

Supporting Information

RNA Control by Photoreversible Acylation

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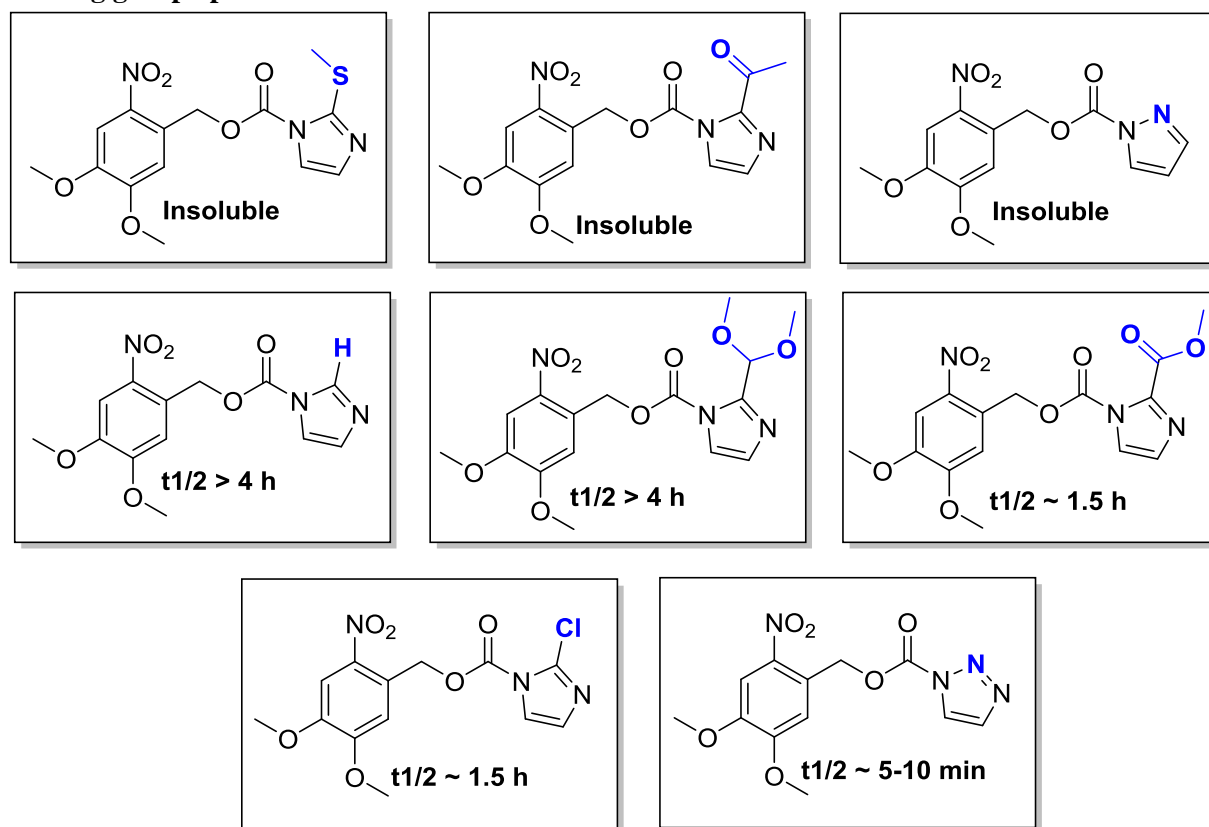
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General cloaking and photo-uncloaking procedure. One μL RNA stock solution in water (1 mM or 100 μM) was dissolved in 8 μL RNase free biological grade water and heated to 95 $^{\circ}\text{C}$ for 2 min and then cooled to room temperature. To this was added 1 μL 1M PCA stock in dry DMSO and the mixture incubated for 4 h at room temperature unless stated otherwise. The solution becomes slightly cloudy initially and then clears after ~ 30 min. After reaction, the RNA was precipitated by adding 1 μL of 3 M NaOAc buffer pH = 5.2, 1 μL glycogen (10 mg/mL) and 36 μL EtOH and storing at -80 $^{\circ}\text{C}$ for 16 h. The resulting suspension was centrifuged at 14,800 rpm for 60 min and the supernatant was removed. The solids were washed with 10 μL 70% EtOH and centrifuged at 14,800 rpm for 5 min. The supernatant was removed and the RNA was dried in air for 10 min and redissolved in RNase free biological grade water at the desired concentration. RNA concentration was determined with a Nanodrop One microvolume UV-VIS spectrophotometer. RNA samples were stored at -20 $^{\circ}\text{C}$.

To photo-uncloak RNA, samples were diluted in RNase free biological grade water to the desired concentration and kept in a 500 μL glass vial placed on top of a 365 nm VWR Scientific Transilluminator LM-20E for up to 60 min, depending on the experiment.

Leaving group optimization



Scheme S1. Structures of the varied leaving groups tested. The ones that were not soluble enough to determine $t_{1/2}$ by NMR spectroscopy were excluded from further consideration.

Half-lives were determined by NMR spectroscopy by dissolving the compounds in a 1:1 mixture of DMSO_{d6} and 50 mM phosphate buffer prepared with deuterium oxide. Compound hydrolysis was observed overtime. Compounds were prepared as described below.

4,5-dimethoxy-2-nitrobenzyl chloroformate (0.36 mmol, 100 mg) was dissolved in DCM (2 mL) and to this was added a solution of 5-membered heterocycle (0.36 mmol or 0.72 mmol, depending on pKa) in DCM (2 mL). The resulting reaction was stirred at room temperature until full conversion. If the desired product precipitated from the reaction it was obtained by filtration. If not, the reaction was filtered and the filtrate concentrated to yield the desired product.

4,5-dimethoxy-2-nitrobenzyl 2-(methylthio)-1H-imidazole-1-carboxylate

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.73 (s, 1H), 7.59 (s, 1H), 7.37 (s, 1H), 7.02 (s, 1H), 5.69 (s, 2H), 3.91 (s, 3H), 3.87 (s, 3H).

4,5-dimethoxy-2-nitrobenzyl 2-acetyl-1H-imidazole-1-carboxylate

¹H NMR (300 MHz, DMSO-*d*₆) δ 7.87 (d, *J* = 1.4 Hz, 1H), 7.76 (s, 1H), 7.33 (s, 1H), 7.23 (d, *J* = 1.5 Hz, 1H), 5.75 (s, 2H), 3.95 (s, 3H), 3.91 (s, 3H), 2.59 (s, 3H).

4,5-dimethoxy-2-nitrobenzyl 1H-pyrazole-1-carboxylate

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.37 (dd, *J* = 2.8, 0.7 Hz, 1H), 7.87 (dd, *J* = 1.6, 0.7 Hz, 1H), 7.74 (s, 1H), 7.38 (s, 1H), 6.58 (dd, *J* = 2.8, 1.6 Hz, 1H), 5.72 (s, 2H), 3.90 (s, 3H), 3.88 (s, 3H).

4,5-dimethoxy-2-nitrobenzyl 1H-imidazole-1-carboxylate

¹H NMR (300 MHz, DMSO-*d*₆) δ 8.83 (s, 1H), 7.83 (s, 1H), 7.77 (s, 1H), 7.38 (s, 1H), 7.34 (s, 1H), 5.78 (s, 2H), 3.95 (s, 3H), 3.91 (s, 3H).

4,5-dimethoxy-2-nitrobenzyl 2-(dimethoxymethyl)-1H-imidazole-1-carboxylate

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.74 (s, 1H), 7.60 (dd, *J* = 1.7, 0.5 Hz, 1H), 7.35 (s, 1H), 6.99 (dd, *J* = 1.7, 0.4 Hz, 1H), 5.76 (s, 1H), 5.69 (s, 2H), 3.91 (s, 3H), 3.88 (s, 3H), 3.29 (d, *J* = 0.5 Hz, 6H).

1-(4,5-dimethoxy-2-nitrobenzyl) 2-methyl 1H-imidazole-1,2-dicarboxylate

¹H NMR (300 MHz, DMSO-*d*₆) δ 7.80 (d, *J* = 1.1 Hz, 1H), 7.78 (s, 1H), 7.35 (s, 1H), 7.19 (d, *J* = 1.1 Hz, 1H), 5.75 (s, 2H), 3.95 (s, 3H), 3.91 (s, 4H), 3.75 (s, 3H).

4,5-dimethoxy-2-nitrobenzyl 2-chloro-1H-imidazole-1-carboxylate

¹H NMR (400 MHz, DMSO-*d*₆) δ 7.73 (s, 1H), 7.71 (d, *J* = 1.9 Hz, 1H), 7.37 (s, 1H), 7.02 (dd, *J* = 1.9, 1.1 Hz, 0H), 5.72 (s, 2H), 3.91 (s, 3H), 3.88 (s, 3H).

4,5-dimethoxy-2-nitrobenzyl 1H-1,2,3-triazole-1-carboxylate

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.76 (dd, *J* = 1.5, 1H), 7.97 (dd, *J* = 1.4, 1H), 7.75 (s, 1H), 7.41 (s, 1H), 5.83 (s, 2H), 3.91 (s, 3H), 3.88 (s, 3H).

Photocloaking agent design and characterization. PCA reagents **1** and **2** were synthesized according to the protocol below. Cloaking and uncloaking was initially evaluated on Cy-5 labeled 12-mer RNA (5' Cy5-GGG UGU AUG GUU) using 30% denaturing PAGE and MALDI-TOF.

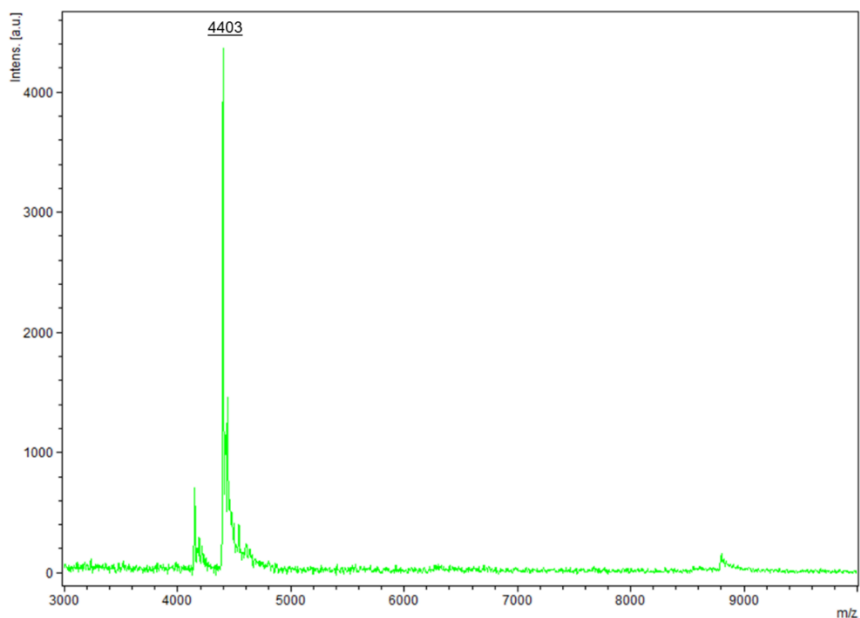


Figure S1. MALDI-TOF spectrum of untreated 12mer RNA. Expected MW=4403, found MW=4403.

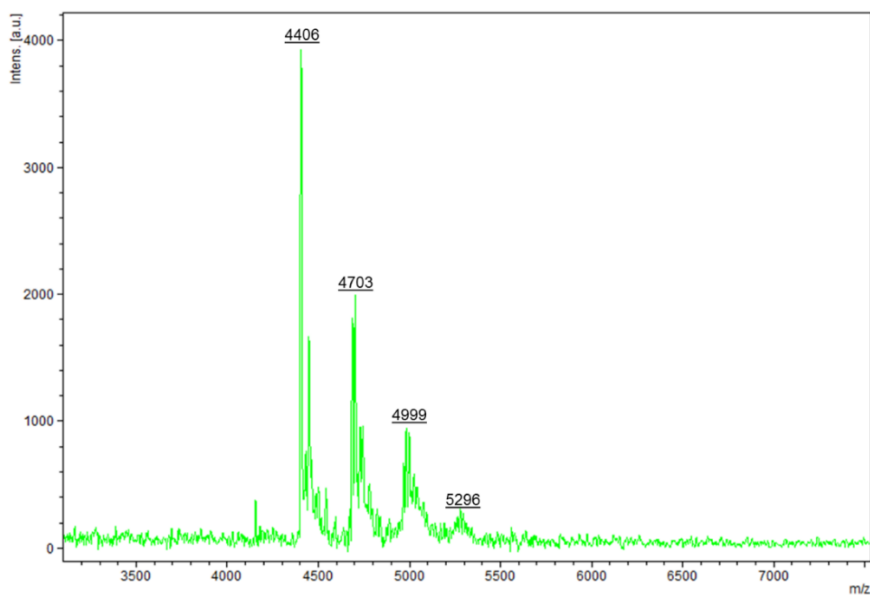


Figure S2. MALDI-TOF spectrum of cloaked RNA. Expected MW one label=4699, found MW=4703; expected MW two labels=4993, found MW=4999; expected MW three labels=5291, found MW=5296. Note that high-power laser used in the laser desorption likely removes many of the PCA groups. See PAGE gel analysis of same RNA in Fig. 1, main manuscript.

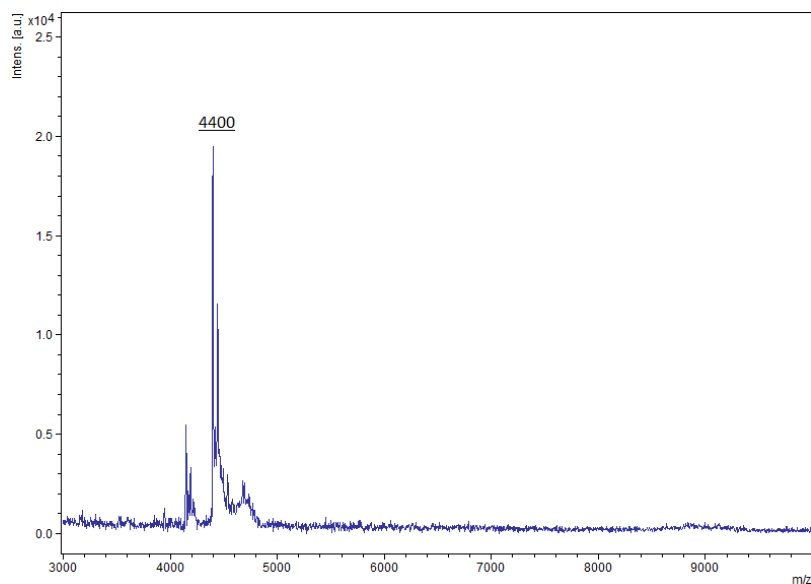


Figure S3. MALDI-TOF spectrum of uncloned RNA. Expected MW=4403, found MW=4400.

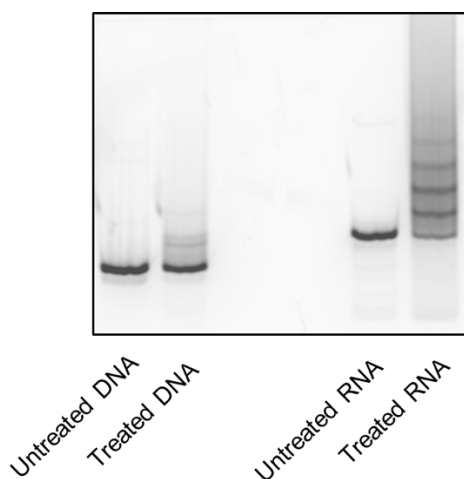


Figure S4. Denaturing PAGE gel analysis of untreated and cloaked DNA and RNA. Very limited cloaking is observed with DNA (est. <5% per strand), whereas up to 5 additional PCA groups are observed in RNA of the same sequence (right). DNA sequence: 5' Cy-5 – GGGTGTATGGTT; RNA sequence: 5' Cy-5 – GGGUGUAUGGUU.

Hybridization studies. Thermal denaturation curves were obtained on a Varian Cary 300 Bio UV-Visible Spectrophotometer with temperature controller. Treated and untreated RNA template were annealed to its complementary DNA strand in phosphate buffer pH = 7.2, 100 mM NaCl at 300 nM by heating the sample to 95 °C for 2 min and then cooling to room temperature. Melting curves were obtained by monitoring the absorbance at 260 nm of the nucleic acid solution while increasing the temperature at a rate of 1 °C/min. Curves were smoothed by adjacent averaging.

Further hybridization studies were conducted using native gel electrophoresis. 800 ng of treated and untreated RNA template were incubated with 800 ng complementary DNA in 1x PBS, 100 mM NaCl and annealed by heating the sample to 96 °C for 5 min and then cooling to room temperature. The samples were loaded on a 15% native polyacrylamide gel and visualized with 0.1% Stains-All in formamide.

RNA 16mer: 5' GCU CCC CAG CUU UCG C

Complementary DNA: 5' GCG AAA GCT GGG GAG C

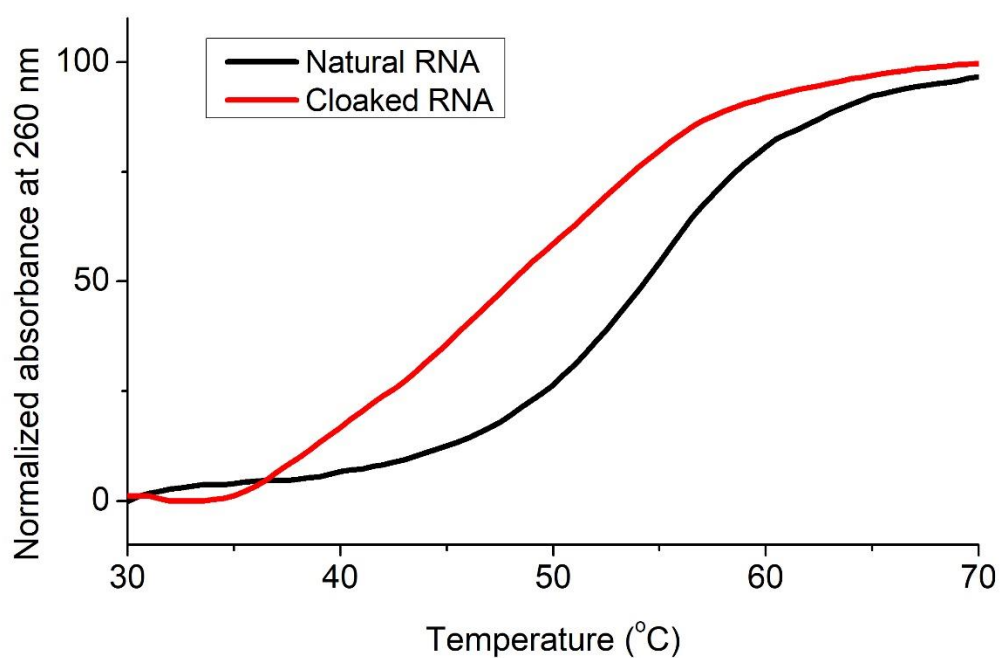


Figure S5. Thermal denaturation curves of unmodified RNA oligomer (black) and cloaked RNA (red) with a complementary DNA strand. Note that the red curve represents an average of the cloaked RNA population having varied numbers and positions of acyl groups. The half-point on each transition indicates a ca. 10 °C average drop in melting temperature due to the polyacylation.

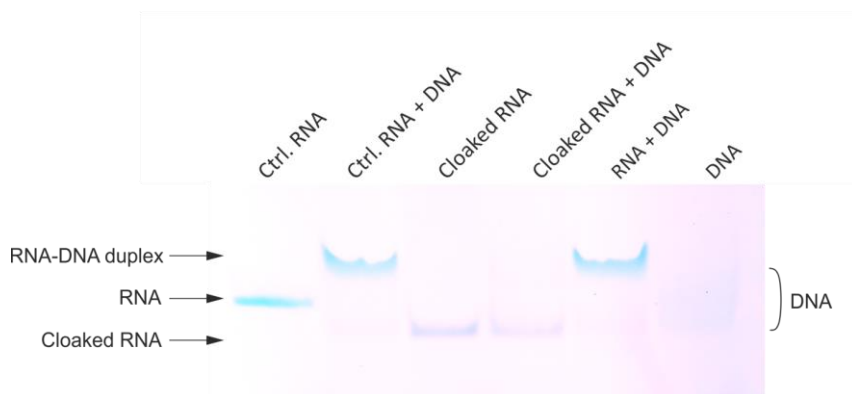


Figure S6. Native electrophoretic analysis of effects of 16nt RNA cloaking on hybridization with a DNA complement. Bands are imaged with Stains-All dye (Sigma Aldrich) according to the manufacturer's instructions. Lane 2 with control (unmodified) RNA + DNA shows a new band for the RNA-DNA duplex, which is absent for cloaked RNA, indicating that hybridization is significantly impacted by cloaking. Electrophoresis was performed with a 15 % polyacrylamide gel, using 1 x TBE buffer (Thermo Scientific) for 1.5 h at room temperature, at 18 mA current. See "Hybridization studies" above for sequences and details. Note that the DNA complement is pure, and reproducibly travels as a smeared band, apparently due to self-association (due to G-rich sequence) at the high concentrations used.

Hybridization QSTAR probe studies. QSTAR probe experiments were performed as described previously.^{1,2} In short, RNA template (untreated, cloaked or photo-uncloaked) was dissolved in 70 mM Tris-Borate buffer pH = 7.0, 10 mM MgCl₂ to a concentration of 200 nM. To this was added QSTAR probe (200 nM) and TPP probe (400 nM) and the fluorescence was monitored overtime at 25 °C on a Fluorolog 3-11 instrument (Jobin Yvon-SPEX). $\lambda_{ex} = 497$ nm and $\lambda_{em} = 519$ nm. Plots were smoothed by adjacent averaging.

QSTAR probe: 5' Dabsyl-azidoetherlinker-T^FGG GGA GC; TPP probe: 5' GCG AAA GC-TPP.
For the detailed preparation of QSTAR and TPP probes see previously reported studies.^{1,2}

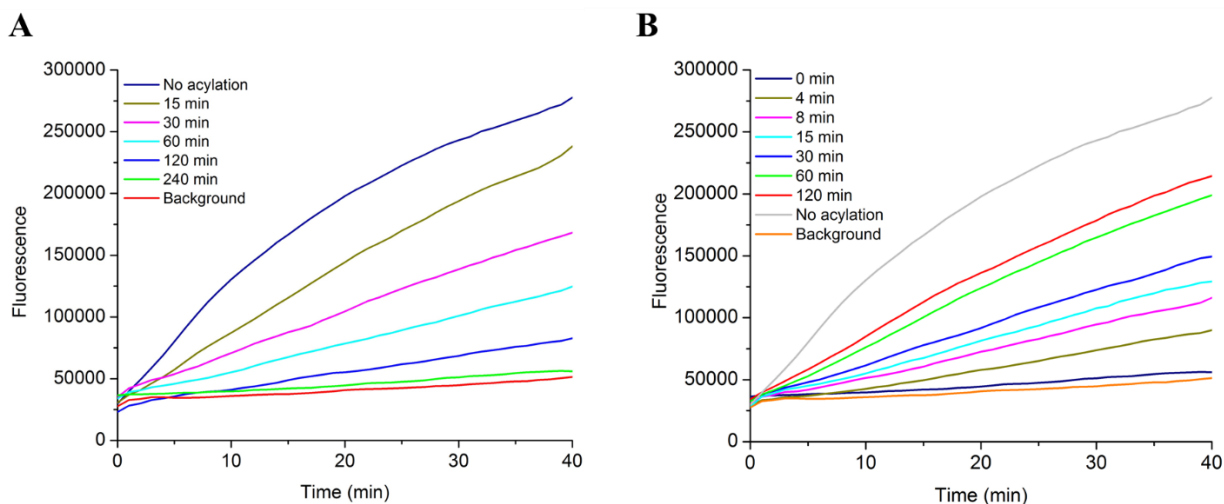


Figure S7. Real-time signals from QSTAR probe reactions after RNA template has been cloaked and photo-uncloaked for varying times. A) Time course of fluorescence increase when 18nt RNA (sequence above) has been

incubated with PCA 1 for increasing times as shown. B) Time course of fluorescence increase after fully cloaked 18nt RNA has been exposed to light for increasing times as shown.

Hammerhead Ribozyme studies. 34 nt hammerhead ribozyme RNA was purchased from Integrated DNA Technologies and purified by standard desalting. Cy5-labeled substrate was purchased from PAN facility, Stanford School of Medicine and purified with GlenPak. The cleavage assay was performed as described.³ In short, 5 μ L ribozyme solution and 5 μ L substrate solution were heated separately at 95 $^{\circ}$ C for 2 min and then cooled to room temperature. The ribozyme and substrate solution were mixed in 10 μ L of $MgCl_2$ solution to final concentrations of 200 nM ribozyme, 500 nM substrate and 10 mM $MgCl_2$ in 50 mM Tris pH = 6.0. The reactions were carried out for 1 h at room temperature unless stated otherwise. Reactions were quenched by the addition of 20 μ L 8 M urea, 100 mM EDTA and analyzed with denaturing PAGE.

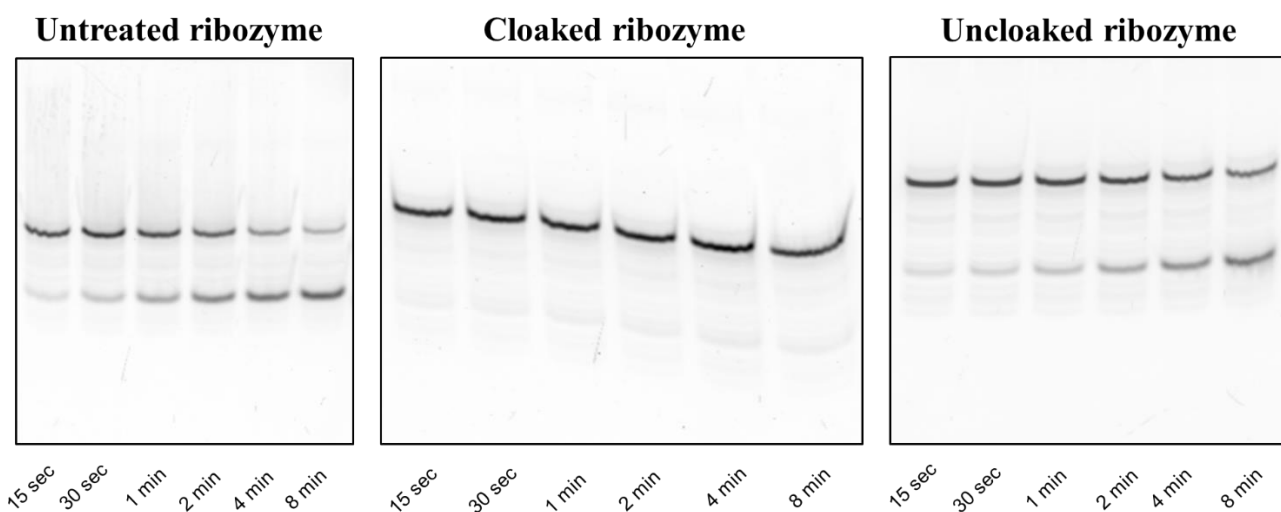


Figure S8. Substrate conversion by hammerhead ribozyme over time when incubated with mock control ribozyme (untreated), cloaked and photo-uncloaked ribozyme analyzed by denaturing PAGE.

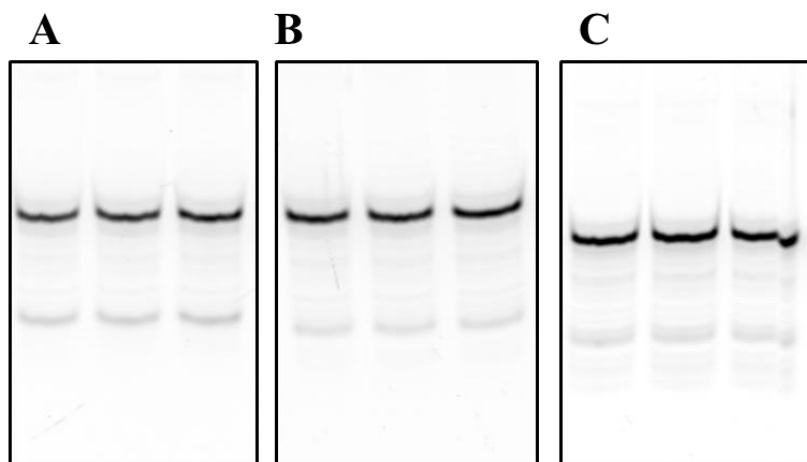


Figure S9. Initial rate determination by denaturing PAGE in triplicate. A) Substrate conversion after incubation with DMSO treated (untreated) ribozyme for 15 sec. B) Substrate conversion after incubation with cloaked ribozyme for 60 min. C) Substrate conversion after incubation with uncloaked ribozyme for 15 sec.

Broccoli aptamer studies F-30 Broccoli DNA template (3 ng) (Table S1) was amplified by PCR (98 °C for 30 s; 30 times: 98 °C for 10 s, 61 °C for 30 s, 72 °C for 30 s; 72 °C for 5 min, 4 °C forever) using Phusion High-Fidelity PCR master Mix (NEB), and primers mix in 50 μ L reaction, according to the NEB protocol. Mini Elute columns, and PNI Buffer (Qiagen) were used to clean up double stranded DNA samples as described in the manufacturer's instructions. DNA was eluted using RNase-free water (Corning), and its purity was verified using electrophoresis in a 2% agarose gel (Sigma Aldrich) in 1 \times TBE Buffer (Life Technologies), 1 h, 80 mA. 250 ng of DNA template was transcribed using MEGAShortScript Kit (Life Technologies) according to the manufacturer's protocol. RNA was purified using Zymo RNA Clean&Concentrator-5 according to the manufacturer's instructions. RNA was eluted in RNase-free water, and stored at -78 °C. RNA purity and quality was verified by electrophoresis in 2% agarose gels. Broccoli aptamer RNA was cloaked for 4 h with PCA 2 (100 mM) in water. DMSO treated (untreated), cloaked and uncloaked RNA samples (500 nM) were incubated in folding buffer (40 mM HEPES, pH = 7.4, 100 mM KCl, 5 mM MgCl₂) with DFHBI (1 μ M) and heated up to 95 °C for 2 min and then allowed to cool to room temperature for 1 h. Fluorescent emission spectra were recorded on a Fluorolog 3-11 instrument (Jobin Yvon-SPEX), $\lambda_{\text{ex}} = 450$ nm at 25 °C.

F-30 Broccoli DNA template:

5'TAATACGACTCACTATAGGGTTGCCATGTGTATGTGGGAGACGGTCGGGTCCAGATATTCCG
TATCTGTGCGAGTAGAGTGTGGGCTCCACATACTCTGATGATCCTTCGGGATCATTTCATGGC

F-30 Broccoli_F primer: 5' TAATACGACTCACTATAGGGTTGC

F-30 Broccoli_R primer: 5' GCCATGAATGATCCCGAAG

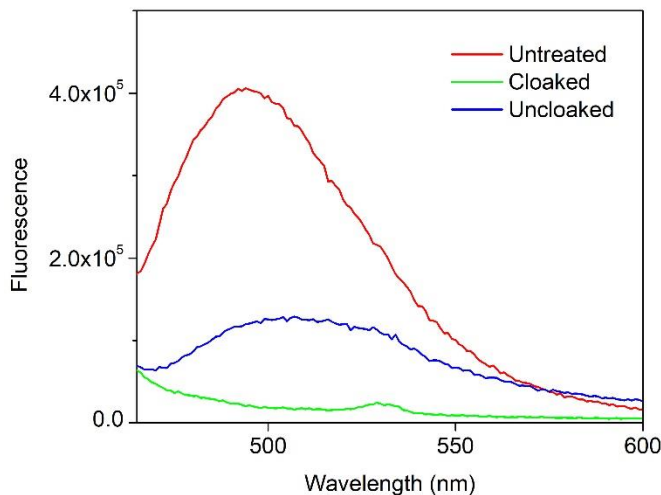


Figure S10. Fluorescence emission spectra of untreated, PCA 1 cloaked and uncloaked Broccoli incubated with DFHBI. Fluorescence is significantly reduced after cloaking. Uncloaking results in only 32% recovery compared to untreated sample. Uncloaking with PCA 2 is more efficient, restoring fluorescence to 98% of original (See Fig. 4, main text).

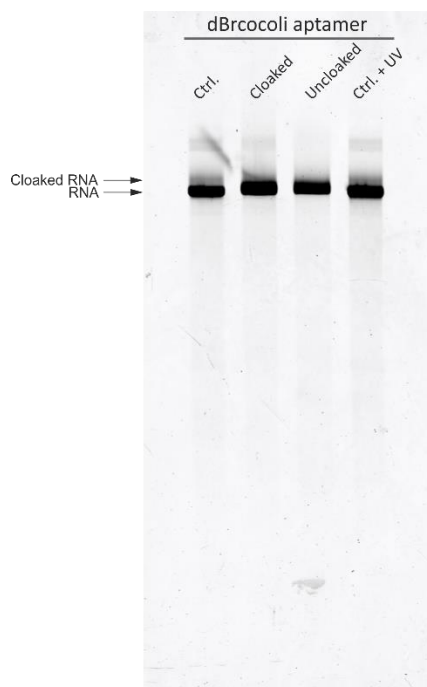


Figure S11. Denaturing PAGE gel analysis of untreated and cloaked dBroccoli aptamer, evaluating whether light exposure degrades RNA. A small band shift is observable after cloaking (lane 2), see “General cloaking procedure” above for details. UV treatment (1h, 365 nm, transilluminator) of the control RNA aptamer shows no observable degradation products on the gel. Electrophoresis was performed with a 10 % denaturing polyacrylamide gel, using 1 x TBE buffer (Thermo Scientific) for 2.5 h at room temperature, 21 mA current. The gel was stained with SYBR Gold used in dilution 1:10,000 in 1xTBE for 5 min. at room temperature with constant rocking. The shade present in the bottom of the third lane is an artifact of the scanner.

Broccoli aptamer studies in cells. F30-2xdB_2 DNA (100 pmol) was 5’ adenylated using Mth Ligase (100 pmol, NEB) according to NEB’s protocol. Mini Elute columns, and PNI Buffer (Qiagen) were used to clean up double stranded DNA samples according to manufacturer’s instructions. ssDNAs F30-2xdB_2 (40 pmol) and F30-2xdB_1 (20 pmol) were ligated according to NEB description, using Thermostable 5’ App DNA/RNA Ligase (40 pmol, NEB). Ligated DNA was purified on DNA Clean&Concentrator 5 columns (Zymo Research) using Zymo’s protocol. F-30 2xd Broccoli ssDNA template (3 ng) was amplified by PCR (98 °C for 30 s; 30 times: 98 °C for 10 s, 60 °C for 30 s, 72 °C for 30 s; 72 °C for 5 min, 4 °C forever) using Phusion High-Fidelity PCR master Mix (NEB), and primers mix in 50 µL reaction, according to the NEB protocol. DNA was purified in 1.8% agarose gel (Sigma Aldrich) in 1× TBE Buffer (Life Technologies), 1 h, 80 mA, next to the length standard Gene Ruler 1kb Plus DNA Ladder (Thermo Fisher Scientific). The band of interest was cut out and dsDNA was extracted using GeneJET Gel Extraction Kit (Thermo Fisher Scientific). DNA was eluted using RNase-free water (Corning), and its purity was verified using electrophoresis in a 2% agarose gel (Sigma Aldrich) in 1× TBE Buffer (Life Technologies), 1 h, 80 mA, next to the length standard Gene Ruler 1kb Plus DNA Ladder (Thermo Fisher Scientific). 250 ng of dsDNA template was transcribed using MEGAShortScript Kit (Life Technologies) according to the manufacturer’s protocol. RNA was purified using Zymo RNA Clean&Concentrator-5 according to the manufacturer’s instructions. RNA was eluted in RNase-free water, and stored at -78 °C. RNA purity and quality were verified by electrophoresis in 2% agarose gels. F30-2xd Broccoli aptamer RNA was cloaked for 4 h with PCA 2 (100 mM) in water or DMSO treated in the same conditions (untreated). HeLa cells were seeded at a concentration of 1.8×10^4 cells per well to an 8-well plate in

supplemented DMEM culture medium (10% FBS, Gibco, 1 × Penicillin/Streptomycin, Thermo Fisher Scientific) and incubated for 16 h at 37°C and 5% CO₂ with 95% humidity. Old medium was removed and replaced with 180 μL of a new supplemented DMEM medium (Gibco). To transfect aptamer RNA to HeLa cells transfection mixture (20 μL of OptiMEM medium (Gibco), 0.6 μL of Lipofectamine MessengerMAX (Thermo Fisher Scientific) and 5 μg of RNA) was prepared. The transfection mixture was added dropwise to each well and incubated for 5 h. The transfection medium was replaced with supplemented DMEM medium and cells exposed to light 375 nm > λ > 325 nm for 30 minutes. In all wells medium was replaced with fresh staining mixture containing: 120 mM sucrose, 5mM Mg SO₄, 25 mM HEPES pH 7.4, 40 μM DFHBI-1T (Lucerna Inc.) and 1 × CellBrite Blue Cytoplasmatic Membrane Staining Kit (Biotium) in DMEM, and cells were incubated for next 30 minutes. The staining mixture was removed and cells washed with fresh DMEM. Medium was removed and plates prepared to visualization using the epifluorescence microscope Nikon Eclipse i80 at room temperature. Visualization and imaging of cells were carried out using bright field and fluorescence filters: blue (375 nm > λ_{ex} >325 nm; λ_{em} > 420 nm) and green (500 nm > λ_{ex} >460 nm; λ_{em} > 520 nm). Images were analyzed and overlapped using Image J software.

F30-2xdB_1:

5'TAATACGACTCACTATAGGGTTGCCATGTGTATGTGGGAGACGGTCGGGTCCATCTGAGAC
GGTCGGGTCCAGATATTCGTATCTGTCTGAGTAGAGTGTGGGCTCAGATGTCGAGTAGAGTGT
GGGCTCCCACATA

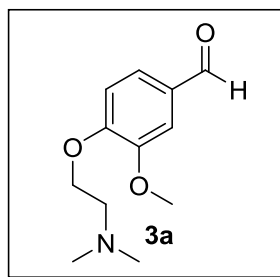
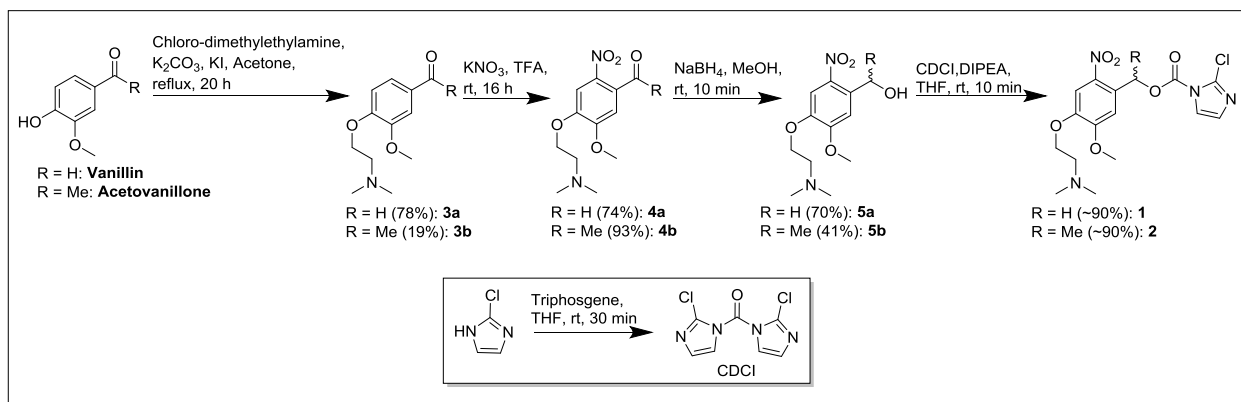
F30-2xdB_2:

5'/5Phos/CTCTGATGATCCAGACGGTCGGGTCCATCTGAGACGGTCGGGTCCAGATATTCGTA
TCTGTCGAGTAGAGTGTGGGCTCAGATGTCGAGTAGAGTGTGGGCTGGATCATTTCATGGCAA

F-30-2xdBroccoli2_R: 5' TTGCCATGAATGATCCAGC

F-30-2xdBroccoli2_F: 5' TAATACGACTCACTATAGGGTTGCC

Synthesis of PCA 1 and 2



4-(2-(dimethylamino)ethoxy)-3-methoxybenzaldehyde (**3a**)

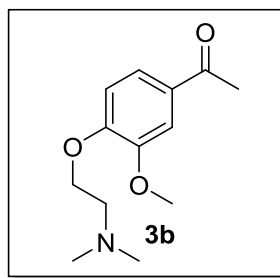
Vanillin (1.52 g, 10 mmol), 2-chloro-dimethylethylamine HCl (2.16 g, 15 mmol), potassium iodide (332 mg, 2 mmol) and potassium carbonate (4.14 g, 30 mmol) were suspended in acetone and heated at reflux for 16 h. Next, volatiles were removed *in vacuo* and the resulting residue is redissolved in water and ethyl acetate. The organic layer was separated and washed with brine and dried with magnesium sulfate. Concentrating

in vacuo resulted in 1.75 g (78%) of a yellow oil.

1H NMR (400 MHz, Chloroform-*d*) δ 9.82 (s, 1H), 7.41 (dd, $J = 8.2, 1.9$ Hz, 1H), 7.37 (d, $J = 1.9$ Hz, 1H), 6.96 (d, $J = 8.2$ Hz, 1H), 4.17 (t, $J = 6.1$ Hz, 2H), 3.89 (s, 3H), 2.79 (t, $J = 6.1$ Hz, 2H), 2.32 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 191.2, 154.0, 150.0, 130.3, 127.0, 111.7, 109.3, 67.4, 58.0, 56.2, 46.3.

ESI-MS [$M+H$]: Calculated: 224.1; Observed: 224.1



1-(4-(2-(dimethylamino)ethoxy)-3-methoxyphenyl)ethan-1-one (**3b**)

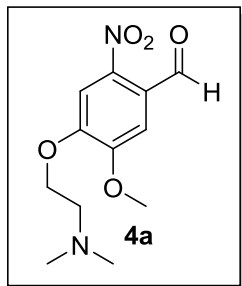
Acetovanillone (1.66 g, 10 mmol), 2-chloro-dimethylethylamine HCl (2.16 g, 15 mmol), potassium iodide (332 mg, 2 mmol) and potassium carbonate (4.14 g, 30 mmol) were suspended in DMF and heated at 70 °C for 16 h. Next, the reaction was diluted with water and ethyl acetate. The organic layer was separated and washed with brine and dried with magnesium sulfate. Concentrating *in vacuo* resulted in 450 g (19%) of a

yellow oil.

1H NMR (300 MHz, Chloroform-*d*) δ 7.53 (dd, $J = 8.3, 2.0$ Hz, 1H), 7.49 (d, $J = 2.0$ Hz, 1H), 6.88 (d, $J = 8.3$ Hz, 1H), 4.15 (t, $J = 6.2$ Hz, 2H), 3.88 (s, 3H), 2.78 (t, $J = 6.2$ Hz, 2H), 2.54 (s, 3H), 2.33 (s, 6H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 197.0, 152.8, 149.5, 130.7, 123.4, 111.5, 110.5, 67.4, 58.1, 56.2, 46.3, 26.5.

ESI-MS [$M+H$]: Calculated: 238.1; Observed: 238.2



4-(2-(dimethylamino)ethoxy)-5-methoxy-2-nitrobenzaldehyde (**4a**)

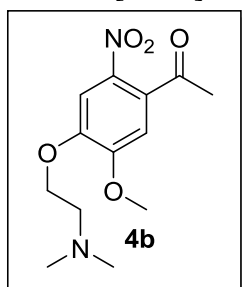
Compound **3a** (1.3 g, 5.8 mmol) was dissolved in TFA (10 mL) and added dropwise to a solution of potassium nitrate (707 mg, 7.0 mmol) in TFA (10 mL) that was cooled on ice. The resulting solution was stirred for 16 h and then concentrated *in vacuo*. The crude was diluted with water and basified with sat. aq. sodium bicarbonate. The aqueous solution was extracted twice with ethyl acetate and the combined organic fractions were washed with brine and dried with magnesium sulfate. Concentrating *in vacuo* resulted in

1.1 g (74%) of a yellow solid.

^1H NMR (300 MHz, Chloroform-*d*) δ 10.47 (s, 1H), 7.66 (s, 1H), 7.43 (s, 1H), 4.36 (d, $J = 5.5$ Hz, 3H), 4.02 (s, 3H), 3.04 (t, $J = 5.5$ Hz, 3H), 2.54 (s, 6H).

^{13}C NMR (75 MHz, CDCl_3) δ 188.1, 153.8, 152.0, 143.9, 125.8, 110.1, 108.4, 68.2, 57.9, 56.9, 46.2.

ESI-MS [$\text{M}+\text{H}$]: Calculated: 269.1; Observed: 269.2



1-(4-(2-(dimethylamino)ethoxy)-5-methoxy-2-nitrophenyl)ethan-1-one (**4b**)

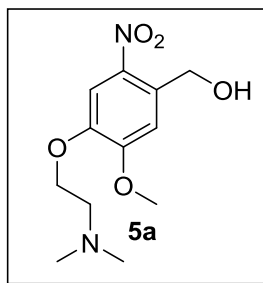
Compound **3b** (270 mg, 1.1 mmol) was dissolved in TFA (1 mL) and added dropwise to a solution of potassium nitrate (139 mg, 1.4 mmol) in TFA (4 mL) that was cooled on ice. The resulting solution was stirred for 16 h and then concentrated *in vacuo*. The crude was diluted with water and basified with sat. aq. sodium bicarbonate. The aqueous solution was extracted twice with ethyl acetate and the combined organic fractions were washed with brine and dried with magnesium sulfate. Concentrating *in*

vacuo resulted in 300 mg (93%) of a beige solid.

^1H NMR (400 MHz, Chloroform-*d*) δ 7.62 (s, 1H), 6.73 (s, 1H), 4.18 (t, $J = 5.8$ Hz, 2H), 3.94 (s, 3H), 2.81 (t, $J = 5.8$ Hz, 2H), 2.48 (s, 3H), 2.35 (s, 6H).

^{13}C NMR (101 MHz, CDCl_3) δ 200.4, 154.6, 149.1, 138.5, 133.2, 108.9, 108.3, 67.9, 58.0, 56.8, 46.28, 30.6.

ESI-MS [$\text{M}+\text{H}$]: Calculated: 283.1; Observed: 283.2



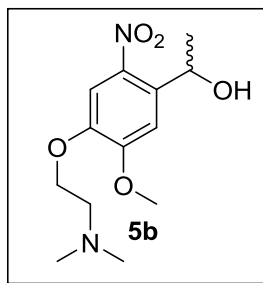
(4-(2-(dimethylamino)ethoxy)-5-methoxy-2-nitrophenyl)methanol (**5a**)

Compound **4a** (1.0 g, 3.7 mmol) was dissolved in methanol (10 mL). Sodium borohydride (284 mg, 7.5 mmol) was added in portions and the resulting solution is stirred for 10 min, when full conversion was observed by TLC. The reaction is concentrated *in vacuo* and the crude purified with flash chromatography (EtOAc:TEA 95:5) and recrystallized from EtOAc, resulting in 700 mg (70%) of yellow crystals.

^1H NMR (400 MHz, Chloroform-*d*) δ 7.64 (s, 1H), 7.16 (s, 1H), 4.95 (s, 2H), 4.11 (t, $J = 5.6$ Hz, 2H), 3.90 (s, 3H), 2.80 (t, $J = 5.6$ Hz, 2H), 2.36 (s, 6H).

^{13}C NMR (101 MHz, CDCl_3) δ 154.4, 147.1, 139.3, 133.7, 110.7, 109.5, 67.7, 62.5, 58.1, 56.5, 46.3.

ESI-MS [$\text{M}+\text{H}$]: Calculated: 271.1; Observed: 271.1



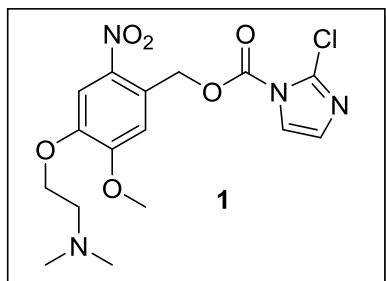
1-(4-(2-(dimethylamino)ethoxy)-5-methoxy-2-nitrophenyl)ethan-1-ol (**5b**)

Compound **4b** (300 mg, 1.1 mmol) was dissolved in methanol (5 mL). Sodium borohydride (81 mg, 2.1 mmol) was added in portions and the resulting solution is stirred for 1 h, when full conversion was observed by TLC. The reaction was concentrated *in vacuo* and the crude purified with flash chromatography (EtOAc:TEA 95:5) and recrystallized from EtOAc resulting in 130 mg (41%) of yellow crystals.

^1H NMR (400 MHz, Chloroform-*d*) δ 7.56 (s, 1H), 7.29 (s, 1H), 5.56 (q, $J = 6.3$ Hz, 1H), 4.11 (t, $J = 5.9$ Hz, 2H), 3.96 (s, 3H), 2.78 (t, $J = 5.8$ Hz, 2H), 2.34 (s, 6H), 1.54 (d, $J = 6.3$, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ 154.3, 146.9, 139.5, 138.17, 109.3, 108.9, 67.7, 65.7, 58.1, 56.5, 46.2, 24.8.

ESI-MS [M+H]: Calculated: 285.2; Observed: 285.2



4-(2-(dimethylamino)ethoxy)-5-methoxy-2-nitrobenzyl 2-chloro-1H-imidazole-1-carboxylate (**1**)

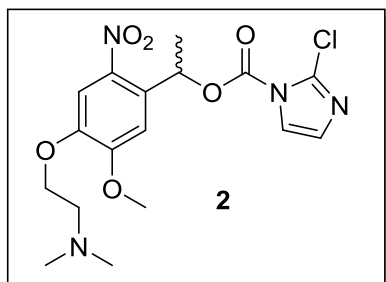
Carbonyldichloroimidazole (CDCI) was freshly prepared by dissolving 2-chloroimidazole (102 mg, 1.0 mmol) in THF (1.5 mL) and triphosgene (25 mg, 0.08 mmol) dissolved in THF (1.0 mL) was added to this. The resulting mixture was stirred for 30 min at room temperature and then filtered. The filtrate was concentrated *in vacuo* and dried under high vacuum for 5 min

resulting in a white powder (^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ 7.70 (d, $J = 1.9$ Hz, 2H), 7.14 (d, $J = 1.9$ Hz, 2H)), which was immediately used without any further purification. The white powder was dissolved in THF (1.0 mL) and to this was added a solution of compound **4a** (8 mg, 0.03 mmol) dissolved in THF (1.0 mL) and the resulting mixture was stirred for 5 min at room temperature. Next, DIPEA (35 μL , 0.20 mmol) was added and the reaction was stirred for an additional 5 min before concentrating *in vacuo*. The obtained yellow solid was immediately dissolved in DMSO (30 μL) resulting in a ~ 1 M solution and stored at -80 $^\circ\text{C}$.

^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ 7.80 (s, 1H), 7.73 (d, $J = 1.9$ Hz, 1H), 7.39 (s, 1H), 7.05 (d, $J = 1.9$ Hz, 1H), 5.75 (s, 2H), 4.19 (t, $J = 5.7$ Hz, 2H), 3.94 (s, 3H), 2.67 (t, $J = 5.6$ Hz, 2H), 2.23 (s, 6H).

^{13}C NMR (101 MHz, DMSO) δ 154.0, 148.0, 147.7, 140.5, 132.0, 124.8, 122.1, 113.1, 109.9, 67.8, 67.4, 58.0, 57.1, 46.2.

HRMS [M+H]: Calculated for $\text{C}_{16}\text{H}_{20}\text{ClN}_4\text{O}_6$: 399.1071; Found: 399.1070



1-(4-(2-(dimethylamino)ethoxy)-5-methoxy-2-nitrophenyl)ethyl 2-chloro-1H-imidazole-1-carboxylate (**2**)

Carbonyldichloroimidazole (CDCI) was freshly prepared by dissolving 2-chloroimidazole (102 mg, 1.0 mmol) in THF (1.5 mL) and triphosgene (25 mg, 0.08 mmol) dissolved in THF (1.0 mL) was added to this. The resulting mixture is stirred for 30 min at room temperature and then filtered. The filtrate was

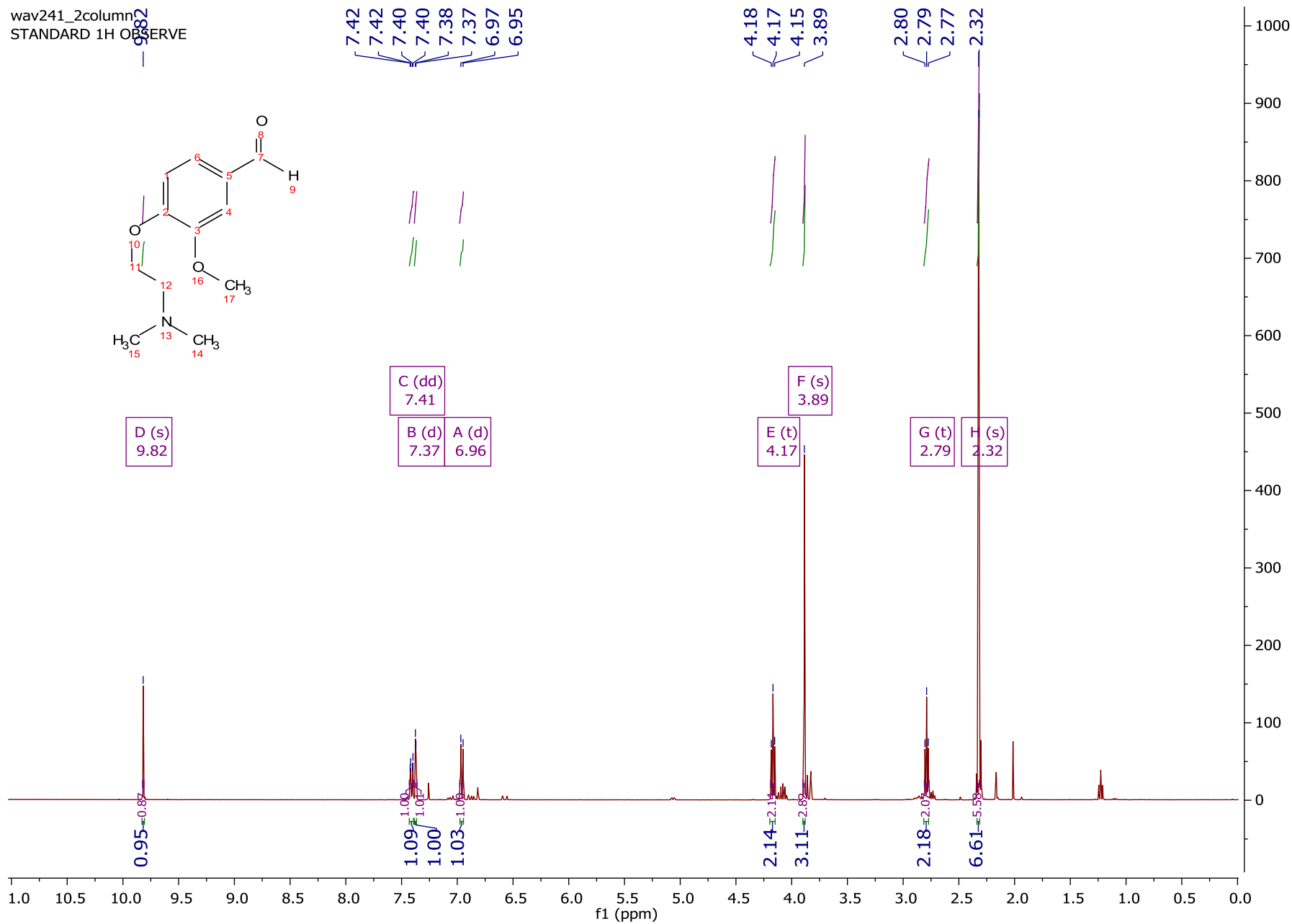
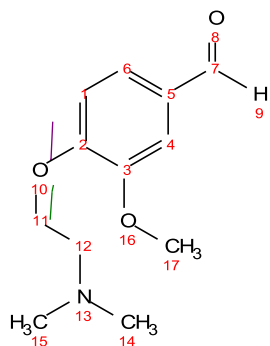
concentrated *in vacuo* and dried under high vacuum for 5 min resulting in a white powder (^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ 7.70 (d, $J = 1.9$ Hz, 2H), 7.14 (d, $J = 1.9$ Hz, 2H)), which was immediately used without any further purification. The white powder was dissolved in THF (1.0 mL) and to this was added a solution of compound **4b** (8 mg, 0.03 mmol) dissolved in THF (1.0 mL) and the resulting mixture was stirred for 5 min at room temperature. Next, DIPEA (35 μL , 0.20 mmol) was added and the reaction was stirred for an additional 5 min before concentrating *in vacuo*. The obtained yellow solid was immediately dissolved in DMSO (30 μL) resulting in a ~ 1 M solution and stored at -80 $^\circ\text{C}$.

^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.82 (d, $J = 1.7$ Hz, 1H), 7.63 (d, $J = 2.0$ Hz, 1H), 7.28 (s, 1H), 7.02 (s, 1H), 6.45 (q, $J = 6.4$ Hz, 1H), 4.15 (t, $J = 6.3$ Hz, 2H), 3.91 (s, 3H), 2.63 (t, $J = 5.7$ Hz, 2H), 2.49 (s, 3H), 2.20 s, 6H).

^{13}C NMR (101 MHz, DMSO) δ 154.3, 148.0, 147.2, 140.4, 132.0, 130.5, 122.2, 109.9, 109.1, 73.3, 67.7, 58.0, 57.1, 46.2, 21.9.

HRMS [M+H]: Calculated for $\text{C}_{17}\text{H}_{22}\text{ClN}_4\text{O}_6$: 413.1228; Found: 413.1225

wav241_2column
STANDARD 1H OBSERVE



wav241_carbon
13C OBSERVE

— 191.14

— 154.04

— 150.05

— 130.32

— 127.00

— 111.69

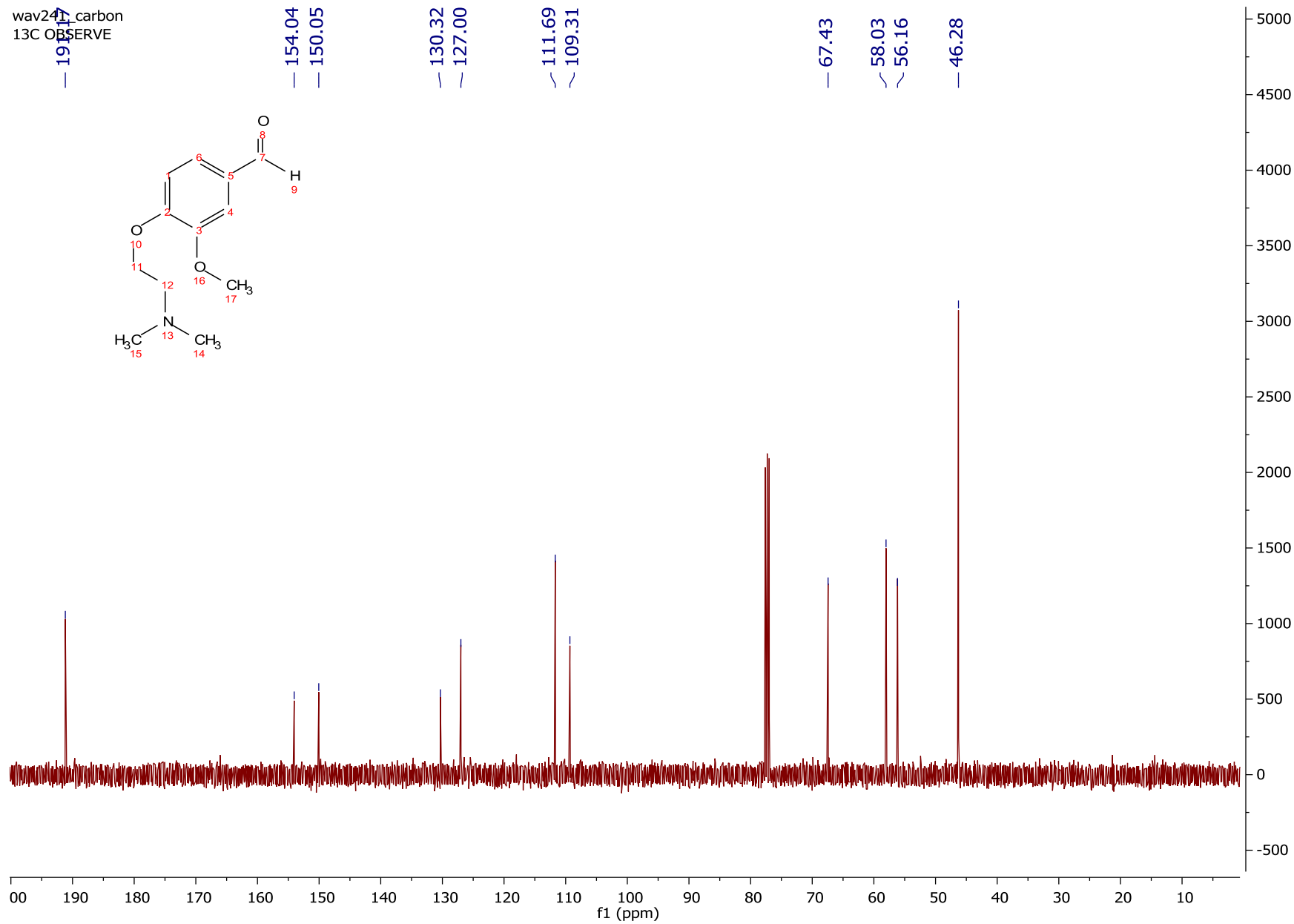
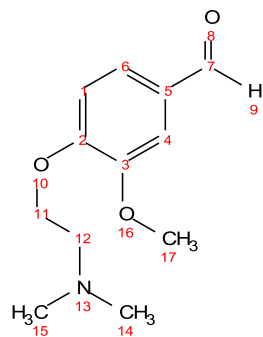
— 109.31

— 67.43

— 58.03

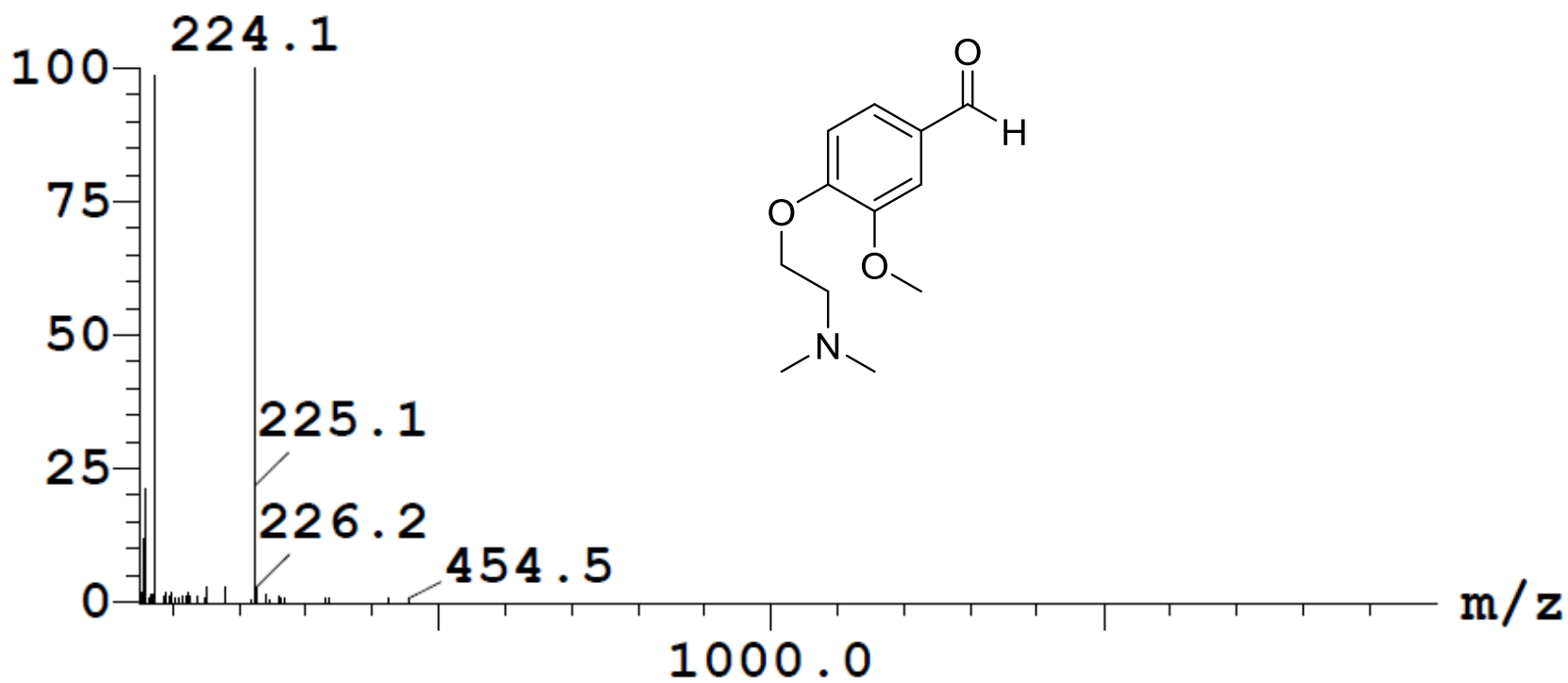
— 56.16

— 46.28

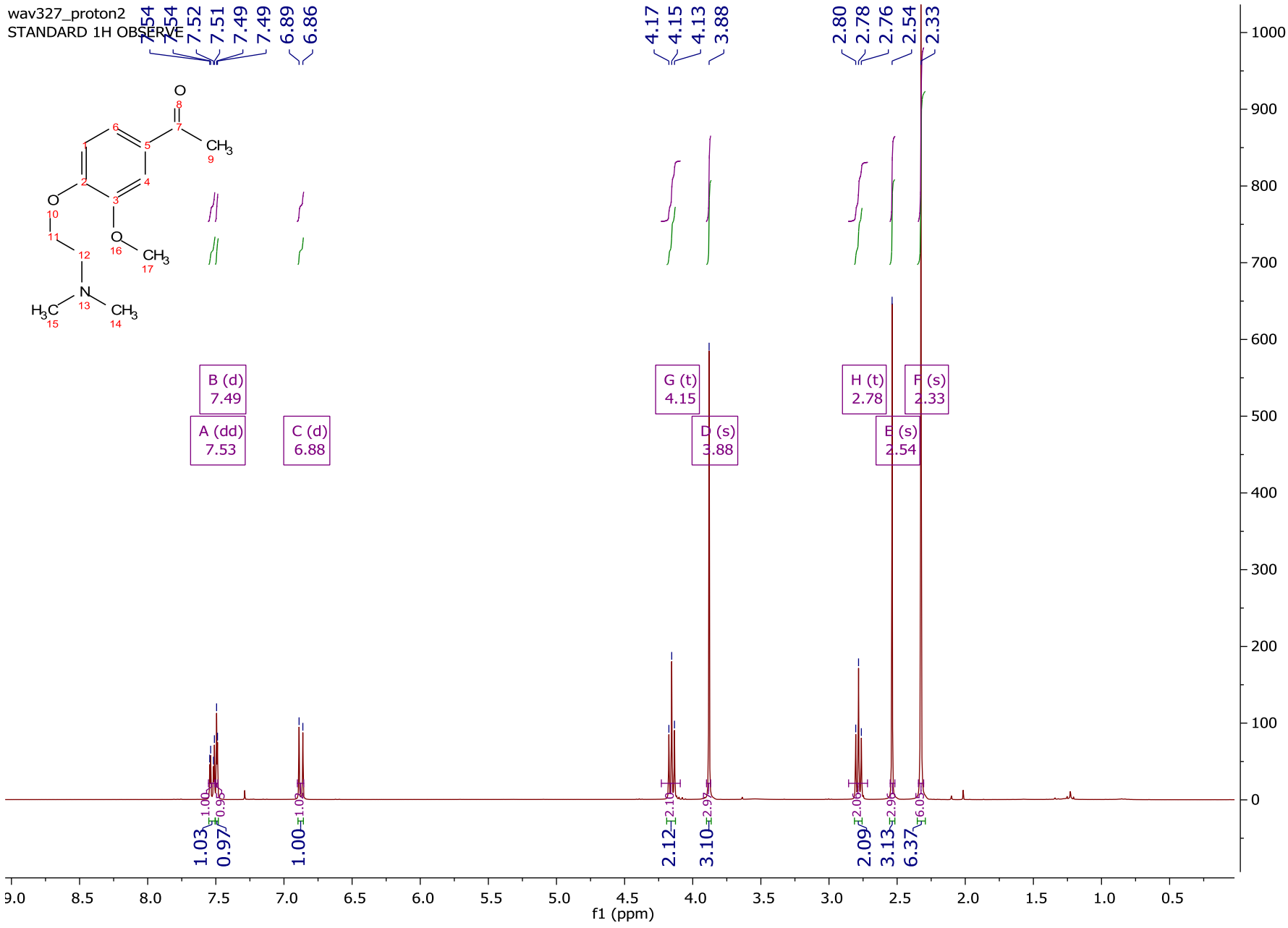
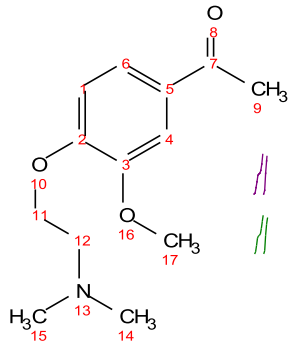


Peak ID	Compound	Time	Mass Found
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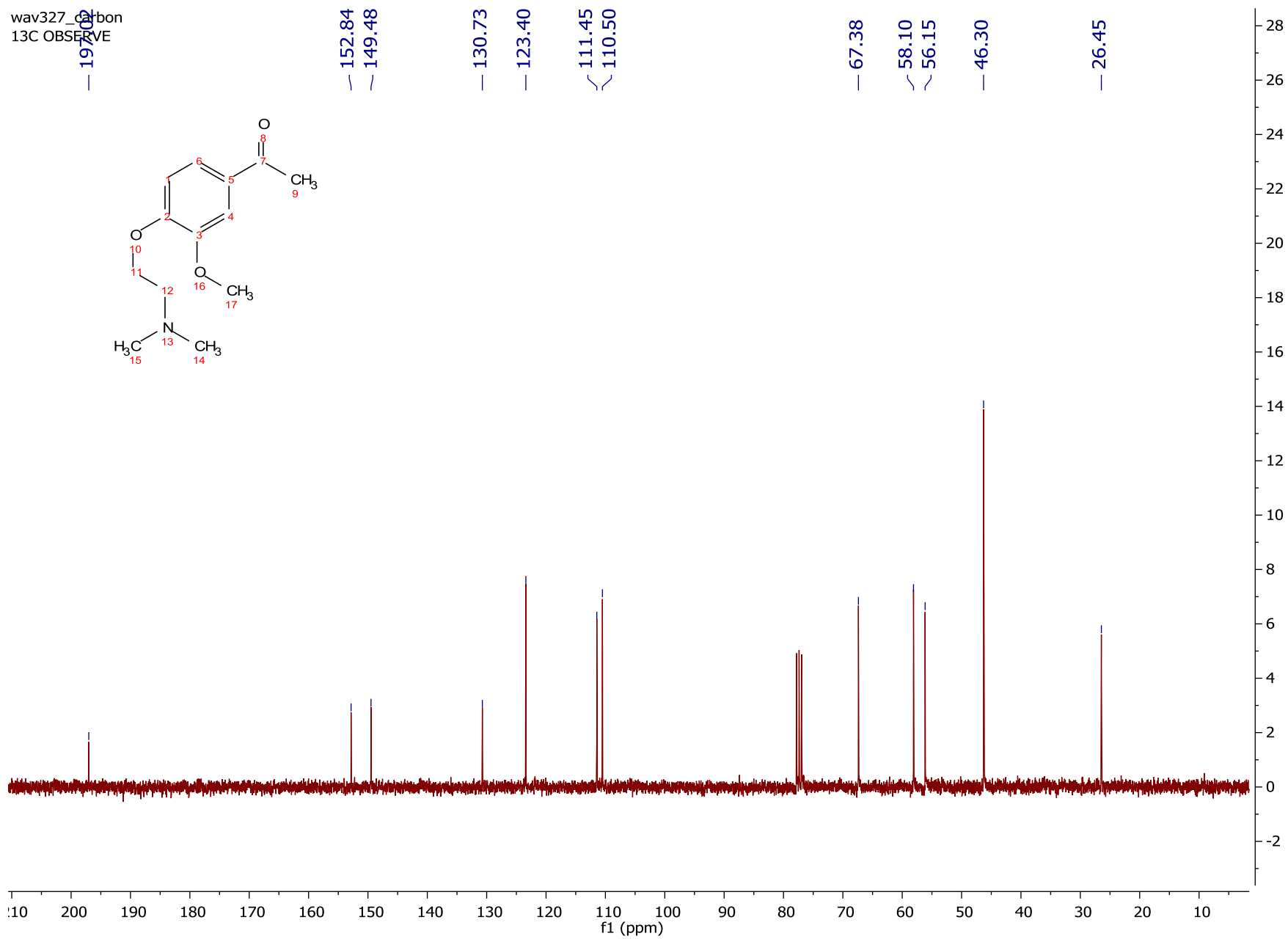
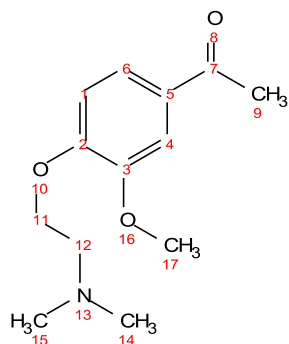
1:MS ES+
1.6e+007



wav327_proton2
STANDARD 1H OBSERVE

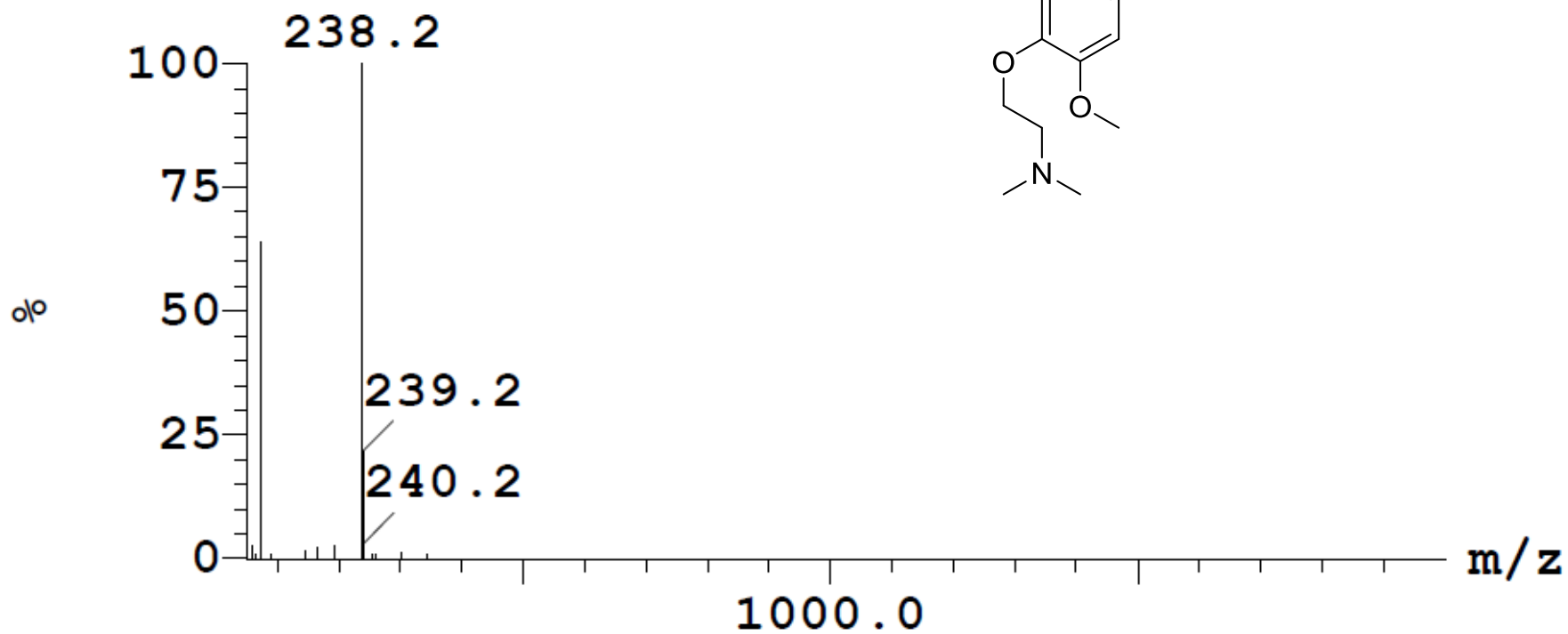
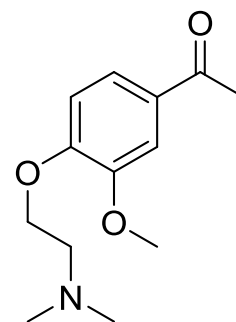


wav327_carbon
13C OBSERVE

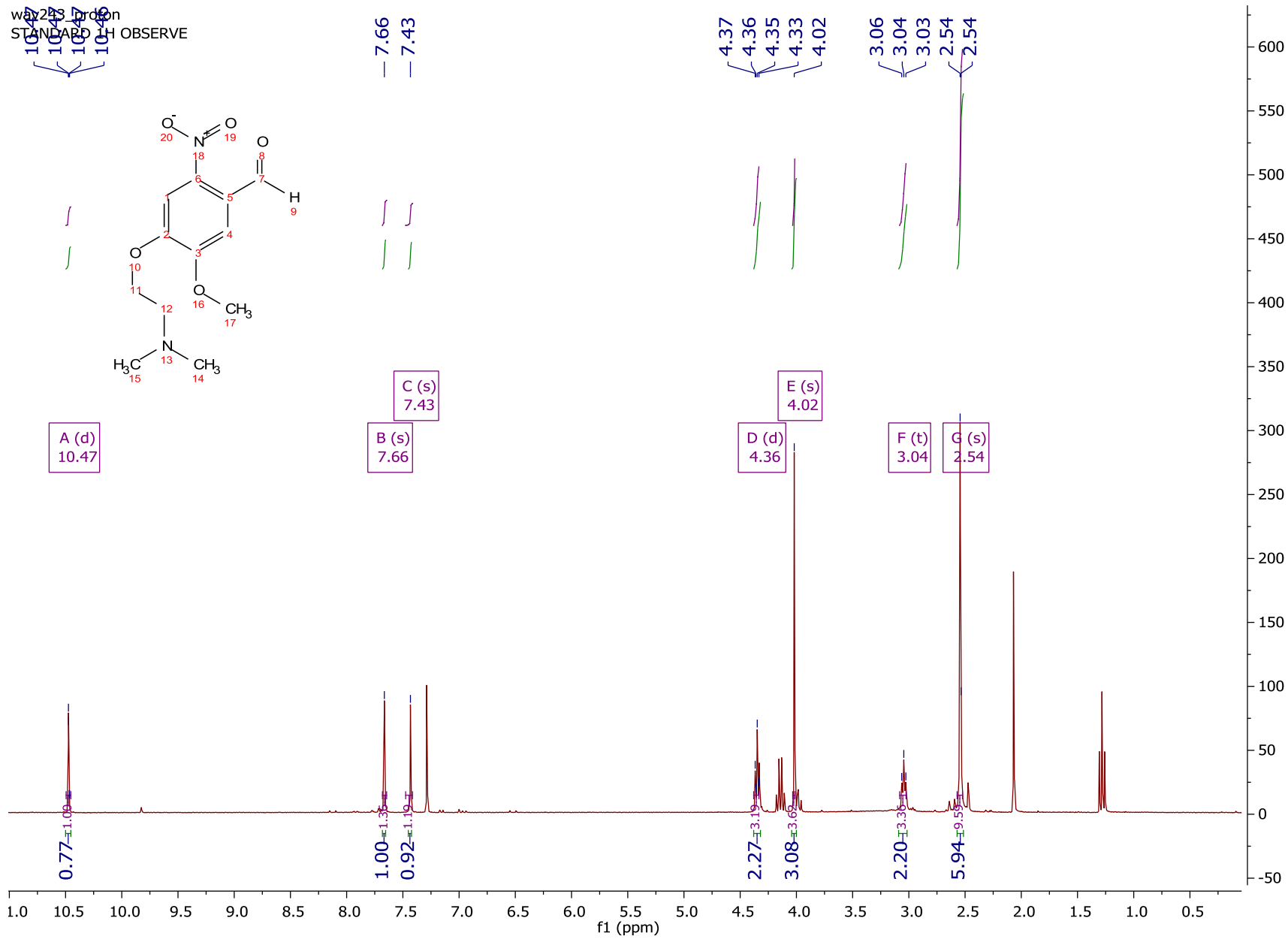


Peak ID	Compound	Time	Mass Found
17		3.12	

1:MS ES+
1.7e+007



way243
S12N143D
CH OBSERVE



wav326_carbon2
13C OBSERVE

— 188.09

— 153.75

— 152.00

— 143.94

— 125.81

— 110.08

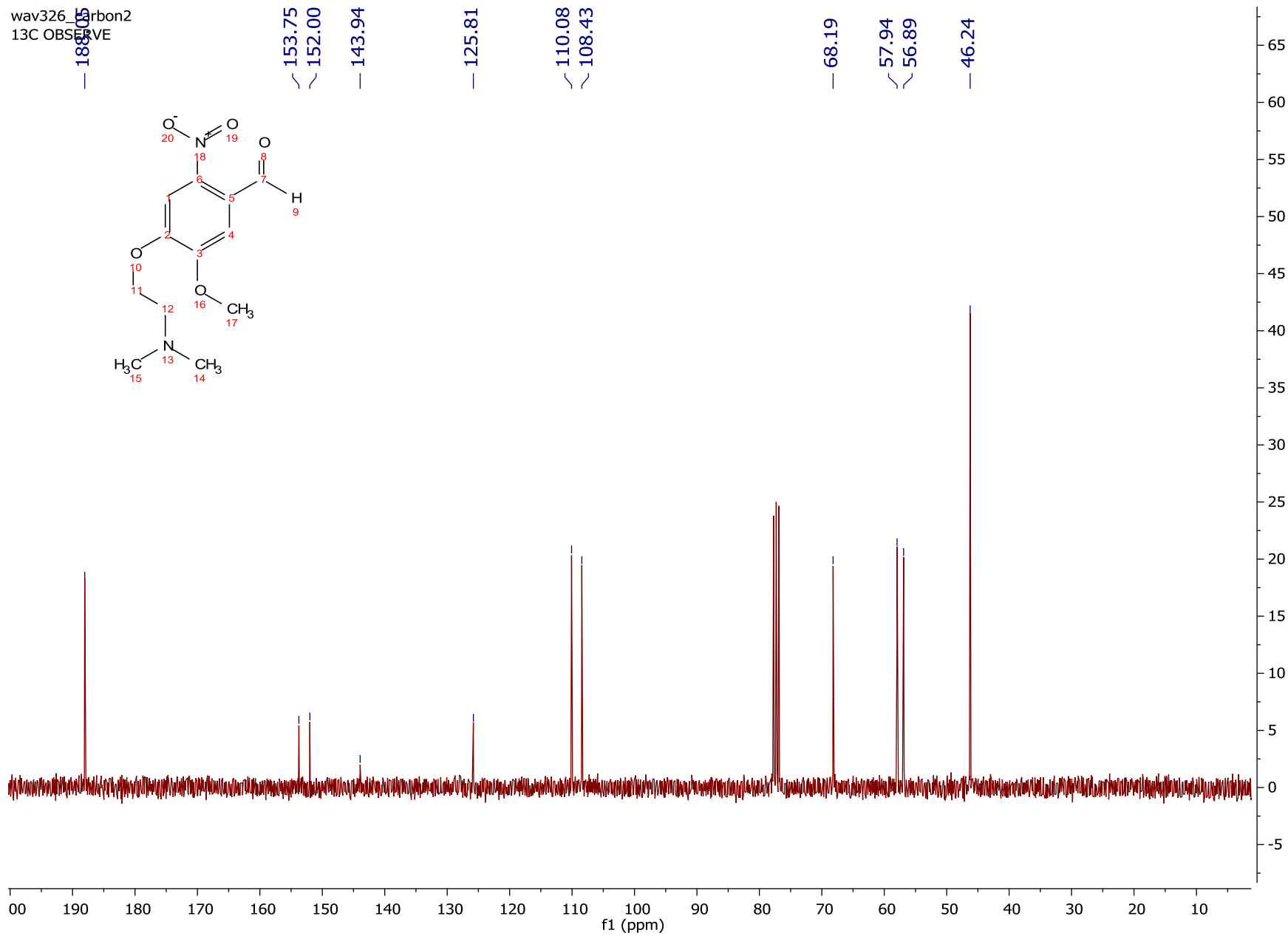
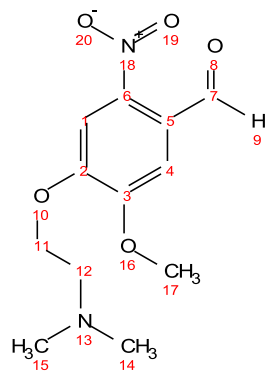
— 108.43

— 68.19

— 57.94

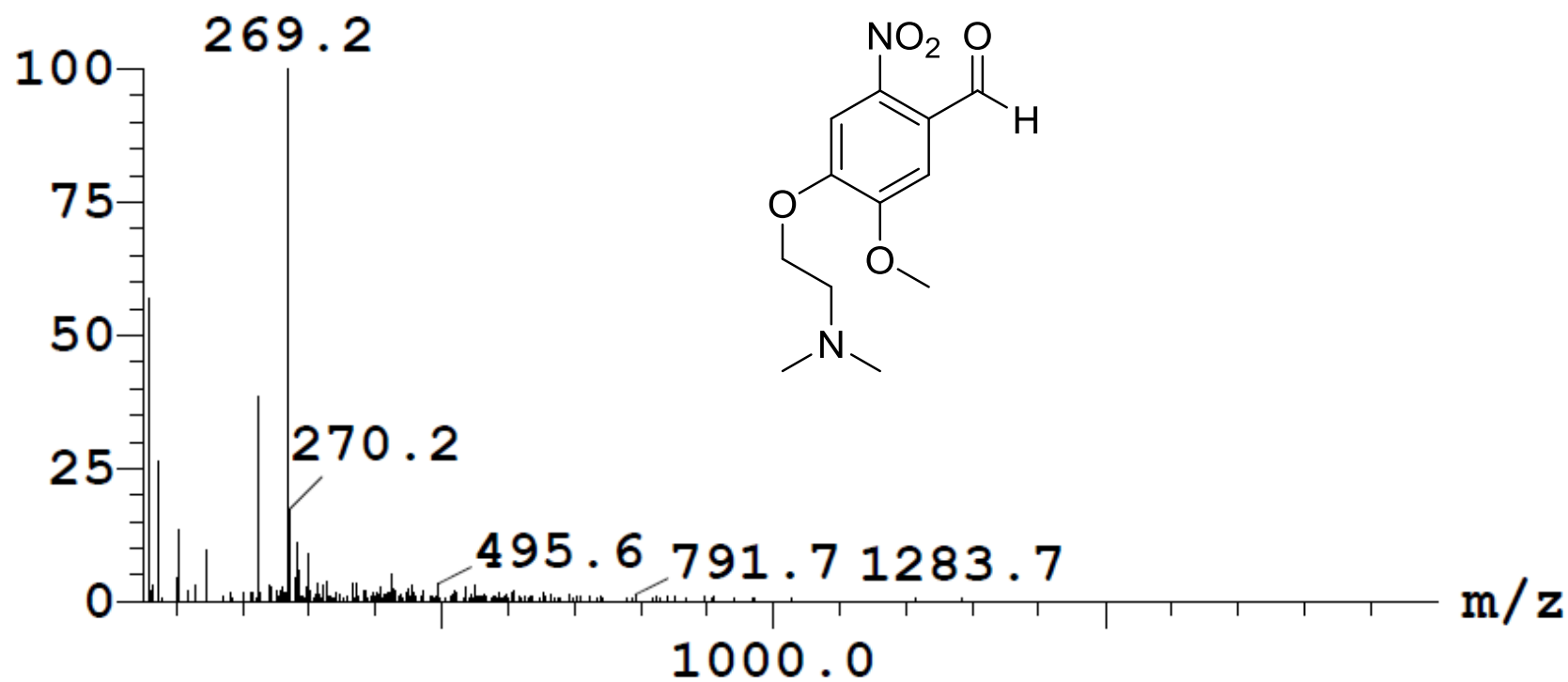
— 56.89

— 46.24

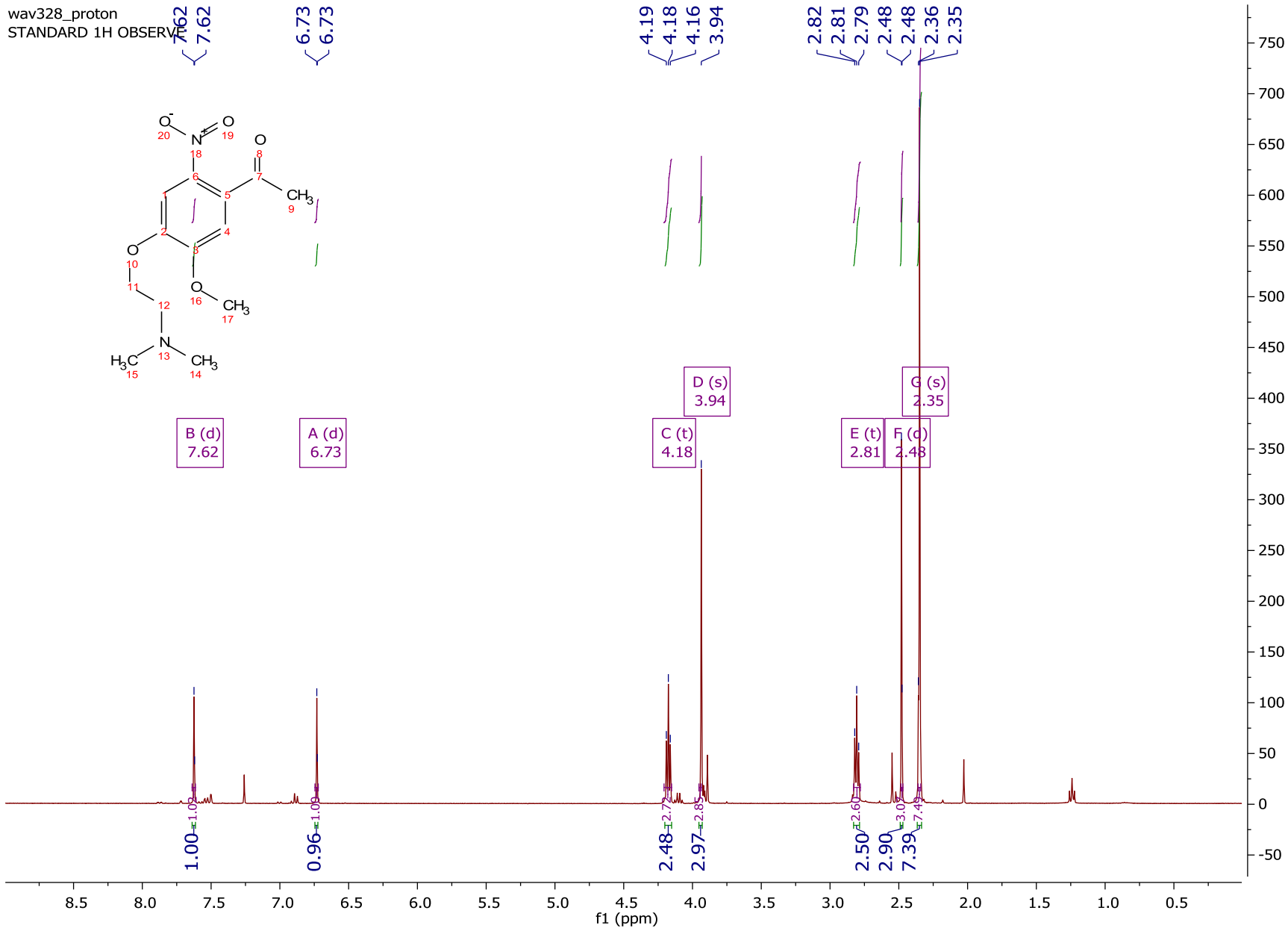
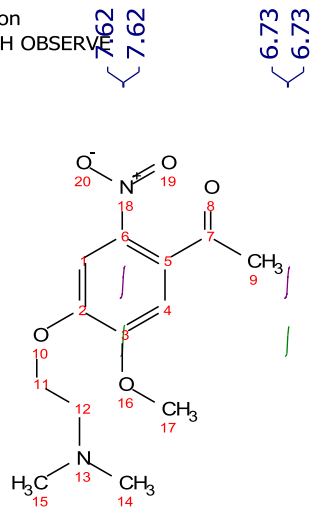


Peak ID	Compound	Time	Mass Found
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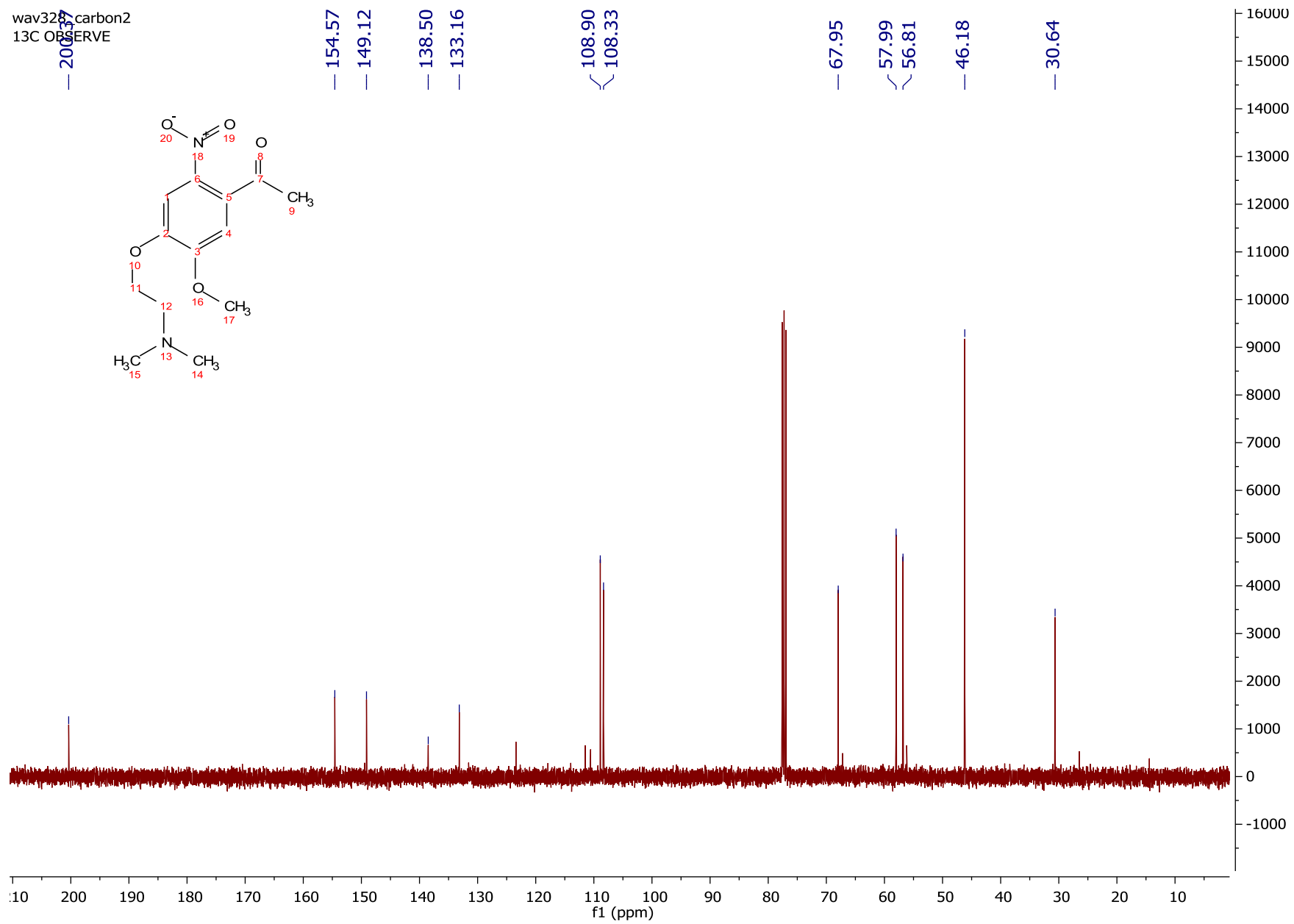
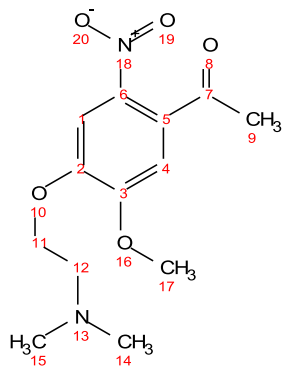
1:MS ES+
1.8e+006



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STANDARD 1H OBSERVE



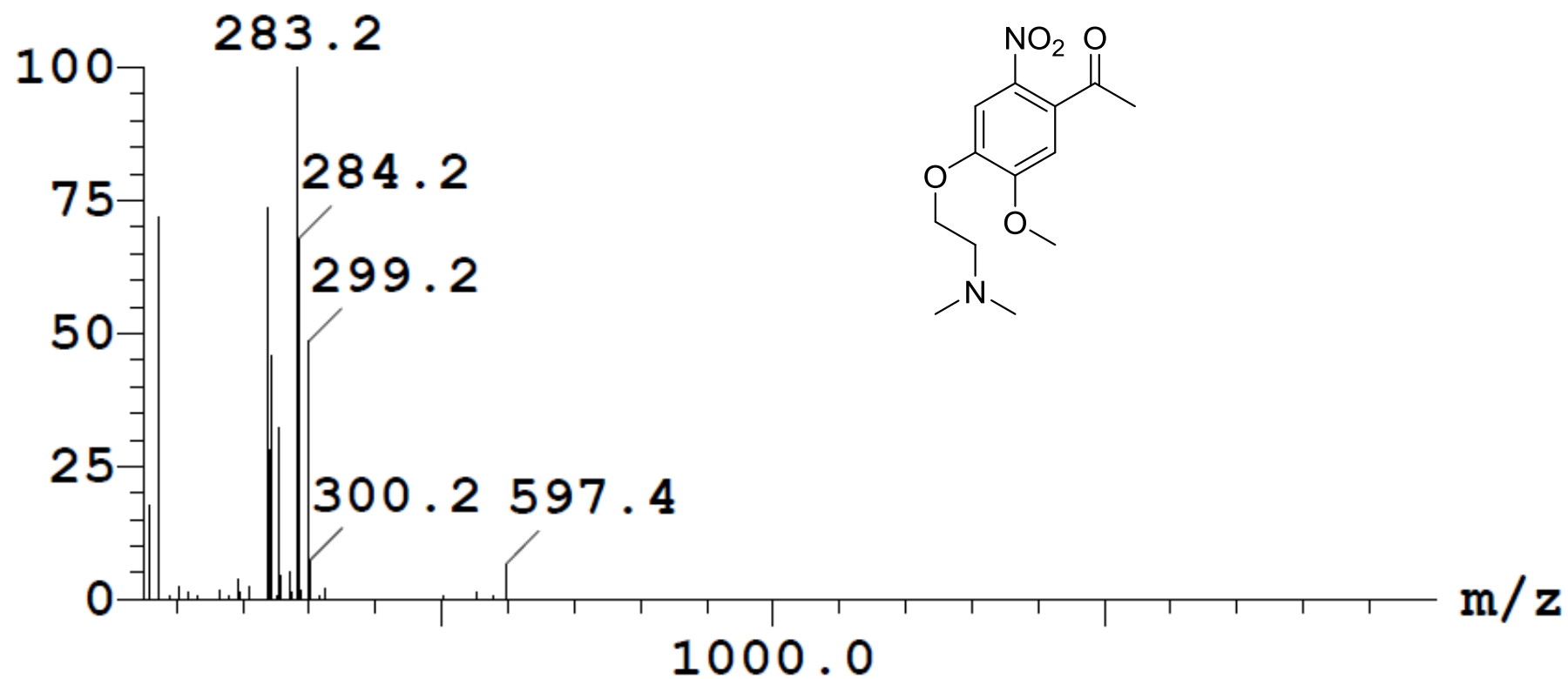
wav328_carbon2
13C OBSERVE



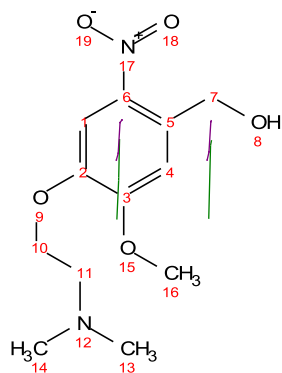
Peak ID Compound Time Mass Found
3 0.43

(Time: 0.43) Combine (10:16-51:54)

1:MS ES+
1.3e+008



wav244_proton
STANDARD 1H OBSERV



A (s)
7.64

B (s)
7.16

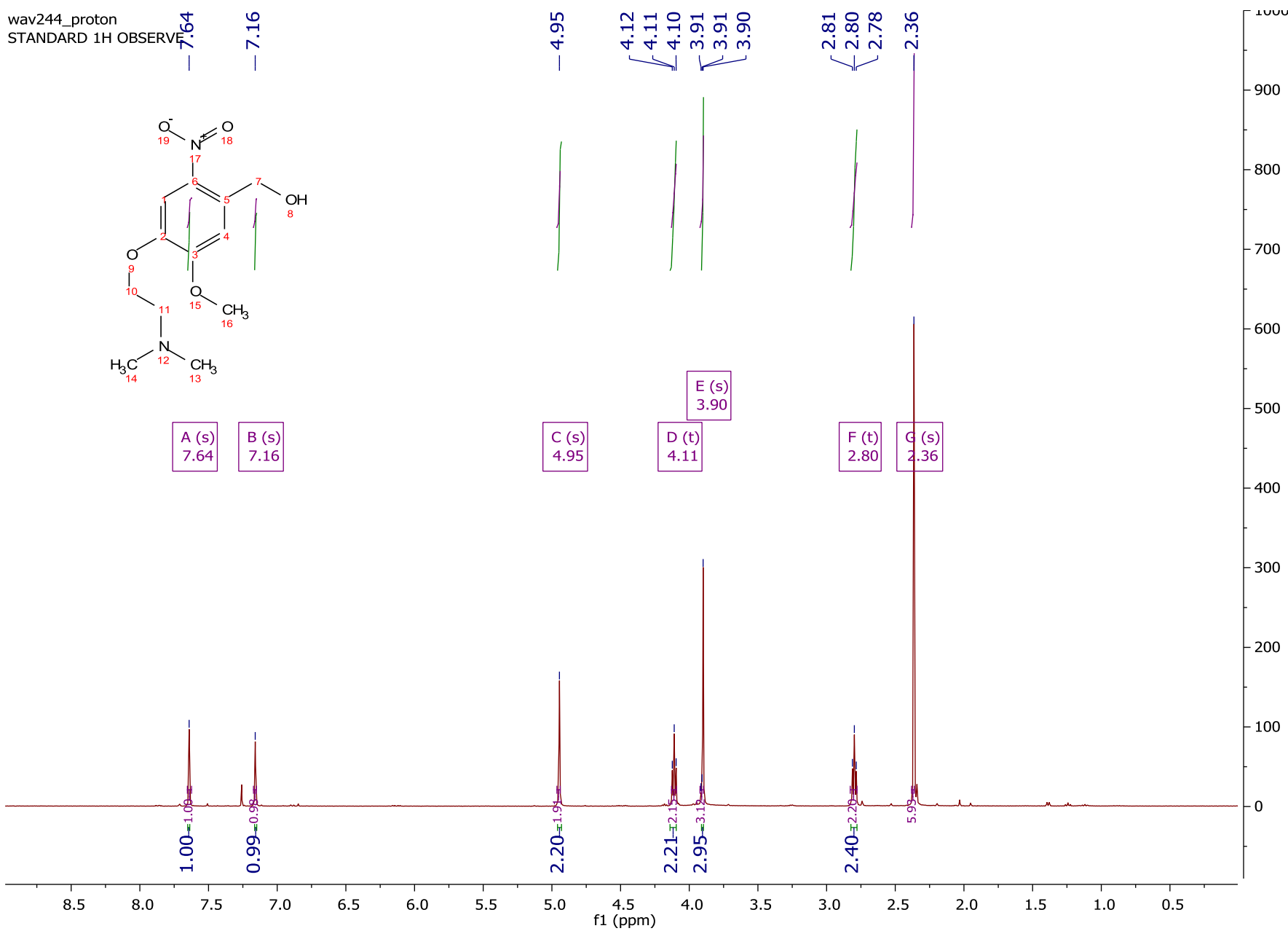
C (s)
4.95

D (t)
4.11

E (s)
3.90

F (t)
2.80

G (s)
2.36



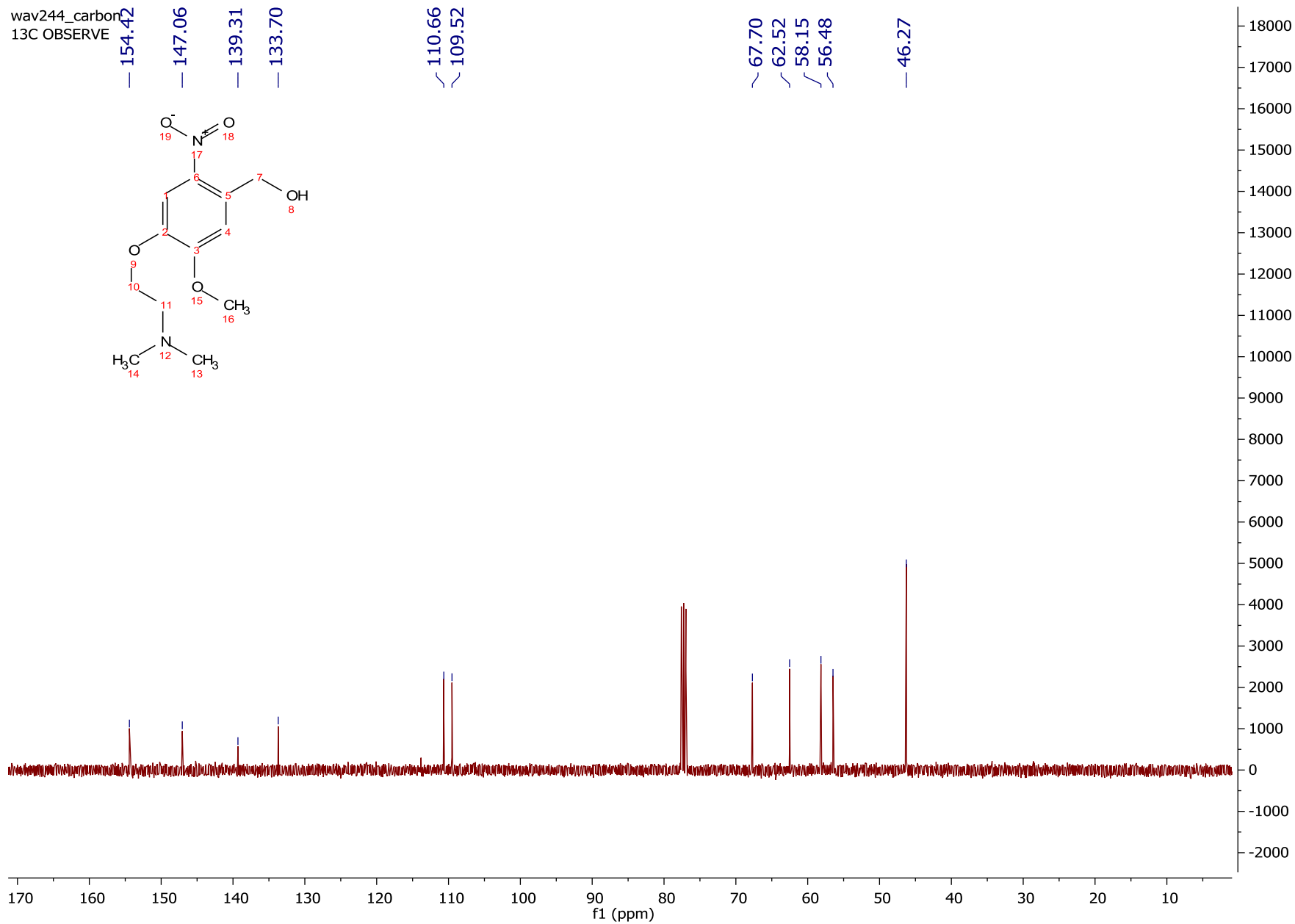
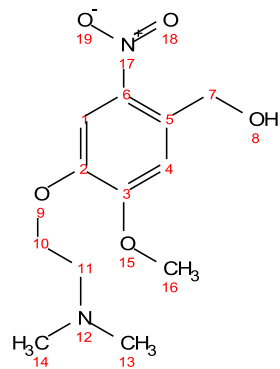
wav244_carbon
13C OBSERVE

— 154.42
— 147.06
— 139.31
— 133.70

— 110.66
— 109.52

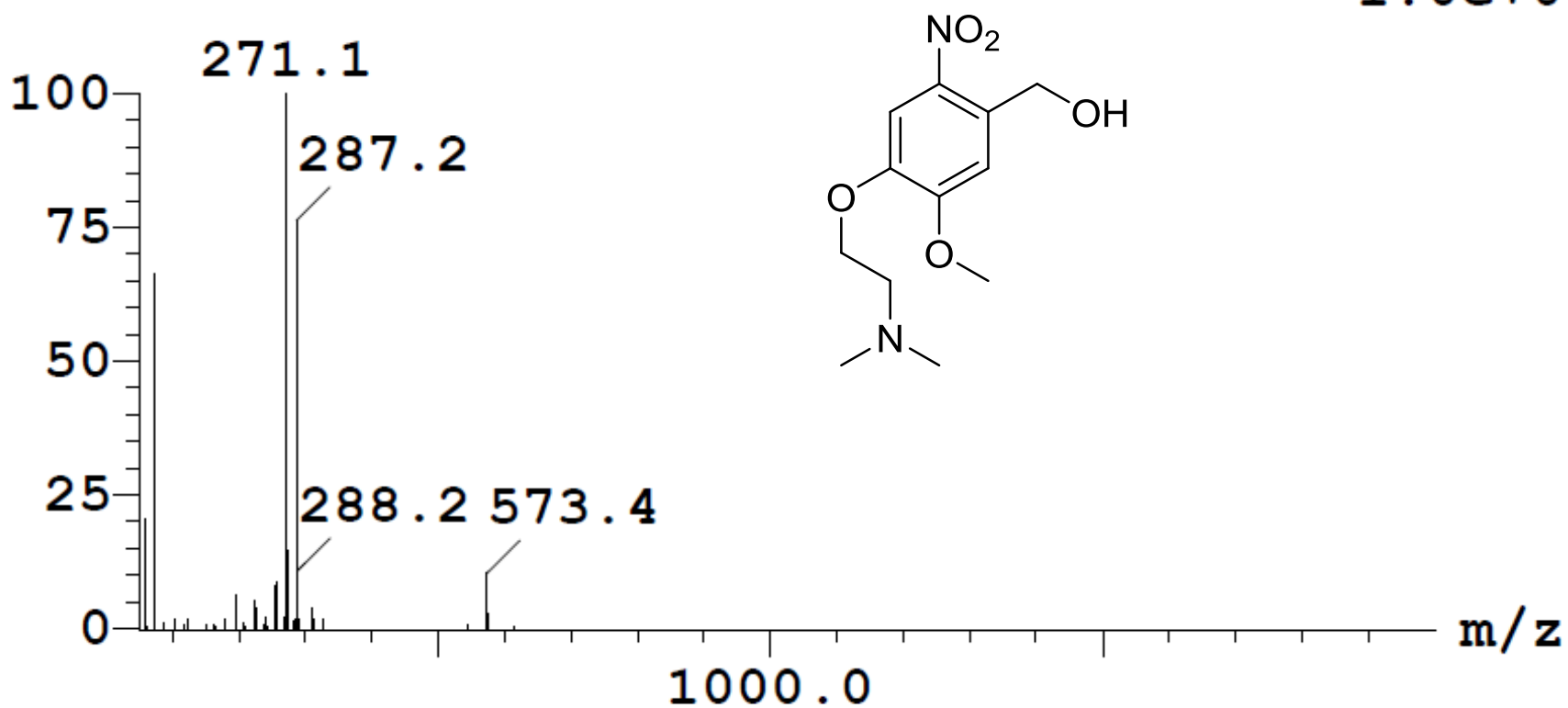
— 67.70
— 62.52
— 58.15
— 56.48

— 46.27

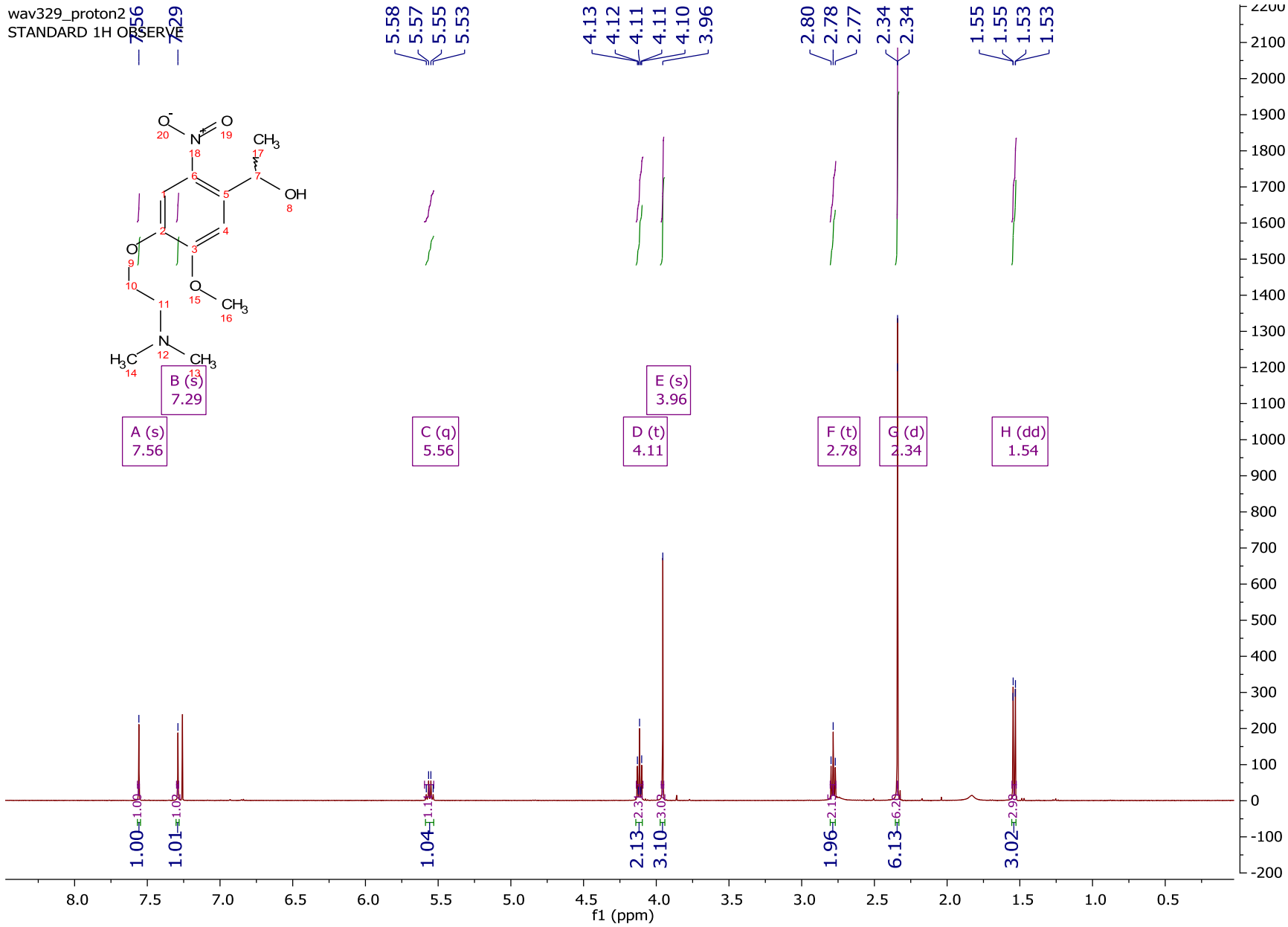


Peak ID	Compound	Time	Mass Found
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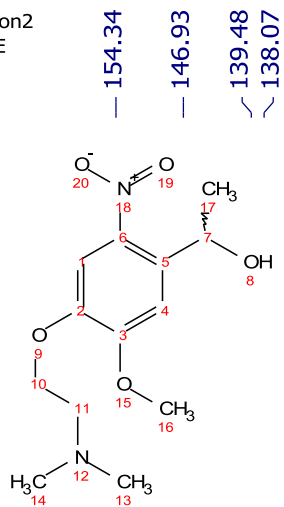
1:MS ES+
1.0e+008



wav329_proton2
STANDARD 1H OBSERVE



wav329_carbon2
13C OBSERVE

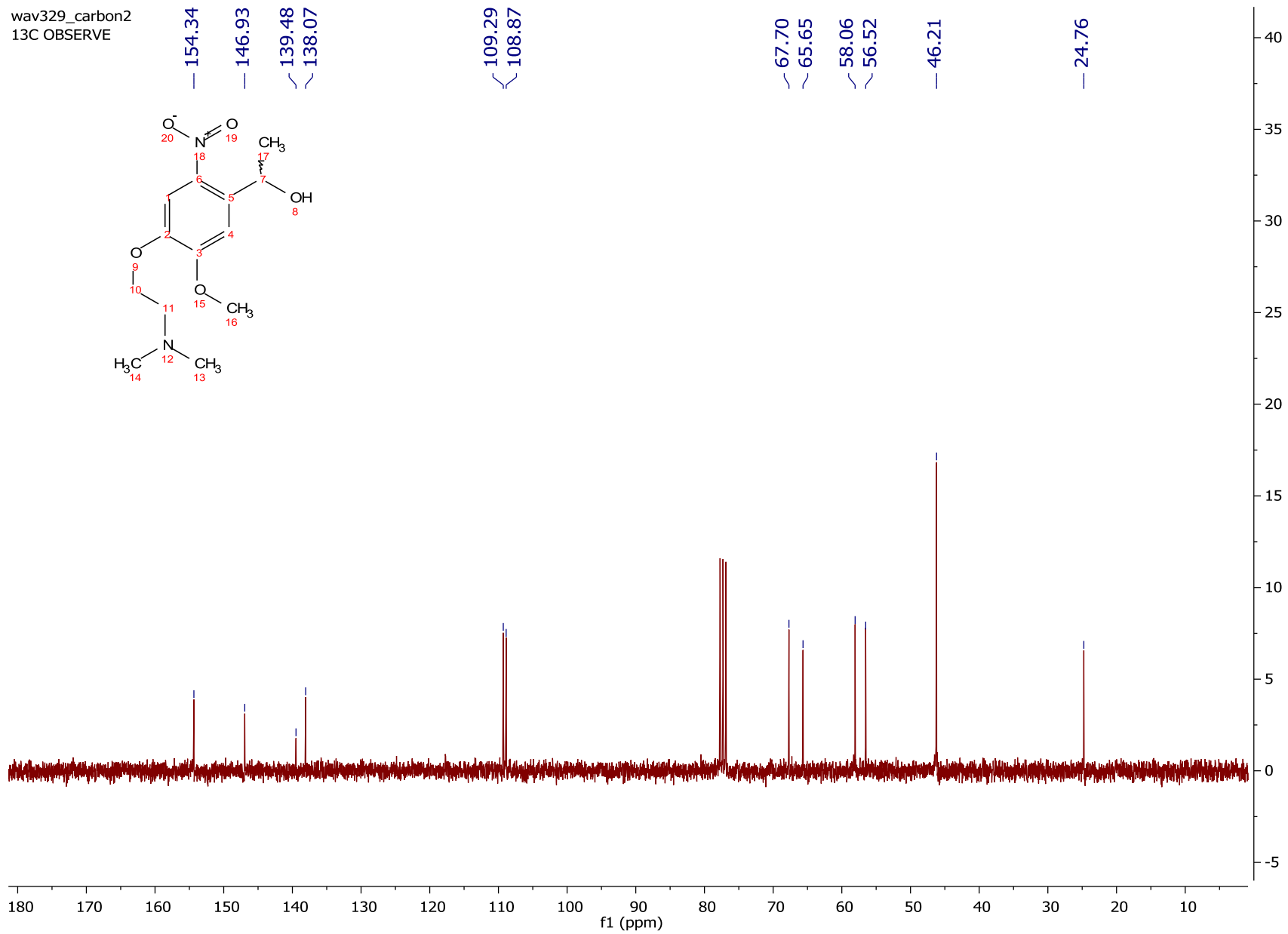


109.29
108.87

67.70
65.65
58.06
56.52

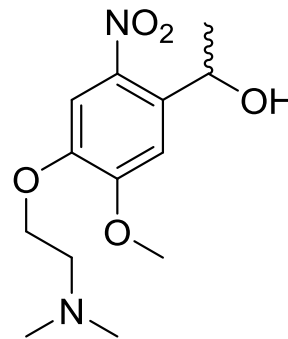
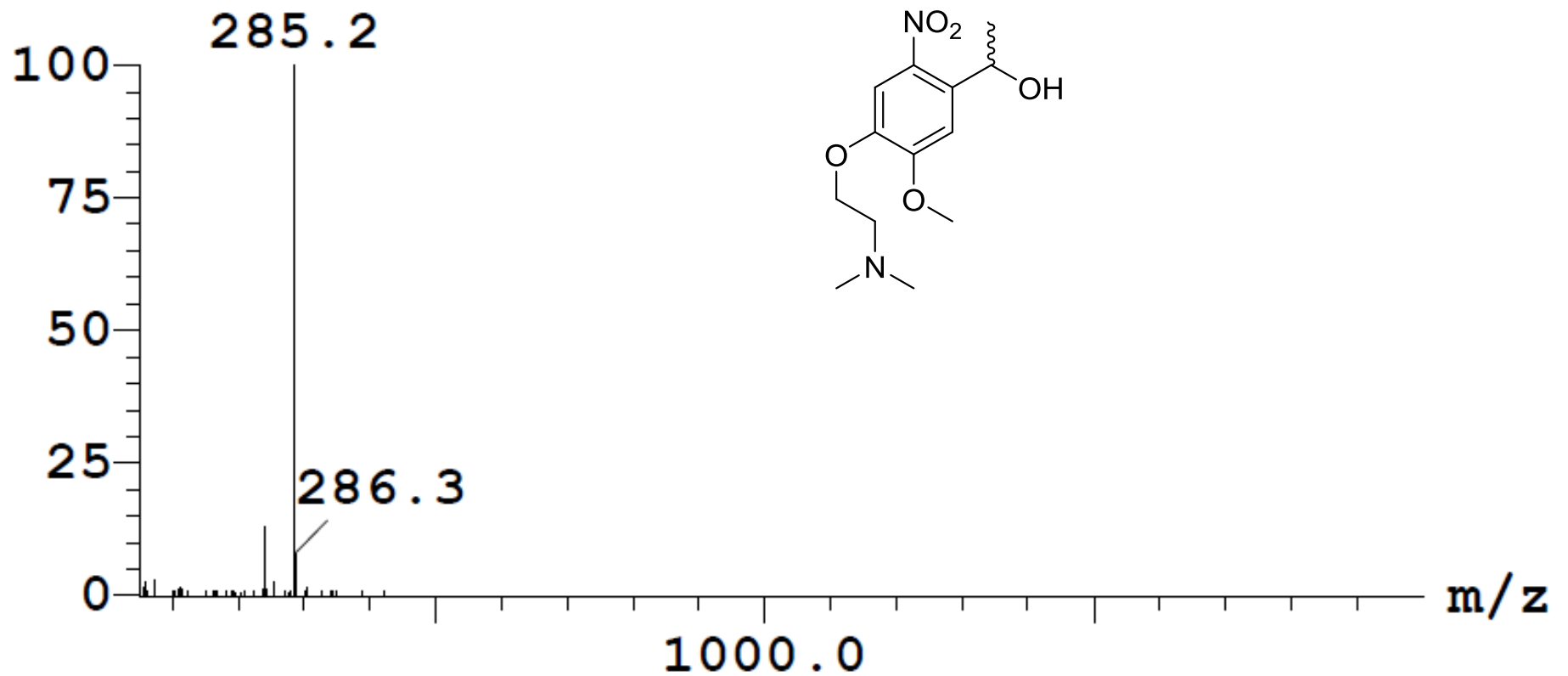
46.21

24.76

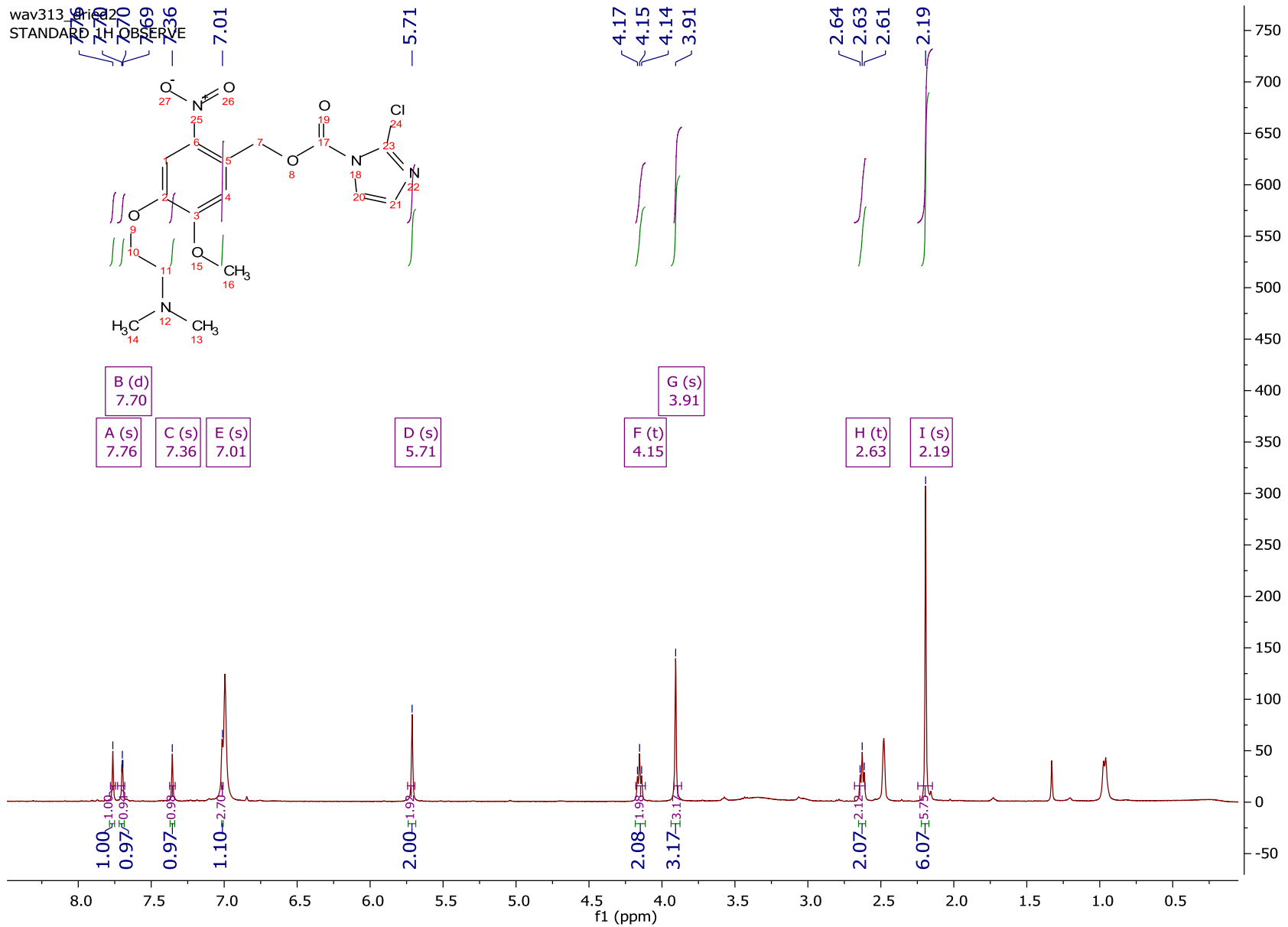


Peak ID	Compound	Time	Mass Found
10		2.43	

1:MS ES+
9.1e+006



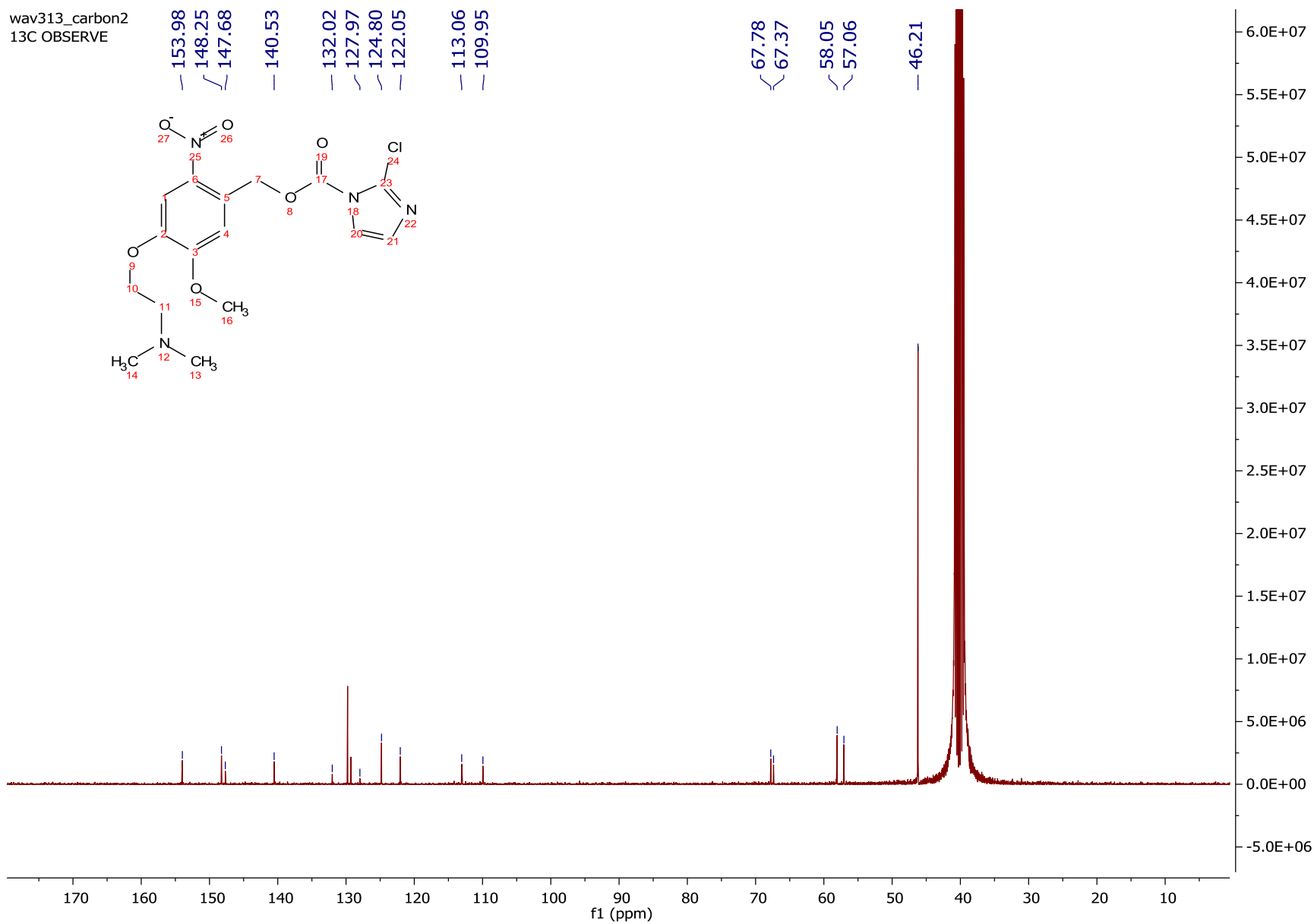
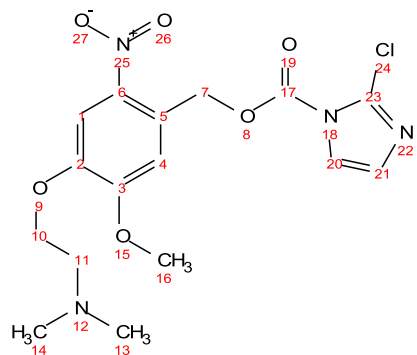
wav313_Std2
STANDARD_1H_OBSERVE

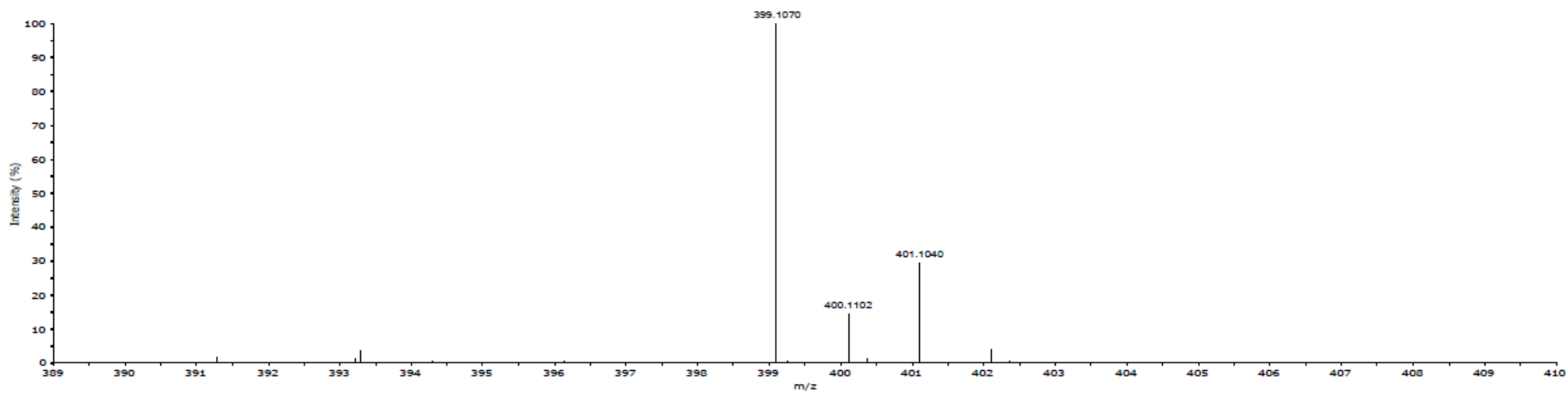
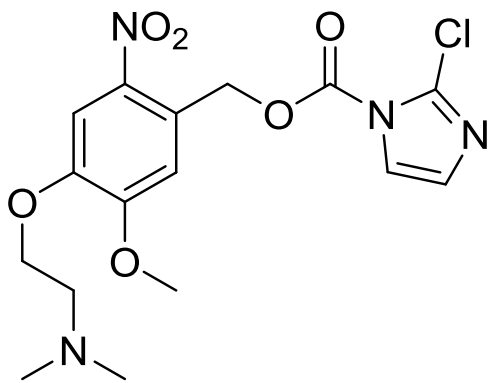


wav313_carbon2
13C OBSERVE

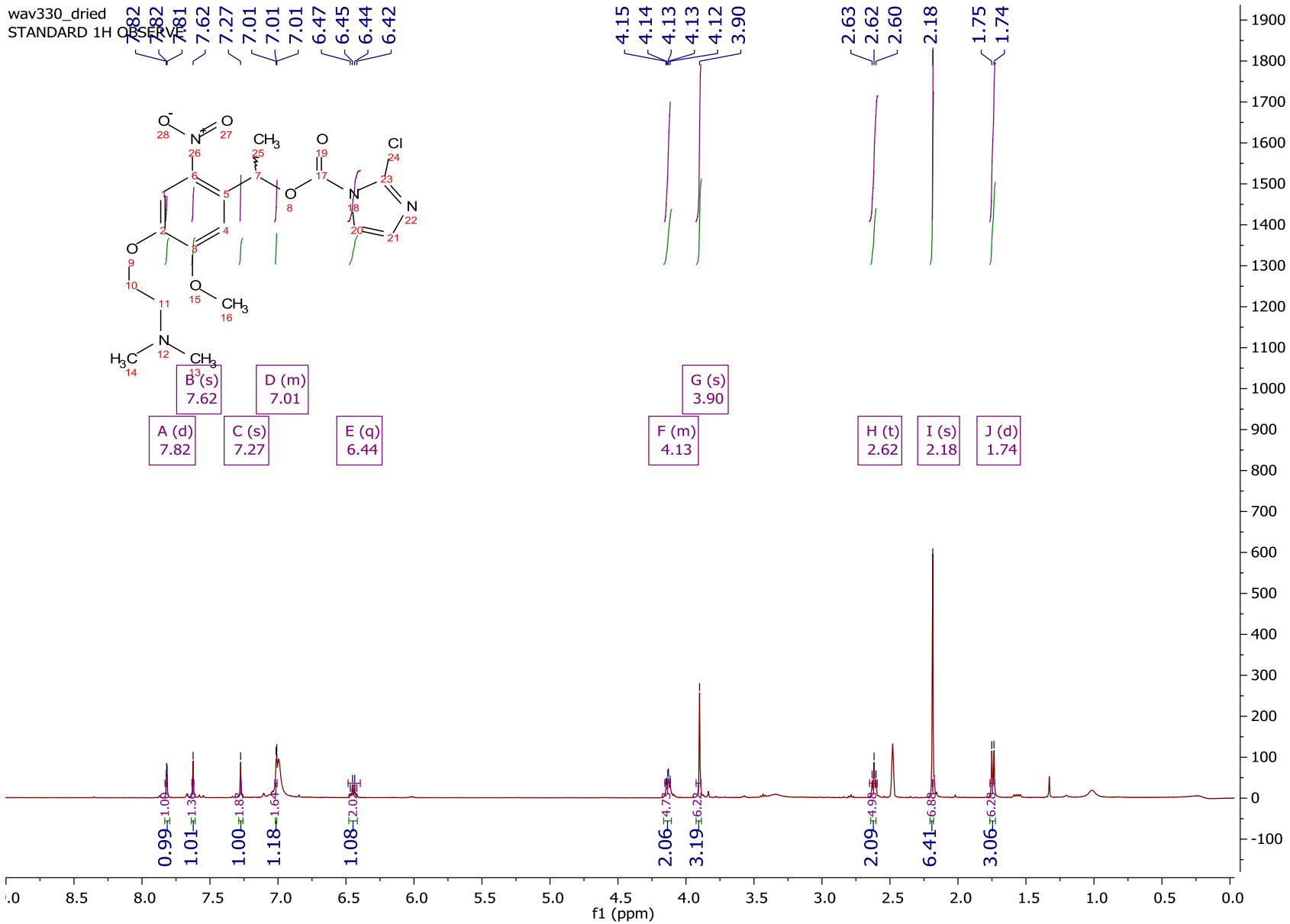
153.98
148.25
147.68
140.53
132.02
127.97
124.80
122.05
113.06
109.95

67.78
67.37
58.05
57.06
46.21





wav330_dried
STANDARD 1H QSPY

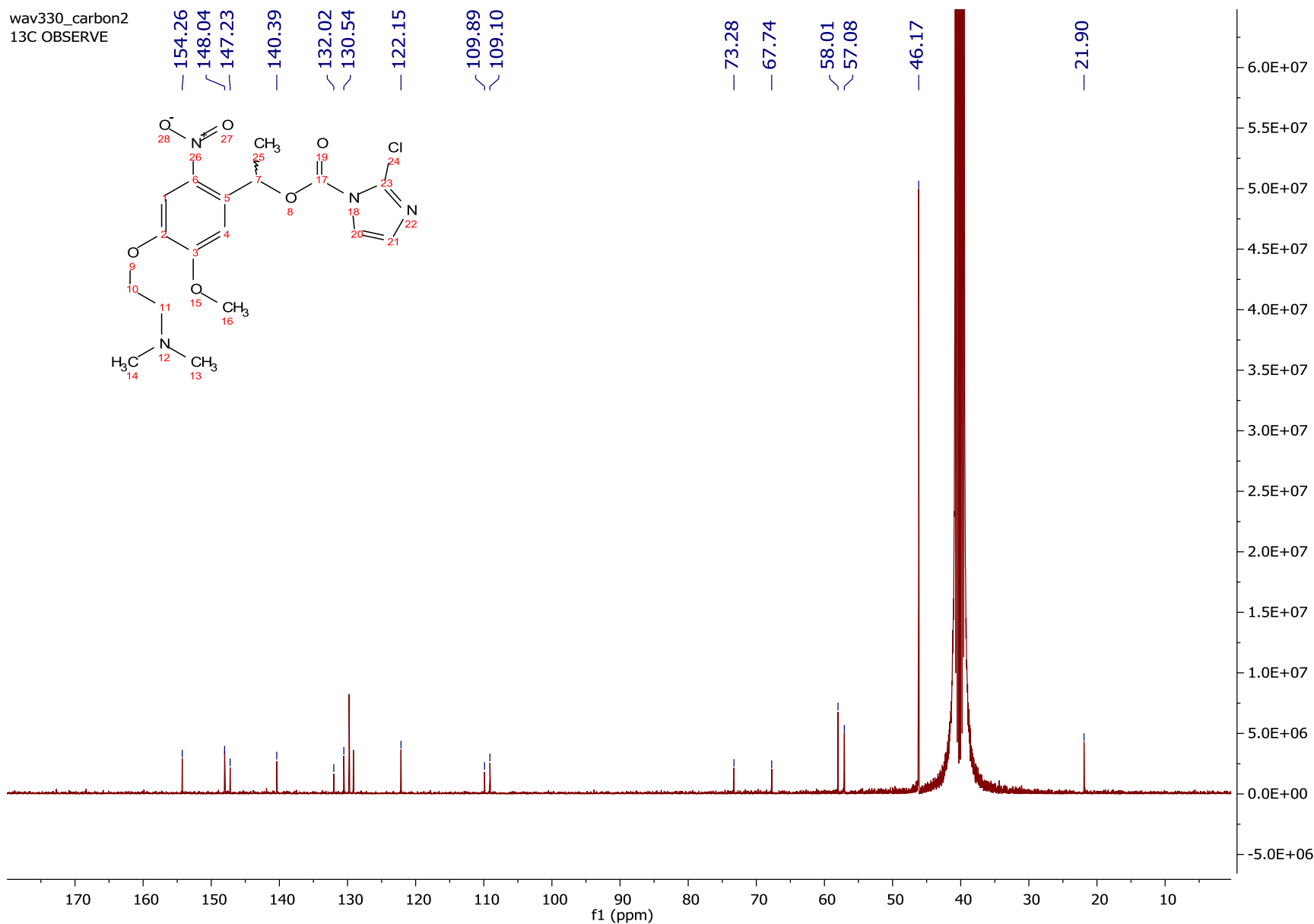
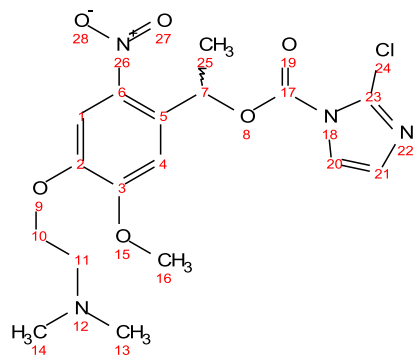


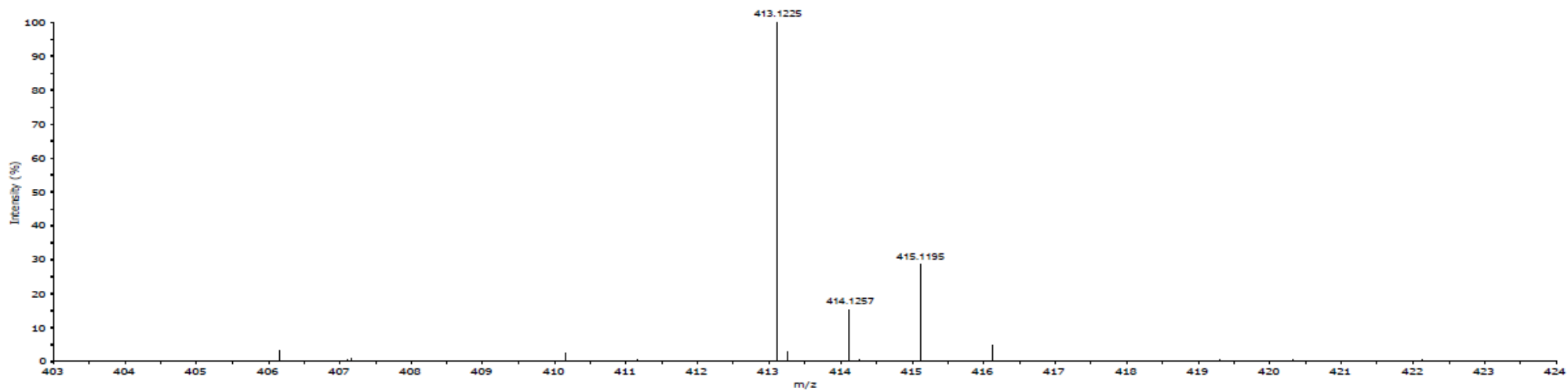
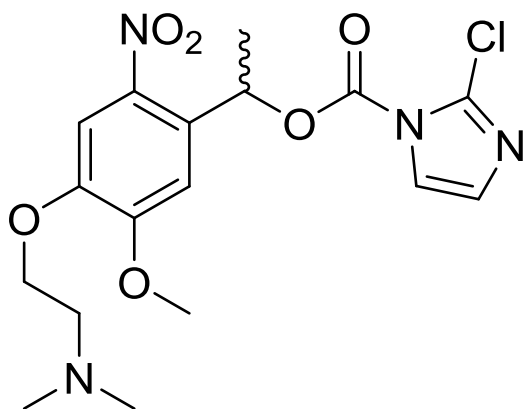
wav330_carbon2
13C OBSERVE

154.26
148.04
147.23
140.39
132.02
130.54
122.15
109.89
109.10

73.28
67.74
58.01
57.08
46.17

21.90





References

- (1) Franzini, R. M.; Kool, E. T. *J. Am. Chem. Soc.* **2009**, *131*, 16021.
- (2) Velema, W. A.; Kool, E. T. *J. Am. Chem. Soc.* **2017**, *139* (15), 5405.
- (3) Chaulk, S. G.; MacMillan, A. M. *Nucleic Acids Res.* **1998**, *26* (13), 3173.