Supplementary Information

Synthetically useful variants of industrial lipases from *Burkholderia cepacia* and *Pseudomonas fluorescens*

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[A] General.

NMR spectra were measured on a Varian 400-MR spectrometer or a JEOL JNM-ECS400 spectrometer, and chemical shifts are reported as the delta scale in ppm using a solvent residual peak as an internal reference (δ = 7.26 ppm ($CDCl_3$) for ^{1}H NMR and δ = 77.0 ppm ($CDCl_3$) for ^{13}C NMR) or using an external reference (δ = –164.9 ppm (C_6F_6) for ^{19}F NMR). IR spectra were recorded on a Shimadzu IRAffinity-1 spectrophotometer. HPLC was performed on a Shimadzu LC-20AT/SPD-20A. Optical rotations were measured on a Horiba SEPA-300 polarimeter at the sodium D line. Column chromatography was carried out using Fuji Silysia BW-127 ZH (100–270 mesh), and thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254}. Secondary alcohols **1f**,[1] **1g**,[2] **1j**,[3] **1k**,[3] **1l**,[4] and **1m**[5,6] were prepared according to the literature, while **1a–e**, **1h**, and **1i** were purchased.

[B] Determination of enantiomeric purities and absolute configurations.

The enantiomeric purities of **1d–g**, **1i**, **1j**, **1m**, **2f**, **2g**, **2i**, **2k**, and **2m** were determined by HPLC using chiral columns (Daicel). Those of **2d**, **2e**, and **2j** were determined after conversion to the corresponding alcohols, and that of **1k** was determined after conversion to the corresponding acetate. Those of **1a**, **1c**, **1h**, **2a–c**, and **2h** were determined by chiral GC (CP-cyclodextrin-β-2,3,6-M-19 column (Varian, ϕ 0.25 mm × 25 m)). That of **1b** was determined after conversion to the corresponding acetate. Those of **1l** and **2l** were determined by ^{19}F NMR after conversion to the corresponding (*R*)-MTPA esters. The absolute configurations were determined by comparison with the signs of the reported specific rotations.

[C] Lipase-catalyzed kinetic resolution.

General procedure. A mixture of alcohol **1** (0.50 mmol), immobilized lipase (200 mg, 0.5% (w/w) enzyme/Toyonite-200M), and molecular sieves 3A (three pieces) in dry *i*-Pr_2O (5.0 mL) in a test tube with a rubber septum was stirred at 30 °C for 30 min. The reaction was started by addition of vinyl acetate (93 μL, 1.0 mmol) via a syringe. The progress of the reaction was monitored by TLC. The reaction was stopped by filtration at an appropriate conversion, and the filtrate was concentrated under reduced pressure. Alcohol **1** and ester **2** were separated by silica gel column chromatography.

Kinetic resolution of 1-phenylethanol (1a). (*S*)-**1a**: Colorless oil; $[\alpha]^{22}_D$ –55.9 (*c* 0.735, $CHCl_3$), >99.9% ee, lit.[3] $[\alpha]^{25}_D$ –56.9 (*c* 0.686, $CHCl_3$) for (*S*)-**1a** with 99.8% ee; GC for **1a**: Inj. 250 °C, Col. 95 °C, Det. 220 °C, (*R*) 29.8 min, (*S*) 32.9 min. (*R*)-**2a**: Colorless oil; $[\alpha]^{22}_D$ +102 (*c* 1.00, $CHCl_3$), 99.6% ee, lit.[3] $[\alpha]^{26}_D$ +110.6 (*c* 1.02, $CHCl_3$) for (*R*)-**2a** with 99.1% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.54 (d, *J* = 6.4 Hz, 3H), 2.07 (s, 3H), 5.88 (q, *J* = 6.7 Hz, 1H), 7.29–7.36 (m, 5H); GC for **2a**: Inj. 250 °C, Col. 95 °C, Det. 220 °C, (*S*) 25.7 min, (*R*) 28.5 min.

Kinetic resolution of 1-cyclohexylethanol (1b). (*S*)-**1b**: Colorless oil; $[\alpha]^{15}_{D}$ +1.99 (*c* 1.01, $CHCl_3$), 59.4% ee, lit.[7] $[\alpha]^{29}_{D}$ +2.90 (*c* 1.00, $CHCl_3$) for (*S*)-**1b** with 81.0% ee. (*R*)-**2b**: Colorless oil; $[\alpha]^{15}_{D}$ +6.77 (*c* 0.842, $CHCl_3$), 99.3% ee, lit.[7] $[\alpha]^{29}_{D}$ +7.45 (*c* 1.02, $CHCl_3$) for (*R*)-**2b** with 97.0% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90–1.76 (m, 14H), 2.03 (s, 3H), 4.69–4.75 (m, 1H); GC for **2b**: Inj. 250 °C, Col. 95 °C, Det. 220 °C, (*S*) 19.3 min, (*R*) 21.8 min.

Kinetic resolution of 4-phenyl-2-butanol (1c). (*S*)-**1c**: Colorless oil; $[\alpha]^{19}_{D}$ +17.7 (*c* 0.926, $CHCl_3$), 93.6% ee, lit.[8] $[\alpha]_{D}$ +17.1 (*c* 1.22, $CHCl_3$) for (*S*)-**1c** with >99% ee; GC for **1c**: Inj. 300 °C, Col. 100 °C, Det. 220 °C, (*S*) 64.7 min, (*R*) 67.9 min. (*R*)-**2c**: Colorless oil; $[\alpha]^{19}_{D}$ +11.5 (*c* 1.04, $CHCl_3$), 92.5% ee, lit.[9] $[\alpha]^{26}_{D}$ +7.82 (*c* 1.00, $CHCl_3$) for (*R*)-**2c** with 99% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (d, *J* = 6.0 Hz, 3H), 1.76–1.85 (m, 1H), 1.89–1.98 (m, 1H), 2.03 (s, 3H), 2.57–2.72 (m, 2H), 4.90–4.98 (m, 1H), 7.16–7.30 (m, 5H); GC for **2c**: Inj. 300 °C, Col. 110 °C, Det. 220 °C, (*S*) 47.1 min, (*R*) 51.7 min.

Kinetic resolution of 1-indanol (1d). (*S*)-**1d**: White solid; $[\alpha]^{21}_{D}$ +32.2 (*c* 1.15, $CHCl_3$), 99.8% ee, lit.[10] $[\alpha]^{22}_{D}$ +30.8 (*c* 0.8, $CHCl_3$) for (*S*)-**1d** with 91% ee; HPLC for **1d**: Chiralcel OB-H, hexane/*i*-PrOH = 9:1, 0.5 mL/min, 254 nm, (*R*) 14.1 min, (*S*) 20.3 min. (*R*)-**2d**: Colorless oil; $[\alpha]^{21}_{D}$ +96.8 (*c* 1.02, $CHCl_3$), 97.4% ee, lit.[9] $[\alpha]^{26}_{D}$ +87.6 (*c* 1.00, $CHCl_3$) for (*R*)-**2d** with 99% ee. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.06 (s, 3H), 2.07–2.15 (m, 1H), 2.45–2.55 (m, 1H), 2.84–2.92 (m, 1H), 3.08–3.16 (m, 1H), 6.20 (dd, *J* = 3.6, 7.2 Hz, 1H), 7.22–7.42 (m, 4H).

Kinetic resolution of *trans*-2-phenylcyclohexanol (1e). (*S*)-**1e**: White solid; $[\alpha]^{18}_{D}$ +20.9 (*c* 1.11, $CHCl_3$), 65.8% ee, lit.[11] $[\alpha]_{D}$ +56 (*c* 1.1, $CHCl_3$) for (*S*)-**1e** with >98% ee; HPLC for **1e**: Chiralcel OD-H, hexane/*i*-PrOH = 100:1, 1.0 mL/min, 254 nm, (*S*) 19.8 min, (*R*) 23.1 min. (*R*)-**2e**:[12] Colorless oil; $[\alpha]^{17}_{D}$ –13.8 (*c* 1.06, $CHCl_3$), >99.9% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26–1.62 (m, 4H), 1.76 (s, 3H), 1.79–1.97 (m, 3H), 2.08–2.14 (m, 1H), 2.62–2.68 (m, 1H), 4.94–5.00 (m, 1H), 7.15–7.29 (m, 5H).

Kinetic resolution of 1-(2-naphthyl)propanol (1f). (*S*)-**1f**: White solid; $[\alpha]^{16}_{D}$ –29.8 (*c* 1.09, $CHCl_3$), 80.4% ee, lit.[1] $[\alpha]^{22}_{D}$ –28.8 (*c* 1.03, $CHCl_3$) for (*S*)-**1f** with 77% ee; HPLC for **1f**: Chiralcel OJ-H, hexane/*i*-PrOH = 9:1, 0.5 mL/min, 254 nm, (*S*) 25.8 min, (*R*) 35.3 min. (*R*)-**2f**: Colorless oil; $[\alpha]^{17}_{D}$ +90.7 (*c* 1.09, $CHCl_3$), 98.9% ee, lit.[1] $[\alpha]^{22}_{D}$ +96.8 (*c* 1.11, $CHCl_3$) for (*R*)-**2f** with 97% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91 (t, *J* = 7.6 Hz, 3H), 1.88–2.03 (m, 2H), 2.10 (s, 3H), 5.83 (t, *J* = 6.8 Hz, 1H), 7.44–7.49 (m, 3H), 7.78–7.84 (m, 4H); HPLC for **2f**: Chiralcel OJ-H, hexane/*i*-PrOH = 9:1, 0.5 mL/min, 254 nm, (*R*) 20.5 min, (*S*) 27.8 min.

Kinetic resolution of 2-chloro-1-phenylethanol (1g). (*R*)-**1g**: Colorless oil; $[\alpha]^{21}_{D}$ –48.2 (*c* 1.02, $CHCl_3$), 64.6% ee, lit.[13] $[\alpha]^{24}_{D}$ –20.5 (*c* 1.20, $CHCl_3$) for (*R*)-**1g** with 23% ee; HPLC for **1g**: Chiralcel OB-H, hexane/*i*-PrOH = 9:1, 0.5 mL/min, 254 nm, (*R*) 19.9 min, (*S*) 27.3 min. (*S*)-**2g**: Colorless oil; $[\alpha]^{20}_{D}$ +50.1 (*c* 1.02, $CHCl_3$), >99.9% ee, lit.[13] $[\alpha]^{23}_{D}$ +84.6 (*c* 0.70, $CHCl_3$) for (*S*)-**2g** with 98% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.14 (s, 3H), 3.73 (dd, *J* = 4.6, 11.4 Hz, 1H), 3.80 (dd, *J* = 8.0, 11.6 Hz, 1H), 5.96 (dd, *J* = 4.6, 7.8 Hz, 1H), 7.34–7.40 (m, 5H); HPLC for **2g**: Chiralcel OB-H, hexane/*i*-PrOH = 9:1, 0.5 mL/min, 254 nm, (*S*) 18.4 min, (*R*) 20.4 min.

Kinetic resolution of 2,2,2-trifluoro-1-phenylethanol (1h). (*R*)-**1h**: Colorless oil; $[\alpha]^{18}_{D}$ –19.0 (*c* 0.920, $CHCl_3$), 63.5% ee, lit.[14] $[\alpha]^{26}_{D}$ –1.77 (*c* 0.12, $CHCl_3$) for (*R*)-**1h** with 62% ee; GC for **1h**: Inj. 250 °C, Col. 100 °C, Det. 220 °C, (*S*) 53.5 min, (*R*) 57.2 min. (*S*)-**2h**: Colorless oil; $[\alpha]^{18}_{D}$ +73.6 (*c* 0.923, $CHCl_3$), 96.4% ee, lit.[14] $[\alpha]^{26}_{D}$ +3.9 (*c* 0.023, $CHCl_3$) for (*S*)-**2h** with 99% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.20 (s, 3H), 6.14 (q, *J* = 7.1 Hz, 1H), 7.40–7.48 (m, 5H); GC for **2h**: Inj. 250 °C, Col. 70 °C, Det. 220 °C, (*R*) 34.3 min, (*S*) 36.2 min.

Kinetic resolution of ethyl mandelate (1i). (*R*)-**1i**: Colorless oil; $[\alpha]^{18}_{D}$ –25.6 (*c* 1.02, $CHCl_3$), 18.0% ee, lit.[15] $[\alpha]^{25}_{D}$ –104 (*c* 1.00, $CHCl_3$) for (*R*)-**1i** with 88% ee; HPLC for **1i**: Chiralcel OB-H, hexane/*i*-PrOH = 9:1, 0.5 mL/min, 254 nm, (*S*) 23.8 min, (*R*) 27.5 min. (*S*)-**2i**:[16] Colorless oil; $[\alpha]^{18}_{D}$ +55.7 (*c* 0.571, $CHCl_3$), 92.6% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.22 (t, *J* = 7.2 Hz, 3H), 2.20 (s, 3H), 4.11–4.27 (m, 2H), 5.91 (s, 1H), 7.38–7.41 (m, 3H), 7.46–7.48 (m, 2H); HPLC for **2i**: Chiralcel OD-H, hexane/*i*-PrOH = 30:1, 0.5 mL/min, 254 nm, (*S*) 15.7 min, (*R*) 16.9 min.

Kinetic resolution of 1-phenyl-1-hexanol (1j). (*S*)-**1j**: Colorless oil; $[\alpha]^{17}_{D}$ –31.5 (*c* 1.04, $CHCl_3$), 96.8% ee, lit.[3] $[\alpha]^{35}_{D}$ –6.4 (*c* 1.18, $CHCl_3$) for (*S*)-**1j** with 18.2% ee; HPLC for **1j**: Chiralcel OB-H, hexane/*i*-PrOH = 9:1, 0.5 mL/min, 254 nm, (*S*) 11.5 min, (*R*) 13.2 min. (*R*)-**2j**: Colorless oil; $[\alpha]^{17}_{D}$ +61.0 (*c* 0.942, $CHCl_3$), 98.3% ee, lit.[3] $[\alpha]^{34}_{D}$ +44.1 (*c* 0.673, $CHCl_3$) for (*R*)-**2j** with 61.3% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (t, *J* = 7.2 Hz, 3H), 1.18–1.34 (m, 6H), 1.72–1.77 (m, 1H), 1.85–1.91 (m, 1H), 2.05 (s, 3H), 5.71 (dd, *J* = 6.4, 7.6 Hz, 1H), 7.26–7.33 (m, 5H).

Kinetic resolution of 1,6-diphenyl-1-hexanol (1k). (*S*)-**1k**: Colorless oil; $[\alpha]^{17}_{D}$ –17.7 (*c* 1.29, $CHCl_3$), 95.9% ee, lit.[3] $[\alpha]^{20}_{D}$ –15.7 (*c* 1.03, $CHCl_3$) for (*S*)-**1k** with 69.1% ee. (*R*)-**2k**: Colorless oil; $[\alpha]^{18}_{D}$ +50.0 (*c* 1.08, $CHCl_3$), >99.9% ee, lit.[3] $[\alpha]^{19}_{D}$ +51.8 (*c* 1.04, $CHCl_3$) for (*R*)-**2k** with >99.5% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.23–1.37 (m, 4H), 1.55–1.63 (m, 2H), 1.71–1.80 (m, 1H), 1.85–1.94 (m, 1H), 2.06 (s, 3H), 2.57 (t, *J* = 7.6 Hz, 2H), 5.71 (dd, *J* = 6.4, 7.6 Hz, 1H), 7.13–7.16 (m, 3H), 7.24–7.33 (m, 7H); HPLC for **2k**: Chiralpak IC, hexane/*i*-PrOH = 75:1, 0.5 mL/min, 254 nm, (*R*) 19.5 min, (*S*) 25.7 min.

Kinetic resolution of 1-trimethylsilyl-1-octyn-3-ol (1l). (*S*)-**1l**: Colorless oil; $[\alpha]^{17}_{D}$ –1.45 (*c* 0.963, $CHCl_3$), 96.6% ee, lit.[17] $[\alpha]^{21}_{D}$ –14.3 (*c* 0.95, $CHCl_3$) for (*S*)-**1l** with >99% ee; ^{19}F NMR for the (*R*)-MTPA esters of **1l**: (*S*) –70.2 ppm, (*R*) –69.9 ppm. (*R*)-**2l**:[18] Colorless oil; $[\alpha]^{17}_{D}$ +94.6 (*c* 1.15, $CHCl_3$), 99.4% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.17 (s, 9H), 0.89 (t, *J* = 7.0 Hz, 3H), 1.30–1.44 (m, 6H), 1.69–1.75 (m, 2H), 2.08 (s, 3H), 5.38 (t, *J* = 6.8 Hz, 1H).

Kinetic resolution of 1-(2-thiazolyl)-1-octanol (1m). (*S*)-**1m**: Colorless oil; $[\alpha]^{18}_{D}$ –14.9 (*c* 0.985, $CHCl_3$), 62.0% ee, lit.[5] $[\alpha]^{25}_{D}$ –18.1 (*c* 1.00, $CHCl_3$) for (*S*)-**1m** with >98% ee; HPLC for **1m**: Chiralpak IA, hexane/*i*-PrOH = 30:1, 0.5 mL/min, 254 nm, (*R*) 39.6 min, (*S*) 45.9 min. (*R*)-**2m**: Colorless oil; $[\alpha]^{18}_{D}$ +54.6 (*c* 1.09, $CHCl_3$), 96.7% ee; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (t, *J* = 6.9 Hz, 3H), 1.24–1.37 (m, 10H), 1.99–2.07 (m, 2H), 2.12 (s, 3H), 6.08 (t, *J* = 6.7 Hz, 1H), 7.28 (d, *J* = 3.3 Hz, 1H), 7.75 (d, *J* = 3.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 20.9, 22.5, 24.9, 29.0, 29.1, 31.6, 35.1, 72.8, 119.0, 142.5, 169.9, 170.0; IR (neat) 3117, 3084, 2955, 2928, 2857, 1749, 1504, 1466, 1458, 1435, 1371, 1229, 1148, 1119, 1026, 959, 930, 725 cm^{-1}; HRMS (EI) calcd for $C_{13}H_{21}NO_2S$ 255.1293, found 255.1294 (M^+); HPLC for **2m**: Chiralpak IA, hexane/*i*-PrOH = 30:1, 0.5 mL/min, 254 nm, (*R*) 20.8 min, (*S*) 24.7 min.

Table S1. Substrate scope of the LPS_L287F/I290A double mutant and the LPS_wild-type enzyme.[a]

		time	L287F/I290A				wild-type			
entry	**1**	(h)	*c* (%)[b]	(*R*)-**2**[c]	(*S*)-**1**[c]	*E*[d]	*c* (%)[b]	(*R*)-**2**[c]	(*S*)-**1**[c]	*E*[d]
1	**1a**	1	50	47 (99.6)	45 (>99.9)	>200	40	42 (99.4)	55 (65.4)	>200
2	**1b**	4	37	34 (99.3)	59 (59.4)	>200	42	37 (96.6)	52 (68.8)	119
3	**1c**	2	50	48 (92.5)	50 (93.6)	90	35	32 (99.3)	63 (52.8)	>200
4	**1d**	1	51	42 (97.4)	44 (99.8)	>200	53	46 (89.2)	38 (>99.9)	>130
5	**1e**	10	40	38 (>99.9)	49 (65.8)	>200	46	43 (>99.9)	47 (85.7)	>200
6	**1f**	15	45	44 (98.9)	46 (80.4)	>200	36	35 (99.1)	50 (56.1)	>200
7	**1g**[e]	2.5	39	35 (>99.9)	52 (64.6)	>200	24	18 (>99.9)	71 (30.9)	>200
8	**1h**[e]	96	40	28 (96.4)	42 (63.5)	105	19	12 (94.4)	71 (22.6)	43
9	**1i**[e]	24	16	16 (92.6)	78 (18.0)	31	49	47 (94.5)	47 (92.2)	117
10	**1j**	2	50	38 (98.3)	47 (96.8)	>200	9[f]	–	–	–
11	**1k**	3	49	43 (>99.9)	48 (95.9)	>200	5[f]	–	–	–
12	**1l**	2	49	43 (99.4)	41 (96.6)	>200	5[f]	–	–	–
13	**1m**	1.5	39	37 (96.7)	59 (62.0)	113	10[f]	–	–	–

[a] Conditions: immobilized lipase (200 mg, 0.5% (w/w) enzyme/Toyonite-200M), **1** (0.50 mmol), vinyl acetate (1.0 mmol), molecular sieves 3A (three pieces), dry *i*-Pr_2O (5 mL), 30 °C. [b] Conversion calculated from c = ee(**1**)/(ee(**1**) + ee(**2**)). [c] Isolated yield (% ee). [d] Calculated from $E = \ln[1 - c(1 + \text{ee}(\mathbf{2}))]/\ln[1 - c(1 - \text{ee}(\mathbf{2}))]$. [e] (*S*)-**2** and (*R*)-**1** were obtained. [f] Conversion calculated from ^{1}H NMR.

Table S2. Substrate scope of the LAK_V287F/I290A double mutant and the LAK_wild-type enzyme.[a]

		time	V287F/I290A				wild-type			
entry	**1**	(h)	*c* (%)[b]	(*R*)-**2**[c]	(*S*)-**1**[c]	*E*[d]	*c* (%)[b]	(*R*)-**2**[c]	(*S*)-**1**[c]	*E*[d]
1	**1a**	1	50	48 (>99.9)	49 (>99.9)	>200	47	46 (98.1)	48 (87.7)	>200
2	**1b**	4	42	31 (99.1)	48 (73.0)	>200	50	30 (92.1)	36 (91.3)	78
3	**1c**	2.5	49	40 (90.2)	48 (87.0)	55	47	37 (>99.9)	54 (89.7)	>200
4	**1d**	0.25	43	41 (>99.9)	54 (74.9)	>200	50	40 (>99.9)	47 (>99.9)	>200
5	**1e**	10	25	46 (>99.9)	43 (33.5)	>200	49	49 (>99.9)	43 (94.5)	>200
6	**1f**	24	43	43 (98.1)	50 (75.2)	>200	35	38 (98.7)	58 (54.3)	>200
7	**1g**[e]	3	43	38 (>99.9)	50 (73.9)	>200	26	25 (>99.9)	66 (34.4)	>200
8	**1h**[e]	60	48	36 (93.9)	51 (86.8)	90	45	29 (43.8)	51 (36.2)	4
9	**1i**[e]	12	41	29 (>99.9)	54 (68.9)	>200	43	31 (87.7)	55 (65.6)	30
10	**1j**	1.5	50	48 (>99.9)	39 (>99.9)	>200	4[f]	–	–	–
11	**1k**	4	46	42 (>99.9)	51 (86.7)	>200	6[f]	–	–	–
12	**1l**	3	50	39 (99.4)	39 (>99.9)	>200	6[f]	–	–	–
13	**1m**	1.5	48	45 (95.8)	52 (87.4)	134	27	28 (71.6)	71 (26.5)	8

[a] Conditions: immobilized lipase (200 mg, 0.5% (w/w) enzyme/Toyonite-200M), **1** (0.50 mmol), vinyl acetate (1.0 mmol), molecular sieves 3A (three pieces), dry *i*-Pr_2O (5 mL), 30 °C. [b] Conversion calculated from c = ee(**1**)/(ee(**1**) + ee(**2**)). [c] Isolated yield (% ee). [d] Calculated from $E = \ln[1 - c(1 + \text{ee}(\mathbf{2}))]/\ln[1 - c(1 - \text{ee}(\mathbf{2}))]$. [e] (*S*)-**2** and (*R*)-**1** were obtained. [f] Conversion calculated from ^{1}H NMR.

Table S3. Temperature effect in the kinetic resolution of **1m** with the LPS_L287F/I290A double mutant and the LPS_wild-type enzyme.[a]

				% yield (% ee)		
LPS	*T* (°C)	time (h)	*c* (%)[b]	(*R*)-**2m**	(*S*)-**1m**	*E*[c]
L287F/I290A	30	2	50	43 (90.0)	47 (89.4)	57.4
L287F/I290A	35	1	33	30 (93.3)	64 (46.3)	45.6
L287F/I290A	40	1	41	35 (90.6)	55 (63.2)	38.8
L287F/I290A	45	1	44	39 (89.1)	55 (68.9)	35.7
L287F/I290A	50	1	50	44 (82.7)	50 (81.3)	26.3
wild-type	30	7	40	34 (55.3)	54 (36.8)	4.92
wild-type	35	5	41	34 (49.6)	53 (34.1)	4.09
wild-type	40	4	39	34 (49.1)	51 (30.9)	3.92
wild-type	45	4	44	40 (44.3)	50 (34.3)	3.57
wild-type	50	4	53	48 (38.1)	48 (43.1)	3.32

[a] Conditions: immobilized lipase (200 mg, 0.5% (w/w) enzyme/Toyonite-200M), **1m** (0.50 mmol), vinyl acetate (1.0 mmol), dry *i*-Pr_2O (5 mL), molecular sieves 3A (three pieces). [b] Conversion calculated from c = ee(**1m**)/(ee(**1m**) + ee(**2m**)). [c] Calculated from $E = \ln[1 - c(1 + \text{ee}(\mathbf{2m}))]/\ln[1 - c(1 - \text{ee}(\mathbf{2m}))]$.

Table S4. Solvent effect in the kinetic resolution of **1m** with the LPS_L287F/I290A double mutant.[a]

					% yield (% ee)		
solvent	log *P*	ε	time (h)	*c* (%)[b]	(*R*)-**2m**	(*S*)-**1m**	*E*[c]
1,4-dioxane	–1.1	2.2	48	38	31 (72.2)	58 (43.7)	9
acetone	–0.23	21	48	11	10 (72.1)	84 (8.75)	7
THF	0.49	7.5	48	19	18 (80.7)	75 (18.9)	11
Et_2O	0.85	4.3	11	37	34 (88.4)	59 (51.2)	27
i-Pr_2O	1.9	3.4	2	50	43 (90.0)	47 (89.4)	57
toluene	2.5	2.4	5	50	47 (87.1)	49 (87.6)	42
cyclohexane	3.2	2.0	1	42	38 (92.2)	54 (67.5)	50
hexane	3.5	1.9	0.5	38	35 (86.7)	60 (52.6)	24

[a] Conditions: LPS_L287F/I290A double mutant (200 mg, 0.5% (w/w) enzyme/Toyonite-200M), **1m** (0.50 mmol), vinyl acetate (1.0 mmol), dry organic solvent (5 mL), molecular sieves 3A (three pieces), 30 °C. [b] Conversion calculated from c = ee(**1m**)/(ee(**1m**) + ee(**2m**)). [c] Calculated from $E = \ln[1 - c(1 + \text{ee}(\mathbf{2m}))]/\ln[1 - c(1 - \text{ee}(\mathbf{2m}))]$.

[D] Genetic engineering and biochemical methods.

General methods. All the enzymes for DNA manipulations such as DNA polymerase and restriction enzymes were purchased from Takara Bio. The lipase genes for LPS and LAK consist of the structural genes for lipase (LipA) and chaperone (LipX), and all the synthetic genes for LPS_wild-type, LPS_L287F/I290A, LAK_wild-type, and LAK_V287F/I290A were purchased from GenScript. PCR was performed on a TaKaRa PCR Thermal Cycler Dice Gradient (Takara Bio). LPS_wild-type and LPS_L287F/I290A were purified as reported previously.[19] The lipase activity was determined by using Lipase Kit S (DS Pharma Biomedical), and the absorbance at 254 nm was measured on a PowerScanHT (DS Pharma Biomedical).

Molecular modeling and design of LPS_L287F/I290A and LAK_V287F/I290A. The structure of *Burkholderia cepacia* lipase (LPS) (PDB: 1OIL)[20] was optimized by QuickPrep function (with Protonate3D module) of MOE (Molecular Operating Environment, MOLSIS), where Amber 10: EHT was used as a force field (the cut-off distance for noncovalent interactions: 10 Å). The structures of the other lipases were constructed with Homology Model function of MOE. The structures of *Burkholderia cepacia* NBRC14595 lipase (BCL14595)[3,19,21] and *Pseudomonas fluorescens* lipase (LAK)[22] were created by using LPS as a template with Homology Model and QuickPrep functions of MOE. LPS_L287F/I290A and LAK_V287F/I290A were designed by molecular modeling. The mutation sites of LPS were determined by superimposing LPS on BCL14595, and Leu287 and Ile290 of LPS were very close to Ile287 and Ile290 of BCL14595, respectively. The mutation sites of LAK were also determined by superimposing LAK on BCL14595, and Val287 and Ile290 of LAK were very close to Ile287 and Ile290 of BCL14595, respectively.

Construction of recombinant *Burkholderia cepacia* strains overproducing lipase. A DNA fragment for LPS_wild-type or LPS_L287F/I290A was amplified by PCR with the primers, LPS-LIP-F (5'-TTTTGCATGCCTCGTTCTATGCGTTCTCG-3') and LPS-LIP-R (5'-TTTTTCTAGAAAACACCCGCCAGTTTCAG-3'), where the restriction sites for *Sph* I and *Xba* I are underlined. The PCR mixture contained PrimeSTAR GXL DNA polymerase (1 μL), 5× PrimeSTAR GXL buffer (10 μL), each primer (10 pmol), 2.5 mM dNTP mixture (4 μL), synthetic gene (50 ng), and sterile distilled water (up to 50 μL). PCR was done for 30 cycles of (98 °C for 10 sec, 60 °C for 30 sec, and 68 °C for 1.5 min). DNA amplification was checked by 1% agarose gel electrophoresis, and the amplified DNA fragment was collected and purified by NucleoSpin DNA Clean-up Kit (Quiagen). The purified DNA fragment and pFL210[23] were digested with *Sph* I and *Xba* I, and both fragments were ligated by using DNA Ligation Kit <Mighty Mix>. The plasmid obtained was used for the transformation of a *Burkholderia cepacia* strain lacking chromosomal lipase genes (Amano Enzyme) by electroporation. A DNA fragment for LAK_wild-type or LAK_V287F/I290A was amplified by PCR with the primers, LAK-LIP-F

(5'-TTTTGCATGCCTCGTTCTATGCGTTCTCG-3') and LAK-LIP-R (5'-TTTTTCTAGA AAACACCCGCCAGTTTCAG-3'), where the restriction sites for *Sph* I and *Xba* I are underlined. The recombinant *Burkholderia cepacia* strains for the production of LAK_wild-type or LAK_V287F/I290A were obtained as shown above.

Cultivation of lipase production strains. Each strain was cultivated in L broth (0.5% yeast extract, 1.0% peptone, 0.5% NaCl, 0.1 mg/1 mL kanamycin, pH 7.0, 5 mL) at 30 °C for 24 h with shaking at 140 rpm. The culture was inoculated into a medium (2.0% soy bean oil, 0.5% peptone, 0.3% meat extract, 0.1% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 1 drop of ADEKA NOL (Adeka), 0.1 mg/1 mL kanamycin, pH 6.2, 50 mL). The cells were incubated at 27 °C for 96 h with shaking at 200 rpm. The culture broth was heat-treated at 52 °C for more than 2 h and centrifuged (7,000 g, 10 min, 4 °C) to give a supernatant containing the recombinant lipase (enzyme extract).

Purification of LAK_wild-type and LAK_V287F/I290A enzymes. The enzyme extract was equilibrated with 10 mM phosphate buffer (pH 7.0, 20 mL) containing 0.2 M $(NH_4)_2SO_4$ by ultrafiltration (Vivaspin 50, 10 kDa), and the lipase was purified by column chromatography. A Butyl Toyopearl 650M column (50 mL) was washed with 10 mM phosphate buffer (pH 7.0, 100 mL) and then equilibrated with 10 mM phosphate buffer (pH 7.0, 200 mL) containing 0.2 M $(NH_4)_2SO_4$. The enzyme solution (20 mL) was loaded, and the column was washed with 10 mM phosphate buffer (pH 7.0, 100 mL) containing 0.2 M $(NH_4)_2SO_4$. The column was further washed with 10 mM phosphate buffer (pH 7.0, 100 mL). The lipase eluted with 10 mM phosphate buffer (pH 7.0, 150 mL) containing 40% EtOH. This eluent was equilibrated with 20 mM phosphate buffer (pH 7.0, 20 mL) by ultrafiltration (Vivaspin 50, 10 kDa). The lipase solution (20 mL) was loaded on a DEAE-Sepharose column (50 mL) equilibrated with 20 mM phosphate buffer (pH 7.0), and 20 mM phosphate buffer (pH 7.0, 150 mL) was passed to give a lipase fraction (150 mL). The solution was concentrated by ultrafiltration (Vivaspin 50, 10 kDa). The purity of the lipase was confirmed by SDS-PAGE (SuperSep ACT, 10–20%, Wako Pure Chemical Industries).

Immobilization of lipases. A solution containing the purified lipase was adjusted to 25 mL at a concentration of 1 mg/1 mL with 20 mM phosphate buffer (pH 7.0) in a centrifuge tube (50 mL), and Toyonite-200M (5.0 g) was then added. The mixture was gently shaken by inversion at 4 °C for 16 h. The mixture was filtered and washed with 20 mM phosphate buffer (pH 7.0, 200 mL). The immobilized lipase (0.5 (w/w) enzyme/Toyonite-200M) was obtained by drying in vacuo, and it was stored in an electric desiccator (humidity 20–25%) with a lid of the vial open.

[E] References.

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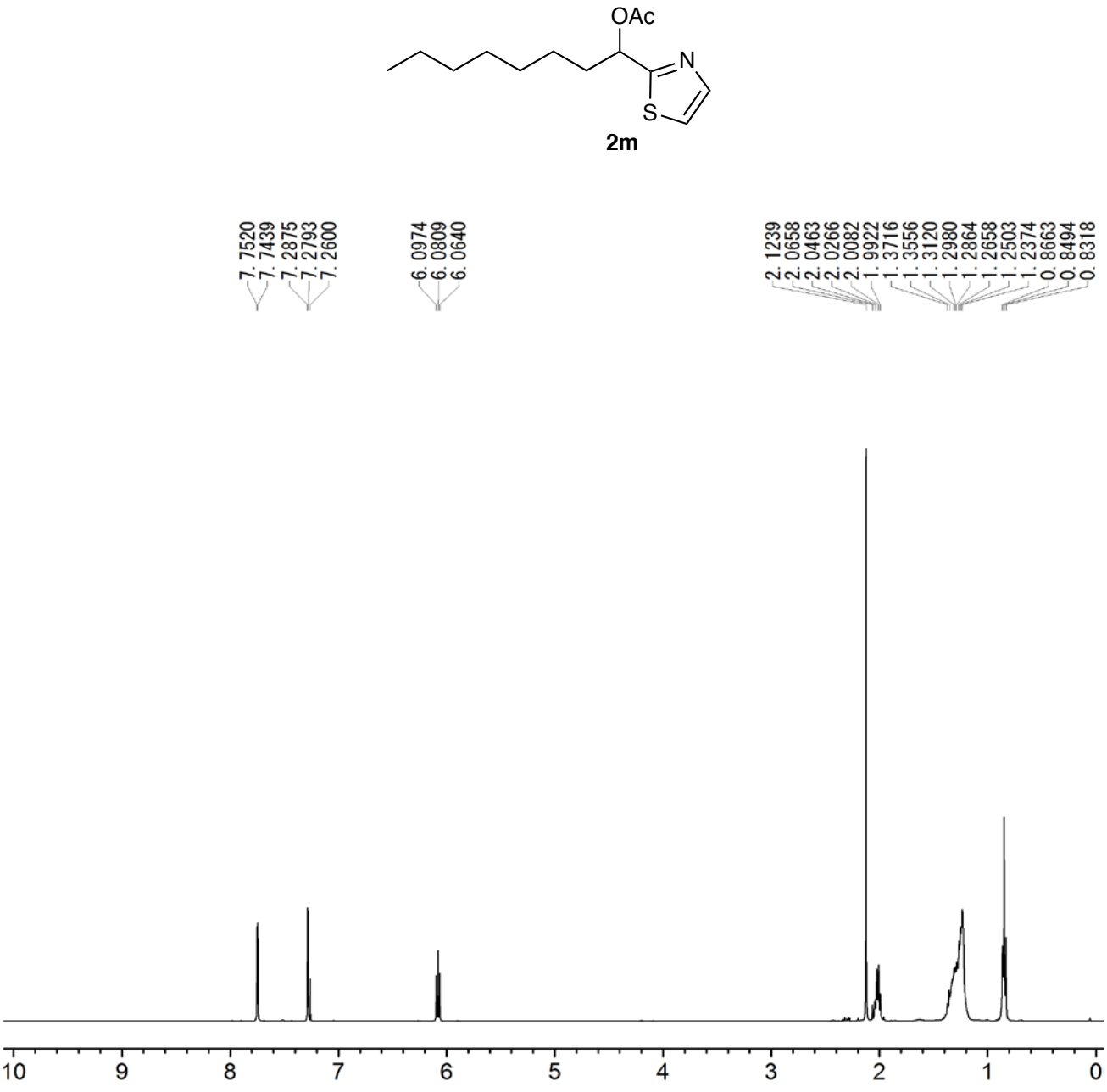
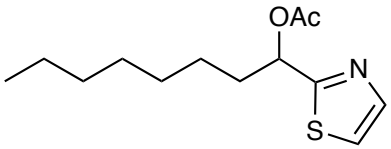
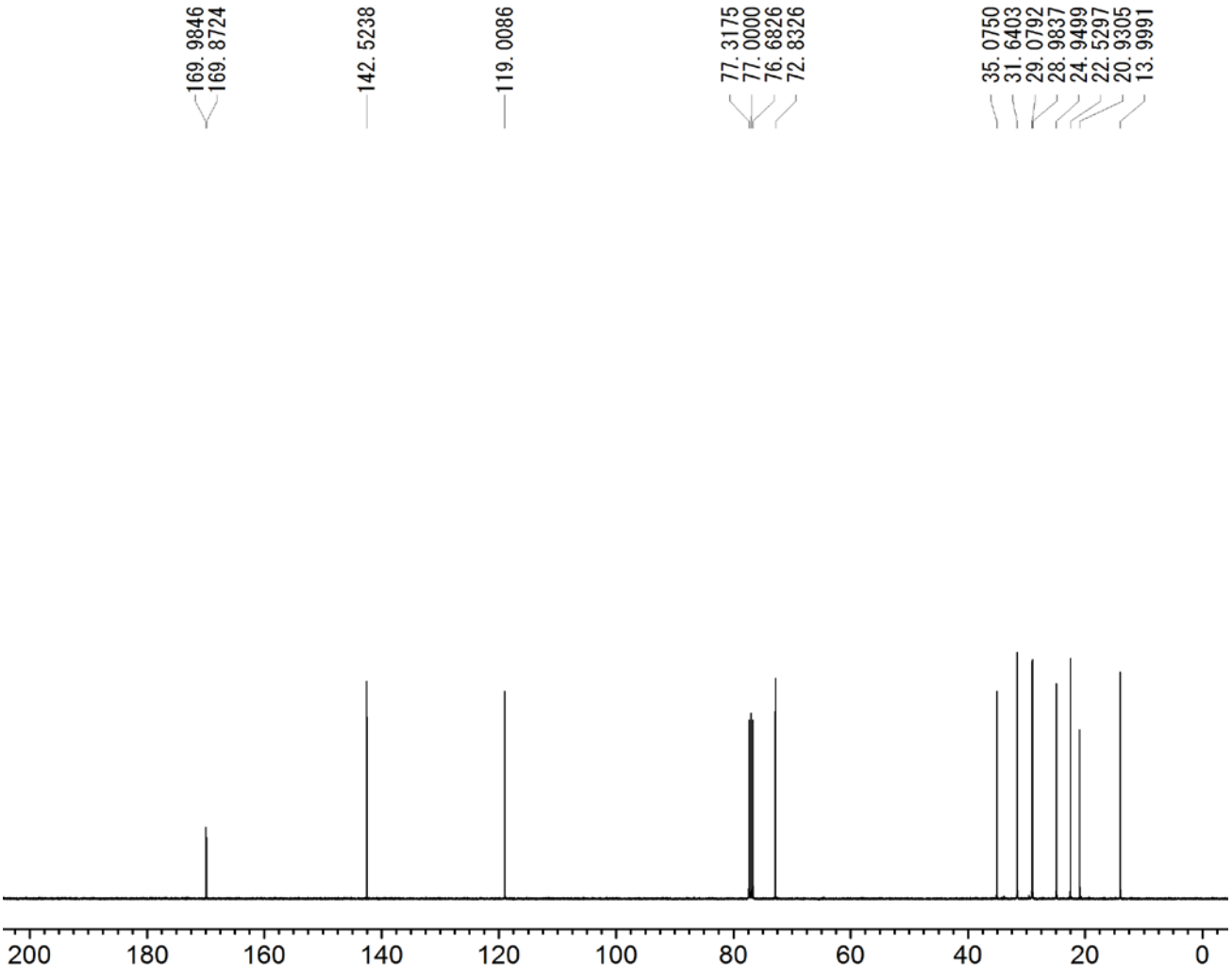
[F] NMR spectra.



400 MHz ^{1}H NMR spectrum of **2m** in $CDCl_3$.



2m



100 MHz ^{13}C NMR spectrum of **2m** in $CDCl_3$.