Fluorinated Solvents

Biotransformations in Low-Boiling Hydrofluorocarbon Solvents**

*Simon Saul, Stuart Corr, and Jason Micklefield**

The scope of biotransformations, particularly in the preparation of homochiral precursors, has been considerably extended through the use of enzymes in organic solvents.[1] Some of the advantages of organic solvents include ease of recovery of products and enzymes, increased solubility and rate of transformation of more lipophilic substrates, increased lifetime of enzymes, increased chemo-, regio-, and enantioselectivity through control of solvent physical properties (solvent engineering), and control of thermodynamic equilibria in favor of synthesis rather than hydrolysis.[1] More recently the use of enzymes in nonaqueous media has been extended to include supercritical fluids[2] and ionic liquids[3] with tuneable solvent properties. The use of these solvents reduces the quantities of waste volatile organic compounds (VOCs), which is an important step in the direction of "green chemistry". One group of potential solvents for biotransfor-

[*] S. Saul, Dr. J. Micklefield
School of Chemistry
The University of Manchester
Institute of Science and Technology (UMIST)
PO Box 88, Manchester M601QD (UK)
Fax: (+44) 161-200-4484
E-mail: j.micklefield@manchester.ac.uk
Dr. S. Corr
INEOS Fluor Ltd.
PO Box 13
The Heath, Runcorn, Cheshire WA7 4QF (UK)

[**] This work is supported by INEOS Fluor Ltd.

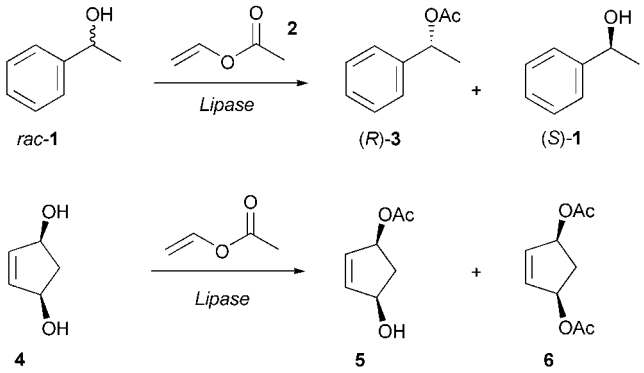
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/ange.200460082

mations that has received little attention to date is that of pressurized liquids with normal boiling points below, and critical temperatures above, room temperature. These fluids are easily handled at moderate pressures and their relative volatility should allow ready removal of solvent residues from the products, in contrast to the situation with many ionic liquids. Such fluids can be readily compressed and reliquified in a closed system, thereby allowing the solvents to be recycled and reused with minimal losses into the environment, which is an important factor in the quest for greener chemical processes.

Here we report the first investigation of biotransformations in liquid-phase, low-boiling hydrofluorocarbon solvents (HFCs).[4] HFCs are generally of low toxicity, do not have ozone depletion potential, and are not classed as VOCs. Many, such as 1,1,1,2-tetrafluoroethane (R-134a) and 1,1,1,2,3,3,3-heptafluoropropane (R-227ea), are also nonflammable. Several HFCs are used as replacements for the chlorofluorocarbons (CFCs) and hydrochlorofluorocarbons (HCFCs) in the refrigeration industry and are manufactured globally on a large scale to high purity. Both R-134a and R-227ea are also manufactured to current good manufacturing practice (cGMP) standards for use in metered-dose inhaler applications in the pharmaceutical industry. The HFCs thus have the potential to be environmentally benign and economically feasible alternatives to conventional organic solvents and supercritical fluids. Whilst a number of the properties of HFCs are also common to supercritical fluids,[4] the moderate absolute pressure of liquid-phase HFCs[5] precludes the need for expensive, specialized high-pressure reaction equipment. A further advantage of using liquid HFCs is their polarities, which are comparable to those of moderately polar organic solvents such as tetrahydrofuran (THF) and dichloromethane.[6a] This increase in polarity over solvents such as hexane and supercritical CO_2 may improve the solubility of a range of desirable substrates and avoid the need for the use of polar cosolvents, which are difficult to remove. Despite this increased polarity, HFCs are relatively hydrophobic,[6b] which should ensure that enzymes retain their essential active-site water molecules and activity.[7]

Initially we chose to investigate the model lipase-catalyzed kinetic resolution of (±)-1-phenylethanol (*rac*-**1**, Scheme 1) in anhydrous[8] R-134a, R-227ea, and difluoromethane (R-32). It is widely accepted that transesterification reactions catalyzed by lipases are most efficient in apolar hydrophobic solvents because more polar solvents can strip the enzymes of their essential water.[7] We therefore chose to compare the resolution of **1** in HFCs with the reaction carried out under identical conditions in anhydrous[8] hexane and methyl *tert*-butyl ether (MTBE), both of which have been shown to be good solvents for this biotransformation.[9] Accordingly, the acylation of (±)-1-phenylethanol (*rac*-**1**) with vinyl acetate **2**, catalyzed by immobilized lipase B from *Candida antarctica* (Novozym 435), was carried out in the five different media and the progress of the reactions was monitored by GC. We found that plastic-coated glass aerosol bottles (10 mL), with teflon-coated aerosol valves that could be crimp sealed, were ideal for small-scale HFC-based reactions and were able to withstand the moderate pressures (up to 19 bar for R-32 at 30 °C)[5] without any loss of solvent. The results (Table 1) clearly show that the reactions in the HFCs are superior, both in terms of rate and degree of conversion observed. In the case of R-32, a near-perfect resolution was achieved with approximately 50% conversion after 5 h resulting in a virtually equimolar mixture of homochiral product ester (*R*)-**3** and unreacted alcohol (*S*)-**1**. Moreover this reaction was easily scaled up by using a one-liter aluminum reaction vessel (see the Supporting Information). By starting with (±)-1-phenylethanol (1.24 g) in R-32 (100 mL), ester (*R*)-**3** (772 mg) was isolated in 46% yield and 99% *ee*, as determined by chiral GC, along with alcohol (*S*)-**1** (595 mg) in 48% yield and 99% *ee*, after 5 h.



Scheme 1. Lipase-catalyzed kinetic resolution of racemic 1-phenylethanol (**1**) and desymmetrization of *meso*-2-cyclopentene-1,4-diol (**4**).

Table 1: Kinetic resolution of 1-phenylethanol (**1**) catalyzed by Novozym 435.

Solvent	t[a] [h]	Conv. [%]	*ee* (*S*)-**1** [%]	*ee* (*R*)-**3** [%]	Initial rates[b] [nmol min^{-1} mg^{-1}]			
					$a_w < 0.01$	$a_w \approx 0.43$	$a_w \approx 0.58$	$a_w \approx 0.75$
R-32	5	50	>99	>99	n.d.[c]	n.d.	n.d.	n.d.
R-227ea	3.5	49	96	>99	n.d.	n.d.	n.d.	n.d.
R-134a	4	49	96	>99	325	387	407	358
hexane	8	46	85	>99	227	241	260	228
MTBE	35	49	96	>99	51	64	68	60

[a] The time point when no further reaction was evident (that is, the rate of the reaction was approaching zero). [b] Initial rates are given in units of nmol of product **3** per minute per mg of enzyme and were determined from the slope of the time-course measurements between 0 and 5% conversion for an approximate water activity (a_w). [c] n.d. = not determined.

It is well known that the activity of enzymes in organic solvents depends on the thermodynamic water activity (a_w) of the system.[10] The reactions described here were all carried out in anhydrous solvents[8] where the a_w value is close to zero, thereby enabling a fair comparison between different media. However, in order to examine how the thermodynamic water

activity affects the lipase-catalyzed acylation of (±)-1-phenylethanol (*rac*-**1**) in an HFC solvent (R-134a) in comparison with the reaction in conventional organic solvents, the initial rates of the reaction were measured at different water activities.[10b] From these measurements it can be seen (Table 1) that the initial rates follow the typical bell-shaped profile,[10a,c] with a maximum rate attained at $a_w \approx 0.58$ in all solvents. Notably, the initial rates in R-134a are always higher than those in hexane or MTBE, irrespective of the a_w value.

The desymmetrization of prochiral or *meso*-diols by monoacylation catalyzed by various hydrolases in nonaqueous solvents can give an enantiomerically pure product in near quantitative yield. As a result, these biotransformations are now widely employed. In order to further investigate the potential of the HFCs as media for biotransformations, the model lipase-catalyzed desymmetrization of *meso*-2-cyclopentene-1,4-diol (**4**) with vinyl acetate **2** was studied. The acylated product (1*R*,4*S*)-1-hydroxy-2-cyclopentene-4-acetate (**5**) and its enantiomer are starting materials for the synthesis of various cyclopentenoid natural products, such as prostaglandins, prostacyclins, and thromboxanes.[11] Previous investigations have found that THF with Et_3N as an additive is the solvent system of choice for this biotransformation.[11a,b] Accordingly, the reaction of *meso*-diol **4** with vinyl acetate, catalyzed by the lipases from *Pseudomonas cepacia* and *C. antarctica* (Novozym 435), was carried out in anhydrous R-134a, R-227ea, and R-32 and compared with identical transformations in anhydrous THF with and without added Et_3N. The time courses for all reactions were followed and the results shown in Table 2 indicate the time point at which the

Table 2: Desymmetrization of *meso*-2-cyclopentene-1,4-diol (**4**) catalyzed by lipase from *P. cepacia* and Novozym 435.

Solvent	*P. cepacia* lipase			Novozym 435		
	t [h][a]	Yield **5** [%]	*ee* **5** [%]	t [h][a]	Yield **5** [%]	*ee* **5** [%]
R-134a	3.5	53	>99	4	55	>99
R-227ea	3	42	>99	3	61	>99
R-32	5	58	>99	5.5	55	>99
THF/Et_3N	17	43	>99	48	42	91
THF	17	53	38	48	56	40

[a] The time point at which the maximum *ee* value of monoacetate product **5** was achieved.

maximum *ee* value was achieved for the monoacetate product **5**. With both lipases it is clear that enzyme activity is far greater in the HFCs than in THF/Et_3N or in THF alone. In the case of *P. cepacia* lipase comparable or higher yields, up to 58%, of enantiopure monoacetate **5** are achieved in around a quarter of the time. For Novozym 435 the use of HFCs similarly increases the yield of **5** with dramatically improved rates and increases the enantioselectivity from 40% *ee* in THF or 91% *ee* in THF/Et_3N to >99% *ee* in all of the HFCs. It is also interesting to note that R-227ea gives the lowest yield of **5** (42%) with *P. cepacia* lipase but gives the highest yield of **5** (61%) with Novozym 435. Clearly HFCs, like conventional solvents, differ in their physical properties (for example, dielectric constants and dipole moments).[6a] Thus, whilst one HFC may be the best solvent for one particular enzyme, it does not necessarily follow that it will be the best for another.

A typical time-course plot for the desymmetrization of *meso*-diol **4** with Novozym 435 in R-134a is shown in Figure 1. The desymmetrization process comprises two reactions. The

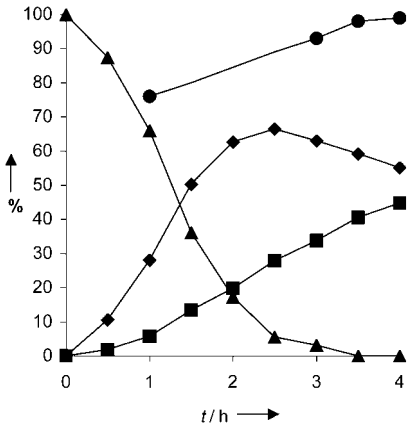
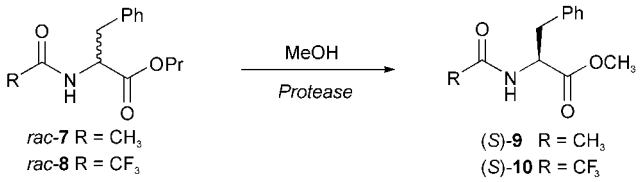


Figure 1. Time-course plot of the desymmetrization of *meso*-2-cyclopentene-1,4-diol (**4**) catalyzed by Novozym 435 in R-134a. The graph shows the percentage of *meso*-diol **4** remaining (▲), the percentage yields of **5** (◆) and **6** (■), and the *ee* value of product **5** (●) versus time.

first reaction produces the monoacylated product **5** (or its enantiomer). In the second step, which constitutes per se a kinetic resolution, the monoacetate product is subject to a second acylation that produces the diacetate **6**. As a result of this, and as is evident from the time-course plot, when all of the diol is consumed the yield of the monoacetate product **5** begins to drop while its *ee* value continues to increase due to the kinetic resolution of the second acylation step. Thus, the final yield of nearly optically pure monoacetate product **5** is dependent on the enantioselectivity of the enzyme in the first acylation step. Clearly, greater enantioselectivities in the first acylation step are achieved when the reaction is undertaken in the HFCs than when it is performed in THF/Et_3N. Furthermore, inspection of the time-course plots for all five solvent systems clearly shows that the rate of reaction with Novozym 435 is much lower in THF/Et_3N or THF alone, with a half life ($t_{1/2}$) of approximately 8–12 h, than in the HFCs ($t_{1/2} = 1.5$–2 h), with the highest rate being observed in R-227ea. With the *P. cepacia* lipase the $t_{1/2}$ value for the reactions in the HFCs was 0.5–1.5 h, which can be compared with the values of 2.5 h for THF/Et_3N and 3.5 h for THF alone. The desymmetrization of *meso*-diol **4** (500 mg) by using Novozym 435 was also successfully carried out on a preparative scale in a one-liter aluminum vessel containing R-227ea (500 mL); this resulted in the isolation of product **5** (425 mg, 59%) in 99% *ee*, as determined by chiral GC, along with diacetate **6** (372 mg, 40%), after 3 h.

Finally, in order to explore the utility of the HFCs as media for other classes of enzyme-catalyzed reactions, the model transesterifications of *N*-acetyl and *N*-trifluoroacetyl phenylalanine propyl esters *rac*-**7** and *rac*-**8** (Scheme 2) with methanol, catalyzed by subtilisin Carlsberg protease, were investigated in anhydrous solvents.[12] In this case, R-134a gave the highest rates and yields of enantiopure methyl esters (*S*)-**9** and (*S*)-**10** and was marginally better than hexane, the



rac-**7** R = CH_3
rac-**8** R = CF_3

(*S*)-**9** R = CH_3
(*S*)-**10** R = CF_3

Scheme 2. Transesterification of racemic N-protected phenylalanine propyl esters catalyzed by subtilisin Carlsberg.

best conventional solvent examined, but significantly better than the more polar solvents, THF and acetonitrile (Table 3). Transesterifications in R-32, on the other hand, exhibited

Table 3: Summary of results from the transesterification of N-protected phenylalanine propyl esters.

Solvent	*rac*-**7** (R = CH_3)			*rac*-**8** (R = CF_3)		
	t [h]	Yield **9** [%]	*ee* **9** [%]	*t* [h]	Yield **10** [%]	*ee* **10** [%]
R-134a	19	23	> 99	72	33	> 99
R-32	19	13	> 99	72 (19)[a]	10 (10)[a]	> 99
hexane	19	20	> 99	72	23	> 99
THF	19	8	> 99	72	1.3	n.d.[b]
CH_3CN	19	4	> 99	72	0	–

[a] The reaction effectively ceased after 19 h. [b] n.d. = not determined.

similar initial rates to the reactions in hexane but stopped at lower yields of (*S*)-**9** (13%) and (*S*)-**10** (10%). This is, however, still better than the results for THF and acetonitrile.

In summary, we have demonstrated the benefits and potential of HFCs as solvents for biotransformations. In the kinetic resolution of model secondary alcohol *rac*-**1** significant increases in rate and product yield were demonstrated in the HFC reactions compared to reactions in the lipase solvents of choice, hexane and MTBE. The desymmetrization of a model *meso*-diol **4** was also achieved with substantially increased rates, yields, and enantioselectivities in the HFCs in comparison with the results in the typical organic solvent system.[11] It is possible that the improved rates of reaction observed are due, in part, to the low viscosity and the consequently increased solute diffusivity in the HFCs, which are mid way between those observed for a typical organic solvent on one hand and a supercritical fluid on the other.[4] Finally, the benefits of HFCs are not limited to lipase-catalyzed reactions, as demonstrated by the improved activity of the subtilisin Carlsberg protease in R-134a. Indeed, recent findings, which will be reported in due course, demonstrate that hydroxynitrile lyases also display activity in the HFC solvents.

Experimental Section

Immobilized lipase B from *Candida antarctica* (Novozym 435) with a specific activity of 10000 U g^{-1} was purchased from Fluka. Lipase from *Pseudomonas cepacia* (92.6 U g^{-1}) and subtilisin Carlsberg protease (10.5 U g^{-1}) were purchased from the Sigma Chemical Co. In all experiments enzymes were used straight from the bottle, unless otherwise stated.

For the kinetic resolution of racemic 1-phenylethanol (**1**) in the HFCs, Novozym 435 (9.5 mg, 95 U) was added to **1** (61.0 mg, 0.50 mmol) and vinyl acetate **2** (861.0 mg, 10.0 mmol) in a plastic-coated aerosol bottle (10 mL). The aerosol was capped, crimp sealed, and immediately charged with the HFC (5.0 mL), then the reaction mixture was stirred magnetically at room temperature. Samples (approximately 20 μL) were discharged periodically through the aerosol valve, dissolved in CH_2Cl_2 (100 μL), and analyzed by GC, or chiral GC where appropriate. Reactions in hexane and MTBE were carried out in an identical fashion except a Supelco graduated screw-top vial (7 mL) was used as the reaction vessel and samples (1 μL) were withdrawn periodically for analysis by using a Hamilton syringe. For initial rate measurements the reactions were carried out in identical fashion, except 1 mg (10 U) of Novozym 435 was used with the same substrate concentrations and total volume (5 mL). The a_w values of hexane or MTBE reaction mixtures with Novozym 435 were all adjusted by preequilibration with saturated salt solutions as described previously.[10b] The a_w value of R-134a reaction mixtures with Novozym 435 was adjusted by mixing appropriate ratios, by weight, of anhydrous and water-saturated R-134a. The relationship between molar ratio of water and a_w value is not always linear so the water activities in R-134a are only approximate. The large-scale kinetic resolution of *rac*-**1** (1.24 g, 10.2 mmol) was carried out in a 1-L aluminum reaction vessel with Novozym 435 (190 mg, 1900 U), vinyl acetate (17.3 g, 0.201 mol), and R-32 (100 mL). After 5 h, the reaction was vented and the resulting mixture was separated by silica gel column chromatography, eluting with 10→1% diethyl ether in petroleum ether, to give ester (*R*)-**3** (772 mg, 46%; $[\alpha]_D = +111$ ($c = 2.0$, CH_3OH); literature value:[13a] $[\alpha]_D = +114$ ($c = 2.0$, CH_3OH)) and alcohol (*S*)-**1** (595 mg, 48%; $[\alpha]_D = +41$ ($c = 2.0$, CH_3OH); literature value:[13a] $[\alpha]_D = +45$ ($c = 2.0$, CH_3OH)). Both products were identical by ^1H and ^{13}C NMR spectroscopy to commercial samples as well as to the characterization data in a previous report.[13b]

The desymmetrization of *meso*-diol **4** in HFCs (5 mL) was carried out and analyzed as described above with **4** (5.0 mg, 0.05 mmol), vinyl acetate (86.1 mg, 1.00 mmol), and *P. cepacia* lipase (5.0 mg, 0.463 U) or Novozym 435 (1.0 mg, 10 U) in aerosol bottles. The reaction was also carried out in an identical fashion with and without Et_3N (10.1 mg, 0.1 mmol) in anhydrous THF (5 mL). The large-scale desymmetrization of **4** (500 mg, 4.99 mmol) was carried out in a 1-L aluminum vessel containing R-227ea (500 mL), vinyl acetate (8.61 g, 0.10 mol), and Novozym 435 (100 mg, 1000 U). After 5 h, the reaction mixture was purified by silica gel column chromatography, eluting with hexane/ethyl acetate (2:1), to give monoacetate **5** (425 mg, 59%; $[\alpha]_D = +65$ ($c = 1.0$, $CHCl_3$); literature value:[11a] $[\alpha]_D = +66$ ($c = 1.0$, $CHCl_3$)) and diactetate **6** (372 mg, 40%). Both products were identical by ^1H and ^{13}C NMR spectroscopy to a commercial samples as well as to the characterization data in a previous report.[11] The kinetic resolutions of *N*-acetyl and *N*-trifluoroacetyl phenylalanine propyl esters *rac*-**7** and *rac*-**8** were carried out as before in aerosol bottles containing anhydrous HFCs (4 mL) or in vials containing anhydrous conventional solvents (4 mL). Reaction mixtures contained *rac*-**7** (9.9 mg, 0.04 mmol) or *rac*-**8** (12.1 mg, 0.04 mmol), MeOH (25.6 mg, 0.80 mmol), and subtilisin Carlsberg (4.0 mg, 0.042 U).

Received: March 22, 2004
Revised: June 11, 2004

Keywords: biotransformations · desymmetrization · hydrofluorocarbons · kinetic resolution · lipases

[1] a) A. M. Klibanov, *Nature* **2001**, *409*, 241–246; b) A. Schmidt, J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, B. Witholt, *Nature* **2001**, *409*, 258–268; c) G. Correa, S. Riva, *Angew. Chem.* **2000**, *112*, 2312–2310; *Angew. Chem. Int. Ed.* **2000**, *39*, 2226–2254; d) S. M. Roberts, *J. Chem. Soc. Perkin Trans. 1* **2000**, 611–633; e) S. M. Roberts, *J. Chem. Soc. Perkin Trans. 1* **1999**, 121; f) C. H. Wong, *Science* **1989**, *244*, 1145–1152; g) C.-S. Chen, C. J.

Sih, *Angew. Chem.* **1989**, *101*, 711 – 723; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 695 – 707.
[2] a) A. J. Mesiano, E. J. Beckman, A. J. Russell, *Chem. Rev.* **1999**, *99*, 623 – 633; b) T. Matsuda, T. Harada, K. Nakamura, *Chem. Commun.* **2000**, 1367 – 1368; c) T. Mori, M. Li, A. Kobayashi, Y. Okahata, *J. Am. Chem. Soc.* **2002**, *124*, 1188 – 1189.
[3] a) M. Erbeldinger, A. J. Mesiano, A. J. Russell, *Biotechnol. Prog.* **2000**, *10*, 1129 – 1131; b) F. Van Rantwijk, R. M. Lau, R. A. Sheldon, *Trends Biotechnol.* **2003**, *21*, 131 – 138; c) S. Park, R. J. Kazlauskas, *Curr. Opin. Biotechnol.* **2003**, *14*, 432 – 437.
[4] S. Corr, *J. Fluorine Chem.* **2002**, *118*, 55 – 67.
[5] The boiling points and absolute pressures at room temperature (20 °C) of liquid phase HFCs used in this study: 1,1,1,2-tetrafluoroethane (R-134a): 26.1 °C, 5.7 bar; difluoromethane (R-32): 51.7 °C, 14.8 bar; 1,1,1,2,3,3,3-heptafluoropropane (R-227ea): 15.6 °C, 3.9 bar.
[6] a) Selected dielectric constants (ε) and dipole moments (μ [D]): R-134a: ε 9.5, μ 2.05; R-32: ε 8.2, μ 1.98; R-227ea: ε 4.1, μ 0.93; hexane: ε 1.9, μ 0.08; THF: ε 7.61, μ 1.63; CH_2Cl_2: ε 9.08, μ 1.55;[4] b) R-134a, R-32, and R-227ea are all totally immiscible with water. The solubility of water in R-134a is around 1100 ppm by weight at 21 °C.
[7] a) G. Kirchner, M. P. Scollar, A. M. Klibanov, *J. Am. Chem. Soc.* **1985**, *107*, 7072 – 7076; b) A. Zaks, A. M. Klibanov, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 3192 – 3196.
[8] Anhydrous solvents were used throughout. The only water in the reaction mixture comes from the enzyme. For example, Novozym 435 contains 14 μg of water per mg of enzyme, as determined by Karl Fischer titration (GRS2000). The majority of the water will remain tightly associated with the enzyme, so reaction mixtures have low thermodynamic water activities ($a_w < 0.1$).
[9] a) S. H. Schöfer, N. Kaftzik, P. Wasserscheid, U. Kragl, *Chem. Commun.* **2001**, 425 – 426; b) M. Persson, U. T. Bornscheuer, *J. Mol. Catal. B* **2003**, *22*, 21 – 27.
[10] a) P. J. Halling, *Enzyme Microb. Technol.* **1994**, *16*, 178 – 206; b) G. A. Hutcheon, P. J. Halling, B. D. Moore, *Methods Enzymol.* **1997**, *286*, 465 – 472; c) G. Bell, A. E. M. Janssen, P. J. Halling, *Enzyme Microb. Technol.* **1997**, *20*, 471 – 477.
[11] a) F. Theil, H. Schick, G. Winter, G. Reck, *Tetrahedron* **1991**, *47*, 7569 – 7582; b) S. R. Ghorpade, R. K. Kharul, R. R. Joshi, U. R. Kalkote, T. Ravindranathan, *Tetrahedron: Asymmetry* **1999**, *10*, 891 – 899; c) C. R. Johnson, S. J. Bis, *Tetrahedron Lett.* **1992**, *33*, 7287 – 7290.
[12] a) J. Partridge, G. A. Hutcheon, B. D. Moore, P. J. Halling, *J. Am. Chem. Soc.* **1996**, *118*, 12873 – 12877; b) G. A. Hutcheon, M. C. Parker, A. James, B. D. Moore, *Chem. Commun.* **1997**, 931 – 932; c) K. Kawashiro, H. Sugahara, S. Sugiyama, H. Hayashi, *Biotechnol. Bioeng.* **1997**, *53*, 26 – 31.
[13] a) M. Bakker, A. S. Spruijt, F. van Rantwijk, R. A. Sheldon, *Tetrahedron: Asymmetry* **2000**, *11*, 1801 – 1808; b) Y. Kita, Y. Takebe, K. Murata, T. Naka, S. Akai, *J. Org. Chem.* **2000**, *65*, 83 – 88.

