

## Supporting information

### Engineering *Pseudomonas dacunhae* L-aspartate $\beta$ -decarboxylase for L-homophenylalanine synthesis

Min Zhang,<sup>a</sup> Pengfei Hu,<sup>b</sup> Yu-Cong Zheng,<sup>a</sup> Bu-Bing Zeng,<sup>b</sup> Qi Chen,<sup>a</sup> Zhi-Jun Zhang<sup>\*a</sup> and Jian-He Xu<sup>\*a</sup>

<sup>a</sup> State Key Laboratory of Bioreactor Engineering, Shanghai Collaborative Innovation Centre for Biomanufacturing, School of Biotechnology, East China University of Science and Technology, Shanghai 200237, China.

<sup>b</sup> Shanghai Key Laboratory of New Drug Design, East China University of Science and Technology, Shanghai 200237, China.

\*Corresponding authors: [zjzhang@ecust.edu.cn](mailto:zjzhang@ecust.edu.cn); [jianhexu@ecust.edu.cn](mailto:jianhexu@ecust.edu.cn)

## **Table of Contents**

**1. Experimental methods**

**2. Additional tables**

**3. Additional figures**

**4. References**

## 1. Experimental methods

### *General methods and materials*

L-Aspartate, L-alanine, 3(*R,S*)-methyl-D,L-aspartate, and L-2-amino butyrate were obtained from Aladdin (Shanghai); 3(*R,S*)-hydroxy-D,L-aspartate and L-serine were purchased from TCI (Shanghai); L-homophenylalanine was supplied by Energy Chemical (Shanghai); PrimerSTAR HS, 2× Taq PCR MasterMix, T<sub>4</sub> DNA ligase, and *Dpn* I were obtained from TIANGEN (Beijing). 3(*R,S*)-Benzyl-L-aspartate was prepared according to the methods reported in the literature.<sup>1</sup> <sup>1</sup>H NMR, and <sup>13</sup>C NMR spectra were recorded on a Bruker AM-400 spectrometer in CDCl<sub>3</sub>, D<sub>2</sub>O. The chemical shift ( $\delta$ ) of <sup>1</sup>H NMR and <sup>13</sup>C NMR was given in ppm relative to solvent residual peak according to the work of Fulmer *et al.*,<sup>2</sup> Fast column chromatography was carried out using 200–300 mesh silica gel. Thin layer chromatography (TLC) analysis was carried out on glass sheets coated with 0.2 mm thickness silica gel, and visualized using alkaline potassium manganate (VII) solution, and/or UV light at 254 nm. All other reagents and compounds were analytical grade and used without further purification.

### *Analytic methods*

Derivatization of amino acids: To 25  $\mu$ L reaction solution was added 225  $\mu$ L acetonitrile-water solution (v/v, 1:1) containing triethylamine (40 mg/mL) and 250  $\mu$ L 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl isothiocyanate (GITC, 4 mM) dissolved in acetonitrile, and the resultant mixture was incubated at 30 °C for 30 min.

Shimadzu LC-2010A<sub>HT</sub> was used for reverse phase HPLC analysis with Hypersil ODS2 column (5 $\mu$ m, 4.6 mm×250 mm). Amino acid derivatives were analyzed using the mobile phase consists of methanol and water (0.005% phosphoric acid) under the following conditions: (1) L-aspartate derivative: 55% (v/v) methanol, 254 nm UV detection, 0.8 mL/min flow rate, and the column temperature was kept at 30°C. (2) 3(*R,S*)-methyl-D,L-aspartate derivative: 45% (v/v) methanol, and the other conditions were the same as (1). (3) 3(*R,S*)-hydroxy-D,L-aspartate derivative: 35% (v/v) methanol, and the other conditions were the same as (1). (4) 3(*R*)-benzyl-L-aspartic acid: 35% (v/v) methanol, 210 nm UV detection, and the other conditions were the same as (1).

### *Construction of AspBDC variants*

The gene sequence of L-aspartate  $\beta$ -decarboxylase (AspBDC) from *Pseudomonas dacunhae*<sup>3</sup> was codon optimized and synthesized by Genscript (Nanjing). The vector pET-28a(+) and

host strain *E. coli* BL21 (DE3) were used for protein expression.

AspBDC variants were created by PCR using the AspBDC gene (pET 28a(+)) as the template. Oligonucleotide primers used for the generation of mutants were listed in Table S1. The PCR mixture (total volume 20  $\mu$ L) contained: template plasmid (1.5  $\mu$ L, 20 ng/ $\mu$ L), forward and reverse primers (1  $\mu$ L, 10  $\mu$ M each), DMSO (1  $\mu$ L), 2 $\times$  PrimeSTAR HS (10  $\mu$ L), and ddH<sub>2</sub>O (5.5  $\mu$ L). The PCR mixture was first subjected to 98  $^{\circ}$ C for 3 min, followed by 16 cycles of denaturing at 98  $^{\circ}$ C for 10 s, annealing at 55  $^{\circ}$ C for 15 s, and elongation at 72  $^{\circ}$ C for 7 min 16 s. A final extension step at 72  $^{\circ}$ C for 5 min was conducted at the end the PCR reaction. To the PCR mixture was added 1  $\mu$ L *Dpn* I and incubated at 37  $^{\circ}$ C for 5 h to eliminate the template plasmid. The digested PCR product was then used to transform the competent *E. coli* BL21 (DE3) cells.

#### *Enzyme expression and purification*

10  $\mu$ L Bacteria store solution was used to inoculate 4 mL LB medium (containing 50  $\mu$ g/mL kanamycin) and incubated at 37  $^{\circ}$ C and 180 rpm overnight. The overnight sub-cultures were transferred to 100 mL LB (1%, v/v) containing 50  $\mu$ g/mL kanamycin and incubated at 37  $^{\circ}$ C and 180 rpm until OD<sub>600</sub> reached 0.6~0.8. Then IPTG (final concentration 0.2 mM) was added to induce AspBDC expression and the culture was then incubated at 16  $^{\circ}$ C for another 24 h with shaking at 180 rpm. Cells were harvested by centrifugation at 7,000 $\times$ g and 4  $^{\circ}$ C for 10 min and the supernatant was discarded. The cells were resuspended in the buffer (20 mM phosphate buffer, 0.5 M NaCl, 10 mM  $\beta$ -mercaptoethanol, 10 mM imidazole; pH 7.4) and the target proteins inside the cells were released by ultrasonication. The cell debris was removed by centrifugation at 16,000 $\times$ g for 30 min at 4  $^{\circ}$ C.

The cell-free extract containing the His-tagged AspBDC was loaded onto a His trap Ni-NTA FF column and eluted with elution buffer (20 mM phosphate buffer, 0.5 M NaCl, 10 mM  $\beta$ -mercaptoethanol, 300 mM imidazole, pH 7.4) to obtain the purified enzyme.

#### *Screening of non-natural substrates*

The pH of the reaction mixture (0.5 mL) containing 100 mM KPB buffer (pH 6.0), 150 mM NaCl, substrate (20 mM), PLP (0.5 mM) was adjusted to 6.0 prior to the addition of purified enzyme (4 mg/mL) to initiate the reaction. The reaction was performed at 30  $^{\circ}$ C and 1000 rpm for 24 h, and the product formed was detected by HPLC and identified by UPLC-MS.

#### *Molecular docking*

The monomer crystal structure (PDB: 3FDD) of *Pseudomonas dacunhae* AspBDC has been reported.<sup>2</sup> The reported hexamer (PDB: 2zy4) crystal structure of *Pseudomonas* sp. ATCC 19121 AspBDC showing 93% sequence identity with *Pseudomonas dacunhae* AspBDC was used for docking using Autodock vina.<sup>4</sup>

#### *Determination of total turnover number (TON)*

The reaction mixture (0.5 mL) consists of 100 mM KPB (pH 6.0) containing 150 mM NaCl, 0.5 mM PLP, 10 mM substrate, and purified enzyme was incubated at 30 °C and 1000 rpm for 24 hours, and the TON was determined according to the following equation: TON = product ( $\mu\text{mol}$ )/enzyme ( $\mu\text{mol}$ ).

#### *Enzyme assay and kinetic parameters determination*

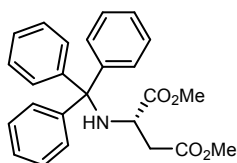
Enzyme assay was performed in a reaction mixture containing 100 mM KPB (pH 6.0), 150 mM NaCl, 10 mM substrate, 0.5 mM PLP, and purified enzyme with different concentrations. The reaction mixture was incubated at 30°C and 1000 rpm for a required period, and the reaction was terminated by metal bath at 95 °C for 10 min to denature the enzyme. The amount of product produced was analyzed by HPLC analysis. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the formation of 1  $\mu\text{mol}$  of product per minute under the assay conditions.

Kinetic constants determination: The reaction mixture (250  $\mu\text{L}$ ) consists of 195  $\mu\text{L}$  purified enzyme in 100 mM KPB (pH 6.0), 5  $\mu\text{L}$  PLP (final concentration 0.5 mM) and 50  $\mu\text{L}$  substrate with different concentrations was incubated at 30°C and 1000 rpm for a required period, and the product formed was measured by HPLC analysis.

#### *Preparation of L-homophenylalanine*

The reaction mixture (15 mL) containing KPB buffer (100 mM, pH 6.0), 3(*R*)-benzyl-L-aspartate (10 mM), 150 mM NaCl and PLP (0.5 mM) was adjusted to a pH value of 6.0, and purified AspBDC R37A/T382G (final concentration 0.07 mg/mL) was added to start the reaction, equal amount of enzyme was supplemented at 5 h. The reaction was stopped by metal bath (95°C for 10 min) for inactivating the enzyme and the denatured protein was removed by centrifugation. L-Homophenylalanine was precipitated by adjusting the pH of the supernatant to 7.0. <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR (100 MHz) spectra were all obtained in D<sub>2</sub>O for L-homophenylalanine.<sup>5</sup>

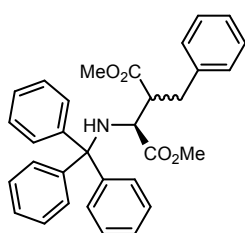
#### *Chemical synthesis of 3(*R,S*)-benzyl-L-aspartate*



**N-Trityl aspartate dimethyl ester:**  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta/\text{ppm}$ :

7.50 – 7.48 (m, 6H), 7.28 – 7.24 (m, 6H), 7.19 (d,  $J = 7.3$  Hz, 3H), 3.68 (s, 3H), 3.26 (s, 3H), 2.68 – 2.48 (m, 2H), 1.67 (br, 1H).  $^{13}\text{C NMR}$  (100

MHz,  $\text{CDCl}_3$ )  $\delta/\text{ppm}$ : 173.9, 171.0, 145.7, 128.8, 127.9, 126.5, 71.2, 53.7, 52.0, 51.8, 40.2.



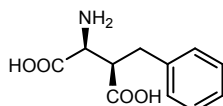
**Dimethyl (3S)-2-benzyl-3-(tritylamino)succinate (3):**  $^1\text{H NMR}$  (400

MHz,  $\text{CDCl}_3$ )  $\delta/\text{ppm}$ : 7.45 (t,  $J = 7.4$  Hz, 6H), 7.28 – 7.26 (m, 5H),

7.24 – 7.14 (m, 10H), 3.58(d,  $J = 16.9$  Hz, 3H), 3.19 (d,  $J = 19.8$  Hz, 3H),

3.06 – 2.81 (m, 2H), 1.64 (br, 1H).  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $^{13}\text{C}$

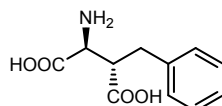
$\text{NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta/\text{ppm}$ : 173.1, 172.8, 145.7, 139.4, 129.1, 129.0, 129.0, 128.9, 128.6, 128.5, 128.1, 128.0, 126.7, 126.6, 71.3, 71.2, 58.3, 58.0, 53.1, 52.0, 51.9, 51.9, 51.8, 34.3, 33.7.



**(R)-benzyl-L-aspartate:**  $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ )  $\delta/\text{ppm}$ : 7.40 – 7.30

(m, 5H), 3.71 (d,  $J = 4.3$  Hz, 1H), 3.21 – 3.18 (m, 1H), 2.90 (ddd,  $J =$

20.8, 13.7, 8.0 Hz, 2H).  $^{13}\text{C NMR}$  (100 MHz,  $\text{D}_2\text{O}$ )  $\delta/\text{ppm}$ : 138.3, 128.9, 128.8, 126.8, 55.2, 49.2, 35.8. **HRMS** (ESI-TOF):  $m/z$  calcd for  $\text{C}_{11}\text{H}_{14}\text{NO}_4^+$  224.0923, found 224.0922.



**(S)-benzyl-L-aspartate:**  $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ )  $\delta/\text{ppm}$ : 7.37 – 7.25

(m, 5H), 3.99 (d,  $J = 3.3$  Hz, 1H), 3.13-3.08 (m, 1H), 2.90-2.76 (m, 2H).

$^{13}\text{C NMR}$  (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  138.6, 128.8, 128.7, 126.7, 55.5, 48.9, 33.2.

$[\alpha]_{\text{D}}^{25} = +14.0$  (c x,  $\text{HCl}_{\text{aq}}$  (1 M)). **HRMS** (ESI-TOF):  $m/z$  calcd for  $\text{C}_{11}\text{H}_{14}\text{NO}_4^+$  224.0923, found 224.0922.

## 2. Additional tables

**Table S1.** List of primers used for the construction of enzyme variants

	Primers	Sequences
Alanine scanning	K17A-F	AGCCCGTTTGAGCTCGCAGATGAGTTGATCAAG
	K17A-R	CTTGATCAACTCATCTGCGAGCTCAAACGGGCT
	L31A-F	GGCGACGGAAACCGCGCAATGCTCAATGCGGGG
	L31A-R	CCCCGCATTGAGCATTGCGCGGTTTCCGTCGCC
	R37A-F	ATGCTCAATGCGGGGGCAGGCAATCCCAATTTT
	R37A-R	AAAATTGGGATTGCCTGCCCCCGCATTGAGCAT
	N39A-F	AATGCGGGGCGGGGCGCACCCAATTTTCTGGCA
	N39A-R	TGCCAGAAAATTGGGTGCGCCCCGCCCGCATT
	Y134A-F	ATTCTGGGCTGCAATGCACCCGTTCCCCCTCGG
	Y134A-R	CCGAGGGGGAACGGGTGCATTGCAGCCCAGAAT
	F204A-F	ATCGGCATGCCGTTGCAACTCCGTACATAGAA
	F204A-R	TTCTATGTACGGAGTTGCAACCGGCATGCCGAT
	K315A-F	GTCTATTCATTCTCCGCATACTTTGGTGCCACT
	K315A-R	AGTGGCACCAAAGTATGCGGAGAATGAATAGAC
	T320A-F	AAATACTTTGGTGCCGCAGGCTGGCGTCTGGGT
	T320A-R	ACCCAGACGCCAGCCTGCGGCACCAAAGTATTT
	T382A-F	GTTGCCTTGAACCACGCAGCCGGTCTGTCCACG
	T382A-R	CGTGGACAGACCGGCTGCGTGGTTCAAGGCAAC
R37X	R37G-F	ATGCTCAATGCGGGGGGTGGCAATCCCAATTTT
	R37G-R	AAAATTGGGATTGCCACCCCCCGCATTGAGCAT
	R37V-F	ATGCTCAATGCGGGGGTGGCAATCCCAATTTT
	R37V-R	AAAATTGGGATTGCCAACCCCCCGCATTGAGCAT
	R37L-F	ATGCTCAATGCGGGGCTGGGCAATCCCAATTTT
	R37L-R	AAAATTGGGATTGCCAGCCCCCGCATTGAGCAT
	R37I-F	ATGCTCAATGCGGGGATTGGCAATCCCAATTTT
	R37I-R	AAAATTGGGATTGCCAATCCCCCGCATTGAGCAT
	R37S-F	ATGCTCAATGCGGGGAGCGGCAATCCCAATTTT
	R37S-R	AAAATTGGGATTGCCGCTCCCCCGCATTGAGCAT
	R37T-F	ATGCTCAATGCGGGGACCGGCAATCCCAATTTT
	R37T-R	AAAATTGGGATTGCCGGTCCCCCGCATTGAGCAT
	R37F-F	ATGCTCAATGCGGGGTTGGCAATCCCAATTTT
	R37F-R	AAAATTGGGATTGCCAAACCCCCGCATTGAGCAT
	R37W-F	ATGCTCAATGCGGGGTGGGGCAATCCCAATTTT
	R37W-R	AAAATTGGGATTGCCCCACCCCCGCATTGAGCAT
	R37P-F	ATGCTCAATGCGGGGCCGGGCAATCCCAATTTT
	R37P-R	AAAATTGGGATTGCCCGGCCCGCATTGAGCAT
R37H-F	ATGCTCAATGCGGGGCATGGCAATCCCAATTTT	

R37H-R	AAAATTGGGATTGCCATGCCCCGCATTGAGCAT	
R37K-F	ATGCTCAATGCGGGGAAAGGCAATCCCAATTTT	
R37K-R	AAAATTGGGATTGCCTTTCCCCGCATTGAGCAT	
R37C-F	ATGCTCAATGCGGGGTGTGGCAATCCCAATTTT	
R37C-R	AAAATTGGGATTGCCACACCCCCGCATTGAGCAT	
R37D-F	ATGCTCAATGCGGGGGATGGCAATCCCAATTTT	
R37D-R	AAAATTGGGATTGCCATCCCCGCATTGAGCAT	
R37E-F	ATGCTCAATGCGGGGGAAGGCAATCCCAATTTT	
R37E-R	AAAATTGGGATTGCCTTCCCCGCATTGAGCAT	
R37Y-F	ATGCTCAATGCGGGGTATGGCAATCCCAATTTT	
R37Y-R	AAAATTGGGATTGCCATACCCCGCATTGAGCAT	
R37M-F	ATGCTCAATGCGGGGATGGGCAATCCCAATTTT	
R37M-R	AAAATTGGGATTGCCATCCCCGCATTGAGCAT	
R37N-F	ATGCTCAATGCGGGGAATGGCAATCCCAATTTT	
R37N-R	AAAATTGGGATTGCCATTCCCCGCATTGAGCAT	
R37Q-F	ATGCTCAATGCGGGGCAGGGCAATCCCAATTTT	
R37Q-R	AAAATTGGGATTGCCCTGCCCGCATGAGCAT	
T382G-F	GTTGCCTTGAACCACGGTGCCGGTCTGTCCACG	
T382G-R	CGTGGACAGACCGGCACCGTGGTTCAAGGCAAC	
T382V-F	GTTGCCTTGAACCACGTTGCCGGTCTGTCCACG	
T382V-R	CGTGGACAGACCGGCAACGTGGTTCAAGGCAAC	
T382L-F	GTTGCCTTGAACCACCTGGCCGGTCTGTCCACG	
T382L-R	CGTGGACAGACCGGCCAGGTGGTTCAAGGCAAC	
T382I-F	GTTGCCTTGAACCACATTGCCGGTCTGTCCACG	
T382I-R	CGTGGACAGACCGGCAATGTGGTTCAAGGCAAC	
T382S-F	GTTGCCTTGAACCACAGCGCCGGTCTGTCCACG	
T382S-R	CGTGGACAGACCGGCGCTGTGGTTCAAGGCAAC	
T382R-F	GTTGCCTTGAACCACCGTGCCGGTCTGTCCACG	
T382R-R	CGTGGACAGACCGGCACGGTGGTTCAAGGCAAC	
T382X	T382F-F	GTTGCCTTGAACCACTTTGCCGGTCTGTCCACG
T382F-R	CGTGGACAGACCGGCAAAGTGGTTCAAGGCAAC	
T382W-F	GTTGCCTTGAACCACTGGGCCGGTCTGTCCACG	
T382W-R	CGTGGACAGACCGGCCAGTGGTTCAAGGCAAC	
T382P-F	GTTGCCTTGAACCACCGGCCGGTCTGTCCACG	
T382P-R	CGTGGACAGACCGGCCGGTGGTTCAAGGCAAC	
T382H-F	GTTGCCTTGAACCACCATGCCGGTCTGTCCACG	
T382H-R	CGTGGACAGACCGGCATGGTGGTTCAAGGCAAC	
T382K-F	GTTGCCTTGAACCACAAAGCCGGTCTGTCCACG	
T382K-R	CGTGGACAGACCGGCTTTGTGGTTCAAGGCAAC	
T382C-F	GTTGCCTTGAACCACTGTGCCGGTCTGTCCACG	
T382C-R	CGTGGACAGACCGGCACAGTGGTTCAAGGCAAC	
T382D-F	GTTGCCTTGAACCACGATGCCGGTCTGTCCACG	



---

T382D-R	CGTGGACAGACCGGCATCGTGGTTCAAGGCAAC
T382E-F	GTTGCCTTGAACCACGAAGCCGGTCTGTCCACG
T382E-R	CGTGGACAGACCGGCTTCGTGGTTCAAGGCAAC
T382Y-F	GTTGCCTTGAACCACTATGCCGGTCTGTCCACG
T382Y-R	CGTGGACAGACCGGCATAGTGGTTCAAGGCAAC
T382M-F	GTTGCCTTGAACCACATGGCCGGTCTGTCCACG
T382M-R	CGTGGACAGACCGGCCATGTGGTTCAAGGCAAC
T382N-F	GTTGCCTTGAACCACAATGCCGGTCTGTCCACG
T382N-R	CGTGGACAGACCGGCATTGTGGTTCAAGGCAAC
T382Q-F	GTTGCCTTGAACCACCAGGCCGGTCTGTCCACG
T382Q-R	CGTGGACAGACCGGCCTGGTGGTTCAAGGCAAC

---

**Table S2.** The decarboxylation of 3(*R*)-benzyl-L-aspartate by AspBDC and its variants<sup>a</sup>

Entry	Mutants	TON ( $\mu\text{mol product}/\mu\text{mol enzyme}$ )
1	WT	9.1 $\pm$ 0.1
2	K17A	91 $\pm$ 0.1
3	L31A	12 $\pm$ 2
4	R37A	53 $\pm$ 5
5	N39A	9.0 $\pm$ 0.1
6	Y134A	21 $\pm$ 0.3
7	F204A	20 $\pm$ 1
8	K315A	4.0 $\pm$ 0.5
9	T320A	7.0 $\pm$ 0.3
10	T382A	184 $\pm$ 3

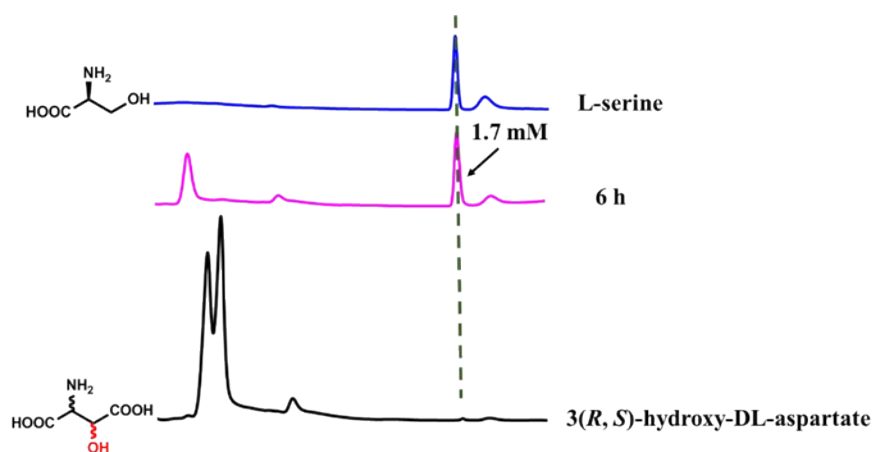
<sup>a</sup> The reaction mixture (0.5 mL) consists of 100 mM KPB (pH 6.0) containing 150 mM NaCl, 0.5 mM PLP, 10 mM substrate, and purified enzyme (6 mg/mL) was incubated at 30°C and 1000 rpm for 24 hours, and the TON was determined according to the following equation: TON = product ( $\mu\text{mol}$ )/enzyme ( $\mu\text{mol}$ ).

**Table S3.** Specific activity of AspBDC and its variants R37A/T382G towards 3(*R*)-benzyl-L-aspartate<sup>a</sup>

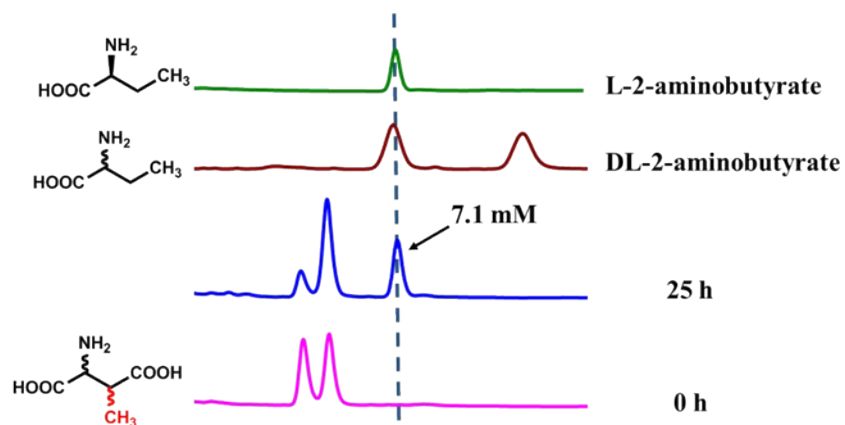
Entry	Enzymes	Specific activity (mU/mg <sub>protein</sub> )	Fold
1	WT	0.014 ± 0.002	1
2	T382G	60.6 ± 0.9	4320
3	T382G/R37A	216 ± 0.3	15400
4	T382G/R37F	37.1 ± 0.5	2650
5	T382G/K17A	145 ± 1.7	10357
6	T382G/Y134A	59.8 ± 0.5	4270
7	T382G/R37A/K17A	198 ± 1.5	14100
8	T382G/R37A/Y134A	14.1 ± 0.5	1000

<sup>a</sup> The reaction mixture containing 100 mM KPB (pH 6.0), 150 mM NaCl, 10 mM substrate, 0.5 mM PLP, and purified enzyme with different concentrations was incubated at 30°C and 1000 rpm for a required period, and the amount of product produced was analyzed by HPLC analysis.

### 3. Additional figures

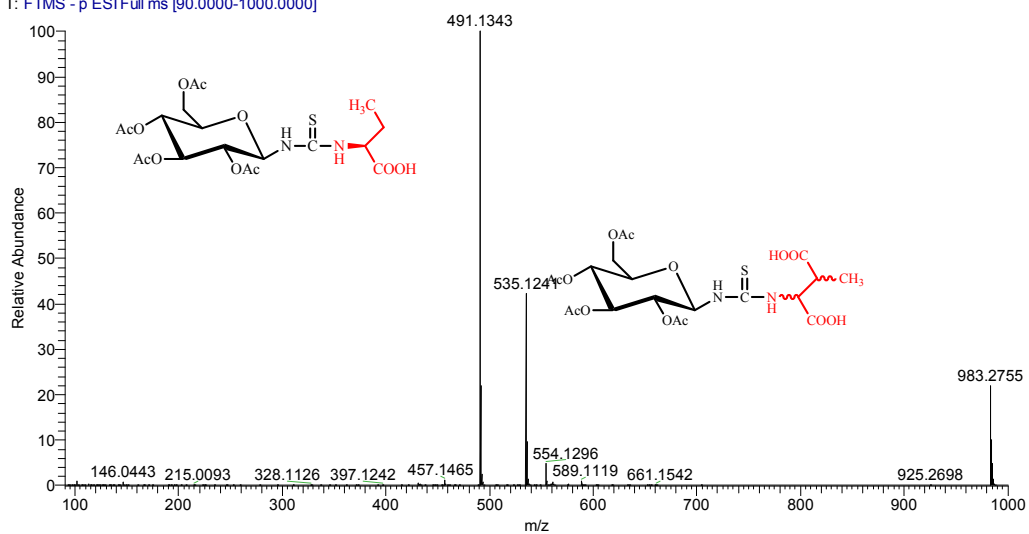


**Figure S1.** HPLC chromatograph of wild-type AspBDC catalyzed decarboxylation of 3(*R*, *S*)-hydroxy-DL-aspartate.

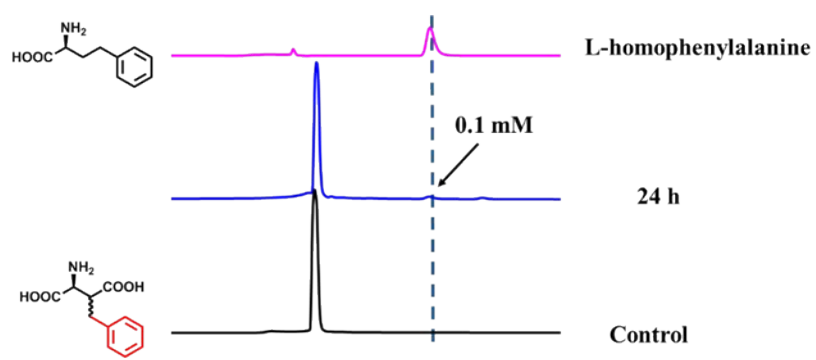


**Figure S2.** HPLC chromatograph of wild-type AspBDC catalyzed decarboxylation of 3(*R*, *S*)-methyl-DL-aspartate.

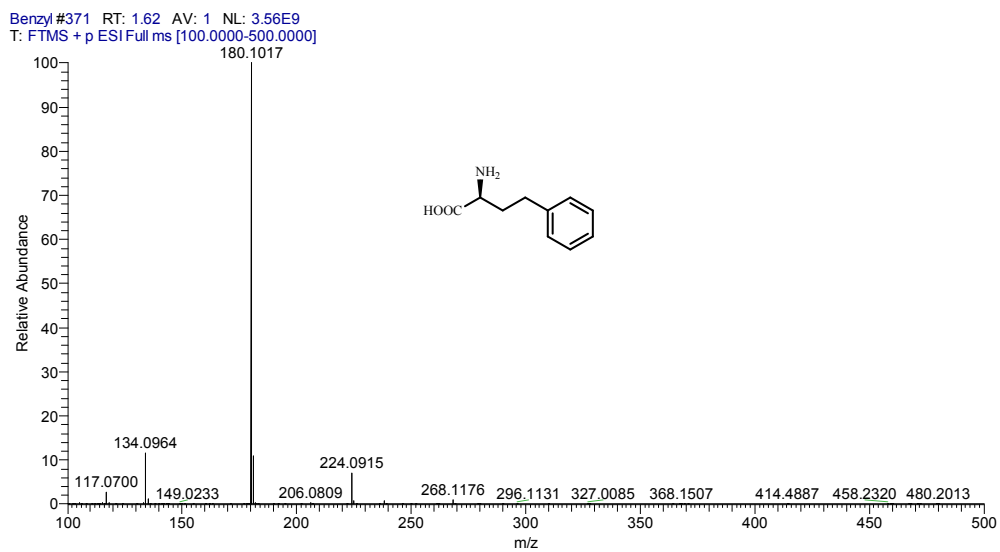
L-ABA #687 RT: 3.01 AV: 1 NL: 5.55E8  
T: FTMS - p ESI Full ms [90.0000-1000.0000]



**Figure S3.** UPLC-MS analysis of the product produced by wild-type AspBDC catalyzed decarboxylation of 3(*R,S*)-methyl-DL-aspartate.

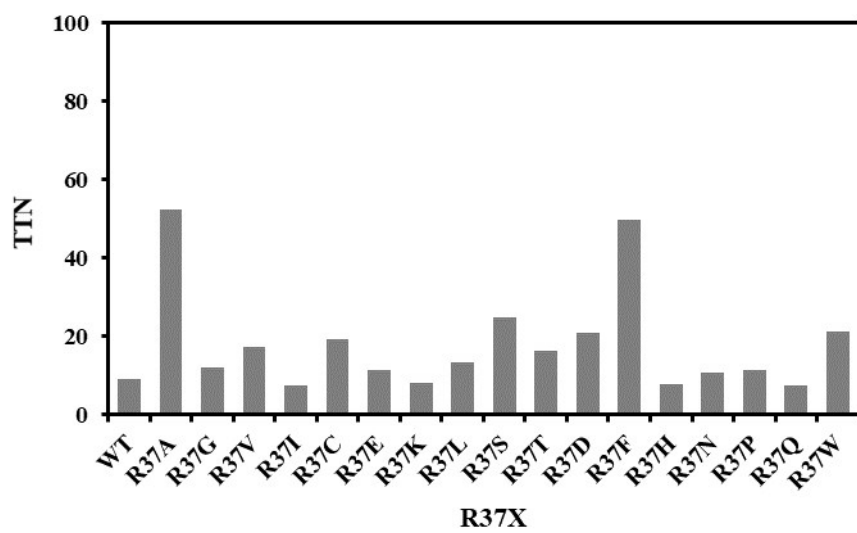


**Figure S4.** HPLC chromatograph of wild-type AspBDC catalyzed decarboxylation of 3(*R*, *S*)-benzyl-L-aspartate.

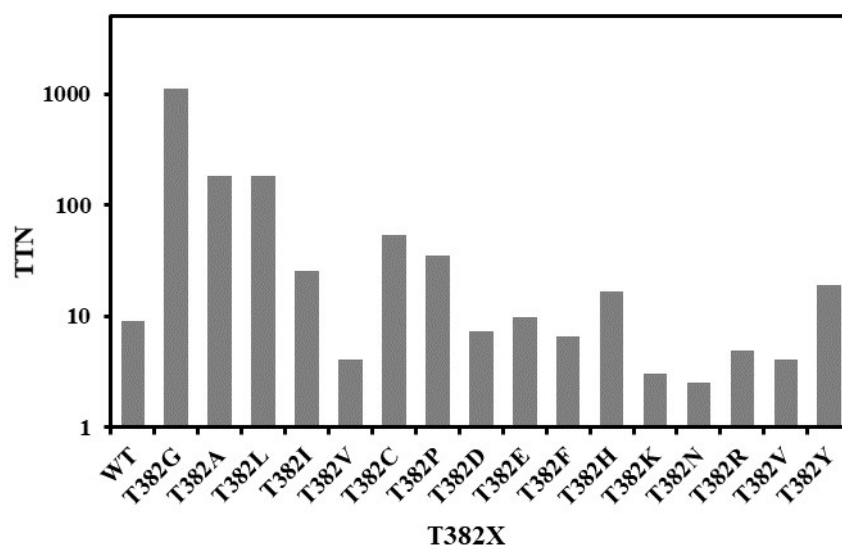


**Figure S5.** UPLC-MS analysis of the product produced by wild-type AspBDC catalyzed decarboxylation of 3(*R,S*)-benzyl-L-aspartate.

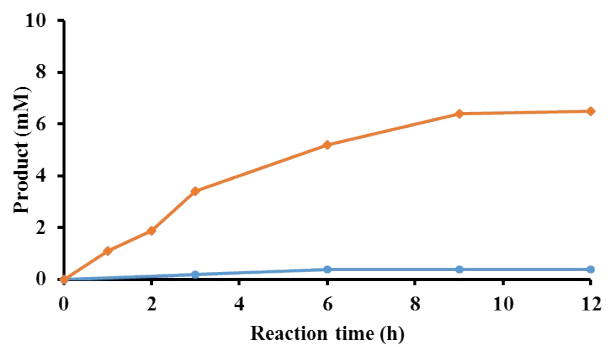




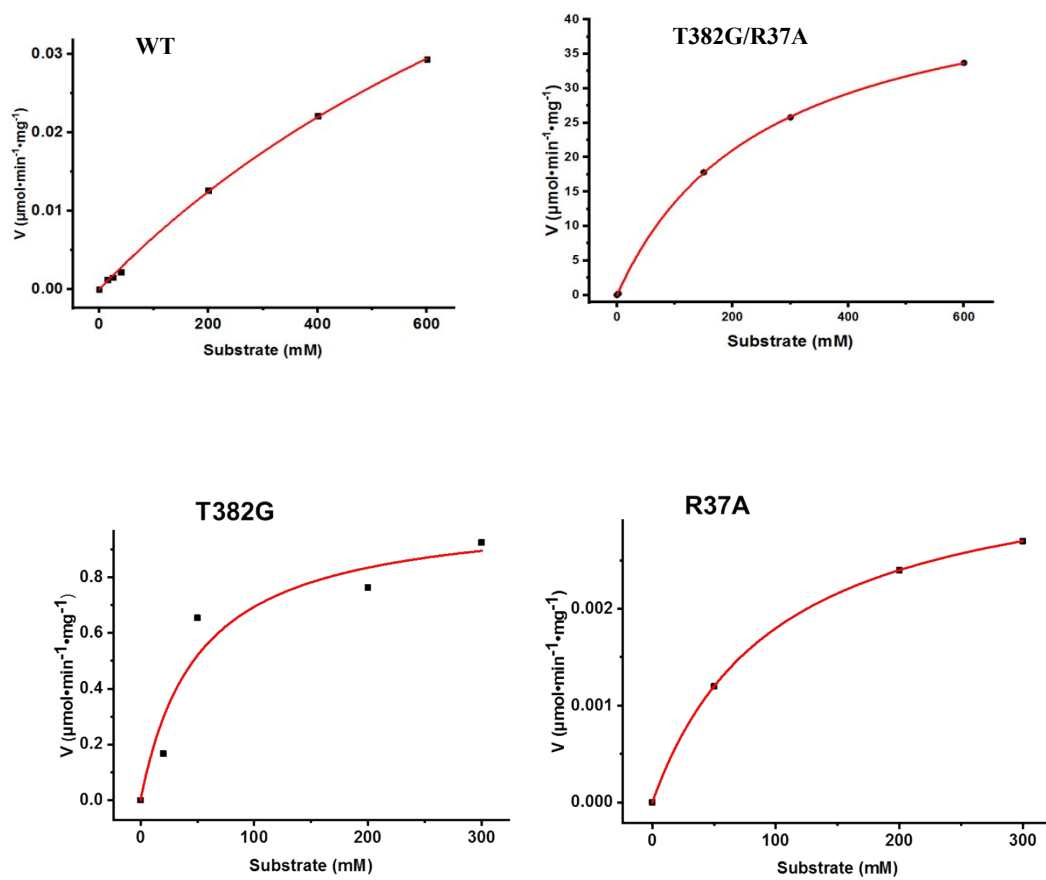
**Figure S6.** Decarboxylation of 3(*R*)-benzyl-L-aspartate by R37X variants of AspBDC.



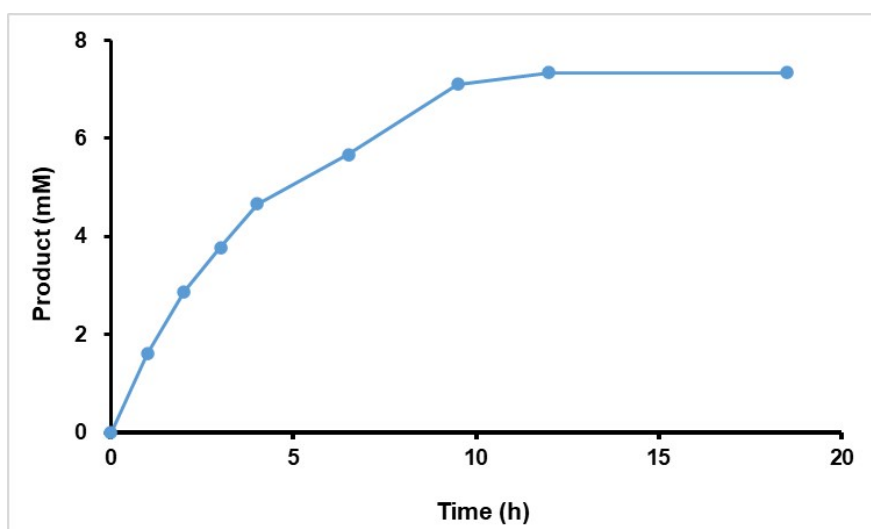
**Figure S7.** Decarboxylation of 3(*R*)-benzyl-L-aspartate by T382X variants of AspBDC.



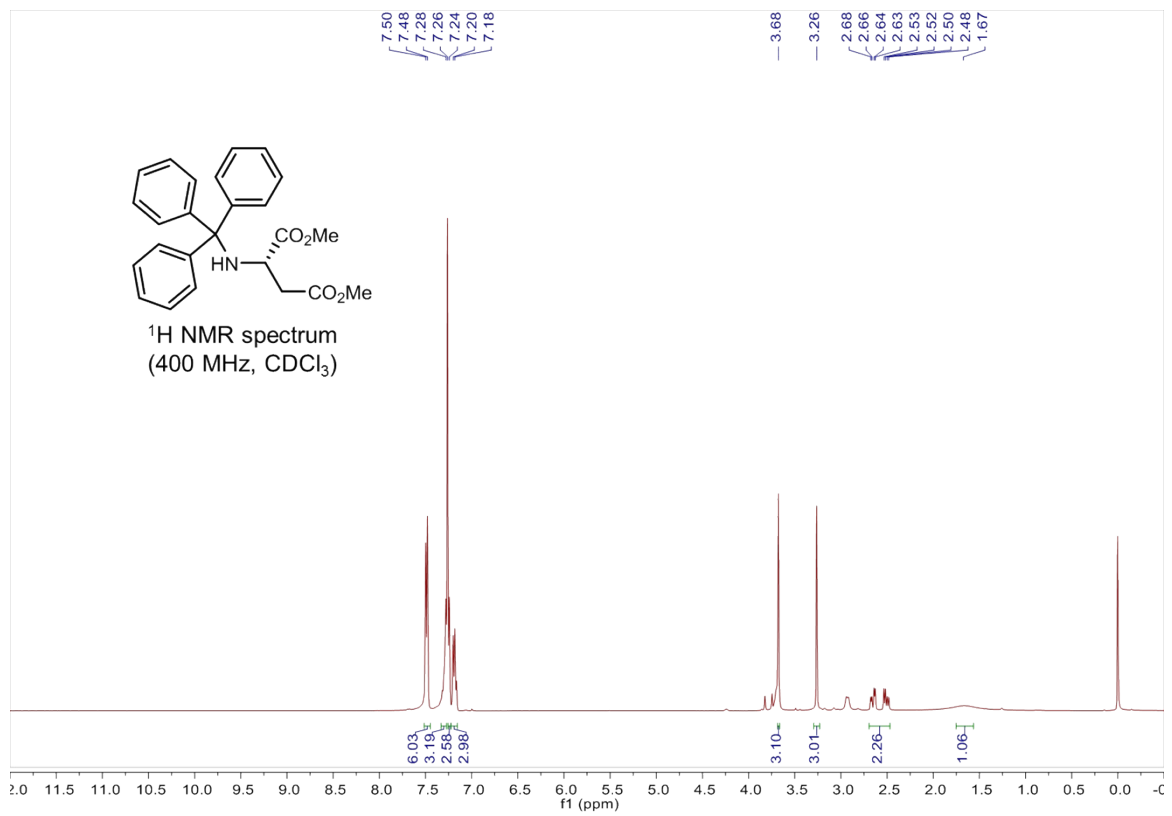
**Figure S8.** Reaction progress curves of 3(*R*)-benzyl-L-aspartate (orange) and 3(*S*)-benzyl-L-aspartate (blue) catalyzed by AspBDC R37A/T382G.

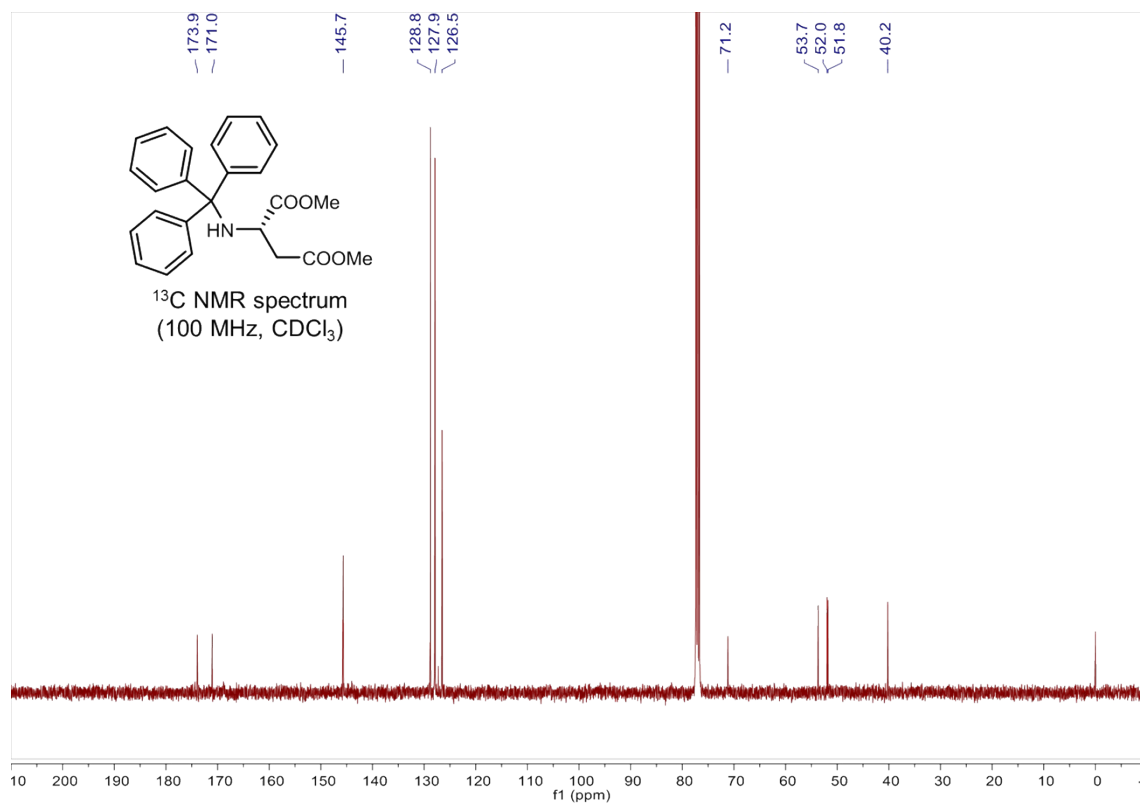


**Figure S9.** Kinetic study of wild-type AspBDC and mutants R37A, T382G, and T382G/R37A towards 3(*R*)-benzyl-L-aspartate.

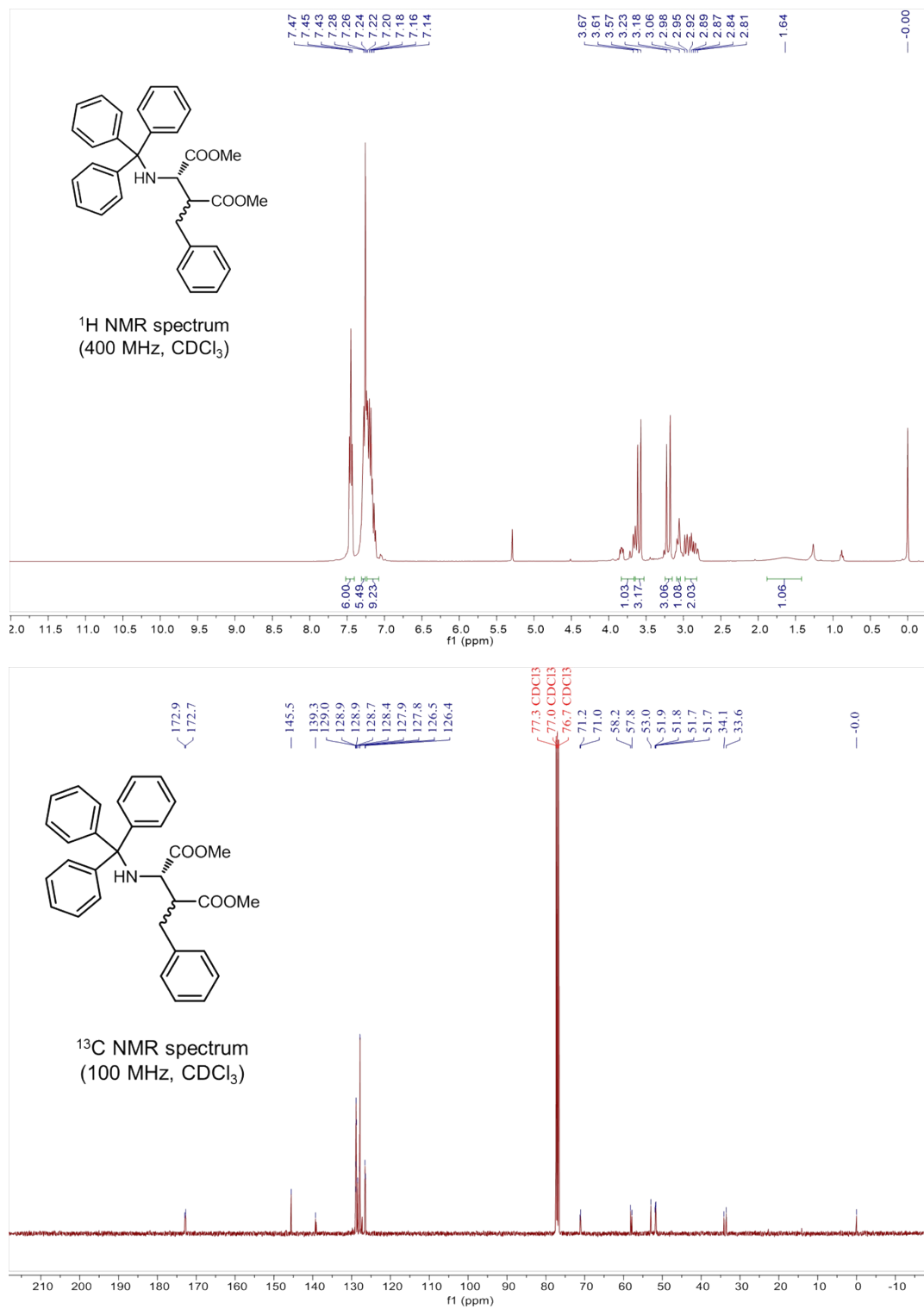


**Figure S10.** Reaction progress curve of AspBDC R37A/T382G catalyzed decarboxylation of 3(*R*)-benzyl-L-aspartate for the synthesis of L-homophenylalanine.



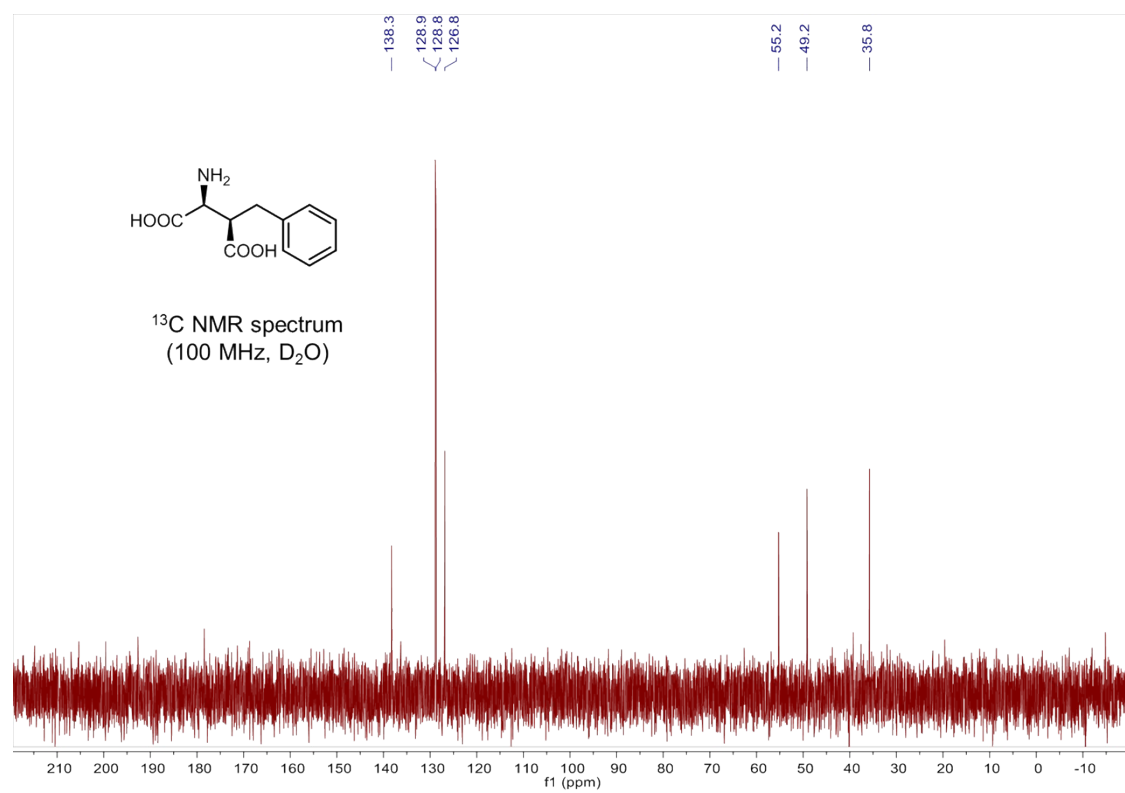
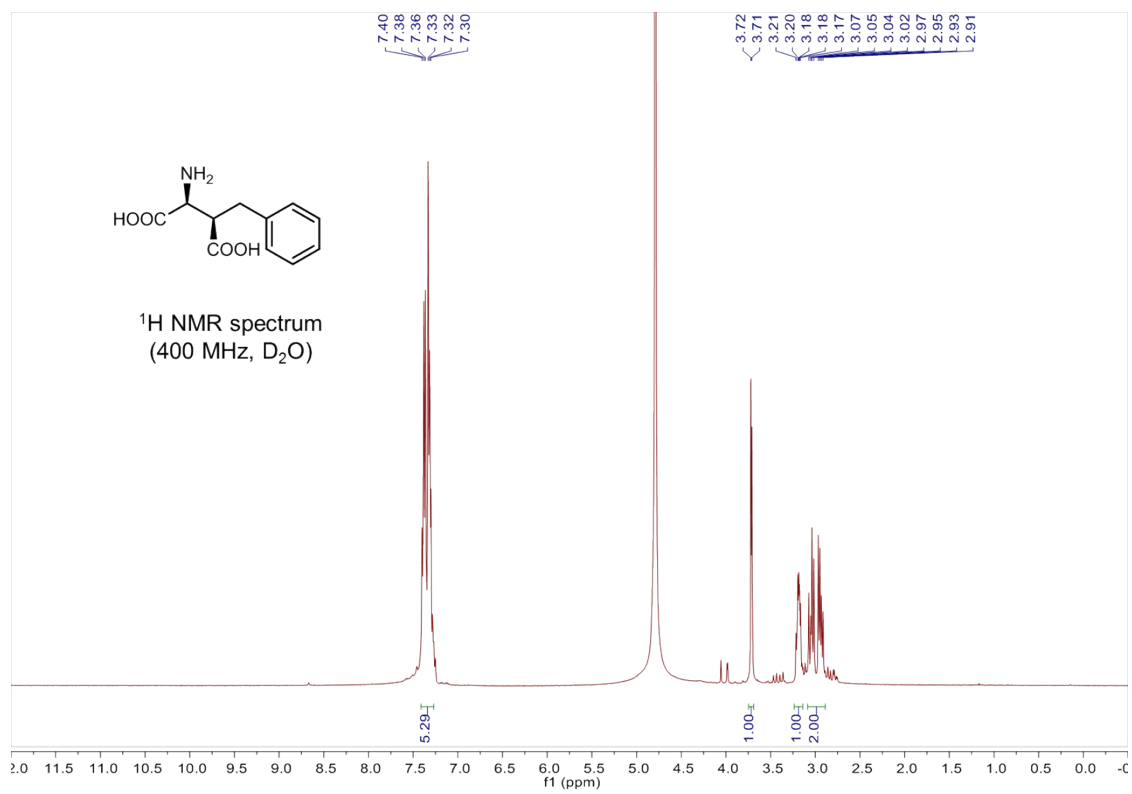


**Figure S11.** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of *N*-trityl aspartate dimethyl ester.

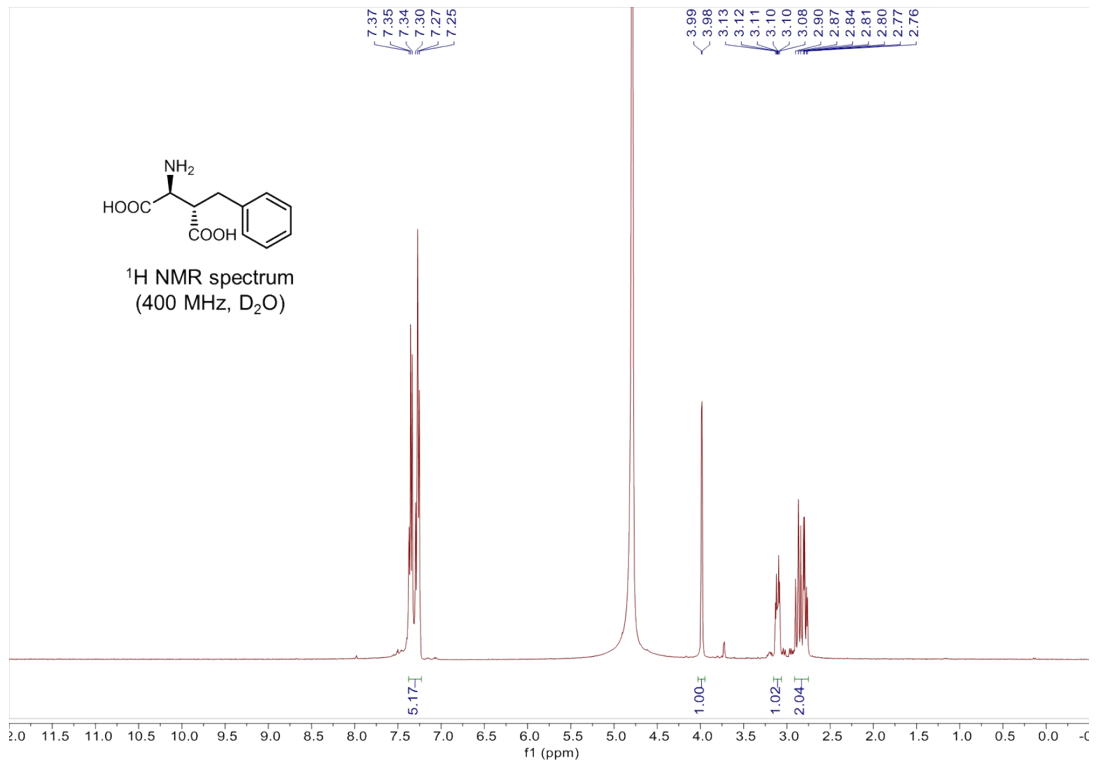


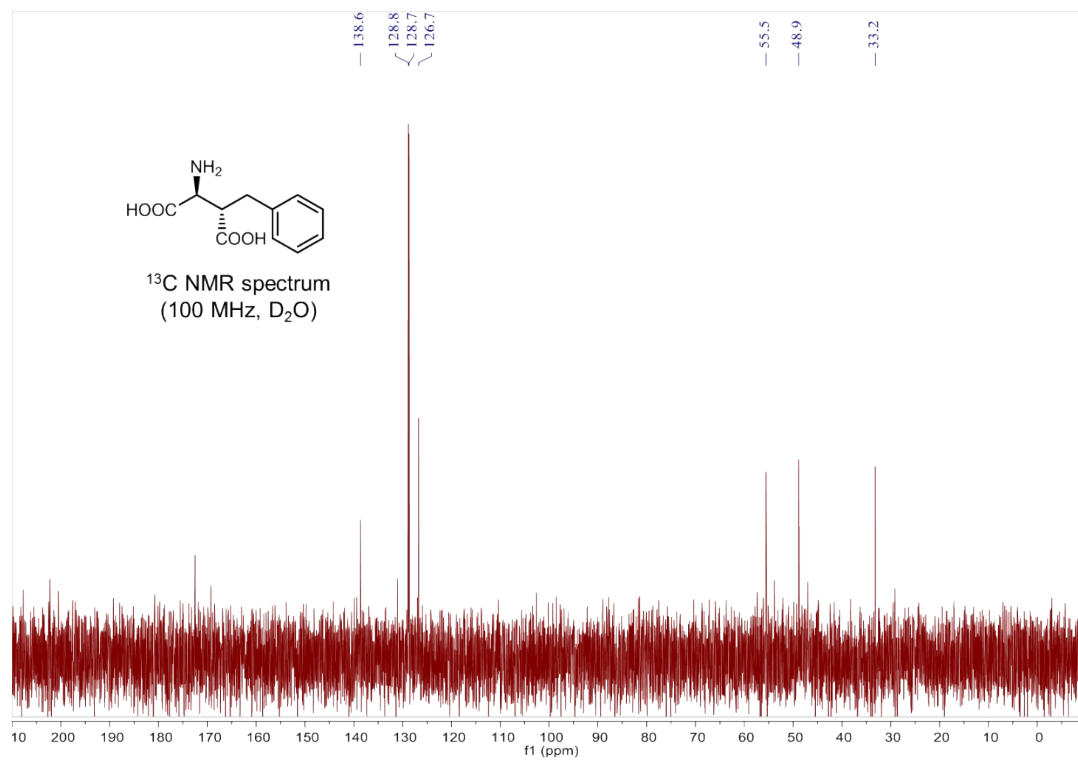
**Figure S12.** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of *N*-tritylamino dimethyl ester  $\beta$ -benzylaspartate.



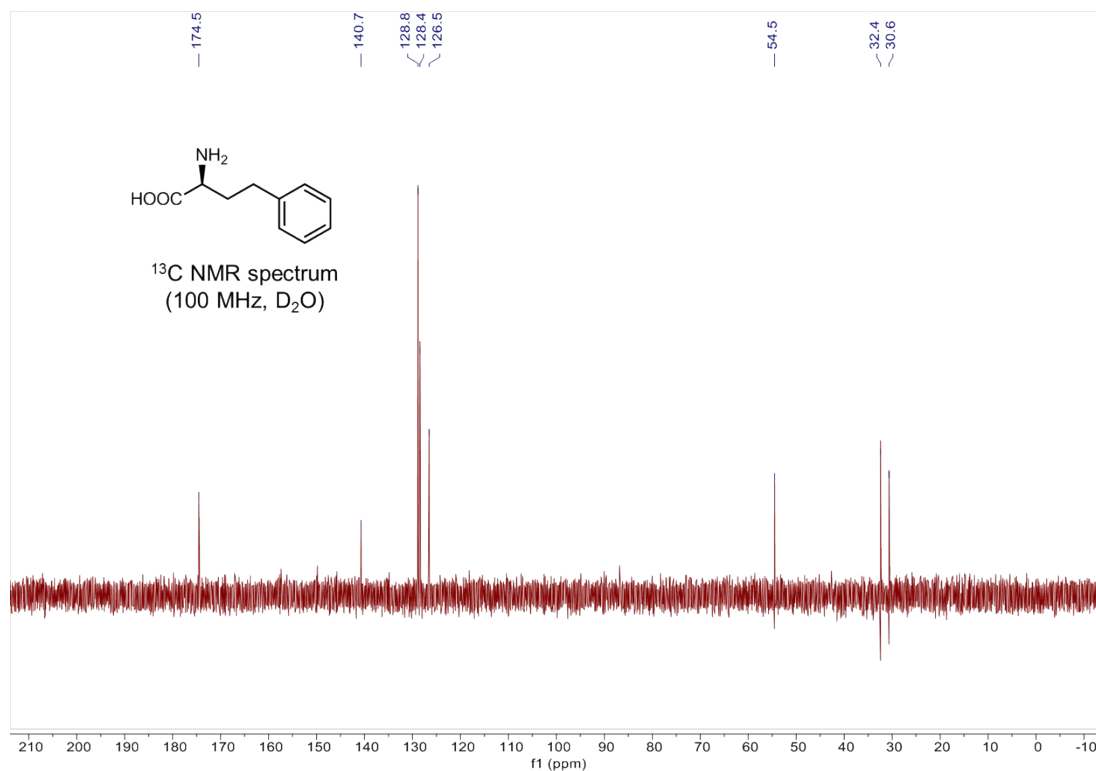
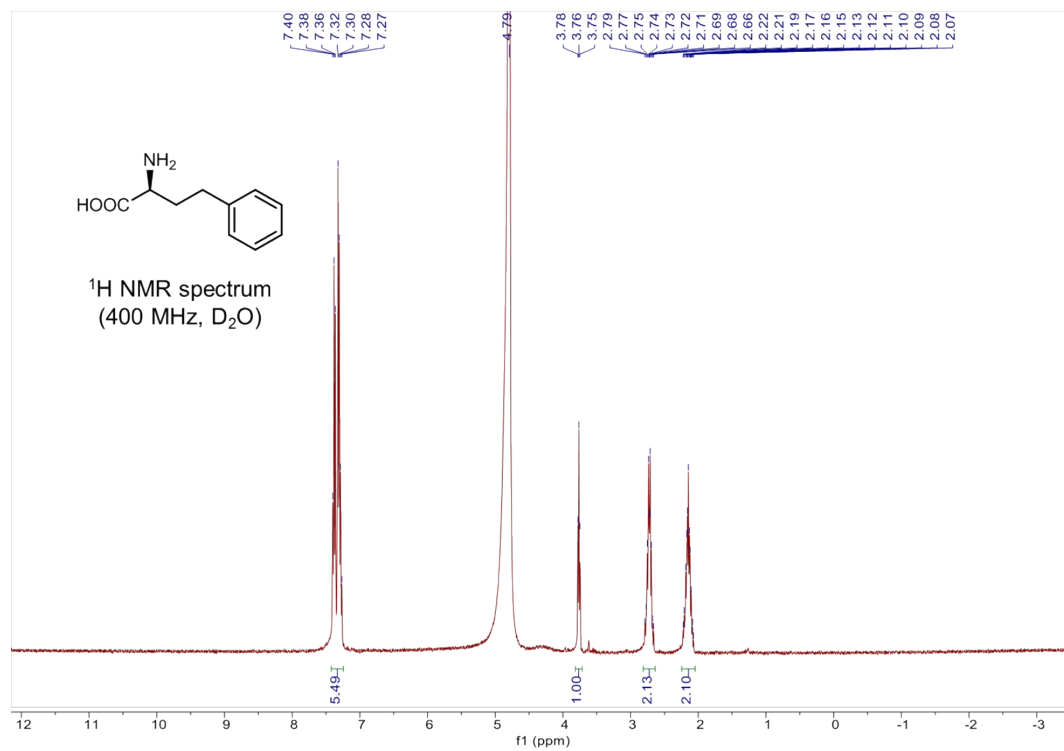


**Figure S13.** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 3(*R*)-benzyl-L-aspartate.

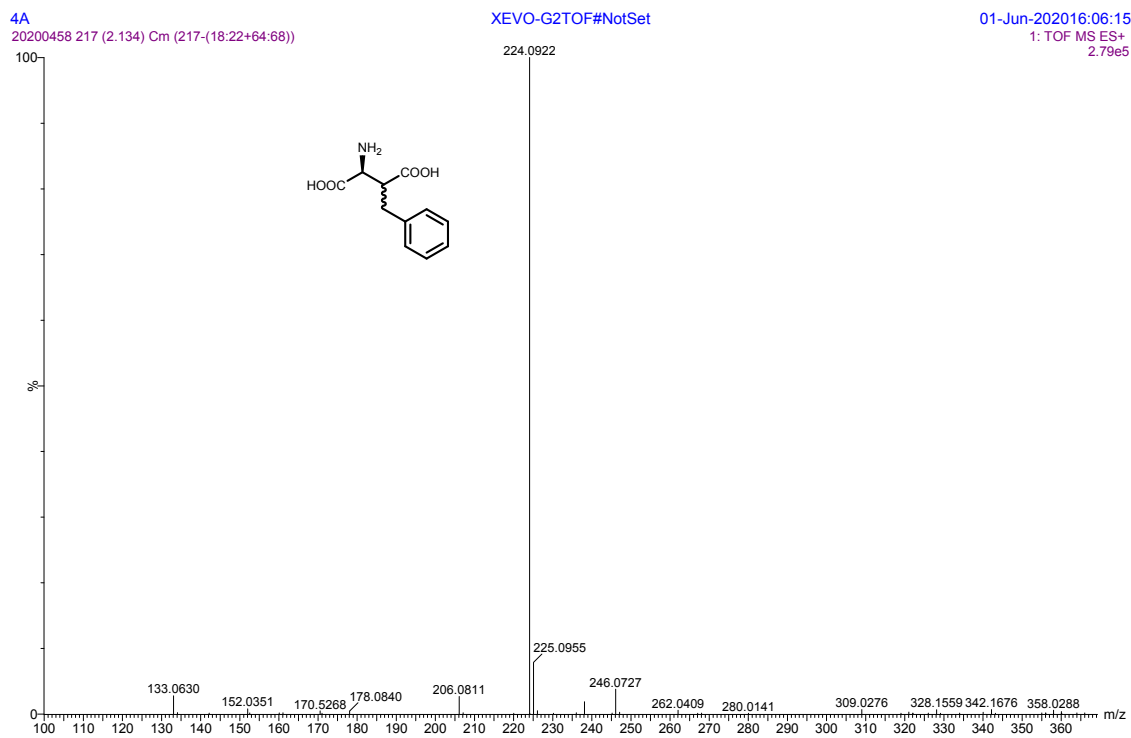




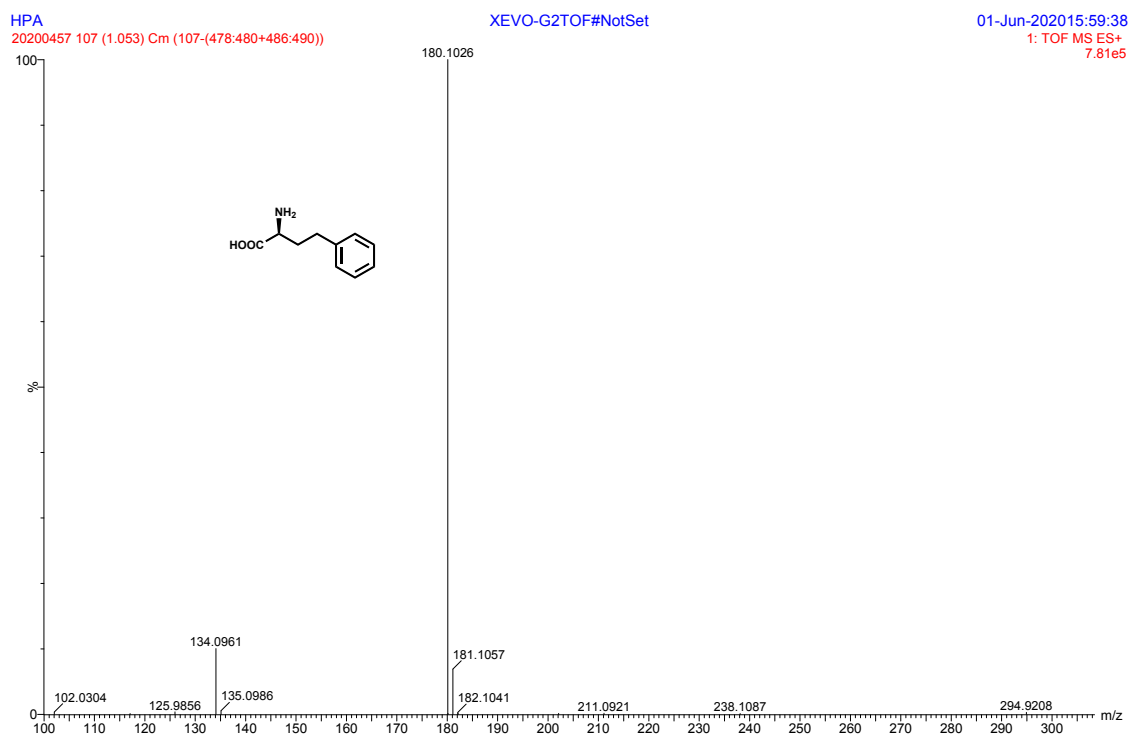
**Figure S14.** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 3(S)-benzyl-L-aspartate.



**Figure S15.** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of L-homophenylalanine.



**Figure S16.** HRMS of chemically synthesized 3(*R,S*)-benzyl-L-aspartate.



**Figure S17.** HRMS of L-homophenylalanine produced by AspBDC catalyzed decarboxylation of 3(*R*)-benzyl-L-aspartate.

#### 4. References

- 1 Mavencamp TL, Rhoderick JF, Bridges RJ, Esslinger CS. *Bioorg. Med. Chem.* 2008, 16: 7740-7748.
- 2 Fulmer, G R, Miller A J M, Sherden N H, Gottlieb H E, Nudelman A, Stoltz B, M.; Bercaw J E, Goldberg K I. *Organometallics* 2010, 29: 2176–2179.
- 3 Lima S, Sundararaju B, Huang C, et al. *J. Mol. Biol.* 2009, 388: 98-108.
- 4 Chen HJ, Ko TP, Lee CY, et al. *Structure* 2009, 17: 517-529.
- 5 Drummond LJ, Sutherland A. *Tetrahedron.* 2010, 66: 5349-5356.