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Supporting Information

On the Antibacterial Activity of Azacarboxylate Ligands: Lowered Metal Ion Affinities for Bis-amide Derivatives of EDTA do not mean Reduced Activity

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Experimental Procedures

Synthesis: general remarks

Materials. Reagents were obtained from commercial sources and used without further purification unless stated otherwise. Solvent extractions were performed in a 100 cm^3 separating funnel with ca. 50 cm^3 for each phase, unless stated otherwise. For procedures involving dry solvent, glassware was oven-dried for at least 8 hours prior to use. Dedicated oxygen-free nitrogen or argon cylinders (BOC, UK) were used to provide an inert atmosphere.

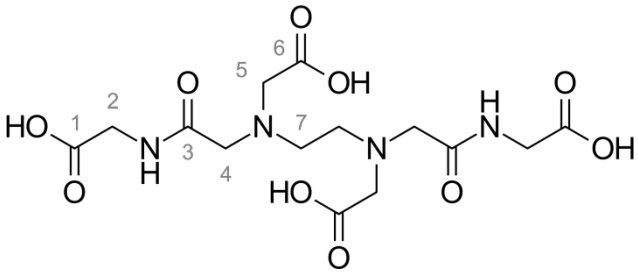
Instrumentation. NMR: Routine ^1H (400 MHz) and ^{13}C NMR (101 MHz) spectra were acquired on Bruker Avance 400 or Varian Mercury 400 NMR spectrometers. Two-dimensional NMR (COSY, HSQC and HMBC), and certain ^1H / ^{13}C NMR spectra, including the ^1H DOSY experiments, were acquired by the solution state NMR service at Durham University on Varian VNMRS-600 (600 MHz) or VNMRS-700 (700 MHz) instruments. Where visible, signals corresponding to $CDCl_3$ were referenced to δ =7.26 ppm in ^1H NMR spectra and δ =77.2 ppm for ^{13}C spectra. For the NMR spectra of **$AmPy_2$**, an appropriate compound was added to the sample to facilitate referencing. Combustion analyses were performed by Stephen Boyer (London Metropolitan University).

Mass spectrometry: ES-MS data (positive and negative ionisation modes) were obtained on a Waters TQD mass spectrometer interfaced with an Acquity UPLC system. GC-MS (EI ionisation) was performed on an Agilent instrument equipped with a 5973 model quadrupole mass spectrometer.

FT-IR*:* All infra-red spectra were recorded on a Perkin-Elmer Spectrum 90 spectrometer equipped with an ATR stage. Substances for analysis were used neat unless indicated otherwise.

Chromatography: TLC (thin layer chromatography) was performed on silica using Merck foil-backed TLC plates. Column chromatography employed a standard glass column with silica gel (Fluorochem). Elution was performed according to the method of Clark Still.[1] Analytical HPLC was performed by the chromatography service at Durham University.

Bis(carboxymethyl)-3,10-dioxo-2,5,8,11-tetraaza-dodecane-1,12-dicarboxylic acid, $AmGly_2$



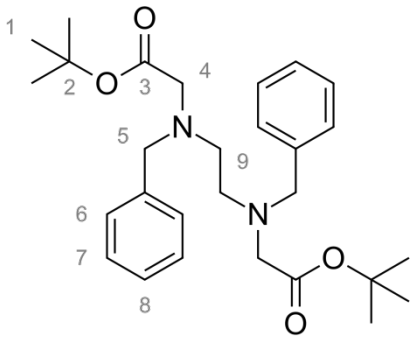
EDTA bis-anhydride[1] (1.50 g, 5.85 mmol), and glycine (1.78 g, 23.7 mmol) were stirred under nitrogen in dry dimethylformamide for 6 hours, initially in an ice bath and then at room temperature. Following solvent removal *in vacuo*, an aqueous solution of hydrochloric acid (7.6% w/w, ca. 20 cm^3) was added to the crude solid. The resulting suspension was boiled, and left to cool overnight to afford a fine white precipitate that was filtered, and dried to constant mass (0.94 g, 2.32 mmol, 40%). Recrystallisation of 0.22g of this material from water afforded a white crystalline solid (0.13 g). Further recrystallisations from water afforded crystals suitable for structural analysis by X-ray crystallography. Elem. Anal. Found: C, 41.43; H, 5.38; N, 13.82;($C_{14}H_{22}N_4O_{10}$ requires C, 41.38; H, 5.46; N, 13.79%); mp 221-225^{o}C (dec.); ν_{max}/cm^{-1} (ATR) 3020 (COOH O-H st), 1730 (carboxylic acid C=O st), 1666 (amide C=O st), 1304 (acid C-O st), 1108 (br, ethylene diamine C-N st); δH (600 MHz, D_2O, pD ≈ 14) 3.79 (s, 4H, ***H2***), 3.31 (s, 4H, ***H4***), 3.22 (s, 4H, ***H5***), 2.72 (s, 4H, ***H7***); δC (151 MHz, D_2O pD ≈ 14) 179 (***C1***) , 177 (***C6***) , 174 (***C3***) , 59 (***C5***) , 58 (***C4***), 52 (***C7***) , 43 (***C2***); m/z (ES-MS^+) 407 (100%, $[M+H]^+$, M= $C_{14}H_{22}N_4O_{10}$); X-ray crystallography: $C_{14}H_{22}N_4O_{10}$, M_r = 406.36, orthorhombic ($P2_12_12$); a = 17.3349(11) Å, b = 9.3065(5) Å, c = 5.3951(4) Å; crystal size = 0.3599 × 0.1611 × 0.0820 mm^3 ; T= 120K.

Preparation of $Mg(AmGly_2)$:

Crystals of **$Mg(AmGly_2)$** ($[Mg(H_{-4}AmGly_2)(H_2O)]Na_3(NO_3).7(H_2O)$) were obtained via slow evaporation of a basic solution (pH 11, basified using NaOH) of a 1:1 mixture of **$AmGly_2$** and $Mg(NO_3)_2$ (10 mg of **$AmGly_2$** in 2 cm^3 of de-ionised water).

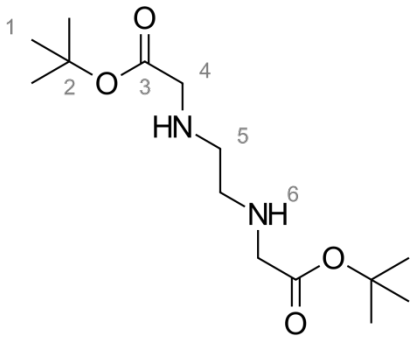
[1] In our experience, the success of this reaction depends on the age of the **EDTA** bis-anhydride used. We recommend preparing the anhydride from **EDTA**, methods for which have been published previously.[2,3]

Tert-Butyl-[(benzyl){2-[(benzyl)(tert-butoxycarbonylmethyl)amino]ethyl}amino]acetate, 1[4]



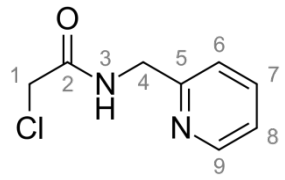
N,N`-dibenzylethylenediamine diacetate (1.25g, 3.47 mmol) was suspended in acetonitrile (20 cm^3 , dried over molecular sieves) in a Schlenk tube equipped with a pressure-equalising funnel. Excess potassium carbonate (2.15g, 15.6 mmol) and sodium sulphate (ca. 0.5g) were then added to the suspension which was stirred at room temperature under nitrogen. t-Butyl bromoacetate (1.07 cm^3 , 7.30 mmol) was subsequently added dropwise to the suspension and addition was complete within 10 minutes. The reaction mixture was then heated to 60^oC and monitored by TLC (silica, ethyl acetate/hexanes 80:20 v/v). Typically, the reaction was complete within 4 hours but could be left overnight with no observable degradation at this temperature. Upon completion, the reaction mixture was cooled to room temperature and filtered through Celite® to give a straw-coloured filtrate which was concentrated *in vacuo* to a viscous oil that was purified by column chromatography (0:100→100:0 v/v ethyl acetate:hexanes, compound eluted in ca. 30:70 v/v ethyl acetate:hexanes). Fractions containing the pure compound were recombined and the solvent removed *in vacuo* to give a colourless oil that crystallised to a white solid upon standing (0.68g, 1.45 mmol, 42%). Slow evaporation of a sample of this solid from wet acetone gave crystals suitable for X-ray crystallography. Similarly, very large crystals may also be grown through recrystallisation of the purified residue in hexanes. mp 62-64^oC; ν_{max} /cm^{-1} (ATR) 2978 (C-H st), 1719 (ester C=O st), 1149 (asymm. C-O st), 1116 (symm. C-O st); δH (700 MHz, $CDCl_3$) 7.66–6.72 (m, 10H, ***H6***, ***H7***, ***H8***), 3.77 (s, 4H, ***H5***), 3.25 (s, 4H, ***H4***), 2.80 (s, 4H, ***H3***), 1.44 (s, 18H, ***H1***); δC (176 MHz, $CDCl_3$) 171 (***C3***) , 139 (***C10***) , 129 (***C7***) , 128 (***C6***) , 127 (***C8***) , 81 (***C2***) , 59 (***C5***) , 55 (***C4***) , 52 (***C9***) , 28 (***C1***); (ES-MS^+) 469 (100%, $[M+H]^+$, M=$C_{28}H_{40}N_2O_4$); X-ray crystallography: $C_{28}H_{40}N_2O_4$, M_r =468.62, tetragonal (P-4); a = 22.2406(6) Å, b = 22.2406(6) Å, c = 5.4929(2) Å; crystal size =0.48 × 0.24 × 0.2 mm^3 ; T= 120K.

Tert-Butyl {2-[(tert-butoxycarbonylmethyl)amino]ethylamino}acetate, 2[4]



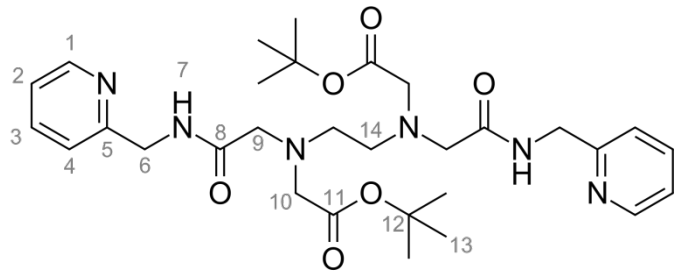
Aminoester **1** (0.63 g, 1.34 mmol) was dissolved in ethanol (ca. 50 cm^3) in a two-necked round bottom flask equipped with a two-way valve secured to the round bottom flask via a Keck clip. The resulting colourless solution was subject to three cycles of vacuum purging and nitrogen filling after which 5 wt. % palladium on charcoal (0.14 g, 0.066 mmol wrt. Pd) was added under a flow of nitrogen. The nitrogen line was then replaced with a balloon containing hydrogen gas, and the reaction mixture was evacuated and backfilled with hydrogen three times and then allowed to stir at room temperature. Reaction progress was monitored by ^{1}H NMR (disappearance of aromatic signals after filtration and evaporation of an aliquot of the reaction mixture) and completion was typically observed within 3 hours. Once complete, the reaction mixture was filtered through Celite® and concentrated *in vacuo*. If necessary, the resulting oil could be purified by column chromatography on silica (isocratic elution in 95:5 v/v methanol:dichloromethane) to afford **2** as a colourless to yellow oil (0.29 g, 1.01 mmol, 74%). If filtration was carried out carefully, column chromatography could be bypassed leading to higher, sometimes quantitative yields. ν_{max} /cm^{-1} (ATR) 3334 (N-H st), 2977 (sp^3 C-H), 1730 (C=O ester), 1148 (C-O); δH (700 MHz, $CDCl_3$) 3.23 (s, 4H, ***H4***), 2.64 (s, 4H, ***H5***), 1.89 (s, 2H, ***H6***), 1.39 (s, 18H, ***H1***); δC (176 MHz, $CDCl_3$) 172 (***C3***) , 81 (***C2***) , 51 (***C4***) , 49 (***C5***) , 28 (***C2***); (ES-MS^+) 289.1 (100%, $[M+H]^+$); HR-MS Found 289.2118 , $C_{14}H_{29}N_2O_4$ [M+H] + requires 289.2127.

2-Chloro-1-{[(2-pyridyl)methyl]amino}-1-ethanone, 3[5]



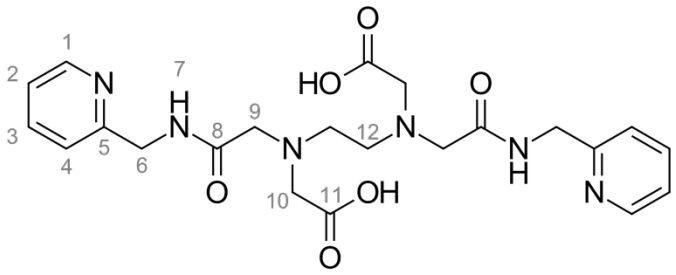
Anhydrous dichloromethane (50 cm^3) was used to dissolve chloroacetyl chloride (1.93cm^3, 24.2 mmol) and the solution cooled using a dry ice/acetone bath under nitrogen. 2-Aminomethylpyridine (2.5 cm^3, 24.5 mmol) was then added dropwise *via* syringe. As addition proceeded, the reaction mixture became purple and a solid precipiated. At this point, the reaction was bought to room temperature and more dichloromethane (as much as was necessary to enable free stirring) was added. The reaction mixture was allowed to stir at room temperature for a further 20 hours and the mixture bought to basic pH by addition of aqueous sodium carbonate (pH ≥ 10) before washing the crude mixture in the same. The organic layer was then dried over magnesium sulphate and the solvent removed *in vacuo*. The residue was purified by column chromatography (dichloromethane:tetrahydrofuran 70:30 v/v) to afford the title compound (3.31g, 17.9 mmol, 75%) as a yellow oil that became a brown solid upon refrigeration. δH (400 MHz, $CDCl_3$) 8.55 (ddd, 1H, ***H9***), 7.86 (s, 1H, ***H3***), 7.66 (ddd, 1H, ***H7***), 7.24 (dd, 1H, ***H6***), 7.20 (ddd, 1H, ***H8***), 4.58 (d, 2H, ***H4***), 4.10 (s, 2H, ***H1***); δC (101 MHz, $CDCl_3$) 166.1 (***C2***), 155.5 (***C9***), 149.2 (***C5***), 136.9 , 122.6 (***C7***), 122.2 (***C8***), 44.6, 42.6 (***C1***, ***C4***).

tert-Butyl-({2-[(tert-butoxycarbonylmethyl) (2-oxo-2-{[(2-pyridyl) methyl]amino} ethyl)amino]ethyl} (2-oxo-2-{[(2-pyridyl)methyl]amino} ethyl)amino)acetate, 4



Aminoester **12** (0.47 g, 1.6 mmol) was dissolved in acetonitrile (4 cm^3 , dried over molecular sieves) and α -haloamide **3** (0.68 g, 3.7 mmol) added at once prior to the addition of potassium carbonate (0.7 g, 5 mmol) and a catalytic amount of potassium iodide (0.027g, 0.16 mmol). Heating was commenced under argon at 60^{o}C for 22 hours, at which point only spots visible by both short-wave UV and PMA staining were observed following TLC analysis (silica, dicholomethane:methanol 90:10 v/v). After cooling to room temperature, the reaction mixture was partitioned between water and dichloromethane and the dichloromethane layer was extracted. The aqueous layer was extracted with dichloromethane a further two times and the combined organic extracts dried over magnesium sulphate and the solvent removed *in vacuo*. The residue was purified via preparative reverse-phase (C18) column chromatography (water:acetonitrile , 0.1% v/v formic acid as additive 0:100 →100:0 compound eluted in ca. 50:50 v/v water:acetonitrile) to afford the title compound (0.479 g, 0.82 mmol, 51%) as a brown oil. δH (600 MHz, $CDCl_3$) 8.49 (ddd, J = 5.0, 2.0, 1.0 Hz, 2H, ***H1***), 8.41 (t, J = 5.5 Hz, 2H, ***H7***), 7.62 (dd, J = 7.5, 2.0 Hz, 2H, ***H3***), 7.25 (d, J = 7.5 Hz, 2H, ***H4***), 7.16 (ddd, J = 7.5, 5.0, 1.0 Hz, 2H, ***H2***), 4.56 (d, J = 5.5 Hz, 4H, ***H6***), 3.36 (s, 4H, ***H9***), 3.30 (s, 4H, ***H10***), 2.82 (s, 4H, ***H14***), 1.41 (s, 18H, ***H13***); δC (151 MHz, $CDCl_3$) 171.6 (***C8***), 170.3 (***C11***), 157.2 (***C5***), 149.1 (***C1***), 137.0 (***C3***), 122.8 (***C2***), 122.1 (***C4***), 81.8 (***C12***), 59.1 (***C9***), 57.1 (***C10***), 53.6 (***C14***), 44.4 (***C6***), 28.3 (***C13***); (ES-MS^+) 585.2 (100 %, $[M+H]^+$); HR-MS Found 585.3403, $C_{30}H_{45}N_6O_6$ $[M+H]^+$ requires 585.3401.

{2-[(Carboxymethyl) (2-oxo-2-{[(2-pyridyl) methyl]amino}ethyl) amino]ethyl} (2-oxo-2-{[(2-pyridyl) methyl]amino} ethyl)amino)acetic acid, $AmPy_2$



Aminoester **4** (0.458 g, 0.78 mmol) was dissolved in dichloromethane (4 cm^3) prior to the addition of anisole (0.16 cm^3, 1.5 mmol) and trifluoroacetic acid (3 cm^3) and the solution stirred under argon for 17 hours under argon. Volatiles were then removed *in vacuo* and the residue redissolved in dichloromethane which was also removed *in vacuo*. Concentrated hydrochloric acid (ca. 5 cm^3) was then used to emulsify the residue, which was also removed *in vacuo* to afford the product as its trifluoroacetate salt, which was a buff solid (0.38 g). The solid was dissolved in the minimum volume of 1 mol dm^{-3} hydrochloric acid and loaded onto DOWEX 1X8 (chloride form) that had been pre-washed with 1 mol dm^{-3} hydrochloric acid and then water. Once loaded onto the resin, elution of the compound was achieved with 1 mol dm^{-3} hydrochloric acid (ca. 100 cm^3). Removal of the solvent *in vacuo* followed by further drying under high vacuum gave the title compound (0.34 g, 0.72 mmol, 92 %) as a light brown solid. t_R (H_2O:MeOH, UV detection); 1.33 min; δH (600 MHz, D_2O, pD ≈ 6) 8.28 (d, 2H, ***H1***), 7.71 (dd, 2H, ***H2***), 7.38 – 7.06 (m, 4H, ***H3***, ***H4***), 4.31 (s, 4H, ***H6***), 3.71 (s, 4H, ***H9***), 3.39 (s, 4H, ***H10***), 3.11 (s, 4H, ***H12***); δC (151 MHz, D_2O, pD ≈ 6) 174.3 (***C11***), 170.1 (***C8***), 155.6 (***C5***), 147.5 (***C1***) , 139.4 (***C2***), 123.4 and 122.1 (***C3, C4***), 57.5 (***C10***), 56.6 (***C9***), 52.1 (***C12***), 43.7 (***C6***); (ES-MS^+) 473.2 (100 %, $[M+H]^+$); HR-MS Found 473.2139, $C_{22}H_{29}N_6O_6$ $[M+H]^+$ requires 473.2149.

Preparation and ^{1}H NMR quantitation of stock solutions of EDTA, $AmGly_2$ and $AmPy_2$ for biological study

EDTA, **$AmGly_2$** or **$AmPy_2$** were assumed to be in their neutral forms (i.e. all carboxylate groups protonated, and all amine groups present as their free bases). A known amount of **EDTA**, **$AmGly_2$** or **$AmPy_2$** was weighed into a suitable container and solutions of either ligand (approximately 0.3 mol dm^{-3}) were prepared using de-ionised water. If the ligand was poorly soluble in water, NaOH (aq. 10% w/w) was added until complete dissolution was observed. The pH of the solution was then adjusted to 7 using hydrochloric acid (aq. 37% v/v) or NaOH (aq. 10% w/w).

An aliquot of these solutions (50µL) was then dissolved in a solution of K_2HPO_4 (0.45 cm^3, 0.2 mol dm^{-3}) and was then added to a solution of t-Butanol in D_2O (0.5cm^3, 1 wt. % t-Butanol) to prepare an NMR sample with a final volume of 1cm^3. A ^{1}H NMR spectrum was then acquired on a Bruker Avance 400 NMR spectrometer using the ROBUST5[6] pulse sequence with a relaxation delay of 15 seconds. The resulting spectrum was integrated using the t-Butanol signal as a reference (integral set to 9H). The ratio of the N-CH_2-COO^- signals of **EDTA**, **$AmGly_2$** or **$AmPy_2$** to t-Butanol could then be used to calculate the concentration of the prepared solutions of **EDTA**, **$AmGly_2$** or **$AmPy_2$**, from which further dilutions could be made.

Bacterial growth inhibition studies with EDTA, $AmGly_2$ and $AmPy_2$

A) *Escherichia coli* K-12 (BW25113 / ATCC 27325 (Δ(*araD-araB*)*567*, Δ(*rhaD-rhaB*)*568, ΔlacZ4787* (::*rrnB-3*), *hsdR514, rph-1*), was used in growth assays alongside mutant derivatives, JW1041 (*lpxL*), JW1115 (*phoQ*), JW1116 (*phoP*), JW1844 (*lpxM*), JW3594 (*hldD*, *rfaD*), JW3596 (*waaC*, *rfaC*), JW3605 (*waaP*, *rfaP*). *Klebsiella pneumoniae* (ATCC 15380) and *Pseudomonas aeruginosa* PA02 (Migula, ATCC 9027) were chosen as additional Gram-negative bacteria, while *Staphylococcus aureus* (3R7089 strain Oxford; ATCC 9144) served as a representative Gram-positive species. Bacteria were streaked on LB agar. Cultures of the bacterium of interest were grown from single colonies in 5 ml of either LS-LB[2] or Iso-sensitest (Oxoid) broth for 16-18 hours at 37°C with gentle agitation. An aliquot (12.5-25 µL) of each culture was transferred into a sterile semi-micro polystyrene cuvette (Sarstedt) containing 2 cm^3 of growth medium and incubated at 37°C with shaking (110 rpm) to an A_{650nm} of ~0.07, equivalent to a 0.5 MacFarland standard (240 µM $BaCl_2$ in 0.18 M H_2SO_4). This culture was diluted ten-fold in fresh medium before use as an inoculum.

B) In parallel, solutions of **EDTA, $AmGly_2$** or **$AmPy_2$** at 2x the concentrations to be studied were prepared aseptically from stock solutions in water as described earlier using the relevant growth medium as the diluent. 50 µL of these 2x solutions in media were transferred to wells in a sterile, round-bottomed, 96-well microtest plate (Sarstedt).

[2] We used low-salt LB with the following constituents: 10 g L^{-1} tryptone, 5 g L^{-1} yeast extract and 0.5 g L^{-1} NaCl. Standard LB contained 10 g L^{-1} tryptone, 5 g L^{-1} yeast extract and 5 g L^{-1} NaCl.

C) 50 µL of the diluted culture prepared in **A)** was added to 50 µL of 2x **EDTA, $AmGly_2$** or **$AmPy_2$** solutions in the 96-well plate prepared in **B)**. Positive controls lacking **EDTA, $AmGly_2$** or **$AmPy_2$** (i.e. 50 µL of media and 50 µl of the diluted bacterial culture) were included in each run. Negative controls containing 100 µL media alone, or in the case of **$AmPy_2$**, two-fold dilutions of the 2x solutions prepared in **B)** were also included. The plate was incubated at 37°C for 16 hours with shaking at 110 rpm and absorbance (A_{650nm}) recorded on a SPECTROstar^{Nano} plate reader (BMG Labtech) to monitor bacterial growth. The measured A_{650nm} values for the positive control and each ligand concentration were corrected by subtracting the A_{650nm} values for the appropriate negative controls. The corrected A_{650nm} values for each ligand concentration were then divided by the corrected A_{650nm} value for the positive control, to give the relative growth.

Bacterial cell integrity and viability

Cultures (5 mL) of *E. coli* BW25113 were grown to an A_{650nm} of 0.2 in standard LB broth. Cells were pelleted by centrifugation at 10,000 x g for 10-15 min and resuspended in 1 mL of sterile PBS. 100 µL of the cell suspension was added to 900 µL of PBS containing either **EDTA** or **$AmGly_2$** and incubated at room temperature for 1 hour with mixing every 15 minutes. Samples were again pelleted by centrifugation at 10,000 x g for 3 minutes, washed in 1 mL PBS before recovery in 1 mL PBS. An A_{650nm} reading was taken and cells stained with SYTO 9 and propidium iodide from the LIVE/DEAD® Viability kit™ (Invitrogen) following the manufacturer's instructions. 30 µL of the stained bacterial suspension was mixed with 10 µL of 80% glycerol and mounted on a 14 mm concave microscope slide (Celestron) covered with an 18 mm square coverslip and sealed with clear nail varnish.

Cells were visualized using a Leica SP5 II microscope using the excitation and emission maxima for SYTO 9 (λ_{ex} = 480 nm; λ_{em} = 500 nm) and propidium iodide (λ_{ex} = 490 nm; λ_{em} = 635 nm). The microscope is equipped with an optical resolution enhancing module called PhMoNa,[7] which allows sub-diffraction limited resolution of 87 x 87 nm to be achieved using a 488 nm laser. Bacterial cells stained with each dye were counted using ImageJ[8] with the Cell Counter plugin (https://imagej.nih.gov/ij/plugins/cell-counter.html).

Potentiometric titrations

Titrations were carried out at 298.1±0.1 K using KCl 0.15 M as the supporting electrolyte. The experimental procedure (burette, potentiometer, cell, stirrer, microcomputer, etc.) has been fully described elsewhere.[9] The acquisition of titration data was performed using the computer program PASAT.[10] An Ag/AgCl electrode in saturated KCl solution was used as the reference electrode and the glass electrode was calibrated as an hydrogen-ion concentration probe by titrating previously standardized amounts of hydrochloric acid with carbonate-free sodium hydroxide solutions, and determining the equivalence point by Gran's

method,[11,12] to give the standard potential, E°', and the ionic product of water (pK_w = 13.73(1)).

The computer program HYPERQUAD[13] was used to fit the protonation and stability constants. Solutions containing the ligand, and the ligand and metal ions, with concentrations 1 x 10^{-3} M were then titrated with sodium hydroxide. Initially, the titration curves for each ligand were treated as separated curves without significant variations in the values of the stability constants. The datasets were then merged and treated simultaneously to give the final stability constants.

X-Ray Crystallography

The X-ray single crystal data for all compounds were collected using graphite-monochromated λMoKα-radiation (λ=0.71073Å) at the temperatures 120.0K maintained by Cryostream (Oxford Cryosystems) open-flow nitrogen cryostats, The data for compound **1** have been collected on a Bruker SMART CCD 6000 (fine-focus sealed tube, MonoCap optics) and for compounds **$AmGly_2$** and **$Mg(AmGly_2)$** on an Agilent Xcalibur-S and Agilent Supernova (Sapphire-3 CCD detectors) diffractometers respectively. The structures were solved by direct method and refined by full-matrix least squares on F^2 for all data using Olex2 [1] and SHELXTL [2] software. All non-hydrogen atoms were refined with anisotropic displacement parameters, H-atoms were located on the difference Fourier maps and refined isotropically (compounds **1** and **$AmGly_2$**) or placed into the calculated positions and refined in riding mode (**$Mg(AmGly_2)$**). The crystal data and details of the refinement are listed in ***Table S1***. Crystallographic data for the structures **1**, **$AmGly_2$** and **$Mg(AmGly_2)$** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC numbers 1814349, 1814350 and 1814351.

Table S1. *Crystallographic data for* ***1****,* ***AmGly$_2$*** *and* ***Mg(AmGly$_2$)***

Compound	**1**	**$AmGly_2$**	**$Mg(AmGly_2)$**
Empirical Formula	$C_{28}H_{40}N_2O_4$	$C_{14}H_{22}N_4O_{10}$	$C_{14}H_{31}MgN_5Na_3O_{21}$
Formula weight	468.62	406.36	698.72
Temperature (K)	120.0	120.0	120.0
Crystal system	tetragonal	orthorhombic	monoclinic
Space group	P-4	$P2_12_12$	C2/c
a	22.2406(6)	17.3349(11)	12.1907(5)
b	22.2406(6)	9.3065(5)	9.6223(5)
b	5.4929(2)	5.3951(4)	23.9144(9)
α	90.0	90.0	90.0
β	90.0	90.0	90.473(4)
γ	90.0	90.0	90.0
Volume ($Å^3$)	2717.03(14)	870.38(10)	2805.1(2)
Z	4	2	4
ρ_{calc} (g/cm^3)	1.146	1.551	1.654
μ/mm^{-1}	0.076	0.133	0.210
F(000)	1016	428	1452
2Θ range (°)	3.66 to 55.00	6.42 to 55.98	6.68 to 50.99
Reflections collected	30203	4500	6788
Independent reflections	3446	2095	2572
R_{int}	0.0501	0.0442	0.0397
Data/restraints/parameters	3446/0/467	2095/0/171	2572/9/225
Goodness-of-fit on F^2	1.066	1.014	1.139
Final R_1 indexes [I≥2σ (I)]	0.0355	0.0449	0.0553
Final wR_2 indexes [all data]	0.0836	0.0793	0.1587

Solid-state structure of 1

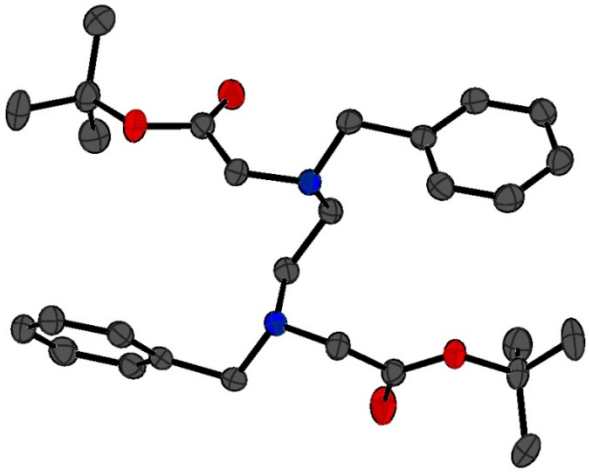


Figure S1. *Molecular structure in the crystal of* ***1****. Thermal ellipsoids are shown at the 50% probability level*

^{1}H-DOSY Diffusion coefficients for EDTA and AmGly$_2$

Table S2. *Diffusion coefficients obtained from ^{1}H-DOSY experiments. All samples had a final ligand concentration of 50 mM and were incubated with shaking (110 rpm) at 37°C for 16 h prior to filtration through a 0.22 µM syringe filter and transferred to an NMR tube containing a coaxial insert. All samples were spiked with NaN_3 to arrest bacterial growth following transfer. A water suppression sequence was used to record all spectra.*

Medium	**10^{10} D/m^2 s^{-1}**	
	EDTA	**AmGly$_2$**
Water	4.63	3.73
Iso-sensitest	4.29	3.82
Iso-sensitest (after bacterial incubation)	4.49	3.41

Low-Salt LB experiments on E. coli mutants

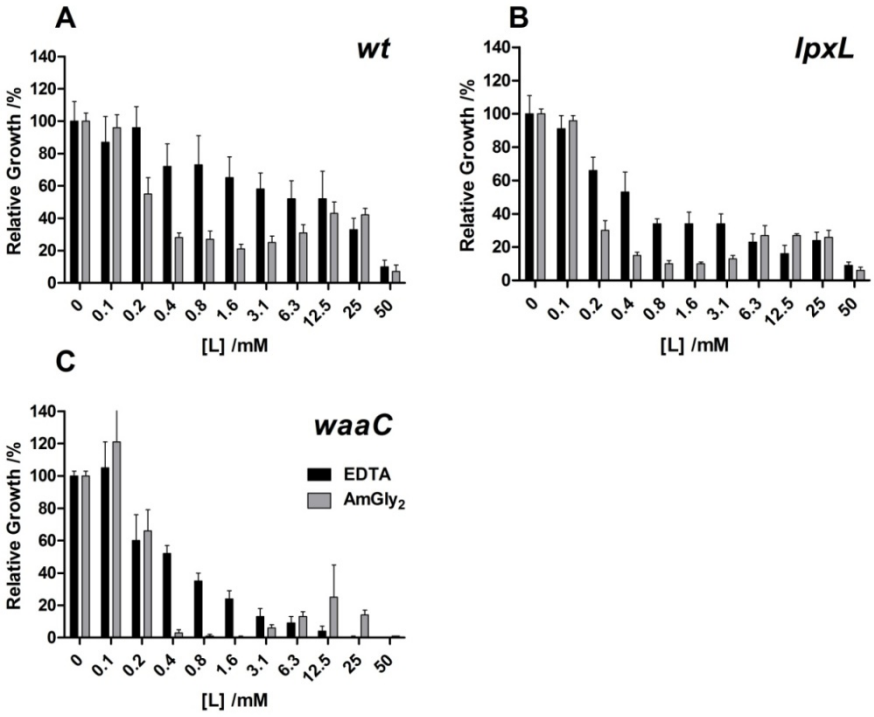


Figure S2 *Growth inhibition studies on* ***A)*** *E. coli wt BW25113 and* ***B)*** *lpxL and* ***C)*** *waaC mutants in low-salt LB (LS-LB) medium. Data are the average of at least three replicates with standard deviation shown.*

E. coli wt and waaC viability

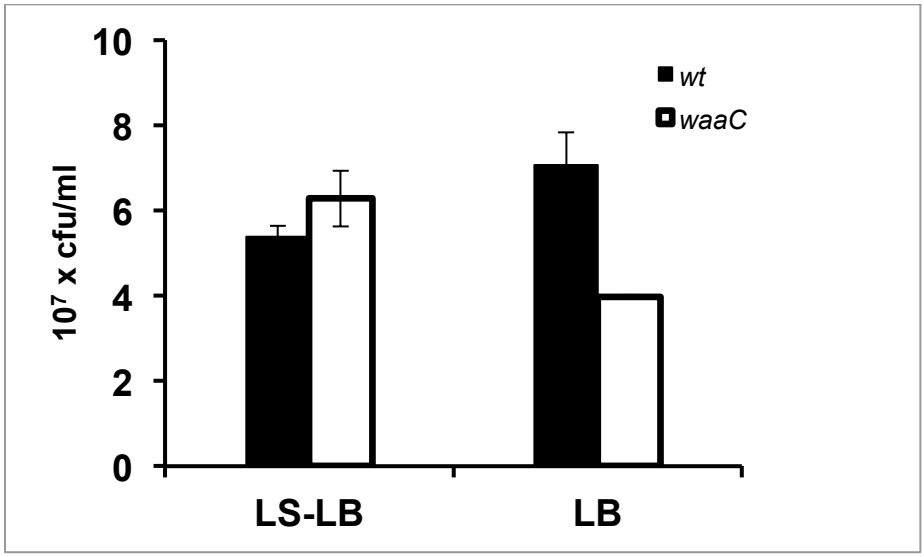


Figure S3 *Viability of E. coli wt (BW25113) and the waaC mutant in low-salt (LS-LB) and standard LB media.*[2] *Cells were grown to an* A_{650nm} *of 0.4 before five 10-fold serial dilutions were performed and small volumes (10 µL) spotted on LS-LB or LB agar plates. Plates were incubated at 37°C for 16 h and colonies counted. Data are the average of three independent experiments with standard deviation shown.*

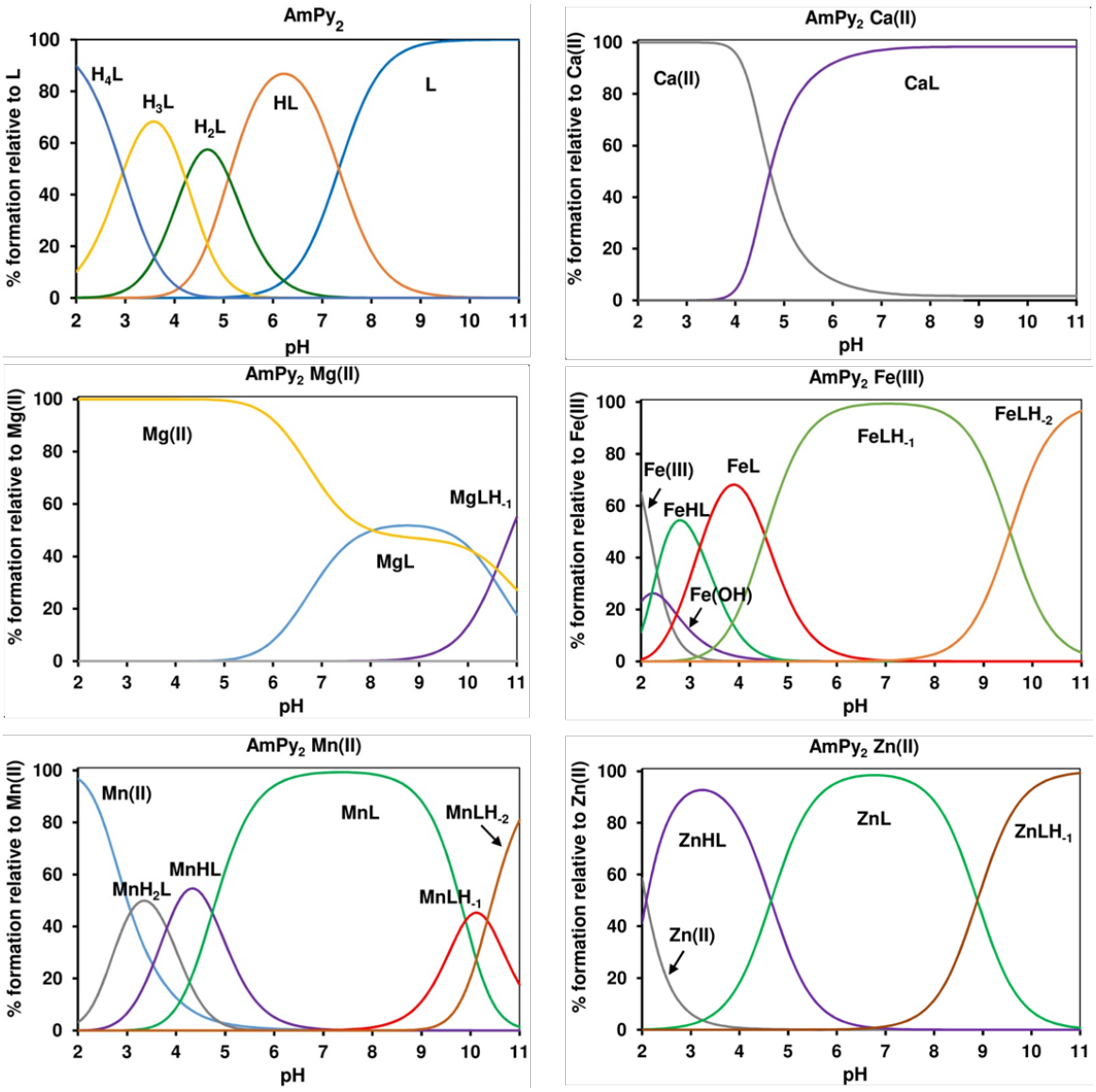


Figure S4 *Distribution plots of the systems L:M (M =H^+, Ca(II), Mg(II), Fe(III), Mn(II) and Zn(II)) in 1:1 molar ratio [L]=[M]=1 × 10^{-3} M (charges omitted)*

Charge states of EDTA, $AmGly_2$ and their metal ion complexes

Table S3 *Charge states of **EDTA**, **$AmGly_2$** and their metal ion complexes at pH 7.4. Charges were calculated from speciation data generated using the formation constants in **Table 2** of the main text using the HySS[16] software.*

	Charge on predominant species at pH 7.4	
M^{n+}	**EDTA**	**$AmGly_2$**
None (ligand only)	-3	-3, -4
Mg^{2+}	-2	-2
Ca^{2+}	-2	-2
Fe^{3+}	-1	-3
Mn^{2+}	-2	-2
Zn^{2+}	-2	-2

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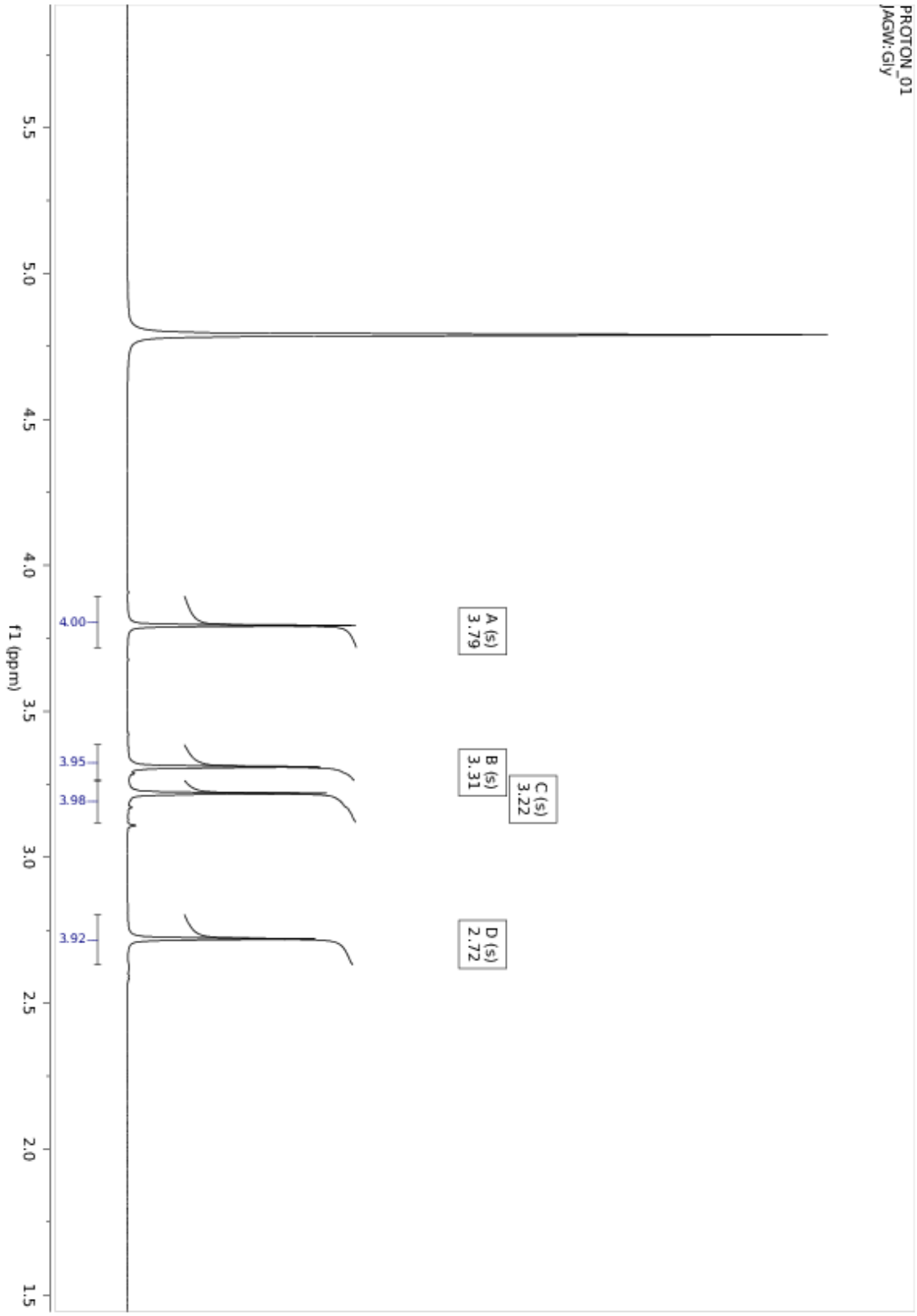
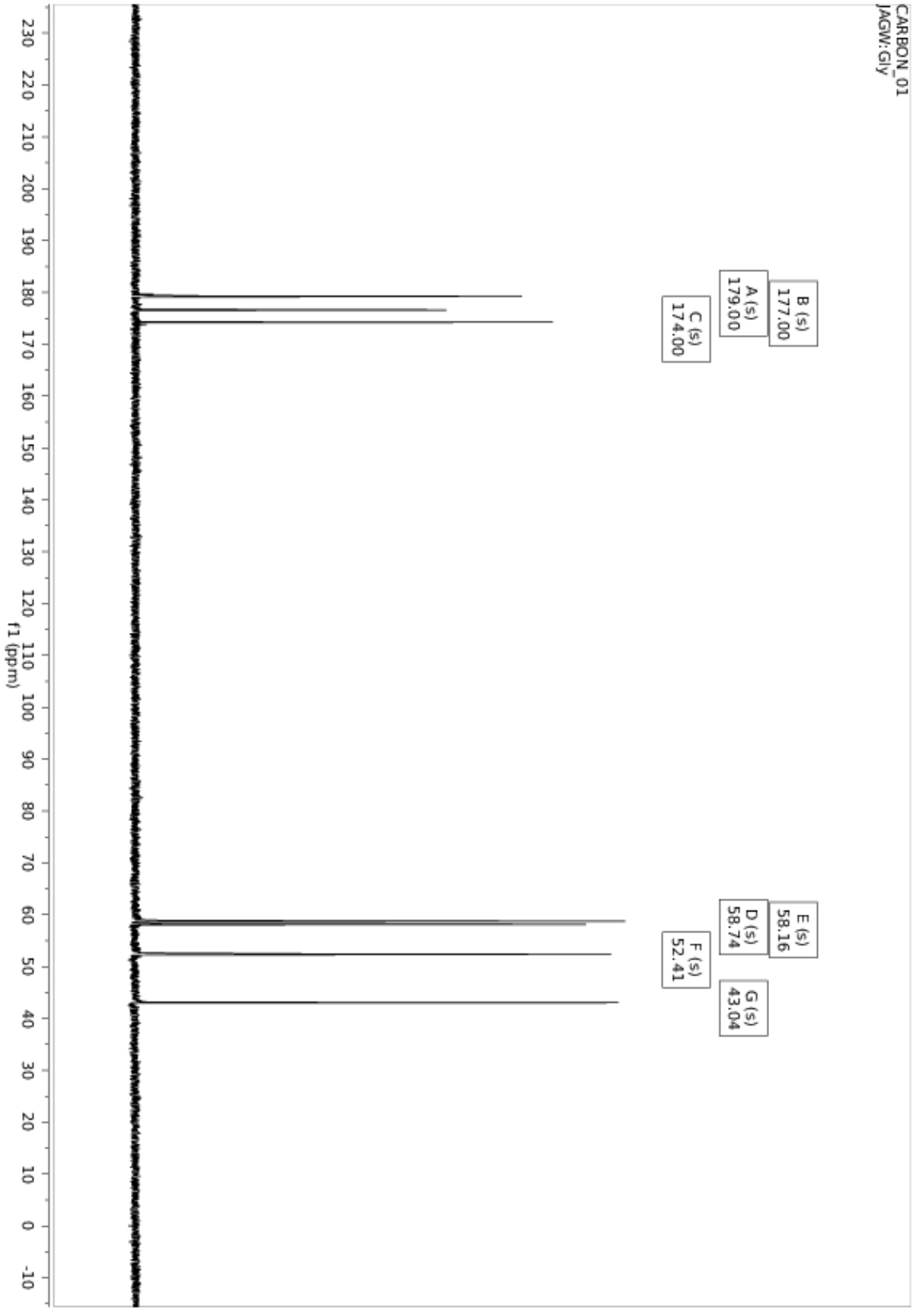
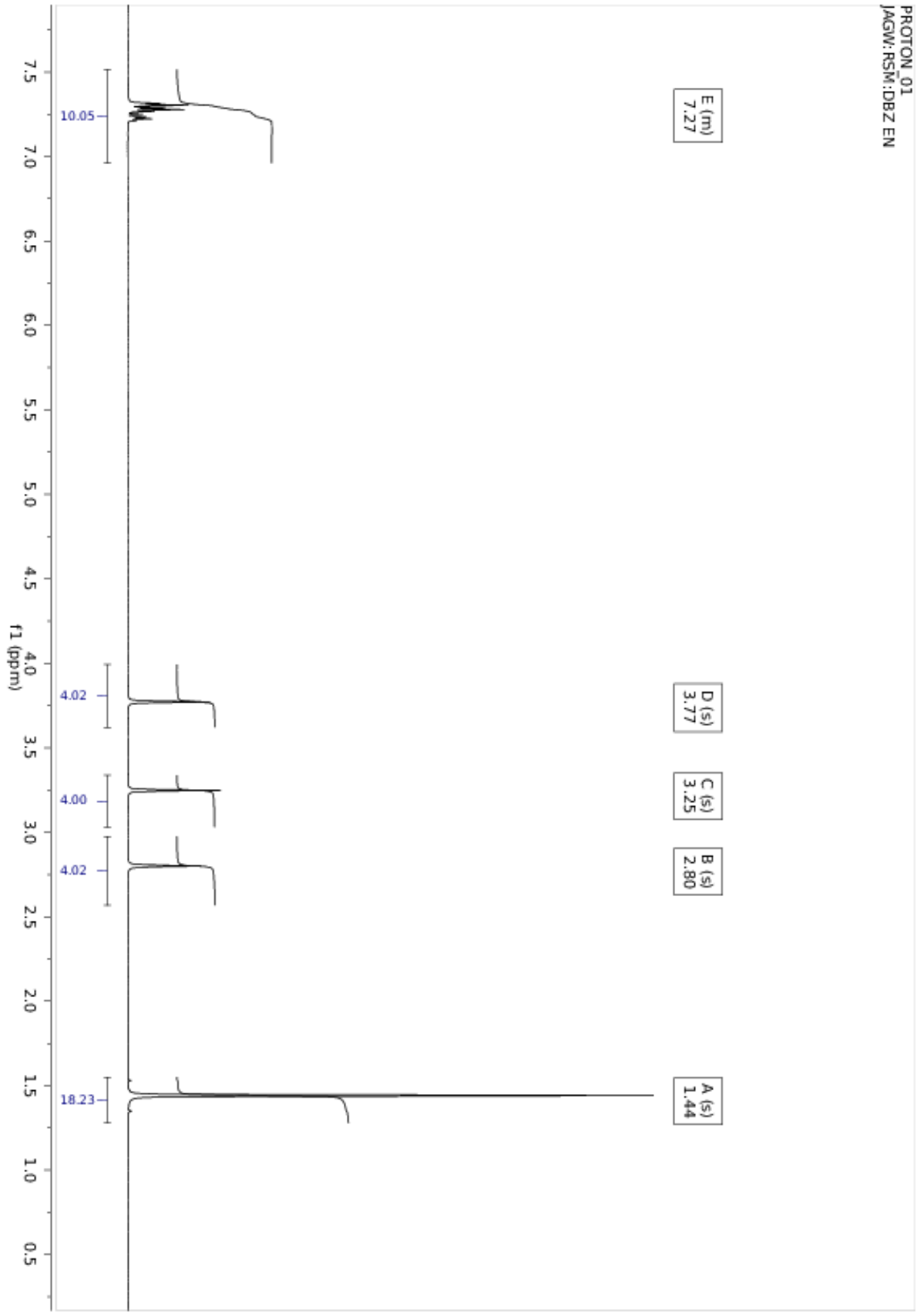
^{1}H NMR of $AmGly_2$



^{13}C NMR of $AmGly_2$



^{1}H NMR of 1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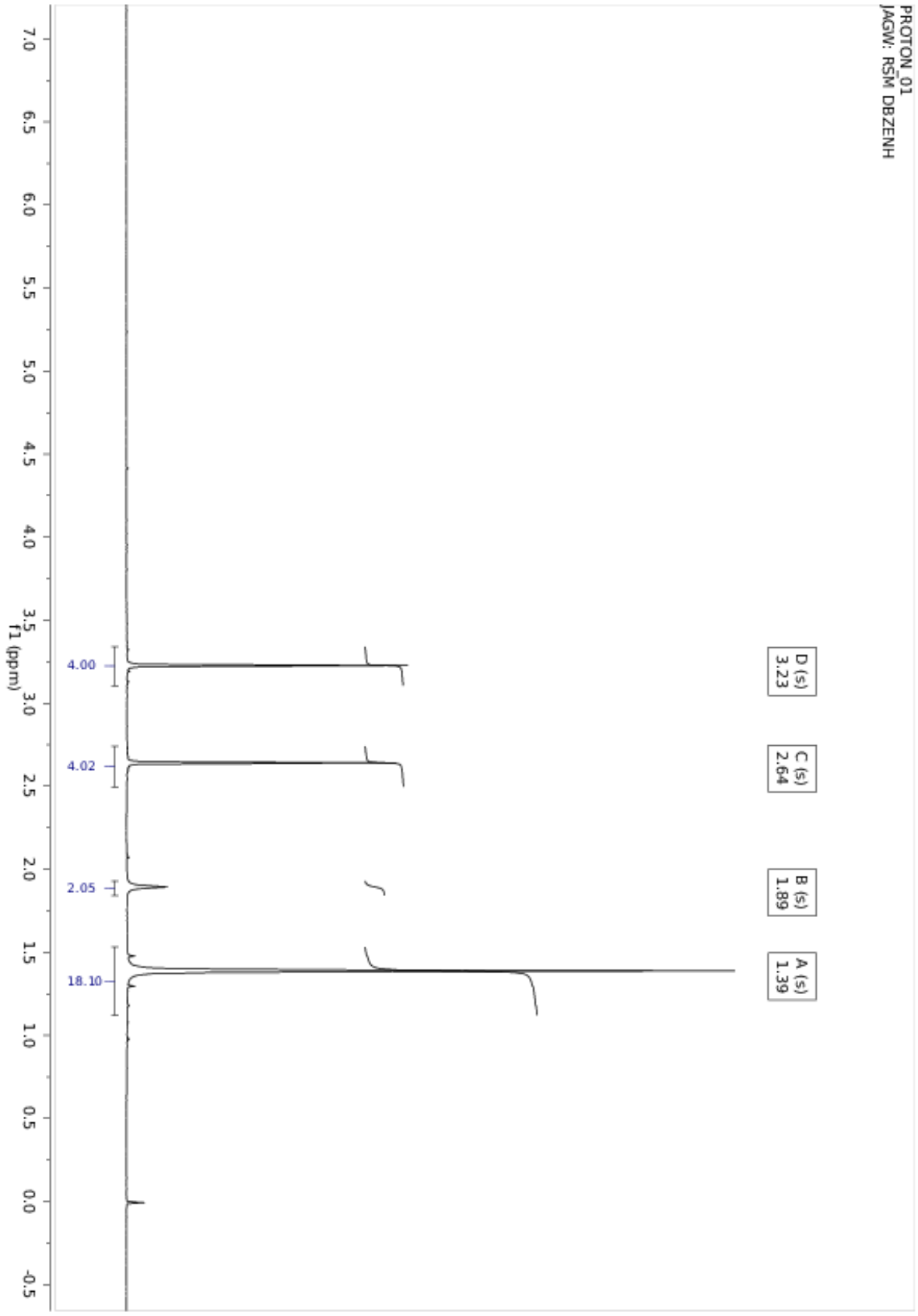
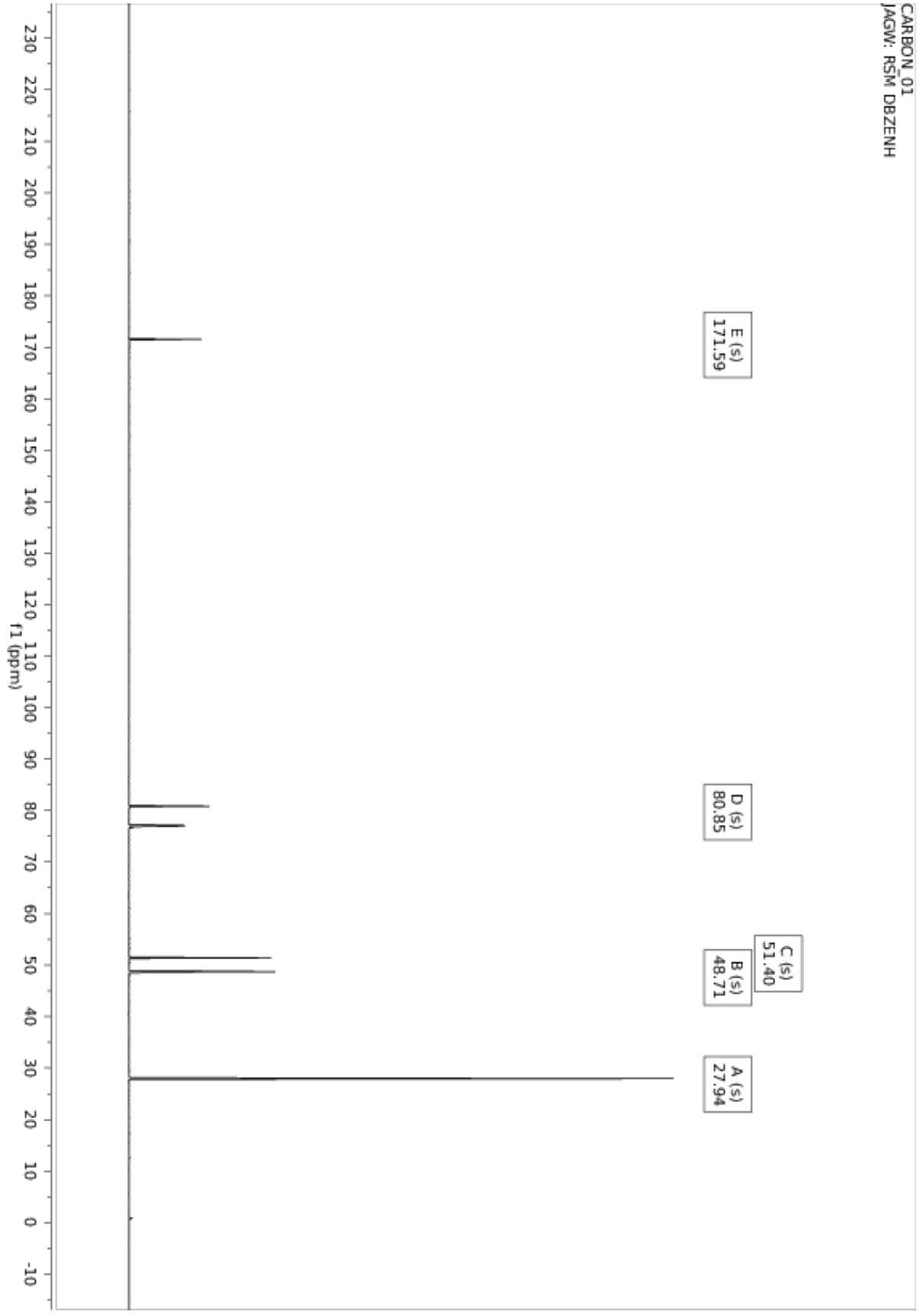
E (m)
7.27
D (s)
3.77
C (s)
3.25
B (s)
2.80
A (s)
1.44
10.05
4.02
4.00
4.02
18.23
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

^{1}H NMR of 2


PROTON_01
JAGW: RSM DBZENH
D (s)
3.23
C (s)
2.64
B (s)
1.89
A (s)
1.39
4.00
4.02
2.05
18.10
f1 (ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5

^{13}C NMR of 2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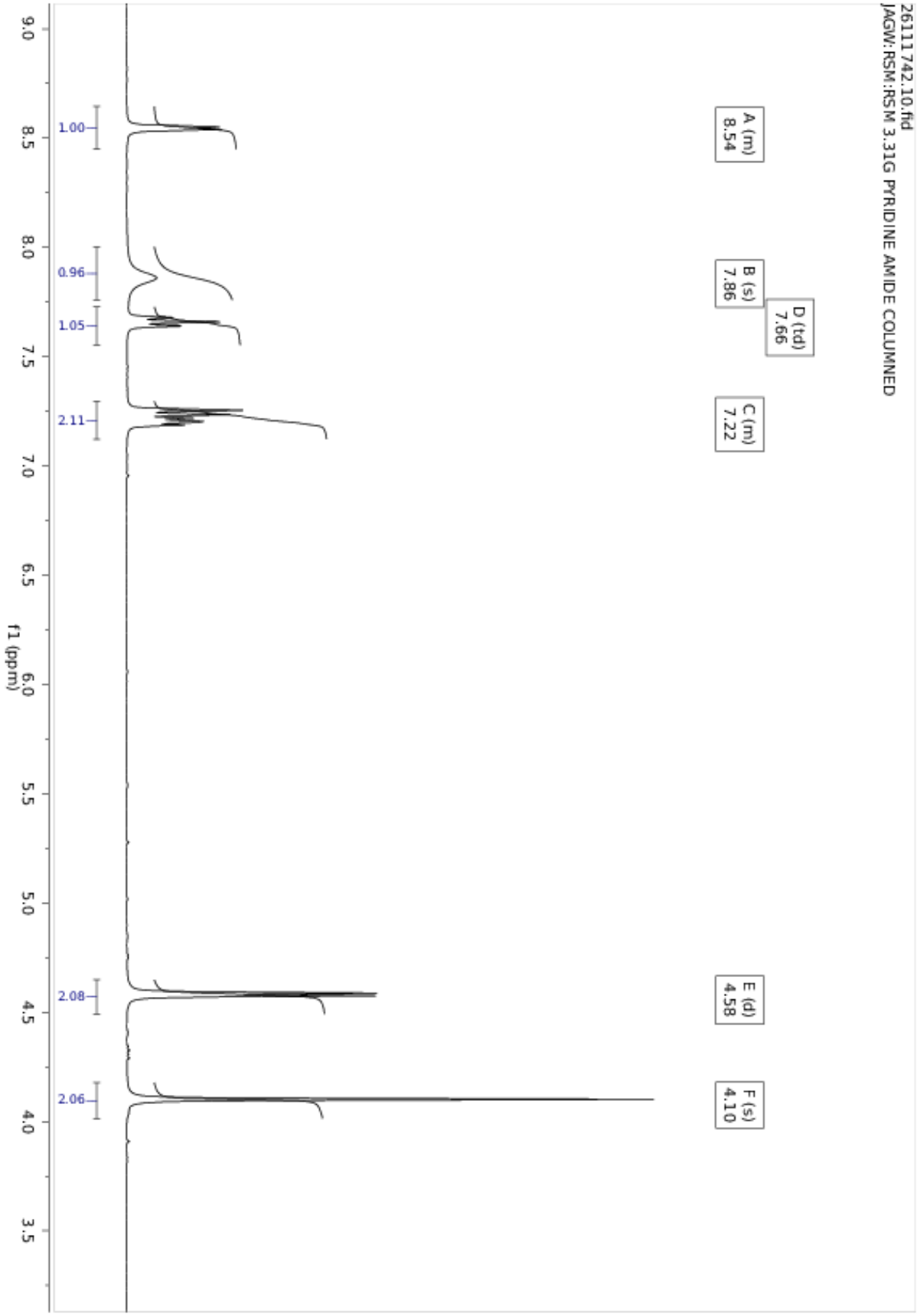
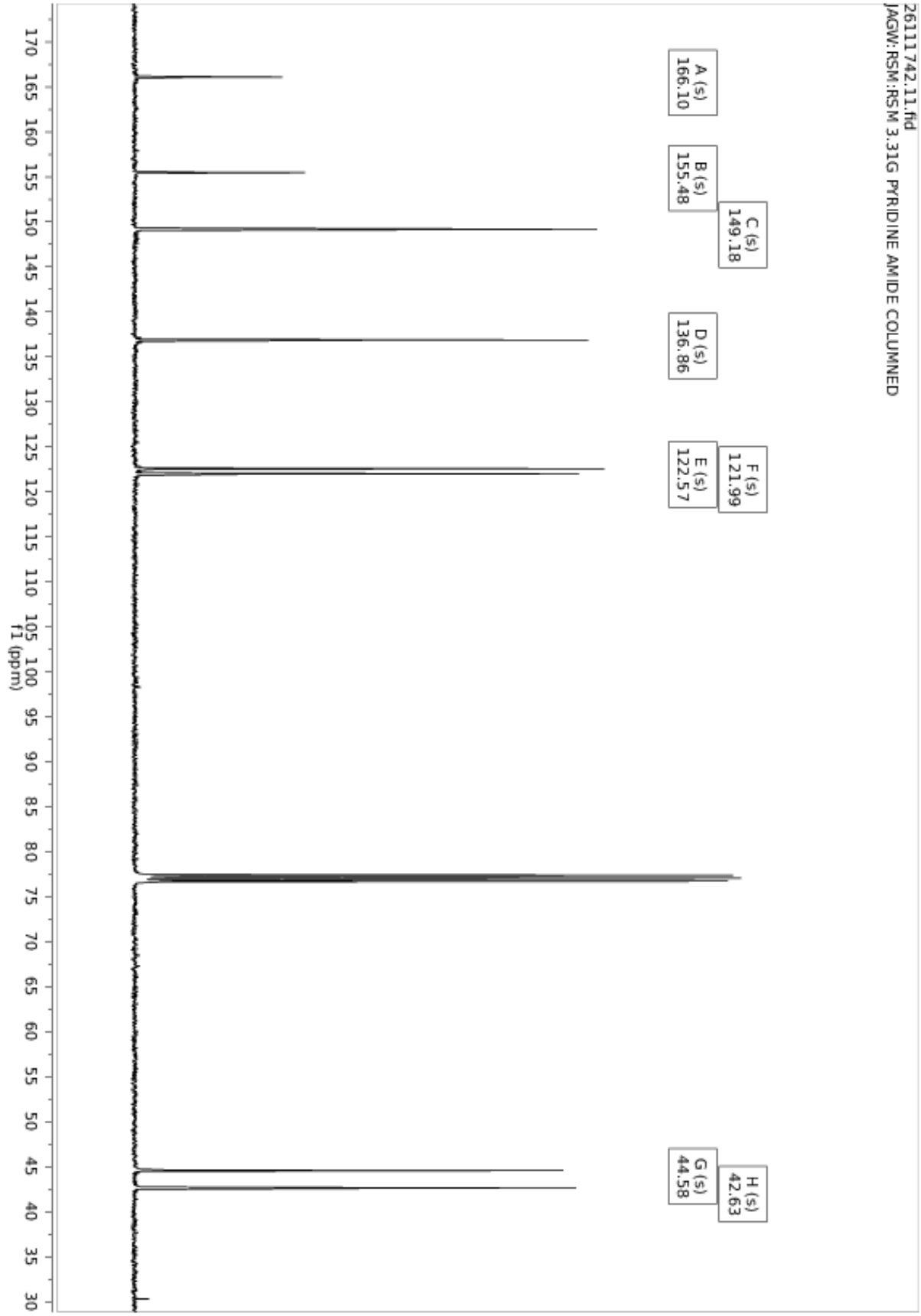
JAGW: RSM DBZENH
E (s)
171.59
D (s)
80.85
C (s)
51.40
B (s)
48.71
A (s)
27.94
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

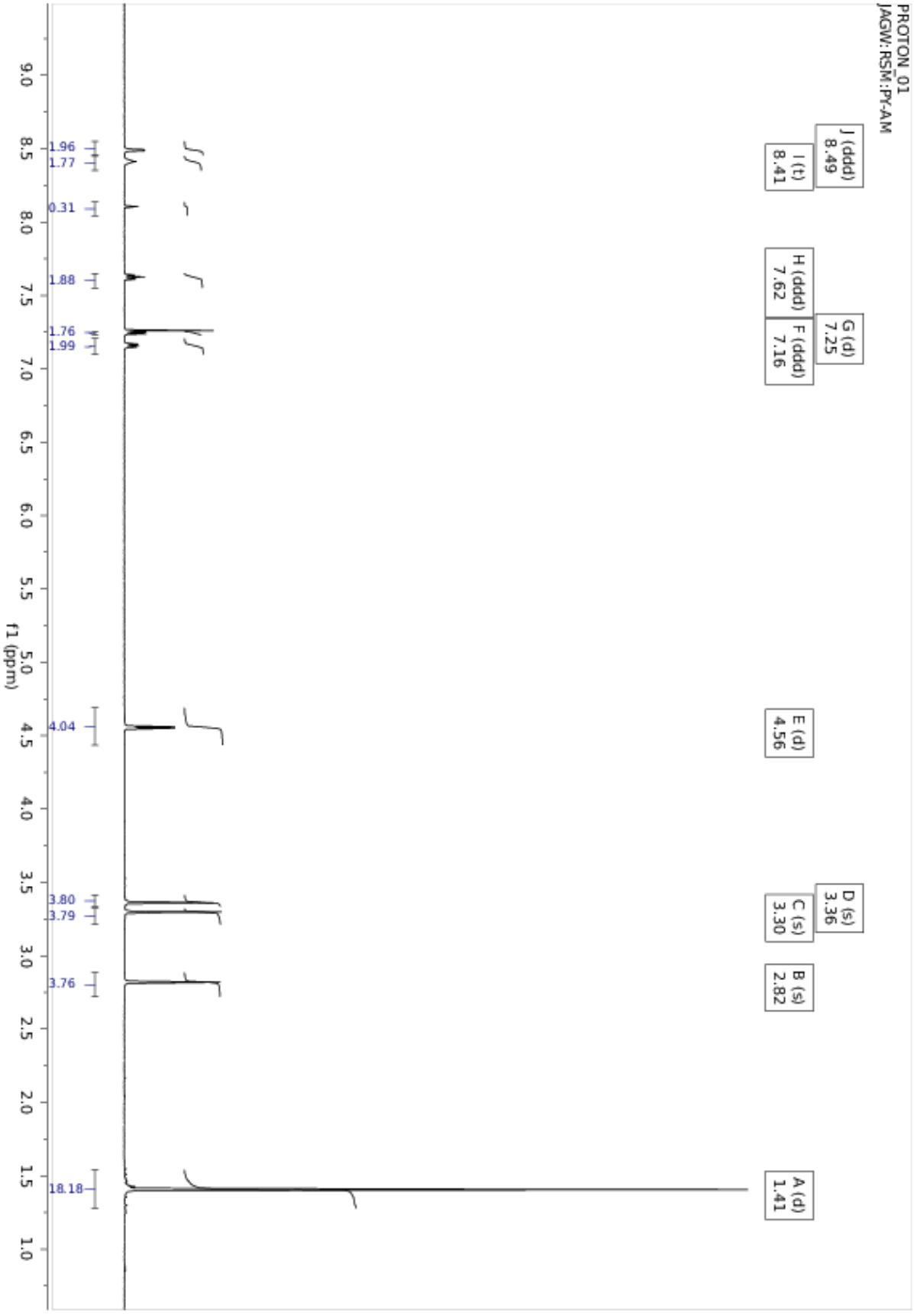
^{1}H NMR of 3


26111742.10.fid
JAGW:RSM:RSM 3.31G PYRIDINE AMIDE COLUMNED
A (m)
8.54
B (s)
7.86
D (td)
7.66
C (m)
7.22
E (d)
4.58
F (s)
4.10
1.00
0.96
1.05
2.11
2.08
2.06
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5

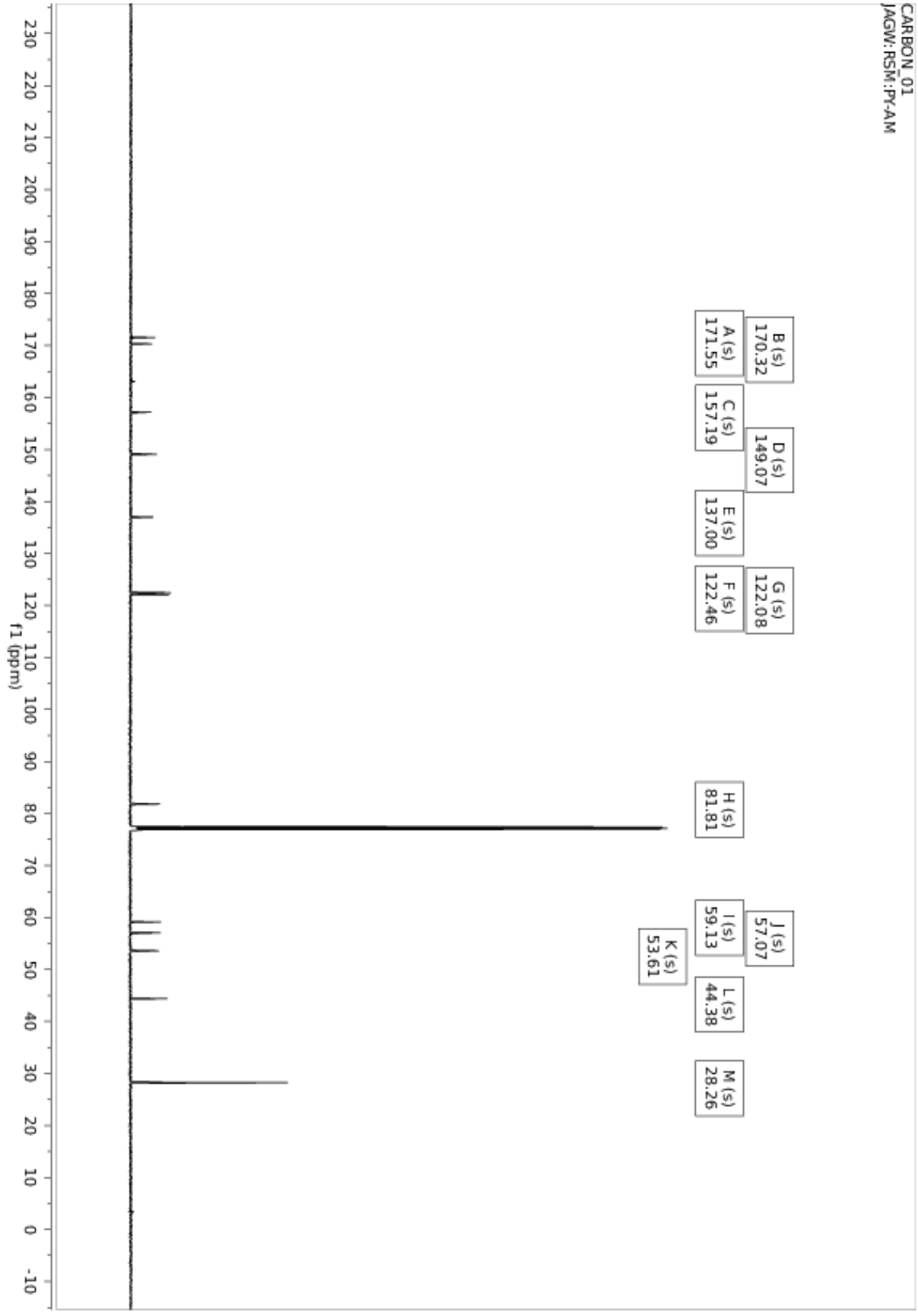
^{13}C NMR of 3


261117 42.11.fid
JAGW:RSM:RSM 3.31G PYRIDINE AMIDE COLUMNED
A (s) 166.10
B (s) 155.48
C (s) 149.18
D (s) 136.86
E (s) 122.57
F (s) 121.99
G (s) 44.58
H (s) 42.63
f1 (ppm)

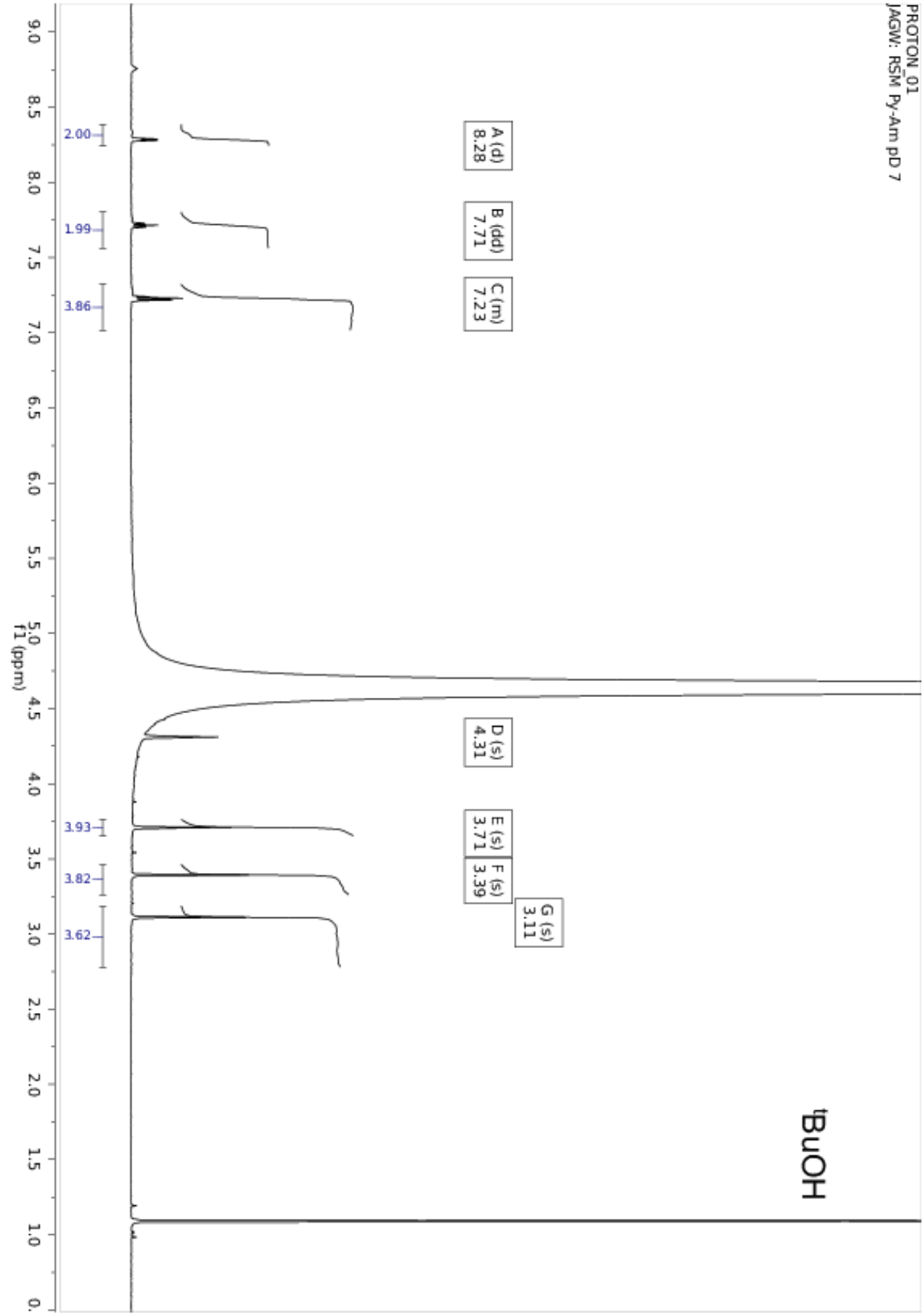
^{1}H NMR of 4


PROTON_01
JAGW:RSM:PY-AM
J (ddd) 8.49
I (t) 8.41
H (dddd) 7.62
G (d) 7.25
F (dddd) 7.16
E (d) 4.56
D (s) 3.36
C (s) 3.30
B (s) 2.82
A (d) 1.41
1.96
1.77
0.31
1.88
1.76
1.99
4.04
3.80
3.79
3.76
18.18
f1 (ppm)

^{13}C NMR of 4


CARBON_01
JAGW:RSM:PY-AM
A (s) 171.55
B (s) 170.32
C (s) 157.19
D (s) 149.07
E (s) 137.00
F (s) 122.46
G (s) 122.08
H (s) 81.81
I (s) 59.13
J (s) 57.07
K (s) 53.61
L (s) 44.38
M (s) 28.26
f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

^{1}H NMR of $AmPy_2$



^{13}C NMR of $AmPy_2$