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# Enzymatic desymmetrisation of (2-hydroxymethyl-oxiranyl)-methanol in organic solvents

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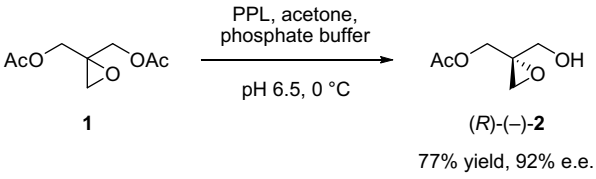
ABSTRACT

The desymmetrisation of (2-hydroxymethyl-oxiranyl)-methanol has been achieved in excellent enantiomeric excess in organic solvents, to access (*S*)-(+)-(2-(hydroxymethyl)oxiran-2-yl)methyl acetate, for the first time. This work provides a complementary method to the desymmetrisation of the corresponding diacetate in aqueous buffer which yields the (*R*)-enantiomer and, as such, this new method should enable sequential chemical reactions to be carried out without the need for isolation of the chiral product.



## 1. Introduction

The enzymatic hydrolysis of acetic acid 2-acetoxymethyl-oxiranylmethyl ester **1** (Scheme 1) to enantiomerically pure mono-acetate (*R*)-(−)-**2** is an important step in the asymmetric synthesis of tertiary alcohols.[1,2] Moreover, protecting group manipulations enable the two enantiomers of **2** to be interconverted, and this feature has provided a useful building block for the enantioselective synthesis of both enantiomers of the insect pheromone frontalin.[1] However, interconverting the enantiomers of **2** inevitably adds extraneous steps to a synthesis which not only increases the time and associated costs, but also increases the waste produced.[3] Moreover, to date, the production of (*R*)-(−)-**2** has solely been carried out in a potassium phosphate buffer, which means that a necessary isolation step must be implemented before subsequent (potentially water sensitive) reactions can be performed.



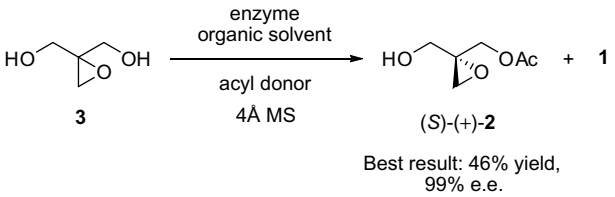

**1** (*R*)-(−)-**2**

77% yield, 92% e.e.

**Scheme 1.** Enzymatic desymmetrisation of *meso*-diacetate **1**.[1]

Consequently, we felt that there was a need to find a suitable biocatalyst to gain direct access to the synthetically useful building block, (*S*)-(+)-**2**, by performing the desymmetrisation of (2-hydroxymethyl-oxiranyl)-methanol **3** in organic solvents.[4] The use of organic solvents would then allow subsequent reactions to be telescoped, and thus prevent the expensive steps of isolation and purification of the chiral epoxides prior to derivatisation.

Herein, we report our results of screening a variety of hydrolases under different experimental conditions for their activity and selectivity in the asymmetric desymmetrisation of (2-hydroxymethyl-oxiranyl)-methanol **3**, Scheme 2.




**3** (*S*)-(+)-**2**

Best result: 46% yield, 99% e.e.

**Scheme 2.** Enzymatic desymmetrisation of *meso*-diol **3**.

## 2. Results and discussion

The syntheses of the substrate, *meso*-diol **3**, and the racemic standard, (±)-**2**, are shown in Scheme 3.

Oxidation of 2-methylene-1,3-propanediol **4** using *meta*-chloroperbenzoic acid gave the desired epoxide **3** in a pleasing 96% yield. Whereas the synthesis of (±)-**2** proved to be less efficient, we still isolated the mono-acetate in good yield (63%) over the two steps as shown in Scheme 3.

For the initial identification of a biocatalyst able to desymmetrise *meso*-diol **3** we screened a number of commercially available enzyme preparations[5] and used them to carry out lab-scale (100 mg of **3**) reactions.[6]

The enantiomeric excess was determined by gas chromatography and the preliminary results, as summarised in Table 1, demonstrated that Amano L, AK was the most selective enzyme against

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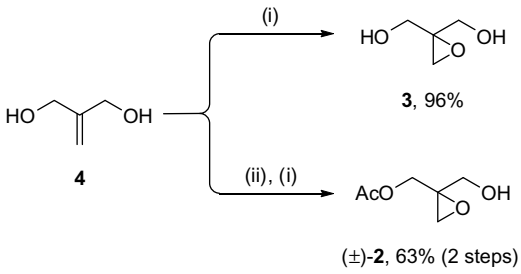



**Scheme 3.** Synthesis of substrate and racemic standard. Reagents and conditions: (i) *m*-CPBA (1.1 equiv), DCM; (ii) AcCl (0.5 equiv), TEA (1.1 equiv), DMAP (0.1 equiv), DCM.

**Table 1**
Results of the enzyme screen

| Entry | Enzyme[a] | Enantiomeric excess[b] (%) | Mono-acetate yield[c] (%) |
|---|---|---|---|
| 1 | CAL-B | 20 | 12 |
| 2 | PPL | 19 | 21 |
| 3 | CRL | 0 | N/D |
| 4 | Amano L, AK | 71 | 31 |
| 5 | Amano L, PS | 66 | 62 |

Reactions were carried out in dichloromethane (0.1 M) at room temperature using *meso*-diol **3** (100 mg) acetic anhydride (1 equiv) and 1 wt equiv of the enzyme preparation.
[a] CAL-B—*Candida antarctica* lipase B; PPL—porcine pancreatic lipase; CRL— *Candida rugosa* lipase; Amano L, AK—*Pseudomonas fluorescens*; Amano L, PS—*Pseudomonas cepacia*.
[b] As determined by GC analysis of purified material.
[c] Isolated yields, N/D = not determined.

this substrate. The reaction took 4 h and gave the mono-acetate (*S*)-(+)-**2** in 31% isolated yield and 71% ee (entry 4).[6]

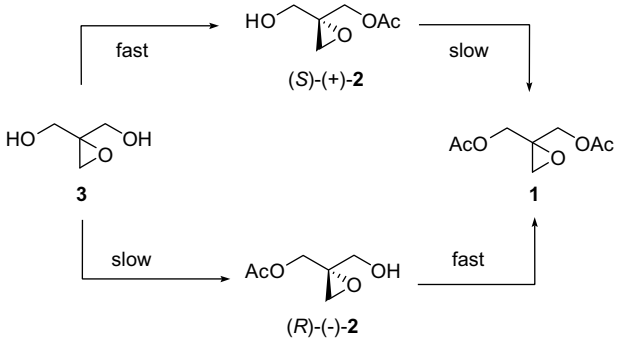
Over the course of this study we performed extensive experiments using these enzymes, and the following general conclusions can be made: (1) the highest ee's obtained for the desymmetrisation were found using dichloromethane as the solvent;[7] (2) the higher the temperature, the better the ee, that is, over the temperature range 0 °C→rt→30 °C→37 °C the ee increased 67%→71%→82%→88% using Amano L, AK; (3) of the acyl donors tested (vinyl acetate, acetic anhydride, *iso*-propyl acetate and ethyl acetate), acetic anhydride turned out to be the acyl donor of choice.

Based on this empirical data we proceeded with this study, using Amano L, AK and monitored the reaction over a 1-h time course (Table 2). In this study it was found that the yield of the mono-acetate (*S*)-(+)-**2** builds up to a maximum conversion of 48% (82% ee) (Table 2, entry 2), the product of an enantioselective biotransformation, before being consumed (entries 3–7) over the course of a subsequent kinetic resolution, Scheme 4.[8] As the result

**Table 2**
Results of the reaction monitored over time

| Entry | Time (min) | Enantiomeric excess[a] (%) | Mono-acetate conversion[a] (%) | Diacetate conversion[a] (%) | Diol conversion[a] (%) |
|---|---|---|---|---|---|
| 1 | 5 | 65 | 32 | 14 | 54 |
| 2 | 10 | 82 | 48 | 46 | 6 |
| 3 | 20 | 95 | 44 | 56 | 0 |
| 4 | 30 | 98 | 40 | 60 | 0 |
| 5 | 40 | 99 | 36 | 64 | 0 |
| 6 | 50 | 99 | 34 | 66 | 0 |
| 7 | 60 | 99 | 31 | 69 | 0 |

Reactions were carried out in dichloromethane (0.1 M) at 37 °C using acetic anhydride (1.8 equiv) and 2 wt equiv of Amano L, AK.
[a] As determined by GC analysis of the crude reaction mixture.




**Scheme 4.** Outline of the two-step process leading to a high enantiomeric excess of (S)-(+)-**2**.

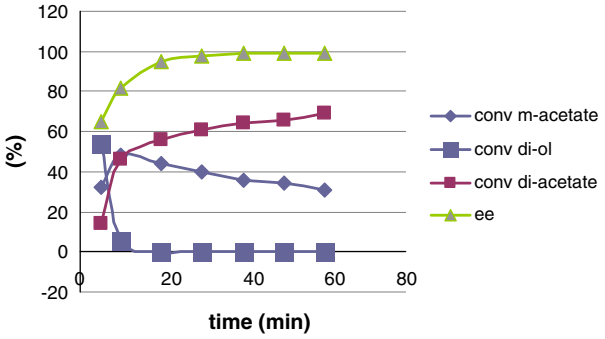



**Figure 1.** Graphical representation of the data in Table 2.

**Table 3**
Results of the reaction monitored varying the molar equivalents of acetic anhydride over 1 h

| Entry | Acyl donor (equiv) | Enantiomeric excess[a] (%) | Mono-acetate yield[b] (%) | Diacetate yield[b] (%) |
|---|---|---|---|---|
| 1 | 1.0 | 67 | 52 | 7 |
| 2 | 1.2 | 75 (74)[c] | 67 | 10 |
| 3 | 1.5 | 98 (99) | 50 | 19 |
| 4 | 1.8 | 97 (99) | 46 | 19 |
| 5 | 2.0 | 99 (99) | 46 | 21 |

Reactions were carried out in dichloromethane (0.1 M) at 37 °C and 2 wt equiv of Amano L, AK.
[a] As determined by GC analysis of purified material.
[b] Isolated yields.
[c] Numbers in parentheses represent the ee from the crude reaction mixture by GC.

of this two-step process, the enantiomeric excess being a function of the conversion, the enantiomeric excess increased to 99% with a conversion of 36% within 40 min (Table 2, entry 5 and Fig. 1).

Further optimisation reactions, in which we varied the molar equivalents of the acyl donor, allowed us to finally isolate the desired mono-acetate (*S*)-(+)-**2** in 46% isolated yield and 99% ee (Table 3, entry 5).

## 3. Conclusions

Herein, we have shown that the enzyme Amano L, AK from *Pseudomonas fluorescens* enables enantiopure mono-acetate (*S*)-(+)-**2** to be isolated in up to 46% yield and 99% enantiomeric excess. This result provides a complementary method for the synthesis of the opposite enantiomer achieved by hydrolysis in aqueous buffer,[1] without resorting to tedious and expensive protecting group manipulations. We predict that the desymmetrisation in organic solvents

will enable subsequent one-pot reactions to be carried out to further streamline syntheses using this highly functionalised building block. Our efforts in this area will be reported in due course.

## 4. Experimental

### 4.1. General

Commercially available reagents were used as received without purification. Analytical thin layer chromatography was performed with Keiselgel 60 $F_{254}$, in a variety of solvents on aluminium-backed plates. The plates were visualised by UV light (254 nm) and *p*-anisaldehyde. Flash column chromatography was conducted with Merck Silica Gel 60H (40–60 μm, 230–400 mesh) under bellows pressure. Nominal mass spectra were recorded on a Waters LCT mass spectrometer connected to a Waters Alliance 1100 LC autosampler and controlled by WATERS MASSLYNX 4.1 and OPENACCESS software using electrospray (ES) ionisation. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance 300 (300 MHz) or a Bruker DPX 400 (400 MHz) spectrometer. All chemical shifts ($\delta$) are quoted in parts per million (ppm) relative to a calibration reference of the residual protic solvent; C*H*Cl$_3$ ($\delta_H$ 7.24, s) was used as the internal standard in $^1$H NMR spectra, and $^{13}$C NMR shifts were referenced using C*D*Cl$_3$ ($\delta_C$ 77.4, t) with broad band decoupling. The following abbreviations were used to define the multiplicities: s, singlet; d, doublet; m, multiplet.

### 4.2. Synthesis of (2-hydroxymethyl-oxiranyl)-methanol, 3

To a solution of 2-methylene-1,3-propanediol **4** (2.47 g, 28.0 mmol) in dichloromethane (280 ml, 0.1 M) were added *m*-CPBA (7.6 g, 30.8 mmol). The solution was stirred at room temperature for 4 h. The mixture was reduced in vacuo and the crude material purified by column chromatography ($SiO_2$; 100% EtOAc) to yield the title compound as a colourless oil (2.8 g, 96 %). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$ 2.12 (2H, dd, $J$ = 5.4 and 7.4 Hz), 2.88 (2H, s), 3.72 (2H, dd, $J$ = 7.4, 12.4 Hz), 3.89 (2H, dd, $J$ = 5.4, 12.4 Hz); $^{13}$C NMR (75 MHz) 49.1 ($CH_2$), 60.1 (C), 62.6 ($CH_2$); MS ES (+ve) found $m/z$ 231 ([2M+$^{23}$Na]$^+$, 100%).

### 4.3. Synthesis of (±)-(2-(hydroxymethyl)oxiran-2-yl)methyl acetate, 2

To a solution of 2-methylene-1,3-propanediol **4** (3.0 g, 34.0 mmol) in dichloromethane (340 ml, 0.1 M) was added 4-dimethylaminopyridine (208 mg, 1.70 mmol), triethylamine (3.6 ml, 25.5 mmol) and acetyl chloride (1.2 ml, 17.0 mmol). The solution was stirred at room temperature for 16 h. The mixture was reduced in vacuo to yield an oil which was purified by column chromatography ($SiO_2$; 60% EtOAc in petroleum ether) to yield the mono-acetylated compound as a colourless oil (1.4 g, 63 % yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta_H$ 2.08 (3H, s), 4.13 (2H, s), 4.63 (2H, s), 5.17 (1H, s), 5.23 (1H, s); $^{13}$C NMR (100 MHz) 21.3 ($CH_3$), 64.1, 65.2, 114.9 ($CH_2$), 143.8, 171.4 (C); MS ES (+ve) found $m/z$ 131 ([M+H]$^+$, 100%). To a solution of the above oil (500 mg, 3.85 mmol) in dichloromethane (0.1 M) was added *m*-CPBA (1.42 g, 5.77 mmol). The solution was stirred at room temperature for 20 h. After this time potassium carbonate (9.4 g, 68.0 mmol) was added to the solution and the slurry was stirred for 15 min. The mixture was filtered and then concentrated in vacuo to yield the title compound as a colourless oil (551 mg, 98% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta_H$ 1.84 (1H, dd, $J$ = 5.7 and 7.8 Hz), 2.09 (3H, s), 2.80 (1H, d, $J$ = 4.6 Hz), 2.92 (1H, d, $J$ = 4.6 Hz), 3.68–3.83 (2H, m), 4.14 (1H, d, $J$ = 12.2 Hz), 4.32 (1H, d, 12.2 Hz); $^{13}$C NMR (75 MHz) 20.9 ($CH_3$), 49.4 ($CH_2$), 57.8 (C), 61.7, 64.4 ($CH_2$), 171.0 (C); MS ES (+ve) found $m/z$ 147 ([M+H]$^+$, 100%).

### 4.4. Enzymatic reactions

To a solution of **3** (100 mg, 0.96 mmol), Amano L, AK (200 mg, 2 wt equiv) and molecular sieves (4 Å) in dichloromethane (10 ml, 0.1 M) was added acetic anhydride (see Tables 1–3). The solution was shaken vigorously in an incubator set at the required temperature for the specified times (see Tables 1–3). The mixture was filtered through Celite®, and the filtrate was reduced in vacuo to yield an oil which was purified by column chromatography ($SiO_2$; 30% EtOAc in petroleum ether). The enantiomeric purity was determined by gas chromatography; CP-Chirasil-Dex CB; 25 m × 0.25 mm × 0.39 mm; 130 °C; split 50:1, He flow 1.0 ml/min; 1 μl injection using FID detection.

## Acknowledgements

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5. The enzymes used were sourced as follows: PPL: Lipase from porcine pancreas type II., Sigma–Aldrich, L3126. Amano L, AK: Amano L, AK from *Pseudomonas fluorescens*, Sigma–Aldrich, 534730. Amano L, PS: Amano L, PS from *Burkholderia cepacia* (*Pseudomonas cepacia*), Sigma–Aldrich, 534641. CRL: Lipase from *Candida rugosa*, Fluka, 90860. CAL-B: Novozyme 435, Novozymes.
6. As well as the work detailed herein, we also carried out the reaction in aqueous buffer following a literature procedure,[1] and demonstrated that the enantiomer isolated from the biotransformation in organic solvents is opposite to that obtained from the hydrolysis in aqueous buffer.
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