Supporting Information

Structure-guided engineering of *meso*-diaminopimelate dehydrogenase for enantioselective reductive amination of sterically bulky 2-keto acids

Xinkuan Cheng,[a,b] Xi Chen,[a,b] Jinhui Feng,[a,b] Qiaqing Wu,[a,b,*] Dunming Zhu[a,b,*]

[a] University of Chinese Academy of Sciences, No.19(A) Yuquan Road, Shijingshan District, Beijing 100049, PR China

[b] National Engineering Laboratory for Industrial Enzymes and Tianjin Engineering Research Center of Biocatalytic Technology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, 32 Xi Qi Dao, Tianjin Airport Economic Area, Tianjin 300308, PR China.

Email: zhu_dm@tib.cas.cn and wu_qq@tib.cas.cn

Table S1. Identification of potential mutagenic sites by molecular docking of distinct D-amino acids.

D-Amino acids	**Amino acid residues around substrate binding pocket within 4 Å**
D-Phenyglycine	D92, W121, F146, M152, H154, T171, H227
D-Homophenylalanine	D92, W121, F146, M152, H154, T171, H227, N253
D-Tryptophan	D92, W121, F146, M152, H227, N253

Table S2. List of Primers

Protein	**Primers (5'→3')**
D92 Forward	CGTTGACTCTTAC**NNK**ATCCACGGTGACCTG
D92 Reverse	CAGGTCACCGTGGAT**MNN**GTAAGAGTCAACG
W121 Forward	CTCTGCGGGT**NNK**GACCCGGGCAC
W121 Reverse	GTGCCCGGGTC**MNN**ACCCGCAGAG
F146 Forward	CACCTACACCAAC**NNK**GGTCCGGGTATGT
F146 Reverse	ACATACCCGGACC**MNN**GTTGGTGTAGGTG
M152 Forward	TCCGGGTATGTCT**NNK**GGTCACTCTGTTGC
M152 Reverse	GCAACAGAGTGACC**MNN**AGACATACCCGGA
H154 Forward	TCCGGGTATGTCTATGGGT**NNK**TCTGTTGC
H154 Reverse	GCAACAGA**MNN**ACCCATAGACATACCCGGA
T171 Forward	GCTGTCTATG**NNK**ATCCCGGCGGGTAT
T171 Reverse	ATACCCGCCGGGAT**MNN**CATAGACAGC
H227 Forward	GATGGACGTTGGT**NNK**GGTGTTGTTATGG
H227 Reverse	CCATAACAACACC**MNN**ACCAACGTCCATC
N253 Forward	GCGTATCAAC**NNK**CCGGCGCTGAC
N253 Reverse	GTCAGCGCCGG**MNN**GTTGATACGC

Table S3. Kinetic parameters of WT and mutants toward 2-oxo-4-phenylbutyric acid

Enzyme	K_M (mM)	k_{cat} (s^{-1})
WT	15.56±4.41	0.23±0.02
T171H	10.59±2.40	0.50±0.03
H227I	24.47±6.68	2.61±0.29
W121L	3.21±1.69	0.32±0.03

Table S4. Kinetic parameters of mutants toward phenylpyruvic acid

Enzyme	K_M (mM)	k_{cat} (s^{-1})
WT	12.5±2.1 [1]	0.11±0.01 [1]
W121L	10.28±2.74	0.78±0.07
T171H	18.31±4.27	0.59±0.05
H227I	12.00±2.62	4.28±0.29

Table S5 Retention time of the FADD derived L/D-amino acids

Amino acids and retention time (min)	Methods [a]
L-alanine: 7.6 D-alanine: 10.7	0.8 mL/min CH_3CN : H_2O (pH 3.0) = 35:65
L-2-aminobutyrate: 6.9 D-2-aminobutyrate: 9.7	0.8 mL/min CH_3CN : H_2O (pH 3.0) = 35:65
L-norvaline: 9.9 D-norvaline: 16.1	0.8 mL/min CH_3CN : H_2O (pH 3.0) = 35:65
L-valine: 6.7 D-valine: 9.6	0.8 mL/min CH_3CN : H_2O (pH 3.0) = 40:60
L-leucine: 9.6 D-leucine: 15.0	0.8 mL/min CH_3CN : H_2O (pH 3.0) = 40:60
L-*tert*-leucine: 9.3 D-*tert*-leucine: 14.5	0.8 mL/min CH_3CN : H_2O (pH 3.0) = 40:60
L-tryptophan: 38.9 D-tryptophan: 59.4	0.8 mL/min CH_3CN : H_2O (pH 3.0) = 30:70
L-homophenylalanine: 12.2 D-homophenylalanie: 20.1	0.8 mL/min CH_3CN : H_2O (pH 3.0) = 40:60
L-phenylglycine: 25.7 D-phenylglycine: 7.9	0.5 mL/min H_2O (perchloric acid 1.5) [b]
L-phenylalanine: 14.4 D-phenylalanine: 11.0	0.5 mL/min H_2O (perchloric acid 1.5) [b]

[a] Standard FDAA derived separation condition.

[b] Performed on CROWNPAK CR(+) column (Daicel Chemical Industries, Japan).

Figure S1. SDS-PAGE analysis of purified WT and mutant StDAPDH. Lane M, protein marker; 1, wild-type; 2, mutant W121L; 3, mutant T171H; 4, mutant H227I; 5, mutant W121L/H227I.

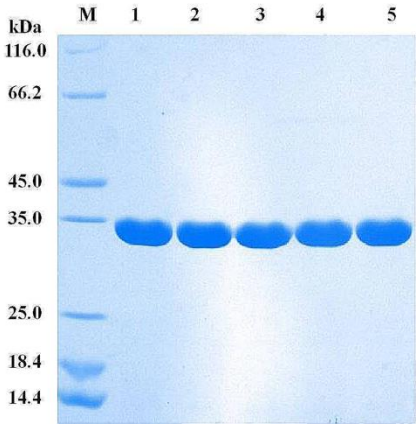
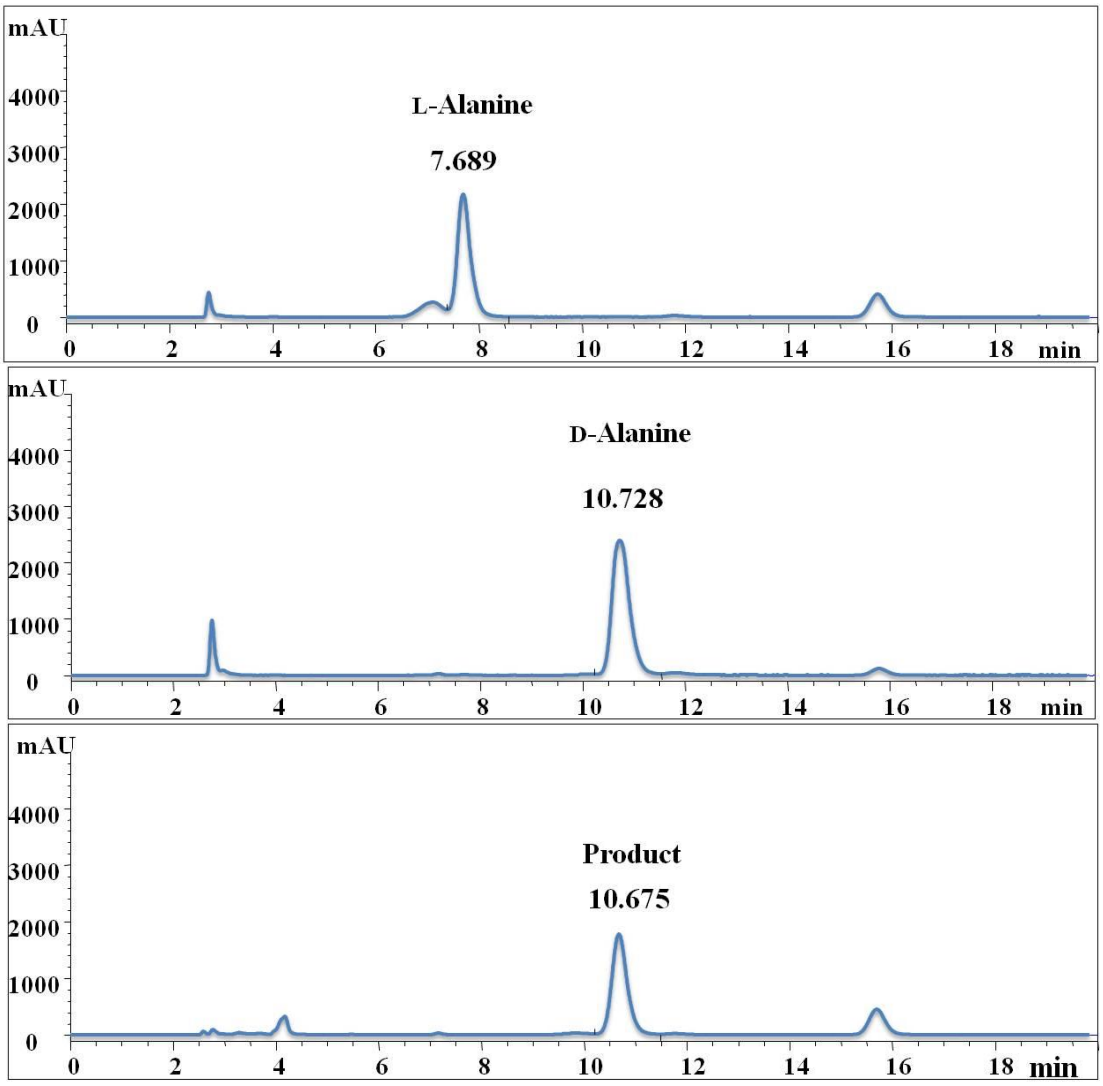
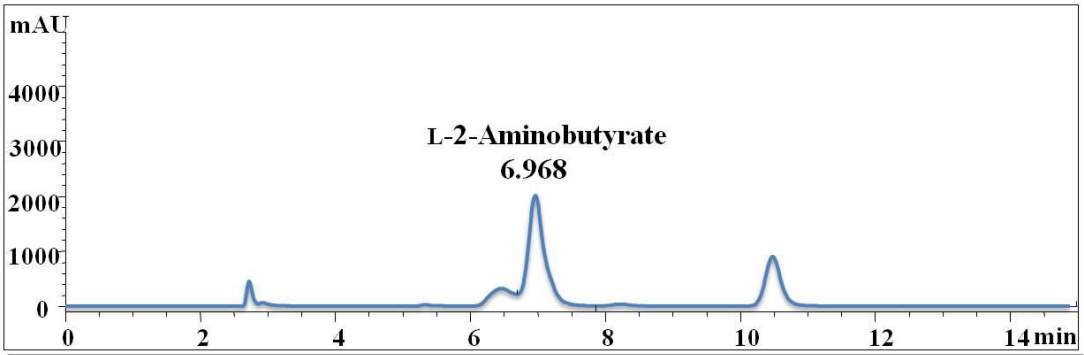


Figure S2. Chiral HPLC analysis of the products for the reductive amination of 2-keto acids.

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8
10
12
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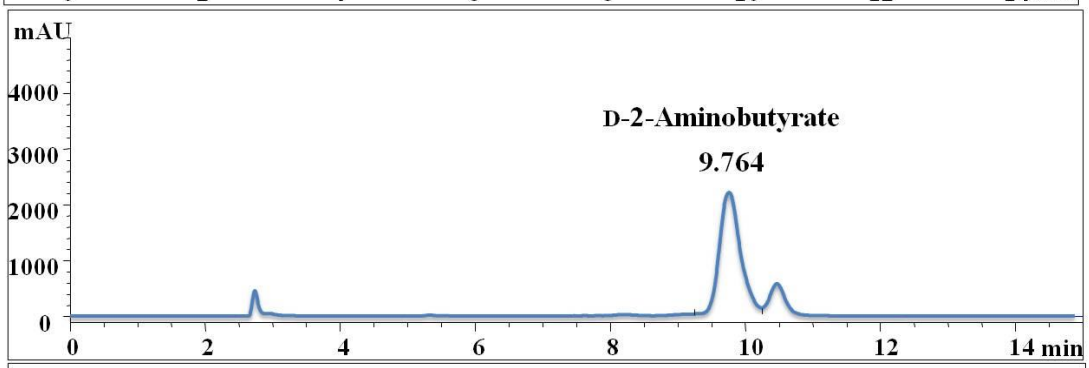
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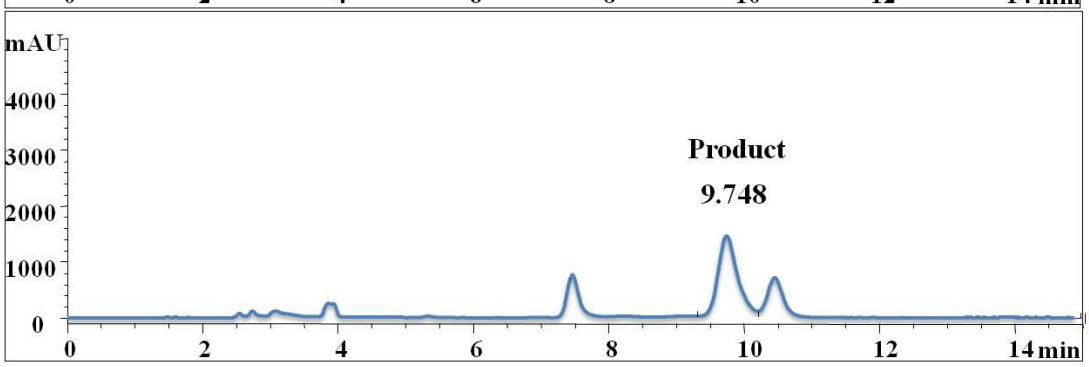
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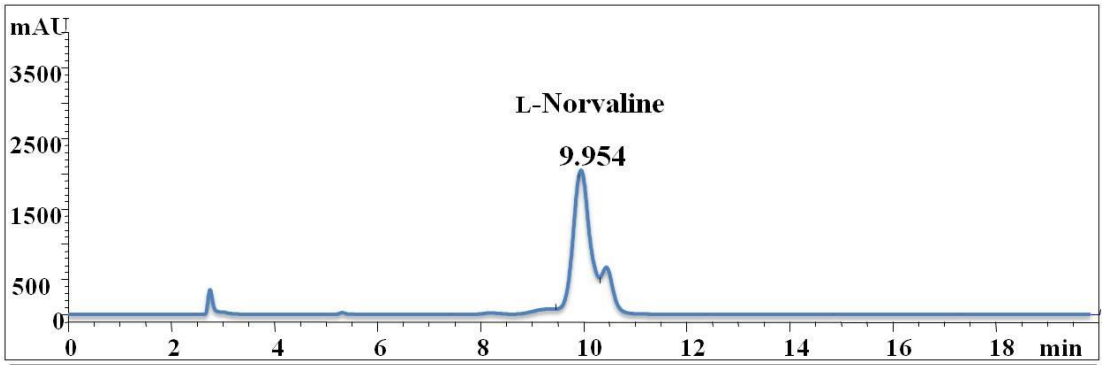
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18
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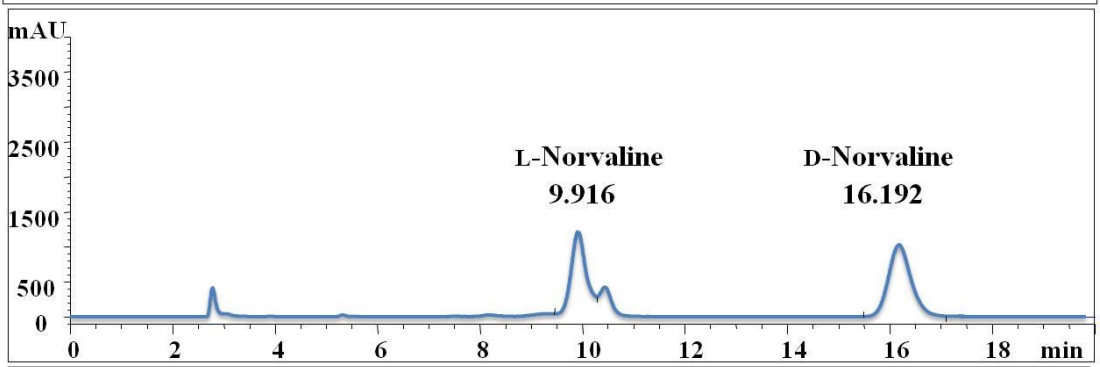
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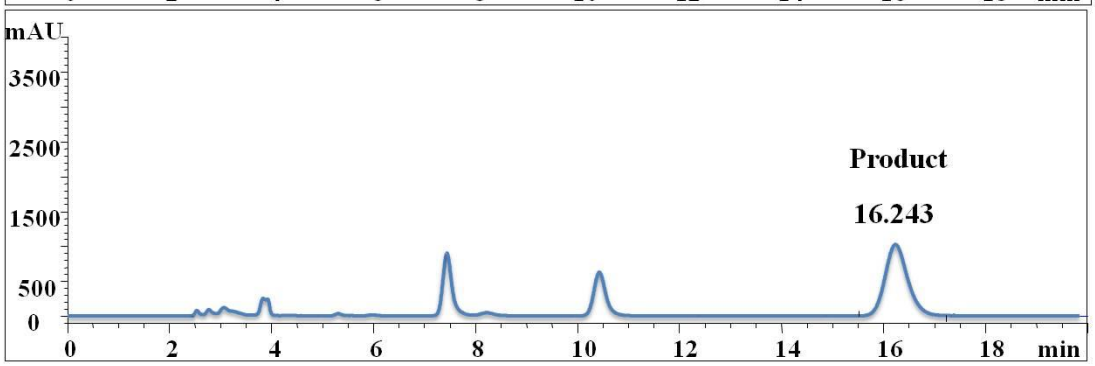
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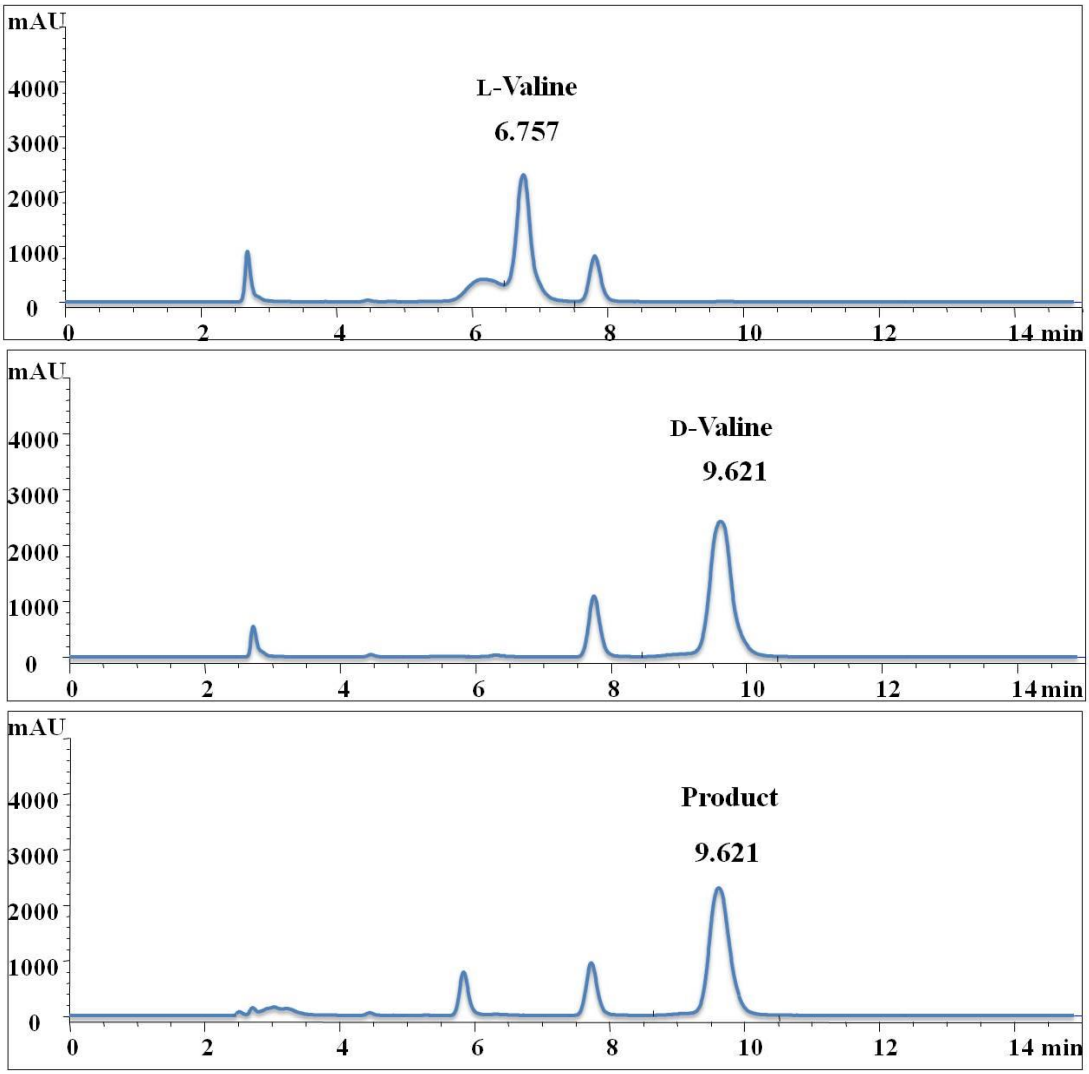
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4
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8
10
12
14 min
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4000
3000
2000
1000
0
D-Valine
9.621
0
2
4
6
8
10
12
14 min
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2000
1000
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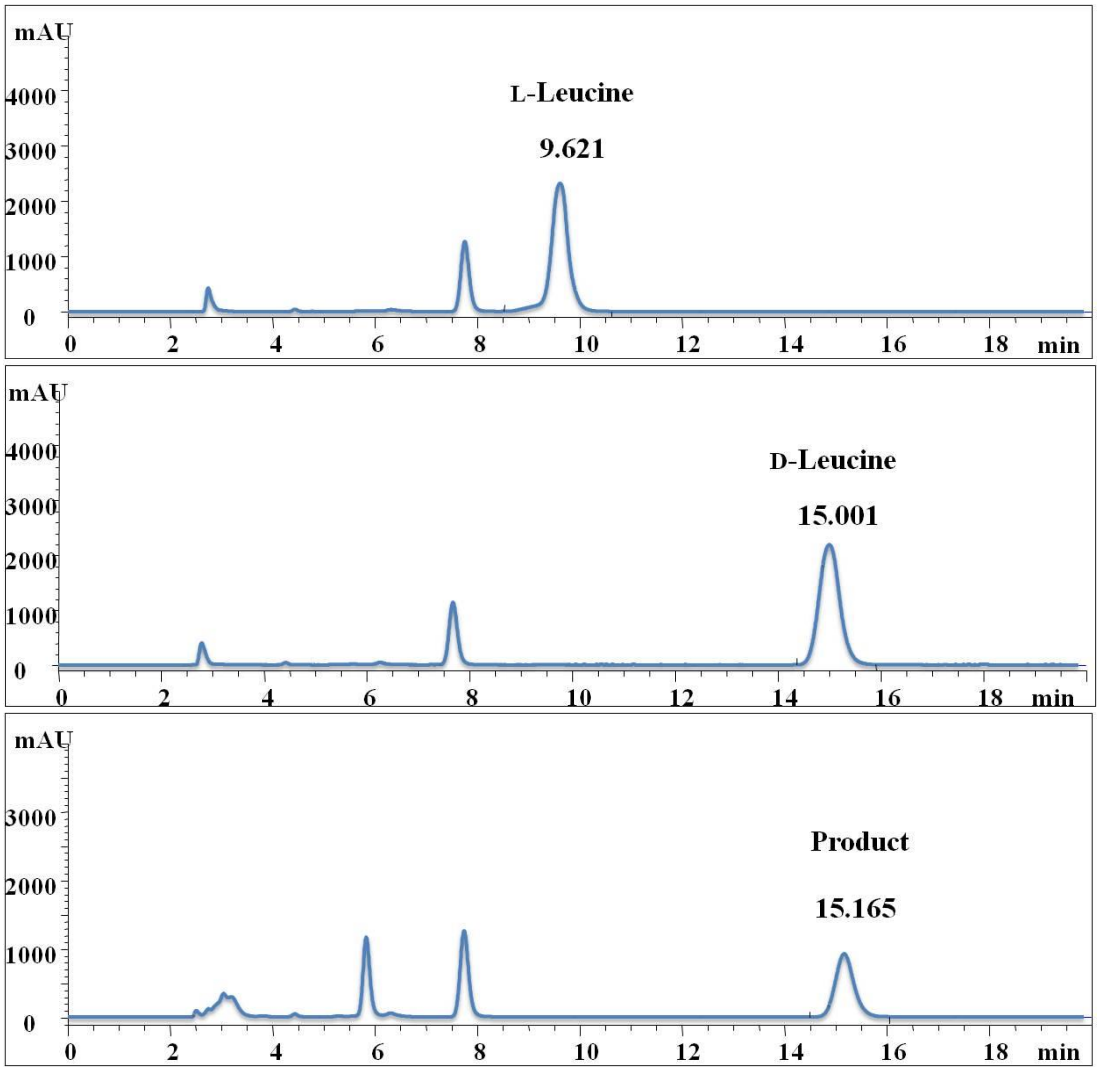
2
4
6
8
10
12
14 min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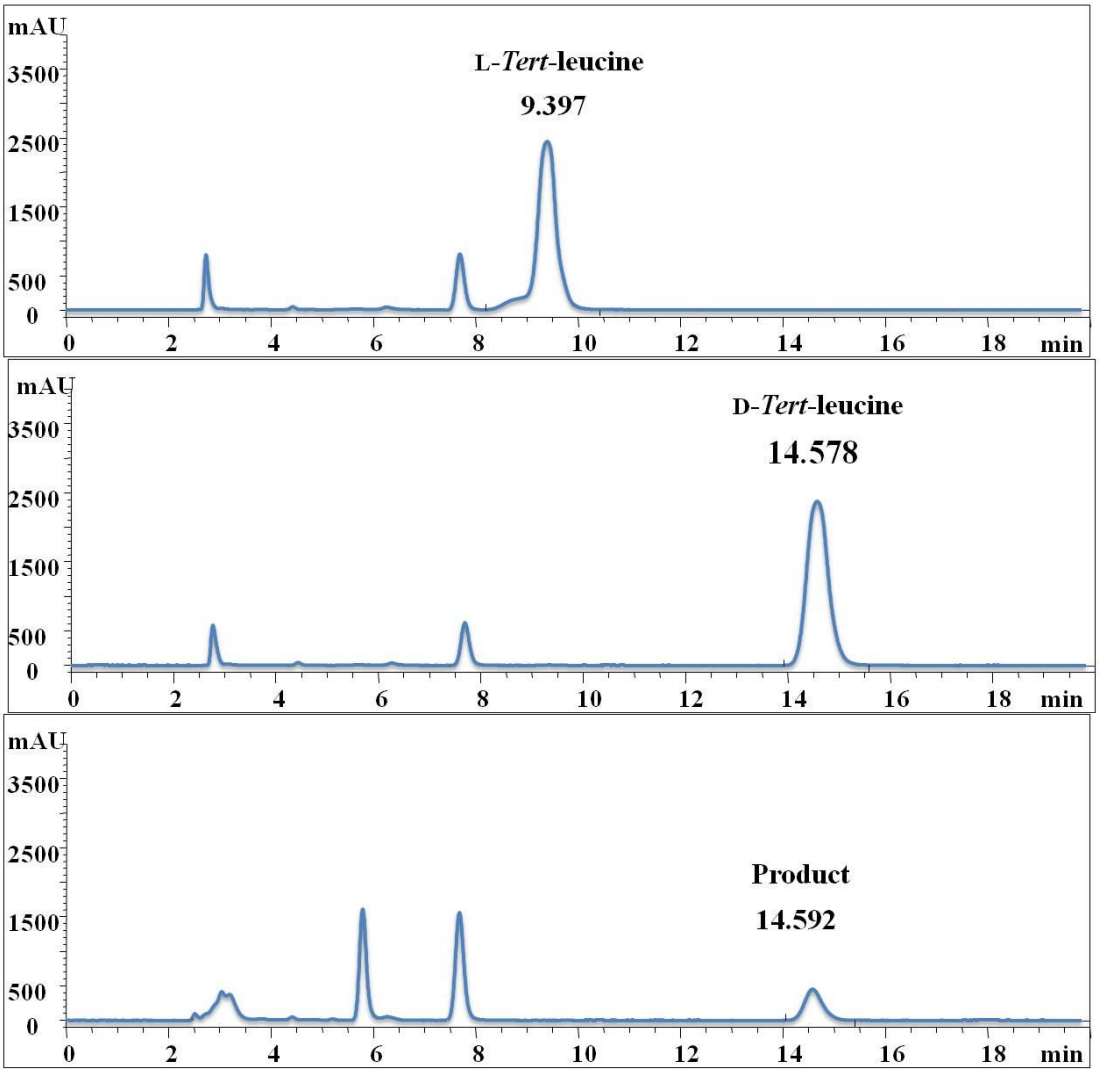
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500
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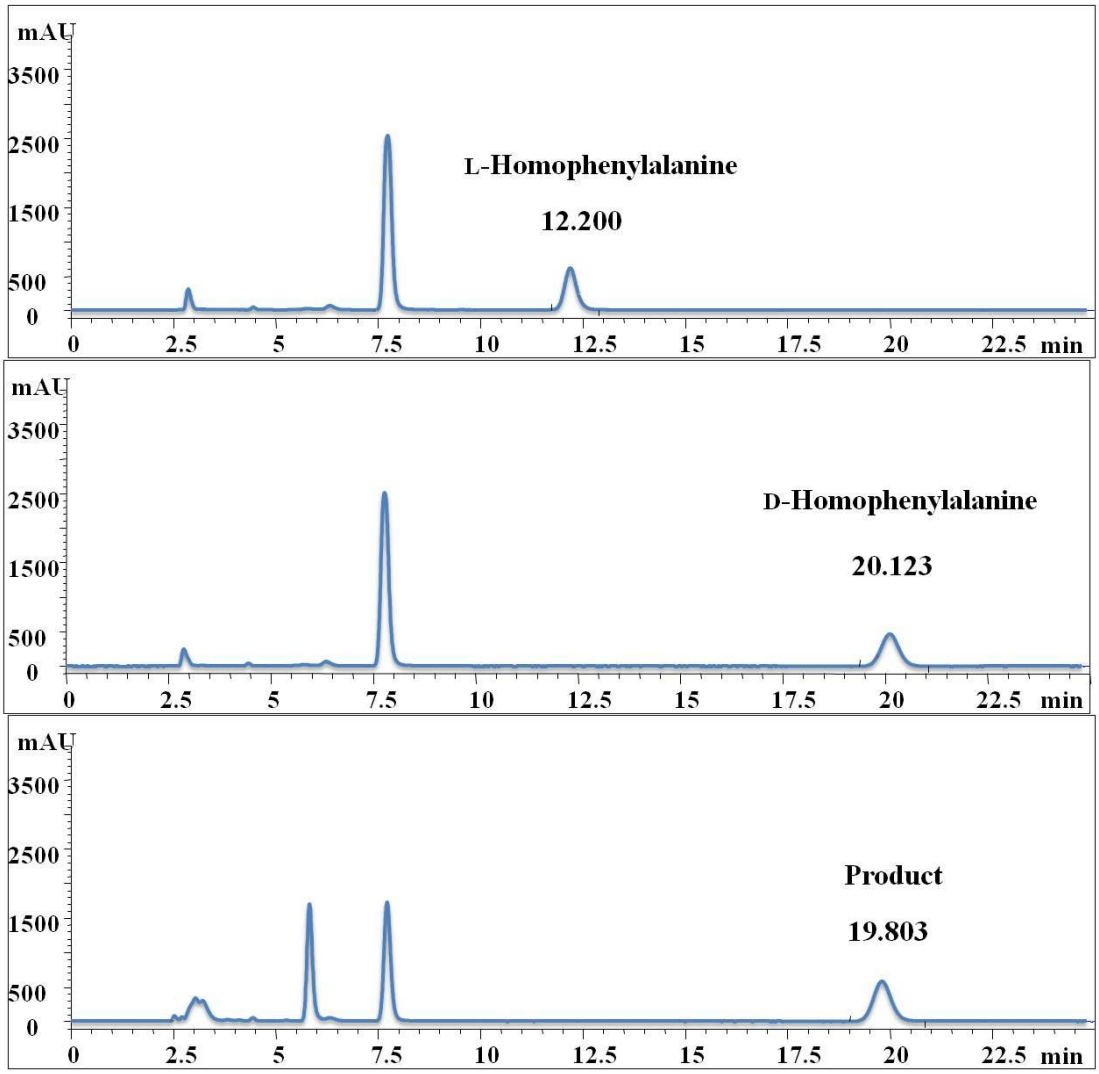
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10
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17.5
20
22.5
min
mAU
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2500
1500
500
0
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5
7.5
10
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17.5
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22.5
min
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17.5
20
22.5
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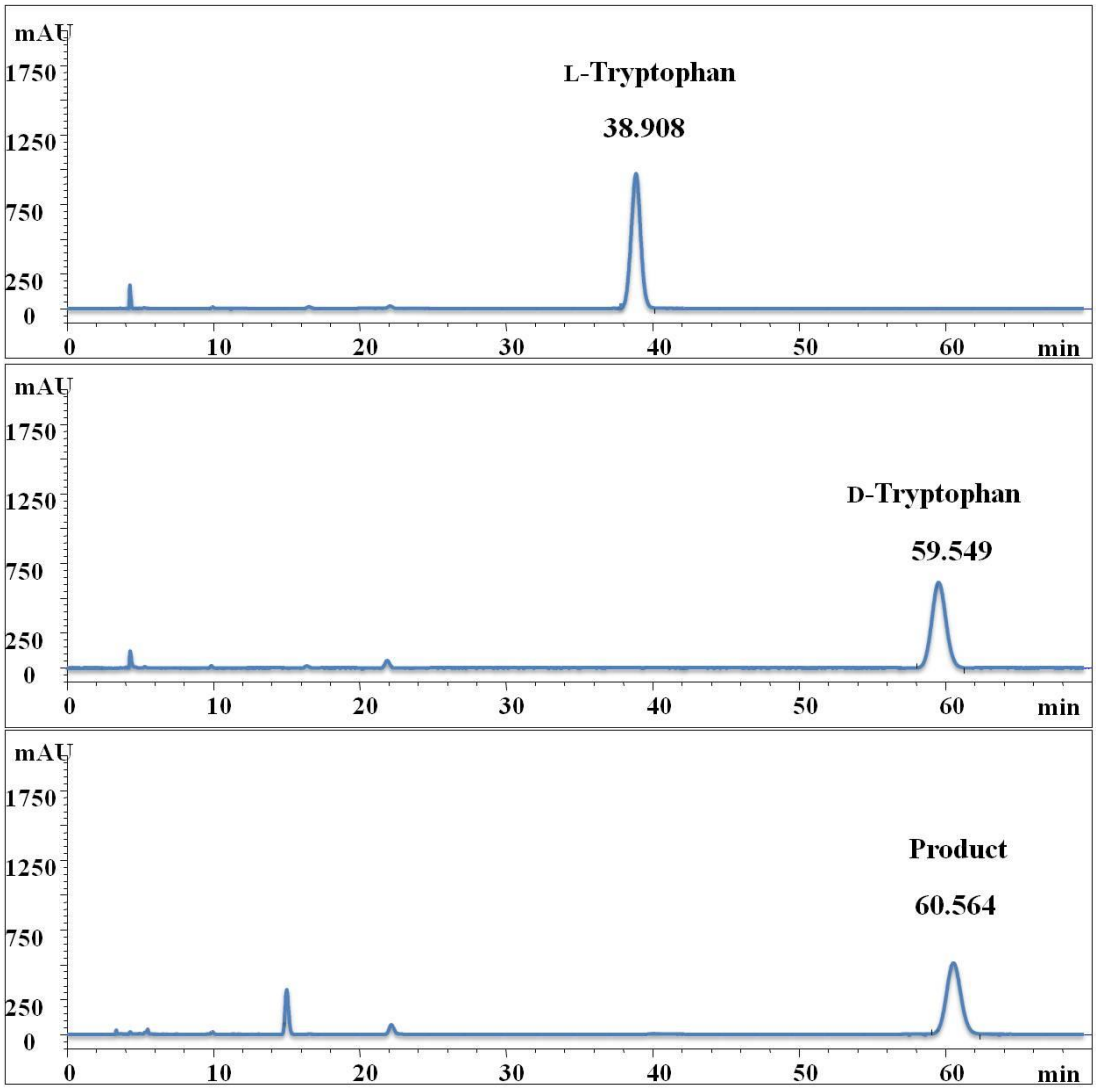
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10
20
30
40
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40
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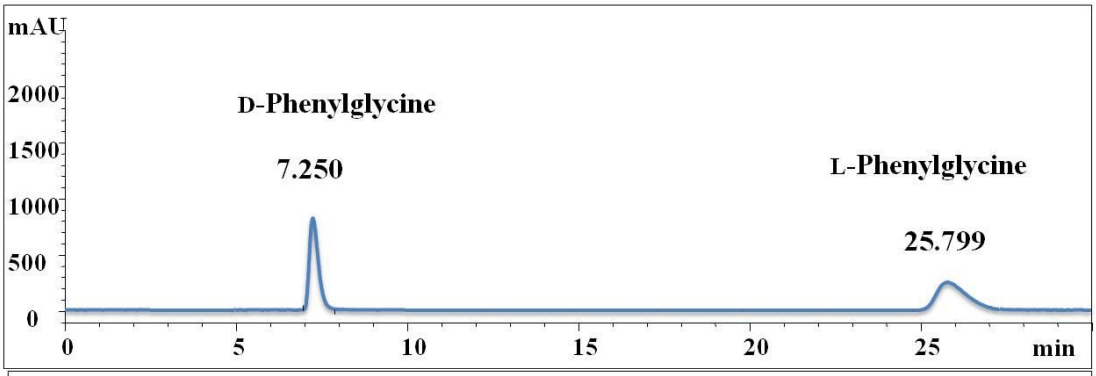
60
min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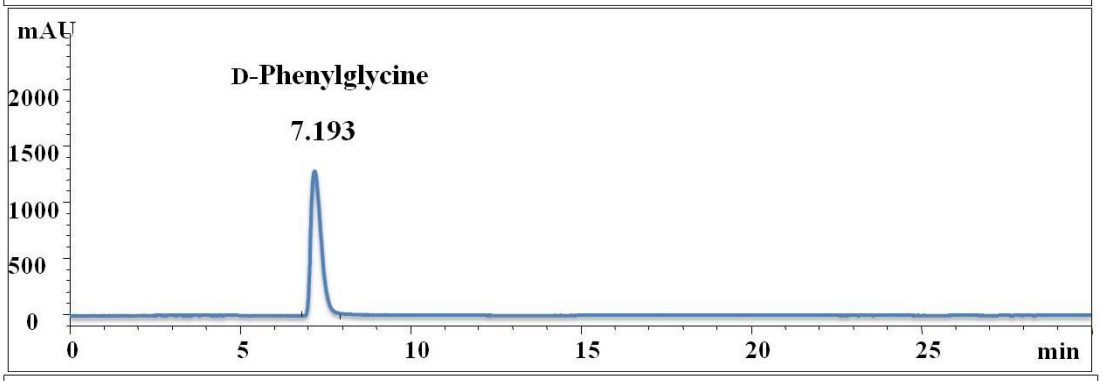
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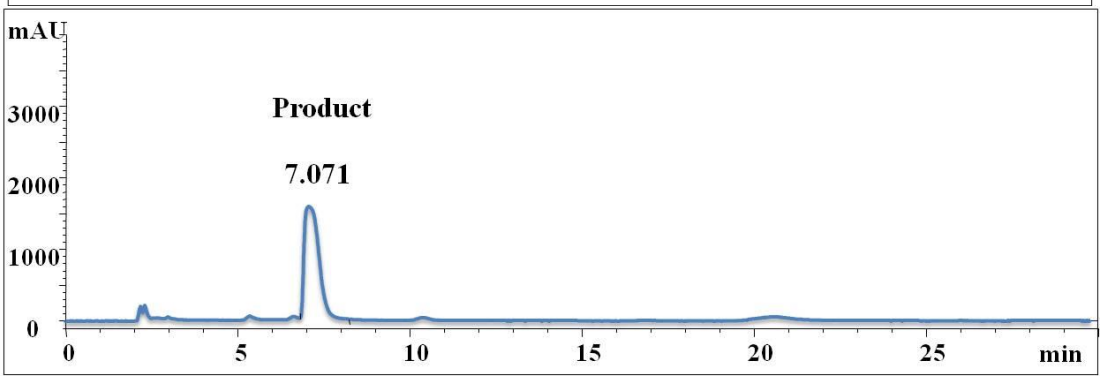
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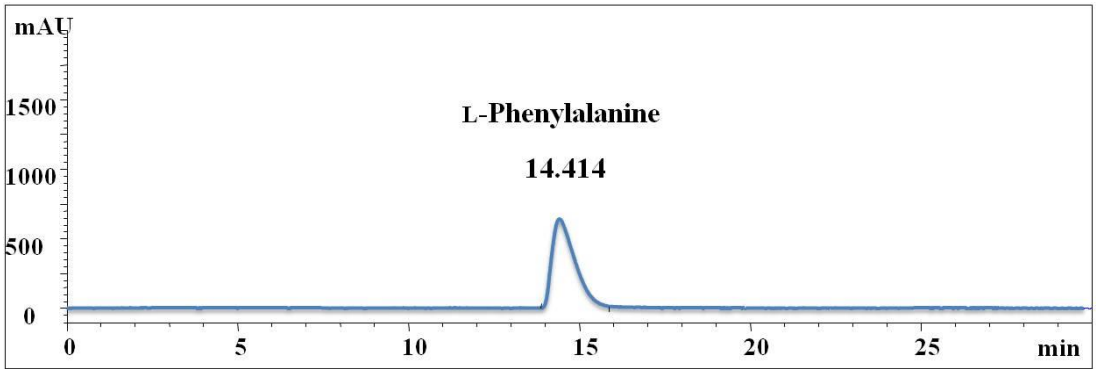
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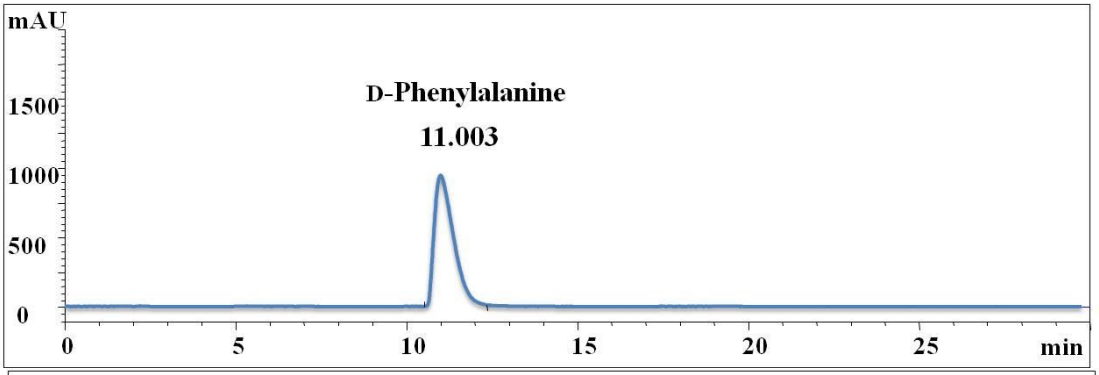
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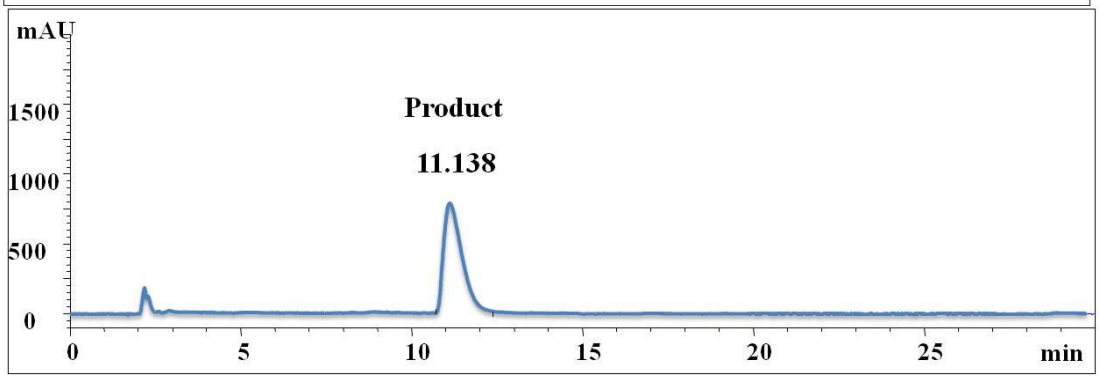
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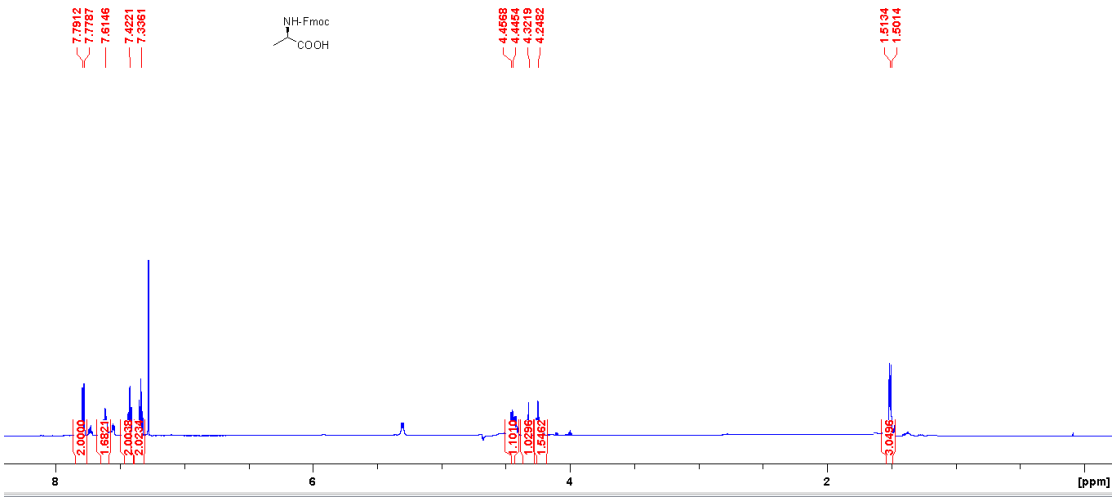
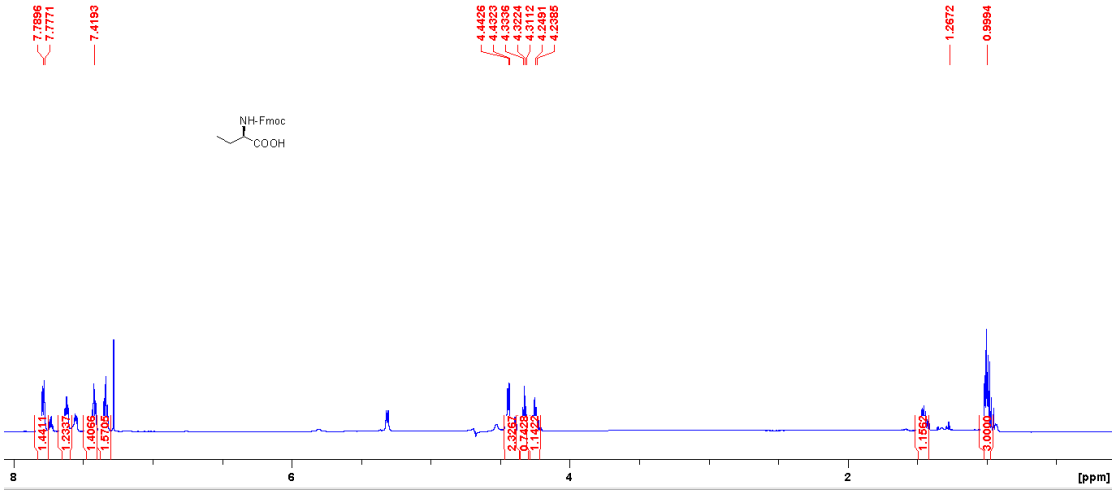
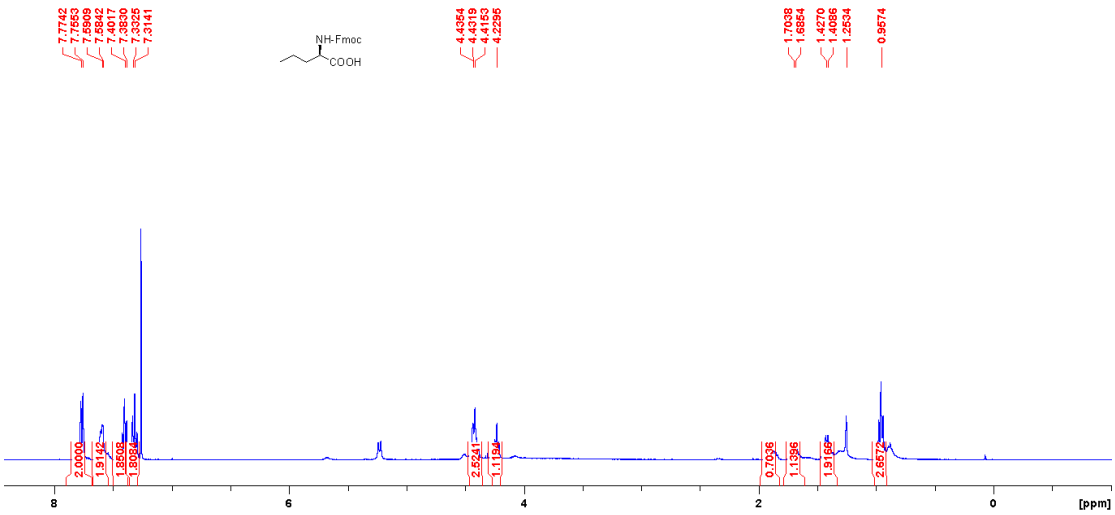
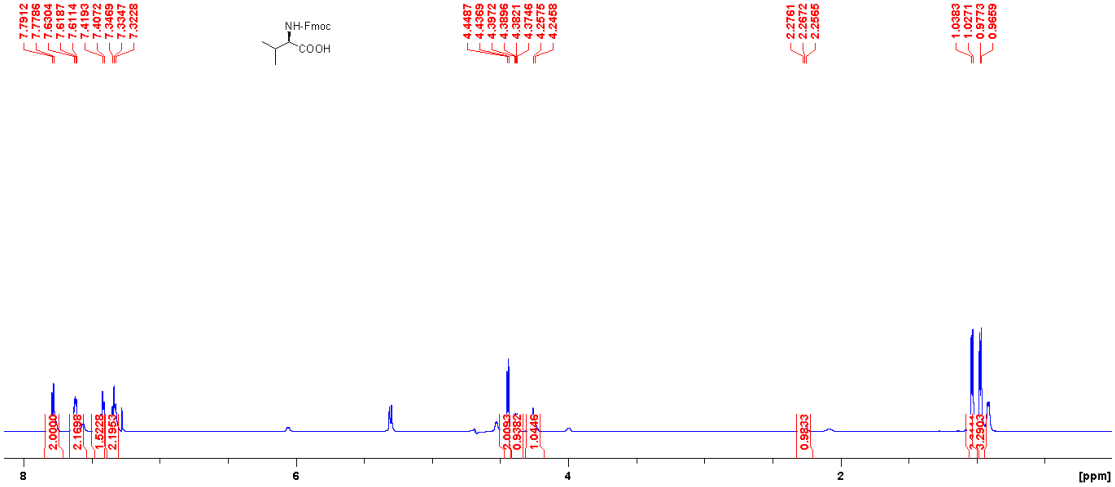
Figure S3. NMR spectra of D-amino acid derivatives





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NH-Fmoc
COOH
4.4487
4.4369
4.3972
4.3896
4.3821
4.3746
4.2575
4.2468
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2.2672
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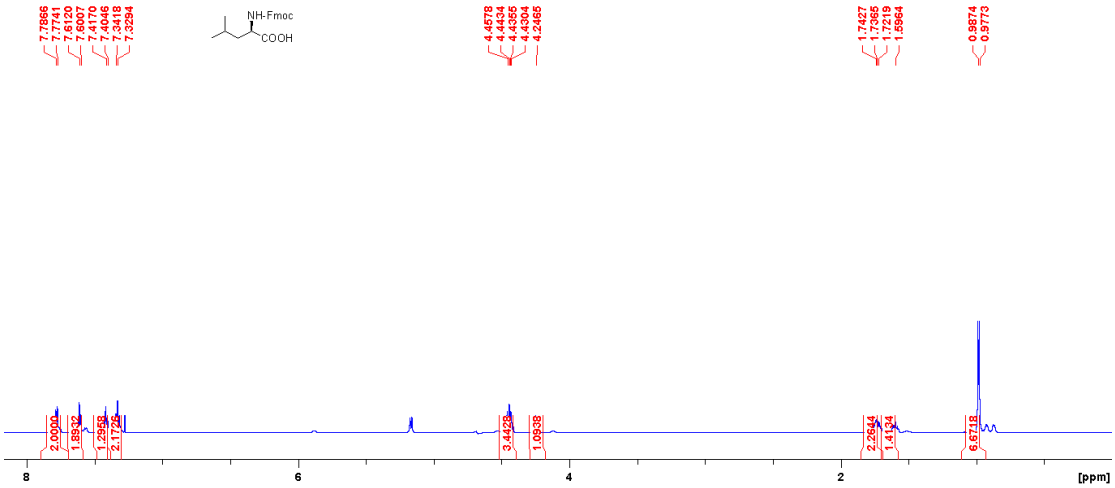
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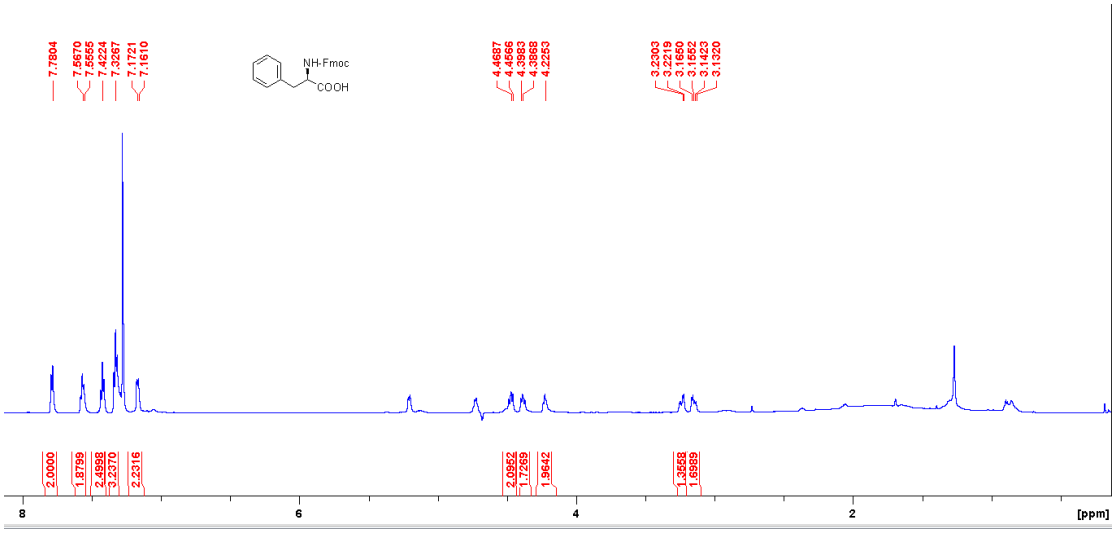
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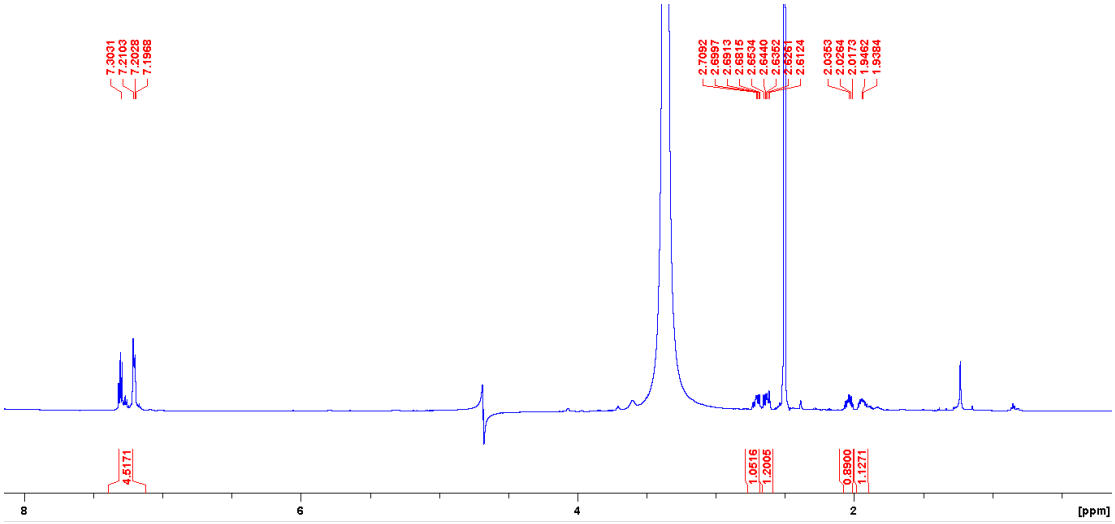

Reference

1. X. Gao, F. Huang, J. Feng, X. Chen, H. Zhang, Z. Wang, Q. Wu and D. Zhu, *Appl. Environ. Microbiol.*, 2013, **79**, 5078-5081.