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Enantioselectivity and CatalysisImprovements of *PseudomenasCepacia* Lipase with Tyr and Asp Modification

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SUPPORTING INFORMATION

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Table S1 The initial rate and the enantioselectivity for the hydrolysis of *sec*-butyl butyrate, *sec*-butyl hexanoate, *hexan*-2-yl acetate and *octan*-2-yl acetate catalyzed by the native and modified *Pc*L.

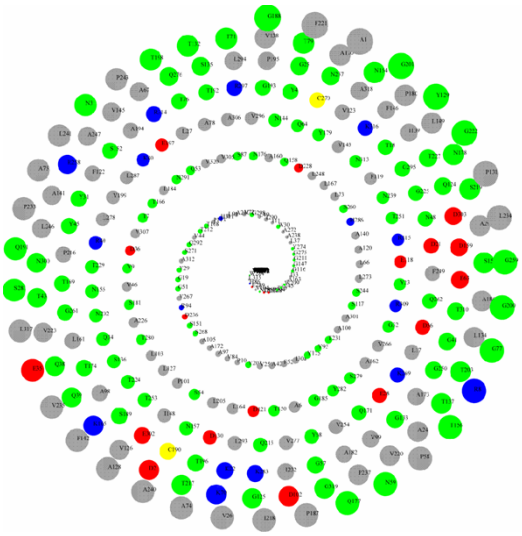
Substrate	Modification reagent	Initial rate(μmol min^{-1}) V_R	*E* value	Enantiopreference
Sec-butyl butyrate	Native *Pc*L	12.0	1.1	R
	NAI alone	10.6	1.2	R
	NAI +*n*-hexane	8.6	3.4	R
	I_3^-alone	11.3	1.7	R
	I_3^-+*n*-hexane	10.2	2.0	R
	EDA alone	14.0	2.2	R
	EDC alone	6.8	3.0	R
Sec-butyl hexanoate	Native *Pc*L	13.1	1.2	R
	NAI alone	9.7	1.2	R
	NAI +*n*-hexane	11.0	3.1	R
	I_3^-alone	12.1	1.8	R
	I_3^-+*n*-hexane	9.3	2.3	R
	EDA alone	14.7	2.3	R
	EDC alone	8.2	3.2	R
Hexan-2-yl acetate	Native *Pc*L	11.2	1.6	R
	NAI alone	10.8	1.8	R
	NAI +*n*-hexane	8.7	3.9	R
	I_3^-alone	10.6	2.6	R
	I_3^-+*n*-hexane	10.3	3.1	R
	EDA alone	12.8	3.0	R
	EDC alone	6.3	3.7	R
Octan-2-yl acetate	Native *Pc*L	12.3	1.9	R
	NAI alone	9.8	2.0	R
	NAI +*n*-hexane	9.1	4.5	R
	I_3^-alone	11.0	3.0	R
	I_3^-+*n*-hexane	11.6	3.5	R
	EDA alone	13.6	3.3	R
	EDC alone	7.8	4.0	R

Table S2 The binding free energy of *Pc*L and *sec*-butyl butyrate, *sec*-butyl hexanoate, *hexan*-2-yl acetate and *octan*-2-yl acetate.

Substrate	Modification reagent	Binding free energy (kJ/mol)		|Δ(ΔG)| (kJ/mol)
		R (ΔG)	S (ΔG)	
	Native *Pc*L	-21.50	-20.34	0.2
	NAI alone	-19.87	-19.68	0.2
Sec-butyl butyrate	NAI +*n*-hexane	-28.88	-13.61	15.3
	I_3^-alone	-17.18	-12.79	4.4
	I_3^-+*n*-hexane	-26.07	-17.36	9.6
	EDA alone	-10.58	-0.26	10.3
	EDC alone	-22.68	-9.15	13.5
	Native *Pc*L	-18.65	-16.49	2.2
	NAI alone	-22.86	-20.20	2.7
	NAI +*n*-hexane	-26.79	-9.69	17.1
Sec-butyl hexanoate	I_3^-alone	-30.19	-18.65	11.5
	I_3^-+*n*-hexane	-30.81	-17.16	13.7
	EDA alone	-22.68	-8.70	14.0
	EDC alone	-21.47	-5.67	15.8
	Native *Pc*L	-18.91	-16.67	2.2
	NAI alone	-15.60	-12.94	2.7
	NAI +*n*-hexane	-28.65	-4.50	24.1
hexan-2-yl acetate	I_3^-alone	-28.65	-17.13	11.5
	I_3^-+*n*-hexane	-18.66	-3.98	14.7
	EDA alone	-20.71	-5.61	15.1
	EDC alone	-17.82	-0.50	17.3
	Native *Pc*L	-11.93	-11.61	0.3
	NAI alone	-7.49	-7.20	0.3
	NAI +*n*-hexane	-18.93	-10.02	8.9
octan-2-yl acetate	I_3^-alone	-13.30	-11.02	2.3
	I_3^-+*n*-hexane	-13.55	-8.74	4.8
	EDA alone	-26.81	-22.75	4.1
	EDC alone	-17.31	-9.40	7.9

Table S3 Modification degree of *Pc*L

Entry	Modification reagent	Modification site	Modification degree
1	NAI + *n*-hexane	Tyr^{4}	61.6
		Tyr^{29}	19.2
		Tyr^{45}	4.1
		Tyr^{95}	1.1
2	NAI alone	Tyr^{4}	56.4
		Tyr^{45}	3.7
		Tyr^{95}	1.2
3	I_3^- + *n*-hexane	Tyr^{29}	12.3
		Tyr^{45}	37.2
		Tyr^{95}	2.1
4	I_3^- alone	Tyr^{45}	53.3
		Tyr^{95}	4.0
5	EDA alone	Asp^{36}	8.2
6	EDC alone	Asp^{55}	5.7



Group	Residue
Carboxyl	Asp^{36}, Asp^{55}, Asp^{303}, Glu^{63}, Glu^{302}
Hydroxyl	Tyr^{4}, Tyr^{23}, Tyr^{29}, Tyr^{31}, Tyr^{45}, Tyr^{95}

Fig.S1 Potential modification sites of *Pc*L scanned by accessible surface analysis (ASA).

Table S4 Theoretical peptide masses of *Pc*L

Mass (*m/z*)	Position	Peptide sequence
2231.0767	23-40	YVGVLEYWYGIQEDLQQR
2138.1604	95-115	YVAAVAPDLVASVTTIGTPHR
2124.9944	41-61	GATVYVANLSGFQSDDGPNGR
1706.8972	284-297	WNHLDEINQLLGVR
1526.8577	9-22	YPIILVHGLTGTDK
1519.7461	270-283	CSALYGQVLSTSYK
1424.7604	81-94	VNLVGHSQGGLTSR
1211.6378	298-309	GANAEDPVAVIR
1047.5065	259-269	GSGQNDGVVSK
1020.5724	62-70	GEGQNDGVVSK
881.4111	1-8	ADNYAATR

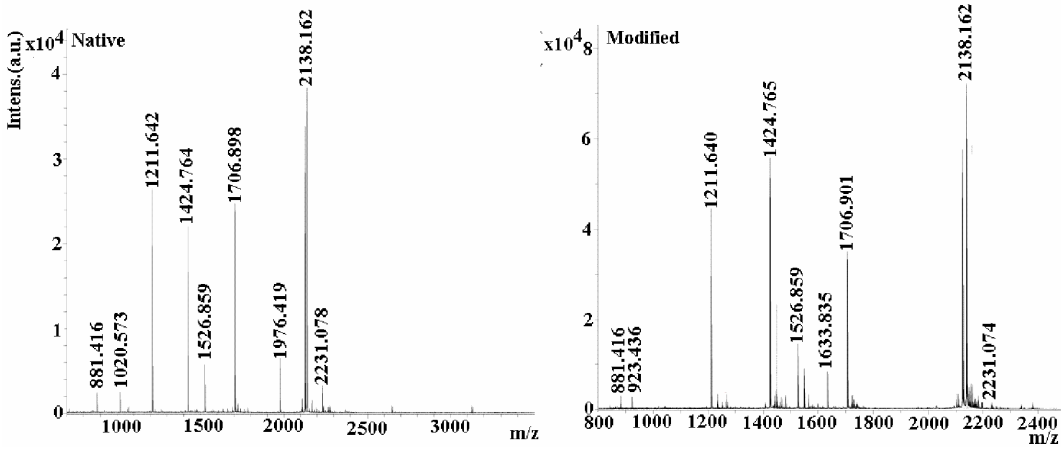


Fig.S2 MALDI-TOF fingerprint mass spectra obtained by tryptic digestion of native and NAI modified *Pc*L in the presence of *n*-hexane.

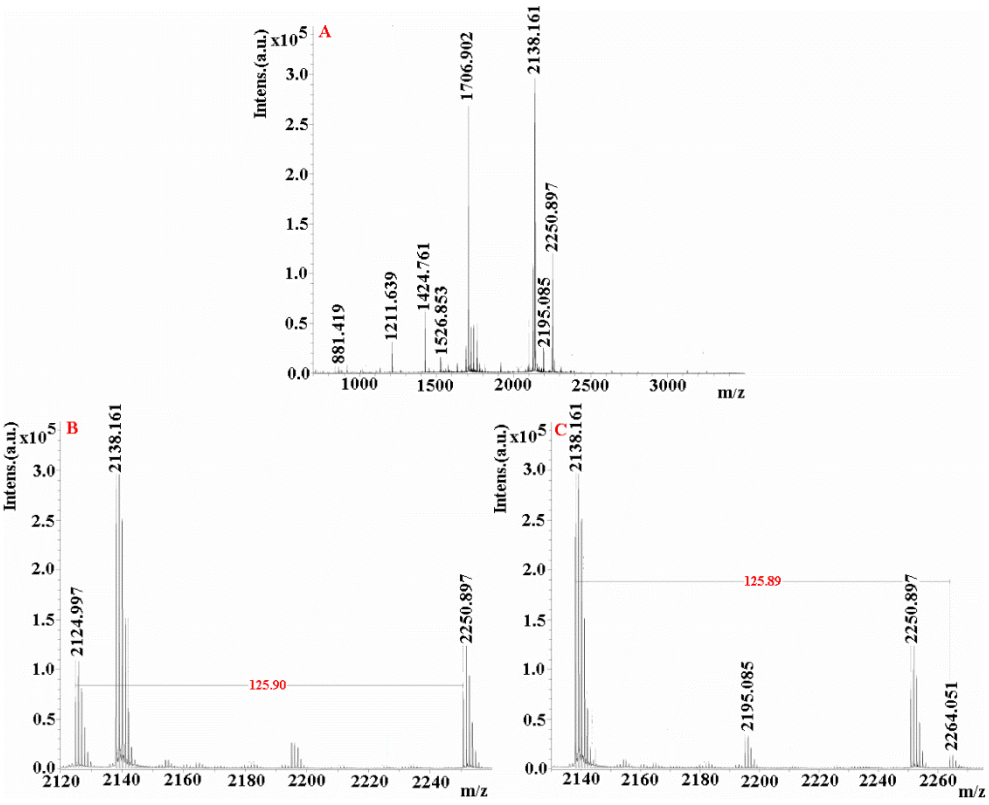


Fig.S3 MALDI-TOF mass spectra by tryptic digestion of I_3^- modified *Pc*Lin the absence of *n*-hexane. (A) mass fingerprint spectrum (B) expanded mass spectrum, Tyr[45] modification, *m/z* change from 2124.997 to 2250.897 (C) expanded mass spectrum, Tyr[95] modification, *m/z* change from 2138.161 to 2264.051.

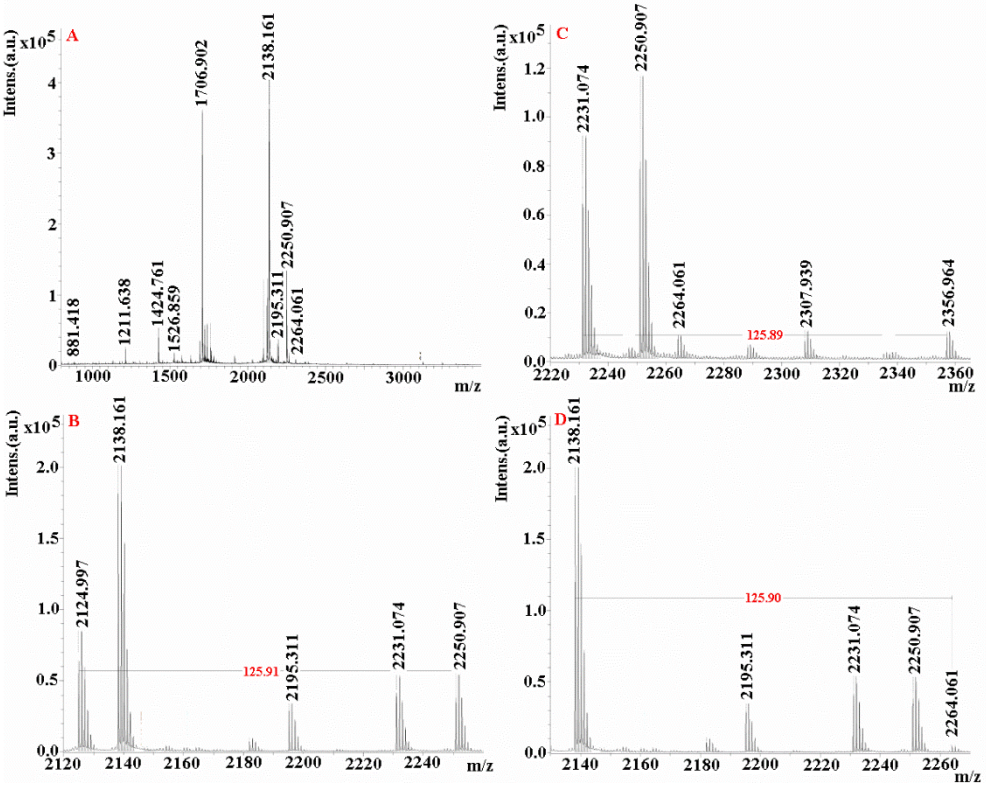


Fig.S4 MALDI-TOF mass spectra by tryptic digestion of I_3^- modified *Pc*Lin the presence of *n*-hexane. (A) mass fingerprint spectrum (B) expanded mass spectrum , Tyr[45] modification, *m/z* change from 2124.997 to 2250.907 (C) expanded mass spectrum, Tyr[29] modification, *m/z* change from 2231.074 to 2356.964 (D) expanded mass spectrum, Tyr[95] modification, *m/z* change from 2138.164 to 2264.064.

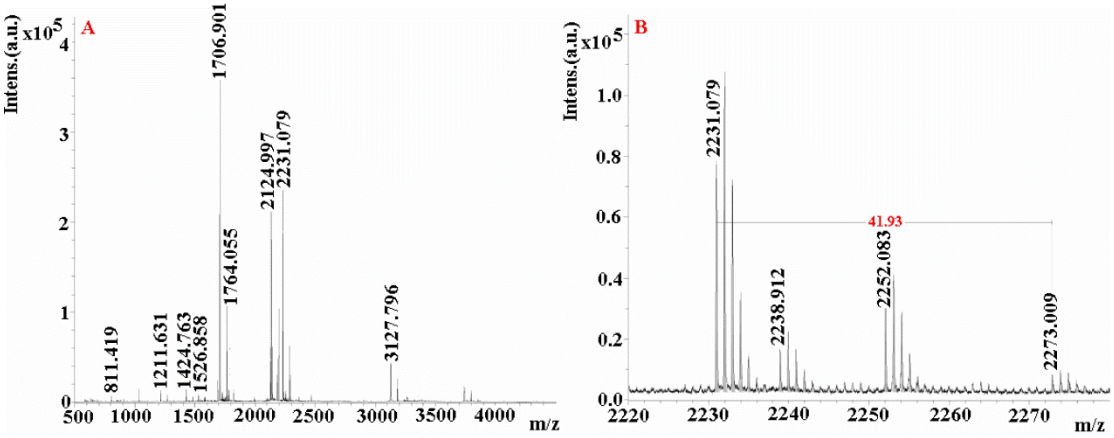


Fig.S5 MALDI-TOF mass spectra by tryptic digestion of EDA modified *Pc*Lin the absence of *n*-hexane. (A) mass fingerprint spectrum (B) expanded mass spectrum, Asp^{36} modification, *m/z* change from 2231.079 to 2273.009.

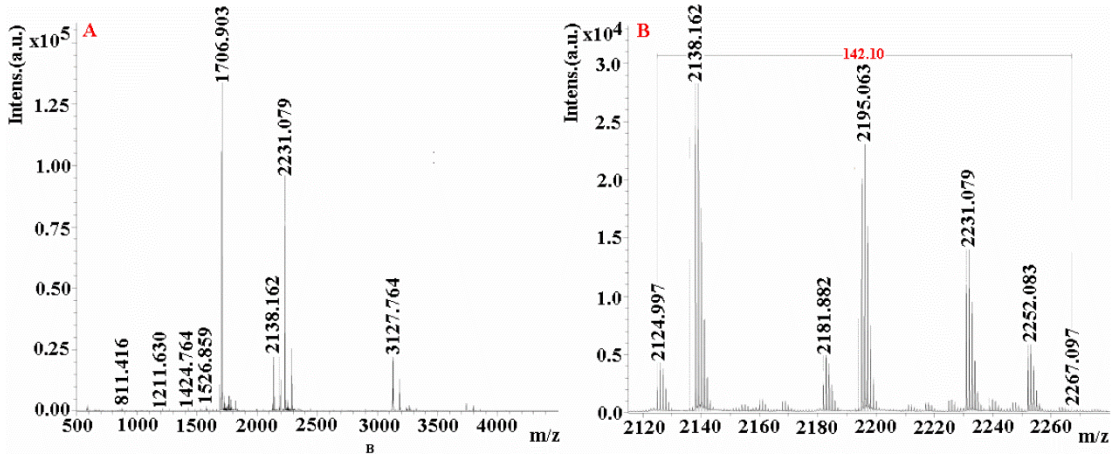


Fig.S6 MALDI-TOF mass spectra by tryptic digestion of EDC modified *Pc*Lin the absence of *n*-hexane. (A) mass fingerprint spectrum (B) expanded mass spectrum, Asp^{55} modification, *m/z* change from 2124.997 to 2267.097.

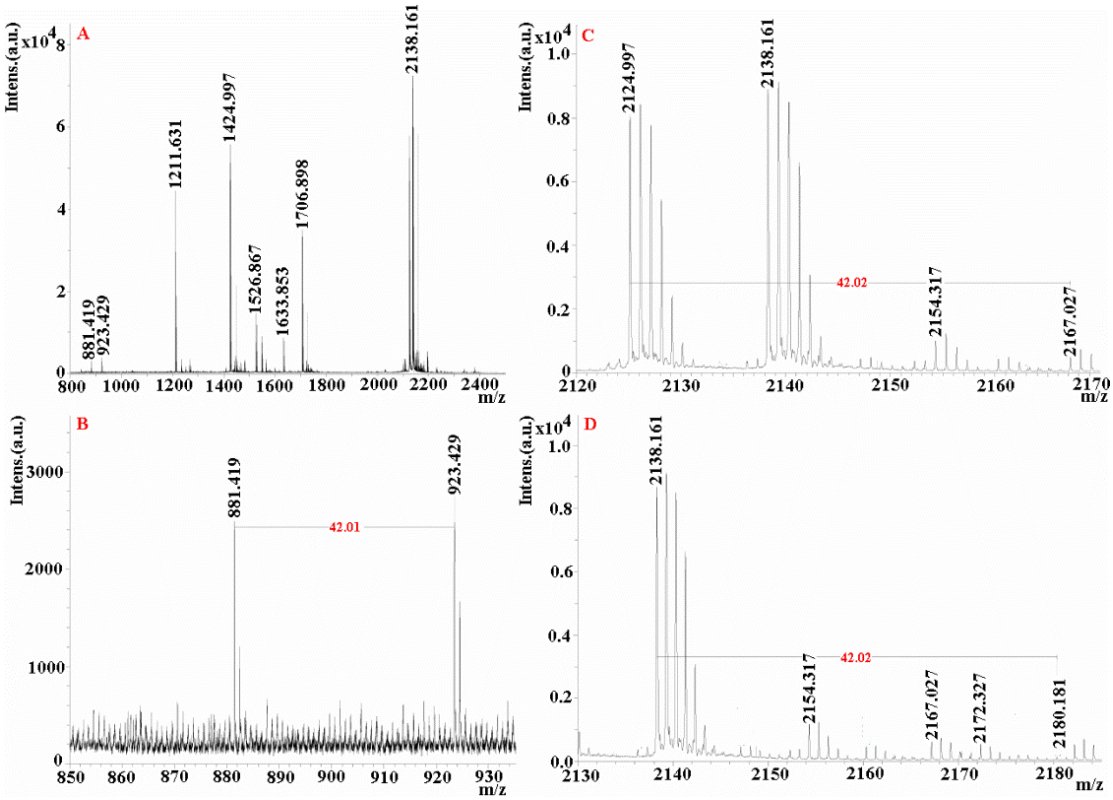


Fig.S7 MALDI-TOF mass spectra by tryptic digestion of NAI modified *Pc*L in the absence of *n*-hexane. (A) mass fingerprint spectrum (B) expanded mass spectrum, Tyr^{4} modification, *m/z* change from to 881.419 to 923.429 (C) expanded mass spectrum , Tyr^{45} modification, *m/z* change from 2124.997 to 2167.027 (D) expanded mass spectrum , Tyr^{95} modification, *m/z* change from 2138.161 to 2180.181.

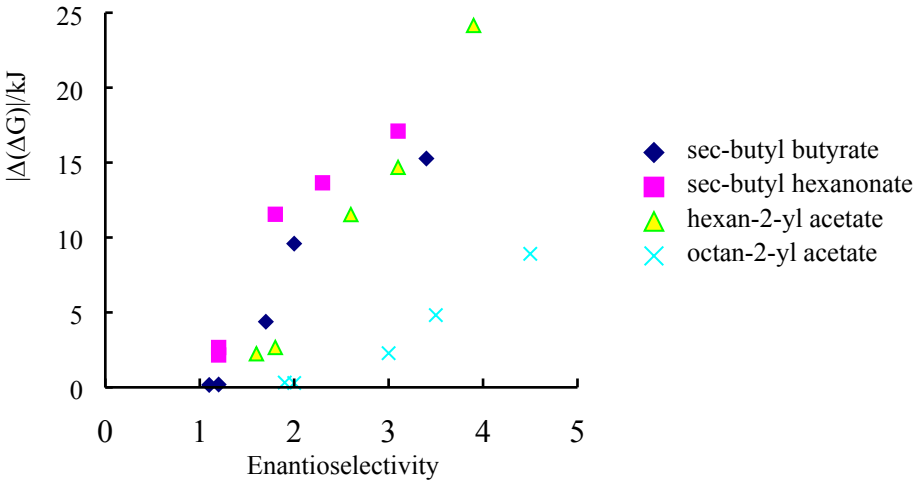


Fig.S8 Correlation of |Δ(ΔG)| and enantioselectivity corresponding to native and modified *Pc*L at Tyr.

Table S5 Activity standard deviation (three parallel experiments) of the *p*-NPP hydrolysis by modification reagents, native and modified *Pc*L in aqueous solution.

Lipase assay substrate	Modification reagent	Concentration (mM/L)	Standard deviation
p-nitrophenyl palmitate(*p*-NPP)	NAI + *n*-hexane	0 (0 mg/L)	0
		0.02 (2 mg/L)	0.02
		0.07 (8 mg/L)	0.01
		0.15 (16 mg/L)	0.01
		0.29 (32 mg/L)	0.02
	NAI alone	0 (0 mg/L)	0
		0.02 (2 mg/L)	0.01
		0.07 (8 mg/L)	0.02
		0.15 (16 mg/L)	0.02
		0.29 (32 mg/L)	0.008
	NAI without *Pc*L	0 (0 mg/L)	0.01
		0.02 (2 mg/L)	0.01
		0.07 (8 mg/L)	0.01
		0.15 (16 mg/L)	0.008
		0.29 (32 mg/L)	0.008
	I_3^-+*n*-hexane	0	0
		20	0.01
		40	0.02
		80	0.01
	I_3^-alone	0	0
		20	0.02
		40	0.02
		80	0.01
	I_3^- without *Pc*L	0	0.01
		20	0.01
		40	0.008
		80	0.008
	EDC alone	0	0
		62.5	0.01
		250	0.01
		500	0.01
		1000	0.02
	EDC without *Pc*L	0	0.01
		62.5	0.01
		250	0.008
		500	0.01
		1000	0.02

	EDA alone	0	0
		31.25	0.02
		125	0.01
		500	0.02
		1000	0.01
		1500	0.01
	EDA without *Pc*L	0	0.008
		31.25	0.008
		125	0.01
		500	0.008
		1000	0.01
		1500	0.008

Table S6 The enantioselectivity standard deviation (three parallel experiments) of the hydrolysis of *sec*-butyl acetate, *sec*-butyl butyrate, *sec*-butyl hexanoate, *hexan*-2-yl acetate and *octan*-2-yl acetate catalyzed by the native and modified *Pc*L.

Substrate	Modification reagent	Enantioselectivity standard deviation
sec-butyl acetate	Native *Pc*L	0.01
	NAI alone	0.02
	NAI + *n*-hexane	0.02
	I_3^- alone	0.02
	I_3^- + *n*-hexane	0.01
	EDA alone	0.02
	EDC alone	0.02
Sec-butyl butyrate	Native *Pc*L	0.009
	NAI alone	0.008
	NAI + *n*-hexane	0.01
	I_3^- alone	0.01
	I_3^- + *n*-hexane	0.02
	EDA alone	0.01
	EDC alone	0.008
Sec-butyl hexanoate	Native *Pc*L	0.008
	NAI alone	0.009
	NAI + *n*-hexane	0.01
	I_3^- alone	0.02
	I_3^- + *n*-hexane	0.01
	EDA alone	0.02
	EDC alone	0.01
Hexan-2-yl acetate	Native *Pc*L	0.01
	NAI alone	0.01
	NAI + *n*-hexane	0.008
	I_3^- alone	0.01
	I_3^- + *n*-hexane	0.01
	EDA alone	0.01
	EDC alone	0.01
Octan-2-yl acetate	Native *Pc*L	0.01
	NAI alone	0.01
	NAI + *n*-hexane	0.008
	I_3^- alone	0.01
	I_3^- + *n*-hexane	0.01
	EDA alone	0.02
	EDC alone	0.02

Table S7 The ee_s of the hydrolysis of *sec*-butyl acetate, *sec*-butyl butyrate, *sec*-butyl hexanoate, *hexan*-2-yl acetate and *octan*-2-yl acetate catalyzed by the native and modified *Pc*L.

Substrate	Modification reagent	ee_s
sec-butyl acetate	Native *Pc*L	7%
	NAI alone	9%
	NAI + *n*-hexane	47%
	I_3^- alone	26%
	I_3^- + *n*-hexane	32%
	EDA alone	36%
	EDC alone	47%
Sec-butyl butyrate	Native *Pc*L	8%
	NAI alone	10%
	NAI + *n*-hexane	48%
	I_3^- alone	27%
	I_3^- + *n*-hexane	34%
	EDA alone	33%
	EDC alone	46%
Sec-butyl hexanoate	Native *Pc*L	8%
	NAI alone	9%
	NAI + *n*-hexane	48%
	I_3^- alone	27%
	I_3^- + *n*-hexane	33%
	EDA alone	38%
	EDC alone	47%
Hexan-2-yl acetate	Native *Pc*L	12%
	NAI alone	16%
	NAI + *n*-hexane	55%
	I_3^- alone	32%
	I_3^- + *n*-hexane	45%
	EDA alone	47%
	EDC alone	53%
Octan-2-yl acetate	Native *Pc*L	15%
	NAI alone	20%
	NAI + *n*-hexane	61%
	I_3^- alone	47%
	I_3^- + *n*-hexane	52%
	EDA alone	49%
	EDC alone	57%

Enantiomeric excess of (*R*)-secondary alcohol (ee_s) and enantioselectivity (E value) were calculated as defined below, where A_R and A_S were the peak area of (*R*)-secondary alcohol and (*S*)-secondary alcohol. Internal standard was n-octane which was used to determine the concentration. Conversion ratio was defined as c, $c = ee_s/(ee_s+ee_p)$.

$$ee_s = \frac{A_R - A_S}{A_R + A_S} \qquad E = \frac{\ln [(1-c)(1-ee_s)]}{\ln [(1-c)(1+ee_s)]}$$