Highly Diastereoselective Inter-esterification Reactions Involving a Racemic Acetate and a Racemic Carboxylic Acid catalysed by Lipase Enzymes

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Reaction of the acetate (±)-**8** with the acid (±)-**2** using *Candida cylindracea* lipase or Lipase AY as catalyst gave mainly the (*R,R*)-diastereoisomer **4** (91 ± 0.5%) with minimal amounts of the other diastereoisomers **3**, **5** and **6** (<5.5%).

Examples of enantioselective hydrolysis of racemic esters using esterases and lipases are well documented.[1] The studies of Klibanov *et al.* showed that by using lipases in organic solvents enantioselective esterification reactions could be accomplished.[2] In Klibanov's seminal work a racemic acid was enantioselectively esterified using an achiral alcohol. Later Sonnet showed that the strategy could be applied to the resolution of a racemic secondary alcohol using an achiral carboxylic acid.[3] More recently we have shown that inter-esterification reactions involving chiral secondary alcohols and achiral acids often exhibit exquisite enantioselectivity owing to the fact that the participating alcohol or the corresponding acetate must approach the enzyme active site on two occasions during the transformation.[4]

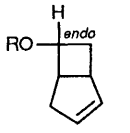
In this paper we describe some lipase-catalysed esterification and inter-esterification reactions using the alcohol (±) **1** and (±)-*p*-chlorophenoxypropanoic acid **2** in hexane.

The immobilized lipase from *Mucor miehei* (Lipozyme^{R}) catalysed the esterification of (±)-bicyclo[3.2.0]hept-2-en-6-*endo*-ol with the (±)-acid **2**; the ratio of the diastereoisomers (*i.e.* **3**, **6**:**4**, **5**) was approximately 5:7 as shown by NMR spectroscopy.

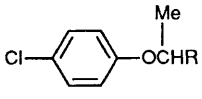
Table 1 Esterification of alcohol (±)-**1** with the acid (±)-**2** and inter-esterification reactions involving the acetate (±)-**8** and the acid (±)-**2**

Entry	Substrate	Enzyme	Time/h	Conversion (%)	Diastereoisomer ratio (**4** + **5**) : (**3** + **6**)	Enantiomeric excess (%)		Diastereoisomer ratio **4** : **5** : **3** : **6**[a]
						Alcohol	Acid	
1	(±)-**1**	Lipozyme	50	28	7 : 5	90	10	54 : 4 : 41 : 1
2	(±)-**1**	CCL	525	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	(±)-**8**	CCL	622	13	12 : 1	93	88	91.5 : 0.5 : 5.5 : 2.5
5	(±)-**8**	Lipase-AY	217	21	13 : 1	88	87	90.5 : 2.5 : 3.5 : 3.5

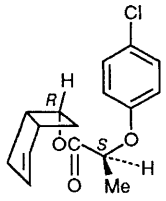
[a] The diastereoisomer ratios were calculated using the equations [*R*(alc.) *R*(acid) **4**] = 1 − ½ (+F_1 + F_2 + F_3); [*S*(alc.) *S*(acid) **5**] = ½ (−F_1 + F_2 + F_3); [*R*(alc.) *S*(acid) **3**] = ½ (+F_1 − F_2 + F_3); [*S*(calc.) *R*(acid) **6**] = ½ (+ F_1 + F_2 − F_3); where F_1 = 1/(1 + f_1); F_2 = 1/(1 + f_2); F_3 = 1/(1 + f_3), and f_1 = [homoesters **4** + **5**]/[heteroesters **3** + **6**]; f_2 = [*R*(calc.)]/[*S*(alc.)]; f_3 = [*R*(acid)/[*S*(acid)].



1 R = H
8 R = COMe



2 R = CO_2H
7 R = CH_2OH



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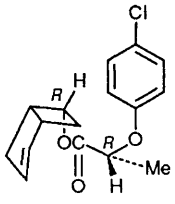

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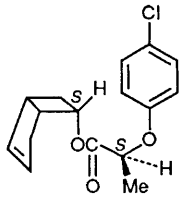

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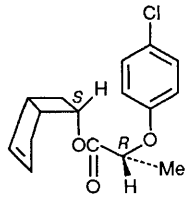

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Treatment of the mixture of diastereoisomers with sodium methoxide gave optically active alcohol **1**, shown to be the (*R*)-alcohol (90% enantiomeric excess, e.e.) by formation of the Mosher's ester and ^{19}F NMR spectroscopy.[4] Obviously the major components of the diastereoisomeric mixture are the esters **3** and **4**; the acid **2** was incorporated with little or no enantioselectivity.

When the same esterification reaction was conducted using *Candida cylindracea* lipase (Sigma) as the catalyst, more encouraging results were obtained. The ratio of diastereoisomers **3, 6** : **4, 5** was 1 : 5 as shown by ^1H NMR spectroscopy. Treatment of this mixture of diastereoisomers with lithium aluminium hydride (LAH) furnished the (*R*)-alcohol **1** (70% e.e.) and the (*R*)-alcohol **7** (83% e.e.). The ratios of the four diastereoisomers **3** : **4** : **5** : **6** was calculated to be 6.0 : 79.0 : 3.0 : 12.0. The increased incorporation of the (*R*)-acid **2** when using *C. cylindracea* lipase over that observed for *M. miehei* lipase was anticipated in earlier work.[2]

The major possible error in this evaluation of the ratios of diastereoisomers lies in the difficulty of the accurate measurement of the diastereoisomer ratio by ^1H NMR spectroscopy. We estimate that an error of ±5% can occur in this measurement. However, the ratios of the diastereoisomers do not change significantly if values of the diastereoisomer ratio at the limits of the error range are used (*vide infra*). The enantiomeric purity of the alcohol **1** was accurately determined (error ± 0.2%) by formation of the Mosher's ester and use of ^{19}F NMR spectroscopy. The optical purity of the alcohol **7** was assessed by ^1H NMR spectroscopy using a chiral shift reagent (estimated error ±2%). The combined uncertainties in measurement produce errors of ±0.5 in the diastereoisomer percentages quoted in the final column of Table 1.

A similar result was obtained on catalysis of the coupling of the alcohol (±)-**1** and the acid (±)-**2** using Lipase AY as the catalyst inasmuch as the ratio of diastereoisomers formed, **3, 6** : **4, 5**, was 1 : 6 and after LAH treatment the alcohols (*R*)-**1** and (*R*)-**7** were obtained with good enantiomeric purity (62 and 76% e.e. respectively). The ratio of the diastereoisomers was calculated to be 3.5 : 77.5 : 8.5 : 10.5.

Modification of the procedure so as to incorporate the interestification 'double-sieving' strategy into this biotransformation brought about the expected increase in diastereoselectivity. Thus, when the acetate (±)-**8** was treated with the acid (±)-**2** using *C. cylindracea* lipase as the catalyst in dry hexane the diastereoisomers **3,6** : **4,5** were formed in the ratio 1 : 12. Following LAH treatment, separation of the primary and secondary alcohols, and Mosher's ester formation as appropriate the (*R*)-alcohol **1** and the (*R*)-alcohol **7** were found to be formed with enantiomeric excesses of 93 and 88% respectively. Therefore, the diastereoisomers **3**, **4**, **5** and **6** had been formed in the ratio 5.5 : 91.5 : 0.5 : 2.5.

Catalysis of the inter-esterification reaction of the ester (±)-**8** and the acid (±)-**2** using Lipase AY gave the diastereoisomer **4** as the major product (90.5%); the other diastereoisomers were formed as impurities (each <3.5%).

In a typical experiment, the ester (±)-**8** (2.5 mmol), 2-(*p*-chlorophenoxy)propanoic acid (±)-**2** (2.5 mmol) and *C. cylindracea* lipase (0.2 g) in anhydrous n-hexane (10 ml) were shaken under an inert atmosphere at 30 °C in an orbital incubator (220 rev./min). The reaction was terminated by removal of the enzyme by filtration. The filtrate was washed with 0.1 mol dm^{-3} aqueous sodium hydroxide (50 ml), water (50 ml) and dried. After removal of the solvent the mixture of diastereoisomeric esters **3–6** was purified by chromatography over silica. The diastereoisomer ratio was determined by ^1H NMR. The mixture of esters **3–6** in dry diethyl ether was treated with LAH (1 equiv.) in diethyl ether for 18 h at room temperature. After work-up in the usual manner, chromatography over silica gave the alcohols **1** and **7**. The optical purity of the alcohol 7 was determined by ^1H NMR spectroscopy using a chiral shift reagent [Eu(hfpc)$_3$] while the optical purity of the alcohol **1** was determined by ^1H and ^{19}F NMR spectroscopy after formation of the Mosher's ester.

In separate experiments we have shown that hydrolysis of the racemic acetate (±)-**8** in water-saturated hexane by *C. cylindracea* lipase or by Lipase AY is selective for the (*R*)-acetate (e.e. 64 and 50% respectively at 10% conversion) and this is reflected in increased amounts of the diastereoisomers **3** and **4** in the product mixture resulting from the inter-esterification reactions.

While diastereoselective hydrolysis reactions have been reported previously[5] we believe that this is the first report of a

doubly enantioselective' enzyme-catalysed reaction involving the synthesis of an ester made up of chiral acid and alcohol moieties.

We are currently working to improve and broaden the scope of the process through finding matching partners for the double enantioselection' and by attempting to increase the rate of the biotransformation.

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