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The scope and limitation of the regio- and enantioselective hydrolysis of aliphatic epoxides using *Bacillus subtilis* epoxide hydrolase, and exploration toward chirally differentiated tris(hydroxymethyl)methanol

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ABSTRACT

The substrate specificity of an engineered *Bacillus subtilis* epoxide hydrolase, which so far had shown high activity and enantioselectivity with 1-benzyloxymethyl-1-methyloxirane, has been studied by altering the methyl substituent into hydrogen, oxygen-containing functionalities, and unsaturated homologs. High enantioselectivity ($E = 44$) was observed with 1-benzyloxymethyl-1-vinyloxirane with a proper catalytic activity. The elaboration of the reaction conditions and work-up procedures enabled a preparative-scale kinetic resolution, to give (*R*)-2-benzyloxymethyl-3-butene-1,2-diol and its antipodal (*R*)-epoxide in high ees.

1. Introduction

Recent extensive studies on epoxide hydrolases (EH) of various microbial origin promote new robust entries for providing enantiomerically enriched epoxides themselves and the corresponding diols which would serve as the starting materials and intermediates for medicines and many biologically active substances. We have investigated a recombinant enzyme from *Bacillus subtilis* (BSEH), which hydrolyzes racemic epoxide **1** in a highly enantioselective manner ($E = 73$).[1]

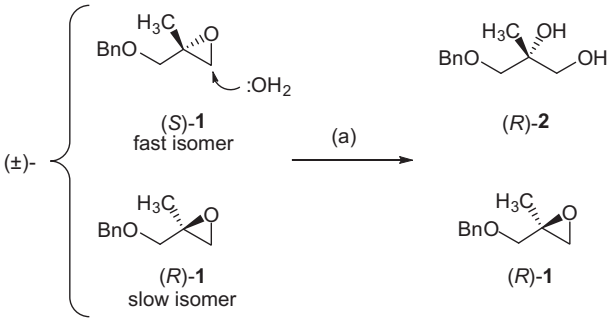
In this epoxide, (*S*)-**1** is more reactive than the (*R*)-isomer and diol (*R*)-**2** is produced with a total retention of absolute configuration at the tertiary stereogenic center, due to the attack of a nucleophile such as water at the less hindered terminal position (Scheme 1). The products, enantiomerically pure epoxide (*R*)-**1** and antipodal diol (*R*)-**2**, were used as the starting materials for the synthesis of (*R*)-bicalutamide and taurospongin A.[1]

One of the most important aspects for any catalyst is its scope and limitation. To clarify, we prepared several aliphatic epoxides **3**–**8** (Fig. 1) by altering the substituents on the oxirane ring and/or the protective group on the hydroxymethyl group, and describe the substrate specificity study in the BSEH-catalyzed hydrolysis.

2. Results and discussion

2.1. Attempts at the kinetic resolution of secondary epoxides

The source of the present epoxide hydrolase was found in *B. subtilis* by screening microorganisms which carry out the hydrolysis on the specific substrate **1**. This enzyme works even at high concentration [1.0 M of (±)-**1**] on the (*S*)-isomer, and is weakly inhibited by (*R*)-**1**, the antipode of the proper substrate (*S*)-**1**.



Scheme 1. Enzyme-catalyzed hydrolysis of racemic epoxide **1**. Reagents and conditions: (a) *Bacillus subtilis* epoxide hydrolase (BSEH), 30 °C, 7 d, 51% conv., $E = 73$.

Our first examination was the BSEH-catalyzed hydrolysis of the less hindered secondary epoxides **3**–**5**, by substituting the methyl group with hydrogen in original substrate **1**. Epoxides (±)-**3**–**5** were subjected to the reaction with harvested whole cells of *B. subtilis* expressing epoxide hydrolases (Tamy 2 strain). We were very surprised to see that the enantioselectivity was quite low when compared with **1**, with only a small change in the structure between **1** and **3**–**5**.

The order of susceptibility of the enzyme-catalyzed hydrolysis for each substrate is summarized in Table 1. It is reasonable to state that the rate of hydrolysis of the sterically less hindered (*R*)-**3** (Scheme 2) was higher than that of (*R*)-**1**, because of the decrease in steric hindrance (entries 4 and 6). In contrast, it was

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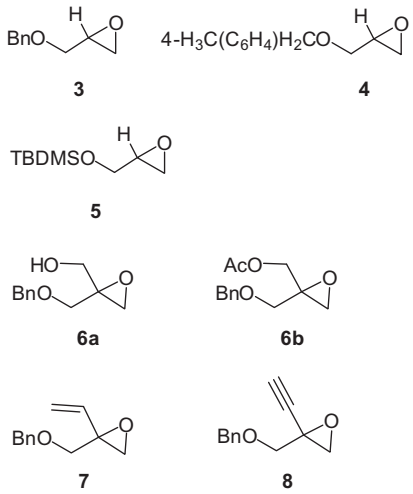
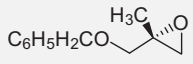
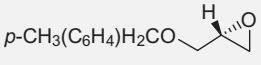
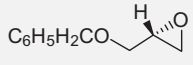
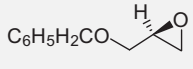
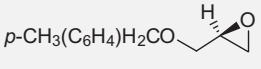
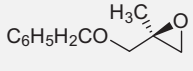


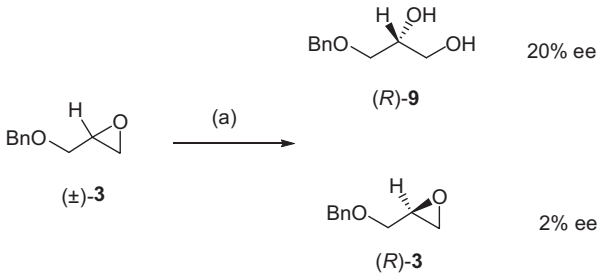
Figure 1. Seven designed substrates as probes for the analysis of the reaction pathway and enantioselectivity.

interesting to note that the rate of hydrolysis of the sterically less hindered (*S*)-**3** (fast isomer) was lower than that of the original tertiary epoxide (*S*)-**1** (entries 1 and 3), which was confirmed by an independent experiment in which (*S*)- or (*R*)-**3** were separately incubated with BSEH. This observation would be ascribable to the 'mobility' of the substrates when they occupied the catalytic site of BSEH. In the case of the 'fast' enantiomer (*S*)-**3**, its sterically less hindered property may help the terminal oxirane ring to 'escape' from the aspartate residue which is responsible for the hydrolysis. On the other hand, even in the case of slow isomer (*R*)-**3**, the terminus can meet the aforementioned catalytic site, through the flexible nature of the substrate.

Table 1
The order of susceptibility of enzyme-catalyzed hydrolysis

Entry	Substrate	Relative rate[a]	*E*-value[b]
1	 (*S*)-**1**	73	73
2	 (*S*)-**4**	20	3.5
3	 (*S*)-**3**	8.6	1.5
4	 (*R*)-**3**	5.7	—
5	 (*R*)-**4**	5.7	—
6	 (*R*)-**1**	1	—

[a] Based on the progress of the reaction and enantioselectivity for racemic substrates **1**, **3**, and **4**.
[b] Calculated for substrates (*S*)-**1**, **3**, and **4** over their enantiomers.



Scheme 2. Enzyme-catalyzed hydrolysis of racemic epoxide **3**. Reagents and conditions: (a) BSEH, 30 °C, 6 d, 10% conv., *E* = 1.5.

A slight modification at the opposite end of the reactive site in (*S*)- and (*R*)-**4**, where a CH_3 group was attached on the *p*-position of the benzyl protective group brought about an increase in the reaction rate for (*S*)-**4** (entries 2 and 3). Disappointingly, the difference in the reaction rates between the enantiomers was still as low as *E* = 3.5 (entries 2 and 5). Finally, both enantiomers of bulky substrates, (*S*)- and (*R*)-**5**, with a TBDMS instead of a benzyl group were very poor toward our BSEH. In summary, our attempts for the enantioselective hydrolysis of the secondary epoxides bearing a similar aliphatic skeleton to the original tertiary epoxide were unsuccessful.

2.2. Tertiary epoxides with further advanced functionalities

The above insufficient results prompted us to design new tertiary epoxides with further advanced functionalities in order to consider new approaches to chirally differentiated tris(hydroxymethyl)methanol and synthetic equivalents **10b**, **11,** and **12** (Fig. 2). These diols would be derived by the enantioselective hydrolysis with *B. subtilis* on epoxides **6b** (for **10b**), **7** (for **11**), and **8** (for **12**). All the substrates are tertiary epoxides and have certain advanced functional group introduced on the methyl group of **1**.

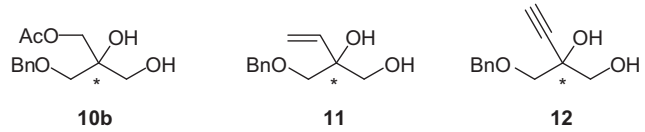
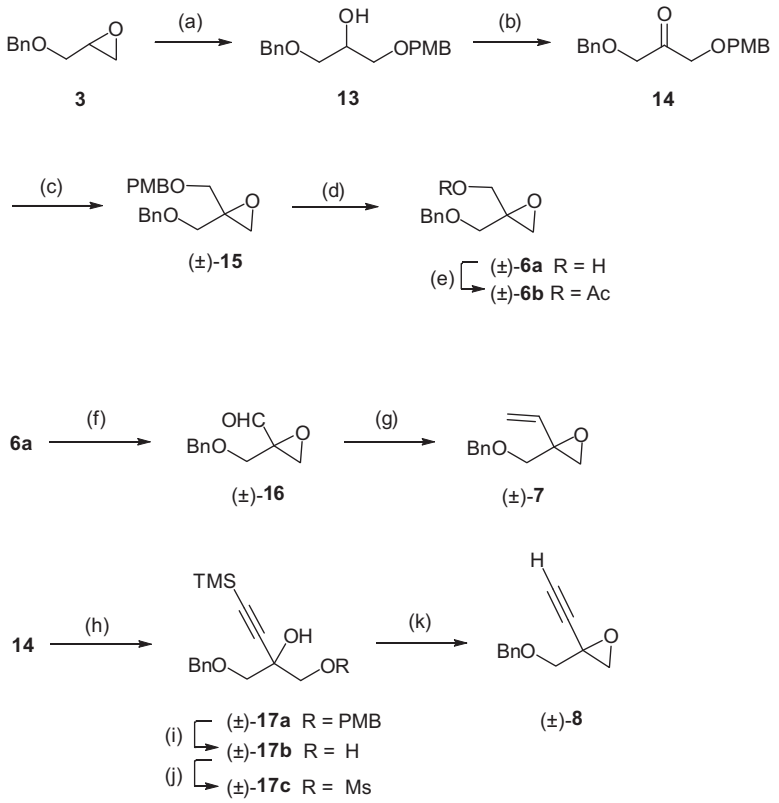


Figure 2. Tris(hydroxymethyl)methanol equivalents to be obtained by enzyme-catalyzed hydrolysis.

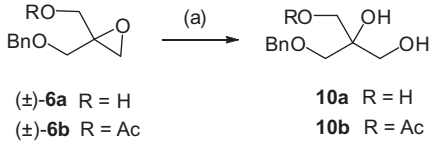
The preparation of the racemic forms of epoxides **6**–**8** is shown in Scheme 3. The key intermediate was one of the substrates itself, **6a**. Starting from benzyl glycidyl ether **3**,[2] the ring opening reaction with *p*-methoxybenzyl alcohol and the subsequent 2-azaadamantane-*N*-oxyl (AZADO) oxidation[3] of the resulting **13** provided an unsymmetrically protected dihydroxyacetone **14** in 73% yield. Although the Wittig reaction of ketone **14** followed by an epoxidation did not proceed efficiently, epoxide ring formation with a concomitant one carbon elongation was achieved by the action of dimethyloxosulfonium methylide.[4] The PMB ether (±)-**15** was treated with DDQ under neutral conditions to give the requisite alcohol (±)-**6a** in 91% yield. Acetylation and Parikh–Doering oxidation[5] produced **6b** and **16** in 84 and 90% yield, respectively. Aldehyde **16** was further derived to alkenyl epoxide **7** by Wittig reaction in 92% yield. Toward the alkynyl epoxide **8**, the first step of Corey–Fuchs acetylene formation, that is, the formation of the terminal dibromoalkene was successful by the action of PPh_3 and CBr_4. The next step, dehydrobromination under basic conditions did not provide any satisfactory results. We then switched to an alternative route; the introduction of a two carbon acetylene unit prior



Scheme 3. Preparation of racemic epoxides **6**–**8**. Reagents and conditions: (a) PMBOH, 40% NaOH aq, THF, 50 °C, 43 h; (b) 1-Me-AZADO, KBr, NaClO, CH_2Cl_2, satd $NaHCO_3$ aq, 0 °C, 30 min (73% over two steps); (c) trimethylsulfoxonium iodide, *n*-BuLi, DMSO, rt, 25 min (93%); (d) DDQ, CH_2Cl_2, 0.1 M phosphate buffer (pH 7.0), rt, 2 h (91%); (e) Ac_2O, pyridine, rt, 45 min (87%); (f) sulfur trioxide-pyridine complex, Et_3N, DMSO, CH_2Cl_2, 0 °C, 2 h (90%); (g) methyltriphenylphosphonium bromide, *t*-BuOK, THF, 0 °C, 15 min (92%), (h) trimethylsilylacetylene, *n*-BuLi, THF, −78 to 0 °C, 1.5 h (97%); (i) CAN, CH_3CN, H_2O, rt, 3 h (94%); (j) MsCl, Et_3N, CH_2Cl_2, −20 to 0 °C, 1 h; (k) K_2CO_3, MeOH, rt, 30 min (70% over two steps).

to the epoxide ring formation. Ketone **14** was treated with lithium trimethylsilylacetylide to give alkyne **17a**. The selective removal of the PMB ether[6] followed by mesylation on the primary alcohol of **17b** and cyclization furnished the desired epoxide **8** in 66% yield from **17a**.

Our first attempts were the incubation of the hydroxymethylated epoxide (±)-**6a** and the corresponding acetate **6b** (Scheme 4). The enzyme-catalyzed reaction, however, was quite slow.



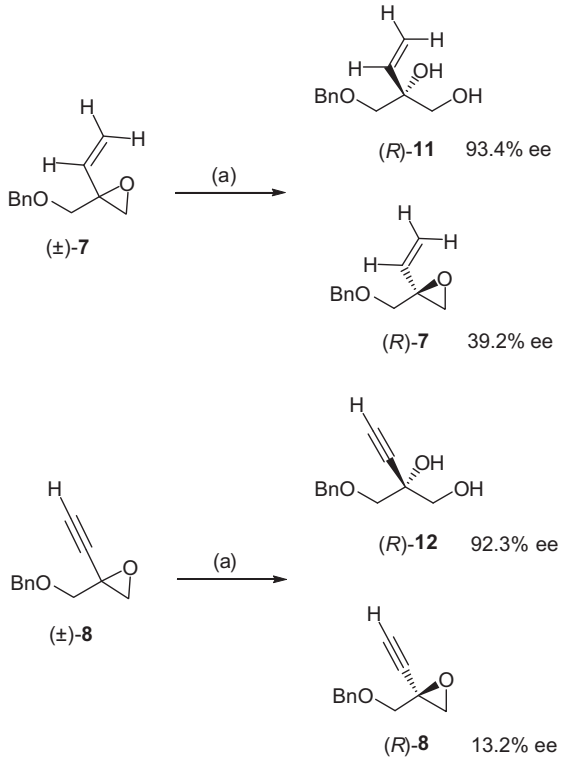
Scheme 4. Enzyme-catalyzed hydrolysis of (±)-**6a** and **6b**. Reagents and conditions: (a) *Bacillus subtilis*, 30 °C, 4 d for (±)-**6a** and 7 d for (±)-**6b**.

The relative activity of **6a** toward BSEH was as low as 7% when compared with **1**. The hydrolysis was very sluggish and after prolonged incubation, the hydrolyzed product **10a** was obtained in as low as 5.1% yield. We realized that a spontaneous, non-enzymatic hydrolysis competed even in the neutral buffer solution. By subtracting the non-enzymatic effect, which was estimated from an independent 'blank' experiment the enantioselectivity of the 'net' enzyme-catalyzed hydrolysis was evaluated; $E = 3$ at 6% conversion. Attempts to apply both acetate **6b** and aldehyde **16** resulted in worse, or almost no reaction.

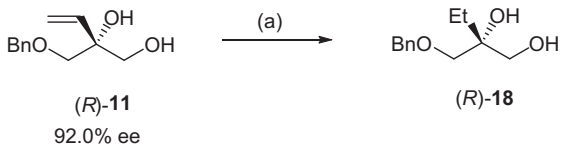
Reetz et al. demonstrated that the rate of the reaction catalyzed by epoxide hydrolase depends upon the proximity between catalytic residues,[7] for example, aspartate and the molecular substrate. Insertion of the methylene group at the opposite or inside position on the secondary epoxide (*S*)-**3**, 'pushes' the terminal of the substrate molecule closer to the catalytic residue, to give (*S*)-**4** and (*S*)-**1** higher reactivity. We expected further enhancement of the reactivity by the insertion of –O– and $–OCOCH_2–$ groups between the methyl C–H bond in (*S*)-**1**, but the results were disappointing. We assumed that some hydrogen bond formation between polar functional groups and the enzyme protein makes the molecules far removed from the catalytic site, in the case of **6a**, **6b,** and **16**.

The above hypothesis was partially correct as was shown by the experiment when the hydrophobic substrates **7** and **8** were applied. In these cases, the enzyme-catalyzed hydrolysis was satisfactory, and the reactions proceeded in enantioselective manners as shown in Scheme 5.

Under the same conditions where the conversion was 51% ($E = 73$) for (±)-**1a**, the hydrolysis proceeded in 29.6% conversion for (±)-**7** ($E = 44$) and in 12.5% for (±)-**8** ($E = 28$). Although the sterically less hindered alkynyl epoxide **8** was anticipated to show a higher reactivity than that of alkenyl epoxide **7**, the hydrolysis of **7** proceeded faster than that of **8**. These results indicated that the vinyl group could reduce steric repulsion between the enzyme protein due to C–C bond rotation compared with a linear and rigid alkynyl group. The absolute configuration of diol **11** from (±)-**7** was determined to be (*R*), after conversion to the saturated diol **18** by chemoselective diimide reduction of the double bond (Scheme 6).[8] The resulting diol **18** showed a negative sign for the specific rotation, whose (*R*)-stereochemistry has been established.[9] This transformation provides an alternative path for the BSEH-catalyzed direct hydrolysis to optically active **18** with an ethyl substituent. The use



Scheme 5. Enzyme-catalyzed hydrolysis of (±)-**7** and (±)-**8**. Reagents and conditions: (a) BSEH, 30 °C; 4 d for (±)-**7**, 29.6% conv., $E = 43.8$; 7 d for (±)-**8**, 12.5% conv., $E = 28.4$.

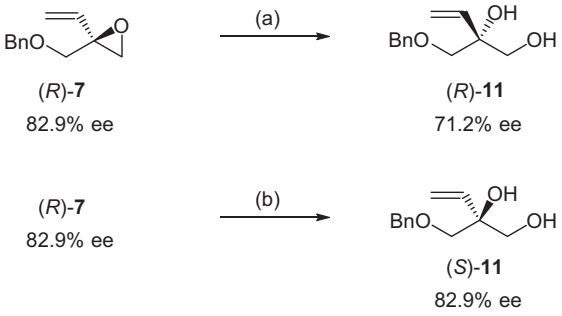


Scheme 6. Determination of the absolute configuration of (*R*)-**11**. Reagents and conditions: (a) hydrazine monohydrate, H_2O_2, EtOH, 0 °C, 3 h (quant.), $[\alpha]_D^{25} = -8.5$.

of (±)-**7** as the substrate for enzyme-catalyzed reaction would be advantageous, in terms of the synthetic utility of product **11** containing a double bond.

An increase in the total amount of enzyme in the hydrolysis of (±)-**7** was straightforward to achieve due to the engineered nature of the enzyme. For example, by doubling the enzyme amount the conversion and ee(S) were improved from 29.6% to 38.0%, and from 39.2% to 56.5%, respectively, without sacrificing either ee(P) (92–93.4% ee) or the *E*-value (42–44). Large-scale hydrolysis also proceeded with good reproducibility. If the conversion reaches near 60%, ee(S) would be over 95%, by a simple calculation under the conditions of $E = 44$.

The repetition of the BSEH-catalyzed kinetic resolution on the recovered epoxide (*R*)-**7** (48.5% ee) enhanced its ee (82.9%), by removing (*S*)-**7**, which had been contaminated. Treatment of (*R*)-**7** (82.9% ee) with diluted H_2SO_4 aq gave (*R*)-**11** with inversion of the stereochemistry and a slight decrease in ee (71.2%) which was suggested by our previous observation with similar substrate **1**.[1] On the other hand, ring opening of (*R*)-**7** with CsOH gave (*S*)-**11** without decreasing its ee (Scheme 7). In all cases, as diols **11** were crystalline, simple recrystallization from cold diethyl ether could increase the ees of both (*R*)- and (*S*)-**11.**



Scheme 7. Ring opening of (*R*)-**7**. Reagents and conditions: (a) H_2SO_4, H_2O, 0 °C, 70 min (39%); (b) $CsOH{\cdot}H_2O$, H_2O, reflux, 12 h (78%).

3. Conclusion

Herein, we have disclosed a new scope and limitation in the *B. subtilis*-catalyzed enantioselective hydrolysis. For the scope, probing the enzyme catalytic site of *B. subtilis* by changing the structure of substituents, an appropriate substrate bearing an advanced functional group was elaborated. (*R*)-1-Benzyloxymethyl-1-vinyloxirane **7** and (*R*)-2-benzyloxymethyl-3-butene-1,2-diol **11** became available in high ees; both of them are promising equivalents for chirally differentiated tris(hydroxymethyl)methanol by proper synthetic transformations, and the efforts to apply them as the starting materials for the synthesis of bioactive natural products will be reported in due course.

4. Experimental

4.1. Materials and methods

Merck Silica Gel 60 F_{254} thin-layer plates (1.05744, 0.5 mm thickness) and Silica Gel 60 (spherical and neutral; 100–210 μm, 37,558–84) from Kanto Chemical Co. were used for preparative thin-layer chromatography and column chromatography, respectively. Preparation of wet cells of *B. subtilis* Tamy 2 was according to the reported procedure.[1]

4.2. Analytical methods

IR spectra were measured as ATR on a Jeol FT-IR SPX60 spectrometer. The ^1H NMR and ^{13}C NMR spectra were measured in $CDCl_3$ or acetone-d_6 at 400 and 100 MHz on a VARIAN 400-MR spectrometer or 600 and 150 MHz on a Jeol JNM-ECP 600 spectrometer. High resolution mass spectra were recorded on a Jeol JMS-700 MStation spectrometer. HPLC data were recorded on Jasco MD-2010 multi-channel detectors and SHIMADZU SPD-20 Å diode array detector. Optical rotation values were recorded on a Jasco P-1010 polarimeter.

4.3. 1-Benzyloxymethyloxirane 3

A mixture of NaOH aq solution (40% w/w, 60 mL), benzyl alcohol (5.00 g, 46.2 mmol), and tetrabutylammonium bromide (745 mg, 2.31 mmol) was vigorously stirred at room temperature and then cooled to 0 °C. To the mixture was added epichlorohydrin (17.1 g, 185 mmol) over 20 min. After stirring for 9 h, the reaction mixture was poured into ice-water (30 mL). The organic materials were extracted with Et_2O twice. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by distillation under reduced pressure to afford **3** (6.90 g, 91%) as a colorless oil, bp

75 °C/11 Torr. Its NMR spectra were identical to that reported previously.[2]

4.4. *B. subtilis*-catalyzed hydrolysis of (±)-3

To epoxide (±)-**3** (21.9 mg, 133 μmol) was added *B. subtilis*, [0.4 g (wet cell weight)/mL, 60 μL] and glycerol (30 μL) and stirred for 6 days at 30 °C. The reaction mixture was saturated with NaCl and diluted with EtOAc. The organic phase was separated and concentrated in vacuo. HPLC [Daicel Chiralcel OD-H, 0.46 cm × 25 cm; hexane/*i*-PrOH (10:1), 0.5 mL/min; detected at 213 nm], t_R (min) = 15.6 [(*S*)-**3**, 43.9%], 17.7 [(*R*)-**3,** 46.1%], 29.2 [(*R*)-**9,** 6.0%], 37.1 [(*S*)-**9,** 4.0%]. The NMR spectra of **3**[2] and **9**[10] were identical to that reported previously.

4.5. *B. subtilis*-catalyzed hydrolysis of (±)-1-(4-methylbenzyloxymethyl)oxirane 4

In a similar manner as described for the hydrolysis of (±)-**3**, (±)-**4**[11] (22.5 mg, 126 μmol) was treated with *B. subtilis* and glycerol (30 μL) for 6 days. The reaction mixture was saturated with NaCl and diluted with EtOAc. The organic phase was concentrated in vacuo and purified by preparative TLC (hexane/EtOAc = 4/3) to afford **4** and 3-(4-methylbenzyloxy)-1,2-propanediol **19**. (*R*)-**4**: HPLC [OD-H; hexane/*i*-PrOH (10:1), 0.5 mL/min, detected at 213 nm]: t_R (min) = 14.6 [(*S*)-, 44.3%], 16.0 [(*R*)-, 55.7%]; 11.4% ee. $[\alpha]_D^{26} = +1.0$ (*c* 0.30, EtOH) {lit.[12] $[\alpha]_D^{25} = +8.6$ (*c* 0.40, EtOH), for (*R*)-**3**}. The NMR spectrum was identical with that of (±)-**4**. (*R*)-**19**: HPLC [OD-H; hexane/*i*-PrOH (10:1), 0.5 mL/min; detected at 212 nm]: t_R (min) = 25.5 [(*R*)-, 75.7%], 41.5 [(*S*)-, 24.3%]; 51.4% ee. $[\alpha]_D^{26} = -1.6$ (*c* 0.30, $CHCl_3$) {lit.[10] $[\alpha]_D^{21} = -1.0$ (*c* 1.02, $CHCl_3$), for (*R*)-**9**}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.25 (1H, br s), 2.33 (3H, s), 2.72 (1H, br s), 3.48 (1H, ddd, *J* = 0.6, 6.3, 9.6 Hz), 3.51 (1H, ddd, *J* = 0.6, 3.9, 9.6 Hz), 3.61 (1H, br dd, *J* = 4.9, 11.4 Hz), 3.67 (1H, br d, *J* = 11.4 Hz), 3.86 (1H, br s), 4.49 (2H, s), 7.14 (2H, d, *J* = 8.0 Hz), 7.19 (2H, d, *J* = 8.0 Hz).

4.6. 1-Benzyloxy-3-(4-methoxybenzyloxy)propan-2-one 14

A mixture of NaOH aq solution (40% w/w, 15 mL), THF (5 mL), *p*-methoxybenzyl alcohol (2.97 mL, 24.0 mmol), **3** (2.62 g, 16.0 mmol), and tetrabutylammonium bromide (257 mg, 0.80 mmol) was vigorously stirred at 50 °C for 43 h. The residue was purified by silica gel column chromatography (60 g) with hexane/EtOAc (10:1–3:1) to afford a 1:1 mixture of **13** and *p*-methoxybenzyl alcohol (5.82 g) as a yellow oil.

To a solution of the above mixture (5.82 g) in CH_2Cl_2 (32 mL) was added saturated $NaHCO_3$ aq solution (16 mL), KBr (2.29 g, 19.2 mmol), 1-methyl-2-aza-adamantan-*N*-oxyl (3.2 mg, 0.02 mmol), and the mixture was vigorously stirred at 0 °C. To the mixture was added 5% NaClO aq solution (60 mL, 48.1 mmol) at 0 °C and vigorously stirred for 30 min. The reaction mixture was quenched with $Na_2S_2O_3$ aq solution and extracted with EtOAc three times. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (300 g) with hexane/EtOAc (1:0–3:1) to afford **14** (3.51 g, 73% over two steps) as a colorless oil. Its NMR spectra were identical with that reported previously.[13]

4.7. (±)-1-Benzyloxymethyl-1-(4-methoxybenzyloxymethyl)-oxirane 15

To a solution of trimethylsulfoxonium iodide (3.08 g, 14.0 mmol) in anhydrous DMSO (30 mL) was added *n*-butyllithium (4.90 mL, 12.8 mmol, 2.6 M in hexane) at room temperature under an argon atmosphere and stirred for 30 min. To the mixture was added a solution of **14** (3.51 g, 11.7 mmol) in anhydrous DMSO (30 mL) via cannula and stirred for 25 min. The reaction was quenched with saturated NH_4Cl aq solution and the organic materials were extracted with EtOAc three times. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by silica gel column chromatography (80 g) with hexane-EtOAc (5:1) to afford **15** (3.40 g, 93%) as a colorless oil. Its NMR spectra were identical with that reported previously.[14]

4.8. (±)-1-Benzyloxymethyl-1-hydroxymethyloxirane 6a

To a solution of **15** (11.6 g, 36.9 mmol) in CH_2Cl_2 (50 mL) and 0.1 M phosphate buffer (pH 7.0, 50 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (10.9 g, 47.9 mmol) and vigorously stirred at room temperature. After 3 h, the reaction was quenched with $Na_2S_2O_3$ aq solution and organic materials were extracted with $CHCl_3$ twice. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by silica gel column chromatography (200 g) with hexane/EtOAc (10:1–1:3) to afford (±)-**6a** (6.52 g, 91%) as a pale yellow oil. Its NMR spectrum was identical with that reported previously.[15]

4.9. (±)-[1-(Benzyloxymethyl)oxiranyl]methyl acetate 6b

To a solution of (±)-**6a** (51.5 mg, 0.27 mmol) in pyridine (1 mL) was added acetic anhydride (50.2 μL, 0.53 mmol) and the mixture was stirred for 45 min at room temperature. After concentration in vacuo, the residue was purified by silica gel column chromatography (2 g) with hexane/EtOAc (5:1) to afford (±)-**6b** (57.3 mg, 87%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.03 (3H, s), 2.79 (2H, s), 2.79 (1H, d, *J* = 11.4 Hz), 2.80 (1H, d, *J* = 11.4 Hz), 4.17 (1H, d, *J* = 12.1 Hz), 4.33 (1H, d, *J* = 12.1 Hz), 4.53 (1H, d, *J* = 11.9 Hz), 4.56 (1H, d, *J* = 11.9 Hz), 7.27–7.35 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5, 137.6, 128.4, 127.8, 127.7, 73.4, 69.8, 63.9, 56.4, 49.2, 20.7; IR ν_{max} 2861, 1743, 1454, 1367, 1228, 1095, 1037, 736, 698, 601 cm^{-1}. Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 66.21; H, 6.83.

4.10. (±)-1-Benzyloxymethyl-1-formyloxirane 16

To a solution of (±)-**6a** (96.9 mg, 0.50 mmol) in anhydrous DMSO (0.5 mL) and anhydrous CH_2Cl_2 (0.5 mL) were added triethylamine (206 μL, 1.50 mmol) and the sulfur trioxide-pyridine complex (175 mg 1.10 mmol) at 0 °C and stirred for 2 h. The reaction was quenched with saturated $NaHCO_3$ aq solution and the organic materials were extracted with EtOAc. The organic phase was washed with brine and dried over anhydrous Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (5 g) with hexane/EtOAc (1:0–5:1) to afford (±)-**16** (86.5 mg, 90%) as a colorless oil. Its NMR spectra were identical with that reported previously.[14]

4.11. (±)-1-Benzyloxymethyl-1-vinyloxirane 7

To a suspension of methyltriphenylphosphonium bromide (444 mg, 1.24 mmol) in anhydrous THF (2.1 mL) was added potassium *t*-butoxide (92.9 mg, 0.83 mmol) at 0 °C under an argon atmosphere. After stirring for 15 min, to the mixture was added a solution of (±)-**16** (79.6 mg, 0.41 mmol) in anhydrous THF (1.2 mL) via cannula and stirred for 15 min. The reaction was quenched with saturated NH_4Cl aq solution and organic materials were extracted twice with EtOAc. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by silica gel col-

umn chromatography (10 g) with hexane/EtOAc (1:0–5:1) to afford (±)-**7** (37.0 mg, 92%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.69 (1H, d, J = 5.5 Hz), 2.92 (1H, d, J = 5.5 Hz), 3.62 (1H, d, J = 11.4 Hz), 3.74 (1H, d, J = 11.4 Hz), 4.56 (1H, d, J = 11.9 Hz), 4.58 (1H, d, J = 11.9 Hz), 5.26 (1H, dd, J = 1.2, 11.0 Hz), 5.44 (1H, dd, J = 1.2, 17.4 Hz), 5.85 (1H, dd, J = 11.0, 17.4 Hz) 7.26–7.33 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 52.9, 57.5, 71.3, 73.2, 117.4, 127.6, 127.7, 128.4, 134.9, 137.8; IR ν_{max} 3033, 2858, 1452, 1367, 1095, 925, 736, 696 cm^{-1}, HRMS (FAB): calcd for $C_{12}H_{15}O_2$: $[M+H]^+$: 191.1072; found: 191.1065.

4.12. (±)-2-Benzyloxymethyl-3-(4-methoxybenzyloxymethyl)-4-trimethylsilylprop-3-yn-2-ol 17a

To a solution of trimethylsilylacetylene (283 μL, 2.00 mmol) in anhydrous THF (5 mL) was added *n*-butyllithium (0.96 mL, 1.50 mmol, 1.6 M in hexane) at −78 °C under an argon atmosphere and stirred for 30 min. To the mixture was added a solution of **14** (300 mg, 1.00 mmol) in anhydrous THF (5 mL) via cannula at −78 °C. After stirring for 1 h, the reaction mixture was warmed to 0 °C and stirred for 30 min. The reaction was quenched with saturated NH_4Cl aq solution and the organic materials were extracted with EtOAc. The organic phase was washed with brine and dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by silica gel column chromatography (20 g) with hexane/EtOAc (1:0–2:1) to afford **17a** (386 mg, 97%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.16 (9H, s), 1.51 (1H, s), 3.56 (1H, d, J = 9.6 Hz), 3.58 (1H, d, J = 9.6 Hz), 3.59 (1H, d, J = 9.6 Hz), 3.61 (1H, d, J = 9.6 Hz), 4.55 (2H, s), 4.62 (2H, s), 6.84 (2H, d, J = 8.6 Hz), 7.23 (2H, d, J = 8.6 Hz), 7.26–7.32 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ = −0.17, 55.2, 70.2, 73.2, 73.4, 73.5, 89.9, 105.2, 113.7, 127.5, 127.6, 128.3, 129.3, 130.0, 137.9, 159.2; IR ν_{max} 3450, 2956, 2904, 2861, 1737, 1612, 1585, 1511, 1454, 1247, 1172, 1091, 1031, 917, 840, 757, 736, 698 cm^{-1}; HRMS (FAB): calcd for $C_{23}H_{30}O_4SiNa$: $[M+Na]^+$: 421.1811; found: 421.1796.

4.13. (±)-2-Benzyloxymethyl-4-trimethylsilyl-but-3-yne-1,2-diol 17b

To a solution of **17a** (6.4 mg, 16 μmol) in CH_3CN:H_2O (9:1) (0.1 mL) was added ceric ammonium nitrate (20.3 mg, 36.9 μmol) and the reaction was vigorously stirred at room temperature. After 3 h, the reaction mixture was diluted with EtOAc (7 mL) and quenched with saturated $NaHCO_3$ aq solution. The organic materials were extracted with EtOAc. The organic phase was washed with brine and dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by silica gel column chromatography (1 g) with hexane/EtOAc (1:0–3:1) to afford **17b** (4.2 mg, 94%) as a colorless solid, mp 88.0–88.5 °C: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.15 (9H, s), 3.61 (1H, d, J = 9.6 Hz), 3.67 (1H, d, J = 9.6 Hz), 3.69 (2H, s), 4.61 (1H, d, J = 12.3 Hz), 4.63 (1H, d, J = 12.3 Hz), 7.24–7.36 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ = −0.17, 67.2, 70.7, 73.7, 74.2, 90.9, 103.9, 127.7, 127.9, 128.5, 137.6; IR ν_{max} 3398, 3222, 2919, 2861, 1403, 1247, 1045, 840 cm^{-1}; HRMS (FAB): calcd for $C_{15}H_{22}O_3SiNa$: $[M+Na]^+$: 301.1236; found: 301.1221.

4.14. (±)-1-Benzyloxymethyl-1-ethynyloxirane 8

To a solution of **17b** (88.1 mg, 0.32 mmol) and triethylamine (88.4 μL, 0.63 mmol) in anhydrous CH_2Cl_2 (1.6 mL) was added methanesulfonyl chloride (29.4 μL, 0.38 mmol) at −20 °C under an argon atmosphere. After stirring for 30 min, the reaction mixture was warmed to 0 °C and stirred for 30 min. The reaction was quenched with saturated NH_4Cl aq solution and the organic materials were extracted with EtOAc twice. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4, and then concentrated in vacuo. This crude **17c** was employed for the next step without further purification.

To a solution of the above crude **17c** (125 mg) in MeOH (3.2 mL) was added potassium carbonate (87.5 mg, 0.63 mmol) and vigorously stirred for 30 min at room temperature. The reaction was quenched with saturated NH_4Cl aq solution and organic materials were extracted with EtOAc twice. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by silica gel column chromatography (2 g) with hexane/EtOAc (1:0–10:1) to afford (±)-**8** (41.4 mg, 70% over two steps) as a yellow oil. Its NMR spectra were identical with that reported previously.[16]

4.15. *B. subtilis*-catalyzed hydrolysis of (±)-7

In a similar manner as described for the hydrolysis of (±)-**3**, (±)-**7** (34.2 mg, 0.18 mmol) were treated with *B. subtilis*, [0.5 g (wet cell weight)/mL, 139 μL] and glycerol (43 μL) for 4 days, to give (*R*)-**7** (18.8 mg, 55%) as a colorless oil along with (*R*)-**11** (10.9 mg, 29%) as a colorless solid.

(*R*)-**7**: HPLC analysis [OD-H; hexane/*i*-PrOH (90:1), 0.5 mL/min, detected at 207 nm], t_R (min) = 22.8 [(*S*)-, 30.4%], 25.6 [(*R*)-, 69.6%]; 39.2% ee. Its NMR spectrum was identical with that of (±)-**7**.

(*R*)-**11**: HPLC [AD-H; hexane/*i*-PrOH (15:1), 0.5 mL/min, detected at 210 nm], t_R (min) = 30.9 [(*R*)-, 96.7%], 34.3 [(*S*)-, 3.3%]; 93.4% ee. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.32 (1H, br s), 3.47 (1H, d, J = 9.4 Hz), 3.50 (1H, d, J = 11.2 Hz), 3.58 (1H, d, J = 9.4 Hz), 3.66 (1H, d, J = 11.2 Hz), 4.53 (1H, d, J = 12.1 Hz), 4.57 (1H, d, J = 12.1 Hz), 5.24 (1H, dd, J = 1.4, 10.9 Hz), 5.41 (1H, dd, J = 1.4, 17.4 Hz), 5.82 (1H, dd, J = 10.9, 17.4 Hz) 7.24–7.36 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 67.2, 73.8, 74.6, 74.7, 116.1, 127.7, 127.9, 128.5, 137.6, 138.0; IR ν_{max} 3419, 2925, 2861, 1454, 1089, 1076, 1043, 1027, 995, 925, 732, 696 cm^{-1}. Anal. Calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 68.92; H, 7.80.

For the large-scale hydrolysis of (±)-**7**, *B. subtilis* [1.0 g (wet cell weight)/mL, 19.8 mL] and glycerol (6.1 mL) were added to epoxide (±)-**7** (4.87 g, 25.6 mmol). The broth was centrifuged (3000 rpm) and the supernatant was saturated with NaCl and diluted with EtOAc. The mixture was stirred for 1 h and filtered through a pad of Celite. The organic layer of the filtrate was separated and the aqueous layer was further extracted with EtOAc. To the cell debris precipitated by the above centrifuge was added acetone (50 mL). The mixture was sonicated for 15 min and filtered. Both of the EtOAc and the acetone extracts were washed with brine, dried over anhydrous Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (100 g) with hexane/EtOAc (10:1–1:3) to afford (*R*)-**7** (3.00 g, 61.6%, 48.5% ee) as a colorless oil along with (*R*)-**11** (1.97 g, 36.7%, 93.3% ee) as a colorless solid.

The secondary hydrolysis of (*R*)-**7** (2.92 g, 15.3 mmol, 48.5% ee) was preformed with *B. subtilis*, [1.0 g (wet cell weight)/mL, 11.9 mL] and glycerol (3.6 mL) for 4 days, to give (*R*)-**7** (2.21 g, 75.9%, 82.9% ee) as a colorless oil along with (*R*)-**11** (0.72 g, 22.5%, 78.4% ee) as a colorless solid. $[\alpha]_D^{24} = +29$ (*c* 1.12, MeOH) for (*R*)-**7** (82.9% ee).

The hydrolyzed product (1.1 g, 90.8% ee) was recrystallized from diethyl ether at −50 °C to give (*R*)-**11** (192.2 mg, 20% recovery, 97.7% ee); mp 30.0–30.5 °C, $[\alpha]_D^{24} = -28$ (*c* 0.65, CH_2Cl_2).

4.16. *B. subtilis*-catalyzed hydrolysis of (±)-8

In a similar manner as described for the hydrolysis of (±)-**3**, (±)-**8** (31.3 mg, 0.17 mmol) was treated with *B. subtilis*, [0.5 g (wet cell

weight)/mL, 128 μL] and glycerol (40 μL) for 7 days, to give (*R*)-**8** (23.6 mg, 75%) as a colorless oil along with (*R*)-**12** (4.0 mg, 12%) as a colorless solid.

(*R*)-**8**: HPLC analysis [AD-H, hexane/*i*-PrOH (90:1), 0.5 mL/min, detected at 207 nm], t_R (min) = 22.3 [(*R*)-, 56.6%], 26.4 [(*S*)-, 43.4%]; 13.2% ee. The NMR spectrum was identical with that of (±)-**8**.

(*R*)-**12**: HPLC [AS-H, hexane-*i*-PrOH (7:1), 0.5 mL/min, detected at 210 nm], t_R (min) = 27.7 [(*R*)-, 96.1%], 30.9 [(*S*)-, 3.9%]; 92.3% ee. ^{1}H NMR (600 MHz, acetone-d_6): δ = 2.85 (1H, s), 3.61 (1H, d, J = 9.7 Hz), 3.63 (1H, d, J = 9.7 Hz), 3.66 (2H, d, J = 6.6 Hz), 3.88 (1H, t, J = 6.3 Hz), 4.41 (1H, s), 4.62 (1H, s), 7.24–7.41 (5H, m); ^{13}C NMR (150 MHz, acetone-d_6): δ = 67.1, 71.2, 74.0, 74.0, 74.4, 85.9, 128.2, 128.3, 129.0, 139.7; IR ν_{max} 3423, 3259, 2923, 2867, 2362, 2343, 1396, 1253, 1178, 1093, 1045, 1025, 1010, 954 cm^{-1}.

4.17. (*R*)-2-Benzyloxymethyl-butane-1,2-diol 18

To a solution of (*R*)-**11** (30.5 mg, 0.15 mmol, 92.0% ee) in EtOH (1.5 mL) were added hydrazine monohydrate (178 μL, 3.66 mmol) and H_2O_2 (239 μL, 30% w/w in water, 2.34 mmol) at 0 °C. After 3 h, the reaction was quenched with $Na_2S_2O_3$ aq solution and the organic materials were extracted twice with Et_2O. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (1 g) with hexane/EtOAc (2:1) to afford diol (*R*)-**18** (33.7 mg, quant.) as a colorless oil: $[\alpha]_D^{25} = -8.5$ (c 1.07, CH_2Cl_2) {lit.[9] $[\alpha]_D = -2.4$ (c 1.52, CH_2Cl_2)}; The NMR spectrum was identical with that reported previously.[9]

4.18. (*R*)-2-Benzyloxymethyl-3-butene-1,2-diol 11

A mixture of (*R*)-**7** (22.2 mg, 0.12 mmol, 82.9% ee), H_2O (0.7 mL), and conc. H_2SO_4 (53 μL, 0.96 mmol) was vigorously stirred at 0 °C. After stirring for 70 min, the reaction mixture was quenched with saturated $NaHCO_3$ aq solution and the organic materials were extracted twice with EtOAc. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by preparative TLC with hexane/EtOAc (1:2) to afford diol (*R*)-**11** (9.5 mg, 39%, 71.2% ee) as a colorless solid. Its NMR spectrum was identical with that of (*R*)-**11** (as shown in Section 4.15).

4.19. (*S*)-2-Benzyloxymethyl-3-butene-1,2-diol 11

A mixture of (*R*)-**7** (16.1 mg, 0.08 mmol, 82.9% ee), $CsOH{\cdot}H_2O$ (92.3 mg, 0.55 mmol), and H_2O (0.4 mL) was refluxed. After 12 h, the reaction was quenched with saturated NH_4Cl aq solution and the organic materials were extracted twice with Et_2O. The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by silica gel column chromatography (1 g) with hexane/EtOAc (2:1–1:1) to afford diol (*S*)-**11** (13.7 mg, 78%) as a colorless solid; $[\alpha]_D^{23} = +24$ (c 0.65, CH_2Cl_2).

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