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Asymmetric epoxidation of styrene derivatives by styrene monooxygenase from *Pseudomonas* sp. LQ26: effects of α- and β-substituents

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ABSTRACT

Recombinant *Escherichia coli* expressing a styrene monooxygenase, StyAB2, from *Pseudomonas* sp. LQ26 was applied to synthesize a range of chiral epoxides from conjugated styrene derivatives with excellent (>99%) enantioselectivity in most cases. The substrate preference was studied with a special focus on the steric effect of α- and β-substituents.

1. Introduction

Enantiopure epoxides are well recognized as extremely important building blocks that can be transformed into numerous biologically active targets. They can be formed from a variety of precursors, among which the asymmetric epoxidation of an olefin is the most attractive. Considerable effort has been made in this area by synthetic chemists.[1–5] Complementary to this, a number of enzymatic approaches have also been studied to provide environmentally friendly alternatives.[6,7]

Styrene monooxygenase (SMO) is an enzyme involved in the upper catabolic pathway of styrene degradation.[8] It shows excellent enantioselectivity in the epoxidation of styrene, to give (*S*)-styrene epoxide with >99% ee. The SMO from *Pseudomonas fluorescens* VLB120 has been used in a pilot-scale production of (*S*)-styrene oxide.[9,10] However, its potential as a tool in organic synthesis remains largely unexplored. Most studies have focused on its characterization from a biochemical point of view.[8,11] Sello et al. and Schmid et al. studied the bioconversion of substituted styrenes by SMO from *P. fluorescens* ST and *P. fluorescens* VLB120. However, in most cases, only substrates with substituents on the benzene ring or α- and β-methyl substituted styrenes were investigated.[12–15]

We recently isolated a novel styrene monooxygenase (designated StyAB2) from *Pseudomonas* sp. LQ26 and functionally expressed it in recombinant *Escherichia coli* BL21 (DE3) (GenBank accession no. GU593979).[16] It is the most distant member of all SMOs originating from the genus *Pseudomonas*. Unlike the SMO from *P. fluorescens* ST, this enzyme displays activity toward both conjugated styrene derivatives and nonconjugated alkenes.[16] The enantioselectivity toward conjugated styrene derivatives remains excellent. Herein we report a systematic study on the performance of this enzyme in the epoxidation of a series of styrene derivatives, with a special focus on the steric effect of α- and β-substituents.

2. Results and discussion

The biotransformations were performed in a biphasic system catalyzed by the whole cells of a recombinant *E. coli* BL21(DE3) bearing a plasmid encoding StyAB2 from *Pseudomonas* sp. LQ26.[16] The results are presented in Table 1. All experiments were performed with the same batch of cells to ensure the same biocatalytic activity. For preparative biotransformations, the cell density used was greater than that in the literature[9,10] to ensure the isolation of all products. This condition might have a negative effect on the catalytic efficiency. Nevertheless, in all cases, (*S*)-epoxides were obtained and their enantiomeric excesses were greater than 99% (except entries 5 and 16). The yield of epoxide, however, varied greatly with the substituents.

As shown in the entries 1–12, the bioconversion of a series of styrene derivatives with substituents ranging from H to propyl clearly demonstrated a significant steric effect. A substrate with a bulky substituent yielded fewer products within the same period of time. In the case of substrates **2a**, **5a**, **6a**, and **9a**, all bearing an R^3 group, the yield rapidly decreased from 92% to 58%, 45%, and 12%, respectively, with an increase in the substituent size. Under the same conditions, styrene **1a** yielded >99% of epoxide. Substrates with an *i*-propyl or *n*-propyl at the R^2 position yielded similar amounts of product (entry 11 vs 9), which might be due to a flexible active pocket in the enzyme. The enzyme catalyzed the epoxidation of cyclic substituted compound **16a** (R^1,R^2 = $-(CH_2)_4-$) with 7% yield and medium enantioselectivity, but could not catalyze the epoxidation of 1-(cyclohexylidenemethyl)benzene (R^2,R^3 = $-(CH_2)_5-$) or (*E*)-stilbene (R^2 = Ph). It can be inferred that the larger substrates are less susceptible toward the enzymatic epoxidation process,

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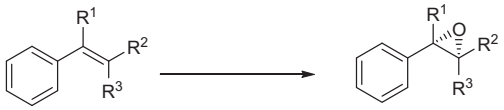
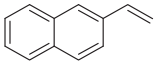
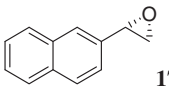
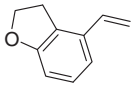
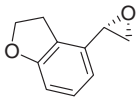
Table 1
Biotransformation of styrene derivatives into the corresponding oxiranes



1a-16a **1b-16b**

Entry	Substrate[a]	Product	Yield[b] (%)	Relative activity[c] (%)	ee (%)
1	Styrene **1a**	**1b**	>99	100	>99
2	R^2 = Me **2a**	**2b**	92	97	>99
3	R^1 = Me **3a**	**3b**	87	93	>99
4	R^2, R^3 = Me **4a**	**4b**	68	71	>99
5	R^2 = –CH=CH_2 **5a**	**5b**	58	64	96
6	R^2 = Et **6a**[d]	**6b**	45	51	>99
7	R^3 = Et **7a**[d]	**7b**	6	10	>99
8	R^1 = Et **8a**	**8b**	35	39	>99
9	R^2 = *n*-Pr **9a**[e]	**9b**	12	15	>99
10	R^3 = *n*-Pr **10a**[e]	**10b**	1	3	>99
11	R^2 = *i*-Pr **11a**	**11b**	11	15	>99
12	R^1 = *n*-Pr **12a**	**12b**	5	7	>99
13	R^2 = –CH(OH)CH_3 *rac*-**13a**	**13b**	>99	167	>99 (*syn*:*anti* 1:1)
14[f]	R^2 = –CH_2OH **14a**	**14b**	>99	271	>99
15	R^2 = –CH_2Cl **15a**	**14b**	73[g]	80	>99
16	R^1,R^2 = –$(CH_2)_4$– **16a**	**16b**	7	9	65
17	 **17a**	 **17b**	76	77	>99
18	 **18a**	 **18b**	65	70	>99

[a] Substituents other than H were listed.
[b] HPLC yield after 24 h reaction.
[c] Relative activity = [specific activity for substrate **a**]/[specific activity for styrene] × 100%; Specific activity for substrate **a** = mmoles of products formed in 1 h by 1 g of wet cells.
[d] Added as a mixture with **6a**:**7a** = 1:0.8.
[e] Added as a mixture with **9a**:**10a** = 1:0.7.
[f] The reaction was performed without BEHP.
[g] *rac*-1-Phenylprop-2-en-1-ol **15c** isolated with a yield of 17%.

which is consistent with a previous report.[12] In contrast, substrates with aromatic rings larger than styrene, such as 2-vinylnaphthalene **17a** and 4-vinyl-2,3-dihydrobenzofuran **18a** were well accepted; both yielded the corresponding (*S*)-epoxides with >99% enantiomeric excesses and high yields (entries 17 and 18). It is worth noting that the product **18b** is a critical intermediate in the synthesis of melatonin agonists. Previously, only indirect biocatalytic methods using epoxide hydrolase or alcohol reductase have been described.[17]

Our results also show that substituents at the α-position (R^1) cause greater steric hindrance than those at the β-position (R^2), as demonstrated by the fact that the bioconversion of substrates **3a**, **8a**, and **12a** resulted in lower product yields than the corresponding β-substituted substrates **2a**, **6a**, and **9a**. The difference was augmented by an increase in the substituent size, as the relative activities of β-substituted over α-substituted substrates were 1.0, 1.3, and 2.1 for methyl-, ethyl-, and propyl-substituted compounds, respectively. Furthermore, the reactivity was also affected by the configuration of the substrates. The (*E*)-isomers **6a** and **9a** performed much better than the corresponding (*Z*)-isomers **7a** and **10a**. The result was contrary to that observed for SMO from *P. fluorescens* ST: a preference for (*Z*)-isomers and accelerated reaction with methyl-substitution at the R^3 position.[13] For the SMO from *Pseudomonas* sp. LQ26, substituents at the R^3 position appeared to be unfavorable in all the cases tested (entries 2, 3, 6, 7, 9, and 10).

Substrates **13a** and **14a** bearing a hydroxy group at the β-position displayed more than 100% relative activity. Compound **13a** had an additional stereogenic center and was used as a racemate. The results show that SMO catalyzes the (*S*)-epoxidation of both enantiomers almost equally well, resulting in the diastereoisomers **13b1** and **13b2** in a ratio of 1:1, each with an ee value >99%. When the reaction was traced by HPLC, a maximal de value of 38% (Fig. 1) was observed, demonstrating a weak ability to distinguish between the (*R*)- and (*S*)-substrates, albeit with steady excellent selectivity during the epoxidation process. When the hydroxyl group in **13a** was replaced with a chloro or bromo group, no product could be detected by HPLC (not listed in Table 1).

Compound **15a** did not yield the corresponding epoxide, but yielded (*S*)-epoxide **14b**. This phenomenon has been reported but not further explained.[12] We found that the real substrate that underwent bioconversion was **14a**. Compound **15a** was unstable in potassium phosphate buffer and would spontaneously transform into **14a** and 1-phenylprop-2-en-1-ol **15c**, with the former further converting into **14b**. As previously reported, StyAB2 can catalyze the epoxidation of both conjugated and nonconjugated olefins,[16] and its activity toward **15c** has recently been confirmed.[18] However, it was apparent that the activity of SMO is higher toward **14a** than **15c**, demonstrating its preference for conjugated substrates, which led to the isolation of unreacted nonconjugated olefin **15c**.

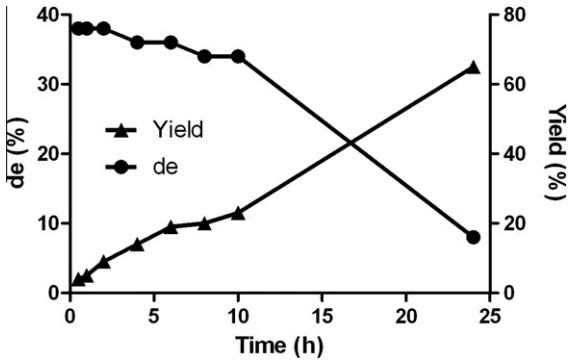


Figure 1. Biotransformation of (*E*)-4-phenylbut-3-en-2-ol **13a** with recombinant *E. coli* expressing StyAB2.

3. Conclusion

Overall, we have applied a styrene monooxygenase from *Pseudomonas* sp. LQ26 to the synthesis of a spectrum of chiral epoxides from conjugated styrene derivatives and studied its substrate preference with a special focus on the steric effects of α- and β-substituents. We found that this enzyme is endowed with remarkable enantioselectivity. To expand its substrate range, the identification of the critical amino acid residues that interact with these substituents is of great interest. Recently, several mutant enzymes with altered substrate preference have been observed and are currently under investigation in this laboratory.

4. Experimental

^{1}H NMR spectra were recorded on a Brucker-600 (600/150 MHz) spectrometer in $CDCl_3$. All signals are expressed as ppm down field from tetramethylsilane. Optical rotations were measured with a Perkin Elmer 341 polarimeter. Chiral HPLC was conducted with a Shimadzu Prominence LC-20AD system connected to a PDA-detector using Daicel Chiralcel OD-H, OJ-H, Chiralpak AD-H, or Whelk-O1(*R*, *R*) columns. Reverse-phase HPLC was conducted using a Luna C18 reverse-phase column (Phenomenex). The absolute configurations of **1b**–**5b**, **8b**, and **12b**–**18b** were established by comparison with the specific rotation reported in the literature. The absolute configurations for all others were based on the analogous chromatographic behavior of racemic mixtures.

Styrene derivatives **1a–4a**, **14a–18a** were obtained from commercial suppliers and used without further purification. Substrates **5a**–**12a** were prepared by a Wittig reaction.[19] Epoxides (*S*)-**1b**, (2*S*,3*S*)-**2b**, and (2*R*,3*R*)-**2b** were obtained from Aldrich. Racemic epoxides were prepared from the corresponding alkenes in accordance with the literature.[20,21]

4.1. Cultivation of recombinant *E. coli* BL21 (DE3)

Recombinant *E. coli* BL21 (DE3) bearing the plasmid encoding StyAB2 from *Pseudomonas* sp. LQ26 was cultivated in Terrific Broth for 3 h at 37 °C, followed by 18 h incubation at 20 °C with gyratory shaking at 220 rpm.[16] Cells were harvested by centrifugation, washed twice with potassium phosphate buffer (0.1 M, pH 7.0), and stored at 4 °C.

4.2. General procedure for biotransformation

Recombinant *E. coli* BL21 cells with a dry weight of 0.2 g were resuspended in 10 ml of 100 mM potassium phosphate buffer (pH 6.5) containing 10% (v/v) bis(2-ethylhexyl) phthalate (BEHP) and 8 mg of substrate. The cells used to transform different substrates were from the same batch of culture. The reactions were carried out at 30 °C for 24 h with shaking at 220 rpm and terminated by extraction with diethyl ether. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure and subjected to reverse phase and chiral HPLC analysis. To determine the specific activity of various substrates, the reactions were terminated after 1 h and the amount of product was analyzed using reverse phase HPLC.

For preparative biotransformations, recombinant *E. coli* BL21 cells with a dry weight of 0.4 g were resuspended in 20 ml of 100 mM potassium phosphate buffer (pH 6.5) containing 10% (v/v) cyclohexane and 30 mg of substrate. The reactions were carried out at 30 °C for 24 h with shaking at 220 rpm, and the products were extracted, concentrated, and subjected to silica gel column chromatography. The purified products were identified with NMR analysis and their specific rotations were measured.

4.2.1. (*S*)-Styrene oxide 1b

Colorless oil, ee >99% (AS-H, hexane/2-propanol = 90:10, 0.5 ml/min, $t_R(R)$ 10.27 min, $t_R(S)$ 10.67 min), $[\alpha]_D^{25} = +32.1$ (*c* 1.02, $CHCl_3$) {lit.[14] $[\alpha]_D^{25} = +21.1$ (*c* 83 mM, $CHCl_3$) for 99% ee, (*S*)} ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.27–7.35 (m, 5H, Ar-H), 3.85 (m, 1H, CH), 3.13 (m, 1H, CH_2), 2.79 (m, 1H, CH_2).

4.2.2. (2*S*,3*S*)-2-Methyl-3-phenyloxirane 2b

Colorless oil, ee >99% (AD-H, hexane/2-propanol = 90:10, 1 ml/min, $t_R(R)$ 4.14 min, $t_R(S)$ 4.78 min), $[\alpha]_D^{25} = -41.8$ (*c* 1.00, $CHCl_3$) {lit.[22] $[\alpha]_D^{20} = +47.2$ (*c* 1.10, $CHCl_3$) for 99% ee, (*R*,*R*)}. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.25–7.34 (m, 5H, Ar-H), 3.56 (d, *J* = 1.92 Hz, 1H, CH), 3.01–3.04 (m, 1H, CH), 1.45 (d, *J* = 5.04 Hz, 3H, CH_3).

4.2.3. (*S*)-2-Methyl-2-phenyloxirane 3b

Colorless oil, ee >99% (OD-H, hexane/2-propanol = 90:10, 0.8 ml/min, $t_R(R)$ 5.17 min, $t_R(S)$ 5.61 min), $[\alpha]_D^{25} = +16$ (*c* 0.20, $CHCl_3$) {lit.[14] $[\alpha]_D^{25} = +19.5$ (*c* 75 mM, $CHCl_3$) for 99% ee, (*S*)}. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.25–7.38 (m, 5H, Ar-H), 2.98 (d, *J* = 5.3 Hz, 1H, CH_2), 2.81 (d, *J* = 5.3 Hz, 1H, CH_2), 1.73 (s, 3H, CH_3).

4.2.4. (*S*)-2,2-Dimethyl-3-phenyloxirane 4b

Colorless oil, ee >99% (AD-H, hexane/2-propanol = 90:10, 1 ml/min, $t_R(R)$ 3.76 min, $t_R(S)$ 4.18 min), $[\alpha]_D^{25} = -23.6$ (*c* 0.85, benzene) {lit.[1] $[\alpha]_D = +36.8$ (*c* 0.63, benzene) for 99% ee, (*R*)}. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.18–7.28 (m, 5H, Ar-H), 3.79 (s, 1H, CH), 1.41 (s, 3H, CH_3), 1.00 (s, 3H, CH_3).

4.2.5. (2*S*,3*S*)-2-Phenyl-3-vinyloxirane 5b

Colorless oil, ee 96% (OD-H, hexane/2-propanol = 95:5, 0.5 ml/min, $t_R(S,S)$ 8.7 min, $t_R(R,R)$ 9.2 min), $[\alpha]_D^{25} = -42.3$ (*c* 0.35, $CHCl_3$) for purity >99% {lit.[23] $[\alpha]_D = -14.2$ (*c* 0.88, $CHCl_3$) for 95% ee, (*S*,*S*)}. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.28–7.36 (m, 5H, Ar-H), 5.70–5.76 (m, 1H, =CH), 5.52 (d, *J* = 17.2 Hz, 1H, $=CH_2$), 5.34 (d, *J* = 10.56 Hz, 1H, $=CH_2$), 3.76 (d, *J* = 1.98 Hz, 1H, CH), 3.35–3.37 (dd, *J* = 1.98 Hz, *J* = 7.62 Hz, 1H, CH).

4.2.6. (*E*)- and (*Z*)-1,2-Epoxy-1-phenyl-butane 6b and 7b

Colorless oil, ee >99% (AD-H, hexane/2-propanol = 99:1, 0.5 ml/min, t_{R1} 9.42 min, t_{R2} 9.98 min (major), t_{R3} 15.28 min (major)) [The peaks of **6b** (*S*,*S*) and **7b** (*R*,*R*) overlapped in the HPLC spectrum, and the ee values were determined by HPLC in combination with NMR, which revealed the ratio of **6b** and **7b**] ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.24–7.34 (m, 5.5H, Ar-H), 4.07 (d, *J* = 4.2 Hz, 0.1H, CH-**7b**), 3.61 (d, *J* = 1.74 Hz, 1H, CH-**6b**), 3.14–3.17 (m, 0.1H, CH-**7b**), 2.92–2.95 (m, 1H, CH-**6b**), 1.69–1.74 (m, 2H, CH_2-**6b**), 1.20–1.27 (m, 0.2H, CH_2-**7b**), 1.06 (t, *J* = 7.44 Hz, 3H, CH_3-**6b**), 1.06 (t, *J* = 7.62 Hz, 0.3H, CH_3-**7b**).

4.2.7. (*S*)-2-Ethyl-2-phenyloxirane 8b

Colorless oil, ee >99% (OD-H, hexane/2-propanol = 90:10, 0.8 ml/min, $t_R(R)$ 4.87 min, $t_R(S)$ 5.38 min), $[\alpha]_D^{25} = +23.1$ (*c* 0.34, $CHCl_3$) {lit.[24] $[\alpha]_D^{25} = -25.2$ (*c* 0.64, $CHCl_3$) for 92% ee, (*R*)}. ^1H NMR (600 MHz, $CDCl_3$): δ 7.25–7.37 (m, 5H, Ar-H), 2.97 (d, J = 5.28 Hz, 1H, CH_2), 2.73 (d, J = 5.28 Hz, 1H, CH_2), 2.15–2.20 (m, 1H, CH_2), 1.78–1.84 (m, 1H, CH_2), 0.94 (t, J = 7.8 Hz, 3H, CH_3).

4.2.8. (*E*)- and (*Z*)-1,2-epoxy-1-phenyl-pentan 9b and 10b

Colorless oil, ee >99% (AD-H, hexane/2-propanol = 99:1, 0.5 ml/min, t_{R1} 9.19 min, t_{R2} 9.63 min, t_{R3} 9.85 min (major), t_{R4} 16.07 min (major)), ^1H NMR (600 MHz, $CDCl_3$): δ 7.24–7.34 (m, 5.3H, Ar-H), 4.06 (d, J = 4.26 Hz, 0.1H, CH-**10b**), 3.61 (d, J = 1.92 Hz, 1H, CH-**9b**), 3.18–3.21 (m, 0.1H, CH-**10b**), 2.93–2.95 (m, 1H, CH-**9b**), 1.58–1.66 (m, 2H, CH_2-**9b**), 1.50–1.56 (m, 2H, CH_2-**9b**), 1.33–1.37 (m, 0.2H, CH_2-**10b**), 1.21–1.25 (m, 0.2H, CH_2-**10b**), 0.99 (t, J = 7.5 Hz, 3H, CH_3-**9b**), 1.06 (t, J = 3.3 Hz, 2.1H, CH_3-**10b**).

4.2.9. (2*S*,3*S*)-2-Isopropyl-3-phenyloxirane 11b

Colorless oil, ee >99% (AD-H, hexane/2-propanol = 90:10, 1 ml/min, $t_R(R,R)$ 3.72 min, $t_R(S,S)$ 4.46 min), $[\alpha]_D^{25} = -62.4$ (*c* 0.25, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.25–7.34 (m, 5H, Ar-H), 3.65 (d, J = 1.92 Hz, 1H, CH), 2.75 (dd, J = 1.92 Hz, J = 6.78 Hz, 1H, CH), 1.65–1.71 (m, 1H, CH), 1.09 (d, J = 6.78 Hz, 3H, CH_3), 1.02 (d, J = 6.78 Hz, 3H, CH_3).

4.2.10. (*S*)-2-Phenyl-2-propyloxirane 12b

Colorless oil, ee >99% (OD-H, hexane/2-propanol = 90:10, 0.8 ml/min, $t_R(R)$ 4.73 min, $t_R(S)$ 5.15 min), $[\alpha]_D^{25} = +32.3$ (*c* 0.26, $CHCl_3$) {lit.[25] $[\alpha]_D^{25} = +26.1$ (*c* 1.4, $CHCl_3$) for 75% ee, (*S*)}. ^1H NMR (600 MHz, $CDCl_3$): δ 7.24–7.37 (m, 5H, Ar-H), 2.94 (d, J = 5.46 Hz, 1H, CH_2), 2.73 (d, J = 5.46 Hz, 1H, CH_2), 2.12–2.17 (m, 1H, CH_2), 1.69–1.74 (m, 1H, CH_2), 1.33–1.44 (m, 2H, CH_2), 0.91 (t, J = 7.26 Hz, 3H, CH_3).

4.2.11. (2*RS*,3*S*,4*S*)-3,4-Epoxy-4-phenyl-butan-2-ol 13b

Light yellow oil, ee >99% (AD-H, hexane/2-propane = 90:10, 1 ml/min, t_{R1} = 6.83 min, t_{R2} = 7.65 min (major), t_{R3} = 8.46 min (major), t_{R4} = 11.39 min), ^1H NMR (600 MHz, $CDCl_3$): δ 7.25–7.36 (m, 7H, Ar-H), 4.08–4.11 (m, 2/5H, CH), 3.94 (d, J = 2.1 Hz, 2/5H, CH), 3.83–3.86 (m, 2H, CH, CH), 3.07–3.08 (m, 2/5H, CH), 3.03–3.04 (m, 1H, CH), 2.21 (br, 7/5H, OH), 1.35 (d, J = 6.54 Hz, 3H, CH_3), 1.31 (d, J = 6.36 Hz, 7/5H, CH_3).

4.2.12. (2*S*,3*S*)-3-Phenyloxiran-2-yl methanol 14b

Colorless oil, ee >99% (OD-H, hexane/2-propane = 95:5, 0.5 ml/min, $t_R(R)$ 21.90 min, $t_R(S)$ 23.58 min), $[\alpha]_D^{25} = -48$ (*c* 0.3, $CHCl_3$) {lit.[26] $[\alpha]_D = -49.6$ (*c* 10.5, $CHCl_3$) for 99% ee, (*S*,*S*)}. ^1H NMR (600 MHz, $CDCl_3$): δ 7.27–7.35 (m, 5H, Ar-H), 3.65 (d, J = 2.1 Hz, 1H, CH), 4.02–4.05 (m, 1H, CH_2), 3.92 (d, 3H, CH), 3.77–3.81 (m, 1H, CH_2), 3.21–3.22 (m, 1H, CH), 2.10 (br, 1H, OH).

4.2.13. (1*S*,2*S*)-1-Phenylcyclohexene oxide (16b)

Colorless oil, ee 65% (OD-H, hexane/2-propane = 99:1, 0.5 ml/min, $t_R(R,R)$ 12.53 min, $t_R(S,S)$ 13.67 min), ee 65%, $[\alpha]_D^{25} = -30.9$ (*c* 1.01, $CHCl_3$) {lit.[1] $[\alpha]_D$ = +116.7 (*c* 1.21, benzene) for 99% ee, (*R*,*R*)}. ^1H NMR (600 MHz, $CDCl_3$): δ 7.23–7.37 (m, 5H, Ar-H), 3.07 (d, 1H, CH), 2.25–2.31 (m, 1H, CH_2), 2.09–2.13 (m, 1H, CH_2), 1.94–2.03 (m, 2H, CH_2), 1.53–1.63 (m, 2H, CH_2), 1.40–1.49 (m, 1H, CH_2), 1.25–1.32 (m, 1H, CH_2).

4.2.14. (*S*)-2-(Naphthalen-2-yl)oxirane 17b

White solid, ee >99% (AD-H, hexane/2-propanol = 90:10, 1 ml/min, $t_R(R)$ 13.42 min, $t_R(S)$ 14.22 min), $[\alpha]_D^{25} = +17$ (*c* 0.72, $CHCl_3$) {lit.[27] $[\alpha]_D^{25} = -9$ (*c* 1.2, $CHCl_3$) for 84% ee, (*R*)}. ^1H NMR (600 MHz, $CDCl_3$): δ 7.79–7.82 (m, 4H, Ar-H), 7.44–7.49 (m, 2H, Ar-H), 7.32 (dd, J = 1.74 Hz, J = 8.4 Hz, 1H, Ar-H), 4.01 (dd, J = 2.52 Hz, J = 4.14 Hz, 1H, CH), 3.20 (dd, J = 4.14 Hz, J = 5.28 Hz, 1H, CH_2), 2.89 (dd, J = 2.52 Hz, J = 5.28 Hz, 1H, CH_2).

4.2.15. (*S*)-4-(Oxiran-2-yl)-2,3-dihydrobenzofuran 18b[28]

Colorless oil, ee >99% (Whelk-O1(*R*,*R*), hexane/2-propane = 90:10, 1 ml/min, $t_R(S)$ 8.10 min, $t_R(R)$ 8.72 min), $[\alpha]_D^{25} = +40.8$ (*c* 1.0, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.09 (t, J = 7.92 Hz, 1H, Ar-H), 6.72 (q, J = 7.92 Hz, 2H, Ar-H), 4.59 (t, J = 7.62 Hz, 2H, CH_2), 3.84 (m, 1H, CH), 3.27 (t, J = 7.62 Hz, 2H, CH_2), 3.12 (m, 1H, CH_2), 2.83 (m, 1H, CH_2).

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