Activation of Pig Liver Esterase in Organic Media with Organic Polymers. Application to the Enantioselective Acylation of Racemic Functionalized Secondary Alcohols

Hans-Joachim Gais,* Manfred Jungen, and Vasudev Jadhav

Institut für Organische Chemie der Rheinisch-Westfälischen Technischen Hochschule Aachen, Professor-Pirlet-Strasse 1, D-52056 Aachen, Germany

Gais@RWTH-Aachen.de

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Pig liver esterase (PLE) shows practically no activity in acylation of alcohols with vinylic esters in organic solvents. However, addition of methoxypoly(ethylene glycol) (MPEG), bovine serum albumin (BSA), TentaGelAmino resin (TGA), or aminomethyl polystyrene (AMPS) confers activity to PLE in acylation of alcohols with vinyl propionate in organic solvents of low water content. Polymer-activated PLE showed high enantioselectivities ($E > 100$) in the acylation of racemic 1-alkoxy-, 1-ethylsulfanyl-, and 1-fluoro-3-aryl-2-propanols as well as racemic 1-phenoxy-2-propanol and racemic 1-methoxy-2-phenoxy-2-propanol. The synthetic utility of polymer-activated PLE has been demonstrated by the gram-scale resolution of 1-methoxy-3-phenyl-2-propanol, 1-ethylsulfanyl-3-phenyl-2-propanol, 1-methoxy-3-*p*-methoxyphenyl-2-propanol, 1-fluoro-3-phenyl-2-propanol, and 1-methoxy-3-phenoxy-2-propanol. In PLE-catalyzed acylation of alcohols with vinyl propionate, acetaldehyde and propionic acids, both being detrimental to the enzyme, are formed as byproducts. In addition, the water content of the system, which is critical for the activity of pig liver esterase, is lowered because of a competing enzymatic hydrolysis of the acyl donor. The polymers TGA, BSA, and AMPS not only scavenge the aldehyde and the acid through imine formation and neutralization, respectively, but replenish at least in part also the water consumed in the competing hydrolysis of the acyl donor. A recovery of PLE together with the polymer was achieved without major loss of activity through their immobilization on a water-saturated polyaramide membrane, which occurs spontaneously in organic solvents.

Introduction

Pig liver esterase (PLE) is one of the most important enzymes for the enantioselective hydrolysis of prochiral or racemic esters in aqueous solution. It has, therefore, seen many applications in asymmetric synthesis.[1−6] However, despite its obvious synthetic potential, the reverse transformation, namely the PLE-catalyzed enantioselective acylation of prochiral or racemic alcohols in organic solvents, has not gained the importance of the lipase-catalyzed acylation method.[3−5,7] This is due to the fact that PLE shows only a very low activity in organic solvents. The esterase differs in this respect considerably from lipases, which are highly active in organic media. Many factors have been found to affect the activity and enzyme stability in organic solvents.[7−14] One important factor is the water activity and the nature of the organic solvent. Another one is the formulation of the enzyme. Enzymes are generally not soluble in organic solvents. Therefore, catalysis is carried out under heterogeneous conditions, which restrict not only the mobility of the enzyme but also of the substrate and products and can, thus, cause mass transfer limitations. Immobilization on solid support, addition of hydrated salts, covalent attachment of methoxpoly(ethylene glycol) (MPEG) residues, lyophilization with organic polymers, cross-linking of enzyme crystals, sol−gel entrapment, coating with amphiphilic molecules, and entrapment in reverse micelles are the most important formulations that have been shown to improve the performance of the enzyme.[5,7−14]

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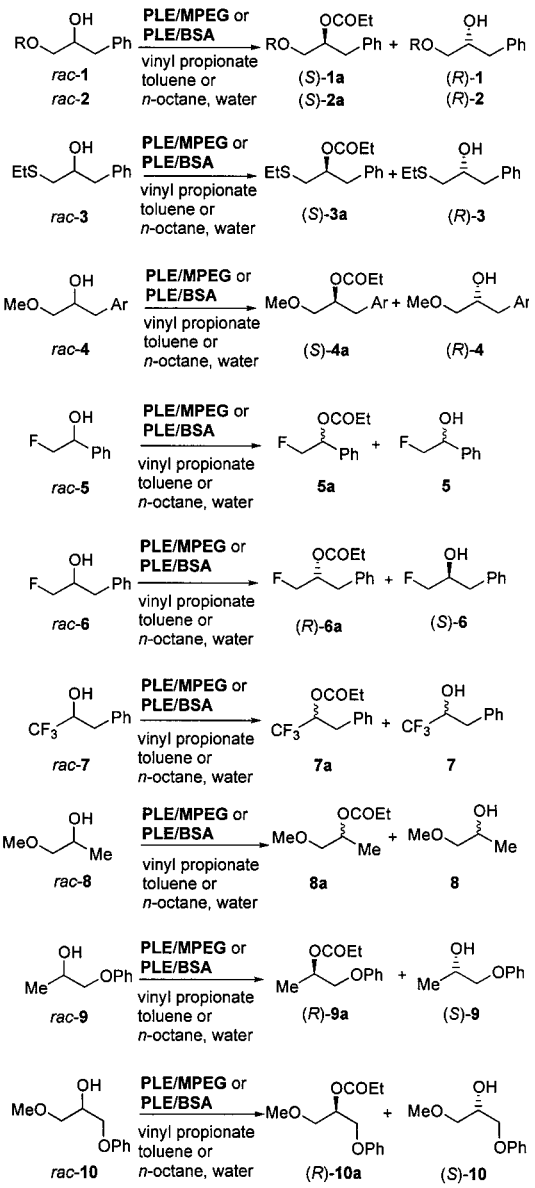
Attempts to confer activity to PLE in organic solvents by entrapment in water-filled porous supports,[15,16] covalent attachment of MPEG residues,[17,18] immobilization on carrageenan[19] and Eupergit,[20] or entrapment in polymers[21] were met with various degrees of success.[22] Recently, we have shown that the simple addition of MPEG to PLE through colyophilization significantly enhances the activity and stability of the enzyme in organic solvents of low to medium polarity and low water content.[23−25] The colyophilisate (CLP) of PLE and MPEG was successfully applied to the kinetic resolution of racemic glycerol derivatives, which bear a primary hydroxy group, through acylation with vinyl and isopropenyl esters in toluene containing less than 1% water (vide infra). We noticed, however, in these experiments also a decrease of the activity of PLE in organic solvents with increasing reaction time. This time dependency of the activity of the enzyme had been attributed in part to a gradual lowering of the critical water content of the system through a competitive enzymatic hydrolysis of the vinylic esters under formation of the corresponding carboxylic acid and carbonyl byproduct.[23,24,26] In addition, earlier studies on the influence of the carbonyl byproducts acetaldehyde and acetone upon PLE had shown that they are detrimental to the activity of the enzyme.[24] Deactivation of PLE by acetaldehyde, which may be caused at least in part by imine formation with the lysine amino groups,[27] was particularly strong. Besides acetaldehyde, the carboxylic acid being formed in the aforementioned hydrolysis seems to cause also a deactivation of PLE in organic solvents.[23] Because of the considerable potential of PLE for enantioselective acylation, it was thus desirable to conduct further studies on its polymer activation in organic solvents in order to circumvent the aforementioned problem. Besides the question of a further activation of PLE that of the substrate specificity of the enzyme in organic solvents was especially important. We were particularly interested to see whether secondary alcohols are also substrates for PLE.[24] In the present contribution we report the results of our studies designed to address these issues. We show that PLE can be (i) activated in organic solvents with a number of special organic polymers, (ii) applied successfully as mixture with these polymers to the enantioselective acylation of racemic secondary alcohols, and (iii) recovered and reused.

Scheme 1. PLE/MPEG- and PLE/BSA-Catalyzed Acylation of Homobenzylic Alcohols, Fluorinated Alcohols, and Methoxy and Phenoxy Group Bearing Alcohols



1: R = Me, **2**: R = *i*Pr, Ar = *p*-MeOC$_6$H$_4$

Results and Discussion

For the determination of the influence of the various activators on the activity and the enantioselectivity of PLE in organic solvents, the racemic functionalized 2-propanols *rac*-**1**−**4**, *rac*-**6**, and *rac*-**7** were selected as potential substrates. These alcohols, which all carry a benzyl group at the sterogenic center (Scheme 1), were chosen for the following reasons. Previous results obtained in the acylation of *rac*-**4** catalyzed by PLE in the presence of MPEG (termed in the following PLE/MPEG-catalyzed acylation) seemed to indicate a particular

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affinity of PLE in organic solvents toward homobenzylic alcohols.[24] Second, the aforementioned alcohols in enantiomerically enriched form are of synthetic value.[28−31] Third, their lipase catalyzed kinetic acylation has to the best of our knowledge not been described. Included into this study were the racemic alcohols *rac*-**5** and *rac*-**8**−**10**. These alcohols were selected because of their structural resemblance to the aforementioned alcohols and because of the synthetic value of the enantiomerically enriched compounds.[32−42] Of the latter alcohols only *rac*-**9**[32,36] and *rac*-**10**[42] had been submitted to a lipase-catalyzed acylation in organic solvents. Native PLE is a mixture of isoenzymes having six major components.[3,5] Commercially available are the native mixture and three different enriched isoenzyme mixtures.[43] In the overwhelming number of cases reported in the literature thus far the native isoenzyme mixture has been applied for enantioselective synthesis.[1−6] We have, thus, used in the work described herein the native isoenzyme mixture because of a better comparison with previous results from our and other laboratories.

Pig Liver Esterase/Methoxypoly(ethylene glycol). Since the colyophilisate PLE/MPEG had shown promise for the enantioselective acylation of racemic primary alcohols in organic solvents, it was of interest to see whether secondary alcohols are also amenable to a PLE/MPEG-catalyzed acylation. In addition, possible activity and selectivity improvements of PLE/MPEG were sought. The commercially available suspension of PLE in ammonium sulfate was first desalted by ultrafiltration in order to exclude any salt effect on the activity and selectivity of the enzyme in organic solvents (vide infra). Lyophilization of desalted PLE (190 U/mg, 30 mg) and $MPEG_{5000}$ (1 g) in aqueous solution gave PLE/MPEG as a fine, free-flowing, white powder, which contained less than 1% water according to a Karl Fischer titration. The enzyme had a specific activity of 170 U/mg as determined by hydrolysis of ethyl butyrate in aqueous solution. Storage of the colyophilisate (CLP) at 4 °C for several weeks did not lead to a decrease of enzyme activity. Thus, preparation of PLE/MPEG was accompanied only by an approximately 10% loss of the activity of the enzyme. We noticed that MPEG acts as a lyoprotectant since lyophilization of PLE in aqueous solution without the polymer was accompanied by a 30% to 50% activity loss. An activity preserving effects of MPEG and PEG upon enzymes had been noticed before in other cases than PLE.[8−14]

Table 1. PLE/MPEG-Catalyzed Acylation of Alcohols *rac*-1−10[a]

entry	substrate	solvent	*t* (d)	convn (%)	alcohol ee (%)	ester ee (%)	*E*[b]
1	*rac*-**1**	toluene	4	47	90	98	>100 (307)
2[c]	*rac*-**2**	toluene	4	4	5	>98	>100
3	*rac*-**3**	toluene	0.8	8	8	>98	>100
4	*rac*-**3**	*n*-octane	2	44	64	93	53
5	*rac*-**4**	toluene	2	39	73	99	>100 (437)
6	*rac*-**4**	*n*-octane	2	51	99	94	>100 (170)
7	*rac*-**5**	toluene	2	3	2	61	4
8	*rac*-**6**	toluene	2	16	21	98	>100
9	*rac*-**6**	*n*-octane	3	39	47	94	51
10	*rac*-**7**	toluene	4	1	*d*	*d*	*d*
11	*rac*-**8**	toluene	1.1	21	26	65	6
12	*rac*-**9**	toluene	2	26	39	>98	>100
13	*rac*-**10**	toluene	5	47	90	97	>100

[a] Conditions: 1 mmol of alcohol, 4 mmol of vinyl propionate, 300 U of PLE, 10 mL of solvent containing 0.4% water. [b] Numbers in parentheses are the calculated values. [c] The substrate concentration was 0.025 mol/L. [d] Not determined.

PLE/MPEG-catalyzed acylation of *rac*-**1**−**10** was generally carried out by dissolving the alcohol in an organic solvent followed by the addition of vinyl propionate as the acyl donor. Then, the water content of the system was adjusted to a total of 0.43% (v/v) whereby a tiny second aqueous phase was formed except in the case of methyl *tert*-butyl ether (MTBE) and dimethoxyethane (DME). Finally, additional solvent and PLE/MPEG, which dissolved mainly in the aqueous phase, were added. Because of the solubility of MPEG in some of the solvents used, part of the polymer may have entered the organic phase. Acylation was started by rapid stirring of the two-phase mixture, which caused the aqueous phase to disperse into small droplets. With increasing reaction time the second liquid phase disappeared, however, and solid PLE/MPEG deposited.

High enantioselectivities were recorded in the case of the 3-phenyl-2-propanols *rac*-**1**, *rac*-**2**, *rac*-**3**, and *rac*-**4**, the fluorinated 3-phenyl-2-propanol *rac*-**6**, and the 3-phenoxy-2-propanols *rac*-**9** and *rac*-**10**[24] (cf. Scheme 1) by using toluene or *n*-octane as solvent (Table 1). All reactions are characterized by a selectivity factor *E* (enantiomer ratio) being in all cases except two greater than 100.[44] In the case of alcohol *rac*-**1** the point of 47% conversion was reached after 4 d using toluene as solvent. The alcohol (*R*)-**1** and the ester (*S*)-**1a** were formed with high selectivity as revealed by an *E* value of greater than 100 (Table 1, entry 1). Acylation of *rac*-**2**, which bears a isopropoxy group, by using PLE/MPEG proceeded also with high selectivity. The activity of the enzyme in toluene toward this substrate was, however, lower than in the case of *rac*-**1** (Table 1, entry 2). The activity loss

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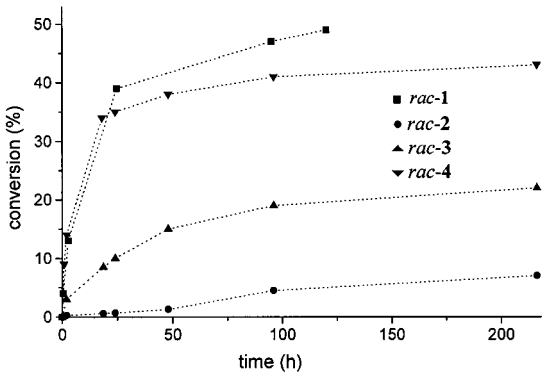

Figure 1. PLE/MPEG-catalyzed acylation of *rac*-**1**−**4** with vinyl propionate in toluene.

could be compensated for by using another polymer for the activation of PLE (vide infra) thus making a preparative scale resolution of this alcohol feasible too. The ethylsulfanyl group bearing alcohol *rac*-**3** could also be acylated with vinyl propionate by using PLE/MPEG. In toluene as solvent the enantioselectivity of the acylation was high ($E > 100$), but the activity of the enzyme was very low (Table 1, entry 3). However, the activity could be increased considerably by using *n*-octane as solvent (Table 1, entry 4). Under these conditions the enzyme exhibited toward *rac*-**3** a good selectivity ($E = 53$) as well as a good activity (44% conversion after 2 d).

To probe the steric requirements of the active site of PLE in organic solvents and because of synthetic reasons, alcohol *rac*-**4**, which bears a methoxy group in *p*-position, was submitted to a PLE/MPEG-catalyzed acylation. The enzyme showed toward this alcohol the highest selectivity ($E > 100$) and a good activity giving ester (*S*)-**4a** and alcohol (*R*)-**4** (Table 1, entries 5 and 6). While in toluene the selectivity of the enzyme was higher than in *n*-octane, in the latter solvent the activity was slightly higher. In the case of the acylation of the fluorine bearing alcohols *rac*-**5**−**7** the activity of the enzyme was the major problem (entries 7−10). In addition the selectivity of PLE toward the benzylic alcohol *rac*-**5** was low. The activity problem could be solved in the case of *rac*-**6** through a solvent change. In toluene the selectivity of PLE toward this alcohol was high ($E > 100$) but its activity was very low (16% conversion after 2 d). A switch to *n*-octane as solvent saw an increase of the activity of the enzyme (39% conversion after 3 d). Although the selectivity ($E = 51$) decreased with this solvent change, it is still high enough for synthetic purposes. In the case of the trifluoromethyl substituted alcohol *rac*-**7** the selectivity of PLE could not be determined because of a very low activity of the enzyme. The activities of PLE/MPEG toward *rac*-**1**−**4** in organic solvents are illustrated by Figure 1.[45] It is surprising that PLE/MPEG shows toward the structurally closely related alcohols *rac*-**1**−**3** such different activities under the conditions employed. We have no convincing explanation at present for this phenomenon. Product inhibition as a possible cause can be excluded since PLE showed toward *rac*-**2** and *rac*-**3** in *n*-octane (cf. Table 1, entry 4) in the presence of MPEG or of another activating polymer (vide infra) much higher activities. The PLE/MPEG-catalyzed resolution of alcohol *rac*-**4** showed the

(45) The dotted lines of Figure 1 and of the following Figures 2 and 3 are only meant to connect the points of measurement and calculation.

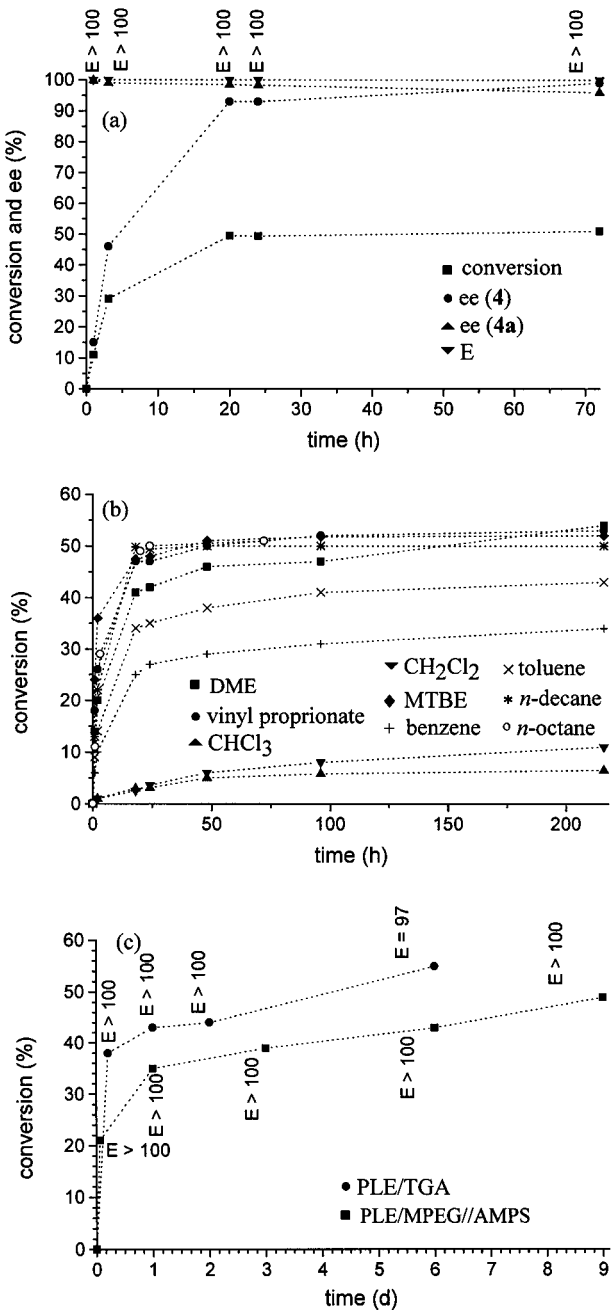

Figure 2. (a) Gram-scale PLE/MPEG-catalyzed kinetic resolution of *rac*-**4** with vinyl propionate in *n*-octane. (b) Solvent dependency of the PLE/MPEG-catalyzed acylation of *rac*-**4** with vinyl propionate. (c) PLE/TGA- and PLE/MPEG//AMPS-catalyzed acylation of alcohol *rac*-**4** with vinyl propionate in organic solvents.

characteristic time dependencies of the conversion and the ee values of a kinetic resolution (Figure 2a), and the selectivity factor *E*, which is high, remained practically constant throughout the acylation.

PLE exhibited toward the alcohols *rac*-**8**−**10**, which lack a benzylic phenyl group, moderate activities and varying selectivities. While the selectivity was only low in the case of alcohol *rac*-**8** ($E = 6$), which does not carry a benzyl group, it was high in the case of the phenoxymethyl group bearing alcohols *rac*-**9** ($E > 100$) and *rac*-**10** ($E > 100$) (Table 1, entries 11−13). The lipases studied so far in the acylation of *rac*-**9** and *rac*-**10** showed lower enantioselectivities than PLE. The *Candida antarctica*

Table 2. PLE/MPEG-Catalyzed Acylation of Alcohol *rac*-4 in Different Organic Solvents[a]

entry	solvent	*t* (d)	convn[b] (%)	alcohol ee (%)	ester ee (%)	*E*[c]
1	toluene	2	39 (35)	73	99	>100 (437)
2	benzene	2	29 (27)	50	99	>100 (327)
3	*n*-decane	2	51 (49)	98	96	>100 (226)
4	*n*-octane	2	51 (49)	99	94	>100 (170)
5	CH_2Cl_2	2	6 (4)	9	≥98	>100
6	$CHCl_3$	2	5 (3)	*d*	≥98	>100
7	DME	2	47 (42)	86	88	43
8	MTBE	2	51 (47)	98	85	55
9[e]	vinyl prop.	2	51 (47%)	95	80	32

[a] Conditions: 1 mmol of alcohol, 4 mmol of vinyl propionate, 300 U of PLE, 10 mL of solvent containing 0.4% water. [b] Numbers in parentheses are those for a reaction time of 1 d. [c] Numbers in parentheses are the calculated values. [d] Not determined. [e] Vinyl propionate was used also as solvent.

lipase and *Pseudomonas* sp. lipase catalyzed acylation of *rac*-**9** features *E* values of 4−139[32] and 35,[36] respectively, while the *Pseudomonas cepacia* lipase catalyzed acylation of *rac*-**10** is characterized by an *E* value of 42.[42] The enantiomer preference of these lipases toward *rac*-**9** and *rac*-**10** is the same as of PLE. However, the rates of the lipase-catalyzed acylations of these alcohols are higher than those of PLE.

Because of the high selectivity and relatively high activity of PLE toward *rac*-**4**, acylation of this alcohol was studied in more detail by variation of the solvent and the temperature. As shown by Table 2and Figure 2b, benzene and toluene are in regard to selectivity the best solvents for the PLE/MPEG-catalyzed kinetic resolution of *rac*-**4** (Table 2, entries 1 and 2). A disadvantage of these solvents is the only moderate activity of the enzyme. Similar solvent dependencies of selectivity and activity have been made previously in the case of lipases.[46] In *n*-decane or *n*-octane the acylation rate of *rac*-**4** was enhanced as compared to the aromatic solvents (Table 2, entries 3 and 4). Although the selectivity decreased slightly by using these solvents, it is still high enough ($E > 100$) for a preparative kinetic resolution. In methylene chloride and chloroform the activity of PLE was low, although the enantioselectivity of the enzyme remained high (entries 5 and 6). A switch to the more polar solvents 1,2-dimethoxyethane (DME), methyl *tert*-butyl ether (MTBE) and vinyl propionate saw decreased but still high selectivities and good activities (Table 2, entries 7−9). From Figure 2b, no clear-cut picture as to the influence of the various solvents upon the activity of PLE emerges. It is obvious, however, that in the case of PLE/MPEG no linear correlation exists between the activity of the enzyme and the log *P* value of the solvent. Such a correlation had been found for a number of lipases, which showed low activity in solvents with log *P* values less than 2 (DME, MTBE), moderate activity in those with log *P* values larger than 2 and less than 4 (toluene, benzene, $CHCl_3$) and high activity in solvents with log *P* values greater than 4 (*n*-octane, *n*-decane).[47] The relatively fast conversion of *rac*-**4** in vinyl propionate is not surprising given the presence of the acyl donor in large excess. Somewhat surprising is the observation that PLE shows in water miscible DME and in water immiscible MTBE similar activities. This may be due to the fact that MPEG has in both solvents only a low solubility. The low activity of PLE in methylene chloride and chloroform may perhaps be related among other things to the high solubility of MPEG in these solvents causing initially a complete separation of PLE and MPEG.

Table 3. PLE/MPEG-Catalyzed Acylation of Alcohol *rac*-4 at Different Temperatures[a]

T (°C)	*t* (d)	convn (%)	alcohol ee (%)	ester ee (%)	*E*[b]
0	1	13	13	≥98	>100
20	1	51	98	96	>100 (226)
40	1	45	99	95	>100 (205)

[a] Conditions: 1 mmol of alcohol, 4 mmol of vinyl propionate, 300 U of PLE, 10 mL of *n*-octane containing 0.4% water. [b] Numbers in parentheses are the calculated values.

Because of the distinct temperature dependencies of the activity and selectivity of some lipases in acylation in organic solvents,[5,12] we looked for this parameter in the PLE/MPEG-catalyzed acylation of *rac*-**4** in *n*-octane containing 0.4% water (Table 3). It was found that the temperature dependence of these parameters is only only weakly expressed. The selectivity did practically not change. The acylation rate increased on going from 0 to 20 °C, and it decreased slightly at higher temperatures, presumably because of a deactivation of the enzyme.

Having recorded favorable selectivities and activities in the acylation of alcohols *rac*-**1**, *rac*-**3**, *rac*-**4**, *rac*-**6**, and *rac*-**10** by using PLE/MPEG, we applied this CLP to their gram-scale resolution. Reaction of 10−20 mmol of alcohols *rac*-**1**, *rac*-**3**, *rac*-**4**, *rac*-**6**, and *rac*-**10** with 4 equivalents of vinyl propionate in toluene or *n*-octane containing 0.43% water for 5 to 10 d afforded the enantiomerically enriched esters (*S*)-**1a**, (*S*)-**3a**, (*S*)-**4a**, (*R*)-**6a**, and (*R*)-**10a**, respectively, and the enantiomerically enriched alcohols (*R*)-**1**, (*R*)-**3**, and (*R*)-**4**, respectively, in good yields (Table 4).

Crucial to the application of PLE/MPEG in enantioselective acylation, besides the choice of the solvent, is the establishment of the optimal water content of the system. In most cases the activity and selectivity of PLE are at maximum if the system contains between 0.2 and 1.4% water. However, as observed previously in the PLE/MPEG-catalyzed acylation of primary alcohols,[23,24] the activity and the selectivity of the enzyme are also in the case of secondary alcohols inversely depended on the water content. Whereas the activity of PLE is the highest at the lower limit of the optimal range of water content, it is the selectivity of the enzyme which is higher at the upper limit. For gram-scale experiments, a water content of 0.43% represents a good compromise.

Pig Liver Esterase/Bovine Serum Albumin. Although PLE/MPEG showed synthetically useful activities in the acylation of *rac*-**1**−**4**, *rac*-**6**, *rac*-**9**, and *rac*-**10**, often the rate of acylation decreased after an initially fast acylation more strongly with increasing reaction time than anticipated (cf. Table 2). This reduction of the rate may have been caused among other things by a decrease of the water content of the system because of a competing PLE/MPEG-catalyzed hydrolysis of vinyl propionate and by a deactivation of the enzyme by acetaldehyde and propionic acid, both being formed as byproducts, through functionalization and protonation of the enzyme (Scheme 2). Such a consumption of water was indicated by the gradual disappearance of the aqueous phase in the aforementioned PLE/MPEG-catalyzed acylation with

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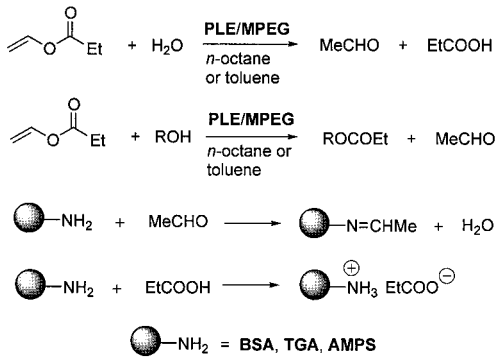
Table 4. Gram-Scale PLE/MPEG-Catalyzed Resolution of Alcohols *rac*-1, *rac*-3, *rac*-4, and *rac*-6[a]

					alcohol		ester		
entry	substrate	solvent[b]	*t* (d)	convn (%)	ee (%)	yield (%)	ee (%)	yield (%)	*E*
1	*rac*-**1**[c]	toluene	5	47	90	41	97	39	>100
2	*rac*-**3**[d]	*n*-octane	5	49	89	42	92	35	71
3	*rac*-**4**[e]	*n*-octane	3	51	99	38	96	39	>100
4	*rac*-**6**[f]	*n*-octane	7	40	61	59	92	39	44
5	*rac*-**10**[g]	toluene	10	31	42	56	97	27	99

[a] Yields are based on the racemic alcohols. [b] Containing 0.4% water. [c] 21 mmol of alcohol, 84 mmol of vinyl propionate, 200 mL of toluene, 5800 U of PLE. [d] 20 mmol of alcohol, 80 mmol of vinyl propionate, 200 mL of *n*-octane, 7700 U of PLE. [e] 20 mmol of alcohol, 80 mmol of vinyl propionate, 200 mL of *n*-octane, 7600 U of PLE. [f] 10 mmol of alcohol, 40 mmol of vinyl propionate, 100 mL of *n*-octane, 4800 U of PLE. [g] 10 mmol of alcohol, 40 mmol of vinyl propionate, 150 mL of *n*-octane, 6300 U of PLE.

Scheme 2. Formation of Side Products in PLE-Catalyzed Acylation of Alcohols with Vinyl Propionate and Their Scavenging by Amino Group Bearing Polymers



increasing reaction time and by the detection of propionic acid in the reaction mixture. Thus, following the PLE/MPEG-catalyzed acylation of *rac*-**4** with vinyl propionate in *n*-octane (0.43% of water) by GC analysis revealed after 1.7 h formation of 6% propionic acid, which increased to 43% after 5 d. The importance of maintaining the critical amount of essential water during the PLE-catalyzed acylation with vinyl propionate in organic solvents as well as the need to scavenge the carbonyl byproducts prompted us to use bovine serum albumin (BSA) as an inexpensive primary amino group containing natural polymer[48] for the activation of PLE. We hoped that the ready reaction of BSA with acetaldehyde under formation of imines and other stable secondary products[49−51] might pave the path for a better activation of PLE through this polymer by suppressing the analogous reaction of acetaldehyde with the enzyme.[52,53] A beneficial feature of the reaction of BSA with acetaldehyde in the present context is the formation of water as a byproduct. This could perhaps serve to maintain to some extend the critical amount of water of the system during acylation with vinyl propionate and thus help to retain the activity of PLE in organic solvents. In addition, BSA would also at least to some extend neutralize the carboxylic acid being formed in the competing enzymatic hydrolysis of the acyl donor.

Lyophilization of desalted PLE (180−200 U/mg, 30 mg) and BSA (1 g) in aqueous solution gave PLE/BSA as a fine, free flowing, white powder, which contained 3−4% water. The enzyme had a specific activity of 150−160 U/mg in aqueous solution, which did not decrease upon storage of the CLP at 4 °C for several weeks. Thus, preparation of PLE/BSA is accompanied only by a slight loss of enzymatic activity.

PLE/BSA-catalyzed acylation was carried out under the same experimental conditions as that with PLE/MPEG. Upon addition of PLE/BSA to the two-phase mixture composed of toluene or *n*-octane and a small amount of water the solid colyophilisate dissolved partly in the aqueous phase. Acylation was started by a rapid stirring of the mixture, which caused the aqueous phase to disperse into small droplets containing some solid material. With increasing reaction time the second phase gradually solidified, presumably because of the consumption of water, leaving behind the solid CLP. The results of the PLE/BSA-catalyzed acylation of *rac*-**1**−**10** in toluene and *n*-octane are compiled in Table 5. The data show that BSA activation of PLE in organic solvents is similarly effective as that by MPEG. In some cases, PLE/BSA showed a higher activity than PLE/MPEG, which was most notable in the case of the acylation of *rac*-**10** (Table 5, entry 20). Furthermore, the rate of acylation did not slow as much as in the case of PLE/MPEG. However, a comparison of the data compiled in Tables 1 and 5 clearly reveals also that in most cases the utilization of BSA as activator for PLE leads to a significantly lower enantioselectivity of acylation as compared to the use of MPEG. A feature of the PLE/BSA-catalyzed acylation of *rac*-**1**−**4**, *rac*-**6**, and *rac*-**8**−**10**, which was also found in the case of PLE/MPEG, is a constant correlation between the reaction rate and the selectivity of the enzyme toward the substrates. For example, in toluene after 3 d the conversion of *rac*-**1** was 41% giving alcohol (*R*)-**1** with 62% ee and ester (*S*)-**1a** 95% ee, which corresponds to an *E* value of 73 (Table 5, entry 1), and in *n*-octane after 3 d the conversion of the alcohol was 56% furnishing (*R*)-**1** with 95% ee and (*S*)-**1a** with 82% ee ($E = 37$) (Table 5, entry 2). During PLE/BSA-catalyzed acylation we noticed a color change of the CLP from colorless to yellow and finally to reddish brown. This phenomenon was also observed in the attempted PLE/BSA-catalyzed acylation of the fluorinated alcohols *rac*-**5** and *rac*-**7** with vinyl propionate, where there was no conversion of the alcohols (Table 5, entries 9, 10, 13, and

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(53) For a study of the activity of lipase/BSA, see: (a) Yamane, T.; Ichiryu, T.; Nagata, M.; Ueno, A.; Shimizu, S.; *Biotechnol. Bioeng*. **1990**, *36*, 1063. (b) Bosley, J. A. EP 424,130, 1991; *Chem. Abstr*. **1991**, *115*, 67527. (c) Matsumoto, Y.; Tsubota, N. JP 06062846, 1994; *Chem. Abstr*. **1994**, *121*, 3961. (d) Inoe, S.; Asahi, N.; Hatanaka, T.; Nanba, T. JP 07289257, 1995; *Chem. Abstr*. **1995**, *124*, 49502. (e) Pencreac'h, G.; Baratti, J. C. *Appl. Microbiol. Biotechnol*. **1997**, *47*, 630. (f) Rotticci, D.; Norin, T.; Hult, K. *Org. Lett*. **2000**, *2*, 1373.

Table 5. PLE/BSA-Catalyzed Acylation of Alcohols *rac*-1−10[a]

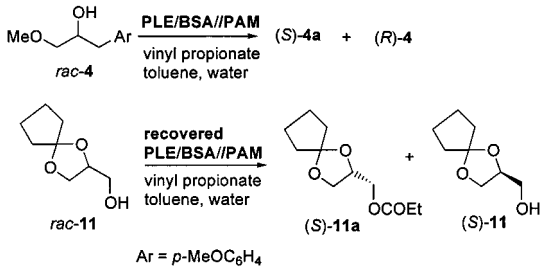
					alcohol		ester		
entry	sub-strate	solvent[b]	*t* (d)	convn (%)	ee (%)	yield (%)	ee (%)	yield (%)	*E*
1[c]	*rac*-**1**	toluene	3	49	62	*d*	95	*d*	73
2[c]	*rac*-**1**	*n*-octane	3	56	95	32	82	44	37
3[c]	*rac*-**2**	toluene	5	16	13	*d*	≥99	*d*	>100
4[c]	*rac*-**2**	*n*-octane	5	40	60	44	≥99	35	>100
5[c]	*rac*-**3**	toluene	2	20	7	*d*	93	*d*	29
6[c]	*rac*-**3**	*n*-octane	2	49	67	*d*	88	*d*	31
7[e]	*rac*-**4**	toluene	2	20	29	*d*	≥99	*d*	>100
8[e]	*rac*-**4**	*n*-octane	2	46	68	*d*	97	*d*	>100
9[c]	*rac*-**5**	toluene	3	<1	0	*d*	*d*	*d*	*d*
10[c]	*rac*-**5**	*n*-octane	3	<1	0	*d*	*d*	*d*	*d*
11[c]	*rac*-**6**	toluene	7	34	35	*d*	90	*d*	26
12[c]	*rac*-**6**	*n*-octane	7	54	>99	37	70	45	28
13[e]	*rac*-**7**	toluene	5	<1	0	*d*	*d*	*d*	*d*
14[e]	*rac*-**7**	*n*-octane	5	<1	0	*d*	*d*	*d*	*d*
15[c]	*rac*-**8**	toluene	1	42	8	*d*	8	*d*	1
16[c]	*rac*-**8**	*n*-octane	1	62	20	*d*	23	*d*	2
17[c]	*rac*-**9**	toluene	1	22	23	*d*	98	*d*	>100
18[c]	*rac*-**9**	*n*-octane	2	52	97	42	91	42	89
19[f]	*rac*-**10**	toluene	1	17	19	*d*	92	*d*	28
20[f]	*rac*-**10**	*n*-octane	1	58	90	*d*	88	*d*	48

[a] Yields are based on the racemic alcohols. [b] Containing 0.4% water. [c] 2 mmol of alcohol, 8 mmol of vinyl propionate, 20 mL of solvent, 600−800 U of PLE. [d] Not determined. [e] 1 mmol of alcohol, 4 mmol of vinyl propionate, 10 mL of solvent, 300−400 U of PLE. [f] 2.6 mmol of alcohol, 10.4 mmol of vinyl propionate, 20 mL of solvent, 600−800 U of PLE.

14). Furthermore, treatment of BSA with acetaldehyde or vinyl propionate alone resulted also in the color change of the protein. Thus, we associate this phenomenon in the case of PLE/BSA to the reaction of BSA with acetaldehyde, which is formed by the enzymatic hydrolysis of vinyl propionate and the enzymatic acylation of the alcohol.[49−51] The observation of a color change upon treatment of BSA with vinyl propionate containing 0.4% water indicates that the acyl donor acylates BSA, with liberation of acetaldehyde, which in turn reacts with the protein. Analysis of the thus obtained colored BSA by the method of Kaiser[54] showed a strong reduction of the number of free primary amino groups.

Immobilization and Recovery of Pig Liver Esterase/Bovine Serum Albumin. We had previously observed that upon placement of a water saturated (approximately 5% water) polyaramide membrane (PAM) in a suspension of PLE/MPEG in toluene a spontaneous and complete deposition of the CLP on the membrane occurs.[23,24] No major differences were observed in acylation of alcohols by using either PLE/MPEG//PAM or PLE/MPEG. Withdrawal of the liquid phase of the reaction mixture containing the alcohol and ester and washing PLE/MPEG//PAM with toluene allowed for its reuse in subsequent batches without major loss of activity. A similar placement of water saturated PAM in a suspension of PLE/BSA in toluene led also to a spontaneous deposition of the CLP on the membrane in form of small droplets. Practically none of the enzyme remained in the suspension. Because of the high activity and selectivity of PLE/BSA toward *rac*-**4** (Table 5, entries 7 and 8), we selected the acylation of this alcohol as a probe for the activity of PLE/BSA//PAM. Enzymatic acylation of *rac*-**4** with vinyl propionate in toluene by using PLE/BSA//PAM led after 5 d to a conversion of the alcohol of 47% giving (*R*)-**4** with 69% ee and (*S*)-4a with 97% ee, which

Scheme 3. Recovery of PLE/BSA through Immobilization on PAM and Reuse of the Enzyme



corresponds to an *E* value of >100 (Scheme 3). Thus, PLE/BSA immobilized on PAM shows in the acylation of this alcohol similar characteristics as the nonimmobilized CLP. To demonstrate that PLE/BSA//PAM can also be reused, the immobilized CLP was subsequently applied to the kinetic resolution of the glycerol derivative *rac*-**11**, which was previously successfully resolved by using PLE/MPEG.[24] PLE/BSA//PAM was washed with toluene following its removal from the reaction mixture containing (*R*)-**4** and (*S*)-**4a** and placed into a solution of *rac*-**11** (1 mM) and vinyl propionate (5 mM) in toluene containing 0.5% water. After 2 d, a 56% conversion of the racemic alcohol had occurred furnishing alcohol (*S*)-**11** with 87% ee and ester (*R*)-**11a** with 90% ee ($E = 52$). The loss of activity of the enzyme after its 2-fold use was estimated to be 20%.

Because of the varying quality of PLE from commercial sources, an activity and a selectivity test of the enzyme prior to its use is required. For the utilization of PLE in hydrolysis in aqueous solution, this can be done by using ethyl butyrate and dimethyl *cis*-1,2-cyclohex-4-ene-1,2-dicarboxylate as substrates.[55] We found that for the application of PLE in acylation in organic solvents, the rate and the enantioselectivity of the acylation of the primary alcohol *rac*-**11** with vinyl propionate in toluene (0.43% water) by using MPEG as activator are good criteria for the performance of the enzyme.

Pig Liver Esterase/TentaGelAmino and Pig Liver Esterase/Methoxypoly(ethylene glycol)//Aminomethyl Polystyrene. Primary amino group containing polymers such as aminomethyl polystyrene (AMPS)[56] and TentaGelAmino (TGA)[57] have been used previously to anchor aldehydes and to scavenge acids or excess electrophiles in solid-phase synthesis.[58] Therefore, we considered AMPS and TGA as interesting alternative candidates for the activation of PLE in acylation with vinylic esters in organic solvents. For the anticipated 3-fold role of the resin, namely to scavenge acetaldehyde, to bind water and to neutralize propionic acid, TGA was selected mainly because of its partial structural resemblance to MPEG. Lyophilization of desalted PLE (180−200 U/mg, 20 mg) in aqueous solution and TGA (2 g) in aqueous suspension afforded PLE/TGA as a creamy free flowing powder. The enzyme had a specific activity of 86 U/mg in aqueous solution, which did not decrease upon storage

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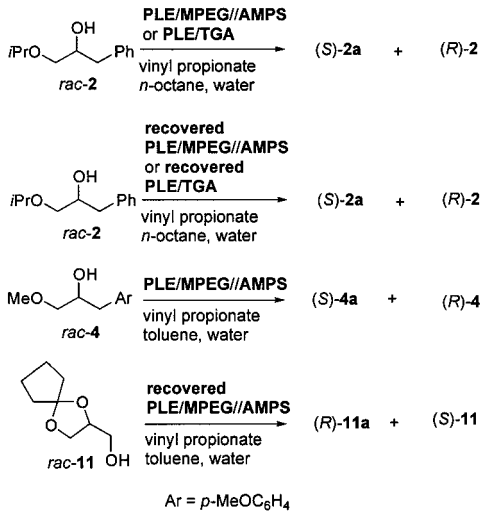
Table 6. PLE-Catalyzed Acylation of Alcohols *rac*-2 and *rac*-4 in the Presence of Amino Group Containing Polymers[a]

						alcohol		ester		
entry	substrate	CLP	solvent[b]	*t* (d)	convn (%)	ee (%)	yield (%)	ee (%)	yield (%)	*E*
1[c]	*rac*-**2**	PLE/MPEG//AMPS	*n*-octane	9	49	80	47	≥99	44	>100
2[d]	*rac*-**2**	PLE/TGA	*n*-octane	6	55	89	38	94	38	97
3[c]	*rac*-**4**	PLE/MPEG//AMPS	toluene	4	43	56	45	98	36	>100

[a] Yields are based on the racemic alcohols. [b] Containing 0.4% water. [c] 1 mmol of alcohol, 4 mmol of vinyl propionate, 10 mL of solvent, 300−400 U of PLE. [d] 2 mmol of alcohol, 4 mmol of vinyl propionate, 20 mL of solvent, 600−800 U of PLE.

Scheme 4. PLE-Catalyzed Acylation of Alcohols in the Presence of Amino Group Bearing Polymers and Re-use of the Enzyme



of the CLP at 4 °C for several weeks. The water content of the CLP was 1−2%. Thus, unlike the preparation of PLE/MPEG and PLE/BSA that of PLE/TGA was accompanied by a significant loss of enzymatic activity. We have no explanation at present for this activity loss.[59] It should be noted, however, that unlike MPEG and BSA the polymer TGA is not soluble in water. This leads to a deposition of PLE on the polymer during lyophilization. In contrast, PLE/MPEG and PLE/BSA are intimate mixtures. The results of the PLE/TGA- and the PLE/MPEG//AMPS-catalyzed acylations, which were carried out under similar experimental conditions as those by using PLE/MPE, PLE/BSA and PLE/MPEG//PAM, are listed in Table 6.

The PLE/MPEG-catalyzed acylation of *rac*-**2** in *n*-octane in the presence of AMPS resulted after 9 d in a 49% conversion of the alcohol giving with excellent selectivity ($E > 100$) (*R*)-**2** with 80% ee and (*S*)-**2a** with ≥99% ee (Table 6, entry 1) (Scheme 4). PLE/MPEG was found adhering to the reaction vessel as it was physically adsorbed by AMPS.[60] PLE/MPEG//AMPS was removed from the reaction mixture containing (*R*)-**2** and (*S*)-**2a** through filtration, washed with *n*-octane and used in the acylation of a second batch of the alcohol (0.67 mM) with vinyl propionate (2.68 mM) in *n*-octane containing 0.4% of water. After 18 d, a 17% conversion of the alcohol was achieved yielding (*R*)-**2** with 9% ee and (*S*)-**2a** with ≥ 99% ee ($E > 100$). AMPS was recovered first by washing with water in order to separate PLE and finally by treatment with oxalic acid in a mixture of tetrahydrofuran and water in order to effect the cleavage of the imines. The loading of the fresh and used AMPS with free amino groups was determined by the Kaiser test.[54] Reaction of *rac*-**2** with vinyl propionate in *n*-octane by using PLE/TGA resulted after 6 d in a 55% conversion of the alcohol furnishing with high selectivity ($E = 97$) (*R*)-**2** with 89% ee and (*S*)-**2a** with ≥99% ee. The CLP was recovered simply by filtration and washing with *n*-octane. Application of recovered PLE/TGA to the acylation of *rac*-**2** (2 mM) in *n*-octane containing 0.4% water saw after 16 d a 36% conversion of the substrate delivering (*R*)-**2** with 50% ee and (*S*)-**2a** with ≥99% ee.

The use of PLE/MPEG//AMPS in the acylation of *rac*-**4** with vinyl propionate in toluene led after 4 d to a 43% conversion of the substrate giving with high selectivity ($E > 100$) (*R*)-**4** with 56% ee and (*S*)-**4a** with 98% ee. PLE/MPEG//AMPS was recovered simply by decanting the liquid phase and washing the solid residue with toluene. The recovered PLE/MPEG//AMPS was used in a subsequent acylation of the glycerol derivative *rac*-**11** (1 mM) with vinyl propionate (5 mM) in toluene containing 0.5% water. After 3 d a conversion of the substrate of 50% was achieved giving with good selectivity ($E = 42$) alcohol (*S*)-**11** with 89% ee and ester (*R*)-**11a** with 87% ee. The loss of activity of the enzyme after its 2-fold use was estimated to be 20%.

A common feature of the PLE-catalyzed acylation in the presence of the aforementioned polymers is that the acylation rates are initially high resulting after 3−4 h in a 30−35% conversion, but slow subsequently more strongly than anticipated on the basis of the selectivity factor *E* (Figure 2c). At present, we have no experimentally verified explanation for this phenomenon. It should be noted, however, that the selectivity factor remains practically constant throughout the acylation.

Acylations by using PLE/TGA and PLE/MPEG//AMPS were, as in the case of PLE/BSA, accompanied by a color change of the CLP from almost colorless via yellow to reddish brown. Such a change was also observed upon treatment of TGA and AMPS with acetaldehyde or vinyl propionate in the absence of the enzyme. TGA and AMPS isolated after this treatment contained no free amino groups according to a Kaiser test.[54] Thus, we ascribe the color change in these cases also to a reaction of the polymers with acetaldehyde (cf. Scheme 4). Reaction of TGA and AMPS with acetaldehyde in the case of the use of vinyl propionate has to be proceeded by an acylation of the polymers.

A comparison of the activity and selectivity of PLE toward *rac*-**4** in the presence of the polymers tested, except TGA, is provided by Figure 3. The enantioselectivity of PLE in the presence of the various polymers is in all cases the same, although there was a slight decrease of the ee value of the ester (*S*)-**4a** with increasing reaction time. Of all the CLPs of PLE and polymers tested PLE/BSA had the lowest activity toward *rac*-**4**.

(59) A reviewer of this paper has suggested that the reason for the reduced activity might be the strong alteration of the hydronium ion activity (pH) of the colyophilisate by these polyamines.

(60) For the immobilization of enzymes on polyaminostyrene, see: Brandenberger, H. *Rev-Ferment. Ind. Aliment.* **1956**, *11*, 237.

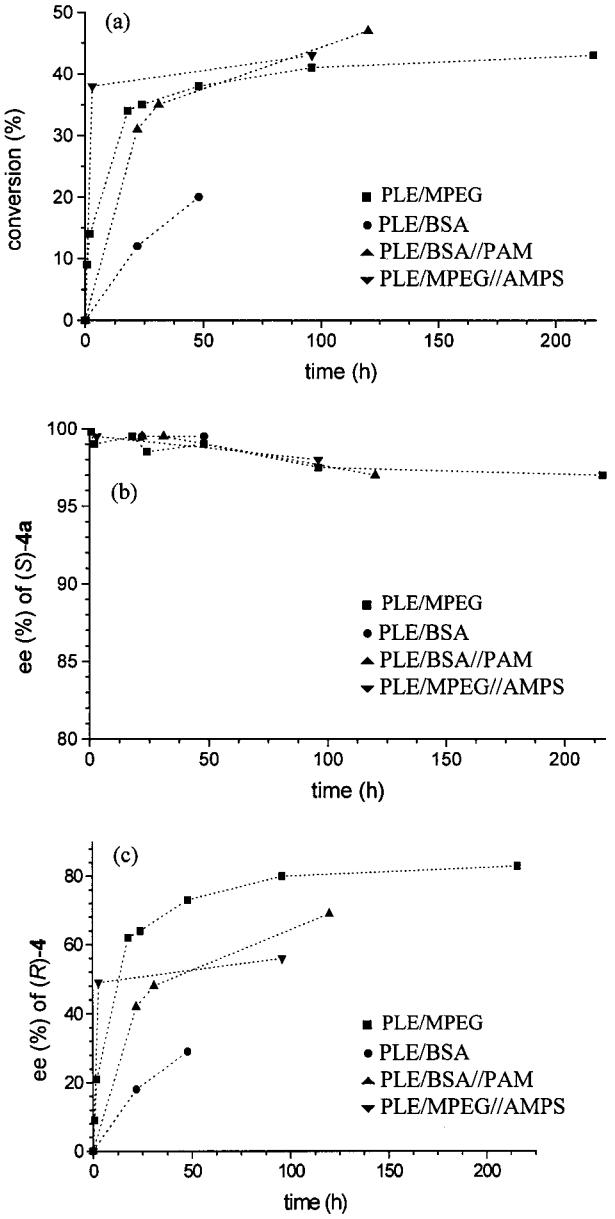
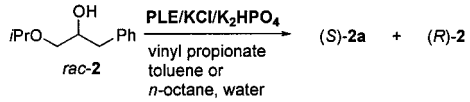


Figure 3. Activity (a) and selectivity (b, c) of PLE/MPEG, PLE/BSA, PLE/BSA//PAM, and PLE/MPEG//AMPS in the acylation of *rac*-**4** with vinyl propionate in toluene.

However, immobilization of PLE/BSA on PAM saw a considerable increase in the activity of the enzyme. Interpretation of this observation is difficult since several parameters such as the water content of the system and the partition of the small PLE/BSA containing phase in the organic phase have been changed. Of all of the PLE/polymer combinations tested PLE/MPEG//AMPS exhibited the highest initial activity toward *rac*-**4**. However, a disadvantage of the use of TGA and AMPS is their competing reaction with the acyl donor, which is also accompanied, as in the case of BSA, by the formation of acetaldehyde.

Pig Liver Esterase/Potassium Chloride/Potassium Hydrogen Phosphate. High concentrations of salts have been used to activate enzymes in organic solvents by maintaining the critical water content as well as the structure of the enzyme during lyophilization.[61] Lyophilization of PLE (180 U/mg, 30 mg), potassium chloride (2.94 g) and potassium hydrogen phosphate (30 mg) in water gave PLE/KCl/K_2HPO_4 as a fine white powder, which contained less than 1% water. The enzyme had a specific activity of only 90 U/mg in aqueous solution, thus, revealing a significant activity loss during colyphilization. The activity of the CLP did, however, not decrease upon its storage at 4 °C for several weeks.

Scheme 5. PLE/KCl/K_2HPO_4-Catalyzed Acylation of Alcohols



The PLE/KCl/K_2HPO_4-catalyzed acylation of *rac*-**2** with vinyl propionate in *n*-octane saw only a very low conversion of the alcohol. In addition, the selectivity was also only low. However, adjustment of the water content of the reaction mixture to 0.4% resulted not only in an increase of the reaction rate but also of the selectivity. Reaction of *rac*-**2** with vinyl propionate in *n*-octane by using PLE/KCl/K_2HPO_4 under these conditions led after 6 d to a 30% conversion of the alcohol affording with high selectivity ($E > 100$) (*R*)-**2** with 39% ee and (*S*)-**2a** with ≥ 99% ee (Scheme 5).

Stereoselectivity of Pig Liver Esterase in Organic Solvents. The absolute configurations of (*S*)-**1a**/(*R*)-**1**,[24,28] (*S*)-**4a**/(*R*)-**4**,[28] (*R*)-**6a**/(*S*)-**6**,[31] (*R*)-**9a**/(*S*)-**9**,[32] and (*R*)-**10a**/(*S*)-**10**[33] were assigned by comparison of their optical rotation with those reported in the literature. Those of (*S*)-**2a**/(*R*)-**2** and (*S*)-**3a**/(*R*)-**3** were assigned on the premise that they have the same sign of optical rotation as (*S*)-**1a**/(*R*)-**1** and (*S*)-**4a**/(*R*)-**4**. It follows, thus, that in the case of *rac*-**1**−**4** the *S*-alcohol reacts with the acyl enzyme faster than the (*R*)-alcohol leading to the (*S*)-ester, whereas in the case of the fluoro derivative *rac*-**6** the (*R*)-alcohol is the faster reaction enantiomer. The replacement of the *p*-methoxyphenyl group in *rac*-**4** by a phenoxy group did not change the enantiomer preference of the enzyme as shown by the acylation of *rac*-**10**. This holds also true for *rac*-**9** where the methoxy group of *rac*-**10** has been replaced by a H-atom. Thus, the isoenzyme mixture of PLE seems to exhibit toward secondary alcohols, which carry a benzyl, *p*-methoxybenzyl, and phenoxymethyl group, not only a high selectivity but also the same enantiomer preferences irrespective of the other group which is attached to the stereogenic center. The only exception is *rac*-**6**, which carries a fluorine atom. Several substrate models have been advanced for the enantiomer and enantiotopic group preference of PLE in the hydrolysis of esters of prochiral or racemic carboxylic acids.[62−64] These models discuss the quality of the binding of the chiral or prochiral carboxylic acid part of an alkyl ester in the active site of PLE during acylation of its serine hydroxy group. The most advanced model is that of Jones et al.[62,63] This model allows for a rationalization of the enantiomer and enantiotopic group preference of PLE in the hydrolysis of most of the esters of prochiral or racemic carboxylic acids investigated thus far. An analogous model, which considers the binding of the chiral or prochiral alcohol part of an ester, whose carboxylic acid part is achiral, in the active site of PLE

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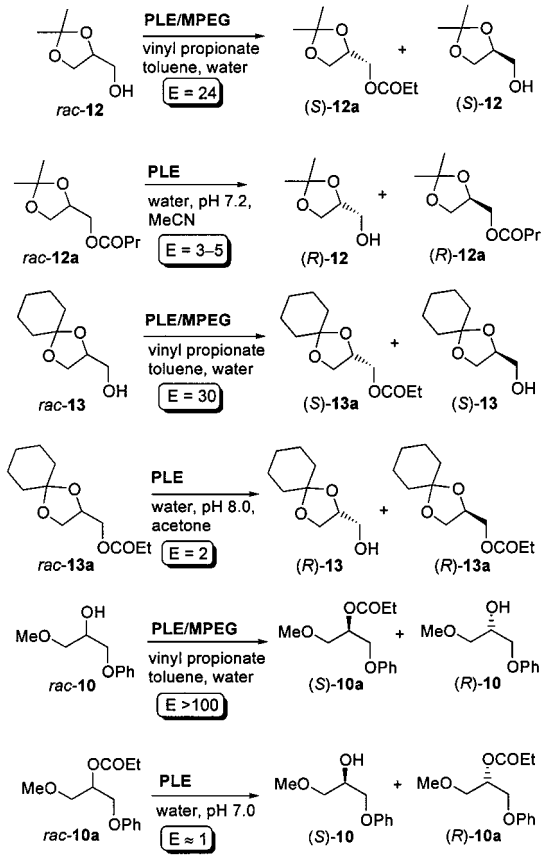
during deacylation of the acyl enzyme or the acylation of the enzyme is not available yet. It has been claimed, however, that the enantiomer preference of PLE in the hydrolysis of the diacetates of cyclic diols can be correctly predicted by the Jones model.[65] Application of this model to the PLE-catalyzed kinetic resolution of the alcohols investigated in this work in a manner as for the aforementioned diacetates led in most cases to a correct prediction of the faster reacting enantiomer.[66] One has to bear in mind, however, that the Jones model has been developed on the basis of the selectivities found by using the native mixture of PLE isoenzymes, which in principle could have different activities and enantioselectivities. Hydrolysis studies of esters in water by using single isoenzymes[67−70] and enriched isoenzyme mixtures[6a,6e,71] had revealed for some substrates indeed such differences. It would be, thus, interesting to see whether the PLE isoenzymes show similar differences also in acylation in organic solvents. Such studies of the isoenzymes are, however, at present not feasible because of their nonavailability from commercial sources and because of the difficulties associated with the separation of the native isoenzyme mixture on a somewhat larger scale.[67−70,72]

PLE in organic solvents shows an interesting selectivity feature (Scheme 6). Previous observations suggested that PLE exhibits in organic solvents a higher acyclation enantioselectivity than for hydrolysis in aqueous solution.[24] For example, we recorded for the PLE/MPEG-catalyzed acylation of the primary alcohol *rac*-**12** with vinyl propionate in toluene (0.43% water) a selectivity factor of $E = 24$, while for the PLE catalyzed hydrolysis of the corresponding butyrate *rac*-**12a** in water an E value of only 3−5 was reported.[73] Similar results were obtained in the case of the related primary alcohol *rac*-**13** and its propionate *rac*-**13a**.[24,66] Whereas the PLE/MPEG-catalyzed acylation of *rac*-**13** is characterized by a selectivity factor of $E = 30$, the PLE catalyzed hydrolysis of *rac*-**1 3a** has an E value of only 2. Having obtained these results, we were interested to see whether such a selectivity difference can be found also in the case of one of the aforementioned 2-propanols. The PLE catalyzed hydrolysis of butyrate *rac*-**10a** in aqueous solution proceeded nonselective according to a selectivity factor of $E \approx 1$.[24,66] In contrast, the PLE/MPEG-catalyzed acylation of alcohol *rac*-**10** was highly selective as expressed by an E value of > 100. Lipases and proteases were previously found to exhibit an analogous selectivity behavior as PLE upon switching from water to an organic solvent.[5] Several explanations for this phenomenon such as differences in the solvation of the transition state are currently being discussed, but no one is completely satisfactory.[5,12]

Finally, Scheme 6 reveals also that in the cases investigated the enantiomer preference of PLE in acylation and hydrolysis of related compounds is the same.

Scheme 6. Enantioselectivity of PLE in Organic Solvents and in Water



Conclusion

Addition of the polymers MPEG, BSA, TGA, and AMPS confers activity to the PLE isoenzymes in the acylation of alcohols with vinyl propionate in toluene and *n*-octane containing less than 1% of water. Whereas in the preparation of PLE/MPEG and PLE/BSA the specific activity of the enzyme is largely retained, colyophilization of PLE with TGA is accompanied by a significant loss of enzymatic activity. In addition, the primary amino group containing polymers BSA, TGA and AMPS are not inert against the acyl donor vinyl propionate. In general, the CLP of choice for a PLE-catalyzed acylation in organic media is PLE/MPEG either as such or immobilized on PAM. PLE shows high enantioselectivity toward the functionalized secondary alcohols *rac*-**1**−**4**, *rac*-**6**, *rac*-**9**, and *rac*-**10**. The activity and selectivity of the enzyme were in the case of *rac*-**1**, *rac*-**3**, *rac*-**4**, *rac*-**6** and *rac*-**10** such as to allow for a kinetic resolution on a gram scale. Recycling of PLE/polymer for a batch-wise application can be achieved through their spontaneous immobilization on a water saturated polyaramide membrane in organic solvents. The activity enhancement observed for PLE in the presence of the primary amino group containing polymers BSA, AMPS, and TGA seems to be associated at least in part with a scavenging of acetaldehyde formed as a byproduct in the acylation with vinyl propionate. However, other factors might contribute too to the enhanced activity of PLE in the presence of the polymers. Colyophilization of PLE with the aforemen-

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tioned polymers leads to an increase of the surface of the enzyme, which should increase its activity. In addition colyophilization may result in a larger intake of water in the surrounding of PLE thus maintaining the water activity of the enzyme during acylation. Although no extensive comparative investigation has thus far been carried out, it seems, that the activity of PLE/polymer in acylation of alcohols in organic solvents is lower than of PLE in hydrolysis of the corresponding esters in aqueous solution. The enantioselectivity of PLE, however, may by occasionally higher in organic solvents.

Experimental Section

General Methods. Chemical shifts are given in ppm relative to Me_4Si: $\delta = 0.00$ as internal standard. Peaks in the ^{13}C NMR spectra are denoted as ×e3u×e3 for carbons with zero or two attached protons or "d" for carbons with one or three attached protons, as determined from the ATP pulse sequence. Specific rotations are given in grad·L/dm·g, *c* in g/100 mL. Enzymatic acylations were run at ca. 22 °C and were monitored by GC analysis on a CP-Sil-8 column. PLE (EC 3.1.1.1, 180−200 U/mg, native mixture of isoenzymes) was purchased from Roche Diagnostics (then Boehringer Mannheim) as a suspension in 3.2 M NH_4SO_4 (30 mg PLE in 3 mL). The specific activity of PLE and of PLE admixed with polymers was determined by hydrolysis of ethyl butyrate in aqueous buffer solution (pH 8.0, 25 °C). Enantiomer compositions were determined by GC analysis on an octakis-(2,3-*O*-dipentyl-6-*O*-methyl)-γ-cyclodextrin column (25 m × 0.25 mm) (Lipodex γ-6-Me, Macherey Nagel), a permethylated β-cyclodextrin column (25 m × 0.25 mm) (CP-Chirasil-Dex-CB, Chrompack), and an octakis-(2,6-di-*O*-pentyl-3-*O*-butyl)-γ-cyclodextrin column (25 m × 0.25 mm) (Lipodex E, Macherey-Nagel) (carrier gas hydrogen, 100 kPa). Column chromatography was done on Merck silica gel 60 (230−400 mesh). $MPEG_{5000}$ was purchased from Sigma, and BSA, fraction V, was obtained from Roche Diagnostics. TGA (TentaGel S NH_2 SS, LC = 0.3 mmol/g, 90 μm) and AMPS (LC = 1.0 mmol/g, 200−400 mesh) were obtained from Advanced ChemTech. The water content of PLE/polymer was determined by Karl Fischer titration with Karl Fischer solutions obtained from Merck and Riedel-de-Haën. The polyaramide ultrafiltration membrane (UF-PA-5H and UF-PA-20H) was from Celanese. Alcohols *rac*-**8** and *rac*-**9** were obtained from Acros Organics. The synthesis of alcohols *rac*-**1**−**7** and *rac*-**10** was carried out according to the literature[24,38,41,74] or as described in the Supporting Information. Standard workup of enzymatic acylation included chromatography in order to separate ester and alcohol following Kugelrohr distillation to remove MPEG.

Desalting of the Suspension of PLE in Aqueous Ammonium Sulfate. A suspension of PLE (30 mg) in aqueous $(NH_4)_2SO_4$ (3 mL) was placed in an Amicon ultrafiltration cell (model 2800, 200 mL, Ø = 62 mm) equipped with a RC-YM30/PM30 membrane (Millipore), and ultrafiltration was carried out under stirring and ice cooling at 1.5 bar positive nitrogen pressure through repetitive concentration and dilution by using distilled water (1000 mL). Five times the aqueous solution/suspension of PLE was concentrated to a volume of 20 mL and taken up again in water (200 mL). Finally a solution/suspension of PLE (30 mg) in water (20 mL) was obtained.

PLE/MPEG. PLE (30 mg, 180−200 U/mg) was desalted by ultrafiltration (cutoff 30 kDa) under ice cooling. The remaining solution/suspension of PLE in water (20 mL) was treated with $MPEG_{5000}$ (1 g), and the mixture was stirred magnetically for 2 h under ice cooling. Then, the mixture was frozen in liquid nitrogen and lyophilized (0.008 mbar, 60 h) to give PLE/MPEG as a white powder, which contained less than 1% water. The enzyme had a specific activity of 160−180 U/mg, which did not decrease upon storage of the CLP at 4 °C for several weeks.

PLE/BSA. PLE (30 mg, 180−200 U/mg) was desalted by ultrafiltration (cutoff 30 kDa) under ice cooling. The remaining solution/suspension of PLE in water (20 mL) was treated with BSA (1 g) and the mixture was stirred magnetically for 2 h under ice cooling. Then, the mixture was frozen in liquid nitrogen and lyophilized (0.004−0.009 mbar, 48 h) to give PLE/BSA as a white powder, which contained 3−4% water. The enzyme had a specific activity of 150−160 U/mg, which did not decrease upon storage of the CLP at 4 °C for several weeks.

PLE/TGA. PLE (20 mg, 180−200 U/mg) was desalted by ultrafiltration (cutoff 30 kDa) under ice cooling. The remaining solution/suspension of PLE in water (20 mL) was treated with TGA resin (2 g) and the suspension was stirred magnetically for 2 h under ice cooling. Then, the mixture was frozen in liquid nitrogen and lyophilized (0.040−0.016 mbar, 48 h) to give PLE/TGA as a free flowing creamy colored powder, which contained 1−2% water. The enzyme had a specific activity of 86 U/mg, which did not decrease upon storage of the CLP at 4 °C for several weeks.

PLE/KCl/K_2HPO_4. PLE (30 mg, 180−200 U/mg) was desalted by ultrafiltration (cutoff 30 kDa) under ice cooling. To the remaining solution/suspension of PLE in water (20 mL) was added KCl (2.94 g) and K_2HPO_4 (30 mg) and the mixture was stirred magnetically for 2 h under ice cooling. Then, the mixture was frozen in liquid nitrogen and lyophilized (0.040−0.016 mbar, 48 h) to give PLE/KCl/K_2HPO_4 as a free flowing colorless powder, which contained less than 1% water. The enzyme had a specific activity of 90 U/mg, which did not decrease upon storage of the CLP at 4 °C for several weeks.

PLE/BSA-Catalyzed Acylation of *rac*-1 in *n*-Octane. To a stirred solution of vinyl propionate (800 mg, 8 mmol) and alcohol *rac*-**1** (322 mg, 2 mmol) in *n*-octane (10 mL) was added water (0.08 mL) (0.4% v/v). Stirring of the mixture was continued for 30 min, and PLE/BSA (127 mg, 580 U) and *n*-octane (10 mL) were then added. After stirring the mixture rapidly for 3 d (57% conversion), $MgSO_4$ (1 g) and $NaHCO_3$ (0.5 g) were added and the mixture was filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified first by Kugelrohr distillation (0.8 mbar, 100 °C) and then by chromatography (cyclohexane/EtOAc, 1:1) to give alcohol (*R*)-**1** (108 mg, 32%) with 95% ee, $[\alpha]^{22}_D = +9.2$ (*c* 1.57, MeOH) and ester (*S*)-**1a** (197 mg, 44%) with 82% ee, $[\alpha]^{22}_D = -3.0$ (*c* 2.23, MeOH).

PLE/BSA-Catalyzed Acylation of *rac*-2 in *n*-Octane. To a stirred solution of vinyl propionate (800 mg, 8 mmol) and alcohol *rac*-**2** (388 mg, 2 mmol) in *n*-octane (10 mL) was added water (80 μL) (0.4% v/v). Stirring of the mixture was continued for 30 min, and PLE/BSA (127 mg, 580 U) and *n*-octane (10 mL) were then added. After stirring the mixture rapidly for 5 d (40% conversion), $MgSO_4$ (1 g) and $NaHCO_3$ (500 mg) were added and the mixture was filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified first by Kugelrohr distillation (0.8 mbar, 100 °C) and then by chromatography (cyclohexane/EtOAc, 3:1) to furnish alcohol (*R*)-**2** (170 mg, 44%) with 60% ee, $[\alpha]^{22}_D = +3.2$ (*c* 1.45, MeOH) and ester (*S*)-**2a** (175 mg, 35%) with ≥99% ee, $[\alpha]^{22}_D = -9.4$ (*c* 1.06, MeOH). (*R*)-**2**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 2H), 7.22 (m, 3 H), 3.98 (m, 1 H), 3.58 (sept, J = 6.04 Hz, 1 H), 3.44 (dd, J = 6.0, 3.3 Hz, 1 H), 3.28 (dd, J = 7.1, 2.2 Hz, 1 H), 2.8 (m, 2 H), 2.48 (s, 1 H), 1.17 (d, J = 4.9 Hz, 3 H), 1.15 (d, J = 4.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.0 (d), 22.1 (d), 39.8 (u), 71.2 (u), 71.4 (d), 72.0 (d), 126.2 (d), 128.2 (d), 129.1 (d), 137.9 (u). Anal. Calcd for $C_{12}H_{18}O_2$ (194.13): C, 74.19; H, 9.34. Found: C, 73.90; H, 9.59. (*S*)-**2a**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 2 H), 7.21 (m, 3 H), 5.16 (m, 1 H), 3.55 (sept, J = 6.0 Hz, 1 H), 3.46 (dd, J = 6.0, 4.4 Hz, 1 H), 3.41 (dd, J = 5.2, 5.2 Hz, 1 H), 2.98 (dd, J = 7.4, 6.3 Hz, 1 H), 2.89 (dd, J = 6.9, 6.9 Hz, 1 H), 2.31 (dd, J = 4.9, 2.5 Hz, 1 H), 2.28 (dd, J = 5.2, 2.5 Hz, 1 H), 1.15 (d, J = 2.5 Hz, 3 H), 1.14 (d, J = 2.5 Hz, 3 H), 1.08 (tr, J = 7.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.2 (d), 22.0 (d), 22.0 (d), 27.7 (u), 37.0 (u), 67.9 (u), 72.0 (d), 73.4 (d), 126.2 (d), 128.1 (d), 129.3 (d), 137.2 (u),

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173.7 (u). Anal. Calcd for $C_{15}H_{22}O_3$ (250.16): C, 71.97; H, 8.86. Found: C, 72.30; H, 9.01.

PLE/MPEG//AMPS-Catalyzed Acylation of *rac*-2. To a stirred solution of vinyl propionate (400 mg, 4 mmol) and alcohol **2** (194 mg, 1 mmol) in *n*-octane (10 mL) was added water (80 μL) (0.4% v/v). The mixture was stirred for 30 min, and PLE/MPEG (71 mg; 290 U) and *n*-octane (10 mL) were then added. After the mixture was stirred for 30 min, aminomethylated polystyrene (1.12 g, 1.45 mmol) was added and rapid stirring was continued for 9 d (49% conversion). Subsequently, the organic phase was decanted and the residue was washed thoroughly with toluene, air-dried and stored for a reuse. The combined organic phases were treated with $MgSO_4$ (1 g) and $NaHCO_3$ (0.5 g) and the mixture was filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified first by Kugelrohr distillation (0.8 mbar, 100 °C) and then by chromatography (cyclohexane/EtOAc, 3:1) to yield alcohol (*R*)-**2** (91 mg, 47%) with 80% ee, $[\alpha]^{22}_D = +5.2$ (*c* 1.44, MeOH) and ester (*S*)-**2a** (111 mg, 44%) with ≥ 99% ee, $[\alpha]^{22}_D = -9.5$ (*c* 1.38, MeOH).

PLE/TGA-Catalyzed Acylation of *rac*-2. To a stirred solution of vinyl propionate (800 mg, 8 mmol) and alcohol **2** (388 mg, 2 mmol) in *n*-octane (20 mL) was added water (80 μL) (0.4% v/v). The mixture was stirred for 30 min, and PLE/TGA (680 mg, 580 U) was then added. After the mixture was stirred rapidly for 6 d (55% conversion), the organic phase was decanted and the residue was washed thoroughly with toluene, air-dried and stored for a reuse. The combined organic phases were treated with $MgSO_4$ (1 g) and $NaHCO_3$ (500 mg), and the mixture was filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified first by Kugelrohr distillation (0.8 mbar, 100 °C) and then by chromatography (cyclohexane/EtOAc, 3:1) to afford alcohol (*R*)-**2** (149 mg, 38%) with 89% ee, $[\alpha]^{22}_D = +5.9$ (*c* 1.38, MeOH) and ester (*S*)-**2a** (187 mg, 38%) with 94% ee, $[\alpha]^{22}_D = -7.4$ (*c* 1.55, MeOH).

PLE/KCl/K_2HPO_4-Catalyzed Acylation of *rac*-2. To a stirred solution of vinyl propionate (800 mg, 8 mmol) and alcohol *rac*-**2** (388 mg, 2 mmol) in *n*-octane (10 mL) was added water (0.08 mL) (0.4% v/v). The mixture was stirred for 30 min, and PLE/KCl/K_2HPO_4 (0.644 g, 580 U) and *n*-octane (10 mL) were then added. After the mixture was stirred rapidly for 6 d (30% conversion), $MgSO_4$ (1 g) and $NaHCO_3$ (0.5 g) were added and the mixture was filtered through Celite. The filtrate was concentrated in vacuo, and the residue was purified first by Kugelrohr distillation (0.8 mbar, 100 °C) and then by chromatography (cyclohexane/EtOAc, 3:1) to give alcohol (*R*)-**2** (208 mg, 53%) with 39% ee, $[\alpha]^{22}_D = +2.15$ (*c* 1.975, MeOH) and ester (*S*)-**2a** (107 mg, 21%) with ≥99% ee, $[\alpha]^{22}_D = -9.4$ (*c* 1.25, MeOH).

PLE/MPEG-Catalyzed Acylation of *rac*-3. To a solution of vinyl propionate (8.0 g, 80 mmol) and alcohol *rac*-**3** (3.9 g, 20 mmol) in *n*-octane (100 mL) was added water (0.9 mL). The mixture was stirred for 30 min, and PLE/MPEG (1.29 g, 7700 U) and *n*-octane (100 mL) were then added. After the mixture was stirred rapidly for 5 d (49% conversion), $MgSO_4$ (10 g) and $NaHCO_3$ (5 g) were added and the mixture was filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified first by Kugelrohr distillation (0.6 mbar, 160 °C) and then by chromatography (cyclohexane/EtOAc, 1:1) to yield alcohol (*R*)-**3** (1.65 g, 42%) with 89% ee, $[\alpha]^{23}_D$ +4.7 (*c* 1.52, EtOH), $[\alpha]^{23}_D = -26.9$ (*c* 1.45, $CHCl_3$) and ester (*S*)-**3a** (1.67 g, 35%) with 92% ee, $[\alpha]^{23}_D = -1.5$ (*c* 1.94, THF). (*R*)-**3**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18−7.29 (m, 5 H), 4.84 (d, J = 5.2 Hz, 1 H), 3.78 (sext, J = 5.8 Hz, 1 H), 2.82 (dd, J = 13.5, 5.0 Hz, 1 H), 2.65 (dd, J = 13.5, 7.4 Hz, 1 H), 2.52 (m, 4 H), 1.15 (tr, J = 7.4 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.7 (d), 25.6 (u), 37.7 (u), 42.0 (u), 70.9 (d), 125.5 (d), 127.7 (d), 129.1 (d), 138.9 (u). Anal. Calcd for $C_{11}H_{16}OS$ (196.31): C, 67.30; H, 8.21. Found: C, 67.50; H, 8.32. (*S*)-**3a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18−7.31 (m, 5 H), 5.17 (trtr, J = 5.9, 5.4 Hz, 1 H), 2.98 (dd, J = 13.7, 9.1 Hz, 1 H), 2.97 (dd, J = 13.8, 5.6 Hz, 1 H), 2.66 (d, J = 6.0 Hz, 2 H), 2.57 (q, J = 7.4 Hz, 2 H), 2.28 (q, J = 7.4 Hz, 2 H), 1.23 (tr, J = 7.4 Hz, 3 H), 1.08 (tr, J = 7.5 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.1 (d), 14.7 (d), 26.5 (u), 27.7 (u), 34.6 (u), 39.1 (u), 73.4 (d), 126.4 (d), 128.2 (d), 129.3 (d), 136.9 (u), 173.6 (u). Anal. Calcd for $C_{14}H_{20}O_2S$ (252.38): C, 66.63; H, 7.99. Found: C, 66.91; H, 8.10.

PLE/MPEG-Catalyzed Acylation of *rac*-4. To a stirred solution of vinyl propionate (8.0 g, 80 mmol) and alcohol *rac*-**4** (3.97 g, 20 mmol) in *n*-octane (100 mL) was added water (0.9 mL). The mixture was stirred for 30 min, and PLE/MPEG (1.27 g, 7600 U) and *n*-octane (100 mL) were then added. After the mixture was stirred rapidly for 3 d (51% conversion), $MgSO_4$ (10 g) and $NaHCO_3$ (5 g) were added and the mixture was filtered through Celite. The filtrate was concentrated in vacuo, and the residue was purified first by Kugelrohr distillation (1.6 mbar, 180 °C) and then by chromatography (cyclohexane/EtOAc, 2:1) to give alcohol (*R*)-**4** (1.50 g, 38%) with ≥99% ee, $[\alpha]^{23}_D = +15.0$ (*c* 1.76, *i*-PrOH) and ester **4a** (2.00 g, 39%) with 96% ee, $[\alpha]^{23}_D = +1.9$ (*c* 2.27, *i*-PrOH). (*R*)-**4**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.12 (m, 2 H), 6.83 (m, 2 H), 4.68 (d, J = 5.4 Hz, 1 H), 3.72 (m, 1 H), 3.71 (s, 3 H), 3.24 (s, 3 H), 3.18 (d, J = 5.4 Hz, 2 H) 2.60 (dd, J = 13.7, 5.4 Hz, 1 H), 2.51 (dd, J = 13.6, 7.6 Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 39.0 (u), 54.8 (d), 58.2 (d), 70.2 (d), 76.1 (u), 113.4 (d), 130.2 (d), 130.9 (u), 157.4 (u). Anal. Calcd for $C_{11}H_{16}O_3$ (196.25): C, 67.32; H, 8.22. Found: C, 67.09; H, 8.01. (*S*)-**4a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13 (m, 2 H), 6.82 (m, 2 H), 5.14 (m, 1 H), 3.78 (s, 3 H), 3.40 (m, 2 H), 3.35 (s, 3H), 2.85 (dd, J = 13.9, 6.9 Hz, 1 H), 2.84 (dd, J = 13.9, 6.9 Hz, 1 H), 2.49 (q, J = 7.5 Hz, 2 H), 1.09 (tr, J = 7.5 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.1 (d), 27.7 (u), 36.1 (u), 55.2 (d), 59.1 (d), 72.6 (u), 73.3 (d), 113.8 (d), 129.2 (u), 130.4 (d), 158.3 (u), 173.9 (u). Anal. Calcd for $C_{14}H_{20}O_4$ (252.31): C, 66.65; H, 7.99. Found: C, 66.91; H, 8.28.

PLE/BSA//PAM-Catalyzed Acylation of *rac*-4. To a stirred solution of vinyl propionate (400 mg, 4 mmol) and alcohol *rac*-**4** (196 mg, 1 mmol) in toluene (20 mL) was added water (80 μL) (0.4% v/v). Subsequently, PLE/BSA (83 mg, 360 U) and the water-saturated polyaramide membrane (ca. 4 cm^2) were added under gentle stirring to the mixture. After 15 min, nearly all of the PLE/BSA had been adsorbed on the membrane and the solution became clear. After the mixture was stirred gently for 5 d (47% conversion), the organic phase was decanted and PLE/BSA//PAM was washed with toluene and stored for a reuse. The combined organic phases were treated with $MgSO_4$ (1 g) and $NaHCO_3$ (500 mg), the mixture was filtered through Celite, and the filtrate was concentrated in vacuo. Purification of the residue first by Kugelrohr distillation (0.8 bar, 100 °C) and then by chromatography (cyclohexane/EtOAc, 2:1) gave alcohol (*R*)-**4** (89 mg, 45%) with 69% ee, $[\alpha]^{22}_D = +1.96$ (*c* 1.37, MeOH) and ester (*S*)-**4a** (104 mg, 41%) with 97% ee, $[\alpha]^{22}_D = -0.81$ (*c* 1.15, MeOH).

PLE/MPEG//AMPS-Catalyzed Acylation of *rac*-4. To a stirred solution of vinyl propionate (400 mg, 4 mmol) and alcohol *rac*-**4** (196 mg, 1 mmol) in toluene (10 mL) was added water (80 μL) (0.4% v/v). The mixture was stirred for 30 min, PLE/MPEG (93 mg, 380 U) and toluene (10 mL) were then added, and stirring was continued for 30 min. Subsequently, aminomethylated polystyrene (1.5 g, 1.5 mmol) was added to the mixture. After the mixture was stirred rapidly for 4 d (43% conversion), the organic phase was decanted and the residue was washed thoroughly with toluene, air-dried, and stored for reuse. The combined organic phases were treated with $MgSO_4$ (1 g) and $NaHCO_3$ (500 mg), the mixture was filtered through Celite, and the filtrate was concentrated in vacuo. Purification of the residue first by Kugelrohr distillation (0.8 mbar, 100 °C) and then by chromatography (cyclohexane/EtOAc, 2:1) afforded alcohol (*R*)-**4** (90 mg, 45%) with 56% ee, $[\alpha]^{22}_D = +1.63$ (*c* 1.41, MeOH) and ester (*S*)-**4a** (90 mg, 36%) with 97% ee, $[\alpha]^{22}_D = -0.83$ (*c* 1.15, MeOH).

PLE/MPEG-Catalyzed Acylation of *rac*-6. To a stirred solution of vinyl propionate (4.0 g, 40 mmol) and alcohol *rac*-**6** (1.49 g, 10 mmol) in *n*-octane (50 mL) was added water (0.4 mL). The mixture was stirred for 30 min, and PLE/MPEG (0.93 g, 4830 U) and *n*-octane (50 mL) were then added. After the mixture was stirred rapidly for 7 d (40% conversion), $MgSO_4$ (5 g) and $NaHCO_3$ (3 g) were added and the mixture was

filtered through Celite. The filtrate was concentrated in vacuo and the residue purified first by Kugelrohr distillation (0.4 mbar, 60 °C) and then by chromatography (cyclohexane/EtOAc, 1:1) to furnish alcohol (*S*)-**6** (880 mg, 59%) with 61% ee, $[\alpha]^{23}_D = +17.1$ (*c* 1.67, $CHCl_3$) and ester (*R*)-**6a** (800 mg, 39%) with 92% ee, $[\alpha]^{23}_D = -14.1$ (*c* 1.64, $CHCl_3$). (*S*)-**6**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20−7.34 (m, 5 H), 4.42 (ddd, J = 47.0, 9.4, 3.5 Hz, 1 H), 4.32 (ddd, J = 47.8, 9.4, 6.1 Hz, 1 H), 4.07 (m, 1 H), 2.81 (dd, J = 6.1, 3.5 Hz, 2 H), 2.14 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.7 (d, J = 7 Hz, u), 71.2 (d, J = 19 Hz, d), 85.5 (d, J = 169 Hz, u), 126.6 (d), 128.5 (d), 129.1 (d), 136.9 (u). Anal. Calcd for $C_9H_{11}OF$ (154.18): C, 70.11; H, 7.19. Found: C, 70.02; H, 7.27. (*R*)-**6a**:^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20−7.34 (m, 5 H), 5.22 (dddtr, J = 23.1, 7.0, 4.4, 3.0 Hz, 1 H), 4.45 (ddd, J = 46.7, 10.4, 3.0 Hz, 1 H), 4.37 (ddd, J = 47.0, 9.9, 4.4 Hz, 1 H), 2.98 (dd, J = 13.7, 7.0 Hz, 1 H), 2.94 (dd, J = 13.7, 7.0 Hz, 1 H), 2.34 (qd, J = 7.4, 3.3 Hz, 2 H), 1.11 (tr, J = 7.6 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.0 (d), 27.6 (u), 35.8 (d, J = 7 Hz, u), 72.7 (d, J = 21 Hz, d), 82.4 (d, J = 173 Hz, u), 126.7 (d), 128.4 (d), 129.2 (d), 136.1 (u), 173.6 (u). Anal. Calcd for $C_{12}H_{15}O_2F$ (210.25): C, 68.55; H, 7.19. Found: C, 68.27; H, 7.36.

PLE/BSA-Catalyzed Acylation of *rac*-6. To a stirred solution of vinyl propionate (800 mg, 8 mmol) and alcohol *rac*-**6** (308 mg, 2 mmol) in *n*-octane (10 mL) was added water (0.08 mL) (0.4% v/v). The mixture was stirred for 30 min, and PLE/BSA (214 mg, 970 U) and *n*-octane (10 mL) were then added. After the mixture was stirred rapidly for 6 d (54% conversion), $MgSO_4$ (1 g) and $NaHCO_3$ (500 mg) were added and the mixture was filtered through Celite. The filtrate was concentrated in vacuo and the residue purified first by Kugelrohr distillation (0.4 mbar, 60 °C) and then by chromatography (cyclohexane/EtOAc, 1:1) to yield alcohol (*S*)-**6** (115 mg, 37%) with ≥99% ee, $[\alpha]^{23}_D = +4.5$ (*c* 1.12, MeOH) and ester (*R*)-**6a** (188 mg, 45%) with 68% ee, $[\alpha]^{23}_D = -5.1$ (*c* 1.28, MeOH).

PLE/BSA-Catalyzed Acylation of *rac*-9. To a solution of vinyl propionate (800 mg, 8 mmol) and alcohol *rac*-**9** (304 mg, 2 mmol) in *n*-octane (10 mL) was added water (80 μL) (0.4% v/v). The mixture was stirred for 30 min, and PLE/BSA (130 mg, 600 U) and *n*-octane (10 mL) were then added. After the mixture was stirred rapidly for 2 d (52% conversion), $MgSO_4$ (1 g) and $NaHCO_3$ (500 mg) were added and the mixture was filtered through Celite. The filtrate was concentrated in vacuo and the residue purified first by Kugelrohr distillation (0.8 mbar, 100 °C) and then by chromatography (cyclohexane/EtOAc, 3:1) to give alcohol (*S*)-**9** (126 mg, 42%) with 97% ee, $[\alpha]^{22}_D = +23.2$ (*c* 1.35, MeOH) and ester (*R*)-**9a** (150 mg, 42%) with 91% ee, $[\alpha]^{22}_D = +10.1$ (*c* 1.87, MeOH). (*S*)-**9**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (m, 2 H), 6.96 (m, 3 H), 4.22 (m, 1 H), 3.93 (dd, J = 6.3, 3.0 Hz, 1 H), 3.82 (dd, J = 7.7, 1.7 Hz, 1 H), 2.36 (s, 1 H), 1.28 (d, J = 6.3 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.1 (u), 66.6 (d), 73.5 (u), 114.8 (d), 121.4 (d), 129.7 (d), 158.4 (u). Anal. Calcd for $C_9H_{12}O_2$ (152.08): C, 71.03; H, 7.95. Found: C, 71.18; H, 7.75. (*R*)-**9a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 2 H), 6.95 (m, 3 H), 5.26 (m, 1 H), 4.03 (dd, J = 5.8, 4.4 Hz, 1 H), 3.97 (dd, J = 5.8, 4.4 Hz, 1 H), 2.34 (q, J = 7.7, 7.4 Hz, 1 H), 1.36 (d, J = 6.3 Hz, 3 H), 1.14 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.0 (d), 16.8 (d), 27.7 (u), 37.0 (u), 68.5 (d), 69.9 (u), 114.5 (d), 120.9 (d), 129.3 (d), 158.4 (u), 173.8 (u). Anal. Calcd for $C_{12}H_{16}O_3$ (208.11): C, 69.21; H, 7.74. Found: C, 68.94; H, 7.52.

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Supporting Information Available: Experimental procedures for the synthesis of compounds *rac*-**2**−**7** and *rac*-**2a**−**7a**, for the acylation of alcohols *rac*-**3** and *rac*-**6**−**10**, and for the determination of enantiomer composition. This material is available free of charge via the Internet at http://pubs.acs.org.

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