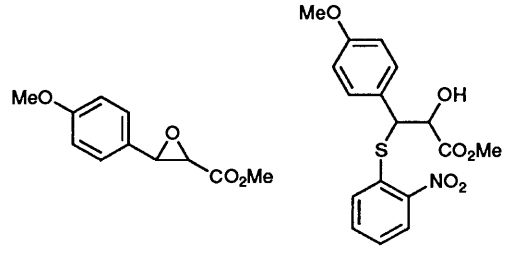
Enzymatic Acylation in the Resolution of Methyl *threo*-2-Hydroxy-3-(4-methoxyphenyl)-3-(2-X-phenylthio)propionates in Organic Solvents

Liisa T. Kanerva * **and Oskari Sundholm**
Department of Chemistry, University of Turku, SF-20500 Turku, Finland

A practical lipase PS (*Pseudomonas cepacia*)-catalysed resolution of methyl *threo*-2-hydroxy-3-(4-methoxyphenyl)-3-(2-X-phenylthio)propionates **3**, the (2*S*,3*S*)-enantiomers of which are important intermediates in the diltiazem synthesis, has been performed using acetone oxime, and isopropenyl or vinyl acetates as acylating reagents in organic solvents. The substituent X in compounds **3** does not have a clear effect on the enzymatic enantioselectivity, but the time needed for the resolution depends on X. The immobilization of the enzyme on Celite or Chromosorb in the presence of sucrose greatly enhances enzymatic activity. The immobilized enzyme is stable for reuse.

In the chemoenzymatic approach to optically active compounds, the resolution of an appropriate intermediate on the synthetic pathway by hydrolytic enzymes has been most practicable.[1–6] In these reactions, the potential of enzymes as catalysts in organic synthesis is often exploited through lipase-catalysed ester hydrolysis in aqueous solutions or trans-esterification in organic solvents.[7] Diltiazem with the (2*S*,3*S*) absolute configuration is a potent calcium channel blocker.[8] In the chemoenzymatic synthesis of diltiazem, the lipase-catalysed resolution of methyl *trans*-3-(4-methoxyphenyl)glycidate **1**[1–3] or methyl *threo*-2-hydroxy-3-(4-methoxyphenyl)-3-(2-nitrophenylthio)propionate **2**[5] have been by far the best methods



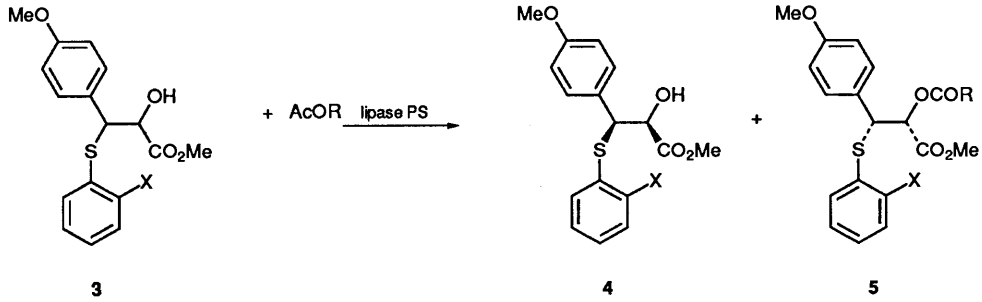
1 **2**

developed. Thus, the lipase PS (*Pseudomonas cepacia*)-catalysed acylation of compound **2** in tetrahydrofuran (THF) was previously shown to be practically enantiospecific.[5] It is well known that enzymatic activity in organic solvents may greatly depend on the nature of the solvent, hydrophobic ones being the best media.[9] The poor solubility of compound **2** in organic solvents other than THF led us to study the possibility of changing the medium by structural modifications of the racemic compound, and consequently, making the method more economical by decreasing the enzyme concentration and reaction times.

In this work, a gram-scale resolution of methyl *threo*-2-hydroxy-3-(4-methoxyphenyl)-3-(2-aminophenylthio)propionate-**3a** and of its analogues **3b–f** has been performed using the lipase PS-mediated acylation of the secondary hydroxy group of hydroxy esters (Scheme 1) in organic solvents. Vinyl, ispropenyl and acetone oxime acetates were used as acylating reagents, and a free as well as an immobilized enzyme was used as a catalyst.

Results and Discussion

Optically pure (2*S*,3*S*)-2-hydroxy-3-(4-methoxyphenyl)-3-(2-nitrophenylthio)propionate and the corresponding (2*R*,3*R*)-acetate were previously prepared by the lipase PS-catalysed acylation of the racemic *threo*-isomer **2** in THF.[5] In the next step of the synthesis of diltiazem, the (2*S*,3*S*)-enantiomer of the hydroxy ester **2** is usually reduced to the (2*S*,3*S*)-isomer **4a**. On the other hand, the reduction of the NO_2 group in racemic **2** or an opening of the oxirane ring in *trans*-glycidate **1** with 2-aminothiophenol with retention of stereochemistry results in the racemic *threo*-compound **3a**.[10] The protection of the NH_2 group by normal procedures leads to the formation of substrates **3b–f**.[11,12] Compounds **3a–e** are more soluble than compound **2** in diethyl ether and toluene, which are more favourable media for lipase activity than THF. Also, vinyl acetate is an appropriate medium for the present enzymatic resolutions (Table 1).



3 **4** **5**

a X = NH_2
b X = NHCOMe
c X = $NHCOCF_3$
d X = $NHCO_2Me$
e X = $NHCO_2Bu^t$
f X = NHCHO

Scheme 1

Table 1 Formation of **4** by the lipase PS-catalysed acylation of **3**

Substrate	R in AcOR	Solvent	T/°C	Enzyme (mg cm^{-3})	t/days	Conversion (%)	E.e.[c] (%) (2*S*,3*S*)-**4**
3a	$CH{=}CH_2$	Et_2O	22	25	3	51	
3a[a]	$CH{=}CH_2$	Et_2O	22	5	3	51	
3a	$N{=}CMe_2$	Et_2O	22	25	2	46	
3a[b]	$N{=}CMe_2$	Et_2O	22	5	2	52	⩾95[d]
3a[a]	$CMe{=}CH_2$	Et_2O	22	5	2	50	
3b	$CH{=}CH_2$	Et_2O	22	5	2	50	⩾95
3b[a]	$CH{=}CH_2$	Et_2O	44	2	1	46	
3b[b]	$CH{=}CH_2$	Et_2O	44	2	1	52	⩾95
3b	$CH{=}CH_2$	$AcOCH{=}CH_2$	44	50	2	48	
3b	$CH{=}CH_2$	Toluene	44	50	3	49	⩾95
3c	$CH{=}CH_2$	Et_2O	44	12.5	4	48	⩾95
3c[b]	$CH{=}CH_2$	Et_2O	44	5	3	53	⩾95
3d	$CH{=}CH_2$	THF	44	100	2	47	99
3d	$CH{=}CH_2$	Et_2O	22	5	2	50	⩾95
3d[a]	$CH{=}CH_2$	Et_2O	22	5	1	50	⩾95
3d	$CH{=}CH_2$	Et_2O	44	5	1	50	⩾95
3d[b]	$CH{=}CH_2$	Et_2O	44	2	1	50	⩾95
3d	$CH{=}CH_2$	$AcOCH{=}CH_2$	44	12.5	2	49	95
3d	$CH{=}CH_2$	$AcOCH{=}CH_2$–Et_2O (1:1)	44	12.5	2	51	98
3d	$CH{=}CH_2$	Toluene	44	50	1	52	⩾95
3e	$N{=}CMe_2$	Et_2O	22	12.5	7		
3f	$CH{=}CH_2$	$AcOCH{=}CH_2$	44	25	2	50[e]	

[a] Lipase PS immobilized in the presence of sucrose on Chromosorb. [b] Lipase PS immobilized in the presence of sucrose on Celite. [c] E.e. ⩾ 95% means that only the (2*S*,3*S*)-enantiomer can be detected by chiral HPLC. [d] Based on optical rotation. [e] At 64% conversion within 8 days.

Table 2 Gram-scale preparation of **4** and **5** by the lipase PS (5 mg cm^{-3})-catalysed acylation of **3** with vinyl acetate in Et_2O at room temperature

			(2*S*,3*S*)-**4**		(2*S*,3*S*)-**3a**		(2*R*,3*R*)-**5**		[2*R*,3*R*)-**3a**]	
Substrate	t/days	Conversion (%)	Yield (g)[a]	E.e. (%)[b]	$[\alpha]_D^{25}$	$[\alpha]_D^{20}$	Yield (g)[a]	E.e. (%)[c]	$[\alpha]_D^{25}$	$[\alpha]_D^{20}$
3a[d]	2	52	0.6 [93%]	⩾95	+159[g]	+294[e]	0.7 [88%]	⩾95[k]	−258[e]	−302[e]
3b[f]	2	50	3.0 [99%]	⩾95	+146[g]	+296[e]	3.4 [100%]	⩾95[k]	−175[g]	−301[e]
3c[h]	3	53	0.7 [81%]	⩾95	+207[g]				−147[g]	
3d	2	47	2.0 [93%]	⩾95	+212[g]		2.1 [100%]	⩾95	−196[g]	
3d[f,h]	1	50	1.9 [82%]	⩾95	+66[j]		2.5 [93%]	⩾95	−196[g]	
2[i]	2	48	0.6 [100%]	⩾95			0.6 [100%]	⩾95	−23[l]	

[a] [ ] the theoretical yield. [b] According to chiral HPLC. [c] According to ^1H NMR in the presence of Eu(hfc)$_3$. [d] Lipase PS immobilized in the presence of sucrose on Celite; AcO–N=CMe_2 as an acylating reagent. [e] 10^{-1} deg cm^2 g^{-1} (*c* 0.5, MeOH); see Experimental section. [f] 44 °C. [g] 10^{-1} deg cm^2 g^{-1} (*c* 1, CH_2Cl_2). [h] Lipase PS immobilized in the presence of sucrose on Celite. [i] In THF with 100 mg cm^{-3} of lipase PS and with Ac_2O as an acylating agent (ref. 5). [j] 10^{-1} deg cm^2 g^{-1} (*c* 3.6, CH_2Cl_2). [k] According to $[\alpha]_D^{20}$ for (2*R*,3*R*)-**3a**. [l] 10^{-1} deg cm^2 g^{-1} (*c* 4.3, CH_2Cl_2).

It is obvious that the high enantioselectivity of lipase PS catalysis observed for the resolution of the hydroxy ester **2** in THF[5] remains unchanged when the hydroxy esters **3** are resolved in organic solvents (Tables 1 and 2). Accordingly, the acylation of compounds **3** (the acylation of compounds **3e** and **f** excluded) practically stops at 50% conversion and at this point the resolution products **4** and **5** are usually optically pure [enantiomeric excess (e.e.) ⩾ 95%]. Compounds **4** and **5** can be almost quantitatively separated by flash chromatography (Table 2). The enantiomers **4b** and **d** can also be fractionally crystallized from the concentrated reaction mixture after the enzyme is filtered off. The enantiomer **4a** and its derivatives **4b–f** with the (2*S*,3*S*) absolute configurations are intermediates for the synthesis of diltiazem. The reaction time needed for the resolution depends on the amount of enzyme, the amounts shown in Table 1 being optimized (except in the case of compound **3d** in THF) so that the reactions reach 50% conversion within a couple of days.

Lipase PS is clearly more effective for catalysing the acylation of compounds **3** (compound **3e** excluded; Table 1) than for the corresponding 2-nitrophenylthio analogue **2**.[5] Part of this is caused by the use of diethyl ether or toluene rather than THF as a solvent, but solvent effects on lipase PS activity are usually less pronounced than now observed.[12] In support of this, to reach *ca.* 50% conversion for the acylations of compounds **2**[5] and **3d** (Table 1) with vinyl acetate in the presence of 100 mg cm^{-3} of lipase PS in THF takes 7 and 2 days, respectively. Also, the X group in hydroxy esters **3** is of importance in the present acylations of Table 1. Thus, for the acylation of compounds **3** the reactivity increases in the order **e** < **c** < **a** < **b** < **d**. The acylation of compound **3f** in vinyl acetate is close to the reactivity of compound **3d**. The simplicity of the diltiazem synthesis in mind, the resolution of compound **3a** is to be recommended because then the protection and the subsequent deprotection of the NH_2 group prior and after the resolution, respectively, is avoided. On the other hand, the reaction times become shorter and the product distribution is more unequivocal when compound **3b** or **3d** is resolved. As a drawback, the deprotection of compound **4d** is difficult and, *e.g.*, its basic hydrolysis to intermediate **4a** results in product racemization.

It is possible to control the reactivity in the lipase PS-catalysed acylation of compounds **3** in organic solvents by a suitable choice of acylating reagent, acetone oxime, isopropenyl

Table 3 Enzymatic activity of lipase preparations for the transesterification of hexanol with propyl acetate (lipase PS and PPL) and of 1-phenylethanol with 2,2,2-trifluoroethyl butyrate in hexane (CCL)

Enzyme	v_0/μmol g^{-1} min^{-1}		
	Commercial	On Celite	On Celite in the presence of sucrose
Lipase PS	0.74	2.3	7.2
CCL	0.34	0.47	0.63
PPL	0.37	0.30	0.32

and vinyl acetates being almost equal in that respect (Table 1). Previously, acid anhydrides were successfully used in the lipase PS-catalysed resolution of the hydroxy ester **2** as well as in the acylations of different α- and β-hydroxy carboxylic acid esters and of some other secondary alcohols.[5,12–14] However, in the acylation of the hydroxy ester **3a** with acid anhydrides extensive nonenzymatic amide formation seriously disturbs enzymatic acylation. Traces (4% or less according to HPLC) of amide formation can still be observed when compound **3a** is acylated with acetone oxime acetate, but not in the case of the other acylating reagents. In accordance with our earlier results, activated esters such as 2,2,2-trifluoroethyl acetate are not effective enough to acylate α-hydroxy carboxylic acid esters.[13]

The preservation of enzymatic activity and enantioselectivity under the reaction conditions as well as the possibility of reusing the biocatalyst are important facilities for industrial processes. These demands are well fulfilled in the present resolutions in organic solvents except for a minor loss of lipase PS activity in every reuse when the commercial enzyme is used as received. The immobilization of lipase PS into Chromosorb or Celite was then tested for better stability of enzymatic activity, and as a result, no loss of catalytic activity was observed in several reuses. In addition to the better stability, immobilization in the presence of sucrose results in an unexpected ten-fold increase in lipase PS activity (Tables 1 and 3). As a consequence, 50% conversion for the acylation of hydroxy ester **3b** with vinyl acetate in diethyl ether at 44 °C is obtained with 2 mg cm^{-3} of the immobilized enzyme within one day while the reaction time is two days with 5 mg cm^{-3} of the untreated lipase PS. An increase in lipase PS activity was previously observed also when the enzyme was immobilized on Hyflo Super gel in the presence of sucrose.[15] When the same immobilization technique on Celite was used with other lipases (CCL or PPL) the enzymatic activity was practically unchanged (Table 3).

In conclusion, the present method provides a practical enzymatic resolution of hydroxy esters **3** in view of the simplicity of the procedure, the high yield (almost 100% of the theory) and optical purity (e.e. ⩾ 95%) of the products (2*S*,3*S*)-**4** and (2*R*,3*R*)-**5** as well as of the convenient recycling of the enzyme especially when lipase PS immobilized on Chromosorb or Celite in the presence of sucrose is used. As a competitive method, the chemical resolution of hydroxy esters **2** or **3a** for the preparation of the optically pure synthon (2*S*,3*S*)-**4a** requires the availability of optically active basic amino acid or tartaric acid and the subsequent recycling of the optically active acid used.[16,17]

Experimental

Materials.—All the solvents were of the highest analytical grade and were dried over molecular sieves (3 Å) before use. Lipase PS from *Pseudomonas cepacia* was purchased from Amano Pharmaceuticals. The lipases from porcine pancreas (PPL, type II) and *Candida cylindracea* (CCL, type VII) were the products of Sigma. Compound **3a** was a generous gift from Orion Corporation/Fermion. The reaction between **3a** and methyl chloroformate, acid anhydrides and ethyl formate produced the compounds **3b–f**.[11,12] Acetone oxime acetate was prepared from acetone oxime and acetyl chloride in the presence of triethylamine in dichloromethane. The other acylating reagents were the products of Aldrich.

Assays.—The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analysing them by HPLC (a C-18 column) using MeOH–water (70:30) as eluent. HPLC (a 25 cm Chiralcel OG column) with hexane–Pr^{i}OH (70:30) as eluent was used to obtain the values of e.e. The absolute configurations of the products were obtained by determining the specific rotations of the resolution products and by comparing the values obtained with literature values.[18] $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}.

Immobilization of Lipase PS.—Lipase PS (0.4 g) was dissolved in Tris-HCl buffer (20 mmol dm^{-3}; 15 cm^3, pH 8.0) at 0 °C and the solutions were added to 4.0 g of Celite (Sigma) or Chromosorb 101 (Aldrich), respectively. For immobilization in the presence of sucrose, sucrose (0.24 g) was dissolved in the enzymatic solution before addition to the solid support. The resulting suspensions were stirred for 10–15 min and thereafter they were left to dry at room temperature.

Enzymatic Resolution of the Hydroxy Esters **3**.—For the gram-scale resolution of compounds **3**, the substrates (usually 0.05 and 0.2 mol dm^{-3} with respect to compounds **3** and an acylating agent, respectively) in an organic solvent were added to a known amount of the enzyme. The reactions were followed by HPLC and stopped by filtration of the enzyme.

As a typical example, diethyl ether (320 cm^3) which contained racemic α-hydroxy methyl carboxylate **3b** (6.0 g, 0.016 mol; 0.05 mol dm^{-3}) and vinyl acetate (5.5 g, 0.064 mol; 0.2 mol dm^{-3}) was added to lipase PS (5 g dm^{-3}; 1.6 g). After sonication the reaction mixture was shaken at 44 °C. Within 48 h the reaction stopped at 50% conversion. The enzyme was filtered off and washed with diethyl ether. The ethereal solution was then concentrated to 200 cm^3 and the product **4b** was left to precipitate at −4 °C overnight. The crude, optically active compound **4b** was recrystallized twice from diisopropyl ether, yielding 2.6 g (0.0070 mol, 87% of the theory) of white crystals $[\alpha]_D^{25}$ +159 (*c* 1, CH_2Cl_2); m.p. 128–129 °C. Using flash chromatography the yield of compound **4b** is almost 100% of the theory.

Flash chromatography on silica using toluene–AcOEt–CH_2Cl_2 (1:1:1) as eluent was used to separate 3.4 g (0.0080 mol; the theoretical yield) of the acetylated reaction product **5b** from the remaining ether solution as a yellowish oil $[\alpha]_D^{25}$ −175 (*c* 1, CH_2Cl_2).

Absolute Configurations of Compounds **4b** *and* **5b**.—Optically pure compound (+)-**4b** (0.50 g, 0.0013 mol) was refluxed for 18 h in methanol (30 cm^3) in the presence of H_2SO_4 (0.0052 mol). The white residue which was left over after neutralization with $NaHCO_3$ and evaporation of methanol was recrystallized from diethyl ether. This procedure yielded 0.33 g (0.0010 mol; 76% of the theory) of the corresponding enantiomer **4a** [(2*S*,3*S*)-**3a**; Table 2] $[\alpha]_D^{20}$ +296 (*c* 0.5, MeOH); m.p. 108–110 °C {lit.[18] (2*S*,3*S*)-**4a**, $[\alpha]_D^{22}$ +294 (*c* 0.5, MeOH), m.p. 110 °C}. Similarly, compound (−)-**5b** (1.1 g, 0.0026 mol) was refluxed in MeOH (50 cm^3), yielding 0.81 g (0.0024 mol; 94%) of the corresponding compound (2*R*,3*R*)-**3a** (Table 2) $[\alpha]_D^{20}$ −301 (*c* 0.5; MeOH); m.p. 108–109 °C. The values of the specific rotations for the enantiomers of compounds **3** observed compared to the known $[\alpha]_D$ values indicate the high optical purity of the resolution products.

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