Lipase Catalysis in the Resolution of Racemic Intermediates of Diltiazem Synthesis in Organic Solvents

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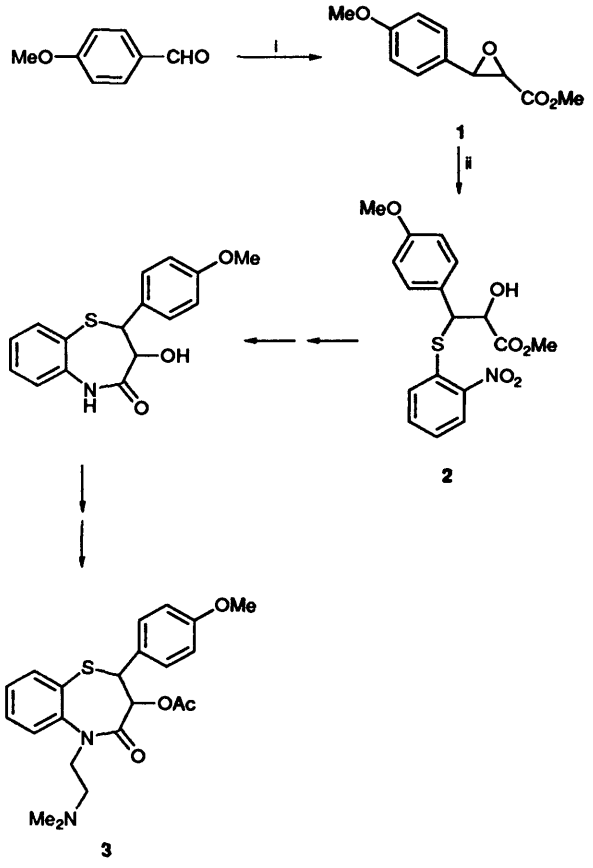
The key intermediates of the diltiazem synthesis, methyl *trans*-3-(4-methoxyphenyl)glycidate **1** and methyl *threo*-2-hydroxy-3-(4-methoxyphenyl)-3-(2-nitrophenylthio)propionate **2**, have been successfully resolved by using lipase catalysis in organic solvents. In the resolution of the glycidate **1**, enzymatic enantioselectivity greatly depends on the solvent, tertiary alcohols leading to the highest optical purities of the products: e.e. close to 90% at 60% conversion for the unchanged (2*R*,3*S*)-enantiomer and over 90% for the new (2*S*,3*R*)-ester, when the first 20% of the product is formed, can be obtained. Ester hydrolysis takes place simultaneously with ester alcoholysis. The enzymatic acylation of compound **2** with acid anhydrides or vinyl esters in THF tends to stop at 50% conversion, yielding the two enantiomers with an e.e. of the order of 100%. The enantiomers can be easily separated by flash chromatography.

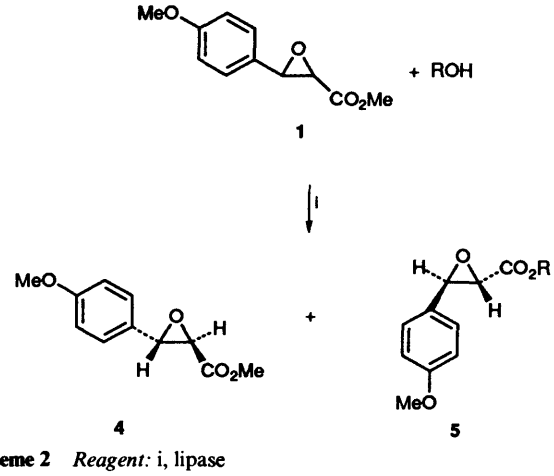
Diltiazem, (+)-*cis*-(2*S*,3*S*)-3-acetoxy-5-[2-(dimethylamino)-ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one **3** (Scheme 1), is one of the most potent calcium-



Scheme 1 *Reagents:* i, $ClCH_2CO_2Me$; ii, 2-nitrothiophenol

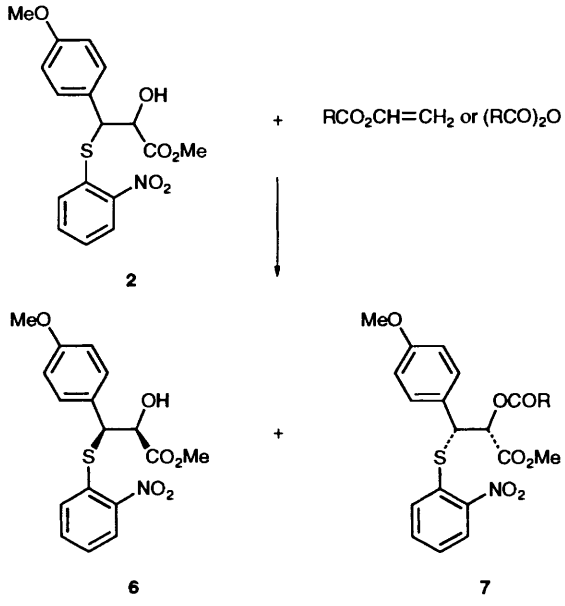
channel blockers.[1] The synthesis of the racemic *cis*-compound is usually based on the Tanabe synthesis,[2] which begins with the Darzens condensation of 4-methoxybenzaldehyde with methyl chloroacetate.[3] The subsequent stereoselective *cis*-opening[4] of the oxirane ring of methyl *trans*-3-(4-methoxyphenyl)glycidate **1** with 2-nitrothiophenol is followed by intramolecular ring closure to *cis*-lactam[5] after the reduction of the NO_2 group of methyl *threo*-2-hydroxy-3-(4-methoxyphenyl)-3-(2-nitrophenylthio)propionate **2** and ester hydrolysis. Finally, acetylation and *N*-alkylation complete the synthesis.[6] An approach to optically active compound **3** usually involves either an asymmetric synthesis or a chemical or biocatalytic resolution of one of the key intermediates **1** or **2**. The asymmetric Darzens condensation,[6,7] the Sharpless asymmetric epoxidation[8] and a stereospecific synthesis through vicinal diols[9] have been successfully used to obtain optically active glycidate **1** or its derivative. The lipase-catalysed hydrolysis of ester **1**[10-12] and the resolution of compound **2** as its diastereoisomeric esters[5,13-15] are among the most appropriate resolution approaches previously described. On the other hand, the lipase-catalysed alcoholysis of compound **1** or hydrolysis of acylated hydroxy ester **2** seem to be less advantageous procedures.[16,17] The (*R*)-oxynitrilase-catalysed asymmetric concensation of 4-methoxybenzaldehyde with HCN to the optically active cyanohydrin has served as an alternative chemoenzymatic way to diltiazem **3**.[18]

In this work, the lipase-catalysed alcoholysis of glycidate **1** (Scheme 2) and acylation of hydroxy ester **2** (Scheme 3) in



Scheme 2 *Reagent:* i, lipase

organic solvents are described. Lipases suspended in organic media have been extensively used to catelyse stereoselective transesterifications.[19,20] The advantage of biocatalytic ways in the place of chemical methods is to perform simple reactions under mild conditions and often with a reduced number of steps. Moreover, substrates and products which are insoluble or



Scheme 3 *Reagent:* i, lipase

Table 1 Formation of (2*R*,3*S*)-substrate 4 by the CCL-catalysed octan-1-olysis of glycidate **1** at 22 °C

Solvent	Time (days)	Yield[a] (%)	e.e.[b] (%)
tert-Pentyl alcohol	11	37	88
3-Methylpentan-3-ol	5	40	85
3-Ethylpentan-3-ol	7	35	82
5-Methylheptan-3-one	14	37	58
Diethyl ether	8	40	45
Diisopropyl ether	1	50	39
Dibutyl ether[c]	2	40	66
Toluene	4	48	51
Hexane–toluene (5:1)	1	41	47
Hexan-1-ol[d]	14	40	67
Acetonitrile	14	79	15

[a] Yield in the reaction mixture according to HPLC (or GLC). [b] According to chiral HPLC. [c] Dried with sodium. [d] A solvent and a nucleophile.

unstable in water can be solubilized and are often stable in organic solvents. An important advantage of working in non-aqueous media is also the possibility of using highly reactive acylating agents, such as acid anhydrides, vinyl esters or activated esters.[20-24]

Results and Discussion

The two intermediates **1** and **2** of the diltiazem synthesis are methyl esters; compound **2** is a secondary alcohol as well. For the enzymatic resolution of racemic alkyl esters through transesterification, a low equilibrium conversion may result in racemization and low optical yields of the products. Different methods, such as the use of an achiral nucleophile in excess or the removal of one of the reaction products as it is formed, can be used to shift the reaction equilibrium in favour of the products. In the resolution of racemic alcohols, a suitable choice of an acylating agent usually results in high equilibrium conversion.

Lipase Catalysis in the Alcoholysis of Compound **1**.—The lipase-catalysed resolution of compound **1** can be performed through enantioselective hydrolysis (R=H) or alcoholysis according to Scheme 2. Several methods for enzymatic hydrolysis, especially in various biphasic systems of water and an organic solvent, have been described in the patent literature.[10-12] Owing to the lability of the resulting *trans*-3-(4-methoxyphenyl)glycidic acid, only the unreactive enantiomer of compound **1** can be prepared by this method. That is also why the resolution based on the lipase-catalysed esterification between the acid and an alcohol is impracticable. To obtain the two enantiomers of compound **1**, the lipase-catalysed alcoholysis of glycidate **1** in anhydrous organic solvents was studied in this work (Scheme 2).

The lipases from *Mucor miehei* (MML), *Pseudomonas cepacia* (lipase PS) and *Candida cylindracea* (CCL or AY 30) effectively catalyse the octanolysis of compound **1**, the conversions of the reactions in *tert*-pentyl alcohol being 76, 72 and 50 or 60% within 2 days, respectively. In the presence of porcine pancreatic or *Rhizopus javanicus* lipases, the corresponding conversions were only 33 and 14%, respectively. As an important observation and independent of the origin of the lipase, the sum of the yields of glycidates **4** and **5** at any time was less than the original concentration of substrate **1**. This was detected by using the GLC and HPLC methods. The loss of the yield was highest (within 2 days the yields of compounds **4** and **5** were 24 and 10%, respectively) in the case of MML, the water content of which is high (~6.7%) compared with the water contents of lipase PS and CCL (1.8 and 3.8%, respectively). Taking into account the slightly enhanced enantioselectivity of CCL over AY 30, and the fact that the enantioselectivity of lipase PS in the present octanolysis is negligible, CCL was used as a biocatalyst to obtain the results shown in Tables 1 and 2.

From the results of Table 1 it is clear that CCL enantioselectively catalyses the octanolysis of compound **1** in different organic solvents, the (2*S*,3*R*)-enantiomer of the racemate reacting preferentially to give the product **5**. This enantioselectivity strongly depends on the solvent, the reactions in tertiary alcohols giving the highest optical purities (e.e. between 80 and 90%) for the less reactive (2*R*,3*S*)-isomer **4** at approximately 60% conversion. The highest optical purity (e.e. > 90%) and yield for the (2*S*,3*R*)-enantiomer **5** is obtained when the enzymatic reaction in the same alcohols is stopped at a much earlier stage of the reaction (Table 2; Fig. 1), the maximal yield of compound **5** being of the order of 20%. The CCL-catalysed butanolysis of compound **1** in the butan-1-ol–n-hexane (1:5) system was published when we were finishing the experiments of this work.[16] In that reaction, a butyl ester with the (2*S*,3*R*) absolute configuration and with 60% e.e. at 50% conversion was obtained. In accord with our results in hexane–toluene (5:1) (Table 1), the optical purity of the less reactive enantiomer **4** in this previous work was low (e.e. 44%).[16b]

From the results of Table 2 it can be seen that not only the enantiomeric purity, but also the yield of (2*S*,3*R*) compound **5** strongly depends on the solvent, the yields in the most hydrophobic solvents (toluene and hexane–toluene) being highest. We propose that the water absorbed in the lipase powder causes enzymatic hydrolysis which takes place parallel with enzymatic alcoholysis. In accordance with proposed enzymatic hydrolysis, the formation of 2-(4-methoxyphenyl)-ethanal, which can be formed through decarboxylation of the epoxy acid,[3,25] was detected in the CCL-catalysed octanolysis of compound **1** in *tert*-pentyl alcohol by the GLC method. Furthermore, compound **1** was incubated in *tert*-pentyl alcohol in the presence of CCL (0.10 g cm^{-3}), and the samples taken at intervals were analysed by HPLC. The results for the disappearance of substrate **1** with time in the presence of (▲) and without (■) octan-1-ol are shown in Fig. 1. Thus, in both cases the concentration of substrate **1** can be seen to diminish enantioselectively. The incubation of the corresponding racemic octyl ester in the absence of an added nucleophile led to an almost identical result with that obtained in the case of compound **1**.

Table 2 Formation of (2*S*,3*R*) octyl ester **5** by the CCL-catalysed octan-1-olysis of glycidate **1** at 22 °C

Solvent	Water content of the solvent[a] (%) (w/w)	Water content after incubation[b] (%) (w/w)	Time (days)	Yield[c] (%)	e.e[c] (%)
tert-Pentyl alcohol	0.035	0.184	1	22	97
3-Methylpentan-3-ol			1	20	96
3-Ethylpentan-3-ol			1	23	90
5-Methylheptan-3-one		0.045	1	13	72
Diethyl ether[d]	0.003	0.068	5	33	22
Diisopropyl ether	0.003	0.061	1	25	50
Dibutyl ether	0.010	0.035	2	32	37
Toluene	0.004	0.010	4	31	44
Hexane–toluene (5:1)	0.003	0.004	4	50	5
Hexan-1-ol[e]			7	24	72
Acetonitrile			14	10	

[a] Dried over molecular sieves (3 Å). [b] Incubated for 2 days in the presence of CCL (0.10 g cm^{-3}). [c] According to HPLC of the reaction mixture. [d] Dried with sodium. [e] A solvent and a nucleophile.

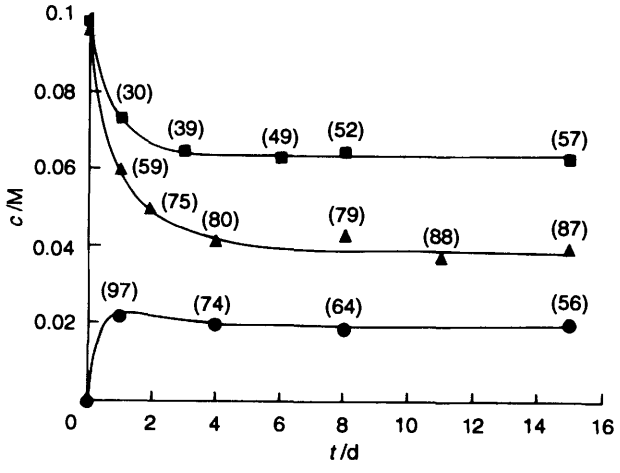


Fig. 1 The disappearance of glycidate **1** upon CCL catalysis in *tert*-pentyl alcohol in the presence (▲) and in the absence (■) of octan-1-ol and the formation of octyl ester **5** (●) with time. The values of e.e. for the prevailing enantiomer with time are shown in parentheses.

The ability of organic solvents to strip water from the enzyme is well recognized.[26] To study this ability in the present case the enzymes were incubated in some of the solvents and the water contents were determined by normal Karl Fischer's water titration. The results in Table 2 show a rough correlation between the yield of compound **5** and the amount of the water stripped.

As shown by the data in Fig. 1 and in accord with the proposed simultaneous CCL-catalysed hydrolysis and alcoholysis of glycidates **1** (▲), the optical purity of the product **4** in *tert*-pentyl alcohol first increases and then is almost unchanged with time because the two reactions preferentially consume the same (2*S*,3*R*)-enantiomer of substrate **1**. The optical purity of compound **5**, on the other hand, decreases with time due to the enantioselectivity of CCL catalysis. Enantioselectivity in the CCL-catalysed hydrolysis of glycidate **1** in originally anhydrous organic solvents (Fig. 1, ■) is only moderate. Evidently this, rather than enzymatic methanolysis of compound **5**, limits the optical purity of the products: the reverse reaction of Scheme 2 should decrease the optical purities of both products **4** and **5** with time. Owing to the competition between water and alcohol molecules acting as the nucleophile, the proportion of hydrolysis in the total reaction (Fig. 1, ▲) must be less, however, than can be expected from the results obtained in the absence of octan-1-ol (■).[27] In accord with the high enantioselectivity observed in the lipase-catalysed hydrolysis of compound **1** in different aqueous systems,[10–12] the optical purity of (2*R*,3*S*)-isomer **4** increases when water is added to an organic solvent, but at the same time the yield and optical purity of (2*S*,3*R*) compound **5** sharply decrease.

To suppress enzymatic hydrolysis and to shift the equilibrium of octanolysis of compound **1** (Scheme 2) in *tert*-pentyl alcohol totally to the formation of products, molecular sieves (4 Å) were added to the reaction mixture. The purpose of this procedure was to remove the water stripped by the solvent from the enzyme and the methanol formed when the reaction proceeds. However, the molecular sieves did not affect the cause of the enzymatic reaction. When the enzyme dried by lyophilization was used to suppress hydrolysis, the yield of compound **5**, somewhat increased, but at the same time enzymatic activity decreased. On the other hand, when the CCL-catalysed hexanolysis of compound **1** was performed in hexan-1-ol as a solvent, low enantioselectivity (e.e. close to 70% for compounds **4** and **5**, Tables 1 and 2) was observed.

The reactivity of different primary alcohols from propan-1-ol to decan-1-ol were studied in the CCL-catalysed resolution of compound **1** (Scheme 1) in *tert*-pentyl alcohol. Independently of the nucleophile the reactions proceeded approximately at the same rates and enantioselectivity (e.e. > 80% with respect to **4** at *ca.* 60% conversion). With enzymatic enantioselectivity in mind, a concentration of octan-1-ol between 0.2 and 0.4 mol dm^{-3} is ideal, but the time needed for the resolution is considerably shorter with higher alcohol concentrations (the reaction times at 60% conversion are 11 and 3 days for 0.2 and 0.4 mol dm^{-3} octan-1-ol, respectively). It is not possible to affect the resolution result by using (*R*)- or (*S*)-butane-1,3-diol as a nucleophile.

Lipase Catalysis in the Acylation of Hydroxy Ester **2**.—The poor solubility of compound **2** in water and in most of the common organic solvents seriously limits the number of biocatalysts which can be used in its resolution.[28,29] As previously shown, lipase PS is catalytically active in a wide variety of organic solvents.[23] It has been shown to be an effective enantioselective catalyst in the acylations of various α- and β-hydroxy carboxylic acid esters.[24] Moreover, it is not sensitive to the nature of acylating agents and it accepts sterically hindered secondary alcohols as its substrates.[23,24] In this work, the lipase PS-catalysed acylation of the secondary HO group in compound **2** was performed with acid anhydrides and vinyl acetate in tetrahydrofuran (THF) (Scheme 3).

It is clear from the results in Table 3 that the lipase PS-

Table 3 Formation of compounds **6** and **7** by the lipase PS-catalysed acylation of hydroxy ester **2** in THF at 22 °C

Acylating agent	Time (days)	Conversion (%)	Yield[a] (%) of substrate **6**	e.e.[b] (%) (2*S*,3*S*)	Yield[a] (%) of acyloxy ester **7**	e.e[b] (%) (2*R*,3*R*)
Ac_2O	2	50	100	⩾95	100	⩾95
$PrCO)_2O$	4	50	100	⩾95	100	⩾95
$AcOCH{=}CH_2$	7	48	100	⩾95	100	⩾95

[a] The yield of the product separated by flash chromatography. [b] According to chiral HPLC.

catalysed acylation of hydroxy ester **2** (Scheme 3) is practically enantiospecific in THF. When acid anhydrides or vinyl acetate are used as acylating reagents the reaction tends to stop at approximately 50% conversion and at that point the two enantiomers of substrate **2** are optically pure (e.e. ⩾95% means that only one enantiomer of the unchanged substrate, **6**, and of the acylated product, **7**, is detected by chiral HPLC). The reaction products **6** and **7** can be easily separated by flash chromatography on silica. According to the optical rotations, the absolute configuration of substrate **6** is (2*S*,3*S*) and consequently that of the acyloxy ester **7** must be (2*R*,3*R*).

The rate of lipase PS-catalysed acylation of hydroxy ester **2** in THF strongly depends on the acylating reagent, the rate in the case of acetic anhydride being highest (Table 3). In accord with our previous results for the lipase PS-catalysed acylations of different α- and β-hydroxy carboxylic acid derivatives, 2,2,2-trifluoroethyl butyrate is a poor substrate for the enzymatic acylation of compound **2**. No reaction takes place in the absence of the lipase.

Conclusions.—The key intermediates **1** and **2** in the synthesis of diltiazem can be conveniently resolved by using lipase catalysis in organic solvents. Enzymatic hydrolysis disturbs the lipase-catalysed alcoholysis of glucidate **1** and the enantioselectivity observed is highly dependent on the solvent, various tertiary alcohols leading to the highest optical purities. Thus, an e.e. of the order of 90% for *trans*-glycidate **4** at approximately 60% conversion and >90% for *cis*-glycidate **5**, when the first 20% of the product is formed, can be obtained. For the production of *trans* compound **4** as an intermediate for the optically active benzothiazepine **3**, this method cannot, however, compete with the lipase-catalysed hydrolysis of glycidate **1** in biphasic systems.[10–12] On the other hand, the lipase PS-catalysed acylation of hydroxy ester **2** in THF proceeds with extremely high enantioselectivity, the two enantiomers, **6** and **7**, being optically pure (e.e. ⩾95%) when the reaction tends to stop at approximately 50% conversion. Moreover, the two products can be easily separated by flash chromatography. Owing to these facts, together with the stability of hydroxy ester **2** compared with the lability of glycidate **1**, enzymatic acylation of compound **2** is among the best methods for the preparation of the optically pure synthons **6** and **7**, the first mentioned being the optically active intermediate for the synthesis of diltiazem **3**.

Experimental

Materials.—All the solvents used were of the highest analytical grade and were dried over molecular sieves (3 Å) before use. MML and *Rhizopus javanicus* lipase were purchased from Biocatalysts, lipases PS and AY 30 from Amano Pharmaceuticals, and CCL and porcine pancreatic lipase were from Sigma Chemicals. Acid anhydrides, vinyl acetate, and the alcohols used as nucleophiles were from Aldrich. Compound **1** and racemic octyl *trans*-3-(4-methoxyphenyl)glycidate were generous gifts from Orion Corporation/Fermion. Compound **2** was prepared by the known method from glycidate **1** and 2-nitrothiophenol.[4] Acylation of compound **2** was performed with the respective acid anhydride in the presence of 4-(dimethylamino) pyridine (DMAP) by the usual procedure.

Assays.—The water content of the enzymes was determined by measuring their loss of mass when kept at 110 °C for 4 h. The water content of the solvents was determined by the Karl Fischer method.

The progress of the reaction was followed by taking samples from the reaction mixture and analysing them by GLC and HPLC (25 m NB-30 capillary and a 25 cm Chiralcel OG column, respectively). The disappearance of the starting ester **1** and formation of the acylated hydroxy ester **2** were followed. The enantiomeric excesses (e.e.s) were determined by the HPLC method using hexane–Pr^{i}OH (95:5) for compounds **4** and **5** and (65:35) for compounds **6** and **7** as eluent.

Enzymatic Resolution of Glycidate **1**.—As a typical example, a solution of racemic compound **1** (0.10 mol dm^{-3}) in *tert*-pentyl alcohol was added to CCL (0.10 g cm^{-3}). After sonication (10 s) octan-1-ol (0.20 mol dm^{-3}) was added to start the reaction, and the reaction mixture was shaken at 22 °C. Samples taken at intervals were analysed as described above. The resolution products **4** and **5** were not usually separated; the yields and the values of e.e. in Tables 1 and 2 were obtained directly from the reaction mixture by using chiral HPLC.

To determine the absolute configurations of the resolved products, the octyl ester **5** (e.e. 87%) was separated from the unchanged substrate **1** by flash chromatography with Et_2O–hexane (2:8) as eluent. The value of $[\alpha]_D^{25}$, $+114 \times 10^{-1}$ deg cm^2 g^{-1} (*c* 3.6, MeOH), in accord with the (2*S*,3*R*)-configuration for compound **5**, was obtained. The same enantioselectivity of CCL catalysis was found in the butan-1-olysis of compound **1**.[16]

Enzymatic Resolution of Hydroxy Ester **2**.—The procedure used in the resolution was the same for different acylating agents. As a typical example, a solution of racemic compound **2** (1.1 g, 0.0030 mol) and Ac_2O (0.80 cm^3, 0.0085 mol) in THF (60 cm^3) was added to lipase PS (6 g). The reaction mixture was shaken at 20 °C until the reaction stopped at 48% conversion within 2 days. The enzyme was filtered off and the solvent was evaporated. The products were separated by flash chromatography with CH_2Cl_2–hexane–AcOEt (63:31:6) as eluent, to give recovered (2*S*,3*S*) substrate **6** {0.0015 mol; e.e. ⩾ 95% by HPLC; $[\alpha]_D^{22}$ + 66 × 10^{-1} deg cm^2 g^{-1} (*c* 3.6, CH_2Cl_2)} and (2*R*,3*R*) compound **7** {0.0015 mol; e.e. ⩾ 95% by HPLC; $[\alpha]_D^{22}$ − 23 × 10^{-1} deg cm^2 g^{-1} (*c* 4.3, CH_2Cl_2)}. Compounds **6** and **7** were optically pure according to ^{1}H NMR spectroscopy in the presence of Eu(hfc)$_3$* (in the case of compound **2**: CO_2Me, d, δ 3.84 and 3.94).

To determine the absolute configuration of the resolution products, compound **6** was hydrolysed in a 2:1 mixture of MeOH–NaOH (0.5 mol dm^{-3}). The product obtained was the

* Europium tris(heptafluorobutyrylcamphorate

corresponding (2*S*,3*S*)-acid {m.p. 113–114 °C (lit.),[13] 111 °C; $[\alpha]_D^{25}$ +112 × 10^{-1} deg cm^2 g^{-1} (*c* 1, $CHCl_3$, lit.,[13] $[\alpha]_D^{25}$ + 121 × 10^{-1} deg cm^2 g^{-1} (*c* 1, $CHCl_3$)}. Accordingly, compound **6** is the intermediate for diltiazem **3**.

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