Supporting Information

A new D-threonine aldolase as promising biocatalyst for highly stereoselective preparation of chiral aromatic β-hydroxy-α-amino acids

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Table S1 Identity between D-low-TAs

	AsDTA	AxDTA	SpDTA	CrDTA	PsDTA	PaDTA	PpDTA	SvDTA
DrDTA	76%	44%	40%	41%	16%	17%	17%	18%
AsDTA		54%	42%	40%	18%	17%	17%	17%
AxDTA			26%	24%	12%	14%	14%	13%
SpDTA				41%	16%	16%	16%	16%
CrDTA					18%	17%	17%	17%
PsDTA						81%	81%	64%
PaDTA							80%	67%
PpDTA								64%

Table S2 Effects of temperature on conversion and de

Entry	Temperature (°C)	Time (h)	Conv. (%)	*threo:erythro*	de (%)
1	4	6	83.6	65.0:35.0	30.0
2	4	12	83.6	55.0:45.0	10.0
3	10	6	81.7	71.1:28.9	42.2
4	10	12	77.8	57.6:42.4	15.2
5	15	6	76.6	44.9:55.1	10.2
6	15	12	75.6	42.7:57.3	14.6
7	25	6	63.9	43.3:56.7	13.4
8	25	12	61.9	42.4:57.6	15.2
9	35	6	69.3	41.1:58.9	17.8
10	35	12	70.9	46.1:53.9	7.8

Reaction conditions: 1 mL solution containing glycine (1 M), benzaldehyde (100 mM), 10 μM PLP, 1 mM $MnCl_2$, 10% (*v/v*) DMSO and DrDTA (57 U) for 6 h and 12 h.

Table S3 Timescale for the synthesis reaction

	5 U				10 U		
Entry	Time (h)	Conv. (%)	de (%)	Entry	Time (h)	Conv. (%)	de (%)
1	6	28.3	>99	5	6	70.4	64.4
2	9	50.9	99.6	6	9	72.6	55.1
3	12	68.3	91.1	7	12	70.1	42.1
4	15	68.4	74.6	8	15	72.5	36.8

Reaction conditions: 1 mL solution containing glycine (1 M), benzaldehyde (100 mM), 10 μM PLP, 1 mM $MnCl_2$, 10% (*v/v*) CH_3CN and DrDTA (5 U or 10 U) at 10°C.

Table S4 Solvent screening for kinetic resolution

Entry	Co-solvent	Conc.[M]	Conv. (%)	ee (%)
1	Non	0.1	50.0	>99
2	Non	0.2	49.4	97.9
3	Dichloromethane	0.1	50.0	>99
4	Dichloromethane	0.2	49.4	97.7
5	1,2-Dichloroethane	0.1	50.0	>99
6	1,2-Dichloroethane	0.2	50.0	>99
7	Cyclohexanone	0.1	50.0	>99
8	Cyclohexanone	0.2	49.3	97.4
9	Ethyl acetate	0.1	29.1	38.3
10	*n*-Butyl acetate	0.1	24.5	33.6
11	MTBE	0.1	41.4	70.4
12	2-Methyltetrahydrofuran	0.1	45.3	84.1
13	*n*-Heptane	0.1	41.1	64.0
14	*n*-Hexane	0.1	36.8	52.3
15	*n*-Octane	0.1	39.0	64.8
16	Isooctane	0.1	41.2	66.3
17	Toluene	0.1	13.6	13.3
18	MeOH	0.1	44.7	80.0
19	EtOH	0.1	44.3	78.4
20	$CNCH_3$	0.1	44.9	81.1
21	Acetone	0.1	47.1	88.3
22	DMSO	0.1	40.0	67.3
23	THF	0.1	29.0	35.8

Kinetic resolution reactions were performed with DL-*threo*-MPS at 30°C in 1 mL of 100 mM HEPES-NaOH buffer (pH 8.0) that contained 10 μM PLP, 1 mM $MnCl_2$, 50% (*v*/*v*) organic solvents and 0.08 g/mL wet cells for 9 h.

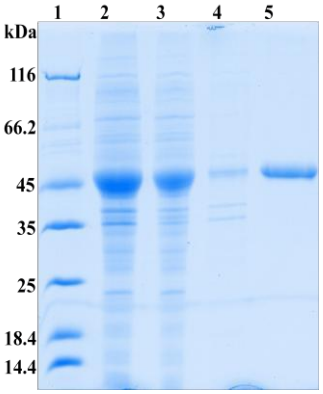

Fig. S1 SDS-PAGE analysis. Lane 1, protein marker; Lane 2, crude extract; Lane 3, supernatant of the lysate; Lane 4, precipitate of the lysate; Lane 5, purified enzyme.