

# Enzymatic synthesis of carbo- al

Dr Richard Wisdom, head of the chiral synthesis group, and Dr Andreas Meudt, global R&D director at Archimica, discuss enzymatic routes to some versatile and highly enantiopure intermediates

**B**iocatalysis plays an increasingly important role in the production of enantiomerically pure molecules for use in the manufacture of APIs. Enzymes are able to carry out highly selective transformations under gentle reaction conditions, resulting in low by-product formation.

Traditionally, the only enzymes available commercially at bulk were various hydrolases, in particular lipases, esterases and proteases, which could be used for the resolution of racemates or selective reactions of pro-chiral molecules. Chemicals companies were restricted in terms of the scope of reactions that could be carried out, unless they had their own capabilities for the identification, development and supply of enzymes.

Now, however, a growing range of biocatalytic activities is commercially available at scale, enabling chemicals companies to use biotransformations successfully without the need for a significant investment in biological capabilities. A number of recombinant alcohol dehydrogenases (ADH), for instance, are now available from various enzyme suppliers.

Whereas yeast bioreductions have been available for many years, they were of limited interest, due to the amount of ADH in yeasts and hence the frequently poor selectivity of such reductions. Biological reductions with other microorganisms were restricted to facilities with fermentation capabilities.

The supply of isolated recombinant ADH preparations with effectively only a single ADH activity now makes it possible to carry out asymmetric reductions of ketones to single enantiomer secondary alcohols with extraordinarily high enantioselectivity. Such enzymes are stable in storage and can readily be used in standard chemical reactors. In addition, as ADHs are available with opposing chiral selectivities, it is possible to access either enantiomer from a specific ketone reduction.

We have investigated the use of such enzymes for the production of (R)- and (S)-oxiranes. Single enantiomer oxiranes are very useful molecules for the pharmaceuticals industry, since they may readily be used to build up complex molecules, producing single enantiomer alcohols.

In our synthesis, an ADH is used to reduce a range of different chloro-ketones selectively to single enantiomer chlorohydrins under mild conditions. *In situ* base treatment then results in the formation of single enantiomer oxiranes without any loss in enantiomeric purity. The product can be iso-

**Table 1 - Summary of epoxides formed**

Epoxide	Ee (%)	Selectivity
4-Hal-phenyl oxiranes (Hal = F, Cl, Br, I)	>99	(R) & (S)
3-Cl-phenyl oxiranes	>99	(R) & (S)
2-Cl-phenyl oxirane	>99	(R)
4-Methoxy-phenyl oxirane	>99	(R) & (S)
2-Oxiranyl-pyridine	>98	(R) & (S)
3-Oxiranyl-pyridine	>98	(S)
2-Thiophen-2-yl oxirane	>98	(R) & (S)
2-Thiophen-3-yl oxirane	>98	(R) & (S)
2-Chloro-1-furan-2-yl-ethanol	>99	(R) & (S)

lated by simple phase separation and distillation, if required, to give highly pure oxiranes in an economic and scalable fashion (Figure 1 & Table 1).

## Substituted phenyl oxiranes

The production of a range of different substituted phenyl oxiranes has been investigated. For instance, the production of 4-halogen substituted phenyl oxiranes starts from 2-chloro-1-(4-halogenated aryl) ethanones, which are generally readily available.

For the preparation of (S)-4-fluoro-phenyloxirane, a biological reduction of 2-chloro-1-(4-fluorophenyl)ethanone was carried out using an ADH from *Lactobacillus brevis*. NADP acts as a co-factor for the enzyme. The reduction of NADP by oxidation of isopropanol to acetone provides reducing equivalents for the reduction of the chloroketone. The oxidised co-factor was then recycled.

As this is an equilibrium reaction, excess isopropanol was used. The reaction was carried out at 20-40°C until >95% conversion was achieved. This gave (S)-2-chloro-1-(4-fluorophenyl)ethanol with an ee of >99%. Due to the mild conditions employed, no other significant by-products were formed.

The oxirane was then formed by the slow addition of concentrated sodium hydroxide under controlled conditions. Any residual starting 2-chloro-1-(4-fluorophenyl) ethanone from incomplete reaction was removed during this step.

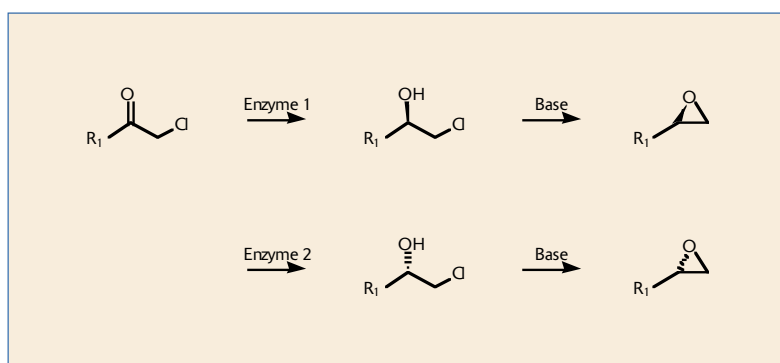
The product was readily isolated by phase separation followed by distillation to give epoxide at >99% ee and purity and with a yield at 20 g scale of 84% from starting 2-chloro-1-(4-fluorophenyl) ethanone. A similar process was readily scaled up into a standard chemical pilot plant with standard equipment to produce this epoxide at a multi tens of kilos scale.

(S)-4-chloro-phenyloxirane was prepared by a similar method. At 60 g scale, the (S)-4-chloro-phenyloxirane was isolated with a yield of 89% and both ee and purity of >99%.

Enzymes of the opposite selectivity are also available. Thus a *Thermoanaerobium sp.* ADH, which is also specific for the co-factor NADP, may be used in a similar fashion to the *L. brevis* ADH described above, but for the preparation of (R)-oxiranes.

Also available with similar selectivity are various ADHs from *Rhodococcus sp.* and *Candida sp.* These are generally specific for NAD as cofactor and alternative methods for

**Figure 1 - General procedure for (R) & (S) oxirane production**



# and heterocyclic aryl oxiranes

cofactor recycle must be used, such as the use of glucose dehydrogenase for the reduction of glucose to gluconic acid or formate dehydrogenase for the reduction of formate to carbon dioxide.

Both of these methods therefore require a second enzyme system. However, the development of reaction schemes for the production of (R)- enantiomers of the above oxiranes with equally good ee's has been possible by careful screening of such enzymes.

Using either the *L. brevis* enzyme or enzymes with the opposite selectivity, it has been possible to prepare both (R)- and (S)-4-halogenated phenyl oxiranes at >99% ee, with the halogen as 4-fluoro, 4-chloro, 4-bromo and 4-iodo. Indeed in many cases the unwanted enantiomer was significantly below 0.1% and difficult to detect.

As well as the 4-halogenated phenyloxiranes, the preparation of 2- and 3-halogenated phenyl oxiranes is equally possible with this technology. Starting 2'-chloro-(halogen-phenyl) ethanones can readily be prepared via chlorination of halogen acetophenones using sulphuryl chloride.<sup>1</sup>

2'-chloro-1-(3-chlorophenyl) ethanone was reduced with ADH from *L. brevis* or from *Thermoanaerobium sp.* or *Rhodococcus sp.* giving either the (R)- or the (S)-chloroalcohols at >99% ee. 2'-chloro-1-(2-chlorophenyl)ethanone could be reduced to the (R)-chloroalcohol. Subsequent formation of the oxirane provides the oxiranes with high yields and >99% ee and purity.

Phenyloxiranes with other substituents are also accessible by this technology. Thus, biological reduction of 2'-chloro-1-(4-methoxyphenyl) ethanone is possible, though at a slower rate than that observed for the 2-chloro-1-(halogenated-phenyl) ethanones. This is presumably due to the electron donating nature of the methoxy substituent.

Using a similar biotransformation with *L. brevis* ADH at 60g scale, 79% conversion was obtained with >99% ee. Base treatment gave the epoxide. The epoxide was isolated with an overall yield of 61%, but with 5% starting chloroketone. Further distillation with a short column could be used to yield the epoxide in high purity.

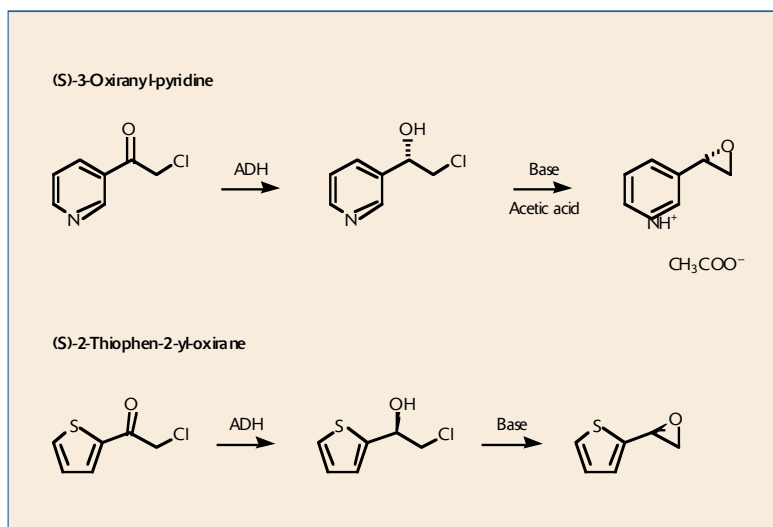
## Heterocyclic oxiranes

Heterocyclic oxiranes are of great interest and these too could readily be accessed using this technology (Figure 2). For example, the 2- and 3-positional isomers of 2-chloro-1-(pyridin-yl) ethanones were prepared by the chlorination of the respective acetyl pyridines.<sup>2</sup>

The products were isolated as their salts, which were reasonably stable white solids at room temperature. Prior to their use in the biotransformation, the salts were broken by suspension in a toluene/water biphasic mixture and the slow addition of alkali. The organic phases were used directly in the biotransformation.

After alkali treatment to form the epoxide, the epoxide was subsequently isolated from the organic phase by formation of the acetate salt. Typical yields were 50%. Selectivity during the biotransformation with these substrates is not quite as high as with the aryl substrates, but is still very acceptable, with ee's in the range 98-99%.

The 2-chloro-1-(pyridin-4-yl) ethanone is not stable at above pH 5. Because of this, the oxiranyl-4-pyridines have not been accessible by this method so far.



**Figure 2 - Preparation of heterocyclic oxiranes**

Thiophenyl oxiranes are likewise readily accessible. 2-Chloro-(1-thiophen-2-yl) ethanone was prepared by Friedel Crafts acetylation,<sup>3,4</sup> which was carried out at low temperature to improve selectivity of the 2 position over the 3 position. The product was isolated by crystallisation (distillation is also possible).

Re-crystallisation was used to reduce the 3-isomer to <2%. 2-Chloro-(1-thiophen-3-yl) ethanone was prepared from 3-bromo thiophene by lithium/halogen exchange, reaction with the Weinreb amide and purification by crystallisation.

Both positional isomers were good substrates for both the (R)- and (S)-selective ADHs and the reduction was carried out to produce the chlorohydrins with ee's for both enantiomers of each isomer of >98%. Subsequent alkali treatment yielded the single enantiomer epoxide ((R)- and (S)-2-thiophen-2-yl oxiranes and (R)- and (S)- 2-thiophen-3-yl oxiranes).

The formation of thiophen-2-yl-oxiranes from the chlorohydrin required mild conditions and had to be carried out at a temperature of <25°C to minimise degradation. However, at the 20g scale under incompletely optimised conditions, single enantiomer oxirane was isolated with a crude yield of 87%, or 55% after distillation, and with a purity of >98%.

Enantiopure furfuryl oxiranes have not been accessible so far. Starting from 2-chloro-(2-furyl) ethanone, biotransformation works well and gives access to both enantiomers of 2-chloro-(2-furyl)ethanol with an ee of >99%. Both are stable at <10°C but subsequent base treatment of the chlorohydrin yields a range of products.

It is clear therefore that this technology is able to access a wide range of epoxides which are highly pure, both chemically and enantiomerically. The technology has been already scaled into the pilot plant without problems. These results show that it can easily be scaled to multi-tonnes of high purity products.

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