Mechanistic Rationalization of Unusual Autoinductive Kinetics in an Aqueous 1,3-Dipolar Cycloaddition

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SUPPORTING INFORMATION

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General Remarks.

Solvents and Reagents.

HPLC grade water and nitromethane were purchased from Fisher Scientific and used without further purification. Ethyl nitroacetate was purchased from Oakwood Chemical. Methylamine (40% w/w aqueous solution) and 1,4-diazabicyclo[2.2.2]octane were purchased from TCI Chemicals. Ethyl glycolate, ethyl glyoxylate (50% in toluene), sodium hypochlorite (10-15% aqueous w/w), dimethyl sulfone, potassium nitrite, tetrabutylammonium nitrite, and hydroxylamine hydrochloride were purchased from Sigma-Aldrich. Sodium hydroxide, potassium hydroxide, acrylamide, trifluoroacetic acid, sodium nitrite, triethylamine, and pyridine were purchased from Acros Organics. Triethylamine was distilled over calcium sulfate before use. The exact concentration of sodium hypochlorite solution was determined via thiosulfate titration. All other reagents were used as received without further purification.

Instrumentation.

NMR spectra were recorded on Bruker DRX-600, DRX-500, and AMX-400 instruments and were calibrated using residual undeuterated solvent or TMS as an internal reference. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m =

multiplet, br = broad. Unit mass spectra were recorded on an Agilent 1100 LC/MSD with an Agilent 1100 SL mass spectrometer (ESI). In-situ FT-IR analysis was performed using a Mettler-Toledo ReactIR^{TM} 45m instrument fitted with a DST 6.35 mm DiComp ATR probe. In-situ pH monitoring was performed using a Hanna Instruments 98160 pH/ORP Meter equipped with a Fisher Scientific Accumet Gel-Filled Pencil-Thin pH combination electrode.

General Procedure for Kinetic Experiments.

Solutions of acrylamide, sodium hydroxide, dimethyl sulfone, and any specific additives in water were prepared using volumetric glassware. A 1 dram vial equipped with magnetic stir bar was charged with water, dimethyl sulfone (DMS) solution (as an internal standard), and N-methyl nitroacetamide (as a solid). Syringes were prepared with the necessary amounts of acrylamide, sodium hydroxide, and additive solution (as measured by mass). The ReactIR instrument's MCT detector was cooled with liquid nitrogen and purged with dry gaseous nitrogen for at least two hours before use. The ReactIR probe was placed in a vial containing water and submerged in an oil bath at 60°C. After allowing approximately 10 minutes for temperature equilibration a background spectrum was recorded.

The probe was then cleaned and placed through a septum into the vial containing N-methyl nitroacetamide and DMS. The vial was sealed onto the probe using several wraps of Teflon tape, lowered into an oil bath at 60°C, and the ReactIR set to begin recording spectra. Temperature equilibration was observed by following the real time progress of the N-methyl nitroacetamide absorption band at 1562 cm^{-1} (NO_2). Once the intensity of this absorption band stabilized the acrylamide solution was injected. This was also allowed to equilibrate and after the signal at 1562 cm^{-1} had stabilized once again (~2-3 min) the sodium hydroxide and additive solutions were injected into the reaction vial. After injection all reagent syringes were massed to determine the exact amount of reagent delivered. The reaction was then allowed to proceed until intensity of the absorption band at 1562 cm^{-1} reached a constant minimum value. At this point an aliquot (~0.05 mL) was removed and placed into an NMR tube containing ~0.8 mL CD_3CN. This sample was then either immediately analyzed by Q-

HNMR, or frozen in an isopropyl alcohol / dry ice mixture until such time as analysis was possible. A delay of 30 seconds was found to be adequate for Q-HNMR analysis as the longest T1 of any species in the reaction mixture was determined to be 6 seconds.

Raw ReactIR absorbance data was transformed with a negative second derivative function to aid in separation of peaks for analysis. N-methyl nitroacetamide and the cycloaddition product were tracked using absorption bands at 1562 cm^{-1} and 913 cm^{-1}, respectively. For some data sets the multi-component analysis algorithm known as ConcIRT, developed by Mettler-Toledo and contained within their iC IR software, was used to extract the time profile corresponding to the cycloaddition product. The initial concentration of N-methyl nitroacetamide was determined from reaction stoichiometry. Final concentrations of N-methyl nitroacetamide and cycloaddition product were determined via integration and comparison of the DMS internal standard peak (2.94 ppm, s), N-methyl nitroacetamide CH_2 peak (5.15 ppm, s), and product CH peak (5.08 ppm, dd) in the Q-HNMR spectra. Raw absorbance data for N-methyl nitroacetamide and product was then normalized, scaled, and shifted so that it fit the initial and final concentrations, creating a concentration vs time profile for the reaction. This method was validated as outlined below.

Validation of ReactIR Method

A reaction with no additives was carried out as described above in the general procedure for kinetic experiments with the only modification being that aliquots (~0.05 mL) were taken at multiple time points during the reaction. These aliquots were placed into NMR tubes containing ~0.8 mL CD_3CN and either immediately analyzed via Q-HNMR (using the same parameters as outlined above) or frozen in a isopropyl alcohol / dry ice mixture until they could be analyzed. The concentration of N-methyl nitroacetamide and product in each of these aliquots was determined by comparison against the DMS internal standard, also as outlined above. This provided a concentration vs time trace independent of all ReactIR measurements. The ReactIR data for this reaction was worked up as described above. Plots of

both ReactIR and Q-HNMR data overlay, indicating that the ReactIR method is valid (see Figure 1 in manuscript).

General Procedure for a "Delayed Addition" Experiment

Experimental setup was carried out identically to that described above in the General Procedure for Kinetic Experiments until the reaction was temperature equilibrated and ready for reagents to be added. At this point *ONLY* sodium hydroxide (or other base) was added and allowed to react with the N-methyl nitroacetamide. The absorbance signal from N-methyl nitroacetamide was observed in real time until a pre-determined amount of decomposition had occurred, at which point the acrylamide solution was injected. The reaction was then allowed to proceed normally with the remainder of the procedure being identical to that described above.

General Procedure for a pH Monitoring Experiment

A two-necked 5 mL round-bottom flask equipped with magnetic stir bar was charged with water, N-methyl nitroacetamide, and DMS solution. The ReactIR instrument was prepared as described in the General Procedure for Kinetic Experiments. The pH meter was calibrated prior to use using 4.01, 7.01, and 10.01 pH solutions. The reaction flask was then fit with the ReactIR probe through one neck and pH probe through the other. The joint between flask and each probe was sealed with several wraps of Teflon tape. The reaction flask was then lowered into a 60°C oil bath into which the pH meter's temperature probe was also placed. A webcam was set to record the pH meter and a timer placed next to it. After temperature equilibration of the reaction flask (described above) the acrylamide and sodium hydroxide solutions were injected and the timer started. The reaction was allowed to proceed normally until deemed complete by real time ReactIR monitoring at which point the webcam recording of the pH meter was terminated and a reaction aliquot was taken and analyzed as described above. A pH vs time trend was constructed using the video recording of the pH meter and timer with a data point extracted every two minutes. ReactIR data was worked up as described above.

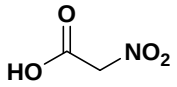
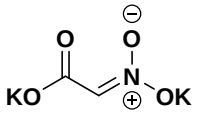
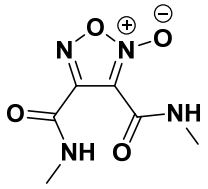
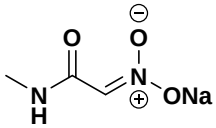
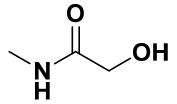
Conditions for Individual Kinetic Experiments

Standard (Figure 1): 0.45 M N-methyl nitroacetamide, 0.30 M acrylamide, 0.03 M NaOH, and ~0.05 M DMS.

Product added (Figure 4): Same as standard conditions with the addition of 0.06 M cycloaddition product.

Different bases (Figure 5): Same as standard conditions, with the exception that 0.03 M NaOH was replaced with 0.03 M of the different base in question for each experiment.

Different additives (Figure 7): Same as standard conditions, with the addition of additives in the following concentrations:

Species	Structure	Concentration
A		0.06 M
B	$MeNH_2$	0.03 M
C		0.03 M
D		0.028 M
E		0.028 M
F		0.024 M
G	$NaNO_2$	0.026 M

Investigation of pH Dependence

The dependence of reaction kinetics on pH was investigated using bases of different strength and through careful adjustments of the reaction's initial pH. It was found that while using bases of different strength may have an effect on the length of induction period, the induction period persisted for all bases used. The pH followed the same general trend for each base used, though somewhat shifted towards an increase or decrease in absolute pH number depending on the strength of the base. There was no single pH value that was found to correspond to a point where there induction period effectively ended and the reaction took off.

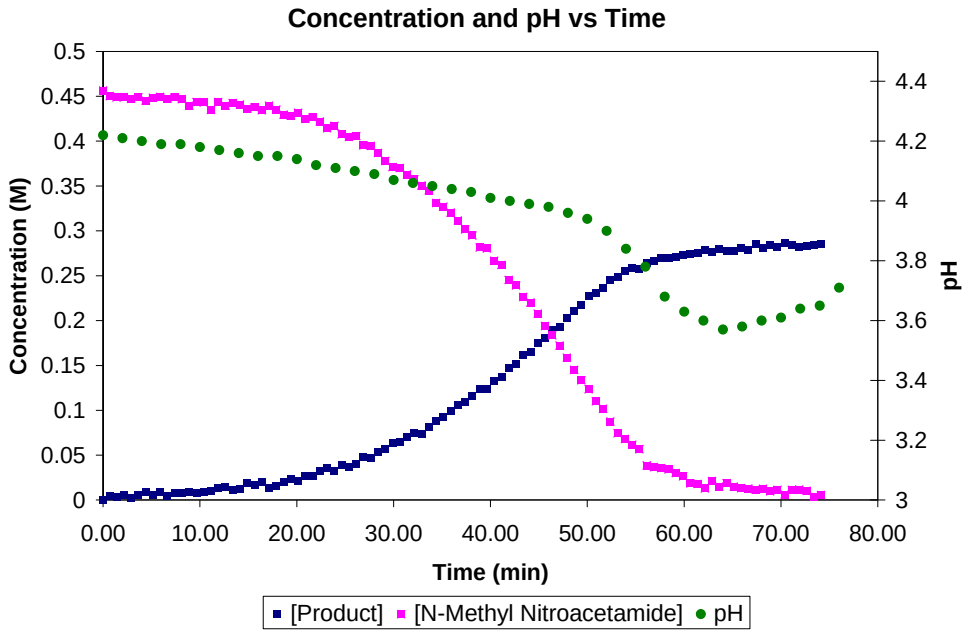


Figure S1 – pH trace of a reaction run using "Standard" conditions

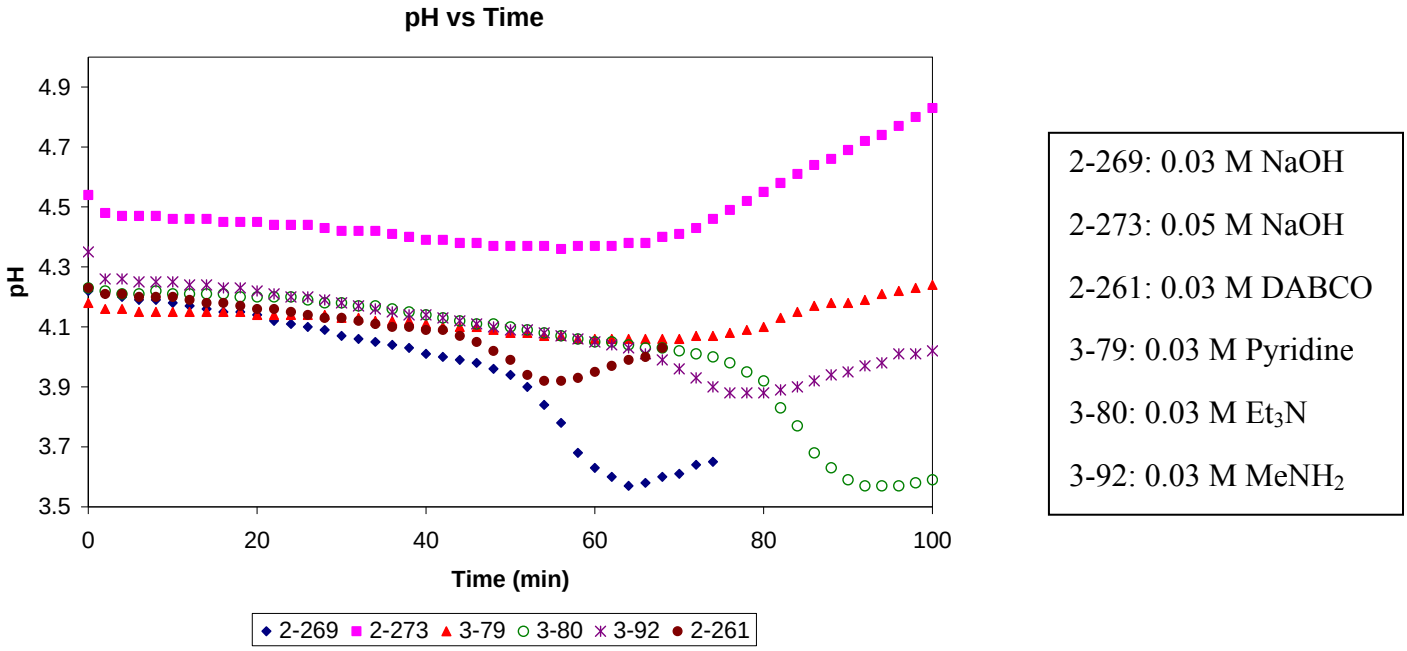


Figure S2 – pH traces from reactions with different bases, all reactions employ "standard" conditions with the base as noted to right of the plot

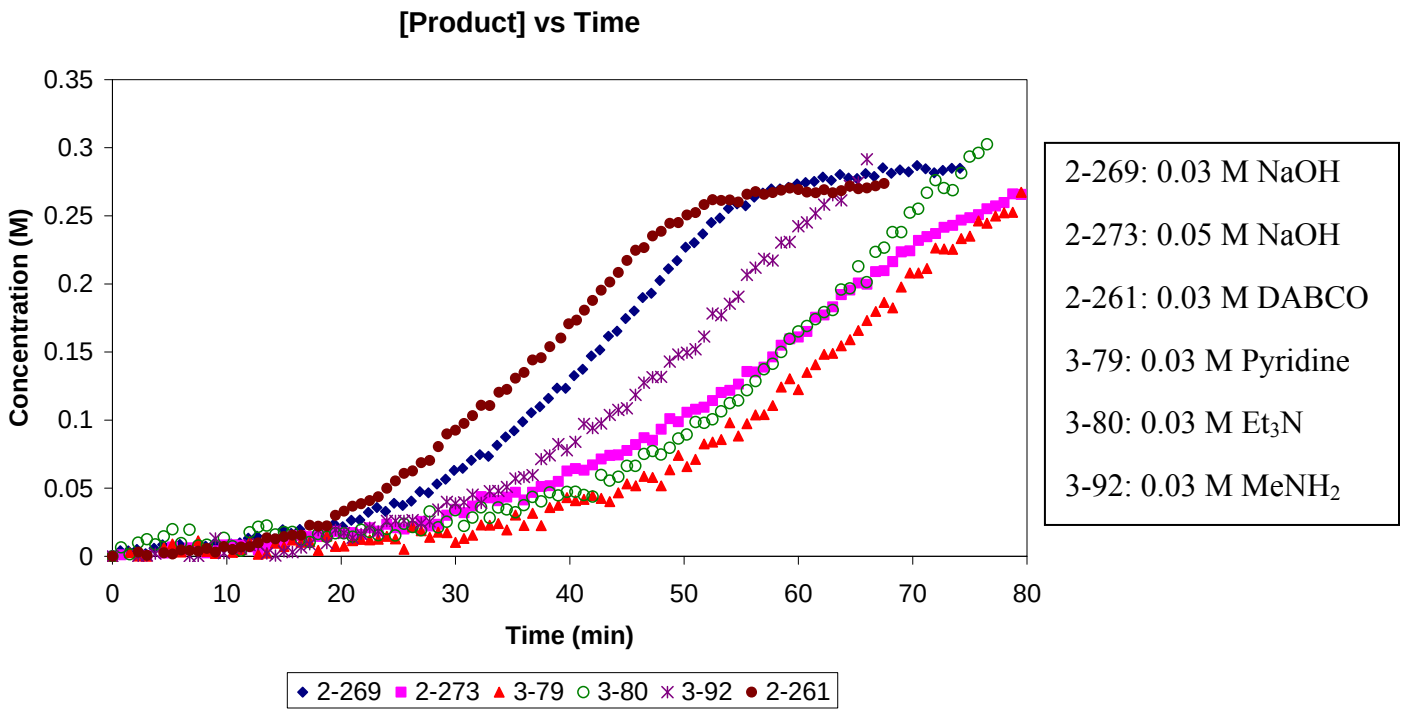


Figure S3 – Product vs time traces for reactions with different bases as depicted in Figure S2

The importance of the slow decrease in pH over the course of reaction was investigated by carefully lowering the initial pH and observing the effect it had on reaction kinetics. To avoid altering any of the reaction dynamics other than pH, a solid supported acid (Amberlyst 15 Hydrogen Form dry) was used to lower the pH. The reaction was setup as described in the General Procedure for a pH Monitoring Experiment, and just after initiation with sodium hydroxide a small amount of Amberlyst was added through a large bore needle to the reaction mixture, lowering the pH slightly. This had no notable affect on the reaction kinetics (Figure S4). The combination of this result with the previously described pH traces indicates that the reaction is not controlled by pH. Rather, pH is just a physical parameter reflecting the changing reaction medium.

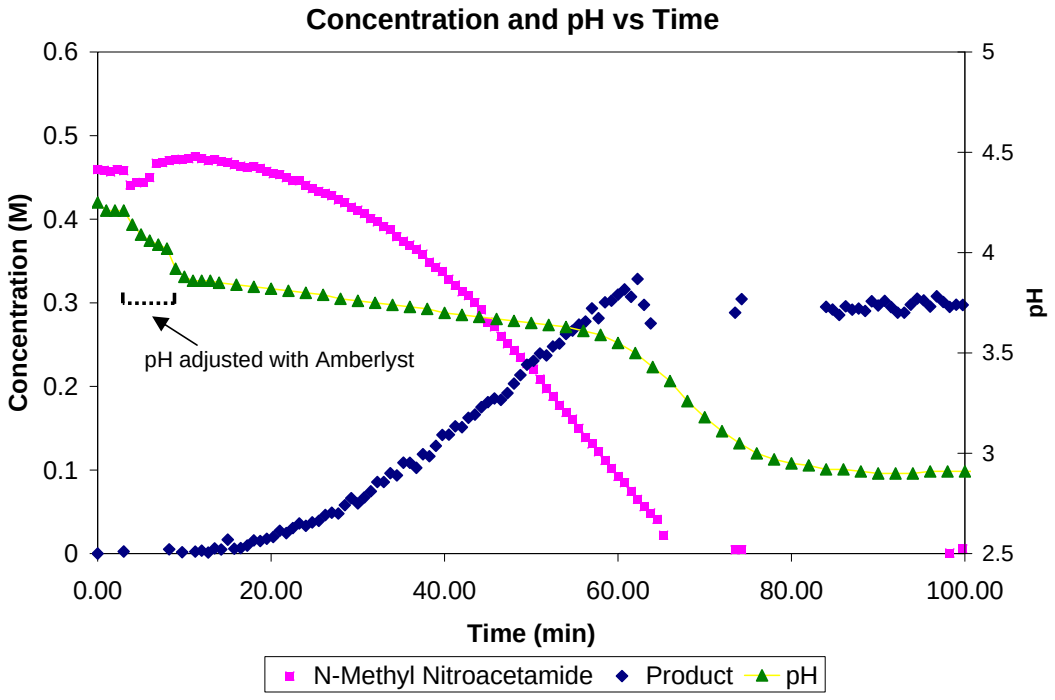


Figure S4 – Concentration and pH traces for a reaction under "standard" conditions for which the initial pH was adjusted with Amberlyst solid supported acid

Detection of NO_2^- during nitroacetamide decomposition

The Griess test (Figure S8) was used to qualitatively check for the presence of NO_2^- the decomposition of N-methyl-2-nitroacetamide.

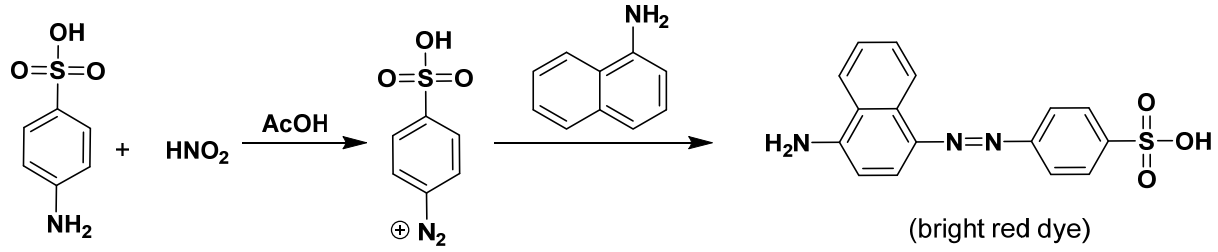


Figure S8 – Chemical reactions comprising the Griess test for NO_2^-

Preparation of Griess Reagents

Solution A: To a 20 mL scintillation vial was added 5 M acetic acid (10 mL) and 1-naphthylamine (0.054 g, 0.38 mmol). This was sonicated and stirred until a homogeneous solution was achieved.

Solution B: To a 20 mL scintillation vial was added 5 M acetic acid (10 mL) and sulfanilic acid (0.080 g, 0.46 mmol). This was sonicated and stirred until a homogeneous solution was achieved.

The addition of five drops of each solution to vial containing 1 mL of water and a few grains of $NaNO_2$ resulted in a bright red solution, representative of a positive test for NO_2^-.

Detection of NO_2^- during a reaction

A decomposition reaction of N-methyl-2-nitroacetamide was prepared using the standard procedure listed above with the exclusion of the acrylamide reagent and initial reagent concentrations of 0.55 M N-methyl-2-nitroacetamide and 0.05 M NaOH. At 6, 25, 52, and 67 minutes a small aliquot (*ca* 0.05 mL) was taken (Figure S9) then mixed with *ca*. 1 mL water and *ca*. 10 drops each of solutions A and B. The solution was shaken and any color change was observed. The first three aliquots all gave a positive result (red / pink color) while the last aliquot did not. The second aliquot also gave a much

darker color than the first and third (See picture in Figure S10). These results confirm that NO_2^- is produced during the decomposition of N-methyl-2-nitroacetamide.

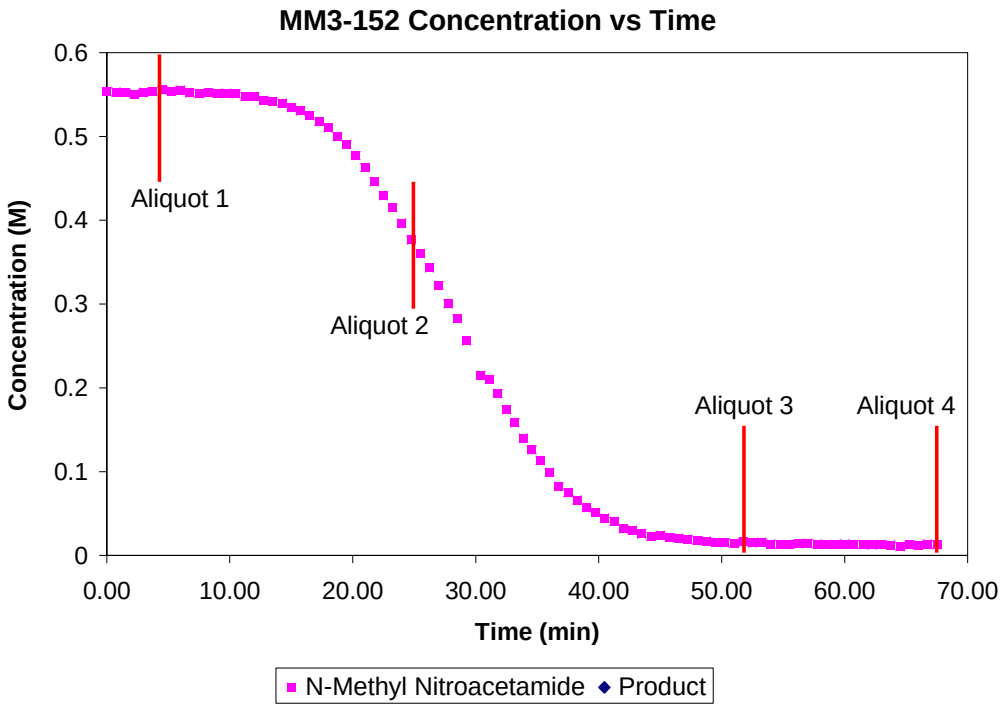


Figure S9 – N-methyl nitroacetamide decomposition and aliquot times

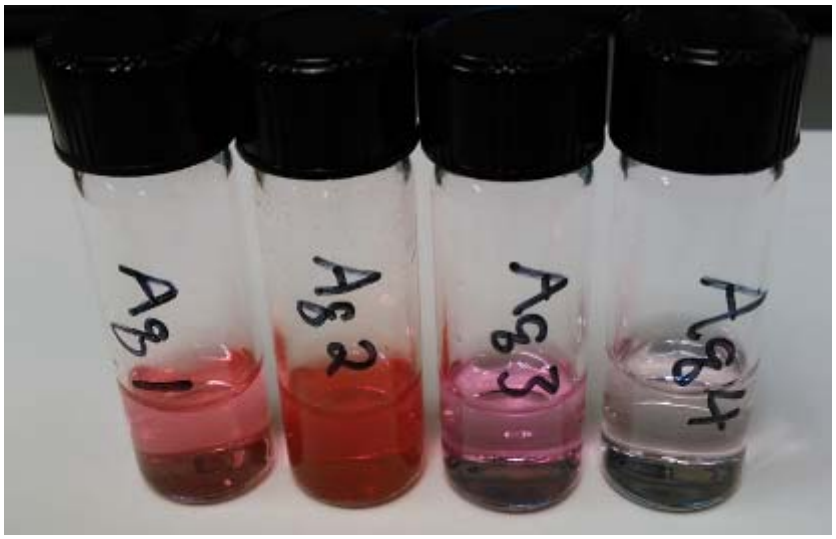


Figure S10 – Results of Griess test from aliquots 1-4 (left to right)

Though the tests were not prepared quantitatively, they can be used as a rough qualitative estimate showing that nitrite concentration builds in the first part of the reaction then decreases as the

reaction continues and eventually is no longer detectable. This decomposition likely occurs through protonation of nitrite to give nitrous acid, which is known to decompose into NO_2, NO, and water.[1]

Each of the reagents involved in the reaction were tested separately and all gave a negative result. This, combined with the fact that the fourth aliquot from the actual reaction also gave a negative result, shows that the results described above are not false positives.

Investigation of reaction dependence on NO_2^- counter-cation

Three reactions were performed using the general procedure for a kinetics experiment outlined above except for the substitution of 0.03 M NaOH with 0.026 M of nitrite source. Sodium nitrite, potassium nitrite, and tetrabutylammonium nitrite were each used individually in one reaction. Kinetic data acquired via ReactIR spectroscopy shows that all three reactions behaved identically (Figure S11), indicating that the counter-cation does not play a kinetically significant role in the reaction mechanism.

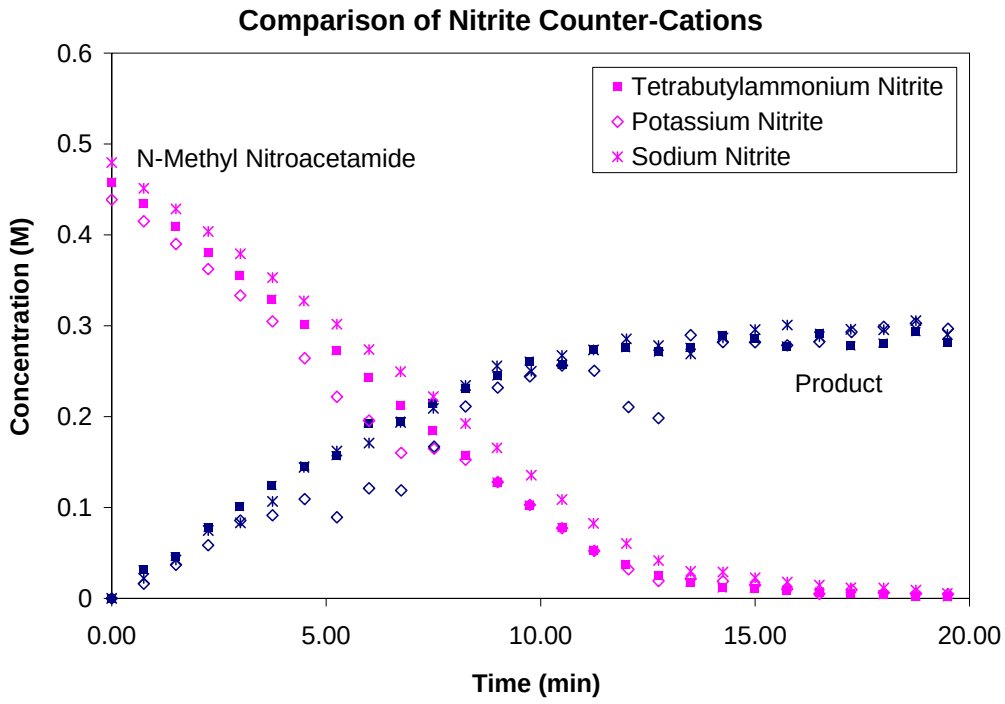


Figure S11 – Comparison of cycloaddition reaction kinetics with different nitrite counter-cations

Mechanistic investigation using ^{15}N labeled sodium nitrite

A vial was charged with N-methyl nitroacetamide (0.080 g, 0.68 mmol), aqueous DMS solution (0.10 mL, 0.055 mmol), and water (1.11 mL). This was lowered into a 60°C oil bath and aqueous acrylamide solution was added (0.19 mL, 0.45 mmol), followed by an aqueous solution of ^{15}N labeled sodium nitrite (0.10 mL, 0.09 mmol). After 12 minutes the reaction vial was removed from the oil bath and all volatiles removed under vacuum to yield a yellow solid. The solid was triturated with cold water to yield a white solid primarily composed of product isoxazoline and minor impurities. A sample of this material was analyzed by HRMS and ^{13}C NMR and compared to similar analyses of normal unlabeled product.

HRMS Analysis for both the unlabeled product and the product from this experiment was performed on a Agilent 1100 Series LC stack with an Agilent LC/MSD ToF detector. The ions m/z of 172.0-172.1 and 173.0-173.1 were extracted, their areas integrated, and a ratio of 172 / 173 calculated for each sample. The ratio for unlabeled product was 100 : 7, compared to a predicted ratio of 100 : 6.7 (based on natural isotopic abundances). The ratio for the product from this experiment was 100 : 28, indicating ~20% incorporation of ^{15}N into the product material, matching the 20% loading of ^{15}N labeled catalyst used in the experiment.

^{13}C NMR Analysis of the product material from this experiment showed small couplings present for the quaternary carbon α to the isoxazoline nitrogen (C5) and the nearby amide carbonyl (C6) consistent with ^{15}N – ^{13}C coupling (Figures S12 and S13). (^{13}C NMR spectra recorded at 600 MHz in DMSO-d_6).

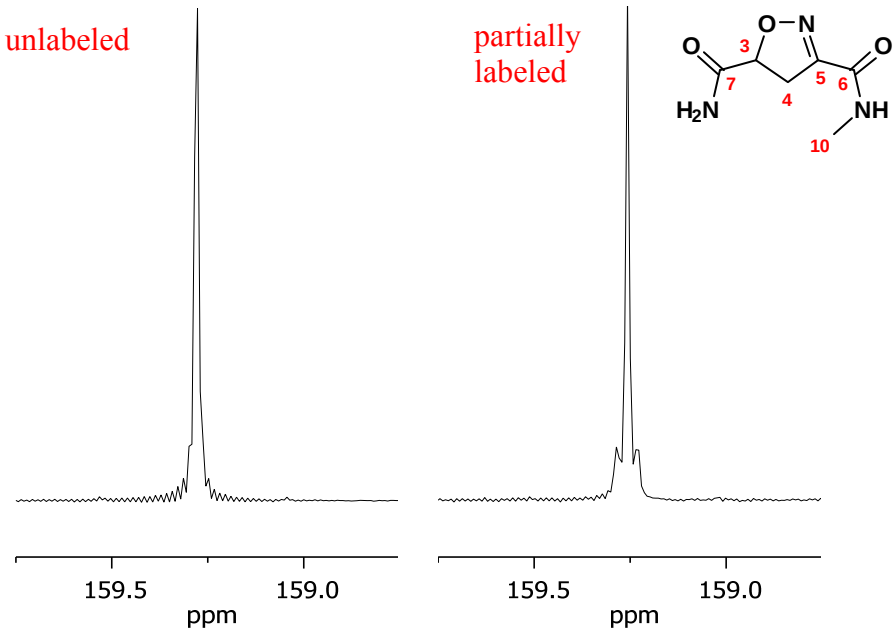


Figure S12 – Small coupling present at the base of ^{13}C signal from C6. The signal in the spectrum of the partially labeled material from this experiment is presumably composed of a singlet for the unlabeled product and a doublet (J = 8.6 Hz) for the ^{15}N labeled product.

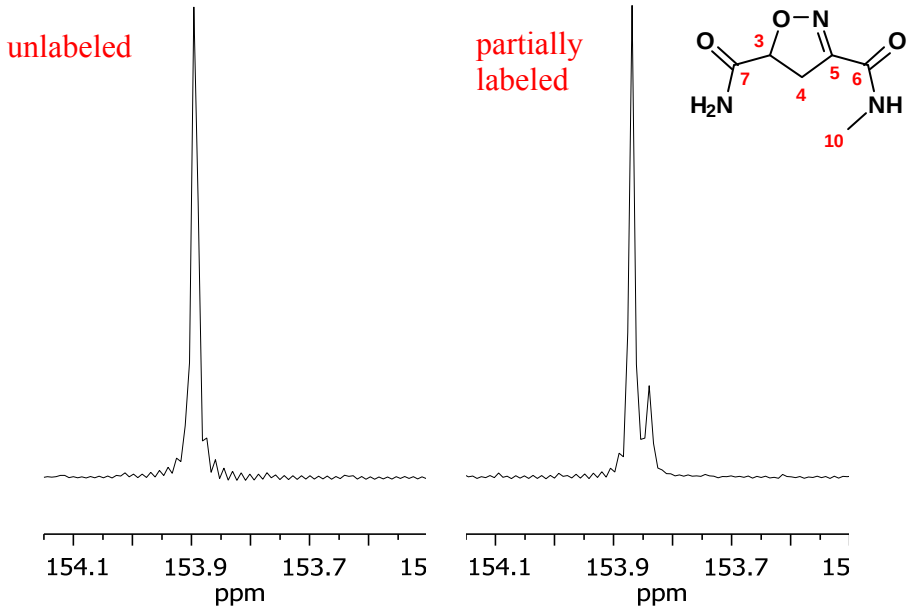


Figure S13 – Small coupling present at the base of ^{13}C signal from C5. The signal in the spectrum of the partially labeled material from this experiment is presumably composed of a singlet for the unlabeled product and a doublet (J = 4.4 Hz) for the ^{15}N labeled product, one of whose signals overlaps in chemical shift with the singlet.

COPASI Kinetic Modeling

Global kinetic modeling was performed using COPASI modeling software (http://www.copasi.org).[2] A model comprised of the chemical steps shown in Figures S5 and S6 was constructed and fit to product and N-methyl nitroacetamide concentration vs time data from a reaction run under standard conditions and one with sodium nitrite added.

#	Name	Reaction
1	reaction_1	A + NaOH = Asalt
2	reaction_2	A = Ataut
3	reaction_3	Ataut + NaOH -> int1
4	reaction_6	int2 -> int3 + cat + H2O
5	reaction_7	int3 + B -> C
6	reaction_8	2 * int3 -> dimer
7	reaction_5	A + cat -> int2
8	reaction_4	int1 -> Alc + cat

Figure S5 – Kinetic model built in COPASI modeling software

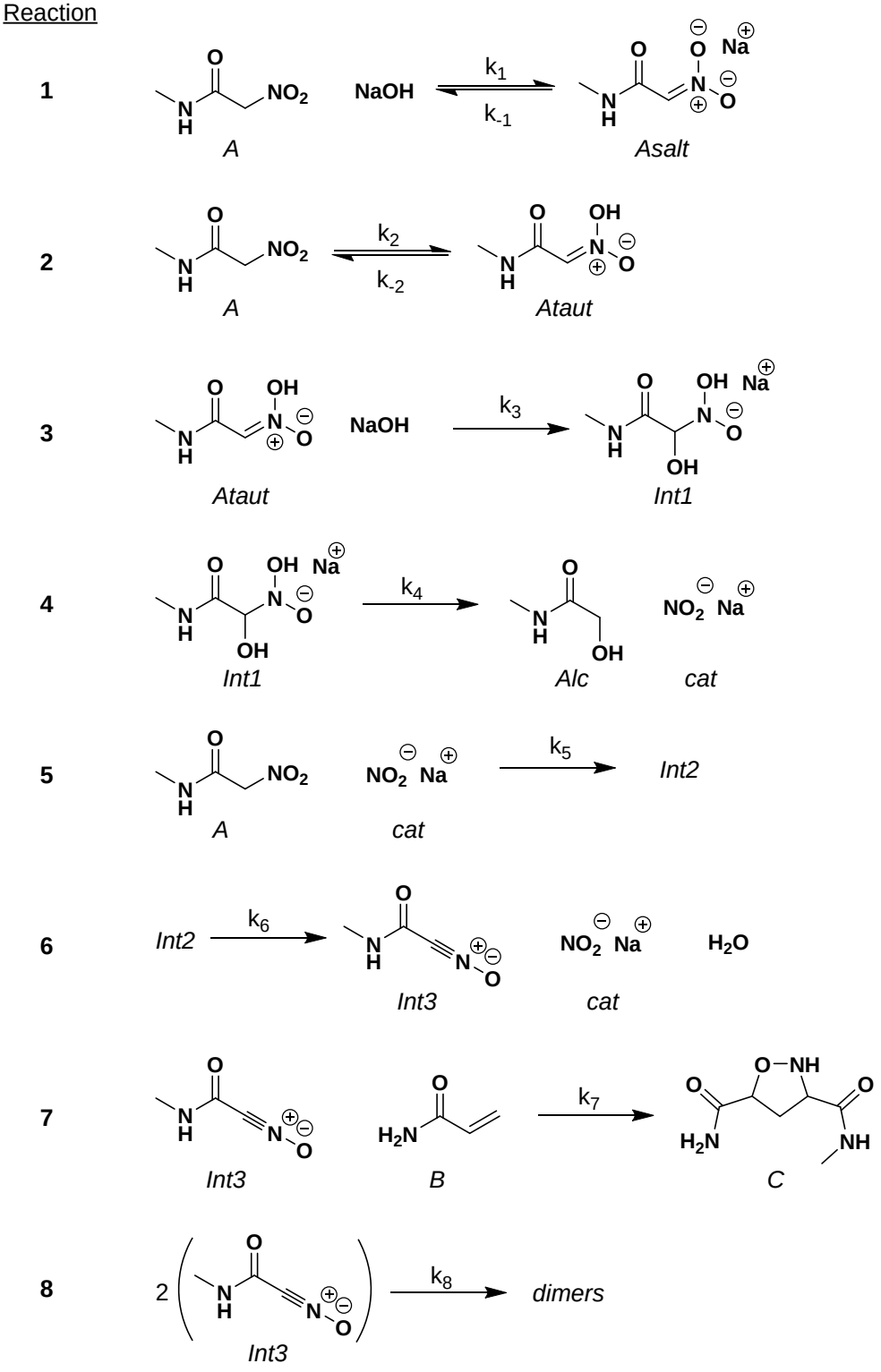


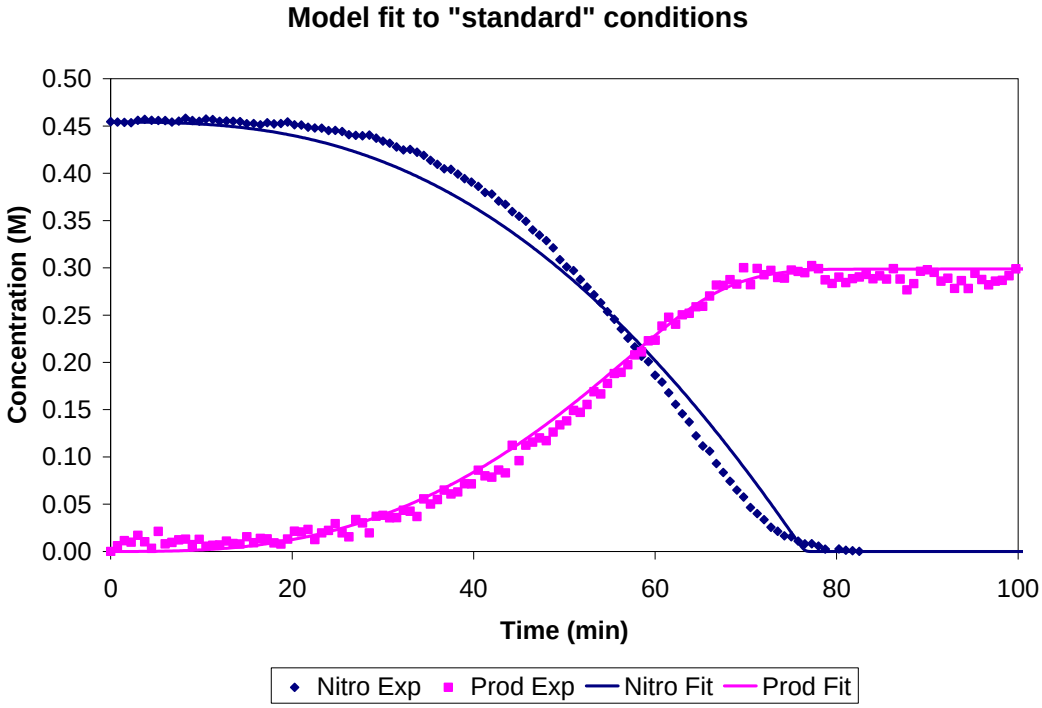
Figure S6 – Schematic diagram of the kinetic model built in COPASI

Parameter estimation was performed on k_3, k_4, k_5, and k_6 with the constraint of: $0 < k_6 < 5$. This constraint was in part used to force the model to meet known chemical reactivity (relative to kinetic constants of other steps) and also used to force the model out of false minima. The remaining kinetic

constants were fixed to the values shown in Table S1. The model was optimized using the Hooke & Jeeves method to yield the kinetic constants shown in Table S1. It is not proposed that individually these constants have any intrinsic significance as the number of kinetic constants being fit results in this model being underdetermined. Rather, this is intended to demonstrate that it is possible for the proposed chemical mechanism to yield kinetics with the same characteristics as those experimentally observed. Fits of the model to the two data sets used to optimize it are shown in Figure S7.

Kinetic Constant	Value	Std. Deviation	Kinetic Constant	Value	Std. Deviation
k_1	200,000	-	**k_4**	**0.018**	**0.093**
k_{-1}	200	-	**k_5**	**5617**	**47284**
k_2	100	-	**k_6**	**1.16**	**0.01**
k_{-2}	100,000	-	k_7	500	-
k_3	**18,057**	**94,780**	k_8	1500	-

Table S1 – Kinetic constants as optimized via COPASI modeling; constants shown in bold were optimized by the model, all others were fixed



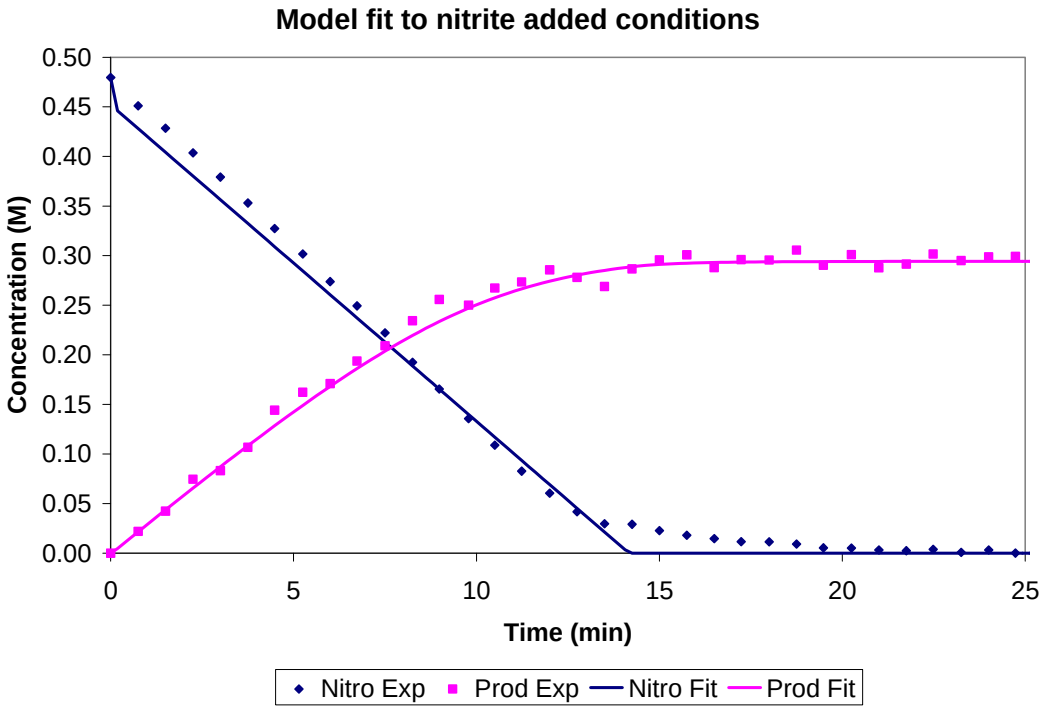
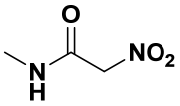


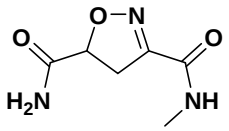
Figure S7 – Fit of COPASI model to reactions under standard and nitrite added conditions

Experimental Procedures

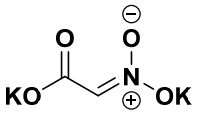
N-methyl-2-nitroacetamide (1). 

A 25 mL Schlenk tube equipped with magnetic stir bar was charged with a 40% w/w aqueous solution of methylamine (10 mL) and ethyl nitroacetate (2.22 mL, 20 mmol) then sealed and stirred at room temperature for 48 hrs. Nitrogen gas was then blown through the solution for 1 hr, followed by transfer to a 100 mL round-bottom flask and addition of aqueous 5% HCl (10 mL). This was then concentrated to *ca.* 2 mL and a second potion of aqueous 5% HCl (10 mL) was added and the solution concentrated to a yellow solid. Dichloromethane (90 mL) was added and the mixture sonicated for 15 minutes then left to stir for 1 hr. The solids were separated via vacuum filtration and the filtrate concentrated to *ca*. 20 mL. This solution was then left to evaporate over several days and form clear crystals. Crystals were separated from the mother liquor via vacuum filtration, washed with cold dichloromethane (2 x 5 mL), and dried under high vacuum to provide **1** (1.98 g, 84 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.11 (s, 2H),

2.90 (d, J = 5 Hz, 3H). ^{13}C NMR (500 MHz, $CHCl_3$) δ 160.82, 77.88, 26.89. MS (ESI): Calcd for $C_3H_6N_2O_3$: 118.04; observed: 119.05 (MH^+).

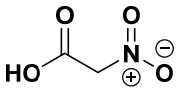
Methyl-4,5-dihydroisoxazole-3,5-dicarboxamide (3). 

A 1 dram vial equipped with magnetic stir bar was charged with water (0.71 mL), N-methyl nitroacetamide (0.054 g, 0.46 mmol), and 0.10 mL of aqueous dimethyl sulfone solution (.005 g, 0.054 mmol). The vial was lowered into a 60°C oil bath, allowed to equilibrate for *ca*. 5 min and then 0.13 mL of aqueous acrylamide (0.023 g, 0.32 mmol) and 0.06 mL of sodium hydroxide solution (0.001 g, 0.03 mmol) were injected. The reaction was allowed to stir for 90 minutes then removed from the oil bath and cooled to room temperature, then in an ice bath. The yellowish white precipitate was separated via vacuum filtration and washed with cold water (2 mL) to yield **3** (0.045 g, 81%). If impurities are present in the precipitate they can be removed via trituration with cold water. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.63 (s, 1H), 7.42 (s, 1H), 5.03 (dd, J = 11.9, 7.1 Hz, 1H) 3.41 (dd, J = 11.8, 17.7 Hz, 1H). 3.27 (dd, J = 17.7, 7.1 Hz, 1H), 2.68 (d, 4.2 Hz, 3H). ^{13}C NMR (600 MHz, $CDCl_3$) δ 171.55, 159.70, 154.32, 80.18, 38.16, 26.23. MS (ESI): Calcd for $C_6H_{11}N_3O_3$: 171.06; observed: 172.07 (MH^+), 194.05 (MNa^+).

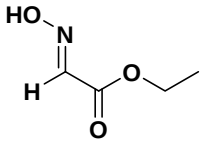
Dipotassium salt of 2-nitroacetic acid (Species C). 

A 150 mL round-bottom flask equipped with magnetic stir bar was charged with water (12 mL) and potassium hydroxide (22.4 g, 400 mmol). The flask was then equipped with a vented reflux condenser and nitromethane (5.37 mL, 100 mmol) was added drop-wise over 30 min via syringe. The reaction was then refluxed for 1 hr with reduced stirring as the reaction mixture thickened and took on a brownish red color. After cooling to room temperature the reaction mixture was filtered and the residue washed with methanol (3 x 15 mL). The resulting pale brown powder was dried under high vacuum to yield pure

Species C (8.13 g, 90 %). ^{1}H NMR (500 MHz, D_2O) δ 6.52 (s, 1H). ^{13}C NMR (500 MHz, D_2O) δ 170.30, 111.96.

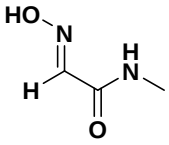
2-Nitroacetic acid (Species A). 

Trifluoroacetic acid (0.92 mL, 12 mmol) was added to HPLC water (5 mL) in a 20 mL scintillation vial. To a separate 20 mL scintillation vial was added dipotassium salt of 2-nitroacetic acid (0.91 g, 5 mmol) and HPLC water (5 mL). Both vials were cooled in a dry ice / ethylene glycol bath until frozen. The TFA solution was allowed to warm just enough to melt, then immediately added to the vial containing the dipotassium salt of nitroacetic acid. The vial was removed from the cold bath and the solutions allowed to mix as they melted. Once mixed and completely homogeneous the solution was saturated with NaCl and extracted with diethyl ether (3 x 20 mL). The organic layers were combined, dried over anhydrous magnesium sulfate, and concentrated from chloroform to give **Species A** as a yellowish white solid (0.126 g, 24%). This material appeared to be stable as a solid, but begins to decompose when in solution. ^{1}H NMR (400 MHz, CD_3CN) δ 5.28 (s, 2H). ^{13}C NMR (400 MHz, CD_3CN) δ 164.69, 77.58.

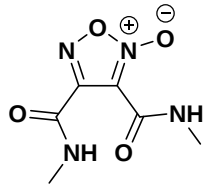
Ethyl 2-(hydroxyimino)acetate. 

A 25 mL round-bottom flask equipped with magnetic stir bar was charged with hydroxylamine hydrochloride (0.69 g, 10 mmol), reagent grade acetonitrile (7.3 mL), and water (0.8 mL). To this was added a solution of 50% ethyl glyoxylate in toluene (1.98 mL, 10 mmol) and the reaction stirred for 5 minutes at room temperature. Triethylamine (1.40 mL, 10 mmol) was then added dropwise over 10 minutes and the reaction stirred for an additional 1 hr before being concentrated via rotary evaporation to a white residue. The residue was dissolved in a 50/50 mixture of water and diethyl ether (30 mL total), the organic layer separated, and the remaining aqueous layer extracted with diethyl ether (2 x 20

mL). The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, and concentrated under high vacuum to yield the product as a clear oil (0.934 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 4.33 (q, *J* = 7.2 Hz, 2H), 1.35 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (500 MHz, $CHCl_3$) δ 162.53, 141.83, 61.98, 14.10.

2-(Hydroxyimino)-N-methylacetamide. 

A schlenk flask was charged with a 40% aqueous methylamine solution (7 mL) to which was added ethanol (7 mL) and ethyl 2-(hydroxyimino)acetate (0.93 g, 7.98 mmol). The flask was sealed and stirred for 5.5 hrs, nitrogen gas bubbled through the solution for 1 hr, and the solution then concentrated under high vacuum to yield the product as a white solid (0.81 g, 99%). ^{1}H NMR (400 MHz, D_2O) δ 7.63 (s, 1H), 2.84 (s, 3H). ^{13}C NMR (400 MHz, D_2O) δ 164.52, 144.02, 25.64. MS (ESI): Calcd for $C_3H_6N_2O_2$: 102.09; observed: 103.2 (MH^+).

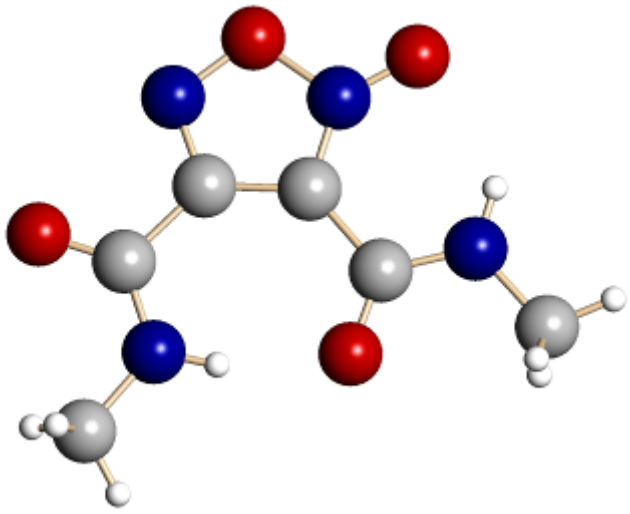
3,4-Bis(methylcarbamoyl)-1,2,5-oxadiazole 2-oxide (Species D). 

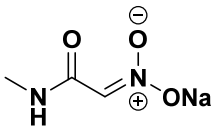
Method A. A 20 mL scintillation vial equipped with magnetic stir bar was charged with 2-(hydroxyimino)-N-methylacetamide (0.051 g, 0.5 mmol) and water (3 mL). This was cooled in an ice bath for *ca*. 20 minutes before an aqueous solution of sodium hypochlorite (1.67 mL, 1 mmol) was added dropwise over 2 minutes. The reaction was stirred for 20 minutes at 0°C before being extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over anhydrous magnesium sulfate and concentrated under high vacuum to give a white powder (0.017 g) containing **Species D** as the major product in addition to several other minor components.

Method B. A 50 mL round-bottom flask equipped with magnetic stir bar was charged with water (18 mL) and N-methyl nitroacetamide (0.981 g, 8.31 mmol). Sodium hydroxide solution (0.24 mL, 0.56

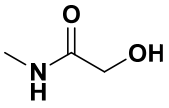
mmol) was injected and the reaction heated at 60°C for 2 hrs. The mixture was then concentrated to a yellow solid. This solid was taken up in water (15 mL) and filtered to yield a white solid (0.059 g) containing **Species D** as well as several other un-identified components. The residue was then purified via flash chromatography (1% MeOH in dichloromethane) to yield pure **Species D** (0.018 g, 2%) (R_f = 0.28, 4% MeOH in DCM). This low yield is representative of the difficulty in separating this species, not in its formation. Crude ^{1}HNMR spectra indicate that **Species D** is formed in approximately 20% or greater yield during the decomposition of N-methyl nitroacetamide. ^{1}H NMR (400 MHz, CD_3CN) δ 2.93 (d, *J* = 4.9 Hz, 3H), 2.91 (d, *J* = 4.8 Hz, 3H). ^{13}C NMR (500 MHz, CD_3CN) δ 157.73, 156.16, 152.07, 111.25, 26.82, 26.69. MS (ESI): Calcd for $C_6H_8N_4O_4$: 200.05; observed: 201.2 (MH^+), 223.1 (MNa^+), 423.1 ($2MNa^+$).

A crystal of **Species D** was grown from slow evaporation in acetonitrile and analyzed via X-ray crystallography. The CCDC deposit number is 1035874. Data were collected at 100K using a Bruker Ultra mini-rotating-anode source on a D8 platform. All data collection and refinement details are contained in Table S1 (see below). All aspects of this structure were routine; all non-hydrogen atoms were refined anisotropically and hydrogen atoms were treated as idealized contributions. All software was contained in the APEX and SHELXTL libraries distributed by Bruker XRD, Madison, WI.



Sodium salt of N-methyl-2-nitroacetamide (Species E). 

A 1 dram vial was charged with N-methyl-2-nitroacetamide (0.059 g, 0.5 mmol), sodium hydroxide solution (0.98 mL, 0.5 mmol), and water (1 mL). Once the solution became homogeneous it was concentrated under high vacuum to yield **Species E** as an off white solid which was used without further purification or spectroscopic characterization.

2-Hydroxy-N-methylacetamide (Species F). 

A 15 mL schlenk flask was charged with a 40% aqueous solution of methylamine (4.33 mL, 50 mmol) and ethyl glycolate (0.95 mL, 10 mmol). The reaction was stirred at room temperature for 3.5 hrs and then nitrogen gas was bubbled through the solution for 1 hr. The remaining solution was then concentrated under high vacuum to yield **Species F** as a white solid (0.84 g, 94%).^{1}H NMR (500 MHz, $CDCl_3$) δ 4.05 (s, 2H), 2.85 (d, J = 5 Hz, 3H). ^{13}C NMR (500 MHz, $CHCl_3$) δ 173.38, 62.10, 25.76. MS (ESI): Calcd for $C_3H_7NO_2$: 89.09; observed: 90.06 (MH^+), 112.04 (MNa^+).

Crystal Structure Information for 3,4-Bis(methylcarbamoyl)-1,2,5-oxadiazole 2-oxide (Species D)

Table S1. Crystal data and structure refinement for black09.

Identification code	black09	
Empirical formula	C6 H8 N4 O4	
Formula weight	200.16	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/n	
Unit cell dimensions	a = 4.6088(5) Å	α= 90°.
	b = 15.9043(18) Å	β= 98.710(3)°.
	c = 11.5802(14) Å	γ = 90°.
Volume	839.04(17) $Å^3$	
Z	4	
Density (calculated)	1.585 Mg/m^3	
Absorption coefficient	0.135 mm^{-1}	
F(000)	416	
Crystal size	0.400 x 0.350 x 0.080 mm^3	
Theta range for data collection	2.192 to 25.327°.	
Index ranges	-5<=h<=5, -17<=k<=19, -13<=l<=13	
Reflections collected	5861	
Independent reflections	1533 [R(int) = 0.0216]	
Completeness to theta = 25.000°	99.9 %	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1533 / 0 / 127	
Goodness-of-fit on F^2	1.083	
Final R indices [I>2sigma(I)]	R1 = 0.0464, wR2 = 0.1204	
R indices (all data)	R1 = 0.0518, wR2 = 0.1241	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.908 and -0.316 $e.Å^{-3}$	

Table S2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for black09. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(4)	-3663(3)	7690(1)	1039(1)	25(1)
O(3)	2031(4)	6222(1)	3082(1)	22(1)
O(2)	5979(4)	6563(1)	4401(1)	24(1)
O(1)	3489(3)	9066(1)	3492(1)	18(1)
N(1)	6802(4)	8291(1)	4660(2)	15(1)
N(3)	-77(4)	6644(1)	2349(2)	19(1)
N(4)	-1235(4)	8835(1)	1857(2)	16(1)
N(2)	3943(4)	6835(1)	3707(2)	16(1)
C(1)	8265(5)	9044(1)	5176(2)	20(1)
C(2)	4455(4)	8376(1)	3846(2)	14(1)
C(3)	2977(4)	7591(1)	3353(2)	13(1)
C(4)	445(4)	7448(1)	2487(2)	14(1)
C(5)	-1681(4)	8018(1)	1717(2)	16(1)
C(6)	-3112(5)	9446(2)	1171(2)	23(1)

Table S3. Bond lengths [Å] and angles [°] for black09.

O(4)-C(5)	1.227(3)
O(3)-N(3)	1.366(2)
O(3)-N(2)	1.434(2)
O(2)-N(2)	1.219(2)
O(1)-C(2)	1.231(3)
N(1)-C(2)	1.330(3)
N(1)-C(1)	1.457(3)
N(3)-C(4)	1.307(3)
N(4)-C(5)	1.322(3)
N(4)-C(6)	1.454(3)
N(2)-C(3)	1.325(3)
C(2)-C(3)	1.494(3)
C(3)-C(4)	1.437(3)
C(4)-C(5)	1.520(3)
N(3)-O(3)-N(2)	107.74(14)
C(2)-N(1)-C(1)	118.92(18)

C(4)-N(3)-O(3)	107.70(17)
C(5)-N(4)-C(6)	121.39(19)
O(2)-N(2)-C(3)	135.68(19)
O(2)-N(2)-O(3)	116.34(17)
C(3)-N(2)-O(3)	107.98(16)
O(1)-C(2)-N(1)	122.76(19)
O(1)-C(2)-C(3)	119.82(19)
N(1)-C(2)-C(3)	117.42(18)
N(2)-C(3)-C(4)	105.81(18)
N(2)-C(3)-C(2)	121.85(18)
C(4)-C(3)-C(2)	132.34(18)
N(3)-C(4)-C(3)	110.77(19)
N(3)-C(4)-C(5)	114.88(18)
C(3)-C(4)-C(5)	134.35(19)
O(4)-C(5)-N(4)	125.7(2)
O(4)-C(5)-C(4)	118.3(2)
N(4)-C(5)-C(4)	116.02(18)

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for black09. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

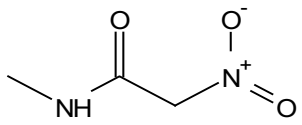
	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(4)	20(1)	25(1)	27(1)	0(1)	-7(1)	-3(1)
O(3)	28(1)	12(1)	24(1)	0(1)	2(1)	-2(1)
O(2)	23(1)	23(1)	23(1)	2(1)	-3(1)	4(1)
O(1)	20(1)	13(1)	21(1)	-1(1)	-2(1)	1(1)
N(1)	16(1)	13(1)	16(1)	-1(1)	0(1)	0(1)
N(3)	20(1)	18(1)	19(1)	1(1)	0(1)	0(1)
N(4)	16(1)	17(1)	16(1)	1(1)	-2(1)	2(1)
N(2)	18(1)	15(1)	15(1)	1(1)	2(1)	1(1)
C(1)	19(1)	18(1)	21(1)	-4(1)	0(1)	-3(1)
C(2)	14(1)	15(1)	14(1)	-1(1)	5(1)	-1(1)
C(3)	14(1)	14(1)	13(1)	0(1)	5(1)	1(1)
C(4)	14(1)	14(1)	14(1)	-2(1)	4(1)	-1(1)
C(5)	13(1)	22(1)	14(1)	-1(1)	4(1)	0(1)
C(6)	24(1)	22(1)	22(1)	5(1)	1(1)	6(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for black09.

	x	y	z	U(eq)
H(1A)	7460	7788	4883	18
H(4A)	229	9013	2377	20
H(1B)	9989	8881	5735	30
H(1C)	6906	9364	5581	30
H(1D)	8885	9392	4559	30
H(6A)	-2780	10003	1526	34
H(6B)	-5172	9286	1152	34
H(6C)	-2648	9459	373	34

[1] Park, J.; Lee, Y. *J. Phys. Chem.*, **1988**, 92, 6294 – 6302.

[2] Hoops S.; Sahle S.; Gauges R.; Lee C.; Pahle J.; Simus N.; Singhal M.; Xu L.; Mendes P.; Kummer U. *Bioinformatics*. **2006**, 22, 3067 – 3074. (2006).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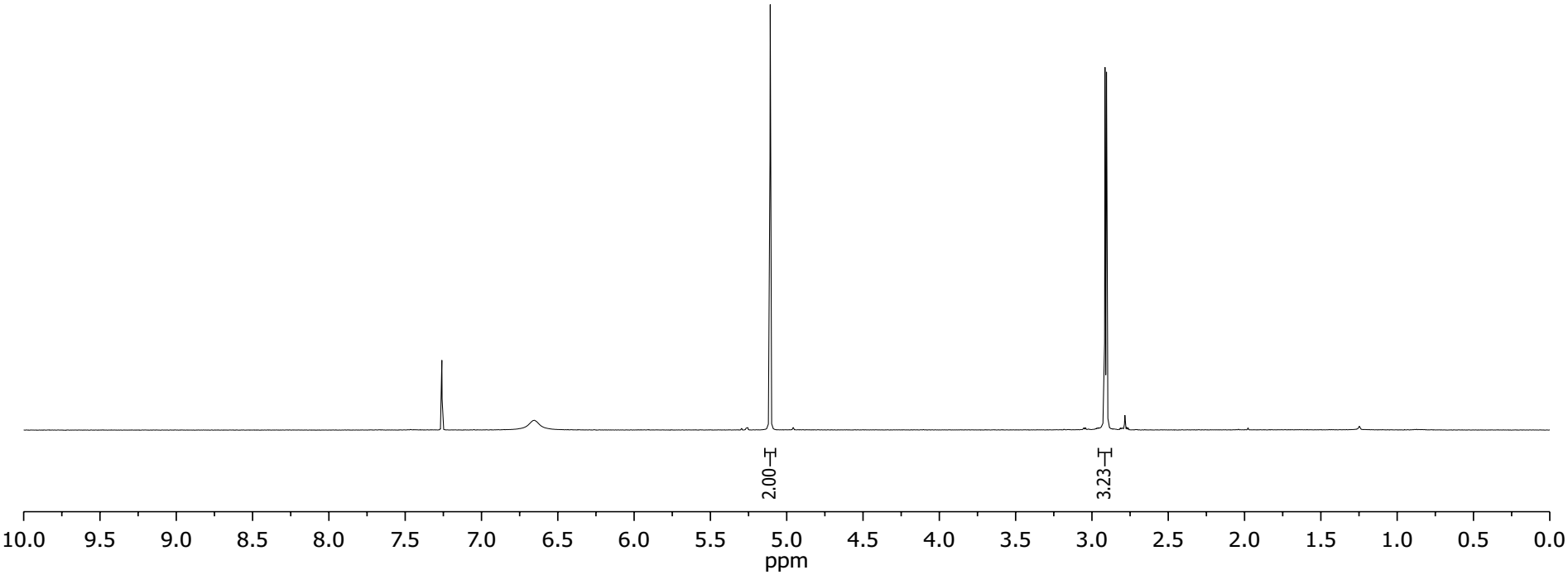
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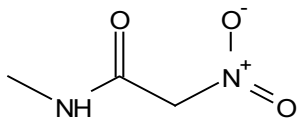
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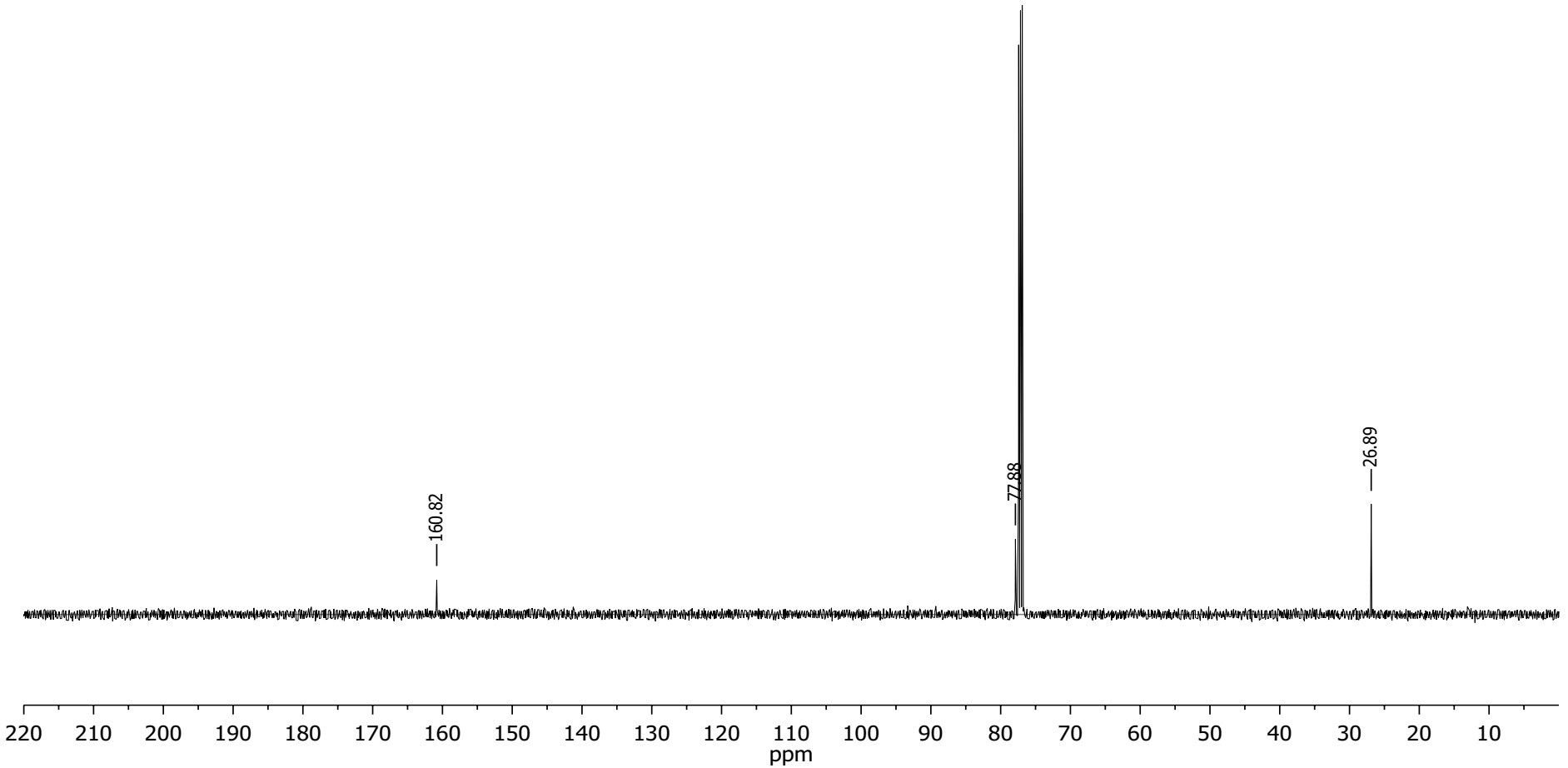
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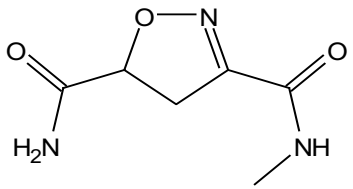
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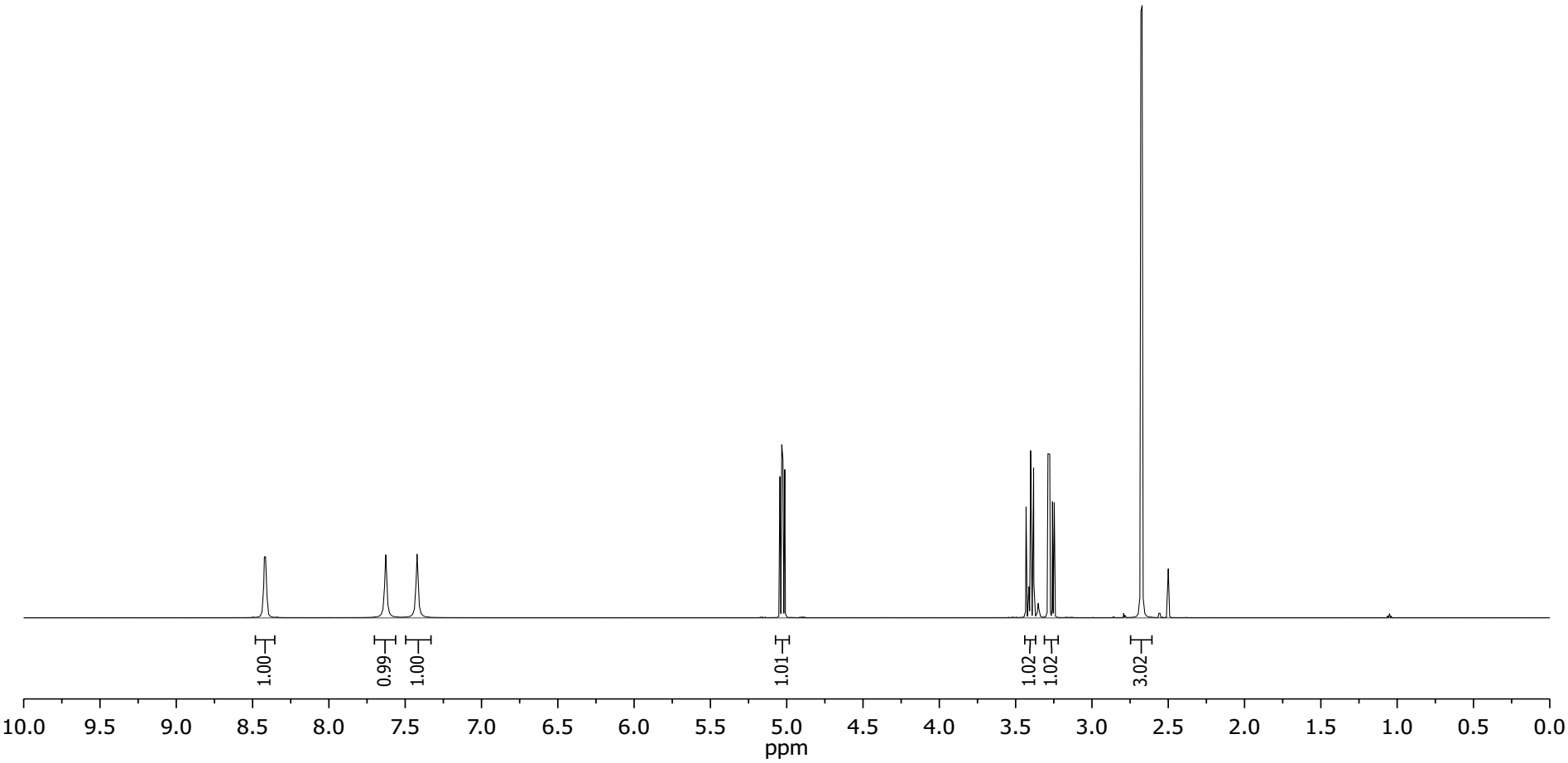
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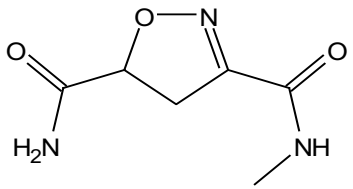
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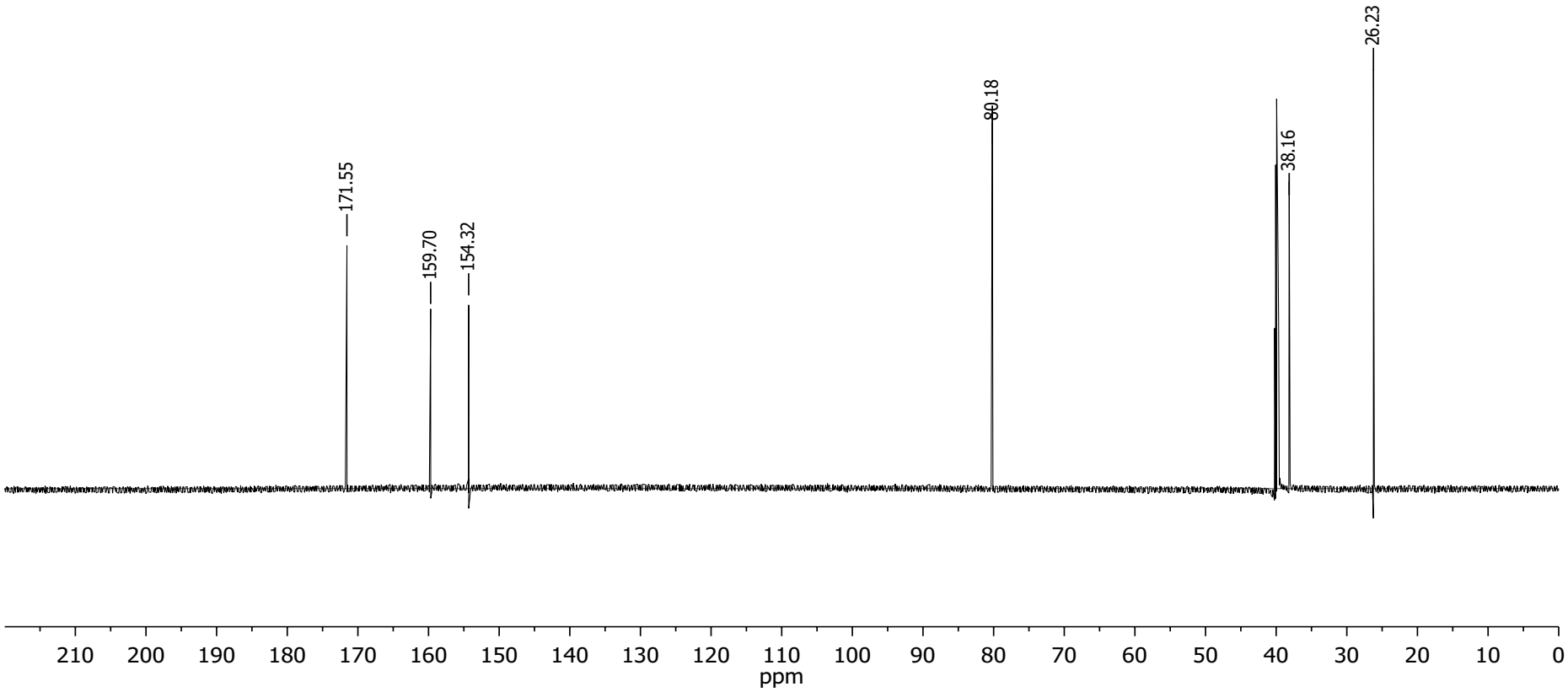
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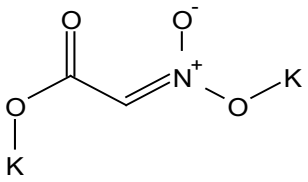
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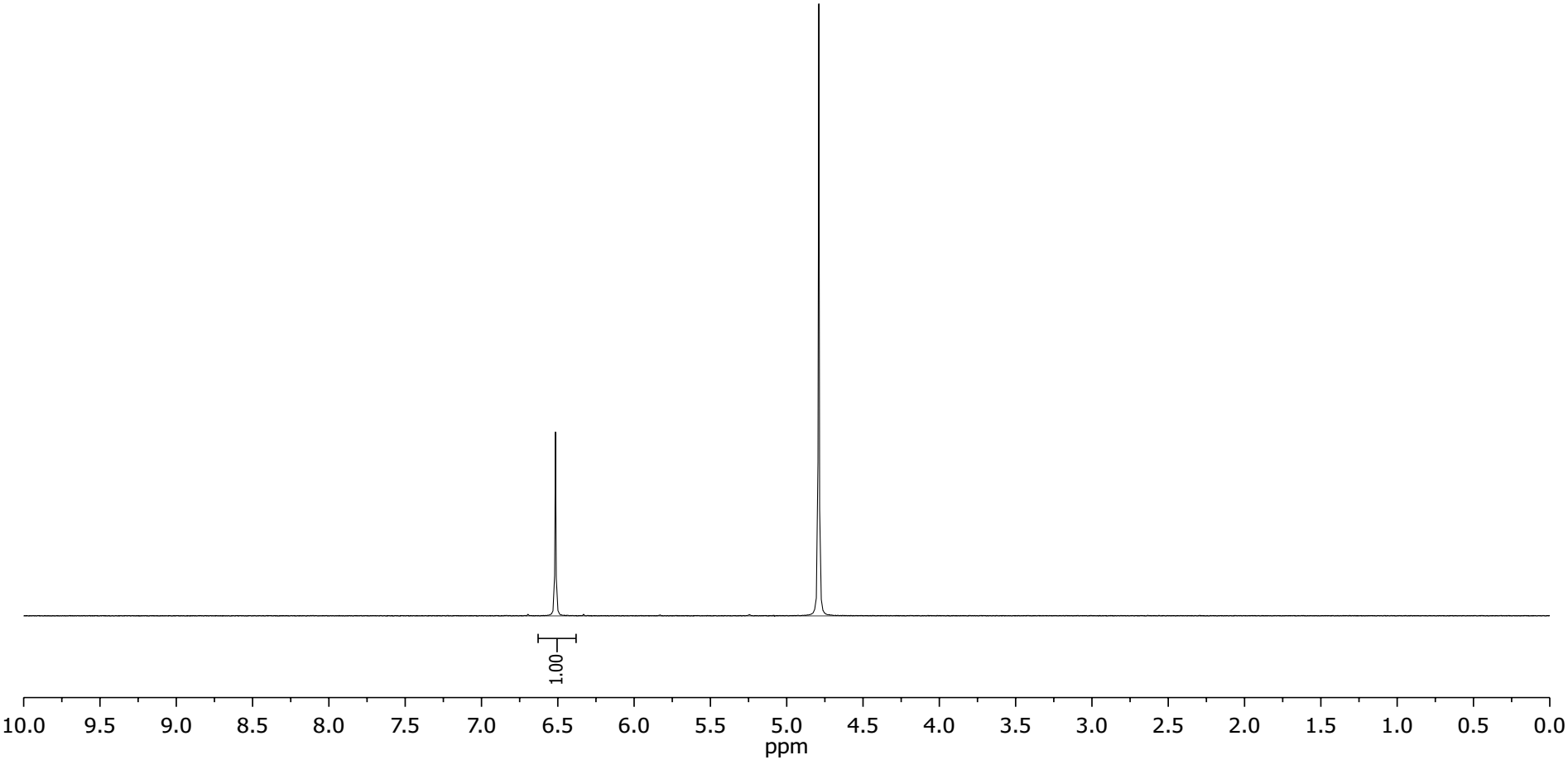
K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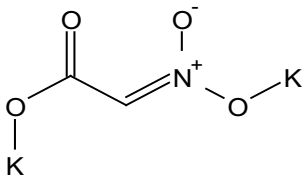
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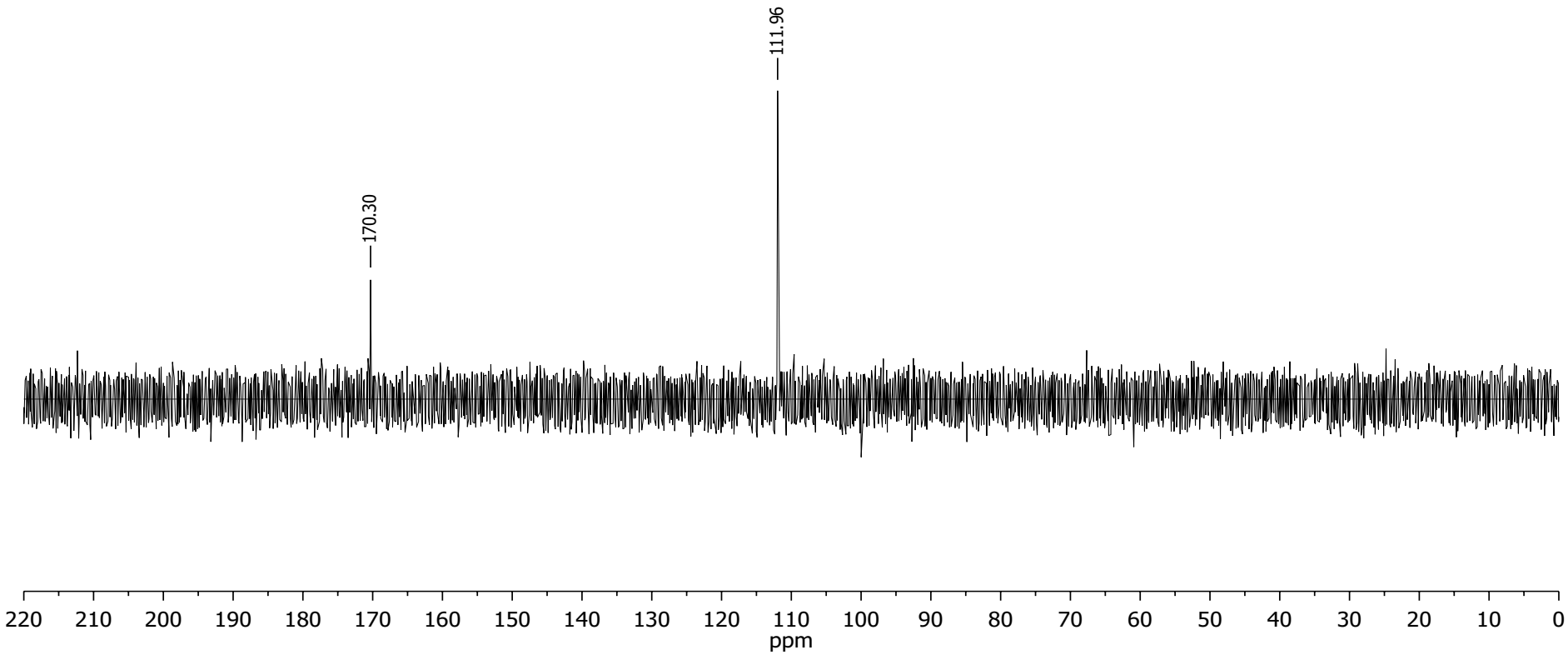
K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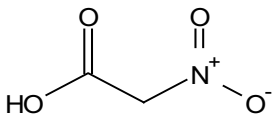
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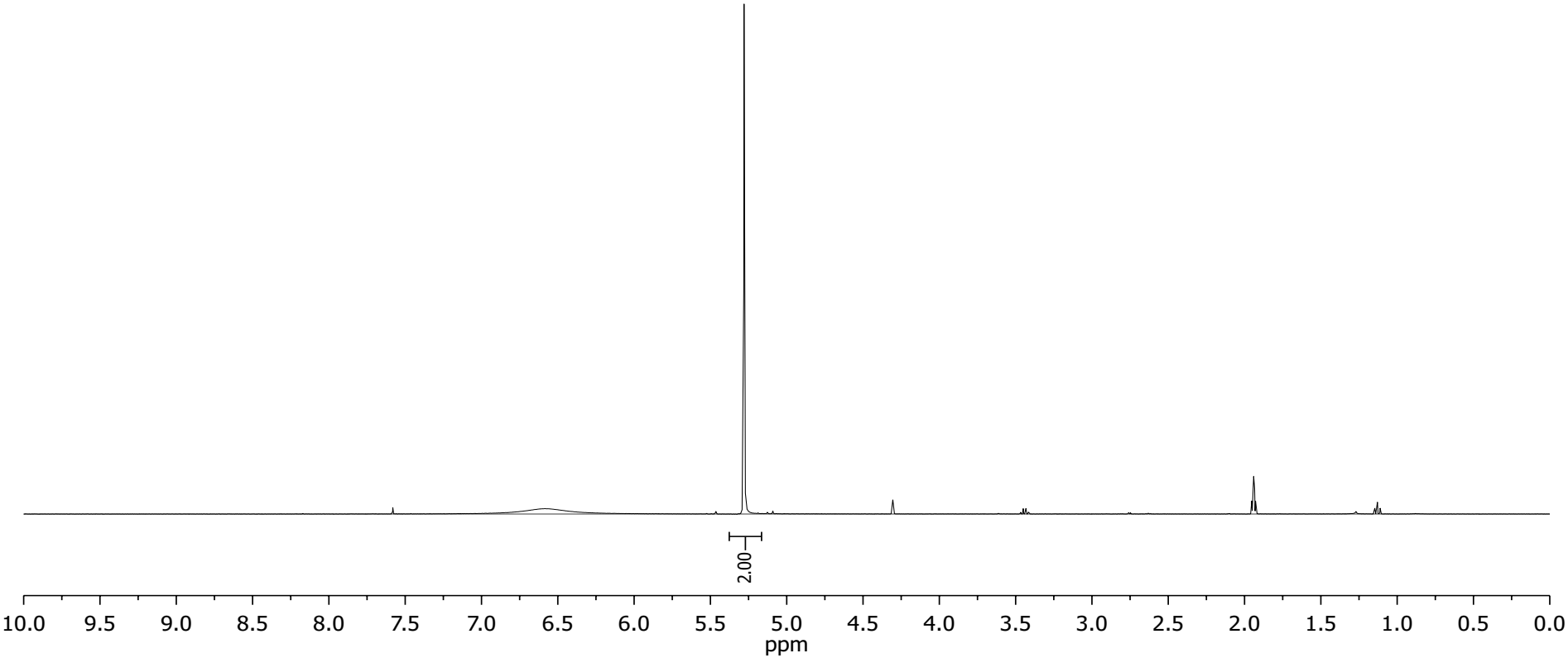
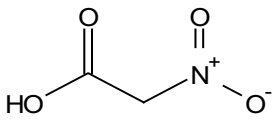
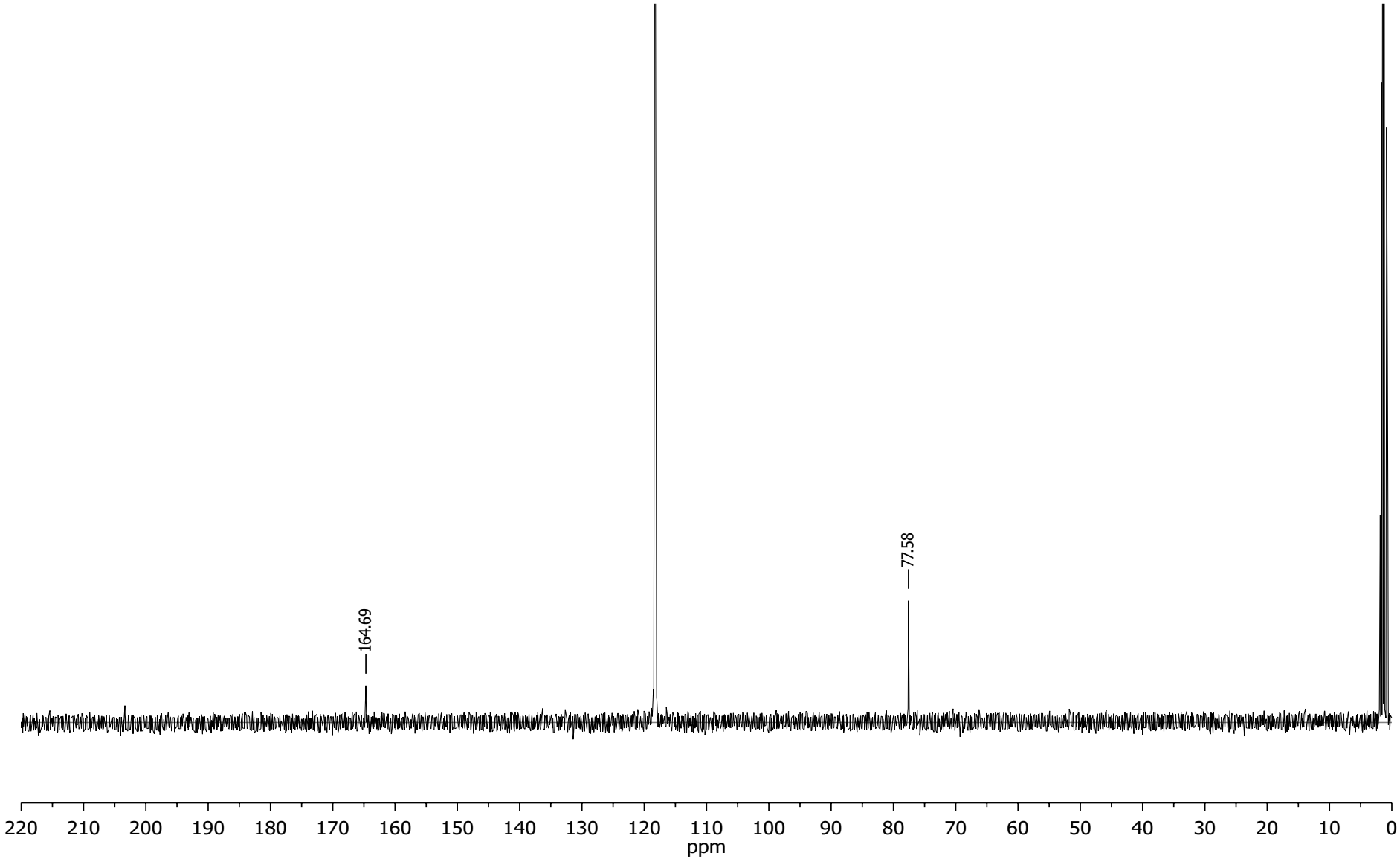
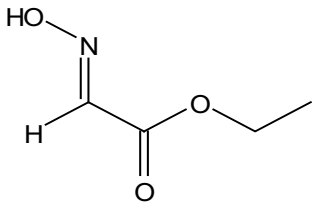
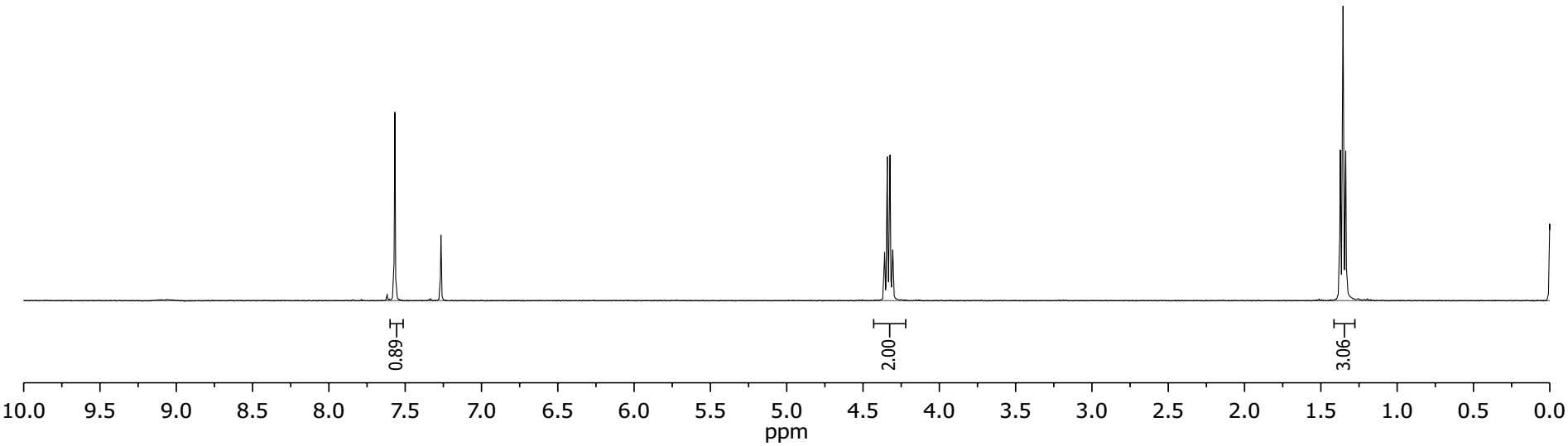
N+
O-

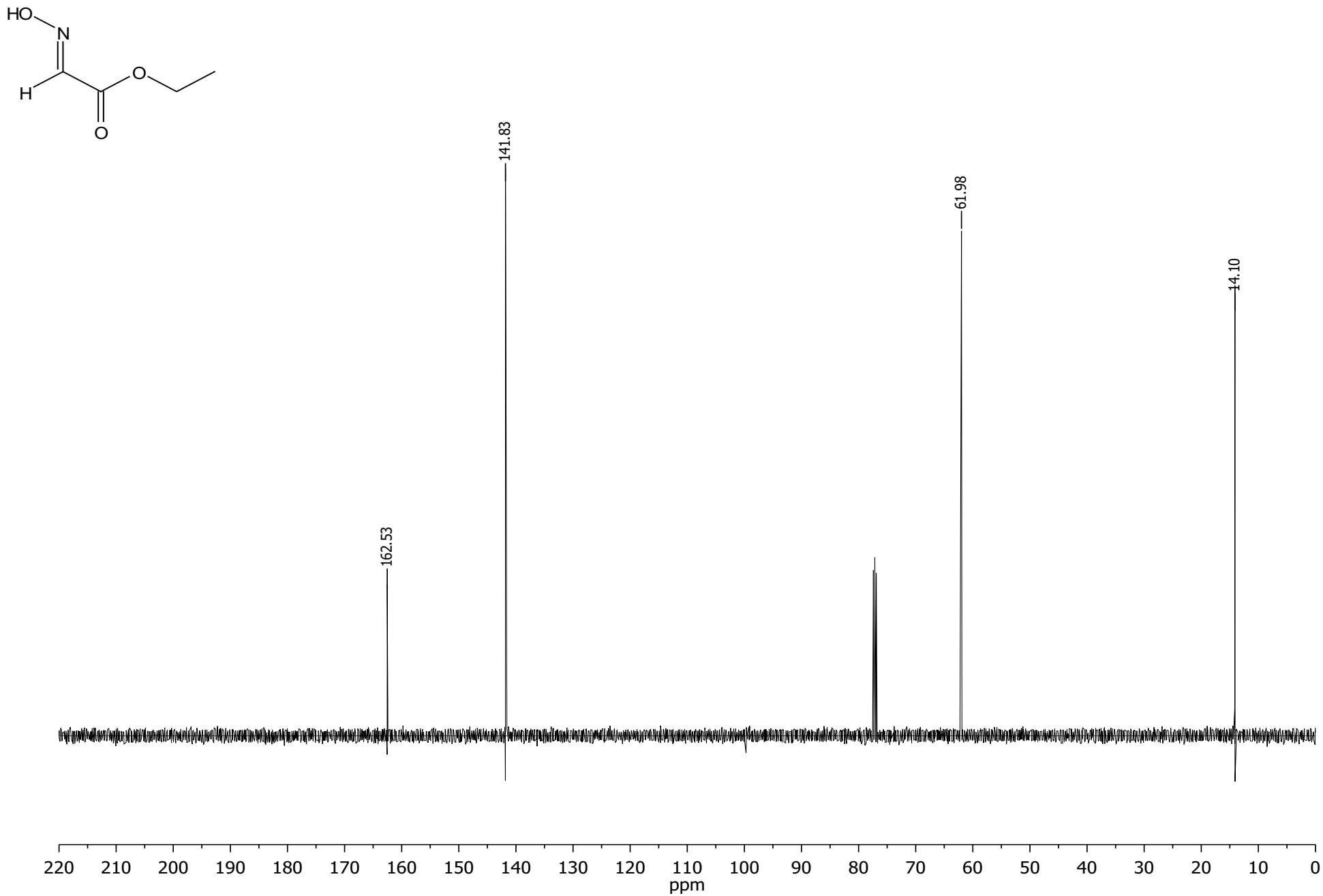

2.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

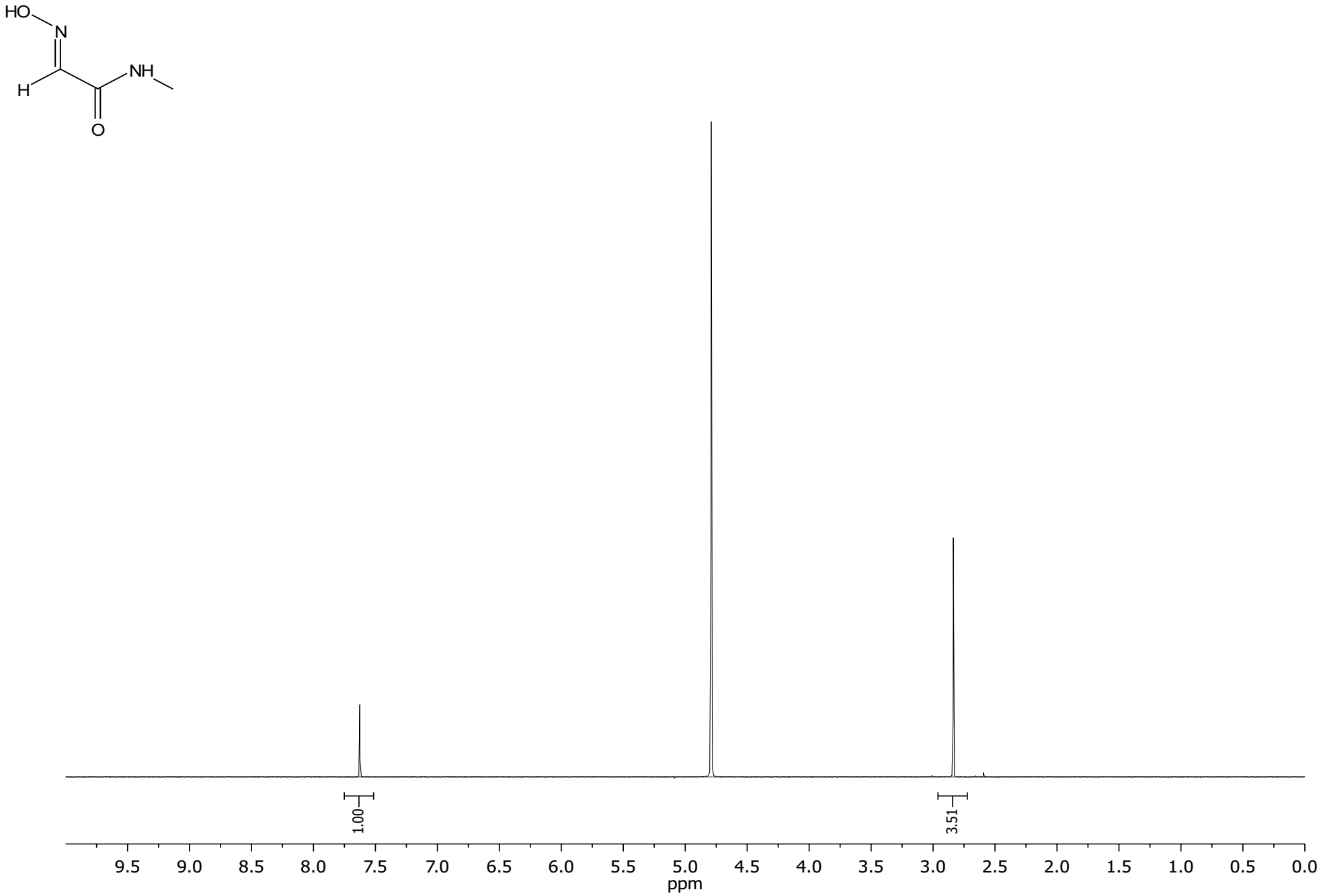

O
O
HO
N+
O-

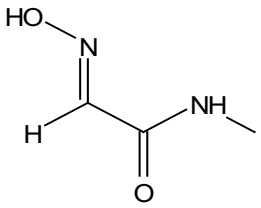

164.69
77.58
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

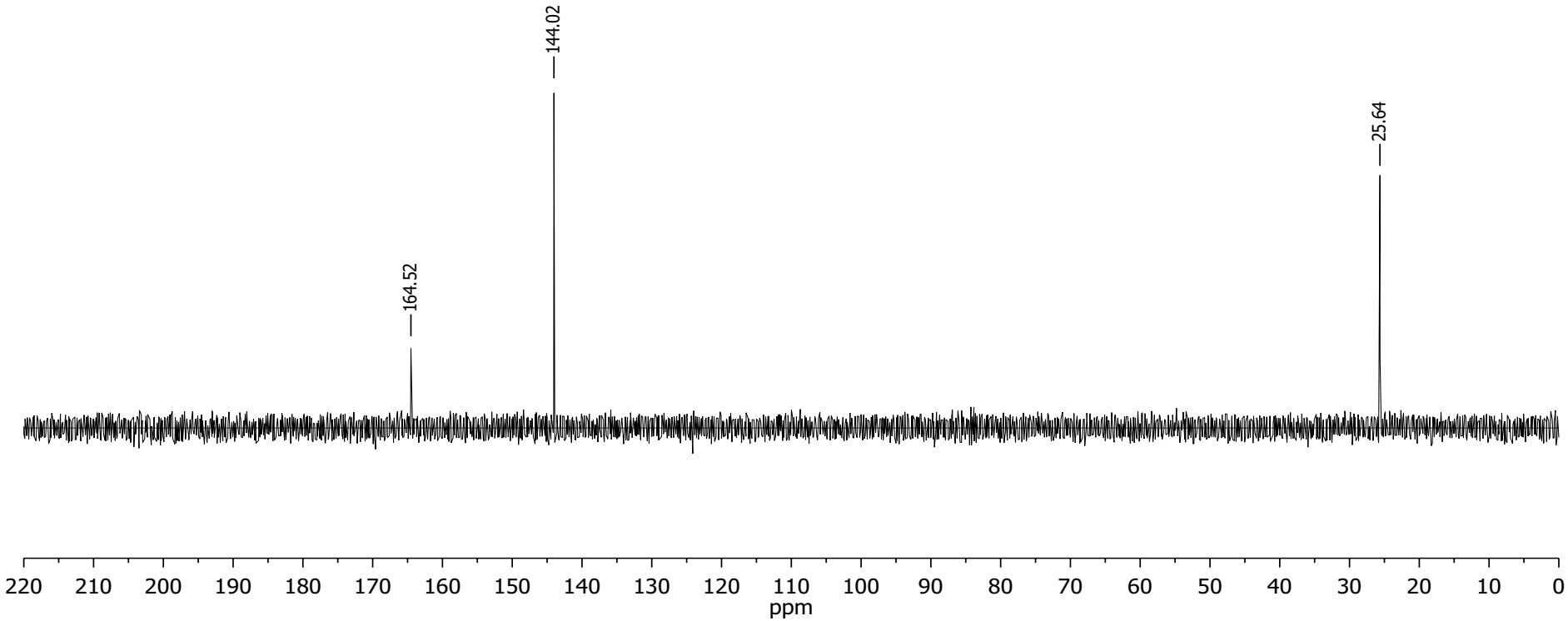

HO
N
H
O
O

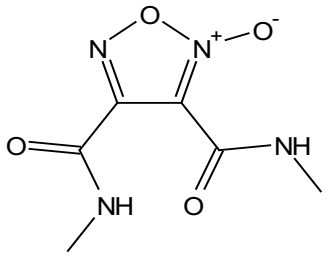

0.89
2.00
3.06
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

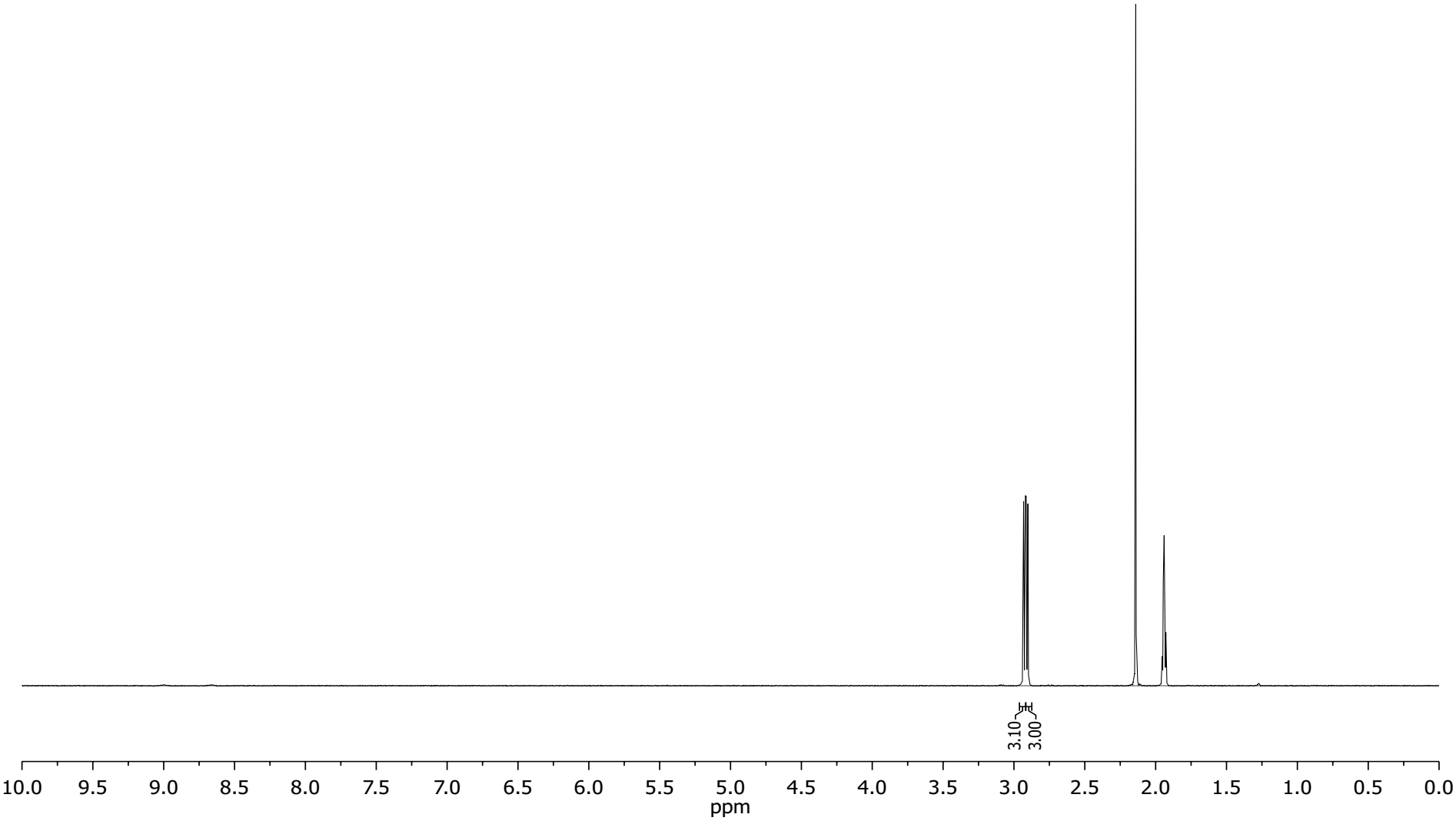

HO
N
H
O
O
141.83
61.98
14.10
162.53
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

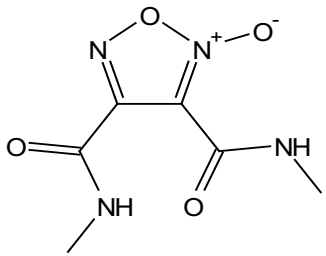

HO
N
H
NH
O
1.00
3.51
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

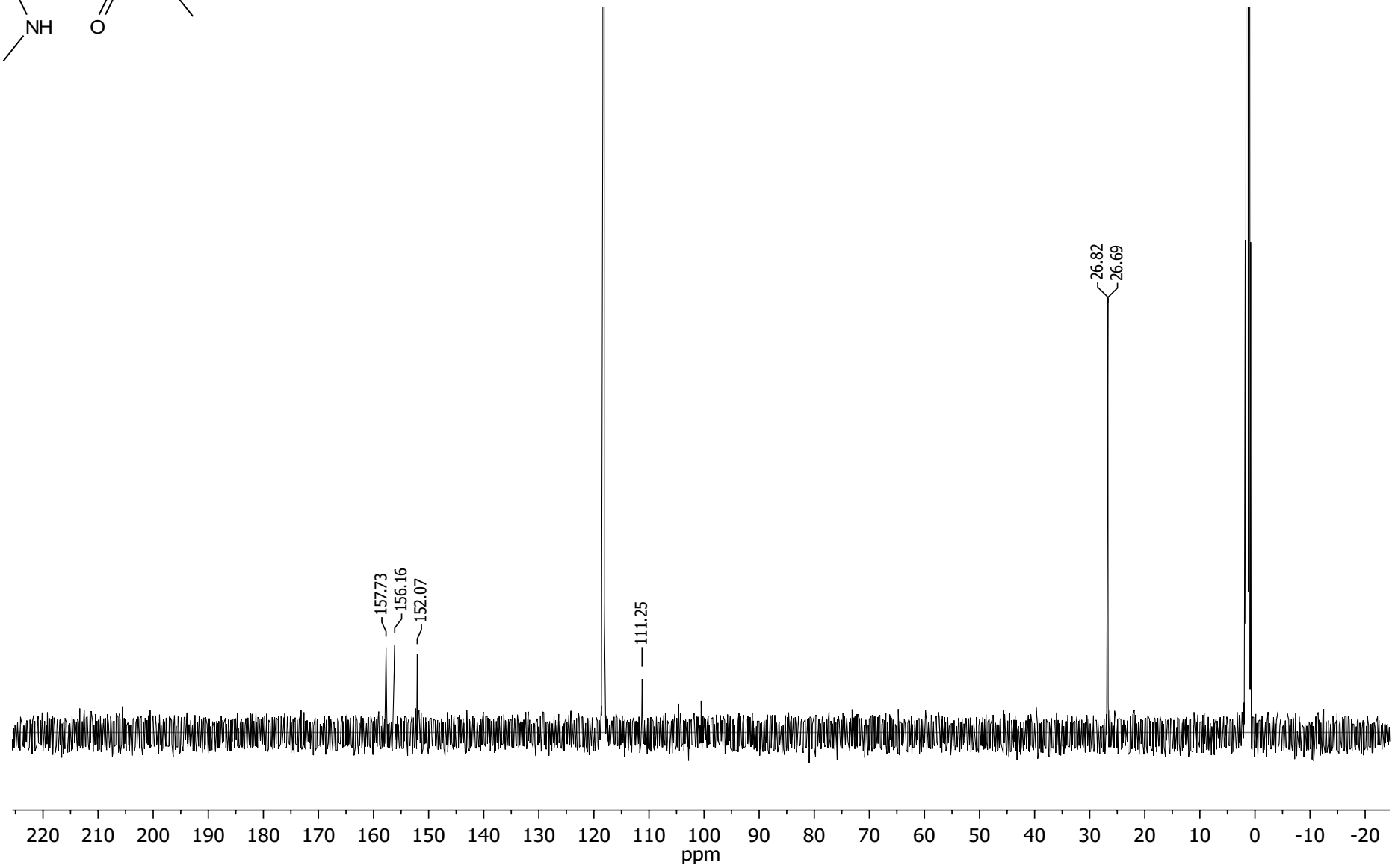

HO
N
NH
H
O

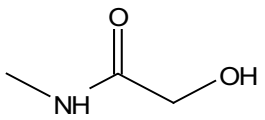

164.52
144.02
25.64
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

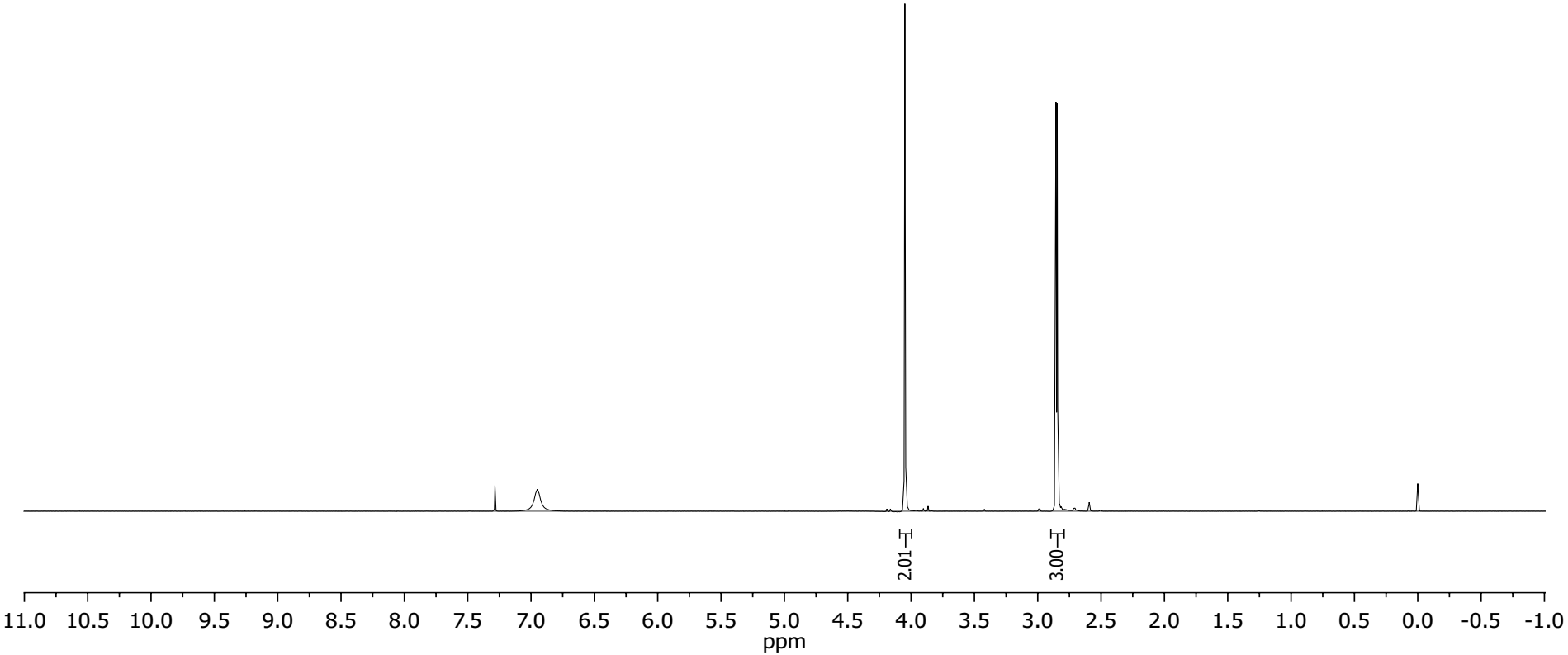

O
N
N+
O-
O
NH
NH
O

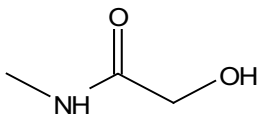

3.10
3.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

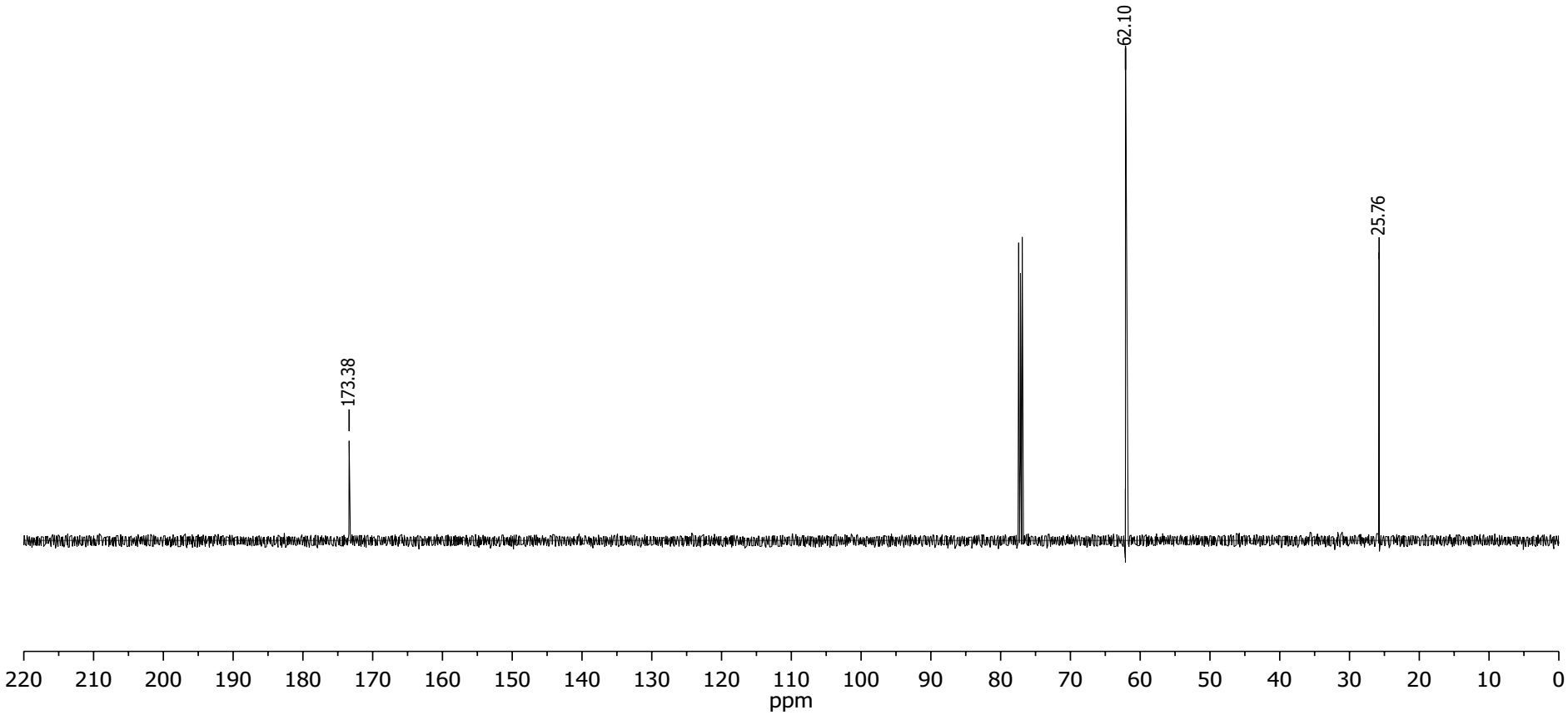

O
N
N+
O-
O
NH
NH
O


157.73
156.16
152.07
111.25
26.82
26.69
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
ppm


O
NH
OH


2.01
3.00
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
ppm


O
NH
OH


173.38
62.10
25.76
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm