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Co-Solvent Enhancement of Enantioselectivity in Lipase-Catalysed Hydrolysis of Racemic Esters. A Process for Production of Homochiral C-3 Building Blocks using Lipase B from *Candida antarctica*

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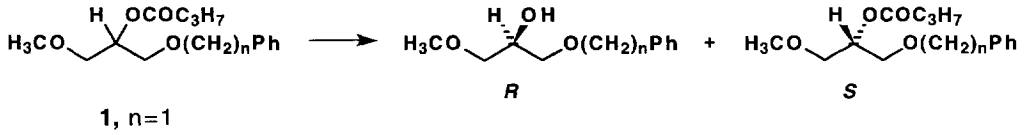
Abstract: Lipase-catalysed hydrolysis of butanoates of 3-methoxy-1-(phenylmethoxy)-2-propanol and 3-chloro-1-(phenylmethoxy)-2-propanol with various lipases gave low enantioselectivity, *E*. By additon of water miscible organic cosolvents, in particular *tert*-butanol and acetone, the *E*-value was raised from 7 to 220 for the useful chloro derivative. This finding has led to proposal of a process for production of homochiral C-3 synthons such as both enantiomers of phenylmethyl glycidyl ether starting from racemic epichlorohydrin. NMR studies of lipase B from *Candida antarctica* show that the conformation most likely is not changed upon addition of up to 50% acetone. Nuclear Overhauser effects were observed upon irradiation of the phenyl protons of the substrate only in the presence of enzyme thus indicating an interaction between the two.

INTRODUCTION

We have for some time been systematically searching for biocatalytic methods to obtain homochiral C-3 synthons by resolution, but so far the enantioselectivity *E* [1]of the reactions has generally been moderate for the most important target molecules. In order to develope a useful process for synthesis, the *E*-value should be above 20, preferable close to or above 100. When the enantioselectivity is low there are in principle three different ways to proceed to improve the

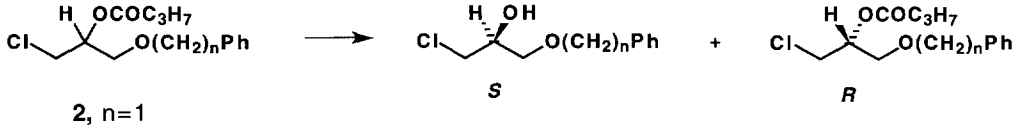
situation, *i)* search for a more suitable biocatalyst either by screening the various available enzymes or by changing the catalytic properties of already excisting enzymes, *ii)* change the substrate preferably by introduction of groups that may easily be removed or substituted afterwards or *iii)* change the reaction conditions such as the solvent system. Previously we have applied the second method, the typical organic chemist's approach, to search for improvements. When primary esters of glycerol with the two other hydroxy groups protected by ketalisation were hydrolysed, the ketalising group influenced the enantioselectivity only to a minor extent and the *E*-value did not exceed 9.[2] For secondary alcohols with various primary substituents the results were as summarized in figure 1 and 2.[3,4]



1, n=1

Figure 1. Lipase catalysed hydrolysis products of secondary butanoates of derivatives of 3-methoxy-1,2-propanediol,[3] n = 0, *E* = 55 (Amano PS), n = 1, *E* = 20, n = 2, *E* > 100, -CH_2CH_2OPh, *E* > 55 (three latter compounds, *Candida antarctica* B).

The absolute stereochemistry of all of the hydrolysis follow the general predictions for lipases.[5] However, it must be mentioned that the *R, S* nomenclature does not directly correspond to the terms "large" and "medium" of the rule. For the present substrates both -CH_2Cl and -CH_2OCH_3 are of "medium size", however, for reasons of nomenclature, the hydrolysis yields predominantly the *R*-alcohol for the methoxy compound and the *S*-alcohol for the chloro derivative.



2, n=1

Figure 2. Lipase catalysed hydrolysis products of secondary butanoates of derivatives of 3-chloro-1,2-propanediol, n = 1, *E* = 15 (PPL), n = 2, *E* = 25 (*Candida antarctica* B).[4]

RESULTS AND DISCUSSION

Our search for an optimized process for resolution started with an investigation of the influence of different cosolvents on the *Candida antarctica* lipase B catalysed hydrolysis of the butanoate of 3-methoxy-1-(phenylmethoxy)-2-propanol **(1)** and the results are shown in *Table 1*. As may be seen some of the solvents give lower *E*-values, but in particular acetone and *tert*-butanol

give considerably higher figures. In subsequent experiments we used these two solvents for the hydrolysis reactions of the two target compounds.

Without cosolvent	20	Diethyl ether	15
Hexane	5	Acetonitrile	21
DMSO	6	Dimethyl formamide	22
Methanol	10	Tetrahydrofuran	29
2-Methyl-2-butanol	14	Ethanol	58
1-Propanol	15	Acetone	70
1-Butanol	15	*tert*-Butanol	98

Table 1. Enantioselectivities after addition of 10% of cosolvents to the *Candida antarctica* lipase B catalysed hydrolysis of the butanoate of 3-methoxy-1-(phenylmethoxy)-2-propanol **(1)**.

The *E*-values obtained for **1** and **2** when the amount of *tert*-butanol and acetone was varied are shown in figures 3a and 3b respectively. For *tert*-butanol the maximum *E*-value was obtained at around 20% (fig. 3a) for both substrates. The enantioselectivity for the butanoate of 3-methoxy-1-(phenylmethoxy)-2-propanol **(1)** increased from 21 to 106 while the corresponding increase for the butanoate of 3-chloro-1-(phenylmethoxy)-2-propanol **(2)** was from 7 to 64.

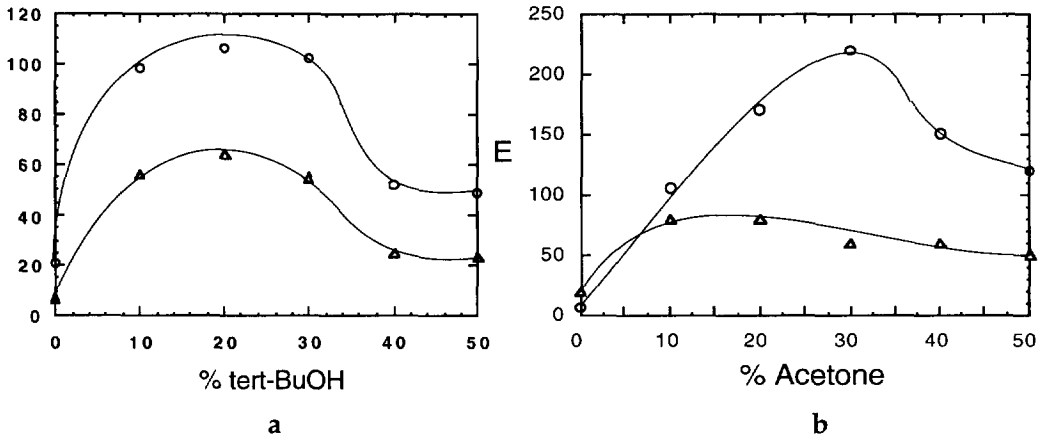


Figure 3. The enantioselectivity *E* of the hydrolysis of butanoates of **1** (-Δ-) and **2** (-O-) as a function of added *tert*-butanol **(a)** and acetone **(b)**.

When acetone was added as the cosolvent the results were not so similar for the two compounds (fig. 3b). The maximum *E*-value for **1** was obtained around 15% acetone in water while the maximum for the chloro compound **2** was at 30%. For this latter compound a tremendous increase in the enantioselectivity was observed. *E* was changed from 7 to 220. These very valuable synthons,[6] the *R* and *S* alcohols of **2**, may under these conditions be prepared easily in good yields and in with very high enantiomeric excesses.

The rate of conversion for the reactions in various amounts of cosolvents have been measured. Generally the rate decreases upon addition of an organic cosolvent. For instance on going from pure water to 20% *tert*-butanol the rate for the two substrates decreased by approximately 10% and in 30% acetone the rate of hydrolysis of **2** was also lowered by 10%. For practical purposes this means that the addition of organic cosolvents in the hydrolysis of these substrates and with lipase B from *Candida antarctica* as catalyst does not represent a serious problem concerning the rate of conversion.

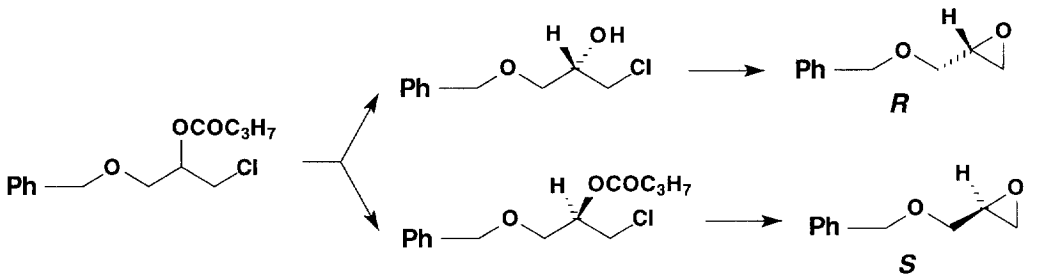


Figure 4. Synthesis of the enantiomers of phenylmethyl glycidyl ether by hydrolysis of the butanoate of 3-chloro-1-(phenylmethoxy)-2-propanol **(2)** catalysed by lipase B from *Candida antarctica.*

We have used our results described here to demonstrate that they may form the basis for a general process to produce homochiral phenylmethyl glycidyl ether by resolution (Figure 4). Both enantiomers had satisfactory *ee* and the overall yield for the reactions in figure 4 was approximately 50% after one attempt.

The basis for the enzyme catalysed resolution is the difference in free energy of activation $\Delta\Delta G^{\neq}$ for reaction with the two enantiomers. This difference which is related to the enantioselectivity *E* by $\Delta\Delta G^{\neq} = -RT\ln E$, also reflects a change in the interaction energy between the enzyme and the two enantiomers. Going from an E-value of 7 to 220 corresponds to an increase in difference of free energy of activation of 2,0 kcal/mole. It may be valid to relate this value to the binding energy of enzyme-substrate interactions measured by Fersht *et al.* for the enzyme tyrosyl tRNA synthetase.[7] The observed energy change may be in the range of changing one or two hydrogen bonds.

We also wanted to investigate whether a positive solvent effect would take place in the transesterification reaction. Reactions with 3-chloro-1-(phenylmethoxy)-2-propanol **(2)** and vinylbutyrate were performed in hexane and in hexane : acetone 70 : 30. The *E* increased from 7 to

15. Also in this case the *S*-alcohol was the faster reacting enantiomer, but the result is not suitable for practical purposes. We have also investigated the effect of water activity on the reaction in hexane, but we conclude that changing the water activity by the salt hydrate method[8] does not influence the enantioselectivity which accord with previous findings.[9] It has been reported, however, that when the chirality lies in the acyl-part of the ester, enantioselectivity of transesterifications in organic media is influenced by the water activity.[10]

In order to trace the origin of the solvent effect we have undertaken preliminary NMR studies of the system. ^{1}H NMR spectra of dissolved lipase B revealed no detectable changes when going from D_2O to acetone-d_6 : D_2O, 50 : 50. This strongly indicates that the conformation of the enzyme at the used acetone concentration does not change. Thus the observed solvent effect is not due to changes in the enzyme. When the phenyl protons were irradiated, no nuclar Overhauser effect was observed on the other resonances. When dissolved enzyme was added a nOe was observed indicating that the correlation time of the substrate had become longer due to interaction with the enzyme. Further NMR studies are in progress. The crystal structure of lipase B from *Candida antarctica* has recently been solved.[11] Molecular modelling of enzyme with the two enantiomeric substrates confirm the observed stereochemical preferences.[12]

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EXPERIMENTAL

Enzymes. Lipase B from *Candida antarctica* Novo-Nordisk SP 435 immobilized on Lewatit had specific activity of 19000 PLU/g. The lipase used for NMR experiments was 50% pure and had specific activity 240 LU/mg. 1 LU releases 1μ mole of fatty acid per minute from a tributyrin emulsion at pH 7.0 and 30°C. 1 PLU is a corresponding unit for immobilized lipase measured during transesterification.[13]

Analytical methods : Enantiomeric excess (%*ee*) of alchols and esters was determined by HPLC using a Varian 9000 system equipped with UV/VIS detector on a chiral column, Chiralcel OB, delivered by J.T. Baker, Deventer, Holland. Optical rotations were determined using an Optical Activity Ltd. AA-10 Automatic Polarimeter, concentrations (c) are given in g/mL. Enantiomeric excess *(ee)* of epoxides was determined by GLC using a chiral column, Chiraldex G-TA, delivered by Astec, Whippany, NJ, USA. NMR-spectroscopy was performed on Bruker AM-500 operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C.

Preparation of 3-methoxy-1-(phenylmethoxy)-2-propanol, 3-chloro-1-(phenylmethoxy)-2-propanol and the corresponding butanoates starting with epichlorohydrin, has been described previously.[3, 4]

General experimental procedure for enzymatic hydrolysis. The butanoates were suspended in 5 mL of 0.20 M phospate buffer at pH 7.2 and the cosolvent was added in various amounts (10, 20, 30, 40 and 50%). Lipase (50 mg) was added and the reaction mixture stirred at room temp. The hydrolysis was stopped by repeated extraction with Et_2O and the reaction mixture was analysed directly by HPLC.

Conversions of R-2 and the corresponding S-alcohol into epoxides. Butanoate **2** (1.5 g) was suspended in phosphate buffer (0.20 M, 140 mL) at pH 7.2, acetone (60 mL) and lipase B (0.5 g) were added. The reaction mixture was stirred for 13h, extracted with Et_2O and the organic phase washed with $NaHCO_3$. After drying with anhyd Na_2SO_4, ester and alcohol were separated on column chromatography (Silica, acetone : hexane 10 : 90). After 51% conversion, this afforded 450 mg of the ester (isolated yield 30%), *ee* >99%, $[\alpha]_D^{20}$ = -9.62 (c 4.30, EtOH) which was stirred with KOH (280 mg, 5mmol) in EtOH (25 mL) at 0°C for 4h. Washing with H_2O, filtering and drying yielded 250 mg of *(S)*-phenylmethyl glycidyl ether, (isolated yield in this step: 92%), *ee* >99%, $[\alpha]_D^{20}$ = +7.82 (c 0.40, EtOH). In a similar manner, the alcohol (380 mg, isolated yield 34 %), *ee* 94%, $[\alpha]_D^{20}$ = +4.38 (c 4.92, EtOH) was stirred with KOH (90 mg) and EtOH (10mL), yielding 220 mg of *(R)*-phenylmethyl glycidyl ether, (isolated yield in this step: 71%), *ee* 94%, $[\alpha]_D^{20}$= -5.81 (c 0.20, EtOH). The overall yields of the enantiomers were 28% of *(S)* and 24% of *(R)*.

REFERENCES

1. Sih, C. J.; Wu, S.-H. *Topics in Stereochemistry* **1989**, *19*, 63 - 125.
2. Partali, V.; Melbye, A.G.; Alvik, T.; Anthonsen, T. *Tetrahedron: Asymmetry* **1992**, *3*, 65-72.
3. Waagen, V.; Hollingsæter, I.; Thorstad O.; Anthonsen, T. *Tetrahedron: Asymmetry* **1993**, *4*, 2265 - 2274.
4. Partali, V.; Waagen, V.; Alvik, T.; Anthonsen, T. *Tetrahedron: Asymmetry* **1993**, *4*, 961 - 968.
5. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656 - 2665.
6. Hanson, R. M. *Chem. Rev.* **1991**, *91*, 437 - 475.
7. Fersht, A. R. *Trends Biochem. Sci.* **1987**, *12*, 301 - 304.
8. Kvittingen, L.; Sjursnes, B. J.; Anthonsen, T.; Halling, P. *Tetrahedron* **1992**, *48*, 2793 - 2802.
9. Bovara, R.; Carrea, G.; Ottolina, G.; Riva, S. *Biotechnol. Lett.* **1993**, *15*, 169 - 174.
10. Högberg, H.-E.; Edlund, H.; Berglund, P.; Hedenström, E. *Tetrahedron: Asymmetry* **1993**, *4*, 2123 - 2126.
11. Uppenberg, J.; Hansen, M. T.; Patkar, S.; Alwyn Jones, T. *Curr. Biol. Structure* **1994**, *2*, 293-308.
12. Uppenberg, J.; Öhrner, N.; Norin, M.; Hult, K.; Patkar, S.; Waagen, V.; Anthonsen, T.; Alwyn Jones, T. EU Bridge-T, Lipase Meeting, Isle de Bendor,France, September 1994.*To be published.*
13. Eigtved, P. Novo-Nordisk A/S, DK-2880 Bagsværd, Denmark. Personal communication.

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