Enzyme-catalysed Inter-esterification Procedure for the Preparation of Esters of a Chiral Secondary Alcohol in High Enantiomeric Purity

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Chiral esters of high optical purity were obtained by an enzyme-controlled inter-esterification procedure in which the constituent hydrolysis and esterification reactions were suitably matched.

The ability of esterases and lipases to produce optically active alcohols by the kinetic resolution of racemic esters is well established.[1] A body of literature has recently been published on the enantioselective esterification of chiral alcohols using lipases in organic solvents.[2] Inter-esterification reactions involving chiral acyl units and achiral alcohols have been described[3] but reports of studies involving the inter-esterification of chiral alcohols and achiral acids are rare.

We have shown that the *endo*-acetate (**1**) is hydrolysed enantioselectively using *Mucor miehei* lipase in phosphate buffer.[4] This robust enzyme is commercially available in immobilized form (Lipozyme^{R}) and the immobilised enzyme shows good stability in non-polar organic solvents.[5] Hydrolysis of the acetate (**1**) in hexane saturated with water gave, after 38 h, a 12% conversion into the (6*R*)-alcohol (+)-(**2**); the enantiomeric excess of the product was modest (e.e. 76%; enantiomer ratio[6] E_R 8) (Table 1).

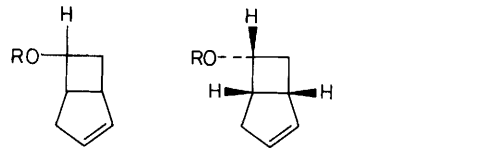
Esterification of the racemic alcohol (**2**) with cyclohexanecarboxylic acid catalysed by Lipozyme in hexane gave, after 29 h, a 48% yield of the ester (−)-(**3**) (e.e. 94.3%, E_R 96) in a reaction displaying good enantioselectivity.

Stirring the immobilised lipase with the racemic ester (**1**) in

Table 1. Hydrolysis, esterification, and inter-esterification reactions on some bicyclo[3.2.0]hept-2-ene derivatives.[a]

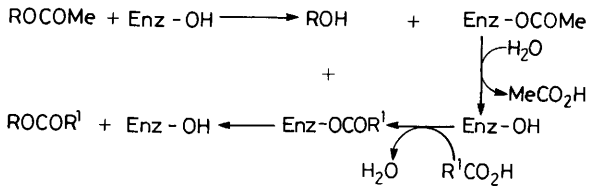
Entry	Substrate (1) or (2)	Acyl donor	Temp./°C	Rate (mmole product/ 12 h/g Lipozyme)	E_R
1	(1)	—	30	0.2	8
2	(2)	cyclo-C_6H_{11}-CO_2H	30	1.0	97
3	(1)	cyclo-C_6H_{11}-CO_2H	30	0.2	~400
4	(2)	$Me[CH_2]_6$-CO_2H	30	15	28
5	(1)	$Me[CH_2]_6$-CO_2H	30	0.09	38
6	(1)	—	60	1.3	10
7	(2)	$PhCO_2H$	60	0.02	58
8	(1)	$PhCO_2H$	60	0.03	90
9	(2)	$PhCO_2Me$	60	1.3	37
10	(1)	$PhCO_2Me$	60	0.4	206

[a] Reaction conditions: substrate (2.5 mmol), acyl donor (2.5 mmol), and Lipozyme (0.5 g) were shaken at 200 rev min^{-1} in either anhydrous hexane or (for hydrolyses, entries 1 and 6) hexane saturated with water (10 ml). Progress of the reaction was monitored by 250 MHz ^1H NMR spectroscopy. Optical purity was determined by NMR spectroscopy using chiral shift reagent and/or through formation of Mosher's esters.



(1) R = COMe
(2) R = H

(+)-(2) R = H
(−)-(3) R = C(:O)C_6H_{11}-cyclo
(−)-(4) R = C(:O)$[CH_2]_6$Me
(−)-(5) R = C(:O)Ph



Scheme 1

hexane containing cyclohexanecarboxylic acid gave, after 66 h, a 27% yield of the optically active ester (−)-(**3**). The product was formed in a highly optically pure state (99.4% e.e.) as determined by separation of the two esters chemically, de-esterification of the cyclohexanoate, and formation of the Mosher's ester from the alcohol (+)-(**2**). The E_R of the inter-esterification reaction was very high (*ca.* 400).

Thus the inter-esterification reaction is considerably more enantioselective than either the corresponding hydrolysis reaction or the equivalent esterification reaction. This is connected with the fact that the bicyclic molecule must visit the enzyme active site twice during the process, first to undergo de-acetylation and then to pick up the cyclohexanoate moiety from the acylated enzyme (Scheme 1). The (acetylated) bicyclic alcohol is doubly sieved through these two enantioselective processes. As expected a small amount (5—10%) of the alcohol (**2**) of low optical purity is obtained as a side product.

The strategy will be useful for the preparation of chiral intermediates with the following constraints.

(a) The ester must be a substrate for the enzyme and the process should show some degree of enantioselectivity: equally the acid should acylate the enzyme and the process of esterification should be enantioselective.

(b) The rate of hydrolysis of the product ester and the rate of acetylation of the alcohol should be very slow.[7]

(c) The two esters should be readily separated by chromatography.

(d) The rate of hydrolysis of the acetate and the rate of esterification of the alcohol must be of the same order of magnitude.

To emphasize the importance of the final point it was shown that esterification of the alcohol (±)-(**2**) with octanoic acid under catalysis by Lipozyme takes place rapidly to give, after 1 h, a 24% yield of ester (−)-(**4**) (e.e. 91%, E_R 28). In the presence of Lipozyme the racemic acetate (**1**) undergoes conversion into the octanoate (−)-(**4**) slowly, with 20% of product (e.e. 94%) being formed over 126 h. The enantiomeric ratio for the latter reaction (E_R 38) is close to the value for the esterification reaction. It is likely that the enzyme active sites are almost fully loaded with octanoate units, and so the acetate (**1**) finds it difficult to get access to the active site, hence when the alcohol *is* formed it is rapidly acylated. Not surprisingly only a trace of alcohol is observed during the inter-esterification involving octanoate.

The hydrolysis of the acetate (**1**) takes place fairly rapidly at 60 °C using Lipozyme as catalyst giving the alcohol (+)-(**2**) (17%; 80% e.e.) over 8 h (E_R 10). Esterification of the racemic alcohol (**2**) using benzoic acid under similar conditions is much slower (6% conversion after 96 h) to give the benzoate (**5**) (96.4% e.e.; E_R 58). Not surprisingly the inter-esterification reaction employing racemic acetate (**1**) and benzoic acid is slow (5% conversion after 114 h) and the optical purity of the product (−)-(**5**) is not exceptionally high (e.e. 97.7%; E_R 90.5). However, and as expected, esterification of the alcohol (**2**) with methyl benzoate is much faster than for the free acid. Indeed on using the aromatic ester the rate of esterification of the alcohol (**2**) was of the same order as the hydrolysis of the acetate (**1**) under similar reaction conditions. The benzoate (−)-(**5**) was found to be formed with an E_R value of 37 (e.e. 88% at 47% conversion) using the more reactive methyl ester. Interestingly, the rate of the hydrolysis and the esterification reactions having been matched, the inter-esterification reaction involving the acetate (**1**) and methyl benzoate gave the ester (−)-(**5**) (21% after 29 h) in a state of high optical purity (e.e. 99%; E_R 206).

We believe the double-sieving involved in a 'matched' inter-esterification process will be useful for obtaining products of high optical purity and may lead to double enantioselection when a chiral racemic acetate and a chiral racemic acid are coupled.

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