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RAPID COMMUNICATION

A highly efficient chemoenzymatic process to produce (*R*)-6,7-dihydroxygeraniol

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ABSTRACT

The efficient asymmetric dihydroxylation of 6,7-epoxygeraniol was performed using a chemoenzymatic process employing a yeast epoxide hydrolase (EH) resolution stereoinversion step, followed by stereoretentive chemical hydrolysis of the remaining epoxide to produce (6*R*)-6,7-dihydroxygeraniol at unprecedented high enantiometic excess (ee) (>97.5%) and high isolated yield (72 mol % overall yield over 5 steps from commercially available geraniol). The enzymatic process was completed within 2 h at 250 g/L substrate loading reaching > 49.5 mass % conversion of the racemic epoxide. The reaction was self-limiting and furnished both the homochiral (6*R*)-triol and the residual (6*R*)-epoxide at > 99% ee, due to enantio-inversion of the (6*S*)-epoxide. The (6*R*)-epoxide was subsequently chemically hydrolysed, without first needing to separate the epoxide and triol products, to afford the desired (6*R*)-triol product in >97.5% ee, at multigram scale. This chemoenzymatic procedure offers an excellent alternative to chemical asymmetric epoxidation or dihydroxylation for the production of enantiopure 6,7-dihydroxygeranyl and 6,7-epoxygeranyl type compounds in general. It exemplifies the benefits of using greener EH bioprocesses to produce such compounds in high ee's and high isolated yields.

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1. Introduction

Many researchers have reported the use of epoxide hydrolases (EHs) for the production of chiral epoxides and chiral diols from racemic mono-, di- and tri-substituted epoxide starting materials to produce valuable chiral intermediates for the synthesis of active pharmaceutical ingredients (APIs) (Bucko et al. 2023). The general reaction typically proceeds by kinetic resolution of the racemic epoxide to form enantiomerically enriched unreacted epoxide and diol product (Figure 1). For EHs, the stereoselective preference for the substrate epoxide and for the diol product absolute stereochemistry will vary depending on the source of EH and the conditions of the reaction.

Enantiomerically enriched epoxides have recently been referred to as the most versatile chiral building blocks known for the asymmetric synthesis of biologically active natural products and APIs (Meninno and Lattanzi 2022). Two chiral centres are highly stereoselectively produced in one hydrolysis step through the bio-resolution of racemic epoxides with EHs. The use of EHs to resolve racemic epoxides to furnish enantiomerically enriched epoxides and diols has received significant attention (Bala and Chimni 2010). However, the major drawback in many of these resolutions is that only a 50% theoretical maximum yield of the desired epoxide or diol product is obtained. In order to realize the full potential of EH mediated biocatalysis, the biological route must increasingly compete with well-known chemical asymmetric epoxidation procedures such as Shi, Jacobsen (also a kinetic resolution, ie maximum yield of 50 mol % of the desired enantiomer is obtained, unless enantioselective Mn epoxidation is applied to produce enantio-enriched epoxides), and Sharpless technology where the recovered product yields are often in the 80–100 mol% range, although it only works on allylic alcohol substrates (Heravi et al. 2015).

(*R*)-6,7-Dihydroxygeraniol (Figure 2, DHG, **1**) was recently identified as a natural product found in

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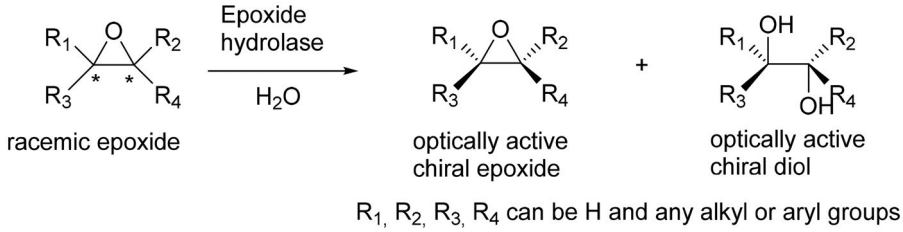

Figure 1. Standard EH mediated resolution of racemic epoxides to produce optically active epoxides and diols.

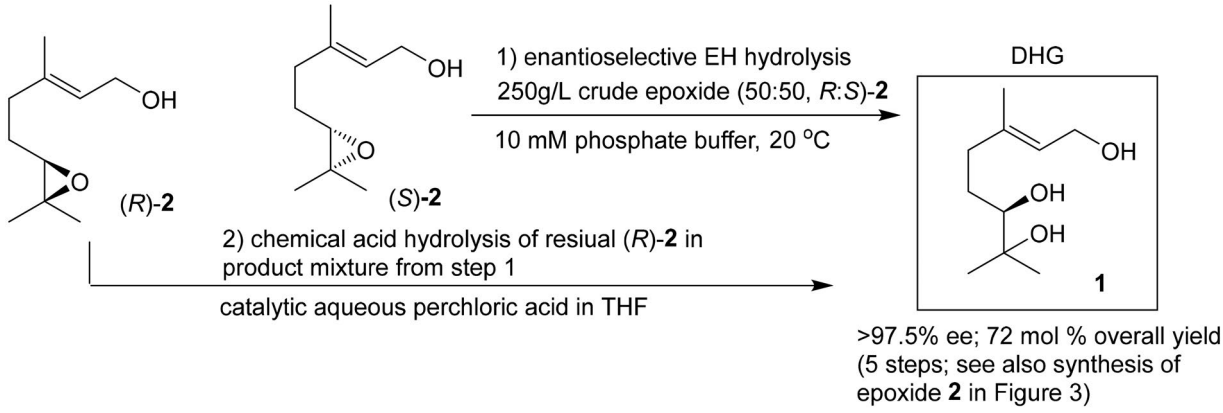

Figure 2. Chemoenzymatic synthesis strategy to produce DHG (**1**).

ginger rhizomes (*Zingiber officinale*) (Wei et al. 2021) and in the leaves of wild betel (*Piper sarmentosum*) (Yang et al. 2023). It has been synthesized by direct Sharpless dihydroxylation of geraniol in moderate yield and 93% ee, as an intermediate to produce its carboxylic acid derivate displaying antifungal properties (Wang et al. 2018). Major disadvantages of Sharpless dihydroxylation include the use of a fatally toxic and highly hazardous oxidizing agent, Osmium tetroxide (MSDS of OsO_4), together with high chemical catalyst costs.

DHG (**1**) can be used to synthesize numerous enantiomerically enriched (*R*)-6,7-dihydroxygeranyl motifs found in a variety of commercially interesting compounds with a multitude of diverse uses. Bowers Compound has been used as an insecticide (Bowers 1971). (*R*)-6,7-Dihydroxybergamottin and (*R*)-Paradisin A are potent inhibitors of the cytochrome P450 3A4 enzyme (Girennavar et al. 2006; Fuhr et al. 2023), and this can be used to enhance the bioavailability of certain drugs, ie as first pass metabolic inhibitors for the effective administration of certain therapeutic drugs. (*R*)-6,7-Dihydroxybergamottin is also an inhibitor of acetylcholinesterase. The presence of the dioxygenated geranyl chain was essential for this inhibition to occur (Youkwan et al. 2010). The well-studied natural product Marmin has many uses, including the treatment of obesity by lowering lipid storage capacity through the induction of lipid cell (3T3-L1) preadipocyte cell apoptosis (Hao et al. 2021). Furthermore, it also displays cytotoxic and antibacterial properties (Oueslati et al. 2021). Finally, excellent acetylcholinesterase inhibitory activity by Marmin has been documented that can result in neuroprotection in, eg Alzheimer disease patients (Kuo et al. 2017). It should be noted that, as the natural products mentioned here clearly all are in the (6*R*)-configuration, the (6*R*)-enantiomer of precursor triol DHG (**1**) is of particular commercial interest.

We sought to develop a simple, commercially viable process for the production of DHG **1** in high ee and high yield. If a highly enantioselective EH that could invert the stereochemistry of the (6*S*)-epoxide **2** upon hydrolysis to produce DHG (**1**) could be found, then subsequent *in situ* acid catalysed hydrolysis of the remaining (6*R*)-epoxide enantiomer would satisfyingly afford more of **1** by well-known stereoretentive chemical acid hydrolysis (Figure 2). To achieve this, we investigated the use of our yeast derived EH technology platform (Pienaar et al. 2008) coupled with chemical hydrolysis, the aim being to produce **1** in 100% theoretical yield from the corresponding racemic epoxide and with isolated yields in excess of 50%. It is essential that this chemoenzymatic process should be sufficiently comparable in efficiency to the best chemical asymmetric methods known, eg Sharpless dihydroxylation (Wang et al. 2018).

For comparison, the stereoselective dihydroxylation of geranyl *N*-phenylcarbamate has been demonstrated

using whole cells of the fungus acidophile *Aspergillus niger* (Chen et al. 1993). The choice of this particular geranyl derivative was based on low observed enantioselectivities when applied directly to the primary alcohol and on geranyl acetate derivatives. The crystalline nature of this *N*-phenylcarbamate geranyl derivative also facilitated enantiopurity improvements by a selective crystallization technique. However, 50% of starting material was lost to the formation of side-products and through its diversion to other metabolites. In addition, the reaction was commercially unattractive due to low observed activity, long biocatalytic reaction duration and a low substrate loading (<1 g/L over 20 h).

Edegger et al. (2004) reported the bio-resolution of trisubstituted geranyloxy compounds such as 6,7-epoxygeranyl chloride, by using EHs from *Rhodococcus* and *Streptomyces*. The reactions proceeded in an enantioconvergent fashion to produce the desired *R*-diol chloride compound with enhanced ee, from both epoxide enantiomers. Conversions above the expected 50% threshold were achieved. This methodology was applied to the chemo-enzymatic total synthesis of (6*R*)marmin from geranyl chloride. Marmin was preparatively synthesized in 95% ee but in very low yield (15%). Our target, DHG (**1**) was also prepared and isolated in the same study, albeit in low ee (73%) and yield (53%), Drawbacks included slow reactions and relatively low substrate loadings (<17 g/L over 24 h).

Vidari et al. (1993) utilized Sharpless dihydroxylation technology to produce the (6*R*) and (6*S*)-6,7-diols of geranyl acetate in 90 and 92% ee, respectively. Acetate ester derivatization of geraniol was required to direct substrate-controlled enantioselective epoxidation of the 6,7-olefin functionality. However, the enantio-enriched acetate diols were oily products that could not be enantiomerically enriched further by selective recrystallization techniques.

DSM Catalytica patented a Shi epoxidation process on the crystalline N-phenylcarbamyl derivative of geraniol to obtain the (*R*)-6,7-dihydroxy geranyl product in 76% ee (Seemayer and Shi 2001). This was subsequently enriched to 88% ee by recrystallization. Such ee enrichment methodologies inherently result in significant yield losses and the creation of undesirable excess waste.

2. Materials and methods

2.1. General

Ethyl acetate (EtOAc) used for chromatographic techniques was distilled prior to use by means of conventional distillation processes, and dichloromethane (DCM) was distilled over calcium hydride. Methanol (MeOH) and diethyl ether (Et_2O) were of analytical grade. All the required reagents and chemicals were obtained from Sigma Aldrich or Fluka, and were used without further purification. Normal chromatography was performed on silica (SiO_2) gel 60 (Macherey-Nagel, particle size 0.063–0.200 mm), employing both isocratic and gradient eluent systems. Thin layer chromatography (TLC) of the compounds was executed on Merck TLC SiO_2 60 F_{254} plates. TLC plates were visualized using a standard vanillin stain solution, ie 1% V/V sulfuric acid in EtOH containing 1% m/V vanillin.

Nuclear magnetic resonance (NMR) spectra were recorded on either a Bruker AVANCE 300 MHz, or a Bruker AVANCE III 500 MHz spectrometer. Spectra were recorded in chloroform-d1 ($CDCl_3$) or DMSO-d6. Chemical shift values (δ) are reported in parts per million referenced against the signal of residual solvent ($CDCl_3$: δ_{1H} = 7.26 ppm, δ_{13C} = 77.16 ppm). Coupling constants (*J*-values) are given in Hertz (Hz).

Chiral GC method: Restek RT-βDEX-SM 30 *m* × 0.25 mm internal diameter column was mounted in a Hewlett Packard 5890 A gas chromatograph. The temperature was set for 1 min at 100 °C, then increased at 5 °C per min from 100 to 180 °C, and held at 180 °C for 3 min. Injection port and flame ionization detector were set at 250 °C. Detector gases: air at 250 kPa, H_2 at 110 kPa. Auxiliary gas: N_2 at 28 cm^3/min. Run time: 20 min. Elution times: (*S*)-**2** 10.87 min., (*R*)-**2** 10.97 min., (*S*)-**1** 17.75 min. and (*R*)-**1** 17.95 min. Polarimetry was used to assign the absolute stereochemistry of the final *R*-epoxide and *R*-triol products, after EH resolution and product separation by SiO_2 gel column chromatography, as described below. A JASCO P-2000 Digital Polarimeter was used to determine the specific optical rotation data quoted in the characterization data of the corresponding enantiomerically resolved epoxide (**2**) and triol (**1**) compounds.

In order to prepare racemic substrate 6,7-epoxy alcohol (±)-**2** for this project, we first needed to convert geraniol into its geranyl acetate derivative **3** (Figure 3). Owing to the resultant inductive electron-withdrawing effect of the *O*-acetyl group in proximity to the 2,3-dialkylated alkene bond (that is already less electron-rich than the target 6,7-trialkylated double bond) is stabilized further and rendered much less reactive towards the *m*-CPBA epoxidation reagent. Hence, chemoselective epoxidation of the more reactive 6,7-alkene could proceed in high yield by standard epoxidation conditions (*m*-CPBA) to afford the desired 6,7-epoxide **4**, with complete disappearance of the geraniol starting material (by TLC). None of the bis-epoxide or the undesired 2,3-epoxide compound was observed. This observation has been well documented

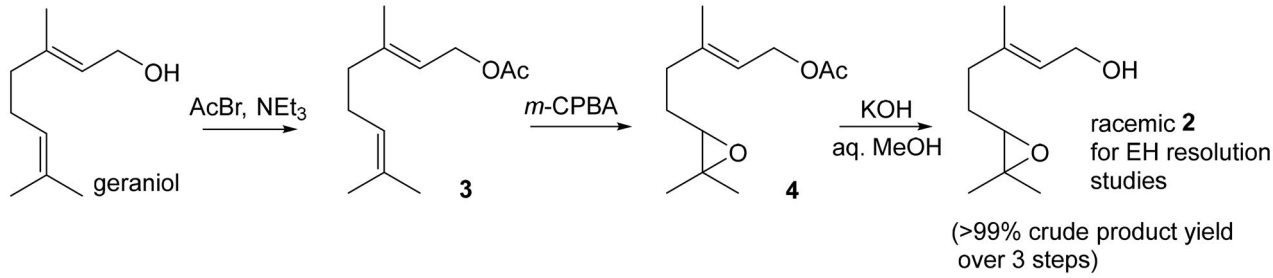

Figure 3. Preparation of the racemic epoxide substrate (±)-**2**. The synthesis could be carried out efficiently without the purification of the intermediate compounds **2**–**4**.

in the literature with quantitative yields of the mono-epoxidized 6,7-epoxygeraniol being reported on several occasions previously under such temperature-controlled (0–10 °C) reaction conditions (Fillion and Beingessner 2003; Muhammet et al. 2006). Subsequent base hydrolysis to deacetylate the alcohol produced the desired (±)-**2** in high overall yield.

2.2. Preparation of geranyl acetate (3)

To a solution of geraniol (98% m/m, 20 g, 0.127 mol) in CH_2Cl_2 (150 mL) was added triethylamine (1,2 eq., 21 mL, 0.15 mol) and anhydrous $MgSO_4$ (0.2 g). The solution was cooled to 0 °C on an ice bath and acetyl bromide (1.1 eq., 99%, 10.4 mL) was added dropwise with vigorous stirring whilst maintaining the temperature between 0 and 10 °C, over a period of 55 min. The reaction was warmed to room temp and, after 1 h, another 0.5 mL acetyl bromide was added. After stirring for another 30 min., water (50 mL) was added and the layers were separated after vigorous shaking in a separatory funnel. The organic layer was dried ($MgSO_4$) and filtered through cotton wool to give a final volume of 170 mL, which is used without further purification in the next step.

2.3. Preparation of epoxy acetate (4)

The vigorously stirred solution obtained from the above procedure was cooled to 0 °C on an ice-bath and $NaHCO_3$ (1.1 eq., 11.7 g) was added, followed by the portionwise addition of *meta*-chloroperoxybenzoic acid (1.1 eq., 70%, 34.5 g), over a period of 55 min. whilst maintaining the reaction mixture temperature between 0 and 10 °C. The reaction was then warmed to room temp and stirred for a further 4 h. After transferring the mixture to a separatory funnel (using another 20 mL CH_2Cl_2 to wash out the product), 50 mL of 10% aq. NaOH was added followed by vigorous shaking. The aqueous layer was extracted with another 20 mL CH_2Cl_2. The combined organic layers was washed with 15 mL H_2O, dried ($MgSO_4$), filtered under suction and roto-evaporated to yield crude product **4**, pure enough by TLC for direct use in the next step.

2.4. Preparation of racemic epoxy alcohol (±)-(2) and characterization of (R)-2

The obtained crude product solution **4** from the above procedure was dissolved in aq. MeOH (MeOH: H_2O, 9:1, 200 mL). Anhydrous K_2CO_3 (1.2 eq., 14.6 g) was added in one portion and the resultant mixture was stirred at room temp for 5 h. After evaporation of most of the MeOH on the roto-evaporator, the residue was partitioned between CH_2Cl_2 (150 mL) and water (50 mL). After shaking and leaving the layers to separate in a separatory funnel, the organic layer was removed and dried ($MgSO_4$), filtered and evaporated to yield crude pre-resolved epoxy alcohol (±)-**2** (*ca.* 22 g, >99% crude yield over three steps); ^{1}H NMR ($CDC1_3$, 200 MHz) 1.26 (s, 3 H), 1.30 (s, 3 H), 1.60–1.70 (m, 2H), 1.70 (overlapping s, 3 H), 2.16 (m, 2 H), 2.71 (t, J = 6.2 Hz, 1 H), 4.16 (br d, J = 6.2 Hz, 2 H), 5.45 (dd, J = 6.8 and 1.4 Hz, 1 H); ^{13}C NMR 16.5, 19.0, 25.1, 27.5, 36.5, 58.5, 59.5, 64.2, 124.2, 138.9. In a separate experiment, a sample of post-resolved (*R*)-**2** was purified by SiO_2 column chromatography and found to display the following optical activity: $[\alpha]^{20}_D$ +8.1° (*c* 2.3, EtOH). The data corresponded well with reported literature NMR spectra (Fillion and Beingessner 2003) and optical rotation data for (*R*)-**2** (Neighbors et al. 2008; Daub et al. 2015).

2.5. Screening of recombinant Yarrowia lipolytica strains for EH activity

The recombinant biocatalysts were constructed as multicopy constructs under control of the HP4D promotor system as described in PCT/IB2005/001034 (Botes et al., 2006, 2008a, 2008b). The active biomass was generated by inoculating the recombinant strains into sterile defined media (250 mL media volume in 1 L Erlenmeyer flask) comprising glucose 20 g/L; ammonium

chloride 4 g/L; casamino acids 2 g/L; Difeo Yeast Nitrogen Base (free of amino acid and ammonium sulphate) 1.7 g/L. All the components are added together and autoclaved at 120 °C for 15 min. Final pH after sterilization was pH 5.2–5.5. The sterile flask medium was inoculated from an agar plate comprising the same defined medium but with 15 g/L Bacteriological agar added. The growth on the plate was one to two weeks old at 28 °C before using it as an inoculum source. The flasks were incubated for five days at 28 °C whilst shaking at 250 rpm. The biomass was then harvested by centrifugation and dry weights calculated by drying samples in the oven at 100 °C. The biomass should be used immediately or stored frozen in 50% m/V glycerol made up in 10 mM phosphate buffer, pH 7.0. Approximately 15 different recombinant EHs biocatalysts have been screened at 1 mL scale. These screens were performed at 2% dry mass/vol cell loading at 100 mM racemic epoxide (±)-**2** substrate concentration in 10 mM phosphate buffer, pH 7.0, over 1 h shaking at room temperature. The reactions were monitored by TLC (silica plates with 66% ethyl acetate in chloroform running solvent; visualization using 1% H_2SO_4 in EtOH containing 1% vanillin. The reactions for chiral analysis were conducted at 1 mL scale in shaken Eppendorfs containing 200 mM racemic 6,7 epoxygeraniol, 1.5% (dry mass equivalent)/volume flask grown recombinant *Yarrowia* biomass in 10 mM phosphate buffer, pH 7.0. The reactions were conducted at 20 °C and 50 µL samples were taken during the resolution reaction, diluted 10x in ethyl acetate, dried over anhydrous magnesium sulphate and the supernatant analysed by Chiral GC-FID as described previously. Elution times: (*S*)-**2** eluted at 10.87 min, (*R*)-**2** at 10.97 min, (*S*)-**1** at 17.75 and (*R*)-**1** at 17.95 min.

2.6. Preparative resolution of racemic epoxy alcohol (2)

20 g of the crude racemic epoxy alcohol (±)-**2**, obtained as described above, was added slowly to 80 g of 10 mM phosphate buffer, pH 7.0, containing a 25 g wet pellet (5g dry mass) of flask grown recombinant *Yarrowia lipolytica* strain expressing the EH from *Rhodosporidium toruloides* NCYC 3181. The reaction vessel comprised a baffled temperature controlled glass vessel fitted with an overhead stirrer of the Rushton turbine-type and the reaction temperature should be controlled at 20 °C (±5 °C) for optimum enzyme performance. The reactor therefore contained approximately 20 g of crude racemic epoxy alcohol (±)-**2** in a total reaction matrix of 80 mL. 50 µL Samples were removed at intervals and diluted 20x in ethyl acetate. The samples were dried over anhydrous magnesium sulphate and analysed by Chiral GC-FID as described previously.

2.7. Processing of the resolved mixture of (R)-1 and (R)-2

After adding three equivalent volumes of acetone and stirring for 30 min, the mixture was filtered by suction filtration and the solids washed with more acetone. The final volume of the filtrate was *ca.* 400 mL. The acetone was then removed by roto-evaporation and NaCl was added to the aqueous residue until the solution was saturated at room temp (final volume = 270 mL). After extraction in three portions, using a total volume of 250 mL EtOAc, the combined organic layers was dried ($MgSO_4$), filtered and evaporated to yield 24 g of the resolved mixture, (*R*)-**1** and (*R*)-**2** as a viscous yellow oil.

2.8. Preparation of pure (R)-1

Approximately 6 g of the above resolved mixture was dissolved in undistilled, undried THF (50 mL) and water (10 mL) was added. The solution was cooled to 0 °C and 60% aq. $HC1O_4$ (perchloric acid, 1.0 mL) was added dropwise with stirring. After 1 h between 0 and 5 °C, $NaHCO_3$ (1.3 eq., 1.0 g) was added and the reaction was vigorously stirred whilst warming it gradually to room temp. NaCl was added until the mixture was saturated and the organic and aqueous layers started separating. Diethyl ether (20 mL) was added and the layers were separated using a separatory funnel. After the addition of *ca.* 10 g $MgSO_4$ to the organic layer, it was filtered and the solids were washed with CH_2Cl_2. The final filtrate (94 mL) was evaporated to yield 5.8 g of crude product. Purification by SiO_2 column chromatography (eluents: 5% EtOAc/CH_2Cl_2, followed by 10% MeOH/EtOAc) afforded the desired triol product (*R*)-**1** (4.7 g, 0.025 mol, 72 mol % adjusted overall yield over 5 steps from 98% m/m geraniol, ee 97.6%) obtained in high chemical purity (>95%) as determined by NMR and GC-FID, as a very viscous, almost colourless oil; ^{1}H NMR ($CDCl_3$, 200 MHz) 1.14 (s, 3 H), 1.19 (s, 3 H), *ca.* 1.4–1.8 (m, 2H), 1.67 (overlapping s, 3 H), *ca.* 2.0–2.4 (m, 2 H), 3.33 (br d, J = 10.6 Hz, 1 H), 4.14 (br d, J = 6.8 Hz, 2 H), 5.45 (tr d, J = 6.4 and 1.2 Hz, 1 H); ^{13}C NMR 16.4, 23.5, 26.6, 29.7, 36.7, 58.3, 59.4, 73.3, 124.2, 139.4; $[\alpha]^{20}_D$ +27.5° (*c* 2.3, EtOH). The NMR and optical rotation data corresponded very well with recent literature data (Wang et al. 2018). Please also see Figure S6 (supporting information) for the ^{1}H NMR spectrum of (*R*)-1 in DMSO-d6.

3. Results and discussion

Chemical synthesis of the prerequisite racemic substrate epoxide (±)-**2**, over 3 steps (acetylation, epoxidation and base hydrolysis) from inexpensive natural geraniol proceeded smoothly and in satisfyingly high yields, with only trace amounts of side-products observable in all of the obtained crude products **2**, **3** and **4**, by TLC and NMR spectroscopy.

After a provisional EH selection process based on observed activity on the substrate (±)-**2** as measured by TLC, we managed to identify microbial isolates that exhibit useful reaction and process characteristics for the production of the enantiopure (6*R*) forms of 6,7-epoxygeraniol (*R*)-**2** and DHG (*R*)-**1,** as summarized in Table 1.

Initially the substrate was screened against recombinant *Yarrowia lipolytica* strains expressing the genes for 15 different EHs obtained from different microbial sources. The recombinant strains were constructed as previously described in PCT/IB2005/001034 (Botes et al. 2006, 2008a, 2008b). Recombinant *Yarrowia* strains showing positive activity, to approximately >10% racemate conversion over 1 h incubation, were identified by TLC as those expressing the EHs from *Rhodosporidium toruloides* strains NCYC 3181 and UFS Y-0471, *Rhodotorula glutinis* UFS Y-0513, *Rhodosporidium paludiginum* NCYC 3179 and the soluble EH from *Solanum tuberosum* (potato) (Monterde et al. 2004). The reactions were then examined in more detail as assessed by chiral GC. Whilst all the above strains exhibited some activity on the (*S*)-epoxide to form the (*R*)-diol, the recombinant EH isolated from *Rhodosporidium toruloides* NCYC 3181 was confirmed to have highest enantioselectivity and highest activity for the (*S*)-epoxy alcohol (*S*)-**2** with near 100% stereoinversion to form the desired (*R*)triol DHG (**1**), as well as the highest specific activity of the strains studied. This recombinant strain was therefore selected as the best hit enzyme during this study and was applied to substrate epoxide (±)-2 in a scaled up process at multigram/L substrate loading. A substantially aqueous (but bilayered liquid phase) batch stirred tank process resulted in the highly efficient stereoinversion of the (*S*)-**2** to form a 50/50 mixture of (*R*)**2** and the desired (*R*)-triol product DHG (**1**).

Upon disappearance of the (*S*)-**2** enantiomer, the reaction halted with no significant loss of either enantiomer during prolonged incubation. As shown in Figure 4, the reaction was observed to achieve >49.5% racemic epoxide conversion (theoretical maximum for kinetic resolution is 50% of the (*S*)-epoxide converted), at which time (*R*)**2** ee >99% and (*R*)-triol (**1**) ee >99%. The reaction was judged complete when the (*S*)**2** peak area is reduced to <1/200 of the (*R*)**2** peak area. The (*R*)-triol (**1**) peak increases in size during the reaction and negligible (*S*)-triol formation was observed. At harvest, a polar solvent (such as MeOH or acetone) was added to the reaction, as described in the Materials & Methods section. The biomass was efficiently removed by precipitation and filtration. The combined recovered yield of (*R*)-epoxide **2** and (*R*)-triol (**1**) products in the harvested filtrate for this step were typically >90 mol % yield of the substrate initially added to the reactor.

Subsequently, the residual highly enantio-enriched (*R*)-**2** epoxide in the biotransformation mixture could be

Table 1. EH screening results of five selected recombinant *Yarrowia* strains expressing EHs isolated from different sources.

	Incubation for 15 min at 20 °C, pH 7.0			Incubation for 120 min at 20 °C, pH 7.0		
EH gene source[a]	m/m % conversion and identity of the converted enantiomer of epoxide 2	m/m % conversion and identity of the residual enantiomer of epoxide 2	% ee and identity of the obtained major triol product 1 enantiomer	m/m % conversion and identity of the converted enantiomer of epoxide 2	m/m % conversion and identity of the residual enantiomer of epoxide 2	% ee and identity of the obtained major triol product 1 enantiomer
Rhodosporidium toruloides UFS[b] Y-0471	9 (*S*)	10 (*R*)	>99 (*R*)	18 (*S*)	22 (*R*)	>99 (*R*)
Rhodosporidium paludiginum NCYC[c] 3179	16 (*S*)	19 (*R*)	>99 (*R*)	49 (*S*)	97 (*R*)	>99 (*R*)
Rhodotorula glutinis UFS[b] Y-0513	9 (*S*)	9 (*R*)	>99 (*R*)	13 (*S*)	15 (*R*)	>99 (*R*)
Solanum tuberosum[d]	8 (*S*)	9 (*R*)	>99 (*R*)	25 (*S*)	33 (*R*)	>99 (*R*)
Rhodosporidium toruloides NCYC[c] 3181	49.3 (*S*)	97.5 (*R*)	>99 (*R*)	50 (*S*)	>99 (*R*)	>99 (*R*)

[a]Expressed in *Yarrowia lipolytica* host.
[b]University of the Free State yeast collection, South Africa.
[c]National Collection of Yeast Cultures, United Kingdom. The sequence for this expressed epoxide hydrolase was obtained from the yeast *Rhodosporidium toruloides* NCYC 3181/CBS 349 under the UNIPROT entry Q8J2N5_RHOTO and the GenBank protein accession number AAO67343.1 as reported in Patent WO2006109198 (Botes et al. 2006).
[d]See Monterde et al. (2004).

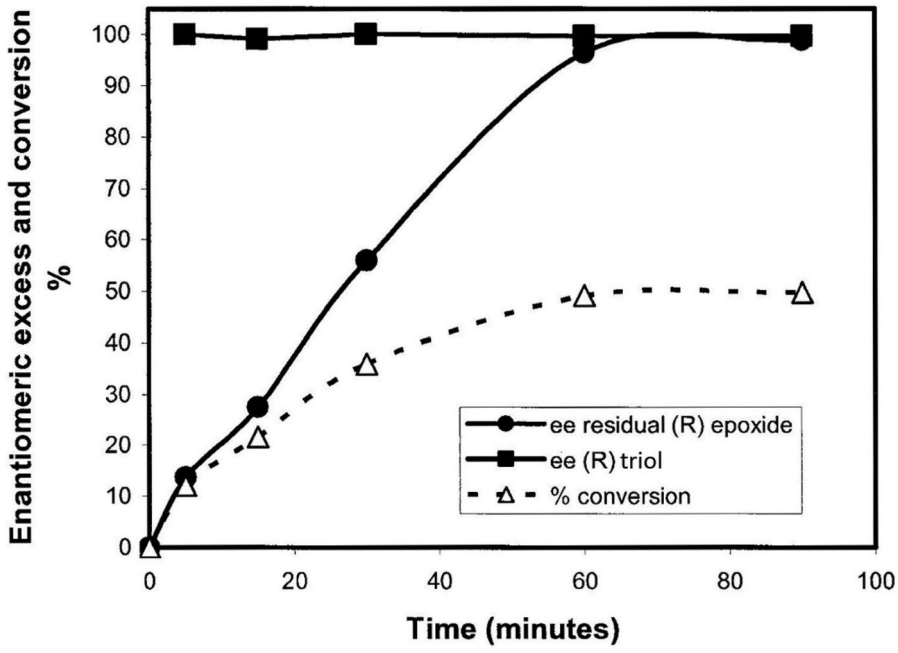

Figure 4. Epoxide (±)-**2** mass % conversion and % enantiomeric excess changes (measured in monoplicate), for both substrate epoxide (**2**) and the (*R*)-triol product (**1**), as observed over time during the scaled up (250 g/L substrate concentration), 80 mL volume biotransformation reaction, conducted at 20 °C, pH 7.0. A *Yarrowia lipolytica* engineered strain was employed that expresses the EH enzyme originally sourced from *Rhodosporidium toruloides* (NCYC 3181), at a biomass loading of 5% dry mass/total volume. Another graphic example of this bio-resolution on a 105 g scale of racemic (±)-**2**, as performed in duplicate experiments, is given in Table S1 and graph S1 (supporting information).

converted through perchloric acid catalysed chemical hydrolysis *in situ* to produce additional DHG product (**1**), with complete retention of stereochemistry. The resulting triol DHG (**1**) displayed ee >97%, as a cumulative result obtained from enzymatic kinetic reaction (ee >99%) and acid chemical hydrolysis (ee >95%).

In conclusion, a highly efficient chemoenzymatic process was developed to produce (*R*)-6,7-dihydroxygeraniol DHG (**1**) in high ee (>97%), at very high substrate loading for the biotransformation step (250 g/L), and in 72 mol % overall yield over 5 facile steps from the inexpensive, commercially available natural product geraniol.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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