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Synthesis of enantiomerically pure glycidol via a fully enantioselective lipase-catalyzed resolution

Jose M. Palomo,[a] Rosa L. Segura,[a] Cesar Mateo,[a] Marco Terreni,[b] José M. Guisan[a,*] and Roberto Fernández-Lafuente[a,*]

[a]*Departamento de Biocatálisis, Instituto de Catálisis (CSIC), Campus UAM Cantoblanco, 28049 Madrid, Spain*
[b]*Dipartimento di Chimica Farmaceutica, Università di Pavia, via Taramelli 12, 27100 Pavia, Italy*

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Abstract—The efficient enzymatic synthesis of enantiopure 2,3-epoxypropanol (glycidol) has been achieved. The racemic glycidyl butyrate was successfully resolved by enzymatic hydrolysis using a strategy that combines different immobilization protocols and different experimental reaction conditions. A new enzyme (25 kDa lipase)—which is a lipase-like enzyme purified from the *pancreatic porcine* lipase (PPL) extract—immobilized on DEAE–Sepharose was selected as the optimal biocatalyst. The optimal results were obtained at pH 7, 25 °C and 10% dioxane using this biocatalyst and a very high enantioselectivity for the enzyme was displayed, obtaining both (*R*)-(−)-glycidyl butyrate and (*R*)-(+)-glycidol with enantiomeric excesses >99% (*E* >100). The hydrolysis of (*R*)-(−)-glycidyl butyrate produced pure (*S*)-(−)-glycidol.

1. Introduction

Optically pure epoxides are versatile intermediates in organic synthesis because the epoxide ring is very reactive towards nucleophiles and easily yields asymmetric alcohols. Particularly, optically active 2,3-epoxypropanol (glycidol) and its derivatives have a compact skeleton of glycerol and wide potential for synthesis. They are considered to be versatile chiral synthesis units.[1–3] In this sense, both enantiomers of glycidol **1** have become widely used as starting materials for the synthesis of many interesting compounds, such as anticancer drugs,[4] protein synthesis inhibitors,[5] as well as a 2-oxazolidinone derivative used against depression.[6]

Therefore, different chemical[7,8] and biological[9–11] methods have been developed in order to get these alcohol epoxides in their enantiomerically enriched form. Thus, from the chemical point of view the asymmetric epoxidation of allyl alcohol with peroxide developed by Sharpless[7] allowed both enantiomers to be obtained and this was a breakthrough in the industrial production of optically active C3 building blocks. However the enantiomeric excess is slightly low (ee = 91% at 65% yield). Recently, Jacobsen et al.[8] have described the use of (salen) Co III complex as catalyst in the preparation of highly enantioenriched terminal epoxides.

Using biological methods, the resolution of racemic epoxides as glycidyl derivatives has been performed by a biocatalytic process using enzymes, especially lipases.[9–11] Indeed, the resolution of (±)-glycidyl esters was developed using a commercial lipase preparation from *Porcine pancreas* (PPL).[9] However, high conversions were necessary to achieve an appropriate enantiomeric excess of (*R*)-glycidyl butyrate **2** for industrial application, which decreased the yield of the enantiomerically pure enantiomer that remained.

Herein, we report an improvement in this area using a new strategy based on the preparation of several biocatalysts from the same lipase with very different immobilization protocols, involving different areas of the enzyme surface, conferring different rigidity levels to the enzyme structure or even generating a special microenvironment surrounding the enzyme, which could somehow modify the shape and features of the lipase active centre (Scheme 1). Moreover, the combination of these methods with the appropriate reaction conditions could improve the selectivity. This strategy

* Corresponding authors. Tel.: +34 91 585 48 09; fax: +34 91 585 47 60; e-mail addresses: jmguisan@icp.csic.es; rfl@icp.csic.es

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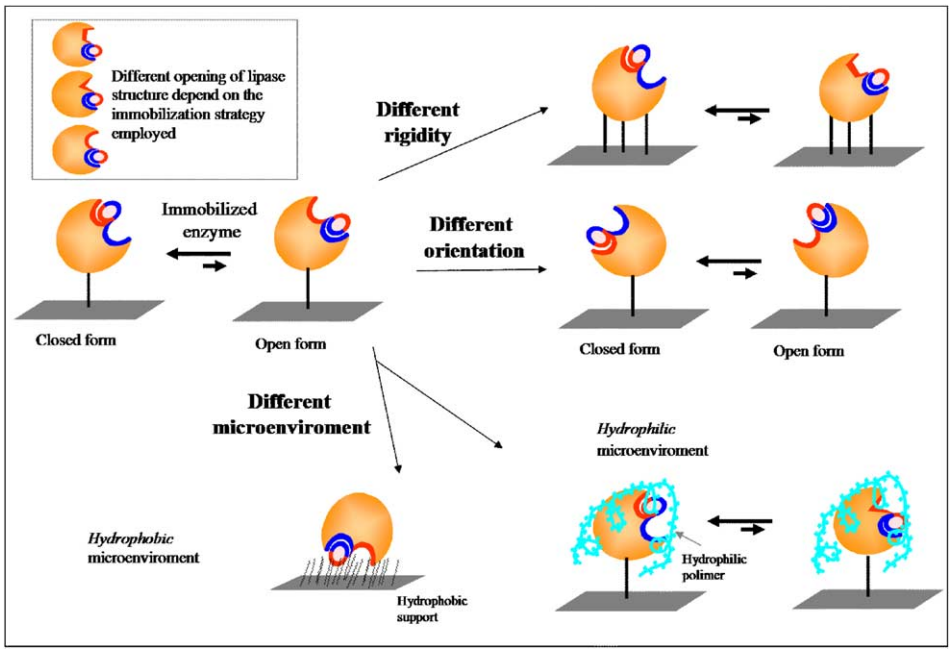


Scheme 1. General representation of how to modulate the catalytic properties of lipases using different immobilization strategies.

has been already successfully used in the design of biotransformations catalyzed by enzymes, which suffer significant changes during catalysis.[12,13] In fact, this technique has demonstrated that the properties of the same lipase immobilized on different supports could be completely different.

This strategy is based on the drastic conformational changes that lipases undergo during catalysis. In aqueous media, they exist mainly in a closed and inactive conformation,[14] where the active site is separated from the reaction medium by an oligopeptide chain acting as a 'lid', in equilibrium with an open and active conformation, where the lid is displaced permitting access of substrates to the active site. However, upon exposure to a hydrophobic interface, the lipase is adsorbed onto it provoking a conformational change shifting the equilibrium to the open form, a phenomenon known as interfacial activation.[15]

Herein, we describe the kinetic resolution of racemic glycidyl butyrate (±)-**2** in aqueous media catalyzed by different lipases. Several immobilized biocatalysts from a 25 kDa lipase (25L)[16]—which is a lipase-like enzyme purified from the *pancreatic porcine* lipase (PPL) extract—were prepared.

2. Results and discussion

2.1. Enzymatic resolution of (±)-2 in aqueous media

We screened different lipases for their ability to catalyze the hydrolysis of racemic **2** (Table 1). The lipases were immobilized on agarose activated with octyl groups on the surface. It was found that the reactivity and the enantioselectivity[17] depended largely on the enzyme used. For instance, the lipase isolated from *Candida antarctica* (CAL-B) displays low E value (Table 1, entry 1) with ee_s of 31%. With the lipase isolated from *Rhizomucor miehei* (RML) better results was obtained $E = 5$ (Table 1, entry 2) although the highest selectivity towards this substrate was found using a lipase purified from PPL crude preparation (25L)[16] reaching 43% yield in 15 min with ee of 54% (Table 1, entry 3). Therefore, the latter was selected to perform the studies in order to get a high enantioselectivity.

2.2. Enantioselective resolution of (±)-2 under different conditions using different 25L-immobilized preparations

The initial rate and enantiomeric ratio (E) values were found to depend on the biocatalyst and the experimental conditions used (Table 2). In this sense, lipase immobilized on octyl agarose (25L-1 biocatalyst) presented the highest initial rate when the reaction was performed at pH 7 and 25 °C (Table 2, entry 1). The activity of the other biocatalysts—prepared using other immobilization strategies—was at least 10-fold lower in relation to the one of the 25L-1 biocatalyst (Table 2, entries 2–4). However, with the adsorption of the lipase on commercial DEAE–Sepharose (25L-3 biocatalyst) it was possible to obtain the most enantioselective catalyst with ee_s of 81% at 52% yield under these experimental conditions (Table 2, entry 3). It is worth noting that this lipase covalently attached on aminoagarose[18] activated with glutaraldehyde (25L-2 biocatalyst) did not exhibit any discrimination between isomers, and 55% of the substrate was converted into a virtually racemic product after 6 h at 25 °C (Table 2, entry 2).

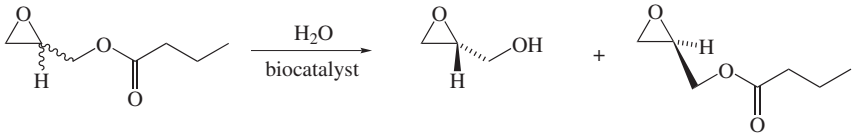
When the pH of the reaction medium was changed from 7 to 5, the activity of the biocatalysts did not improve (Table 2, entries 5–8), even the initial rate for the lipase immobilized on dextran-sulfate agarose

Table 1. Enzymatic resolution screening of (±)-**2** in aqueous media

Entry	Enzyme[a]	T (°C)	pH	m[b] (g)	V (mL)	t (min)	c (%)	ee_s[c] (%)	E[d]
1	CAL-B	25	7	0.01	8	15	58	31*	2
2	RML	25	7	0.05	20	5	41	39	5
3	25L	25	7	0.03	5	15	43	54	10

[a] Lipases were immobilized on octyl-agarose support.
[b] m: amount of catalyst added.
[c] Determined by chiral HPLC: ee_s = ee [(*R*)-**2** or *(*S*)-**2**].
[d] Determined from c and ee_s as in Ref. 17.

Table 2. Kinetic resolution of (±)-**2** catalyzed by different 25L-biocatalysts



(±)-2 **(*R*)-(+)-1** **(*R*)-(-)-2**

Entry	Biocatalyst[a]	T (°C)	pH	Initial rate[b]	t (h)	c (%)	ee_s[c] (%)	E[d]
1	25L-1	25	7	1200	0.30	61	89	10
2	25L-2	25	7	81	6	55	06	1.1
3	25L-3	25	7	63	6	52	81	17
4	25L-4	25	7	114	3	56	50	4
5	25L-1	25	5	850	0.33	53	94	38
6	25L-2	25	5	51	8	51	0	1
7	25L-3	25	5	54	8	50	47	4
8	25L-4	25	5	5.4	60	47	38	3.6

[a] Different biocatalysts were prepared as described in experimental.
[b] The initial rate ($\mu mol \times mg_{prot}^{-1} \times min^{-1}$) was calculated at 10–15% of conversions.
[c] Determined by chiral HPLC: ee_s = ee [(*R*)-**2**].
[d] Determined from c and ee_s as in Ref. 17.

(25L-4 biocatalyst) decreased up to 20-fold (Table 2, entry 8). Nevertheless, the 25L-1 biocatalyst displayed the highest E value when the reaction was performed at pH 5 with 94% of ee_s at 53% conversion (Table 2, entry 5). The selectivity in the case of the other biocatalysts was similar or lower.

On the other hand, the use of different percentages of solvent (1,4-dioxane) in the aqueous solution displayed interesting results on the enantioselectivity of the biocatalysts (Fig. 1). The addition of 10% solvent to the aqueous phase promoted an improvement in the E value for most of the biocatalysts (Fig. 1), emphasizing the excellent enantioselectivity reached by the 25L-3 biocatalyst in these conditions ($E \geqslant 100$, in fact we cannot detect hydrolysis of the (*R*)-enantiomers of remaining glycidyl butyrate after 24 h even using pure compounds). The increase in dioxane concentration to 20% promoted a very dissimilar effect on the enantioselectivity of the different biocatalysts. The 25L-4 biocatalyst improved the E value slightly from 4 (0% dioxane) to 11 (Fig. 1). However, the 25L-3 biocatalyst suffered a considerable decrease in the enantiomeric ratio from $\geqslant 100$ (10% dioxane) to 4.

A similar study was performed at pH 5, although the results did not improve on the addition of dioxane (results not shown).

Therefore, these immobilization strategies combined with the appropriate reaction conditions have permitted to obtain E values very different from 1 (25L-2 biocatalyst) to >100 (25L3 biocatalyst) for the same lipase.

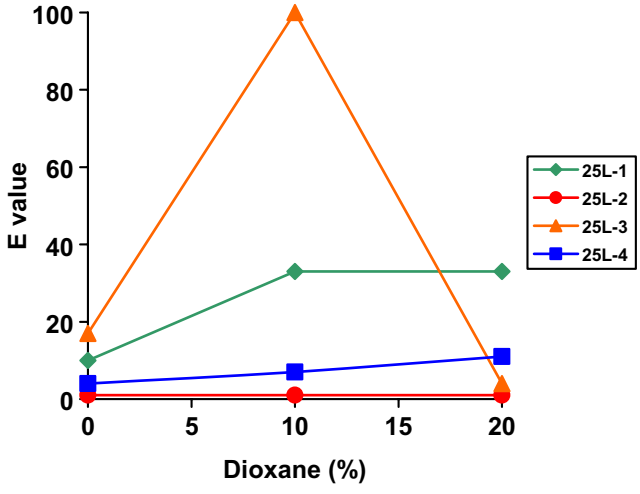


Figure 1. Diagram of the co-solvent effect on the enantiomeric ratio of 25L-biocatalysts in the resolution of (±)-**2**. Experiments were performed at pH 7 using 1,4-dioxane as co-solvent. E values were determined from c and ee_s as in Ref. 17.

2.3. Preparative resolution of (*R*)-glycidol

Taking into account the fact that when the 25L-3 biocatalyst was used none of the (*S*)-product was detected at 50% conversion as previously described (even when the reaction time was double), we carried out the hydrolytic resolution of (±)-**2** (3 mmol) using 10% dioxane (Scheme 2). Hydrolyses were carried out up to 50% conversion (over 10 h when using 1 g of 25L-biocatalyst and 100 mL of substrate) and the ee_p and ee_s were analyzed at different conversions (Table 3). Thus, the ee-**1** was always >99% at 10–50% conversions (Table 3, entries 1–3) whereas the ee-**2** was >99% at 50% conversion (Table 3). They were isolated in enantiopure form from the same reaction by semi-preparative HPLC and characterized, obtaining (*R*)-(+)-**1** ($[\alpha]_D^{20} = 36.9$) in 45% overall yield.

Moreover, a simple hydrolytic reaction, using, for example, 25L-2 biocatalyst, converted all of the (*R*)-**2** enantiomer into (*S*)-**1** providing the other enantiomer of glycidol in enantiopure form (Scheme 2). Nine cycles could be performed with identical results with respect to the catalytic properties of the biocatalyst (activity, enantioselectivity).

Table 3. Hydrolytic resolution of (±)-**2** catalyzed by 25L-3 immobilized preparation under the optimized reaction conditions

Entry	Solvent[a] (%)	pH	*T* (°C)	Ee_p[b] (%)	Ee_s[c] (%)	*c* (%)	*E*[d]	$[\alpha]_D^{20}$
1	10	7.00	25	>99	22	18	>100	—
2	10	7.00	25	>99	66	40	>100	—
3	10	7.00	25	>99	>99	50	>100	+36.9

[a] The solvent was 1,4-dioxane.
[b] Determined by chiral GC: ee_p = ee[(*R*)-**1**].
[c] Determined by chiral HPLC: ee_s = ee [(*R*)-**2**].
[d] Determined from *c* and ee_s as in Ref. 17.

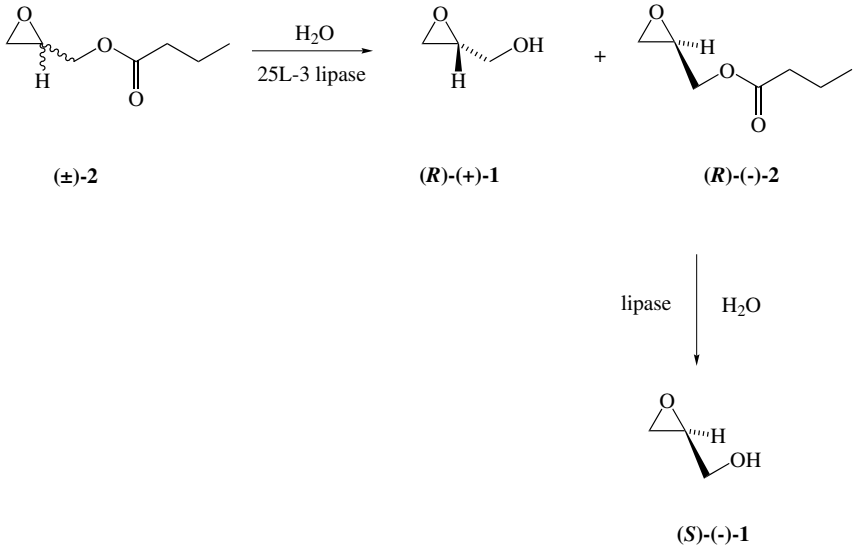
3. Conclusions

These results show the full resolution of (±)-glycidyl butyrate (±)-**2** via a hydrolysis reaction. This was possible by selecting the best lipase (25L), the best biocatalyst from different ones, 25L immobilized on DEAE–Sepharose, and the best reaction medium conditions—pH 7, 25 °C and 10% 1,4-dioxane. Under these conditions, both products, (*R*)-(+)-glycidol as well as (*R*)-(−)-glycidyl butyrate were obtained with enantiomeric excesses >99% (*E* >100). Moreover, the hydrolytic reaction of (*R*)-(−)-glycidyl butyrate permitted to obtain the (*S*)-(−)-glycidol in enantiopure form (Scheme 2). This easy and rapid biocatalytic method competes with the results in enantiomeric purity obtained by Jacobsen[8] or by other enzymatic processes.[9,10]

4. Experimental

4.1. General

p-Nitrophenyl propionate (pNPP), 1,4-dioxane, dextran sulfate (Mr 10,000), glutaraldehyde, *Porcine pancreatic* lipase (type II) (PPL) and Triton X-100 were obtained from Sigma–Aldrich St. Louis, EEUU. Fully purified 25 kDa lipase from PPL crude was prepared as previously described.[16] The lipase from *Candida antarctica* (*fraction B*) (Novozym 525 L) and from *Rhizomucor Miehei* (Novozym 388) were from Novo Nordisk (Denmark). Glyoxyl-agarose 6BCL and 10 BCL was kindly donated by the company Hispanagar SA (Burgos, Spain). Octyl-agarose 4BCL and DEAE–Sepharose were purchased from Pharmacia Biotech (Uppsala, Sweden). Glutaraldehyde agarose[18,19] was prepared as previously described. (±)-Glycidyl butyrate (±)-**2** was kindly donated by Dr. Pregnolato (University of Pavia, Italy). Protein concentration was determined by the Bradford's method.[20] pH-stat Mettler Toledo DL50 graphic was used to maintain a constant pH value during the



(±)-2 (*R*)-(+)-1 (*R*)-(-)-2

(*S*)-(-)-1

Scheme 2. Synthesis of (*R*)- and (*S*)-glycidol by using a biocatalytic process.

reactions. Conversion was analyzed by RP-HPLC (Spectra Physic SP 100 coupled with an UV detector Spectra Physic SP 8450) using a kromasil C_{18} (25 × 0.4 cm) column. Products were eluted at flow rate of 1.5 mL min^{-1} using acetonitrile–10 mM ammonium phosphate buffer at pH 2.95 (35:65, v/v) and UV detection performed at 225 nm. The enantiomeric excess (ee) of the remaining ester was determined by Chiral Phase HPLC analysis. The column was a Chiracel OD, the mobile phase was an isocratic mixture of isopropanol and hexane (2:98 v/v) at a flow rate of 0.4 mL/min and UV detection was performed at 225 nm. The retention times of the enantiomers were: (*S*)-**2** (17.32 min), ($\alpha = 0.92$); (*R*)-**2** (18.60 min), ($\alpha = 1.08$). Otherwise, the enantiomeric excess of the released glycidol was evaluated by gas chromatographic. GC Varian 3800 analyses were performed using a Cyclosilb 112-6632 chiral column (30 m × 0.25 mm, I&J Scientific). Chromatographic conditions were: injector temperature 230 °C, initial oven temperature 60 °C for 3 min to 76 °C (1 °C/min) and then to 240 °C (25 °C/min). Flame Ionization detector was maintained at 200 °C. Gas carrier:Helio (1.5 mL/min). The retention times of the enantiomers were: (*R*)-**1** (9.8 min), ($\alpha = 0.965$); (*S*)-**1** (10.1 min) ($\alpha = 1.036$), ($R_s = 1.02$). ^1H and ^{13}C NMR data were recorded on a Varian Mercury 400 spectrometer. Optical rotations were measured with a Perkin–Elmer Polarimeter 341.

4.2. Enzymatic activity assay

This assay was performed by measuring the increase in the absorbance at 348 nm produced by the released *p*-nitrophenol in the hydrolysis of 0.4 mM pNPP in 25 mM sodium phosphate buffer at pH 7 and 25 °C. To initialize the reaction, 0.05 mL of lipase solution or suspension was added to 2.5 mL of substrate solution. One international unit of pNPP activity was defined as the amount of enzyme necessary to hydrolyze 1 μmol of pNPP per minute (IU) under the conditions described above.

4.3. Biocatalysts preparation

Four different immobilization protocols were used. The yields from the immobilization process were >95% in all cases.

4.4. 25L1 Biocatalyst: immobilization on octyl agarose

Ten grams of octyl-agarose support was added to 100 mL of 25 mM sodium phosphate buffer lipase solution (0.1 mg protein/mL) at pH 7.[21] The mixture was then shaken at 4 °C and 250 rpm for 3 h. After that, the solution was removed by filtration and the supported lipase washed several times with distilled water.

4.5. 25L2 Biocatalyst: immobilization on glutaraldehyde agarose

Eight grams of activated agarose gel modified with glutaraldehyde[18,19] was added to 40 mL of 25 mM sodium phosphate buffer lipase solution (0.1 mg protein/mL) at pH 7. The mixture was then shaken at 4 °C and 250 rpm for 24 h. After that, the solution was removed by filtration. $NaBH_4$ (2.65 mmol) dissolved in 100 mL of 100 mM sodium carbonate buffer at pH 8.5 was added to the solid supported lipase. The mixture was then shaken at 4 °C for 30 min. After that, the solution was removed by filtration and the supported lipase washed properly with distilled water to remove the reduction agent excess and keep it at 4 °C.

4.6. 25L3 Biocatalyst: immobilization on DEAE–Sepharose

Ten grams of DEAE–Sepharose gel was added to 50 mL of 25 mM sodium phosphate buffer lipase solution (0.1 mg protein/mL) at pH 7. The mixture was then shaken at 4 °C and 250 rpm for 4 h. After that, the solution was removed by filtration and the supported lipase washed several times with distilled water.

4.7. 25L4 Biocatalyst: immobilization on dextran sulfate agarose

Nine grams of activated agarose gel with amino groups coated with dextran sulfate[22] was added to 45 mL of 25 mM sodium acetate buffer lipase solution (0.1 mg protein/mL) at pH 5. The mixture was then shaken at 4 °C and 250 rpm for 3 h. After that, the solution was removed by filtration and the supported lipase washed several times with distilled water.

4.8. Enzymatic hydrolysis of (±)-glycidyl butyrate (±)-2

(±)-**2** (0.6 mmol) was dissolved in 25 mM sodium phosphate buffer (6 mL) at pH 7 or 25 mM sodium acetate buffer (6 mL) at pH 5 with different percentage of 1,4-dioxane from 0% to 20% (v/v) and biocatalyst (60 mg) was added. The mixture was then shaken at 25 °C and 250 rpm for the time shown in Table 2. The biocatalyst was filtered and the conversion was analyzed by RP-HPLC. Enantiomeric excesses were determined using a chiral phase-column previous extraction 0.2 mL of aqueous phase with hexane in HPLC or ethyl acetate in GC (4 × 0.2 mL). After that, the enantiomeric ratio (*E*) was calculated in all cases using the equations reported by Chen et al.[17]

4.9. Separation protocol of compounds (*R*)-1 and (*R*)-2

One hundred millilitres of reaction mixture at 50% of conversion (432 mg of initial substrate) was separated of immobilized preparation by vacuum filtration. The reaction mixture was extracted with dichloromethane (3 × 100 mL) and the organic solvent was dried over sodium sulfate and evaporated under vacuum. The residue was dissolved in acetonitrile and chiral products, alcohol as well as ester were isolated by reverse-phase HPLC on a 10–250 mm C18 column (Vydac, 5 μm particles and 300 Å pore size) using a Bio-Rad model 2800 HPLC system equipped with a Linear model 205 dual wavelength UV detector with an acetonitrile–water (65:35) mobile phase. Reversed-phase HPLC was used to confirm the purity of the products (>99%).

The remaining (*R*)-**2** was hydrolyzed using the 25L-2 biocatalyst to get enantiomerically pure (*S*)-**1**.

(*R*)-glycidol **1**: (45%). $[\alpha]_D^{20} = +36.9$ (*c* 1, $CHCl_3$) ^1H NMR (400 MHz, $CDCl_3$): $\delta = 3.78$ (dd, $J = 4.89$ Hz, 1H, CH_2), 3.75 (dd. $J = 5.47$ Hz, 1H, CH_2), 2.85 (s, 1H; OH), 2.67 (m, 2H; CHoxi, CH_2oxi), 2.57 (dd, $J = 4.89$ Hz, 1H, CH_2oxi). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 63.6$, 52.6, 44.5.

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