Electronic Supplementary Material (ESI) for Molecular BioSystems.

A Tyrosine-Reactive Irreversible Inhibitor for Glutathione *S*-Transferase Pi (GSTP1)

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Supplemental Figures, Schemes, and Tables

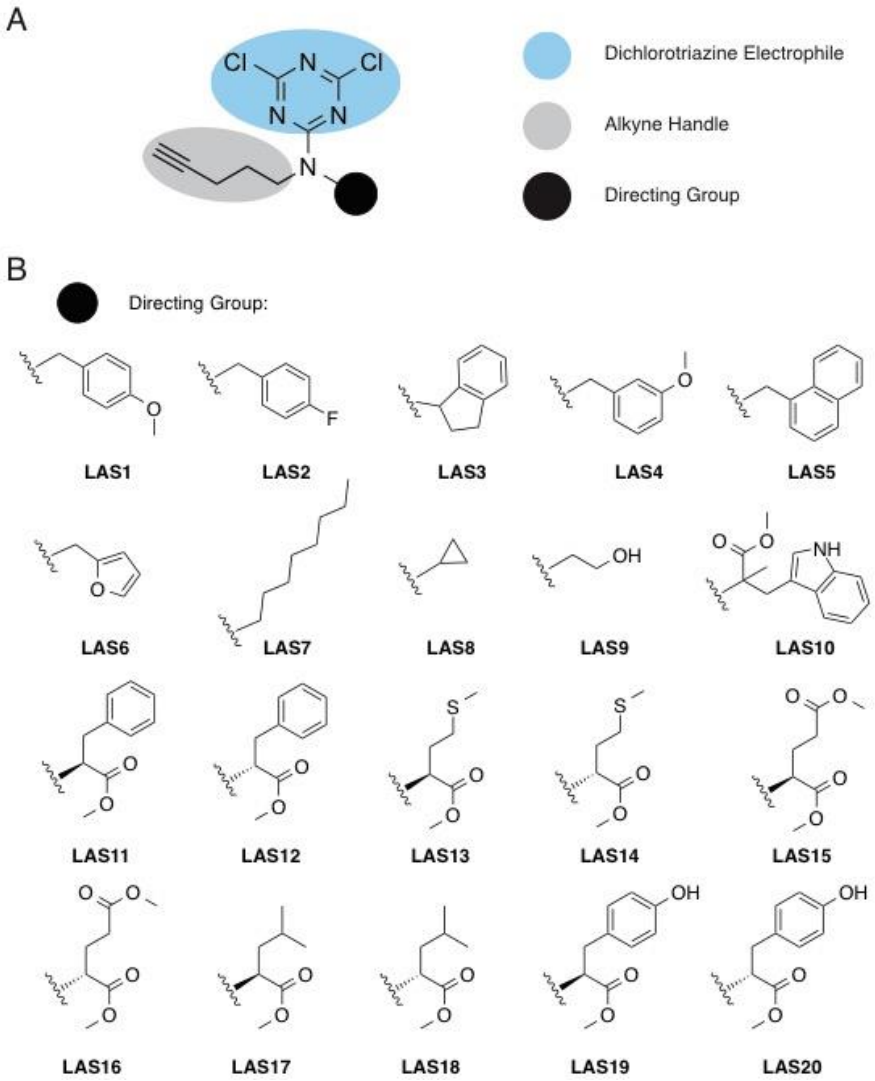
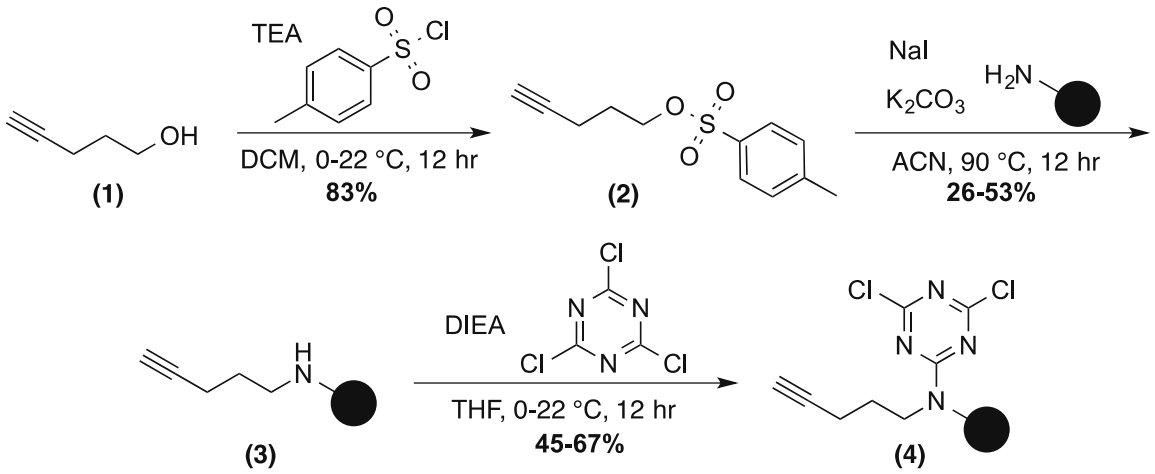


Figure S1. Dichlorotriazine probe design and library members. (A) All library members contain the dichlorotriazine electrophile to participate in nucleophilic aromatic substitution, an alkyne handle for further functionalization by a reporter group, and a directing group to fine tune for selectivity in a diverse proteome. (B) Chemical structures of the directing groups.



Scheme S1. General synthetic scheme of LAS1-20.

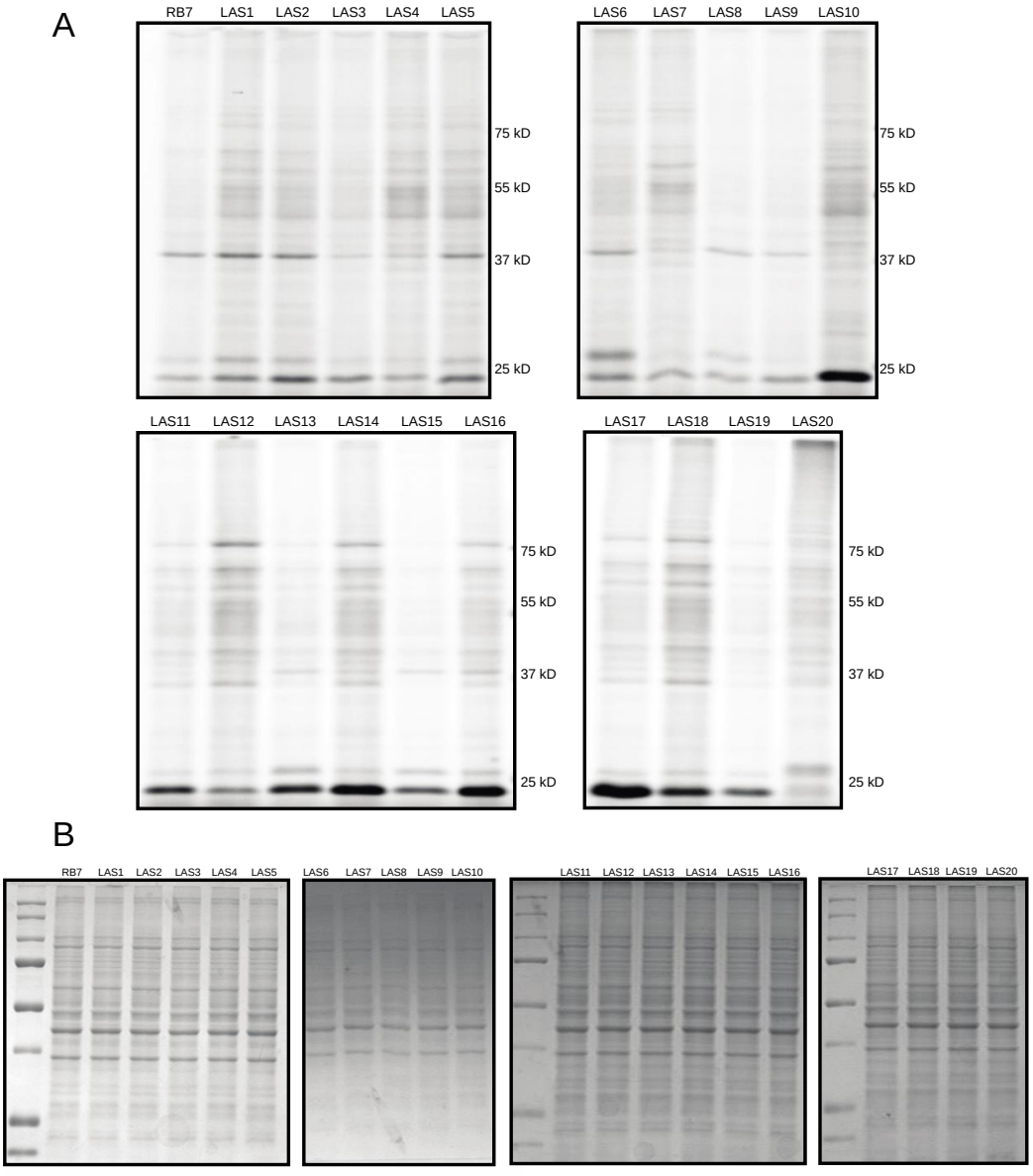


Figure S2. Evaluating the dichlorotriazine library in HeLa cell lysates. (A) HeLa lysates (50 µL, 2 mg/mL) were pretreated with LAS1-20 (1 µM) at RT for 1 hr. Samples were subjected to CuAAC with TAMRA-azide, separated by SDS-PAGE and analyzed by in-gel fluorescence. (B) Coomassie Blue stained gels. The RB7 probe has been previously reported.[1]

Table S1. Proteins identified in DMSO and LAS17-treated HeLa cell lysates. Data are shown for entries with greater than 5 average spectral counts for LAS17-treated samples across the three trials. Data are displayed in the table below from highest to lowest average spectral counts in LAS17-treated samples.

Protein	Mol Weight (Da)	DMSO_1	DMSO_2	DMSO_3	LAS17_1	LAS17_2	LAS17_3
GSTP1 Glutathione S-transferase	23342	0	0	0	36	23	13
HSP90AB1 Heat shock protein HSP 90-beta	83264	11	0	0	8	11	53
TKT Transketolase	67878	7	0	3	15	8	46
TUBA4A Tubulin alpha-4A chain	49924	8	0	5	4	13	48
PKM Pyruvate kinase isozymes M1/M2	57937	7	5	5	7	5	55
EEF1A1 Elongation factor 1-alpha 1	50141	12	0	4	4	0	57
FLNA Filamin-A	280737	12	0	5	9	9	42
TUBB Tubulin beta chain	49671	5	8	4	15	10	33
PGK1 Phosphoglycerate kinase 1	44615	3	0	0	3	8	31
HSP90AA1 Heat shock protein HSP 90-alpha	84660	0	0	0	5	0	31
ACTB Actin, cytoplasmic 1	41737	14	2	10	10	3	48
TUBB4B Tubulin beta-4B chain	49831	5	8	0	0	10	33
EEF1A2 Elongation factor	50470	0	0	4	4	0	26

1-alpha 2							
HSPA5 78 kDa glucose-regulated protein	72333	0	0	0	0	2	19
MYH9 Myosin-9	226530	5	0	4	5	3	21
YWHAE 14-3-3 protein epsilon	29174	0	0	0	0	0	19
LMNA Prelamin-A/C	74140	2	0	0	0	3	17
PGAM1 Phosphoglycerate mutase 1	28804	0	0	0	0	0	18
GAPDH Glyceraldehyde-3-phosphate dehydrogenase	36053	2	0	0	2	0	16
PFKP 6-phosphofructokinase type C	85596	2	0	0	7	5	6
CLTC Clathrin heavy chain 1	191613	4	0	4	5	4	14
EEF2 Elongation factor 2	95338	0	0	2	6	0	11
HSPD1 60 kDa heat shock protein, mitochondrial	61055	0	0	0	4	0	11
XRCC6 X-ray repair cross-complementing protein 6	69843	0	0	0	0	2	13

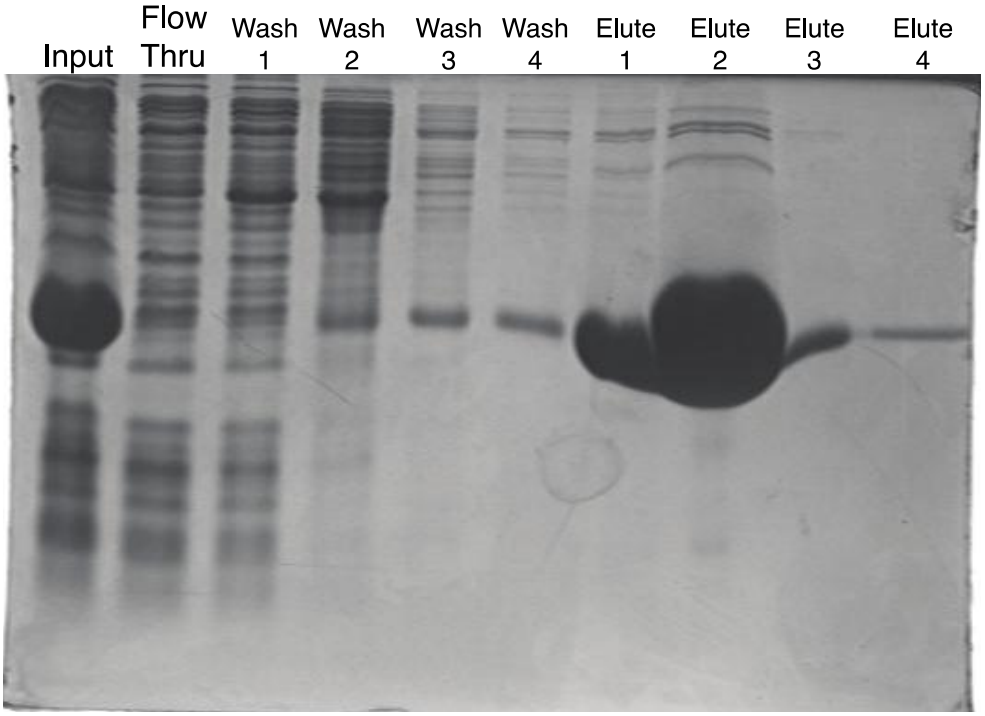


Figure S3. Sample purification gel of wild-type GSTP1.

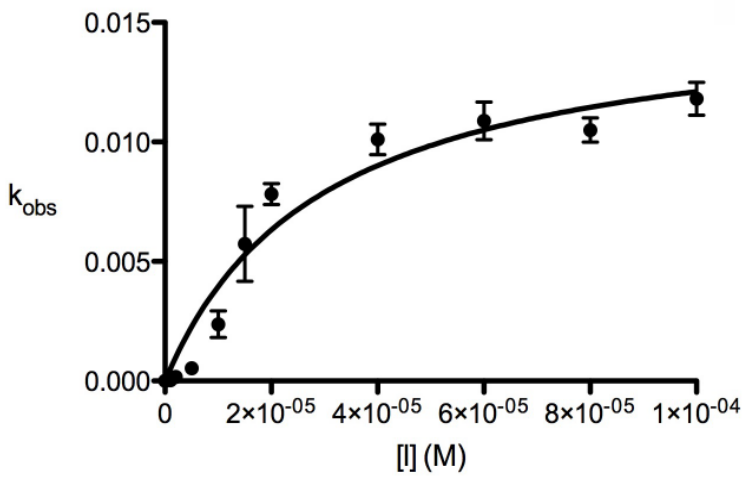


Figure S4. Time-dependent inhibition of GSTP1 activity demonstrated using an *in vitro* activity assay that monitors GSTP1-catalyzed conjugation of GSH to BDNB. LAS17 inhibits inhibits GSTP1 with a second-order rate constant of inactivation (k_{inact}/K_I) of 31,200 $M^{-1}s^{-1}$.

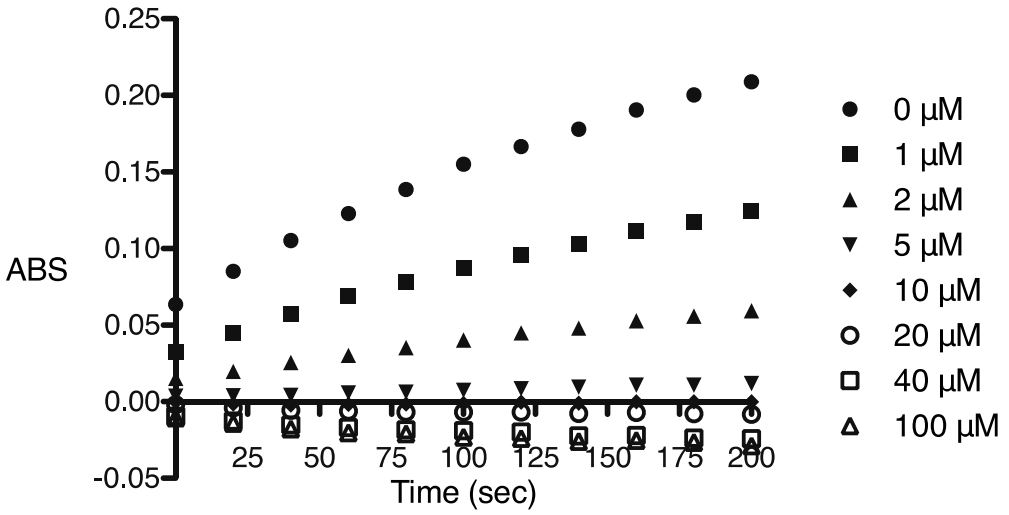


Figure S5. LAS17 inhibits the activity of GSPT1 in the presence of HeLa cell lysate background (1.0 mg/mL).

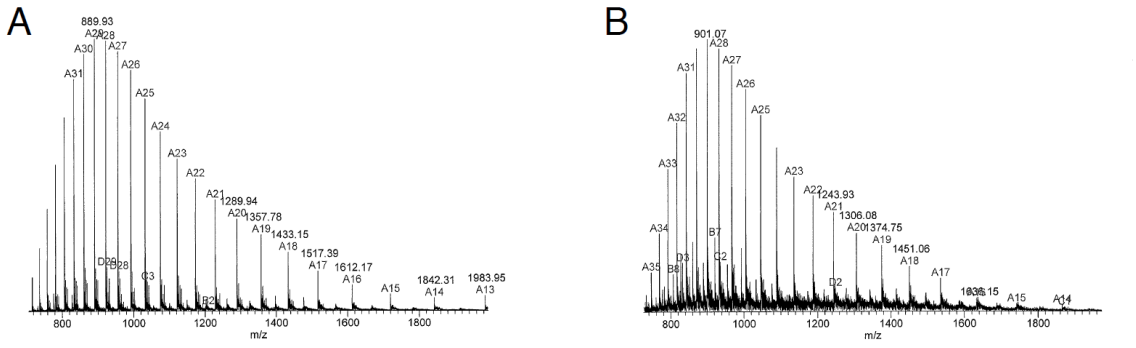


Figure S6. Intact-protein MS of (A) DMSO and (B) LAS17-treated GSTP1 prior to deconvolution.

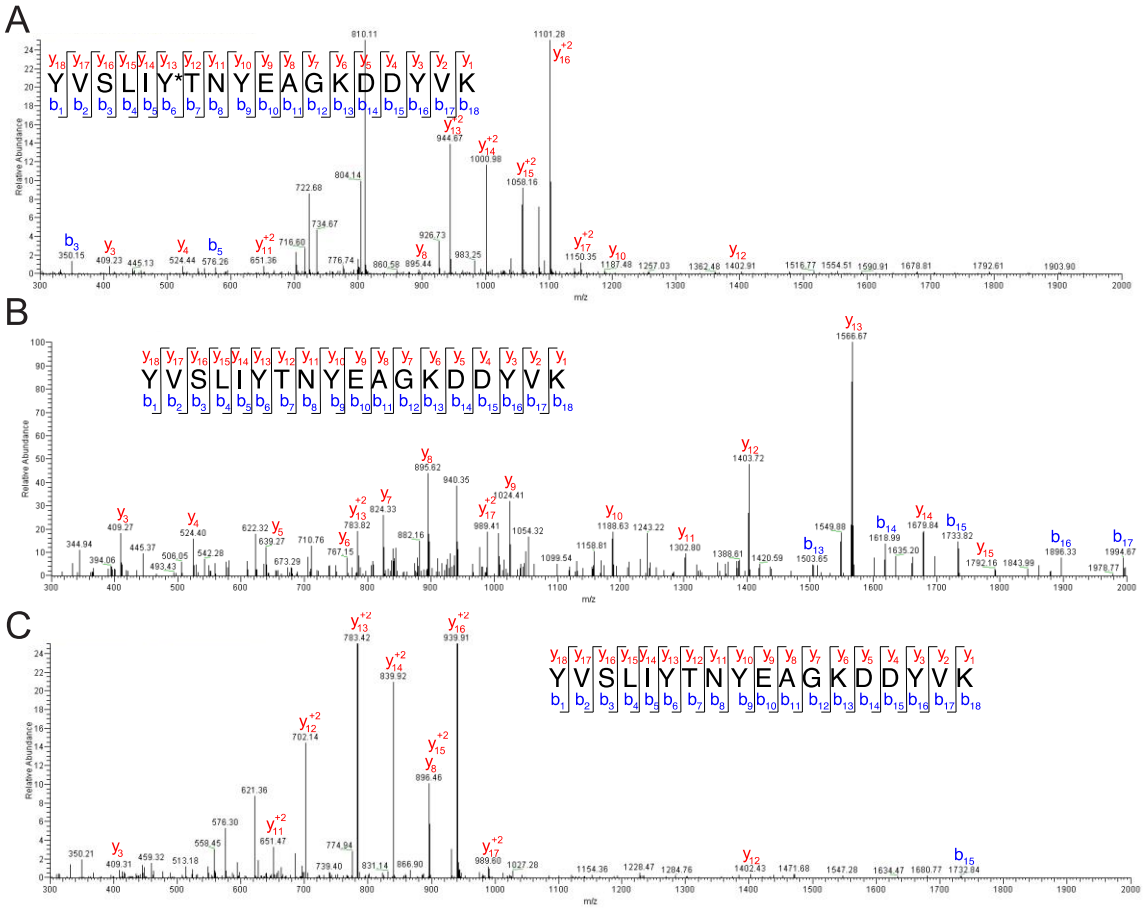


Figure S7. (A) Annotated MS2 fragmentation spectra for the GSTP1 LAS17-modified peptide (+3). (B) Annotated MS2 fragmentation spectra for the unmodified GSTP1 peptide (+2). (C) Annotated MS2 fragmentation spectra for the unmodified GSTP1 peptide (+3). The fragmentation spectra for the +2 LAS17-modified peptide is shown in Figure 3.

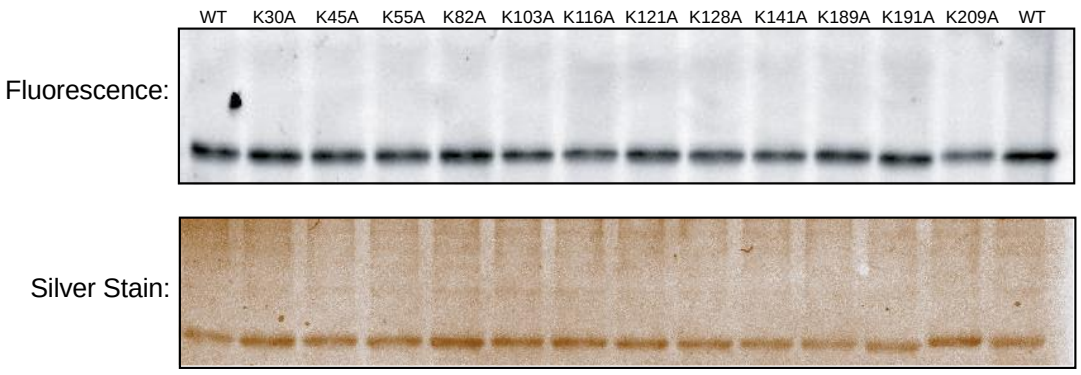
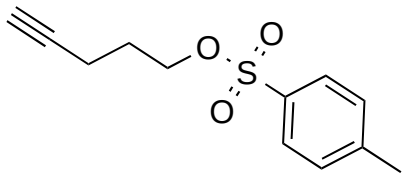


Figure S8. Screening of GSTP1 lysine mutants for LAS17 labeling.

General Procedures and Materials

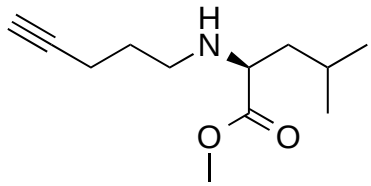
All reagents were purchased from Sigma Aldrich unless otherwise noted. All compounds were characterized by ^{1}H and ^{13}C NMR on either a Varian (Palo Alto, CA) 500 MHz or 600 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) with chemical shifts referenced to internal standards: $CDCl_3$ (7.26 ppm for ^{1}H, 77.8 ppm for ^{13}C). Coupling constants (*J*) are reported in Hertz (Hz) and multiplicities are abbreviated as singlet (s), broad singlet (bs), doublet (d), triplet (t), pentet (p), multiplet (m), doublet of doublets (dd), and doublet of triplets (dt). High resolution mass spectra (HRMS) were obtained at the Mass Spectrometry Facility at Boston College (Chestnut Hill, MA). Analytical thin layer chromatography (TLC) was performed on Sorbent Technologies Silica G TLC Plates w/UV354 (0.25 mm). All compounds were visualized on TLC by UV and/or $KMnO_4$ staining. Column chromatography was carried out using forced flow of indicated solvent on Sorbent Technology Standard Grade Silica Gel, 40-63 μm particle size, 60 Å pore size (Sorbent Technologies). PBS buffer, DMEM/High glucose media, and penicillin streptomycin (Pen/Strep) were purchased from Thermo Scientific (Waltham, MA). Primers were ordered from Eurofins MWG Operon (Hunstville, AL), and sequencing was performed by Genewiz (Cambridge, MA).

Synthesis of LAS17



Synthesis of 1-methyl-4-(pent-4-yn-1-ylsulfonyl)benzene (2)

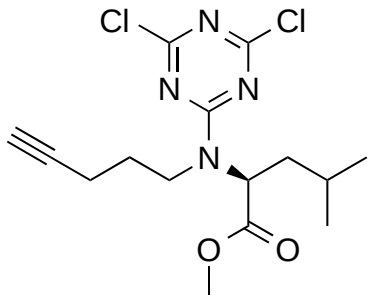
To an oven dried flask equipped with a stir bar, 4-pentyn-1-ol (11.8 mmol, 1.0 eq.), triethylamine (TEA) (23 mmol, 1.95 eq.), and dichloromethane (DCM) (33 mL) was added under N_2. The resulting solution was cooled to 0 °C using an ice bath. Next, the 4-toluenesulfonyl chloride (12.5 mmol, 1.06 eq.) was added in one portion. The reaction vessel was purged with N_2 and allowed to slowly warm to 22 °C . The reaction was quenched after 12 hrs with water (20 mL) and extracted with DCM (3 X 20 mL). The combined organic layers were dried over sodium sulfate (Na_2SO_4) and then concentrated *in vacuo*. The crude oil was purified by silica column chromatography (9:1, Hexanes (Hex):Ethylacetate (EtOAc)). The product was isolated as a clear oil (83 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.3 Hz, 2H), 7.35 (dd, *J* = 8.7, 0.7 Hz, 2H), 4.15 (t, *J* = 6.1 Hz, 2H), 2.45 (d, *J* = 0.7 Hz, 3H), 2.26 (td, *J* = 6.9, 2.7 Hz, 2H), 1.88 (t, *J* = 2.6 Hz, 1H), 1.88-1.83 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.9, 133.1, 130.0, 128.1, 82.24, 77.16, 69.56, 68.85, 27.87, 21.78, 14.84. HRMS m/z calculated for $C_{12}H_{14}O_2S$ $[M+H]^+$: 239.0664. Found: 239.0739.



Synthesis of methyl pent-4-yn-1-yl-L-leucinate (3)

To an oven dried flask equipped with a stir bar and reflux condenser, methyl *L*-leucinate hydrochloride (0.95 mmol 1.0 eq.), sodium iodide (0.47 mmol, 0.5 eq.), and potassium carbonate (2.37 mmol, 2.5 eq.) in acetonitrile (ACN) (1.6 mL) was added under N_2. The resulting mixture was allowed to heat to 90 °C and stirred for 1 hr before the drop-wise addition of tosylated alcohol (1) (1.04 mmol, 1.1 eq.). The reaction was allowed to mix for 12 hrs before being cooled and diluted with DCM. The suspension was filtered to remove solid precipitate. The remaining supernatant was concentrated *in vacuo*. The crude oil was purified by silica column chromatography (9:1–1:1 Hex:EtOAc) after dry loading. The product was isolated as a clear oil (36% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.72 (d, *J* = 1.4, 3H), 3.33 – 3.24 (m, 1H), 2.77 – 2.65 (m, 1H), 2.53 (dt, *J* = 12.71,

7.10, 1H), 2.26 (td, *J* = 7.5, 7.1, 2.4, 2H), 1.93 (dt, *J* = 4.2, 1.8, 1H), 1.79 – 1.61 (m, 3H), 1.46 (tt, *J* = 7.4, 1.7, 2H), 0.91 (ddd, *J* = 11.9, 6.6, 1.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.7, 84.20, 77.16, 68.57, 60.10, 51.72, 46.98, 42.97, 29.06, 25.07, 22.84, 22.46, 16.30.



Synthesis of methyl N-(4,6-dichloro-1,3,5-triazin-2-yl)-N-(pent-4-yn-1-yl)-L-leucinate (LAS17) (4)

To an oven dried flask equipped with a stir bar was added, cyanuric chloride (0.211 mmol, 1 eq.) and tetrahydrofuran (THF) (6 mL), under N_2. Diisopropylethylamine (DIEA) (0.222 mmol, 1.05 eq.) and (2) (0.211 mmol, 1 eq.) were dissolved in THF (8 mL) and the resulting solution was added drop-wise to the cyanuric chloride solution. The reaction vessel was purged with N_2.The resulting mixture was allowed to cool to 0 °C in an ice bath. The reaction was allowed to warm to 22 °C over 12 hrs while stirring. The crude product was purified by silica column chromatography (1:1 DCM:Hex – DCM). The product was isolated as a yellow-white solid (52% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.16 (dd, *J* = 10.0, 4.8, 1H), 3.79 (ddd, *J* = 14.1, 10.5, 5.2, 1H), 3.73 (s, 3H), 3.41 (ddd, *J* = 14.1, 10.5, 5.4, 1H), 2.32 (dtd, *J* = 16.4, 6.7, 2.7, 1H), 2.25 (dddd, *J* = 17.0, 7.7, 6.2, 2.7, 1H), 2.01 (t, *J* = 2.7, 1H), 2.00 – 1.90 (m, 2H), 1.88 – 1.74 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.4, 170.7, 170.1, 165.8, 83.28, 69.74, 58.55, 52.96, 46.45, 38.28, 30.07, 26.51, 25.21, 23.42, 22.09, 16.50. HRMS m/z calculated for $C_{15}H_{20}Cl_2N_4O_2$ $[M+H]^+$: 359.0963. Found 359.1047.

LAS1-20 were synthesized following a similar general protocol as detailed here for LAS17.

Evaluating Protein Labeling in HeLa Cell Lysates

HeLa cells were grown in complete DMEM media with FBS, penicillin, and streptomycin on 20 cm cell culture plates. Cells were harvested and sonicated to lyse to form whole cell lysates. These lysates were separated by centrifugation at 45,000 rpm for 45 min at 4 °C to yield soluble and membrane proteins. The supernatant was collected and the pellet was discarded. Protein concentrations for soluble lysates were determined using a standard Bradford Assay (Bio-Rad DC Protein Assay). HeLa soluble protein lysates (50 µL, 2 mg/mL) were pretreated with probe (1 µM, 50X stock in DMSO) at RT for 1 hr. Samples then underwent click chemistry with TAMRA-azide (Lumiprobe, 25 µM, 50X stock in DMSO), TCEP (1 mM, 50X fresh stock in water), TBTA ligand (100 µM, 17X stock in DMSO:*t*-butanol = 1:4), and copper(II) sulfate (1 mM, 50X stock in water) followed by incubation at RT for 1 hr. SDS-PAGE loading buffer 2X (reducing, 50 µL) was added to the samples and 25 µL of this solution was separated by SDS-PAGE at 100 volts for 130 minutes on a 10% polyacrylamide gel. Gels were visualized on a Hitachi FMBIO II multiview flatbed laser-induced fluorescent scanner (Figure S2A). After analysis, gels underwent a typical procedure for Coomassie staining and destaining. Stained gels were visualized on a Stratagene Eagle Eye apparatus by COHU High performance CCD camera (Figure S2B).

Protein Target Identification by LC/LC-MS/MS

HeLa soluble protein lysates in DPBS (pH 7.4) (500 µL, 2 mg/mL) were aliquoted and LAS17 (1 µM) or DMSO was added to the appropriate samples. Two aliquots were made for each inhibitor concentration or DMSO equaling 2 tubes for one final sample. All samples were then treated with biotin-azide (100 µM, 50X stock in DMSO), TCEP (1 mM, 50X fresh stock in water), TBTA ligand (100 µM, 17X stock in DMSO:*t*-butanol = 1:4), and copper(II) sulfate (1 mM, 50X stock in water) followed by incubation at 22 °C for 1 hr. Samples were combined pairwise and centrifuged (6500 g, 4 min, 4 °C) to pellet the precipitated proteins. The pellets were resuspended in cold methanol by sonication and the two samples were combined. Centrifugation was followed by a second cold methanol wash, after which the pellet was solubilized in DPBS containing 1.2% SDS via sonication and heating (90 °C, 5 min).

The SDS-solubilized proteome samples were diluted by 5 mL of DPBS for a final SDS concentration of 0.2%. The solution was incubated with 100 µL of streptavidin-agarose beads (Thermo Scientific, washed 3X with DPBS to remove storage buffer) overnight at 4 °C. Samples were rotated at 22 °C for 2 hr before

being washed by 5 mL 0.2 % SDS/DPBS, 3 X 5 mL DPBS, and 3 X 5 mL water. The beads were pelleted by centrifugation (1400 X g, 3 min) between washes.

The washed beads were suspended in 500 uL of 6 M urea/DPBS and 10 mM DTT (from 20X stock in water) and placed in a 65 °C heat block for 15 min. Iodoacetamide (20 mM from 50X stock in water) was then added and the samples were allowed to react at 37 °C for 30 min while shaking. Following reduction and alkylation, the beads were pelleted by centrifugation and resuspended in 200 µL of 2 M urea/DPBS, 1 mM $CaCl_2$ (100X stock in water), and sequencing-grade trypsin (2 µg). The digestion was allowed to proceed overnight at 37 °C while shaking. The beads were pelleted by centrifugation and washed with 2 X 50 µL water. The washes were combined with the supernatant from the trypsin digestion step. Formic acid (15 µL) was added to the samples, which were stored at -20 °C until mass spectrometry analysis.

LC/LC-MS/MS analysis was performed on an LTQ-Orbitrap Discovery mass spectrometer (ThermoFisher) coupled to an Agilent 1200 series HPLC. Peptide digests were pressure loaded onto a 250 µm fused silica desalting column packed with 4 cm of Aqua C18 reverse phase resin (Phenomenex). The peptides were eluted onto a biphasic column (100 µm fused silica with a 5 µ, tip, packed with 10 cm C18 and 4 cm Partisphere strong cation exchange resin (SCX, Whatman) using a gradient 5-100% Buffer B in Buffer A (Buffer A: 95% water, 5% acetonitrile, 0.1% formic acid; Buffer B: 20% water, 80% acetonitrile, 0.1% formic acid). The peptides were then eluted from the SCX onto the C18 resin and into the mass spectrometer using 4 salt steps previously described.[2] The flow rate through the column was set to ~0.25 µL/min and the spray voltage was set to 2.75 kV. One full MS scan (FTMS) (400-1800 MW) was followed by 8 data dependent scans (ITMS) of the n^{th} most intense ions.

The tandem MS data were searched using the SEQUEST algorithm using a concatenated target/decoy variant of the human UniProt database. A static modification of +57.02146 on cysteine was specified to account for alkylation by iodoacetamide. SEQUEST output files were filtered using DTASelect.

Overexpression and Purification of GSTP1

The cDNA for WT-GSTP1 was subcloned into a pET-47b N-term His Tag expression vector using a forward primer (5'-CCAGGATCCGCCGCCCTACA-3') containing a BamH1 restriction site and a reverse primer (5'-AGCCTCGAGTCACTGTTTCCCG) containing a Xho1 restriction site. All constructs were verified by sequencing (Genewiz, Cambridge, MA). Constructs were transformed into BL21 competent *E. coli* (New England Biolabs). From an overnight LB culture with antibiotics at 37 °C, 5 mL were added to 500 mL LB (pH 7.0) with antibiotics and were grown to OD600 of 0.8. Protein expression was induced with IPTG (400 μM, 250X stock in water) for 5 hrs at 37 °C. Soluble cell lysates in DPBS (pH 7.4) were purified using Ni-NTA chromatography with imidazole concentrations of 25 mM and 500 mM in DPBS (pH 7.4) for the wash and elution steps, respectively. Purification fractions were analyzed for purity using SDS-PAGE (Figure S3). Imidazole was removed from pure protein fractions using NAP-5 desalting columns that had been buffer exchanged with DPBS.

LAS17 Labeling of Purified GSTP1

Purified GSTP1 (50 μL, 0.2 mg/mL) was pretreated with LAS17 (50 nM, 50X stock in DMSO) at 22 °C for 1 hr. Samples then underwent click chemistry with TAMRA-azide (Lumiprobe, 25 μM, 50X stock in DMSO), TCEP (1 mM, 50X fresh stock in water), TBTA ligand (100 μM, 17X stock in DMSO:*t*-butanol = 1:4), and copper(II) sulfate (1 mM, 50X stock in water) followed by incubation at 22 °C for 1 hr. SDS-PAGE loading buffer 2X (reducing, 50 μL) was added to the samples and 25 μL of this solution was separated by SDS-PAGE at 100 volts for 145 minutes on a 12% polyacrylamide gel. Gels were visualized on a Bio-Rad ChemiDoc MP Imaging System using the rhodamine setting (Figure 2D). After analysis, gels underwent a typical procedure for Coomassie staining and destaining. Stained gels were visualized on a Bio-Rad ChemiDoc MP Imaging System.

Intact-protein MS Analysis

Purified GSTP1 (50 μL, 0.2 mg/mL) was pretreated with DMSO or probe (50 μM, 50X stock in DMSO) at 22 °C for 1 hr. Samples were centrifuged at 13,000 rpm for 5 min at RT to remove any insoluble particles. Samples were diluted by half in

water and analyzed by LC/MS. 10 µL samples were injected onto a Aeris WIDEPORE 3.6u XB-C18 using an Agilent 1260 Infinity and were analyzed by an Agilent 6230 TOF Mass Spectrometer. Peak masses were extracted using Agilent MassHunter Qualitative Analysis B.06.00 software. Deconvolution software MagTran was used to determine the mass of the protein from the peak lists generated by mMass software (Figure S6).

Evaluating the Site of Modification

100 µg of GSTP1 in DPBS was treated with DMSO or LAS17 (10 µM, 50X stock in DMSO) for 1 hr at 22 °C. Protein was precipitated by addition of 100% trichloroacetic acid in PBS and incubated at -80 °C overnight. Thawed samples were centrifuged at 15K for 10 min at 22 °C and the supernatant was discarded. The remaining protein pellet was washed with 500 µL of cold acetone, vortexed to resuspend the pellet and centrifuged at 15K for 10 min at 22 °C. The supernatant was again discarded and the pellet was allowed to air dry until trace amounts of acetone were gone. The pellet was resuspended in 30 µL of 8M urea in PBS, then 70 µL 100 mM ammonium bicarbonate in PBS and 1.5 µL of 1 M DTT in PBS were added. Samples were incubated at 65 °C for 15 minutes. Samples were alkylated for 30 minutes at room temperature with the addition of 2.5 µL of 500 mM iodoacetamide in PBS. Sample volume was increase to 224 µL by addition of 120 µL PBS, then 2 µg of sequencing-grade trypsin (Promega) and 2.5 µL of 100 mM $CaCl_2$ was added. Samples were agitated over night at 37 °C. Then trypsin was quenched with 10 µL of formic acid (~5% of final volume) and were centrifuged at 15K for 20 minutes at room temperature to pellet undigested protein. Supernatant was transferred to a new tube and stored at -20 °C. Samples were analyzed by LC-MS/MS using a LTQ Orbitrap XL mass spectrometer (ThermoFisher) coupled to a EASY-nLC 1000 nanoLC (ThermoFisher). 10 µL of peptide digests were loaded onto 100 µm fused silica column with a 5 µm tip packed with 10 cm of Aqua C18 reverse phase resin (Phenomenex) using the EASY-nLC 1000 autosampler. The digests were eluted using a gradient 0-100% Buffer B in Buffer A (Buffer A: 95% water, 5% acetonitrile, 0.1% formic acid; Buffer B; 20% water, 80% acetonitrile, 0.1% formic acid). The flow rate through the column was set to 400 nL/min and the spray voltage was set to 3.5 kV. One full MS scan (FTMS) (400-1800 MW) was followed by 7 data dependent scans (ITMS) of the nth most intense ion with dynamic exclusion. The tandem MS data were searched using the SEQUEST algorithm using a concatenated target/decoy variant of the human IPI databases. A static modification of +57.02146 on cysteine was specified to account for

iodoacetamide alkylation and differential modification of +322.12 (LAS-17) were specified on tyrosine to account for probe modification. Modification searches on cysteine and lysine confirmed cysteine and lysine residues were not modified by LAS17. SEQUEST output files were filtered using DTASelect 2.0 (Figure S7).

GSTP1 Mutants

To verify the site of modification identified by mass spectrometry, tyrosine 108 was mutated to phenylalanine using an adopted QuikChange lightening MSDS protocol.
(primer: 5'-CCCTCATCTTCACCAACTATGAGGCGGGCAAGGATGACTATGTGAAGGCACTGCC -3').

To confirm LAS17 was not modifying any of the lysine residues on GSTP1 we used the QuikChange Kit (Agilent Technologies, Santa Clara, California) to mutate each of the lysines to alanine (Figure S8).

Primers:
K30A Forward:
5'-GAGCTGGGCGGAGGAGGTGGTGACCGTGGAGACGTGGC-3'
K30A Reverse:
5'-CCTCCTCCGCCCAGCTCTGGCCCTGATCTGCCAGCAG-3'
K45A Forward:
5'-CTCACTCGCAGCCTCCTGCCTATACGGGCAGCTCCCC-3'
K45A Reverse:
5'-GAGGCTGCGAGTGAGCCCTCCTGCCACGTCTCCACG-3'
K55A Forward:
5'-GCTCCCCGCGTTCCAGGACGGAGACCTCACCCTG-3'
K55A Reverse:
5'-CCTGGAACGCGGGGAGCTGCCCGTATAGGCAGGAGG-3'
K82A Forward:
5'-CTATGGGGCGGACCAGCAGGAGGCAGCCCTGGTG-3'
K82A Reverse:
5'-GGCCGCACCCTTGGGCTCTATGGGGCGGACCAG-3'
K103A Forward:
5'-GGCGTGGAGGACCTCCGCTGCGCATACGTCTCCC-3'
K103A Reverse:
5'-GCCCGCCTCATAGTTGGTGTAGATGAGGGAGCGTATGCGCAGCG-3'
K116A Forward:
5'-GAGGCGGGCGCGCGGATGACTATGTGAAGGCACTGCCCGG-3'
K116A Reverse:
5'-GTCATCCGCGCCCGCCTCATAGTTGGTGTAGATGAGGGAGACGTATTTGCAGCGG-3'
K121A Forward:
5'-GACTATGTGGAGGCACTGCCCGGGCAACTGAAGCC-3'
K121A Reverse:

5’-CAGTGCCTCCACATAGTCATCCTTGCCCGCCTCATAGTTGGTGTAGATGAGGG-3’

K128A Forward:

5’-GCCCGGGCAACTGGCGCCTTTTGAGACCCTGC-3’

K128A Reverse:

5’-GCAGGGTCTCAAAAGGCGCCAGTTGCCCGGGC-3’

K141A Forward:

5’-CCAGGGAGGCGCGACCTTCATTGTGGGAGACCAGATCTCCTTCG-3’

K141A Reverse:

5’-GAAGGTCGCGCCTCCCTGGTTCTGGGACAGCAGGG-3’

K189A Forward:

5’-CGGCCCGCGCTCAAGGCCTTCCTGGCCTCCC-3’

K189A Reverse:

5’-CCTTGAGCGCGGGCCGGGCGCTGAGGCG-3’

K191A Forward:

5’-CCAAGCTCAAGGCCTTCCTGGCCTCCCCTGAGTACG-3’

K191A Reverse:

5’-GAAGGCCGCGAGCTTGGGCCGGGCACTGAGG-3’

K209A Forward:

5’-CCTCCCCATCAATGGCAACGGGGCACAGTGACTCGAGGC-3’

K209A Reverse:

5’-CGAGTCACTGTGCCCCGTTGCCATTGATGGGGAGGTTCACGTACTC-3’

Verification of the Site of Labeling

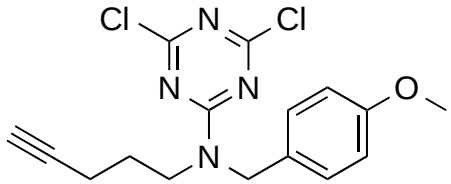
Purified GSTP1 tyrosine mutant (50 µL, 0.2 mg/mL) or lysine mutants (50 µL, 0.5 mg/mL) were pretreated with LAS17 (50 µM, 50X stock in DMSO) at 22 °C for 1 hr. Samples then underwent click chemistry with TAMRA-azide (Lumiprobe, 25 µM, 50X stock in DMSO), TCEP (1 mM, 50X fresh stock in water), TBTA ligand (100 µM, 17X stock in DMSO:*t*-butanol = 1:4), and copper(II) sulfate (1 mM, 50X stock in water) followed by incubation at RT for 1 hr. SDS-PAGE loading buffer 2X (reducing, 50 µL) was added to the samples and 25 µL of this solution was separated by SDS-PAGE at 100 volts for 145 minutes on a 12% polyacrylamide gel. Gels were visualized on a Bio-Rad ChemiDoc MP Imaging System using the rhodamine setting (Figure 2D). After analysis, gels underwent a typical procedure for Coomassie staining and destaining. Stained gels were visualized on a Bio-Rad ChemiDoc MP Imaging System.

GSTP1 Activity Assay

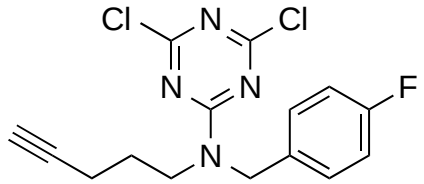
Purified recombinant GSTP1 was diluted to 0.02 mg/mL in Assay Buffer (100 mM NaH_2PO_4, pH 7.0). Samples were then incubated with LAS17 or DMSO for

varying time points. A prepared stock of 250 mM L-Glutathione, reduced (GSH) substrate in water was diluted to 4 mM in Assay Buffer. Equal volumes of treated GSTP1 (25 µL) and GSH (25 µL, 4mM) were combined to afford final GSTP1 concentrations of 0.01 mg/mL and GSH concentrations of 2 mM . A substrate stock solution of 75 mM 1-bromo-2,4-dinitrobenzene (BDNB) in ethanol was diluted to 2 mM in Assay buffer. 50 uL of GSTP1/GSH mixture was aliquoted into wells of a 96 well plate (Costar, Catalog # 3595). The reaction was started by adding 50 µL of the 2 mM BDNB in Assay Buffer to each well. Reaction was monitored by kinetic mode for 5 minutes at an absorbance of 340 nm.

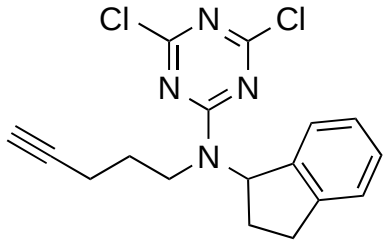
Characterization of LAS1-20



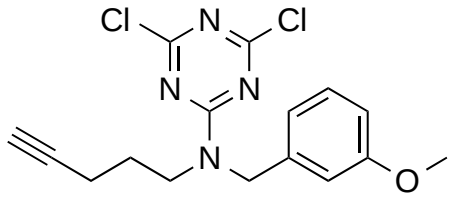
Compound LAS1: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.21 (d, *J* = 8.7, 2H), 6.87 (d, *J* = 8.7, 2H), 4.80 (s, 2H), 3.81 (s, 3H), 3.64 (t, *J* = 7.4, 2H), 2.23 (td, *J* = 6.9, 2.7, 2H), 2.01 (t, *J* = 2.6, 1H), 1.82 (q, *J* = 7.2, 2H). ^{13}C NMR (120 MHz, $CDCl_3$): δ 170.7, 170.5, 165.4, 159.9, 129.9, 128.2, 114.6, 83.36, 69.77, 55.71, 50.68, 46.37, 25.96, 16.39. HRMS m/z calculated for $C_{16}H_{16}Cl_2N_4O$ $[M+H]^+$: 351.0701. Found: 351.0767.



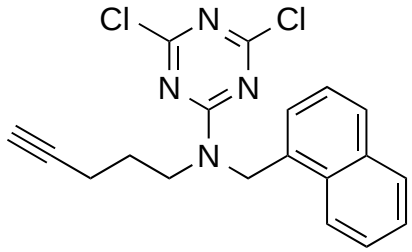
Compound LAS2: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (m, 2H), 7.06 (t, *J* = 8.6, 2H), 4.86 (s, 2H), 3.68 (t, *J* = 7.4, 2H), 2.70 (td, *J* = 6.8, 2.6, 2H), 2.03 (t, *J* = 2.6, 1H), 1.86 (q, *J* = 7.2, 2H). ^{13}C NMR (120 MHz, $CDCl_3$): δ 165.3, 163.7, 161.8, 131.8, 130.0, 115.9, 83.05, 69.67, 50.39, 46.36, 25.75, 16.16. HRMS m/z calculated for $C_{15}H_{13}Cl_2FN_4$ $[M+H]^+$: 339.0501. Found 339.0584.



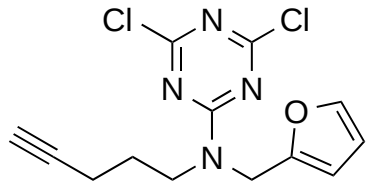
Compound LAS3: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28-7.27 (m, 2H), 7.20 (td, *J* = 6.7, 2.6, 1H), 7.09 (d, *J* = 7.5, 1H), 6.36 (t, *J* = 7.6, 1H), 3.42 (ddd, *J* = 13.8, 10.5, 5.46, 1H), 3.31-3.27 (m, 1H), 2.95 (dt, *J* = 16.1, 8.0, 1H), 2.52 (td, *J* = 8.8, 4.3, 1H), 2.14-2.06 (m, 3H), 1.88 (t, *J* = 2.7, 1H), 1.79-1.73 (m, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.2, 169.9, 165.1, 143.8, 140.0, 128.5, 126.9, 125.2, 124.2, 82.78, 69.01, 61.72, 44.01, 30.51, 29.83, 26.89, 16.20. HRMS m/z calculated for $C_{17}H_{16}Cl_2N_4$ $[M+H]^+$: 347.0752. Found 347.0841.



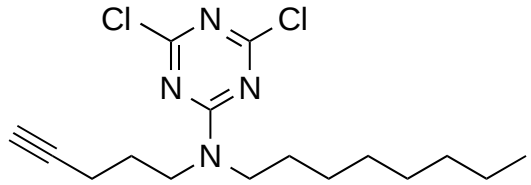
Compound LAS4: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.26 (t, *J* = 7.9, 1H), 6.86-6.81 (m, 3H), 4.84 (s, 2H), 3.80 (s, 3H), 3.67 (t, *J* = 7.4, 2H), 2.24 (td, *J* = 6.9, 2.7, 2H), 2.00 (t, *J* = 2.7, 1H), 1.84 (q, *J* = 7.2, 2H). ^{13}C NMR (120 MHz, $CDCl_3$): δ 170.3, 170.1, 165.1, 159.9, 137.2, 129.9, 120.2, 113.9, 113.1, 82.90, 69.39, 55.23, 50.76, 46.25, 25.55, 15.96. HRMS m/z calculated for $C_{16}H_{16}Cl_2N_4O$ $[M+H]^+$: 351.0701. Found 351.0780.



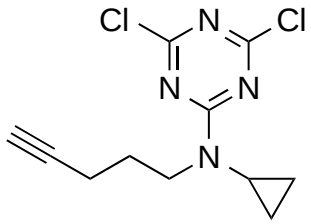
Compound LAS5: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91-7.89 (m, 1H), 7.84 (dd, *J* = 6.2, 3.2, 1H), 7.78 (d, *J* = 8.2, 1H), 7.50-7.45 (m, 2H), 7.37 (t, *J* = 7.7, 1H), 7.22 (d, *J* = 7.0, 1H), 5.30 (s, 2H), 3.58 (t, *J* = 7.5, 2H), 2.13 (td, *J* =6.9, 2.6, 2H), 1.86 (t, *J* = 2.6, 1H), 1.72 (q, *J* = 7.2, 2 H). ^{13}C NMR (120 MHz, $CDCl_3$): δ 172.7, 170.6, 134.1, 131.7, 130.9, 129.2, 129.1, 127.0, 126.3, 125.5, 123.2, 83.04, 69.55, 48.90, 46.15, 25.73, 16.18. HRMS m/z calculated for $C_{19}H_{16}Cl_2N_4$ $[M+H]^+$: 371.0752. Found 371.0830.



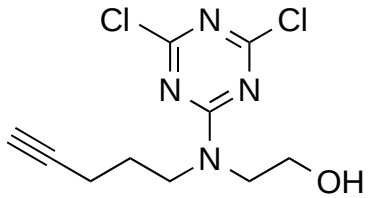
Compound LAS6: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37 (s, 1H), 6.35 (dd, *J* = 4.5, 2.0, 2H), 4.82 (s, 2H), 3.73 (t, *J* = 7.3, 2H), 2.32 (td, *J* = 6.9, 2.3, 2H), 2.00 (t, *J* = 2.1, 1H), 1.82 (q, *J* = 7.1, 2H). ^{13}C NMR (120 MHz, $CDCl_3$): δ 172.5, 149.1, 142.7, 110.6, 109.6, 105.0, 82.88, 69.30, 46.64, 44.04, 25.64, 15.95. HRMS m/z calculated for $C_{13}H_{12}Cl_2N_4O$ $[M+H]^+$: 311.0388. Found 311.0471.



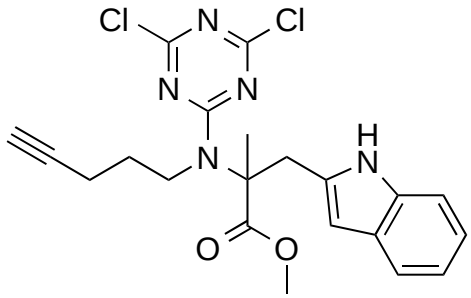
Compound LAS7: ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.66 (t, *J* = 7.4, 2H), 3.57 (t, *J* = 7.59, 2H), 2.26 (td, *J* = 6.90, 2.63, 2H), 2.00 (t, *J* = 2.65, 1H), 1.88-1.79 (m, 2H), 1.31-1.23 (m, 12H), 0.88 (t, *J* = 6.91, 3H). ^{13}C NMR (120 MHz, $CDCl_3$): δ 169.9, 164.6, 82.91, 69.34, 48.23, 46.93, 31.78, 29.55, 29.14, 26.66, 25.98, 23.84, 22.63, 16.00, 14.08. HRMS m/z calculated for $C_{16}H_{24}Cl_2N_4$ $[M+H]^+$: 343.1378. Found 343.1452.



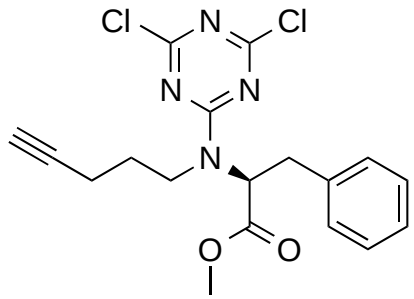
Compound LAS8: ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.72 (t, *J* = 7.39, 2H), 2.89-2.84 (m, 1H), 2.26 (td, *J* = 6.94, 2.66, 2H), 2.00 (t, *J* = 2.67, 2H), 1.87 (q, *J* = 7.24, 2H), 1.01 (m, 2H), 0.78-0.74 (m, 2H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 170.8, 167.4, 83.66, 69.97, 47.60, 31.40, 27.09, 16.84, 8.94. HRMS m/z calculated for $C_{11}H_{12}Cl_2N_4$ $[M+H]^+$: 271.0439. Found 271.0519.



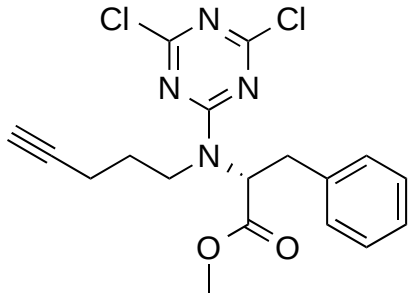
Compound LAS9: ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.74 (t, *J* = 5.24, 2H), 4.06 (t, *J* =5.26, 2H), 3.79 (t, *J* = 7.25, 2H), 2.83 (td, *J* = 6.83, 2.54, 2H), 2.01 (t, *J* = 2.65, 1H), 1.90 (q, *J* = 7.00, 2H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 172.9, 171.1, 82.88, 69.77, 67.05, 48.33, 46.82, 26.08, 16.05. HRMS m/z calculated for $C_{10}H_{12}Cl_2N_4O$ $[M+H]^+$: 275.0388. Found 275.0468.



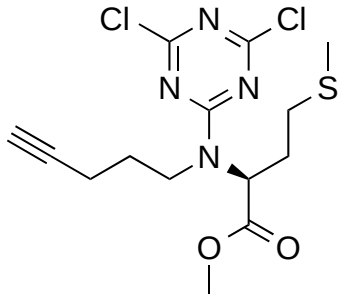
Compound LAS10: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (s, 1H), 7.47 (d, *J* = 7.89, 1H), 7.39 (d, 8.20, 1H), 7.21 (t, *J* = 7.62, 1H), 7.14 (t, *J* = 7.49, 1H), 6.20 (dd, *J* = 9.69, 5.21, 1H), 3.51 (d, *J* = 16.57, 1H), 3.48 (s, 3H), 3.19 (d, *J* = 16.40, 1H), 2.45-2.39 (m, 2H), 2.32-2.25 (m, 2H), 2.20 (d, *J* = 2.63, 1H), 2.00-1.93 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 173.6, 164.8, 136.0, 132.7, 126.2, 122.5, 120.3, 118.2, 111.6, 105.4, 82.72, 70.89, 63.51, 53.18, 52.96, 33.30, 33.00, 26.91, 16.01. HRMS m/z calculated for $C_{21}H_{21}Cl_2N_5O_2$ $[M+H]^+$: 446.1072. Found 444.0995.



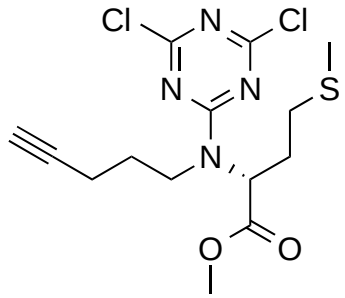
Compound LAS11: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32-7.26 (m, 2H), 7.24-7.21 (m, 1H), 7.15-7.11 (m, 2H), 4.83-4.79 (m, 1H), 3.77 (s, 3H), 3.71-3.65 (m, 1H), 3.52-3.48 (m, 1H), 3.34-3.28 (m ,1H), 3.02-2.95 (m, 1H), 2.26-2.19 (m, 1H), 2.16-2.09 (m, 1H), 1.99-1.96 (m, 1H), 1.75-1.62 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5, 170.1, 165.1, 136.9, 129.2, 129.0, 127.3, 83.21, 69.53, 63.23, 52.97, 48.28, 35.07, 25.93, 15.99. HRMS m/z calculated for $C_{18}H_{18}Cl_2N_4O_2$ $[M+H]^+$: 393.0807. Found 393.0895.



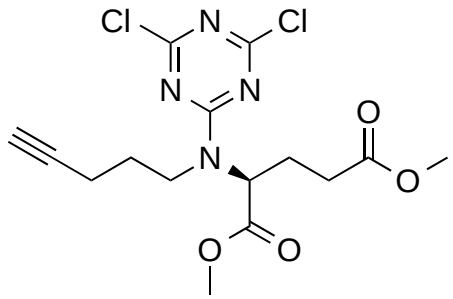
Compound LAS12: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (t, *J* = 7.57, 2H), 7.23 (t, *J* = 7.32, 1H), 7.13 (d, *J* = 7.48, 2H), 4.81 (dd, *J* = 10.23, 4.88, 1H), 3.76 (s, 3H), 3.69 (ddd, *J* = 14.32, 8.81, 5.70, 1H), 3.51 (dd, *J* = 14.38, 4.97, 1H), 3.32 (dd, *J* = 14.37, 10.36, 1H), 3.00 (ddd, *J* = 14.33, 8.75, 5.73, 1H), 2.26-2.20 (m, 1H), 2.17-2.12 (m, 1H), 1.98 (td, *J* = 2.64, 0.83, 1H), 1.75-1.63 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$); δ 170.7, 170.2, 169.8, 165.2, 137.1, 129.4, 129.1, 127.5, 83.36, 77.16, 69.66, 63.37, 53.12, 48.43, 35.23, 26.09, 16.15.HRMS m/z calculated for $C_{18}H_{18}Cl_2N_4O_2$ $[M+H]^+$: 393.0807. Found 393.0899.



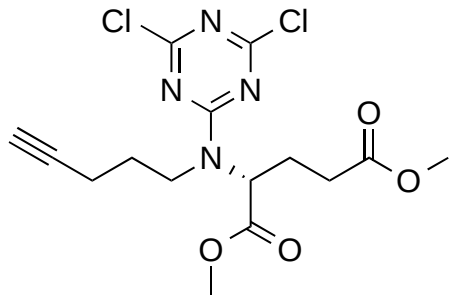
Compound LAS13: ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.89 (dd, *J* = 8.58,4.88, 1H), 3.97 (ddd, *J* = 14.31, 9.27, 5.39, 1H), 3.74 (s, 3H), 3.49 (ddd, *J* = 14.28, 9.23, 5.35, 1H), 2.64 (dq, *J* = 15.90, 5.83, 1H), 2.57-2.50 (m, 2H), 2.37-2.29 (m, 2H), 2.27-2.20 (m, 1H), 2.13 (s, 3H), 2.03-2.02 (m, 1H), 1.98-1.87 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.6, 170.4, 169.7, 165.2, 82.99, 77.16, 69.60, 59.76, 52.88, 48.02, 31.01, 28.55, 26.33, 16.16, 15.52. HRMS m/z calculated for $C_{14}H_{18}Cl_2N_4O_2S$ $[M+H]^+$: 377.0528. Found 377.0590.



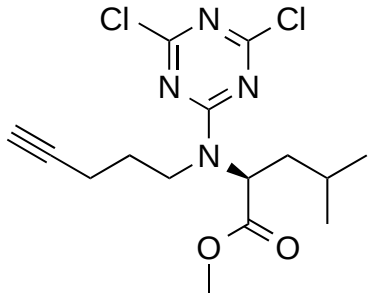
Compound LAS14: ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.88 (dd, *J* = 8.58, 4.84, 1H), 3.96 (ddd, *J* = 14.31, 9.24, 5.40, 1H), 3.74 (s, 3H), 3.48 (ddd, *J* = 14.27, 9.25, 5.32, 1H), 2.61 (dd, 11.21, 5.56, 1H), 2.55-2.50 (m, 2H), 2.31 (dtd, *J* = 17.82, 7.12, 2.80, 2H), 2.24-2.19 (m, 2H), 2.12 (s, 1H), 2.02 (t, *J* = 2.62, 1H), 1.99-1.86 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 170.4, 83.04, 69.65, 59.81, 53.61, 52.92, 48.06, 31.06, 28.60, 26.37, 16.20, 15.56. HRMS m/z calculated for $C_{14}H_{18}Cl_2N_4O_2S$ $[M+H]^+$: 377.0528. Found 377.0616.



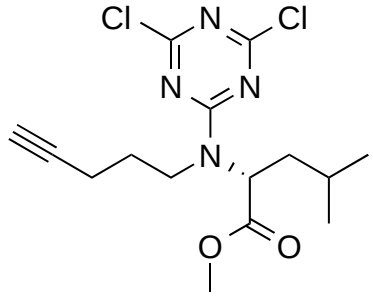
Compound LAS15: ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.86 (dd, *J* = 8.72, 6.02, 1H), 3.92 (ddd, *J* = 14.09, 9.72, 5.62, 1H), 3.76 (s, 3H), 3.72 (s, 3H), 3.44 (ddd, *J* = 14.06, 9.67, 5.60, 1H), 2.62-2.55 (m, 1H), 2.51-2.42 (m, 2H), 2.35-2.29 (m, 2H), 2.27-2.19 (m, 1H), 2.03 (t, *J* = 2.65, 1H), 1.97-1.87 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.0, 170.7, 170.2, 169.9, 165.4, 83.02, 69.66, 60.03, 52.93, 52.11, 47.62, 30.55, 26.31, 24.58, 16.23. HRMS m/z calculated for $C_{15}H_{18}Cl_2N_4O_4$ $[M+H]^+$: 389.0705. Found 389.0768.



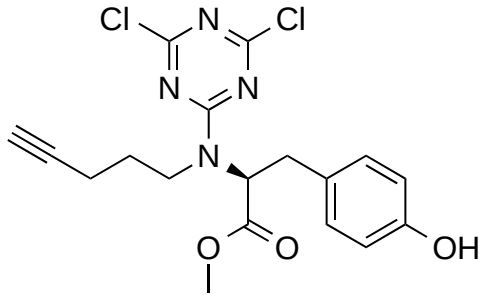
Compound LAS16: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.83 (dd, *J* = 8.8, 5.9, 1H), 3.89 (ddd, *J* = 14.1, 9.7, 5.6, 1H), 3.73 (s, 3H), 3.69 (s, 3H), 3.41 (ddd, *J* = 14.1, 9.7, 5.6, 1H), 2.62 – 2.50 (m, 1H), 2.50 – 2.37 (m, 2H), 2.37 – 2.25 (m, 2H), 2.25 – 2.15 (m, 1H), 2.01 (td, *J* = 2.7, 0.6, 1H), 1.98 – 1.80 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 173.2, 170.8, 170.3, 170.0, 165.5, 83.16, 69.80, 60.16, 53.07, 52.25, 47.76, 30.69, 26.45, 24.73, 16.37. HRMS m/z calculated for $C_{15}H_{18}Cl_2N_4O_4$ $[M+H]^+$: 389.0705. Found 389.0775.



Compound LAS17: ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.16 (dd, *J* = 10.0, 4.8, 1H), 3.79 (ddd, *J* = 14.1, 10.5, 5.2, 1H), 3.73 (s, 3H), 3.41 (ddd, *J* = 14.1, 10.5, 5.4, 1H), 2.32 (dtd, *J* = 16.4, 6.7, 2.7, 1H), 2.25 (dddd, *J* = 17.0, 7.7, 6.2, 2.7, 1H), 2.01 (t, *J* = 2.7, 1H), 2.00 – 1.90 (m, 2H), 1.88 – 1.74 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 171.4, 170.7, 170.1, 165.8, 83.28, 69.74, 58.55, 52.96, 46.45, 38.28, 30.07, 26.51, 25.21, 23.42, 22.09, 16.50. HRMS m/z calculated for $C_{15}H_{20}Cl_2N_4O_2$ $[M+H]^+$: 359.0963. Found 359.1047.

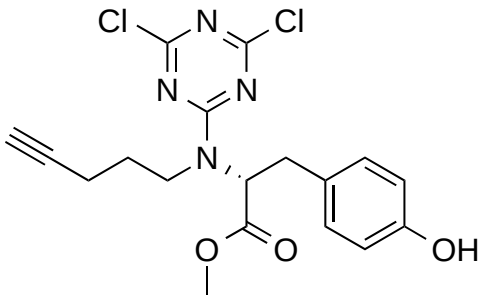


Compound LAS18: ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.79 (ddd, *J* = 14.4, 9.6, 5.3, 1H), 3.75 – 3.69 (m, 3H), 3.42 (ddd, *J* = 14.3, 10.5, 5.5, 1H), 2.37 – 2.20 (m, 2H), 2.01 (q, *J* = 3.5, 3.0, 1H), 1.98 – 1.89 (m, 2H), 1.89 – 1.73 (m, 2H), 0.97 (td, *J* = 7.2, 2.2, 6H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 171.0, 165.4, 82.89, 69.35, 58.16, 52.57, 46.07, 37.89,26.12, 24.82, 23.03, 21.70, 16.11. HRMS m/z calculated for $C_{15}H_{20}Cl_2N_4O_2$ $[M+H]^+$: 359.0963. Found 359.1034.



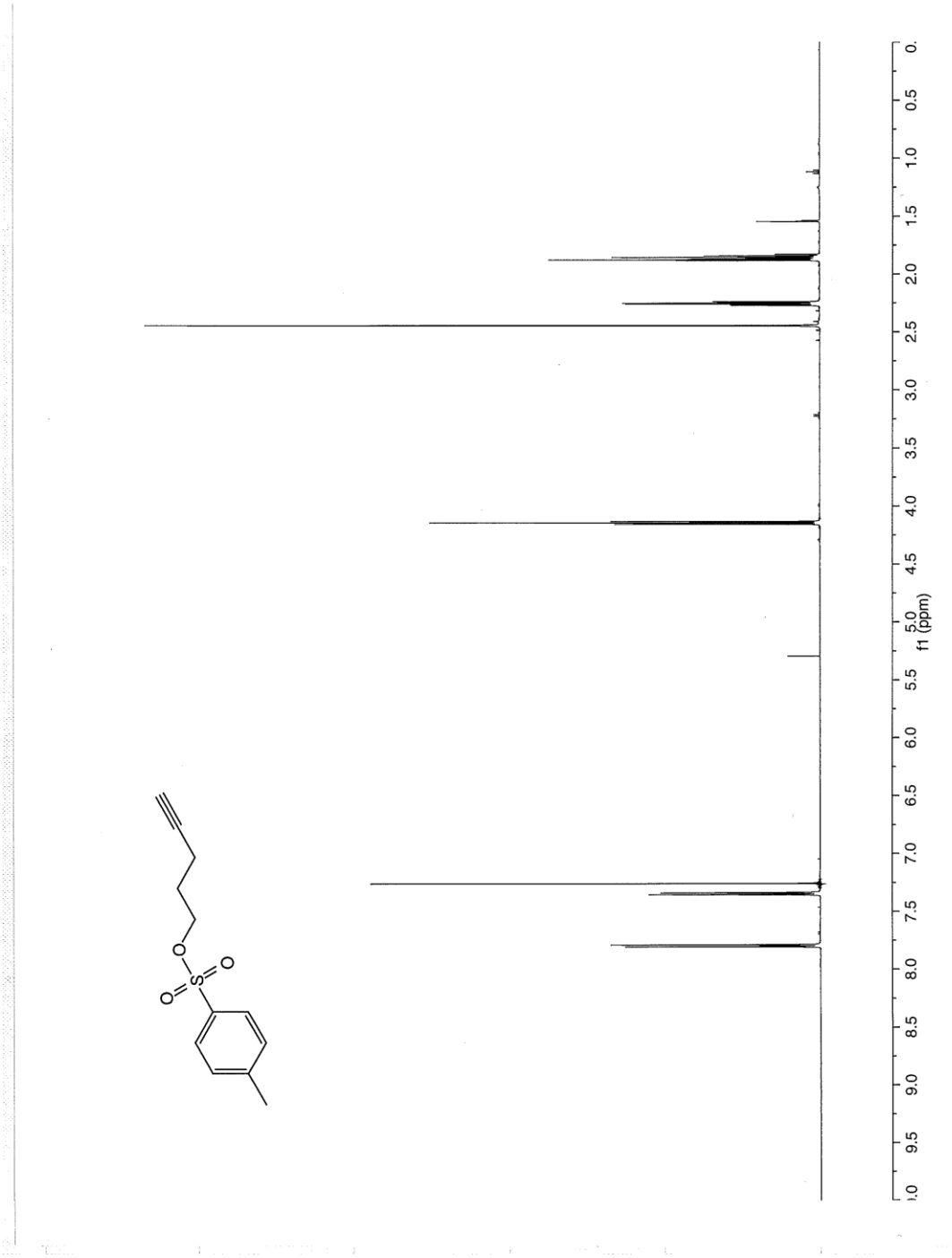
Compound LAS19: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.00 (d, *J* = 8.28, 2H), 6.75 (d, *J* = 8.10, 2H), 4.75 (dd, *J* = 10.15, 5.06, 1H), 3.76 (s, 3H), 3.70 (ddd, *J* = 14.33,

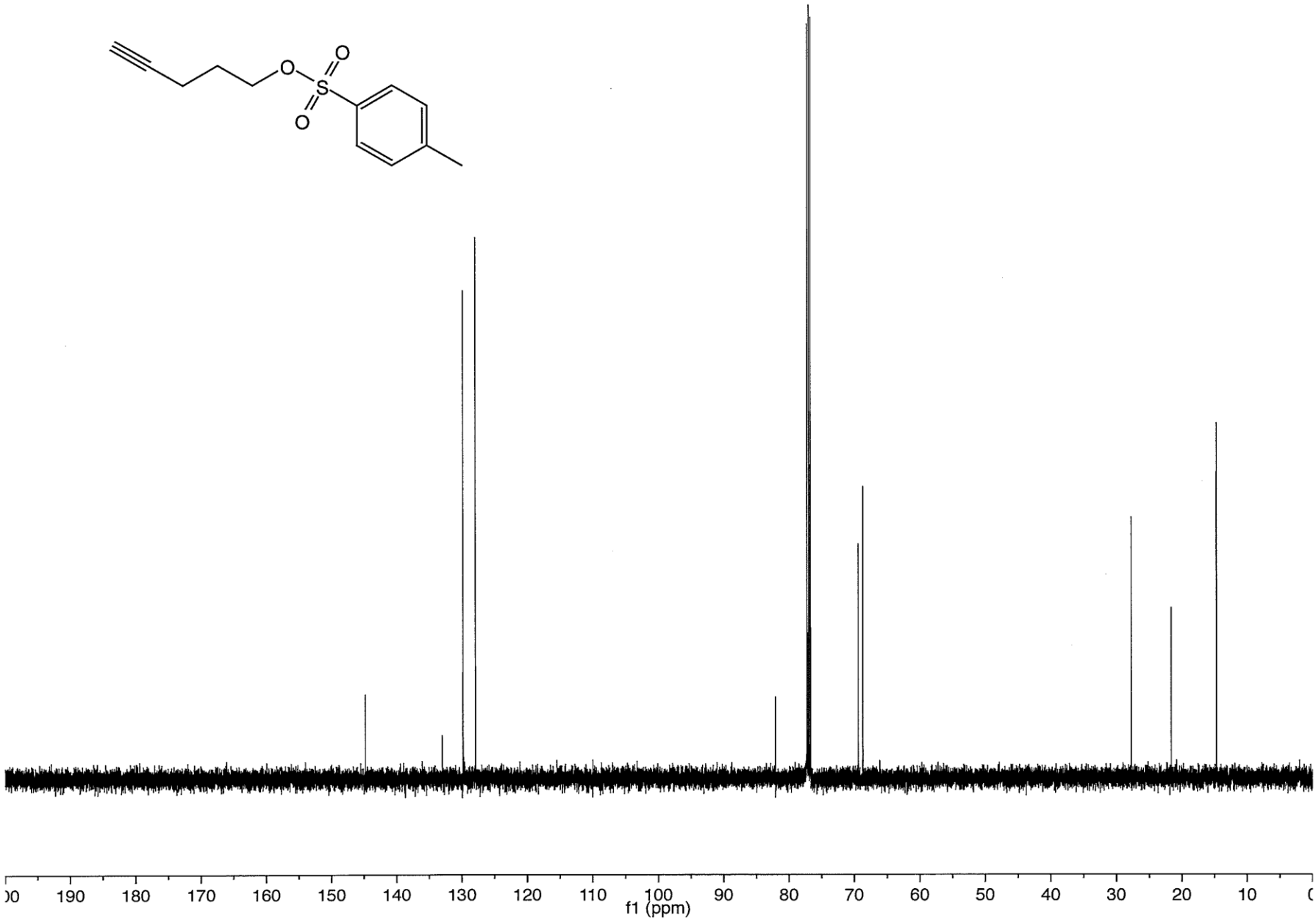
8.67, 5.84 1H, 3.43 (dd, *J* = 14.53, 5.08, 1H), 3.25 (dd, 14.53, 10.30, 1H), 3.03 (ddd, *J* = 14.29, 8.69, 5.85, 1H), 2.24 (dtd, *J* = 10.02, 6.95, 2.84, 1H), 2.19-2.12 (m, 1H), 1.99 (t, *J* = 2.65, 1H), 1.76-1.67 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5. 170.2, 169.6, 165.0, 154.8, 130.4, 128.9, 115.9, 83.24, 69.53, 63.34, 52.96, 48.30, 34.18, 25.96, 16.01. HRMS m/z calculated for $C_{18}H_{18}Cl_2N_4O_3$ $[M+H]^+$: 409.0756. Found 409.0839.



Compound LAS20: ^{1}H NMR (500 MHz, CDCl3) δ 7.20 (d, *J* = 8.6, 2H), 7.10 (d, *J* = 8.6, 1H), 4.78 (dd, *J* = 10.0, 5.6, 1H), 3.76 (d, *J* = 5.4, 3H), 3.73 – 3.63 (m, 1H), 3.54 (dd, *J* = 14.5, 5.3, 1H), 3.33 (dd, *J* = 14.5, 9.9, 1H), 3.09 (ddd, *J* = 14.5, 9.1, 5.9, 1H), 2.32 – 2.09 (m, 1H), 1.98 (td, *J* = 2.6, 1.2, 1H), 1.70 (dddd, *J* = 16.0, 13.2, 9.3, 6.5, 2H).^{13}C NMR (151 MHz, $CDCl_3$) δ 170.8, 170.0, 169.9, 165.2, 150.7, 135.7, 130.8, 130.6, 122.0, 116.0, 83.30, 77.16, 69.79, 63.33, 53.21, 48.51, 34.79, 26.13, 16.17. HRMS m/z calculated for $C_{18}H_{18}Cl_2N_4O_3$ $[M+H]^+$: 409.0756. Found 409.0819.

NMR Spectra

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S
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O
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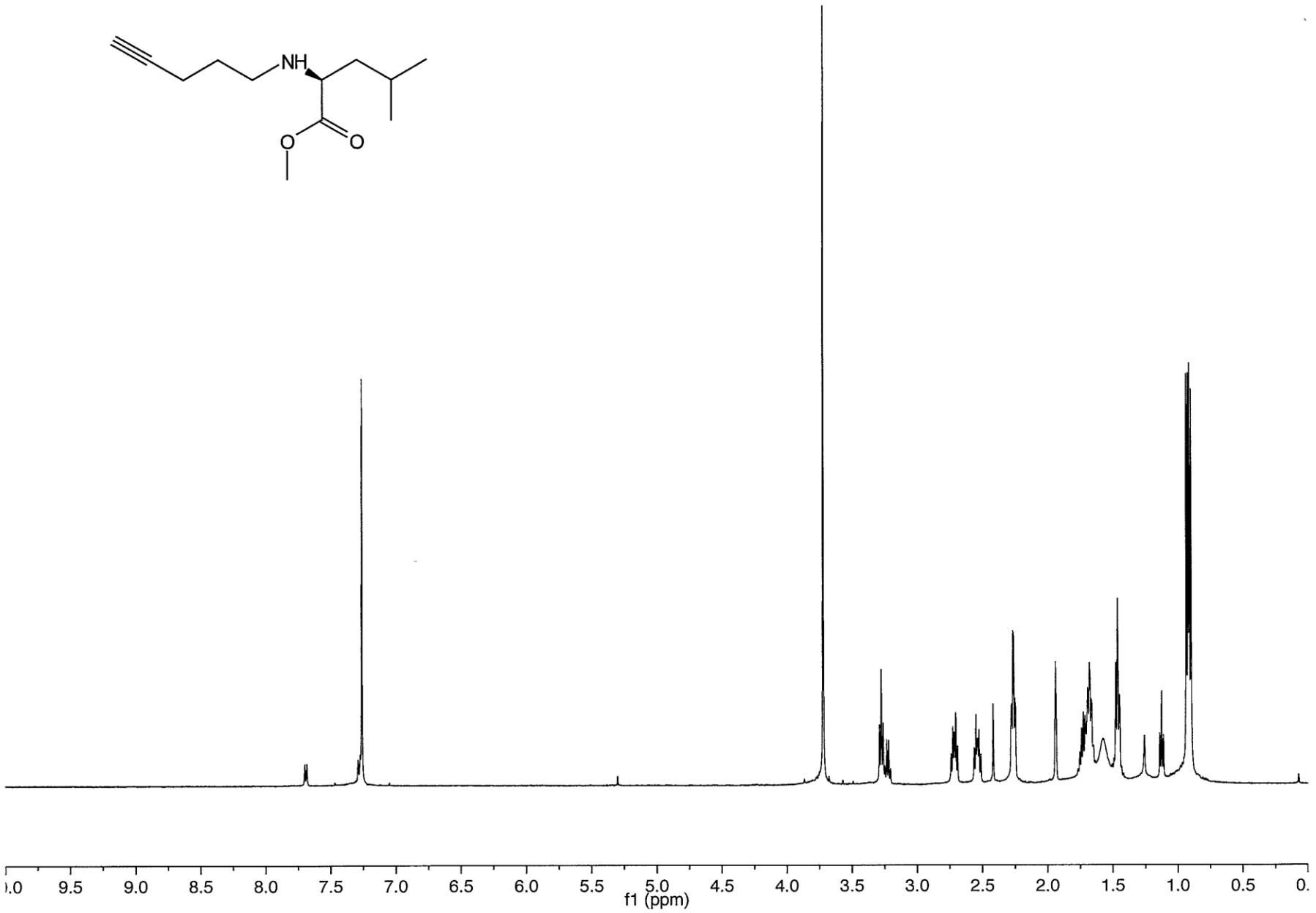
f1 (ppm)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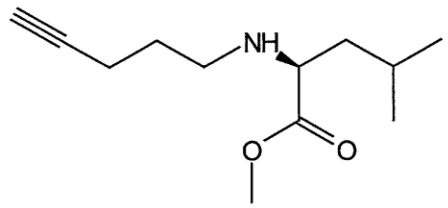
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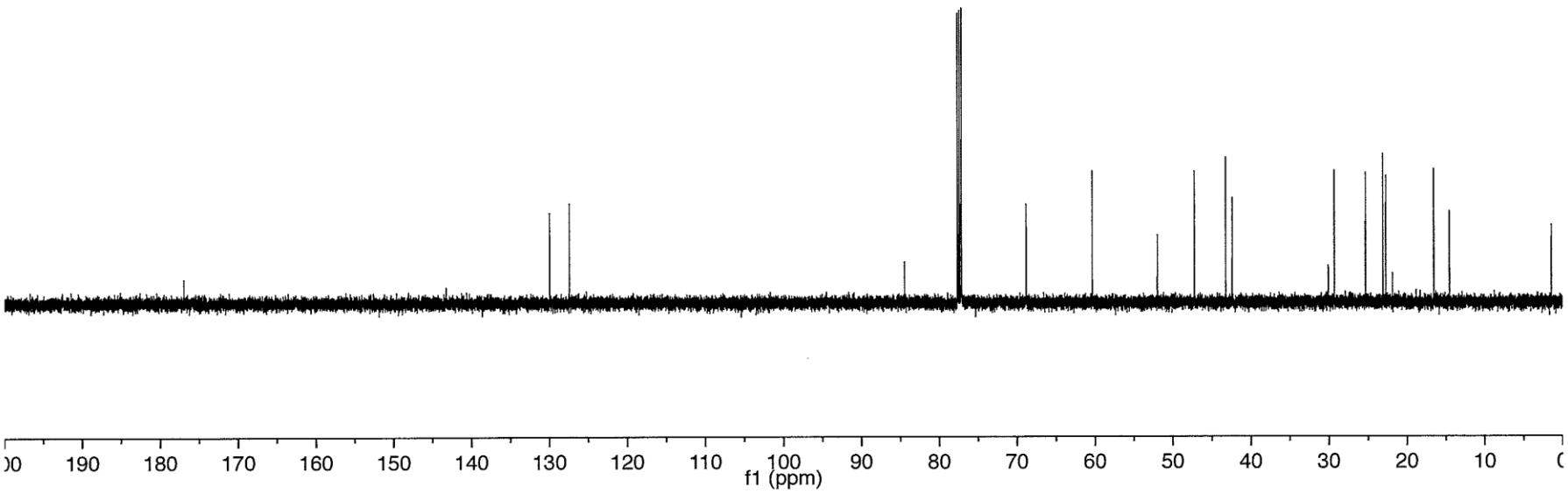
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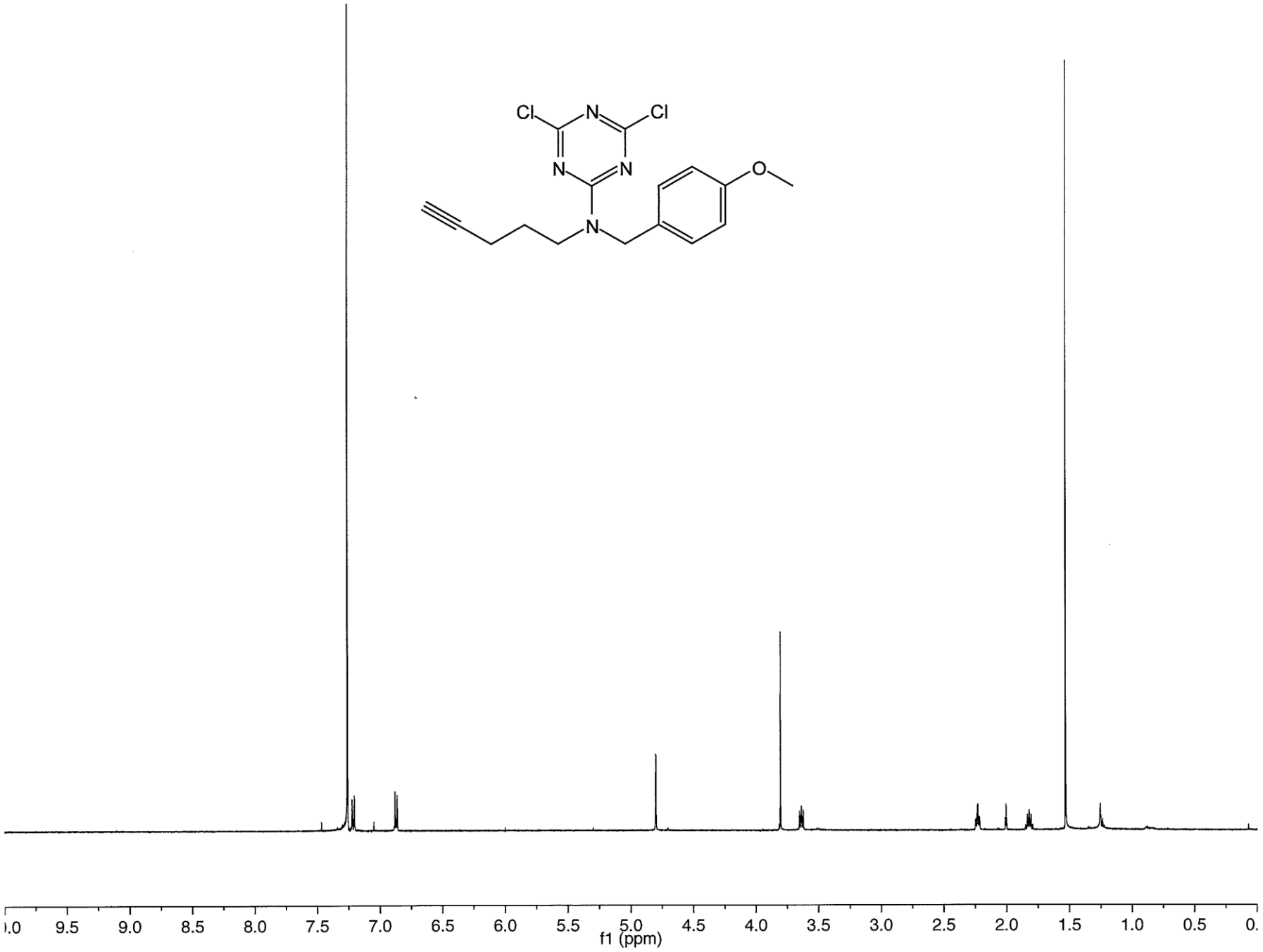
f1 (ppm)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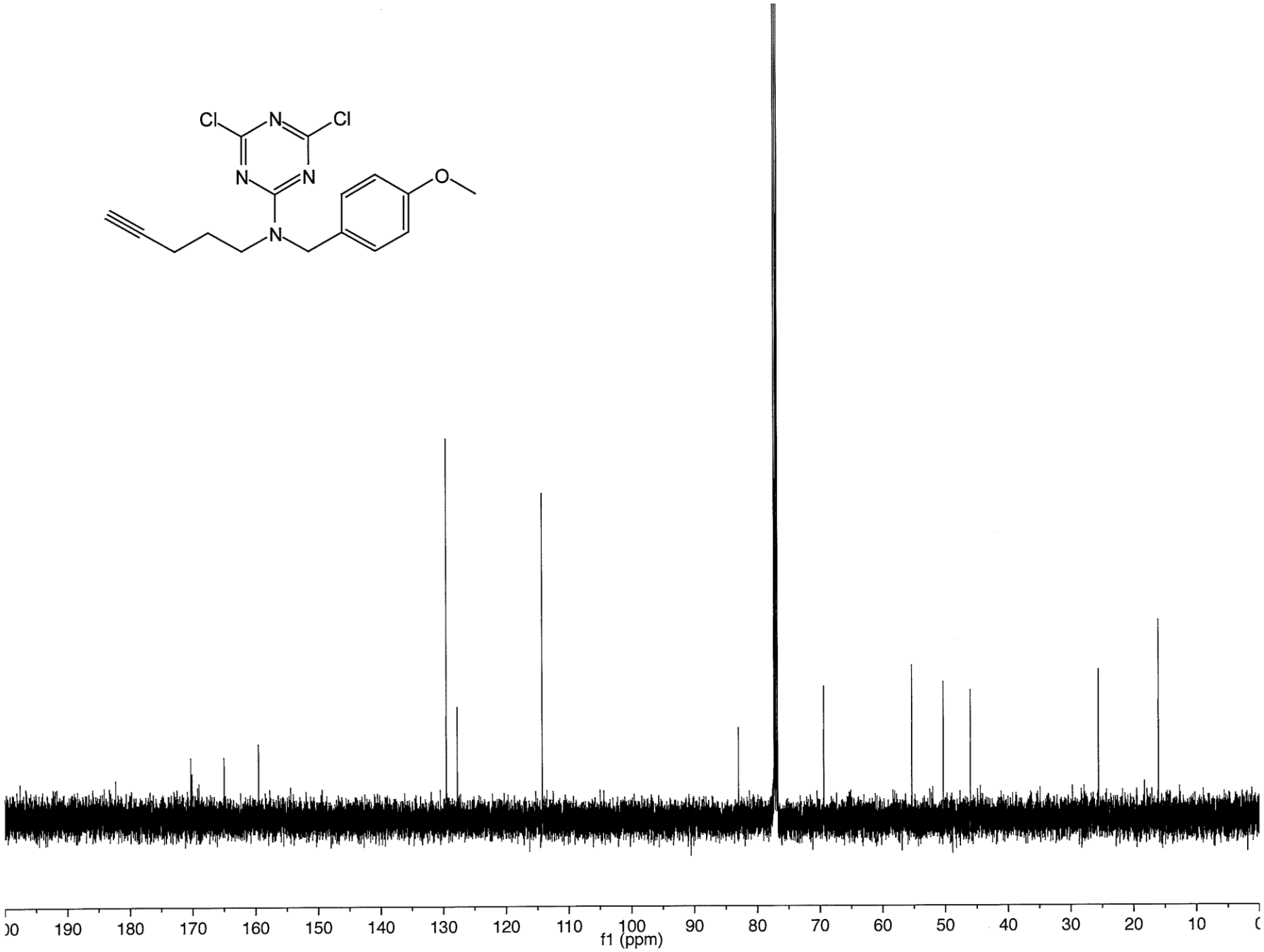
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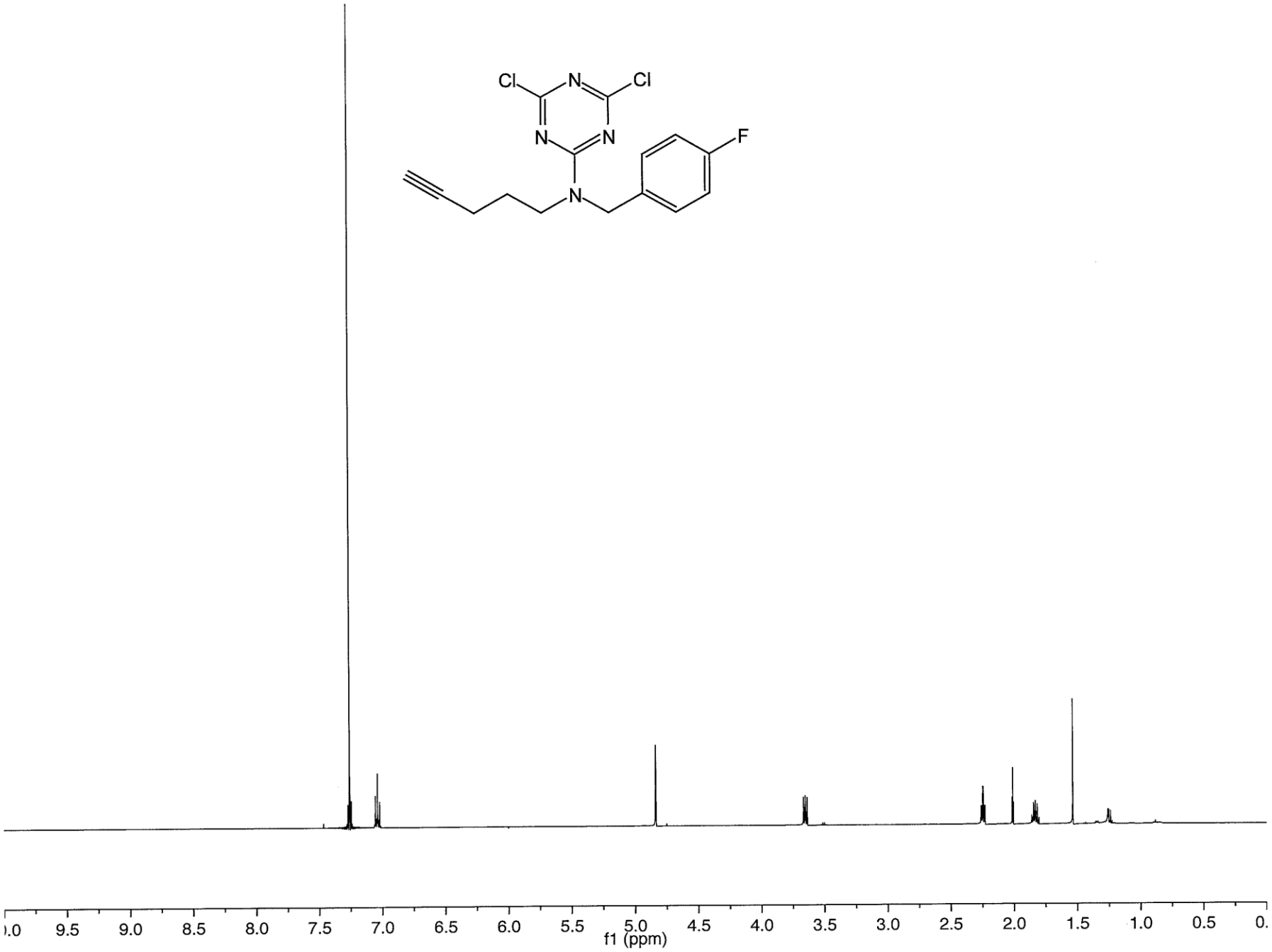
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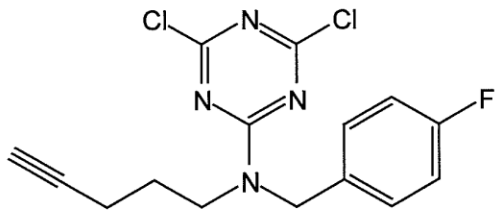
f1 (ppm)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F
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20
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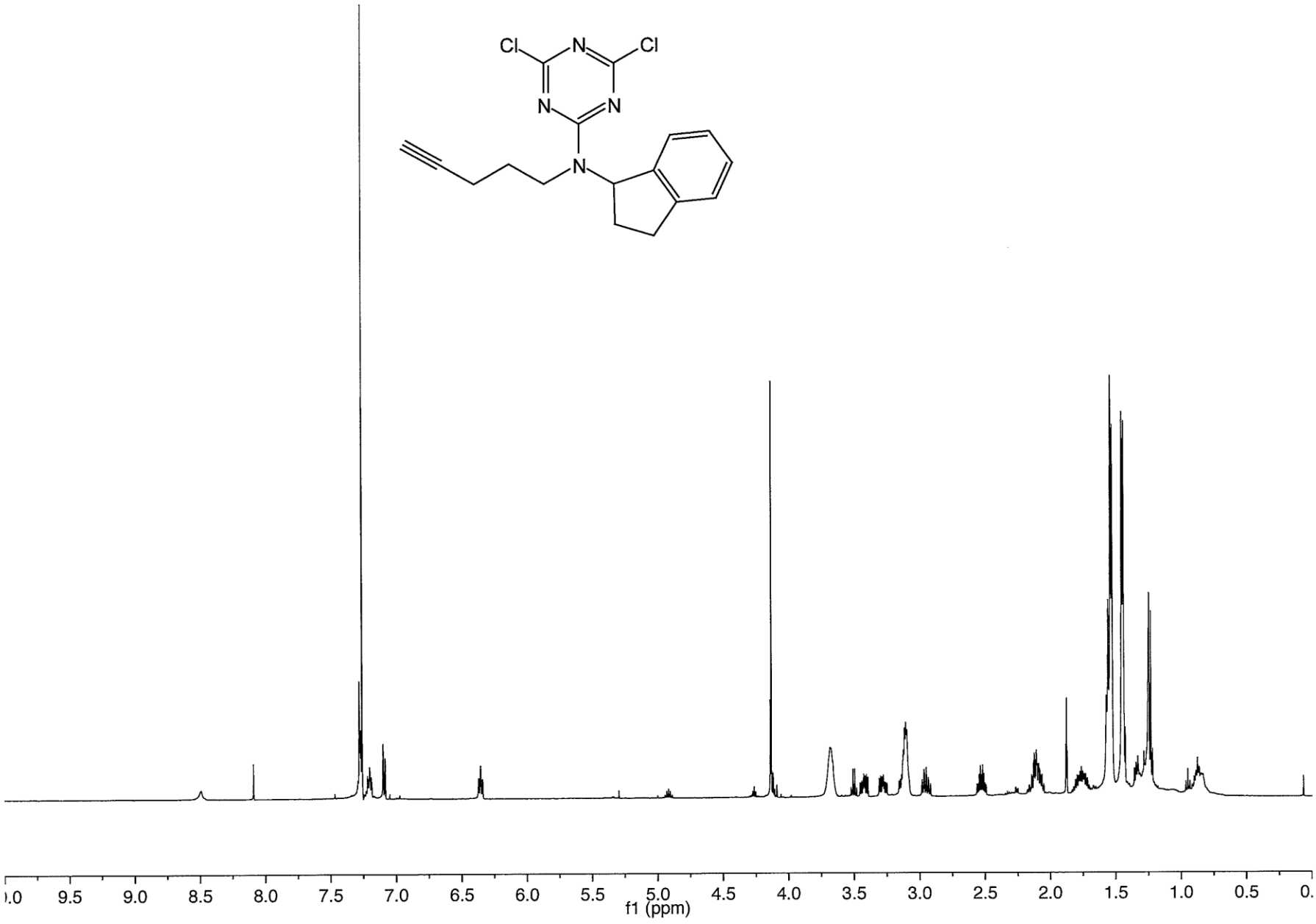
f1 (ppm)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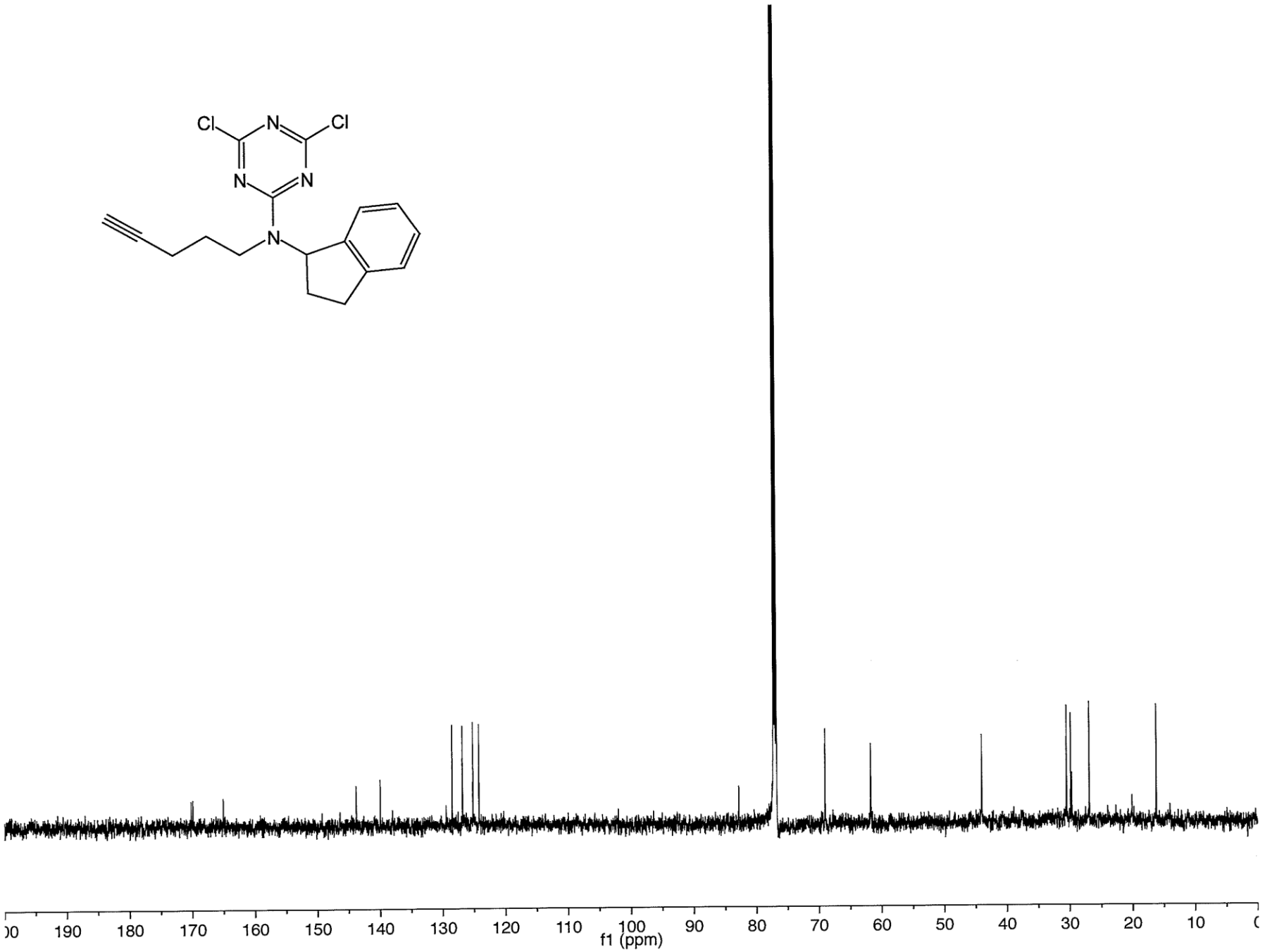
f1 (ppm)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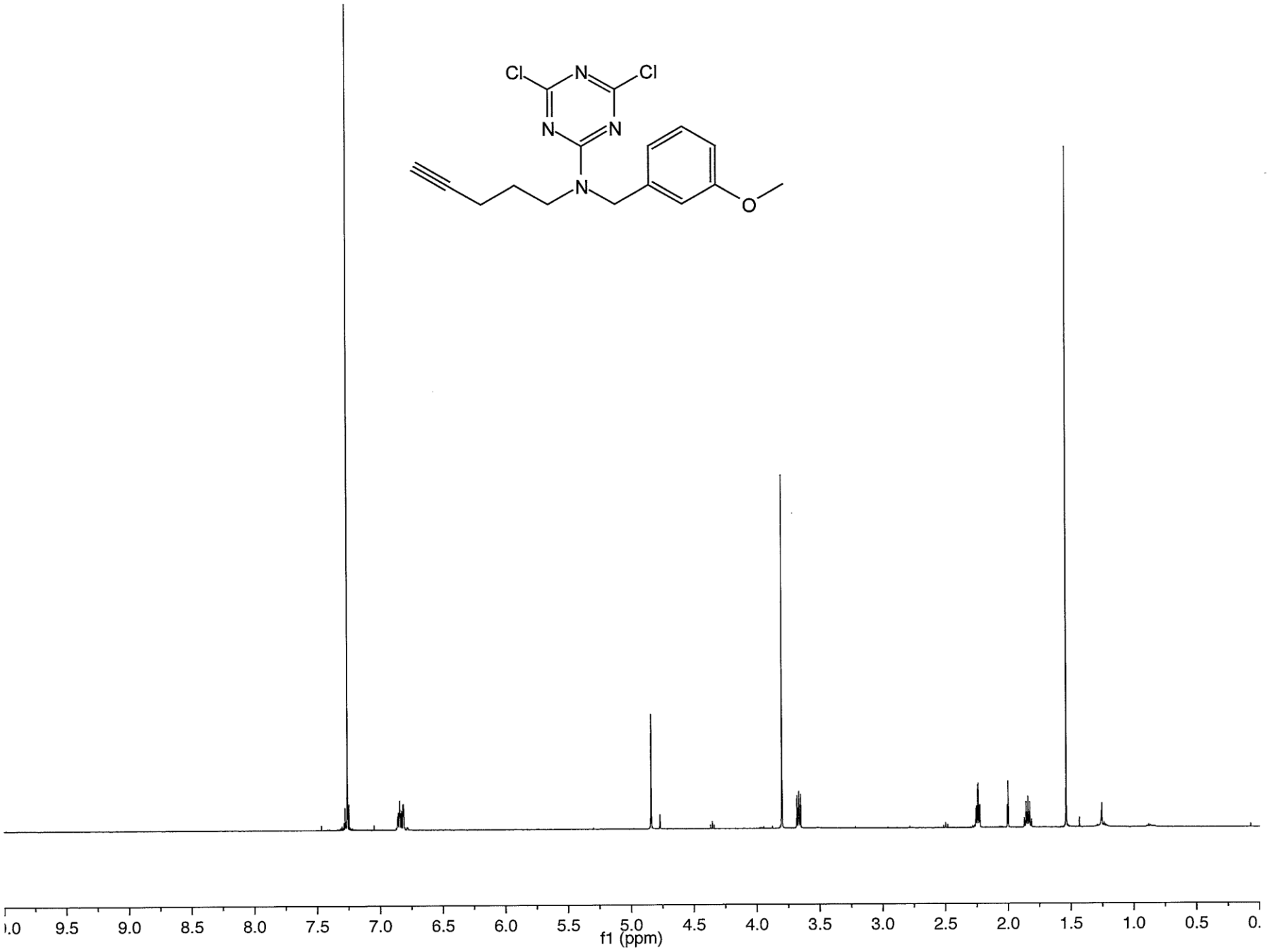
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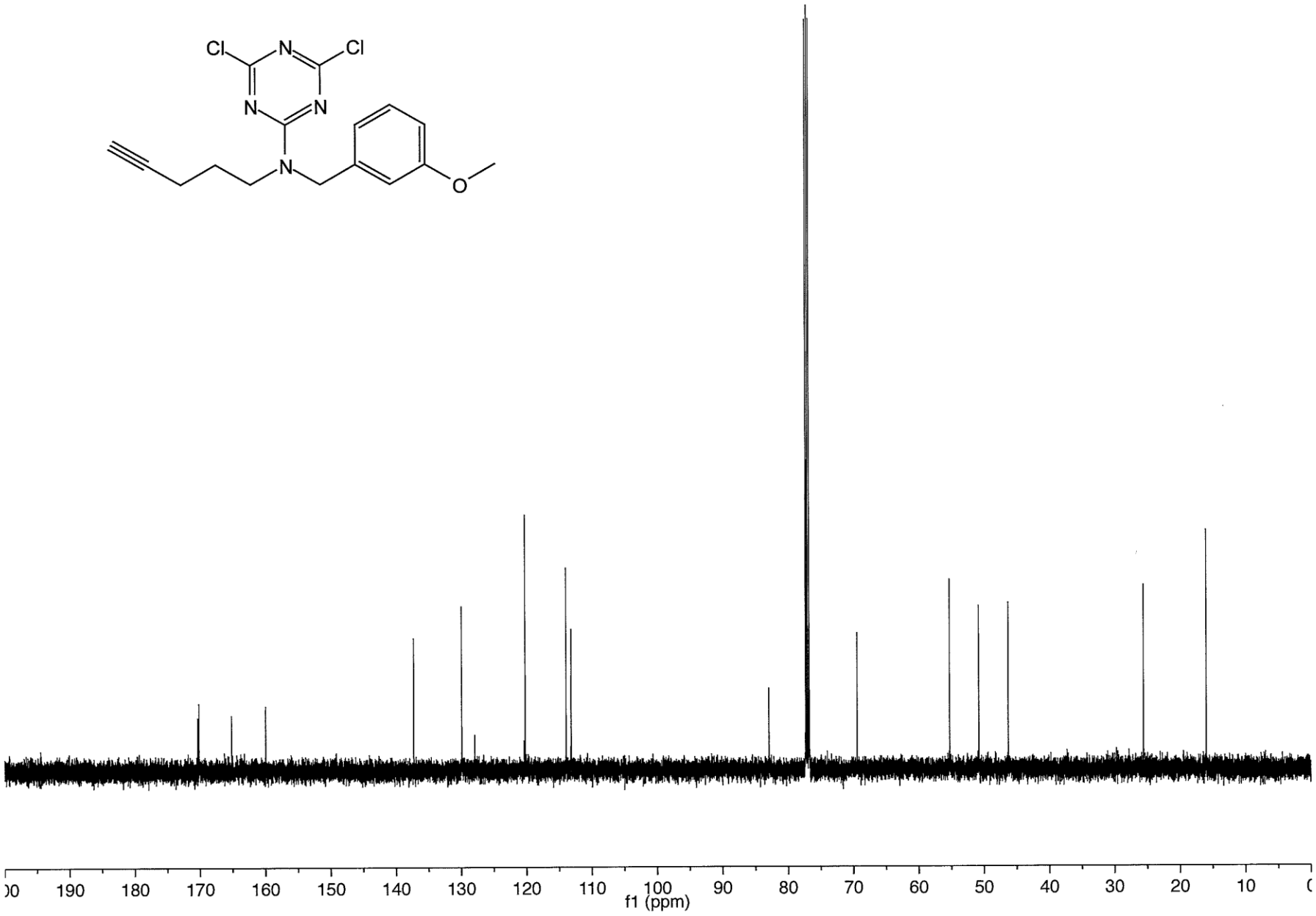
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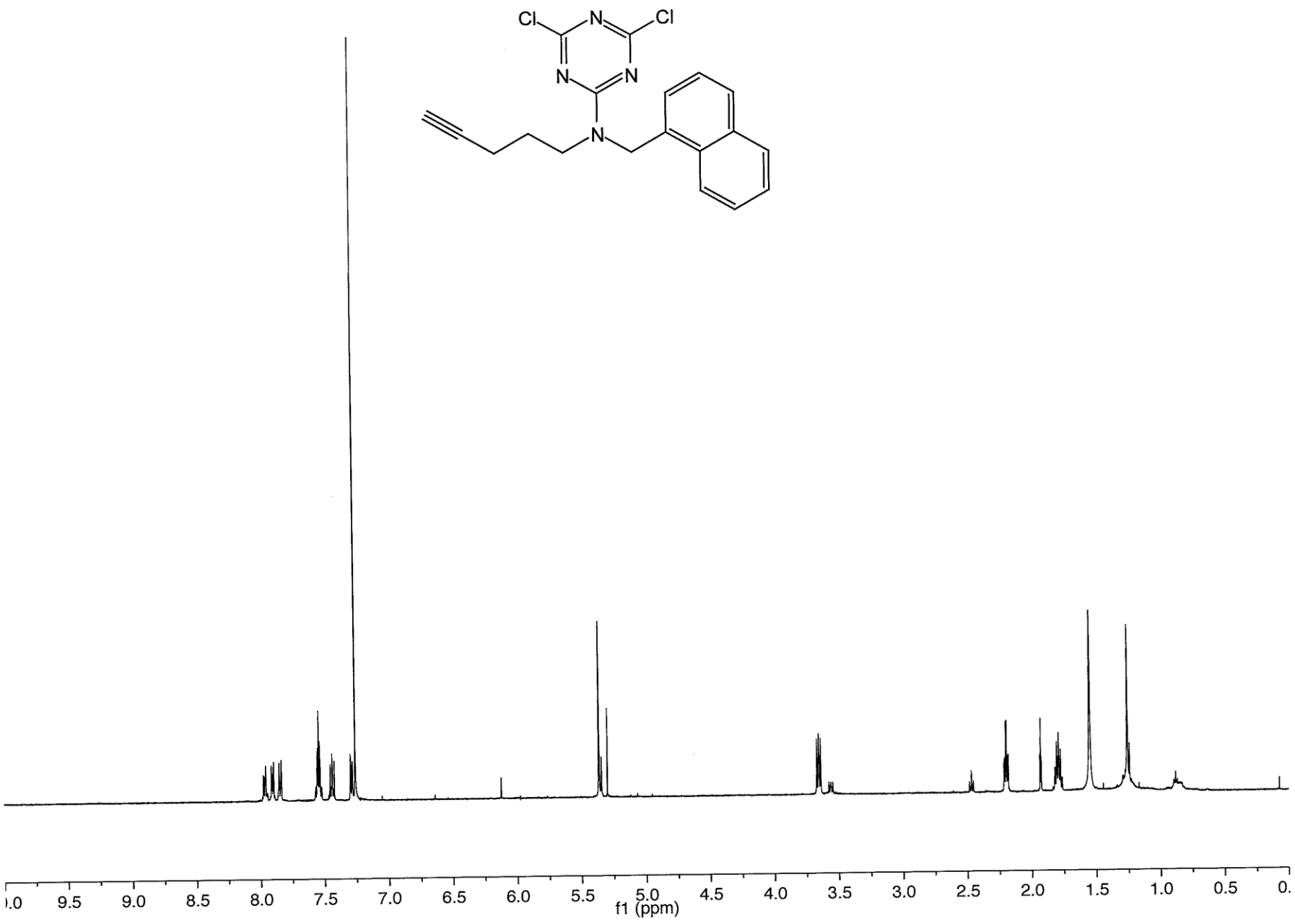
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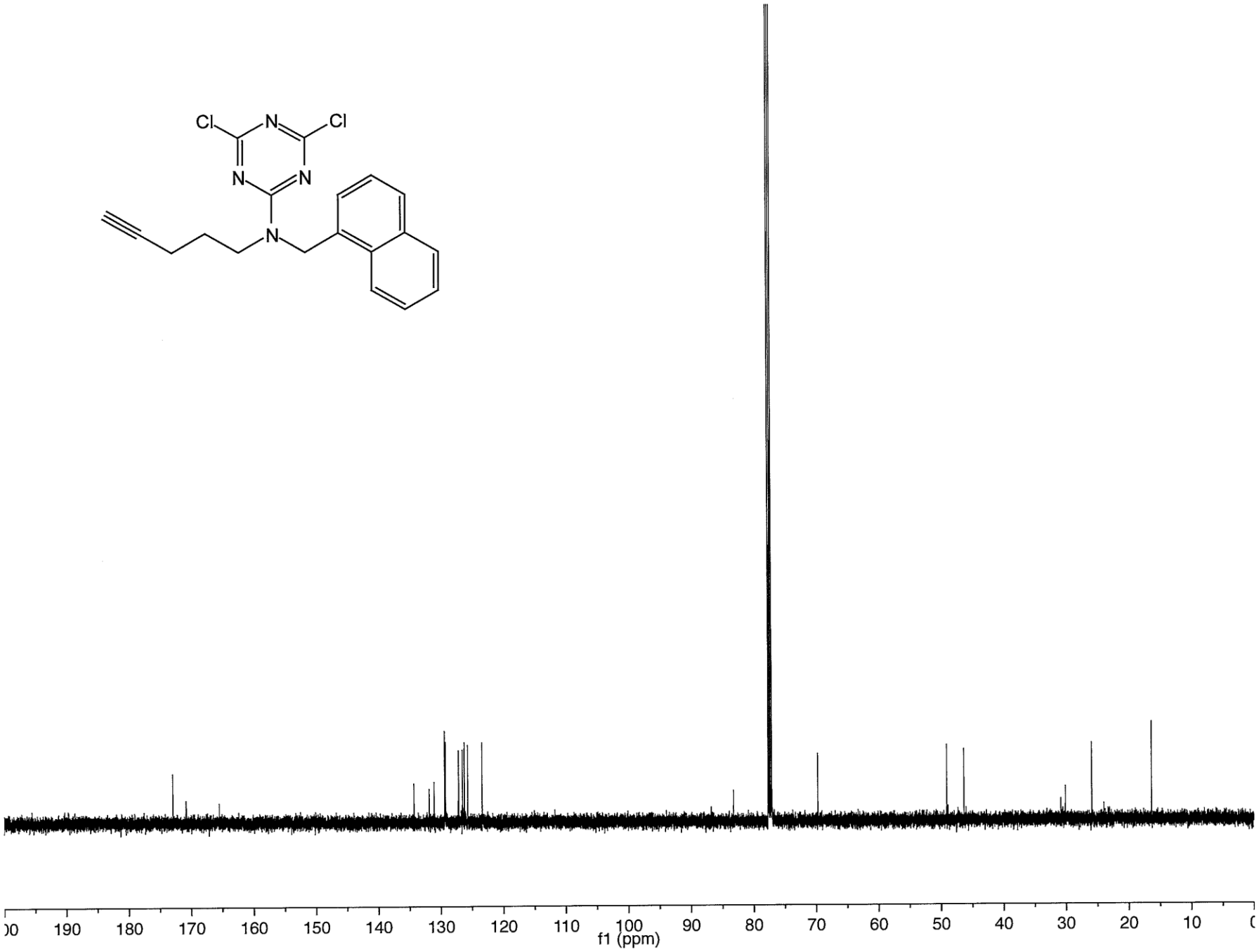
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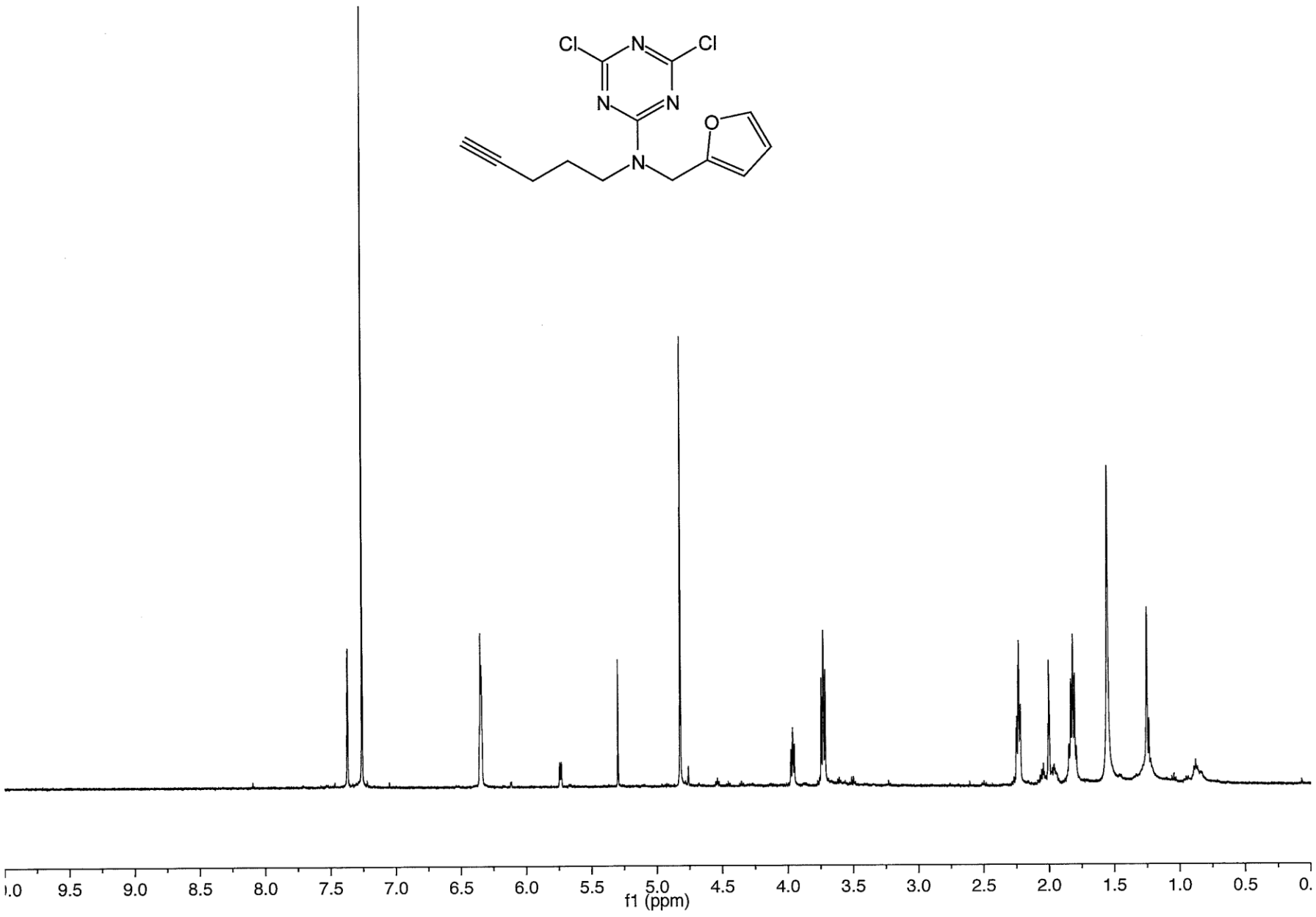
f1 (ppm)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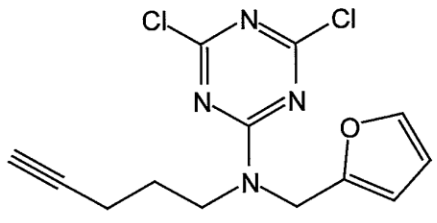
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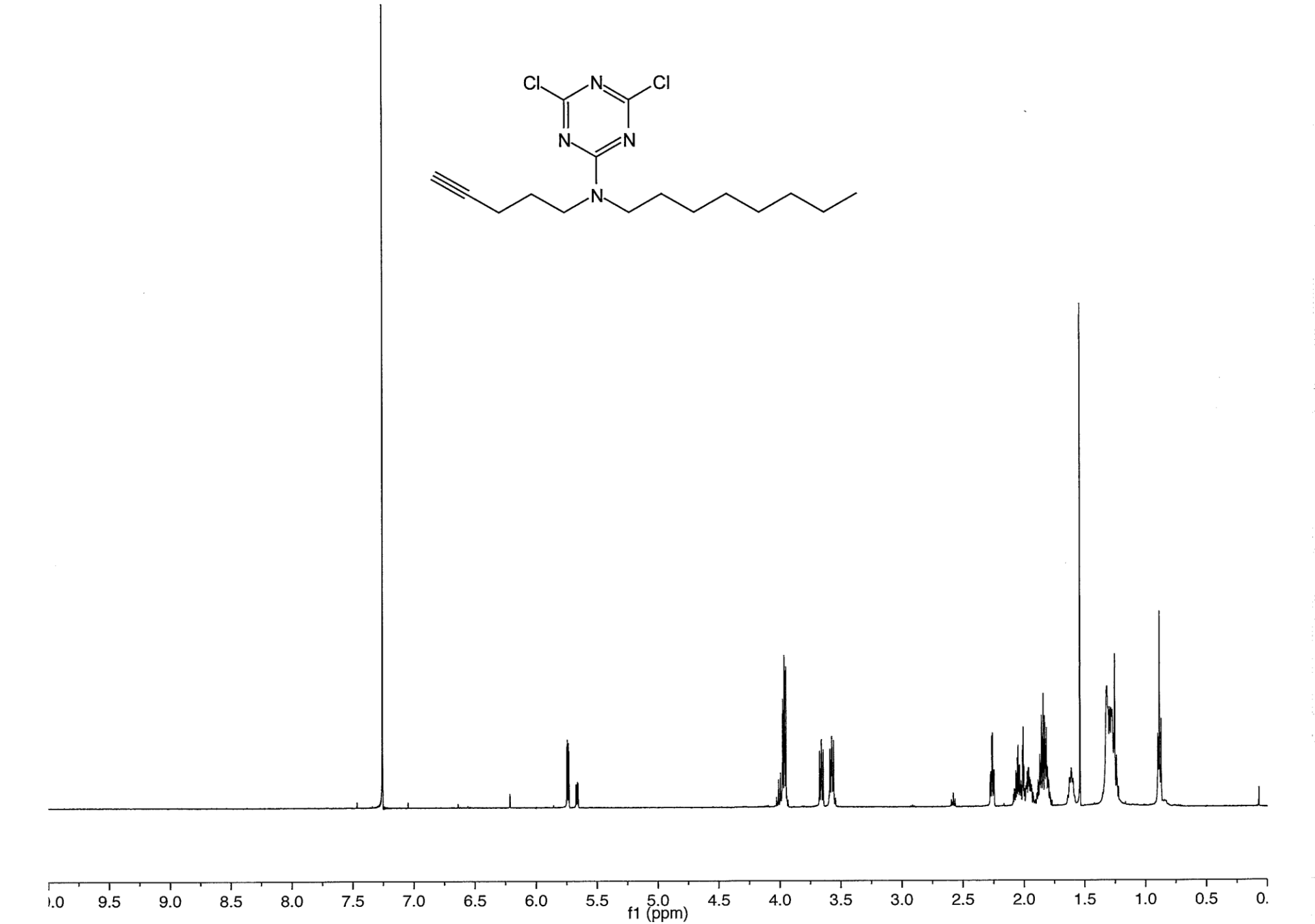
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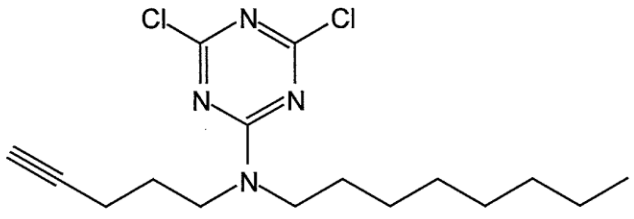
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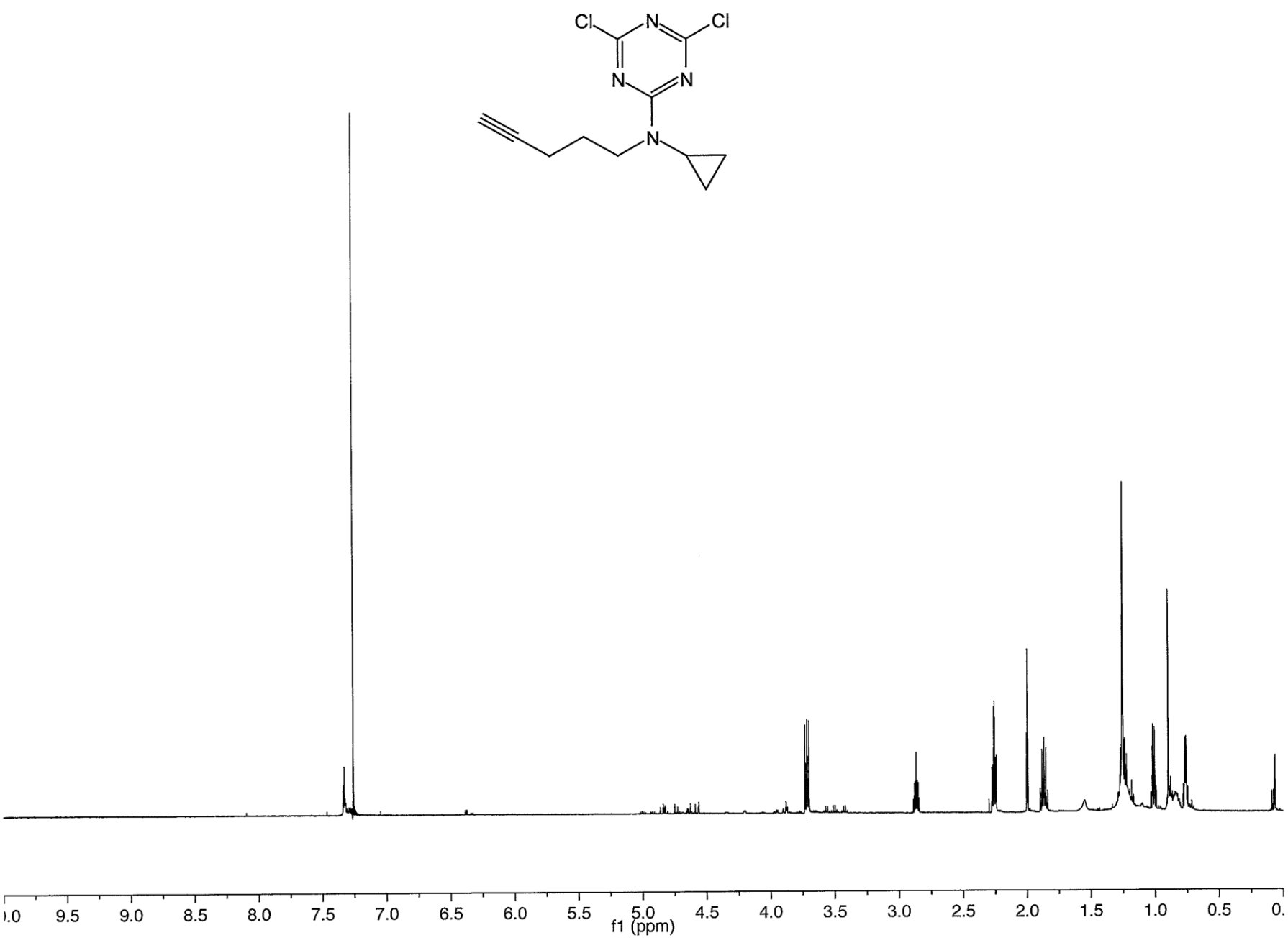
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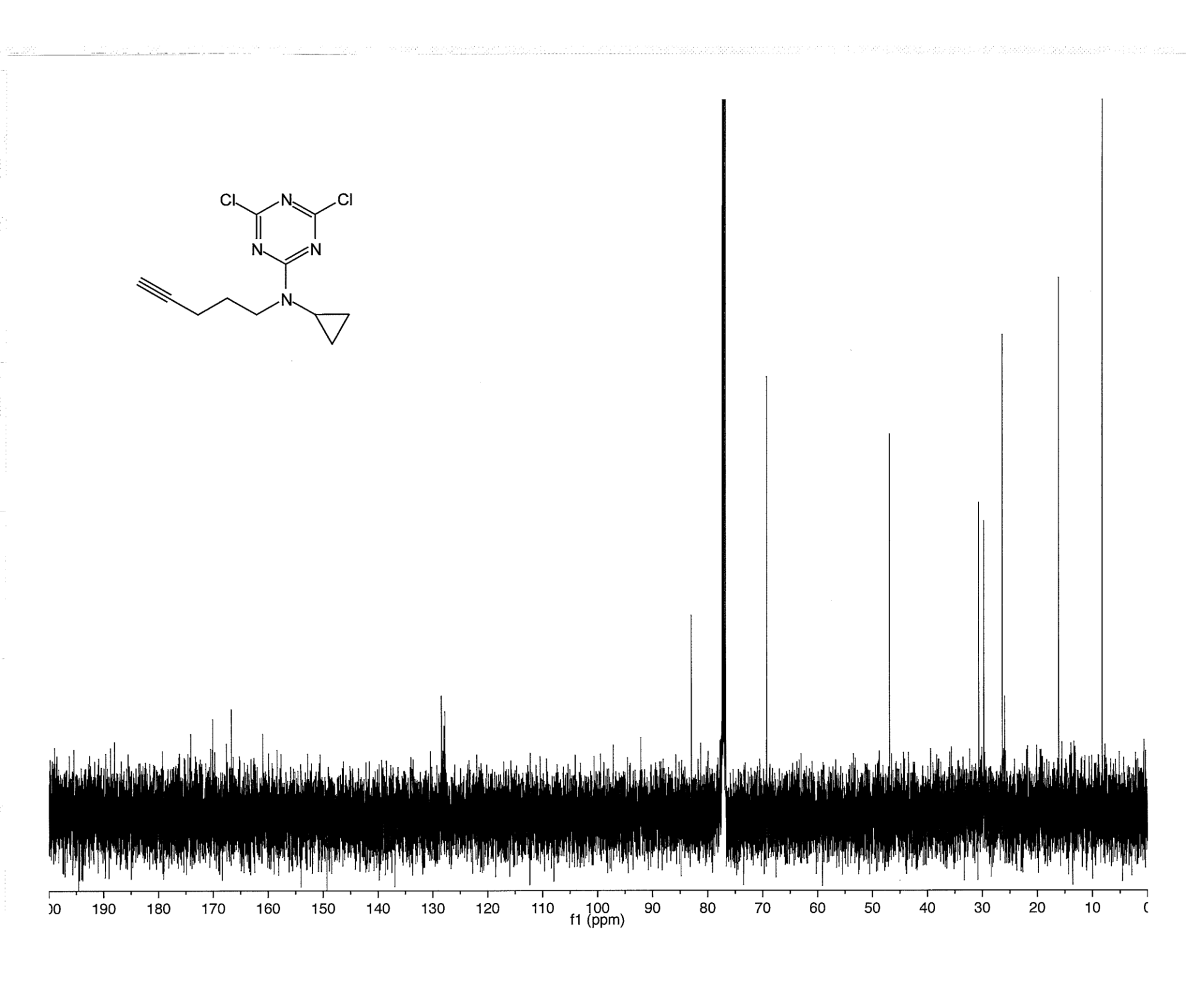
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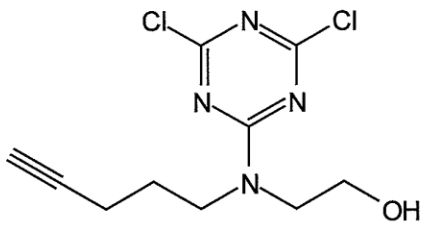
f1 (ppm)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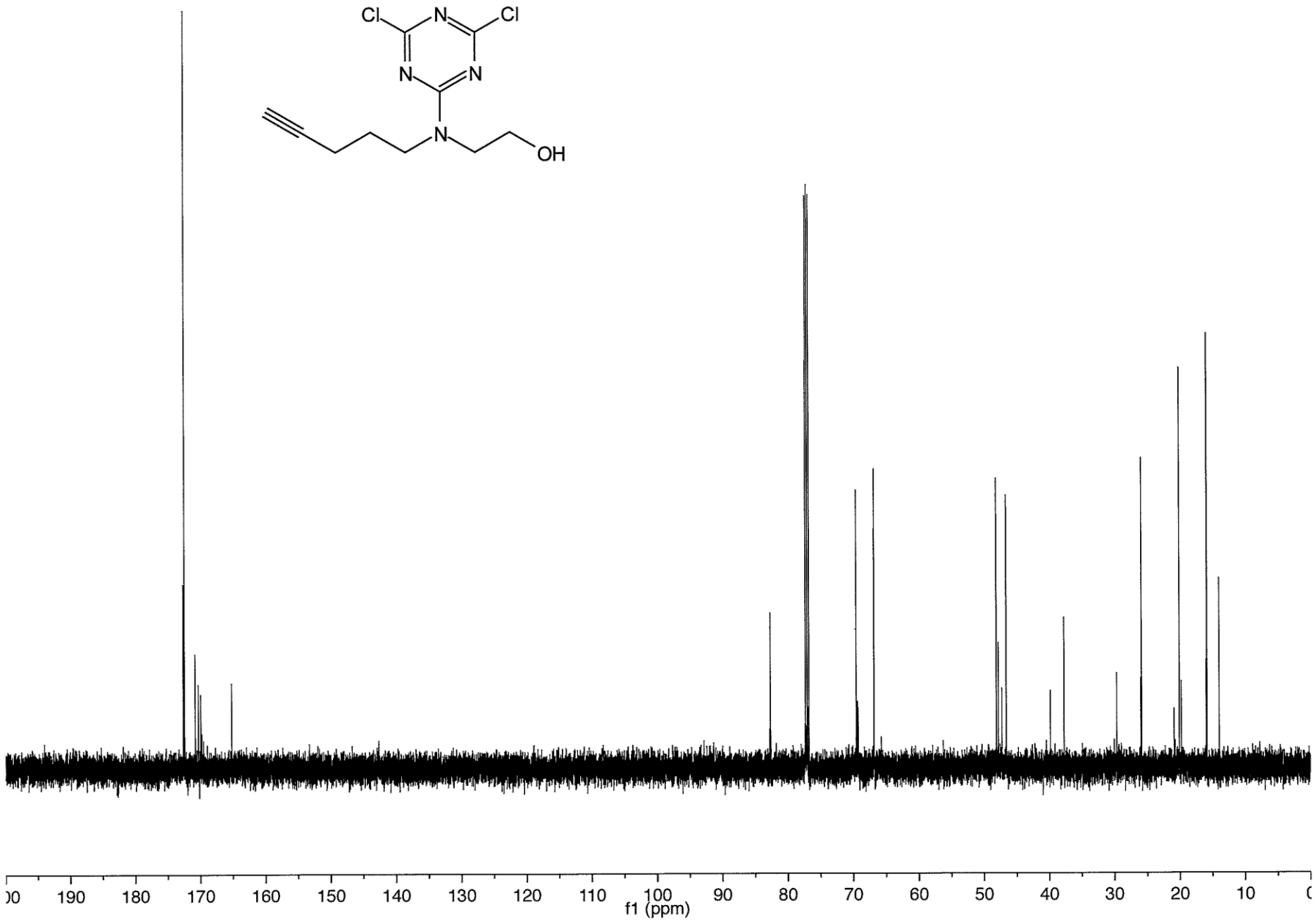
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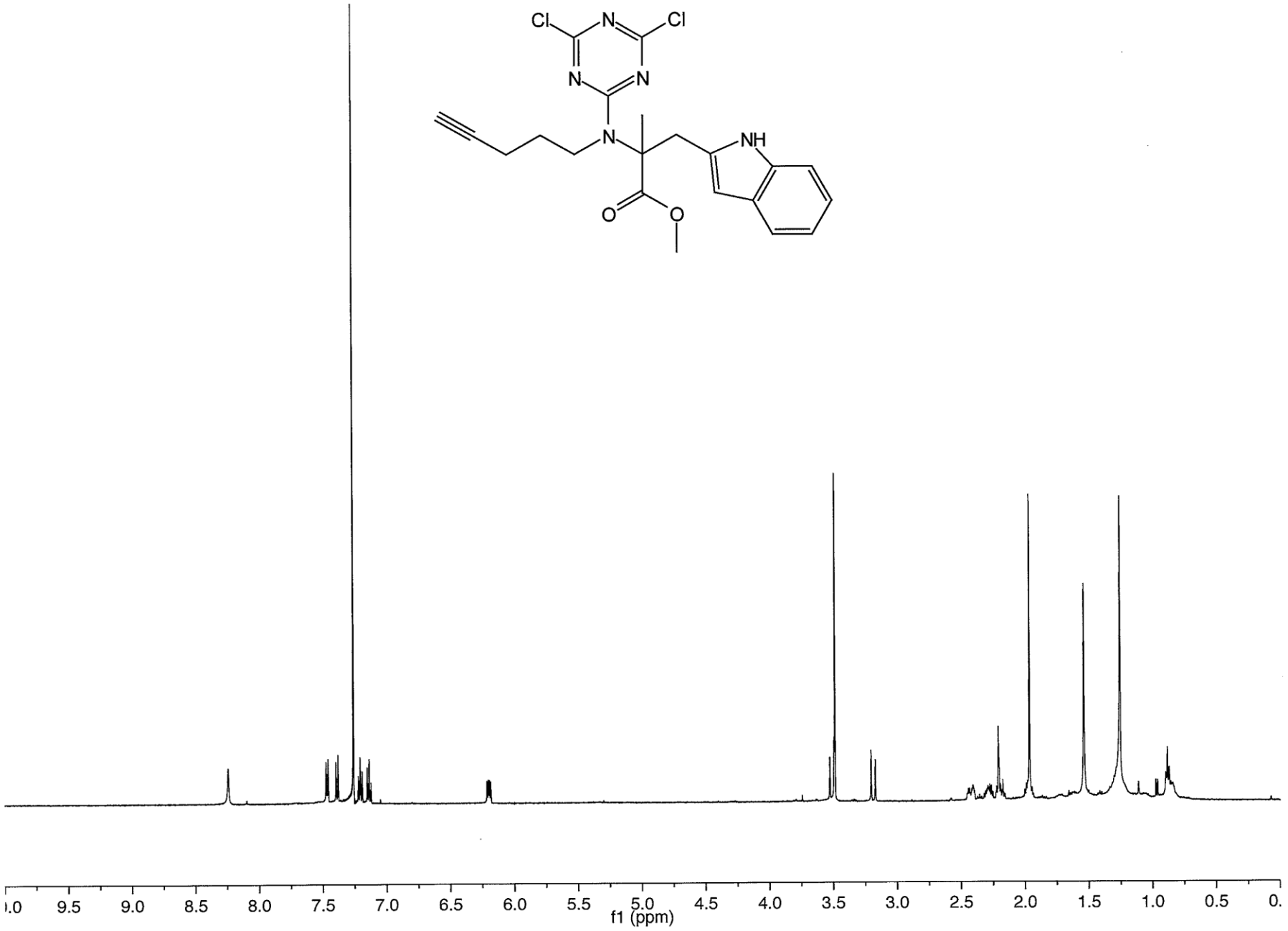
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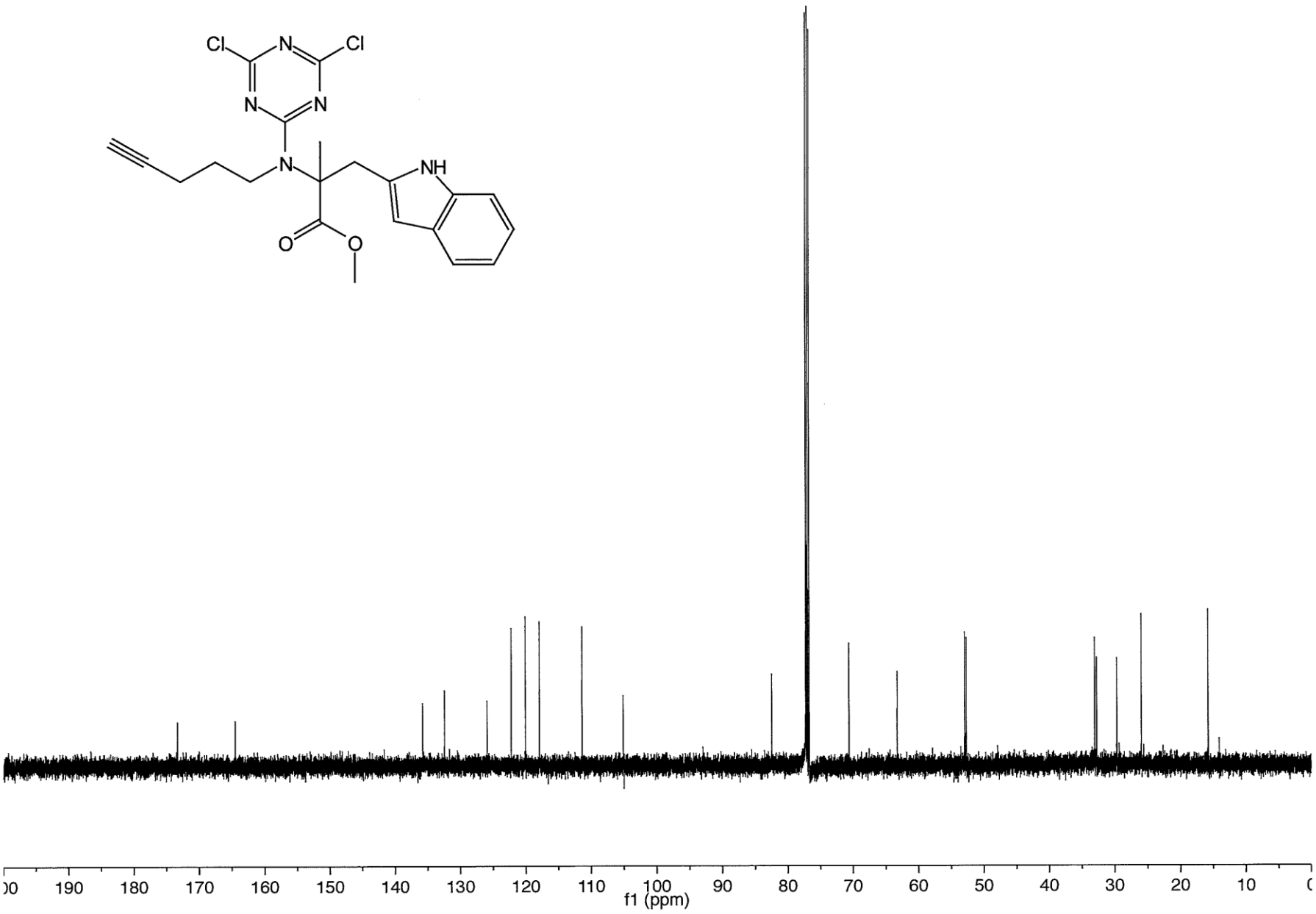
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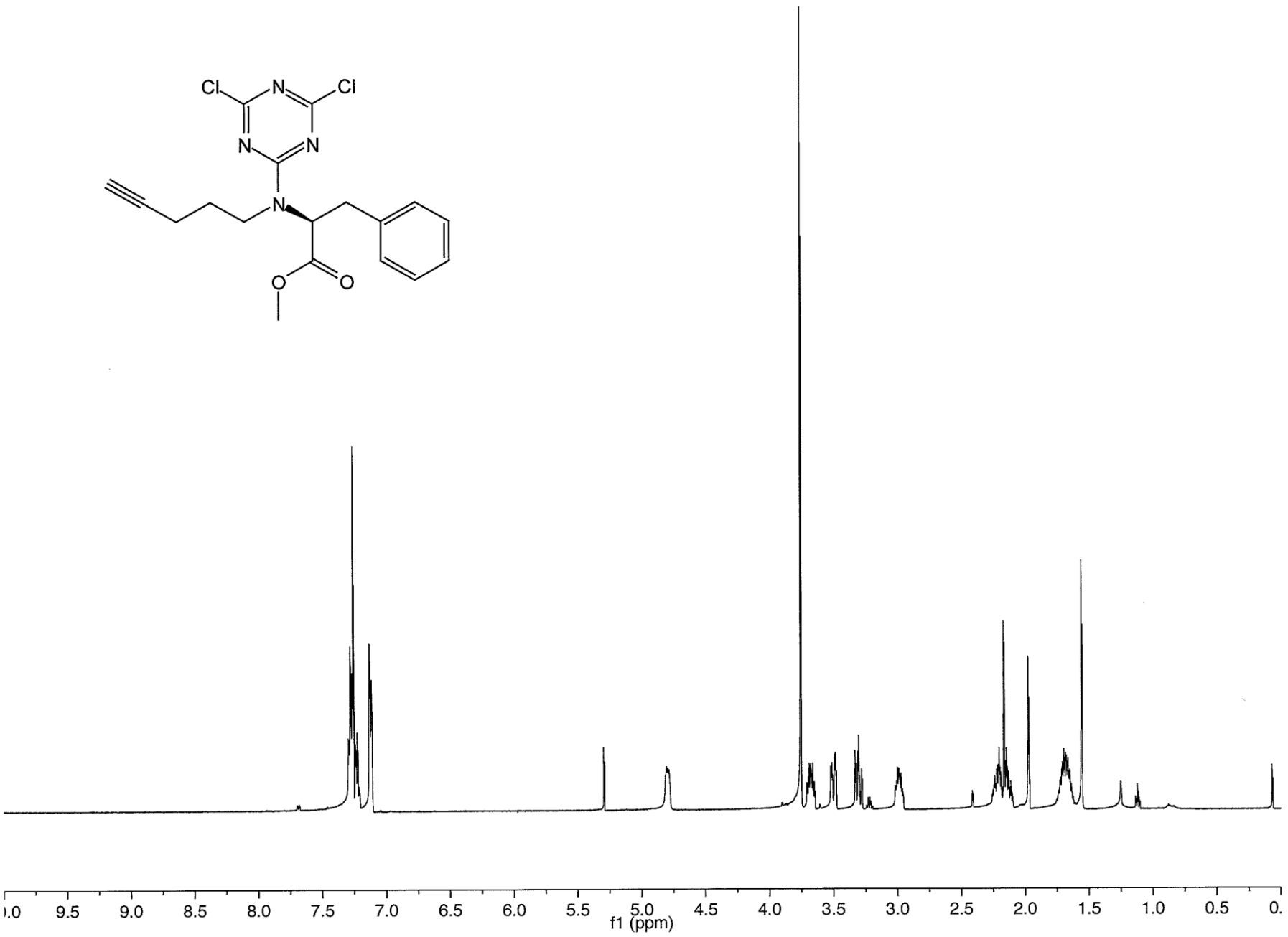
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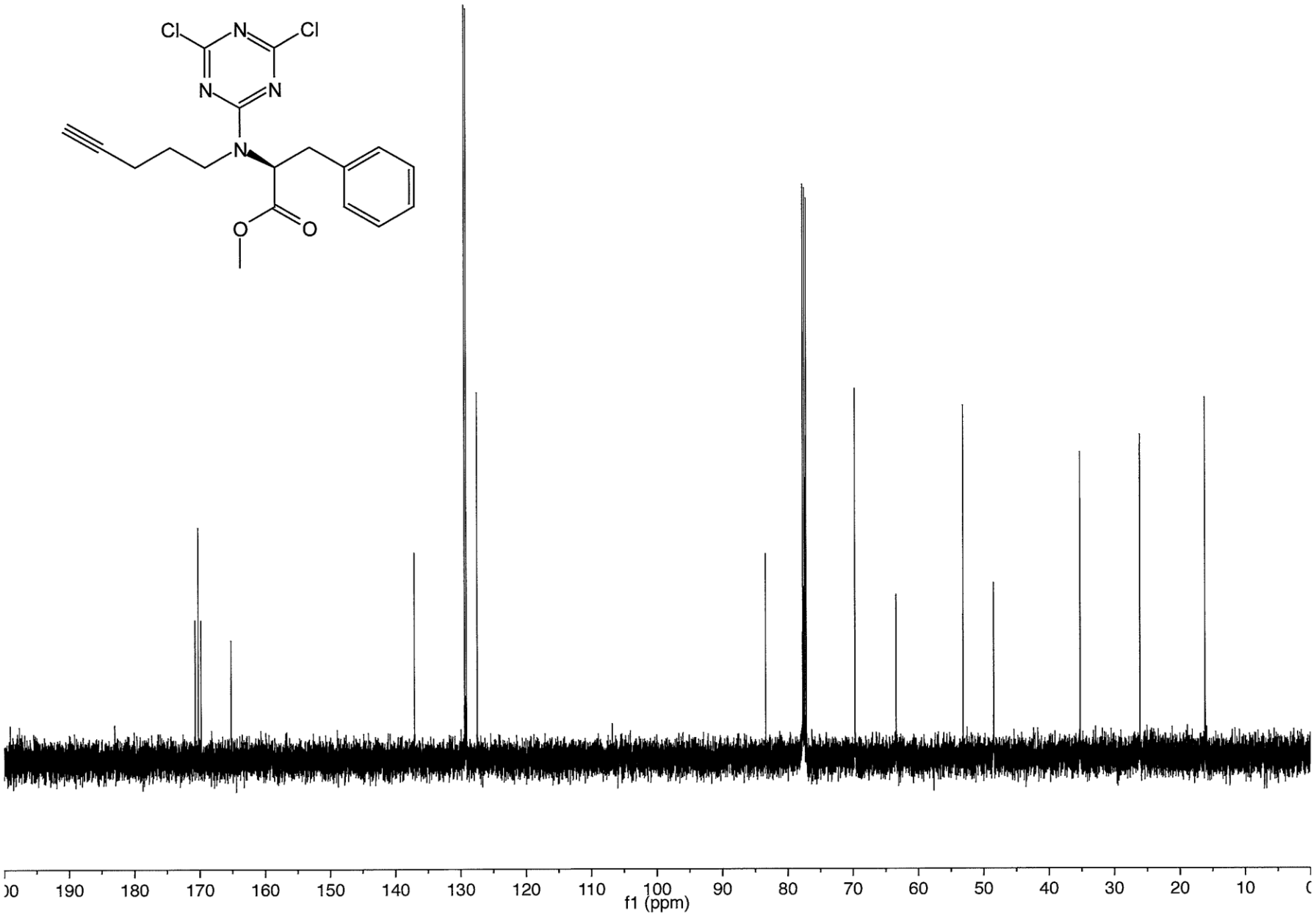
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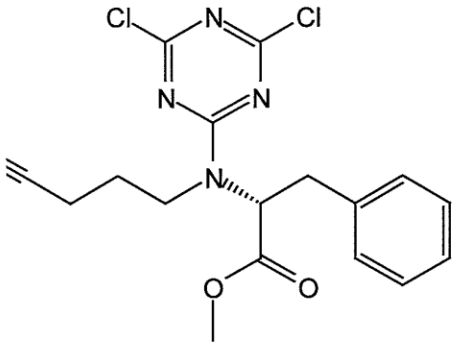
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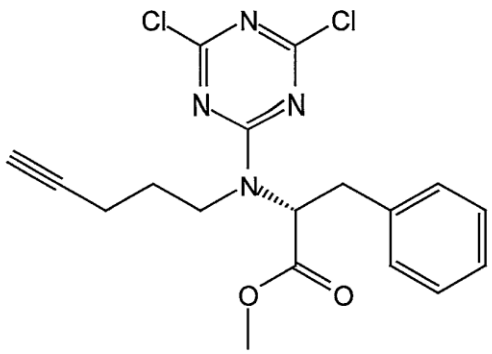
f1 (ppm)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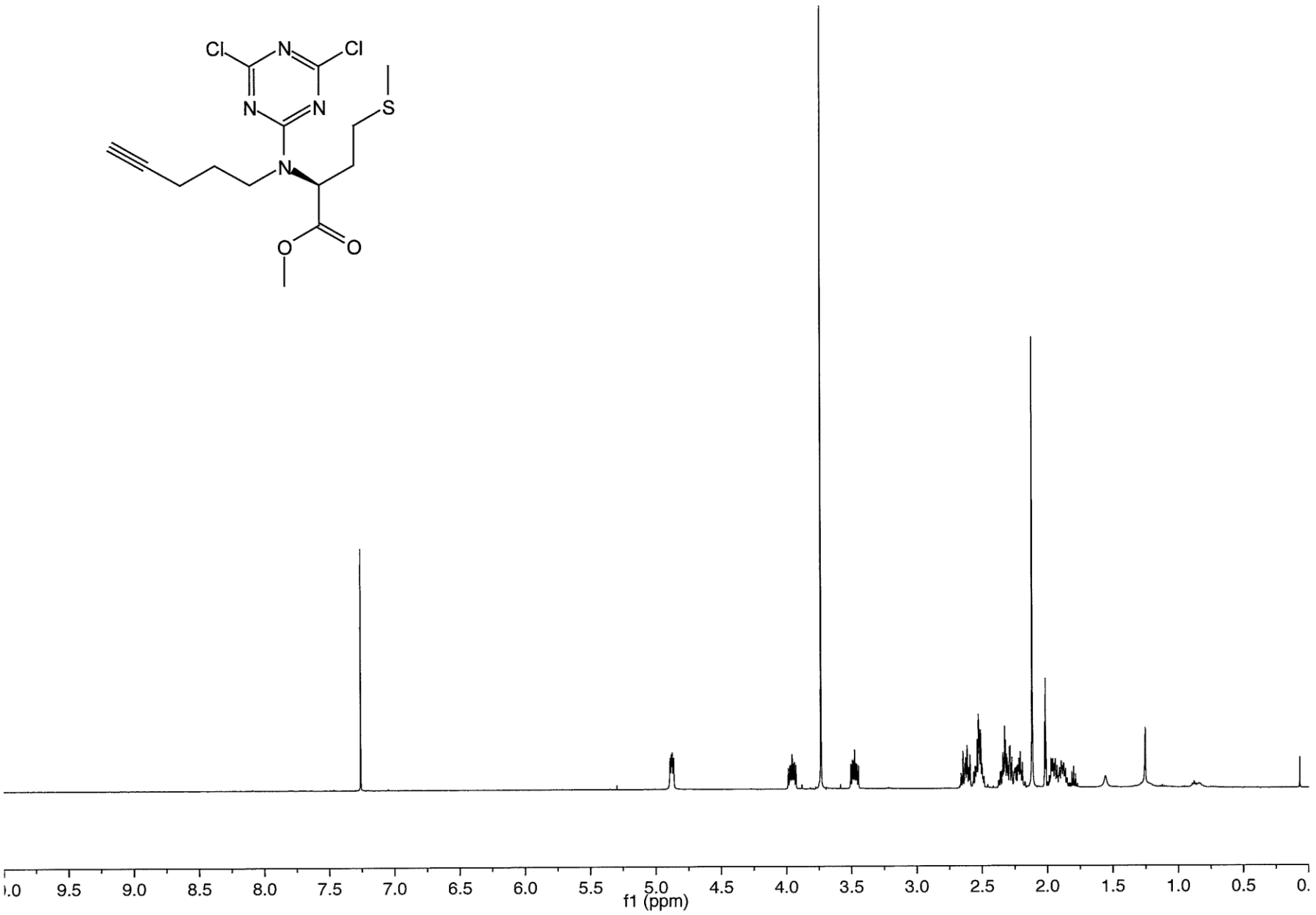
f1 (ppm)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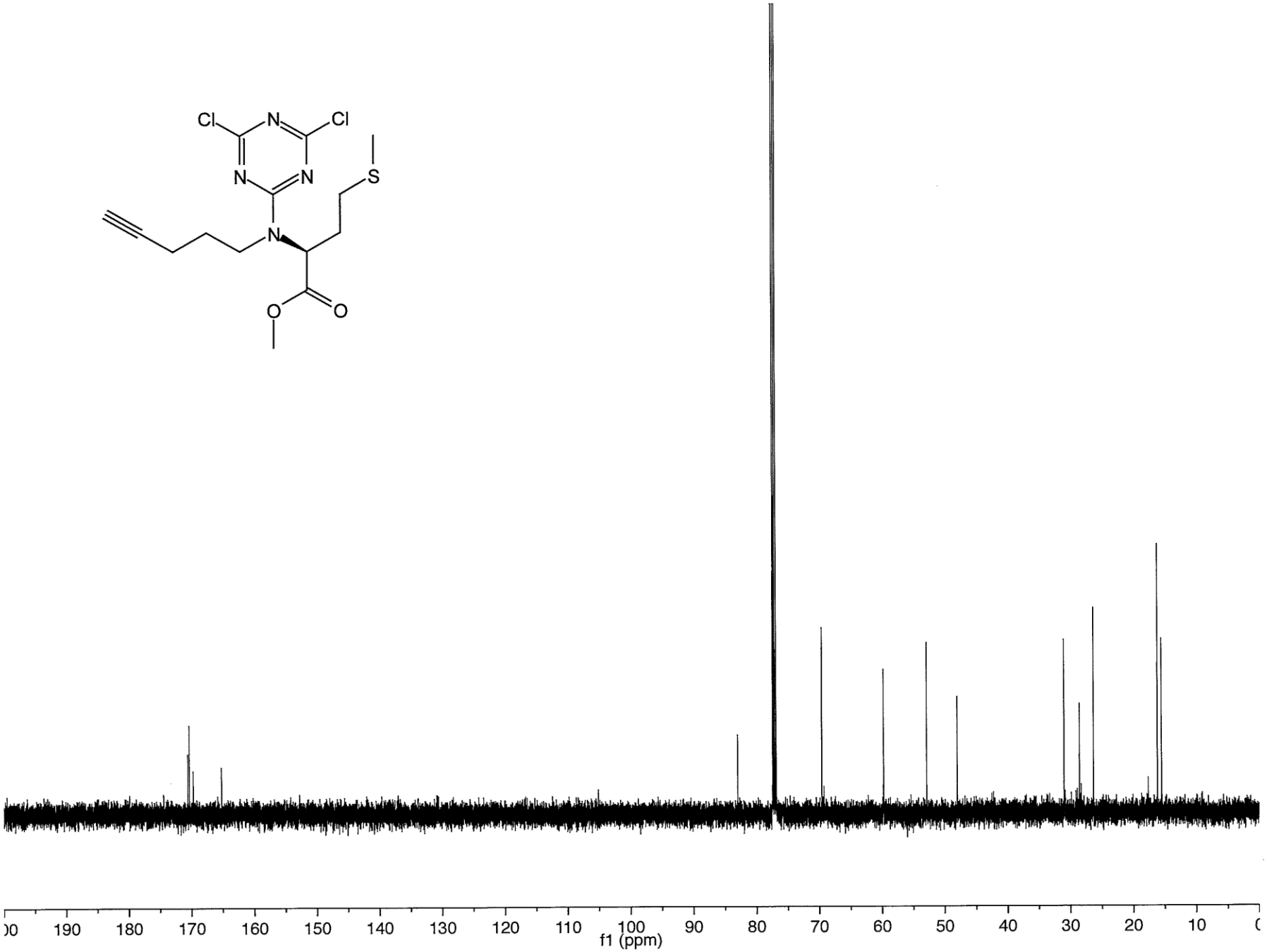
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f1 (ppm)
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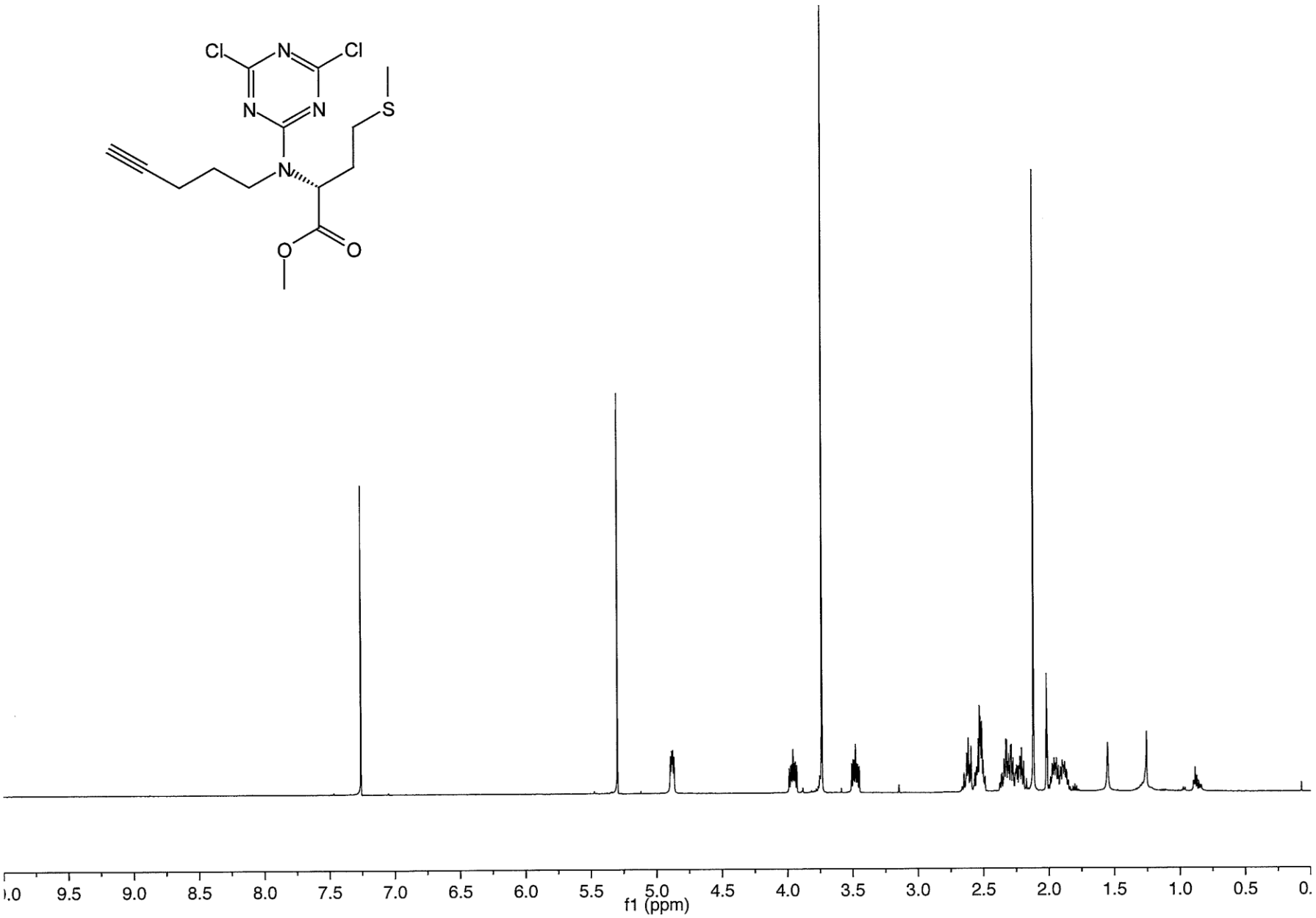
40
30
20
10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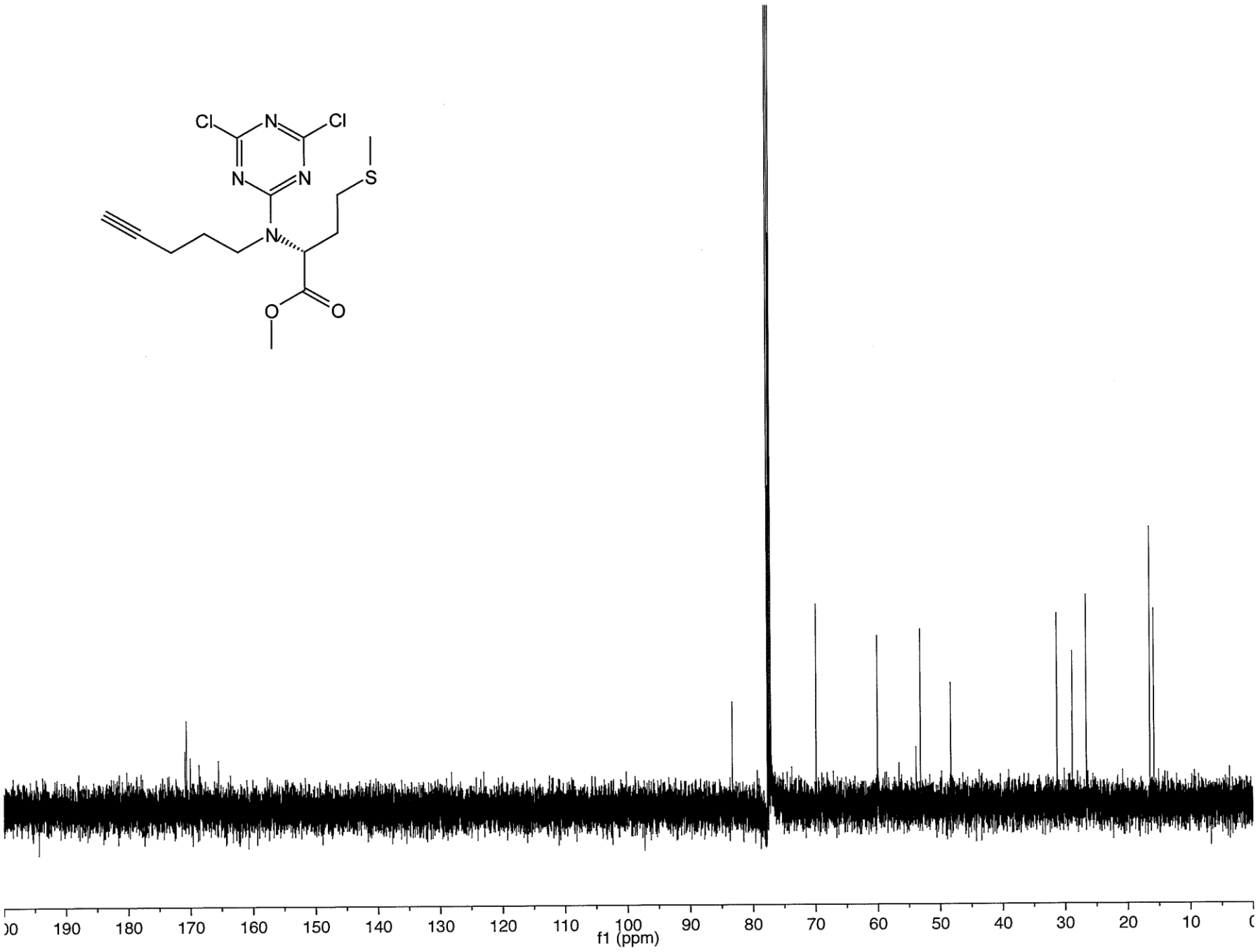
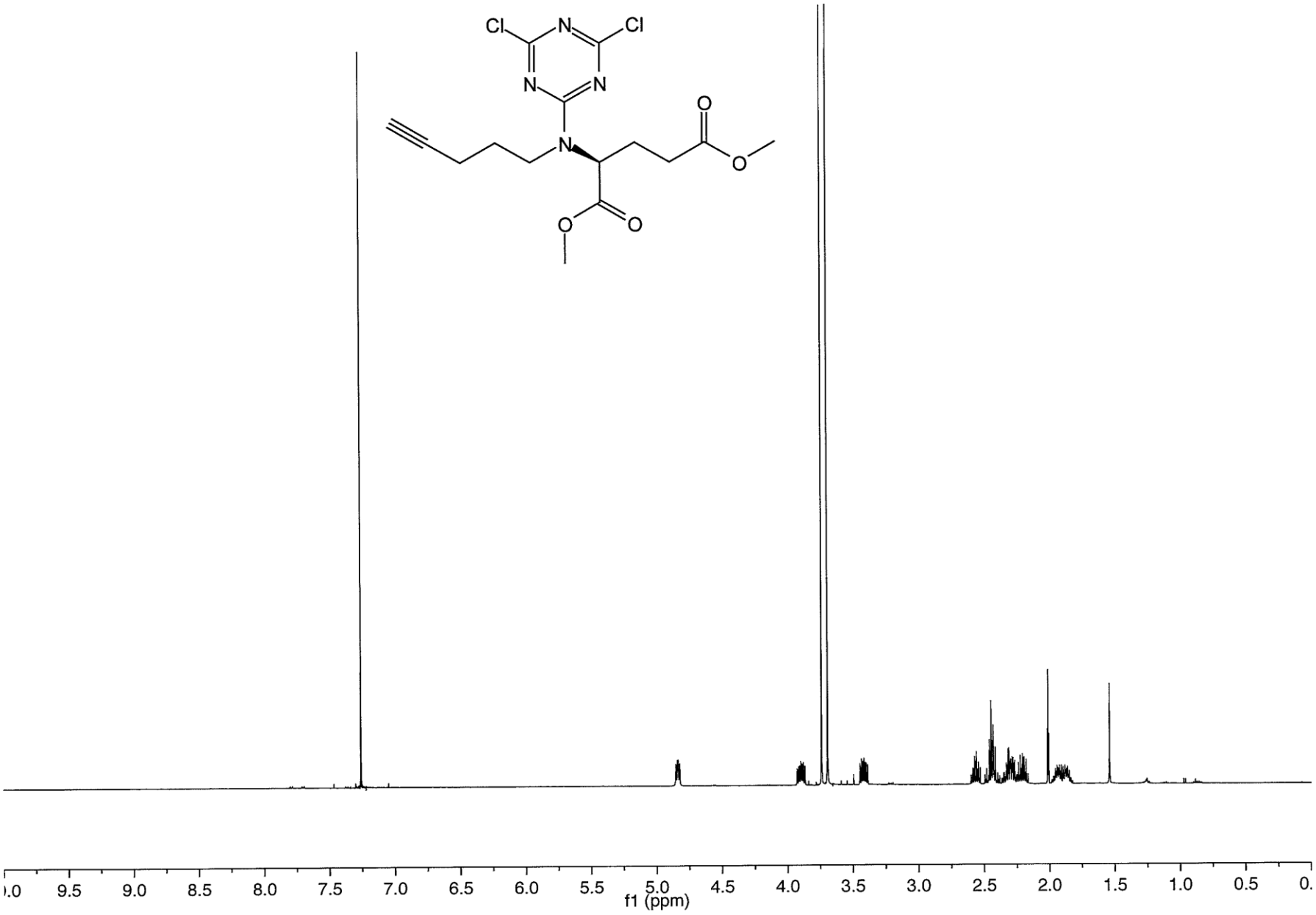
N
Cl
N
N
S
N
O
O
O
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)


Cl
N
Cl
N
N
S
N
O
O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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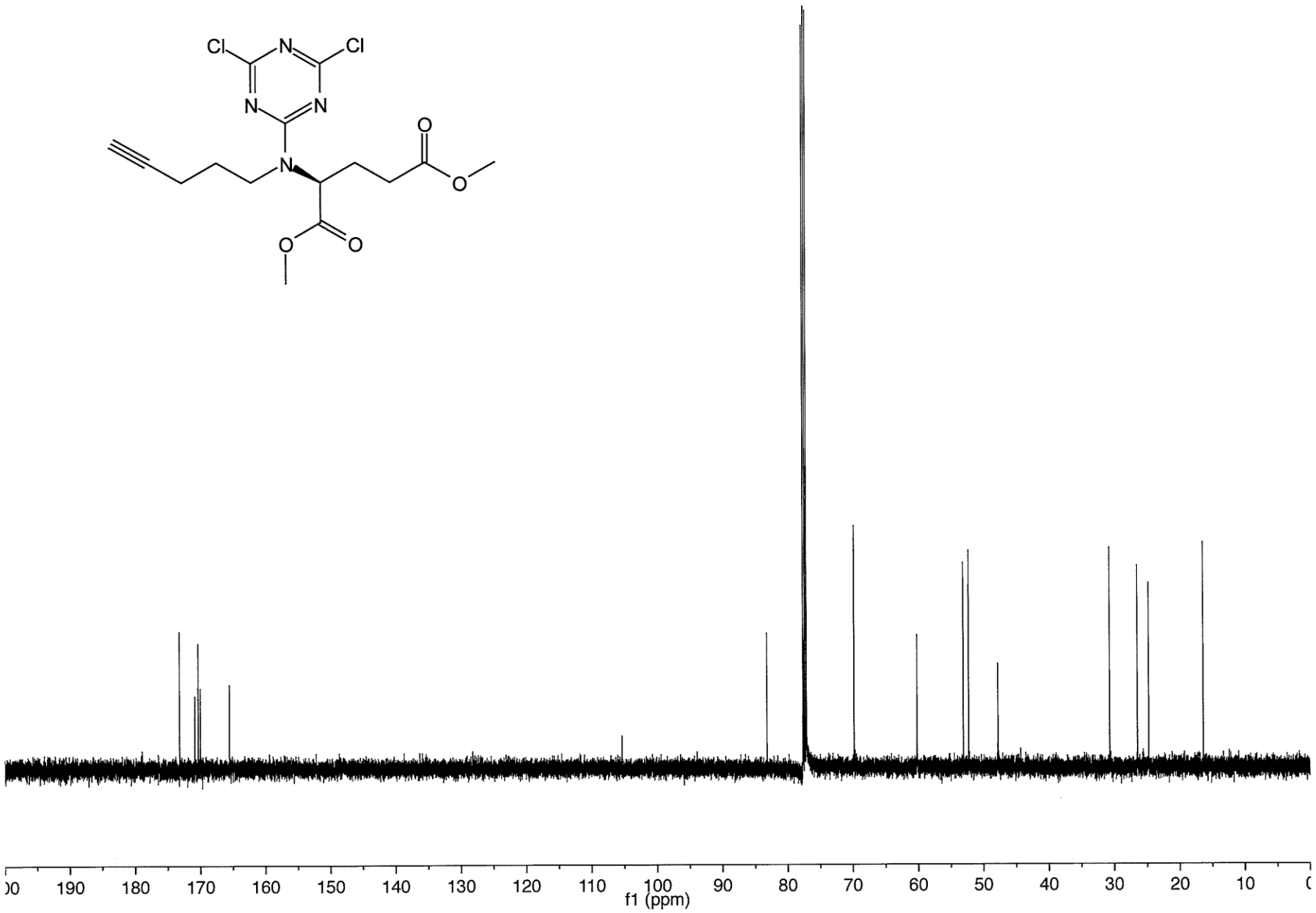
Cl
N
N
N
O
O
O
O
f1 (ppm)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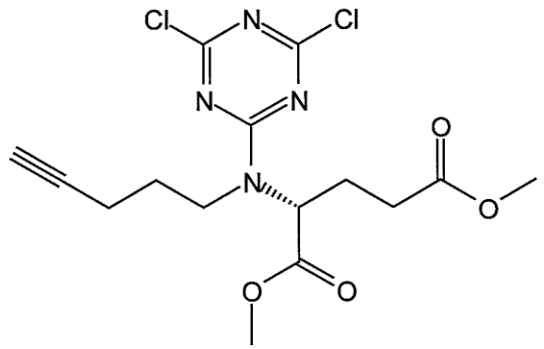
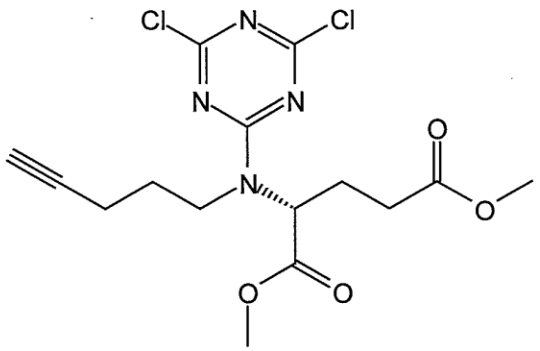
Cl
N
N
N
O
O
O
O
f1 (ppm)


Cl
N
Cl
N
N
N
O
O
O
O

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
f1 (ppm)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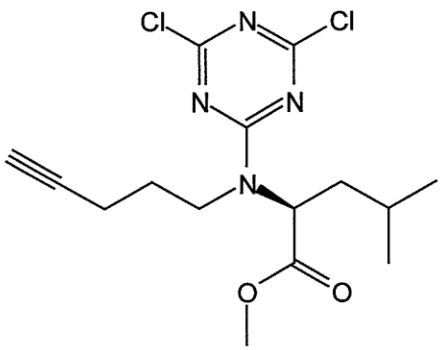
Cl
N
N
N
O
O
O
O
O

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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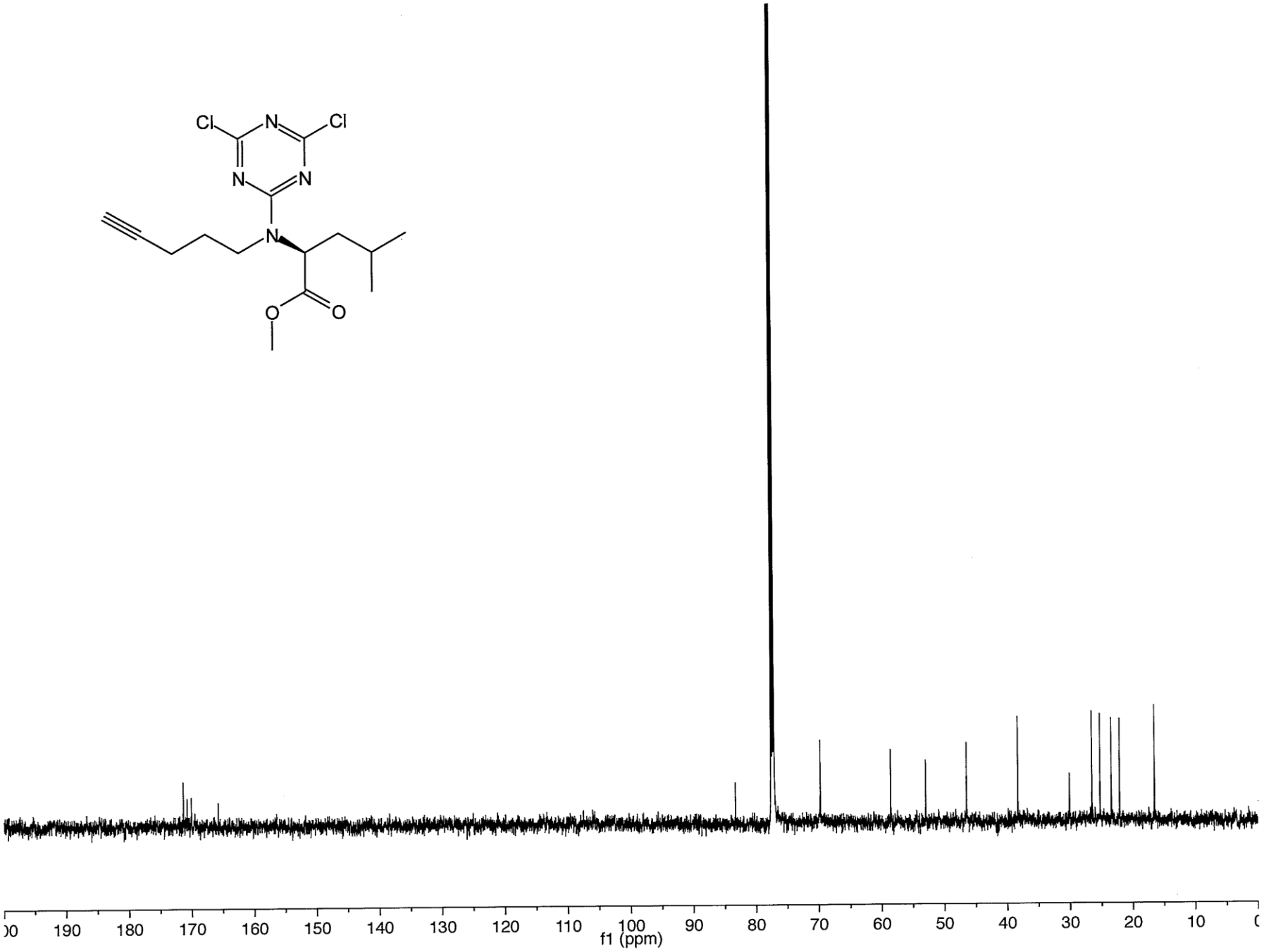
Cl
N
N
N
O
O

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
f1 (ppm)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Cl
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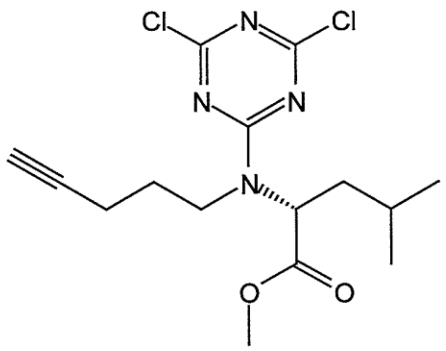
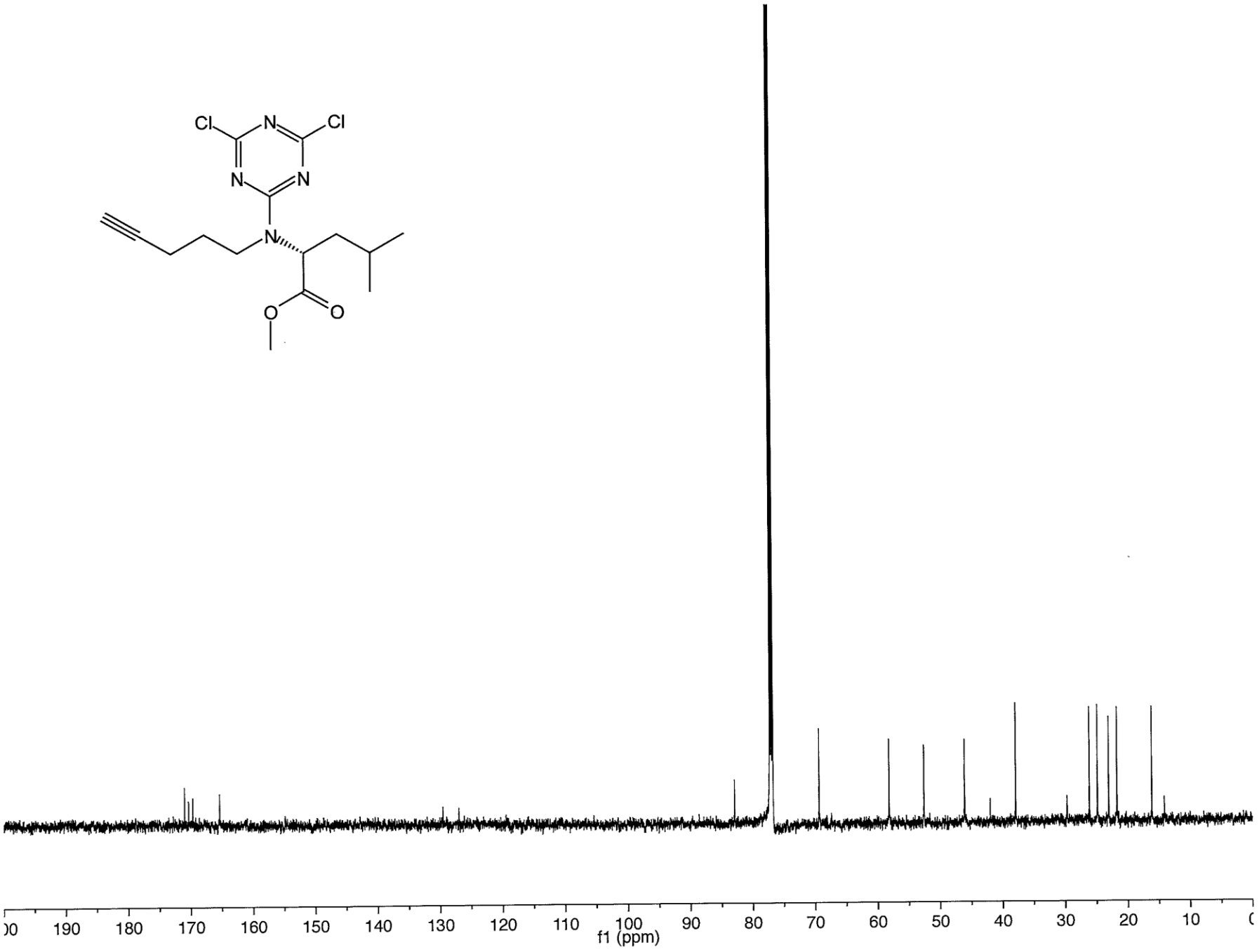
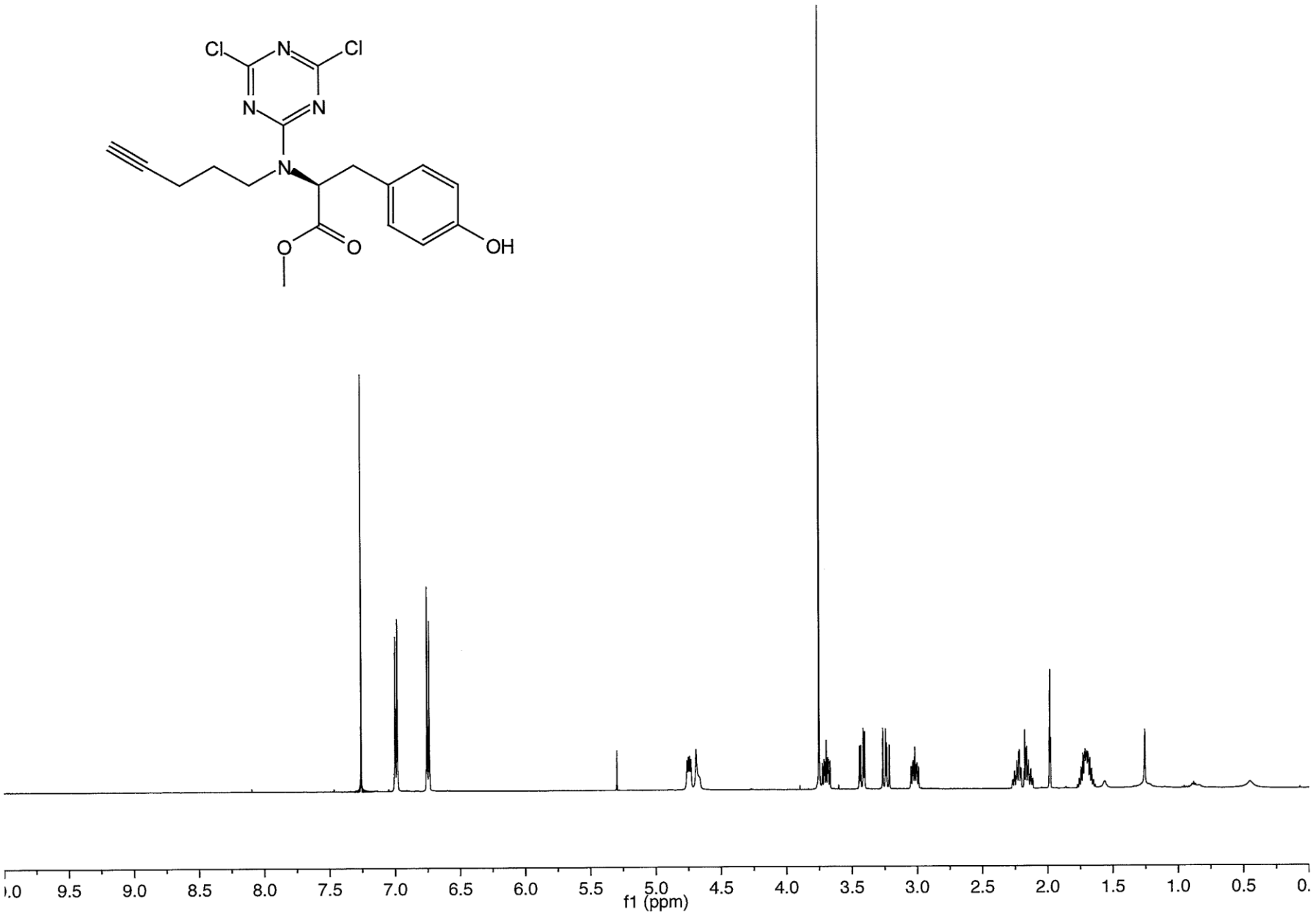
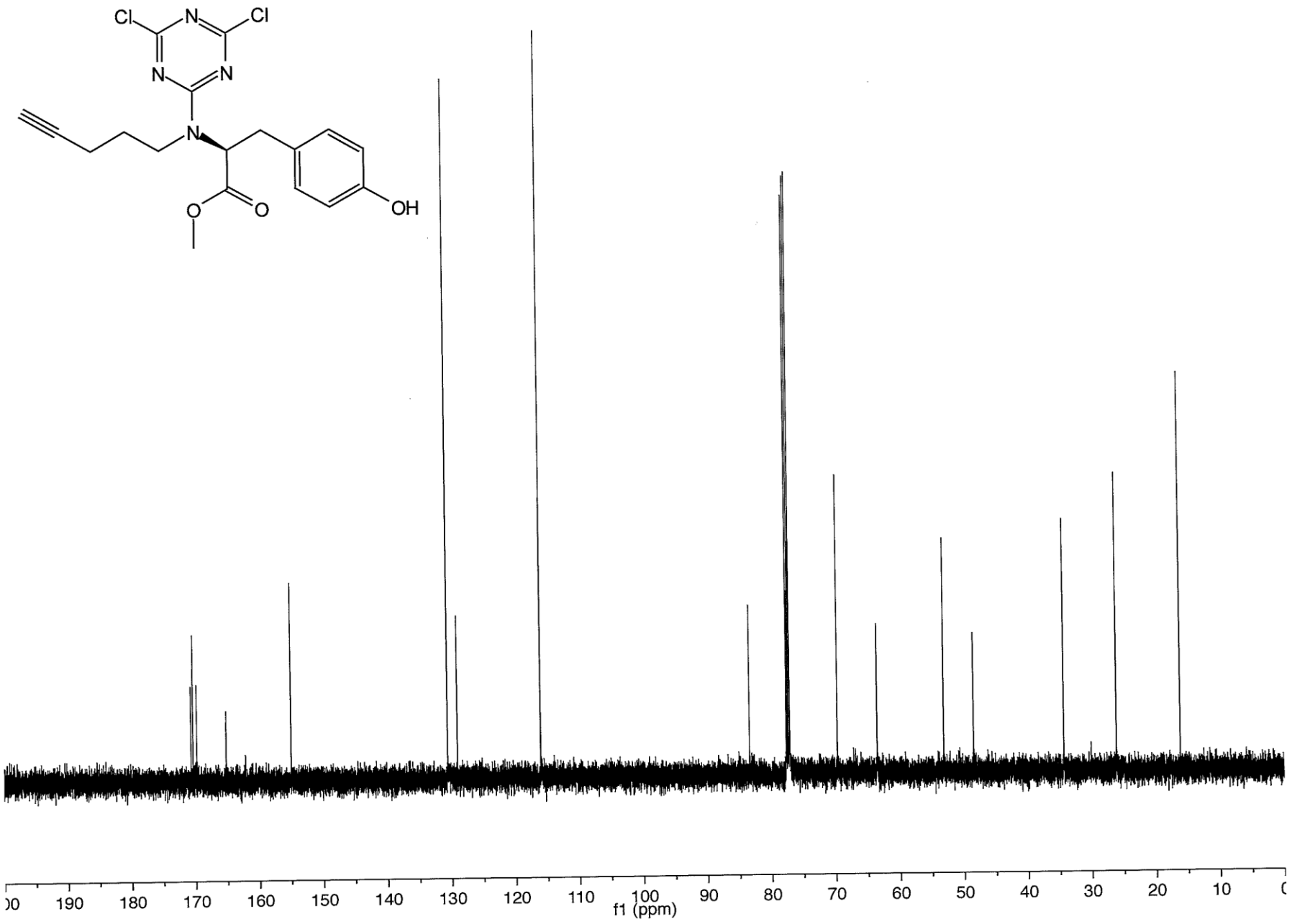
O
O
f1 (ppm)

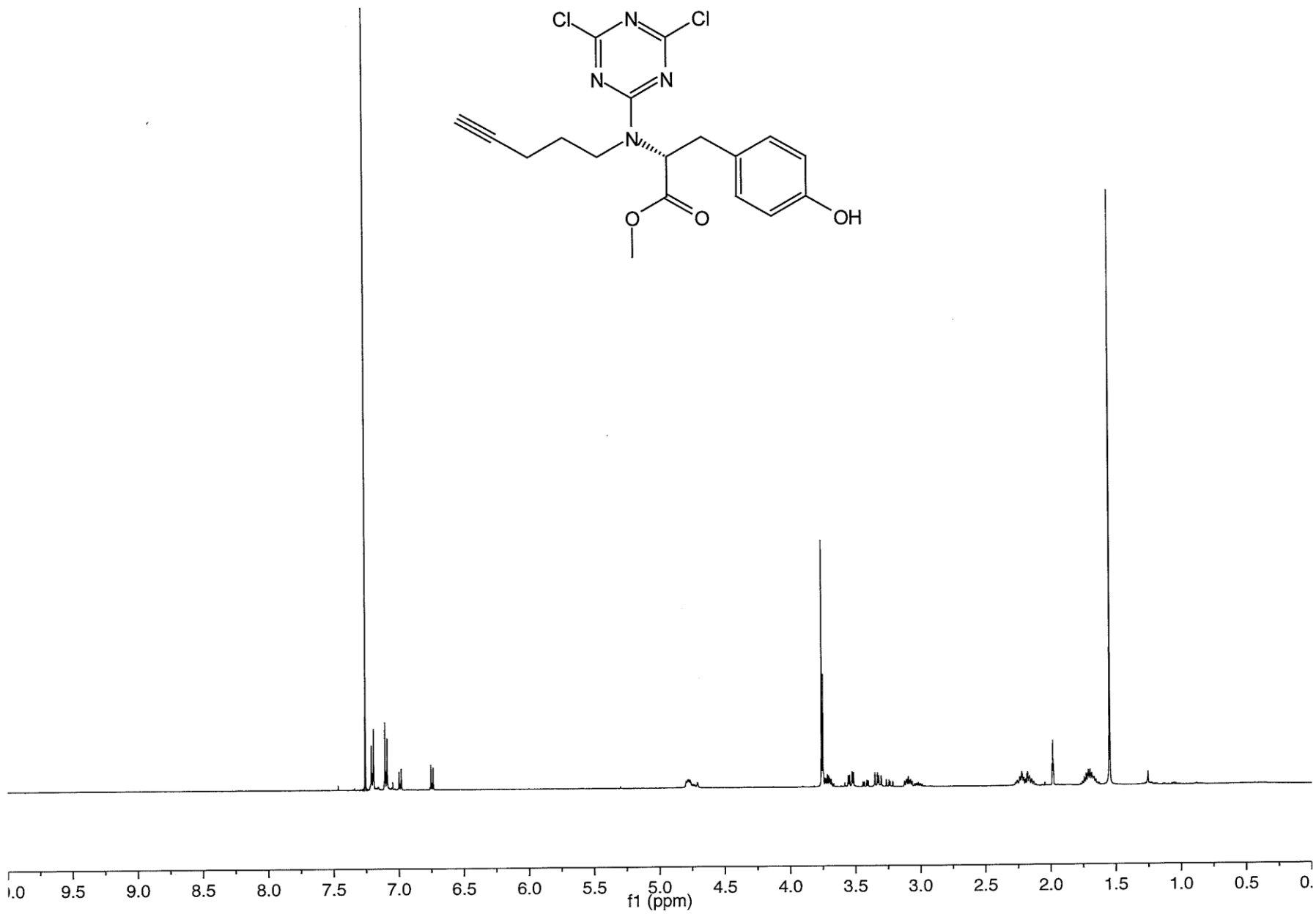

Cl
N
Cl
N
N
N
O
O

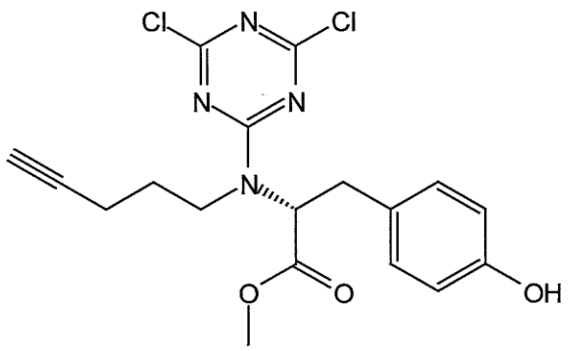
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


Cl
N
Cl
N
N
N
O
O
f1 (ppm)


Cl
N
Cl
N
N
N
O
O
OH
f1 (ppm)


Cl
N
Cl
N
N
N
O
O
OH
f1 (ppm)


Cl
N
Cl
N
N
N
O
O
OH
f1 (ppm)


Cl
N
Cl
N
N
N
O
O
OH

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

[1] D. A. Shannon, R. Banerjee, E. R. Webster, D. W. Bak, C. Wang, E. Weerapana, *Journal of the American Chemical Society* **2014**, *136*, 3330-3333.