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Supporting Information

Characteristic of a L-threonine transaldolase for asymmetric synthesis of β -

hydroxy-α-amino acids

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Running title: L-threonine transaldolase for synthesis of β -hydroxy- α -amino acids

Protein	GenBank No.	Sequence identity	
PsLTTA	WP_065936857	100%	
EuSHMT	TLZ64415	31.7%	
DeSHMT	OYT59861	30.1%	
ThSHMT	RLG85937	32.5%	
CaSHMT	OYT34991	29.2%	
ArSHMT	RUM35137	33.5%	
EcSHMT	1EQB:A	26.9%	

 Table S1. Sequence identity of PsLTTA and SHMTs.

Substrate	$K_{\rm m}$ (mM)	k_{cat} (min ⁻¹)	$k_{\text{cat}}/K_{\text{m}} (\text{mM}^{-1} \min^{-1})$
L-threonine	9.85	63.4	6.44
D-threonine	ND	ND	ND
L-allo-threonine	ND	ND	ND
D-allo-threonine	ND	ND	ND
L-serine	ND	ND	ND
glycine	ND	ND	ND

 Table S2 Kinetic parameters of PsLTTA on donor substrates.

ND, Not detected.

Figure S1.

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PSLTTA	MSNVKQQTAQIVDW	LSSTLGKDHQYREDS	SLTANEN YFSALVRLTSO	STAGAFYHCSFFFEVPA	GEWHFPEPGHMNAIADQVRDL	G <mark>KT<mark>LI</mark>GAQAFDWRFN<mark>GG</mark>ST</mark>	105
EuSHMT	MDENVSFIE	EEQVDR <mark>H</mark> HELFSR.S <mark>I</mark>	P <mark>MIAS</mark> ENVI <mark>SPLA</mark> REMLI	SDFGDRYAEGLFGER	YYEGNVEVERRVTAL	ARELFRCRLADVRFISCTV	93
DaSHMT	MSEFEKILKEFLPEEVVKV	IKLTAS <mark>H</mark> NRW <mark>R</mark> KREC <mark>I</mark>	N <mark>MIPS</mark> ENCM <mark>SPLA</mark> EMVY <mark>I</mark>	TEMGHRYAEGLEYKE	YYQGLIYVDEMEVYAQEL	MSK <mark>LF</mark> HVKYV <mark>DLRFIS</mark> GTI	105
TaSHMT	MSHYPPELAKV	IELTRSHNKWRRFECI	N <mark>LIAS</mark> ENVM <mark>SPLA</mark> EAAYI	TEMMHRYAEGKERKR	YYQGNIYTDEVELYAMEL	MSE <mark>LL</mark> KVRYV <mark>ELRFIS</mark> GTI	97
CaSHMT	MSNNI	LQLLEQ <mark>H</mark> NNWRKN.CI	N <mark>LIAS</mark> ENVM <mark>SPIA</mark> EKIY <mark>V</mark>	SDLMHRYAEGMEYKR	YYQ <mark>G</mark> LKYFDKIEDIATEF	FKCHFCCDFADLRFISCTL	90
ArSHMT	MKE.IHEI	LTFIKAHHKFIDS.AI	P <mark>LIAS</mark> ENVTSLAVRNAL <mark>I</mark>	SDFQHRYAEGLEMHR	VYAGCEYIDKIELKAIEL	AKD <mark>IF</mark> DAEHANVQ <mark>FTSG</mark> SV	92
ECSHMT	MLKREMNIADYDAE	LWQAME <mark>C</mark> EKVRQEEHI	ELIASEN YTSPRVMQAQO	SCLTNKYAEGYEGKR	YFGGCEYVDIVEQLAIDR	AKELFGADY <mark>ANVQ</mark> FH <mark>SG</mark> SQ	100
Consensu	IS		en s	у р		p g	
PSLTTA	AE CALMIAACKEGE GFVHF	AHRD <mark>GGH</mark> FALESLA	QK <mark>MGI</mark> EIFHL <mark>P</mark> VNPISLI	IDVAKLDEMVRRNPHIR	IVILDOSFKLRWOPLAEIRSV	LPDS.CTLTYDMSHDG <mark>GLI</mark>	212
EuSHMT	ANLAVYYALLEFGD IMTSVA	ALSH <mark>GAH</mark> I <mark>S</mark> SAKFGGA	GMRGINNVNYPFEVERM	IDIDGSAKLIR.HVKPK	VAAF <mark>G</mark> O <mark>SVFLFFT</mark> FL <mark>KELRD</mark> A	FQ <mark>EAG</mark> CHVW <mark>Y</mark> IGA <mark>HVMGLI</mark>	202
DaSHMT	ANLTAYYAMARY <mark>GD</mark> KAVVVI	PVQA <mark>GAH</mark> VSHTKYGGI	GALGIQQIEMPFSLEDM	IDVDKAAKVIR.EVKPK	FVTF <mark>G</mark> G <mark>SVWLFPHP</mark> VKELSEV	AHEVGAKVMY <mark>DAAHVLGLI</mark>	214
TaSHMT	ANAIVFRNLANEGO KALIA	PVQA <mark>GAH</mark> VSHTKFGTI	GALGIEHIELPFNQEEWN	IDIDKAVKMIR.EVKPK	F <mark>VTLGA</mark> SVYLFPHPTKEISEA	AHEVGAKVVH <mark>UVAHVLGLI</mark>	206
CaSHMT	ANMAVESALAKTCONIITS	GIDG <mark>GAH</mark> ISHENP <mark>G</mark> AA	GLLGFKITHFKFDQN.YN	LELKDAKFKIK.KLKPK	F <mark>IVLGA</mark> SVIPFPF <mark>P</mark> VKELKEI	CKTTNTKII <mark>Y</mark> DAA <mark>HVLGLI</mark>	198
ArSHMT	ANLALYSAFTKEGDTIITLS	SIRD <mark>GGH</mark> ITMN <mark>KVG</mark> LT	GKLSLNVINFFFDRDEM	IDVDKAEKVLR.NAEPK	L <mark>VLF</mark> GA <mark>SVFLFPHP</mark> VKELRDV	AKEIGAIMAY <mark>D</mark> AS <mark>HVIGLI</mark>	201
ECSHMT	AN FAVYTALLEFGD TVLGMN	NLAH <mark>GGH</mark> LTHGSPVNE	SGKLYNIVPYGIDAT.G	IDYADLEKQAK.EHKPK	MIIG <mark>G</mark> F <mark>S</mark> AYSGVVDWAKMREI	ADSI <mark>GA</mark> YLFV <mark>DMAHV</mark> AGL <mark>V</mark>	208
Consensu	isa g	gh		d	s	d h gl	
PSLTTA	MGCVFDSELSCGADIVHGN	THKTIFGPCKCYIGFK	SACHPLLVDTSLWVCEHI	.QSNCHAEQLPPMWVAFK	EMEL <mark>FG</mark> .RDYAACIVSNAKTL	ARHIHELCIDWTGESFCFT	321
EuSHMT	AGC CFCD PLREGADVITGS	THKT <mark>LP</mark> GP <mark>C</mark> H <mark>GILL</mark> SI	SADDKEVKRLMKGVEEGV	V <mark>SNHHLH</mark> AM <mark>AALGITL</mark> A	EHLO <mark>FG</mark> .R <mark>DYA</mark> A <mark>CIVK</mark> NAKAL	GCALVECCFKVLAEKFCFT	311
DaSHMT	AGCCFCDFIKEGADIITSS1	THKT <mark>FF</mark> GF <mark>C</mark> G <mark>QVIL</mark> TN	DEELYKKVRKI <mark>VF</mark> EIH	V <mark>SNHHLH</mark> RL <mark>PATAI</mark> VAL	ELLY <mark>FG.KEYA</mark> A <mark>CIIKNAK</mark> AL	AEALAAE <mark>G</mark> FN <mark>VLGE</mark> KH <mark>GYT</mark>	321
TaSHMT	IGGVWPNEIHEGADVATSS	THKT <mark>FP</mark> GP <mark>C</mark> GCLVYTN	DEKLYKAISKT <mark>IF</mark> FWB	V <mark>SNHHLH</mark> RL <mark>PALAV</mark> TAV	EMKY <mark>FG</mark> .EEYAR <mark>CIVR</mark> NAKAF	AEALAEE <mark>G</mark> FK <mark>VLAENLGY</mark> T	313
CaSHMT	CNGFFCNFLEEGADIITSS	THKT <mark>FP</mark> GP <mark>C</mark> G <mark>CLIL</mark> GN	I. DNNLOKKIRNK <mark>IF</mark> FGV	L <mark>SNHHLH</mark> RI <mark>PSLYIAL</mark> S	EMKK <mark>FG</mark> .KDY <mark>A</mark> S <mark>CIIKNAK</mark> TL	AEE <mark>L</mark> YNLEFD <mark>VL</mark> VKER <mark>GFT</mark>	306
ArSHMT	AGKQFQDPLKEGIDVVTSS	IHKT <mark>FP</mark> GP <mark>C</mark> H <mark>G</mark> MILCF	AEFAEAMDRAVE	L <mark>SNHHLH</mark> NVT <mark>ALAIAL</mark> C	EMKV <mark>FG</mark> .REY <mark>A</mark> K <mark>CIVKNAK</mark> AL	AEALANE <mark>G</mark> FNVIAEHK <mark>GFT</mark>	307
EcSHMT	AAGVYPNEVPH.AHVVTTT	THKT <mark>LA</mark> GP <mark>RG</mark> GL <mark>IL</mark> AK	GGSEE <mark>l yk</mark> klnsa <mark>vf</mark> ego	SQ <mark>G</mark> GPL <mark>MH</mark> VI <mark>AGKAVAL</mark> K	<mark>B</mark> AMEP <mark>E</mark> FKT <mark>YQQ<mark>QV</mark>AKNAKAM</mark>	VEVELER <mark>GYKVV</mark> SGGT	314
Consensu	is p t	thkt gp g	p		e yq nak	v gt	
PSLTTA	QTHOUHFAVGDLQKALDLCV	VNS <mark>L</mark> HAGG <mark>I</mark> RSTNIEI	PGKPGVHGIRI	GVCAMTRRGMKE KDFEV	VARFIADLYFKKTEPAKVAQQ	IKEFLQAF PLAPLAYSFD	423
EuSHMT	ESHALAIDVSAHG.GGAEIS	SLE <mark>FEKANIITNKNLI</mark>	PWDTSSVH <mark>PS</mark> GVRV	GTCELTRLGMREAQMKD	VSD <mark>lm</mark> ar <mark>vai</mark> kre <mark>dp</mark> rr <mark>v</mark> aad	VRELERE NTVRFCFQPG	415
DaSHMT	KSHQVLLDVRALG.GGAKCA	A <mark>KM<mark>L</mark>EDAN<mark>IIVN</mark>KNLI</mark>	PY <mark>D</mark> KPEMIKD <mark>PSGLR</mark> I	GACELTRWGMKEDDMKE	IARFFRRVLIDKEDPAKVRKD	VIEFRKNFQKIHYTFDVP	427
TaSHMT	KSHTIVVEVENLG, GGAKVA	AKKLEEANIIVNKNLI	PWDPPEAVKDPSCIRI	CTCEMTR FGMKE SDFKE	VARFMREVLIDGKDPKSIKEK	VIEFRKOFIEVKYT <mark>F</mark> PLT	419
CaSHMT	Q <mark>SHQVLVNM</mark> KNK <mark>G.GG</mark> DFV	A <mark>KKLEEQNIIVNKN</mark> II	QG <mark>D</mark> K.LNIAN <mark>PSGIR</mark> I	GV <mark>CEMTRFLMKC</mark> DE <mark>MK</mark> C	IAEFIKKIIIDNK <mark>DI</mark> KQE	VI <mark>EFR</mark> SK <mark>F</mark> QDIGFC	404
CaSHMT	CSHCVLVNMKNKG.GGDFV ESHCVLVDLIPSGLSGLKAN	A <mark>KKLEEQNIIVNKNII</mark> EKMLEQAG <mark>ILVNRN</mark> LI	QG <mark>D</mark> K.LNIAN <mark>PSGI</mark> RI PWDKERNRNFRD <mark>PSGI</mark> RI	GV <mark>CE</mark> MTRFLMKCDEMKC GVCEVTRL <mark>GMRE</mark> DEMED	I <mark>AEFI</mark> KK <mark>III</mark> DNK <mark>DI</mark> KQE I <mark>ARFMAEVLL</mark> RKK <mark>DP</mark> VVVKK	VIEFRSKFQDIGFC VAEFRREFVRVEYS <mark>F</mark> DKG	404 416
CaSHMT ArSHMT EcSHMT	QSHQVLVNMKNKG.GGDFV ESHQVLVDLIPSGLSGLKAH DNHLFLVDLVDKNLTGKEAI	A <mark>KKLEEQNIIVNKNII</mark> EKMLECAGIIVNRNLI DAA <mark>LGRANI</mark> TVNKNSV	QG <mark>D</mark> K.LNIAN <mark>PS</mark> GIRI PWDKERNRNFRD <mark>PSGIR</mark> I PNDPKSPFVT <mark>S</mark> GIRV	GV <mark>CENTRFLMKCDEMK</mark> O GVCEVTRLGMREDEMED GTPAITR <mark>RGFKE</mark> AEAKE	I <mark>AEFI</mark> KK <mark>III</mark> DNKDIKQE IARFMAEVLLRKKDPVVVVKK L <mark>AGWM</mark> CD <mark>VL</mark> DSINDEAV	VIEFRSKFQDIGFC VAEFRREFVRVEYSFDKG IERIKGKVLDICARYPVY	404 416 416

Figure S1. Sequence alignments of PsLTTA and SHMTs. The PLP binding sites were

indicated as (\bullet) .

Figure S2.



Figure S2. (A) The purified PsLTTA was pink in solution. (B) SDS-PAGE analysis of PsLTTA. Lane 1, whole cell extract; Lane 2, supernatant of the lysate; Lane 3, precipitate of the lysate; Lane 4, purified enzyme.

Figure S3.



Figure S3. Trans-aldehyde reaction catalyzed by PsLTTA.

Figure S4.



Figure S4. Kinetic analysis of PsLTTA. Kinetic analysis of PsLTTA reaction with 10 mM *p*-methylsulfonyl benzaldehyde and variable L-threonine (0.1-200 mM). All data were mean values of five independent experiments and error bars represent the SEM.

Figure S5.



Figure S5. Determination of stereospecificity of PsLTTA and ClLTA by HPLC analysis after OPA/NAC derivatization. Product L-*p*-methylsulfonylphenylserine ($t_{L-threo}=7.2 \text{ min}, t_{L-erythro}=8.6 \text{ min}$).

Figure S6.



Figure S6. Determination of reverse activities of PsLTTA and CILTA. (A) L-threo-*p*methylsulfonylphenylserine (L-threo-MPS) and acetaldehyde were as substrates in PsLTTA catalyzed-assay while L-threo-MPS was as substrate in CILTA catalyzedassay. The reaction products were detected by HPLC at 236 nm ($t_{L-threo-MPS} = 7.2 \text{ min}$, $t_{methylsulfonylbenzaldehyde} = 11.5 \text{ min}$. (B) Time course of L-threo-MPS catalyzed by PsLTTA and CILTA.

Figure S7.



Figure S7. Determination of stereospecificity of PsLTTA and ClLTA. Product L-*p*nitrophenylserine by PsLTTA ($t_{L-threo}$ =17.1 min, $t_{L-erythro}$ =20.7 min) and by ClLTA ($t_{L-threo}$ =16.9 min, $t_{L-erythro}$ =20.4 min).

Figure S8.

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Figure S8. Determination of stereospecificity of PsLTTA and ClLTA. Product Lphenylserine by PsLTTA ($t_{L-threo}$ =10.6 min, $t_{L-erythro}$ =13.8 min) and by ClLTA ($t_{L-threo}$ =10.5 min, $t_{L-erythro}$ =13.7 min).





Figure S9. Determination of stereospecificity of PsLTTA and ClLTA. Product L-o-fluorophenylserine by PsLTTA ($t_{L-threo}$ =11.4 min, $t_{L-erythro}$ =15.1 min) and by ClLTA ($t_{L-threo}$ =11.4 min, $t_{L-erythro}$ =15.1 min).

Figure S10.



Figure S10. Determination of stereospecificity of PsLTTA and ClLTA. Product L-*m*-fluorophenylserine by PsLTTA ($t_{L-threo}$ =15.3 min, $t_{L-erythro}$ =18.8 min) and by ClLTA ($t_{L-threo}$ =15.2 min, $t_{L-erythro}$ =18.6 min).

Figure S11.



Figure S11. Determination of stereospecificity of PsLTTA and ClLTA. L-ochlorophenylserine by PsLTTA ($t_{L-threo}$ =14.5 min, $t_{L-erythro}$ =25.0 min) and by ClLTA ($t_{L-threo}$ =14.5 min, $t_{L-erythro}$ =24.9 min).

Figure S12.



Figure S12. Determination of stereospecificity of PsLTTA and ClLTA. L-obromophenylserine by PsLTTA ($t_{L-threo}=15.9$ min, $t_{L-erythro}=29.3$ min) and by ClLTA ($t_{L-threo}=15.8$ min, $t_{L-erythro}=29.2$ min).

Figure S13.



Figure S13. Determination of stereospecificity of PsLTTA and ClLTA. L-onitrophenylserine by PsLTTA ($t_{L-threo}=11.2 \text{ min}$, $t_{L-erythro}=14.7 \text{ min}$) and by ClLTA ($t_{L-threo}=11.1 \text{ min}$, $t_{L-erythro}=14.6 \text{ min}$).

Figure S14.



Figure S14. Determination of stereospecificity of PsLTTA and ClLTA. L-*m*-nitrophenylserine by PsLTTA ($t_{L-threo}$ =16.1min, $t_{L-erythro}$ =19.4 min) and by ClLTA ($t_{L-threo}$ =15.8 min, $t_{L-erythro}$ =19.1 min).

Figure S15.



Figure S15. HRMS analysis of L-threo-*p*-methylsulfonylphenylserine ethyl ester. HRMS (m/z) (M⁺): calcd. for C₁₂H₁₈NO₅S, 288.0897, found 288.0900.

Figure S16.



Figure S16. ¹H- NMR spectra of L-threo-*p*-methylsulfonylphenylserine ethyl ester. ¹H NMR (400 MHz, CDCl₃) δ 7.96 (d, *J* = 8.1 Hz, 2H), 7.62 (d, *J* = 8.1 Hz, 2H), 4.98 (d, *J* = 4.5 Hz, 1H), 4.19 (q, *J* = 7.0 Hz, 2H), 3.64 (d, *J* = 4.7 Hz, 1H), 3.08 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).



Figure S17. ¹³C NMR spectra of L-threo-*p*-methylsulfonylphenylserine ethyl ester. ¹³C NMR (101 MHz, CDCl₃) δ 172.79, 147.58, 139.91, 127.48, 127.26, 73.41, 61.64, 60.22, 44.53, 14.08.