PROCESS DEVELOPMENT WITH BIFUNCTIONAL CHIRAL EPOXIDES TO ACCESS SINGLE ENANTIOMERS OF PHARMACEUTICAL INTERMEDIATES

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ABSTRACT

*Two selected case studies on process development will be discussed: one is on the enantiocontrolled synthesis of (S)-3-hydroxytetradecanoic acid (**2**), an intermediate of ONO-4007 (**3**) possessing anti-tumour activity, which employs double homologation of (S)-epichlorohydrin (ECH) (**1b**) with its termini being differentiated. The other is on the enantioselective access to N-4-cyano-3-trifluoromethylphenyl-(S)-2,3-dihydroxy-2-methylpropanamide (**5a**), an intermediate of (R)-bicultamide (**5b**) exhibiting potent anti-androgen activity, which starts with enantioconvergent preparation of (R)-3-benzyloxy-2-methylpropane-1,2-diol (**4**) from O-benzyl (±)-2-methylglycidol (**1c**) by the enantiocomplementary hydrolysis using* Bacillus subtilis *epoxide hydrolase (BSEH) and H_2SO_4 in sequence. In each case study, emphasis will be placed on how to select viable synthetic routes on the basis of availability of single enantiomers of chiral starting materials and preparative methods thereof. In the chemoenzymatic synthesis of (S)-**5a**, BSEH indispensable for building its quaternary stereogenic centre is developed from scratch, which culminates in successful overexpression of its gene from* B. subtilis *JCM 10629 under the influence of an amylase promoter and terminator of* B. amyloliquefaciens *NBRC 15535 in an engineered strain of* B. subtilis *MT-2 deficient in neutral protease. The discussion should help to develop process chemistry in producing value-added fine chemicals from glycerol, one of its natural sources being palm oil.*

Keywords: (S)-epichlorohydrin, (R)-3-benzyloxy-2-methylpropane-1,2-diol, (S)-3-hydroxytetradecanoic acid, N-4-cyano-3-trifluoromethylphenyl (S)-2,3-dihydroxy- 2-methylpropanamide, epoxide hydrolase, palm oil, glycerol.

Date received: 14 March 2008; **Sent for revision:** 18 March 2008; **Received in final form:** 11 August 2008; **Accepted:** 15 August 2008.

INTRODUCTION

Glycerol, 1,2,3-trihydroxypropane [$HOCH_2CH(OH)CH_2OH$], is a colorless, odourless, hygroscopic, viscous, sweet-tasting liquid and is a tonnage chemical due to its numerous applications and long record of safety in use. While glycerol has long been produced from petroleum, the depleting carbon resource, the bulk of its world's supply today is coming from natural sustainable resources, such as palm oil and palm kernel oil. In addition, a recent global shift from fossil fuels to biofuels has brought about a huge surplus of glycerol as it is generated as a genuine by-product of the biodiesel manufacturing that depends on methanolysis of triglyceride: $R^1CO_2CH_2CH(OCOR^2)CH_2OCOR^3 + 3\ MeOH \rightarrow HOCH_2CH(OH)CH_2OH + R^1CO_2Me + R^2CO_2Me + R^3CO_2Me$. Hence, glycerol has now emerged as a renewable three-carbon platform chemical from which a range of industrial materials can be produced, including 1,3-propanediol [$HO(CH_2)_3OH$], acrolein [CH_2=CHCHO], dihydroxyacetone [$HOCH_2COCH_2OH$], and (±)-epichlorohydrin (**1b**) (*Figure 1*) (Pagliaro, *et al*., 2008).

Under such circumstances, it is invaluable to devise synthetic methods by which glycerol-derived materials can be converted into value-added

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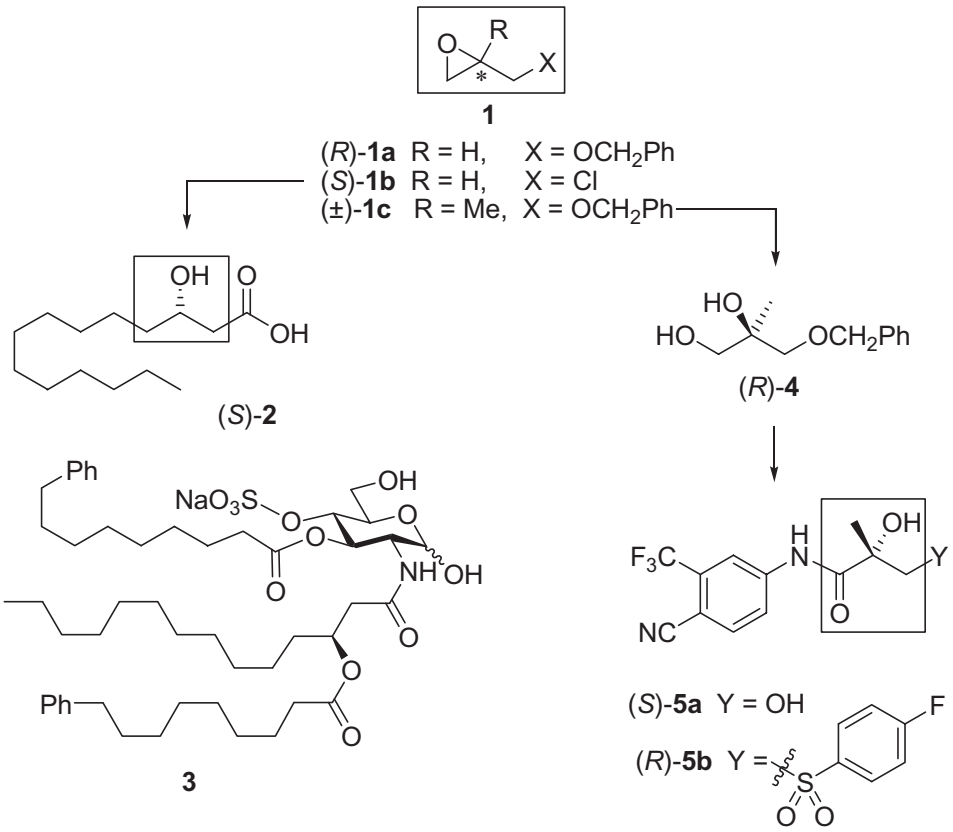

*Figure 1. Bifunctional chiral epoxide (**1**) and its synthetic application.*

products. Thus, the present article will deal with the process chemistry developed around bifunctional chiral C_3 epoxide (**1a**) and (**1b**) (*Figure 1*). What will be covered in this context is not only comparative discussion on the available methods to produce (*R*)-*O*-benzylglycidol (OBG) (**1a**) (Chikusa *et al*., 2003) and (*S*)-epichlorohydrin (ECH) (**1b**) (Kasai *et al*., 2004; Kasai *et al*., 1992; Aouni *et al*., 2004; Larrow *et al*., 2003) but also application of (*S*)-ECH (**1b**) to the enantiocontrolled synthesis of (*S*)-3-hydroxytetradecanoic acid (**2**), a side chain component of ONO-4007 (**3**) that enhances the immunological response to neoplasmic cells (Ikunaka *et al*., 1999; Ikunaka, 2003).

In addition, while racemic *O*-benzyl-2-methylglycidol (**1c**), a branched C_4 epoxide, is not a direct descendant of glycerol, light will be shed on the enantioconvergent hydrolysis of (±)-(**1c**) into a single (*R*)-configured enantiomer of 3-benzyloxy-2-methylpropane-1,2-diol (**4**) (Hellström *et al*., 2001; Simeó *et al*., 2006), a terminally differentiated derivative of 2-methylpropane-1,2,3-triol, by the enantiocomplementary catalysis of epoxide hydrolase and H_2SO_4 for two reasons that follow: (1) the basic concept that underpins such enantioconvergent synthetic manoeuvre would be helpful in exploring new and improved approaches to differentiation between two enantiotopic hydroxy groups in glycerol-related derivatives. (2) On functional group manipulations, (*R*)-**4** should be elaborated to *N*-4-cyano-3-trifluoromethylphenyl-(*S*)-2,3-dihydroxy-2-methylpropanamide (**5a**) (Soros *et al*., 2002), an advanced intermediate for the (*R*)-isomer of bicultamide (**5b**) that represents the active enantiomer of (±)-bicultamide (casodex®) (**5b**) (Tucker *et al*., 1998; Tucker and Chesterson, 1998), a non-steroidal anti-androgen for the treatment of prostate cancer (Fujino *et al*., 2007).

ENANTIOCONTROLLED SYNTHESIS OF (*S*)-3-HYDROXYTETRADECANOIC ACID (2)

Enantiomers of 3-Hydroxytetradecanoic Acid (2) in Nature and Pharmaceuticals

Being covalently linked to both 2-amino and 3-hydroxy groups of the D-glucosamine unit in lipid A, (*R*)-3-hydroxytetradecanoic acid **(2)** imparts a lipophilic nature to lipid A and hence to lipopolysacharide (LPS) in which lipid A is incorporated; thus, (*R*)-**2** helps LPS to be anchored to outer membranes of Gram-negative bacteria, such as *Escherichia coli* (*Figure 2*). While O antigen polysaccharide situated on the periphery of LPS functions as antigenic determinants of Gram-negative bacteria, the principal endotoxic activities of LPS, such as macrophage activation, are ascribed to the structural characteristics of lipid A (Kusumoto *et al*., 1996).

ONO-4007 (**3**) is a synthetic immunoadjuvant designed to mimic lipid A (**6**) stimulating

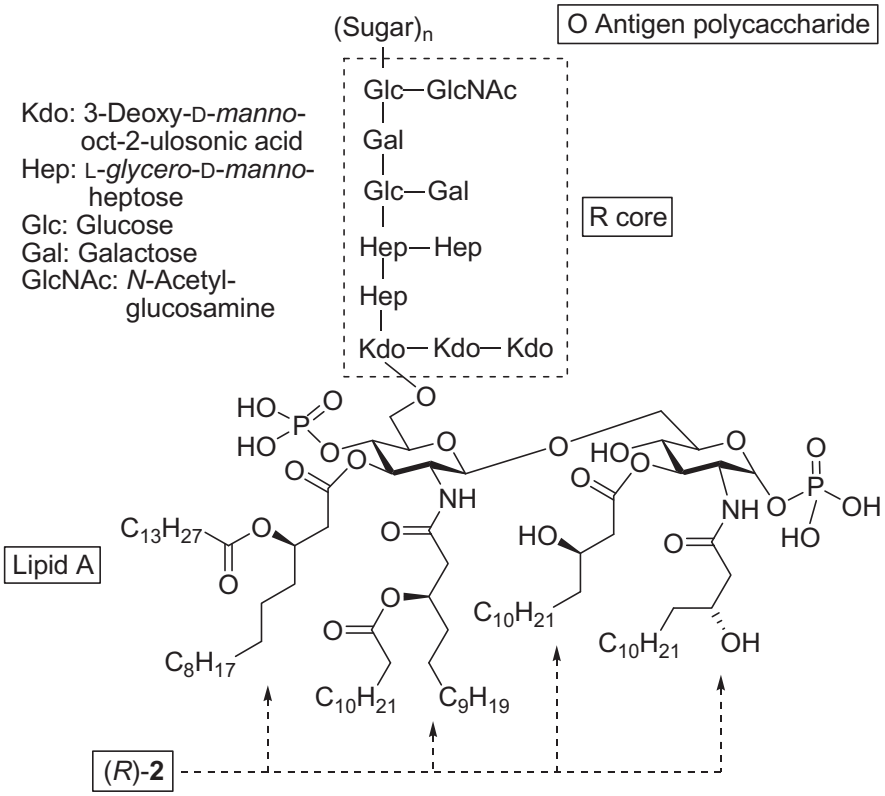

Figure 2. Structure of lipopolysaccharide (LPA) from Escherichia coli.

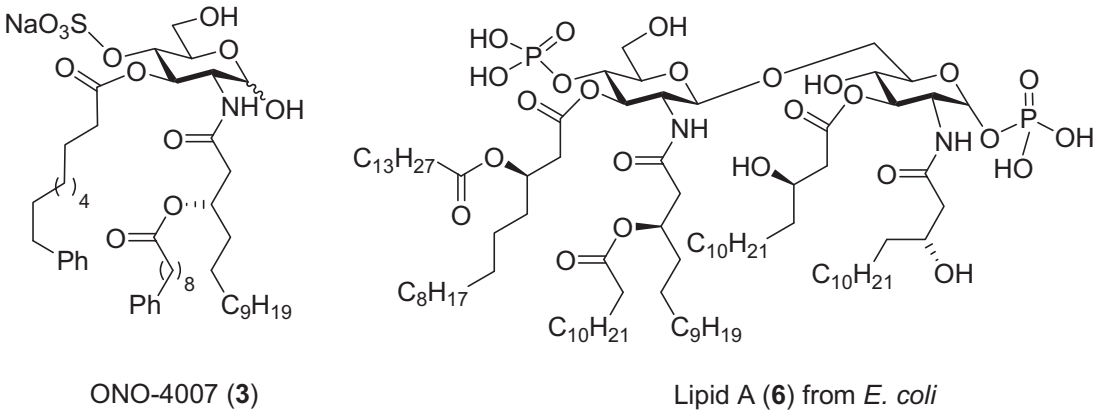

*Figure 3. Structural comparison between ONO-4007 (**3**) and Lipid A (**6**).*

macrophages to attack cancerous cells and as such, was once nominated as a clinical candidate for cancer chemotherapy (*Figure 3*) (Imaki *et al*., 1994; Kusumoto *et al*., 1996; other references cited by Ikunaka *et al*., 1999). When viewed from a perspective of structure activity relationship (SAR), ONO-4007 (**3**) deserves noticing in that it requires (***S***)-**2** for its potent immunoadjuvant activity rather than the natural counterpart, (*R*)-**2**.

Synthetic Plan to Access a Single Enantiomer of β-Hydroxy Acid (2)

To help medicinal chemists gain deeper insight into the SAR around lipid A analogues, a synthetic programme was set up to develop a scalable enantioselective route to a single enantiomer of chiral β-hydroxy acid (**2**) at Nagase & Co., Ltd. However, preliminary literature search indicated that little, if any, would remain to be explored since a range of methods to access a single enantiomer of **2** had been explored to such an extent as itemized below (Ikunaka, 2003): (1) resolution via diastereomeric salt formation (Nakamoto *et al*., 1986; Ikawa *et al*., 1953; Kiso *et al*., 1986); (2) lipase-mediated kinetic resolution (Sugai *et al*., 1993; Liu *et al*., 1997; Fukase *et al*., 1995; Feicher *et al*., 1990; 1989); (3) asymmetric microbial reduction (Utaka *et al*., 1990; Feicher *et al*., 1990); (4) asymmetric reduction over Izumi-Tai's catalyst (tartaric acid-NaBr-modified Raney Ni) (Tai *et al*., 1980; Nakahata *et al*., 1982); (5) asymmetric reduction over Noyori's catalyst (BINAP-Ru) (Case-Green *et al*., 1991; Keegan *et al*., 1996); (6) Sharpless asymmetric epoxidation (Kamireddy *et al*., 1993); (7)

Sharpless asymmetric dihydroxylation (Oikawa *et al*., 1995); (8) asymmetric allylboration with allyl-B(^{d}Ipc)$_2$ (Jadav, 1989); and (9) chiral pool synthesis starting with (*S*)-β- hydroxy-γ-butyrolactone (Huang *et al*., 1998).

The above listed overwhelming track records notwithstanding, a viable alternative to assemble a single enantiomer of **2** was designed as outlined in *Figure 4* using the synthesis of (*S*)-**2** as an illustrative example (Ikunaka *et al*., 1999). The key to this newly devised approach is to start with C_3 chiral bifunctional epoxide (**7**) and to allow a C_{10} organometallic species, such as decyl magnesium bromide (**8**), to attack **7** chemo- and regioselectively at its epoxide end such that homologated C_{13} secondary (*S*)-alcohol (**9**) is generated without affecting the functionality (Y) at another terminus. Once this nucleophilic substitution has completed with **7**, C_1 homologation on (*S*)-**9** via nucleophilic substitution of a cyanide anion (CN^-) for Y should provide (*S*)-β-hydroxy nitrile **10** (C_{14}), alkaline hydrolysis of which would then furnish (*S*)-**2**.

(*R*)-*O*-Benzylglycidol (OBG) (1a) Versus (S)-Epichlorohydrin (ECH) (1b)

To put into practice the above elaborated synthetic plan, a molecular entity of **7** was needed and it was envisioned that (*R*)-*O*-benzylglycidol (OBG) **(1a)** (Chikusa *et al*., 2003) and (*S*)-epichlorohydrin (ECH) **(1b)** (Kasai *et al*., 2004; 1992; Aouni *et al*., 2004; Larrow *et al*., 2003) should well function as synthetic equivalents to **7** (*Figure 5*).

(*R*)-OBG (**1a**) was obtained by the chemoenzymatic method established at Nagase & Co., Ltd. as outlined in *Figure 6* (Chikusa *et al*., 2003): When (±)-ECH (**1b**) was treated with benzyl alcohol in the presence of $BF_3{\cdot}OEt_2$, it underwent nucleophilic epoxide opening regioselectively to give racemic chlorohydrin (**11**), which, on acylation with succinic anhydride in pyridine, was converted into (±)-hemisuccinate (**12**), a substrate for the hydrolase-mediated kinetic resolution, uneventfully.

Cells of *Bacillus lichenformis* were cultivated, harvested and incubated with (±)-**12** at pH 8 and 30°C for 18 hr at which point its (*S*)-isomer underwent selective hydrolysis to give (*S*)-chlorohydrin (**11**) while (*R*)-**12** remained unaffected. Being neutral in its nature, the digested (*S*)-alcohol (**11**) was removed from the spent aqueous mixture by extracting it into AcOEt at pH 8. The left-over (*R*)-ester (**12**) that remained dissolved in the aqueous layer with its acidic functionality being dissociated was treated with 2 M NaOH (5 equiv) at 5°C for 5 hr to cleave the hemisuccinate linkage and to close an epoxide ring *in situ*. Finally, the usual extractive workup afforded (*R*)-OBG (**1a**) of > 98% ee in 34% overall yield from (±)-**12**.

With regard to (*S*)-ECH (**1b**), two industrial methods for its production have been developed so far, as summarized in *Figure 7*: one is the microbial enantioselective assimilation (catabolic degradation) of (±)-2,3-dichloro-1-propanol (DCP) (**13**) which was developed and commercialized at Daiso Co., Ltd. (Kasai *et al*., 2004; 1992). The other is Jacobsen's hydrolytic kinetic resolution (HKR) of (±)-ECH (**1b**)

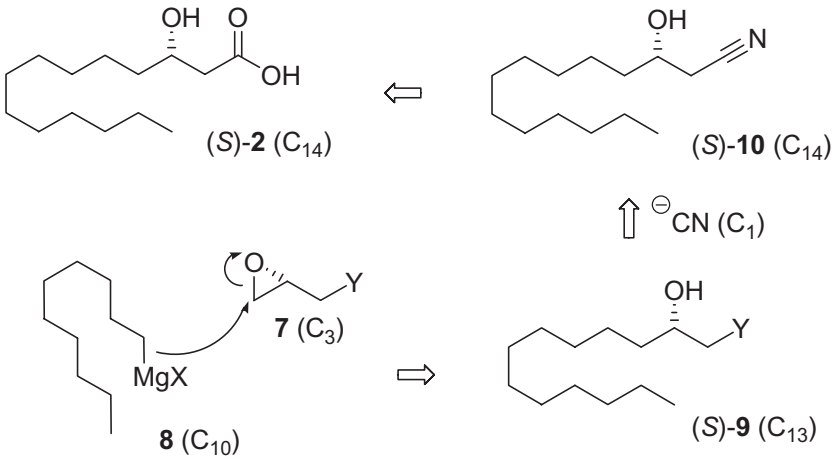

*Figure 4. Synthetic plan to assemble (S)-**2** in a $C_{10} + C_3 + C_1$ fashion.*

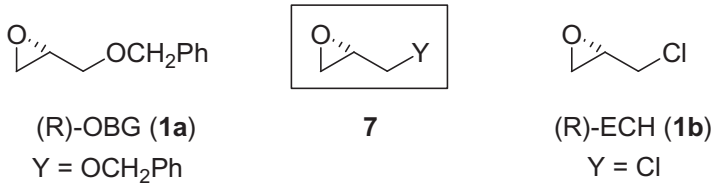

*Figure 5. The C_3 chiral bifunctional epoxide (**7**) and its molecular entities.*

which was commercialized at Rhodia Pharma Solutions (Aouni *et al*., 2004; Larrow *et al*., 2003).

When cultivation of *Alcaligenes* sp. DS-K-S38 is fed with (±)-DCP (**13**), its (*S*)-isomer is consumed catabolically while (*R*)-DCP (**13**) remains unaffected. On treatment with $Ca(OH)_2$, (*R*)-DCP (**13**) is converted into (*S*)-ECH (**1b**) (*Figure 7*) (Kasai *et al*., 2004; 1992). To obtain (*S*)-ECH (**1b**) via Jacobsen's HKR, (±)-ECH (**1b**) is exposed to H_2O (0.75 equiv) in the presence of catalytic (*S,S*)-(salen)Co(III)OAc (**14**) (0.5 mol%) to hydrolyze its (*R*)-isomer to (*R*)-3-chloro-1,2-propanediol (**15**) with (*S*)-ECH (**1b**) being unaffected (Aouni *et al*., 2004; Larrow *et al*., 2003). Separation between (*S*)-ECH (**1b**) and (*R*)-diol (**15**) is then effected by distillation *in vacuo* and (*S*)-ECH (**1b**) is collected as lower boiling fractions in 41% yield. Because of its superior operational simplicity and higher space-time yield, Jacobsen's HKR seemed to be a better process than the microbial enantioselective assimilation with the production of (*S*)-ECH (**1b**) in terms of production cost.

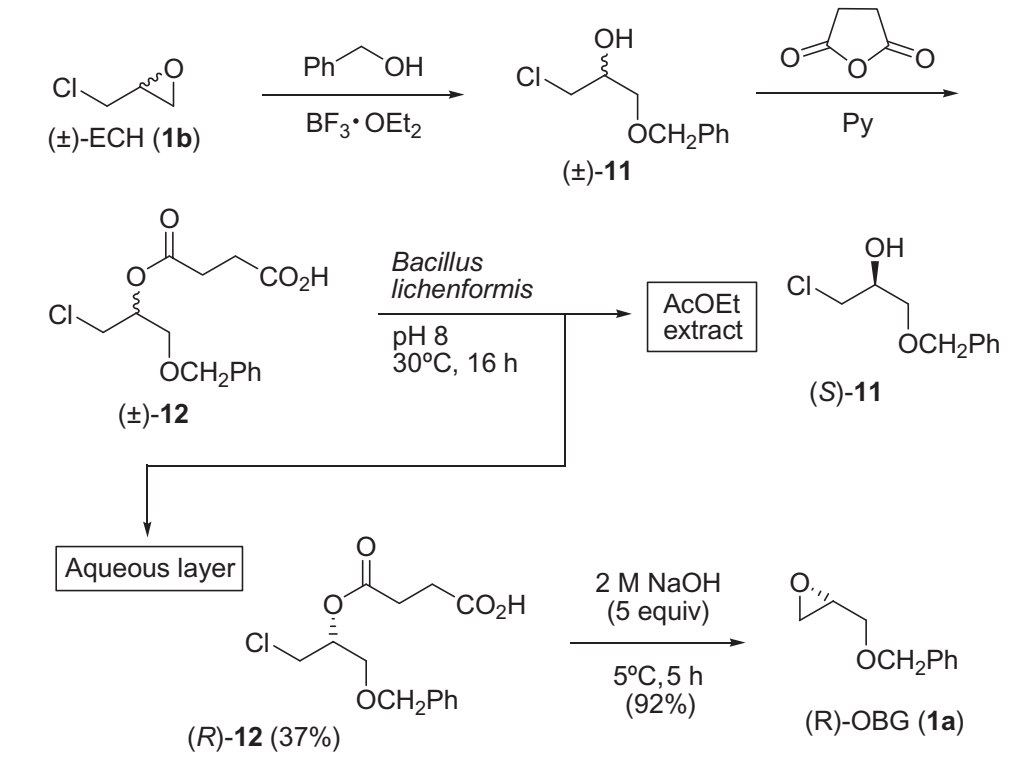

*Figure 6. Chemoenzymatic synthesis of (R)-OBG (**1a**).*

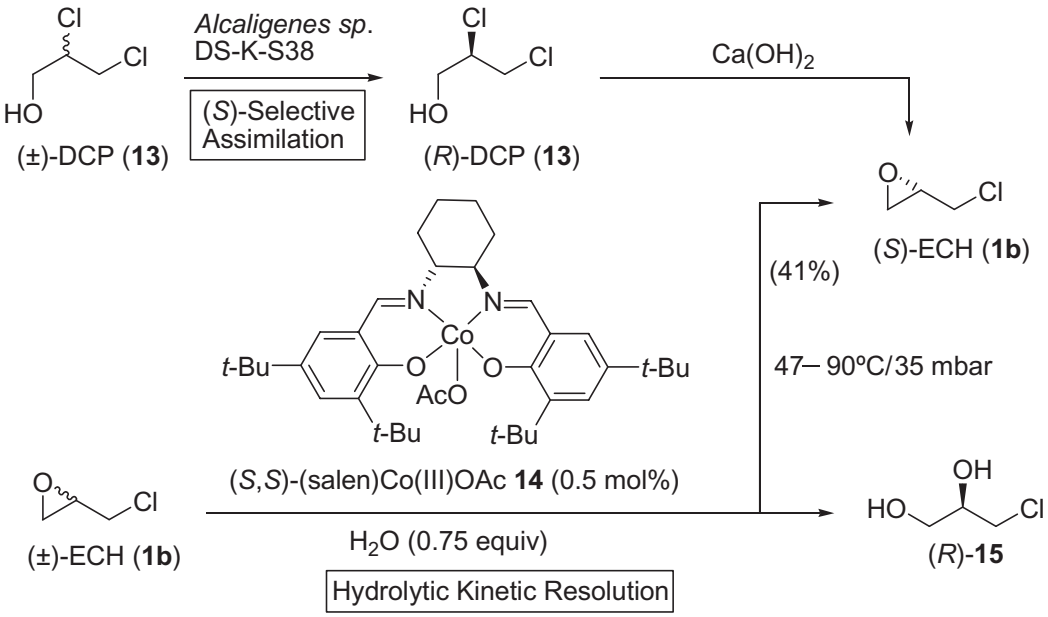

*Figure 7. Industrial methods to produce (S)-ECH (**1b**).*

To choose between (*R*)-OBG (**1a**) and (*S*)-ECH (**1b**), careful comparison was made from the two viewpoints that follow: (1) procurement cost of those materials and (2) stereochemical unambiguousness in the reaction(s) involved. At first, it proved less costly to purchase (*S*)-ECH (**1b**) from Rhodia Pharma Solutions than to produce (*R*)-OBG (**1a**) in house at Nagase & Co., Ltd.

On second thought, however, (*R*)-OBG (**1a**) seemed superior to (*S*)-ECH (**1b**) in the unambiguousness for the loci of nucleophilic substitution. In fact, possessing two electrophilic sites, (*S*)-ECH (**1b**) may well take two alternative courses in the nucleophilic substitution reaction, which would detract from stereochemical integrity of the product by the plausible mechanism outlined in *Figure 8*: when the chloride in (*S*)-ECH (**1b**) is displaced by a nucleophile (Nu-H), epoxide (**16**) is produced without affecting the original configuration of (*S*)-ECH (**1b**) (path 1 in *Figure 8*). In contrast, when (*S*)-ECH (**1b**) suffers the nucleophilic attack at its epoxide functionality, the resulting chlorohydrin (**17**) undergoes epoxide ring formation *in situ* to give *ent*-**16** with overall inversion of configuration (path 2 in *Figure 8*). As a result, racemization should occur to a varying degree depending on the ratios of the two reaction courses actually taken. Contrary to (*S*)-ECH (**1b**), (*R*)-OBG (**1a**) is free from such stereochemical ambiguity since it has no locus for nucleophilic substitution other than the epoxide functionality (*Figure 8*).

Despite such a sensible concern about (*S*)-ECH (**1b**) suffering from some stereochemical scrambling during its nucleophilic substitution, (*S*)-glycidyl tosylate (**19**) was reported to undergo Li_2CuCl_4-catalyzed nucleophilic attack of 4-chlorobenzylmagnesium chloride (**20**) exclusively at its epoxide terminus to provide *(S)*-hydroxy tosylate (**21**) (*Figure 9*) (Rotstein *et al*., 1993). Chloride being a less reactive leaving group than tosylate, an organometallic nucleophile, such as **8** (*Figure 4*), should attack (*S*)-ECH (**1b**) preferentially at its epoxide terminus. Therefore, because of its availability and presumed preferential reactivity of the epoxide functionality towards carbon nucleophiles, (*S*)-ECH (**1b**) was eventually chosen as the substrate to which nucleophilic homologation was to be applied two-fold in the synthesis of (*S*)-**2** as shown schematically in *Figure 4*.

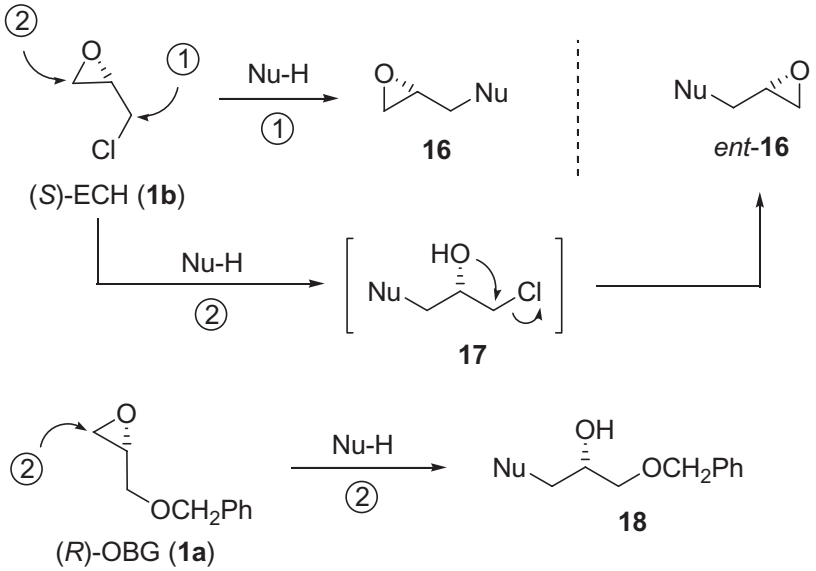

*Figure 8. Stereochemical ambiguity for nucleophilic substitution on (S)-ECH (**1b**).*

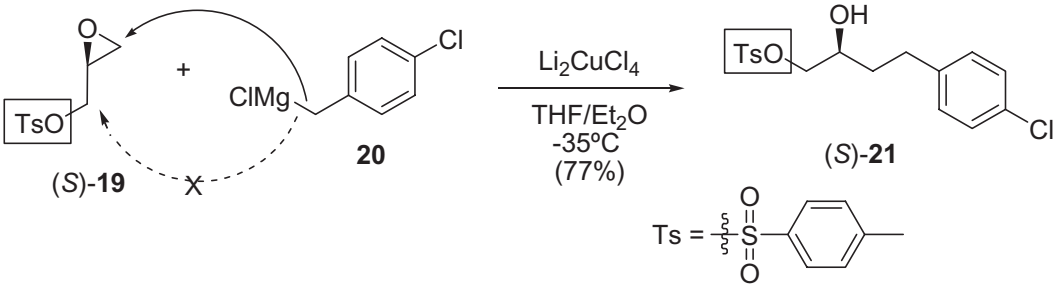

*Figure 9. Chemoselective nucleophilic substitution on (S)-glycidyl tosylate (**19**).*

Use of (*S*)-ECH (1b) as a Starting Material for the Synthesis of (*S*)-2

(*S*)-ECH (**1b**) (> 99.9% ee; purchased from Rhodia Pharma Solutions) was treated with decylmagnesium bromide (**22**) (1.1 equiv; prepared from decyl bromide, Mg turnings, and catalytic 1,2-dibromoethane) in the presence of CuI (0.9 mol%) in PhMe/THF (3:2) at temperatures between 3°C and 15°C for 5 hr to give crude homologated (*S*)-chlorohydrin (**23**) (*Figure 10*). Without isolation or purification, the resulting (*S*)-**23** was exposed to aqueous NaOH (1.1 equiv) in PhMe/MeOH (2:1) at ambient temperature for 4.5 hr to rebuild an epoxide ring (Ikunaka *et al*., 1999). On distillation *in vacuo*, (*S*)-epoxide (**24**) (bp 104°C-107°C/4-5 mmHg) was obtained in a two-step overall yield of 55% from (*S*)-ECH (**1b**).

To assess whether the nucleophilic substitution reaction [(*S*)-ECH (**1b**) + **22** → (*S*)-**23**] had been plagued with any stereochemical scrambling, it was attempted to determine the enantiomeric purity of the isolated (*S*)-epoxide (**24**). However, no separation was achieved between enantiomers of **24** on any chiral stationary phase of HPLC or GLC employed. Thus, (±)-tridecane-1,2-diol 1-methyl ether (**25**), prepared by treatment of (±)-epoxide (**24**) with NaOMe, was analysed by 400 MHz ^{1}H-NMR in the presence of a range of chiral shift agents for the chiral discrimination between enantiomers of **25**. After experimentation, europium tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorate] {Eu[(+)-tfc]} (0.3 equiv) was found to induce down field shifts (Δδ) with a significant difference (ΔΔδ) in the resonances due to the OCH_3 groups of enantiomers of **25**: δ 4.68 (s) for (*S*)-**25** and δ 4.53 (s) for (*R*)-**25** in $CDCl_3$ under the influence of Eu[(+)-tfc] (0.3 equiv). When (*S*)-**25**, prepared via (*S*)-**24** from (*S*)-ECH (**1b**) and the Grignard reagent (**22**), was subjected to the 400 MHz ^{1}H-NMR analysis under the above-specified conditions, no signal due to (*R*)-**25** was detected, which demonstrated that little, if any, stereochemical scrambling had afflicted the nucleophilic substitution in question. When considering the error of measurement inherent in the ^{1}H-NMR spectroscopy, the enantiomeric purity of (*S*)-**24** was estimated at not less than 97% ee (Ikunaka *et al*., 1999).

When (*S*)-epoxide (**24**) was treated with NaCN (1.2 equiv) in aqueous 80% MeOH at pH 11–12 (adjusted with H_2SO_4) at 50°C for 5 hr, nucleophilic epoxide opening occurred with exclusive regioselectivity (Ikunaka *et al*., 1999) (*Figure 11*). Without purification, the resulting crude (*S*)-β-hydroxy nitrile (**26**) was subjected to mild alkaline hydrolysis [aqueous NaOH, 30% H_2O_2, MeOH, 60°C, 7.5 hr] to afford crude (*S*)-**2** in 74% overall yield from (*S*)-**24**. To determine its enantiomeric purity, a portion of crude (*S*)-**2** was converted into (*S*)-*p*-bromophenacyl ester (**27**); the latter was then

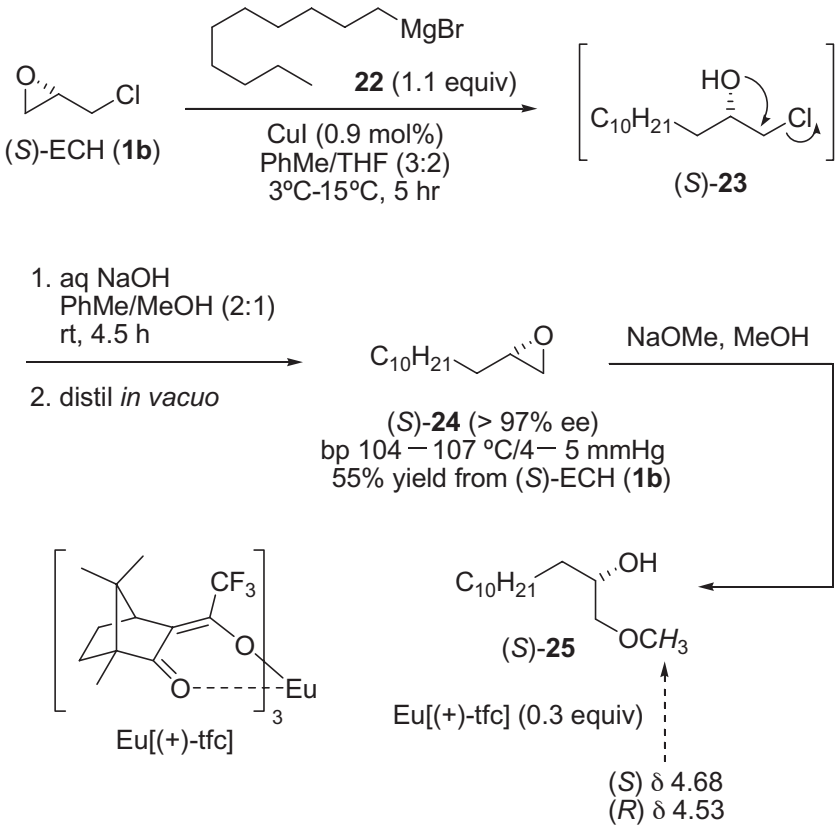

*Figure 10. Conversion of (S)-ECH (**1b**) into (S)-1,2-epoxytridecane (**24**).*

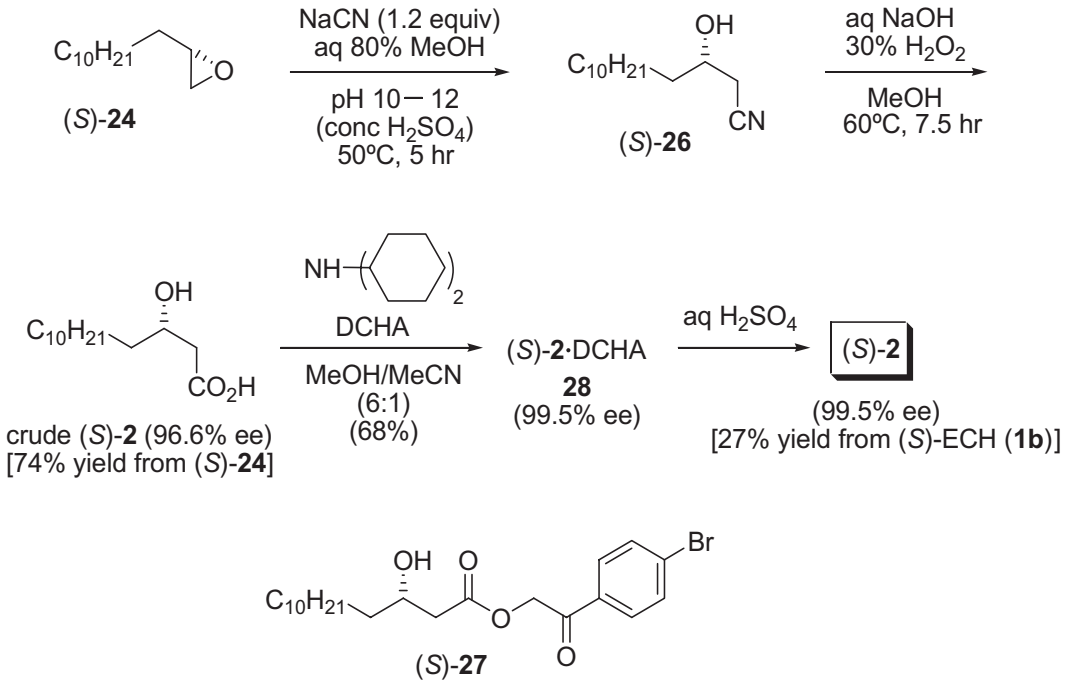

*Figure 11. Completion of the synthesis of (S)-**2** via its DCHA salt (**28**).*

analysed by HPLC [Chiralpack AS, *n*-hexane/*i*-PrOH (97:3 v/v)] for the enantiomeric composition. As a result, the enantiomeric purity of (*S*)-**27**, hence, that of the parent (*S*)-**2** in the crude state, was determined to be 96.6% ee (Ikunaka *et al*., 1999).

The crude (*S*)-**2** thus obtained was purified via crystalline salt formation: when it was combined with dicyclohexylamine (DCHA) in MeOH/MeCN (6:1), solid precipitates were formed. Filtration provided salt (**28**) [(*S*)-**2**·DCHA] in 68% yield with increase in the enantiomeric purity of (*S*)-**2** contained in it to 99.5% ee as determined by the method mentioned above. Finally, the DCHA salt (**28**) was treated with aqueous H_2SO_4 solution to liberate (*S*)-**2** of 99.5% ee as free carboxylic acid (mp 72.8°C – 73.1°C) in an overall of 27% from (*S*)-ECH (**1b**) (Ikunaka *et al*., 1999).

CHEMOENZYMATIC ENANTIOSELECTIVE SYNTHESIS OF (*S*)-*N*-4-CYANO-3-TRIFLUOROMETHYLPHENYL-(*S*)-2,3-DIHYDROXY-2-METHYLPROPANAMIDE (5a), AN ADVANCED INTERMEDIATE OF (*R*)-BICULTAMIDE (5b)

Bicultamide [(±)-(5b)], a Non-steroidal Antiandrogen, and its Active Isomer of (*R*)-Configuration

Bicultamide (casodex®), a racemate of *N*-(4-cyano-3-trifluoromethylphenyl)-3-(4-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamide [(±)-**5b**] (Tucker and Chesterson, 1988; James *et al*., 2002b; Chen *et al*., 2003), is a potent non-steroidal anti-androgen prescribed to treat prostate cancer (*Figures 1* and *12*) (Tucker, 1990). However, its actual therapeutic effect resides in the (*R*)-isomer, the anti-androgen activity of which exceeds that of the (*S*)-isomer by 60 times (Tucker *et al*., 1988). In addition, the (*S*)-isomer undergoes metabolic degradation in the liver and elimination from the body much faster than the (*R*)-isomer (James *et al*., 2002a). Thus, a racemic switch of bicultamide [redevelopment of its active isomer, (*R*)-**5b**, as a new drug entity] should be of much benefit to the patients because of reduction in both dosage and metabolic burden on the liver (James *et al*., 2002a).

To synthesize (*R*)-bicultamide (**5b**), two enantioselective approaches had been explored (*Figure 12*): one took advantage of diastereoselective bromolactonization on *N*-methacryloyl D-proline (**29**) to access (*R*)-3-bromo-2-hydroxy-2-methylpropanoic acid (**30**), which was converted into (*R*)-**5b** after functional group manipulations (Tucker *et al*., 1988; Kirkovsky *et al*., 2000; Nair *et al*., 2005; 2006). The other employed Barton's decarboxylative bromination on (*S*)-5-oxo-1,3-dioxolane- 4-acetic acid (**32**), prepared from (*S*)-citramalic acid (**31**) and bromal (CBr_3CHO), to build (*R*)-4-bromomethyl-5-oxo-1,3-dioxolane (**33**), a synthetic equivalent to (*R*)-**30** (James *et al*., 2002a).

SYNTHETIC PLAN FOR (*R*)-BICULTAMIDE (5b) AND ITS SYNTHETIC PRECURSOR [(*S*)-5a]

Once a latent element of symmetry is recognized in the structure of (*R*)-bicultamide (**5b**), enantiomers

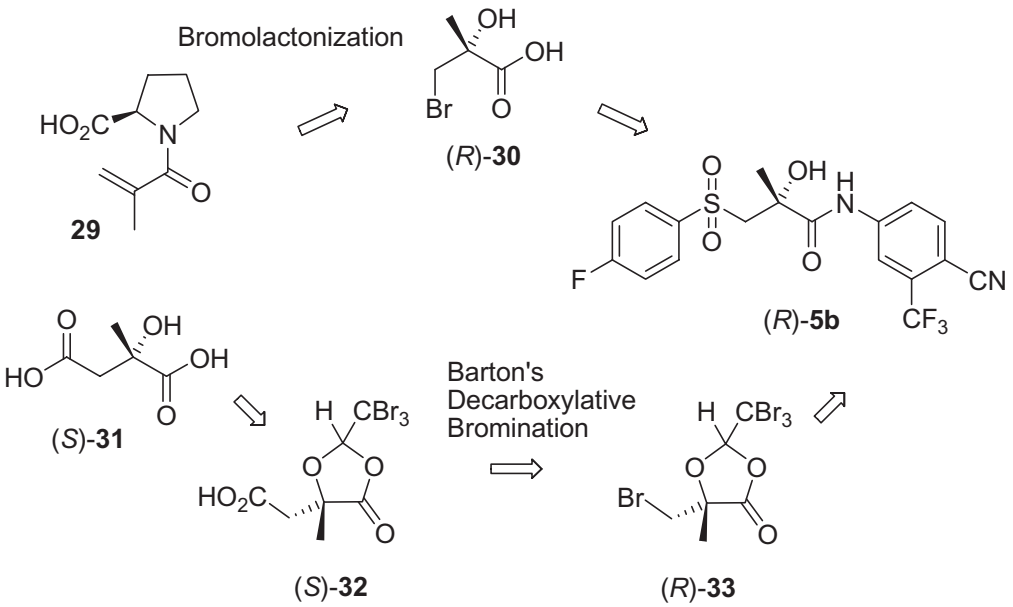

*Figure 12. Two hitherto-developed alternative methods to access (R)-bicultamide (**5b**).*

of a 2-methyl-2-hydroxypropane derivative (**34**) with its termini being properly differentiated (X ≠ Y), would emerge as two alternative chiral starting points in the synthesis of (*R*)-**5b** as depicted in *Figure 13*. In fact, such retrosynthetic analysis should help to devise two approaches that are opposite to each other in terms of the order of functional group manipulations and the sense of chirality involved (Fujino *et al*., 2007): One approach is to install the sulphur-carbon linkage to (*S*)-anilide (**5a**) at a later stage of the synthesis and to trace it back to (*R*)-3-benzyloxy-2-methylpropane-1,2-diol (**4**) via (*S*)-carboxylic acid (**35**). The other is to form the anilide function on (*S*)-carboxylic acid (**36**), equipped with the required sulphur functionality, at the later stage of the synthesis and to prepare (*S*)-**36** from (*R*)-*O*-benzyl 2-methylglycidol (**1c**), which, in fact, is antipodal to (*R*)-diol (**4**) with respect to the central stereogenic centers. Thus, the issue to be addressed would boil down to making a sensible choice between *O*-benzyl (*R*)-2-methylgycidol (**1c**) and (*R*)-3-benzyloxy-2-methylpropane-1,2-diol (**4**).

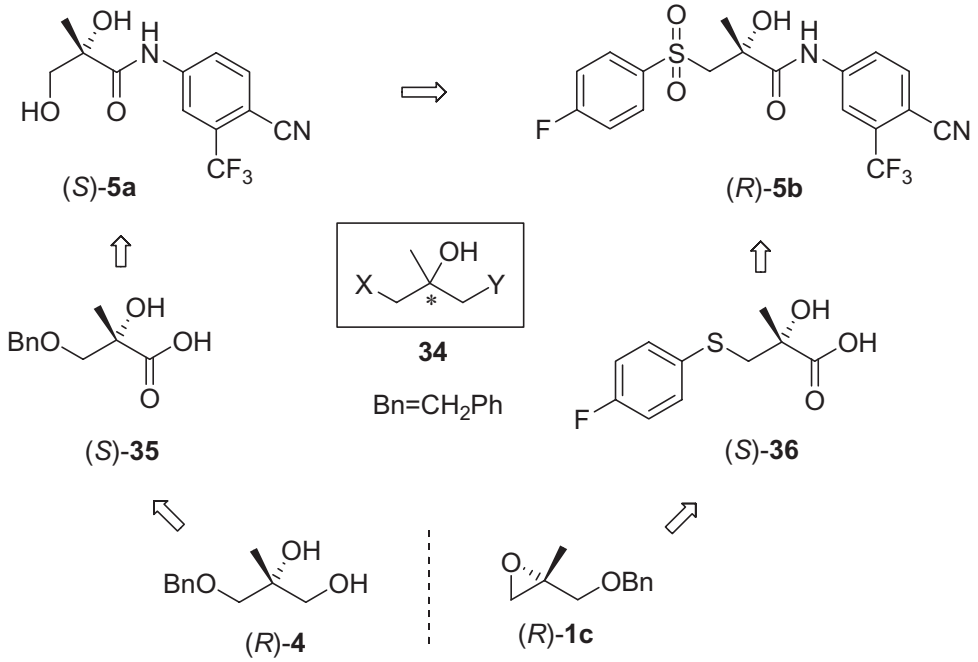

*Figure 13. Retrosynthetic analysis of (R)-bicultamide (**5b**).*

Chemoenzymatic Preparation of (*R*)-3-Benzyloxy-2-Methylpropane-1,2-Diol (4) Via Enantiocomplementary Hydrolysis of *O*-Benzyl (±)-2-Methylglycidol (1c)

When (±)-epoxide (**1c**) is subjected to hydrolytic kinetic resolution by the catalysis of epoxide hydrolase (EH) (Orru *et al*., 2000), every enantiomer of chiral epoxide (**1c**) and diol (**4**) can be obtained using the enzyme of an appropriate origin as summarized in *Figure 14* (Simeó *et al*., 2006). For instance, when (±)-**1c** is treated with EH from soyabean or *Rhodotorula* sp., its (*R*)-isomer undergoes selective hydrolysis with retention of configuration to give (*S*)-diol (**4**) with (*S*)-epoxide (**1c**) being left unaffected. In contrast, EH from limonene is unique in that it acts on (*S*)-epoxide (**4**), hydrolyzing it with inversion of configuration to afford (*R*)-diol (**4**) with (*S*)-epoxide (**1c**) being left untouched (*Figure 14*) (Simeó *et al*., 2006).

On exposure to EH from potato (Stapleton *et al*., 1994) or *Rhodococcus* sp. (Steinreiber *et al*., 2000), (±)-epoxide (**1c**) undergoes (*S*)-selective hydrolysis with retention of configuration (*Figure 14*) to afford the hydrolyzed (*R*)-diol (**4**) and the unaffected (*R*)-epoxide (**1c**), the two possible chiral starting materials in question (*Figure 13*). What is more, the resulting mixture of (*R*)-**4** and (*R*)-**1c** is allowed to converge solely to (*R*)-**4** on treatment with mineral acid, such as H_2SO_4 (Orru *et al*., 1988), which helps to hydrolyze a 2,2-disubstituted epoxide ring, such as (*R*)-**1c**, with inversion of configuration (*Figure 14*). As a result, (±)-epoxide (**1c**) as a whole is converted into the (*R*)-configured single enantiomer of (**4**), providing that EH can be secured in hand which hydrolyzes (*S*)-epoxide (**1c**) with retention of configuration in the following manner: (±)-epoxide (**1c**) → (*R*)-epoxide (**1c**) + (*R*)-diol (**4**). Hence, because of atom economy (Trost, 1995; 1991) being gained from its enantioconvergent elaboration, (*R*)-diol (**4**) was eventually chosen as the starting material of choice and as such, (*S*)-anilide (**5a**) was identified as a primary target in the synthetic endeavor to access (*R*)-bicultamide (**5b**); for the synthesis of (*R*)-**5b** from (*R*)-**1c** via (*S*)-carboxylic acid (**36**) (*Figure 13*), see Fujino *et al*. (2007).

Development of *Bacillus subtilis* Epoxide Hydrolase (BSEH)

The EH fulfilling (*S*)-selective hydrolysis of (±)-**1c** with retention of configuration was developed from scratch at Nagase & Co., Ltd. since no such enzyme was available in quantity (Yamaguchi *et al*., 2004; 2005). Thus, the terrestrial microbial flora was explored intensively for strains that could produce the desired EH; *Bacillus subtilis* JCM 10629 was then identified as producing, albeit in minute amounts, the EH that could convert (±)-epoxide (**1c**) into a mixture of (*R*)-diol (**4**) of 84% ee and (*R*)-epoxide (**1c**) of > 99% ee. To effect the EH-catalyzed kinetic resolution in question on an industrial scale, its gene (*yhf*) was cloned to a plasmid of pUB 110, so that the gene was flanked by an amylase promoter (P_{amy}) and terminator (T_{amy}) both of which had been isolated from *B. amyloliquefaciens* NBRC 15535 (Saito *et al*., 1975). The expression vector thus constructed (pUB 110-P_{amy}-EH-T_{amy}) was transformed into *B. subtilis* MT-2, a mutant strain deficient in neutral protease, to deliver an EH-overproducing strain, *B. subtilis* $PT_{amy}EH$, which acquired 400 times increase in the EH productivity compared to *B. subtilis* JCM 10629.

To isolate *B. subtilis* EH (BSEH) thus produced, much effort was made, but to no avail. It was probably because the enzyme was too labile to survive the regular conditions for protein purification. Thus, instead of using the isolated BSEH, cultivated cells of *B. subtilis* $PT_{amy}EH$

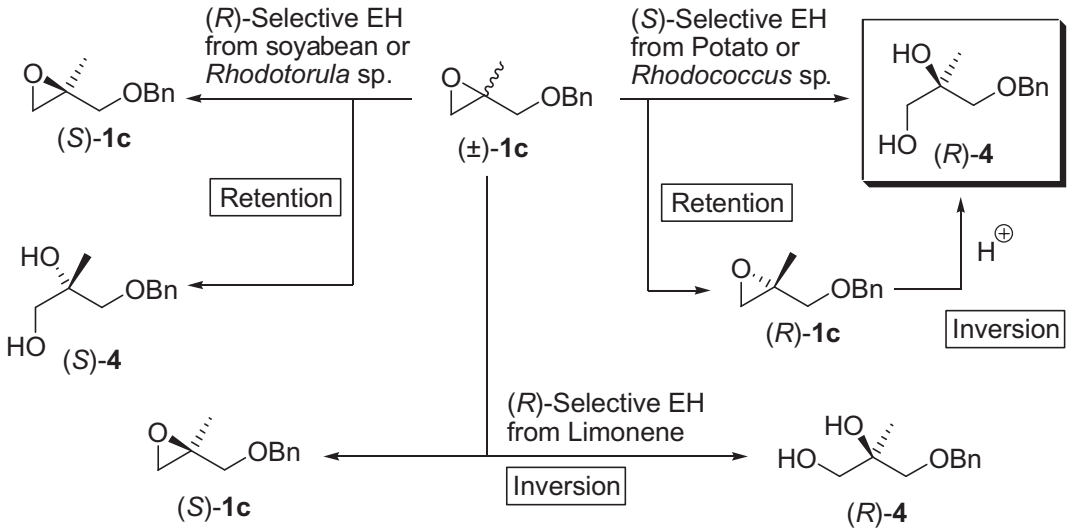

*Figure 14. Epoxide hydrolases (EH's) acting on O-benzyl (±)-2-methylgycidol (**1c**).*

engineered to overproduce BSEH were to be harvested and then applied to the enantioselective hydrolysis (±)-epoxide (**1c**). Eventually, under the optimized conditions, it proved to be possible to digest (±)-**1c** (1 g) using a suspension (20 ml) of harvested cells of *B. subtilis* $PT_{amy}EH$ (Fujino *et al*., 2007).

Enantioconvergent Chemoenzymatic Preparation of (*R*)-3-Benzyloxy-2-Methylpropane-1,2-Diol (4)

Now that the *B. subtilis* $PT_{amy}EH$ strain engineered to produce the desired EH in quantities had been secured in hand, the chemoenzymatic enantioconvergent preparation of (*R*)-diol (**4**) and its application to the enantioselective synthesis of (*S*)-anilide (**5a**) were both explored in collaboration between Keio University and Nagase & Co., Ltd. Benzyl alcohol (**37**) was treated with methallyl chloride (**38**) in the presence of NaH in DMF to give ether (**39**), which, on epoxidation under Payne's conditions (H_2O_2, $KHCO_3$, MeCN, MeOH), was converted into *O*-benzyl (±)-2-methylgycidol (**1c**), the substrate for the EH-mediated hydrolysis, in 90% overall yield from **37** (*Figure 15*) (Fujino *et al*., 2007; Yamaguchi *et al*., 2005).

When (±)-**1c** was treated with harvested cells of *B. subtilis* $PT_{amy}EH$ at 30°C for a week, selective hydrolysis of (*S*)-**1c** proceeded giving (*R*)-diol (**4**) in 53% conversion with (*R*)-epoxide (**1c**) being unaffected. After the spent cellular bodies were filtered off, the aqueous mixture containing (*R*)-**4** and (*R*)-**1c** was treated with aqueous H_2SO_4 to hydrolyze (*R*)-epoxide (**1c**) with inversion of configuration, which allowed (±)-epoxide (**1c**) as a whole to be converted into (*R*)-diol (**4**) of 82.3% ee in an enantioconvergent manner in 83% overall yield (Fujino *et al*., 2007).

When (*R*)-**4** thus prepared was crystallized from Et_2O at -30°C, enantiomerically pure (*R*)-**4** was obtained as a low melting solid (mp 30ºC-31°C) in 52% yield (*Figure 16*) (Fujino *et al*., 2007). From the mother liquor, (*R*)-**4** of 68% ee was recovered in 48% yield which was then subjected to the following procedures to further gain enantiomerically pure (*R*)-diol (**4**) (*Figure 16*) (Fujino *et al*., 2007): (*R*)-Diol (**4**) of 68% ee was converted into (*S*)-epoxide (**1c**) of 68% ee in 94% overall yield after tosylation (TsCl, Py) followed by base treatment (K_2CO_3, MeOH). The regenerated (*S*)-**1c** was subjected to the BSEH-mediated hydrolysis again. Finally, chromatographic separation provided enantiomerically pure (*R*)-**4** in 82% yield along with (*S*)-epoxide (**1c**) of 68% ee in 18% yield. When those two crops of enantiomerically pure (*R*)-diol (**4**) were combined, its total yield from (±)-epoxide (**1c**) amounted to 73%.

COMPLETION OF THE SYNTHESIS OF (*S*)-ANILIDE (5a)

The enantiomerically pure (*R*)-diol (**4**) thus secured was oxidized according to Zhao's procedures [NaClO, $NaClO_2$, catalytic 2, 2, 6, 6-tetramethyl-1-piperidyniloxy, free radical (TEMPO), pH 6.7, 37 °C] (Fujino *et al*., 2007; Zhao, *et al*., 1999) to give (*S*)-carboxylic acid (**35**) in 97% yield (*Figure 17*). On treatment with $SOCl_2$ in THF, the resulting acid chloride was treated with 3-cyano-4-trifluoromethylaniline (**40**) in slightly excess amounts (1.2 equiv) in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 5 equiv) to give (*S*)-anilide (**41a**) as an inseparable mixture with the unreacted **40**. After experimentation, *O*- and *N*-acetylation turned out to be an effective means to achieve the

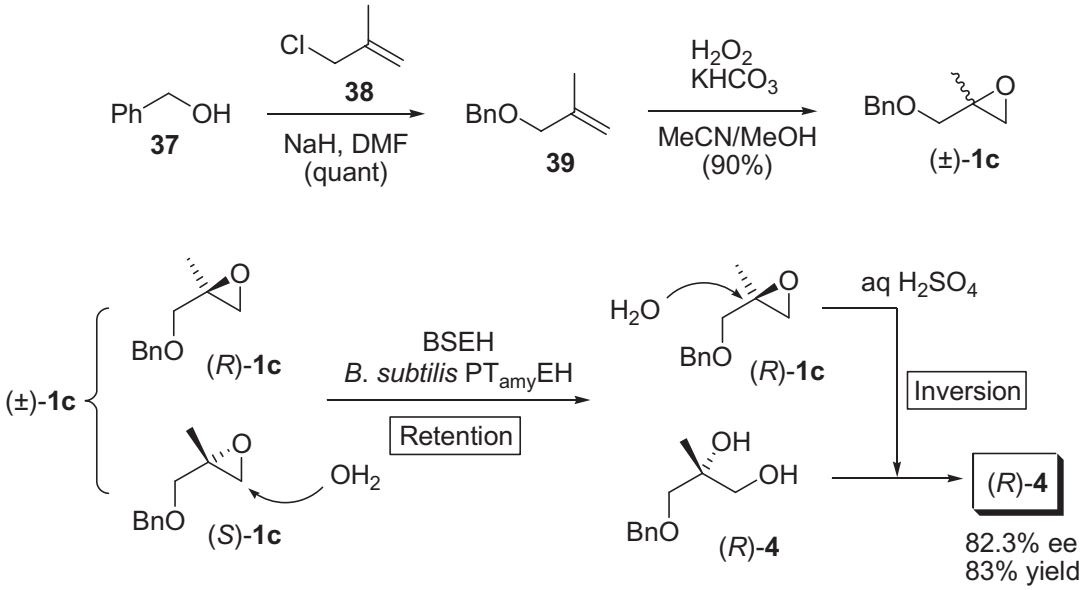

*Figure 15. Stereoconvergent preparation of (R)-diol (**4**) from (±)-epoxide (**1c**).*

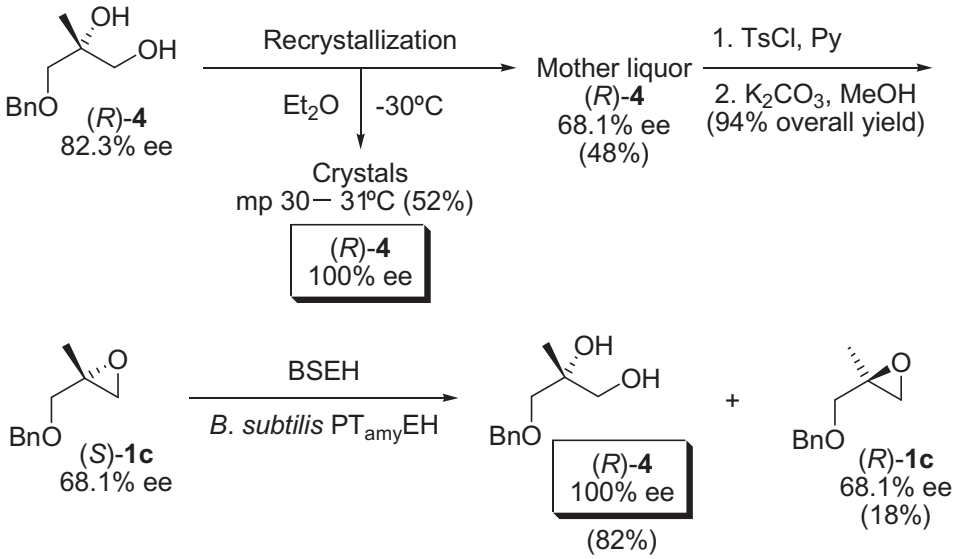

*Figure 16. Isolation of enantiomerically pure (R)-diol (**4**).*

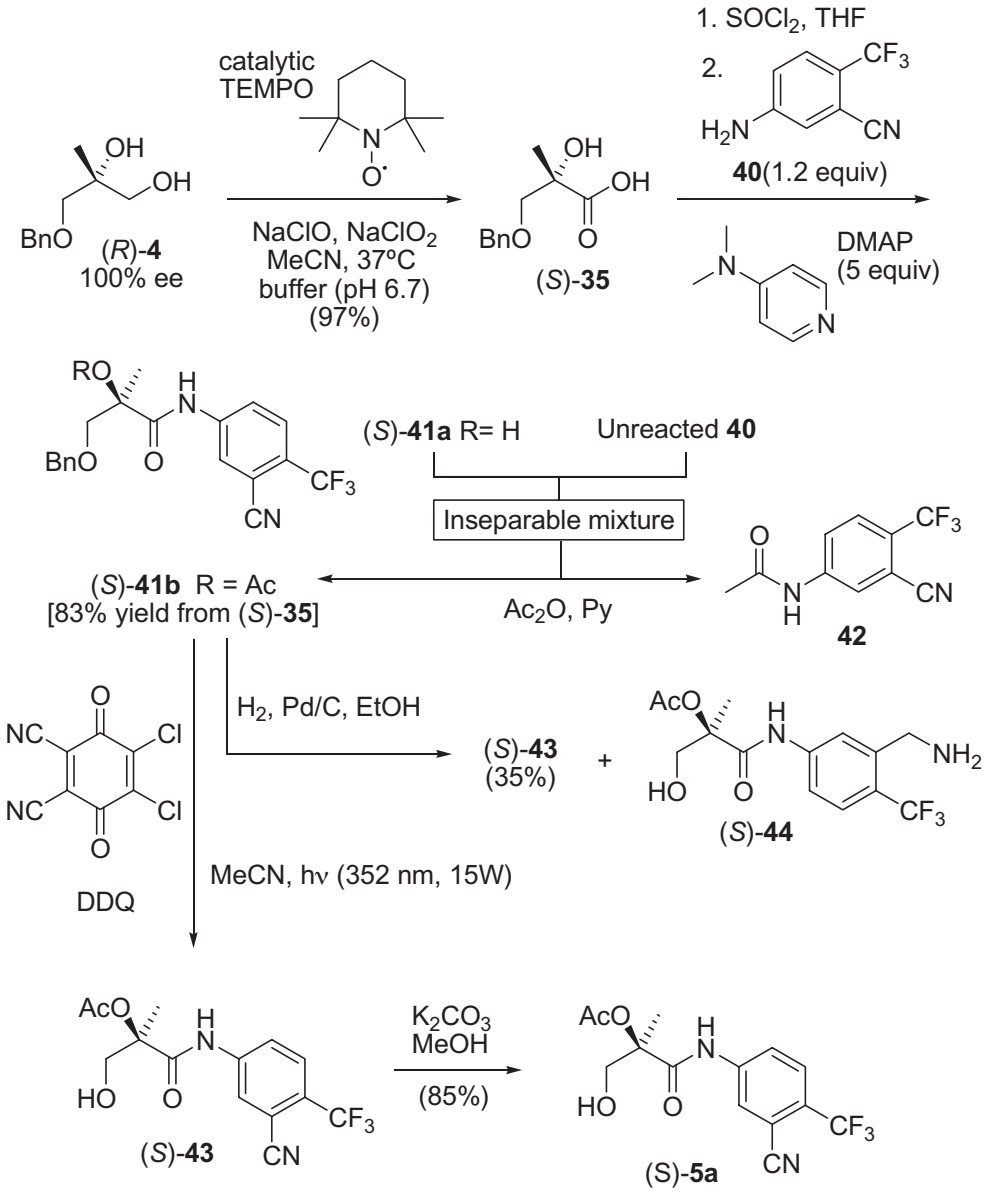

*Figure 17. Conversion of (R)-diol (**4**) into (S)-anilide (**5a**).*

required separation: crude (*S*)-α-hydroxy anilide (**41a**) contaminated with aniline (**40**) was treated with Ac_2O in Py to afford a mixture of (*S*)-acetate (**41b**) and acetanilide (**42**). The mixture was then chromatographed on a short pad of silica gel to give pure (*S*)-acetate (**41b**) in 83% overall yield from (*S*)-**35**.

To deprotect the *O*-benzyl group from (*S*)-**41b**, catalytic hydrogenolysis (H_2, Pd/C, EtOH) was attempted, but the desired (*S*)-alcohol (**43**) was obtained in a poor yield of 35% due to the cyano group of (*S*)-acetate (**41b**) suffering from hydrogenation to (*S*)-benzylamine (**44**) (Fujino *et al*., 2007). Thus, removal of the *O*-benzyl group in question was explored under oxidative conditions. When (*S*)-benzyl ether (**41b**) was treated with 2, 3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 1.5 equiv) in MeCN under UV irradiation (352 nm, 15W) (Fujino *et al*., 2007; Rahim *et al*., 2005), the *O*-debenzylation proceeded smoothly without affecting other functional groups to give (*S*)-**43** in 85% yield (for other oxidative conditions to remove *O*-benzyl protection, see: Adinolfi *et al*., 1999; 2000). Finally, K_2CO_3-mediated methanolysis completed the synthesis of (*S*)-anilide (**5a**), delivering it in 85% yield (Fujino *et al*., 2007).

CONCLUSION

The ultimate objectives of contemporary process chemistry should be defined in two ways: one is to maximize sustainability with little, if any, resort to petroleum, a non-renewable resource on earth. The other is to minimize E-factor, a ratio in weight of by-products/main products and thereby to reduce wastes in fine chemicals manufacturing, in particular (Anastas *et al*., 1994).

From a viewpoint of synthetic chemistry, an efficacious way to attain such goals is to streamline manufacturing processes as a whole by the strategic use of chemo- and biocatalysis as exemplified by the two case studies discussed above: (1) production of (*S*)-ECH (**1b**) by Jacobsen's HKR (Aouni *et al*., 2004; Larrow *et al*., 2003) and tactical application of (*S*)-ECH (**1b**) to the enantiocontrolled synthesis of (*S*)-3-hydroxytetradecanoic acid (**2**) (Ikunaka *et al*., 1999); and (2) enantioconvergent preparation of (*R*)-3-benzyloxy-2-methylpropane-1,2-diol (**4**) from *O*-benzyl (±)-methylglycidol (**1c**) using the enantiocomplementary hydrolytic action of the *B. subtilis* epoxide hydrolase (BSEH) followed by H_2SO_4 and the ensuing conversion of (*R*)-**4** into (*S*)-anilide (**5a**), an advanced intermediate of (*R*)-bicultamide (**5b**) (Fujino *et al*., 2007).

Besides such development effort to reduce E-factor directly, the following two measures may well be taken to make manufacturing processes greener: (1) to use to a full advantage chemicals available from renewable feedstocks, such as palm oils, starch and cellulose (Draths *et al*., 1994); and (2) to improve manufacturing efficiency by changing batchwise processes to continuous ones with the help of microreactors, for instance (Mason *et al*., 2007).

ACKNOWLEDGEMENT

Thanks are due to all who were involved in the work discussed above: Dr K Otsuka, Mr S Kamiyama, Ms C Yamamoto, Ms T Umezato, Mr K Matsuyama, D H Yamaguchi, Mr N Shirasaka, and Ms A Sakoda each at Research & Development Centre, Nagase & Co., Ltd. and Ms A Fujino, Mr M Asano, Ms R Obata, and Dr S Nishiyama each at Department of Chemistry, Keio University.

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