Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

The Mutagenesis of a Single Site for Enhancing or Reversing the Enantio- or Regiopreference of Cyclohexanone Monooxygenases

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KEYWORDS: Baeyer-Villiger monooxygenase, directed evolution, stereoselectivity, chirality, biocatalysis.

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1. Experimental methods

Materials.

Substrates (**1a**-**1f**, **3a**-**3c**, **6a-6c**) were purchased from Energy-Chemical (China). Substrates (**1g**-**1l**) were prepared according to the methods reported in the literature.[1] Hot Start DNA polymerase was purchased from TOKYO (Japan); *Dpn* I was purchased from Thermo Fischer Scientific Inc. All solvents and other reagents were analytical grade and used without further purification.

Analytical methods.

Gas chromatographic analyses (GC) was conducted on a Shimadzu GC-1024C chromatograph equipped with a flame ionization detector (FID) and a CP-chirasil-DEX CB 25cm×0.25cm column (Agilent). Chiral HPLC was performed with Chiralpak AS-H column an OB-H column (250 mm×4.6 mm, *n*-hexane/2-propanol as the mobile phase) and a UV detector (220 nm). The ^{1}HNMR spectra were recorded using a Bruker DRX 400 NMR spectrometer (Rheinstetten, Germany) and chemical shifts were expressed in ppm.

Generation of mutant libraries

CHMO genes from *Acinetobacter* sp. NCIMB 9871[2a] were cloned into vector pET-22b (+) in Sangon Biological Technology (China). CHMO gene from *Rhodococcus* sp. HI-31*(chnB1)*[2b]*, Rhodococcus* sp. HI-31-(chnB2)[2b] and *Thermocrispum municipale*[2c] were cloned into vector pET-22b (+) in TSINGKE Biological Technology (China).

PCR were performed using CHMOs genes (pET-22b (+)) as the template for mutagenesis. Table S1 (Supporting Information) provides the oligonucleotide primers used for the generation of mutant libraries. PCR mixtures (50 μL final volume) contained: ddH_2O (25 μL), 10KOD buffer (5 μL), $MgSO_4$ (3 μL, 25 mM), dNTP (5 μL, 2 mM each), forward and reverse primers (5 μL, 2.5 μM each), template plasmid (1 μL, 50 ng/μL) and 1 μL of KOD Hot Start DNA polymerase. The PCR mixtures were initially subjected to 94°C for 5 min, followed by 18 cycles of denaturing step at 94°C for 1 min, annealing at 60°C for 1 min and elongation at 72°C for 8 min. And final extension step at 72°C for 10 min was performed. To ensure the elimination of template plasmid, PCR mixtures were digested at 37°C overnight after adding 2 μL *Dpn* I (10 U/μL). The digested product was purified with an Omega PCR purification spin column, and then an aliquot of 20 μL was used to transform electrocompetent *E. coli* BL21 (DE3) cells. The transformation mixture was shaken with 1 mL of LB medium at 37°C for 1 h, and spread on LB-agar plates containing 100 μg/mL ampicillin.

Expression of CHMO variants

Single colony was picked into 5.0 mL LB media with 100 μg/mL ampicillin, and then incubated at 37°C under shaking of 200 rpm for 12h. After DNA sequencing, the target mutants were conserved at -80°C with 30% glycerol aliquot. A fresh 20.0 mL of TB media in 50 mL erlenmeyer flasks containing 100 μg/mL ampicillin mixed with 200 μL preculture was inoculated at 37°C, 200 rpm until the OD_{600} reached between 0.6 and 0.7. Then isopropyl β-thiogalactopyranoside (IPTG) used to induce CHMO expression was added to a final concentration of 0.2 mM and the incubation was continued for additional 16 h at 17°C, 200rpm. Then the cells were harvested by centrifugation (30 min, 5000 rpm, 4°C) and were flushed by 50 mM PBS (pH 7.4) three times. The weighing wet cells were resuspended in the fresh 50 mM PBS (pH 7.4) to obtain a final concentration of 0.1g/mL.

Mutant library screening

In the whole cell screening protocol, the reaction system contained 1 mL cell culture (0.1 g/mL), 7 μL of

a stock solution of 0.5 M ketones or sulfides in acetonitrile and D-glucose (3 equiv). The mixture in 10 mL glass tube with a sealed cap was shaken at 200 rpm and 17°C. For the desymmetrization, the reaction time was 10-24 h. For the oxidative kinetic resolution of ketones, the conversion was limited less than 50%. The reaction was stopped by adding sodium chloride and the mixture was extracted with 1 mL ethyl acetate three times. The sample was analyzed by chiral gas chromatographic analyses (GC) or high performance liquid chromatography (HPLC) to determine the conversion and the enantiomeric excess of the residues and products.

Determination of kinetic parameters

Kinetic parameters of $CHMO_{Acineto}$ were measured by monitoring the decrease of NADPH following the absorbance at 340 nm using spectrophotometry method (SHIMADZU-UV-2550). The activity assay was performed in a mixture (0.5 mL) containing 50 mM sodium phosphate buffer (pH 7.4), an appropriate amount of the enzyme and varying concentration of 3-Phenylcyclobutan-1-one (0-5 mM) with 5% (final) acetonitrile as a cosolvent. NADPH was added to a final concentration of 150 µM as the last component to start the reaction.

Scaling-up reaction

The scaling-up reaction was performed with 0.5 L cell culture (0.1 g/mL), 2.9g/L **1a** and D-glucose (3 equiv). The mixture was shaken at 200 rpm and 17°C. The reaction was stopped after 16h by adding sodium chloride and the mixture was extracted with 0.5 L ethyl acetate three times. The sample was analyzed by chiral gas chromatographic analyses (GC) and high performance liquid chromatography (HPLC) to determine the conversion and the enantiomeric excess of the residues and products.

Computational modelling

The crystal structure of $CHMO_{Acineto}$ was not reported so far. Recently, the structure of a CHMO mutant from *Acinetobacter* calcoaceticus (with 70% identity to WT $CHMO_{Acineto}$) has been obtained,[3] however, considering 10 residues have been substituted, it remains uncertain whether this structure would be a good alternative for structural analysis. Here, $CHMO_{Thermo}$ (from *Thermocrispum municipale*)[4] and $CHMO_{Rhodo}$ (from *Rhodococcus* sp. HI-31(chnB))[5] both with a bound ligand were referred. The homology model of WT CHMO was built based on the crystal structure of CHMO from *Rhodococcus* sp. strain HI-31 (PDB code: 4RG3) [5]. The computational methods referred to the literature[6].

2. Additional tables and figures

Table S1. List of Forward and Reverse Primers

	Primers	Sequence
$CHMO_{Acineto}$	Forward F277A	CAGGTGGCGGTGCTCGTTTCATGTTTG
	Forward F277C	CAGGTGGCGGTTGTCGTTTCATGTTTG
	Forward F277D	CAGGTGGCGGTGATCGTTTCATGTTTG
	Forward F277E	CAGGTGGCGGTGAACGTTTCATGTTTG
	Forward F277G	CAGGTGGCGGTGGTCGTTTCATGTTTG
	Forward F277H	CAGGTGGCGGTCATCGTTTCATGTTTG
	Forward F277I	CAGGTGGCGGTATTCGTTTCATGTTTG
	Forward F277K	CAGGTGGCGGTAAACGTTTCATGTTTG
	Forward F277L	CAGGTGGCGGTCTTCGTTTCATGTTTG
	Forward F277M	CAGGTGGCGGTATGCGTTTCATGTTTG
	Forward F277N	CAGGTGGCGGTAATCGTTTCATGTTTG
	Forward F277P	CAGGTGGCGGTCCGCGTTTCATGTTTG
	Forward F277Q	CAGGTGGCGGTCAGCGTTTCATGTTTG
	Forward F277R	CAGGTGGCGGTCGTCGTTTCATGTTTG
	Forward F277S	CAGGTGGCGGTTCTCGTTTCATGTTTG
	Forward F277T	CAGGTGGCGGTACACGTTTCATGTTTG
	Forward F277V	CAGGTGGCGGTGTTCGTTTCATGTTTG
	Forward F277W	CAGGTGGCGGTTGGCGTTTCATGTTTG
	Forward F277Y	CAGGTGGCGGTTATCGTTTCATGTTTG
	Reverse primer	GCGGCCGCTCTGGATCCATGC
$CHMO_{Rhodo2}$	Forward F282V	GATAAAGGTGGTGGTGTGCAGTTTATGTTTGG
	Reverse F282V	CCAAACATAAACTGCACACCACCACCTTTATC
	Forward F282I	GGGATAAAGGTGGTGGTATTCAGTTTATGTTTGG
	Reverse F282I	CCAAACATAAACTGAATACCACCACCTTTATCCC
	Forward F282P	GATAAAGGTGGTGGTCCGCAGTTTATGTTTGG
	Reverse F282P	CCAAACATAAACTGCGGACCACCACCTTTATC
	Forward F282W	GATAAAGGTGGTGGTTGGCAGTTTATGTTTGGTAC
	Reverse F282W	GTACCAAACATAAACTGCCAACCACCACCTTTATC
$CHMO_{Thermo}$	Forward F279V	GATAAAGGCAATGGTGTGCGTTTTATGTTTGG
	Reverse F279V	CCAAACATAAAACGCACACCATTGCCTTTATC
	Forward F279I	GGGATAAAGGCAATGGTATTCGTTTTATGTTTGGC
	Reverse F279I	GCCAAACATAAAACGAATACCATTGCCTTTATCCC
	Forward F279P	GATAAAGGCAATGGTCCGCGTTTTATGTTTGG
	Reverse F279P	CCAAACATAAAACGCGGACCATTGCCTTTATC
	Forward F279W	GATAAAGGCAATGGTTGGCGTTTTATGTTTGGC
	Reverse F279W	GCCAAACATAAAACGCCAACCATTGCCTTTATC
$CHMO_{Rhodo}$	Forward F279V	CATGGTGGTGGTGTGCGTTTTATGTTTG
	Reverse F279V	CAAACATAAAACGCACACCACCACCATG
	Forward F279I	GATCATGGTGGTGGTATTCGTTTTATGTTTGG
	Reverse F279I	CCAAACATAAAACGAATACCACCACCATGATC
	Forward F279P	CATGGTGGTGGTCCGCGTTTTATGTTTG
	Reverse F279P	CAAACATAAAACGCGGACCACCACCATG
	Forward F279W	CATGGTGGTGGTTGGCGTTTTATGTTTG
	Reverse F279W	CAAACATAAAACGCCAACCACCACCATG 3'

Table S2. The Desymmetrization of Ketones **1a** by 277X Mutants of $CHMO_{Acineto}$[a]

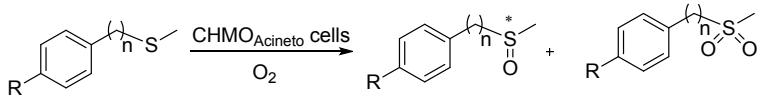
Entry	Enzymes	ee_p/% [b, c]	Conv./%[d]
1	WT	60(*R*)	99
2	277W	99(*R*)	99
3	277Y	38(*R*)	99
4	277A	96(*S*)	99
5	277C	92(*S*)	99
6	277D	39(*S*)	76
7	277E	40(*S*)	83
8	277G	91(*S*)	89
9	277H	42(*S*)	60
10	277I	97(*S*)	99
11	277K	44(*S*)	40
12	277L	49(*S*)	99
13	277M	36(*S*)	99
14	277N	87(*S*)	78
15	277P	93(*S*)	99
16	277Q	87(*S*)	99
17	277R	93(*S*)	99
18	277S	93(*S*)	99
19	277T	86(*S*)	83
20	277V	96(*S*)	99

[a] The whole cell experiments are described in Experimental section. [b] The enantiomeric excess (ee) values of lactones were calculated by HPLC data. [c] The absolute configurations of lactones were confirmed by comparison with the literature[7]. [d] The conversion was determined by GC. Note that the *R/S* assignment is according to the Cahn-Ingold-Prelog convention.

Table S3 Kinetic data of WT-$CHMO_{Acineto}$ and its mutants, 3-phenylcyclobutan-1-one (**1a**) was used as substrate for the kinetic analysis

Entry	Enzyme	V_{max}(μmol s^{-1})	K_m(mM）	k_{cat}(s^{-1})	k_{cat}/K_m(mM^{-1} s^{-1})
1	WT	0.013±0.00037	26.45±2.27	4.26±0.40	0.16±0.0014
2	277W	0.020±0.003	14.47±2.72	9.78±1.51	0.68±0.023
3	277I	0.025±0.0018	8.56±1.86	12.54±0.92	1.49±0.22

Table S4 The Sulfoxidation of Sulfides **6a-6c** by WT $CHMO_{Acineto}$ and Selected Variants[a].



6a-c

a R = H, n=1
b R = Cl, n=0
c R = H, n=0

(*S*)- or (*R*)-**7a-c**

8a-c

Entry	Sub	Enzymes	Major product	Conv. /%[b]	ee_p /%[c]	Config.[d]	Sulfone **8**/%
1	**6a**	WT(F277)	**7a**	92	60	*R*	<1
2	**6a**	F277V	**7a**	87	70	*S*	<1
3	**6a**	F277P	**7a**	85	64	*S*	<1
4	**6a**	F277I	**7a**	74	57	*S*	<1
5	**6b**	WT(F277)	**7b**	99	22	*S*	2.9
6	**6b**	F277V	**7b**	88	43	*R*	1.2
7	**6b**	F277P	**7b**	93	41	*R*	2.5
8	**6b**	F277I	**7b**	82	25	*R*	<1
9	**6c**	WT(F277)	**7c**	99	94	*R*	<1
10	**6c**	F277V	**7c**	89	37	*R*	<1
11	**6c**	F277P	**7c**	80	34	*R*	<1
12	**6c**	F277I	**7c**	71	10	*R*	<1

[a] The whole cell experiments are described in Experimental section. [b] The conversion was calculated by HPLC data. [c] The enantiomeric excess (ee) values of lactones were calculated by HPLC data. [d] The absolute configurations of lactones were confirmed by comparison with the literature[8]. Note that the *R/S* assignment is according to the Cahn-Ingold-Prelog convention.

Table S5. The Conversation of Baeyer-Villiger Oxidation of Ketones **1a**-**1f** by CHMOs and Their Variants[a,b].

CHMOs	Variants	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**
$CHMO_{Thermo}$	WT(F279)	99	99	99	99	99	99
	F279W	99	99	99	99	99	99
	F279P	99	99	99	99	99	99
	F279V	99	99	99	99	99	99
	F279I	99	99	99	99	99	99
$CHMO_{Rhodo}$	WT(F279)	99	99	99	99	99	99
	F279W	99	99	99	99	46	40
	F279P	99	99	99	99	76	99
	F279V	99	99	99	99	73	99
	F279I	99	99	99	99	69	99
$CHMO_{Rhodo2}$	WT(F282)	99	99	99	99	85	99
	F282W	99	99	99	99	25	59
	F282P	99	99	99	99	79	93
	F282V	99	99	99	99	83	91
	F282I	99	99	99	99	70	80

[a] The whole cell experiments are described in Experimental section. [b] The conversion was calculated by HPLC or GC data.

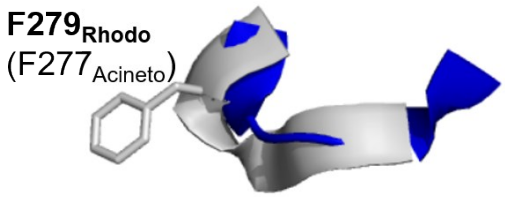


Figure S1. Protein structure alignment of $CHMO_{Rhodo}$ (PDB ID: 4RG3, 276-280 (274-278 numbered in $CHMO_{Acineto}$), pale gray cartoon) and PAMO (PDB ID: 2YLT, 282-286, blue cartoon).

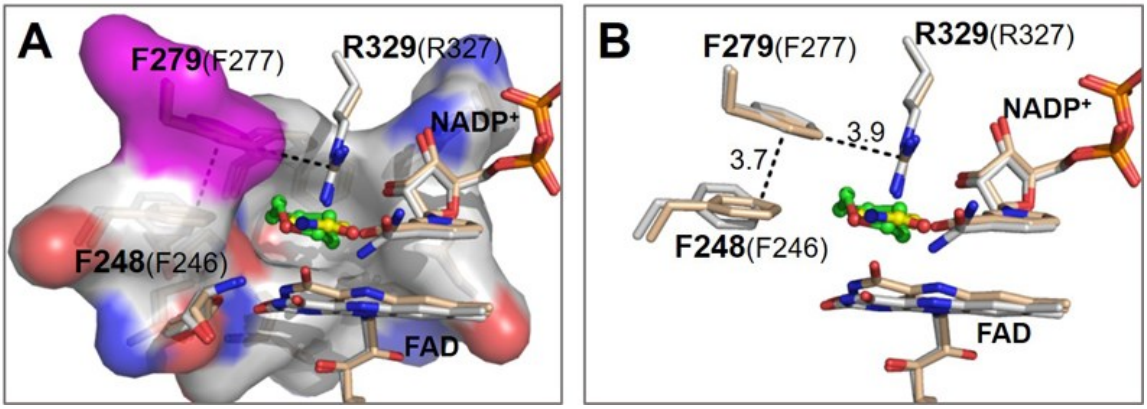


Figure S2. Superposition of active sites and ligands in $CHMO_{Thermo}$ (PDB ID: 5M10)[4] and $CHMO_{Rhodo}$ (PDB ID: 4RG3)[5]. (A) The active sites of $CHMO_{Rhodo}$ shown with surface; (B) The active sites of $CHMO_{Thermo}$ (pale orange carbon atoms) and $CHMO_{Rhodo}$ (pale gray carbon atoms). The unit of the critical distance is Å.

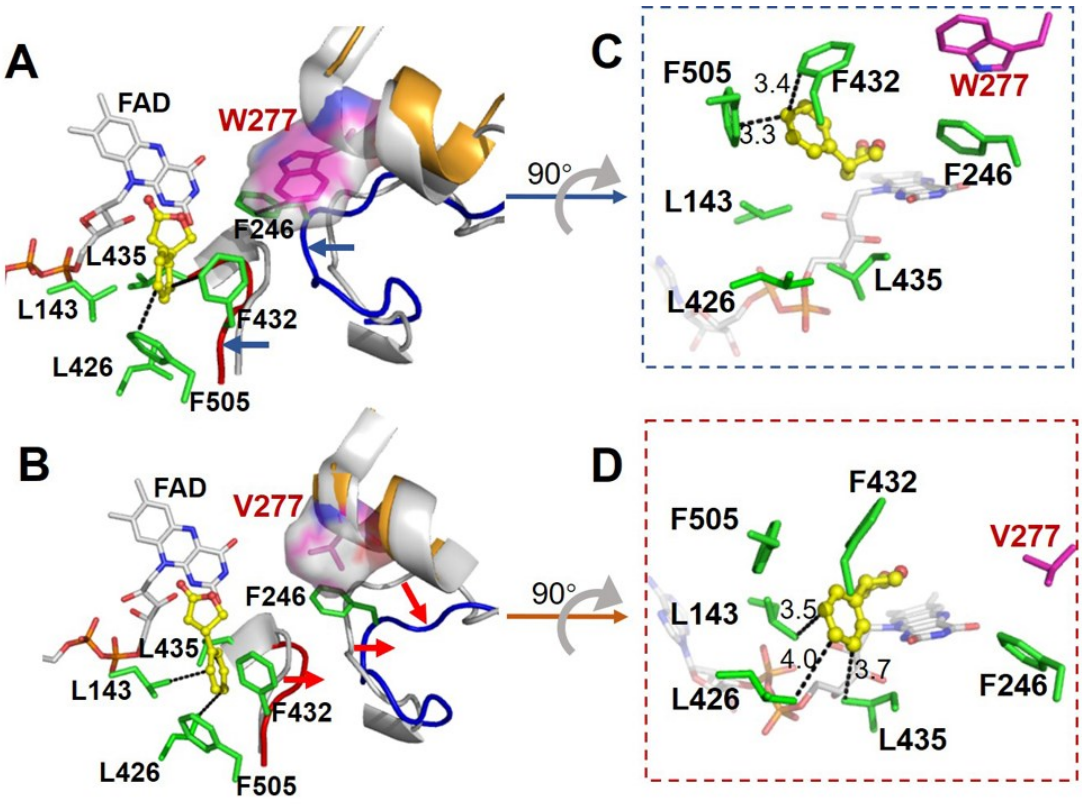


Figure S3. Comparison of MD structures of WT CHMO and its mutants. (A) F277W complexed with (R)-**2a**; (B) F277V complexed with (S)-**2a**; (C), (D) A rotation about 90 degrees into the page from the perspective of (A), (B). WT is shown with pale gray cartoon as a reference; Ligand **2a** is shown with ball and sticks (yellow carbon atoms); The crucial position 277 is shown with sticks and surface. The unit of distances is in Å.

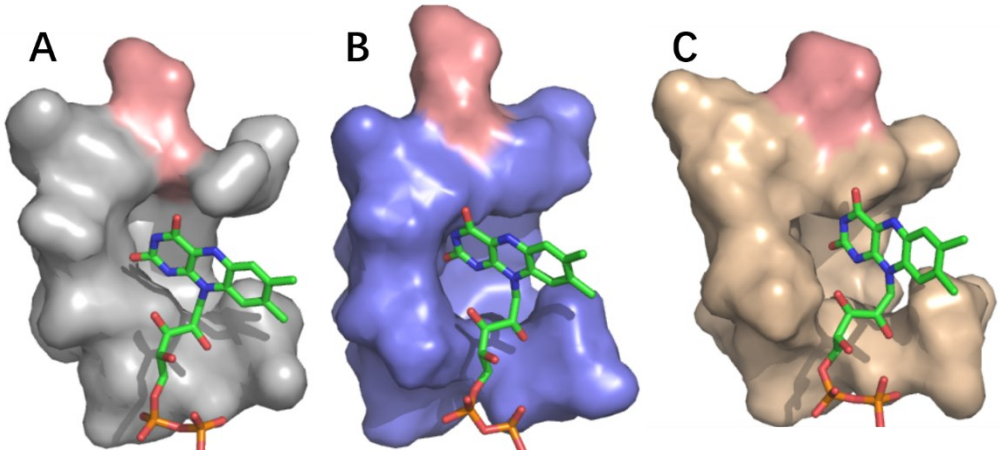


Figure S4. The reshaped binding pocket (surface) caused by the mutagenesis of F277 (pink). (A) WT; (B) F277W; (C) F277V.

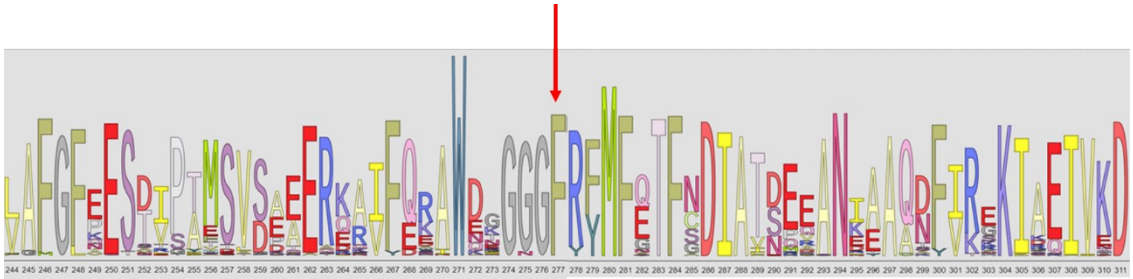


Figure S5. Protein sequence alignment of $CHMO_{Acineto}$ and other BVMOs.

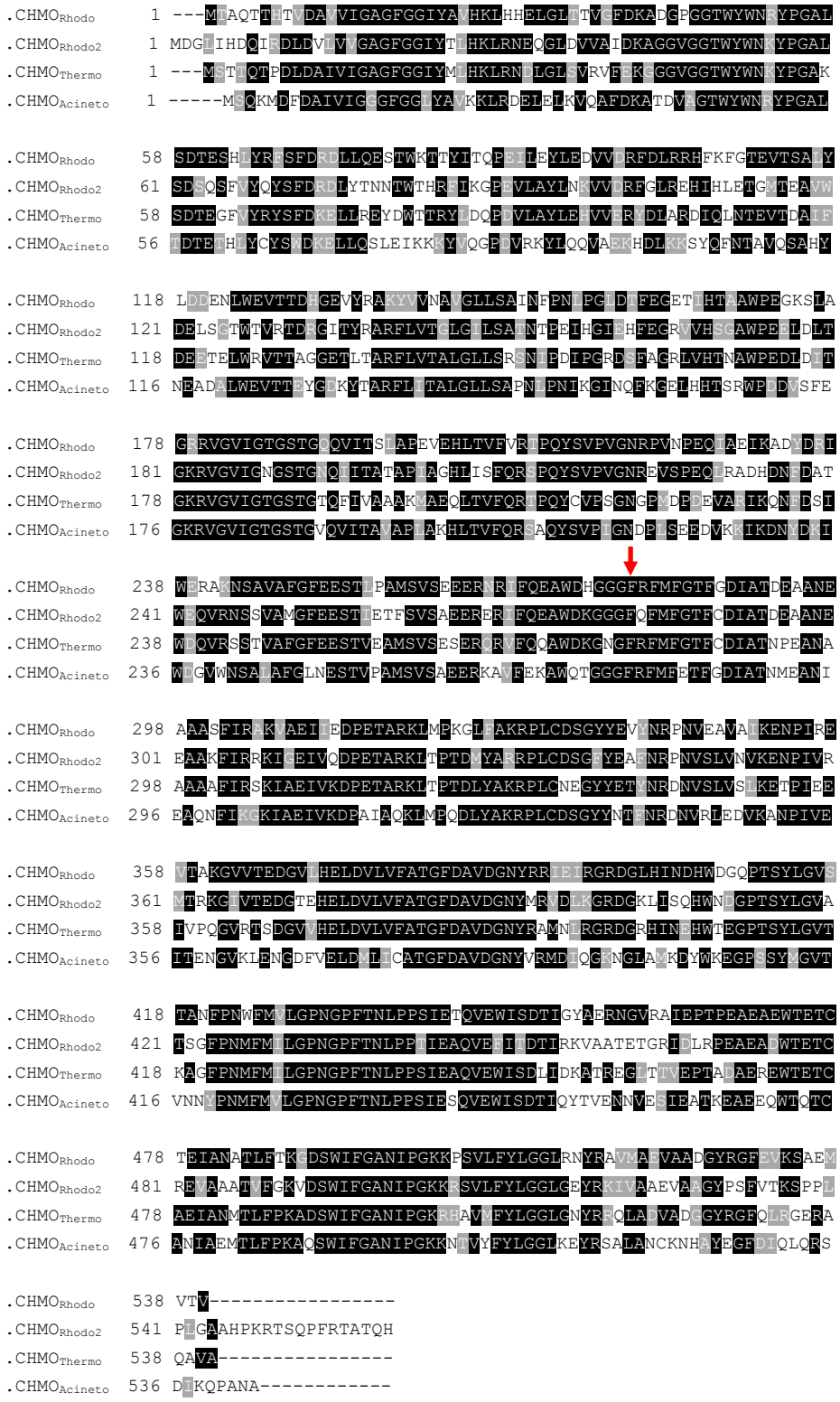
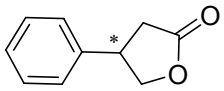


Figure S6. Protein sequence alignment of CHMO$_{Acineto}$ and other CHMOs. The red arrow points the position 277 of CHMO$_{Acineto}$ and corresponding positions of other CHMOs. The amino acid sequences were fetched from the NCBI database. CLUSTERALW was used to perform the alignment and the BoxShade server was used to edit it. Sequence similarity to CHMO$_{Acineto}$: CHMO$_{Rhodo}$ (50.1%), CHMO$_{Rhodo2}$ (50.6%), CHMO$_{Thermo}$ (56.7%).

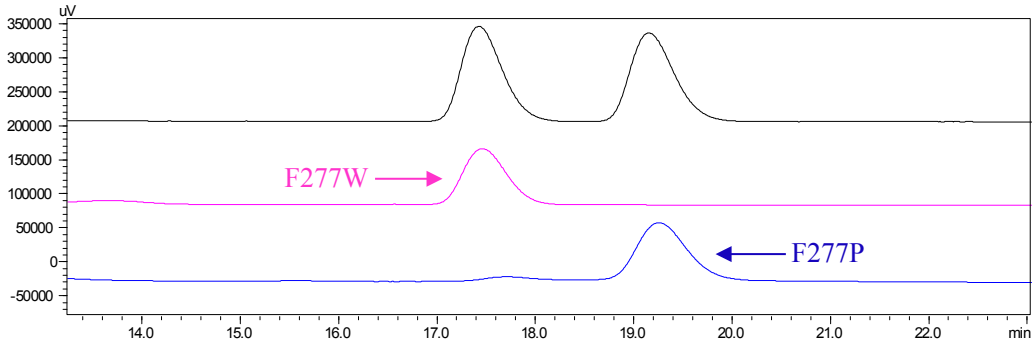
3. Chiral GC and HPLC data of enantiopure lactones.

4-phenyldihydrofuran-2(3H)-one (2a):

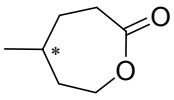


2a

F277W: (*R*), 99% ee; F277P: (*S*), 93% ee. The ee value was determined by HPLC analysis using a chiralcel AS-H column (hexane/2-propanol = 80/20, 1.0 mL/min, 220 nm), t_r (*R*) = 17.421 min, t_r (*S*) = 19.148min.

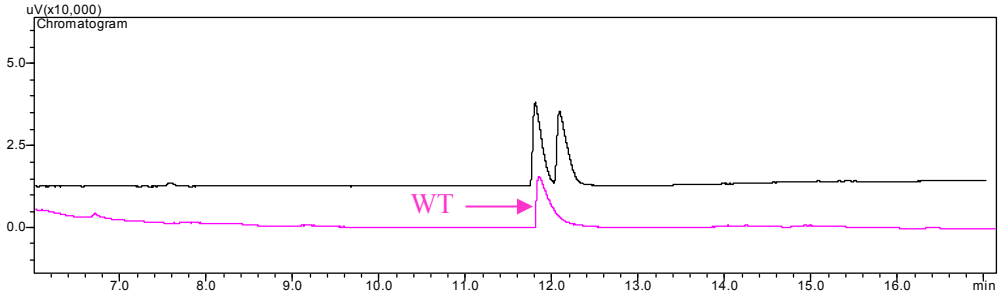


5-methyloxepan-2-one (2b):

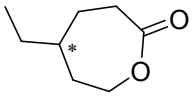


2b

WT: (*S*), 99% ee. The ee value was determined by chiral GC using CP-chirasil-DEX CB 25*0.25 column (110°C, iso 110°C, 20 min, 2°C/min to 200°C), t_r (*S*) = 11.875min, t_r (*R*) = 12.195 min.

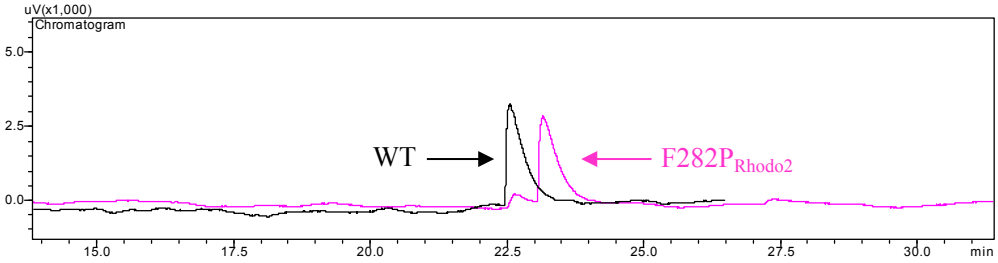


5-ethyloxepan-2-one (2c):

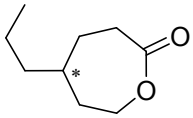


2c

WT: (*S*), 98% ee; F282P ($CHMO_{Rhodo2}$): (*R*), 80% ee. The ee value was determined by chiral GC using CP-chirasil-DEX CB 25*0.25 column (110°C, iso 110°C, 20 min, 2°C/min to 200°C), t_r (*S*) = 22.643 min, t_r (*R*) = 23.156 min.

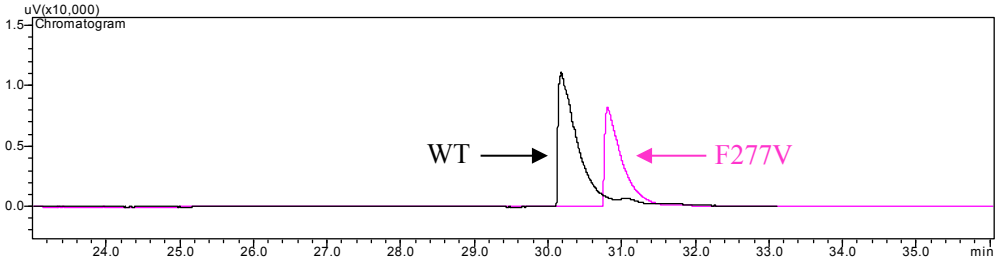


5-propyloxepan-2-one (2d):

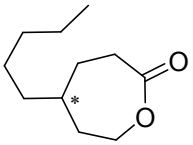


2d

WT: (*S*), 94% ee; F277V: (*R*), 98% ee. The ee value was determined by chiral GC using CP-chirasil-DEX CB 25*0.25 column (110°C, iso 110°C, 20 min, 2°C/min to 200°C), t_r (*S*) = 30.186 min, t_r (*R*) = 31.061 min.

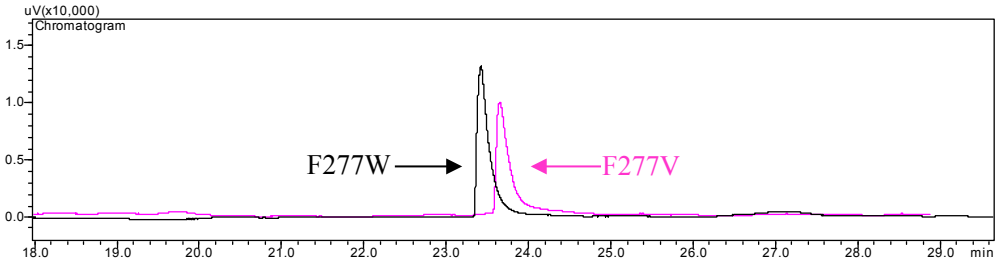


5-pentyloxepan-2-one (2e):

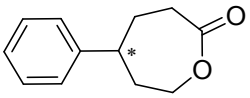


2e

F277W: (-), 99% ee; F277V: (+), 98% ee. The ee value was determined by chiral GC using CP-chirasil-DEX CB 25*0.25 column (110°C, 2/min 200°C, 10min), t_r (-) = 23.497 min, t_r (+) = 23.652 min.

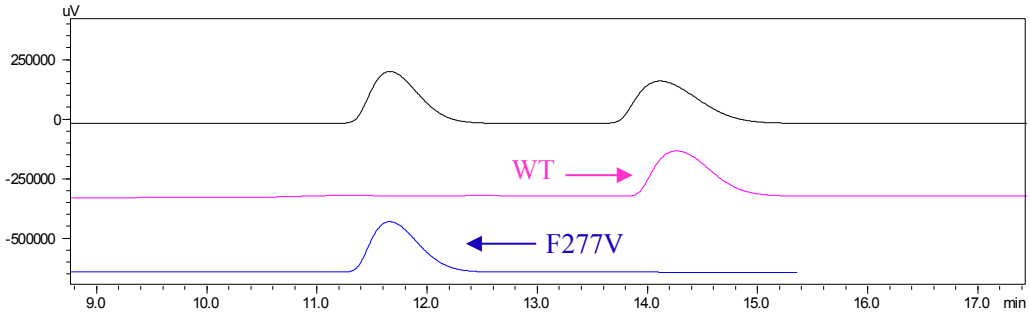


5-phenyloxepan-2-one (2f):

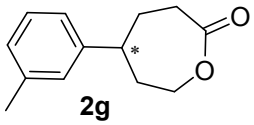


2f

WT: (*S*), 98% ee; F277V: (*R*), 99% ee. The ee value was determined by HPLC analysis using a chiralcel AS-H column (hexane/2-propanol = 70/30, 1.0 mL/min, 220 nm), t_r (*R*) = 11.657 min, t_r (*S*) = 14.113 min.

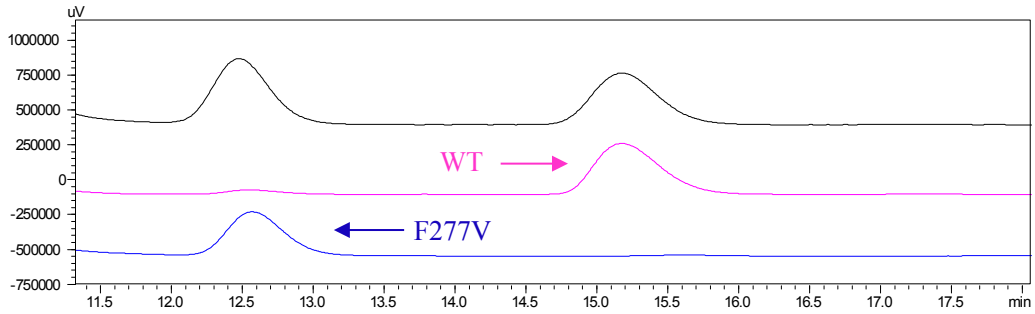


5-(*m*-tolyl)oxepan-2-one (2g):

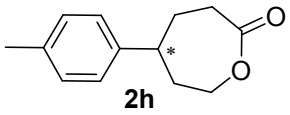


2g

WT: (-), 88% ee; F277V: (+), 98% ee. The ee value was determined by HPLC analysis using a chiralcel AS-H column (hexane/2-propanol = 80/20, 1.0 mL/min, 220 nm), t_r (+) = 12.470 min, t_r (-) = 15.170 min.

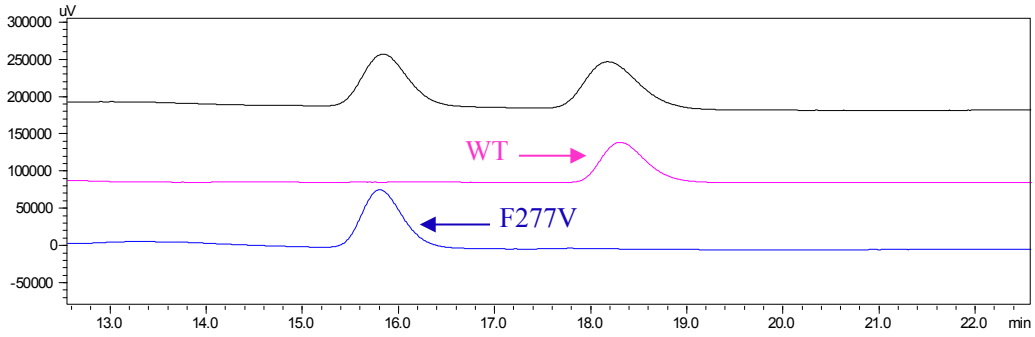


5-(*p*-tolyl)oxepan-2-one (2h):

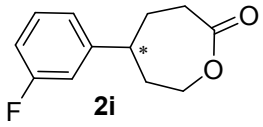


2h

WT: (-), 97% ee; F277V: (+), 96% ee. The ee value was determined by HPLC analysis using a chiralcel AS-H column (hexane/2-propanol = 80/20, 1.0 mL/min, 220 nm), t_r (+) = 15.834 min, t_r (-) = 18.173 min.

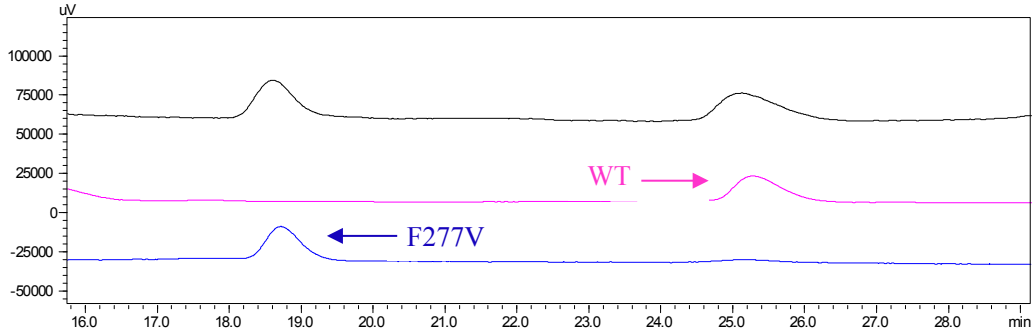


5-(3-fluorophenyl)oxepan-2-one (2i):

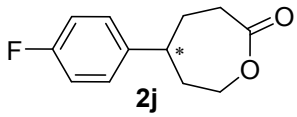


2i

WT: (-), 96% ee; F277V: (+), 99% ee. The ee value was determined by HPLC analysis using a chiralcel AS-H column (hexane/2-propanol = 80/20, 1.0 mL/min, 220 nm), t_r (+) = 18.610 min, t_r (-) = 25.128 min.

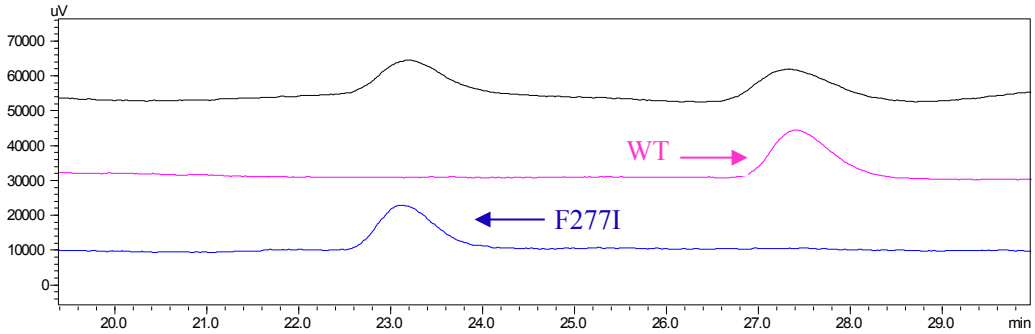


5-(4-fluorophenyl)oxepan-2-one (2j):

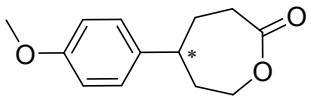


2j

WT: (-), 93% ee; F277I: (+), 99% ee. The ee value was determined by HPLC analysis using a chiralcel AS-H column (hexane/2-propanol = 80/20, 1.0 mL/min, 220 nm), t_r (+) = 23.175 min, t_r (-) = 27.330 min.

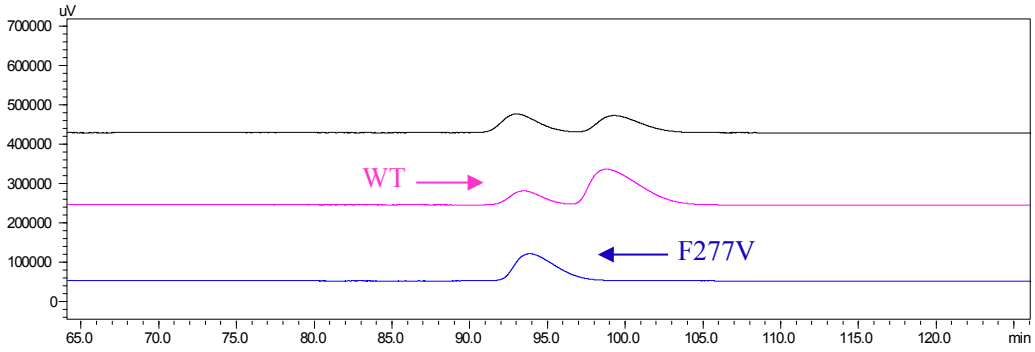


5-(4-methoxyphenyl)oxepan-2-one (2k):

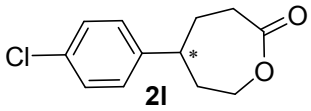


2k

WT: (-), 60% ee; F277V: (+), 99% ee. The ee value was determined by HPLC analysis using a chiralcel AS-H column (hexane/2-propanol = 90/10, 1.0 mL/min, 220 nm), t_r (+) = 92.594 min, t_r (-) = 98.827 min.

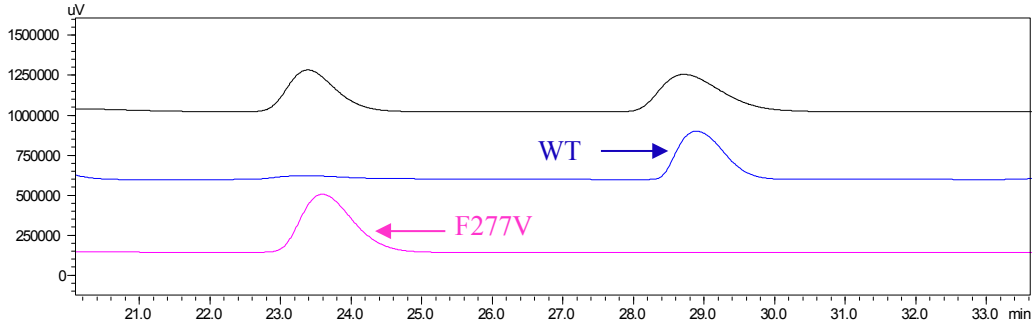


5-(4-chlorophenyl)oxepan-2-one (2l):

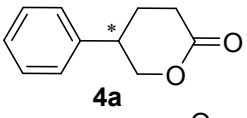


2l

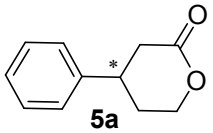
WT: (-), 85% ee; F277V: (+), 99% ee. The ee was determined by HPLC analysis using a chiralcel AS-H column (hexane/2-propanol = 80/20, 1.0 mL/min, 220 nm), t_r (+) = 23.384 min, t_r (-) = 28.708 min.



5-phenyltetrahydro-2H-pyran-2-one (4a), 4-phenyltetrahydro-2H-pyran-2-one (5a):

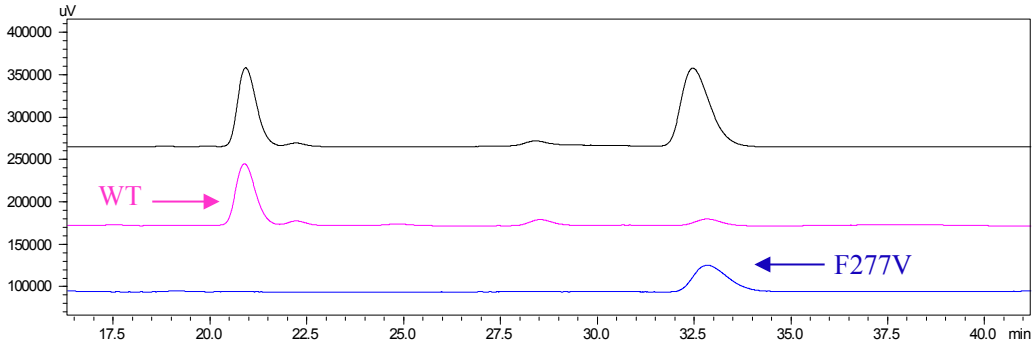


4a

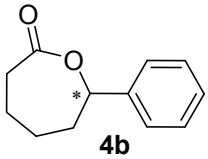


5a

WT: 87% ee (*R*) of **4a**; F277V: 99% ee (*S*) of **5a**. The ee was determined by HPLC analysis using a chiralcel AS-H column (hexane/2-propanol = 80/20, 1.0 mL/min, 220 nm), t_r (**4a**, *R*) = 20.919 min, t_r (**4a**, *S*) = 22.237 min, t_r (**5a**, *R*) = 28.357 min, t_r (**5a**, *S*) = 32.463 min.

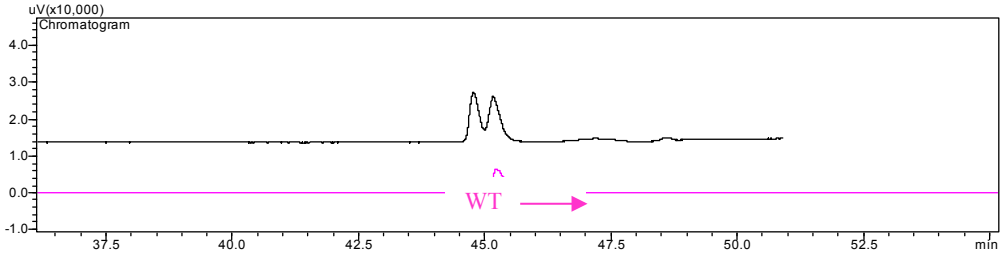


7-phenyloxepan-2-one (4b):

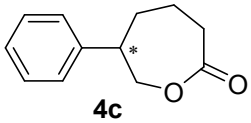


4b

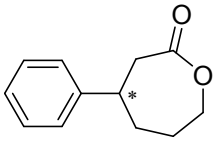
WT: (*R*), 97% ee. The ee value was determined by chiral GC using CP-chirasil-DEX CB 25*0.25 column (110°C, 1°C/min to 200°C), t_r (*S*) = 44.581 min, t_r (*R*) = 45.283 min.



6-phenyloxepan-2-one (4c), 4-phenyloxepan-2-one (5c):

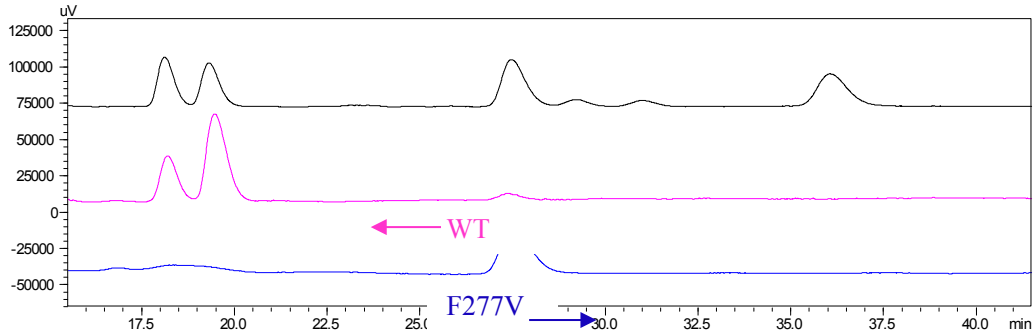


4c

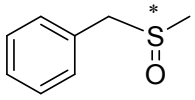


5c

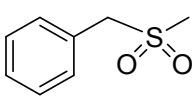
WT: 45% ee (*S*) of **4c**; F277V: 99% ee (*S*) of **5c**. The ee was determined by HPLC analysis using a chiralcel AS-H column (hexane/2-propanol = 80/20, 1.0 mL/min, 220 nm), t_r (**4c**, *R*) = 18.113 min, t_r (**4c**, *S*) = 19.306 min, t_r (**5c**, *S*) = 27.472 min, t_r (**5c**, *R*) = 36.050 min.



((methylsulfinyl)methyl)benzene (7a), ((methylsulfonyl)methyl)benzene (8a):

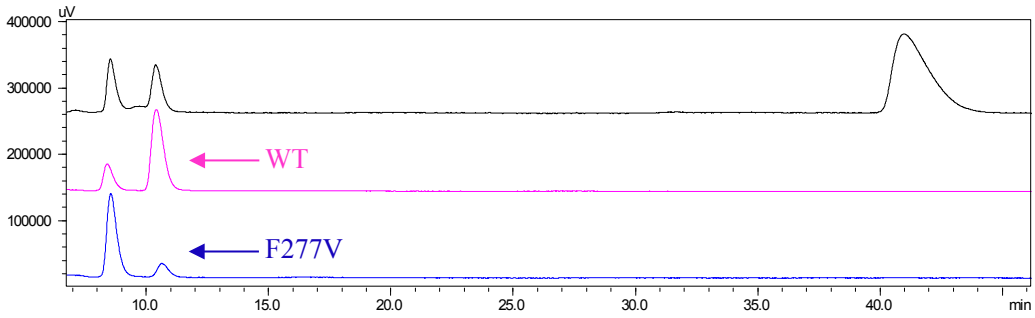


7a

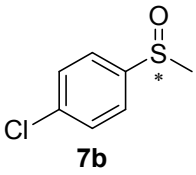


8a

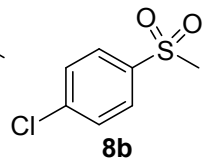
WT: 60% ee (*R*) of **7a**; F277V: 70% ee (*S*) of **7a**. The ee was determined by HPLC analysis using a chiralcel OB-H column (hexane/2-propanol = 70/30, 1.0 mL/min, 220 nm), t_r (**7a**, *S*) = 8.530 min, t_r (**7a**, *R*) = 10.385 min, t_r (**8a**) = 41.005 min.



1-chloro-4-(methylsulfinyl)benzene (7b), 1-chloro-4-(methylsulfonyl)benzene (8b):

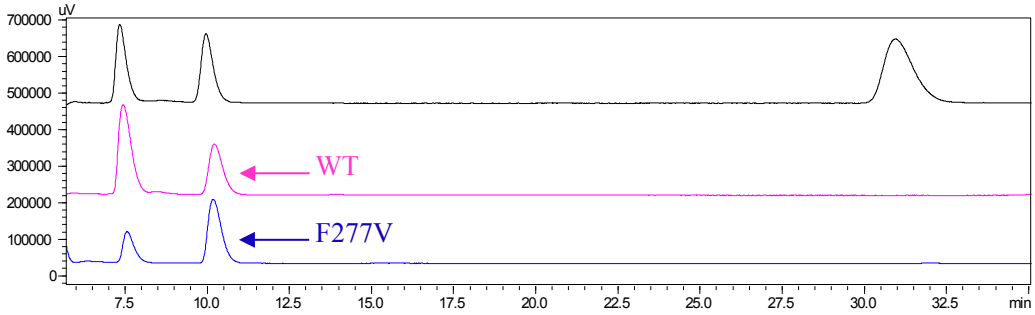


7b

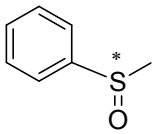


8b

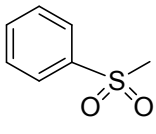
WT: 22% ee (*S*) of **7b**; F277V: 43% ee (*R*) of **7b**. The ee was determined by HPLC analysis using a chiralcel OB-H column (hexane/2-propanol = 70/30, 1.0 mL/min, 220 nm), t_r (**7b**, *S*) = 7.336 min, t_r (**7b**, *R*) = 9.958 min, t_r (**8b**) = 30.959 min.



(methylsulfinyl)benzene (7c), (methylsulfonyl)benzene (8c):

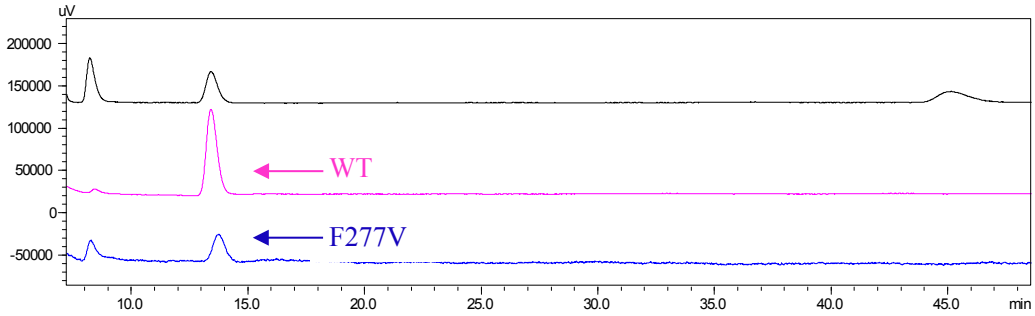


7c



8c

WT: 94% ee (*R*) of **7c**; F277V: 34% ee (*R*) of **7c**. The ee was determined by HPLC analysis using a chiralcel OB-H column (hexane/2-propanol = 70/30, 1.0 mL/min, 220 nm), t_r (**7c**, *S*) = 8.217 min, t_r (**7c**, *R*) = 13.411 min, t_r (**8c**) = 45.156 min.



4. References

1 Y. Zhang and G. B. Schuster, *J. Org. Chem.*, 1994**, 59**, 1855.

2 (*a*) N. A. Donoghue, D. B. Norris and P. W. Trudgill, *Eur. J. Biochem.*, 1976, **63**,175; (*b*) I. A. Mirza, B. J. Yachnin, S. Wang, S. Grosse, H. Bergeron, A. Imura, H. Iwaki, Y. Hasegawa, P. C. K. Lau and A. M. Berghuis, *J. Am. Chem. Soc.*, 2009, **131**, 8848; (*c*) F. Korn-Wendisch, F. Rainey, R. M. Kroppenstedt, A. Kempf, A. Majazza, H. J. Kutzner, E. Stackebrandt, *Int. J. Syst. Bacteriol.*, 1995, **45**, 67.

3 Y. Zhang, Y.-Q. Wu, N. Xu, Q. Zhao, H.-L. Yu, J.-H. Xu, *ACS Sustain. Chem. Eng.*, 2019, **7**, 7218.

4 E. Romero, J. R. G. Castellanos, A. Mattevi and M. W. Fraaije, *Angew. Chem., Int. Ed.*, 2016, **55**, 15852.

5 B. J. Yachnin, M. B. McEvoy, R. J. D. MacCuish, K. L. Morley, P. C. K. Lau and A. M. Berghuis, *ACS Chem. Biol.*, 2014, **9**, 2843.

6 Y. Hu, J. Wang, Y. Cen, H. Zheng, M. Huang, X. Lin and Q. Wu, *Chem. Commun.*, 2019, **55**, 2198.

7 L. Zhou, X. Liu, J. Ji, Y. Zhang, X. Hu, L. Lin and X. Feng, *J. Am. Chem. Soc.*, 2012, **134**, 17023.

8 Z. G. Zhang, R. Lonsdale, J. Sanchis and M. T. Reetz, *J. Am. Chem. Soc.*, 2014, **136**, 17262.