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TETRAHEDRON: *ASYMMETRY*

Enzymatic transformations. Part 53: Epoxide hydrolase-catalysed resolution of key synthons for azole antifungal agents

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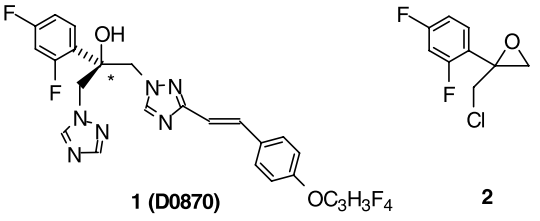
Abstract—The biocatalysed hydrolytic kinetic resolution (BHKR) of a key building block allowing the synthesis of an enantiopure azole antifungal compound is described. This is based on the epoxide hydrolase-catalysed resolution of a racemic epoxide. Using epoxide hydrolase from *Aspergillus niger*, both the unreacted epoxide and the formed diol were obtained with excellent yield and very high ee (>98%). Interestingly, both products can be used for the synthesis of the target molecule, thus allowing the theoretical 50% yield limitation linked to such resolution processes to be overcome.

1. Introduction

Recently new triazole derivatives, such as D0870 **1**,[1,2] were shown to display very interesting activity against infections such as fluconazole-resistant oro-oesophageal candidiasis for example.[3,4] Furthermore it has been shown, for these potential new drugs, that the desired activity was essentially—if not totally—linked to their (*R*) enantiomer. Therefore, various chemical approaches have been explored allowing the preparation of these targets in enantiopure form (Scheme 1).[5–8]

Herein, we propose another strategy allowing the synthesis of enantiopure **1**, based on the biocatalysed hydrolytic kinetic resolution (BHKR) of the racemic chloro-epoxide (±)-**2** using a 'new' type of hydrolytic enzyme, epoxide hydrolases. Starting from this substrate, we expected to obtain the unreacted chloro-epoxide **2** on one hand and the formed chloro-diol **3** on the other—both in enantiomerically enriched form. Interestingly it has previously been shown that by using appropriate chemical strategies,[2,5] both of these products could be elaborated to allow further syntheses of (*R*)-**1**, whatever their absolute configuration is. This theoretically circumvents the 50% yield limitation obtained from a normal resolution process, which is a major drawback for industrial application of such an approach. Two important criteria for the validity of our approach were therefore: (a) the necessity to find an appropriate enzyme offering a high enantiomeric ratio (a criteria directly linked to the overall yield of the two products obtained); (b) the necessity to achieve this resolution with an enzyme offering a reasonable enzymatic activity, i.e. offering an acceptable compromise between the quantity of enzyme used and the necessary reaction time. In order to fully characterise the enzymatic process, it was also important to determine the absolute configuration of the products formed as well as the regioselectivity of the hydrolytic reaction. Herein, we describe the results obtained from this study.



Scheme 1.

Racemic epoxide (±)-**2** was prepared as described previously[5] by condensation of 1,3-dichloroacetone with 1-bromo-2,4-difluorobenzene, followed by intramolecular cyclisation of the thus obtained chlorohydrin. Several epoxide hydrolases available in our laboratory were tested using this substrate. Once again,

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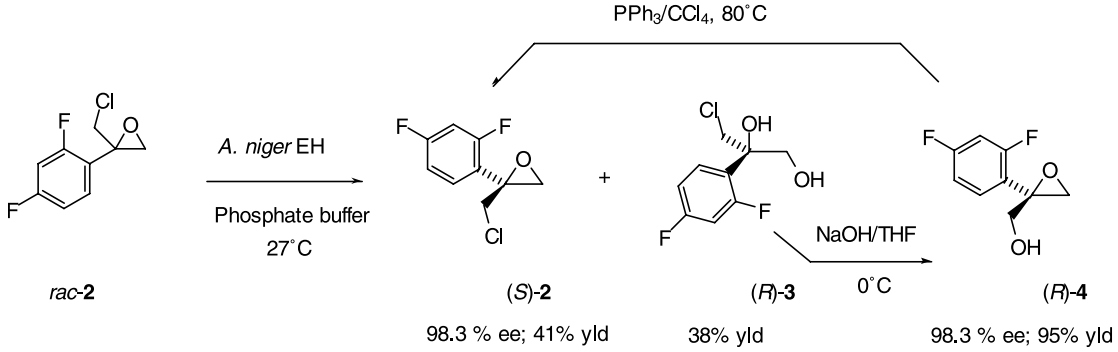
the fungal *Aspergillus niger* (*An*EH) epoxide hydrolase, which we have recently cloned and overexpressed,[9] gave the best results. Interestingly, this enzyme exhibited an excellent enantioselectivity as well as a very high activity towards **2**. Thus, a preparative scale experiment carried out using (±)-**2** (400 mg, 2 mM) and *An*EH crude enzyme (13 mg, specific activity 8 U/mg protein)[10] in phosphate buffer solution (100 mM containing 10% DMSO, pH 7, 1 L) led, after a reaction time of 4 h 45 min, to a conversion ratio of nearly 50%. Stopping the reaction at this point allowed recovery of the unreacted epoxide (*S*)-**2**, which showed an excellent ee (98.3%) in good yield (41%) as well as of the formed chloro-diol **3** in nearly enantiopure form (98.3% ee, 38% yield) (Scheme 2).

The conversion ratio and the ee of the recovered epoxide (*S*)-**2** were determined by chiral GC analysis (Lipodex G, 110°C, Macherey–Nagel). The ee of the formed diol **3** could be determined (using the same chiral column, 120°C) after intramolecular cyclisation into the corresponding epoxy-alcohol **4**. It was shown to be also higher than 98%. The *E* value, calculated on the basis of the ee of the residual epoxide and either the ee of the formed diol or the conversion ratio, appeared to be higher than 200.

The absolute configuration of the obtained chloro-diol **3** could be established by chemical correlation with the previously described epoxy-alcohol (*S*)-**4**.[5] Thus, intramolecular cyclisation of **3** (dry THF/NaH, 0°C, 1.5 H) led to **4** ($[\alpha]_D^{26}=+44.6$ (*c* 1; THF); lit. for (*S*)-**4**: $[\alpha]_D=42$ (*c* 1; THF)). This allowed us to conclude that the chloro-diol product **3** ($[\alpha]_D^{26}=+3.6$ (*c* 1; THF)) had *R* absolute configuration. As a consequence it could be deduced that, most probably, this (*R*)-chloro-diol **3** was formed by hydrolysis of the *R* enantiomer of **2**, assuming that nucleophilic attack by water occurred at the less substituted carbon atom of the epoxide moiety. (Theoretically, formation of (*R*)-**3** could also result from attack of (*S*)-**2** at the more substituted carbon atom.) In this case, the absolute configuration of the unreacted chloro-epoxide **2** ($[\alpha]_D^{26}=+45$ (*c* 1.2; THF)) should be of *S* absolute configuration. This assignment was confirmed by treatment of the epoxy-alcohol (*R*)-**4** (obtained from (*R*)-**3** as described above) with triphenylphosphine in carbon tetrachloride (80°C, 7 h).[5] Indeed, this essentially afforded a single enantiomer of the corresponding chloro-epoxide **2**, which proved to be the same (identical retention time on chiral GC) as the one recovered after biohydrolysis. These experiments serve to confirm that the products obtained from this biohydrolysis were, without doubt, the unreacted chloro-epoxide (*S*)-**2** and the diol (*R*)-**3**, respectively.

Since determination of the regioselectivity is also an important factor in this type of reaction, we have also determined the $\alpha(S)/\beta(S)$ and $\alpha(R)/\beta(R)$ ratios for each enantiomer of **2**. These were calculated on the basis of the ee of the diol **3** obtained upon biohydrolysis of enantiopure (*S*)-**2** and the ee of the diol **3** formed after total conversion of the racemic epoxide.[11] The results indicate that attack by water occurred almost exclusively at the β-carbon atom for each enantiomer [$\alpha(S)/\beta(S)=5/95$ and $\alpha(R)/\beta(R)=1/99$]. As a result, this biohydrolysis essentially led to retention of configuration at the stereogenic carbon atom of **2**, an observation consistent with the fact that the ee of the formed diol **3** decreased throughout conversion of (±)-**2**, down to around 4% at total conversion.

The aim of this work was to explore the possibility of developing a new strategy allowing further preparative scale syntheses of enantiopure building blocks usable for elaboration of the potentially important antifungal drug D0870. Our results show that this goal could, in principle, be reached by performing the resolution of (±)-**2** using our recombinant *A. niger* epoxide hydrolase. Indeed, this enzyme shows good activity against **2** and the observed *E* value was shown to be excellent (>200). This therefore allows a very effective resolution of substrate **2**. Both the recovered chloro-epoxide (*S*)-**2** and the formed chloro-diol (*R*)-**3** were thus obtained in very good yields and in nearly enantiopure form at a conversion ratio of about 50%. Since both these products can be used in the synthesis of our target, this approach interestingly allows the industrially very important '100% yield/100% ee' criteria to be fulfilled. Further work is going on in our laboratory in order to improve this resolution for preparative scale application, and this will be described in due course.



Scheme 2.

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