Some Diastereoselective Enzyme-catalysed Esterifications and Interesterifications

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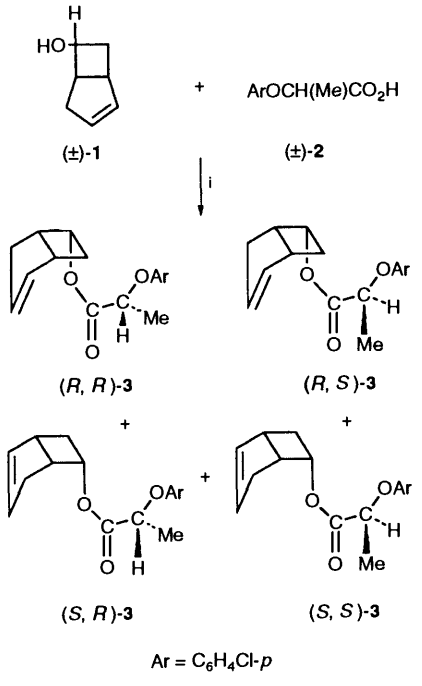
The alcohol bicycloheptenol (±)-**1** or the corresponding acetate (±)-**5** couple with 2-(*p*-chlorophenoxy)propionic acid (±)-**2** using lipases in organic solvents to give the ester bicycloheptenyl 2-(*p*-chlorophenyl)propionate (*R,R*)-**3** with good/excellent selectivity. Similarly, 2-methoxycyclohexanol (±)-**6** or the corresponding acetate (±)-**7** undergo enzyme-induced coupling to the acid (±)-**2** to form the (*R,R,R*)-ester 2-methoxycyclohexyl *p*-chlorophenoxyacetate **8** almost exclusively in a doubly enantioselective process.

The formation of optically active esters using enzyme-catalysed reactions is now almost a standard technology in synthetic organic chemistry.[1] The Exeter group have made contributions to this area investigating esterification[2] and interesterifications[3] following the seminal work of Klibanov,[4] Sonnet[5] *et al.* Recently we have concentrated our attention on 'doubly enantioselective' esterifications and interesterifications using racemic alcohols and racemic acids as substrates. We detail the outcome of these studies in this paper.

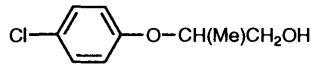
Results and Discussion

Ester formation from the racemic alcohol **1**[6] and the racemic acid **2** can obviously lead to four compounds (*R,R*)-**3**, (*S,R*)-**3**, (*R,S*)-**3** and (*S,S*)-**3** (Scheme 1). The coupling of (±)-**1** and



Scheme 1 *Reagents and conditions:* i, lipase, hexane

(±)-**2** is catalysed by *Candida cyclindracea* lipase (CCL), immobilized *Mucor miehei* lipase (Lipozyme) and lipase AY using almost anhydrous hexane as the solvent. Thus, after 50 h the Lipozyme-catalysed reaction had accumulated 28% of the ester **3**. This ester was purified by column chromatography. The diastereoisomer ratio was judged to be 7:5 on the basis of ^{1}H NMR spectroscopy, attention being concentrated on the doublet due to the methyl group. Lithium aluminium hydride reduction of this diastereoisomeric mixture gave the alcohols **1** and **4**. The absolute configuration of the major enantiomer of the bicyclic *endo*-alcohol was assigned (1*R*,5*S*,6*R*) by comparison with an authentic sample[7] and the enantiomeric excess was shown to be 90% using NMR spectroscopy and employing a chiral shift reagent. The configuration of the major enantiomer of the alcohol **4** was assigned as *R* by formation of the corresponding Mosher's ester and application of Tsuda's



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methodology.[6] The enantiomeric excess was low (10%): this figure was determined by NMR experiments on the Mosher's ester derivative of the alcohol **4** or, more conveniently, by ^{1}H NMR spectroscopy of compound **4** in the presence of chiral shift reagent. Obviously *Mucor miehei* lipase is acylated by both enantiomers of the acid **2** with very little selectivity. Using the values for the diastereoisomer ratio and the enantiomeric excesses as determined above, the proportion of the four different isomers of **3** in the mixture can be calculated using the equation:

$$(R,R)\text{-}\mathbf{3} = 1 - \tfrac{1}{2}(+F_1 + F_2 + F_3)$$
$$(S,S)\text{-}\mathbf{3} = \tfrac{1}{2}(-F_1 + F_2 + F_3)$$
$$(R,S)\text{-}\mathbf{3} = \tfrac{1}{2}(+F_1 - F_2 + F_3)$$
$$(S,R)\text{-}\mathbf{3} = \tfrac{1}{2}(+F_1 + F_2 - F_3)$$

where $F_1 = \dfrac{1}{(1+f_1)}$, $F_2 = \dfrac{1}{(1+f_2)}$, $F_3 = \dfrac{1}{(1+f_3)}$

and $$f_1 = \frac{[(R,R)\text{-}\mathbf{3}\ (S,S)\text{-}\mathbf{3}]}{[(S,R)\text{-}\mathbf{3}\ (R,S)\text{-}\mathbf{3}]}$$

$$f_2 = \frac{[(R)\text{-}\mathbf{1}]}{[(S)\text{-}\mathbf{1}]};\quad f_3 = \frac{[(R)\text{-}\mathbf{2}]}{[(S)\text{-}\mathbf{2}]}$$

The amounts of the different isomers of **3** in the mixture are shown in Table 1 (Entry 1).

Candida cylindracea lipase catalyses a slower but more selective combination of the alcohol **1** and the acid **2**. Thus, after

Table 1 Coupling of the alcohol (±)-**1** or the ester (±)-**5** with the acid (±)-**2** using lipases in hexane

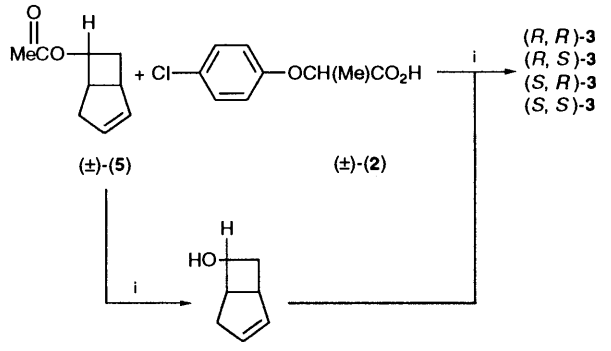
Entry	Substrate	Enzyme	Time (h)	Convn. (%)	Diastereoisomer ratio	Enantiomeric excess (%) of components of ester Alcohol	Acid	Diastereoisomer ratio *RR*:*SS*:*RS*:*SR*
1	(±)-**1**	Lipozyme	50	28	7:5	90	10	54:4:41:1
2	(±)-**1**	CCL	96	32	5:1	70	83	79:3:6:12
3	(±)-**1**	Lipase-AY	290	34	6:1	62	76	77.5:8.5:3.5:10.5
4	(±)-**5**	CCL	622	13	12:1	93	88	91.5:0.5:5.5:2.5
5	(±)-**5**	Lipase AY	402	19	12:1	90	88	90:2:4:4
6	(±)-**5**	Lipase-AY[1]	217	21	12:1	88	87	90.5:2.5:3.5:3.5

[1] The amount of catalyst was increased three-fold in this run.

4 days, 32% of esters **3** had been formed. The diastereoisomer ratio was 5:1 and, after reduction and subsequent analysis, the major alcohol component in the ester was again (1*R*,5*S*,6*R*) (70% e.e.) The acid moiety was mainly made up of the *R*-enantiomer (83% e.e.) and the ratio of *R*,*R*-, *S*,*S*-, *R*,*S*-, and *S*,*R*-**3** was 79:3:6:12 (Entry 2).

Lipase AY produced very similar results. Thus, the diastereoisomer ratio was 6:1 and the enantiomeric excesses of the bicyclic moiety and the aryloxyalkyl unit of the ester were 62 and 76%, respectively, giving the corresponding ratio of the diastereoisomers of **3** as follows: 77.5, 8.5, 3.5 and 10.5 (Table 1, Entry 3).

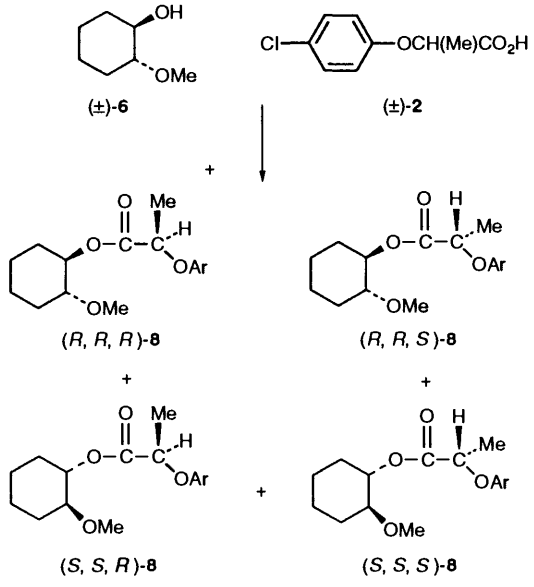
The rationale for utilizing interesterifications for the production of chiral esters centres on the fact that the unit that forms the alcohol part of the product ester is forced to visit the active site of the enzyme on two occasions, amplifying any intrinsic enantioselectivity. For example, consider the interesterification of the racemic acetate (±)-**5** and the racemic acid (±)-**2** under the influence of CCL or lipase AY in hexane containing a trace quantity of water (Scheme 2). The first stage involves



Scheme 2 *Reagents and conditions:* i, lipase, hexane, trace H_2O

enzyme-catalysed hydrolysis of the ester (±)-**5**. In separate control experiments this can be shown to take place in a modestly enantioselective fashion giving the (1*R*,5*S*,6*R*)-alcohol **1** (65% e.e. for CCL, 50% e.e. for lipase AY at 10% conversion). The alcohol released in this way is the substrate for the esterification reaction, involving the enzyme acylated with the aryloxypropanoyl unit. The interesterification strategy leads to a slow reaction of the components but the diastereoisomer ratio is considerably enhanced (to 12/13:1) due to the improved selectivity towards the alcohol component rather than, of course, a change in the acid component (Table 1, Entries 4–6). As expected, a small amount of alcohol of low optical purity was often isolated as a side-product. The amount of enzyme employed can be increased to speed up the interconversion without affecting the product ratio (Table 1, Entry 6).

A similar study was conducted using *trans*-2-methoxycyclohexanol (±)-**6** (compound of interest in connection with the preparation of antibacterial agents[8]) and the corresponding acetate (±)-**7** (Scheme 3).



Scheme 3 *Reagents and conditions:* i, lipase, hexane

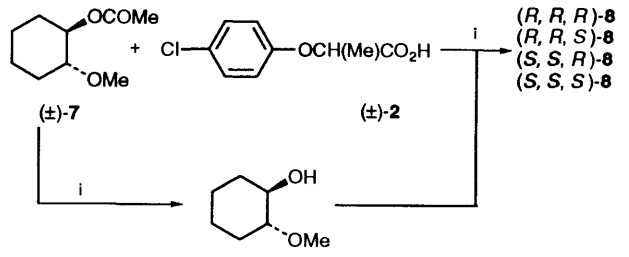
Coupling of the alcohol (±)-**6** and the acid (±)-**2** using CCL as the catalyst gave the esters **8**. The ratio of diastereoisomers (6:1) was assessed by ^{13}C NMR. Lithium aluminium hydride reduction of the esters **8** gave the alcohol (*R*)-**4** (> 98% e.e.) and the (*R*,*R*)-methoxycyclohexanol **6** (71% e.e.). The absolute configuration of (*R*,*R*)-**6** was elucidated by comparison with authentic material[9] and the enantiomeric excess was determined by GC using a chiral stationary phase. These results suggest that the ratio of the four isomers (*R*,*R*,*R*)-**8**, (*S*,*S*,*S*)-**8**, (*R*,*R*,*S*)-**8** and (*S*,*S*,*R*)-**8** is 84:1:1:13 (Table 2, Entry 1).

Hydrolysis of the acetate (±)-**7** in hexane using CCL as the catalyst proceeded slowly and with modest selectivity giving, after 7 days, a 39% conversion into the (*R*,*R*)-alcohol (70% e.e.). The interesterification involving the acetate (±)-**7** and the acid (±)-**2** gave, after 14 days, a 15% yield of the (*R*,*R*,*R*)-ester **8** (Scheme 4) contaminated with just a small amount of the (*R*,*R*,*S*)- and the (*S*,*S*,*R*)-isomer (Table 2, Entry 2). In addition, 28% of (*R*,*R*)-alcohol **6** was isolated (64% e.e.) together with 57% of (*S*,*S*)-acetate **7** (44% e.e.). Thus, in the interesterification process the coupling takes place, essentially, between one enantiomer of the alcohol and one enantiomer of the acid.

It is to be expected that the interesterification strategy does not succeed for all combinations of (±)-alcohol (acetate) and (±)-acid. Indeed, this is the case and one example, involving low selectivity towards the acid component, has been cited above. Another example involves the coupling of the acetate (±)-**9** and the acid (±)-**2** which afforded a 17% yield of the esters **10** [in addition to recovered (1*R*,2*S*,4*S*)-acetate **9** (68% yield) (8% e.e.) and (1*S*,2*R*,4*R*)-norbornanol (15% yield, 13% e.e.)]. Lithium aluminium hydride reduction of the diastereoiso-

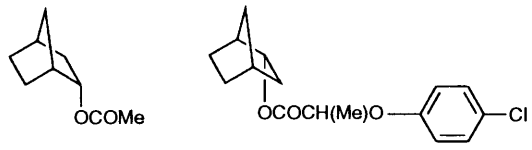
Table 2 Formation of the esters **8** and **10** from the acid (±)-**2** and the compounds (±)-**6**, (±)-**7** and (±)-**9** using CCL as catalyst and hexane as the solvent

Entry	Substrate	Time (h)	Convn. %	Diastereoisomer ratio	Enantiomeric excess of components of ester Alcohol	Acid	Ratio of isomers *RRR*:*SSS*:*RRS*:*SSR*
1	(±)-**6**	24	21.5	6:1	71	*ca.* 98	85:1:1:13
2	(±)-**7**	336	15.0	13:1	94	*ca.* 98	94:0:3:3
3	(±)-**9**	96	17.4	2:1	32	*ca.* 98	65:2:1:32



Scheme 4 *Reagents and conditions:* i, lipase, hexane

meric mixture of esters **10** gave (*R*)-acid **2** of high optical purity (>98% e.e.) but gave (1*S*,2*R*,4*R*)-norbornanol of low optical purity (32% e.e.) indicating a process which is poorly selective towards the alcohol component (Table 2, Entry 3).



Conclusions

'Doubly-enantioselective' enzyme-catalysed esterifications can be effected. The coupling reactions are slow and a good deal of process research and process engineering would be necessary before suitably interesting and suitably structured compounds could be manufactured on a large scale.

Experimental

Ethyl acetate and light petroleum (b.p. 60–80 °C) were distilled prior to use. Diethyl ether was dried and distilled from sodium metal. Hexane was dried and distilled from phosphorus pentoxide. Thin layer chromatography was performed on pre-coated glass plates (Merck silica gel 60F 254). The plates were visualised using UV light (254 nm) and/or *p*-anisaldehyde. Flash chromatography was performed over silica (Merck silica 60, 40–63 rm) and this method was used to purify all products. IR spectra were obtained using a Perkin-Elmer 881 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker AM 250 machine operating at 250 and 62.9 MHz respectively. For the esters **3** the diastereoisomer ratio was calculated from the ^{1}H 250 MHz NMR spectrum by determination of the relative heights of the pair of doublets at δ 1.51 and 1.49 due to the protons within the diastereoisomeric methyl groups. Estimated maximum error ± 5%.*

For the esters **8** the diastereoisomeric ratio was calculated from the ^{13}C 62.9 MHz NMR spectra by determination of the intensities of the appropriate pairs of signals; estimated maximum error ± 7.5%.*

The ratio of enantiomers of the alcohols **1** and **4** were assessed by NMR spectroscopy using {tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III)} as the chiral shift reagent. Estimated maximum error ± 5%.* The enantiomeric excess of the alcohol **6** was measured using a PYE UNICAM PU 4500 chiral GC. (FS-lipodex A, 25 m × 0.25 mm); estimated maximum error ± 2%.*

Both low and high resolution mass spectra were obtained using the SERC Mass Spectroscopy Service at Swansea. Spectra were obtained by electron impact (EI) at 70 eV or chemical ionisation (CI) using ammonia.

Esterification: General Procedure.—Anhydrous hexane (10 cm^3) was added to the alcohol (or acetate) (2 mmol), the 2-(*p*-chlorophenoxy)propionic acid (2 mmol, 401 mg) and the enzyme (200 mg) which had been placed in a flask under argon. The reaction mixture was then shaken in an incubator (220 rev min^{-1}, 30 °C).

The reaction was stopped by filtering off the enzyme. The filtrate was washed with a saturated aqueous sodium hydrogen carbonate, dried ($MgSO_4$), filtered and evaporated and the residue was purified by column chromatography over silica. The physical data on the ester **3** were as follows: ν_{max}(film)/cm^{-1} 2941, 1742, 1486; δ_H (250 MHz, $CDCl_3$) 7.28–7.18 (2 H, m, ArH), 6.85–6.78 (2 H, m, ArH), 5.82–5.71 (2 H, m, 2-H, 3-H), 5.30–5.15 (1 H, m, 6-H), 4.72 (1 H, q, *J* 7, CHMe), 3.34–3.21 (1 H, m, 5-H), 3.10–2.98 (1 H, m, 1-H), 2.82–2.65 (1 H, m, 7-H), 1.62–1.60 (3 H, 2d, *J* 7, CH_3); δ_C (250 MHz, $CDCl_3$) 171.22, 171.13 (C, C=O), 156.33 (C, C–Ar), 133.92, 132.16, 132.08 (CH, C-2, C-3), 129.38, 129.35 (CH, C-Ar), 126.52 (C, C-Ar), 116.66, 116.61 (CH, C-Ar), 72.96, 72.87 (CH, C-9), 70.04, 69.98 (CH, C-6), 41.13, 41.04, 40.78, 40.73 (CH, C-1, C-5), 36.67, 36.55, 32.43, 32.38 (CH_2, C-4, C-7) and 18.56 and 18.48 (CH_3, C-10) (Found: M$^+$, 292.0866, $C_{16}H_{17}ClO_3$ requires *M*$^+$, 292.0862). The ester **8** gave the following data: ν_{max}(film)/cm^{-1}, 2943, 1757 and 1493; δ_H (250 MHz, $CDCl_3$), 7.21 (2 H, d, *J* 8.5, ArH), 6.82 (2 H, d, *J* 8.5, ArH), 4.76 (1 H, m, 2-H), 4.68 (1 H, q, *J* 6.7, CHMe), 3.16 (3 H, s, OCH_3), 3.04 (1 H, m, 1-H), 2.00, 1.68, 1.42 (8 H, 3 × m, 3-H, 4-H, 5-H, 6-H) and 1.61 (3 H, d, *J* 6.7, $CHCH_3$); δ_C (250 MHz, $CDCl_3$), 171.49 (C, C=O), 156.37 (C, C-Ar), 129.33, 129.31 (CH, C-Ar), 126.36 (C, C-Ar), 116.44 (CH, C-Ar), 80.55, 80.40, 75.84, 75.70, 73.15, 73.10 (CH, C-1, C-2, CHMe), 56.76, 56.67 (CH_3, OCH_3), 29.74, 29.58, 28.99, 23.31, 23.25, 23.12 (CH_2, C-3, C-4, C-5, C-6) and 18.59 and 18.50 (CH_3, $CHCH_3$) (Found: M$^+$, 312.1128. $C_{16}H_{21}ClO_4$ requires *M*$^+$, 312.1128].

Reduction of the Esters: General Procedure.—Lithium aluminium hydride (1.1 equiv., 1.1 mmol) was stirred in anhydrous diethyl ether (3 cm^3) for 5 min, at room temperature under argon. A solution of the ester (1 mmol) in anhydrous diethyl ether (3 cm^3) was added. The reaction was stirred at room temperature under argon and checked by TLC. Deionised water was added dropwise until no further reaction occurred. The mixture was filtered through Celite, dried ($MgSO_4$) and

* Incorporation of values at the limit of the experimental errors for the diastereoisomer/enantiomer ratios produced little change in the calculated ratios of the four isomers.

filtered. The residue was purified by column chromatography on silica to give the required alcohols.

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