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Asymmetric Synthesis by Enzymatic Hydrolysis of Prochiral Dienol Diacetate.

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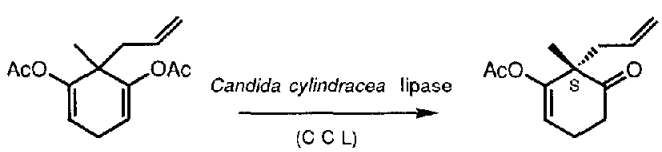
Abstract: Enzymatic hydrolysis of prochiral dienol diacetate **1** by *Candida cylindracea* lipase (C C L) leads to the keto enol acetate **2** in high yield with an enantiomeric excess > 98 %. The S configuration of the asymmetric center was assigned.

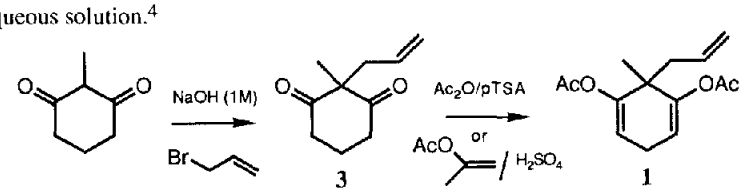
Enzymes have been widely used as chiral catalysts in organic synthesis.[1] Hydrolytic enzymes especially lipases and esterases are among the most useful enzymes for enantioselective transformations. In most cases the hydrolysis of racemic ester or the esterification of racemic alcohol leads to the resolution of the starting material. There are only a few reports of hydrolysis of enol esters.[2,3] The kinetic resolution of enol acetates [2a,b] and an asymmetric synthesis by an enantioface-differenciating enzymatic hydrolysis leading to a chiral center from a sp^2 carbon [2c-e] have been reported.

In this paper we report an asymmetric synthesis of the keto enol acetate **2** bearing a chiral center on a sp^3 carbon, in high yield and high enantiomeric excess, by enzymatic hydrolysis of the prochiral dienol diacetate **1** .



1 **2**

The dienol diacetate **1** was prepared from the commercially available 2-methylcyclohexane-1,3-dione as described above. Diketone **3** was prepared by allylation of the starting diketone in high yield in basic aqueous solution.[4]

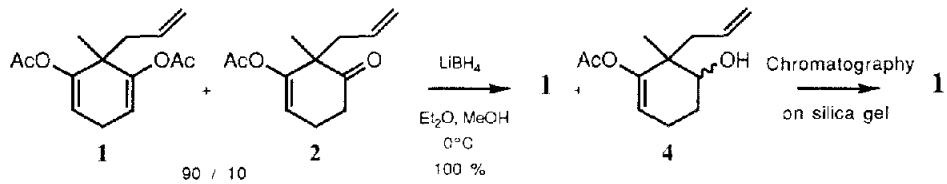


3 **1**

87 % 76 %

The ketone **3** treated with acetic anhydride in the presence of pTSA (in a catalytic amount) or with isopropenyl acetate in the presence of H_2SO_4 (in a catalytic amount) leads to the dienol diacetate **1**.

There was also a few percent of keto enol acetate **2** present. The separation of compound **1** from **2** was not obvious by chromatography on silica gel, so we chose to reduce the carbonyl moiety of compound **2** . $LiBH_4$ proved to be a selective reagent since only the carbonyl function was reduced. The alcohol **4**[5] obtained was easily separated from the dienol diacetate **1** by flash chromatography on silica gel (overall yield of dienol diacetate **1** : 60 - 66 %).



For the enzymatic hydrolysis, two lipases and one esterase[6] were tried. The hydrolysis was performed in phosphate buffer with the use of a pHstat to control the pH. The acetic acid produced during the hydrolysis was neutralized by an aqueous solution of sodium hydroxide (1N)[7]. The progress of the reaction was followed by GC analysis of samples of the reaction mixture (previously extracted with ethyl ether) and from the quantity of the aqueous solution of sodium hydroxide introduced into the suspension. Results are summarized in the Table.

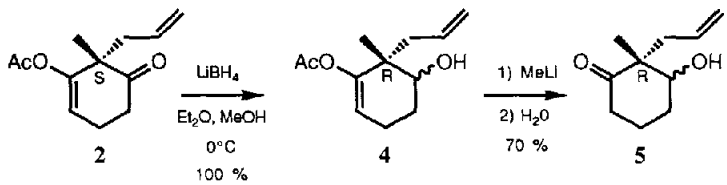
Table : Enzymatic hydrolysis of dienol diacetate **1**.

Entry	Enzyme[a]	Temp. °C	Conversion %	Time (hours)	Yield (%)	ee %	Absolute configuration
1	H L E	RT	50	2	45	< 5	S
2	P F L	30	45	18	40	30	R
3	C C L	28	80	23	75	> 98	S
4	C C L	32	80	14	74	> 98	S
5	C C L	35	100	19	80	> 98	S
6	C C L	40	100	14	**80**	**> 98**	**S**

a) H L E : Hog Liver esterase (3.1.1.1) 108,5 U / mg; P F L : *Pseudomonas Fluorescens* Lipase SAM-2 EC (3.1.1.3) 42 U / mg; C C L : *Candida cylindracea* Lipase EC (3.1.1.3) 36 U / mg. All the experiments were performed with the following enzyme units for one mmol of substrate : H L E : 80 U, P F L : 160 U, C C L : 225 U .

Hog liver esterase (H L E) and *Pseudomonas fluorescens* lipase (P F L) (entry 1-2) gave poor enantiomeric excess. But *Candida cylindracea* lipase (C C L) (entry 3-6) afforded in good yield the keto enol acetate **2** with a very high enantiomeric excess. As expected, the reaction time is reduced when the temperature is increased. The best result was obtained at 40°C (entry 6) leading to an enantiomeric excess > 98%. The enantiomeric excess for the keto enol acetate **2** was determined by ^1H NMR spectroscopy.[8]

The absolute configuration of compound **2** was established as described in the following scheme. The optically active keto enol acetate **2** was reduced by $LiBH_4$ leading to the alcohol **4** in a 73/27 diastereomeric ratio. The preparation of the known optically active allyl ketols **5**,[9] was performed by addition of 3 equivalents of methyl lithium[10] to the non-purified enol acetate **4** followed by an aqueous treatment.[11] The same diastereomeric ratio was observed for compounds **4** and **5**. Comparison with the literature leads us to assign the S configuration to the sp^3 carbon of compound **2**.[12]



2R,3R/2R,3S : 73 / 27 **2R,3R/2R,3S** : 73 / 27

In conclusion, a new type of asymmetric synthesis by enzymatic hydrolysis of a prochiral dienol diacetate **1** has been achieved. The prochiral dienol diester **1** is hydrolysed by *Candida cylindracea* lipase to give the optically pure (S) enol ester **2**. This work is currently being developed with some other structures, particularly with benzyl dienol diacetate; first results are also showing high enantiomeric excess, demonstrating the versatility of this procedure. The application to synthesis of these valuable intermediates is now in progress.

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5. Diastereoisomeric ratio erythro/threo : 27/73

6. Enzymes supplier : Fluka.

7. General procedure for enzymatic hydrolysis :

The enzyme was added to a suspension of dienol diacetate **1** (1 mmol-250 mg) in phosphate buffer KH_2PO_4/Na_2HPO_4 0.1 M (5 mL). The reaction was performed at pH 7 (with a pHstat to maintain the pH value) and at the desired temperature (the temperature was maintained with the aid of a thermostated bath). When the desired % conversion was obtained the mixture was extracted with Et_2O, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residual oil was chromatographied on silica gel (eluent ethyl ether/petroleum ether : 0/100 to 5/100) to give the keto enol acetate **2.** For the entry 6 : $[\alpha]_{436}^{25} = 21$, c = 1, CH_2Cl_2.

An enzymatic hydrolysis was also performed on a larger scale (1 g - 4 mmol of dienol diacetate **1** with 25 mg of C C L) at 40 °C. The reaction time was longer (68 h) than the one corresponding to the hydrolysis of 1 mmol of substrate but the yield and enantiomeric excess were the same in both cases.

8. The enantiomeric excess was determined by ^1H NMR (360 MHz) in the presence of a chiral NMR shift reagent : tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato] euro-pium (III) or tris[3-(heptafluoropropylhydroxymethylene)-(d)-camphorato] prasco-dymium (III).

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11. Try to hydrolyse enol acetate **4** by HCl 3N does not lead to the ketol **5**, compound **4** was recovered in high yield.

12. Absolute configuration of ketol **5** : the compound **5** was obtained in a 73 / 27 diastereomeric ratio (determined by GC). According to the literature [9] the calculed optical activity of the mixture is - 24.6 [$\alpha = 0.73(-32) + 0.27(-4.7)$] in $CHCl_3$ at 23 °C The optical rotation found was : $[\alpha]_D^{23} = -24$, c = 0.6, $CHCl_3$. So we have assigned the R configuration to the ketol **5** and thus the S configuration to the keto enol acetate **2**.