molecules

MDPI

Supplementary Materials: Production of Enantiopure Chiral Epoxides with *E. coli* Expressing Styrene Monooxygenase

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1. Expression of SMO

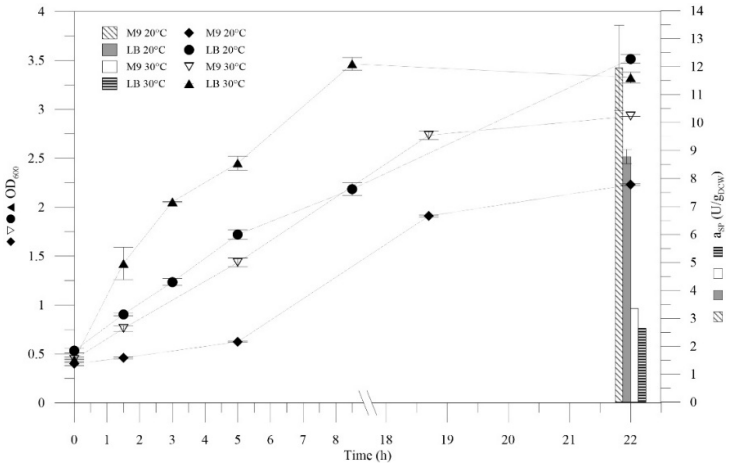


Figure S1. Cell growth after induction of SMO expression (0.25 mM IPTG) in LB and M9 medium and final specific activity of SMO.

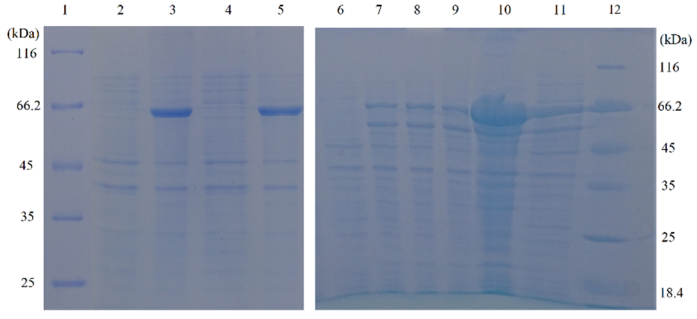


Figure S2. SDS-PAGE electrophoresis of induction of SMO expression by different concentration of IPTG. OD_{600} = 0.7 – 0.8. Lane 1 – protein ladder; Lane 2, 3 – 0.25 mM IPTG 0, 4 h; Lane 4, 5 – 0.5 mM IPTG 0, 4 h; Lane 6, 7, 8, 9 – 1 mM IPTG 0, 2, 3, 4 h; Lane 10 – cell pellet (inclusion bodies); Lane 11 – cell extract (soluble SMO); Lane 12 – protein ladder.

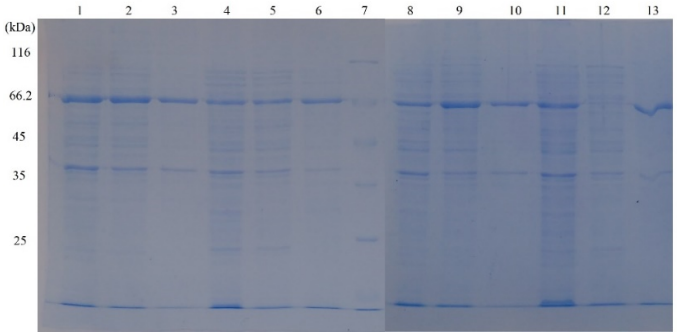


Figure S3. SDS-PAGE electrophoresis of induction of SMO expression at 20 and 30 °C in LB and M9 medium. OD_{600} = 0.4 – 0.5. Lane 1, 2, 3 – LB, 20 °C whole cells, crude extract, pellet; Lane 4, 5, 6 – LB, 30 °C whole cells, crude extract, pellet; Lane 7 – protein ladder, Lane 8, 9, 10 – M9, 20 °C whole cells, crude extract, pellet; Lane 11, 12, 13 – M9, 30 °C whole cells, crude extract, pellet.

2. High cell density fermentation

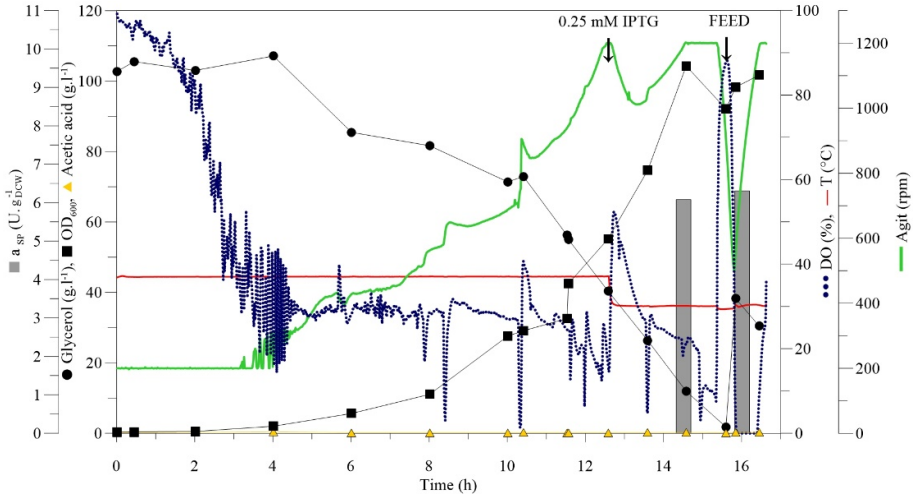


Figure S4. HCD batch fermentation of *E. coli* expressing SMO performed on the 0.5 L scale.

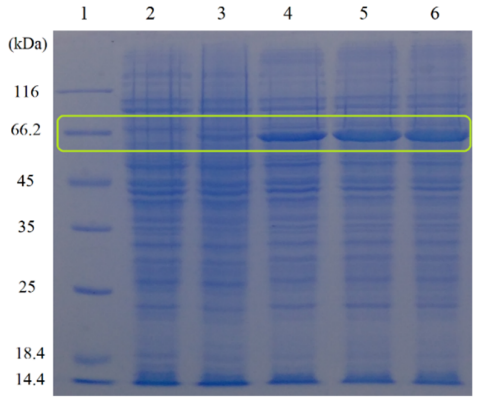


Figure S5. Protein profile of *E. coli* after induction of SMO expression during HCD fermentation. Lane 1: protein ladder, Lane 2: 0 h, Lane 3: 2 h, Lane 4: 4 h, Lane 5: 6 h, Lane 6: 7.5 h after induction.

3. Purification of recombinant SMO

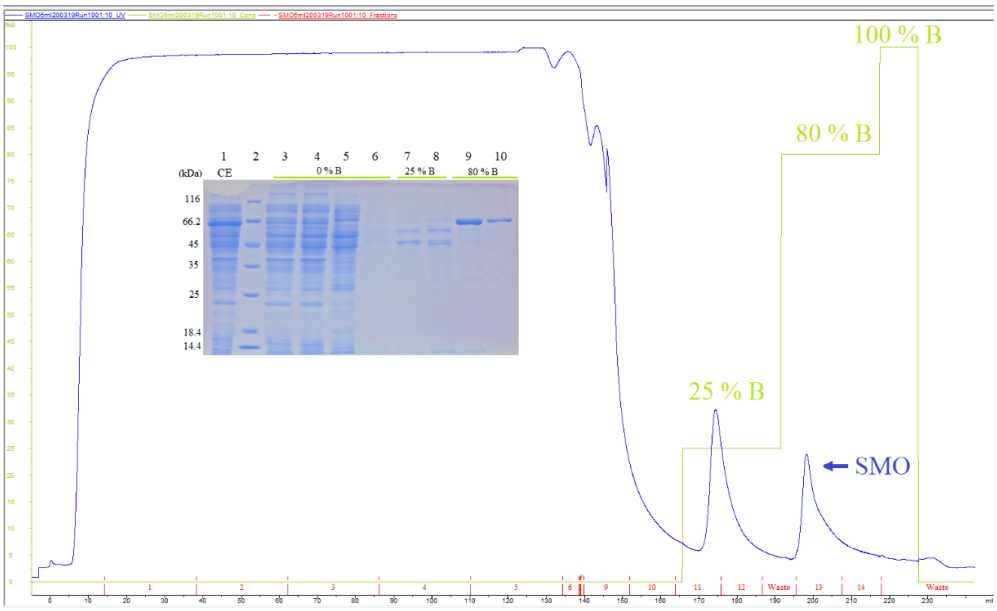


Figure S6. Isolation of SMO by immobilised Ni^{2+} affinity chromatography.

Table S1. Summarised results of SMO purification.

Resin volume (ml)	Loaded crude extract volume (ml)	Recovered SMO amount (mg)	Yield (mg/mL$_{CE}$)
5	50	8.4	0.2
50	300	34	0.1

4. Biotransformation of alkenes

Table S2. The evaluation of SMO specific activity during purification.

The form of SMO	Specific SMO activity (U/g$_{DCW}$)
whole-cell	9.3 ± 0.5
CE	8.1 ± 0.4
purified	0.03 ± 0.003

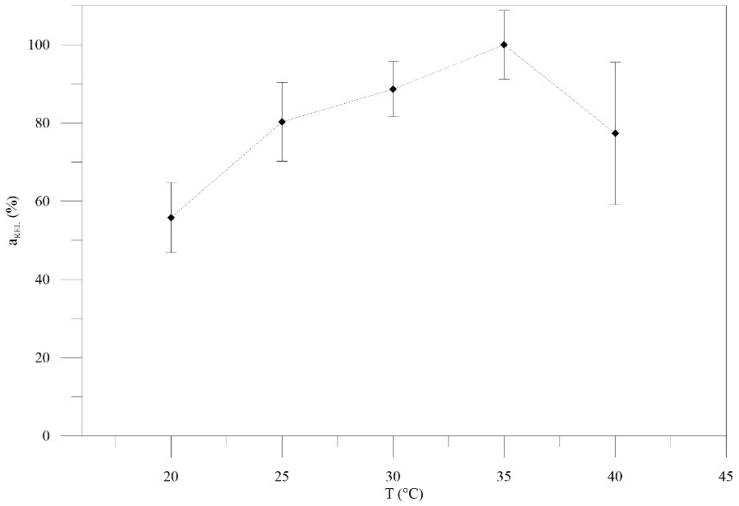


Figure S7. Temperature profile of SMO in form of crude extract.

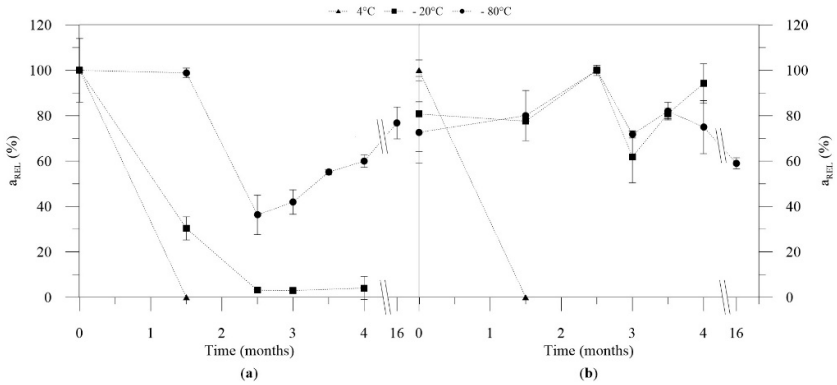


Figure S8. Storage of SMO in form of whole cells (**a**) and crude extract (**b**).

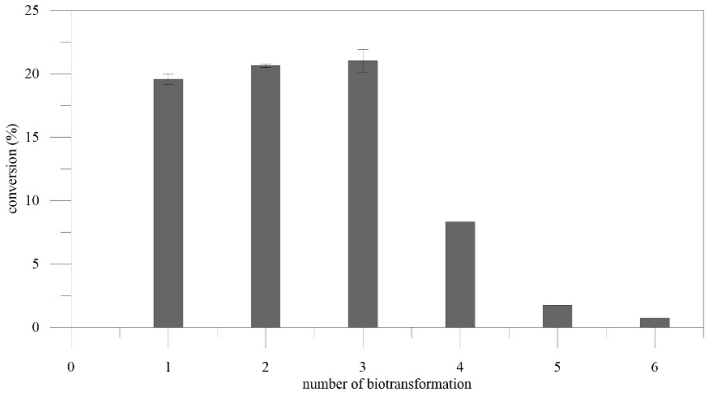
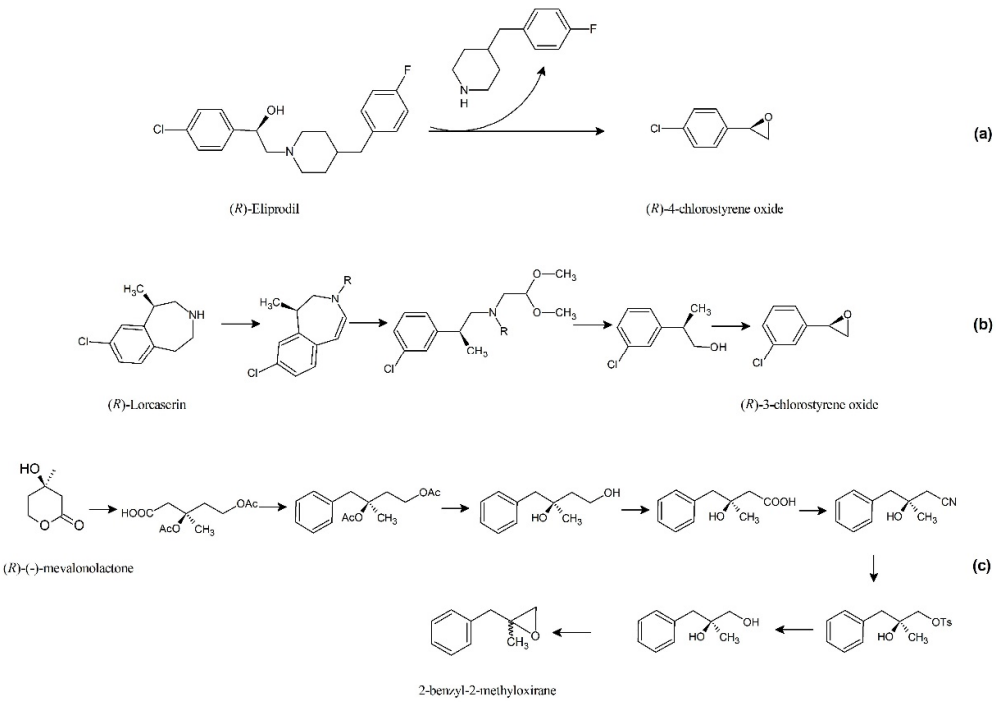


Figure S9. Repeated biotransformation of styrene by whole-cell SMO.

5. Upscale production of chiral epoxides



Scheme S1. Retrosynthesis of (*R*)-4-chlorostyrene oxide **(a)** [37], (*R*)-3-chlorostyrene oxide **(b)** [35], and 2-benzyl-2-methyloxirane **(c)** [35].

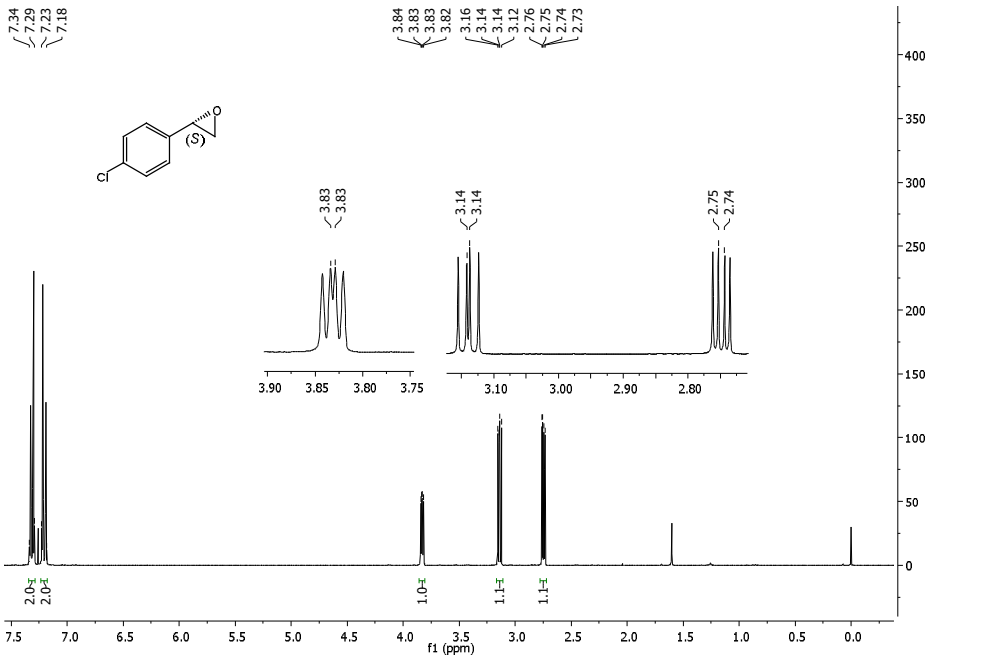


Figure S10. ^{1}H NMR spectrum of (*S*)-4-chlorostyrene oxide.

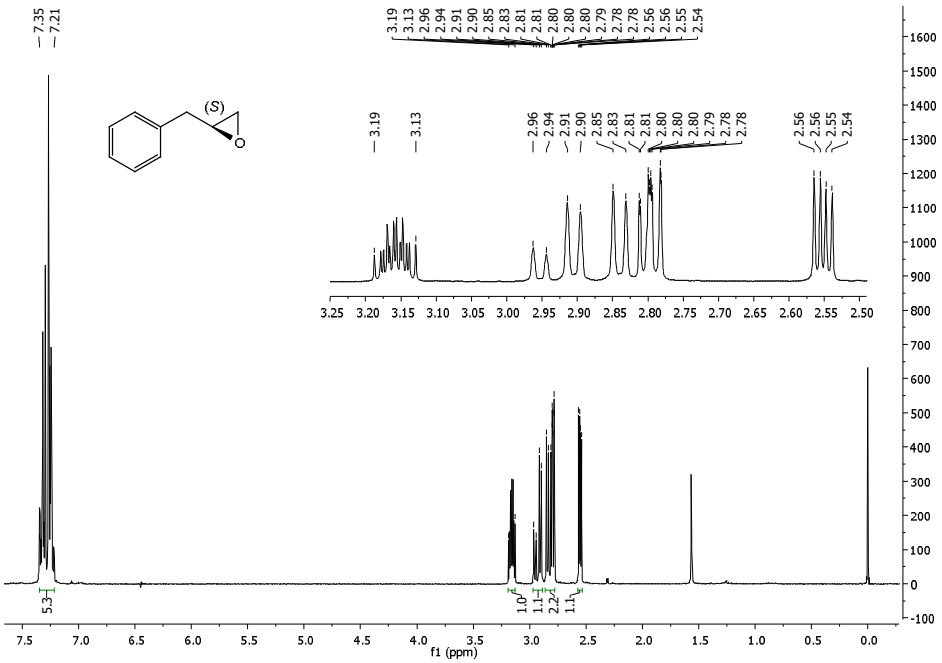


Figure S11. ^{1}H NMR spectrum of (*S*)-allylbenzene oxide.

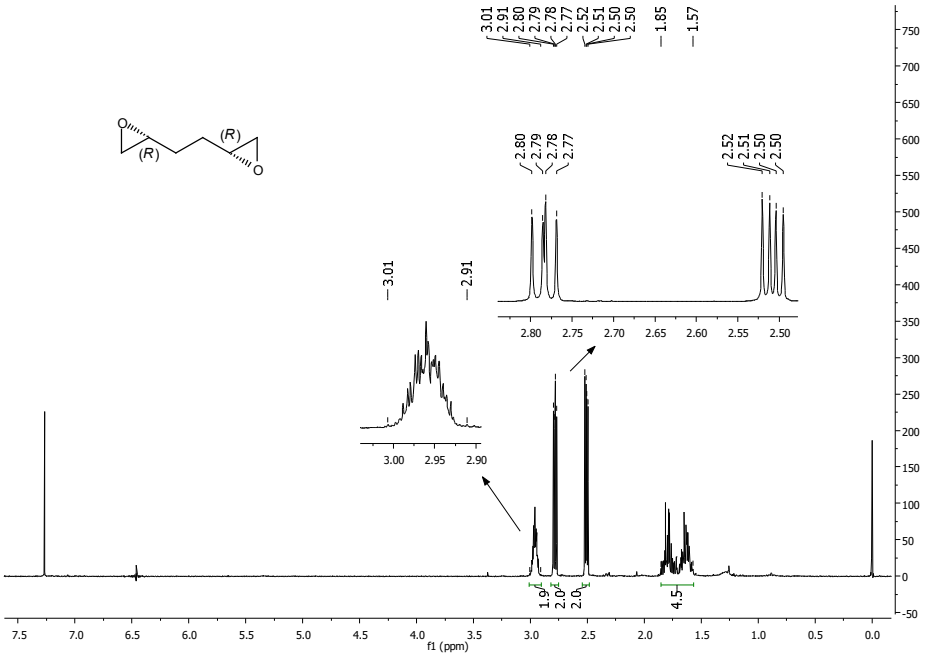


Figure S12. ^{1}H NMR spectrum of (2*R*,5*R*)-1,2:5,6-diepoxyhexane.

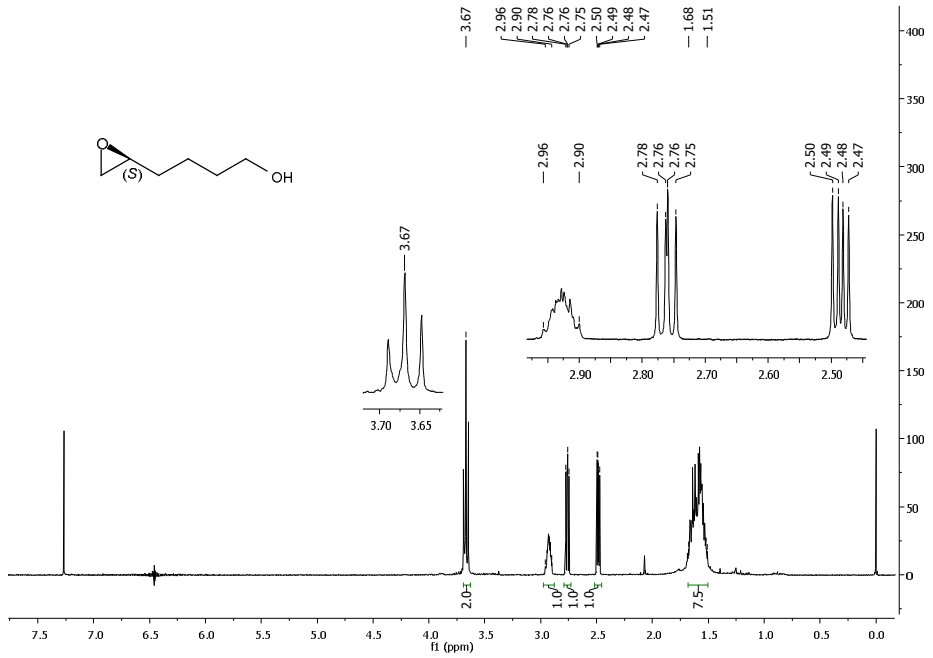


Figure S13. ^{1}H NMR spectrum of (*S*)-4-(oxiran-2-yl)butan-1-ol.

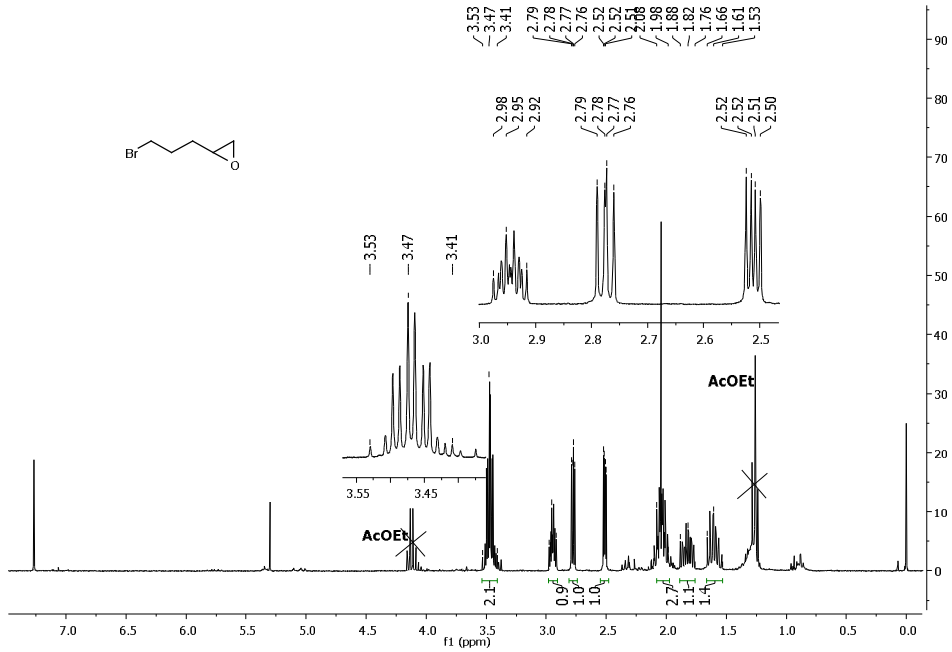


Figure S14. ^{1}H NMR spectrum of 2-(3-bromopropyl)oxirane.