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

Table S1. Sequence identity of PsLTTA and SHMTs.

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 S2 Kinetic parameters of PsLTTA on donor substrates.

Substrate	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m (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

ND, Not detected.

Figure S1.

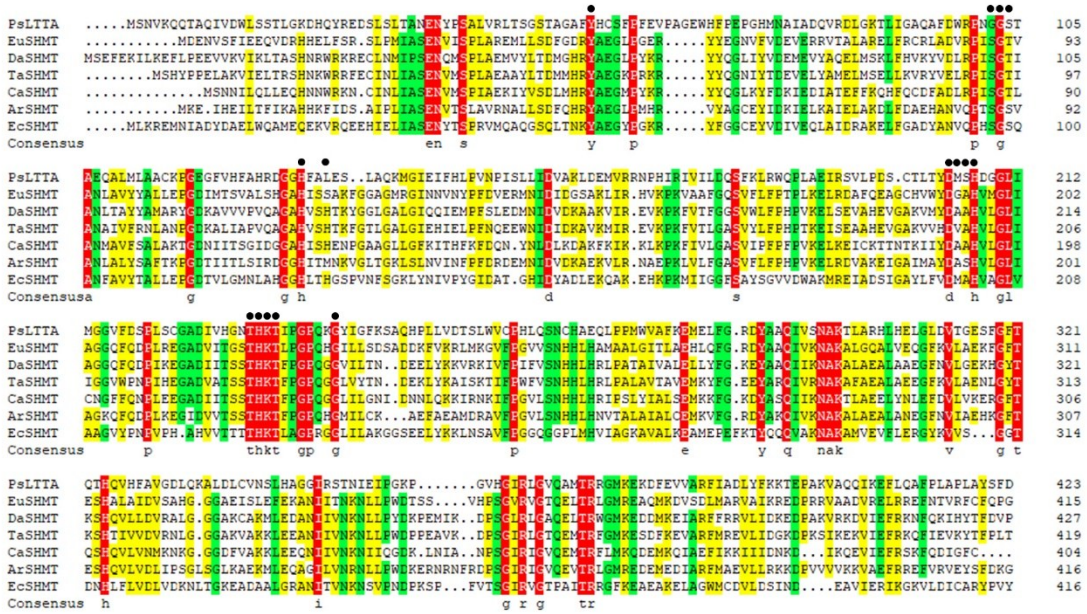

Figure S1. Sequence alignments of PsLTTA and SHMTs. The PLP binding sites were indicated as（●）.

Figure S2.

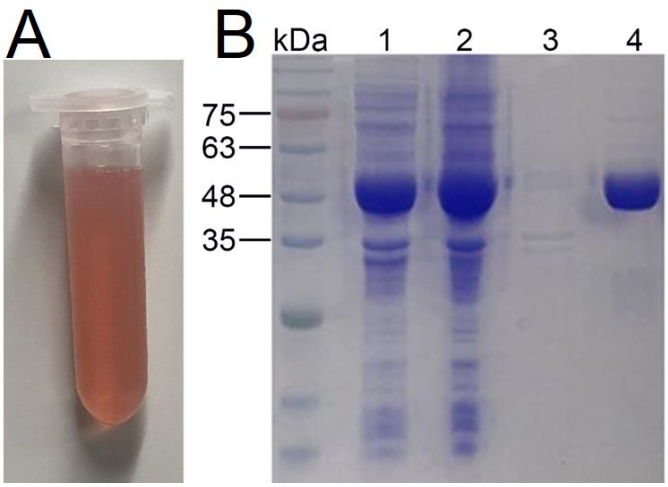

Figure S2. (A) The purified PsLTTA was pink in solution. (B) SDS-PAGE analysis of PsLTTA. Lane 1, whole cell extract; Lane 2, supernatant of the lysate; Lane 3, precipitate of the lysate; Lane 4, purified enzyme.

Figure S3.

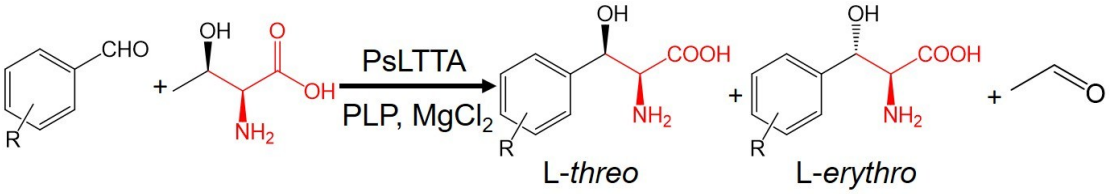

Figure S3. Trans-aldehyde reaction catalyzed by PsLTTA.

Figure S4.

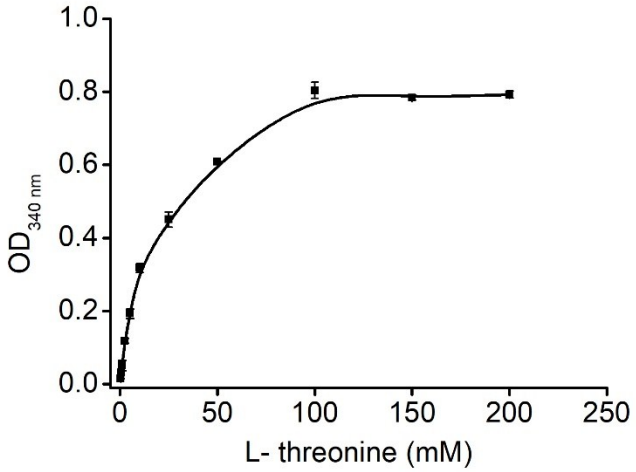

Figure S4. Kinetic analysis of PsLTTA. Kinetic analysis of PsLTTA reaction with 10 mM *p*-methylsulfonyl benzaldehyde and variable L-threonine (0.1-200 mM). All data were mean values of five independent experiments and error bars represent the SEM.

Figure S5.

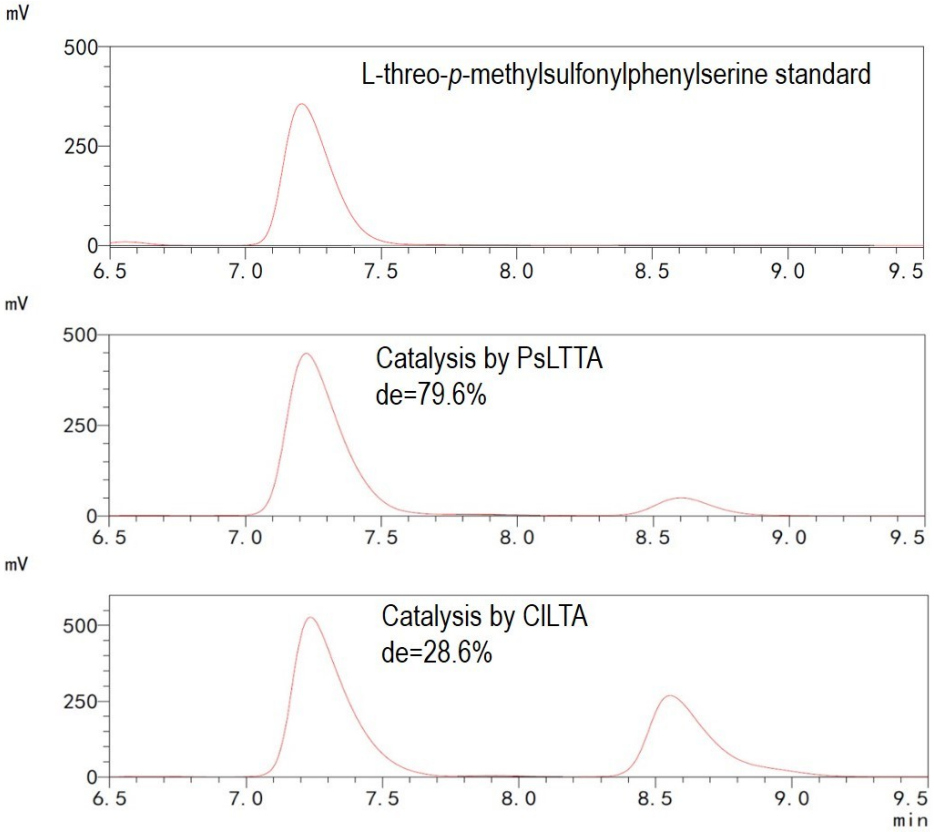

Figure S5. Determination of stereospecificity of PsLTTA and ClLTA by HPLC analysis after OPA/NAC derivatization. Product L-*p*-methylsulfonylphenylserine ($t_{L\text{-}threo}$=7.2 min, $t_{L\text{-}erythro}$=8.6 min).

Figure S6.

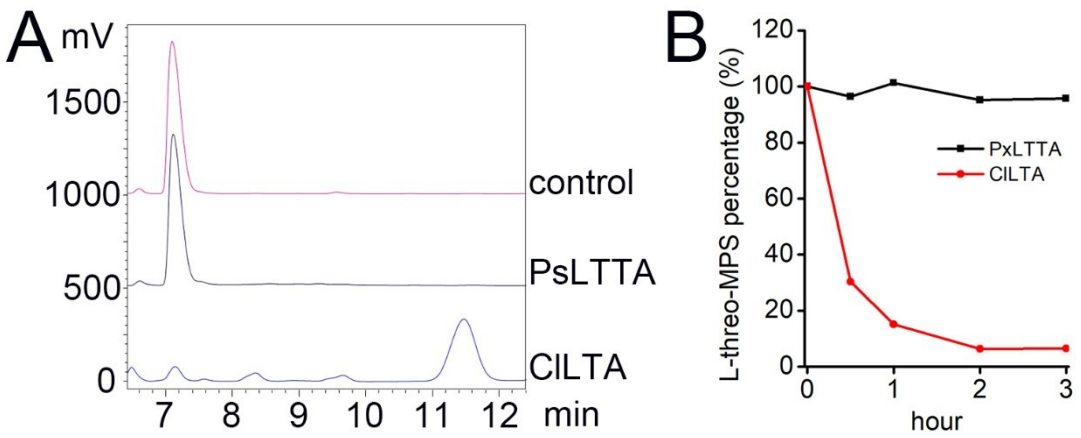

Figure S6. Determination of reverse activities of PsLTTA and ClLTA. (A) L-threo-*p*-methylsulfonylphenylserine (L-threo-MPS) and acetaldehyde were as substrates in PsLTTA catalyzed-assay while L-threo-MPS was as substrate in ClLTA catalyzed-assay. The reaction products were detected by HPLC at 236 nm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

Figure S7.

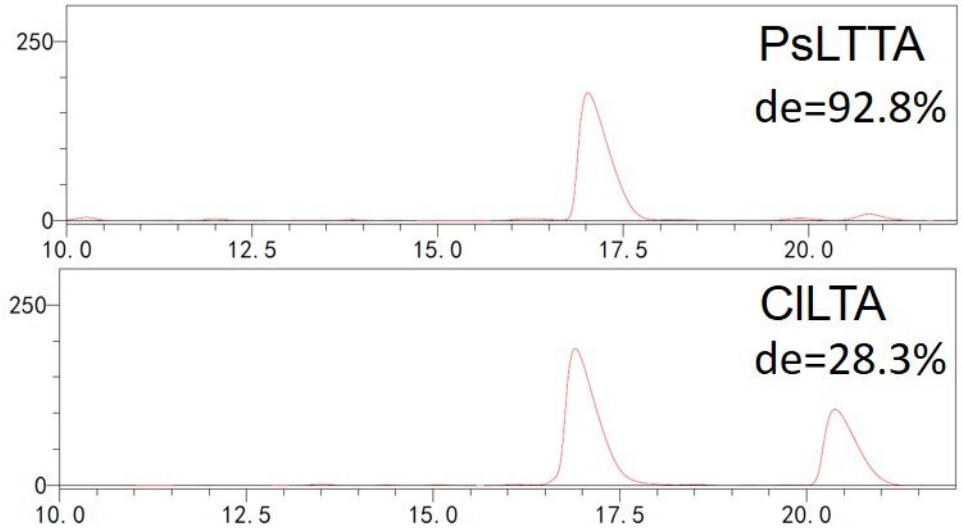

Figure S7. Determination of stereospecificity of PsLTTA and ClLTA. Product L-*p*-nitrophenylserine by PsLTTA ($t_{L\text{-}threo}$=17.1 min, $t_{L\text{-}erythro}$=20.7 min) and by ClLTA ($t_{L\text{-}threo}$=16.9 min, $t_{L\text{-}erythro}$=20.4 min).

Figure S8.

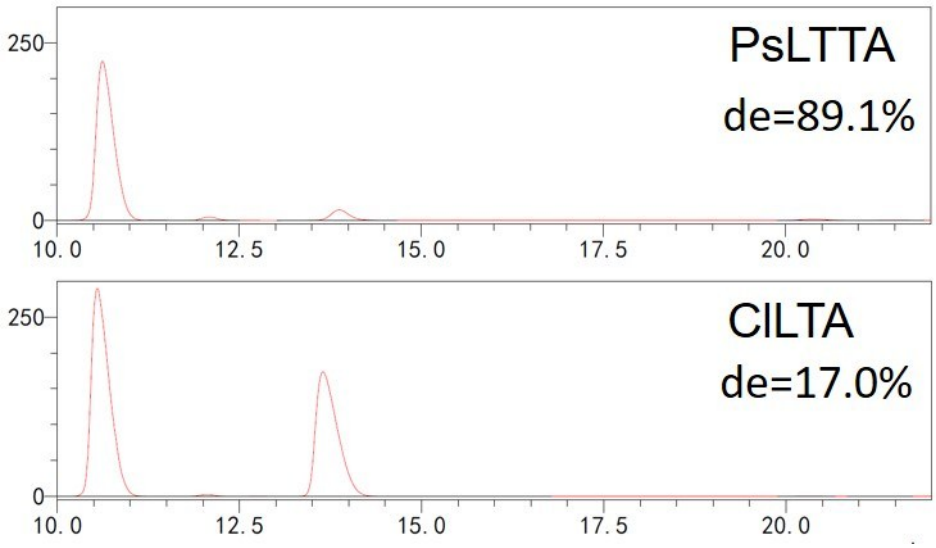

Figure S8. Determination of stereospecificity of PsLTTA and ClLTA. Product L-phenylserine by PsLTTA ($t_{L\text{-}threo}$=10.6 min, $t_{L\text{-}erythro}$=13.8 min) and by ClLTA ($t_{L\text{-}threo}$=10.5 min, $t_{L\text{-}erythro}$=13.7 min).

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Figure S9.

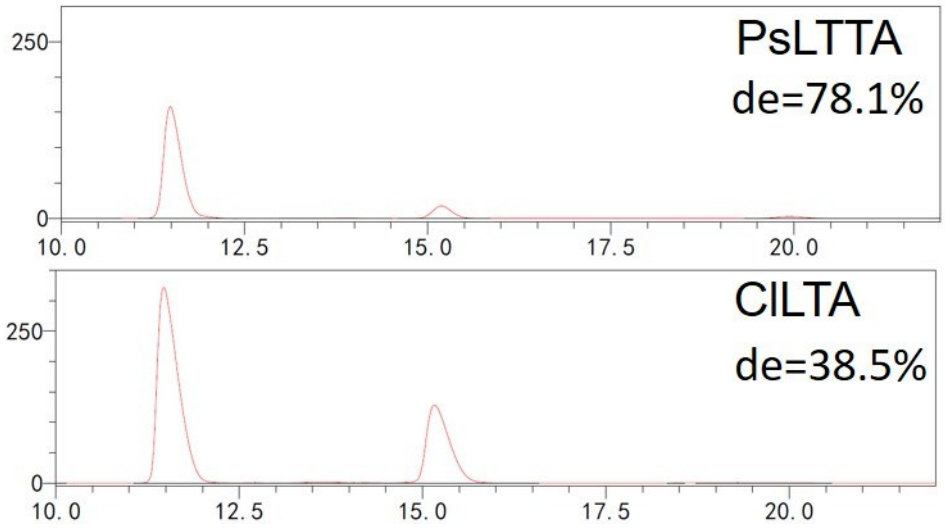

Figure S9. Determination of stereospecificity of PsLTTA and ClLTA. Product L-*o*-fluorophenylserine by PsLTTA ($t_{L\text{-}threo}$=11.4 min, $t_{L\text{-}erythro}$=15.1 min) and by ClLTA ($t_{L\text{-}threo}$= 11.4 min, $t_{L\text{-}erythro}$=15.1 min).

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Figure S10.

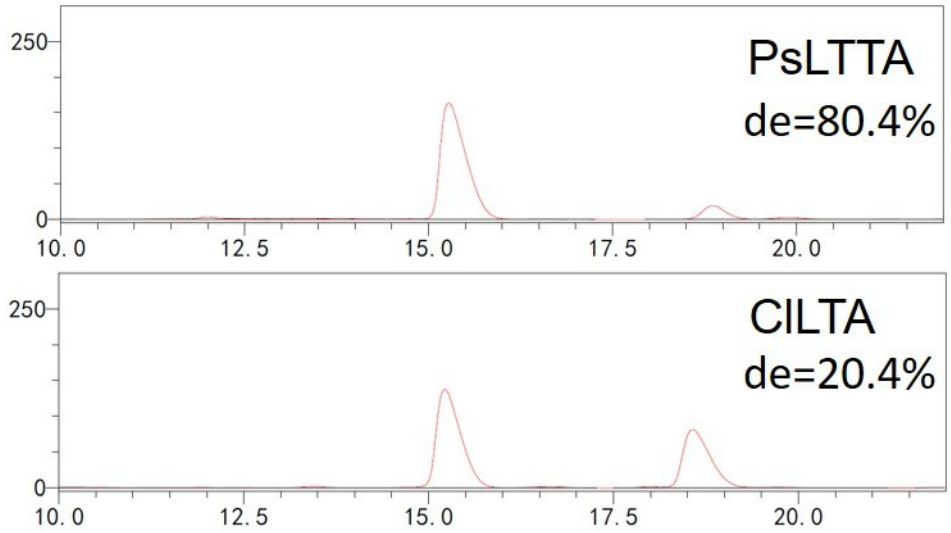

Figure S10. Determination of stereospecificity of PsLTTA and ClLTA. Product L-*m*-fluorophenylserine by PsLTTA ($t_{L\text{-}threo}$=15.3 min, $t_{L\text{-}erythro}$=18.8 min) and by ClLTA ($t_{L\text{-}threo}$=15.2 min, $t_{L\text{-}erythro}$=18.6 min).

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Figure S11.

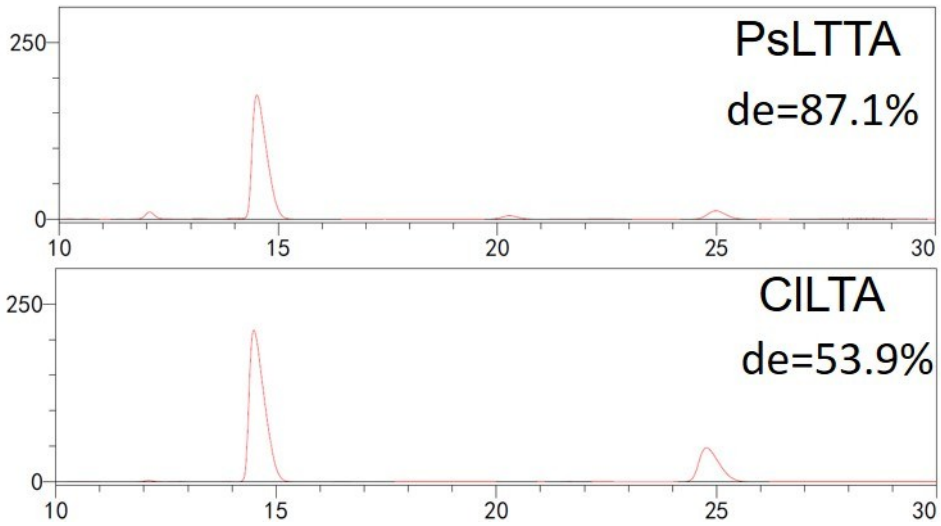

Figure S11. Determination of stereospecificity of PsLTTA and ClLTA. L-*o*-chlorophenylserine by PsLTTA ($t_{L\text{-}threo}$=14.5 min, $t_{L\text{-}erythro}$=25.0 min) and by ClLTA ($t_{L\text{-}threo}$=14.5 min, $t_{L\text{-}erythro}$=24.9 min).

Figure S12.

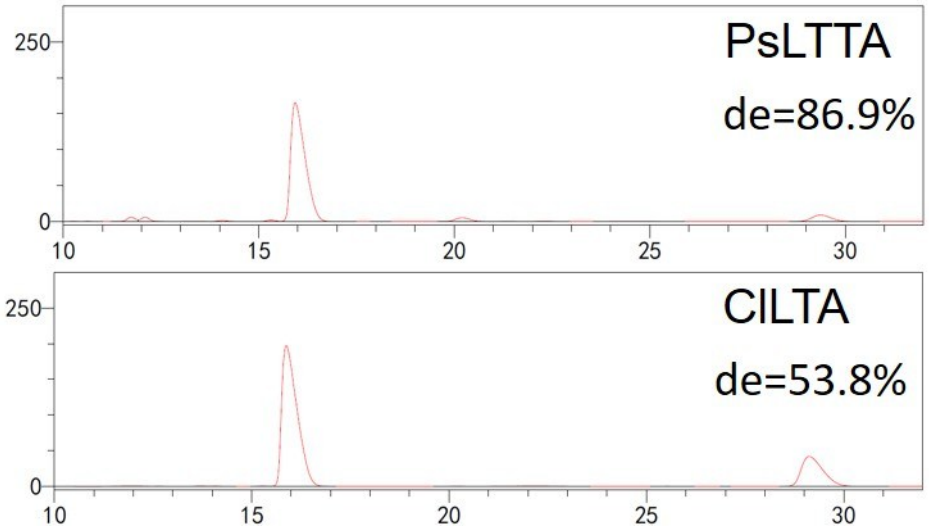

Figure S12. Determination of stereospecificity of PsLTTA and ClLTA. L-*o*-bromophenylserine by PsLTTA ($t_{L\text{-}threo}$=15.9 min, $t_{L\text{-}erythro}$=29.3 min) and by ClLTA ($t_{L\text{-}threo}$=15.8 min, $t_{L\text{-}erythro}$=29.2 min).

Figure S13.

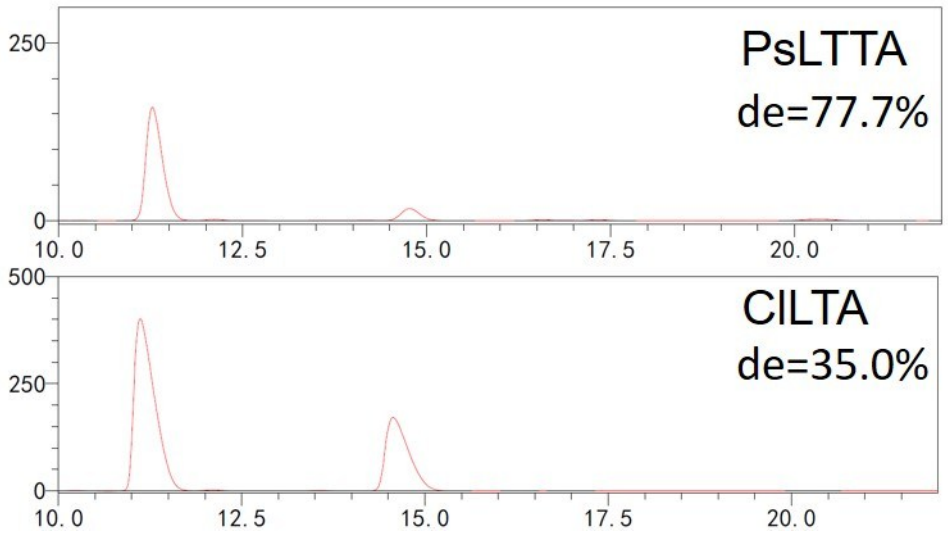

Figure S13. Determination of stereospecificity of PsLTTA and ClLTA. L-*o*-nitrophenylserine by PsLTTA ($t_{L\text{-}threo}$=11.2 min, $t_{L\text{-}erythro}$=14.7 min) and by ClLTA ($t_{L\text{-}threo}$=11.1 min, $t_{L\text{-}erythro}$=14.6 min).

Figure S14.

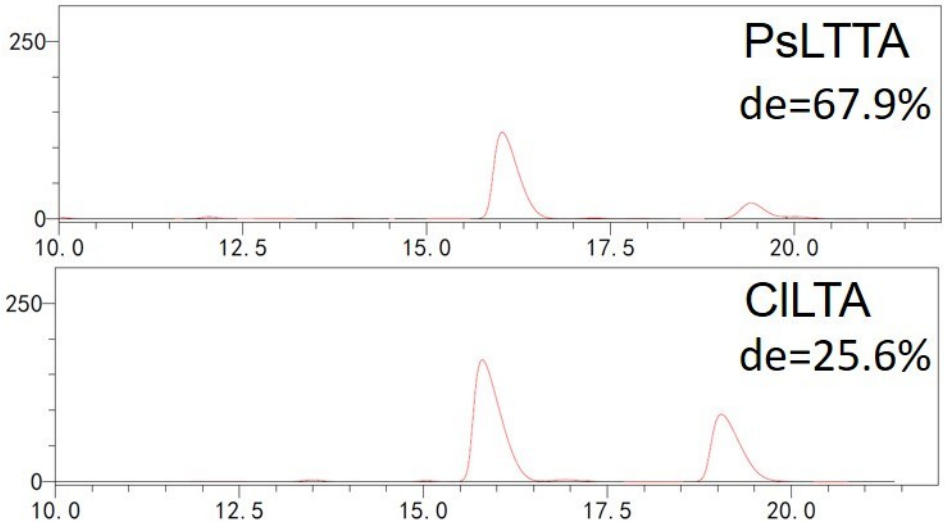

Figure S14. Determination of stereospecificity of PsLTTA and ClLTA. L-*m*-nitrophenylserine by PsLTTA ($t_{L\text{-}threo}$=16.1min, $t_{L\text{-}erythro}$=19.4 min) and by ClLTA ($t_{L\text{-}threo}$=15.8 min, $t_{L\text{-}erythro}$=19.1 min).

Figure S15.

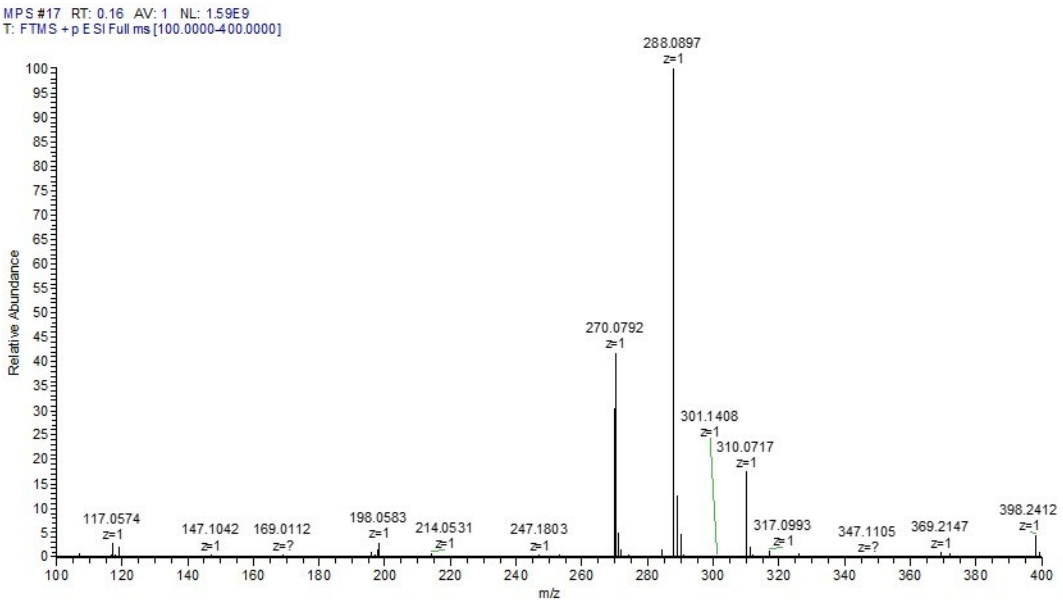

Figure S15. HRMS analysis of L-threo-*p*-methylsulfonylphenylserine ethyl ester. HRMS (*m/z*) (M^+): calcd. for $C_{12}H_{18}NO_5S$, 288.0897, found 288.0900.

Figure S16.

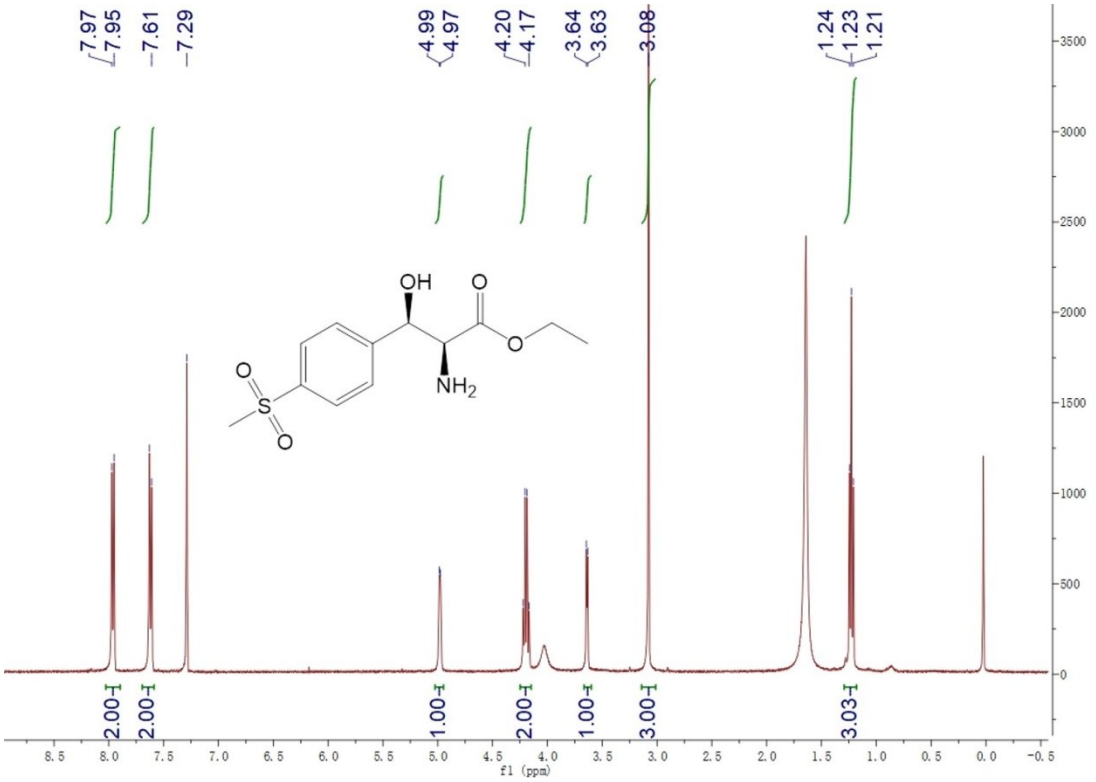

Figure S16. ^{1}H- NMR spectra of L-threo-*p*-methylsulfonylphenylserine ethyl ester. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.1 Hz, 2H), 7.62 (d, *J* = 8.1 Hz, 2H), 4.98 (d, *J* = 4.5 Hz, 1H), 4.19 (q, *J* = 7.0 Hz, 2H), 3.64 (d, *J* = 4.7 Hz, 1H), 3.08 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

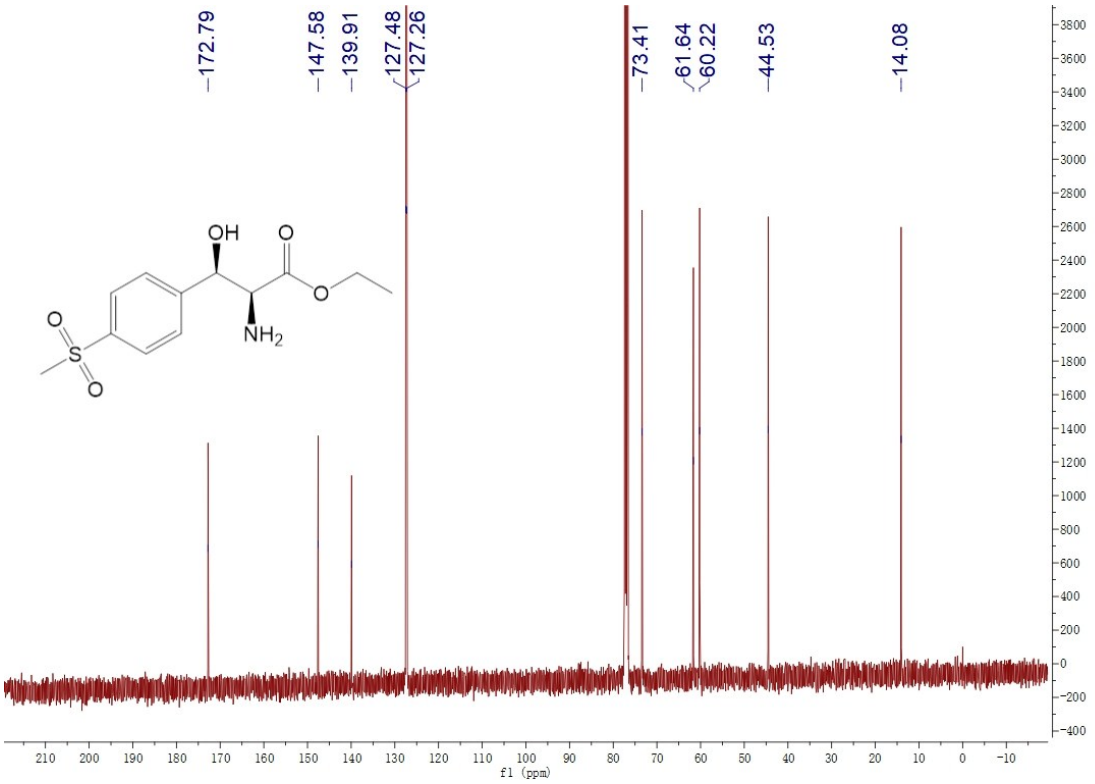

Figure S17. ^{13}C NMR spectra of L-threo-*p*-methylsulfonylphenylserine ethyl ester. ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.79, 147.58, 139.91, 127.48, 127.26, 73.41, 61.64, 60.22, 44.53, 14.08.