Epoxide Hydrolases: Process Applications

Scientists at Oxyrane (UK) Ltd discuss the application of their cutting-edge epoxide hydrolase (EH) technology to the production of several chiral products, as illustrated by selected case studies.

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In the previous edition of Innovations in Pharmaceutical Technology, epoxide hydrolase (EH) technologies were reviewed as an alternative to asymmetric chemical methods for the synthesis of chiral intermediates. Here, we provide details of several case studies illustrating how Oxyrane’s in-house EH technology has been applied up to kilogram scale in a range of racemic epoxide resolutions. These case studies have been selected to demonstrate how the traditional constraints of biocatalytic technologies – such as restricted substrate flexibility, specialised reactor requirement and low volumetric yields – have been overcome by the inherent robustness of the EH platform.

EH TECHNOLOGY: APPLICABILITY FOR INDUSTRIAL-SCALE PROCESSES

Enantiomerically pure epoxides and vicinal diols are key chiral synthons in the production of small molecule pharmaceuticals. Over the last two decades, much effort has thus been devoted to developing routes for their synthesis – these routes being dominated by chemo-catalytic methodologies. In order to compete with these commercially established chemical technologies, a biocatalytic technology has to fulfil very stringent process and economic requirements. At Oxyrane, we have succeeded in developing an EH-mediated synthetic capability that offers a viable alternative to these chemical technologies, while still being easily integrated into existing chemical manufacturing plants.

The following examples illustrate the potential of EH technology as an industrially useful tool in chiral syntheses.
Case study 1: Synthesis of cis-(1S, 2R)-aminoindanol as a key chiral intermediate for the production of antiretroviral compounds

Cis-(1S, 2R)-aminoindanol is a key chiral intermediate in the synthesis of several anti-retroviral compounds (such as Merck’s Crixivan). A number of biocatalytic routes for its synthesis have been patented and published, but none of these biocatalytic technologies are economically feasible when compared with the current commercial route to cis-(1S, 2R)-aminoindanol via Jacobsen’s asymmetric epoxidation of indene followed by a Ritter reaction. However, indene is expensive as a starting material, and, as such, the resolution of racemic indene oxide (obtained chemically from low cost bromo-indanol) offers a potentially attractive alternative. Although utilising the epoxide is a potentially lower cost route, the aqueous conditions required for the biocatalytic resolution promote chemical instability of this substrate.

To overcome these difficulties, Oxyrane has developed a proprietary process whereby the biocatalytic resolution is performed on solid indene oxide particulates in an aqueous suspension, thereby enabling high volumetric substrate loading with minimal substrate degradation. The solid form of the substrate imparts a high degree of chemical stability, while still enabling a high rate of reaction, and the selected biocatalyst yields the trans-diol with the required (2R)-configuration. In a typical bench-scale reaction, the resolution is complete within two hours at 10% m/v racemic indene oxide substrate loading. The formed diol precipitates in situ and (1R, 2S)-indane oxide substrate loading. The formed diol precipitates in situ and (1R, 2R)-trans-diol is isolated in good yield (>90% mass yield from epoxide racemate) and purity (enantiomeric excess [ee] >99%, chemical purity >95%) after a selective one-step crystallisation (see Figure 1). The (1R, 2R)-trans-diol is then reacted through a Ritter reaction to afford the required (1S, 2R)-cis-aminoindanol.

The substrate-loading of the biocatalytic resolution is dictated solely by the mixing efficiency required to reach completion at the high solid content of reactant and product. However, the use of mechanical stirred reactors typical of most chemical plants has enabled the resolution of substrate concentrations up to 250g/l. The lower cost of starting materials and the high yields obtained with this route represent a potentially useful biocatalytic alternative to the existing production process.

Case study 2: Chemo-enzymatic enantioconvergent production of (R)-6,7-dihydroxygeraniol from geraniol on a multi-kilogram scale

(R)-6,7-dihydroxygeranyl compounds are synthetic intermediates used in the production of several natural first-pass inhibitors that increase the oral bioavailability of certain drugs. A viable route to the (R)-6,7-dihydroxygeranyl moiety, has been developed from geraniol as an inexpensive starting material (see Figure 2). In its simplest form, the biocatalytic resolution step is integrated into a six-step synthesis without the necessity to isolate intermediate products. The synthesis has...
been successfully performed to produce kilogram quantities of \((6R)\)-dil, incorporating a 6-litre batch biocatalytic step to resolve 1.5 kg racemic epoxide (25% m/v substrate loading).

The selected EH catalyst is absolutely enantiospecific \((E>200)\) with inversion of configuration (see Figure 3), and the kinetic resolution ceases without intervention after two hours’ reaction time as illustrated in Figure 3 (final ee \((6R)\)-epoxide >99.7% and final ee \((6R)\)-dil >99.5%). The subsequent acid-mediated chemo-hydrolysis of the remaining \((6R)\)-epoxide results in hydrolysis to the \((6R)\)-dil with retention of configuration, high yield and minimal chiral slippage. This chemo-hydrolysis is applied without the requirement for prior separation of the epoxide from the dil, thereby simplifying downstream operations and yielding the desired \((6R)\)-dihydroxygeraniol in high chiral purity as the sole cumulative chiral species (ee >97.5%). Subsequent facile purification procedures provide the final \((6R)\)-dihydroxygeraniol product at an overall isolated mass yield of 70-75% from the racemic epoxide.

**Case study 3: Applications of catalysts with opposite enantiopreferences for the kinetic resolution of styrene oxides or enantioconvergent one-pot production of phenyl ethanediols**

Styrene oxides and phenyl ethanediols (including 3- and 4-phenyl substituted derivatives such as meta-chlorostyrene oxide and para-nitrostyrene oxide) are valuable intermediates in the synthesis of a wide range of pharmaceuticals. Oxyrane has developed processes whereby either enantiomer of these epoxides can be produced using EH’s with opposite selectivities, or the (substituted) \((R)\)-phenyl ethanediols can be produced in a one-pot enantioconvergent fashion in 100% theoretical yield by combining two catalysts with matching opposite selectivities in one reaction. The resolution of \(p\)-NSO at high substrate levels is particularly challenging due to the insoluble nature of this epoxide.

EH technology has been successfully applied to the resolution of 85g/L \(p\)-NSO to produce either enantiomer of the epoxide, together with the \((R)\)-dil, or alternatively through an enantioconvergent hydrolysis to furnish only the \((R)\)-dil at high isolated yield and high chiral purity. In all these instances the bioresolution is complete within 3 hours (see Figure 4). The various chiral products of these reactions are then isolated at ee >99% through the careful use of controlled recrystallisation techniques.

**CONCLUSION**

As demonstrated here, many of the typical constraints regarding the integration of biocatalytic processes into the chemical industry do not apply to this robust and versatile platform. Developed specifically to utilise existing chemical plant equipment, the technology has significant commercial potential – both as a competitor to existing chemo-catalytic technologies and as a route to the many chiral epoxides and diols where no viable commercial alternative exists.

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