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Protocol

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A practical guide for the preparation of C1-labeled α-amino acids using aldehyde catalysis with isotopically labeled CO_2

In the format provided by the authors and unedited

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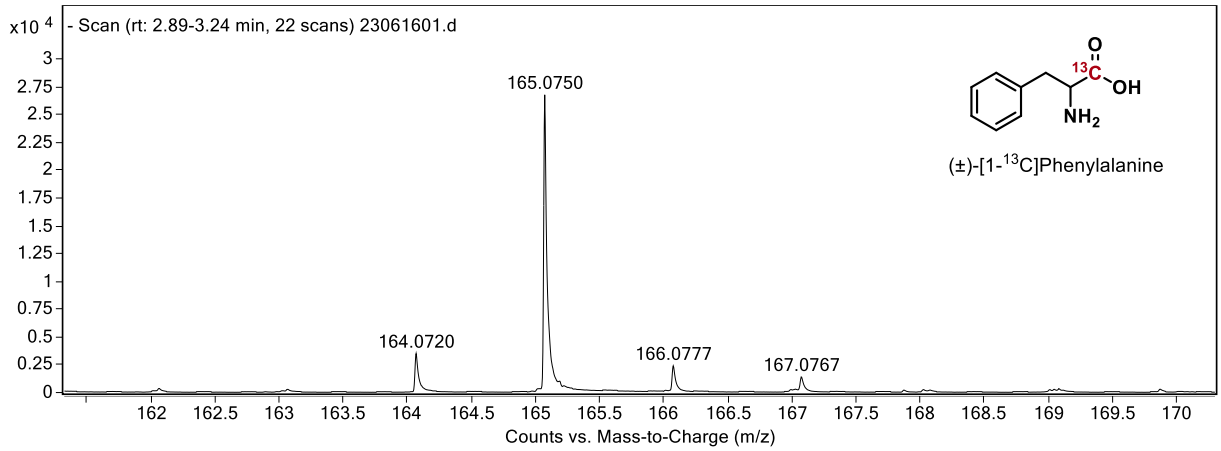
I. (±)-[1-^{13}C]Phenylalanine HRMS Spectrum and Incorporation Calculation



m/z	Relative Natural Abundance (%)	Observed Abundance (%)	Corrected Abundance (%)	Isotopic Enrichment Relative (%)
[M-H] + 0	100	3501	3501	12
[M-H] + 1	10.29	26785	26425	88

Determination of ^{13}C Incorporation: Percent ^{13}C isotope incorporation was determined by comparison of the mass spectral patterns of carbon-13 labelled product versus authentic starting material and calculated by the expressions below.

% ^{13}C Incorp. = [(Corrected abundance of ^{13}C) / (Corrected abundance of ^{13}C + Observed abundance of ^{12}C)] × 100, where observed abundance of ^{12}C is obtained from the mass signal intensities at M (m/z);

Corrected Abundance of ^{13}C = Observed abundance of ^{13}C – (Observed abundance of ^{12}C × Relative Natural Abundance of ^{13}C / 100), where observed abundance of ^{13}C is obtained from the mass signal intensities at M+1 (m/z).

Representative Example (Phenylalanine):
Corrected abundance of ^{13}C of phenylalanine = 26785 – [(3501 × 10.29) / 100] = 26425
% ^{13}C incorp. of phenylalanine = [26425 / (26425 + 3501)] × 100 = **88%**

II. (±)-[1-^{11}C]Phenylalanine Characterization Data and Additional Information

Radiochemical Yield and Quality Control

Radiochemical product identity was confirmed by co-injection of the labelled compound and corresponding non-radioactive standard. Radiochemical purity (RCP) was determined by calculating the ratio of the decay-corrected product signal area over the sum of all signal areas on the radio-HPLC chromatogram. Trapping efficiency (TE) was determined by measuring the activity remaining in the reaction vessel at the end of the reaction (after bubbling helium for 1 min), correcting it for decay and calculating the ratio over the starting activity value. Radiochemical yield (RCY) was calculated as the product of RCP and TE values.

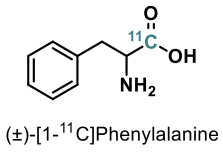
Radiolabelling Experimental Data

Grey and red arrows indicate peaks identified as the title compound in the UV and radiation chromatograms, respectively, based on co-elution with nonradioactive standards. As detectors are connected in series, there remains an uncorrected delay between signals in the UV and radiation chromatograms.

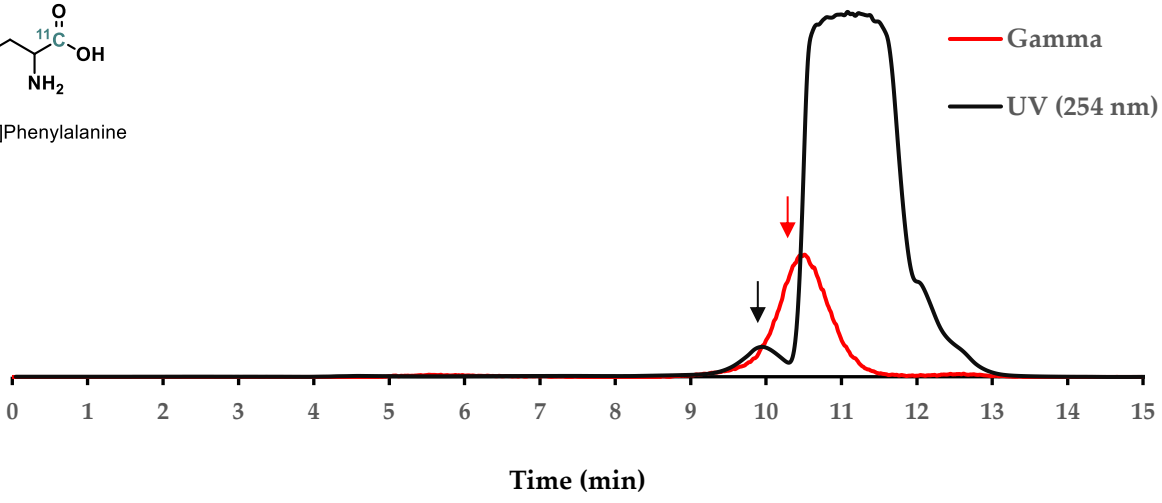
(±)-[1-^{11}C]Phenylalanine Crude Reaction Radio-HPLC Analysis

Column: Phenomenex Aqua C18, 4.6 × 250 mm, 5 µm, 125 Å pore size

TE = 25%; **RCP** = 95%; **RCY** = 24% (n = 3)



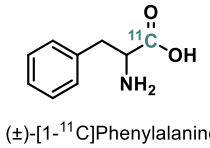
(±)-[1-^{11}C]Phenylalanine



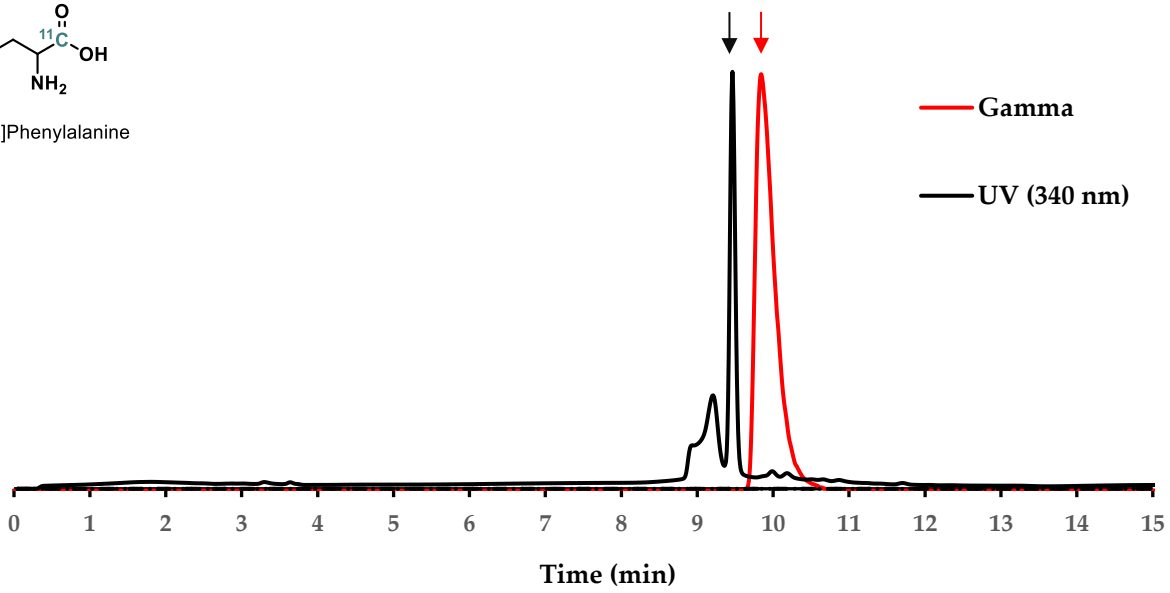
(±)-[1-^{11}C]Phenylalanine Isolation Radio-HPLC Analysis

Column: Phenomenex Synergi Hydro-RP, 4.6 × 250 mm, 10 μm, 80 Å pore size

TE = 45%; **RCP** = 30%; **RCY** = 13%

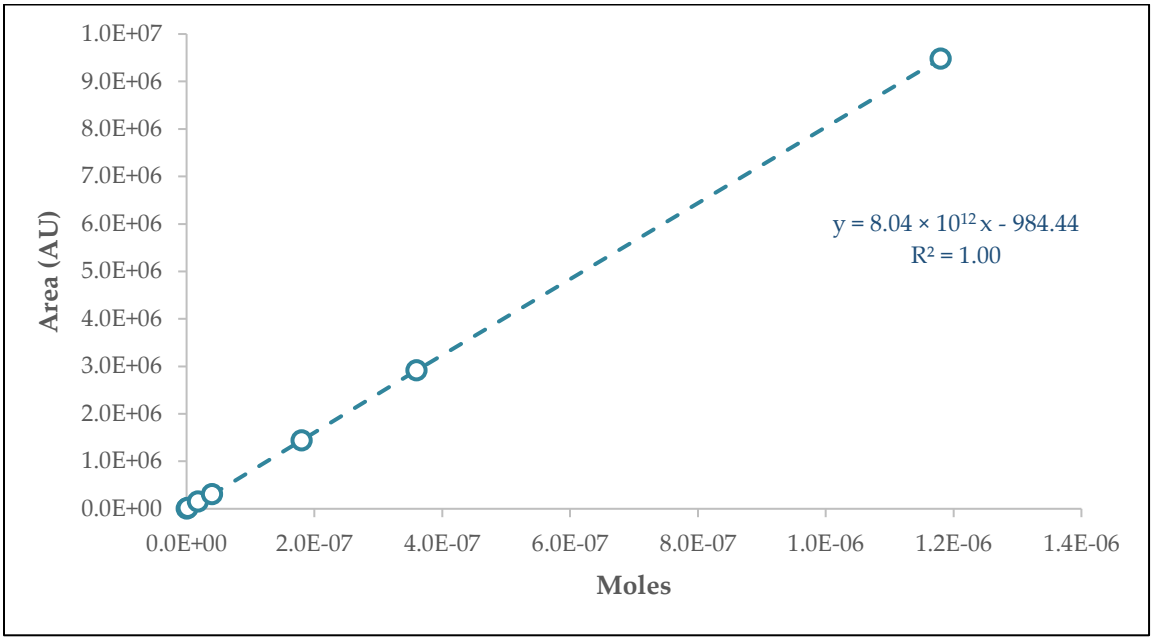


(±)-[1-^{11}C]Phenylalanine



Starting activity	12.7 GBq	@ 16:34
Activity in reactor	5.55 GBq	@ 16:35 (5.74 GBq @ 16:34; 45% TE)
Product activity	0.69 GBq	@ 17:00 (1.66 GBq @ 16:34; 13% RCY)
Activity concentration	107 MBq/mL	@ 17:11 (156 MBq @ 17:00)
Injection volume	50 μL	
Activity injected	5.36 MBq	@ 17:11 (7.79 MBq @ 17:00)

(±)-[1-^{11}C]Phenylalanine Isolation Calibration Curve



UV area	7.34 × 10^6 AU	
Calibration equation	y = 8.04 × 10^{12} x – 984.44	
n (moles)	0.913 μmol	
Molar activity	8.4 GBq/mmol	@ 17:00

Additional Details on Radiochemical Yield Determinations

Decay Correction Formula for Carbon-11:

$$A = A_0 \times e^{(\frac{ln\ 2}{20.364})\times(t-t_0)}$$

where:

A_0 is activity at reference time

A is activity at measurement time

$(t - t_0)$ is the interval between measurement time and reference time in minutes

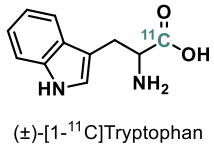
20.364 is the half-life of carbon-11 in minutes

Trapping Efficiency Calculation (Using (±)-[1-^{11}C]Tryptophan as an Example)[1]:

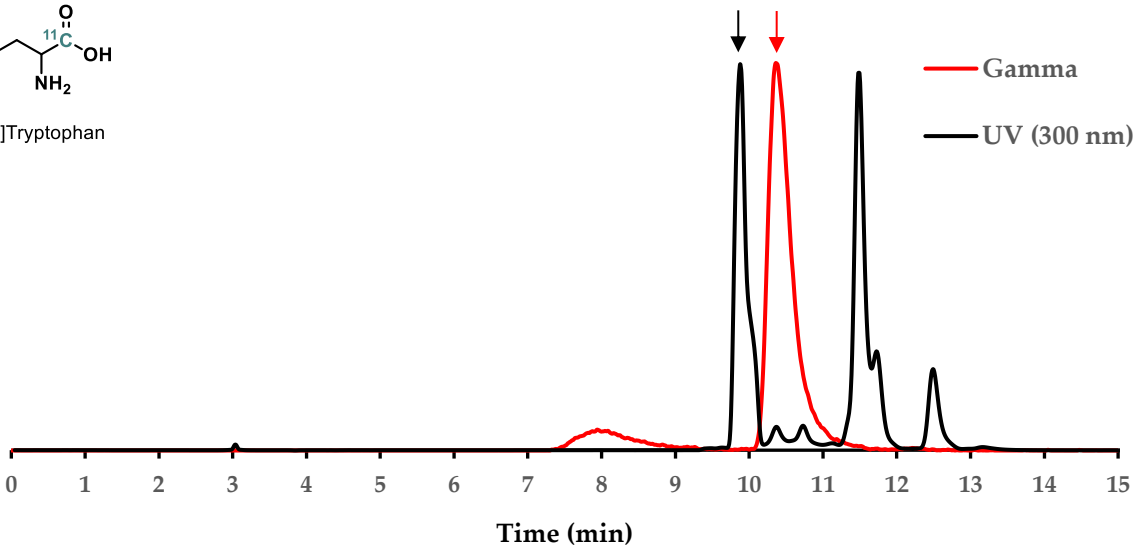
Note: this data was collected from the key reference using this protocol. It will be used as the detailed example to illustrate trapping efficiency, radiochemical purity, and radiochemical yield calculations.

Column: Phenomenex Aqua C18, 4.6 × 250 mm, 5 μm, 125 Å pore size

TE = 18%; **RCP** = 91%; **RCY** = 16% (n = 2)



(±)-[1-^{11}C]Tryptophan



[^{11}C]CO_2 trap activity:	115 mCi (4255 MBq)	@	14:39
Reactor activity at the end of the reaction:	9.5 mCi (352 MBq)	@	15:01
Decay-corrected reactor activity:	20 mCi (740 MBq)	@	14:39

TE = (decay corrected reactor activity / trap activity) × 100

= (20 mCi/ 115 mCi) × 100%

= 17%

Radiochemical Purity Calculation:

Radio-HPLC integrations:

Elution time (min)	Area (AU)	Decay-corrected area (AU)†
8.418	86386	115091
10.407‡	799087	1139283
12.206	4118	6242

†Corrected to time of injection (14:44). ‡[^{11}C]Tryptophan.

RCP = (decay corrected product area / sum of decay corrected areas) × 100

= [1139283 / (115091 + 1139283 + 6242)] × 100

= 90%

Radiochemical Yield Calculation:

RCY = TE × RCP

= (17 × 90) / 100

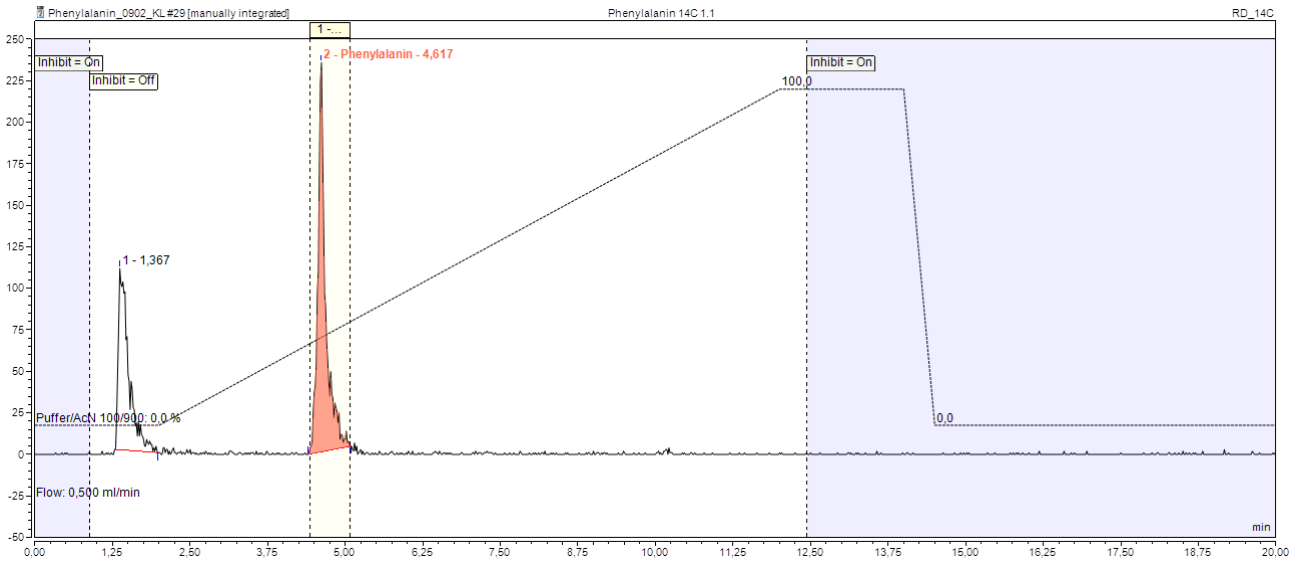
= 15%

Average Values:

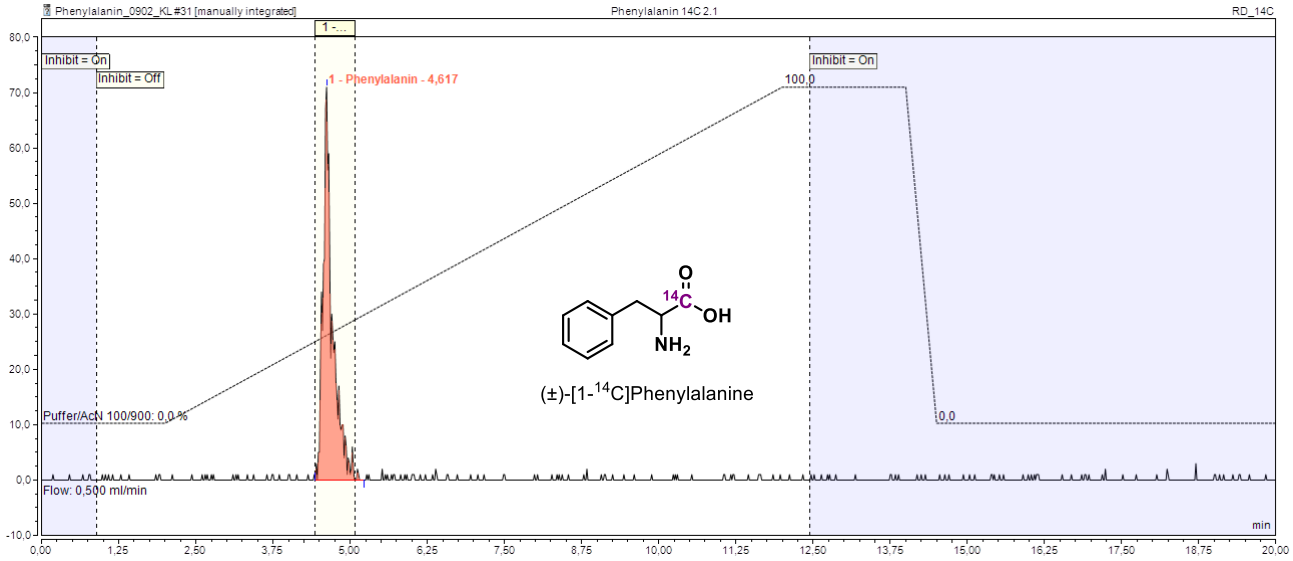
	Run 1	**Run 2**	**Average**
TE	17%	19%	18%
RCP	90%	91%	91%
RCY	15%	17%	16%

III. (±)-[1-^{14}C]Phenylalanine Characterization Data

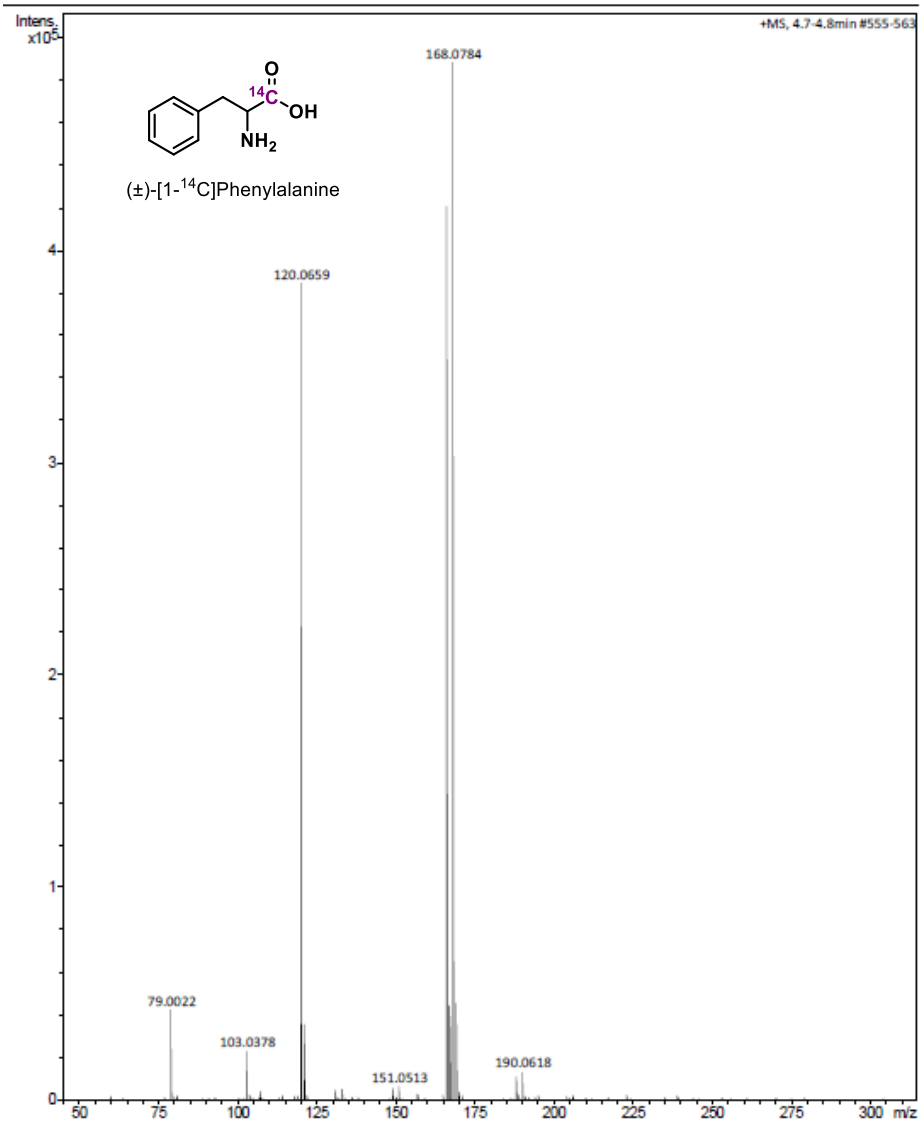
Radio-HPLC Detection: After Reaction Before Purification



Radio-HPLC Detection: After Purification



HRMS and Activity Data for (±)-[1-^{14}C]Phenylalanine

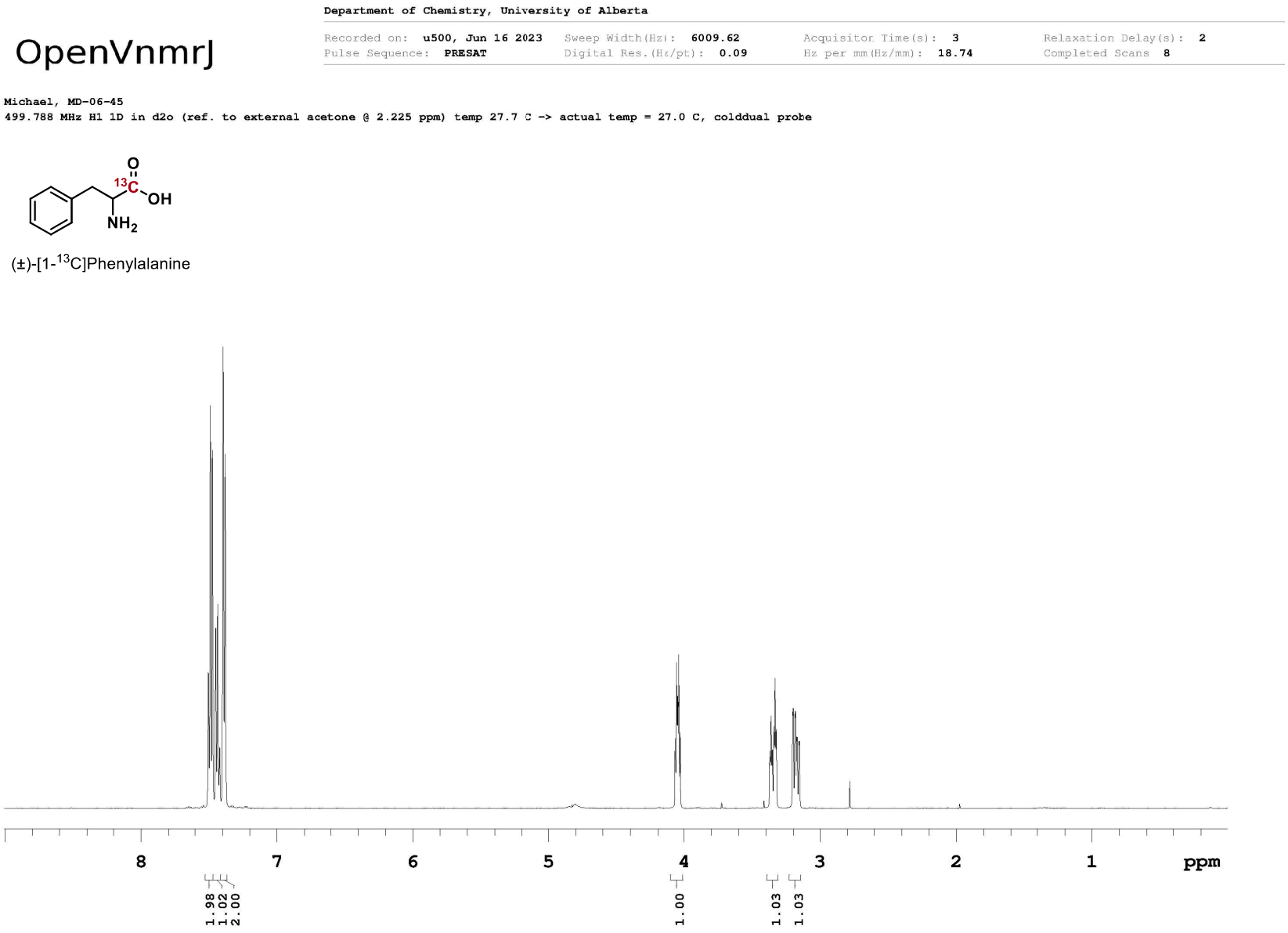


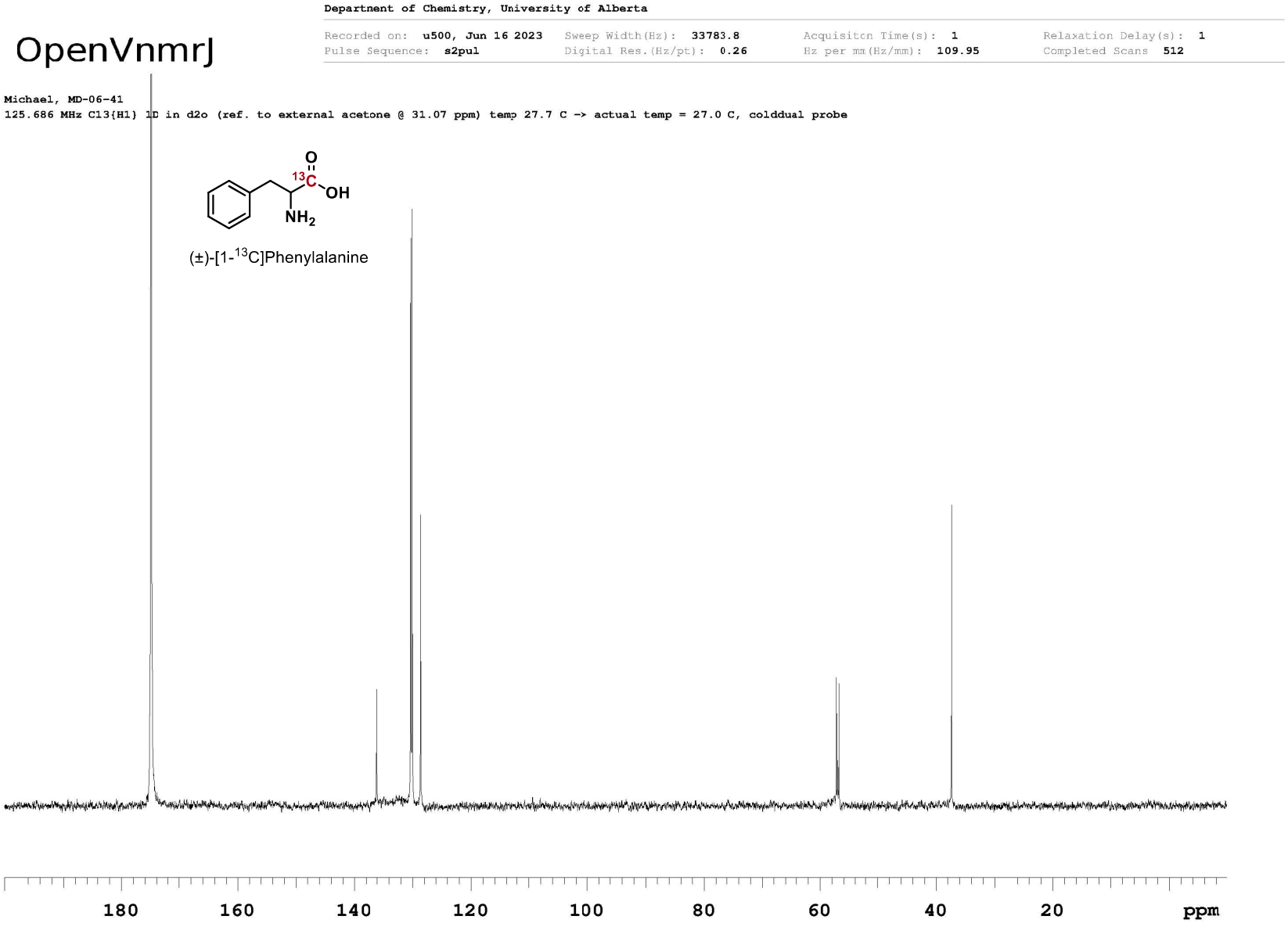
46,5 % 0 14C
53,6 % 1 14C → x 62,5 Ci/mmol → **33,5 mCi/mmol**

IV. References

[1] Bsharat, O.; Doyle, M. G. J.; Munch, M.; Mair, B. A.; Cooze, C. J. C.; Derdau, V.; Bauer, A.; Kong, D.; Rotstein, B. H.; Lundgren, R. J. Aldehyde-catalyzed carboxylate exchange in α-amino acids with isotopically labelled CO_2. *Nat. Chem.* **2022**, *14*, 1367–1374.

V. ^{1}H and ^{13}C NMR Spectra for (±)-[1-^{13}C]Phenylalanine




OpenVnmrJ
Department of Chemistry, University of Alberta
Recorded on: u500, Jun 16 2023
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Acquisition Time(s): 1
Relaxation Delay(s): 1
Pulse Sequence: s2pul
Digital Res.(Hz/pt): 0.26
Hz per mm(Hz/mm): 109.95
Completed Scans 512
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O
13C
OH
NH2
(±)-[1-13C]Phenylalanine
180
160
140
120
100
80
60
40
20
ppm