

Supporting information to

Direct biosynthetic cyclization of a distorted paracyclophane highlighted by double isotopic labelling of L-tyrosine

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General information

All reactions were carried out in oven-dried reaction vessels under an atmosphere of argon and in anhydrous solvents, unless otherwise stated. Reactions and purifications were monitored by TLC on Merck silica gel 60 F254 aluminium, using UV absorption then vanillin–H₂SO₄ (1% vanillin in ethanol + 2% H₂SO₄), or ninhydrin (3% ninhydrin in EtOH) as staining system. The products were purified by flash chromatography on Geduran silica gel Si 60, 40–63 μm. NMR spectra were recorded on Bruker Avance III HD 400 MHz and 600 MHz spectrometers. Chemical shifts (δ) are quoted in ppm with internal calibration from the residual solvent peak (CHCl₃: 7.27, 77.0 ppm; DMSO-d₅: 2.49, 39.5 ppm; H₂O: 4.79 ppm for ¹H and ¹³C NMR, respectively). All coupling constants (*J*) are quoted in Hertz. The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. High resolution mass spectra (HR-MS) of synthesized compounds were measured on an Applied Biosystem QSTAR Pulsar-i spectrometer, using electrospray ionization (ESI). Infrared spectra were recorded on a Shimadzu 8400S FTIR spectrometer. Specific rotations were recorded on a Perkin Elmer 341 polarimeter at 20°C (589 nm, Na lamp). The enantiomeric excess of compound **16** was measured by gas chromatography (GC control: Varian 3400) on a chiral column (see synthetic procedure). The enantiomeric excess of tyrosine **17** was measured by UHPLC (Shimadzu Nexera coupled to DAD) on a standard reverse phase column after derivatization (see synthetic procedure). Melting points were measured on a Büchi B-545 apparatus. Detection of pyrrocidines in samples from mycelium extracts were done with the high performance liquid chromatography (Shimadzu, UHPLC Nexera) coupled with mass spectrometry (Shimadzu, LCMS2020) using atmospheric pressure chemical ionization (APCI).

Synthetic procedures and compound data

4-(¹⁸O)Hydroxybenzaldehyde (**10**)

To a solution of 4-formylphenylboronic acid **9** (1.0 g, 6.67 mmol) in DMF (20 mL) was added the catalyst [Ru(bpy)₃Cl₂]·6H₂O (10 mg, 0.13 mmol) and iPr₂NEt (2.32 mL, 13.3 mmol) at room temperature. The reaction was irradiated at 420 nm (blue led)¹ at room temperature during 24 h and under an atmosphere of 97.1% isotopically enriched ¹⁸O₂. The solution was cooled in an ice bath at 0°C before quenching with 10% aqueous HCl (25 mL). The solution was extracted with Et₂O (25 mL x 3). The organic phase was washed with brine (50 mL) and drought over Na₂SO₄. The crude extract was purified by flash chromatography on silica gel (EtOAc/cyclohexane 1/3) to furnish the compound (**10**) as a colourless solid (340 mg, 41% or 89 % based on recovered starting material). A loss of isotopic enrichment was

¹ Y.-Q. Zou, J.-R. Chen, X.-P. Liu, L.-Q. Lu, R. L. Davis, K. A. Jørgensen, W.-J. Xiao, *Angew. Chem. Int. Ed.* **2012**, *51*, 784–788.

found at 86% yield (measured by mass spectrometry, Figure S1), probably due to slight air dilution during the experiment.

^1H NMR (400 MHz, CDCl_3), δ (ppm): 9.89 (s, 1H, $\underline{\text{CHO}}$), 7.83 (d, $J = 8.6$ Hz, 2H, \textit{ortho} ArH), 6.99 (d, $J = 8.6$ Hz, 2H, \textit{meta} ArH), 6.61 (brs, 1H, OH).

^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 191.0 ($\underline{\text{CHO}}$), 164.5 ($\underline{\text{COH}}$), 132.5 (\textit{ortho} C_{Ar}), 129.0 ($\underline{\text{C}}_{\text{Ar}}\text{CHO}$), 116.1 (\textit{meta} C_{Ar}).

IR (thin film on NaCl pellets) ν (cm^{-1}): 3187, 1682, 1601, 1495, 1296, 1200, 1142, 802.

HRMS (ESI+), m/z calcd. for $\text{C}_7\text{H}_7\text{O}^{18}\text{O}$ [$\text{M}+\text{H}$] $^+$: 125.0488; found: 125.0493.

m.p.: 114-115 $^\circ\text{C}$ (lit.² 115-116 $^\circ\text{C}$).

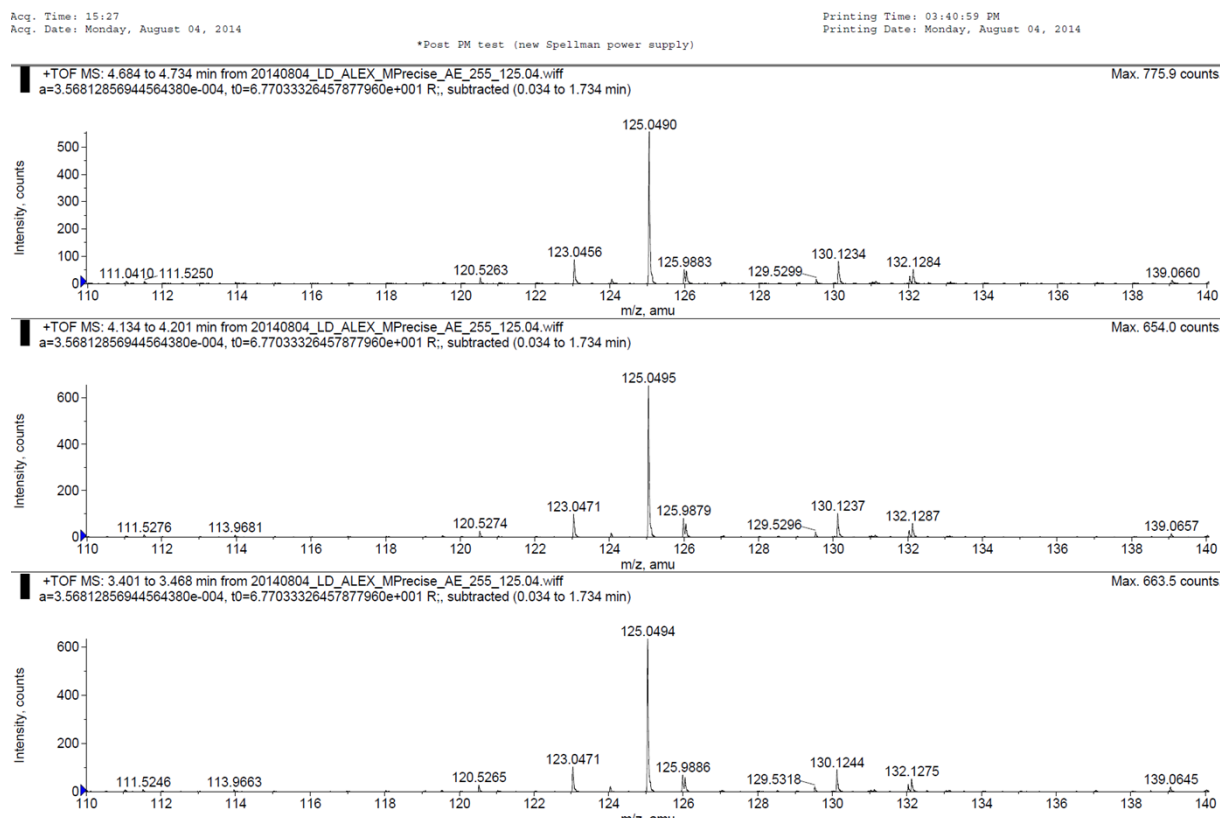


Figure S1: Mass spectra ^{18}O -labelled p -hydroxybenzaldehyde (**10**) showing the presence of unlabelled compound.

4-[(^{18}O)Methoxy]benzaldehyde (**11**)

To a solution of KOH (470 mg, 8.40 mmol) in DMSO (5 mL) was added, at room temperature, the benzaldehyde **10** (260 mg, 2.10 mmol) and MeI (259 μL , 4.20 mmol). The reaction is stirred at room temperature for 3 h, and H_2O (15 mL) was then added. The reaction mixture was extracted with DCM (15 mL x 2). The organic layer was drought over Na_2SO_4 and evaporated to give 4-[(^{18}O)methoxy]benzaldehyde or (4- ^{18}O)anisaldehyde (**11**) as a clean oily product without further purification (270 mg, 93 %).

^1H NMR (400 MHz, CDCl_3), δ (ppm): 9.89 (s, 1H, $\underline{\text{CHO}}$), 7.84 (d, $J = 8.8$ Hz, 2H, \textit{ortho} ArH), 7.02 (d, $J = 8.7$ Hz, 2H, \textit{meta} ArH), 3.89 (s, 3H, OCH_3).

² H.Yang, Y. Li, M. Jiang, J. Wang, H. Fu, *Chem. Eur. J.*, **2011**, *17*, 5652-5660.

^{13}C NMR (100 MHz, CDCl_3), δ (ppm): 190.8 ($\underline{\text{C}}\text{HO}$), 164.6 ($\underline{\text{C}}_{\text{Ar}}^{18}\text{OMe}$), 132.0 (*ortho* C_{Ar}), 130.0 ($\underline{\text{C}}_{\text{Ar}}\text{CHO}$), 114.3 (*meta* C_{Ar}), 55.6 ($^{18}\text{O}\underline{\text{C}}\text{H}_3$).

IR (film on NaCl pellets) ν (cm^{-1}): 2913, 2820, 2707, 1685, 1601, 1579, 1500, 1452, 1403, 1260, 1173, 1002, 810.

HRMS (ESI+), m/z calcd. for $\text{C}_8\text{H}_8\text{O}^{18}\text{O}$ $[\text{M}+\text{H}]^+$: 139.0644; found 139.0640

2-((*Tert*-butoxycarbonyl)amino)-(1- ^{13}C)acetic acid (**12**)

To a solution of 1- ^{13}C glycine (1 g, 13.2 mmol) in a 1:1 mixture of dioxane/ H_2O (32 mL) was added Boc_2O (1.45 g, 6.67 mmol) and Et_3N (1.77 mL, 13.2 mmol) at room temperature. After the reaction was left for 2 h at room temperature, dioxane was evaporated and $\text{H}_2\text{O}/\text{Et}_2\text{O}$ (50 mL/80 mL) were added for extraction. The aqueous phase was recovered and its pH was adjusted to 2 with a 1 N solution of HCl. Extraction was performed with AcOEt (50 mL x 2) and the organic layer was drought over MgSO_4 and concentrated to furnish the protected *N*-Boc-(1- ^{13}C)glycine **12** as a colorless solid (2.16 g, 93 %) which was used for the next step without further purification.

^1H NMR (400 MHz, $\text{DMSO}-d_6$), δ (ppm): 12.43 (brs, 1H, $\underline{\text{C}}\text{OOH}$), 7.03 (t, $J = 6.1$ Hz, 1H, $\underline{\text{N}}\text{H}$), 3.58 (d, $J = 6.1$ Hz, 2H, $\underline{\text{C}}\text{H}_2$), 1.39 (s, 9H, $\text{O}(\underline{\text{C}}\text{H}_3)_3$).

^{13}C NMR (100 MHz, D_2O), δ (ppm): 174.7 ($^{13}\underline{\text{C}}\text{OOH}$), 158.3 ($\underline{\text{N}}\text{H}\underline{\text{C}}\text{O}$), 81.6 ($\text{O}\underline{\text{C}}(\underline{\text{C}}\text{H}_3)_3$), 42.0 (d, $J_{\text{C-C}} = 56.3$ Hz, $\underline{\text{C}}\text{H}_2$), 28.1 ($(\underline{\text{C}}\text{H}_3)_3$).³

IR (thin film on NaCl pellets) ν (cm^{-1}): 3323, 2985, 2913, 1700, 1502, 1360, 1358, 1221, 1178, 1020.

HRMS (ESI+), m/z calcd. for $^{13}\text{CC}_6\text{H}_{14}\text{NO}_4$ $[\text{M}+\text{H}]^+$: 177.0956; found: 177.0958.

m.p.: 85-86 °C (lit.⁴ 85-88°C).

Methyl (*Z*)-2-acetamido-3-[(4-(^{18}O)methoxyphenyl)-(1- ^{13}C)acrylate (**13**)

The reaction mixture of the ^{18}O -labelled methoxybenzaldehyde **11** (270 mg, 2.00 mmol), CH_3COONa (160 mg, 2.00 mmol), the ^{13}C -labelled *N*-Boc-Gly **12** (344 mg, 2.00 mmol) and Ac_2O (1.18 mL, 12.5 mmol) was stirred at 40°C for 40 min and then heated to 140 °C for 18 h.⁵ The reaction was cooled down at room temperature and water (25 mL) was added before stirring at the same temperature for 1 h. The solution was filtrated, the brown precipitate was washed with water (5 mL x 2) and then diluted in MeOH (20 mL). After addition of Et_3N (0.3 mL, 2.00 mmol), the solution was heated at 80°C for 3 h, then solvents were evaporated. Water (50 mL) and AcOEt (3 x 30 mL) were added for extraction. The organic phase was drought over MgSO_4 , evaporated and the concentrated crude extract was purified by flash chromatography on silica gel (cyclohexane/ EtOAc 1:2), to furnish the desired compound **13** as a brown resin (122 mg, 25 % or 48 % based on recovered starting material).

^1H NMR (400 MHz, CDCl_3), δ (ppm): 7.44 (d, $J = 8.4$ Hz, 2H, *ortho* ArH), 7.40 (s, 1H, $\underline{\text{N}}\text{H}$), 7.07 (s, 1H, = $\underline{\text{C}}\text{H}$ -), 6.88 (d, $J = 8.4$ Hz, 2H, *meta* ArH), 3.81 (s, 6H, $\text{ArO}\underline{\text{C}}\text{H}_3$, $\text{CO}_2\underline{\text{C}}\text{H}_3$), 2.15 (s, 3H, $\underline{\text{N}}\text{HCO}\underline{\text{C}}\text{H}_3$).

³ Isotope shifts were observed, to be compared with unlabelled compound: ^{13}C NMR (100 MHz, D_2O), δ (ppm): 171.8 ($\underline{\text{C}}\text{OOH}$), 155.8 ($\underline{\text{N}}\text{H}\underline{\text{C}}\text{O}$), 78.0 ($\text{O}\underline{\text{C}}(\underline{\text{C}}\text{H}_3)_3$), 41.8 ($\underline{\text{C}}\text{H}_2$), 28.2 ($(\underline{\text{C}}\text{H}_3)_3$).

⁴ G.W. Anderson, A.C. McGregor, *J. Am. Chem. Soc.*, **1957**, *79*, 6180.

⁵ M. Takeda, J. Jee, A. M. Ono, T. Terauchi, M. Kainosho, *J. Am. Chem. Soc.* **2009**, *131*, 18556–18562.

¹³C NMR (100 MHz, CDCl₃), δ (ppm): 169.3 (NCOCH₃), 166.1 (¹³COOCH₃), 165.5 (C_{Ar}¹⁸OCH₃), 131.8 (*ortho* C_{Ar}), 126.3 (C_{Ar}CH=), 122.4 (d, J = 60 Hz, ArCH=C), 121.5 (ArCH=C), 114.2 (*meta* C_{Ar}), 55.4 (Ar¹⁸OCH₃), 52.7 (¹³CO₂CH₃), 23.7 (NHCOCH₃).⁶

IR (thin film on NaCl pellets) ν (cm⁻¹): 3260, 2953, 2847, 1747, 1732, 1651, 1558, 1543, 1508, 1249, 1176, 1033, 823.

HRMS (ESI+), *m/z* calcd. for ¹³CC₁₂H₁₆NO₃¹⁸O [M+H]⁺: 253.1154; found: 253.1152.

Methyl (2S)-2-acetamido-3-[4-(¹⁸O)methoxyphenyl]-(1-¹³C)propanoate (15)

To a solution of degassed compound **13** (110.0 mg, 0.44 mmol) in degassed MeOH (3 mL) was added the degassed DuPhos-Rh catalyst **14** (Alfa Aesar ref. 44546, 16 mg, 0.02 mmol). After 1 h of reaction at room temperature under a pressure of 2 bars, the mixture was filtered through a short pad of silica gel with MeOH elution. The solution was evaporated to furnish quantitatively the desired product **15** as a beige solid (111 mg, ee > 99 %).

¹H NMR (400 MHz, CDCl₃), δ (ppm): 7.01 (d, J = 8.7 Hz, 2H, *ortho* ArH), 6.83 (d, J = 8.6 Hz, 2H, *meta* ArH), 5.87 (d, J = 5.5 Hz, 1H, NH), 4.86 (pseudo-dt, J = 7.8, 5.6, 5.6 Hz, 1H, ArCH₂CH), 3.80 (s, 3H, ArOCH₃), 3.74 (s, 3H, CO₂CH₃), 3.08 (m, 2H, ArCH₂CH), 2.00 (s, 3H, NHCOCH₃).

¹³C NMR (100 MHz, CDCl₃), δ (ppm): 172.3 (¹³CO₂CH₃), 169.6 (NHCOCH₃), 158.8 (C_{Ar}¹⁸OCH₃), 130.4 (*ortho* C_{Ar}), 127.8 (C_{Ar}CH₂), 114.1 (*meta* C_{Ar}), 54.5 (d, J = 61.2 Hz, ArCH₂CH), 53.0 (Ar¹⁸OCH₃), 52.4 (¹³CO₂CH₃), 37.1 (ArCH₂CH), 23.3 (NHCOCH₃).⁷

IR (thin film on NaCl pellets) ν (cm⁻¹): 3286, 3076, 3017, 2959, 1746, 1678, 1608, 1549, 1216, 1183, 1126, 1183, 1126, 1017, 832.

HRMS (ESI+), *m/z* calcd. for ¹³CC₁₂H₁₈NO₃¹⁸O [M+H]⁺: 255.1306; found: 255.1290.

[α]_D²⁰ = + 18 ° (c 0.3, MeOH; lit.⁸ + 17.8 °, c 0.5, MeOH).

m.p.: 103 °C (lit.⁹ 104°C).

The enantiomeric excess of **15** was measured by comparison with the racemic mixture, by GC on the chiral column CP chirasil-DEX-CB/25 m-0.32 mm- 0.25 μm, with helium as the carrier gas at 16 psi. A 40 min program was used: 0-25 min (isocratic 150 °C), 25-29 min (gradient of 5 °C/min from 150 to 170 °C), 29-40 min (isocratic 175 °C). The racemic product was injected in GC and two retention times were obtained (rt = 31.345 and 31.731 min). Injection of compound **15** gave a single peak at 31.701 min.

(2S)-2-Acetamido-3-[4-(¹⁸O)hydroxyphenyl]-(1-¹³C)propanoic acid

To the protected doubly labelled L-tyrosine **15** (111 mg, 0.44 mmol) was added a solution of NaI (72 mg, 0.48 mmol) in 48% aqueous HBr (6 mL, used as received from Aldrich) at room temperature. The mixture was heated at 94°C during 2 h. The reaction mixture was then filtered through a pad of resin Amberlite IR120 (H⁺) washed with H₂O. The solvent was evaporated to give quantitatively the desired N-acetyl-L-tyrosine as a colourless solid (99 mg).

⁶ Isotope shifts were observed, to be compared with unlabelled compound: ¹³C NMR (100 MHz, CDCl₃), δ (ppm): 169.0 (NCOCH₃), 166.1 (COOCH₃), 160.7 (C_{Ar}OCH₃), 133.2 (*ortho* C_{Ar}), 131.8 (C_{Ar}CH=), 126.3 (ArCH=C), 122.0 (ArCH=C), 114.2 (*meta* C_{Ar}), 55.4 (ArOCH₃), 52.7 (CO₂CH₃), 23.6 (NHCOCH₃).

⁷ Isotope shifts were observed, to be compared with unlabelled compound: ¹³C NMR (100 MHz, CDCl₃), δ (ppm): 171.5 (CO₂CH₃), 170.7 (NHCOCH₃), 157.8 (C_{Ar}OCH₃), 129.8 (*ortho* C_{Ar}), 128.9 (C_{Ar}CH₂), 114.2 (*meta* C_{Ar}), 56.4 (ArCH₂CH), 55.8 (ArOCH₃), 51.9 (CO₂CH₃), 36.8 (ArCH₂CH), 23.3 (NHCOCH₃).

⁸ K. Noureddine, N. Raquel, S. Belen, A. Eleuterio, F. Inmaculada, *J. Am. Chem. Soc.*, **2008**, *130*, 3697-3700.

⁹ L. Navarre, R. Martinez, J-P. Genet, S. Darses, *J. Am. Chem. Soc.*, **2008**, *130*, 6159-6169.

¹H NMR (400 MHz, D₂O), δ (ppm): 7.17 (d, *J* = 8.3 Hz, 2H, *ortho* ArH), 6.86 (d, *J* = 8.3 Hz, 2H, *meta* ArH), 4.61 (pseudo-dt, *J* = 8.6, 5.7, 5.7 Hz, 1H, ArCH₂CH), 3.15 (dd, *J* = 14.2, 5.8 Hz, 1H, ArCH₂CH), 2.96 (dd, *J* = 14.2, 5.9 Hz, 1H, ArCH₂CH), 1.96 (s, 3H, NHCOCH₃).

¹³C NMR (100 MHz, D₂O), δ (ppm): 175.2 (¹³C=O), 172.5 (NHCO), 154.4 (C_{Ar}¹⁸OH), 130.5 (*ortho* C_{Ar}), 128.5 (C_{Ar}CH₂), 115.4 (*meta* C_{Ar}), 54.3 (d, *J* = 58.8 Hz, ArCH₂CH), 35.8 (ArCH₂CH), 21.5 (NHCOCH₃).¹⁰

IR (thin film on NaCl pellets) ν (cm⁻¹): 3354, 2890, 1705, 1602, 1556, 1498, 1412, 1206, 800.

[α]_D²⁰ = + 44 ° (*c* = 0.5, MeOH; lit.¹¹ + 44.9 °, *c* = 0.5, MeOH).

(1-¹³C)-(4-phenyl-¹⁸O)-L-Tyrosine hydrochloride (17)

A solution of the labelled *N*-acetyl-L-tyrosine (148 mg, 0.66 mmol) in 6N aqueous HCl (15 mL) was heated at 110°C during 24 h. The reaction was then directly evaporated, furnishing quantitatively the labelled L-tyrosine hydrochloride **17** as a white powder (145 mg, ee>99%). The ee was measured by derivatization with OPTA-NAC¹² and HPLC-UV chromatography (Figure S2): Agilent Extend C18 column, 5 μm, 4.6 x 250 mm; binary A/B solvent system (A = percent of AcONa at 50 mM and pH= 5.6; B= percent of MeOH mixed with AcONa 50 mM at pH= 5.6); 60 min program (0-10 min [isocratic 30/70], 10-60 min [gradient up to 70/30]; flow rate at 1 mL/min; Gilson, UV detector-119 at 360 nm.

¹H NMR (400 MHz, D₂O), δ (ppm): 7.22 (d, *J* = 8.5 Hz, 2H, *ortho* ArH), 6.92 (d, *J* = 8.6 Hz, 2H, *meta* ArH), 4.08 (pseudo-dt, *J* = 7.6, 5.3, 5.3 Hz, 1H, CH₂CH), 3.25 (dd, *J* = 14.7, 7.6 Hz, 1H, CH₂CH), 3.11 (dd, *J* = 14.8, 7.6 Hz, 1H, CH₂CH).

¹³C NMR (100 MHz, D₂O), δ (ppm): 172.4 (¹³C=O), 155.0 (C_{Ar}¹⁸OH), 130.8 (*ortho* C_{Ar}), 126.1 (C_{Ar}CH₂), 115.9 (*meta* C_{Ar}), 54.9 (d, *J* = 58.1 Hz, ArCH₂CH), 35.0 (ArCH₂CH).¹³

IR (KBr) ν (cm⁻¹): 3205, 2930, 1728, 1608, 1560, 1515, 1361, 1304, 825.

HRMS (ESI+), *m/z* calcd. for ¹³CC₈H₁₁NO₂¹⁸O [M+H]⁺: 185.0888; found: 185.0886.

[α]_D²⁰ = - 8 ° (*c* = 0.1, 1N HCl; lit.¹⁴ [- 8.4 °], HCl 20 %).

m.p.: 216 °C (lit.¹⁵ 215 °C).

¹⁰ Isotope shifts were observed, to be compared with unlabelled compound: ¹³C NMR (100 MHz, D₂O), δ (ppm): 175.0 (¹³C=O), 173.9 (NHCO), 154.4 (C_{Ar}OH), 130.5 (*ortho* C_{Ar}), 128.3 (C_{Ar}CH₂), 115.4 (*meta* C_{Ar}), 54.3 (ArCH₂CH), 35.7 (ArCH₂CH), 21.5 (NHCOCH₃).

¹¹ S. Selvera, C. Anju, *Tetrahedron: Asymmetry*, **2010**, *21*, 457-460.

¹² N. Nimura, T. Kinoshita, *J. Chromato.*, **1986**, *352*, 169-177.

¹³ Isotope shifts were observed, to be compared with unlabelled compound: ¹³C NMR (100 MHz, D₂O), δ (ppm): 174.9 (C=O), 156.2 (C_{Ar}OH), ¹³ 130.2 (*ortho* C_{Ar}), 129.5 (C_{Ar}CH₂), 115.8 (*meta* C_{Ar}), 56.7 (ArCH₂CH), 37.4 (ArCH₂CH).

¹⁴ L. Gunter, *Journal für praktische Chemie*, **1958**, *7*, 141.

¹⁵ Y. Liwshitz, A. Izikha, I. Shahak, *J. Org. Chem.*, **1956**, *21*, 1530.

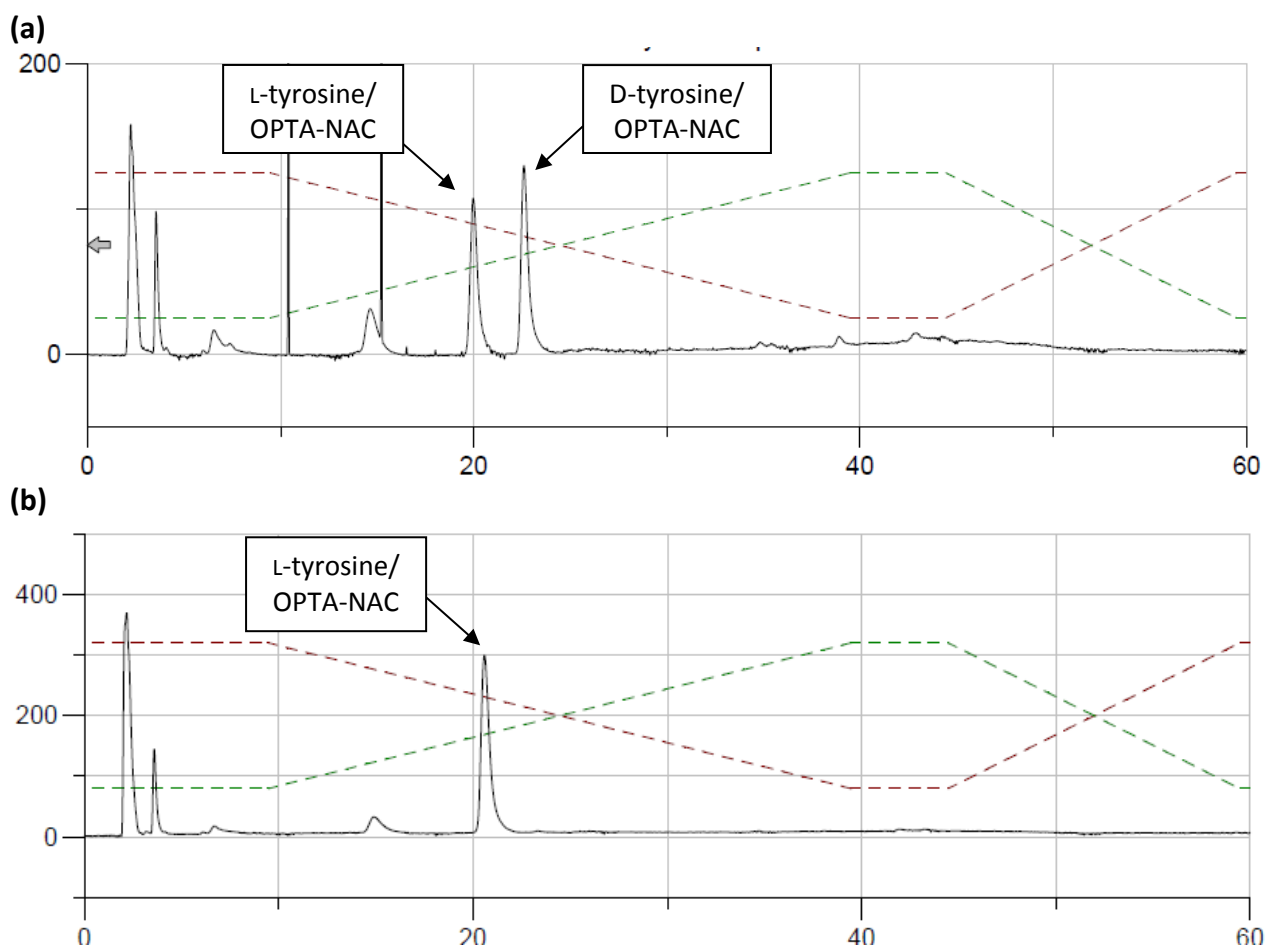


Figure S2: HPLC-UV chromatogram (detection at 360 nm) of (a) an equimolar mixture of APTA-NAC-derivatized L-tyrosine and D-tyrosine [RT (L-tyrosine) = 20.56 min; RT (D-tyrosine) = 23.16 min] (see text for conditions) and (b) OPTA-NAC-derivatized synthetic L-tyrosine (**17**) showing the enantiomeric purity of this compound.

Supplementation of culture broths of *Acremonium zeae* NRRL 13540 with labelled L-tyrosine and metabolic analyses

A preculture of *A. zeae* NRRL 13540 was grown on slants of PDA at 25°C during 7 days. A mixture of hyphal fragments and spores at a density of 10^6 propagules/mL in sterile filtrated glycerol (20 % in water) was prepared from the slants of PDA and served as the inoculum. For a 20 mL culture of CLGB (prepared from an autoclaved then filtered mixture of corn leaves 20 g/L and glucose 20 g/L) in a 50 mL Erlenmeyer, 0.2 mL of the inoculum was added. At 48, 72 and 96 h of culture, 20 mg of the doubly labelled L-tyrosine **17** was supplemented each time into the medium under sterile conditions.

After 1 week of culture, the medium was centrifuged at 4000 rpm and 4°C during 40 min, before separation of the mycelium and the medium in two Erlenmeyers. The mycelium was suspended in AcOEt (25 mL) and sonicated during 30 min. Brine (30 mL) was added into the mixture for extraction. The procedure from the suspension in AcOEt was repeated once. The organic extracts were drought over $MgSO_4$ and the solvent was evaporated to give the crude fungal extract (2 mg) which was analyzed. The supplementation experiment was performed twice, with a 3-month interval.

Detection of pyrrocidines in the mycelium extracts were performed using a coupled UHPLC-MS instrumentation (UHPLC Nexera and MS2020 from Shimadzu) with an atmospheric pressure chemical ionization (APCI source; ESI was ineffective for ionization of pyrrocidines). Separation was performed on a Kinetex column 1.7 μ , C18 (100 Å), 100 x 2.1 mm, from Phenomenex. A 12 min program was used with binary A/B solvent system (solvent A: acetonitrile containing 0.1 % of formic acid; solvent B: deionized water containing 0.1 % of formic acid): 0-1 min [isocratic 30/70], 1-6 min [gradient up to 100/0], 6-12 min [isocratic 100/0], with a flow rate of 0.5 mL/min. The optimum MS parameters for detection of pyrrocidines were set with a Q-array voltage of +52 V, and both DL and Q-array DC voltages at 0 V.

To explain the isotope distribution in pyrrocidines A and B, calculation were performed with the open source mass spectrometry tool mMass (<http://www.mmass.org/>). The spectra for each isotopomer of pyrrocidines A and B were calculated, taking into account their isotope enrichments and relative proportions (pyrrocidine A is at 71% of pyrrocidine B while peaks at m/z 488 and 493 were adjusted with reference to experimental data). They were combined to get the full spectrum of Figure S3, demonstrating the origin of the intensity of the peak at m/z 489. Experimental and calculated peak intensities are provided in Table S1.

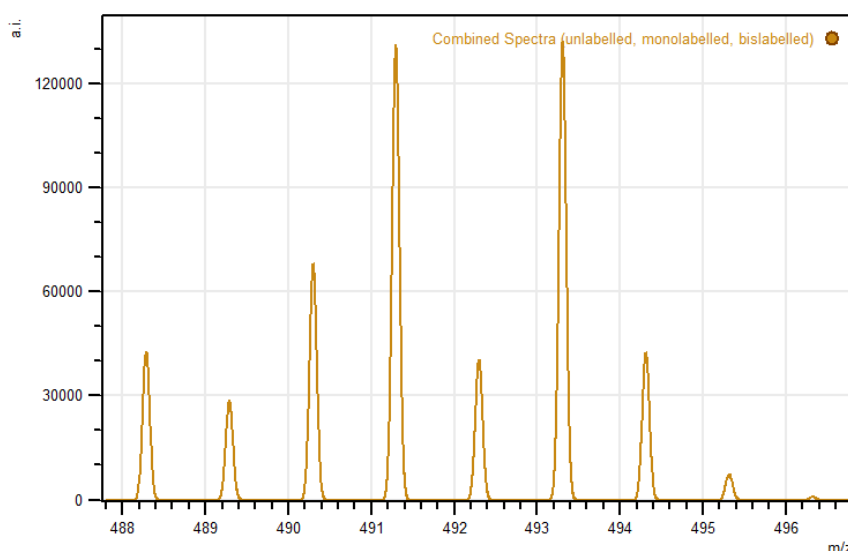


Figure S3: Calculated isotopic distribution of pyrrocidines A and B.

Table S1: Values of peaks intensities in experimental and calculated spectra.

m/z	Experimental intensities	Calculated intensities
488	43340	43340 ^a
489	26772	28700
490	76014	68900
491	100057	131000
492	44403	41100
493	132703	132703 ^a
494	56057	42700
495	15188	7700

^a Fixed as experimental (considered unaffected by the isotope pattern of neighbour peaks).

Isolation of pyrrocidines B (4) and C (5)

A CLGB culture medium (1 L) was inoculated with *A. zeae* (10 mL of inoculum) as previously described. After 14 days of culture at 25 °C, the medium was centrifuged at 4000 rpm and 4°C during 40 min, before separation of the mycelium and the medium in two Erlenmeyers. The mycelium was suspended in AcOEt (250 mL) and sonicated during 30 min. Brine (200 mL) was added into the mixture for extraction. The procedure from the mycelium suspension in AcOEt was repeated once. The organic extracts were drougth over MgSO₄ and the solvent was evaporated to give the fungal extract. The AcOEt extract (1.3 g) was obtained after evaporation of volatiles. It was degreased with cyclohexane (3 x 100 mL), providing 577 mg of the crude extract.

Pyrrocidine B (4) and C (5) were purified by semi-preparative HPLC (Agilent Technologies 1260 Infinity) coupled to Diode Array Detector, using the following gradient on a C18 Eclipse XDB column from Agilent (21.2 x 150 mm, 5 µm). Mobile phases were solvent A: 95% water (0.05% trifluoroacetic acid)–5% acetonitrile; and solvent B: 5% water (0.05% trifluoroacetic acid)–95% acetonitrile. The separation was achieved at a flow rate of 15 mL/min with the following gradient (A/B): 0-2 min [isocratic 30/70], 2-10 min [gradient up to 3/97], 10-14 min [gradient up to 0/100], 14-17 min [isocratic 0/100], 17-18 min [back to 30/70]. From 577 mg of the crude extract diluted in MeOH (2 injections / volume injected: 200 µL), a mixture of compounds 3 and 4 and pure compound 5 were obtained respectively at retention times 10.2 (7.9 mg, ratio 3/4: 60:40), 10.8 (2.2 mg) minutes. Pyrrocidine C (5) was stable upon storage and could be crystallized from acetonitrile (see below). Apart from crystallography which is described therein for the first time, all data of 5 were consistent with those of the literature.¹⁶ The mixture of pyrrocidines 3 and 4 spontaneously evolved as a mixture of 4 and 5 upon storage in the freezer at –30°C (see note 20 in the article). This new mixture could be separated by the same method as before, providing pyrrocidine B (4) (2.3 mg) whose data were consistent with those of the literature¹⁷ and an additional batch of pyrrocidine C (5) (3 mg).

¹⁶ Y. Shiono, A. Kosukegawa, T. Koseki, T. Murayama, E. Kwon, S. Uesugi, K.-i. Kimura, *Phytochem. Lett.* **2012**, *5*, 91–95.

¹⁷ H. He, H. Y. Yang, R. Bigelis, E. H. Solum, M. Greenstein, G. Carter, *Tetrahedron Lett.* **2002**, *43*, 1633–1636.

Crystallographic data for pyrrocidine C (5)

Figure S4 displays the X-ray crystal structure of pyrrocidine C (5). Single crystals suitable for X-ray diffraction were obtained by recrystallization from CH₃CN. The data analysis showed it to be monoclinic crystals with space group C2.

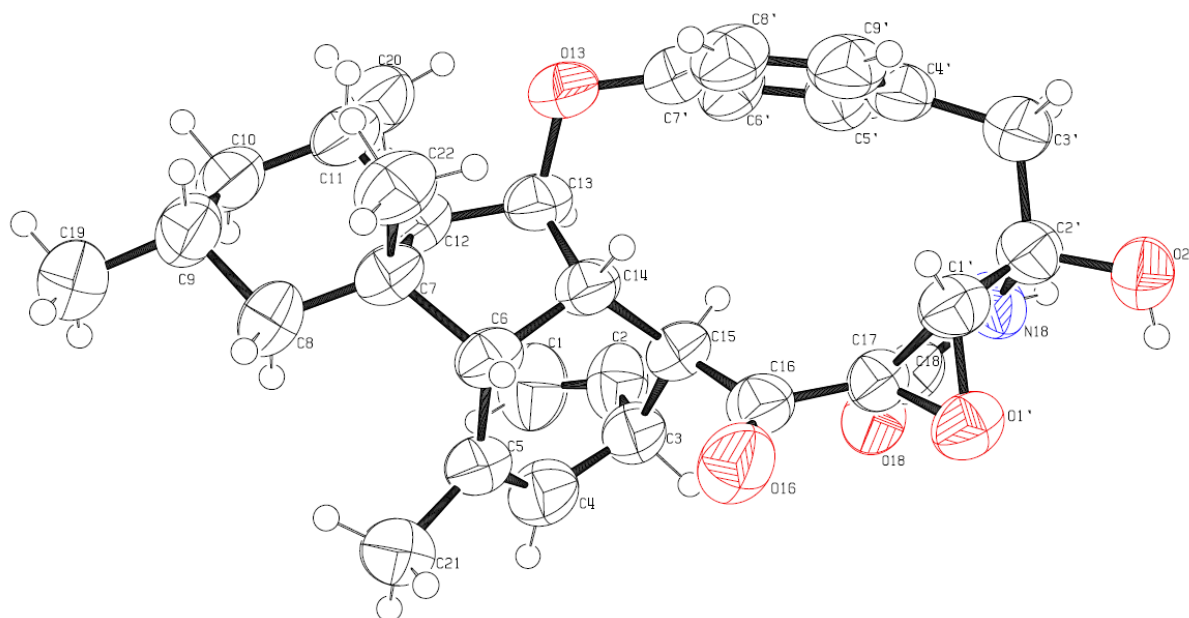


Figure S4: ORTEP-3 plot of pyrrocidine C. Ellipsoids are drawn at the 50% probability level and H atoms are shown as spheres of arbitrary radius.

The absolute configuration was analysed by using Hooft methods,^{18,19} and the results indicated that absolute structure had been correctly assigned. According to these methods, the probability that the structure is inverted is 0.5×10^{-8} (Flack parameter is -0.14(16), 3279 Bijvoet pairs, Bijvoet coverage = 0.65. P2(true) = 1.00. P3(true) = 0.987. P3(rac-twin) = 0.003. P3(false) = 0.5×10^{-8} . Correlation coefficient = 0.995).

X-ray data were collected with a Rigaku Rapid II diffractometer equipped with a rotating anode mm007 HF generator and Osmic mirrors (Cu K α radiation, $\lambda = 1.54187 \text{ \AA}$) using ω -scans. The structure was solved by direct methods with SHELXS-97, and refined with SHELXL-2014/6 (Table S2).²⁰ The model was refined using full-matrix least-squares, all non-hydrogen atoms, except those from solvent molecules, were refined with anisotropic displacement parameters and H atoms have been added geometrically and treated as riding on their parent atoms. CH₃CN solvent molecule was divided into two parts due to large anisotropic displacement parameters for C24 and N1 atoms, using PART, DFIX and EADP commands. Molecular graphics were computed with Ortep 3.

¹⁸ R. W. W. Hooft, L. H. Straver, A. L. Spek, *J. Appl. Crystallogr.* **2008**, *41*, 96.

¹⁹ R. W. W. Hooft, L. H. Straver, A. L. Spek, *J. Appl. Crystallogr.* **2010**, *43*, 665.

²⁰ G. M. Sheldrick, *Acta Crystallogr. A* **2008**, *64*, 112.

CCDC 1031563 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

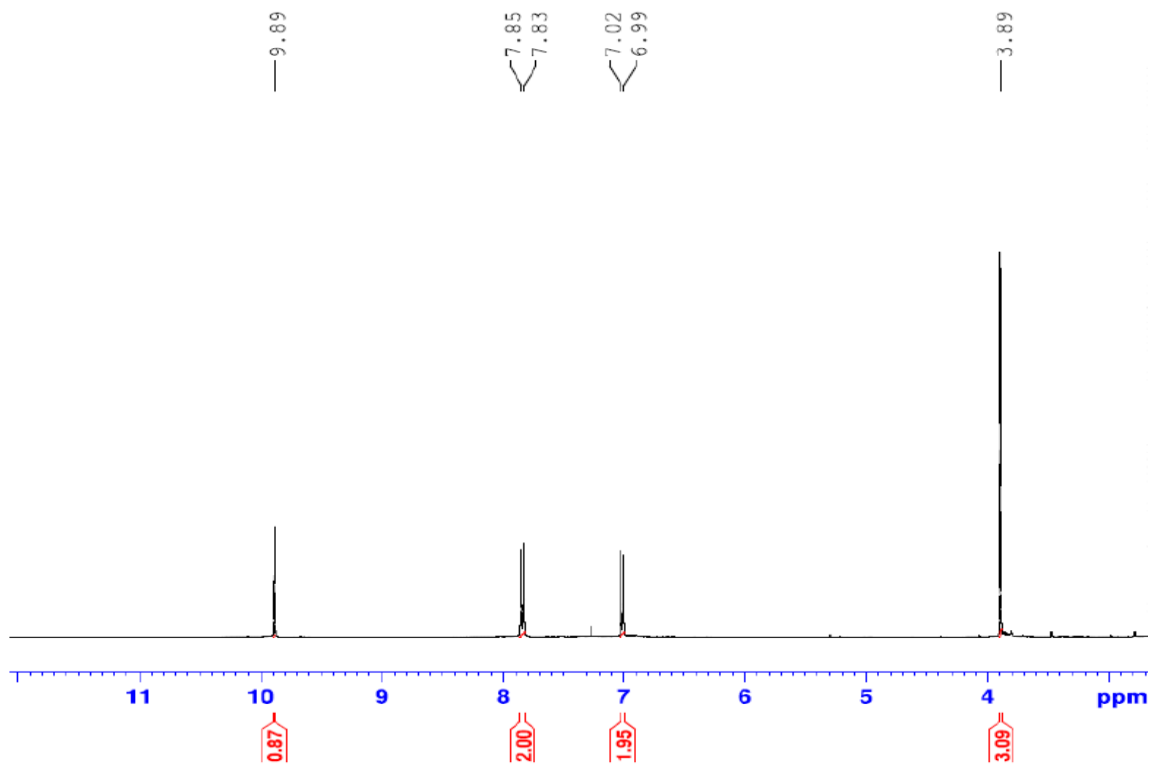
Table S2: Crystal data and structure refinement for pyrrocidine C (compound 5)

Compound name	Pyrrocidine C
Formula	C ₆₄ H ₈₁ N ₃ O ₁₂
Formula weight	1084.31
Temperature/K	293
Crystal color	Colorless
Crystal size/mm	0.22 x 0.16 x 0.11
Crystal system	Monoclinic
Space group	C2
a/Å	34.802(2)
b/Å	8.7586(5)
c/Å	22.5029(16)
β/°	120.418(9)
Volume/Å ³	5915.2(8)
Z, ρ _{calculated} /g.cm ⁻³	4, 1.218
μ/mm ⁻¹	0.674
Θ range/°	2.277 – 68.245
Limiting indices	-41 ≤ h ≤ 36 -7 ≤ k ≤ 10 -27 ≤ l ≤ 27
Collected reflections	26382
Unique reflections	9069 [R(int) = 0.0894]
Parameters	711
Goodness-of-fit on F ²	1.026
Final R indices [I > 2σ(I)]	R1 = 0.0658 wR2 = 0.1511
R indices (all data)	R1 = 0.0927 wR2 = 0.1897
Largest diff. peak and hole/e.Å ⁻³	0.292 and -0.279

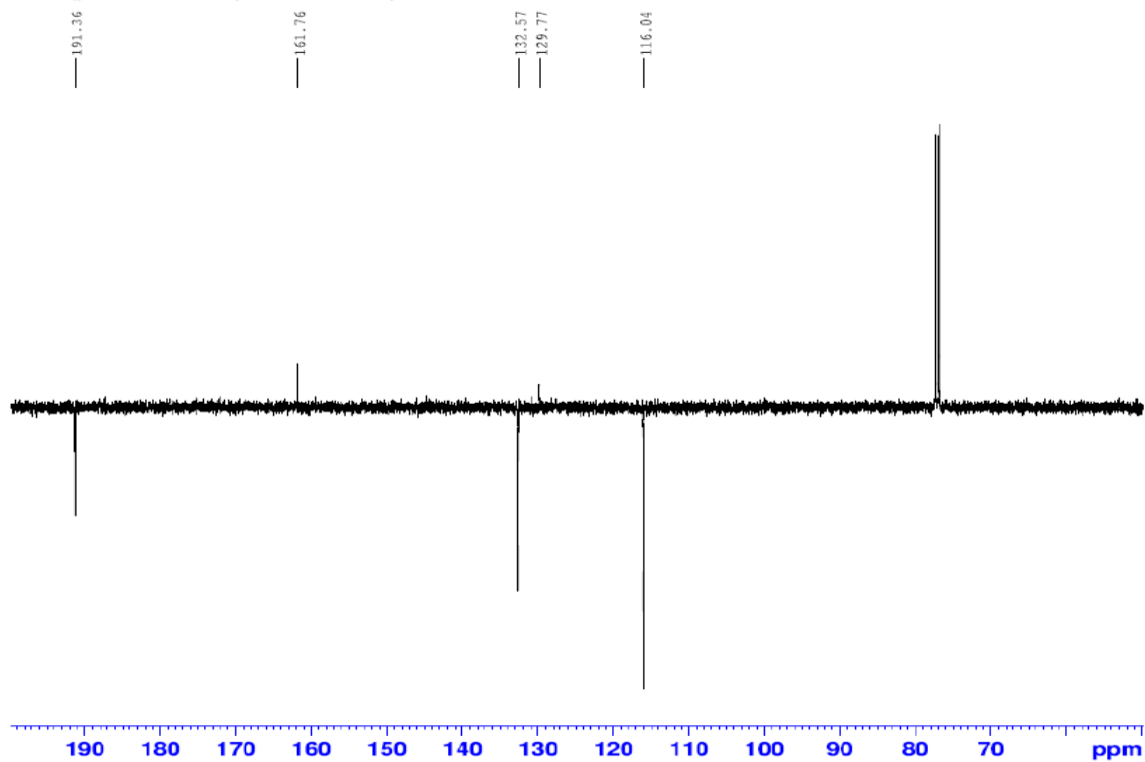
Copies of proton and carbon NMR spectra

4-[¹⁸O]Hydroxybenzaldehyde (10)

AE256-CDCL3
1H-400MHZ
PROTON CDCL3 {X:\AV400HD} CBAF 11

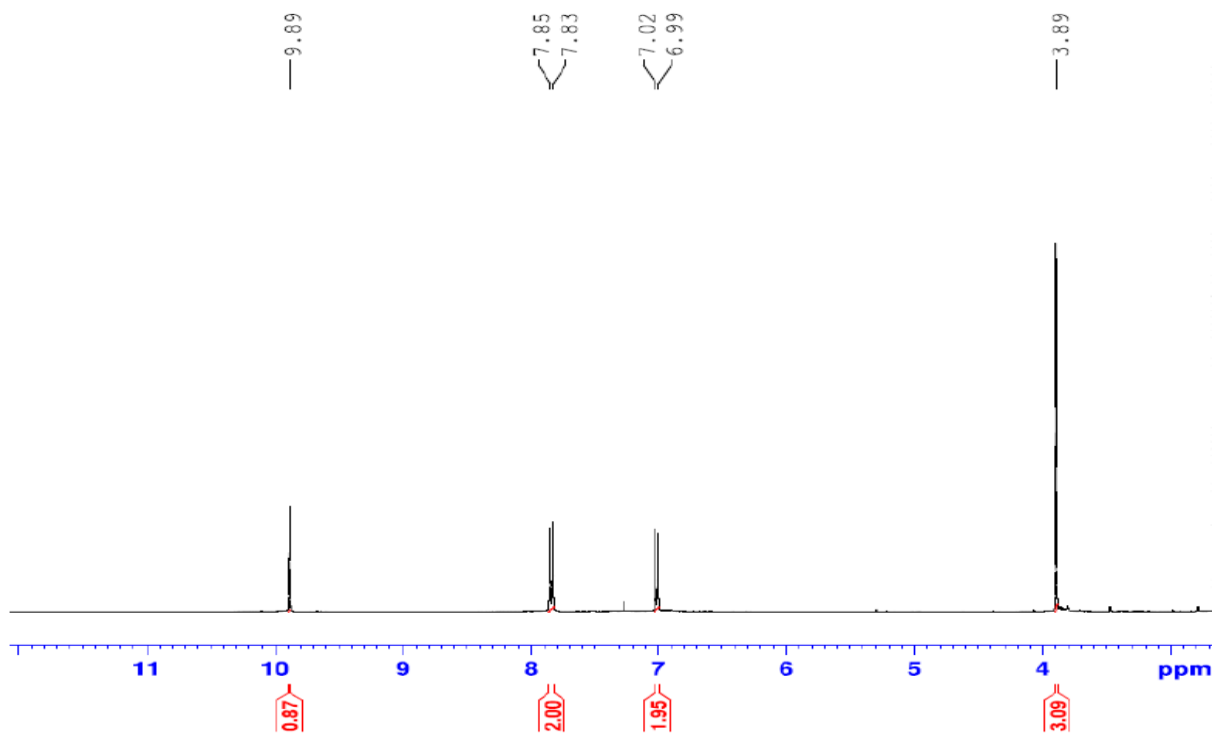


AE255-CDCL3
13C-400MHZ
DEPTQall CDCL3 {X:\AV400HD} CBAF 10

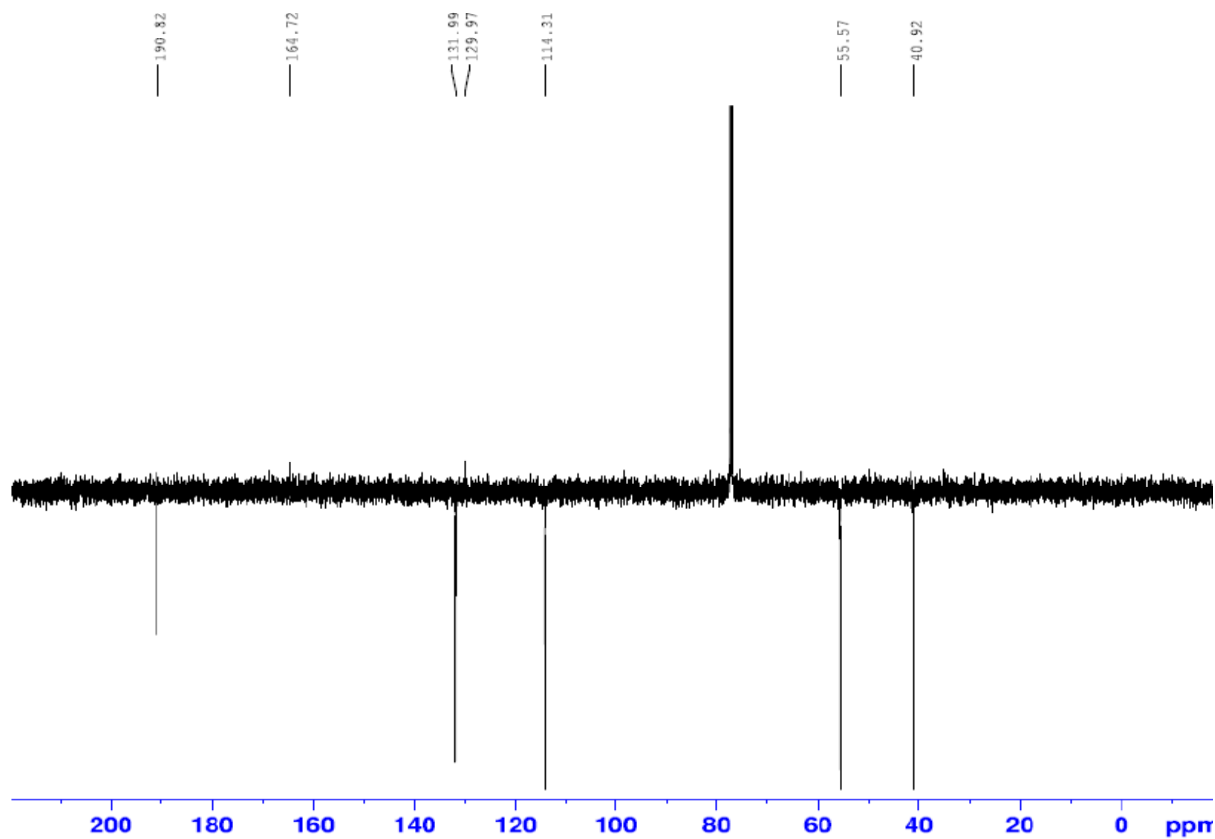


4-([¹⁸O]Methoxy)benzaldehyde (11)

AE256-CDCL3
1H-400MHZ
PROTON CDC13 {X:\AV400HD} CBAF 11



AE256-CDCL3
13C-100MHZ
DEPTQall CDC13 {X:\AV400HD} CBAF 2

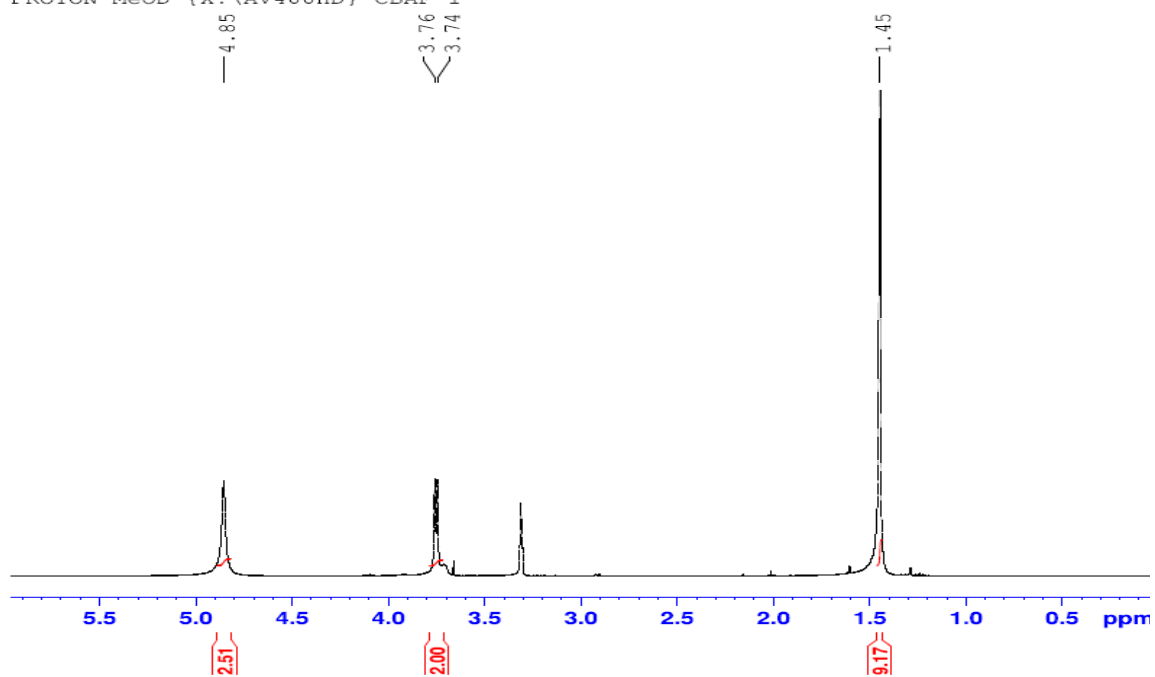


2-((*Tert*-butoxycarbonyl)amino)-1-[¹³C]acetic acid (12)

AE249-MEOD

400MHZ-1H

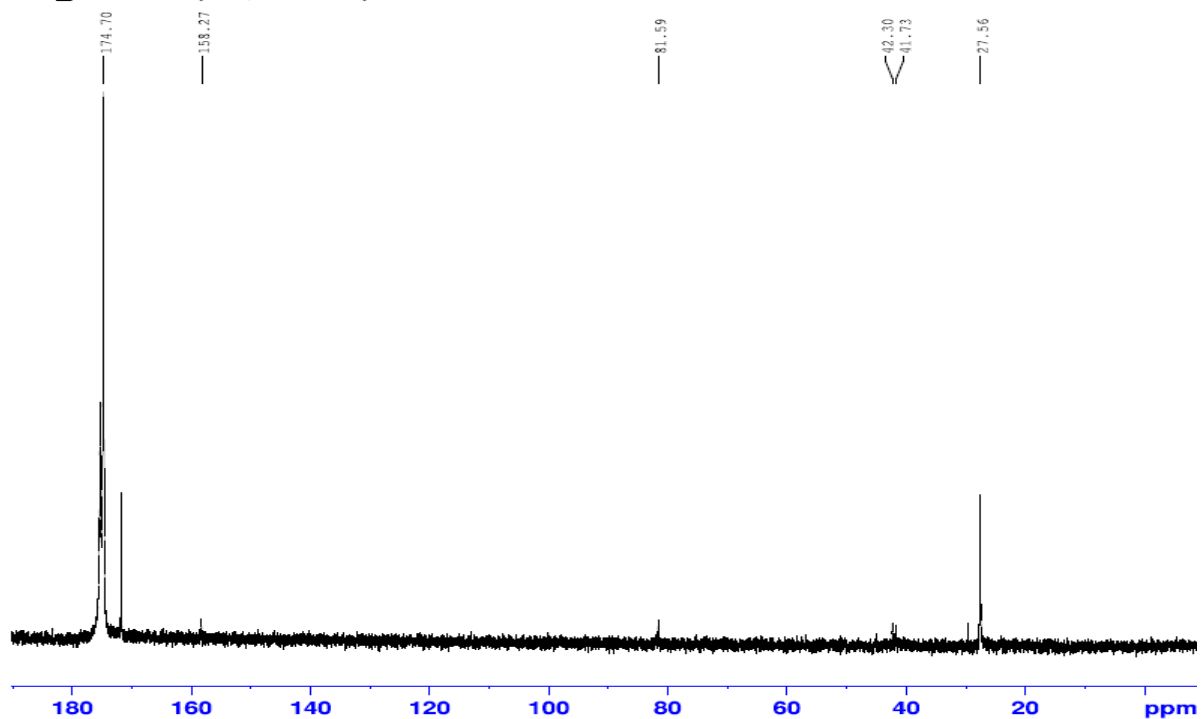
PROTON MeOD {X:\AV400HD} CBAF 1



AE249-D2O

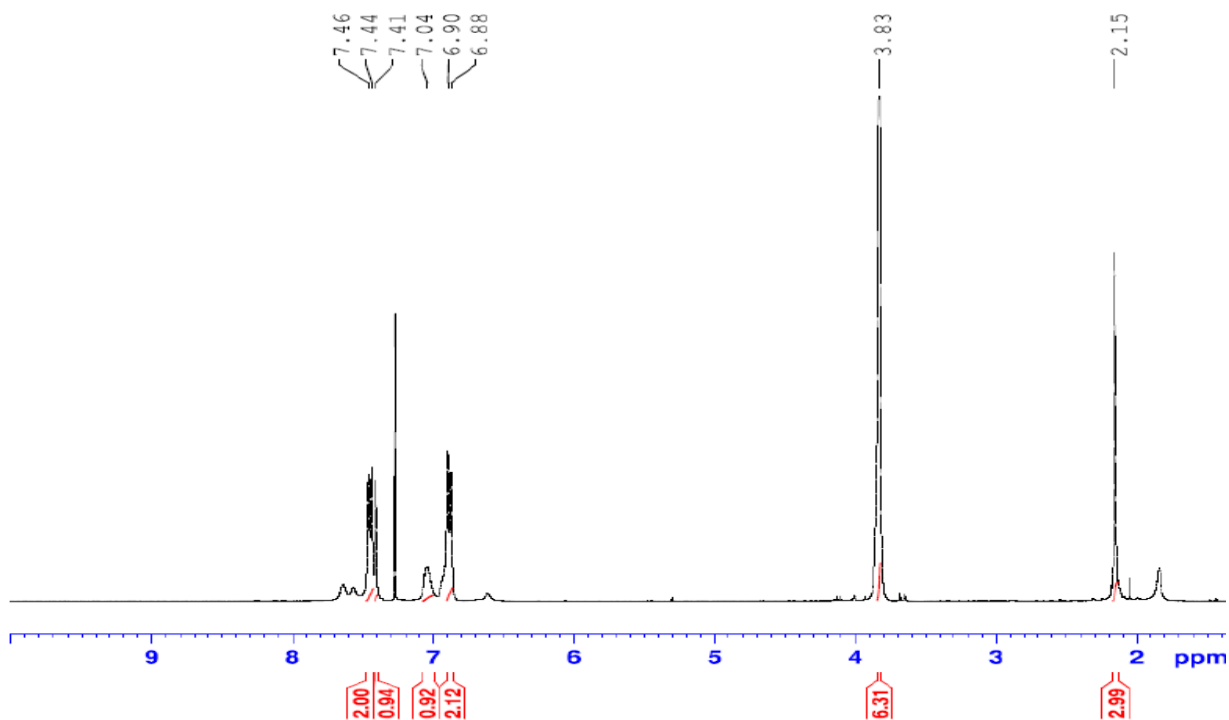
13C-100MHZ

C13_CPD D2O {X:\AV400HD} CBAF 1

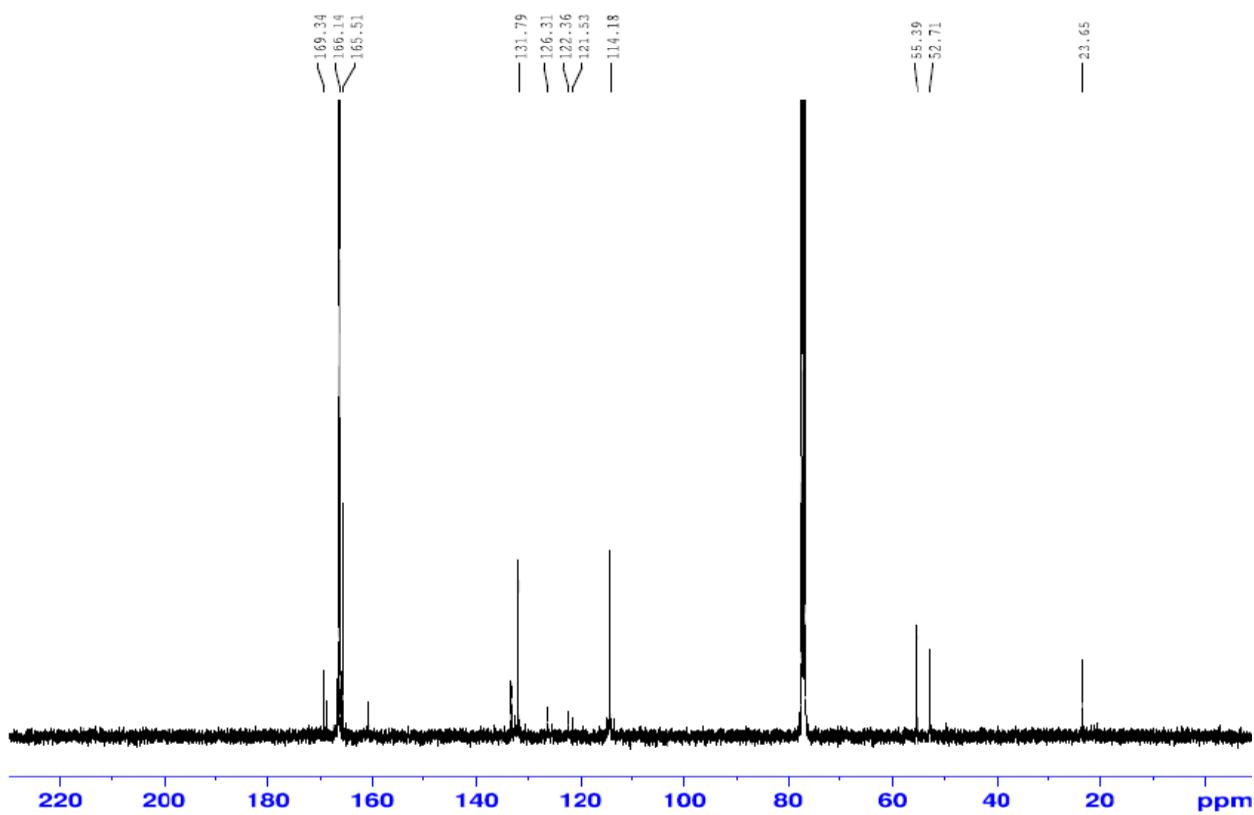


Methyl (Z)-2-acetamido-3-(4-[¹⁸O]methoxyphenyl)-1-[¹³C]acrylate (13)

AE241-CDCL3
400MHZ-1H
PROTON CDC13 {X:\AV400HD} CBAF 13

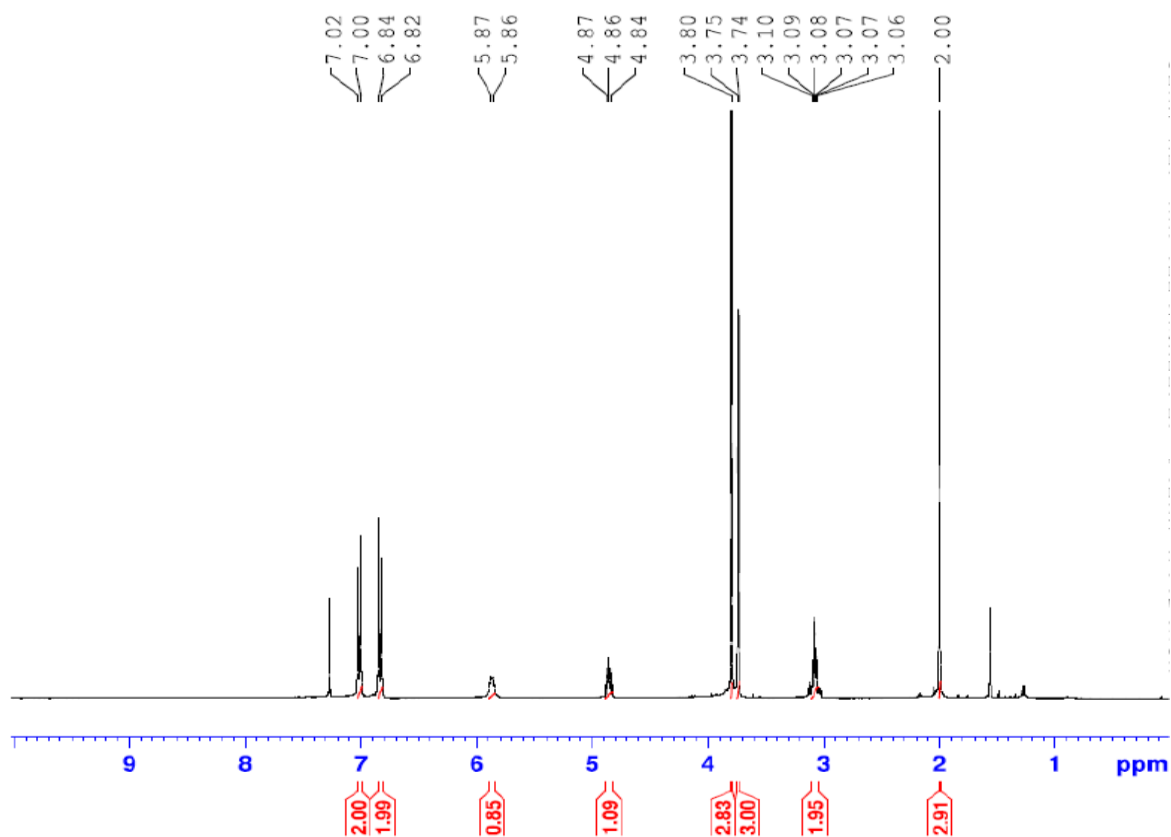


AE258-CDCL3
13C-100MHZ
C13_CPD CDC13 {X:\AV400HD} CBAF 1

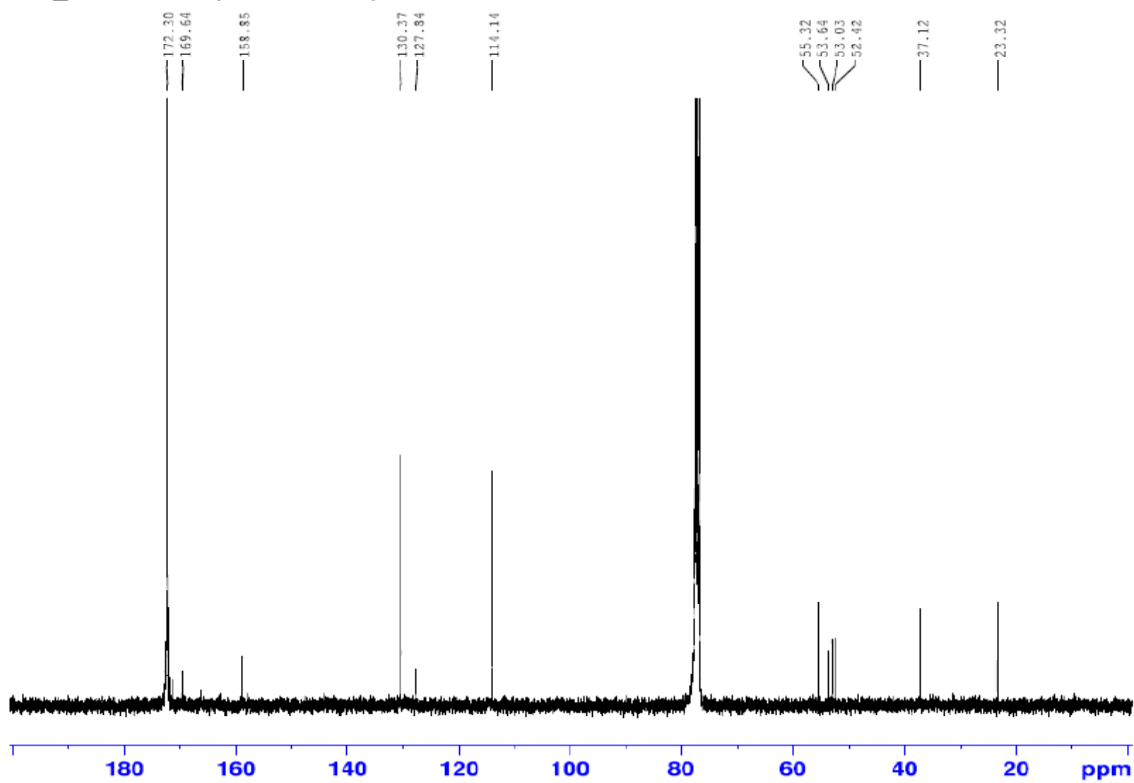


Methyl (2S)-2-acetamido-3-(4-[¹⁸O]methoxyphenyl)-1-[¹³C]propanoate (15)

AE268-CDCl3
1H-400MHZ
PROTON D2O {X:\AV400HD} CBAF 12



AE268-CDCl3
13C-100MHZ
C13_CPD CDCl3 {X:\AV400HD} CBAF 5

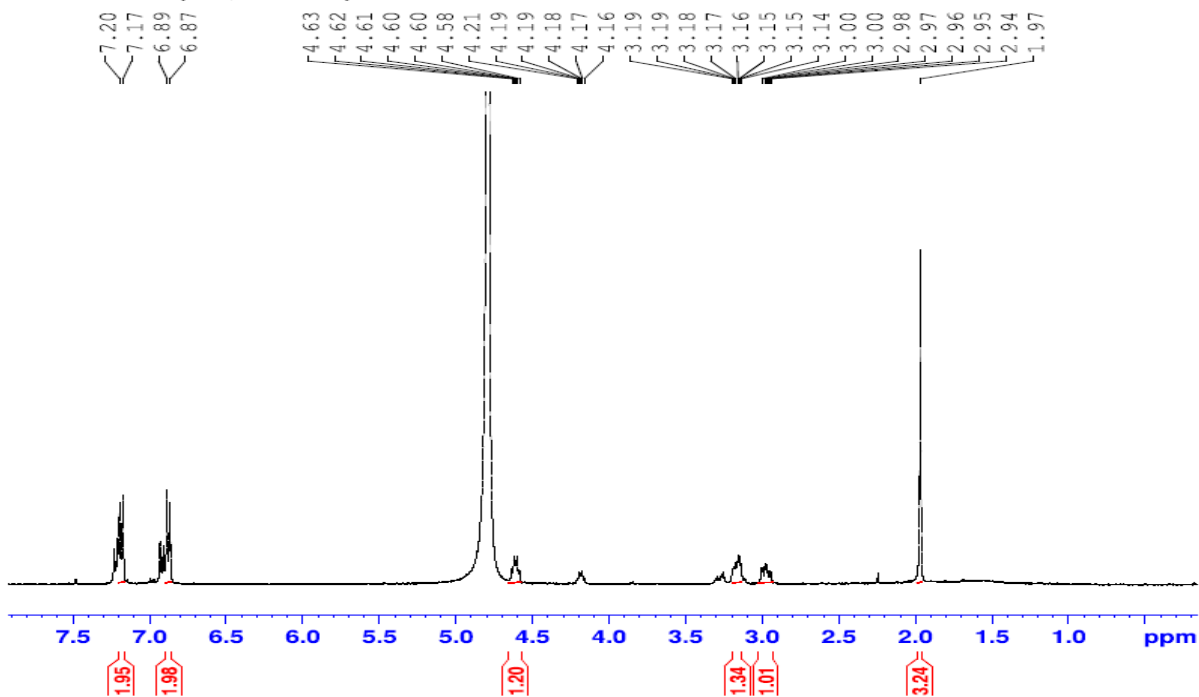


(N-acetyl)-1-[¹³C]-4-phenyl-[¹⁸O]-L-tyrosine

AE271B-D2O

1H-400MHZ

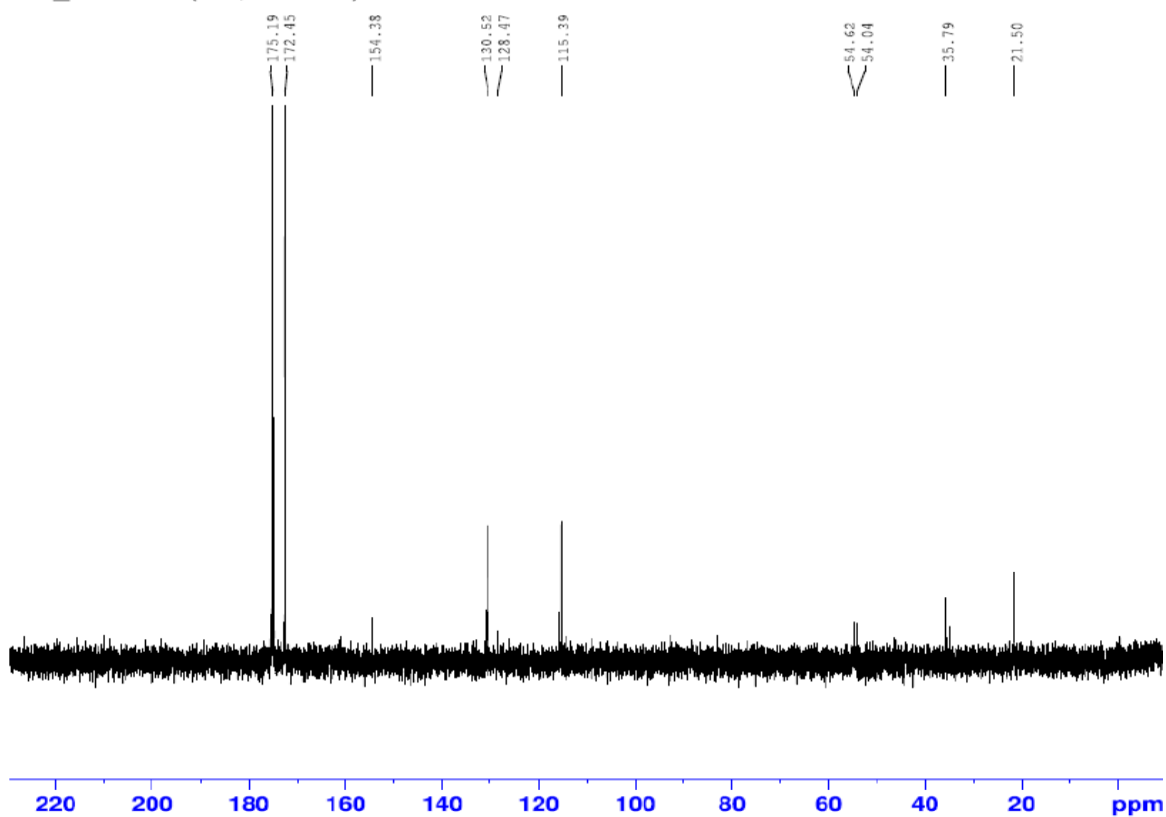
PROTON D2O {X:\AV400HD} CBAF 6



AE271-D2O

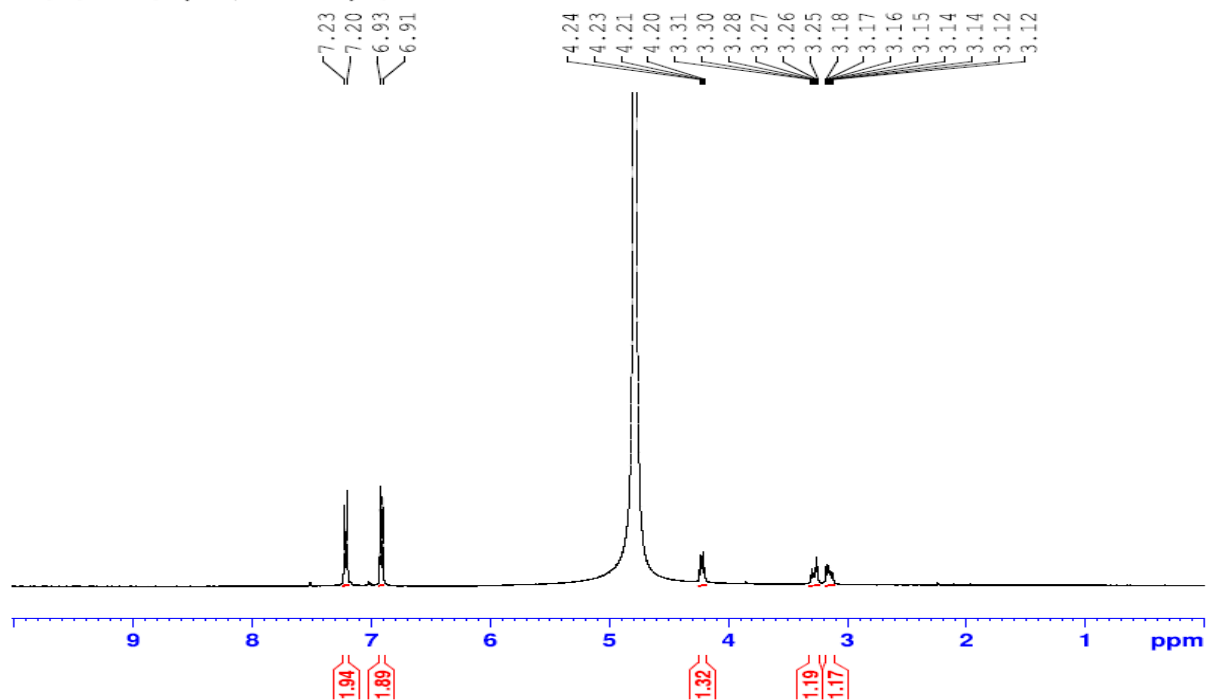
13C-100MHZ

C13_CPD D2O {X:\AV400HD} CBAF 10



L-Tyrosine hydrochloride, 1-[¹³C]-4-phenyl-[¹⁸O] (17)

AE275D-D2O
1H-400MHZ
PROTON D2O {X:\AV400HD} CBAF 1



AE275D-D2O
13C-100MHZ
C13_CPD D2O {X:\AV400HD} CBAF 2

