Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Affinity- and activity-based probes synthesized from structurally diverse hops-derived xanthohumol flavonoids reveal highly varied protein profiling in *Escherichia coli*

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General Procedures and Materials

NMR spectra were recorded at 25 °C on a Varian Oxford 500 MHz spectrometer at the following frequencies: 499.8 MHz (^{1}H) and 125.7 MHz (^{13}C); spectra were calibrated to the chemical shift of residual undeuterated solvent. Spectra were assigned with appropriate ^{1}H and ^{13}C NMR experiments. Chemical shifts are reported in parts per million, coupling constants in Hertz (Hz), and multiplicities indicated with: singlet (s), doublet (d), triplet (t), doublet of doublets (dd), triplet of doublets (td), triplet of triplets (tt), and multiplet (m). High-resolution mass spectra were obtained with a QExactive Orbitrap mass spectrometer (Thermo Scientific). Protein concentration measurements were made using a SpectraMax 96-well UV/vis microplate reader. Fluorescent ABP-labeled proteins were separated by SDS-PAGE on Invitrogen 10-20% Tris-Glycine precast gels and imaged with a Protein Simple FluorchemQ: 534 nm excitation, 606 nm emission filter. Unless otherwise noted, silica gel flash column chromatography was used to purify all compounds using Biotage purification system and prepacked columns for the same were obtained from Luknova. Other reagents were purchased from Sigma-Aldrich, TCI, Acros, or Alfa Aesar and used as received unless stated otherwise. Dry solvents, if not purchased, were obtained via an LC Technology Solutions, Inc., SP-1 solvent drying system; reactions were carried out in an inert nitrogen or argon environment.

Analytical Sample Techniques & Bacterial Cultivation Methods

Bacterial Cloning and Expression of CHI from Eubacterium ramulus[1]

Eubacterium ramulus ATCC 29099 (Taxon:1256908) was cultured in chopped meat medium K19 purchased from Hardy Diagnostics (Santa Maria, CA, USA). Bacterial DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The gene encoding for the chalcone isomerase enzyme (CHI, GenBank accession: KF154734.1) was amplified by PCR using the iProof High-fidelity DNA polymerase from Bio-Rad (Hercules, CA, USA). Primers were purchased from Eurofins Genomics (Louisville, KY, USA) and their sequences are as follows:

Primers	Gene	Sequence	Restriction site
CHI-F	Chalcone isomerase	5'-AGAGCAAAGCTTATGGCAGATTTCAAATTCGA-3'	HindIII
CHI-R	Chalcone isomerase	5'-CTAAGACTCGAGTTATCTCATGGTGATGTATCC-3'	XhoI

The PCR products were cleaned using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and inserted individually into Novagen pET22b (+) plasmid with T4 DNA ligase from New England BioLabs (Ipswich, MA, USA). Heat-shock transformation was performed by adding the ligation reaction to 100 μL of BL21-AI (Invitrogen, Waltham, MA, USA) competent cells pre-treated with $CaCl_2$. The transformants were grown in LB broth containing 50 mg/ml of Carbenicillin and plasmids were extracted using Zyppy Plasmid Miniprep Kit (Zymo Research, Irvine, CA, USA). Sequencing was performed at the Center for Genome research and Biocomputing (CGRB) Center for Genome research and Biocomputing (CGRB), Oregon State University, Corvallis, OR.

Bacterial Culture and Whole Cell Lysate Preparation

E. Coli BL21 (AI) mutant containing CHI from *E. ramulus* cloned into pET22b (no HIS tag) was initiated from a single colony grown on LB agar into 20 mL LB broth (Lennox) containing 50 μg/mL carbenicillin and grown with shaking at 37 °C for 24 hours until reaching an OD of 1.0. Induction of the protein expression was achieved by adding 0.5 mM IPTG and 0.2% arabinose, incubating at 37 °C for an additional 24 hours. Biomass was harvested between mid- and late-log phases by centrifugation at 7,000 × g for 5 min. Cell pellets were resuspended in 600 μL of 1x PBS and transferred to 2.0 mL bead beater tubes with 100 μL glass beads. Cells were lysed on a Bead Ruptor Elite (Omni, Inc.) (6.60 m/s, 30 s, 4x, 2 min ice between runs). Beads and debris were pelleted by centrifugation at 14,000 × g for 5 min. Supernatant was removed, aliquoted, and protein concentrations were determined using a bicinchoninic acid (BCA) assay (Thermo Scientific Pierce). Colorimetric assays were analyzed using a Molecular Devices plate reader.

Photoaffinity Probe Enabled Fluorescent SDS-PAGE Gel Electrophoresis

Bacterial culture stocks were prepared as previously mentioned above. Whole cell lysate gel samples (50 μg/μL) in 1X PBS, were labeled with each individual probe, and incubated in the dark at 37 °C for 30 min.

Following probe incubation, lysates were transferred to a 96-well UV treated polystyrene microwell plates, and irradiated using a 15 W 365 nm lamp (with removal of cover lid), bacterial whole cell lysates (BTO:0004304) were exposed to longwave UV-irradiation (365 nm, 15W, 10 mm) using a UV benchtop lamp (Fisher Scientific, UVP Blak-Ray™) on ice for 7 min. Labeled proteome samples were treated with picolyl-azide rhodamine fluorescent reporter tag (30 μM in DMSO) followed by addition of freshly prepared sodium ascorbate (2.5 mM in H_2O), tris-hydroxypropyltriazolylmethylamine (1 mM THPTA in H_2O), and copper (II) sulfate (2 mM H_2O). Samples were vortexed and incubated in the dark at 37 °C for 1 h. Protein targets were separated (0.25 μg/μL maximum protein loading), using a 6-200 kDa size exclusion range, on precast Invitrogen Novex™ WedgeWell™ 10 to 20%, Tris-Glycine, 1.0 mm, Mini Protein Gel, 15-well (Thermo Fisher), using 1X SDS sample buffer and reducing agent, heated at 85 °C for 2 min, and loaded into precast polyacrylamide gels (~200V, 55 min). The gels were imaged for fluorescence activity using the Typhoon FLA 9500 (GE Healthcare)). After fluorescence imaging, gels were fixed in 50% MeOH and 7% acetic acid in MilliQ water for at least 30 min and then stained with SYPRO™ Ruby Protein Gel Stain reagent (Thermo Fisher) overnight. After destaining in MilliQ water, the gel was imaged using the Typhoon FLA 9500.

Activity-based Probe Enrichment for Mass Spectrometry Analysis[2]

Whole cell bacterial lysate samples were similarly prepared for liquid chromatography mass spectrometry proteome enrichment collections for protein labeled target identification (93 μg, in 450 μL 1XPBS). Bacterial samples were cultivated in technical and biological replication (4 technical replicates x 3 biological cellular stocks; replicate #4 represents pooled samples for each treatment). Probe-labeled samples were incubated with each probe in parallel (5 μM final probe concentration) across a suite of Xanthohumol activity-based probes (XN-ABP1 throμgh XN-ABP8) or an equivalent volume of DMSO for the no probe controls, for 1 h at 37 °C with agitation at 500 rpm. Samples being photoaffinity labeled were irradiated using a 15 W 365 nm lamp for 7 min on ice. After labeling, samples were treated with picoyl biotin-azide (30 μM, in DMSO), sodium ascorbate (2.5 mM, in H_2O), trishydroxypropyltriazolylmethylamine (THPTA, 1 mM, in H_2O), and copper (II) sulfate (2 mM, in H_2O). Samples were vortexed and incubated in the dark at 37 °C for 1.5 h. MeOH (800 μL, cold) was added to each sample, then placed in a -80 °C freezer for 18 h to induce protein precipitation. Samples were centrifuged at 14,000 × *g* at 4 °C for 5 min, and the supernatant was discarded. 520 μL of 1.2% SDS in PBS was added to each sample, vortexed to dissolve. Samples were then heated at 95 °C for 2 min, and probe sonicated for 6 s, 1 s pulses, 60% amplitude with a Sonic Dismembrator (Fisher Scientific). Samples were centrifuged at 14,000 × g for 5 min, and then transferred to fresh Eppendorf tube, leaving any insoluble protein behind. BCA was performed on the samples for normalization (final protein concentration of 0.180 mg/mL) prior to enrichment.

All washes were performed utilizing vacuum filtration. BioSpin Disposable Chromatography Columns (Bio-Rad Laboratories) were rinsed twice with 1 mL of 1x PBS. 100 μL of Streptavidin-agarose beads (Fisher Scientific) were added to each column (Thermo Fisher). The beads were rinsed twice with 1 mL of 0.5% SDS in 1x PBS, twice with 1 mL of 6 M Urea (prepared fresh in 25 mM NH_4HCO_3, pH 8), and four times in 1 mL of 1x PBS. Beads were transferred to 4 mL cryovials using two 1 mL aliquots of 1x PBS. Then 500 μL of normalized, biotin-labeled sample was added to the corresponding Eppendorf tubes and another 500 μL of 1x PBS was added following this. The cyrovials were rotated end over end for 1 h at 37 °C. Samples were added back onto BioSpin Disposable Chromatography columns. Cryovials were rinsed twice with 1 mL of 1x PBS and added to corresponding columns. Samples were then washed twice with 1 mL of 0.5 % SDS in 1x PBS, twice with 1 mL of 6 M Urea (prepared fresh in 25 mM NH_4HCO_3, pH 8), twice with 1 mL of MilliQ water, eight times with 1 mL of 1x PBS, and four times with 1 mL of 25 mM NH_4HCO_3 (pH 8). Samples were then transferred to DNA low-bind Eppendorf tubes (Fisher Scientific)

using 2 aliquots of 500 μL 1x PBS. Eppendorf tubes were centrifuged at 10,500 × g for 5 min at RT. The supernatant was discarded, and beads were re-suspended in 200 μL 25 mM NH_4HCO_3 (pH 8). Trypsin (0.125 μg) was added to each bead mixture followed by incubation overnight at 37 °C with 1200 rpm agitation. The following morning, samples were spun down at 10,500 × *g* for 5 min and supernatant transferred to individually wrapped Eppendorf tubes. The samples were placed on the vacuum concentrator (Savant SC110) until dry (~3 h). Samples were resuspended by adding 40 μL of 25 mM NH_4HCO_3 and heating samples at 37 °C for 5 min with agitation. 40 μL of sample was transferred to ultracentrifuge tubes (Beckman Coulter 5/16" × 1 3/8") and spun at 53,000 × g for 20 min. 25 μL of sample were then transferred to MS vial inserts and stored in glass MS vials at -20 °C until MS analysis was performed.

Mass Spectrometry and Data Analysis

Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) Data Acquisition

Mass spectrometry data acquisition (MS:1003213) for identification of peptide-level quantification data (MS:1002737) collections for both probe-labeled (ABP) and no probe-labeled (NP) datasets and were analyzed using a mass spectrometry capability instrument QExactive Plus (Thermo Scientific Instrument Model, MS:1000494) collection method (https://data.pnnl.gov/group/nodes/data-source/13385) using a standard 2-pump LC-MS cart configuration (Waters, nanoACQUITY) for trapping with reverse elute to analytical C18 columns (70 cm x 75 μm, i.d. 3 μm) and constant flow (formic acid) for a 60 min separation collection method in which data were acquired 65 min after sample injection (5 μg/μL) and 20 min into the LC gradient. Spectra were collected between *m*/*z* 300 to 2000 1,800 at 50K resolution of 100k, followed by data-dependent ion trap generation of tandem MS (MS/MS) spectra of the 20 most abundant ions using 35% collision energy and a dynamic exclusion time of 30 s.

MaxQuant Proteomic Data Analysis

Cheminformatic (EDAM topic_2258) mass spectrometry data analysis for identification of enzyme mimics (CHEBI:78152) activity-based probe (ABP) biotin enriched bacterial whole cell lysate dataset samples were analyzed independently using MaxQuant software (version 1.6.17.0) for feature detection and subsequent protein/peptide quantification (HMS-HCD-HMSn). Thermo Scientific RAW datasets files (MS:1000563) were grouped by probe-labeled treatment, and spectra were searched using a standard LCMS run type against *Eubacterium ramulus* ATCC 29099 chalcone isomerase (CHI/U2Q8X2_EUBRA; UniProt Proteome: UP000016608), component from an unassembled WGS sequence (UP000016608; downloaded on 2021-02-02), containing reviewed overexpression protein entry (U2Q8X2_EUBR) providing in sequence fasta (BL21_AI_wCI.fasta, https://massive.ucsd.edu/ProteoSAFe/DownloadResultFile?file=f.MSV000088789/sequence/BL21_AIwCI.fasta&forceDownload=true). N-Terminal protein acetylation and methionine oxidation were selected as variable modifications for all datasets. Peptides and proteins were processed using a maximum false discovery rate (FDR) of 0.01 (~1%). For increased peptide/protein (unique + razor) identification using small tissue sample collections, match between runs (MBR) was applied within an alignment time window of 20 min (3 min match window). Unique peptides (peptide fragment is unique to a single protein sequence in the proteome file) were used for continued analysis, requiring a minimum peptide length of 7 amino acids for matching to a protein. Additional MaxQuant parameters were ran at software default entries. Label-free quantification (iBAQ) relative intensity values were $\log_2$ transformed using the "proteinGroups.txt" output file and required unique peptide fragments to match to a single protein sequence in fasta file (protein count = 1).). For more 792 proteins were identified as being ≥ 2 replicate observations across all 32 ABP datasets within each probe group (4 subgroups for diazirine linker probes & 4 subgroups for alkyne linker probes). 732 proteins were identified as having a p value of 0.05 and a ≥1.5-fold change compared to the NP control prior to being evaluated for ABP statistical comparative analysis for structural probe affinity profiling. Protein intensities for each probe group additionally required peptide count observations for ≥50% of the biological replicates to be included for further qualitative analysis. peptide sequences and identified proteins associated with each ABP enriched sample were compiled and tabulated to generate a dataset (SI) detailing the degree to which each ABP and sample type enriched for a specific identified protein, sorted by iBAQ (Intensity Based Absolute Quantification) relative abundance values. Given the diverse suite of ABPs applied, probe-specific structural insights regarding how CHI or other proteins recognize XN and IXN can be determined.

Data Availability

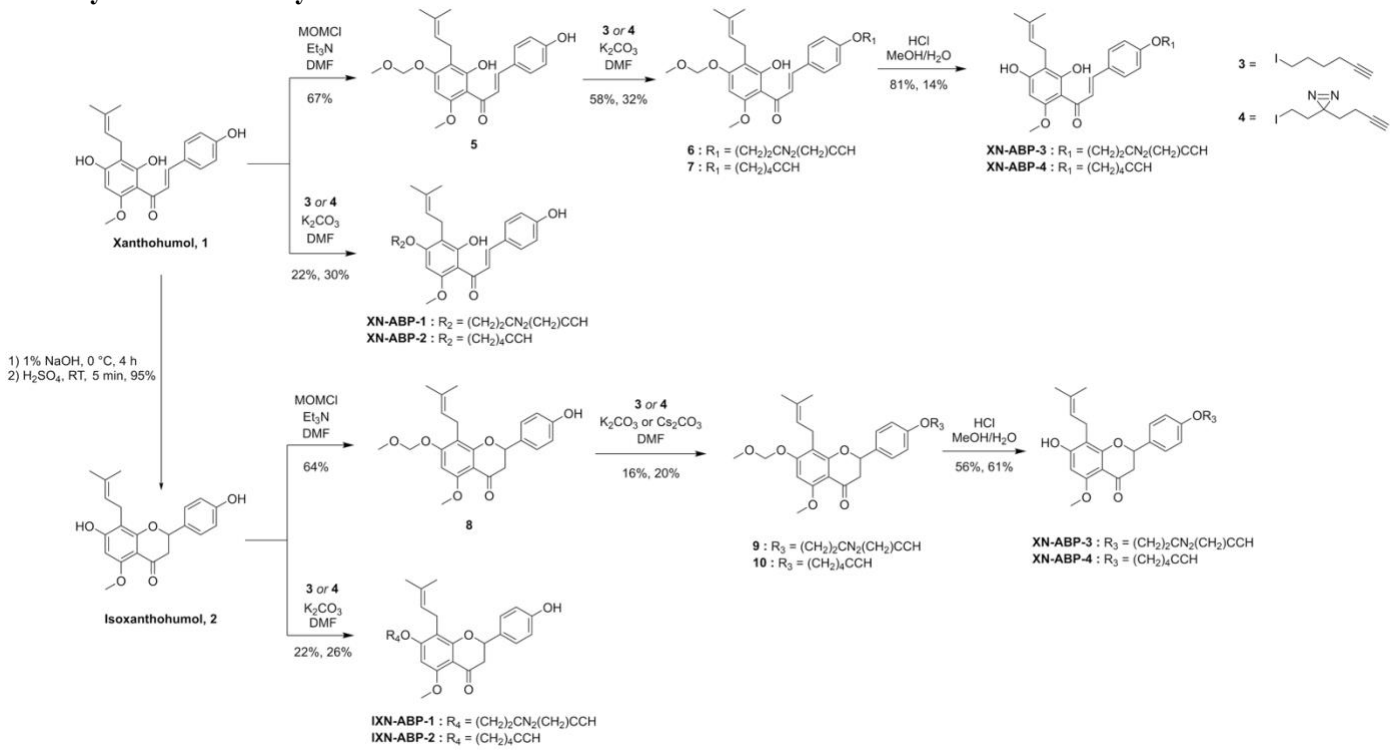
Proteomics was performed at Pacific Northwest National Laboratory (PNNL) (https://ror.org/05h992307). User facility data acquisition was performed under the EMSL project award 51663 (10.46936/reso.proj.2020.51663/60000245) at the Environmental Molecular Sciences Laboratory (https://ror.org/04rc0xn13), a DOE Office of Science User Facility sponsored by the Biological and Environmental Research program under Contract No. DE-AC05-76RL01830. The data underlying this reported study (are openly available (including data analysis parameters and summary filtration methods) at the MassIVE database under the DOI: 10.25345/C55295. For MassIVE database submission direct access, visit: https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?accession=MSV000088789; ftp://massive.ucsd.edu/MSV000088789/).

Dataset Naming Key

Probe Name	.RAW File Naming Schema
XN-ABP-1	CHI_DA_XN_9-12
XN-ABP-2	CHI_ALKY_XN_33-36
XN-ABP-3	CHI_XN_DA_25-28
XN-ABP-4	CHI_XN_ALKY_41-44
IXN-ABP-1	CHI_DA_IXN_17-20
IXN-ABP-2	CHI_ALKY_IXN_37-40
IXN-ABP-3	CHI_IXN_DA_29-32
IXN-ABP-4	CHI_IXN_ALKY_45-48
No Probe (No UV)	CHI_NP_NoUVCTR_5-8
No Probe (UV)	CHI_NP_UVCTR_1-4

(https://ror.org/00ysfqy60).

Activity-Based Probe Synthesis



Isoxanthohumol (2)

XN (100 mg, 0.282 mmol, 1 eq) was dissolved in 100 mL of aqueous NaOH (1%) to generate a clear orange solution, which was stirred at 0 °C for 4 h, then acidified to pH 3 using H_2SO_4 (50% in H_2O). The yellow precipitate was removed by vacuum filtration, and washed with minimal H_2O, before being dried overnight to yield IXN (**2**) as a yellow powder (95 mg, 95%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 7.39 (d, 2H, J = 8.5 Hz), 6.89 (d, 2H, J = 8.6 Hz), 6.21 (s, 1H), 5.35 (dd, 1H, J_1 = 12.5 Hz, J_2 = 3.1 Hz), 5.20 (tt, 1H, J_1 = 7.2 Hz, J_2 = 1.4 Hz), 3.73 (s, 3H), 3.26 (d, 2H, J = 7.2 Hz), 2.91 (dd, 1H, J_1 = 16.3 Hz, J_2 = 12.7 Hz), 2.61 (dd, 1H, J_1 = 16.5 Hz, J_2 = 3.0 Hz), 1.61 (s, 3H), 1.59 (s, 3H).

6-iodo-hexyne (4)

To an oven-dried 50 mL flask, PPh_3 (1.70 g, 6.63 mmol, 1.3 eq), imidazole (450 mg, 6.63 mmol, 1.3 eq), and a stir bar were added followed by DCM (anhydrous, 15 mL) and stirred for a few min to produce a clear and colorless solution. Then, iodine (1.67 g, 6.63 mmol, 1.3 eq) was added to produce a brownish-orange suspension, before 5-hexyn-1-ol (500 mg, 5.09 mmol, 1 eq) in DCM (10 mL) was slowly added dropwise. The reaction was stirred at RT for 90 min and the reaction progress was monitored by TLC. The whole reaction mixture was quenched with saturated $Na_2S_2O_3$ (30 mL). The organic layer was separated, and the aqueous solution was further extracted with DCM. The combined organic layers were wahsed with saturated NaCl (10 mL) and dried over anhydrous Na_2SO_4, and evaporated in vacuo to yield a white solid, which was subsequently purified using automated column chromatography (5% EtOAc/hexanes) to yield **4** as a clear oil (526 mg, 46%). Spectra matched literature references.[3]

***(E)-1-(4-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethoxy)-2-hydroxy-6-methoxy-3-(3methylbut-2-en-1-yl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one* (XN-ABP-1)**

To an oven-dried 10 mL RBF was added XN (50 mg, 0.141 mmol, 1 eq), K_2CO_3 (30 mg, 0.212 mmol, 1.5 eq), DMF (anhydrous, 3 mL), and a stir bar. To the deep orange mixture went **3** (40 mg, 25 μL, 0.162

mmol, 1.15 eq) in one portion via syringe. The reaction was stirred at RT for 24 h, then diluted with DCM (30 mL), washed with LiCl (aq, 5%, 3 x 50 mL) to remove DMF, and dried with NaCl (aq, saturated, 20 mL) and Na_2SO_4. The organic solution was purified by flash column chromatography (0 – 30% acetone/hexanes) to yield **XN-ABP-1** as a yellow solid (15 mg, 22%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 14.34 (s, 1H), 7.88 (d, 1H, J = 15.5 Hz), 7.76 (d, 1H, J = 15.6 Hz), 7.63 (d, 2H, J = 8.8 Hz), 6.92 (d, 2H, J = 8.8 Hz), 6.30 (s, 1H), 5.26 (tt, 1H, J_1 = 7.1 Hz, J_2 = 1.4 Hz), 4.08 (t, 2H, J = 6.3 Hz), 4.03 (s, 3H), 3.34 (d, 2H, J = 7.6 Hz), 2.41 (t, 1H, J = 2.7 Hz), 2.08 (td, 2H, J_1 = 7.5 Hz, J_2 = 2.7 Hz), 2.01 (t, 2H, J = 6.3 Hz), 1.78 (s, 3H), 1.76 (t, 2H, J = 7.5 Hz), 1.64 (s, 3H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 193.8, 164.9, 163.3, 162.4, 160.7, 143.3, 131.3, 131.3, 131.0, 128.0, 125.4, 124.0, 116.8, 116.8, 110.3, 107.0, 88.6, 83.5, 70.7, 64.0, 56.5, 33.4, 33.1, 27.6, 25.9, 22.1, 18.0, 13.6.

(E)-1-(4-(hex-5-yn-1-yloxy)-2-hydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3(4--hydroxyphenyl)prop-2-en-1-one **(XN-ABP-2)**

To an oven dried 25 mL round-bottomed flask was added XN (100 mg, 0.282 mmol, 1 eq), K_2CO_3 (43 mg, 0.310 mmol, 1.1 eq), DMF (anhydrous, 10 mL), and a stir bar. Reaction was stirred to produce a deep orange solution. **4** (69 mg, 0.310 mmol, 1.1 eq) in DMF (anhydrous, 1 mL) was slowly added via syringe. The orange solution was stirred at RT overnight, at which point the reaction was diluted with EtOAc (50 mL) and H_2O (30 mL). The organic was separated, and the aqueous layer was acidified to pH 4 with 1 M HCl, and extracted with EtOAc (3 x 20 mL). The organic extracts were combined and residual DMF was removed with LiCl (5% aq, 3 x 25 mL). The organic extract was then washed with saturated NaCl (20 mL), dried over Na_2SO_4, and evaporated *in vacuo*, to yield the crude product, which was purified using automated silica column chromatography (0-100% acetone/hexanes) to yield **XN-ABP-2** as a yellow solid (37 mg, 30%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 14.36 (s, 1H), 8.89 (s, 1H), 7.88 (d, 2H, J = 15.5 Hz), 7.75 (d, 2H, J = 15.5 Hz), 7.61 (d, 2H, J = 8.7 Hz), 6.92 (d, 2H, J = 8.6 Hz), 6.29 (s, 1H), 5.20 (tt, 1H, J_1 = 7.2 Hz, J_2 = 1.3 Hz), 4.20 (t, 2H, J = 6.4 Hz), 4.03 (s, 3H), 3.28 (d, 2H, J = 7.4 Hz), 2.37 (t, 1H, J = 2.6 Hz), 2.29 (td, 2H, J_1 = 7.0 Hz, J_2 = 2.7 Hz), 1.95 (m, 2H), 1.76 (s, 3H), 1.74 (m, 2H), 1.63 (s, 3H); **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 192.8, 164.0, 162.9, 161.6, 159.8, 142.4, 130.4, 130.4, 129.9, 127.2, 124.5, 123.2, 115.9, 109.2, 105.9, 87.8, 83.7, 69.3, 67.6, 55.5, 25.1, 25.0, 21.2, 17.6, 17.1.

Protection of XN with chloromethyl methyl ether (5)

To an oven-dried 10 mL round bottomed flask was added XN (50 mg, 0.141 mmol, 1 eq), MOMCl (17 mg, 16 μL, 0.21 mmol, 1.5 eq), triethylamine (28 mg, 38 μL, 0.28 mmol, 2 eq), DMF (anhydrous, 3 mL), and a stir bar. The clear orange solution was stirred on ice at 0 ºC for 30 min, warmed to RT, then stirred overnight. After the reaction was complete, the solution was diluted with EtOAc (30 mL), and H_2O (20 mL), and the organic layer was separated. The aqueous layer was acidified to pH 3 with 1 M HCl, and extracted with EtOAc (3 x 20 mL). The organic extracts were combined and washed with acidified (pH 4) LiCl (aqueous, 5%, 3 x 20 mL), saturated NaCl (10 mL), dried over Na_2SO_4, and evaporated *in vacuo* to yield a yellow residue. The crude product was purified by automated flash silica column chromatography (0100% acetone/hexanes) to yield **5** as a yellow solid (38 mg, 67%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 14.33 (br s, 1H), 7.88 (dd, 1H, J = 15.6 Hz), 7.62 (d, 2H, J = 8.9 Hz), 6.92 (d, 2H, J = 8.6 Hz), 6.39 (s, 1H), 5.36 (s, 2H), 5.21 (tt, 1H, J_1 = 7.3 Hz, J_2 = 1.5 Hz), 3.99 (s, 3H), 3.47 (s, 3H), 3.30 (br d, 2H, J = 7.4 Hz), 1.77 (s, 3H), 1.63 (s, 3H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 193.0, 164.1, 161.1, 161.0, 159.8, 142.7, 130.5, 130.5, 130.2, 127.1, 124.4, 122.9, 115.9, 115.9, 110.1, 106.5, 93.9, 89.4, 55.6, 55.5, 25.0, 21.3, 17.0.

7-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethoxy)-2-(4-hydroxyphenyl)-5-methoxy-8-(3methylbut-2-en-1-yl)chroman-4-one **(IXN-ABP-1)**

To an oven-dried 10 mL RBF was added IXN (50 mg, 0.141 mmol, 1 eq), K_2CO_3 (30 mg, 0.212 mmol, 1.5 eq), DMF (anhydrous, 3 mL), and a stir bar. To the yellow mixture went **3** (40 mg, 25 μL, 0.162 mmol, 1.15 eq) in one portion via syringe. The reaction was stirred at RT for 24 h, then diluted with DCM (30 mL), washed with LiCl (aq, 5%, 3 x 50 mL) to remove DMF, and dried with NaCl (aq, saturated, 20 mL) and Na_2SO_4. The organic solution was dried *in vacuo,* and the crude product was purified by flash column chromatography (0 – 100% acetone/hexanes) to yield **IXN-ABP-1** as a white solid (15 mg, 22%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 8.46 (br s, 1H), 7.39 (d, 2H, J = 9.0 Hz), 6.89 (d, 2H, J = 8.9 Hz), 6.33 (s, 1H), 5.37 (dd, 1H, J_1 = 12.5 Hz, J_2 = 3.1 Hz), 5.22 (tt, 1H, J_1 = 7.1 Hz, J_2 = 1.4 Hz), 4.05 (t, 2H, J = 6.3 Hz), 3.84 (s, 3H), 3.32 (d, 2H, J = 6.8 Hz), 2.94 (dd, 1H, J_1 = 16.4 Hz, J_2 = 12.4 Hz), 2.64 (dd, 1H, J_1 = 16.2 Hz, J_2 = 3.1 Hz), 2.39 (t, 1H, J = 2.7 Hz), 2.07 (td, 2H, J_1 = 7.6 Hz, J_2 = 2.6 Hz), 1.99 (t, 2H, J = 6.1 Hz), 1.75 (t, 2H, J = 7.5 Hz), 1.62 (s, 3H), 1.61 (s, 3H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 188.8, 162.7, 161.9, 161.5, 158.4, 131.5, 131.1, 128.7, 128.7, 123.9, 116.1, 116.1, 110.4, 107.0, 90.8, 83.5, 79.6, 70.7, 64.0, 56.2, 46.3, 33.4, 33.1, 27.5, 25.9, 22.6, 18.1, 13.6.

7-(hex-5-yn-1-yloxy)-2-(4-hydroxyphenyl)-5-methoxy-8-(3-methylbut-2-en-1yl)chroman-4-one **(IXN-ABP-2)**

To an oven-dried 25 mL round bottomed flask was added IXN (100 mg, 0.282 mmol, 1 eq), K_2CO_3 (46 mg, 0.336 mmol, 1.2 eq), **4** (69 mg, 0.310 mmol, 1.1 eq), DMF (anhydrous, 10 mL), and a stir bar. The deep orange solution was stirred at RT overnight. After the reaction was complete, EtOAc (50 mL) and H_2O (30 mL) were added, and the organic layer was separated. The aqueous layer was acidified to pH 3 with 1 M HCl, and extracted with EtOAc (3 x 20 mL). The organic extracts were combined and washed with acidified (pH 4) LiCl (aqueous, 5%, 3 x 20 mL), saturated NaCl (10 mL), dried over Na_2SO_4, and evaporated *in vacuo* to yield a yellow oil. The crude product was purified by automated flash silica column chromatography (0100% acetone/hexanes) to yield **IXN-ABP-2** as an off white powder (32 mg, 26%). ^{1}H NMR (Acetoned_6; 500 MHz) δ 8.46 (s, 1H), 7.38 (d, 2H, J = 8.5 Hz), 6.89 (d, 2H, J = 8.5 Hz), 6.35 (s, 1H), 5.36 (dd, 1H, J_1 = 12.5 Hz, J_2 = 3.0 Hz), 5.16 (tt, J_1 = 7.2 Hz, J_2 = 1.3 Hz), 4.17 (t, 2H, J = 6.3 Hz), 3.84 (s, 3H), 3.26 (d, 2H, J = 7.3 Hz), 2.94 (dd, 1H, J_1 = 8.1 Hz, J_2 = 12.6 Hz), 2.63 (dd, 1H, J_1 = 16.5 Hz, J_2 = 2.9 Hz), 2.36 (t, 1H, J = 2.7 Hz), 2.28 (td, 2H, J_1 = 7.1 Hz, J_2 = 2.7 Hz), 1.94 (m, 2H), 1.73 (m, 2H), 1.62 (s, 3H), 1.61 (s, 3H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 188.8, 163.2, 161.8, 161.6, 158.4, 131.6, 131.0, 128.7, 128.7, 123.9, 116.1, 116.1, 110.3, 106.7, 90.9, 84.6, 79.5, 70.2, 68.5, 56.2, 46.4, 29.0, 26.0, 25.9, 22.6, 18.5, 18.0.

(E)-3-(4-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethoxy)phenyl)-1-(2-hydroxy-6-methoxy4-(methoxymethoxy)-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (6)

To an oven-dried 10 mL RBF was added **5** (35 mg, 0.088 mmol, 1 eq), K_2CO_3 (25 mg, 0.18 mmol, 2 eq), DMF (anhydrous, 3 mL), and a stir bar. The deep orange solution was placed on ice and stirred at 0 °C for 5 min, before **3** (33.5 mg, 21 μL, 0.135 mmol, 1.5 eq) was added in one portion via syringe. The solution was stirred at 0 °C for 30 min, then RT for 18 h. After the reaction was complete, the reaction mixture was diluted with DCM (30 mL), washed with LiCl (aq, 5%, 3 x 50 mL) to remove DMF, and dried with NaCl (aq, saturated, 20 mL) and Na_2SO_4. The organic solution was dried *in vacuo,* and the crude product was purified by flash column chromatography (0 – 50% acetone/hexanes) to yield **6** as a yellow solid (27.1 mg, 58%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 14.28 (s, 1H), 7.90 (m, 1H, J_1 = 15.5 Hz, J_2 = 5.6 Hz), 7.77 (m, 1H, J_1 = 15.6 Hz, J_2 = 3.3 Hz), 7.70 (d, 2H, J = 8.8 Hz), 7.03 (d, 2H, J = 8.9 Hz), 6.39 (s, 1H), 5.37 (s, 2H), 5.21 (tt, 1H, J_1 = 7.2 Hz, J_2 = 1.5 Hz), 4.00 (t, 2H, J = 6.6 Hz), 3.99 (s, 3H), 3.48 (s, 3H), 3.29 (d, 2H, J = 7.3 Hz), 2.40 (t, 1H, J = 2.7 Hz), 2.10 (td, 2H, J_1 = 7.5 Hz, J_2 = 2.6 Hz), 1.95 (t, 2H, J = 6.1 Hz), 1.77 (s, 3H), 1.74 (t, 2H, J = 7.5 Hz), 1.63 (s, 3H). **^{13}C NMR** (Acetone-d_6,

125 MHz): δ 193.9, 165.0, 162.1, 162.0, 161.5, 143.1, 131.1, 131.1, 129.3, 126.3, 126.2, 123.8, 115.9, 115.9, 111.0, 107.4, 94.8, 90.3, 83.6, 70.6, 63.7, 56.5, 56.4, 33.3, 27.6, 25.9, 22.2, 17.9, 13.6.

(E)-3-(4-(hex-5-yn-1-yloxy)phenyl)-1-(2-hydroxy-6-methoxy-4-(methoxymethoxy)-3-(3methylbut-2-en-1-yl)phenyl)prop-2-en-1-one (7)

To an oven-dried 10 mL round-bottomed flask was added **5** (35 mg, 0.088 mmol, 1 eq), K_2CO_3 (25 mg, 0.18 mmol, 2 eq), DMF (anhydrous, 2.5 mL), and a stir bar. To the clear orange solution was added **4** (28 mg, 17.5 μL, 0.135 mmol, 1.5 eq) via syringe. After the reaction was complete, DCM (30 mL) was added, and the organic layer was separated. The organic extract was washed with acidified (pH 4) LiCl (aqueous, 5%, 3 x 20 mL), saturated NaCl (10 mL), dried over Na_2SO_4, and evaporated *in vacuo* to yield a yellow residue. The crude product was purified by automated flash silica column chromatography (0-50% acetone/hexanes) to yield **7** as a yellow solid (13.6 mg, 32%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 14.31 (s, 1H), 7.90 (dd, 1H, J_1 = 15.8 Hz, J_2 = 5.8 Hz), 7.77 (dd, 1H, J_1 = 15.5 Hz, J_2 = 3.0 Hz), 7.69 (d, 2H, J = 8.7 Hz), 7.01 (d, 2H, J = 8.8 Hz), 6.41 (s, 1H), 5.39 (s, 3H), 5.21 (br tt, 1H, J_1 = 7.2 Hz, J_2 = 1.2 Hz), 4.13 (t, 2H, J = 6.3 Hz), 4.00 (s, 3H), 3.48 (s, 3H), 3.30 (d, 2H, J = 7.1 Hz), 2.36 (t, 1H, J = 2.6 Hz), 2.29 (td, 2H, J_1 = 7.1 Hz, J_2 = 2.6 Hz), 1.92 (m, 2H), 1.77 (s, 3H), 1.71 (m, 2H), 1.63 (s, 3H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 193.9, 165.0. 162.1, 162.0, 162.0, 143.2, 131. 5, 131.5, 128.9, 126.0, 123.8, 115.9, 115.9, 111.0, 107.4, 94.8, 90.3, 84.6, 70.1, 68.3, 56.5, 56.4, 28.9, 25.9, 25.9, 22.2, 18.5, 17.8.

***(E)-3-(4-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethoxy)phenyl)-1-(2,4-dihydroxy-6methoxy-3-(3-methylbut-2-en-1-yl)phenyl)prop-2-en-1-one* (XN-ABP-3)**

To a 20 mL scintillation vial was added **6** (13 mg, 0.025 mmol, 1 eq), MeOH (12 mL), H_2O (1 mL), and a stir bar. The yellow solution was warmed to 40 ºC, then HCl (12 drops, 12 M) was added dropwise with stirring. The solution was stirred in the dark for 18 h at RT. Once the reaction was complete, the solvent was removed *in vacuo*, taken up in DCM (20 mL), washed with $NaHCO_3$ (saturated, 25 mL), brine, dried over Na_2SO_4, and then purified using automated silica column chromatography (0 – 50% acetone/hexanes) to yield **XN-ABP-3** as a yellow solid (9.6 mg, 81%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 14.62 (s, 1H), 7.91 (m, 1H, J_1 = 15.6 Hz, J_2 = 5.3 Hz), 7.75 (m, 1H, J_1 = 15.5 Hz, J_2 = 3.6 Hz), 7.69 (d, 2H, J = 8.8 Hz), 7.02 (d, 2H, J = 8.9 Hz), 6.14 (s, 1H), 5.24 (tt, 1H, J_1 = 7.2 Hz, J_2 = 1.5 Hz), 3.99 (t, 2H, J = 6.2 Hz), 3.92 (s, 3H), 3.28 (d, 2H, J = 7.1 Hz), 2.40 (t, 1H, J = 2.7 Hz), 2.10 (td, 2H, J_1 = 7.5 Hz, J_2 = 2.6 Hz), 1.94 (t, 2H, J = 6.1 Hz), 1.76 (s, 3H), 1.73 (t, 2H, J = 7.4 Hz), 1.63 (s, 3H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 193.3, 166.4, 162.9, 162.0, 161.4, 142.5, 131.0, 131.0, 129.4, 126.5, 126.4, 123.9, 115.9, 115.9, 108.9, 106.3, 91.7, 83.6, 70.6, 63.7, 56.2, 33.3, 33.3, 27.5, 25.9, 22.0, 17.9, 13.6

***(E)-1-(2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-en-1-yl)phenyl)-3-(4-(hex-5-yn-1yloxy)phenyl)prop-2-en-1-one* (XN-ABP-4)**

To a 20 mL scintillation vial was added **7** (13.6 mg, 0.0285 mmol, 1 eq), MeOH (10 mL), and a stir bar. The clear yellow solution was warmed at 40 ºC for 10 min, before 6 drops of 12 M HCl were added. The reaction mixture was stirred at 35 ºC for 15 min, then for 18 h at RT. Once complete, the crude product was removed *in vacuo*, redissolved in DCM (25 mL), washed with $NaHCO_3$ (saturated, 25 mL), brine, dried over Na_2SO_4, and the purified using automated silica column chromatography (0 – 50% EtOAc/hexanes) to yield **XN-ABP-4** as a yellow solid (1.7 mg, 14%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 14.64 (s, 1H), 7.91 (d, 2H, J = 15.6 Hz), 7.74 (d, 1H, J = 15.8 Hz), 7.67 (d, 2H, J = 8.8 Hz), 7.01 (d, 2H, J = 8.9 Hz), 6.14 (s, 1H), 5.24 (tt, 1H, J_1 = 7.2 Hz, J_2 = 1.4 Hz), 4.11 (t, 2H, J = 6.5 Hz), 3.92 (s, 3H), 3.28 (d, 2H, J = 7.2 Hz), 2.36 (t, 1H, J = 2.6 Hz), 2.28 (td, 2H, J_1 = 7.1 Hz, J_2 = 2.6 Hz), 1.92 (m, 2H), 1.75 (s, 3H), 1.71 (m, 2H), 1.63 (s, 3H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 193.2, 166.4, 163.0, 162.0, 161.9, 142.7, 131.0, 131.0, 129.0, 126.1, 124.0, 115.8, 115.8, 108.9, 106.3, 91.7, 84.6, 70.2, 68.3, 56.2, 29.0, 25.9, 25.9, 22.1, 18.5, 17.9.

Protection of isoxanthohumol with chloromethyl methyl ether (8)

To an oven-dried 10 mL round bottomed flask was added IXN (95 mg, 0.254 mmol, 1 eq), MOMCl (32 mg, 30 μL, 0.38 mmol, 1.5 eq), DIPEA (65 mg, 88 μL, 0.51 mmol, 2 eq), DMF (anhydrous, 5 mL), and a stir bar. The slightly yellow solution was stirred on ice at 0 ºC for 30 min, warmed to RT, then stirred overnight. After the reaction was complete, the solution was diluted with DCM (30 mL), and H_2O (20 mL), and the organic layer was separated. The aqueous layer was acidified to pH with 1 M HCl and extracted with DCM (3 x 20 mL). The organic extracts were combined and washed with acidified (pH 4) LiCl (aqueous, 5%, 3 x 20 mL), saturated NaCl (10 mL), dried over Na_2SO_4, and evaporated *in vacuo* to yield a yellow residue. The crude product was purified by automated flash silica column chromatography (0-100% acetone/hexanes) to yield **8** as a white solid (63 mg, 64%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 8.47 (br s, 1H), 7.96 (s, 1H), 7.39 (d, 2H, J = 8.9 Hz), 6.89 (d, 2H, J = 8.5 Hz), 6.45 (s, 1H), 5.38 (dd, 2H, J_1 = 12.6 Hz, J_2 = 2.8 Hz), 5.34 (s, 2H), 5.17 (tt, 1H, J_1 = 7.2 Hz, J_2 = 1.3 Hz), 3.81 (s, 3H), 3.46 (s, 3H), 3.28 (br d, 2H, J = 7.3 Hz), 2.95 (dd, 1H, J_1 = 8.1 Hz, J_2 = 12.6 Hz), 2.65 (dd, 1H, J_1 = 16.4 Hz, J_2 = 2.4 Hz), 1.62 (s, 3H), 1.60 (s, 3H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 188.9, 162.0, 161.3, 161.2, 158.5, 131.4, 131.3, 128.7, 128.7, 123.7, 116.1, 116.0, 111.2, 107.4, 94.9, 92.6, 79.6, 56.5, 56.1, 46.3, 25.9, 22.7, 17.9.

2-(4-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethoxy)phenyl)-5-methoxy-7(methoxymethoxy)-8-(3-methylbut-2-en-1-yl)chroman-4-one (9)

To an oven-dried 20 mL scintillation vial was added **8** (76 mg, 0.19 mmol, 1 eq), K_2CO_3 (100 mg, 0.72 mmol, 3.8 eq), DMF (anhydrous, 4 mL), and a stir bar. The slightly yellow mixture was stirred at 0 ºC for 5 min, and **3** (74 mg, 46 μL, 0.3 mmol, 1.6 eq) was added via syringe in two portions at 0 ºC. The reaction was closely monitored by TLC, and after 6 h was stopped to prevent reversion to the chalcone by addition of 30 mL of DCM, washed with LiCl (aq, 5%, 3 x 50 mL) to remove DMF, and dried with NaCl (aq, saturated, 20 mL) and Na_2SO_4. The organic solution was dried *in vacuo,* and the crude product was purified by flash column chromatography (0 – 75% acetone/hexanes) to yield **9** as an off white solid (16.2 mg, 16%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 7.49 (d, 2H, J = 8.8 Hz), 7.00 (d, 2H, J = 8.9 Hz), 6.46 (s, 1H), 5.43 (dd, 1H, J_1 = 12.5 Hz, J_2 = 2.9 Hz), 5.34 (s, 2H), 5.17 (tt, 1H, J_1 = 7.2 Hz, J_2 = 1.5 Hz), 3.95 (t, 2H, J = 6.1 Hz), 3.81 (s, 3H), 3.47 (s, 3H), 3.28 (d, 2H, J = 7.2 Hz), 2.96 (dd, 1H, J_1 = 16.5 Hz, J_2 = 12.5 Hz), 2.67 (dd, 1H, J_1 = 16.7 Hz, J_2 = 3.1 Hz), 2.39 (t, 1H, J = 2.7 Hz), 2.10 (td, 2H, J_1 = 7.4 Hz, J_2 = 2.6 Hz), 1.93 (t, 2H, J = 6.4 Hz), 1.73 (t, 2H, J = 7.5 Hz), 1.63 (s, 3H), 1.61 (s, 3H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 188.7, 162.0, 161.3, 161.2, 159.6, 132.9, 131.3, 128.6, 128.6, 123.7, 115.3, 115.3, 111.2, 107.4, 95.0, 92.6, 83.6, 79.4, 70.6, 63.5, 56.5, 56.1, 46.3, 33.4, 27.6, 25.9, 22.7, 19.3, 17.9, 13.6.

2-(4-(hex-5-yn-1-yloxy)phenyl)-5-methoxy-7-(methoxymethoxy)-8-(3-methylbut-2-en-1yl)chroman-4-one (10)

To a 20 mL scintillation vial was added **8** (35 mg, 0.088 mmol, 1 eq), **4** (73 mg, 46 μL, 0.352 mmol, 4 eq), DMF (anhydrous, 3 mL), and a stir bar. The clear and colorless solution was placed on ice to cool for 5 min, before Cs_2CO_3 (28 mg, 0.088 mmol, 1 eq) was added. The deep orange solution was closely monitored by TLC, and after 16 h was stopped to prevent reversion to the chalcone by addition of 30 mL of DCM, washed with LiCl (aq, 5%, 3 x 50 mL) to remove DMF, and dried with NaCl (aq, saturated, 20 mL) and Na_2SO_4. The organic solution was purified by flash column chromatography (0-100% EtOAc/hexanes) to yield **10** as a white solid (8.5 mg, 20%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 7.47 (d, 2H, J = 8.5 Hz), 6.99 (d, 2H, J = 8.5 Hz), 6.45 (s, 1H), 5.42 (dd, 1H, J_1 = 12.5 Hz, J_2 = 2.9 Hz), 5.34 (s, 2H), 5.17 (tt, 1H, J_1 = 7.3 Hz, J_2 = 1.4 Hz), 4.06 (t, 2H, J = 6.4 Hz), 3.81 (s, 3H), 3.46 (s, 3H), 3.28 (d, 2H, J = 7.7 Hz), 2.96 (dd, 1H, J_1 = 16.4 Hz, J_2 = 12.6 Hz), 2.67 (dd, 1H, J_1 = 16.3 Hz, J_2 = 2.3 Hz), 2.35 (t, 1H, J = 2.7 Hz), 2.28 (td, 2H, J_1 = 7.1 Hz, J_2 = 2.6 Hz), 1.90 (m, 2H), 1.71 (m, 2H), 1.62 (s, 3H), 1.61

(s, 3H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 188.7, 162.0, 161.3, 161.2, 160.1, 132.6, 131.3, 128.7, 128.7, 123.7, 115.3, 115.3, 111.3, 107.7, 95.0, 92.6, 84.7, 79.5, 70.1, 68.1, 56.5, 56.1, 46.3, 29.1, 26.0, 25.9, 22.7, 18.5, 17.9.

2-(4-(2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethoxy)phenyl)-7-hydroxy-5-methoxy-8-(3methylbut-2-en-1-yl)chroman-4-one **(IXN-ABP-3)**

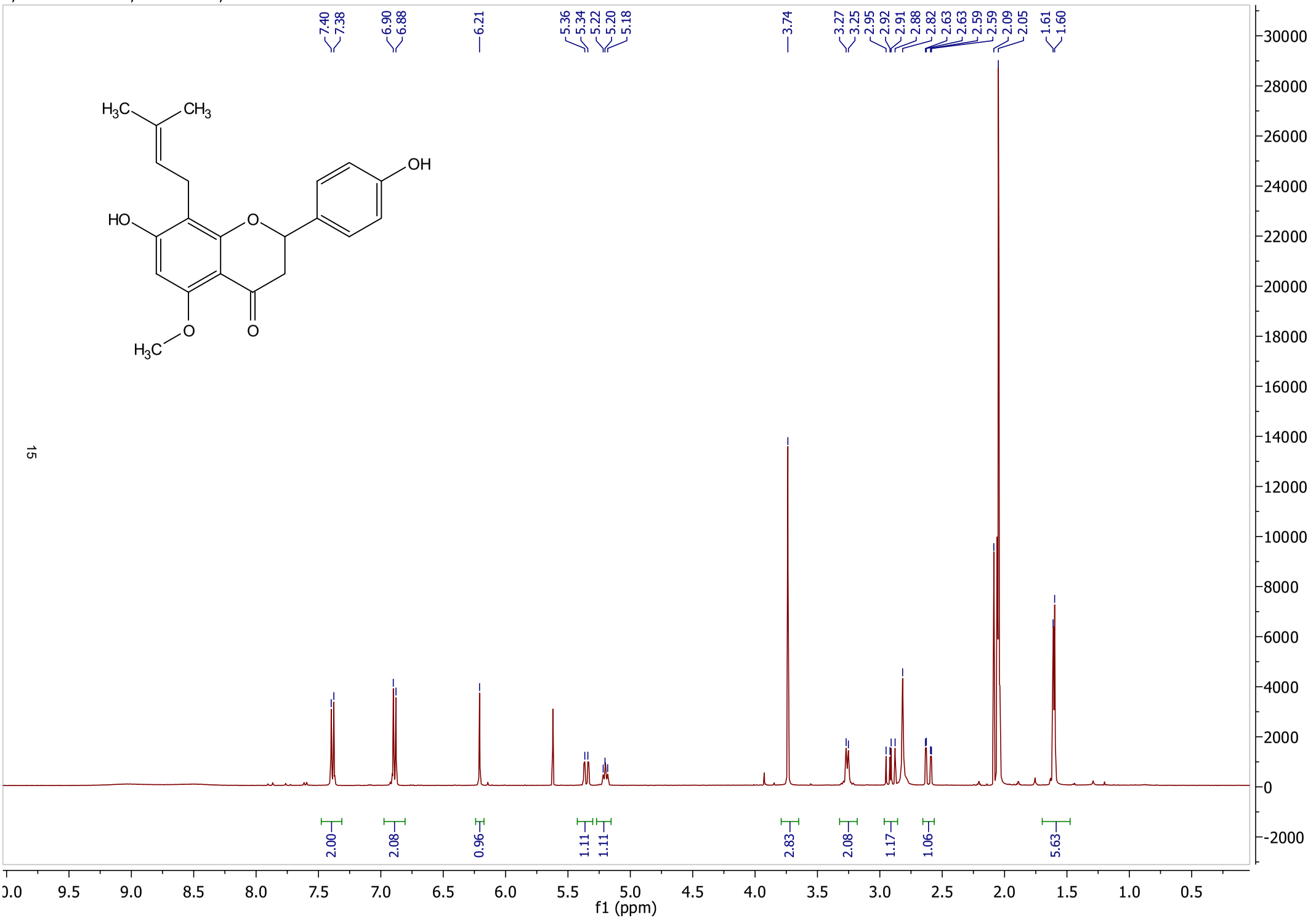
To a 20 mL scintillation vial was added **9** (13 mg, 0.025 mmol, 1 eq), MeOH (10 mL), and a stir bar. The clear solution was warmed to 40 ºC, then HCl (20 drops, 12 M) was added dropwise with stirring. The solution was stirred in the dark for 18 h at RT. Once the reaction was complete, the solvent was removed *in vacuo*, taken up in DCM (20 mL), washed with brine, dried over Na_2SO_4, and then purified using automated silica column chromatography (0 – 100% acetone/hexanes) to yield **IXN-ABP-4** as an off white solid (6.6 mg, 56%). **^{1}H NMR** (Acetone-d_6; 500 MHz) δ 9.16 (br s, 1H), 7.48 (d, 2H, J = 8.7 Hz), 7.00 (d, 2H, J = 8.8 Hz), 6.22 (s, 1H), 5.40 (dd, 1H, J_1 = 12.6 Hz, J_2 = 3.0 Hz), 5.21 (tt, 1H, J_1 = 7.3 Hz, J_2 = 1.3 Hz), 3.95 (t, 2H, J = 6.1 Hz), 3.73 (s, 3H), 3.27 (d, 2H, J = 7.1 Hz), 2.91 (dd, 1H, J_1 = 16.3 Hz, J_2 = 12.5 Hz), 2.64 (dd, 1H, J_1 = 16.3 Hz, J_2 = 3.1 Hz), 2.39 (t, 1H, J = 2.7 Hz), 2.10 (td, 2H, J_1 = 7.5 Hz, J_2 = 2.5 Hz), 1.93 (t, 2H, J = 6.1 Hz), 1.74 (t, 2H, J = 7.4 Hz), 1.61 (s, 6H). **^{13}C NMR** (Acetone-d_6, 125 MHz): δ 188.4, 162.8, 162.0, 161.1, 159.6, 133.1, 131.1, 128.7, 128.7, 123.9, 115.4, 115.4, 109.4, 106.3, 93.8, 83.6, 79.3, 70.6, 63.5, 55.9, 46.2, 33.4, 33.3, 27.6, 25.9, 22.5, 17.9, 13.6.

2-(4-(hex-5-yn-1-yloxy)phenyl)-7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1yl)chroman-4-one **(IXN-ABP-4)**

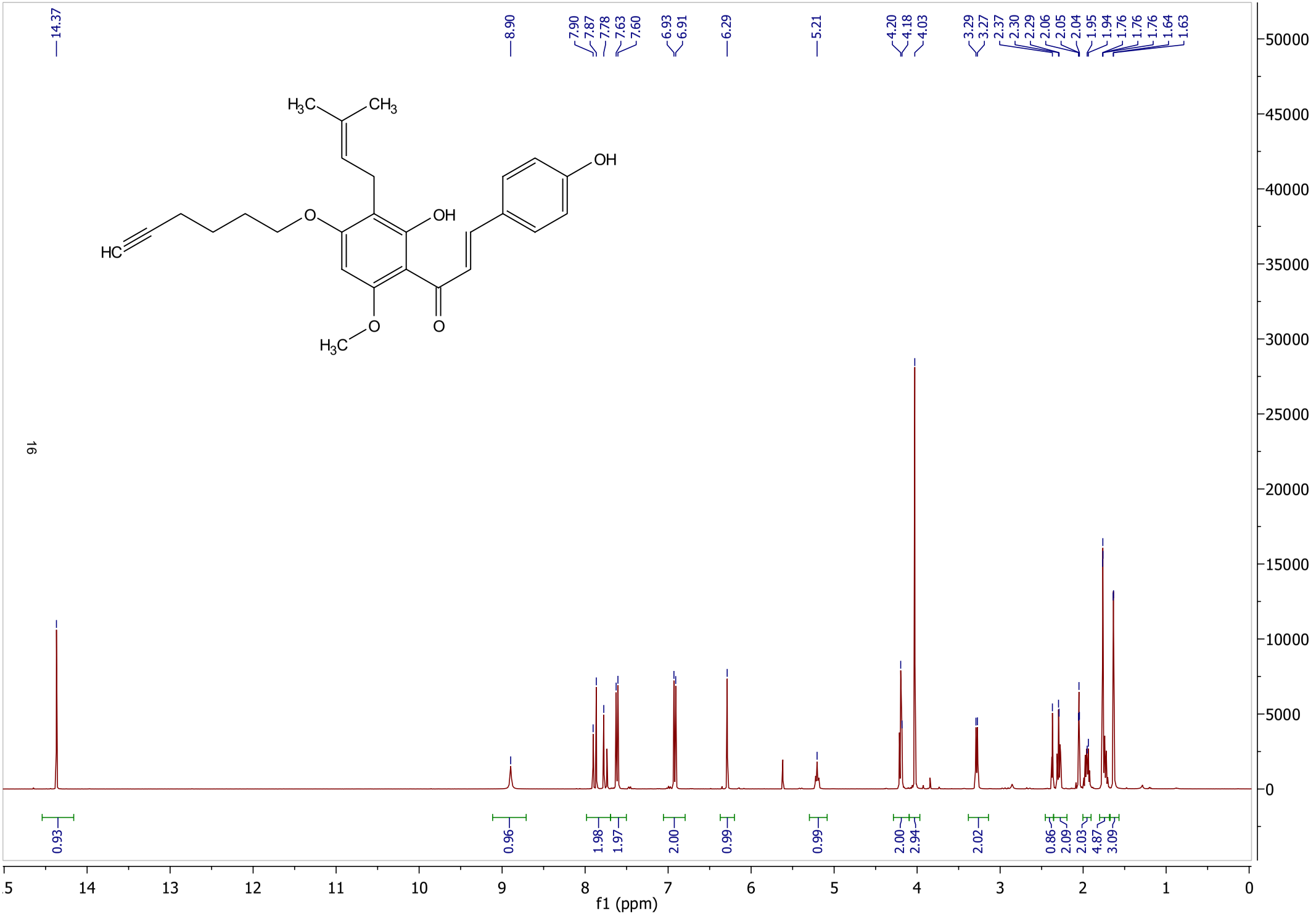
To a 20 mL scintillation vial was added **10** (12.5 mg, 0.026 mmol, 1 eq), MeOH (10 mL), H_2O (1 mL), and a stir bar. To the clear solution then went HCl (12 M, 0.5 mL) dropwise with stirring. The reaction mixture reacted at RT overnight. Once complete, the solvent was removed, and the yellowish residue was purified by column chromatography (0-100% EtOAc/hexanes) to yield **IXN-ABP-4** as a white solid (6.9 mg, 61%). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.35 (d, 2H, J = 8.7 Hz), 6.92 (d, 2H, J = 8.9 Hz), 6.09 (s, 1H), 5.33 (dd, 1H, J_1 = 13.0 Hz, J_2 = 2.9 Hz), 5.24 (tt, 1H, J_1 = 7.2 Hz, J_2 = 1.4 Hz), 4.00 (t, 2H, J = 6.1 Hz), 3.85 (s, 3H), 3.35 (d, 2H, J = 7.5 Hz), 2.99 (dd, 1H, J_1 = 16.3 Hz, J_2 = 13.3 Hz), 2.79 (dd, 1H, J_1 = 16.5 Hz, J_2 = 3.0 Hz), 2.28 (td, 2H, J_1 = 7.0 Hz, J_2 = 2.8 Hz), 1.97 (t, 1H, 2.7 Hz), 1.91 (m, 2H), 1.74 (br s, 6H), 1.71 (m, 2H). **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 190.4, 161.9, 161.8, 160.9. 159.3, 135.6, 131.2, 127.6, 127.6, 121.7, 114.7, 114.7, 106.8, 106.0, 93.5, 84.2, 78.8, 68.8, 67.5, 56.2, 45.5, 28.4, 26.0, 25.1, 22.3, 18.3, 18.0.

NMR Structural Validation

2, isoxanthohumol, Acetone-d6, 500 MHz



5, Alkyne-XN, Acetone-d6, 500 MHz



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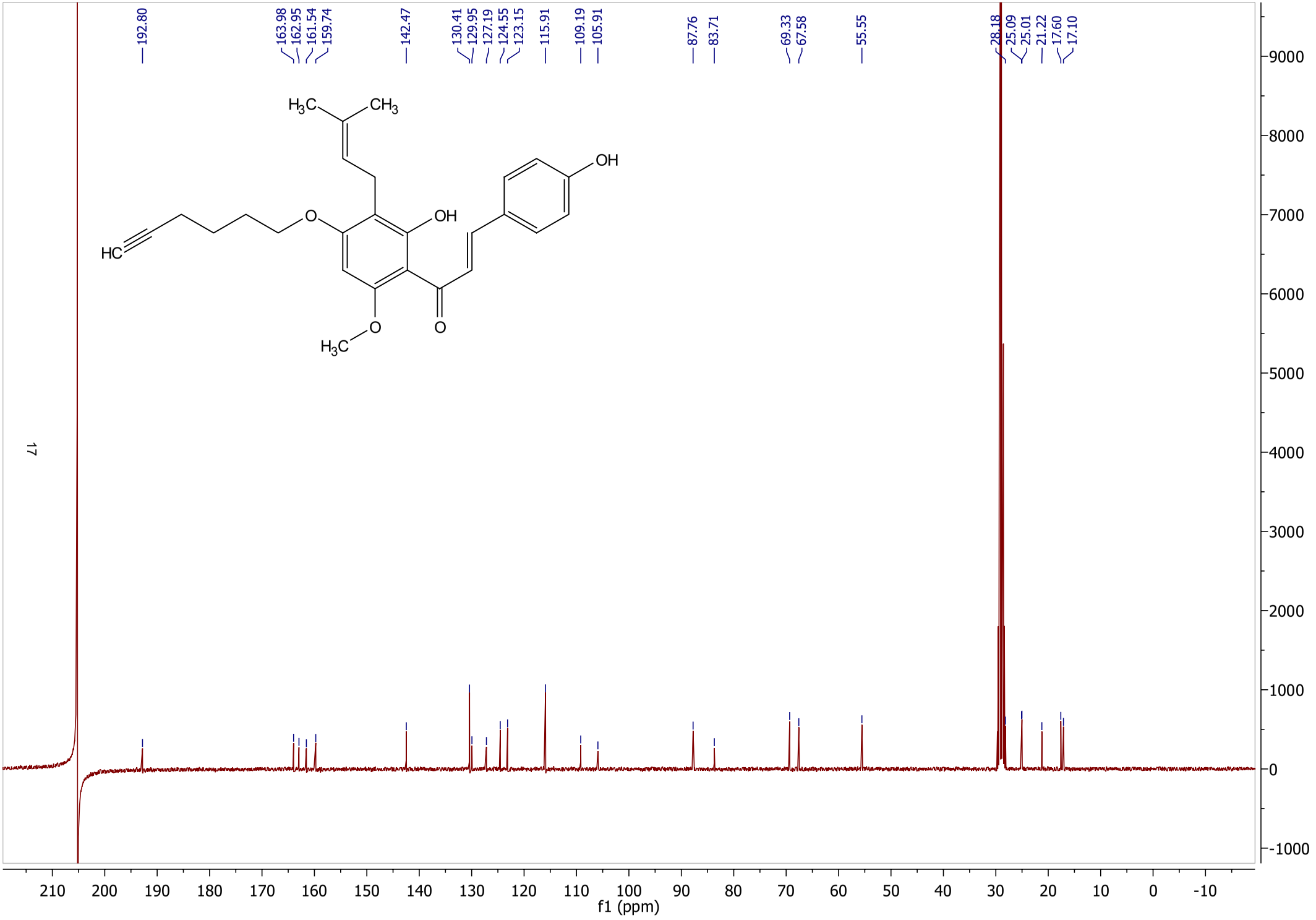

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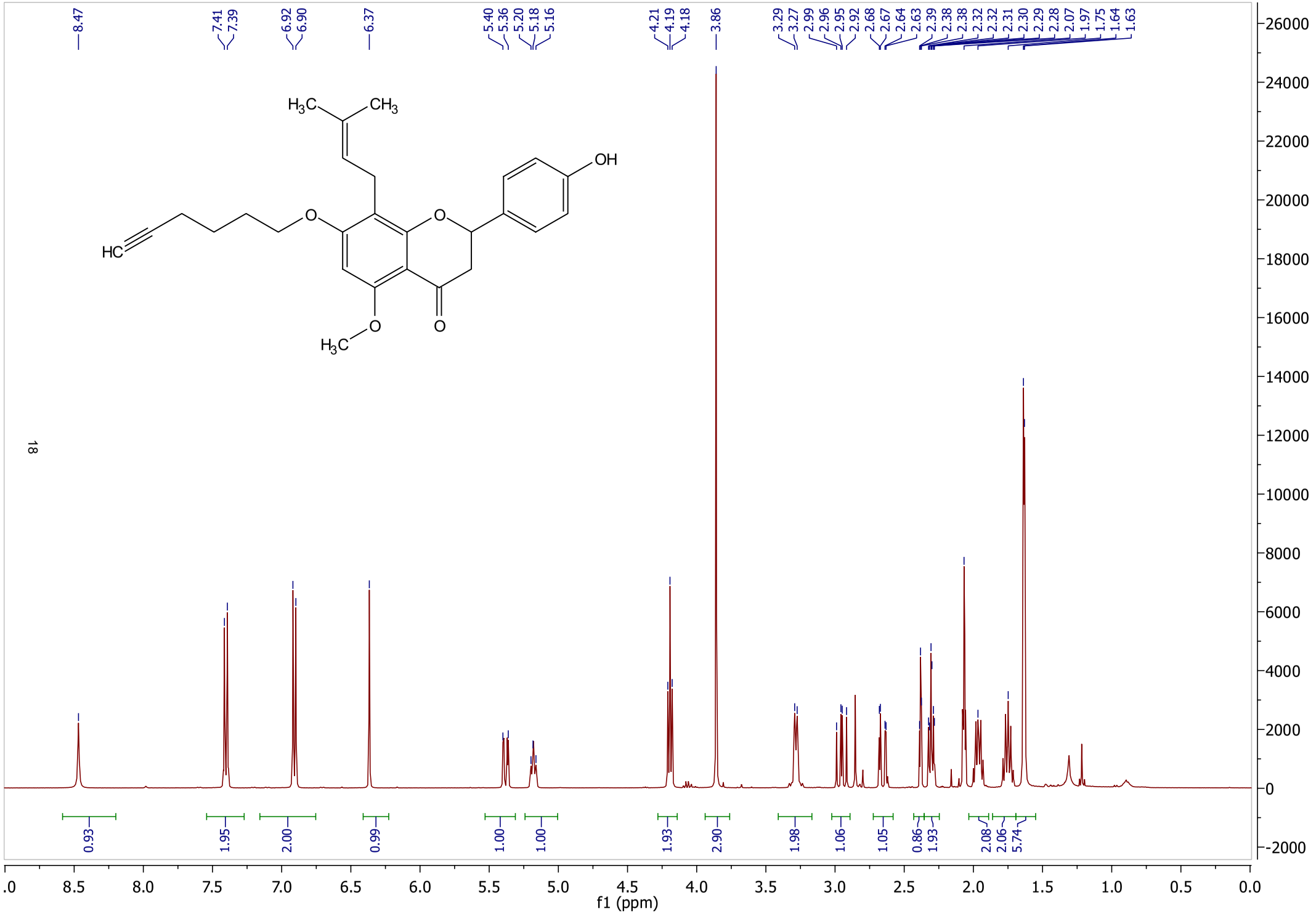
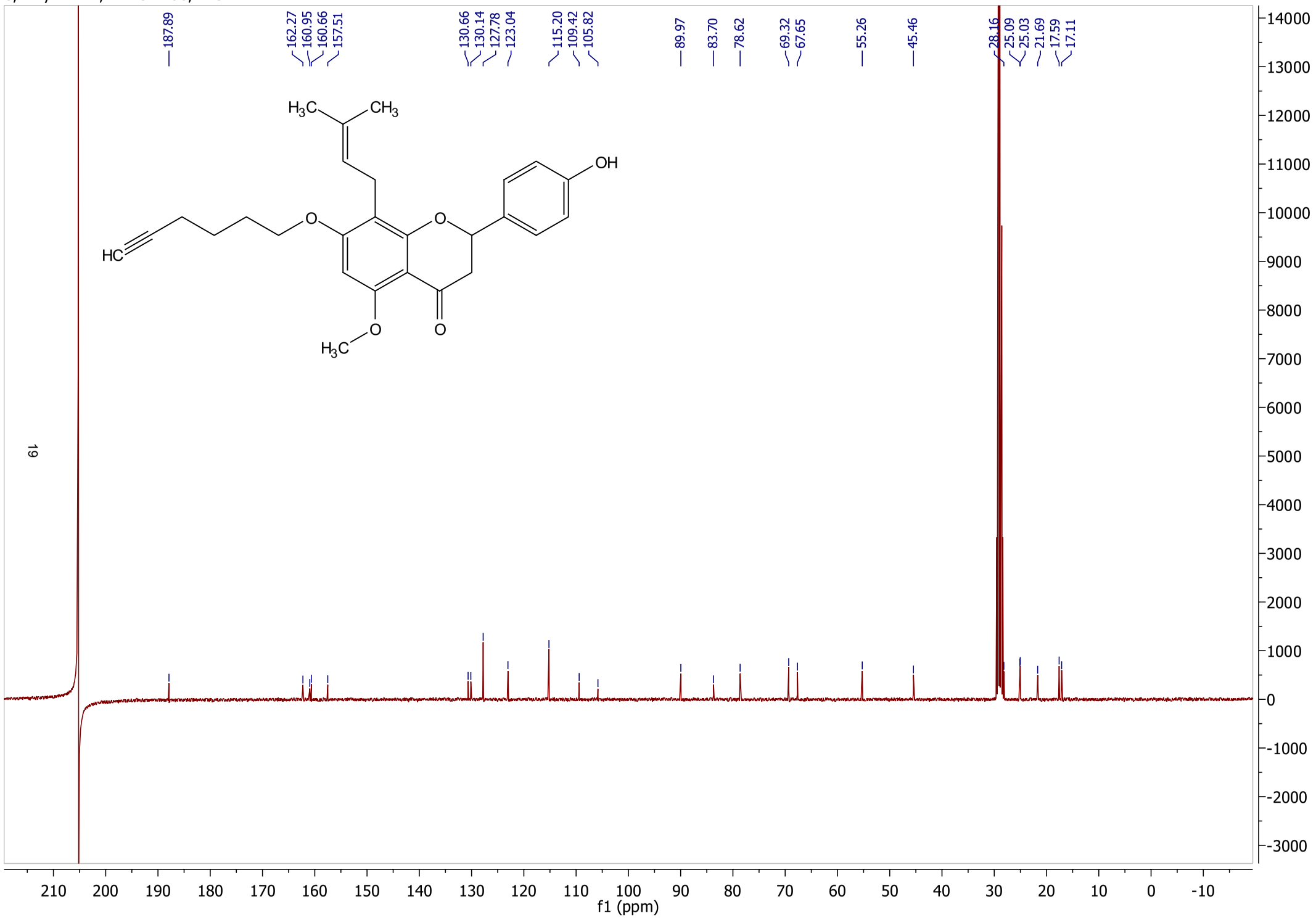
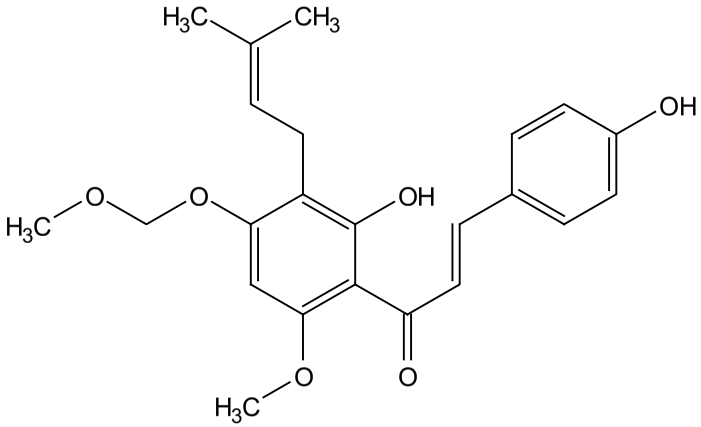


6, Alkyne-IXN, Acetone-d6, 125 MHz



7, MOM-XN, Acetone-d6, 500 MHz



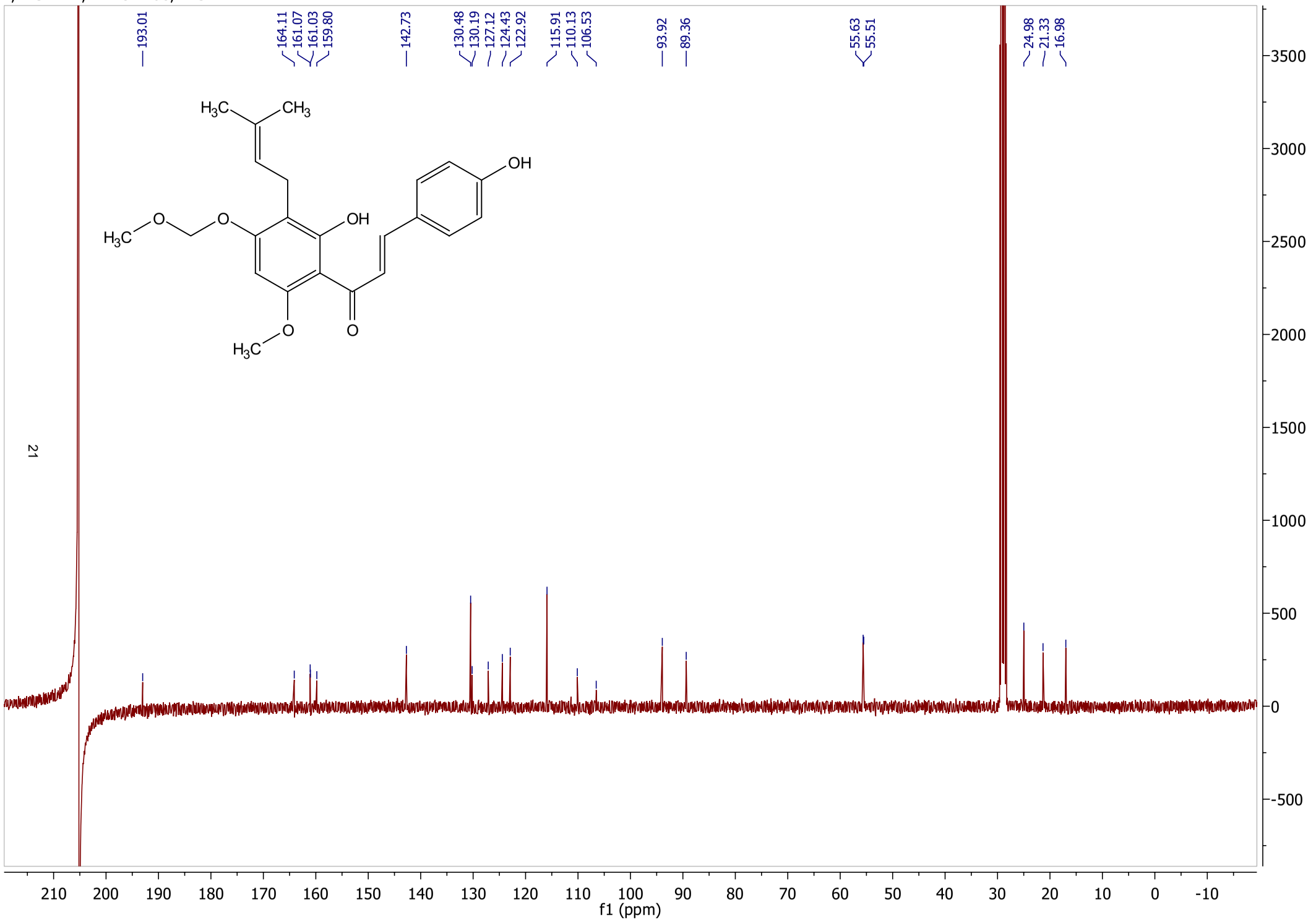
14.34

7.90 7.86 7.79 7.75 7.64 7.62 6.93 6.91 6.39 5.37 5.21 3.99 3.48 3.31 3.29 2.04 1.77 1.63

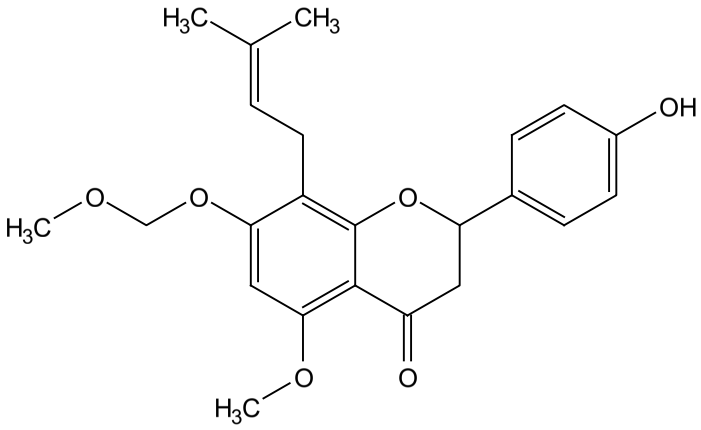
0.90 2.13 2.04 2.00 1.04 1.95 1.10 2.89 2.86 2.08 2.96 3.14

f1 (ppm)

7, MOM-XN, Acetone-d6, 125 MHz



8, MOM-IXN, Acetone-d6, 500 MHz

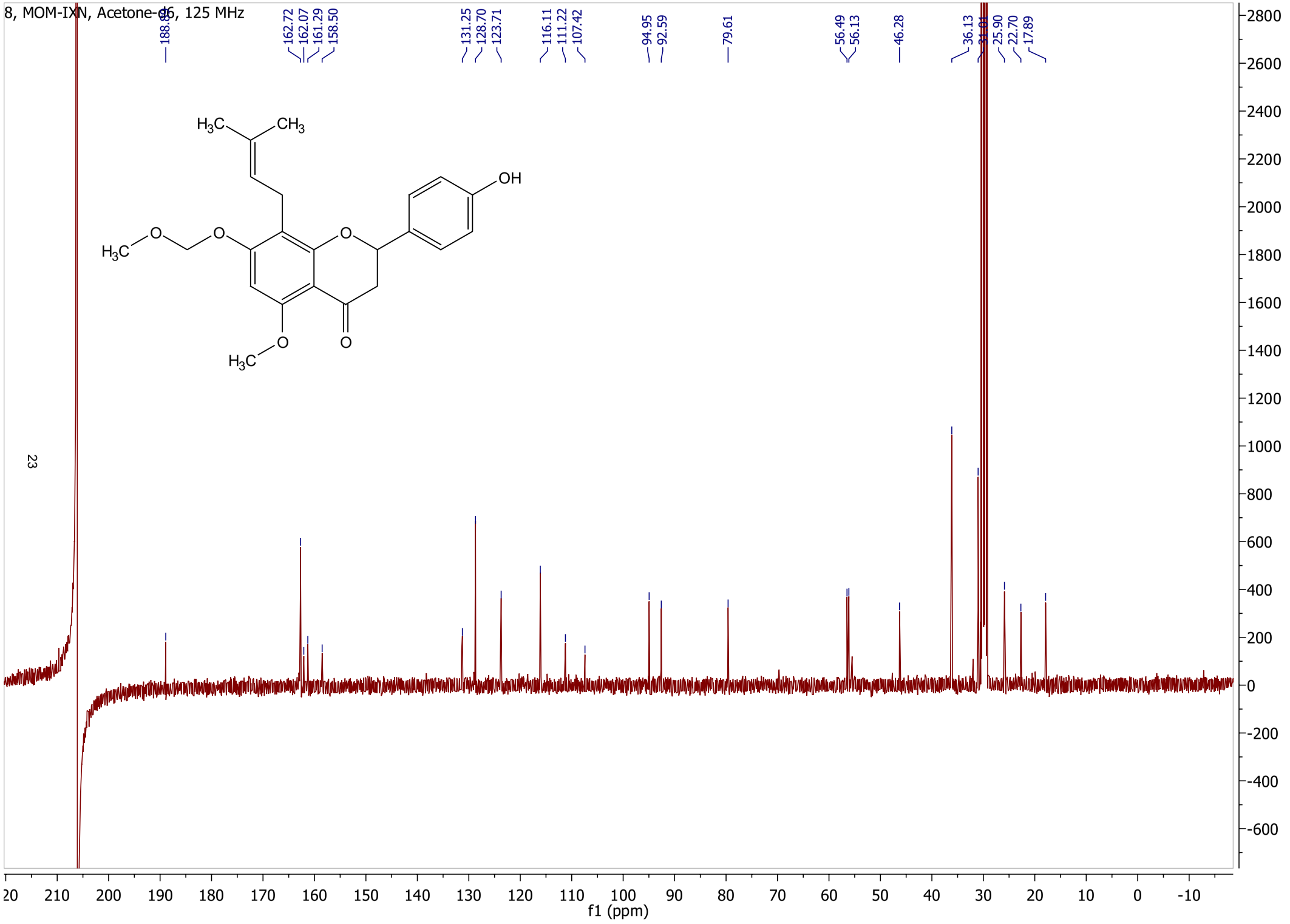


22

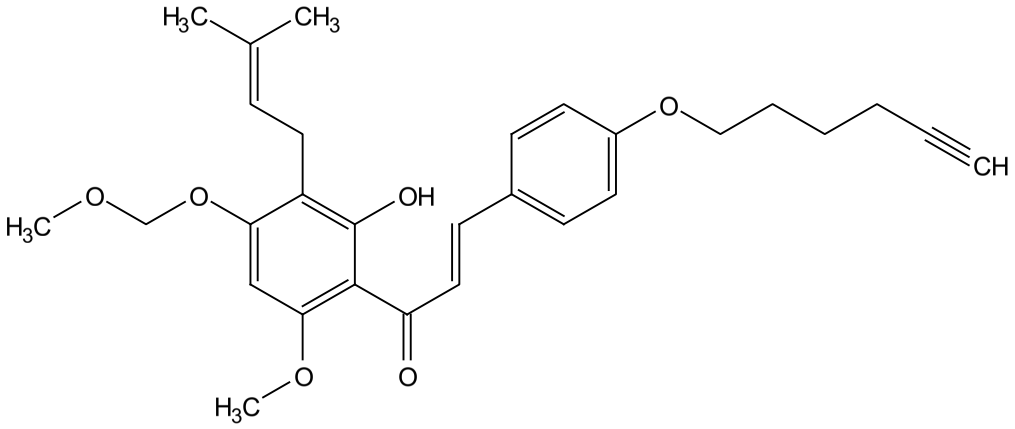
8.48 7.96 7.40 7.38 6.90 6.88 6.45 5.40 5.36 5.34 5.17 3.81 3.47 3.29 3.27 2.94 2.83 2.78 2.67 2.62 1.62 1.61

1.00 1.11 2.01 2.02 1.15 1.07 1.85 1.06 2.91 2.98 2.03 0.44 3.89 1.12 6.14

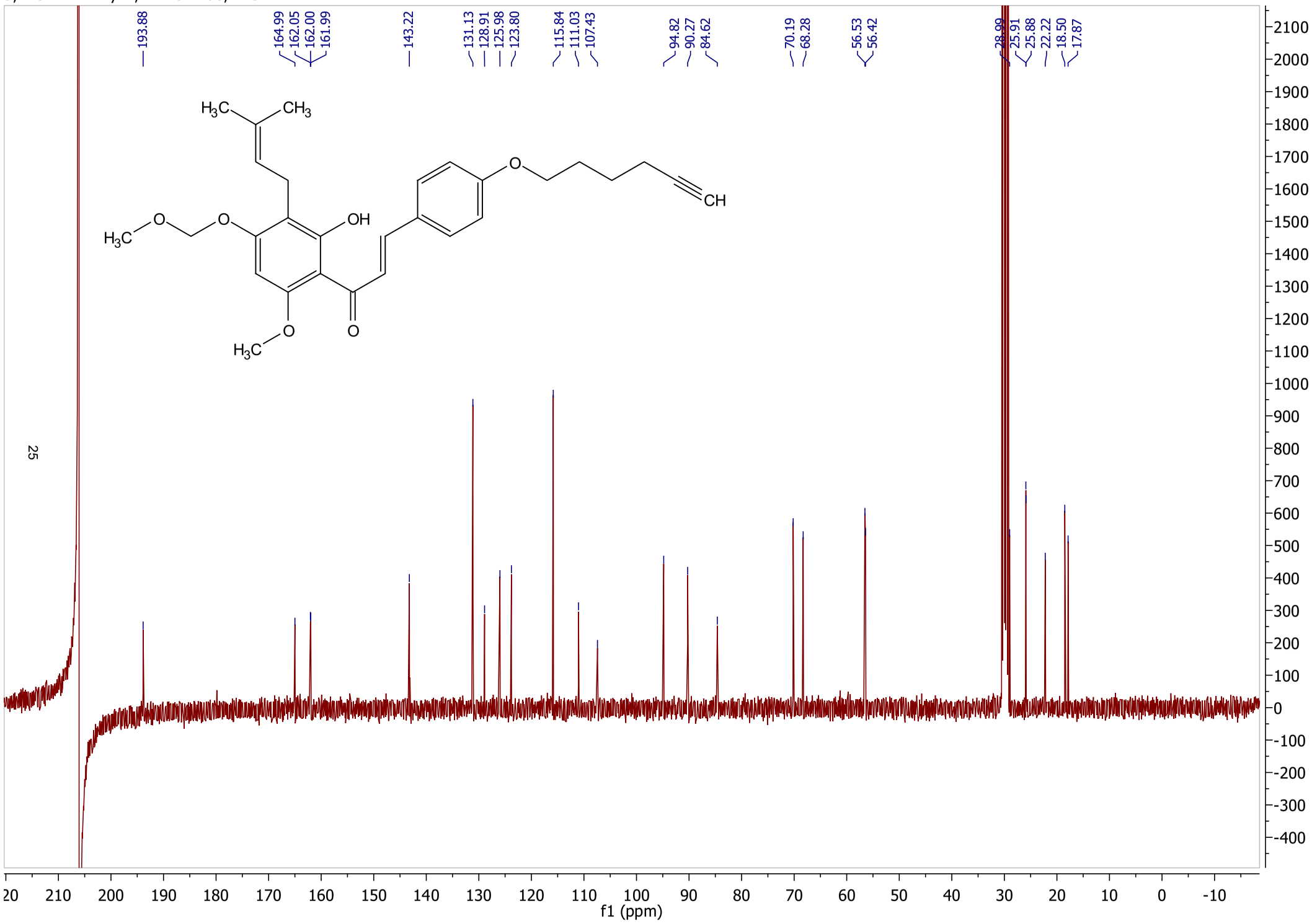
f1 (ppm)


8, MOM-IXN, Acetone-d6, 125 MHz
188.85
162.72
162.07
161.29
158.50
131.25
128.70
123.71
116.11
111.22
107.42
94.95
92.59
79.61
56.49
56.13
46.28
36.13
31.01
25.90
22.70
17.89
H3C
CH3
OH
O
H3C
O
O
O
H3C
O
O
23
f1 (ppm)

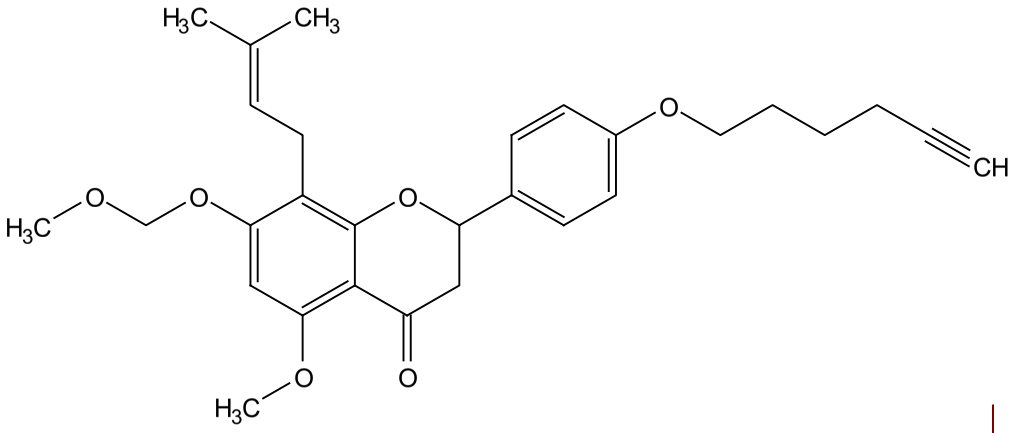
9, MOM-XN-Alkyne, Acetone-d6, 500 MHz



9, MOM-XN-Alkyne, Acetone-d6, 125 MHz



10, MOM-IXN-Alkyne, Acetone-d6, 500 MHz

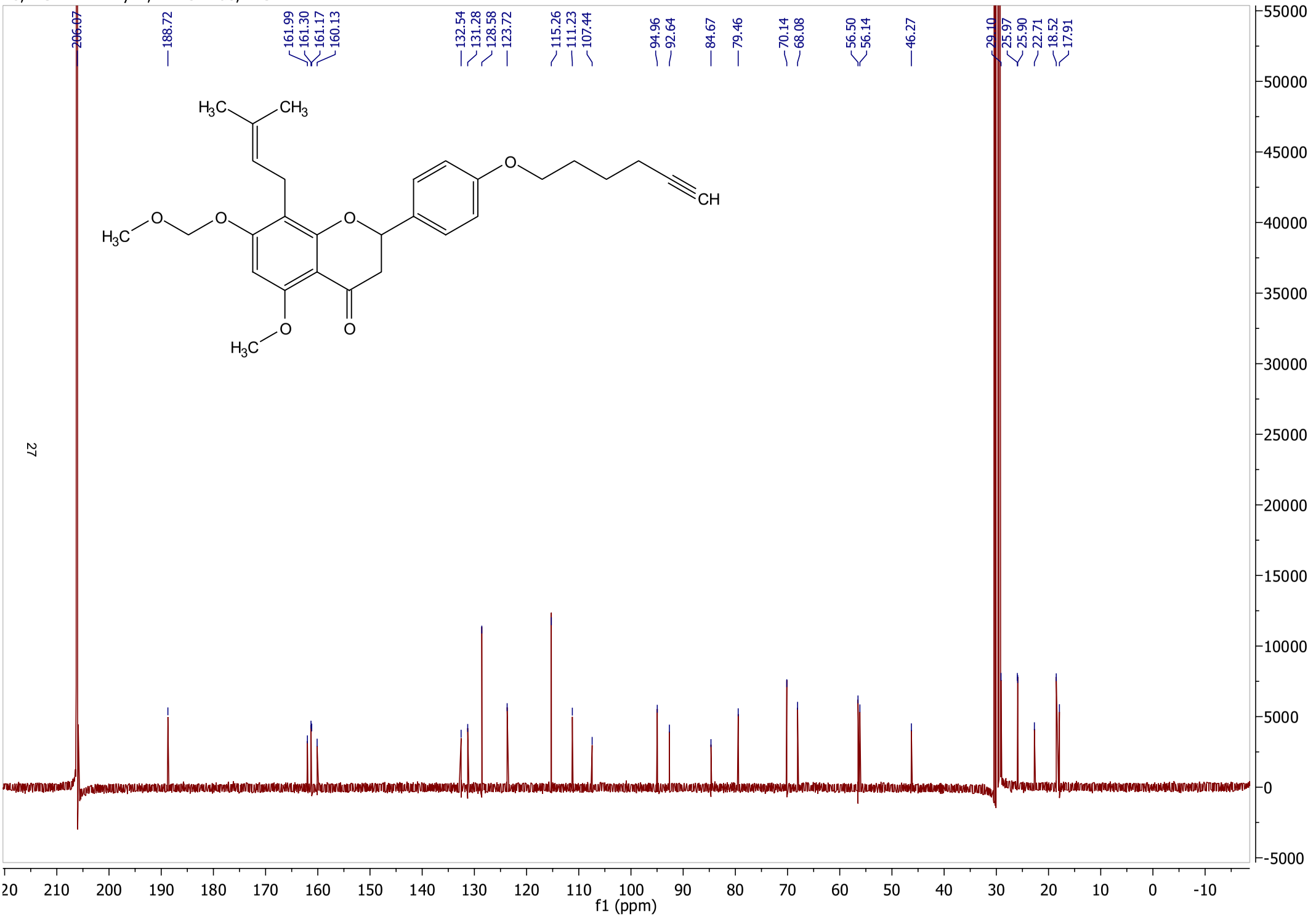


7.49 7.46 7.01 6.98 6.46 5.45 5.41 5.35 5.17 4.08 4.07 4.05 3.81 3.29 3.28 3.00 2.95 2.81 2.69 2.65 2.35 2.29 2.05 1.89 1.73 1.63

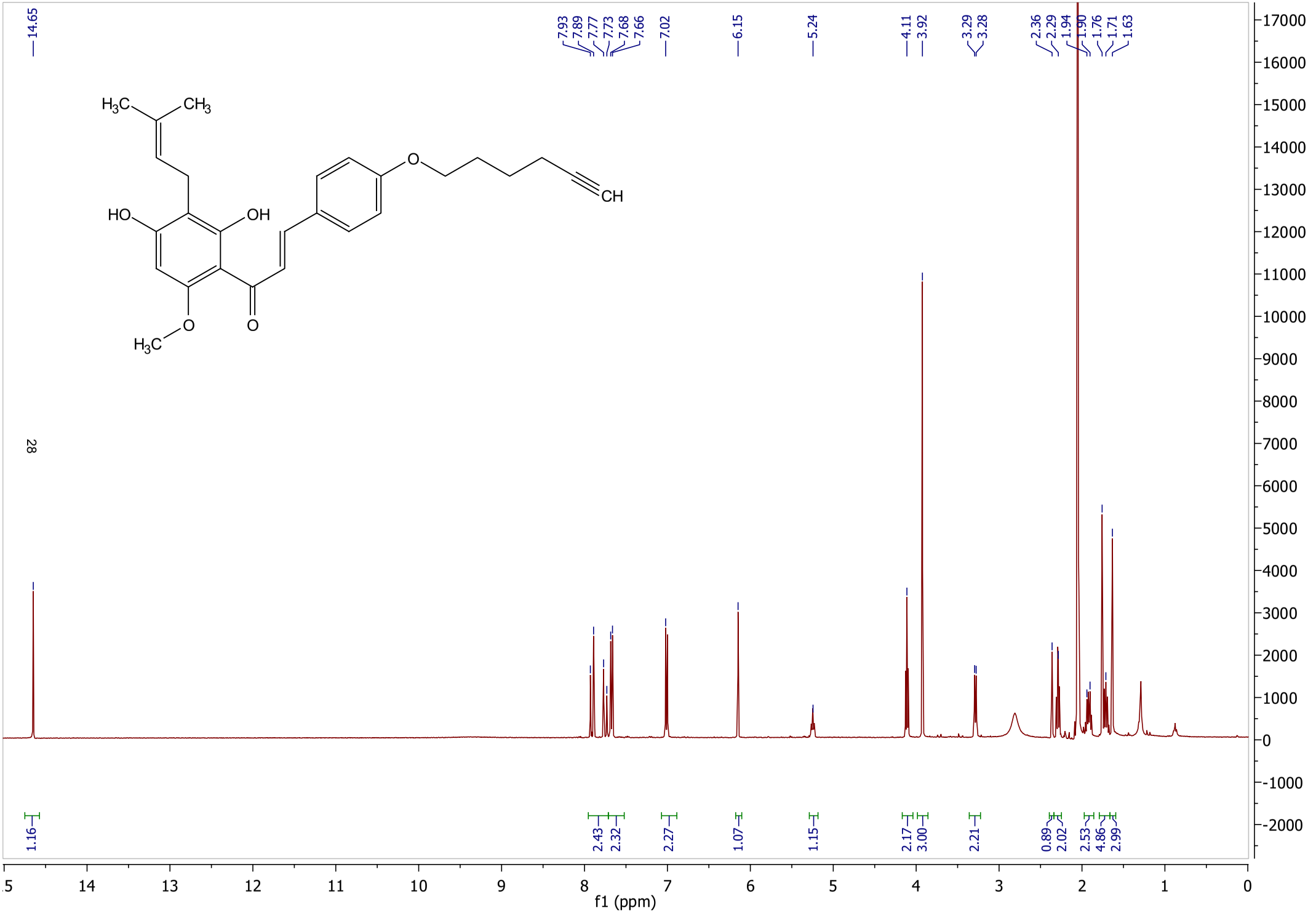
2.00 1.87 1.12 0.98 1.85 0.97 1.85 2.80 2.69 1.93 1.08 1.08 0.81 1.84 2.08 2.06 5.25

f1 (ppm)

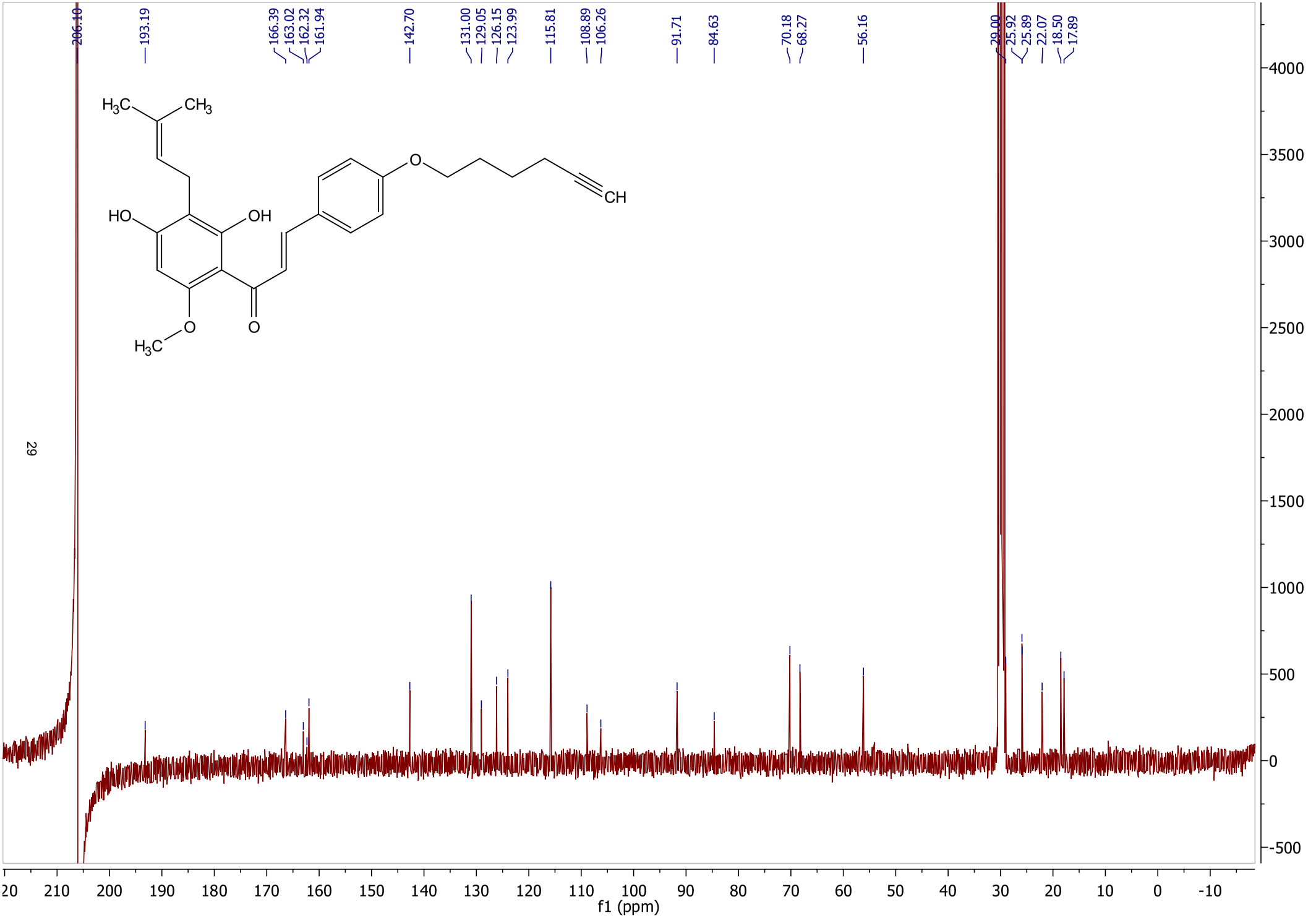
10, MOM-IXN-Alkyne, Acetone-d6, 125 MHz



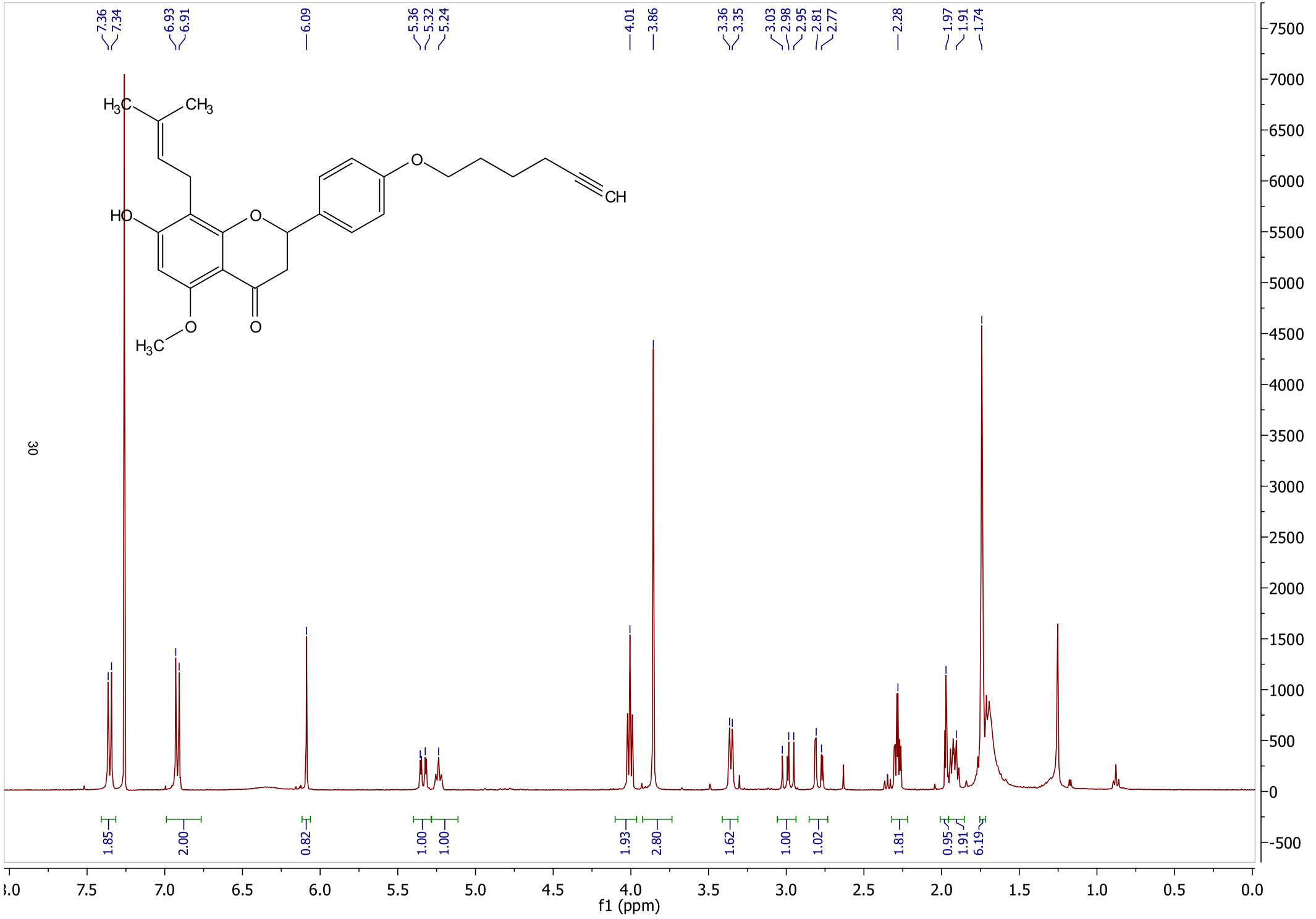
11, XN-Alkyne, Acetone-d6, 500 MHz



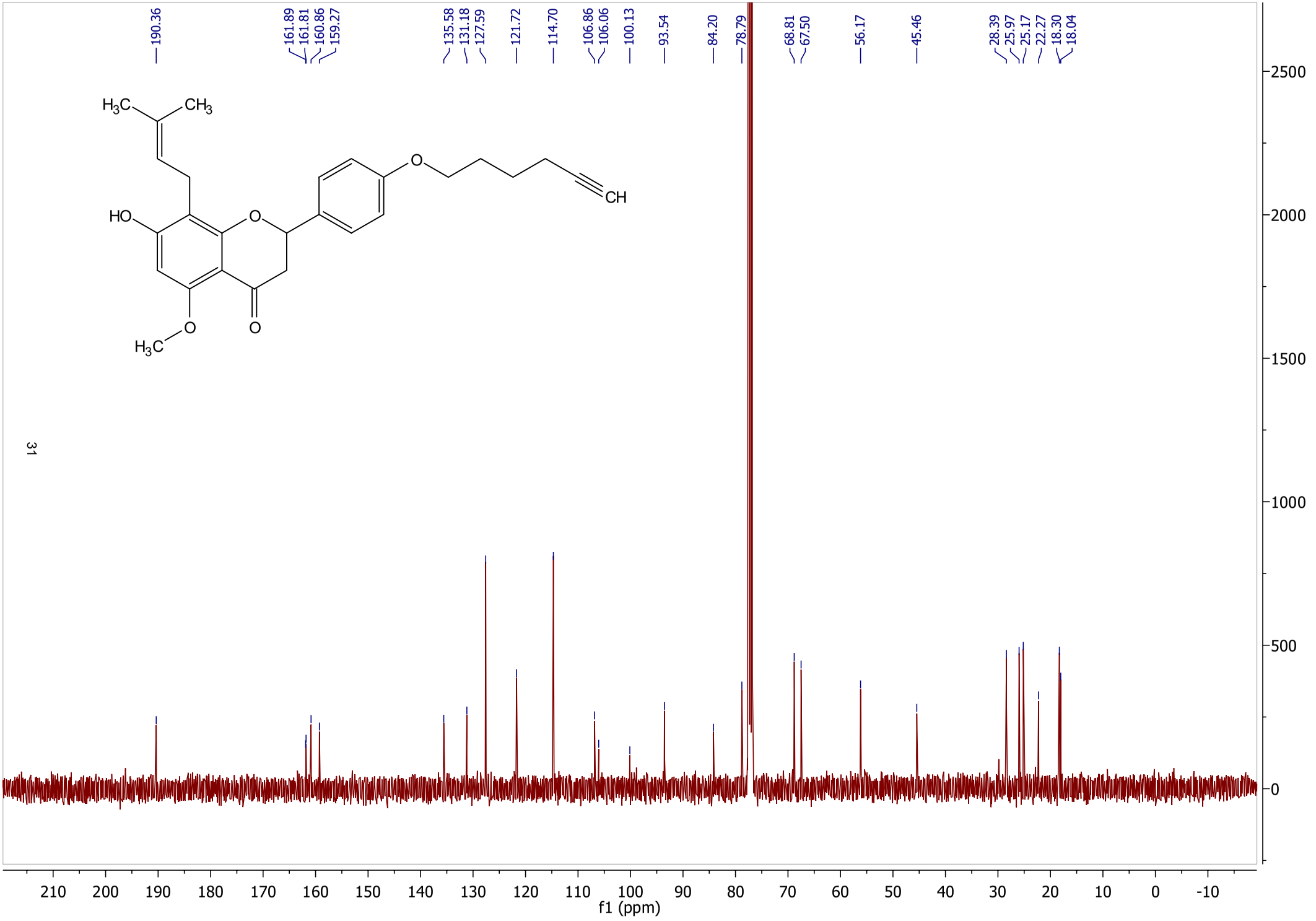
11, XN-Alkyne, Acetone-d6, 125 MHz



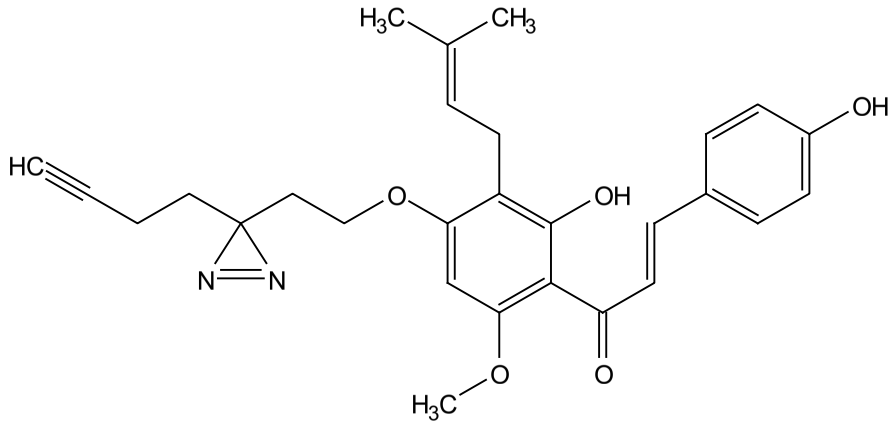
12, IXN-Alkyne, CDCl3, 500 MHz



12, IXN-Alkyne, CDCl3, 125 MHz



13, DA-XN, Acetone-d6, 500 MHz



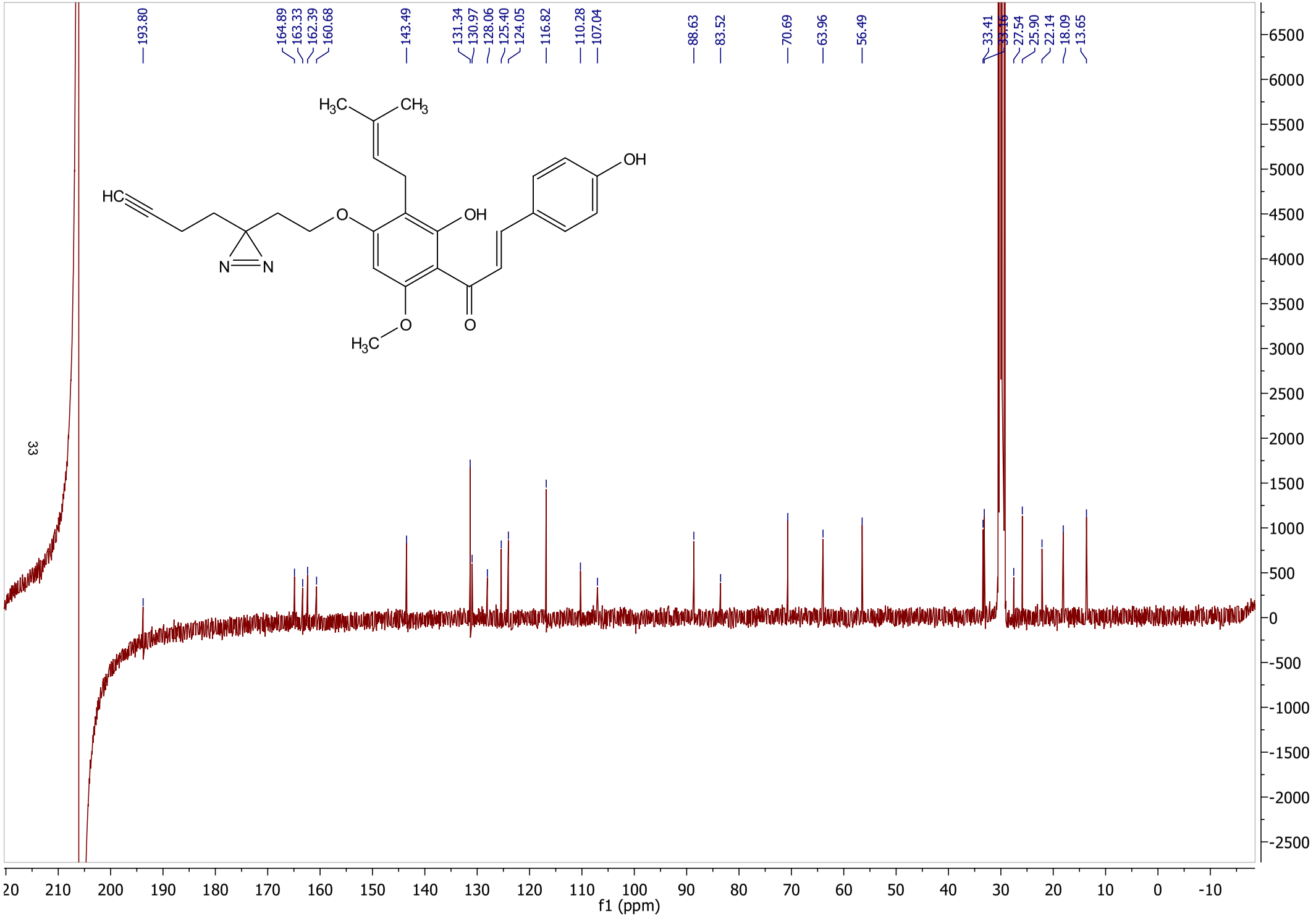
14.35
7.92
7.88
7.80
7.65
7.63
6.95
6.93
6.31
5.28
4.10
4.05
3.37
2.43
2.10
2.02
1.80
1.78
1.66

32

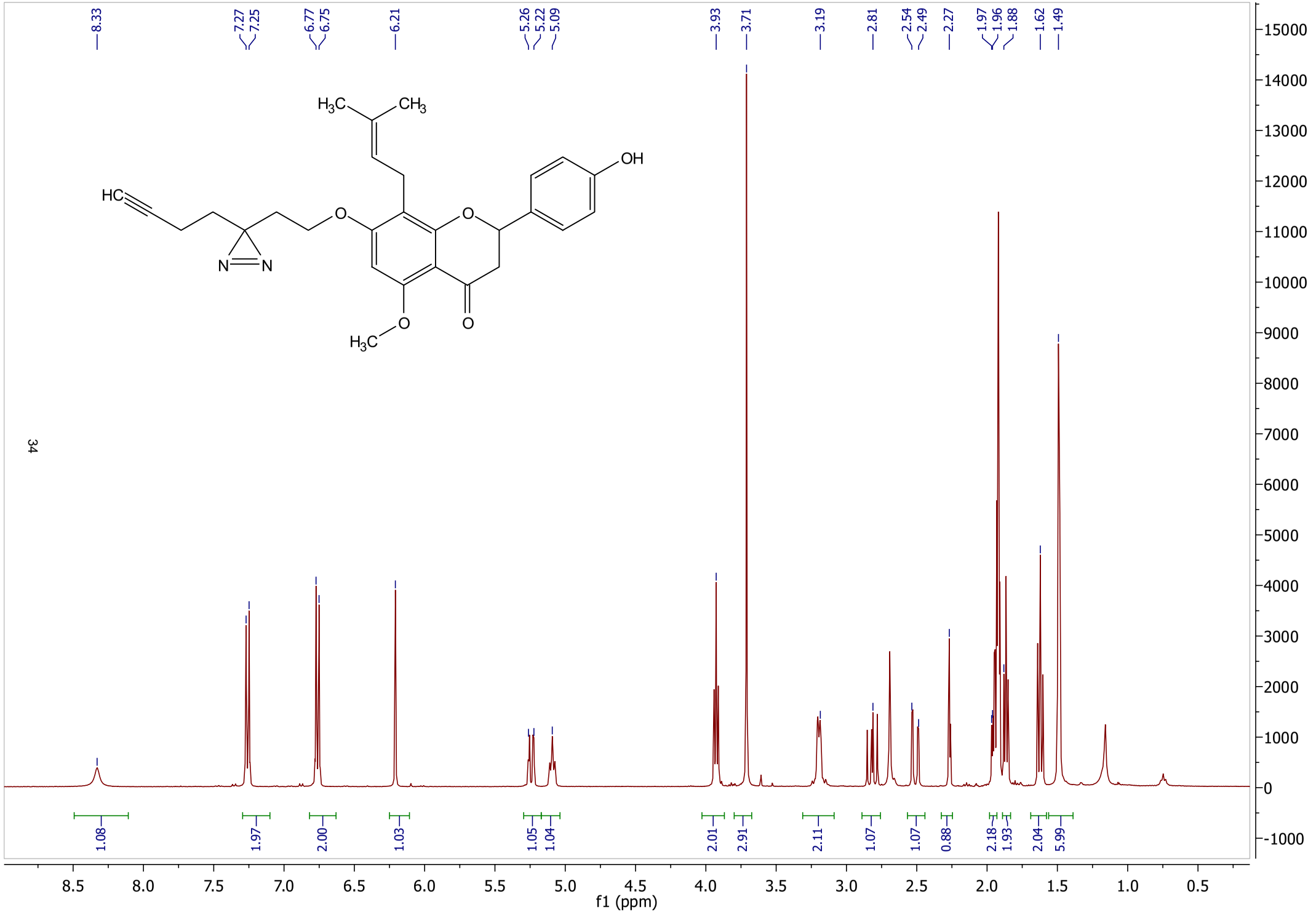
0.91
2.00
2.00
1.98
0.94
0.91
2.03
3.03
2.07
0.90
2.08
2.28
3.54
1.54
3.23

f1 (ppm)

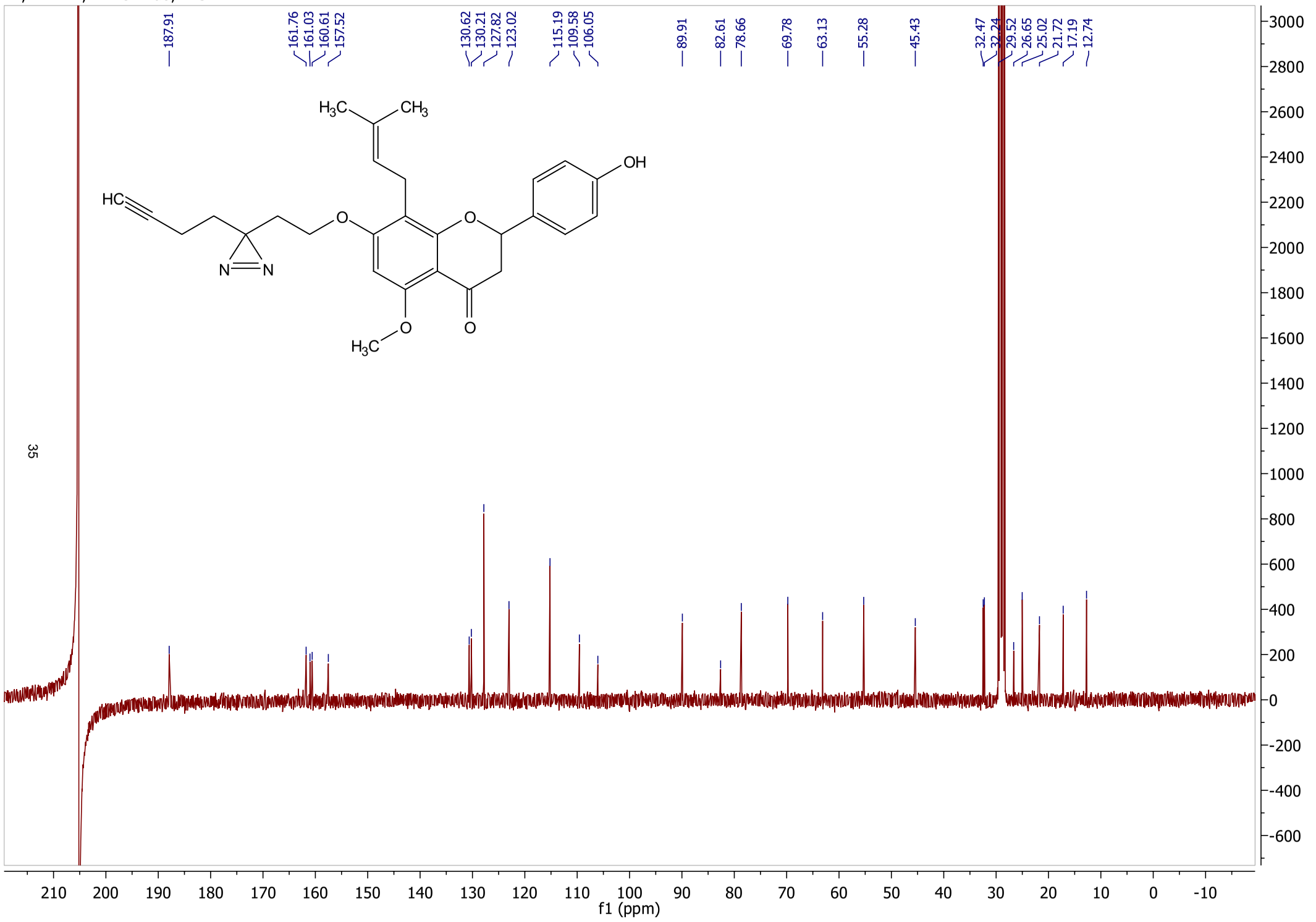
12, DA-XN, Acetone-d6, 125 MHz



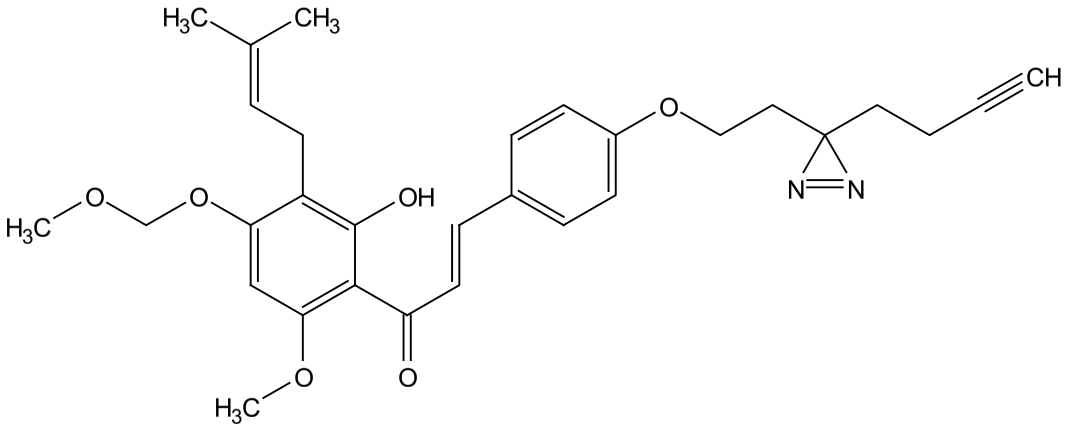
14, DA-IXN, Acetone-d6, 500 MHz



14, DA-IXN, Acetone-d6, 125 MHz



15, MOM-XN-DA, Acetone-d6, 500 MHz

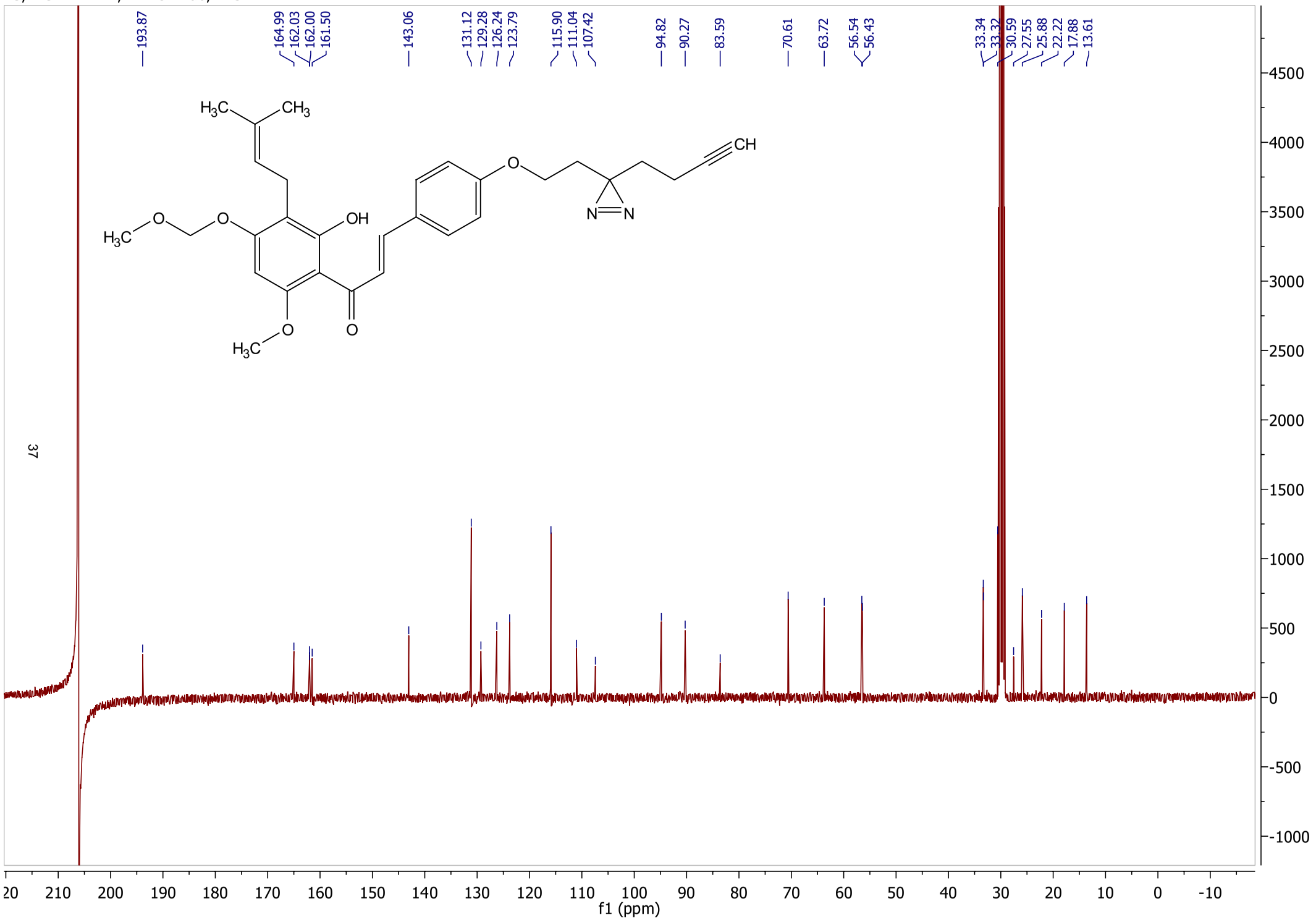


14.28 | 7.93 | 7.80 | 7.72 | 7.70 | 7.04 | 7.02 | 6.39 | 5.37 | 5.21 | 3.98 | 3.48 | 3.31 | 2.82 | 2.39 | 2.11 | 2.09 | 2.04 | 1.97 | 1.77 | 1.74 | 1.63 | 1.63

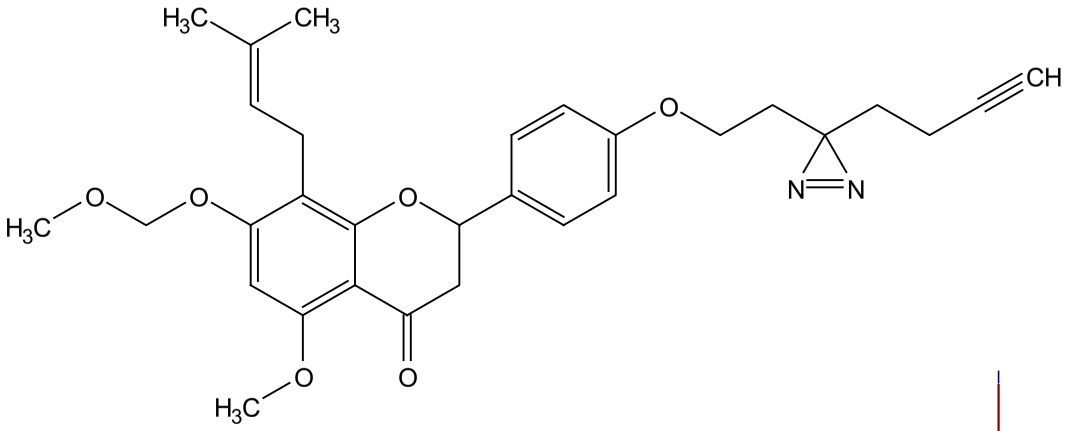
0.85 | 2.05 | 2.03 | 2.00 | 0.98 | 2.01 | 1.07 | 5.07 | 2.99 | 2.03 | 0.88 | 1.85 | 6.68 | 2.09 | 2.81 | 2.26 | 3.01

f1 (ppm)

15, MOM-XN-DA, Acetone-d6, 125 MHz



16, MOM-IXN-DA, Acetone-d6, 125 MHz

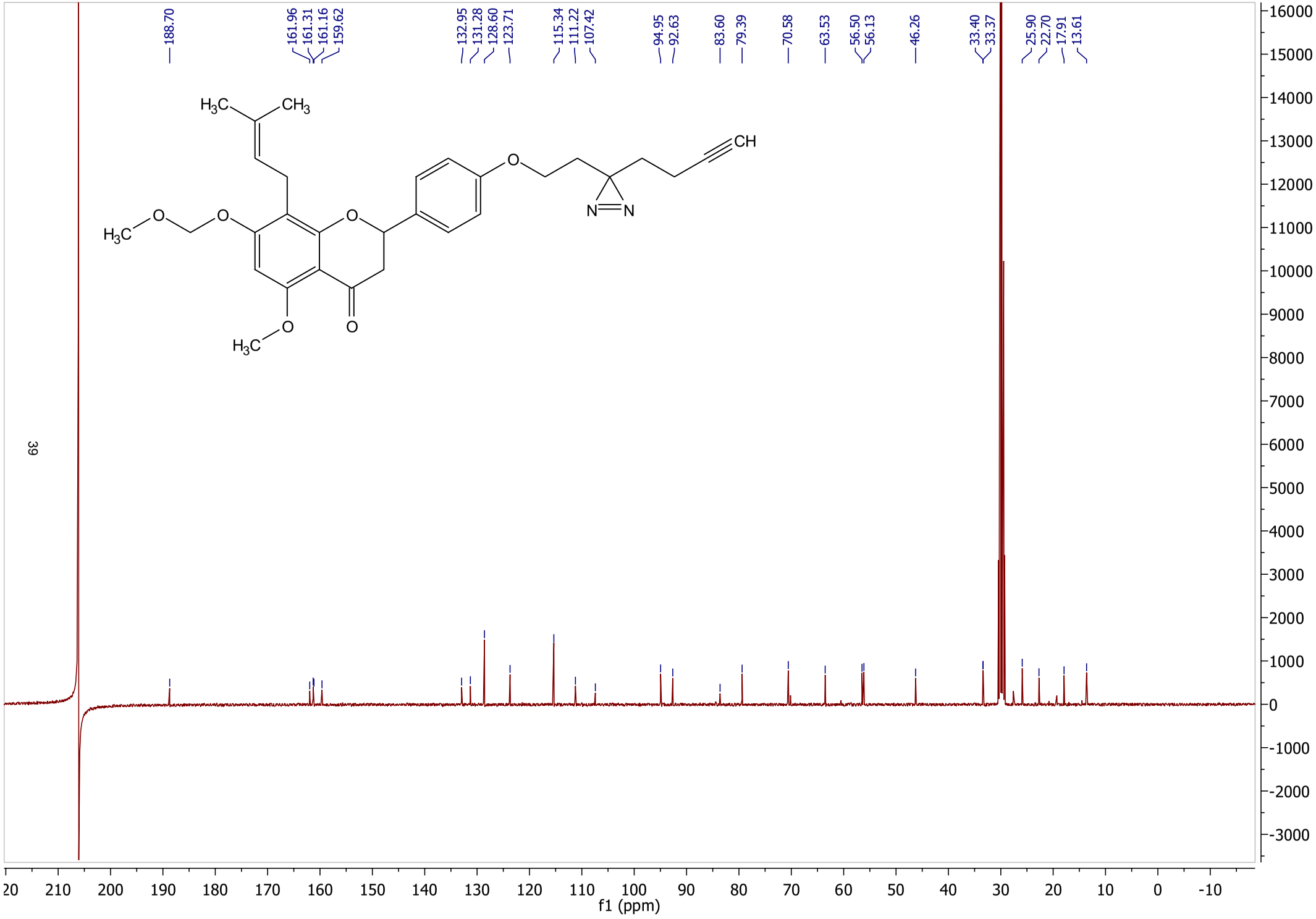


7.50 7.48 7.02 7.00 6.46 5.46 5.42 5.35 5.17 3.95 3.47 3.30 2.96 2.82 2.70 2.66 2.65 2.40 2.12 2.10 1.93 1.92 1.76 1.74 1.63 1.61

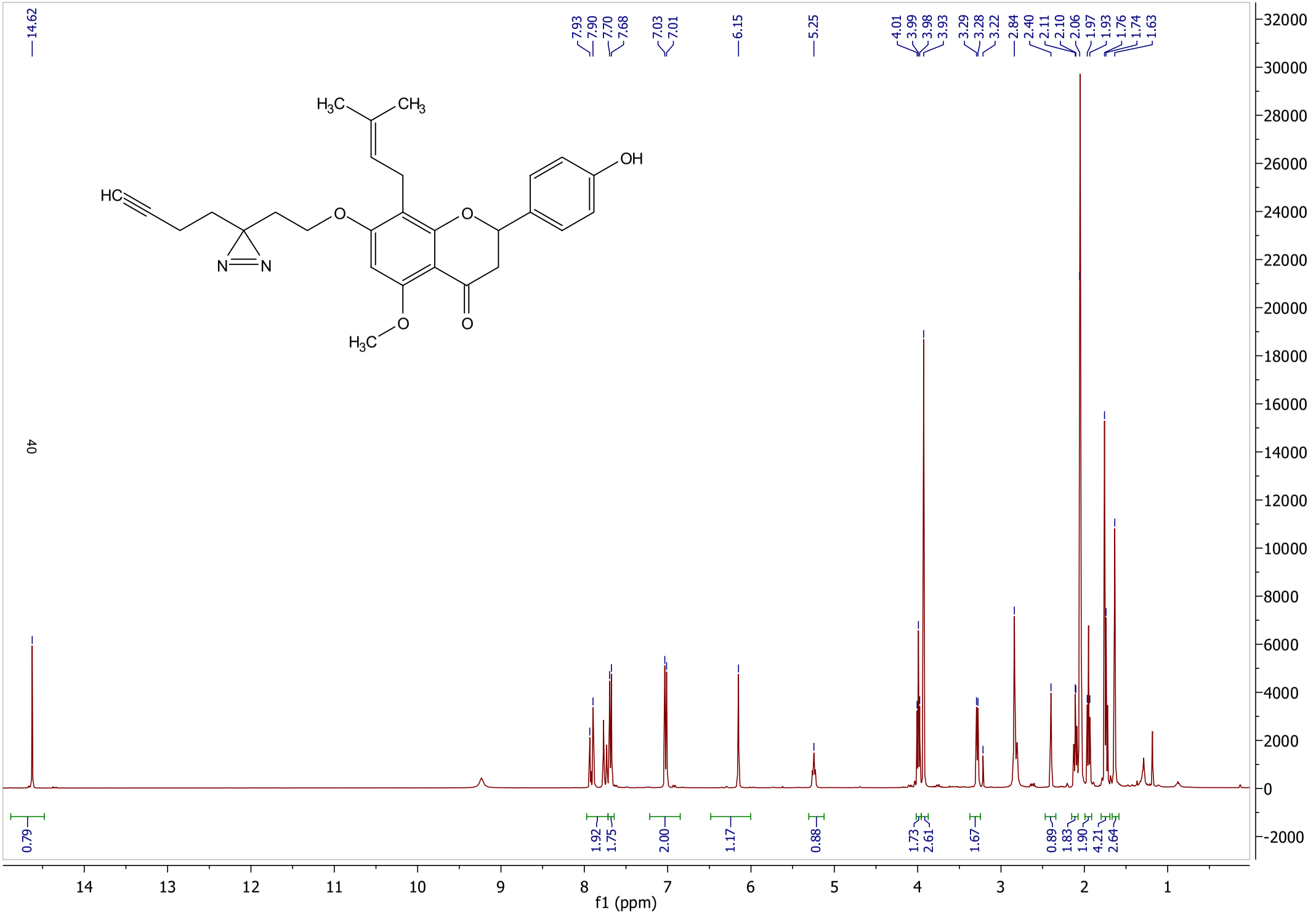
2.16 2.10 1.00 0.94 1.78 0.97 1.86 2.81 2.89 1.95 1.00 1.03 0.39 0.80 1.70 1.81 1.83 5.52

f1 (ppm)

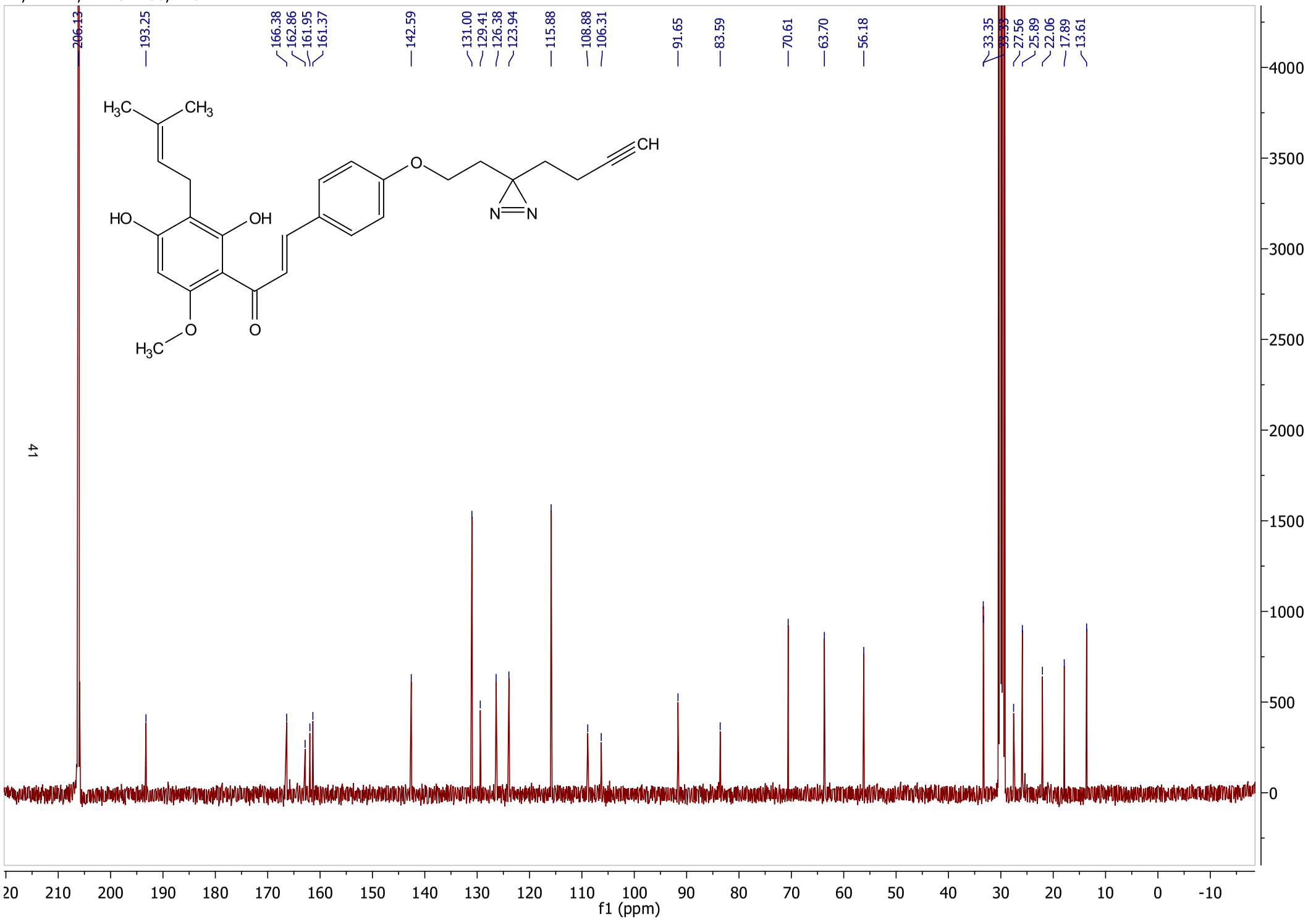
16, MOM-IXN-DA, Acetone-d6, 125 MHz



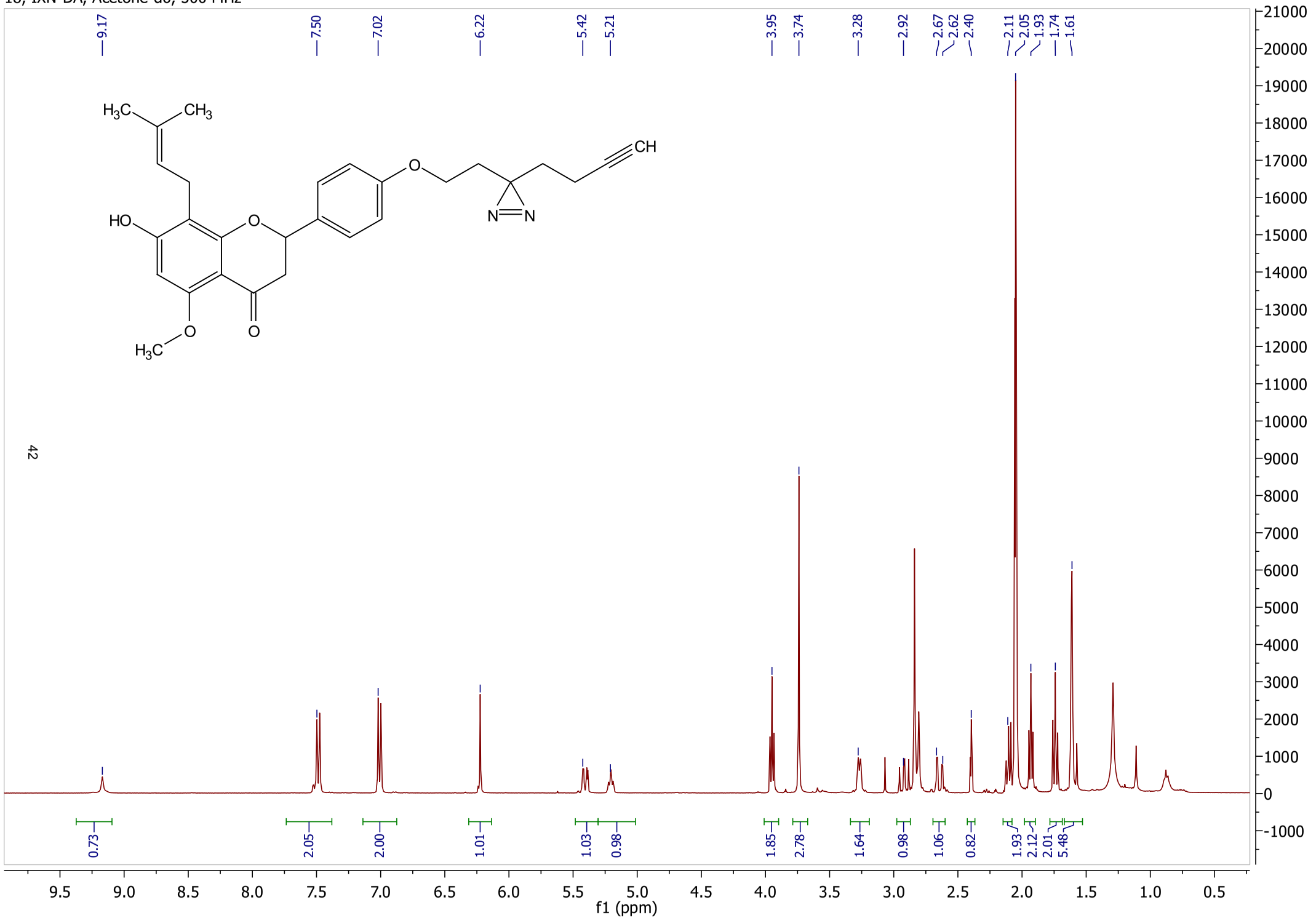
17, XN-DA, Acetone-d6, 500 MHz



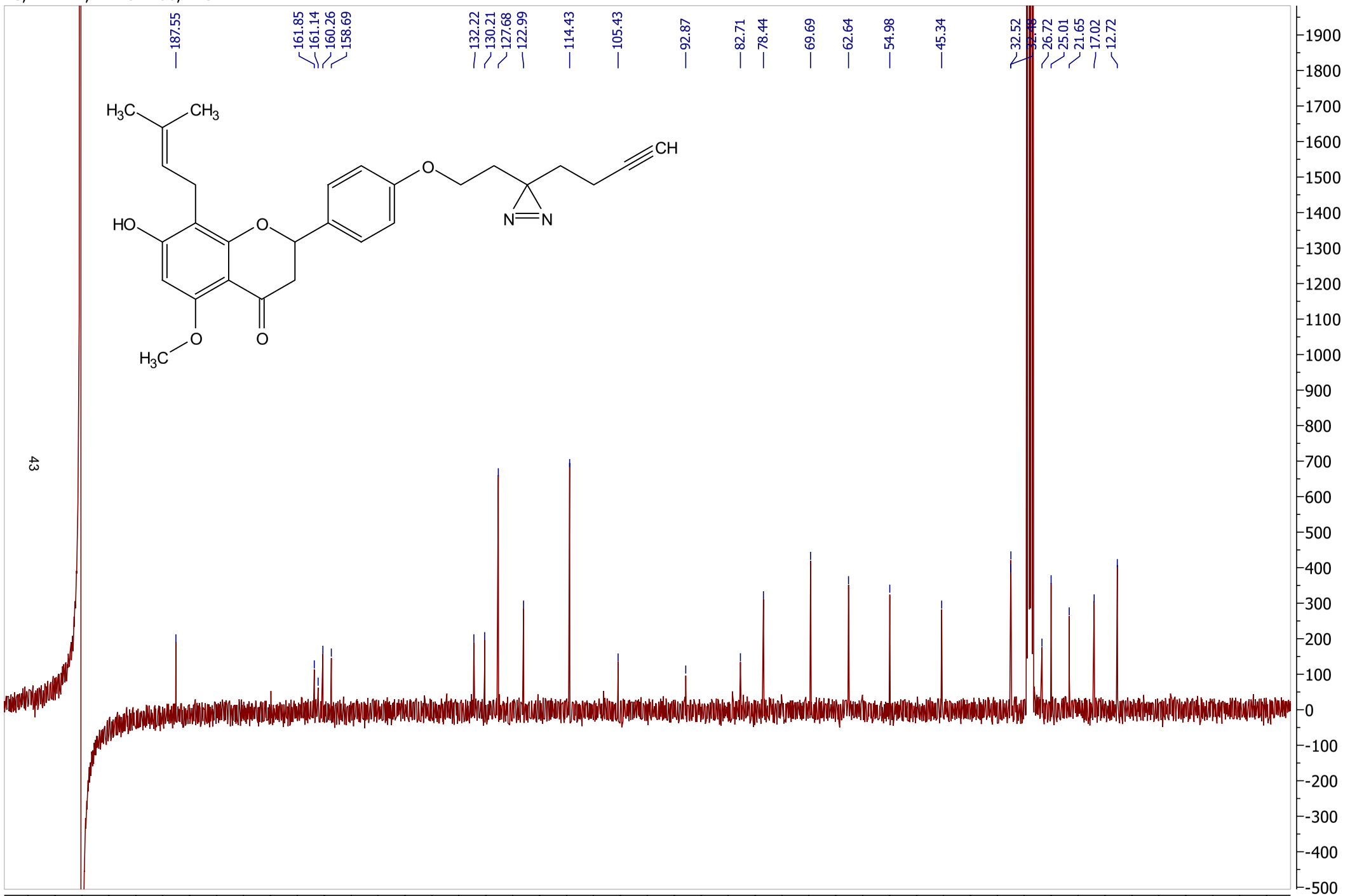
14, XN-DA, Acetone-d6, 125 MHz



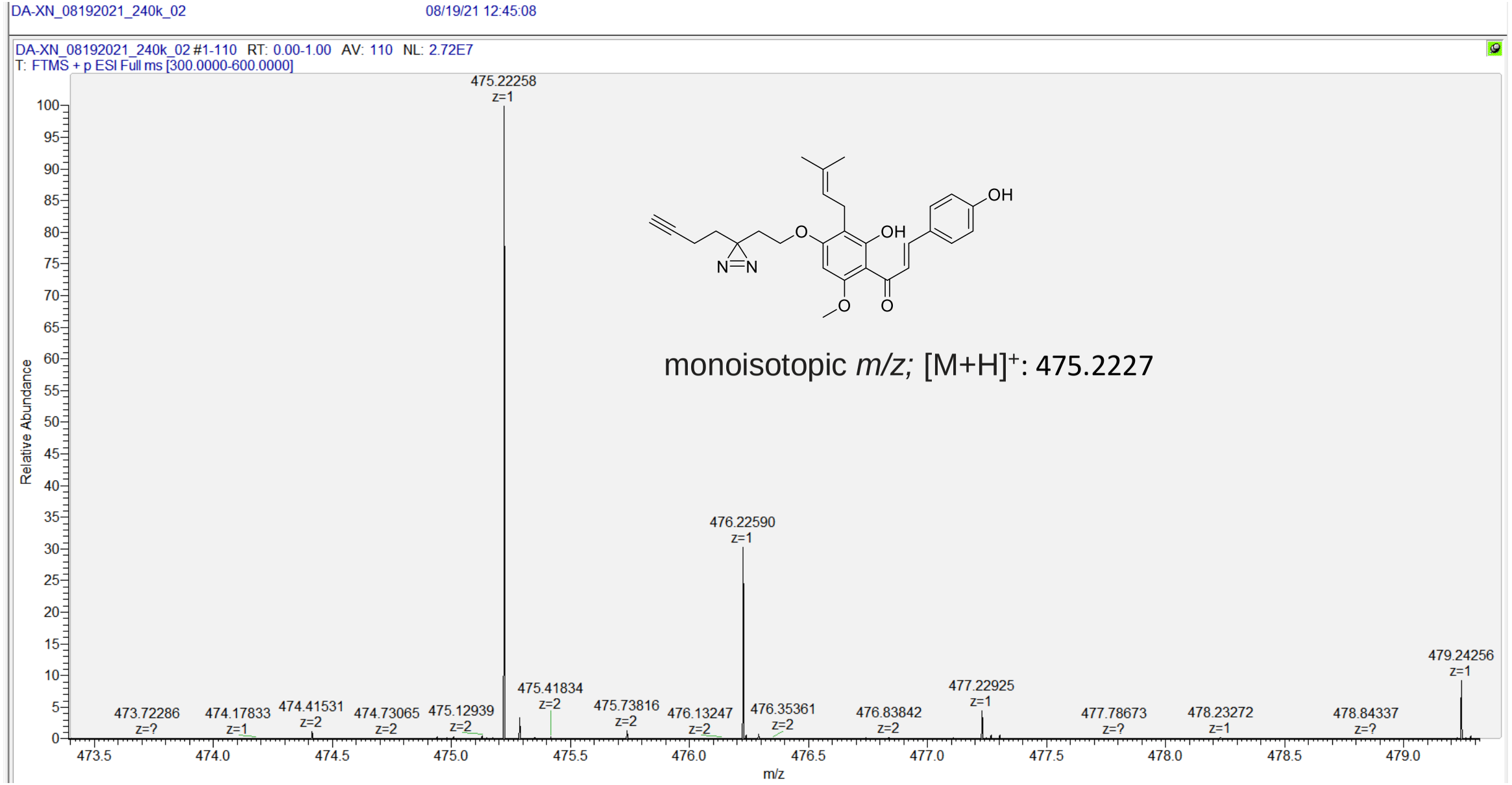
18, IXN-DA, Acetone-d6, 500 MHz



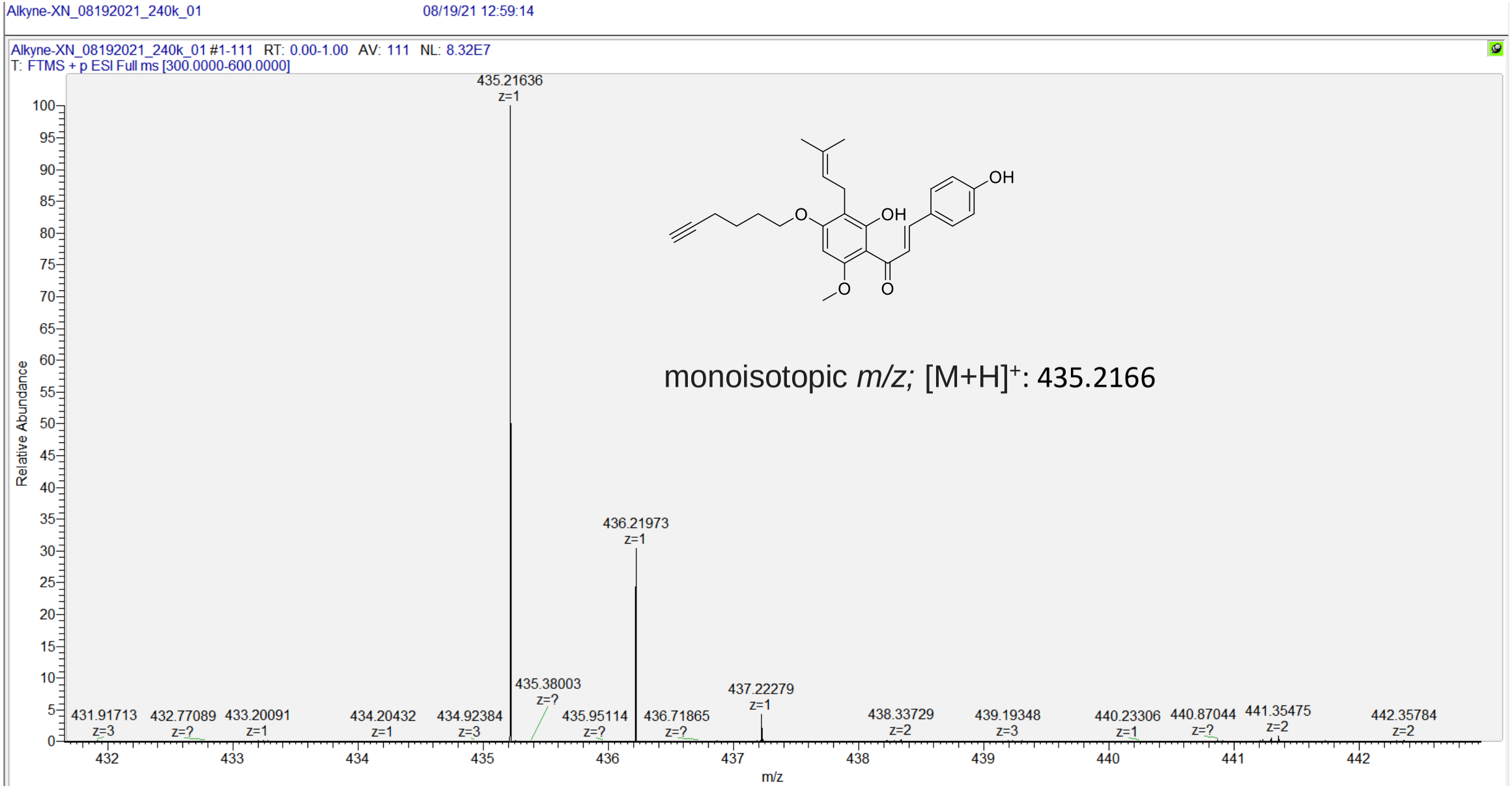
18, IXN-DA, Acetone-d6, 125 MHz



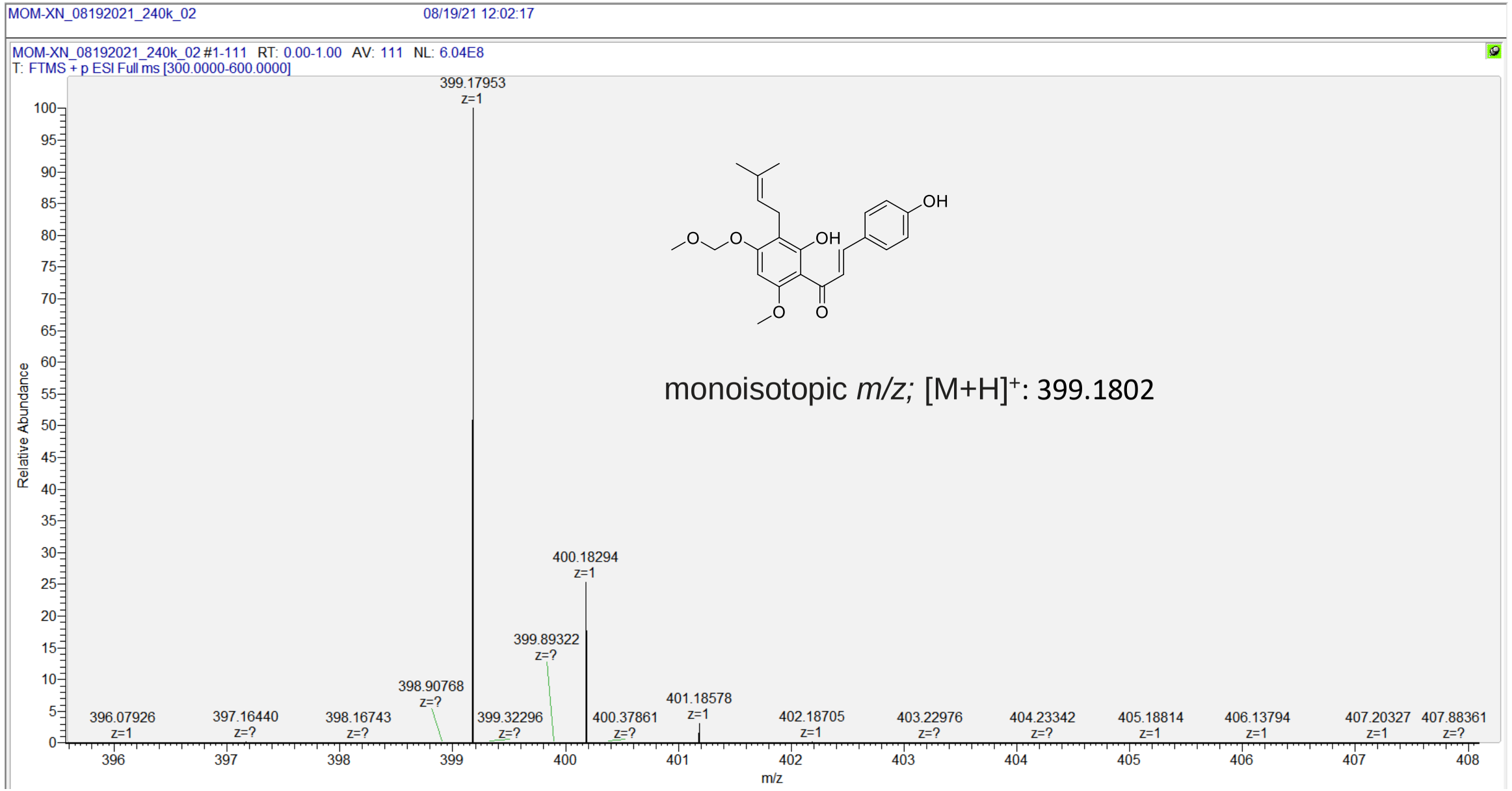
XN-ABP-1


DA-XN_08192021_240k_02
08/19/21 12:45:08
DA-XN_08192021_240k_02 #1-110 RT: 0.00-1.00 AV: 110 NL: 2.72E7
T: FTMS + p ESI Full ms [300.0000-600.0000]
Relative Abundance
m/z
475.22258 z=1
476.22590 z=1
473.72286 z=?
474.17833 z=1
474.41531 z=2
474.73065 z=2
475.12939 z=2
475.41834 z=2
475.73816 z=2
476.13247 z=2
476.35361 z=2
476.83842 z=2
477.22925 z=1
477.78673 z=?
478.23272 z=1
478.84337 z=?
479.24256 z=1
OH
OH
O
N=N
O
O
monoisotopic *m/z;* [M+H]+: 475.2227

XN-ABP-2


Alkyne-XN_08192021_240k_01
08/19/21 12:59:14
Alkyne-XN_08192021_240k_01 #1-111 RT: 0.00-1.00 AV: 111 NL: 8.32E7
T: FTMS + p ESI Full ms [300.0000-600.0000]
435.21636
z=1
436.21973
z=1
435.38003
z=?
437.22279
z=1
431.91713
z=3
432.77089
z=?
433.20091
z=1
434.20432
z=1
434.92384
z=3
435.95114
z=?
436.71865
z=?
438.33729
z=2
439.19348
z=3
440.23306
z=1
440.87044
z=?
441.35475
z=2
442.35784
z=2
Relative Abundance
m/z
OH
O
OH
O
O
monoisotopic m/z; [M+H]+: 435.2166

Compound **5**

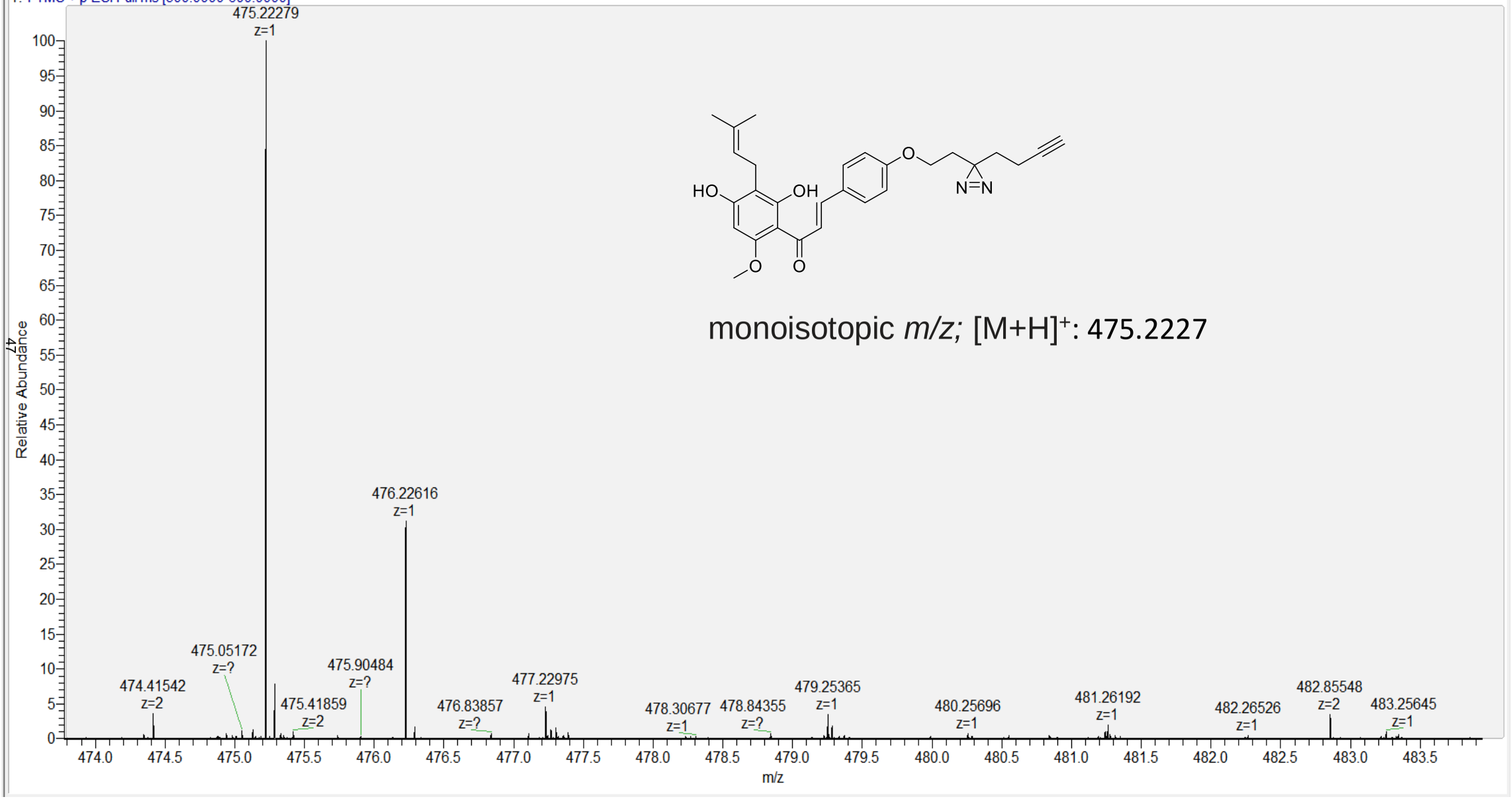


monoisotopic *m/z;* $[M+H]^+$: 399.1802

XN-ABP-3 474 Run 01

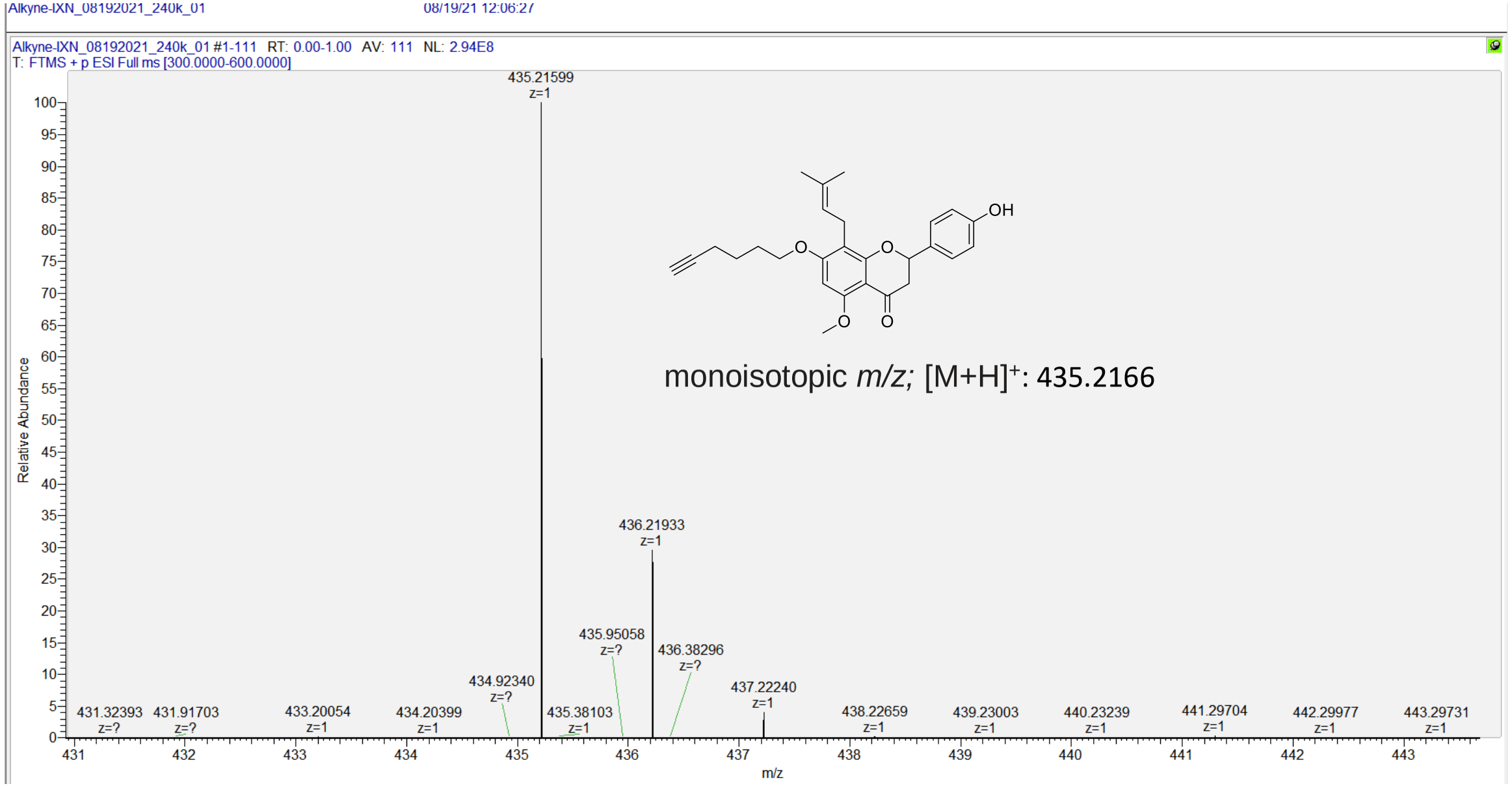
XN-DA_08192021_240k_01 08/19/21 11:49:38

XN-DA_08192021_240k_01 #1-110 RT: 0.00-1.00 AV: 110 NL: 1.06E7
T: FTMS + p ESI Full ms [300.0000-600.0000]

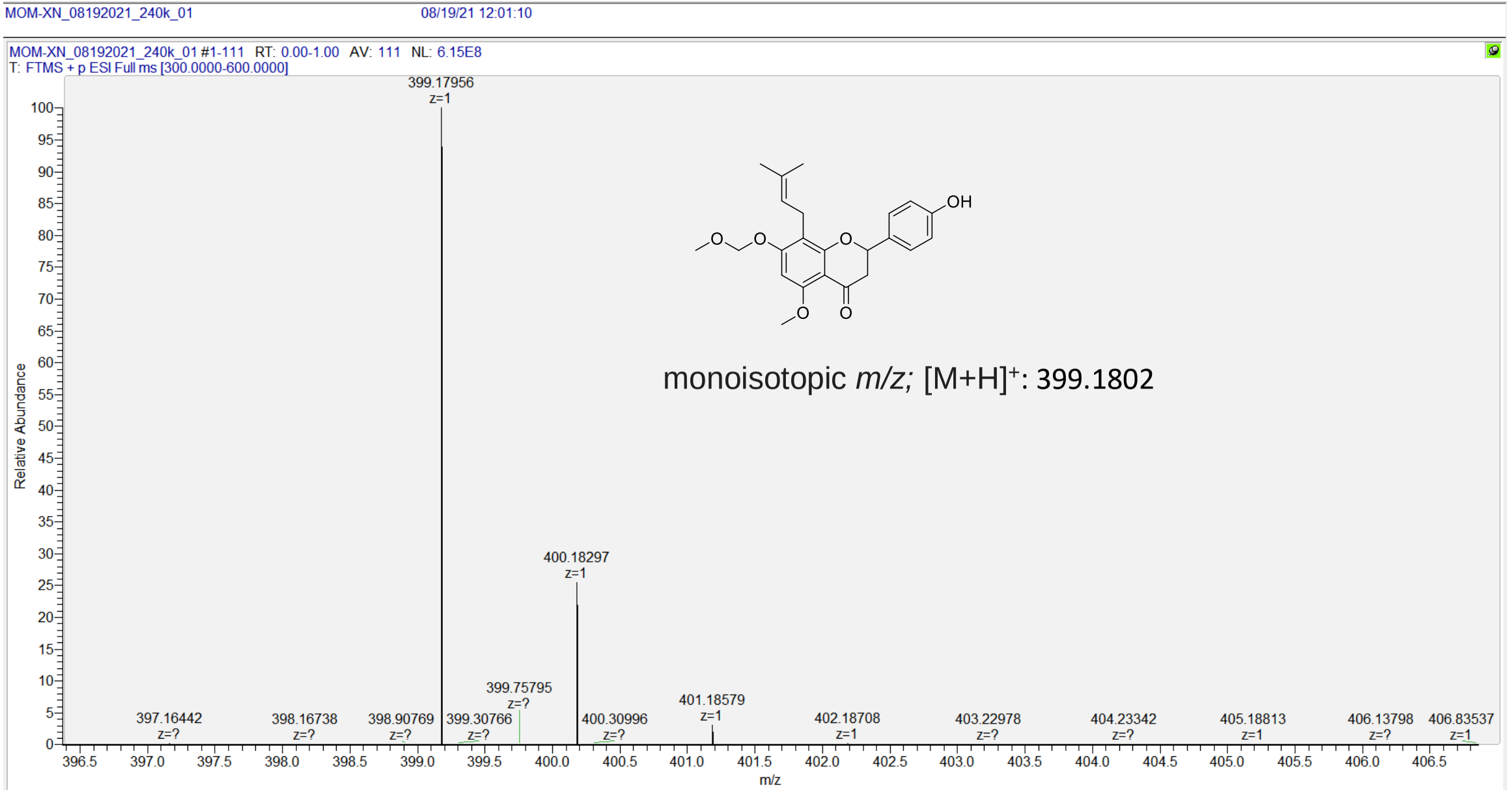


monoisotopic *m/z;* $[M+H]^+$: 475.2227

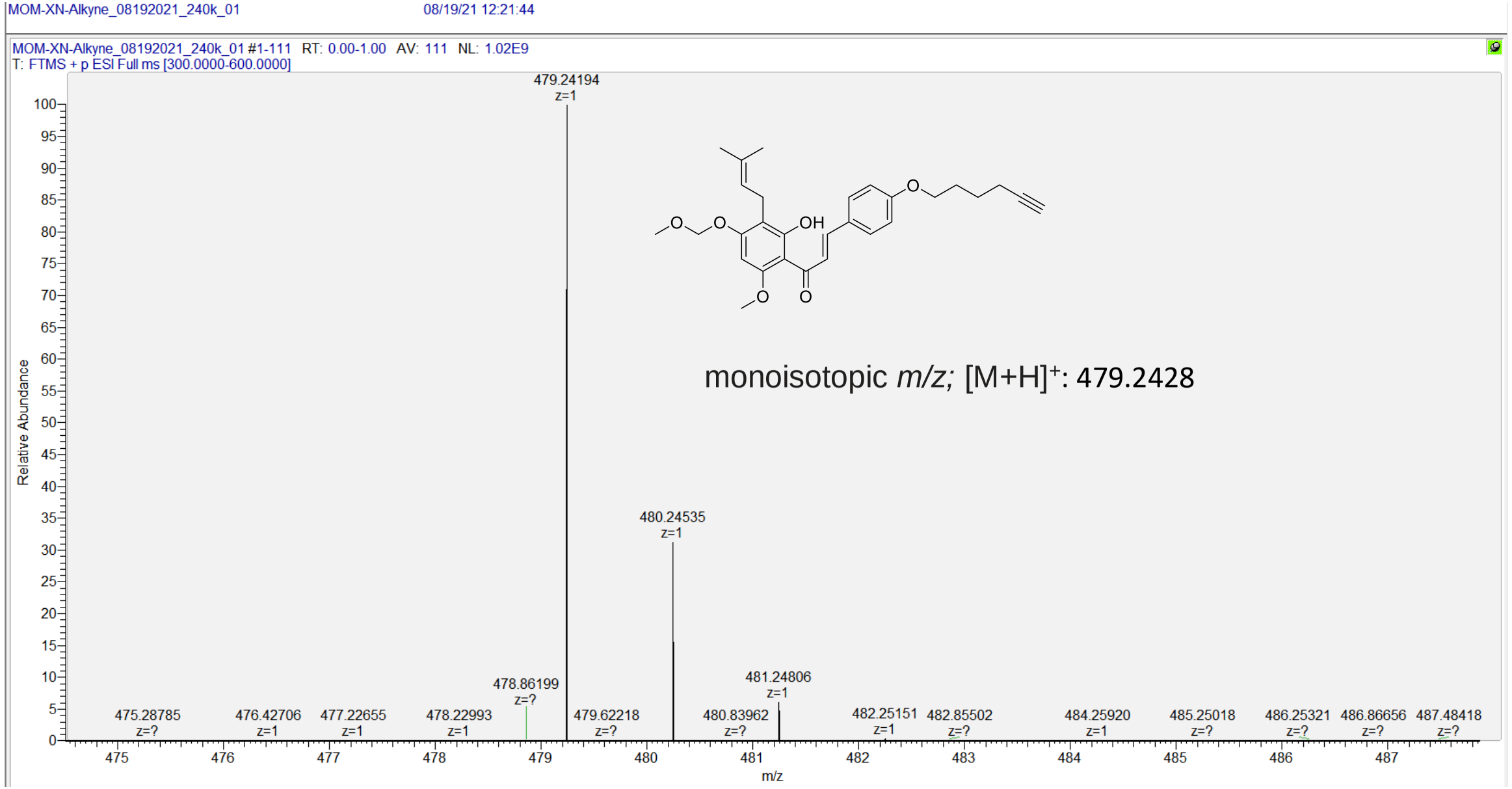
IXN-ABP-2 434 Run 01



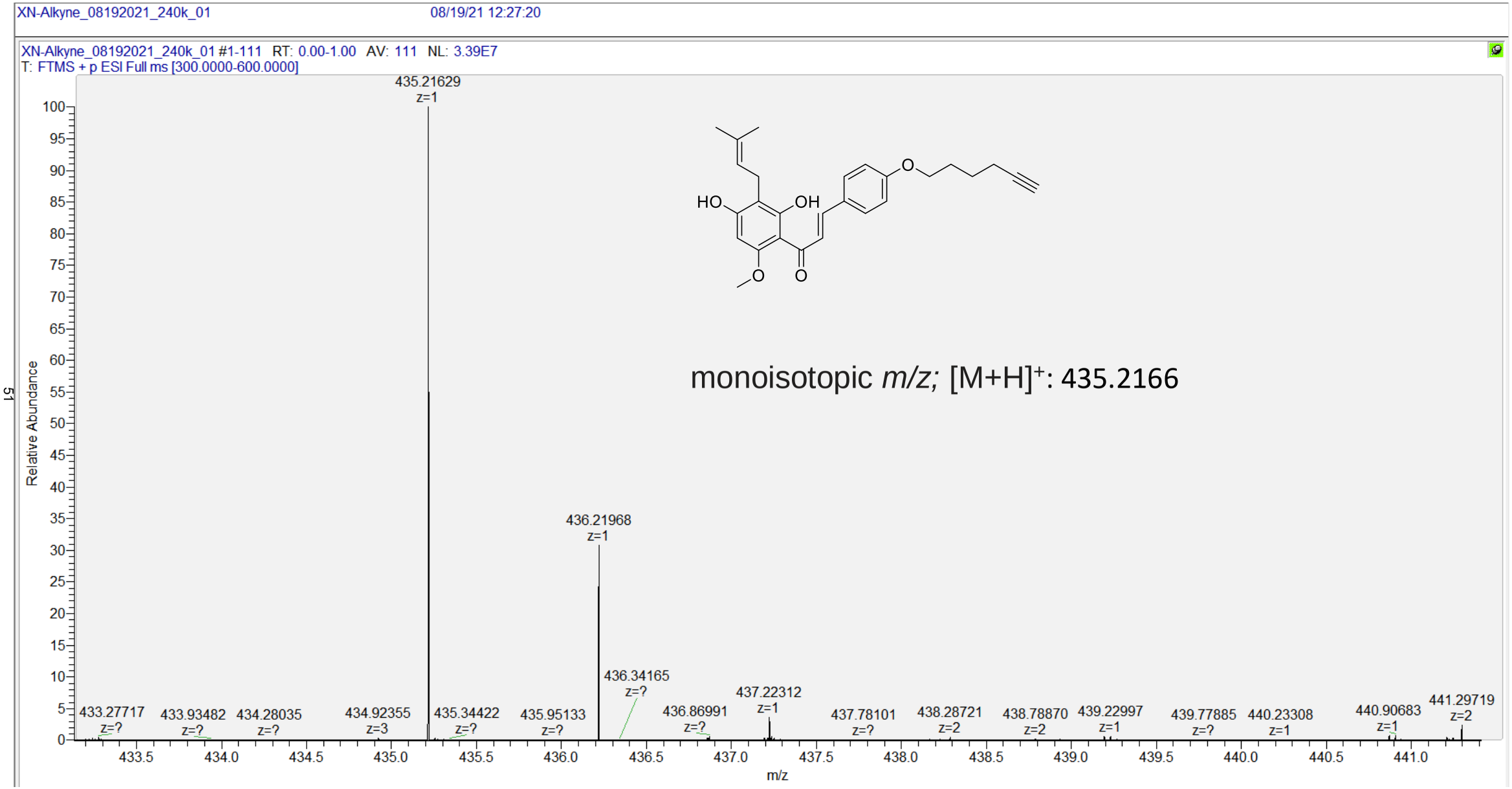
MOM-IXN 398 Run 01



MOM-XN-Alkyne 478 Run 01



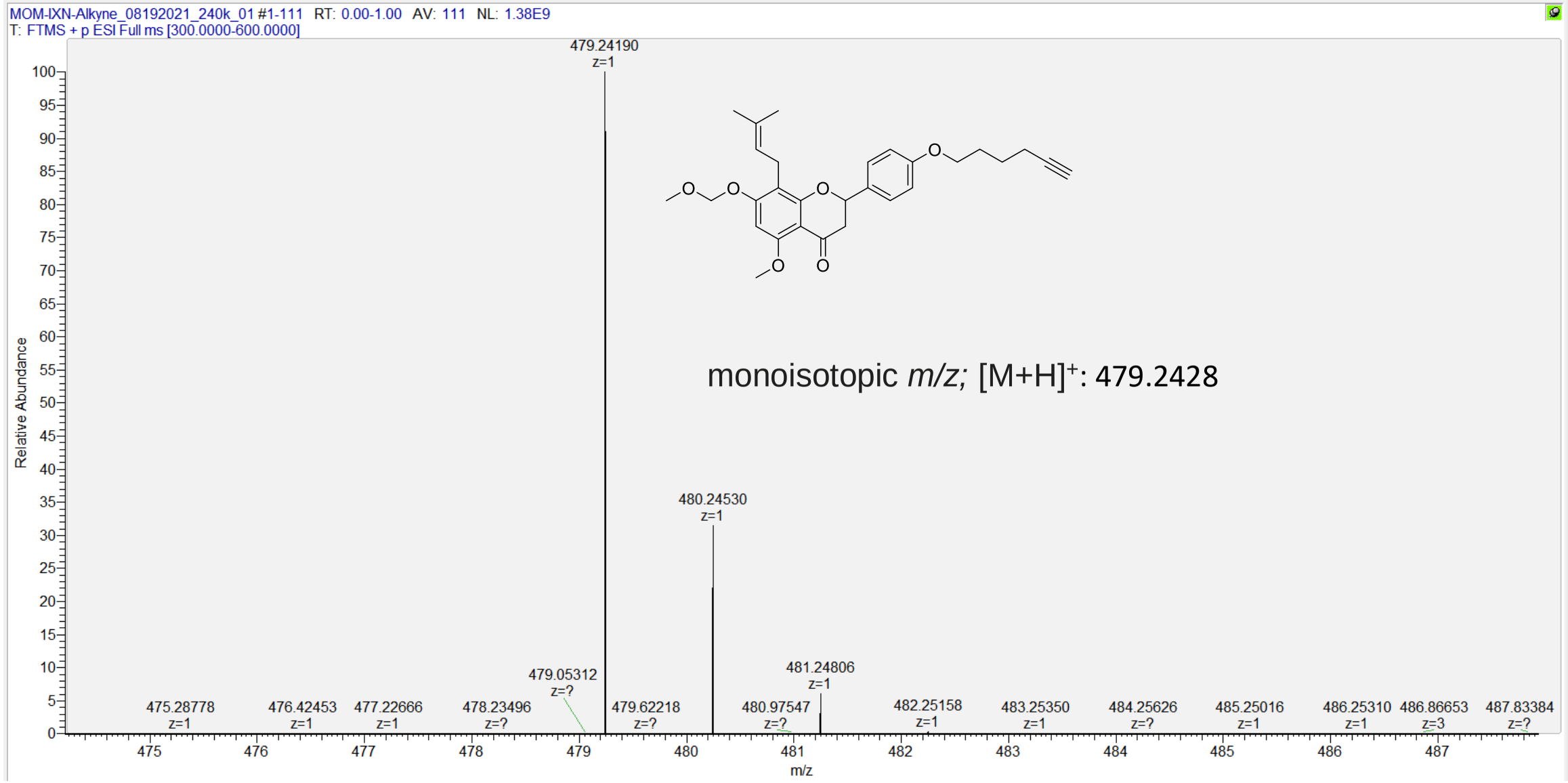
XN-ABP-4 434 Run 01



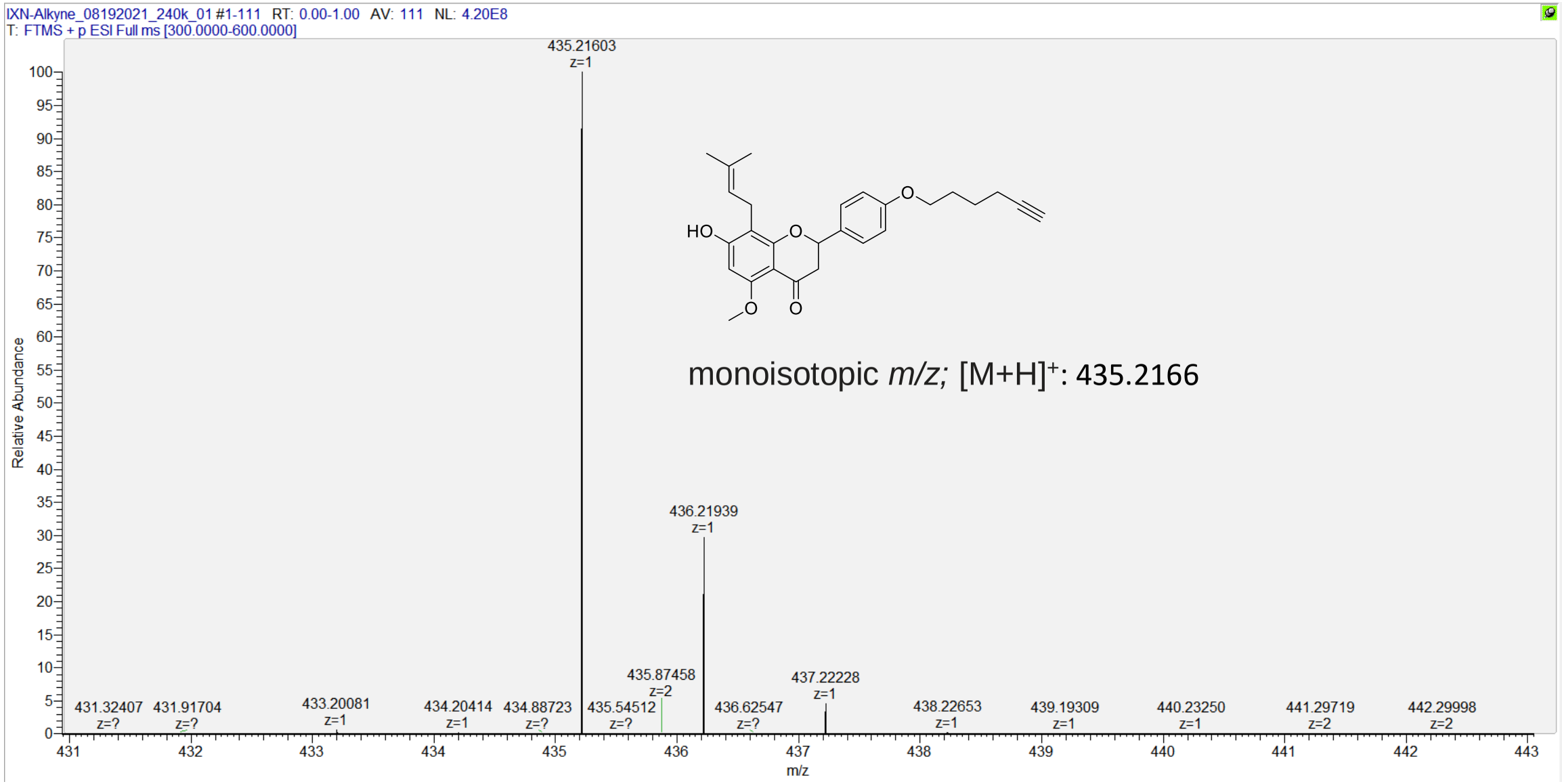
monoisotopic *m/z;* $[M+H]^+$: 435.2166

MOM-IXN-Alkyne 478 Run 01

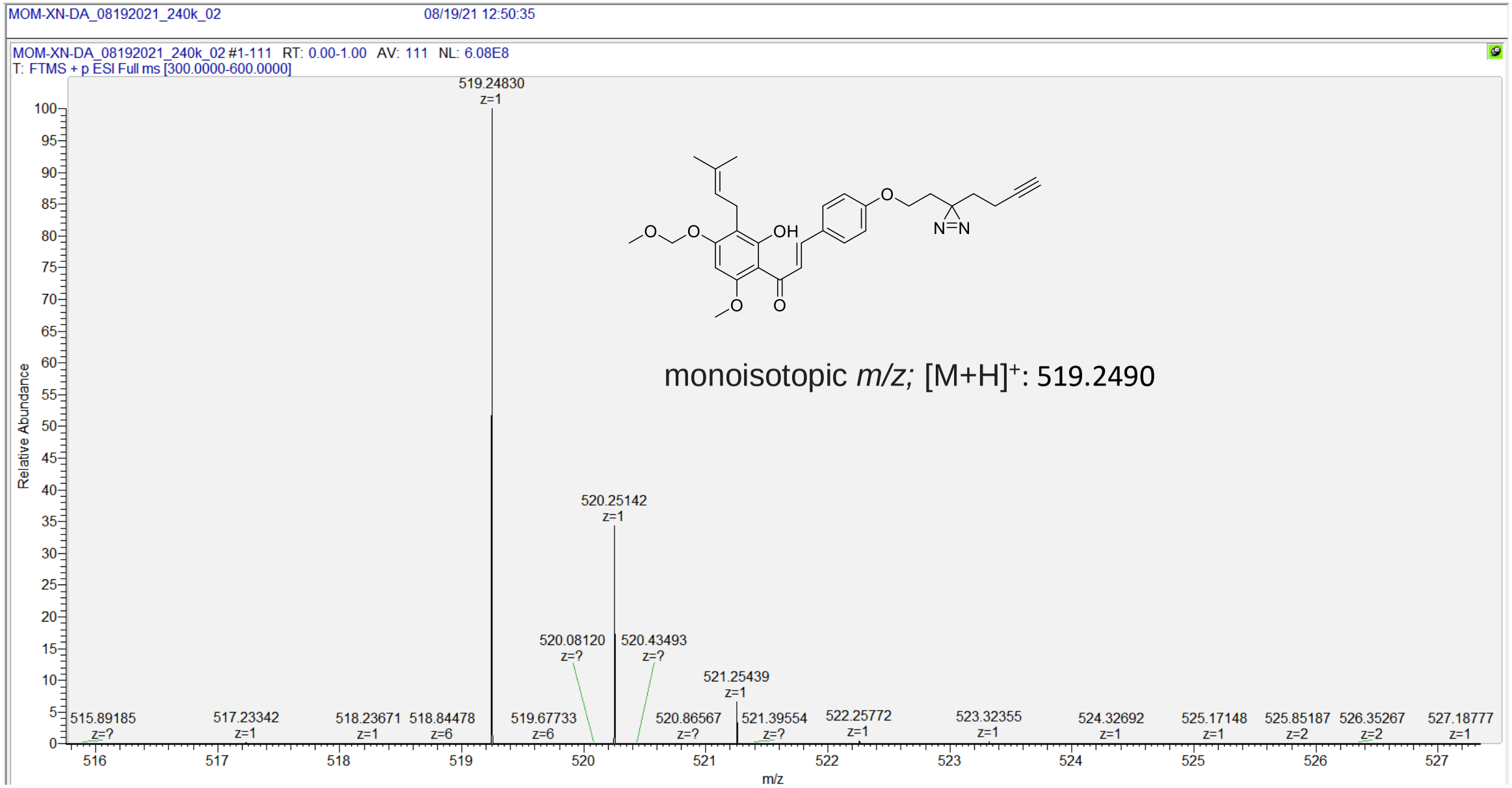
MOM-IXN-Alkyne_08192021_240k_01 08/19/21 12:32:21



IXN-ABP-4 434 Run 01



MOM-XN-DA 519 Run 02

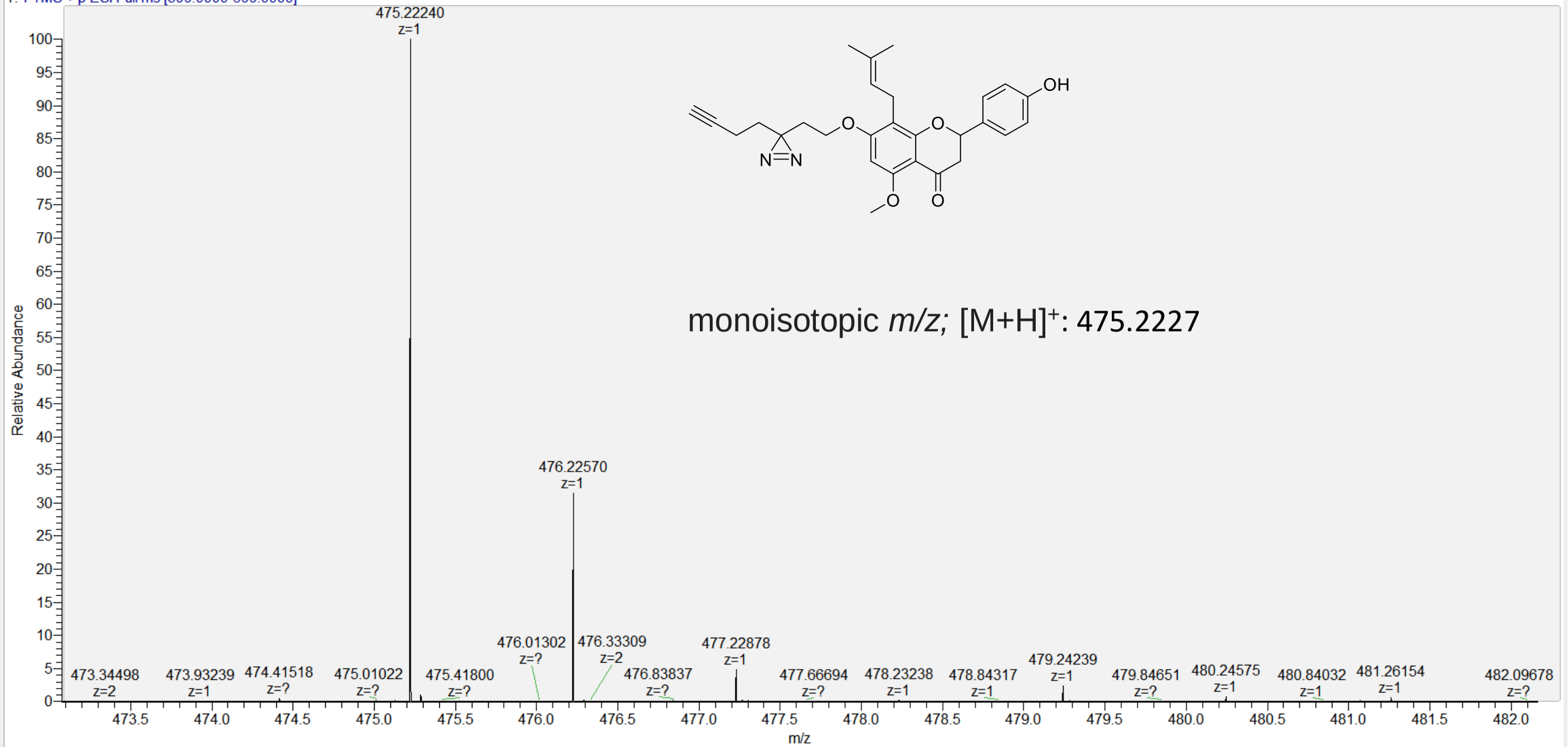


monoisotopic *m/z;* $[M+H]^+$: 519.2490

IXN-ABP-1 474 Run 01

DA-IXN_08192021_240k_01 08/19/21 12:54:10

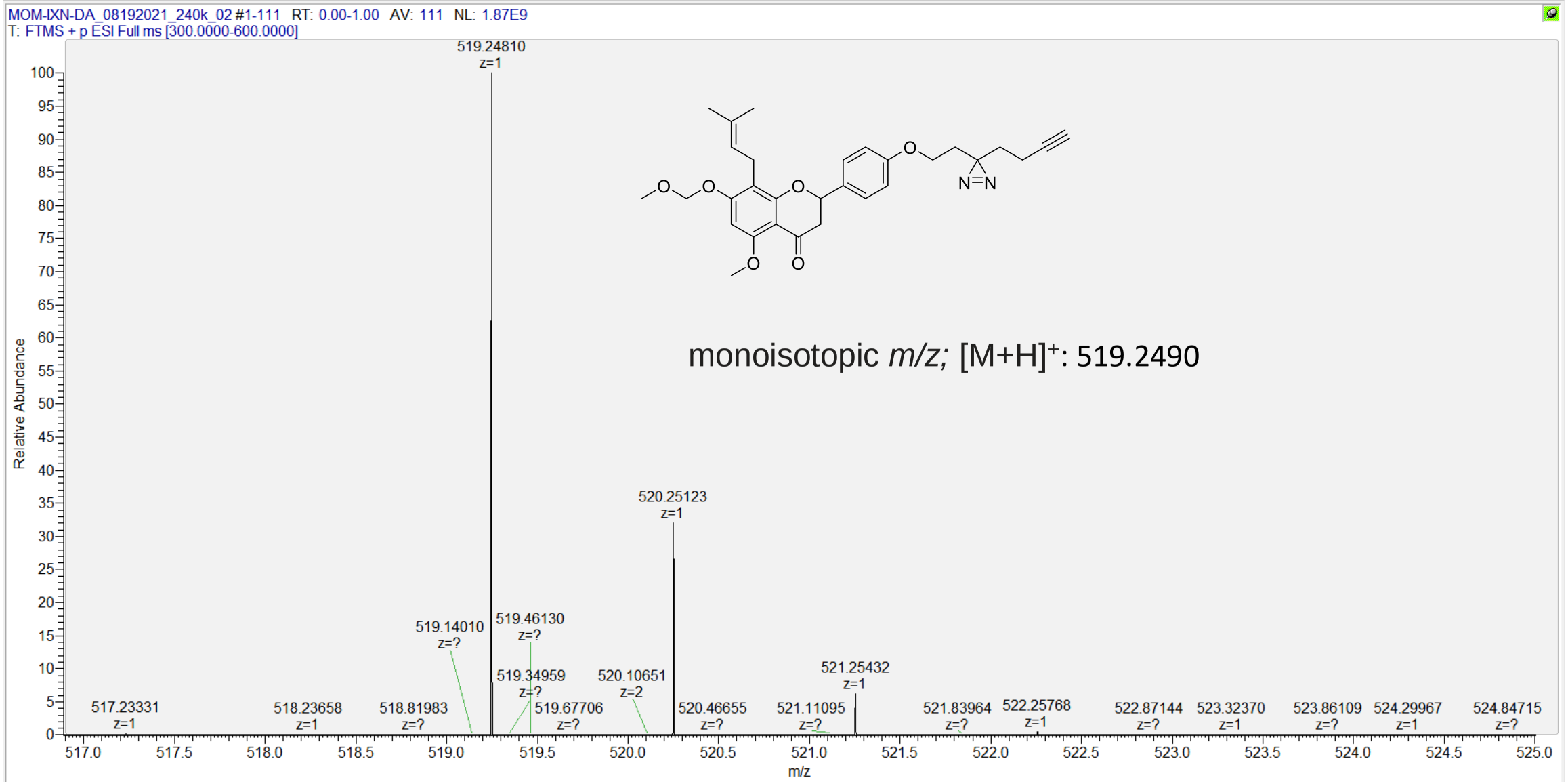
DA-IXN_08192021_240k_01 #1-111 RT: 0.00-1.00 AV: 111 NL: 7.08E7
T: FTMS + p ESI Full ms [300.0000-600.0000]



monoisotopic *m/z;* $[M+H]^+$: 475.2227

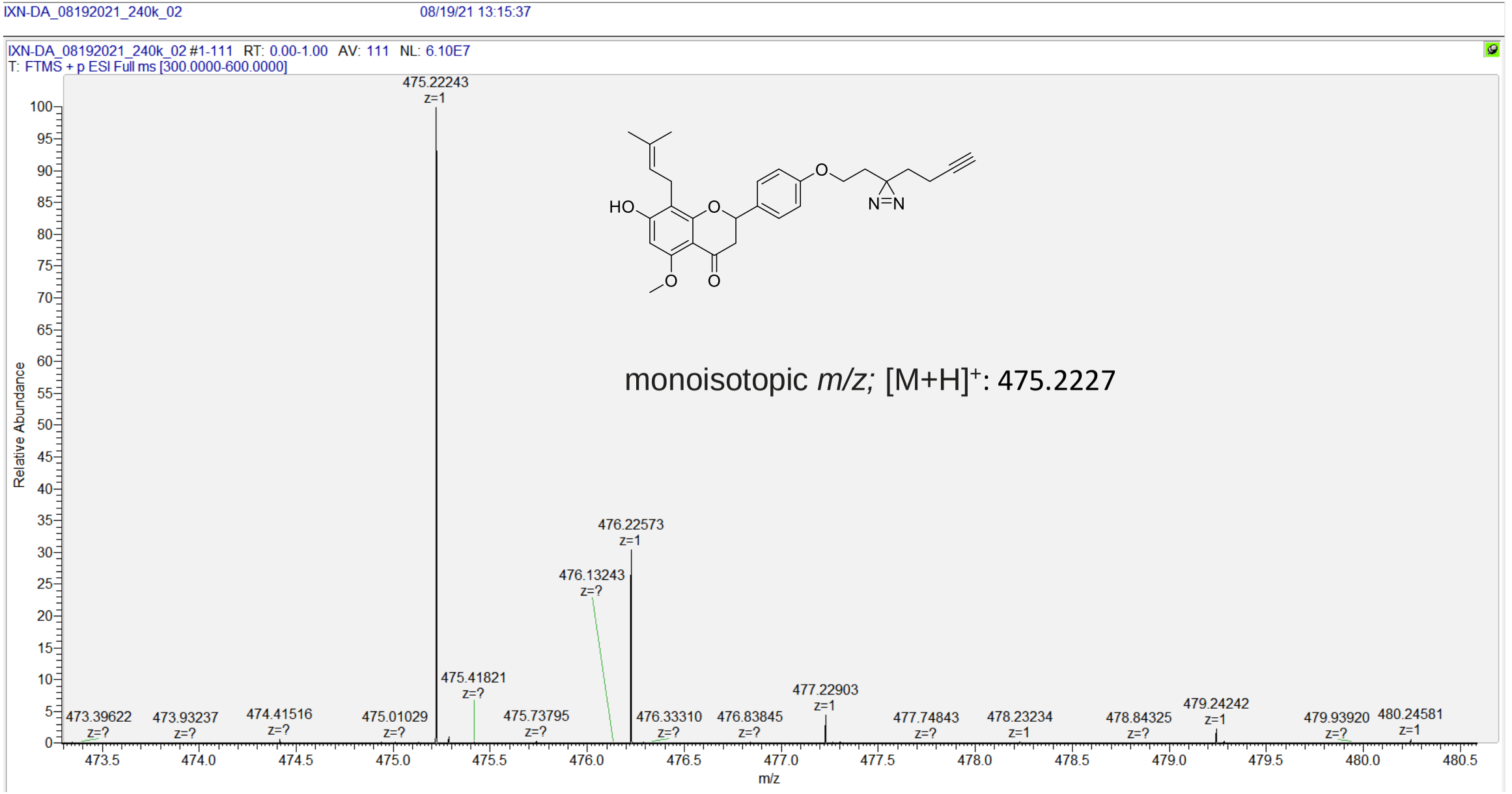
MOM-IXN-DA 518 Run 02

MOM-IXN-DA_08192021_240k_02 08/19/21 13:07:47

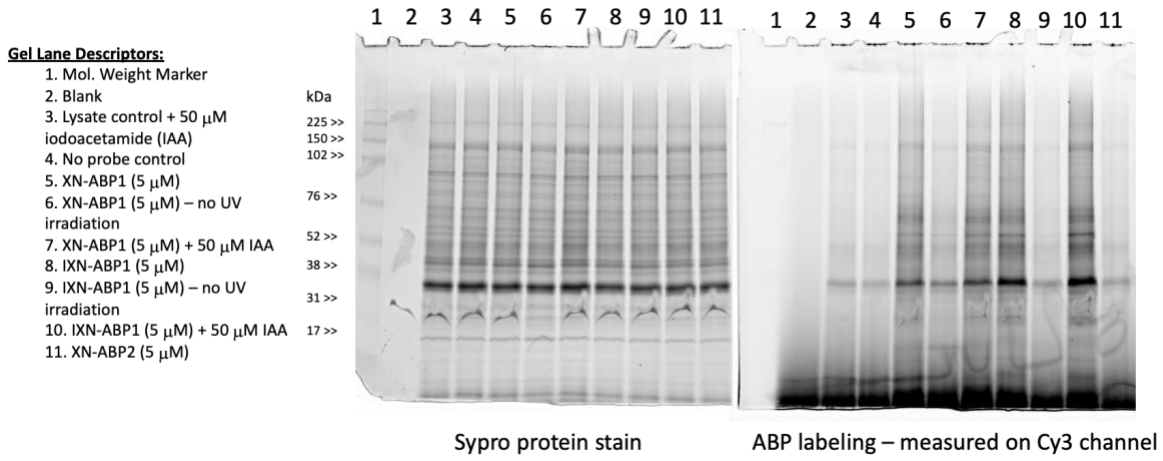


monoisotopic *m/z;* $[M+H]^+$: 519.2490

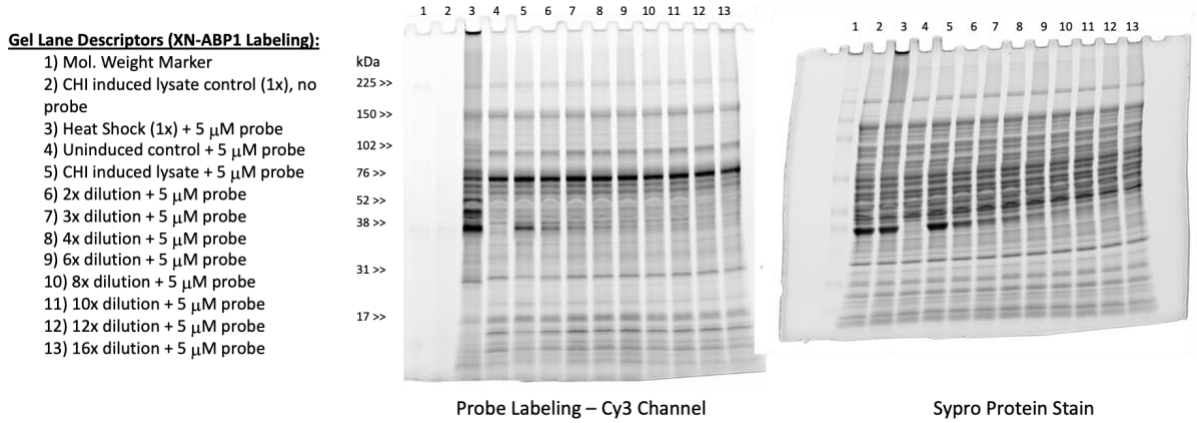
IXN-ABP-3 474 Run 02



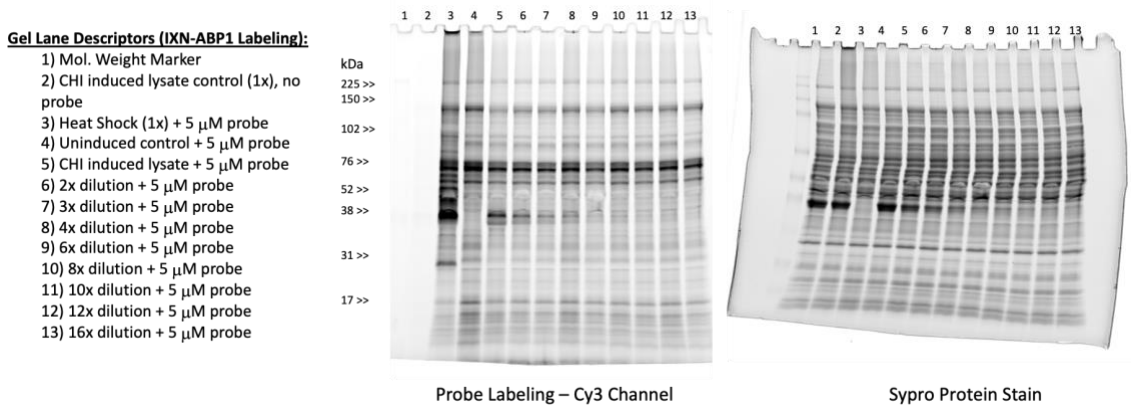
Supplemental Figures



Supplemental Figure S1. Full gel of labeling shown in manuscript Figure 3. Chalcone isomerase overexpressing *E. coli* BL21. Left – protein staining of all sample lanes to show consistent protein loading across all lanes. Right – affinity- and activity-based XN and IXN labeling of proteins.



Supplemental Figure S2a. Full gel of labeling shown in manuscript Figure 4 (top panel). Chalcone isomerase overexpressing *E. coli* BL21. Right – protein staining of all sample lanes to show consistent protein loading across all lanes and the dilution affect. Left – affinity-based XN-ABP1 labeling of proteins.



Supplemental Figure S2b. Full gel of labeling shown in manuscript Figure 4 (bottom panel). Chalcone isomerase overexpressing *E. coli* BL21. Right – protein staining of all sample lanes to show consistent protein loading across all lanes and the dilution affect. Left – affinity-based IXN-ABP1 labeling of proteins.

References

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2) L. N. Anderson, P. K. Koech, A. E. Plymale, E. V. Landorf, A. Konopka, F. R. Collart, M. S. Lipton, M. F. Romine and A. T. Wright, Live Cell Discovery of Microbial Vitamin Transport and Enzyme-Cofactor Interactions. *ACS Chem. Biol.* 2016, **11**, 34554.

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