Table of Contents

Reagents and Procedures:

Substrates, deuterium oxide (D_2O) and ruthenium on carbon 5 wt. % loading (RuC 5%) purchased from Sigma-Aldrich and Cambridge Isotope Laboratories were used without further purification. The OX063 radical was purchased from GE Healthcare and DOTAREM® gadolinium from Guerbet. Other chemicals and solvents were purchased from Aldrich. ^1H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded on a Bruker Avance 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from residual solvent peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), triplet (t). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). High resolution mass spectrometry was performed by the Notre Dame mass spectrometry facility, on a microTOF instrument (Notre Dame, IN). For compounds **1** to **8** HPLC chromatograms were recorded using a Chirex 3126 chiral column (250 × 4.6 mm) and the mobile phase consisted of 1 mM sulfate copper(II) in water, at room temperature, with UV detection at 254 nm.

Procedures for deuteration:

HD exchange reaction of the starting material was carried out in a capped vial (Hollister-Stier Laboratories 10mL/20mm) with RuC 5% (40 wt. %) in D_2O, under H_2 (1 atm) at 80°C overnight. The reaction mixture was cooled down to room temperature and filtered on a syringe-driven filter (Millex-HN 0.45µm Nylon Membrane). After treatment on activated carbon (1 g stirred overnight) then filtration, concentrated HCl was added to the filtrate to pH 3. 5 g of Dowex 50WX8 hydrogen form were pre-treated with 0.001 N HCl before elution of the filtrate with 0.001 N HCl then water. Final elution with ammonia solution 25%, evaporation then lyophilization gave the deuterated product. The ^2H (or D) content was determined by ^1H, ^{13}C NMR analyses and mass spectrometry.

Procedure	Amount of starting material	D_2O Volume	Treatment	Deuterated products
A	2 mmol	5 mL	Activated carbon, Dowex resin, lyophilization	[1-^{13}C,2-^{2}H]alanine 1
B	1 mmol	5 mL	Activated carbon, Dowex resin, lyophilization	[1-^{13}C,2,3-^{2}H]serine 2, [2-^{13}C,2,3-$^{2}H_3$]serine 7
C	3 mmol	5 mL	Lyophilization only	[1-^{13}C,2-^{2}H]sodium lactate 3
D	3 mmol	7 mL	Activated carbon, Dowex resin, lyophilization	[1-^{13}C,2-^{2}H]valine 4
E	3 mmol	5 mL	Activated carbon, Dowex resin, lyophilization	[1-^{13}C,2-$^{2}H_2$]glycine 5
F	2.5 mmol	5 mL	Activated carbon, Dowex resin, lyophilization	[2-^{13}C,2-^{2}H]alanine 6
G	1.4 mmol	5 mL	Lyophilization only	[2-^{13}C,2-^{2}H]sodium lactate 8

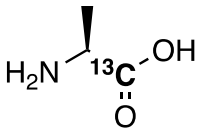
Table S1: Procedures for H/^{2}H (or D) exchange

Deuterium incorporation quantification:

Deuterium incorporation was quantified by mass spectrometry and by the decrease of ^{1}H NMR integral intensities at the specified positions compared to the starting material. Integral intensities were calibrated against hydrogen signals that did not undergo H/D exchange. In cases with multiple sites of deuteration, we analyzed the product with 1 equivalent of commercially available serine or glycine used as internal standard. Mass spectrometry quantification was performed by subtraction of the mean molecular masses of the product and substrate isotopologue clusters.

Experimental Details and Characterization for Compounds 1 to 8:

Starting material: commercially available [1-^{13}C]alanine:



^{1}H NMR (400 MHz, D_2O) δ 1.46 (dd, *J*=7.18, 4.26 Hz, 3H), 3.76 (qd, *J*=7.22, 5.11 Hz, 1H)

^{13}C NMR (101 MHz, D_2O) δ 16.07, 50.37 (d, *J*=53.55 Hz), 175.75 (enriched)

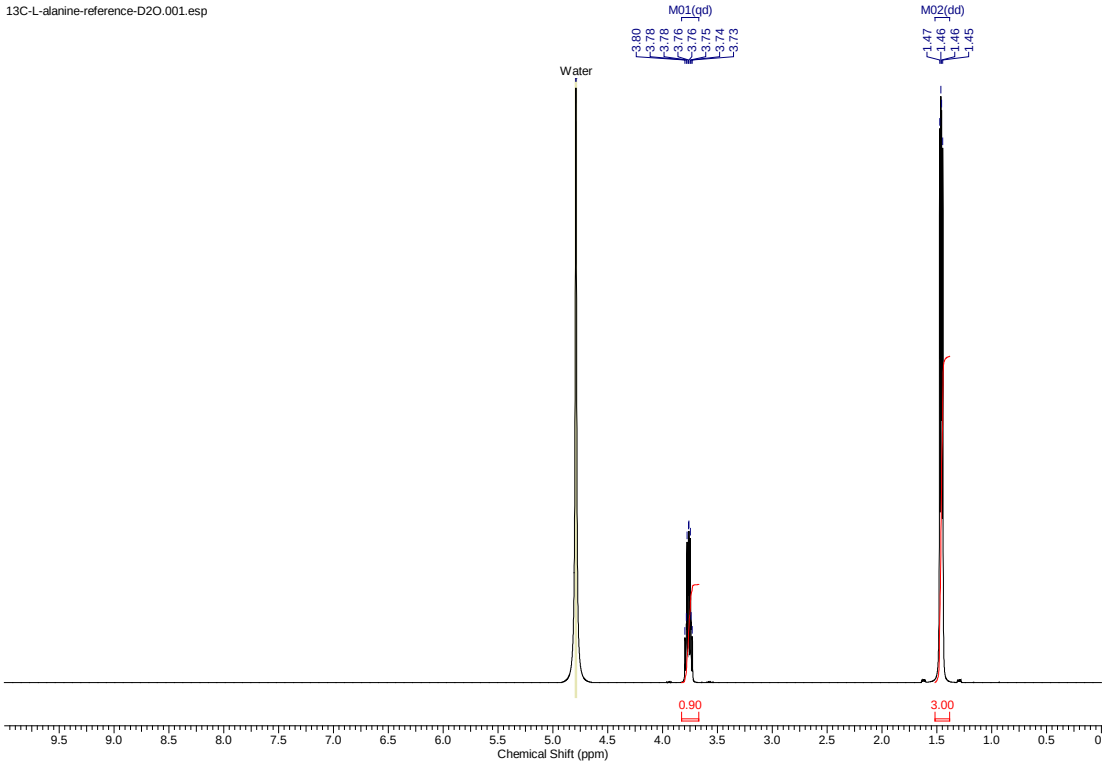


Figure S1: ^{1}H NMR spectrum of commercially available [1-^{13}C]alanine

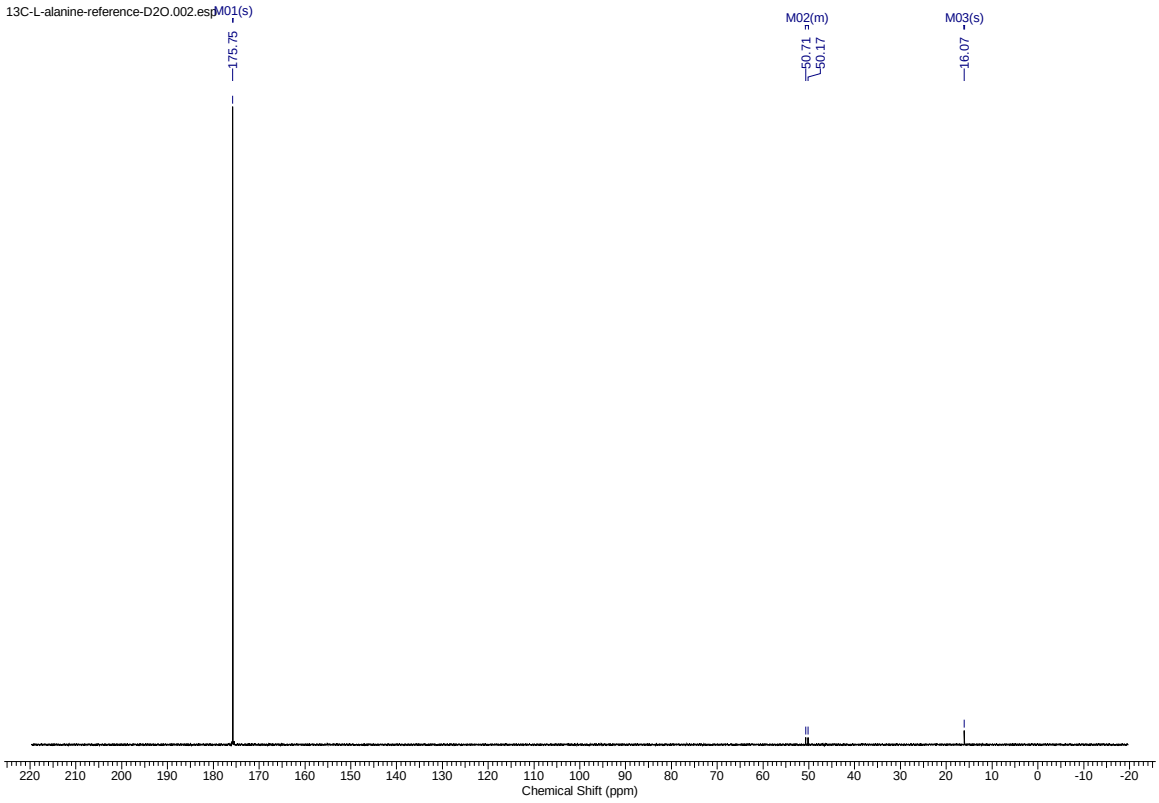


Figure S2: ^{13}C NMR spectrum of commercially available [1-^{13}C]alanine

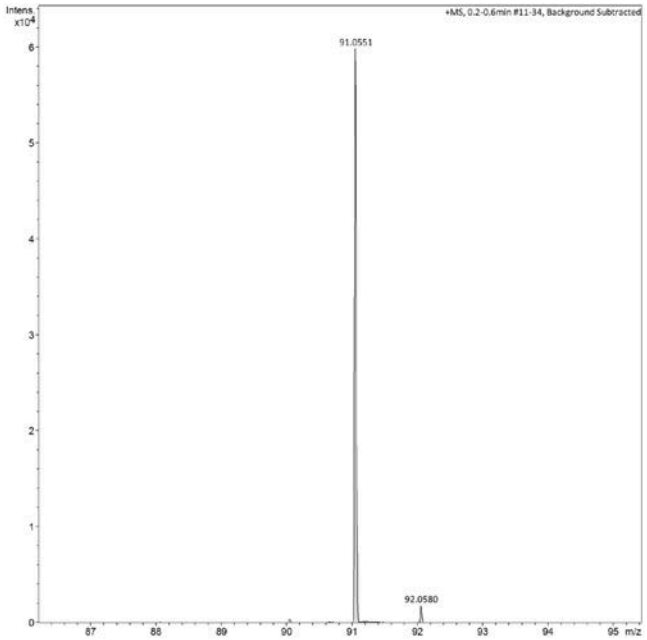
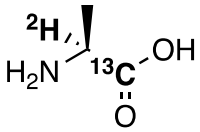


Figure S3: Mass spectrum of commercially available [1-^{13}C]alanine

[1-^{13}C,2-^{2}H]alanine 1 (procedure A):

[97]



After treatment [1-^{13}C,2-^{2}H]alanine **1** was lyophilized to give a white solid (185 mg; 0.49 mmol; 99% yield).

^{1}H NMR (400 MHz, D_2O) δ 1.47 (d, *J*=3.90 Hz, 3H), 3.72 - 3.82 (m, 0.03H)

^{13}C NMR (101 MHz, D_2O) δ 15.96, 175.77 (enriched)

HRMS (ESI): $[M+H]^+$ calcd. for $C_2{}^{13}CH_6{}^{2}HNO_2$: 92.0651, found: 92.0615.

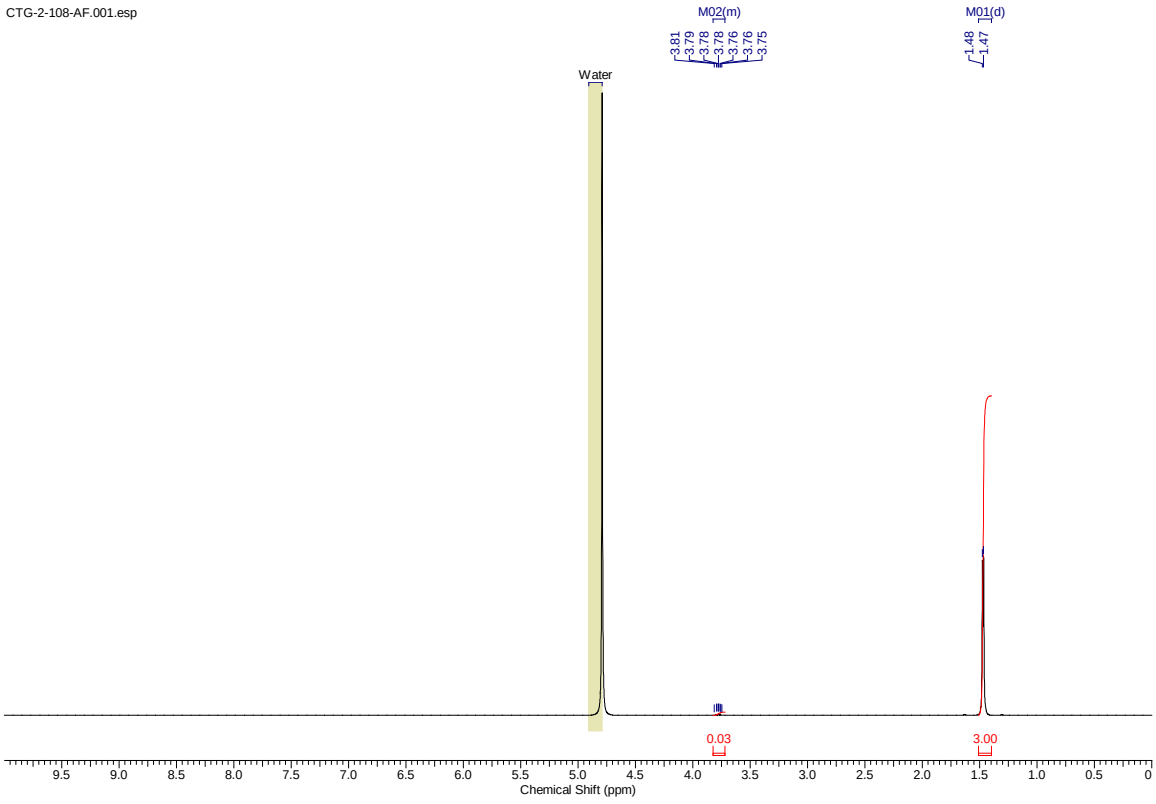


*Figure S4: ^{1}H NMR spectrum of [1-^{13}C,2-^{2}H]alanine **1***

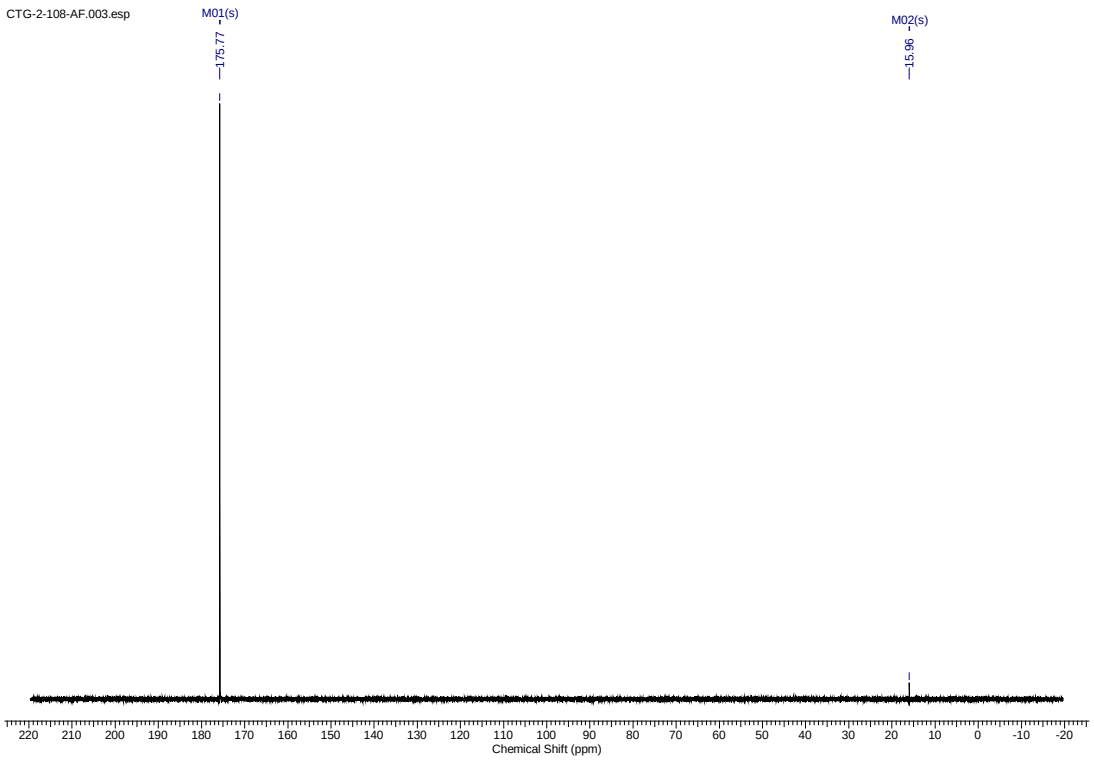


*Figure S5: ^{13}C NMR spectrum of [1-^{13}C,2-^{2}H]alanine **1***

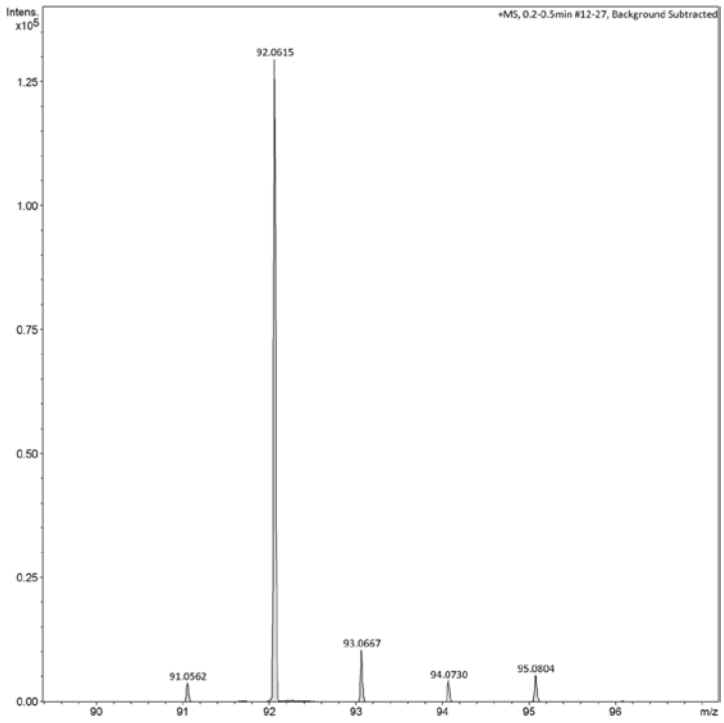


*Figure S6: Mass spectrum of [1-^{13}C,2-^{2}H]alanine **1***

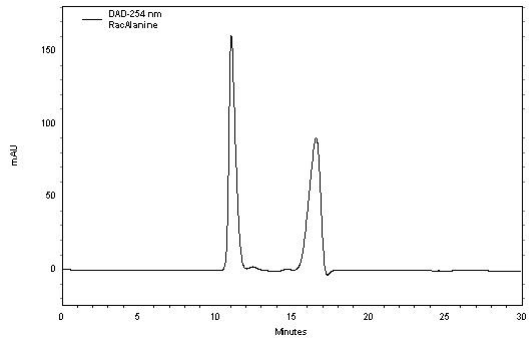


Figure S7: Chiral HPLC on commercially available racemic Alanine

Mobile phase: 1 mM sulfate copper(II) in water.
Flow rate: 1.0 ml.min^{-1}.
Injection volume: 10 µL solution of substrate in 1 mM sulfate copper(II) in water (1 mg.mL^{-1})

Time (min)	Area	%
11.045	19187800	52
16.584	17761100	48

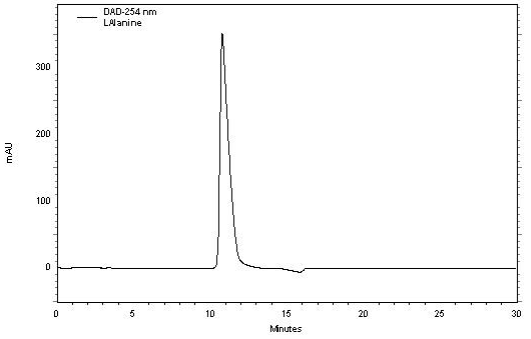


Figure S8: Chiral HPLC on commercially available L-Alanine

Time (min)	Area	%
10.774	53011800	>99
16.138	20970	<1

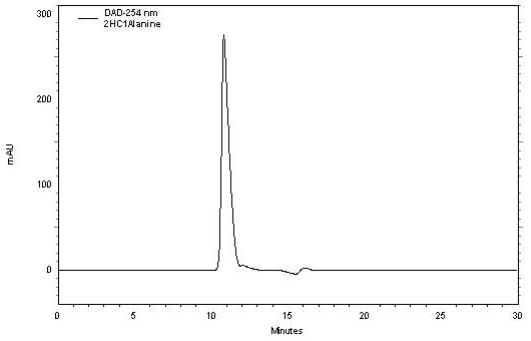
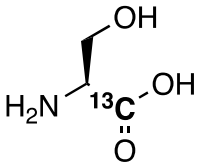


*Figure S9: [1-^{13}C,^{2}-2H]alanine **1***

Time (min)	Area	%
10.821	36846200	>99
16.145	152529	<1

Starting material: commercially available [1-^{13}C]serine:



^{1}H NMR (400 MHz, D_2O) δ 3.81-3.88 (m, 1H), 3.90-4.03 (m, 2H)

^{13}C NMR (101 MHz, D_2O) δ 56.60 (d, *J*=52.82 Hz), 60.11, 172.29 (enriched)

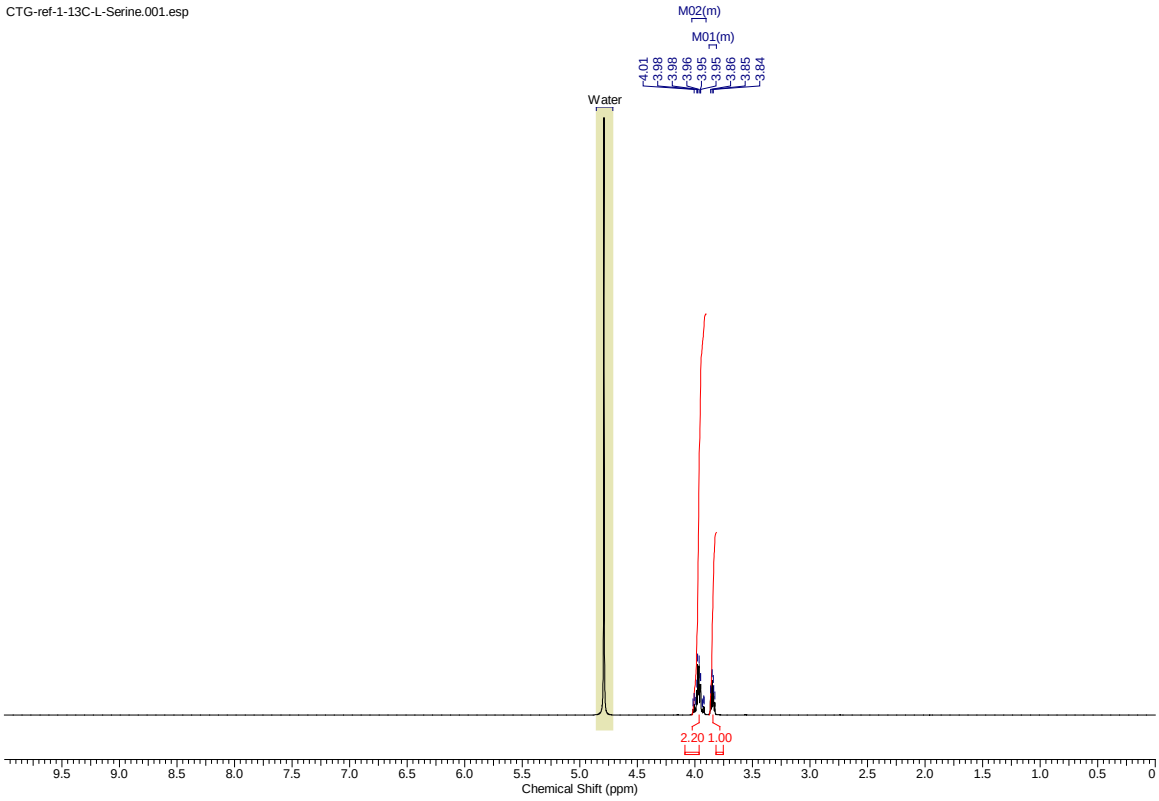


Figure S10: ^{1}H NMR spectrum of commercially available [1-^{13}C]serine

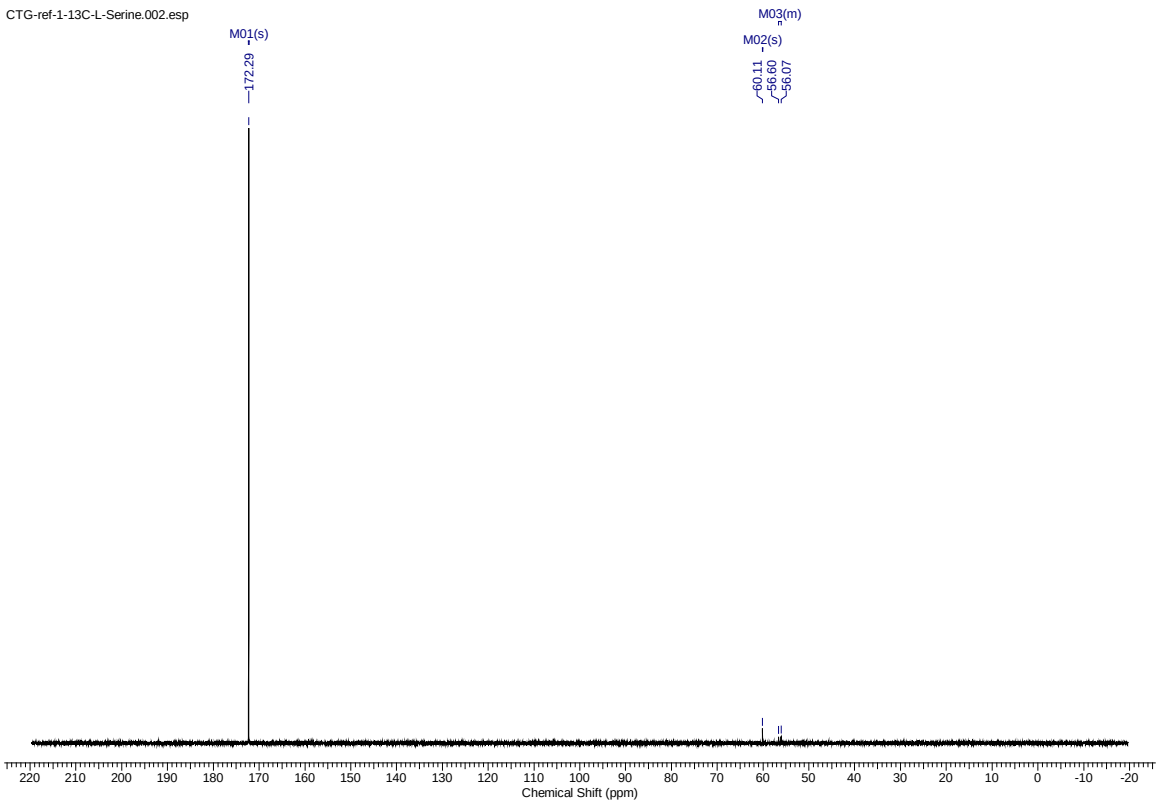


Figure S11: ^{13}C NMR spectrum of commercially available [1-^{13}C]serine

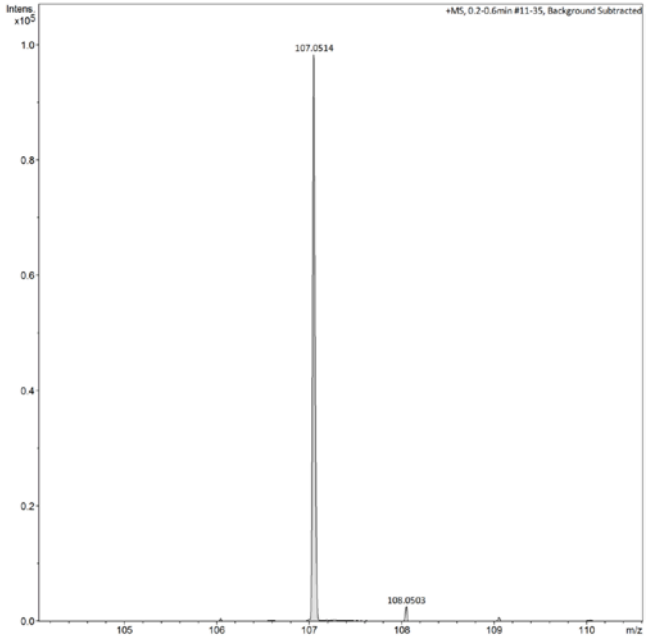
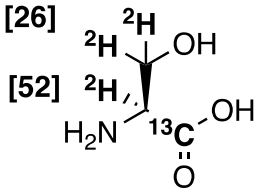


Figure S12: Mass spectrum of commercially available [1-^{13}C]serine

[1-^{13}C,2,3-^{2}H]serine 2 (procedure B):



After treatment [1-^{13}C,2,3-^{2}H]serine **2** was lyophilized to give a white solid (82 mg; 0.75 mmol; 78% yield).

^{1}H NMR (400 MHz, D_2O) δ 3.71-3.79(m, 1.54H), 3.80-3.95 (m, 0.48H)

^{13}C NMR (101 MHz, D_2O) δ 172.31 (enriched)

HRMS (ESI): $[M+H]^+$ calcd. for $C_2{}^{13}CH_6{}^{2}HNO_3$: 108.0600, found: 108.0572 (more abundant isotope).

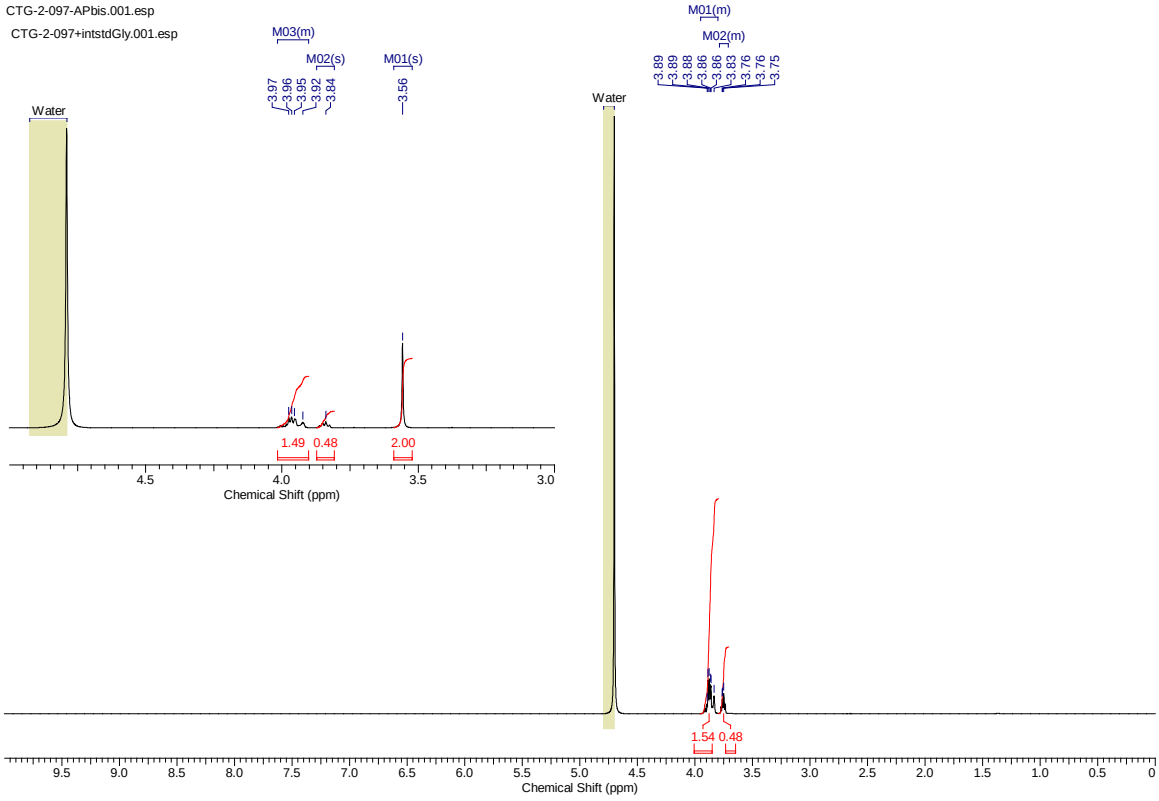


*Figure S13: ^{1}H NMR spectrum of [1-^{13}C,2,3-^{2}H]serine **2** (as shown in the upper left inset, use of 1 equivalent of commercially available glycine enables to determine isotopic enrichment of [1-^{13}C,2,3-^{2}H]serine **2**)*

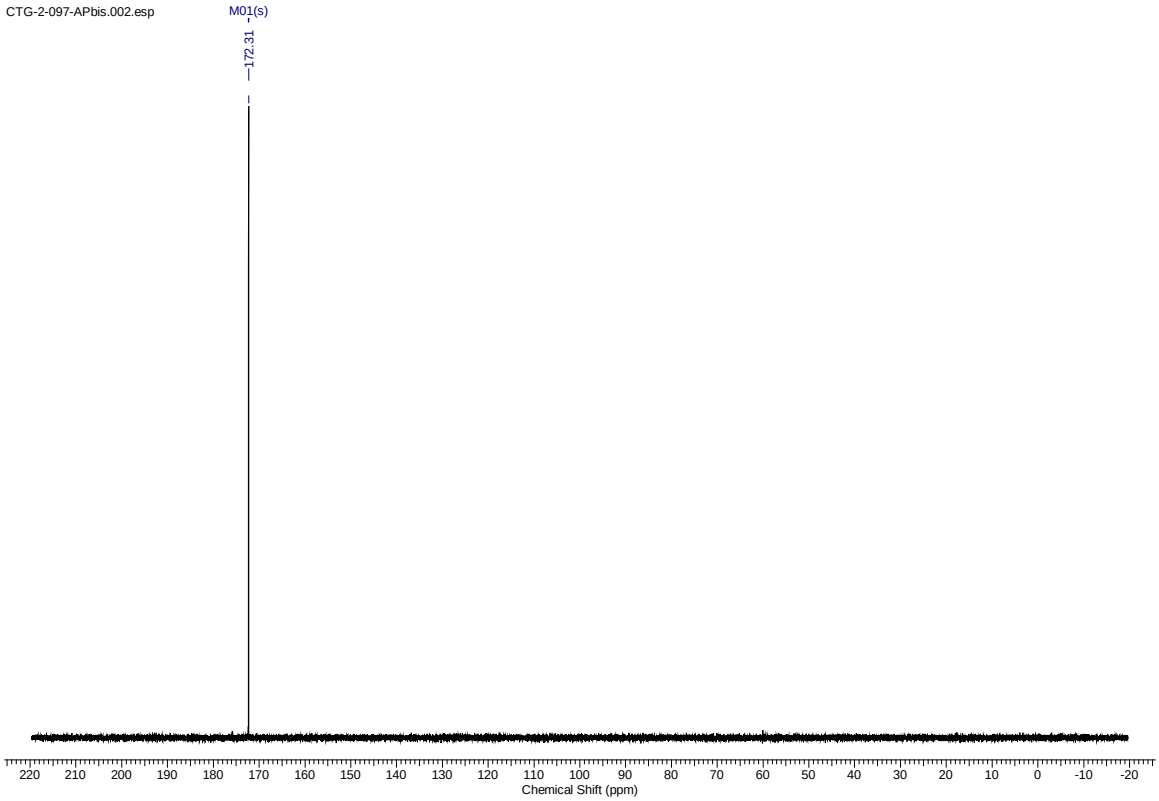


*Figure S14: ^{13}C NMR spectrum of [1-^{13}C,2,3-^{2}H]serine **2***

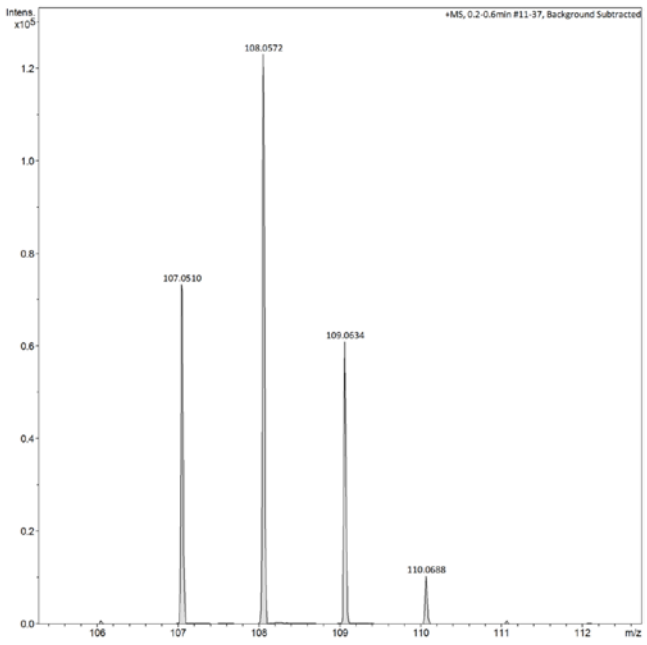


*Figure S15: Mass spectrum of [1-^{13}C,2,3-^{2}H]serine **2***

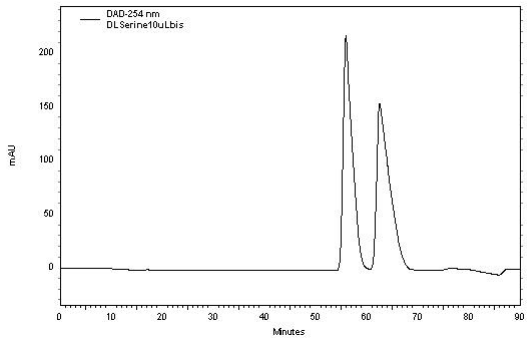


Figure S16: Chiral HPLC on commercially available racemic Serine
Mobile phase: 1 mM sulfate copper(II) in water.
Flow rate: 0.2 ml.min^{-1}.
Injection volume: 10 µL solution of substrate in 1 mM sulfate copper(II) in water (1 mg.mL^{-1})

Time (min)	Area	%
55.914	105471000	51
62.560	102312000	49

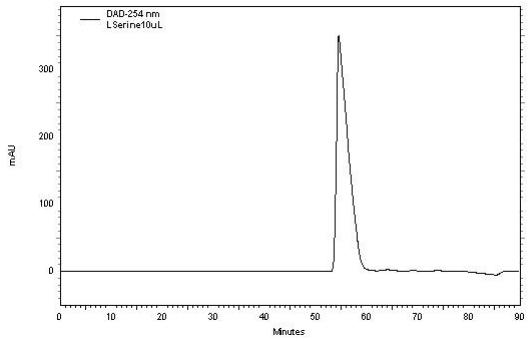


Figure S17: Chiral HPLC on commercially available L-Serine

Time (min)	Area	%
54.576	215314000	>99
64.112	199702	<1

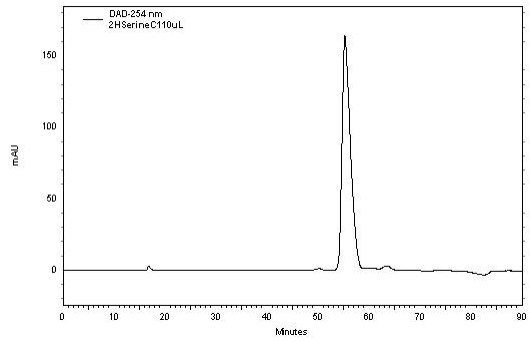
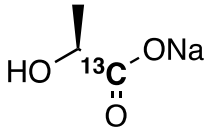


*Figure S18: [1-^{13}C,2-$^{2}H_3$]serine **2***

Time (min)	Area	%
55.300	72220100	>99
63.503	603651	<1

Starting material: commercially available [1-^{13}C]sodium lactate:



^{1}H NMR (400 MHz, D_2O) δ 1.32 (dd, *J*=6.82, 4.14Hz, 3H), 4.10 (qd, *J*=6.90, 3.17 Hz, 1H)

^{13}C NMR (101 MHz, D_2O) δ 20.03, 68.44 (d, *J*=55.02 Hz), 182.51 (enriched)

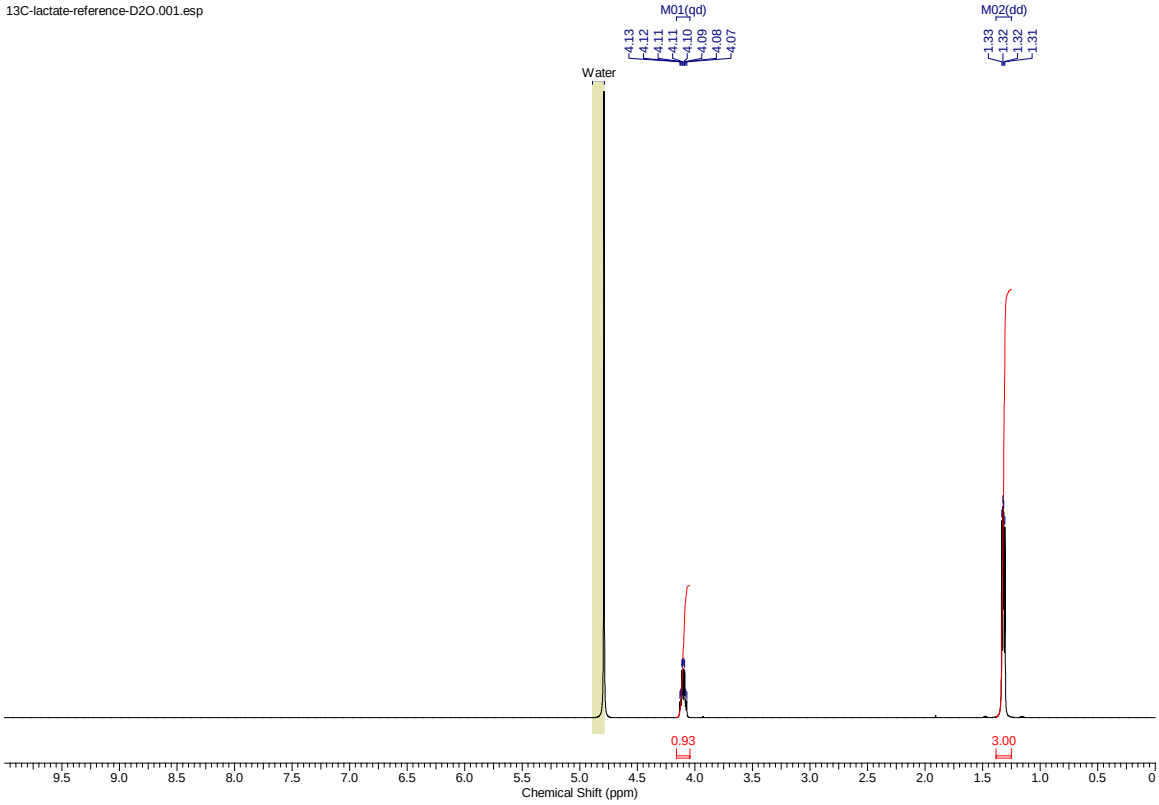


Figure S19: ^{1}H NMR spectrum of commercially available [1-^{13}C]sodium lactate

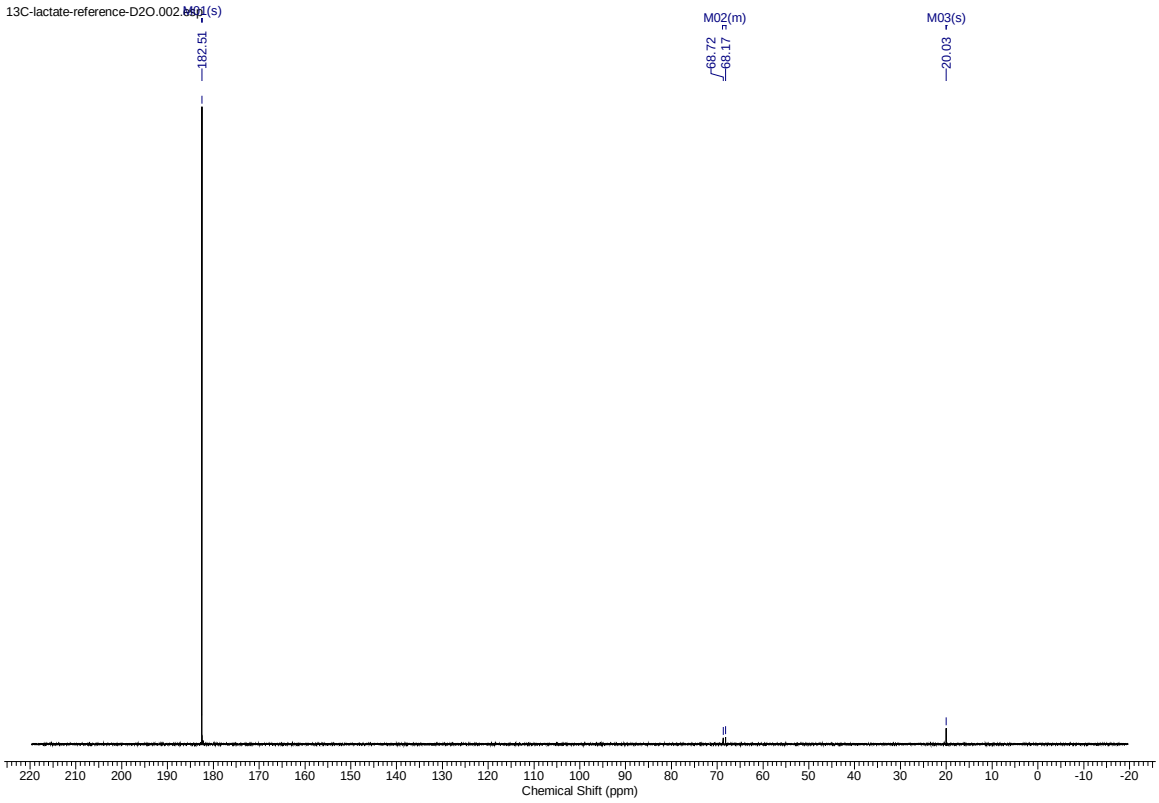


Figure S20: ^{13}C NMR spectrum of commercially available [1-^{13}C]sodium lactate

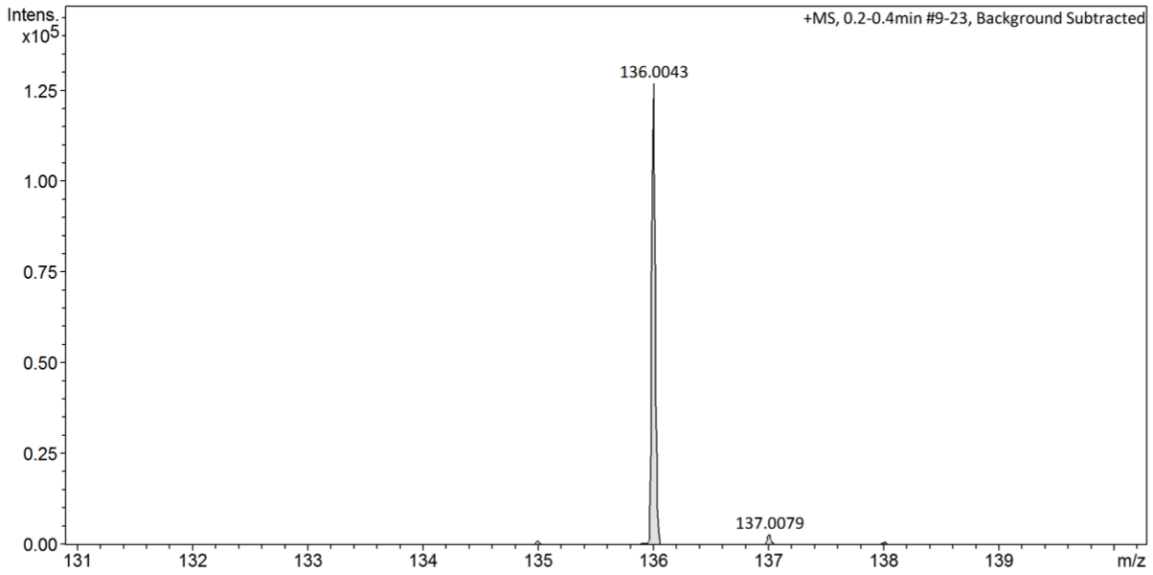
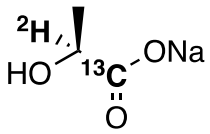


Figure S21: Mass spectrum of commercially available [1-^{13}C]sodium lactate

[1-^{13}C,2-^{2}H]sodium lactate 3 (procedure C):

[97]



After treatment [1-^{13}C,2-^{2}H]sodium lactate **3** was lyophilized to give a clear solid (332 mg; 2.91 mmol; 98% yield).

^{1}H NMR (400 MHz, D_2O) δ 1.20-1.43 (m, 3H), 4.07-4.17 (m, 0.03H)

^{13}C NMR (101 MHz, D_2O) δ 19.83, 182.15 (enriched)

HRMS (ESI): $[M+Na]^+$ calcd. for $C_2{}^{13}CH_4{}^{2}HO_3Na$: 137.0130, found: 137.0105 (more abundant isotope).

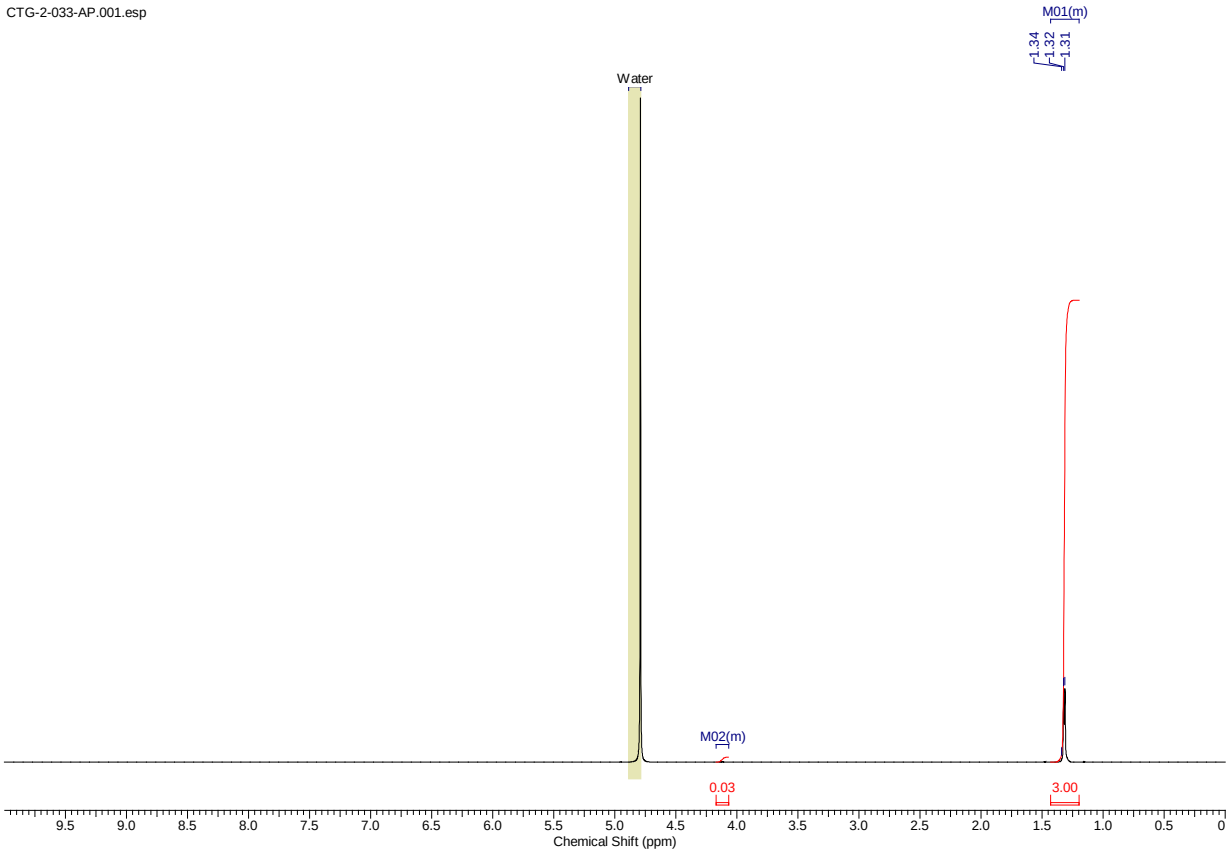


*Figure S22: ^{1}H NMR spectrum of [1-^{13}C,2-^{2}H]sodium lactate **3***

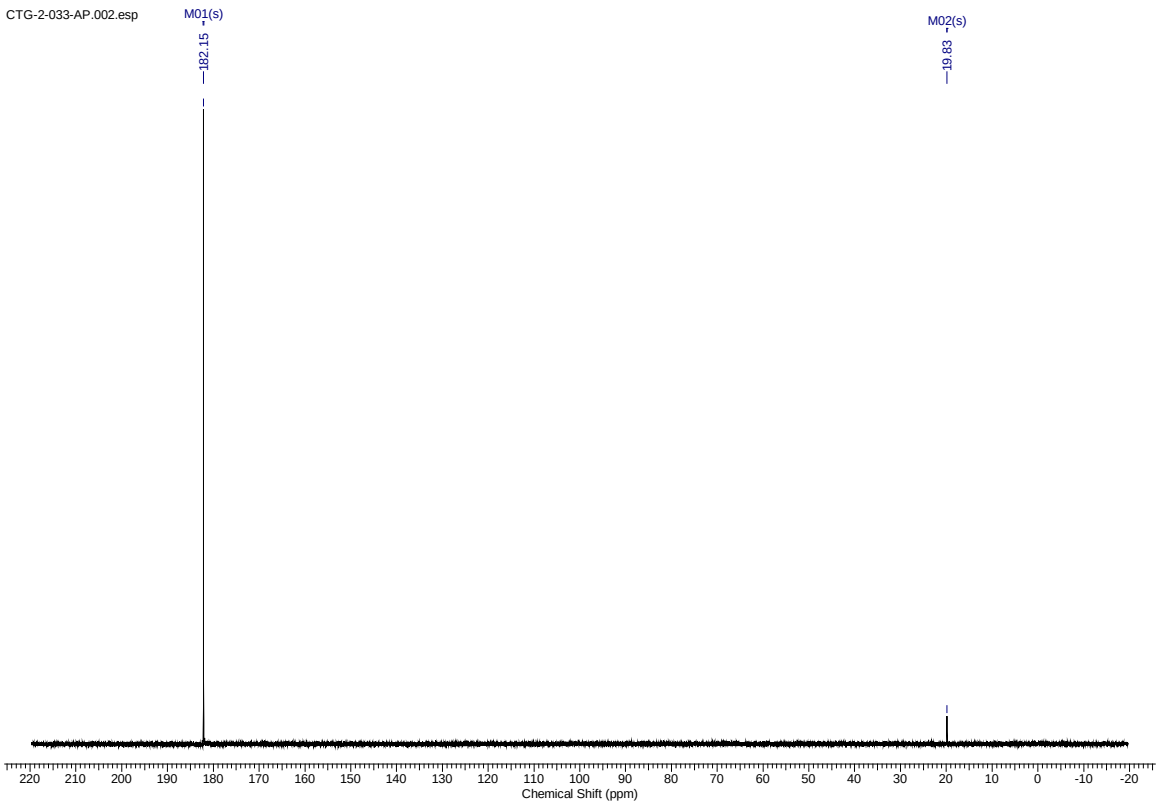


*Figure S23: ^{13}C NMR spectrum of [1-^{13}C,2-^{2}H]sodium lactate **3***

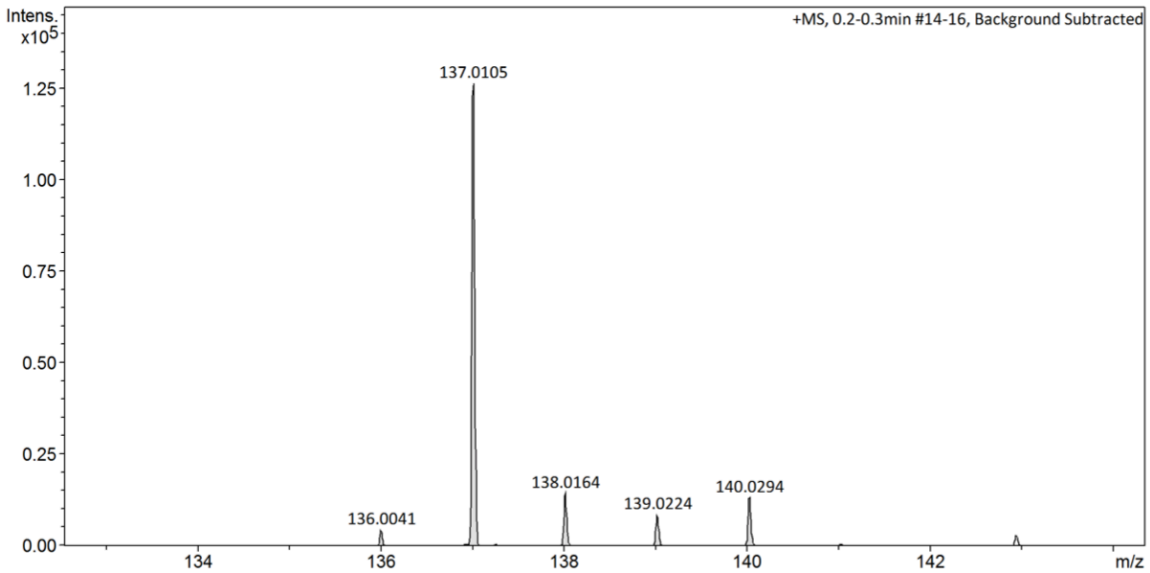


*Figure S24: Mass spectrum of [1-^{13}C,2-^{2}H]sodium lactate **3***

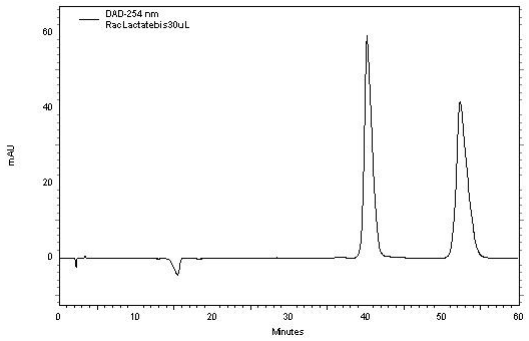


Figure S25: Chiral HPLC on commercially available racemic Lactate

Mobile phase: 1 mM sulfate copper(II) in water.
Flow rate: 1 ml.min^{-1}.
Injection volume: 30 µL solution of substrate in 1 mM sulfate copper(II) in water (1 mg.mL^{-1})

Time (min)	Area	%
40.160	16034900	50
52.308	15896900	50

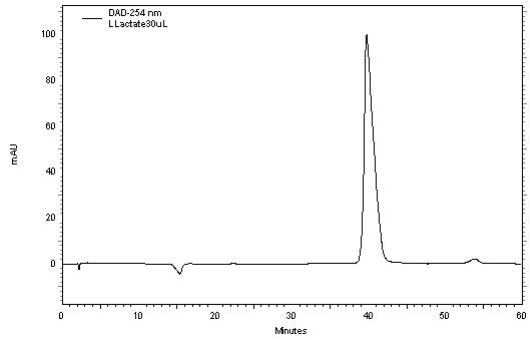


Figure S26: Commercially available [1-^{13}C]-L-lactate

Time (min)	Area	%
39.7329	33221700	>99
53.8242	419696	<1

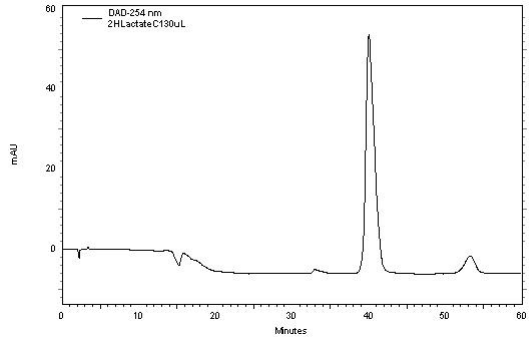
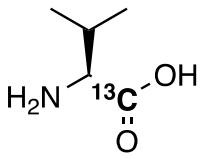


*Figure S27: [1-^{13}C,2-^{2}H]lactate **3***

Time (min)	Area	%
40.033	18258900	93
53.266	1461490	7

Starting material: commercially available [1-^{13}C]valine:



^{1}H NMR (400 MHz, D_2O) δ 0.90-1.08 (m, 1H), 2.15-2.37 (m, 1H), 3.60 (t, *J*=4.75 Hz, 9H)

^{13}C NMR (101 MHz, D_2O) δ 16.58, 17.92, 29.03, 60.32 (d, *J*=53.55 Hz, 174.22 (enriched)

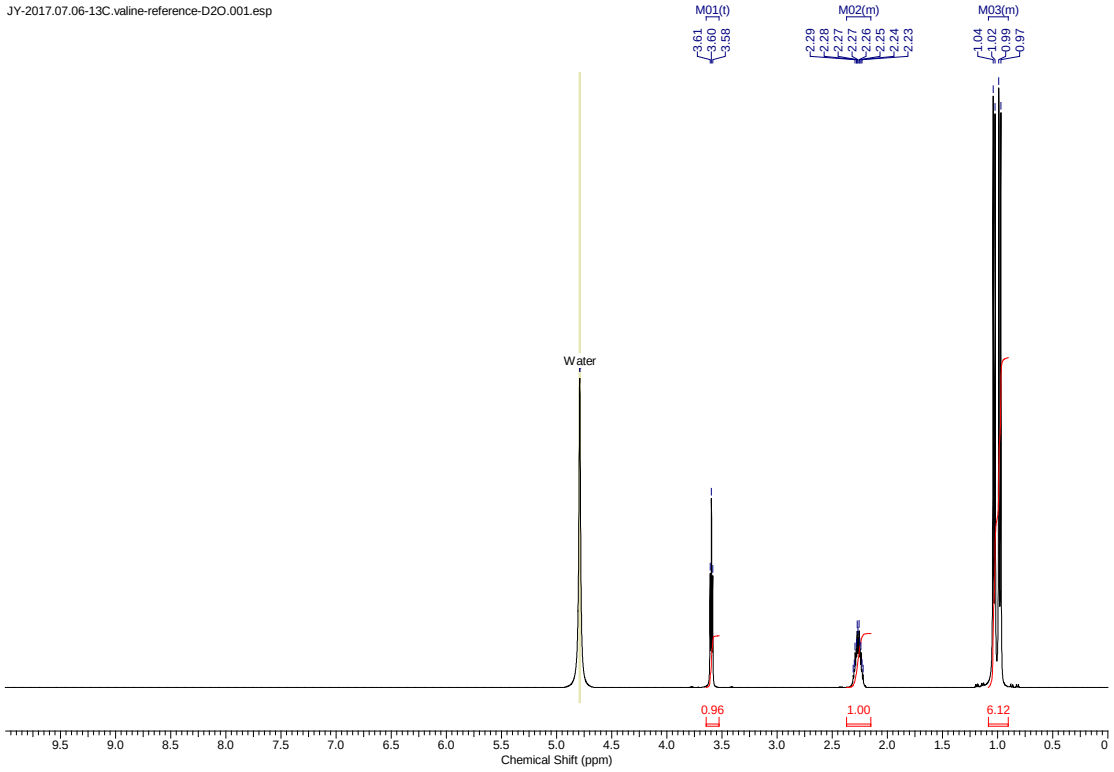


Figure S28: ^{1}H NMR spectrum of commercially available [1-^{13}C]valine

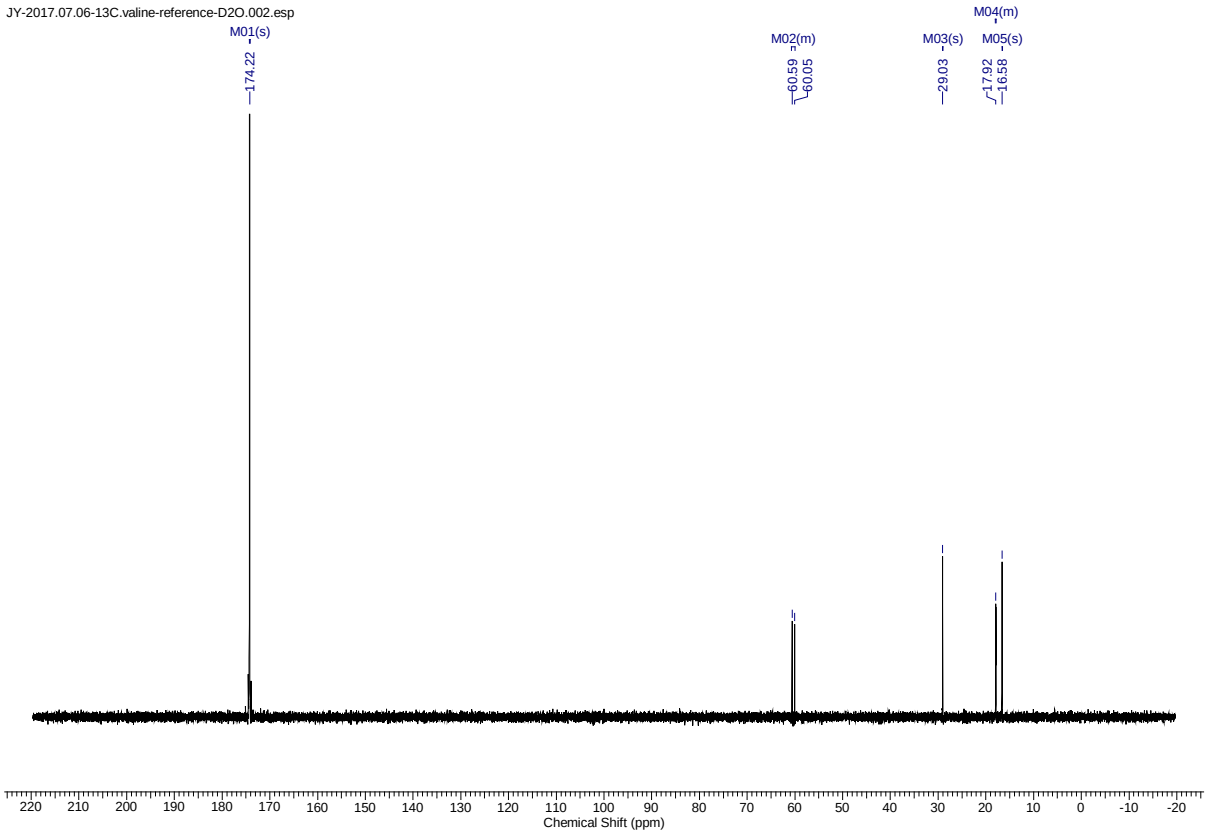


Figure S29: ^{13}C NMR spectrum of commercially available [1-^{13}C]valine

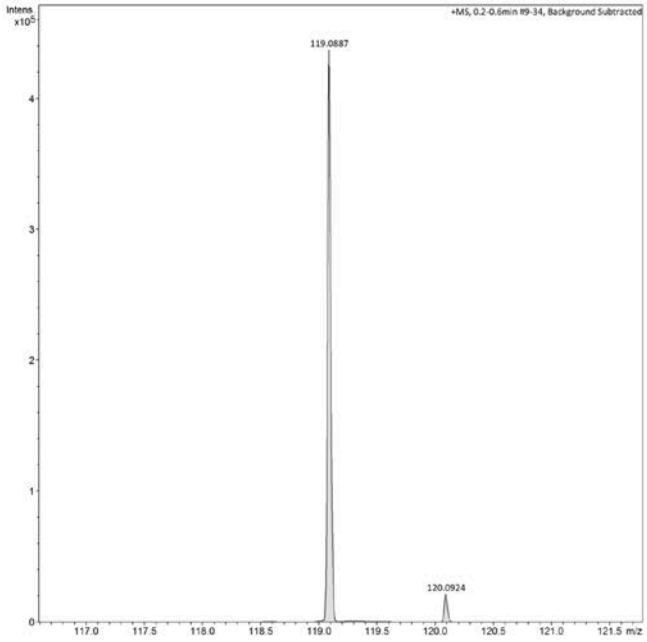
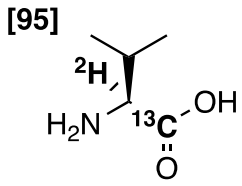


Figure S30: Mass spectrum of commercially available [1-^{13}C]valine

[1-^{13}C,2-^{2}H]valine 4 (procedure D):



After treatment [1-^{13}C,2-^{2}H]valine **4** was lyophilized to give a white solid (188 mg; 1.58 mmol; 53% yield).

^{1}H NMR (400 MHz, D_2O) δ 0.89-1.10 (m, 6H) 2.25 (m, 1H), 3.56-3.63 (m, 0.05H)

^{13}C NMR (101 MHz, D_2O) δ 16.56, 17.88, 28.94, 174.25 (enriched)

HRMS (ESI): $[M+H]^+$ calcd. for $C_4{}^{13}CH_{10}{}^{2}HNO_2$: 120.0964, found: 120.0947 (more abundant isotope).

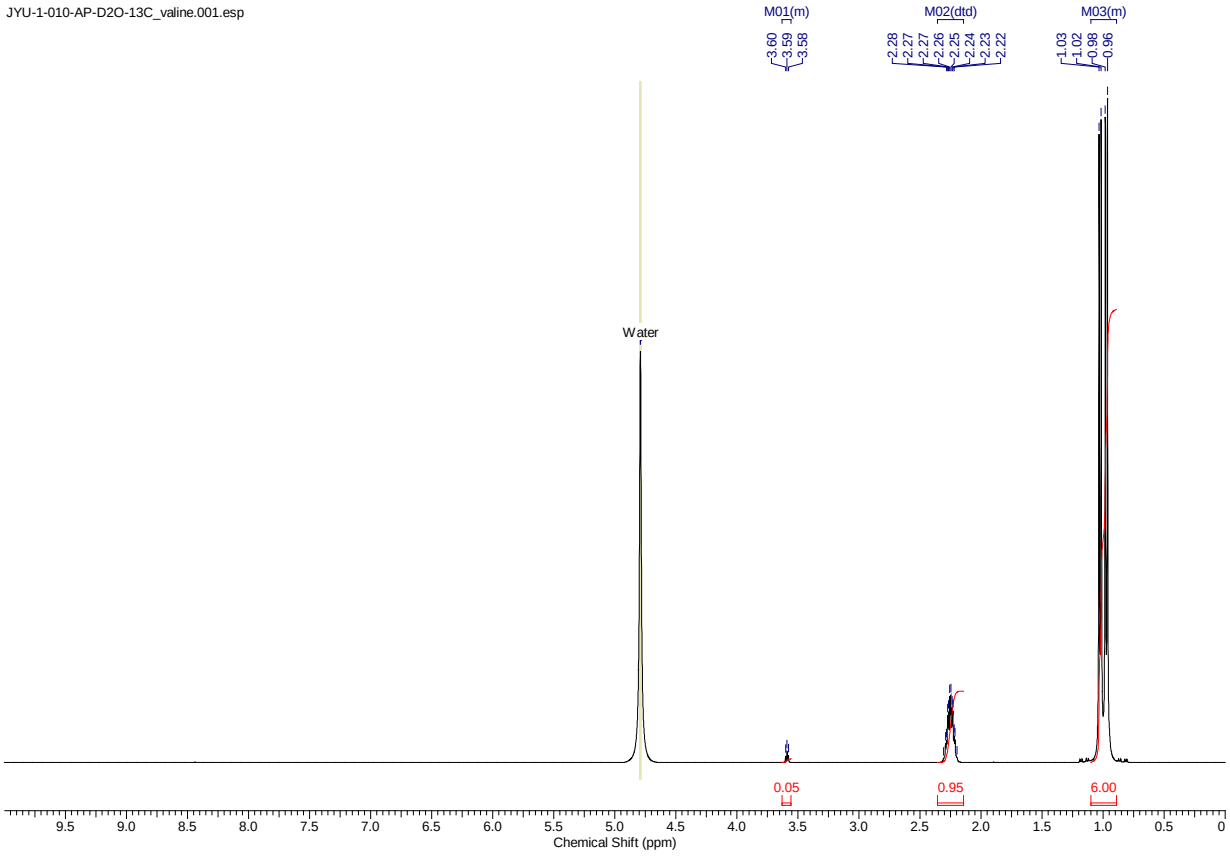


*Figure S31: ^{1}H NMR spectrum of [1-^{13}C,2-^{2}H]valine **4***

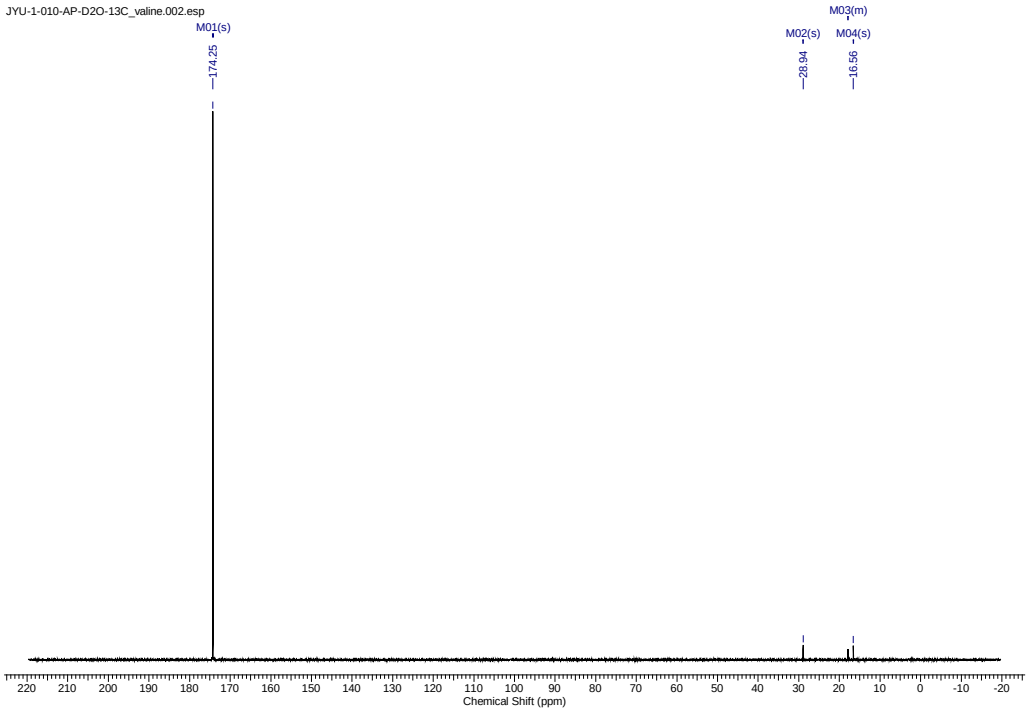


*Figure S32: ^{13}C NMR spectrum of [1-^{13}C,2-^{2}H]valine **4***

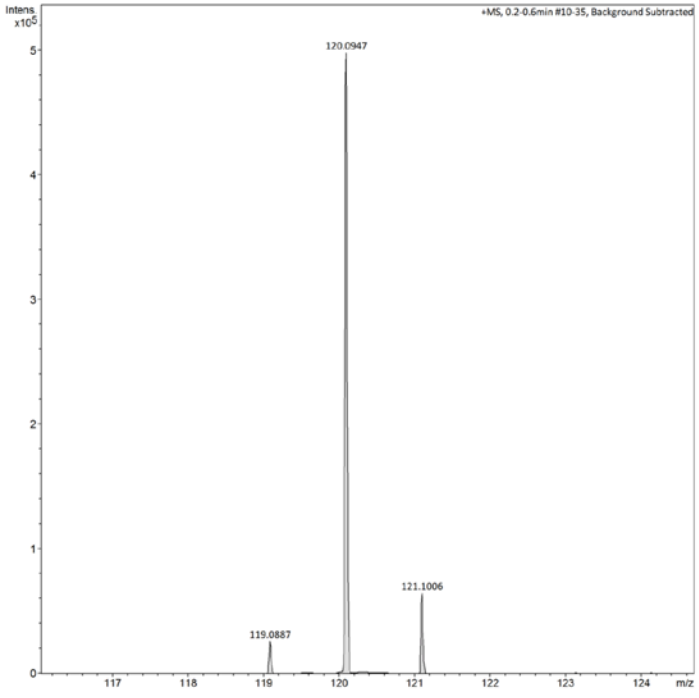


*Figure S33: Mass spectrum of [1-^{13}C,2-^{2}H]valine **4***

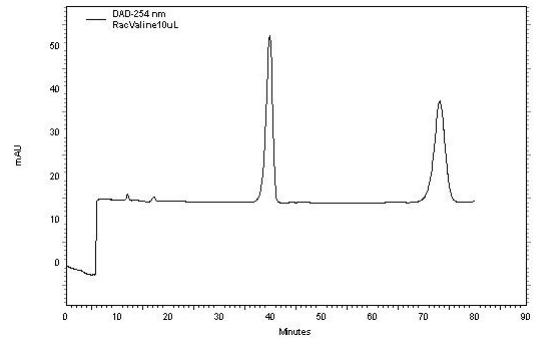


Figure S34: Chiral HPLC on commercially available racemic Valine

Mobile phase: 1 mM sulfate copper(II) in water.
Flow rate: 1 ml.min^{-1}.
Injection volume: 10 µL solution of substrate in 1 mM sulfate copper(II) in water (1 mg.mL^{-1})

Time (min)	Area	%
40.160	16034900	50
52.308	15896900	50

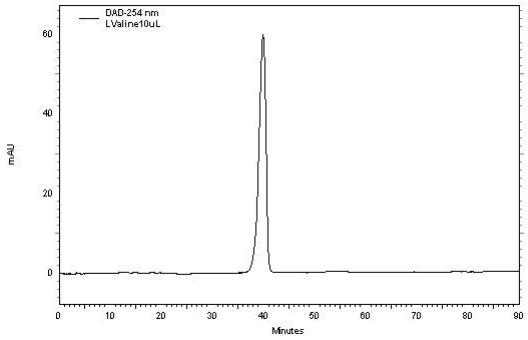


Figure S35: Commercially available L-Valine

Time (min)	Area	%
39.733	33221700	>99
53.824	76428	<1

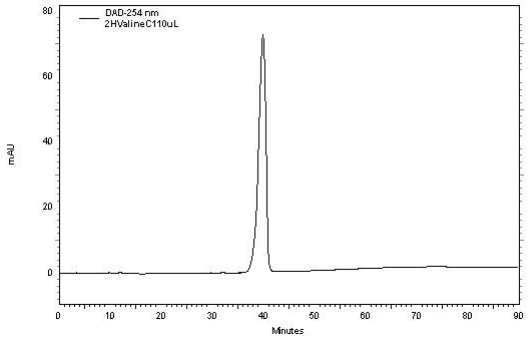
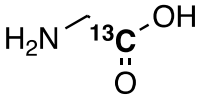


*Figure S36: [1-^{13}C,2-^{2}H]valine **4***

Time (min)	Area	%
39.896	28811800	>99
51.502	17764	<1

Starting material: commercially available [1-^{13}C]glycine:



^{1}H NMR (400 MHz, D_2O) δ 3.55 (d, *J*=5.36 Hz, 2H)

^{13}C NMR (101 MHz, D_2O) δ 41.37 (d, *J*=53.55 Hz), 172.37 (enriched)

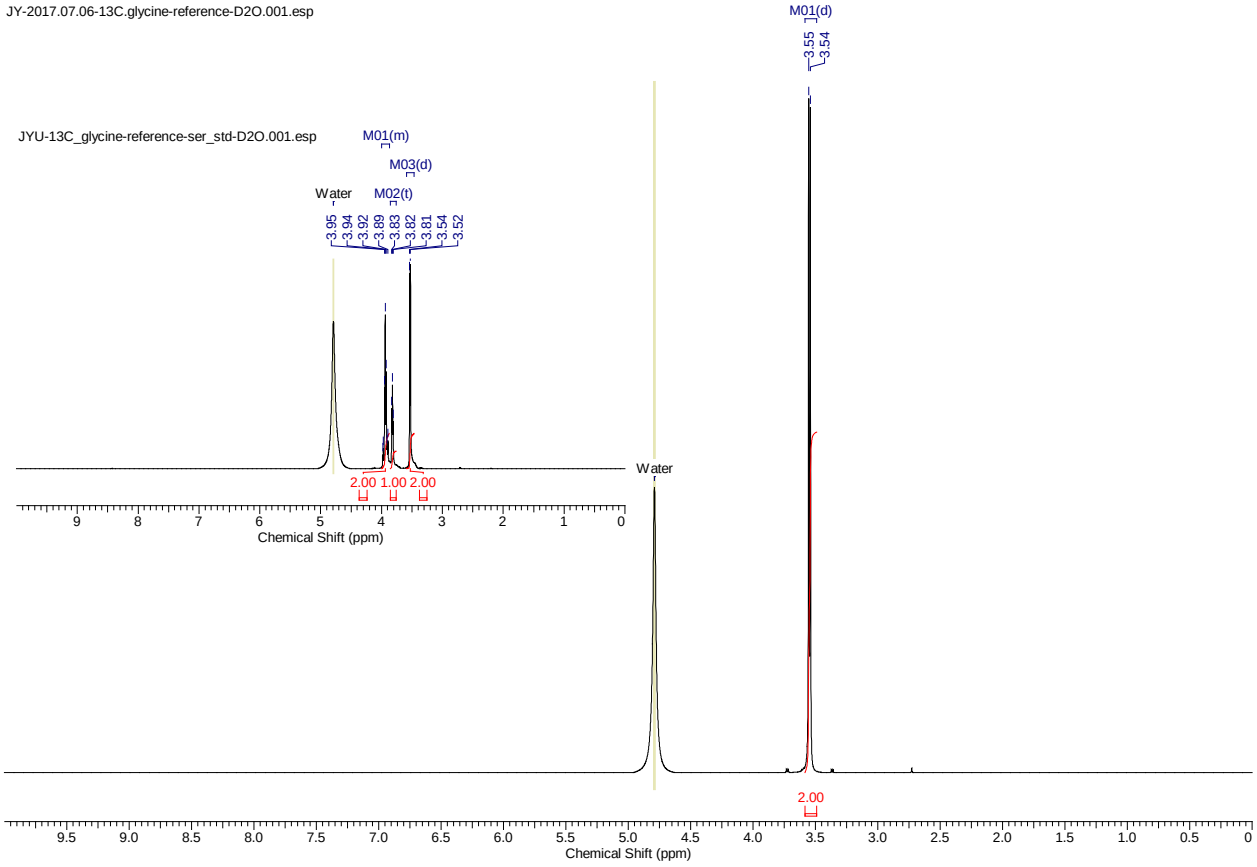


*Figure S37: ^{1}H NMR spectrum of commercially available [1-^{13}C]glycine (as shown in the upper left inset, use of commercially available serine enables to determine isotopic enrichment of [1-^{13}C,2-D_2]glycine **5**)*

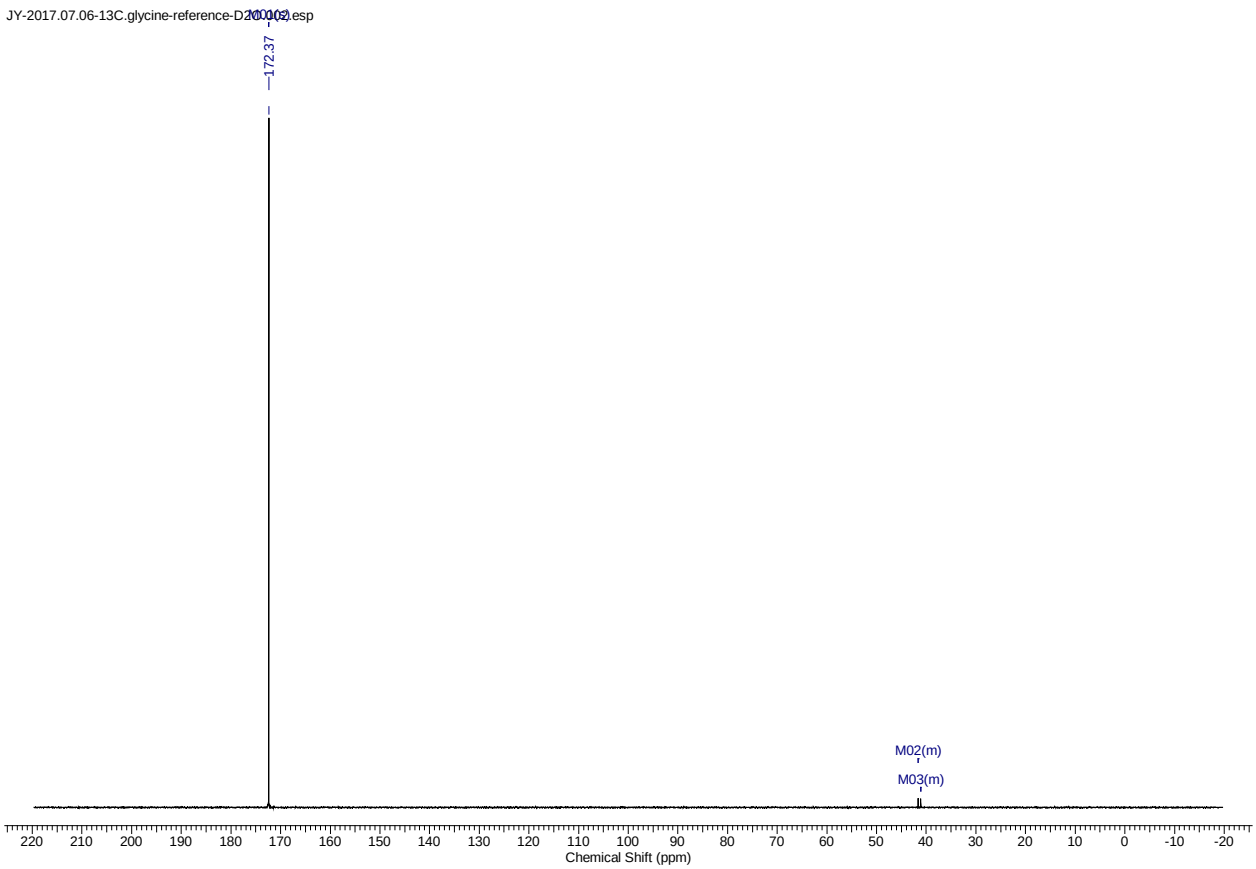


Figure S38: ^{13}C NMR spectrum of commercially available [1-^{13}C]glycine

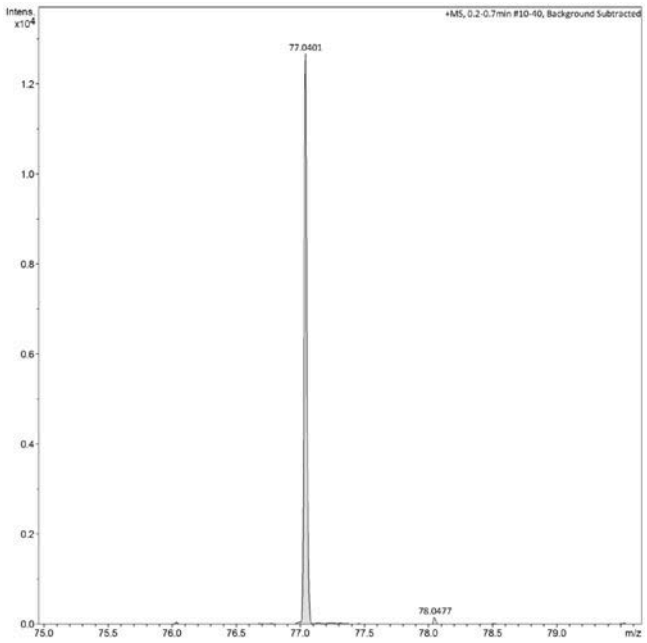
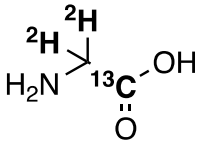


Figure S39: Mass spectrum of commercially available [1-^{13}C]glycine

[1-^{13}C,2-^{2}H$_2$]glycine 5 (procedure E):

[97]



After treatment [1-^{13}C,2-^{2}H$_2$]glycine **5** was lyophilized to give a white solid (181 mg; 2.32 mmol; 79% yield).

^{1}H NMR (400 MHz, D$_2$O) δ 3.52 (dt, *J*=4.99, 2.37 Hz, 0.07H)

^{13}C NMR (101 MHz, D$_2$O) δ 172.39 (enriched)

HRMS (ESI) : [M+H]$^{+}$ calcd. for C^{13}CH$_3$^{2}H$_2$NO$_2$: 79.0558, found : 79.0525 (more abundant isotope).

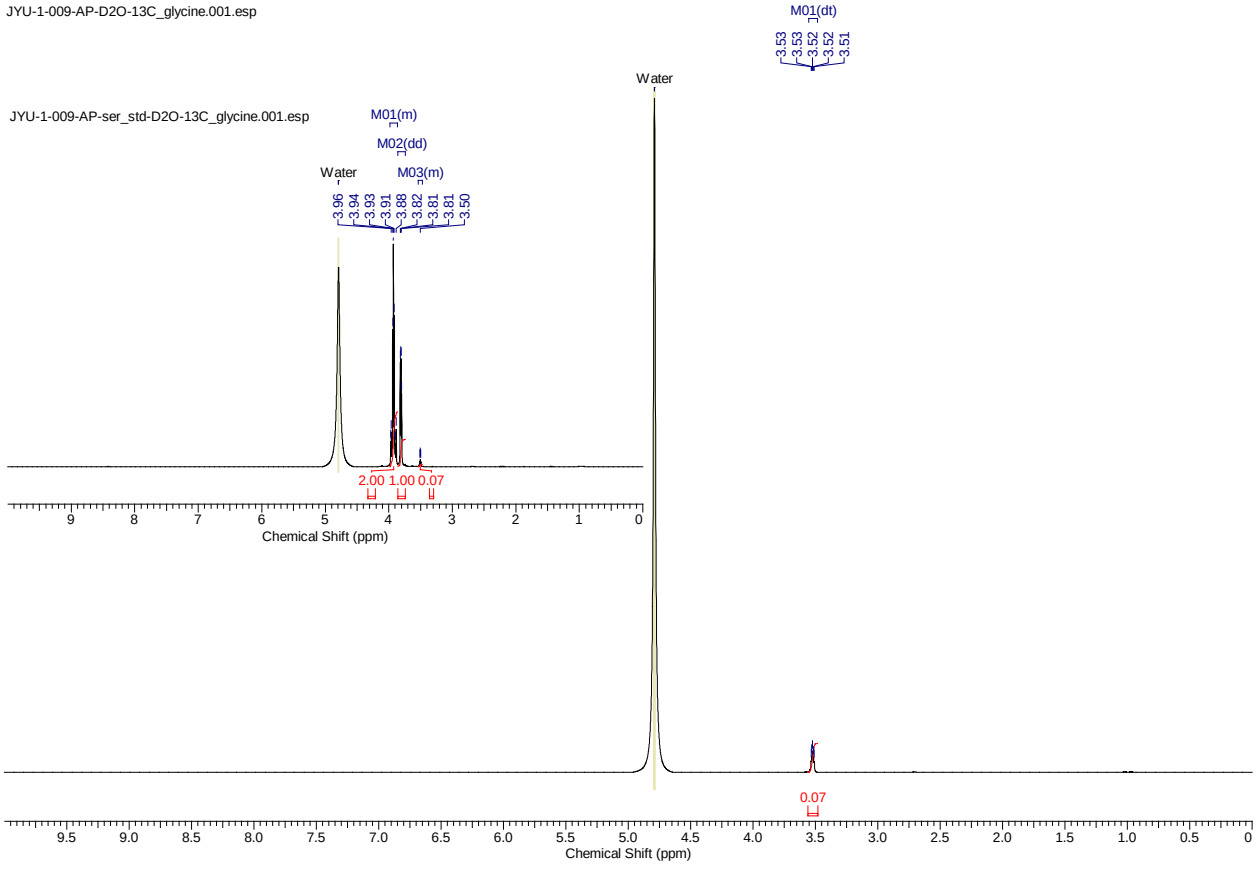


*Figure S40: ^{1}H NMR spectrum of [1-^{13}C,2-^{2}H$_2$]glycine **5** (as shown in the upper left inset, use of 1 equivalent of commercially available serine enables to determine isotopic enrichment of [1-^{13}C,2-^{2}H$_2$]glycine **5**)*

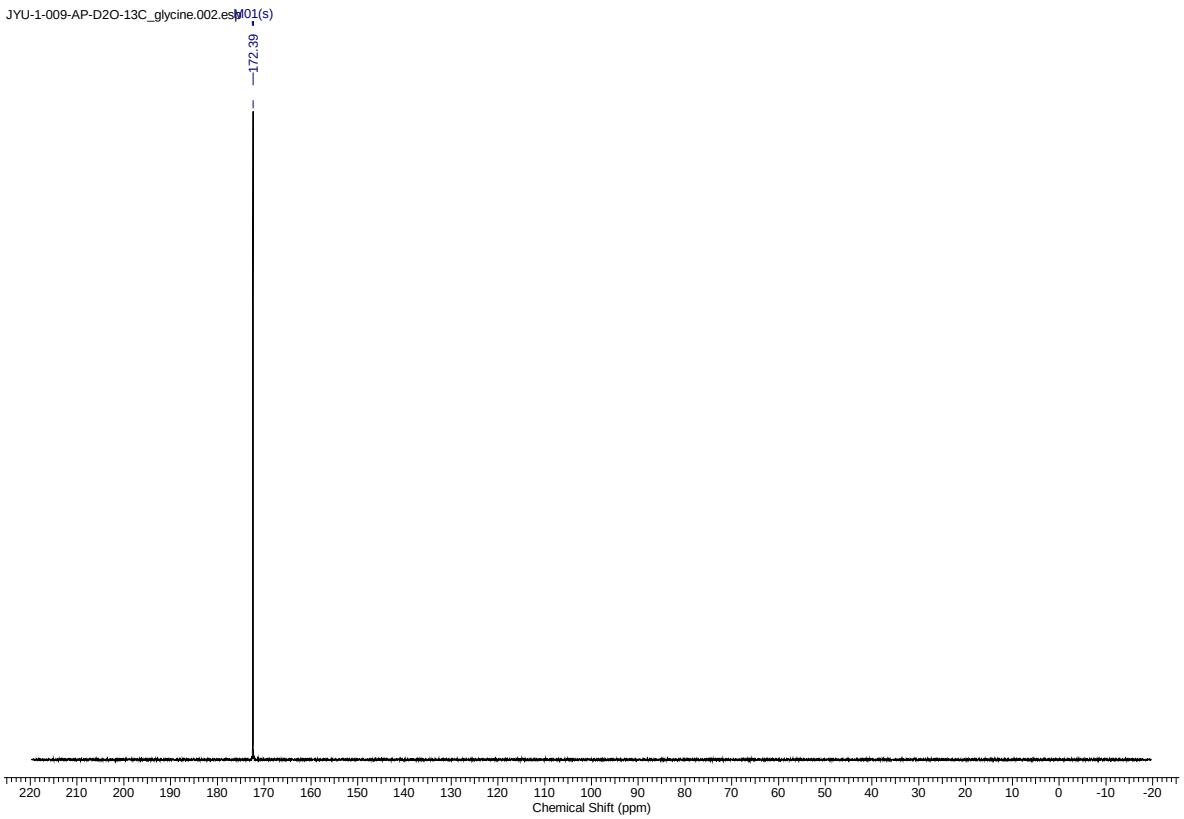


*Figure S41: ^{13}C NMR spectrum of [1-^{13}C,2-$^{2}H_2$]glycine **5***

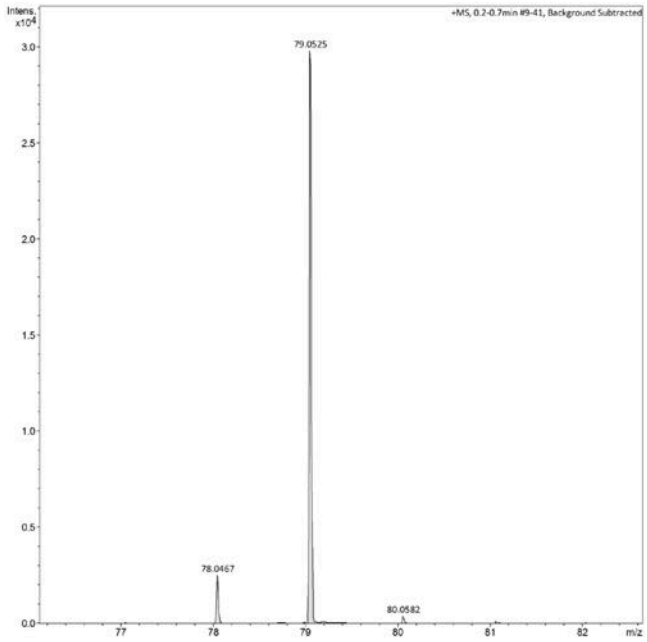
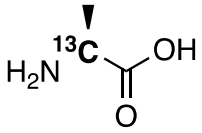


*Figure S42: Mass spectrum of [1-^{13}C,2-$^{2}H_2$]glycine **5***

Starting material: commercially available [2-^{13}C]alanine:



^{1}H NMR (400 MHz, D$_2$O) δ 1.48 (dd, *J*=7.30, 4.38 Hz, 3H), 3.51-4.03 (m, 1H)

^{13}C NMR (101 MHz, D$_2$O) δ 15.91-16.26 (d, *J*=35.21 Hz), 50.49 (enriched)

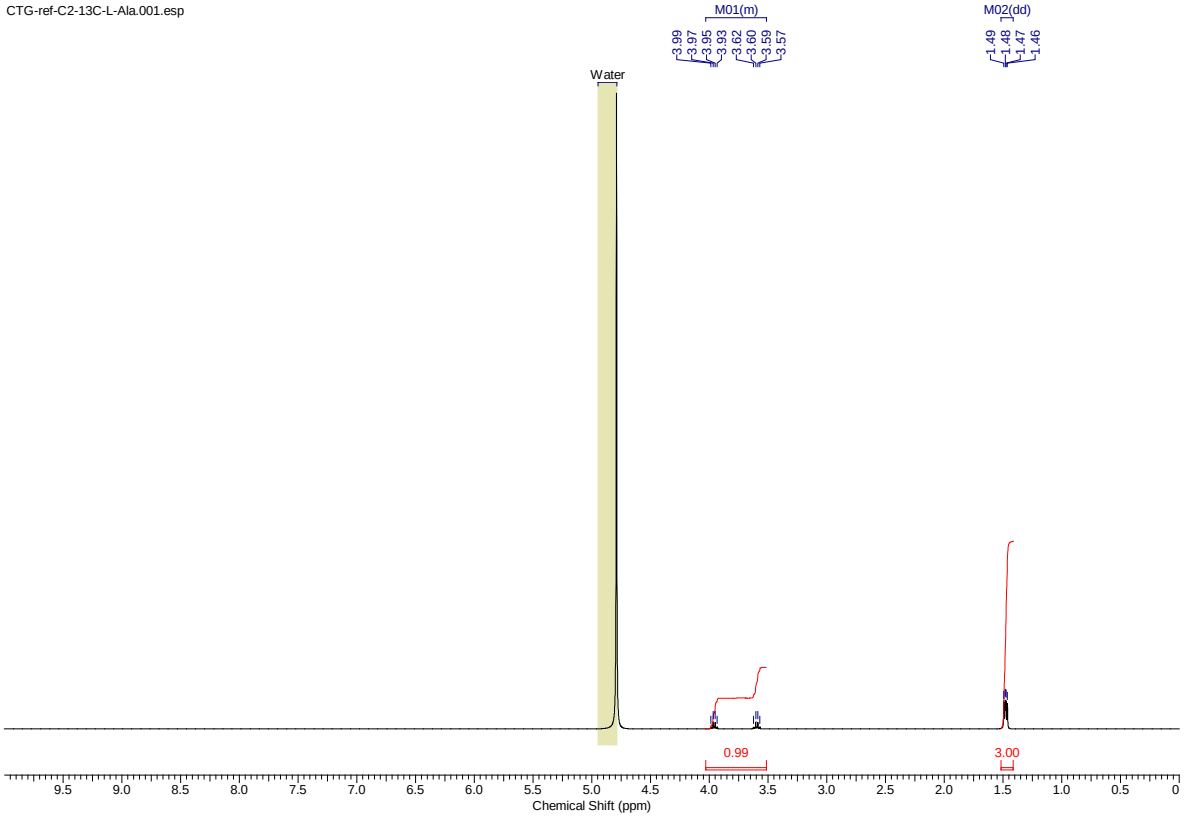


Figure S43: ^{1}H NMR spectrum of commercially available [2-^{13}C]alanine

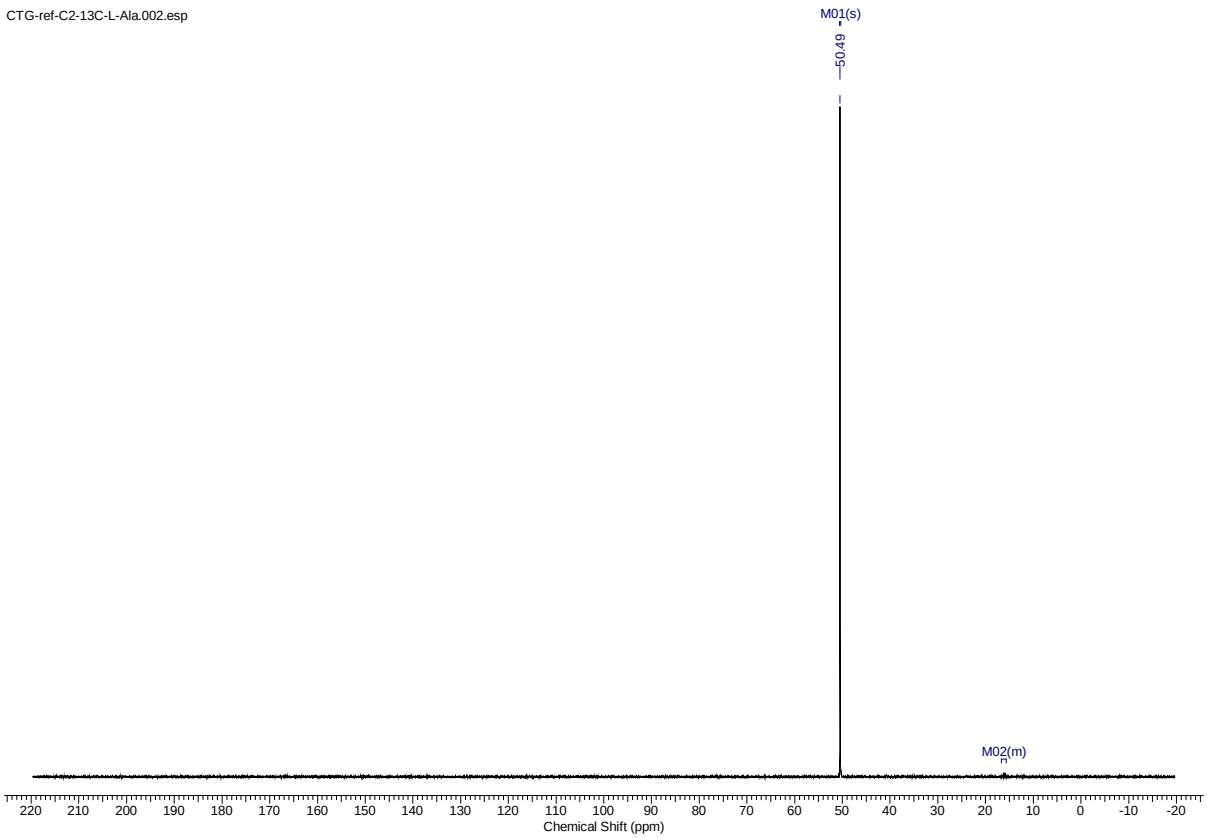


Figure S44: ^{13}C NMR spectrum of commercially available [2-^{13}C]alanine

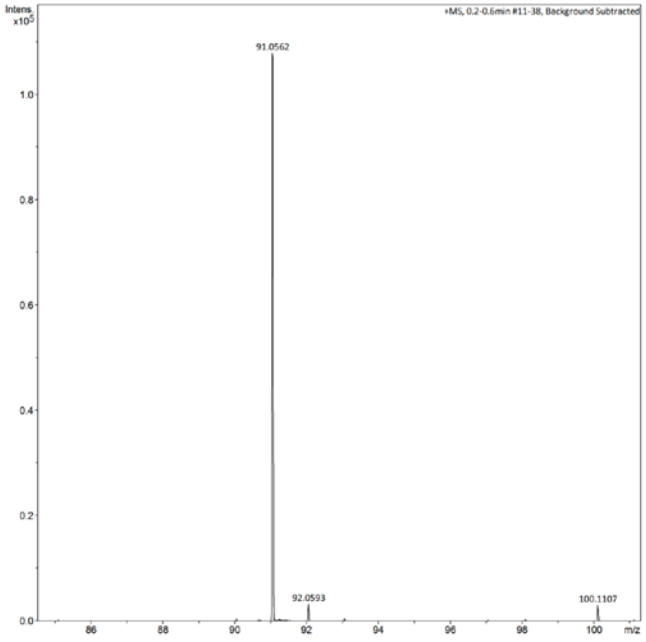
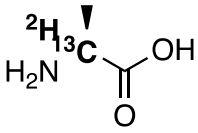


Figure S45: Mass spectrum of commercially available [2-^{13}C]alanine

[2-^{13}C,2-^{2}H]alanine 6 (procedure F):

[97]



After treatment [2-^{13}C,2-^{2}H]alanine **6** was lyophilized to give a white solid (205 mg; 2.25 mmol; 89% yield).

^{1}H NMR (400 MHz, D_2O) δ 1.47 (d, *J*=4.14 Hz, 3H), 3.55-4.00 (m, 0.03H)

^{13}C NMR (101 MHz, D_2O) δ 15.97 (d, *J*=34.48 Hz), 50.22 (t, *J*=22.01 Hz) (enriched)

HRMS (ESI) : $[M+H]^+$ calcd. for $C_2{}^{13}CH_6{}^{2}HNO_2$: 92.0651, found : 92.0626 (more abundant isotope).

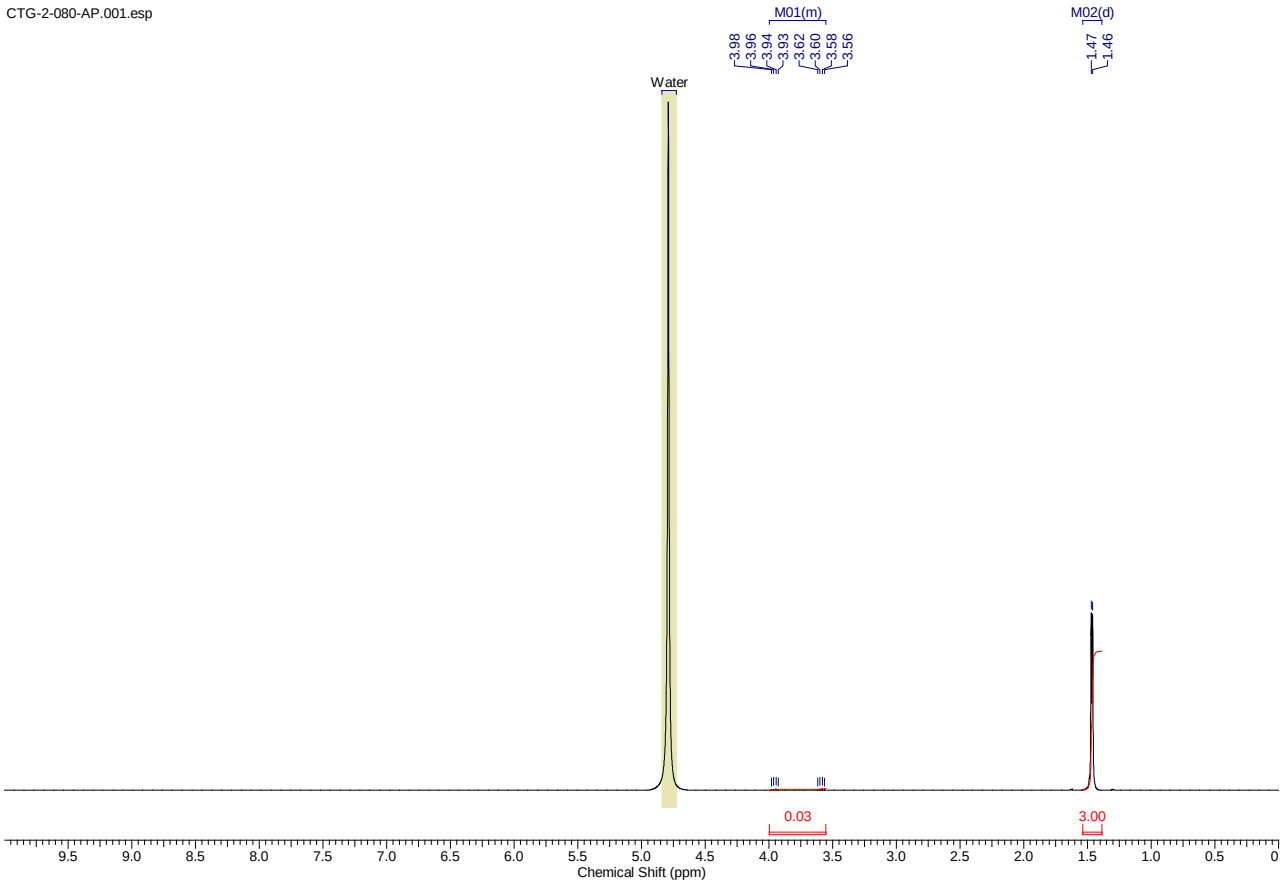


*Figure S46: ^{1}H NMR spectrum of [2-^{13}C,2-^{2}H]alanine **6***

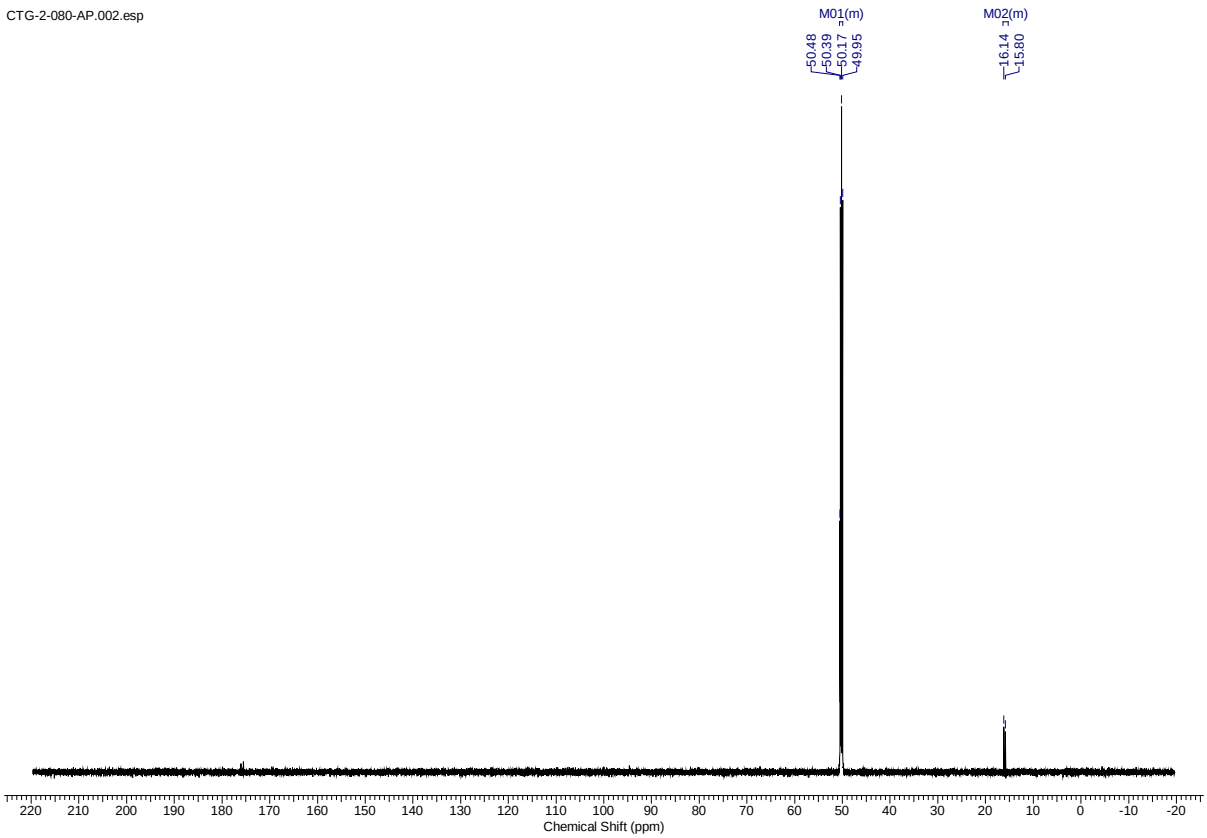


*Figure S47: ^{13}C NMR spectrum of [2-^{13}C,2-^{2}H]alanine **6***

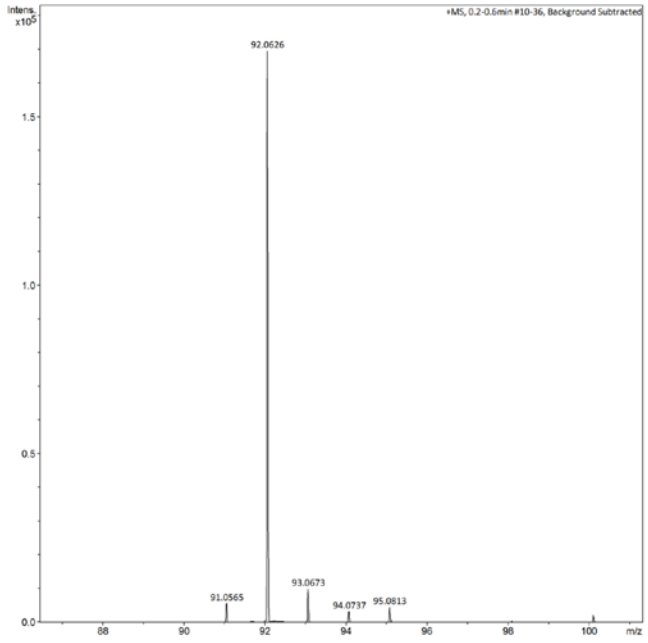


*Figure S48: Mass spectrum of [2-^{13}C,2-^{2}H]alanine **6***

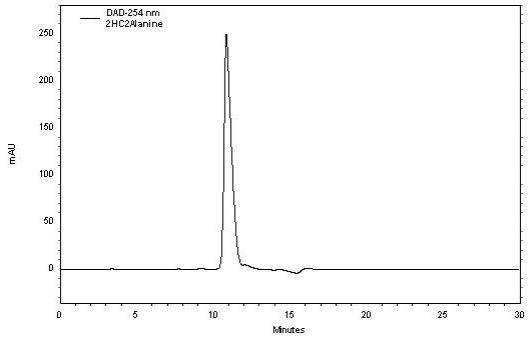
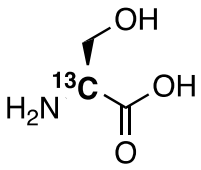


Figure S49: [2-^{13}C,^{2}H]alanine ***6***
Mobile phase: 1 mM sulfate copper(II) in water.
Flow rate: 1 ml.min^{-1}.
Injection volume: 10 µL solution of substrate in 1 mM sulfate copper(II) in water (1 mg.mL^{-1})

Time (min)	Area	%
10.852	31558200	>99
16.145	57270	<1

Starting material: commercially available [2-^{13}C]serine:



^{1}H NMR (400 MHz, D_2O) δ 3.66 (dd, *J*=5.48, 4.02 Hz, 1H), 3.91-4.05 (m, 3H)

^{13}C NMR (101 MHz, D_2O) δ 56.34 (enriched), 60.12 (d, *J*=37.41 Hz), 172.31

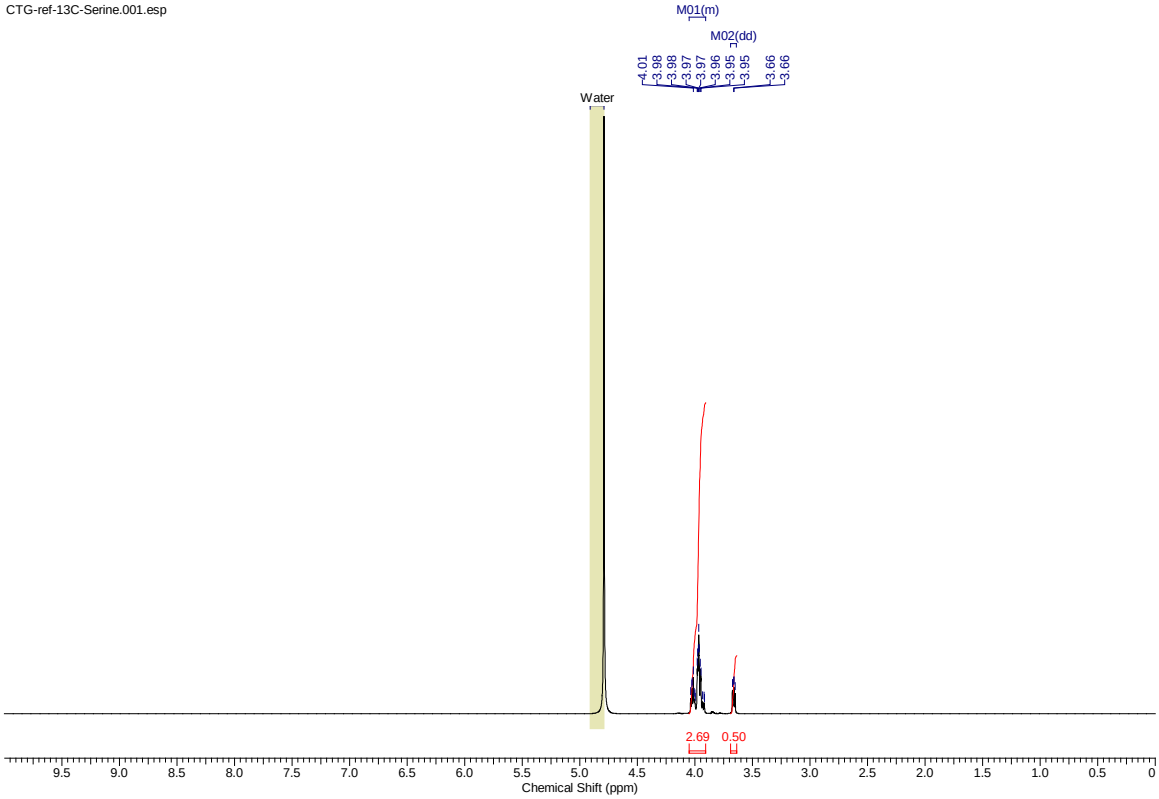


Figure S50: ^{1}H NMR spectrum of commercially available [2-^{13}C]serine

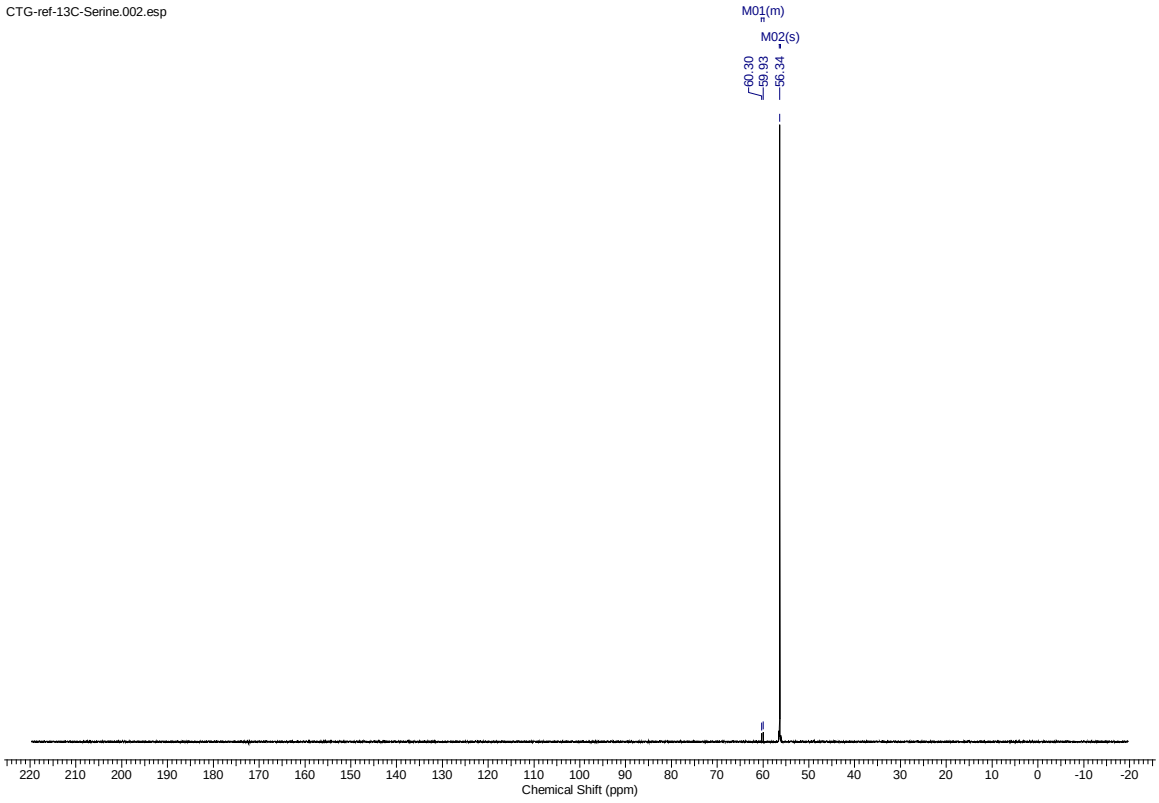


Figure S51: ^{13}C NMR spectrum of commercially available [2-^{13}C]serine

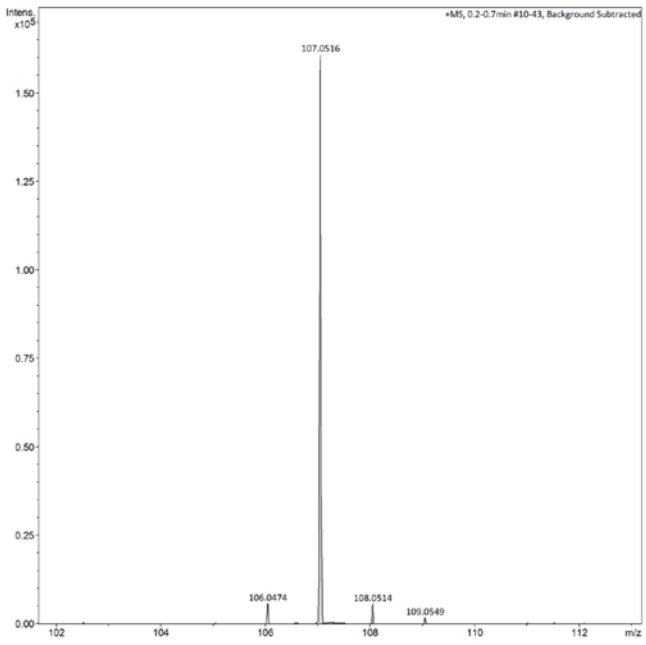
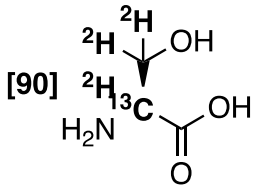


Figure S52: Mass spectrum of commercially available [2-^{13}C]serine

[2-^{13}C,2,3-^{2}H$_3$]serine 7 (procedure B):

[65]



After treatment [2-^{13}C,2,3-^{2}H$_3$]serine **7** was lyophilized to give a white solid (81 mg; 0.74 mmol; 77% yield).

^{1}H NMR (400 MHz, D$_2$O) δ 3.63-3.67 (m, 0.05H), 3.92 (s, 0.93H)

^{13}C NMR (101 MHz, D$_2$O) δ 56.35 (m, *J*=6.60, 7.34, 22.74 Hz, enriched)

HRMS (ESI): [M+H]$^+$ calcd. for $C_2{}^{13}CH_5{}^2H_2NO_3$: 109.0663, found: 109.0644 (more abundant isotope).

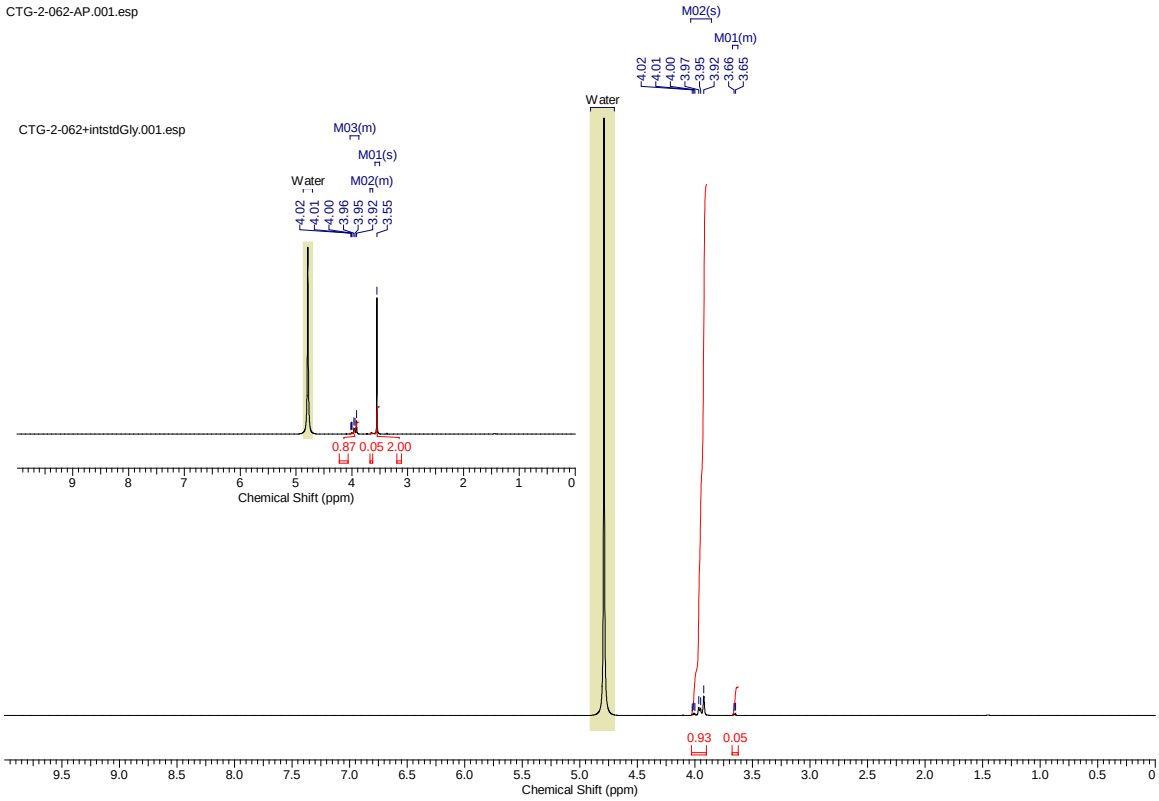


*Figure S53: ^{1}H NMR spectrum of [2-^{13}C,2,3-^{2}H$_3$]serine **7** (as shown in the upper left inset, use of 1 equivalent of commercially available glycine enables to determine isotopic enrichment of [2-^{13}C,2,3-^{2}H$_3$]serine **7**)*

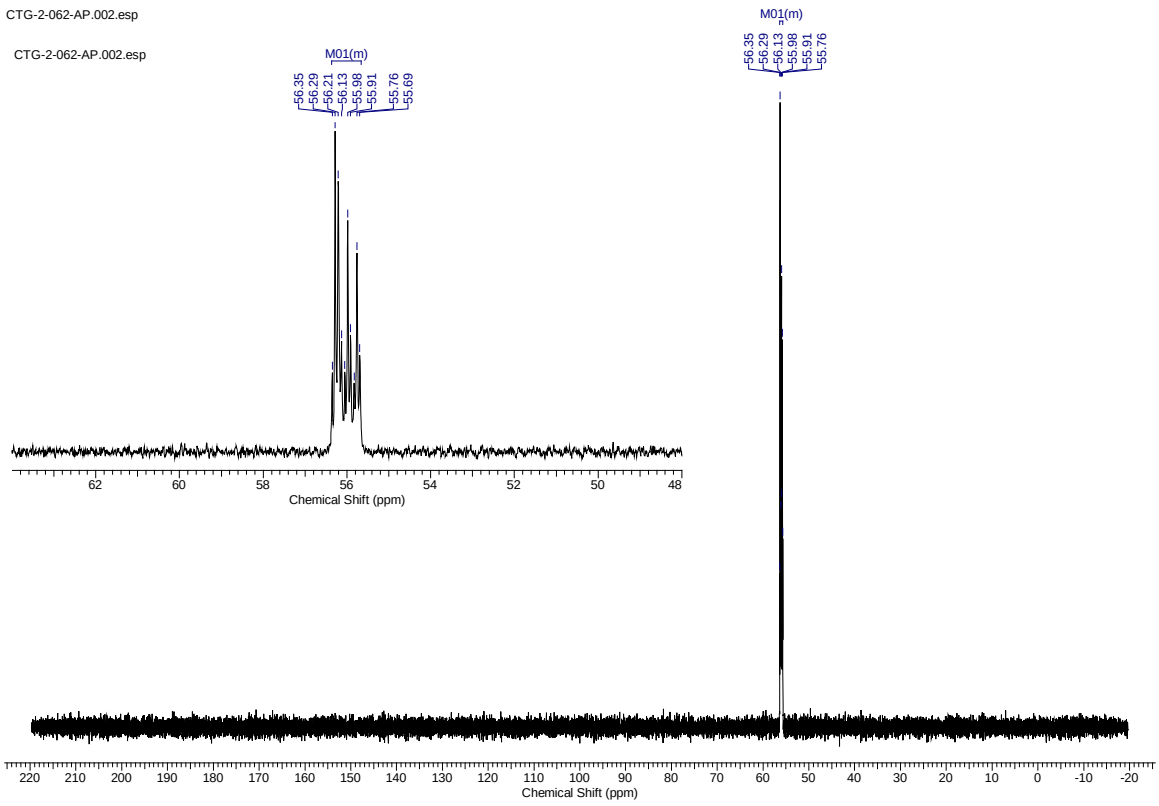


*Figure S54: ^{13}C NMR spectrum of [2-^{13}C,2,3-$^{2}H_3$]serine **7***

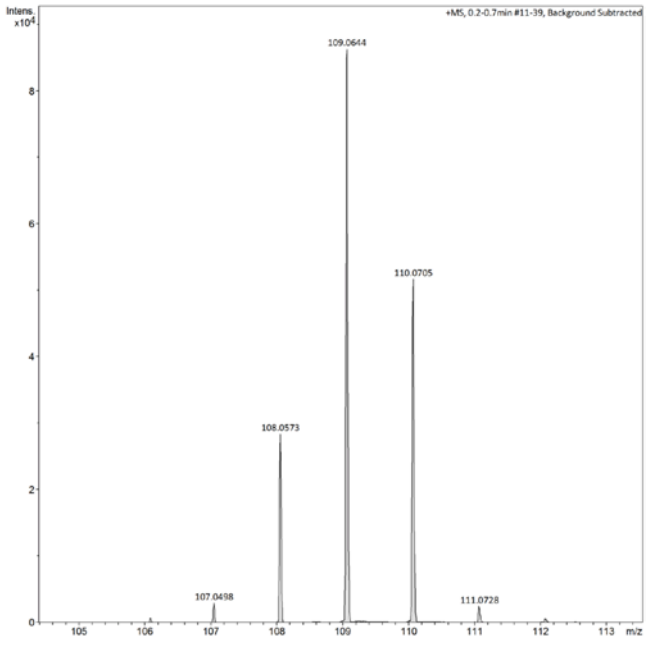


*Figure S55: Mass spectrum of [2-^{13}C,2,3-$^{2}H_3$]serine **7***

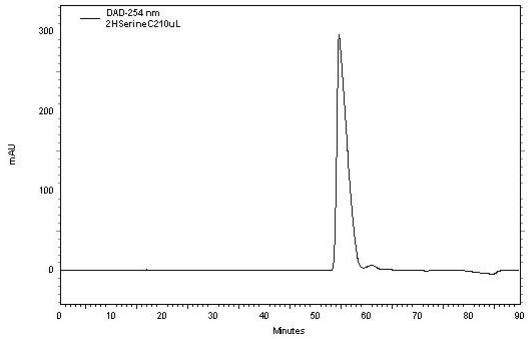
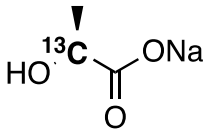


*Figure S56: [2-^{13}C,$^{2}H_3$]serine **7***
Mobile phase: 1 mM sulfate copper(II) in water.
Flow rate: 0.2 ml.min^{-1}.
Injection volume: 10 µL solution of substrate in 1 mM sulfate copper(II) in water (1 mg.mL^{-1})

Time (min)	Area	%
54.631	159605000	>99
61.023	638924	<1

Starting material: commercially available [2-^{13}C]sodium lactate:



^{1}H NMR (400 MHz, D_2O) δ 1.33 (dd, *J*=7.06, 4.38 Hz, 3H), 3.87-4.35 (m, 1H)

^{13}C NMR (101 MHz, D_2O) δ 20.03 (d, *J*=36.68 Hz), 68.47 (enriched)

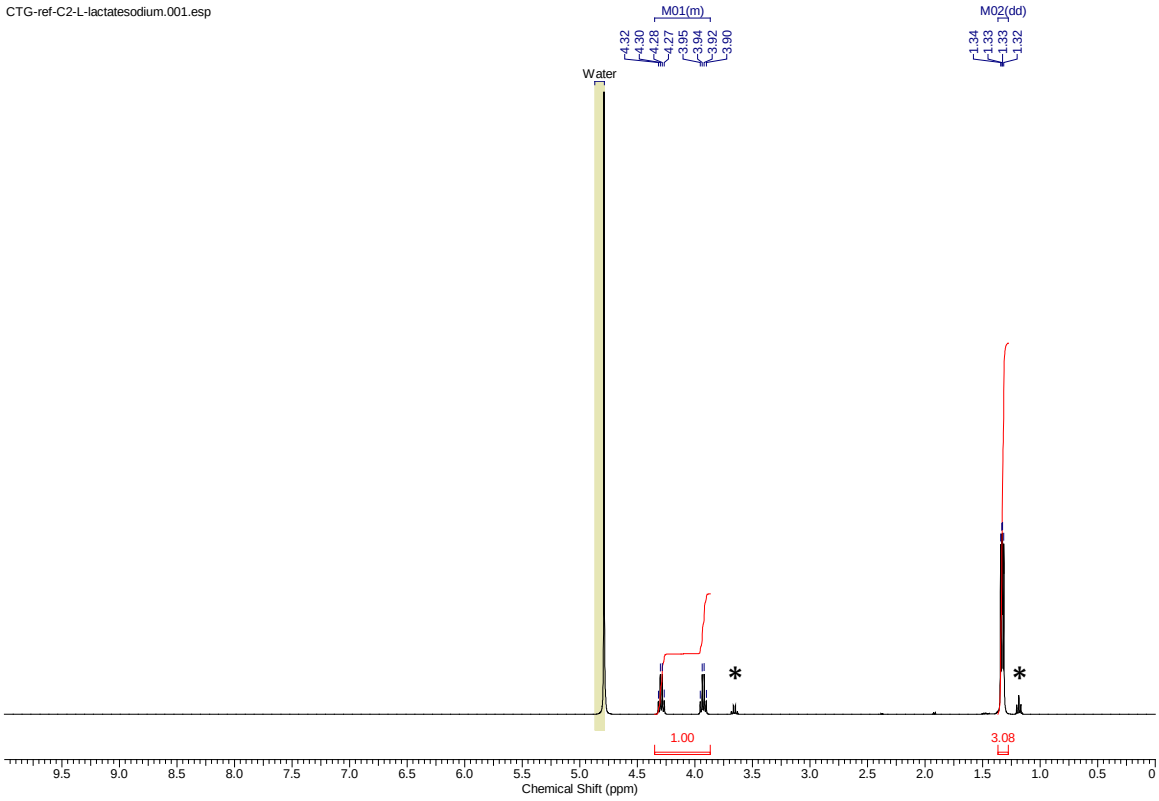


Figure S57: ^{1}H NMR spectrum of commercially available [2-^{13}C]sodium lactate

**Presence of residual ethanol*

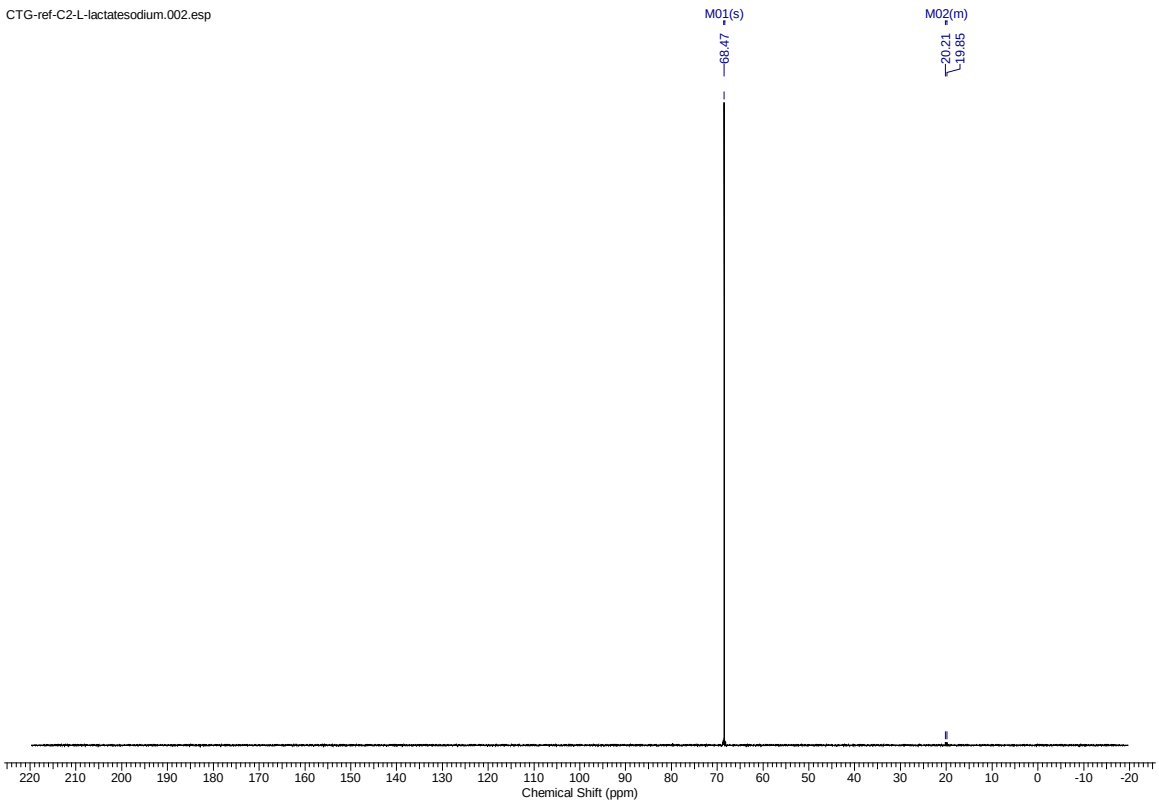


Figure S58: ^{13}C NMR spectrum of commercially available [2-^{13}C]sodium lactate

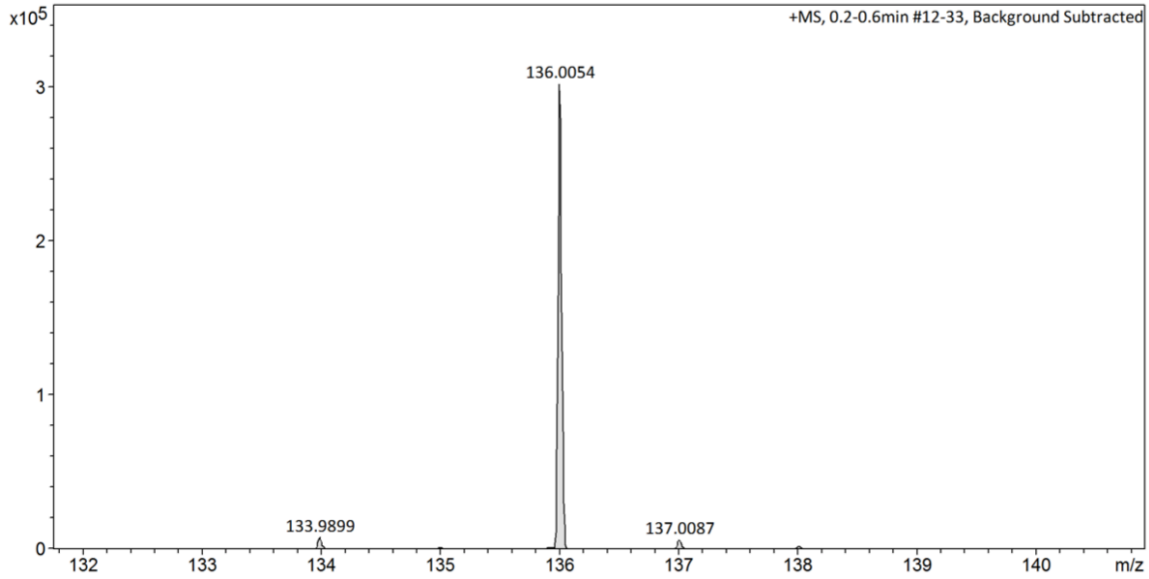
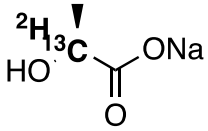


Figure S59: Mass spectrum of commercially available [2-^{13}C]sodium lactate

[2-^{13}C,2-^{2}H]sodium lactate 8 (procedure G):

[98]



After treatment [2-^{13}C,2-^{2}H]sodium lactate **8** was lyophilized to give a clear solid (163 mg; 1.43 mmol; 99% yield).

^{1}H NMR (400 MHz, D_2O) δ 1.33 (d, *J*=4.38 Hz, 3H), 3.91-4.38 (m, 0.02H)

^{13}C NMR (101 MHz, D_2O) δ 19.81 (d, *J*=36.68 Hz), 67.83 (t, *J*=22.01 Hz, enriched)

HRMS (ESI): $[M+Na]^+$ calcd. for $C_2{}^{13}CH_4{}^{2}HO_3Na$: 137.0130, found: 137.0121 (more abundant isotope).

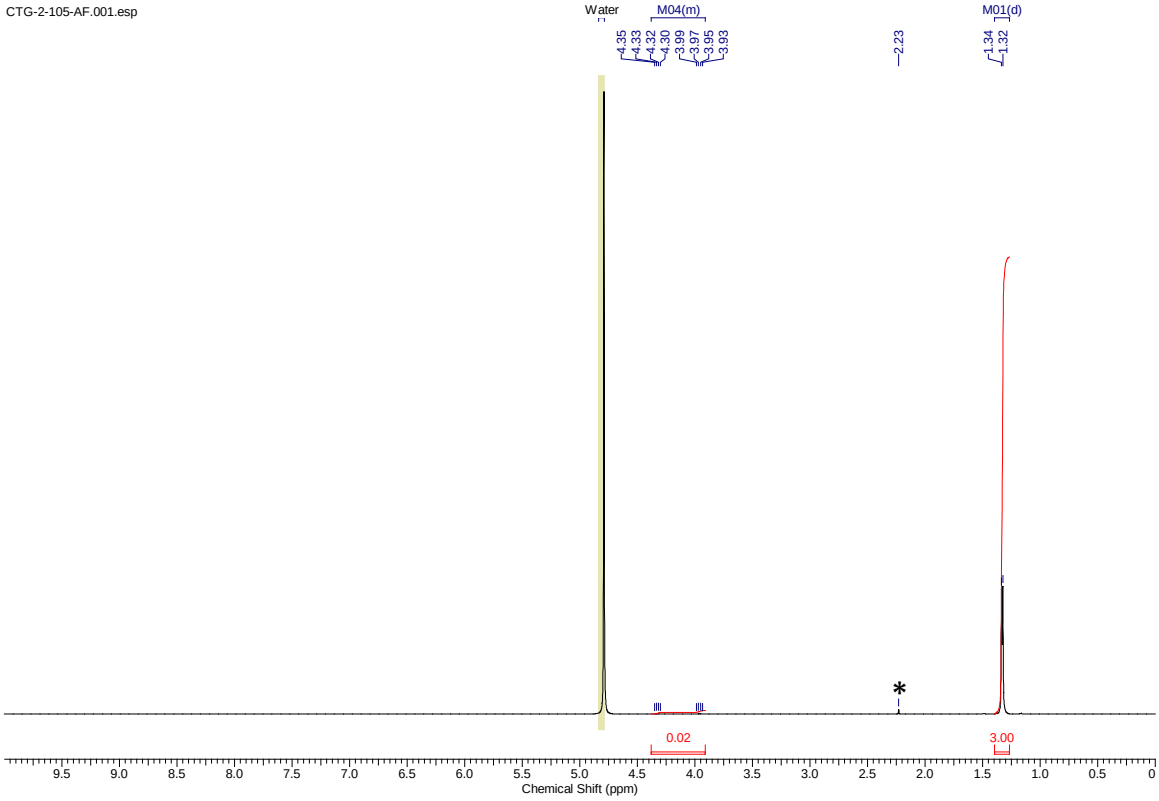


*Figure S60: ^{1}H NMR spectrum of [2-^{13}C,2-^{2}H]sodium lactate **8***

**Presence of residual acetone*

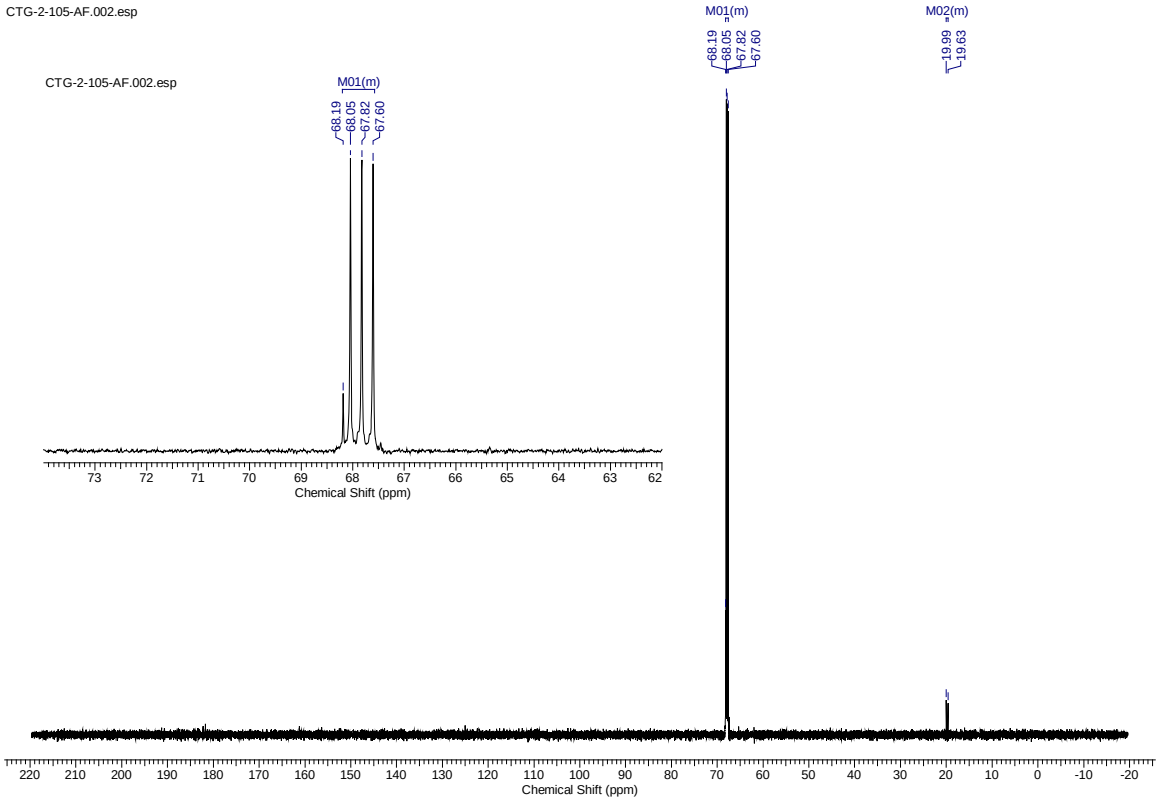


*Figure S61: ^{13}C NMR spectrum of [2-^{13}C,2-^{2}H]sodium lactate **8***

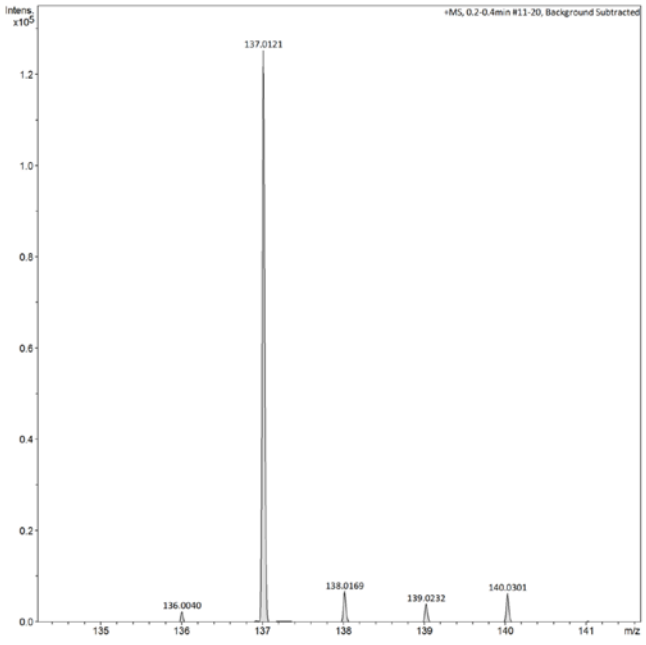


*Figure S62: Mass spectrum of [2-^{13}C,2-^{2}H]sodium lactate **8***

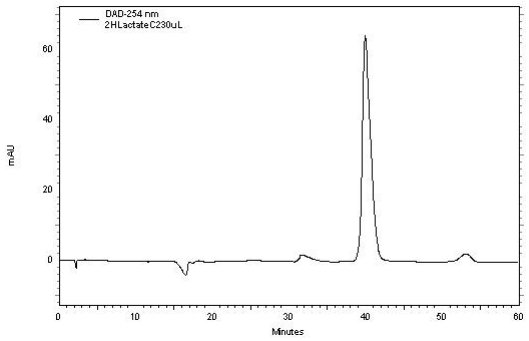


Figure S63: [2-^{13}C,^{2}H]lactate ***8***

Mobile phase: 1 mM sulfate copper(II) in water.
Flow rate: 1 ml.min^{-1}.
Injection volume: 30 µL solution of substrate in 1 mM sulfate copper(II) in water (1 mg.mL^{-1})

Time (min)	Area	%
39.939	18698600	97
53.172	668692	3

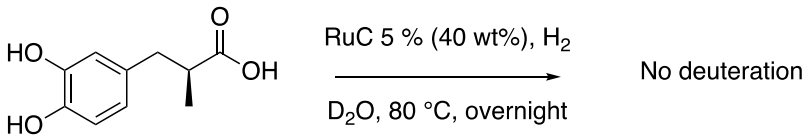
Data of mass analysis for compounds 1 to 8

Compound	M_0	M_{+1}	M_{+2}	M_{+3}	M_{+4}	M_{+5}
1	3	87	5	2	3	
2	28	47	22	3	<1	
3	3	78	7	4	8	
4	5	88	7			
5	0	8	92			
6	3	91	3	1	2	
7	2	17	51	30	<1	<1
8	2	89	3	2	4	

*Table S2: Relative amount in percent of the non-deuterated (M_0) and deuterated ($M_{(0+x)}$) isotopologues for compounds **1** to **8**.*

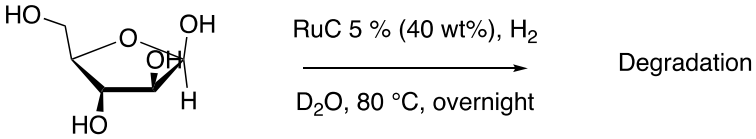
Limitations

3,4-Dihydroxy-L-phenylalanine (L-DOPA):



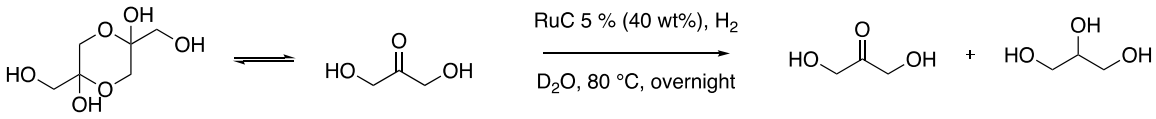
HD exchange reaction of the starting material L-DOPA (49 mg, 0.25 mmol) was carried out in a capped vial (Hollister-Stier Laboratories 10mL/20mm) with RuC 5% (20 mg, 40 wt%) in D_2O (2 mL), under H_2 (1 atm) at 80°C overnight. The reaction mixture was cooled down to room temperature and filtered on a syringe-driven filter (Millex-HN 0.45µm Nylon Membrane). After evaporation, no deuteration was observed. This may be due to instability of L-DOPA in aqueous alkali solution. It can rapidly be oxidized by air and may darken on exposure to air and light.[1]

D-(-)-Fructose:



HD exchange reaction of the starting material D-(-)-Fructose (45 mg, 0.25 mmol) was carried out in a capped vial with RuC 5% (18 mg, 40 wt%) in D_2O (2 mL), under H_2 (1 atm) at 80°C overnight. The reaction mixture was cooled down to room temperature and filtered on a syringe-driven filter (Millex-HN 0.45µm Nylon Membrane). After evaporation, a complex mixture was obtained presumably due to the hydrolysis of the hemiacetal functionality and following hydrogenation of the corresponding aldehyde in D_2O. Sajiki *et al.* showed that HD exchange reaction led to the regio- and chemoselective deuterium incorporation on the carbons adjacent to the corresponding hydroxyl groups with moderate deuterium efficiencies without hydrolysis and byproducts on methyl-protected sugars.[2]

1,3-Dihydroxyacetone dimer (DHA):



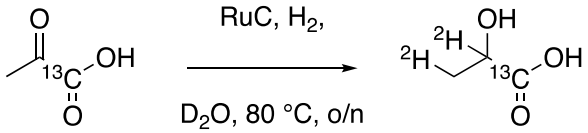
HD exchange reaction of the starting material DHA (45 mg, 0.25 mmol) was carried out in a capped vial with RuC 5% (18 mg, 40 wt%) in D_2O (2 mL), under H_2 (1atm) at 80°C overnight. The reaction mixture was cooled down to room temperature and filtered on a syringe-driven filter (Millex-HN 0.45µm Nylon Membrane). After evaporation, a complex mixture containing glycerol was obtained due to reduction on DHA.

---

[1]A. E. Stroomer *et al.*, *Clin. Chem.*, 1990, **36**, 1834–1837.

[2]H. Sajiki *et al.*, *Chem. Commun.*, 2010, **46**, 4977-4979.

Pyruvic acid:



HD exchange reaction of the starting material pyruvic acid (88 mg, 1 mmol) was carried out in a capped vial with RuC 5% (35 mg, 40 wt%) in D_2O (5 mL), under H_2 (1atm) at 80°C overnight. The reaction mixture was cooled down to room temperature and filtered on a syringe-driven filter (Millex-HN 0.45µm Nylon Membrane). As in the case of DHA, after evaporation, lactate was obtained due to reduction on cetone.

T_1 Measurements in Solution

Preparations

Each compound in the study was formulated at the maximum concentration that formed a glassy state upon immediate immersion in liquid nitrogen. Formulation glassing was determined by visual inspection. If the flash-frozen formulation without radical added was transparent, then it was determined to be in a glassy state. The same recipes, based on previous studies by Vigneron *et al.*[3] and Comment *et al.*[4], were used for the ^{13}C-enriched starting material used before deuteration and its corresponding ^{13}C,^{2}H-enriched molecule.

*[1-13C]alanine and [1-^{13}C,2-^{2}H]alanine **1**:* 6.2 mg of OX63 trityl radical were added to 100.5 mg of alanine, 71.5µL of 18.9M NaOH and 28.7µL DMSO. This yielded a preparation with 6.0 M alanine and 24 mM OX63 radical. The mixture was vortexed and sonicated for 5 minutes at room temperature.

*[1-^{13}C]serine and [1-^{13}C,2,3-$^{2}H_3$]serine **2**:* 3.2 mg of OX63 trityl radical were added to 60 mg of serine, 36.2µL of 18.9M NaOH and 13.5µL DMSO. This yielded a preparation with 5.6 M serine and 23 mM OX63 radical. The mixture was vortexed and sonicated for 5 minutes at room temperature.

*[1-^{13}C]sodium lactate and [1-^{13}C,2-^{2}H]sodium lactate **3**:* 5.1 mg of OX63 trityl radical were added to 86.6 mg of alanine and 185µL of H2O:glycerol solution 37.5:62.5 w:w. This yielded a preparation with 4.2 M sodium lactate. The mixture was vortexed and sonicated for 5 minutes at room temperature.

*[1-^{13}C]valine and [1-^{13}C,2-^{2}H]valine **4**:* 4.7 mg of OX63 trityl radical were added to 108 mg of valine, 48.6 µL of 18.9M NaOH and 20 µL DMSO, to a final concentration of 18 mM. This yielded a preparation with 5.6 M valine. The mixture was vortexed and sonicated for 5 minutes at room temperature.

*[1-^{13}C]glycine and [1-^{13}C,2-$^{2}H_2$]glycine **5**:* 4.3 mg of OX63 trityl radical were added to 65 mg of valine (5.7 M) and 85 µL of 10 M NaOH. This yielded a preparation with 5.7 M glycine. The mixture was vortexed and sonicated for 5 minutes at room temperature.

[2-^{13}C]alanine and [2-^{13}C,2-^{2}H]alanine 6: 6.2 mg of OX63 trityl radical were added to 100.5 mg of alanine (5.6 M), 71.5µL of 18.9M NaOH and 28.7µL DMSO. This yielded a

---

[3] S. Hu, M. Zhu, H. A. I. Yoshihara, D. M. Wilson, K. R. Keshari, P. Shin, G. Reed, C. von Morze, R. Bok, P. E. Z. Larson, J. Kurhanewicz and D. B. Vigneron, *Magn. Reson. Imaging*, 2011, **29**, 1035–1040.

[4] J. A. M. Bastiaansen, H. A. I. Yoshihara, Y. Takado, R. Gruetter and A. Comment, *Metabolomics*, 2014, **10**, 986–994.

preparation with 5.6 M alanine. The mixture was vortexed and sonicated for 5 minutes at room temperature.

*[2-^{13}C]serine and [2-^{13}C,2-$^{2}H_3$]serine **7**:* 3.2 mg of OX63 trityl radical were added to 60 mg of alanine, 36.2µL of 18.9M NaOH and 13.5µL DMSO, to a final concentration of 23 mM. This yielded a preparation with 5.6 M serine. The mixture was vortexed and sonicated for 5 minutes at room temperature.

*[2-^{13}C]sodium lactate and [2-^{13}C,2-D]sodium lactate **8**:* 5.1 mg of OX63 trityl radical were added to 86.6 mg of alanine and 185µL of H2O:glycerol solution 37.5:62.5. This yielded a preparation with 4.2 M sodium lactate. The mixture was vortexed and sonicated for 5 minutes at room temperature.

Polarizations and Dissolutions

Before polarization, a microwave sweep was performed for each formulation to find the peak polarization frequency to be 0.012-0.014GHz lower than the peak polarization frequency for neat pyruvic acid + trityl radical on the same polarizer. 40 to 50 µL of each formulation were polarized using a HyperSense dissolution-DNP polarizer (Oxford Instruments, Abingdon, UK), at 1.40 K for 25 minutes. The sample was then dissolved with 3.5 mL of a 50 mM, Tris 0.3mM EDTA solution, and 1.5 M HCl preloaded in the collection flask, to a final concentration of 65 mM. The resulting pH of the dissolution was about 7 (pH strip).

MR Studies and Data Processing

After transfer to a capped 5mL syringe, the sample was placed inside the Bruker 3T Biospec scanner equipped with two channels (^{1}H and broadband) and a gradient strength of 960 mT/m. Dynamic HP ^{13}C NMR spectra were immediately acquired with 30 5° hard RF pulses and a TR of 3 seconds. The hyperpolarized peak areas were measured using MestreNova. T_1 was then calculated from the peak areas using a MATLAB function. The function automatically applies tip angle correction, where S_n is the n^{th} signal intensity, θ is the flip angle, and $S_{n,uncorrected}$ is the uncorrected signal intensity:

$$S_n = \frac{S_{n,uncorrected}}{\cos^{n-1}\theta}$$

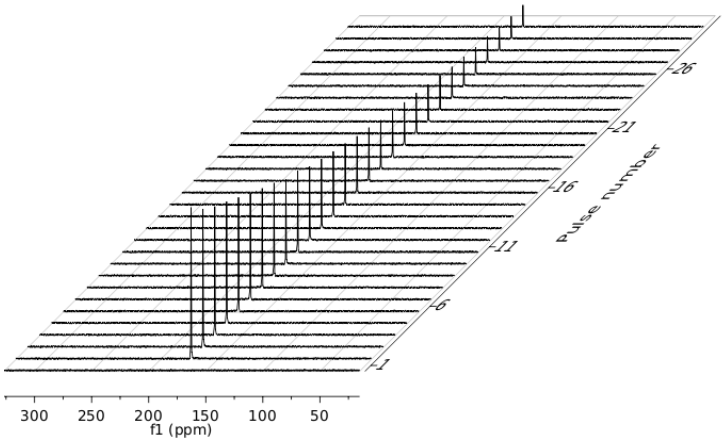


Figure S64 : Representative HP [1-^{13}C]serine spectra acquired at 3T (173 ppm).

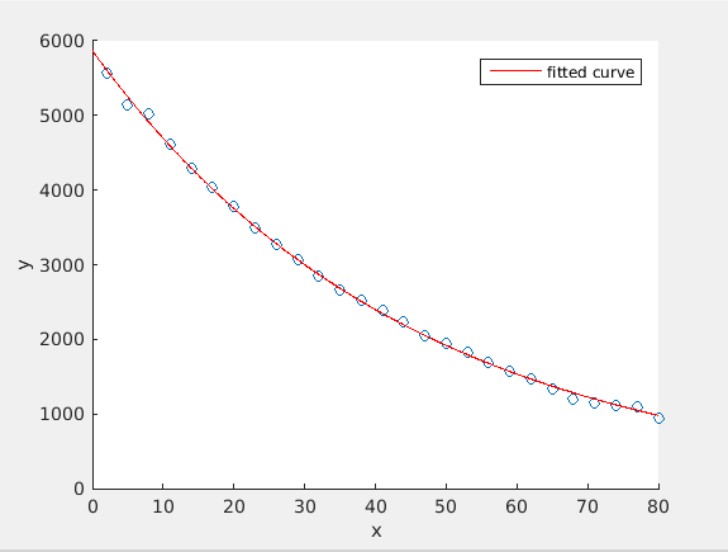


Figure S65 : Example of a hyperpolarized signal with T_1 relaxation equation fit. This measurement of [1-^{13}C]serine yielded a T_1 of 41.3s. Calculation and figure were processed using MATLAB.

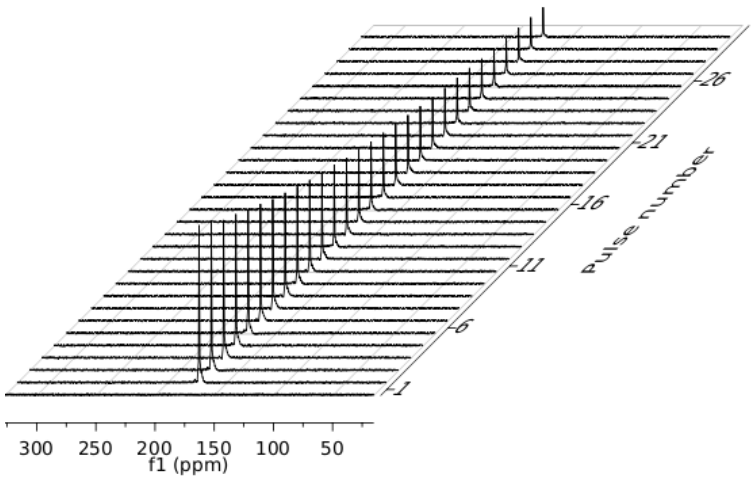


*Figure S66: Representative HP [1-^{13}C,2,3-$^{2}H_3$]serine **2** spectra acquired at 3T (173 ppm).*

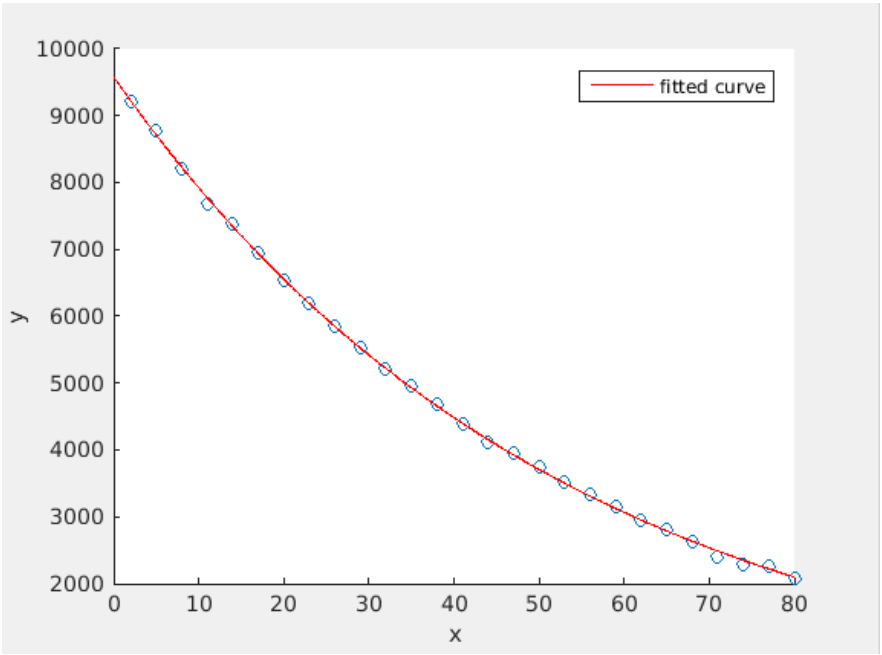


*Figure S67: Example of a hyperpolarized signal with T_1 relaxation equation fit. This measurement of [1-^{13}C,2,3-$^{2}H_3$]serine **2** yielded a* T_1 *of 53.2s. Calculation and figure were processed using MATLAB*

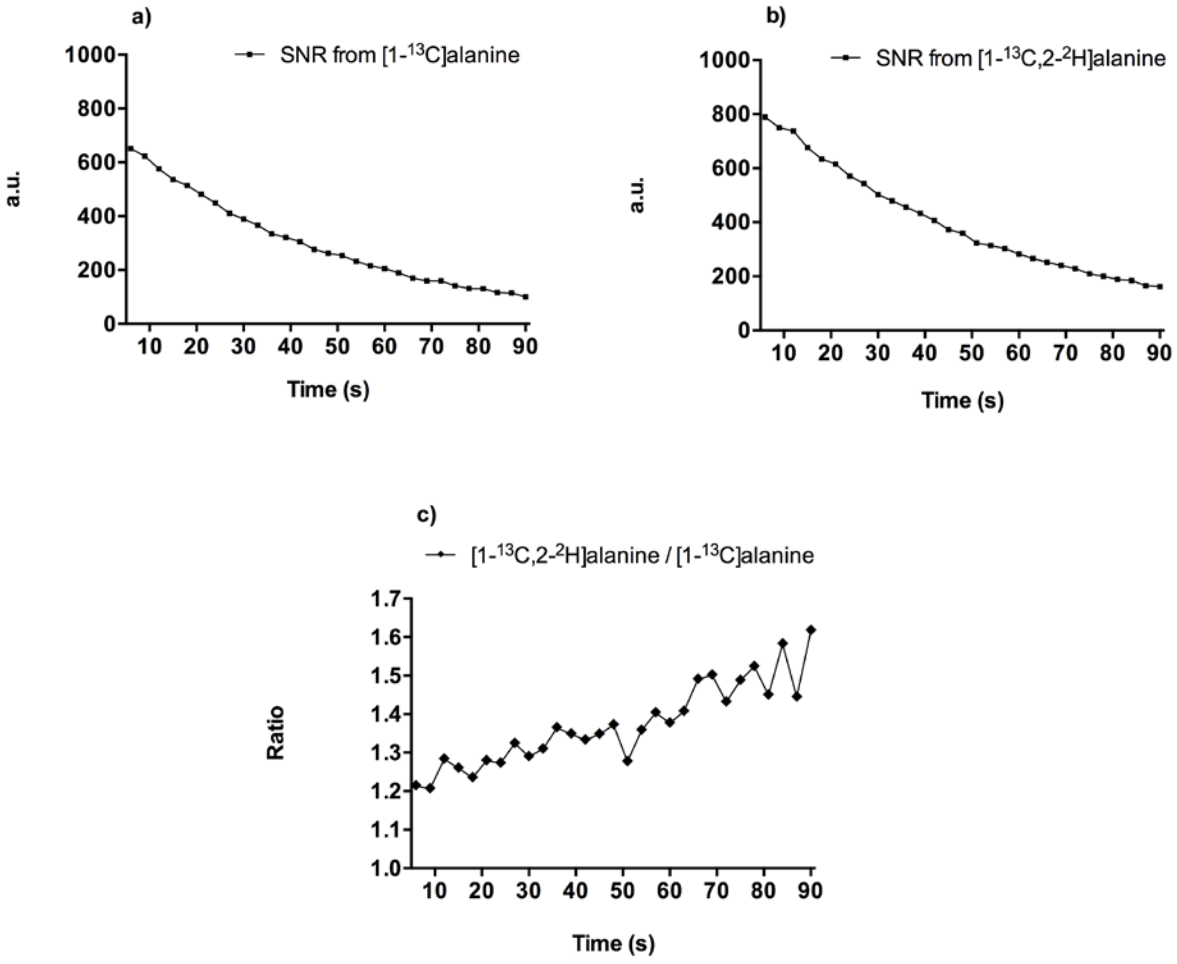
n=3 for each set of protonated and deuterated ^{13}C-labeled compounds allowed to determine T_1 and uncertainty as standard deviation:

	^{1}H T_1 (s)	**^{2}H T_1 (s)**
[1-^{13}C]alanine	52.9 ± 2.2	66.4 ± 1.7
[1-^{13}C]serine	43.3 ± 1.8	52.7 ± 0.7
[1-^{13}C]sodium lactate	55.2 ± 2.8	64.0± 2.1
[1-^{13}C]valine	38.1 ± 1.1	49.2 ± 0.4
[1-^{13}C]glycine	52.0 ± 3.2	65.0 ± 1.2
[2-^{13}C]alanine	4.9[a]	25.2 ± 0.1
[2-^{13}C]serine	3.6[a]	23.2 ± 0.9
[2-^{13}C]sodium lactate	7.2[a]	33.8 ± 0.4

Table S3: Summary of T_1's for ^{2}H-unlabeled and ^{2}H-labeled ^{13}C compounds measured with hyperpolarized decay at 3T (n = 3, p < 0.02).

[a]Due to very low HP signal, T_1 was measured by inversion recovery method.

Influence of deuterium enrichment on signal gain - Example of [1-^{13}C]alanine



*Figures S68: Time courses of spectra showing the evolution of SNR of HP [1-^{13}C]alanine (a) and [1-^{13}C,2-^{2}H]alanine **1** (b). c) Measurements of [1-^{13}C,2-^{2}H]alanine / [1-^{13}C]alanine signals ratio.*

Inversion Recovery Method

A solution of 50 mM of each protonated ^{13}C-labelled compound was placed in a 5 mm NMR tube and inserted into a 11.7 T Varian INOVA spectrometer (Agilent Technologies, Palo Alto, CA) equipped with a 5 mm triple-tuned, direct-detect, triple-axis gradient-equipped broadband probe (Agilent Technologies, Palo Alto, CA). The transmit-receive frequency was placed within ±50 Hz of the [2-^{13}C] metabolite peak, and the 90° hard pulse width was calibrated for ^{13}C using the zero-crossing at 360°. An inversion-recovery pulse sequence was then performed (10 points, 180°-90° delay incremented by 0.5s), and the T_1 was calculated by fitting the data to an exponential model of the form $S = A\left(1 - 2e^{-\frac{t}{T_1}}\right) + B$, where S is the signal at each 180°-90° delay t and A, B, and T_1 are determined via the "fit" function in MATLAB. The TR for both pulse width calibration and inversion-recovery was retrospectively verified to be at least 5-fold greater than the measured T_1, ensuring full relaxation between acquisitions.

Mouse Experiment

Animal Handling

The mouse study was carried out under a protocol approved by the UCSF Instutitional Animal Care and Use Committee. The mouse was placed on a heated pad and anesthetized with isoflurane (2-3%). A catheter was introduced into the tail vein for the eventual intravenous administration of [1-^{13}C]alanine and [1-^{13}C,2-^{2}H]alanine solutions, then the mouse was transferred to a heated pad in the RF coil in the Bruker BioSpec 3T MR scanner, strapped in place, kept under anesthesia with continuous delivery of isoflurane (1-2%) through a nose cone at an oxygen flow of 2 mL/min and periodically monitored for respiratory rate and general tissue perfusion.

Preparations

*[1-^{13}C]alanine and [1-^{13}C,2-^{2}H]alanine **1**:* 6.3 mg of OX63 trityl radical were added to 100.2 mg of alanine, 71.3μL of 18.9M NaOH and 28.6μL DMSO, to a final concentration of 23 mM. This yielded a preparation with 5.6 M alanine. The mixture was vortexed and sonicated for 5 minutes at room temperature.

Polarizations and Dissolutions

50 μL of each formulation were polarized at 94.082 GHz on Test bed DNP polarizer and 94.068 GHz on HyperSense (Oxford Instruments, Abingdon, UK), at 1.40 K for 2 hours. The sample was then dissolved with 3.5 mL of a 50 mM, Tris 0.3mM EDTA solution, and 220 μL 1.5 M HCl preloaded in the collection flask. The resulting pH of the dissolution was about 7 (pH strip). Time constant and solid state polarizations were similar for both [1-^{13}C]alanine and [1-^{13}C,2-^{2}H]alanine **1** samples.

MR Studies and Data Processing

Experiments were performed on a Bruker BioSpec 3T scanner equipped with multi-nuclear spectroscopy capability. The RF coil used in these experimentse was a dual-tuned ^{1}H-^{13}C coil for mice.

A CD-1 white mouse was cannulated via the portal vein and flushed with 50 µL of 8 U/mL heparin in normal saline solution every 8-12 minutes to prevent clotting. [1-^{13}C]alanine and [1-^{13}C,2-^{2}H]alanine were polarized as described in a previous section and dissolved to a final concentration of 80 mM. Shortly after dissolution, 300-400 µL of the dissolution solution was injected into the mouse over a period of 15 s. A slice-selective ^{13}C MRS pulse-acquire sequence was initiated along with start of injection (8 mm slice thickness, 10° tip angle, TR = 3 s, spectral width = 5 kHz, 4096 spectral points, 30 timepoints).

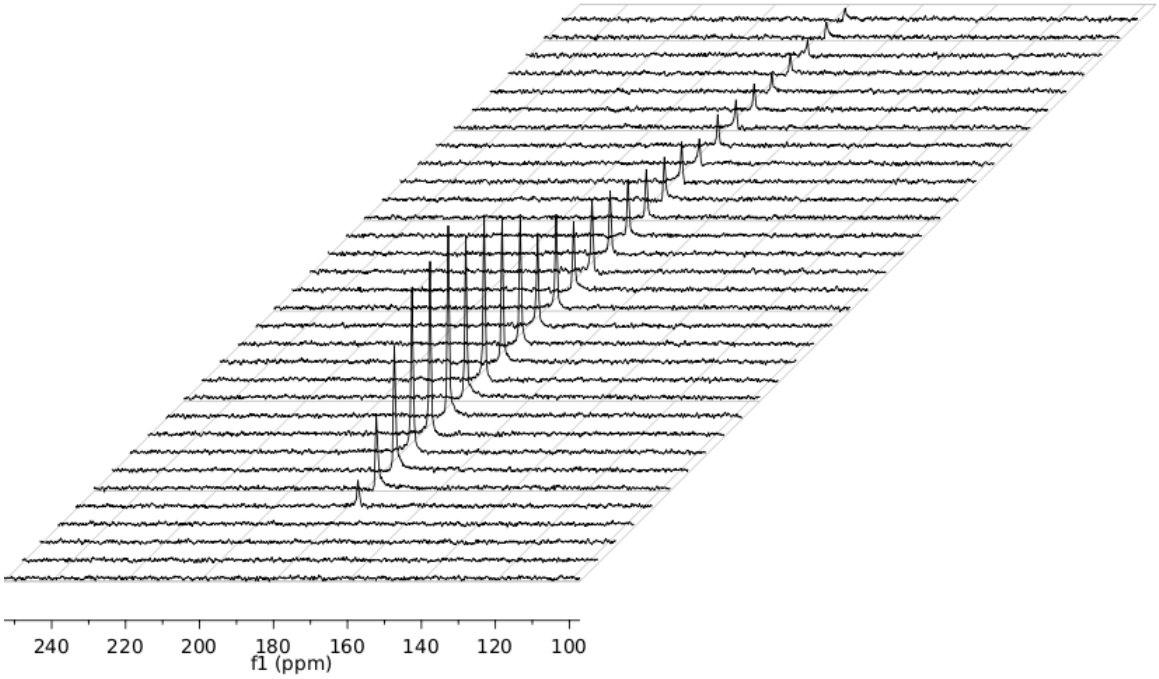


Figure S69: Stacked plot of individual spectra from each time point at 3T after a normal mouse injection of HP [1-^{13}C]alanine (176 ppm).

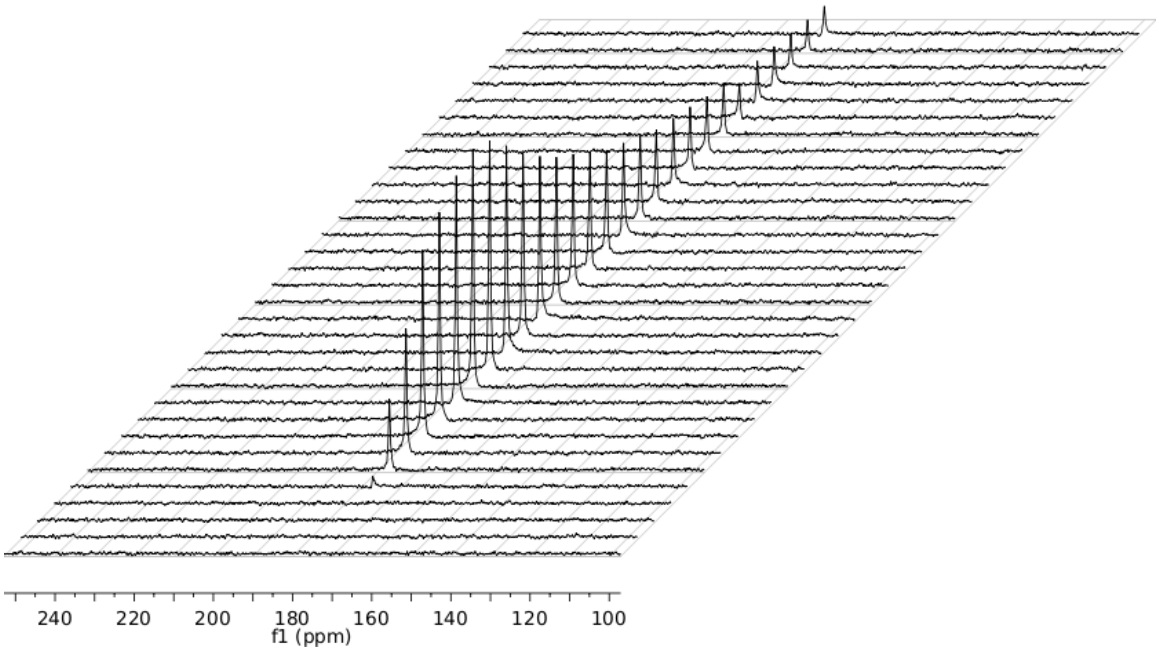


*Figure S70: Stacked plot of individual spectra from each time point at 3T after a normal mouse injection of HP [1-^{13}C,2-^{2}H]alanine **1** (176 ppm).*

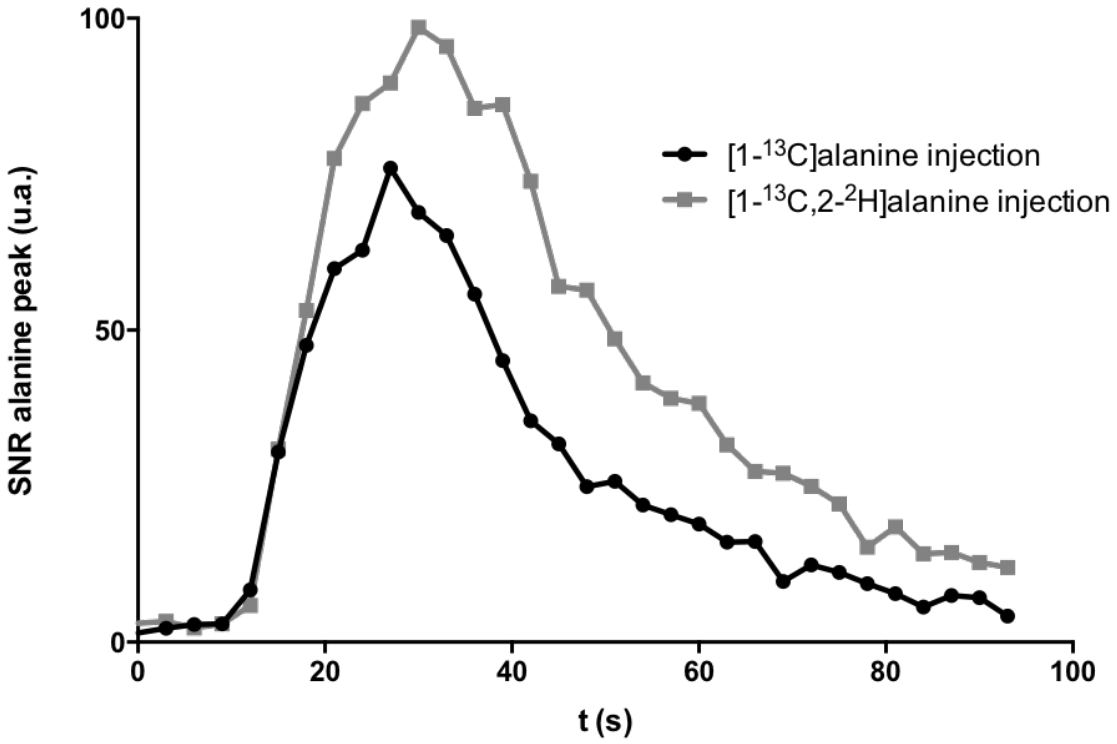


*Figure S71: Time courses of alanine were obtained by measuring SNR values at each point of spectra acquired from a representative mouse liver with a temporal resolution of 3 s after an injection of 80 mM hyperpolarized [1-^{13}C]alanine and [1-^{13}C,2-^{2}H]alanine **1***

Enzyme Experiments

Preparations

*[1-^{13}C]alanine and [1-^{13}C,2-^{2}H]alanine **1**:* 3.1 mg of OX63 trityl radical were added to 50.3 mg of alanine, 35.8 µL of 18.9M NaOH and 14.4 µL DMSO. This yielded a preparation with 5.6 M alanine. The mixture was vortexed and sonicated for 5 minutes at room temperature.

Polarizations, Dissolutions, and Acquisitions

[1-^{13}C]alanine or [1-^{13}C,2-^{2}H]alanine was polarized as described in a previous section, dissolved in 200 mM phosphate buffer plus 50 mM each of α-ketoglutarate and glutamate, and neutralized with 1.5 M HCl. The resulting dissolution was quickly mixed in a 5 mm NMR tube preloaded with 15 U of porcine alanine aminotransferase (ALT, Sigma-Aldrich) and inserted into a 11.7 T Varian INOVA spectrometer (Agilent Technologies, Palo Alto, CA) equipped with a 5 mm triple-tuned, direct-detect, triple-axis gradient-equipped broadband probe (Agilent Technologies, Palo Alto, CA). Dynamic ^{13}C NMR was performed on the sample to measure change in HP signal (5° hard pulses, TR = 3 s, 50 timepoints, spectral width = 20 kHz, 40k complex spectral points) The delay between addition of alanine dissolution to enzyme and start of acquisition was 8-9 seconds. The final volume in the NMR tube was about 800 µL. The resulting pH of the dissolution was about 6.5 (pH strip).

Data Processing and Kinetic Modeling

Using MestReNova 11 (Mestrelab Research S.L., Santiago de Compostela, Spain), spectral data were 10 Hz apodized and Fourier transformed. HP [1-^{13}C]alanine and [1-^{13}C]pyruvate peaks were fitted to Lorentzian-Gaussian lineshapes in order to determine peak integrals. The kinetic data were then fitted in MATLAB to differential equations, similarly to as was previously described[5]:

$$\frac{dM_{aH}}{dt} = -k_{aHp}M_{aH} + k_{pa}M_p - \frac{1}{T_{1a}}M_{aH}$$

$$\frac{dM_{aD}}{dt} = -k_{aDp}M_{aD} - \frac{1}{T_{1a}}M_{aH}$$

$$\frac{dM_p}{dt} = k_{aHp}M_{aH} + k_{aDp}M_{aD} - k_{pa}M_p - \frac{1}{T_{1p}}M_p$$

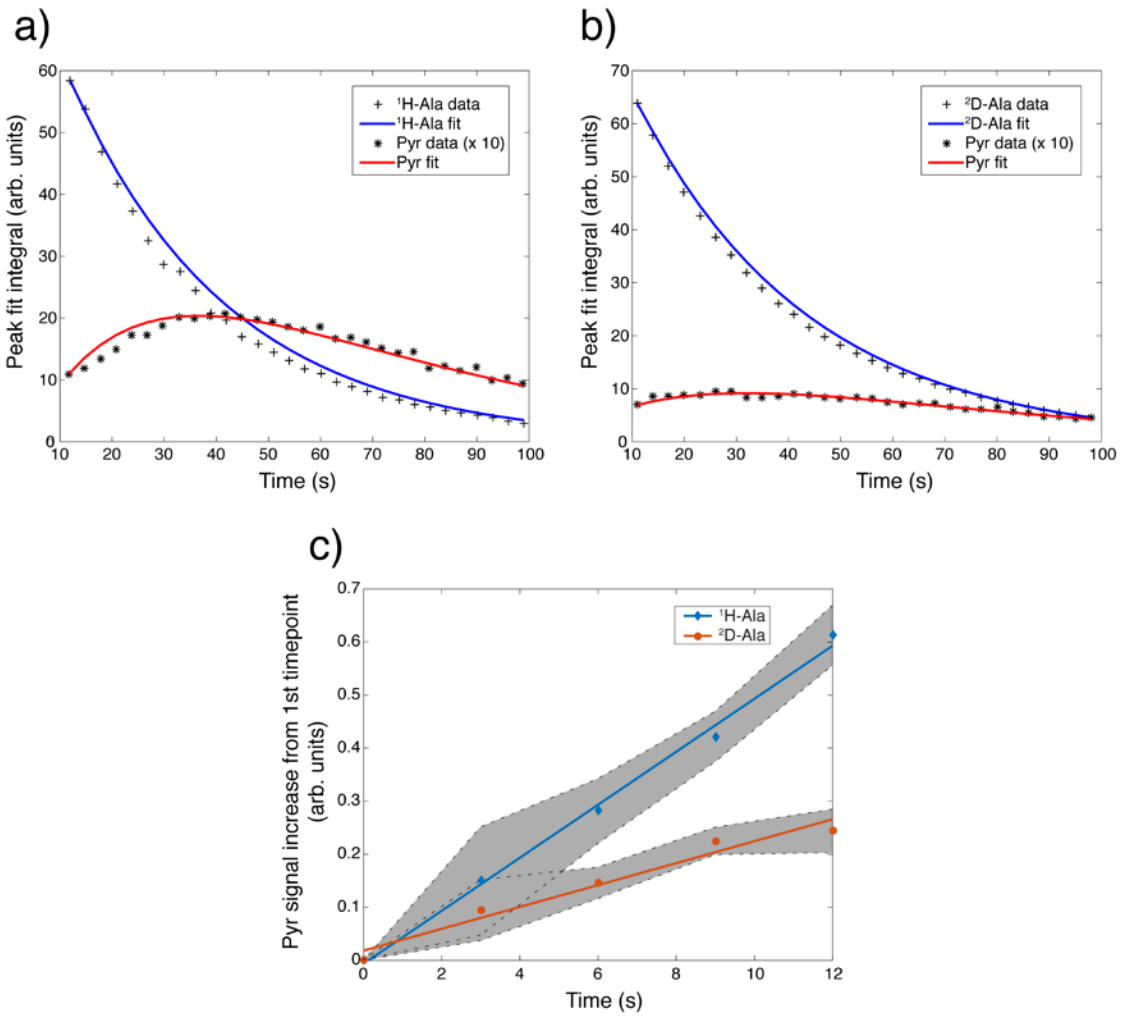
[5] H. Y. Chen, P. E. Z. Larson, R. A. Bok, C. Von Morze, R. Sriram, R. D. Santos, J. D. Santos, J. W. Gordon, N. Bahrami, M. Ferrone, J. Kurhanewicz and D. B. Vigneron, *Cancer Res.*, 2017, **77**, 3207–3216.

$$M_m^+[n] = M_m^-[n] \cos \alpha$$

$$S_m[n] = M_m^-[n] \sin \alpha$$

In the equations above, $M_m(t)$ and $S_m(t)$ represent the longitudinal magnetization and measured MR signal intensity, respectively, for a given metabolite m, with + and – superscripts denoting the values before and after the nth excitation, respectively. For the [1-^{13}C,2-^{2}H]alanine, the total HP alanine longitudinal magnetization was split into two separate pools M_{aH} and M_{aD} to account for the protonated and deuterated metabolites, respectively. Importantly, the deuterium is lost when [1-^{13}C,2-^{2}H]alanine is converted to [1-^{13}C]pyruvate. The initial fraction of deuterated alanine to total alanine at the start of acquisition was assumed to equal 1 (ie. all HP alanine deuterated at acquisition start). The alanine pyruvate reaction was assumed to be first-order in each direction (assuming no change in α-ketoglutarate or glutamate pools) with a reverse rate constant k_{pa} and forward rate constants k_{aHp} and k_{aDp} for protonated and deuterated alanine, respectively. Both protonated and deuterated alanine were modeled to have the same spin-lattice relaxation constant T_{1a}, similar to what we observed experimentally at 11.7 T. T_{1p} was the spin-lattice relaxation constant for pyruvate. The starting T_1 values for iteration were determined by fitting the last 20-30 points to a monoexponential decay, and the fitted T_1 was constrained to be within ± 10 % of this value. The first 30 points were then used with the full kinetic model.

Figures S72a and S72b display the best-fit kinetic curves for representative data sets acquired from HP [1-^{13}C]alanine and [1-^{13}C,2-^{2}H]alanine, respectively. All alanine fits had a coefficient of determination $R^2 > 0.99$, and all pyruvate fits exhibited $R^2 > 0.84$. Figure S72c shows the results for linear fitting of the first five points of the pyruvate dynamic curve in order to determine the initial rate of pyruvate conversion. This should correspond closely to k_{aHp} or k_{aDp} for the protonated and deuterated alanine experiments, since at early timepoints $M_p \ll M_{aH} + M_{aD}$ (see differential equations above). The slopes of the best-fit lines to the data are as reported in the main body of the paper: 0.050 for the protonated alanine, versus 0.0206 for the deuterated alanine (units: integral area/s).



Figures S72: *a-b) Representative kinetic curve fitting for HP ^{13}C-NMR experiments with (a) HP [1-^{13}C]alanine and (b) HP [1-^{13}C,2-^{2}H]alanine **1** to measure ALT kinetics. Note that the pyruvate signal is scaled up in order to properly display goodness of fit. (c) Initial slope analysis of pyruvate conversion for protonated and deuterated HP alanine (n = 3 each). Grey shaded regions denote standard deviation, and markers indicate mean values at each timepoint.*