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On the Optimization of Pig Pancreatic Lipase Catalyzed Monoacetylation of Prochiral Diols

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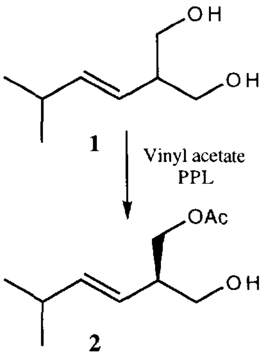
Abstract: The effect of various experimental variables (solvent, methodology of supportation on celite, presence of water, conversion and so on) on the rate and on the enantioselectivity of monoacetylation of some prochiral 2-substituted-1,3-propanediols with vinyl acetate catalyzed by crude PPL (pig pancreatic lipase) was analyzed. This study allowed to assess the best conditions for performing these transformations, providing an efficient methodology for the synthesis of valuable chiral building blocks with moderate amounts of inexpensive PPL, which can also be easily recycled.

Enzyme catalyzed acetylation of alcohols or diols in organic solvents has rapidly become one of the best methods for the preparation of chiral building blocks, either *via* kinetic resolution of chiral racemic substrates, or by asymmetrization of prochiral or *meso* diols.[1]

Crude steapsin (PPL), isolated from porcine pancreas, represents probably the cheapest commercially available lipase.[1h] Although it is a complex mixture of different hydrolases, many successful applications of this crude preparation for the resolution of chiral alcohols or for the asymmetrization of prochiral diols by selective hydrolysis of esters in water have been achieved.[1]



On the other hand, its synthetic utility in the complementary acylation of alcohols or diols in organic solvents[2-6] seems to be less widespread, compared to other lipases of microbial origin, like those from *Pseudomonas sp.*[1,7] or *Candida sp.*[1] strains. The main reason for this fact is probably related to the low catalytic efficiency of crude steapsin when used as such in ordinary organic solvents.[5a] In the last five years, during the preparation of several enantiomerically enriched 2-substituted-1,3-propanediol monoacetates, we found that in most cases crude PPL was the catalyst of choice for the enantioselective monohydrolysis of the diacetyl derivatives.[8] The complementary acetylation reactions in organic solvents were however more troublesome, since PPL catalyzed reactions turned out to be very sluggish and little reproducible, while other enzymes furnished unsatisfactory e.e.s. In the attempt to optimize these acetylation reactions, we have recently discovered, and preliminary reported,[9] that the problem of low reactivity could be resolved by supporting crude PPL on celite and by using vinyl acetate as both solvent and acylating agent: under these conditions a 7 to 20 fold acceleration was achieved, compared to "normal" conditions, thus making PPL catalyzed acetylations in organic solvents synthetically useful.

In view of this large difference of reactivity achieved by changes in the experimental conditions, we decided to undertake a deeper study on the effect of various parameters on the rate and selectivity of these acetylation, using, as model reaction, the conversion of diol **1** into (*R*) monocetate **2**. The (*S*) enantiomer of **2** was previously employed by us in several synthetic applications.[10]

Table 1: Influence of solvent in monoacetylation of **1** with vinyl acetate, catalysed by crude unsupported or supported PPL[a]

Entry	Enzyme	Solvent	Relative rate
1	PPL[b]	$CHCl_3$[d]	0.037
2	PPL[b]	*n*-Hexane[d]	0.085
3	PPL[b]	Dioxane[d]	0.124
4	PPL[b]	MeOAc[d]	0.133
5	PPL[b]	CH_2Cl_2[d]	0.135
6	PPL[b]	Et_2O[d]	0.227
7	PPL[b]	MeO*t*Bu[d]	0.258
8	PPL[b]	THF[d]	0.352
9	PPL[b]	*i*Pr_2O[d]	0.359
10	PPL[b]	vinyl acetate (VA)	1
11	SPPL-7[c]	*i*Pr_2O[d]	0.98
12	SPPL-7[c]	*i*Pr_2O / VA 3:1	2.43
13	SPPL-7[c]	*i*Pr_2O / VA 1:1	2.76
14	SPPL-7[c]	vinyl acetate (VA)	2.54

[a] Reactions carried out under N_2 at 20°C in the presence of 2.5 mg of 3 Å mol. sieves per mL of solvent. The concentration of **1** was 0.1 M. For other details see the experimental part. [b] PPL "straight from the jar". [c] PPL supported on celite (see Table 2 for details). [d] 0.5 mL (5.5 mmol) vinyl acetate per mmol of **1** was used.

We first studied the effect of solvent in the acetylation with commercial crude PPL ("straight from the jar")(Table 1, entries 1-10).[11] As can be seen, striking differences in reactivity were found, vinyl acetate being by far the best solvent. This increase in rate is not obvious, since in other cases the use of vinyl acetate as solvent was not beneficial.[12] The increase in rate was particularly evident by comparison with solvents like CH_2Cl_2 or methyl acetate, which have been often used for acylation in organic solvents. Apart from vinyl acetate, the best solvents, among the ones tested, turned out to be THF and di-*iso*-propyl ether. The same difference in rate between vinyl acetate and di-*iso*-propyl ether was confirmed on using a supported preparation of PPL (Table 1, entries 11 and 14). Interestingly, mixtures of these two solvents gave results comparable to those realized with vinyl acetate alone (entries 12 and 13).

We next studied the effect of the conditions of supportation of crude PPL on celite. The use of supported PPL on celite was already reported in the literature by various groups,[2a,13,14] although the exact procedure followed was often not specified. A procedure often quoted is that of Ramos-Tombo,[2a] which involves partial purification of the crude enzyme. In our hands[9] the catalyst prepared in that way turned out to be, however, less efficient than crude PPL "straight from the jar". On the contrary, by using a simpler procedure, similar to that first described by Carrea,[5d] which avoids any fractionation of the crude enzyme, the activity of the catalyst was remarkably increased. However, subsequently to our preliminary report,[9] we noticed that slight differences in the supportation procedure could lead to large differences in efficiency. Thus, in order to better understand these subtle effects, we carried out a comparison among supported preparations of PPL (here called SPPL-n) prepared under different conditions. Table 2 shows some selected data. First we verified that no increase in reactivity was achieved by simply mixing crude PPL with celite in the reaction media (entries 2,3). Furthermore, it is crucial that PPL and celite be allowed to stand for a certain amount of time prior to freeze and lyophilize the resulting suspension (compare entry 5 with entry 4). This "conditioning" is best carried out at room temperature (see entries 7 and 10) and without stirring (entry 9). The addition of acetone to the reaction mixture[2a] has only a slight negative effect. Also the relative quantities of PPL, celite, and buffer are important. Similar activities were found by using the conditions of entries 5 and 8, but increasing or reducing the celite / buffer ratio was deleterious (entries 11-13). ***Thus, the best conditions were those of entry 5[15] (SPPL-4) and of entry 8 (SPPL-7). The increase in activity was ≈ 2.5 fold compared to commercial PPL.[16,17]*** Another important advantage of the use of these supported preparation is shown in entry 14: the enzyme can be easily recovered after reaction and reused with no loss of activity.

Table 3 shows the effect of concentration on the rate of reaction. It is interesting to note that a remarkable decrease of reactivity is observed when the concentration of substrate in vinyl acetate is increased over 0.1 M (entries 4,5). A slight decrease was also observed by diluting too much (entry 1).[18]

The importance of little amount of water on the rate of enzymatic reactions has been recently pointed out.[11b-h,18] Thus we were puzzled by the possibility that the differences in reactivity among the various

Table 2. Effect of supportation conditions on the rate of monoacetylation of **1** with PPL in vinyl acetate[a]

		Supportation conditions[b]			
Entry	Enzyme	g Celite/ g PPL	Buffer (mL/g PPL)	Notes on procedure for supportation	Relative rate
1	PPL[c]	-	-	-	1
2	SPPL-1	3.33	-	PPL dry-mixed with celite	1.25
3	SPPL-2	3.33	-	PPL dry-mixed with celite + K_2HPO_4 -KH_2PO_4[d]	0.86
4	SPPL-3	3.33	16.66	Buffer, PPL, and celite mixed and stirred for 15min.; then freezed and lyophilized	0.65
5	**SPPL-4**	3.33	16.66	Buffer, PPL, and celite mixed and stirred for 15 min.; then allowed to stand 9h at r.t., freezed and lyophilized	**2.54**
6	SPPL-5	3.33	16.66	As SPPL-4, but adding 10 mL of acetone	2.19
7	SPPL-6	3.33	16.66	As SPPL-4 but allowed to stand 9h at 0°C	1.25
8	**SPPL-7**	5	25	As SPPL-4	**2.54**
9	SPPL-8	5	25	As SPPL-7, but stirring 9h instead than standing still	2.11
10	SPPL-9	5	25	As SPPL-7, but allowed to stand 9h at 40°C	0.81
11	SPPL-10	2	16.66	As SPPL-4	1.09
12	SPPL-11	3.33	30	As SPPL-4	1.61
13	SPPL-12	3.33	10	As SPPL-4	1.41
14	SPPL-13	3.33	16.66	SPPL-4 recovered after a 5 mmol scale reaction[e]	2.58

[a] Reactions carried out under N_2 at 20°C on 0.1 M **1** in vinyl acetate in the presence of 2.5 mg of 3Å mol. sieves per mL of vinyl acetate. For further details see the experimental part [b] Commercial PPL was suspended in 0.067 M pH buffer (K_2HPO_4-KH_2PO_4), treated with Hyflo-Supercel, stirred for 15 min. and, after standing for the requisite time, rapidly freezed to -25°C, lyophilized, and finally stripped overnight at 10^{-2} mbar over P_2O_5. For further details see the experimental part. [c] PPL "straight from the jar". [d] Phosphates were in the same amount contained in 16.66 mL buffer/g PPL. [e] The reaction was stopped at 50% conversion, the supported enzyme was filtered through buchner, washed with diethyl ether, and dried overnight at 10^{-2} mbar over P_2O_5.

supported enzymes could be in some way related to small quantities of water retained by the catalyst even after lyophilization and further stripping at 10^{-2} mbar to constant weight. The data collected in Table 4, however, do not seem to support this hypothesis. The differences observed in the presence or in the absence of molecular sieves are indeed negligible. Moreover, exposure of the thoroughly dried SPPL-4 preparation to moisture, which resulted to weight increases between 0.5 and 1% (depending on the time of exposure), gave catalyst which showed the same activity than the dried one (see entries 5,6). On the other hand, the addition of a relatively large amount of water to the solvent led to a decrease in rate.

Table 3. Effect of concentration on the monoacetylation of **1** with supported PPL SPPL-4[a]

Entry	Substrate conc.	rel. rate
1	0.02 M	1.97
2	0.05 M	**2.62**
3	0.1 M	**2.54**
4	0.2 M	2.11
5	0.4 M	1.26

[a] Reactions performed at 20°C in vinyl acetate as described in note a of Table 2.

It has been suggested that the rate increase observed by supporting hydrolytic enzymes may be due to protection against deactivation provoked by acetaldehyde formed during the reaction.[19] In order to check this possibility, we examined the effect of added acetaldehyde on the relative reaction rates using both supported and unsupported PPL (Table 5). The results show that both supported and unsupported preparations are not remarkably deactivated by the presence of acetaldehyde.

Having found the optimal conditions for maximum rate, we then decided to study the effect of conversion on the enantioselectivity. In the asymmetrization of prochiral diols by enantioselective esterification,

Table 4. Effect of molecular sieves and water on the rate of monoacetylation of **1** with supported PPL SPPL-4[a]

Entry	3 Å mol. sieves (mg/mL)	added H_2O (mg/mL)	Rel. rate
1	14	0[b]	2.08
2	6	0[b]	2.21
3	3	0[b]	2.54
4	0	0[b]	1.98
5	0	0.005[c]	2.62
6	0	0.01[d]	2.67
7	0	0.275[e]	1.73

[a] Reactions performed at 20°C in vinyl acetate as described in note a) of Table 2. [b] The enzyme was thoroughly dried over P_2O_5 at 10^{-2} mbar. [c] The enzyme was expose to moisture until a 0.5% increase in weight was achieved. [d] The enzyme was allowed to equilibrate in presence of moisture to constant weight. The increase of weight was 1%. [e] Water was added to the solvent just before starting the reaction.

Table 5. Effect of added acetaldehyde on the rate of monoacetylation of **1**[a]

Entry	Enzyme	Added CH_3-CHO (equiv.)	Rel. rate
1	PPL[b]	0	1
2	PPL[b]	1	0.87
3	PPL[b]	2	0.85
4	SPPL-7[c]	0	2.54
5	SPPL-7[c]	1	2.54
6	SPPL-7[c]	2	2.42

[a] Reactions carried out as described in note a of Table 2, adding CH_3CHO before enzyme addition. [b] Crude PPL "straight from the jar". [c] Supported PPL on celite (see Table 2).

usually the e.e. tends to increase at higher conversions,[1e,h] thanks to the kinetic resolution in the second acylation step. However we have previously noted an exception to this general behaviour.[2g] Thus it was interesting to check whether in the case of **1**, higher conversions were helpful or deleterious. Table 6 clearly shows that in this case the kinetic resolution plays an important role in the achievement of high e.e.s. Stopping the reaction at 33.3% conversion (entry 1), when no diacetate has yet formed, the e.e. was indeed only 87%. It was raised to very high values by simply allowing the reaction to reach conversions slightly higher than 50%. This improvement of e.e. required only a minor loss in the isolated yield. As it is evident from Table 6, the rate of reaction (which remains practically constant for conversions up to 32-33%),[20] drops sharply after 50% conversion. It is also worth noting that only traces of diacetate are formed before consumption of all diol (as determined by ^{1}H n.m.r. analysis). A similar behaviour was observed by carrying out the reaction in iPr$_2$O / vinyl acetate (entries 6-8).[21]

In conclusion (*R*) monoacetate **2** can be synthesized in good yield and high e.e. in few hours, by using a small amount of inexpensive crude commercial PPL (only 25 mg per mmol of **1**). This amount is less than that required for the preparation of (*S*) **1** by the complementary hydrolysis of the corresponding diacetate.[8c,22] Moreover the work-up is in this case simpler, and the catalyst can be recycled without loss of activity. This reaction has been by now scaled up to 50 mmol without problems.

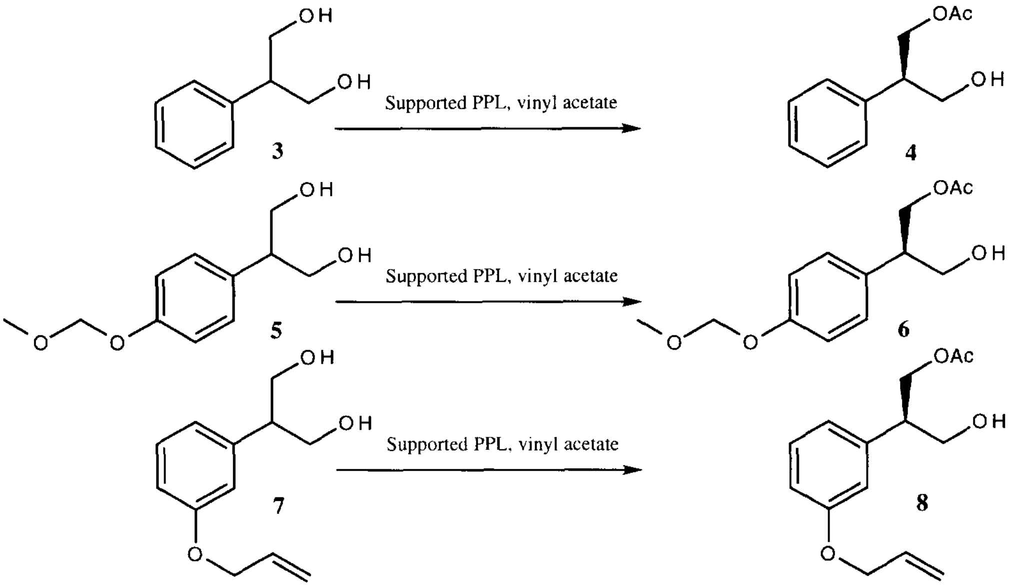
We next examined the asymmetrization of the three 2-aryl-1,3-propanediols **3**, **5**, and **7** (Scheme 1), which are useful synthetic intermediates.[2b,8b,23] The results are depicted in Table 7. First it should be noted

Table 6. Effect of conversion on yield and enantioselectivity in the monoacetylation of **1** with SPPL-7[a]

Entry	Solvent	Time (min.)	Conversion[b]	Yield of **2** (nmr)	Yield (isolated)[c]	e.e.[d]	$[\alpha]_D$[e]
1	Vinyl acetate (VA)	105	33.3%	66.5%	60%	87.0%	+ 23.4
2	Vinyl acetate (VA)	220	50.4%	88.5%	86%	92.0%	+ 25.3
3	Vinyl acetate (VA)	252	52.0%	91.6%	89%	92.8%	+ 25.5
4	Vinyl acetate (VA)	320	55.5%	88.5%	82%	96.0%	+ 26.7
5	Vinyl acetate (VA)	420	57.4%	85.5%	80%	**97.0%**	+ 27.3
6	VA / iPr$_2$O 1:3	99	29.6%	59%	57%	88.7%	+ 24.8
7	VA / iPr$_2$O 1:3	217	51.8%	91%	81%	94.0%	+ 26.1
8	VA / iPr$_2$O 1:3	385	59.6%	81%	76%	**98.0%**	+ 27.9

[a] Reactions carried out under N_2 at 21°C with 0.1 M **1**, using 155 mg of SPPL-7 (corresponding to 25.1 mg of original crude PPL) per mmol of **1**, in the presence of 3 mg 3 Å mol. sieves per mL of vinyl acetate. [b] For a definition of conversion see ref. 8c. [c] After silica gel chromatography. [d] Determined by ^{1}H n.m.r. in the presence of Eu(hfc)$_3$. [e] (c 2, $CHCl_3$).

Scheme 1



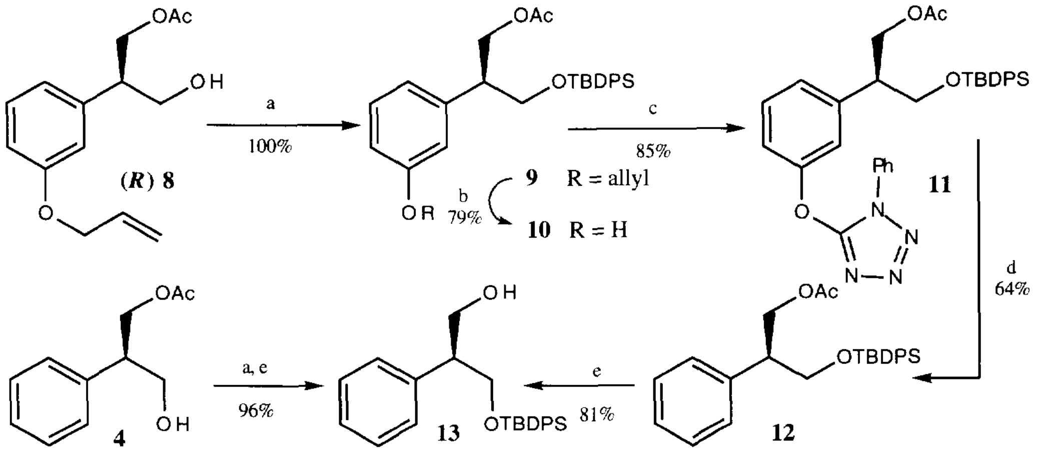
that the order of reactivity is **1** > **3** > **5** > **7**. This order is in agreement with the reactivity of the corresponding diacetates in the PPL catalyzed hydrolysis.[8] The striking difference in reactivity between the *para*-substituted diol **5** and the *meta*-substituted compound **7** is especially noteworthy. Also for these three diols higher conversions seem to afford better enantioselectivities. The effect is however less pronounced. Furthermore,

Table 7. Effect of conversion on yield and enantioselectivity in the monoacetylation of **3,5,7** with SPPL-7[a]

Entry	Substrate	Time (min.)	Conversion[b]	Yield (nmr) of monoacetates **4,6,8**	Yield (isolated)[c]	e.e.[d]	$[\alpha]_D$[e]
1	**3**	72	16.0%	31.9%	24%	94.9%	n.d.
2	**3**	136	24.9%	49.9%	45%	93.1%	+ 16.9
3	**3**	221	33.5%	63.6%	62%	95.0%	+ 15.5
4	**3**	307	44.3%	83.8%	72%	94.2%	+ 17.2
5	**3**	414	52.3%	94.9%	87%	94.1%	+ 16.9
6	**3**	808	55.2%	88.0%	81%	**96.9%**	+ 16.5
7	**5**	120	34.2%	not determined	57%	86.4%	+ 12.3
8	**5**	240	47.9%	not determined	78 %	96.8%	+ 13.2
9[f]	**5**	390	51.5%	not determined	82 %	**97.0%**	+ 13.5
10	**7**	128	10.0%	20.1%	18 %	81.3%	+ 10.2
11	**7**	256	27.2%	52.5%	42%	86.7%	+ 13.1
12	**7**	450	34.2%	65.1%	55 %	87.9%	+ 13.4
13	**7**	1540	47.4%	91.2%	72 %	**89.1%**	+ 14.1
13	**7**	3077	49.5%	90.0%	72 %	87.0%	+ 13.1

[a] Reactions carried out (on 0.3-0.5 mmol) in vinyl acetate under N_2 at 15°C with 0.065 M **3** or 0.048 M **5** and **7**, using 380 mg of SPPL-7 (corresponding to 61.6 mg of original crude PPL) per mmol of **3**, and 730 mg of SPPL-7 (corresponding to 118 mg of original crude PPL) per mmol of **5** or **7**, in the presence of 3 mg 3 Å mol. sieves per mL of vinyl acetate. [b] For a definition of conversion see ref. 8c. [c] After silica gel chromatography. [d] Determined by ^1H n.m.r. in the presence of $Eu(hfc)_3$. [e] (c 2, $CHCl_3$). [f] This reaction was also upscaled to 10 mmols.

Scheme 2



a) *tert*-Butyldiphenylsilyl chloride, imidazole, DMF, r.t.; **b)** *n*Bu$_3$SnH, AcOH, Pd(PPh$_3$)$_4$, toluene, 80°C; **c)** 5-Chloro-1-phenyltetrazole, K$_2$CO$_3$, DMF, 80°C; **d)** H$_2$, Pd-C, EtOH, 45°C; **e)** KOH, MeOH, 0°C.

especially for **5** and **7**, it is difficult to achieve conversions higher than 50%, since the reaction tends to become very sluggish. In the case of **7** too long reaction times have only a deleterious effect on the enantioselectivity.[24]

Under the best conditions, **4** and **6** could be prepared in excellent e.e. In particular, the e.e. obtained for **4** (entry 6) is higher than that previously achieved both in diacetate monohydrolysis or in diol monoacetylation.[25,2a,2b,8a] On the other hand, the best e.e. obtained for **8** is only ≈ 89%, thus worse than what achievable by the complementary hydrolysis.[8b]

The absolute configurations of the new compounds **6** and **8** was determined as follows. In the case of **6** the configuration was established as (*R*) by the sign of the optical rotation. Indeed in a previous work we have found a close correlation between the configuration and the sign of $[\alpha]_D$ for a series of 2-(4-substituted-phenyl)-3-acetoxypropan-1-ols, including the 4-methoxyphenyl derivative, structurally very similar to **6**.[8a]

In the case of **8** we carried out a chemical correlation with monoacetate **4**, whose absolute configuration was previously established,[2a,8a] taking advantage of a method for the reductive deoxygenation of phenols, which involves the hydrogenolysis of the corresponding O-(1-phenyltetrazol-5-yl) derivatives (Scheme 2). In this way we could prepare **13** from the diprotected phenol **10**. Compound **13** was also prepared from (*R*) **4** and the two compound correlated by the sign of the $[\alpha]_D$.

The preference for the (*R*) enantiomer for all 4 substrates examined is in agreement with the empirical model for the PPL active site previously proposed by us.[8c]

In conclusion with the present work we have demonstrated that several factors can affect the rate and the enantioselectivity of monoacetylation in organic solvents of prochiral 2-substituted-1,3-propanediols catalyzed by crude steapsin (PPL). Thanks to this study we are able now to perform these reactions in acceptable time, without the need to use large quantities of enzyme, and with the added possibility to recycle the catalyst. We think that this optimization study can be valuable to all those who, notwithstanding the low cost of crude PPL, have decided not to employ it for this type of reaction because of low efficiency or unreproducible results.

We thank C.N.R. and M.U.R.S.T. for financial assistance.

EXPERIMENTAL

N.m.r. spectra were recorded on a Varian Gemini 200 spectrometer in $CDCl_3$. Tetramethylsilane was used as internal standard. Coupling constants are reported in Hz. Elemental analyses were performed with a Perkin-Elmer 240 instrument. Chromatographies were carried out on 220-400 mesh silica gel. Thin layer chromatographies were carried out on 0.25 mm silica gel F 254 plates (Merck). Spots were detected at U.V. or, unless otherwise noted, through immersion in a solution of 21 g $(NH_4)_4MoO_4 \cdot 4\ H_2O$ and of 1 g $Ce(SO_4)_2 \cdot 4\ H_2O$ in 469 mL H_2O and 31 mL conc. H_2SO_4, followed by warming on a hot plate. PE = petroleum ether 40-60°C. Celite used in this work was Hyflo-Supercel (Fluka cat. no. 56678). Crude PPL was purchased from Sigma (lot no. 41-HO954, 12.5 U/mg protein using triacetin as standard). Lyophylizations were carried out with an Edwards Micromodulyo apparatus at 10^{-2} mbar. All reactions employing dry solvents were run under a nitrogen atmosphere. Tetrahydrofuran (THF) was dried over K/benzophenone; dry diethyl ether, methylene chloride, acetonitrile, dimethylformamide, toluene, ethanol and methanol were purchased from Fluka. Organic extracts, if not otherwise indicated, were finally washed with brine, dried over Na_2SO_4 and filtered before removal of solvent under reduced pressure.

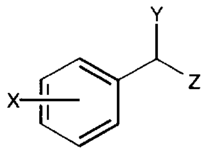
Preparation of supported catalysts SPPL-4 and SPPL-7. SPPL-4: Crude PPL (3 g) was suspended in 0.067 M pH 7 buffer (purchased from Fluka)(50 mL) and immediately treated with Hyflo Supercel (10 g). The suspension was magnetically stirred for 15 min. at r.t. and then allowed to stand still for 9 h. The resulting suspension was freezed at -25°C while gently manually stirring. The freezed mixture was then lyophilized at 10^{-2} mbar. The resulting solid was removed from the flask, gently grinded, and further stripped at 10^{-2} mbar in a dessicator over P_2O_5 (a weight loss of about 0.5-0.7% was observed). Yield= 13.452 g. Thus 1 g of SPPL-4 corresponds to 0.223 g of crude PPL. **SPPL-7**: It was prepared exactly as for SPPL-4, but using 75 mL of buffer solution and 15 g of Hyflo Supercel. 1 g of this preparation corresponds to 0.162 g of crude PPL.

(*E*)-2-(3-Methyl-1-buten-1-yl)-1,3-propanediol 1.[8c] A suspension of $LiAlH_4$ (20 g, 0.53 mol) in dry diethyl ether (500 mL) was cooled to 0°C and slowly treated, during 30 min., with a solution of diethyl (*E*) 2-(3-methyl-1-buten-1-yl)malonate[8c] (30 g, 0.13 mol). After stirring for 20 min. at 0°C and 5h at r.t., the suspension was cooled to 0°C and quenched (with caution) by slow addition of a solution of NaOH (2.33 g) in H_2O (79.2 mL). The resulting suspension was stirred overnight at r.t., and filtered. The white solid was thoroughly washed with Et_2O and the combined filtrates evaporated to dryness to give crude **1** as a solid (18.2 g, 96 %). Crystallization (Et_2O / PE) gave pure **1** as a white solid (13.1 g, 69%). The mother liquors still contained > 50% **1**, as judged by ^1H n.m.r. R_f 0.29 (PE / Et_2O 1:9). Anal. found C, 66.5; H, 11.3 %. $C_8H_{16}O_2$ requires: C, 66.63; H, 11.18%. M.p. 49.4-49.8°C. ^1H n.m.r.: δ 0.99 [6 H, d, CH_3, J= 6.7]; 2.03 [2 H, broad s, O*H*]; 2.29 [1 H, doublet of octuplet, $CH(CH_3)_2$, J=1.3 (d) and 6.8 (oct)]; 2.47 [1 H, sextuplet, $CH(CH_2OH)_2$, J=6.7]; 3.70 [4 H, d, CH_2OH, J=6.2]; 5.21 [1 H, ddd, $CH{=}CH{-}CH(CH_2OH)_2$, J=1.3, 8.1, and 15.7]; 5.61 [1 H, dd, $CH{=}CH{-}CH(CH_3)_2$, J=6.5, 15.7].

General procedure for determination of catalyst activity. A solution of **1** (60 mg, 0.416 mmol) in the appropriate solvent (see tables) was treated (except where indicated in Table 4) with powdered 3 Å molecular sieves (10 mg) and stirred for 15 min. at 20°C. Then the enzyme [in the case of commercial PPL (entries 1-10 of Table 1 and entries 1-3 of Table 5): 29 mg. In the case of supported PPL preparations (SPPLn) a quantity corresponding to 10.4 mg of commercial PPL] was added in one portion, and the mixture magnetically stirred under N_2 for a certain amount of time (usually 1-4 h) in order to achieve a conversion between 10% and 30% (qualitatively judged by tlc analysis). The mixture was then suddenly filtered and the filtrate evaporated to dryness. ^1H n.m.r. allowed easy determination of the conversion, by integrating the signals due to CH_2OH and to CH_2OAc groups. The ratio between the conversion achieved and that of a control experiment, carried out with crude PPL "straight from the jar", corrected by the amount of enzyme employed, and by the reaction time, corresponds to the relative rates indicated in tables 1-5. This calculation was made under the assumption that: **a**) the reaction follows a *pseudo*-zero order kinetic; **b**) the relationship between rate and enzyme amount is linear. The first assumption was verified to be correct, in the case of the reactions catalyzed by SPPL-7, or by commercial PPL, by kinetic experiments which indicated that the rate was constant up to 32-33% conversion, when it started to decrease. Also the second assuption was verified, both for commercial and supported PPL (SPPL-4 and SPPL-7), by experiments which demonstrated a linear relationship between rate and enzyme amount (at least in the range between 15 mg enzyme/mmol substrate to 70 mg enzyme/mmol substrate).

2-Phenyl-1,3-propanediol 3. This compound was prepared from commercially available diethyl phenylmalonate (30 mL, 0.139 mol) by using a the same procedure employed for **1**. Chromatography (PE : Et_2O 8:2 → 1:1; then AcOEt) gave **3** as a white solid (17.98 g, 85%). For spectroscopic characterization see ref. 8a.

Ethyl (4-hydroxyphenyl)acetate 14. Concentrated sulfuric acid (6.5 mL, 122.04 mmol) was added to a solution of 4-hydroxyphenylacetic acid (26.65 g, 0.175 mol) in absolute ethanol (400 mL) and the mixture refluxed overnight. The solvent was removed under reduced pressure and the residue diluted with water and neutralized with solid $NaHCO_3$. After extraction with Et_2O, the combined organic extracts were washed with saturated $NaHCO_3$. Evaporation of the solvent gave crude product as a colourless oil. Crude product was distilled (b.p. 130-145°C at 0.18 torr) to give **14** (29.10 g, 92%). R_f 0.13 (PE : Et_2O 8:2). Anal. found C, 66.85; H, 6.80. $C_{10}H_{12}O_3$ requires C, 66.65; H, 6.71. ^{1}H-n.m.r.: δ 1.25 [3H, t, $-CO_2CH_2CH_3$, J=7.1]; 3.54 [2H, s, $-CH_2CO_2Et$]; 4.15 [2H, q, $-CO_2CH_2CH_3$, J=7.1]; 5.04 [1H, s, Ar-O*H*]; 6.76 [2H, app. d, *H* ortho to -OH, J=8.5]; 7.14 [2H, app. d, *H* ortho to $-CH_2CO_2Et$, J=8.4].



compound	X	Y	Z
14	4-OH	H	CO_2Et
15	4-OMOM	H	CO_2Et
16	4-OMOM	CO_2Et	CO_2Et
17	3-OH	H	CO_2Et
18	3-allyl	H	CO_2Et
19	3-allyl	CO_2Et	CO_2Et

Ethyl [4-(methoxy)methoxy]phenylacetate 15. Methoxymethyl chloride [(4.22 + 2.11 + 2.11) mL, 0.111 mol] and triethylamine [(8.52 + 4.26 + 2.70) mL, 0.111 mmol] were added portionwise to a solution of **14** (5.01 g, 27.29 mmol) in dry CH_3CN (50 mL) and the resulting mixture was refluxed for 2 days. Water and saturated $NaHCO_3$ were added and the reaction was extracted with Et_2O. The extracts were concentrated *in vacuo* to give crude **15**. Chromatography (PE : Et_2O 95:5 → 7:3) gave pure **15** (5.97 g, 96%). R_f 0.41 (PE : Et_2O 8:2). Anal. found C, 64.35; H, 7.25. $C_{12}H_{16}O_4$ requires C, 64.27; H, 7.19. ^{1}H-n.m.r.: δ 1.25 [3H, t, $-CO_2CH_2CH_3$, J=7.1]; 3.47 [3H, s, $-OCH_3$]; 3.55 [2H, s, $-CH_2CO_2Et$]; 4.14 [2H, q, $-CO_2CH_2CH_3$, J=7.1]; 5.16 [2H, s, $-OCH_2OCH_3$]; 6.99 [2H, app. d, 2*H* ortho to -OMOM, J=8.6]; 7.20 [2H, app. d, 2*H* ortho to $-CH_2CO_2Et$, J=8.7].

Diethyl [4-(methoxy)methoxy]phenylmalonate 16. A solution of **15** (5.96 g, 26.58 mmol) in anhydrous THF (15 mL) was cooled to -78°C and treated with a 0.56 M solution of LDA (71.1 mL, 39.88 mmol) in *n*-hexane-THF 2:1, prepared in the presence of 2,2'-bipyridyl and previously cooled to the same temperature. After 15 min stirring at -78°C the red solution was added *via* syringe to a solution of ethyl chloroformate (3.30 mL, 34.56 mmol) in THF (10 mL). The resulting yellow solution was allowed to react for about 30 min and then quenched with saturated aqueous NH_4Cl (20 mL). After extraction with Et_2O, the combined organic phases were evaporated to dryness. Chromatography (PE : Et_2O 9:1 → 7:3) gave pure **16** (4.40 g, 56%, 86% based on unrecovered **15**) together with pure unreacted **15** (2.08 g, 35%). R_f 0.18 (PE : Et_2O 85:15). Anal. found C, 60.95; H, 7.70. $C_{15}H_{20}O_6$ requires C, 60.80; H, 6.80. ^{1}H-n.m.r.: δ 1.26 [6H, t, $-CO_2CH_2CH_3$, J=7.1]; 3.47 [3H, s, $-OCH_3$]; 4.21 [4H, centre of m, $-CO_2CH_2CH_3$]; 4.56 [1H, s, $-CH(CO_2CH_2CH_3)_2$]; 5.17 [2H, s, $-OCH_2OCH_3$]; 7.02 [2H, app. d, *H* ortho to -OMOM, J=8.8]; 7.32 [2H, app. d, *H* ortho to $-CH_2CO_2Et$, J=8.8].

2-[4-(Methoxy)methoxy]phenyl-1,3-propanediol 5. It was prepared from **16**, following the same procedure reported for compound **1**. Chromatography (PE : AcOEt 3:7 → 0:10; then AcOEt : MeOH 9:1) gave **5** as a pale yellow oil in 72% yield. R_f 0.31 (Et_2O). Anal. found C, 62.00; H, 7.75. $C_{11}H_{16}O_4$ requires C, 62.25; H, 7.60. ^{1}H-n.m.r.: δ 2.02 [2H, broad s, -O*H*]; 3.07 [1H, centre of m, $-CH(CH_2OH)_2$]; 3.48 [3H, s, $-OCH_3$]; 3.89-4.06 [4H, m, $-CH(CH_2OH)_2$]; 5.16 [2H, s, $-OCH_2OCH_3$]; 7.01 [2H, app. d, *H* ortho to -OMOM, J=8.7]; 7.16 [2H, app. d, *H* ortho to $-CH(CH_2OH)_2$, J=8.7].

Ethyl (3-hydroxyphenyl)acetate 17. This compound was prepared starting from 3-hydroxyphenylacetic acid (20.64 g, 135.68 mmol) using the same procedure reported for **14**. The crude product was distilled (b.p. 130-140°C at $6\cdot10^{-2}$ torr) to give pure **17** (23.02 g, 94%). R_f 0.49 (PE : Et_2O 1:1). Anal. found C, 66.75; H, 6.85. $C_{10}H_{12}O_3$ requires C, 66.65; H, 6.71. ^{1}H n.m.r.: δ 1.26 [3H, t, $-CO_2CH_2CH_3$, J=7.1]; 3.57 [2H, s, $ArCH_2CO_2Et$]; 4.16 [2H, q, $-CO_2CH_2CH_3$, J=7.1]; 5.25 [1H, s, -O*H*]; 6.71 - 6.86 [3H, m, *H* para and ortho to -OH]; 7.19 [1H, t, *H* meta to -OH, J=8.0].

Ethyl (3-allyloxy)phenylacetate 18. A solution of **17** (2.12 g, 11.76 mmol) in dry DMF (15 mL) was treated with anhydrous K_2CO_3 (3.25 g, 23.51 mmol); after stirring for 15 min. at r.t., allyl bromide (2.04 mL, 23.51 mmol) was added and the reaction mixture heated at 55°C for 4 hours. The reaction was diluted with water and the pH adjusted to 4-5 with a 5% aqueous solution of $NH_4H_2PO_4$; after extraction with Et_2O, the combined organic phases were evaporated and crude product was chromatographed (PE : Et_2O 95:5 → 9:1) to give **18** 2.49 g, 96%) as a colourless oil. R_f 0.34 (PE:Et_2O 9:1). Anal. found C, 70.70; H, 7.45. $C_{13}H_{16}O_3$ requires C, 70.89 H, 7.32. ^{1}H-n.m.r.: δ 1.25 [3H, t, $-CO_2CH_2CH_3$, J=7.1]; 3.56 [2H, s, $-CH_2CO_2Et$]; 4.15 [2H, q, $-CO_2CH_2CH_3$, J=7.1]; 4.54 [2H, dt, $-OCH_2CH{=}CH_2$, J=5.3(d), 1.5(t)]; 5.29 [1H, dq, $-OCH_2CH{=}CHH$ trans to CH_2, J=10.5(d), 1.5(q)]; 5.41 [1H, dq, $-OCH_2CH{=}CHH$ cis to CH_2, J=17.3(d), 1.5(q)]; 6.06 [1H, ddt, $-OCH_2CH{=}CH_2$, J=17.3(d), 10.5(d), 5.3(t)]; 6.80 - 6.90 [3H, m, *H* para and ortho to -OAllyl]; 7.19 - 7.28 [1H, m, *H* meta to -OAllyl].

Diethyl (3-allyloxy)phenylmalonate 19. This compound was prepared starting from **18** (2.09 g, 9.50 mmol), by using the same procedure employed for **16**. Chromatography (PE : Et_2O 8:2 → 7:3) gave pure **19** (1.73 g, 62%, 90% on unrecovered **18**) together with pure unreacted **19** (648 mg, 31%). R_f 0.36 (PE : Et_2O 8:2). Anal. found C, 65.95; H, 7.00. $C_{16}H_{20}O_5$ requires C, 65.74; H, 6.90. ^{1}H-n.m.r.: δ 1.26 [6H, t, $-CH(CO_2CH_2CH_3)_2$, J=7.1]; 4.22 [4H, m, $-CH(CO_2CH_2CH_3)_2$]; 4.54 [2H, dt, $-OCH_2CH{=}CH_2$, J=5.3(d), 1.5(t)]; 4.58 [1 H, s, $-CH(CO_2Et)_2$]; 5.29 [1H, dq, $-OCH_2CH{=}CHH$ trans to CH_2, J=10.5(d), 1.5(q)]; 5.42 [1H, dq, $-OCH_2CH{=}CHH$ cis to CH_2, J=17.3(d), 1.5(q)]; 6.06 [1H, ddt, $-OCH_2CH{=}CH_2$, J=17.3(d), 10.5(d), 5.3(t)]; 6.86 - 7.02 [3H, m, *H* para and ortho to -OAllyl]; 7.27 [1H, broad t, *H* meta to -OAllyl, J=7.9].

2-(3-Allyloxy)phenyl)-1,3-propanediol 7. A 1M solution of DIBALH in CH_2Cl_2 (37.84 mL, 37.84 mmol) was cooled to 0°C and treated with a solution of **19** (1.62 g, 5.54 mmol) in dry CH_2Cl_2 (30 mL); the reaction was stirred at 0°C for 30 min and at r. t. for 2 hours. After cooling the reaction flask in an ice bath MeOH (15 mL) and HCl 6 N (about 15 mL) were cautiously added. The mixture was extracted with AcOEt; the combined organic layers were dried over K_2CO_3 and evaporated to dryness. Chromatography (PE : AcOEt 8:2 → 100% AcOEt) gave **8** as a pale yellow oil (7.27 mg, 63%). R_f 0.27 (Et_2O). Anal. found C, 69.00; H, 7.85. $C_{12}H_{16}O_3$ requires C, 69.21; H, 7.74. ^{1}H-n.m.r.: δ 2.15 [2H, broad s, $-CH_2OH$]; 3.07[1H, centre of m, $-CH(CH_2OH)_2$]; 3.88-4.04 [4H, m, $-CH(CH_2OH)_2$]; 4.53 [2H, dt, $-OCH_2CH{=}CH_2$, J=5.3(d), 1.4(t)]; 5.30 [1H, dq, $-OCH_2CH{=}CHH$ trans to CH_2, J=10.4(d), 1.4(q)]; 5.42 [1H, dq, $-OCH_2CH{=}CHH$ cis to CH_2, J=17.3(d), 1.6(q)]; 6.06 [1H, ddt, $-OCH_2CH{=}CH_2$, J=17.3(d), 10.4(d), 5.3(t)]; 6.80 - 6.85 [3H, m, *H* para and ortho to -OAllyl]; 7.21-7.29 [1H, m, *H* meta to -OAllyl, J=7.9].

General procedure for the preparative synthesis of (*R*) 2, 4, 6, and 8. Essentially the same procedure employed for the determination of catalyst activity was followed (see also notes of Tables 6,7). The crude product was chromatographed (PE : Et_2O) to give pure monoacetates **2**, **4**, **6**, **8** as colourless liquids. Yields, e.e.s, and $[\alpha]_D$ are listed in Tables 6-7. Spectroscopic data were already reported for (*S*) **2**[8c] and for (*S*) **4**.[8a]

(*R*)-3-Acetoxy-2-[[4-(methoxy)methoxy]phenyl]propan-1-ol 6. R_f 0.60 (Et_2O). Anal. found C, 61.65; H, 7.25; $C_{13}H_{18}O_5$ requires C, 61.41; H, 7.13. ^{1}H-n.m.r.: δ 1.75 [1H, bs, -O*H*]; 2.06 [3H, s, $-OCOCH_3$]; 3.07 [1H, quintuplet, $-CH(CH_2OH)CH_2OAc$, J=6.4]; 3.48 [3H, s, $-OCH_3$]; 3.83 [2H, broad t, $-CH(CH_2OH)CH_2OAc$, J=5.9]; 4.35 [2H, d, $-CH(CH_2OH)CH_2OAc$, J=6.5]; 5.17 [2H, s, $-OCH_2OCH_3$]; 7.02 [2H, app. d, *H* ortho to -OMOM, J=8.1]; 7.18 [2H, app. d, *H* ortho to $-CH(CH_2OH)CH_2OAc$, J=8.7].

(*R*)-3-Acetoxy-2-[(3-allyloxy)phenyl]propan-1-ol 8. R_f 0.35 (PE : Et_2O 3:7). Anal. found C, 67.35; H, 7.45; $C_{14}H_{18}O_4$ requires C, 67.18; H, 7.25. ^{1}H-n.m.r.: δ 1.76 [1H, t, $-CH_2OH$, J=6.4]; 2.06 [3H, s, $-CH_2OCOCH_3$]; 3.13 [1H, quintuplet, $-CH(CH_2OH)CH_2OAc$, J=6.4]; 3.85 [2H, t, $-CH_2OH$, J = 6.2]; 4.37 [2H, d, $-CH_2OAc$, J=6.4]; 4.54 [2H, dt, $-OCH_2CH{=}CH_2$, J=5.3(d), 1.5(t)]; 5.30 [1H, dq, $-OCH_2CH{=}CHH$ trans to CH_2, J=10.5(d), 1.5(q)]; 5.42 [1H, dq, $-OCH_2CH{=}CHH$ cis to CH_2, J=17.3(d), 1.5(q)]; 6.06 [1H, ddt,

$-OCH_2CH=CH_2$, J=17.3(d), 10.5(d), 5.2(t)]; 6.80 - 6.87 [3H, m, *H* para and ortho to -OAllyl]; 7.20-7.30 [1H, m, *H* meta to -OAllyl].

(*S*)-1-Acetoxy-2-[(3-allyloxy)phenyl]-[3-(*t*-butyldiphenylsilyl)oxy]propane 9. Note: Monoacetate (*R*) **8** employed in the preparation of (*S*) **9** was stored for some months at r.t. and thus was partially racemized. It had $[\alpha]_D$ = + 7.78°and the e.e. (determined by ^{1}H-n.m.r.) was 47.6%. A solution of **8** [200 mg, 799 μmol] in dry DMF (5 mL) was treated with *t*-butyldiphenylsilylchloride (353 μL, 1.36 mmol) and imidazole (109 mg, 1.60 mmol). The reaction was stirred overnight at r. t., then diluted with H_2O and extracted with Et_2O. Partial purification by chromatography (PE : Et_2O 95:5 → 8:2) gave **9**, used as such in the next reaction. R_f 0.46 (PE : Et_2O 85:15). ^{1}H-n.m.r.: δ 1.02 [9H, s, *t*-Bu]; 1.97 [3H, s, $-CH_2OCOCH_3$]; 3.12 [1H, quintuplet, $-CH(CH_2OTBDPS)CH_2OAc$, J=6.3]; 3.84 [2H, d, $-CH_2OTBDPS$, J = 5.9]; 4.36-4.54 [4H, m, $-CH_2OAc$ + $-OCH_2CH=CH_2$]; 5.27 [1H, dq, $-OCH_2CH=CHH$ trans to CH_2, J=10.4(d), 1.4(q)]; 5.39 [1H, dq, $-CH=CHH$ cis to CH_2, J=17.3(d), 1.2(q)]; 6.04 [1H, ddt, $-OCH_2CH=CH_2$, J=17.3(d), 10.4(d), 5.2(t)]; 6.77-6.82 [3H, m, *H* para and ortho to -OAllyl]; 7.19 [1H, centre of m, *H* meta to -OAllyl]; 7.30-7.61 [10 H, m, *H* of Ph_2Si].

(*S*)-1-Acetoxy-[3-(*t*-butyldiphenylsilyl)oxy]-2-[(3-hydroxy)phenyl]propane 10. A solution of **9** (≤ 799 μmol) in dry toluene (10 mL) was treated with acetic acid (69 μL, 1.19 mmol), *n*-Bu_3SnH (430 μL, 1.60 mmol) and $Pd(PPh_3)_4$ (92 mg, 79.9 μmol) and the mixture was heated to 80°C for 45 min. The solution was diluted with H_2O/Et_2O and filtered over a celite pad. After extraction with Et_2O crude product was chromatographed twice (PE : Et_2O 8:2 → 3:7) to give nearly pure **10** (276 mg, 77%) as a colourless oil. R_f 0.40 (PE : Et_2O 1:1). ^{1}H-n.m.r.: δ 1.02 [9H, s, *t*-Bu]; 1.98 [3H, s, $-CH_2OCOCH_3$]; 3.11 [1H, quintuplet, $-CH(CH_2OTBDPS)CH_2OAc$, J=6.2]; 3.84 [2H, d, $-CH_2OTBDPS$, J=5.9]; 4.41 & 4.47 [2H, AB part of ABX system, $-CH_2OAc$, J_{AB}=11.0, J_{AX} & J_{BX}=6.9, 6.5]; 4.82 [1H, s, -O*H*]; 6.65-6.79 [3H, m, *H* para and ortho to -OH]; 7.16 [1H, t, *H* meta to -OH, J=7.8]; 7.30-7.61 [10 H, m, *H* of Ph_2*t*-BuSi-].

(*S*)-1-Acetoxy-[3-(*t*-butyldiphenylsilyl)oxy]-2-[[3-(1-phenyl-1*H*-tetrazol-5-yl)oxy)]phenyl]propane 11. A solution of **10** (80.7 mg, 180 μmol) in dry DMF (2mL) was treated with anhydrous K_2CO_3 (50.0 mg, 362 μmol) and 5-chloro-1-phenyl-1*H*-tetrazole (65.0 mg, 359.76 μmol) and the suspension heated at 80°C for 9 hours. The mixture was diluted with water, neutralized with 5% aqueous $NH_4H_2PO_4$ and extracted with Et_2O. Chromatography (PE : Et_2O 7:3 → 4:6) gave pure **11** (90.6 mg, 85%) as a pale yellow solid. R_f 0.26 (PE : Et_2O 7:3). ^{1}H-n.m.r.: δ 1.00 [9H, s, *t*-Bu]; 1.99 [3H, s, $-CH_2OCOCH_3$]; 3.19 [1H, quintuplet, $-CH(CH_2OTBDPS)CH_2OAc$, J=6.1]; 3.87 [2H, d, $-CH_2OTBDPS$, J = 5.6]; 4.42 & 4.48 [2H, AB part of ABX system, $-CH_2OAc$, J_{AB}=11.0, J_{AX} & J_{BX}=6.9, 6.7]; 6.65-6.79 [3H, m, *H* para and ortho to -OH]; 7.16 [1H, t, *H* meta to -OC<, J=7.8]; 7.14-7.82 [15 H, m, aromatics].

(*S*)-[1-(*t*-Butyldiphenylsilyl)oxy]-3-hydroxy-2-(phenyl)propane 13. a) From (*S*)-**11**: 60 mg of Pd/C 10% were added portion wise to a solution of **11** (80.0 mg, 134.96 μmol) in EtOH 96% (5 mL) and the suspension hydrogenated at r. t. for 19.5 hours and then at 45°C for 20 hours. The catalyst was filtered off and the solvent evaporated. The crude product was purified by preparative tlc (PE : Et_2O 8:2) to give **12** (37.5 mg, 64%) as a colourless oil. Potassium hydroxide (132 μL of a 1 N solution in dry MeOH) was added to a solution of **12** (37.5 mg, 86.68 μmol) in dry MeOH (1 mL), previously cooled to 0°C. After 1 hour the solution was stirred for an additional 25 min at r.t.. $NH_4H_2PO_4$ (5 mL of 5% aqueous solution) was added and the mixture extracted with Et_2O. Preparative chromatography (PE : Et_2O 6:4) furnished pure **13** (27.4 mg, 81%). R_f 0.27 (PE : Et_2O 65:35). Anal. found C, 76.95; H, 7.50; $C_{25}H_{30}O_2Si$ requires C, 76.88; H, 7.74. $[\alpha]_D$ = - 5.48° (c 1.305, $CHCl_3$) (see the note in the preparation of **9**).
b) From (*S*)-**4**: Compound **4** (e.e. = 95% by ^{1}H n.m.r.) (752 mg, 3.87 mmol) was converted into crude **12** following the same procedure reported for the synthesis of compound **9**. Hydrolysis of **12** was performed as described above to give pure **13** (1.26 g, 83% from **4**) as a colourless oil. $[\alpha]_D$ = - 8.37°(c 2.040, $CHCl_3$). ^{1}H-n.m.r.: δ 1.05 [9H, s, *t*-Bu]; 3.12 [1H, centre of m, $-CH(CH_2OTBDPS)CH_2OH$]; 3.35 [1H, dd, $-CH_2OH$, J=6.7, 5.4]; 3.85-4.18 [4H, m, $-CH(CH_2OTBDPS)CH_2OH$]; 7.12-7.68 [15 H, m, aromatics].

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13. Use of PPL supported according to ref. 2a: see refs. 2c, 2e. Use of PPL supported as described in ref. 14: see refs. 3a,3b. Use of PPL supported in a way similar to ours: see refs. 5d, 5o, 11f.
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15. Supportation conditions of entry 5 are similar to those preliminary reported.[9] The commercial PPL used in this work (purchased from Sigma) was however of a different lot (No. 41-H0954) than the one used in ref. 9. The supported PPL used in ref. 9 was about 1.42 fold more active. The relative ratios between supported and unsupported enzyme were however very close to those here reported.
16. We prepared several batches of supported PPL under these optimized conditions, with no appreciable differences in reactivity. Furthermore, for each batch, several reactions have been carried out and the reactivity (and the e.e.) were quite reproducible, also after storing the supported enzyme for several weeks in the refrigerator.
17. Supportation on celite in the presence of sucrose was recently found to increase the activity of PS lipase.[5o] In our case, as anticipated,[5o] addition of sucrose to the mixture of celite and PPL did not bring about any special effect.
18. One may think that the effect of concentration is related to the presence of small amounts of water in the solvent. However, this hypothesis seems in contrast with the data of table 4. On the effect of water amount on PPL reactions see refs. 11f, 5d, 5h,2f.
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20. This was demonstrated by a kinetic experiments, which showed that, up to 32-33% conversion, the reaction followed a pseudo-zero order kinetic.
21. In the case of **1** no particular advantage was noted by carrying out the reaction at lower temperatures.
22. The $[\alpha]_D$ reported in ref. 8c for the (*S*) enantiomer were somewhat lower compared to those reported in Table 6 for similar e.e.s. This fact is probably due to the lower purity of diacetate used in ref. 8c, compared to the recrystallized diol employed in the present work.
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