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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 1091–1102

A study of the enantiopreference of lipase PS (*Pseudomonas cepacia*) towards diastereomeric dihydro-5-alkyl-4-hydroxymethyl-2(3*H*)-furanones

Federico Berti, Cristina Forzato, Patrizia Nitti, Giuliana Pitacco and Ennio Valentin*

Dipartimento di Scienze Chimiche, Università di Trieste, via Licio Giorgieri 1, I-34127 Trieste, Italy

Received 12 November 2004; revised 10 January 2005; accepted 11 January 2005

Abstract—Lipase PS acetylation of diastereomeric dihydro-4-hydroxymethyl-2(3*H*)-furanones bearing a methyl or a pentyl group at C-5 have been studied. Higher enantioselectivities were found for the *cis*-isomers with respect to the *trans*-isomers. They were also higher for the systems bearing the longer alkyl chain. Lipase PS-catalyzed hydrolyses of racemic acetates were found to follow the same trend, although the efficiency of the enzyme was lower than in the acetylation reactions. These results were supported by molecular modelling studies that correctly predicted the maximum stereoselectivity for the *cis*-isomer of 5-pentyl substituted lactones both in acetylation and in hydrolysis reactions.

1. Introduction

The γ-lactone ring, either isolated or fused, is present in many natural compounds exhibiting biological activity.[1] This peculiarity is in some cases strictly related to the nature and position of the substituents at the heterocycle.[2] Interesting compounds, possessing a hydroxymethyl group at the lactonic β-carbon atom, are known to be involved in the production of secondary metabolites such as antibiotics. The best compound studied is the A-factor,[3] but other molecules have been isolated, such as factor I,[4] factors from *S. bikiniensis* and *S. cyaneofuscatus* and virginiae butanolides[5] from *S. virginiae*, all effective in initiating the synthesis of different antibiotics (Fig. 1).

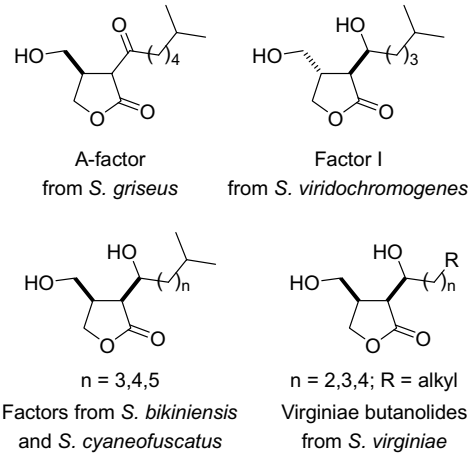

Figure 1. Factors from *Streptomyces*.

In some cases,[6] the importance of the presence of the hydroxymethyl group at β-position for the biological activity of these molecules has been well evidenced. Within the frame of a general study directed towards the synthesis of enantiomerically pure functionalized γ-lactones, we turned our attention to β-hydroxymethyl-γ-lactones bearing a methyl and pentyl group at the γ-carbon atom, namely **1** and **2** (Fig. 2), in both configurations, using the lipase-catalyzed transesterification in the enantiodifferentiating step. Lipases are flexible biocatalysts both for the acylation and deacylation of a wide range of unnatural compounds[7] and in particular they are able to resolve chiral racemic primary alcohols.[8] In this regard lipase PS (*Pseudomonas cepacia*) has been the enzyme of choice in many cases as it has proven to be particularly effective.

* Corresponding author. Tel.: +39 040 5583917; fax: +39 040 5583903; e-mail: valentin@dsch.units.it

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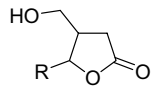
doi:10.1016/j.tetasy.2005.01.036



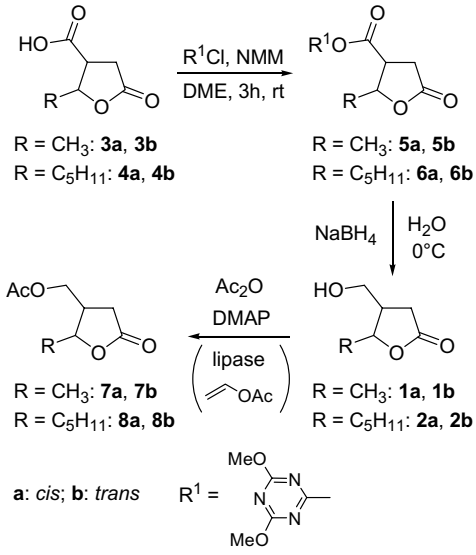
R = CH_3: **1a**,**b**
R = C_5H_{11}: **2a**,**b**
a: *cis*; **b**: *trans*

Figure 2. Substrates **1** and **2**.

2. Results and discussion

2.1. Synthesis of substrates

Racemic lactonic alcohols **1a**, **1b** and **2a**, **2b**[9] were prepared from the corresponding diastereomerically pure lactonic acids **3a**,[10] **3b**[10] and **4a**,[11] **4b**,[12] respectively, following a procedure involving the preliminary formation of the corresponding activated lactonic esters **5a**, **5b** and **6a**, **6b**, by the use of 2-chloro-4,6-dimethoxy[1,3,5]triazine and *N*-methylmorpholine (NMM) in dimethoxyethane[13] (Scheme 1). Subsequent reduction of the activated esters thus obtained with sodium borohydride in water afforded the lactonic alcohols **1a**, **1b** and **2a**, **2b**. This particular methodology was required by the fact that direct reduction of the lactonic acids **3a**, **3b** and **4a**, **4b** with borane in tetrahydrofuran,[9] was less clean and resulted in the formation of a variety of compounds. Acetylation of the lactonic alcohols **1a**, **1b** and **2a**, **2b** with acetic anhydride afforded the corresponding racemic acetates **7a**, **7b** and **8a**, **8b**.

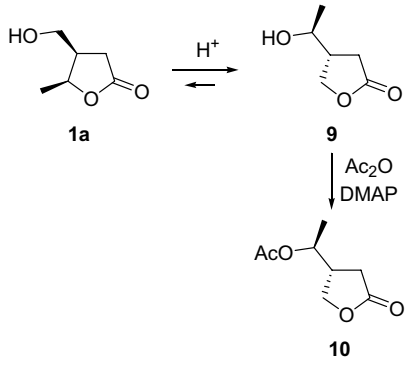


R = CH_3: **3a**, **3b**
R = C_5H_{11}: **4a**, **4b**
R = CH_3: **5a**, **5b**
R = C_5H_{11}: **6a**, **6b**
R = CH_3: **7a**, **7b**
R = C_5H_{11}: **8a**, **8b**
R = CH_3: **1a**, **1b**
R = C_5H_{11}: **2a**, **2b**
a: *cis*; **b**: *trans*
R^1 =

Scheme 1. Synthesis of substrates **1**, **2**, **7** and **8**.

The enantiomerically enriched lactonic alcohols (4*R*,5*S*)-(−)-**1a**, (4*S*,5*S*)-(−)-**1b**, (4*R*,5*S*)-(−)-**2a** and (4*S*,5*S*)-(−)-**2b** have also been prepared, in order to evaluate the enantiomeric purity of the biotransformation products and to determine their absolute configurations, by HRGC analysis. They were obtained from the already known enantiomerically enriched lactonic acids (2*S*,3*S*)-(−)-**3a** (99% ee),[10] (2*S*,3*R*)-(−)-**3b** (99% ee),[10] (2*S*,3*S*)-(−)-**4a** (66% ee),[11] and (2*S*,3*R*)-(−)-**4b** (90% ee),[12] using the same procedure as above. No loss of enantiomeric excess was observed. For the same purpose the corresponding acetates (4*R*,5*S*)-(−)-**7a**, (4*S*,5*S*)-(−)-**7b**, (4*R*,5*S*)-(−)-**8a** and (4*S*,5*S*)-(−)-**8b** were also prepared, by acetylation, either chemical or enzymatic, of the appropriate alcohol.

A comment must be made about the stability of the lactonic alcohols **1a**, **1b** and **2a**, **2b**. Purification of each diastereomer by column chromatography was easily accomplished for all compounds with the exception of the *cis*-lactone **1a**. In fact, under the weak acidic conditions of silica gel, **1a** underwent a partial internal transesterification reaction into the isomeric lactone **9**[14] (Scheme 2). No internal transesterification product derived from the *trans* derivative **1b** was ever detected. An equilibration reaction under acidic conditions carried out on a 4:1 mixture of **1a** and **9** afforded a 3:7 mixture of the two components. From the two mixtures both **1a** and **9**, which were characterized by very similar R_f's, could be isolated as almost pure compounds by flash chromatography, although in low yields. Racemic acetate derivative **10** was also prepared for chiral HRGC analysis.



Scheme 2. Synthesis of substrates **9** and **10**.

2.2. Lipase catalyzed acylation of alcohols 1a, 1b, 2a, 2b and 9

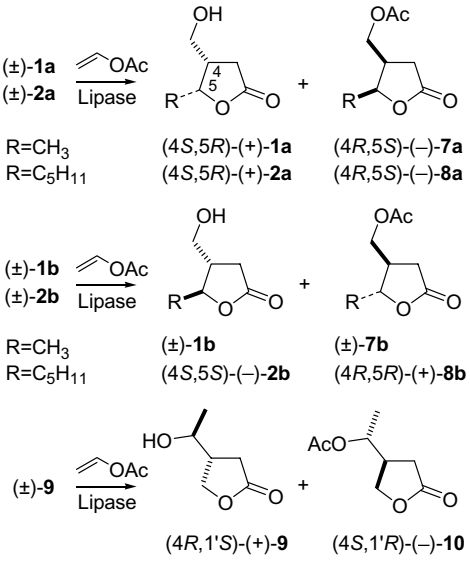
Enzymatic kinetic resolutions of the primary lactonic alcohols **1a**, **1b** and **2a**, **2b** and that of the secondary lactonic alcohol **9** were performed by a transesterification reaction with vinyl acetate catalyzed by lipase PS (from *Pseudomonas* sp., PS, Amano, 5100 U/mmol of substrate). The reactions were monitored by chiral HRGC analysis, quenching the reactions at fixed times. The results of interest are reported in Table 1 and in Scheme 3.

Within the primary alcohols, the racemic *cis*-diastereomers **1a** and **2a** are better substrates for the lipase than the *trans* counterparts **1b** and **2b**, as indicated by their respective E[15] values. Furthermore, **2a**, bearing the pentyl chain at C-γ, seems to interact more favourably (higher E value, Table 1) with the enzyme with respect to **1a**, in which the substituent at C-γ is methyl. Acetylation of the secondary alcohol **9** was highly enantio-

Table 1. Enzymatic acetylation of compounds **1a**, **1b**, **2a**, **2b** and **9** with lipase PS

Substrate	E[a]	Conv. (%)	Time (h)	Ee_s (%)[b]/ config.[c]	Ee_p (%)[b]/ config.[c]
1a	36	24[d]	2	30/(4*S*,5*R*)	93/(4*R*,5*S*)
1b	1	57[b]	1	0	0
2a	150	30[d]	3	43/(4*S*,5*R*)	98/(4*R*,5*S*)
2b	5	36[d]	2	34/(4*S*,5*S*)	60/(4*R*,5*R*)
9	88	24[d]	40	30/(4*R*,1′*S*)	97/(4*S*,1′*R*)

[a] Ref. 15.
[b] Determined by chiral HRGC analysis.
[c] Absolute configurations were determined by chiral HRGC, by comparison with authentic enantiomerically pure samples.
[d] Calculated values, Ref. 16.



Scheme 3. Enzymatic acetylation of compounds **1a**, **1b**, **2a**, **2b** and **9** with lipase PS.

selective but much slower than those of the primary alcohols.

Examining the results in detail, acetylation mediated by lipase PS of the racemic *cis* diastereomer **1a**, which was contaminated by 5% of **9**, owing to the difficulty found in chromatographic separation, afforded the corresponding product (4*R*,5*S*)-(−)-**7a** with 93% ee, after 2 h and the unreacted alcohol (4*S*,5*R*)-(+)-**1a** with 30% ee. Therefore, in order to measure the specific rotations of chemically and enantiomerically pure compounds, a mixture of **1a** and **9**, prepared from lactonic acid (2*S*,3*S*)-(−)-**3a** (99% ee) by reduction, was silylated with *t*-butyldimethylsilyl chloride to the corresponding compounds (4*R*,5*S*)-(−)-**11** and (4*R*,1′*S*)-(+)-**12** (Fig. 3), which were separated by flash chromatography.

Under the same conditions, *cis*-diastereomer (±)-**2a** afforded, after 3 h and 30% calculated conversion,[16] the corresponding acetate (4*R*,5*S*)-(−)-**8a** with 98% ee and 15% isolated yield. The remaining alcohol (4*S*,5*R*)-(+)-**2a**, which was 43% ee, was then subjected to the same enzymatic transesterification, which

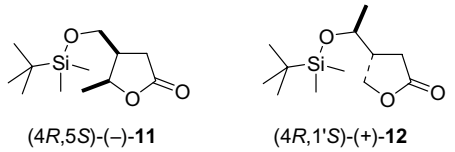


Figure 3. *t*-Butyldimethylsilyl derivatives (−)-**11** and (+)-**12**.

allowed, after 18 h, the isolation of (4*S*,5*R*)-(+)-**2a** with >99% ee and 40% yield.

As anticipated, no selectivity was found for the *trans* diastereomer (±)-**1b**, which however acetylated to furnish **7b** as a racemate at 57% conversion, after 1 h. Also acetylation of (±)-**2b** was unsatisfactory, as, owing to the very low E value, at low conversion value, the resulting acetate (4*R*,5*R*)-(+)-**8b** was obtained with only 60% ee. However, at 70% conversion value, the unreacted alcohol (4*S*,5*S*)-(−)-**2b** with 82% ee was isolated in 30% yield, after 3.5 h.

Finally, lipase-catalyzed acetylation of the secondary lactonic alcohol (±)-**9** was slower than those of the primary lactonic alcohols but the enantioselectivity was high, affording, after 40 h at 24% conversion, (4*S*,1′*R*)-(−)-**10** with 97% ee and the unreacted alcohol (4*R*,1′*S*)-(+)-**9** with 30% ee.

2.3. Lipase catalyzed hydrolyses of the acetate derivatives 7a, 7b, 8a, 8b and 10

The efficiency of the lipase PS was also verified in the enzymatic hydrolyses of the lactonic acetates (±)-**7a**, (±)-**7b**, (±)-**8a**, (±)-**8b** and (±)-**10**, to make a comparison with the acetylation reactions of the corresponding lactonic alcohols, previously discussed. The reactions were carried out in phosphate buffer at pH 7.4. As in transesterification reactions, in the hydrolyses, the *cis* derivatives proved better substrates than the *trans* ones. However, while at very low conversion values (5%) hydrolysis of compound (±)-**7a** gave the corresponding alcohol (4*R*,5*S*)-(−)-**1a** as a pure enantiomer (E >200); during the course of the reaction the product isomerized into the secondary alcohol **9** as a racemate. However, at 60% conversion, after 72 h, the unreacted acetate (4*S*,5*R*)-(+)-**7a** was recovered with 90% ee, thus indicating that hydrolysis was enantioselective.

Hydrolsyses of the *trans* isomers (±)-**7b** and (±)-**8b** resulted in the formation of the corresponding alcohols **1b** and **2b**, the former as a racemate, the latter with 34% ee in favour of the (4*R*,5*R*)-(+)-enantiomer. The remaining acetate (4*S*,5*S*)-(−)-**8b** had 17% ee (33% calculated conversion, $E = 2$). The enantioselectivity observed for the enzymatic resolution of the system (±)-**2b**, containing the longer alkyl chain, although low, indicates an easier accommodation of the molecule within the active site of the enzyme. This observation is supported by the results obtained for hydrolysis of (±)-**8a**, which gave, at 12% calculated conversion, the enantiomeric alcohol (4*R*,5*S*)-(−)-**2a** with 97% ee in 10% yield and the unreacted acetate (4*S*,5*R*)-(+)-**8b** with 13% ee

in 50% yield. The *E* value for hydrolysis was 74, lower than the *E* value found in the case of the acetylation reaction. Evidently, the presence of water decreases the discriminating power of the enzyme. This result will be supported by theoretical calculations.

It is interesting to compare these results with those reported for lipase PS catalyzed hydrolyses of *cis* and *trans* γ-lactones bearing the hydroxymethyl group at the γ-carbon atom and the methyl group at the β-carbon atom.[8b] In this case, enantioselectivity was high for both diastereomers but with opposite enantiopreference. Evidently, an exchange of the two substituents between the β- and γ-position completely changes the interactions with the enzyme.

Finally, it was rather surprising to find out that the hydrolysis of the acetate derived from the secondary alcohol (±)-**10** was not hydrolyzed by lipase PS, even after two days.

2.4. Analysis of the CD spectra

As known, in γ-lactones the sign of the Cotton effect of the n → π* transition band does depend on the configuration of the stereocentres and on the conformation of the lactone ring, which is determined by the relative position and configuration of the substituents at the ring itself.[17] In general γ-lactones bearing a single alkyl chain at their γ-carbon atom exhibit a positive Cotton effect when its configuration is *S* (see compound **13** in Fig. 4).[18] The CD curves for compound (*S*)-(−)-**13**[19] and the *cis* diastereomers (4*R*,5*S*)-(−)-**1a**, (4*R*,5*S*)-(−)-**2a**, (4*R*,5*S*)-(−)-**8a**, and (4*S*,5*S*)-(−)-**14**,[20] all having the same (*S*)-configuration at their C-5, are reported in Figure 4. All compounds are characterized by a positive Cotton effect at about the same wavelength.

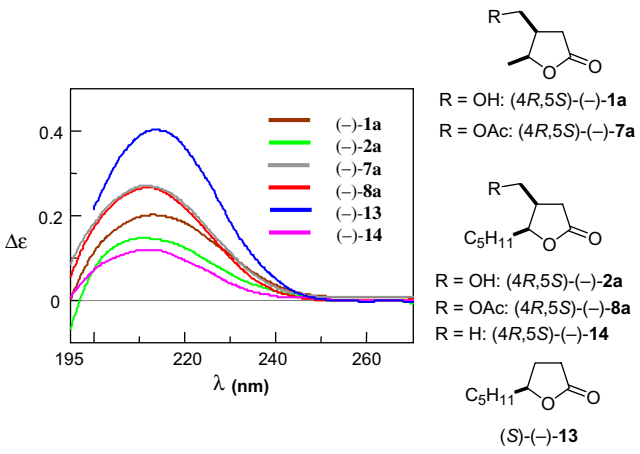


Figure 4. CD spectra.

The CD curves of the *trans* diastereomers (4*S*,5*S*)-(−)-**1b**, (4*S*,5*S*)-(−)-**2b**, (4*S*,5*S*)-(−)-**7b** and (4*S*,5*S*)-(−)-**8b**, reported in Figure 5, are compared with those of (*S*)-(−)-**13**[19] (Fig. 4) and (4*R*,5*S*)-(−)-**15**.[20] Again the positive Cotton effect is related to the configuration of C-5. The bisignate curve exhibited by (4*R*,5*S*)-(−)-**15** is probably due to the presence of a second equally populated conformation of the ring.

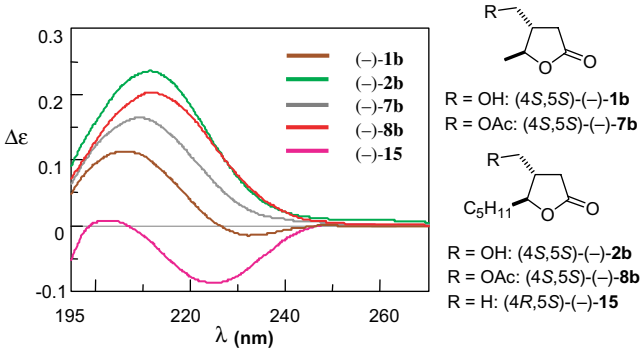
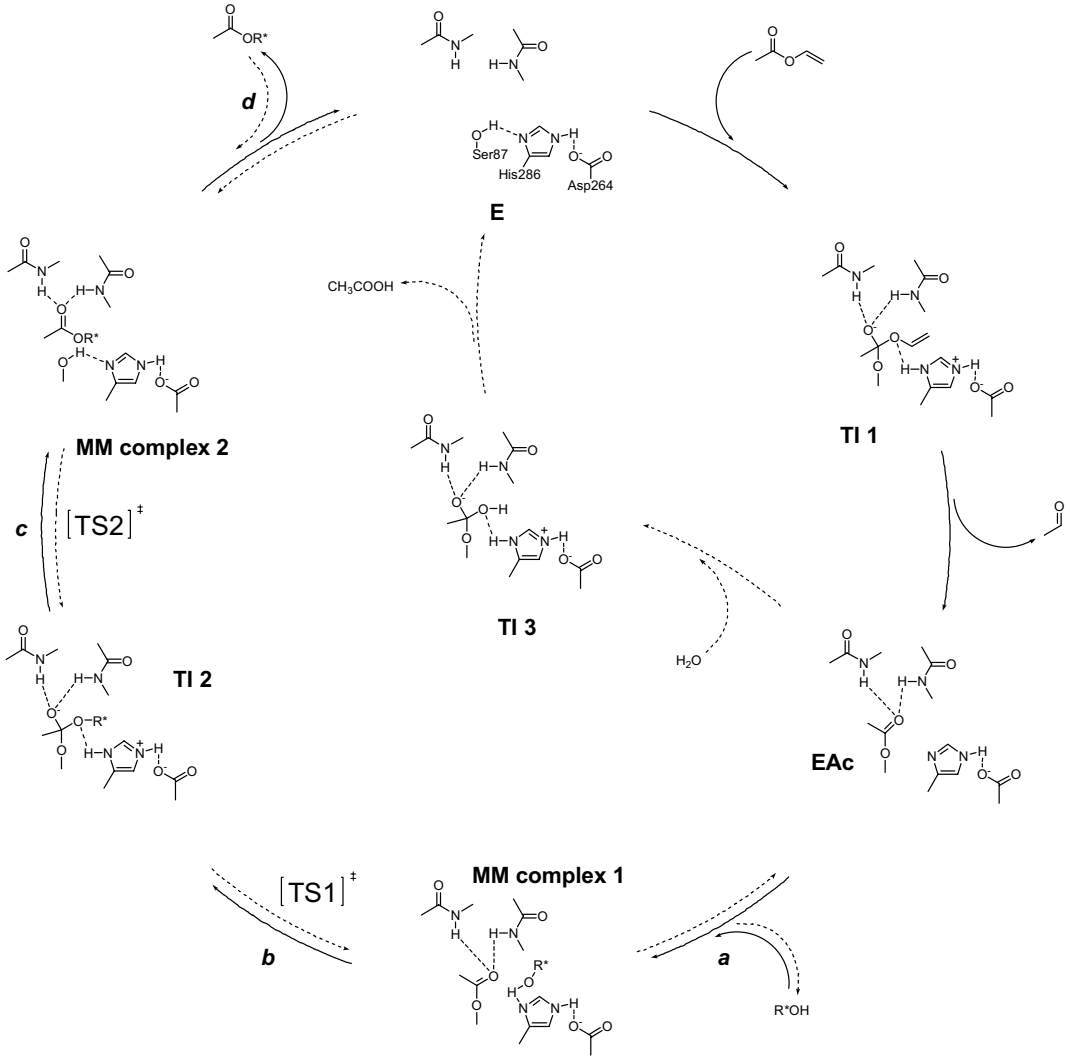


Figure 5. CD spectra.

2.5. Molecular modelling

The enantioselectivity of lipase-catalyzed acyl transfer has been widely studied in the past, and a large number of models, either empirical or structure based, have been proposed. The empirical rules are focused on the size of the substituents at the stereogenic centre of the substrate, and on the definition of favoured orientations of such groups, within a frame of 'box' binding subsites; they lead to satisfactory predictions for the selectivity of hydrolases towards secondary alcohol esters, while they are less consistent if the substrate is a primary alcohol, even if the stereogenic centre is far from the reacting carbon, or if the substrate bears more than one stereogenic centre.[21] However, the mechanism of lipase-acyl transfer is well depicted,[22] and many serine hydrolase structures are now available, thus allowing a deep insight into the structural origin of their selectivity.[23] The lipase catalytic cycle is reported in a simplified way in Scheme 4 for both the synthetic path operating under non-aqueous conditions and with an excess of acyl donor, and for the hydrolytic path, which takes place in water, provided that interfacial activation of the lipase switches the enzyme on.

All the diastereomeric stationary points along the cycle, namely the Michaelis–Menten complexes **MM1** and **MM2**,[24] the tetrahedral intermediate **TI2**[22b,24,25] and the saddles **TS1** and **TS2**,[22b,26] as well as the diastereomeric trajectories of the enantiomeric alcohols incoming to the binding site **a**, and those of the outcoming enantiomeric esters **d**,[27] should, and indeed have been, considered to give a full explanation of the lipase stereoselectivity. Nevertheless, the relative stability of diastereomeric tetrahedral intermediates **TI2** is expected to be the dominating factor, since its formation/decomposition is almost always rate determining, and several evaluations carried out at high levels of theory on model systems have shown that saddle points **TS1** and **TS2** are closely similar to the intermediate, rather than to the Michaelis–Menten complexes.[26] We have thus chosen to evaluate first the relative stability of the tetrahedral intermediates of our reactions, and used as reference structure for building and optimizing the intermediates

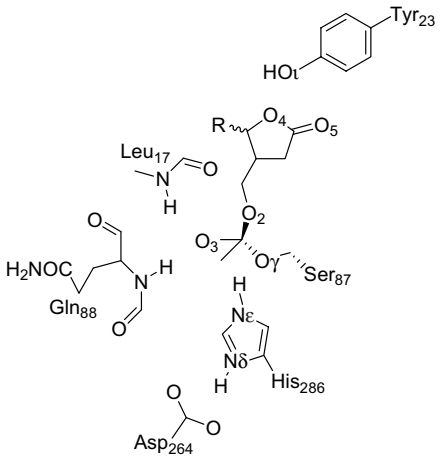


Scheme 4. Catalytic cycle for the lipase-catalyzed acyl transfers.

models, the crystallographic structure of the covalent complex of the lipase from *Burkholderia cepacia* (formerly *P. cepacia*, that is the enzyme used in the experiments) with a phosphonate transition state analogue of 1-phenoxy-2-acetoxybutane, which has recently been solved by Sunjic et al.[23n] The structure of the lipase was downloaded from the Protein Data Bank (PDB id: 1HQD), and after the usual work-up (removal of crystallization water, addition of hydrogens, multistage relaxation,[28] molecular dynamics/mechanics optimization of the initial geometry with the Cornell version of the Amber force field[29]), the eight tetrahedral intermediates corresponding to the acetyl transfer to/from all the stereoisomers of our *cis*/*trans* alcoholic substrates (Table 2) were built by docking their structures over that of the inhibitor. Its phosphorus atom was replaced by a carbon, the methyl group of the inhibitor left in its original place in the initial geometries, and the lactone/alkyl chain of the substrates were built over the phenoxybutane moiety of the inhibitor. In this way the stereochemistry of the tetrahedral intermediates at the reacting carbon was resembling that of the inhibitor at phosphorus, and was thus *R* for all the structures; inversion at this centre was also considered, but it always lead to higher energy geometries. The starting structures of all intermediates were then submitted to an extensive conformational search carried out with a modification of the method developed by Zuegg et al. for a similar problem on this same lipase.[25d] The conformations corresponding to 'productive' intermediates were then selected by structural analysis: the stereoelectronic theory of Deslongchamps predicts that in a tetrahedral intermediate, a C–O bond can be cleaved efficiently only if both of the two remaining oxygens of the intermediate have a lone pair orbital antiperiplanar to the breaking bond.[30] In the lipase-transesterification, this condition is satisfied only if the geometry around the O(Ser87)–Ct–O(alcohol)–R dihedral angle is *gauche* (Fig. 6).

This condition has proven to be effective in the lipase reactions,[31] and it also allows us to establish the optimal hydrogen bond network within the catalytic triad and around the intermediate. It has been thus used as a criterion to select reactive conformations in most of the previous studies.[24b,25d,27,32] We followed the same

Table 2. Energetic and structural parameters for the optimized tetrahedral intermediates TI2



Intermediate	E_{rel} Tot (bonded, *VdW*, Elst) (Kcal mol^{-1})[a]	$H_{\varepsilon His286}$–$O_{\gamma Ser87}$ (Å)	$H_{\varepsilon His286}$–O_2 (Å)	H_{NGln88}–O_γ (Å)	$E_{w,rel}$ (Kcal mol^{-1})[b]	SASA[c] Pol. *hydrof.* (Å^2)
(4*R*,5*S*)-TI2-**1a**	0.00 (0.00, *0.00*, 0.00)	2.36	1.99	2.27	0.00	20.84 *31.56*
(4*S*,5*R*)-TI2-**1a**	4.70 (2.23, *−0.14*, 2.61)	2.40	2.10	2.30	6.00	8.35 *34.77*
(4*R*,5*R*)-TI2-**1b**	0.00 (0.00, *0.00*, 0.00)	2.29	2.04	2.26	0.00	10.40 *11.9*
(4*S*,5*S*)-TI2-**1b**	1.46 (0.45, *−2.12*, 3.13)	2.31	2.07	2.29	1.49	9.11 *36.09*
(4*R*,5*S*)-TI2-**2a**	0.00 (0.00, *0.00*, 0.00)	2.37	1.89	2.28	0.00	7.34 *77.02*
(4*S*,5*R*)-TI2-**2a**	7.03 (−1.96, *3.79*, 5.2)	2.38	2.10	2.32	0.78	5.48 *64.37*
(4*R*,5*R*)-TI2-**2b**	0.00 (0.00, *0.00*, 0.00)	2.29	2.03	2.27	0.00	6.54 *64.34*
(4*S*,5*S*)-TI2-**2b**	1.92 (1.74, *−5.76*, 5.58)	2.31	2.16	2.29	1.80	2.93 *66.12*

[a] Total, bonded, Van der Waals and electrostatic Amber energies for the optimized complexes, expressed relatively to the energies of the 4*R*-isomer for any enantiomeric pair.

[b] Total Amber energy for the complexes optimized within a periodic box of TIP water molecules.

[c] Solvent accessible surface area, calculated as the Connoly surface lined by a spherical probe of 1.4 Å radius, for the polar and hydrophobic atoms of the substrates.

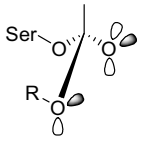


Figure 6. Productive conformation of the tetrahedral intermediates.

method with the lower energy productive conformations selected and further optimized. Energies and selected structural parameters for the optimized intermediates are reported in Table 2.

The relative energy values of Table 2, column 2, show that the model accounts in an excellent way for the observed stereoselectivity. A remarkable preference for the substrates with a (*R*)-configuration at carbon 4 of the lactone ring can be observed within the whole series; this is consistent with the experimental results. Moreover, the energy difference between the enantiomeric substrates is larger for the *cis* compounds, and at a maximum for **2a**, which possesses the longer alkyl chain at position 5. A linear correlation ($r^2 = 0.94$) can be found between the energy difference among the enantiomers and the log of the enantiomeric ratio (Fig. 7).

The contributions of electrostatic, van der Waals and bonded interactions to the total energy difference between the enantiomers of substrate **2a** show that the intermediate formed by the (4*S*,5*R*)-enantiomer is less stable because both the electrostatic/hydrogen bonding and van der Waals interactions established within the intermediate are less favourable, while a lower strain of the whole structure is not sufficient to counterbalance these effects (Table 2, column 2). There are no major differences between the two complexes at the catalytic site level, although hydrogen bonds are somewhat more tight for the (4*R*,5*S*)-complex (Table 2, columns 3, 4,

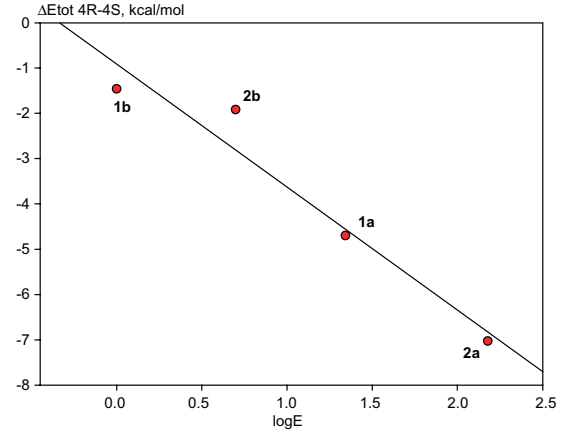


Figure 7. Energy difference among the enantiomers versus log *E*.

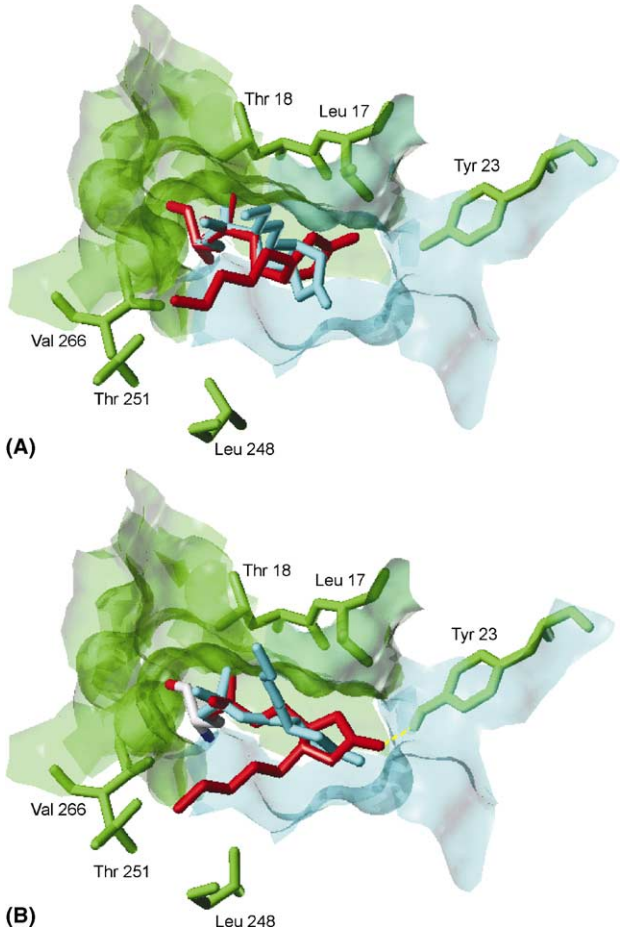


Figure 8. Top view of the overlay of the *cis* (A) and *trans* (B) tetrahedral intermediates into the lipase catalytic site. Red: 4*R*, blue: 4*S*; green surface: hydrophobic channel; blue surface: mixed cavity.

5). Figure 8 shows an overall picture of the binding area, with an overlay of the *cis* intermediates **2a** (Fig. 8A) and of the *trans* intermediates **2b** (Fig. 8B): the lactone ring is always placed into the 'cavity',[25b] a subsite, which contains both hydrophobic and polar residues, while the hydrophobic side of the channel leading to the catalytic site, which hosts the alkyl chain of the glyceride lipase substrates, is here occupied by the pentaatomic alkyl chain of the substrates.

The orientation of the chain is less favourable for the (4*S*,5*R*)-complex than for its enantiomer (4*R*,5*S*) (Fig. 8A): in the first case the pentyl residue establishes hydrophobic contacts only with one side of the site, namely with Leu 17 and Thr 18, while in the (4*R*,5*S*)-complex the chain is also in contact with the other side of the hydrophobic channel, through Leu 248, Val 266 and Thr 251.

If we compare the intermediates formed by *trans*-enantiomers **2b**, we find that the energy difference is smaller because of the electrostatic term: the lactone ring of the (4*R*,5*R*)-complex is placed in a very favourable way, and its carbonyl oxygen is here hydrogen-bonded to the phenolic hydrogen of tyrosine 23 (Fig. 8B).

The intermediates of the γ-methyl compounds **1a** and **1b** resemble the same structural differences described for their long-chain analogues, and the smaller differences in energy appear simply due to the presence of a smaller alkyl chain.

We also investigated the effect of water on the stereoselectivity of the hydrolytic reactions, and the productive intermediates were reoptimized also within a periodic box of TIP water molecules, in order to simulate the aqueous environment (Table 2, column 6). Solvation appears to play an opposite role in the methyl and pentyl series of substrates. Within the long-chain series, water acts in a compensating way and reduces the energy difference between the enantiomeric intermediates, while within the short-chain substrates, the effect of water reinforces the trend already obtained in the absence of solvent. Thus the model correctly predicts the maximum stereoselectivity for the hydrolysis of the *cis* γ-methyl ester **7a**. The water effect could be related to the extent of hydrophobic surface, which remains exposed to water: this is in fact larger for the (4*R*,5*S*)-intermediate (Table 2, column 7) than for the (4*S*,5*R*) one, while it is always larger for the 4*S* intermediates for all the other substrates.

3. Conclusion

In conclusion, the diastereo- and enantiopreference of *P. cepacia* observed in the acetylation reaction of γ-methyl and γ-pentyl-β-hydroxymethyl-γ-lactones has been clearly supported by theoretical calculations.

Conversely, the same enzyme proved less efficient in the hydrolyses of the racemic acetylated alcohol substituted lactones, showing however again a diastereopreference for the *cis* systems.

4. Experimental

4.1. General

IR spectra were recorded on a Jasco FT/IR 200 spectrophotometer and on a Thermo Nicolet Avatar 320 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were run on a Jeol EX-400 spectrometer (400 MHz for proton, 100.5 MHz for carbon), and on a Jeol EX-270 (270 MHz for proton, 67.94 MHz for carbon) using deuteriochloroform as a solvent and tetramethylsilane as the internal standard. Coupling constants are given in Hz. Optical rotations were determined on a Perkin–Elmer Model 241 polarimeter. CD spectra were obtained on a Jasco J-700A spectropolarimeter (0.1 cm cell). GLC analyses were run on a Carlo Erba GC 8000 instrument and on a Shimadzu GC-14B instrument, the capillary columns being OV 1701 (25 m × 0.32 mm) (carrier gas He, 40 KPa, split 1:50) and a Chiraldex^{TM} type G-TA, trifluoroacetyl γ-cyclodextrin (40 m × 0.25 mm) (carrier gas He, 180 KPa, split 1:100) or DiMePe β-cyclodextrin (25 m × 0.25 mm) (carrier gas He, 110 KPa, split 1:50). Enzymic hydrolyses were

performed using a pH-stat Controller PHM290 Radiometer Copenhagen. Mass spectra were recorded on a VG 7070 (70 eV) spectrometer and on an ion trap instrument Finnigan GCQ (70 eV). TLC's were performed on Polygram® Sil G/UV$_{254}$ silica gel pre-coated plastic sheets (eluant: light petroleum-ethyl acetate). Flash chromatography was run on silica gel 230–400 mesh ASTM (Kieselgel 60, Merck). Light petroleum refers to the fraction with bp 40–70 °C and ether to diethyl ether. Lipase PS (*Pseudomonas* species, 30,000 U/g) was purchased from Amano. Compounds **3a**,[10] **3b**,[10] **4a**[11] and **4b**[12] were prepared by hydrolysis of the corresponding ethyl or methyl esters in 6 N HCl under reflux for 2 h.

4.2. Syntheses of lactonic alcohols 1a, 1b, 2a and 2b[13]

To a solution of 2-chloro-4,6-dimethoxy[1,3,5]triazine (0.96 g, 5.5 mmol) dissolved in DME (32 mL), N-methylmorpholine (0.6 mL, 5.5 mmol) was added at room temperature under stirring. A white suspension was formed and to this mixture a solution of the appropriate lactonic acid **3a**, **3b**, **4a** or **4b** (5.5 mmol) in DME (11 mL) added. After 3 h at room temperature the mixture was filtered. The filtrate was cooled to 0 °C and $NaBH_4$ (0.308 g, 8.1 mmol) having been dissolved in water (16 mL) was added. The mixture was stirred for 5 min and acidified (5% $KHSO_4$). Since the lactonic alcohols **1a** and **1b** are very soluble in water, the reaction mixture was evaporated to dryness and treated with ethyl acetate. The solid was filtered and the solvent evaporated. The resulting product **1a** (or **1b**) was purified by flash chromatography. For compounds **2a** and **2b**, the mixture was extracted with ether and the organic layer washed with a solution of 10% Na_2CO_3, brine and dried over anhydrous Na_2SO_4 to give, after evaporation of the solvent, the crude alcohol **2a** (or **2b**), which was purified by flash chromatography (light petroleum/ethyl acetate, gradient from 70:30 to 65:35).

4.2.1. *cis*-Dihydro-4-hydroxymethyl-5-methyl-2(3*H*)-furanone 1a. Purification on silica gel of the crude reaction mixture afforded fractions of different composition in **1a** and **9** (57% overall yield), from which **1a** was separated in admixture (5%) with **9**. Oil; IR cm^{-1}: 3421 (OH), 1762 (COO); ^{1}H NMR, δ, ppm: 4.78 (1H, quintet, *J* 6.6, H-5), 3.73 (2H, apparent d, part AB of an ABX system, C*H*$_2$OH), 2.77 (1H, br s, OH), 2.75–2.40 (3H, m), 1.40 (3H, d, *J* 6.6, CH_3); ^{13}C NMR, δ, ppm: 177.3 (s), 78.7 (d, C-5), 60.5 (t, CH_2OH), 40.1 (d, C-4), 31.5 (t, C-3), 15.3 (q); MS (*m*/*z*): 131 (MH^+, 100), 113 (37), 97 (16), 85 (22), 69 (22), 58 (23), 57 (65), 55 (31); Chiral HRGC, trifluoroacetyl γ-cyclodextrin, 150 °C, retention time: (4*R*,5*S*)-**1a**, 60.8 min; (4*S*,5*R*)-**1a**, 65.3 min. The same procedure carried out on (2*S*,3*S*)-(−)-tetrahydro-2-methyl-5-oxo-3-furancarboxylic acid **3a**[10] with >99% ee to give (4*R*,5*S*)-(−)-**1a** in an admixture with 5% of (4*R*,1′*S*)-(+)-**9** after purification by flash-chromatography. $[\alpha]_D^{25} = -27.3$ (*c* 0.88, CH_3CN), $\Delta\varepsilon_{214} = +0.2$ (CH_3CN).

4.2.2. Dihydro-4-(1-hydroxyethyl)-2(3*H*)-furanone 9. Purification of the crude reaction mixture by flash chromatography furnished **9** as a pure compound in only 10% yield. Oil; IR, cm^{-1}: 3430 (OH), 1766 (COO); ^{1}H NMR, δ, ppm: 4.44 (1H, dd, J_1 7.9, J_2 9.3, H-5), 4.31 (1H, dd, J_1 6.8, J_2 9.3, H-5), 3.80 (1H, quintet, *J* 6.6, C*H*OH), 2.57 (2H, m), 2.42 (1H, vbr s, OH), 2.38 (1H, m), 1.24 (3H, d, *J* 6.6, CH_3); ^{13}C NMR δ, ppm: 177.4 (s), 70.2 (t, C-5), 68.6 (d, CHOH), 42.2 (d, C-4), 31.2 (t, C-3), 21.7 (q); MS (*m*/*z*): 131 (MH^+, 100), 113 (33), 97 (14), 86 (61), 69 (28), 58 (60), 57 (72), 55 (38); Chiral HRGC, trifluoroacetyl γ-cyclodextrin, 150 °C, retention time: (4*R*,1′*S*)-**9**, 40.5 min; (4*S*,1′*R*)-**9** 43.0 min. The same procedure carried out on (2*S*,3*S*)-(−)-tetrahydro-2-methyl-5-oxo-3-furancarboxylic acid **3a**[10] with >99% ee gave (4*R*,1′*S*)-(+)-**9**. $[\alpha]_D^{25} = +20.8$ (*c* 0.24, CH_3CN), $\Delta\varepsilon_{221} = -0.1$ (CH_3CN).

4.2.3. *trans*-Dihydro-4-hydroxymethyl-5-methyl-2(3*H*)-furanone 1b. Oil (51% yield); IR, cm^{-1}: 3431 (OH), 1761 (COO); ^{1}H NMR, δ, ppm: 4.52 (1H, quintet, *J* 6.2, H-5), 3.68 (2H, apparent d, part AB of an ABX system, C*H*$_2$OH), 3.30 (1H, br s, OH), 2.65 (1H, dd, J_1 9.0, J_2 17.8, H-3), 2.49 (1H, dd, J_1 8.0, J_2 17.8, H-3), 2.33 (1H, m, H-4), 1.44 (3H, d, *J* 6.6, CH_3); ^{13}C NMR, δ, ppm: 177.1 (s), 79.4 (d, C-5), 61.8 (t, CH_2OH), 44.4 (d, C-4), 31.4 (t, C-3), 20.3 (q); MS (*m*/*z*): 131 (MH^+, 100), 113 (76), 97 (20), 88 (21), 69 (30), 57 (65), 55 (80); Chiral HRGC, trifluoroacetyl γ-cyclodextrin, 150 °C, retention times: (4*R*,5*R*)-(+)-**1b**, 47.9 min; (4*S*,5*S*)-(−)-**1b**, 63.0 min. DiMePe β-cyclodextrin; 150 °C, retention times: (4*R*,5*R*)-(+)-**1b**, 23.2 min (4*S*,5*S*)-(−)-**1b**, 25.1 min. The same procedure, carried out on (2*S*,3*R*)-(−)-tetrahydro-2-methyl-5-oxo-3-furancarboxylic acid **3b**[10] with >99% ee, gave the alcohol (4*S*,5*S*)-(−)-**1b** with the same enantiomeric excess. (4*S*,5*S*)-(−)-**1b**: $[\alpha]_D^{25} = -23.2$ (*c* 0.41, MeOH); $[\alpha]_D^{25} = -22.5$ (*c* 0.36, MeCN); $\Delta\varepsilon_{206}$ +0.2 (MeOH); $\Delta\varepsilon_{206}$ +0.1 (MeCN).

4.2.4. *cis*-Dihydro-4-hydroxymethyl-5-pentyl-2(3*H*)-furanone 2a. Oil (60% yield), IR, cm^{-1}: 3457 (OH), 1773 (COO); ^{1}H NMR, δ, ppm: 4.54 (1H, m, H-5), 3.72 (2H, m, C*H*$_2$OH), 3.20 (1H, br s, OH), 2.66 (2H, m), 2.50 (1H, m), 1.75–1.50 (3H, m), 1.43–1.25 (5H, m), 0.90 (3H, t, CH_3); ^{13}C NMR, δ, ppm: 177.1 (s), 82.7 (d, C-5), 60.5 (t, CH_2OH), 40.0 (d, C-4), 32.6 (t), 31.5 (t), 29.8 (t), 25.8 (t), 22.4 (t), 13.9 (q); MS (*m*/*z*): 158 (39), 116 (100), 97 (27), 86 (44), 85 (20), 83 (10), 69 (21), 58 (21), 57 (37), 55 (29). Chiral HRGC, DiMePe β-cyclodextrin, 150 °C, retention times: (4*R*,5*S*)-(−)-**2a**, 153.6 min; (4*S*,5*R*)-(+)-**2a**, 163.7 min. The same procedure carried out on (2*S*,3*S*)-(−)-tetrahydro-5-oxo-2-pentyl-3-furancarboxylic acid **4a**[11] with 66% ee gave the alcohol (4*R*,5*S*)-(−)-**2a** with the same enantiomeric excess.

4.2.5. *trans*-Dihydro-4-hydroxymethyl-5-pentyl-2(3*H*)-furanone 2b. Oil (60% yield); all spectroscopic data are in accordance to the literature;[9] MS (*m*/*z*): 144 (10), 116 (100), 99 (17), 97 (58), 86 (41), 85 (42), 83 (12), 71 (12), 69 (39), 68 (10), 58 (22), 57 (63), 56 (12), 55 (43). Chiral HRGC, DiMePe β-cyclodextrin, 150 °C, retention times: for (4*R*,5*R*)-(+)-**2b**, 131.0 min, (4*S*,5*S*)-(−)-**2b**, 142.7 min.

The same procedure carried out on (2*S*,3*R*)-(−)-tetrahydro-5-oxo-2-pentyl-3-furancarboxylic acid **4b**[12] with 90% ee gave the alcohol (4*S*,5*S*)-(−)-**2b** with the same enantiomeric excess. (4*S*,5*S*)-(−)-**2b**: $[\alpha]_D^{25} = -39.7$ (*c* 0.88, MeOH); $[\alpha]_D^{25} = -37.4$ (*c* 0.82, MeCN); $\Delta\varepsilon_{209}$ +0.2 (MeOH); $\Delta\varepsilon_{211}$ +0.2 (MeCN).

4.3. Synthesis of acetates 7a, 7b, 8a, 8b and 10

To a solution of the appropriate alcohol (1.2 mmol) in dioxane (5 mL), (dimethylamino)pyridine (DMAP) (0.290 g, 2.4 mmol) and acetic anhydride (0.36 mL, 3.6 mmol) were added. The mixture was stirred at room temperature overnight, the solvent was evaporated, 10% HCl then added and the aqueous layer extracted with ether or ethyl acetate. The organic phase was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded the acetate products as pure compounds.

4.3.1. *cis*-Dihydro-4-acetoxymethyl-5-methyl-2(3*H*)-furanone 7a. Oil (98% yield); IR, cm^{-1}: 1780, 1740 (COO); ^1H NMR, δ, ppm: 4.77 (1H, quintet, *J* 6.6, H-5), 4.16 (2H, m, C*H*$_2$OCOCH$_3$), 2.84 (1H, m, H-4), 2.69 (1H, dd, J_1 8.6, J_2 17.5, H-3), 2.43 (1H, dd, J_1 5.9, J_2 17.5, H-3), 2.08 (3H, s, CH_3), 1.37 (3H, d, *J* 6.6, CH_3); ^{13}C NMR δ, ppm: 175.6 (s), 170.6 (s), 77.6 (d, C-5), 62.3 (t, CH_2O), 37.5 (d, C-4), 32.1 (t, C-3), 20.6 (q, CH_3), 15.4 (q, CH_3); MS (*m*/*z*): 173 (MH^+, 100), 131 (28), 112 (45), 97 (24), 86 (19), 68 (58), 69 (41).

Lipase PS catalyzed acetylation of lactonic alcohol (4*R*,5*S*)-(−)-**1a** obtained from (2*S*,3*S*)-(−)-2-methyl-5-oxo-tetrahydro-3-furancarboxylic acid **3a**[10] with >99% ee afforded (4*R*,5*S*)-(−)-**7a** with >99% ee: $[\alpha]_D^{25} = -24.3$ (*c* 0.30, MeCN); $\Delta\varepsilon_{212}$ +0.3 (MeCN).

4.3.2. *trans*-Dihydro-4-acetoxymethyl-5-methyl-2(3*H*)-furanone 7b. Oil (76% yield); IR, cm^{-1}: 1775, 1741 (COO); ^1H NMR, δ, ppm: 4.43 (1H, quintet, *J* 6.2, H-5), 4.12 (2H, part AB of an ABX system, J_{AB} 11.3, C*H*$_2$OCOCH$_3$), 2.71 (1H, m), 2.50 (2H, m), 2.09 (3H, s, CH_3), 1.45 (3H, d, *J* 6.2, CH_3); ^{13}C NMR, δ, ppm: 175.4 (s), 170.7 (s), 79.0 (d, C-5), 63.8 (t, *C*H$_2$O-COCH$_3$), 41.9 (d, C-4), 32.0 (t, C-3), 20.7 (q, CH_3), 20.4 (q, CH_3); MS (*m*/*z*): 173 (MH^+, 100), 172 ($M^{+\cdot}$, 26), 144 (21), 143 (18), 131 (24), 103 (15), 95 (12), 84 (36), 69 (17); Chiral HRGC: trifluoroacetyl γ-cyclodextrin, 150 °C, retention times: (4*S*,5*S*)-(−)-**7b**, 21.9 min; (4*R*,5*R*)-(+)-**7b**, 23.3 min. Lipase PS catalyzed acetylation of lactonic alcohol (4*S*,5*S*)-(−)-**1b** obtained from (2*S*,3*R*)-(−)-2-methyl-5-oxo-tetrahydro-3-furancarboxylic acid **3b**[10] with >99% ee afforded (4*S*,5*S*)-(−)-**7b** with >99% ee: $[\alpha]_D^{25} = -16.2$ (*c* 0.47, MeOH); $[\alpha]_D^{25} = -18.1$ (*c* 0.47, MeCN); $\Delta\varepsilon_{208}$ +0.2 (MeOH); $\Delta\varepsilon_{210}$ +0.2 (MeCN).

4.3.3. *cis*-4-Dihydro-4-acetoxymethyl-5-pentyl-2(3*H*)-furanone 8a. Oil (90% yield); IR, cm^{-1}: 1782, 1745 (COO); ^1H NMR, δ, ppm: 4.54 (1H, m, H-5), 4.21 (1H, dd, J_1 5.9, J_2 11.3, C*H*OCOCH$_3$), 4.06 (1H, dd, J_1 6.6, J_2 11.3, C*H*OCOCH$_3$), 2.81 (1H, m, H-4), 2.69 (1H, dd, J_1 8.0, J_2 17.2, H-3), 2.44 (1H, dd, J_1 4.4, J_2 17.2, H-3), 2.07 (3H, s, CH_3), 1.68 (2H, m), 1.54 (2H, m), 1.35–1.20 (4H, m), 0.90 (3H, t, CH_3), ^{13}C NMR, δ, ppm: 175.6 (s), 170.7 (s), 81.8 (d, C-5), 62.3 (t, *C*H$_2$O-COCH$_3$), 37.4 (d, C-4), 32.8 (t), 31.5 (t), 30.0 (2 t), 22.4 (t), 20.8 (q), 13.9 (q); MS (*m*/*z*): 168 (13), 157 (28), 128 (18), 116 (100), 99 (12), 98 (75), 86 (36), 85 (19), 71 (12), 69 (18), 68 (46), 57 (12), 55 (28). Chiral HRGC, DiMePe β-cyclodextrin, 150 °C, retention times: (4*R*,5*S*)-(−)-**8a**, 79.6 min; (4*S*,5*R*)-(+)-**8a**, 81.4 min.

4.3.4. *trans*-Dihydro-4-acetoxymethyl-5-pentyl-2(3*H*)-furanone 8b. Oil (90% yield); IR, cm^{-1}: 1778, 1743 (COO); ^1H NMR, δ, ppm: 4.28 (1H, q, *J* 6.2, H-5), 4.12 (2H, part AB of an ABX system, J_{AB} 11.3, C*H*$_2$OCOCH$_3$), 2.71 (1H, dd, J_1 8.8 J_2 17.3, H-3), 2.51 (1H, m, H-4), 2.40 (1H, dd, J_1 7.3, J_2 17.3, H-3), 2.09 (3H, s, $COCH_3$), 1.67 (2H, m), 1.53–1.25 (6H, m), 0.90 (3H, t, *J* 7.0, CH_3); ^{13}C NMR, δ, ppm: 175.5 (s), 170.6 (s), 82.6 (d, C-5), 64.2 (t, *C*H$_2$OCOCH$_3$), 39.7 (d, C-4), 35.0 (t), 31.7 (t), 31.4 (t), 25.0 (t), 22.4 (t), 20.7 (q), 13.9 (q); MS (*m*/*z*): 157 (21), 140 (33), 116 (100), 99 (37), 98 (91), 86 (49), 85 (33), 84 (10), 83 (13), 81 (10), 71 (25), 70 (11), 69 (35), 68 (27), 67 (12), 58 (22), 57 (47), 56 (13), 55 (48), 54 (11). Chiral HRGC, DiMePe β-cyclodextrin, 150 °C, retention times: (4*R*,5*R*)-(+)-**8b**, 72.5 min; (4*S*,5*S*)-(−)-**8b** 74.9 min.

Acetate (4*S*,5*S*)-(−)-**8b** with 82% ee was obtained, by the procedure described above, from alcohol (4*S*,5*S*)-(−)-**2b** (82% ee), isolated from lipase PS-catalyzed acetylation of (±)-**2b** (Section 4.5.5).

(4*S*,5*S*)-(−)-**8b**: $[\alpha]_D^{25} = -25.3$ (*c* 0.49, MeOH); $[\alpha]_D^{25} = -25.8$ (*c* 0.40, MeCN); $\Delta\varepsilon_{211}$ +0.3 (MeOH); $\Delta\varepsilon_{211}$ +0.2 (MeCN).

4.3.5. Dihydro-4-(1-acetoxyethyl)-2(3*H*)-furanone 10. Oil (98% yield); IR, cm^{-1}: 1780, 1730 (COO); ^1H NMR, δ, ppm: 4.96 (1H, quintet, *J* 6.2, C*H*OCOCH$_3$), 4.41 (1H, dd, J_1 7.7, J_2 9.4, H-5), 4.18 (1H, dd, J_1 6.8, J_2 9.4, H-5), 2.76 (1H, m, H-4), 2.61 (1H, dd, J_1 9.1, J_2 17.5, H-3), 2.32 (1H, dd, J_1 7.6, J_2 17.5, H-3), 2.06 (3H, s, CH_3), 1.27 (3H, d, *J* 6.2, CH_3); ^{13}C NMR, δ, ppm: 176.1 (s), 170.2 (s), 70.7 (d, CH_3*C*HO), 69.3 (t, C-5), 39.8 (d, C-4), 30.7 (t, C-3), 20.8 (q), 17.7 (q); MS (*m*/*z*): 173 (MH^+, 52), 172 ($M^{+\cdot}$, 100), 112 (34), 68 (34).

Chemical acetylation of (4*R*,1′*S*)-(+)-**9** with 99% ee afforded (4*R*,1′*S*)-(+)-**10**. $[\alpha]_D^{25} = +23.3$ (*c* 0.51, MeCN); $\Delta\varepsilon_{211}$ −0.6 (MeCN).

4.4. Derivatization of 1a and 9 with *t*-butyldimethylsilyl chloride (TBDMSCl)

TBDMSCl (0.414 g, 2.7 mmol) and imidazole (0.375 g, 5.5 mmol) were added to a 65:35 mixture of **1a** and **9** (0.120 g, 0.9 mmol) in DMF (3 mL). The mixture was stirred at room temperature for 48 h. At the end of the reaction, water was added and the mixture extracted with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4 and the solvent evaporated. The crude reaction mixture was purified by flash-chromatography

(light petroleum/ethyl acetate, gradient from 95:5 to 90:10) to afford compounds **11** and **12**.

4.4.1. *cis*-Dihydro-4-(*t*-butyldimethylsilyloxy)methyl-5-methyl-2(3*H*)-furanone 11. Oil (50% yield); IR ($CHCl_3$), cm^{-1}: 1766 (COO); ^1H NMR, δ, ppm: 4.73 (1H, quintet, J 6.6, H-5), 3.68 (2H, part AB of an ABX system, J 10.2, CH_2O), 2.64–2.57 (2H, m), 2.44–2.37 (1H, m), 1.39 (3H, d, J 7.0, CH_3), 0.89 (9H, s, $C(CH_3)_3$), 0.06 (3H, s, CH_3), 0.05 (3H, s, CH_3); ^{13}C NMR, δ, ppm: 176.5 (s), 78.6 (d), 61.4 (t), 40.4 (d), 32.2 (t), 25.8 (3q), 18.1 (s), 15.5 (q), −5.6 (2q); MS (*m*/*z*): 245 (MH^+, 57), 187 (10), 169 (10), 95 (11), 81 (10), 75 (22), 57 (15).

The same procedure carried out on (4*R*,5*S*)-**1a** with 99% ee gave (4*R*,5*S*)-(−)-**11**; $[\alpha]_D^{25} = -19.0$ (c 0.30, CH_3CN); $\Delta\varepsilon_{208}$ +0.2 (CH_3CN).

4.4.2. Dihydro-4-(1-(*t*-butyldimethylsilyloxy)ethyl)-2(3*H*)-furanone 12. Oil (18% yield); IR ($CHCl_3$), cm^{-1}: 1773 (COO); ^1H NMR, δ, ppm: 4.37 (1H, dd, J_1 7.7, J_2 9.0, H-5), 4.24 (1H, dd, J_1 6.8, J_2 9.0, H-5), 3.82 (1H, quintet, J 5.9, C*H*CH_3), 2.65–2.45 (2H, m), 2.33 (1H, dd, J_1 7.5, J_2 16.6, H-3), 1.16 (3H, d, J 6.2, CH_3), 0.88 (9H, s, $C(CH_3)_3$), 0.08 (3H, s, CH_3), 0.06 (3H, s, CH_3); ^{13}C NMR, δ, ppm: 177.2 (s), 69.8 (t), 69.1 (d), 42.9 (d), 31.4 (t), 25.8 (3q), 22.0 (q), 18.0 (s), −4.0 (q), −4.9 (q); MS (*m*/*z*): 245 (MH^+, 27), 188 (13), 187 (70), 175 (26), 170 (20), 169 (100), 167 (14), 159 (30), 157 (18), 149 (21), 143 (20), 97 (12), 95 (18), 85 (12), 77 (14), 76 (15), 75 (90), 73 (12), 67 (10), 57 (11).

The same procedure carried out on (4*R*,1′*S*)-**9** gave (4*R*,1′*S*)-(+)-**12**; $[\alpha]_D^{25} = +29.4$ (c 0.17, CH_3CN); $\Delta\varepsilon_{224}$ −0.1 (CH_3CN).

4.5. Enzymatic transesterification of 1a, 1b, 2a, 2b and 9

4.5.1. General procedure. To a solution of the appropriate alcohol (2.4 mmol) in vinyl acetate (4.4 mL), lipase PS (0.400 g, 12036 U) was added under stirring. The reaction was monitored by chiral HRGC. After stirring for the period indicated below, the mixture was filtered and the solid washed with ethyl acetate. After evaporation of the solvent, the crude reaction mixture was purified by flash chromatography (eluent: light petroleum/ethyl acetate, gradient from 80:20 to 65:35) to afford the unreacted alcohol and acetate.

4.5.2. Lipase-catalyzed acetylation of 1a. Enzymatic resolution afforded, after 2 h, the unreacted alcohol (4*S*,5*R*)-(+)-**1a** with 30% ee and the acetate (4*R*,5*S*)-(−)-**7a** with 93% ee at 24% calculated conversion, E = 36.

4.5.3. Lipase-catalyzed acetylation of 1b. After 1 h, both the unreacted alcohol **1b** and the acetylated product **7b** were recovered as racemates and after 8 h only (±)-**7b** was recovered.

4.5.4. Lipase-catalyzed acetylation of 2a. Enzymatic resolution afforded, after 3 h, the unreacted alcohol (4*S*,5*R*)-(+)-**2a** with 43% ee and 42% yield and the acetate (4*R*,5*S*)-(−)-**8a** with 98% ee and 15% yield: $[\alpha]_D^{25} = -41.9$ (c 0.37, MeOH); $[\alpha]_D^{25} = -42.5$ (c 0.40, MeCN); $\Delta\varepsilon_{209}$ +0.3 (MeOH); $\Delta\varepsilon_{212}$ +0.2 (MeCN); 30% calculated conversion, E = 150.

The unreacted alcohol (+)-**2a** (43% ee) was submitted to acetylation under the same conditions as above. After 18 h (+)-**2a** was obtained in 40% yield and >99% ee $[\alpha]_D^{25} = +48.9$ (c 0.54, MeOH); $[\alpha]_D^{25} = +48.8$ (c 0.56, MeCN); $\Delta\varepsilon_{209}$ −0.2 (MeOH); $\Delta\varepsilon_{212}$ −0.2 (MeCN). The acetate (−)-**8a** was recovered in 17% yield and 76% ee.

4.5.5. Lipase-catalyzed acetylation of 2b. After 2 h, at 36% calculated conversion, the unreacted alcohol (4*S*,5*S*)-(−)-**2b** with 34% ee was formed, while the acetate (4*R*,5*R*)-(+)-**8b** had 60% ee; E = 5.

At 70% calculated conversion, after 3.5 h, the unreacted alcohol (4*S*, 5*S*)-(−)-**2b** was recovered with 82% ee in 30% yield and the acetate (4*R*,5*R*)-(+)-**8b** with 38% ee isolated in 50% yield.

4.6. Lipase-catalyzed hydrolyses

4.6.1. General procedure. To a suspension of the appropriate acetate (0.43 mmol) in 10 mL of phosphate buffer (pH 7.4, 0.1 M) was added the lipase PS (0.073 g, 2190 U), under vigorous stirring. The course of the reaction was monitored with a pH-STAT, with continuous addition of 1.0 M NaOH. At about 20% conversion, the reaction mixture was filtered, extracted with ether and the organic phase dried over anhydrous Na_2SO_4. After evaporation of the solvent, the crude was purified by flash chromatography to afford the corresponding lactonic alcohol and the unreacted acetate.

4.6.2. Lipase-catalyzed hydrolysis of (±)-7a. After 5 h, at about 5% conversion (E >200), the resulting alcohol (4*R*,5*S*)-**1a** with 99% ee was formed (E >200), as shown by chiral HRGC analysis. However, after 72 h, the secondary alcohol **9** as a racemate was formed at the expense of **1a**. At 60% conversion the unreacted acetate (4*S*,5*R*)- **7a** was recovered with 90% ee.

4.6.3. Lipase-catalyzed hydrolysis of (±)-7b. After 4 h, at about 20% conversion, the reaction mixture was filtered, evaporated to dryness and treated with ethyl acetate. Chiral HRCG analysis of the crude indicated the presence of (±)-**1b** (30%) and (±)-**7b** (70%).

4.6.4. Lipase-catalyzed hydrolysis of (±)-8a. After 17 min, the alcohol (4*R*,5*S*)-(−)-**2a** with 97% ee was isolated in 10% yield; $[\alpha]_D^{25} = -47.3$ (c 0.11, MeOH); $[\alpha]_D^{25} = -47.3$ (c 0.11, MeCN); $\Delta\varepsilon_{210}$ +0.2 (MeOH); $\Delta\varepsilon_{212}$ +0.1 (MeCN). The acetate (4*S*,5*R*)-(+)-**8a** with 13% ee was recovered in 50% yield (12% calculated conversion, E = 74).

4.6.5. Lipase-catalyzed hydrolysis of (±)-8b. After 7.5 min, the usual work-up furnished the unreacted acetate (4*S*,5*S*)-(−)-**8b** with 17% ee and the alcohol (4*R*,5*R*)-(+)-**2b** with 34% ee (33% calculated conversion, E = 2).

4.7. Molecular modelling

Calculations were carried out on two Silicon Graphics Octane 1 R12000 workstations and on a Pentium4 2.53 GHz/Red Hat Linux machine. The Cornell version of the Amber force field[29] as implemented in Sybyl6.8 (Tripos Inc.) was used in all energy minimizations and dynamics runs. New Amber force constants for the lactone system of the substrates and for the non-standard structural features of the anionic tetrahedral intermediates were developed according to Geremia and Calligaris,[33] while charge distribution and equilibrium bond lengths were calculated following the original Amber protocol on the substrates and on a reduced model of the tetrahedral intermediates, represented by the intermediate for the transesterification of *O*-acetyl-serine and isopropyl alcohol. The required ab initio calculations were carried out at the HF-6.31G* level with Gaussian 03.[34]

All the energy minimizations were carried out until a convergence criterion of 0.001 Kcal/mol/Å was achieved for all the energy gradients. The conjugate gradient minimization algorithm was always used after running 20 initial steps of Simplex linear minimization. In the absence of solvent, all the calculations were carried out in a continuum dielectric of relative permittivity $\varepsilon = 4rij$.[35] The water effect on the hydrolytic reactions was modelled by a discrete model obtained by adding a seven layer periodic box of 3178 water molecules on the catalytic site region of the optimized structures.

The crystallographic coordinates of the reference complex of *Pseudomonas cepacia* lipase with a phosphonate transition state analogue of 1-phenoxy-2-acetoxy butane were obtained from the Protein Data Bank, Brookhaven National Laboratory (Pdb id 1HQD[23n]). All the hydrogen atoms were added, assuming a pH environment of 7.5, all the crystallization water molecules were removed, and the reference complexes was then allowed to relax by the multistage minimization procedure described by Levit and Lifson.[28]

The initial geometries of the enzyme tetrahedral intermediates were built by docking the substrate structures onto the structure of the phosphonate inhibitor as found in the optimized reference complex. The structures were first optimized by running an energy minimization to a 0.05 Kcal/mol/Å energy gradient. The conformational space accessible to the tetrahedral intermediates was then explored following a simplified protocol derived from that described by Zuegg et al. for similar intermediates on the same lipase.[25d] A set of sterically tolerable conformations was generated by a systematic search carried out on all the rotatable bonds of the serine-bound intermediates, and each member of the set then submitted to Monte–Carlo minimization. The best minimum thus found was then subjected to a molecular dynamics run in the NTV ensemble; the systems were gradually heated to 300 K, in three steps, allowing a 25 ps interval per each 100 K, then equilibrated for 25 ps at 300 K, and finally submitted to a 400 ps collection run at 300 K. The lowest potential energy equilibrium geometries were finally reoptimized.

Acknowledgements

Financial supports by the M.I.U.R. (Rome), PRIN 2002–2003 and the University of Trieste are gratefully acknowledged. The 'Centro di Eccellenza in Biocristallografia' of the University of Trieste is acknowledged for having provided all the softwares used in this work.

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