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# A two-step enzymatic resolution of glycidyl butyrate

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**Abstract**

A two-step enzymatic resolution process for production of (*R*)- and (*S*)-glycidyl butyrate was investigated and the lipases were screened. The first step involved a hydrolysis of (*R*,*S*)-glycidyl butyrate catalyzed by porcine pancreatic lipase (*S*-favored) with an *E* of 21 for production of (*R*)-glycidyl butyrate (13.2 mmol, 98% ee, 36% yield) under the optimal conditions (pH 7.4, 30 °C, 30 mg/ml CTAB). Then, the recovered (*R*)-enriched glycidol (19.8 mmol, 65% ee, 56% yield) was used for transesterification catalyzed by Novozym 435 (*R*-favored) with an *E* of 69 to obtain (*S*)-glycidyl butyrate (15.1 mmol, 98% ee, 42% yield) under the optimum conditions ($a_W$ = 0.24, *n*-heptane, 80 min).


*Keywords:* Two-step; Resolution; Glycidyl butyrate; Porcine pancreatic lipase; Novozym 435

## 1. Introduction

Optically pure 2,3-epoxy-1-propanol (glycidol) and its derivatives are versatile intermediates in organic synthesis because the epoxide ring is reactive towards nucleophiles for the synthesis of asymmetric alcohols [1–3]. Particularly, both enantiomers of the (*R*)- and (*S*)-glycidyl butyrate are chiral synthesis units for the production of biologically active compounds of commercial interest. (*R*)-Glycidyl butyrate has been used to introduce a stereogenic center in the synthesis of Linezolid [4,5], which is currently marketed for the treatment of multidrug resistant Gram-positive infections such as nosocomial, community-acquired pneumonia, and skin infections. (*S*)-Glycidyl butyrate is also very useful as starting material in the synthesis of many drugs, such as (*R*)-argentilactone [6] which exhibits both antileishmanial activity and cytotoxic activity against mouse leukemia cells [7]. Recently, lipases have been widely used in the resolution of (*R*,*S*)-glycidyl butyrate [8–10]. Due to the existence of a lid in most lipases [11–14], the open–close status of the conformation may be changed dramatically under various experimental conditions, which in turn strongly affects the activity and enantioselectivity of lipases [15,16]. Many methods (such as medium engineering, immobilization techniques et al.) are often used to improve the activity and the enantioselectivity of the lipase. However, the enantioselectivity of most lipases toward (*R*,*S*)-glycidyl butyrate is still low with a poor yield [17,18]. Although the (*R*)-glycidyl butyrate can be obtained from hydrolysis of (*R*,*S*)-glycidyl butyrate in ton quantities, it is necessary to reach high conversions for obtaining adequate enantiomeric excesses (ee). It is more difficult to prepare (*S*)-glycidyl butyrate on a large scale because most lipases present (*S*)-stereochemical preference in hydrolysis of (*R*,*S*)-glycidyl butyrate, producing (*R*)-glycidyl butyrate and (*R*)-glycidol. Palomo et al. [19] have reported the enzymatic hydrolytic resolution of (*R*,*S*)-glycidyl butyrate by using Novozym 525L, which presents an enantioselectivity towards the (*R*)-glycidyl butyrate, and obtained the (*S*)-glycidyl butyrate. However, the (*S*)-glycidyl butyrate in their reports is only obtained at 90% ee and less than 36% yield for its low enantioselectivity of Novozym 525L.

Contrary to the transformations based on the classic kinetic resolution (KR) catalyzed by enzymes, where one enantiomer can be obtained with high enantiomeric excess [20], two-step enzymatic resolution is a method to obtain both enantionmers with high enantiomeric excess by subjecting the isolated enantiomerically enriched product of the first enzymatic resolution to the second [21], which results in the increase

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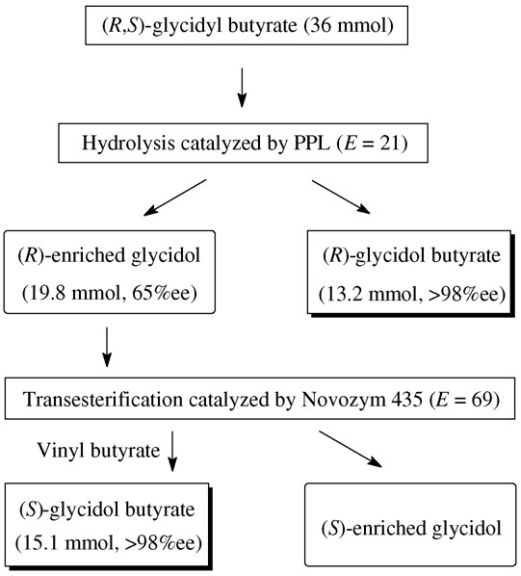


Scheme 1. Outline of the process developed for the production of (*R*)- and (*S*)-glycidyl butyrate.

of the enantiomeric excess of the product in the second step. A good example has been proposed by Fishman et al. [22], who use CALB as catalyst for a two-step enzymatic resolution of (*R*,*S*)-ethyl-3-hydroxybutyrate (HEB) and both enantiomers of (*R*)- and (*S*)-HEB are obtained at over 96% enantiomeric excess, with a total process yield of 73%. Similarly, Cong et al. [23] have reported the double kinetic resolution of the (*R*,*S*)-2-octanol through transesterification by *Pseudomonas* cp. lipase. The produced (*S*)-2-octanol and (*R*)-2-octanol in their results have been obtained with 98% ee and 95% ee, respectively and 80% racemic substrate are converted to enantiopure products. Zada et al. [24] also reported the production of (*R*)-lavandulol with 96.7% ee and (*S*)-lavandulol with 92.6% ee by combining the two-cycle enzymatic transesterification of racemic lavandulol with vinyl acetate as acyl donor.

In the present study, an efficient method to produce (*R*)- and (*S*)-glycidyl butyrate with high enantiomeric purity is performed by using a two-step enzymatic resolution with sequential hydrolysis and transesterification by porcine pancreatic lipase (PPL) and the immobilized *Candida antarctica lipase B* (Novozym 435) with the opposite enantioselectivity, respectively (Scheme 1). The reaction conditions for the hydrolysis and transesterification are also optimized.

## 2. Materials and methods

### 2.1. Materials

(*R*,*S*)-Glycidyl butyrate was prepared according to the procedure previously described [25]. Lipase from *Pseudomonas* sp. (PSL) and *Candida cylindracea* A.Y. lipase (AYL) was purchased from Amano Pharmaceutical Co. Ltd. (Japan). Novozym 435 was purchased from Novo (Bagsvaerd, Denmark). *Candida rugosa* lipase (CRL) was purchased from Sigma (St. Louis, MO). Porcine pancreatic lipase (PPL) was purchased from Shanghai Dongfeng Biochemical Reagent Co. Ltd. (China). *Candida lipolytic* lipase (CLL) was provided by Wuxi enzyme preparation plant (China). (*R*)-Glycidol, (*S*)-glycidol, (*R*)-glycidyl butyrate and (*S*)-glycidyl butyrate were purchased from Sigma (St. Louis, MO) to determine the retention times. Tween-80 was purchased from Tianjin Chemical Reagent Company (China). Bis-(2-ethylhexyl) sodium sulfosuccinate (AOT), hexadecyltrimethyl ammonium bromide (CTAB), (*R*,*S*)-glycidol, vinyl butyrate and other reagents of analytical grade were purchased from Shanghai Chemical Reagent Company (China).

### 2.2. Enantioselective hydrolysis of (R,S)-glycidyl butyrate

The hydrolysis was performed by adding (*R*,*S*)-glycidyl butyrate (5 ml, 36 mmol) and the lipase (100 mg) into sodium phosphate buffer (5 ml, pH 7.4, 10 mmol) containing surfactant CTAB (30 mg/ml), with magnetically stirred at 200 rpm and 30 °C. And the pH was kept constant by automatic titration of aqueous sodium hydroxide (NaOH, 4 M) by using a pH controller. The activity (μmol/min $mg_{prot}$) of the lipases in hydrolysis of (*R*,*S*)-glycidyl butyrate and the conversion were calculated based on the consumption of NaOH. Samples were recovered and analyzed by chiral high performance liquid chromatography (HPLC) at various time intervals. The hydrolysis was stopped at about 60% conversion by removing the lipase through centrifugation (11197 × *g*, 5 min), and then the reaction mixture was extracted with ether (10 ml) over three times. The combined organic extracts were subsequently washed with 10% (v/v) aqueous HCl (20 ml), saturated aqueous sodium bicarbonate (20 ml), brine (20 ml) and finally dried with anhydrous magnesium sulfate. The remained glycidyl butyrate was in ether layer while the generated glycidol was in the aqueous layer after extraction. The remained glycidyl butyrate (13.2 mmol) was obtained after the removal of ether by rotary evaporation (50 °C, 150 mbar).

### 2.3. Recovery of the generated glycidol

The generated glycidol in the first step was isolated by extraction of the aqueous layer with dichloromethane (20 ml) after prior removal of the glycidyl butyrate with ether. The organic phase concentrated by rotary evaporation (30 °C, 200 mbar) was analyzed by chiral gas chromatography (GC) and 19.8 mmol glycidol (65% ee) was recovered.

### 2.4. Enantioselective transesterification of the recovered glycidol

The transesterification were performed by using the recovered glycidol (19.8 mmol, 65% ee), vinyl butyrate (40 mmol), *n*-heptane (5 ml), water activity ($a_W = 0.24$) and lipase (10 mg) with magnetically stirred at 40 °C and 200 rpm. The transesterification was stopped at around 80% conversion by removing the lipase through centrifugation (11197 × *g*, 5 min). After the removal of the vinyl butyrate by rotary evaporation (50 °C, 100 mbar), the mixture was extracted with saturated aqueous sodium bicarbonate (10 ml) over three times to wash out the remaining glycidol and dried with anhydrous magnesium sulfate. (*S*)-Glycidyl butyrate (15.1 mmol) was obtained. All experiments were repeated three times with the measurement errors less than 5%, and the illustrative graphs were based on the average values.

### 2.5. Water activity setting

All the reaction mixture components were pre-equilibrated to the water activity ($a_W$) of the experiment through the vapor phase with saturated salt solutions at 25 °C [26,27]: LiBr ($a_W = 0.06$), LiCl ($a_W = 0.11$), $KCH_3COO$ ($a_W = 0.24$), $MgCl_2$ ($a_W = 0.33$), $Mg(NO_3)_2$ ($a_W = 0.53$), NaCl ($a_W = 0.75$), $K_2SO_4$ ($a_W = 0.97$). Equilibration was performed overnight.

### 2.6. Analytical methods

The extracted glycidyl butyrate were diluted by the mobile phase of *n*-hexane and ethanol (90:10, v/v) at a flow rate of 0.4 ml/min and analyzed by chiral HPLC using a Chiralpak AD column. UV detection was performed at 254 nm. The retention time of the (*R*)- and (*S*)-glycidyl butyrate were 14.3 min and 13.6 min, respectively.

The recovered glycidol was analyzed directly by GC on an Agilent 6890 instrument equipped with a flame ionization detector (FID) and a chiral column (HP-Chiral Cap. 30 m × 0.25 mm × 0.25 μm). The temperatures of the injector and the detector were 200 and 280 °C, respectively. Nitrogen was used as the