Summary

Development of Biocatalytic Methods for procuring Enantiomerically Pure Alcohols

Enantiomerically pure compounds play a significant role in the present day life as they find applications in the synthesis of numerous bioactive molecules such as agrochemicals, pharmaceuticals, pheromones, fragrances, diagnostics, bio-fuels and performance chemicals. Among the enantiomerically pure compounds, chiral alcohols, epoxides and diols formed represent important classes of molecules in organic chemistry. The chiral alcohols bearing functional substituent at α-carbon are valuable due to their transformation of hydroxyl group to amines, azides, chlorides and epoxides. The sulfur atom placed at an appropriate position from the hydroxyl group provides unique synthetic versatility, so the chiral hydroxy sulfides also occupy a special place among the enantiopure alcohols. They can be used as the synthons of a variety of chiral organic compounds, such as chiral oxiranes, allylic alcohols, lactones, macrolides, pheromones and tetrahydrofurans. On the other hand, chiral epoxides and their diols find many applications in the synthesis of complex molecules due to their ability to undergo nucleophilic attack by a variety of nucleophiles such as carbanions, alcohols, amines, and thiols leading to the formation of new C-C, C-O, C-N and C-S σ-bond, respectively. These classes of enantiomerically pure compounds have been synthesized

2 Kaluzna, I.A; Rozzell, J.D; Kambourakis, S. Tetrahedron Asymmetry 2005, 16, 3682-3689
using different synthetic approaches such as chiral pool synthesis, chiral auxiliary directed asymmetric synthesis and asymmetric catalysis.

Asymmetric catalysis is advantageous in comparison to the other methods due to the requirement of catalyst in sub-stoichiometric amount, easy purification, high turnover number, low cost of the process and reusability. The asymmetric catalysis has been further divided into transition metal complexes (metal catalysis), organocatalysis and biocatalysis depending on the use of different catalysts. Among these, biocatalysis involves the use of nature’s catalysts i.e. enzymes, which provides a greener approach towards chiral molecules to obtain enantiomerically pure compounds. It offers advantages over the other two owing to the milder reaction conditions such as ambient temperature and atmospheric pressure, exquisite catalytic efficiency, non-toxic effluents and good substrate selectivity.\(^\text{14}\)

The significance of these chiral alcohols and our interest in biocatalysis, directed us towards employment of biocatalytic reduction and kinetic resolution approach for procuring chiral alcohols. Hence, the present research work is presented in three chapters:

**Chapter 1: Introduction**

**Chapter 2:** Bioreduction of phenacyl bromide derivatives with whole cell of bacteria *Pseudomonas gessardii* which consists of screening of bacterial isolates, optimization of reaction conditions for improvement of enantioselectivity and conversion as well as screening of various substrates under the obtained condition.

**Chapter 3:** Synthesis and enantioselective resolution of \(\beta\)-hydroxy sulfides containing selection of lipase and optimization of reaction conditions, enantioselective resolution of aryl \(\beta\)-hydroxy sulfides under optimized conditions. A further oxidation to \(\beta\)-hydroxy sulfoxides was also studied

**Chapter 4:** Kinetic resolution of glycidyl ethers by recombinant epoxide hydrolase from *Streptomyces grisius* (SGEH) consisting of biohydrolysis of glycidyl ethers catalyzed by purified epoxide hydrolases.

In each chapter, the discussion of the present work is preceded by a brief review of literature.

Chapter 1: Introduction

This chapter gives an account of organization of the thesis along with general introduction and application of various biocatalytic methods for procuring enantiomerically pure alcohols.

Chapter 2: Bioreduction approach for procuring enantiomerically pure alcohols

Section 2.1 Introduction

Section 2.2 Review of literature

Section 2.3 Result and Discussion

Section 2.1 Introduction

This section incorporates the general introduction about the biocatalytic methods for achieving chiral alcohols.

Section 2.2 Review of literature

This section incorporates the recent developments in the whole cell microorganism catalyzed bioreduction of ketones to provide enantiopure alcohols, which are useful synthons and pharmaceutical intermediates.

Section 2.2 Result and discussion

The results of the investigation provided an efficient bacterial strain, *Pseudomonas gessardii* out of the twenty four bacterial strains that were screened for their ability to transform 3-nitrophenacylbromide (3) to 2-bromo-1-(3-nitrophenyl) ethanol (3a). After getting a potent bacterial strain for the desired bioreduction, the reaction conditions were optimized in terms of different reaction parameters, e.g., pH, temperature, substrate concentration, organic supplements types and their concentrations and reaction time. All these parameters were optimized one by one, keeping the all others constant and were verified by response surface methodology. Optimized condition obtained by varying above parameters provides us a protocol of bioreduction including MSM medium supplemented with 1% glucose as carbon source, 0.3% yeast extract as nitrogen source, substrate concentration of 0.5mg/mL, at incubation temperature of 30°C and 7.0 pH of medium.
**Scheme 1**: Enzymatic conditions for bioreduction of various phenacyl bromide derivatives

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<th>Substrate</th>
<th>Conversion (a) (%)</th>
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<td><img src="image" alt="Substrate 1" /></td>
<td>&gt;99</td>
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Further, the substrate scope was studied under the optimized conditions. It was found that the phenacyl bromide (1) was converted to its corresponding alcohol in 72% ee. Irrespective of the electronic nature of the group, ortho substituted phenacyl bromide (4, 13) gave enantioselectivity equivalent to that of unsubstituted phenacyl bromide (1) while meta substituted and para substituted phenacyl bromide derivatives gave product with enantioselectivity greater than ortho. It was thus concluded that the para substituted phenacyl bromides are good substrates for carbonyl reductions with this enzymatic system. In case of halo substitution at para position of the aromatic moiety, it was observed that p-Bromo phenacyl bromide yielded the corresponding alcohol in 95% ee while p-chloro phenacyl bromide in 73% ee. On the other hand, p-iodo-substituted phenacyl bromide (8) and p-fluoro phenacyl bromides (7) gave the corresponding alcohol in 27% ee and 32% ee, respectively. Carrying out the reduction of α-chloroacetophenone (14) and α-azidoacetophenone (15), the corresponding alcohols were obtained in 21% ee.
and 32% ee, respectively. In case of cyano derivative (16), neither substrate nor product was detected, which may be due to the possibility of degradation of both the substrate and the formed product.

In conclusion we have developed a biocatalytic methodology using growing cells of *Pseudomonas gessardii* for procuring enantiomERICally enriched halohydrin derivatives. The process has been optimized by OFAT and further verified by RSM. The scope and limitation of this methodology has also been explored.

Chapter 3: Lipase mediated resolution of \( \beta \)-hydroxy sulfides containing selection of lipase and optimization of reaction conditions, enantioselective resolution.

Section 3.1 Introduction and review of literature

Section 3.2 Result and Discussion

Section 3.1 Introduction and review of literature

Organosulfur compounds are important building blocks in synthetic organic chemistry. They can be used as intermediates as well as chiral auxiliaries both for analytical and synthetic applications.\(^{15}\) The brief review of literature on lipase resolution of cyclic hydroxy compounds has been discussed.

Section 3.2 Result and discussion

The results of the finding of this chapter are discussed in the following sections:

Section 3.2.1 Selection of lipase and optimization of reaction conditions

Section 3.2.2 *Candida antarctica* lipase catalyzed enantioselective resolution of aryl \( \beta \)-hydroxy sulfides

3.2.1 Selection of lipase and optimization of reaction conditions:

In the present investigations, lipase based methodology has been developed for the resolution of \( \beta \)-hydroxy sulfides. The selection of the lipase for kinetic resolution is based upon the screening of the available lipases for their suitability.

In the preliminary study, different lipases were used to resolve \( \text{trans-2-} (\text{phenylthio})\text{cyclohexanol} \), (±)-14 using vinyl acetate as acylating agent under solvent

free conditions at room temperature (Scheme 2). The progress of the reactions was monitored at regular intervals for 24 h by chiral HPLC.

Scheme 2

Candida antartica B lipase (CAL B) was found to be most efficient in resolving (±)-14 in 4 h to provide (1R, 2R)-14a in >99% ee and (1S, 2S)-14 in 92% ee, so it was selected for further optimization. Further, studying the effect of different solvents, it was found that using methyl tert-butyl ether (MTBE) as solvent both the acetylated product as well as unreacted alcohol have >99% ee. Varying the amount of lipase, it was found that conversion as well as enantioselectivity increases from 0.2 to 1.00 of lipase: substrate ratio while the lipase substrate ratio 1:1 was optimum to achieve 50% conversion and >99% ee. The amount of vinyl acetate was optimized and found giving best results using 10 equiv. of the vinyl acetate with respect to 1 equiv. of the substrate. The reaction temperature of 30°C was selected for running all further reactions. Thus, the optimized condition for the resolution of trans-2-(phenylthio)cyclohexanol (±)-14 consist of stirring a mixture of (±)-14 (100 mg) in MTBE (1 mL) and vinyl acetate (10 equiv.) with CAL-B (100 mg) as the catalyst.

3.2.2 Candida antartica B lipase catalyzed enantioselective resolution of β-hydroxy sulfides

In this section Candida antartica B lipase (CAL-B) catalyzed resolution of trans-2-(phenylthio)cyclohexanol derivatives has been discussed.
It had been observed that conversion of 50% and >99% enantioselectivity with $E > 500$ was achieved for both the acetylated and unreacted enantiomers in all \textit{trans}-2-(phenylthio)cyclohexanol derivatives. However, a slight variation in reaction times (3-9 h) was observed. It has been found that the resolution of \textit{trans}-2-(phenylthio)cyclohexanol can be obtained with >99% ee in 4 hours while the \textit{trans}-2-(phenylthio)cyclopentanol (24) was resolved in 3 hours affording (1R,2R)-2-(phenylthio)cyclopentyl acetate and (1S,2S)-2-(phenylthio)cyclopentanol in >99% ee. Varying the substitution at phenyl ring of the thiol part of the molecule, it was observed that \textit{p}-halo substituted \textit{β}-hydroxy sulfides took longer time for the transformation than \textit{p}-methyl- and \textit{p}-methoxy- substituted derivatives (20, 21). It was observed that \textit{ortho}-substituted derivative took 9 h to resolve while \textit{meta}- and \textit{para}- were resolved in 7 h and 6 h, respectively. \textit{Trans}-2-(Cyclohexylthio)cyclohexanol (22) provided the corresponding acetate and alcohol in 50% conversion in 8 hours. Immobilized CAL-B gave reproducible results with 50% conversion and >99% enantioselectivity for the kinetic resolution of \textit{trans}-2-(phenylthio)cyclohexanol with vinyl acetate in MTBE over the four reuse cycles investigated. The same enantioselectivity and conversion was observed over the four sequentially performed reactions, however the time taken to achieve 50% conversion was longer. The second cycle requires 12 h in order to reach the full 50% conversion instead of 4 h in the first cycle while the third cycle requires almost 24 h to achieve complete conversion.

Further, sulfoxidation of the isolated enantiopure (1R,2R)-14a and (1S,2S)-14 was studied with \textit{m}-CPBA in CH$_2$Cl$_2$ at -20°C (Scheme 3.19). The oxidation of (1R,2R)-14a provided a mixture of (1R, 2R, $S_s$)- and (1R, 2R, $R_s$)-2-(phenylsulfinyl)cyclohexyl acetate 14a. The fractional crystallization of this diastereomeric mixture provided (1R, 2R, $S_s$)-25a as pure colorless crystals (>99% ee, mp 153°C) having $[\alpha]_D^{27} = +112.9$ (c 0.50, CH$_2$Cl$_2$) and mother liquor (25a) as a colorless liquid with $[\alpha]_D^{27} = -110.0$ (c 1.0, CH$_2$Cl$_2$). The crystalline diastereomer (1R, 2R, $S_s$)-25a was assigned the configuration on the basis of its X-ray data. Similarly oxidation of (1S,2S)-2-(phenylthio)cyclohexanol (17) followed by crystallization afforded (1S, 2S, $S_s$)-25 and (1S, 2S, $R_s$)-25 (>99% ee, mp: 156°C) having $[\alpha]_D^{27} = -129.2$ (c 1.0, CH$_2$Cl$_2$).
In conclusion, CAL-B mediated kinetic resolution of cyclic β-hydroxyulfides provides the chiral β-hydroxyulfides and their acetates in good yield and excellent enantioselectivity (>99%). The products can be further oxidized to their corresponding sulfoxides using \( m-\text{CPBA} \) at low temperature.

**Chapter 4: Kinetic resolution of glycidyl ethers by recombinant epoxide hydrolase from *Streptomyces griseus*.

**Section 4.1 Introduction**

Chiral epoxides and vicinal diols are important intermediates in the synthesis of pharmaceutical compounds, drugs and agrochemicals.\(^{16}\) This section contains general introduction about the biocatalytic pathways for obtaining these molecules.

**Section 4.2 Review of literature**

Among chiral epoxides, aryl glycidyl ethers are potentially important intermediate for the synthesis of chiral amino alcohols\(^ {17} \) and β-blockers.\(^ {18} \) Vicinal diols obtained by hydrolysis of glycidyl ethers are also used as intermediates in pharmaceuticals such as


guaiifenesin, mephenesin and clorphene.\(^{19}\) A brief review of literature on the biohydrolysis of glycidyl ethers catalyzed by epoxide hydrolases as whole cell and recombinant enzyme is presented in this section.

**Section 4.2 Result and discussion**

In this section the result of investigation on the substrate scope of the recombinant EH gene from *Streptomyces grisius* by carrying out the enzymatic hydrolysis of aryl glycidyl ethers has been done. The resolutions were studied by carrying out blank, test and control experiments for all the substrates. The reaction was quenched by extracting with diethylether (3x500 µL), dried over anhydrous sodium sulfate and concentrated to obtain the resulting residue. The obtained residue was dissolved in 150 µL isopropanol and the samples were injected to HPLC (Shimadzu) on Chiralcel OD–H column or and Chiralcel IB (Diacel) column having PDA detector.

The stereoselective hydrolysis of all the glycidyl ethers (26-33) by SGEH can yield optically pure \((R)\)-epoxides and \((S)\)-diols in 0.5 hour indicating that the substituents do not influence the positioning of the substrate in the active site of the enzyme. In order to study the effect of substituent on the hydrolytic kinetic resolution by SGEH, different substituted aryl glycidyl ethers were screened. Glycidyl ethers having both the *ortho* positions substituted were well recognized by the recombinant enzyme. 2,6-dichlorophenyl glycidyl ether (30) provide the corresponding diol with 89% enantioselectivity at 41% conversion in 0.5h while the remaining epoxide have enantioselectivity of 65%. On the other hand, 2,6-Dimethylphenyl glycidyl ether (29)

undergo 48% conversion to corresponding diol with 73% ee, providing 64% ee for the residual epoxides. However, aryl glycidyl ether substituted with methyl group at one ortho position (28) resulted in 70% enantioselectivity in epoxides and 62% in corresponding diol. Replacing the methyl with nitro group at one ortho position slower down the hydrolysis as only 18% conversion was found in case of nitro substituted glycidyl ether (31) in comparison to 54% conversion in 2-methyl glycidyl ether. In case of α-Naphthyl glycidyl ether (33), a conversion of 30% was obtained in 0.5 h with 34% enantiomeric excess of epoxide and 57% enantiomeric excess for corresponding diol.

Comparing the results for benzyl glycidyl ether (26) and phenyl glycidyl ether (27), it had been found that the introduction of CH$_2$ spacer between the phenyl ring and ethereal oxygen slow down the hydrolysis as only 10% conversion of benzyl glycidyl ether was observed in comparison to 51% conversion of phenyl glycidyl ether to its corresponding diol in 0.5 h. The 42% enantiomeric excess of 3-(benzyloxy)propan-1,2-diol and 5% ee for benzyl glycidyl ether was obtained. On the other hand, PGE, the enantiomeric excess of remaining epoxide was 40% and ee of its corresponding diol was 69% at conversion of 51%.

Replacing the ethereal oxygen with sulfur atom (32) under similar conditions resulted in very fast hydrolysis hence decreasing the enantiomeric excess for diol as well as epoxide. Here, the conversion of 91% was obtained with only 3% ee for corresponding diol and 32% for the remaining epoxides. Hence, it was observed that arylglycidyl ethers containing O are much suitable substrates rather than the sulfur containing aryl glycidyl thioethers.

Thus, application of recombinant epoxide hydrolase from Streptomyces griseus (SGEH) in the kinetic resolution of glycidyl ethers has been successfully developed. This protocol provides a convenient and environmentally benign route for the synthesis of chiral vicinal diols and epoxides in an enantioselectivity of up to 89%.