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One-pot deracemisation of an enol acetate derived from a prochiral cyclohexanone

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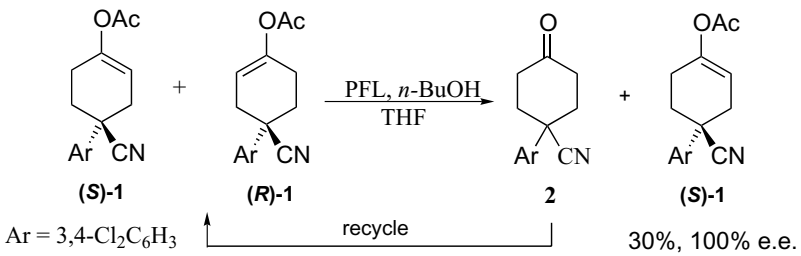
Abstract—A one-pot method for the deracemisation of the enol acetate **1** derived from the prochiral 4,4-disubstituted cyclohexanone **2** has been developed using the combination of *Pseudomonas fluorescens* lipase and potassium *t*-butoxide/isopropenyl acetate to give the enantiomerically pure enol acetate (*S*)-**1** in 82% yield. Calculations based on the inherent enantioselectivity of the lipase (*E*) allowed an estimation of the optimum theoretical conversion for each enzyme step prior to recycling the ketone.

1. Introduction

The resolution of esters using hydrolytic enzymes such as lipases, proteases and esterases is well established for the production of enantiomerically enriched esters, acids and alcohols.[1] These enzymes can be used in organic solvents to catalyse the reverse reaction, formation of esters, in which case it is advisable to use an irreversible acyl donor such as isopropenyl acetate (IPPA) to drive the reaction.[2] Dynamic kinetic resolution (DKR) has been employed successfully where the substrate is amenable to in situ racemisation, resulting in high yields of single enantiomer products.[3] We have been exploring the resolution of enol esters **1** derived from prochiral ketones **2** such that the enol ester **1** is resolved in an enantioselective step forming an enol as the initial product, which then tautomerises to the parent prochiral ketone **2** (Scheme 1).[4,5] Depending on the inherent enantioselectivity of the enzyme (*E*), the reaction can be stopped at an appropriate point to recover enantiomerically pure enol ester **1**. In principle a very high *E* value for a given reaction is not a prerequisite for achieving high yields of the enol ester since the prochiral ketone can be recycled to the racemic enol ester. We envisaged that if this recycle could be achieved in an efficient manner in one-pot then the two-step process becomes equivalent to a dynamic kinetic resolution of the enol ester and a desymmetrisation of the prochiral ketone.

2. Results and discussion

In order to achieve the proposed cyclic deracemisation we considered three options: (1) the enzyme and chemical recycle are performed simultaneously, (2) the enzyme and recycle reactions are performed in a two stage process without separation of the enol ester **1** and the ketone **2** and (3) the ketone is separated from the



Scheme 1.

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enol ester after each enzyme reaction and the ketone is recycled. Initial experiments focussed on the most desirable option (1), in which case the conditions for recycling the ketone would need to be compatible with the enzyme. To this end we investigated the use of solid acids (Nafion, Dowex 50X8-100 cation exchange resin, Montmorillonite clay KSF or K10) for the enol ester formation with isopropenyl acetate or acetic anhydride as the acyl donor in a range of solvents. Unfortunately, we observed little (<10%) or no conversion at room temperature or at elevated temperature with these catalysts. Another serious concern was the competition for the enzyme between the acylating agent and the enol ester **1**. We then turned our attention to option (2), which would allow us to temporarily remove the enzyme and recycle the ketone using conditions that we knew would give near quantitative conversion to the enol ester. In this case, upon recycling the ketone after each enzyme step we would be generating an enantiomerically enriched enol ester as the substrate for the next enzyme cycle. For a cyclic enzyme resolution of this type it can be derived[6] that the maximum theoretical enantiomeric excess for 100% yield is:

$$\text{e.e.}_{\max} = (E-1)/(E+1)$$

Thus, for an enzyme resolution with an E value of 13, the $\text{e.e.}_{\max} = 85.7\%$. A higher e.e. in a stepwise process is possible only if the yield is compromised. The question then arises, at which point should each kinetic resolution of each cycle be stopped in order to minimise the number of cycles needed for deracemisation? In the first enzyme reaction the e.e. reaches a theoretical 85.7% ($\text{e.e.}_{\max}$) at 56% conversion. It has been shown mathematically[7] that to obtain the highest e.e. after the ketone recycling step the preceding kinetic resolution should be stopped at $\text{e.e.}_{\max}$ of a cyclic process, thus for $E=13$ this corresponds to a theoretical e.e. of 85.7% at 56.5% conversion. From the simulation (Fig. 1) it can be seen that in fact the optimum window is quite broad. It can easily be calculated that after the first ketone recycle the e.e. of **1** equals 37.3%. Thus, the second cycle starts with a theoretical e.e. of 37.3%, which corresponds to a conversion of 31.6% in a kinetic resolution when $E=13$ (see Fig. 1). To reach an e.e. of 85.7% this time, the reaction must be run to a conversion of 24.9% (56.5–31.6). From this data the e.e. after the second cycle can be calculated, and so forth. The theoretical values for cycles 1–5 for this E value are given in Table 1 and Fig. 1.

On considering option (3) we felt that losses due to chromatographic separation of the enol ester and ketone would be too great. Before putting the proposed cyclic reaction scheme into practice it was important to establish that the ketone could be recycled efficiently under conditions which would not be deleterious to subsequent enzyme reactions in terms of diminished enzyme activity or selectivity. In addition it was necessary to show that the enzyme could be re-used over a number of cycles with reproducible selectivity. Formation of the enol ester **1** was carried out with potassium *tert*-butoxide and isopropenyl acetate in THF at room temperature in 95–100% yield. Crucially, when the enzyme reaction was run in the presence of up to 6 molar equivalents of *tert*-butanol and acetone (expected by-products from the enol ester formation after acidification), there was no change in activity or selectivity of

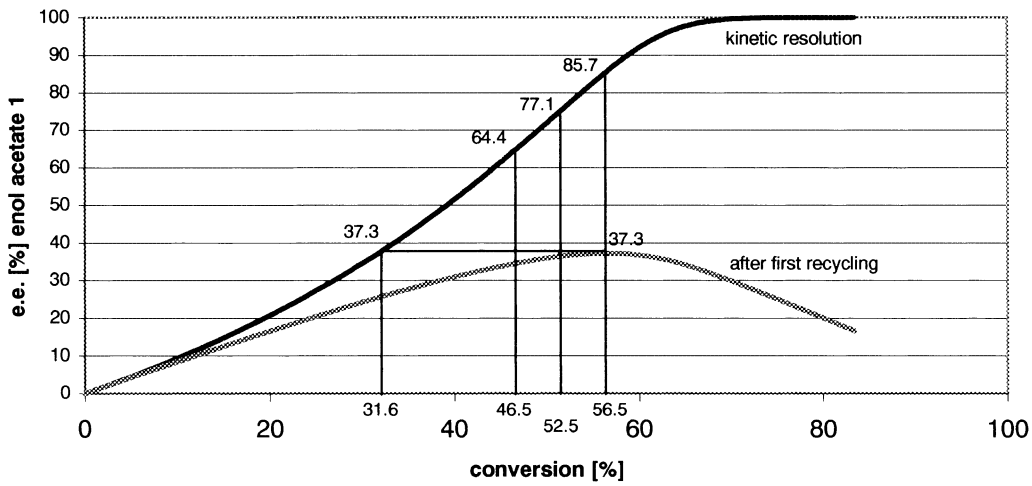
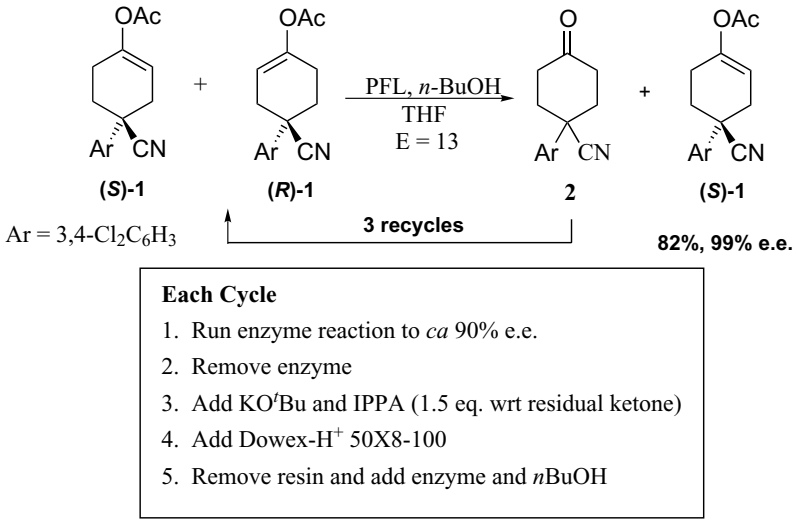


Figure 1. Enantiomeric excess (e.e.) of enol acetate **1** after recycling of the ketone **2** versus conversion for $E=13$.

Table 1. Theoretical conversions and e.e.'s for deracemisation

Cycle	% e.e. **1** (start)	% e.e. **1** (end)	% Conversion to ketone **2** needed
1	0	85.7	56.5
2	37.3	85.7	24.9
3	64.36	85.7	10
4	77.1	85.7	4
5	82.27		



Scheme 2.

Table 2. Deracemisation of enol ester **1** over four cycles

Cycle	% e.e. residual enol ester **1**	% Conversion to ketone **2**
1	92	61
2	86	28
3	87	16
4	>99	12

For the ketone recycle, KO'Bu and IPPA (1.5 equiv. with respect to residual ketone) was used.

the enzyme. The enzyme could be re-used in free or immobilised form with no loss in activity or enantioselectivity after four cycles.

In practice we were able to carry out the deracemisation as planned with some slight variation in the predicted values for conversion and e.e. after each cycle (Scheme 2, Table 2).[8] These variations were due to the difficulty in stopping the reaction at the precise conversion predicted in Table 1 and the incomplete conversion of ketone to enol ester (ca. 5% residual ketone) in the recycle, meaning that the starting e.e. for the subsequent enzyme reaction would be higher than the theory. The enzyme reaction was run to the required degree of conversion. The enzyme was removed by filtration, the appropriate amount of potassium *tert*-butoxide and isopropenyl acetate added and the reaction stirred for 3 h. Dowex H^+ 50X8-100 cation exchange resin was added to neutralise the basic conditions and then removed by filtration. The enzyme was then replaced along with *n*-butanol for the next enzyme reaction.

On the fourth cycle, the enzyme reaction was run beyond the calculated end-point in order to obtain enantiomerically pure material. In this way we obtained 330 mg (82%) of the enol ester **1** in >99% e.e. starting from 400 mg of the racemic material over four cycles. Obviously, with a higher *E* value the number of cycles could be reduced and the yield would be higher. We are currently exploring the use of directed evolution to create PFL mutants with higher enantioselectivity for this class of substrates, which are key intermediates in the synthesis of an important class of tachykinin NK-2 antagonists.[9]

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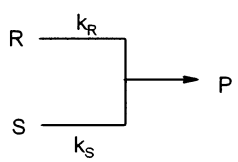
7. 

For a kinetic resolution, assuming that it follows first order kinetics and is irreversible, no enzyme inhibition or enzyme destruction occurs, and the temperature is not varied, the following equations describe the above scheme: S, R, and P are the concentrations of (S)-**1** and (R)-**1** and ketone **2** at time t.

S_0 and R_0 are the initial concentrations, whereby $R_0 = S_0$ for the first cycle (starting from a racemate)

k_S and k_R are the first order rate constants (arbitrary $k_R > k_S$)

$$S = S_0 * \exp(-k_S^* t) \quad (1)$$

$$R = R_0 * \exp(-k_R^* t) \quad (2)$$

$$P = S_0[1-\exp(-k_S^* t)] + R_0[1-\exp(-k_R^* t)] \quad (3)$$

$$\text{e.e.}_{\text{after recycling}} = (S+P/2-R-P/2)/(S+R+P) \quad (4)$$

Substituting S, R and P with Eqs. (1)–(3) and with $R_0 = S_0$ one obtains:

$$\text{e.e.}_{\text{after recycling}} = [\exp(-k_S^* t) - \exp(-k_R t)]/2 \quad (5)$$

Setting the first derivation

$$\text{d(e.e.}_{\text{after recycling}})/\text{d}t = [-k_S \exp(-k_S^* t) + k_R \exp(-k_R t)]/2$$

equal to zero to calculate the optimum one obtains:

$$k_R/k_S = \exp[(-k_S + k_R)t] \quad (6)$$

Since k_R/k_S equals E (enantioselectivity) and the right side equals S/R one obtains with $S/R = (\text{e.e.}+1)/(1-\text{e.e.})$: e.e. (optimum to stop the reaction) $= (E-1)/(E+1)$.

8. Typical in situ recycle protocol: To a solution of enol acetate (400 mg, 1.29 mmol) in dry THF (50 cm^3) was added n-butanol (0.15 cm^3) and lipase from *Pseudomonas fluorescens* (50 mg). The solution was shaken at 32°C until the desired extent of conversion was obtained (see Table 2) (GC: column SE-30, 255°C, carrier gas helium 70 kPa, FID detection: Retention time; ketone 11.2 min, enol acetate 16.2 min). The solution was then filtered and 1 cm^3 removed, concentrated under reduced pressure and re-suspended in propan-2-ol. The enantiomeric excess of enol acetate was determined (Chiral HPLC: Chiralpak AD column, eluant 100% ethanol, flow rate 0.35 cm^3/min, $\lambda_{obs} = 220$ nm: Retention time (R) enantiomer 28.9 min, (S) enantiomer 34.8 min). The analysed sample was re-suspended in THF and returned to the reaction mixture. To the solution was added isopropenyl acetate (1.5 equiv. wrt ketone **2**) followed by potassium *tert*-butoxide (1.5 equiv. wrt ketone **2**). The mixture was stirred at rt for 3 h, the reaction quenched by the addition of Dowex H^+ 50X8-100 cation exchange resin and the resin removed by filtration through a pad of flash chromatography silica gel.
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