Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION

Biocatalytic Enantioselective Hydroaminations Enabling Synthesis of *N*-Arylalkyl-Substituted L-Aspartic Acids

Mohammad Z. Abidin[1,2], Thangavelu Saravanan[*1,3], Laura Bothof[1], Pieter G. Tepper[1],
Andy-Mark W.H. Thunnissen[4], and Gerrit J. Poelarends[*1]

[1]Department of Chemical and Pharmaceutical Biology, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands.

[2]Department of Animal Product Technology, Faculty of Animal Science, Gadjah Mada University, Bulaksumur, Yogyakarta, 55281, Indonesia.

[3]School of Chemistry, University of Hyderabad, P.O. Central University, Hyderabad-500046, India. E-mail: tsaravanan@uohyd.ac.in

[4]Molecular Enzymology Group, Groningen Institute of Biomolecular Sciences and Biotechnology, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands.

*Address correspondence to Prof. Dr. Gerrit J. Poelarends. Tel: +31-50-3633354; Fax: +31-50-3633000; E-mail: g.j.poelarends@rug.nl.

Table of contents

1. General information
2. Detailed experimental procedures
    - 2.1 Analytical scale synthesis
    - 2.2 Conversion analysis by ^{1}H NMR
    - 2.3 Enzymatic synthesis of *N*-arylalkyl-substituted L-aspartic acids
    - 2.4 Chemical synthesis of *N*-arylalkyl-substituted D- and L-aspartic acids
3. NMR spectra
    - 3.1 ^{1}H and ^{13}C NMR spectra of enzymatically obtained *N*-arylalkyl-substituted *L*-aspartic acids
    - 3.2 ^{1}H and ^{13}C NMR spectra of chemically obtained *N*-arylalkyl-substituted D- and L-aspartic acids
4. LC-HRMS analysis
5. Chiral HPLC analysis
6. Additional figures
7. Supplementary references

1. General information

2-Phenethylamine (**2a**), 4-phenylbutylamine (**2b**), 4-fluorobenzylamine (**2c**), 4-chlorobenzylamine (**2d**), 4-nitrobenzylamine (**2e**), *p*-xylylenediamine (**2f**), 4-methylphenethylamine (**2g**), 4-hydroxyphenethylamine (**2h**), 4-fluorophenethylamine (**2j**), 3-fluorophenethylamine (**2k)**, 2-fluorophenethylamine (**2l**), 4-chlorophenethylamine (**2m**), *rac*-β-methylphenethylamine (**2n**), *rac*-2-amino-1-phenylethanol (**2o**), tryptamine (**2p**), *N*-methyl-phenethylamine (**2q**), 3-methylbenzylamine (**2r**), 3-chlorobenzylamine (**2s**), 3-methylphenethylamine (**2t**), and 3-chlorophenethylamine (**2u**) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The compound 3-hydroxyphenethylamine (**2i**) was purchased from AURUM Pharmatech (USA). Solvents were purchased from Biosolve (Valkenswaard, The Netherlands) or Sigma-Aldrich Chemical Co. Ingredients for buffers and media were obtained from Duchefa Biochemie (Haarlem, The Netherlands) or Merck (Darmstadt, Germany). Dowex® 50W X8 resin (100-200 mesh) was purchased from Sigma-Aldrich Chemical Co. Ni sepharose 6 fast flow resin and HiLoad® 16/600 Superdex® 200 pg prep grade column were purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden).

Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions on precast gels (NuPAGE™ 12% Bis-Tris protein gels). The gels were stained with Instant Blue. NMR analysis was performed on a Brucker 500 MHz NMR machine at the Drug Design Laboratory of the University of Groningen. Chemical shifts (δ) are reported in parts per million (ppm). Electrospray ionization orbitrap high-resolution mass spectrometry (HRMS) was performed by the Mass Spectrometry core facility of the University of Groningen.

For calculation of the specific activity, one unit (nmol/min) was defined as the amount of biocatalyst required for the hydroamination of 1 nmol fumaric acid per minute (50 mM amine and 10 mM fumarate in 20 mM NaH_2PO_4-NaOH buffer, pH 8.5).

2. Detailed experimental procedures

The EDDS lyase enzyme was overexpressed and purified to homogeneity by following previously described protocols.[1,2]

2.1 Analytical scale synthesis

The initial reaction mixture (2.5 mL) consisted of fumarate (sodium salt, 10 mM) and an amine or amino acid (**2a**-**u**; 50 mM) in NaH_2PO_4-NaOH buffer (50 mM; pH 8.5) and the pH of the reaction mixture was adjusted to pH 8.5. The enzymatic reaction was started by addition of freshly purified EDDS lyase (15 µM, 1.3 U based on ethylenediamine addition to fumarate) and the final volume of the reaction mixture was adjusted to 3 mL with the same buffer. The reaction mixture was incubated at room temperature for 24 h (except for **2c**, **2e**, **2f**, and **2n**; 48h). The enzyme was inactivated by heating at 70 °C for 10 min. The reaction mixture was filtered to remove the precipitated enzyme, and the filtrate was evaporated under vacuum. The resulting residue was dissolved in D_2O (0.5 mL) and analyzed by ^1H NMR spectroscopy. The conversion was estimated by comparing the signals of fumaric acid (**1**: olefinic protons) and corresponding products (**2**: methylene protons or **1**: methine proton) in ^1H NMR spectra as shown in Figures **S1-S21**.

2.2 Conversion analysis by ^1H NMR

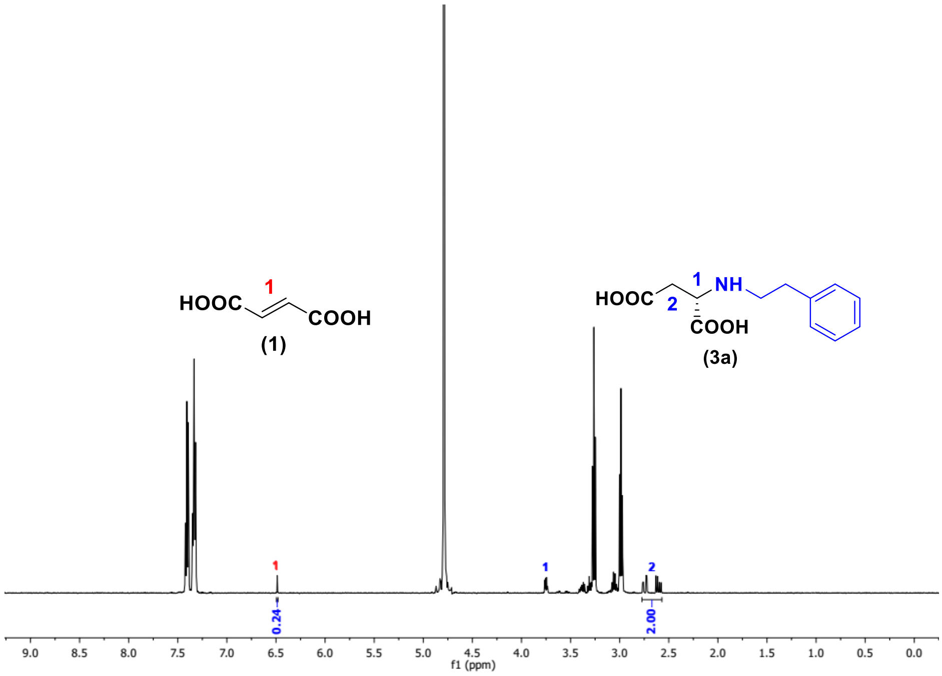


Figure S1. ^1H NMR spectrum monitoring the EDDS lyase catalyzed asymmetric addition of **2a** to fumarate (**1**). The conversion of substrates into corresponding product (**3a**) is 89%.

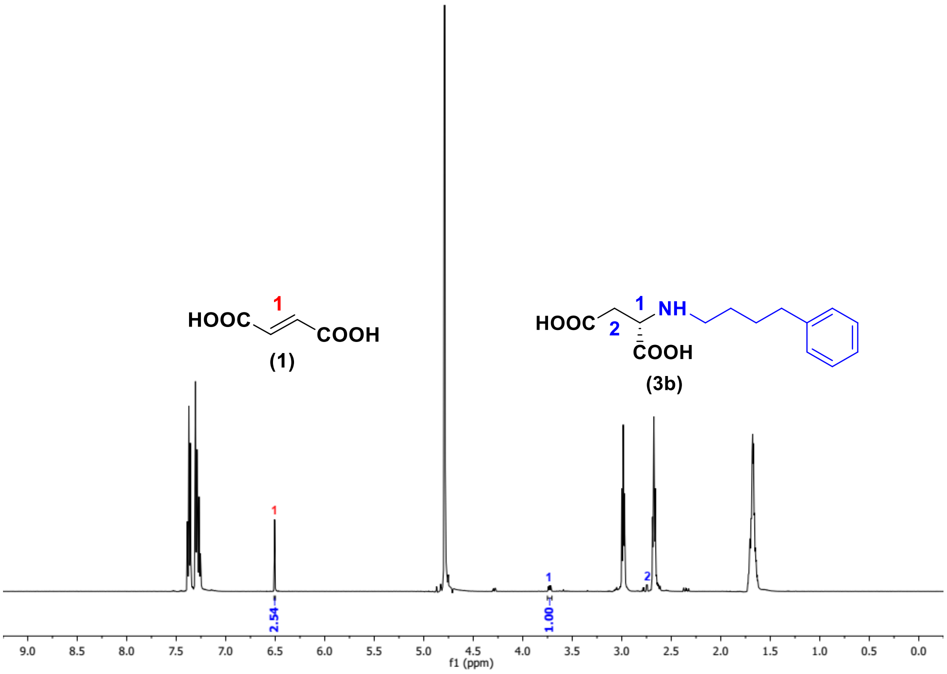


Figure S2. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2b** to fumarate (**1**). The conversion of substrates into corresponding product (**3b**) is 44%.

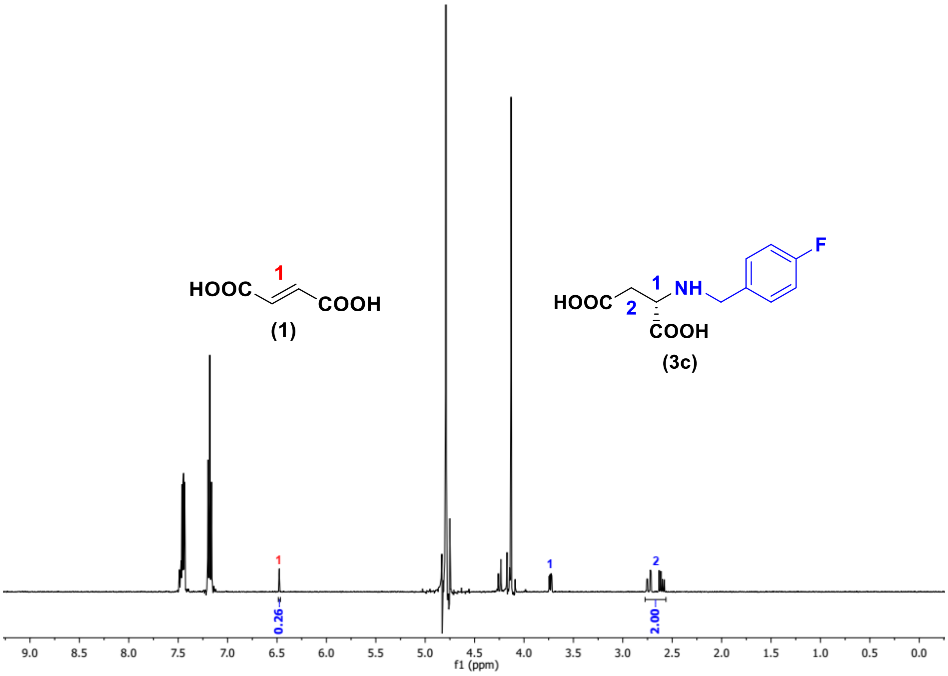


Figure S3. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2c** to fumarate (**1**). The conversion of substrates into corresponding product (**3c**) is 88%.

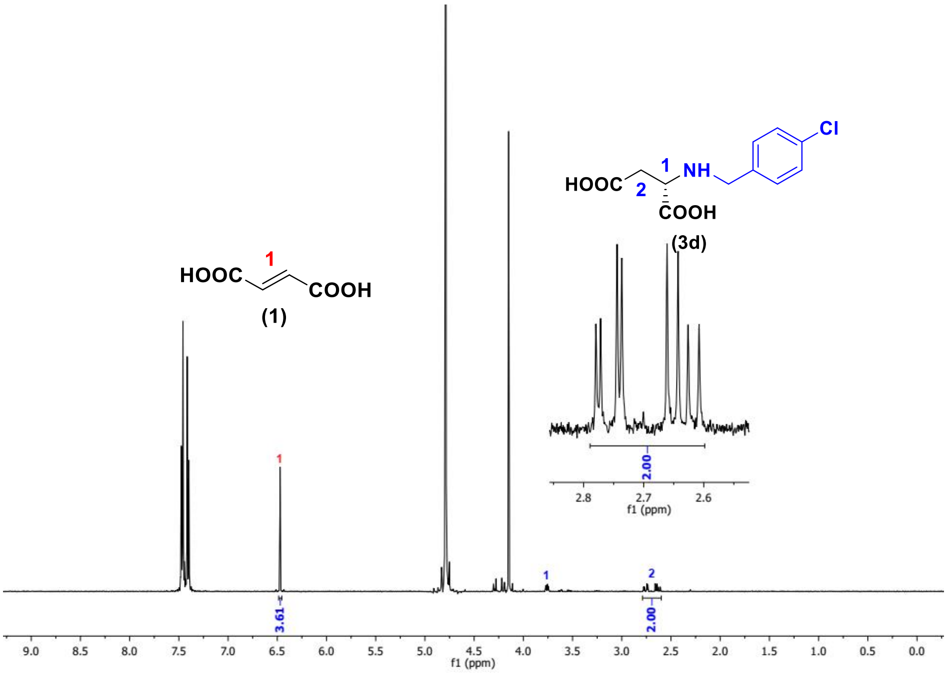


Figure S4. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2d** to fumarate (**1**). The conversion of substrates into corresponding product (**3d**) is 36%.

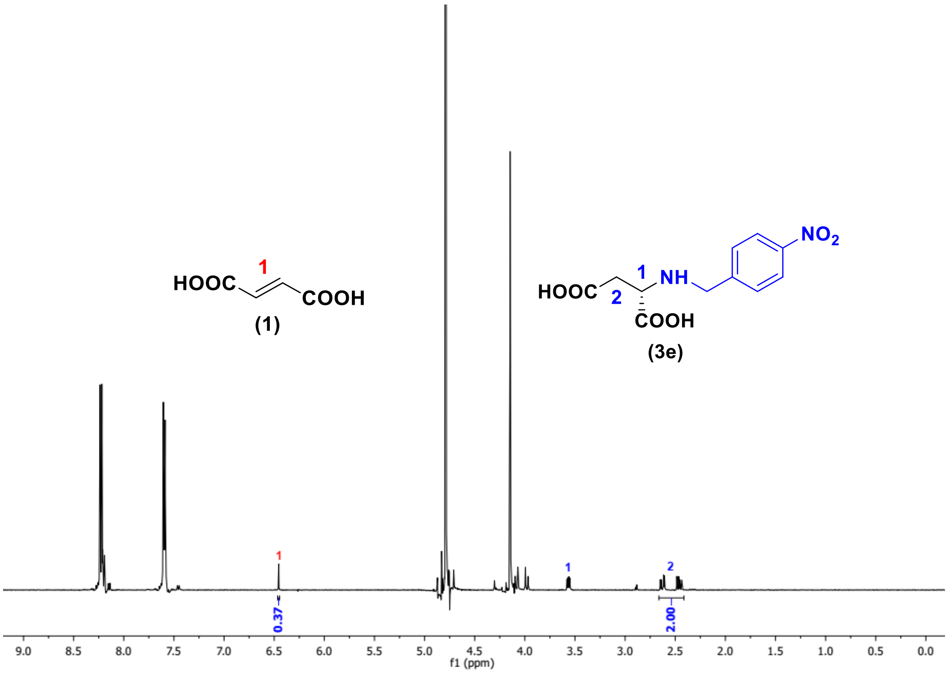


Figure S5. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2e** to fumarate (**1**). The conversion of substrates into corresponding product (**3e**) is 84%.

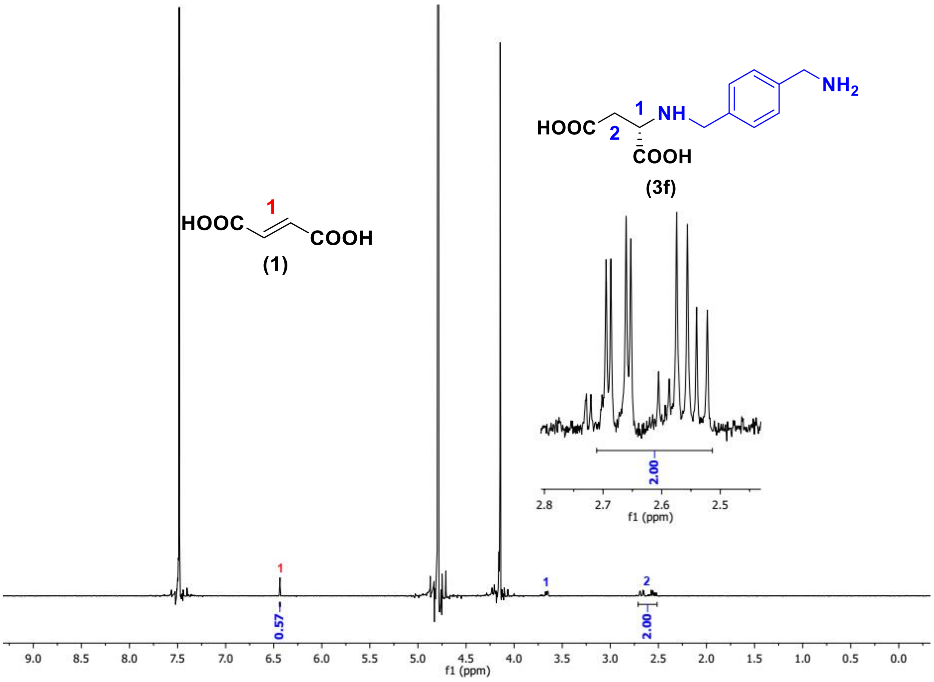


Figure S6. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2f** to fumarate (**1**). The conversion of substrates into corresponding product (**3f**) is 78%.

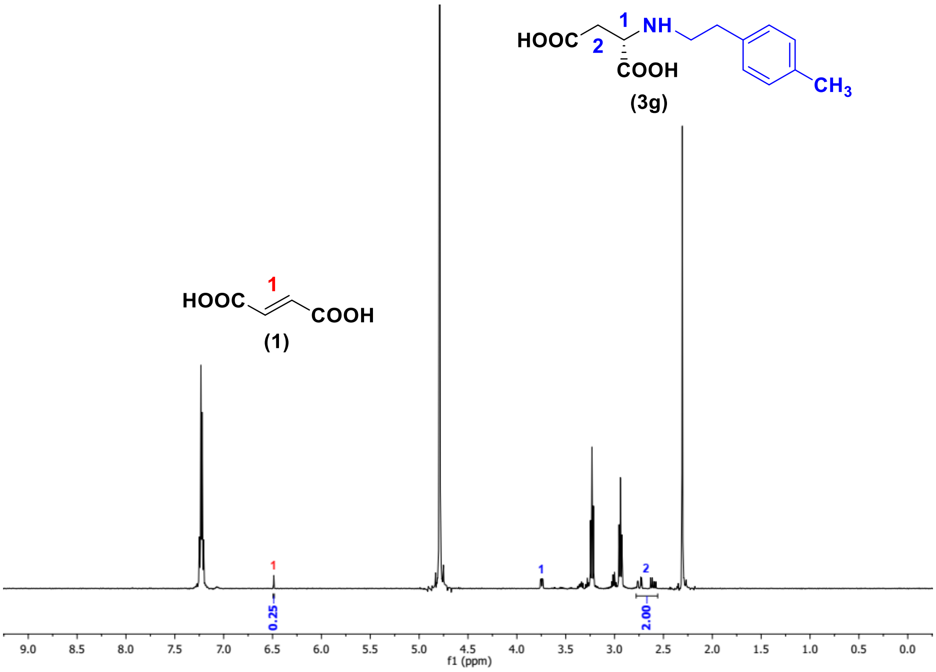


Figure S7. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2g** to fumarate (**1**). The conversion of substrates into corresponding product (**3g**) is 89%.

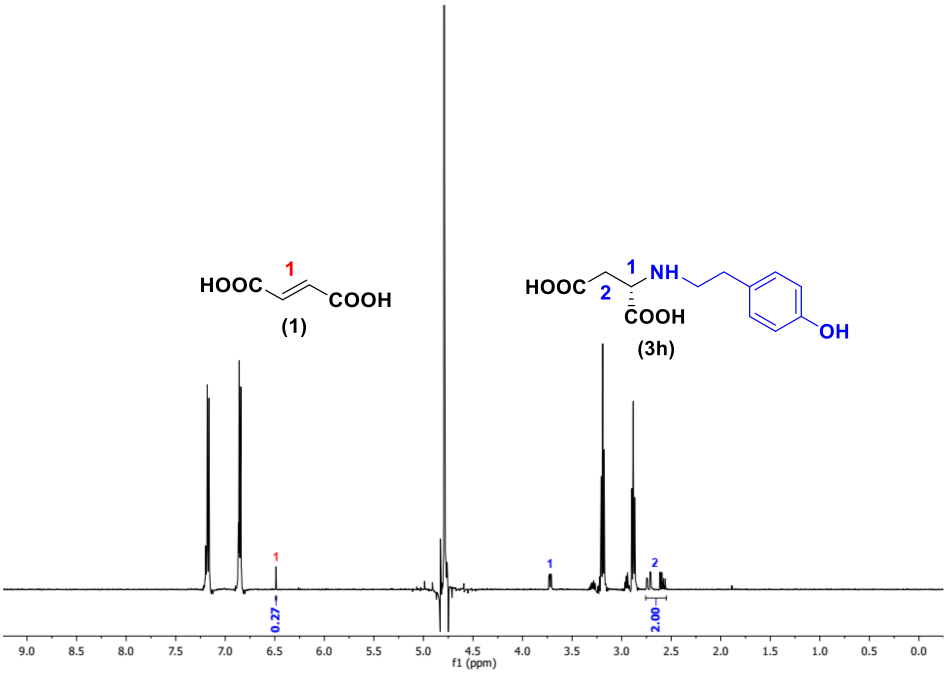


Figure S8. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2h** to fumarate (**1**). The conversion of substrates into corresponding product (**3h**) is 88%.

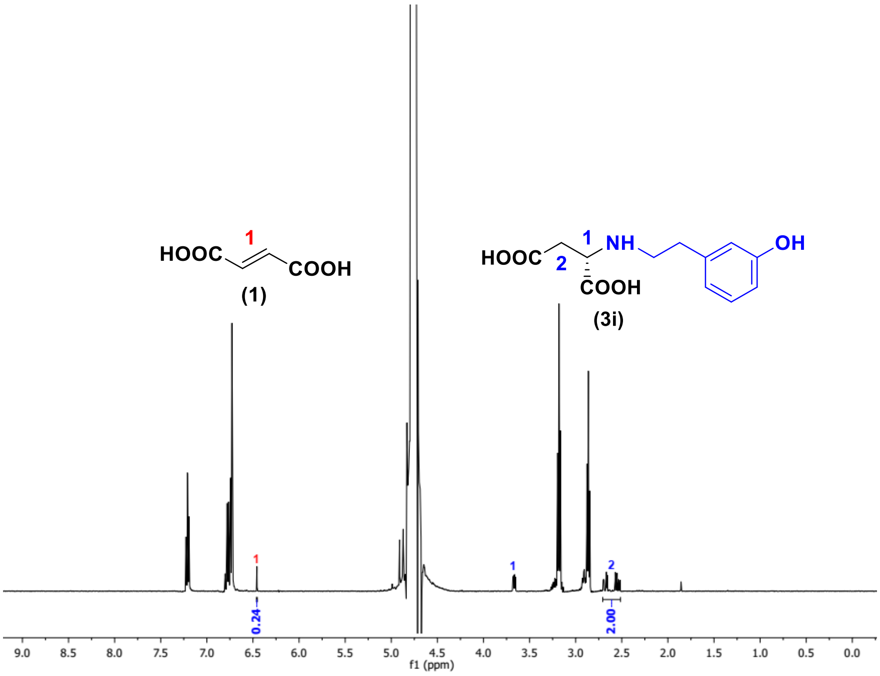


Figure S9. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2i** to fumarate (**1**). The conversion of substrates into corresponding product (**3i**) is 89%.

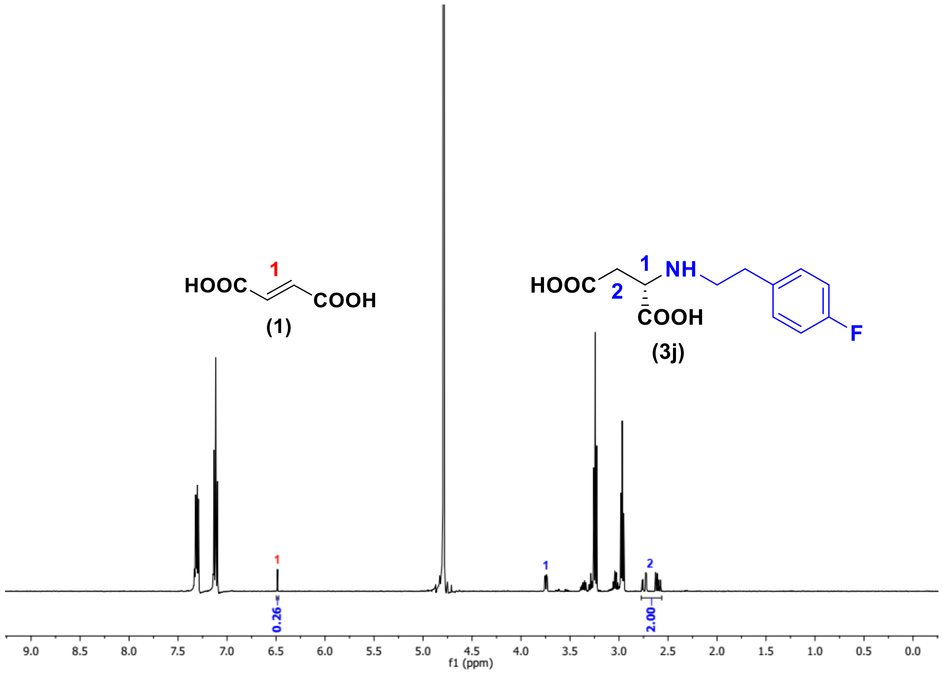


Figure S10. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2j** to fumarate (**1**). The conversion of substrates into corresponding product (**3j**) is 88%.

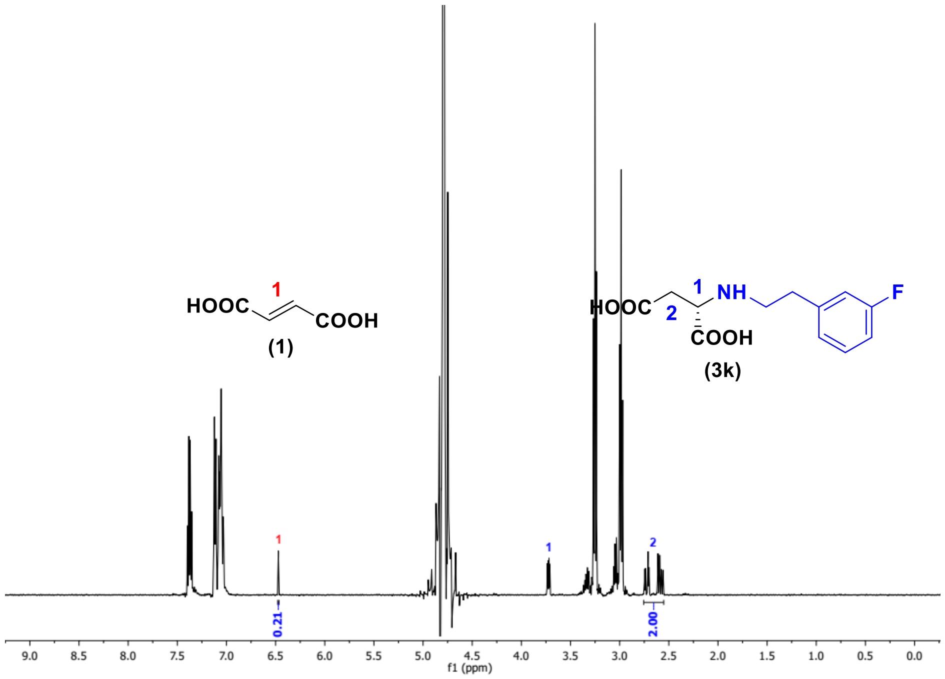


Figure S11. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2k** to fumarate (**1**). The conversion of substrates into corresponding product (**3k**) is 90%.

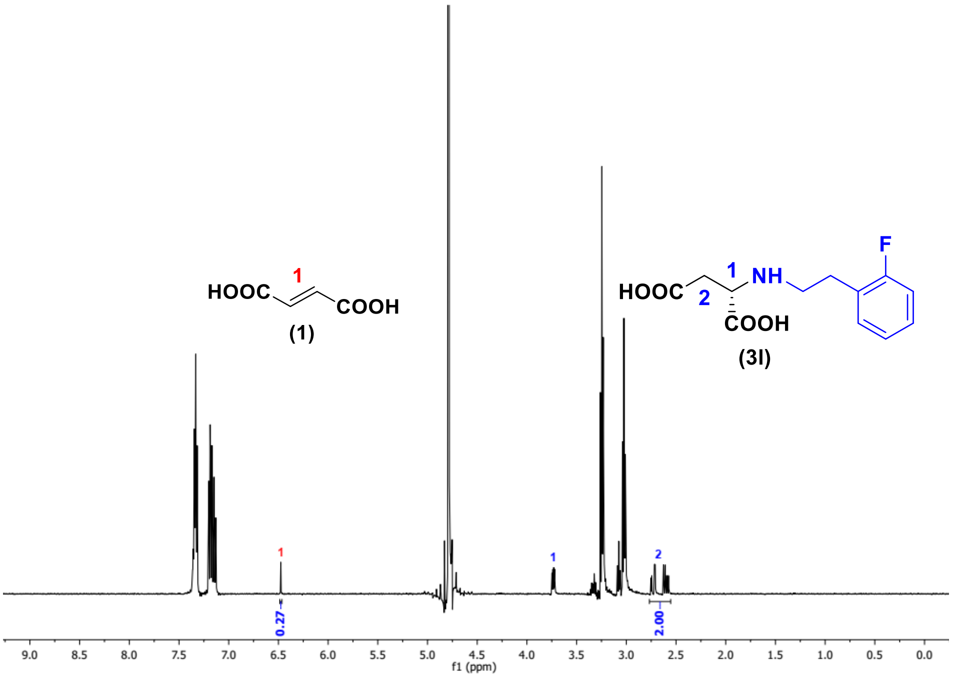


Figure S12. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2l** to fumarate (**1**). The conversion of substrates into corresponding product (**3l**) is 88%.

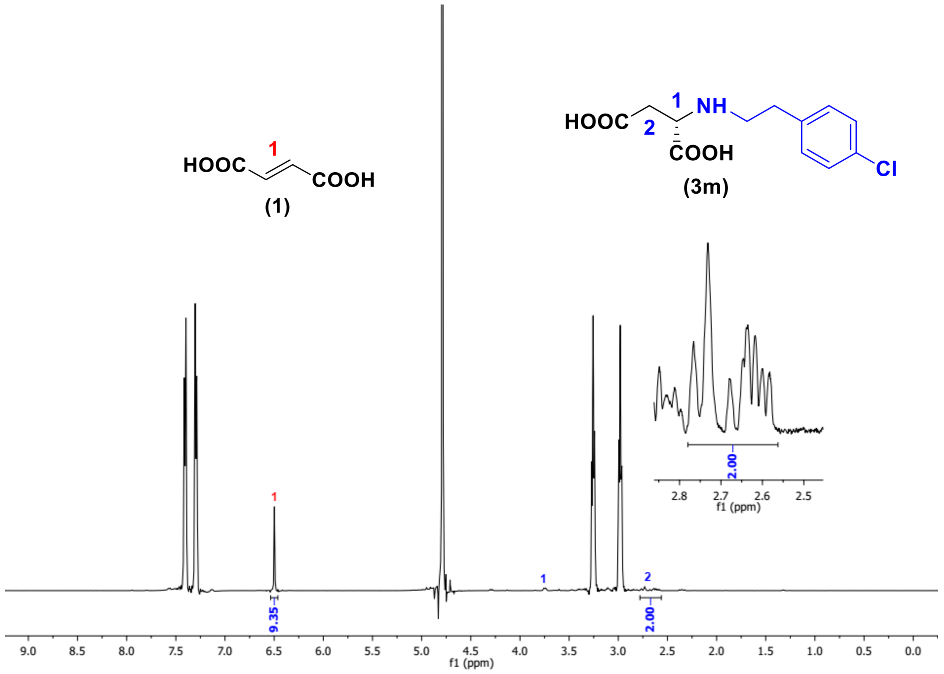


Figure S13. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2m** to fumarate (**1**). The conversion of substrates into corresponding product (**3m**) is 18%.

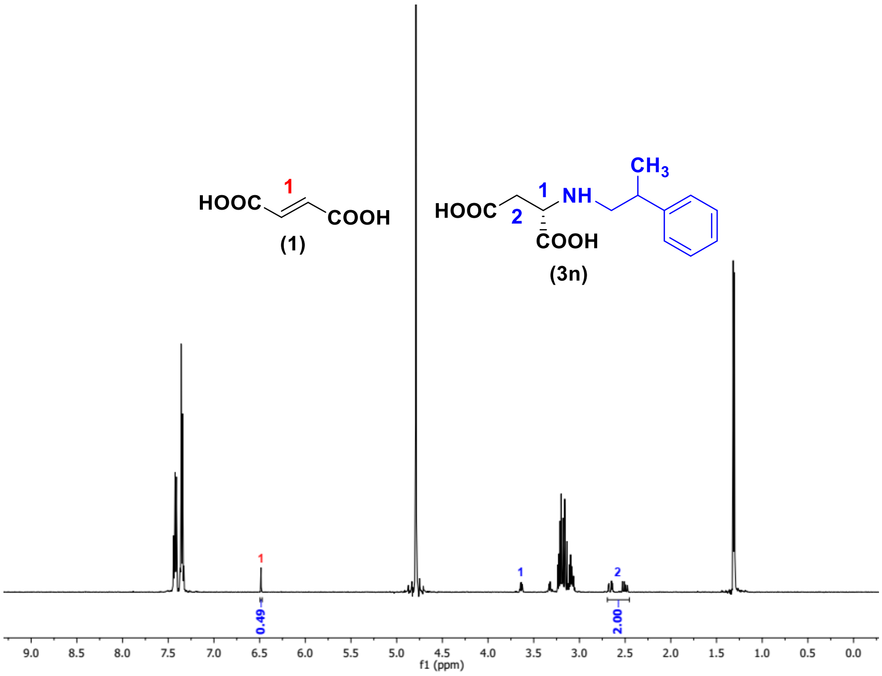


Figure S14. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2n** to fumarate (**1**). The conversion of substrates into corresponding product (**3n**) is 80%.

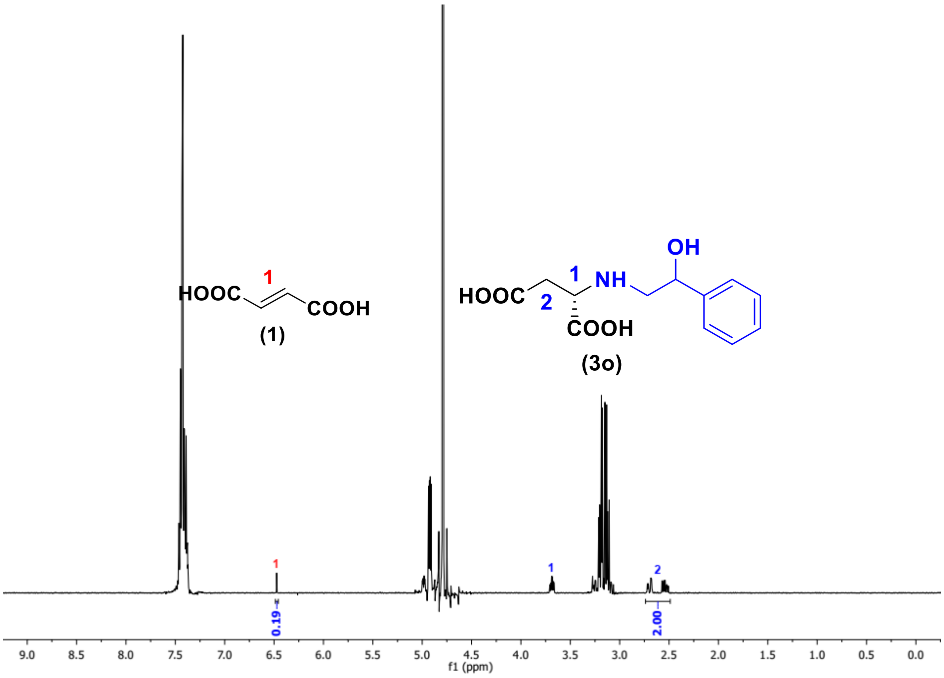


Figure S15. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2o** to fumarate (**1**). The conversion of substrates into corresponding product (**3o**) is 91%.

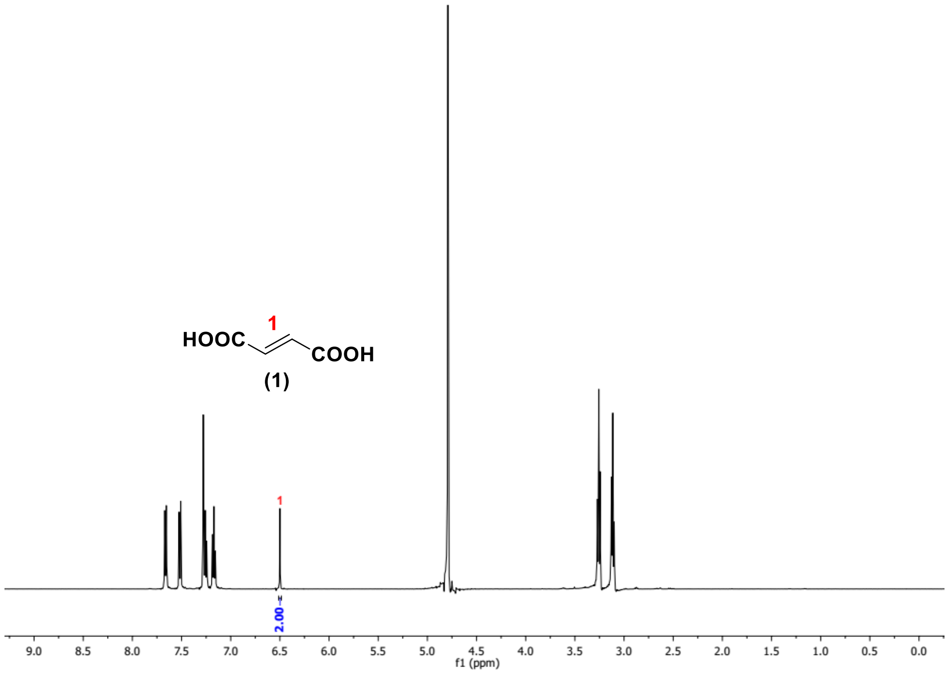


Figure S16. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2p** to fumarate (**1**). No product formation was observed.

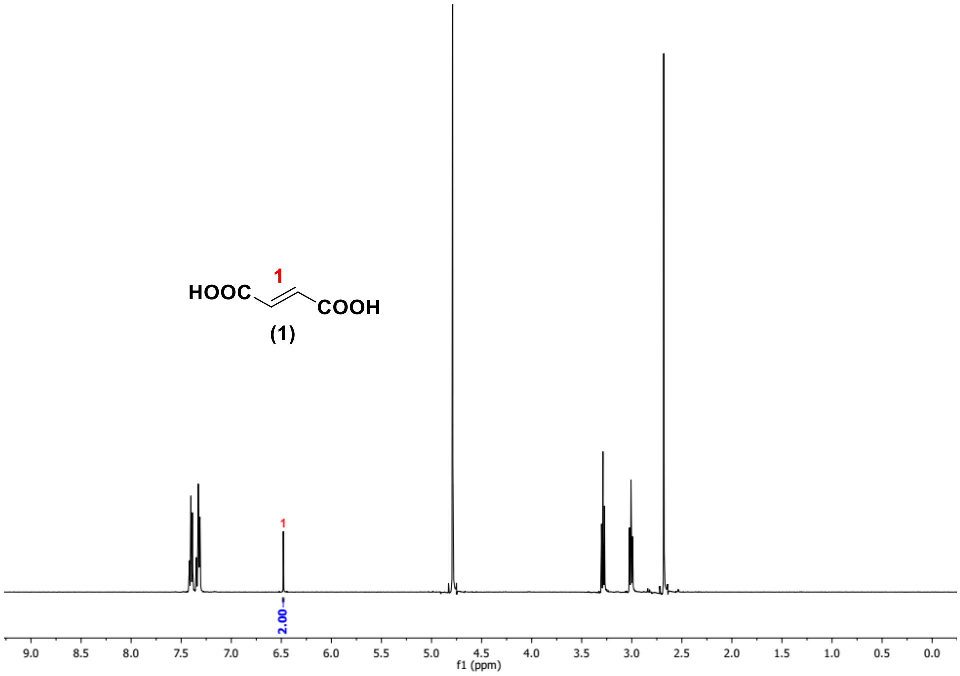


Figure S17. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2q** to fumarate (**1**). No product formation was observed.

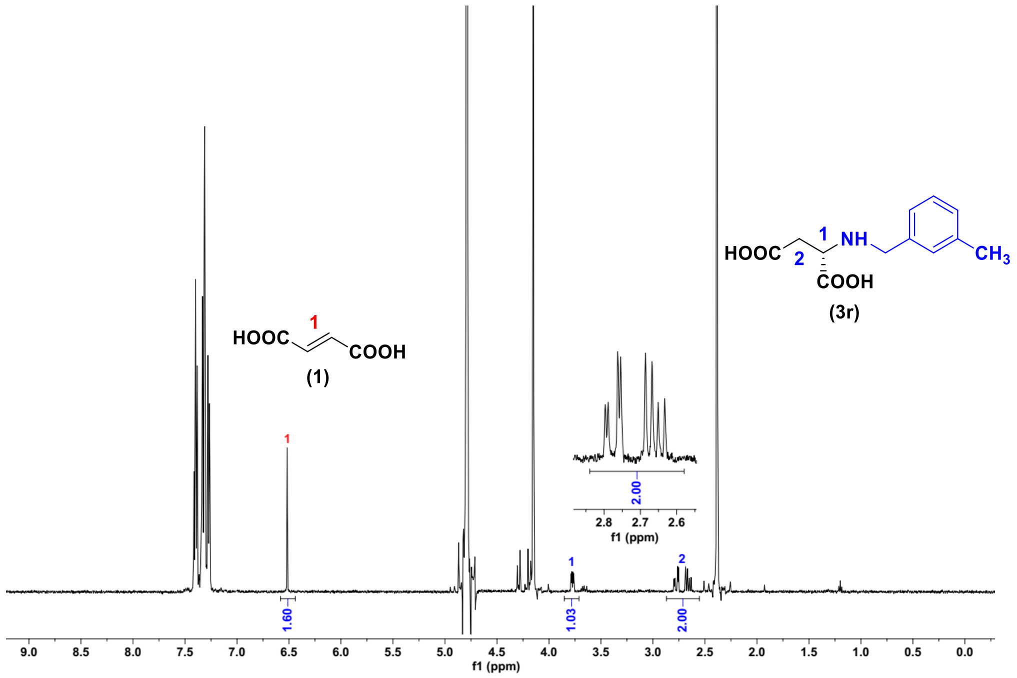


Figure S18. ^{1}H NMR spectrum monitoring the EDDS lyase catalyzed asymmetric addition of **2r** to fumarate (**1**). The conversion of substrates into corresponding product (**3r**) is 56%.

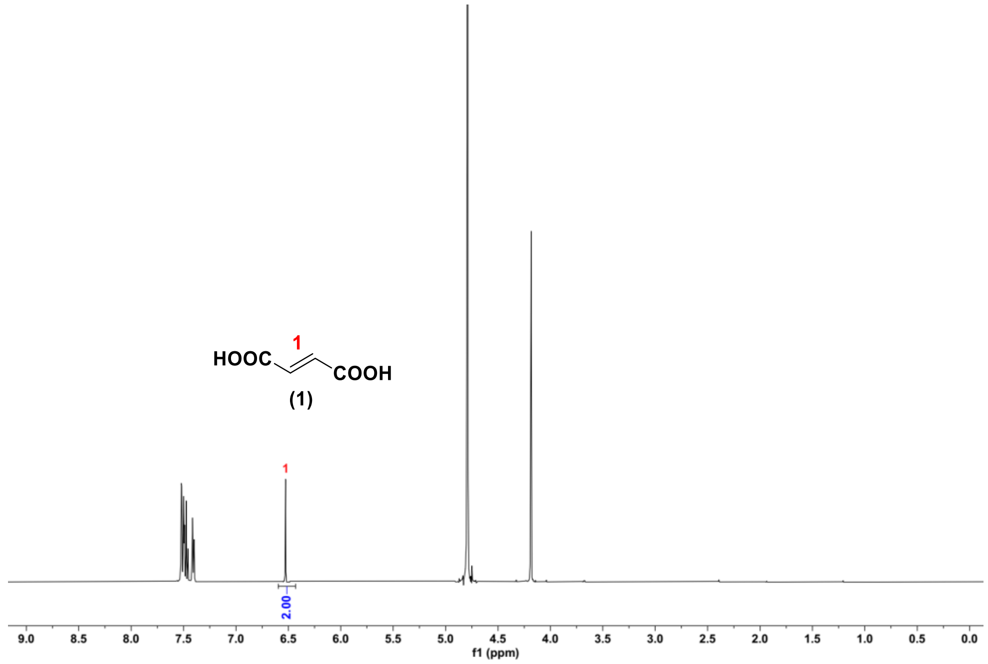


Figure S19. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2s** to fumarate (**1**). No product formation was observed.

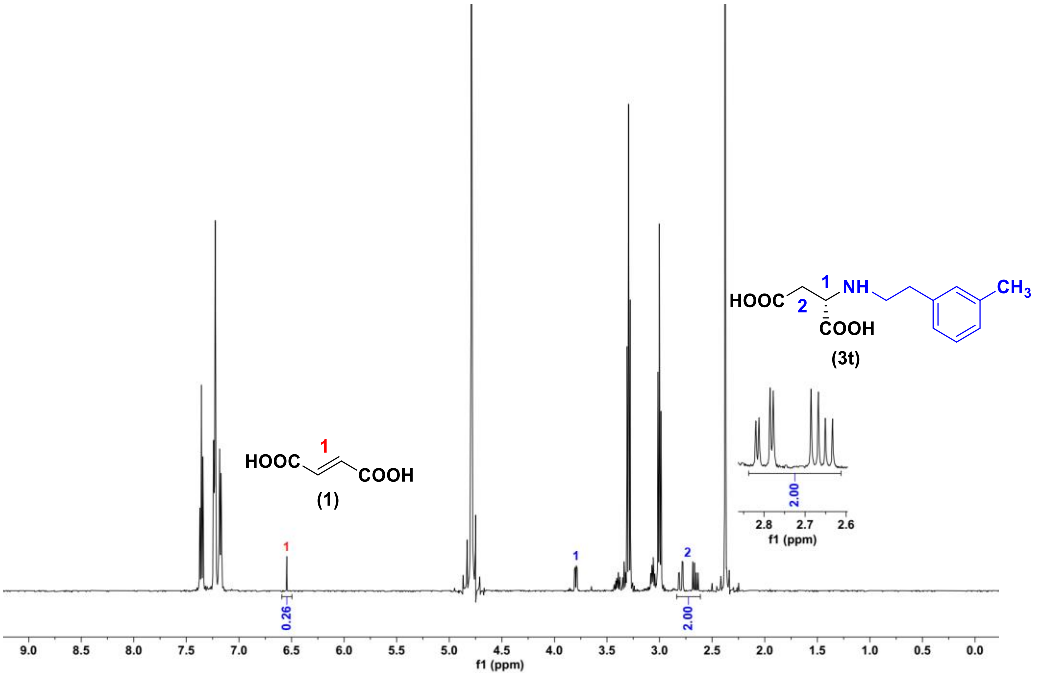


Figure S20. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2t** to fumarate (**1**). The conversion of substrates into corresponding product (**3t**) is 89%.

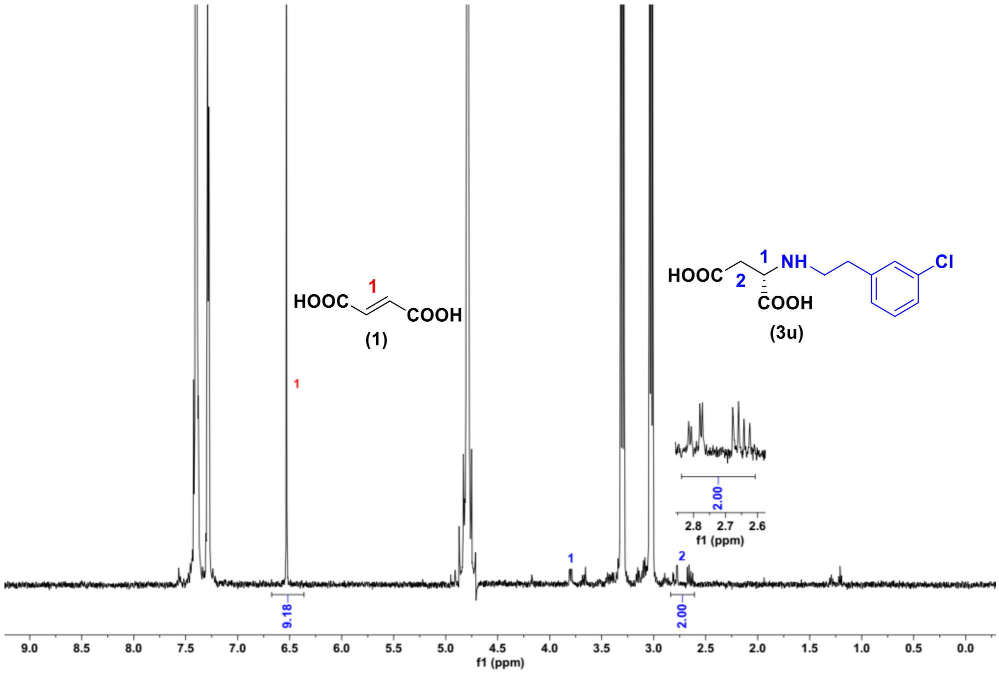
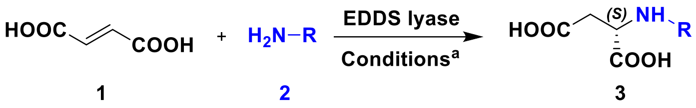


Figure S21. ^{1}H NMR spectra monitoring the EDDS lyase catalyzed asymmetric addition of **2u** to fumarate (**1**). The conversion of substrates into corresponding product (**3u**) is 18%.

2.3 Enzymatic synthesis of *N*-arylalkyl-substituted L-aspartic acids

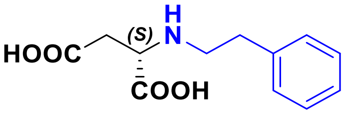


The initial reaction mixture (10 mL) consisted of fumarate (**1**; 10 mM; 0.15 mmol) and a selected amine substrate (**2a**, **2c**, **2d**, and **2g-j**; 50 mM) in NaH_2PO_4-NaOH buffer (20 mM; pH 8.5). The pH of the reaction mixture was adjusted to pH 8.5. The enzymatic reaction was started by the addition of freshly purified EDDS lyase (15 µM, 6.5 U), and the final volume of the reaction mixture was adjusted to 15 mL with the same buffer. The reaction mixture was then incubated at room temperature for 24 h (except for **2c**; 48 h). The progress of the enzymatic reaction was monitored by ^{1}H NMR spectroscopy by comparing signals of substrates and corresponding products. After completion of the reaction, the enzyme was inactivated by heating to 70 °C for 10 min.

Enzymatic products were purified by two steps of ion-exchange chromatography, as described previously.[1] For a typical purification procedure, the precipitated enzyme was removed by filtration (membrane filter, 0.45 µm pore size). The filtrate was loaded slowly onto an anion-exchange column (5 g of AG 1-X8 resin, acetate form, 100-200 mesh), which was pretreated with 1 M aqueous acetic acid (5 column volumes) and then water (until pH was neutral). The column was washed with water (3 column volumes), and then 0.1 M acetic acid (3 column volumes) until all the excess starting amine substrate was washed out. The product was eluted with 2 M acetic acid. The ninhydrin-positive fractions were collected and loaded onto a cation-exchange column (5 g of Dowex 50W X8 resin, 100-200 mesh), which was pretreated with 2 M aqueous ammonia (5 column volumes), 1 M HCl (3 column volumes), and water (5 column volumes). The column was washed with water (3 column volumes) to remove the remaining fumaric acid and eluted with 2 M aqueous ammonia until the desired product was collected. The ninhydrin-positive fractions were collected, concentrated under vacuum and lyophilized to provide the desired products as ammonium salts. The purified products were lyophilized and characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. The enantiomeric excess and absolute configuration of the product was determined by HPLC analysis on a chiral stationary phase using chemically synthesized authentic standards.

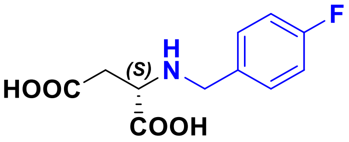
NMR data

N-phenethyl-L-aspartic acid (3a)



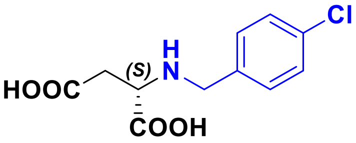
White powder. 25 mg (70% yield, *ee* >99%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.45 – 7.40 (m, 2H), 7.37 – 7.32 (m, 3H), 3.80 (dd, *J* = 8.7, 4.0 Hz, 1H), 3.45 – 3.30 (m, 2H), 3.12 – 3.02 (m, 2H), 2.80 (dd, *J* = 17.6, 4.0 Hz, 1H), 2.67 (dd, *J* = 17.6, 8.7 Hz, 1H).^{13}C NMR (126 MHz, Deuterium Oxide) δ 176.80, 172.89, 136.34, 129.06, 128.77, 127.33, 59.34, 47.51, 35.09, 31.87. HRMS (ESI$^+$): calcd. for $C_{12}H_{16}NO_4$, 238.1001 [M+H]$^+$; found: 238.1073.

N-(4-fluorobenzyl)-L-aspartic acid (3c)



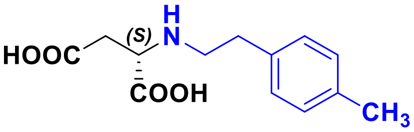
White powder. 27 mg (75% yield, *ee* >99%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.43 – 7.38 (m, 2H), 7.15 – 7.06 (m, 2H), 4.22 (d, *J* = 13.2 Hz, 1H), 4.14 (d, *J* = 13.2 Hz, 1H), 3.70 (dd, *J* = 8.9, 4.0 Hz, 1H), 2.70 (dd, *J* = 17.6, 4.0 Hz, 1H), 2.58 (dd, *J* = 17.6, 8.9 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 176.94, 172.92, 164.05, 162.10, 131.93, 126.81, 116.12, 58.48, 49.28, 35.48. HRMS (ESI$^+$): calcd. for $C_{11}H_{13}FNO_4$, 242.0750 [M+H]$^+$; found: 242.0822.

N-(4-chlorobenzyl)-L-aspartic acid (3d)



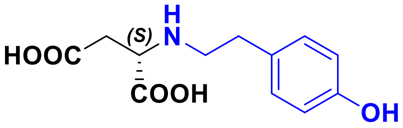
White powder. 11 mg (28% yield, *ee* >99%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.48 – 7.42 (m, 4H), 4.27 (d, *J* = 13.2 Hz, 1H), 4.19 (d, *J* = 13.2 Hz, 1H), 3.75 (dd, *J* = 9.1, 3.9 Hz, 1H), 2.75 (dd, *J* = 17.4, 3.9 Hz, 1H), 2.62 (dd, *J* = 17.5, 9.1 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.33, 173.33, 134.83, 131.25, 129.84, 129.17, 58.82, 49.29, 35.84. HRMS (ESI$^+$): calcd. for $C_{11}H_{13}ClNO_4$, 258.0454 [M+H]$^+$; found: 258.0528.

N-(4-methylphenethyl)-L-aspartic acid (3g)



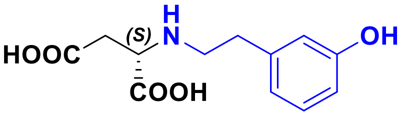
White powder. 25 mg (66% yield, *ee* >99%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.25 – 7.19 (m, 4H), 3.82 (dd, *J* = 7.9, 4.2 Hz, 1H), 3.38 – 3.25 (m, 2H), 3.06 – 2.95 (m, 2H), 2.85 (dd, *J* = 17.7, 4.2 Hz, 1H), 2.75 (dd, *J* = 17.7, 7.9 Hz, 1H), 2.30 (s, 3H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 175.87, 172.59, 137.41, 133.15, 129.60, 128.73, 58.89, 47.70, 34.66, 31.36, 20.05. HRMS (ESI^{+}): calcd. for $C_{13}H_{18}NO_4$, 252.1157 [M+H]$^{+}$; found: 252.1230.

N-(4-hydroxyphenethyl)-L-aspartic acid (3h)



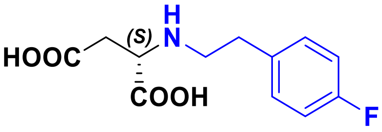
White powder. 23 mg (61% yield, *ee* >99%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.23 – 7.18 (m, 2H), 6.91 – 6.86 (m, 2H), 3.79 (dd, *J* = 8.4, 4.1 Hz, 1H), 3.38 – 3.31 (m, 1H), 3.30 – 3.24 (m, 1H), 3.02 – 2.93 (m, 2H), 2.81 (dd, *J* = 17.6, 4.1 Hz, 1H), 2.69 (dd, *J* = 17.6, 8.4 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 176.56, 172.84, 154.49, 130.16, 128.11, 115.74, 59.18, 47.71, 34.96, 30.99. HRMS (ESI^{+}): calcd. for $C_{12}H_{16}NO_5$, 254.0950 [M+H]$^{+}$; found: 254.1022.

N-(3-hydroxyphenethyl)-L-aspartic acid (3i)



White powder. 24 mg (63% yield, *ee* >99%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.28 – 7.23 (m, 1H), 6.86 (d, *J* = 7.7 Hz, 1H), 6.79 (dd, *J* = 5.3, 2.8 Hz, 2H), 3.74 (dd, *J* = 8.8, 3.9 Hz, 1H), 3.37 – 3.32 (m, 1H), 3.29 – 3.24 (m, 1H), 3.03 – 2.93 (m, 2H), 2.74 (dd, *J* = 17.5, 3.9 Hz, 1H), 2.60 (dd, *J* = 17.5, 8.8 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.33, 173.11, 155.89, 138.31, 130.46, 120.83, 115.61, 114.28, 59.54, 47.28, 35.35, 31.81. HRMS (ESI^{+}): calcd. for $C_{12}H_{16}NO_5$, 254.0950 [M+H]$^{+}$; found: 254.1022.

N-(4-fluorophenethyl)-L-aspartic acid (3j)

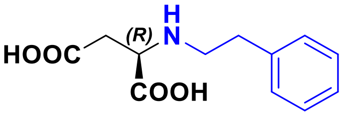


White powder. 29 mg (76% yield, *ee* >99%). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.35 – 7.27 (m, 2H), 7.12 – 7.09 (m, 2H), 3.79 (dd, *J* = 8.2, 3.9 Hz, 1H), 3.37 (dt, *J* = 13.8, 7.2 Hz, 1H), 3.33 – 3.25 (m, 1H), 3.08 – 2.98 (m, 2H), 2.80 (dd, *J* = 17.6, 3.8 Hz, 1H), 2.68 (dd, *J* = 17.6, 8.4 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 176.53, 172.78, 162.72, 160.79, 132.07, 130.48, 115.71, 59.20, 47.54, 34.94, 31.07. HRMS (ESI$^+$): calcd. for $C_{12}H_{15}FNO_4$, 256.0906 [M+H]$^+$; found: 256.0976.

2.4 Chemical synthesis of *N*-arylalkyl-substituted D- and L-aspartic acids

Chemical synthesis of *N*-substituted L- or D-aspartic acids was performed using a previously described procedure with a slight modification.[1,3] In general, the reaction was performed in a vial (10 mL). A mixture of L- or D-Aspartic acid (104 mg, 0.78 mmol, 1 eq) and an appropriate aldehyde (1.2 eq) in dry methanol (2 mL) was added to a solution (1 mL) of sodium cyanoborohydride (72.5 mg, 1.15 mmol, 1.5 eq) in dry methanol. The mixture was stirred for 24 – 48 hours at room temperature. The reaction mixture was coated first on silica and the desired product purified by flash (silica) chromatography [dichloromethane/methanol (50%)]. The fractions containing purified product were combined, concentrated, and dried under vacuum.

N-phenethyl-D-aspartic acid (D-3a)

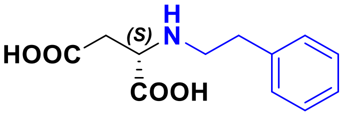


Compound D-**3a** was obtained by reacting phenylacetaldehyde (108 mg, 0.90 mmol) with D-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol). The desired product was further purified by silica column chromatography (DCM/MeOH: 50:50, v/v).

81 mg (44% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.44 – 7.38 (m, 2H), 7.36 – 7.31 (m, 3H), 3.76 (dd, *J* = 8.7, 3.9 Hz, 1H), 3.39 (dt, *J* = 13.1, 6.8 Hz, 1H), 3.31 (dd, *J* = 13.4,

5.9 Hz, 1H), 3.10 – 3.00 (m, 2H), 2.76 (dd, *J* = 17.5, 3.9 Hz, 1H), 2.63 (dd, *J* = 17.5, 8.8 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.16, 173.01, 136.36, 129.07, 128.78, 127.33, 59.47, 47.46, 35.26, 31.90. HRMS (ESI$^+$): calcd. for $C_{12}H_{16}NO_4$, 238.1001 [M+H]$^+$; found: 238.1071.

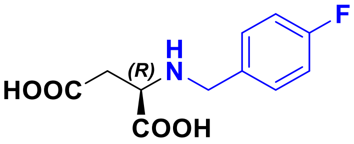
***N*-phenethyl-L-aspartic acid (L-3a)**



Compound L-**3a** was obtained by reacting phenylacetaldehyde (108 mg, 0.90 mmol) with L-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol). The desired product was further purified by silica column chromatography (DCM/MeOH: 50:50, v/v).

74 mg (40% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.46 – 7.43 (m, 2H), 7.40 – 7.36 (m, 3H),, 3.80 (dd, J = 8.7, 3.9 Hz, 1H), 3.47 – 3.39 (m, 1H), 3.38 – 3.30 (m, 1H), 3.14 – 3.04 (m, 2H), 2.81 (dd, J = 17.5, 3.9 Hz, 1H), 2.67 (dd, J = 17.5, 8.7 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.08, 172.99, 136.39, 129.10, 128.81, 127.36, 59.42, 47.49, 35.24, 31.92. HRMS (ESI$^+$): calcd. for $C_{12}H_{16}NO_4$, 238.1001 [M+H]$^+$; found: 238.1073.

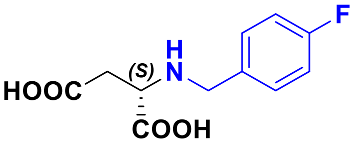
***N*-(4-fluorobenzyl)-D-aspartic acid (D-3c)**



Compound D-**3c** was obtained by reacting 4-fluorobenzaldehyde (111.5 mg, 0.90 mmol) with D-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

69 mg (37% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.52 – 7.46 (m, 2H), 7.22 – 7.18 (m, 2H), 4.32 (d, J = 13.2 Hz, 1H), 4.23 (d, J = 13.1 Hz, 1H), 3.78 (dd, J = 9.0, 3.9 Hz, 1H), 2.78 (dd, J = 17.6, 3.9 Hz, 1H), 2.66 (dd, J = 17.5, 9.1 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.14, 172.99, 164.06, 162.11, 131.93, 126.85, 115.95, 58.55, 49.26, 35.58. HRMS (ESI+): calcd. for $C_{11}H_{13}FNO_4$, 242.0750 [M+H]$^+$; found: 242.0821.

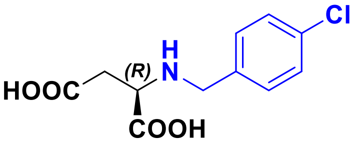
N-(4-fluorobenzyl)-L-aspartic acid (L-3c)



Compound L-**3c** was obtained by reacting 4-fluorobenzaldehyde (111.5 mg, 0.90 mmol) with L-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

51 mg (27% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.45 – 7.37 (m, 2H), 7.14 – 7.09 (m, 2H), 4.23 (d, J = 13.1 Hz, 1H), 4.14 (d, J = 13.2 Hz, 1H), 3.70 (dd, J = 9.0, 3.7 Hz, 1H), 2.69 (dd, J = 17.5, 3.6 Hz, 1H), 2.57 (dd, J = 17.6, 8.9 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.14, 172.99, 164.06, 162.10, 131.94, 126.85, 116.13, 58.56, 49.26, 35.58. HRMS (ESI+): calcd. for $C_{11}H_{13}FNO_4$, 242.0750 [M+H]$^+$; found: 242.0820.

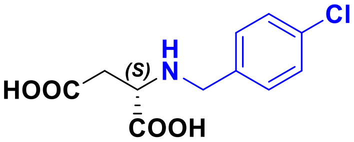
N-(4-chlorobenzyl)-D-aspartic acid (D-3d)



Compound D-**3d** was obtained by reacting 4-chlorobenzaldehyde (126.5 mg, 0.90 mmol) with D-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

54 mg (27% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.50 – 7.48 (m, 2H), 7.47 – 7.45 (m, 2H), 4.32 (d, J = 13.1 Hz, 1H), 4.23 (d, J = 13.1 Hz, 1H), 3.79 (dd, J = 9.6, 3.3 Hz, 1H), 2.78 (dd, J = 17.6, 3.8 Hz, 1H), 2.66 (dd, J = 17.5, 9.2 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.16, 173.01, 136.36, 129.07, 128.78, 127.33, 59.47, 47.46, 35.26. HRMS (ESI$^+$): calcd. for $C_{11}H_{13}ClNO_4$, 258.0454 [M+H]$^+$; found: 258.0527.

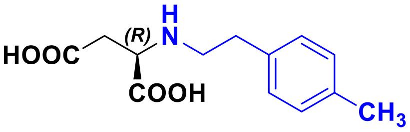
N-(4-chlorobenzyl)-L-aspartic acid (L-3d)



Compound L-**3d** was obtained by reacting 4-chlorobenzaldehyde (126.5 mg, 0.90 mmol) with L-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

53 mg (26% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.50 (d, *J* = 7.9 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 4.33 (d, J = 13.2 Hz, 1H), 4.24 (d, J = 13.2 Hz, 1H), 3.85 – 3.75 (m, 1H), 2.80 (dd, J = 17.6, 3.8 Hz, 1H), 2.68 (dd, J = 17.6, 9.1 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.13, 172.94, 134.99, 131.34, 129.49, 129.24, 58.71, 49.28, 35.55. HRMS (ESI$^+$): calcd. for $C_{11}H_{13}ClNO_4$, 258.0454 [M+H]$^+$; found: 258.0526.

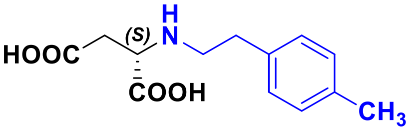
***N*-(4-methylphenethyl)-D-aspartic acid (D-3g)**



Compound D-**3g** was obtained by reacting 2-(4-methylphenyl)acetaldehyde (121 mg, 0.90 mmol) with D-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

81 mg (41% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.26 – 7.20 (m, 4H), 3.76 (dd, J = 8.6, 4.0 Hz, 1H), 3.41 – 3.32 (m, 1H), 3.31 – 3.23 (m, 1H), 3.07 – 2.94 (m, J = 7.6 Hz, 2H), 2.77 (dd, J = 17.5, 4.0 Hz, 1H), 2.64 (dd, J = 17.5, 8.7 Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.02, 172.97, 137.40, 133.22, 129.62, 128.74, 59.37, 47.54, 35.23, 31.47, 20.06. HRMS (ESI$^+$): calcd. for $C_{13}H_{18}NO_4$, 252.1157 [M+H]$^+$; found: 252.1230.

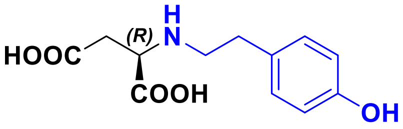
***N*-(4-methylphenethyl)-L-aspartic acid (L-3g)**



Compound L-**3g** was obtained by reacting 2-(4-methylphenyl)acetaldehyde (121 mg, 0.90 mmol) with L-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

81 mg (41% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.24 – 7.20 (m, 4H), 3.75 (dd, J = 8.6, 3.9 Hz, 1H), 3.36 – 3.30 (m, 1H), 3.28 – 3.20 (m, 1H), 3.01 – 2.97 (m, 2H), 2.77 (dd, J = 17.5, 3.9 Hz, 1H), 2.64 (dd, J = 17.5, 8.7 Hz, 1H), 2.30 (s, 3H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.14, 173.00, 137.37, 133.21, 129.61, 128.73, 59.45, 47.50, 35.27, 31.47, 20.07. HRMS (ESI+): calcd. for $C_{13}H_{18}NO_4$, 252.1157 [M+H]$^+$; found: 252.1229.

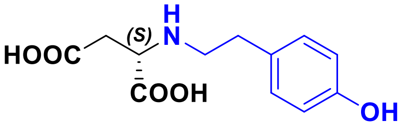
N-(4-hydroxyphenethyl)-D-aspartic acid (D-3h)



For the synthesis of compound D-**3h**, the appropriate starting material 2-(4-hydroxyphenyl)acetaldehyde was first prepared using a previously described method[4]. Subsequently, the synthesis of compound D-**3h** was achieved by reacting 2-(4-hydroxyphenyl)acetaldehyde (122.5 mg, 0.90 mmol) with D-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

92 mg (47% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.20 (d, J = 7.5 Hz, 2H), 6.88 (d, J = 8.2 Hz, 2H), 3.76 (dd, J = 8.7, 3.7 Hz, 1H), 3.33 – 3.28 (m, 1H), 3.25 – 3.20 (m, 1H), 3.02 – 2.91 (m, 2H), 2.76 (dd, J = 17.4, 3.7 Hz, 1H), 2.62 (dd, J = 17.5, 8.8 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.07, 172.99, 154.48, 130.16, 128.10, 115.84, 59.39, 47.62, 35.19, 31.04. HRMS (ESI+): calcd. for $C_{12}H_{16}NO_5$, 254.0950 $[M+H]^+$; found: 254.1021.

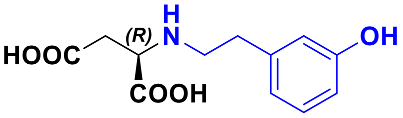
N-(4-hydroxyphenethyl)-L-aspartic acid (L-3h)



For the synthesis of compound L-**3h**, the appropriate starting material 2-(4-hydroxyphenyl)acetaldehyde was first prepared using a previously described method[4]. Subsequently, the synthesis of compound L-**3h** was achieved by reacting 2-(4-hydroxyphenyl)acetaldehyde (122.5 mg, 0.90 mmol) with L-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

74 mg (37% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.17 (d, J = 8.2 Hz, 2H), 6.86 (d, J = 8.0 Hz, 2H), 3.75 (dd, J = 8.5, 3.7 Hz, 1H), 3.30 (dd, J = 13.1, 6.5 Hz, 1H), 3.26 – 3.18 (m, 1H), 3.02 – 2.91 (m, 2H), 2.77 (dd, J = 17.4, 3.9 Hz, 1H), 2.64 (dd, J = 17.3, 8.5 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.07, 172.99, 154.48, 130.16, 128.10, 115.84, 59.39, 47.62, 35.19, 31.04. HRMS (ESI+): calcd. for $C_{12}H_{16}NO_5$, 254.0950 $[M+H]^+$; found: 254.1023.

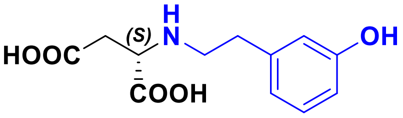
N-(3-hydroxyphenethyl)-D-aspartic acid (D-3i)



For the synthesis of compound D-**3i**, the starting material 2-(3-hydroxyphenyl)acetaldehyde was first prepared using a previously described method[4]. Subsequently, the synthesis of compound D-**3i** was obtained by reacting 2-(3-hydroxyphenyl)acetaldehyde (122.5 mg, 0.90 mmol) with D-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

84 mg (43% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.33 – 7.30 (m, 1H), 6.94 – 6.92 (m, 1H), 6.87 – 6.85 (m, 2H), 3.80 (dd, J = 8.8, 3.8 Hz, 1H), 3.44 – 3.39 (m, 1H), 3.36 – 3.29 (m, 1H), 3.09 – 2.99 (m, 2H), 2.80 (dd, J = 17.5, 3.7 Hz, 1H), 2.67 (dd, J = 17.5, 8.8 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.26, 173.05, 155.87, 138.33, 130.49, 120.90, 115.62, 114.30, 59.60, 47.33, 35.31, 31.82. HRMS (ESI+): calcd. for $C_{12}H_{16}NO_5$, 254.0950 [M+H]$^+$; found: 254.1022.

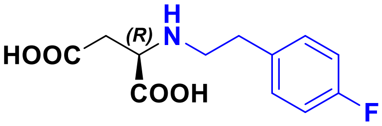
N-(3-hydroxyphenethyl)-L-aspartic acid (L-3i)



For the synthesis of compound L-**3i**, the starting material 2-(3-hydroxyphenyl)acetaldehyde was first prepared using a previously described method.[4] Subsequently, the synthesis of compound L-**3i** was obtained by reacting 2-(3-hydroxyphenyl)acetaldehyde (122.5 mg, 0.90 mmol) with L-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

93 mg (47% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.29 – 7.26 (m, 1H), 6.90 – 6.87 (m, 1H), 6.84 – 6.81 (m, 2H), 3.76 (dd, J = 8.7, 3.8 Hz, 1H), 3.39 – 3.34 (m, 1H), 3.32 – 3.25 (m, 1H), 3.05 – 2.96 (m, 2H), 2.77 (dd, J = 17.8, 4.1 Hz, 1H), 2.64 (dd, J = 17.6, 8.7 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.15, 172.99, 155.81, 138.25, 130.45, 120.89, 115.63, 114.31, 59.46, 47.29, 35.22, 31.77. HRMS (ESI+): calcd. for $C_{12}H_{16}NO_5$, 254.0950 [M+H]$^+$; found: 254.1023.

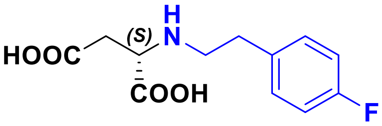
N-(4-fluorophenethyl)-D-aspartic acid (D-3j)



Compound D-**3j** was obtained by reacting 2-(4-fluorophenyl)acetaldehyde (124 mg, 0.90 mmol) with D-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

89 mg (45% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.37 – 7.30 (m, 2H), 7.14 – 7.11 (m, 2H), 3.79 – 3.76 (m, 1H), 3.42 – 3.36 (m, 1H), 3.34 – 3.27 (m, 1H), 3.11 – 3.00 (m, 2H), 2.78 (dd, J = 17.4, 3.5 Hz, 1H), 2.65 (dd, J = 17.5, 8.7 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.02, 172.94, 162.73, 160.80, 132.09, 130.49, 115.73, 115.56, 59.45, 47.48, 35.18, 31.12. HRMS (ESI$^+$): calcd. for $C_{12}H_{15}FNO_4$, 256.0906 [M+H]$^+$; found: 256.0979.

N-(4-fluorophenethyl)-L-aspartic acid (L-3j)



Compound L-**3j** was obtained by reacting 2-(4-fluorophenyl)acetaldehyde (124 mg, 0.90 mmol) with L-aspartic acid (104 mg, 0.78 mmol) and sodium cyanoborohydride (72.5 mg, 1.15 mmol).

69 mg (35% yield). ^{1}H NMR (500 MHz, Deuterium Oxide) δ 7.26 – 7.21 (m, 2H), 7.05 – 7.01 (m, 2H), 3.68 (dd, J = 8.7, 3.9 Hz, 1H), 3.32 – 3.26 (m, 1H), 3.25 – 3.16 (m, 1H), 3.03 – 2.90 (m, 2H), 2.69 (dd, J = 17.5, 3.9 Hz, 1H), 2.56 (dd, J = 17.6, 8.7 Hz, 1H). ^{13}C NMR (126 MHz, Deuterium Oxide) δ 177.06, 172.95, 162.72, 160.79, 132.09, 130.48, 115.72, 59.38, 47.47, 35.18, 31.12. HRMS (ESI$^+$): calcd. for $C_{12}H_{15}FNO_4$, 256.0906 [M+H]$^+$; found: 256.0978.

3. NMR spectra

3.1 ^{1}H and ^{13}C NMR spectra of enzymatically obtained *N*-arylalkyl-substituted *L*-aspartic acids

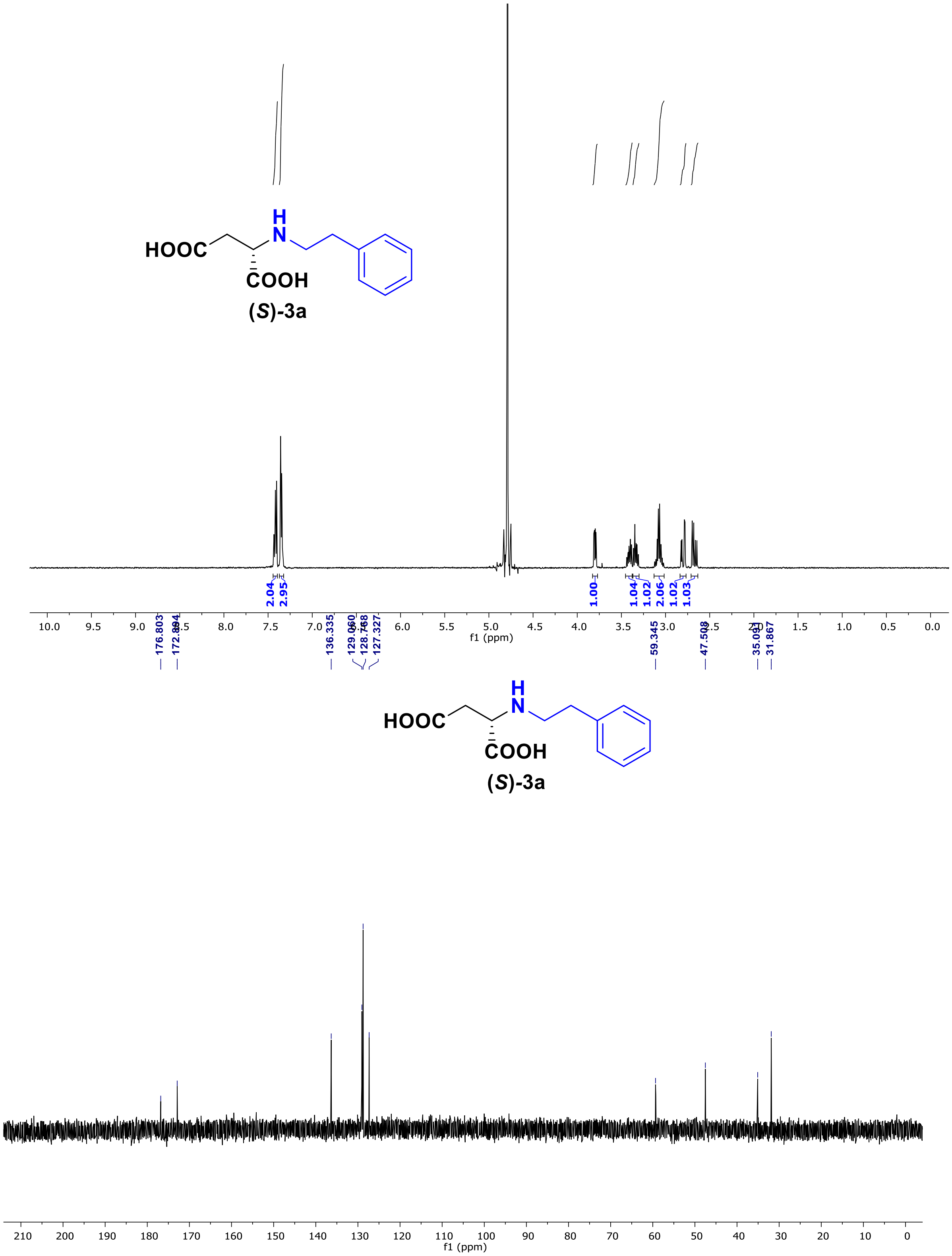


Figure S22. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(phenethyl)-L-aspartic acid [(*S*)-**3a**].

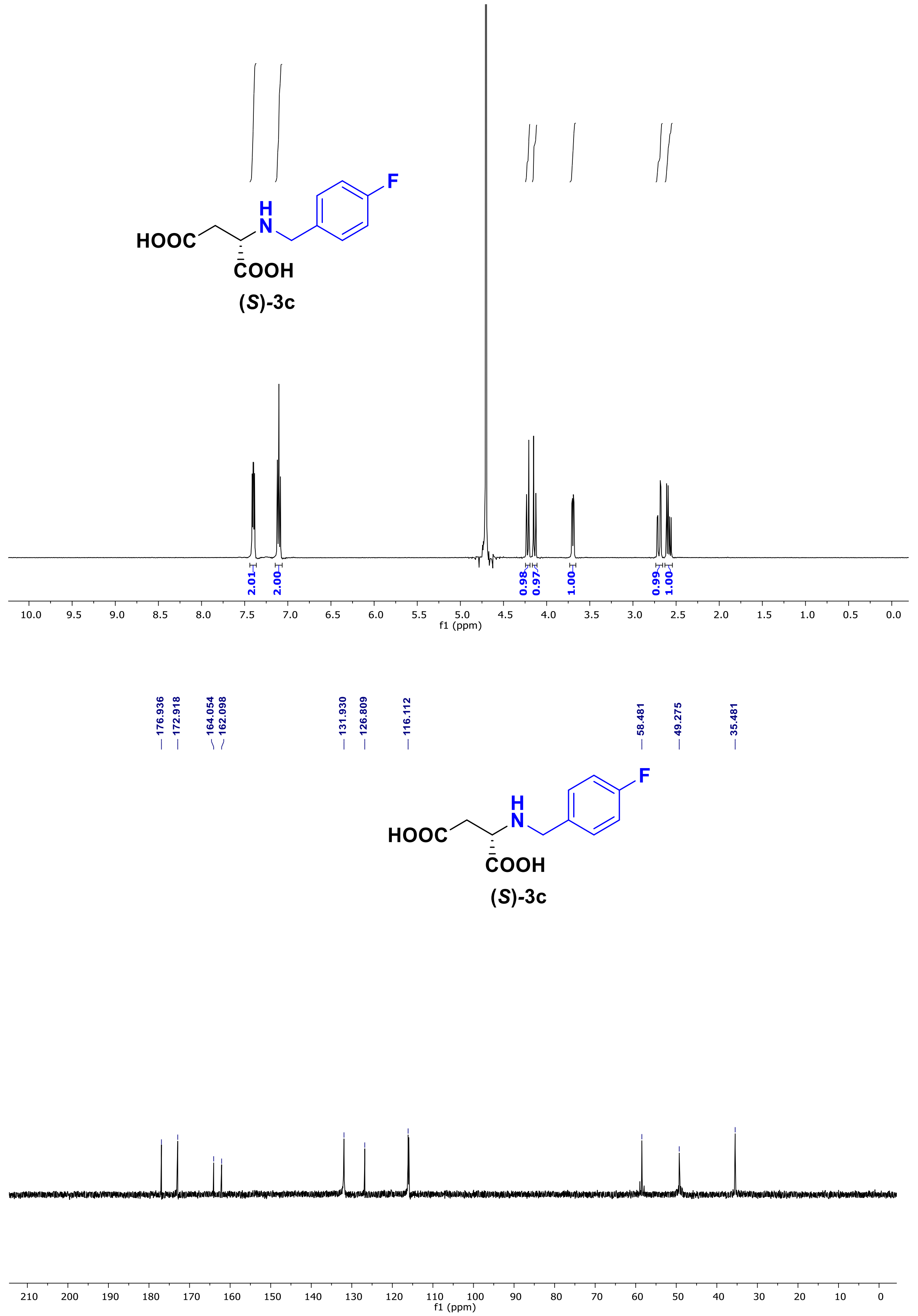


Figure S23. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-fluorobenzyl)-L-aspartic acid [(*S*)-**3c**].

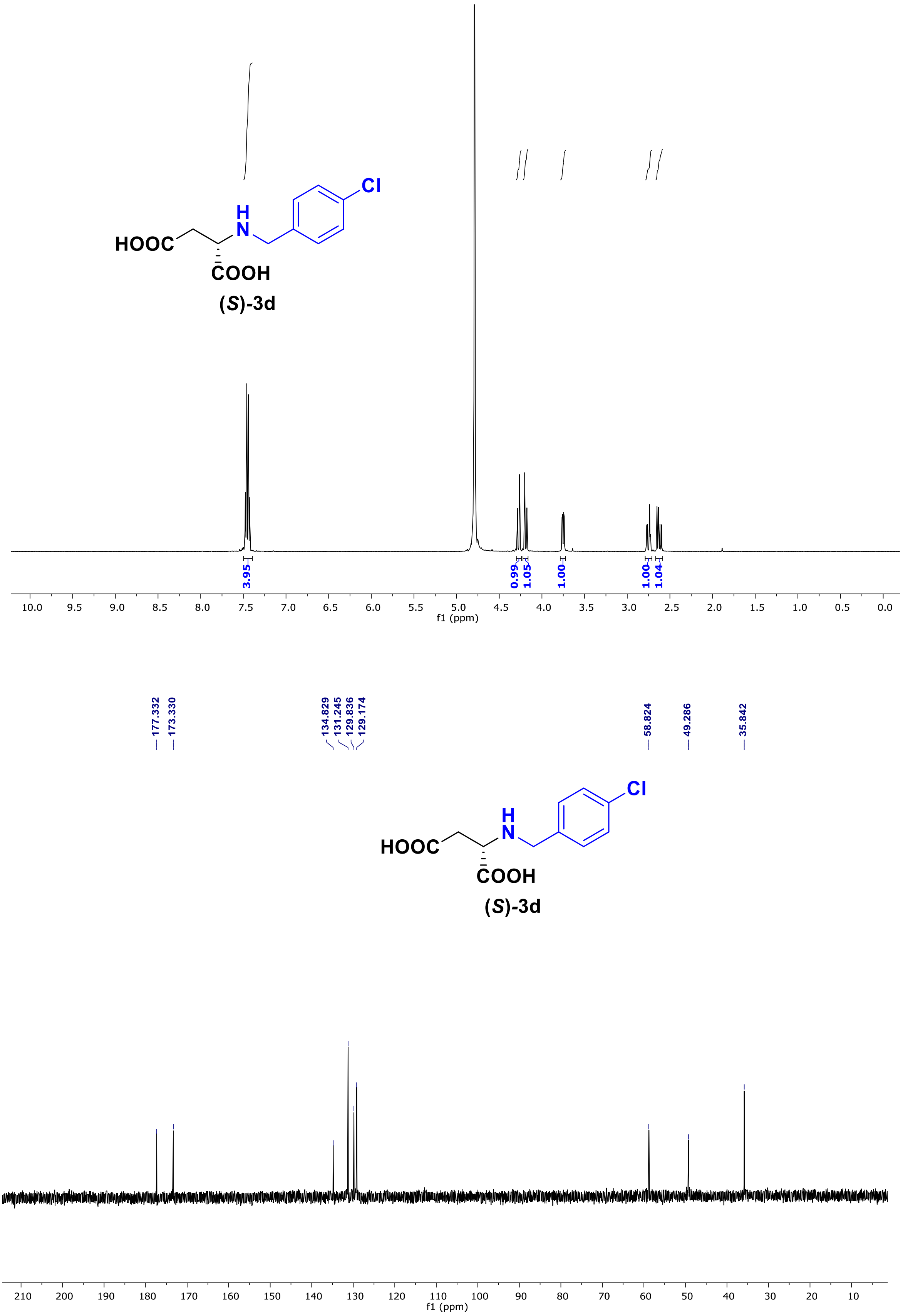


Figure S24. ^1H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-chlorobenzyl)-L-aspartic acid [(*S*)-**3d**].

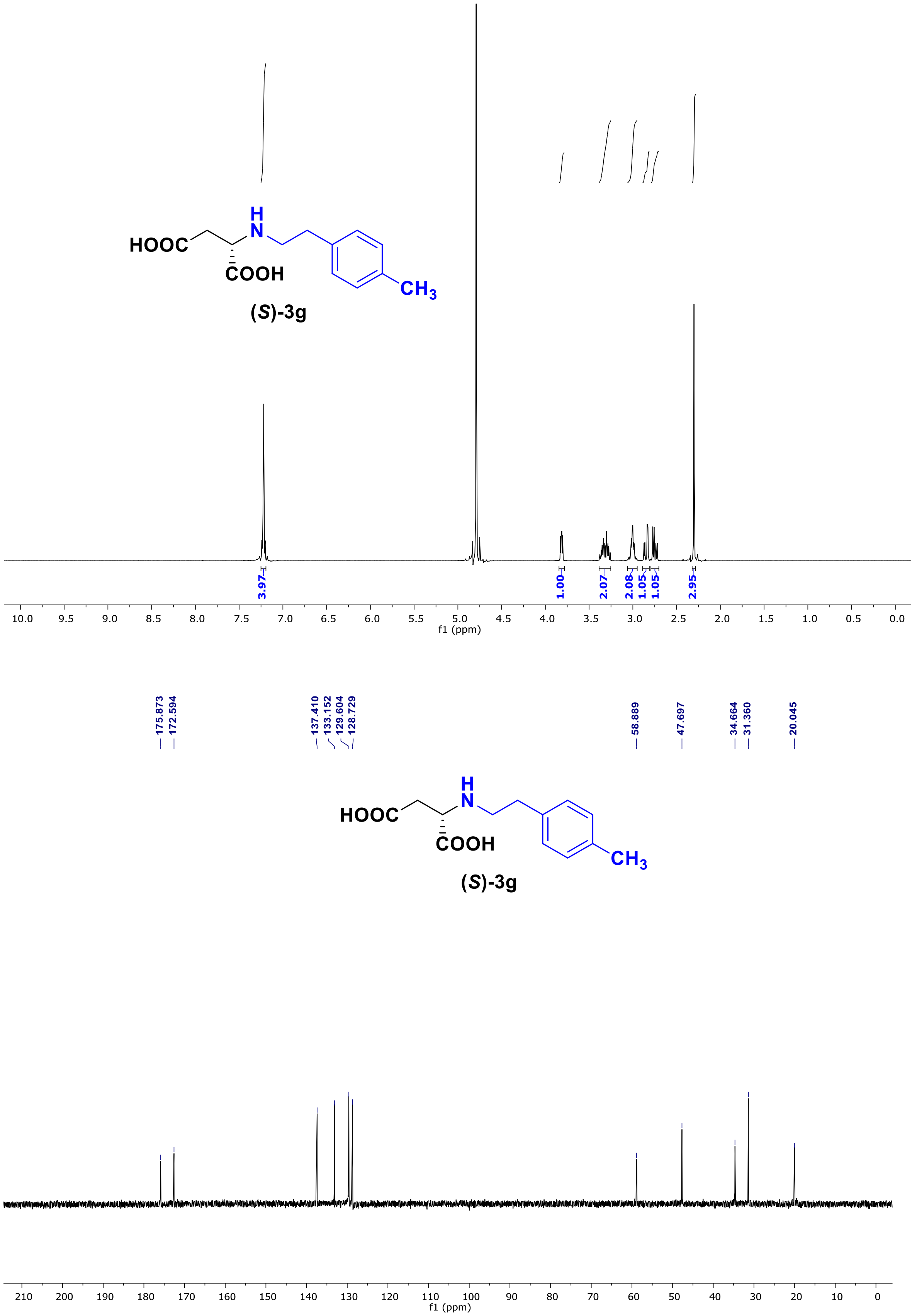


Figure S25. ${}^{1}H$ NMR (top) and ${}^{13}C$ NMR (bottom) of *N*-(4-methylphenethyl)-L-aspartic acid [(*S*)-**3g**].

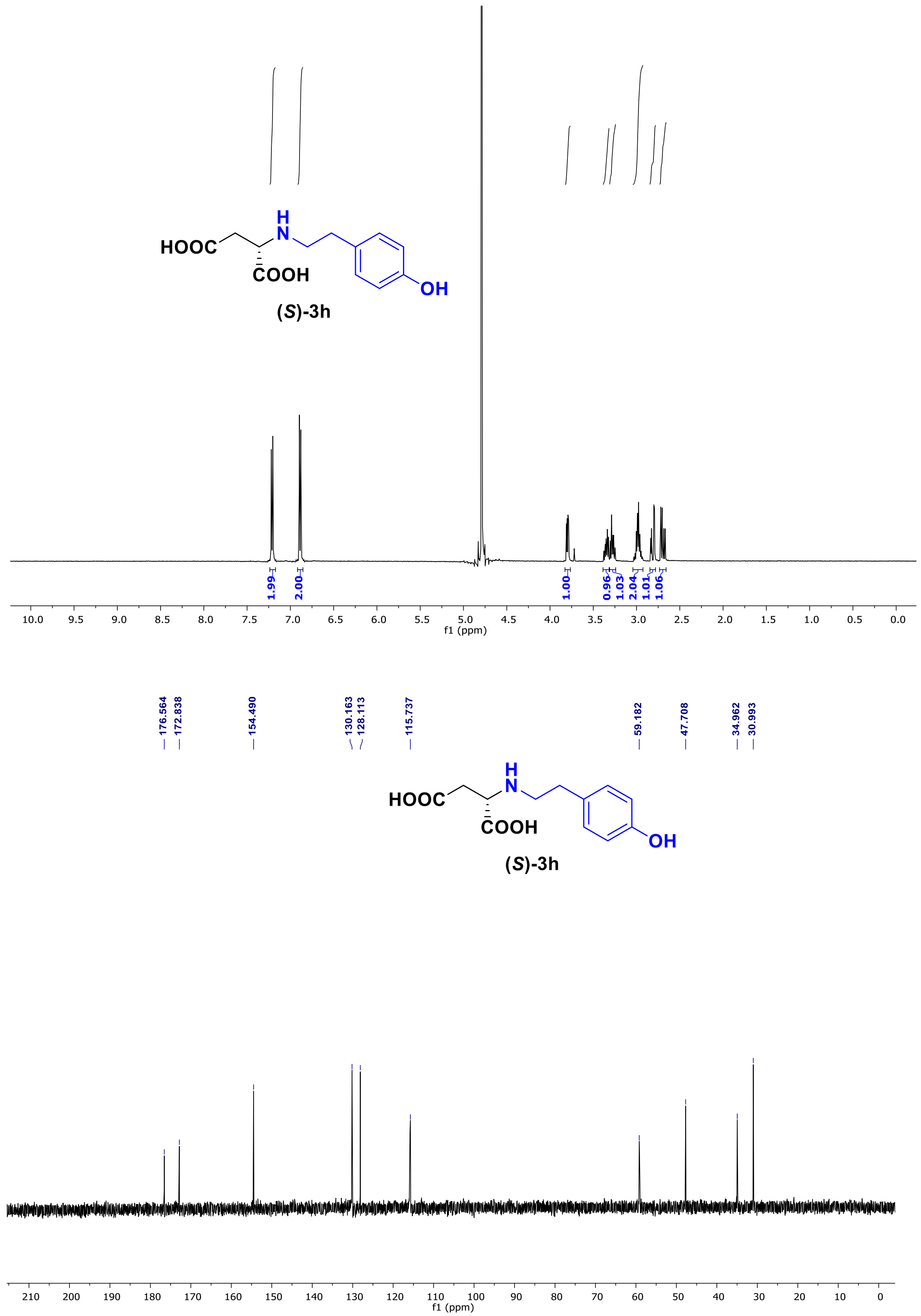


Figure S26. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-hydroxyphenethyl)-L-aspartic acid [(*S*)-**3h**].

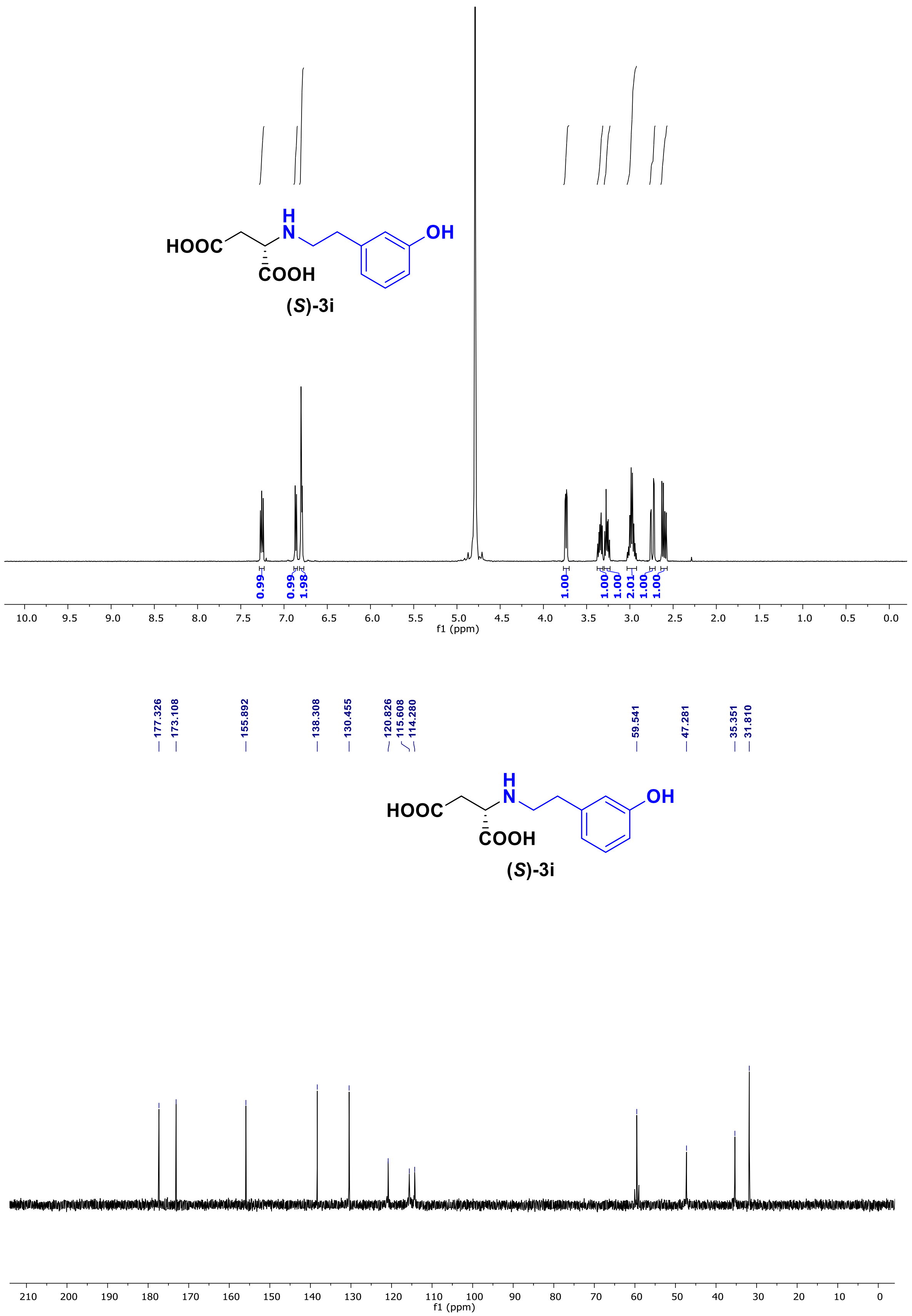


Figure S27. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(3-hydroxyphenethyl)-L-aspartic acid [(*S*)-**3i**].

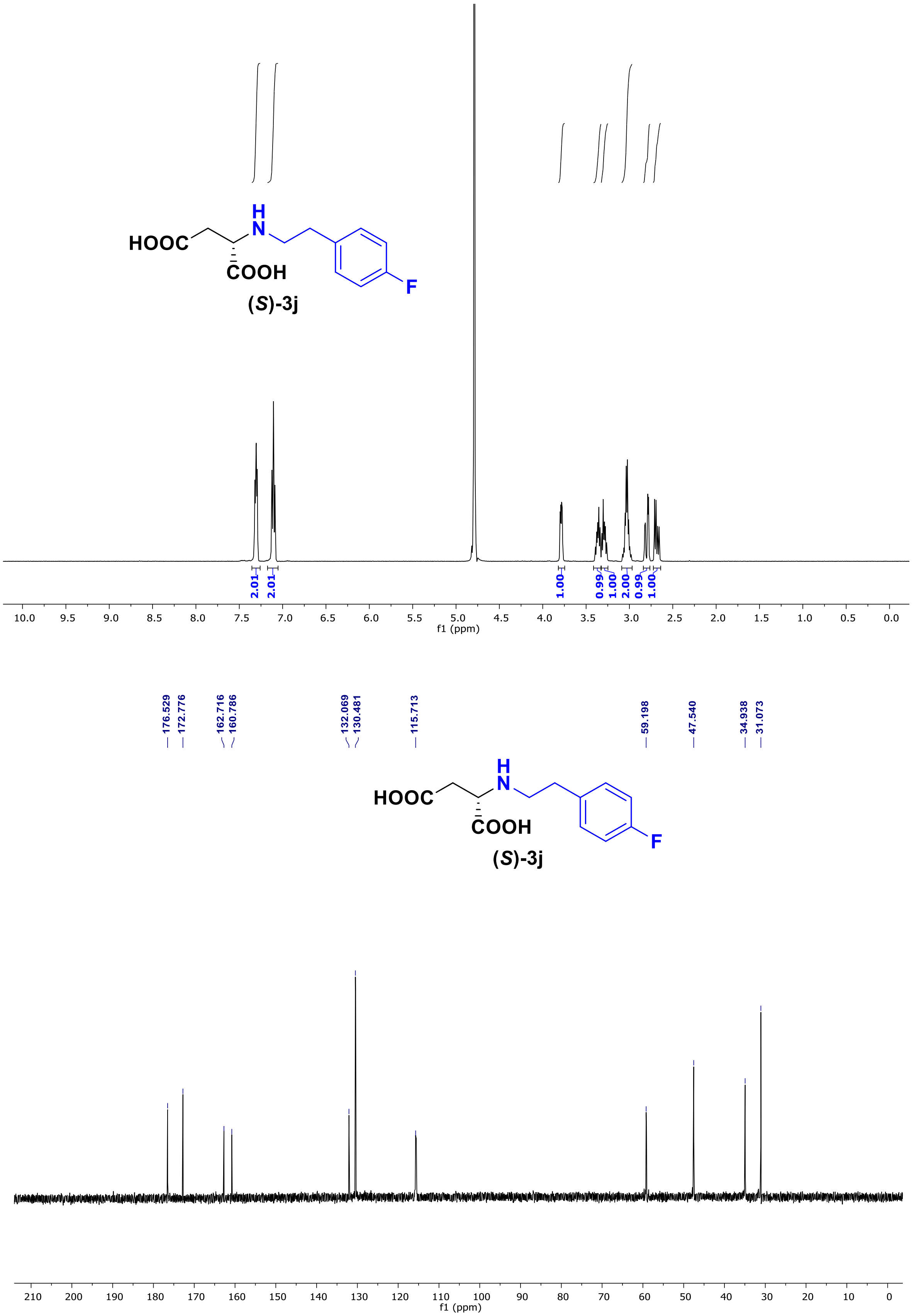


Figure S28. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-fluorophenethyl)-L-aspartic acid [(*S*)-**3j**].

3.2 ^{1}H and ^{13}C NMR spectra of chemically obtained *N*-arylalkyl-substituted D- and L-aspartic acids

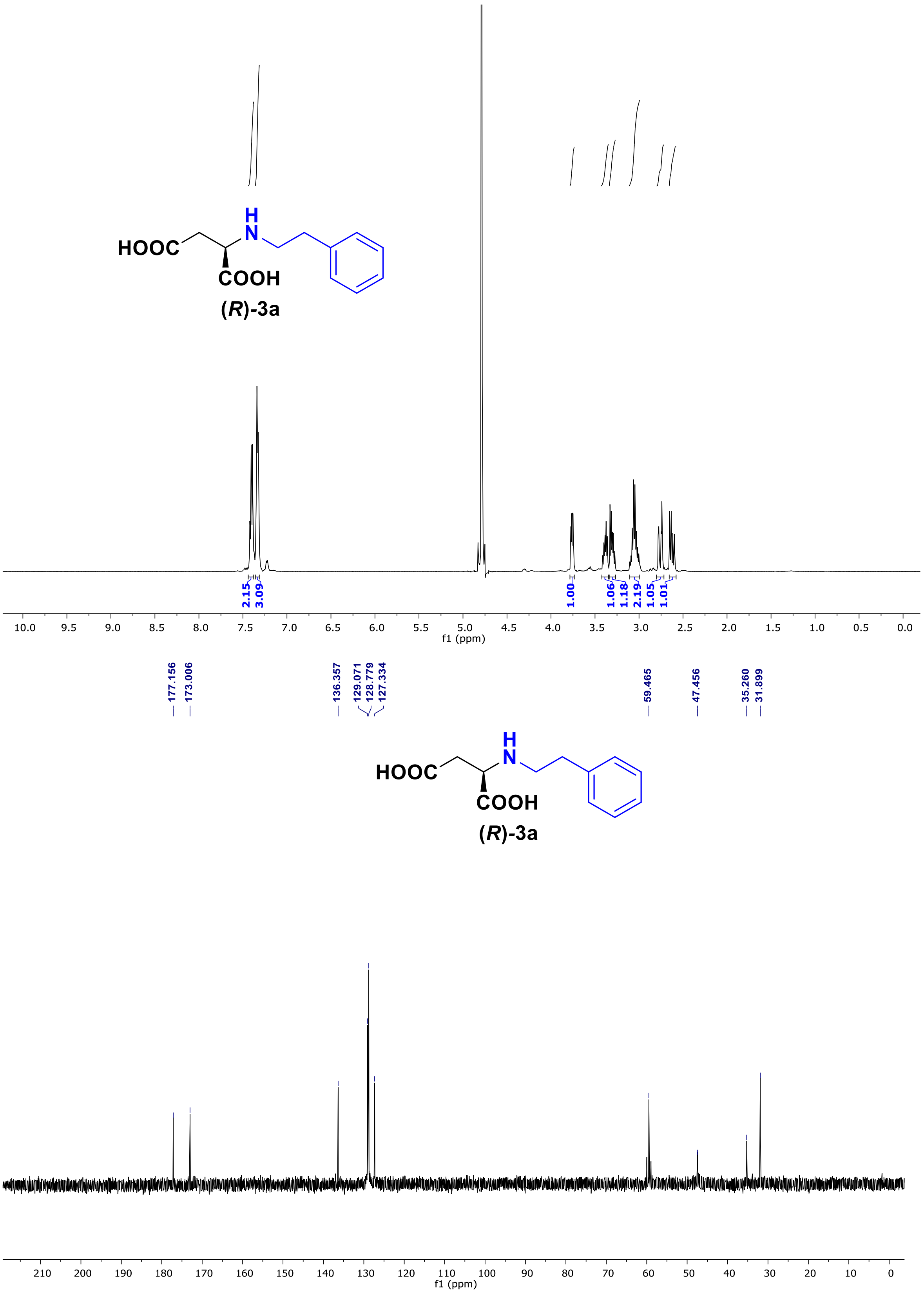


Figure S29. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(phenethyl)-D-aspartic acid [(*R*)-**3a**].

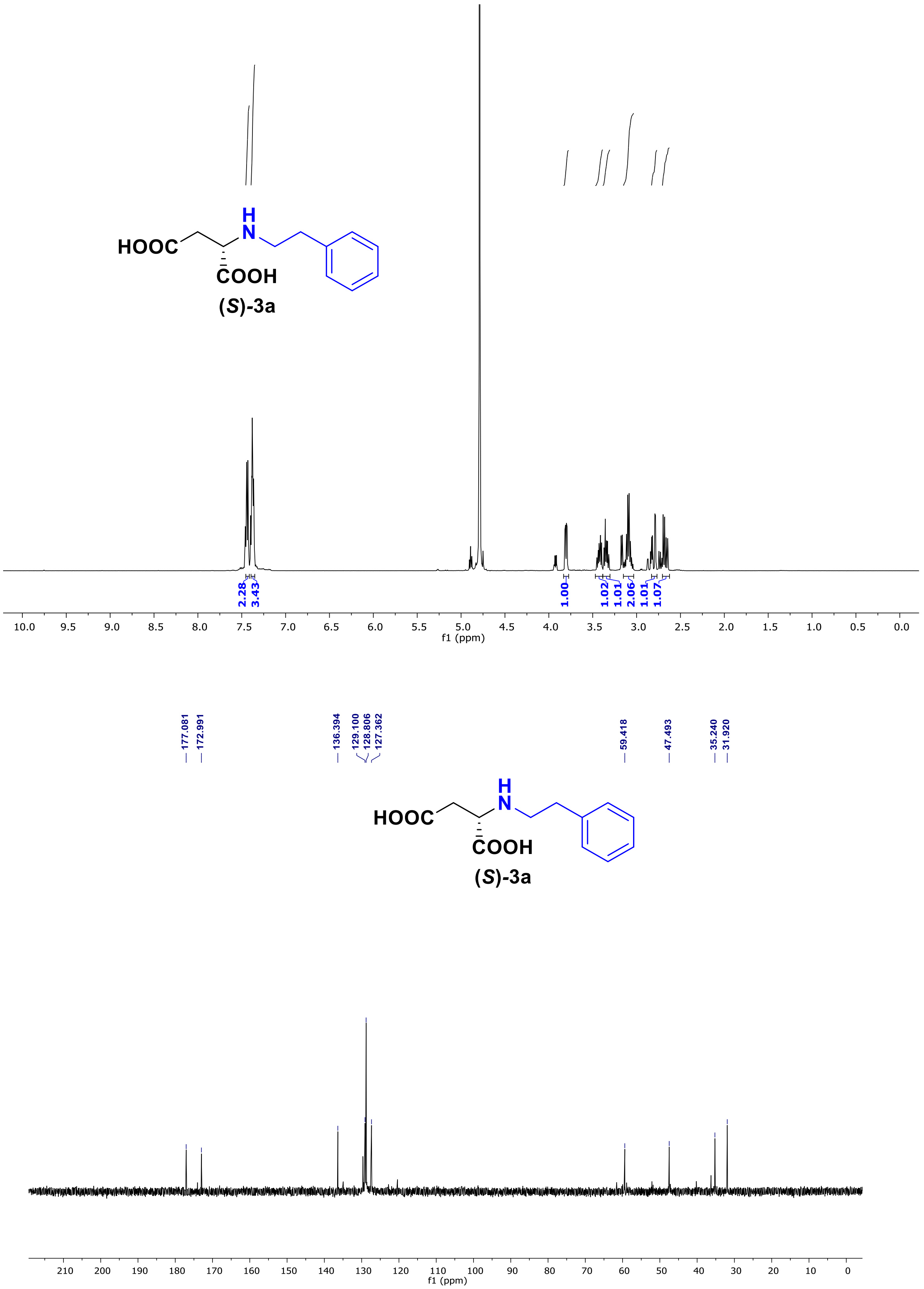


Figure S30. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(phenethyl)-L-aspartic acid [(*S*)-**3a**].

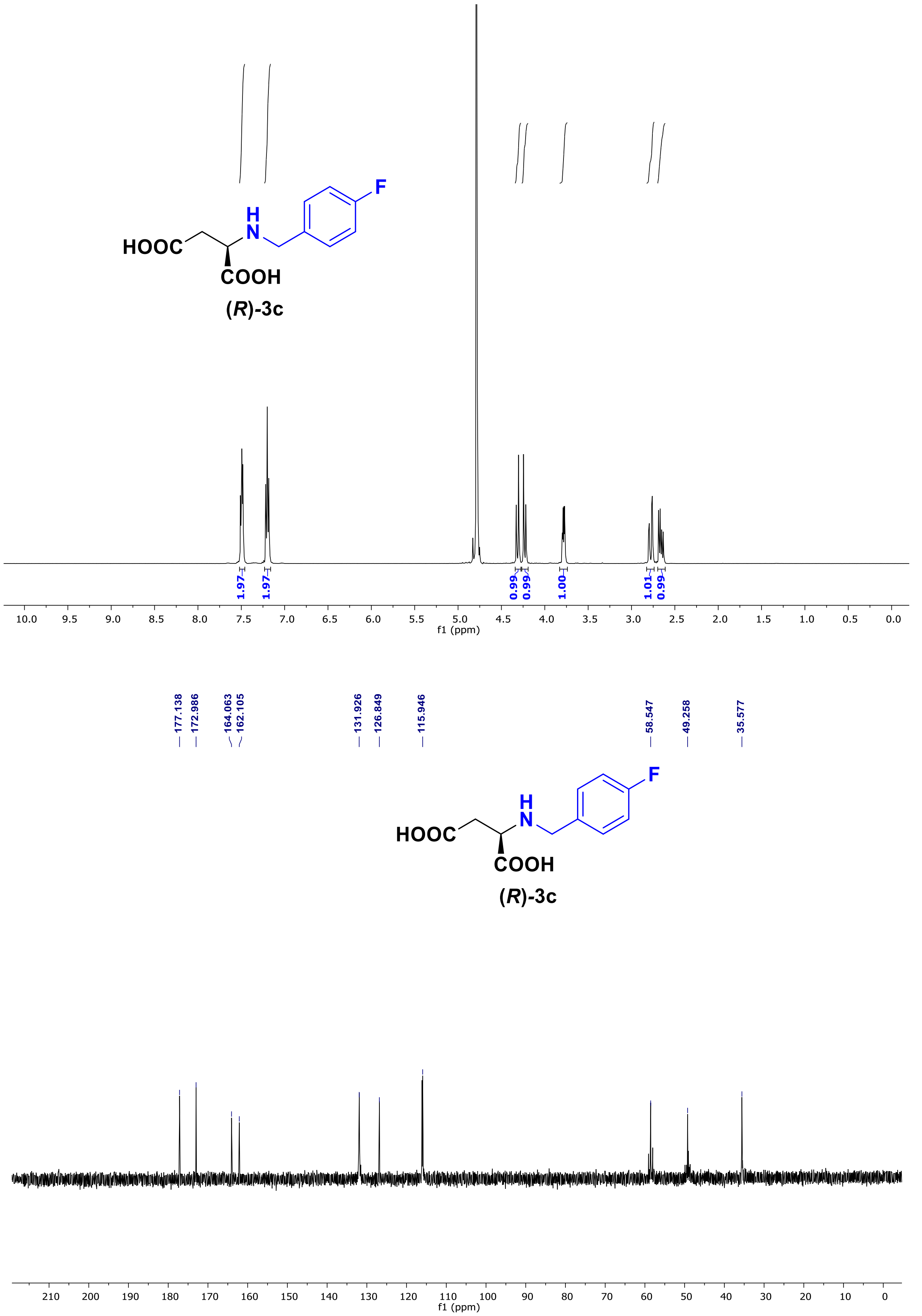


Figure S31. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-fluorobenzyl)-D-aspartic acid [(*R*)-**3c**].

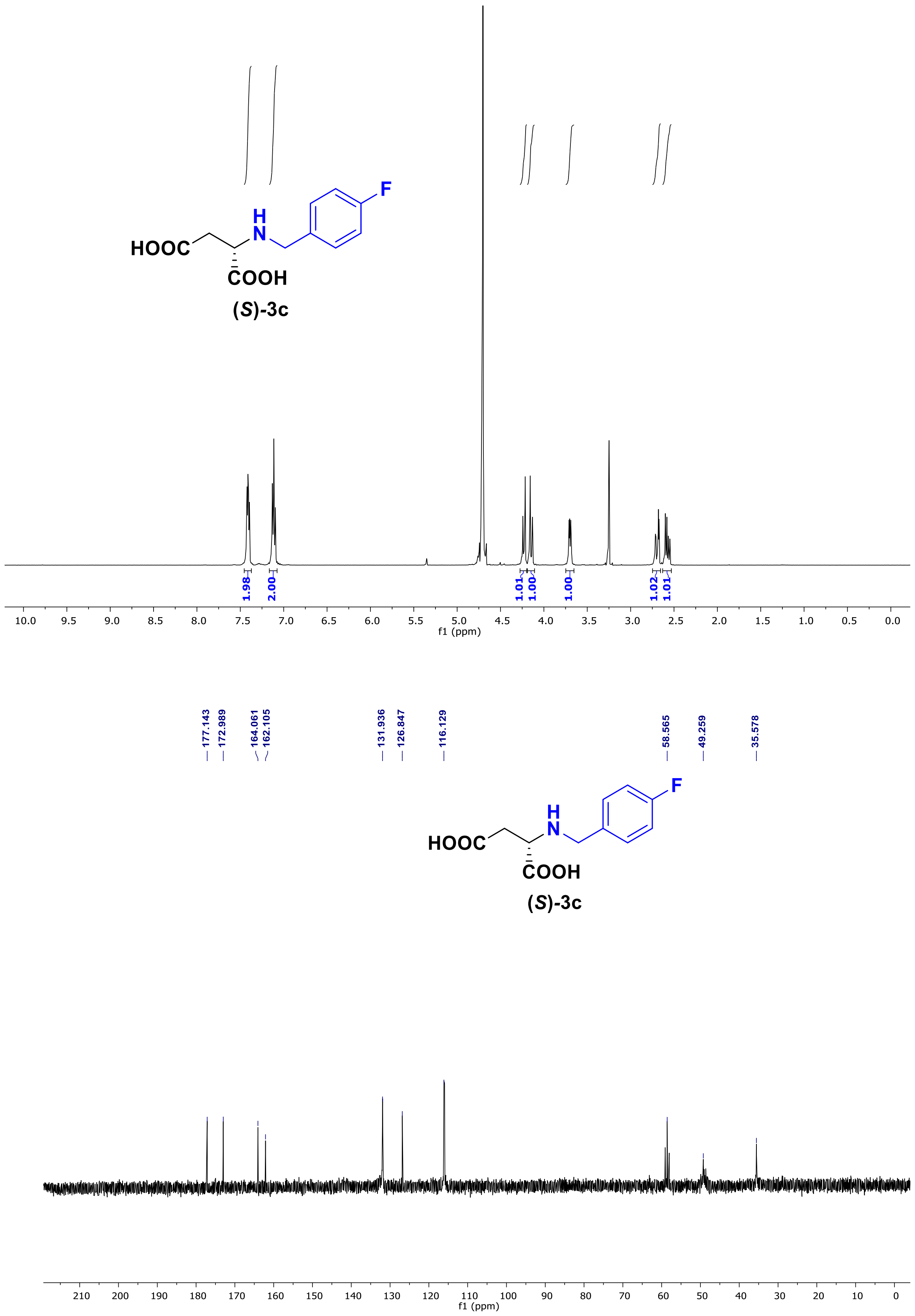


Figure S32. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-fluorobenzyl)-L-aspartic acid [(*S*)-**3c**].

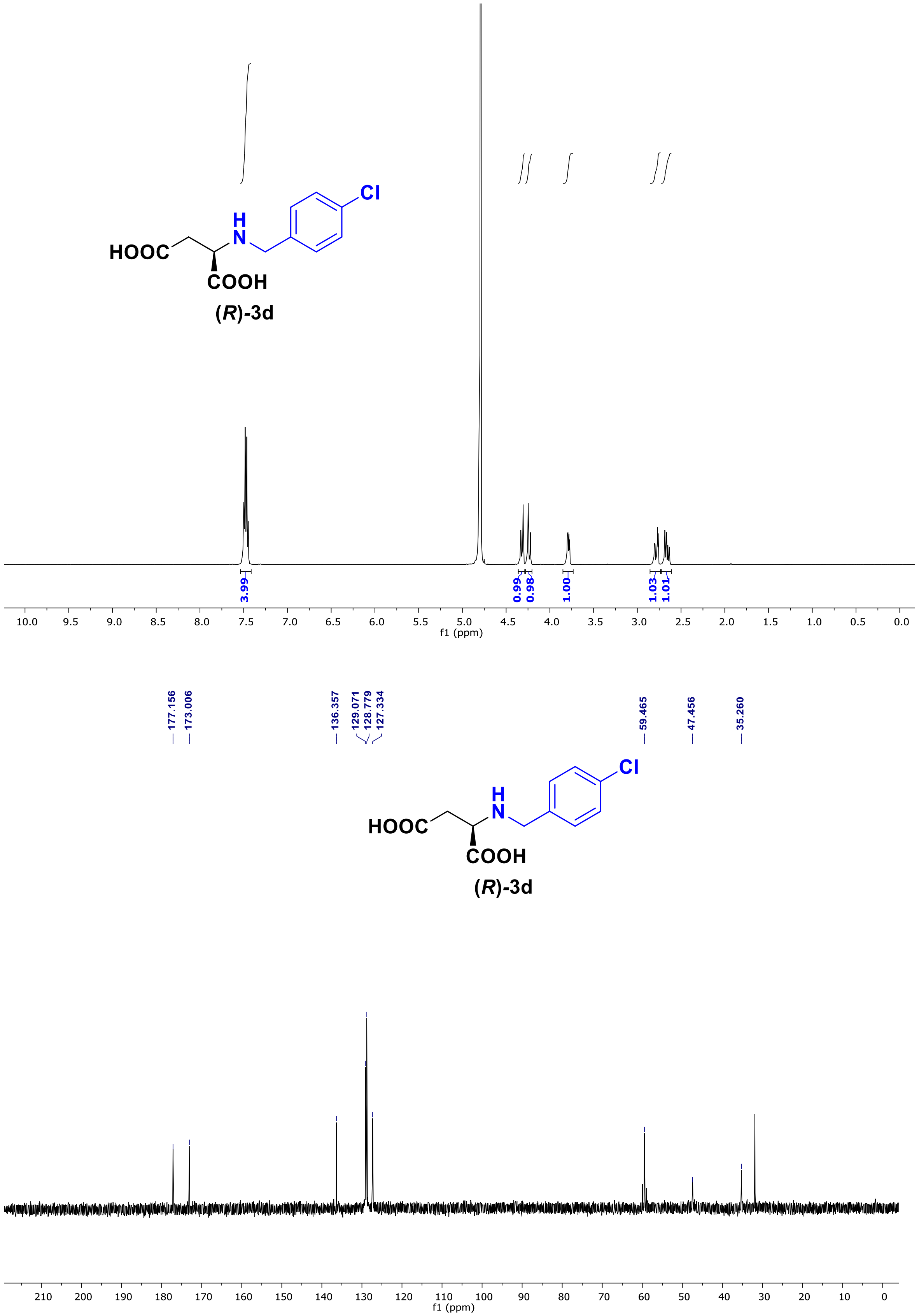


Figure S33. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-chlorobenzyl)-D-aspartic acid [(*R*)-**3d**].

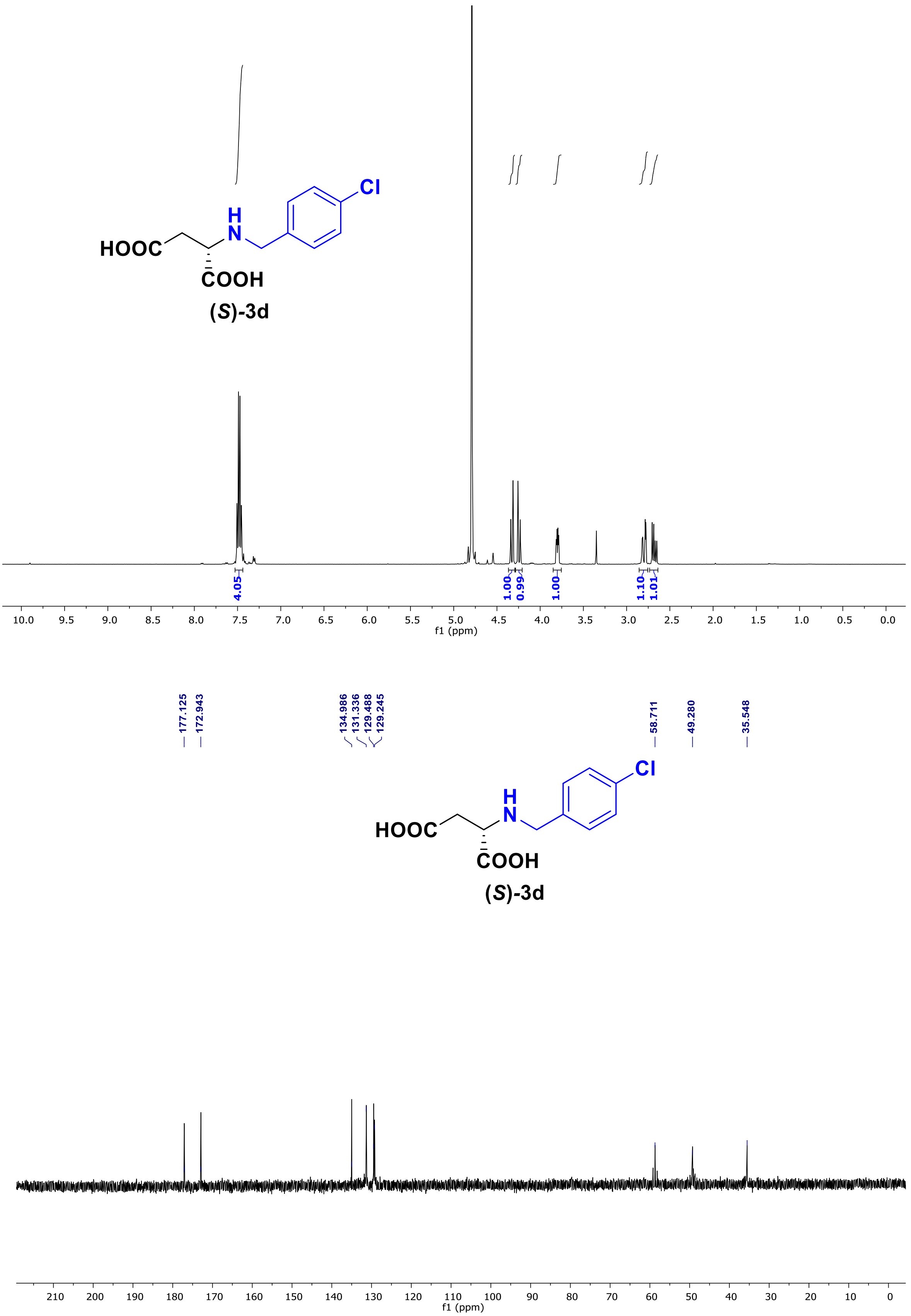


Figure S34. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-chlorobenzyl)-L-aspartic acid [(*S*)-**3d**].

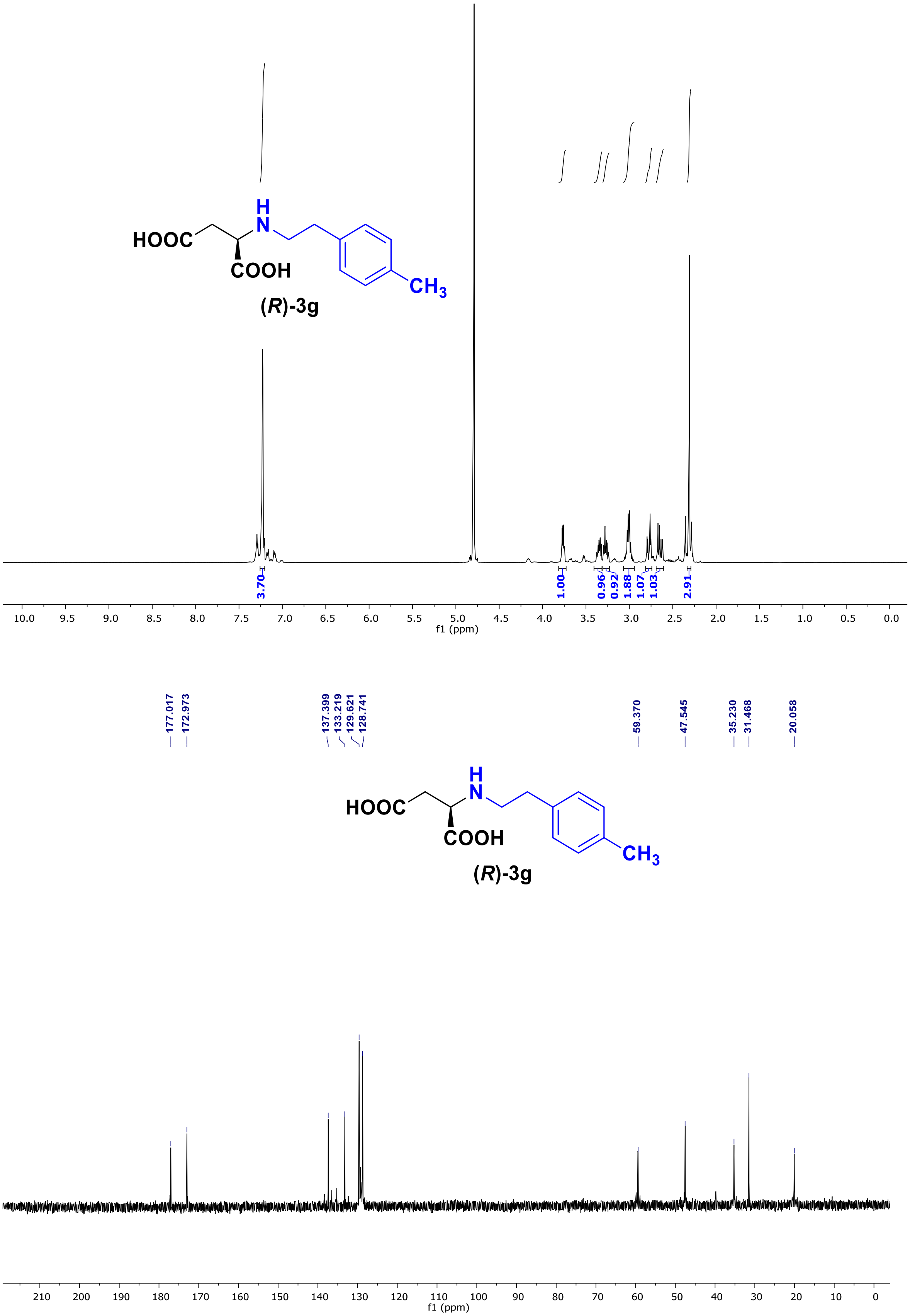


Figure S35. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-methylphenethyl)-D-aspartic acid [(*R*)-**3g**].

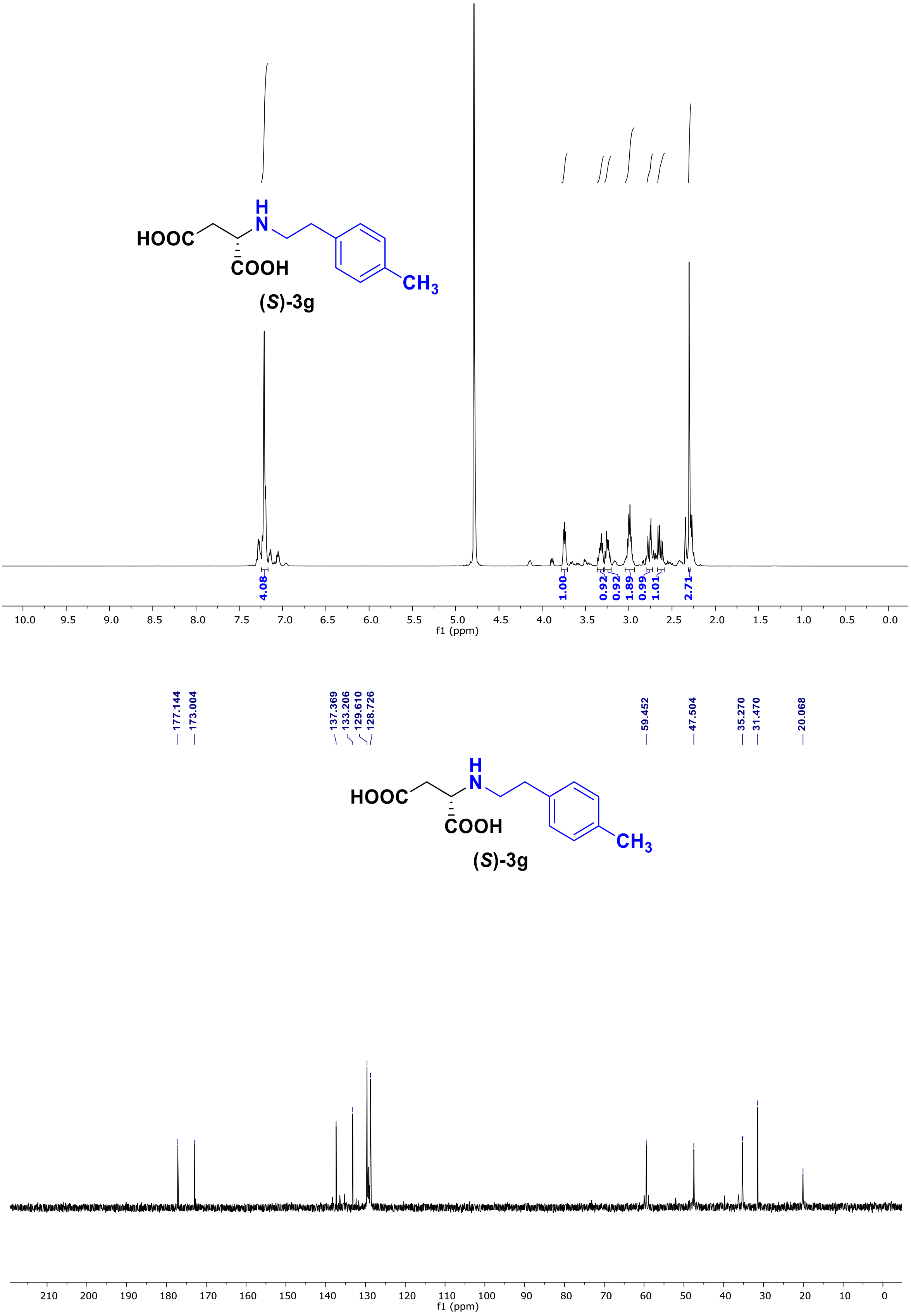


Figure S36. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-methylphenethyl)-L-aspartic acid [(*S*)-**3g**].

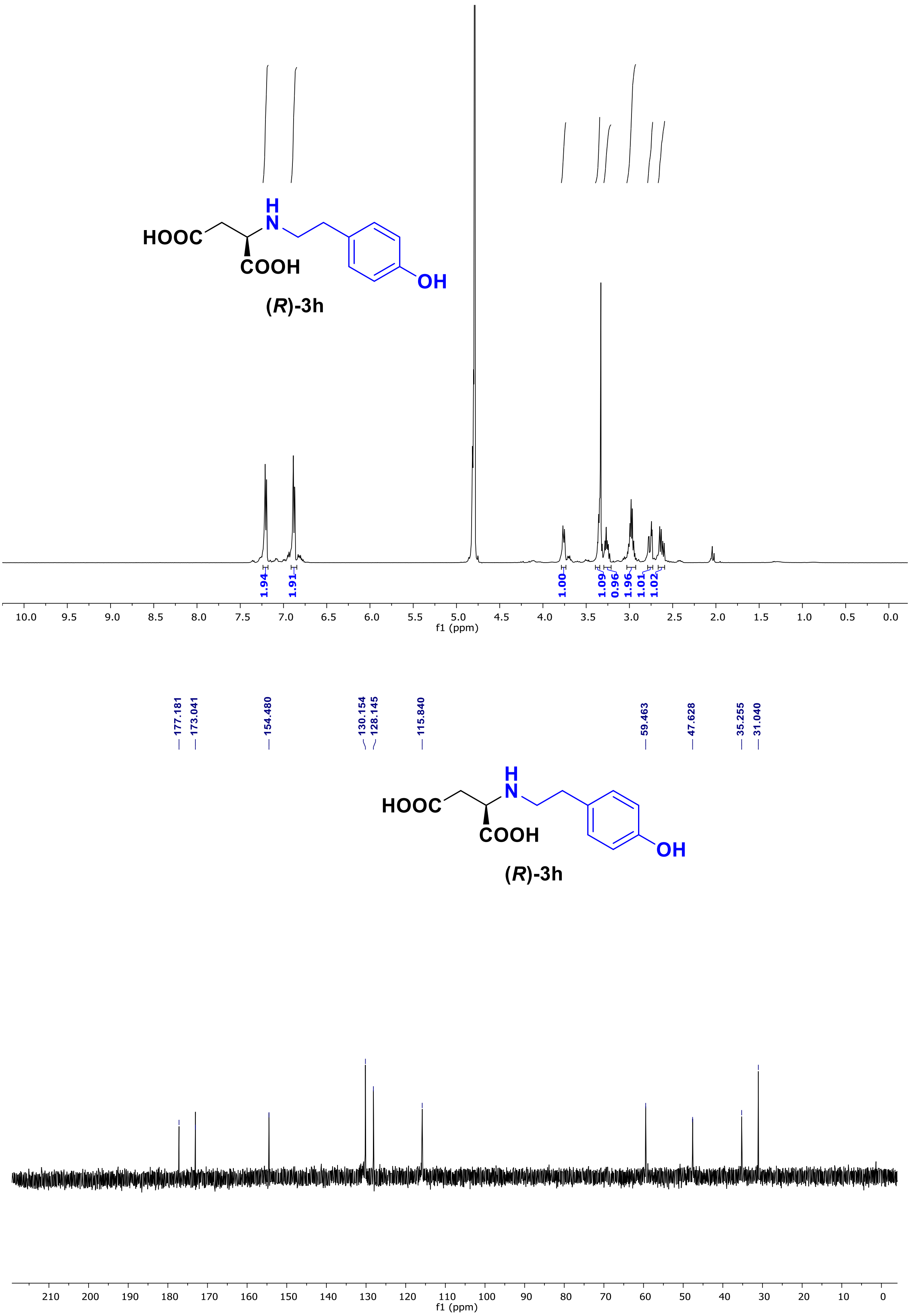


Figure S37. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-hydroxyphenethyl)-D-aspartic acid [(*R*)-**3h**].

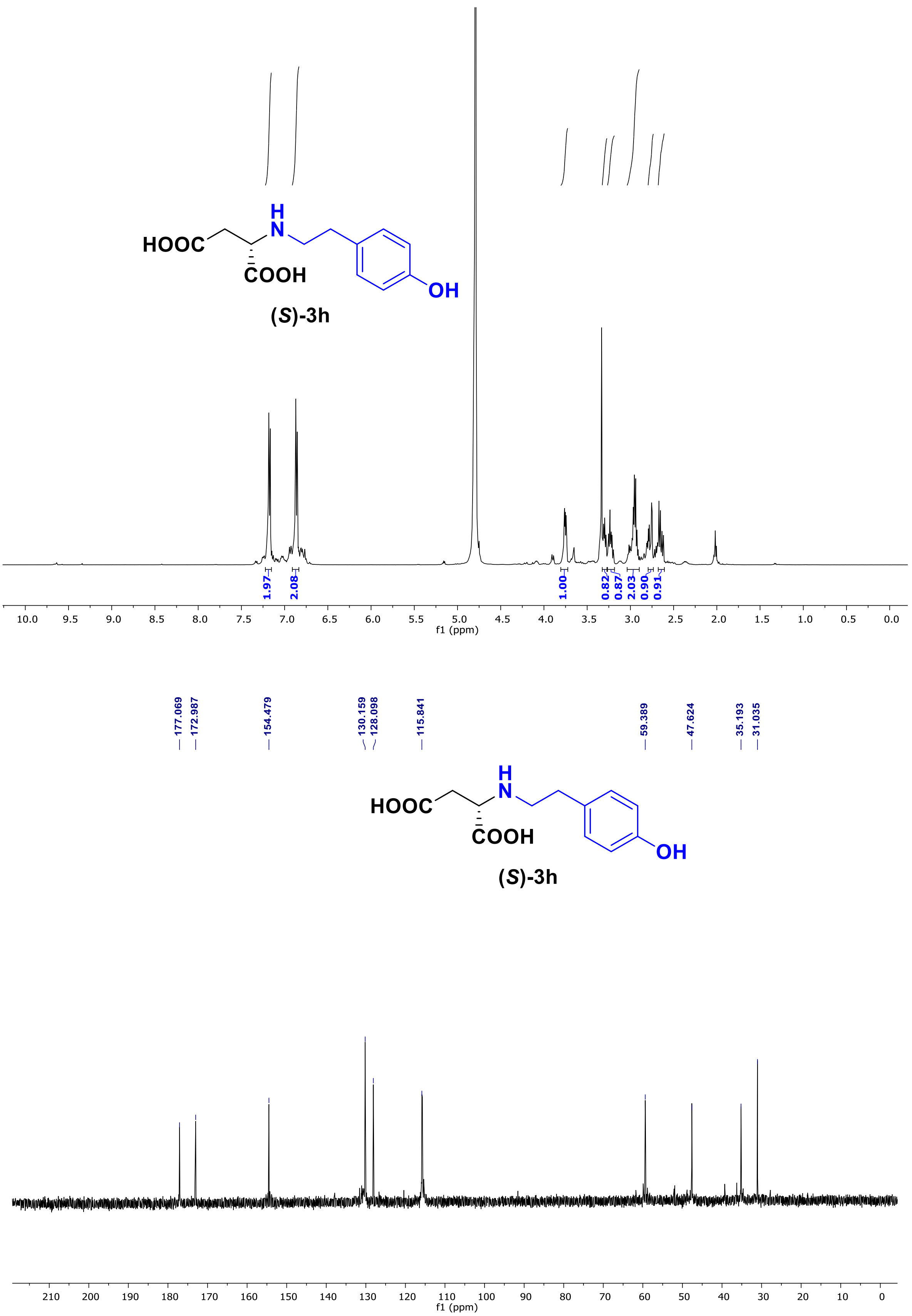


Figure S38. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-hydroxyphenethyl)-L-aspartic acid [(*S*)-**3h**].

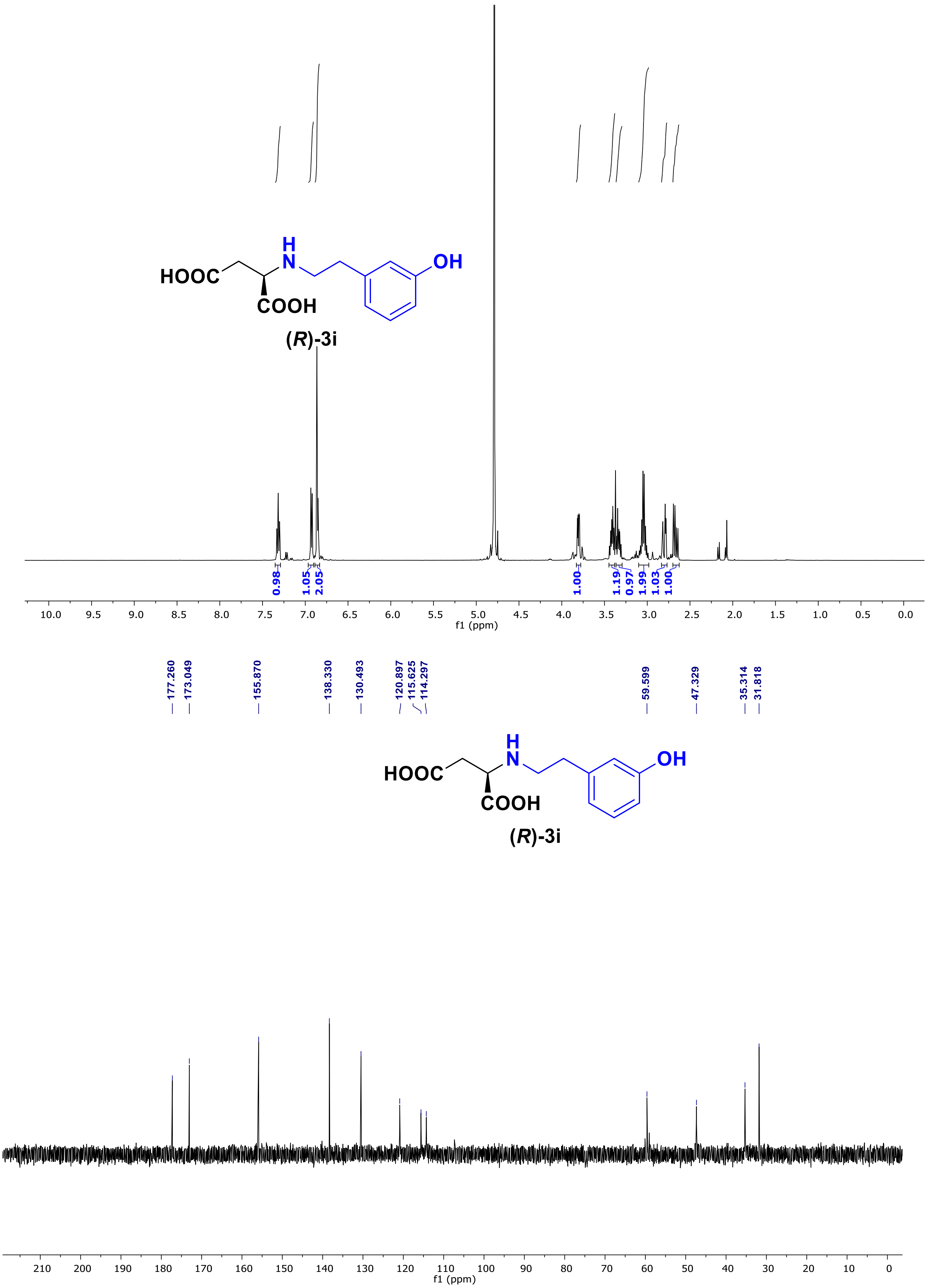


Figure S39. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(3-hydroxyphenethyl)-D-aspartic acid [(*R*)-**3i**].

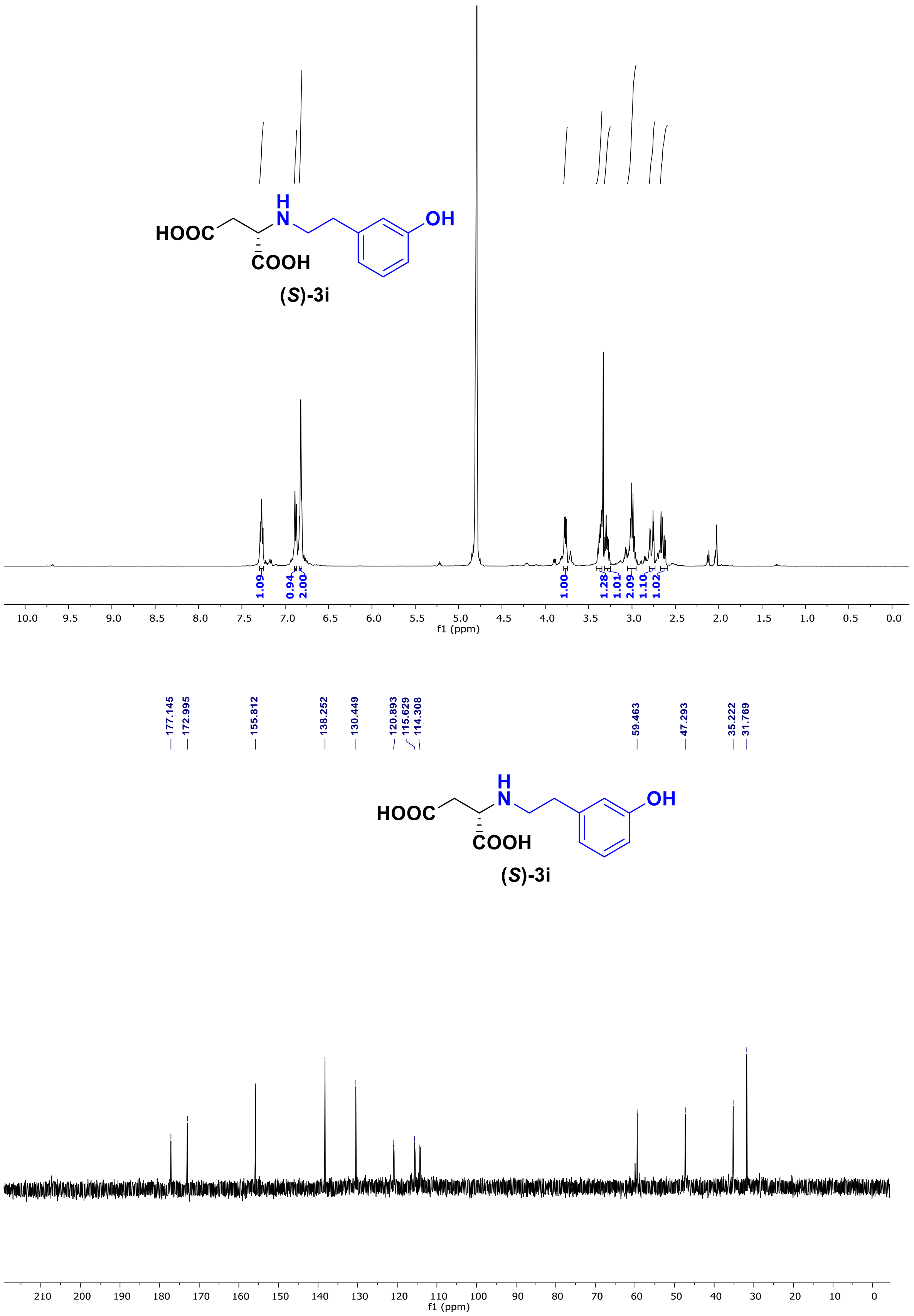


Figure S40. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(3-hydroxyphenethyl)-L-aspartic acid [(*S*)-**3i**].

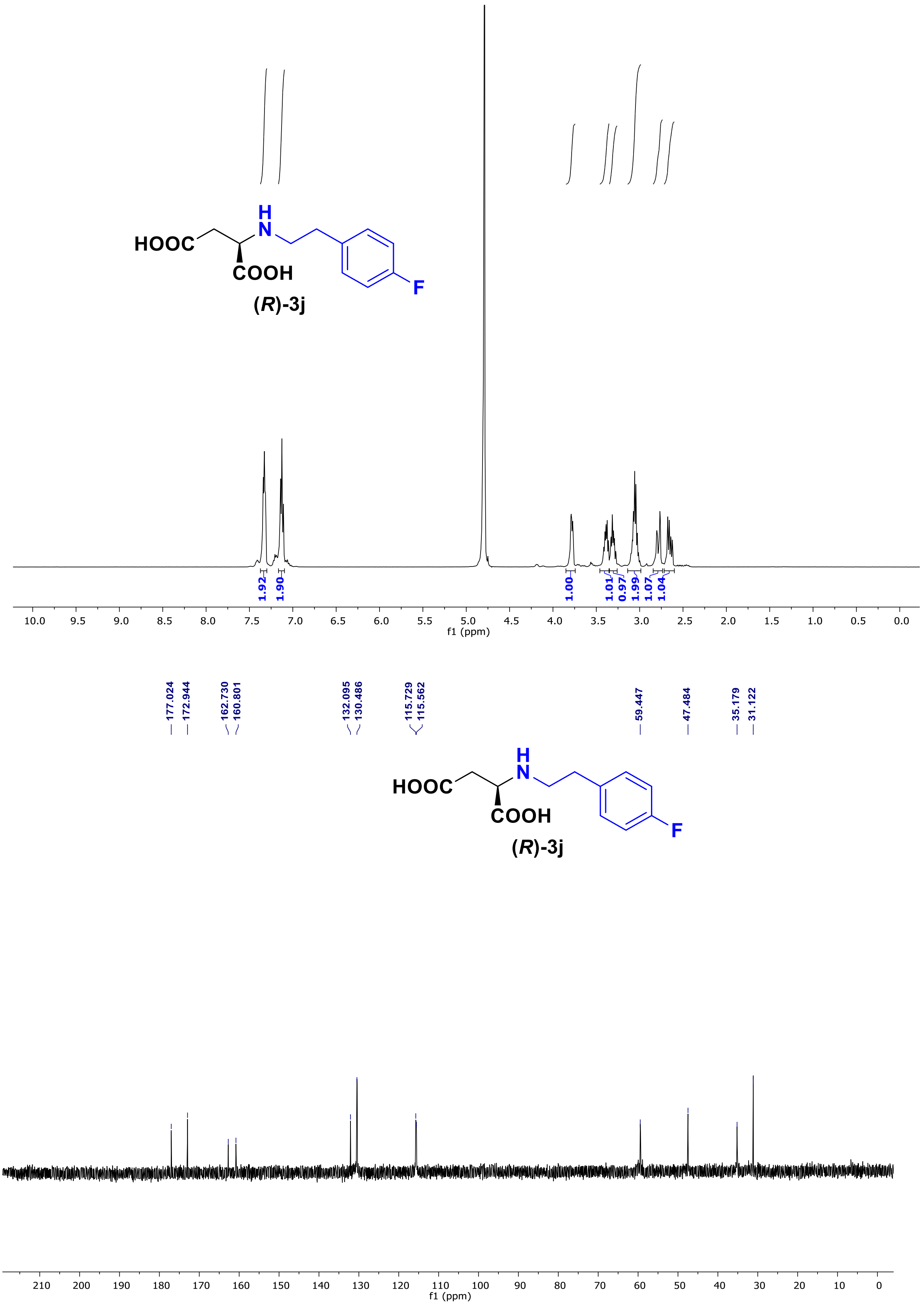


Figure S41. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-fluorophenethyl)-D-aspartic acid [(*R*)-**3j**].

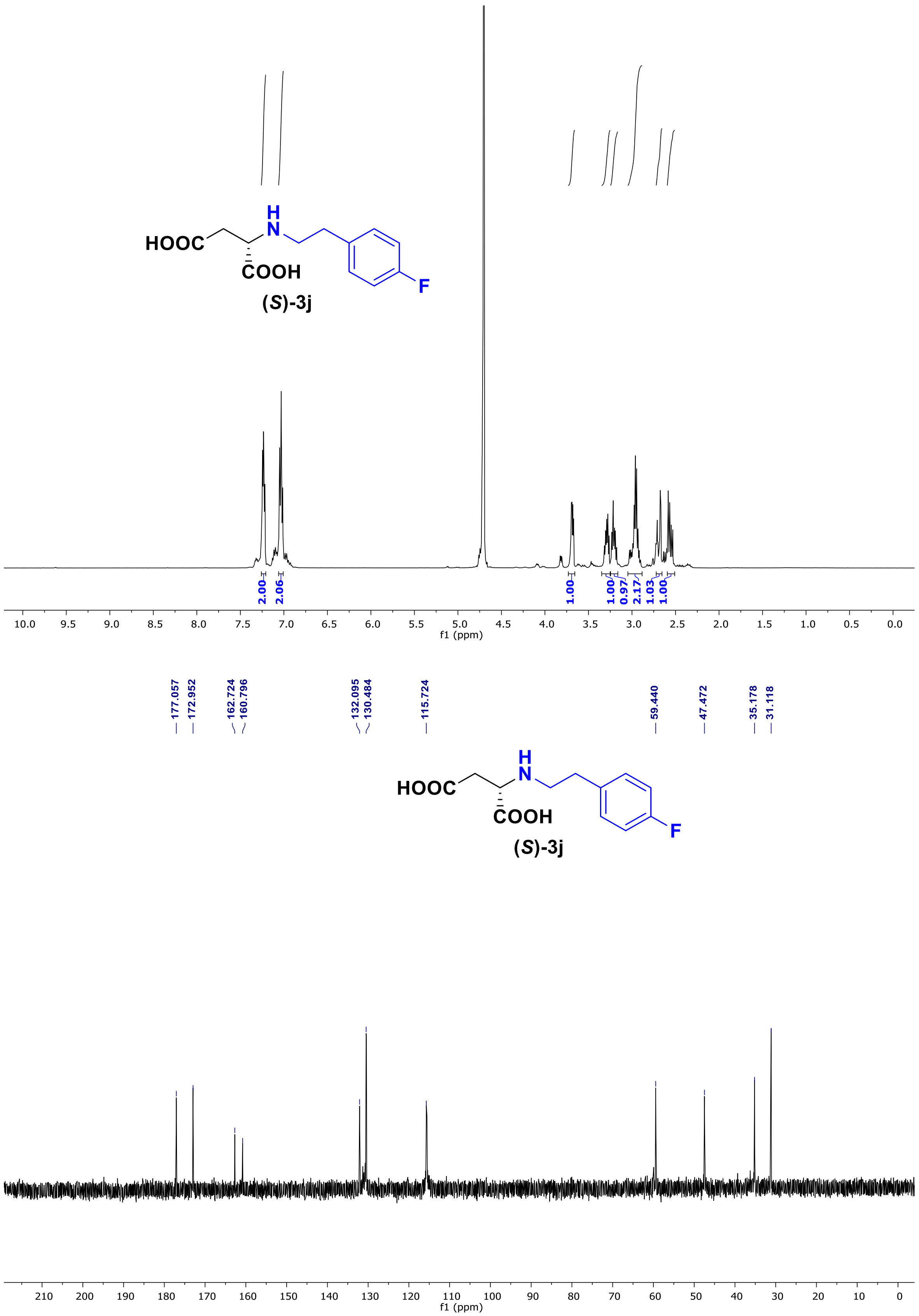


Figure S42. ^{1}H NMR (top) and ^{13}C NMR (bottom) of *N*-(4-fluorophenethyl)-L-aspartic acid [(*S*)-**3j**].

4. LC-HRMS analysis

LC-HRMS data of enzymatically obtained *N*-arylalkyl-substituted L-aspartic acids

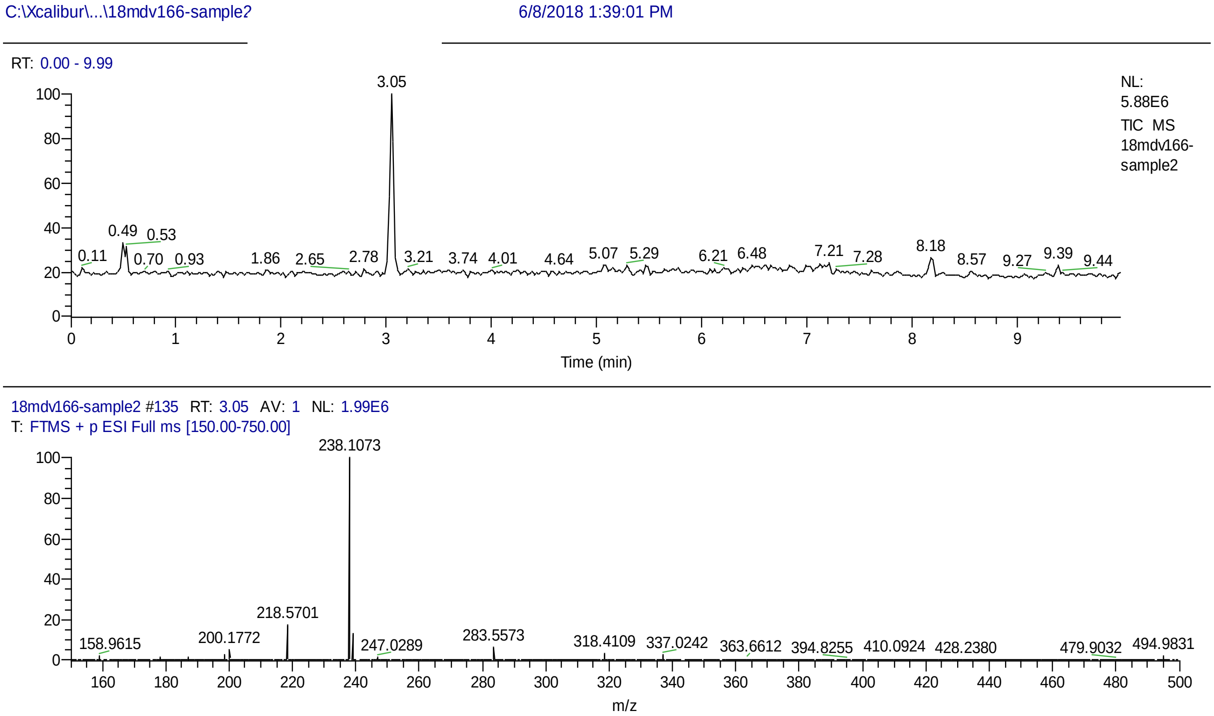


Figure S43. LC-HRMS spectrum of enzymatic product (*S*)-**3a**.

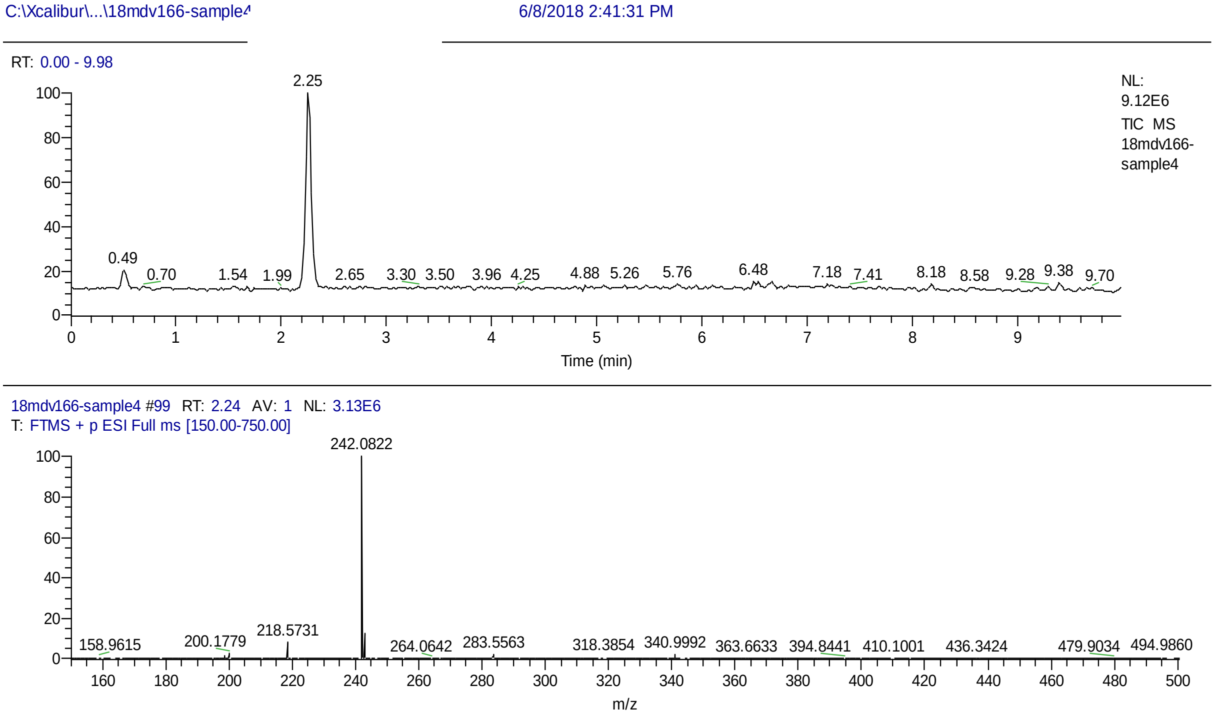


Figure S44. LC-HRMS spectrum of enzymatic product (*S*)-**3c**.

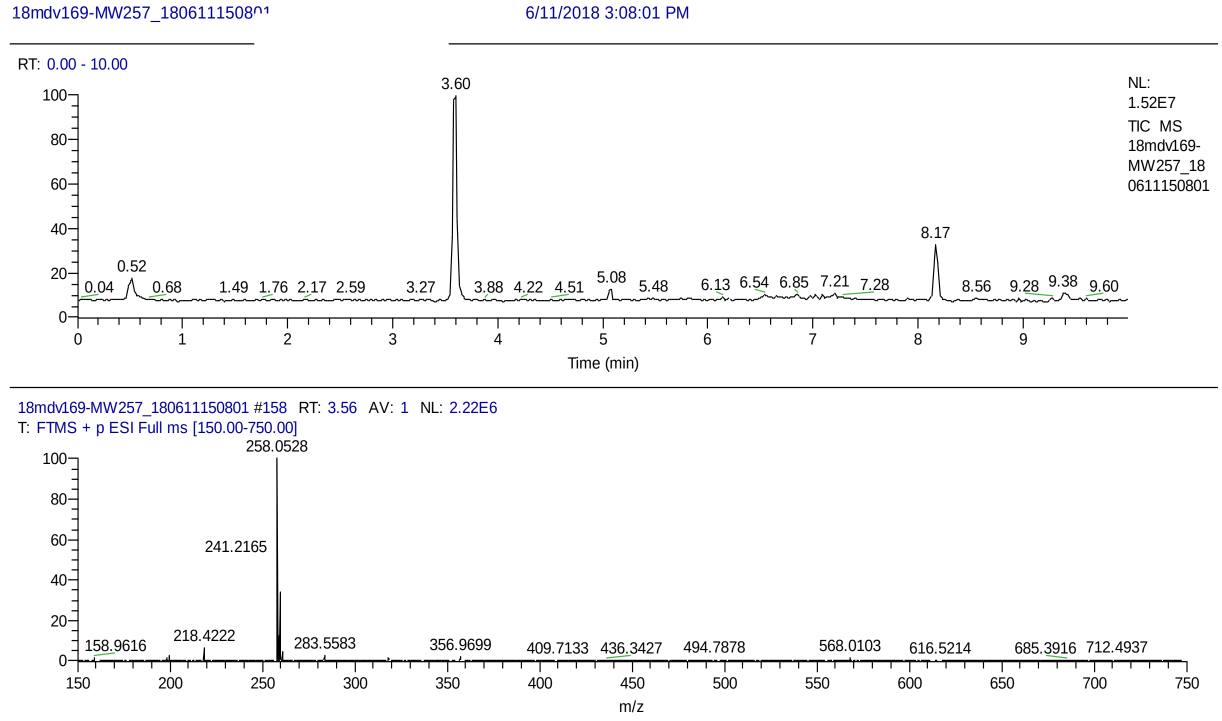


Figure S45. LC-HRMS spectrum of enzymatic product (*S*)-**3d**.

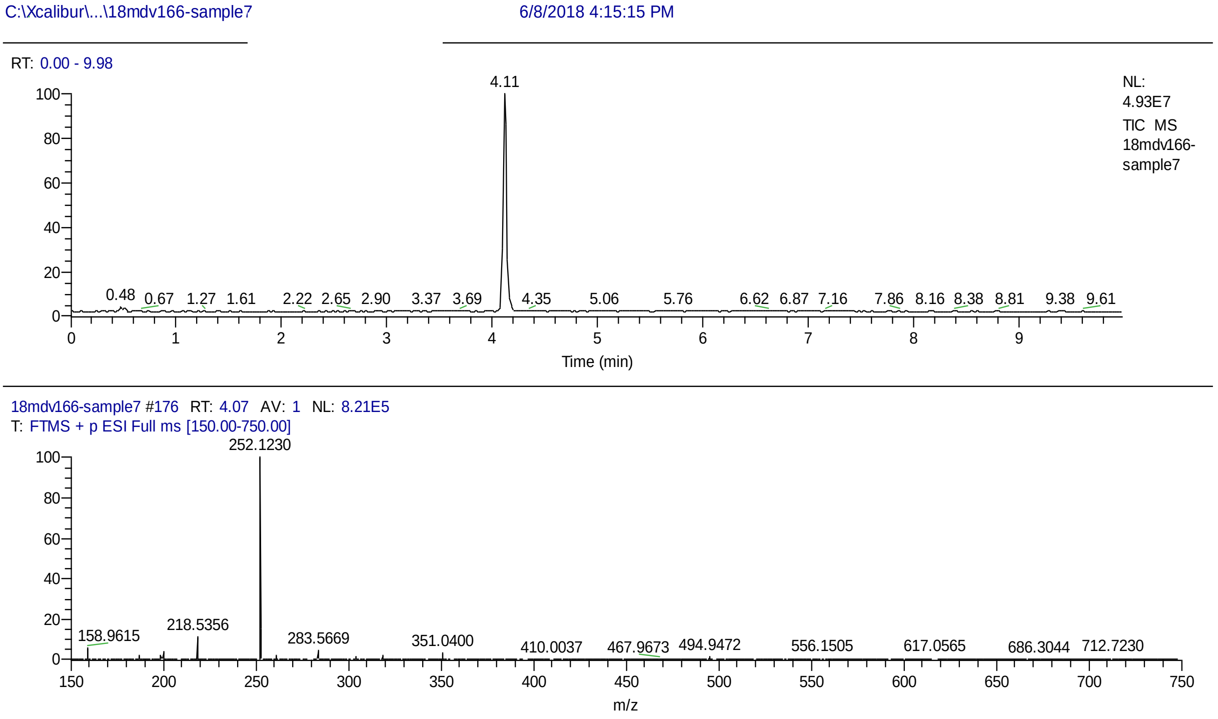


Figure S46. LC-HRMS spectrum of enzymatic product (*S*)-**3g**.

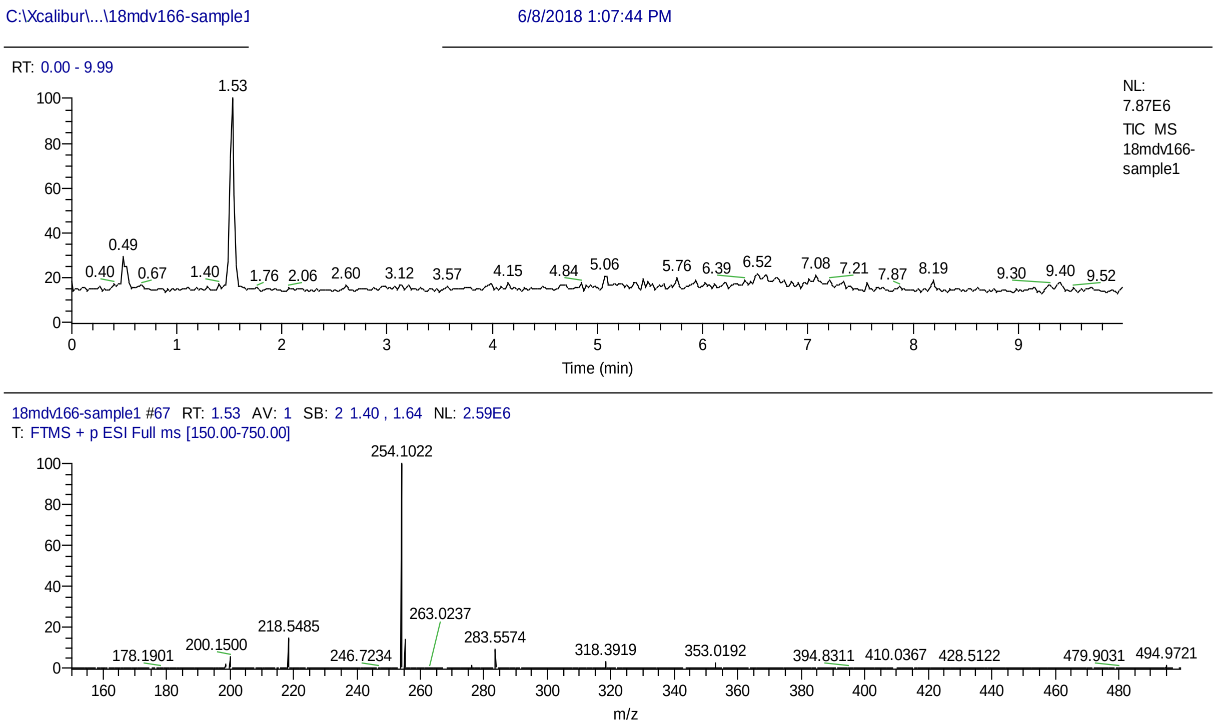


Figure S47. LC-HRMS spectrum of enzymatic product (*S*)-**3h**.

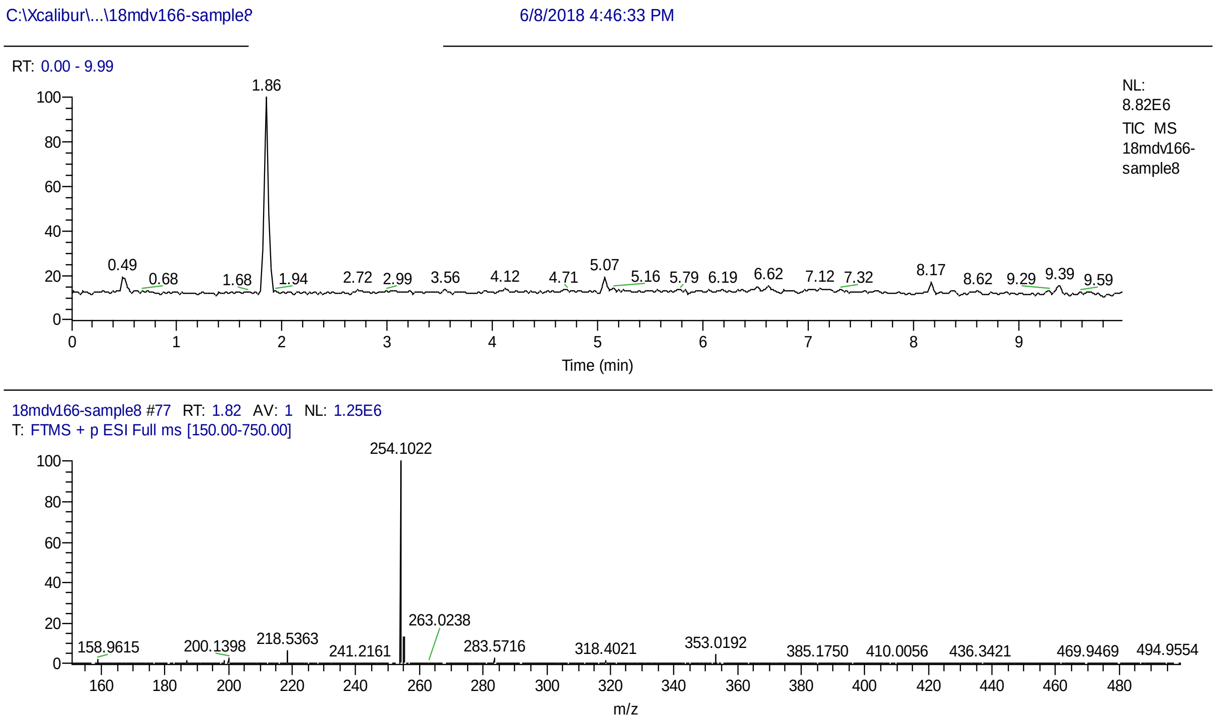


Figure S48. LC-HRMS spectrum of enzymatic product (*S*)-**3i**.

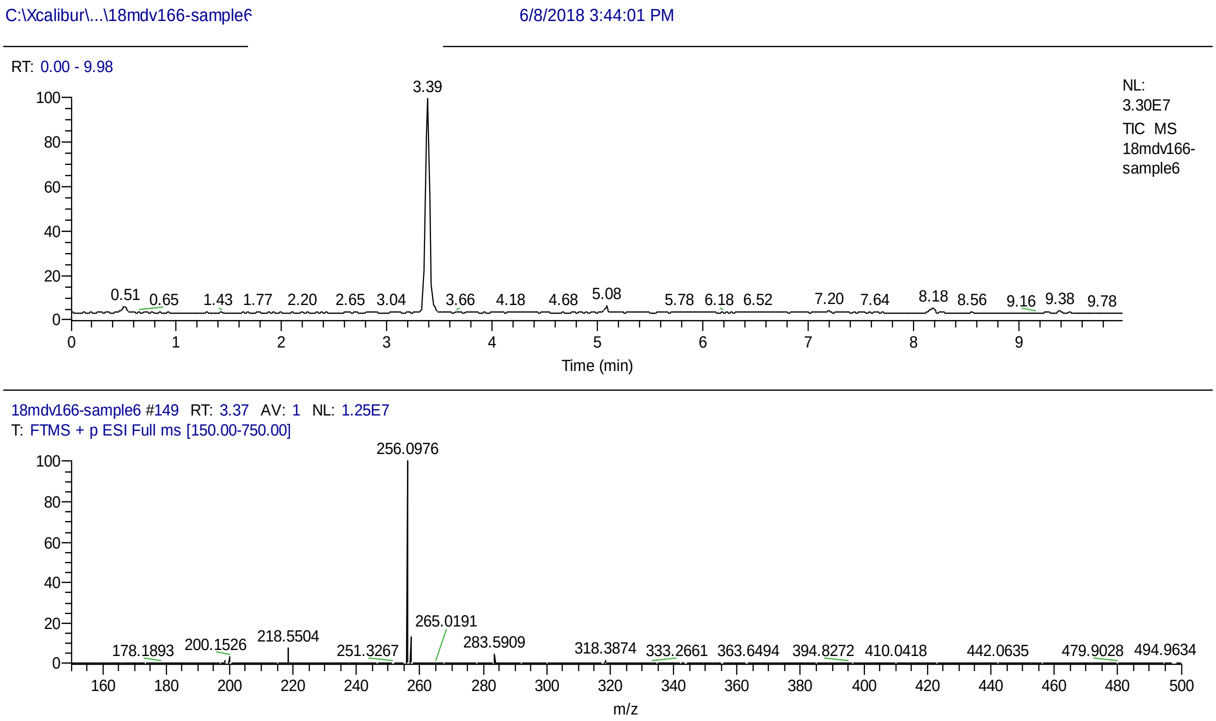


Figure S49. LC-HRMS spectrum of enzymatic product (*S*)-**3j**.

LC-HRMS data of chemically synthesized *N*-arylalkyl-substituted L- or D-aspartic acids

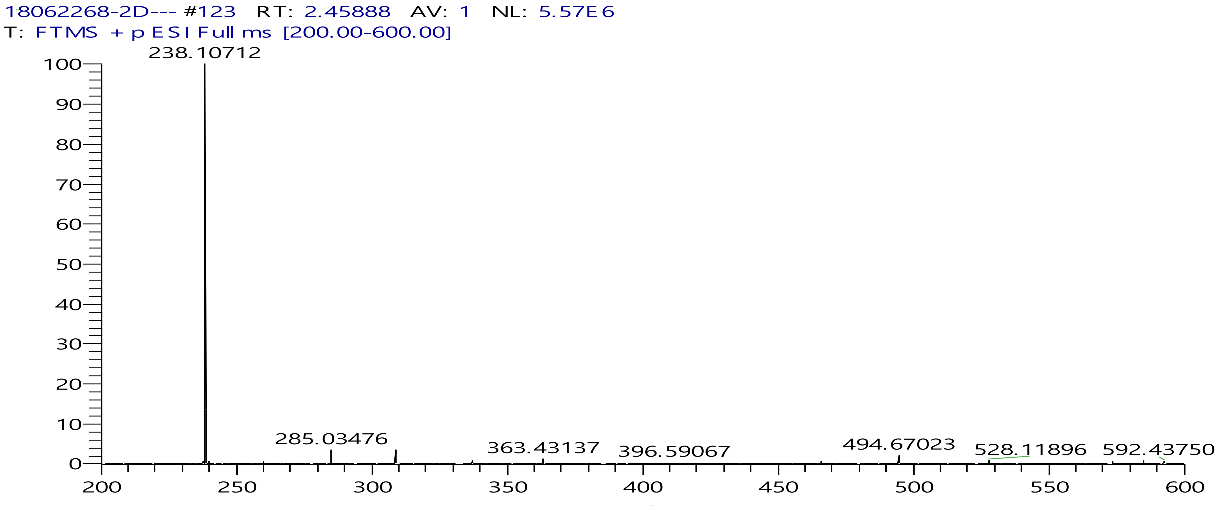


Figure S50. LC-HRMS spectrum of chemical reference (*R*)-**3a**.

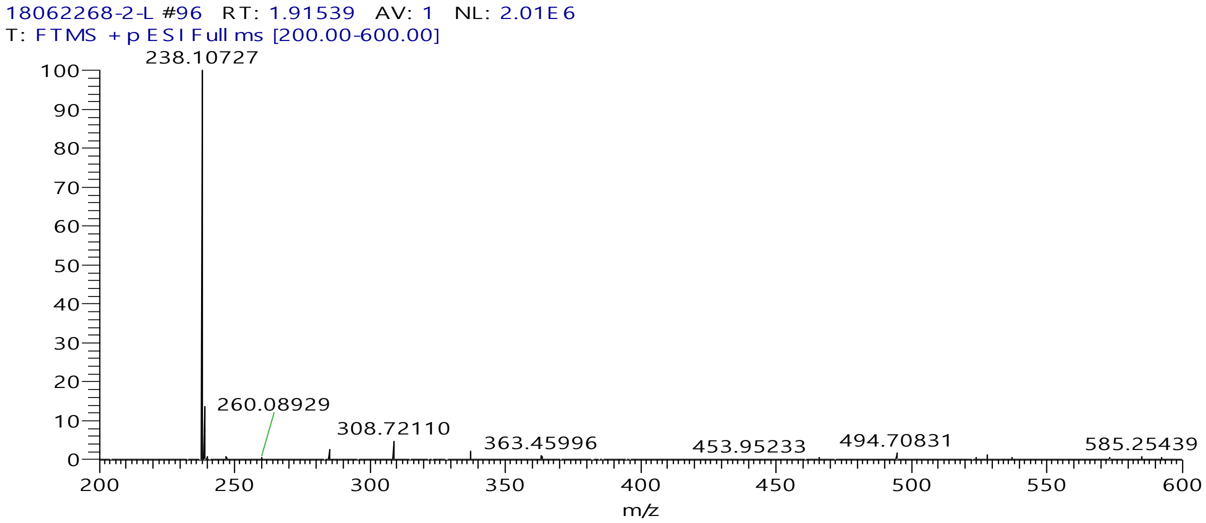


Figure S51. LC-HRMS spectrum of chemical reference (*S*)-**3a**.

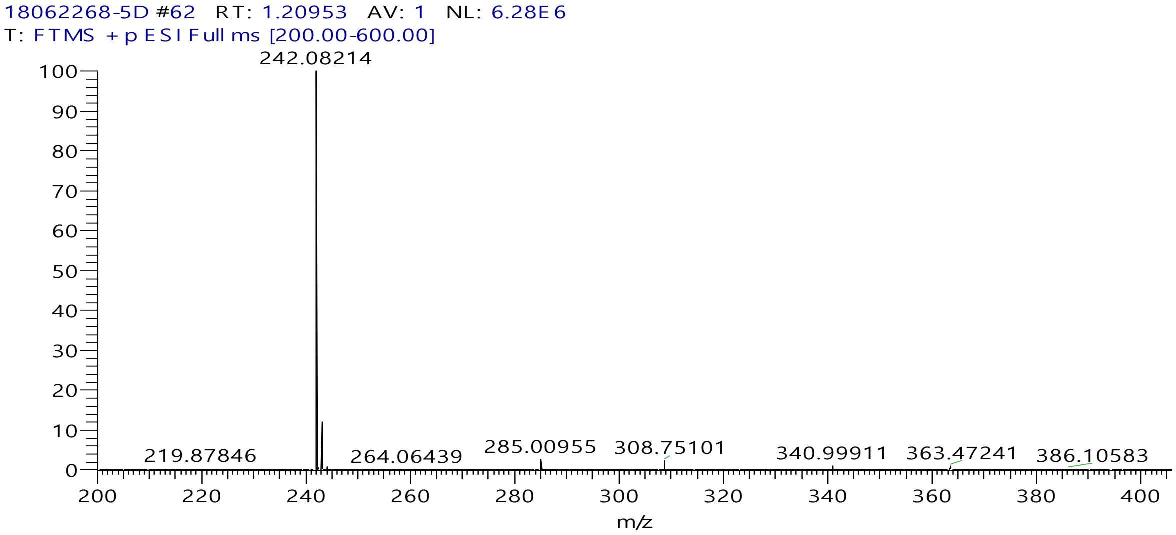


Figure S52. LC-HRMS spectrum of chemical reference (*R*)-**3c**.

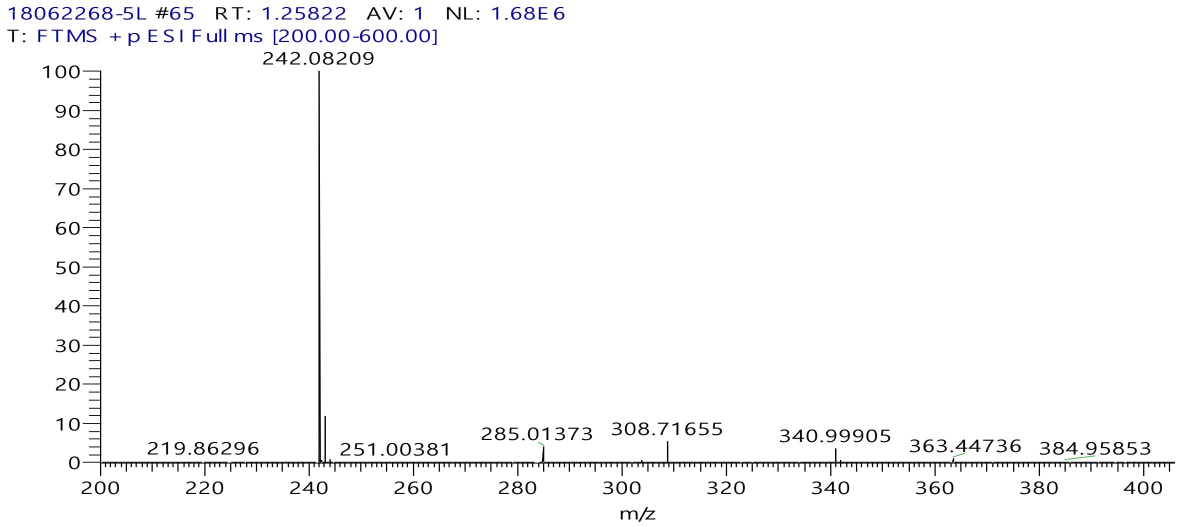


Figure S53. LC-HRMS spectrum of chemical reference (*S*)-**3c**.

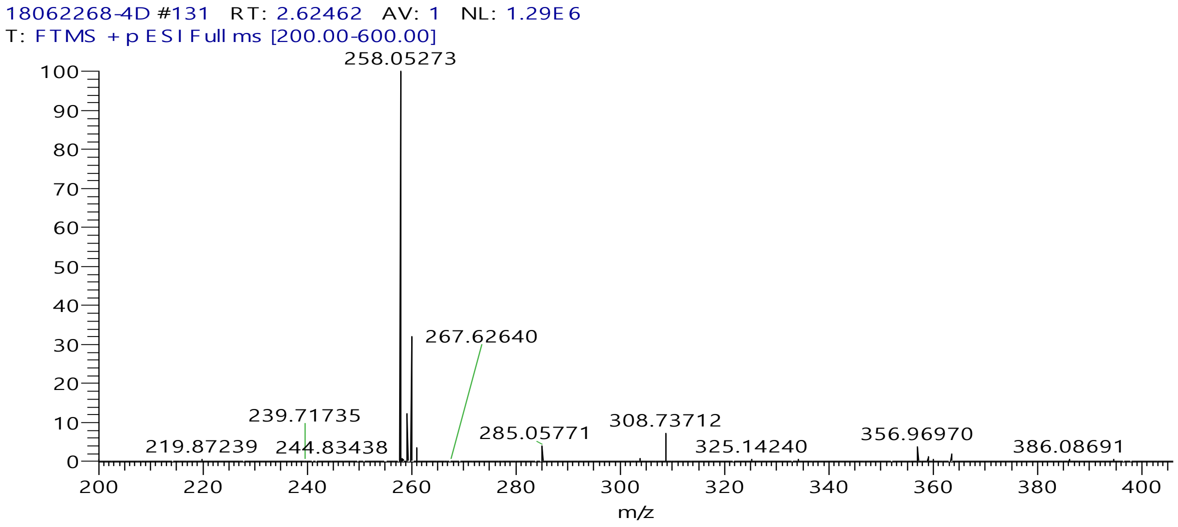


Figure S54. LC-HRMS spectrum of chemical reference (*R*)-**3d**.

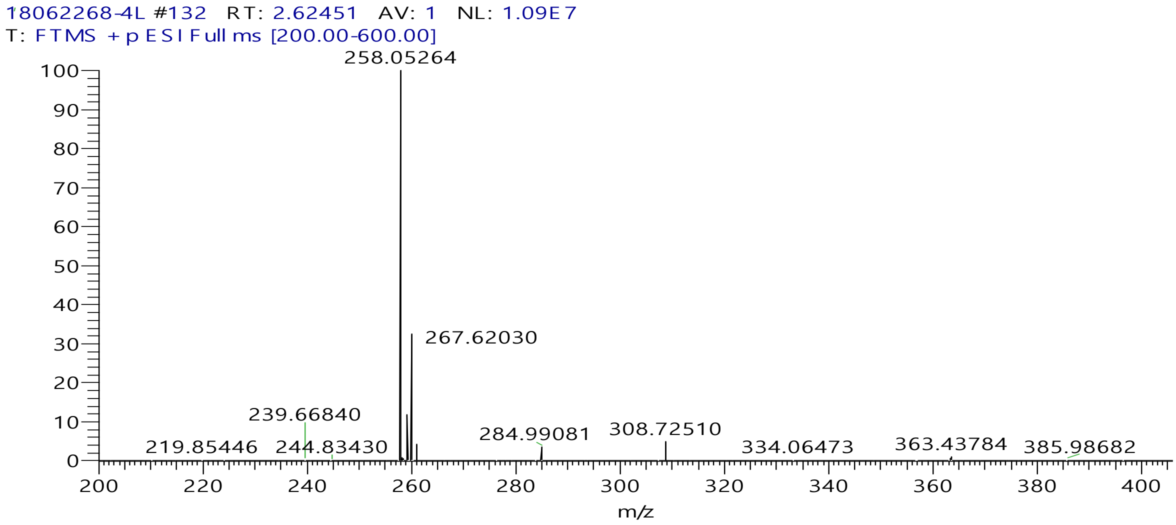


Figure S55. LC-HRMS spectrum of chemical reference (*S*)-**3d**.

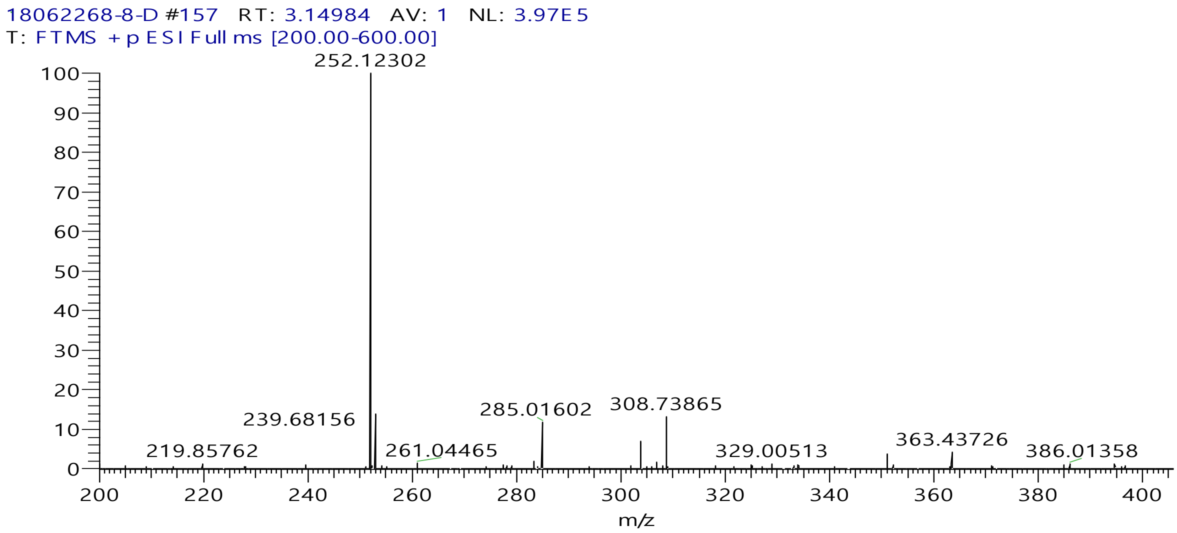


Figure S56. LC-HRMS spectrum of chemical reference (*R*)-**3g**.

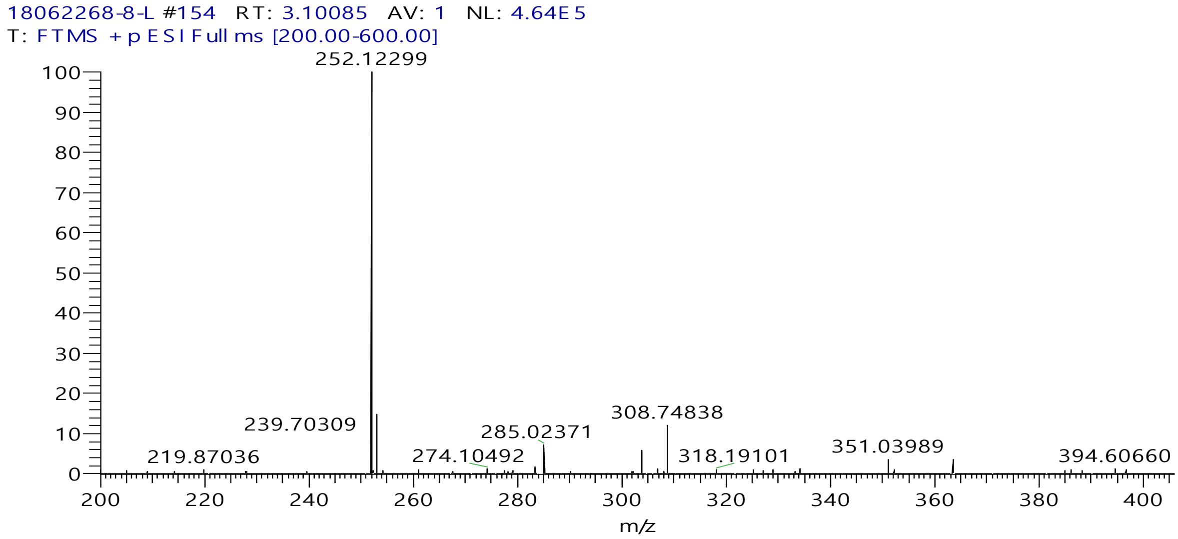


Figure S57. LC-HRMS spectrum of chemical reference (*S*)-**3g**.

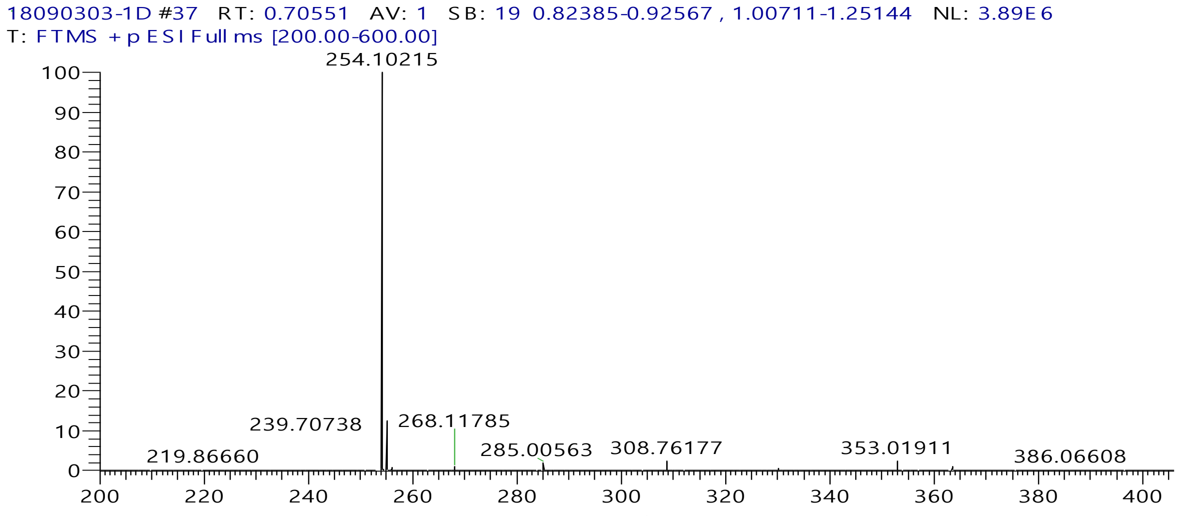


Figure S58. LC-HRMS spectrum of chemical reference (*R*)-**3h**.

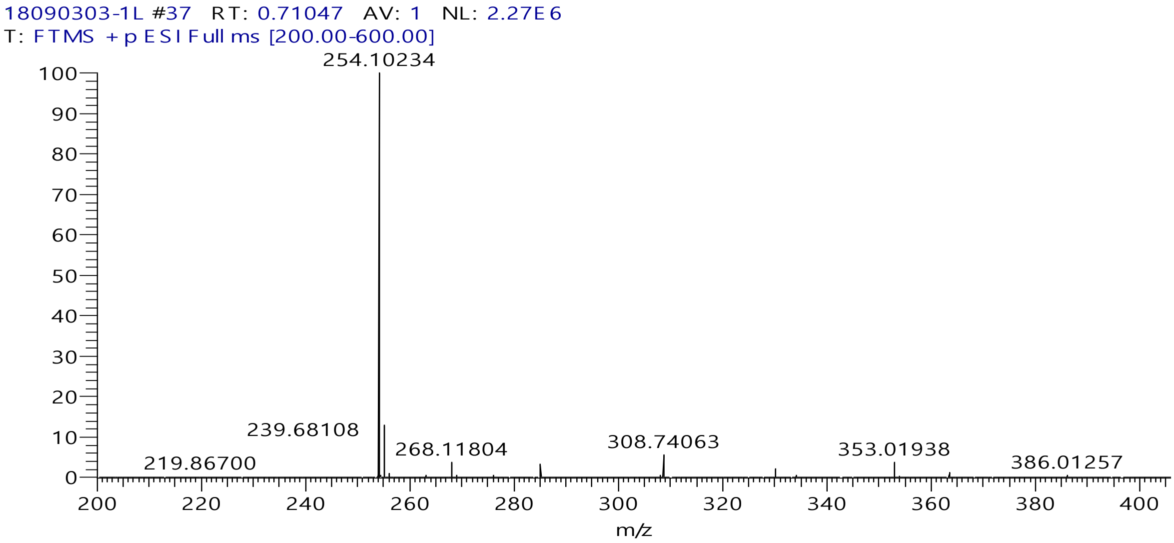


Figure S59. LC-HRMS spectrum of chemical reference (*S*)-**3h**.

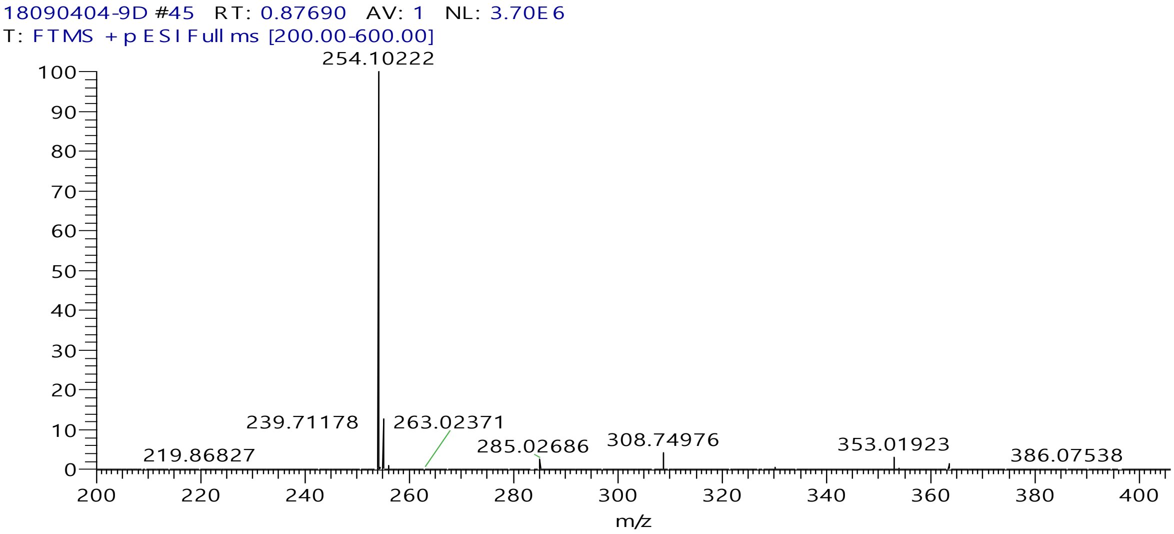


Figure S60. LC-HRMS spectrum of chemical reference (*R*)-**3i**.

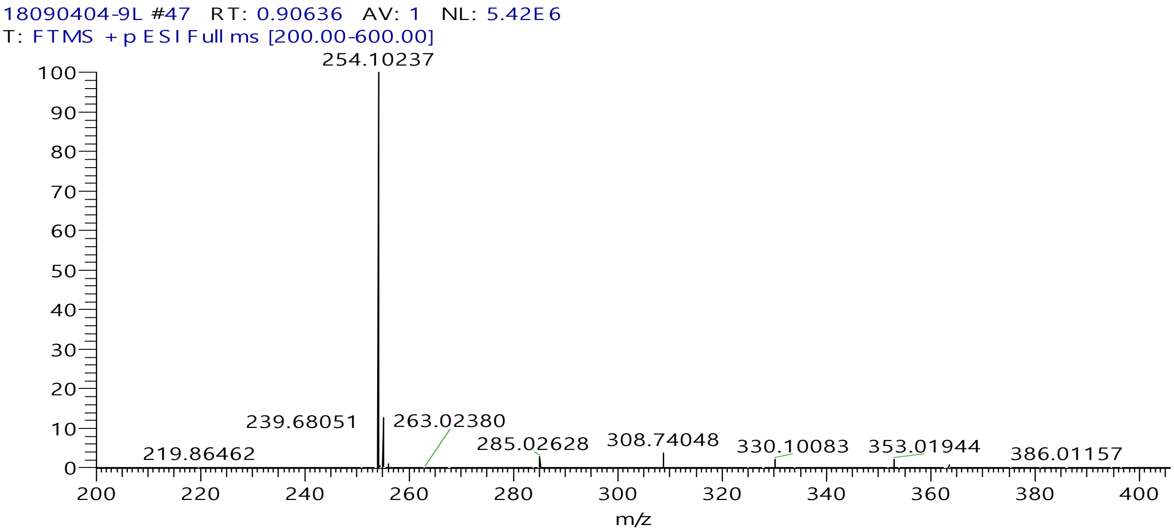


Figure S61. LC-HRMS spectrum of chemical reference (*S*)-**3i**.

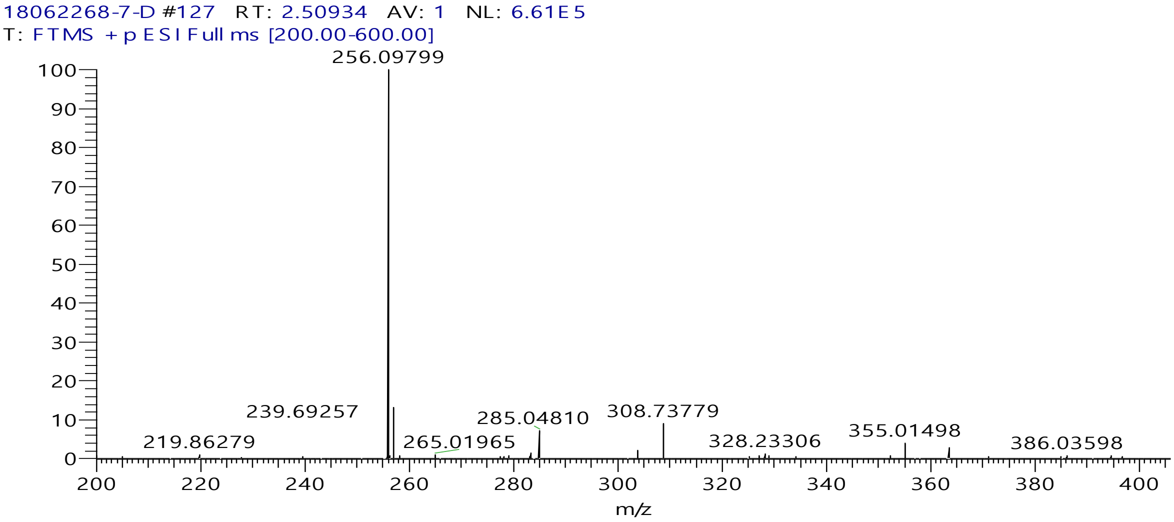


Figure S62. LC-HRMS spectrum of chemical reference (*R*)-**3j**.

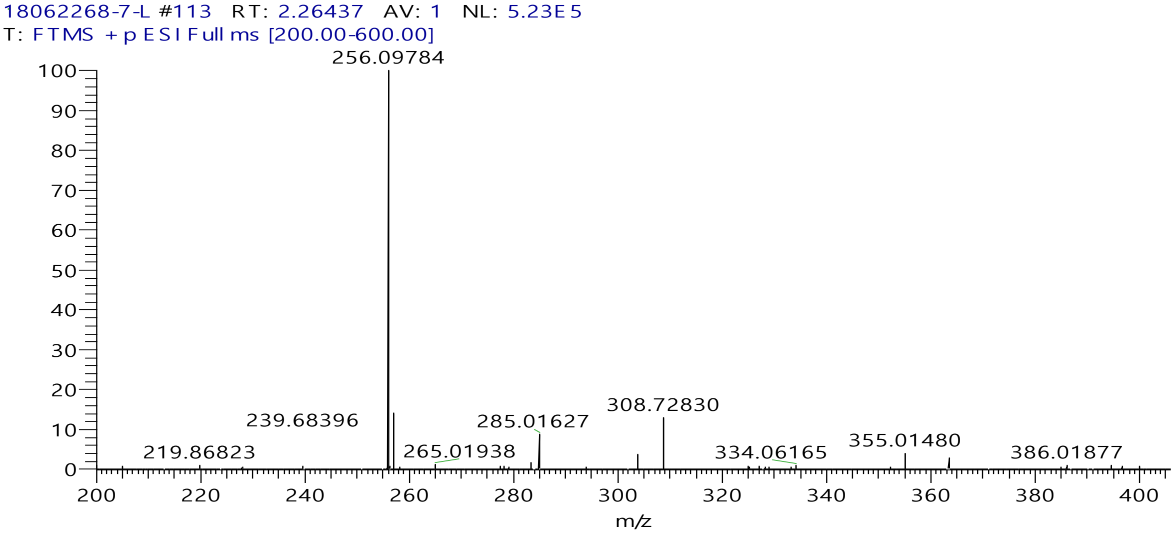
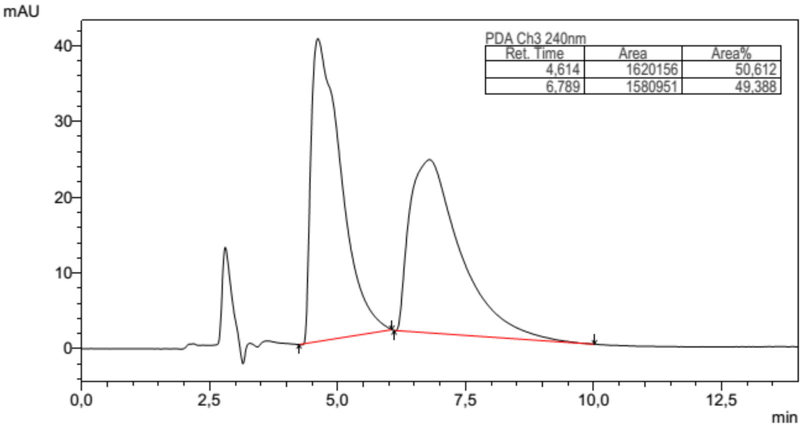


Figure S63. LC-HRMS spectrum of chemical reference (*S*)-**3j**.

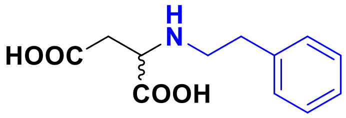
5. Chiral HPLC analysis

Chiral HPLC analysis was performed on a Nucleosil 5µ chiral-1 120A (250 x 4.6 mm) column, using isocratic 0.5 mM $CuSO_4$ at 1 ml/min at 60°C, detection at 240 nm. A racemic mixture was prepared by mixing chemically synthesized *N*-arylalkyl-substituted L- and D-aspartic acids in a 1:1 ratio.

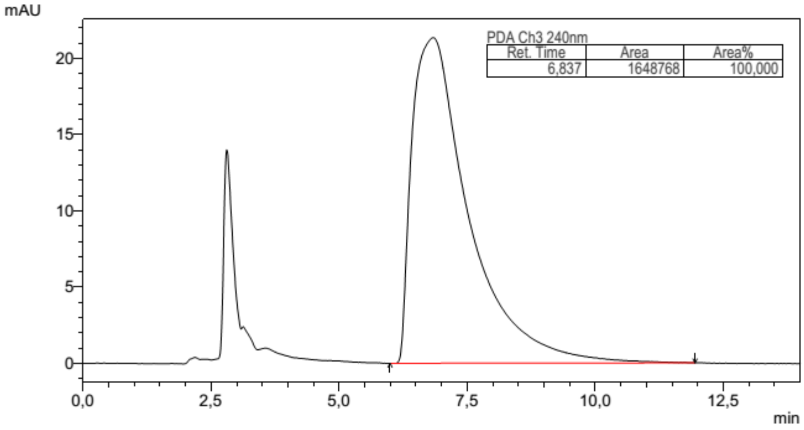


PDA Ch3 240nm

Ret. Time	Area	Area%
4,614	1620156	50,612
6,789	1580951	49,388

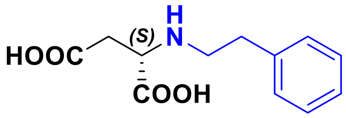


(*rac*)-**3a**

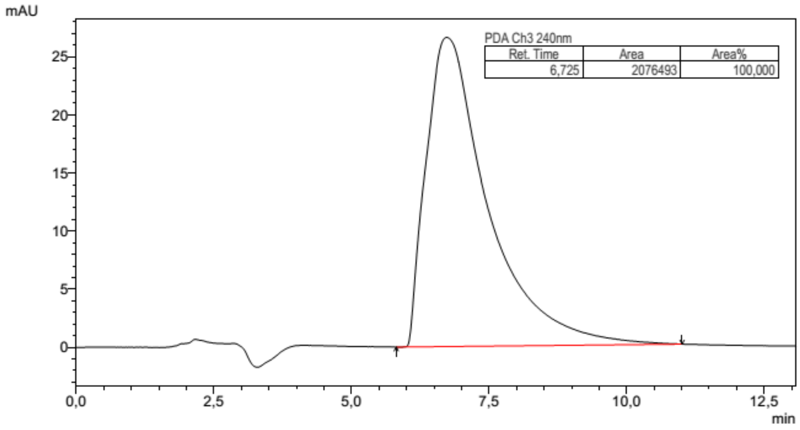


PDA Ch3 240nm

Ret. Time	Area	Area%
6,837	1648768	100,000

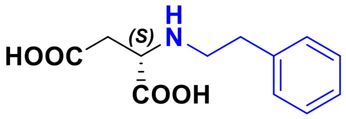


authentic standard (*S*)-**3a**



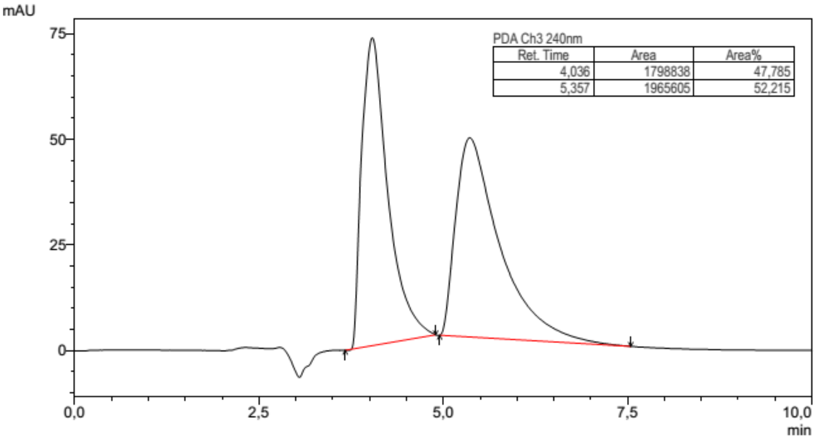
PDA Ch3 240nm

Ret. Time	Area	Area%
6,725	2076493	100,000

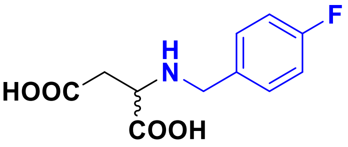


enzymatic product (*S*)-**3a**

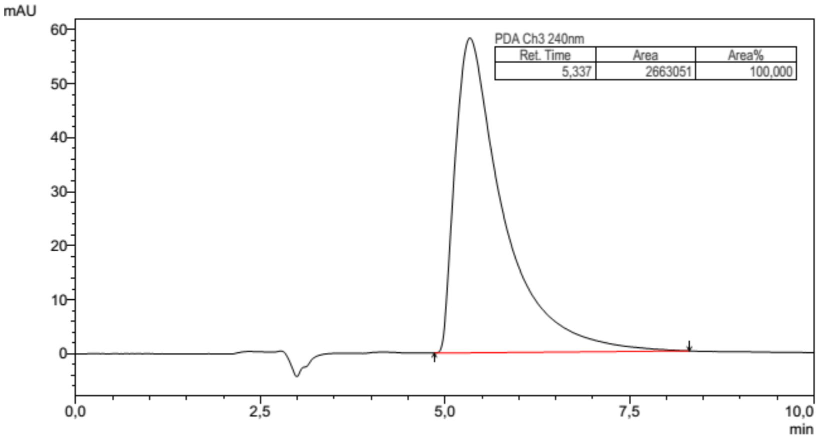
Figure S64. Chiral HPLC analysis of product **3a**.



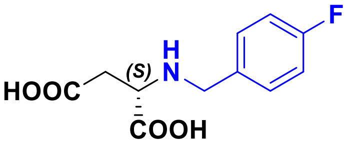
Ret. Time	Area	Area%
4,036	1798838	47,785
5,357	1965605	52,215



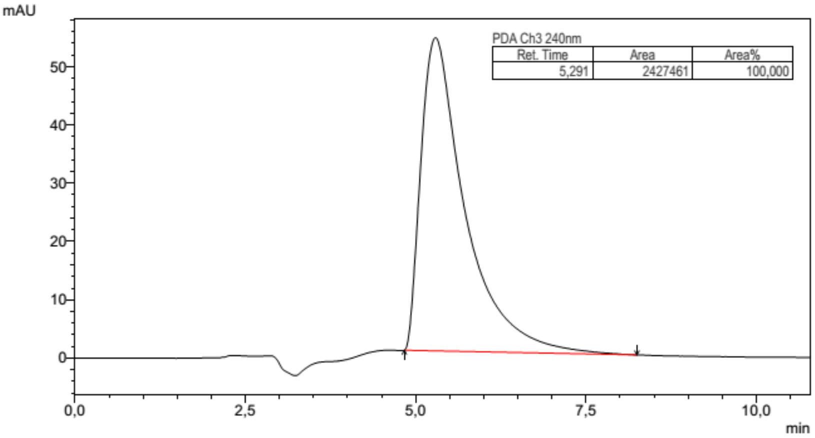
(*rac*)-**3c**



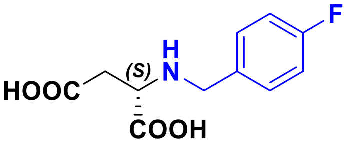
Ret. Time	Area	Area%
5,337	2663051	100,000



authentic standard (*S*)-**3c**

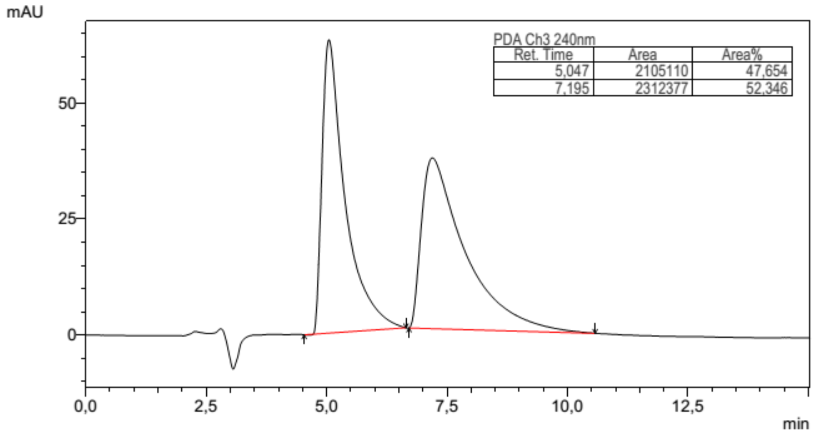


Ret. Time	Area	Area%
5,291	2427461	100,000



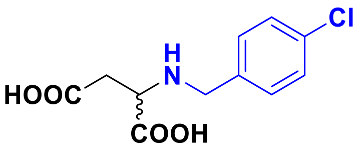
enzymatic product (*S*)-**3c**

Figure S65. Chiral HPLC analysis of product **3c**.

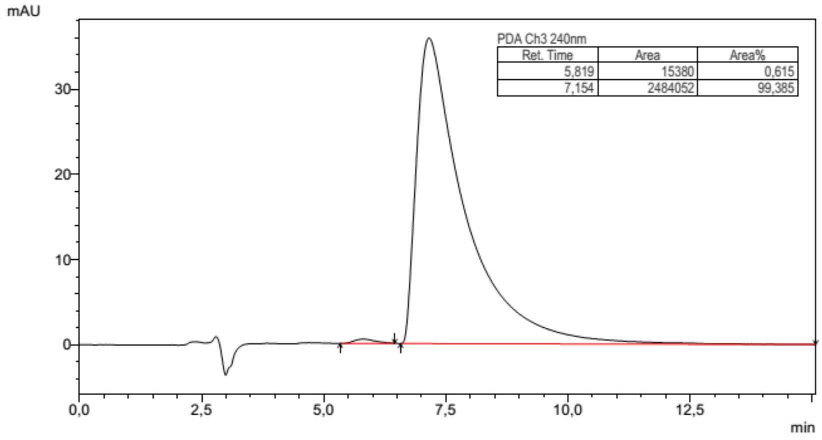


PDA Ch3 240nm

Ret. Time	Area	Area%
5,047	2105110	47,654
7,195	2312377	52,346

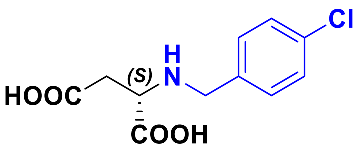


(*rac*)-**3d**

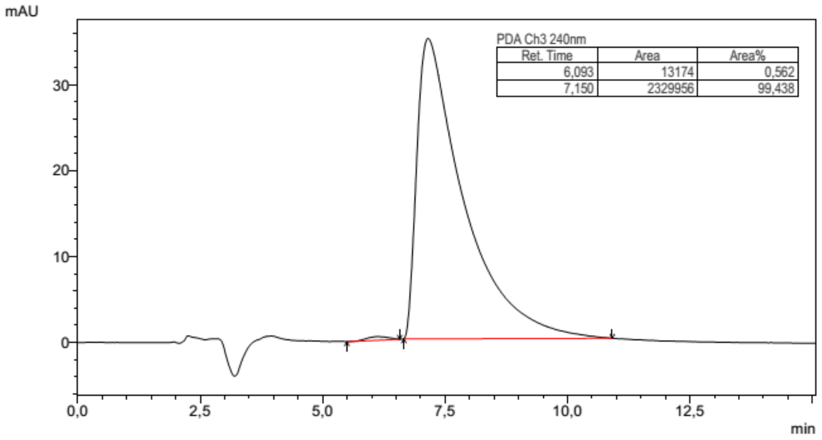


PDA Ch3 240nm

Ret. Time	Area	Area%
5,819	15380	0,615
7,154	2484052	99,385

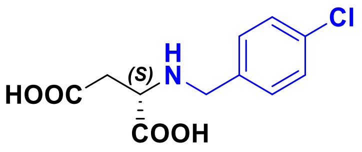


authentic standard (*S*)-**3d**



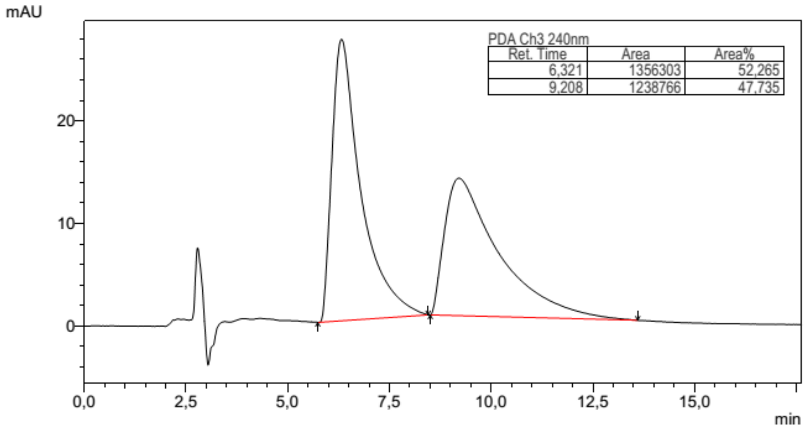
PDA Ch3 240nm

Ret. Time	Area	Area%
6,093	13174	0,562
7,150	2329956	99,438

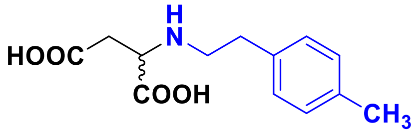


enzymatic product (*S*)-**3d**

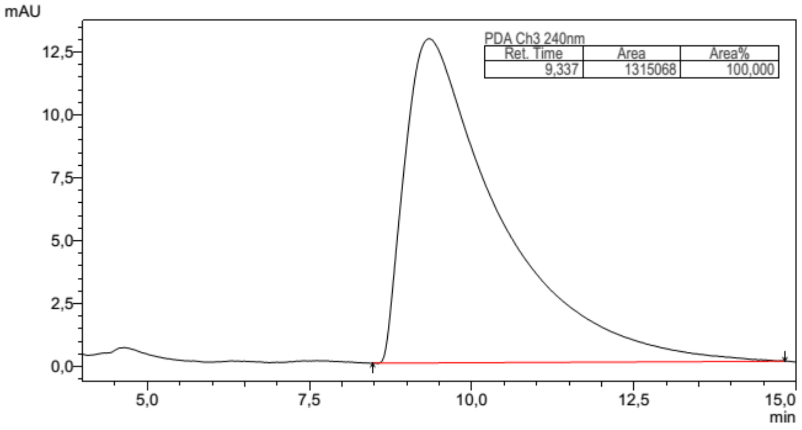
Figure S66. Chiral HPLC analysis of product **3d**.



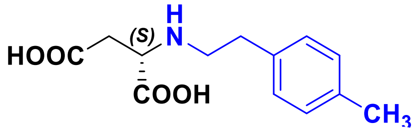
Ret. Time	Area	Area%
6,321	1356303	52,265
9,208	1238766	47,735



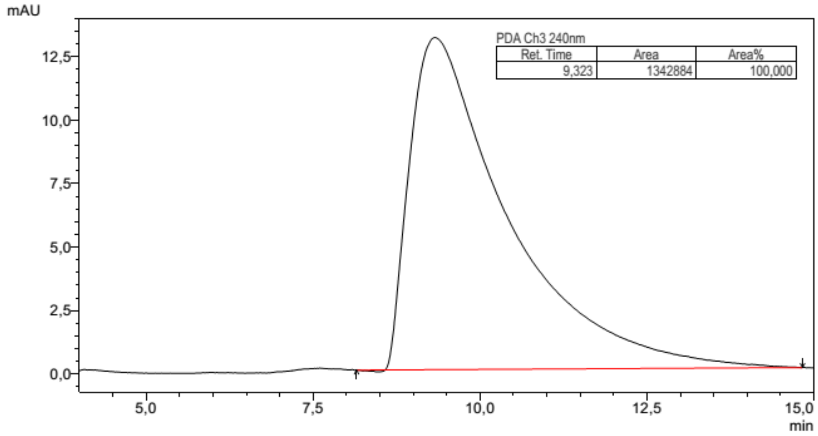
(*rac*)-**3g**



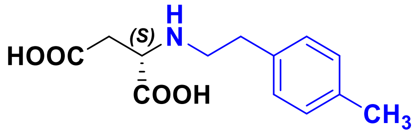
Ret. Time	Area	Area%
9,337	1315068	100,000



authentic standard (*S*)-**3g**

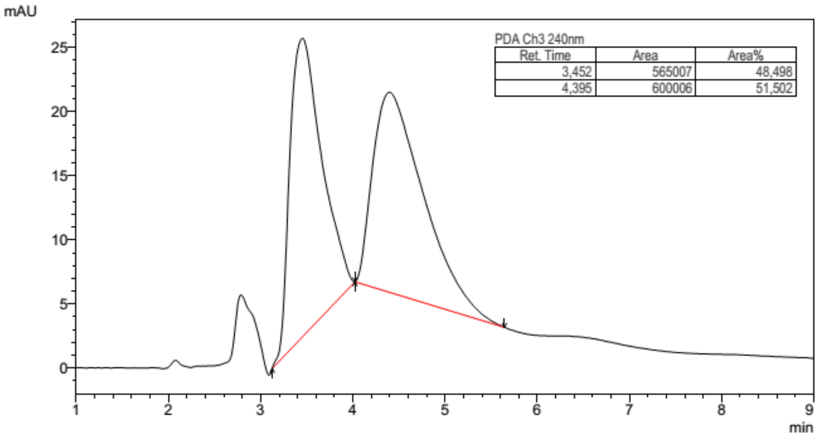


Ret. Time	Area	Area%
9,323	1342884	100,000

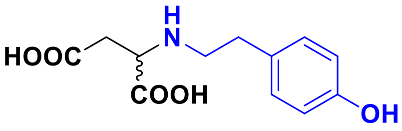


enzymatic product (*S*)-**3g**

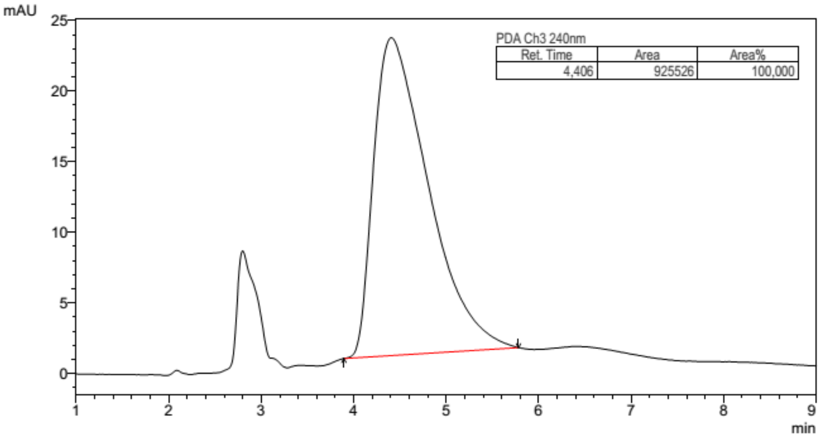
Figure S67. Chiral HPLC analysis of product **3g**.



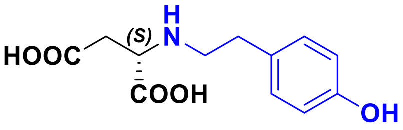
Ret. Time	Area	Area%
3,452	565007	48,498
4,395	600006	51,502



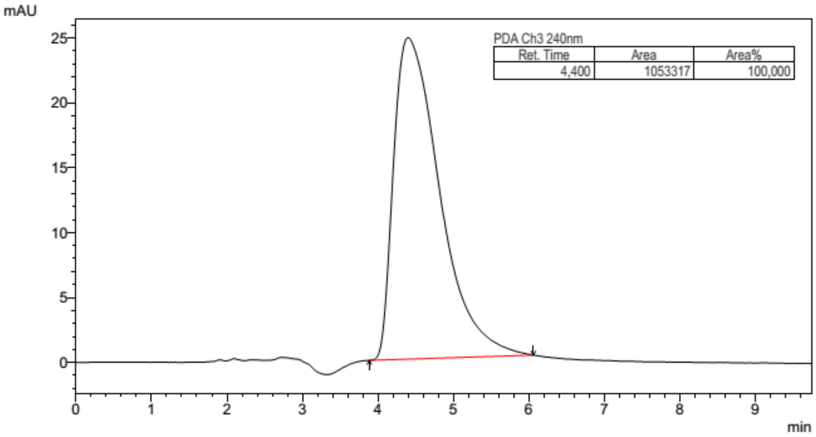
(*rac*)-**3h**



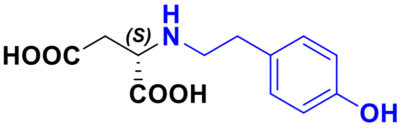
Ret. Time	Area	Area%
4,406	925526	100,000



authentic standard (*S*)-**3h**

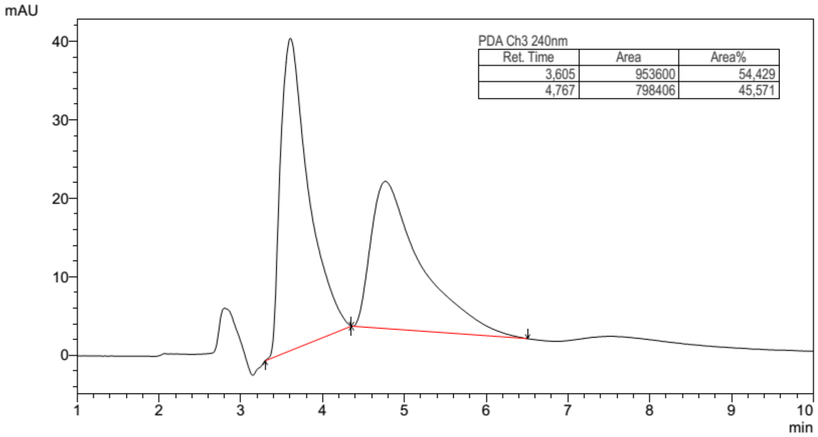


Ret. Time	Area	Area%
4,400	1053317	100,000

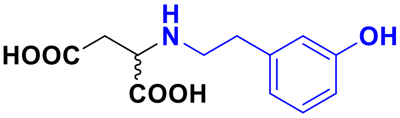


enzymatic product (*S*)-**3h**

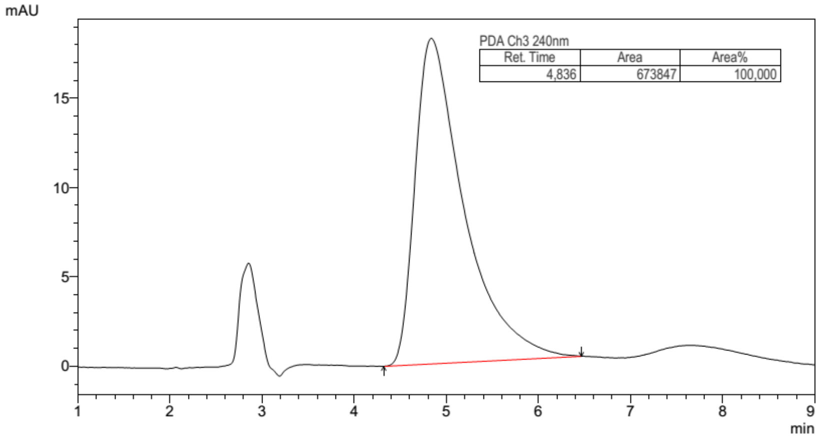
Figure S68. Chiral HPLC analysis of product **3h**.



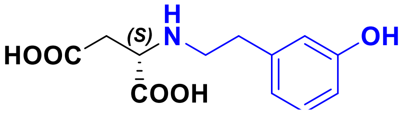
Ret. Time	Area	Area%
3,605	953600	54,429
4,767	798406	45,571



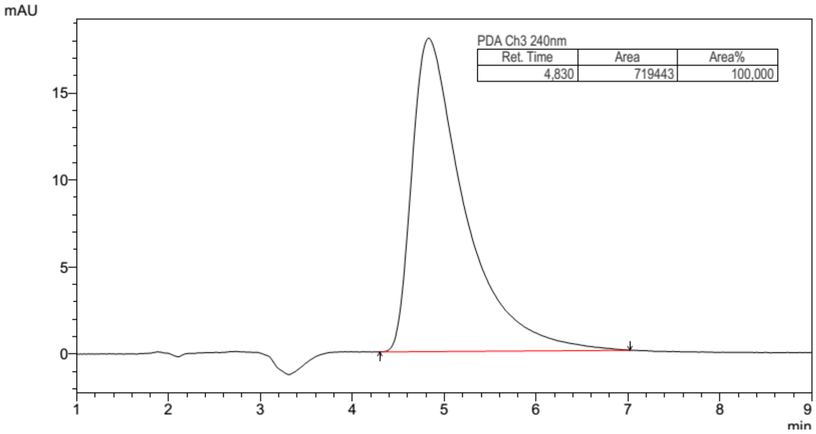
(*rac*)-**3i**



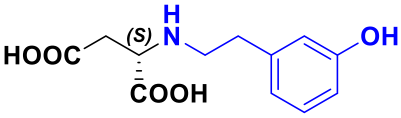
Ret. Time	Area	Area%
4,836	673847	100,000



authentic standard (*S*)-**3i**

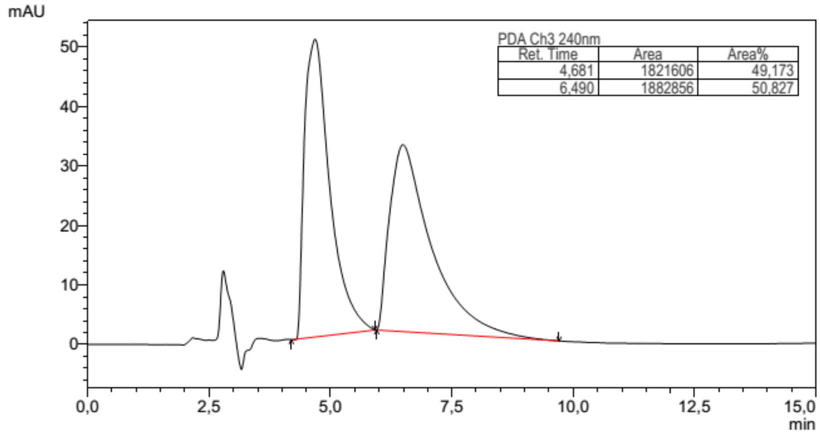


Ret. Time	Area	Area%
4,830	719443	100,000



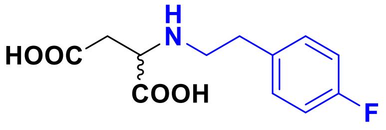
enzymatic product (*S*)-**3i**

Figure S69. Chiral HPLC analysis of product **3i**.

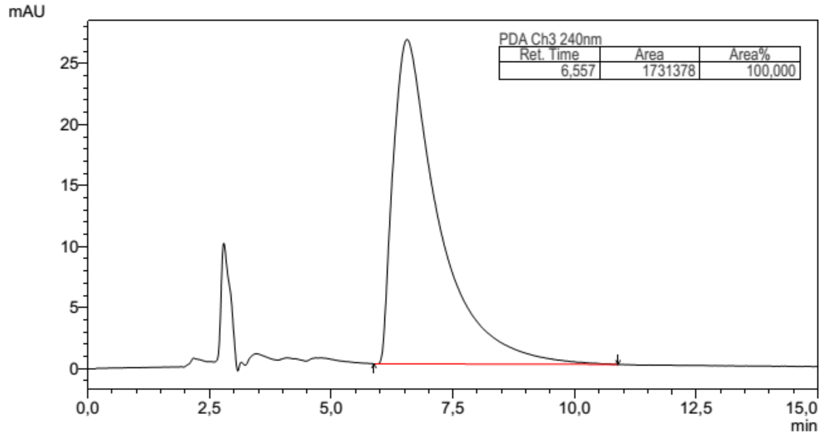


PDA Ch3 240nm

Ret. Time	Area	Area%
4,681	1821606	49,173
6,490	1882856	50,827

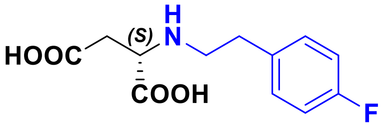


(*rac*)-**3j**

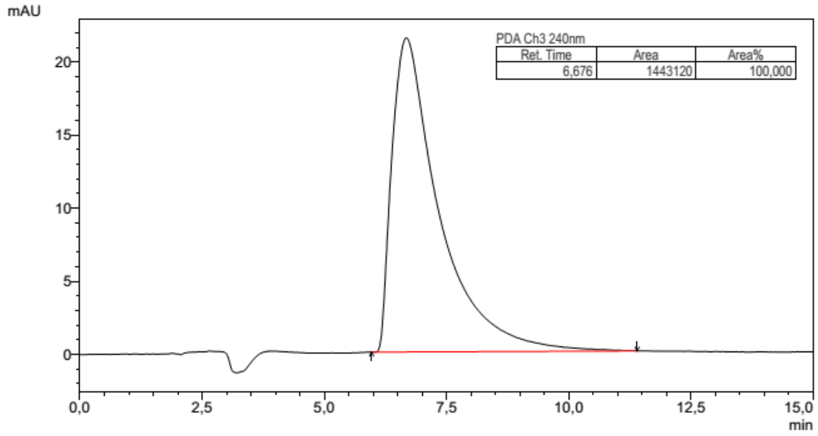


PDA Ch3 240nm

Ret. Time	Area	Area%
6,557	1731378	100,000

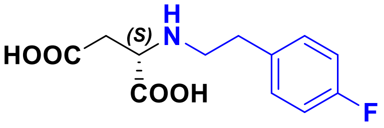


authentic standard (*S*)-**3j**



PDA Ch3 240nm

Ret. Time	Area	Area%
6,676	1443120	100,000



enzymatic product (*S*)-**3j**

Figure S70. Chiral HPLC analysis of product **3j**.

6. Additional figures

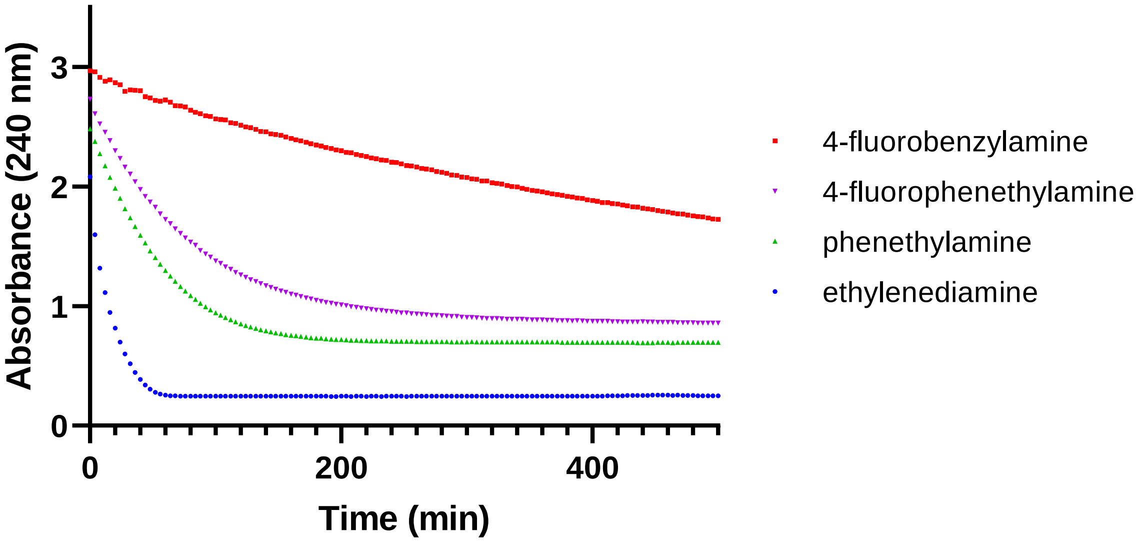


Figure S71. Progress curves for the EDDS-lyase (15 µM, 0.15 mol%) catalyzed addition of 4-fluorobenzylamine (**2c**, 50 mM), 4-fluorophenethylamine (**2j**, 50 mM), phenethylamine (**2a**, 50 mM) or ethylenediamine (50 mM) to fumarate (**1**, 10 mM) in 20 mM NaH_2PO_4-NaOH buffer (pH 8.5), as monitored by UV spectroscopy.

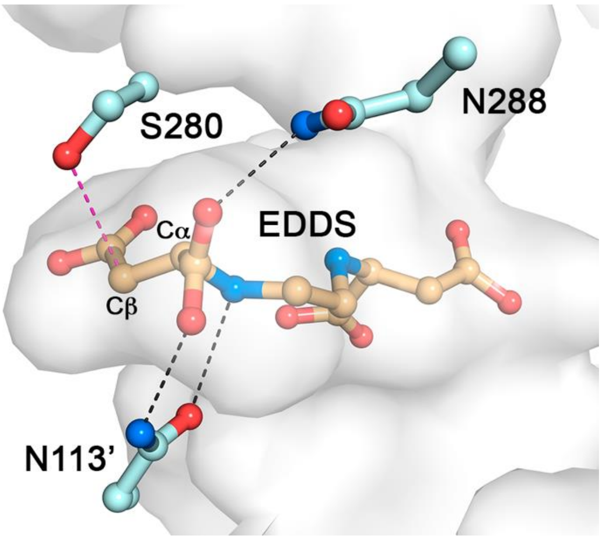


Figure S72. Binding mode of (*S,S*)-EDDS in the active site pocket of EDDS lyase, as based on the EDDS lyase crystal structure (PDB entry 6G3H).[5] Selected hydrogen bonds are shown with black dashed lines. The dashed line in magenta indicates the contact of Ser280 (the catalytic base) with the Cβ carbon of the proximal aspartyl moiety. Asn113’ (the apostrophe indicates that this residue belongs to a different polypeptide chain in the EDDS lyase tetramer) forms stabilizing hydrogen bonds with both the α-amino and α-carboxylate group of the proximal aspartyl moiety. The relative positions of Ser280 and Asn113' at opposite sides of the aspartyl-moiety are a key feature that govern the enantioselectivity of the reaction catalyzed by EDDS lyase. This figure was generated with PyMOL version 2.4.2 (Schrödinger, LLC).

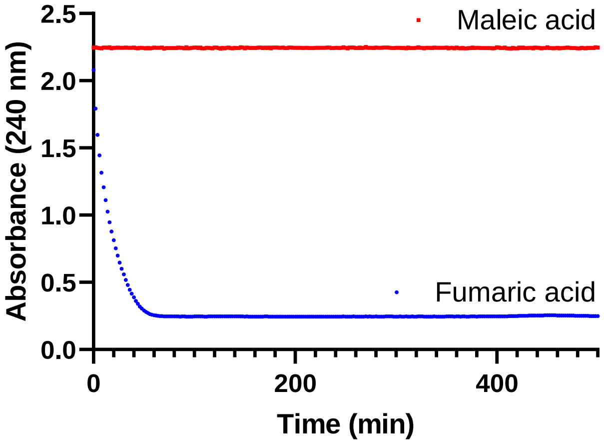


Figure S73. Progress curves for the EDDS-lyase (15 µM, 0.15 mol%) catalyzed addition of ethylenediamine (50 mM) to maleic acid (10 mM) or fumaric acid (10 mM) in 20 mM NaH_2PO_4-NaOH buffer (pH 8.5), as monitored by UV spectroscopy.

7. Supplementary references

1 V. Puthan Veetil, H. Raj, M. de Villiers, P. G. Tepper, F. J. Dekker, W. J. Quax and G. J. Poelarends, *ChemCatChem*, 2013, **5**, 1325–1327.

2 H. Raj, B. Weiner, V. Puthan Veetil, C. R. Reis, W. J. Quax, D. B. Janssen, B. L. Feringa and G. J. Poelarends, *ChemBioChem*, 2009, **10**, 2236–2245.

3 Y. Ohfune, N. Kurokawa, N. Higuchi, M. Saito, M. Hashimoto and T. Tanaka, *Chem. Lett.*, 1984, **13**, 441–444.

4 B. R. Lichman, E. D. Lamming, T. Pesnot, J. M. Smith, H. C. Hailes and J. M. Ward, *Green Chem.*, 2015, **17**, 852–855.

5. H. Poddar, J. de Villiers, J. Zhang, V. Puthan Veetil, H. Raj, A.-M. W. H. Thunnissen and G. J. Poelarends, *Biochemistry*, 2018, **57**, 3752–3763.