Enzymatic Transformations; 61. Preparation of Enantiopure Trifluoromethyl-Substituted Aromatic Epoxides and Vicinal Diols using the *Aspergillus niger* **Epoxide Hydrolase-Catalysed Resolution**

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Abstract: The resolution of eight differently substituted trifluoromethylstyrene oxide derivatives was explored using the *Aspergillus niger* epoxide hydrolase. The obtained results indicate that all (but one) epoxides were efficiently processed by this enzyme, under very mild experimental conditions. The specific activity and the enantioselectivity of the enzyme against each substrate were determined. Circular dichroism analysis was also performed, allowing us to establish the absolute configuration of the products.

Keywords: *A. niger* epoxide hydrolase; circular dichroism; enantiopure epoxides; enzyme catalysis; fluorinated derivatives; kinetic resolution

Asymmetric synthesis of selectively fluorinated compounds, driven initially by the necessity of preparing biologically relevant compounds in an enantiomerically pure form, has gained tremendous impetus over the recent years.^[1] As a consequence, the development of practical methods for preparing selectively fluorinated yet stereochemically defined compounds is definitely critical to the further advances of fluorine chemistry at the biomedical interface. Fluorinated organic compounds are, however, well known for their unexpected and generally unusual reactivity. Thus, due to the specific fluorine factors such as steric, electrostatic and electronic features, as well as to the ability of fluorine in coordinating positively charged and electron-deficient species, many of the methods established for the asymmetric synthesis of fluorine-free compounds do not work or give impractical outcomes when applied to prepare fluorine-containing targets.

In this context, we were strongly interested in exploring the possibility to use the overexpressed (and commercially available)^[2] Aspergillus niger (A. niger) epoxide hydrolase to perform the biocatalysed hydrolytic kinetic resolution of some selected trifluoromethylsubstituted styrene oxide derivatives. We here describe our results in this context.

Over the last decades we and others have demonstrated that preparation of various enantiomerically pure epoxides and vicinal diols, including aliphatic as well as aromatic substrates, can be very efficiently achieved by carrying out a so-called biocatalysed hydrolytic kinetic resolution (BHKR) of the corresponding racemate.^[3] These "green chemistry" resolutions were performed using various epoxide hydrolases from different origins, thus offering an easy, cheap and environmentally gentle alternative to substrate limited and potentially toxic transition metal-based methodologies. Our studies using the A. niger enzyme demonstrated that this enzyme was able to operate in plain water at room temperature and in two-phase reactors allowing us to run the reaction within a few hours, at substrate concentrations as high as 2.5 M (i.e., 500 gL^{-1}).^[4,5] We have now raised the question whether this type of process could be applied to some fluorine-bearing substrates. The trifluoromethyl-substituted styrene oxide derivatives *rac*-**1–8** were selected to explore this possibility and, more precisely, to determine the relative influence of (a) their ortho-, meta- and para- substitution, (b) the intrinsic nature of different para substituents, (c) the presence of an additional gem-methyl group on the (thus trisubstituted) epoxide moiety (Scheme 1).

The racemic epoxides **1–8** used in this study were synthesised with moderate to excellent (46–99%) yields by reaction of the corresponding commercially available aldehyde or ketone with either trimethylsul-



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Scheme 1. Structure of the racemic epoxides used in this study.

fonium or trimethyloxosulfonium iodide, following the procedure previously described by Corev.^[6] Each of these epoxides was also quantitatively hydrolysed in H₂O/THF solution in the presence of catalytic amounts of sulphuric acid, thus affording racemic probes of the corresponding vicinal diols rac-1d-8d. The solubility of each of the epoxides in plain water and room temperature was estimated as being in the order of 1 mM. However, using an aqueous solution containing 20 to 30% DMSO allowed enhancement of this solubility to about 2.5 mM. Therefore, the experiments described in this exploratory study were achieved, at 27°C, using this solvent mixture, owing to our previous observation indicating that the A. niger epoxide hydrolase was not noticeably affected in these conditions over a few hours period.^[7]

The precise experimental conditions used to run each one of the experiments, including the concentration of substrate, the percentage of DMSO, the spontaneous consumption and the specific activity of the enzyme against each epoxide are indicated in Table 1. Blank experiments allowing us to accurately measure the spontaneous consumption of each specific substrate were performed under these experimental conditions, indicating that some epoxides (for instance, **1**) were rather sensitive to spontaneous hydrolysis

whereas some other ones (for instance, 7) were very stable. Initial velocity of the EH-catalysed resolution was also determined and allowed us to quantify the specific activity of the enzyme against each epoxide. As usual, the activity was dependent on the structure of each substrate. Thus, ortho-substituted epoxides 1 and 6 showed much lower reactivity as compared to styrene oxide itself (11 U·mg⁻¹),^[8] whereas the other (meta- and para-substituted) substrates exhibited comparable - if not better - reactivity. Surprisingly enough, epoxides 7 and 8, bearing an additional gemmethyl group showed the highest activity. This is a very interesting and noteworthy observation since even the Jacobsen's salen (Co) catalysts, claimed to be the best transition metal-based catalysts for achieving the hydrolytic kinetic resolution of epoxides, are inoperative on such trisubstituted substrates.^[9]

Analytical scale resolutions: Each one of the epoxides rac-1-8 was submitted to small-scale experiments in order to (a) establish the kinetic profile of the resolution, (b) follow the evolution of the conversion ratio c in correlation with the ee of the less reactive (recovered) epoxide enantiomer, and (c) obtain a small amount of the corresponding formed diol, in order to determine the absolute configuration (see below). The (weight to weight) substrate over enzymatic powder R ratio used is indicated in Table 1. It is to be stressed that these R ratios can be valuably compared since all the experiments were conducted using the same enzymatic preparation (showing an activity of 11 $U \cdot mg^{-1}$ as measured by UV spectroscopy against styrene oxide^[8]). Thus, by using R ratios of 58 or 120 (i.e.,1 g of enzyme powder for 58 or 120 g of racemic epoxide) the resolution was complete within 20 to 60 min, depending on the substrate.

Our results indicate that all – but one (rac-6) – epoxides were indeed efficiently processed by the enzyme, following a classical one-phase resolution profile.^[10] As an example, the kinetic profile of rac-4 is illustrated in Figure 1. The apparent E value (E_{app})

| Substrate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|------------------|-------------------|------|------|-------------|-----|-------------|-------------|
| Epoxide concentration [mM] | 2.5 | 2.66 | 2.5 | 2.5 | 2 | 2.5 | 2.5 | 1.8 |
| % DMSO | 25 | 20 | 20 | 30 | 30 | 20 | 30 | 30 |
| % Spontaneous consumption [h ⁻¹] | 44.4 | 0 | 11.3 | 8.8 | 2 | 4 | 0 | 18.9 |
| Specific activity [U·mg _{powder} ⁻¹] | 4.9 | 8.9 | 16.0 | 10.8 | 12.1 | 2.3 | 29.9 | 16.9 |
| R Pre Createry | 58 | 67 | 58 | 116 | 116 | 58 | 58 | 58 |
| Reaction time ^[a] [min] | 120 | 50 | 30 | 60 | 60 | - | 20 | 40 |
| Absolute configuration (epoxide) | (S) | (S) | (S) | (S) | (S) | - | (S) | (S) |
| Absolute configuration (diol) | (\hat{R}) | (\hat{R}) | (R) | (R) | (\hat{R}) | - | (\hat{R}) | (\hat{R}) |
| $E_{\rm app}^{[b]}$ | 5 ^[c] | 10 ^[c] | 50 | 30 | 160 | 1 | 25 | 30 |

Table 1. Experimental results obtained upon hydrolytic kinetic resolution of rac-1-8 catalysed by the A. niger EH.

^[a] Reaction time to reach an epoxide of ee > 99%.

^[b] The *ee* was determined by chiral GC on a Chirasil-Dex CB Chrompack column.

^[c] The *ee* determined by chiral GC on a Lipodex G column.



Figure 1. Kinetic profile of the resolution of *rac*-4 catalysed by the *A. niger* EH. Epoxide enantiomeric excess, ee_{epox} (triangles); conversion ratio *c* (circles); chemical hydrolysis = blank experiment (diamonds). *Experimental conditions:* 27°C; H₂O/DMSO 30% (v/v); R = 116.

of each resolution was calculated by using both the c and ee_{epox} values on the basis of the appropriate Sih's equation, following a curve-fitting methodology.^[11] Interestingly, it can be observed that this E_{app} value appeared to be very low for the *ortho*-substituted compounds 1 and 6, moderate for the *meta*-substituted substrates 2 and 7 and good to even excellent for the *para*-substituted epoxides 3, 4, 5 and 8.

Determination of the absolute configuration of the obtained epoxides and diols: As far as the absolute configuration of the obtained enantiomerically enriched epoxides and diols is concerned, examination of the literature indicated that, except for epoxide (S)-2^[12] and diol (R)-3d,^[13] their absolute configuration was unknown. We therefore had to achieve this determination. This was performed by using circular dichroism spectroscopy, following a methodology developed by Schnatzke and co-workers.^[14] and recently revisited by Di Bari and co-workers.^[15] According to these authors, the absolute configuration of various vicinal diols can be determined on the basis of the

sign of the Cotton effect of the CD band around 305 nm ("band IV") observed for a complex formed between a given vicinal diol and dimolybdenum tetraacetate. According to their study, a negative sign of the "band IV" is associated with the (R) absolute configuration of the diol and, although being an empirical approach, no exception to this rule is known up to now. Thus, each one of our obtained diols 1d-8d was submitted to CD spectroscopy measurements following this methodology. The results obtained are described in Table 2 and Figure 2. These measurements indicate that all our diols led to a negative sign for the band IV, leading to the conclusion that they all were of (R) absolute configuration. This is in perfect agreement with our previous observations that, as a general feature, the A. niger epoxide hydrolase (a) preferably hydrolyses the (R) epoxide of styrene oxide derivatives by attack at the terminal - less substituted – carbon atom of the oxirane ring (i.e., with retention of configuration at the benzylic carbon atom), thus leading to the (R) diol and (b) that the para substitution allows the most efficient resolution



Figure 2. Circular dichroism spectra of enantioenriched diol 1d-8d in the presence of Mo₂(AcO₄) (DMSO solution).

| Table 2. Circular dichroism studies of enantioenriched diols 1d-8d in the pre- | presence of $Mo_2(AcO_4)$ (DMSO) | solution). |
|--|----------------------------------|------------|
|--|----------------------------------|------------|

| Diol | $ee^{[a]}$ | [diol] [mM] | Ratio diol/Mo ₂ (AcO) ₄ | Bands ICD; λ_{ext} (nm), ($\Delta \varepsilon_{ext}$) | | | | |
|------|-------------------|-------------|---|---|-------------|-------------|-------------|--|
| | | | | V | IV | III | II | |
| 1d | 77 ^[b] | 0.375 | 1 | 280 (0.12) | 305 (-0.66) | 348 (0.06) | 385 (-0.18) | |
| 2d | 13.2 | 0.75 | 1 | 279 (0.04) | 301(-0.14) | 352 (-0.01) | 375(-0.05) | |
| 3d | 84.3 | 0.375 | 1 | 270 (0.07) | 308 (-0.60) | 350 (-0.12) | 381 (-0.20) | |
| 4d | 94.5 | 0.375 | 1 | 272 (0.26) | 310 (-1.22) | 352 (-0.22) | 375 (-0.33) | |
| 5d | 85 | 0.375 | 1 | 271 (0.22) | 308 (-1.12) | 352 (-0.18) | 379 (0.30) | |
| 7d | 59 | 0.75 | 1 | 277 (0.15) | 316(-0.22) | 342(-0.01) | 385(-0.05) | |
| 8d | 88.3 | 0.375 | 1 | 273 (0.15) | 308 (-0.18) | 350 (0.02) | 379 (-0.06) | |

^[a] Determined by chiral GC on a Lipodex column after derivatisation into the corresponding acetonide.

^[b] Determined by chiral GC on a Chirasil-Dex CB Chromback column after dimethylation.

(unpublished results). Each one of the obtained diols was further cyclised back, without loss of stereochemical integrity, into the corresponding epoxide. Comparative chiral GC analysis confirmed that the unreacted (recovered) epoxide was, as expected, of (S) absolute configuration.

As an overall result, it thus appears that this methodology allowed us to prepare both the epoxides of (S) configuration and the diols of (R) configuration. To the best of our knowledge, most – if not all – of these products have never been prepared previously in enantiopure form. Moreover since, in principle, the enantiopure (S) epoxides can be chemically hydrolysed to the corresponding (S) diol, and the enantioenriched (R) diols cyclised back to the (R) epoxide, this methodology opens the way to all the possible epoxide and diol enantiomers.^[5a,16] Indeed, a second resolution cycle applied to the thus obtained enantioenriched (R) epoxides would afford the corresponding diols in enantiomerically pure form.

Further experimental improvements: We have previously described several examples illustrating the fact that, by using a two-phase reactor, such a hydrolytic kinetic resolution could be performed on different styrene oxide derivatives at a substrate concentration as high as several hundred grams per litre.^[4] This very interesting achievement, paving the way to possible industrial-scale application, was in fact based on the possibility to assure a sufficient phase-transfer rate between the aqueous and organic (the substrate itself) phases by creating an emulsion under vigorous stirring. Unfortunately, our preliminary experiments aimed at applying this methodology to either substrate 1-8 did not lead to satisfactory results. This is obviously due to the presence of fluorine atoms, as it is well known that fluorinated derivatives do exhibit rather surprising (and specific) physico-chemical properties.^[17] As a consequence, this interestingly illustrates the fact that, although chemical reactivities of fluorine-bearing epoxides toward the A. niger epoxide hydrolase are not altered, practical limitations to scale-up may be encountered due to such specific properties. Obviously, more elaborated methodologies will have to be set up in order to allow efficient resolution to be performed.

The aim of this study was to explore the possibility to perform the hydrolytic kinetic resolution of various trifluoromethyl-substituted styrene oxide derivatives using the *A. niger* epoxide hydrolase. Our results described in this paper indicate that, in spite of the presence of fluorine atoms, which very often disturb chemical reactivity, this is indeed possible. Thus, we have shown that, for all (but one) of the epoxides we have studied, such a resolution can be performed very efficiently under gentle experimental conditions, i.e., at room temperature and in plain water/DMSO solution. Moreover, by using a (w/w) substrate over enzyme powder ratio of about 60 to 120, these resolutions could be performed within one hour (or less). The reactivity as well as the enantioselectivity of the selected substrates was in perfect agreement with the previous tendency we have observed using this enzyme. Thus, the para-substituted derivatives exhibited the highest E value (up the 160) and, interestingly, the 1,1-gem-disubstituted epoxides were also processed. The HKR of such disubstituted epoxides has never been described using Jacobsen's salen(Co)OAc catalysts, and control experiments we have attempted following this methodology on our substrates were unsuccessful. The described methodology thus allowed us to prepare all (but two) of the fluorinated epoxides or diols in enantioenriched form, most (but two) of them for the first time. Preliminary experiments aimed at increasing the substrate concentration by using a two-phase reactor approach, however, did not lead to satisfactory results. Further work toward this goal is ongoing in our laboratory and will be described later on.

Experimental Section

Analytical Scale BHKR Experiments

In a typical experiment, 5.1 mg (2.5 mM) of 4-(trifluoromethoxyphenyl)-oxirane 4 were dissolved in 3 mL of DMSO. Distilled water (7.9 mL) as well as 3 µL of 3-(trifluoromethyl)-acetophenone (as an internal standard) were added. A solution of A. niger epoxide hydrolase was prepared separately by dissolving 2 mg of recombinant enzymatic powder (about 25% purity) in 4.55 mL of distilled water. The two solutions were placed at 27°C for half an hour in order to equilibrate the temperature. The reaction was started by addition of 100 µL of the enzymatic solution to the epoxide solution. Aliquots (400 µL) were withdrawn at definite time intervals and mixed with 200 µL of acetonitrile to stop the reaction, under vortex agitation. This sample was further extracted with 400 µL of isooctane and the organic phase analysed by chiral GC for conversion ratio and ee of the epoxide.

Circular Dichroism Measurements

A DMSO solution of $Mo_2(OAc)_4$ (2 mL) is placed in a 3 mL UV cuvette and a DMSO solution (200 µL) of diol is added. The final concentrations of reactant and diol are between 0.375 and 0.750 mM depending on the specific diol. The cuvette is shaken and the ICD spectra observed immediately (20 °C; 50 nmmin⁻¹; time constant 1 s; bandwith 2 nm). A new spectrum was recorded each 10 min until stabilisation of the signal.

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