SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Facial selection of the carbonyl donor pyruate enolate in aldol addition reaction of 4-hydroxy-2-oxopentanoate (HOPA) by HpaI and BphI. BphI is stereospecific, and the pyruvate enolate can only attack the R_e face of acetaldehyde to form (*S*)-HOPA. HpaI lacks stereospecific control and allows the carbonyl donor to attack either face.

Fig. S2. Lineweaver-Burk plots of HpaI and BphI in the aldol addition reaction to produce 4-hydroxy-2-oxopentanoate (HOPA). (A) HpaI aldol addition reaction. Reaction rates were determined using 5 mM (●), 10 mM (○), 20 mM (▼) and 300 mM (△) of acetaldehyde. (B) BphI aldol addition reaction. Reaction rates were determined using 5 mM (●), 10 mM (○), 20 mM (▼), 50 mM (△) and 300 mM (□) of acetaldehyde.

Fig. S3. Lineweaver-Burk plots of the product inhibition of HpaI and BphI in the HOPA aldol cleavage reaction. (A) Inhibition of HpaI with pyruvate (K_{ic} = 0.51 ± 0.07 mM). Reaction rates were determined using 0 mM (●), 0.5 mM (○), 1 mM (▼), 2 mM (△) and 3 mM (■) of pyruvate. (B) Inhibition of HpaI with acetaldehyde (K_{ic} = 20.0 ± 0.96 mM). Reaction rates were determined using 0 mM (●), 15 mM (○), 30 mM (▼), 50 mM (△) 75 mM (■) of acetaldehyde. (C) Inhibition of BphI with pyruvate (K_{ic} = 0.34 ± 0.04). Reaction rates were determined using 0 mM (●), 2 mM (○), 4 mM (▼), 6 mM (▽) and 10 mM (■) pyruvate. (D) Inhibition of BphI acetaldehyde (K_{ic} = 59.1 ± 5.0 mM, K_{iu} 94.3 ± 5.0). Reaction rates were determined using 0 mM (●), 20 mM (○), 50 mM (▼), 75 mM (△) 100 mM (■) acetaldehyde. Inhibition was calculated using the competitive inhibition for HpaI and mixed inhibition equation in Leonora. Reaction conditions contained; 2 mM divalent metal ions and either 20 U of ADH or 20 LDH in 100 mM HEPES buffer pH 8.0 at 25 °C.

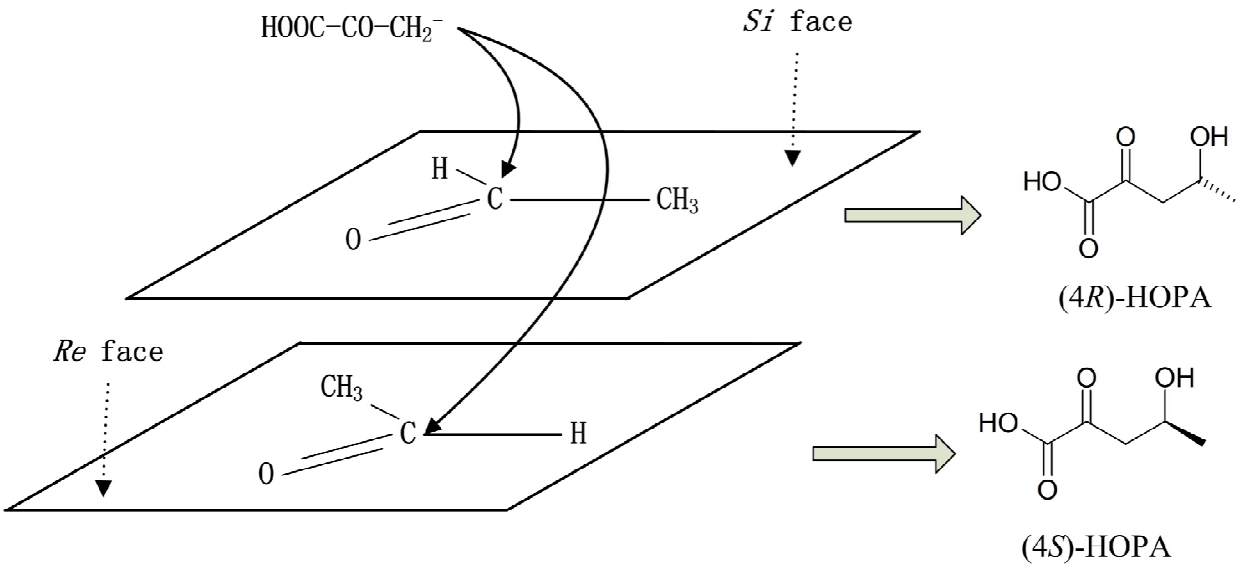
Table S1. HPLC and Q-Tof-MS analysis of products synthesized by aldolases. M represents the aldol addition product of pyruvate or 2-ketoanoate with various aldehydes. The retention time was determined by HPLC using an Aminex® HPX-87X ion exchange column. Products were eluted using in 0.1% formic acid at a flow rate of 0.5 mL/min and detected at 215 nm.

Carbonyl Donor		Pyruvate								2-Ketobutanoate
Aldehyde		Acetaldehyde	Glycolaldehyde	Propionaldehyde	(D,L)-Glyceraldehyde	Butyraldehyde	Isobutyraldehyde	Succinic Semialdehyde	Pentaldehyde	Acetaldehyde
Retention time (min)		40.04	13.29	53.23	12.69	85.55	60.35	36.7	137.96	42.01
Total Mass		132	148	146	178	160	160	190	174	146
Ions	**Mass change**									
$[M-H]^{-1}$	-1	131	147	145	177	159	159		173	
$[M-2H+Na^{+}-H_2O]^{-1}$	+3									
$[M-2H+Na^{+}-CO_2-H_2O]^{-1}$	-41							149		
$[M-H-C_2H_2O]^{-1}$	-43	89								
$[M-H-CO_2]^{-1}$	-45	87		101		115				
$[M-H-2CO_2]^{-1}$	-89	43								
$[M-H-H_2O]^{-1}$	-19	113	129	127	159	141	141	171	155	127
$[M-H-2H_2O]^{-1}$	-37		111	109					137	
$[M-H-2H_2O-CO_2]^{-1}$	-81		67		97			109		65
$[M-H-2H_2O-CO_2-CO]^{-1}$	-109							81		
$[M-H-2H_2O-CO]^{-1}$	-65		83	81					109	
$[M-H-2H_2O-2CO]^{-1}$	-93		55		85					
$[M-H-H_2O-CO]^{-1}$	-47	85	101	99		113	113		127	
$[M-H-H_2O-CO_2]^{-1}$	-63	69	85	83		97	97	127		83
$[M-H-H_2O-CO_2-CO]^{-1}$	-91		57	55		69	69	99	83	55
$[M-H-H_2O-CO_2-C_2H_2O]^{-1}$	-105		43							41
$[M-H-CO_2-CO-CH_2]^{-1}$	-87					73				
$[M-H-H_2O-CO-C_2H_2O]^{-1}$	-89									
$[CH_3COCO_2]^{-1}$			87	87	87	87	87		87	

Table S2. Properties of HOPA produced in aldol addition reaction by HpaI and BphI with pyruvate and acetaldehyde. Optical rotation was determined using an Autopol® III automatic polarimeter at 25°C. NMR spectrum was recorded with a Bruker Avance 600 MHz at 25°C.

Enzyme used in synthesis of HOPA	**Properties of HOPA from aldol addition reaction by HpaI and BphI**			
	NMR	HPLC Retention Time (min)	Optical rotation	% of HOPA degraded by BphI (%)
HpaI	^{1}H NMR (600 MHz,D_2O): δ=1.19(d, 3 H, CH_3, J=6.39Hz), 2.88(d, 2 H, CH_2, J=6.32Hz), 4.26(m, 1 H, CH, J=6.29Hz) ^{13}C NMR (600 MHz,D_2O): δ=24.9(CH_3), 50.8(CH_2), 66.4(CHOH), 172.8(C=O), 207.3(C=O(OH)).	40.04	$[a]_D^{25}$ =0.11	53.1 ± 2.9
BphI	^{1}H NMR (600 MHz,D_2O): δ=1.16(d, 3 H, CH_3, J=6.36Hz), 2.86(d, 2 H, CH_2, J=6.24Hz), 4.23(m, 1 H, CH, J=6.30Hz) ^{13}C NMR (600 MHz,D_2O): δ=23.8(CH_3), 49.1(CH_2), 65.0(CHOH), 171.1(C=O), 205.9(C=O(OH)).	40.00	$[a]_D^{25}$ =+15.4	96.3 ± 4.7
XylE, TodF, BphH	^{1}H NMR (600 MHz,D_2O): δ=1.18(d, 3 H, CH_3, J=6.30Hz), 2.86(d, 2 H, CH_2, J=6.31Hz), 4.27(m, 1 H, CH, J=6.36Hz) ^{13}C NMR (600 MHz,D_2O): δ=22.7(CH_3), 48.6(CH_2), 63.9(CHOH), 170.9(C=O), 206.1(C=O(OH)).	39.24	$[a]_D^{25}$ =+12.3	91.7 ± 5.1
HpaI product treated by BphI	^{1}H NMR (600 MHz,D_2O): δ=1.17(d, 3 H, CH_3, J=6.36Hz), 2.86(d, 2 H, CH_2, J=6.30Hz), 4.24(m, 1 H, CH, J=6.66Hz) ^{13}C NMR (600 MHz,D_2O): δ=22.1(CH_3), 47.9(CH_2), 63.6(CHOH), 168.1(C=O), 204.6(C=O(OH)).	39.43	$[a]_D^{25}$ =-11.3	16.9 ± 2.0

Fig S1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C
CH3
O
Re face
CH3
C
H
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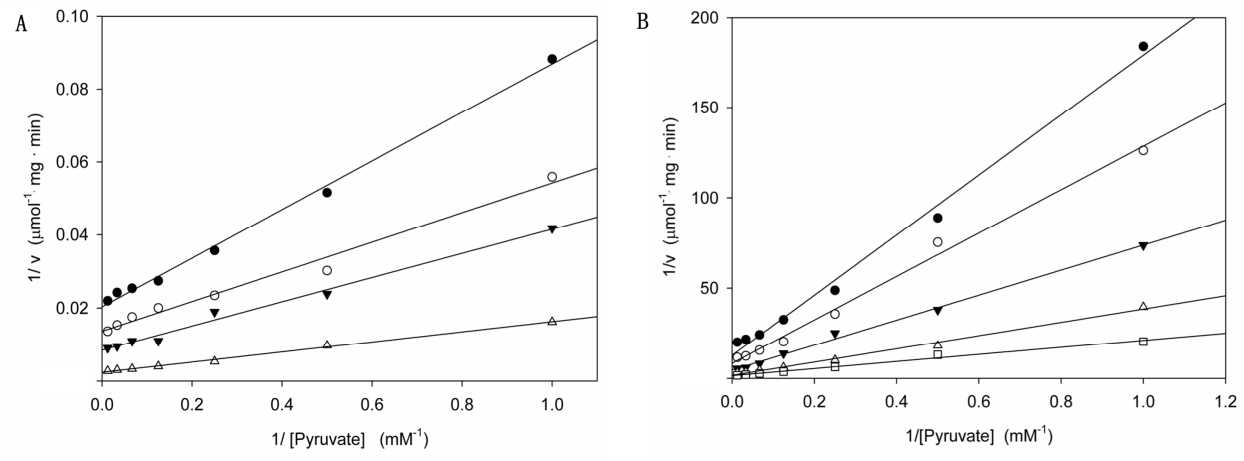
HO
O
OH
O
(4R)-HOPA
HO
O
OH
O
(4S)-HOPA

Fig S2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0.08
0.06
0.04
0.02
1/ v (μmol-1 mg · min)
0.0
0.2
0.4
0.6
0.8
1.0
1/ [Pyruvate] (mM-1)
B
200
150
100
50
1/v (μmol-1 mg · min)
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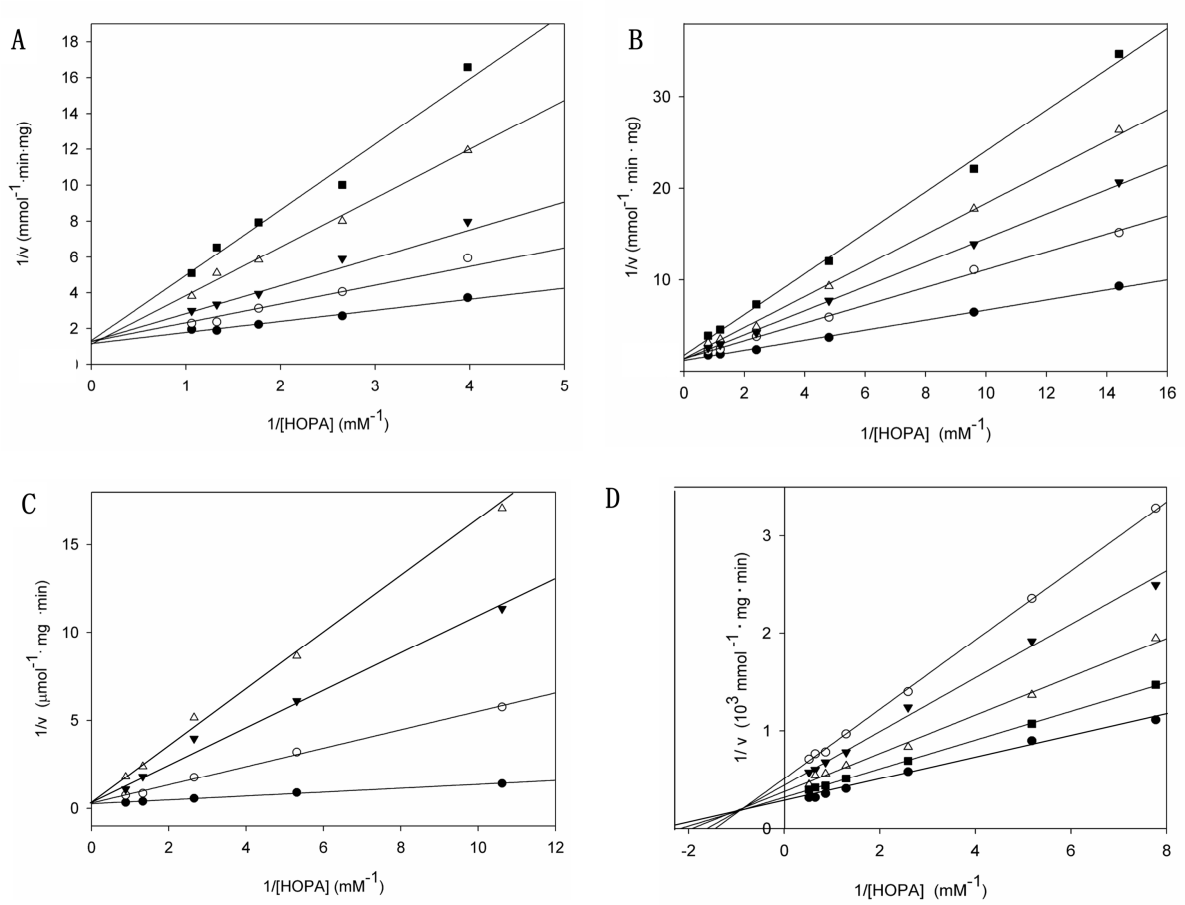
0.2
0.4
0.6
0.8
1.0
1.2
1/[Pyruvate] (mM-1)

Fig S3


A
1/v (mmol^{-1}·min·mg)
1/[HOPA] (mM^{-1})
B
1/v (mmol^{-1}· min · mg)
1/[HOPA] (mM^{-1})
C
1/v (μmol^{-1}· mg ·min)
1/[HOPA] (mM^{-1})
D
1/ v (10^3 mmol^{-1} · mg · min)
1/[HOPA] (mM^{-1})