

Regioselective resolution of 1,*n*-diols catalysed by lipases: a rational explanation of the enzymatic selectivity

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Abstract

The regioselective acylation of different phenylalkanedioles catalysed by porcine pancreatic lipase (PPL) was used for modelling the enzymatic substrate recognition. Thus, different racemic or prochiral (1,*n*)-diols, with *n* ranging from 2 to 6, were resolved via transesterification with vinyl acetate, and the results obtained (yield, reaction rate, enantioselectivity) were explained according to the microcrystalline enzyme structure. A logical model for explaining the enzyme regio- and stereoselectivity is proposed, based on literature data reporting similar recognition patterns for some other lipases; our model is built on three residues of the active site (Ser153, Phe216 and His264), which turned out to be crucial for the substrate binding and transformation. Furthermore, some other tentative models proposed for PPL recognition are explained with our criterion. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Porcine pancreatic lipase; Phenylalkanedioles; Transesterification; Active site model.

1. Introduction

Lipase-mediated resolution of chiral alcohols, either by acyl transfer methods or by hydrolysis of their corresponding esters, is probably the biotransformation most commonly described in modern literature [1–3]. If there are two hydroxy groups, the possibility of exploiting both the regio- and the

enantioselectivity of the biocatalyst makes the process even more attractive. When the diol includes a primary and a secondary hydroxy group, the primary one is resolved much faster than the secondary, which generally remains unaltered [4–6]. On the other hand, in the lipase regioselection between two primary hydroxy groups, the discrimination normally is high for prochiral diols [7], although only moderate for racemic mixtures [8], while between two secondary hydroxylic functions, the stereobias generally is acceptable for *meso* [9,10] or prochiral diols [7,11,12], although some *meso* diols are very poor substrates for enzymatic-catalysed transesterification with vinyl acetate [13].

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Several references in lipase literature propose empirical rules (trial and error) for the prediction of the enantioselectivity of different lipases on chiral secondary [14–24] or primary alcohols [25–27], generally based upon the relative site of the substituents around the substrate stereocentre. More recently, some structural basis for explaining these empirical rules have been published, either by studying the 3D structure of different lipases complexed with transition-state mimicking inhibitors (determined by X-ray diffraction) [28,29] or by molecular modelling [30–33].

Nevertheless, the rules proposed for explaining the stereobias of porcine pancreatic lipase (PPL) on primary alcohols are not very convincing because enantiomeric models are proposed [34–37]. Recently, the crystal structure of the ternary porcine lipase–colipase–tetraethylene glycol monoethyl ether (TGME) complex has been determined by X-ray diffraction at 2.8 Å resolution [38,39]. We present the results obtained in the resolution of 1,*n*-phenylalkanediols ($n = 2,3,4,5,6$) by means of PPL-catalysed transesterification with vinyl acetate. The regio- and enantioselectivity is discussed, introducing an active site model based on the crystalline enzyme structure. This qualitative model enables a rational explanation of the enzymatic stereocontrol at a higher level than other previously proposed models.

2. Experimental

2.1. Materials

Lipases (E.C.3.1.1.3) from Porcine Pancreas, crude (Steapsin), type II, and purified, type VI, were obtained from Sigma. The racemic alcohol 1-phenyl-1,2-ethanediol ((±)-**1**) (for numeration of products see Fig. 1) and all the reagents and solvents used were purchased from Aldrich Chemical, Alcobendas, Spain.

2.2. PPL-catalysed transesterification of racemic 2-phenyl-1,*n*-alkanediol

2.2.1. General procedure

A solution of diols **1–5** (6 mmol) and vinyl acetate (48 mmol, 4.4 ml), in diisopropyl ether (15

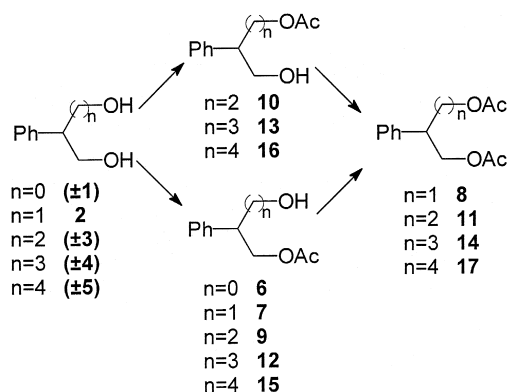


Fig. 1. Overall reaction scheme for the PPL-catalysed transesterification of the substrates.

ml) was stirred at 25°C with PPL (300 mg commercial powder). The water activity of this system, measured using a rotronic hygroskop BT-RS1, was 0.159. This low value, maintained throughout the reaction course as long as no water is produced in transesterifications, ensures the irreversibility of the acyl-transfer process, avoiding any undesired hydrolysis of the esters produced. Then, aliquots of 0.1 ml were taken from the solution (at different times) and added to 0.9 ml of a 80/20 *n*-hexane/isopropanol mixture; after microfiltration, they were analysed by HPLC, using a chiral column Chiralcel OD (cellulose carbamate, 25 cm × 0.46 cm i.d.). The spectrophotometrical quantification ($\lambda = 254$ nm) of product concentration and the enantiomeric excess of the products were calculated using an external standard method.

The following are analysis conditions for the products.

(i) For the resolution of mixtures of (±)-**1**, *R*-**6** and *S*-**6**: isocratic mixture of *n*-hexane/isopropanol (97/3), flow rate = 0.7 ml/min (P₄₀₀ psi). Retention time: *S*-**1**, $t = 46$ min; *R*-**1**, $t = 41$ min; *R*-**6**, $t = 34$ min; *S*-**6**, $t = 30$ min.

(ii) For the resolution of mixtures of **2**, (±)-**7** and **8**: isocratic mixture of *n*-hexane/isopropanol (97/3), flow rate = 0.7 ml/min (P₄₀₀ psi). Retention times: **2**, $t = 34$ min; *R*-**7**, $t = 24$ min; **8**, $t = 13$ min.

(iii) For the resolution of mixtures of (±)-**3**, *R*-**10**, *S*-**10**, *S*-**9**, *R*-**11** and *S*-**11**: *n*-hexane/isopropanol gradient: $t = 0$ min, flow rate = 0.5 ml/min,

98/2 *n*-hexane/isopropanol; $t = 30$ min, flow rate = 1 ml/min, 97/3 *n*-hexane/isopropanol. Retention times: *R*-3, $t = 58$ min; *S*-3, $t = 56$ min; *R*-10, $t = 42$ min; *S*-10, $t = 40$ min; *S*-9, $t = 48$ min; *R*-11, $t = 28$ min; *S*-11, $t = 21$ min.

(iv) For the resolution of mixtures of (\pm)-4, *R*-13, *S*-13, *S*-12, *R*-14 and *S*-14: *n*-hexane/isopropanol gradient: $t = 0$ min, flow rate = 0.5 ml/min, 98/2 *n*-hexane/isopropanol; $t = 25$ min, flow rate = 0.6 ml/min, 97/3 *n*-hexane/isopropanol; $t = 29$ min, flow rate = 1 ml/min, 97/3 *n*-hexane/isopropanol. Retention times: 4, $t = 57$ min (only one peak); 13, $t = 40$ min (only one peak); *S*-12, $t = 46$ min; *R*-14, $t = 22$ min; *S*-14, $t = 20$ min.

(v) For the resolution of mixtures of (\pm)-5, *R*-16, *S*-16, *R*-15, *S*-15, *R*-17 and *S*-17, the same above mentioned solvent gradient was used. Retention times: *S*-5, $t = 68$ min; *R*-5, $t = 64$ min; *S*-16, $t = 47$ min; *R*-16, $t = 45$ min; *S*-15, $t = 53$ min; *R*-15, $t = 50$ min, *S*-17, $t = 19$ min; *R*-17, $t = 17$ min.

At a convenient fixed reaction time, the crude reaction mixture, after removal of the enzyme by filtration, was concentrated and the remaining residue was chromatographically separated on a silica gel column (hexane:EtOAc, 1:2), obtaining fractions containing the monoacetates (major and minor), the diacetates and the remnant diols, structures of which were confirmed by $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ and microanalysis. The absolute configuration of the reaction product was established by comparison with literature data, by $^1\text{H-NMR}$ spectra of the corresponding MTPA (α -methoxy- α -(trifluoromethyl)-phenylacetate) esters and by chiroptical methods (CD technique), as we have recently described [40].

3. Results and discussion

Fig. 1 shows the overall reaction scheme, with the numeration of all the products cited throughout the paper. As can be seen, these compounds may be divided in three groups:

- (a) racemic diols possessing two chemically different (primary and secondary) hydroxy groups, such as (\pm)-1
- (b) prochiral primary diols, such as 2-phenyl-1,3-propanediol (2), and

- (c) racemic diols possessing two primary hydroxy groups, placed at different distance from the stereocentre, such as 2-phenyl-1,4-butanediol ((\pm)-3), 2-phenyl-1, 5-pentanediol ((\pm)-4) and 2-phenyl-1, 6-hexanediol ((\pm)-5).

Therefore, because of their distinct nature, their resolution (or desymmetrisation) will be differently considered.

3.1. Resolution of racemic (\pm)-1

Different compounds possessing the 1,2-diol functionality are useful as synthetic intermediates [8] or as drug intermediates [41,42]. As a consequence, in the literature some enantioselective chemical methods for their preparation can be found [43–45]. The enzymatic preparation of homochiral 1,2-diols have also been reported, either catalysed by lipases [4,46–48] or epoxide-hydrolases [49,50]. In the enantioselective acylation of racemic diols, two cases are described in literature: reaction termination after the first acylation step [51,52], or a first acylation step that quickly leads (with low enantioselectivity) to a racemic monoacetate, which is a better substrate for the enzymatic catalysis, therefore obtaining (with better enantioselectivity) a monoacylated derivative and the corresponding diacylated compound [53,54].

In our case, Fig. 2 shows the progress curves (yield vs reaction time) obtained for the PPL-catalysed acylation of racemic (\pm)-1 with vinyl acetate; for a better visual following of the reaction course, the generation of monoacetate 6 is displayed as two separate curves, corresponding to the single enantiomers, *R*-6 and *S*-6, as calculated by chiral HPLC measurements. As can be seen, we are reporting an example of the above-mentioned first case, due to the fact that no diacetate was observed, and because of the low enantioselectivity obtained in the monoacylation. In fact, from the progress curve of both enantiomers it can be deduced that both enantiomeric monoacetates (*R*-6 and *S*-6) are simultaneously being produced through the parallel and competitive pathway depicted in Fig. 3. As can be seen, the monoacylation of (\pm)-1 only takes place on the primary alcohol, leaving the secondary hydroxy group unaltered, without any trace of diacetate. Al-

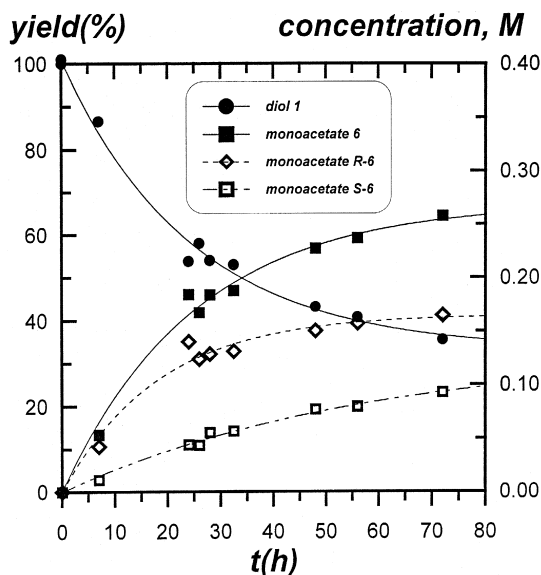


Fig. 2. Progress curve for the resolution of (\pm) -1. Conditions as described in Section 2.

though some examples of resolution of secondary alcohols catalysed by PPL can be found in literature [55–57], in the presence of both $-\text{OH}$ groups only the primary one is acylated.

3.2. Desymmetrisation of 2

Prochiral diols (*meso*-compounds or molecules possessing pseudo-asymmetric centres) are very useful synthetic intermediates due to the fact that the maximum feasible yield upon its enzymatic transformation is not limited to 50%, as what happens when resolving racemic mixtures. In particular, 2-substituted 1,3-propanediols have been extensively used as substrates in lipase-catalysed acylation [7,12,58–60].

In Fig. 4 we present the progress curve obtained in the acylation of **2** with vinyl acetate, following the path shown in Fig. 5. This acylation showed a high *R*-stereopreference in the monoacylation (*ee* > 98% *R*-monoacetate (*R*-7), detecting only traces of *S*-monoacetate (*S*-7); the fitting shown in Fig. 4 for *S*-7 would be explained posteriorly), in agreement with the data reported in the literature [61]. Comparing Figs. 2 and 4, we can observe that **2** is more reactive than (\pm) -1 because the prochiral compound

is completely consumed after 24 h, while 40% of (\pm) -1 remains unaltered after 80 h (Fig. 2). Thus, we can assume that **2** has better steric and electronic properties for being recognized by the active site of the lipase. On the other hand, *R*-7 could be considered as an unstable primary compound, following the classical nomenclature of Campbell and Wojciechowski [62] because it is produced from the earlier reaction steps and consumed as the reaction progresses, as deduced from the slow decrease of its concentration at $t > 20$ h; the diacetate **8**, using the same above-mentioned nomenclature, is a stable primary compound because it is produced from the very beginning of the reaction, and its yield increases with the reaction time. Therefore, we must conclude that *R*-7 and **8** are consecutively produced, but the first acylation is faster than the production of **8**.

3.3. Resolution of (\pm) -3, (\pm) -4 and (\pm) -5

The resolution of racemic acyclic (1,*n*)-diols, with $n > 3$, is not very well documented. Many references can be found in the literature dealing with the asymmetrisation of cyclic primary *meso*-diols, cyclic (generally six- and seven-membered cycles) secondary *meso*-diols, or acyclic *meso* diols possessing

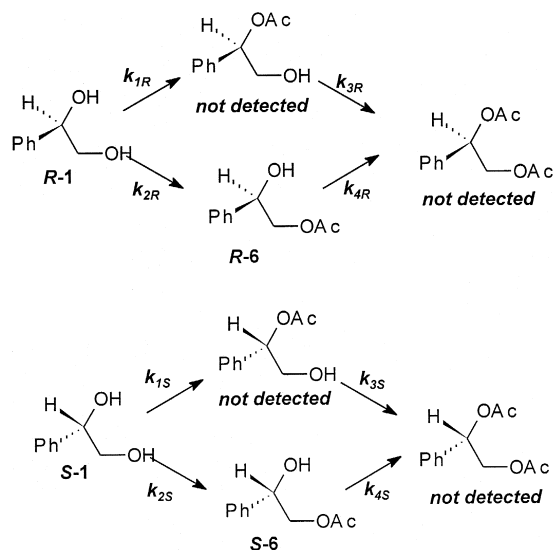


Fig. 3. Reaction pathway for the resolution of both enantiomers of **1**.

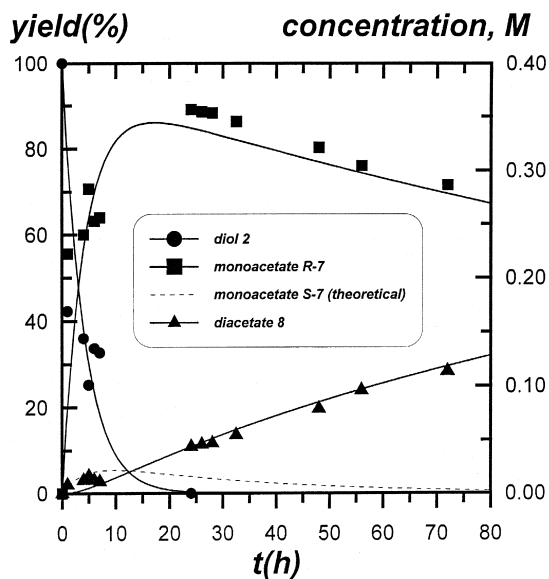


Fig. 4. Progress curve for the desymmetrisation of **2**. Conditions as described in Section 2.

hydroxy groups separated by more than two carbon atoms (for an excellent review about all these compounds, see the work of Kazlauskas and Bornscheuer [63]); nevertheless, the resolution of these «long-distance» racemic diols has only been studied in depth for cyclic compounds [64,65] and very little is described about the resolution of racemic acyclic diols [66,67]. Thus, the resolution of (\pm)-**3**, (\pm)-**4** and (\pm)-**5** may help contribute to this field.

First, these diols were synthesized as described in a previous paper [40] and subsequently used as substrates for the PPL-catalysed acylation with vinyl acetate. The reaction progress was followed by chiral HPLC (See Section 2), and the overall pathway is depicted in Fig. 6.

In all these cases, the regioselective acylation in the primary hydroxy group that is further from the stereocentre was the main process, yielding the monoacetates (major products) **10**, **13**, and **16** (see Fig. 6), with slight *R*-stereopreference, although this decreases as 1,4 goes to the 1,6-diol. On the other hand, the acylation of the primary hydroxy group that is closer to the stereogenic centre rendered the «minor» monoacetates **9**, **12** and **15** (Fig. 6), in lower yields compared to the major compounds, although with higher optical purity (*S*-stereoprefer-

ence, with no traces of the *R*-compounds except for **15**). A second acylation in their free –OH groups led to the *S*-enriched diacetates **11**, **14** and **17** (Fig. 6). Only in the acylation of (\pm)-**3**, the concentration of the major monoacetate **10** slightly decreased with the reaction time, which was mainly due to the *S*-**10** transformation into *S*-**11**, because *R*-**10** remained unaltered at high reaction time. In the other diols (\pm)-**4** and (\pm)-**5**, both major monoacetates achieve a stable yield (55–60%). Thus, comparing the global reactivity of (\pm)-**3**, (\pm)-**4** and (\pm)-**5** with (\pm)-**1** and **2**, we deduced this relative activity:

$$2 > (\pm\text{-}3), (\pm\text{-}4), (\pm\text{-}5) \gg (\pm\text{-}1)$$

In order to quantify the stereoselectivity of PPL in the asymmetrisation of the prochiral diol **2**, we used the mathematical model proposed by Kroutil et al. [68] for sequential two-step asymmetrisation/kinetic resolutions, which is summarised in Fig. 7. In this scheme, *S* is a prochiral (or *meso*) substrate, *P* and *Q* are the enantiomeric products obtained in the asymmetrisation step, while *R* is the prochiral (or *meso*) product obtained via the enzymatic kinetic resolution of *P* and *Q*. The selectivity of this type of reaction is solely governed by the so-called selectivity factor (α), which is equal to the ratio of the apparent first-order constants k_1 and k_2 , while E_2 (the ratio of k_3 and k_4) is the enantioselectivity ratio of the kinetic resolution. Thus, by simply fitting the experimental data to the equations shown in Fig. 7 (the fittings are those indicated in Fig. 4), the values of α and E_2 for the PPL-catalysed asymmetrisation of **2** could be estimated, and they are shown in Table 1. In Fig. 4 we have presented the calculated curve for *S*-**7**, obtained by Eq. 3 (shown in Fig. 7), because

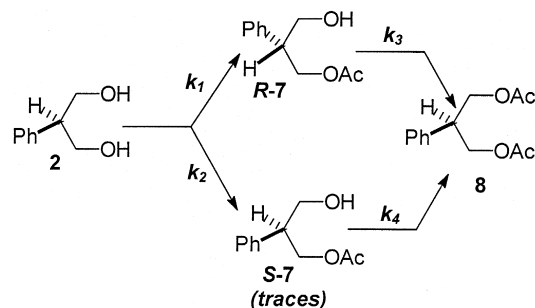


Fig. 5. Reaction pathway for the desymmetrisation of **2**.

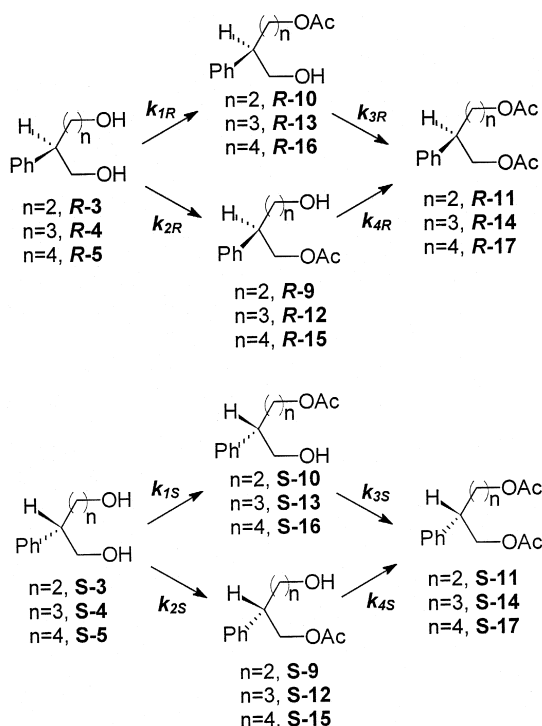


Fig. 6. Reaction pathways for the resolution of 3, 4 and 5.

as we indicated before, the low amount of this compound made its determination impossible.

Fitting the curves obtained for the resolution of the racemic diols with this same model, due to the similarity of the Kroutil's model equations and the behaviour of the catalysed system, some similar constants can be obtained, but now with different meanings, which can be envisaged as follows (taking Fig. 6 as reference):

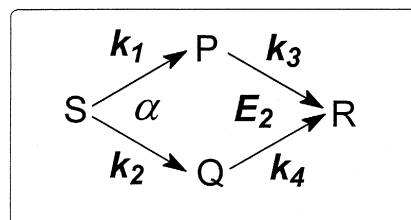
$E_1 = (k_{1R}/k_{1S})$ and $E_2 = (k_{2R}/k_{2S})$ represents the enantioselectivity ratios for the formation of the major and minor monoacetates (acetoxy group further or closer from the stereocentre, respectively).

$E_{DIAC} = (k_{3S} + k_{4S}) / (k_{3R} + k_{4R})$ would quantify the enantioselectivity of the diacetates, because it describes the ratio between the two pathways leading to them.

$rr = (k_{1R} + k_{1S}) / (k_{2R} + k_{2S})$ stands for the regioselectivity ratio, as a quantification of the

overall ratio between the formation of the major and minor monoacetates.

The values of the constants and these three parameters are shown in Table 1, and they are useful to describe the stereo and regioselectivity of the transesterifications. Thus, the acylation of the prochiral diol 2 leads to a *R*-enriched monoacetate 7, because only traces of *S*-7 (< 5%) were detected. So, in order to explain the formation of 8, we assumed the double-step kinetics shown in Fig. 5. In these kind of processes, an «opposite» selectivity is always observed for the second kinetic step compared to that of the first step [68] that is, if $k_1 \gg k_3$, then $k_4 \gg k_2$. This would mean that 8 is formed mainly from the *S*-monoacetate, which is transformed into 8 so quickly that the accumulation of *S*-7 is impossible (and, because of this fact, only traces of *S*-7 are obtained), ($k_4 \gg k_2$). The formation of 8 from the *R*-monoacetate is produced only when the first acylation step is reaching the end, and, because of this fact, the concentration of *R*-7 decreases at long reaction times, as experimentally observed (Fig. 4). This means that $k_1 \gg k_3$. Thus, PPL displays an excellent asymmetrisation capability, and the best enantiomeric excess of the *R*-monoacetate would be obtained by simply stopping the reaction before its consumption gets noticeable ($t_{max} = [1 / (k_3 - (k_1 + k_2))] \times \ln[k_3 / (k_1 + k_2)]$, 17.2 h).



$$S = S_0 e^{-(k_1+k_2)t} \quad \text{Equation 1}$$

$$P = S_0 \frac{k_1}{k_3(k_1+k_2)} [e^{-(k_1+k_2)t} - e^{-k_3t}] \quad \text{Equation 2}$$

$$Q = S_0 \frac{k_2}{k_4(k_1+k_2)} [e^{-(k_1+k_2)t} - e^{-k_4t}] \quad \text{Equation 3}$$

$$R = S_0 - S - P - Q \quad \text{Equation 4}$$

Fig. 7. Model and mathematical equations of Kroutil et al. [68] for the desymmetrisation of pro-chiral compounds.

Table 1
Kinetic constants for the PPL-catalysed transesterification of (1,*n*)-diols

Prochiral diol												
Diol	k_1, h^{-1}	k_2, h^{-1}	α	k_3, h^{-1}	k_4, h^{-1}	E_2						
2	2.2×10^{-1}	1.8×10^{-2}	12.3	4.2×10^{-3}	3.2×10^{-2}	0.13						
Racemic diols												
Diol	k_{1R}, h^{-1}	k_{2R}, h^{-1}	k_{3R}, h^{-1}	k_{4R}, h^{-1}	k_{1S}, h^{-1}	k_{2S}, h^{-1}	k_{3S}, h^{-1}	k_{4S}, h^{-1}	E_1	E_2	E_{DIAC}	rr
1	5.5×10^{-2}	$< 10^{-5}$	$< 10^{-5}$	$< 10^{-5}$	1.7×10^{-2}	$< 10^{-5}$	$< 10^{-5}$	$< 10^{-5}$	3.2	–	–	> 1000
3	9.0×10^{-2}	$< 10^{-5}$	1.3×10^{-3}	$< 10^{-5}$	7.1×10^{-2}	7.6×10^{-2}	2.9×10^{-2}	8.0×10^{-3}	1.3	> 1000	29	2.1
4	1.4×10^{-1}	$< 10^{-5}$	1.8×10^{-2}	$< 10^{-5}$	5.4×10^{-2}	5.7×10^{-2}	$< 10^{-5}$	5.5×10^{-2}	2.6	> 1000	3.1	25
5	6.9×10^{-2}	6.9×10^{-2}	$< 10^{-5}$	3.6×10^{-3}	6.1×10^{-2}	4.0×10^{-2}	$< 10^{-5}$	1.1×10^{-2}	1.1	1.1	3.1	1.2

If we try to explain the first acylation of the racemic diols, we observe how $k_{1R} > k_{1S}$ in all the diols, that is, there is a slightly higher *R*-stereobias in the discrimination between the major monoacetates, which is reversed (*S*-stereopreference) if we consider k_{2S} and k_{2R} , except for the resolution of **5**. On the other hand, $k_{1R} > k_{2R}$, while $k_{2S} \geq k_{1S}$, that is, the process leading to the major monoacetates is faster than that leading to the minor ones, specially for **4** ($rr = 25$). It is noticeable that k_{2R} is practically 0 for **1**, **3** and **4**, (no formation of minor *R*-monoacetates), which obviously implies that $k_{4R} = 0$ in these cases. In the *S*-reaction pathway (Fig. 6) k_{3S} is practically 0 for **4** and **5**. This fact indicates that these *S*-major monoacetates do not suffer a second acylation process.

If we try to explain the second acylation, we must consider that the diacetates can be obtained through two different pathways, that is, from the acylation of both the major and the minor monoacetates, so that in Table 1, for quantifying the enantioselectivity of this second step, we must use E_{DIAC} . On the other hand, the second acylation is governed by the first step, as we indicated in the previous paragraph. As a general rule, we could establish that this second acylation step is generally slower than the first one.

The results obtained for the enzymatic transesterification of the different (1,*n*)-diols are thus demanding a qualitative explanation of the mechanism of the enantioselectivity for this enzyme.

3.4. Proposal of a qualitative model for the interpretation of the PPL regio- and stereoselectivities

With this aim, in order to explain the good regioselectivity in the enzymatic acylation of the OH groups further from the stereocentre, the minimum-energy conformers of the different (1,*n*)-diols were calculated using the HYPERCHEM program [71] (with an AMBER Force Field, and Fletcher–Reeves algorithms (RMS gradient of 0.01 kcal/(Å mol)) to minimise the energy), and were compared with the dimensions of the PPL active site, recently described in literature [38,39]. Fig. 8 shows the geometry of the active site of this enzyme (taken from the PDB structure of the enzyme) highlighting two of the

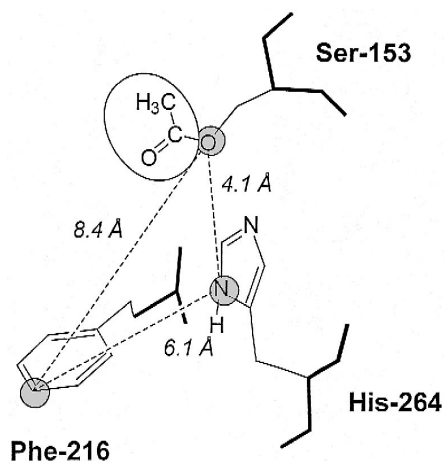


Fig. 8. Dimensions of the active site of PPL [38].

three essential residues from the catalytic triad (Ser153 and His264) as well as Phe216.

Thus, it is possible, according to the dimensions shown in this figure, to postulate the enzymatic recognition of the substrates as exemplified in the structure shown in Fig. 9, obtained by a manual docking of the previously minimised structure of **2** into the active site pocket, keeping the reported geometries [38,39] of the complexes pancreatic lipases–substrates; in this sense, the recognition would be caused by a face to face π – π stacking interaction between Phe216 and the aromatic moiety of the substrates (red colour), as well as by the creation of hydrogen bonds between the non-acylated hydroxy group of the diol and the imidazole ring of His264 (green colour). With these interactions, the –OH group to be acylated should be placed near the catalytic Ser 153 (blue colour). This hypothesis is solidly based on literature data, because from the study of the crystalline structure of different pancreatic lipases (human [70,71] and porcine [38,39]) complexed with different substrates or inhibitors, it can be noticed how in all cases the only residue of the active site that suffers a change upon binding with the inhibitors is Phe216 for pancreatic lipase [38,39] and its equivalent residue Phe215 for human lipase [69,70]. Therefore, these facts indicate the crucial role of this residue for the correct binding of the substrates. On the other hand, in literature some other evidences can be found for the interaction (face to face π – π stacking) of aromatic residues nearby

the active sites of lipases with the aromatic moiety of different substrates, e.g., 2-aryl or 2-aryloxypropionic acids with Trp-88 residue of *Rh. miehei* lipase [72] or with Phe216 for *C. rugosa* lipase [70,72]. Furthermore, the interaction of substrates with His263 of human pancreatic lipase (equivalent to His264 for the porcine lipase) has also been described [70] via a hydrogen bond between oxygen atoms of inhibitors and the *N ϵ* of the imidazole ring.

Good enantioselectivities are obtained only for the monoacetate *R*-7 and the minor monoacetates *S*-9 and *S*-12. Nevertheless, the high optical purity of *S*-9 and *S*-12 may be caused by kinetic reasons due to the low concentration detected, and fitting **2** in the active site would be the most appropriate, because the distances between the three points that we are using as references are very similar to the triangle of the active site. The first acylation of **2** takes place on the *pro-R* hydroxy group because, as can be seen from Fig. 9, even in vacuum (where the modelling of the substrates was carried out), both –OH groups are not placed at the same distance from the aromatic ring, and therefore the substrate does not have to suffer any distortion when reaching the active site. We think that this is the reason why PPL is the most

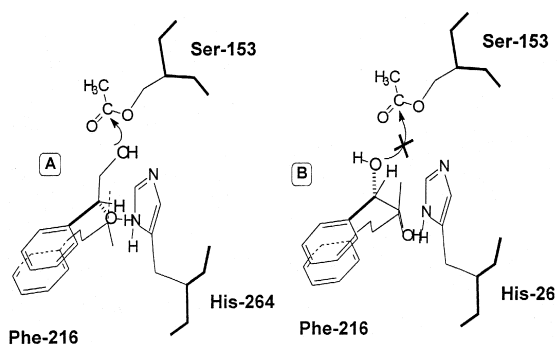


Fig. 10. Schemes for the acylation of **1**. (A) Observed acylation on the primary hydroxy group. (B) Not detected acylation of the secondary hydroxy group.

effective catalyst for the enantiotopic asymmetrisation of 2-substituted 1,3-propanediols [63].

Thus, using our qualitative model, the high regioselectivity in the acylation of (\pm)-**1** can be understood comparing their distances to those of the active site; thus, as represented in Fig. 10, the acylation takes place only at the primary alcohol because it is the only possibility for the correct fit: in fact, in the hypothetical acylation of the secondary –OH group (Fig. 10B), while the distance between the

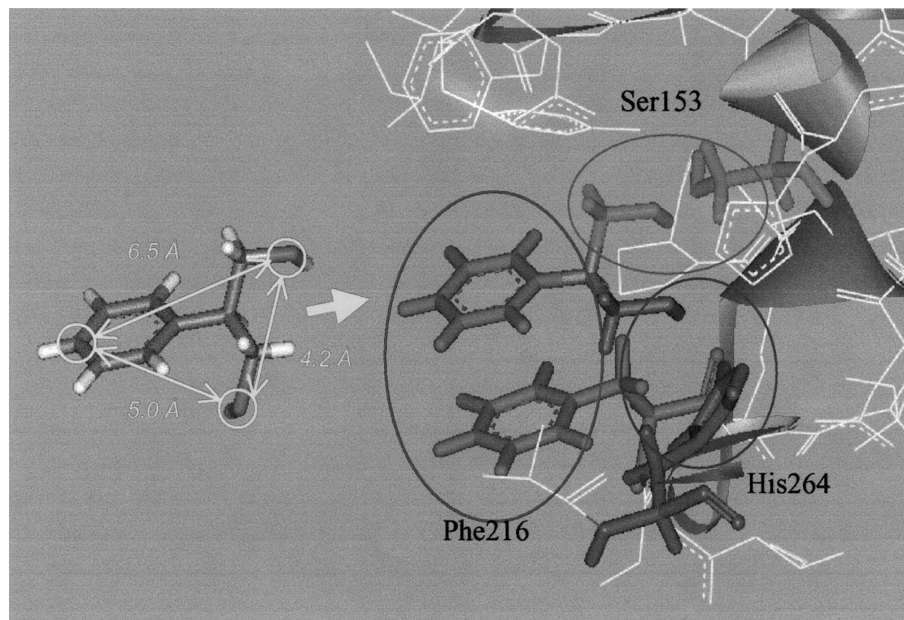


Fig. 9. Manual docking of **2** into the active site of PPL. Red colour, face to face aromatic interaction. Green, H-bonding. Blue, Catalytic Ser153.

aromatic ring and the primary –OH (5.1 Å) could emulate that one between Phe216–His264 (6.1 Å), the distance between the aromatic ring and the secondary –OH (5Å) is absolutely fixed because of the complete lack of conformational flexibility of that moiety, and this distance would never be close to that required for the acylation (around 8.4 Å, according to Fig. 8). On the contrary, the acylation of the primary alcohol of *R*-1 (Fig. 10A) would be produced because this –OH group can reach the acylated serine without any problem.

For the rest of the substrates, their conformational flexibility would allow them to be acylated in the

active site on both hydroxy groups, although the preference observed in the transformation of the longer hydroxyalkyl chain is produced by the best matching of the distance between the aromatic ring and the shorter hydroxyalkyl moiety and the distance between the residues Phe216 and His264; this first recognition of the substrates would enable the larger hydroxyalkyl chain to rotate and flex to reach the acylated serine. This is possible due to the large dimensions of the PPL active site [38].

As we show in Fig. 11, the stereobias observed in the PPL-catalysed transesterification of (1,*n*)-diols would favour the previously reported model A of the

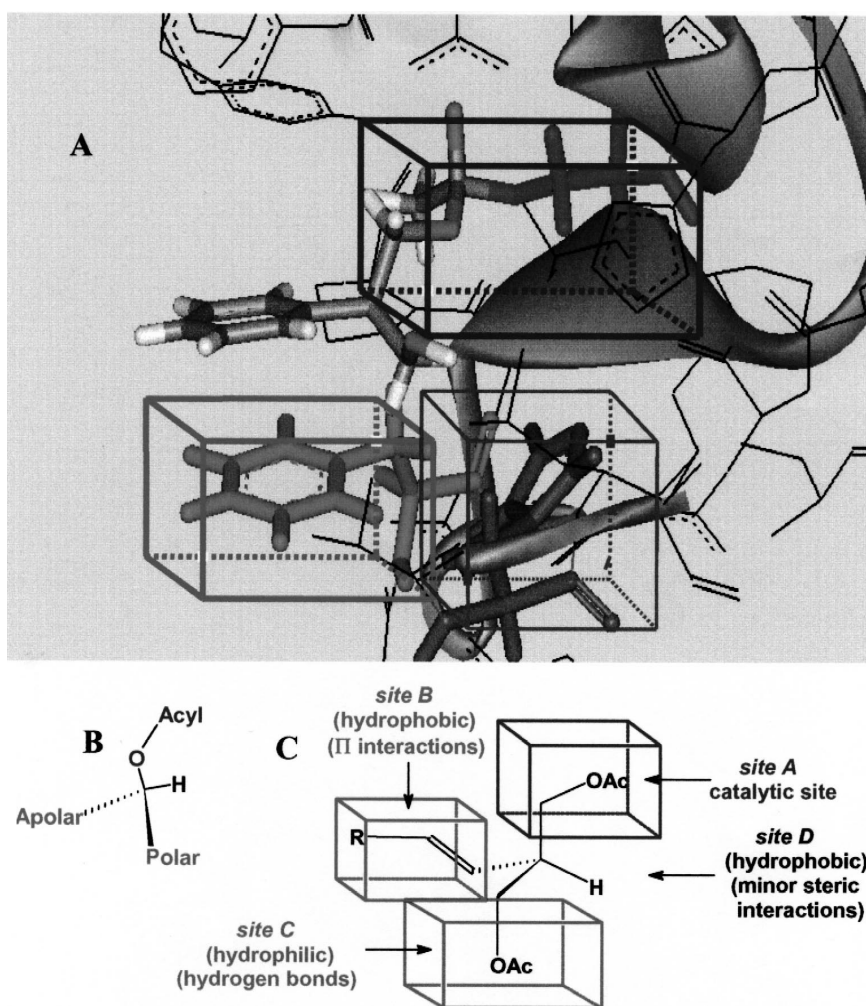


Fig. 11. (A) Our «Box-type» model for the substrate recognition by PPL. (B) Model A of Wimmer [36]. (C) Model of Guanti et al. [37].

PPL active site proposed for hydrolytic processes by Wimmer [36] (Fig. 11B), assuming that the benzene ring is the apolar residue and the hydroxyalkyl group which will not be acylated in the first enzymatic step is the polar moiety; on the other hand, the model defined by Guanti et al. [37] (Fig. 11C) for the hydrolysis of different *Z* or *E*-alkenyl-1,3-diacetoxypropanes could also be explained in terms of our active site study, which is depicted in Fig. 11A for the theoretical transition state structure of **2** covalently linked to PPL via the acylated Ser153, where we propose the residues which would be correlated with the classical subsites models.

Thus, from all the experimental results presented, we propose the following criteria for determining the correct fitting of the diols in the enzymatic active site, based upon its structure:

- (a) Correct orientation of the longer hydroxyalkyl moiety facing the acylated serine and the shorter one facing His264. This criterium would explain the regioselective acylation of the –OH group further from the stereocenter.
- (b) Correct orientation of the phenyl group over the Phe216, which would lead to mainly *R*-compounds in the first acylation (monoacetates) and *S*-compounds in the second step (diacetates).

For the second step in the acylation (leading to the diacetates), the criteria are not that straightforward. Although it seems clear that an acetoxyalkyl group can be also recognized in the polar subsite (His264), as described, [37] this recognition must be poorer than that observed for a hydroxyalkyl moiety, because the second acylation step generally proceeds more slowly than the first one. Nevertheless, this could induce some modifications of the previously mentioned criteria.

As a final conclusion, we have proposed a rational hypothesis for understanding the enzymatic recognition of substrates for PPL, and located the residues responsible for the lipase regio- and stereobias. In order to confirm all these hypothesis, different experiments of molecular dynamics, minimising the enzyme–substrate transition state binding energies, are currently in progress.

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References

- [1] K. Faber, *Biotransformations in Organic Chemistry*, Springer-Verlag, Heidelberg, 1992.
- [2] C.H. Wong, G.M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Elsevier Science, Trowbridge, 1994.
- [3] K. Drauz, H. Waldmann, *Enzyme Catalysis in Organic Synthesis*, VCH, Weinheim, 1995.
- [4] F. Theil, K. Lemke, S. Ballschuh, A. Kunath, H. Schick, *Tetrahedron: Asymmetry* 6 (1995) 1323.
- [5] K. Lemke, F. Theil, A. Kunath, H. Schick, *Tetrahedron: Asymmetry* 7 (1996) 971.
- [6] V. Di Bussolo, G. Catelani, E. Mastroilli, C. di Bugno, R. Giorgi, *Tetrahedron: Asymmetry* 7 (1996) 3585.
- [7] G. Guanti, R. Riva, *Tetrahedron: Asymmetry* 6 (1995) 2921.
- [8] S.P. Ferraboschi, E. Casati, E. Verza, E. Santaniello, *Tetrahedron: Asymmetry* 6 (1995) 1027.
- [9] F. Theil, *Tetrahedron: Asymmetry* 6 (1995) 1693.
- [10] M.S. Nair, A.T. Anilknmar, *Tetrahedron: Asymmetry* 7 (1996) 511.
- [11] T. Eguchi, K. Mochida, *Biotechnol. Lett.* 15 (1993) 955.
- [12] L. Banfi, G. Guanti, R. Riva, *Tetrahedron: Asymmetry* 6 (1995) 1345.
- [13] F. Levayer, C. Rabiller, C. Tellier, *Tetrahedron: Asymmetry* 6 (1995) 1675.
- [14] R.J. Kazlauskas, A.N.E Weissfloch, A.T. Rappaport, L.A. Cuccia, *J. Org. Chem.* 56 (1991) 2656.
- [15] A.E.M. Janssen, C. Klabbbers, M.C.R. Franssen, K. vant Reit, *Tetrahedron* 47 (1991) 7645.
- [16] K. Burgess, L.D. Jennings, *J. Am. Chem. Soc.* 113 (1991) 6129.
- [17] D. Lutz, M. Huffer, D. Gerlach, P. Schreier, in: R. Teranishi, G.R. Takeoka, M. Guentert (Eds.), *Flavor Precursors: Thermal and Enzymatic Conversions*, American Chemical Society, Washington, DC, 1992, p. 32.
- [18] M.J. Kim, H. Cho, *J. Chem. Soc., Chem. Commun.* (1992) 1411.
- [19] K. Naemura, H. Ida, R. Fukuda, *Bull. Chem. Soc. Jpn.* 66 (1993) 573.
- [20] K. Naemura, R. Fukuda, M. Murata, M. Konishi, K. Hirose, Y. Tobe, *Tetrahedron: Asymmetry* 6 (1995) 2385.
- [21] C. Orrenius, N. Örhner, D. Rotticci, A. Mattson, K. Hult, T. Norin, *Tetrahedron: Asymmetry* 6 (1995) 1217.
- [22] K. Naemura, M. Murata, R. Tanaka, M. Yano, K. Hirose, Y. Tobe, *Tetrahedron: Asymmetry* 7 (1996) 1581.

- [23] E. Janes, R.J. Kazlauskas, *Tetrahedron: Asymmetry* 8 (1997) 3719.
- [24] K. Lemke, M. Lemke, F. Theil, *J. Org. Chem.* 62 (1997) 6268.
- [25] G. Carrea, M. De Amici, C. De Micheli, P. Liverani, M. Carnielli, S. Riva, *Tetrahedron* 41 (1993) 1063.
- [26] A.N.E. Weissfloch, R.J. Kazlauskas, *J. Org. Chem.* 60 (1995) 6959.
- [27] O. Goj, A. Burchardt, G. Haufe, *Tetrahedron: Asymmetry* 8 (1997) 399.
- [28] M. Cygler, P. Grochulski, R.J. Kazlauskas, J.D. Schrag, F. Bouthillier, B. Rubin, A.N. Serrequei, A.N.A.K. Gupta, *J. Am. Chem. Soc.* 46 (1994) 3180.
- [29] J. Uppenberg, N. Öhrner, M. Norin, K. Hult, G.J. Kleywegt, S. Patkar, V. Waagen, T. Anthonsen, T.A. Jones, *Biochemistry* 34 (1995) 16838.
- [30] M. Holmquist, F. Haefner, T. Norin, K. Hult, *Protein Sci.* 5 (1996) 83.
- [31] J. Zuegg, H. Höning, J.D. Schrag, M. Cygler, *J. Mol. Catal. B: Enzymatic* 3 (1997) 83.
- [32] P. Berglund, M. Holmquist, K. Hult, *J. Mol. Catal. B: Enzymatic* 5 (1998) 283.
- [33] D. Rotticci, F. Haefner, C. Orrenius, T. Norin, K. Hult, *J. Mol. Catal. B: Enzymatic* 5 (1998) 267.
- [34] J. Ehrler, D. Seebach, *Liebigs Ann. Chem.* (1990) 379.
- [35] G. Hultin, B. Jones, *Tetrahedron Lett.* 33 (1992) 1399.
- [36] Z. Wimmer, *Tetrahedron* 48 (1992) 8431.
- [37] G. Guanti, L. Banfi, E. Narisano, *J. Org. Chem.* 57 (1992) 1540.
- [38] J. Hermoso, D. Pignol, B. Kerfelec, I. Crenon, C. Chapus, C.J.C. Fontecilla-Camps, *J. Biol. Chem.* 271 (1996) 18007.
- [39] J. Hermoso, D. Pignol, S. Penel, M. Roth, C. Chapus, *J.C. Fontecilla-Camps, EMBO J.* 16 (1997) 5531.
- [40] A. Rumbero, I. Borreguero, J.V. Sinisterra, A.R. Alcántara, *Tetrahedron* 55 (1999) 14947.
- [41] J.N. Denis, A. Correa, A.E. Greene, *J. Org. Chem.* 55 (1990) 1957.
- [42] Z.M. Wang, H.C. Kolb, K.B. Sharpless, *J. Org. Chem.* 59 (1994) 5104.
- [43] H.L. Kwong, C. Sorato, H. Chen, Y. Ogino, K.B. Sharpless, *Tetrahedron Lett.* 31 (1990) 2999.
- [44] H.C. Kolb, M.S. van Nieuwenhze, K.B. Sharpless, *Chem. Rev.* 94 (1994) 2483.
- [45] Q.H. Fan, C.H. Yeung, A.S.C. Chan, *Tetrahedron: Asymmetry* 8 (1997) 4041.
- [46] S. Parida, J.S. Dordick, *J. Am. Chem. Soc.* 113 (1991) 2253.
- [47] M.J. Kim, Y.K. Choi, *J. Org. Chem.* 57 (1992) 1605.
- [48] D. Lee, M.J. Kim, *Tetrahedron Lett.* 39 (1998) 2163.
- [49] R.V.A. Orru, S.F. Mayer, N. Kroutil, K. Faber, *Tetrahedron* 54 (1998) 859.
- [50] A. Archelas, *J. Mol. Catal. B: Enzymatic* 5 (1998) 79.
- [51] V.S. Parmar, R. Sinha, K.S. Bishit, S. Gupta, A.K. Prasad, P. Taneja, *Tetrahedron* 49 (1993) 4107.
- [52] R.N. Patel, A. Banerjee, C.G. McNamee, L.J. Szarka, *Tetrahedron: Asymmetry* 6 (1995) 123.
- [53] F. Theil, J. Weidner, S. Ballschuh, A. Kunath, H. Schick, *Tetrahedron Lett.* 34 (1993) 305.
- [54] J. Weidner, F. Theil, H. Schick, *Tetrahedron: Asymmetry* 5 (1994) 751.
- [55] L. Poppe, L. Novák, M. Katjár-Peredy, C. Szántay, *Tetrahedron: Asymmetry* 4 (1993) 2211.
- [56] K. Matsumoto, S. Fuwa, H. Kitajima, *Tetrahedron Lett.* 36 (1995) 6499.
- [57] K. Matsumoto, S. Fuwa, M. Shimojo, H. Kitajima, *Bull. Chem. Soc. Jpn.* 69 (1996) 2977.
- [58] F.R. Alexandre, F. Huet, *Tetrahedron: Asymmetry* 9 (1998) 2301.
- [59] L. Banfi, G. Guanti, A. Mugnoli, R. Riva, *Tetrahedron: Asymmetry* 9 (1998) 2481.
- [60] K. Shishido, T. Bando, *J. Mol. Catal. B: Enzymatic* 5 (1998) 183.
- [61] F. Theil, *Catal. Today* 22 (1994) 517.
- [62] D.R. Campbell, B.W. Wojciechowski, *J. Catal.* 20 (1971) 217.
- [63] R. Kazlauskas, U.T. Bornscheuer, in: H.J. Rehm, G. Reed, A. Pühler, P. Stadler, D.R. Kelly (Eds.), *Biotechnology, 2nd edn., Biotransformations I, Biotransformations with Lipases vol. 8a, Wiley-VCH, Weinheim, 1998, cap. 3.*
- [64] S.M. Roberts, V.G.R. Stenkers, P.L. Taylor, *Tetrahedron: Asymmetry* 4 (1993) 969.
- [65] K. Lemke, S. Ballschuh, A. Kunath, F. Theil, *Tetrahedron: Asymmetry* 8 (1997) 2051.
- [66] P. Grisenti, P. Ferraboschi, S. Casati, E. Santaniello, *Tetrahedron: Asymmetry* 4 (1993) 997.
- [67] K.F. Hsiao, F.L. Yang, S.H. Wu, K.T. Wang, *Biotechnol. Lett.* 18 (1996) 1277.
- [68] W. Kroutil, A. Kleewein, K. Faber, *Tetrahedron: Asymmetry* 8 (1997) 3251.
- [69] HYPERCHEM V. 5.1 Pro for Windows™. Molecular modelling system. Hypercube, and Autodesk, 1997.
- [70] M.P. Egloff, F. Marguet, G. Buono, R. Verger, C. Cambillau, H. van Tilbeurgh, *Biochemistry* 34 (1995) 2751.
- [71] H. van Tilbeurgh, M.P. Egloff, C. Martinez, N. Rugani, R. Verger, R.C. Cambillau, *Nature* 362 (1993) 814.
- [72] M. Botta, E. Cernia, F. Corelli, F. Manetti, S. Soro, *Biochim. Biophys. Acta* 1337 (1997) 302.