Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 2959–2963

The first hydroxynitrile lyase catalysed cyanohydrin formation in ionic liquids

Richard P. Gaisberger, Martin H. Fechter and Herfried Griengl*

Institute of Organic Chemistry, Graz University of Technology, Research Centre Applied Biocatalysis, Stremayrgasse 16, A-8010 Graz, Austria

Received 26 May 2004; accepted 10 June 2004
Available online 3 August 2004

Abstract—Benzaldehyde, decanal, undecanal and dodecanal were reacted with hydrogen cyanide in a two phase solvent system aqueous buffer and ionic liquids EMIM·BF_4, PMIM·BF_4 and BMIM·BF_4 in the presence of the hydroxynitrile lyases from *Prunus amygdalus* and *Hevea brasiliensis*. When compared to the use of organic solvents as the nonaqueous phase, the reaction rate was significantly increased whereas the enantioselectivity remained good.

1. Introduction

Cyanohydrins are easily available by HCN addition to aldehydes or ketones[1,2] with several biocatalytic approaches to this class of compounds already described and reviewed.[3–6] The catalytic application of hydroxynitrile lyases (HNLs) has been investigated for more than forty years.[7] In particular the (*R*)-selective HNL of *Prunus amygdalus* (*Pa*HNL, E.C. 4.1.2.10) has been used over a long period as a defatted almond meal[8] or purified enzyme preparations adsorbed on various carriers.[9] This *Pa*HNL has been extensively investigated and has recently been successfully overexpressed in *Pichia pastoris*.[10] The (*S*)-selective enzyme isolated from *Hevea brasiliensis* (*Hb*HNL, E.C. 4.1.2.39) and also overexpressed in Graz[11,12] shows a comparably high stereoselection towards various substrates.

The enzyme-catalysed cyanohydrin formation can be performed in aqueous solution with an appropriate acidic component and alkali cyanide for in situ development of the required HCN.[13] A significant advancement in cyanohydrin production was made by performing the transformation with immobilised HNL in organic solvents, which are immiscible with water. It has been observed that there is virtually no spontaneous chemical addition of HCN to the carbonyl moiety.[8,9,14–23] Biphasic solvent mixtures (buffer/ether) were reported employing both (*R*)-[24,25] and (*S*)-HNL.[26–28]

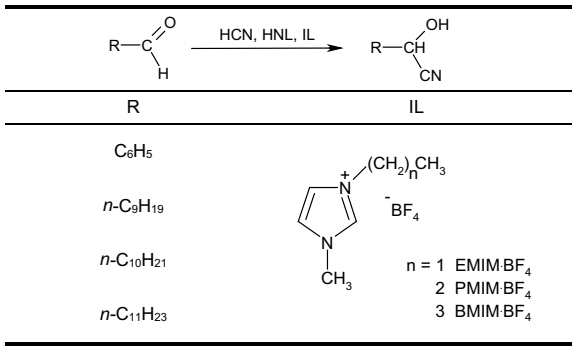
Ionic liquids (ILs) are generally considered to be highly polar and have been used to a large extent as solvents for chemical transformations and for enzyme-catalysed reactions as well.[29–33] Initially we were unsuccessful; the first cyanide additions to benzaldehyde in IL catalysed by immobilised HNL only gave racemic mandelonitrile. These reactions were performed in pure ILs as described for lipase catalysed conversions.[34,35] However, on the basis of results of Kragl et al.[36] with IL/water-mixtures, the application of this methodology to enzyme catalysed cyanohydrin reactions using both *Pa*HNL and *Hb*HNL in a two phase system IL/aqueous buffer was successful as will be reported below. Recently, ILs have also been applied as solvents for the enantioselective synthesis of cyanohydrins, in this case catalysed by VO(Salen) complexes.[37]

2. Results and discussion

To evaluate the methodology first, benzaldehyde was used as the substrate (Scheme 1). Initial experiments with *Pa*HNL and *Hb*HNL immobilised on Celite in neat IL containing 1% of water, which is necessary for enzyme activity, showed no promising results. In all of the three ILs used, only racemic mandelonitrile was

* Corresponding author. Tel.: +43-316-873-8240; fax: +43-316-873-8740; e-mail: sekretariat@orgc.tu-graz.ac.at

0957-4166/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2004.06.028



Scheme 1. *Pa*HNL and *Hb*HNL catalysed cyanohydrin reactions using EMIM·BF_4, PMIM·BF_4, BMIM·BF_4, respectively.

formed after 2 h of reaction. Conversely, when toluene was used instead of the ILs under the same conditions, 87% and 80% ee were obtained (Table 1).

Table 1. Conversion and ee of mandelonitrile after 2 h reaction at room temperature at pH 7 (HNL immobilised on Celite, 2000 U/mmol aldehyde)

Entry	HNL	Solvent	Conv. (%)[a]	Ee (%)[a]
1	*Hb*HNL	Toluene	82	80
2	*Hb*HNL	EMIM·BF_4	95	*rac*
3	*Hb*HNL	PMIM·BF_4	64	*rac*
4	*Hb*HNL	BMIM·BF_4	88	*rac*
5	*Pa*HNL	Toluene	56	87
6	*Pa*HNL	EMIM·BF_4	96	*rac*
7	*Pa*HNL	PMIM·BF_4	95	*rac*
8	*Pa*HNL	BMIM·BF_4	97	*rac*

[a] Determined by chiral GC after acetylation.

A change of the solvent system to IL/aqueous buffer 1:1 vol/vol lead to positive results (Table 2). It can be seen, that for both *Pa*HNL and *Hb*HNL excellent conversion rates and enantiomeric excesses were achieved after 1 h reaction time. Since *Hb*HNL is not as stable at lower pHs as *Pa*HNL,[10] the former reaction was run at pH 4.8. When performing enzyme catalysed cyanohydrin reactions in solvent systems containing an aqueous buffer, the nonenzymatic normal cyanohydrin formation has to be taken into consideration. Usually, this reaction is slow at low pH-values. However, both the enzymatic and the nonenzymatic transformation is accelerated by ILs, which results in a drop of the ee in case of *Hb*HNL.

Table 2. Conversion and ee of mandelonitrile after 1 h reaction at room temperature in ionic liquid/aqueous reaction media (2000 U HNL/mmol aldehyde)

Entry	HNL	Solvent	Conv. (%)[a]	Ee (%)[a]
1	*Hb*HNL[b]	EMIM·BF_4	98	80
2	*Hb*HNL[b]	PMIM·BF_4	86	74
3	*Hb*HNL[b]	BMIM·BF_4	98	24
4	*Pa*HNL[c]	EMIM·BF_4	98	93
5	*Pa*HNL[c]	PMIM·BF_4	95	96
6	*Pa*HNL[c]	BMIM·BF_4	97	97

[a] Determined by chiral GC after acetylation.
[b] Reaction done at pH 4.8.
[c] Reaction done at pH 3.7.

Figures 1 and 2 show the increased rate of the cyanohydrin formation with the ionic liquid PMIM·BF_4 as the co-solvent when compared to the well investigated *tert*-butylmethyl ether (TBME)/aqueous conditions.

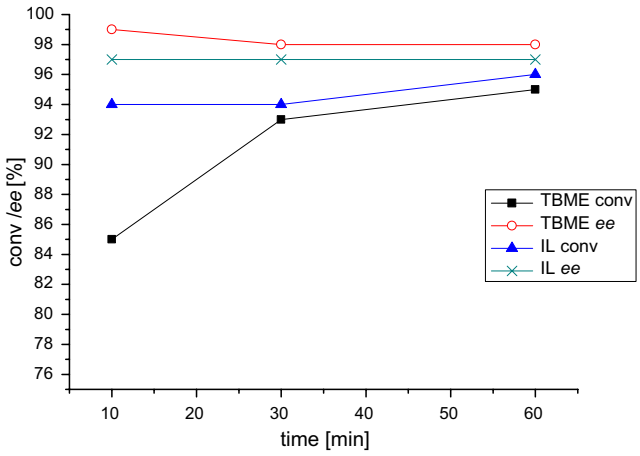


Figure 1. (*S*)-*Hb*HNL catalysed mandelonitrile formation in TBME/buffer = 1/1 and PMIM·BF_4/buffer = 1/1.

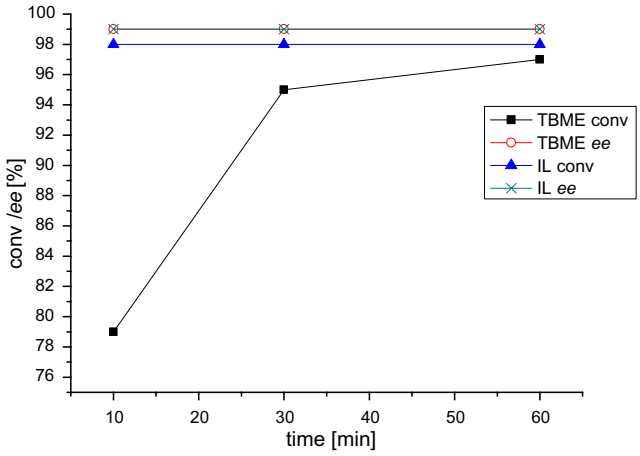


Figure 2. (*R*)-*Pa*HNL catalysed mandelonitrile formation in TBME/buffer = 1/1 and PMIM·BF_4/buffer = 1/1.

With this result in mind, we decided to investigate substrates for the hydroxynitrile lyase catalysed cyanohydrin formation where very long reaction times were necessary to obtain satisfactory conversion and enantioselectivity[38,39] when using the standard solvent mixture of organic solvent/aqueous buffer. Thus, we examined the reaction of long chain aldehydes, such as decanal, undecanal and dodecanal, with prussic acid using *Hb*HNL and *Pa*HNL as biocatalysts (Scheme 1).

As shown in Figure 3, after only 1.5 h the conversion of all three aldehydes was very much higher with PMIM·BF_4 when compared to TBME. The lower ee-values can be explained by the concurring chemical reaction, as mentioned. Excellent results were achieved using *Pa*HNL at pH 3.7 (Fig. 4).

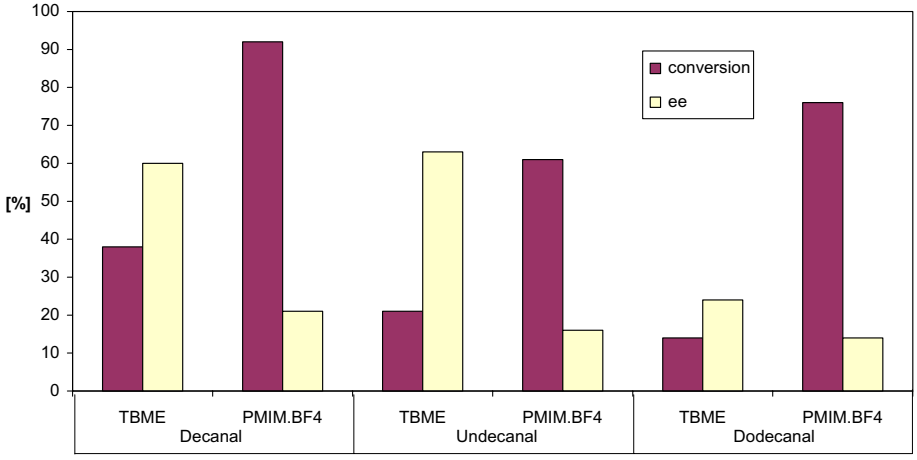


Figure 3. (*S*)-*Hb*HNL catalysed cyanohydrin formation of long-chain aldehydes in TBME/buffer = 1/1 and PMIM·BF_4/buffer = 1/1 after 1.5 h.

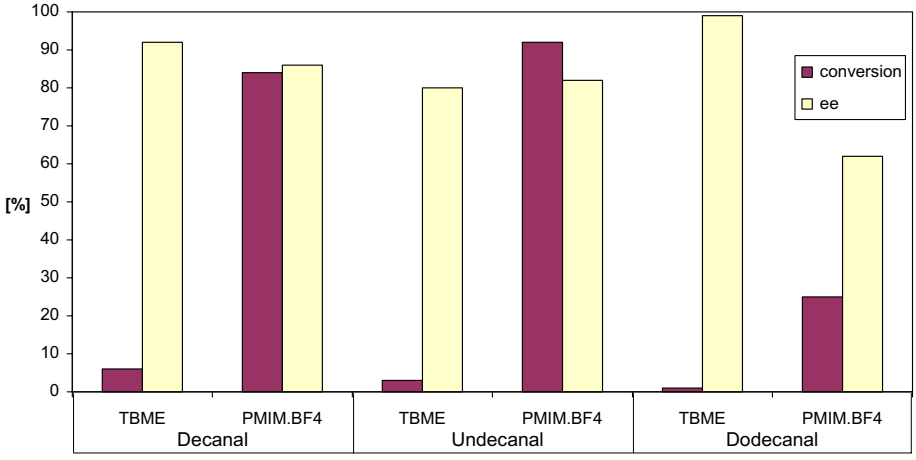


Figure 4. (*R*)-*Pa*HNL catalysed cyanohydrin formation of long-chain aldehydes in TBME/buffer = 1/1 and PMIM·BF_4/buffer = 1/1 after 1.5 h.

Here the chemical side reaction is suppressed and the cyanohydrins formed with very high enantiomeric excess and high conversion after only 1.5 h in IL/aqueous buffer. At the same time hardly any conversion was detected using TBME/aqueous buffer.

3. Conclusions

As shown, the rate of the enzyme catalysed cyanohydrin reaction in a biphasic system IL/aqueous buffer is greatly enhanced. This opens up the possibility of achieving acceptable transformation times where the normal protocol using either buffer alone or together with a water immiscible organic solvent does not lead to satisfying results.

4. Experimental

4.1. Materials and methods

All solvents and materials were commercially available and purified if necessary. *Hb*HNL and *Pa*HNL were a kind gift of DSM Fine Chemicals Austria. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 500 (^{1}H 499.82 MHz, ^{13}C 125.69 MHz) with TMS as an internal reference. Enantiomeric purities and conversions were analysed using a Hewlett–Packard 6890 instrument equipped with an FID and a Chirasil-DEX CB column (25 m × 0.32 mm, 0.25 μm film). The cyanohydrins were acetylated using standard methods prior to analysis. For analytical data vide infra.

4.2. HCN formation–caution

All reaction equipment in which cyanides were used or produced were placed in a well ventilated hood. The required amount of HCN was freshly formed by dropping a saturated NaCN solution into aqueous sulfuric acid (60%) at 80 °C and trapping HCN at −12 °C in a cooling trap. For continuous warning, an electrochemical sensor for HCN detection was used. Waste solutions containing cyanides were treated with aqueous sodium hypochlorite (10%). Subsequently, the pH was adjusted to 7.0 with aqueous sulfuric acid.

4.3. Synthesis of ionic liquids

EMIM·BF_4, PMIM·BF_4, BMIM·BF_4 were prepared and purified according to literature procedures and the ^{1}H NMR data were consistent with those reported.[35]

EMIM·BF_4 ^{13}C NMR (acetone-d_6): δ 137.31; 124.69; 123.02; 45.62; 36.48; 15.62.

PMIM·BF_4 ^{13}C NMR (acetone-d_6): δ 136.93; 124.10; 122.71; 51.17; 35.90; 23.51; 10.14.

BMIM·BF_4 ^{13}C NMR (acetone-d_6): δ 136.93; 124.09; 122.71; 49.48; 35.90; 32.11; 19.31; 13.04.

4.4. General procedure for the synthesis of racemic cyanohydrins

To a solution of aldehyde (5%) in TBME, weakly basic ion-exchange resin (Amberlyst A-21) and freshly generated HCN (5 equiv) were added. The reaction mixture was stirred at room temperature, and after quantitative conversion (1–24 h), the mixture was filtered over a bed of Na_2SO_4. Evaporation of the solvent yielded the crude cyanohydrins. Structures were proven by NMR analysis.

4.4.1. (*RS*)-Mandelonitrile. GC 1 bar H_2; 60 °C, 2 min, 10 °C/min, 140 °C, 2 min; 5.2 min (aldehyde), 9.8 min (*R*), 10.7 min (*S*).

4.4.2. (*RS*)-2-Hydroxy-undecanenitrile. NMR data were consistent with those reported.[40] GC 1 bar H_2; 120 °C, 2 min, 5 °C/min, 160 °C, 2 min; 2.1 min (aldehyde), 7.8 min (*R*), 8.7 min (*S*).

4.4.3. (*RS*)-2-Hydroxy-dodecanenitrile. ^{1}H NMR data slightly different to literature.[41] ^{1}H NMR ($CDCl_3$): δ 4.40 (1H, t); 2.83 (1H, br s); 1.77 (2H, m); 1.42 (2H, m); 1.24 (14H, m); 0.81 (3H, t). ^{13}C NMR ($CDCl_3$): δ 120.31; 61.58; 35.42; 32.12; 29.79; 29.71; 29.58; 29.54; 29.16; 24.77; 22.92; 14.36. GC 1 bar H_2; 120 °C, 2 min, 5 °C/min, 160 °C, 2 min; 3.2 min (aldehyde), 9.6 min (*R*), 10.3 min (*S*).

4.4.4. (*RS*)-2-Hydroxy-tridecanenitrile. ^{1}H NMR ($CDCl_3$): δ 4.40 (1H, t); 2.83 (1H, br s); 1.77 (2H, m); 1.42 (2H, m); 1.24 (14H, m); 0.81 (3H, t). ^{13}C NMR ($CDCl_3$): δ 120.31; 61.58; 35.42; 32.12; 29.79; 29.71; 29.58; 29.54; 29.16; 24.77; 22.92; 14.36. GC 1 bar H_2; 120 °C, 2 min, 5 °C/min, 160 °C, 2 min; 4.7 min (aldehyde), 11.5 min (*R*), 12.4 min (*S*).

4.5. HNL catalysed cyanohydrin formation

4.5.1. Enantioselective mandelonitrile formation in organic solvent. *Hb*HNL and *Pa*HNL were immobilised on Celite® according to literature procedure[42] (5.1 U/mg carrier). To a suspension of 40 mg of immobilised enzyme in 990 μL of organic solvent, respectively, IL and 10 μL of phosphate buffer [pH 7.0, 1% (v/v)] 10 μL of benzaldehyde (0.10 mmol) and 19 μL of HCN (0.49 mmol) were added. The reaction mixture was stirred on a Thermomixer (1400 rpm) at 24 °C. After 2 h of reaction, the conversion and enantiomeric excess were analysed by GC.

4.5.2. Enantioselective mandelonitrile formation in ionic liquid/aqueous buffer solvent system. (*S*)-*Hb*HNL solution (700 μL, 2965 U/mL) was diluted with 600 μL of sodium citrate buffer (pH 4.8, 100 mM) and the pH value adjusted by the addition of a citric acid solution to exactly 4.8, which gave enzyme solution 1. 11.5 mL of (*R*)-*Pa*HNL solution (175 U/mL) were freeze dried and dissolved in 1300 μL of sodium citrate buffer (pH 3.7, 100 mM), which gave enzyme solution 2. The corresponding enzyme mixtures were then added to a solution of 100 μL of benzaldehyde (0.99 mmol) in 1300 μL of ionic liquid and vigorously stirred for 20 min. After addition of 200 μL of freshly prepared HCN (5.17 mmol), stirring at room temperature was continued for 1h, after which conversion and enantiomeric excess were analysed by GC.

4.5.3. Enantioselective mandelonitrile formation in PMIM·BF_4/aqueous buffer and TBME/buffer solvent system. (*S*)-*Hb*HNL solution (34 μL, 2965 U/mL) was diluted with 1266 μL of sodium citrate buffer (pH 4.8, 100 mM) and the pH value adjusted by the addition of a citric acid solution to exactly 4.8, which gave enzyme solution 1. 573 μL of (*R*)-*Pa*HNL solution (175 U/mL) was diluted with 727 μL of sodium citrate buffer (pH 3.7, 100 mM) and the pH value adjusted again to exactly 3.7, which gave enzyme solution 2. The corresponding enzyme mixtures were then added to a solution of 100 μL of benzaldehyde (0.99 mmol) in 1300 μL of TBME or PMIM·BF_4 and vigorously stirred for 20 min. After addition of 100 μL of freshly prepared HCN (2.59 mmol), stirring at room temperature was continued. The course of the reaction was followed by GC after acetylation.

4.5.4. Enantioselective cyanohydrin formation of long chain aldehydes in PMIM·BF_4/aqueous buffer and TBME/buffer solvent system. (*S*)-*Hb*HNL solution (675 μL, 2965 U/mL) was diluted with 625 μL of sodium citrate buffer (pH 4.8, 100 mM) and the pH of 4.8 adjusted by the addition of a citric acid solution (enzyme solution 1). (*R*)-*Pa*HNL solution (11.5 mL, 175 U/mL) were freeze dried and dissolved in 1300 μL of sodium citrate buffer (pH 3.7, 100 mM), which gave enzyme solution 2. The corresponding enzyme mixtures were then added to a solution of decanal, undecanal and dodecanal (1.0 mmol) in 1300 μL of TBME or PMIM·BF_4, and this was cooled to 0 °C. After vigorous stirring for 20 min, freshly prepared HCN (5.17 mmol) was added. After 1 h of reaction time, the conversion and enantiomeric purity were analysed by GC.

Acknowledgements

Financial support by DSM Fine Chemicals Austria is gratefully acknowledged. Research Centre Applied Biocatalyis is funded by TIG, Technology Impulse GmbH, Vienna, the Province of Styria and the City of Graz.

References

1. Gregory, R. J. H. *Chem. Rev. (Washington, DC)* **1999**, *99*, 3649–3682.
2. North, M. *Tetrahedron: Asymmetry* **2003**, *14*, 147–176.
3. Brussee, J.; van der Gen, A. Biocatalysis in the Enantioselective Formation of Chiral Cyanohydrins, Valuable Building Blocks in Organic Synthesis. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker: New York/Basel, 2000, pp 289–320.
4. van der Gen, A.; Brussee, J. Stereoselective Biocatalytic Formation of Cyanohydrins, Versatile Building Blocks for Organic Synthesis. In *Enzymes in Action*; Zwanenburg, B., Mikolajczyk, M., Kielbasinski, P., Eds.; Kluwer Academic: Netherlands, 2000, pp 365–396.
5. Effenberger, F. Hydroxynitrile Lyases in Stereoselective Synthesis. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker: New York/Basel, 2000, pp 321–342.
6. Fechter, M. H.; Griengl, H. Enzymatic Synthesis of Cyanohydrins. In *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002, pp 974–989.
7. Becker, W.; Benthin, U.; Eschenhof, E.; Pfeil, E. *Angew. Chem.* **1963**, *75*, 93.
8. Zandbergen, P.; van der Linden, J.; Brussee, J.; van der Gen, A. *Synth. Commun.* **1991**, *21*, 1387–1391.
9. Ziegler, T.; Hoersch, B.; Effenberger, F. *Synthesis* **1990**, 575–578.
10. Glieder, A.; Weis, R.; Skranc, W.; Poechlauer, P.; Dreveny, I.; Majer, S.; Wubbolts, M.; Schwab, H.; Gruber, K. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 4815–4818.
11. Hasslacher, M.; Schall, M.; Hayn, M.; Bona, R.; Rumbold, K.; Luckl, J.; Griengl, H.; Kohlwein, S. D.; Schwab, H. *Protein Expr. Purif.* **1997**, *11*, 61–71.
12. Hasslacher, M.; Schall, M.; Hayn, M.; Griengl, H.; Kohlwein, S. D.; Schwab, H. *J. Biol. Chem.* **1996**, *271*, 5884–5891.
13. Schmidt, M.; Hervé, S.; Klempier, N.; Griengl, H. *Tetrahedron* **1996**, *52*, 7833–7840.
14. Effenberger, F.; Ziegler, T.; Foerster, S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 458–460.
15. Wehtje, E.; Adlercreutz, P.; Mattiasson, B. *Biotechnol. Bioeng.* **1990**, *36*, 39–46.
16. Effenberger, F.; Förster, S.; Hörsch, B.; Ziegler, T. *Biochem. Eng. Stuttgart, [Proc. Int. Symp.]* **1991**, *2*, 134–136.
17. Effenberger, F.; Hoersch, B.; Weingart, F.; Ziegler, T.; Kuehner, S. *Tetrahedron Lett.* **1991**, *32*, 2605–2608.
18. Wehtje, E.; Adlercreutz, P.; Mattiasson, B. *Biotechnol. Bioeng.* **1993**, *41*, 171–178.
19. Van den Nieuwendijk, A. M. C. H.; Warmerdam, E. G. J. C.; Brussee, J.; van der Gen, A. *Tetrahedron: Asymmetry* **1995**, *6*, 801–806.
20. Wajant, H.; Foerster, S.; Sprauer, A.; Effenberger, F.; Pfizenmaier, K. *Ann N.Y. Acad. Sci.* **1996**, *799*, 771–776.
21. Costes, D.; Wehtje, E.; Adlercreutz, P. *Enzyme Microb. Technol.* **1999**, *25*, 384–391.
22. Gotor, V. *Molecules* **2000**, *5*, 290–292.
23. Hickel, A.; Radke, C. J.; Blanch, H. W. *Biotechnol. Bioeng.* **2001**, *74*, 18–28.
24. Loos, W. T.; Geluk, H. W.; Ruijken, M. M. A.; Kruse, C. G.; Brussee, J.; van der Gen, A. *Biocatal. Biotransform.* **1995**, *12*, 255–266.
25. Effenberger, F.; Schwaemmle, A. *Biocatal. Biotransform.* **1997**, *14*, 167–179.
26. Griengl, H.; Hickel, A.; Johnson, D. V.; Kratky, C.; Schmidt, M.; Schwab, H. *Chem. Commun. (Cambridge)*, **1997**, 1933–1940.
27. Griengl, H.; Klempier, N.; Pöchlauer, P.; Schmidt, M.; Shi, N.; Zabelinskaja-Mackova, A. A. *Tetrahedron* **1998**, *54*, 14477–14486.
28. Fröhlich, R. F. G.; Zabelinskaja-Mackova, A. A.; Fechter, M. H.; Griengl, H. *Tetrahedron: Asymmetry* **2003**, *14*, 355–362.
29. Kragl, U.; Eckstein, M.; Kaftzik, N. *Curr. Opin. Biotechnol.* **2002**, *13*, 565–571.
30. Park, S.; Kazlauskas, R. *J. Curr. Opin. Biotechnol.* **2003**, *14*, 432–437.
31. Sheldon, R. A.; Lau, R. M.; Sorgetrager, M. J.; van Rantwijk, F.; Seddon, K. R. *Green Chem.* **2002**, *4*, 147–151.
32. Van Rantwijk, F.; Lau, R. M.; Sheldon, R. A. *Trends Biotechnol.* **2003**, *21*, 131–138.
33. Park, S.; Viklund, F.; Hult, K. *ACS Symp. Ser.* **2003**, *856*, 225–238.
34. Suarez, P. A. Z.; Dullius, J. E. L.; Einloft, S.; De Souza, R. F.; Dupont, J. *Polyhedron* **1996**, *15*, 1217–1219.
35. Park, S.; Kazlauskas, R. J. *J. Org. Chem.* **2001**, *66*, 8395–8401.
36. Kragl, U.; Eckstein, M.; Kaftzik, N. In *Ionic Liquids in Synthesis*; Wasserscheid, P., Welton, T., Eds.; Wiley-VCH: Weinheim, 2003, pp 336–347.
37. Baleizao, C.; Gigante, B.; Garcia, H.; Corma, A. *Green Chem.* **2002**, *4*, 272–274.
38. Huuhtanen, T. T.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1992**, *3*, 1223–1226.
39. Kiljunen, E.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1997**, *8*, 1225–1234.
40. Miura, T.; Masaki, Y. *J. Chem. Soc., Perkin Trans.* **1995**, *17*(1), 2155–2158.
41. Noe, C. R.; Knollmüller, M.; Kürner, H.; Steinbauer, G.; Koberg, H.; Gärtner, P. *Monatsh. Chem.* **1991**, *122*, 101–110.
42. Purkarthofer, T.; Skranc, W.; Weber, H.; Griengl, H.; Wubbolts, M.; Scholz, G.; Pöchlauer, P. *Tetrahedron* **2004**, *60*, 735–739.