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Resolution of Racemic Sterically Hindered Secondary Alcohols via Enzymatic Alcoholysis of Their Esters. The First Enzymatic Preparation of Optically Pure 2,2,2-Trifluoro-1-(9-Anthryl)Ethanols

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Abstract—An approach has been developed which exploits the non aqueous enzymatic alcoholysis reaction for resolution of racemic sterically hindered secondary alcohols. The method was used effectively in the first enzymatic preparation of both enantiomers of the title compound via porcine pancreatic lipase catalysed alcoholysis of its butyrate ester. A considerable enhancement of the reaction rate was achieved by dispersion of the powdered enzyme preparation on aluminium oxide. A facile procedure was developed for separating the (*R*)-alcohol product from the unreactive (*S*)-butyrate ester and for the hydrolysis of the latter into the (*S*)-alcohol. The preparative usefulness of the resolution procedure is demonstrated by the convenience of the scaled-up enzymatic experiment carried out on 370 g of substrate in an ordinary flat bottom flask.

Introduction

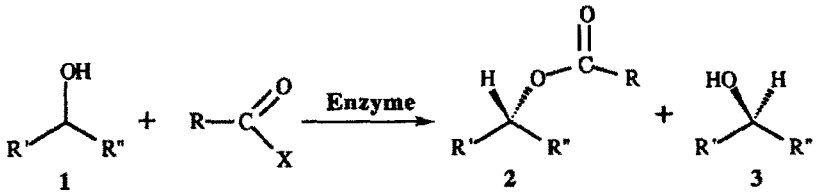
Enantiomerically pure secondary alcohols are very common as aroma and flavour enhancing compounds, as intermediates and as valuable chiral auxiliaries in organic synthesis.[1] Some complex aromatic compounds of this class also have important analytical applications as chiral shift reagents in NMR and as key ingredients of stationary phases for chiral chromatography columns.[2] It is therefore not surprising that over the last two decades much effort has been devoted to the enzymatic resolution of racemic alcohols. Enzymatic hydrolysis of racemic esters has been used extensively for this purpose.[3] Klibanov's first reports on lipase catalysed stereoselective esterifications and transesterifications in organic solvents,[4] prompted much work on the kinetic resolution of racemic secondary alcohols via enzymatic acylation in organic solvents.

The acylating agents used include carboxylic acids,[4] various activated and enol esters,[5] or acyclic aliphatic acid anhydrides.[6] The enzymatic reactions amounted to conversion of one enantiomer of the alcohol **1** into an ester **2** with the other one remaining as the unreactive alcohol **3** (Scheme I). Separation between the unreactive alcohol and ester, followed by hydrolysis of the latter, enabled the resolution of racemic mixtures into their enantiomers.

Very recently we extended the enzymatic acylation approach to develop a convenient, large-scale method for the preparation of optically pure alkyl–aryl secondary alcohols using succinic anhydride in organic solvents. The advantage of using such a cyclic anhydride is that the resultant (*R*) half ester (*R*)-**5** can be easily separated from the unreactive (*S*) alcohol (*S*)-**4** by a simple extraction with an aqueous solution of $NaHCO_3$. Base hydrolysis of the half ester (*R*)-**5** afforded the (*R*) enantiomer of the alcohol (*R*)-**4** (Scheme II).[7]

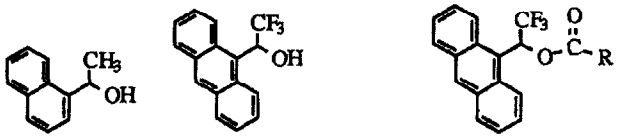
Results and Discussion

The enzymatic acylation approach turned out to be either ineffective or totally unsuitable in cases of secondary alcohols that are highly sterically hindered, such as 1-(1-naphthyl)ethanol (**6**) or 2,2,2-trifluoro-1-(9-anthryl)ethanol (**7**). The only report on the enzymatic acylation of **6** is that by Heathcock *et al.*,[8] that describes a tedious low-yield procedure, using the active ester 2,2,2-trichloroethyl butyrate as an acylating agent and involving 14 days of reflux under an argon atmosphere, with frequent additions of large quantities of fresh porcine pancreatic lipase. To our knowledge there have been no reports whatsoever on enzymatic acylation of **7**, and very considerable efforts of our own to achieve its enzymatic acylation under various reaction conditions, were disappointing. These unsuccessful attempts were carried out with different combinations of three active acylating agents (vinyl acetate, 2,2,2-



Scheme I.

trifluoroethyl butyrate and succinic anhydride), five commonly used enzyme preparations,[9] and in several organic solvents. In all cases the acylation was negligible, with the 'best' result of 10 % conversion achieved after **7** was shaken for 24 days (!) at 40 °C and 200 rpm with large quantities of PPL and 2,2,2-trifluoroethyl butyrate in *tert*-butyl methyl ether.



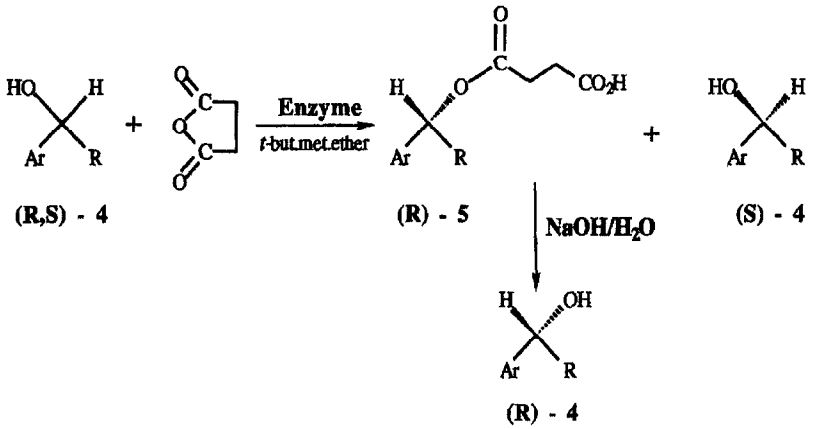
6 **7** **8**

a=CH_3-
b=$CH_3CH_2CH_2$-

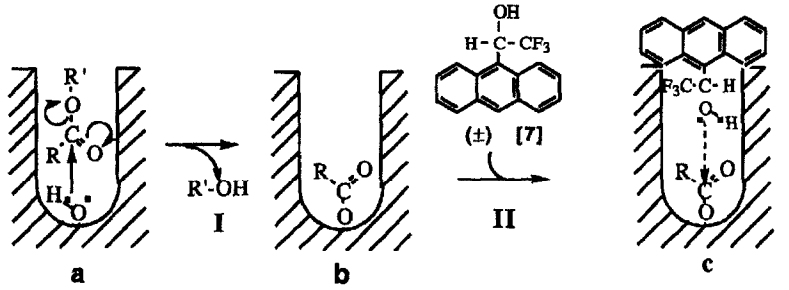
The inefficiency of enzymatic acylation of these alcohols can be explained by steric and electronic considerations effecting the mechanism of the enzymatic process. In the first step an acyl–enzyme intermediate is formed between the enzyme and the acylating agent (Step I, Scheme III). In the second (critical) step the carbonyl of this intermediate has to undergo a nucleophilic attack by the alcohol oxygen of **7** which is accompanied by the breakup of the acyl enzyme link, resulting in the release of the free enzyme and the formation of the desired ester. It is at this second stage, which is critical from the point of view of enantiodiscrimination, that the steric hindrance around the hydroxyl group plays a crucial role by blocking the approach of nucleophile to the carbonyl that is positioned deep inside the enzyme pocket (Scheme III). The electronic effect of the CF_3 group, which strongly reduces the nucleophilicity of the alcohol oxygen, is also responsible for the inefficiency of step II.

In the present work we attempted to develop an enzymatic procedure in organic solvents that would be suitable for resolution of sterically hindered hydrophobic alcohols. The anthryl alcohol **7** was chosen as the most challenging target molecule for this study because of its practical usefulness and its total unsuitability for the conventional methods of enzymatic resolution. The very high cost of the enantiomerically pure isomers of **7** (U.S. $ 28 for 100 mg from Aldrich Chemical Co.) emphasises the practical challenge of developing a scalable enzymatic process for this compound.

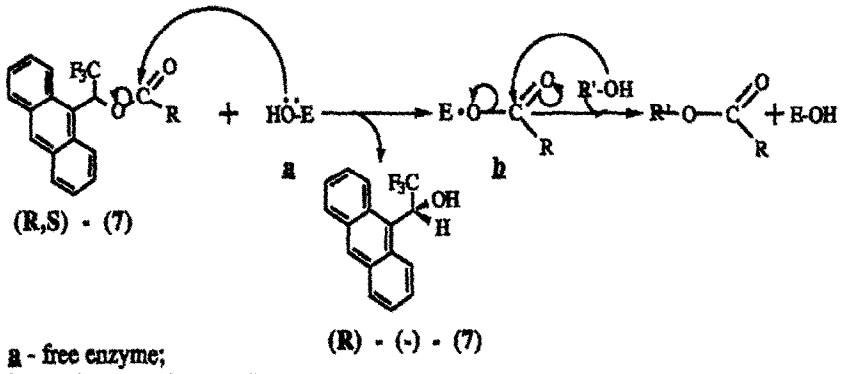
We predicted that it may be possible to reverse the unfavourable electronic effect, by changing the sequence of mechanistic events (Scheme IV). It was expected that enantiodiscrimination would be exercised during the first enzymatic step and that the reacting ester (and hence the released alcohol) would be one enantiomer, whereas the other one would be left behind as the unreactive ester. The second step along this route, would simply involve the breakup of the acyl–enzyme intermediate by an achiral nucleophile, and is of no stereochemical interest. According to this approach, the electron withdrawing effect of the CF_3 group should help, as it creates a better leaving group.



Scheme II.

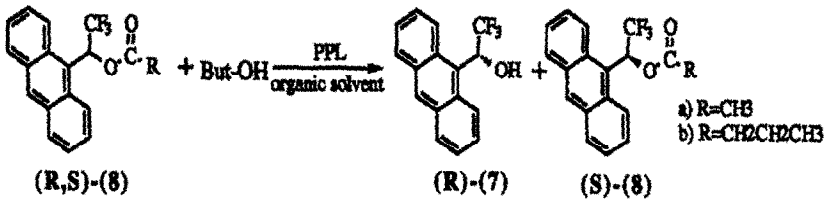


Scheme III. **a** - Formation of acyl-enzyme intermediate via nucleophilic attack of serine oxygen on the acylating agent; **b** - acyl-enzyme intermediate; **c** - sterically hindered interaction between acyl-enzyme and racemic alcohol.



a - free enzyme;
b - acyl-enzyme intermediate.

Scheme IV.



Scheme V.

This general mechanism is followed in reactions of enzymatic ester hydrolysis (R' = H). Since the anthryl ester **8** would be totally insoluble in water, it seemed advantageous to consider carrying out the reaction in an organic solvent, and use a simple aliphatic alcohol instead of water. We prepared the respective acetate and butyrate esters **8** by simple acylation procedures with acetyl chloride or butyryl chloride, and studied their reaction with ethanol and butanol in a variety of organic solvents, in the presence of several commercially available lipases.[9] These experiments revealed that porcine pancreatic lipase (PPL) catalysed the alcoholysis reaction in hexane, *tert*-butyl methyl ether, diisopropyl ether and toluene (Scheme V).

It turned out, that the butyrate ester **8b** reacted faster than the acetate ester **8a** and that the reaction rate was strongly dependent on the alcohol concentration. In the case of ethanol, there is a limit to the maximal allowable concentration, beyond which the hydrophilic alcohol inactivates the enzyme by stripping off its essential water layer.[10] This problem does not exist with the considerably less hydrophilic *n*-butanol. We have reported recently (for a different enzymatic reaction), that its concentration can be raised infinitely without any detrimental effect on the enzyme.[11] As expected, it was possible to increase the butanol concentration with impunity in the present case as well, the best results being obtained when butanol was used as the only solvent.

Having chosen the enzyme, the substrate and the solvent, we undertook to study in detail the enzymatic alcoholysis reaction of the butyrate ester **8b** with *n*-butanol illustrated in Scheme V. The crude commercial preparation of porcine pancreatic lipase (PPL) was highly stereospecific towards the (*R*)-enantiomer of **8b** which underwent reaction in preference to the (*S*)-enantiomer. Periodic examination of aliquots from the reaction mixture by HPLC on a chiral column Chiracel OJ, enabled us to detect both enantiomers of the substrate ester **8b** and product alcohol **7**, and thus (using appropriate calibration), to follow simultaneously the extent of the enzymatic reaction and the optical purity of starting material and product. It was revealed, that the extent of enantiodiscrimination of this reaction was extremely high and no detectable quantity of the 'wrong' enantiomer (*S*)-**7** was formed. Thus, unlike in most cases of kinetic resolution, there was no need to worry about stopping the experiment in time to prevent the other enantiomer from reacting.[12]

Although highly stereospecific with regard to the optical purity of the resultant (*R*)-**7**, the reaction was very slow and even at an enzyme-to-substrate ratio of 2:1, up to 15 days at 40 °C were necessary to achieve 40 % conversion. Furthermore, it was virtually impossible to push the reaction beyond the point of 40 % conversion, which made it very difficult to obtain the unreacted (*S*)-enantiomer of the ester **8b** with high optical purity. One of the factors contributing to the inefficiency of an enzyme suspended in organic solvent is the problem of intraparticle diffusional limitations.[13] Immobilisation of the enzyme on a solid support may often remove this problem, as well as make the enzyme more stable and thus improve its overall efficiency. In our previous work on subtilisin catalysed aminolysis we showed that the reaction rate could be increased nearly 20-fold by non-covalent immobilisation of the enzyme on glass beads.[14]

In the present work we further simplified the immobilisation procedure and achieved adequate dispersion of PPL by simply mixing the solid enzyme preparation of

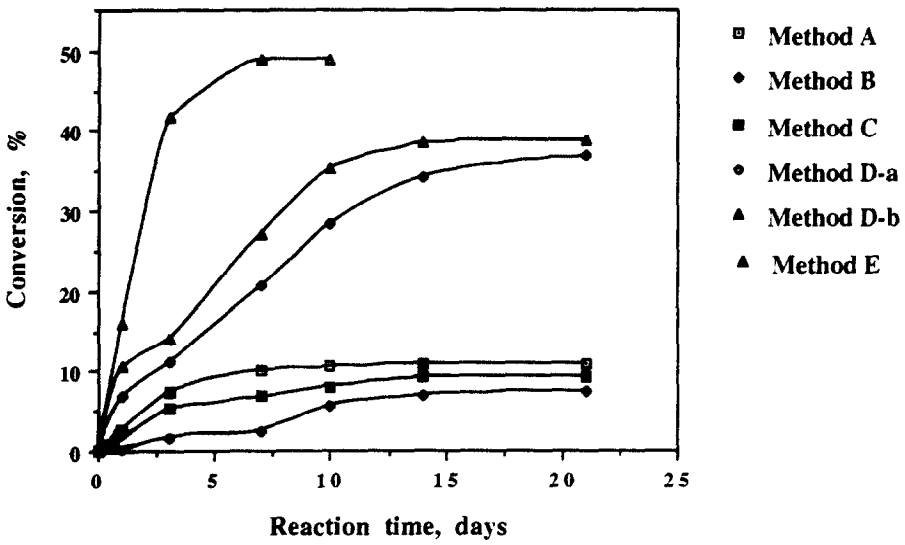


Figure 1.
Method A: 40 mg of PPL was added to a solution of 10 mg (0.036 mmol) of **7** and 0.1 mL 2,2,2-trifluoroethylbutyrate in 20 mL *tert*-butyl methyl ether (TBME). The mixture was shaken at 40 °C and 200 rpm and the reaction monitored by HPLC.
Method B: 50 mg of PPL was added to a solution of 20 mg (0.072 mmol) of **7** and 0.1 mL 2,2,2-trifluoroethylbutyrate in 20 mL hexane. The mixture was refluxed and the reaction monitored by HPLC.
Method C: 20 mg (0.072 mmol) of **7**, 0.86 g (8.6 mmol) of succinic anhydride and 40 mg of lipase in 8 mL TBME were shaken at 40 °C and 200 rpm and the reaction monitored by HPLC.
Method D-a: Carried out in the same way as in method D-b with **8a** as substrate.
Method D-b: 20 mg of PPL and 20 mg (0.055 mmol) of **8b** in 2 mL of *n*-butanol were shaken at 40 °C and 200 rpm and the reaction was monitored by HPLC. The enzyme was filtered off, washed with ether and the filtrate evaporated at reduced pressure. The mixture of optically pure alcohol **7** and unreacted ester **8b** was separated by preparative TLC eluting with hexane:ethyl acetate 5:1.
Method E: 0.6 g of PPL dispersed on aluminium oxide and 0.3 g (0.83 mmol) **8b** in 6 mL *n*-butanol were magnetically stirred at 40 °C. The reaction was treated in the same way as in method D-b to give after separation by preparative TLC 0.1 g (86 %) of optically pure (*R*)-(–)-**7**, and 0.148 g of the unreacted (*S*)-**8b**. Hydrolysis of the latter with aqueous KOH gave 0.09 g (79 %) of (*S*)-(+)-**7**.

crude commercial PPL with neutral aluminium oxide at the ratio of 1:1[15] In the resultant powder, the enzyme was dispersed between the small particles of aluminium oxide which considerably increased its active surface. The use of this powder instead of the native enzyme enabled us to achieve nearly 50 % conversion within a 4–10 day period at an enzyme to substrate ratio of 1:1.

As can be seen from the comparison of the reaction profiles under different enzymatic conditions (Figure 1), the PPL–aluminium oxide mixture (method E) provided the best results. Under these conditions the reaction proceeded in a smooth manner until it came to a halt at 50 % conversion, when all of the (*R*)-ester had undergone alcoholysis. This reaction profile indicates that: (a) the immobilised enzyme remained stable and active throughout the reaction and could probably be reused, and (b) that the stereospecificity was complete, as no progress was observed after the 50 % conversion stage.

In conclusion, the enzymatic alcoholysis approach described in this work enabled us to carry out a preparative resolution of the heretofore enzymatically unresolved 2,2,2-trifluoro-1-(9-anthryl)ethanol. It was shown that the stability of PPL and the rate of the enzymatic reaction could be enhanced considerably by simple dispersion of the enzyme on neutral aluminium oxide. The combination of an inexpensive and stable enzyme and of a relatively efficient reaction makes the economics of the process feasible for the practical preparation of this and other sterically hindered alcohols. Work in our laboratory is currently underway to determine the generality of this approach to other sterically hindered alcohols.

Experimental

General

Porcine pancreatic lipase (PPL) was purchased from Sigma Chemical Co. 9-Anthryl trifluoromethyl ketone was prepared by Friedel–Crafts acylation of anthracene with trifluoroacetic anhydride following the previously described procedure by Pirkle *et al.*[2] Aluminium oxide (Al_2O_3) used for enzyme dispersion was aluminium oxide 90 active neutral (E. Merck). Thin-layer chromatography (TLC) were performed on plates coated with 0.25 mm thick silica gel 60F-254 (E. Merck). Preparative-layer chromatography (PLC) were performed on plates coated with 0.5 mm thick layer of silica gel 60 PF 254. Column chromatography and "filtration through silica" were performed on silica gel kieselgel 40, 0.063–0.200 mm (E. Merck). Unless otherwise stated, all enzymes, solvents and other chemicals were obtained from commercial suppliers and were used

without further purification. Solvent extracts of aqueous solutions were dried over anhydrous Na_2SO_4. Solutions were concentrated under reduced pressure on a rotary evaporator.

^1H NMR spectra were recorded on a Bruker AM 200-MHz spectrometer in $CDCl_3$. All chemical shifts were reported in ppm with tetramethylsilane as internal standard. Distillations were performed on a glass tube oven Buchi GKR-50. The shaker used for enzymatic experiments was a G 24 environmental shaker incubator from New Bronswick Scientific Co. Chemical purity of substrates and products was determined by HP 5890 Series II GC on DB-210 column. Optical rotations were determined on a Jasco digital polarimeter model DIP-370.

Determination of optical purity and degree of conversion

The optical purities of the alcohol **7** and esters **8a** and **8b** were determined with a Merck Hitachi high-performance liquid chromatography (HPLC) system equipped with a chiral column Chiralcel OJ 4.6 × 250 mm (Daicel) eluting with a mixture of hexane and 2-propanol 85:15 at a flow rate of 0.9 mL/min with detection at 280 nm. The retention times (R_t) were: for **7**, 11 min for the *R*- and 13 min for the *S*-enantiomer; for **8a**, 5 min for the *R*- and 7 min for the *S*-enantiomer; for **8b**, 4.35 min for the *R*- and 5.01 min for the *S*-enantiomer. The peak assignments were confirmed by co-injections with the commercially available standard of (*R*)-(–)-**7** and its esters (*R*)-**8a** and (*R*)-**8b**.

The degree of ester-to-alcohol conversion during the enzymatic experiments was also determined at the above HPLC conditions. The exact ester-to-alcohol ratios were calculated using a calibration curve obtained with analytical samples of racemic alcohol **7** and racemic esters **8a** and **8b** respectively. Thus, a single injection enabled us to determine both the reaction progress and optical purities of reactant and product.

Synthesis of racemic substrates

(R,S)-2,2,2-Trifluoromethyl-1-(9-anthryl)ethanol (7). 9-Anthryl trifluoromethyl ketone (335 g, 1.22 mol)[2a] was dissolved in 2 L of boiling methanol in a 10 L beaker equipped with a magnetic stirrer. The solution was cooled to room temperature and sodium borohydride (20 g, 0.52 mol) was added portionwise with vigorous stirring over a period of 30 min. The suspension was stirred for an additional period of 10 min. Water (2 L) and dichloromethane (1 L) were added and, after separation of the phases, the organic layer was washed with water (2 × 500 mL), dried and evaporated to give crude (*R,S*)-**7**, 320 g (95 %) as off-white crystals. Melting point 140–142 °C; ^1H NMR δ 3.06 (1H, br s), 6.6 (1H, q, J = 8.06 Hz), 7.46–7.55 (4H, m), 8.0 (2H, d, J = 7.5 Hz), 8.1 (1H, br s), 8.49 (1H, s, ArH at position 10), 8.9 (1H, br s).

(R,S)-2,2,2-Trifluoromethyl-1-(9-anthryl)ethyl butyrate (8b). The foregoing dry alcohol **7** (320 g, 1.16 mol) was placed into a 2 L flask cooled by an ice–water bath, and 400 mL *n*-butyryl chloride were dropwise added through a funnel with cooling and strong magnetic stirring. After the solid was dissolved, the solution was stirred at 50 °C overnight. Completion of the reaction was verified by the absence of alcohol **7** by HPLC. The excess of butyryl chloride was removed by distillation (40 °C, 0.5 mm Hg), the residue was diluted with 500 mL of cold ethyl alcohol and allowed to crystallise at –15 °C overnight to give after filtration white crystals of **8b** 372 g (89 %). Melting point 79–81 °C; ^1H NMR δ 0.87 (3H, t, J = 7.4 Hz), 1.63 (2H, m), 2.44 (2H, m), 7.44–7.62 (4H, m), 7.62 (1H, q, J = 8.08 Hz), 8.02 (2H, d, J = 8.1 Hz), 8.35 (1H, d, J = 8.83 Hz), 8.55 (1H, s, ArH at position 10), 8.72 (1H, d, J = 8.43 Hz).

(R,S)-2,2,2-Trifluoromethyl-1-(9-anthryl)ethyl acetate (8a). This was prepared by esterification with acetyl chloride from **7**, as described for **8b**: mp = 99–102 °C; ^1H NMR δ 2.19 (3H, s), 7.42–7.58 (4H, m), 7.70 (1H, q, J = 8.23), 8.01 (2H, d, J = 8.3), 8.34 (1H, d, J = 8.8), 8.55 (1H, s, ArH at position 10), 8.72 (1H, d, J = 8.36).

Preparation of dispersed porcine pancreatic lipase (PPL)

PPL (370 g) was added to neutral aluminium oxide (370 g) and thoroughly mixed in a stoppered Erlenmeyer flask by gentle inversions of the flask for about 15 min. The resultant homogeneous white powder was stored in the cold and used in the preparative enzymatic resolution experiments.

Preparative resolution of (R,S)-2,2,2-trifluoromethyl-1-(9-anthryl)ethanol by enzymatic alcoholysis

(*R,S*)-2,2,2-Trifluoromethyl-1-(9-anthryl)ethyl butyrate (**8b**) (370 g, 1.03 mol) was dissolved in 2.5 L of boiled *n*-butanol in a 5 L flat bottom flask equipped with a powerful magnetic stirrer. The solution was cooled to 40 °C and 400 g of the dispersed PPL preparation (containing 200 g PPL) were added. The resultant suspension was vigorously stirred at 40 °C and reaction progress was followed by periodically examining aliquots of the suspension by HPLC on the chiral column. Integration of the appropriate HPLC signals enabled us to determine the ratio between starting material and product as well as their optical purity. Thus, both the reaction rate and *e.e.* values of remaining ester and formed alcohol were followed simultaneously during the course of the reaction. On the fourth day of the reaction an additional 340 g of the dispersed enzyme and 500 mL butanol were added and the reaction was continued for another 6 days (10 days altogether). After 9 days it was observed that the reaction came to a virtual standstill at 47 % conversion, at which point the remaining (*S*)-ester had an enantiomeric excess value *e.e.* = 98.3 %, and the resultant alcohol had *e.e.* = 96.5 %. The butanol solution was filtered off, the solid dispersed PPL was washed with 5 L hot acetone and the combined filtrates evaporated at reduced pressure to remove most of the acetone. To the remaining butanol solution, hexane (350 mL) was added and evaporated to remove azeatropically part of the butanol. This operation was repeated 4 times and most of the butanol was removed in this way. An additional 500 mL hexane were then added to the remaining butanol solution, and the new solution was left overnight at –15 °C. Crystallisation occurred and the precipitate was filtered off and washed with hexane to

give the crystalline alcohol (*R*)-**7** (55 g, 39 %), with *e.e.* = 100 %. The mother liquor was evaporated to dryness to give 281 g of light-brown oily residue containing the ester (*S*)-**8b** and alcohol (*R*)-**7** at a ratio of approximately 2.5: 1. This was mixed with 250 g silica gel in 400 mL hexane and partially evaporated until a thick brown slurry was obtained. The slurry was placed onto a 300 g silica gel column and eluted with hexane. TLC and HPLC examination indicated that the eluent (7 L) contained only the ester (*S*)-**8b**. After all the ester was eluted the column was washed with diethyl ether (1.5 L) to remove the alcohol (*R*)-**7**. Evaporation of the hexane fractions resulted in the crude ester (*S*)-**8b**, which was crystallised from ethanol to give (*S*)-2,2,2-trifluoromethyl-1-(9-anthryl)ethyl butyrate (*S*)-**8b** (171 g, 92 %), as white crystalline powder. The *e.e.* of the ester as determined by HPLC was *e.e.* = 99.2 %. Evaporation of the ether fractions resulted in the crude alcohol (*R*)-**7**, which was washed with hexane to give (*R*)-2,2,2-trifluoromethyl-1-(9-anthryl) (*R*)-**7** (62 g, 43 %). Together with the first crop 117 g of alcohol (*R*)-**7** was obtained (yield of 82 %) with optical purity of *e.e.* = 99.4 % by HPLC. $[\alpha]_D^{20} = -32\,^\circ$ (c = 6, $CHCl_3$).

Hydrolysis of (S)-8b

To a solution of the ester (*S*)-**8b** (171 g, 0.475 mol) in 600 mL methanol were added KOH (27 g, 0.49 mol) in 60 mL water and the mixture was stirred at room temperature for 1 h. Water (400 mL) was then added forming a white suspension which was stirred for another 1 h and filtered to afford a white precipitate, which was dried and washed with hexane to give a white crystalline powder of (*S*)-**7** (130 g, 99 %). Optical purity of *e.e.* = 99 % by HPLC, $[\alpha]_D^{20} = +32.5\,^\circ$ (c = 6, $CHCl_3$).

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