This journal is (c) The Royal Society of Chemistry 2011

Supplementary Information

Highly Diastereo- and Enantio-selective Epoxidation of Secondary Allylic Alcohols Catalyzed by Styrene Monooxygenase

*Hui Lin, Yan Liu, and Zhong-Liu Wu**

Materials and General Methods S2

Bacterial strains and growth conditions S2

Substrate preparation (**1b-1p**) S3

Spectral data for products **2a-2p** and (*R*)-**1a-1p** S7

Docking studies S17

Construction of point mutations S17

Table S1 Epoxidation of α-hydroxyl olefins (*rac*)-**1a-1p**. S18

Table S2 Primers used in site-directed mutagenesis. S19

Figure S1 Kinetic resolution of **1a**. S20

Figure S2 Kinetic resolution of **1p** S21

Figure S3 Orientation of (*R*)-**1a** docked into the putative active site of SMO S22

References S23

Materials and General Methods

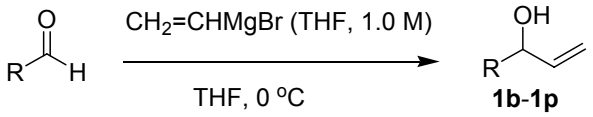
1-phenylprop-2-en-1-ol (**1a**) was purchased from Sigma-Aldrich. Other substrates were prepared from the corresponding aldehydes and vinylmagnesium bromide.[1] Racemic epoxides were prepared from the corresponding olefins using *m*-CPBA as the oxidant.[2] All chemicals were in analytical grade and used without further purification.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker-600 (600/150 MHz) spectrometer in $CDCl_3$ or CD_3OD. All signals are expressed as ppm down field from tetramethylsilane. High-resolution mass spectrometry (HRMS) was obtained on a Bruker Daltonics Bio-TOF-Q mass spectrometer equipped with an electron-spray ionization (ESI) source. 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 CHIRALPAK® AS-H, Daicel CHIRALCEL® OD-H or OJ-H columns. Chiral GC was conducted with a Fuli 9790 system connected to a FID-detector using CHIRASIL-DEX CB (Varianinc, US).

Bacterial strains and growth conditions

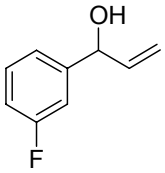
The organism used in this study was a recombinant *Escherichia coli* BL21 harboring the plasmid pET28a(+) encoding the *styAB* gene from *Pseudomonas* sp. LQ26 (StyAB2). The strain was cultivated over night at 37 °C in LB media containing kanamycin (50 μg/ml). Two-ml of overnight culture was then inoculated into 200 ml of Terrific Broth containing kanamycin (50 μg/ml) in a 500 ml flask, and incubated at 37 °C for 3 h, followed by incubation at 20 °C for another 20 h with gyratory shaking at 220 rpm. Cells were harvested by centrifugation, washed twice with potassium phosphate buffer (0.1 M, pH 7.0) and stored at 4 °C.

Substrate preparation (1b-1p)



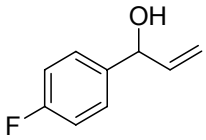
To the solution of aldehyde (5 mmol) in absolute THF (20 ml) was added vinylmagnesium bromide (6 ml, 1.0 M in THF) slowly at -10 °C. After 2 h, the reaction was quenched with saturated aqueous NH_4Cl (40 ml), followed by the addition of 60 ml ether. The organic phase was washed with salina, dried with anhydrous sodium sulfate and removed under reduced pressure. The crude product was purified by silica-gel column chromatography.

1-(3-fluorophenyl)prop-2-en-1-ol (**1b**)



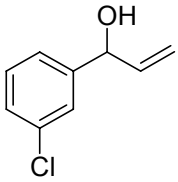
Colorless liquid; 72% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.95-7.33 (m, 4H, Ar-H), 5.98-6.04 (m, 1H, =CH), 5.36 (d, J = 16.86, 1H, -CH), 5.22 (d, J = 10.26, 1H, =CH_2), 5.19 (d, J = 6.24, 1H, =CH_2), 1.96 (br, 1H, OH).

1-(4-fluorophenyl)prop-2-en-1-ol (**1c**)



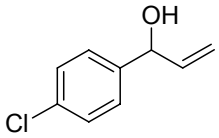
Colorless liquid, 85% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.23-7.35 (m, 2H, Ar-H), 7.02-7.04 (m, 2H, Ar-H), 5.99-6.04 (m, 1H, =CH), 5.34 (d, J = 17.22, 1H, -CH), 5.18-5.21 (m, 2H, =CH_2), 1.98 (br, 1H, OH).

1-(3-chlorophenyl)prop-2-en-1-ol (**1d**)



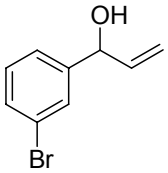
Light yellow liquid; 63% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.37 (s, 1H, Ar-H), 7.23-7.29 (m, 3H, Ar-H), 5.97-6.02 (m, 1H, =CH), 5.35 (d, *J* = 17.22, 1H, -CH), 5.22 (d, *J* = 10.26, 1H, =CH_2), 5.18 (d, *J* = 6.24, 1H, =CH_2), 1.93 (br, 1H, OH).

1-(4-chlorophenyl)prop-2-en-1-ol (**1e**)



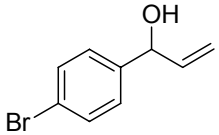
Light yellow liquid; 77% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.25-7.33 (m, 4H, Ar-H), 5.97-6.02 (m, 1H, =CH), 5.34 (d, *J* = 17.28, 1H, -CH), 5.20 (d, *J* = 11.28, 1H, =CH_2), 5.18 (br, 1H, =CH_2), 1.98 (br, 1H, OH).

1-(3-bromophenyl)prop-2-en-1-ol (**1f**)



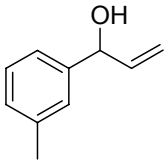
Light yellow liquid; 61% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.08-7.24 (m, 4H, Ar-H), 6.00-6.06 (m, 1H, =CH), 5.33 (d, *J* = 17.22, 1H, -CH), 5.22 (d, *J* = 10.62, 1H, =CH_2), 5.18 (br, 1H, =CH_2), 2.34 (s, 3H, -CH_3), 2.04 (br, 1H, OH).

1-(4-bromophenyl)prop-2-en-1-ol (**1g**)



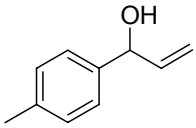
Light yellow liquid; 63% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.47-7.48 (br, 2H, Ar-H), 7.23-7.25 (br, 2H, Ar-H), 5.96-6.02 (m, 1H, =CH), 5.34 (d, *J* = 16.86, 1H, -CH), 5.20 (d, *J* = 10.26, 1H, =CH_2), 5.16 (br, 1H, =CH_2), 2.02 (br, 1H, OH).

1-*m*-tolylprop-2-en-1-ol (**1h**)



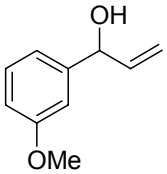
Colorless liquid; 82% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.08-7.24 (m, 4H, Ar-H), 6.00-6.06 (m, 1H, =CH), 5.33 (d, *J* = 17.22, 1H, -CH), 5.16 (d, *J* = 10.62, 1H, =CH_2), 5.14 (br, 1H, =CH_2), 2.34 (s, 3H, -CH_3), 2.03 (br, 1H, OH).

1-*p*-tolylprop-2-en-1-ol (**1i**)



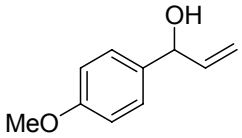
Colorless liquid; 87% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.25 (d, *J* = 8.04, 2H, Ar-H), 7.15 (d, *J* = 6.3, 2H, Ar-H), 6.01-6.06 (m, 1H, =CH), 5.32 (d, *J* = 17.28, 1H, -CH), 5.16-5.18 (m, 2H, =CH_2), 2.33 (s, 3H, -CH_3), 1.76 (br, 1H, OH).

1-(3-methoxyphenyl)prop-2-en-1-ol (**1j**)



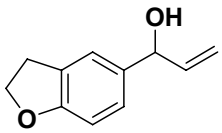
Light yellow solid; 82% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.25-7.28 (m, 1H, Ar-H), 6.93-6.95 (m, 2H, Ar-H), 6.81-6.83 (m, 1H, Ar-H), 6.01-6.06 (m, 1H, =CH), 5.35 (d, *J* = 17.28, 1H, -CH), 5.17-5.20 (m, 2H, =CH_2), 3.81 (s, 3H, -CH_3), 1.95 (br, 1H, OH).

1-(4-methoxyphenyl)prop-2-en-1-ol (**1k**)



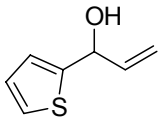
Light yellow solid; 79% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.25-7.29 (m, 2H, Ar-H), 6.87-6.89 (m, 2H, Ar-H), 6.01-6.07 (m, 1H, =CH), 5.31-5.34 (m, 1H, -CH), 5.15-5.31 (m, 2H, =CH_2), 3.79 (s, 3H, -CH_3).

1-(2,3-dihydrobenzofuran-5-yl)prop-2-en-1-ol (**1l**)



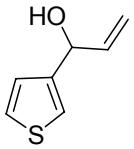
Light yellow liquid; 83% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.25 (s, 1H, Ar-H), 7.09-7.10 (m, 1H, Ar-H), 6.75 (d, *J* = 6.96, 1H, Ar-H), 6.02-6,07 (m, 1H, =CH), 5.32-5.35 (m, 1H, -CH), 5.14-5.18 (m, 2H, =CH_2), 4.56 (t, *J* = 8.4, 2H, -CH_2), 3.19 (t, *J* = 8.4, 2H, -CH_2).

1-(thiophen-2-yl)prop-2-en-1-ol (**1m**)



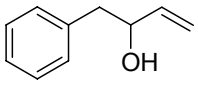
Yellow solid; 71% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.26-7.27 (m, 1H, Ar-H), 6.96-6.99 (m, 2H, Ar-H), 5.97-6.02 (m, 1H, =CH), 5.39-5.44 (br, 2H, =CH_2, -CH), 5.25 (d, *J* = 10.26, 1H, =CH_2), 2.15 (br, 1H, OH).

1-(thiophen-3-yl)prop-2-en-1-ol (**1n**)



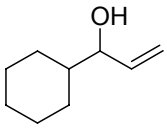
Yellow solid; 88% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.29-7.30 (m, 1H, Ar-H), 7.21 (m, 1H, Ar-H), 7.07 (d, *J* = 4.8, 1H, Ar-H), 6.05-6.11 (m, 1H, =CH), 5.34 (d, *J* = 17.28, 1H, -CH), 5.20 (d, *J* = 11.28, 1H, =CH_2), 5.18 (br, 1H, =CH_2), 1.98 (br, 1H, OH).

1-phenylbut-3-en-2-ol (**1o**)



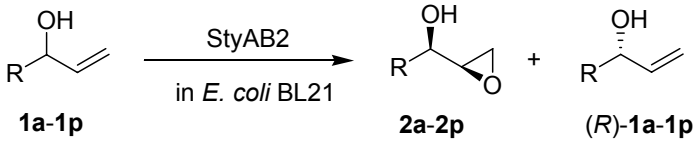
Colorless liquid; 91% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.22-7.32 (m, 5H, Ar-H), 5.90-5.96 (m, 1H, =CH), 5.26 (d, *J* = 2.58, 1H, =CH_2), 5.23 (d, *J* = 2.58, 1H, =CH_2), 4.33-4.35 (m, 1H, -CH), 2.88 (dd, *J* = 5.16, *J* = 13.56, 1H, -CH_2-), 2.79 (dd, *J* = 7.02, *J* = 13.56, 1H, -CH_2-), 1.62 (br, 1H, OH).

1-cyclohexylprop-2-en-1-ol (**1p**)

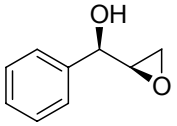


Colorless liquid; 61% yield; ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.83-5.89 (m, 1H, =CH), 5.20 (d, *J* = 17.22, 1H, =CH_2), 5.20 (d, *J* = 10.26, 1H, =CH_2), 3.84 (t, *J* = 6.24, 1H, -CH), 1.83-1.85 (br, 1H, cyclohexanyl), 1.72-1.79 (br, 2H, cyclohexanyl), 1.65-1.68 (br, 2H, cyclohexanyl), 1.37-1.43 (m, 1H, cyclohexanyl), 1.10-1.30 (m, 3H, cyclohexanyl), 1.72-1.79 (m, 2H, cyclohexanyl).

Spectral data for products 2a-2p and (*R*)-1a-1p



(1*R*, 2*R*)-phenyl glycidol (**2a**)[3]

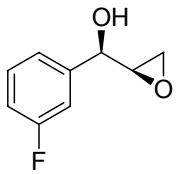


Colorless liquid, $[\alpha]_D^{20}$ = -27 (c 0.6 in $CHCl_3$) (lit,[4] $[\alpha]_D^{24}$ = -11.5 (c 2.4 in $CHCl_3$) 90% ee for (1*R*, 2*R*)); diastereomeric ratios (dr): 98:2 determined by chiral HPLC and ^{1}H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.5 ml min^{-1}): t_{R1} = 21.1 min (minor), t_{R2} = 25.1 min (minor), t_{R3} = 30.0 min (major); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.32-7.43 (m, 5H, Ar-H), 4.98 (d, *J* = 5.52, 1H, -CH), 3.22-3.24 (m, 1H, -CH), 2.85-2.87 (br, 1H, -CH_2), 2.83 (dd, *J* = 2.7, *J* = 4.8, 1H, -CH_2), 2.40 (br, 1H, OH).

(*R*)-1-phenylprop-2-en-1-ol ((*R*)-**1a**)

Colorless liquid, $[\alpha]_D^{20}$ = +1.3 (c 1.0 in $CHCl_3$) , ee >99% determined by HPLC (Daicel CHIRALCEL® OD-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_R = 7.4 min.

(1*R*, 2*R*)-3-fluorophenyl glycidol (**2b**)

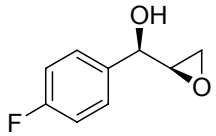


Colorless liquid, $[\alpha]_D^{20}$ = -6.8 (c 0.35 in $CHCl_3$); diastereomeric ratios (dr): 96:4 determined by chiral HPLC and ^{1}H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.5 ml min^{-1}): t_{R1} = 20.9 min (minor), t_{R2} = 24.7 min (minor), t_{R3} = 26.3 min (major); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.00-7.36 (m, 4H, Ar-H), 4.48 (br, 1H, -CH), 3.18-3.21 (m, 1H, -CH), 2.87-2.88 (br, 1H, -CH_2), 2.83-2.84 (br, 1H, -CH_2), 2.58 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 162, 142, 130, 121, 115, 113, 73, 55, 45; HRMS (ESI) exact mass calculated for $(C_9H_9O_2NaF)^+$ requires m/z 191.0484, found m/z 191.0491.

(*R*)-1-(3-fluorophenyl)prop-2-en-1-ol ((*R*)-**1b**)

Colorless liquid, $[\alpha]_D^{20}$ = -14.4 (c 0.75 in $CHCl_3$) , ee 98% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}) : t_{R1} = 9.2 min (minor), t_{R2} = 9.6 min (major).

(1*R*, 2*R*)-4-fluorophenyl glycidol (**2c**)

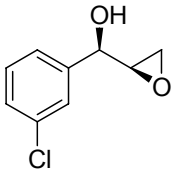


Colorless liquid, $[\alpha]_D^{20}$ = -9.7 (c 0.4 in $CHCl_3$); diastereomeric ratios (dr): 96:4 determined by chiral HPLC and ^{1}H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 19.8 (minor), t_{R2} = 23.0 (major); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.37-7.41 (m, 2H, Ar-H), 7.05-7.08 (br, 2H, Ar-H), 4.48 (d, *J* = 5.16, 1H, -CH), 3.18-3.20 (m, 1H, -CH), 2.86-2.87 (m, 1H, -CH_2), 2.82-2.83 (m, 1H, -CH_2), 2.39 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 161, 135, 128, 115, 73, 55, 45; HRMS (ESI) exact mass calculated for $(C_9H_9O_2NaF)^+$ requires m/z 191.0484, found m/z 191.0490.

(*R*)-1-(4-fluorophenyl)prop-2-en-1-ol ((*R*)-**1c**)

Colorless liquid, $[\alpha]_D^{20}$ = -9.8 (c 0.55 in $CHCl_3$), ee >99% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_R = 10.7 min (major).

(1*R*, 2*R*)-3- chlorophenyl glycidol (**2d**)

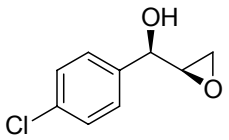


Colorless liquid, $[\alpha]_D^{20}$ = -11. (c 0.25 in $CHCl_3$); diastereomeric ratios (dr): 89:11 determined by chiral HPLC and ^{1}H NMR analysis; enantiomeric excess: 97% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 13.5 (minor), t_{R2} = 14.7 (minor), t_{R3} = 15.2 (minor), t_{R4} = 15.7 (major); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.27-7.43 (m, 4H, Ar-H), 4.48 (d, *J* = 5.52, 1H, -CH), 3.19-3.22 (m, 1H, -CH), 2.87-2.89 (br, 1H, $-CH_2$), 2.83-2.84 (br, 1H, $-CH_2$), 2.41 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 142, 134, 129, 128, 126, 124, 70, 55, 45; HRMS (ESI) exact mass calculated for $(C_9H_9O_2NaCl)^+$ requires m/z 207.0189, found m/z 207.0188.

(*R*)-1-(3-chlorophenyl)prop-2-en-1-ol ((*R*)-**1d**)

Light yellow liquid, $[\alpha]_D^{20}$ = -2.5 (c 0.85 in $CHCl_3$) , ee 55% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 95: 5, flow rate 0.8 ml min^{-1}): t_{R1} = 14.1 min (minor), t_{R2} = 15.0 min (major).

(1*R*, 2*R*)-4- chlorophenyl glycidol (**2e**)



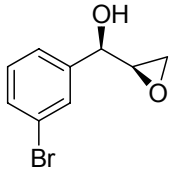
Colorless liquid, $[\alpha]_D^{20}$ = -5.6 (c 0.75 in $CHCl_3$); diastereomeric ratios (dr): >99:1 determined by chiral HPLC and ^{1}H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.5 ml min^{-1}): t_{R1} = 21.8 (minor), t_{R2} = 29.4 (minor), t_{R3} = 32.1 (major); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.33-7.35 (m, 4H, Ar-H), 4.48 (br, 1H, -CH), 3.17-3.19

(m, 1H, -CH), 2.82-2.87 (m, 2H, $-CH_2$), 2.41 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 138, 134, 128, 127, 73, 55, 45; HRMS (ESI) exact mass calculated for $(C_9H_9O_2NaCl)^+$ requires m/z 207.0189, found m/z 207.0193.

(*R*)-1-(4-chlorophenyl)prop-2-en-1-ol ((*R*)-**1e**)

Light yellow liquid, $[\alpha]_D^{20}$ = -7.2 (c 0.85 in $CHCl_3$) , ee 80% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 95: 5, flow rate 0.8 ml min^{-1}): t_{R1} = 14.7 min (minor), t_{R2} = 16.2 min (major).

(1*R*, 2*R*)-3- bromophenyl glycidol (**2f**)

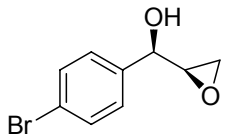


Colorless liquid, $[\alpha]_D^{20}$ = -4 (c 0.2 in $CHCl_3$); diastereomeric ratios (dr): 87:13 determined by chiral HPLC and ^1H NMR analysis; enantiomeric excess: 92% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.5 ml min^{-1}): t_{R1} = 23.7 (minor), t_{R2} = 25.6 (minor), t_{R3} = 26.8 (minor), t_{R4} = 27.7 (major); ^1H NMR (600 MHz, $CDCl_3$): δ 7.24-7.58 (m, 4H, Ar-H), 4.47 (br, 1H, -CH), 3.18-3.21 (m, 1H, -CH), 2.83-2.89 (m, 1H, -CH2), 2.83-2.84 (m, 1H, -CH2), 2.36 (br, 1H, OH); ^{13}C NMR (150 MHz, CDCl3): δ 142, 131, 130, 129, 124, 122, 73, 55, 45; HRMS (ESI) exact mass calculated for $(C_9H_8O_2Br)^-$ requires m/z 226.9708, found m/z 226.9705.

(*R*)-1-(3-bromophenyl)prop-2-en-1-ol ((*R*)-**1f**)

Light yellow liquid, $[\alpha]_D^{20}$ = +6.7 (c 1.0 in $CHCl_3$) , ee 28% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 9.5 min (minor), t_{R2} = 10.0 min (major).

(1*R*, 2*R*)-4- bromophenyl glycidol (**2g**)

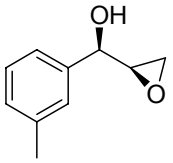


Colorless liquid, $[\alpha]_D^{20}$ = -9.4 (c 0.9 in $CHCl_3$); diastereomeric ratios (dr): >99:1 determined by chiral HPLC and ^1H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 14.7 (minor), t_{R2} = 19.9 (minor), t_{R3} = 22.6 (major); ^1H NMR (600 MHz, $CDCl_3$): 7.51-7.52 (m, 2H, Ar-H), 7.29-7.31 (m, 2H, Ar-H), 4.48 (br, 1H, -CH), 3.17-3.19 (m, 1H, -CH), 2.86-2.87 (m, 1H, $-CH_2$), 2.82-2.83 (m, 1H, $-CH_2$), 2.32 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 139, 131, 127, 122, 73, 55, 45; HRMS (ESI) exact mass calculated for $(C_9H_8O_2Br)^-$ requires m/z 226.9708, found m/z 226.9707.

(*R*)-1-(4-bromophenyl)prop-2-en-1-ol ((*R*)-**1g**)

Light yellow liquid, $[\alpha]_D^{20}$ = +4.8 (c 2.0 in $CHCl_3$) , ee 50% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 95: 5, flow rate 0.8 ml min^{-1}): t_{R1} = 16.1 min (minor), t_{R2} = 17.8 min (major).

(1*R*, 2*R*)-*m*-tolyl glycidol (**2h**)

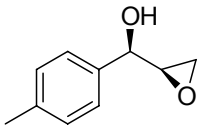


Colorless liquid, $[\alpha]_D^{20}$ = -10.9 (c 0.5 in $CHCl_3$); diastereomeric ratios (dr): 98:2 determined by chiral HPLC and ^1H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 95: 5, flow rate 0.8 ml min^{-1}): t_{R1} = 19.0 (minor), t_{R2} = 23.1 (minor), t_{R3} = 24.8 (major); ^1H NMR (600 MHz, $CDCl_3$): δ 7.13-7.28 (m, 4H, Ar-H), 4.43 (br, 1H, -CH), 3.21-3.23 (m, 1H, -CH), 2.84-2.86 (m, 1H, $-CH_2$), 2.82-2.83 (m, 1H, $-CH_2$), 2.40 (br, 1H, OH), 2.37 (s, 3H, $-CH_3$); ^{13}C NMR (150 MHz, $CDCl_3$): δ 140, 138, 129, 128, 126, 123, 74, 55, 45; HRMS (ESI) exact mass calculated for $(C_{10}H_{12}O_2Na)^+$ requires m/z 187.0735, found m/z 187.0740.

(*R*)-1-*m*-tolylprop-2-en-1-ol ((*R*)-**1h**)

Colorless liquid, $[\alpha]_D^{20}$ = +6.4 (c 0.45 in $CHCl_3$) , ee 87% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 95: 5, flow rate 0.8 ml min^{-1}): t_{R1} = 14.1 min (minor), t_{R2} = 15.4 min (major).

(1*R*, 2*R*)-*p*-tolyl glycidol (**2i**)

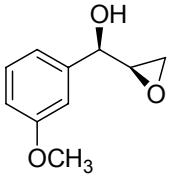


Colorless liquid, $[\alpha]_D^{20}$ = -1.5 (c 0.65 in $CHCl_3$); diastereomeric ratios (dr): >99:1 determined by chiral HPLC and ^1H NMR analysis; enantiomeric excess: 98% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 12.4 (minor), t_{R2} = 16.7 (minor), t_{R3} = 19.1 (major); ^1H NMR (600 MHz, $CDCl_3$): δ 7.18-7.31 (m, 4H, Ar-H), 4.44 (d, *J* = 5.1, 1H, -CH), 3.20-3.22 (m, 1H, -CH), 2.80-2.85 (m, 2H, $-CH_2$), 2.35 (s, 3H, $-CH_3$), 2.31 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 138, 137, 129, 126, 74, 56, 45; HRMS (ESI) exact mass calculated for $(C_{10}H_{12}O_2Na)^+$ requires m/z 187.0735, found m/z 187.0733.

(*R*)-1-*p*-tolylprop-2-en-1-ol ((*R*)-**1i**)

Colorless liquid, $[\alpha]_D^{20}$ = -13 (c 0.2 in $CHCl_3$) , ee 98% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 95: 5, flow rate 0.8 ml min^{-1}): t_{R1} = 17.5 min (minor), t_{R2} = 20.9 min (major).

(1*R*, 2*R*)-3-methoxyphenyl glycidol (**2j**)



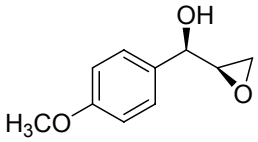
Colorless liquid, $[\alpha]_D^{20}$ = -9 (c 0.1 in $CHCl_3$); diastereomeric datios (dr): 92:8 determined by chiral HPLC and ^1H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 80: 20, flow rate 0.8 ml min^{-1}): t_{R1} = 19.8 min (minor), t_{R2} = 21.1 min (minor), t_{R3} = 25.8 min (minor), t_{R4} = 27.0 min (major); ^1H NMR (600 MHz, $CDCl_3$): δ 7.28-7.31 (m,

1H, Ar-H), 6.96-6.99 (m, 2H, Ar-H), 6.86-6.88 (m, 1H, Ar-H), 4.45 (d, J = 4.74, 1H, -CH), 3.82 (s, 3H, $-OCH_3$), 3.21-3.23 (m, 1H, -CH), 2.82-2.86 (m, 2H, $-CH_2$), 2.36 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 159, 141, 129, 118, 113, 111, 74, 55, 55, 45; HRMS (ESI) exact mass calculated for $(C_{10}H_{12}O_2Na)^+$ requires m/z 203.0684, found m/z 203.0686.

(*R*)-1-(3-methoxyphenyl)prop-2-en-1-ol ((*R*)-**1j**)

Light yellow solid, ee 15% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 15.4 min (minor), t_{R2} = 16.8 min (major).

(1*R*, 2*R*)-4-methoxyphenyl glycidol (**2k**)

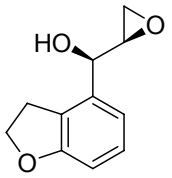


Colorless liquid, $[\alpha]_D^{20}$ = -5 (c 0.1 in $CHCl_3$); diastereomeric ratios (dr): 98:2 determined by chiral HPLC and ^1H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 14.8 (minor), t_{R2} = 27.3 (major); ^1H NMR (600 MHz, $CDCl_3$): δ 7.35 (d, J = 8.46, 2H, Ar-H), 6.91 (d, J = 8.46, 2H, Ar-H), 4.43 (br, 1H, -CH), 3.81 (s, 3H, $-OCH_3$), 3.20-3.22 (m, 1H, -CH), 2.80-2.85 (m, 2H, $-CH_2$), 2.24 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 159, 132, 127, 114, 74, 55, 45; HRMS (ESI) exact mass calculated for $(C_{10}H_{12}O_2Na)^+$ requires m/z 203.0684, found m/z 203.0683.

(*R*)-1-(4-methoxyphenyl)prop-2-en-1-ol ((*R*)-**1k**)

Light yellow solid, ee 20% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 23.0 min (minor), t_{R2} = 24.7 min (major).

(1*R*, 2*R*)-2,3-dihydrobenzofuran-4-yl glycidol (**2l**)

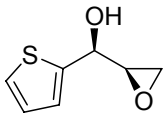


Colorless liquid, $[\alpha]_D^{20}$ = -11 (c 0.15 in $CHCl_3$); diastereomeric ratios (dr): 98:2 determined by chiral HPLC and ^{1}H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 21.7 (minor), t_{R2} = 28.4 (major); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.29 (s, 1H, Ar-H), 7.14 (d, *J* = 8.1, 1H, Ar-H), 6.78 (d, *J* = 8.1, 1H, Ar-H), 4.58 (d, *J* = 8.7, 2H, -CH_2), 4.41 (br, 1H, -CH), 3.20-3.23 (m, 3H, -CH_2, -CH), 2.79-2.85 (m, 2H, -CH_2), 2.21 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 160, 132, 127, 126, 123, 109, 71, 56, 45, 29; HRMS (ESI) exact mass calculated for $(C_{11}H_{12}O_3Na)^+$ requires m/z 215.0684, found m/z 215.0689.

(*R*)-1-(2,3-dihydrobenzofuran-5-yl)prop-2-en-1-ol ((*R*)-**1l**)

Light yellow liquid, ee 13% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 22.5 min (minor), t_{R2} = 22.9 min (major).

(1*S*, 2*R*)-thiophen-2-yl glycidol (**2m**)

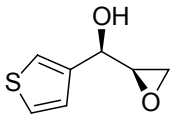


Yellow liquid, $[\alpha]_D^{20}$ = +15.2 (c 0.85 in $CHCl_3$); diastereomeric ratios (dr): 62:38 determined by chiral HPLC and ^{1}H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 80: 20, flow rate 0.8 ml min^{-1}): t_{R1} = 10.0 (minor), t_{R2} = 11.2 (minor), t_{R3} = 15.7 (major); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.34-7.35 (m, 1H, Ar-H), 7.06-7.08 (m, 1H, Ar-H), 6.98-7.00 (m, 1H, Ar-H), 4.83 (d, 1H, -CH), 3.17-3.21 (m, 1H, -CH), 2.73-2.83 (m, 2H, -CH_2); ^{13}C NMR (150 MHz, $CDCl_3$): δ 142, 124, 123, 122, 69, 54, 42; HRMS (ESI) exact mass calculated for $(C_7H_7O_2S)^-$ requires m/z 155.0167, found m/z 155.0171.

(*R*)-1-(thiophen-2-yl)prop-2-en-1-ol ((*R*)-**1m**)

Yellow solid, $[\alpha]_D^{20}$ = -6.0 (c 0.25 in $CHCl_3$), ee 82% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 80: 20, flow rate 0.8 ml min^{-1}): t_{R1} = 8.0 min (minor), t_{R2} = 9.7 min (major).

(1*R*, 2*R*)-thiophen-3-yl glycidol (**2n**)

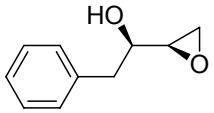


Yellow liquid, $[\alpha]_D^{20}$ = -8.4 (c 0.5 in $CHCl_3$); diastereomeric ratios (dr): 92:8 determined by chiral HPLC and ^1H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 80: 20, flow rate 0.8 ml min^{-1}): t_{R1} = 10.6 (minor), t_{R2} = 11.6 (minor), t_{R3} = 21.1 (major); ^1H NMR (600 MHz, $CDCl_3$): δ 7.12-7.35 (m, 3H, Ar-H), 4.61 (d, *J* = 4.74, 1H, -CH), 3.26-3.27 (m, 1H, -CH), 2.83-2.88 (m, 2H, $-CH_2$), 2.38 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 142, 125, 125, 121, 71, 55, 44; HRMS (ESI) exact mass calculated for $(C_7H_7O_2S)^-$ requires m/z 155.0167, found m/z 155.0166.

(*R*)-1-(thiophen-3-yl)prop-2-en-1-ol ((*R*)-**1n**)

Yellow solid, ee 58% determined by HPLC (Daicel CHIRALCEL® OJ-H, hexane/ 2-propanol = 80: 20, flow rate 0.8 ml min^{-1}): t_{R1} = 7.9 min (minor), t_{R2} = 9.5 min (major).

(1*R*, 2*R*)-benzyl glycidol (**2o**)



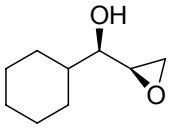
Colorless liquid, $[\alpha]_D^{20}$ = -5.6 (c 0.95 in $CHCl_3$); diastereomeric ratios (dr): 86:14 determined by chiral HPLC and ^1H NMR analysis; enantiomeric excess: >99% determined by HPLC (Daicel CHIRALPAK® AS-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 14.2 (minor), t_{R2} = 16.6 (minor), t_{R3} = 24.3 (minor), t_{R4} = 26.6 (major); ^1H NMR (600 MHz, $CDCl_3$): δ 7.23-7.32 (m, 5H, Ar-H), 3.71-3.74 (m, 1H, -CH), 3.03-3.05 (m, 1H, -CH), 2.95 (dd, *J* = 7.32, *J* = 13.92, 1H, $-CH_2$), 2.90 (dd, *J* = 6.6, *J* = 13.56, 1H, $-CH_2$), 2.74-2.76 (m, 1H, $-CH_2$), 2.62-2.63 (m, 1H, $-CH_2$), 1.93 (br, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 137, 129, 128, 126, 72,

54, 45, 41; HRMS (ESI) exact mass calculated for $(C_{10}H_{12}O_2Na)^+$ requires m/z 187.0735, found m/z 187.0735.

(*R*)-1-phenylbut-3-en-2-ol ((*R*)-**1o**)

Colorless liquid, ee 30% determined by HPLC (Daicel CHIRALCEL® OD-H, hexane/ 2-propanol = 90: 10, flow rate 0.8 ml min^{-1}): t_{R1} = 6.4 min (minor), t_{R2} = 7.1 min (major).

(1*R*, 2*R*)-cyclohexyl glycidol (**2p**)



Colorless liquid, $[\alpha]_D^{20}$ = +1.8 (c 0.8 in $CHCl_3$); diastereomeric ratios (dr): 79:21 determined by chiral GC and ^1H NMR analysis; enantiomeric excess: 78% determined by GC (Flame ionization detector, nitrogen was used as the carrier gas at 1.0 ml min^{-1}, split ratio was 1:30 (v/v), the injector and the detector temperatures were set at 260 °C and 280 °C, the column temperature was programmed as being kept at 100 °C for 1 min and then upgraded to 160 at a rate of 5 °C min^{-1}, CHIRASIL-DEX CB, Varian, USA): t_{R1} = 12.9(minor), t_{R2} = 13.1(minor), t_{R3} = 13.2(minor), t_{R4} = 13.4(major); ^1H NMR (600 MHz, $CDCl_3$): δ 3.16 (br, 1H), 3.03-3.05 (m, 1H), 2.82 (dd, *J* = 2.94, *J* = 4.74, 1H), 2.70 (dd, *J* = 2.94, *J* = 4.74, 1H), 1.92-1.94 (m, 1H), 1.83-1.86 (m, 1H), 1.75-1.77 (m, 1H) 1.67-1.69 (m, 1H), 1.53-1.56 (m 2H), 1.24-1.28 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 75, 54, 45, 42, 28, 28, 26, 26, 26; HRMS (ESI) exact mass calculated for $(C_9H_{16}O_2Na)^+$ requires m/z 179.1048, found m/z 179.1053.

(*R*)-1-cyclohexylprop-2-en-1-ol ((*R*)-**1p**)

Colorless liquid, $[\alpha]_D^{20}$ = +14 (c 0.1 in $CHCl_3$), ee 95%。

This product was transformed into the corresponding oxide using *m*-CPBA as the oxidant, and the enantiomeric excess was determined by chiral GC (*vide supra*).

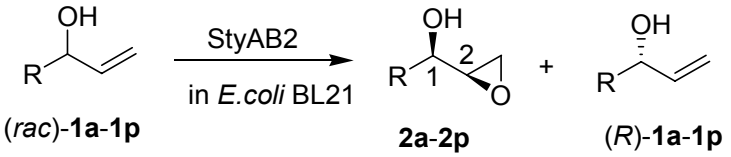
Docking studies

Docking studies were performed using the program AutoDock 4.0 following the User Manual of the program.[5] The X-ray crystal structure of the epoxidase component of SMO from *Pseudomonas putida* S12 (PDB code 3IHM) was used as a rigid receptor for the docking of (*S*)-**1a** and (*R*)-**1a**. Docked conformations were ranked automatically by the Autodock 4.0 program using a free-energy scoring function. The results were showed in the program Pymol.

Construction of point mutations

Plasmid pCDFDuet-1 (Novagen, Madison, WI, USA) encoding the two subunits of StyAB2 was used as the template for all site-directed mutagenesis reactions. PCRs were carried out with the QuikChange® Mutagenesis Kit (Stratagene, La Jolla, CA) following the manufacture's protocol with primers listed in Table S3; the PCR products were purified by DpnI treatment to remove the wild type template. Successful mutations were confirmed by DNA sequencing (Invitrogen, Shanghai, China).

Table S1 Epoxidation of α-hydroxyl olefins (*rac*)-**1a**-**1p**



Entry[a]	Substrate R =	t (h)	*dr*[b]	*ee*[c] (%)	*de*[d] (%)	de_1[e] (%)	Yield[f] (%)	*E*[g]	$ee_{R\text{-}1}$[h] (%)
1	Ph (**1a**)	2	98:2	>99	98	98	50	>200	>99
2	*m*-FC_6H_4 (**1b**)	16	96:4	>99	94	98	51	140	98
3	*p*-FC_6H_4 (**1c**)	16	96:4	>99	96	96	50	190	>99
4	*m*-ClC_6H_4 (**1d**)	48	89:11	97	89	87	42	34	55
5	*p*-ClC_6H_4 (**1e**)	48	>99:1	>99	98	>99	46	>200	80
6	*m*-BrC_6H_4 (**1f**)	48	87:13	92	91	83	28	30	28
7	*p*-BrC_6H_4 (**1g**)	48	>99:1	>99	98	>99	35	170	50
8	*m*-MeC_6H_4 (**1h**)	16	98:2	>99	96	>99	48	150	87
9	*p*-Me C_6H_4 (**1i**)	16	>99:1	98	98	>99	50	>200	98
10	*m*-$OMeC_6H_4$ (**1j**)	48	92:8	>99	90	94	17	22	15
11	*p*-$OMeC_6H_4$ (**1k**)	48	98:2	>99	96	>99	17	59	20
12	2,3-dihydrobenzofuran-5-yl (**1l**)	48	98:2	>99	>99	96	12	>200	13
13	Thiophen-2-yl (**1m**)	3	62:38	98	31	85	58	3	82
14	Thiophen-3-yl (**1n**)	3	92:8	>99	86	98	40	23	95
15	Benzyl (**1o**)	24	86:14	>99	85	85	25	16	30
16[i]	Cyclohexyl (**1p**)	24	79:21	78	66	83	52	10	95

[a] The absolute configuration of **2a** was established by comparing the ^{1}H-NMR spectrum and $[\alpha]_D^{20}$ with those in literature, and the others based on the analogous chromatographic behavior of racemic mixtures. [b] Diastereoisomeric ratios (*dr*) = *erythro*: *threo*, determined *via* ^{1}H-NMR. [c] Determined *via* chiral HPLC analysis. [d] The diastereomeric excess for the hydroxyl group: de = (|[1*R*, 2*R*]-[1*S*, 2*R*]|)/([1*R*, 2*R*]+[1*S*, 2*R*]), determined *via* chiral HPLC analysis. [e] The diastereomeric excess for the oxiranyl group de_1 = (|[1*R*, 2*R*]-[1*R*, 2*S*]|)/([1*R*, 2*R*]+[1*R*, 2*S*]), determined *via* chiral HPLC analysis. [f] Determined *via* reverse-phase HPLC analysis, and defined as (moles of product formed) / (moles of total added substrate). [g] Enantiomeric ratio *E* = ln[1-Yield(1+de)]/ ln[1-Yield(1-de)]. [h] $ee_{R\text{-}1}$: Enantiomeric excess of product *R*-**1a**, determined *via* chiral HPLC. [i] Determined *via* chiral GC analysis.

Table S2 Primers used in site-directed mutagenesis

Mutation	Primer
Y73F	Forward 5′- CCATCTGATGAATTCGGTTTCTTTGGCCACTACTACTATG -3′
	Reverse 5′- CATAGTAGTAGTGGCCAAAGAAACCGAATTCATCAGATGG -3′
T200V	Forward 5′- GAGCTGATCGAAATTCCAGTGCTCTCGTTCAACGGCATG -3′
	Reverse 5′- CATGCCGTTGAACGAGAGCACTGGAATTTCGATCAGCTC -3′

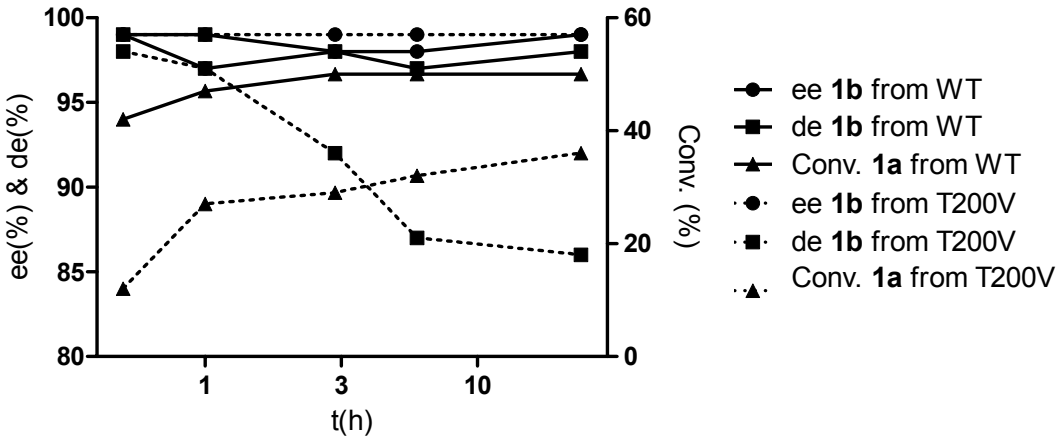


Fig. S1 Kinetic resolution of **1a** (0.6 g L^{-1}) with the resting cells of *E. coli* BL21 expressing the wild type SMO and the mutant T200V (100 g wet weight L^{-1})

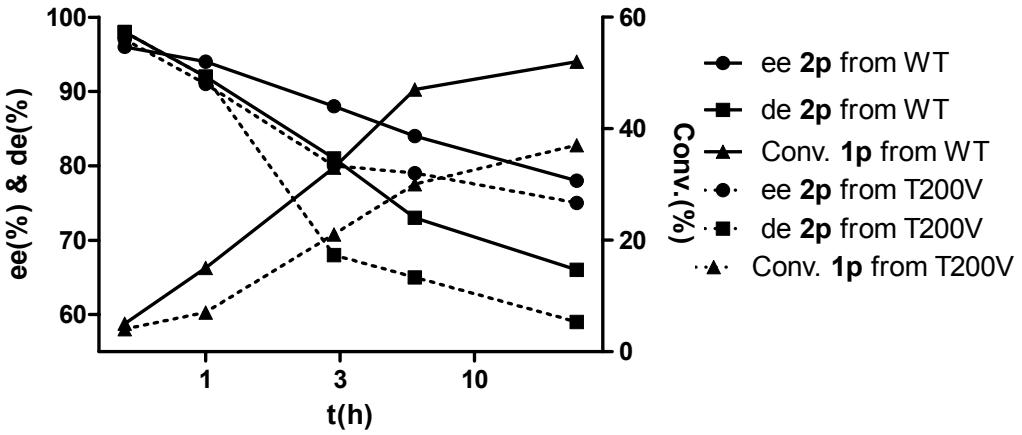


Fig. S2 Kinetic resolution of **1p** (0.6 g L^{-1}) with the resting cells of *E. coli* BL21 expressing the wild type SMO and the mutant T200V (100 g wet weight L^{-1})

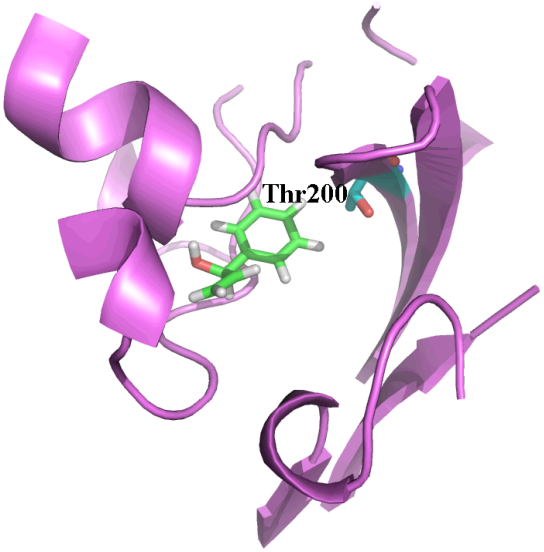


Fig. S3 Orientation of (*R*)-**1a** docked into the putative active site of SMO (PDB code 3IHM). Substrate is shown in the stick mode. The figure was generated using the program Autodock and displayed using Pymol

References

1. D. Gryko, Z. Urbanczyk-Lipkowska and J. Jurczak, *Tetrahedron: Asymmetry*, 1997, **8**, 4059-4067.
2. L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis*, 1967, **1**, 135.
3. D. R. Kelly, E. Caroff, R. W. Flood, W. Heal and S. M. Roberts, *Chem. Commun.*, 2004, 2016-2017.
4. M. Takeshita, R. Yaguchi and N. Akutsu, *Tetrahedron: Asymmetry*, 1992, **3**, 1369-1372.
5. G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, *J. Comput. Chem.*, 1998, **19**, 1639-1662.