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Biocatalytic Synthesis of Chiral Polyoxygenated Compounds: Modulation of the Selectivity upon Changes in the Experimental Conditions†

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Abstract: Derivatives of both enantiomers of butane-1,2,4-triol have been obtained through a transesterification reaction catalyzed by *Pseudomonas fluorescens* lipase (PFL) in organic solvents. The influence of the solvent on the enantioselectivity has been thoroughly examined. It has been found that the enantioselectivity depends on both the hydrophobicity and the polarity of the solvent in a semi-quantitative way. The influence of other experimental variables (temperature, amounts of lipase and acylating agent, addition of water, addition of molecular sieves) on the enantioselectivity has also been investigated.

INTRODUCTION

Enzymes catalyze processes with remarkable levels of selectivity under very mild experimental conditions. On the basis of these properties, it is not surprising that enzymes have attracted the attention of synthetic organic chemists who wish to perform selective reactions on unnatural substrates.[1,2] Although chemoselective,[3] ambidoselective,[4] regioselective,[5] and diastereoselective[6] transformations mediated by enzymes have become important from a synthetic point of view, the most valuable characteristic is their ability to promote enantioselective transformations (EPC-synthesis[7]).

Although the majority of the properties of enzymes are useful for the purpose of organic synthesis, two are disadvantageous. The natural environment for enzymatic reactions is water, which confines the applicability of enzymes to a few organic substrates. On the other hand, it is difficult to predict whether an enzyme will catalyze the transformation of an unnatural substrate with a satisfactory rate and with the desired level of selectivity. A major breakthrough in the synthetic applications of enzymes was the discovery that certain enzymes are stable in organic solvents,[8,9] which expanded very much the synthetic usefulness of enzymes.[2]

When a synthetic organic chemist wishes to perform a selective transformation on an unnatural substrate two approaches are most frequently used. The first one involves ***variation of the substrate structure***, by attaching protective and modifier groups of different sizes and polarities up to the desired level of selectivivity is achieved. This approach is time consuming, and often there is an undesirable deviation from the initial substrate structure. The second approach is to ***screen different enzymes*** up to a selective enough

† Dedicated to the memory of Narciso de la Hoz.

transformation is attained. Although this strategy is frequently successful, it is usually empirical. Furthermore, the set of available enzymes from a given group may be narrow.

Alterations in selectivity or substrate specificity of an enzyme can be induced by modifications in either the enzyme's structure or the nature of the reaction medium. Although the tools of molecular biology are being more and more sucessfully applied to protein engineering,[10] the method is not applicable to all the enzymes of synthetic interest,[11] and can be expensive for the standard of organic synthesis.[12,13] On the other hand, it is well known from "traditional" organic chemistry that the solvent can influence the outcome of organic reactions;[14] this fact, along with the stability of some enzymes in organic media, provides the synthetic chemists the opportunity to use a variety of solvents to alter the substrate specificity and the level of selectivity[2,15,16] of enzymatic reactions. Furthermore, the experimental procedure in organic solvents is easier than in water.[17] As an additional bonus when using nearly anhydrous organic solvents, undesired side reactions caused by water are avoided.[18]

The use of different organic solvents to modulate the selectivity of enzymes is straighforward. Furthermore, this kind of studies provides data on the behavior of enzymes in non-aqueous solvents (non-aqueous enzymology).[8b,c] If this approach is successful, the number of enzymes needed for synthetic applications may be reduced considerably. With these goals in mind, we have initiated investigations[19] on the influence of the nature of the solvent on the selectivity of enzyme-mediated synthetic transformations.

Lipases are noteworthy among enzymes. They are readily available, do not require cofactors, can accept a wide structural variety of organic compounds as substrates, and are remarkably stable in organic solvents.[2a,20] Although these enzymes under natural circumstances catalyze the reaction of water with triesters of glycerol, in a hydrolytic reaction; it has been shown that the reaction can be reversed in media with low water-content, furnishing esters by the reaction of an alcohol and an acylating agent.[21]

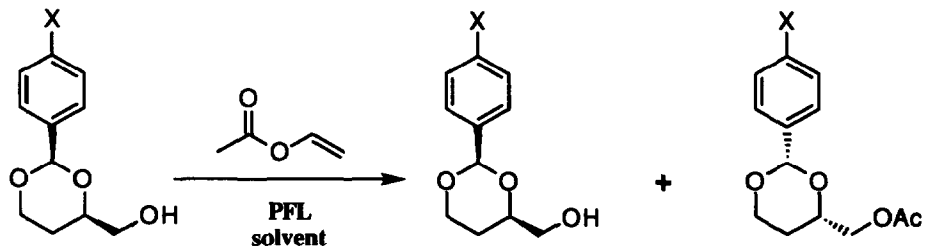
Although the synthetic potential of lipases in organic solvents is considerable, the field is still in its infancy, and a deeper knowledge of the factors which influence the selectivity of the transformations catalyzed by these enzymes is needed.

Derivatives of both enantiomers of butane-1,2,4-triol [e. g., (-)-**1** and (+)-**2**, **Scheme 1**] are useful chiral building blocks for the synthesis of natural products,[22] as well as chiral auxiliaries for asymmetric synthesis.[23] In the course of our work on the EPC-syntheses of polyoxygenated compounds[24] and branched chain nucleosides[25] we need practical routes to these chiral building blocks. Although derivatives of (S)-butane-1,2,4-triol can be prepared from natural (S)-malic acid,[26] the synthesis of derivatives of the (R)-enantiomer involves the use of expensive (R)-malic acid.[27] Due to this inconvenience we searched for an alternative route to these compounds, finding that the transesterification reaction of readily available derivatives of racemic butane-1,2,4-triol [(±)-**1a** and (±)-**1b**] with vinyl acetate[28] catalyzed by *Pseudomonas fluorescens* lipase (PFL)[29] allows to reach this goal efficiently (**Scheme 1**).

The effectiveness of the kinetic resolution has been calibrated by the value of the enantioselectivity (E-values as defined as Sih and Wu[1b]). In order to optimize the enantioselectivity of the transformation and with the goal of unveiling the factors which influence the selectivity of the process, we have carried out an extensive study of the influence of the experimental conditions on the enantioselectivity of the reaction. We have observed an interesting dependence of the enantioselectivity on both the hydrophobicity of the solvent (represented by the values of the logarithm of the partition coefficient of the solvent between 1-octanol and water,[30] lg P) as well as the polarity of the solvent (as indicated by the dielectric constant,[14,31] ε). From a

practical point of view, it is demonstrated that the enantioselectivity is modulated by changes in the solvent, which allows to obtain the target molecules in high enantiomeric purities by simply varying the solvent.

Scheme 1



(±)-1a; X= H
(±)-1b; X= OMe

(-)-1a; X= H
(-)-1b; X= OMe

(+)-2a; X= H
(+)-2b; X= OMe

RESULTS

Lipase catalyzed acetylation of (±)-*cis*-4-(hydroxymethyl)-2-phenyl-1,3-dioxane [(±)-1a] in anhydrous organic solvents.

The results of the kinetic resolution of (±)-**1a** in anhydrous organic solvent[32] (**Scheme 1**) are indicated in **Table 1**. The values of the logarithm of the partition coefficient between water and 1-octanol (lg P) and the dielectric constant (ε) for each solvent are also indicated in **Table 1**. In all the solvents tested, the enzyme recognizes preferently the (S,S)-enantiomer of (±)-**1a**, giving the acetate (+)-**2a** and the alcohol (-)-**1a**. The absolute configurations of the reaction products were assigned by comparison with the compounds prepared from (R)- and (S)-malic acid.[24c]

As shown in **Table 1**, both the enantioselectivity and the velocity (as indicated by the time necessary to achieve a 25% conversion, $t_{1/4}$) of the reaction are highly dependent on the nature of the solvent. The enantioselectivity is from modest (E = 4.5, using chloroform as solvent, entry 7) to very good (E > 40, using tetrahydrofuran as solvent, entries 18 and 19, **Table 1**).

Several features are worth noting. The speed of the process is highly dependent on the nature of the solvent. The reaction is very slow in most halogenated solvents (**Table 1**, entries 7, 12, 13, and 14; the less polar halogenated solvent carbon tetrachloride is an exception, entry 3), being necessary higher amounts of PFL to reach acceptable conversion rates (entries 12 and 14, **Table 1**). On the contrary, the reaction is quite fast in hydrocarbon solvents (entries 1, 2, 4, 5, and 6, **Table 1**), in ethereal solvents (entries 8, 9, 15, and 17-20), in vinyl acetate (entry 21), and in acetone (entry 23, **Table 1**). In some of the more polar solvents as well as in the hydroxylic solvent 3-methyl-3-pentanol the reaction is quite slow (**Table 1**, entries 10, 16, 22 and 24, acetone being an exception). More polar solvents (dioxane, DMF and DMSO) have been also tried, but the reaction is extremely slow (data not shown).[33]

Table 1. Results of the *Pseudomonas Fluorescens* Lipase Catalyzed Acetylation of (±)-**1a** in Anhydrous Solvents.[a]

Entry	Amount[b]	Solvent[c]	lg P	ε	$t_{1/4}$ (h)[d]	Time (h)	%c[e]	(-)-**1a** %ee (%y)[f]	(+)-**2a** %ee (%y)[f]	E[g]
1	205	tetralin	4.18	2.77	3	5.5	48	70 (40)	76 (40)	15.2
2	200	tetralin	4.18	2.77	3	16	60	>95 (32)	64 (53)	16.0
3	205	CCl_4	3.0	2.24	3	11.5	56	95 (36)	76 (49)	26.8
4	195	toluene	2.5	2.38	3.5	9	54	90 (35)	77 (46)	23.3
5	210	benzene	2.0	2.27	6	22	47	72 (46)	82 (44)	21.7
6	430	benzene[h]	2.0	2.27	3	9	55	>95 (32)	78 (49)	>29
7	410	$CHCl_3$	2.0	4.81	22	72	64	39 (30)	71 (46)	4.5
8	150	*t*-BuOMe	1.9	4.50	6	20	63	80 (32)	46 (50)	6.4
9	240	*i*-Pr_2O	1.9	3.88	16	43	45	58 (31)	71 (44)	10.4
10	200	$Et_2C(OH)Me$	1.9	4.3	216	480	45	67 (43)	81 (39)	19.0
11	570	$Et_2C(OH)Me$	1.9	4.3	6	23.25	58	96.8 (38)	69 (48)	21.7
12	400	$(ClCH_2)_2$	1.48	10.37	6	14	41	60 (51)	88 (32)	29.2
13	200	CH_2Cl_2	1.25	8.93	240	504	36	43 (47)	78 (31)	12.3
14	525	CH_2Cl_2	1.25	8.93	28	88	52	77 (36)	70 (44)	12.9
15	220	Et_2O	0.85	4.20	3	9	48	68 (36)	74 (39)	13.3
16	250	pyridine	0.71	12.91	70	264	43	66 (41)	86 (35)	26.4
17	230	DME	< 0.6	7.20	13	32	49	78 (41)	74 (42)	24.0
18	210	THF	0.49	7.58	5.5	45	54	>97 (39)	82 (48)	44.0
19	230	THF	0.49	7.58	4.5	5	31	42 (49)	93 (29)	42.0
20	360	THF	0.49	7.58	2	6	46	74 (46)	86 (41)	31.5
21	230	vinyl acetate[i]	0.42	5.80	5	23	59	95 (33)	66 (52)	17.3
22	200	butanone	0.29	18.51	90	336	47	69 (44)	78 (38)	16.5
23	130	acetone	-0.23	20.90	6	23	49	72 (38)	74 (36)	14.4
24	120	acetonitrile	-0.33	35.95	39	44	27	23 (52)	64 (24)	5.4

a) All the reactions were carried out at room temperature. b) The enzyme used was *Pseudomonas fluorescens* lipase (PFL) purchased from FLUKA. The specific activity of this enzyme is 31.5 U/mg. The amounts refer to units of PFL per mmol of (±)-**1a**. An unit corresponds to the quantity of enzyme which liberates 1 μmol oleic acid per minute at pH 8.0 and 40°C (as defined in FLUKA catalogue). c) All the solvents were of purissimum quality (FLUKA). Unless otherwise indicated, all the reactions were carried out using 2.5 mol equiv of vinyl acetate. d) $t_{1/4}$ indicates the time at which 25% conversion was achieved. e) The conversion degree was calculated by the expression $c = ee_S/(ee_S+ee_P)$ (ref 1b). f) %ee were determined by ^{1}H-NMR spectroscopy of either (-)-**1a** or (+)-**2a** in the presence of 0.35-0.50 mol equiv of Eu(hfc)3; all the yields refer to isolated compounds after flash-chromatography. g) Calculated according to ref 1b. h) 1.5 mol equiv of vinyl acetate were used. i) 48 mol equiv of vinyl acetate was used.

The effect of the amount of PFL on the enantioselectivity of the acetylation of (±)-**1a** in anhydrous solvent have been briefly examined in four solvents (benzene, 3-methyl-3-pentanol, methylene chloride and

tetrahydrofuran).[35] Meanwhile the enantioselectivity slightly increases with the amount of PFL in benzene (entries 5 and 6, **Table 1**), there is a slight decrease in THF (entries 19 and 20). In 3-methyl-3-pentanol and methylene chloride the values of E are relatively insensitive to the amount of the enzyme, and only increases in the velocities of the reactions are observed (entries 10 and 11, and entries 13 and 14, **Table 1**).

The most remarkable characteristic of this reaction is that the enantioselectivity is dependent on the nature of the solvent in a semi-quantitative way, and this fact can be tentatively rationalized on the basis of the properties of the solvent, the structure of the enzyme and the course of the reaction (see **Discussion** section).

Lipase catalyzed acetylation of (±)-*cis*-4-(hydroxymethyl)-2-phenyl-1,3-dioxane [(±)-1a] under "modified" experimental conditions.

The influence of some experimental variables (temperature, addition of water and molecular sieves, amounts of enzyme and acylating agents, behavior in mixture of solvents) on the outcome of the *Pseudomonas fluorescens* lipase catalyzed transesterification between vinyl acetate and **(±)-1a** (**Scheme 1**) has been studied. The results are shown in **Table 2**.

For the sake of comparison, the results obtained in some anhydrous solvents are also included. Several features deserve to be commented:

a) The reaction is slightly faster and the enantioselectivity is slightly lower in wet toluene[36] than in dry toluene (entries 1 and 2, **Table 2**). The enzyme sticks to the wall of the flask when the reaction is conducted in wet toluene. This enzyme, "immobilized" on the glassware, has been used in the experiment reported in entry 3; possessing essentially the same activity and enantioselectivity as the "unmodified" enzyme.[37]

b) The effect of the addition of water[38] and molecular sieves,[39] as well as the influence of the temperature[43] and the amounts of vinyl acetate[44] and enzyme[35] have been examined using chloroform as solvent (entries 4-10, **Table 2**). When the PFL-catalyzed acetylation is carried out in the presence of 3 Å molecular sieves the reaction is very slow; and, after *ca* 80 hours, the enzyme became completely deactivated (entry 5, **Table 2**). It have been claimed[28b,40] that molecular sieves is beneficial to lipase catalyzed transesterification using vinyl esters as acyl donors, because the sieves will retain acetaldehyde generated during the acylation, avoiding its reaction with the enzyme. Due to the scarcity of examples[39] where molecular sieves have been added to lipase catalyzed transesterification, no generalization can be made; although we think that, in this particular case, the role of the sieve is to strip-off most of the *essential water* from the enzyme's surface, disturbing seriously the most active conformation of the enzyme (see **Discussion** section).[8b,c] As it is indicated below, the effect of molecular sieves on the velocity and enantioselectivity of the process depends on the solvent used.

The effect of adding water to chloroform is very significant: faster and more selective transformations are obtained in wet chloroform than in dry chloroform (compare entry 4 with entries 6-9, **Table 2**). The selectivity is dependent of the amount of enzyme used (entries 6 and 8), decreasing the value of E with the quantity of enzyme used. The effect of the amount of vinyl acetate have briefly been examined (entries 6 and 7, **Table 2**), finding a slightly better selectivity when a higher dose of acetylating agent was used. With the idea that a small amount of enzyme and a high quantity of vinyl acetate increase the selectivity, the conditions of entry 9 in **Table 2** have been tried; although the enantioselectivity is pretty good, the reaction is very slow to be practical.

Table 2. Results of the *Pseudomonas Fluorescens* Lipase Catalyzed Acetylation of (±)-**1a** under "Modified" Experimental Conditions.[a]

Entry	Amount[b]	Vinyl acetate[c]	Solvent[d]	$t_{1/4}$ (h)[e]	Time (h)	%c[f]	(-)-**1a** %ee (%y)[g]	(+)-**2a** %ee (%y)[g]	E[h]
1	195	2.5	toluene	3.5	9	54	90 (35)	77 (46)	23.3
2	200	2.5	wet toluene	1.75	6	52	81 (39)	76 (49)	18.1
3	200[i]	2.5	wet toluene	2	3	30	37 (66)	85 (29)	17.7
4	410	2.5	$CHCl_3$	22	72	64	39 (30)	71 (46)	4.5
5	200	2.5	$CHCl_3$[k]		480	20[l]	n d	n d	n d
6	120	1.0	wet $CHCl_3$	13.75	13.75	25	36 (56)	86 (32)	21.2
7	120	5.0	wet $CHCl_3$	19	80	47	74 (41)	84 (41)	25.7
8	420	1.08	wet $CHCl_3$	4	13	48	64 (40)	68 (43)	10.0
9	30	10.0	wet $CHCl_3$	163	163	25	30 (58)	84 (27)	25.5
10	120	5.0	wet $CHCl_3$[m]	2	5.75	38	49 (51)	80 (29)	14.3
11	200	2.5	CH_2Cl_2	240	504	36	43 (47)	78 (31)	12.3
12	230	2.5	wet CH_2Cl_2	9.5	28	50	70 (42)	69 (46)	11.2
13	220	2.5	Et_2O	3	9	48	68 (36)	74 (39)	13.3
14	220	1.5	wet Et_2O	25	67	45	59 (34)	72 (38)	11.1
15	210	2.5	THF	5.5	45	54	>97 (39)	82 (48)	44.0
16	360	2.5	THF	2	6	46	74 (46)	86 (41)	31.5
17	330	2.5	THF[m]	1.5	4.75	46	75 (46)	89 (44)	38.6
18	230	2.5	THF-H_2O (200:1)	12	32	49	84 (41)	88 (45)	42.0
19	200	2.5	THF-H_2O (50:1)	69	168	25[n]	n. d.	n. d.	n. d.
20	210	1.5	THF-hexane (1:1)	3	10.5	52	93 (33)	87 (42)	48.6
21	230	1.5	THF-hexane (1:1)	3	15	56	>99 (32)	77 (50)	45.8
22	210	1.5	THF-hexane (1:2)	7	22	51	91 (41)	87 (46)	45.8
23	180	2.5	acetone	6	23	49	72 (38)	74 (36)	14.4
24	205	2.5	acetone[k]	22	79.5	51	84 (37)	82 (47)	25.5

a) Unless otherwise indicated, all the reactions were performed at room temperature. b) The enzyme used was *Pseudomonas fluorescens* lipase (PFL) purchased from FLUKA. The specific activity of this enzyme is 31.5 U/mg. The amounts refer to units of PFL per mmol of (±)-**1a**. An unit corresponds to the quantity of enzyme which liberates 1 μmol oleic acid per minute at pH 8.0 and 40°C (as defined in FLUKA catalogue). c) The indicated amount is in mol equiv relative to (±)-**1a**. d) All the solvents were of *purissimum* quality (FLUKA). "Wet solvent" means water-saturated solvent. The contents of water of wet toluene and wet $CHCl_3$ were determined by the modified Karl Fischer method, being 0.03% in toluene and 0.13% in $CHCl_3$. e) $t_{1/4}$ indicates the time at which 25% conversion was achieved. f) The conversion degree was calculated by the expression $c = ee_S/(ee_S+ee_P)$ (ref 1b). g) %ee were determined by ^{1}H-NMR spectroscopy of either (-)-**1a** or (+)-**2a** in the presence of 0.35-0.50 mol equiv of Eu(hfc)$_3$; all the

yields refer to isolated compounds after flash-chromatography. h) Calculated according to ref 1b. i) PFL recovered from the reaction in entry 2 was used (see text). k) The reaction was carried out in the presence of 0.5 mass equiv [relative to (±)-**1a**] of powered 3 Å molecular sieves. l) The reaction stopped after *ca* 80 hours. The reaction products were not isolated. The conversion degree was estimated by ^{1}H-NMR. m) The reaction was carried out at 40°C. n) The reaction stopped after *ca*. 70 hours. Acetic acid was detected. The reaction products were not isolated. The conversion was estimated by ^{1}H-NMR.

Finally, the effect of the temperature have been essayed (entry 10, **Table 2**), finding that higher temperature causes faster reactions and lower selectivity (compare entries 7 and 10, **Table 2**). Although this is the expected consequence from the principles of physical organic chemistry; this have not been always the rule[43] when enzymes have been applied in organic synthesis (*see below*).

c) Analogously to the case of chloroform, there have been a considerable rate increment when wet methylene chloride is used instead of anhydrous methylene chloride, although the selectivity is nearly the same (entries 11 and 12, **Table 2**).

d) The opposite tendency is observed when dry diethyl ether is replaced by wet diethyl ether. The reaction is slower in the wet solvent than in the anhydrous solvent, but no appreciable change in the enantioselectivity is observed (entries 13 and 14, **Table 2**).

e) The effect of the amount of PFL, the temperature, and the addtion of water on the enantioselectivity of the reaction using THF as solvent have been studied. It is found that higher dose of lipase diminishes the enantioselectivity (entries 15 and 16, **Table 2**). When the reaction is carried out at 40°C instead of room temperature, a slightly better selectivity is obtained (entries 16 and 17, **Table 2**), being this a case where the enantioselectivity increases with the temperature.[43] Small addition of water to THF does not have any effect on the enantioselectivity of the reaction (compares entries 15 and 18, **Table 2**), although the reaction is slightly slower. When the proportion of water increases (entry 19) the reaction is very slow, and after *ca*. 70 hours the reaction does not progress anymore, acetic acid being detected in the reaction media.[45]

f) Hexane and pentane have frequently been used[33] as solvents in lipase catalyzed transesterifications. Due to problems of solubility of our starting materials in these hydrocarbon solvents, we could not use them in our study. We have found that this problem does not exist in mixtures of THF and hexane, discovering that the kinetic resolution of (±)-**1a** is quite efficient in THF-hexane mixtures (entries 20-22, **Table 2**). Comparing with THF, the enantioselectivity is roughly the same (contrast with the data in entry 15). The enantioselectivity is insensitive to the ratio of hexane in the mixture, and the reaction rate decreases slightly when the proportion of hexane increases.

g) Finally, the effect of the addition of molecular sieves to a hydrophilic and polar solvent (acetone) has been examined (entries 23 and 24, **Table 2**). The reaction in the presence of molecular sieves is slower, but the enantioselectivity is higher.

Lipase catalyzed acetylation of (±)-*cis*-4-(hydroxymethyl)-2-(4-methoxyphenyl)-1,3-dioxane [(±)-1b].

With the goal to elucidate the structural requirements of the substrates in the reactions catalyzed by *Pseudomonas fluorescens* lipase in organic solvents, we have studied the PFL-catalyzed acetylation of (±)-**1b** with vinyl acetate as acylating agent (**Scheme 1**). Although the structural difference between (±)-**1a** and (±)-**1b** is far away from the reacting center, we have observed some interesting differences. The results are collected in **Table 3**.

Similarly to the kinetic resolution of (±)-**1a**, the (S,S)-enantiomer of (±)-**1b** is also preferently recognized by the enzyme, giving the acetate (+)-**2b** and the alcohol (-)-**1b**. The enantioselectivity E ranges from modest (E = 7.5) in toluene in the presence of a high proportion of molecular sieves (entries 4 and 5, **Table 3**) to excellent (E > 40) in a variety of solvents: toluene (entries 1 and 2, **Table 3**), benzene (entry 7), wet chloroform (entries 9-12), pyridine (entries 16 and 17), and THF-hexane (1:2) (entry 19, **Table 3**). In general, the enantioselectivity is higher in the acetylation of (±)-**1b** than in the acetylation of (±)-**1a**.[46]

Analogous to the reaction with (±)-**1a**, the effectiveness of the kinetic resolution of (±)-**1b** as well as the velocity of the reaction (as indicated by $t_{1/4}$) are dependent on the experimental conditions, especially on the nature of the solvent.[47] Athough not so thoroughly studied as in the acetylation of (±)-**1a**, the enantioselectivity of the PFL-acetylation of (±)-**1b** in anhydrous solvent (entries 1, 7, 8, 13-18, and 20-23, **Table 3**) is also dependent on both the hydrophobicity and the polarity of the solvent in a semi-quantitative way (see **Discussion** section).

The influence of other experimental variables (addition of water and molecular sieves) on the enantioselectivity of the acetylation of (±)-**1b** in toluene and chloroform has been studied. When the reaction is carried out in toluene in the presence of molecular sieves, both the selectivity and the velocity of the reaction diminish when the amount of molecular sieves increases (entries 2-5, **Table 3**). The addition of water to toluene has hardly any effect on the selectivity and the velocity of the reaction (compare entries 6 and 1, **Table 3**). On the contrary, and similarly to the acetylation of (±)-**1a**, the use of wet chloroform instead of dry chloroform as solvent makes the reaction faster and more selective (entries 8 and 9, **Table 3**). When wet choroform is used the enzyme sticks on the wall of the flask (*see above*), being possible to reuse the enzyme without loss in enantioselectivity (entries 11 and 12, **Table 3**).[37]

Similarly to the acetylation of (±)-**1a**, the reaction is also highly enantioselective in a THF-hexane mixture (entry 19, **Table 3**), finding that higher selectivity and faster reaction are obtained in this solvent mixture than in pure THF (entry 18, **Table 2**).

Summarizing, from a practical point of view, the results reported in **Tables 1-3** show that it is possible to obtain the target molecules in high enantiomeric purities and good chemical yields. Although most of the experiments reported in **Tables 1-3** have been carried out in 1-2 mmol scales, some of the more selective and faster transformations have been carried out in higher scales (up to 40 mmol) with the same selectivity; furthermore, most of the enzyme have been recovered and reused.

Table 3. Results of the *Pseudomonas Fluorescens* Lipase Catalyzed Acetylation of (±)-**1b**.[a]

Entry	Amount[b]	Solvent[c]	lg P	ε	$t_{1/4}$ (h)[d]	Time (h)	%c[e]	(-)-**1b** %ee (%y)[f]	(+)-**2b** %ee (%y)[f]	E[g]
1	200	toluene	2.5	2.38	1.5	4.5	50	91 (38)	90 (40)	>50
2	200	toluene[h]			2	6	52	>96 (43)	88 (41)	>50
3	200	toluene[i]			7	22.5	54	91 (43)	77 (41)	24.1
4	200	toluene[k]			48	192	48	62 (44)	67 (46)	7.5
5	200	toluene[l]			200	552	44	55 (39)	69 (32)	9.4
6	215	wet toluene			1	3.5	58	>98 (41)	72 (47)	>30
7	210	benzene	2.0	2.27	1.5	5	47	80 (38)	90 (46)	46.6
8	440	$CHCl_3$	2.0	4.81	6.5	26	52	84 (47)	78 (47)	21.2
9	240	wet $CHCl_3$			2.5	8	48	86 (52)	94 (40)	>50
10	240	wet $CHCl_3$			4	20	54	>98 (44)	83 (44)	>50
11	1100[m]	wet $CHCl_3$			1.5	19.5	60	>99 (33)	66 (49)	>40
12	800[n]	wet $CHCl_3$			2.5	12	44	73 (41)	92 (35)	>50
13	210	*i*-Pr_2O	1.9	3.88	1	2.75	51	86 (42)	84 (41)	31.7
14	190	CH_2Cl_2	1.25	8.93	22	113	46	73 (48)	86 (42)	28.9
15	200	Et_2O	0.85	4.20	2.25	5.75	52	86 (45)	81 (46)	26.1
16	200	pyridine	0.71	12.91	42	193	48	82 (48)	90 (40)	48.4
17	200	pyridine			44	74	32	45 (61)	>94 (30)	>50
18	225	THF	0.49	7.58	2.5	9	48	79 (50)	85 (39)	29.7
19	200	THF-hexane (1:2)			1.5	4.5	51	90 (47)	86 (43)	39.2
20	215	vinyl acetate[o]	0.42	5.80	1.5	6.75	48	76 (50)	84 (41)	26.2
21	220	acetone	-0.23	20.90	8	19.5	45	70 (44)	87 (37)	30.1
22	230	acetone			8	51	54	>96 (40)	82 (45)	>30
23	205	acetonitrile	-0.33	35.95	42	50	27	31 (53)	82 (18)	13.7

a) Unless otherwise indicated, all the reactions were carried out at room temperature. b) The enzyme used was *Pseudomonas fluorescens* lipase (PFL) purchased from FLUKA. The specific activity of this enzyme is 31.5 U/mg. The amounts refer to units of PFL per mmol of (±)-**1b**. An unit corresponds to the quantity of enzyme which liberates 1 µmol oleic acid per minute at pH 8.0 and 40°C (as defined in FLUKA catalogue). c) All the solvents were of *purissimum* quality (FLUKA). "Wet solvent" means water-saturated solvents. Unless otherwise indicated, all the reactions were carried out using 2.5 mol equiv of vinyl acetate. d) $t_{1/4}$ indicates the time at which 25% conversion was achieved. e) The conversion degree was calculated by the expression $c = ee_S/(ee_S+ee_P)$ (ref 1b). f) %ee were determined by ^{1}H-NMR spectroscopy of either (-)-**1b** or (+)-**2b** in the presence of 0.50-0.65 mol equiv of Eu(hfc)3; all the yields refer to isolated compounds after flash-chromatography. g) Calculated according to ref 1b. h) 0.09 mass equiv [relative to (±)-**1b**] of powered 3 Å molecular sieves was used. i) 0.22 mass equiv [relative to (±)-**1b**] of powered 3 Å molecular sieves was used. k) 0.46 mass equiv [relative to (±)-**1b**] of powered 3 Å molecular sieves was used. l) 1.0 mass equiv [relative to (±)-**1b**] of powered 3 Å molecular sieves was used. m) The enzyme recovered from the reaction in entry 10 was used (see text). n) The enzyme recovered from the reaction in entry 11 was used (see text). o) *ca.* 48 mol equiv of vinyl acetate was used.

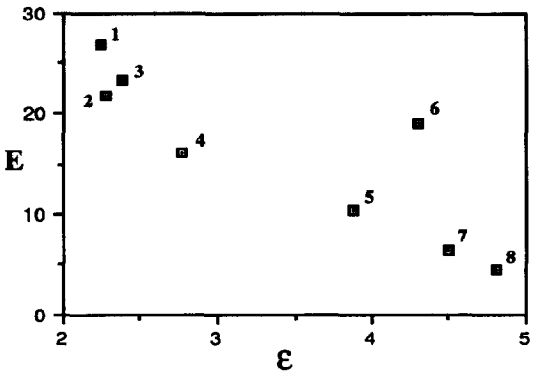
DISCUSSION

On analyzing the results of the kinetic resolutions of (±)-**1a** and (±)-**1b** in anhydrous organic solvents [**Table 1** for (±)-**1a** and entries 1, 7, 8, 13, 14, 15, 16, 18, 20, 21 and 23 of **Table 3** for (±)-**1b**], we observe a dependence of the enantioselectivity E on both the hydrophobicity (as an indication of the miscibility with water)[48] and the polarity of the solvent.

First, the solvents have been divided in two groups according to low miscibility (high lg P) and high miscibility (low lg P) with water. Then, the influence of the dielectric constant ε of the solvent on the values of the enantioselectivity E has been studied. The border line to separate the solvents has been established between 3-methy-3-pentanol (entry 11, **Table 1**) and 1,2-dichloroethane (entry 12, **Table 1**) for the kinetic resolution of (±)-**1a**, and between isopropyl ether (entry 13, **Table 3**) and methylene chloride (entry 14, **Table 3**) for the kinetic resolution of (±)-**1b**, because these pairs of solvents have relatively high differences in both the values of lg P and ε. We realize that there is not a clear cut-off in the values of lg P and the division we have done is arbitrary. It is evident that some solvents are border-line (entries 8-14 in **Table 1**, and entries 13 and 14 in **Table 3**) and it is difficult to unequivocally include them into any of the two groups indicated. We have found that some of these solvents fit both behaviors (*see below*).

The plot of E in the kinetic resolution of (±)-**1a** versus ε for the solvents with high lg P (entries 1-12 in **Table 1**) is depicted in **Figure 1**. Except for 3-methyl-3-pentanol (solvent in the border line!), it shows a very good correlation; the value of E decreases almost linearly with ε.

Figure 1. Plot of E *vs.* ε for solvents with high lg P for the kinetic resolution of (±)-**1a**.

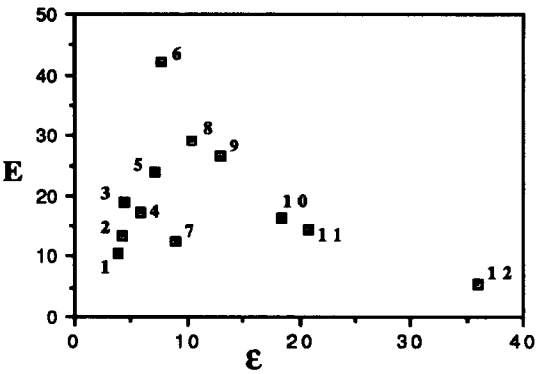


1, CCl_4; **2**, benzene; **3**, toluene; **4**, tetralin; **5**, iPr_2O; **6**, 3-methyl-3-pentanol; **7**, tBuOMe; **8**, $CHCl_3$

The plot of E in the kinetic resolution of (±)-**1a** versus ε of the solvents with low lg P (entries 13-24 in **Table 1**) is shown in **Figure 2**. For this group of solvents, two different behaviors are observed: with relatively non-polar solvents the value of E increases with ε, reaching the maximum in THF. With relatively polar solvents, the value of E decreases almost exponentially with ε. The only exception to this behaviour is

CH_2Cl_2 (again a solvent in the border line!). Furthermore, two solvents of the first group, diisopropyl ether and 3-methyl-3-pentanol, fit this behavior (points number 1 and 3 in the plot of **Figure 2**).

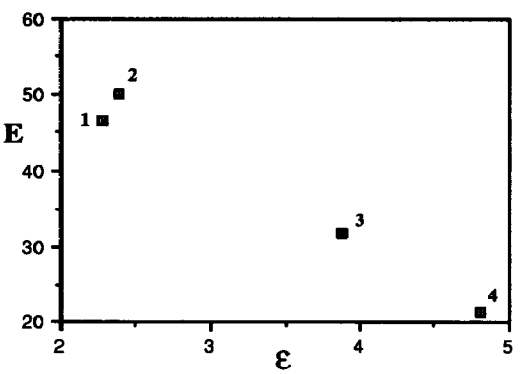
Figure 2. Plot of E *vs.* ε for solvents with low lg P for the kinetic resolution of (±)-**1a**.



1, iPr_2O; **2**, Et_2O; **3**, 3-methyl-3-propanol; **4**, vinyl acetate; **5**, DME; **6**, THF; **7**, CH_2Cl_2; **8**, $(ClCH_2)_2$; **9**, pyridine; **10**, butanone; **11**, acetone; **12**, acetonitrile

The plot of E in the kinetic resolution of (±)-**1b** versus ε for the more hydrophobic solvents (toluene, benzene, chloroform and isopropyl ether, entries 1, 7, 8 and 13, **Table 3**) is shown in **Figure 3**. Although the solvents examined are few, it is observed that similarly to the kinetic resolution of (±)-**1a** (compare with **Figure 1**), the effectiveness of the resolution of (±)-**1b** in hydrophobic solvents decreases with the polarity of the solvent.

Figure 3. Plot of E *vs.* ε for solvents with high lg P for the kinetic resolution of (±)-**1b**.

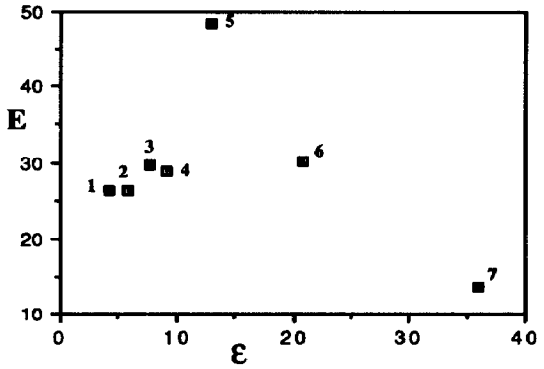


1, benzene; **2**, toluene; **3**, iPr_2O; **4**, $CHCl_3$

The plot of E in the kinetic resolution of (±)-**1b** versus ε for the less hydrophobic solvents (methylene chloride, diethyl ether, pyridine, tetrahydrofuran, vinyl acetate, acetone and acetonitrile, entries 14, 15, 16, 18,

20, 21 and 23, **Table 3**) is shown in **Figure 4**. For the less polar solvents, the enantioselectivity increases with the polarity of the solvent, reaching the maximum when pyridine is the solvent. For more polar solvents the enantioselectivity decreases with the polarity of the solvent. Comparing with the kinetic resolution of (±)-**1a** (see **Figure 2**), the same tendency is observed; although the maximum of the curve is reached at a higher value of ε.

Figure 4. Plot of **E** *vs.* ε for solvents with low lg P for the kinetic resolution of (±)-**1b**.

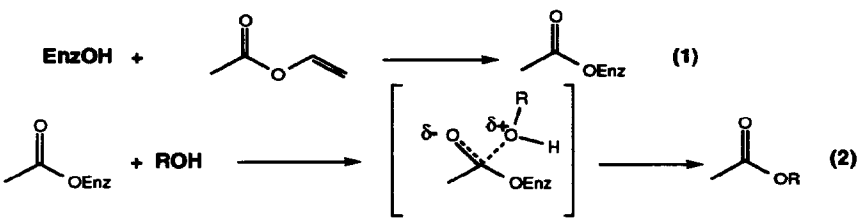


1, Et_2O; **2**, vinyl acetate; **3**, THF; **4**, CH_2Cl_2; **5**, pyridine; **6**, acetone; **7**, acetonitrile

Although a precise explanation of the solvent's influence on the PFL catalyzed transesterification of (±)-**1** in organic solvents can not be given with the present knowledge,[49] we speculate that **the conformation of the molecule of *Pseudomonas fluorescens* lipase is modified upon a change in the nature of the solvent**. The fact that both the hydrophobicity and the polarity of the solvent influence the outcome of the reaction relies on the currently available data on the structure of lipases and its mechanism of action.

It is believed[8b,c] that the lipase molecule is surrounded by water (*essential water*) which keeps the enzyme in a conformation identical or very close to the one it has in its natural environment. Recently, the first crystal structures of the lipases from human pancreas, *Rhizomucor miehei* and *Geotrichum candidum* have been resolved,[52] which have shed light on the mechanism of lipolysis;[53] and in turn suggests that the transesterification goes through the mechanism indicated in **Scheme 2**.

Scheme 2



Although these enzymes are presumably not homologous (as indicated by their primary structures), their active center regions are very similar; being formed by the catalytic triad Ser-His-Asp (or Glu), characteristic of the serine proteases[54] and acetylcholinesterases.[55] Furthermore, although the sequence similarity among the three lipases is limited to the region near the active-site serine, their three dimensional structures are quite similar. The hydrolytic sites in these lipases are buried under a surface loop, referred to as the 'lid',[53a] and are therefore inaccesible to the solvent in the natural environment (water). While the underside of the 'lid' is hydrophobic in nature, the upper surface of the 'lid' (in contact with the media) is polar, and therefore susceptible to be modified by the solvent. When the lipase molecule is in contact with its natural substrate in an oil-water interface, the 'lid' moves leaving a large hydrophobic cavity exposed to the media; this cavity contains the catalytic site.[53a]

Although the tertiary structure of *Pseudomonas fluorescens* lipase is not yet known,[56] preliminary results on the crystal structure as well as inhibition studies of *Pseudomonas* lipases[57] indicate a similar arrangement around the active center. An additional structural feature found in the lipases from *Pseudomonas* species is that these molecules present a high contents of serine and threonine residues, which are mainly located in the surface. Some authors[56a] have observed that this enzyme adhered to glassware, and serine residues were atributed to be responsible of this fact. We have observed the same behavior with the lipase used in the present work (see **Results** section).[37]

On basis to the data reported in the present paper we hypothesize that the organic solvent modifies the conformation of the *Pseudomonas fluorescens* lipase molecule,[2a,16d,58] especially the regions near to the active center and the 'lid'. The change in the conformation of the enzyme is the responsible of the variation in the enantioselectivity upon changes in the solvent.

The rationalization of the influence of both the hydrophobicity and the polarity of the solvent on the conformation of the molecule of *Pseudomonas fluorescens* lipase can be as follows.

It is expected that when water is relatively soluble in the organic solvent, the solvent can strip off *essential water* from the enzyme surface, modifying the conformation of the enzyme. One roughly indicator of the miscibility of organic solvents and water is lg P, which has been mentionated above, and which has been frequently invoked to explain the enantioselectivity of enzymatic reactions in non-aqueous media.[8]

Based on the proposed mechanistic picture of the lipase catalyzed triacylglycerol hydrolysis,[53] we can consider that the transesterification reaction proceeds in two separate steps as indicated in **Scheme 2**. The step 2 is the enantioselectivity-determining step, and it goes through an intermediate where charge separation is produced. The enzyme could influence, stabilizing or destabilizing, the charge separation; but, overall a net charge separation would be produced in the whole system. It is expected that the factors which influence the charge development will be involved in the outcome of the process. A physical property which indicate the capacity of a solvent to stabilize electrical charges is the dielectric constant ε. Futhermore, as it has been pointed out above, the surface of the molecules of lipases from *Pseudomonas* are relatively polar; thus, it is not unexpected that the polarity of the solvent influences the conformation of the enzyme and, therefore, the selectivity of the reaction.

Based on the ideas pointed out above, we have considered that these two physical properties (lg P and ε) will be involved in determining the degree of enantioselectivity in the acetylation catalyzed by *Pseudomonas fluorescens* lipase; and indeed we have found the correlations shown in the present paper.

Also the results of the reactions carried out in the presence of molecular sieves (entries 5 and 24 from **Table 2**, and entries 2-5 from **Table 3**) points towards that the interaction between the solvent and *essential water* is important to determine the activity and selectivity of the enzyme. In all the cases studied, the velocity of the reaction decreases when the reaction is carried out in the presence of molecular sieves, what seems to indicate that *essential water* is removed from the enzyme's surface, modifying the most active conformation of the enzyme. The change in the conformation of the lipase molecule makes the reaction more [such as in the acetylation of (±)-**1a** in acetone, entry 24, **Table 2**] or less enantioselective [such as in the acetylation of (±)-**1a** in chloroform (entry 5, **Table 2**) or in the acetylation of (±)-**1b** in toluene (entries 2-5, **Table 3**].

Also it is interesting to compare the results of the kinetic resolutions of (±)-**1a** and (±)-**1b** (**Scheme 1**). Although the structural dissimilarity between these two alcohols is far away from the reacting center, there is a relatively high difference in the values of the enantioselectivity E in the PFL-catalyzed acetylation of these two substrates; what indicates that relatively far away from the active site, there is a region that has some influence on the outcome of the reaction. Although we do not know the reason of this difference, we speculate that the effect is steric in nature, although we can not be ruled out an electronic origin.[59]

CONCLUSION

This paper reports one of the most comprehensive studies on the influence of the solvent on the selectivity of an enzyme-mediated synthetic transformation. The solvents span through a broad range of polarities (as indicated by the dielectric constant, ε) and hydrophobicities (as indicated by the partition coefficient between 1-octanol and water, lg P), and the enantioselectivities range from modest (4.5) to excellent (> 50). It has been found that the enantioselectivity is dependent on both the hydrophobicity and polarity of the solvent in a semi-quantitative way.

Although we can not give a precise explanation on the solvent's influence on the selectivity of the transformations reported in the present paper, the results show that the selectivity of PFL-catalyzed transesterifications can be modulated upon changes in the nature of the solvent. Because the published papers on the influence of the nature of the solvent on the selectivity in related transformations are scarce,[50] no generalization on the behavior of lipases can be done. On analyzing the results reported in the literature, we think that the reactions catalyzed by lipases from *Pseudomonas* species are the most sensitive to the nature of the solvent, this behavior is not found in the synthetic applications of other lipases.[60] This conduct seems to reflect the high polarity of the surface of the *Pseudomonas* lipase molecules, as well as the different amino acid sequences of the 'lids' of each enzyme.[61]

For the purpose of organic synthesis, it is demonstrated that synthetically useful transformations (i.e., high enantioselectivity) are achieved by variations in the nature of the solvents; this strategy, which have been termed *solvent engineering* elsewhere,[8b] provides a more straighforward way to reach high selectivities than the approaches more frequently used up to now (see **Introduction** section). Furthermore, the results reported in the present paper are also significant to understand the behavior of enzymes in organic solvents (*non-aqueous enzymology* [8b,c]). Although it have been claimed that only high hydrophobic solvents are the most suitable for biocatalyzed transformations,[33] we show in the present paper that a broad variety of solvents, with very different hydrophobicities and polarities, can be used.

EXPERIMENTAL PART

General. Lipase from *Pseudomonas fluorescens* (SAM-2) was purchased from FLUKA and used as received. All the solvents and chemicals are commercially available (Fluka or Aldrich), and unless otherwise indicated were used as received. Pyridine was distilled over CaH_2 under argon and kept over molecular sieves. Benzaldehyde was freshly distilled under a reduced pressure of argon. ^{1}H-NMR and ^{13}C-NMR spectra were measured in *Varian-XL-300* or *Varian-Gemini-200*; chemical shift are reported in parts per million (δ) relative to TMS, and the coupling constants are indicated in Hz. The multiplicity of the signals in the ^{13}C-NMR spectra have been determined by ATP or DEPT experiments. Low-resolution electron-impact (70eV) mass spectra were recorded in a *RMU-GMG* spectrometer from *Hitachi-Perkin-Elmer*. Microanalysis were performed by E. Barbero in a *Carlo Erba EA 1108-Elemental Analyzer*. The optical rotations were measured in a *Perkin-Elmer 241 MC* polarimeter; all the optical rotations were measured in $CHCl_3$ solution at room temperature (21-24°C). Enantiomeric excesses were determined by ^{1}H-NMR spectra of **(+)-2** and **(-)-1** in the presence of 0.4-0.55 molar equivalents of Eu(hfc)$_3$.[62]

Synthesis of (±)-*cis*-4-(hydroxymethyl)-2-phenyl-1,3-dioxane [(±)-1a]. A mixture of 90% purity (±)-butane-1,2,4-triol (6.9g, 60.2 mmol), PhCHO (9.5 ml, 90.3 mmol) and TsOH·H_2O (1.16g, 6.0 mmol) in 600 ml of dry toluene was heated at reflux under a *Soxhlet* containing 20 g of freshly activated powered 4Å molecular sieves under an argon flow for 4.5 hours. After cooling, 38% aqueous $NaHSO_3$ (100 ml) was added and stirred at rt for 1 hour. The mixture was extracted with CH_2Cl_2, and the combined organic extracts were washed with saturated aqueous $NaHCO_3$ and water. After drying ($MgSO_4$); evaporation of the solvents gave **(±)-1a**, which contained *ca.* 5-10% of the regioisomeric dioxolanes (as a *ca.* 1:1 mixture of *trans*- and *cis*-diastereoisomers) and a trace amount of the *cis*- diastereoisomer of **(±)-1a** (^{1}H-NMR evidence). Pure **(±)-1a** (10.16 g, 87% yield), identical to the previously reported,[27a,b,63] was obtained by flash-chromatography (hexane/EtOAc, from 55:45 to 40:60).

The reaction was carried out at different scales [from 5 to 250 mmol of starting butane-1,2,3-triol] without appreciable decrease in neither yield (> 75%) nor selectivity (> 90% regioselectivity; > 98% diastereoselectivity).

Synthesis of (±)-*cis*-4-(acetoxymethyl)-2-phenyl-1,3-dioxane [(±)-2a]. Ac_2O (2.5 ml, 25.75 mmol) was added to a solution of **(±)-1a** (999 mg, 5.15 mmol) in 4.5 ml of dry pyridine at 0°. The mixture was allowed to reach room temperature for one hour, and additionally stirred at rt for 11 hours. The mixture was poured over saturated aqueous $NaHCO_3$ at 0°, stirred for 30 minutes and extracted with CH_2Cl_2. The combined organic extracts were washed with 2N aqueous HCl, H_2O, half saturated aqueous $CuSO_4$ and H_2O and dried ($MgSO_4$). Evaporation of the solvent gave **(±)-2a** (1.16 g, 96% yield). An analytical pure sample was obtained by flash-chromatography (hexane/EtOAc, 4:1).

Synthesis of (±)-*cis*-4-(hydroxymethyl)-2-(4-methoxyphenyl)-1,3-dioxane [(±)-1b]. A mixture of 90% purity (±)-butane-1,2,4-triol (15.1 g, 128 mmol), p-anisaldehyde dimethylacetal (28.0 ml, 165 mmol) and TsOH·H_2O (2.4 g, 12.8 mmol) in 650 ml of dry toluene was heated at reflux under a *Soxhlet* containing 35 g of freshly activated powered 4Å molecular sieves under an argon flow for 12 hours. After cooling, excess solid anhydrous K_2CO_3 was added and stirred at rt for 1 hour. The mixture was diluted with CH_2Cl_2, and filtered. The solid was thorougly washed with CH_2Cl_2. The solvent was evaporated to give a crude material, which on analyzing by ^{1}H-NMR showed the presence of 8-10% of the two regioisomers (*cis*-

and *trans*-, in a *ca* 1:1 ratio) of (±)-**1b** and a trace of the diastereoisomer of (±)-**1b**. Pure (±)-**1b** (19.6 g, 69% yield) was obtained by flash-chromatography (hexane/EtOAc, from 50:50 to 20:80).

Synthesis of (±)-*cis*-4-(acetoxymethyl)-2-(4-methoxyphenyl)-1,3-dioxane [(±)-2b]. Ac_2O (1.5 ml, 15.63 mmol) was added to a solution of (±)-**1b** (700 mg, 3.13 mmol) in 3.0 ml of dry pyridine at 0°. The mixture was allowed to reach room temperature for one hour, and additionally stirred at rt for 15 hours. The mixture was poured over saturated aqueous $NaHCO_3$ at 0°, stirred for 30 minutes and extracted with CH_2Cl_2. The combined organic extracts were washed with 2N aqueous HCl, H_2O, half saturated aqueous $CuSO_4$ and H_2O and dried ($MgSO_4$). Evaporation of the solvent gave (±)-**2b** (818 mg, 98% yield). An analytical pure sample was obtained by flash-chromatography (hexane/EtOAc, 65:35).

General procedure for the PFL-catalyzed acetylation of (±)-1a and (±)-1b. Vinyl acetate (the mol equiv. indicated in either **Table 1** or **Table 2** or **Table 3**) was added to a mixture of (±)-**1** and PFL (the amount indicated in **Table 1-3**) in the corresponding solvent (when the reaction was carried out in the presence of powdered molecular sieves, it was added before vinyl acetate). The mixture was stirred at room temperature. The reactions were readily followed by ^{1}H-NMR. When the desired conversion degree was achieved, the mixture was diluted with CH_2Cl_2. The enzyme was filtered off and thoroughly washed with CH_2Cl_2. Evaporation of the solvents gave a crude material, which was chromatographed [hexane-EtOAc, 3:1 to 2:3] affording the ester (+)-**2** and the alcohol (-)-**1**. The analytical and spectroscopic data of compounds (-)-**1a**, (+)-**2a**, (-)-**1b** and (+)-**2b** are indicated below.

(R,R)-4-(Hydroxymethyl)-2-phenyl-1,3-dioxane [(-)-1a] (> 98% ee). $[\alpha]_D$ = -10.0 ($CHCl_3$, c = 1.18). MS: m/e = 194 (41.7), 193 (65.1), 163 (72.5), 123 (7.8), 117 (7.2), 107 (44.7), 106 (18.4), 105 (100), 91 (47.5), 79 (75.2), 78 (24.8), 77 (68.8), 71 (47.7), 57 (35.8), 43 (24.8). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.53-7.33 (m, 5H), 5.56 (s, 1H), 4.31 (ddd, J = 1.3, 5.2, 11.4, 1H), 4.09-3.92 (m, 2H), 3.72-3.65 (m, 2H), 2.10 (broad s, exchange with D_2O, 1H), 2.04-1.83 (m, 1H), 1.51-1.41 (m, 1H) ppm.. ^{13}C-NMR (50.3 MHz, $CDCl_3$): δ = 138.4 (s), 128.9 (d), 128.4 (2C, d), 126.1 (2C, d), 101.3 (d), 77.6 (d), 66.6 (t), 65.6 (t), 26.8 (t). Anal. Calcd. for $C_{11}H_{14}O_3$: C, 68.02%; H, 7.27%. Found: C, 67.78%; H, 7.60.

(S,S)-4-(Acetoxymethyl)-2-phenyl-1,3-dioxane [(+)-2a] (> 98% ee). $[\alpha]_D$ = +27.1 ($CHCl_3$, c = 1.2). MS: m/e = 236 (19.1), 235 (22.2), 193 (2.7), 176 (3.8), 163 (28.0), 114 (39.4), 105 (100), 91 (33.2), 79 (29.9), 77 (44.2), 43 (76.4). ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 7.61-7.46 (m, 2H), 7.32 (m, 3H), 5.53 (s, 1H), 4.25-4.09 (m, 1H), 4.25-4.09 (m, 3H), 4.00 (dt, J = 4.0, 12.7, 1H), 2.11 (s, 3H), 2.05-1.80 (m, 1H), 1.62-1,49 (m, 1H). ^{13}C-NMR (50.3 MHz, $CDCl_3$): δ = 171.4 (s), 138.7 (s), 129.2 (d), 128.6 (2C, d), 126.5 (2C, d), 101.5 (d), 75.1 (d), 66.8 (2C, t), 27.7 (t), 21.1 (q). Anal. Calcd. for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 66.16; H, 6.80.

(R,R)-4-(Hydroxymethyl)-2-(4-methoxyphenyl)-1,3-dioxane [(-)-1b]. (> 98% ee). $[\alpha]_D$ = -10.3 ($CHCl_3$, c = 1.45). MS: m/e = 224 (17.6), 223 (27.3), 193 (27.6), 135 (100), 109 (22.4), 108 (23.8), 77 (45.1), 71 (48.6), 57 (46.5), 55 (30.5), 43 (71.5), 41 (55.2). ^{1}H-NMR (200 MHz, acetone-d_6): δ = 7.39 (m, 2H), 6.90 (m, 2H), 5.49 (s, 1H), 4.25-3.90 (m, 3H), 3.79 (s, 3H), 3.59 (m, 2H), 2.86 (s, 1H), 1.78-1.69 (m, 1H), 1.57 (m, 1H). ^{13}C-NMR (50.3 MHz, $CDCl_3$): δ = 160.5 (s), 131.3 (s), 127.8 (2C, d), 114.0 (2C, d), 101.5 (s), 77.8 (d), 66.8 (t), 66.0 (t), 55.6 (q), 27.0 (t). Anal. Calcd. for $C_{12}H_{16}O_4$: C, 64.27%; H, 7.19%. Found: C, 64.62%; H, 7.29%.

(S,S)-4-(Acetoxymethyl)-2-(4-methoxyphenyl)-1,3-dioxane [(+)-2b]. (> 94% ee). $[\alpha]_D$ = +26.6 ($CHCl_3$, c = 0.8). MS: m/e = 266 (17.2), 265 (20.0), 193 (26.3), 152 (21.0), 135 (100), 43 (64.7). ^{1}H-NMR

(200 MHz, $CDCl_3$): δ = 7.42 (m, 2H), 6.90 (m, 2H), 5.49 (s, 1H), 4.30 (dd, J= 11.5, 4.0, 1H), 4.20-4.08 (m, 3H), 3.97 (dt, J = 2.5, 11.8, 1H), 3.80 (s, 3H), 2.09 (s, 3H), 1.89 (m, 1H), 1.51 (m, 1H).^{13}C-NMR (50.3 MHz, $CDCl_3$): δ = 171.3 (s), 160.4 (s), 131.2 (s), 127.8 (2C, d), 113.9 (2C, d), 101.4 (d), 75.0 (d), 66.9 (t), 66.8 (t), 55.5 (q), 27.7 (t), 21.1 (q). Anal. Calcd. for $C_{14}H_{18}O_5$: C, 63.14%; H, 6.81%. Found: C, 63.30%; H, 7.21%.

Determination of the enantiomeric excess of (R,R)-4-(hydroxymethyl)-2-phenyl-1,3-dioxane [(-)-1a]. A typical procedure is as follows. A mixture of 4.4 mg of enantiomerically enriched (-)-**1a** (of 60% ee, from the reaction reported in entry 12, **Table 1**) and 13.6 mg (0.5 mol equiv) of Eu(hfc)$_3$ was dissolved in *ca.* 0.6 ml of $CDCl_3$. After 15-20 minutes, a ^{1}H-NMR spectrum was taken, showing peaks (1:4.03 ratio) at 7.095 ppm (for the minor diastereoisomer) and at 7.047 ppm (for the main diastereoisomer) for the acetalic protons.

Determination of the enantiomeric excess of (S,S)-4-(acetoxymethyl)-2-phenyl-1,3-dioxane [(+)-2a]. A typical procedure is as follows. A mixture of 8.8 mg of enantiomerically enriched (+)-**2a** (of 78% ee, from the reaction reported in entry 6, **Table 1**) and 17.9 mg (0.4 mol equiv) of Eu(hfc)$_3$ was dissolved in *ca.* 0.6 ml of $CDCl_3$. After 15-20 minutes, a ^{1}H-NMR spectrum was taken, showing peaks (8.09:1 ratio) at 3.790 ppm (for the main diastereoisomer) and at 3.730 ppm (for the minor diastereoisomer) for the methyl groups.

Determination of the enantiomeric excess of (R,R)-4-(hydroxymethyl)-2-(4-methoxyphenyl)-1,3-dioxane [(-)-1b]. A typical procedure is as follows. A mixture of 3.6 mg of enantiomerically enriched (-)-**1b** (of 76% ee, from the reaction reported in entry 20, **Table 3**) and 10.6 mg (0.55 mol equiv) of Eu(hfc)$_3$ was dissolved in *ca.* 0.6 ml of $CDCl_3$. After 15-20 minutes, a ^{1}H-NMR spectrum was taken, showing peaks (1:7.23 ratio) at 7.053 ppm (for the minor diastereoisomer) and at 7.003 ppm (for the main diastereoisomer) for the acetalic protons.

Determination of the enantiomeric excess of (S,S)-4-(acetoxymethyl)-2-(4-methoxyphenyl)-1,3-dioxane [(+)-2b]. A typical procedure is as follows. A mixture of 1.4 mg of enantiomerically enriched (+)-**2b** (of 84% ee, from the reaction reported in entry 20, **Table 3**) and 9.57 mg (0.5 mol equiv) of Eu(hfc)$_3$ was dissolved in *ca.* z0.6 ml of $CDCl_3$. After 15-20 minutes, a ^{1}H-NMR spectrum was taken, showing peaks (10.95:1 ratio) at 2.982 ppm (for the main diastereoisomer) and at 2.949 ppm (for the minor diastereoisomer) for the methyl of the acetyl groups.

REFERENCES AND NOTES

(1) For recent books and reviews, see: (a) Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformations in Preparative Organic Chemistry. The Use of Isolated Enzymes and Whole Cell Systems in Synthesis*; Academic Press: London, 1988. (b) Sih, C. J.; Wu, S. H. *Topics in Stereochemistry* **1989**, *19*, 63-125. (c) Crout, D. H. G.; Christen, M. In *Modern Synthetic Methods*; vol. 5; Scheffold, R., Ed.; Springer-Verlag: Berlin, 1989; pp 1-114. (d) Wong, C.-H. *Science* **1989**, *244*, 1145-1152. (e) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365-5422. (f) Ohno, M.; Otsuka, M. *Org. React.* **1989**, *37*, 1-55. (g) Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. *Crit. Rev. Biotechnology.* **1990**, *10*, 129-153. (h) Wong,

C.-H. CHEM TRACTS-*Organic Chemistry* **1990**, 91-111. (i) Zhu, L. M.; Tedford, M. C. *Tetrahedron* **1990**, *46*, 6587-6611. (j) Boland, W.; Frößl, C.; Lorenz, M. *Synthesis* **1991**, 1049-1072. (k) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071-1140. (l) Haraldsson In *The Chemistry of Functional Groups. Supplement B: The Chemistry of Acid Derivatives*; Patai, S., Ed.; John Wiley and Sons: Chichester, 1992; vol. 2, part 2, pp 1395-1473. (l) Poppe, L.; Novák, L. *Selective Biocatalysis. A Synthetic Approach*; VCH: Weinheim, 1992. (m) Faber, K. *Biotransformations in Organic Chemistry*; Springer-Verlag: Berlin, 1992. (n) Danieli, B.; Lesma, G.; Passarella, D.; Riva, S. *Advances in the Use of Sybthons in Organic Chemistry* **1993**, *1*, 143-219.

(2) (a) Chen, C. S.; Sih, C. J. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 695-797. (b) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114-120.

(3) Kamal, A.; Rao, M. V. *Tetrahedron: Asymmetry* **1991**, *2*, 751-754. Moris, F.; Gotor, V. *Tetrahedron* **1992**, *48*, 9869-9876. VanMiddlesworth, F.; López, M.; Zweerink, M.; Edison, A. M.; Wilson, K. *J. Org. Chem.* **1992**, *57*, 4753-4754. Khan, R.; Gropen, L.; Konowicz, P. A.; Matulovà, M.; Paoletti, S. *Tetrahedron Lett.* **1993**, *34*, 7767-7770. Basak, A.; Bhattacharya, G.; Nag, A. *Biotechnology Lett.* **1993**, *15*, 469-470.

(4) Rebolledo, F.; Brieva, R.; Gotor, V. *Tetrahedron Lett.* **1989**, *30*, 5345-5346.

(5) Herradón, B.; Cueto, S.; Morcuende, A.; Valverde, S. *Tetrahedron: Asymmetry* **1993**, *4*, 845-864; and references cited therein.

(6) Mulzer, J.; Greifenberg, S.; Beckstett, A.; Gottwald, M. *Liebigs Ann. Chem.* **1992**, 1131-1135. Morgan, B.; Oehlschlager, A. C. *Tetrahedron: Asymmetry* **1993**, *4*, 907-910. Adamczyk, M.; Chen, Y.-Y.; Fishpaugh, J. R.; Gebler, J. C. *Tetrahedron: Asymmetry* **1993**, *4*, 1467-1468. Schirmeister, T.; Otto, H.-H. *J. Org. Chem.* **1993**, *58*, 4819-4822.

(7) Seebach, D.; Hungerbühler, E. In *Modern Synthetic Methods*; Scheffold, R; Ed.; Salle and Sauerländer: Berlin, 1980; vol. 2, pp 93-171.

(8) (a) Laane, C.; Tramper, J.; Lilly, M. D.; Eds.; *Biocatalysis in Organic Media*; Elsevier: Amsterdam, 1986. (b) Klibanov, A. M. *Trends Biochem. Sci.* **1989**, *14*, 141-144. (c) Dordick, J. S. In *Applied Biocatalysis*, Blanch, H. W.; Clark, D. S. Eds.; Marcel Dekker, Inc.: New York, 1991; vol. 1, pp 1-51.

(9) Some enzymes are even more stable in organic solvents than in water; for an example, see: Zaks, A.; Klibanov, A. M. *Science* **1984**, *224*, 1249-1251. For a discussion on the factors affecting enzyme's stability, see: Klibanov, A. M. In *Biotechn 1. Microbial Genetic Engineering and Enzyme Technology*; Hollenberg, C. P.; Sahm, H., Eds.; Gustav Fischer: Stuttgart, 1987; pp. 131-136.

(10) See, for instance: Dodson, G. In *Microbial Products: New Approaches*; Baumberg, S; Hunter, I.; Rhodes, M.; Eds.; Cambridge University Press: Cambridge, 1989; pp 293-307.

(11) Some synthetically useful enzymes have been engineered; for some leading references, see: α-Chymotrypsin: West, J. B.; Hennen, W. J.; Lalonde, J. L.; Bibbs, J. A.; Zhong, Z.; Meyer, Jr., E. F.; Wong C.-H. *J. Am. Chem. Soc.* **1990**, *112*, 5313-5320. Subtilisin: Graycar, T. P. In *Biocatalysts for Industry*; Dordick, J. S.; Ed.; Plenum Press: New York, 1991; pp 257-283. Lactate dehydrogenase: Casy, G.; Lee, T. V.; Lovell, H.; Nichols, B. J.; Sessions, R. B.; Holbrook, J. J. *J. Chem. Soc., Chem. Comm.* **1992**, 924-926. Aldolases: Fessner, W.-D. In *Microbial Reagents in Organic Synthesis*; Servi, S., Ed.; Kluwer Academic Publishers: Dordrecht; 1992; pp 43-55. Glycosyltransferases: Wong,

C.-H.; Dumas, D. P.; Ichikawa, Y.; Koseki, K.; Danishefsky, S. J.; Weston. B. W.; Lowe, J. B. *J. Am. Chem. Soc.* **1992**, *114*, 7321-7322.

(12) Chemical modifications of enzymes might be a cheaper solution, but it is difficult to predict the outcome. For some references, see: Wu, S. H.; Guo, Z. W.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 1990-1995. Zhong, Z.; Bibbs, J. A.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 2259-2263. Bianchi, D.; Battistel, E.; Bosetti, A.; Cesti, P.; Fekete, Z. *Tetrahedron: Asymmetry* **1993**, *4*, 777-782.

(13) Enantioselective inhibition promises to be an useful strategy to increase the enantioselectivity of enzyme catalyzed reactions, see: Guo, Z. W.; Sih, C. J. *J. Am. Chem. Soc.* **1989**, *111*, 6836-6841.

(14) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, 2nd. ed.; VCH: Weinheim, 1988.

(15) For some recent examples, see: Parida, S.; Dordick, J. S. *J. Am. Chem. Soc.* **1991**, *113*, 2253-2259. Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1991**, *113*, 3166-3171. Wescott, C. R.; Klibanov, A. M. *J. Am. Chem. Soc.* **1993**, *115*, 1629-1631. Parida, S.; Dordick, J. S. *J. Org. Chem.* **1993**, *58*, 3238-3244.

(16) Up to the best of our knowledge, the first examples on the reversal of enzyme enantioselectivity upon a change in the solvents have just been reported, see: (a) Wu, S. H.; Chu, F. Y.; Wang, K. T. *Bioorg, Med. Chem. Lett.* **1991**, *1*, 339-342. (b) Tawaki, S.; Klibanov, A. M. *J. Am. Chem. Soc.* **1992**, *114*, 1882-1884. (c) Hirose, Y.; Kariya, K.; Sasaki, I; Kurono, Y.; Ebiike, H.; Achiwa, K. *Tetrahedron Lett.* **1992**, *33*, 7157-7160. (d) Ueji,S.; Fujino, R,; Okubo, N.; Miyazawa, T.; Kurita, S.; Kitadani, M.; Muromatsu, A. *Biotechnology Lett.* **1993**, *15*, 163-168. (e) We have observed the same behavior in the lipase-mediated acylations of (±)-1-benzyloxy-2,3-propanediol[5] and 1-*tert*butoxycarbonyl-2-hydroxymethylpiperidine (Molina, M. T.; del Valle, C.; Herradón, B. unpublished results).

(17) For a significant example where the use of an organic solvent have been critical for the success of the transformation, see: Bevinakatti, H. S.; Banerji, A. A. *J. Org. Chem.* **1992**, *57*, 6003-6005.

(18) Kazandjian, R. Z.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 5448-5450. Ricca, J. M.; Crout, D. H. G. *J. Chem. Soc. Perkin Trans. 1* **1989**, 2126-2127.

(19) Herradón, B. *Synlett* **1993**, 108-110.

(20) Alberghina, L.; Schmid, R. D.; Verger, R., Eds.; *Lipases: Structure, Mechanism and Genetic Engineering*; VCH: Weinheim, 1991.

(21) For a review discussing the different acylating agents in lipase-catalyzed transformations, see: Faber, K.; Riva, S. *Synthesis* **1992**, 895-910.

(22) Hizuka, M.; Hayashi, N.; Kamashita, T.; Suemune, H.; Sakai, K. *Chem. Pharm. Bull.* **1988**, *36*, 1550-155. Hizuka, M.; Fang, C.; Suemune, H.; Sakai, K. *Chem. Pharm. Bull.* **1989**, *37*, 1185-1187. Schmidt, U.; Kleefeldt, A.; Mangold, R. *J. Chem. Soc., Chem. Comm.* **1992**, 1687-1689.

(23) Thiam, M.; Chastrette, F. *Tetrahedron Lett.* **1990**, *31*, 1429-1432. Thiam, M.; Slassi, A.; Chastrette, F.; Amouroux, R. *Synth. Comm.* **1992**, *22*, 83-95. Riant, O.; Samuel, O.; Kagan, H. B. *J. Am. Chem. Soc.* **1993**, *115*, 5835-5836.

(24) (a) Valverde, S.; Herradón, B.; Martín-Lomas, M. *Tetrahedron Lett.* **1985**, *26*, 3731-3734. (b) Valverde, S.; Hernández, A.; Herradón, B.; Rabanal, R. M.; Martín-Lomas, M. *Tetrahedron* **1987**, *43*, 3499-3504. (c) Herradón, B. *Tetrahedron: Asymmetry* **1991**, *2*, 191-194.

(25) Herradón, B. Communication to the "V Jornadas de Química Orgánica", Poblet, 1991. Arslan, T.; Herradón, B.; Schweizer, B. W.; Benner, S. A. *Helv. Chim. Acta* **1993**, *76*, 2969-2975.

(26) Hanessian, S.; Ugolini, A.; Dube, D.; Glamyan, A. *Can. J. Chem.* **1984**, *62*, 2146-2147.

(27) Derivatives of (R)-malic acid have been synthesized from cheap (R,R)-tartaric acid, see: (a) Hungerbühler, E.; Seebach, D.; Wasmuth, D. *Angew. Chem. Int. Ed. Engl.* **1979**, *18*, 958-960. (b) Hungerbühler, E.; Seebach, D.; Wasmuth, D. *Helv. Chim. Acta* **1981**, *64*, 1467-1487. (c) Gao, Y.; Zepp, C. M. *Tetrahedron Lett.* **1991**, *32*, 3155-3158. For recent biocatalytic syntheses of (R)- and (S)-malic acid derivatives, see: (d) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Aragozzini, F.; Maconi, E. *J. Chem. Soc. Perkin 1* **1991**, 601-605. (e) Miyama, M.; Nakayama, K. *Biotechnology Lett.* **1993**, *15*, 23-28. (f) Nakayama, K.; Ushijima, M. *Biotechnology Lett.* **1993**, *15*, 271-276.

(28) (a) The use of enol ester as acylating agents in lipase catalyzed esterifications was used for the first time by: Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 6421-6422. (b) For an early application to the reaction between achiral esters and alcohols, see: Degueil-Castaing, M.; De Jeso, B.; Drouillard, S.; Maillard, B. *Tetrahedron Lett.* **1987**, *28*, 953-954.

(29) For a review on the applications of *Pseudomonas fluorescens* lipase in asymmetric synthesis, see: Xie, Z. F. *Tetrahedron Asymmetry* **1991**, *2*, 733-750.

(30) Hansch, C.; Leo, A. *Substituent Constant for Correlation Analysis in Chemistry and Biology*; John Wiley and Sons: New York, 1979.

(31) Riddick, J. A.; Bunger, W. B.; Sakano, T. K. *Organic Solvents. Physical Properties and Methods of Purification*, 4th. ed.; John Wiley and Sons: New York, 1986.

(32) All the solvents were of the best quality available (Fluka, Merck or Aldrich) and kept over molecular sieves. The estimated water-content is lower than 0.5%. For the shake of comparison, when freshly dried toluene and THF (distilled over sodium/benzophenone ketyl) were used as solvents the same results were obtained.

(33) Hexane and pentane (dry and water-saturated), which have frequently been used as solvents in lipase catalyzed transesterification,[34] have been tried; but the insolubility of (±)-**1a** and (±)-**1b** in these solvents hampered their uses.

(34) It has been stated that solvents with high log P values are the most convenient for lipase catalyzed transesterifications; for a discussion, see: Laane, C.; Boeren, S.; Vos, K.; Veeger, C. *Biotechn. Bioeng.*, **1987**, *30*, 81-87.

(35) Up to the best of our knowledge the effect of the amount of enzyme on the selectivity of lipase catalyzed transformations has not been extensively studied.

(36) Thoroughly the text, wet solvent means water-saturated solvent.

(37) This fact has provided a straightforward method to immobilize the enzyme. We are currently investigating the synthetic applications of this immobilized enzyme.

(38) For a recent paper dealing with the influence of water-content of the solvent on the lipase catalyzed esterifications, see: Kitaguchi, H.; Itoh, I.; Ono, M. *Chem. Lett.* **1990**, 1203-1206; and references cited therein.

(39) Molecular sieves have been claimed either to absorb the acetaldehyde generated during the reaction,[28b,40] or to keep the water activity constant,[41a]. For some recent uses of molecular sieves in lipase catalyzed acylations see ref 42.

(40) Izumi, T.;Tamura, F.; Sasaki, K. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2784-2788.

(41) (a) Ottolina, G.; Carrea, G.; Riva, S.; Sartore, L.; Veronese, F. M. *Biotechnology Lett.* **1992**, *14*, 947-952. (b) For a discussion on the influence of water activity in enzyme catalyzed transformations in biphasic systems, see: Halling, P. J. In *Biocatalysis in Organic Media*; Laane, C.; Tramper, J.; Lilly, M. D., Eds.; Elsevier: Amsterdam, 1986; pp 125-132. (c) For an outstanding example on the influence of water activity on the enantioselectivity of lipase-catalyzed esterifications, see: Högberg, H.-E.; Edlund, H.; Berglund, P.; Hendenström, E. *Tetrahedron: Asymmetry*: **1993**, *4*, 2123-2126.

(42) Holla, E. W. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 220-221. Carretero, J. C.; Dominguez, E. *J. Org. Chem.* **1992**, 57, 3867-3873. Molecular sieves have been used to absorb methanol generated in a transesterification reaction with methyl esters, see: Henkel, B.; Kunath, A.; Schik, H. *Tetrahedron: Asymmetry*: **1993**, *4*, 153-156.

(43) The effect of the temperature on the selectivity has not been frequently studied; for a discussion, see: Holmberg, E.; Hult, K. *Biotechnology Lett.* **1991**, *13*, 323-326. For a recent example where the enantioselectivity increases with the temperature, see: Parmar, V. S.; Prasad, A. K.; Singh, P. K.; Gupta, S. *Tetrahedron: Asymmetry* **1992**, *3*, 1395-1398.

(44) The effect of the amount of acylating agent on the selectivity and velocity of lipase catalyzed transformations has not been extensively studied; for an exception, see: Mischitz, M.; Pöschl, U.; Faber, K. *Biotechnology Lett.* **1991**, *13*, 653-656. The importance of the amount of acylating agent has been noted elsewhere, see: Chen, C.-S.; Liu, Y.-C. *J. Org. Chem.* **1991**, *56*, 1966-1968.

(45) Most likely acetic acid is formed from vinyl acetate, although the hydrolysis of **2a** can not be ruled out. We have also observed by ^{1}H-NMR spectroscopy that the ratio **2a**:**1a** decreases slightly with the time.

(46) The only exception is when THF is the solvent. A slightly better enantioselectivity is obtained in the kinetic resolution of (±)-**1a** than in the kinetic resolution of (±)-**1b** (compare entry 18, **Table 1** with entry 18, **Table 3**).

(47) Only in the most polar solvents (acetone and acetonitrile), the PFL-acetylation of (±)-**1a** is faster than the acetylation of (±)-**1b** (compare entries 23 and 24, **Table 1** with entries 21 and 23, **Table 3**).

(48) More appropriately, the parameter we should have to use is the amount of water that the organic solvent can dissolve, but we have not found this data in any of the reference books we have checked. In any case, we think that log P gives a good indication of this property.

(49) Only a few comprehensive studies on the influence of the solvent on the selectivity of lipase catalyzed transesterification have been reported.[50,51] Due to the scarcity of experimental data, it is not easy to make any generalization on the behavior of lipases in organic solvents.

(50) Nakamura, K.; Takebe, Y.; Kitayama, T.; Ohno, A. *Tetrahedron Lett.* **1991**, *32*, 4941-4944. Secundo, F.; Riva, S.; Carrea, G. *Tetrahedron Asymmetry*, **1992**, *3*, 267-280. Carrea, G.; Ottolina, G.; Riva, S.; De Amici, M.; De Micheli, C. In *Microbial Reagents in Organic Synthesis*; Servi, S., Ed.; Kluwer Academic Publishers: Dordrecht; 1992; pp 225-238. Miyazawa, T.; Kurita, S.; Ueji, S.; Yamada, T.; Kuwata, S. *Biotechnology Lett.* **1992**, *14*, 941-946. Terradas, F.; Teston-Henry, M.; Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1993**, *115*, 390-396.

(51) For a comprehensive study on the influence of the nature of the solvent on the rate of lipase-catalyzed transesterification of tributyrin with various alcohols, see: Hirata, H.; Higuchi, K.; Yamashina, T. *J. Biotechnology* **1990**, *14*, 157-167.

(52) Human pancreatic lipase (hPL): Winkler, F. K.; D'Arcy, A.; Hunziker, W. *Nature* **1990**, *343*, 771-774. *Rhizomucor miehei* lipase (Rml): Brady, L.; Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E.; Dodson, G.; Tolley, S.; Turkenburg, J. P.; Christiansen, L.; Huge-Jensen, B.; Norskov, L.; Thim, L.; Menge, U. *Nature* **1990**, *343*, 767-770. *Geotrichum candidum* (GCL): Schrag, J. D.; Li, Y.; Wu, S.; Cygler, M. *Nature* **1991**, *351*, 761-764.

(53) For recent discussions on the mechanism of the lipolysis, see: (a) Lawson, D. M.; Brzozowski, A. M.; Dodson, G. G. *Current Biology* **1992**, *2*, 473-475. (b) Dodson, G. G., Lawson, D. M., Winkler, F. K. *Faraday Discuss.* **1992**, *93*, 95-105. (c) Derewenda, Z. S.; Sharp, A. M. *Trends Biochem. Sci.* **1993**, *18*, 20-25. For a recent mechanistic study, see: (d) Kawase, M.; Sonomoto, K.; Tanaka, A. *Biocatalysis* **1992**, *6*, 43-50.

(54) Fersht, A. *Enzyme Structure and Mechanism*, 2nd. ed.; W. H. Freeman and Company: New York, 1985; pp 405-413.

(55) Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L. L.; Silman, I. *Science* **1991**, *253*, 872-879.

(56) For a discussion on the primary structures of some *Pseudomonas* lipases, and assignment of the amino acids of the active site, see: (a) Nishioka, T.; Chihara-Shiomi, M.; Yoshikawa, K.; Inagaki, M.; Yamamoto, Y.; Hiratake, J.; Baba, N.; Oda, J. In *Lipases: Structure, Mechanism and Genetic Engineering*; Alberghina, L.; Schmid, R. D.; Verger, R., Eds.; VCH: Weinheim, 1991; pp 253-262. (b) Chun, G. H.; Lee, Y. P.; Jeohn, G. H.; Yoo, O. J.; Rhee, J. S. *Agric. Biol. Chem.* **1991**, *55*, 2359-2365.

(57) Sarma, R.; Dauberman, J.; Poulose, A. J.; van Bielen, J.; Power, S.; Shew, B.; Gray, G.; Norton, S.; van Kimmenade, A.; Jarnagin, A.; Ashizawa, E.; Boston, M.; Requadt, C.; Kolattakudy, P. E.; Bott, R. In *Lipases: Structure, Mechanism and Genetic Engineering*; Alberghina, L.; Schmid, R. D.; Verger, R., Eds.; VCH: Weinheim, 1991; pp 71-76. Kordel, M.; Schmid, R. D. In *Lipases: Structure, Mechanism and Genetic Engineering*; Alberghina, L.; Schmid, R. D.; Verger, R., Eds.; VCH: Weinheim, 1991; pp 385-388.

(58) Sih, C. J.; Gu, R. L. In *Microbial Reagents in Organic Synthesis*; Servi, S., Ed.; Kluwer Academic Publishers: Dordrecht; 1992; pp 79-92.

(59) Up to the best of our knowledge, electronic effects have not been generally invoked to explain the selectivity of synthetic enzymatic reactions. For discussions on stereoelectronic effect in enzymatic reactions, see: Gorenstein, D. G. *Chem. Rev.* **1987**, *87*, 1047-1077. Dugas, H. *Bioorganic Chemistry. A Chemical Approach to Enzyme Action*, 2nd. ed.; Springer-Verlag: New York, 1989; pp 222-242. We are currently investigating the putative electronic influence of the substituents on the selectivity in this kind of transformations.

(60) Herradón, B. *Tetrahedron: Asymmetry* **1992**, *3*, 209-212. Kanerva, L. T.; Vänttinen, E. *Tetrahedron: Asymmetry* **1993**, *4*, 85-90. Antus, S.; Gottsegen, A.; Kajtár, J.; Kovács, T.; Tóth, T. S.; Wagner, H. *Tetrahedron: Asymmetry* **1993**, *4*, 339-344. Herradón, B. *J. Org. Chem.* in the press.

(61) Derewenda, U.; Brzozowski, A. M.; Lawson, D. M.; Derewenda, Z. S. *Biochemistry* **1992**, *31*, 1532-1541.

(62) Parker, D. *Chem. Rev.* **1991**, *91*, 1441-1457.

(63) Pawlak, K.; Nakanishi, K.; Iwashita, T.; Borowski, E. *J. Org. Chem.* **1987**, *52*, 2896-2901.

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