Enzymatic Resolution of Oxalate Esters of a Tertiary Alcohol Using Porcine Pancreatic Lipase

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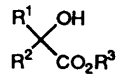
The tertiary alcohol **5** has been resolved to 90% ee (42% yield) by hydrolysis of its oxalate **6b** with porcine pancreatic lipase in pH 7 buffer containing 13% *tert*-butyl alcohol.

The hydrolysis of racemic esters using esterases and lipases is a well-documented approach to the synthesis of secondary alcohols and α-monosubstituted carboxylic acids in optically active form. In contrast, relatively few examples of the resolution of the more substituted tertiary systems have been reported. α-Hydroxy-α-methyl carboxylates **1** have been successfully resolved using pig liver esterase, a lipase from *Candida cylindracea* and a protease from *Aspergillus oryzae*,[1] while substituted *N*-acylamino acids **2** have been hydrolysed enantioselectively using acylase I fron hog kidney[2] to afford the corresponding amino acid in high optical purity.



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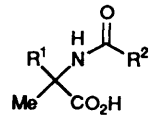
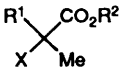


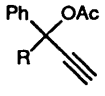
2

Recently a group at Genzyme[3] isolated an esterase enzyme from *Candida lipolytica* and used this to resolve a series of α-methyl carboxylates possessing a range of α-heteroatom substituents **3**, including α-hydrazino, α-amino and α-hydroxy functions.



X = $NHNH_2$, NH_2, OH

3

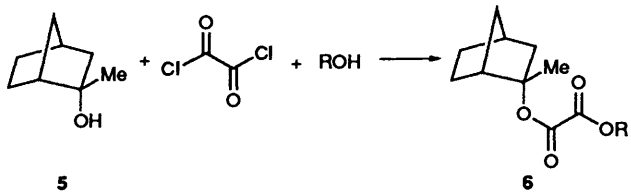


4

In complementary studies, O'Hagan has recently reported the hydrolysis of a series of tertiary acetylenic acetate esters **4** using the lipase from *Candida cylindracea*.[4] When R = CF_3 the resultant alcohol and residual acetate were recovered with respectable enantiomeric excesses (ee's 87 and 75%, respectively). Other R groups (*e.g.* Me, Et, Pr) gave less satisfactory results, findings in agreement with some of our unpublished work on the hydrolysis of tertiary acetylenic acetates. O'Hagan also found that replacement of the acetylenic moiety by methyl, vinyl or nitrile units resulted in compounds inert to lipase-catalysed hydrolysis.

Prompted by these recent publications we report the resolution of a tertiary alcohol *via* the formation of a series of mixed oxalates. The idea behind the use of a mixed oxalate was that the enzyme could hydrolyse the carboxy group distal from the chiral centre, but still benefit from differentiation in the binding of the two enantiomers at the active site, hence effecting a kinetic resolution. Mixed oxalates **6** were readily prepared from the tertiary alcohol **5**, oxalyl chloride and a second alcohol (ROH; R = Me, Pr^i, c-C_6H_{12}, Bu^t) in high yields (80–90%) (Scheme 1).

Attempts at resolving the oxalate esters **6a** and **6b** using a selection of lipases [porcine pancreatic lipase (PPL) *C. cylindracea* lipase, *Pseudomonas fluorescens* lipase and Lipozyme®] were not encouraging initially. In both cases PPL gave the best result. For the methyl oxalate **6a** hydrolysis was rapid but recovered oxalate was only 26% enantiomeric excess (ee) at 20% yield. The isopropyl oxalate **6b** was recovered in the same ee but 40% yield. The cyclohexyl oxalate **6c** gave a slow reaction with little enantiospecificity while the *tert*-butyl oxalate **6d** was inert. Monitoring all of these reactions by TLC indicated very little alcohol **5** had been produced, indicative of hydrolysis at the less-hindered carboxy group.



5 **6**
a R = Me
b R = Pr^i
c R = cyclohexyl
d R = Bu^t

Scheme 1

It has been reported in the literature[5] that the addition of organic cosolvents to the reaction media employed for enzyme-catalysed hydrolyses can considerably alter the stereochemical outcome of the reaction. Thus, the oxalate **6b** was hydrolysed using porcine pancreatic lipase in water containing organic cosolvents. Addition of 10% methanol, acetonitrile or dimethyl sulfoxide to the reaction mixture had little effect on the enantioselectivity. However, addition of 10% *tert*-butyl alcohol to the medium had a dramatic effect on the ee of the recovered oxalate. After a 14 h biotransformation a 51% yield of the oxalate was obtained with 53% ee. A longer reaction time of 18 h increased the ee to 76%, for a 33% recovery of the oxalate. The effect of varying the amount of added *tert*-butyl alcohol to the reaction medium was then studied in greater detail and the results are shown in Table 1. Clearly the addition of 13% *tert*-butyl alcohol had a particularly beneficial effect on the outcome of the hydrolysis, affording both the recovered oxalate and the alcohol in *ca.* 90% ee and acceptable yield. The addition of *tert*-butyl alcohol appeared to switch the site of hydrolysis to the carboxy group nearer to the chiral centre, thus producing both the alcohol **5** and oxalate in good ee. Further optimization of the reaction conditions was sought by adjustment of the pH and the temperature of the reaction. The results of studying these effects are shown in Table 2. Each reaction was carried out in buffer containing 13% *tert*-butyl alcohol. Raising the pH to 8 had no significant effect on the rate of hydrolysis, but it did have a detrimental effect on the enantioselectivity of the reaction. Reducing the initial pH to 6 resulted in a very slow reaction rate, with an accompanying decrease in the enantioselectivity. An increase in the temperature to 40 °C effectively gave racemic

Table 1 Effect of addition of *tert*-butyl alcohol to the reaction medium for the resolution of oxalate (**6b**), using porcine pancreatic lipase (PPL) at room temp.

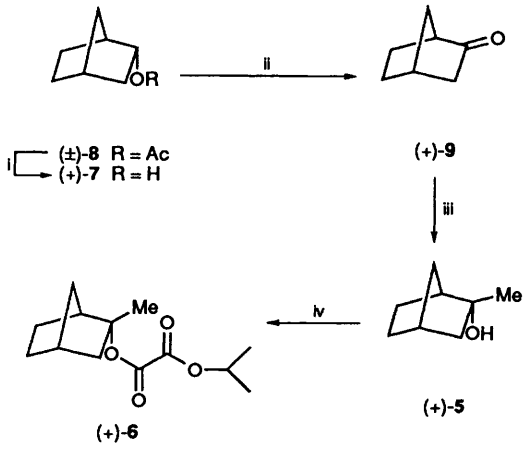
% Bu^{t}OH added to buffer	Reaction time (h)	pH	Alcohol **5**		Oxalate **6**		E*
			Yield (%)	ee (%)	Yield (%)	ee (%)	
7	19	7	41	74	29	77	4
13	22	7	42	90	33	88	7
15	17	7	35	85	39	63	4
18	27	7	45	62	33	79	5

* E = enantiomeric ratio: see C.-S. Chen, Y. Fujimoto, G. Gidaukas and C. J. Sik, *J. Am. Chem. Soc.*, 1982, **104**, 7294.

Table 2 Effect of pH and temperature on the porcine pancreatic lipase catalysed hydrolysis of the oxalate **6b**

Reaction time (h)	pH	Temp. (°C)	Alcohol **5**		Oxalate **6**		E*
			Yield (%)	ee (%)	Yield (%)	ee (%)	
96	6	RT	24	82	62	28	3.5
25	8	RT	43	80	36	67	4
25	7	5	13	96	56	42	5
90	7	40	23	0	36	6	—

* See footnote to Table 1.



Scheme 2 *Reagents and conditions:* i, CCL, pH 7.2; ii, pyridinium dichromate; iii, MeMgI; iv, $(COCl)_2$, then Pr^{i}OH.

alcohol and oxalate, possible due to non-enzymic degradation at the higher temperature. Lowering the temperature resulted in a reduced rate of reaction, but with no significant enhancement in selectivity.

In order to verify the absolute stereochemistry of the major enantiomer of the alcohol that was produced, an authentic sample of secondary *endo*-alcohol **7** was required. This was obtained by hydrolysis of the corresponding acetate **8** at pH 7.2 using the lipase from *C. cylindracea*.[6] After 20 h, a 34% yield of the (+)-enantiomer {$[\alpha]_D^{25}$ +1.3 (*c* 7.6 in $CHCl_3$)} of the secondary alcohol was obtained, with an ee of 60%, as measured by chiral shift NMR spectroscopy. Oxidation of the alcohol afforded an 81% yield of the (+)-ketone **9** {$[\alpha]_D^{27}$ +19.2 (*c* 1.07 in $CHCl_3$)}, indicative of the (1*S*,4*R*) enantiomer.[7] Treatment of this ketone with methylmagnesium iodide gave a 74% yield of the (+)-alcohol **5** {$[\alpha]_D^{25}$ +11.1 (*c* 2.96 in $CHCl_3$)},[8] which was antipodal to that produced by the PPL-catalysed hydrolysis of the oxalate ester **6b**, indicating that the enzyme PPL preferentially hydrolysed the (1*R*,2*S*,4*S*) enantiomer of the oxalate ester. This was confirmed by conversion of the (+)-alcohol **5** into the oxalate **6b**, in 90% yield {$[\alpha]_D^{28}$ +17.0 (*c* 1.01 in $CHCl_3$)}.

We are currently exploring the applicability of this methodology to the resolution of other tertiary alcohols, in an effort to find a general method for the production of tertiary alcohols in high enantiomeric purity.

Experimental

Hydrolysis of the Oxalate **6b**, *using Porcine Pancreatic Lipase.*—PPL (Sigma Chemical Co. Type II) (52.8 mg, 53 units mg^{-1} solid) was added to the oxalate **6b** (180.3 mg, 0.75 mmol) in solvent (13% *tert*-butyl alcohol in 0.1 mol dm^{-3} phosphate buffer, pH 7.0; 15 cm^3) and the mixture stirred at room temperature. After 22 h the mixture was filtered through Celite and extracted with diethyl ether (4 × 20 cm^3). The extract was dried ($MgSO_4$) and evaporated under reduced pressure to afford a mixture of unchanged oxalate and alcohol. Purification of the mixture by flash chromatography [eluent, light petroleum–diethyl ether (4:1–2:1)] afforded the (+)-oxalate **6** (60.2 mg, 33%) and the (−)-alcohol **5** (39.6 mg, 42%). Enantiomeric excesses were determined directly on the oxalate **6**, and by derivatization of the alcohol **5** to the oxalate **6** (R = Me), by ^{1}H NMR spectroscopy in $CDCl_3$ with added Eu(hfc)$_3$.

References

1 H. Moorlag, R. M. Kellogg, M. Kloosterman, B. Kaptein, J. Kamphuis and H. E. Shoemaker, *J. Org. Chem.*, 1990, **55**, 5878; T. Sugai, H. Kakeya and H. Ohta, *J. Org. Chem.*, 1990, **55** 4643; C. Feichter, K. Faber and H. Griengl, *J. Chem. Soc., Perkin Trans. 1*, 1991, 653.
2 H. K. Chenault, J. Dahmer and G. M. Whitesides, *J. Am. Chem. Soc.*, 1989, **111**, 6354; J. W. Keller and B. J. Hamilton, *Tetrahedron Lett.*, 1986, **27**, 1249.
3 C. Yee, T. A. Blythe, T. J. McNabb and A. E. Walts, *J. Org. Chem.*, 1992, **57**, 3525.
4 D. O'Hagan and N. A. Zaidi, *J. Chem. Soc., Perkin Trans. 1*, 1992, 947.
5 A. J. H. Khinder, W. B. Huizinga, A. J. M. Hulshof and B. Zwanenburg, *Tetrahedron Lett.*, 1986, **27**, 2543; L. K. P. Lam, R. A. H. F. Hui and J. B. Jones, *J. Org. Chem.*, 1986, **51**, 2047; F. Björkling, J. Boutelje, M. Hjalmarsson, K. Hult and T. Norin, *J. Chem. Soc., Chem. Commun.*, 1987, 1041.
6 T. Oberhauser, M. Bodenteich, K. Faber, G. Penn and H. Griengl, *Tetrahedron*, 1987, **43**, 3931.
7 R. K. Hill and A. G. Edwards, *Tetrahedron*, 1965, **21**, 1501.
8 J. A. Berson, J. S. Walia, A. Remanick, S. Suzuki, P. Reynolds-Warnhoff and D. Willner, *J. Am. Chem. Soc.*, 1961, **83**, 3986.

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