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Bioconversion of substituted styrenes to the corresponding enantiomerically pure epoxides by a recombinant *Escherichia coli* strain

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Abstract

Enantiomerically pure epoxides are produced by bioconversion of the corresponding styrenes using a recombinant *Escherichia coli* strain containing the styrene monooxygenase gene cloned from *Pseudomonas fluorescens* ST. Different procedures were used to optimise yields and to permit product isolation. Conversion rates depend on the position and nature of the styrene substituent. © 2000 Elsevier Science Ltd. All rights reserved.

The use of microbial bioconversions to synthesise chemical compounds is becoming an everyday alternative to classical chemical synthesis. The specificity and the regio- and stereoselectivity of enzymatic transformations combined with the mild reaction conditions and the use of inexpensive reagents represent sound advantages that must be considered each time a transformation can be realised microbiologically. Moreover, the continuous development of genetic methodologies has permitted the achievement of controlled and highly productive engineered microorganisms.

We are interested in the production of enantiomerically pure compounds that can be used as chiral auxiliaries and ligands. In this perspective, we are exploiting the capability of enzymes in the preparation of geometrically defined compounds that can be successively transformed into the desired products. Most of our preceding work has been done using 1,2-dihydro-1,2-dihydroxy naphthalenes obtained from recombinant *E. coli* strains.[1] A promising alternative is represented by chiral epoxides that can evidently be transformed into interesting derivatives (Fig. 1).[2,3]

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Figure 1. Chiral ligands from enantiomerically pure epoxides

The recombinant *E. coli* strain contains a fragment of 1.9 Kb carrying *styA* and *styB* genes encoding for monooxygenase activity derived from *P. fluorescens* ST.[4] *StyA* and *styB* genes are responsible for the production of two enzymes, the styrene monooxygenase (1.3 Kb) and a putative flavine reductase (0.6 Kb), respectively. The strain has been successfully used to convert some substrates with good results.[5] Nevertheless, some problems still remained unsolved; in particular, the enzyme selectivity and the product isolation. Concerning this second point, it is in fact difficult to avoid some side reactions that give rise to unwanted by products, mainly diols and aldehydes (Fig. 2).

Figure 2. By products of biosynthesised epoxides

We used the recombinant strain to perform several conversions that generate epoxides in good overall isolated yields.[6] To this goal we developed procedures suited to each compound set:

- water soluble compounds: group 1
- water insoluble compounds:
- isooctane soluble compounds: group 2
- organic solvent mixture soluble compounds: group 3

The procedure for group 1 is very simple and requires only the addition of the substrate to the culture broth and extraction of the product using a suitable organic solvent (CH_2Cl_2, EtOAc). To our pleasure, the epoxides obtained were stable in water and the low boiling points of the extraction solvents guarantee easy recovery.

The procedure for group 2 requires the use of a mixture of two phases; water/isooctane (75/25). The water phase contains the microorganism in the usual culture broth; the hydrocarbon phase contains the substrate (at 1 g/L concentration) and the product. At the end of the conversion the phases are separated and the organic phase is extracted using acetonitrile (MeCN) that is not miscible with isooctane and which extracts the epoxides with good efficiency. Due to the low MeCN boiling point, it is always possible to recover the product by solvent evaporation under reduced pressure without heating.

However, isooctane is not a good solvent for all substrates. To overcome this problem we considered the possibility of using solvent mixtures. One additional problem is presented by the sensitivity of the microorganism to organic solvents.[7] In fact, it is known that many solvents are deleterious to cells. We are still working on this aspect to have a complete understanding of the reactions involved; but, we wish to report some preliminary results.

Table 1 reports the list of the substrates used together with the products, the enantiomeric excesses, and the conversion rates. To measure the conversion rates we organised conversions that guaranteed the correct comparison between experiments performed under different conditions. Thus, we always operated measuring the cell activity using the conversion of styrene as a reference. Table 2 shows the substrates that were not transformed.[8]

Table 1
Bioconversion results using *E. coli* JM109(pTAB19)

Substrate	Product	Conversion rates[a]	E. e. and absolute configuration	Procedure
N	O, N	95 %	> 99%[b] S[c]	1
	O	28 %	> 99 %[b] 1S,2S[c]	2
	O	21 %	> 99 %[b] S[c]	2
	O	65 %	> 99 %[b] 1S,2R[c]	2
	O	18 %	> 95 %[c] S[c]	2
COOMe	O, COOMe	3 %	> 95 %[c] 2R,3S[c]	2
O	O, O	n.d.	> 99 %[b] n.d.	3
CH_2X; X = Cl, Br	O, CH_2OH 80%; HO 20%	n.d.	> 95 %[c] 1S,2S[c]	2

[a]Rates relative to styrene bioconversion.
[b]Determined by chiral GLC in comparison with synthetic racemic samples.
[c]Determined by specific optical rotation.

Table 2
Substrates not transformed by *E. coli* JM109(pTAB19)

NC OMe O O Et HO

From Tables 1 and 2 it is clear that both the nature and the position of the styrene substituents affect the conversion rate. In particular, electron withdrawing substituents decrease the rate, an expected result considering that oxidation reactions are usually easier on electron rich substrates. The position of the substituent has a more complex effect. α- and β-Substituted styrenes react at a slower rate than unsubstituted styrenes, but not at a rate as slow as styrenes substituted at position 4 on the phenyl ring. A cyclic styrene equivalent (indene) reacts as fast as styrene, whilst the introduction of a substituent on the five membered ring in the β position inhibits the reaction. Finally, all the rates are affected by the substituent size, as expected.

Two final points are worth noting. First, 4-methoxystyrene contains an electron donating substituent, yet is completely transformed into the corresponding epoxide only if the conversion is performed using a solvent mixture that permits both dissolution of the substrate and its transfer to the cells. Secondly, there are substrates that are apparently not converted; however, the limitation is sometimes related to their limited availability inside the cell. This is confirmed by the reactions of the cinnamyl derivatives. The alcohol is converted extremely slowly to the product; on the contrary, both the halogen derivatives are transformed very well into the phenyl glycidol, i.e. they are transferred inside the cell, where they are transformed into the glycidol.[9]

In conclusion, we now have available a good procedure that gives access to a series of enantiomerically pure epoxides.

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6. A typical preparation is performed using a 500 mL flask containing 100 mL culture with ~1 g/L DWC in phosphate buffer (pH 7) and glucose (0.1%), at 30°C on a rotatory shaker. The substrate is added at a concentration of 1 g/L, either directly to the culture or dissolved into the organic solvent.
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8. All products have been isolated and identified by ^1H NMR spectroscopy. The epoxide production has been monitored by GLC or HPLC. Chiral GLC analyses have been performed using the column Chrompack Chiraldex-CB at 100°C and racemic standards were synthesised in our laboratory when not commercially available. Some substrates have been prepared from the corresponding aldehydes.
9. This experiment uses Procedure 2. The product is recovered from the water phase; the direct use of the alcohol using the same procedure does not give the product.