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Factors affecting the lipase catalyzed transesterification reactions of 3-hydroxy esters in organic solvents.

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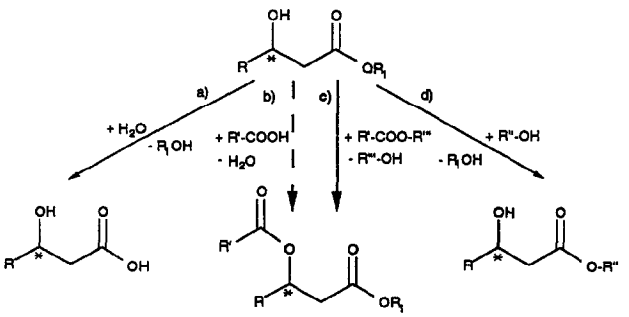
Abstract: Chiral resolutions of racemic 3-hydroxy esters were performed in organic phases with lipases from *Pseudomonas cepacia*, *Chromobacterium viscosum* and Porcine pancreas. The reaction conditions have been optimized with 3-hydroxy octanoic acid methyl ester. Different organic solvents have been tested showing a tendentious correlation with the hydrophobicity of the solvents expressed as log P. The reaction time was shortened six fold by using irreversible acylating agents. We have found solvent type, lipase type and acylating agent acting as tools for changing the enantioselectivity. Lipase from *Pseudomonas cepacia* was lyophilized at different pH and the influence of the amount of water added was investigated, resulting in the highest activity at the pH optimum and a denaturation of the lipase above 1 % water (w/w_{lipase}) . The water activity was measured on-line with a humidity sensor. Water activities greater than 0.4 led to a decrease in enantioselectivity and reaction rate. In the optimized system the resolutions of other 3-hydroxy esters were tested. Aliphatic compounds reacted with lower enantioselectivity, only the substrates could be isolated in high enantiomeric purity. In contrast, aromatic 3-hydroxy esters were acylated by lipases with high stereoselectivity. A model of the active site of lipase from *Pseudomonas sp.* explained these experimental observations.

INTRODUCTION

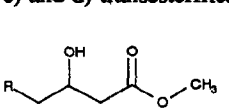
The use of organic media for enzymatic reactions is now well documentated[1]. The demand of health authorities for enantiomerically pure, pharmaceutically active substances, lead to an increasing use of biocatalysts, especially lipases and esterases.

Beside biphasic systems and reverse micelles, organic solvents with low water content have been successfully employed in the synthesis of *e.g.* t-Sobrerol[2] and Isoserine[3]. When working in microaqueous systems, an investigation and optimization of the factors influencing the catalytic activity and the reaction rate is necessary.

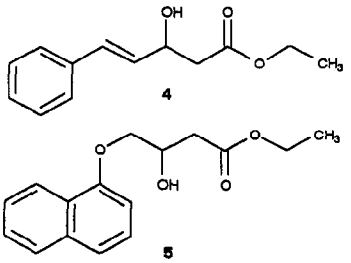
We have chosen 3-hydroxy esters as substrates (Scheme 2). These compounds are possible precursors in the synthesis of natural compounds and ß-adrenergic blockers like propranolol. 3-hydroxy octanoic acid methyl esters was used as model substrate. In this paper the influence of acyl donor, organic solvent, water content and water activity on enantioselectivity and reaction rate have been studied in detail. The results were transferred to the resolution of other aliphatic and aromatic 3-hydroxy esters. A model of the active site of lipase from *Pseudomonas sp.*[4] was used for the explanation of the experimental results. A system for the continuous lipase-catalyzed resolution of 3-hydroxy esters in a fixed-bed reactors was developed[1i] and kinetic studies were performed[1k]. The possible reaction ways for enzymatic reactions of 3-hydroxy esters are shown in Scheme 1. The results of hydrolysis, esterification and transesterification at the acid group have been published in an earlier paper[5].



Scheme 1: Possibilities for lipase-catalyzed reactions: a) hydrolysis, b) esterification, c) and d) transesterification



1: R = Ethyl-
2: R = Butyl-
3: R = Hexyl-



Scheme 2: 3-hydroxy esters used as substrates in the transesterification reactions

RESULTS AND DISCUSSION

Screening of the lipase

About 125 lipases are now commercially available[6a]. We have chosen those lipases which either have been employed successfully in hydrolysis or transesterification reactions or are isolated new. Table 1 shows the results in the transesterification reaction of (R,S)-2 with vinyl acetate or cyclohexyl acetate as acyl donor and different lipases.

Table I: Conversion, enantiomeric excess and configuration obtained with several lipases in the resolution of (R,S)-2 (for abbreviation see text, for calculation of data see experimental section):

lipase	acyl donor		solvent		conversion	enantiomeric excess		time
	name	[ml]	name	[ml]	[%]	substrate[%ee]	product[%ee]	[h]
PCL	cyclohexyl acetate	2	-	-	49	(S) 78	(R) 82	200
PCL	vinyl acetate	0,03	dodecane	2	58	(R) 92	(S) 67	117
PCL (pure)	vinyl acetate	0,03	dodecane	2	16	(R) 13	(S) 68	100
CVL	vinyl acetate	0,1	dodecane	2	68	(R) 99	(S) 46	168
PSL	vinyl acetate	2	-	-	24	(R) 11	(S) 33	191
PEL	vinyl acetate	0,1	dodecane	4	40	(S) 13	(R) 20	433
PPL	cyclohexyl acetate	2	-	-	50	(R) 50	(S) 50	330

As shown in Table 1, high enantiomeric excesses for the substrate were obtained with the lipases from *Pseudomonas cepacia* (PCL), *Chromobacterium viscosum* (CVL) and Porcine pancreas (PPL). The new lipases from *Penicillium expansum* (PEL) and *Penicillium simplicissimum* (PSL) were stable in organic solvents, but only low reaction rates have been determined. PEL has shown a different stereoselectivity. In this case, R-2 was the preferably converted enantiomer. The purified PCL was nearly unstable in organic solvents (even after covalent immobilization, data not shown) compared to the crude lipase. In the pH-stat-assay we observed a very high activity (5800 U/mg) for the pure lipase (data not shown)[6b]. All other lipases listed in the experimental section exhibited only low conversions and/or stereoselectivity. In Table 1 reactions with two acylating agents were also listed. The use of vinyl acetate led to higher reaction rates and higher enantiomeric purities (*e.g.*: lipase from *Pseudomonas cepacia*) compared to the experiments with cyclohexyl acetate. This was also observed with isopropenyl acetate (data not shown). Surprisingly the enantioselectivity changed in the case of PCL using cyclohexyl acetate instead of vinyl acetate as acylating agent. In both cases the same acyl group was transferred to the enzyme, thus there is no obvious reason for this observation. As described by Chen et al.[1c] one would expect no change in the stereochemical behaviour, if the same acyl group is transferred. In all reactions the enantioselectivity of the lipases used was low, thus it was impossible to obtain the product in high optical purity.

The following experiments have been performed with lipase from *Pseudomonas cepacia* and vinyl acetate.

Influence of the organic solvent

Several attempts were made to find a correlation between the nature of the organic solvent and the reaction rate[1a]. We have found the best correlation with hydrophobicity (expressed as log P in Table 2) with the exception of the reaction in pure vinyl acetate. This is in accordance with the results of Bovara et al.[2] for the resolution of *trans*-sobrerol. We think that the high concentration of the acyl donor is responsible for this observation, because more active sites of the enzyme could be acylated. On the other hand, we observed a substrate inhibition caused by high concentrations of vinyl acetate and the slow reacting R-

enantiomer[1k]. In contrast to the results of Hirata et al.[7d] and Kitaguchi et al.[7e] we found an increase in the reaction rate with increasing hydrophobicities.

The enantiomeric excess and thus the enantiomeric ratio were markedly influenced by the organic solvent. This was also reported by other groups[7a-c]. In methylen chloride we have found an inversion in the stereoselectivity. Ueji et al.[7h] described the same observation for the *Candida cylindracea* lipase catalyzed esterification of 2-phenoxypropanoic acid in either carbon tetrachloride or acetone. In contrast to the results of Fitzpatrick et al.[7g] we found no correlation with the dielectric constant or the dipole moment.

In summary, the results show that it is hard to predict in which solvent one would obtain the best results. If the log P concept of Laane is true and highly hydrophobic solvents strip the essential water from the layer around the enzyme and lead to a change in enzyme activity (and stereoselectivity), one would probably find a better prediction if the water activity in the system is measured and/or controlled to a certain level.

Table II: Enantiomeric excess, enantiomeric ratio (E), configuration and conversion obtained in different organic solvents*.

solvent	logP	ϵ	D	conversion [%]	enantiomeric excess substrate[%ee]	enantiomeric excess product[%ee]	E
methylen chloride	- 0.5	8.93	5.2	34	(S) 31	(R) 60	5
1,4-dioxane	- 1.1	2.2	1.5	40	(R) 35	(S) 53	4
acetone	- 0.23	20.6	9	44	(R) 63	(S) 79	17
vinyl acetate	0.3	-	-	57	(R) 81	(S) 60	10
tetrahydrofuran	0.49	7.6	5.8	12	(R) 9	(S) 66	5
toluene	2.5	2.4	1	38	(R) 35	(S) 58	5
hexane	3.5	1.88	0	56	(R) 89	(S) 70	16
dodecane	6.6	-	-	58	(R) 92	(S) 67	16

* logP values see: 1a,7f,7j; ϵ and D-values: see 7f,7j; E: see experimental section;

Influence of the water content and the pH

For the following experiments the crude, untreated lipase from *Pseudomonas cepacia* was prepared as described in the experimental section. Subsequently a certain amount of water was added to the standard reaction mixture (Table III). Figure 1 and Table III show the results of the reactions with lipase lyophilized at pH 5, 7 and 9 with different amounts of water added. The reaction was also carried out with crude lipase resulting in the highest reaction rate. Increasing the water content led to a decrease in the enantioselectivity. In the case of pH adjusted lipase the highest activity was achieved with enzyme lyophilized at pH 7 (the pH optimum in hydrolysis) and water contents below 1 % water (w/w_{lipase}). This is in accordance with Cao et al.[7i] and Klibanov[7k]. Higher water contents also led to a nearly complete denaturation of the lipase (Table III). At high water contents the lipase was no more suspended in the reaction mixture, but stuck to the walls of the flask and was dark coloured. This was also observed by Hirata et al[8d].

The water content of a system gives no real information about the distribution of the water between solvent, substrates and enzyme. Thus, one would expect a better understanding, considering the water

activity as a key parameter[8b-e]. Hence, for transesterification reactions in organic solvents at low water contents it was also of interest, whether there is a change in the water activity, although there is no water produced during the reaction. Instead of other methods like reactions at fixed water activities[8d,8g] or the addition of salt hydrates[8h] we have used a humidity sensor (Rotronic, München) for the on-line measurement of water activity. As described by Khan et al.[8f] the relative humidity in the gas phase above the reaction mixture is directly related to the water acitivity a_w. In a reaction mixture containing 0.6 mmol (R,S)-**2**, 2.2 mmol vinyl acetate and 100 mg PCL in 10 ml dodecane a certain amount of water was added after equilibration of the water activity between the phases (approx. 30 min). The water activity was monitored with the sensor. Samples for GC analysis were drawn through a septum to avoid the penetration of air humidity.

Table III: Influence of pH and water content on reaction rate, enantioselectivity and remaining activity of the lipase (see also experimental section).

pH	water added [%w/w$_{lipase}$]	conversion [%]	enantiomeric excess substrate[%ee]	E	remaining activity [%]
-	-	58	92	16	80
5	0	28	27	7	71
5	3	12	9	5	< 1
5	16	1.5	1	5	< 1
7	0	55	61	5	70
7	0.5	39	44	5	65
7	1	22	14	3	< 1
7	3	9	5	3	10
9	0	29	28	7	32
9	2	1	< 1	5	1
9	16	< 1	< 1	3	1

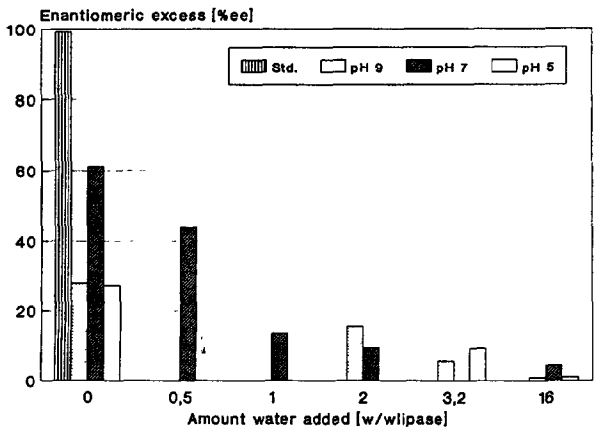


Figure 1: Enantiomeric excess (substrate) as a function of pH (before lyophilization) and amount of water added.

When water was added, the a_w increased suddenly to a higher level and after further equilibration the water activity increased slightly until the end of the reaction (Figure 2). At water concentrations of > 0.1 % we observed a higher increase in the water activity together with a strong decrease in the reaction rate represented in lower enantiomeric excesses of the substrates (Figure 2b). The critical a_w value was determined to approximately 0.4.

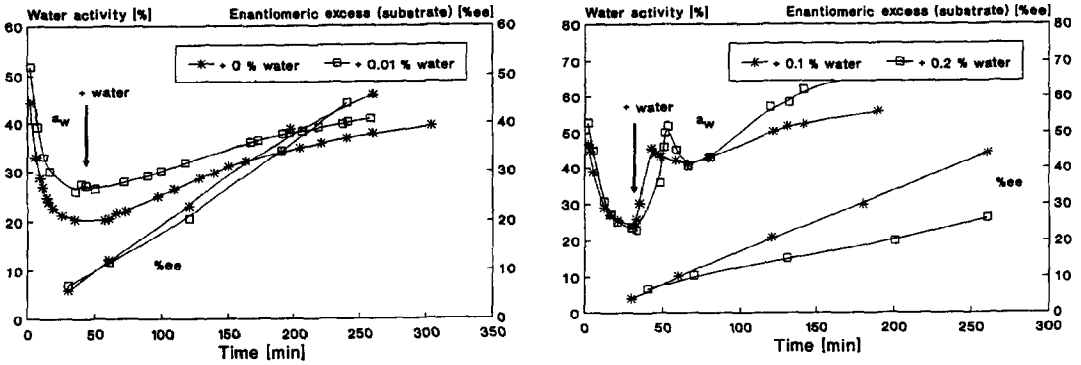


Figure 2: Water activity [a_w] and enantiomeric excess (substrate) [%ee] monitored during the reactions at different water contents a) 0, 0.01 (%w/w_{lipase}) b) 0.1, 0.2 (%w/w_{lipase}).

Reactions of the other 3-hydroxy esters

For compounds (R,S)-**1** and (R,S)-**3** we also developed a monitoring of the reaction progress via chiral gas chromatography[9b]. For the aromatic 3-hydroxy esters (R,S)-**4** and (R,S)-**5** the enantiomeric excess of substrate and product were determined by ^{1}H-NMR spectroscopy. As seen in Table IV the enantioselectivity of the lipases from *Pseudomonas cepacia* and *Chromobacterium viscosum* towards the aromatic compounds was much higher compared to the aliphatic 3-hydroxy esters. We have used the model of the active site from *Pseudomonas sp.* derived by Burgess et al.[4] to explain the experimental observation. Figure 3 shows both enantiomers of the aliphatic compound **1** fitted into the two dimensional structure of the active site (with the other aliphatic compounds (R,S)-**2** and (R,S)-**3** the figures are comparable). Both enantiomers have enough space in the active site, thus one would expext only a low enantioselectivity. This is in accordance with the calculated E-values between 5 and 16. In contrast only one enantiomer of the 3-hydroxy esters with the bulky aromatic groups fits well into the active site resulting in high E-values from 150 to 350 (Table IV and Figure 4). Hence it was possible to synthesize the enantiomerically pure product esters. Figure 4 only shows the enantiomers of compound **5**, but regarding the aromatic compound (R,S)-**4** the figures are comparable.

Table IV; Conversion, enantiomeric excess, configuration and E-values obtained with the other 3-hydroxy esters

compound	conversion [%]	enantiomeric excess substrate[%ee]	product[%ee]	E
1	73	(R) 99	(S) 37	6
3	44	(R) 60	(S) 75	10
4	30	(S) 43	(R) 98	150
5	33	(S) 49	(R) 99	320

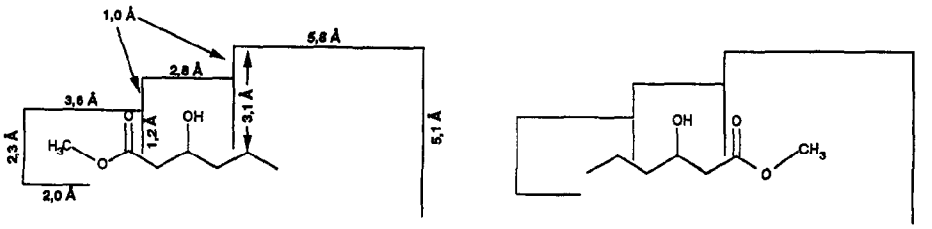


Figure 3: Enantiomers of the compound **1** fitted into the model of the active site of *Pseudom. sp.*

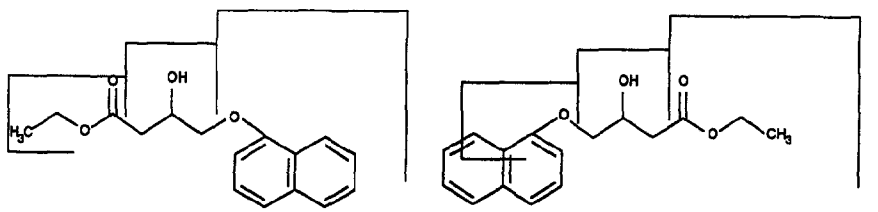


Figure 4: Enantiomers of compound **5** fitted into the model of the active site of *Pseudomonas sp.*

CONCLUSION

The lipase catalyzed transesterifications studied were greatly influenced by the reaction conditions. The stereoselectivity of the reaction of 3-hydroxy octanoic acid methyl ester was affected by the solvent, the lipase and the acylating agent. Especially in the case of the solvent, we do not know the reasons responsible for the inversion of the stereoselectivity yet. The structure of the substrate also led to a change in stereoselectivity. Hence it would be useful to know the shape of the active site of the lipase. Three dimensional structures of the biocatalyst together with computer modelling would certainly help in the prediction of the reaction behaviour and the stereoselectivity of a lipase. With lipase of *Pseudomonas cepacia* it was not required to optimize the biocatalyst. The crude untreated lipase showed the highest acitivities in all cases. Neither lyophilization at the pH optimum nor immobilization or purification could enhance stability or reactivity. Reactions at controlled water content or water activity resp. have shown the same observation, that for transesterification reactions in hydrophobic organic solvents the addition of water is not necessary.

EXPERIMENTAL SECTION

Materials

The following commercially available lipases were used: *Pseudomonas cepacia* lipase (Amano PS, A001526, A08530), *Humicola lanuginosa* lipase (Amano CE, 01023TM5)), *Penicillium cyclopium* lipase (Amano G, 80222T), *Chromobacterium viscosum* (Toyo Jozo Co., LP-251-S), Lypozym (NOVO), Lipolase 100 T (NOVO), *Pseudomonas sp.* lipase (Röhm, immobilized, EI 220-88), Porcine pancreas lipase (Solvay, PA-2225), *Mucor miehei* lipase (Gist-Brocades, E 30.000). *Penicillium simplicissimum* lipase (PSL) and *Penicillium expansum* lipase (PEL) were gifts from Dr. Menge, Gesellschaft für Biotechnologische Forschung, Braunschweig, Germany. The lipase from *Pseudomonas cepacia* was also used after purification up to 99%[6b]. All solvents used were of analytical grade and dried over 0.4 nm molecular sieves.

General methods:

Lipase activity assay: The activity of all lipases was measured in a Metrohm autotitration system (pH-stat-assay) by hydrolysis of 3 g tricaprylin (Fluka) in 50 ml 0.05 M potassium phosphate buffer (pH 8) without emulsifier. The solution was stirred vigorously and the temperature was maintained at 25 °C. At the end of a reaction the lipase was separated from the reaction mixture via centrifugation and the activity was determined as described above to calculate the remaining activity. The crude lipase, fresh from the bottle contained 2.7 % water (w/w_{lipase}). For studies at different pH the lipase from *Pseudomonas cepacia* was solubilized in 0.05 M potassium phosphate buffer at fixed pH (5, 7, 9) centrifuged and the supernatant lyophilized to enzyme powder.

Determination of enantiomeric excess and conversion: Both were performed by gas chromatography using a chiral stationary phase[9a] (Lipodex E^R 50 m, 0.25 mm ID, Macherey & Nagel) under the following conditions: compounds **2** and **3**: oven temperature from 80 °C (initial time 15 min) to

120 °C for 20 min (heating rate 10 °C/min) to 180 °C (20 °C/min), H_2 as carrier gas and a split of 1:100. Compound **1**: analogous but initial temperature 60 °C. All samples were derivatized with trifluoroacetic acid anhydride (see below). The data obtained via GC were verified using ^{1}H-NMR spectroscopy (Bruker, 90 and 200 Mhz resp.) with (+) Eu(hfc)$_3$ as chiral auxiliary in $CDCl_3$. The specific rotation was determined by polarimetry (Perkin-Elmer model 241) in methylen chloride. Substrates and products were isolated from the reaction mixture via flash chromatography with diethyl ether/petrol ether mixtures, controlled by thin layer chromatography. The conversion and the enantiomeric ratio were calculated as described by Chen et al.[1c]. The absolute configuration was determined with enantiomerically pure compounds with known configuration on GC and NMR.

Transesterification reactions: All reactions were carried out at 40°C in 10 ml glass stoppered round bottom flasks (exception: reactions at controlled water activity where conducted in a 50 ml two neck round bottom flask). The magnetic stirrer speed was kept at 400 rpm. In a typical experiment 50 mg (0.3 mmol) (R,S)-**2**, 30 µl (0.3 mmol) vinyl acetate, 100 mg PCL and 2 ml solvent were added. Samples withdrawn during the reaction were centrifuged to separate them from the enzyme. The solution was then derivatized as follows: 200 µl dichloro methane, 50 µl trifluoroacetic acid anhydride and 5 µl of the reaction solution were mixed. After evaporation of solvent and excess reagent, n-hexane was added for analysis.

The measurement of water activity was performed with a humidity sensor (Rotronic). The water content was determined in an autotitration system (Metrohm) using the Karl-Fischer-method.

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REFERENCES

1: For reviews and articles see: a) Laane, C.: *Biocatalysis* **1** (1987), 17-22. b) Klibanov, A. M.: *Acc. Chem. Res.* **23** (1990), 114-120. c) Chen, C. S.; C. J. Sih: *Angew. Chem. Int. Ed. Engl.* **29** (1989), 695-708. d) Xie, Z. F.: *Tetrahedron: Asymmetry* **2**(8) (1991), 733-750. e) Schapöhler, S.: **17**(66) *FORTSCHRITTS-BERICHT VDI Verlag*, Düsseldorf (1990). f) Zaks, A.: *Biocatal. Ind.* (Ed.: J. S. Dordick), New York, (1991), 161-180. g) Bornscheuer, U.; S. Schapöhler; T. Scheper; K. Schügerl: *Tetrahedron: Asymmetry* **2**(10) (1991), 1011-1014. h) Bornscheuer, U.: **17**(87), *FORTSCHRITTS-BERICHT VDI*, Düsseldorf (1993). i) Kreye, L.; U. Bornscheuer; A. Herar; T. Scheper; H. H. Meyer; F. N. Kolisis: *Enz. Microb. Technol.*, submitted. k) Bornscheuer, U.; A. Herar; H. H. Meyer; T. Scheper; F. N. Kolisis: in preparation.

2: Bovara, R.; G. Carrea; L. Ferrara; S. Riva: *Tetrahedron: Asymmetry* **2**(9) (1991), 931-938.

3: Liu, Y.; C. Miet; N. Kunesch; J. E. Poisson: *Tetrahedron: Asymmetry* **2**(9) (1991), 871-872.

4: Burgess, K.; L. D. Jennings: *J. Am. Chem. Soc.* **113** (1991), 6129-6139.

5: Scheper, T.; U. Bornscheuer; A. Capewell; A. Herar; H. G. Hundeck; H. H. Meyer; F. Schuber; F. Kolisis; (in Biocatalysis in Non-Conventional Media; ed. J. Tramper): *Elsevier, Amsterdam* **8** (1992), 37-44.

6: a) Gais, H.; H. Hemmerle: *Chemie in unserer Zeit* **24**(5) (1990), 239-248. b) Bornscheuer, U.; O.-W. Reif; R. Lausch; R. Freitag; Th. Scheper; F. Kolisis; U. Menge: *Biochim. Biophys. Acta*, submitted

7: a) Manjon, A.; J. L. Iborra; A. Arocas: *Biotechnol. Lett.* **13** (1991), 339-344. b) Parida, S.; J. S. Dordick: *J. Am. Chem. Soc.* **113** (1991), 2253-2259. c) Sakurai, T.; A. L. Margolin; A. J. Russell; A. M. Klibanov: *J. Am. Chem. Soc.* **110**(21) (1988), 7236-7237. d) Hirata, H.; K. Sakaki; T. Yamashina; K. Higuchi; H. Yanagishita; I. Iida; K. Toyota; M. Sugiura: *Chem. Express* **7** (1992), 293-296. e) Kitaguchi, H.; P. A. Fitzpatrick; J. E. Huber; A. M. Klibanov: *JACS* **111** (1989), 3094-3095. f) C. Reichardt: VCH, Weinheim (1988 2.Ed.). g) Fitzpatrick, P. A.; A. M. Klibanov: *J. Am. Chem. Soc.* **113** (1991), 3166-3171. h) Ueji, S.; R. Fujino; N. Okubo; T. Miyazawa; S. Kurita; M. Kitadani; A. Muromatsu: *Biotechnol. Lett.* **14**(3) (1992), 163-168. i) Cao, S. G.; Y. Feng; Z. B. Liu; Z. T. Ding; Y. H. Cheng: *Appl. Biochem. Biotechnol.* **32** (1992), 7-13. j) Rekker, R.F., Elsevier, Amsterdam (1977) k) Klibanov, A. M.: *Chem. Tech.* (1986), 354-359

8: a) Hirata, H.; K. Higuchi; T. Yamashina: *J. Biotechnol.* **14** (1990), 157-167. b) Touraine, F.; R. Drapron: *Can. Inst. Food Sci. Technol. J.* **21** (1988), 255-259. c) Manjon, A.; J. M. Obon; M. Canovas; J. L. Iborra; (in Biocatalysis in Non-Conventional Media; ed. J. Tramper): *Elsevier, Amsterdam* **8** (1992), 121-128. d) Goldberg, M.; D. Thomas; M. D. Legoy: *Enzyme Microb. Technol.* **12** (1990), 976-981. e) Goderis, H. L.; G. Ampe; M. P. Feyten; B. L. Fouwe; W. M. Guffens; S. M. Van Cauwenbergh; P. P. Tobback: *Biotechnol.Bioeng.* **30** (1987), 258-266. f) Khan, S. A.; P. J. Halling; G. Bell: *Enzyme Microb. Technol.* **12** (1990), 453-458. g) Valivety, R. H.; P. J. Halling; A. R. Macrae: *FEBS* **301**(3) (1992), 258-260. h) Sjursnes, B.; L. Kvittingen; T. Anthonsen; P. Halling (in Biocatalysis in Non-Conventional Media; ed. J. Tramper): *Elsevier, Amsterdam* (1992), 451-457.

9: a) Bornscheuer, U.; S. Schapöhler; Th. Scheper; K. Schügerl; W. A. König: *J.Chrom.* **606** (1992), 288-290. b) Bornscheuer, U.; Th. Scheper; W. A. König: in preparation