Supporting Information

Efficient access to L-phenylglycine using newly identified amino acid dehydrogenase from *Bacillus clausii*

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Table S1 Primers used in this study.

Name	Source microorganism	Identity /%	Primers
*Bc*AADH	*Bacillus clausii*	50.6	F: CGCGGATCCATGGAATTATTTGCAAAGA
			R: CCGCTCGAGTTACTTTTTCCTCGAA
*Xc*AADH	*Xanthomonas campestris*	51.0	F: CGCGGATCCATGCTTTTCGAAACCCTCGAT
			R: CCGCTCGAGTTACTCGCCGCGCAACTTGT
*Bp*AADH	*Bacillus sphaericus*	51.5	F:CGCGGATCCATGGAAAAGTATGATTATGAAC
			R: CCGCTCGAGTTAAGAACTGACTACGCGATTTCG
*Bs*AADH	*Bacillus subtilis*	50.9	F: CGCGGATCCATGGAAATTTTTAAATATAT
			R:CCGCTCGAGCTATCGTCTGCTTAATACACTT

Table S2 Screening result of four potential amino acid dehydrogenases.

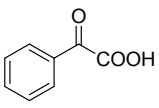
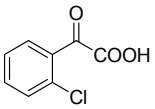
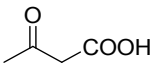
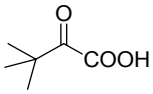
AADH				
	[U/mg]	[U/mg]	[U/mg]	[U/mg]
*Bc*AADH	0.461	0.400	0.898	0.159
*Xc*AADH	0.452	0.304	2.965	0.1261
*Bp*AADH	0.494	0.193	0.491	0.037
*Bs*AADH	0.375	0.453	1.086	0.253

Table S3 Effect of metal ions on the activity of purified *Bc*AADH

Entry	Metal ions [1 mM]	Relative activity [%]
1	Control	100±1.5
2	Co^{2+}	113±0.7
3	Mg^{2+}	108±2.0
4	Ni^{2+}	105±1.6
5	Cu^{2+}	97.2±0.7
6	Zn^{2+}	96.0±0.9
7	Mn^{2+}	80.1±0.1
8	Ba^{2+}	74.0±0.8
9	Ca^{2+}	68.4±0.4
10	Al^{3+}	41.3±2.1
11	Fe^{3+}	36.4±2.0
12	Li^{+}	27.5±1.6
13	Ag^{+}	0±0
14	EDTA	101±1.2

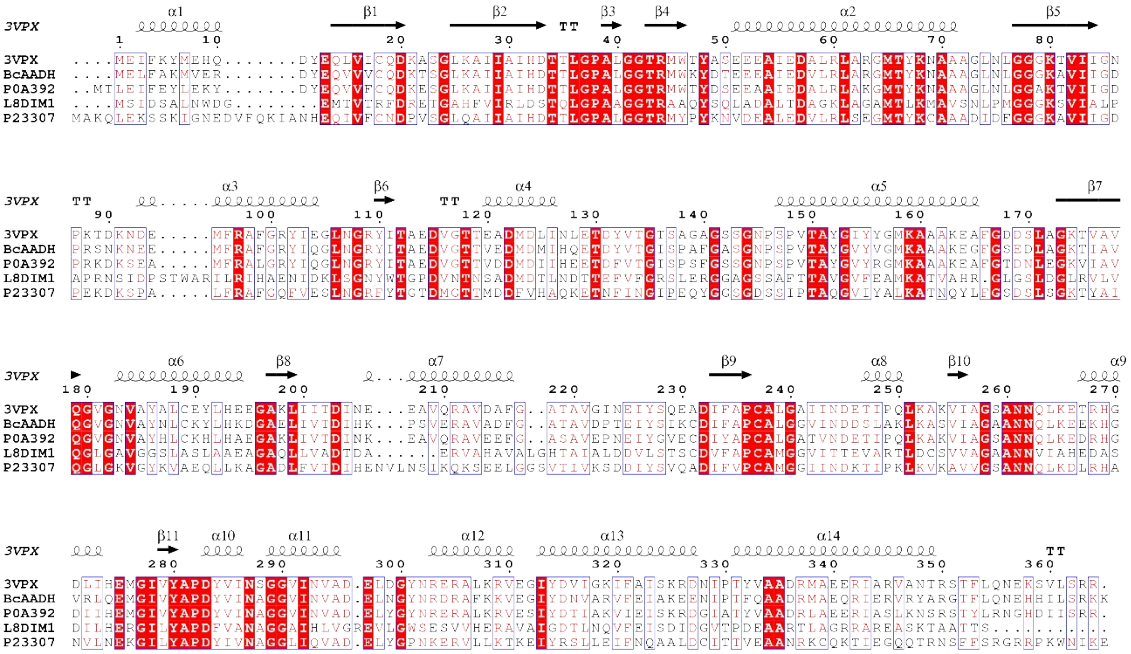
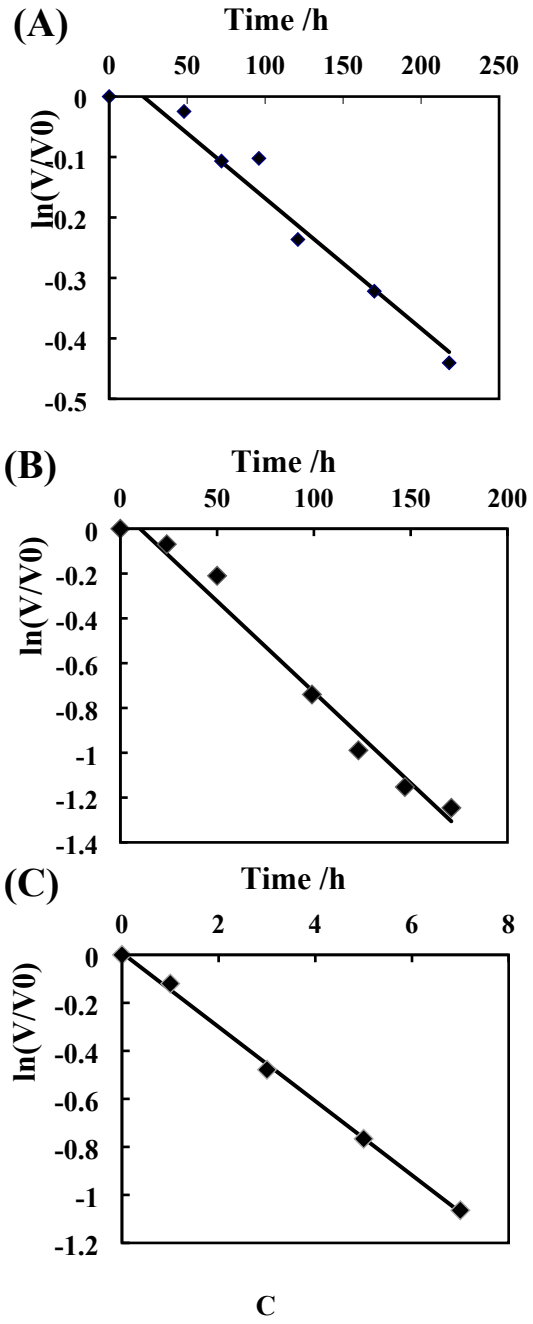


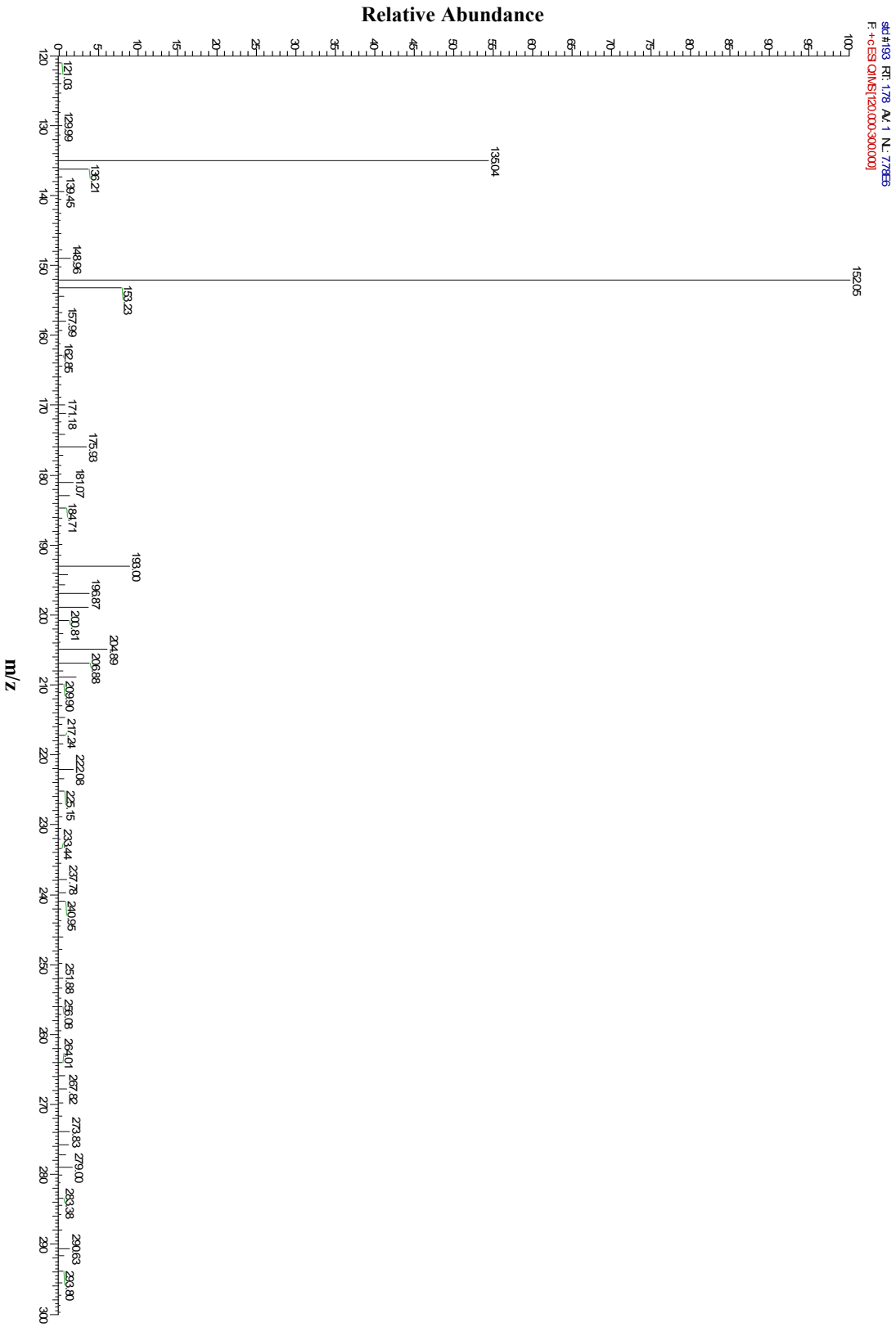
Figure S1 Sequence alignment of *Bc*AADH and similar amino acid dehydrogenase. **3VPX**, AADH from *Sporosarcina psychrophila*; **P0A392**, AADH from *Bacillus cereus*; **L8DIM1**, phenylalanine dehydrogenase from *Rhodococcus* sp.; **P23307**, phenylalanine dehydrogenase from *Lysinibacillus sphaericus*.



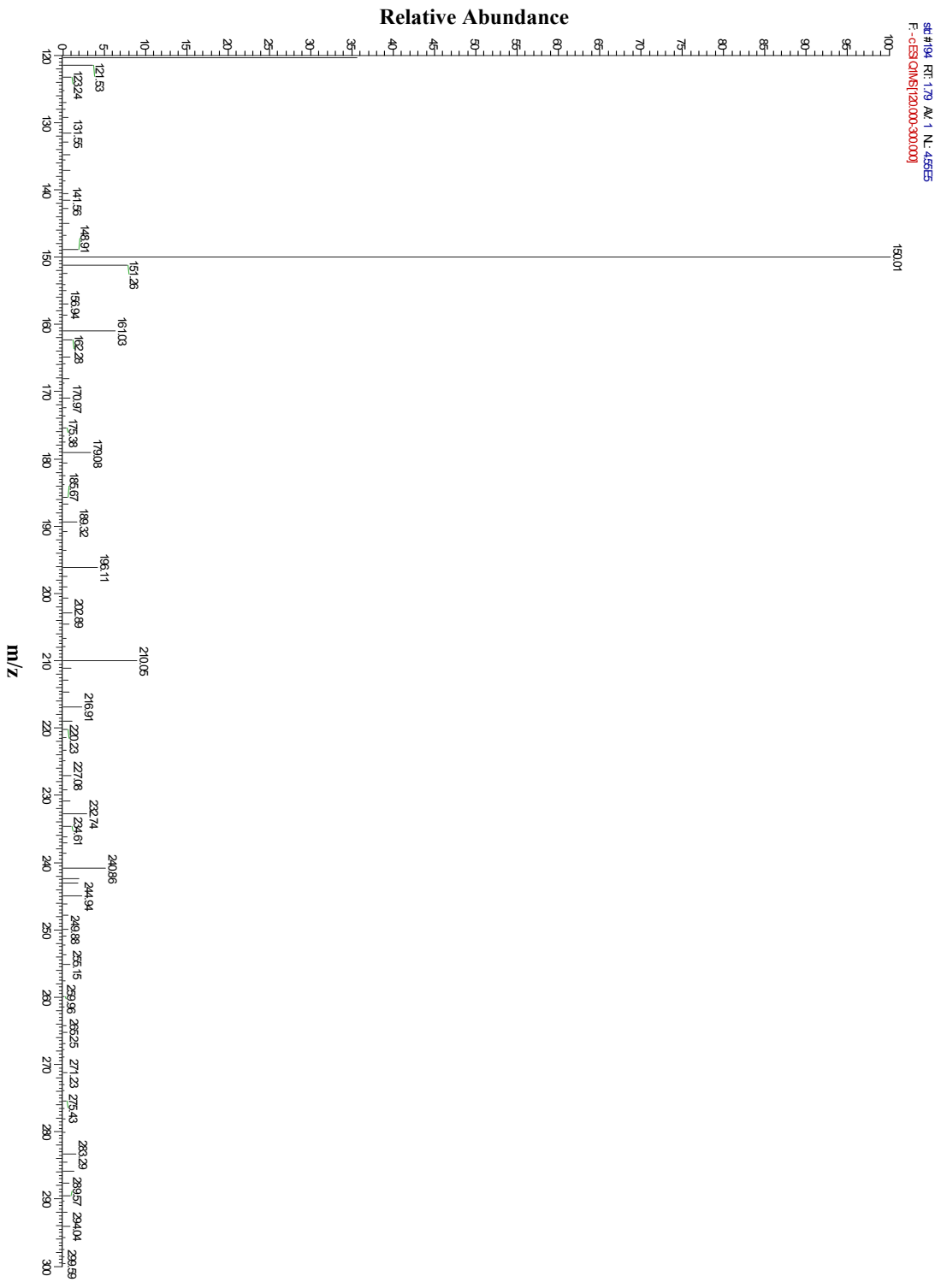
C

Figure S2 Thermostability of *Bc*AADH. Purified enzyme was incubated at various temperatures, 30 °C (A), 40 °C (B) and 50 °C (C).

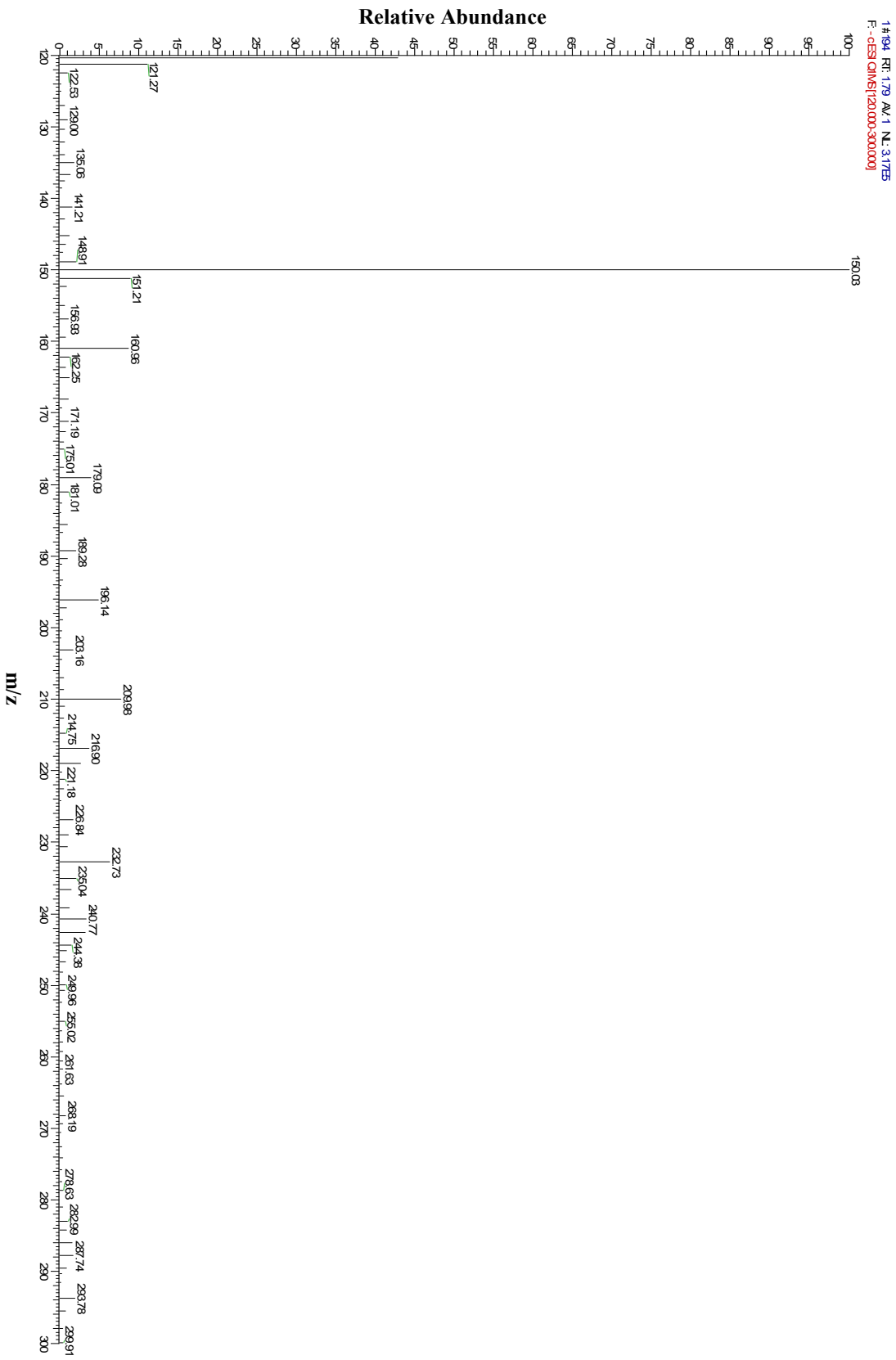
(A1)


Relative Abundance
std #193 RT: 1.78 AV: 1 NL: 7.78E6
F: + c ESI CIMS[120.000-300.000]
135.04
152.05
193.00
m/z

(A2)


Relative Abundance
m/z
scan #194 RT: 1.79 AV: 1 NL: 4.55E5
F: - c ESI Q1MS [120.000-300.000]
150.01
121.53
123.24
131.55
141.56
148.91
151.26
155.94
161.03
162.28
170.97
175.38
179.08
185.67
189.32
196.11
202.89
210.05
216.91
220.23
227.08
232.74
234.61
240.86
244.94
249.88
255.15
259.96
265.25
271.23
275.43
283.29
289.57
294.04
299.59

(B1)


Relative Abundance
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F: - cESI CIMS[120.000-300.000]
m/z
121.27
150.03
122.53
129.00
135.06
141.21
148.91
151.21
156.93
160.96
162.25
171.19
175.01
179.09
181.01
189.28
196.14
203.16
209.98
214.75
216.90
221.18
226.84
232.73
235.04
240.77
244.38
249.96
255.02
261.63
268.19
278.63
282.99
287.74
293.78
299.91

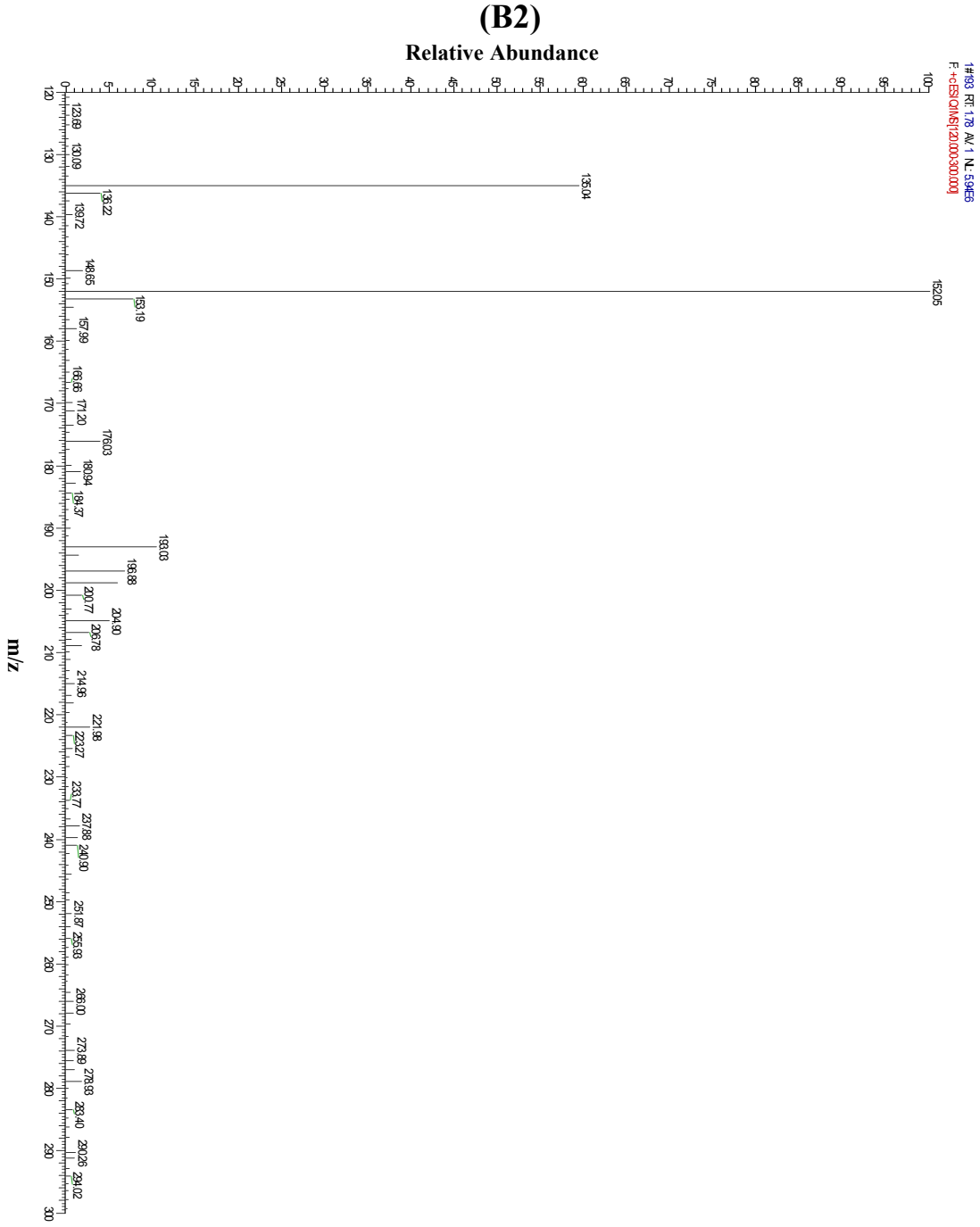

Fig. S3 Mass spectra of standard and synthesized L-phenylglycine by *Bc*AADH. A1 & A2: positive and negative modes of standard L-phenylglycine; B1 & B2: positive and negative modes of synthesized L-phenylglycine.

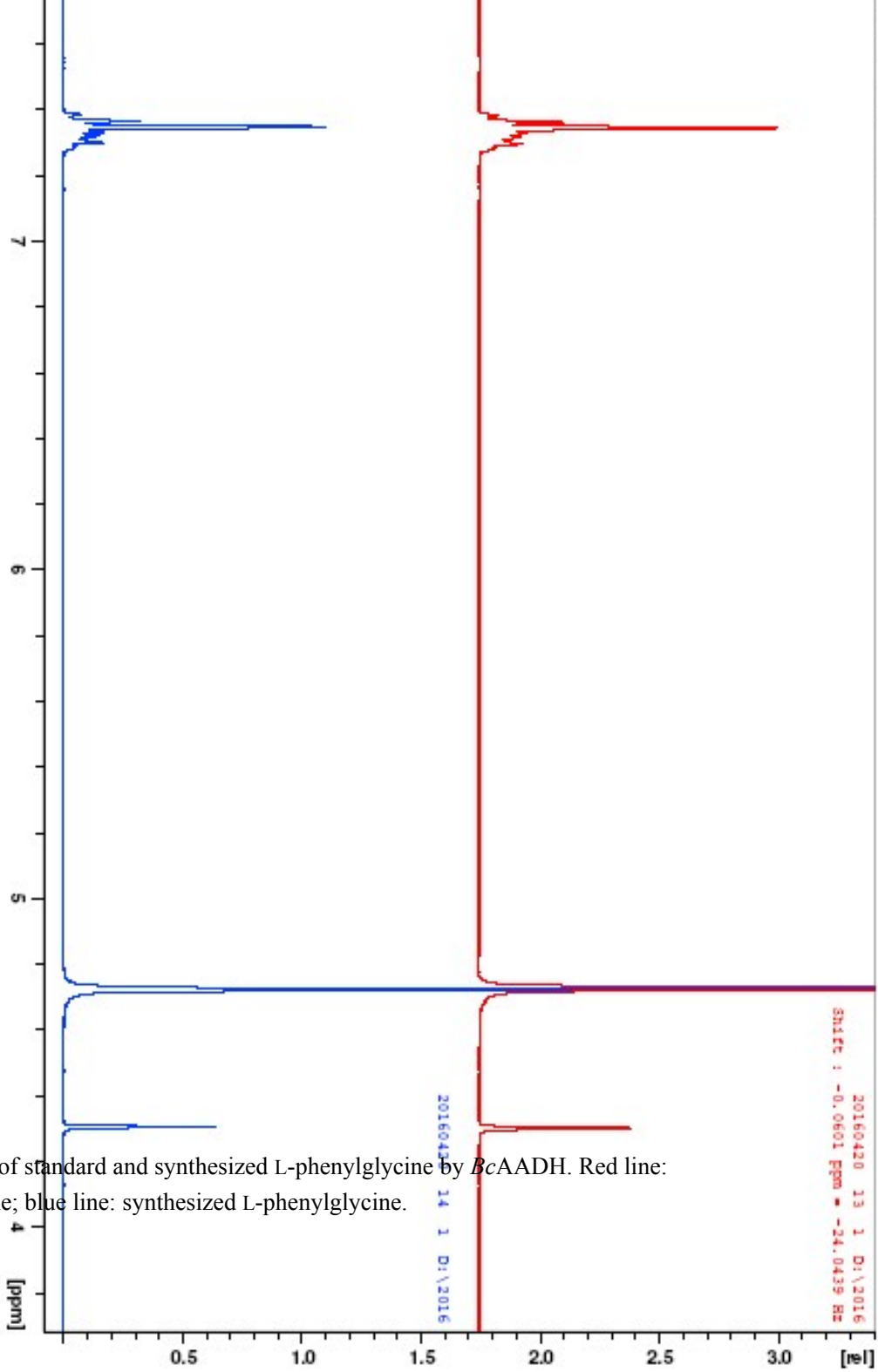

Fig. S4 ^{1}H-NMR spectra of standard and synthesized L-phenylglycine by *Bc*AADH. Red line: standard L-phenylglycine; blue line: synthesized L-phenylglycine.

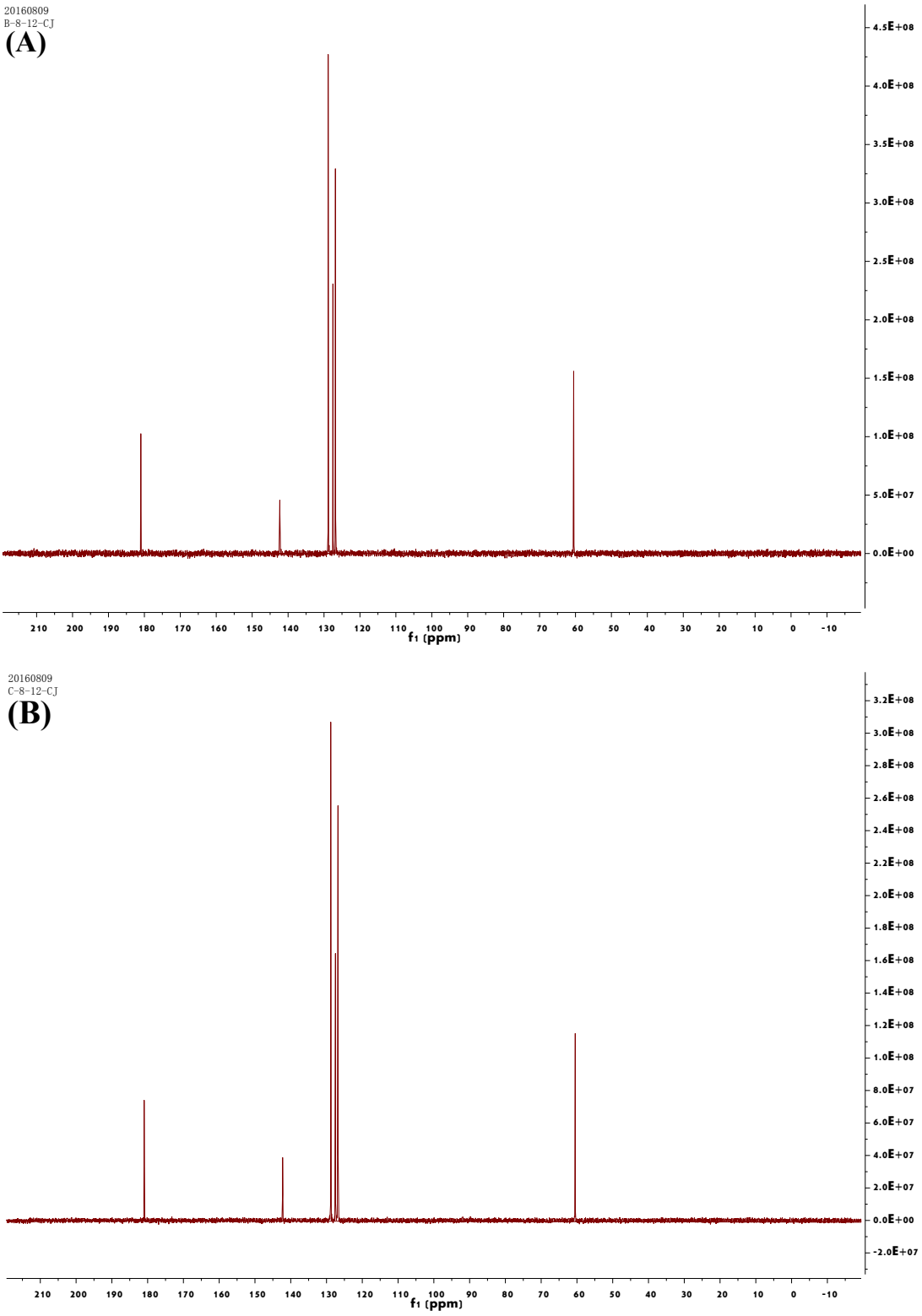

Fig. S5 ^{13}C-NMR spectra of standard and synthesized L-phenylglycine by *Bc*AADH. (A): standard L-phenylglycine; (B): synthesized L-phenylglycine.