Supporting Information

Dual-enzyme-Catalyzed Synthesis of Enantiocomplementary Polyesters

Yujing Hu,† Yu Zhang,† Weihua Xu,† Jian Xu,† Xianfu Lin,† Qi Wu*,†

†Department of Chemistry, Zhejiang University, Hangzhou, 310027, People's Republic of China

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1. Experimental Section

1.1 Experimental methods

Materials.

4-methylcyclohexanone, 4-ethylcyclohexanone, 4-propylcyclohexanone, 4-pentylcyclohexanone, 4-phenylcyclohexanone were purchased from Energy-Chemical (China). The lipase immobilized on acrylic resin from *Candida antarctica* (CALB; EC 3.1.1.3, ≥10 000 U g^{-1}), and lipozyme®, immobilized from *Mucor miehei* (MML, 42 U g^{-1}, 1 U corresponds to the amount of enzyme which liberates 1 mol oleic acid at pH 8.0 and 40°C per minute) were purchased from Sigma-Aldrich (Shanghai, China). All solvents and other reagents were analytical grade and used without further purification.

Analytical Methods. Gas chromatographic analyses (GC) was used to analyze the conversion and enantiomeric excess of samples, which 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). Optical rotation data were measured on a Perkin-Elmer 341 polarimeter equipped with a Na-lamp. The ^{1}HNMR spectra were recorded using a Bruker DRX 400 NMR spectrometer (Rheinstetten, Germany) and chemical shifts were expressed in ppm. The number- and weight-average molecular weights (*M*n and *M*w, respectively) of polyesters were measured by gel permeation chromatography (GPC) with a system equipped with a refractive-index detector (Waters 2414) and Waters Styragel GPC columns (Massachusetts, USA). The GPC columns were standardized with narrow-dispersity polystyrene in molecular weights ranging from 1×10^5 to 162. The mobile phase was THF at a flow rate of 1.0 $mLmin^{-1}$.

Mutant Library Screening.

The generation of mutant libraries and expression of $CHMO_{Acineto}$ variants were performed as the previous work reported[1]. In the whole cell screening protocol, the reaction system contained 1 mL of cell culture (0.1 g wet cell/mL), 4 μL of a stock solution of 0.5 M ketones in acetonitrile and D-glucose (3 equiv.). The mixture in 10 mL of glass tube with a sealed cap was shaken at 200 rpm and 22°C for desymmetrization, and the reaction time is 32 h. The reaction was stopped by adding sodium chloride and the mixture was extracted with 1 mL of ethyl acetate three times. The sample was analyzed by chiral gas chromatographic analyses (GC) (CP-chirasil-DEX CB 25 cm×0.25 cm) to determine the conversion and the enantiomeric excess of the residues and products.

Identifying the Optimal Concentration of Baeyer–Villiger Oxidation in Biphasic System.

The reaction system was performed in 10 mL of cell culture (0.1 g wet cell/mL) with different concentrations of ketones and D-glucose (3 equiv.), and 30 mL of organic solvents. The mixture was shaken at 200 rpm and 20°C for 40 h. The reaction was stopped by adding sodium chloride. Samples (1 mL of emulsified reaction mixture) were taken with a pipette and extracted with ethyl acetate three times. The sample was analyzed by chiral GC (CP-chirasil-DEX CB 25 cm×0.25 cm) to determine the conversion and the enantiomeric excess of the residues and products.

General Procedure for Scaling-up Baeyer–Villiger Oxidation in Biphasic System.

The scaling-up reaction was performed in 150 mL of cell culture (0.1 g wet cell/mL) with limited concentrations of ketones and D-glucose (3 equiv.), and 450 mL of organic solvents. The mixture was shaken at 20°C. Oxygen supply was increased by fixing a balloon filled with oxygen to a syringe and then passing it through Diaphragm seal shaker. After 40 h, samples (1 mL of emulsified reaction mixture) were taken with a pipette and then extracted with ethyl acetate three times. The samples were detected by GC. If the conversion was not complete, additional fresh cell culture would be added until that the substrate was completely transformed. The reaction was stopped by adding sodium chloride. At the end of the reaction (40 h), the emulsified reaction mixture was added with sodium chloride and additional ethyl acetate, and then shook for another 24h in order to extract all of the remaining product in aqueous medium. Then, the organic solvent was dried with anhydrous magnesium sulfate and then evaporated in vacuum at 40°C. The purified lactones were used for subsequent ring-opening polymerization.

General Procedure for Synthesis of the Substituted Chiral Polyesters.

Lipase CALB and MML were added to a Schlenk tube. The tube was put in a vacuum oven (10 mm Hg) for 12h at 45°C in presence of P_2O_5. The tube was removed from the oven at the atmosphere of nitrogen. The polymerization mixture containing chiral lactones obtained from BV Oxidation, initiator butanol (1:100 molar ratio of lactones), extra dry toluene (1 mL) and dry molecular sieves (3 Å) was stirred at 50°C for 18 hours in nitrogen to remove traces of water. The small-scaling reaction was then started by the addition of quantitative dried lipase CALB under the atmosphere of nitrogen at 80°C for 7 days. The samples were taken out by syringe at intervals and monitored by GC. The large-scaling reaction was started by the addition of quantitative dried lipase CALB (or MML) under the atmosphere of nitrogen at 80°C (or 70°C) for 7 days. Then the enzymatic reaction was stopped by filtration and flushed with dichloromethane. The conversion of monomers was calculated by ^{1}H NMR of crude product. The filtrate was concentrated and precipitated from ethyl acetate and *n*-hexane. The isolated yield of polyesters after purification was between 50%-60%. The analysis of purified product was performed by GPC and ^{1}H NMR.

1.2 Computational methods

The crystal structure of $CHMO_{Acineto}$ is not available, so we build a homology model (named as $CHMO_{homo}$) based on the crystal structure of CHMO from *Rhodococcus* sp. strain HI-31 (PDB code: 3GWD), which exhibits 55% sequence similarity and thus would represent the enzyme's substrate scope and degree of selectivity.[2] The CHMO mutants were generated using Discovery Studio (version 2.5). The processes of molecular docking and molecular dynamics were performed as previous work reported.[1]

2. Additional tables and figures

Table S1. List of Forward and Reverse Primers

Primers	Sequence
Forward L435A	GTTTACCAACGCTCCGCCATCAATTG
Forward L435C	GTTTACCAACTGTCCGCCATCAATTG
Forward L435D	CCCGTTTACCAACGACCCGCCATCAATTG
Forward L435E	CCCGTTTACCAACGAGCCGCCATCAATTG
Forward L435G	GTTTACCAACGGTCCGCCATCAATTG
Forward L435H	GTTTACCAACCATCCGCCATCAATTG
Forward L435I	CCCGTTTACCAACATCCCGCCATCAATTG
Forward L435K	CCCGTTTACCAACAAACCGCCATCAATTG
Forward L435F	GTTTACCAACTTTCCGCCATCAATTG
Forward L435M	GTTTACCAACATGCCGCCATCAATTG
Forward L435N	CCCGTTTACCAACAACCCGCCATCAATTG
Forward L435P	GTTTACCAACCCTCCGCCATCAATTG
Forward L435Q	CCCGTTTACCAACCAGCCGCCATCAATTG
Forward L435R	CCCGTTTACCAACCGGCCGCCATCAATTG
Forward L435S	CCCGTTTACCAACAGCCCGCCATCAATTG
Forward L435T	GTTTACCAACACCCCGCCATCAATTG
Forward L435V	CCCGTTTACCAACGTGCCGCCATCAATTG
Forward L435W	GTTTACCAACTGGCCGCCATCAATTG
Forward L435Y	GTTTACCAACTATCCGCCATCAATTG
Forward F432L	GAATGGCCCGCTTACCAACCTGCCGCCATCA
Forward F432I	GCTTGGACCGAATGGCCCGATTACCAAC
Forward F432I/L435A	GAATGGCCCGATTACCAACGCGCCGCCATC
Forward F432L/L435A	GAATGGCCCGCTGACCAACGCGCCGCCATC
Forward F432V/L435A	GAATGGCCCGGTGACCAACGCGCCGCCATC
Forward F432M/L435A	GAATGGCCCGATGACCAACGCGCCGCCATC

Forward F432I/L435G	GAATGGCCCGATTACCAACGGCCCGCCATC
Forward P431A/F432I/L435A	ACCGAATGGCGCTATTACCAACGCTCCGCCATC
Forward P431L/F432I/L435A	ACCGAATGGCCTGATTACCAACGCGCCGCCATC
Forward P431F/F432I/L435A	ACCGAATGGCTTTATTACCAACGCGCCGCCATC
Forward F432I/T433A/L435A	ACCGAATGGCCCGATTGCGAACGCGCCGCCATC
Forward F432I/T433L/L435A	ACCGAATGGCCCGATTCTGAACGCGCCGCCATC
Forward F432I/T433I/L435A	ACCGAATGGCCCGATTATTAACGCGCCGCCATC
Forward F432I/T433V/L435A	ACCGAATGGCCCGATTGTGAACGCGCCGCCATC
Forward F432I/T433M/L435A	ACCGAATGGCCCGATTATGAACGCGCCGCCATC
Forward F432I/T433C/L435A	ACCGAATGGCCCGATTTGCAACGCGCCGCCATC
Forward F432I/T433S/L435A	ACCGAATGGCCCGATTTCTAACGCGCCGCCATC
Silent reverse primer	GCGGCCGCTCTGGATCCATGC

Table S2. WT and Single $CHMO_{Acineto}$ Mutants as Catalysts in the Desymmetrization of Prochiral Cyclohexanones **1**[a]

entry	substrate	variants	conv.(%)[b]	ee_p(%)[c]
1	**1d**	WT	99	33(+)
2	**1a**	F432L	99	99(*S*)
3	**1b**	F432L	99	98(*S*)
4	**1c**	F432L	99	77(*S*)
5	**1d**	F432L	99	85(+)
6	**1e**	F432L	99	95(+)
7	**1a**	F432I	99	99(*S*)
8	**1b**	F432I	99	98(*S*)
9	**1c**	F432I	99	81(*S*)
10	**1d**	F432I	99	82(+)
11	**1e**	F432I	99	85(+)
12	**1a**	L435G	99	70(*S*)
13	**1b**	L435G	99	28(*S*)
14	**1c**	L435G	99	70(*R*)
15	**1d**	L435G	83	35(+)
16	**1a**	L435A	99	62(*S*)
17	**1b**	L435A	99	31(*S*)
18	**1c**	L435A	99	60(*R*)
19	**1d**	L435A	99	30(-)
20	**1e**	L435A	99	98(+)

[a] The whole cell screening experiments are described in Experiment section. [b, c] Determined by chiral GC. [c] The absolute configuration was confirmed by comparison with the literature[3-4].

Table S3. 435X $CHMO_{Acineto}$ Mutants as Catalysts in the Desymmetrization of Prochiral Cyclohexanones **1d**[a]

entry	variants	conv.(%)[b]	ee_p(%)[c]
1	L435C	99	40(-)
2	L435D	<3	-
3	L435E	6	4(-)
4	L435F	<3	-
5	L435H	93	40(+)
6	L435K	<3	-
7	L435N	<3	-
8	L435P	16	66(-)
9	L435Q	<3	-
10	L435R	<3	-
11	L435S	99	38(-)
12	L435T	44	22(-)
13	L435Y	22	89(+)

[a] The whole cell screening experiments are described in Experiment section. [b, c] Determined by chiral GC. [c] The absolute configuration was confirmed by comparison with the literature[3-4].

Table S4. Double $CHMO_{Acineto}$ Mutants as Catalysts in the Desymmetrization of Prochiral Cyclohexanones **1**[a]

entry	substrate	variants	conv.(%)[b]	ee_p(%)[c]
1	**1a**	F432I/L435A	99	20(*R)*
2	**1a**	F432I/L435G	99	17(*S*)
3	**1b**	F432I/L435A	99	73(*R)*
4	**1b**	F432I/L435G	99	55(*R)*
5	**1c**	F432I/L435A	99	94(*R*)
6	**1d**	F432I/L435A	99	80(+)

[a] The whole cell screening experiments are described in Experiment section. [b, c] Determined by chiral GC. [c] The absolute configuration was confirmed by comparison with the literature[3-4].

Table S5. Triple $CHMO_{Acineto}$ Mutants as Catalysts in the Desymmetrization of Prochiral Cyclohexanones **1**[a]

entry	substrate	variants	conv.(%)[b]	ee_p(%)[c]
1	**1b**	F432I/L435A/T433A	99	29(*R*)
2	**1b**	F432I/L435A/T433V	99	89(*R*)
3	**1b**	F432I/L435A/T433M	99	85(*R*)
4	**1b**	F432I/L435A/T433I	99	85(*R*)

[a] The whole cell screening experiments are described in Experiment section. [b, c] Determined by chiral GC. [c] The absolute configuration was confirmed by comparison with the literature[3-4].

Table S6. The Amplified Reaction of Substrate **1a**-**1e** by the WT and the Best Mutants in Biphasic System[a]

entry	variants	substrate	M/mmol	conv./%[b]	ee_p%[c]	yield/%[d]
1	WT	**1a**	6.75	99	99(*S*)	74
2	WT	**1b**	5.25	99	98(*S*)	72
3	WT	**1c**	2.25	99	94(*S*)	64
4	L435V	**1d**	2.40	99	99(-)	65
5	WT	**1e**	2.40	99	99(-)	86
6	F432I/L435A/T433L	**1a**	3.90	99	82(*R*)	61
7	F432I/L435A/T433L	**1b**	3.00	99	94(*R*)	67
8	F432I/L435G	**1c**	3.80	99	98(*R*)	68
9	F432I/L435G	**1d**	1.80	99	97(-)	62
10	L435G	**1e**	2.40	99	99(+)	89

[a] The large-scale experiments are described in Experiment section. [b, c] Determined by chiral GC. [c] The absolute configuration was confirmed by comparison with the literature[3-4]. [d] Isolated yield calculated by isolation of products using column chromatography.

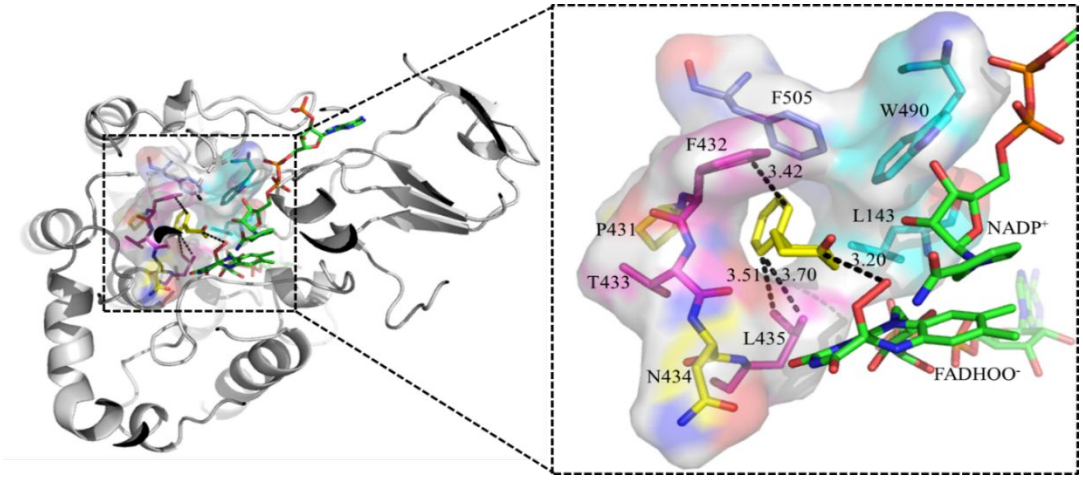


Figure S1. MD reference structure of WT CHMO in complex with ketone **1e**. The active center residues are represented by sticks and surfaces. Cofactors ($FADHOO^-$ and $NADP^+$) are colored in green. The substrate is shown in yellow. The unit of the critical distance is Å. The MD reference structure corresponds to the structure with the lowest RMSD (α-C atoms), relative to the average structure of the MD trajectory. The homology model based on the crystal structure of CHMO from *Rhodococcus* sp. strain HI-31 (PDB code: 3GWD)

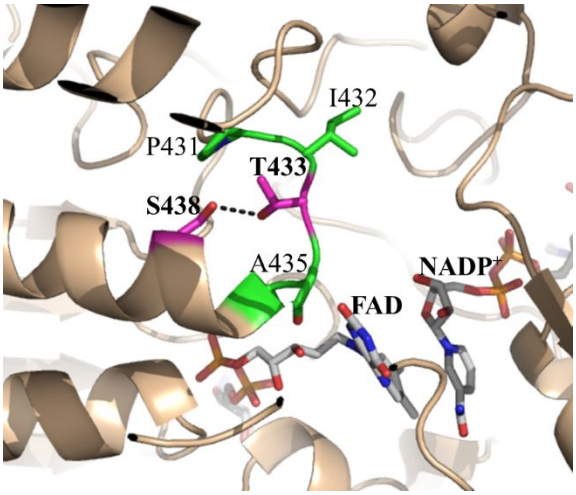


Figure S2. The residues T433 adjacent to the residue 432 in MD references structure of F432I/L435A are shown in magenta. Cofactors (FAD and $NADP^+$) are colored in light gray.

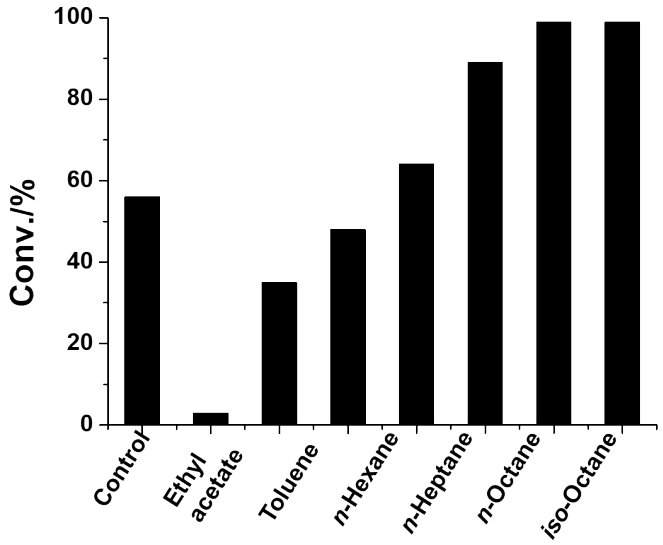
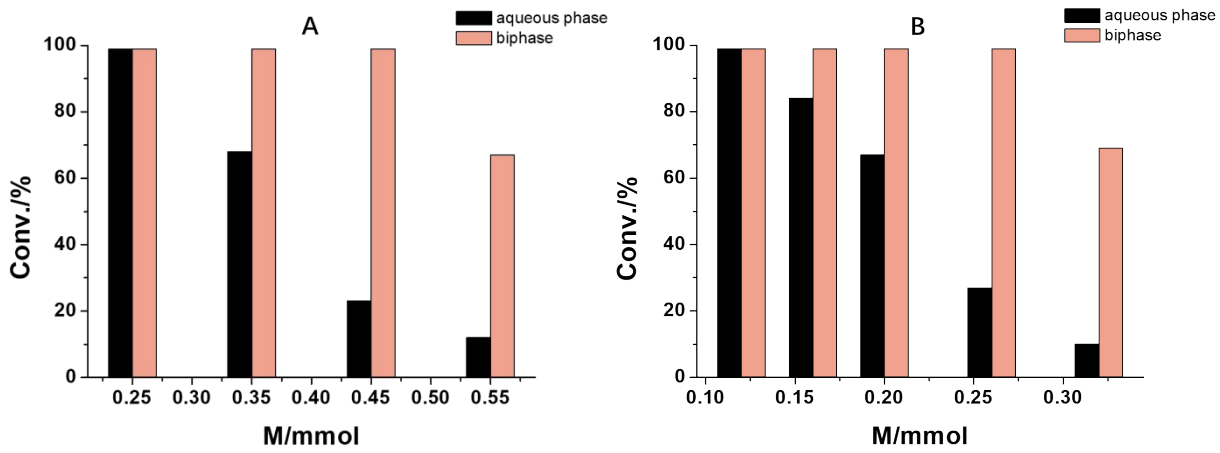
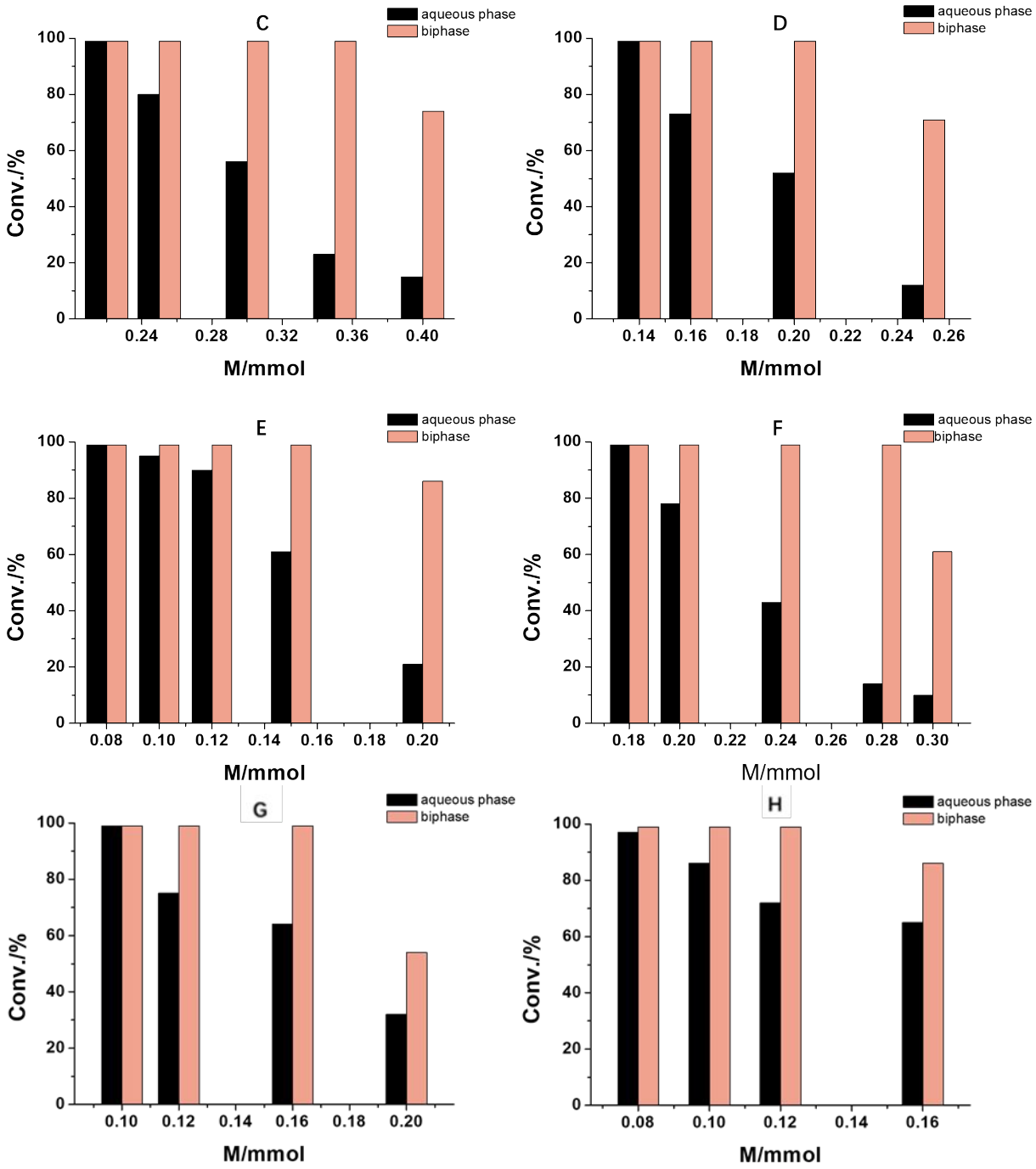


Figure S3. The effect of solvents in biphasic reaction.




C
aqueous phase
biphase
Conv./%
M/mmol
D
E
F
G
H

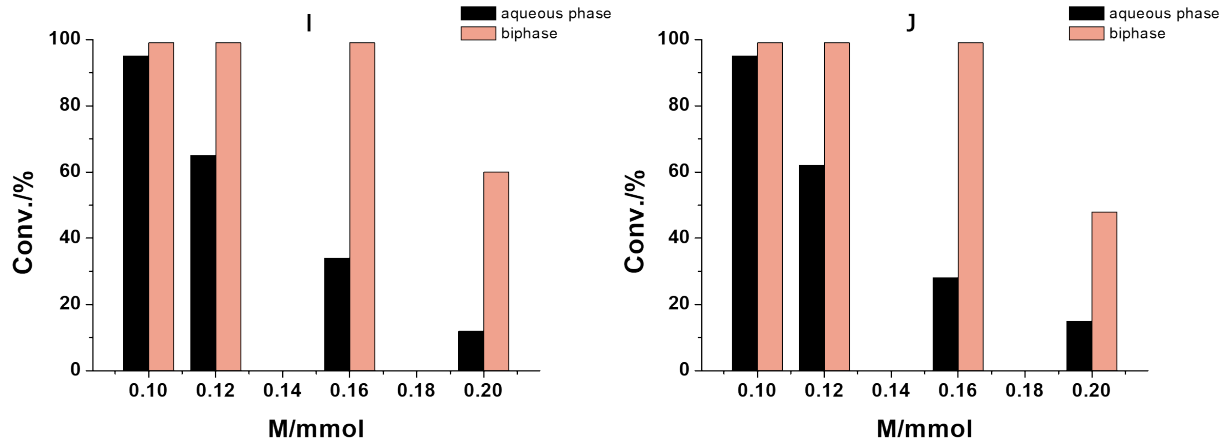


Figure S4. The limited mass of substrates **1a**-**1e** in the biphasic medium (10 mL cell culture and 30 mL *iso*-octane) in the reactions catalyzed by WT and selected mutants. (A) WT-**1a**; (B) F432I/L435A/T433L-**1a**; (C) WT-**1b**; (D) F432I/L435A/T433L-**1b**; (E) WT-**1c**; (F) F432I/L435G-**1c**; (G) WT-**1d**; (H) F432I/L435G-**1d**; (I) WT-**1e**; (J) L435G-**1e**.

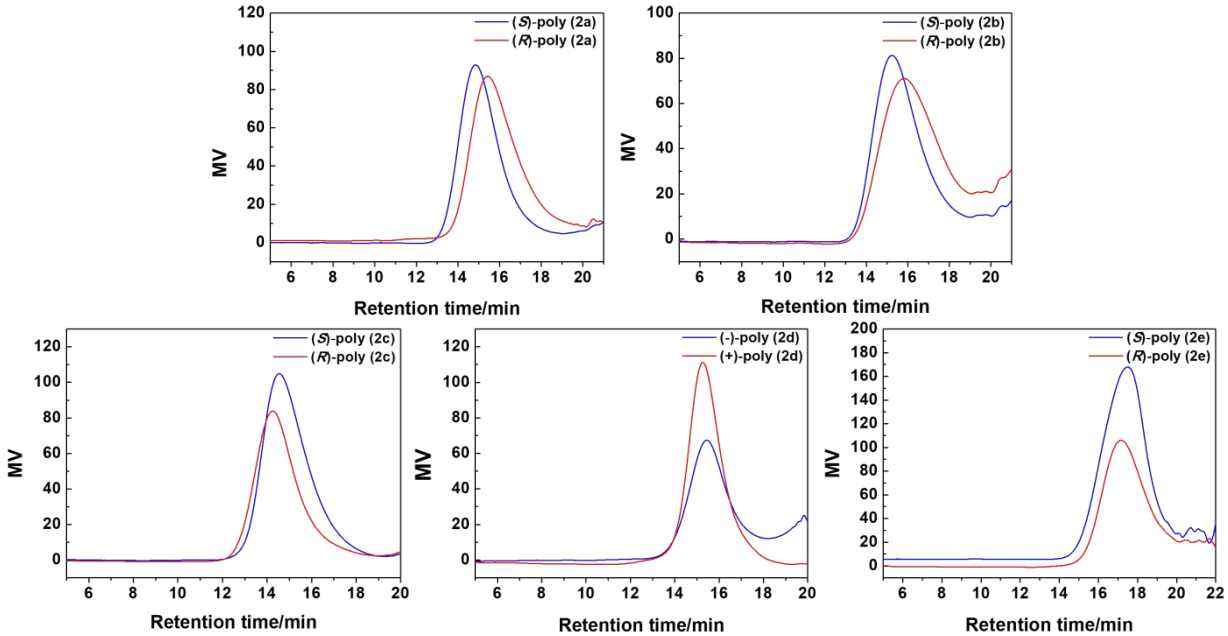
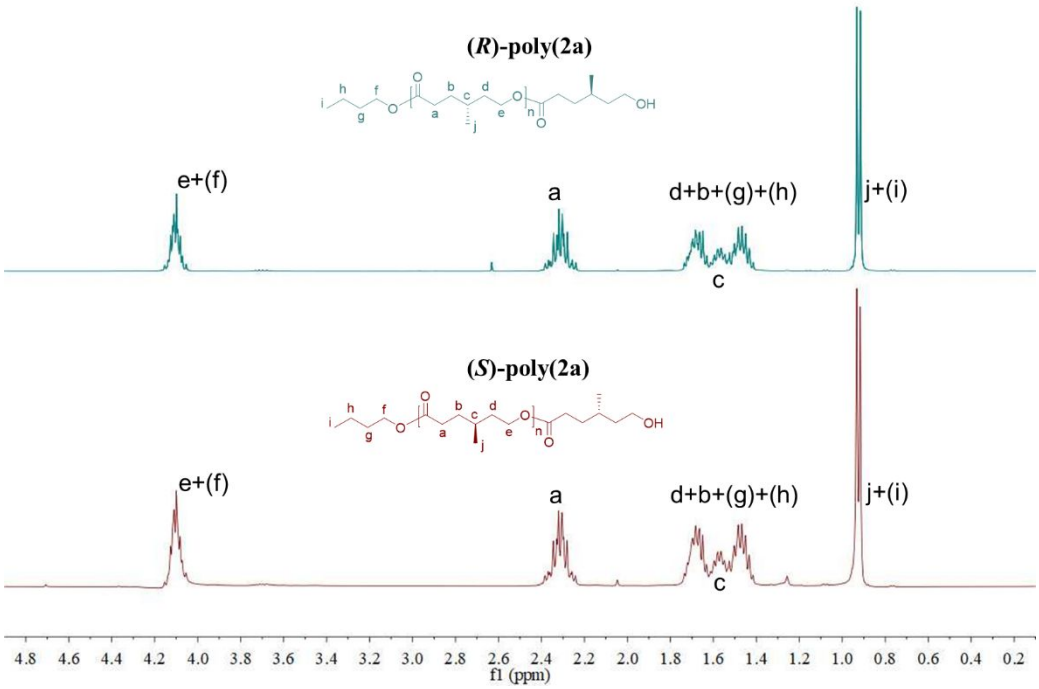
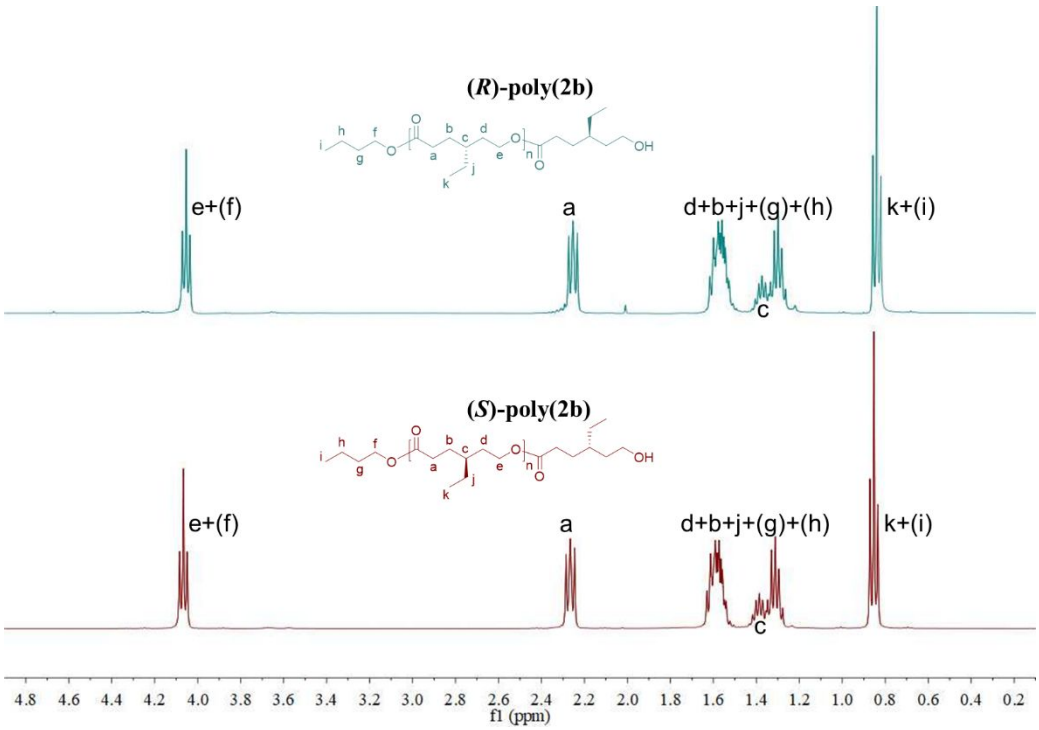
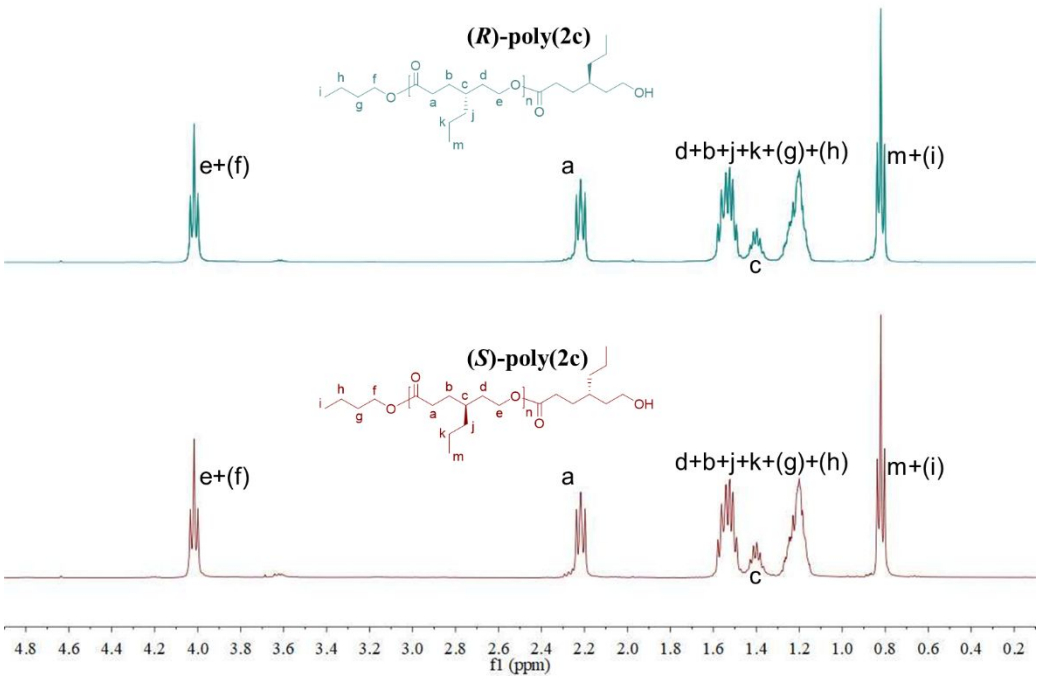


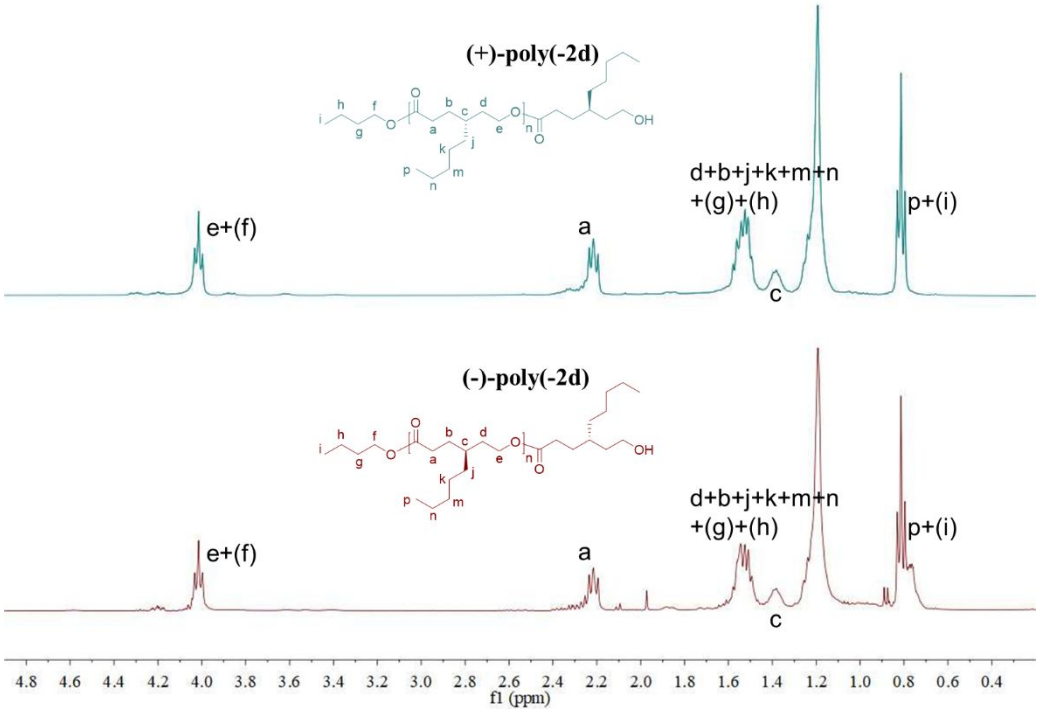
Figure S5. GPC traces of the obtained chiral polyesters. (A) (*S*)-poly(**2a**) and (*R*)-poly(**2a**); (B) (*S*)-poly(**2b**) and (*R*)-poly(**2b**); (C) (*S*)-poly(**2c**) and (*R*)-poly(**2c**); (D) (-)-poly(**2d**) and (+)-poly(**2d**); (E) (*S*)-poly(**2e**) and (*R*)-poly(**2e**).

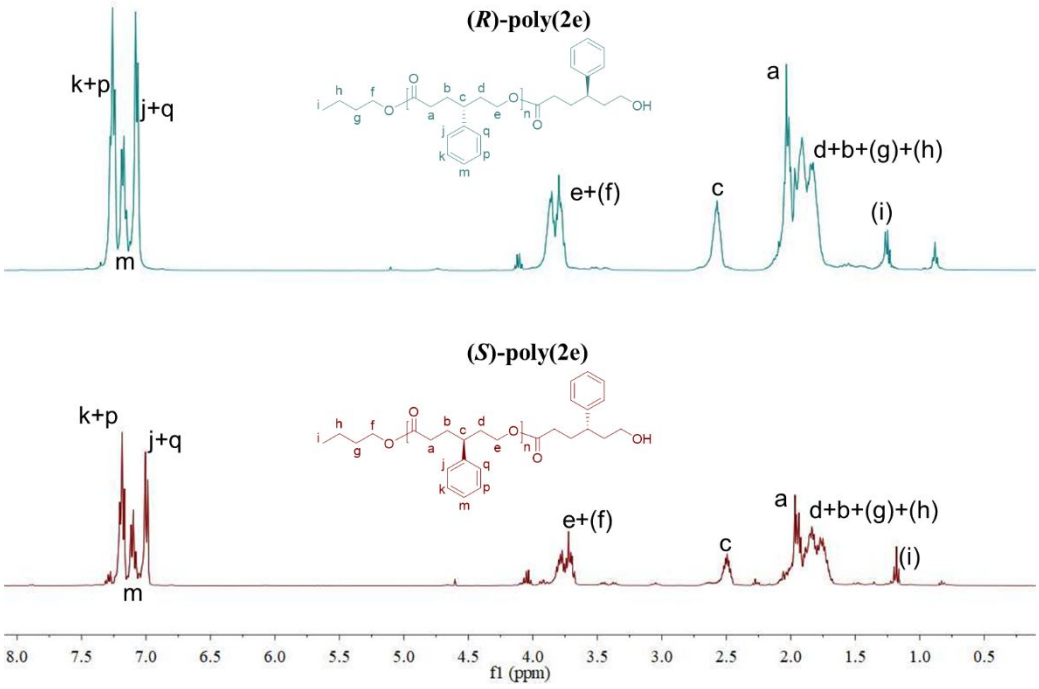
3. ^{1}H NMR spectra of chiral polyesters.





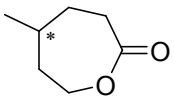

(R)-poly(2c)
e+(f)
a
d+b+j+k+(g)+(h)
m+(i)
c
(S)-poly(2c)
e+(f)
a
d+b+j+k+(g)+(h)
m+(i)
c
4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2
f1 (ppm)


(+)-poly(-2d)
e+(f)
a
d+b+j+k+m+n
+(g)+(h)
p+(i)
c
(-)-poly(-2d)
e+(f)
a
d+b+j+k+m+n
+(g)+(h)
p+(i)
c
4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4
f1 (ppm)

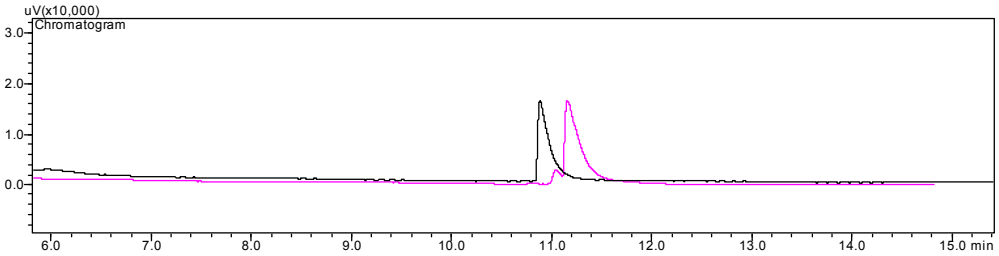


4. GC data of chiral lactones and optical rotation data

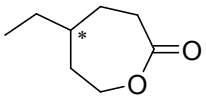
5-methyloxepan-2-one 2a: (*S*)-enantiomer: 99% ee, $[\alpha]_D^{20}$ = -50.3 (c 0.99, $CHCl_3$); (*R*)-enantiomer: 82% ee, $[\alpha]_D^{20}$ = +31.2 (c 0.87, $CHCl_3$). The ee was determined by GC analysis, 100°C, 2°C/min, to 140°C. t_r (*S*) = 11.043 min, t_r (*R*) = 11.158min.



2a

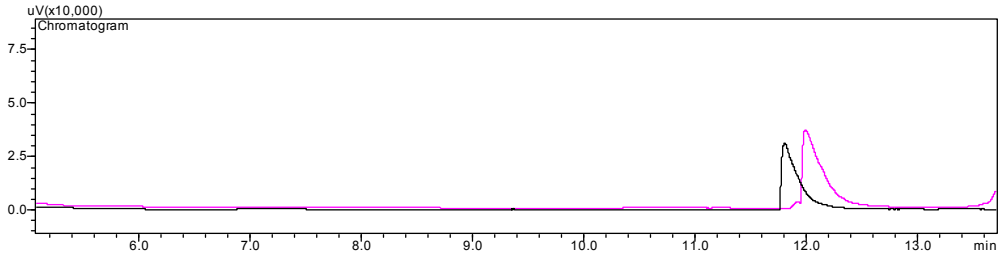


5-ethyloxepan-2-one 2b: (*S*)-enantiomer: 98% ee, $[\alpha]_D^{20}$ = -47.4 (c 0.98, $CHCl_3$); (*R*)-enantiomer: 94% ee, $[\alpha]_D^{20}$ = +43.4 (c

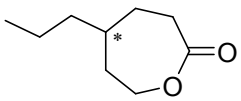


2b

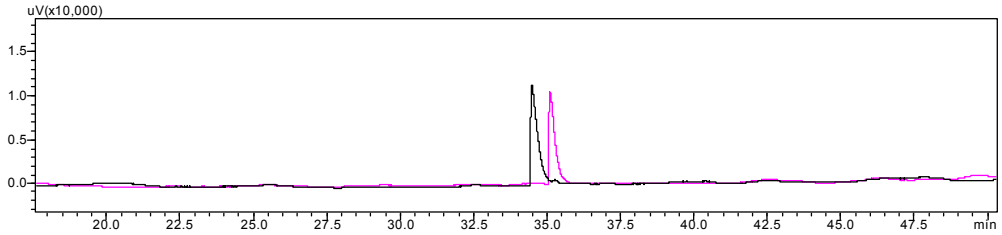
1.13, $CHCl_3$). The ee was determined by GC analysis, 110°C, 2°C/min, to 140°C. t_r (*S*) = 11.921 min, t_r (*R*) = 11.999 min.



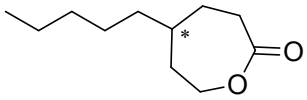
5-propyloxepan-2-one 2c: (*S*)-enantiomer: 94% ee, $[\alpha]_D^{20}$ = -44.0 (c 1.23, $CHCl_3$); (*R*)-enantiomer: 98% ee, $[\alpha]_D^{20}$ = +46.1 (c 1.09, $CHCl_3$). The ee was determined by GC analysis, 110°C, isotherm, 30 min, 5°C/min, 200°C, 20 min. t_r (*S*) = 34.491 min, t_r (*R*) = 35.100 min.



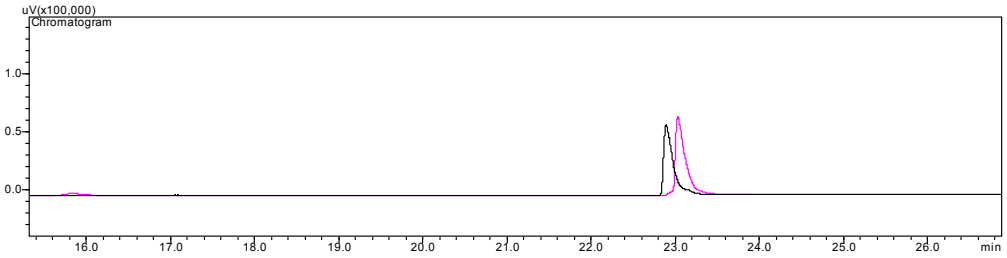
2c



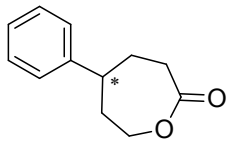
5-pentyloxepan-2-one 2d: (*S*)-enantiomer: 99% ee, $[\alpha]_D^{20}$ = -33.7 (c 1.01, $CHCl_3$); (*R*)-enantiomer: 97% ee, $[\alpha]_D^{20}$ = +32.3 (c 1.14, $CHCl_3$). The ee was determined by GC analysis, 110°C, 2°C/min, 170°C, 2 min. t_r (-) = 22.592 min, t_r (+) = 23.096 min.



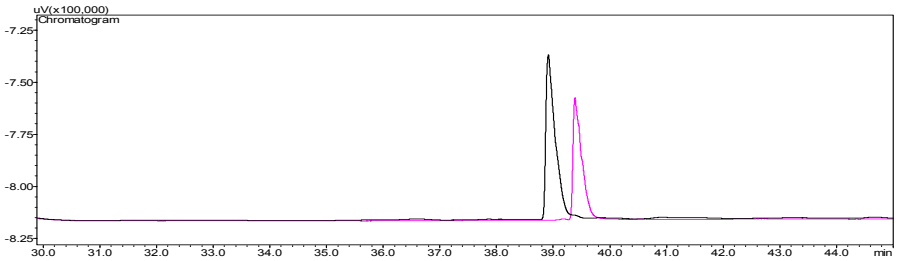
2d



5-phenyloxepan-2-one 2e: (-)-enantiomer: 98% *ee*, $[\alpha]_D^{20}$ = -57.0 (c 1.03, $CHCl_3$); (+)-enantiomer: 99% *ee*, $[\alpha]_D^{20}$ = +58.6 (c 1.18, $CHCl_3$). The ee was determined by GC analysis, 110°C, 2°C/min, to 200°C, 10 min. t_r(-) = 39.001 min, t_r(+) = 39.461 min.



2e



5. References

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