Integrated Biocatalytic Synthesis on Gram Scale: The Highly Enantioselective Preparation of Chiral Oxiranes with Styrene Monooxygenase

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Abstract: Enantiopure oxiranes have been prepared on a gram scale by recombinant *E. coli* JM101 (pSPZ10) containing styrene monooxygenase in an emulsion process including facile and efficient downstream processing. This biocatalyst was compared to the chemical catalysts introduced by Jacobsen et al. with respect to its performance for epoxidations of vinyl groups.

Keywords: asymmetric synthesis; emulsion bioprocess; enzyme catalysis; epoxidations; oxygenation

Introduction

Chiral oxiranes are important building blocks for organic synthesis since a broad range of interesting products is accessible via selective nucleophilic attack on the epoxide function, or opening of the epoxide function to yield a non-functionalized, chiral carbon center or an alkene of defined configuration.[1] Much effort has been directed towards the development of suitable catalysts for the synthesis of such building blocks by the direct epoxidation of unfunctionalized hydrocarbons.[2] The best chemical catalysts for the direct epoxidation of olefins developed thus far are transition metal-salen complexes introduced by Jacobsen and coworkers[3] (see Table 4). Limitations of these catalysts are poor catalytic efficiencies for the epoxidation of *trans* and terminal olefins.[4] An interesting alternative is the use of biocatalysts to obtain chiral oxiranes. These methods were reviewed recently[5,6,7] and comprise two general strategies: resolution of a racemic mixture with stereospecific epoxide hydrolases[1] and direct epoxidation of olefins at theoretical quantitative yields using peroxidases or oxygenases.[8,9] Peroxidases use H_2O_2 as an oxidant which makes the reaction independent of cofactors but this results in very limited operational stability.[10,11] Oxygenases use molecular oxygen as oxidant and have considerable potential in asymmetric synthesis. However, examples of processes based on biooxidations are still few,[12] compared to bioreductive[13] or hydrolytic reactions. A major reason for this is the shortage of available methods that result in high product concentrations at high volumetric productivities. In part, this is due to substrate and/or product inhibition of the enzymes used for biooxidations, but the lack of biological enzyme systems that allow high and sustainable reaction rates also limits the application of oxygenases.

The molecular oxygen used by oxygenases is activated only in the active site of an enzyme at a heme or non-heme iron complex, or in flavin monooxygenases as peroxoflavin. This specific formation of activated oxygen species allows highly selective reactions with few by-products formed and little enzyme inactivation. On the other hand, such enzymes are dependent on cofactors as donors of reduction equivalents, mostly reduced NAD(P)H, which necessitates cofactor regeneration solutions for economical applications on a larger scale. Today, this can most easily be achieved by using oxygenases in metabolically active cells, thus allowing a recycling of NAD(P)H via the central cellular metabolism from cheap sources of reduction equivalents like glucose. In order to maximally exploit this regeneration potential, growing cells containing the oxygenase of interest are used as catalysts.[4] Especially in recombinant whole cell catalysts containing high amounts of overexpressed oxygenases, turnover frequencies may not be dependent on the amount of oxygenase present in the cell, but on other cellular parameters.[14] In the present paper we relate activity to the total amount of cells and consider the whole microbial cell as a catalytic unit.

The use of growing microbial cells is mainly limited by the toxicities of reactants for cells. These toxicities

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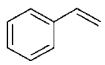
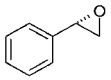
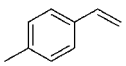
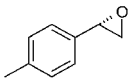
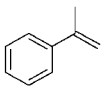
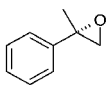
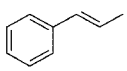
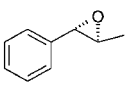
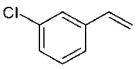
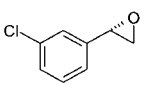
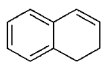
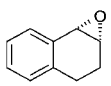
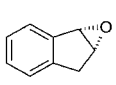
limit not only the spectrum of reactants, but also the concentrations in which they can be applied. One possible solution is the well known application of aqueous/organic reaction mixtures consisting of a buffer system and an apolar organic solvent,[15,16] acting as reservoir for toxic substrates and toxic, water–labile products. Suitable organic solvents are selected based on their toxicity to cells and on favorable partition coefficients for reactants.

Results and Discussion

Reported methods for the isolation of gram amounts of biocatalytically produced epoxides are few. A method for the preparation of *(S)*-styrene oxide from styrene, based on styrene monooxygenase in recombinant *E. coli* and a buffer/dioctyl phthalate reaction mixture was described recently.[17] Here, we characterize the practicability and synthetic application of this method for the preparation of a range of enantiomerically pure oxiranes (Table 1) in gram amounts.

Table 1. Substrates and products of biotransformations using the styrene monooxygenase in *E. coli* JM101 (pSPZ10).

Substrates	Products[a]
1 	1a 
2 	2a 
3 	3a 
4 	4a 
5 	5a 
6 	6a 
7 	7a 

[a] Absolute configuration was shown for **1a** and **7a**. Styrene (**1**), 4-methylstyrene (**2**), α-methylstyrene (**3**), *trans*-β-methylstyrene (**4**), 3-chlorostyrene (**5**), 1,2-dihydronaphthalene (**6**) and indene (**7**) were substrates for the biotransformation. (*S*)-Styrene oxide (**1a**), 4-(*S*)-methylstyrene oxide (**2a**), (*S*)-α-methylstyrene oxide (**3a**), (*S*)-*trans*-β-methylstyrene oxide (**4a**), (*S*)-3-chlorostyrene oxide (**5a**), (*S*)-1,2-dihydronaphthalene oxide (**6a**), and (*1S,2R*)-indene oxide (**7a**) were the corresponding epoxides prepared biocatalytically.

The use of growing recombinant *E. coli* cells as catalysts in organic/aqueous emulsions can be limited by the toxicity of the solvent and the apolar substrate in the organic phase. We therefore tested the growth of *E. coli* JM101 without the plasmid pSPZ10 carrying the styrene monooxygenase expression casette, in the presence of the organic solvent dioctyl phthalate (50% v/v) containing different amounts of substrate (Figure 1).

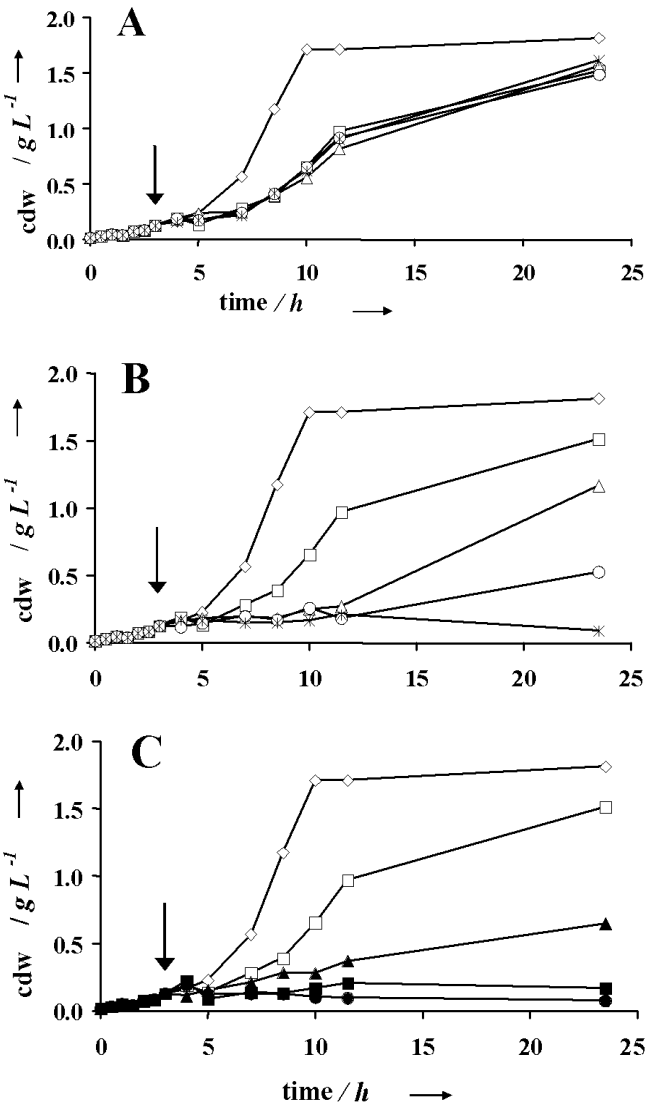


Figure 1. Influence of reaction substrates on the growth rate of *E. coli* JM101. Growth in 20 mL M9 medium, in the absence (◊) or in the presence (□) of additional 20 mL dioctyl phthalate and different amounts of substrate in the apolar phase: (**A**) α-methylstyrene, [1% (Δ), 2% (O), and 5% (*)]; (**B**) 3-chlorostyrene [1% (Δ), 2% (O), and 5% (*)]; (**C**) 4-vinylpyridine [0.1% (▲),0.5% (■), 1% (●)]. The arrow shows the addition of the second phase with or without substrate.

Figure 1 shows that the compounds tested can be divided in three groups: α-methylstyrene present in the organic solvent dioctyl phthalate up to 5% (v/v) does not significantly influence the growth rate of *E. coli* JM101 in shake flasks. The same is true for styrene, β-methylstyrene, 4-methylstyrene, and 1,2-dihydronaphthalene (data not shown). In contrast, increasing amounts of 3-chlorostyrene in the organic phase decrease the growth of *E. coli* JM101. Finally, 4-vinylpyridine inhibited growth of *E. coli* JM101 even at low concentrations in the organic phase and can therefore not be epoxidized efficiently in this system. For this substrate we detected conversion to a putative epoxide with isolated styrene monooxygenase (data not shown). A cell-free biotransformation system based on styrene monooxygenase could therefore give access to an even broader range of chiral oxiranes. In shake flasks, the presence of dioctyl phthalate in the reaction medium resulted in a decrease of the cell growth rate. We attribute this to an artifact in shake flasks (e.g., oxygen limitation due to poor mixing) as this solvent has no effect on *E. coli* JM101 in well mixed reactors.[17]

Based on these results, the compounds shown in Table 1 were selected for biotransformation studies on a two-liter scale [50% (v/v) dioctyl phthalate] in a bioreactor. Biotransformation times and biocatalyst growth for the different reactions are listed in Table 2. Biocatalyst growth and the corresponding yield of biomass as a by-product was not the same for all reactions, compared to growth characteristics shown in Figure 1. This points to different toxicities of reaction products for *E. coli* JM101. The single biotransformations were also quantified with respect to yield, enantiomeric excess, and catalytic efficiency of *E. coli* JM101 (pSPZ10) (Table 3).

Reaction times varied between 17 hours for the preparation of *(S)*-styrene oxide and 45.5 hours for 1,2-dihydronaphthalene oxide with an increase of biomass during the reaction up to 28 g L^{-1} cell dry weight (CDW). This corresponds to a range of 0.4 g product formed overall per g CDW of biocatalyst for 1,2-dihydronaphthalene oxide up to 1.8 g product g^{-1} CDW for *(S)*-styrene oxide. Isolation of epoxides via distillation from the high boiling dioctyl phthalate phase under reduced pressure was straightforward after separation of the biomass via centrifugation. Separate experiments have shown that the cells are not inactivated after the biotransformation time and could be reused as catalysts. The enantiomeric excess obtained was usually over 98%. The average specific activities over the total reaction time were in the range

Table 2. Biomass production during biotransformations.[a]

Substrate	Time of biotransformation [h]	Cell density (in aqueous phase)		Amount of cells produced [g]	Growth rate (exponential) μ [h^{-1}]
		Initial CDW [g L^{-1}]	Final CDW [g L^{-1}]		
1	17	0.8	7.1	6.3	0.13
2	17	1.1	8.6	7.5	0.12
3	23.5	5.4	28.5	23.1	0.07
4	20	4.0	17.1	13.1	0.07
5	17.5	3.5	12.3	8.8	0.07
6	45.5	0.9	28.8	27.9	0.08
7	19	0.7	8.5	7.8	0.13

[a] Biotransformations were performed at a phase ratio of 0.5 (second phase = dioctyl phthalate) in a volume of 2 L, a substrate concentration of 2% (v/v) in the apolar phase, an aeration rate of 1 L min^{-1}, a stirrer speed of 2000 rpm and at a temperature of 30 °C. pH was regulated at 7.1 and the feed rate during fed batch cultivation was 10 g h^{-1} of glucose (45% w/v), 9 g L^{-1} of $MgSO_4 \cdot 7\,H_2O$.

Table 3. Catalytic efficiencies of *E. coli* JM101 (pSPZ10) for the epoxidation of olefins.

Substrate	Yield [%]	ee [%]	Total product [g]	Biocatalyst activity[a] [U g^{-1}]	Product ratio[b] [g g^{-1}]	Productivity[c] [g g^{-1} h^{-1}]
1	76.3	99.5	12.6	14.5 (20.0)	1.8	0.10
2	46.5	99.9	8.7	7.4 (7.7)	1.0	0.06
3	74.8	96.7	11.3	2.1 (4.0)	0.4	0.02
4	87.2	99.8	15.4	5.6 (15.9)	0.9	0.05
5	87.3	99.4	18.3	9.2 (10.1)	1.5	0.09
6	53.0	98.5	10.2	0.9 (0.9)	0.4	0.01
7	47.9	98.0	11.0	8.6 (12.5)	1.3	0.07

[a] Biocatalyst activity = total product × final cell $mass^{-1}$ × total conversion $time^{-1}$ [μmole g^{-1} min^{-1}]; in parentheses: values for at least 65% conversion.
[b] Product ratio = total product × final cell $mass^{-1}$ [g g^{-1}]
[c] Productivity = product ratio × conversion $time^{-1}$ [g g^{-1} h^{-1}].

Table 4. Catalytic efficiency of chemical catalysts for the epoxidation of olefins.[a]

Substrate	Catalyst	Yield [%]	ee [%]	Catalyst activity[b] [U g^{-1}]	Product ratio[c] [mg mg^{-1}]	Productivity[d] [mg mg^{-1} h^{-1}]	TTN[e]	TF[f] [s^{-1}]
1	Ru-brucin[24]	64	43 (*S*)	180	31.3	1.30	213	9
	Mn-salen[25]	86	n.d.[g]	91	3.3	0.66	57	11
2	Ru-brucin[24]	68	48 (*S*)	192	37.1	1.55	226	10
4	Mn-salen[25]	75	n.d.[g]	79	3.2	0.64	53	10
6	Mn-salen[26]	70	73 (1*S*,2*R*)	360	3.2	3.16	14	14
	Mn-salen[25]	72	50 (1*S*,2*R*)	382	3.4	3.37	18	18
	Ru-brucin[24]	76	57 (1*S*,2*R*)	214	45.2	1.88	253	11
	Mn-salen[25]	70	10	62	3.3	0.54	46	8
7	Mn-salen[26]	50	80 (1*S*,2*R*)	514	2.0	4.04	10	20
	Mn-salen[25]	78	43 (1*S*,2*R*)	414	3.3	3.28	20	20
	Mn-salen[25]	83	92	219	3.5	1.74	55	27
	Ru-brucin[24]	57	48 (1*S*,2*R*)	161	30.6	1.28	190	8

[a] Catalytic efficiencies and product amounts were calculated based on literature data. Examples were selected to compare turnover frequencies and are not intended to be complete.
[b] Catalyst activity = total product × catalyst $amount^{-1}$ × $time^{-1}$ [μmole g^{-1} min^{-1}].
[c] Product ratio = total product × $catalyst\ amount^{-1}$ [mg mg^{-1}].
[d] Productivity = product ratio × $time^{-1}$ [mg mg^{-1} h^{-1}].
[e] TTN = total product × $catalyst\ amount^{-1}$ [mole $mole^{-1}$].
[f] TF = TTN × $time^{-1}$ [s^{-1}].
[g] n.d. = not determined.

of 0.9 international units (U) per g CDW of cells for 1,2-dihydronaphthalene up to 14.5 U g^{-1} CDW for styrene (Table 3). Highest specific activities were determined for styrene, followed by *trans*-β-methylstyrene, indene, 3-chlorostyrene, and 4-methylstyrene. Specific activities for α-methylstyrene and 1,2-dihydronaphthalene were about 20% and 4% of styrene turnover, respectively. Recently, the substrate spectrum of another styrene monooxygenase has been characterized based on initial reaction rates,[18,19] with highest initial activities for styrene and indene. Interestingly and in contrast to our enzyme system, rather low activities were reported for 4-methylstyrene and α-methylstyrene.

Turnover numbers were also calculated based on the concept of a single *E. coli* JM101 (pSPZ10) cell as catalytic unit. The relative amount of cells in mole per mole substrate was in the range of 10^{-13}. Here, total turnover numbers (TTN) were calculated in the range of 10^{12} mole product formed per mole of cells with turnover frequencies (TF) of about 10^{8} mole product formed per mole cells per second. These high numbers result from the presence of numerous active styrene monooxygenase enzymes in a single cell which, based on initial estimations of the k_{cat} value of styrene monooxygenase, are expected to be around 10^{5} per cell.

During biocatalytic epoxidation 0.5 g to 2.5 g of biomass are formed per g products (Table 2 and 3). In contrast, catalysts used for the chemical epoxidation of indene such as Mn-salen or Ru-brucin complexes are used in amounts of 4 – 7 mole % with respect to the substrate. This is also equal to 0.02 g to 0.5 g of catalyst needed for the formation of 1 g product (Table 4). These reactions were performed on small scales (50 to 500 mg substrate) and turnover frequencies were in the range of 10 to 30 per hour.

By rational ligand design, the ee values obtained with chemical catalysis can be increased for **6a** and **7a**, but the total turnover numbers are still in the range of a few hundred.[3] This corresponds to a specific activity in the range of 80 to about 500 U g^{-1} chemical catalyst. These activities are about 10 times higher than those reported for optimized biological systems based on styrene monooxygenase.[17] Yet, biomass thus produced is non-hazardous as compared to heavy metal catalysts and high value components like, e.g., amino acids may even be recycled.

Conclusions

The combination of using growing recombinant *E. coli* cells containing overexpressed styrene monooxygenase in a suitable organic/aqueous reaction medium and recovery of oxiranes from the organic phase via distillation gives easy access to gram amounts of chiral oxiranes. These reactions are characterized by a high selectivity and very high reaction rates based on single cells as catalytic units.

Yet, the application of living microbial cells as catalysts was shown to be limited by the toxicity of reactants, even in non-toxic, two liquid phase reaction mixtures. One solution is the use of cell-free styrene monooxygenase as a catalyst, an approach which would also allow the optimization of catalyst preparation separate from its application. To follow this strategy we will now focus on aspects like enzyme stabili-

zation and cofactor regeneration of the isolated oxygenase.

Experimental Section

Chemicals were purchased from Fluka and Aldrich and used without further purification.

Analytical Methods

Reaction progress was followed by gas chromatography (Fisons HRGC Mega2 gas chromatograph with FID) on a Chrompack Cp-Sil 5CB column (30 m × 0.32 mm × 0.25 μm, T = 40 – 140 °C at 10 °C min^{-1}, splitless injection) with hexadecane as internal standard. At intervals, 2 mL samples were taken from the reaction emulsion. The two phases were separated by 7 min centrifugation at 10300 *g* and 4 °C. Prior to analysis, the aqueous phase was extracted exhaustively with an aliquot diethyl ether.

Optical purities were determined by chiral GC (styrene oxide, on a Supelco β-DEX 120 column, 30 m × 0.25 mm × 0.25 μm, isothermal at 90 °C, split injection 20:1) or chiral HPLC on a Merck/Hitachi system connected to a UV-detector set to 210 nm [β-methylstyrene oxide and 3-chlorostyrene oxide on a CC200/4 Nucleodex α-PM column (25 cm × 0.46 cm) with 50:50 methanol/water (1% TEAA, pH 4) or, respectively, α-methylstyrene oxide, 4-methylstyrene oxide, indene oxide, and 1,2-dihydronaphthalene oxide on a DIACEL OB-H chiracel column (25 cm) with 98:2 hexane/2-propanol]. The absolute *(S)*-configuration of biocatalytically prepared *(S)*-styrene oxide could be proven via comparison with commercially available, enantiopure *(S)*-styrene oxide (Aldrich). Based on the analogous chromatographic behavior of α-methylstyrene oxide, 4-methylstyrene oxide, indene oxide, and 1,2-dihydronaphthalene oxide to literature data for *(S)*-indene oxide,[20] we assume the *(S)*-configuration for these epoxides. The direct proof however remains to be made.

Mass spectra were obtained from GC-MS using a Fisons 8000 gas chromatograph equipped with an Optima-5 column (Macherey-Nagel, 30 m × 0.32 mm × 0.25 μm, T = 40 – 140 °C at 10 °C min^{-1}), that was directly connected to a Fisons MD800 quadropole mass spectrometer (EI = 70 eV). Optical rotations were measured with a Perkin Elmer 341 polarimeter. NMR spectra were recorded on a Bruker AC 400 (^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz). All signals are expressed as ppm down field from tetramethylsilane.

General Procedure for Biotransformation

Biotransformations were conducted in a 3-L stirrer tank reactor at 30 °C, pH 7.1 (controlled by addition of 25% NH_4OH) and aeration with air. The preculturing was performed by adding 1 mL of an overnight LB culture of freshly transformed *E. coli* JM101 (pSPZ10) in 100 mL M9* media. 900 mL M9* medium in the reactor were inoculated with the preculture.[17] Incubation took place until glucose starvation at an aeration rate of 1 L min^{-1} and a stirrer speed of 1500 rpm. Afterwards the culture was supplemented with 4 mL UStrace solution and 4 mL thiamine solution (1% w/v). A feed of 10 g h^{-1}, consisting of 45% (w/v) glucose and 9 g L^{-1} $MgSO_4 \cdot 7\,H_2O$, was applied. The actual biotransformation was initiated by addition of substrate (2% v/v) and octane (1% v/v to induce the *alk* promotor system)[21] dissolved in 1 L of bis(2-ethylhexyl) phthalate (BEHP, dioctyl phthalate). The stirrer speed was increased to 2000 rpm. Oxygen concentration was measured, temperature (30 °C) and pH 7.1 were regulated automatically. Cell growth was followed by measuring the OD_{450nm} and calculating the cell dry weight (CDW) with 1 OD_{450nm} = 0.29 g L^{-1} CDW = 4.8 × 10^8 cells L^{-1}.

Downstream Processing

The biotransformations were terminated by harvesting the reactor contents. After phase separation by centrifugation (3 – 4 times for 20 min at 12200 *g* and 4 °C), the organic phase was dried over Na_2SO_4. Product purification was achieved by distillation at reduced pressure (0.04 – 0.1 mbar). Fractions containing the product were obtained between 60 °C and 85 °C. Thus, recovery rates of typically 90% (calculated on the final product concentration in the organic phase) were obtained.

General Procedure for Chemical Preparation of Racemic Epoxides

The olefin (58 mmole) was slowly added to a cooled solution of 3-chloroperbenzoic acid (63 mmole) in 100 mL diethyl ether. The reaction mixture was stirred at 0 °C for 24 h. Afterwards it was washed several times with 100 mL portions of NaOH solution (10% w/v) and water. The ether phase was dried over anhydrous $MgSO_4$. The products were obtained by fractionated distillation at reduced pressure.[22]

***(S)*-Styrene oxide (1a):** Colorless fluid; $[\alpha]_D^{20}$: +21.1° (83 mM, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.42 – 7.22 (m, 5H), 3.85 (dd, *J* = 2.6, 4.1 Hz, 1H), 3.13 (dd, *J* = 4.1, 5.5 Hz, 1H), 2.79 (dd, *J* = 2.6, 5.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 51.2, 52.4, 125.5, 128.2, 128.5, 137.7; MS (EI): *m/z* (%) = 120 (39), 91 (100), 65 (23), 51 (10).

4-Methylstyrene oxide (2a): Colorless fluid; $[\alpha]_D^{20}$: –50.5° (75 mM, $CHCl_3$); product identification was achieved via comparative GC-analysis; MS (EI): *m/z* (%) = 134 (15), 133 (23), 105 (76), 89 (96), 77 (79), 51 (100).

α-Methylstyrene oxide (3a): Colorless fluid; $[\alpha]_D^{20}$: +19.5° (75 mM, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 – 7.19 (m, 5H), 2.96 (d, *J* = 5.4 Hz, 1H), 2.79 (dq, *J* = 0.6, 5.4 Hz, 1H), 1.71 (d, *J* = 0.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9, 56.8, 57.8, 125.3, 125.5, 127.5, 128.2, 128.4, 141.2; MS (EI): *m/z* (%) = 134 (6), 105 (100), 91 (8), 77 (52), 51 (54).

β-Methylstyrene oxide (4a): Colorless fluid; $[\alpha]_D^{20}$: –15.6° (75 mM, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.26 – 7.14 (m, 5H), 3.47 (d, *J* = 1.9 Hz, 1H), 2.93 (dq, *J* = 1.9, 5.2 Hz, 1H), 1.35 (d, *J* = 5.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.0, 59.1, 59.6, 125.6, 128.1, 128.5, 137.8.

3-Chlorostyrene oxide (5a): Yellowish fluid; $[\alpha]_D^{20}$: +10.8° (65 mM, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29 – 7.09 (m, 4H), 3.83 (dd, *J* = 2.5, 4.1 Hz, 1H), 3.14 (dd, *J* = 4.1, 5.5 Hz, 1H), 2.75 (dd, *J* = 2.5, 5.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 51.2, 51.7, 123.7, 125.6, 128.3, 129.8, 134.6,

139.9; MS (EI): *m/z* (%) = 154 (5), 125 (20), 119 (17), 89 (100), 63 (79), 50 (47).

1,2-Dihydronaphthalene oxide (6a): Colorless solid; $[\alpha]_D^{20}$: –134.5° (68 mM, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 – 7.11 (m, 4H), 3.87 (d, *J* = 4.1 Hz, 1H), 3.75 (dd, *J* = 3.2, 4.1 Hz, 1H), 2.83 (ddd, 0.7, 6.4, 15.0 Hz, 1H), 2.57 (ddd, *J* = 1.7, 5.7, 15.0 Hz, 1H), 2.43 (dddd, *J* = 1.7, 3.2, 6.4, 14.5 Hz, 1H), 1.78 (ddd, *J* = 0.7, 5.7, 14.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 136.7, 132.5, 129.5, 128.3, 128.2, 126.1, 55.1, 52.7, 24.4, 21.9; MS (EI): *m/z* (%) = 146 (50), 104 (100), 91 (35), 78 (54), 65 (32), 63 (77) 51 (71).

***(1S,2R)*-Indene oxide (7a):** Light brown oil; $[\alpha]_D^{20}$: –3.1° (76 mM, $CHCl_3$, due to poor stability of this compound, optical rotation was found to erratic[23]); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.52 – 7.21 (m, 4H), 4.26 (d, *J* = 5.8 Hz, 1H), 4.11 (dd, *J* = 2.9, 5.8 Hz, 1H), 3.21 (d, *J* = 18 Hz, 1H), 2.96 (dd, *J* = 2.9, 18 Hz, 1H); MS (EI): *m/z* (%) = 132 (11), 104 (47), 78 (42), 63 (68), 51 (100).

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