Hydrolytic Resolution of Tertiary Acetylenic Acetate Esters With the Lipase from *Candida cylindracea*

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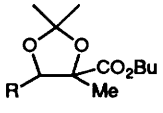
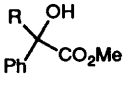
The kinetic resolution of a series of tertiary acetylenic acetate esters using the lipase from *Candida cylindracea* has been explored. Compounds **6c** and the trifluoromethyl acetate **6e** have been resolved with high enantiomeric enrichments. Several other tertiary acetate esters carrying a CF_3 group have been investigated which proved inert to enzymatic hydrolysis. From these results and published data, we are able to propose a predictive model for identifying the preferred enantiomer of secondary and tertiary trifluoromethyl acetate esters for this lipase.

The kinetic resolution of racemic esters by employing hydrolytic enzymes is a well established protocol for the synthesis of alcohols and carboxylic acids of high optical purity.[1] In general, the method is used to generate secondary alcohols or carboxylic acids in enantiomerically enriched form from either secondary acetates or α-monosubstituted carboxylic acid esters respectively. Resolutions of more highly substituted tertiary systems are limited although a number of examples of α,α-disubstituted carboxylic acid esters[2–4] such as **1**[5] and **2**,[6] and α-fluorinated malonate diesters[7,8] such as **3**[8] have been successfully hydrolysed and resolved using a variety of different lipases. α-Substituted *N*-acylamino acids **4**[9] have also been hydrolytically



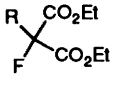
R = H, Me

1



R = CF_3, Me

2



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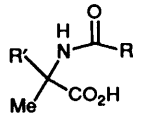

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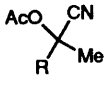

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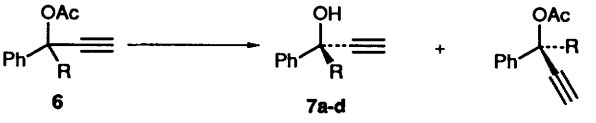
cleaved using acylases to generate α-substituted amino acids of high enantiomeric purity. On the other hand, examples of the resolution of tertiary acyl esters are rare. Recently,[10] however, a range of racemic α-acetoxy nitriles **5** were shown to be selectively hydrolysed using the micro-organism *Pichia miso* resulting in the enantiomeric enrichment of the residual acetate with the hydrolysed cyanohydrin collapsing to a ketone.

In this communication we report our results with the lipase from *Candida cylindracea* [Sigma Chem. Co. Type (VII)] where we have explored the hydrolysis of a range of tertiary acetylenic acetate esters giving rise to tertiary acetylenic alcohols. We have prepared as substrates the tertiary acetylenic acetates **6b–e** from ethynylmagnesium bromide[11] and the appropriate ketones. The resultant alcohols were acetylated[12] to afford **6b–e** and these compounds were then incubated in buffer (pH 7) with *C. cylindracea* lipase. For comparison, we have also included in Table 1 data on the secondary acetate ester **6a** prepared in the same way from benzaldehyde. The hydrolysis was followed in each case by ^{1}H NMR spectrometry until 40% conversion and it can be seen from Table 1 that the enzymatic hydrolysis gets progressively slower as the size of the R group increases. The secondary acetate **6a** was rapidly hydrolysed using the lipase, however with very poor resolution. Apparently little distinction is made by the enzyme between the hydrogen and acetylene functionalities. This is consistent with a previous study for a much wider range of secondary acetylenic acetate esters using the *C. cylindracea* lipase.[13] For substrates **6b–d** there was a competing non-enzymatic hydrolysis **6b** > **6c** = **6d**. This was particularly deleterious in the case of **6d** where the rate of enzymatic hydrolysis was slow (70 h/40%), however for **6c**, although the rate of non-enzymatic hydrolysis is comparable to **6d**, the increased rate of the enzymatic reaction allowed the alcohol **7c** to be recovered with a respectable enantiomeric enrichment (75% ee).† The most successful compound of the series was the trifluoromethyl acetate **6e** which was hydrolytically stable in buffer and proved to be an excellent substrate for the lipase. Both the resultant alcohol **7a** and residual acetate could be recovered in high ee after 40% conversion. The absolute stereochemistry of the predominant enantiomer of alcohol **7e** was determined to be *R* after conversion into methyl ether **8** and then oxidative cleavage of the acetylene with RuO_4[14] to afford predominantly the *S* enantiomer of Mosher's

† The absolute configuration was not determined.



6 **7a-d**

a R = H
b R = Me
c R = Et
d R = Pr
e R = CF_3

R **7e** *S* **6e**

Table 1

Substrate	Time (h) to achieve 40% conversion	% Non-enzymatic hydrolysis	E.e (%) alcohol	E.e (%) acetate	Enantiomeric ratio E
6a	1	—	—	9	
6b	7	11	0	0	
6c	11	3	75	31	>12
6d	70	25	11	8	
6e	23	0	87	75	>20

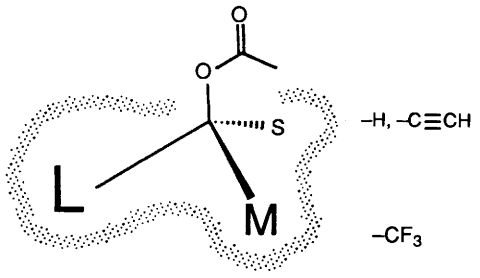
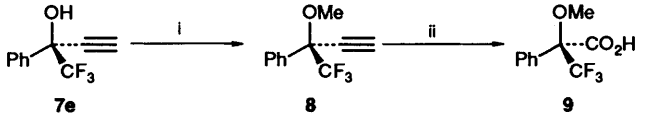


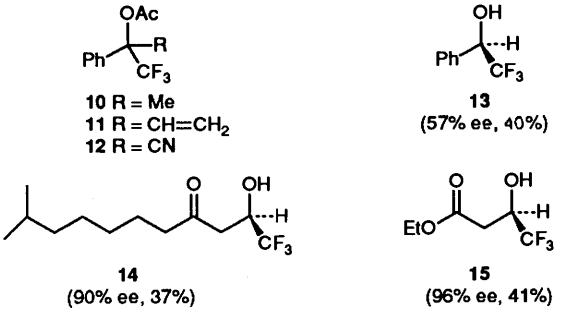
Fig. 1

acid **9** {$[\alpha]_D^{20}$ −42* (*c* 1.6, CH_2Cl_2), lit.,[15] −71.8 (*c* 1.6, EtOH)} (Scheme 1). Further, we have been able to obtain enantiomerically pure residual (*S*)-acetate **6e** (>98% ee) {$[\alpha]_D^{20}$ −27.37 (*c* 1.2, CH_2Cl_2)} by proceeding to a 60% conversion, although the reaction slows down considerably at *ca.* 50% conversion.



Scheme 1 i, NaH, MeI; ii, RuO_4, KIO_4, (CCl_4–MeCN–H_2O; 1:1:4)

It is noteworthy that replacement of the acetylene functionality of **6e** by methyl **10** vinyl **11**, or nitrile **12** resulted in compounds which were inert to lipase hydrolysis even after extended periods of time (>48 h). From our survey only hydrogen (secondary alcohols) and acetylene are accommodated with CF_3 and phenyl at the enzyme active site. That the acetylene of **6e** occupies the same space on the enzyme as the α-hydrogen atom of secondary trifluoromethyl acetate esters is consistent with the predominant *R* configuration of a range of trifluoromethyl alcohols[16] such as **13**,[16] **14**[16] and **15**[17] (ee's and extents of conversion are bracketed), which result after *C. cylindracea* (Lipase-MY) hydrolysis of their corresponding acetates. From these observations we can deduce a simple but useful model for the binding of the preferred enantiomer of secondary and tertiary trifluoromethyl acetate esters with the *C. cylindracea* lipase. The model has three binding sites, small (S), medium (M) and large (L) in the configuration shown in Fig. 1. The S-site is very specific. It accepts hydrogen and acetylene but would appear not to accommodate methyl, vinyl or nitrile groups as **10**, **11** and **12** were inert to enzymatic hydrolysis. The M-site accommodates the CF_3 group and the L-site is spacious and can accept a wide variety of substituents.[16] This model is consistent with and extends a previous one[18] which was deduced after analysis of a series of bicyclic secondary esters as substrates for the *C. cylindracea* lipase. The previous model could be considered to map the spacial constraints of the L-site in Fig. 1. We are currently exploring the extent to which non CF_3-containing compounds apply to our model such that a wider range of substrates can be tuned in a predictive way to provide further tertiary alcohols in high enantiomeric purity.



* $[\alpha]_D$ Values are expressed in units 10^{-1} deg cm^2 g^{-1}.

Experimental

Synthesis of Racemic Tertiary Acetates.—A solution of the appropriate ketone (29 mmol) in THF (20 cm^3) was added to a refluxing solution of either methyl-, vinyl- or ethynylmagnesium bromide (1.2 equiv.) in tetrahydrofuran (THF) (70 cm^3) and the mixture heated under reflux for 4 h or until all the ketone had been consumed (TLC). Water (20 cm^3) was added to the cooled reaction mixture after which the product was extracted into ether (3 × 100 cm^3). The combined ether extracts were washed with water (2 × 100 cm^3) and saturated brine (100 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was distilled to give the alcohol as a colourless oil (50–75%).

A solution of butyllithium (0.7 g, 11 mmol) in ether was added to a solution of the alcohol (10 mmol) in THF (50 cm^3) and the mixture stirred. After 15 min, acetyl chloride (0.9 g, 11 mmol) was added and the reaction mixture heated under reflux for 1 h before being cooled and quenched with water (20 cm^3). The reaction mixture was extracted into ether (2 × 50 cm^3), and the combined organic extracts were washed successively with water (2 × 50 cm^3), and brine (100 cm^3), dried ($MgSO_4$) and evaporated under reduced pressure. The residual material was distilled to provide the required acetates **6b–e** as colourless oils (55–70%).†

Lipase Hydrolyses.—The lipase from *Candida cylindracea* (Sigma Chem. Co. Type VII) (250 mg, 1010 units mg^{-1} solid) was added to a suspension of the acetates **6a–e** (100 mg) in phosphate buffer (pH 7) (10 cm^3) and the suspension was efficiently stirred at 25 °C. The course of the reaction could be monitored by extraction and work-up of aliquots (2 cm^3), followed by NMR or GC analysis. After a 40% conversion has been achieved, the reaction mixture was then extracted into ether (4 × 100 cm^3), and the combined organic extracts were washed with water (2 × 100 cm^3) dried ($MgSO_4$), and evaporated under reduced pressure to afford a mixture of alcohol and unhydrolysed acetate. Each component was purified using flash silica gel (Kieselgel 60) which was eluted with methylene dichloride to afford optically active products. Enantiomeric excesses were determined either directly on the residual acetates, or by derivatisation of the resultant alcohols **7** back to their acetates, by ^1H NMR spectrometry (and in the case of **7e** by ^{19}F NMR) in CCl_4 with added Eu(hfc)$_3$.

Acknowledgements

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† All alcohols and acetates gave satisfactory spectroscopic analysis.

References

1 H. G. Davis, R. H. Green, D. R. Kelly and S. M. Roberts, *Biotransformations in Preparative Organic Chemistry. The Use of Isolated Enzymes and Whole Cell Systems in Synthesis*, Academic Press, London, 1989.
2 M. Schneider, N. Engel and H. Boensmann, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 66.
3 T. Sugai, H. Kakeya and H. Ohta, *J. Org. Chem.*, 1990, **55**, 4643.
4 H. Moorlag, R. M. Kellogg, M. Kloosterman, B. Kaptein, J. Kamphuis and H. E. Schoemaker, *J. Org. Chem.*, 1990, **55**, 5878.
5 M. Pottie, J. Van der Eycken, M. Vandewalle, J. M. Dewanckele and H. Roper, *Tetrahedron Lett.*, 1989, **30**, 5319.

6 C. Feichter, K. Faber and H. Griengl, *J. Chem. Soc., Perkin Trans. 1*, 1991, 653.
7 T. Kitazume, K. Murata and T. Ikeya, *J. Fluorine Chem.*, 1986, **32**, 233.
8 T. Kitazume, T. Sato, T. Kobayashi and J. T. Lin, *J. Org. Chem.*, 1986, **51**, 1003.
9 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, 1249.
10 H. Ohta, Y. Kimura, Y. Sugano and T. Sugai, *Tetrahedron*, 1989, **45**, 5469.
11 J. March, *Advanced Organic Chemistry; Reactions, Mechanisms and Structure*, Wiley, New York, 3rd edn., 1985, p. 816.
12 E. M. Kaiser and R. A. Woodruff, *J. Org. Chem.*, 1970, **35**, 1198.
13 T. Oritani and Y. Yamashita, *Agric. Biol. Chem.*, 1980, **44**, 2407.
14 R. Zibuck and D. Seebach, *Helv. Chim. Acta*, 1988, **71**, 237.
15 J. A. Dale, D. L. Dull and H. S. Mosher, *J. Org. Chem.*, 1969, **34**, 2543.
16 J. T. Lin, T. Yamazaki and T. Kitazume, *J. Org. Chem.*, 1987, **52**, 3211.
17 T. Kitazume and J. T. Lin, *J. Fluorine Chem.*, 1987, **34**, 461.
18 Th. Oberhauser, K. Faber and H. Griengl, *Tetrahedron*, 1989, **45**, 1679.

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