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Synthesis of a variety of optically active hydroxylated heterocyclic compounds using epoxide hydrolase technology

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ABSTRACT

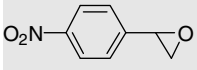
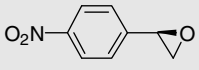
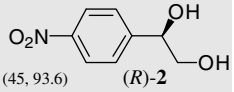
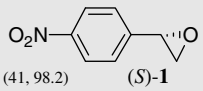
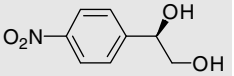
Novel epoxide hydrolases in *Yarrowia lipolytica* have been shown to hydrolyse a variety of functionalised epoxides with good to excellent stereoselectivity and at high volumetric productivities. Individual biotransformation products have been converted into optically active (*R*)-(tetrahydrofuran-2-yl)methanol (**6**), (*S*)-*N*-benzyl-3-hydroxypyrrolidine (**7**), (*S*)-3-hydroxytetrahydrothiophene (**8**), (*S*)-*N*-benzyl-3-acetoxypiperidine (**10**), (*S*)-3-hydroxytetrahydrofuran (**16**) and (*R*)-[(*S*)-*N*-benzylpyrrolidin-2-yl](phenyl)-methanol (**20**).

The preparation of optically active epoxides and optically active vicinal diols using epoxide hydrolase (EH) technology has attracted significant attention.[1] The work of Furstoss is of particular note.[2] However, to date EH technology has suffered from limitations in substrate concentration (and hence volumetric productivity) often rendering this approach inferior to alternative chemical (e.g., Jacobsen) technology.[3]

Recently, a portfolio of EHs has been engineered in the yeast *Yarrowia lipolytica*.[4] This novel biotransformation platform is extremely robust, and can be applied to a wide range of substrates at high initial concentration. The availability of a library of different EH catalysts allows the selection of the appropriate enzyme to furnish the required optically active epoxide and/or diol from a racemic epoxide. This is illustrated by the transformation of a standard substrate, namely *para*-nitrostyrene oxide (**1**), with two EHs (Oxy-4 and Oxy-10, see Table 1). Incubation of racemic **1** with Oxy-10 affords the (*R*)-epoxide **1**[2b] and the (*R*)-diol **2**[2b] through attack by the incoming water molecule at the benzylic position with inversion of configuration (⩾97% selectivity). In contrast, transformation of racemic epoxide **1** with the EH Oxy-4 afforded the (*S*)-epoxide **1** and the (*R*)-diol **2**[2b] reflecting enzyme-catalysed attack by water at the terminal position (⩾94% selectivity).

It was of commercial interest to subject less well-studied non-aromatic substrates to the new biotransformation system in order to provide products which, on further transformation, would provide noteworthy hydroxylated heterocyclic compounds. Initial experiments concentrated on obtaining the epoxides in high optical purity, and hence the biotransformations were run to relatively high conversion (62–73%). The results are summarised in Table 2. Two bromo-epoxyalkanes **3** and **4** and an epoxyester **5** were

Table 1
Epoxide hydrolase biotransformations on (±)-epoxide **1**

Substrate	Enzyme	% Conversion[a]	Product epoxide (yield[b] %, ee %)	Product diol (yield[b] %, ee %)
 **1**	Oxy-10	50	 (43, 99.3) (*R*)-**1**	 (45, 93.6) (*R*)-**2**
1	Oxy-4	49	 (41, 98.2) (*S*)-**1**	 (46, 89.3) (*R*)-**2**

[a] Based on HPLC analysis.
[b] The resolved epoxides and diols were isolated by column chromatography over silica gel.

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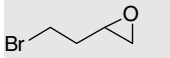
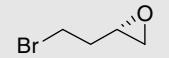
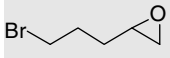
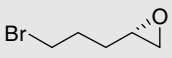
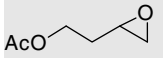
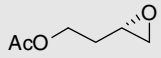
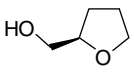
Table 2
Epoxide hydrolase biotransformations on non-aromatic, functionalised epoxides

Substrate	Enzyme	% Conversion[a]	Product epoxide (yield[b] %, ee %)
 **3**	Oxy-9	68	 (26, 98.6)[5] (*S*)-**3**
 **4**	Oxy-1	62	 (26, 98.7)[6,c] (*S*)-**4**
 **5**	Oxy-3	73	 (20, 97.8)[7,d] (*S*)-**5**

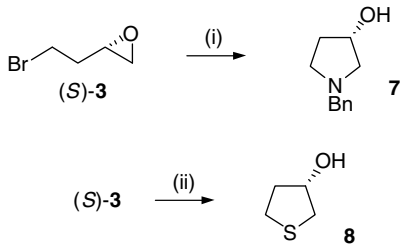
[a] Based on GC analysis.
[b] The resolved epoxides were isolated by column chromatography over silica gel.
[c] Configuration is deduced from the optical data literature reference for the 3-acetoxypiperidine product **10**[6] (see text).
[d] Configuration is deduced from the optical data reference for the deacetylated product **13**[7] (see text).

screened against a panel of EH catalysts to identify strains exhibiting chiral activity suitable for preparative resolutions. A typical procedure, which demonstrates the high substrate loading that can be achieved, is described for epoxide **3**. Thus, for a final reaction mixture volume of ca. 100 cm^3, 5.0 g of wet cells of the Oxy-9 *Y. lipolytica* strain (i.e., ca. 1 g of dry weight equivalent) was suspended in 100 mM phosphate buffer (pH 7.5) made up to a volume of 60 $cm.^3$ The cell suspension was chilled in a baffled stirred vessel to 2–4 °C. Then racemic 4-bromo-1,2-epoxybutane **3** (60 g, 0.40 mol, ca. 40 cm^3) was added over 1 min with vigorous stirring. Samples were removed and extracted into 20 volumes of ethyl acetate, and dried over anhydrous $MgSO_4$. The epoxide was analysed by chiral GC, using a β-Dex 225 (Supelco) column. At the required enantiomeric excess (after 90 min), the reaction mixture was quenched by the addition of two volumes of chilled acetone, and mixed for 30 min at 2–4 °C. The precipitated biomass was removed by suction filtration through a Büchner funnel. After removing the acetone under reduced pressure, the products were extracted twice into ethyl acetate. The combined organic layer was dried ($MgSO_4$), filtered and evaporated to yield a crude product, which was purified by column chromatography over silica gel (using hexane–ethyl acetate mixtures as eluents) to yield epoxide (*S*)-**3** (17.8 g; ee 98.6%).[5]

In the studies summarised in Table 2, no effort was made to optimise the production and isolation of the optically active diols, though it was interesting to note that the hydrolysis product derived from the epoxide (±)-**4** spontaneously cyclised in aqueous solution to give (*R*)-(tetrahydrofuran-2-yl)methanol **6** (ee 67% on the basis of optical data).[8]


6

Obviously, the biotransformation system is tolerant of a distal bromine atom and a remote acetate group, which is useful because the readily available optically active epoxides could then be manipulated by simple procedures to afford optically active heterocyclic compounds. In the first example, (*S*)-**3** was reacted with 2 equiv of benzylamine, in the presence of 1.5 equiv of triethylamine, to afford (*S*)-*N*-benzyl-3-hydroxypyrrolidine **7**[9] as the exclusive product, which was isolated in 86% yield (Scheme 1). Pyrrolidinol **7** is a versatile pharmaceutical intermediate, for example,


Scheme 1. Reagents and conditions: (i) $BnNH_2$, NEt_3, THF, reflux 4 h, 86%; (ii) $Na_2S{\cdot}9H_2O$, CH_3CN, rt, 14 h, 89%.

it is used for the preparation of the calcium antagonist *Barnidipine*, amongst other applications.[10]

Owing to practical issues[11a] associated with an alternative published route to pyrrolidine **7** (and the deprotected heterocycle) from (*S*)-malic acid,[11b] other methodologies have been developed for the preparation of **7**,[12] which are complementary to the simple route described above.

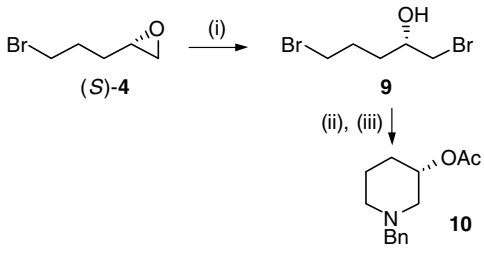
Similarly, the reaction of (*S*)-**3** with sodium sulfide resulted in the exclusive formation of (*S*)-3-hydroxytetrahydrothiophene **8** (Scheme 1).[13] Compound **8** and its derived sulfone have been utilised as novel P_2 ligands for the development of HIV-1 protease inhibitors.[14]

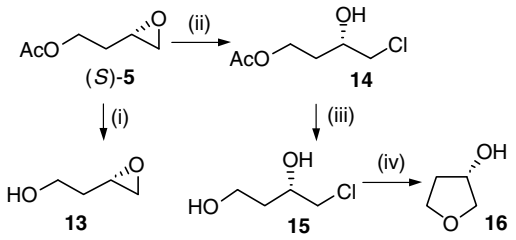
The reaction of (*S*)-**4** with benzylamine under conditions similar to those described in Scheme 1 resulted in a mixture of products. However, ring-opening of the epoxide (*S*)-**4** with LiBr,[15] followed by acetylation of the bromohydrin intermediate **9** and subsequent reaction with benzylamine in THF provided the known compound (*S*)-3-acetoxypiperidine **10**[6] in 64% overall yield (Scheme 2). Piperidine **10**, in its *N*- and/or *O*-deprotected form, has been used for the preparation of cholinotoxic agents.[16]

The epoxide (*S*)-**5** was deacetylated efficiently in the presence of a lipase enzyme (from *Rhizomucor miehei*, expressed in *Aspergillus oryzae*) to afford the versatile chiral building block, (*S*)-3,4-epoxy-1-butanol **13** in 92% yield (Scheme 3). Several asymmetric metal-catalysed epoxidations of 3,4-buten-1-ol to produce optically active **13** [and its (*R*)-enantiomer] have been reported.[17] However, such procedures result in low yields and moderate ee's for the desired product **13**.

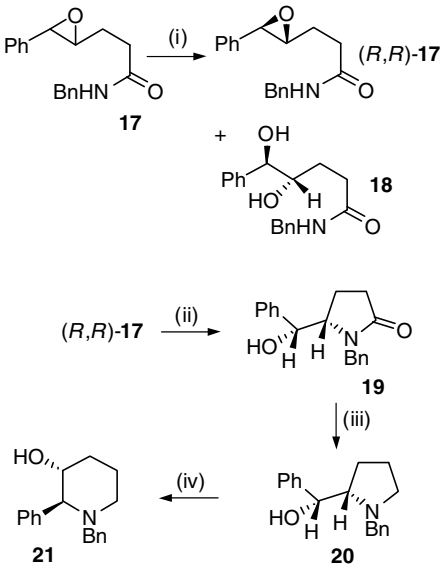
Optically active **13**, for example, has been recently utilised to develop the first total syntheses of the cell division inhibitors, 11-α- and 11-β-methoxycurvularin.[18]

In another application, ring-opening of epoxide (*S*)-**5** with LiCl,[15] followed by deacetylation of the intermediate chlorohydrin **14** under the same conditions as described before, afforded the optically active 1,3-diol **15** in good yield (Scheme 3). It is known that **15** can be transformed efficiently, by acidic catalysis in aqueous medium, to produce (*S*)-3-hydroxytetrahydrofuran **16**,[19] which is used for the production of the well-known HIV protease inhibitors amprenavir and fosamprenavir.[11a]


Scheme 2. Reagents and conditions: (i) LiBr, AcOH, THF, 91%; (ii) AcCl, pyridine, CH_2Cl_2; (iii) $BnNH_2$, pyridine, THF, reflux 7 h, 70% over 2 steps.



Scheme 3. Reagents and conditions: (i) lipase (see text), phosphate buffer (pH 7.5), 14 h, 92%; (ii) LiCl, AcOH, THF, 77%; (iii) same as (i), 48 h, 75%; (iv) see Ref. 19, 79%.



Scheme 4. Reagents and conditions: (i) Oxy-10 EH; (ii) K^{t}BuO, ^{t}BuOH, reflux 2 h, 89%; (iii) LAH, THF, reflux 1 h, 96%; (iv) see Refs. 22 and 23.

In addition, *Y. lipolytica* EH (Oxy-10) was employed to selectively hydrolyse the epoxyamide **17** (Scheme 4). In this instance, the biotransformation (which was run to 50% substrate conversion) was highly enantioselective with optically pure (⩾99.9% ee) epoxide and equally pure diol **18** (⩾99.9% ee) being isolated in unoptimised yields of 32% and 22%, respectively. The configuration of the epoxide was established as (4*R*,5*R*) by further chemical transformations (see below). X-ray crystallographic analysis revealed that the diol (**18**) possessed the *threo* stereochemistry (see Fig. 1), but the absolute stereochemistry of the compound was not determined.

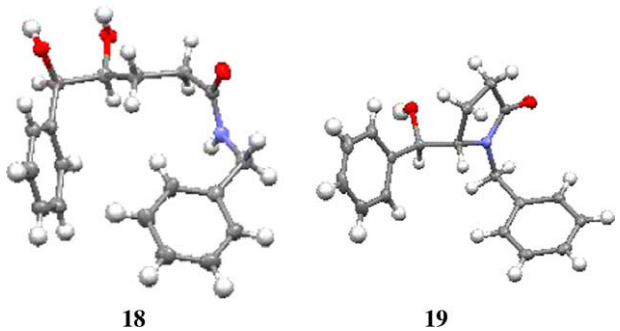


Figure 1. Mercury generated representations of the structures of compounds **18** and **19**.

The optically active *trans*-epoxyamide (*R*,*R*)-**17** was heated at reflux in *tert*-butanol in the presence of potassium *tert*-butoxide to afford pyrrolidone **19** in 89% yield (Scheme 4). This 5-*exo*-*tet* cyclisation is similar to a previously reported reaction of an analogous *cis*-epoxyamine.[20] The structure of **19** was confirmed by NMR spectroscopy and by X-ray crystallography (see Fig. 1). Reduction of **19** with lithium aluminium hydride afforded the known pyrrolidine **20** in high yield. The absolute stereochemistry of **20** (and hence, that of the precursor pyrrolidone **19**) could be deduced by comparing the NMR and optical data with those in recent literature.[21]

Pyrrolidinol **20** is a valuable chiral intermediate for the synthesis of a wide range of pharmacologically active products and intermediates. For example, the transformation of **20** under well-defined conditions (trifluoroacetic acid anhydride, followed by NEt_3 and NaOH)[22] affords ring-expanded 3-hydroxypiperidine **21**, without loss of stereochemical integrity.[23] The piperidine **21** can be utilised for the synthesis of non-peptidic substance P inhibitors,[24] and also for the preparation of *trans*-(2*R*,3*R*)-3-hydroxypipecolic acid, which is a precursor for the α-D-mannosidase inhibitor (−)-swainsonine.[25] The new synthesis of pyrrolidinol **21** described above circumvents the requirement for separation of diastereomers, a problem with earlier synthetic routes to this material.[21,23,26]

In conclusion, a robust epoxide hydrolase technology has been developed and applied to the preparation of optically active, functionalised epoxides and vicinal diols. These chiral products are applicable to the synthesis of pharmacologically important heterocycles. The biocatalytic resolutions were successfully applied at unprecedented substrate loading and were complete within hours, demonstrating the efficiencies of the technology and competitiveness with chemical methods.

Crystallographic data (excluding structure factors) for the structures of compounds **18** and **19** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 688482 and 688481, respectively.

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