Supporting Information

Electrosynthesis of ^{15}N-labeled amino acids from ^{15}N-nitrite and ketonic acids

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1. Experimental Section

1.1 Chemicals

K_2HPO_4, KH_2PO_4, $NaNO_2$, $^{15}NH_4Cl$, D_2O, $^{15}NH_2OH$, $Na^{15}NO_2$ pyruvic acid, glyoxylic acid, phenylpyruvic acid, and maleic acid were purchased from Aladdin Ltd. (Shanghai, China). NO gas was purchased from Lian Bo (Tianjin) Co., Ltd. Monolithic metal foils of Co, Fe, Ni, Cu, Ti, and Zn were obtained from Qingyuan Metal Materials (Hebei, China). Pt foil was obtained from Gaossunion Ltd. (Wuhan, China). Deionized water was used in all the experimental processes. All chemicals were of analytical grade and used without further purification.

1.2 Electrochemical measurements

The electrochemical measurement was carried out on an Ivium-n-Stat electrochemical workstation (Ivium Technologies B.V.) with an H-type cell. Ni foam (NF), carbon rod, and Ag/AgCl electrode were adopted as the working electrode (the working area is 1.4 cm^2), the counter electrode, and the reference electrode, respectively. A Nafion117 proton exchange membrane was applied to separate the anode and cathode compartments of the H-type cell. Each compartment contained 20 ml of electrolyte solution. Typically, 20 mL 0.5 M pH 5.8 phosphate buffer solution (PBS) with 0.2 mmol ketonic acids and 0.1 M $NaNO_2$ or $Na^{15}NO_2$ were used as the electrolytes (all substrates were directly dissolved in PBS except phenylpyruvic acid, which was first dissolved in 3 mL 1,4-dioxane and then added to 17 mL PBS.). For the linear sweep voltammetry (LSV) test, the potential was set as -0.2 V to -0.7 V (vs. Ag/AgCl) with a scan rate of 10 mV s^{-1}. For the constant current electrolysis test, the current densities were set as -13 to -65 mA cm^{-2}.

1.3 Product identification and quantification

The organic products in the electrolyte were first identified by NMR spectroscopy and further confirmed by LC-HRMS. The products were quantified using ^1H NMR in the water suppression mode. When the electrolysis finished, 1 mL of maleic acid aqueous solution (0.1 M) was first added to the electrolyte, and then 450 μL of the above mixture was mixed with 50 μL of D_2O for NMR tests. The amount of the analyte was calculated based on the area ratio of the analyte peak to that of the internal standard (maleic acid). For the identification and quantification of $^{15}NH_4^+$ from $^{15}NO_2^-$ electroreduction, an extra 20 μL of 4.0 M H_2SO_4 was added to the as-prepared NMR sample to reach a pH value of ~3, and the concentration was calculated based on the area ratio of the $^{15}NH_4^+$ peak ($^{15}NH_4^+$, ~6.90 ppm, double peak) to that of maleic acid using calibration curves. NO_2^- was quantified by ultraviolet–visible (UV–Vis) absorbance spectra, and the specific detection methods followed the previous work of our group. [1]

The amount of amino acid and oxime was calculated by the equation,

$$n(\text{mmol}) = \frac{\text{the peak area of the product} \times 2}{\text{the peak area of the standard samples} \times \text{a}} \times 0.1$$

The yield rate was calculated by the equation

$$\text{Yield (\%)} = \frac{\text{mol of the formed product}}{\text{mol of the initial substrate}} \times 100\%$$

where *a* is the number of H atoms of the characteristic peak.

In this paper, error bars correspond to the standard deviation of three independent measurements.

1.4 ^{15}N-amino acid purification

^{15}N-alanine (**2a**), ^{15}N-glycine (**2d**), and 4-(amino-^{15}N)benzoic acid (**2c**) were purified by the following procedures: The reaction mixture (containing ~0.5 mmol ^{15}N-amino acids) was first freeze-dried and then dissolved in EtOH (2 mL) containing NaOH (245 mg, 920 μmol, 15% purity) and Boc_2O (120 mg, 552 μmol, 126 μL). The mixture was stirred at 25°C for 2 hr. The residue was diluted with H_2O (10 mL) and extracted with DCM (10 mL*3). The combined organic layers were washed with brine (10 mL), dried by Na_2SO_4, filtered, and concentrated under reduced pressure to give ^{15}N-(tert-butoxycarbonyl)alanine, ^{15}N-(tert-butoxycarbonyl)glycine, or 4-[(tert-butoxycarbonyl amino-^{15}N)methyl]benzoic acid. Then, the obtained product was dissolved in DCM (1 mL) containing HCl/dioxane (4 M, 1 mL). The mixture was stirred at 25 ℃ for 0.5 hr and then concentrated under reduced pressure.

^{15}N-Phenylalanine (**2b**) and 2-(amino-^{15}N)-2-phenylacetic acid (**2f**) were purified by prep-HPLC (column: Waters Xbridge C18 150 * 50 mm * 10 μm; mobile phase: water containing 0.1 mol% NH_4HCO_3; isocratic elution).

2-(Amino-^{15}N)-2-(furan-2-yl)acetic acid (**2e**) was purified by prep-HPLC (column: Waters Xbridge C18 150 * 50 mm *10 um; mobile phase: water containing 0.1 mol % NH_4HCO_3; isocratic elution), and then the residue was purified by prep-HPLC (column: Phenomenex C18 150 * 25 mm * 10 um; mobile phase: water containing 0.1 mol % NH_4HCO_3 and 0%~13% MeOH; gradient elution).

1.5 Electrochemical in situ ATR-FTIR spectra measurements

The in situ ATR-FTIR was performed on a Nicolet 6700 FTIR spectrometer equipped with an MCTA detector with silicon as the prismatic window. First, Ni ink (pure ethanol as a dispersant) was carefully dropped on the surface of the gold film, which was chemically deposited on the surface of the silicon prismatic before each experiment. Then, the deposited silicon prismatic served as the working electrode. The Pt foil and Ag/AgCl electrode containing saturated KCl solution were used as the counter and reference electrodes, respectively. The 0.5 M PBS solution (pH = 5.8) with ketonic acids, and $NaNO_2$/$Na^{15}NO_2$ was employed as the electrolyte. Spectra were recorded at -0.7 V vs. Ag/AgCl. The background spectrum of the catalyst electrode was acquired at an open-circuit voltage before each systemic measurement.

1.6 Online DEMS measurement.

A mass spectrometer (QAS 100) and an electrochemical workstation (Chenhua, Shanghai) were used for online differential electrochemical mass spectrometry (DEMS) measurements. 0.5 M PBS containing 0.1 M $^{15}NaNO_2$ was used as the electrolyte. Ar was bubbled into the electrolyte constantly before and during the DEMS measurements. NF, Pt wire, and Ag/AgCl electrodes were used as the working electrode, counter electrode, and reference electrode, respectively. The potentiostatic test at -0.7 V vs. Ag/AgCl was performed, and the corresponding mass signals could be detected during this period. After the electrochemical test was finished and the mass signal returned to baseline, the next cycle was started using the same test conditions to avoid accidental error during DEMS measurements. The measurement was ended after five cycles.

1.7 Synthesis procedures of ^{15}N-tiopronin[2]

1.7.1 Synthesis of a saline solution of thiobenzoic acid (1)

A total of 1.38 g thiobenzoic acid was slowly added to 10 mL potassium carbonate aqueous solution, dissolved, and filtered to obtain a yellow aqueous solution.

1.7.2 Synthesis of α-benzoyl mercaptopropionylglycine (2)

First, 0.3 g (4 mmol) ^{15}N-glycine was dissolved in 2 mL dilute sodium hydroxide solution, the ice salt was cooled to below 0 ℃, and 0.68 g (4 mmol) 2-bromopropionyl chloride and 2 mL dilute sodium hydroxide solution were added at the same time under fierce stirring. Stirring was maintained for 1 h. Then, 4 mmol (1) aqueous potassium salt was added and kept overnight at room temperature. After that, the solution was acidified to pH 3 by hydrochloric acid, and the solid was precipitated, filtered, washed, and dried. Finally, a white solid was obtained with a yield of 58%.

1.7.3 Synthesis ^{15}N-tiopronin

First, 0.5 g (1.8 mmol) of compound (2) was suspended in 2 mL of water and neutralized with sodium bicarbonate. Then, 0.3 mL of concentrated ammonia was added, and the mixture was kept overnight at room temperature. The precipitated benzamide was then removed by filtration. Then, the residue was further purified by pre-HPLC (column: Phenomenex luna C18 150*50 mm*10 μm; mobile phase: water containing 0.1% formic acid and 1%~15% acetonitrile; gradient elution) to give ^{15}N-tiopronin as a white solid. Finally, the product was identified and quantified by NMR, and a yield of 31% ^{15}N-tiopronin was obtained.

2. Supplementary figures

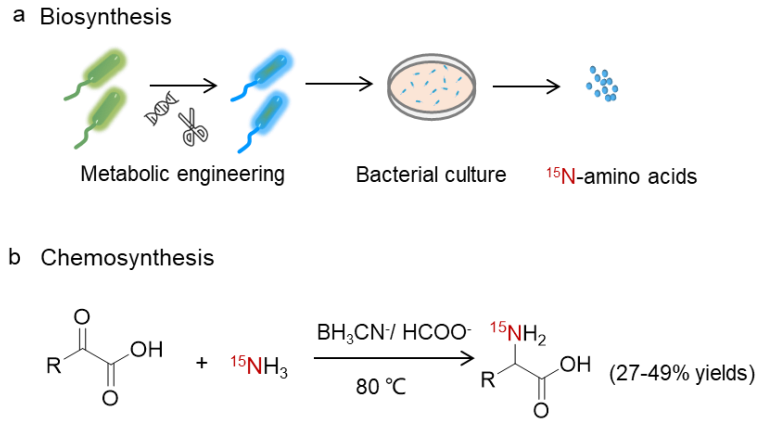


Figure S1. Schematic diagram of (a) biosynthesis and (b) chemosynthesis of amino acids.

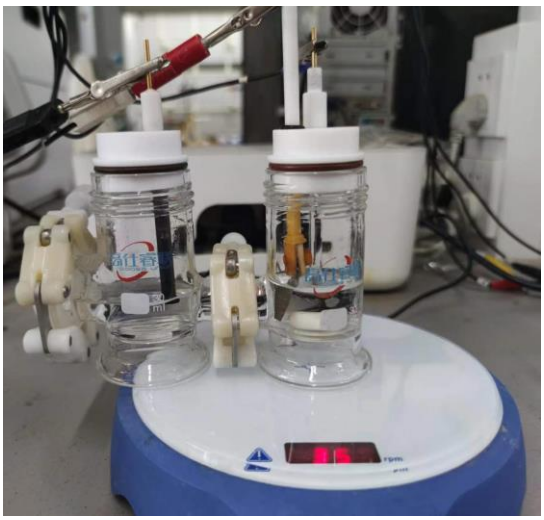


Figure S2. Reaction setup for the electrochemical synthesis of amino acids over a Ni NF cathode.

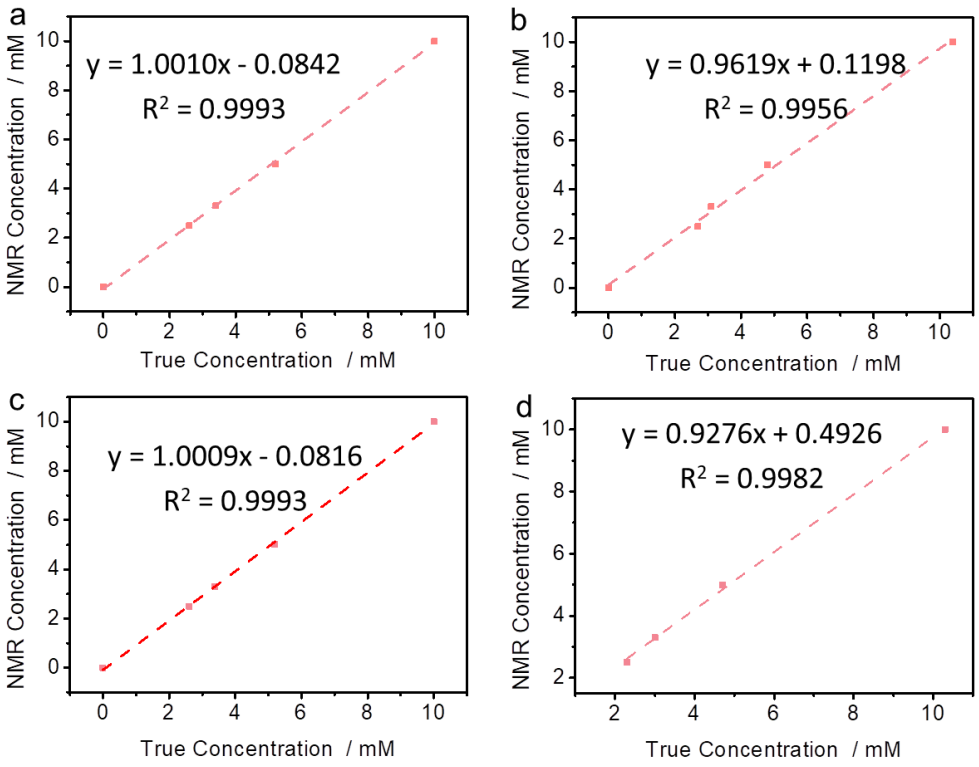


Figure S3. The calibration curve for (a) pyruvate, (b) alanine, (c) lactic acid, and (d) pyruvate oxime in the range of 0.00 mM~10 mM.

The organic products were quantified by ^{1}H NMR using maleic acid as the internal standard. To verify the accuracy of this quantification method, we configured substrate and product standard sample solutions with different concentrations and found that the tested concentration was highly consistent with the actual concentration through the internal standard nuclear magnetic quantification method, indicating the accuracy of our quantitative method.

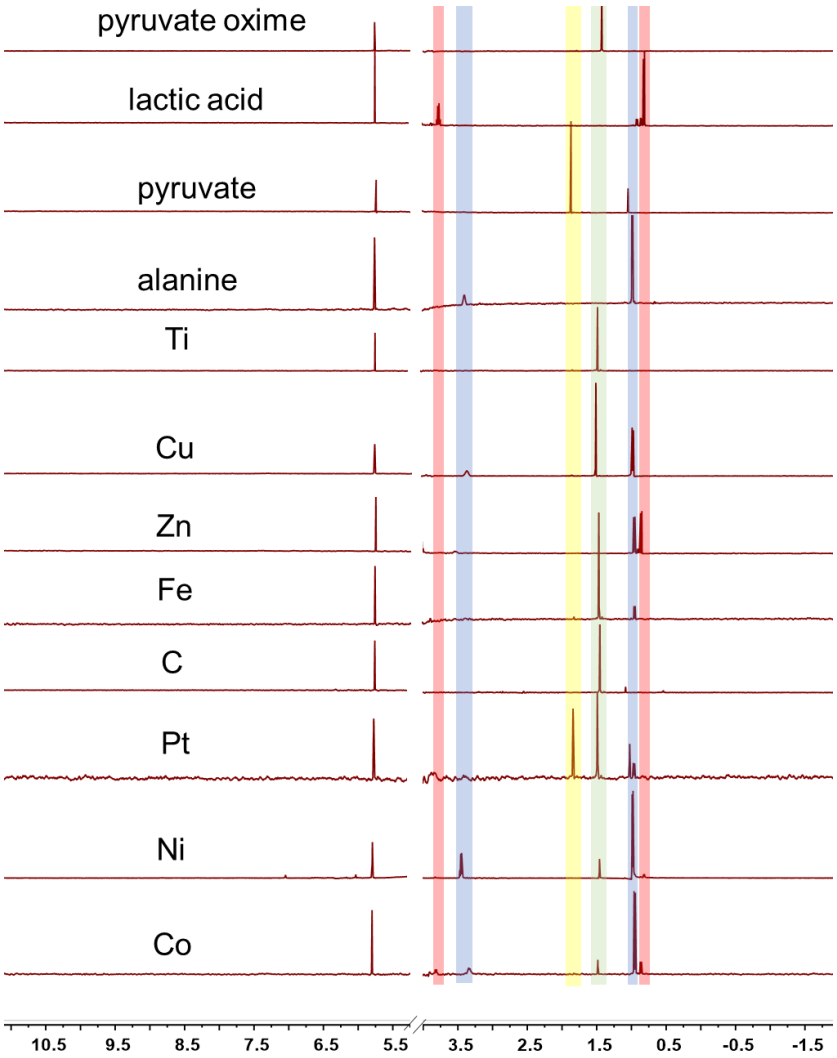


Figure S4. ^{1}H NMR spectra of the reaction mixture and the standard with maleic acid (5.8 ppm) as the internal standard.

Characteristic peaks are displayed in color backgrounds: pyruvate-yellow, alanine-blue, lactic acid-red, and pyruvate oxime-green.

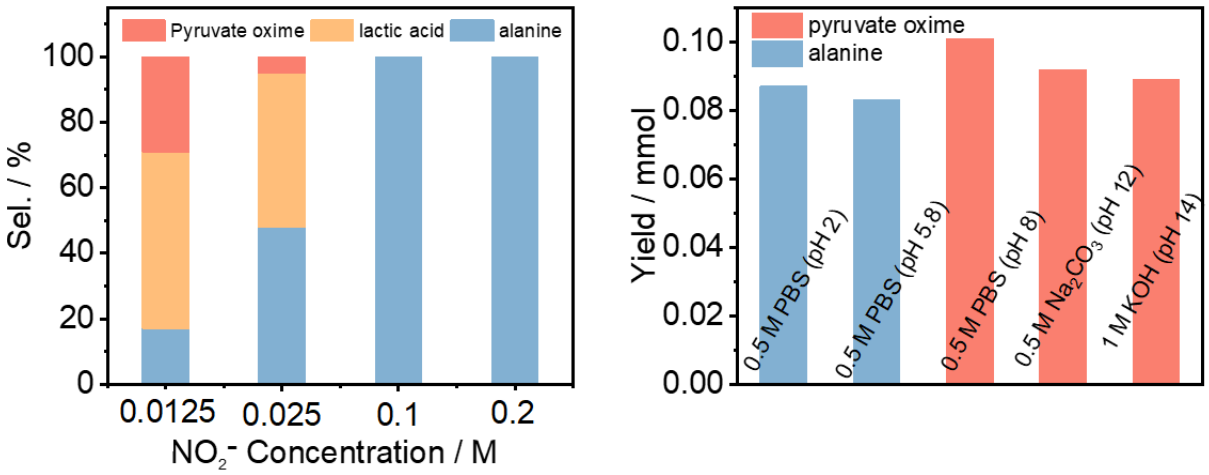


Figure S5. (a) Product selectivity over a Ni foam cathode by using different concentrations of NO_2^- and (b) pH-dependent yields of products over a Ni foam cathode.

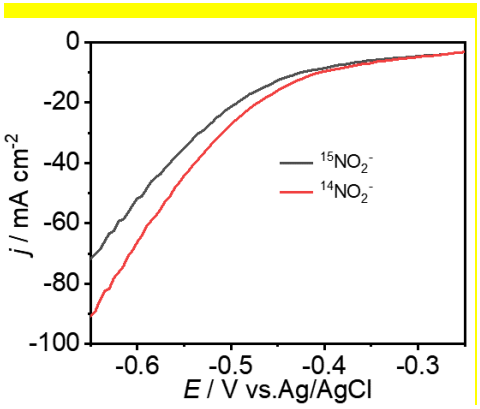


Figure S6. LSV curves of $^{14}NO_2^-$ and $^{15}NO_2^-$ electroreduction over NF.

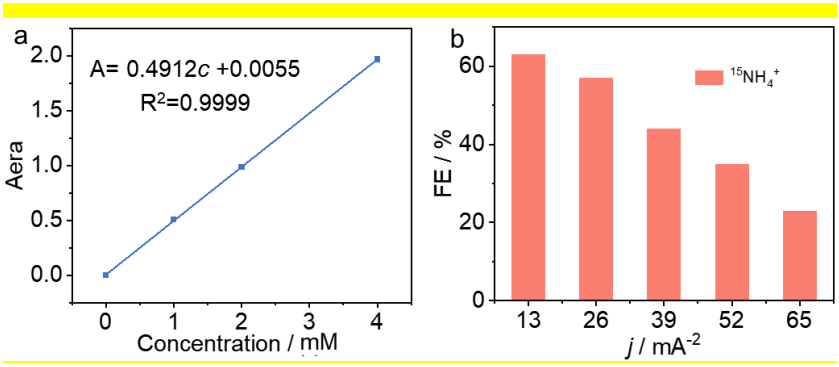


Figure S7. (a) The calibration curve for NH_4^+ in the range of 0.00 mM~4 mM and the typical ^1H NMR spectrum of the acidified electrolyte after electrocatalysis.

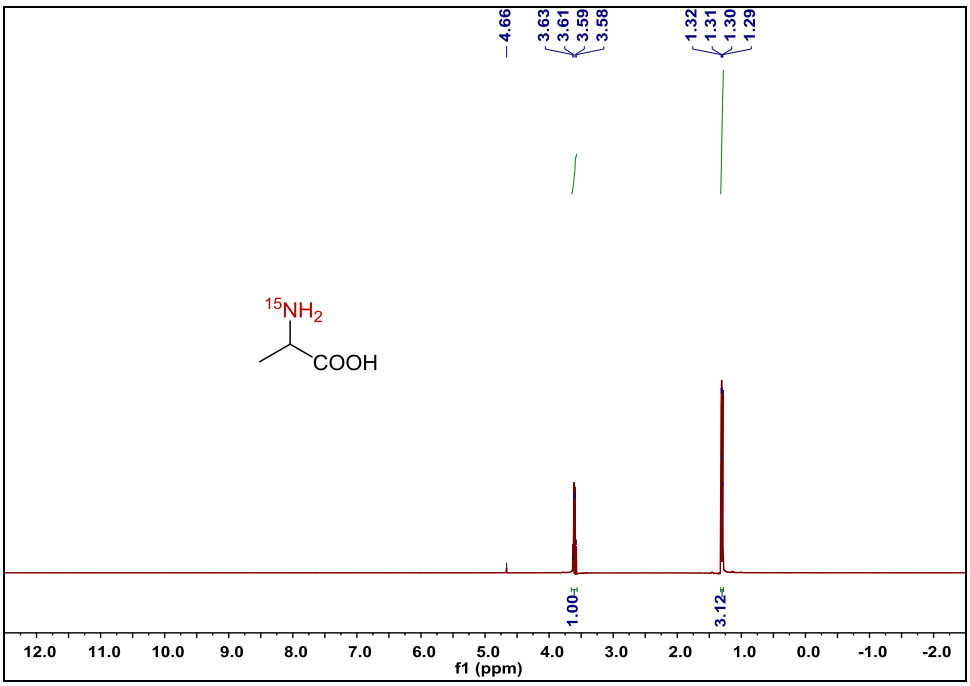


Figure S8. ^{1}H NMR spectrum of ^{15}N-alanine.

^{1}H NMR (400 MHz, D_2O) δ [ppm] 3.60 (dd, J = 14.8, 7.6 Hz, 1H), (dd, J = 7.2, 2.8 Hz, 3H). HRMS [M+H^{+}] 91.0515, theoretical value for $C_3H_7{}^{15}NO_2$ [M+H^{+}] is 91.0525. 93% NMR yield, 47% isolated yield.

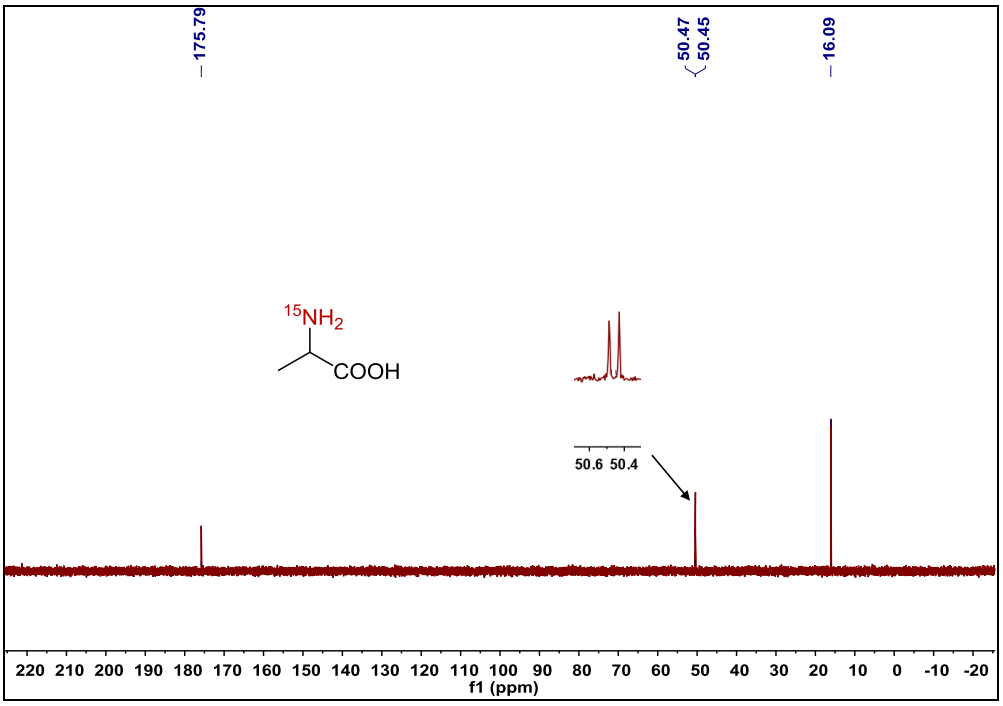


Figure S9. ^{13}C NMR spectrum of ^{15}N-alanine.

^{13}C NMR (101 MHz, D_2O) δ [ppm] 175.79, 50.46 (d, J = 5.7 Hz), 16.09.

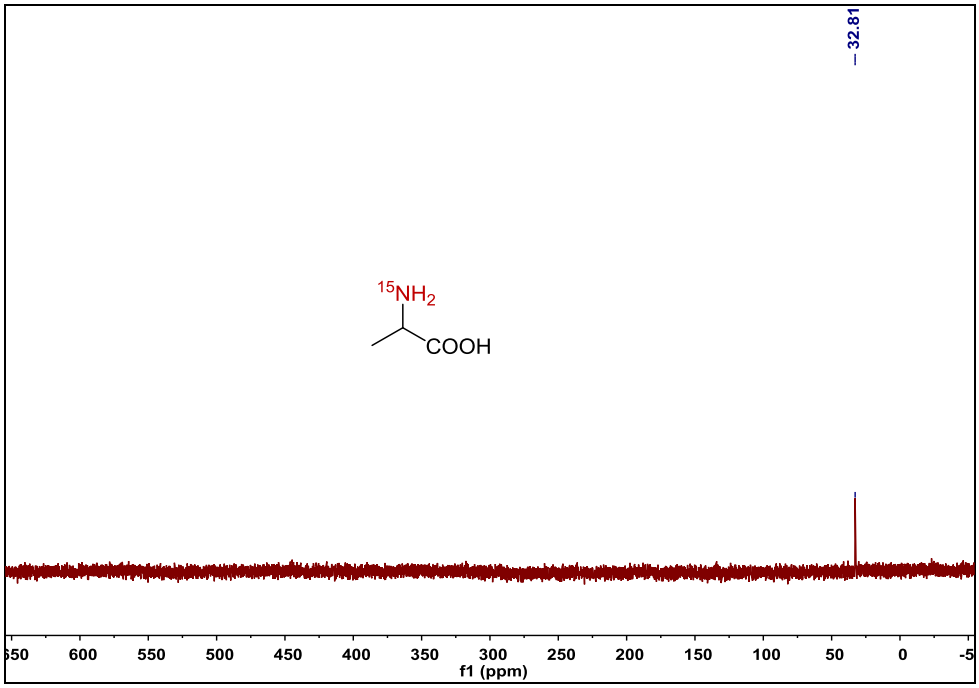


Figure S10. ^{15}N NMR spectrum of ^{15}N-alanine.

^{15}N NMR (61 MHz, D_2O) δ [ppm] 32.81.

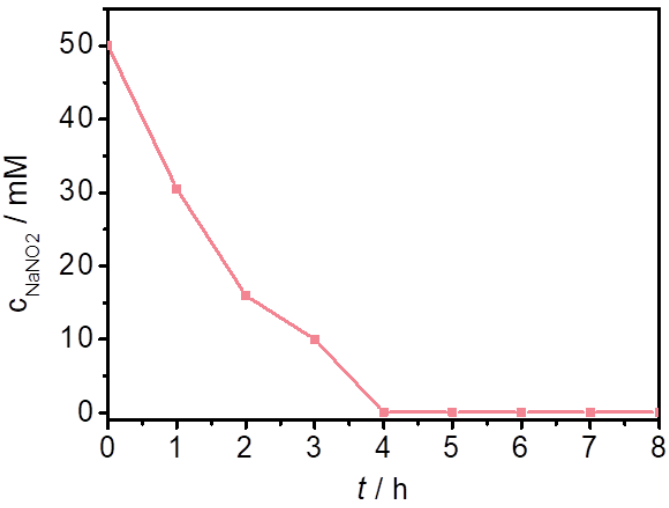


Figure S11. Time-dependent concentration variation of $NaNO_2$ during the reaction.

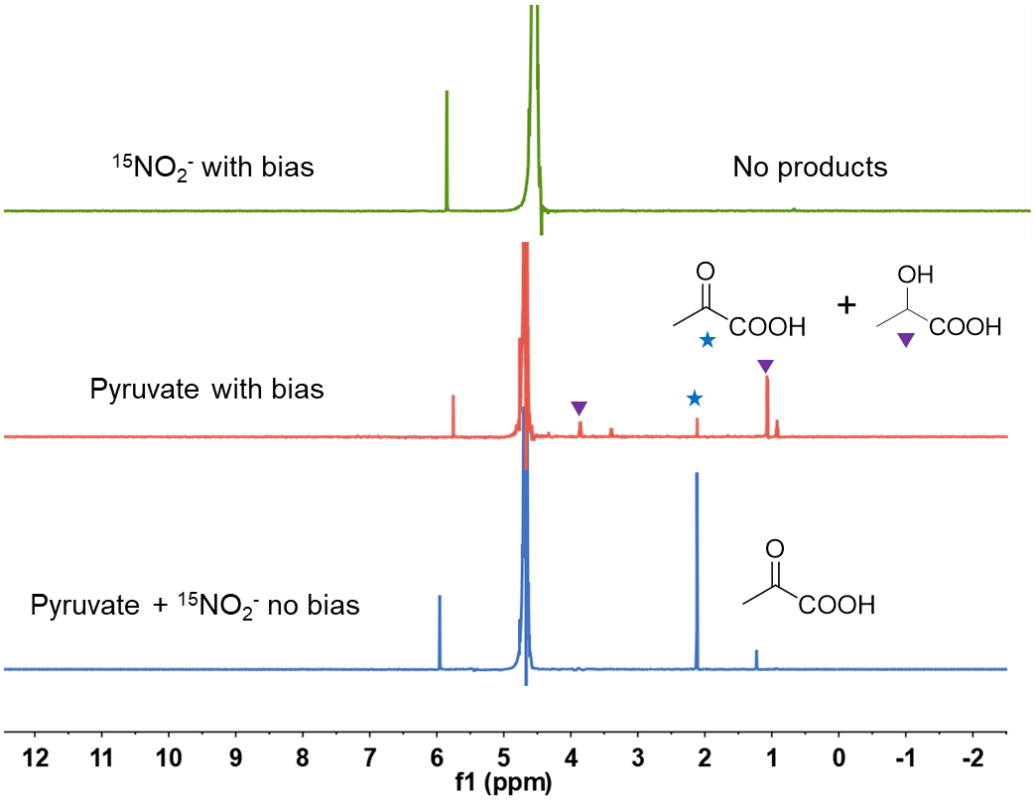


Figure S12. Typical ^{1}H NMR spectra of control experiments.

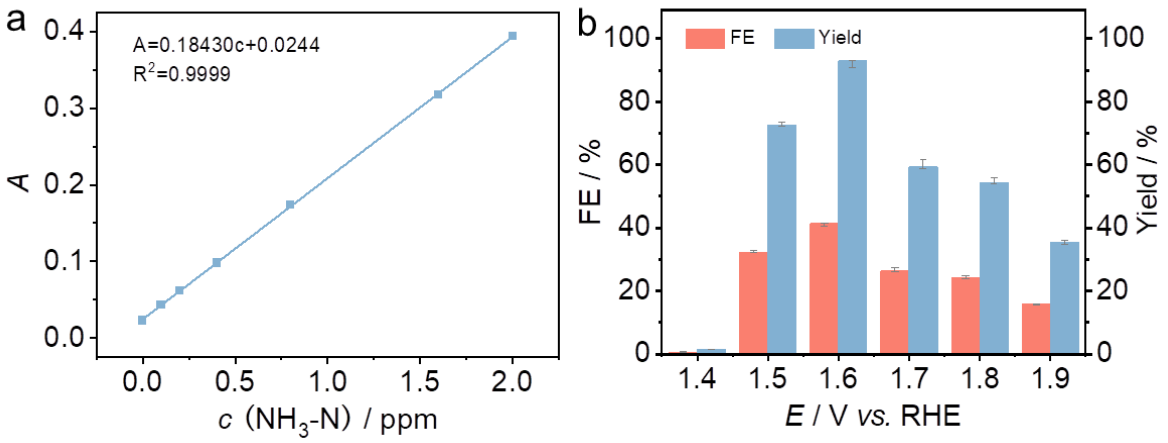


Figure S13. (a) The calibration curves of ammonia-N based on the absorbance of different ion concentrations[3] and (b) the FEs and yields of $^{15}NH_4^+$ for $^{15}NO_2^-$ electrooxidation at different potentials over a $Cu(OH)_2$ electrode. Error bars correspond to the standard deviation of three independent measurements.

The electrooxidation of $^{15}NH_4^+$ to $^{15}NO_2^-$ is conducted in a two-compartment three-electrode system. 0.1 M KOH solution containing 10 mM $^{15}NH_4Cl$ was used as the electrolyte. $Cu(OH)_2$/Cu NF was used as the working electrode. The electrolytic reaction proceeded at potentials of 1.4 to 1.9 V vs. RHE. The produced $^{15}NH_4^+$ was quantified by the UV–vis absorption spectrum after passing a charge of 260 C. Impressively, 45% FE and 93% yield of $^{15}NH_4^+$ were obtained at the optimal potential of 1.6 V vs. RHE, rationalizing the recycling idea of the ^{15}N source.

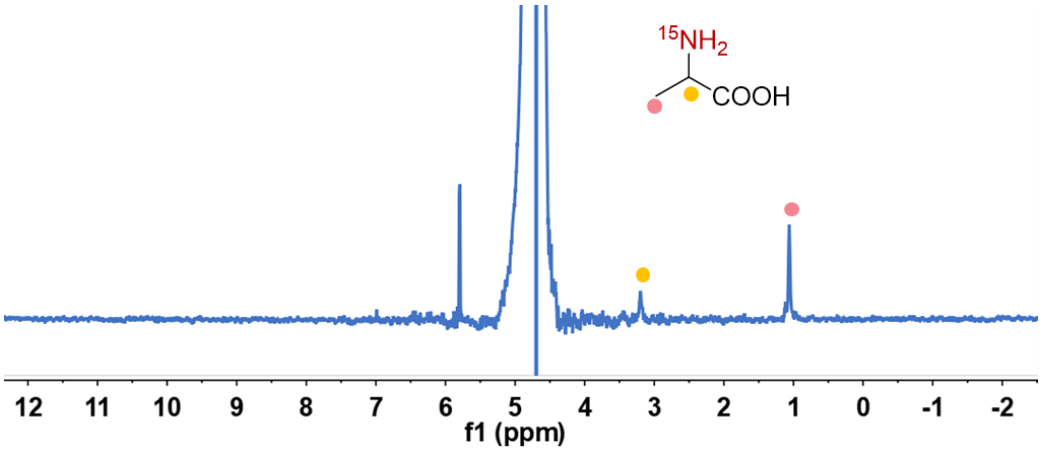


Figure S14. Typical ^{1}H NMR spectra of the electrolyte when using ^{15}N-pyruvate oxime as the reactant.

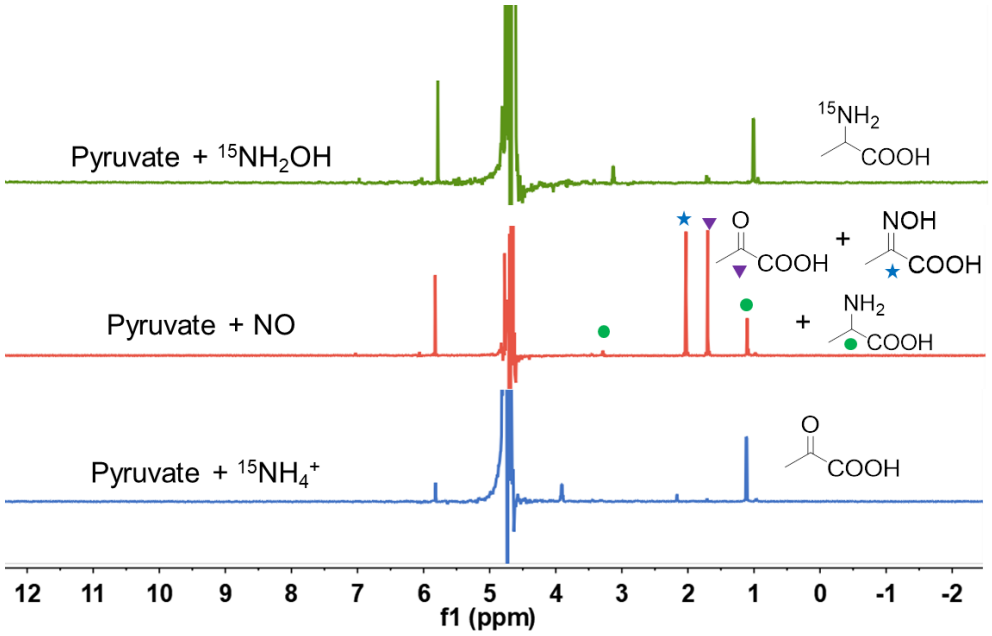


Figure S15. Typical ^{1}H NMR spectra of the electrolyte when using ^{15}N-NH_4^+ (blue), NO (red), and $^{15}NH_2OH$ (green) as the reactant.

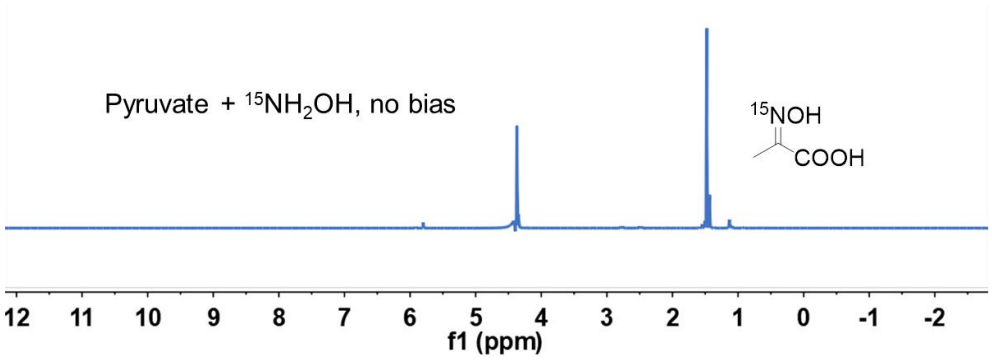
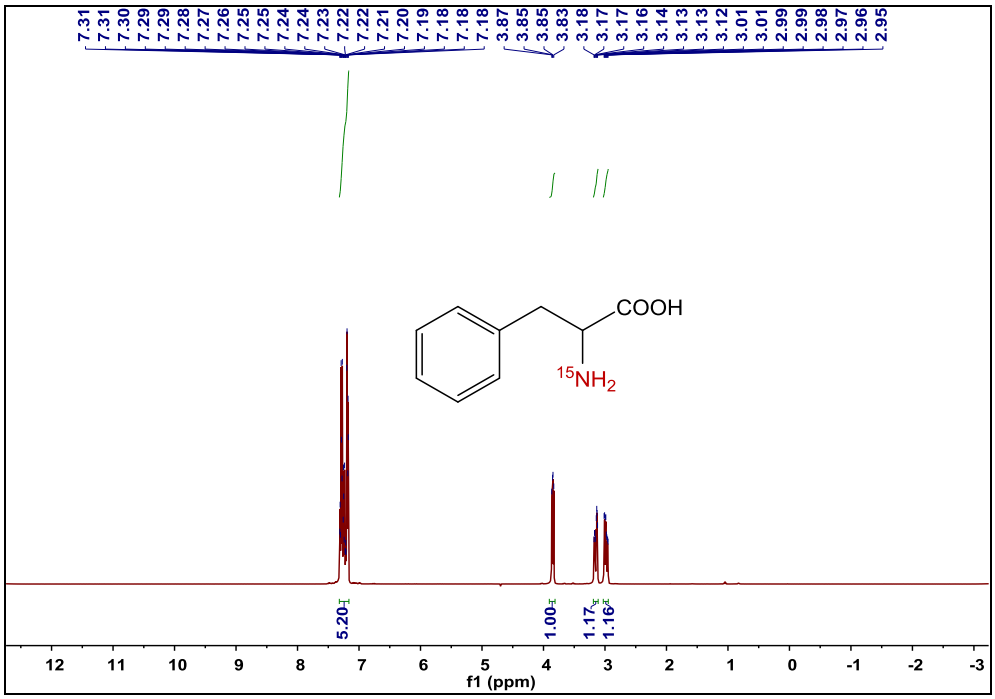
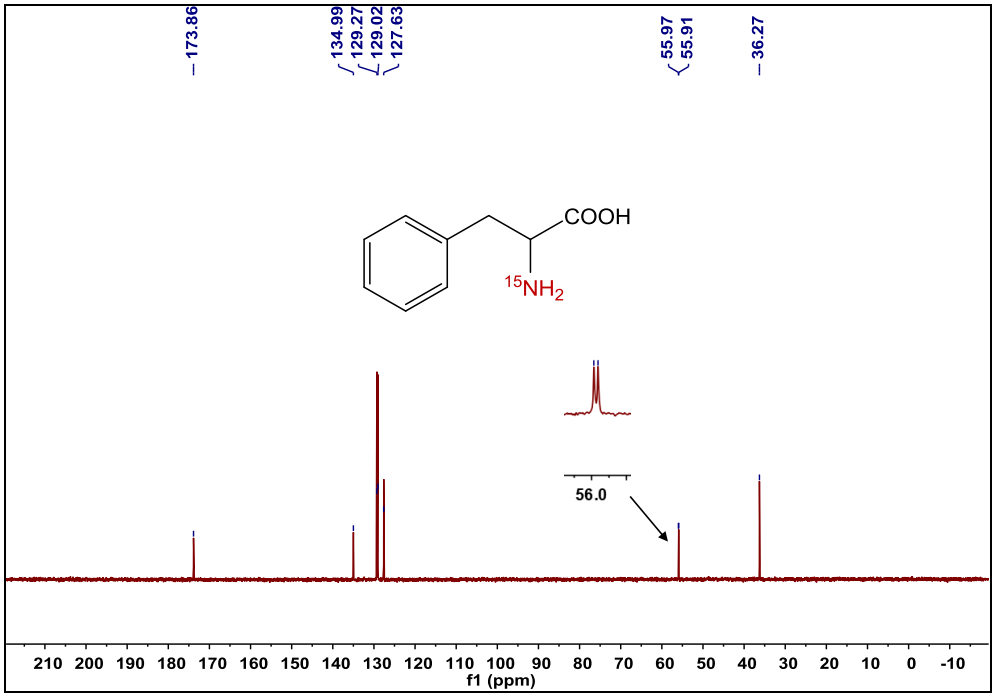


Figure S16. ^{1}H NMR spectrum of the product obtained by mixing pyruvate and $^{15}NH_2OH$ under ambient conditions.


7.31 7.31 7.30 7.29 7.29 7.28 7.27 7.26 7.25 7.25 7.24 7.24 7.23 7.22 7.22 7.21 7.20 7.19 7.18 7.18 7.18
3.87 3.85 3.85 3.83 3.18 3.17 3.17 3.16 3.14 3.13 3.13 3.12 3.01 3.01 2.99 2.99 2.98 2.97 2.96 2.95
COOH
15NH2
5.20
1.00
1.17
1.16
12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)


173.86
134.99
129.27
129.02
127.63
55.97
55.91
36.27
COOH
15NH2
56.0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

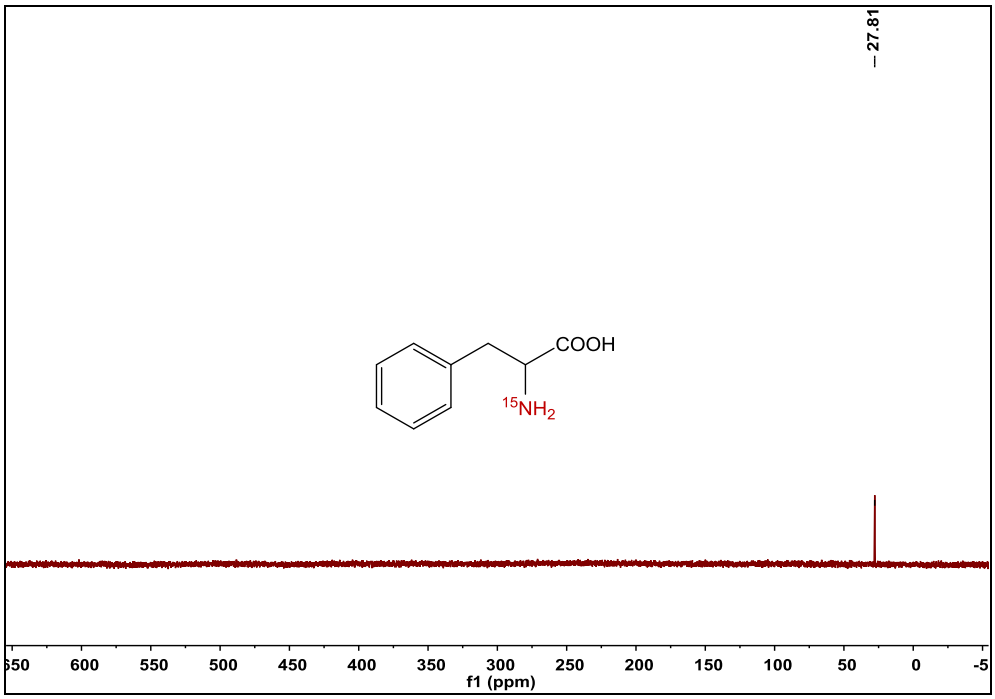
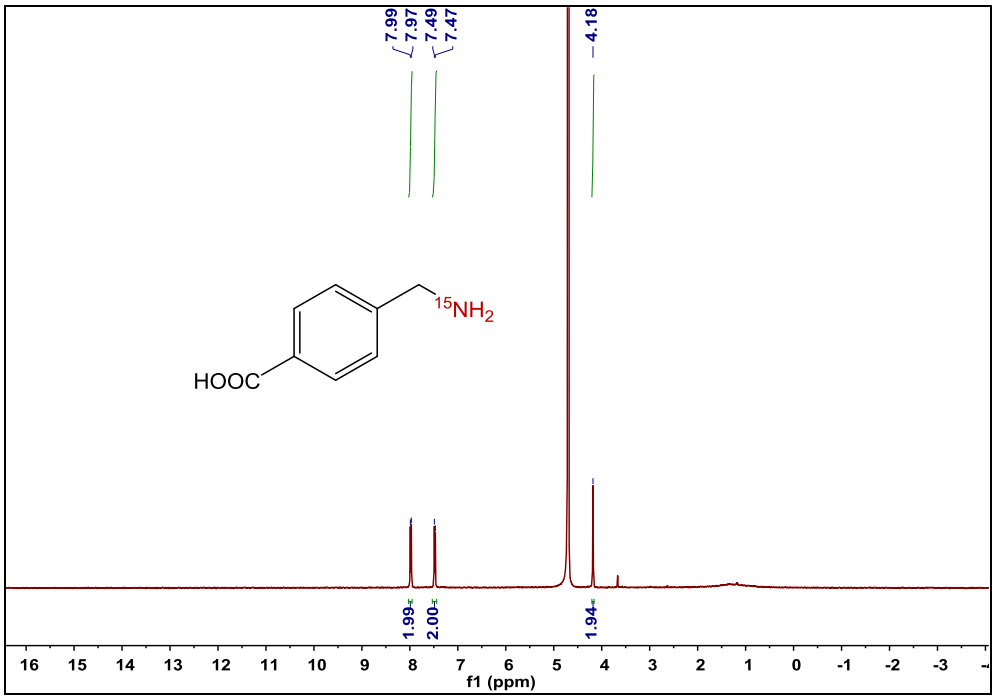
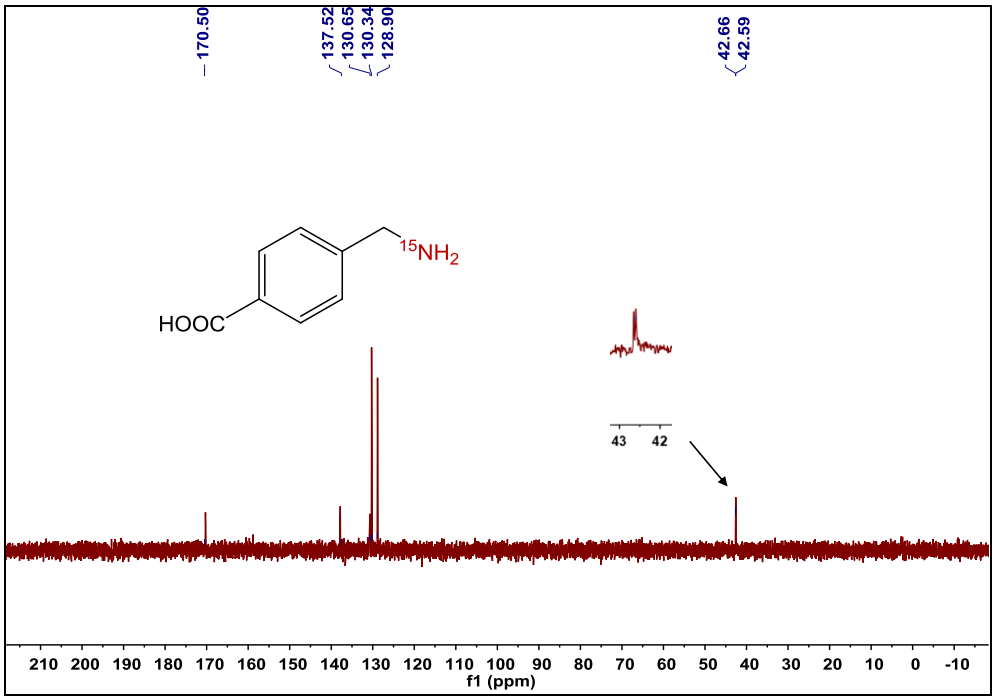


Figure S17. ^{1}H, ^{13}C, and ^{15}N NMR spectra of ^{15}N-phenylalanine.

^{1}H NMR (400 MHz, D_2O) δ [ppm] 7.32-7.17 (m, 5H), (dd, *J* = 8.4, 5.2 Hz, 1H), 3.19-3.11 (m, 1H), 3.02-2.94 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ [ppm] 173.86, 134.99, 129.27, 129.02, 127.73, 55.4 (d, *J* = 5.9 Hz), 36.27. ^{15}N NMR (61 MHz, D_2O) δ [ppm] 27.81. HRMS [$M+H^+$] 167.0826, the theoretical value for $C_2H_{11}{}^{15}NO_2$ [$M+H^+$] is 167.0838. 68% NMR yield, 33% isolated yield.


7.99
7.97
7.49
7.47
4.18
$^{15}NH_2$
HOOC
1.99
2.00
1.94
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)


170.50
137.52
130.65
130.34
128.90
42.66
42.59
$^{15}NH_2$
HOOC
43
42
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

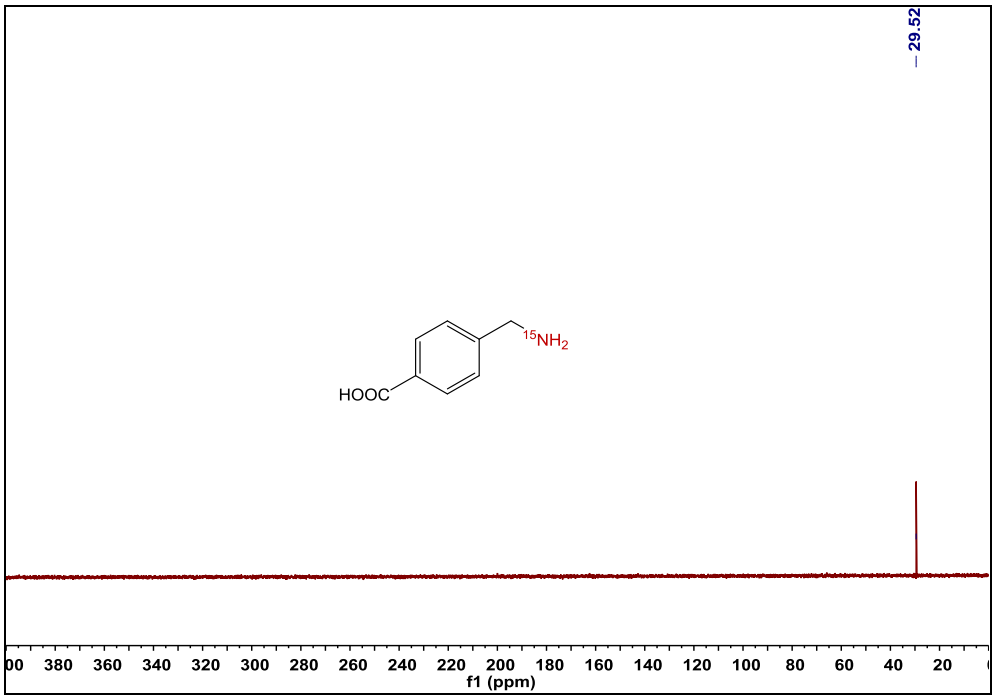
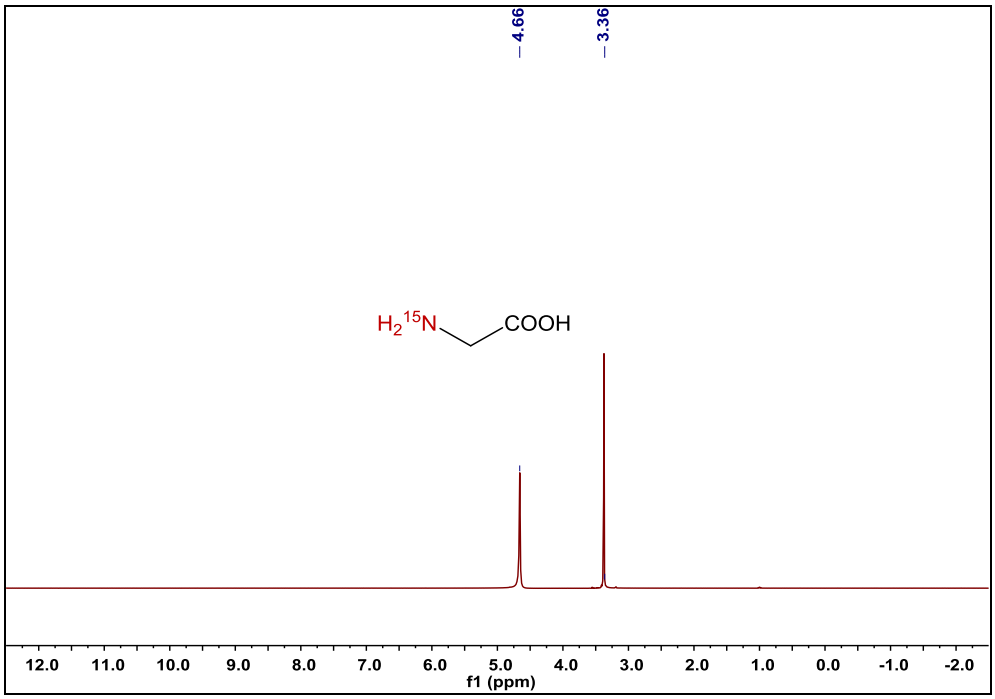
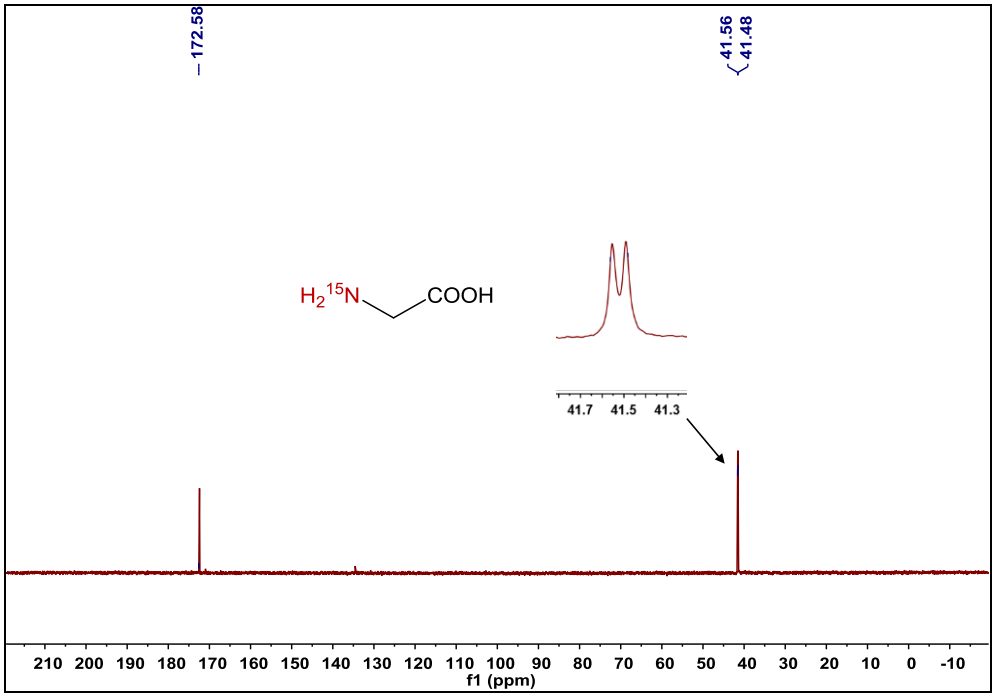


Figure S18. ^{1}H, ^{13}C, and ^{15}N NMR spectra of 4-(amino-^{15}N)benzoic acid.

^{1}H NMR (400 MHz, D_2O) δ [ppm] 7.98 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 8.0 Hz, 2H), 4.18 (s, 2H). ^{13}C NMR (101 MHz, D_2O) δ [ppm] 170.50, 137.52, 130.65, 130.34, 128.90, 42.62 (d, J = 6.7 Hz). ^{15}N NMR (61 MHz, D_2O) δ [ppm] 29.52. HRMS [M+H$^+$] 153.0656, theoretical value for $C_8H_9{}^{15}NO_2$ [M+H$^+$] is 153.0682. 81% NMR yield, 45% isolated yield.


4.66
3.36
$H_2{}^{15}N$
COOH
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0 -1.0 -2.0
f1 (ppm)


172.58
41.56
41.48
$H_2{}^{15}N$
COOH
41.7 41.5 41.3
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

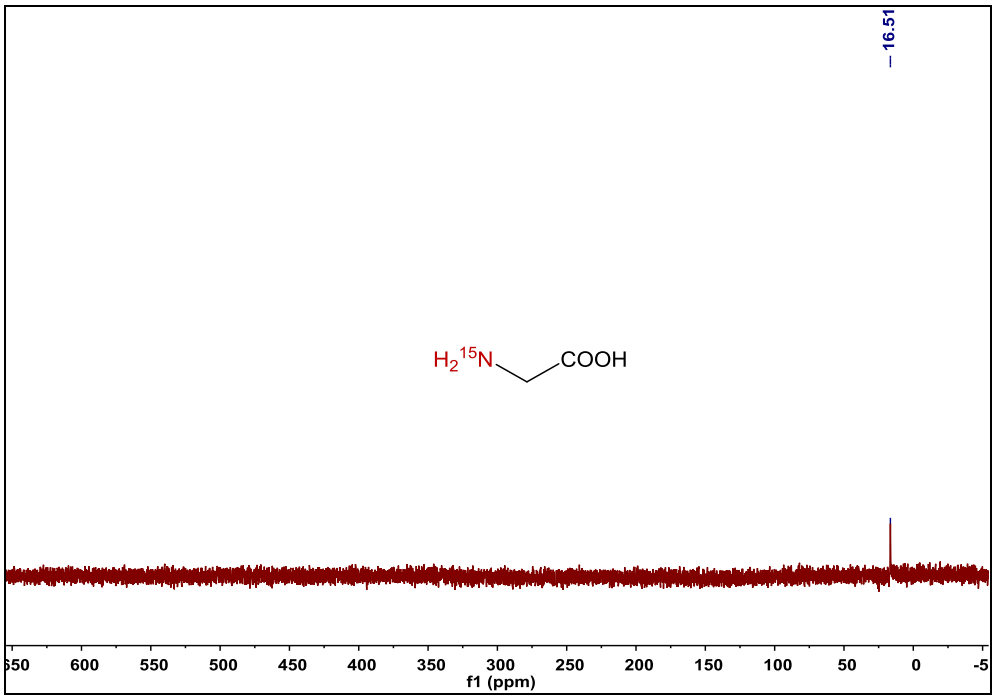
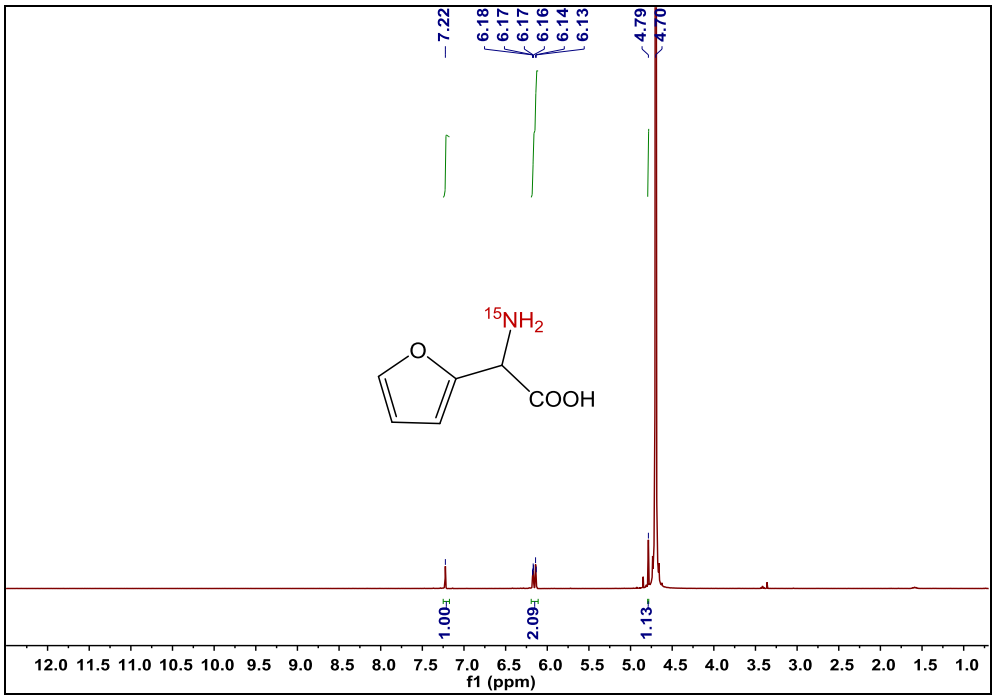
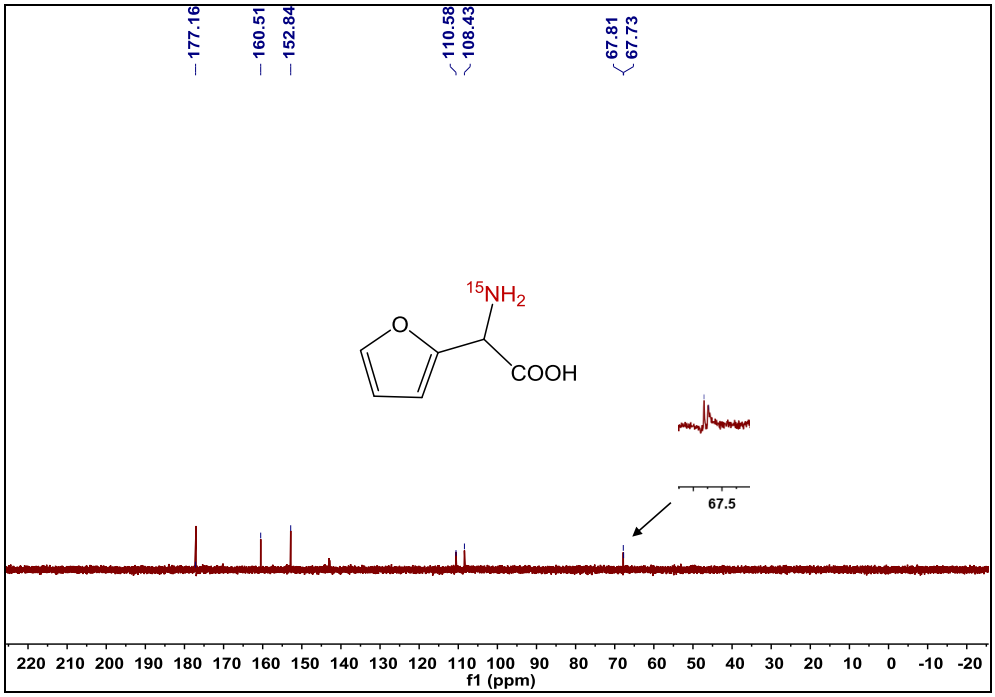


Figure S19. ^{1}H, ^{13}C, and ^{15}N NMR spectra of ^{15}N-glycine.

^{1}H NMR (400 MHz, D_2O) δ [ppm] 3.36 (s, 2H). ^{13}C NMR (101 MHz, D_2O) δ [ppm] 172.58, 41.52 (d, J = 6.3 Hz.) ^{15}N NMR (61 MHz, D_2O) δ [ppm] 16.51. HRMS [M+H^{+}] 77.0365, the theoretical value for $C_2H_5{}^{15}NO_2$ [M+H^{+}] is 77.0369. 95% NMR yield, 41% isolated yield.


7.22
6.18
6.17
6.17
6.16
6.14
6.13
4.79
4.70
15NH2
O
COOH
1.00
2.09
1.13
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f1 (ppm)


177.16
160.51
152.84
110.58
108.43
67.81
67.73
15NH2
O
COOH
67.5
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

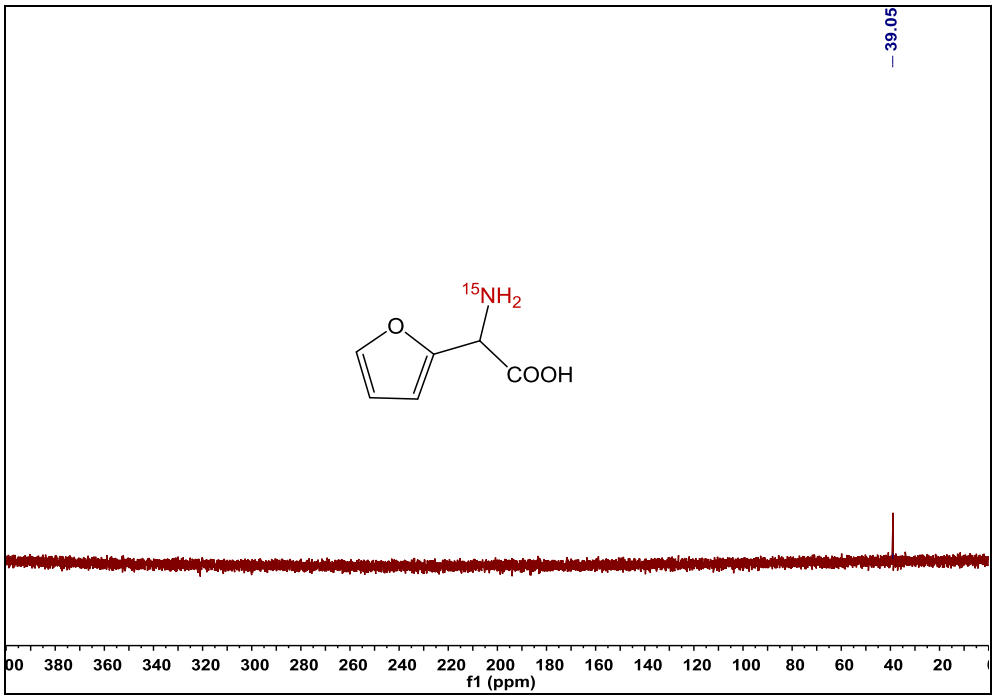
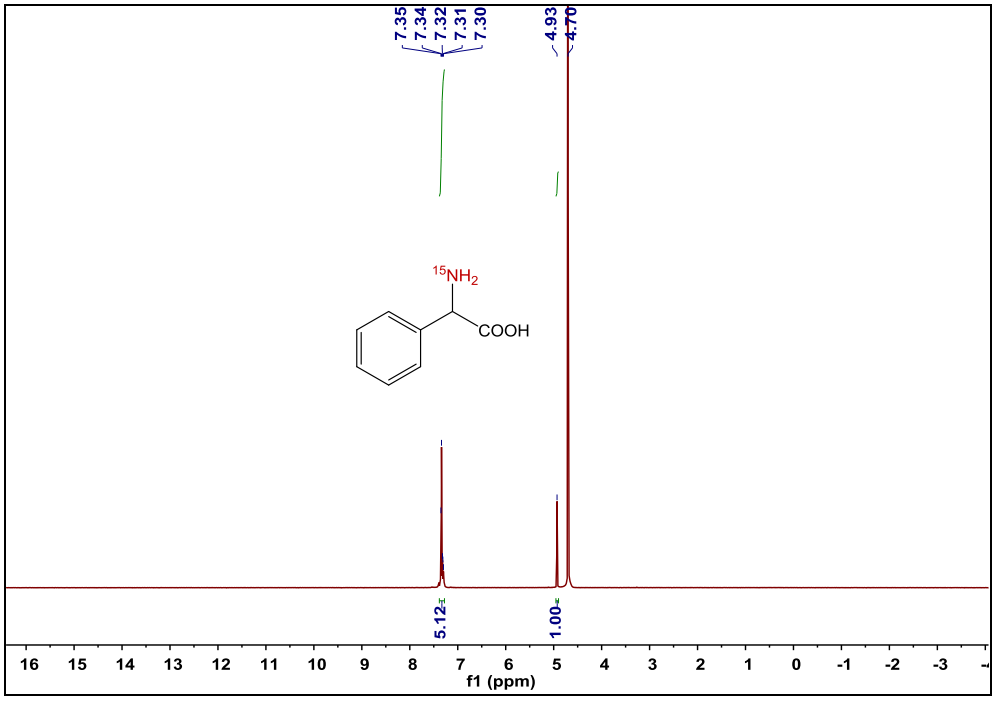
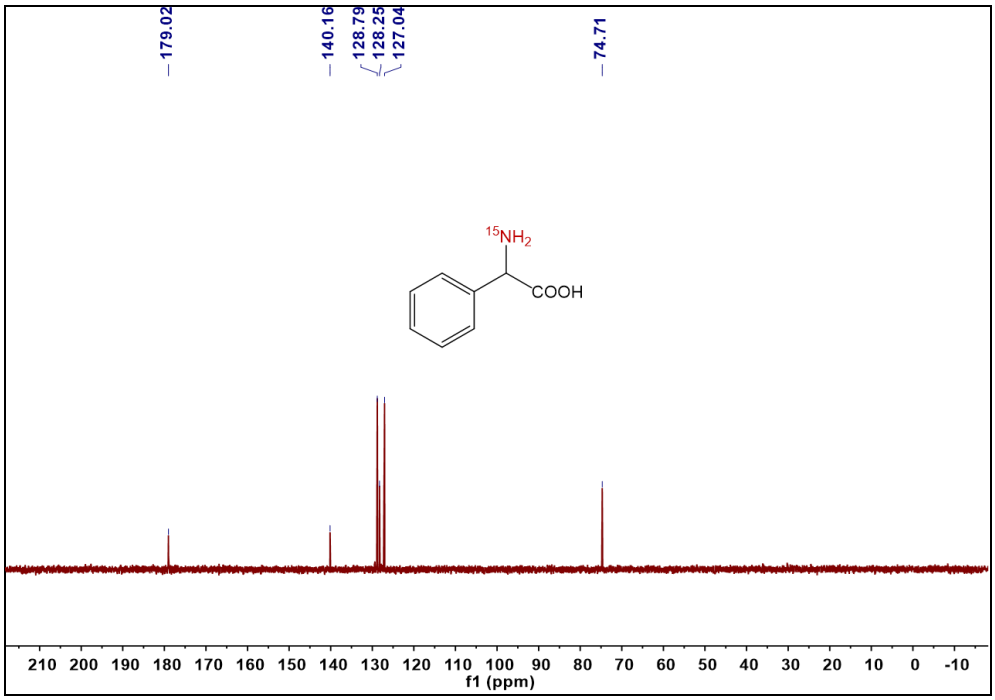


Figure S20. ^{1}H, ^{13}C, and ^{15}N NMR spectra of 2-(amino-^{15}N)-2-(furan-2-yl)acetic acid.

^{1}H NMR (400 MHz, D_2O) δ [ppm] 7.22 (s, 1H), 6.19-6.12 (m, 2H), 4.79 (s, 1H); **^{13}C NMR** (101 MHz, D_2O) δ [ppm] 177.16, 160.51, 152.84, 110.58, 108.43, 67.77 (d, J = 8.1 Hz); **^{15}N NMR** (61 MHz, D_2O) δ [ppm] 39.05; **HRMS** *m/z* 141.0180, the theoretical value for $[C_6H_7{}^{15}NO_2 + H]^+$ is 141.0318. 83% NMR yield, 41% isolated yield.


7.35
7.34
7.32
7.31
7.30
4.93
4.70
$^{15}NH_2$
COOH
5.12
1.00
f1 (ppm)


179.02
140.16
128.79
128.25
127.04
74.71
$^{15}NH_2$
COOH
f1 (ppm)

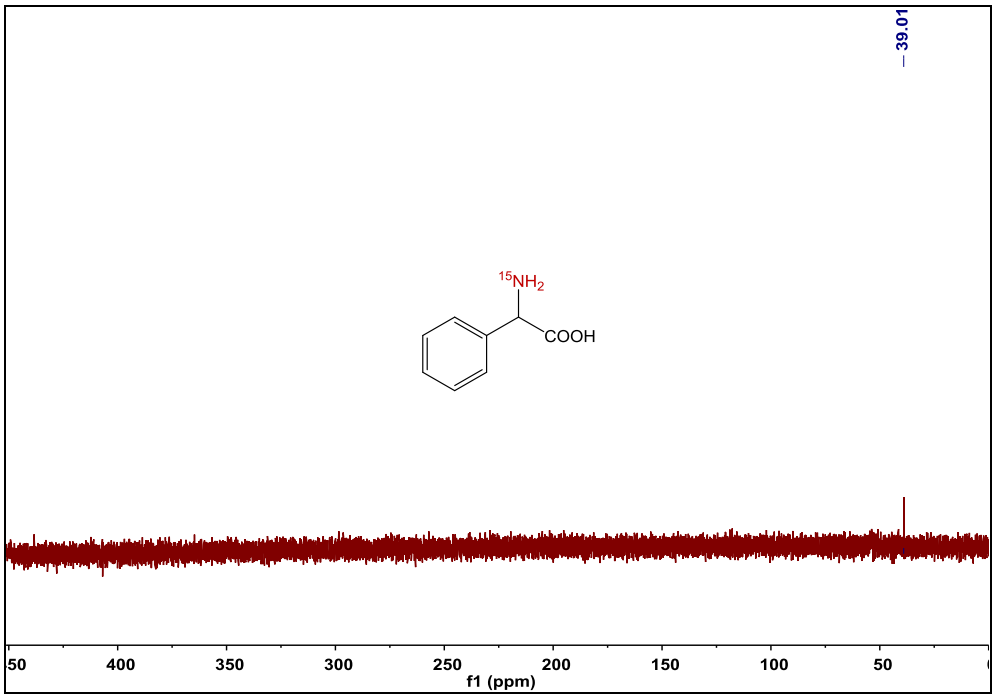
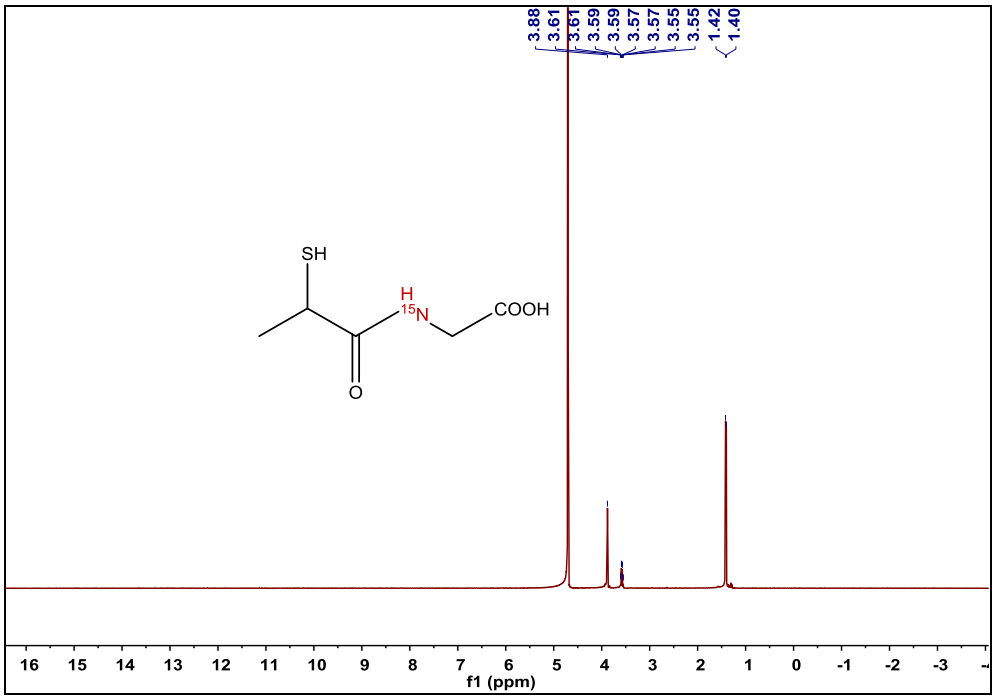
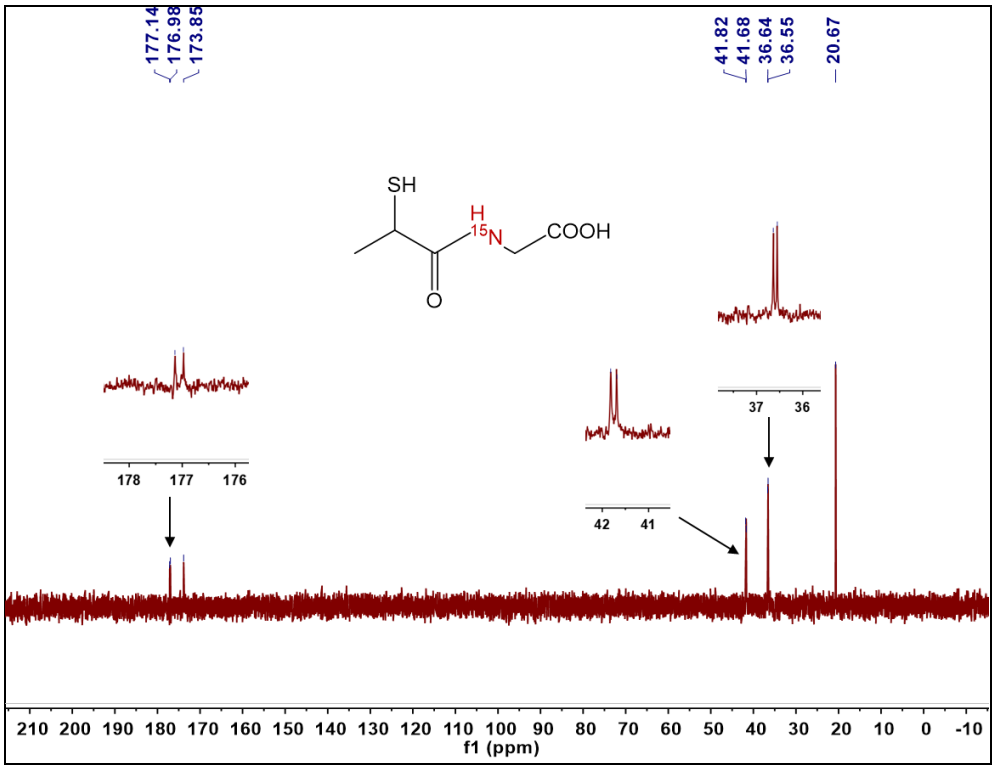


Figure S21. ^{1}H, ^{13}C, and ^{15}N NMR spectra of 2-(amino-^{15}N)-2-phenylacetic acid.

^{1}H NMR (400 MHz, D_2O) δ [ppm] 7.36-7.29 (m, 5H), 4.93 (s, 1H); **^{13}C NMR** (101 MHz, D_2O) δ [ppm] 179.02, 140.16, 128.79, 128.25, 127.04, 74.71; **^{15}N NMR** (61 MHz, D_2O) δ [ppm] 39.01; **HRMS** *m/z* 153.0670, the theoretical value for $[C_8H_9{}^{15}NO_2 + H]^+$ is 153.0671. 74% NMR yield, 52% isolated yield.


3.88
3.61
3.61
3.59
3.59
3.57
3.57
3.55
3.55
1.42
1.40
SH
H
15N
COOH
O
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)


177.14
176.98
173.85
41.82
41.68
36.64
36.55
20.67
SH
H
15N
COOH
O
178 177 176
42 41
37 36
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

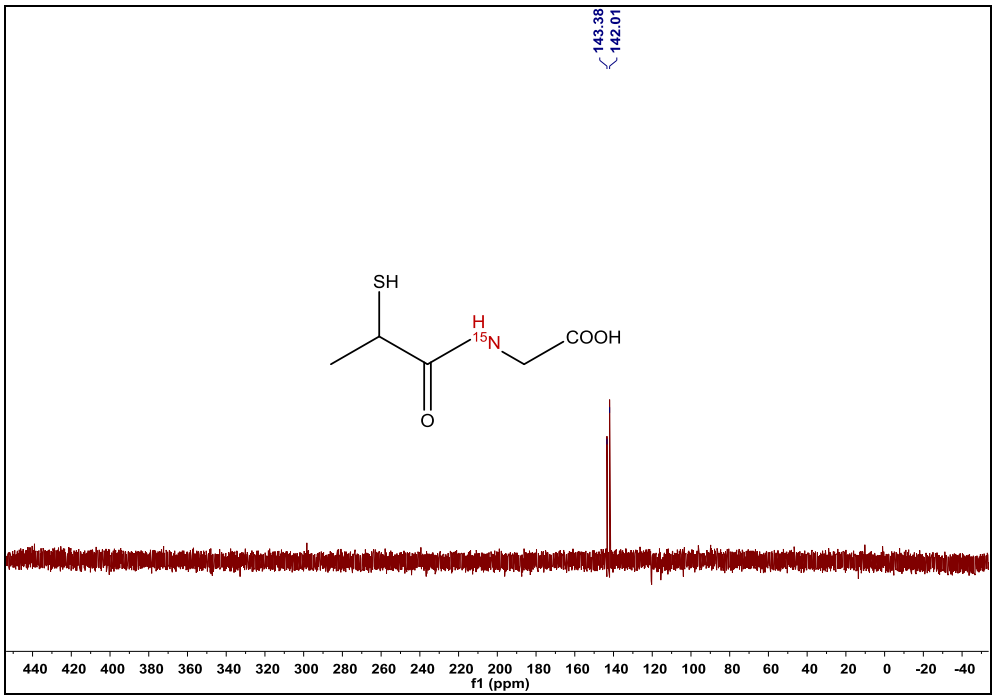


Figure S22. ^{1}H, ^{13}C and ^{15}N NMR spectra of ^{15}N-tiopronin.

^{1}H NMR (400 MHz, D_2O) δ [ppm] 3.88 (s, 2H), 3.62-3.54 (m, 1H), 1.41 (d, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ [ppm] 177.06 (d, J = 16 Hz), 173.85, 41.75 (d, J = 14 Hz), 36.60 (d, J = 9 Hz), 20.67. ^{15}N NMR (61 MHz, D_2O) δ [ppm] 143.38, 142.01. HRMS [M+H$^+$] 165.0335, theoretical value for $C_5H_9{}^{15}NO_3S$ [M+H$^+$] is 165.0352. 30% isolated yield.

3. References

1. Wang, Y., Yu, Y., Jia, R., Zhang, C., Zhang, B. Electrochemical Synthesis of Nitric Acid from Air and Ammonia through Waste Utilization. *Natl. Sci. Rev.* **2019**, 6, 730-738.
2. Yang, J., Chen, X., Zhang, Y., Zhen, W. Studies on Synthetic Process of Tiopronin. *China Pharmacist*. **2005**, *8*, 631-633.
3. Wang, Y., Li, H., Zhou, W., Zhang, X., Zhang, B., Yu, Y. Structurally Disordered RuO_2 Nanosheets with Rich Oxygen Vacancies for Enhanced Nitrate Electroreduction to Ammonia, *Angew. Chem. Int. Ed.*, **2022**, 61, e202202604.