase was dissolved in [bmim][NO_3], [bmim][lactate], [emim][$EtSO_4$] and

[$EtNH_3$][NO_3] and the solution allowed to stand for 24 h at 40 °C. The solution was then diluted with a large excess of water forming an homogeneous system and the activity of the enzyme was determined in the hydrolysis of triacetin. In all cases I observed a substantial recovery of activity, varying from 33% in [EtNH3][NO3] to 73% in [bmim][NO_3]. Taken together with the observed lack of transesterification in these solvents these results suggest that the enzyme denatures (unfolds) on dissolving in the ionic liquid and that on the addition of water it refolds into its active form. It is known[21] that denaturated enzymes are able to refold when put into an aqueous medium. An alternative explanation is that the loss of activity on dissolution is caused by strong coordination of the anions to the enzyme (see above). Investigations are underway, e.g. using circular dichroism, to confirm whether or not the enzyme unfolds on dissolution in an ionic liquid and on methods to stabilise the enzyme in solution.

It is also evident from Table 4 that both the nature of the ionic liquid and the form of the enzyme have an influence on the enantioselectivity of the reaction. However, the enantioselectivity was modest at best (E = 9) and the hoped-for dramatic improvements were not observed.

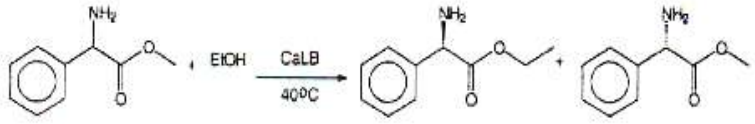
Thermal stability of CaLB in ionic liquids

I studied the thermal stability of different CaLB preparations by suspending them in an organic solvent or [bmim][PF_6], allowing the mixture to stand at 80 °C and taking aliquots at various time intervals and measuring the residual activity, after dilution with water, in triacetin hydrolysis.

Incubation of free enzyme (SP 525) in [bmim][PF_6] at 80 °C resulted in an increase in activity. After an incubation time of 20 h, the maximum activity found was 120% of the activity of the native untreated enzyme. This activity value did not decrease up to an incubation time of 100 h, while in Bu^tOH a nearly linear deactivation in time was observed. Incubation of Novozym 435 in [bmim][PF_6] at 80 °C showed a maximum activity of 350% compared to the activity of the untreated SP 435 after an incubation time of 40 h. After 5 days this preparation still exhibited 210% of the initial activity. Here again, incubation in Bu^tOH showed a linear deactivation in time. Recently, Iborra and coworkers[22] have similarly

reported a stabilising effect of ionic liquids on CaLB. Interestingly, they found that the halflife of the enzyme was three orders of magnitude greater when it was incubated in the presence versus in the absence of the substrate. In their study experiments were performed in the presence of 2% (v/v) water.

Table 4 Transesterification of phenylglycine methyl ester with ethanol by different preparations of CaLB



(8)

	Novozym 435		SP525		CLEA		CLEC	
	Conv. after		Conv. after		Conv. After		Conv. After	
Solvent/CaLBpreparation	24 h (%)	E	24 h (%)	E	24 h (%)	E	24 h (%)	E
[bmim][BF4]	42	1	36	9	24	9	50	2
[bmim][PF6]	39	1	32	9	47	4	40	1
[bmim][OTf]	41	1	44	3	—	—	44	1
Bu^t OH	46	1	40	4	52	7	50	1
[bmim][NO3]	< 5	—	< 5	—	< 5	—	< 5	—
[bmim][Lactate]	< 5	—	—	—	—	—	—	—
[emim][EtSO4]	< 5	—	—	—	—	—	—	—
[EtNH3][NO3	< 5	—	< 5	—	< 5	—	—	—

In contrast to the results obtained with free CaLB and Novozyme 435, no stabilising effect was observed when a CLEC or CLEA from CaLB was incubated in [bmim][PF6] at 80°C. A decrease in activity was observed in both the ionic liquid and tert-butyl alcohol which was comparable to that found for CaLB and Novozyme 435 in tert-butyl alcohol. The stabilising

effect of the ionic liquid on the free and supported enzyme possibly results from a protecting action of a coating of ionic liquid on the microenvironment (hydration layer) of the enzyme. The reason for the lack of a stabilising effect on the CLEA and CLEC preparations is not clear and forms the subject of further investigations.

Concluding remarks & future prospects

As the preceding discussion hopefully has shown, performing biocatalytic conversions in ionic liquids can be beneficial with regard to activity, (enantio)selectivity and stability. Indeed, the use of enzymes in ionic liquids opens up new possibilities for non-aqueous enzymology. Ionic liquids could have added benefits for performing biotransformations with highly polar substrates, e.g. carbohydrates23 and amino acids, which are sparingly soluble in most organic solvents. I am currently investigating the scope with regard to type of enzyme and biotransformation and the origins of the observed dependence of activity and stability of the enzyme on the structure (both cation and anion) of the ionic liquid and will report my results in due course.

Acknowledgments

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References

1. M. J. Earle and K. R. Seddon, Pure Appl. Chem., 2000, 72, 1391–1398; T. Welton, Chem. Rev., 1999, 99, 2071–2084; J. D. Holbrey and K. R. Seddon, Clean Prod. Process, 1999, 1, 223–236.
2. Y. Chauvin and H. Olivier-Bourbigou, CHEMTECH, 1995, 26–30; P. Wasserscheid and W. Keim, Angew. Chem., Int. Ed., 2000, 39, 3772–3789; R. A. Sheldon, Chem. Commun., 2001, 2399–2407.
3. M. Fremantle, Chem. Eng. News, March 30, 1998, p. 32; M. Fremantle, Chem. Eng. News, May 15, 2000, p. 37; M. Fremantle, Chem. Eng. News, January 1, 2001, p. 21; H. Carmichael, Chem. Br., January 2000, p. 36.

4. S. G. Cull, J. D. Holbrey, V. Vargas-Mora, K. R. Seddon and G. J. Lye, Biotechnol. Bioeng., 2000, 69, 227–233.
5. (a) A. G. Fadeev and M. M. Meagher, Chem. Commun., 2001, 295–296; (b) M. Freemantle, Chem. Eng. News, April 2, 2001, p. 57.
6. M. Erbeldinger, M. Mesiano and A. J. Russell, Biotechnol. Prog., 2000, 16, 1129–1131.
7. R. Madeira Lau, F. van Rantwijk, K. R. Seddon and R. A. Sheldon, Org. Lett., 2000, 2, 26, 4189–4191.
8. F. van Rantwijk, A. C. Kock-van Dalen and R. A. Sheldon, in Stability and stabilisation of enzymes, ed. A. Ballasteros, F. J. Plou, P. Iborra and P.Halling, Progress in Biotechnology, Elsevier Science, Amsterdam, 1998, vol. 15, pp. 447–452.
9. M. C. de Zoete, A. C. Kock-Van Dalen, F. van Rantwijk and R. A. Sheldon, Biocatalysis, 1995, 10, 307–316; M. C. de Zoete, A. C. Kock-van Dalen, F. van Rantwijk and R. A. Sheldon, J. Chem. Soc., Chem. Commun., 1993, 1832–1832.
10. R. Opshal, Fatty acid amides, ed. J. I. Koschwitz and M. Howe-Grant, Kirk-OthmerEncyclopedia of Chemical Technology, Wiley, New York, 1992, vol. 2, pp. 346–356.
11. M. J. Litjens, A. J. J. Straathof, J. A. Jongejan and J. J. Heijnen, Chem. Commun., 1999, 1255–1256.
12. F. Björkling, S. E. Godtfredsen and O. Kirk, J. Chem. Soc., Chem. Commun., 1990, 1301–1303; F. Björkling, H. Frykman, S. E. Godtfredsen and O. Kirk, Tetrahedron, 1992, 48, 4587–4592.
13. M. C. de Zoete, PhD thesis, Delft University of Technology, Delft, The Netherlands, 1995.
14. S. H. Schöfer, N. Kaftzik, P. Wasserscheid and U. Kragl, Chem. Commun., 2001, 425–426.
15. S. Park and R. J. Kazlauskas, J. Org. Chem., 2001, 66, 8395–8401.
16. T. Itoh, E. Akasaki, K. Kudo and S. Shirakami, Chem. Lett., 2001, 262–263.
17. K.-W. Kim, B. Song, M.-Y. Choi and M.-J. Kim, Org. Lett., 2001, 3, 10, 1507–1509.
18. R. Madeira Lau, M. J. Sorgedrager, F. van Rantwijk and R. A. Sheldon, manuscript in preparation.

19. (a) St. Clair, L. Nancy and M. Navia, J. Am. Chem. Soc., 1992, 114, 18, 7314–7316; (b) A. L. Margolin and M. Navia, Angew. Chem., Int. Ed., 2001, 40, 2204–2222; (c) A. L. Margolin, Trends Biotechnol., 1996, 14, 7, 219–259.
20. (a) L. Cao, L. M. van Langen, F. van Rantwijk and R. A. Sheldon, J. Mol. Catal. B: Enzym., 2001, 11, 665–670; (b) L. Cao, F. van Rantwijk and R. A. Sheldon, Org. Lett., 2000, 2, 1361–1364.
21. J. M. Guisan, R. Fernandez-Lafuente, A. Bastide, R. M. Blanco, G. Soler and E. Garcia-Junceda, in Engineering of/with Lipases, ed. F. X. Malcata, Kluwer, Dordrecht, 1996, pp. 257–271.
22. P. Lozano, T. De Diego, D. Carnie, M. Vaultier and J. L. Iborra, Biotechnol.Lett., 2001, 23, 1529–1533.
23. The use of a glycosidase in ionic liquid/water mixtures has recently been reported: N. Kaftzik, P. Wasserscheid and U. Kragl, Poster presented at Enzyme Engineering XVI, Potsdam, October, 2001.