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Desymmetrisation of Prochiral Ketones by Catalytic Enantioselective Hydrolysis of their Enol Esters using Enzymes.

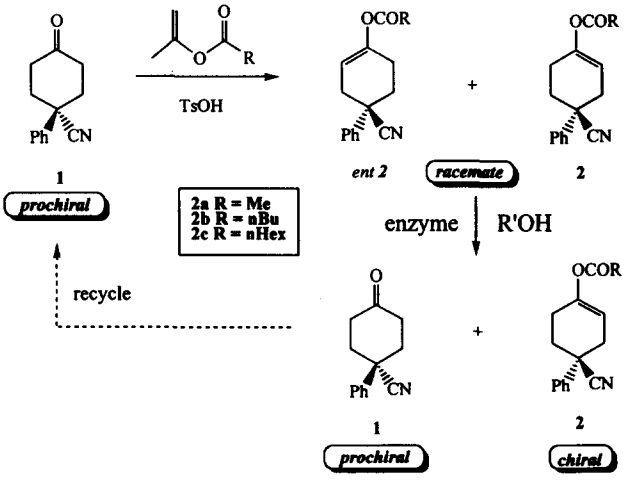
Andrew J. Carnell*, Jim Barkley and Amarjit Singh

Department of Chemistry, Robert Robinson Laboratories, University of Liverpool, Liverpool L69 7ZD

Abstract: Desymmetrisation of 4-cyano-4-phenylcyclohexanone **1** has been achieved by enzyme-catalysed enantioselective alcoholysis with n-butanol of the derived racemic enol acetate **2** in tetrahydrofuran. The absolute configuration of the enol acetate (-)-(S)-**2** (100% e.e.) obtained was determined by X-ray analysis of the camphanyl derivative **7**. © 1997 Elsevier Science Ltd.

The desymmetrization of prochiral ketones has become an important method for asymmetric synthesis. The use of chiral lithium amides for the asymmetric deprotonation of 4-substituted cyclohexanones and prochiral bicyclic ketones has been developed by several groups and Koga has recently described a catalytic version of the reaction.[1-3] The chiral product silyl enol ethers or enol esters are useful intermediates, the enol double bond providing a handle for maintaining the newly introduced asymmetry, for example by oxidative cleavage.[2] In connection with our synthetic programme we were interested in finding an enzymatic method for the desymmetrization of prochiral 4,4'-disubstituted cyclohexanones. allowing for the generation of a new remote chiral quaternary centre. Initial attempts to carry out an enzymatic Baeyer Villiger reaction on the commercially available model substrate 4-cyano-4-phenylcyclohexanone **1** with cyclohexanone monooxygenase or related enzymes were unsuccesful with no lactone formation observed.

Enzymatic hydrolysis of enol esters derived from α-substituted ketones has been described by Ohta and constitutes a process for asymmetric protonation.[4] Duhamel has recently reported lipse catalysed asymmetric hydrolysis of prochiral 3,3-disubstituted dienol diacetates derived from 2,2-disubtituted cyclohexa-1,3-diones to afford chiral keto enol esters.[5] We have developed a related strategy for the desymmetrization of prochiral ketone **1** whereby it is initially chemically converted to a racemic enol ester **2** (Scheme 1).



Scheme 1

Enzyme-catalysed enantioselective hydrolysis of **2** would regenerate the prochiral ketone leaving unreacted chiral enol ester. This approach constitutes the reverse of the asymmetric deprotonation - enolate trapping strategy and takes advantage of the wide availability of hydrolytic enzymes and mild reaction conditions typically associated with biotransformations. Enol esters **2a-c** were synthesised by acid catalysed reaction of the ketone **1** with the corresponding isopropenyl alkanoate. Initial screening of commercially available lipases for the hydrolysis of enol acetate **2a** (R = CH_3) in aqueous buffer (pH 7) resulted in conversion to the ketone with little or no enantioselectivity. However, transesterification with nBuOH (2 eq.) in tetrahydrofuran gave more promising results. Lipases from *Candida rugosa*, *C. antartica*, *Humicola* sp. and *Aspergillus niger* gave little or no conversion (<5%) over extended reaction times. *Burkholderia* sp. *Pseudomonas* sp. and Porcine pancreatic lipases (Boehringer Mannheim Chirazyme L-1, L-6 and L-7) showed limited selectivity, L-7 giving a very slow rate of conversion (Table 1). Of those enzymes screened, *Pseudomonas fluoresens* lipase (Amano AK) gave the most encouraging results giving, after 18h, a 67 % conversion to the ketone and 33% yield of optically pure (100% e.e. by Chiral HPLC, Chiracel OJ) enol acetate **2a**.

Table 1

Lipase[a]	Time (h)	Conversion to ketone **1** (%)	Enol acetate **2a** remaining (%)	E.e. (%) Enol acetate **2**
Burkholderia sp. (Chirazyme L-1)	15	70	30	51.4
Pseudomonas sp. (Chirazyme L-6)	45min 90min	53 95	47 5	28.8 100
Porcine pancreatic (Chirazyme L-7)	301	74	26	50.5
Pseudomonas fluorescens (Amano AK)[b]	9 18	49 67	51 33	75 100

a) Reactions were carried out in dry tetrahydrofuran with 2eq. nBuOH, enol acetate **2a** (10mg/ml) at room temperature
b) Reaction done under same conditions as (a) and enol acetate concentration of 100mg/ml.

We then examined the effect of solvent on selectivity with *Pseudomonas fluorescens* lipase. Reaction in each of the solvents shown in Table 2 gave enol acetate **2a** in the same enantiomeric series, but reaction times in order to obtain high e.e. varied considerably. All solvents were dried before use to minimise water activity (a_W) effects.[6] The optimum solvent was tetrahydrofuran giving optically pure enol acetate of 100% e.e. after 67% conversion. The substrate was insoluble in hexane and the reaction did not proceed at a useful rate. Interestingly, although the substrate was fully soluble in acetonitrile the reaction time was long (9 days) perhaps reflecting competitive binding of the cyano group of a solvent molecule in the active site.

Table 2

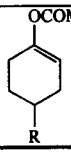
Solvent	Time (h)	Conversion to ketone **1** (%)	Enol acetate **2a** remaining (%)	E.e (%) Enol acetate
THF	18	67	33	100
Toluene	11.3	65	35	92.4
$^iPr_2O$	2.75	72	28	94.2
CH_3CN	9 days	62	38	89.4

Reactions were carried out in dry solvent with PFL, 1eq. nBuOH, enol acetate **2** (10mg/ml) at room temperature

In a preparative scale reaction in THF we were able to isolate the enol acetate **2a** in 23 % yield with a 95% e.e., $[\alpha]_D = -4.5^\circ$ (c = 1,$CHCl_3$) after flash column chromatography. The ketone produced as the hydrolysis product may be recycled through re-formation of the enol acetate giving, after two recycles, a 55% yield of optically active enol acetate.

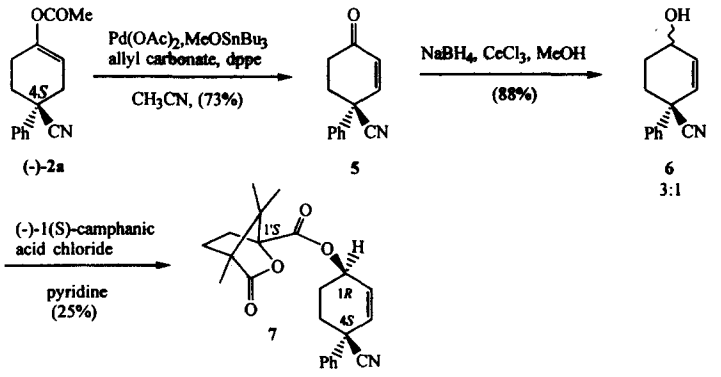
We have also examined the effect of chain length of the carboxylic acid component of the enol ester. With the n-butyl and n-hexyl substrates **2b** and **2c** the enantioselectivity was very similar to that obtained for the enol acetate **2a** requiring around 70% conversion to obtain optically pure enol ester. The possibility that the selectivity observed for this biotransformation depends on having a large and a small group in the 4-position of the parent ketone was probed by using substrates **3** and **4**. The enantioselectivity for substrate **3** was significantly lower and for substrate **4** only 12% at 50 % conversion as shown in Table 3. Chiral discrimination may well depend on having a large and a small (polar) group in the 4-position of the parent ketone.

Table 3

substrate	Time (h)	Conversion to ketone (%)	Enol ester **3** or **4** remaining (%)	E.e (%) Enol ester **3** or 4
 **3** R = Ph	57	84	16	100
4 R = Me	39.5	50.5	49.5	12

Reactions were carried out in dry tetrahydrofuran with 1eq. nBuOH, enol ester **3** or **4** (10mg/ml) at room temperature

In order to establish the absolute configuration of the optically active enol ester **2a** we converted it into the crystalline camphanyl derivative **7** *via* the series of reactions shown in Scheme 2. Initially the enol ester (-)-**2a** was converted using a modification of Tsuji's conditions[7] to the enone **5** in 73 % yield with no loss of optical purity as evidenced by HPLC. Luche reduction ($NaBH_4$, $CeCl_3$) of the enone **5** afforded an inseparable 3:1 mixture of two diasteriomeric alcohols **6** in 88 % yield. Subsequent derivatisation with (-)-(1S) camphanic acid chloride afforded **7** (25%) which was crystallized from hexane/ethyl acetate to provide a suitable crystal for X-ray analysis (Fig 1). By reference to the 1'(*S*) camphanyl moiety the absolute configuration of the cyclohexenyl system is 1*R*, 4*S*. It was thus possible to assign by correlation the absolute configuration of the biotransformation product (-)-**2a** as 4*S*.



Scheme 2

The selectivity which is observed for this reaction raises important questions about the nature of the enzyme-substrate interaction upon binding. If one assumes that the phenyl group occupies an equatorial position and binds to a large hydrophobic pocket in the active site and that a smaller binding site accommodates the more polar cyano group, then enantiomeric differentiation is solely dependent on the position of the enol double bond and the resultant small change in

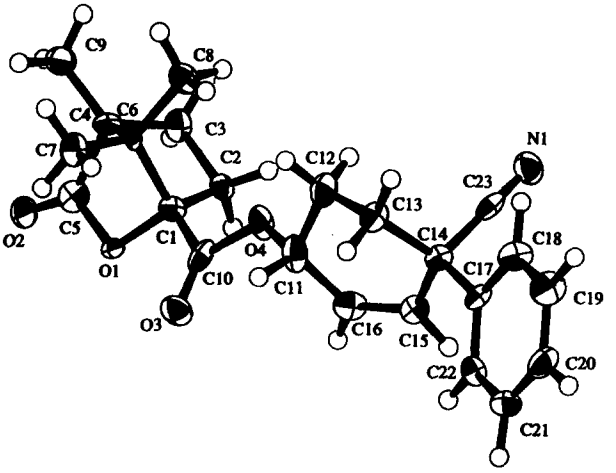


Fig. 1. X-ray Crystal Structure of Camphanyl Derivative 7 showing the crystallographic numbering.

the ring conformation "experienced" by the enzyme. Further work in our group will be aimed at exploring the scope of this reverse enzymatic desymmetrization reaction for other symmetrical ketones and exploitation of the chiral enol esters and derived enones in synthesis.

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