

## Supporting Information

### Rational Chemical Design of Molecular Glue Degraders

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## **Supporting Methods**

### **Cell Culture**

C33A cells were purchased from the American Type Culture Collection (ATCC) and were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS) and maintained at 37 °C with 5% CO<sub>2</sub>. 22RV1 cells were purchased from the ATCC and were cultured in RPMI-1640 Medium containing 10% (v/v) FBS and maintained at 37 °C with 5% CO<sub>2</sub>. HEK293T cells were obtained from the UC Berkeley Cell Culture Facility and were cultured in DMEM containing 10% (v/v) FBS and maintained at 37 °C with 5% CO<sub>2</sub>. K562 cells were obtained from the UC Berkeley Cell Culture Facility and were cultured in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% (v/v) FBS and maintained at 37 °C with 5% CO<sub>2</sub>. MV-4-11 cells were obtained from the ATCC and were cultured in IMDM containing 10% (v/v) FBS and maintained at 37 °C with 5% CO<sub>2</sub>. A549 cells were obtained from the ATCC and were cultured in F-12K Medium containing 10% (v/v) FBS and maintained at 37 °C with 5% CO<sub>2</sub>. Mino cells were obtained from the ATCC and were cultured in RPMI-1640 Medium containing 10% (v/v) FBS and maintained at 37 °C with 5% CO<sub>2</sub>. LNCaP cells were obtained from the UC Berkeley Cell Culture Facility and were cultured in RPMI-1640 Medium containing 10% (v/v) FBS and maintained at 37 °C with 5% CO<sub>2</sub>. Unless otherwise specified, all cell culture materials were purchased from Gibco. It is not known whether HEK293T cells are from male or female origin.

### **Expression and purification of recombinant RNF126 protein**

RNF126 mammalian expression plasmid with a C-terminal FLAG tag was purchased from Origene (Origene Technologies Inc., RC204986). The plasmid was transformed into NEB 5-alpha Competent E. coli (DH5 $\alpha$ ) cells (NEB product no. C2987H). The following day, a single transformed colony was used to inoculate 50 ml of nutrient rich LB medium containing kanamycin (50  $\mu$ g/mL) and was incubated at 37 °C overnight, with agitation (250 rpm). A Miniprep (Qiagen) kit was used to isolate the plasmid before sequence verification with appropriate primers.

HEK293T cells were grown to 30-50% confluency in DMEM supplemented with 10% FBS (Corning) and maintained at 37 °C with 5% CO<sub>2</sub>. Immediately before transfection, media was replaced with DMEM

containing 10% FBS. Each plate was transfected with 24 µg of overexpression plasmid with 24 µL Lipofectamine 2000 (Invitrogen) in Opti-MEM. After 48 h cells were collected in PBS, lysed by sonication, and batch bound with anti-DYKDDDDK resin (GenScript, L00432) for 2 hours. Lysate and resin were washed with PBS and eluted with 133.33 µg/mL 3X FLAG peptide (ApexBio, A6001) in PBS. Five elutions were performed for 15 minutes each. Elutions were concentrated and the protein was stored in PBS. Concentration and purity was determined using the BCA assay and Western blotting.

### **Preparation of Cell Lysates**

Cells were washed twice with cold PBS, scraped, and pelleted by centrifugation (700 g, 5 min, 4 °C). Pellets were resuspended in PBS, lysed by sonication or RIPA lysis buffer (Thermo Scientific), clarified by centrifugation (12,000 g, 10 min, 4 °C), and lysate was transferred to new low-adhesion microcentrifuge tubes. Proteome concentrations were determined using the BCA assay and lysate was diluted to appropriate working concentrations.

### **Western Blotting**

Proteins were resolved by SDS/PAGE and transferred to nitrocellulose membranes using the Trans-Blot Turbo transfer system (Bio-Rad). Membranes were blocked with 5% BSA in Tris-buffered saline containing Tween 20 (TBS-T) solution for 1 hr at RT, washed in TBS-T, and probed with primary antibody diluted in recommended diluent per manufacturer overnight at 4 °C. After 3 washes with TBS-T, the membranes were incubated in the dark with IR680- or IR800-conjugated secondary antibodies at 1:10,000 dilution in 5 % BSA in TBS-T at RT for 1 h. After 3 additional washes with TBST, blots were visualized using an Odyssey Li-Cor fluorescent scanner. The membranes were stripped using ReBlot Plus Strong Antibody Stripping Solution (EMD Millipore, 2504) when additional primary antibody incubations were performed. Antibodies used in this study were CDK4 (Abcam ab108357), CDK6 (Cell Signaling Technology DCS83), Vinculin (Abcam ab129002), GAPDH (Cell Signaling Technology 14C10), RNF126 (Santa Cruz Biotechnology sc-376005), BRD4 (Abcam ab128874), BRD2 (Cell Signaling Technology D89B4), BRD3 (Abcam ab300106), Beta Actin (Cell Signaling Technology 13E5), PDE5 (Abcam ab259945), AR-V7 (Abcam ab273500), c-Abl (Santa Cruz Biotechnology sc-23), SMARCA2 (Abcam ab240648), BRG1 (SMARCA4) (Cell Signaling Technology D1Q7F), LRRK2 (Abcam

ab133474), BTK (Cell Signaling Technology D3H5), Androgen Receptor (Cell Signaling Technology D6F11), HDAC1 (Cell Signaling Technology 10E2), HDAC2 (Cell Signaling Technology 3F3), HDAC3 (Cell Signaling Technology 7G6C5), and HDAC6 (Cell Signaling Technology D2E5).

### **Knockdown studies**

Short-hairpin oligonucleotides were used to knock down the expression of RNF126 in C33A cells. For lentivirus production, lentiviral plasmids and packaging plasmids (pMD2.G, Addgene catalog no. 12259 and psPAX2, Addgene catalog no. 12260) were transfected into HEK293T cells using Lipofectamine 2000 (Invitrogen). Lentivirus was collected from filtered cultured medium and used to infect the target cell line with 1:1000 dilution of polybrene. Target cells were selected over 3 d with 1  $\mu$ g/mL of puromycin for C33A cells. The short-hairpin sequences which were used for generation of the knockdown lines were:

RNF126: TGCCATCATCACACAGCTCCT (Sigma RNF126 MISSION shRNA Bacterial Glycerol Stock, TRCN0000368954). MISSION TRC1.5 pLKO.1- or TRC2 pLKO.5-puro Non-Mammalian shRNA Control (Sigma) was used as a control shRNA.

### **IsoDTB-ABPP Cysteine Chemoproteomic Profiling of EST1027**

C33A cells were treated with either EST1027 (20  $\mu$ M) or DMSO for 2 h before cell collection and lysis. The proteome concentrations were determined using BCA assay and adjusted to 2 mg/mL. For each biological replicate, 2 aliquots of 1 mL of 2 mg/mL were used (i.e. 4 mg per condition). Each aliquot was treated with 20  $\mu$ L of IA-alkyne (26.6 mg/mL in DMSO, 200  $\mu$ M final concentration) for 1 h at RT. Two master mixes of the click reagents were prepared in the meanwhile, each containing 510  $\mu$ L TBTA (0.9 mg/mL in 4:1 tBuOH/DMSO), 165  $\mu$ L CuSO<sub>4</sub> (12.5 mg/mL in H<sub>2</sub>O), 165  $\mu$ L TCEP (14.0 mg/mL in H<sub>2</sub>O) and 160  $\mu$ L of either heavy or light isoDTB tags (4 mg in DMSO, Click Chemistry Tools, 1565). The samples were then treated with 120  $\mu$ L of the heavy (DMSO treated) or light (compound treated) master mix for 1 h at RT. After incubation, one light and one heavy-labeled samples were combined and acetone-precipitated overnight at -20 °C. The samples were then centrifuged at 3,500 rpm for 10 min, acetone was removed, and the protein pellets resuspended in cold MeOH by sonication. The samples were centrifuged at 3,500 rpm for 10 min and MeOH was removed (repeated 3 $\times$  in

total). The pellets were dissolved in 600  $\mu$ L urea (8M in 0.1 M TEAB) by sonication and the urea concentration was then adjusted to 2M by adding 1800  $\mu$ L of TEAB (0.1 M). Two tubes containing solubilized proteins were combined, further diluted with 2400  $\mu$ L 0.2% NP40 in PBS, and bound to high-capacity streptavidin agarose beads (200  $\mu$ L/sample, ThermoFisher, 20357) for 1 h at RT with mixing. The beads were then centrifuged for 1 min at 1,000 g, the supernatant was removed, and the beads were washed 3 times with 0.1% NP40 in PBS, 3 times with PBS and 3 times with H<sub>2</sub>O. The samples were then resuspended in 8M urea (600  $\mu$ L in 0.1 M TEAB) and treated with DTT (30  $\mu$ L, 31 mg/mL in H<sub>2</sub>O) for 45 min at 37 °C. They were then reacted with iodoacetamide (30  $\mu$ L, 74 mg/mL in H<sub>2</sub>O) for 30 min at RT, followed by DTT (30  $\mu$ L, 31 mg/mL in H<sub>2</sub>O) for 30 min at RT. The samples were diluted with 1800  $\mu$ L TEAB (0.1 M), centrifuged for 1 min at 1,000 g, and the supernatant was removed. The beads were resuspended in 400  $\mu$ L urea (2M in 0.1 M TEAB), and trypsin (8  $\mu$ L, 0.5 mg/mL) was added and incubated for 20 h at 37 °C. The samples were then diluted with 800  $\mu$ L 0.1% NP40 in PBS and the beads were washed 3 times with 0.1% NP40 in PBS, 3 times with PBS, and 3 times with H<sub>2</sub>O. Peptides were then eluted with 0.1% formic acid in 50% acetonitrile (3  $\times$  400  $\mu$ L). The samples were then dried using a vacuum concentrator at 30 °C, resuspended in 300  $\mu$ L 0.1% TFA in H<sub>2</sub>O, and fractionated using high pH reversed-phase peptide fractionation kits (ThermoFisher, 84868) according to the manufacturer's protocol.

### **IsoDTB-ABPP Mass Spectrometry Analysis**

Mass spectrometry analysis was performed on an Orbitrap Eclipse Tribrid Mass Spectrometer with a High Field Asymmetric Waveform Ion Mobility (FAIMS Pro) Interface (Thermo Scientific) with an UltiMate 3000 Nano Flow Rapid Separation LCnano System (Thermo Scientific). Off-line fractionated samples (5  $\mu$ L aliquot of 15  $\mu$ L sample) were injected via an autosampler (Thermo Scientific) onto a 5  $\mu$ L sample loop which was subsequently eluted onto an Acclaim PepMap 100 C18 HPLC column (75  $\mu$ m  $\times$  50 cm, nanoViper). Peptides were separated at a flow rate of 0.3  $\mu$ L/min using the following gradient: 2 % buffer B (100 % acetonitrile with 0.1 % formic acid) in buffer A (95:5 water:acetonitrile, 0.1 % formic acid) for 5 min, followed by a gradient from 2 to 40 % buffer B from 5 to 159 min, 40 to 95 % buffer B from 159 to 160 minutes, holding at 95 % B from 160-179 min, 95 % to 2 % buffer B from 179 to 180 min, and then 2 % buffer B from 180 to 200 min. Voltage applied to the nano-LC electrospray ionization source was 2.1 kV. Data was acquired through an MS1 master scan (Orbitrap analysis, resolution 120,000, 400-1800 m/z, RF lens 30 %, heated capillary temperature 250 °C) with dynamic exclusion

enabled (repeat count 1, duration 60 s). Data-dependent data acquisition comprised a full MS1 scan followed by sequential MS2 scans based on 2 s cycle times. FAIMS compensation voltages (CV) of -35, -45, and -55 were applied. MS2 analysis consisted of: quadrupole isolation window of 0.7 m/z of precursor ion followed by higher energy collision dissociation (HCD) energy of 38 % with a orbitrap resolution of 50,000.

Data were extracted in the form of MS1 and MS2 files using Raw Converter (Scripps Research Institute) and searched against the Uniprot human database using ProLuCID search methodology in IP2 v.3-v.5 (Integrated Proteomics Applications, Inc.)<sup>1</sup>. Cysteine residues were searched with a static modification for carboxyamino-methylation (+57.02146) and up to two differential modifications for methionine oxidation and either the light or heavy isoDTB tags (+561.33872 or +567.34621, respectively). Peptides were required to be fully tryptic peptides. ProLUCID data were filtered through DTASelect to achieve a peptide false-positive rate below 5%. Only those probe-modified peptides that were evident across two out of three biological replicates were interpreted for their isotopic light to heavy ratios. Light versus heavy isotopic probe-modified peptide ratios are calculated by taking the mean of the ratios of each replicate paired light versus heavy precursor abundance for all peptide-spectral matches associated with a peptide. The paired abundances were also used to calculate a paired sample *t*-test *P* value in an effort to estimate constancy in paired abundances and significance in change between treatment and control. *P* values were corrected using the Benjamini–Hochberg method.

### **Gel-Based ABPP**

Recombinant RNF126 (0.1 µg/sample) was pre-treated with either DMSO vehicle or covalent ligand at 37 °C for 30 min in 25 µL of PBS, and subsequently treated with of IA-Rhodamine (concentrations designated in figure legends) (Setareh Biotech) at room temperature for 1 h in the dark. The reaction was stopped by addition of 4×reducing Laemmli SDS sample loading buffer (Alfa Aesar). After boiling at 95 °C for 5 min, the samples were separated on precast 4–20% Criterion TGX gels (Bio-Rad). Probe-labeled proteins were analyzed by in-gel fluorescence using a ChemiDoc MP (Bio-Rad).

### **NMR Spectroscopy**

We recorded all NMR spectra on Bruker Avance III 600 MHz (using 3 mm tubes filled with 160  $\mu$ L sample) and Bruker Neo 600 MHz (using 1.7 mm tubes filled with 40  $\mu$ L of sample) spectrometers, equipped with either a 5 mm QCI-F cryo probe with z-gradient or a 1.7 mm TCI cryo probe, and kept the temperature constant at 298K during our experiments. Standard Bruker  $^1\text{H}$ -1D (with excitation sculpting water suppression) and  $^1\text{H}$ ,  $^{15}\text{N}$ -SOFAST-HMQC experiments were conducted while using traditional or 50% non-uniform sampling<sup>2</sup>. Our samples contained 50  $\mu$ M or 100  $\mu$ M human wt-(U)- $^{15}\text{N}$ -RNF126(1-40), unlabeled 50  $\mu$ M BRD4(44-168) (for protein-protein binding experiments only), 5% (3 mm tubes) or 10% (1.7 mm tubes)  $\text{D}_2\text{O}$ , 25 mM Hepes pH 7.5, 150 mM NaCl, 11.1  $\mu$ M DSS, 2%  $\text{d}_6$ -DMSO, 0-50  $\mu$ M d-TCEP, and 100  $\mu$ M or 200  $\mu$ M of compounds (for samples with compound; DMSO-matched). All spectra were recorded at two time points (incubation times of 30 minutes and 24 hours) to detect time-dependent ligand binding effects.

We assigned most peaks by transferring the published resonance assignments onto our amide spectrum<sup>3</sup>. To localize the ligand-interaction site, we clustered compound-induced chemical shift perturbations and line broadening effects in groups with very strong, strong, medium, weak and no residue-specific spectral changes. Clusters were subsequently mapped onto the three-dimensional structure of RNF126(1-40) (PDB code: 2N9O)<sup>3</sup>.

### **Protein Expression for NMR Studies**

Plasmid construct 8xHis-Thioredoxin-TEV-RNF126(1-40) was transformed into BL21(DE3) competent cells and grown overnight on LB-agar plates. A single colony was used to inoculate a 3 mL LB + kanamycin starter culture, which was grown shaking at 37 °C for 4-6 hours. For each liter of culture, 50 mL of expression media (per liter: 50 mL 20X M9 salts dissolved in sterile water, 2 mM  $\text{MgSO}_4$ , 1 mL vitamin solution [Sigma R7256], 0.1 mL 1000X trace elements [made in-house], 1 g  $^{15}\text{N}$ - $\text{NH}_4\text{Cl}$ , 3 g glucose, 50 mg kanamycin, 0.05 mM  $\text{ZnCl}_2$ , 0.25 g ISOGRO- $^{15}\text{N}$  [Sigma 606871], sterile water to volume) was inoculated with 0.5 mL of the starter culture and grown overnight shaking at 37 °C. The 50 mL culture was used to inoculate 950 mL of expression media until the  $\text{OD}_{600}$  reached 0.7-0.8, at which point the culture was induced with 0.5 mM IPTG and the temperature reduced to 18 °C. The culture was grown overnight (~18 hours) and harvested via centrifugation. Pellets were stored at -80 °C.

A pET vector containing 6xHis-TEV-BRD4(44-168) was used to transform BL21(DE3) *E. coli* using standard techniques. 1 L cultures were grown in Terrific Broth supplemented with 50 mM Sodium Phosphate pH 7.0 and 50 µg/mL kanamycin. Cells were induced when the OD600 reached 1.0-1.5 and were left to continue to grow overnight at 19 °C. Cells were harvested by centrifugation and lysed via cell homogenizer (Lysis Buffer: 50 mM Tris pH 8.0, 400 mM NaCl, 1 mM TCEP).

### **Protein Purification for NMR Studies**

RNF126(1-40) cell pellets were thawed and resuspended in cold lysis buffer (20 mM HEPES, pH 8.0, 500 mM NaCl, 30 mM Imidazole) at a ratio of 1 g cells to 5 mL buffer. Cells were disrupted via sonication and separated from the supernatant via centrifugation. 1 mL of loose Ni Sepharose 6 Fast Flow resin per 50 mL of supernatant was washed three times in lysis buffer, then incubated with the supernatant for 2-3 hours, rotating at 4 °C. The slurry was washed with lysis buffer in a drip column until the flow-through no longer contained protein (measured via NanoDrop). The protein was eluted in 10 x 4 mL steps with the imidazole concentration increasing by 30 mM each step. Cleavage with TEV protease was performed via overnight dialysis in a 1000 MWCO dialysis cassette against dialysis buffer (20 mM HEPES, pH 8.0, 500 mM NaCl) spinning at 4 °C. The protein solution was run through a HisTrap FF 5 mL on AKTA to separate the tag. 0.05 mM ZnCl<sub>2</sub> was added to the eluate which was concentrated using an Amicon 1K centrifugal filter. The sample was run on a Superdex 30 size exclusion column with SEC buffer (20 mM HEPES, pH 7.5, 150 mM NaCl). Protein yield was estimated to be between 0.5 and ~1 mg per liter according to a NanoDrop readout.

BRD4 cell lysate was clarified via centrifugation (45,000 x g for 30 minutes) prior to IMAC purification using 5 mL Ni-NTA bulk resin pre-equilibrated with Lysis Buffer. The resin was then washed with 5 CV of Lysis Buffer followed by Lysis Buffer supplemented with increasing concentrations of imidazole (20 mM and 40 mM). The protein was eluted with 5 CV of Elution Buffer (50 mM Tris pH 8.0, 400 mM NaCl, 1 mM TCEP, 500 mM imidazole) and subsequently treated with TEV protease while dialyzing against 3 L of Lysis Buffer at 4 °C overnight. Cleaved protein was subject to reverse IMAC purification with 5 mL of Ni-NTA bulk resin pre-equilibrated with Lysis Buffer. The flow-through was collected, concentrated, and subjected to size exclusion chromatography using a Superdex 200 16/60 column attached to an AKTA FPLC. The column was pre-equilibrated with SEC Buffer (25 mM HEPES pH 7.5, 150 mM NaCl, 1 mM TCEP). Fractions containing protein



were pooled, concentrated, and aliquoted. Correct mass was confirmed by ESI-LC/MS and purity was confirmed by SDS-PAGE.

### **JP-2-196-Alkyne Pulldown Quantitative Proteomics**

Cells were treated with either DMSO vehicle or compound (JP-2-196-alkyne, 10  $\mu$ M) for 6 h. Cells were harvested and lysed by probe sonication in PBS. The proteome concentrations were determined using the BCA assay and adjusted to 5 mg/mL in a volume of 500  $\mu$ L PBS. For each biological replicate, 4 aliquots were used (i.e. 10 mg per condition). Copper-catalyzed azide-alkyne cycloaddition (CuAAC) was performed by sequential addition of 10  $\mu$ L TCEP (14.4 mg/mL in H<sub>2</sub>O), 30  $\mu$ L TBTA (0.9 mg/mL in 4:1 tBuOH/DMSO), 10  $\mu$ L CuSO<sub>4</sub> (12.5 mg/mL in H<sub>2</sub>O) and 10  $\mu$ L biotin picolyl azide (10 mM in DMSO). After 1 h, proteomes were precipitated by centrifugation at 6,500 g, washed in ice-cold methanol, combined to attain 10 mg per sample, washed again, then denatured and resolubilized by heating in 1.2% SDS-PBS (1 mL) to 90 °C for 5 min. The soluble proteome was diluted with 4 mL of PBS and labeled proteins were bound to high-capacity streptavidin-agarose beads (170  $\mu$ L/sample, Thermo Fisher Scientific, 20357) while rotating overnight at 4 °C. Bead-linked proteins were enriched by washing once with 0.2% SDS-PBS and 3 times each in PBS and water, then resuspended in 6 M urea/PBS (500  $\mu$ L), reduced in DTT (25  $\mu$ L, 30 mg/mL in H<sub>2</sub>O), and alkylated with iodoacetamide (74 mg/mL in H<sub>2</sub>O), before being washed and resuspended in 50 mM Triethylammonium bicarbonate (TEAB) (100  $\mu$ L) and trypsinized overnight with sequencing grade trypsin (4  $\mu$ L, 0.5 mg/mL). Tryptic peptides were eluted off through centrifugation. Individual samples were then labeled with isobaric tags using commercially available TMTsixplex (Thermo Fisher Scientific, P/N 90061) kits, in accordance with the manufacturer's protocols. Tagged samples (20  $\mu$ g per sample) were combined, dried using a vacuum concentrator at 30 °C, resuspended with 300  $\mu$ L 0.1% TFA in H<sub>2</sub>O, and fractionated using high pH reversed-phase peptide fractionation kits (Thermo Fisher Scientific, P/N 84868) according to the manufacturer's protocol. Fractions were dried with using a vacuum concentrator at 30 °C, resuspended with 50  $\mu$ L 0.1% FA in H<sub>2</sub>O, and analyzed by LC-MS/MS as described below.

Quantitative TMT-based proteomic analysis was performed as previously described using a Thermo Eclipse with FAIMS LC-MS/MS<sup>4</sup>. Acquired MS data was processed using ProLuCID search methodology in IP2 v.3-v.5 (Integrated Proteomics Applications, Inc.)<sup>1</sup>. Trypsin cleavage specificity (cleavage at K, R except if followed by P) allowed for up to 2 missed cleavages. Carbamidomethylation of cysteine was set as a fixed modification, methionine oxidation, and TMT-modification of N-termini and lysine residues were set as variable modifications. Reporter ion ratio calculations were performed using summed abundances with most confident centroid selected from 20 ppm window. Only peptide-to-spectrum matches that are unique assignments to a given identified protein within the total dataset are considered for protein quantitation. High confidence protein identifications were reported with a <1% false discovery rate (FDR) cut-off. Differential abundance significance was estimated using ANOVA with Benjamini-Hochberg correction to determine p-values.

### **Quantitative TMT Proteomics Analysis**

Cells were treated with either DMSO vehicle or compound (EST1027, 5  $\mu$ M, or JP-2-197, 1  $\mu$ M) for 24 h and lysate was prepared as described above. Briefly, 25-100  $\mu$ g protein from each sample was reduced, alkylated and tryptically digested overnight. Individual samples were then labeled with isobaric tags using commercially available TMTsixplex (Thermo Fisher Scientific, P/N 90061) kits, in accordance with the the manufacturer's protocols. Tagged samples (20  $\mu$ g per sample) were combined, dried using a vacuum concentrator at 30 °C, resuspended with 300  $\mu$ L 0.1% TFA in H<sub>2</sub>O, and fractionated using high pH reversed-phase peptide fractionation kits (Thermo Fisher Scientific, P/N 84868) according to the manufacturer's protocol. Fractions were dried using a vacuum concentrator at 30 °C, resuspended with 50  $\mu$ L 0.1% FA in H<sub>2</sub>O, and analyzed by LC-MS/MS as described below.

Quantitative TMT-based proteomic analysis was performed as previously described using a Thermo Eclipse with FAIMS LC-MS/MS<sup>4</sup>. Acquired MS data was processed using ProLuCID search methodology in IP2 v.3-v.5 (Integrated Proteomics Applications, Inc.)<sup>1</sup>. Trypsin cleavage specificity (cleavage at K, R except if followed by P) allowed for up to 2 missed cleavages. Carbamidomethylation of cysteine was set as a fixed modification, methionine oxidation, and TMT-modification of N-termini and lysine residues were set as variable modifications.

Reporter ion ratio calculations were performed using summed abundances with most confident centroid selected from 20 ppm window. Only peptide-to-spectrum matches that are unique assignments to a given identified protein within the total dataset are considered for protein quantitation. High confidence protein identifications were reported with a <1% false discovery rate (FDR) cut-off. Differential abundance significance was estimated using ANOVA with Benjamini-Hochberg correction to determine p-values.

### **Data Availability Statement**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **Code Availability Statement**

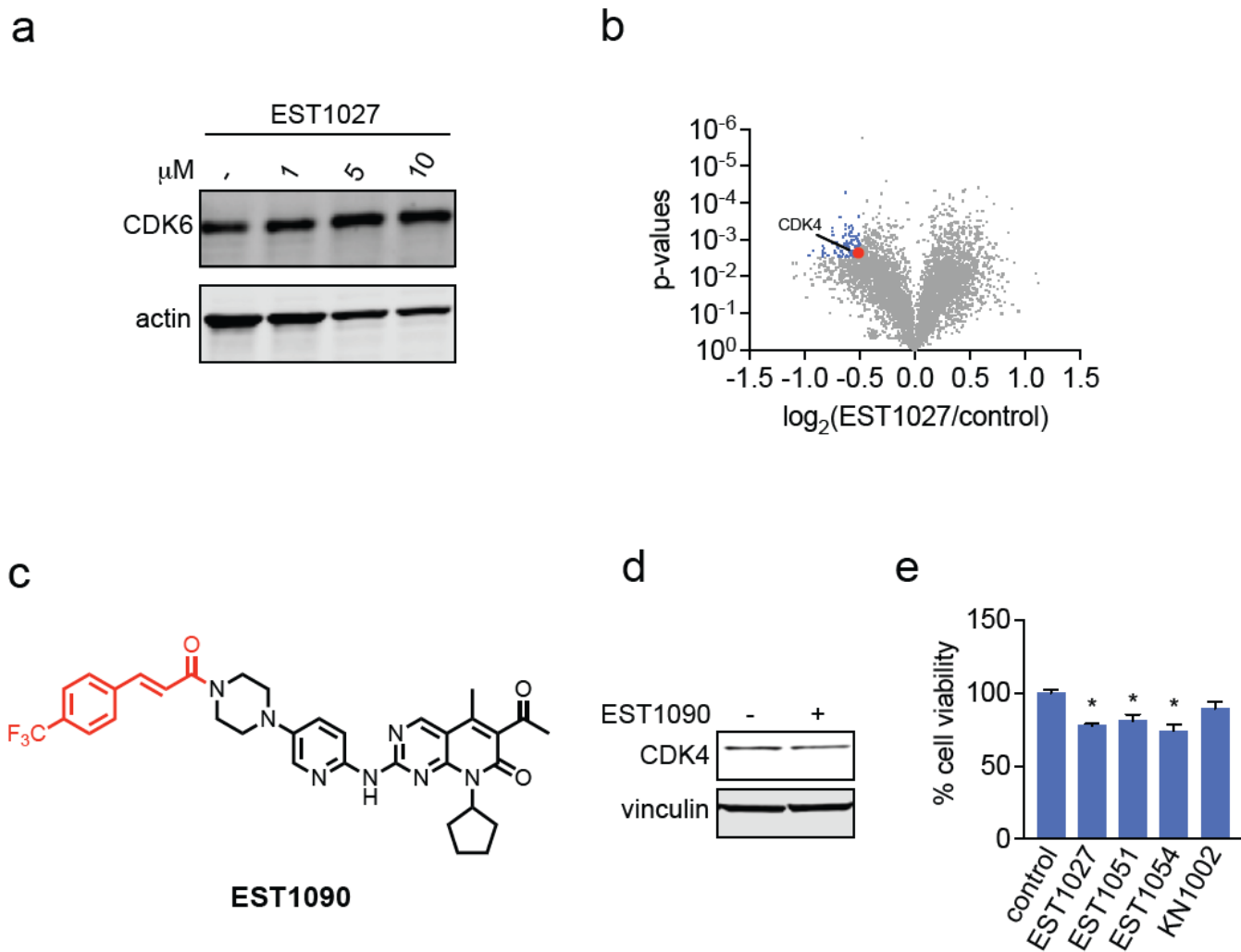
Data processing and statistical analysis algorithms from our lab can be found on our lab's Github site: <https://github.com/NomuraRG>, and we can make any further code from this study available at reasonable request.

### **Safety Statement**

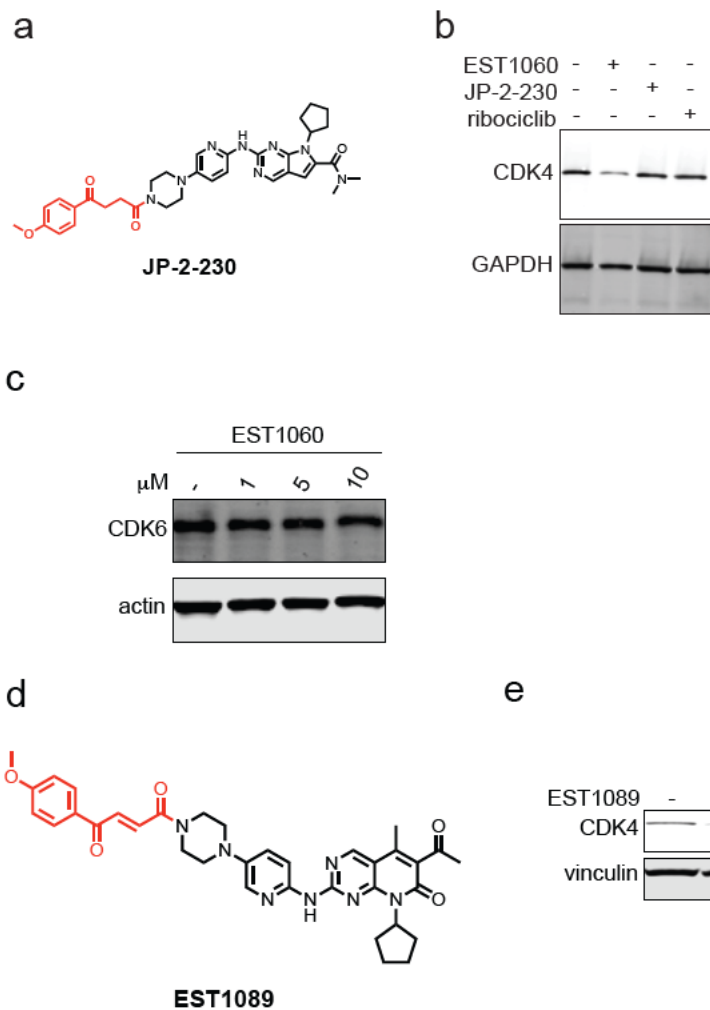
No unexpected or unusually high safety hazards were encountered.

### **Supporting References**

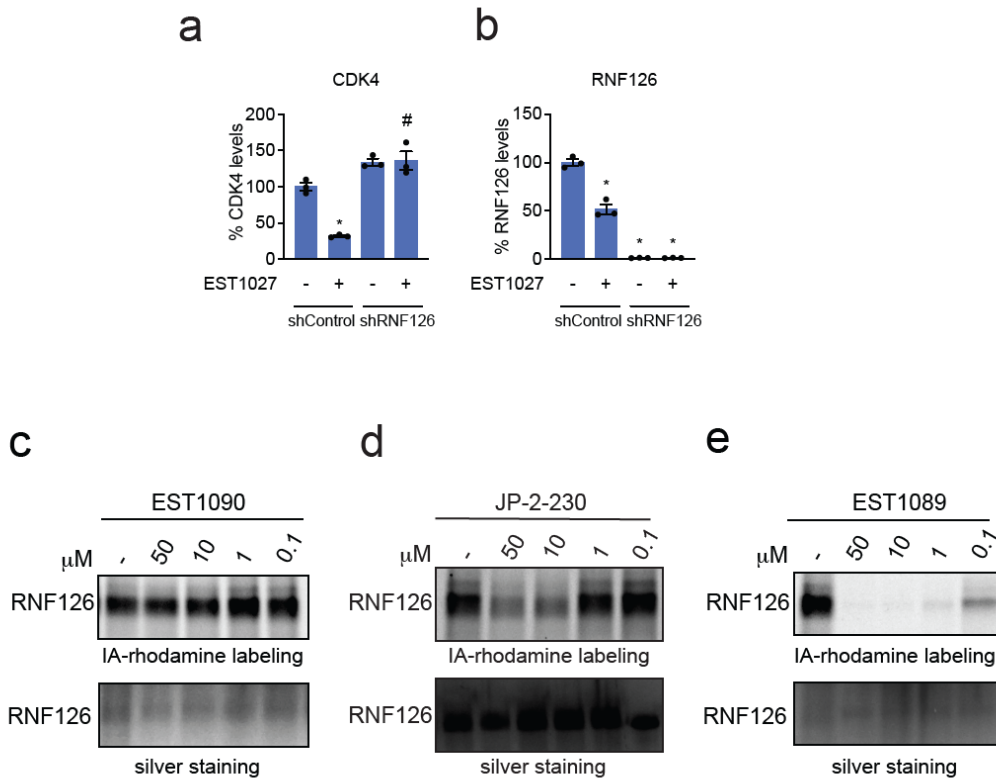
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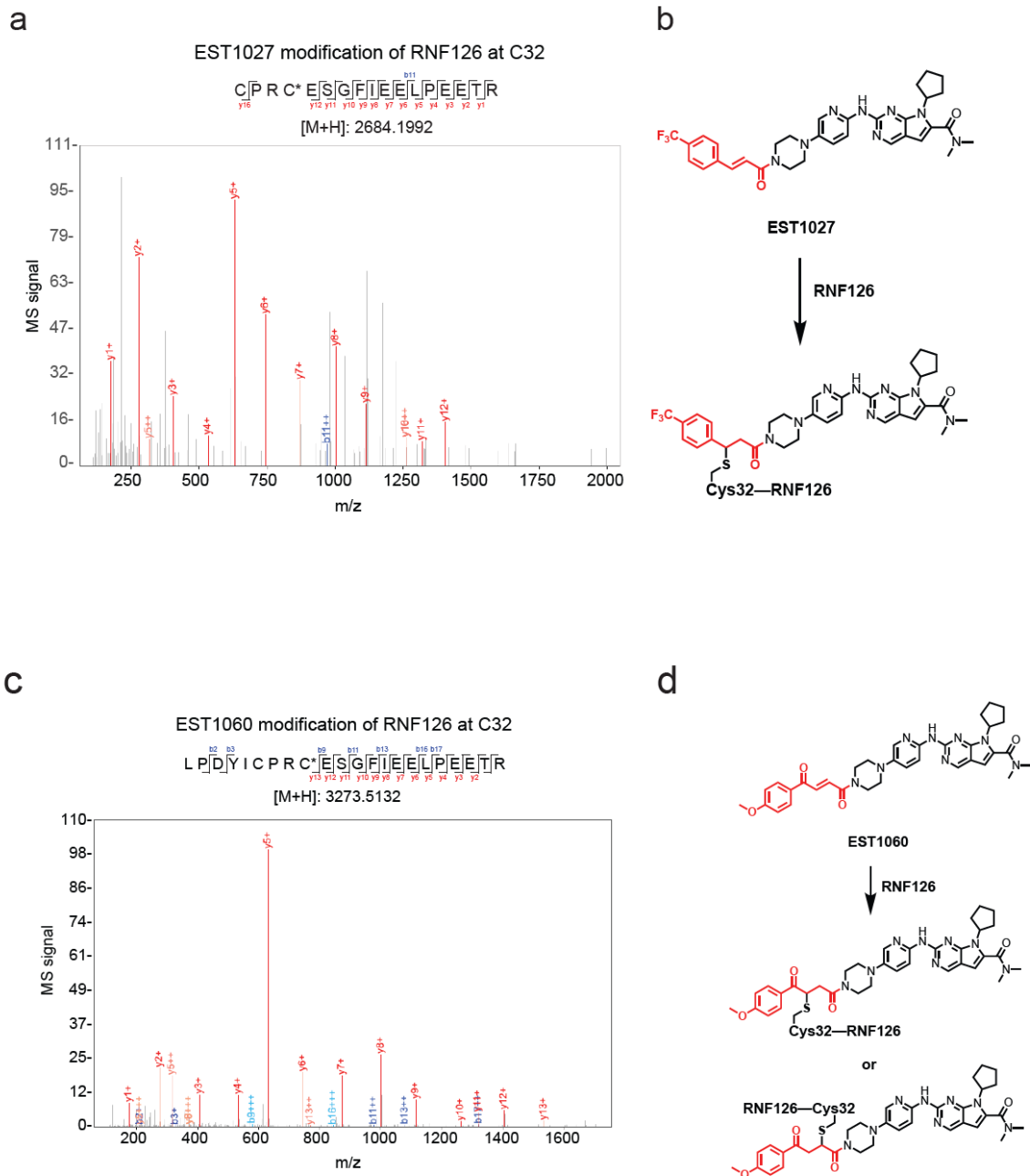
**Figure S1.** Palbociclib derivatives and their ability to degrade CDK4 and CDK6. **(a)** CDK6 levels in C33A cells. C33A cells were treated with DMSO vehicle or EST1027 for 24 h and CDK6 and loading control actin levels were detected by Western blotting. **(b)** TMT-based quantitative proteomic profiling of EST1027 in C33A cells. C33A cells were treated with DMSO vehicle or EST1027 (5  $\mu\text{M}$ ) for 24 h and cell lysates were subjected to TMT-based proteomic analyses. Shown are average EST1027/control TMT protein level ratios with points in blue or red noting proteins that showed ratios  $<0.7$  and  $p < 0.003$  from  $n=3$  biologically independent replicates/group. Shown in red is CDK4. **(c)** Structure of EST1090 with the trifluoromethyl propenamide handle linked to the CDK4/6 inhibitor Palbociclib. **(d)** CDK4 levels in C33A cells. C33A cells were treated with DMSO vehicle or EST1090 (10  $\mu\text{M}$ ) for 24 h and CDK4 and loading control vinculin levels were detected by Western blotting. **(e)** Cell viability assessed by Cell Titer Glo in C33A cells treated with DMSO vehicle or compounds (5  $\mu\text{M}$ ) for 24 h. Gels and blots in **(a, d)** are representative of  $n=3$  biologically independent replicates/group. Bar graph in **(e)** show average  $\pm$  sem. Statistical significance in **(e)** is calculated as  $*p < 0.05$  compared to DMSO vehicle.



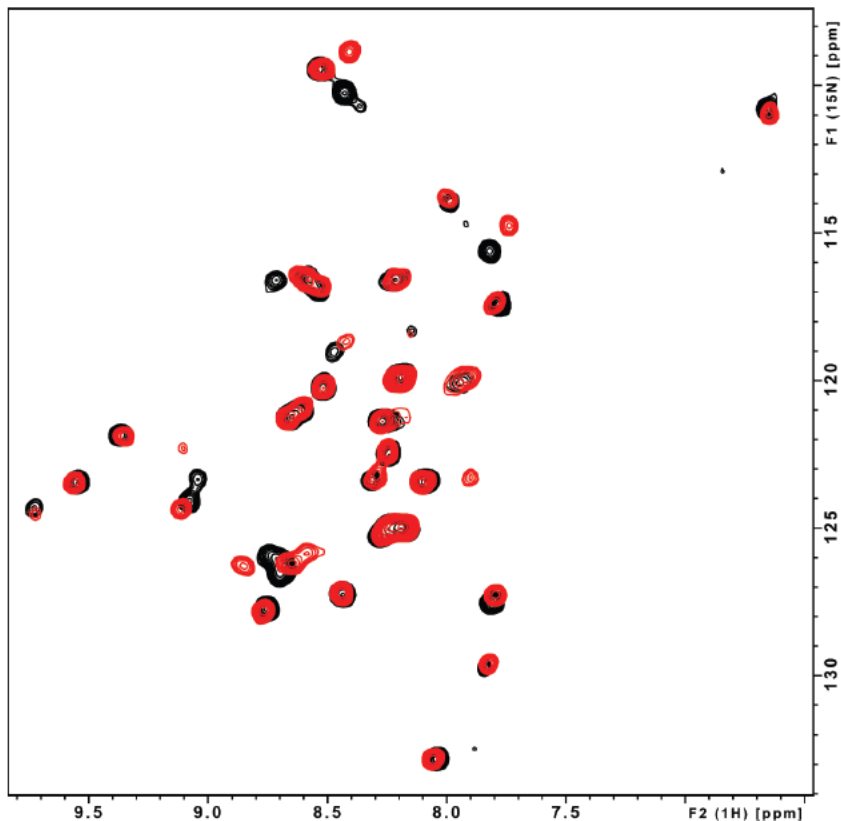
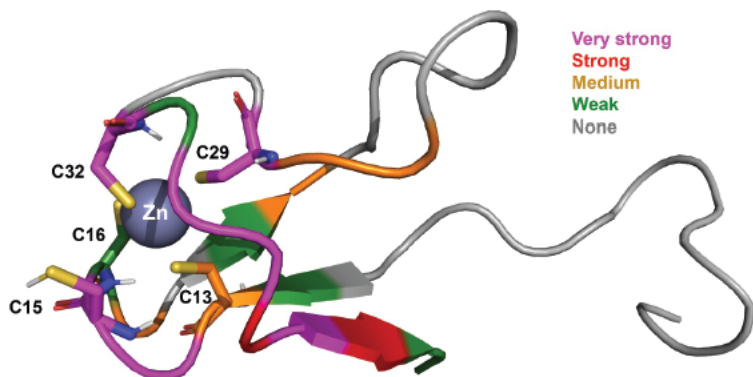
**Figure S2. Ribociclib and Palbociclib derivatives and their ability to degrade CDK4 and CDK6.** (a) Structure of JP-2-230 with non-reactive handle linked to the CDK4/6 inhibitor Ribociclib. (b) CDK4 levels in C33A cells. C33A cells were treated with DMSO vehicle, EST1060, or JP-2-230 (10  $\mu\text{M}$ ) for 24 h and CDK4 and loading control GAPDH levels were detected by Western blotting. (c) CDK6 levels in C33A cells. C33A cells were treated with DMSO vehicle or EST1060 for 24 h and CDK6 and loading control actin levels were assessed by Western blotting. (d) Structure of EST1089 with fumarate handle linked to the CDK4/6 inhibitor Palbociclib. (e) CDK4 levels in C33A cells. C33A cells were treated with DMSO vehicle or EST1089 (10  $\mu\text{M}$ ) for 24 h and CDK4 and loading control vinculin levels were detected by Western blotting. Gels and blots in (b, c, e) are representative of n=3 biologically independent replicates/group.



**Figure S3. Characterization of CDK4 degraders.** (a, b) Quantification of experiment described in Figure 3c. (c, d, e) Gel-based ABPP of EST1090, JP-2-230, EST1089 against RNF126. Recombinant RNF126 was pre-incubated with DMSO vehicle or compounds for 30 min prior to labeling of RNF126 with IA-rhodamine (250 nM) for 1 h. Gels were visualized by in-gel fluorescence and protein loading was assessed by silver staining. Shown are representative gels from n=3 biologically independent replicates/group. Bar graphs in (a, b) show individual replicate values and average  $\pm$  sem. Statistical significance in (a, b) is calculated as \* $p < 0.05$  compared to DMSO vehicle and # $p < 0.05$  compared to EST1027-treated group.

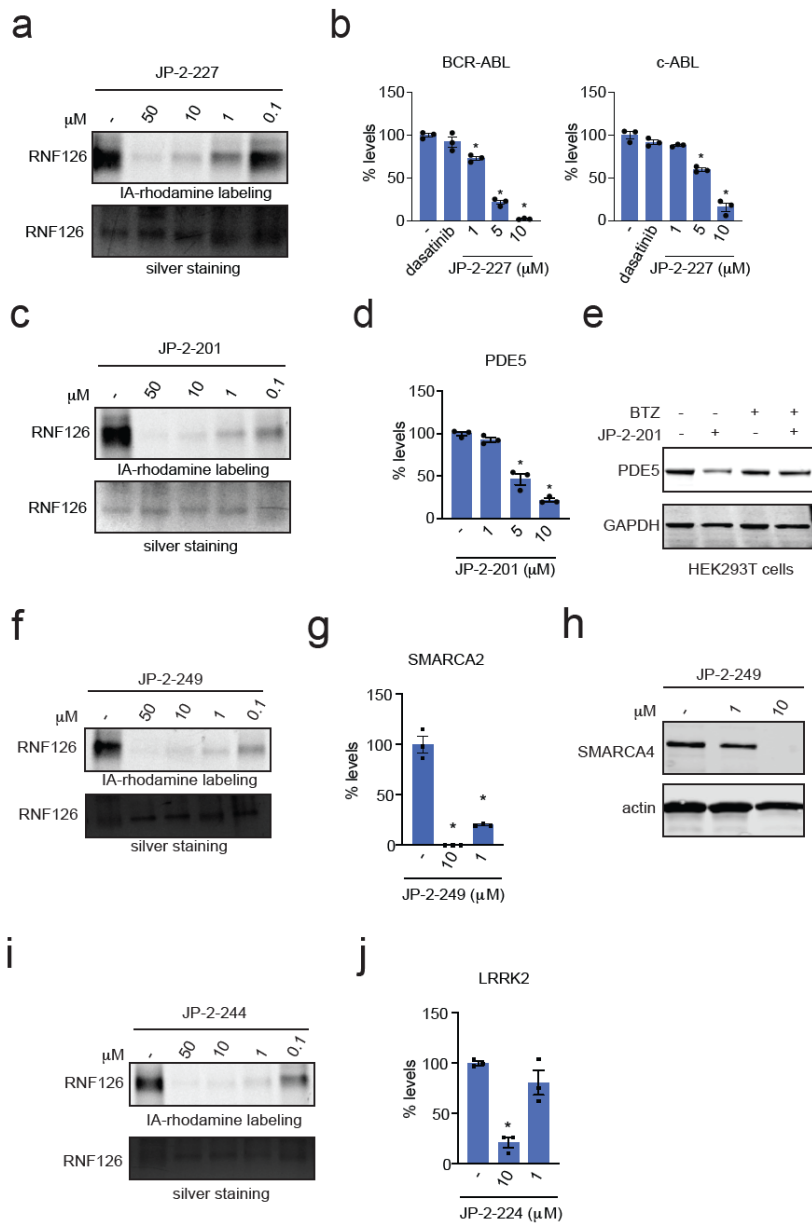


**Figure S4. Site of modification analysis of EST1027 and EST1060 with pure RNF126 protein.** RNF126 protein (24  $\mu$ g) was incubated with EST1027 (**a, b**) or EST1060 (**c, d**) (50  $\mu$ M) for 30 min and tryptic digests were analyzed by LC-MS/MS. (**b, d**) Structure of presumed adduct of EST1027 and EST1060 with C32 of RNF126.

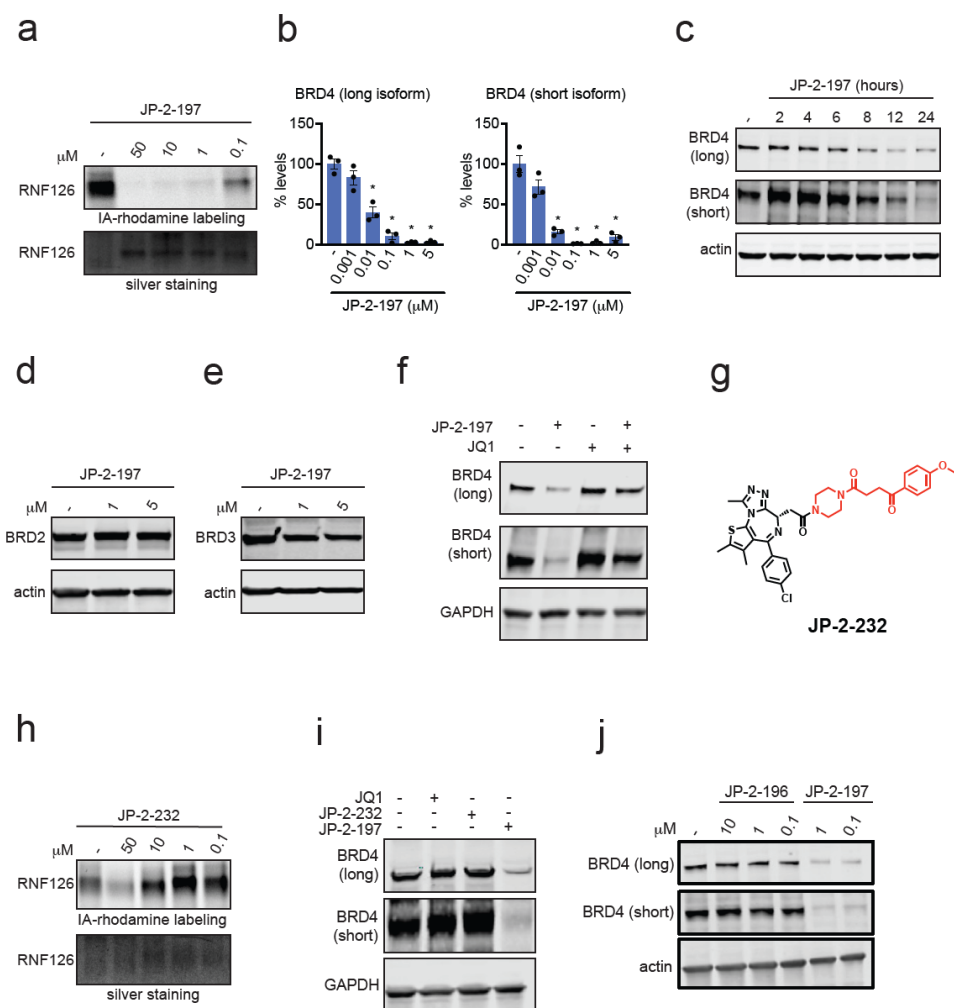
**a****b**

**Figure S5. Fumarate binding to RNF126(1-40) is specific and does not lead to protein unfolding or aggregation. (a)** Overlay of two  $^1\text{H}$ ,  $^{15}\text{N}$ -HMQC spectra, with peaks shown in black corresponding to  $\sim 75 \mu\text{M}$  of apo-RNF126 and signals in red representing protein amide groups in the presence of  $200 \mu\text{M}$  of JP-2-196. The compound-induced, peak-dependent chemical shift perturbations are proof of specific binding of the small molecule to RNF126. Overall protein signal intensities and the spectral dispersion are unchanged upon compound addition, indicating that the ligand interactions do not lead to protein unfolding or aggregation. **(b)** Three-dimensional structure of RNF126(1-40) with color-coded mapping of chemical shift perturbations and residue-specific line broadening. The ligand binds at or close to the zinc (“Zn”) coordination site while the other areas of RNF126(1-40) remain unchanged.

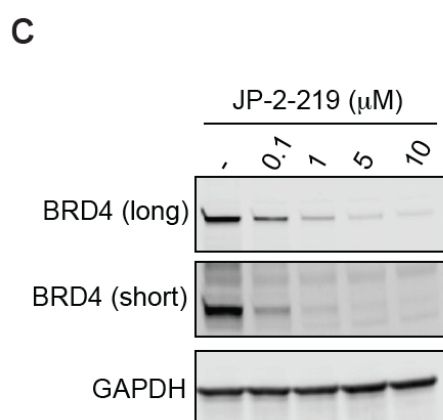
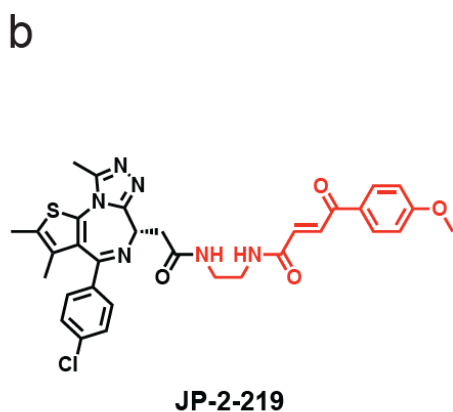
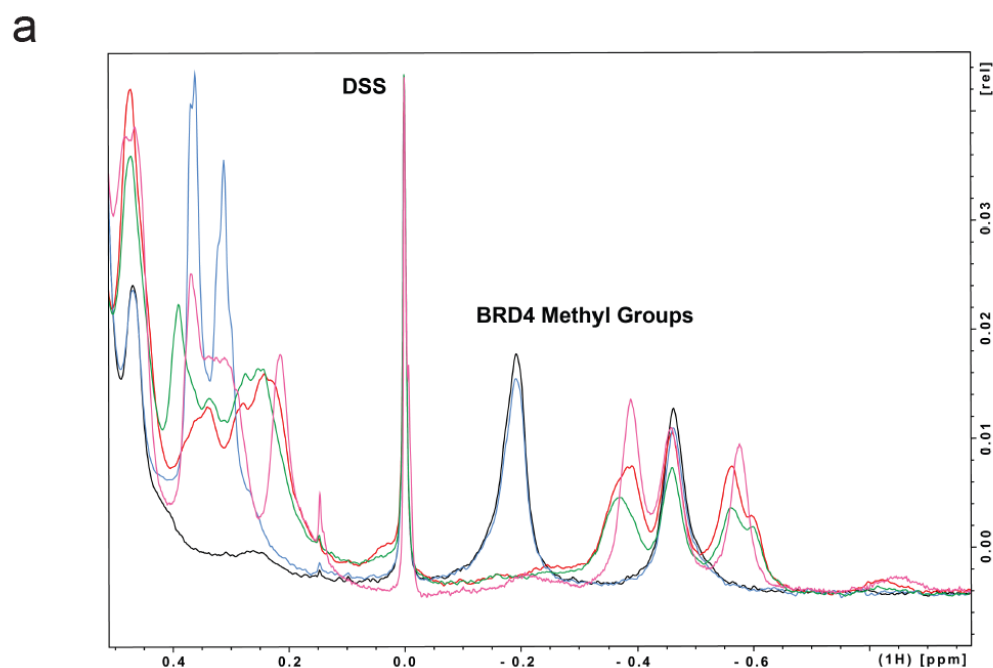




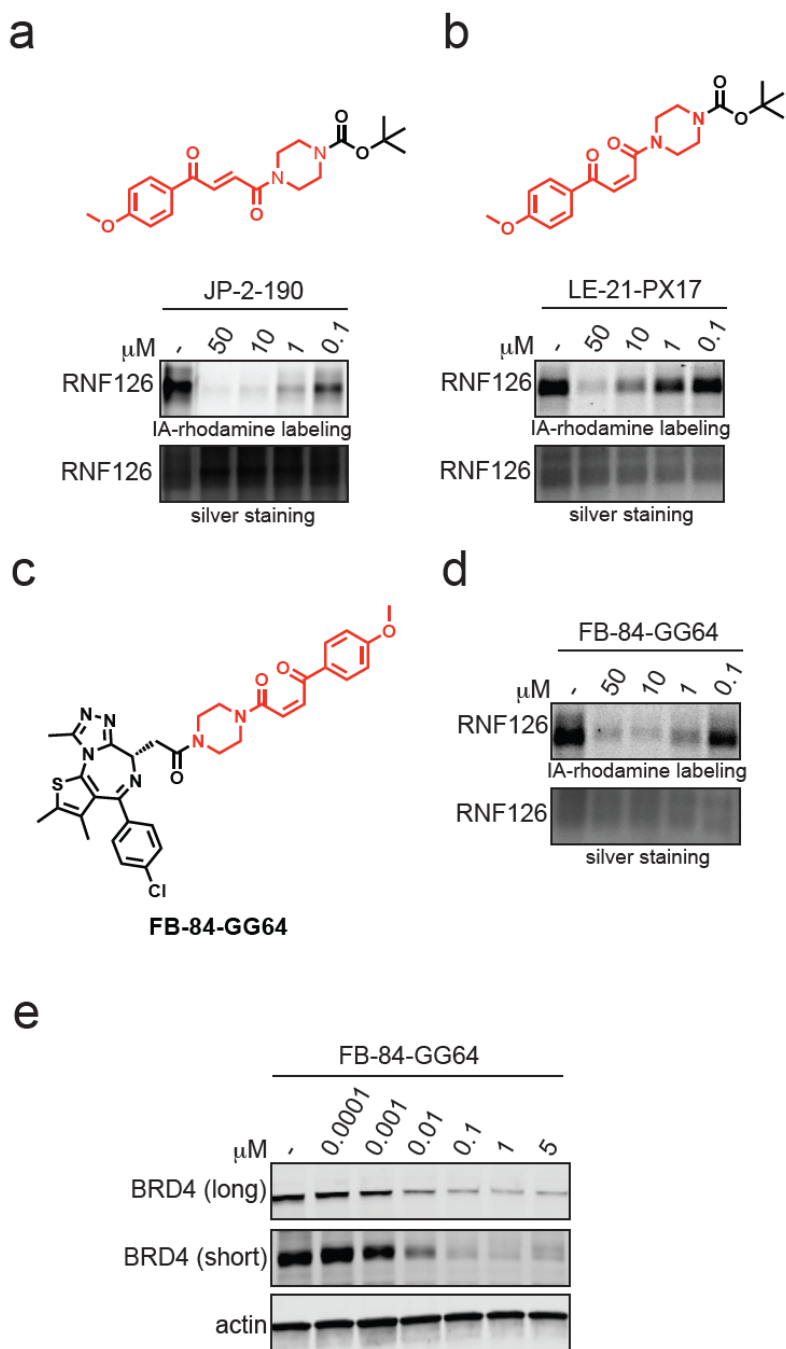
**Figure S6. Characterizing Degraders Against Additional Targets.** (a) Gel-based ABPP analysis of JP-2-227 against RNF126. Recombinant RNF126 was pre-incubated with DMSO vehicle or JP-2-227 for 30 min prior to labeling of RNF126 with IA-rhodamine (250 nM) for 1 h. Gels were visualized by in-gel fluorescence and protein loading was assessed by silver staining. (b) Quantitation of data in **Figure 4b**. (c) Gel-based ABPP analysis of JP-2-201 as described in (a). (d) Quantitation of data in **Figure 4d**. (e) JP-2-201-mediated degradation of PDE5 is proteasome-dependent. HEK293T cells were pre-treated with DMSO vehicle or bortezomib (1  $\mu$ M) for 1 h prior to treating cells with DMSO or JP-2-201 (10  $\mu$ M). PDE5 and loading control GAPDH levels were assessed by Western blotting. (f) Gel-based ABPP analysis of JP-2-249 against RNF126 as described in (a). (g) Quantitation of data in **Figure 4f**. (h) MV-4-11 cells were treated with DMSO vehicle or JP-2-249 for 24 h and SMARCA4 and loading control actin levels were assessed by Western blotting. (i) Gel-based ABPP of JP-2-244 against RNF126 as described in (a). (j) Quantitation of data in **Figure 4h**. Gels and blots shown in (a, c, e, f, h, i) are representative images from  $n=3$  biological replicates. Bar graphs in (b, d, g, j) show individual replicate values and average  $\pm$  sem. Statistical significance is calculated as  $*p < 0.05$  compared to DMSO vehicle.



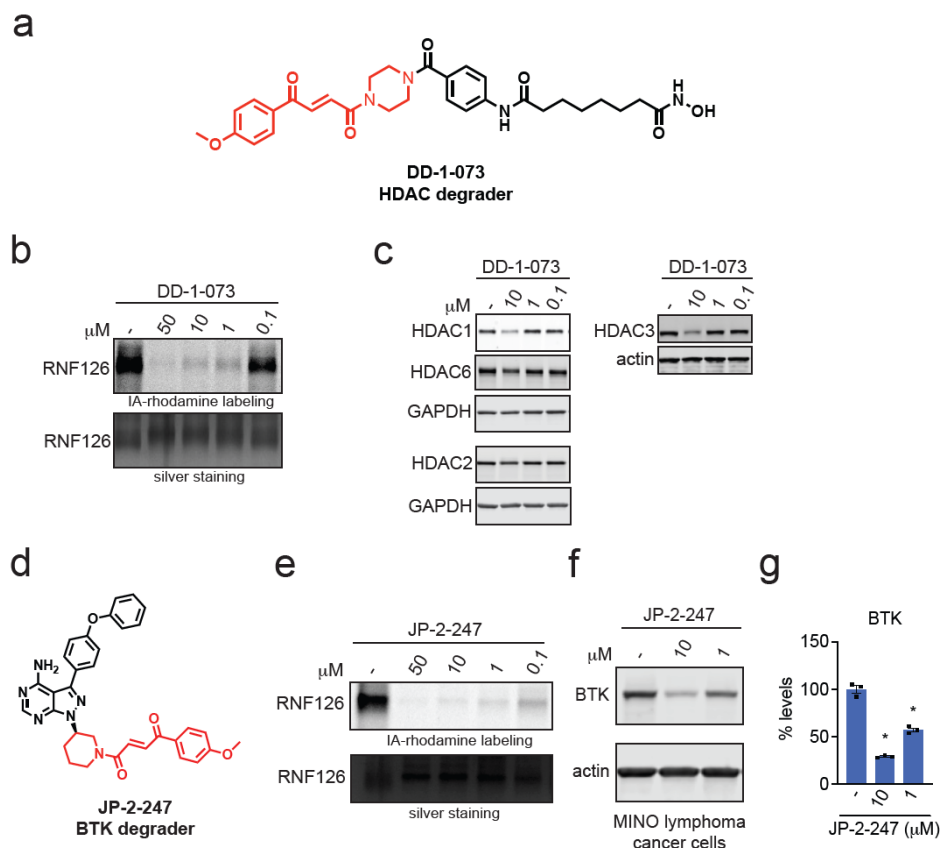
**Figure S7. Characterization of BRD4 degraders and their derivatives.** (a) Gel-based ABPP analysis of JP-2-197 against RNF126. Recombinant RNF126 was pre-incubated with DMSO vehicle or JP-2-197 for 30 min prior to labeling of RNF126 with IA-rhodamine (250 nM) for 1 h. Gels were visualized by in-gel fluorescence and protein loading was assessed by silver staining. (b) Quantitation of data in Figure 5b. (c) Time-course of BRD4 degradation with JP-2-197. HEK293T cells were treated with DMSO vehicle or JP-2-197 (1  $\mu\text{M}$ ) and BRD4 and loading control actin levels were assessed by Western blotting. (d, e) BRD2 (d) and BRD3 (e) levels in HEK293T cells. HEK293T cells were treated with DMSO vehicle or JP-2-197 for 24 h and BRD2, BRD3, and loading control actin levels were assessed by Western blotting. (f) HEK293T cells were pre-treated with either DMSO vehicle or JQ1 (1  $\mu\text{M}$ ) for 1 h prior to treating cells with DMSO or JP-2-197 (0.1  $\mu\text{M}$ ) for 24 h and BRD4 and loading control GAPDH levels were assessed by Western blotting. (g) Structure of non-reactive derivative of JP-2-197, JP-2-232. (h) Gel-based ABPP of JP-2-232 against RNF126. Recombinant RNF126 was pre-incubated with DMSO vehicle or JP-2-232 for 30 min prior to labeling of RNF126 with IA-rhodamine (250 nM) for 1 h. Gels were visualized by in-gel fluorescence and protein loading was assessed by silver staining. (i) BRD4 levels in HEK293T cells. HEK293T cells were treated with DMSO vehicle, JP-2-197, or JP-2-232 (1  $\mu\text{M}$ ) for 24 h and BRD4 and loading control GAPDH levels were detected by Western blotting. (j) HEK293T cells were treated with DMSO vehicle, JP-2-196, or JP-2-197 for 24 h and BRD4 and loading control actin levels were assessed by Western blotting. Gels and blots in (a, c, d, e, f, h, i, j) are representative of n=3 biologically independent replicates/group. Bar graphs in (b) show individual replicate values and average  $\pm$  sem. Statistical significance is calculated as \*p<0.05 compared to DMSO vehicle.



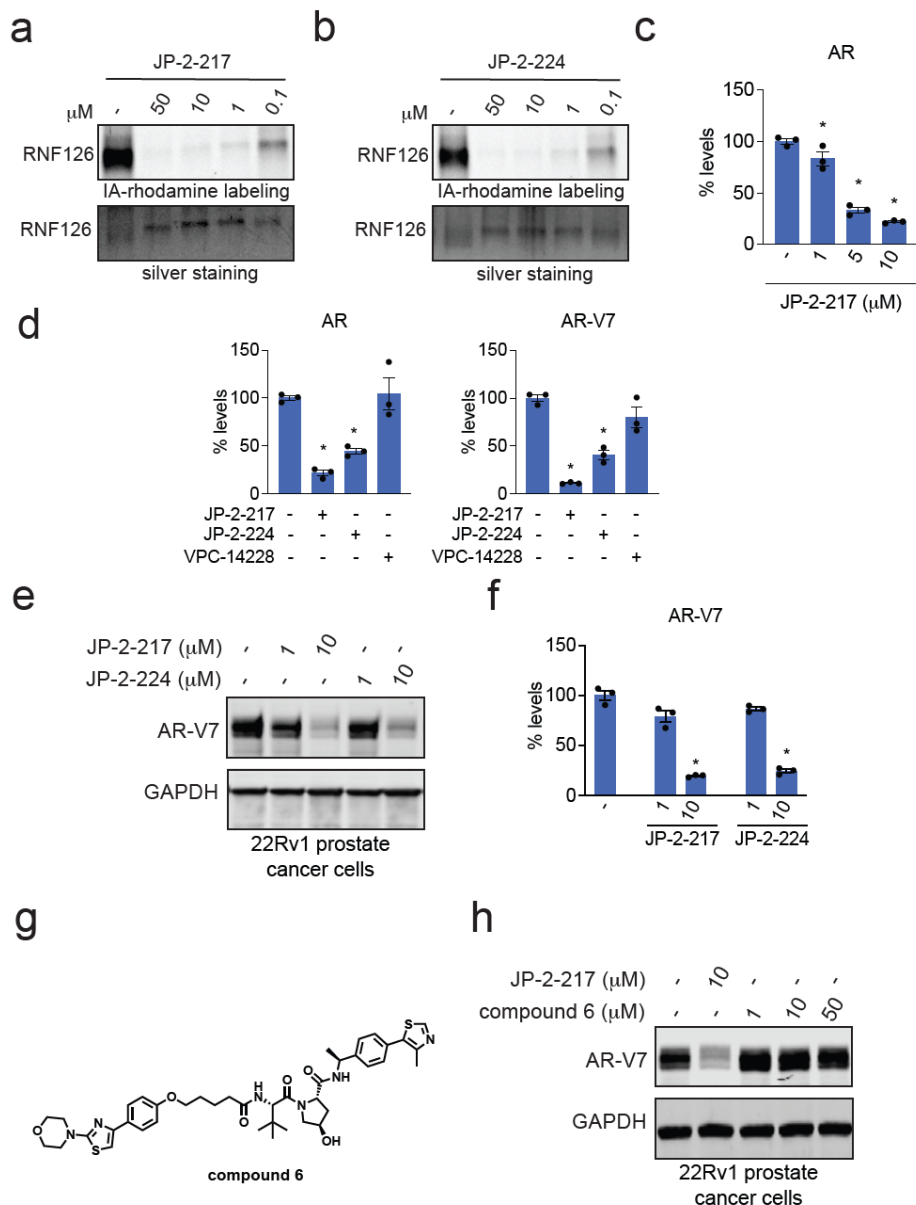
**Figure S8. Characterization of BRD4 Degraders.** **(a)** Overlay of  $^1\text{H}$ -1D spectra of the following samples: 50  $\mu\text{M}$  BRD4(44-168) (black); 50  $\mu\text{M}$  BRD4(44-168) + 200  $\mu\text{M}$  JP-2-197 (red); 50  $\mu\text{M}$  BRD4(44-168) + RNF126(1-40) + JP-2-197 (green); 50  $\mu\text{M}$  BRD4(44-168) + RNF126(1-40) (blue); 50  $\mu\text{M}$  BRD4(44-168) + RNF126(1-40) + 100  $\mu\text{M}$  JQ1 + 100  $\mu\text{M}$  JP-2-196 (magenta). BRD4 (black spectrum) and JP-2-197 strongly interact with each other as indicated by the drastic perturbation of one of the upfield shifted BRD4 methyl signals ( $\delta \approx -0.19$  ppm) and the appearance of additional signals (red spectrum). When RNF126(1-40) is present, the perturbations are further pronounced as small additional chemical shifts and line broadening are detected, suggesting slowed down protein tumbling due to the formation of a ternary protein-protein-glue complex (green spectrum). There is hardly any effect on the BRD4 methyl peaks in the absence of the glue molecule (blue spectrum). No line broadening is observed when the unlinked protein ligands JQ1 (BRD4 ligand) and JP-2-196 (RNF126 ligand) are added to the protein mixture (magenta). **(b)** Shown is a structure of JP-2-219, a BRD4 degrader with an alkyl linker rather than a piperazine linking the fumarate handle to the BRD4 inhibitor JQ1. **(c)** BRD4 levels in HEK293T cells. HEK293T cells were treated with DMSO vehicle or JP-2-219 (1  $\mu\text{M}$ ) for 24 h and BRD4 and loading control GAPDH levels were detected by Western blotting. Blot is a representative of  $n=3$  biologically independent replicates/group.



**Figure S9. Characterizing the *cis*-isomer covalent handle and degrader. (a, b)** Gel-based ABPP of JP-2-190 and LE-21-PX17 against RNF126. Recombinant RNF126 was pre-incubated with DMSO vehicle or compounds for 30 min prior to labeling of RNF126 with IA-rhodamine (250 nM) for 1 h. Gels were visualized by in-gel fluorescence and protein loading was assessed by silver staining. **(c)** Structure of the *cis* isoform of the JP-2-197 BRD4 degrader, FB-84-GG64. **(d)** Gel-based ABPP of FB-84-GG64 against RNF126. Recombinant RNF126 was pre-incubated with DMSO vehicle or FB-84-GG64 for 30 min prior to labeling of RNF126 with IA-rhodamine (250 nM) for 1 h. Gels were visualized by in-gel fluorescence and protein loading was assessed by silver staining. **(e)** BRD4 degradation by FB-84-GG64 in HEK293T cells. HEK293T cells were treated with DMSO vehicle or FB-84-GG64 for 24 h and BRD4 and loading control actin levels were assessed by Western blotting. Gels and blots shown in **(a, b, d, e)** are representative of  $n=3$  biologically independent replicates/group.



**Figure S10. Characterization of HDAC and BTK Degradors.** (a) Structure of DD-1-073 HDAC degrader linking our fumarate JP-2-196 handle to the HDAC inhibitor vorinostat. (b) Gel-based ABPP of DD-1-073 against RNF126. Recombinant RNF126 was pre-incubated with DMSO vehicle or DD-1-073 for 30 min prior to labeling of RNF126 with IA-rhodamine (250 nM) for 1 h. Gels were visualized by in-gel fluorescence and protein loading was assessed by silver staining. (c) HDAC1, HDAC2, HDAC3, and HDAC6 levels in HEK293T cells. HEK293T cells were treated with DMSO vehicle or DD-1-073 for 24 h and HDAC and loading control GAPDH and actin levels were detected by Western blotting. (d) Structure of a BTK degrader JP-2-247 consisting of the fumarate handle incorporated into the BTK inhibitor ibrutinib. (e) Gel-based ABPP analysis of JP-2-247 against RNF126. Recombinant RNF126 was pre-incubated with DMSO vehicle or JP-2-247 for 30 min prior to labeling of RNF126 with IA-rhodamine (250 nM) for 1 h. Gels were visualized by in-gel fluorescence and protein loading was assessed by silver staining. (f) MINO lymphoma cancer cells were treated with DMSO vehicle or JP-2-247 for 24 h and BTK and loading control actin levels were detected by Western blotting. (g) Quantitation of the data shown in (f). Gels and blots shown in (b, c, e, f) are representative of n=3 biologically independent replicates/group. Bar graph in (g) shows individual replicate values and average  $\pm$  sem. Statistical significance is calculated as \*p<0.05 compared to DMSO vehicle.



**Figure S11. Characterization of AR-V7 Degraders.** (a, b) Gel-based ABPP analysis of JP-2-217 (a) and JP-2-224 (b) against RNF126. Recombinant RNF126 was pre-incubated with DMSO vehicle or JP-2-217 or JP-2-224 for 30 min prior to labeling of RNF126 with IA-rhodamine (250 nM) for 1 h. Gels were visualized by in-gel fluorescence and protein loading was assessed by silver staining. (c) Quantitation of data in Figure 5g. (d) Quantitation of data in Figure 5h. (e) 22Rv1 cells were treated with DMSO vehicle, JP-2-217, or JP-2-224 for 24 h and AR-V7 and loading control GAPDH levels were detected by Western blotting. (f) Quantified AR-V7 levels from experiment described in (e). (g) Structure of a previously reported AR-V7 degrader linking the AR DNA binding domain ligand VPC-14228 to a VHL recruiter, compound 6. (h) AR-V7 levels in 22Rv1 cells. 22Rv1 cells were treated with DMSO vehicle, JP-2-217, or compound 6 for 24 h and AR-V7 and loading control GAPDH levels were detected by Western blotting. Gels and blots in (a, b, e, h) are representative of n=3 biologically independent replicates/group with individual replicates. Bar graphs in (c, d, f) show individual replicate values and average  $\pm$  sem. Statistical significance is calculated as \*p<0.05 compared to DMSO vehicle.

## Supporting Table Legends

**Table S1. TMT-based quantitative proteomic profiling of EST1027 in C33A cells.** C33A cells were treated with DMSO vehicle or EST1027 (5  $\mu$ M) for 24 h. Data are from n=3 biological replicates per group.

**Table S2. Cysteine chemoproteomic profiling of EST1027 in C33A cells using isoDTB-ABPP.** C33A cells were treated with DMSO vehicle or EST1027 (20  $\mu$ M) for 2 h. Resulting lysates were labeled with an alkyne-functionalized iodoacetamide probe (IA-alkyne) (200  $\mu$ M) for 1 h, after which isotopic desthiobiotin tags were appended by CuAAC and taken through the isoDTB-ABPP procedure. Experiment was from n=3 biologically-independent replicates.

**Table S3. Quantitative Proteomics of JP-2-196-Alkyne Pulldown in HEK293T cells.** JP-2-196 pulldown proteomics showing significant and moderately selective engagement of RNF126 with less significant engagement of 5 additional E3 ubiquitin ligases LRSAM1, RNF40, MID2, RNF219, and RNF14. HEK293T cells were treated with DMSO vehicle or JP-2-196-alkyne (10  $\mu$ M) for 6 h. Subsequent lysates were subjected to copper-catalyzed azide-alkyne cycloaddition (CuAAC) with an azide-functionalized biotin handle, after which probe-modified proteins were avidin-enriched, eluted, and digested, and analyzed by TMT-based quantitative proteomics. Data shown are ratios of JP-2-196-alkyne vs DMSO control enriched proteins and p-values from n=3 biologically independent replicates/group.

**Table S4. TMT-based quantitative proteomic profiling of JP-2-197 in HEK293T cells.** HEK293T cells were treated with DMSO vehicle, JP-2-196 (1  $\mu$ M), or JP-2-197 (1  $\mu$ M) for 24 h. Data are from n=2 biological replicates per group.

## Synthetic Methods and Characterization

All chemical reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. The key building block, *trans*-3-(4-methoxybenzoyl)acrylic acid, was acquired from Sigma-Aldrich (Catalog Number: 650765) at a reported purity of 99%, however upon further investigation this purity was found to vary between batches, consisting of anywhere from 0-10% of the *cis*-isomer. Reactions were stirred magnetically and monitored by thin layer chromatography (TLC) carried out on Merck glass silica gel plates (60 F254) using UV light as a visualizing agent and iodine and/or phosphomolybdic acid stain as developing agents. Solvents were removed *in vacuo* using either a Buchi R-300 Rotavapor (equipped with an I-300 Pro Interface, B-300 Base Heating Bath, Welch 2037B-01 DryFast pump, and VWR AD15R-40-V11B Circulating Bath). Solvents for silica gel chromatography were used as supplied by Sigma-Aldrich. Automated flash chromatography was performed on a Biotage Isolera instrument, equipped with a UV detector. Chromatograms were recorded at 254 and 280 nm. Analytical ultra-performance liquid chromatography (UPLC) was performed on a Waters Acquity UPLC-MS(SQD) with a Phenomenex Kinetix EVO-C18 2.6  $\mu\text{m}$ , 100  $\text{\AA}$ , 2.1  $\times$  50 mm column. Solvents (water and acetonitrile) containing 0.1% trifluoroacetic acid (TFA) were used. A gradient of 2-88% acetonitrile in water, 0–10 min was used at ambient temperature. Purification was run with a flow rate of 1.2 mL/min with detection at 220 nm. Certain fumarate analogs were sensitive to acidic aqueous purification conditions, and a small fraction was found to isomerize between the *E* and *Z*-isomers therefore a neutral, SFC analytical method was also employed using an Amino Phenyl SFC 5  $\mu\text{m}$ , 120  $\text{\AA}$ , 30 mm  $\times$  25 cm column. Solvents ( $\text{CO}_2$  and MeOH) were used at a flow rate of 2.5 mL/min with detection at 220 nm. The purity of final compounds was evaluated using the analytical systems described above, characterized by MS and nuclear magnetic resonance (NMR) and ratios of *E/Z*-isomers were reported as detected in UPLC analysis. Isomerization rates were analyzed for key final compounds by preparing small amounts of material in a 1:1 mixture of water:acetonitrile, filtering through a 0.2  $\mu\text{m}$  syringe filter for UPLC analysis at days 1 and 6. Low-resolution mass spectra were obtained using Agilent 6460 Triple Quad LC/MS instrument. High-resolution mass spectra (HRMS) were obtained at the Catalysis Center at the College of Chemistry, University of California, Berkeley.  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectra were recorded on BRUKER AV (600 MHz and 700 MHz), AVB (400 MHz), AVQ (400 MHz) and NEO (500 MHz)



spectrometers. Measurements were carried out at ambient temperature. Chemical shifts ( $\delta$ ) are reported in ppm with the residual solvent signal as internal standard (chloroform at 7.26 and 77.00 ppm for  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopy, respectively). The data is reported as (s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, m = multiplet or unresolved, br = broad signal, coupling constant(s) in Hz, integration).  $^{13}\text{C}$  NMR spectra were recorded with broadband  $^1\text{H}$  decoupling.

## **General Procedures**

### **Amide Couplings**

#### **General Procedure A**

A mixture of the corresponding carboxylic acid (1.1 equiv.) and HATU (1.2 equiv.) was purged with  $\text{N}_2$  for 5 minutes. The mixture was dissolved in *N,N*-dimethylformamide (DMF) (0.1 M), *N,N*-diisopropylethylamine (DIPEA) (3 equiv.) was added and the reaction mixture was allowed to stir at ambient temperature for 30 minutes. The corresponding amine (1 equiv.) was dissolved in DMF (0.1 M) then added dropwise and the reaction mixture was stirred at ambient temperature overnight. The reaction was quenched with 5 times the reaction volume of 5%  $\text{LiCl}_{(\text{aq})}$  and extracted 3 times with dichloromethane (DCM) or ethyl acetate (EtOAc). The organic extracts were dried over  $\text{Na}_2\text{SO}_4$ , vacuum filtered, and concentrated *in vacuo*. The resultant residue was purified by silica gel flash chromatography to afford the title compound.

#### **General Procedure B**

The corresponding carboxylic acid (1.1 equiv.) was added to a vessel and purged with  $\text{N}_2$  for 5 minutes. The acid was then dissolved in DCM (0.1 M) and cooled to 0 °C in an ice bath. Oxalyl chloride (1.2 equiv.) was added dropwise at 0 °C. A few drops of DMF were added and the reaction mixture was allowed to stir and come to ambient temperature over 2 hours. The volatiles were removed *in vacuo* and the resultant residue was redissolved in DCM (0.1 M) and cooled to 0 °C in an ice bath. The corresponding amine (1.1 equiv.) was dissolved in DCM (0.1M). DIPEA (3 equiv.) was added, and the mixture was stirred for 5 minutes at ambient temperature before being added to the acyl chloride dropwise at 0 °C. The reaction mixture was allowed to stir and come to ambient temperature overnight. The reaction was quenched with water and extracted 3 times with

EtOAc. The organic extracts were dried over  $\text{Na}_2\text{SO}_4$ , vacuum filtered, and concentrated *in vacuo*. The resultant residue was purified by silica gel flash chromatography to afford the title compound.

### **General Procedure C**

The corresponding carboxylic acid (1.1 equiv.) was added to a vessel and purged with  $\text{N}_2$  for 5 minutes. The acid was dissolved in acetonitrile (0.1 M) and DIPEA (2 equiv.) was added. Pentafluoropyridine (1.1 equiv.) was added dropwise, and the reaction mixture was stirred at ambient temperature for 30 minutes. The corresponding amine was dissolved in acetonitrile (0.1 M) and added to acyl fluoride. The reaction mixture was stirred at 100 °C overnight. The volatiles were removed *in vacuo*, and the resultant residue was purified by silica gel flash chromatography to afford the title compound.

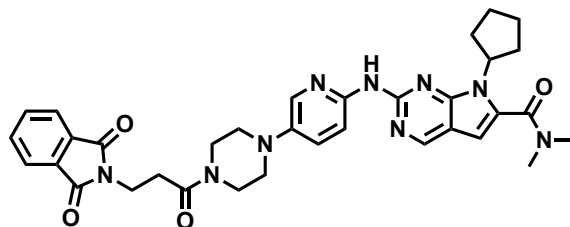
### **General Procedure D**

The corresponding carboxylic acid (1.0 equiv.) was added to a vessel and purged with  $\text{N}_2$  for 5 minutes. The acid was dissolved in DMF (0.1 M) and DIPEA (3 equiv.) was added. A >50% wt. solution of propylphosphonic anhydride (T3P) in EtOAc (1.5 equiv.) was added dropwise, and the reaction mixture was stirred at ambient temperature for 30 minutes. The corresponding amine (1.2 equiv.) was dissolved in DMF (0.1 M) then added dropwise and the reaction mixture was stirred at ambient temperature overnight. The reaction was quenched with 5 times the reaction volume of 5%  $\text{LiCl}_{(\text{aq})}$  and extracted 3 times with EtOAc. The organic extracts were washed once with 5%  $\text{LiCl}_{(\text{aq})}$ , dried over  $\text{Na}_2\text{SO}_4$ , vacuum filtered, and concentrated *in vacuo*. The resultant residue was purified by silica gel flash chromatography to afford the title compound.

### **Tert-butyloxycarbonyl Deprotection**

#### **General Procedure E**

The corresponding tert-butyloxycarbonyl protected amine (1 equiv.) was dissolved in DCM (0.1 M). Trifluoroacetic acid (32 equiv.) was added, and the reaction mixture was stirred at ambient temperature for 30 minutes to overnight. The volatiles were removed *in vacuo* and the crude residue was used without further purification, unless otherwise noted.



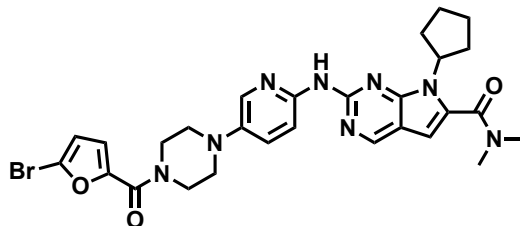
**EST1001**

**General Procedure A** was followed with 3-phthalimidopropionic acid (21.6 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 14.9 mg (41%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.70 (s, 1H), 8.37 (d, *J* = 9.0 Hz, 1H), 8.03 – 7.95 (m, 2H), 7.89 – 7.81 (m, 2H), 7.75 – 7.68 (m, 2H), 7.32 (dd, *J* = 9.1, 3.0 Hz, 1H), 6.44 (s, 1H), 4.79 (p, *J* = 9.0 Hz, 1H), 4.09 – 4.03 (m, 2H), 3.80 (t, *J* = 5.2 Hz, 2H), 3.64 (t, *J* = 5.1 Hz, 2H), 3.15 (s, 6H), 3.14 – 3.08 (m, 4H), 2.85 – 2.78 (m, 2H), 2.65 – 2.52 (m, 2H), 2.06 (m 4H), 1.77 – 1.70 (m, 2H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 168.4, 168.2, 164.1, 154.5, 151.9, 151.8, 147.6, 142.1, 137.6, 134.0, 132.1, 132.1, 127.4, 123.3, 112.7, 112.4, 101.0, 57.9, 50.6, 50.3, 45.4, 41.5, 34.3, 31.6, 30.2, 24.7.

**LRMS (ESI)** *m/z* calcd for [C<sub>34</sub>H<sub>37</sub>N<sub>9</sub>O<sub>4</sub> + H]<sup>+</sup> = 636.3, found 636.4.



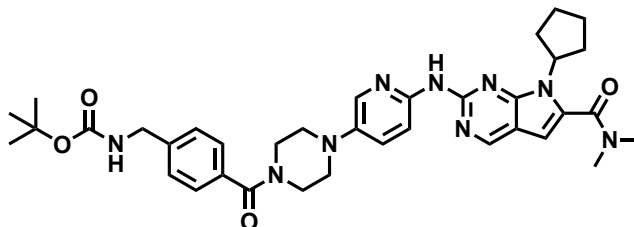
**EST1004**

**General Procedure A** was followed with 5-Bromo-2-furoic acid (11.0 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-15% MeOH in DCM) to afford 23.9 mg (63%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.72 (s, 1H), 8.39 (d, *J* = 9.0 Hz, 1H), 8.25 (s, 1H), 8.06 (d, *J* = 3.0 Hz, 1H), 7.34 (dd, *J* = 9.1, 3.0 Hz, 1H), 7.03 (d, *J* = 3.5 Hz, 1H), 6.48 – 6.42 (m, 2H), 4.79 (p, *J* = 8.9 Hz, 1H), 3.98 (s, 4H), 3.24 – 3.18 (m, 4H), 3.15 (s, 6H), 2.58 (m, 2H), 2.11 – 2.01 (m, 4H), 1.76 – 1.68 (m, 2H).

<sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 164.1, 157.8, 154.6, 151.9, 151.9, 149.5, 147.7, 142.0, 137.5, 132.0, 127.3, 124.4, 119.3, 113.5, 112.6, 112.5, 101.0, 57.9, 50.6, 30.2, 24.7.

LRMS (ESI) *m/z* calcd for [C<sub>28</sub>H<sub>31</sub>BrN<sub>8</sub>O<sub>3</sub> + H]<sup>+</sup> = 607.3, found 607.2.



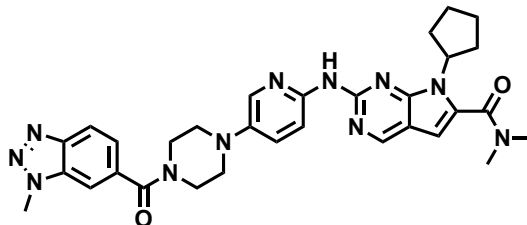
**EST1007**

**General Procedure A** was followed with 4-(Boc-aminomethyl)benzoic acid (14.5 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-15% MeOH in DCM) to afford 29.5 mg (77%) of the title compound as a yellow film.

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.72 (s, 1H), 8.37 (d, *J* = 9.1 Hz, 1H), 8.26 (s, 1H), 8.04 (d, *J* = 2.9 Hz, 1H), 7.41 (d, *J* = 7.9 Hz, 2H), 7.36 – 7.30 (m, 3H), 6.44 (s, 1H), 4.99 (d, *J* = 6.5 Hz, 1H), 4.79 (p, *J* = 9.0 Hz, 1H), 4.35 (d, *J* = 6.1 Hz, 2H), 3.94 (s, 2H), 3.62 (s, 2H), 3.20 (br s, 2H), 3.15 (s, 6H), 3.09 (br s, 2H), 2.58 (m, 2H), 2.11 – 2.00 (m, 4H), 1.76 – 1.67 (m, 2H), 1.46 (s, 9H).

<sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 170.2, 164.1, 156.0, 154.6, 151.9, 151.9, 147.7, 142.0, 141.1, 137.6, 134.4, 132.0, 127.5, 127.4, 112.7, 112.5, 101.0, 79.7, 57.9, 50.6, 44.3, 39.4, 35.2, 30.2, 28.4, 24.7.

LRMS (ESI) *m/z* calcd for [C<sub>36</sub>H<sub>45</sub>N<sub>9</sub>O<sub>4</sub> + H]<sup>+</sup> = 668.4, found 668.5.



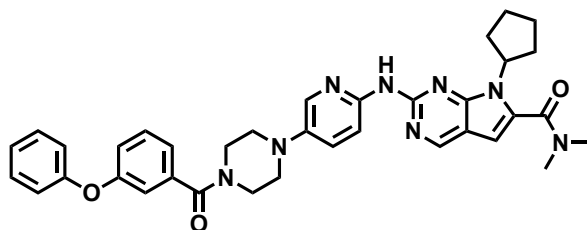
**EST1012**

**General Procedure A** was followed with 1-Methyl-1h-1,2,3-benzotriazole-6-carboxylic acid (10.2 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-15% MeOH in DCM) to afford 23.7 mg (69%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.72 (s, 1H), 8.34 (s, 1H), 8.21 (s, 1H), 8.15 (d, *J* = 1.1 Hz, 1H), 8.04 (d, *J* = 2.9 Hz, 1H), 7.68 – 7.58 (m, 2H), 7.35 (dd, *J* = 9.0, 2.9 Hz, 1H), 6.44 (s, 1H), 4.79 (p, *J* = 8.9 Hz, 1H), 4.34 (s, 3H), 3.94 (br s, 2H), 3.70 (br s, 2H), 3.15 (m, 10H), 2.57 (m, 2H), 2.12 – 1.99 (m, 4H), 1.71 (d, *J* = 4.1 Hz, 2H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 169.7, 164.0, 151.9, 145.3, 142.0, 134.1, 131.3, 127.1, 119.2, 112.9, 112.6, 109.9, 101.0, 57.9, 50.6, 34.5, 30.2, 24.7.

**LRMS (ESI)** *m/z* calcd for [C<sub>31</sub>H<sub>35</sub>N<sub>11</sub>O<sub>2</sub> + H]<sup>+</sup> = 594.3, found 594.3.



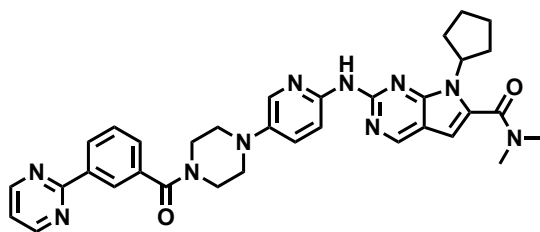
**EST1018**

**General Procedure A** was followed with 3-phenoxybenzoic acid (12.3 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-15% MeOH in DCM) to afford 30.1 mg (83%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.72 (s, 1H), 8.39 (d, *J* = 9.0 Hz, 1H), 8.30 (s, 1H), 8.05 (d, *J* = 2.9 Hz, 1H), 7.47 – 7.42 (m, 2H), 7.42 – 7.31 (m, 3H), 7.19 – 7.12 (m, 1H), 7.04 (ddd, *J* = 13.1, 7.6, 1.6 Hz, 4H), 6.44 (s, 1H), 4.79 (p, *J* = 8.9 Hz, 1H), 3.96 – 3.62 (m, 4H), 3.15 (m, 10H), 2.64 – 2.53 (m, 2H), 2.11 – 2.00 (m, 4H), 1.76 – 1.65 (m, 2H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 170.1, 164.1, 159.1, 156.2, 154.5, 151.9, 151.8, 147.6, 142.1, 137.3, 132.1, 130.0, 129.8, 129.3, 127.5, 124.1, 119.7, 118.1, 112.7, 112.5, 101.0, 57.9, 50.7, 39.5, 35.2, 30.2, 24.7. (Mixture of rotamers)

**LRMS (ESI)** *m/z* calcd for [C<sub>36</sub>H<sub>38</sub>N<sub>8</sub>O<sub>3</sub> + H]<sup>+</sup> = 631.3, found 631.3.



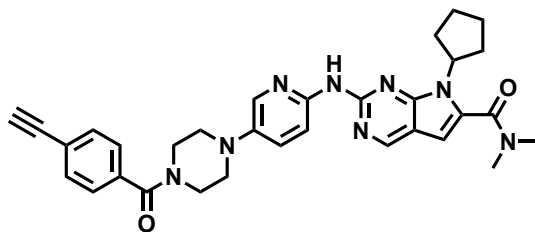
**EST1021**

**General Procedure A** was followed with 3-(2-pyrimidinyl)benzoic acid (11.5 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-15% MeOH in DCM) to afford 12.2 mg (34%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.83 (d, *J* = 4.8 Hz, 2H), 8.69 (s, 1H), 8.54 (dt, *J* = 8.9, 1.7 Hz, 2H), 8.38 (d, *J* = 9.1 Hz, 1H), 8.01 (d, *J* = 2.9 Hz, 1H), 7.92 (s, 1H), 7.63 – 7.54 (m, 2H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.23 (t, *J* = 4.8 Hz, 1H), 6.44 (s, 1H), 4.79 (p, *J* = 8.9 Hz, 1H), 4.01 (br s, 2H), 3.69 (br s, 2H), 3.25 (br s, 2H), 3.15 (s, 6H), 3.13 (br s, 2H), 2.64 – 2.52 (m, 2H), 2.12 – 1.99 (m, 4H), 1.77 – 1.69 (m, 2H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 170.1, 164.1, 163.9, 157.4, 154.4, 152.0, 151.8, 147.5, 142.2, 137.9, 135.9, 132.2, 129.5, 129.5, 129.1, 127.6, 126.8, 119.5, 112.8, 112.6, 101.0, 57.9, 42.1, 39.5, 35.2, 30.2, 24.7.

**LRMS (ESI)** *m/z* calcd for [C<sub>34</sub>H<sub>36</sub>N<sub>10</sub>O<sub>2</sub> + H]<sup>+</sup> = 617.3, found 617.3.



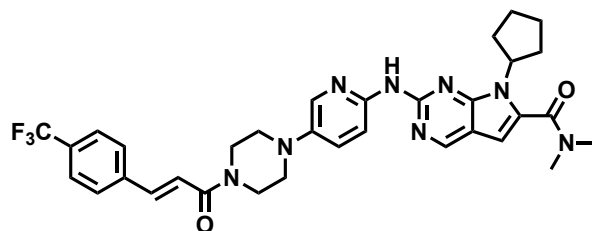
**EST1026**

**General Procedure A** was followed with 4-Ethynylbenzoic acid (8.4 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-15% MeOH in DCM) to afford 13.6 mg (42%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.70 (s, 1H), 8.35 (d, *J* = 9.1 Hz, 1H), 8.16 (s, 1H), 7.99 (d, *J* = 2.9 Hz, 1H), 7.58 - 7.53 (m, 2H), 7.44 - 7.39 (m, 2H), 7.35 (dd, *J* = 9.1, 3.0 Hz, 1H), 6.45 (s, 1H), 4.79 (p, *J* = 9.0, 9.0, 9.0, 9.0 Hz, 1H), 3.93 (s, 2H), 3.61 (s, 2H), 3.16 (d, *J* = 3.9 Hz, 10H), 2.61 (s, 1H), 2.56 (dt, *J* = 16.4, 8.3, 8.3 Hz, 2H), 2.11 - 2.01 (m, 4H), 1.74 - 1.68 (m, 2H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 169.7, 164.0, 154.2, 151.9, 151.6, 147.4, 142.1, 135.6, 132.3, 128.9, 127.2, 123.9, 112.9, 112.7, 101.0, 82.8, 78.8, 57.9, 47.6, 39.4, 35.2, 31.1, 30.2, 29.7, 24.8.

**LRMS (ESI)** *m/z* calcd for [C<sub>32</sub>H<sub>34</sub>N<sub>8</sub>O<sub>2</sub> + H]<sup>+</sup> = 563.3, found 563.3.



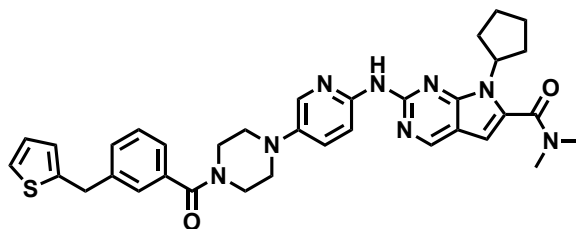
**EST1027**

**General Procedure A** was followed with *trans*-4-(trifluoromethyl)cinnamic acid (12.4 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-15% MeOH in DCM) to afford 24.7 mg (68%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.73 (s, 1H), 8.17 (s, 1H), 8.00 (d, *J* = 2.9 Hz, 1H), 7.71 (d, *J* = 15.4 Hz, 1H), 7.64 (s, 4H), 7.44 – 7.37 (m, 1H), 7.01 (d, *J* = 15.5 Hz, 1H), 6.46 (s, 1H), 4.77 (q, *J* = 8.9 Hz, 1H), 3.99 – 3.78 (m, 4H), 3.19 (br s, 4H), 3.15 (s, 6H), 2.53 (m, 2H), 2.10 – 1.97 (m, 4H), 1.69 (m, 2H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 164.9, 164.1, 154.8, 152.1, 152.0, 148.0, 141.9, 141.3, 138.6, 137.7, 131.9, 131.2 (q, <sup>4</sup>*J*<sub>CF</sub> = 32.5 Hz), 127.9, 127.3, 125.8 (q, <sup>3</sup>*J*<sub>CF</sub> = 3.7 Hz), 125.0, 122.8 (q, <sup>1</sup>*J*<sub>CF</sub> = 272.2 Hz), 119.4, 112.5, 101.1, 57.9, 50.9, 50.3, 45.9, 42.2, 39.4, 35.2, 30.1, 24.7.

**HRMS (ESI)** *m/z* calcd for [C<sub>33</sub>H<sub>35</sub>F<sub>3</sub>N<sub>8</sub>O<sub>2</sub> + H]<sup>+</sup> = 633.2835, found 633.2906.



**EST1030**

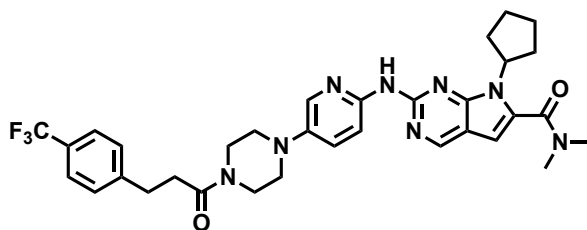
**General Procedure A** was followed with 4-(2-thienylmethyl)benzoic acid (12.6 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-15% MeOH in DCM) to afford 32.3 mg (88%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.65 (s, 1H), 8.35 (d, *J* = 9.1 Hz, 1H), 8.30 (s, 1H), 7.89 (d, *J* = 2.9 Hz, 1H), 7.35 – 7.28 (m, 3H), 7.27 – 7.21 (m, 2H), 7.10 (dd, *J* = 5.2, 1.2 Hz, 1H), 6.87 (dd, *J* = 5.2, 3.4 Hz, 1H), 6.75 (dd, *J* = 3.3,

1.2 Hz, 1H), 6.38 (s, 1H), 4.73 (p,  $J = 8.8$  Hz, 1H), 4.12 (s, 2H), 3.94 – 3.50 (m, 4H), 3.08 (s, 10H), 2.54 – 2.43 (m, 2H), 2.06 – 1.91 (m, 4H), 1.70 – 1.60 (m, 2H).

$^{13}\text{C NMR}$  (126 MHz,  $\text{CDCl}_3$ )  $\delta$  170.4, 164.0, 154.4, 151.9, 151.7, 147.5, 143.0, 142.5, 142.1, 133.6, 132.3, 128.8, 127.8, 127.6, 126.9, 125.5, 124.2, 112.8, 112.7, 101.0, 57.9, 50.6, 39.5, 35.8, 30.2, 24.7.

**LRMS (ESI)**  $m/z$  calcd for  $[\text{C}_{35}\text{H}_{38}\text{N}_8\text{O}_2\text{S} + \text{H}]^+ = 635.3$ , found 635.4.



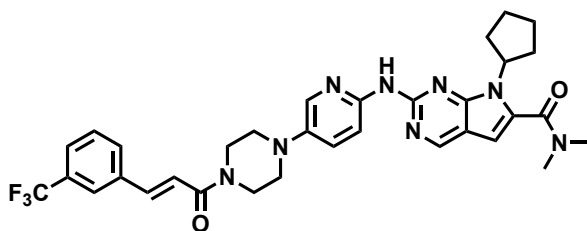
**EST1036**

**General Procedure A** was followed with 4-(trifluoromethyl)hydrocinnamic acid (99.5 mg, 0.46 mmol), HATU (350.2 mg, 0.92 mmol), DIPEA (0.32 mL, 1.4 mmol), and ribociclib (200.1 mg, 0.46 mmol). The crude residue was purified by reverse phase silica gel chromatography (5-95% MeCN in  $\text{H}_2\text{O}$ ) to afford 133.0 mg (46%) of the title compound as a yellow film.

$^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  9.93 (s, 1H), 8.76 (s, 1H), 7.79 (s, 1H), 7.54 (d,  $J = 8.2$  Hz, 2H), 7.47 (d,  $J = 7.9$  Hz, 2H), 7.32 (d,  $J = 7.9$  Hz, 2H), 6.48 (s, 1H), 4.73 (p,  $J = 8.8$  Hz, 1H), 3.74 (t,  $J = 5.0$  Hz, 2H), 3.58 (t,  $J = 4.6$  Hz, 2H), 3.12 (d,  $J = 13.6$  Hz, 6H), 3.08 – 3.00 (m, 6H), 2.71 (t,  $J = 7.5$  Hz, 2H), 2.38 (m, 2H), 2.05 – 1.91 (m, 4H), 1.64 – 1.56 (m, 2H).

$^{13}\text{C NMR}$  (126 MHz,  $\text{CDCl}_3$ )  $\delta$  170.1, 164.1, 154.7, 152.0, 151.9, 147.9, 145.4, 141.9, 137.4, 132.0, 128.9, 128.4 (q,  $^2J_{\text{CF}} = 32.2$  Hz), 127.5, 127.4, 125.4 (q,  $^3J_{\text{CF}} = 3.7$  Hz), 124.3 (q,  $^1J_{\text{CF}} = 271.8$  Hz), 112.5, 101.1, 57.9, 50.6, 50.3, 45.4, 41.6, 39.4, 35.2, 34.4, 31.0, 30.1, 24.7.

**HRMS (ESI)**  $m/z$  calcd for  $[\text{C}_{33}\text{H}_{37}\text{F}_3\text{N}_8\text{O}_2 + \text{H}]^+ = 635.2992$ , found 635.3061.



**EST1051**

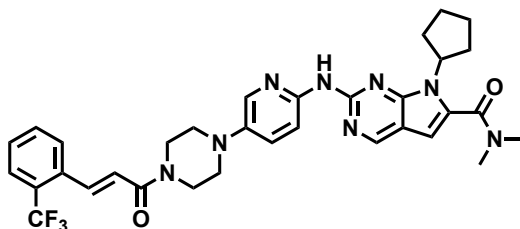


**General Procedure A** was followed with 3-(trifluoromethyl)cinnamic acid (12.4 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 30.3 mg (83%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.65 (s, 1H), 8.32 (d, *J* = 9.0 Hz, 1H), 8.31 (s, 1H), 8.12 (s, 1H), 7.97 (d, *J* = 2.9 Hz, 1H), 7.72 (d, *J* = 2.1 Hz, 1H), 7.66 (d, *J* = 15.4 Hz, 1H), 7.62 (d, *J* = 7.7 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.45 (t, *J* = 7.8, 7.8 Hz, 1H), 7.29 (dd, *J* = 9.1, 3.0 Hz, 1H), 6.92 (d, *J* = 15.4 Hz, 1H), 6.38 (s, 1H), 4.73 (p, *J* = 9.0, 9.0, 8.9, 8.9 Hz, 1H), 3.91 - 3.76 (m, 4H), 3.13 (t, *J* = 5.0, 5.0 Hz, 4H), 3.09 (s, 6H), 2.57 - 2.46 (m, 2H), 2.05 - 1.95 (m, 4H), 1.69 - 1.62 (m, 2H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 164.9, 164.0, 154.2, 151.9, 151.6, 147.3, 142.0, 141.6, 136.0, 132.5, 131.4 (q, <sup>2</sup>*J*<sub>CF</sub> = 31.7 Hz), 131.3, 129.5, 126.2 (q, <sup>3</sup>*J*<sub>CF</sub> = 3.7 Hz), 123.9 (q, <sup>3</sup>*J*<sub>CF</sub> = 3.8 Hz), 122.8 (q, <sup>1</sup>*J*<sub>CF</sub> = 272.6 Hz), 118.7, 113.0, 113.0, 101.0, 57.9, 50.3, 45.9, 42.2, 39.5, 35.2, 30.3, 24.8.

**HRMS (ESI)** *m/z* calcd for [C<sub>33</sub>H<sub>35</sub>F<sub>3</sub>N<sub>8</sub>O<sub>2</sub> + H]<sup>+</sup> = 633.2835, found 633.2906.



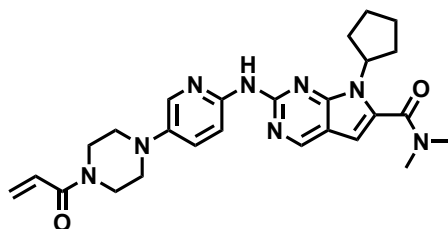
**EST1054**

**General Procedure A** was followed with *trans*-2-(trifluoromethyl)cinnamic acid (12.4 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 26.1 mg (72%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.65 (s, 1H), 8.36 (d, *J* = 9.2 Hz, 1H), 8.30 (s, 1H), 7.96 – 7.87 (m, 2H), 7.66 – 7.60 (m, 2H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.43 – 7.32 (m, 2H), 6.76 (d, *J* = 15.3 Hz, 1H), 6.39 (s, 1H), 4.73 (p, *J* = 8.7 Hz, 1H), 3.92 – 3.71 (m, 4H), 3.12 (t, *J* = 5.1 Hz, 4H), 3.08 (s, 6H), 2.48 (m, 2H), 2.06 – 1.95 (m, 4H), 1.71 – 1.59 (m, 2H).

<sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 165.0, 164.0, 154.2, 151.9, 151.6, 147.4, 142.0, 138.4, 134.4, 132.4, 132.0, 129.1, 128.5 (q, <sup>2</sup>J<sub>CF</sub> = 30.3 Hz), 128.0, 126.2 (q, <sup>3</sup>J<sub>CF</sub> = 5.5 Hz), 122.9 (d, <sup>1</sup>J<sub>CF</sub> = 274.0 Hz), 121.9, 112.9, 112.9, 101.0, 57.9, 50.2, 46.0, 42.1, 39.5, 35.2, 30.3, 24.8.

**HRMS (ESI)** *m/z* calcd for [C<sub>33</sub>H<sub>35</sub>F<sub>3</sub>N<sub>8</sub>O<sub>2</sub> + H]<sup>+</sup> = 633.2835, found 633.2906.



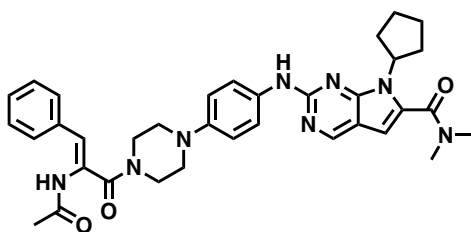
**EST1057**

**General Procedure A** was followed with acrylic acid (0.004 mL, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 15.2 mg (54%) of the title compound as a yellow film.

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.7 (s, 1H), 7.9 (br s, 2H), 7.6 – 7.4 (m, 1H), 6.6 (dd, *J* = 16.8, 10.6 Hz, 1H), 6.5 (s, 1H), 6.2 (d, *J* = 16.8 Hz, 1H), 5.7 (d, *J* = 10.6 Hz, 1H), 4.7 (p, *J* = 9.0 Hz, 1H), 3.8 (t, *J* = 5.2 Hz, 2H), 3.7 (q, *J* = 9.4 Hz, 2H), 3.1 – 3.1 (m, 8H), 3.0 – 2.9 (m, 2H), 2.4 (p, *J* = 8.3 Hz, 2H), 2.0 – 2.0 (m, 2H), 2.0 (q, *J* = 7.7 Hz, 2H), 1.6 (h, *J* = 8.0 Hz, 2H).

<sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 165.4, 164.0, 154.4, 151.9, 151.8, 147.5, 142.1, 132.2, 128.3, 127.8, 127.3, 112.8, 112.7, 101.0, 57.9, 50.8, 50.3, 45.8, 41.9, 39.5, 30.2, 24.8.

**LRMS (ESI)** *m/z* calcd for [C<sub>26</sub>H<sub>32</sub>N<sub>8</sub>O<sub>2</sub> + H]<sup>+</sup> = 489.3, found 489.4.



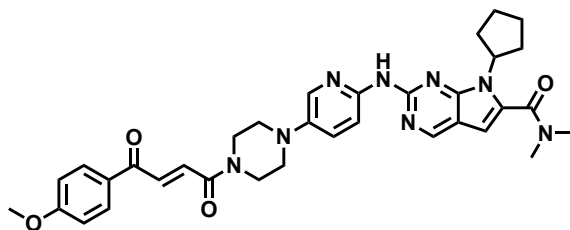
**EST1059**

**General Procedure A** was followed with 2-(acetylamino)-3-phenyl-2-propenoic acid (12.7 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 34.2 mg (96%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (700 MHz, DMSO)  $\delta$  10.84 (s, 1H), 10.11 (s, 1H), 8.93 (s, 1H), 7.95 (d,  $J$  = 2.6 Hz, 2H), 7.75 (d,  $J$  = 8.9 Hz, 1H), 7.68 (d,  $J$  = 8.4 Hz, 2H), 7.63 (d,  $J$  = 8.3 Hz, 2H), 7.49 (d,  $J$  = 15.2 Hz, 1H), 7.23 (d,  $J$  = 15.3 Hz, 1H), 6.76 (s, 1H), 4.80 (p,  $J$  = 8.9 Hz, 1H), 3.91 (s, 2H), 3.77 (s, 2H), 3.22 (s, 4H), 3.06 (s, 6H), 2.40 – 2.31 (m, 2H), 2.07 (s, 3H), 2.05 – 2.00 (m, 2H), 1.99 – 1.93 (m, 2H), 1.66 (m, 2H).

**<sup>13</sup>C NMR** (151 MHz, DMSO)  $\delta$  169.0, 165.2, 162.9, 153.0, 152.0, 149.7, 145.1, 142.4, 142.1, 141.1, 134.9, 130.2, 129.3, 119.3, 116.5, 115.0, 113.9, 101.4, 79.6, 57.5, 49.5, 49.1, 48.8, 45.1, 41.7, 39.2, 35.0, 30.5, 24.7, 24.5.

**LRMS (ESI)**  $m/z$  calcd for  $[C_{35}H_{40}N_8O_3 + H]^+$  = 621.8, found 622.3.



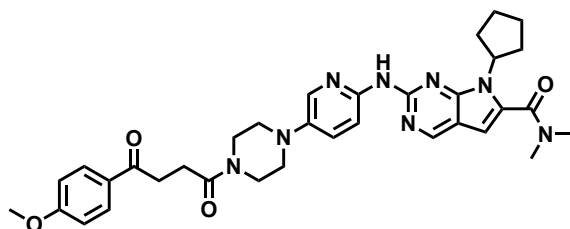
**EST1060**

**General Procedure D** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (99.6 mg, 0.48 mmol), T3P (0.46 mL, 0.72 mmol), DIPEA (0.25 mL, 1.5 mmol), and ribociclib (216.0 mg, 0.57 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM). The resultant dark yellow oil was triturated in diethyl ether to afford 199.3 mg (83% - *E:Z* = >99:<1) of the title compound as an orange powder.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.75 (s, 2H), 8.28 (d,  $J$  = 8.1 Hz, 1H), 8.06 – 8.01 (m, 3H), 7.98 (d,  $J$  = 14.9 Hz, 1H), 7.50 (d,  $J$  = 15.0 Hz, 1H), 7.36 (dd,  $J$  = 9.1, 2.9 Hz, 1H), 7.00 – 6.93 (m, 2H), 6.45 (s, 1H), 4.78 (p,  $J$  = 8.9 Hz, 1H), 3.92 (t,  $J$  = 5.2 Hz, 2H), 3.88 (s, 3H), 3.82 (t,  $J$  = 5.1 Hz, 2H), 3.17 (t,  $J$  = 5.2 Hz, 4H), 3.14 (s, 6H), 2.60 – 2.49 (m, 2H), 2.11 – 1.97 (m, 4H), 1.73 – 1.63 (m, 2H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>)  $\delta$  187.7, 164.2, 164.1, 164.1, 154.5, 151.9, 151.8, 147.7, 141.9, 134.7, 132.1, 131.3, 131.3, 130.0, 127.6, 114.1, 112.7, 112.5, 101.0, 57.9, 55.6, 50.9, 50.3, 46.0, 42.2, 39.5, 35.2, 30.2, 24.7.

**HRMS (ESI)**  $m/z$  calcd for  $[C_{34}H_{38}N_8O_4 + H]^+$  = 623.3016, found 623.3086.



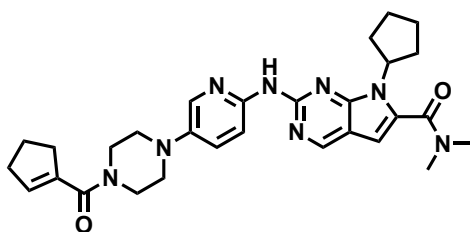
### JP-2-230

A modified **General Procedure A** was followed with 3-(4-Methoxybenzoyl)propionic acid (15.9 mg, 0.08 mmol), HATU (25.5 mg, 0.07 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (27.6 mg, 0.06 mmol). A precipitate had formed during the reaction which was vacuum filtered and washed with hexanes. The yellow precipitate was dissolved in DCM and dry loaded onto silica gel and was purified by silica gel chromatography (0-7% MeOH in DCM) to afford 28.9 mg (73%) of the title compound as a yellow-white powder.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.74 (s, 1H), 8.43 (s, 1H), 8.38 (d, *J* = 9.0 Hz, 1H), 8.07 (d, *J* = 2.9 Hz, 1H), 8.02 – 7.98 (m, 2H), 7.33 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.95 – 6.91 (m, 2H), 6.44 (s, 1H), 4.79 (p, *J* = 8.9 Hz, 1H), 3.86 (s, 3H), 3.81 (t, *J* = 5.1 Hz, 2H), 3.76 (t, *J* = 5.1 Hz, 2H), 3.34 (t, *J* = 6.6 Hz, 2H), 3.18 (t, *J* = 5.1 Hz, 2H), 3.15 (s, 6H), 3.11 (t, *J* = 5.2 Hz, 2H), 2.82 (t, *J* = 6.6 Hz, 2H), 2.62 – 2.54 (m, 2H), 2.11 – 2.00 (m, 4H), 1.75 – 1.67 (m, 2H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 197.6, 170.4, 164.1, 163.5, 154.6, 152.0, 151.9, 147.6, 142.1, 137.5, 132.0, 130.4, 129.9, 127.3, 113.7, 112.6, 112.4, 101.0, 57.9, 55.5, 50.6, 50.3, 45.4, 41.7, 39.5, 35.2, 33.2, 30.2, 27.1, 24.7.

**HRMS (ESI)** *m/z* calcd for [C<sub>34</sub>H<sub>40</sub>N<sub>8</sub>O<sub>4</sub> + H]<sup>+</sup> = 625.3173, found 625.3242.



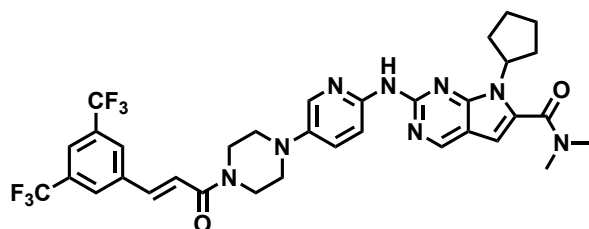
### EST1061

**General Procedure A** was followed with 1-cyclopentenecarboxylic acid (6.5 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 23.3 mg (77%) of the title compound as a yellow powder.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.75 (s, 1H), 8.02 (s, 1H), 7.94 (d, *J* = 2.9 Hz, 1H), 7.50 – 7.42 (m, 1H), 6.47 (s, 1H), 5.93 (p, *J* = 2.2 Hz, 1H), 4.77 (p, *J* = 8.9 Hz, 1H), 3.78 (s, 4H), 3.21 – 3.08 (m, 10H), 2.63 (tq, *J* = 7.5, 2.4 Hz, 2H), 2.54 – 2.42 (m, 4H), 2.12 – 1.99 (m, 4H), 1.96 (p, *J* = 7.6 Hz, 2H), 1.67 (h, *J* = 8.4 Hz, 2H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 168.6, 163.9, 154.1, 152.0, 151.4, 147.1, 142.2, 138.1, 133.1, 132.7, 113.0, 101.0, 58.0, 53.8, 50.5, 39.5, 35.2, 34.5, 33.3, 30.3, 24.8, 22.9, 18.7, 17.5.

**LRMS (ESI)** *m/z* calcd for [C<sub>29</sub>H<sub>36</sub>N<sub>8</sub>O<sub>2</sub> + H]<sup>+</sup> = 529.3, found 529.4.



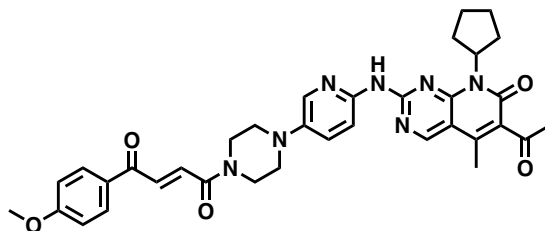
**KN1002**

**General Procedure A** was followed with *trans*-3,5-bis(trifluoromethyl)cinnamic acid (14.6 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and ribociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 14.5 mg (40%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.65 (s, 1H), 8.28 (s, 1H), 7.97 (d, *J* = 3.0 Hz, 2H), 7.87 (s, 2H), 7.79 (s, 1H), 7.68 (d, *J* = 15.4 Hz, 1H), 7.30 (dd, *J* = 9.2, 2.9 Hz, 1H), 6.99 (d, *J* = 15.4 Hz, 1H), 6.38 (s, 1H), 4.73 (t, *J* = 8.9 Hz, 1H), 3.88 (s, 2H), 3.82 (d, *J* = 13.7 Hz, 2H), 3.13 (d, *J* = 8.6 Hz, 4H), 3.09 (s, 6H), 2.50 (dq, *J* = 15.6, 7.3 Hz, 2H), 2.06 – 1.92 (m, 4H), 1.65 (dt, *J* = 12.6, 5.1 Hz, 2H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 164.4, 164.2, 154.4, 151.9, 151.8, 147.6, 142.0, 139.9, 137.5, 137.2, 132.4 (q, <sup>2</sup>*J*<sub>CF</sub> = 33.4 Hz), 132.1, 127.5, 126.3, 124.1, 122.9 (q, <sup>1</sup>*J*<sub>CF</sub> = 273.0 Hz), 122.0, 120.7, 119.8, 112.7, 112.6, 112.6, 101.1, 57.9, 50.8, 50.3, 49.9, 49.8, 49.6, 49.4, 49.3, 45.9, 42.3, 39.5, 35.2, 30.1, 24.7.

**LRMS (ESI)** *m/z* calcd for [C<sub>34</sub>H<sub>34</sub>F<sub>6</sub>N<sub>8</sub>O<sub>2</sub> + H]<sup>+</sup> = 701.3, found 701.4.



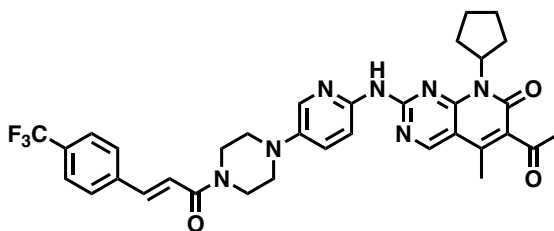
### EST1089

**General Procedure A** was followed with 4-methoxycinnamic acid (13.8 mg, 0.06 mmol), HATU (42.5 mg, 0.11 mmol), DIPEA (0.04 mL, 0.23 mmol), and palbociclib (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 26.1 mg (74% - *E:Z* = >99:<1) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.86 (s, 1H), 8.22 (d, *J* = 9.0 Hz, 1H), 8.09 (d, *J* = 2.9 Hz, 1H), 8.08 – 8.02 (m, 2H), 8.00 (d, *J* = 14.9 Hz, 1H), 7.52 (d, *J* = 14.8 Hz, 1H), 7.35 (dd, *J* = 9.1, 3.0 Hz, 1H), 7.01 – 6.95 (m, 2H), 5.88 (p, *J* = 8.9 Hz, 1H), 3.94 (t, *J* = 5.2 Hz, 2H), 3.89 (s, 3H), 3.85 (t, *J* = 5.1 Hz, 2H), 3.22 (t, *J* = 5.2 Hz, 4H), 2.54 (s, 3H), 2.38 (s, 3H), 2.36 – 2.30 (m, 1H), 2.12 – 2.01 (m, 2H), 1.89 (dddd, *J* = 15.4, 13.0, 7.6, 4.7 Hz, 2H), 1.75 – 1.64 (m, 3H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 202.6, 187.6, 164.3, 164.1, 161.4, 158.0, 157.2, 155.5, 145.8, 143.0, 141.7, 137.3, 134.9, 131.3, 131.1, 130.9, 129.9, 126.9, 114.2, 113.6, 107.9, 55.6, 54.1, 53.4, 50.4, 49.8, 45.8, 42.1, 31.5, 28.1, 25.8, 22.7, 14.0.

**HRMS (ESI)** *m/z* calcd for [C<sub>35</sub>H<sub>37</sub>N<sub>7</sub>O<sub>5</sub> + H]<sup>+</sup> = 636.2929, found 636.2935.



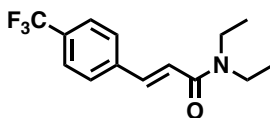
### EST1090

**General Procedure A** was followed with *trans*-4-(trifluoromethyl)cinnamic acid (14.5 mg, 0.07 mmol), HATU (42.5 mg, 0.11 mmol), DIPEA (0.04 mL, 0.23 mmol), and palbociclib (0.25, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-100% EtOAc in Hexanes) to afford 22.8 mg (63%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.87 (s, 1H), 8.74 (s, 1H), 8.22 (d, *J* = 9.1 Hz, 1H), 8.09 (d, *J* = 2.9 Hz, 1H), 7.72 (d, *J* = 15.4 Hz, 1H), 7.64 (s, 4H), 7.37 (dd, *J* = 9.1, 3.0 Hz, 1H), 7.00 (d, *J* = 15.5 Hz, 1H), 5.88 (p, *J* = 8.9 Hz, 1H), 4.01 – 3.81 (m, 4H), 3.23 (t, *J* = 5.1 Hz, 4H), 2.54 (s, 3H), 2.38 (s, 3H), 2.37 – 2.31 (m, 2H), 2.07 (tdd, *J* = 11.9, 9.9, 5.2 Hz, 2H), 1.92 – 1.84 (m, 2H), 1.69 (tdd, *J* = 10.9, 6.6, 4.0 Hz, 2H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 202.6, 164.9, 161.4, 158.0, 157.2, 155.6, 145.8, 143.1, 141.8, 141.6, 138.5 (d, <sup>5</sup>*J*<sub>CF</sub> = 1.4 Hz), 137.1, 131.4 (q, <sup>2</sup>*J*<sub>CF</sub> = 32.7 Hz), 130.9, 128.0, 126.9, 125.8 (q, <sup>3</sup>*J*<sub>CF</sub> = 3.8 Hz), 123.9 (d, <sup>1</sup>*J*<sub>CF</sub> = 272.1 Hz), 119.2, 113.7, 107.9, 54.1, 50.3, 49.8, 45.7, 42.1, 31.5, 28.1, 25.8, 14.0.

**HRMS (ESI)** *m/z* calcd for [C<sub>34</sub>H<sub>34</sub>F<sub>3</sub>N<sub>7</sub>O<sub>3</sub> + H]<sup>+</sup> = 646.2748, found 646.2741.



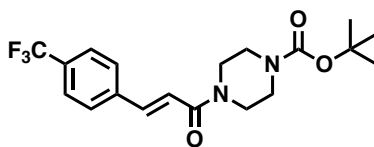
**KN1026**

**General Procedure A** was followed with *trans*-4-(trifluoromethyl)cinnamic acid (100.0 mg, 0.06 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and *N,N*-diethylamine (0.05, 0.48 mmol). The crude residue was purified by silica gel chromatography (0-60% EtOAc in Hexanes) to afford 66.0 mg (50%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 7.63 (d, *J* = 15.4 Hz, 1H), 7.54 (s, 4H), 6.83 (d, *J* = 15.4 Hz, 1H), 3.42 (dq, *J* = 9.8, 7.1 Hz, 4H), 1.19 (t, *J* = 7.2 Hz, 3H), 1.12 (t, *J* = 7.1 Hz, 3H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 165.1, 140.5, 138.9 (d, <sup>5</sup>*J*<sub>CF</sub> = 1.4 Hz), 131.0 (q, <sup>2</sup>*J*<sub>CF</sub> = 32.5 Hz), 127.9, 125.7 (q, <sup>3</sup>*J*<sub>CF</sub> = 3.8 Hz), 123.9 (q, <sup>1</sup>*J*<sub>CF</sub> = 272.1 Hz), 120.3, 42.4, 41.2, 15.1, 13.1.

**LRMS (ESI)** *m/z* calcd for [C<sub>14</sub>H<sub>16</sub>F<sub>3</sub>NO + H]<sup>+</sup> = 272.1, found 272.2.



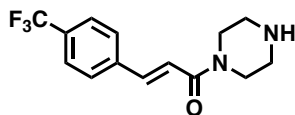
**EST1096**

**General Procedure A** was followed with *trans*-4-(trifluoromethyl)cinnamic acid (100.0 mg, 0.46 mmol), HATU (43.8 mg, 0.12 mmol), DIPEA (0.04 mL, 0.23 mmol), and 1-boc-piperazine (94.7 mg, 0.51 mmol). The crude residue was purified by silica gel chromatography (0-70% EtOAc in Hexanes) to afford 72.5 mg (41%) of the title compound as a white powder.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 7.62 (d, *J* = 15.4 Hz, 1H), 7.55 (s, 4H), 6.87 (d, *J* = 15.4 Hz, 1H), 3.60 (s, 4H), 3.42 (dd, *J* = 6.6, 3.9 Hz, 4H), 1.41 (s, 9H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 165.0, 154.5, 141.4, 138.5, 131.3 (q, <sup>2</sup>*J*<sub>CF</sub> = 32.6 Hz), 127.9, 125.8 (q, <sup>3</sup>*J*<sub>CF</sub> = 3.7 Hz), 123.9 (q, <sup>1</sup>*J*<sub>CF</sub> = 272.3 Hz), 119.3, 80.4, 45.7, 42.0, 28.4.

**LRMS (ESI)** *m/z* calcd for [C<sub>19</sub>H<sub>23</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub> + Na]<sup>+</sup> = 407.2, found 407.2.



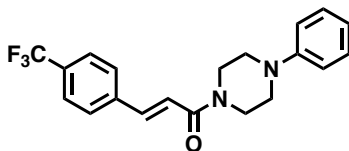
**EST1102**

**General Procedure E** was followed with **EST1096** (47.3 mg, 0.12 mmol), and TFA (0.3 mL, 3.94 mmol) for 2 hours. The crude residue was purified by silica gel chromatography (0-17% MeOH in DCM) using a Biotage® Sfär KP-Amino D cartridge to afford 14.5 mg (41%) of the title compound as a white film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 7.64 (d, *J* = 15.5 Hz, 1H), 7.59 (s, 4H), 6.93 (d, *J* = 15.4 Hz, 1H), 3.72 – 3.66 (m, 2H), 3.64 – 3.58 (m, 2H), 2.89 (t, *J* = 5.0, 5.0 Hz, 4H), 1.80 (s, 1H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 164.9, 141.3, 138.6, 131.2 (q, <sup>2</sup>*J*<sub>CF</sub> = 32.6 Hz), 127.9, 125.8 (q, <sup>3</sup>*J*<sub>CF</sub> = 3.8 Hz), 125.0, 122.8 (q, <sup>1</sup>*J*<sub>CF</sub> = 272.0 Hz), 119.4, 46.5, 46.1, 45.5, 42.7.

**LRMS (ESI)** *m/z* calcd for [C<sub>14</sub>H<sub>15</sub>F<sub>3</sub>N<sub>2</sub>O + H]<sup>+</sup> = 285.1, found 285.2.



**KN1023**

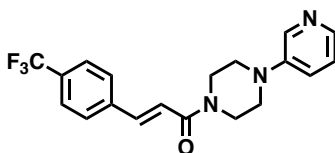
**General Procedure A** was followed with *trans*-4-(trifluoromethyl)cinnamic acid (200.0 mg, 0.93 mmol), HATU (703.6 mg, 1.85 mmol), DIPEA (0.64 mL, 3.7 mmol), and 1-phenylpiperazine (150.1 mg, 0.93 mmol). The crude residue was purified by silica gel chromatography (15-55% EtOAc in Hexanes) to afford 125.0 mg (37%) of the title compound as a yellow powder.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 7.62 (d, *J* = 15.4 Hz, 1H), 7.53 (s, 4H), 7.23 – 7.16 (m, 2H), 6.91 (d, *J* = 15.4 Hz, 1H), 6.83 (dd, *J* = 16.5, 7.9 Hz, 3H), 3.80 (br s, 2H), 3.72 (br s, 2H), 3.13 (m, 4H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 164.9, 150.9, 141.3, 138.7, 131.2 (q, <sup>2</sup>*J*<sub>CF</sub> = 32.5 Hz), 129.3, 128.0, 125.8 (q, <sup>3</sup>*J*<sub>CF</sub> = 3.8 Hz), 124.0 (q, <sup>1</sup>*J*<sub>CF</sub> = 272.2 Hz), 120.6, 119.6, 116.7, 49.9, 49.4, 45.9, 42.2.



**LRMS (ESI)**  $m/z$  calcd for  $[C_{20}H_{19}F_3N_2O + H]^+$  = 361.1, found 361.2.



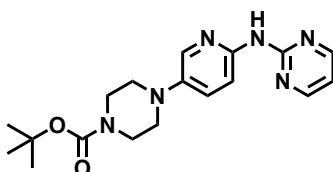
**KN1017**

**General Procedure A** was followed with *trans*-4-(trifluoromethyl)cinnamic acid (200.0 mg, 0.93 mmol), HATU (703.6 mg, 1.85 mmol), DIPEA (0.64 mL, 3.7 mmol), and 1-(3-pyridinyl)piperazine (151.1 mg, 0.93 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 53.1 mg (16%) of the title compound as a yellow powder.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.34 – 8.29 (m, 1H), 8.14 (dd,  $J$  = 3.9, 2.1 Hz, 1H), 7.70 (d,  $J$  = 15.4 Hz, 1H), 7.61 (s, 4H), 7.23 – 7.14 (m, 2H), 6.98 (d,  $J$  = 15.5 Hz, 1H), 3.90 (br s, 2H), 3.83 (br s, 2H), 3.25 (m, 4H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 164.9, 146.5, 141.7, 141.5, 139.1, 138.5, 131.3 (q,  $^2J_{CF}$  = 32.6 Hz), 128.0, 125.8 (q,  $^3J_{CF}$  = 3.8 Hz), 123.9 (q,  $^1J_{CF}$  = 272.2 Hz), 123.6, 123.1, 119.2, 49.2, 48.7, 45.6, 41.9.

**LRMS (ESI)**  $m/z$  calcd for  $[C_{19}H_{18}F_3N_3O + H]^+$  = 362.1, found 362.1.



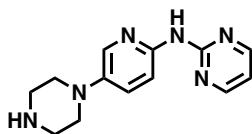
**JP-2-195**

A combination of 2-chloropyrimidine (100.0 mg, 0.95 mmol), tert-butyl 4-(6-aminopyridin-3-yl)piperazine-1-carboxylate (265.2 mg, 0.95 mmol), palladium (II) acetate (21.4 mg, 0.10 mmol), caesium carbonate (465.6 mg, 1.4 mmol), and Xantphos (82.7 mg, 0.14 mmol) was suspended in dioxane (10 mL) and heated to 120 °C overnight. The volatiles were removed *in vacuo*, and the crude residue was purified by silica gel chromatography (0-5% MeOH in DCM) to afford 282.1 mg (91%) of the title compound as a beige solid.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.50 (d,  $J$  = 4.8 Hz, 2H), 8.30 (d,  $J$  = 9.0 Hz, 1H), 8.19 (s, 1H), 8.07 (d,  $J$  = 2.9 Hz, 1H), 7.35 (dd,  $J$  = 9.1, 3.0 Hz, 1H), 6.78 (t,  $J$  = 4.8 Hz, 1H), 3.63 (t,  $J$  = 5.1 Hz, 4H), 3.11 (t,  $J$  = 5.2 Hz, 4H), 1.51 (s, 9H).

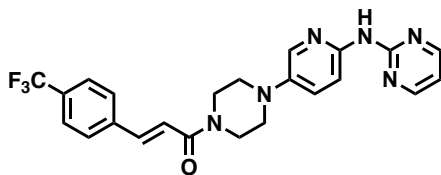
**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 159.3, 158.0, 154.7, 146.6, 143.0, 137.4, 127.2, 113.0, 112.9, 80.0, 50.1, 28.4.

**LRMS (ESI)**  $m/z$  calcd for  $[C_{18}H_{24}N_6O_2 + H]^+$  = 375.2, found 375.3.



**JP-2-198**

**General Procedure E** was followed with **JP-2-195** (152.4 mg, 0.43 mmol), and TFA (1.1 mL, 13.7 mmol) for 2 hours. The crude material was used without further purification.



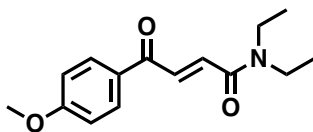
**JP-2-200**

**General Procedure A** was followed with *trans*-4-(trifluoromethyl)cinnamic acid (43.0 mg, 0.20 mmol), HATU (82.6 mg, 0.22 mmol), DIPEA (0.09 mL, 0.54 mmol), and **JP-2-198** (46.4 mg, 0.18 mmol). The crude residue was purified by silica gel chromatography (50-100% EtOAc in Hexanes followed by 0-5% MeOH in DCM) to afford 13.3 mg (16%, 2 steps) of the title compound as a yellow residue.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.48 (dd, *J* = 4.8, 1.6 Hz, 2H), 8.30 (d, *J* = 8.9 Hz, 1H), 8.14 (s, 1H), 8.05 (t, *J* = 2.2 Hz, 1H), 7.72 (dd, *J* = 15.5, 1.7 Hz, 1H), 7.64 (d, *J* = 1.7 Hz, 3H), 7.35 (dt, *J* = 9.1, 2.3 Hz, 1H), 7.00 (dd, *J* = 15.5, 1.6 Hz, 1H), 6.77 (td, *J* = 4.8, 1.7 Hz, 1H), 3.94 (br s, 2H), 3.85 (br s, 2H), 3.19 (m, 3H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 164.9, 159.2, 158.0, 147.0, 142.5, 141.5, 138.6 (d, <sup>5</sup>*J*<sub>CF</sub> = 1.3 Hz), 137.5, 131.3 (q, <sup>2</sup>*J*<sub>CF</sub> = 32.8), 127.9, 127.4, 125.8 (q, <sup>3</sup>*J*<sub>CF</sub> = 3.8 Hz), 123.9 (q, <sup>1</sup>*J*<sub>CF</sub> = 272.2 Hz), 119.3, 113.1, 113.0, 50.7, 50.1, 45.8, 42.1, 29.7, 28.4.

**LRMS (ESI)** *m/z* calcd for [C<sub>23</sub>H<sub>21</sub>F<sub>3</sub>N<sub>6</sub>O + H]<sup>+</sup> = 455.2, found 455.2.



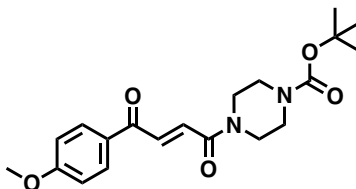
**KN1025**

**General Procedure A** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (100.1 mg, 0.47 mmol), HATU (175.9 mg, 0.48 mmol), DIPEA (0.08 mL, 0.46 mmol), and *N,N*-diethylamine (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-55% EtOAc in Hexanes) to afford 16.3 mg (13%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.06 – 8.04 (m, 2H), 7.99 (d, *J* = 14.8 Hz, 1H), 7.43 (d, *J* = 14.8 Hz, 1H), 6.99 – 6.95 (m, 2H), 3.89 (s, 3H), 3.52 – 3.45 (m, 4H), 1.24 (t, *J* = 7.2 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 187.9, 164.5, 164.1, 134.0, 132.2, 131.3, 131.0, 130.1, 114.1, 55.6, 42.5, 41.2, 15.1, 13.0.

**LRMS (ESI)** *m/z* calcd for [C<sub>15</sub>H<sub>19</sub>NO<sub>3</sub> + H]<sup>+</sup> = 262.1, found 262.3.



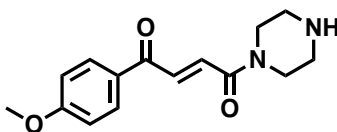
**JP-2-190**

**General Procedure A** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (977 mg, 7.3 mmol), HATU (3.1 g, 8.1 mmol), DIPEA (3.8 mL, 21.8 mmol), and 1-boc-piperazine (1.5 g, 8.0 mmol). The crude residue was purified by silica gel chromatography (0-50% EtOAc in Hexanes) to afford 1.59 g (58%) of the title compound as a yellow solid.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.04 (d, *J* = 8.6 Hz, 2H), 7.96 (dd, *J* = 15.4, 3.9 Hz, 1H), 7.46 (d, *J* = 14.9 Hz, 1H), 6.98 (d, *J* = 8.7 Hz, 2H), 3.89 (s, 3H), 3.72 (t, *J* = 5.4, 5.4 Hz, 2H), 3.62 (t, *J* = 5.2, 5.2 Hz, 2H), 3.49 (t, *J* = 5.2, 5.2 Hz, 4H), 1.48 (s, 9H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 187.6, 164.2, 154.5, 134.8, 131.3, 131.2, 131.1, 129.9, 114.1, 114.0, 80.5, 55.6, 45.9, 42.1, 28.4.

**LRMS (ESI)** *m/z* calcd for [C<sub>20</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub> + Na]<sup>+</sup> = 397.2, found 397.2.



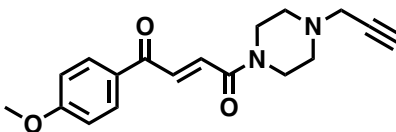
**JP-2-196**

**General Procedure E** was followed with **JP-2-190** (78.6 mg, 0.21 mmol), and TFA (0.51 mL, 6.7 mmol) for 1.5 hours. The reaction mixture was concentrated *in vacuo*, redissolved in DCM, and stirred with a saturated solution of NaHCO<sub>3</sub> (2 mL) for 30 min. The organic layer was separated, washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (0-12% MeOH in DCM) to afford 20.8 mg (81% - *E:Z* = 98.5:1.5) of the title compound as a white solid.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 7.99 (dd, *J* = 8.9, 1.9 Hz, 2H), 7.93 (dd, *J* = 14.9, 1.8 Hz, 1H), 7.37 (dd, *J* = 14.9, 1.8 Hz, 1H), 6.94 (dd, *J* = 9.0, 2.0 Hz, 2H), 3.97 (t, *J* = 5.4 Hz, 2H), 3.92 (t, *J* = 5.4 Hz, 2H), 3.85 (d, *J* = 1.9 Hz, 3H), 3.22 (t, *J* = 5.2 Hz, 4H), 2.73 (s, 1H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 187.5, 164.5, 136.0, 131.5, 130.0, 129.7, 114.3, 55.7, 43.4, 43.1, 42.8, 38.9.

**LRMS (ESI)** *m/z* calcd for [C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub> + H]<sup>+</sup> = 275.1, found 275.1.



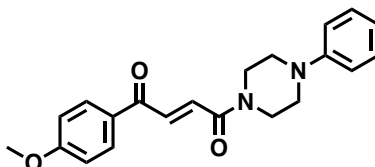
**JP-2-253**

A combination of **JP-2-196** (25.9 mg, 0.094 mmol) and potassium carbonate (15.7 mg, 0.11 mmol) was suspended in DMF (4 mL) and stirred at ambient temperature for 5 minutes. Propargyl bromide (0.01 mL, 0.13 mmol) was added, and the reaction mixture was heated to 90 °C overnight. The reaction mixture was concentrated *in vacuo*, and the crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 11.7 mg (40% - *E:Z* = 96:4) of the title compound as a yellow oil.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.05 – 8.02 (m, 2H), 7.95 (d, *J* = 14.9 Hz, 1H), 7.47 (d, *J* = 14.9 Hz, 1H), 6.99 – 6.95 (m, 2H), 3.89 (s, 3H), 3.79 (t, *J* = 5.1 Hz, 2H), 3.68 (t, *J* = 5.1 Hz, 2H), 3.35 (d, *J* = 2.4 Hz, 2H), 2.60 (q, *J* = 4.6 Hz, 4H), 2.28 (d, *J* = 2.4 Hz, 1H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 187.7, 164.2, 164.0, 134.4, 131.5, 131.3, 130.0, 114.1, 78.0, 73.8, 55.6, 51.9, 51.3, 46.8, 45.9, 42.1.

**LRMS (ESI)** *m/z* calcd for [C<sub>18</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> + H]<sup>+</sup> = 213.1, found 213.2.



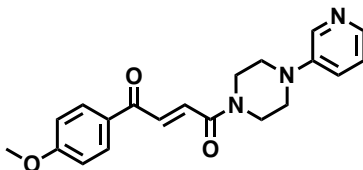
**KN1021**

**General Procedure A** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (201.2 mg, 0.07 mmol), HATU (737.6 mg, 1.94 mmol), DIPEA (0.68 mL, 3.88 mmol), and 1-phenylpiperazine (25.0 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (15-55% EtOAc in Hexanes) to afford 255.6 mg (75%) of the title compound as an orange film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.05 (d, *J* = 8.6 Hz, 2H), 7.99 (d, *J* = 14.9 Hz, 1H), 7.52 (d, *J* = 14.9 Hz, 1H), 7.30 (t, *J* = 7.7 Hz, 2H), 7.01 – 6.89 (m, 5H), 3.92 (m, 2H), 3.90 (s, 3H), 3.81 (t, *J* = 5.1 Hz, 2H), 3.23 (m, 4H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 187.7, 164.2, 164.1, 150.8, 134.6, 131.4, 131.3, 130.0, 129.3, 120.8, 116.8, 114.1, 55.6, 50.0, 49.4, 46.0, 42.2.

**LRMS (ESI)** *m/z* calcd for [C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> + H]<sup>+</sup> = 351.2, found 351.1.



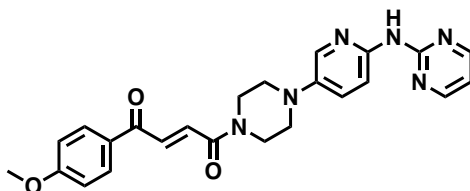
**KN1018**

**General Procedure A** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (200.2 mg, 0.97 mmol), HATU (740.2 mg, 1.96 mmol), DIPEA (0.68 mL, 3.90 mmol), and 1-(3-pyridinyl)piperazine (158.3 mg, 0.97 mmol). The crude residue was purified by silica gel chromatography (0-8% MeOH in DCM) to afford 40.4 mg (12%) of the title compound as a yellow film.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.33 – 8.27 (m, 1H), 8.14 (dd, *J* = 3.8, 2.2 Hz, 1H), 8.06 – 8.00 (m, 2H), 7.97 (d, *J* = 14.9 Hz, 1H), 7.49 (d, *J* = 14.8 Hz, 1H), 7.22 – 7.14 (m, 2H), 6.99 – 6.92 (m, 2H), 3.90 (m, 2H), 3.86 (s, 3H), 3.80 (t, *J* = 5.2 Hz, 2H), 3.27 – 3.21 (m, 4H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 187.6, 164.2, 164.1, 146.5, 141.7, 139.2, 134.8, 131.3, 131.1, 129.9, 123.6, 123.1, 114.1, 55.6, 49.2, 48.7, 45.7, 42.0.

**LRMS (ESI)** *m/z* calcd for [C<sub>20</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub> + H]<sup>+</sup> = 352.2, found 352.1.



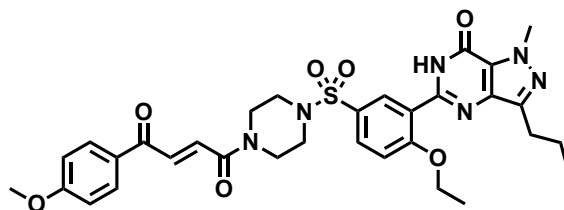
**JP-2-199**

**General Procedure A** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (39.2 mg, 0.19 mmol), HATU (78.9 mg, 0.21 mmol), DIPEA (0.09 mL, 0.52 mmol), and **JP-2-198** (44.3 mg, 0.17 mmol). The crude residue was purified by silica gel chromatography (50-100% EtOAc in Hexanes followed by 0-5% MeOH in DCM) to afford 37.9 mg (49%) of the title compound as a yellow residue.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.77 (s, 1H), 8.50 (d, *J* = 4.8 Hz, 2H), 8.31 (d, *J* = 9.1 Hz, 1H), 8.10 (d, *J* = 2.9 Hz, 1H), 8.05 (d, *J* = 8.4 Hz, 2H), 7.99 (d, *J* = 14.8 Hz, 1H), 7.52 (d, *J* = 14.6 Hz, 1H), 7.34 (dd, *J* = 9.0, 2.9 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.76 (t, *J* = 4.9 Hz, 1H), 3.93 (t, *J* = 5.4 Hz, 2H), 3.89 (s, 3H), 3.83 (t, *J* = 5.1 Hz, 2H), 3.18 (q, *J* = 4.5 Hz, 4H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 187.6, 164.2, 164.1, 159.3, 158.0, 147.2, 142.3, 137.5, 134.7, 131.3, 131.3, 130.0, 127.5, 114.1, 113.2, 112.9, 55.6, 50.7, 50.1, 45.9, 42.2, 29.7, 28.4.

**LRMS (ESI)** *m/z* calcd for [C<sub>24</sub>H<sub>24</sub>N<sub>6</sub>O<sub>3</sub> + H]<sup>+</sup> = 445.2, found 445.2.



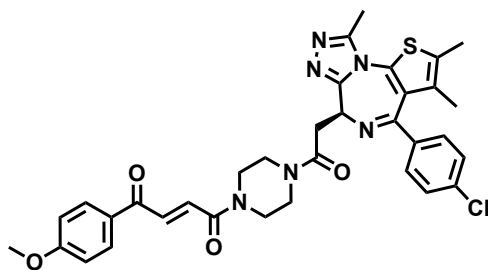
**JP-2-201**

The secondary amine **JP-2-196** (23.2 mg, 0.08 mmol) was dissolved in DCM (1 mL). DIPEA (0.1 mL, 0.5 mmol) was added, and the reaction mixture was stirred at ambient temperature for 5 minutes. A solution of 5-(5-Chlorosulfonyl-2-ethoxyphenyl)-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (34.7 mg, 0.08 mmol) in DCM (0.5 mL) was added dropwise to the reaction mixture and allowed to stir at ambient temperature for 2 hours. The volatiles were removed *in vacuo*, and the crude residue was purified by silica gel chromatography (25-100% EtOAc in Hexanes) to afford 49.5 mg (90% - *E:Z* = 90:10) of the title compound as a yellow oil.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 10.73 (s, 1H), 8.73 (d, *J* = 2.4 Hz, 1H), 7.92 – 7.89 (m, 2H), 7.82 (d, *J* = 14.9 Hz, 1H), 7.75 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.27 (d, *J* = 14.8 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 1H), 6.89 – 6.84 (m, 2H), 4.30 (q, *J* = 7.0 Hz, 2H), 4.19 (s, 3H), 3.80 (s, 3H), 3.79 – 3.76 (m, 2H), 3.68 (t, *J* = 5.0 Hz, 2H), 3.05 (q, *J* = 5.4 Hz, 4H), 2.86 (t, *J* = 7.6 Hz, 2H), 1.79 (h, *J* = 7.4 Hz, 2H), 1.57 (t, *J* = 7.0 Hz, 3H), 0.96 (t, *J* = 7.4 Hz, 3H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 187.3, 164.3, 164.0, 159.6, 153.6, 147.0, 146.2, 138.3, 135.2, 131.5, 131.3, 131.1, 130.5, 129.7, 128.5, 124.5, 121.4, 114.1, 113.3, 77.3, 66.2, 55.6, 46.4, 45.8, 45.4, 41.6, 38.2, 27.7, 22.3, 14.5, 14.1.

**HRMS (ESI)** *m/z* calcd for [C<sub>32</sub>H<sub>36</sub>N<sub>6</sub>O<sub>7</sub>S + H]<sup>+</sup> = 649.2366, found 649.2450.



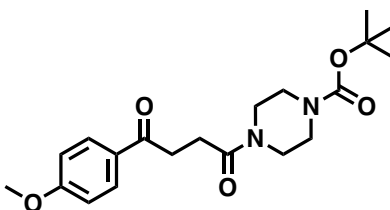
**JP-2-197**

**General Procedure A** was followed with (6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine-6-acetic acid (JQ1-Acid) (92.5 mg, 0.23 mmol), HATU (93.0 mg, 0.24 mmol), DIPEA (0.1 mL, 0.6 mmol), and **JP-2-196** (55.0 mg, 0.2 mmol). The crude residue was purified by silica gel chromatography (0-5% MeOH in DCM) to afford 108.6 mg (83%, 2 steps - *E:Z* = 89:11) of the title compound as a yellow powder.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.08 (d, *J* = 8.6 Hz, 2H), 8.02 (dd, *J* = 14.9, 6.5 Hz, 1H), 7.51 (dd, *J* = 14.9, 6.7 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 2H), 7.39 – 7.34 (m, 2H), 7.01 (d, *J* = 8.5 Hz, 2H), 4.83 (td, *J* = 6.9, 2.9 Hz, 1H), 4.08 – 3.95 (m, 2H), 3.93 (s, 3H), 3.91 – 3.70 (m, 5H), 3.71 – 3.49 (m, 3H), 2.69 (s, 3H), 2.43 (s, 3H), 1.70 (s, 3H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 187.8, 187.7, 169.3, 169.2, 164.5, 164.2, 164.2, 164.1, 155.6, 150.0, 136.7, 136.5, 134.9, 131.9, 131.3, 131.0, 131.0, 131.0, 130.9, 130.5, 129.7, 129.7, 128.6, 114.1, 114.0, 55.5, 54.2, 54.1, 45.8, 45.8, 45.6, 45.2, 42.1, 42.0, 41.8, 41.4, 35.1, 29.6, 14.2, 13.0, 11.6.

**HRMS (ESI)** *m/z* calcd for [C<sub>34</sub>H<sub>33</sub>ClN<sub>6</sub>O<sub>4</sub>S + H]<sup>+</sup> = 657.1973, found 657.2054.



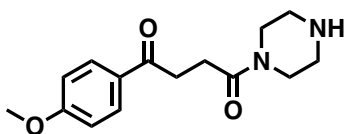
**JP-2-229**

**General Procedure A** was followed with 3-(4-methoxybenzoyl)propionic acid (100.7 mg, 0.48 mmol), HATU (206.8 mg, 0.54 mmol), DIPEA (0.25 mL, 1.45 mmol), and 1-boc-piperazine (104.7 mg, 0.56 mmol). The crude residue was purified by silica gel chromatography (0-100% EtOAc in Hexanes) to afford 132.8 mg (73%) of the title compound as a white powder.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 7.93 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 3.80 (s, 3H), 3.58 – 3.52 (m, 2H), 3.52 – 3.47 (m, 2H), 3.44 (d, *J* = 7.5 Hz, 2H), 3.35 (t, *J* = 5.5 Hz, 2H), 3.26 (t, *J* = 6.5 Hz, 2H), 2.71 (t, *J* = 6.6 Hz, 2H), 1.43 (s, 9H).

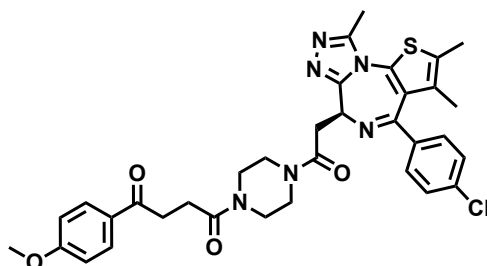
**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 197.5, 170.6, 163.5, 154.5, 130.3, 129.8, 113.7, 80.2, 55.4, 45.2, 41.6, 33.1, 29.7, 28.4, 27.1.

**LRMS (ESI)** *m/z* calcd for [C<sub>20</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub> + H]<sup>+</sup> = 377.5, found 377.2.



**JP-2-231**

**General Procedure E** was followed with **JP-2-229** (23.6 mg, 0.06 mmol), and TFA (0.13 mL, 1.7 mmol) for 2.5 hours. The crude material was used without further purification.



**JP-2-232**

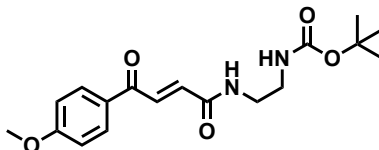
**General Procedure A** was followed with (6*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine-6-acetic acid (JQ1-Acid) (26.1 mg, 0.07 mmol), HATU (27.3 mg, 0.07 mmol), DIPEA (0.4 mL, 0.19 mmol), and **JP-2-231** (17.3 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-7% MeOH in DCM) to afford 38.8 mg (94%, 2 steps) of the title compound as a yellow-white foam.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 7.99 (d, *J* = 8.8 Hz, 2H), 7.39 (d, *J* = 8.2 Hz, 2H), 7.32 (dd, *J* = 8.6, 4.2 Hz, 2H), 6.95 – 6.91 (m, 2H), 4.79 (q, *J* = 6.7 Hz, 1H), 4.01 – 3.94 (m, 1H), 3.86 (s, 3H), 3.85 – 3.80 (m, 2H), 3.79 – 3.69 (m, 4H), 3.69 – 3.63 (m, 1H), 3.60 – 3.55 (m, 1H), 3.54 – 3.48 (m, 1H), 3.42 – 3.35 (m, 1H), 3.32 – 3.26 (m, 1H), 2.84 (tt, *J* = 16.0, 6.5 Hz, 1H), 2.78 – 2.73 (m, 1H), 2.65 (d, *J* = 2.8 Hz, 3H), 2.39 (s, 3H), 1.67 (s, 3H).



<sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 197.5, 170.7, 169.4, 169.2, 163.9, 163.8, 163.6, 155.8, 149.9, 149.9, 136.8, 136.7, 132.2, 130.9, 130.9, 130.7, 130.7, 130.5, 130.4, 129.9, 129.8, 128.7, 113.7, 55.5, 54.6, 54.4, 45.9, 45.6, 45.5, 45.2, 41.8, 41.7, 41.6, 35.4, 35.3, 33.2, 33.2, 31.9, 29.7, 27.2, 27.1, 22.7, 14.4, 14.1, 13.1, 11.8.

**HRMS (ESI)** *m/z* calcd for [C<sub>34</sub>H<sub>35</sub>ClN<sub>6</sub>O<sub>4</sub>S + H]<sup>+</sup> = 659.2129, found 659.2207.



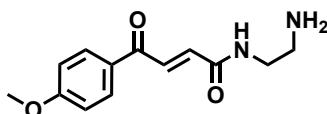
**JP-2-216**

**General Procedure A** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (54.2 mg, 0.26 mmol), HATU (124.1 mg, 0.33 mmol), DIPEA (0.13 mL, 0.79 mmol), and *N*-*boc*-ethylenediamine (0.1 mL, 0.62 mmol). The crude residue was purified by silica gel chromatography (0-5% MeOH in DCM followed by 0-100% EtOAc in Hexanes) to afford 38.0 mg (42%) of the title compound as a brown residue, and 101.8 mg of a double addition byproduct. LRMS (ESI) of byproduct *m/z* calcd for [C<sub>25</sub>H<sub>40</sub>N<sub>4</sub>O<sub>7</sub> + H]<sup>+</sup> = 509.3, found 509.3.

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.1 – 8.0 (m, 2H), 7.9 (d, *J* = 14.96 Hz, 1H), 7.1 (s, 1H), 7.0 – 6.9 (m, 3H), 5.0 (d, *J* = 6.18 Hz, 1H), 3.9 (s, 3H), 3.5 (q, *J* = 5.48 Hz, 2H), 3.4 (q, *J* = 5.77 Hz, 2H), 1.4 (s, 9H).

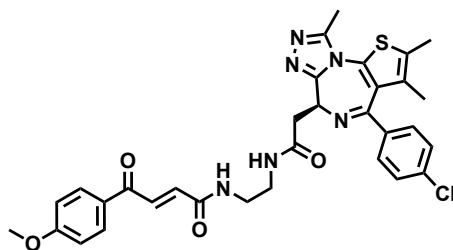
<sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 187.9, 164.9, 164.2, 157.1, 134.6, 133.2, 131.3, 130.0, 114.1, 80.0, 55.6, 41.4, 40.1, 28.4.

**LRMS (ESI)** *m/z* calcd for [C<sub>18</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub> + Na]<sup>+</sup> = 371.2, found 371.1.



**JP-2-218**

**General Procedure E** was followed with **JP-2-216** (19.0 mg, 0.05 mmol), and TFA (0.13 mL, 1.8 mmol) for 40 minutes. The crude material was used without further purification.



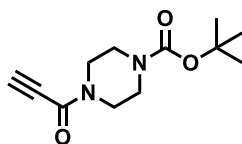
**JP-2-219**

**General Procedure A** was followed with (6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine-6-acetic acid (JQ1-Acid) (24.6 mg, 0.06 mmol), HATU (27.0 mg, 0.07 mmol), DIPEA (0.04 mL, 0.23 mmol), and **JP-2-218** (13.5 mg, 0.05 mmol). The crude residue was purified by silica gel chromatography (0-6% MeOH in DCM) to afford 17.4 mg (51%, 2 steps - *E:Z* = 98:2) of the title compound as a yellow foam.

**<sup>1</sup>H NMR** (600 MHz, CDCl<sub>3</sub>) δ 8.02 – 7.98 (m, 2H), 7.95 (t, *J* = 5.6 Hz, 1H), 7.88 (s, 1H), 7.82 (q, *J* = 5.4 Hz, 1H), 7.39 – 7.36 (m, 2H), 7.29 (d, *J* = 7.4 Hz, 2H), 6.96 – 6.91 (m, 2H), 6.89 (d, *J* = 15.0 Hz, 1H), 4.71 (dd, *J* = 7.9, 6.2 Hz, 1H), 3.87 (s, 3H), 3.55 (qdt, *J* = 10.9, 6.8, 3.5 Hz, 5H), 3.46 (dd, *J* = 14.7, 6.2 Hz, 1H), 2.73 (s, 3H), 2.39 (s, 3H), 1.66 (s, 3H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 188.0, 171.3, 164.8, 164.3, 164.0, 156.0, 150.4, 136.9, 136.4, 135.1, 132.6, 132.0, 131.2, 131.2, 131.0, 130.6, 130.2, 129.9, 128.7, 114.0, 55.5, 54.2, 40.3, 39.0, 38.9, 29.7, 14.4, 13.1, 11.8.

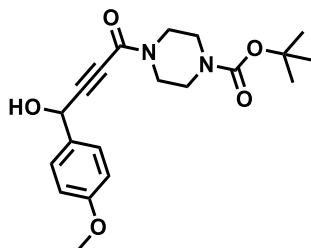
**HRMS (ESI)** *m/z* calcd for [C<sub>32</sub>H<sub>31</sub>ClN<sub>6</sub>O<sub>4</sub>S + H]<sup>+</sup> = 631.1816, found 631.1899.



**S1**

To a solution of Boc-piperazine (7.2 g, 38.94 mmol) and DMAP (237 mg, 1.95 mmol) in DCM (45 mL) was added propionic acid (3.0 g, 42.83 mmol) at ambient temperature. Next, DCC (5.6 g, 27.25 mmol) in DCM (5 mL) was added dropwise to the mixture at 0 °C, the mixture was stirred and warmed to ambient temperature over 1 hour. Once LCMS monitoring showed complete consumption of starting material, the reaction mixture was quenched with saturated NaHCO<sub>3</sub> aq. (20 mL) and extracted with EtOAc (20 mL x 3). The combined organic layers were washed by brine (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (0-100% EtOAc in Petroleum Ether) to afford 7.2 g (78%) of the title compound as a yellow solid.

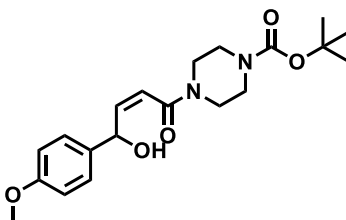
**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 3.75 (dd, *J* = 4.3, 6.2 Hz, 2H), 3.65 - 3.59 (m, 2H), 3.49 (dd, *J* = 4.3, 6.1 Hz, 2H), 3.45 - 3.40 (m, 2H), 3.16 (s, 1H), 1.48 (s, 9H).



**S2**

To a solution of **S1** (2.0 g, 8.40 mmol) in THF (30 mL) was added dropwise n-BuLi (4.0 mL, 10.08 mmol) at -70 °C. The reaction was stirred at -70 °C for 30 mins. Then 4-methoxybenzaldehyde (914 mg, 6.72 mmol) and TMSCl (1.8 g, 16.80 mmol) was added dropwise to the mixture at -70 °C, the mixture was stirred and warmed to ambient temperature over 3 hours. The reaction mixture was quenched with ice water (200 mL), and extracted with EtOAc (200 mL x 2). The combined organic extracts were washed with brine (300 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (0-100% EtOAc in Petroleum ether) to afford 1.3 g (41%) of the title compound as a white solid.

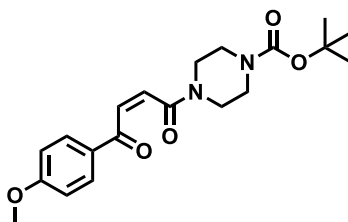
**LCMS** Rt = 0.454 min, M+23 (397.0), 71.7%.



**S3**

A mixture of **S2** (300 mg, 0.80 mmol) and Lindlar's catalyst (30 mg) in THF (10 mL) was stirred at ambient temperature under a H<sub>2</sub> atmosphere (10 psi) for 2 hours. The reaction mixture was filtered and concentrated under reduced pressure to afford 300 mg of the title compound as a yellow solid, which was used without further purification.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 7.37 (d, J = 8.6 Hz, 2H), 6.90 - 6.86 (m, 2H), 6.28 (dd, J = 6.6, 11.9 Hz, 1H), 6.18 - 6.11 (m, 1H), 5.55 (br d, J = 5.1 Hz, 1H), 4.42 (br s, 1H), 3.81 - 3.79 (m, 3H), 3.68 - 3.61 (m, 2H), 3.49 - 3.41 (m, 6H), 1.48 (s, 9H).



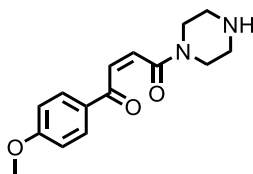
**LE-21-PX17**

To a mixture of **S3** (150 mg, 0.40 mmol) in DCM (5 mL) was added DMP (186 mg, 0.44 mmol) at 0 °C. The mixture was stirred and warmed to ambient temperature over 2 hours. The reaction mixture was filtered and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (0-100% EtOAc in Petroleum Ether) to afford 53 mg (35%- *E:Z* = >99:<1) of the title compound as a white solid.

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 7.98 - 7.93 (m, 2H), 7.03 (d, J = 11.9 Hz, 1H), 6.98 - 6.92 (m, 2H), 6.56 (d, J = 12.0 Hz, 1H), 3.89 (s, 3H), 3.65 (br s, 2H), 3.52 - 3.44 (m, 6H), 1.49 - 1.47 (m, 9H).

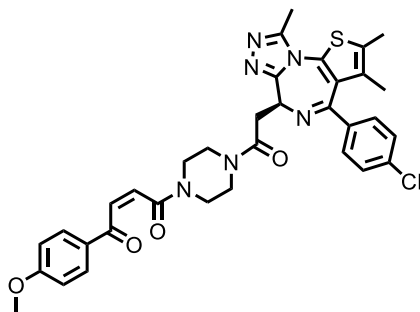
**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 188.6, 166.7, 164.0, 154.5, 134.4, 131.1, 129.7, 128.7, 114.0, 80.2, 55.5, 46.1, 41.1, 28.4.

**HRMS (ESI)** *m/z* calcd for [C<sub>20</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub> + Na]<sup>+</sup> = 379.1820, found 379.1735.



**S4**

**General Procedure E** was followed with **LE-21-PX17** (100.0 mg, 0.26 mmol), and TFA (0.4 mL) for 1 hour. The crude material was used without further purification.



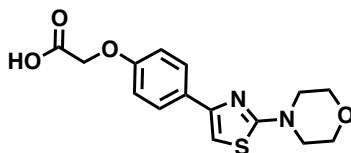
**FB-84-GG64**

**General Procedure A** was followed with (6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine-6-acetic acid (JQ1-Acid) (144.0 mg, 0.36 mmol), HATU (152.1 mg, 0.40 mmol), DIPEA (0.16 mL, 0.94 mmol), and **S4** (100.0 mg, 0.36 mmol). The crude residue was purified by Prep-HPLC (NH<sub>4</sub>HCO<sub>3</sub>) to afford 31.2 mg (13%, 2 steps - *E:Z* = >99:<1) of the title compound as a white solid.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 7.89 (d, *J* = 8.5 Hz, 2H), 7.36 – 7.30 (m, 2H), 7.28 (d, *J* = 8.2 Hz, 2H), 6.99 (s, 1H), 6.93 – 6.85 (m, 2H), 6.52 (d, *J* = 11.8 Hz, 1H), 4.76 – 4.70 (m, 1H), 3.81 (s, 5H), 3.73 (s, 2H), 3.65 – 3.39 (m, 6H), 2.60 (s, 3H), 2.35 – 2.31 (m, 3H), 1.61 (d, *J* = 0.9 Hz, 3H).

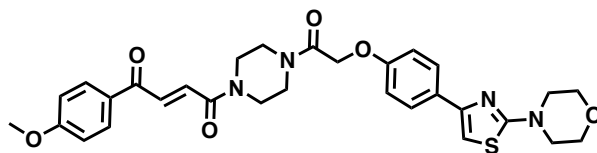
**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 188.6, 169.3, 166.8, 164.0, 155.8, 149.9, 136.8, 136.7, 134.6, 134.5, 132.2, 131.1, 130.9, 130.7, 130.5, 129.8, 129.7, 128.7, 114.1, 55.6, 54.6, 54.4, 46.4, 45.9, 45.3, 41.7, 41.4, 41.2, 35.3, 14.4, 13.1, 11.9.

**HRMS (ESI)** *m/z* calcd for [C<sub>34</sub>H<sub>33</sub>ClN<sub>6</sub>O<sub>4</sub>S + Na]<sup>+</sup> = 679.2020, found 679.1867.



**JP-2-215**

A solution of *tert*-butyl 2-(4-(2-morpholinothiazol-4-yl)phenoxy)acetate (40.0 mg, 0.11 mmol) in 4 M hydrochloric acid in dioxane (0.7 mL) was stirred at ambient temperature overnight. The reaction mixture was diluted with toluene and the volatiles were removed *in vacuo* to yield a yellow-white solid which was used without further purification.



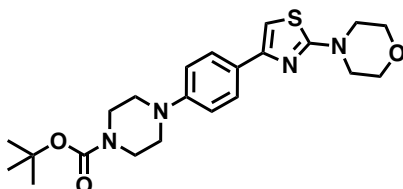
**JP-2-217**

**General Procedure A** was followed with 2-(4-(2-morpholinothiazol-4-yl)phenoxy)acetic acid (35.00 mg, 0.11 mmol), HATU (49.9 mg, 0.13 mmol), DIPEA (0.06 mL, 0.33 mmol), and **JP-2-196** (36.9 mg, 0.13 mmol). The crude residue was purified by silica gel chromatography (0-5% MeOH in DCM) to afford 34.6 mg (55%, 2 steps - *E:Z* = 93:7) of the title compound as a beige powder.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.03 (d, *J* = 8.6 Hz, 2H), 7.96 (dd, *J* = 14.9, 4.6 Hz, 1H), 7.79 – 7.76 (m, 2H), 7.43 (d, *J* = 14.7 Hz, 1H), 6.99 – 6.94 (m, 4H), 6.68 (s, 1H), 4.76 (d, *J* = 3.3 Hz, 2H), 3.89 (s, 3H), 3.85 – 3.82 (m, 4H), 3.73 (q, *J* = 6.3 Hz, 1H), 3.68 (p, *J* = 5.5 Hz, 5H), 3.64 – 3.59 (m, 2H), 3.54 – 3.50 (m, 4H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 187.5, 171.2, 166.9, 164.4, 164.3, 157.1, 151.2, 135.1, 131.3, 130.8, 129.9, 129.3, 127.6, 114.5, 114.1, 100.5, 68.2, 68.0, 66.2, 55.6, 48.6, 46.1, 45.7, 45.2, 42.4, 42.0.

**HRMS (ESI)** *m/z* calcd for [C<sub>30</sub>H<sub>32</sub>N<sub>4</sub>O<sub>6</sub>S + H]<sup>+</sup> = 577.2043, found 577.2122.



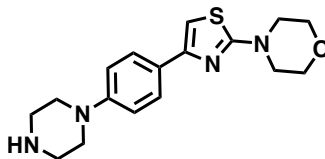
**JP-2-221**

A mixture of 4-(4-bromo-2-thiazolyl)morpholine (120.0 mg, 0.51 mmol), 4-(4-*tert*-butoxycarbonylpiperazinyl)phenylboronic acid pinacol ester (210.6, 0.54 mmol), caesium carbonate (485.9 mg, 1.5 mmol), and tetrakis(triphenylphosphine)palladium(0) (66.4 mg, 0.05 mmol) was suspended in DMF (12 mL) and stirred at 100 °C overnight. The reaction was quenched with 5% LiCl (aq) (60 mL) and extracted 3 times with DCM. The organic extracts were washed again with 5% LiCl (aq) and the organic extract was dried over Na<sub>2</sub>SO<sub>4</sub>, vacuum filtered, and the volatiles were removed *in vacuo*. The crude residue was purified by silica gel chromatography (0-50% EtOAc in Hexanes) to afford 28.8 mg (32%, BRSM) of the title compound as a white solid.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 7.75 – 7.72 (m, 2H), 6.93 – 6.90 (m, 2H), 6.65 (s, 1H), 3.85 – 3.81 (m, 4H), 3.58 (t, *J* = 5.2 Hz, 4H), 3.52 (dd, *J* = 5.9, 3.9 Hz, 4H), 3.16 (t, *J* = 5.1 Hz, 4H), 1.49 (s, 9H).

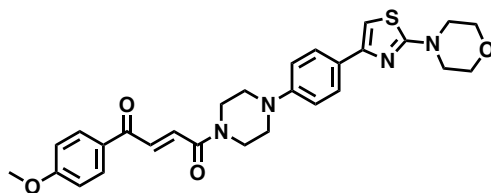
**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 171.1, 151.8, 150.7, 127.3, 127.2, 127.0, 116.8, 116.3, 99.7, 79.9, 66.3, 49.2, 48.6, 28.5.

**LRMS (ESI)** *m/z* calcd for [C<sub>22</sub>H<sub>30</sub>N<sub>4</sub>O<sub>3</sub>S + H]<sup>+</sup> = 431.6, found 431.2.



**JP-2-223**

**General Procedure E** was followed with **JP-2-221** (33.6 mg, 0.07 mmol), and TFA (0.18 mL, 2.4 mmol) for 30 minutes. The crude material was used without further purification.



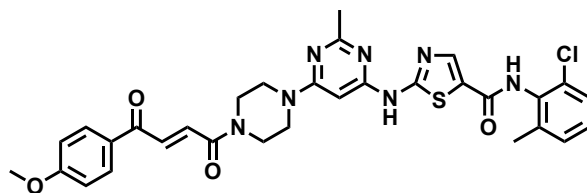
**JP-2-224**

**General Procedure A** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (19.3 mg, 0.09 mmol), HATU (43.8 mg, 0.10 mmol), DIPEA (0.04 mL, 0.23 mmol), and **JP-2-223** (23.0 mg, 0.07 mmol). The crude residue was purified by silica gel chromatography (0-100% EtOAc in Hexanes) to afford 18.6 mg (51%, 2 steps - *E:Z* = 47:53) of the title compound as a light orange foam.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.07 – 8.03 (m, 2H), 7.99 (d, *J* = 14.8 Hz, 1H), 7.77 – 7.73 (m, 2H), 7.54 – 7.50 (m, 1H), 7.00 – 6.96 (m, 2H), 6.95 – 6.91 (m, 2H), 6.66 (s, 1H), 3.91 (t, *J* = 5.2 Hz, 2H), 3.89 (s, 3H), 3.83 (t, *J* = 4.9 Hz, 4H), 3.81 (d, *J* = 5.1 Hz, 2H), 3.52 (t, *J* = 4.9 Hz, 4H), 3.26 (q, *J* = 4.9 Hz, 4H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 187.7, 171.2, 164.2, 164.1, 151.6, 150.1, 134.6, 131.3, 130.0, 127.7, 127.1, 116.4, 114.1, 99.9, 66.3, 55.6, 49.7, 49.1, 48.6, 45.9, 42.1.

**HRMS (ESI)** *m/z* calcd for [C<sub>28</sub>H<sub>30</sub>N<sub>4</sub>O<sub>4</sub>S + H]<sup>+</sup> = 519.1988, found 519.2067.



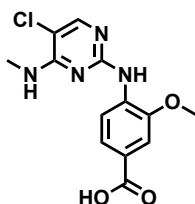
**JP-2-227**

**General Procedure A** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (7.0 mg, 0.03 mmol), HATU (12.8 mg, 0.03 mmol), DIPEA (0.02 mL, 0.13 mmol), and *N*-deshydroxyethyl dasatinib (10.0 mg, 0.02 mmol). The crude residue was purified by silica gel chromatography (0-6% MeOH in DCM) to afford 12.8 mg (90% - *E:Z* = 93:7) of the title compound as a yellow solid.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.00 – 7.95 (m, 3H), 7.92 (d, *J* = 14.9 Hz, 1H), 7.42 (d, *J* = 14.9 Hz, 1H), 7.24 (d, *J* = 7.7 Hz, 1H), 7.15 – 7.07 (m, 2H), 6.95 – 6.91 (m, 2H), 5.87 (s, 1H), 3.83 (s, 3H), 3.77 (t, *J* = 5.3 Hz, 2H), 3.70 (p, *J* = 5.2 Hz, 4H), 3.62 (d, *J* = 5.5 Hz, 2H), 2.45 (s, 3H), 2.26 (s, 3H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 188.0, 166.5, 164.6, 164.4, 162.8, 156.9, 140.4, 138.6, 135.0, 132.4, 131.4, 131.0, 129.7, 129.1, 128.1, 127.1, 125.6, 114.2, 83.1, 55.5, 45.5, 43.9, 43.6, 41.8, 29.6, 25.4, 18.7.

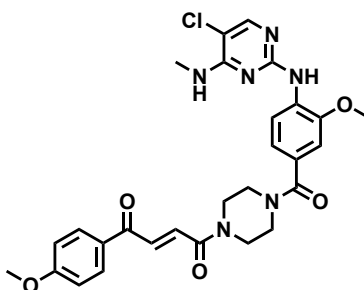
**HRMS (ESI)** *m/z* calcd for [C<sub>31</sub>H<sub>30</sub>ClN<sub>7</sub>O<sub>4</sub>S + H]<sup>+</sup> = 632.1769, found 632.1848.



**JP-2-241**

A mixture of 2,5-dichloro-N-methyl pyrimidin-4-amine (106.2 mg, 0.60 mmol) and 4-amino-3-methoxybenzoic acid (111.8 mg, 0.67 mmol) was dissolved in a 1:1 mixture of Dioxane:H<sub>2</sub>O (4 mL). A solution of 4 M HCl in dioxane (0.15 mL) was added and the reaction mixture was stirred at 100 °C overnight. A white precipitate formed upon cooling which was filtered and washed with H<sub>2</sub>O to afford 78.7 mg (43%) of the title compound as a white powder which product was used without further purification.

**<sup>1</sup>H NMR** (500 MHz, DMSO) δ 9.17 (s, 1H), 8.55 (d, *J* = 5.8 Hz, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 8.18 (s, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.55 (s, 1H), 3.93 (s, 3H), 2.98 (d, *J* = 4.6 Hz, 3H).



**JP-2-244**

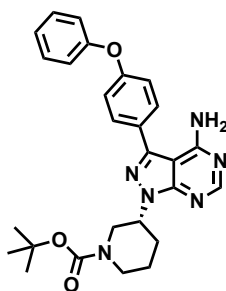
**General Procedure A** was followed with **JP-2-241** (21.3 mg, 0.07 mmol), HATU (34.2 mg, 0.07 mmol), DIPEA (0.05 mL, 0.3 mmol), and **JP-2-196** (20.8 mg, 0.07 mmol). The crude residue was purified by silica gel chromatography (0-8% MeOH in DCM) to afford 23.3 mg (60%, 2 steps - *E:Z* = 91:9) of the title compound as a yellow film.



**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.58 (d, *J* = 8.2 Hz, 1H), 8.06 – 8.02 (m, 2H), 7.99 (d, *J* = 14.9 Hz, 1H), 7.93 (s, 1H), 7.67 (s, 1H), 7.47 (d, *J* = 14.9 Hz, 1H), 7.04 (d, *J* = 1.8 Hz, 1H), 7.02 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.00 – 6.96 (m, 2H), 5.34 (q, *J* = 4.9 Hz, 1H), 3.93 (s, 3H), 3.89 (s, 3H), 3.81 – 3.59 (m, 8H), 3.11 (d, *J* = 4.9 Hz, 3H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 187.5, 170.9, 164.3, 164.3, 158.6, 147.6, 135.1, 131.7, 131.4, 130.9, 129.9, 120.3, 116.9, 114.2, 109.7, 105.7, 56.0, 55.6, 46.1, 42.5, 28.2.

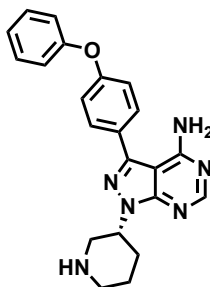
**HRMS (ESI)** *m/z* calcd for [C<sub>28</sub>H<sub>29</sub>ClN<sub>6</sub>O<sub>5</sub> + H]<sup>+</sup> = 565.1888, found 565.1968.



**JP-2-242**

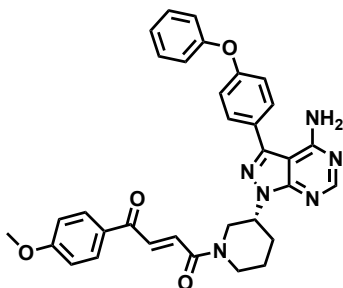
A mixture of (4-phenoxyphenyl)boronic acid (60.6 mg, 0.28 mmol), *tert*-butyl (*R*)-3-(4-amino-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)piperidine-1-carboxylate (117.1 mg, 0.26 mmol), tetrakis(triphenylphosphine)palladium (28.4 mg, 0.03 mmol), and potassium carbonate (105.0 mg, 0.79 mmol) was suspended in a 1:1 mixture of Dioxane:H<sub>2</sub>O (4 mL) and the reaction mixture was stirred at 90 °C for 5 hours. The solvent was removed *in vacuo*, and the crude residue was purified by silica gel chromatography (0-70% EtOAc in Hexanes) to afford 127.1 mg (99%) of the title compound as a yellow foam.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 8.37 (s, 1H), 7.65 (dd, *J* = 8.1, 6.1 Hz, 2H), 7.41 – 7.36 (m, 2H), 7.15 (dd, *J* = 8.4, 6.4 Hz, 3H), 7.08 (d, *J* = 7.9 Hz, 2H), 5.64 (s, 2H), 4.84 (tt, *J* = 10.9, 4.4 Hz, 1H), 4.47 – 4.03 (m, 2H), 2.85 (td, *J* = 13.0, 3.0 Hz, 1H), 2.32 – 2.14 (m, 2H), 1.88 (d, *J* = 7.9 Hz, 1H), 1.75 – 1.63 (m, 1H), 1.44 (s, 9H).



**JP-2-246**

**General Procedure E** was followed with **JP-2-242** (37.2 mg, 0.08 mmol), and TFA (0.19 mL, 2.5 mmol) for 30 minutes. The crude material was used without further purification.



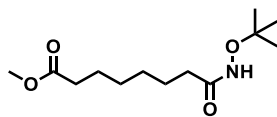
**JP-2-247**

**General Procedure A** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (24.5 mg, 0.09 mmol), HATU (40.7 mg, 0.11 mmol), DIPEA (0.05 mL, 0.29 mmol), and **JP-2-246** (27.8 mg, 0.07 mmol). The crude residue was purified by silica gel chromatography (0-5% MeOH in DCM) and then by reverse phase silica gel chromatography (5-95% MeCN in H<sub>2</sub>O) to afford 16.9 mg (41%, 2 steps - *E:Z* = 85:15) of the title compound as a white solid.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.38 – 8.31 (m, 1H), 8.07 – 8.03 (m, 1H), 8.00 – 7.97 (m, 1H), 7.97 – 7.85 (m, 1H), 7.67 – 7.62 (m, 2H), 7.55 – 7.41 (m, 1H), 7.40 – 7.36 (m, 2H), 7.17 (t, *J* = 7.3 Hz, 1H), 7.16 – 7.13 (m, 2H), 7.08 (d, *J* = 8.0 Hz, 2H), 7.00 – 6.96 (m, 1H), 6.96 – 6.92 (m, 1H), 5.64 (s, 2H), 4.96 – 4.87 (m, 2H), 4.55 – 4.49 (m, 1H), 4.22 (dd, *J* = 13.6, 4.1 Hz, 1H), 4.11 (d, *J* = 13.8 Hz, 1H), 3.92 (dd, *J* = 13.4, 9.9 Hz, 1H), 3.88 (d, *J* = 13.3 Hz, 3H), 3.48 (dd, *J* = 12.6, 10.6 Hz, 1H), 3.28 (td, *J* = 13.2, 2.9 Hz, 1H), 3.08 (ddd, *J* = 13.7, 11.1, 3.2 Hz, 1H), 2.45 – 2.33 (m, 1H), 2.28 (dd, *J* = 13.7, 4.1 Hz, 1H), 2.05 (ddt, *J* = 12.1, 7.5, 3.7 Hz, 1H), 1.81 – 1.71 (m, 1H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 187.7, 187.6, 164.7, 164.4, 164.2, 164.1, 158.6, 158.6, 157.8, 156.4, 156.3, 155.8, 155.7, 154.3, 154.3, 144.1, 144.0, 134.4, 134.1, 132.0, 131.9, 131.3, 131.3, 130.0, 127.8, 127.6, 124.1, 119.6, 119.1, 114.1, 114.0, 98.6, 98.6, 55.6, 55.6, 53.4, 52.4, 50.1, 46.3, 46.2, 42.5, 30.3, 30.0, 25.3, 23.7.

**HRMS (ESI)** *m/z* calcd for [C<sub>33</sub>H<sub>30</sub>N<sub>6</sub>O<sub>4</sub> + H]<sup>+</sup> = 575.2329, found 575.2406.

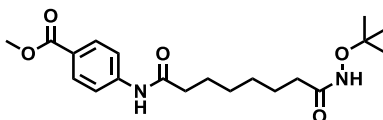


**DD-1-42-P1**

**General Procedure D** was followed with suberic acid monomethyl ester (500 mg, 2.66 mmol), T3P (1.66 mL, 2.79 mmol), DIPEA (1.38 mL, 7.98 mmol), and *O*-*tert*-Butylhydroxylamine hydrochloride (400 mg, 3.19 mmol). The reaction was quenched with 5% LiCl (aq) (60 mL) and extracted 3 times with EtOAc. The organic extracts were washed again with 5% LiCl (aq) and the organic extract was dried over Na<sub>2</sub>SO<sub>4</sub>, vacuum filtered, and the volatiles were removed *in vacuo*. The crude residue was purified by silica gel chromatography (0-100% EtOAc in Hexanes) to afford 573 mg (83%) of the title compound as a slightly yellow, thick oil.

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 3.65 (s, 3H), 2.29 (t, J = 7.5 Hz, 2H), 2.14 – 2.01 (m, 1H), 1.62 (qd, J = 12.9, 8.0 Hz, 4H), 1.33 (p, J = 3.7 Hz, 4H), 1.25 (s, 9H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 174.4, 172.2, 81.9, 51.6, 34.1, 33.4, 28.9, 26.4, 25.4, 24.8, 14.3.



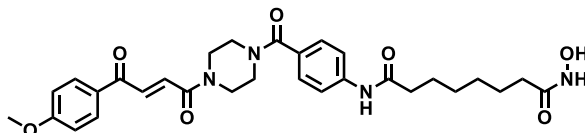
**DD-1-47-P1**

Methyl ester **DD-1-42-P1** (100 mg, 0.39 mmol) was dissolved in MeOH (2.0 mL) and 2M LiOH (aq, 0.77 mL) was added dropwise. The reaction mixture was stirred at ambient temperature overnight, and upon completion was quenched with 1M HCl (10 mL) and extracted 3 times with DCM. The organic extracts were washed again with brine and the organic extract was dried over Na<sub>2</sub>SO<sub>4</sub>, vacuum filtered, and the volatiles were removed *in vacuo*. The crude material was used without further purification.

A modified **General Procedure A** was followed with crude residue, HATU (147 mg, 0.39 mmol), DIPEA (0.2 mL, 1.16 mmol), and methyl 4-aminobenzoate (64 mg, 0.42 mmol). The reaction mixture was stirred at 100 °C for two days. The reaction was quenched with 5% LiCl (aq) (20 mL) and extracted 3 times with EtOAc. The organic extracts were washed again with 5% LiCl (aq) and the organic extract was dried over Na<sub>2</sub>SO<sub>4</sub>, vacuum filtered, and the volatiles were removed *in vacuo*. The crude residue was purified by silica gel chromatography (0-100% EtOAc in Hexanes) to afford 55 mg (38%) of the title compound as a beige solid.

<sup>1</sup>H NMR (500 MHz, DMSO) δ 10.20 (s, 2H), 7.89 (d, J = 3.0 Hz, 2H), 7.72 (d, J = 3.0 Hz, 2H), 3.81 (s, 3H), 2.34 (t, J = 8.3 Hz, 2H), 1.99 (t, J = 7.9 Hz, 2H), 1.30 – 1.24 (m, 5H), 1.13 (s, 9H).

<sup>13</sup>C NMR (126 MHz, DMSO) δ 171.9, 170.4, 165.8, 143.7, 130.2, 123.6, 118.3, 80.1, 51.8, 36.4, 32.3, 28.3, 26.4, 25.0, 24.9, 20.6.



### DD-1-073-P1

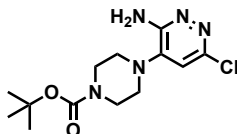
Methyl ester **DD-1-47-P1** (55 mg, 0.15 mmol) was dissolved in MeOH (6.0 mL) and 2M LiOH (aq, 0.90 mL) was added dropwise. The reaction mixture was stirred at ambient temperature for 7 days. The volatiles were removed *in vacuo*, and the off-white residue was dissolved in water and acidified with 1M HCl until a beige precipitate was formed that was vacuum filtered and dried under vacuum (40 mg) and the resultant material was used without further purification.

A modified **General Procedure A** was followed with crude residue, HATU (42 mg, 0.11 mmol), DIPEA (0.08 mL, 1.16 mmol), and **JP-2-196** (33 mg, 0.12 mmol) and stirred overnight. The reaction was quenched with 5% LiCl (aq) (20 mL) and extracted 3 times with EtOAc. The organic extracts were washed again with 5% LiCl (aq) and the organic extract was dried over Na<sub>2</sub>SO<sub>4</sub>, vacuum filtered, and the volatiles were removed *in vacuo*. The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 62 mg (66%) of the *tert*-butyl protected hydroxamic acid, which was dissolved in TFA (5.0 mL) and stirred at 35 °C overnight. The volatiles were removed *in vacuo* and the brown residue was dissolved in DCM, adsorbed onto silica gel and was purified by silica gel chromatography (0-10% MeOH in DCM, 0.1% AcOH) to afford 27mg (49%, 2 steps - *E:Z* = 97:7) of the title compound as a beige film.

<sup>1</sup>H NMR (500 MHz, MeOD) δ 8.07 (dd, J = 8.9, 3.0 Hz, 2H), 7.92 (d, J = 15.1 Hz, 1H), 7.71 (d, J = 8.1 Hz, 2H), 7.52 (d, J = 15.2 Hz, 1H), 7.45 (d, J = 8.1 Hz, 2H), 7.11 – 7.06 (m, 2H), 3.91 (s, 3H), 3.85 – 3.45 (m, 8H), 2.41 (t, J = 7.4 Hz, 2H), 2.11 (t, J = 7.4 Hz, 2H), 1.69 (dp, J = 36.6, 7.0 Hz, 4H), 1.48 – 1.35 (m, 4H).

<sup>13</sup>C NMR (126 MHz, MeOD) δ 189.4, 174.8, 172.6, 166.7, 166.0, 142.1, 135.9, 132.9, 132.4, 132.3, 131.3, 131.0, 129.3, 120.7, 115.3, 56.2, 47.1, 43.2, 37.9, 33.7, 29.9, 29.8, 26.6, 26.6.

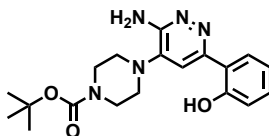
**HRMS (ESI)** *m/z* calcd for [C<sub>30</sub>H<sub>36</sub>N<sub>4</sub>O<sub>7</sub> + Na]<sup>+</sup> = 587.2476, found 587.2482.



**JP-2-238**

A mixture of 3-amino-4-bromo-6-chloropyridazine (108.5 mg, 0.52 mmol) and 1-boc-piperazine (461.9 mg, 2.5 mmol) was dissolved in a THF (1 mL) and the reaction mixture was stirred at 80 °C for overnight. The solvent was removed *in vacuo*, and the crude residue was purified by silica gel chromatography (0-100% EtOAc in Hexanes) to afford 147.4 mg (90%) of the title compound as a yellow foam.

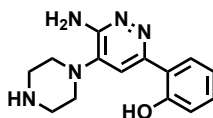
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 6.73 (s, 1H), 5.12 (s, 2H), 3.60 – 3.55 (m, 4H), 3.00 (t, *J* = 5.0 Hz, 4H), 1.48 (s, 9H).



**JP-2-243**

A mixture of **JP-2-238** (122.3 mg, 0.39 mmol), 2-hydroxyphenyl boronic acid (138.4 mg, 1.00 mmol), [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (34.5 mg, 0.05 mmol), and potassium carbonate (186.1 mg, 1.35 mmol) was suspended in a 1:1 mixture of MeCN:H<sub>2</sub>O (2.5 mL) in a sealed tube, and the reaction mixture was stirred at 120 °C for 45 minutes. The solvent was removed *in vacuo*, and the crude residue was purified by silica gel chromatography (0-100% EtOAc in Hexanes) to afford 21.2 mg (15%) of the title compound as a white-yellow powder.

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.57 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.29 (ddd, *J* = 8.5, 7.2, 1.6 Hz, 1H), 7.05 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.91 (td, *J* = 7.6, 1.3 Hz, 1H), 4.88 (s, 2H), 3.65 (t, *J* = 5.0 Hz, 4H), 3.10 (t, *J* = 5.0 Hz, 4H), 1.50 (s, 9H).

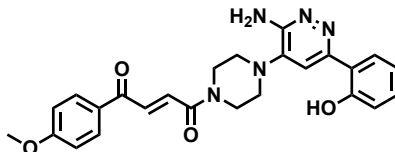


**JP-2-248**

**General Procedure E** was followed with **JP-2-243** (11.2 mg, 0.03 mmol), and TFA (0.09 mL, 0.96 mmol) for 1 hour. The solvent was removed *in vacuo*, and the crude residue was purified by silica gel chromatography (0-20% MeOH in DCM with 0.1% Et<sub>3</sub>N) to afford 6.2 mg (76%) of the title compound as a yellow oil.

**<sup>1</sup>H NMR** (600 MHz, DMSO) δ 7.92 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.48 (s, 1H), 7.24 (ddd, *J* = 8.4, 7.3, 1.6 Hz, 1H), 6.89 (dtd, *J* = 8.2, 3.6, 1.2 Hz, 2H), 6.23 (d, *J* = 6.0 Hz, 1H), 4.01 (s, 1H), 3.04 (t, *J* = 5.0 Hz, 4H), 2.98 – 2.91 (m, 4H).

**LRMS (ESI)** *m/z* calcd for [C<sub>14</sub>H<sub>17</sub>N<sub>5</sub>O + H]<sup>+</sup> = 272.3, found 272.1.



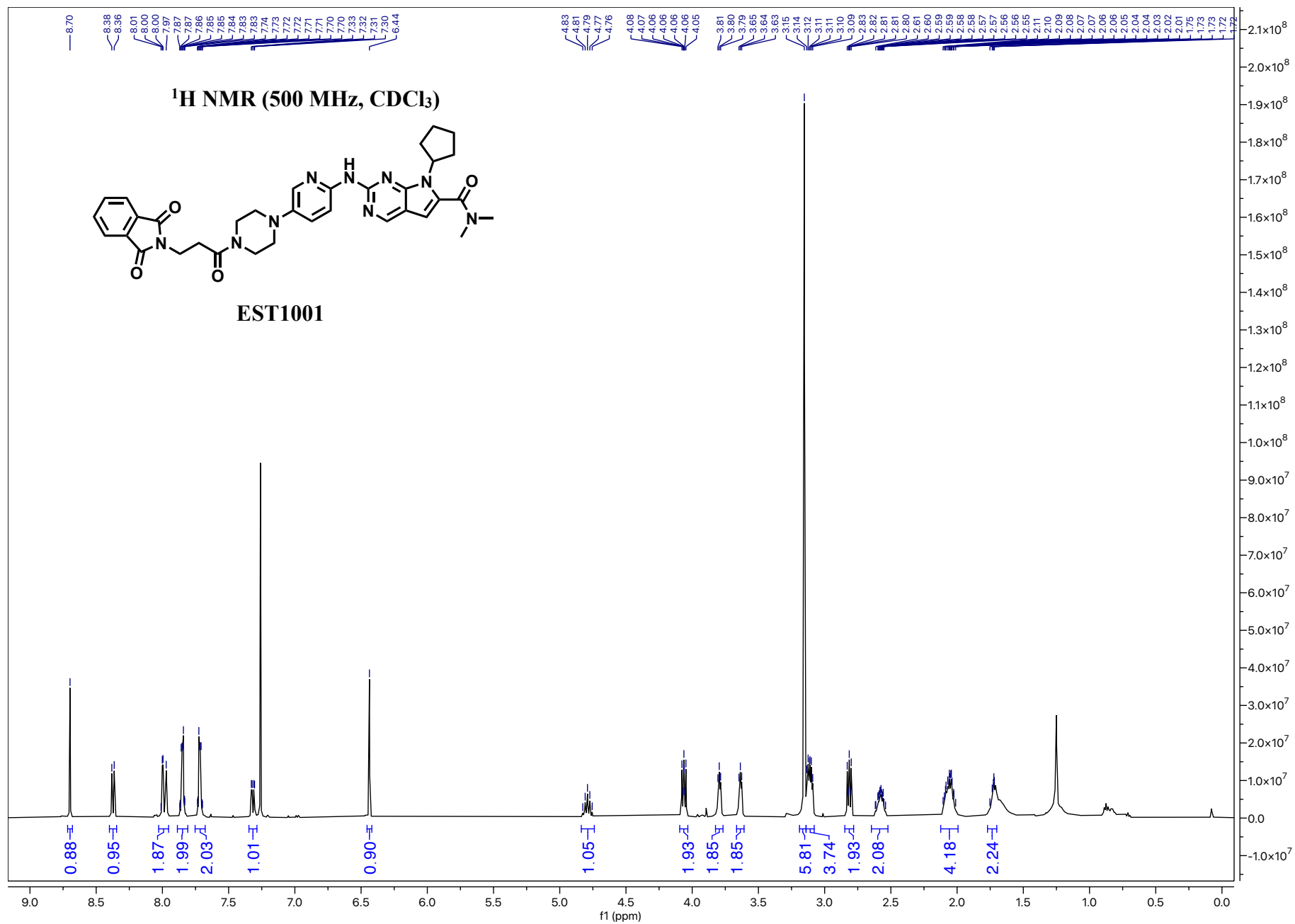
**JP-2-249**

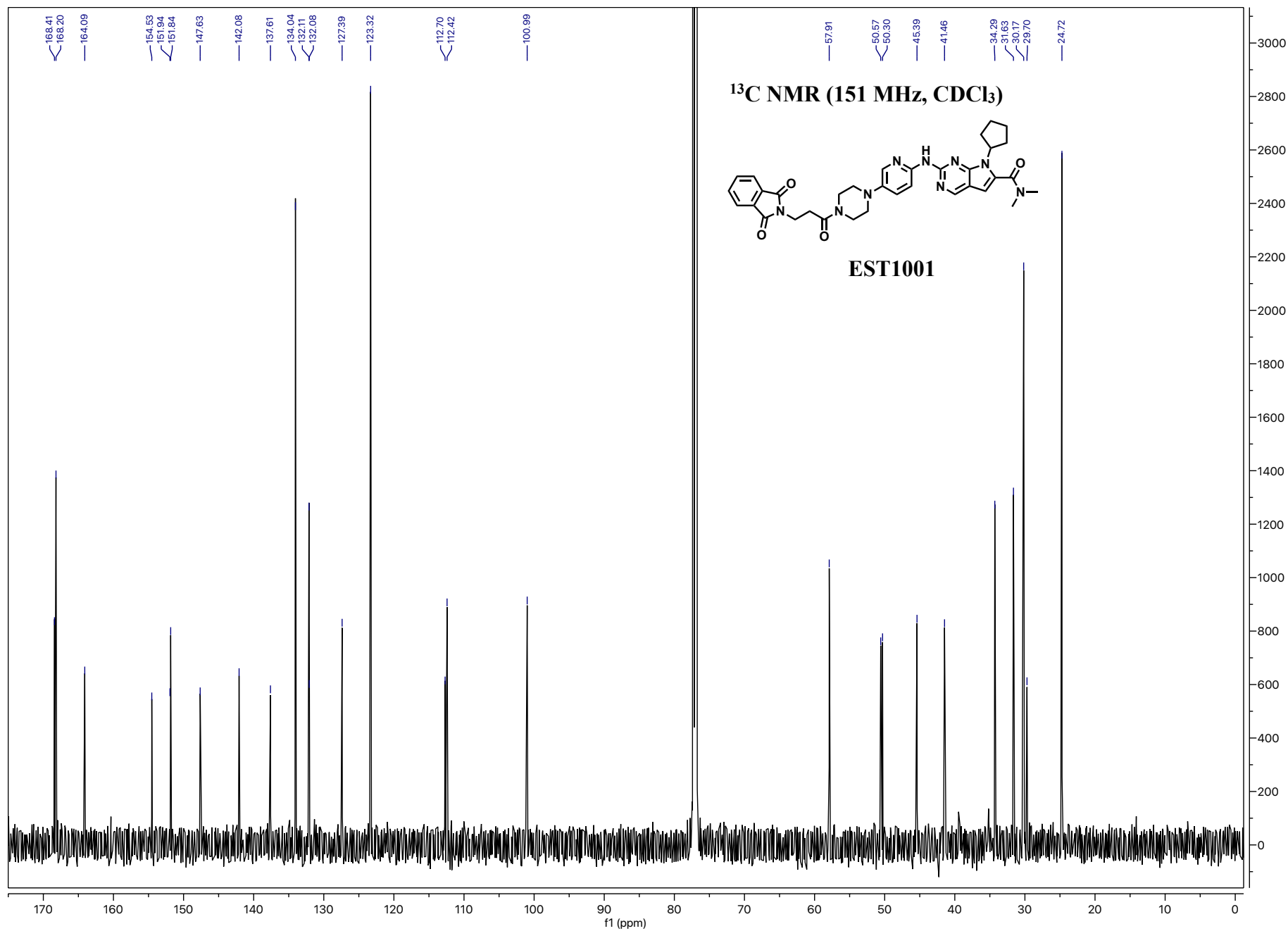
**General Procedure A** was followed with *trans*-3-(4-methoxybenzoyl)acrylic acid (12.5 mg, 0.06 mmol), HATU (27.7 mg, 0.07 mmol), DIPEA (0.05 mL, 0.29 mmol), and **JP-2-248** (16.4 mg, 0.06 mmol). The crude residue was purified by silica gel chromatography (0-10% MeOH in DCM) to afford 25.5 mg (92%, 2 steps - *E:Z* = 92:8) of the title compound as a white-yellow powder.

**<sup>1</sup>H NMR** (700 MHz, CDCl<sub>3</sub>) δ 8.02 (d, *J* = 8.5 Hz, 2H), 7.97 (d, *J* = 14.9 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.48 (d, *J* = 14.9 Hz, 1H), 7.30 (s, 1H), 7.26 (t, *J* = 1.7 Hz, 1H), 7.00 (d, *J* = 8.1 Hz, 1H), 6.96 (d, *J* = 8.6 Hz, 2H), 6.89 (t, *J* = 7.5 Hz, 1H), 3.92 (t, *J* = 5.0 Hz, 2H), 3.87 (s, 3H), 3.86 – 3.83 (m, 2H), 3.16 (t, *J* = 5.2 Hz, 4H), 2.47 (br s, 2H).

**<sup>13</sup>C NMR** (151 MHz, CDCl<sub>3</sub>) δ 187.8, 164.6, 164.5, 158.7, 155.0, 154.1, 140.6, 135.3, 131.5, 131.1, 130.9, 129.8, 125.3, 119.1, 118.4, 117.5, 114.3, 111.6, 55.7, 49.6, 49.1, 49.1, 45.9, 42.1.

**HRMS (ESI)** *m/z* calcd for [C<sub>25</sub>H<sub>25</sub>N<sub>5</sub>O<sub>4</sub> + H]<sup>+</sup> = 460.1979, found 460.1983.









HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

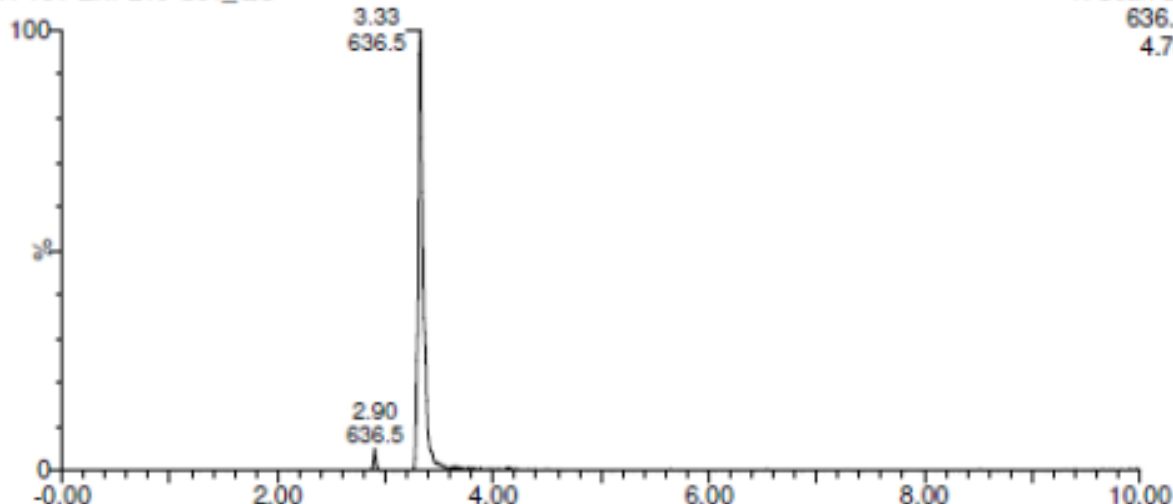
EM23001594423

08-Feb-2023  
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strubhe1-0208A-v04

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HESSEMA4-101-EXP219-S01\_QC

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4.78e7

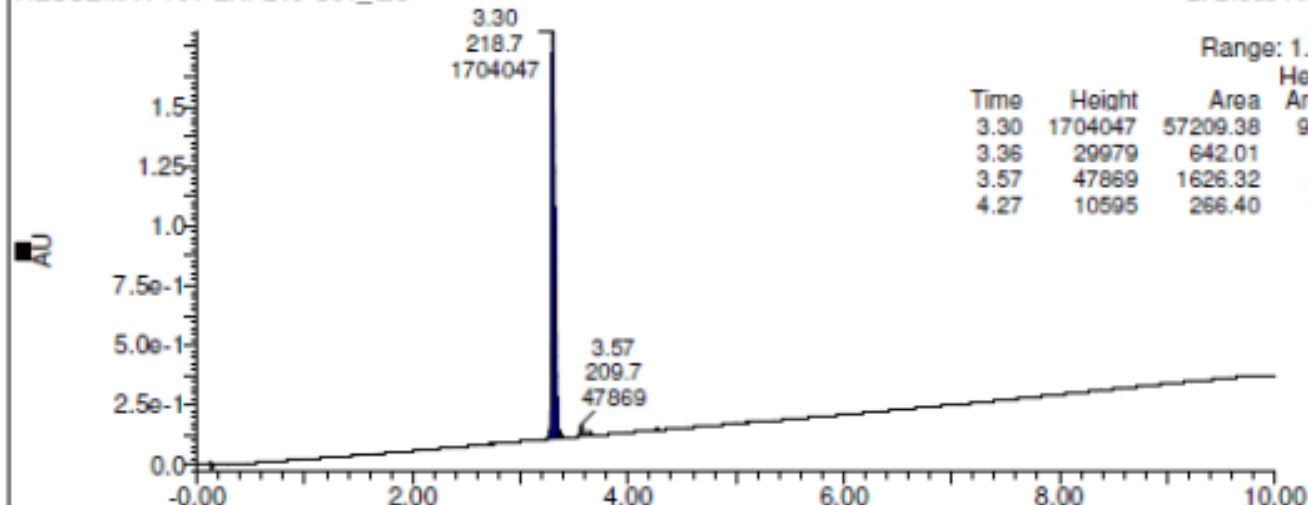


HESSEMA4-101-EXP219-S01\_QC

2: Diode Array  
220

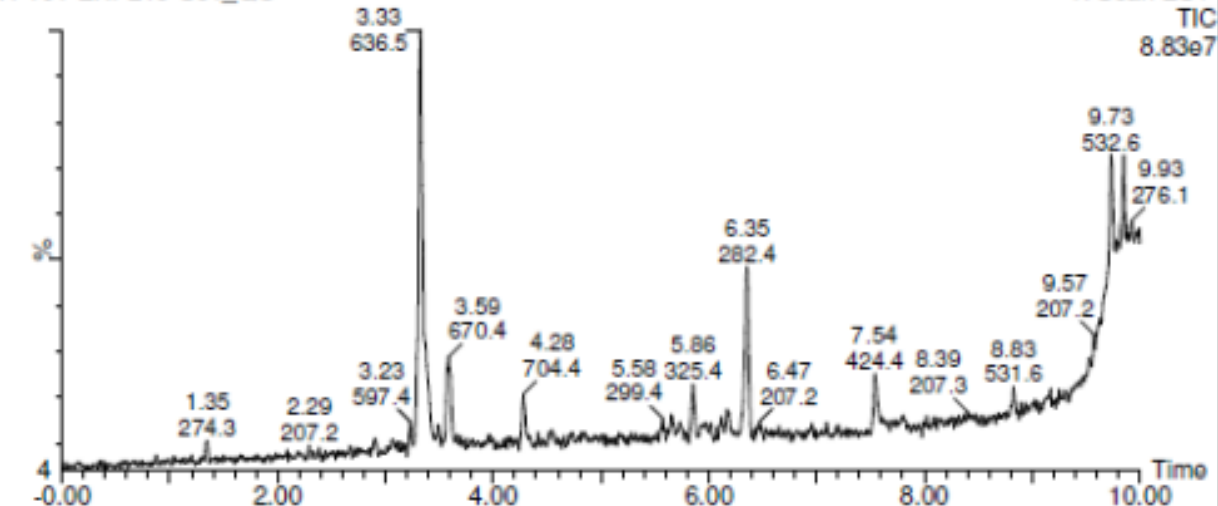
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Height

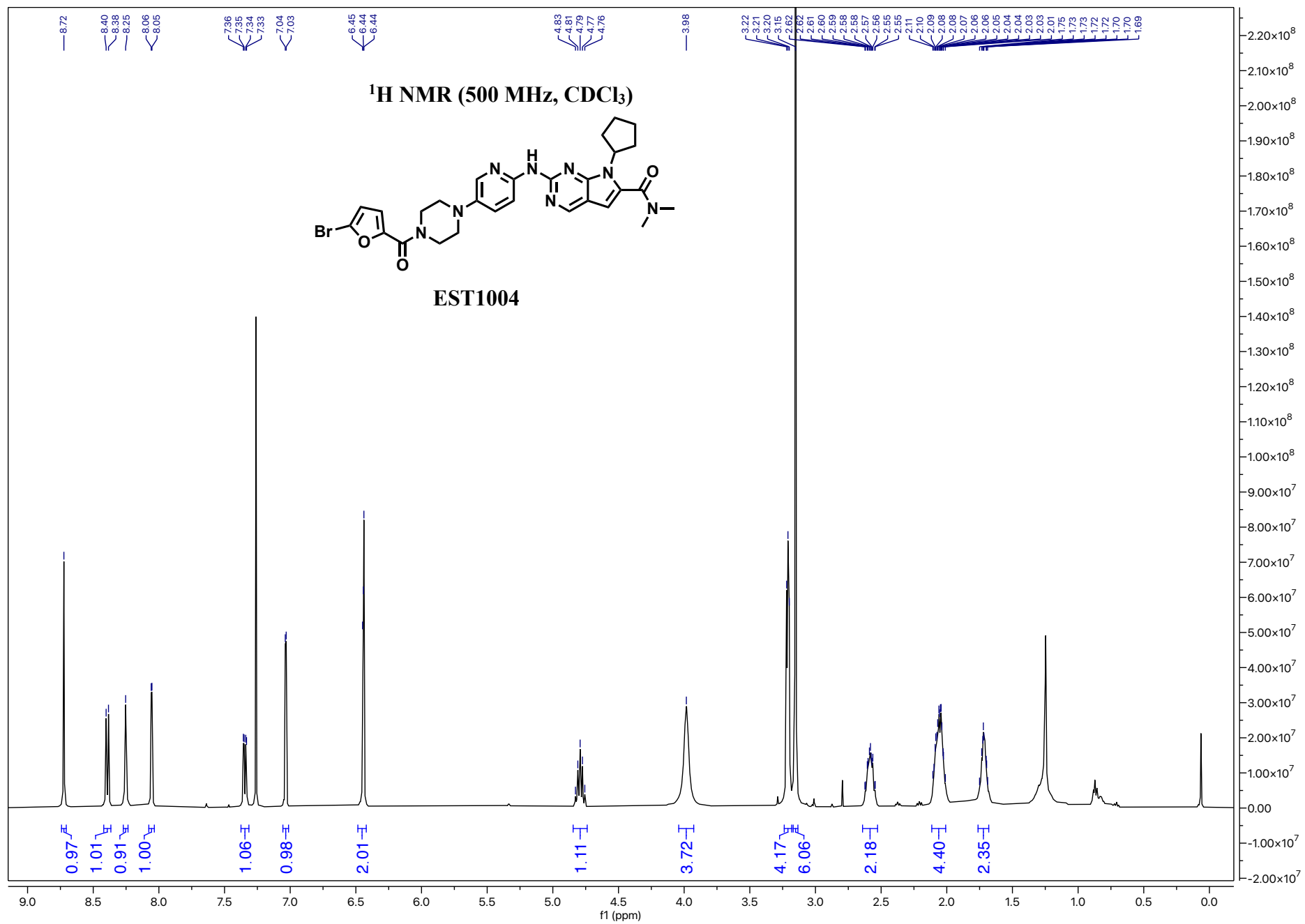
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3.57	47869	1626.32	2.72
4.27	10595	266.40	0.45

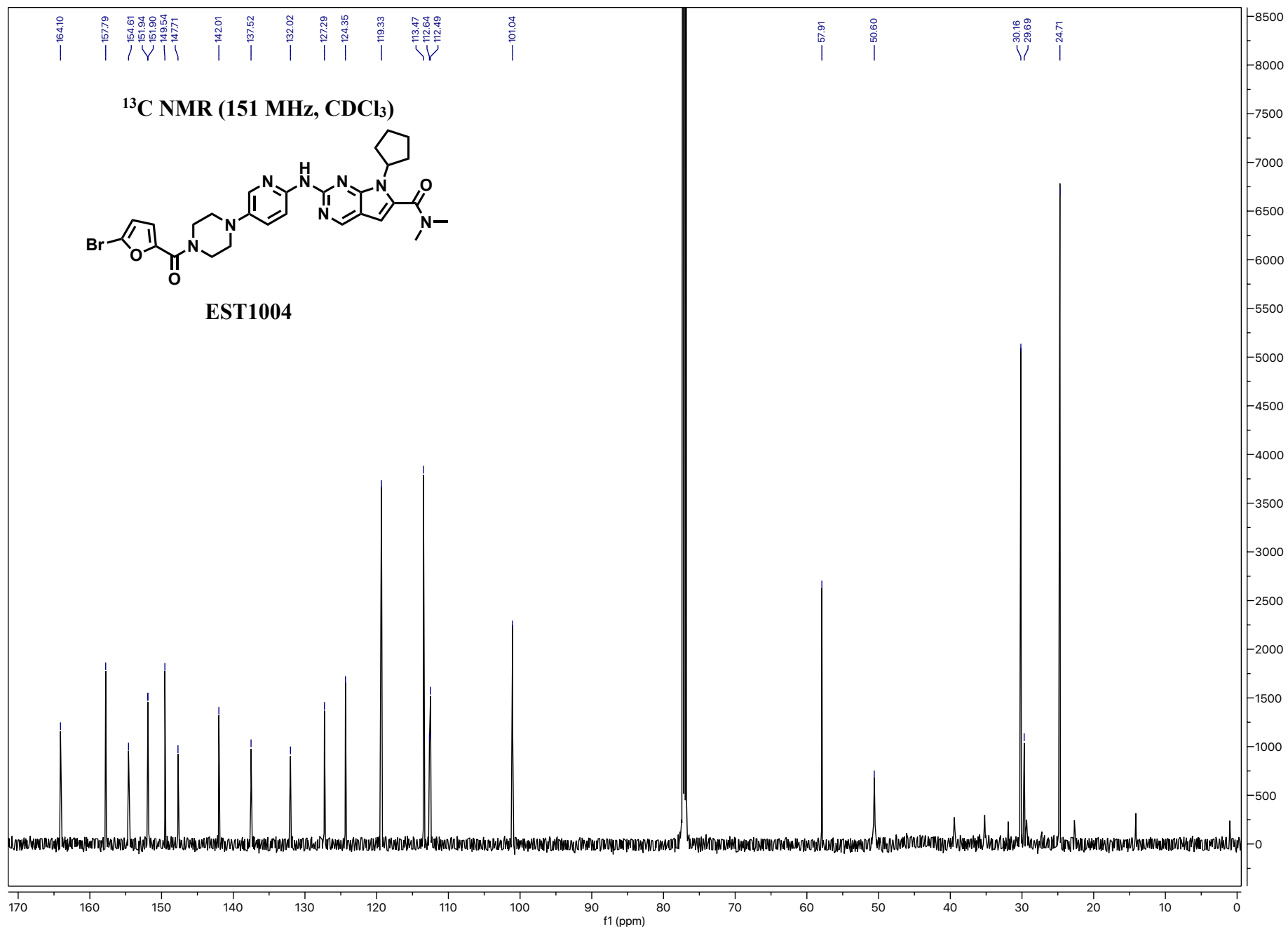


HESSEMA4-101-EXP219-S01\_QC

1: Scan ES+  
TIC  
8.83e7







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

EM23001594424

08-Feb-2023  
14:31:26  
strubhe 1-0208A-v05

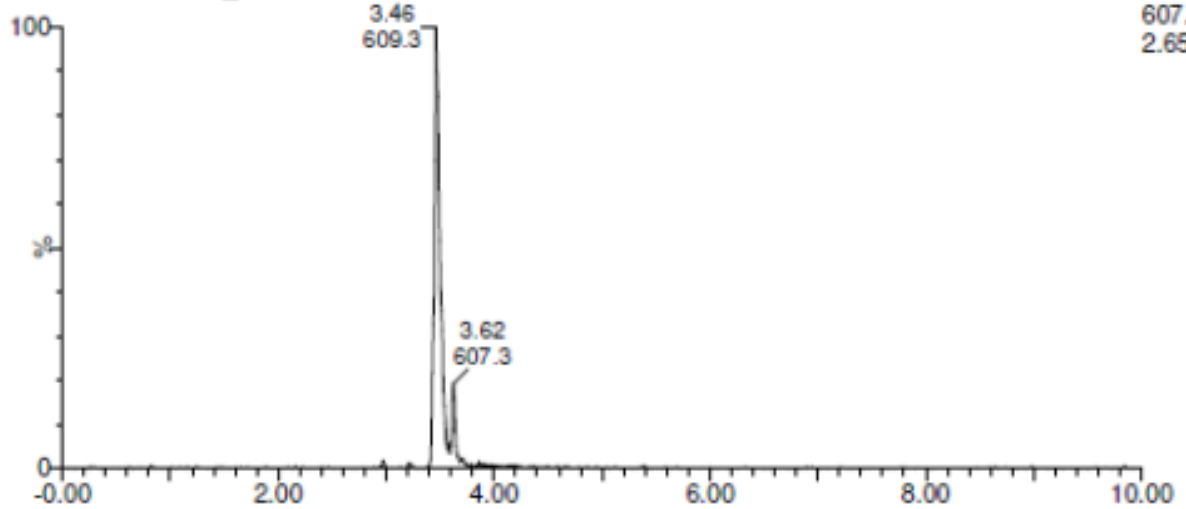
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HESSEMA4-101-EXP220-S01\_QC

1: Scan ES+

607.17

2.65e7



HESSEMA4-101-EXP220-S01\_QC

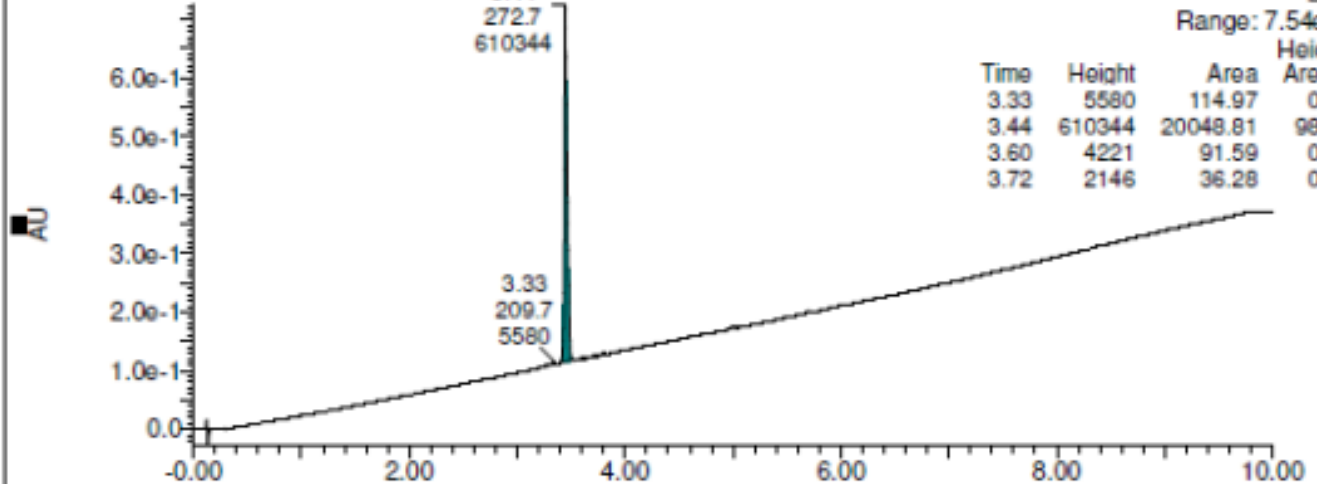
2: Diode Array

220

Range: 7.54e-1

Height

Time	Height	Area	Area%
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3.72	2146	36.28	0.18

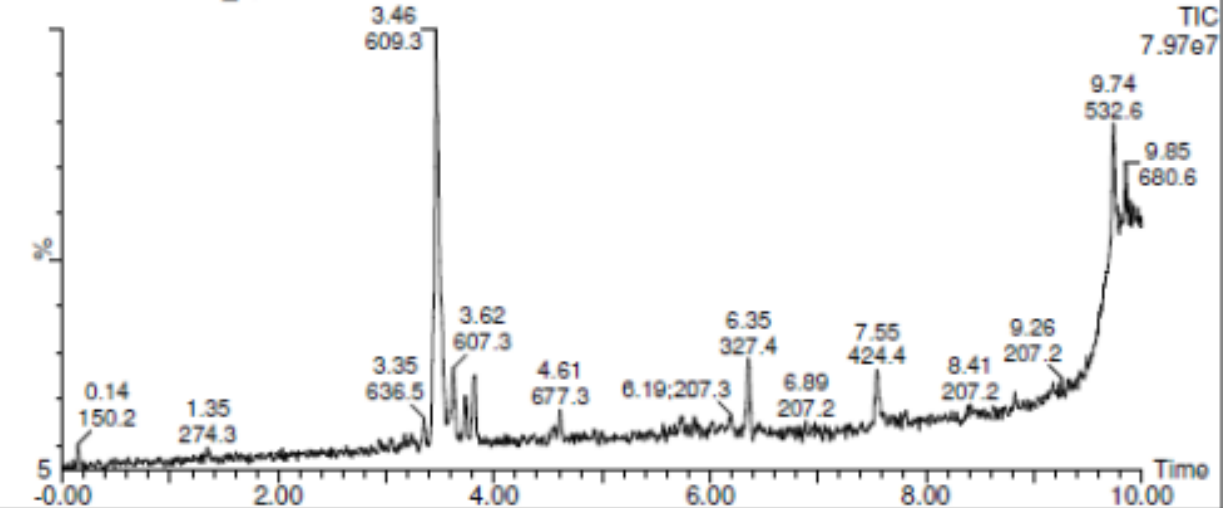


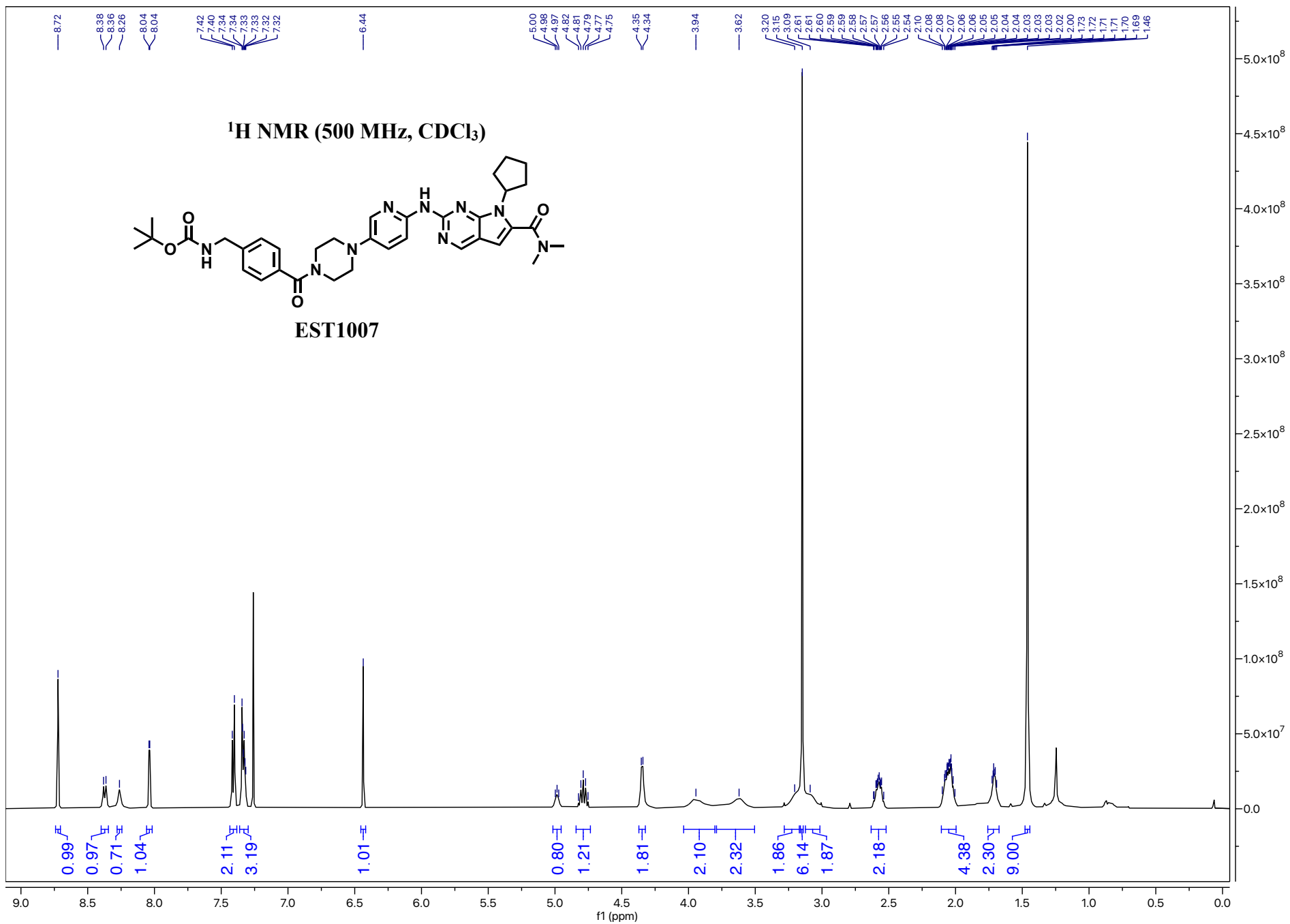
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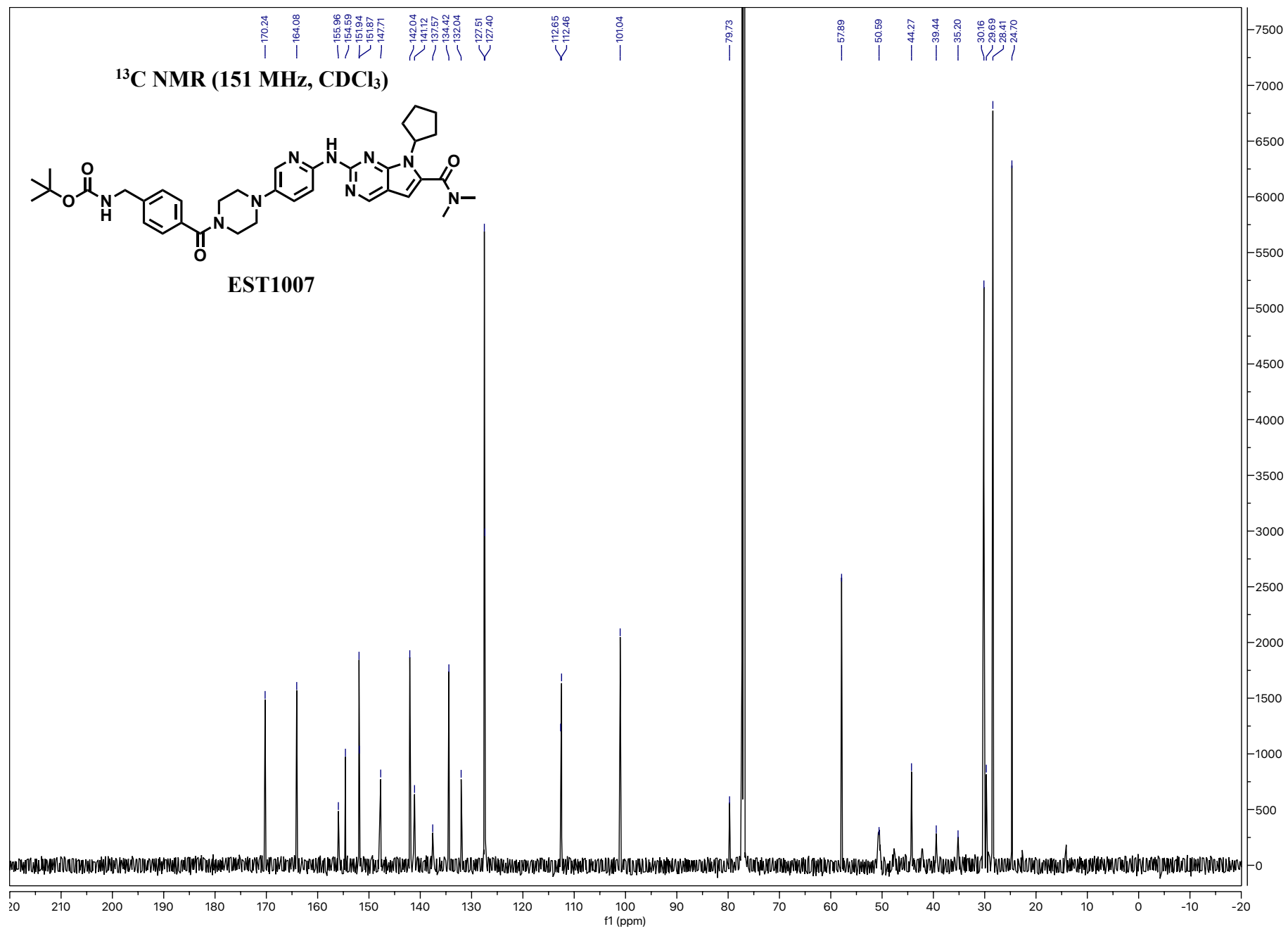
1: Scan ES+

TIC

7.97e7







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

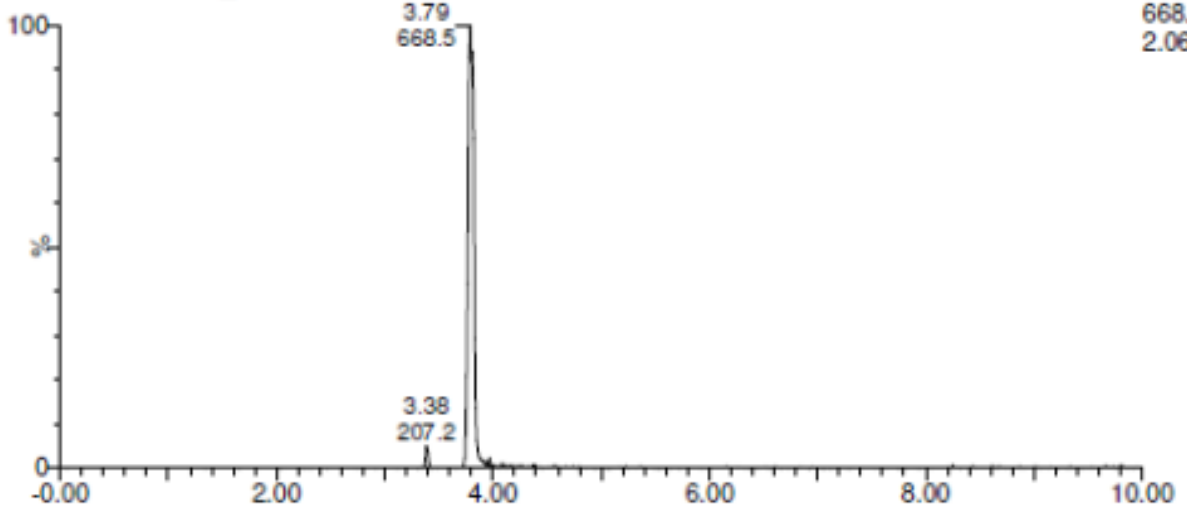
EM23001594425

08-Feb-2023  
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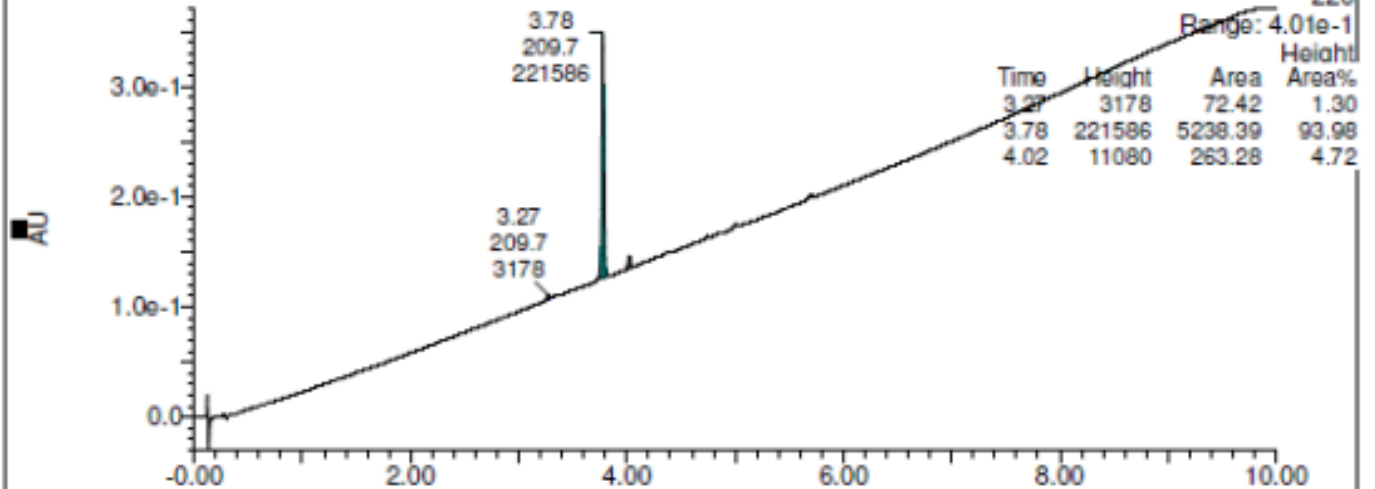
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2.06e7



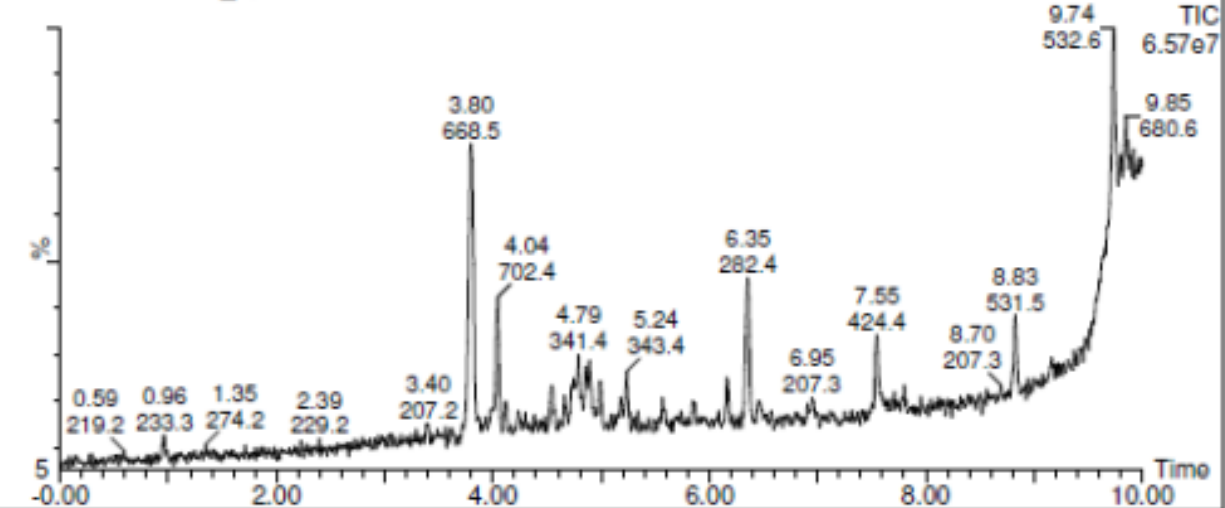
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2: Diode Array  
220  
Range: 4.01e-1

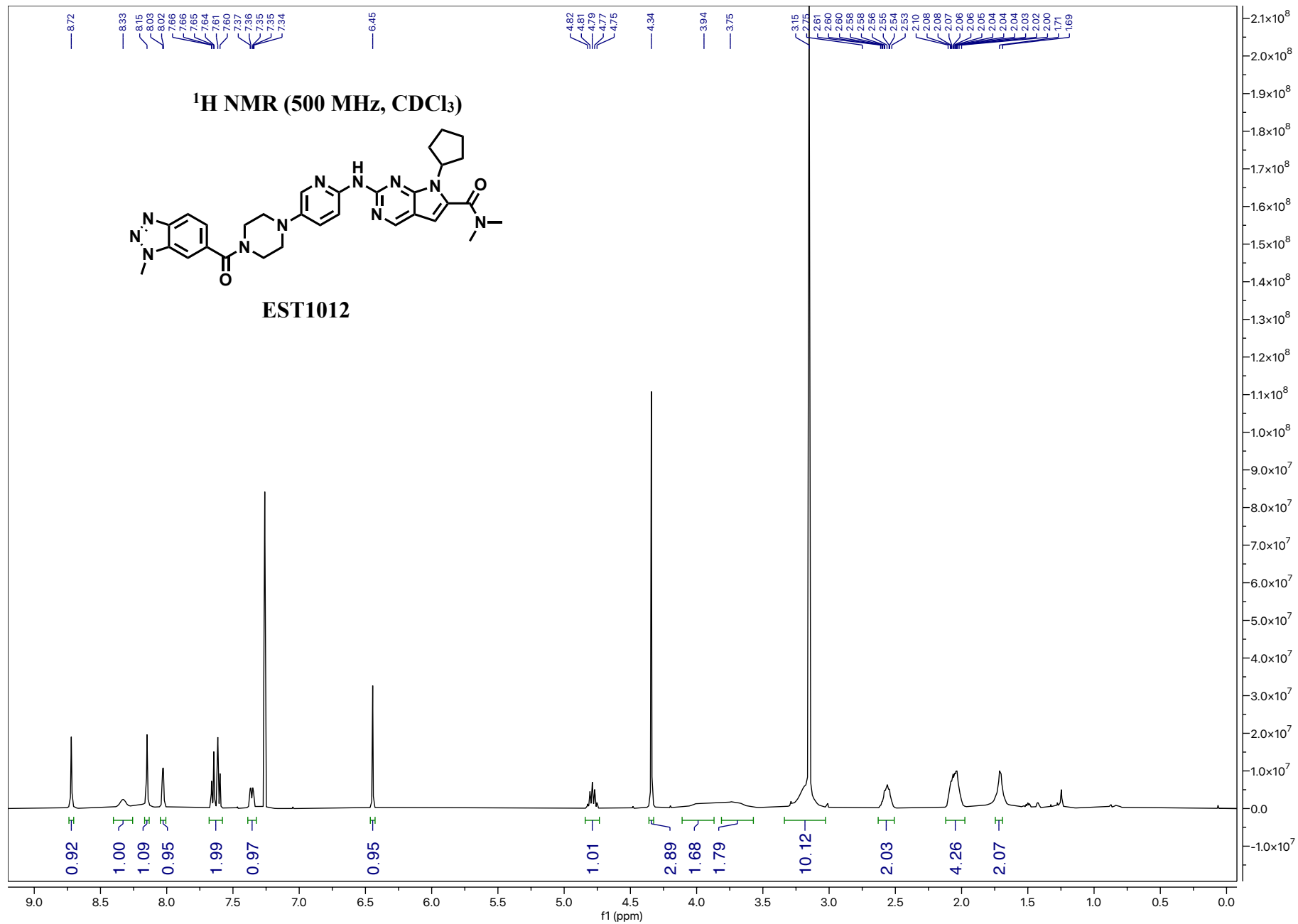


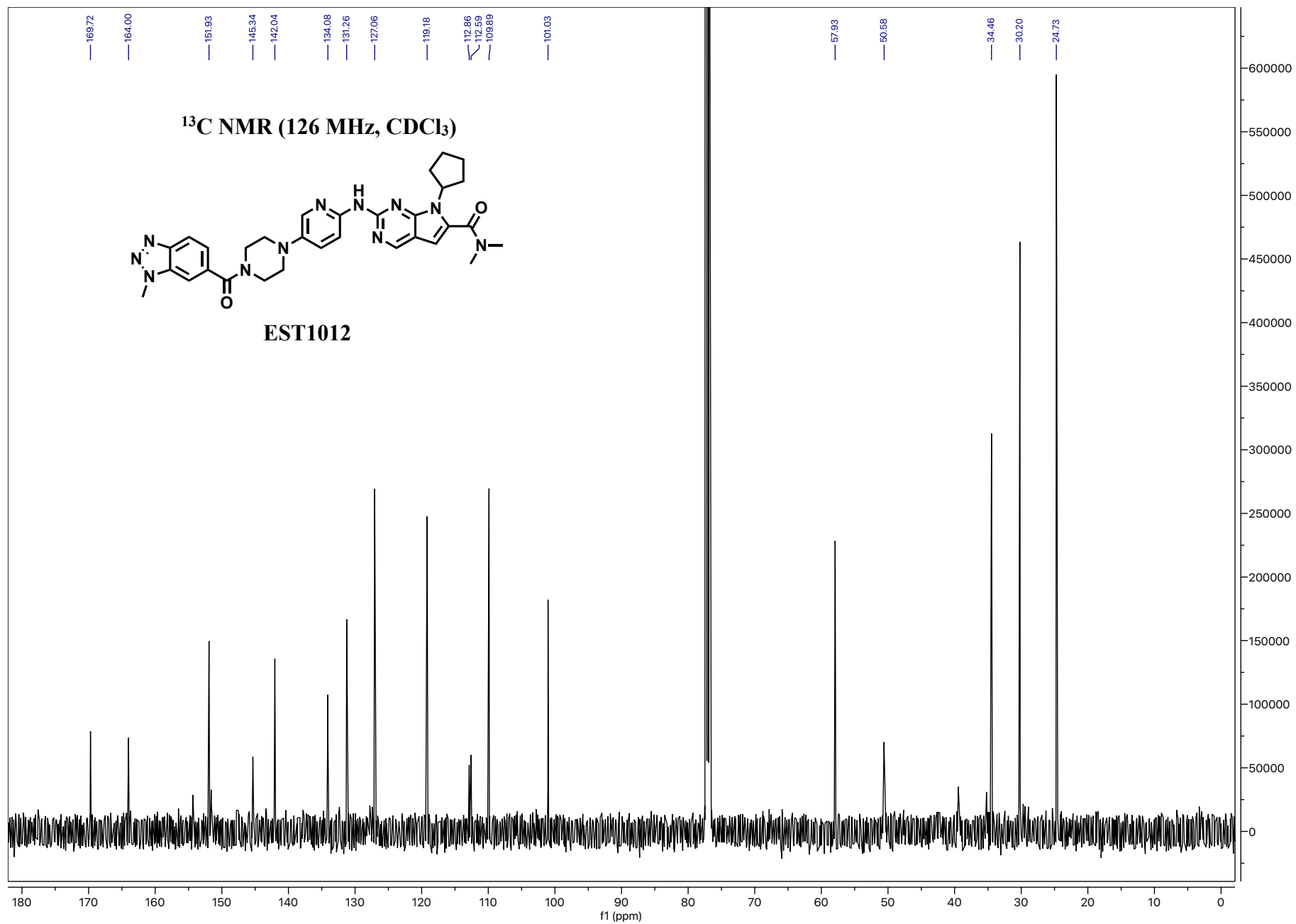
HESSEMA4-101-EXP221-S01\_OC

1: Scan ES+  
9.74  
532.6  
TIC  
6.57e7









HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

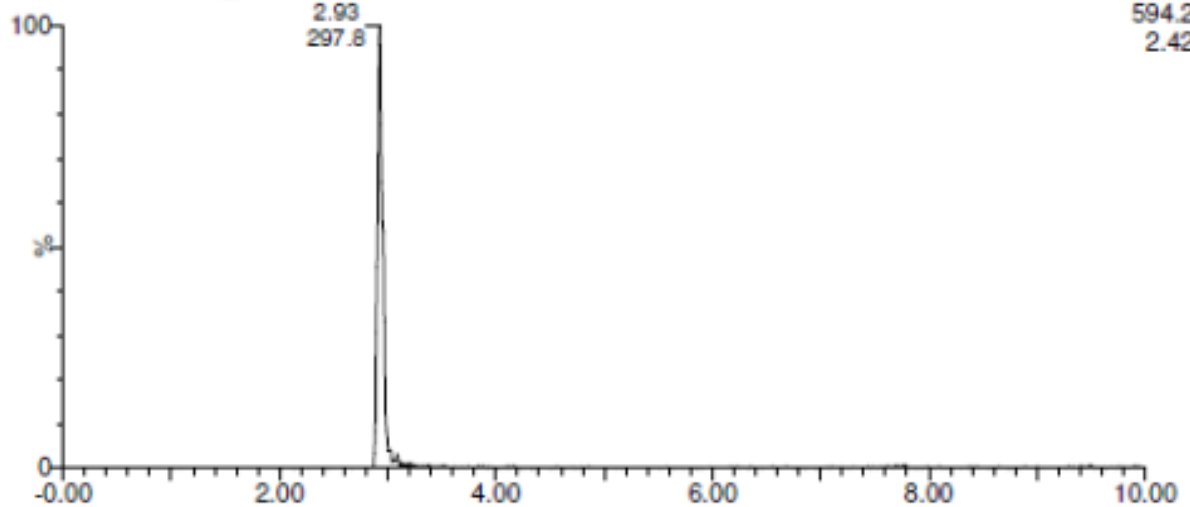
EM23001594427

08-Feb-2023  
14:53:33  
strubhe1-0208A-v07

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

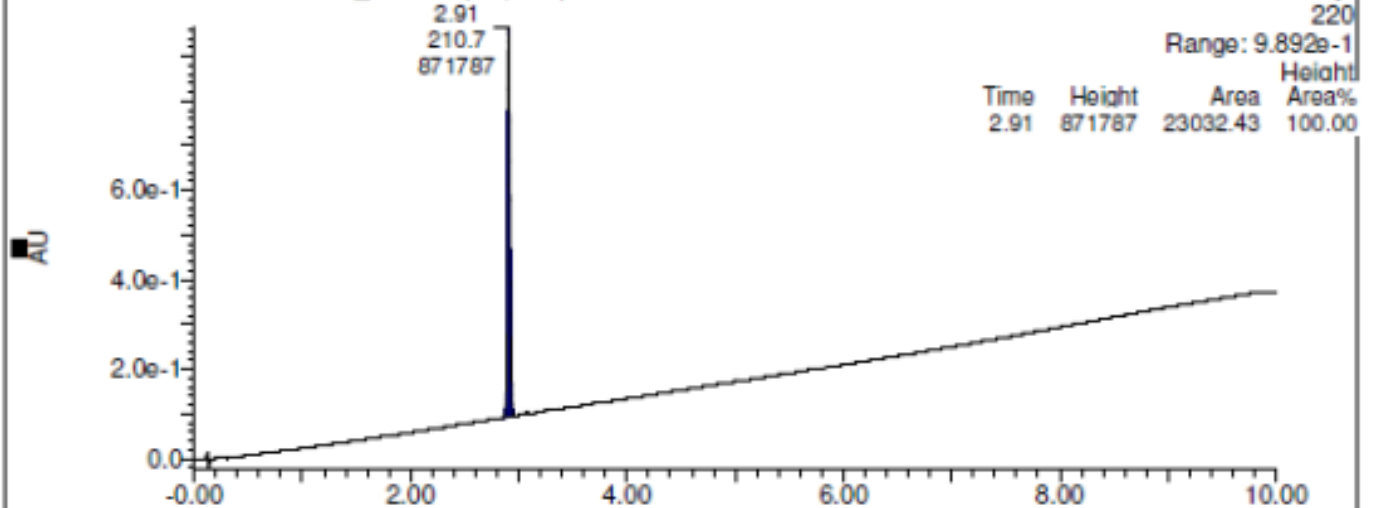
1: Scan ES+  
594.298  
2.42e7

HESSEMA4-101-EXP222-S01\_OC



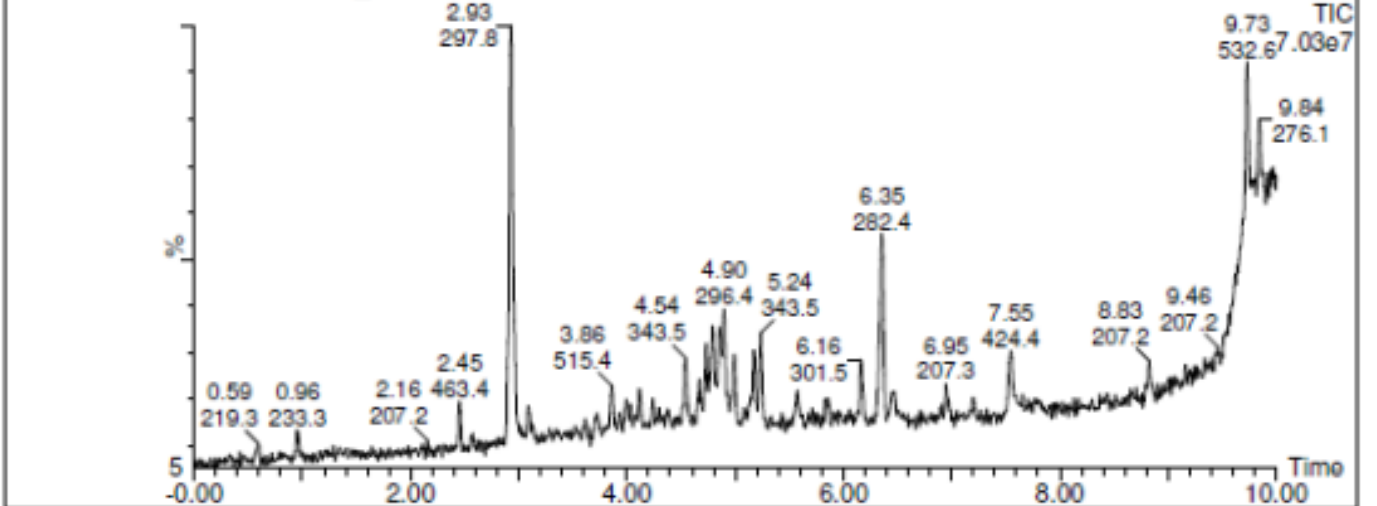
HESSEMA4-101-EXP222-S01\_OC Sm (Mn, 2x3)

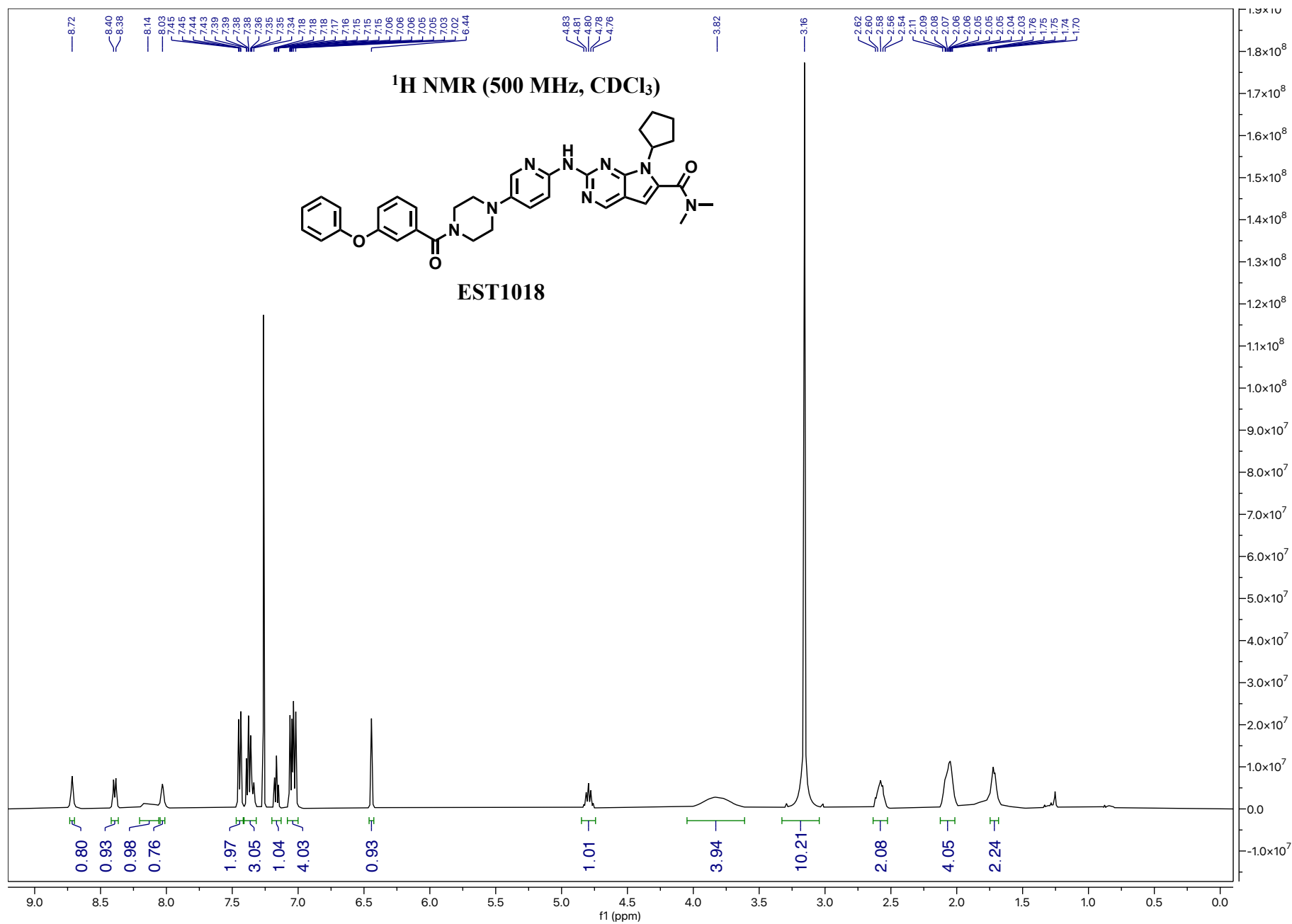
2: Diode Array

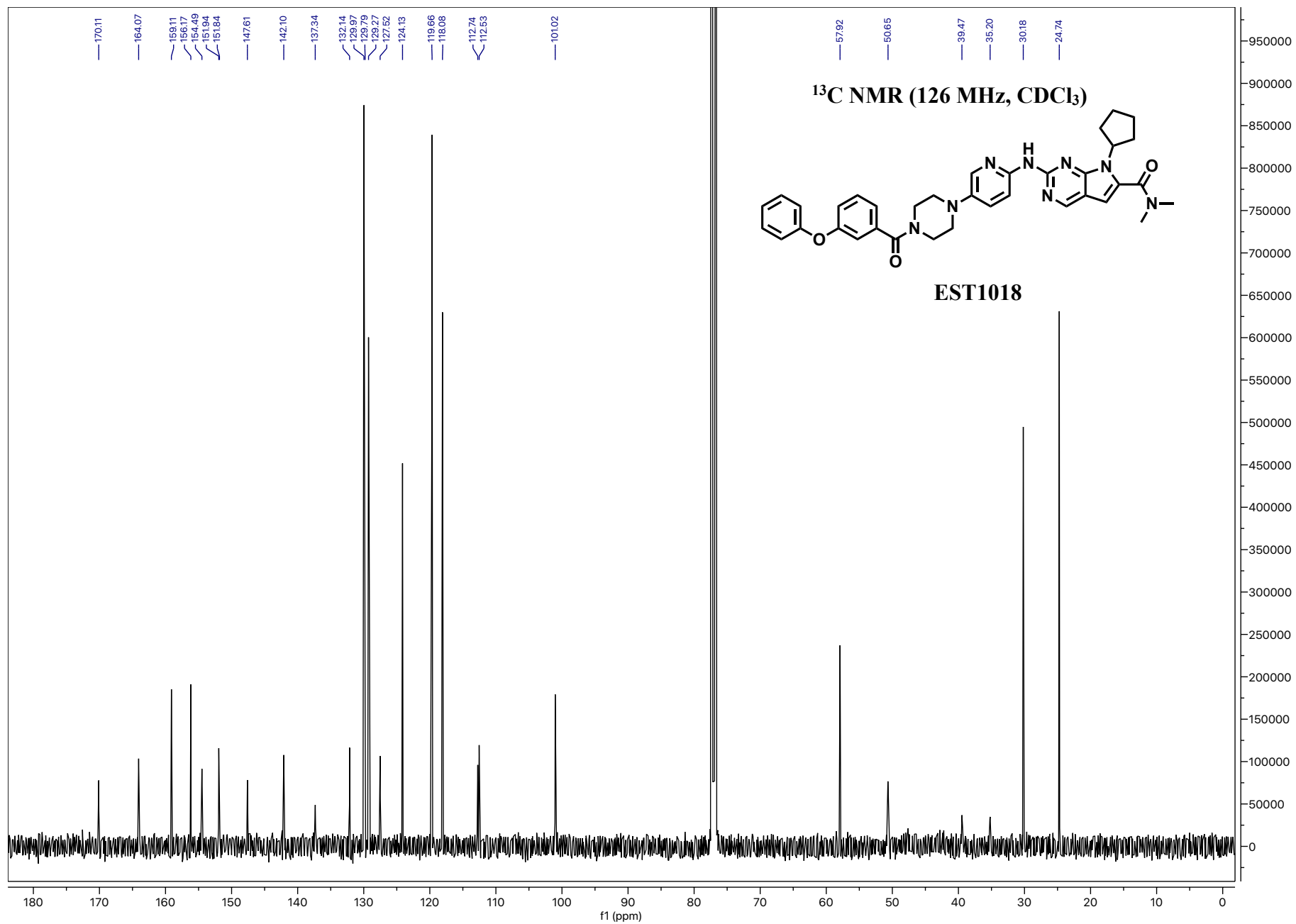


HESSEMA4-101-EXP222-S01\_OC

1: Scan ES+ TIC







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

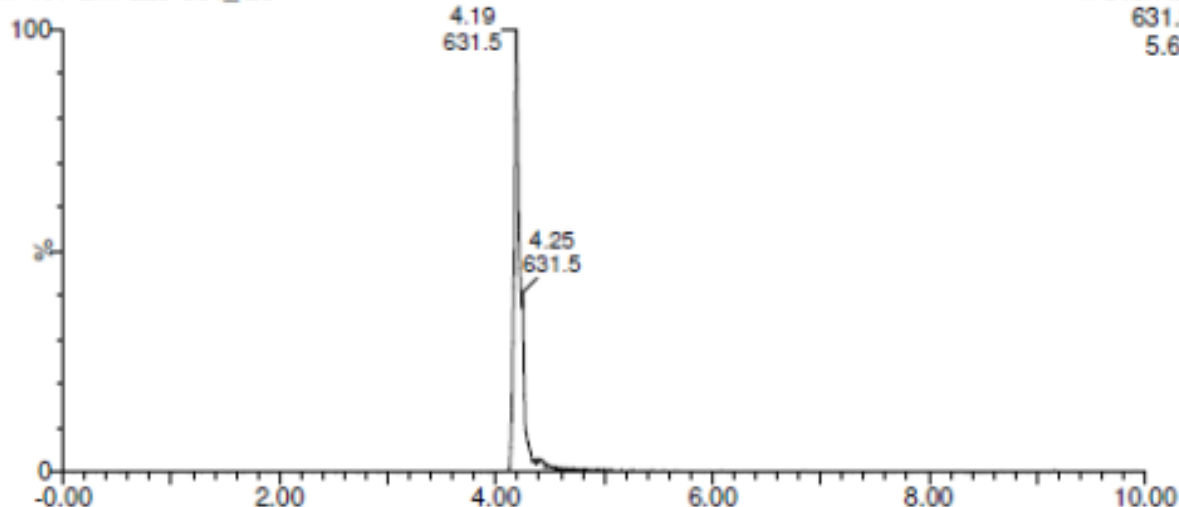
EM23001594428

08-Feb-2023  
15:04:37  
strubhe1-0208A-v08

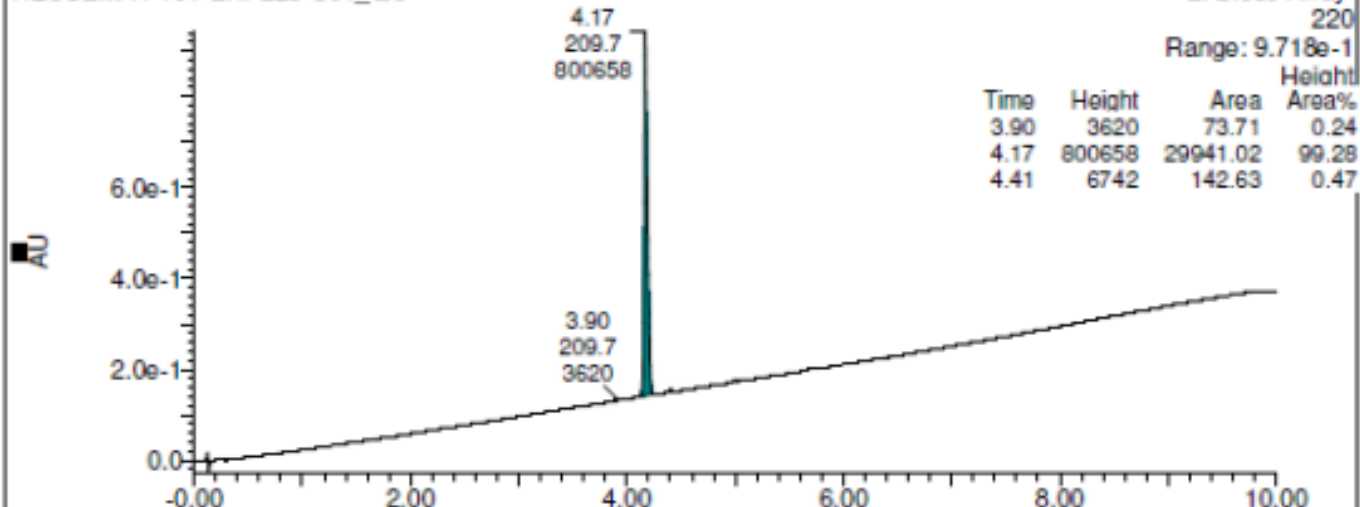
LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
631.307  
5.61e7

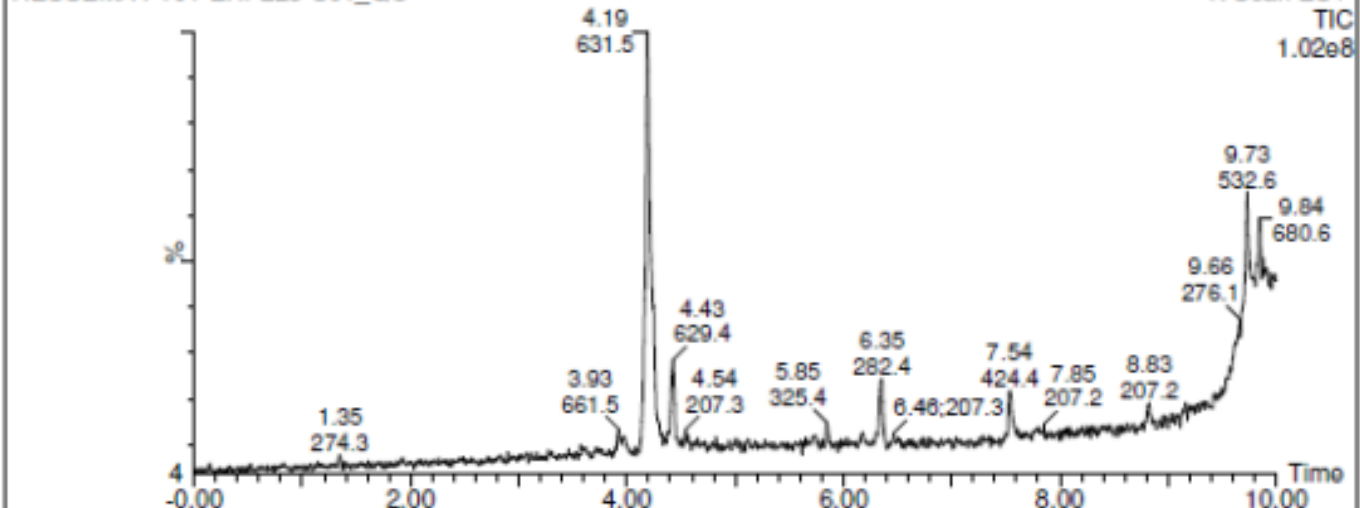
HESSEMA4-101-EXP223-S01\_OC

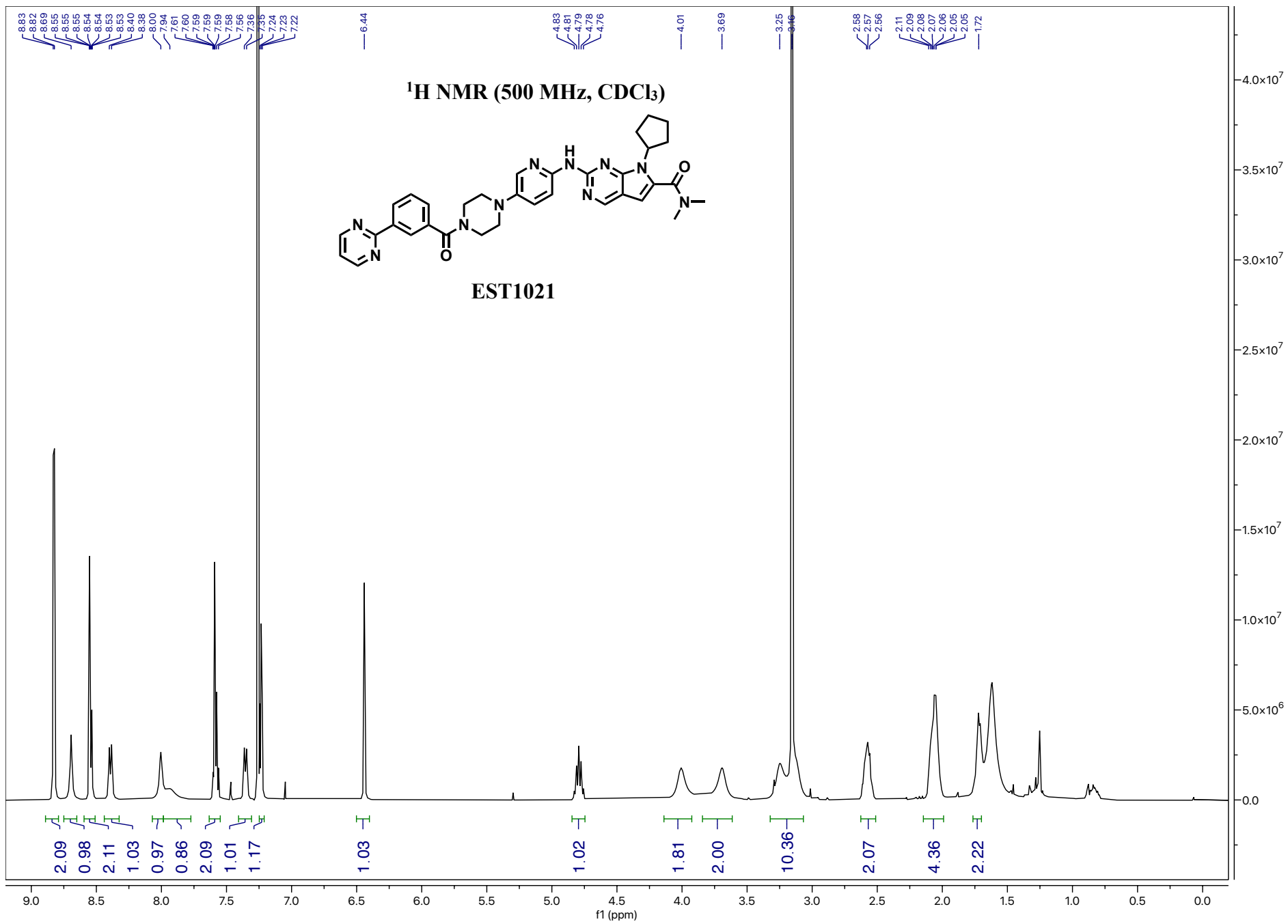


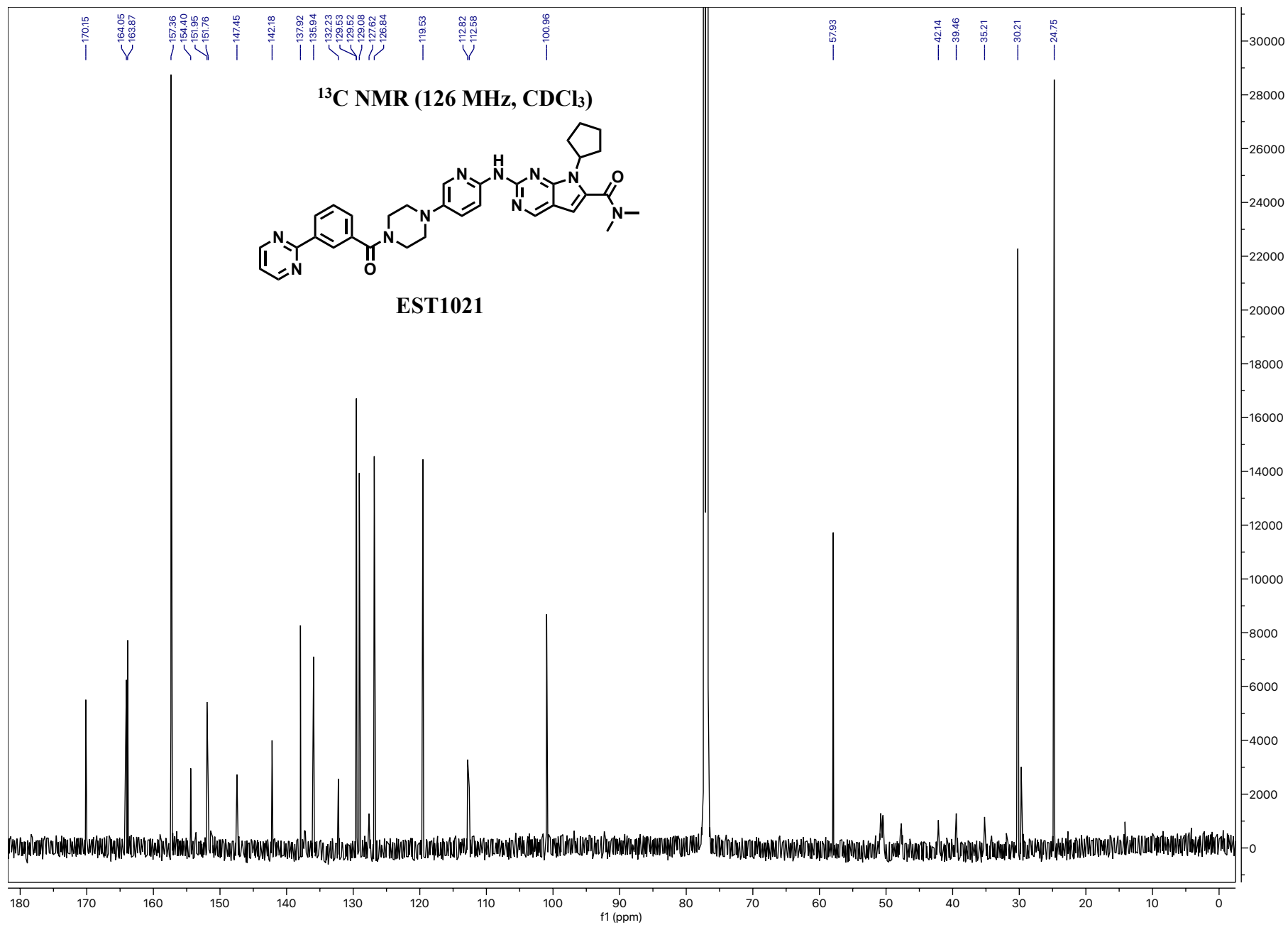
HESSEMA4-101-EXP223-S01\_OC



HESSEMA4-101-EXP223-S01\_OC









HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

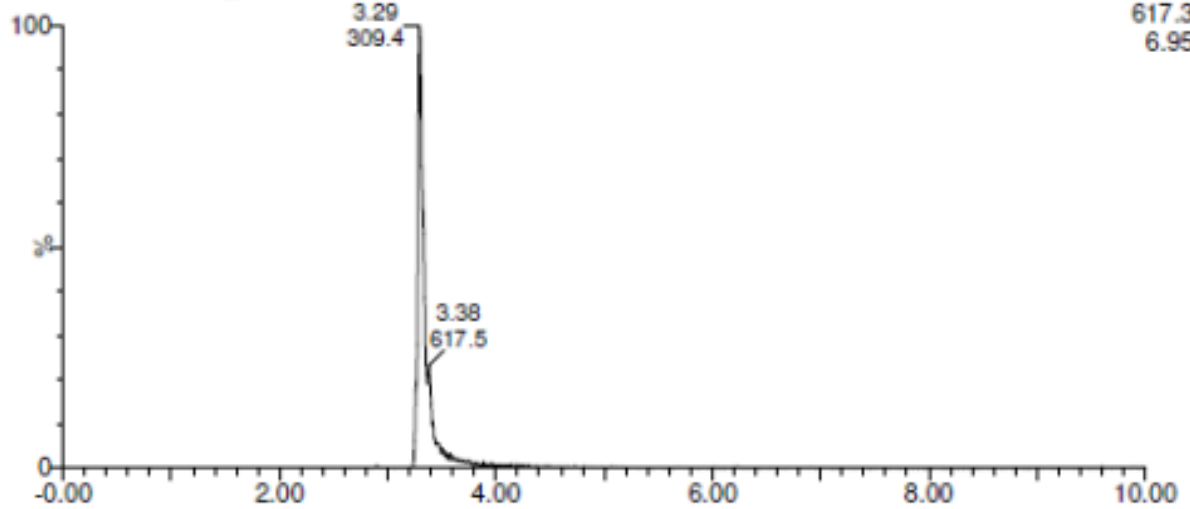
EM23001594429

08-Feb-2023  
15:15:41  
strubhe1-0208A-v09

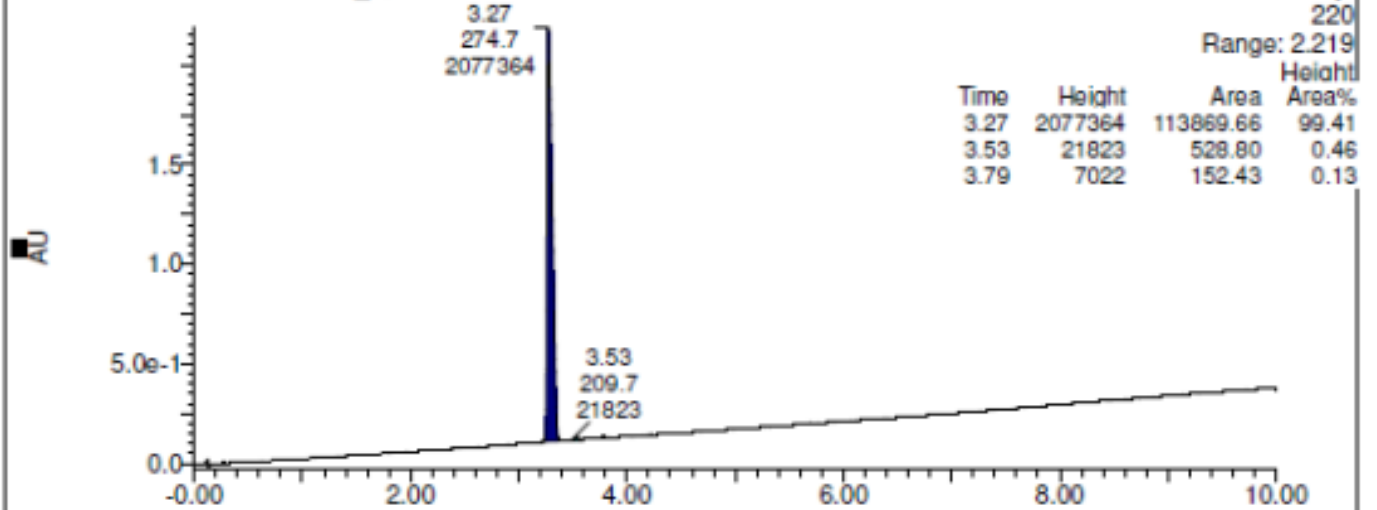
LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
617.302  
6.95e7

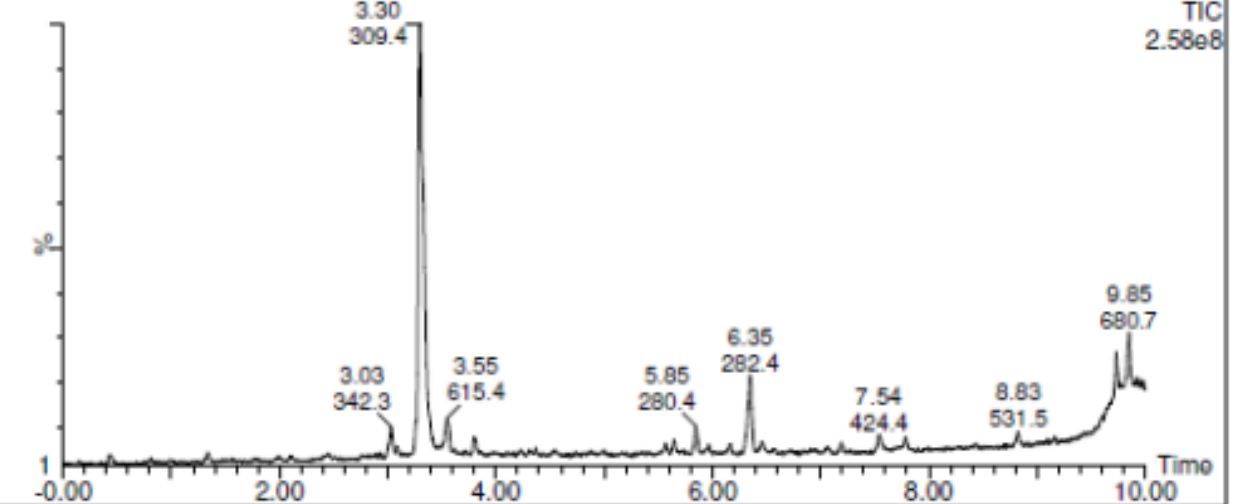
HESSEMA4-101-EXP224-S01\_OC

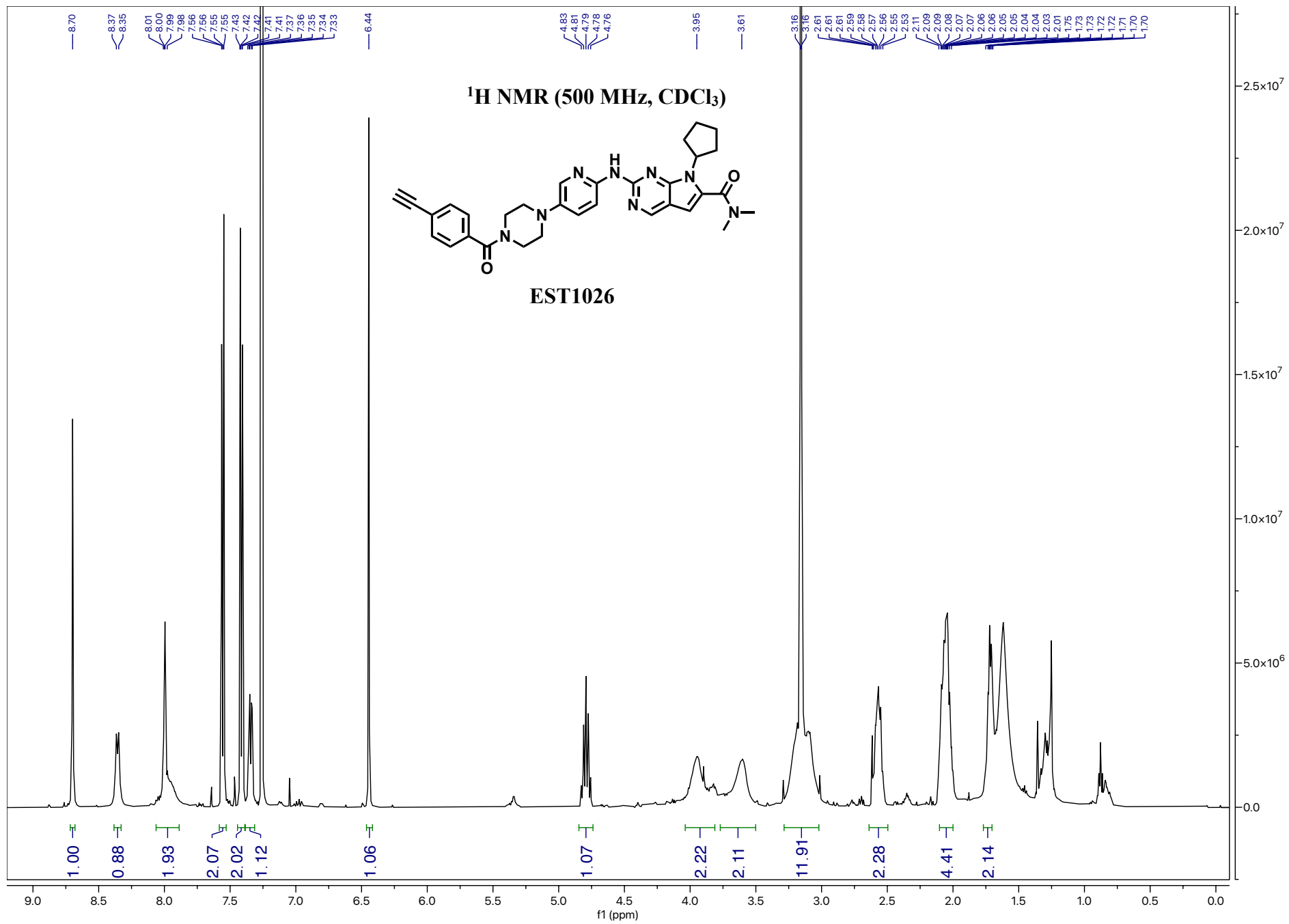


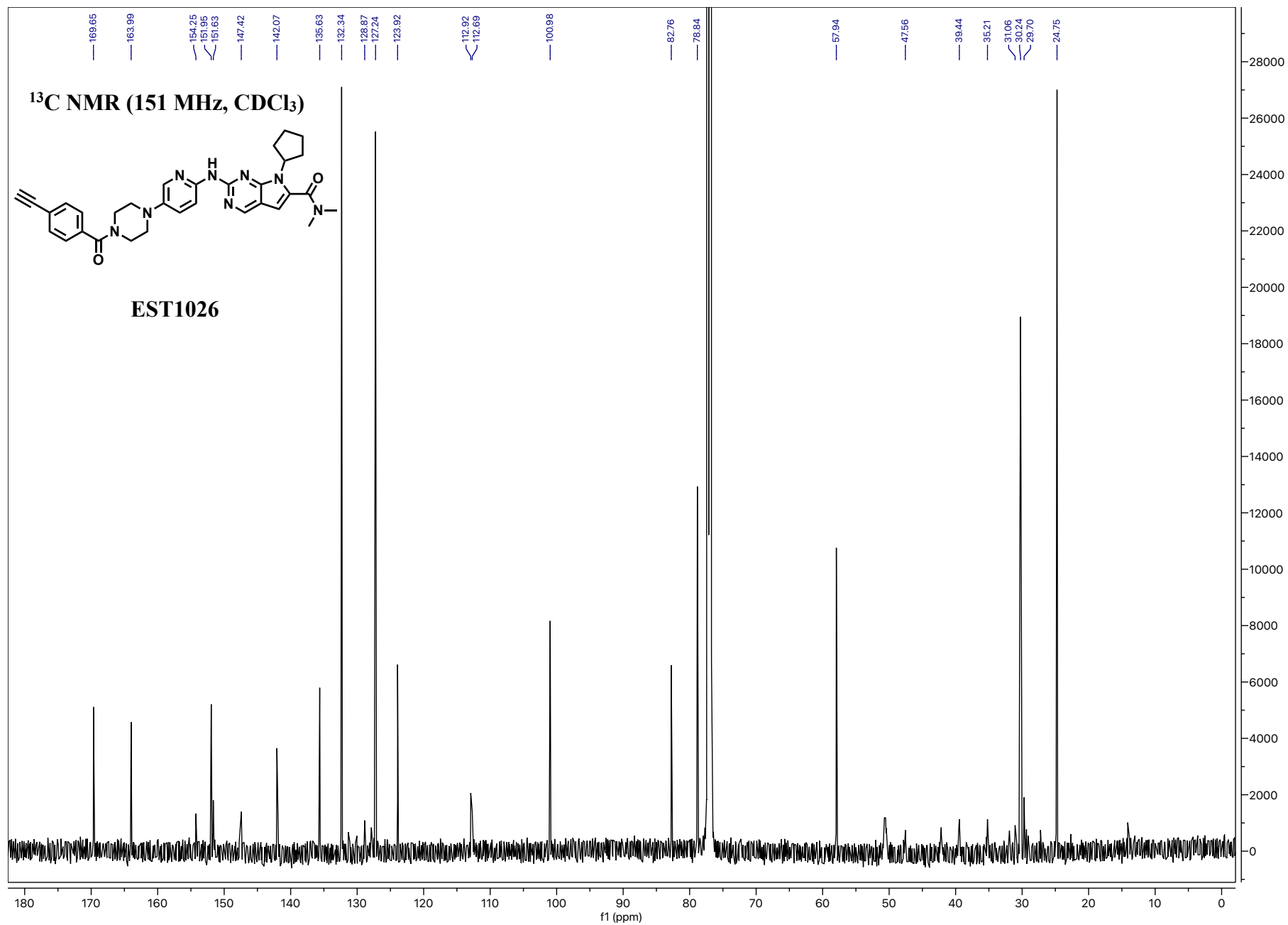
HESSEMA4-101-EXP224-S01\_OC



HESSEMA4-101-EXP224-S01\_OC







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

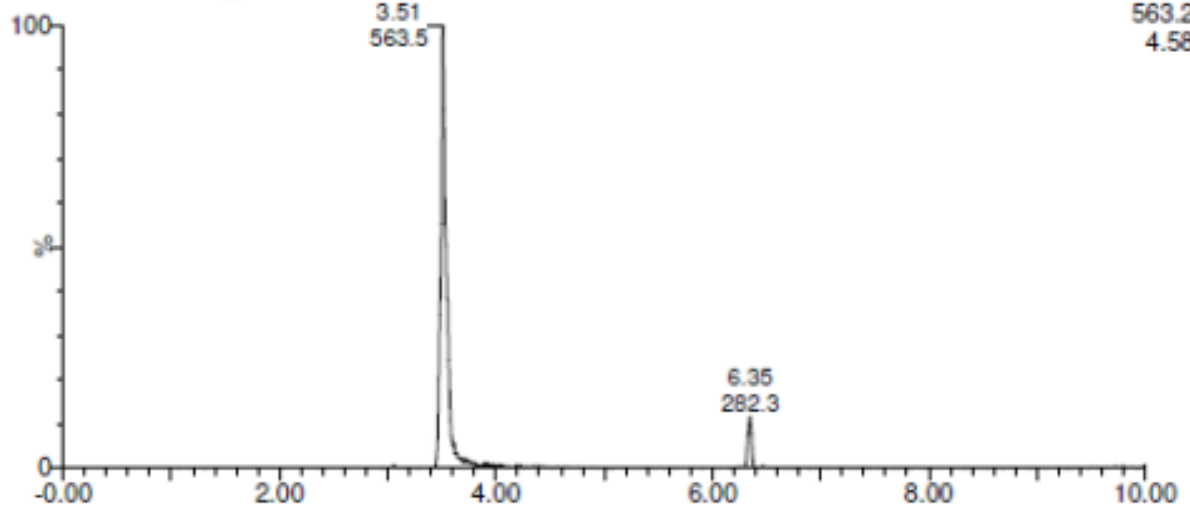
EM23001594430

08-Feb-2023  
15:26:43  
strubhe1-0208A-v10

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

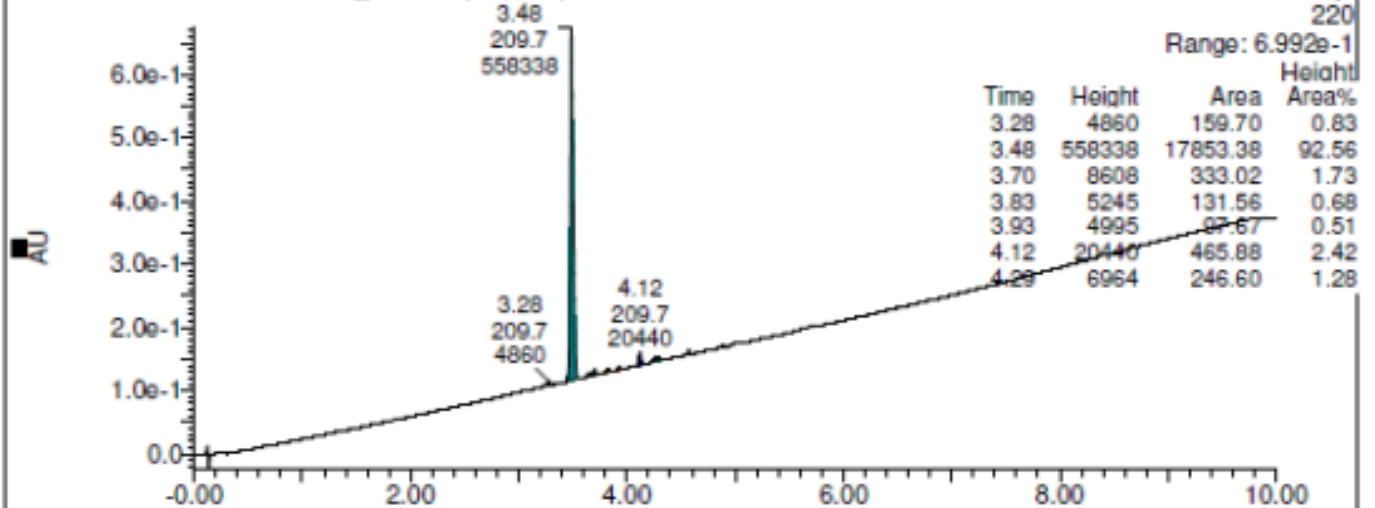
1: Scan ES+  
563.281  
4.58e7

HESSEMA4-101-EXP225-S01\_OC



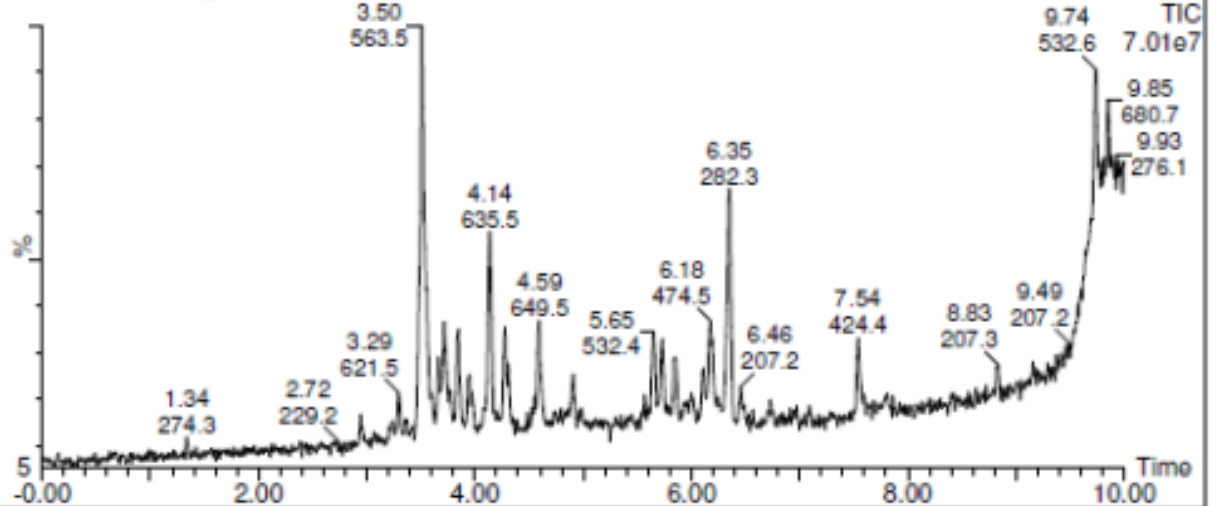
HESSEMA4-101-EXP225-S01\_OC Sm (Mn, 2x3)

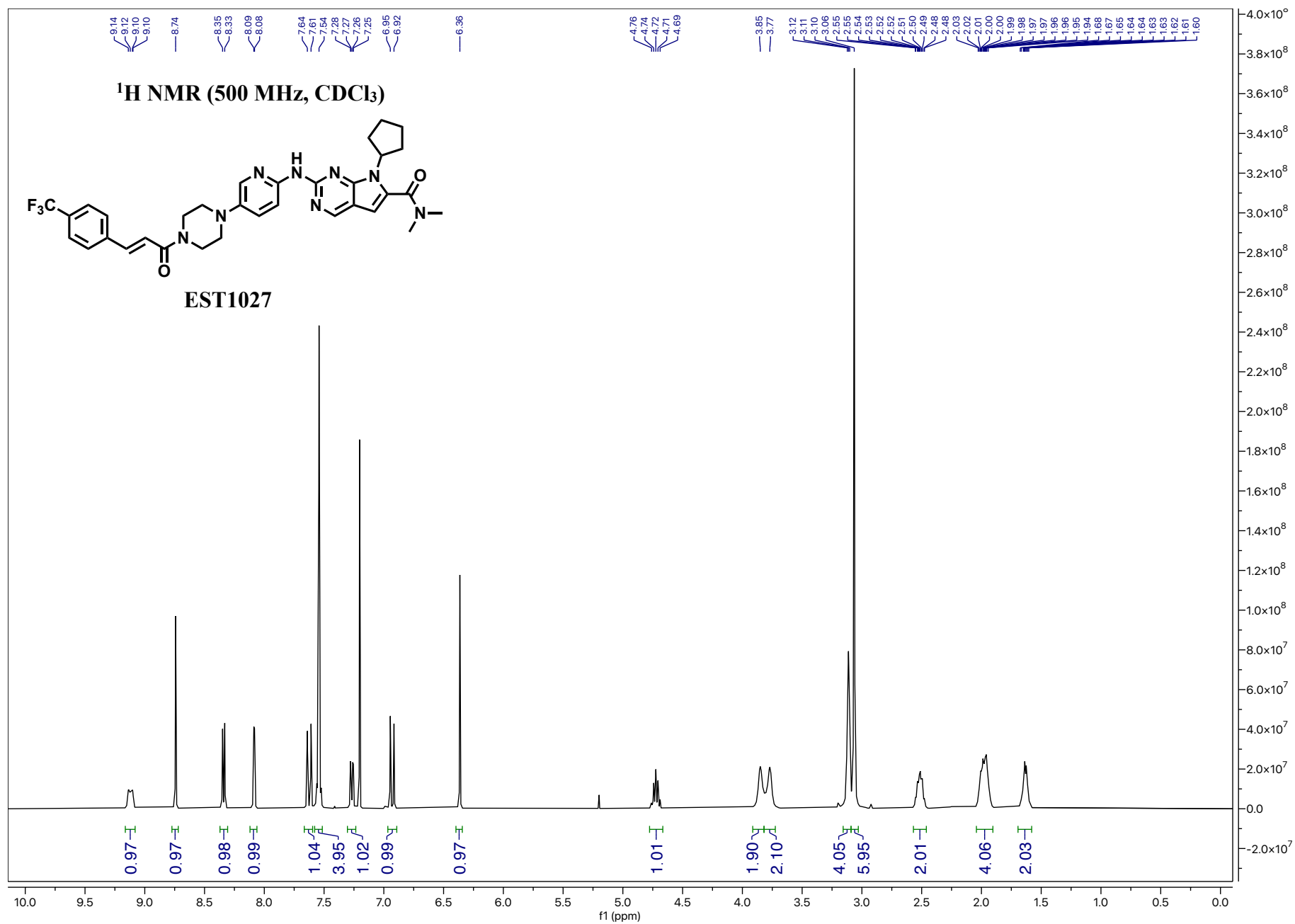
2: Diode Array  
220

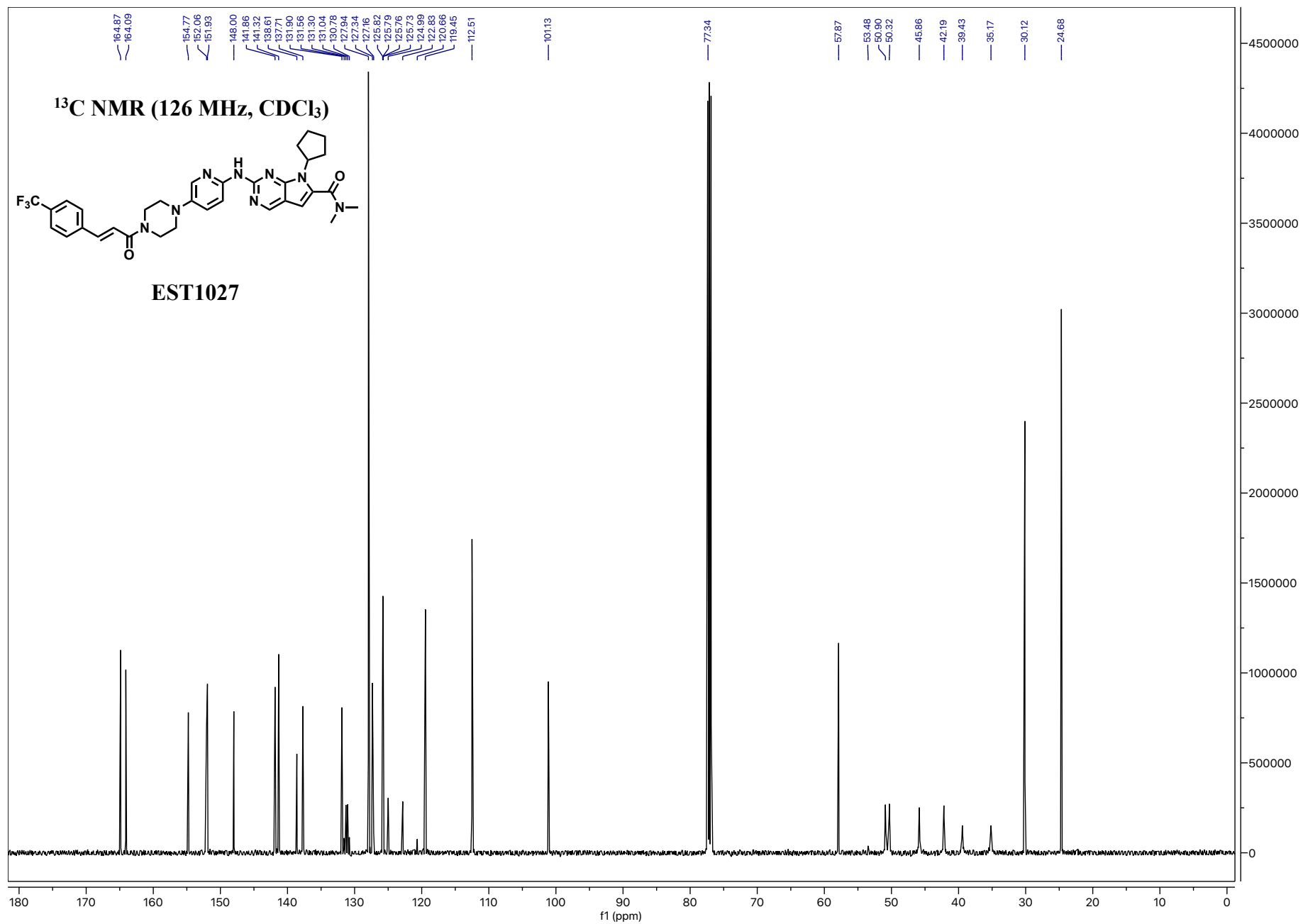


HESSEMA4-101-EXP225-S01\_OC

1: Scan ES+  
9.74 TIC  
532.6 7.01e7







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

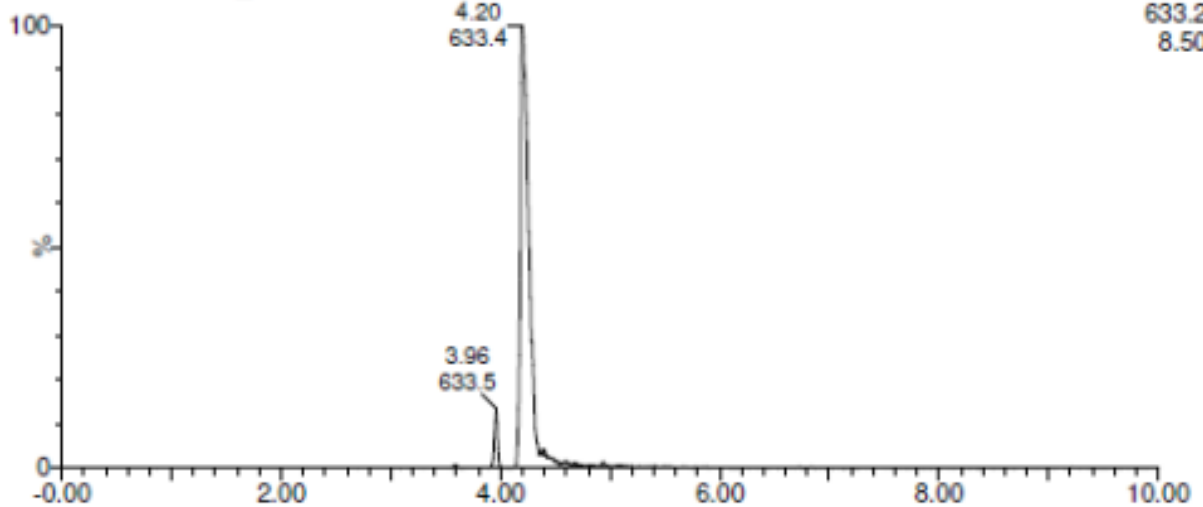
EM23001594419

08-Feb-2023  
13:46:34  
strubhe1-0208A-v01

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
633.284  
8.50e7

HESSEMA4-101-EXP216-S01\_OC



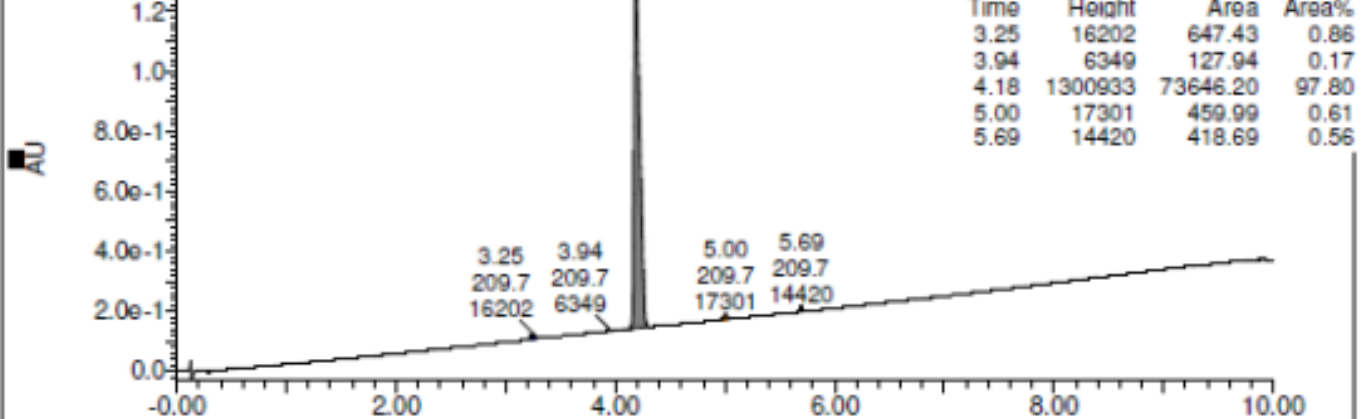
HESSEMA4-101-EXP216-S01\_OC Sm (Mn, 2x3)

2: Diode Array

220

Range: 1.474

Height

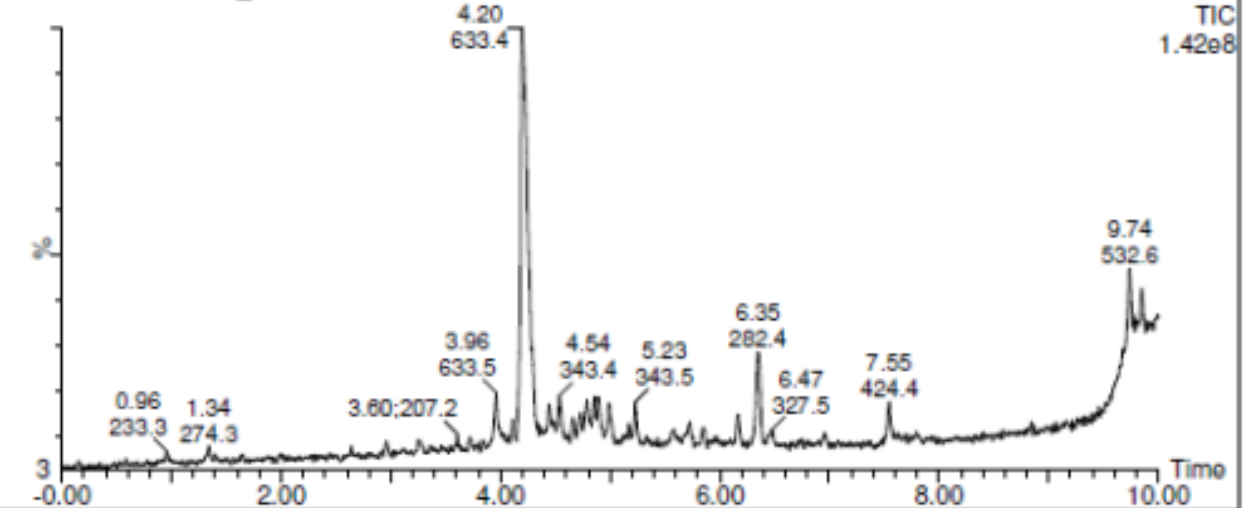


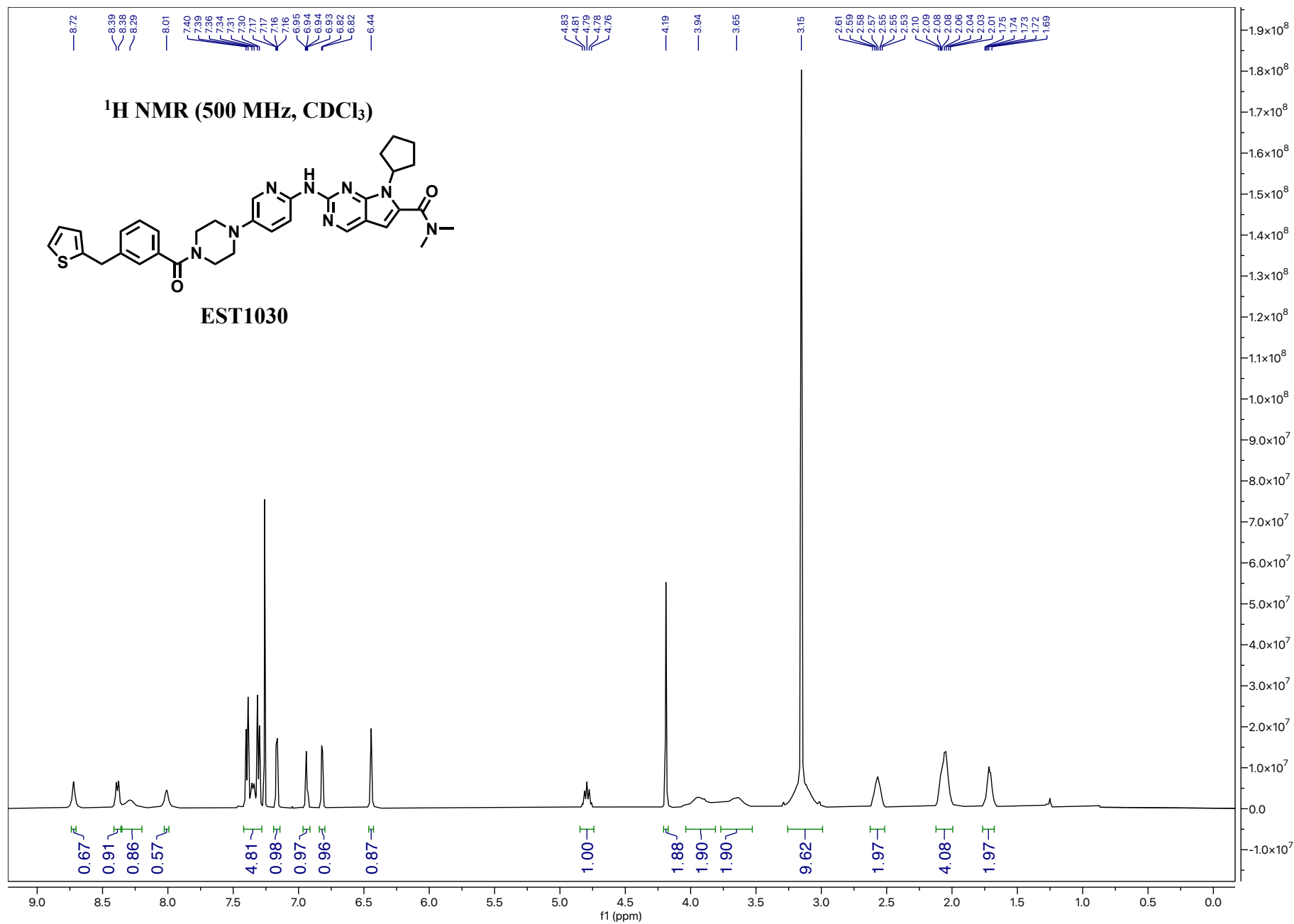
HESSEMA4-101-EXP216-S01\_OC

1: Scan ES+

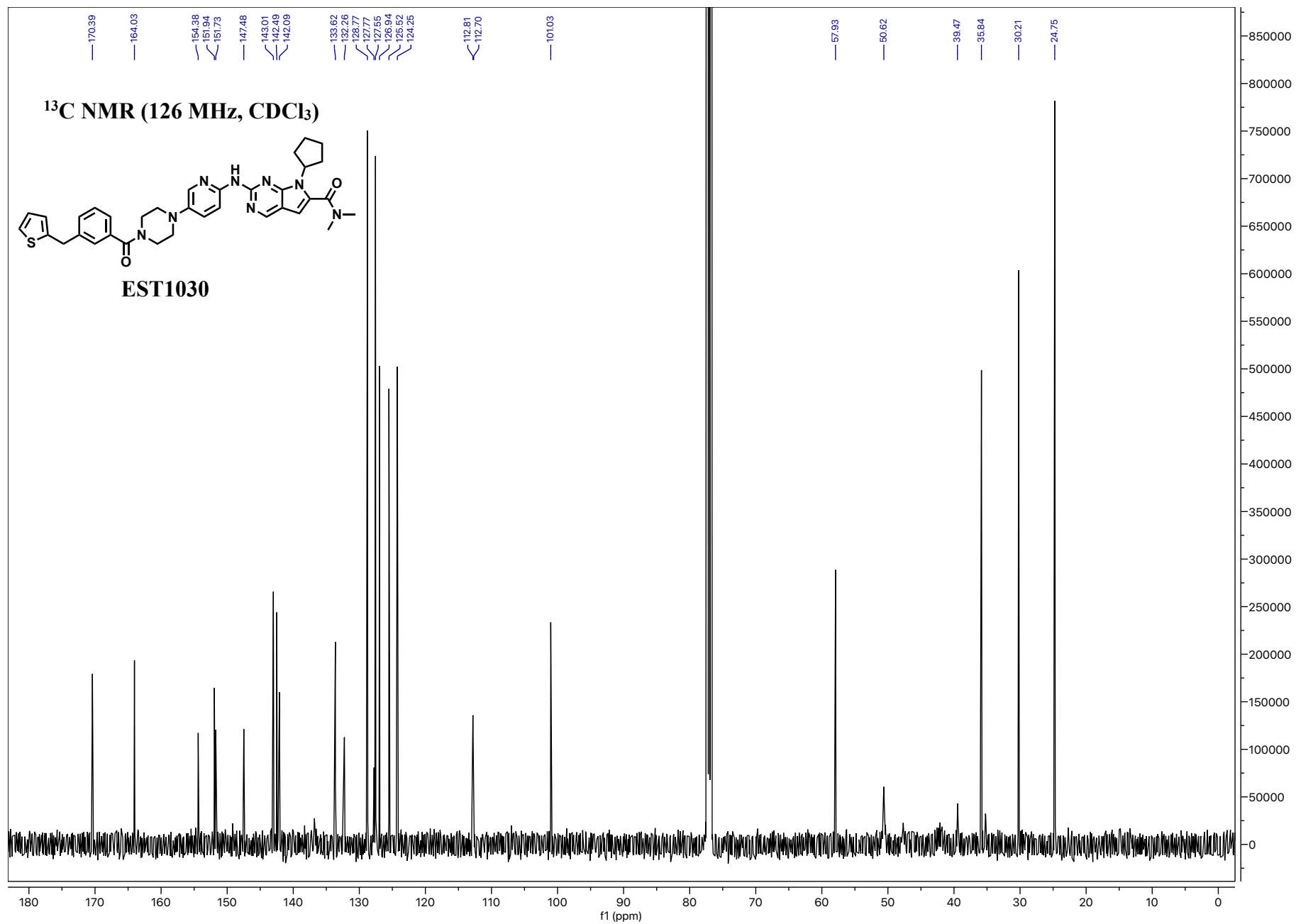
TIC

1.42e8









HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

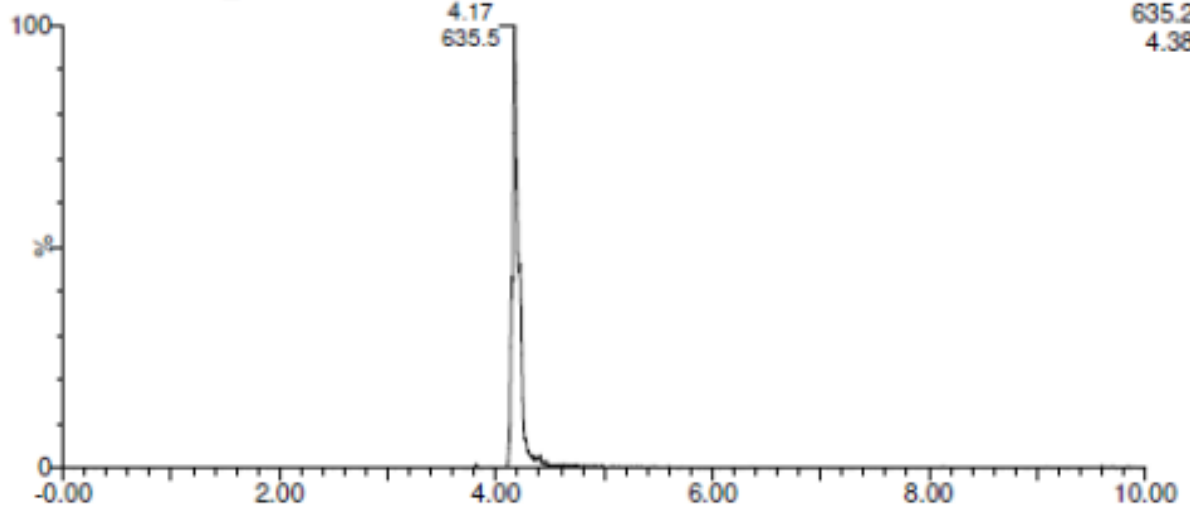
EM23001594431

08-Feb-2023  
15:37:45  
strubhe1-0208A-v11

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

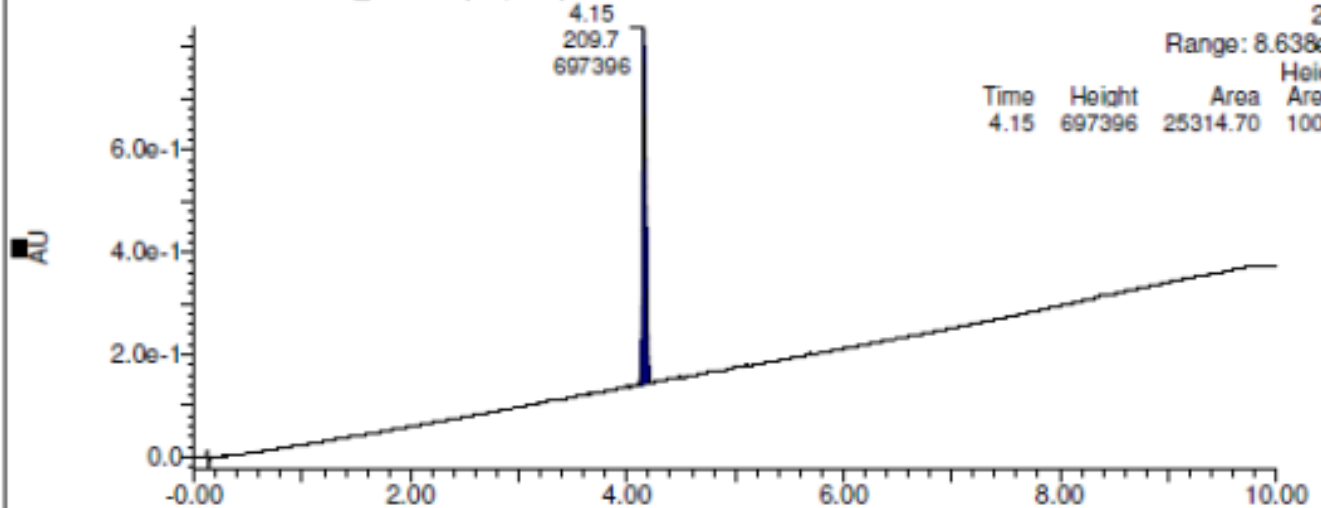
HESSEMA4-101-EXP226-S01\_OC

1: Scan ES+  
635.284  
4.38e7



HESSEMA4-101-EXP226-S01\_OC Sm (Mn, 2x3)

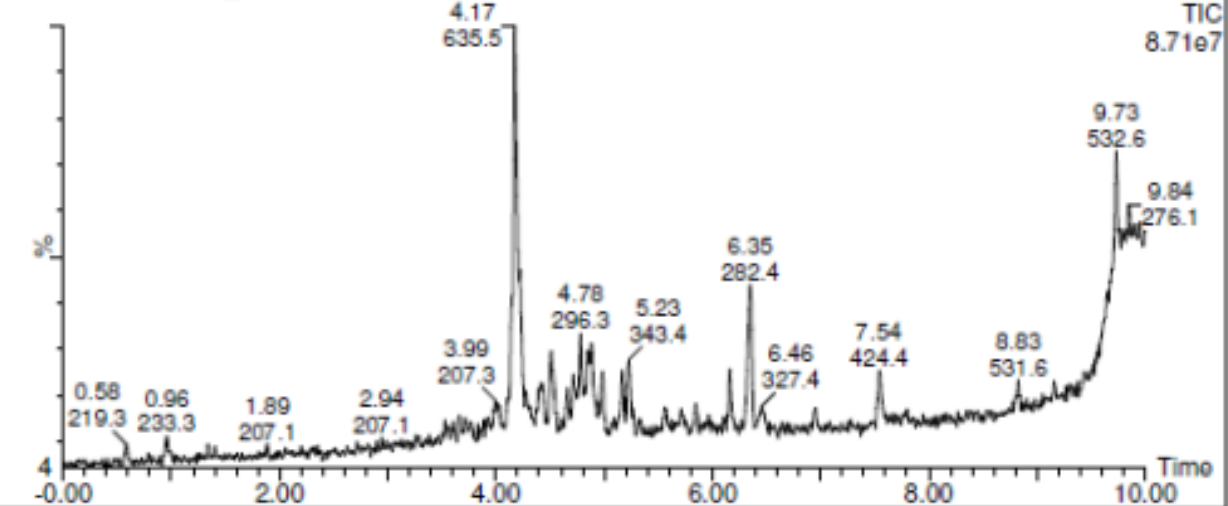
2: Diode Array  
220

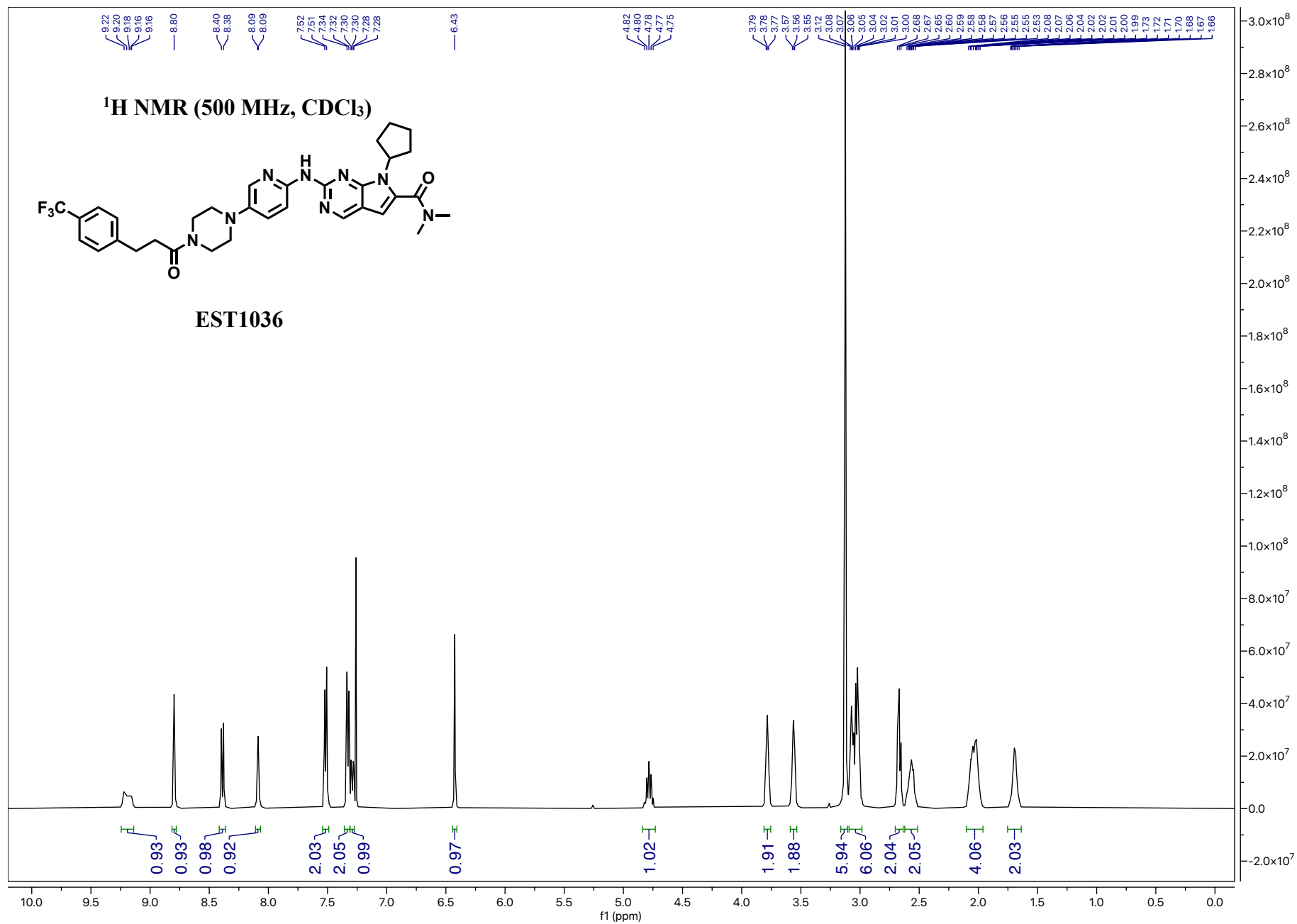


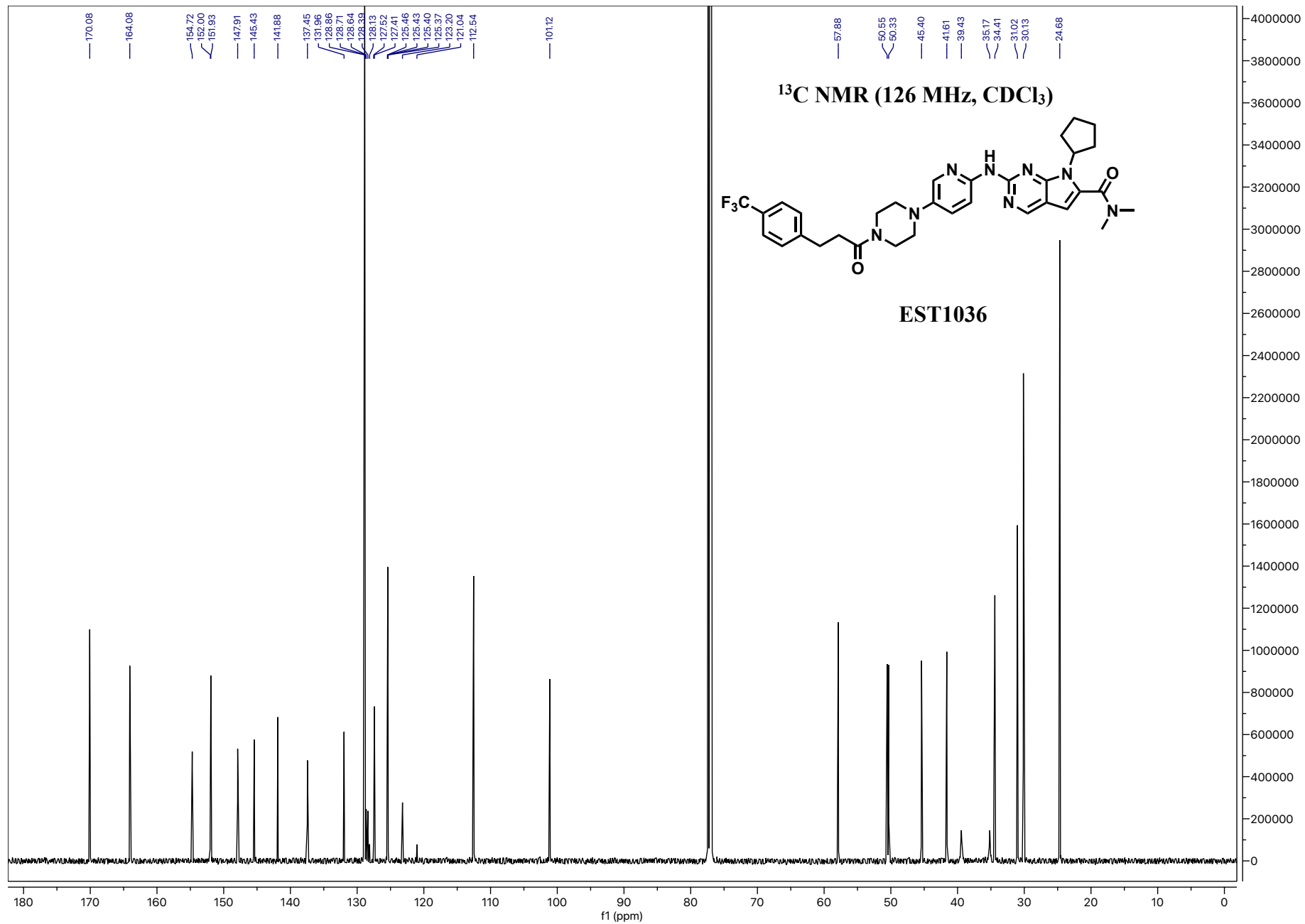
Time	Height	Area	Area%
4.15	209.7	25314.70	100.00

HESSEMA4-101-EXP226-S01\_OC

1: Scan ES+  
TIC  
8.71e7







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

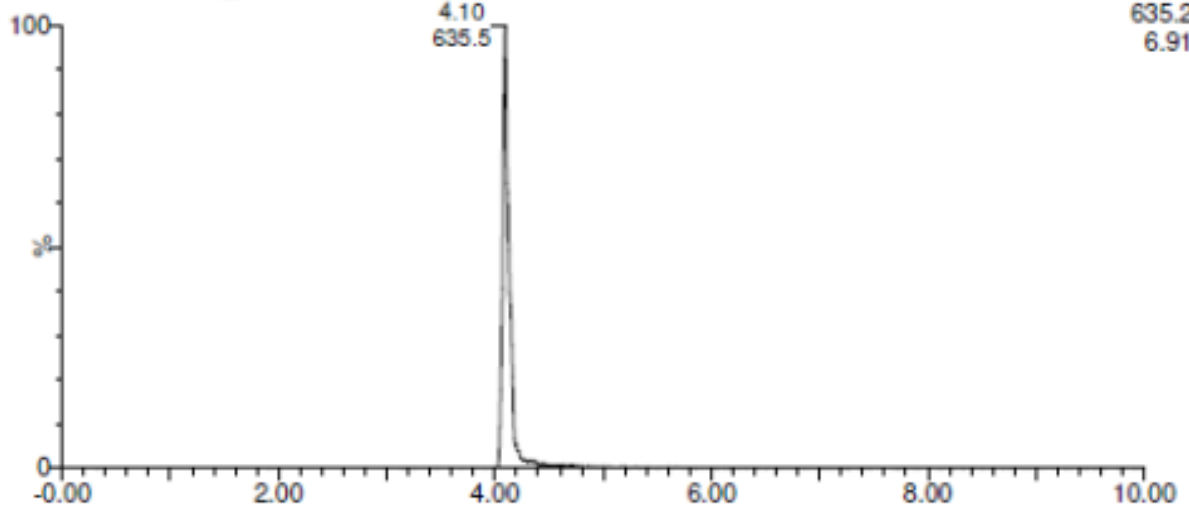
EM23001594432

08-Feb-2023  
15:48:47  
strubhe1-0208A-v12

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
635.299  
6.91e7

HESSEMA4-101-EXP227-S01\_OC

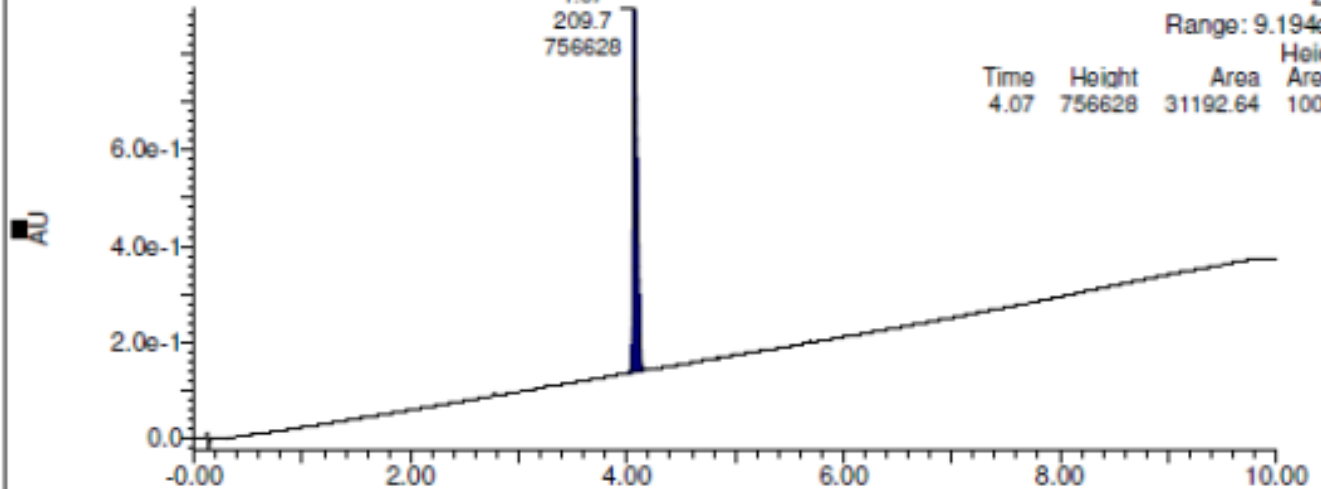


HESSEMA4-101-EXP227-S01\_OC Sm (Mn, 2x3)

2: Diode Array  
220

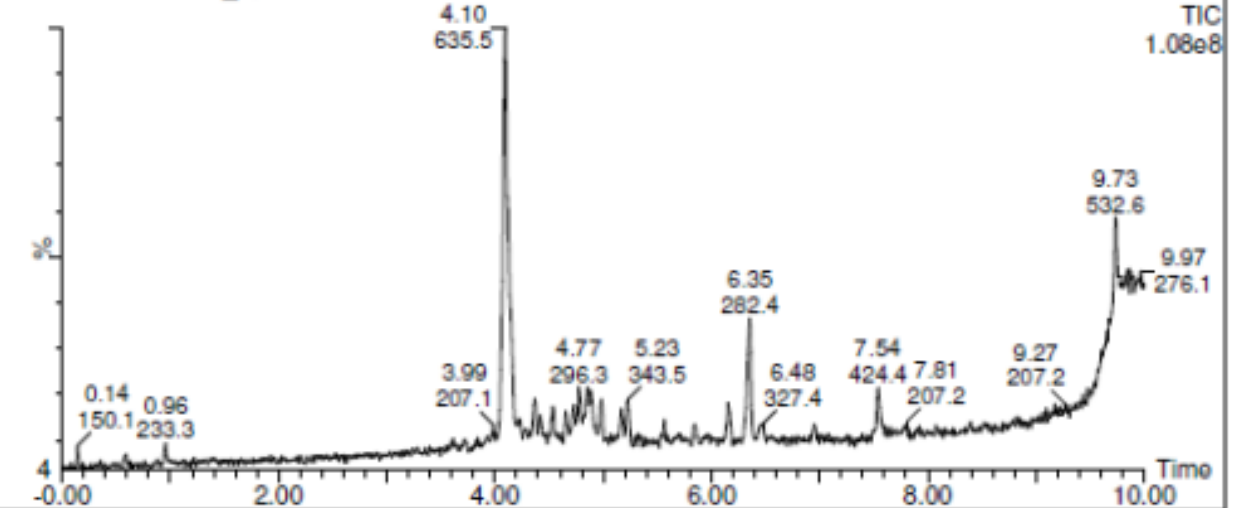
Range: 9.194e-1  
Height

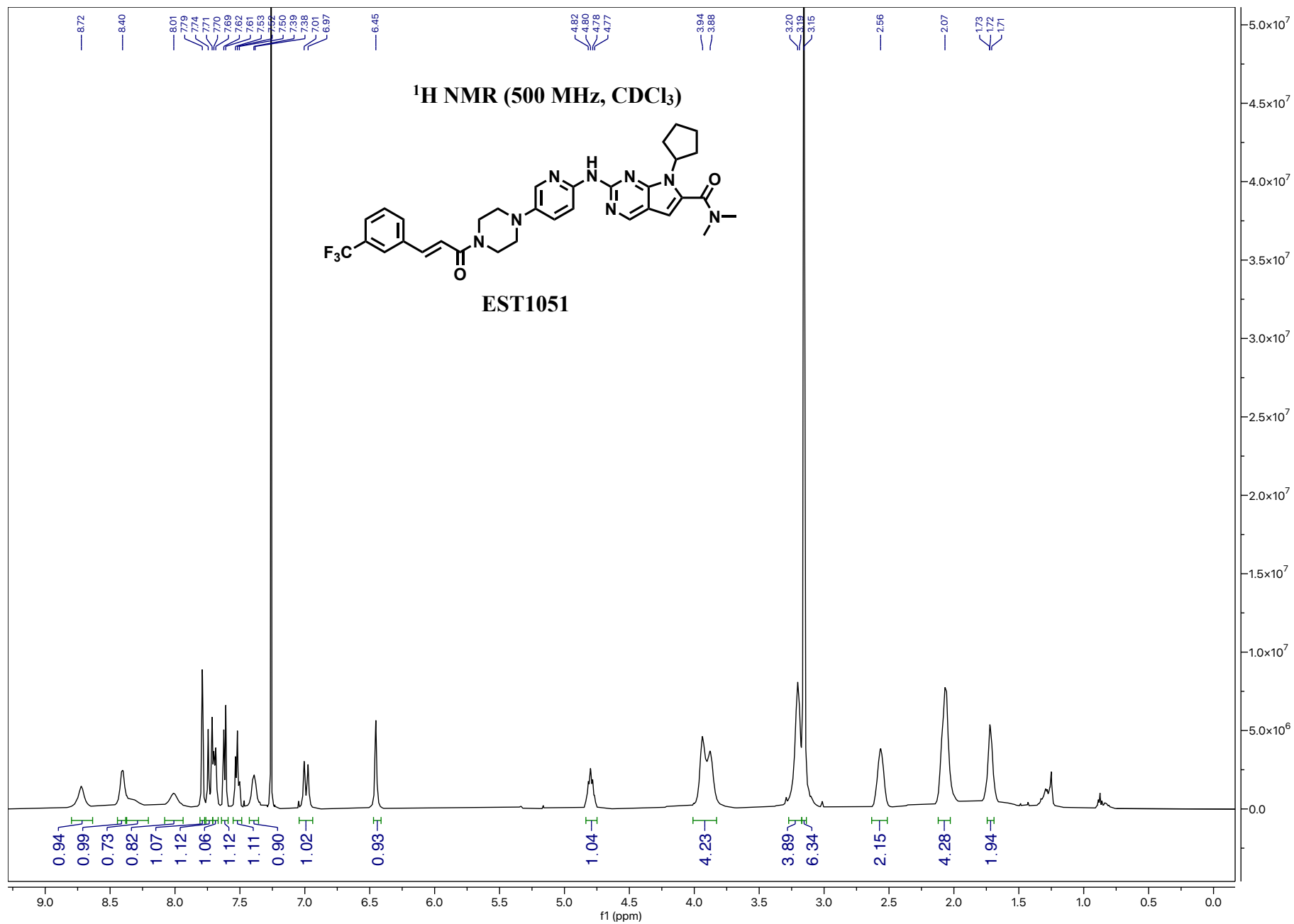
Time	Height	Area	Area%
4.07	756628	31192.64	100.00

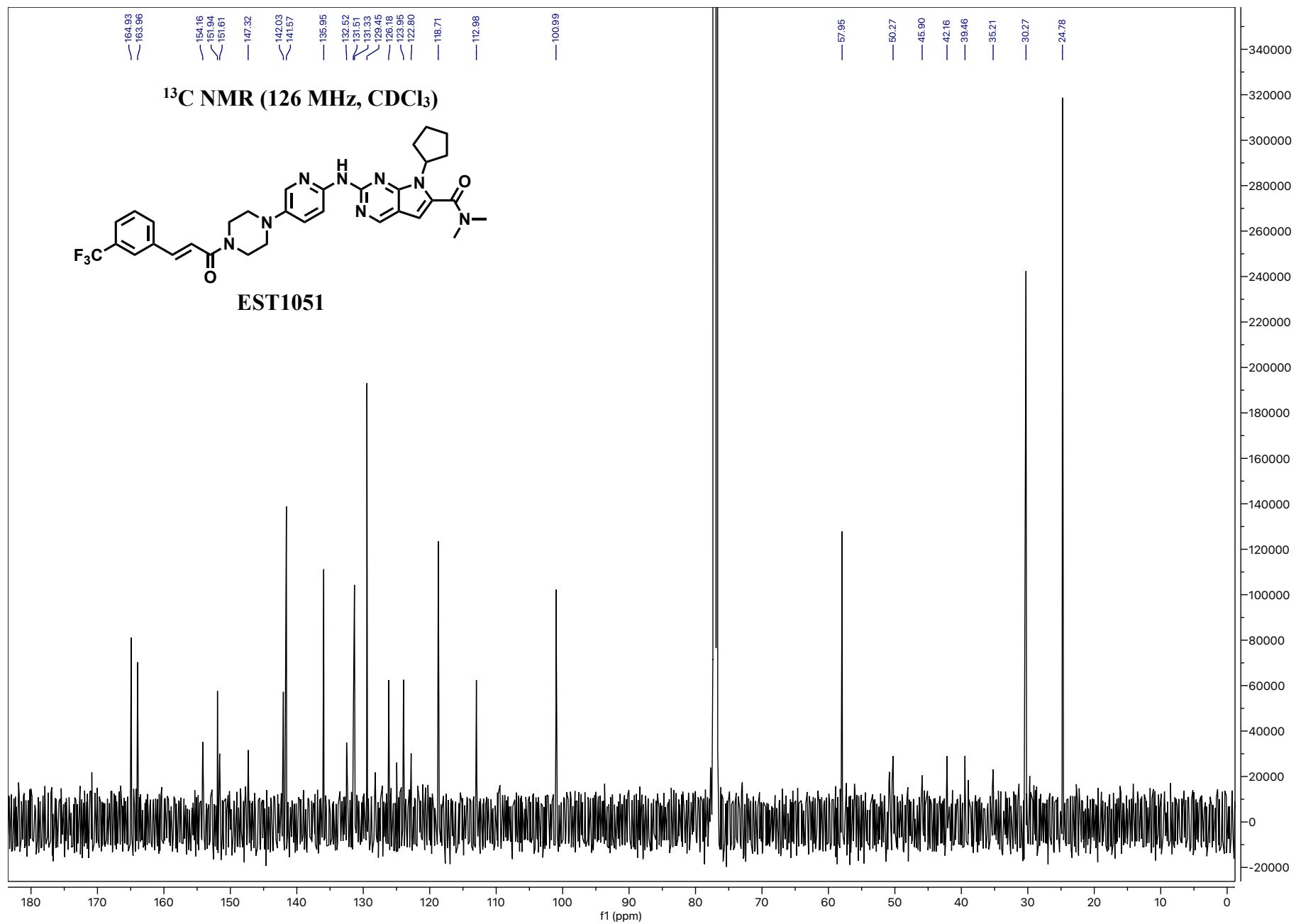


HESSEMA4-101-EXP227-S01\_OC

1: Scan ES+  
TIC  
1.08e8







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

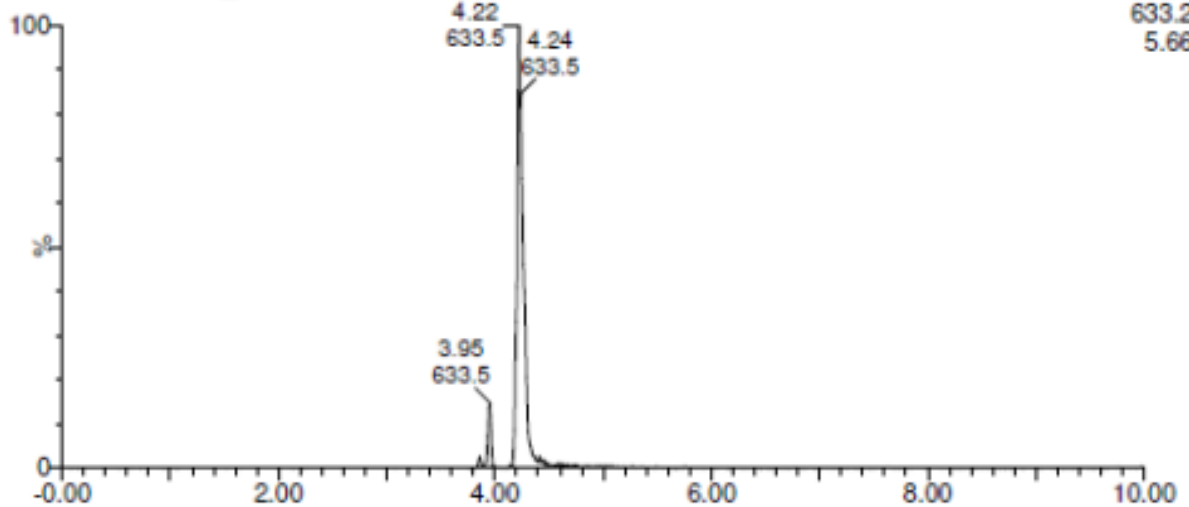
EM23001594434

08-Feb-2023  
15:59:50  
strubhe1-0208A-v13

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acid

1: Scan ES+  
633.284  
5.66e7

HESSEMA4-101-EXP228-S01\_OC



HESSEMA4-101-EXP228-S01\_OC

2: Diode Array

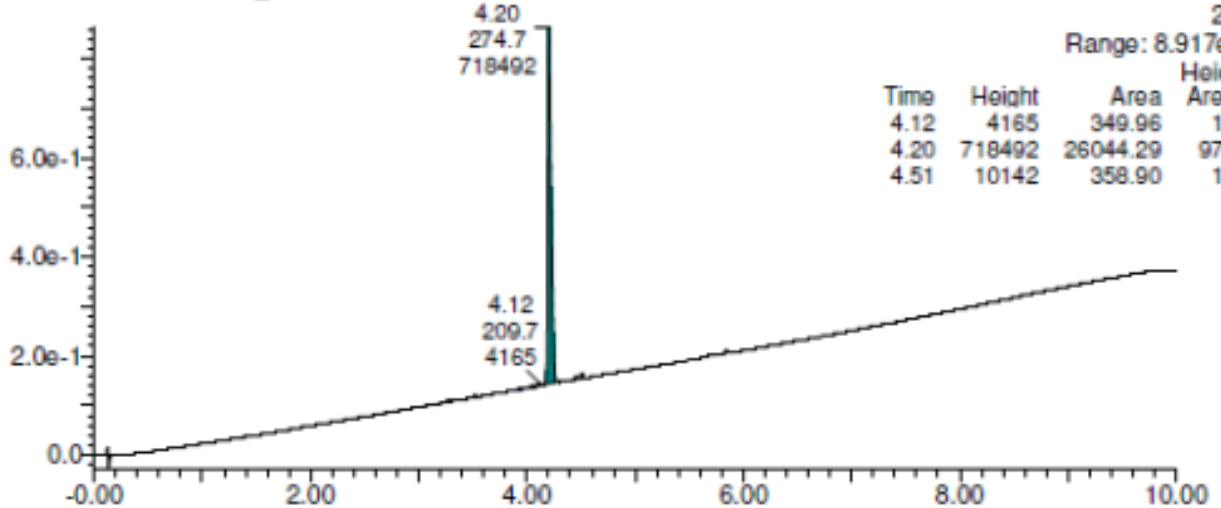
220

Range: 8.917e-1

Height

Time	Height	Area	Area%
4.12	4165	349.96	1.31
4.20	718492	26044.29	97.35
4.51	10142	358.90	1.34

AU

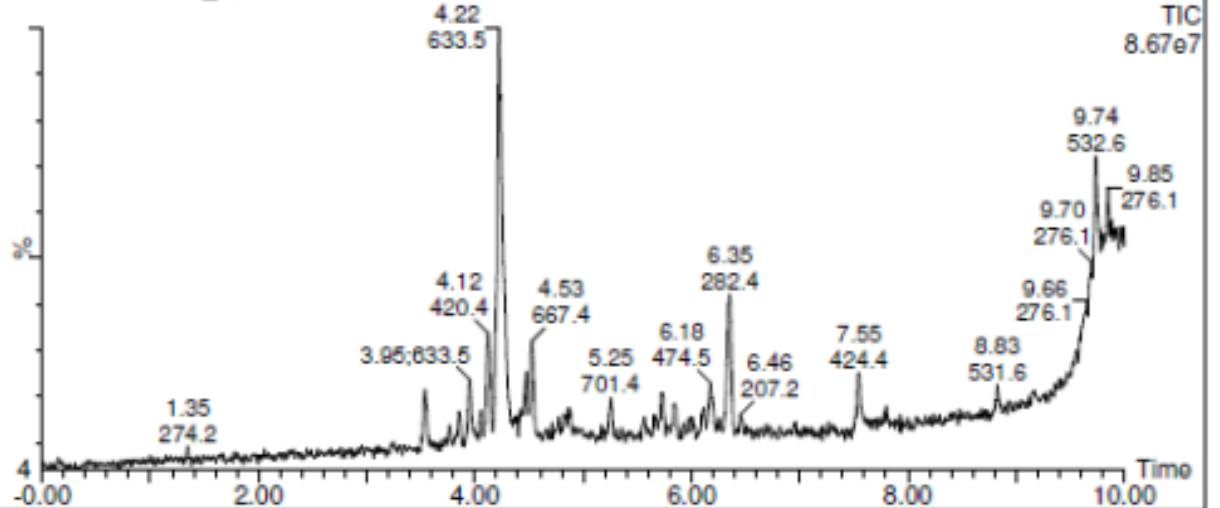


HESSEMA4-101-EXP228-S01\_OC

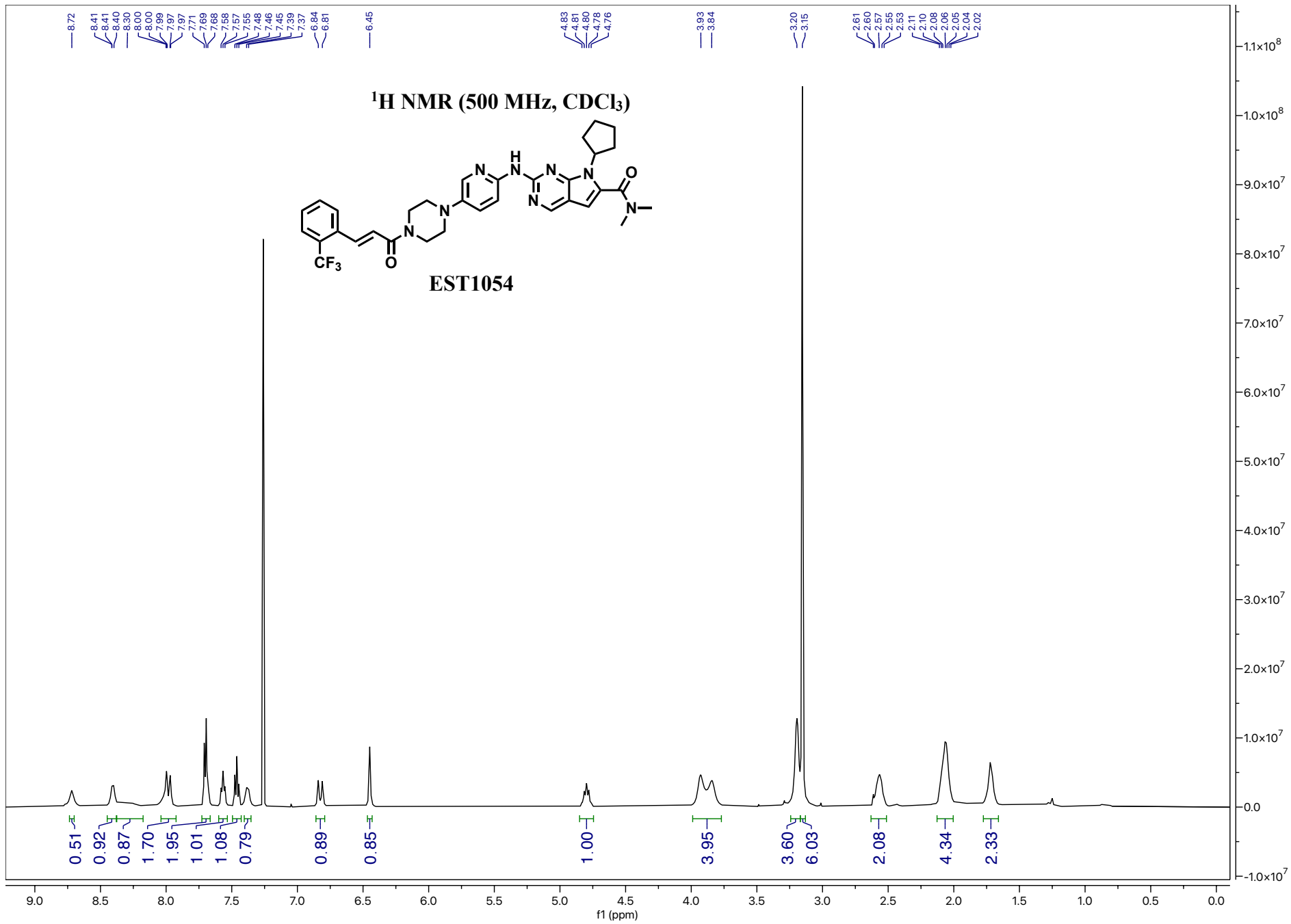
1: Scan ES+

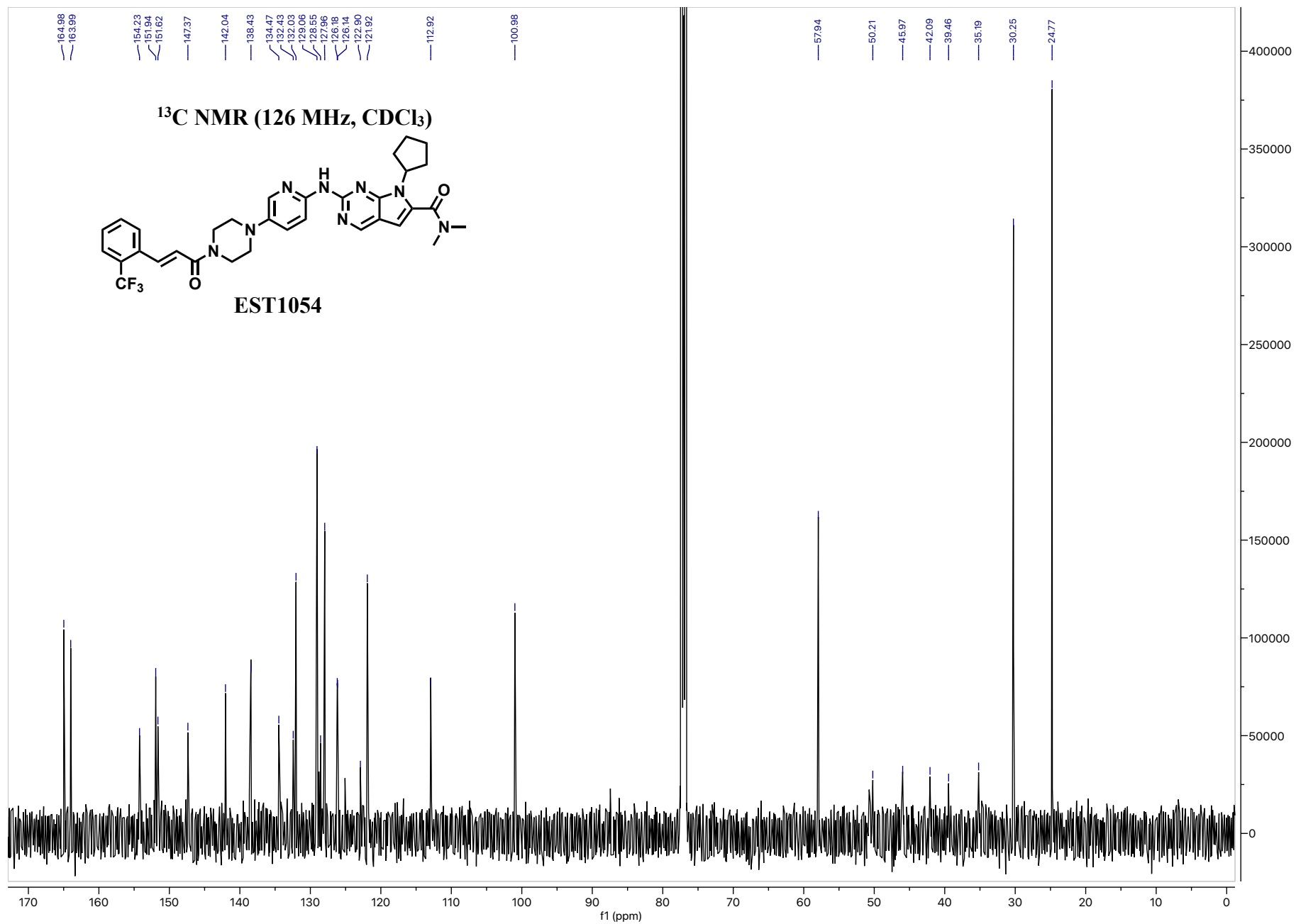
TIC

8.67e7









HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

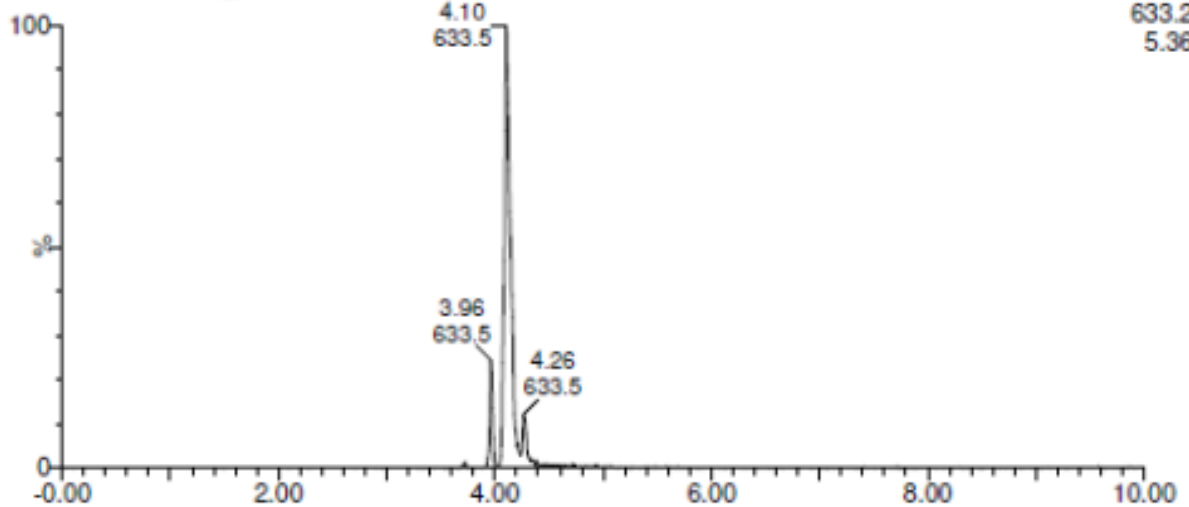
EM23001594435

08-Feb-2023  
16:10:53  
strubhe1-0208A-v14

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
633.284  
5.36e7

HESSEMA4-101-EXP229-S01\_OC



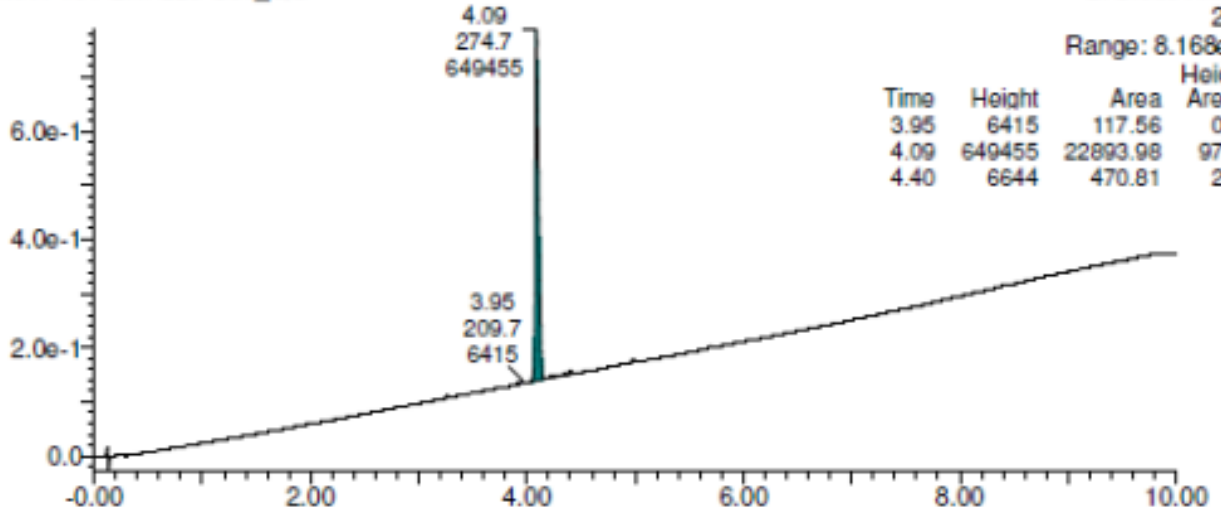
HESSEMA4-101-EXP229-S01\_OC

2: Diode Array  
220

Range: 8.168e-1  
Height

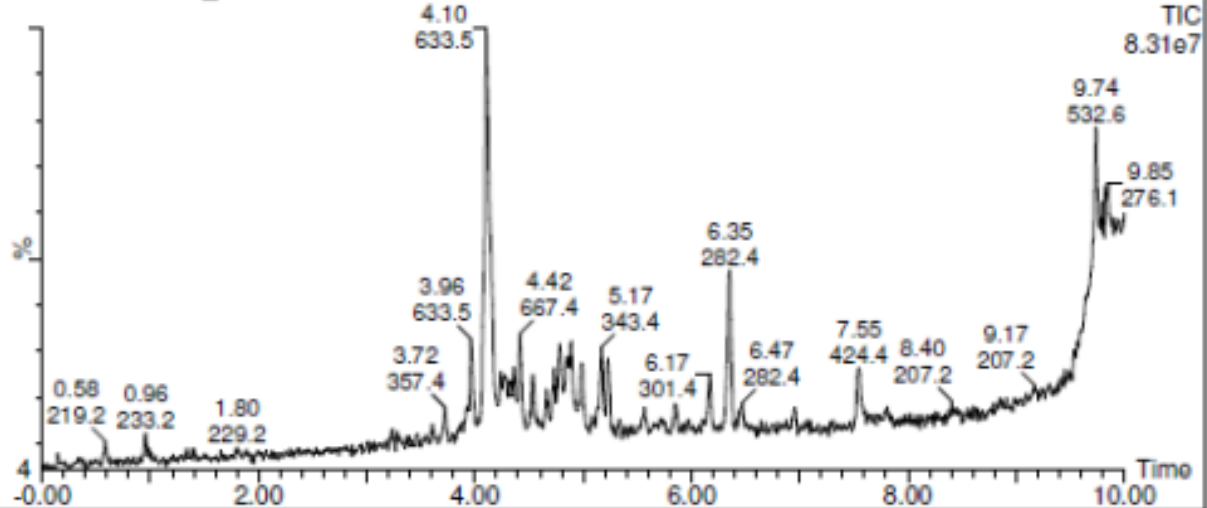
Time	Height	Area	Area%
3.95	6415	117.56	0.50
4.09	649455	22893.98	97.49
4.40	6644	470.81	2.00

AU

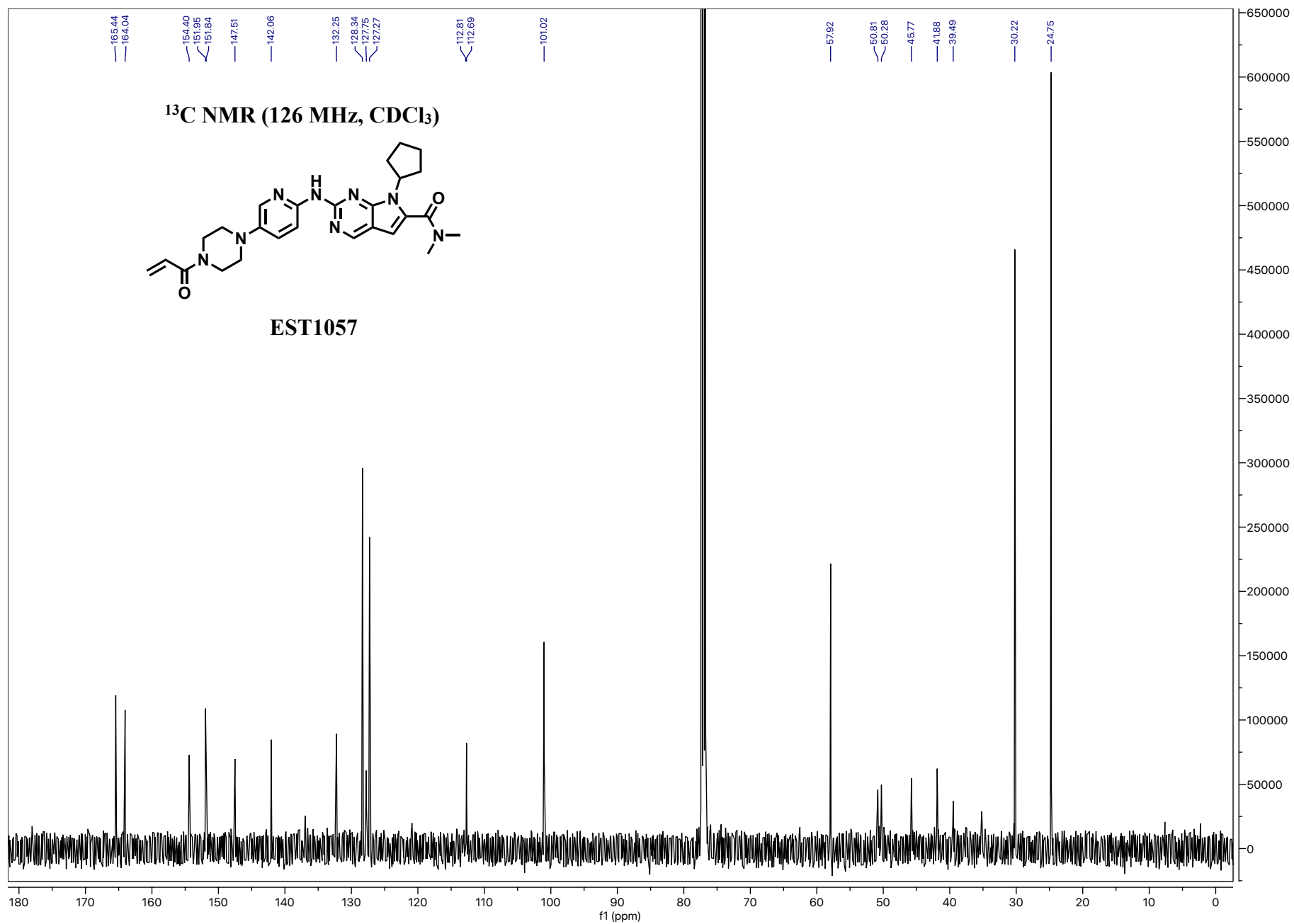


HESSEMA4-101-EXP229-S01\_OC

1: Scan ES+  
TIC  
8.31e7







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

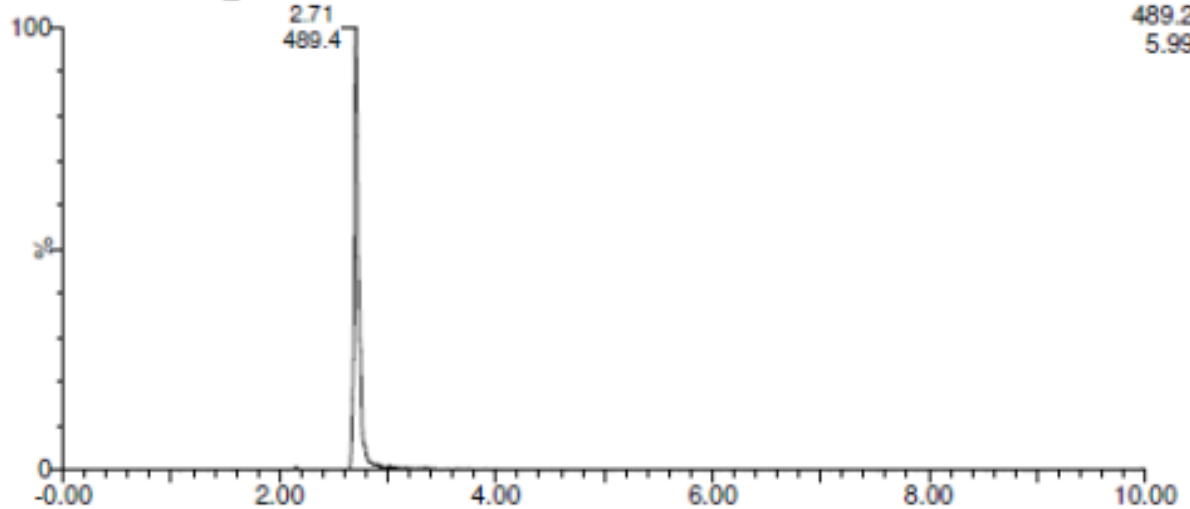
EM23001594436

08-Feb-2023  
16:21:57  
strubhe1-0208A-v15

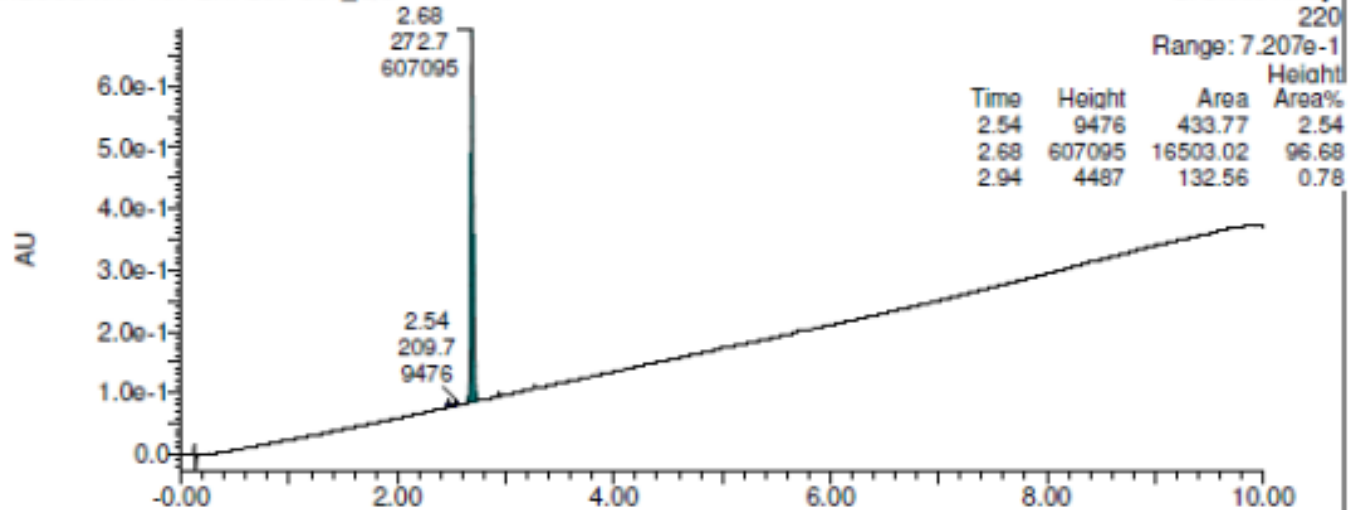
LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
489.265  
5.99e7

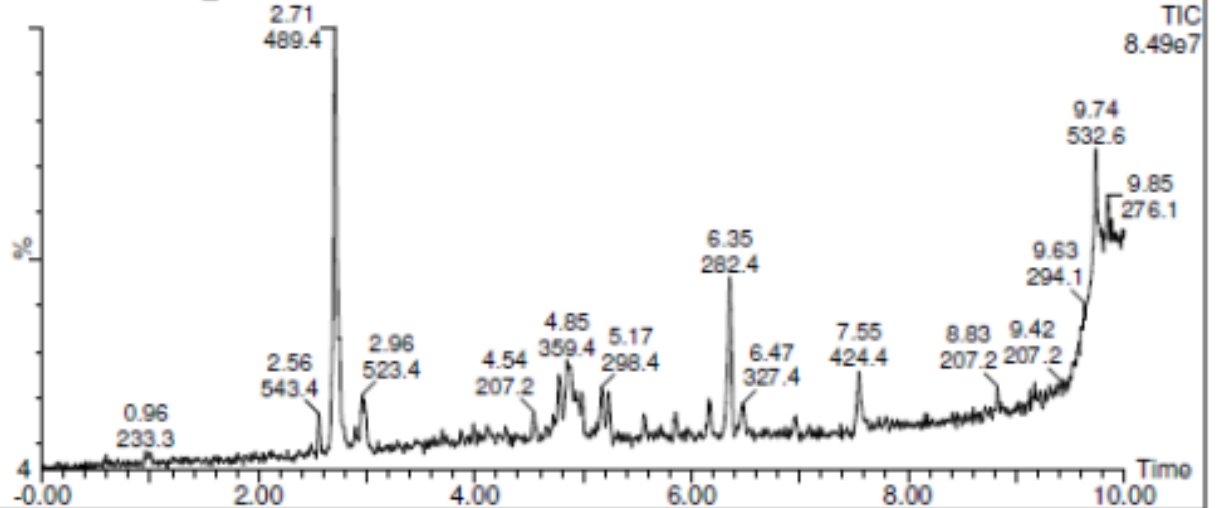
HESSEMA4-101-EXP230-S01\_OC

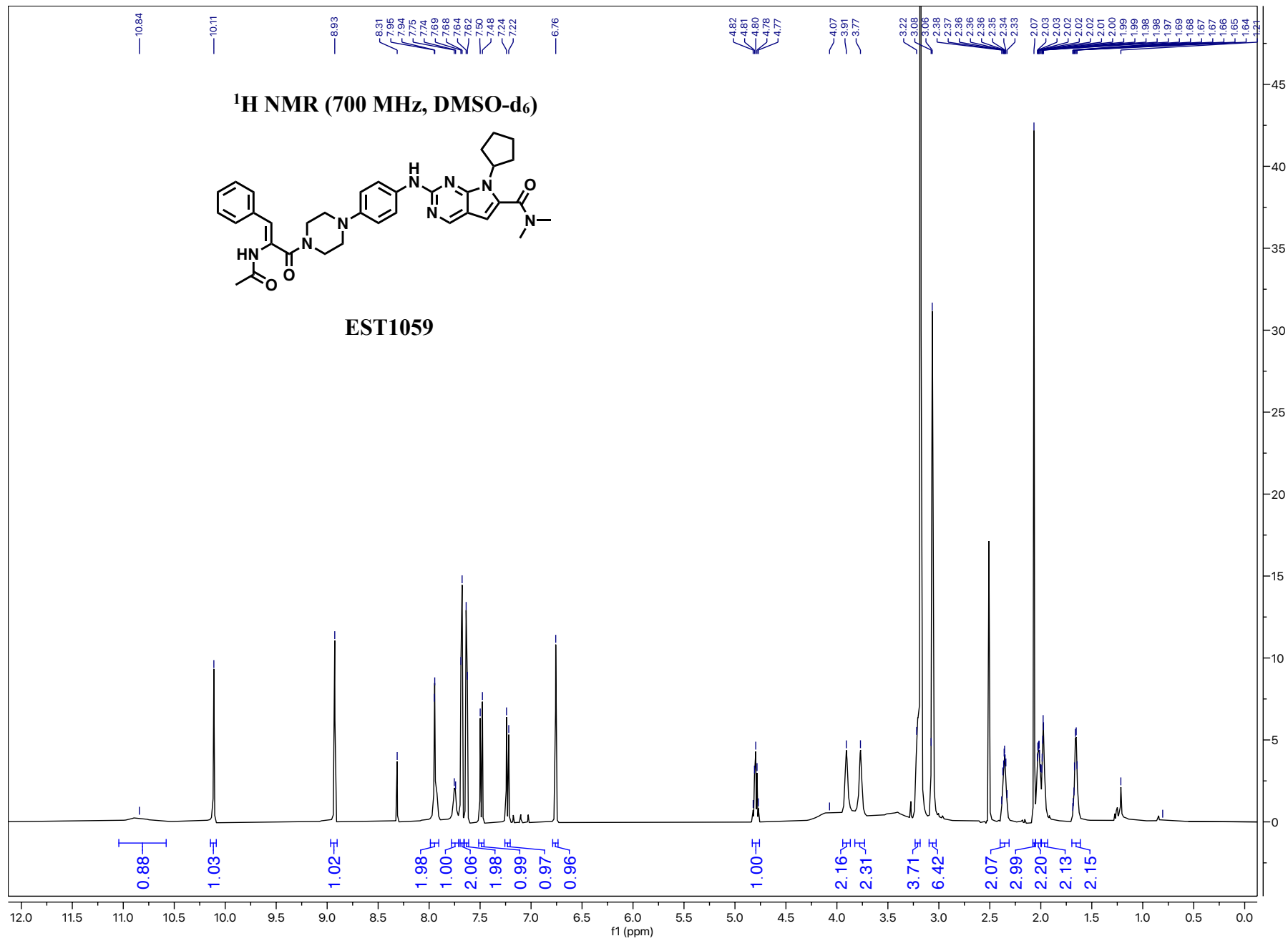


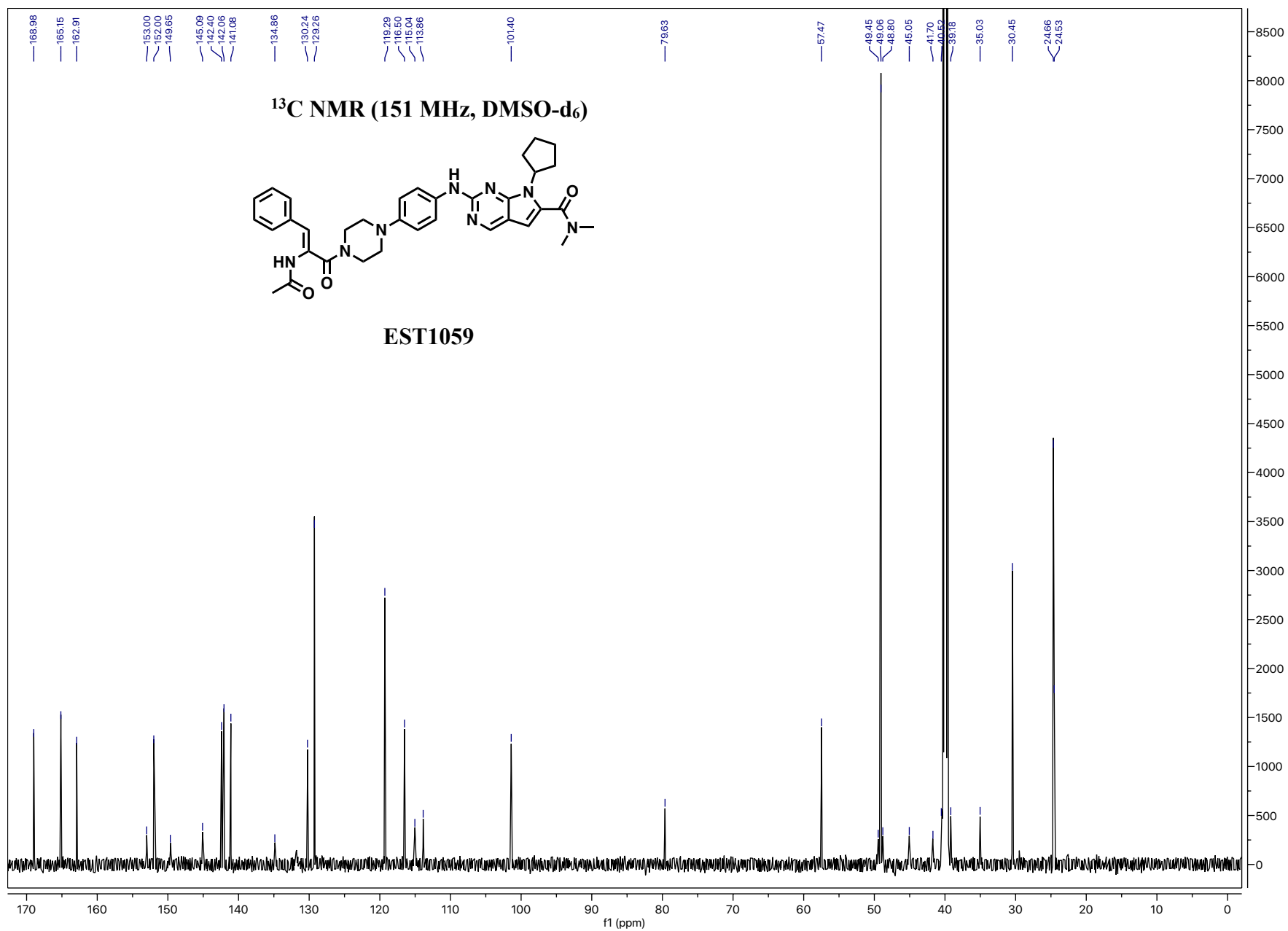
HESSEMA4-101-EXP230-S01\_OC



HESSEMA4-101-EXP230-S01\_OC









HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

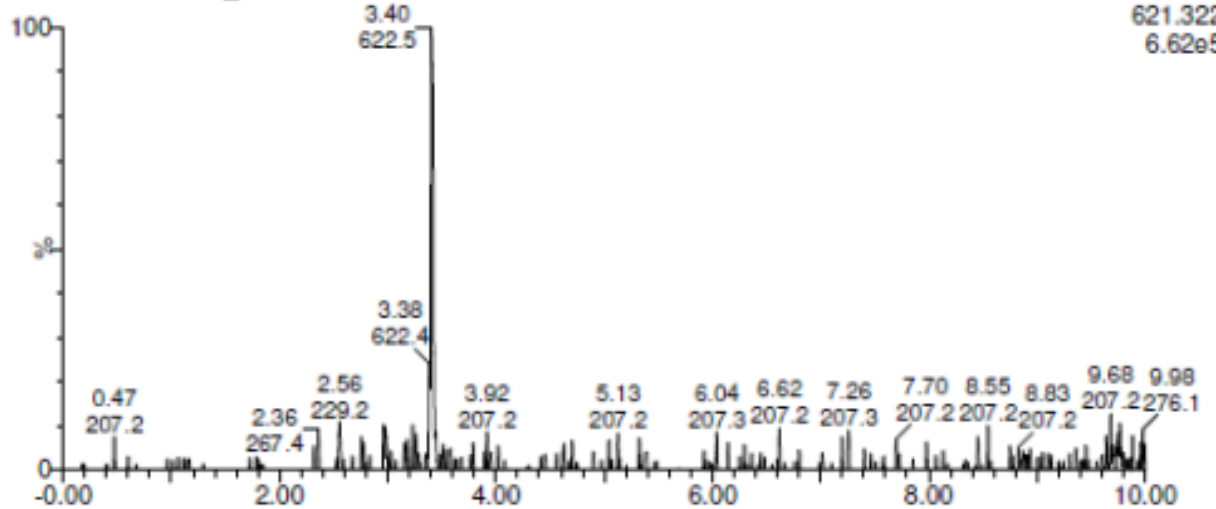
EM23001594437

08-Feb-2023  
16:33:02  
strubhe1-0208A-v16

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

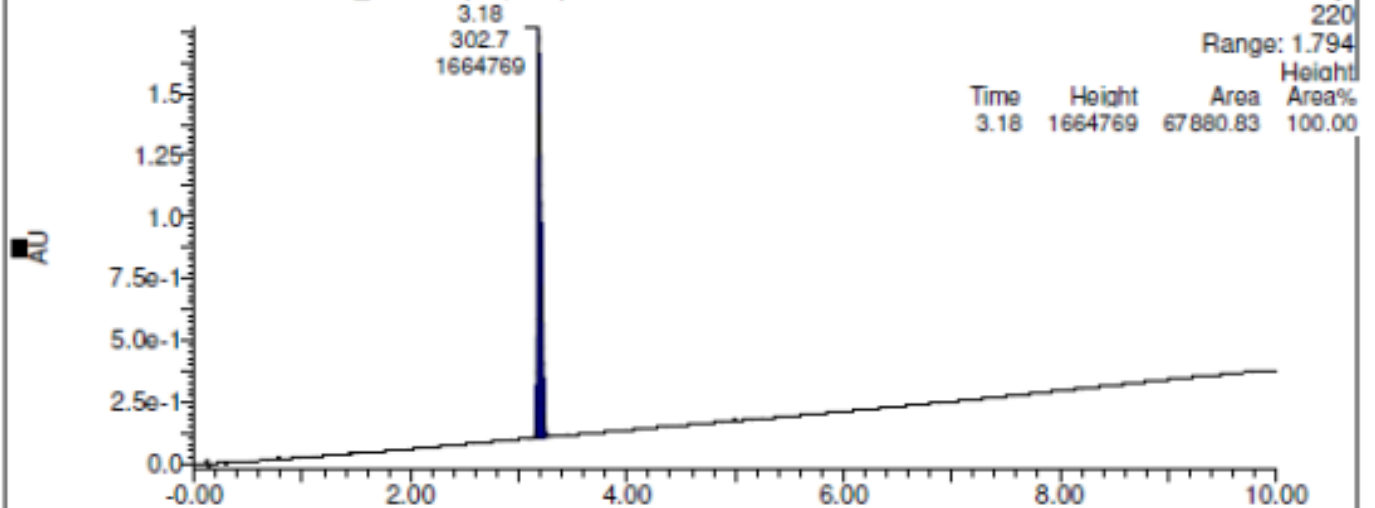
1: Scan ES+  
621.322  
6.62e5

HESSEMA4-101-EXP231-S01\_OC



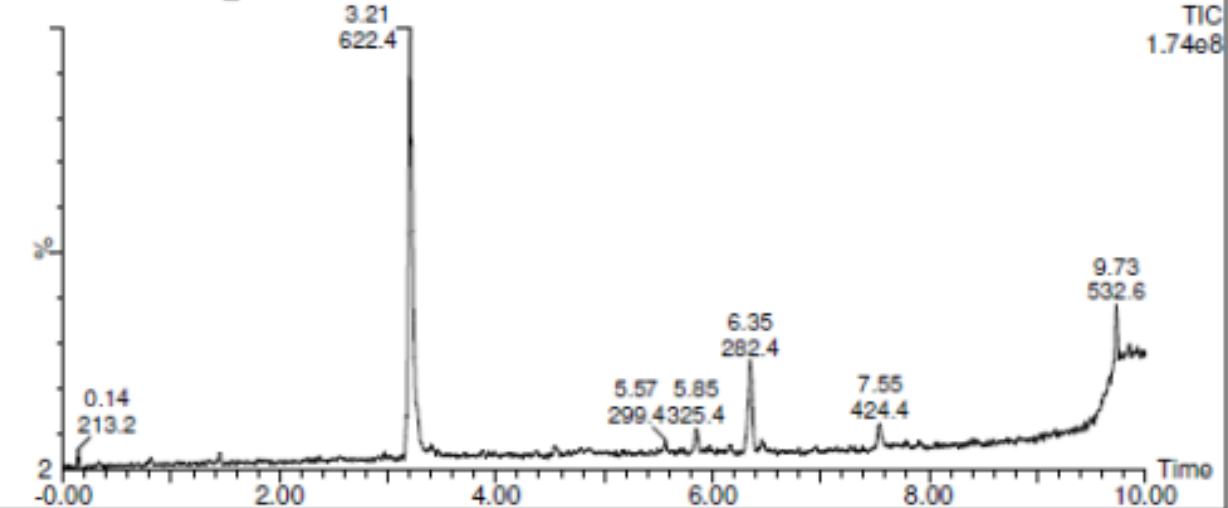
HESSEMA4-101-EXP231-S01\_OC Sm (Mn, 2x3)

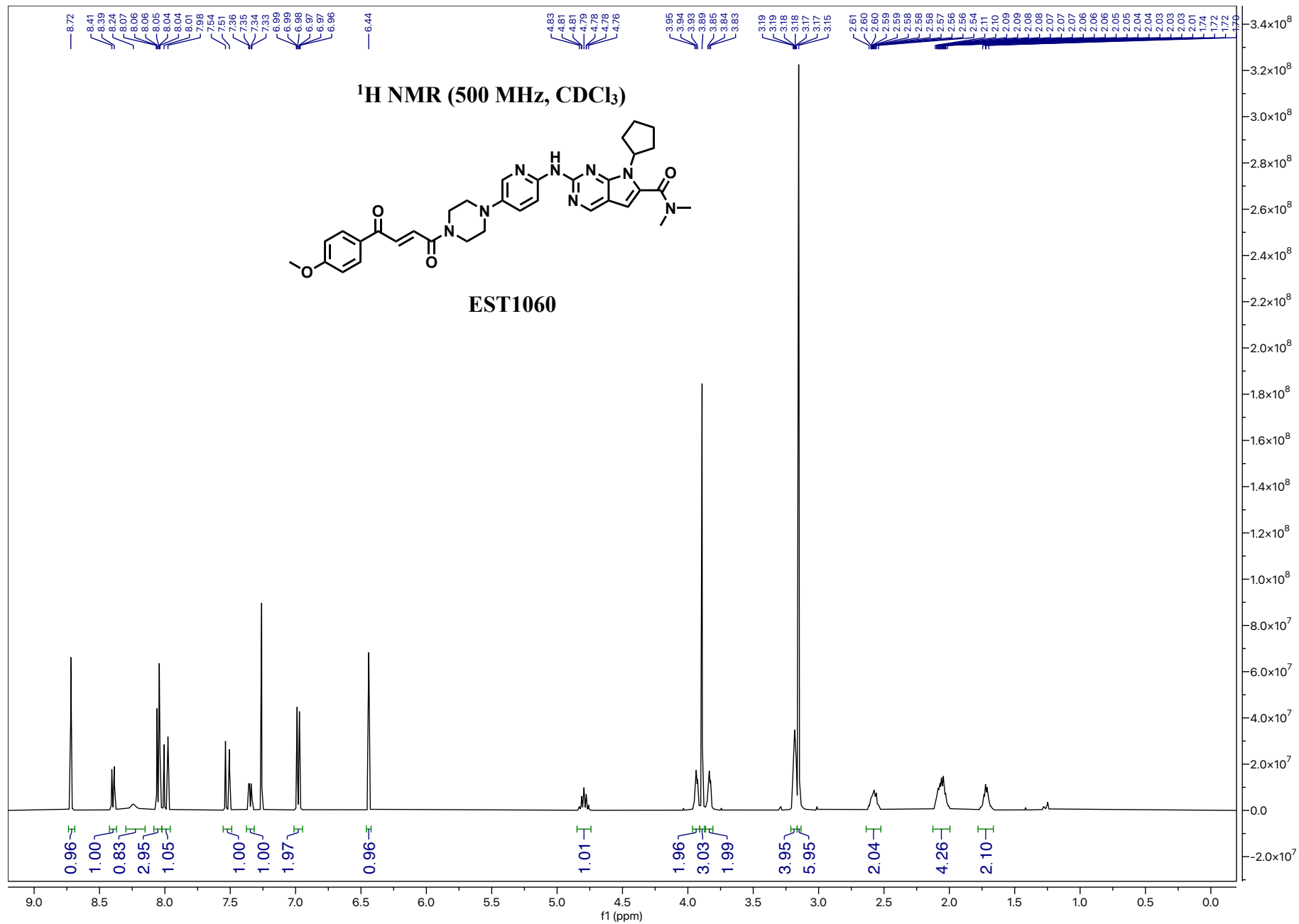
2: Diode Array  
220  
Range: 1.794  
Height

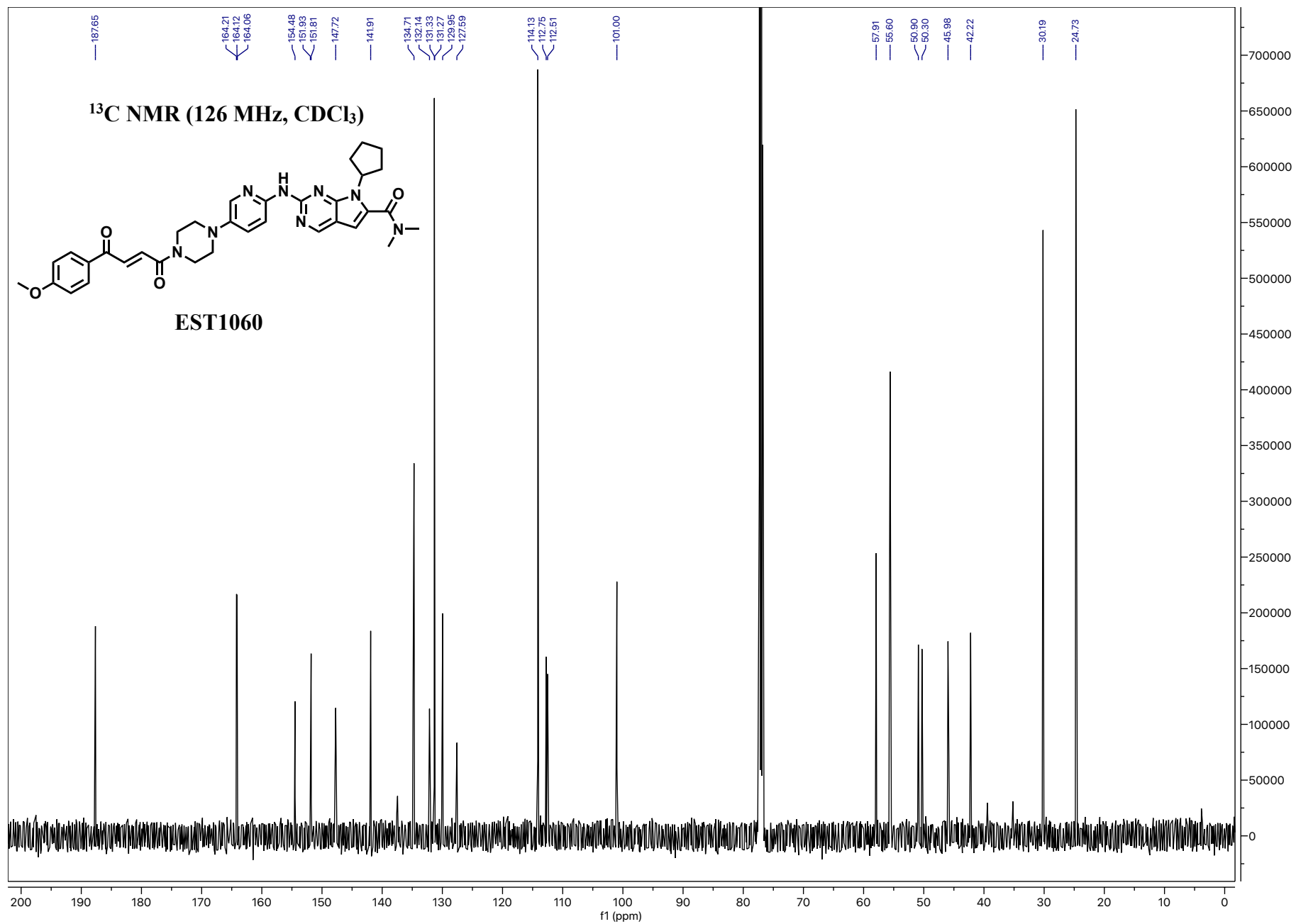


HESSEMA4-101-EXP231-S01\_OC

1: Scan ES+  
TIC  
1.74e8







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

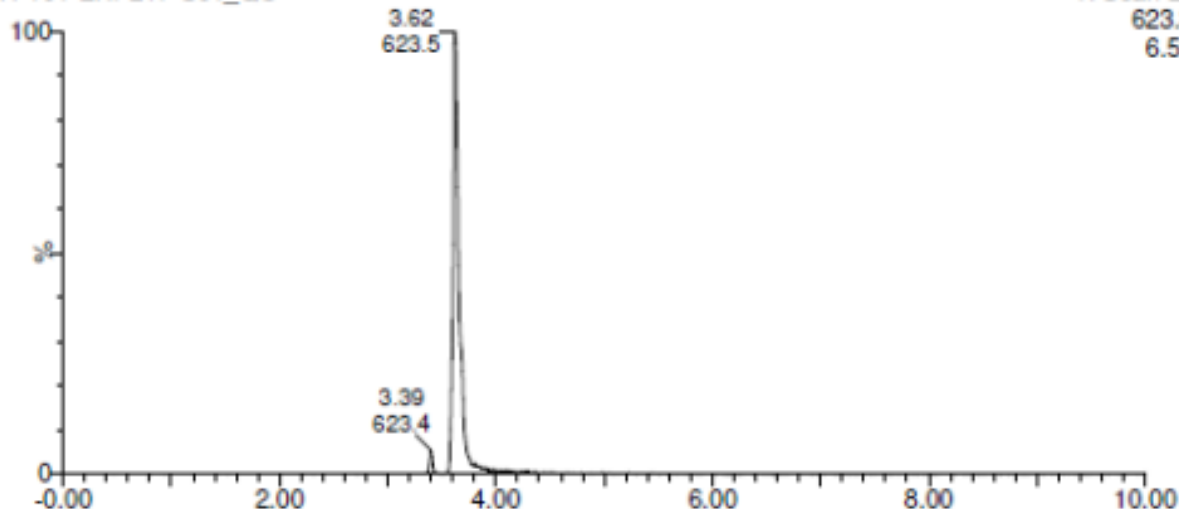
EM23001594421

08-Feb-2023  
13:58:19  
strubhe1-0208A-v02

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acid

1: Scan ES+  
623.302  
6.55e7

HESSEMA4-101-EXP217-S01\_OC

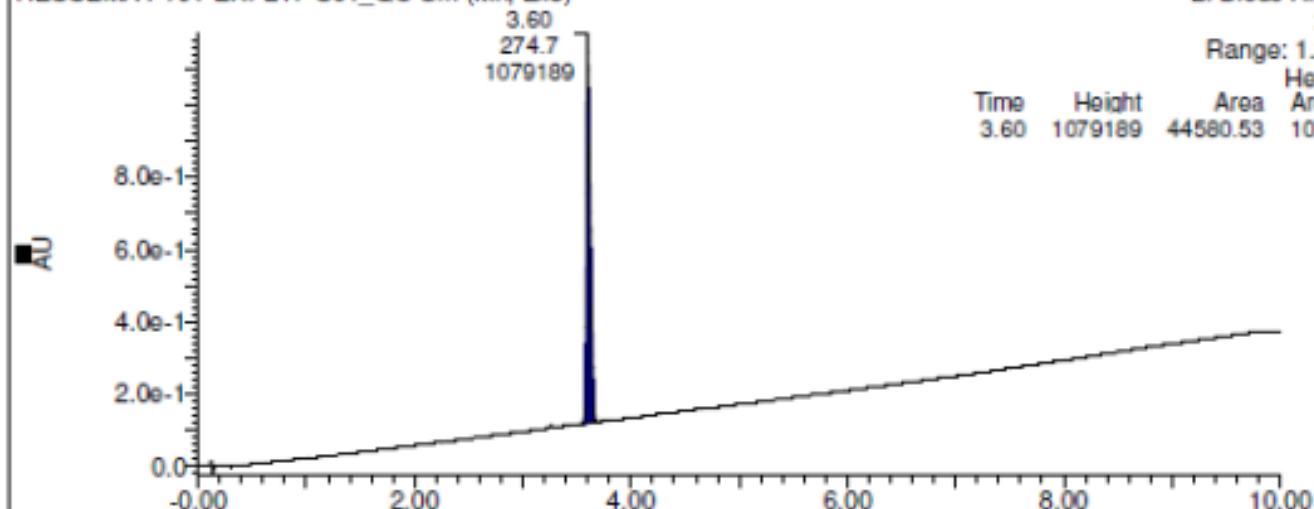


HESSEMA4-101-EXP217-S01\_OC Sm (Mn, 2x3)

2: Diode Array  
220

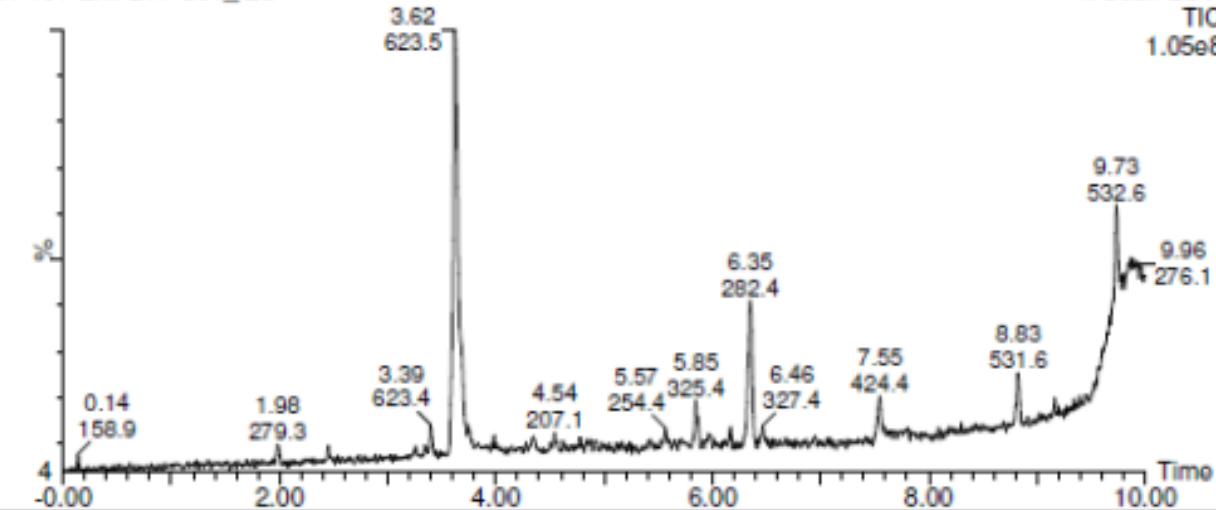
Range: 1.223  
Height

Time	Height	Area	Area%
3.60	1079189	44580.53	100.00



HESSEMA4-101-EXP217-S01\_OC

1: Scan ES+  
TIC  
1.05e8



HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

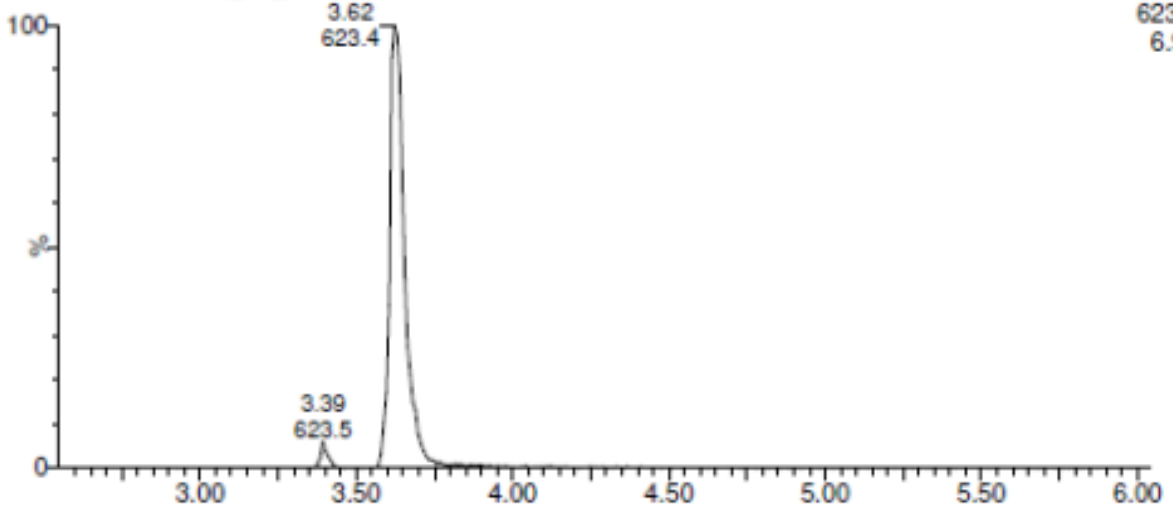
EM23001594421

14-Feb-2023  
13:06:02  
strubhe1-0208A-v02

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
623.302  
6.93e7

HESSEMA4-101-EXP217-S01\_6d\_QCr



HESSEMA4-101-EXP217-S01\_6d\_QCr Sm (Mn, 2x3)

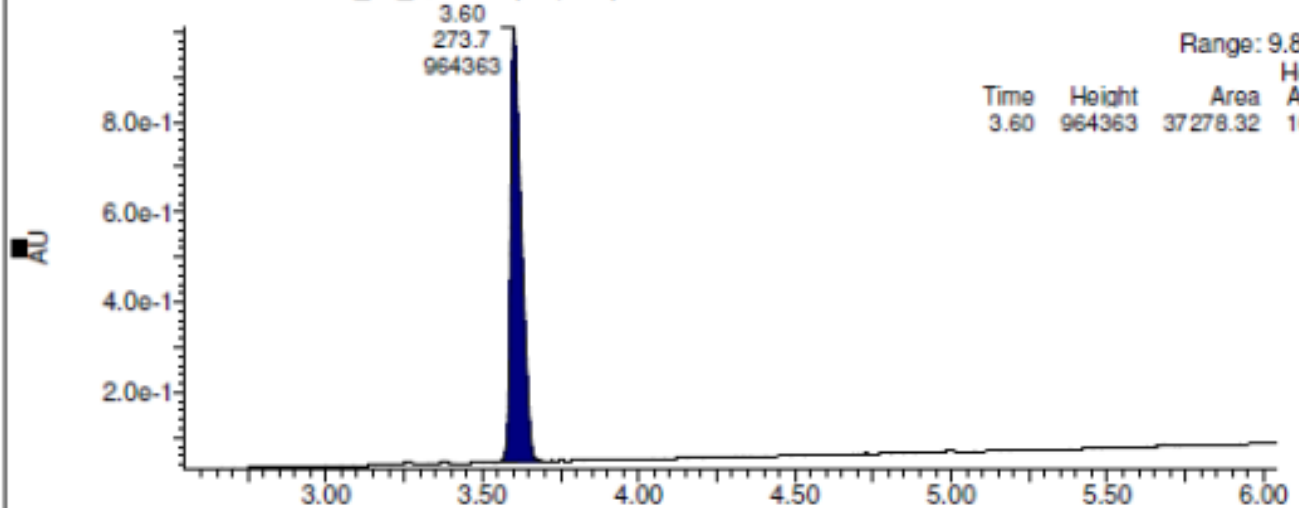
2: Diode Array

220

Range: 9.81e-1

Height

Time	Height	Area	Area%
3.60	964363	37278.32	100.00

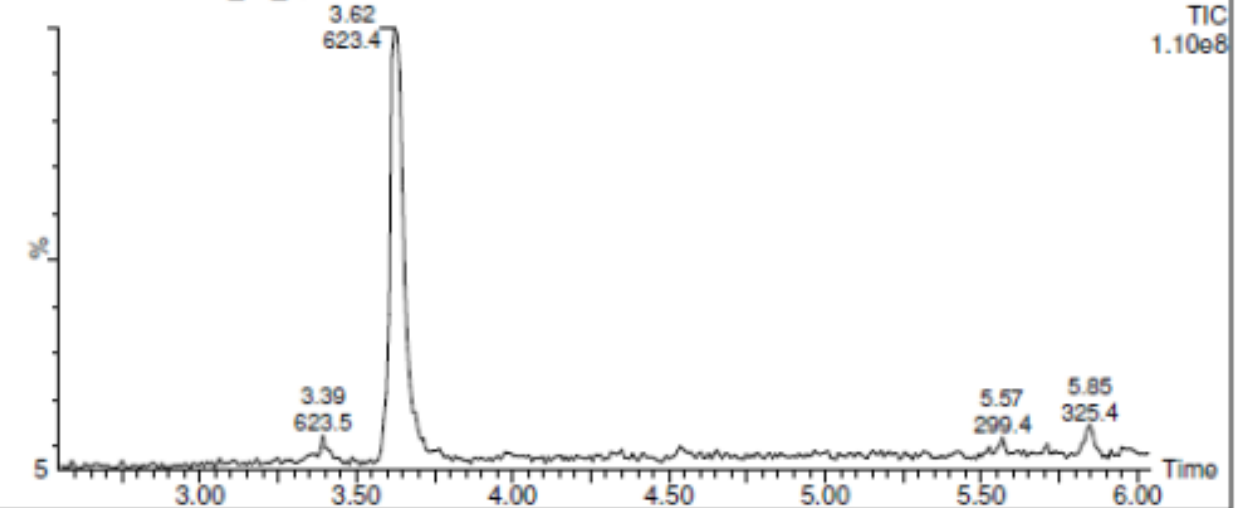


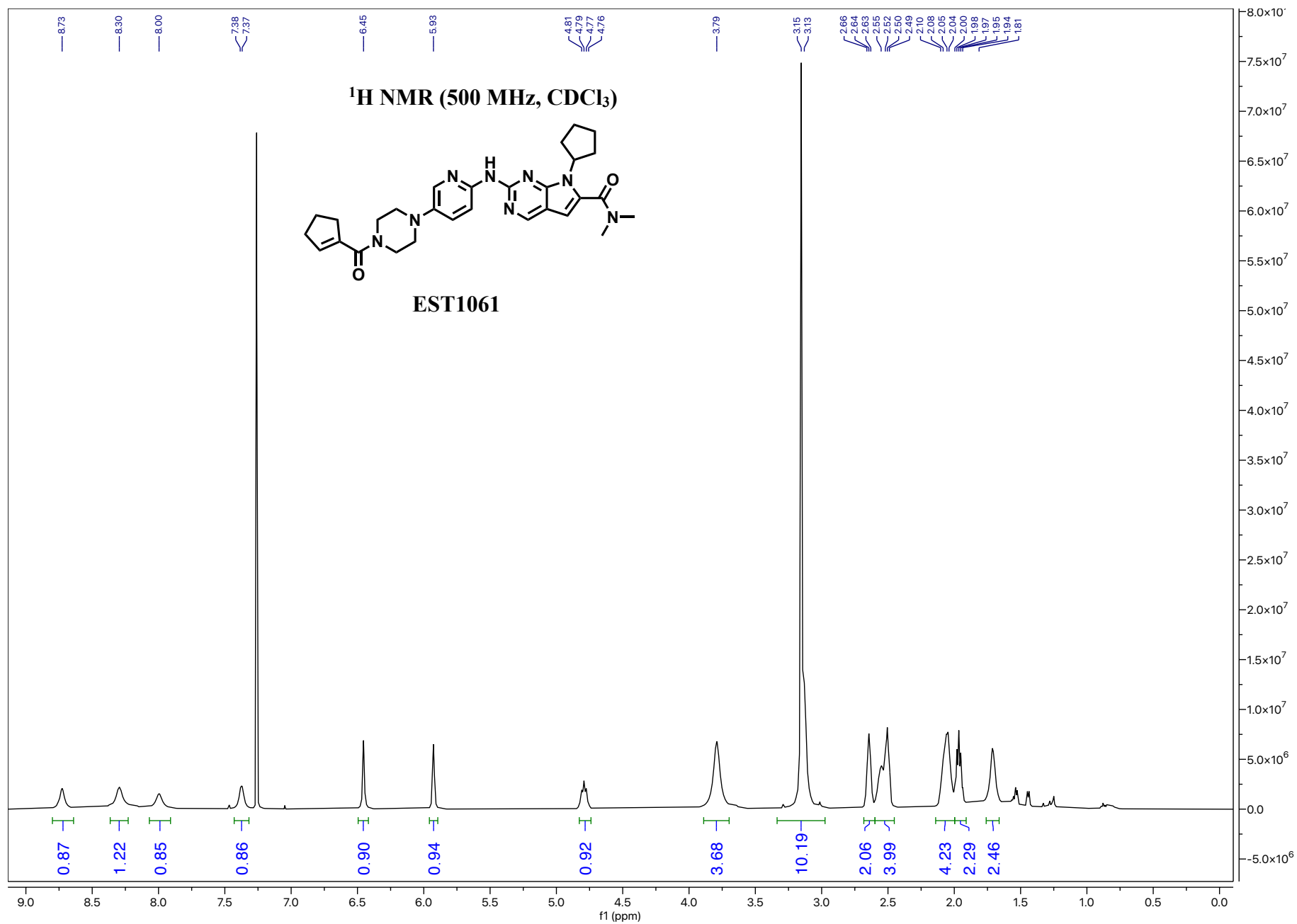
HESSEMA4-101-EXP217-S01\_6d\_QCr

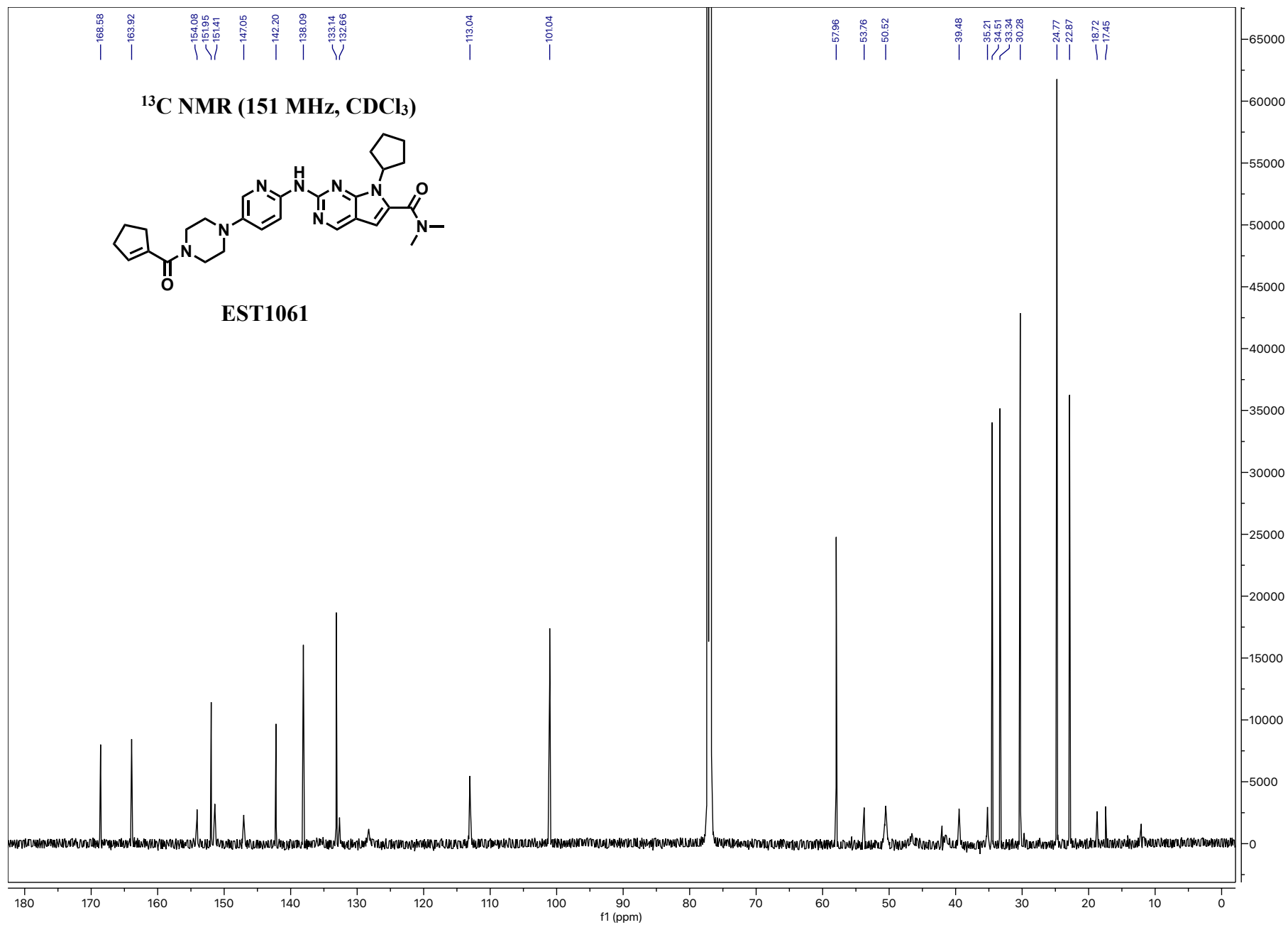
1: Scan ES+

TIC

1.10e8







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

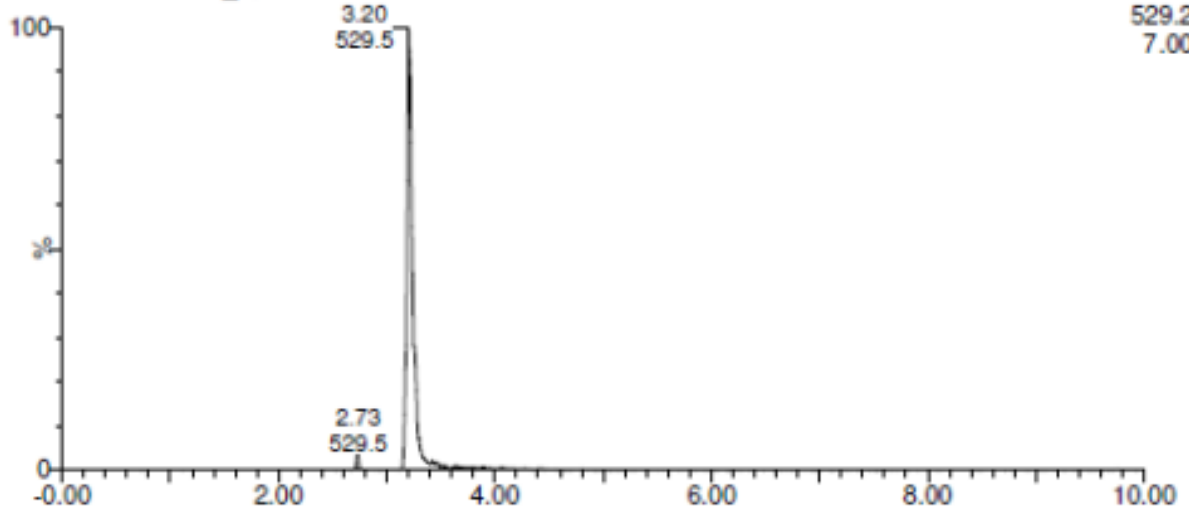
EM23001594438

08-Feb-2023  
16:44:08  
strubhe1-0208A-v17

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
529.296  
7.00e7

HESSEMA4-101-EXP232-S01\_OC



HESSEMA4-101-EXP232-S01\_OC

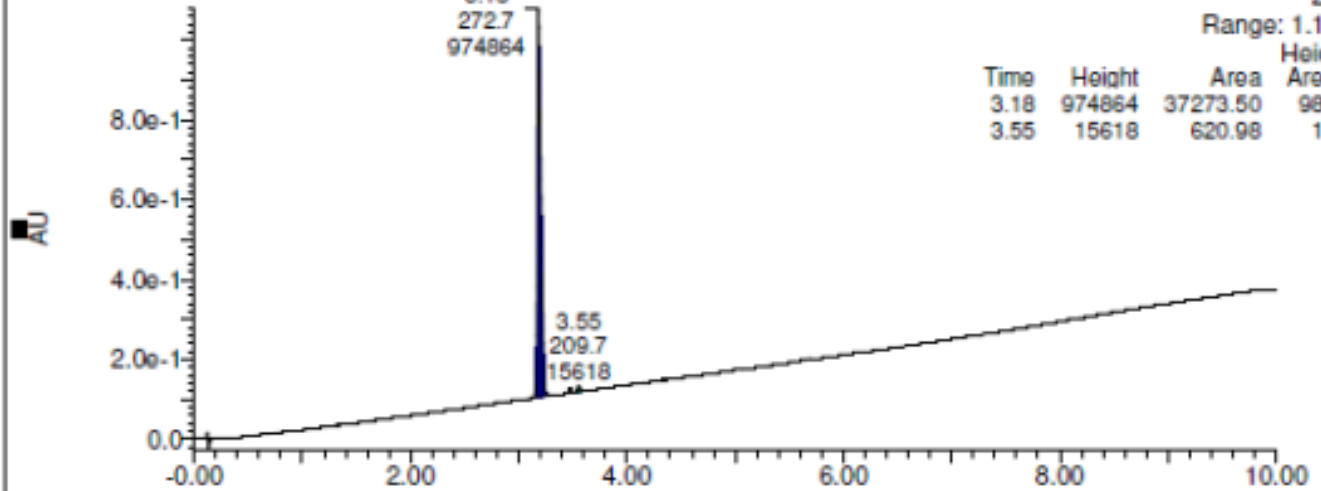
2: Diode Array

220

Range: 1.109

Height

Time	Height	Area	Area%
3.18	974864	37273.50	98.36
3.55	15618	620.98	1.64

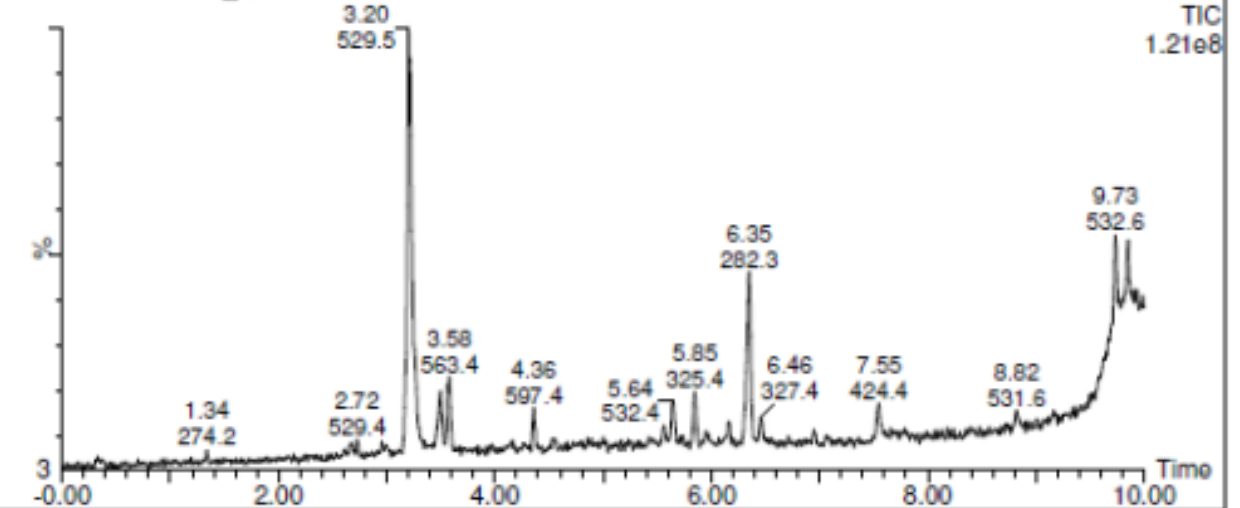


HESSEMA4-101-EXP232-S01\_OC

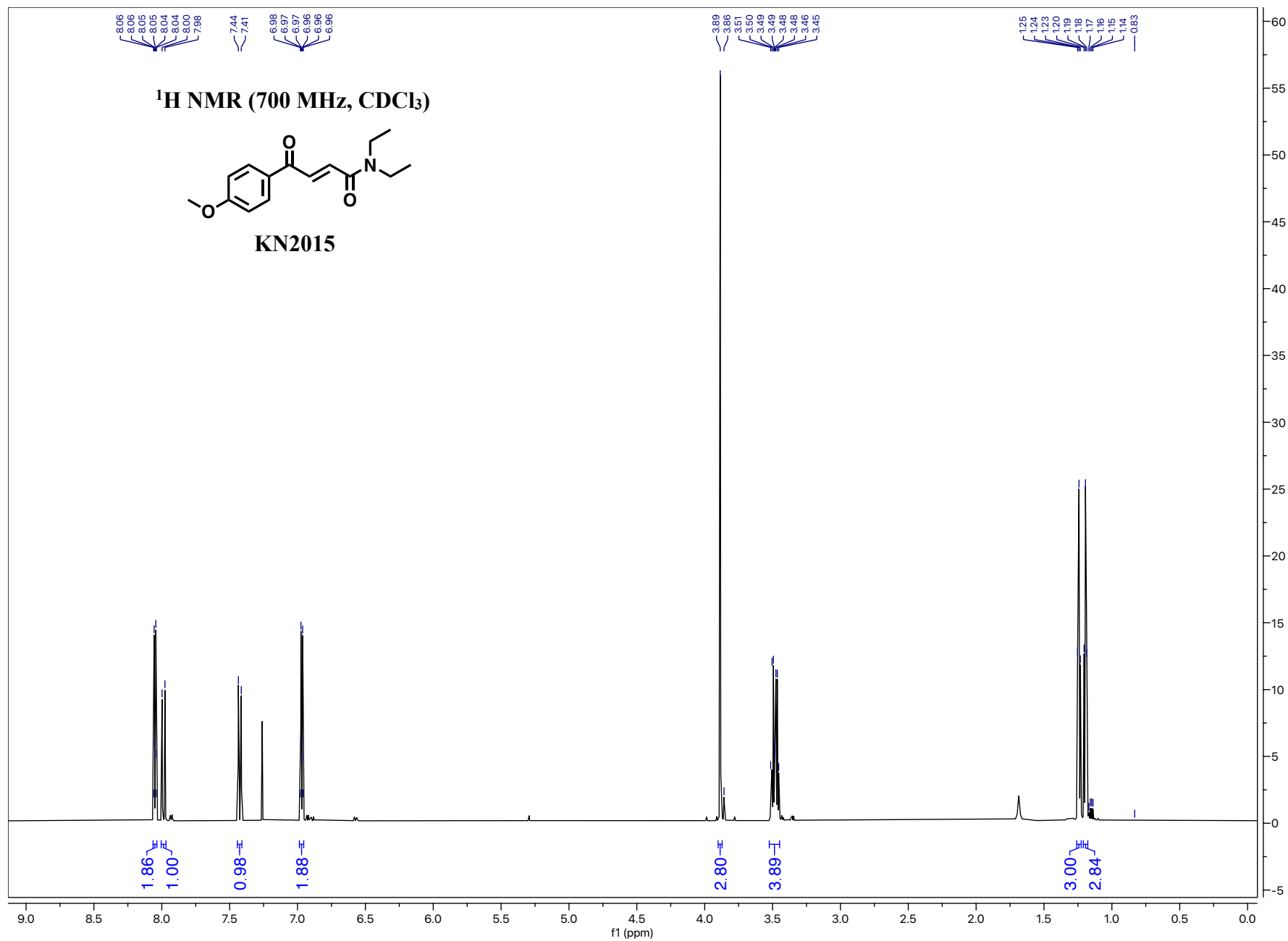
1: Scan ES+

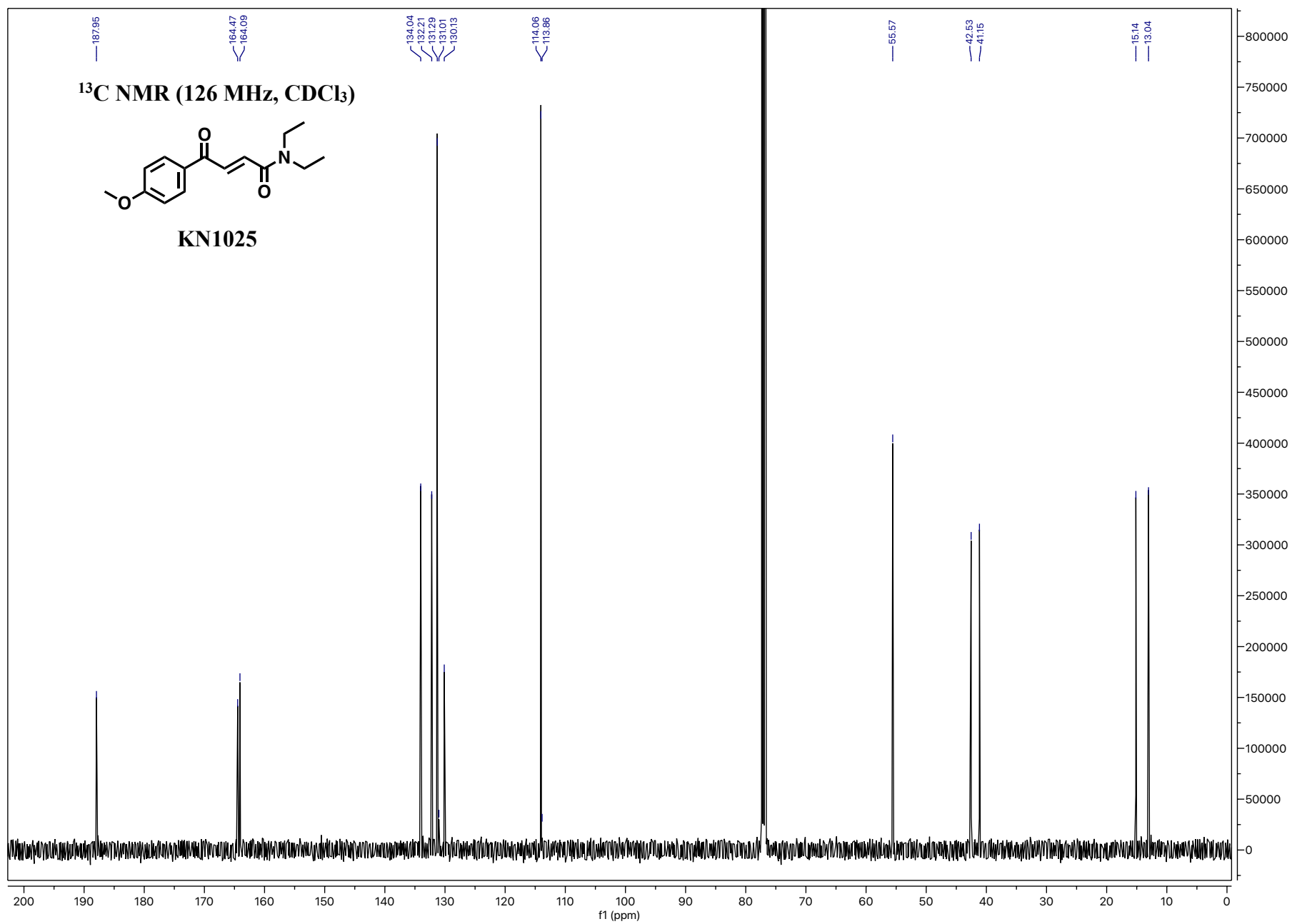
TIC

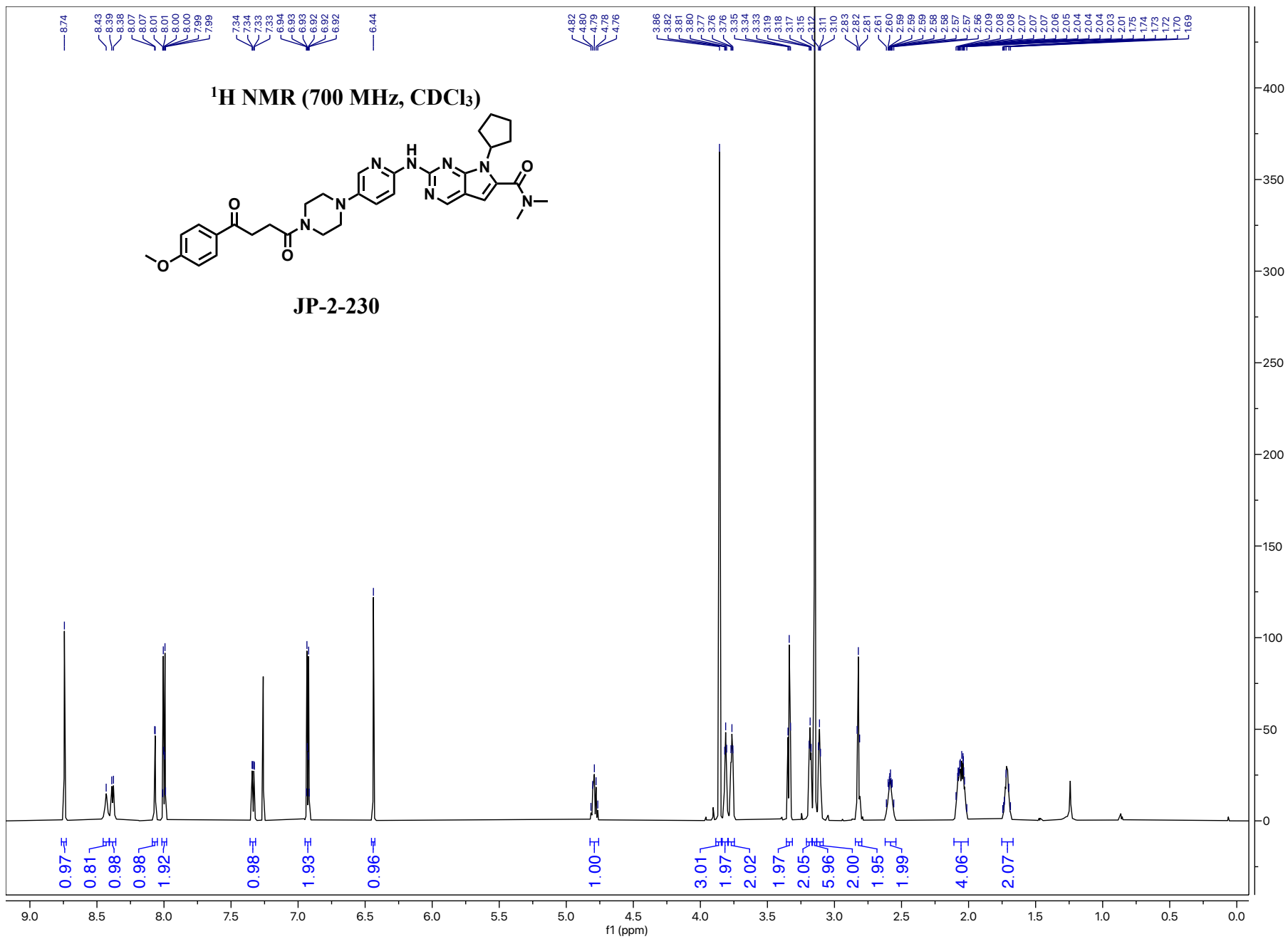
1.21e8

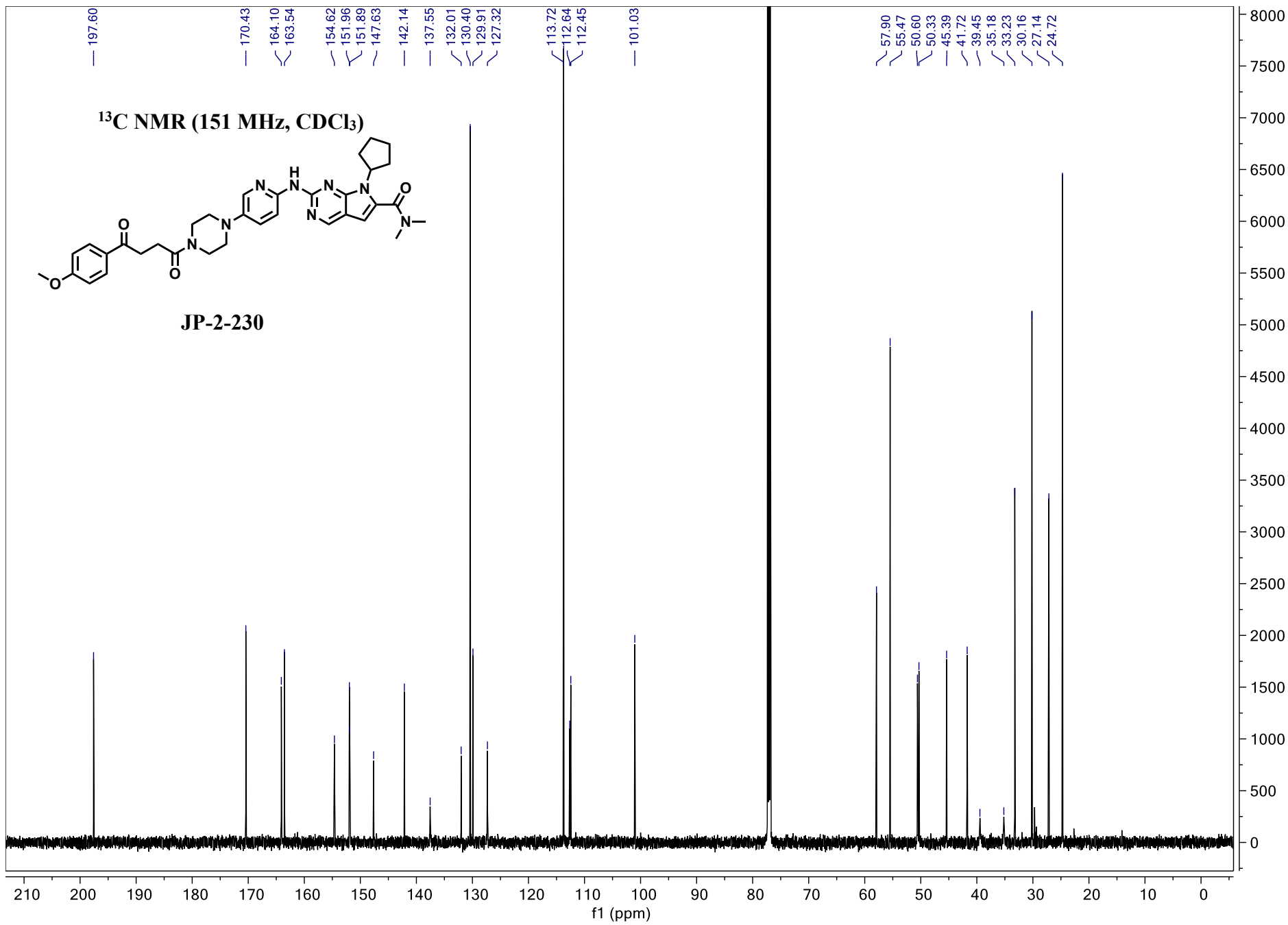












HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

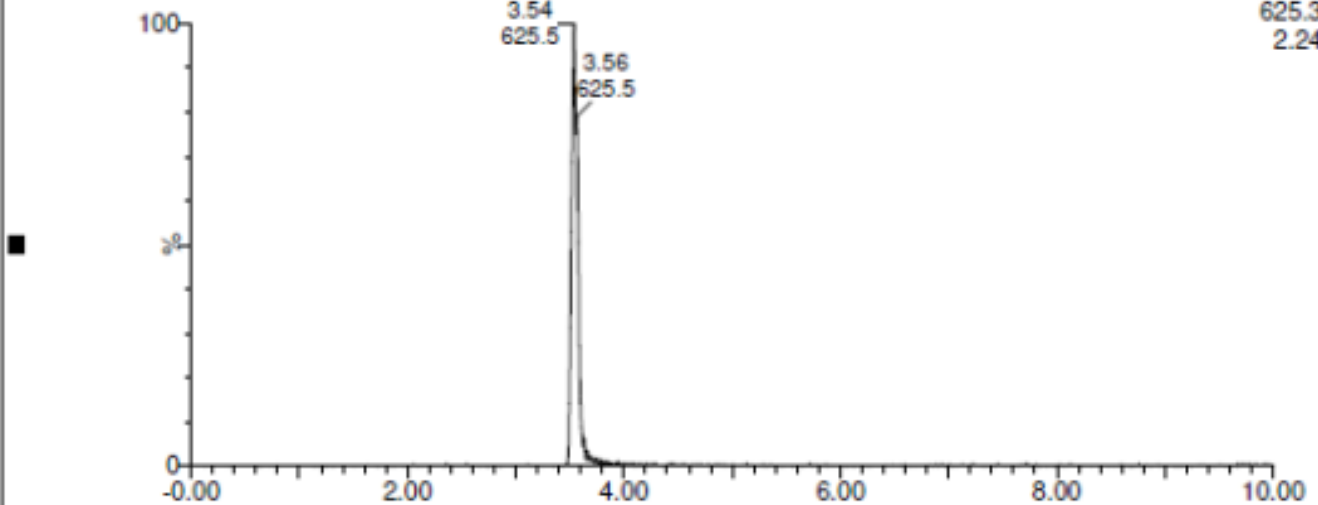
EM23001594439

08-Feb-2023  
16:55:09  
strubhe1-0208A-v18

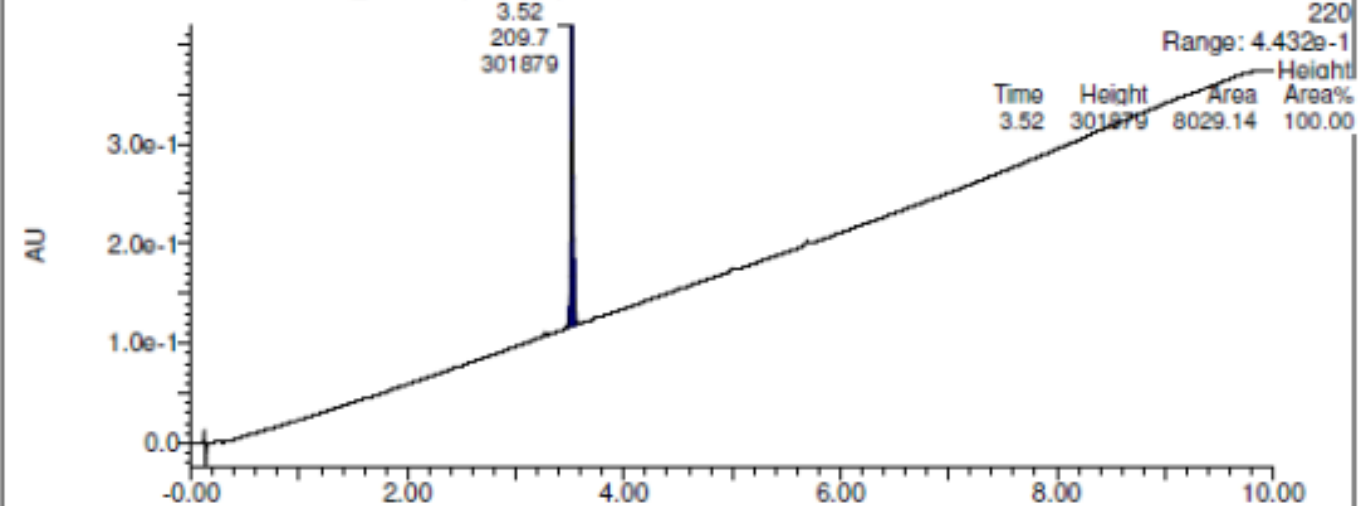
LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
625.317  
2.24e7

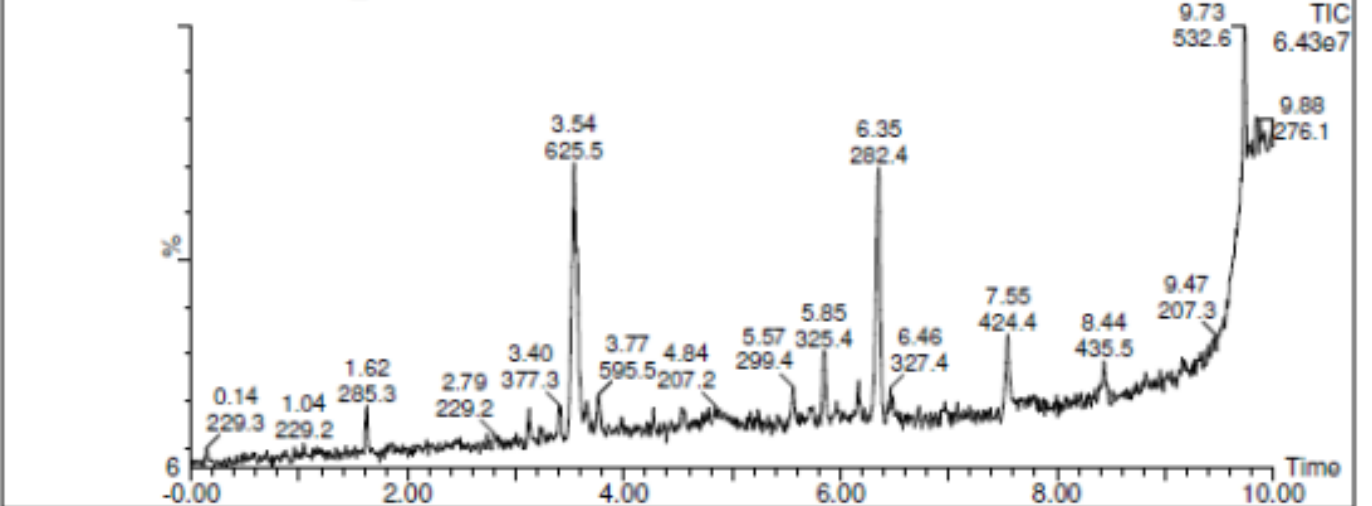
HESSEMA4-101-EXP233-S01\_OC

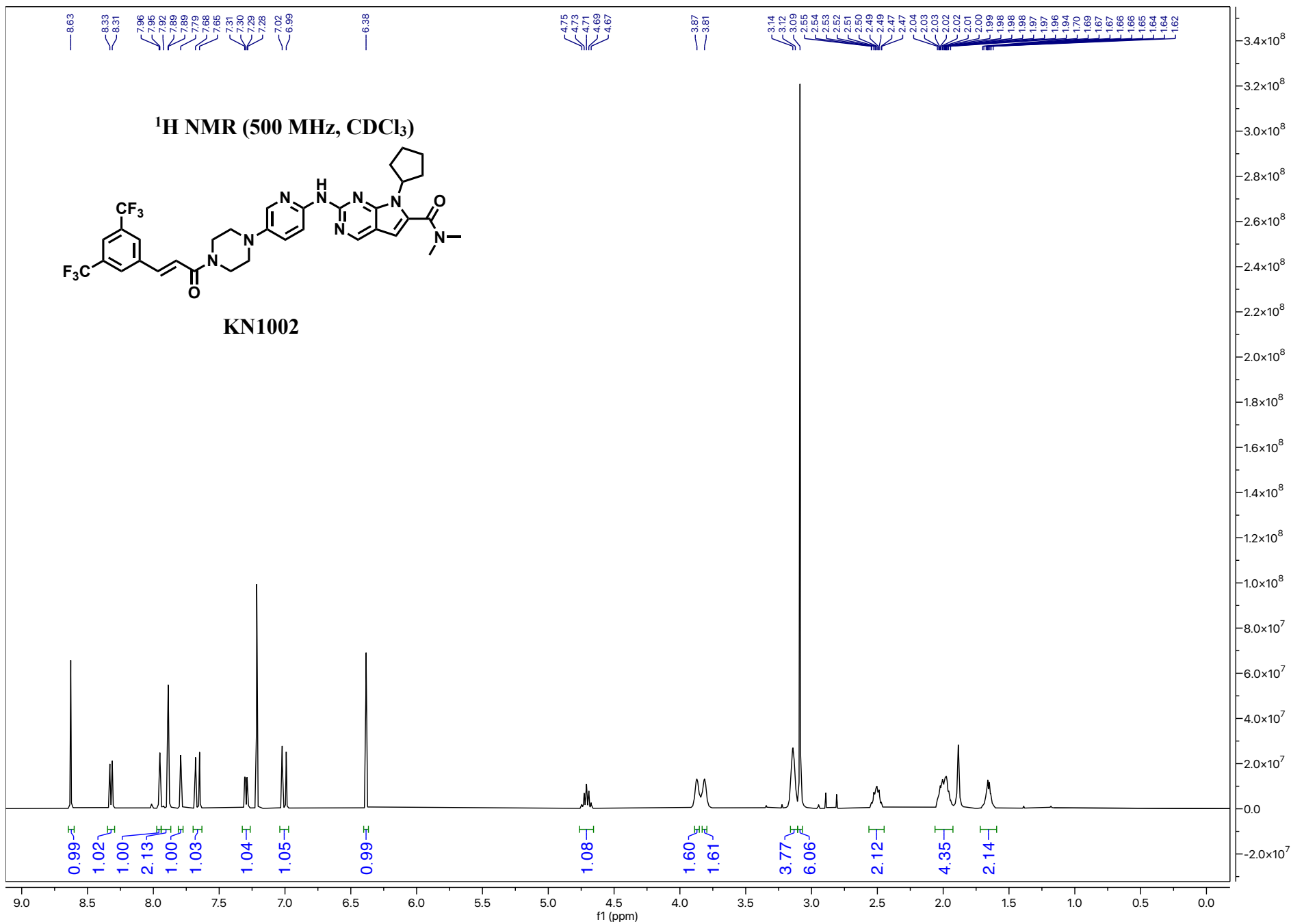


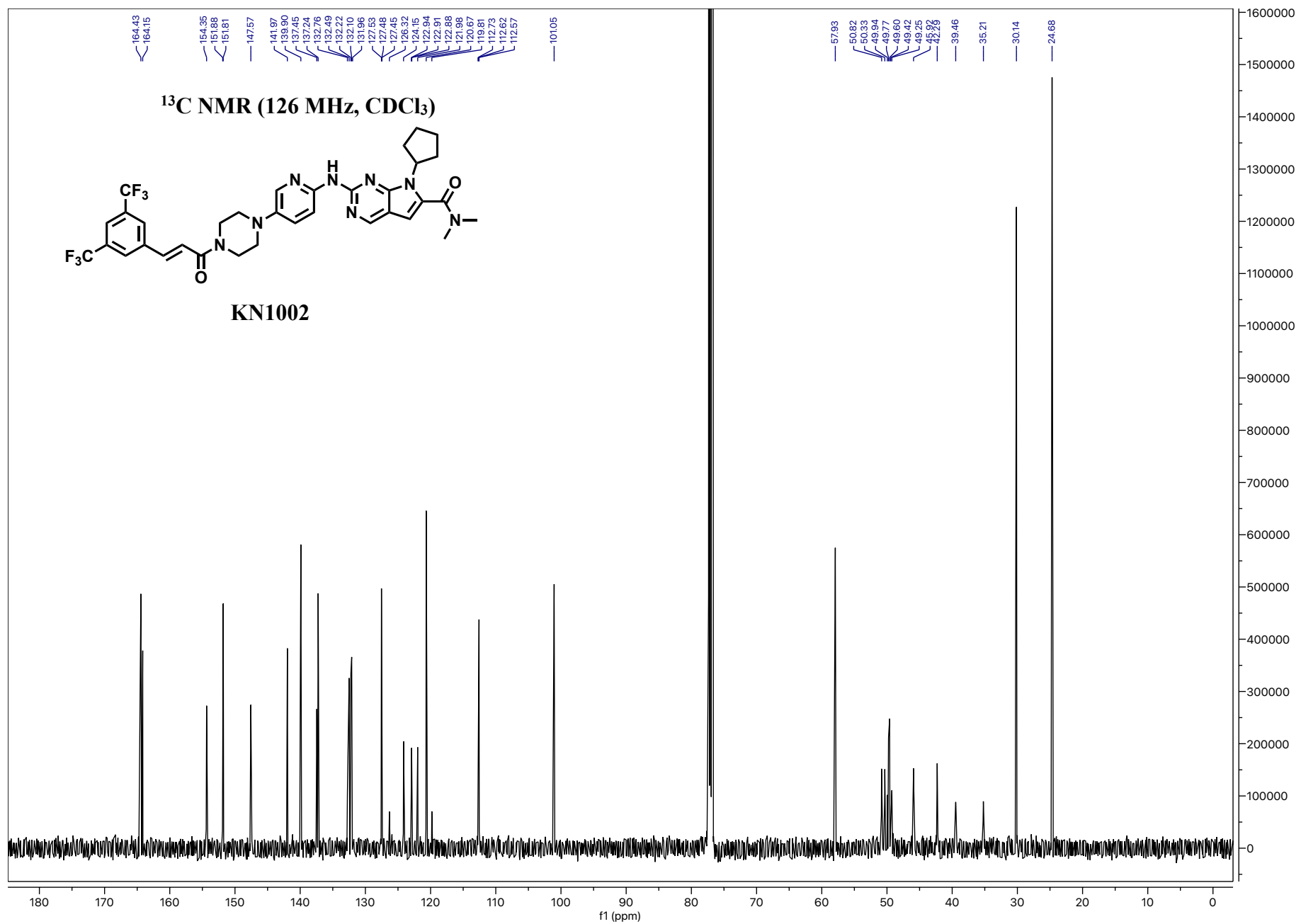
HESSEMA4-101-EXP233-S01\_OC Sm (Mn, 2x3)



HESSEMA4-101-EXP233-S01\_OC







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

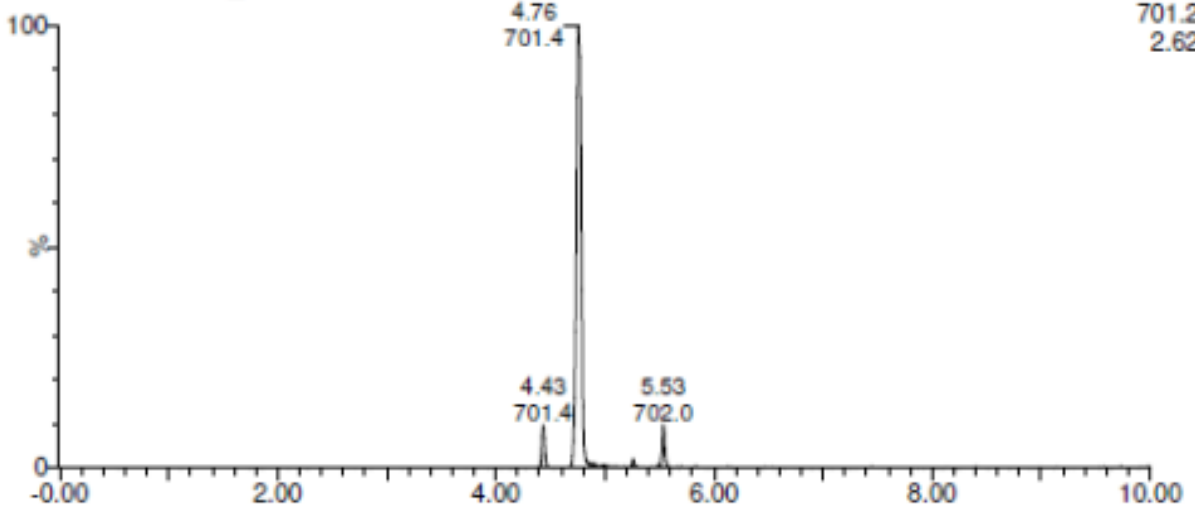
EM23001594442

08-Feb-2023  
17:06:11  
strubhe1-0208A-v19

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

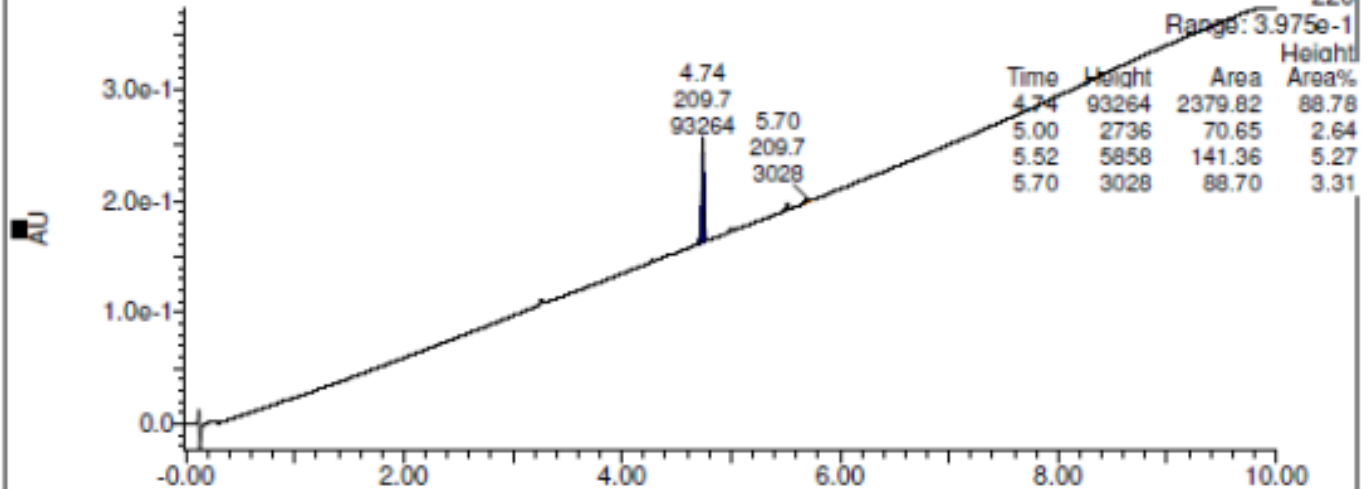
1: Scan ES+  
701.271  
2.62e7

HESSEMA4-101-EXP234-S01\_OC



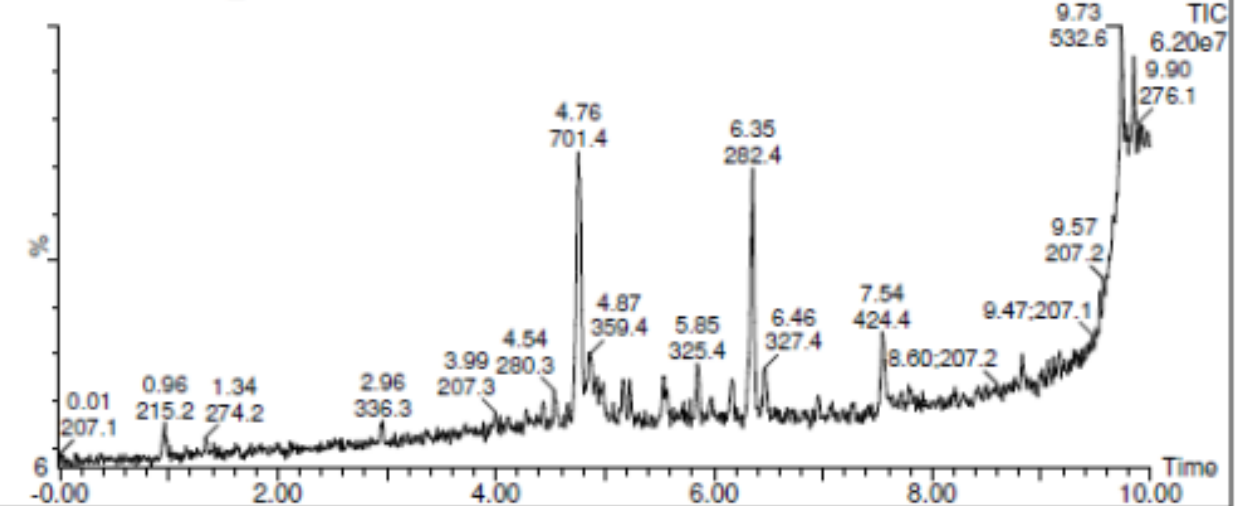
HESSEMA4-101-EXP234-S01\_OC Sm (Mn, 2x3)

2: Diode Array  
220  
Range: 3.975e-1

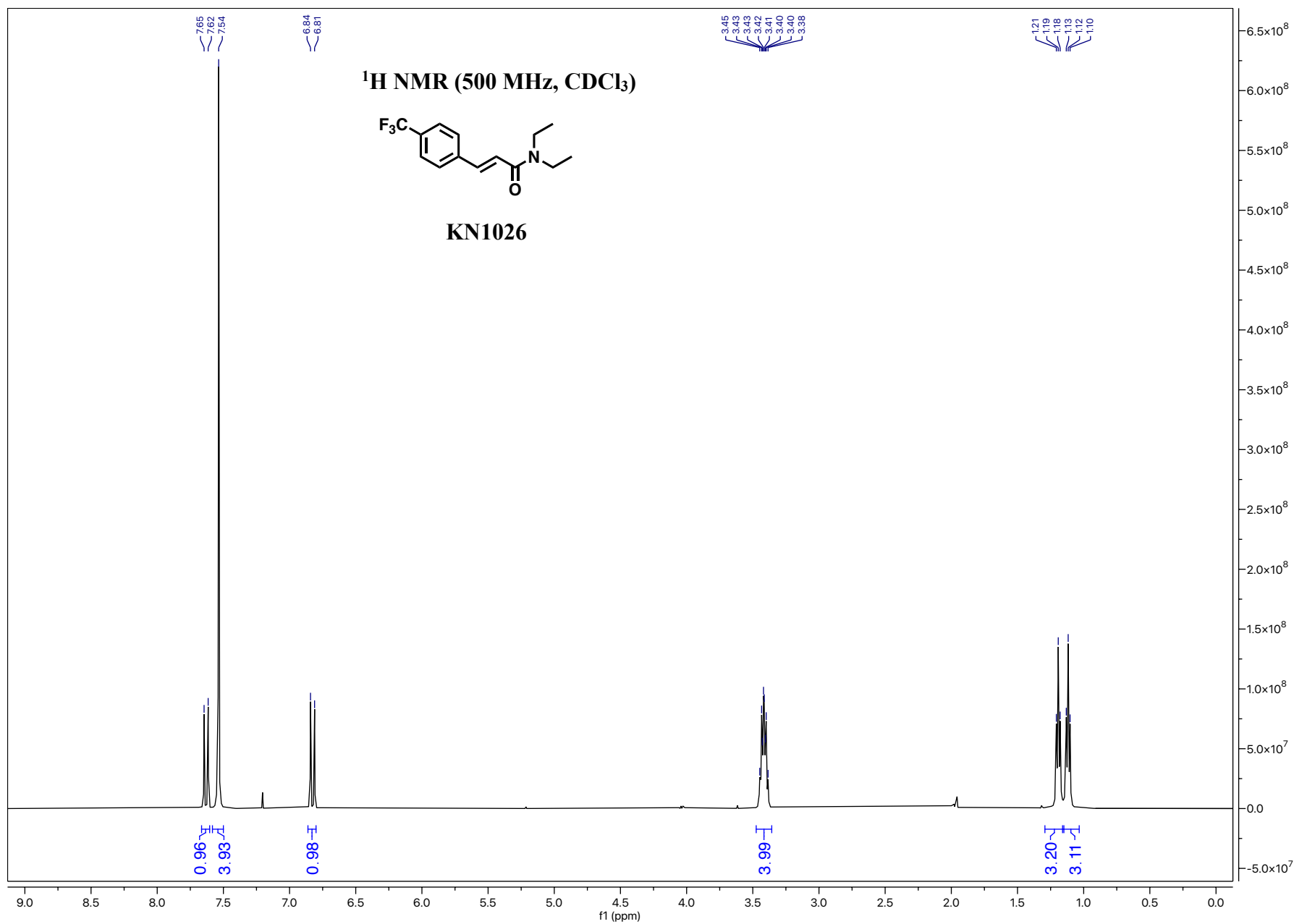


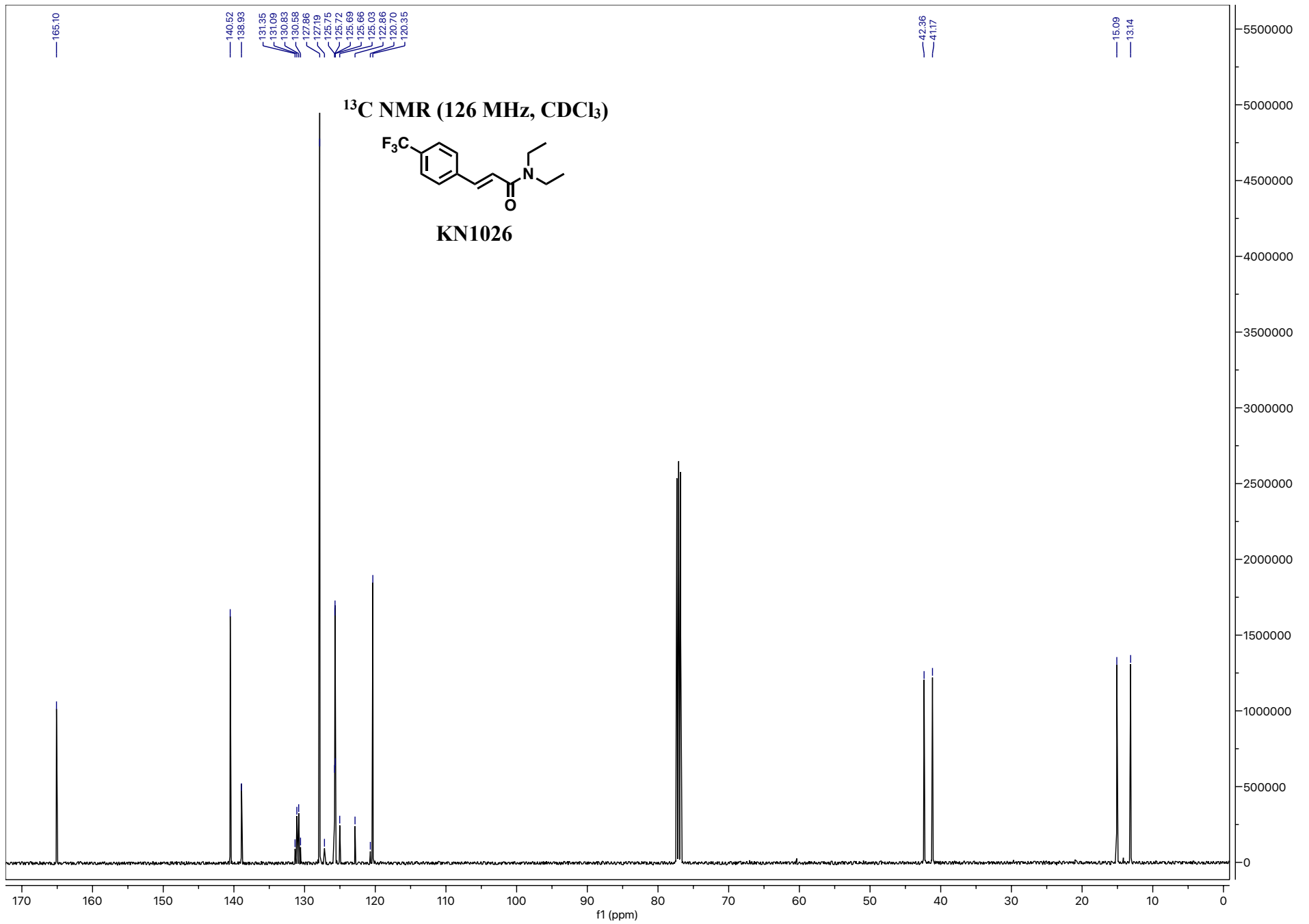
HESSEMA4-101-EXP234-S01\_OC

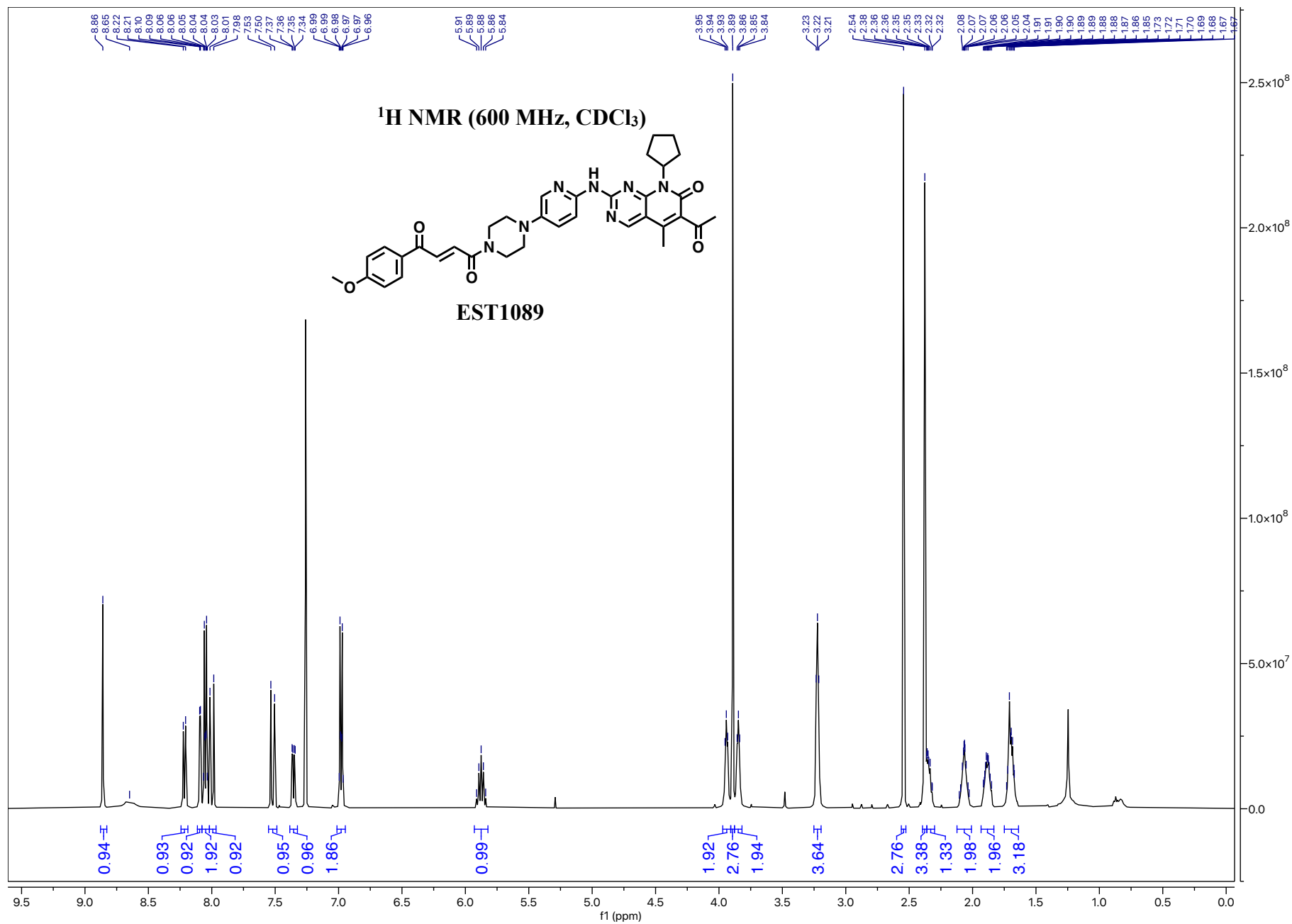
1: Scan ES+  
TIC

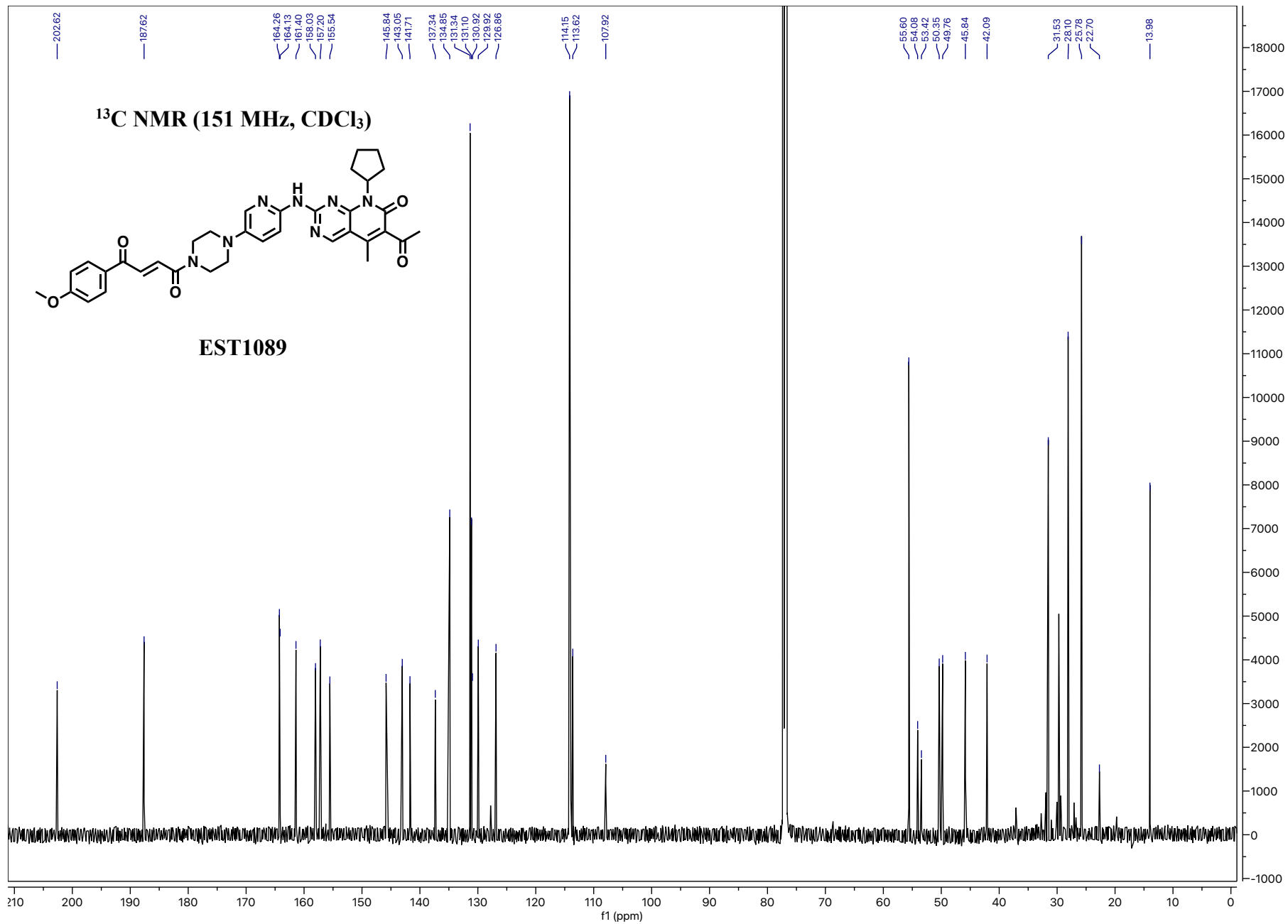












HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

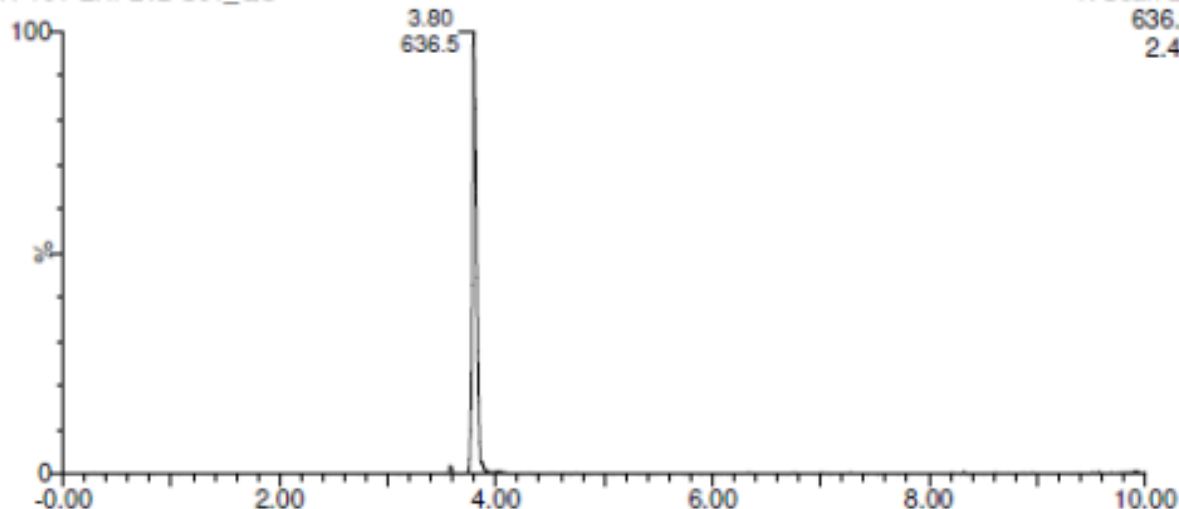
EM23001590295

13-Jan-2023  
16:38:55  
strubhe1-0112A-v09

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

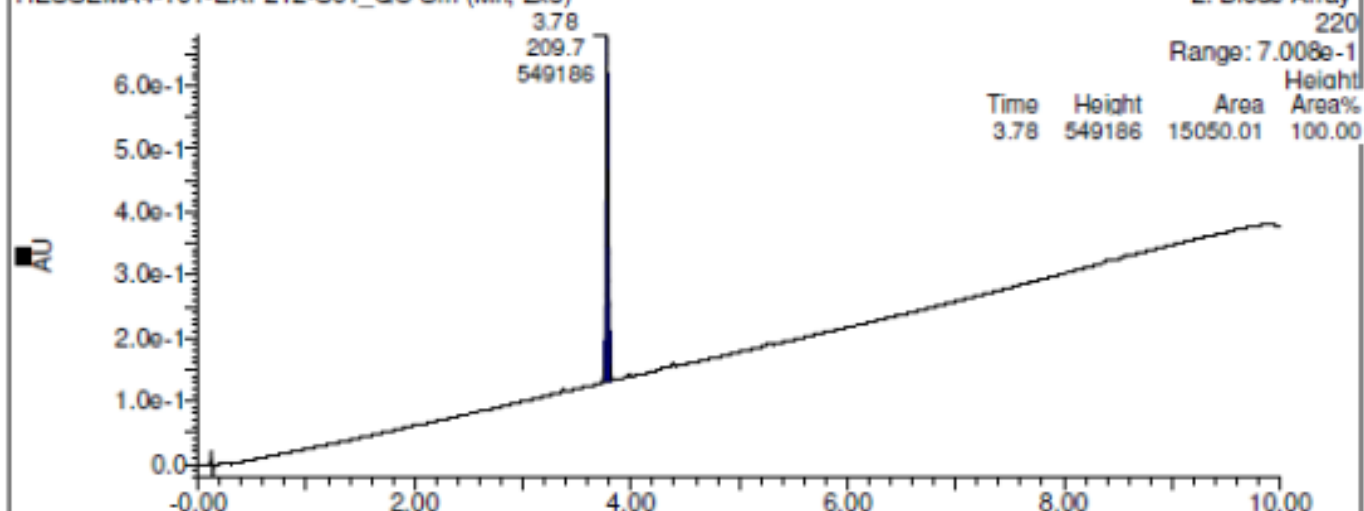
1: Scan ES+  
636.286  
2.43e7

HESSEMA4-101-EXP212-S01\_OC



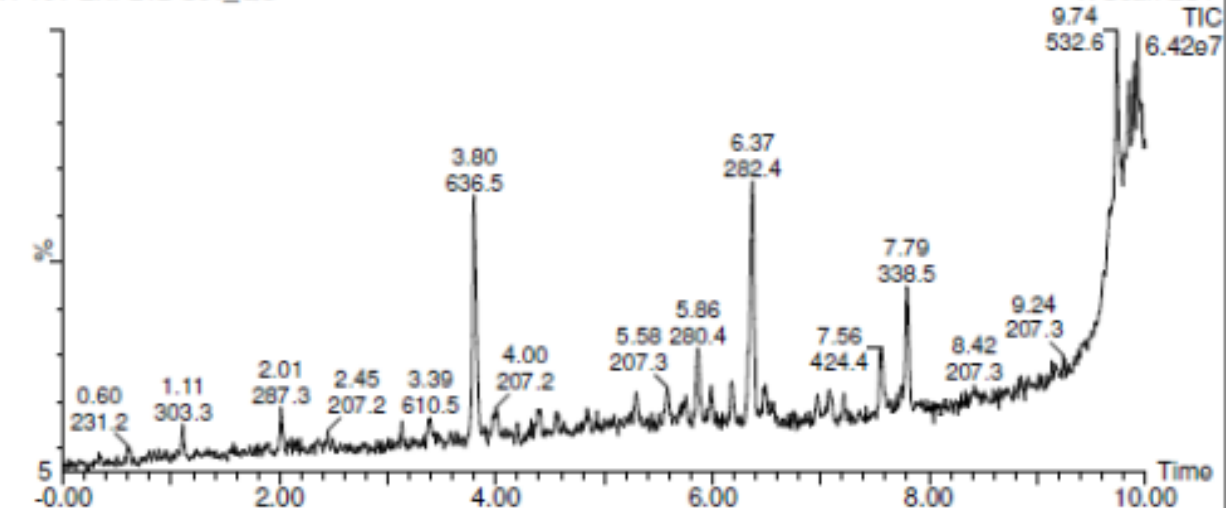
HESSEMA4-101-EXP212-S01\_OC Sm (Mn, 2x3)

2: Diode Array



HESSEMA4-101-EXP212-S01\_OC

1: Scan ES+ TIC



HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

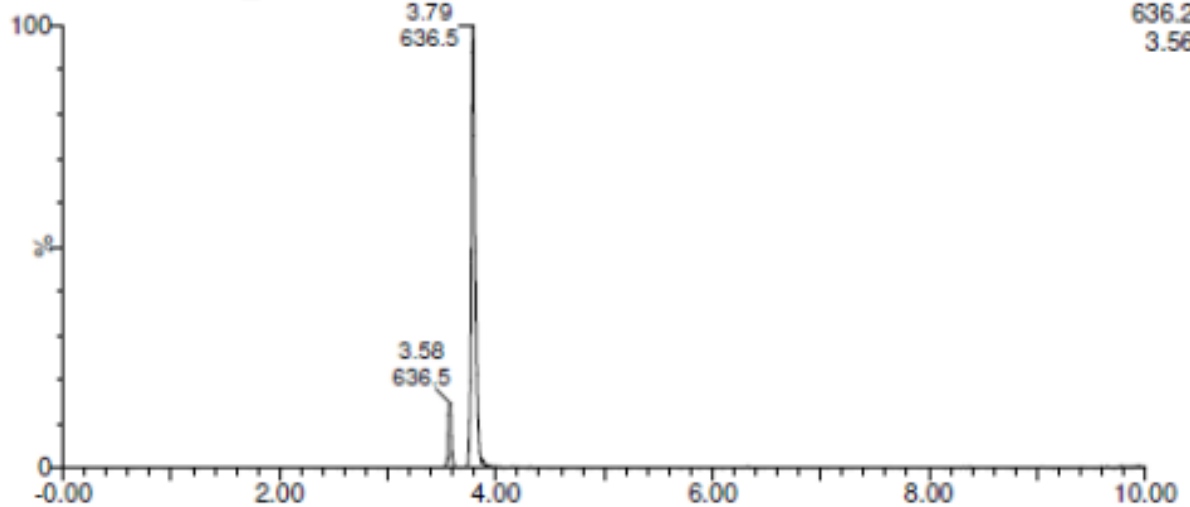
EM23001590295

19-Jan-2023  
17:04:54  
strubhe1-0118A-v09

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
636.286  
3.56e7

HESSEMA4-101-EXP212-S01R\_OC

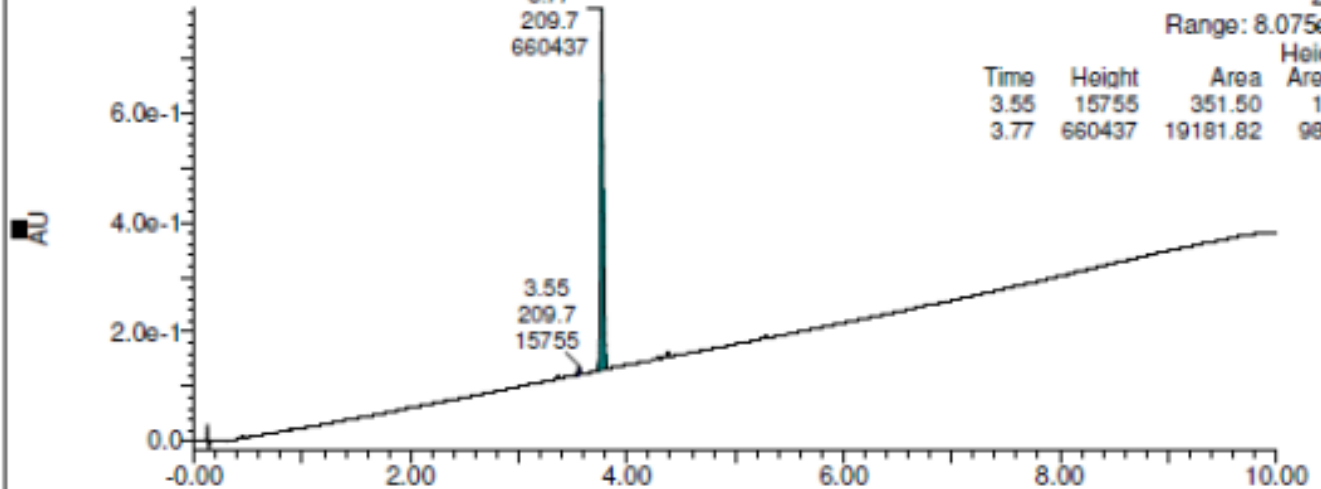


HESSEMA4-101-EXP212-S01R\_OC Sm (Mn, 2x3)

2: Diode Array  
220

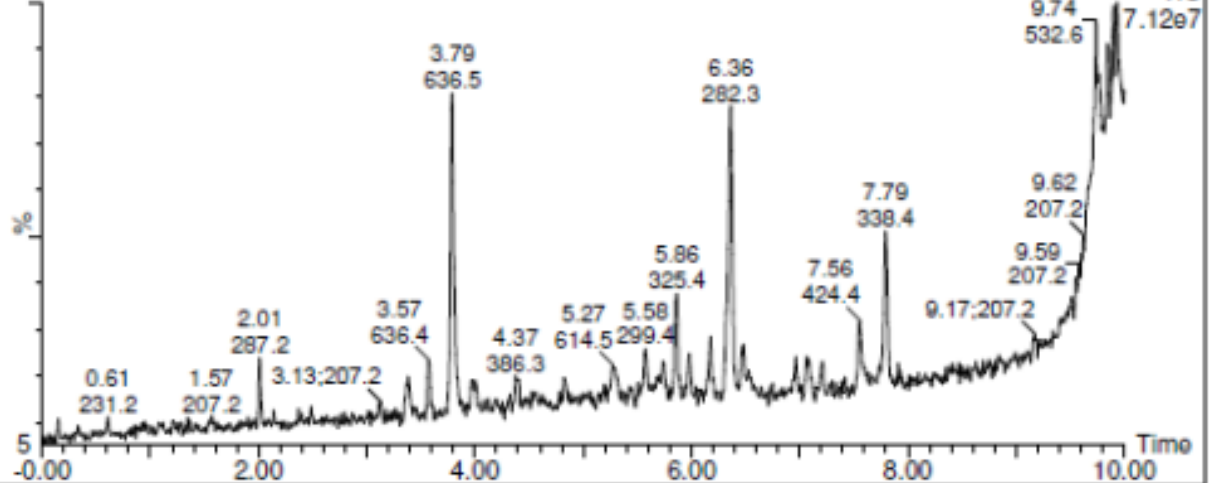
Range: 8.075e-1  
Height

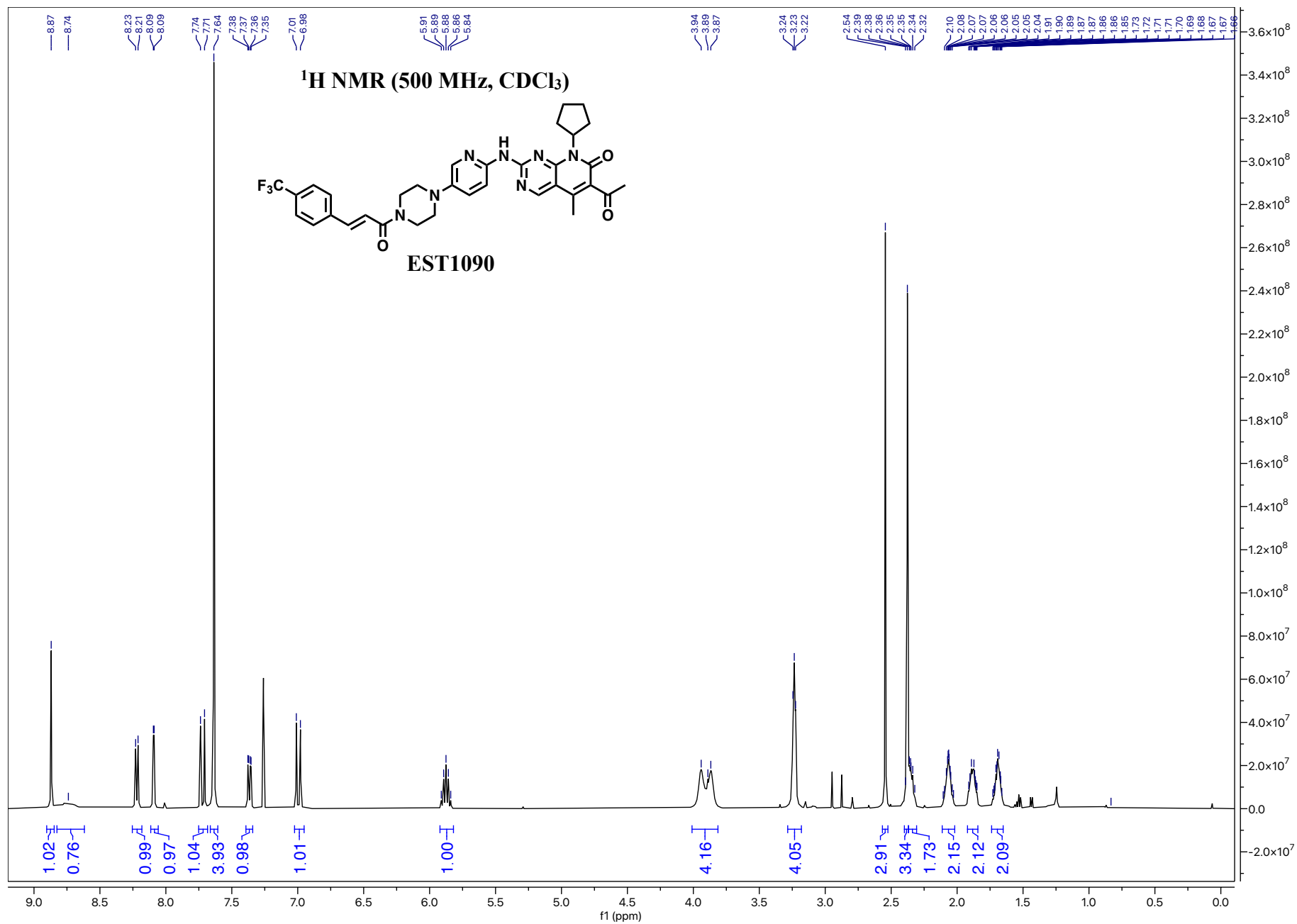
Time	Height	Area	Area%
3.55	15755	351.50	1.80
3.77	660437	19181.82	98.20

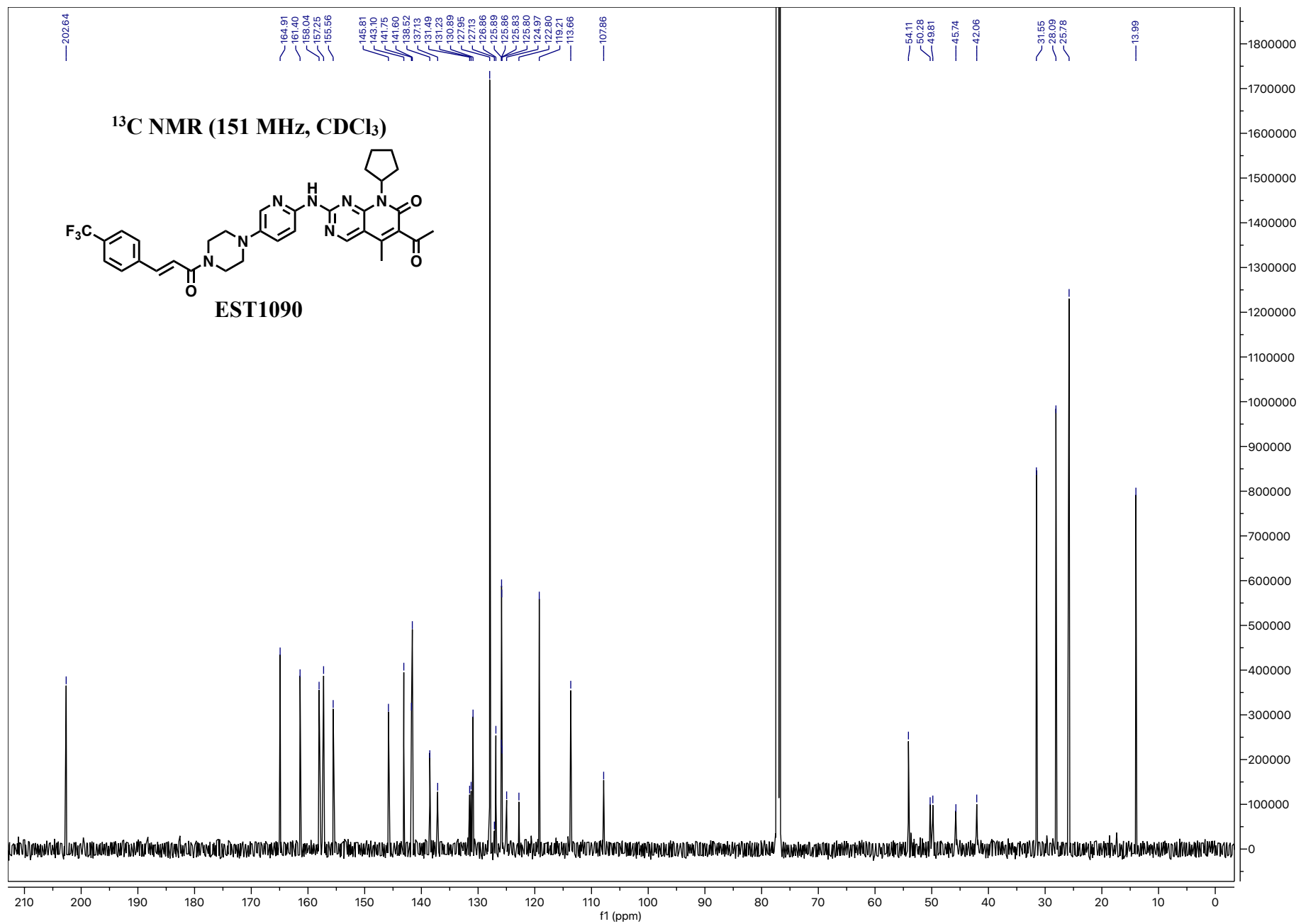


HESSEMA4-101-EXP212-S01R\_OC

1: Scan ES+  
TIC









HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

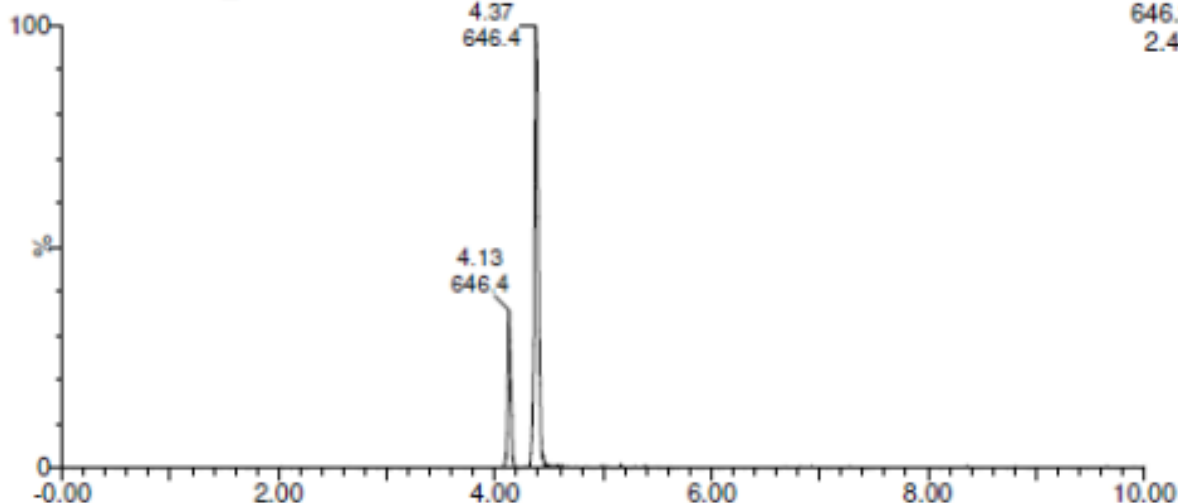
EM23001594444

08-Feb-2023  
17:17:14  
strubhe1-0208A-v20

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
646.268  
2.44e7

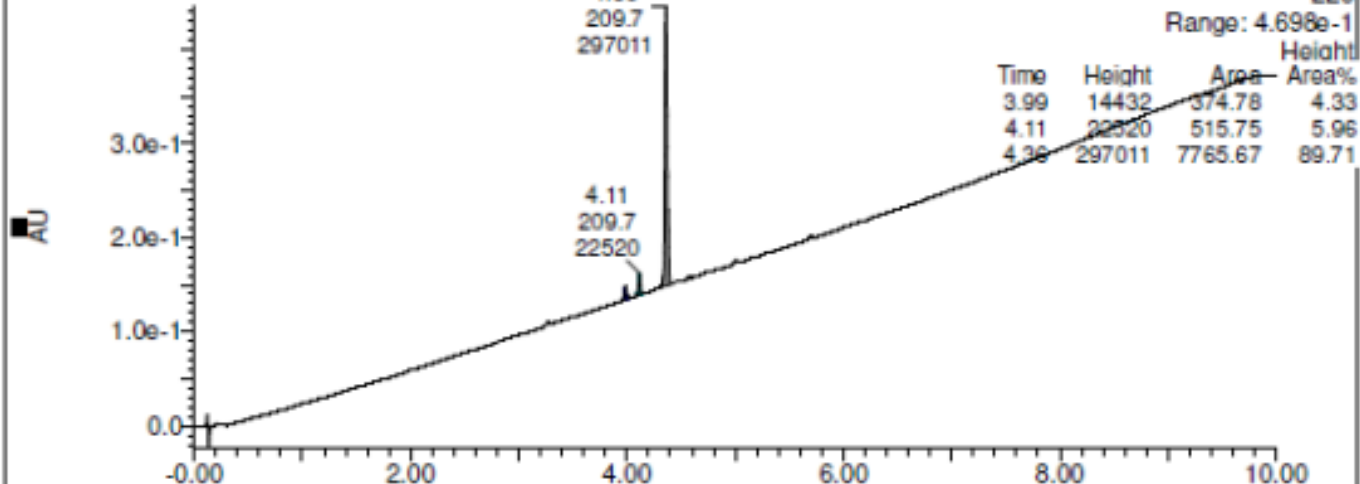
HESSEMA4-101-EXP235-S01\_OC



HESSEMA4-101-EXP235-S01\_OC Sm (Mn, 2x3)

2: Diode Array  
220

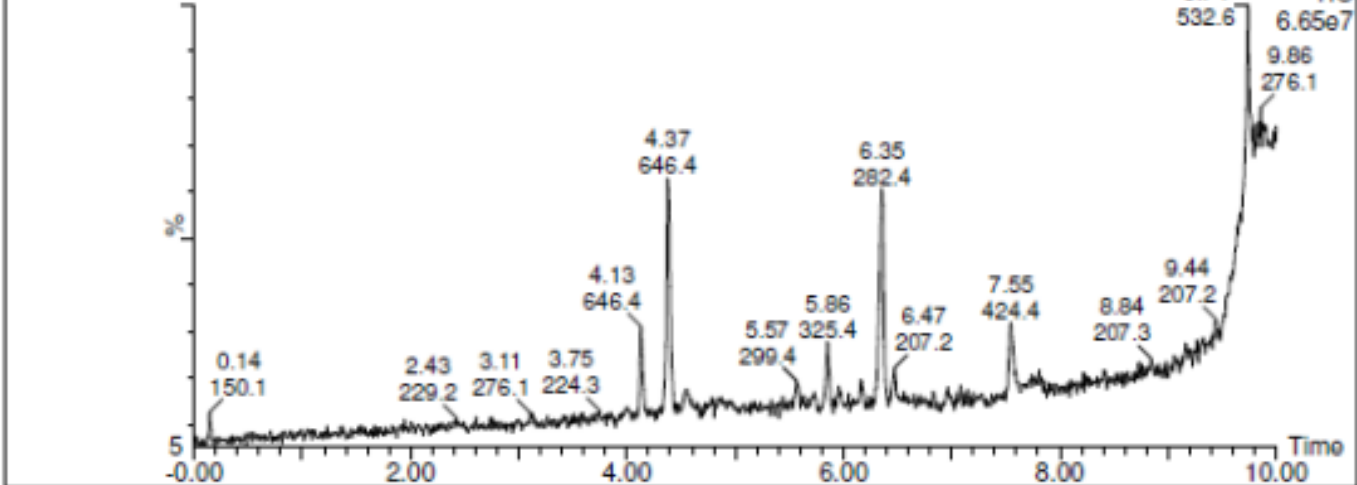
Range: 4.698e-1  
Height

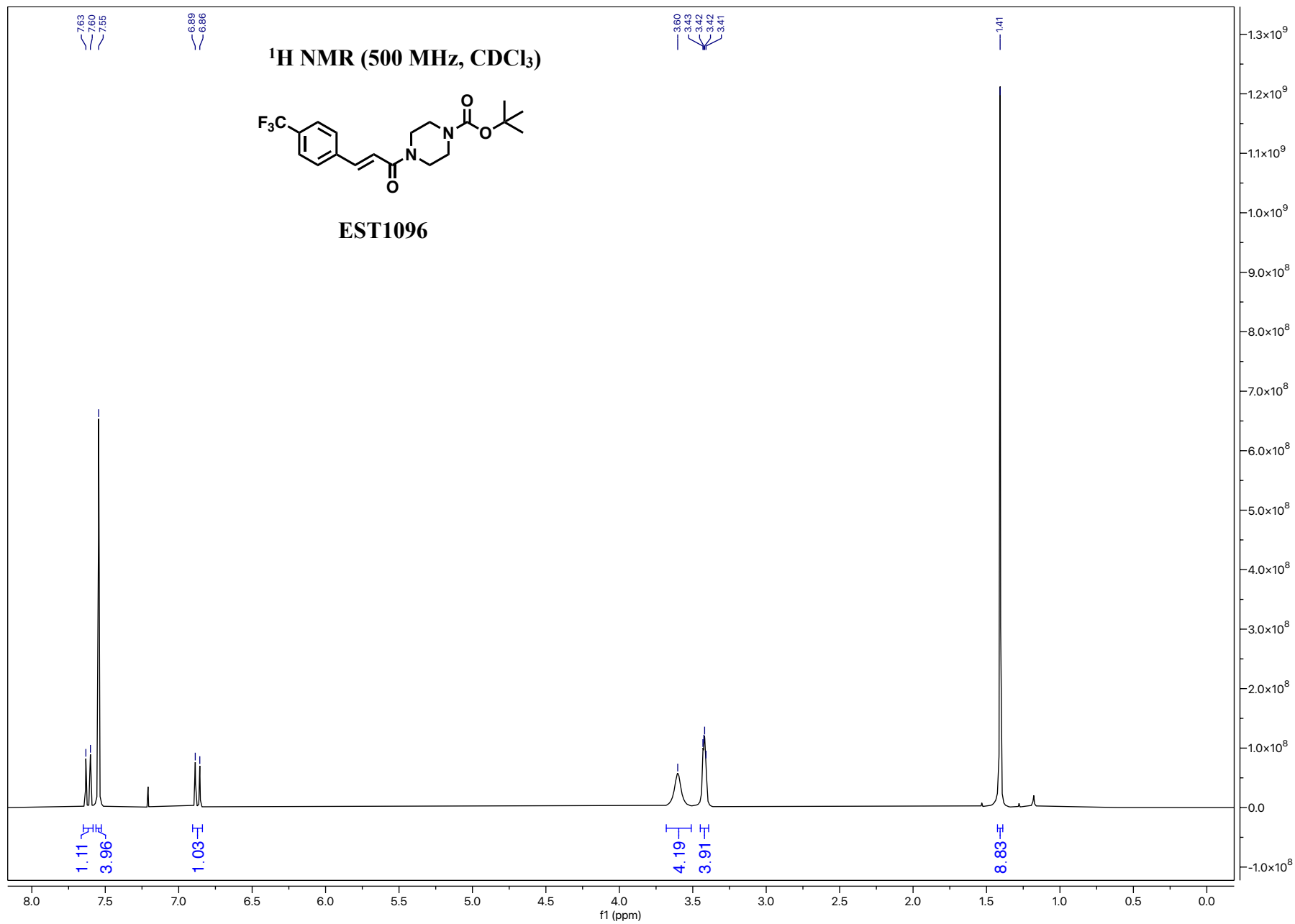


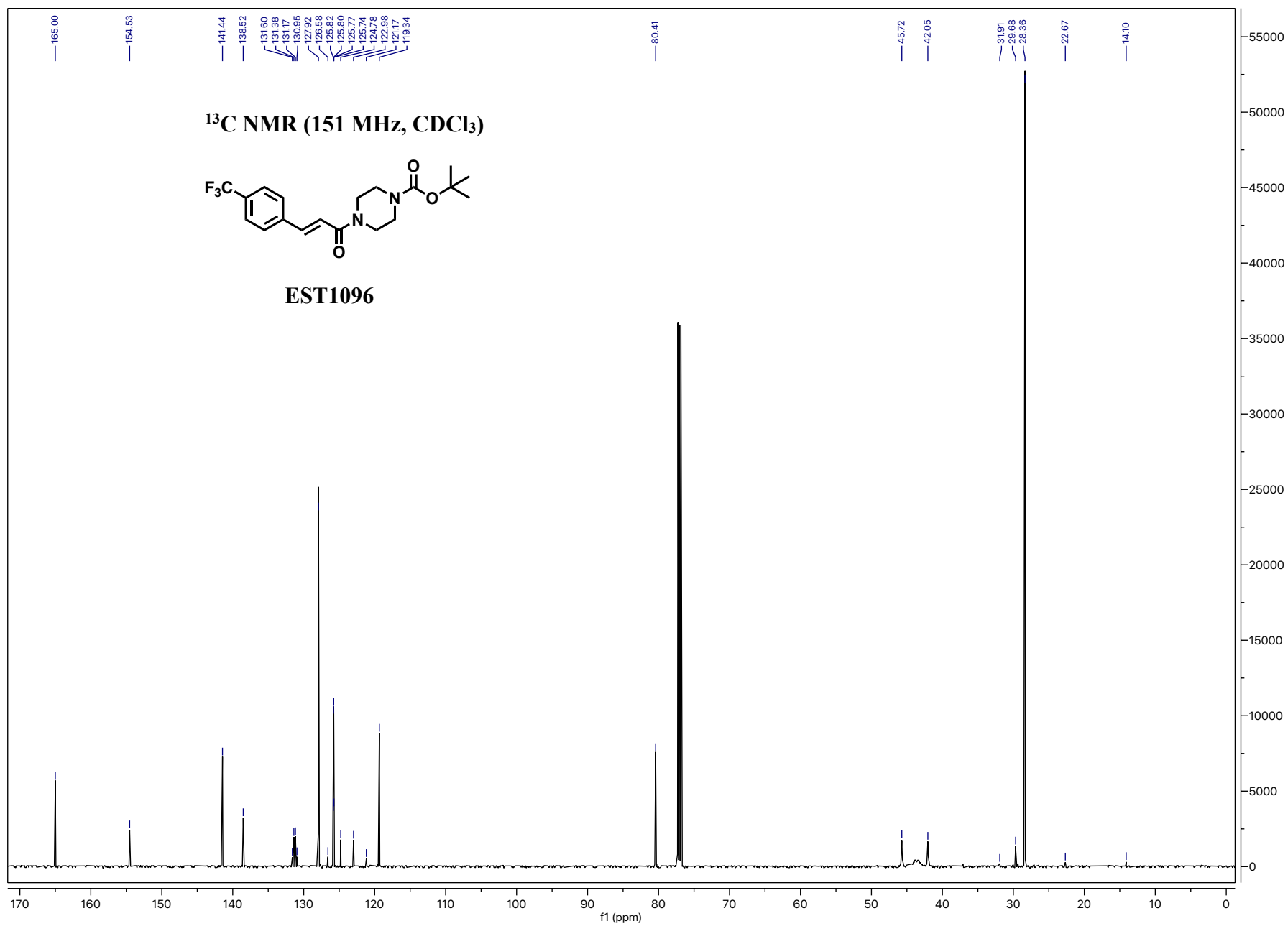
HESSEMA4-101-EXP235-S01\_OC

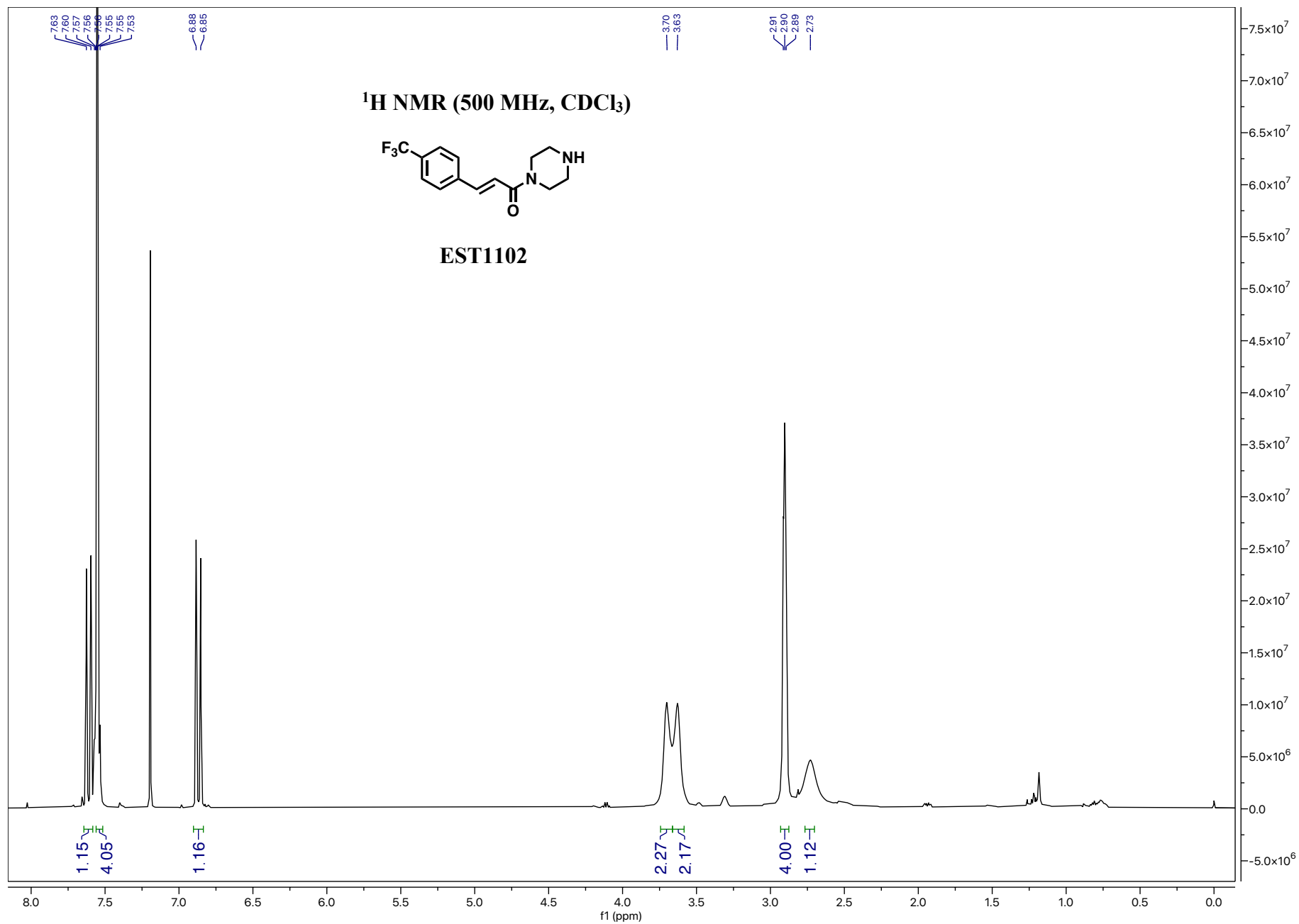
1: Scan ES+  
TIC

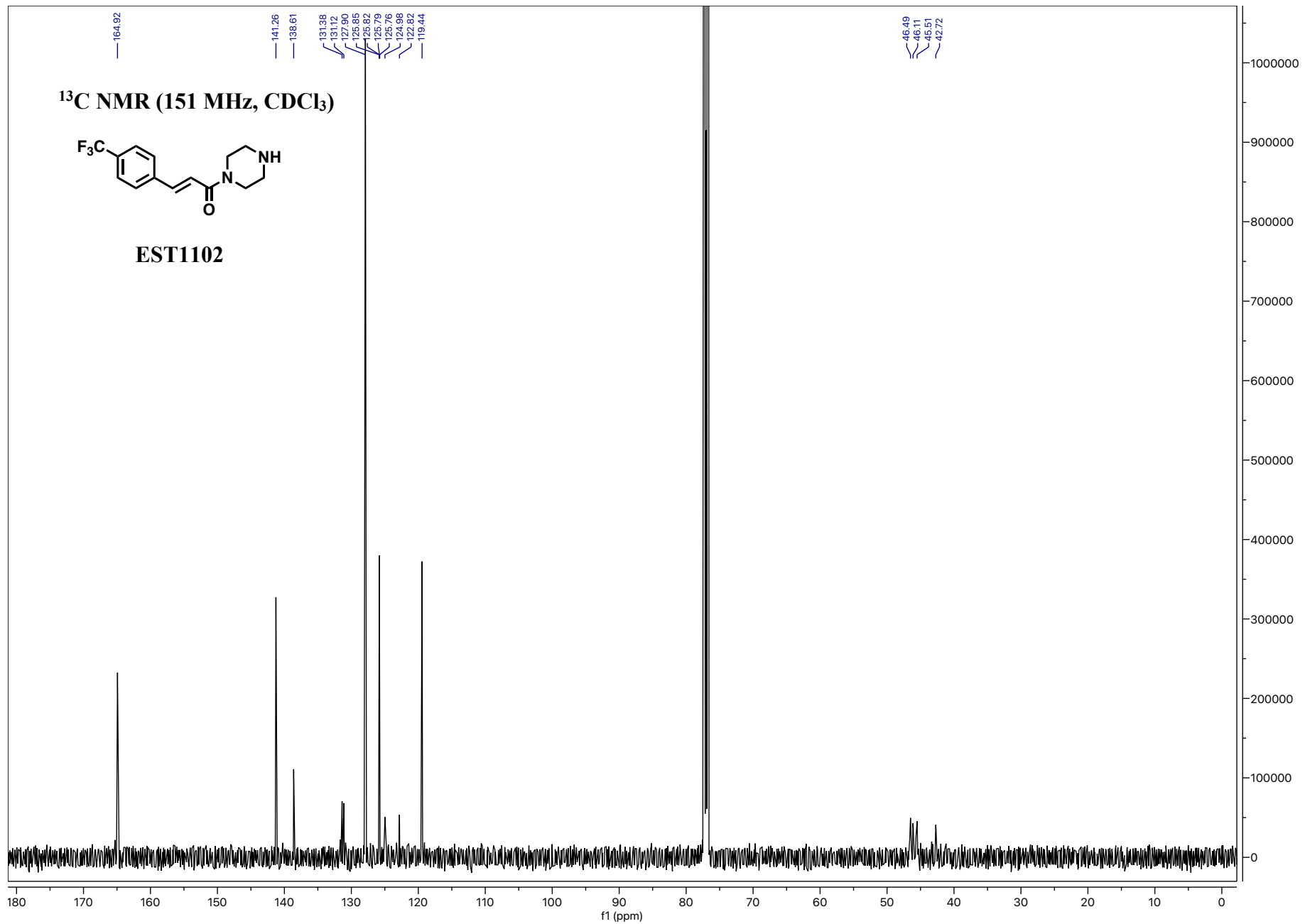
9.74  
532.6  
6.65e7

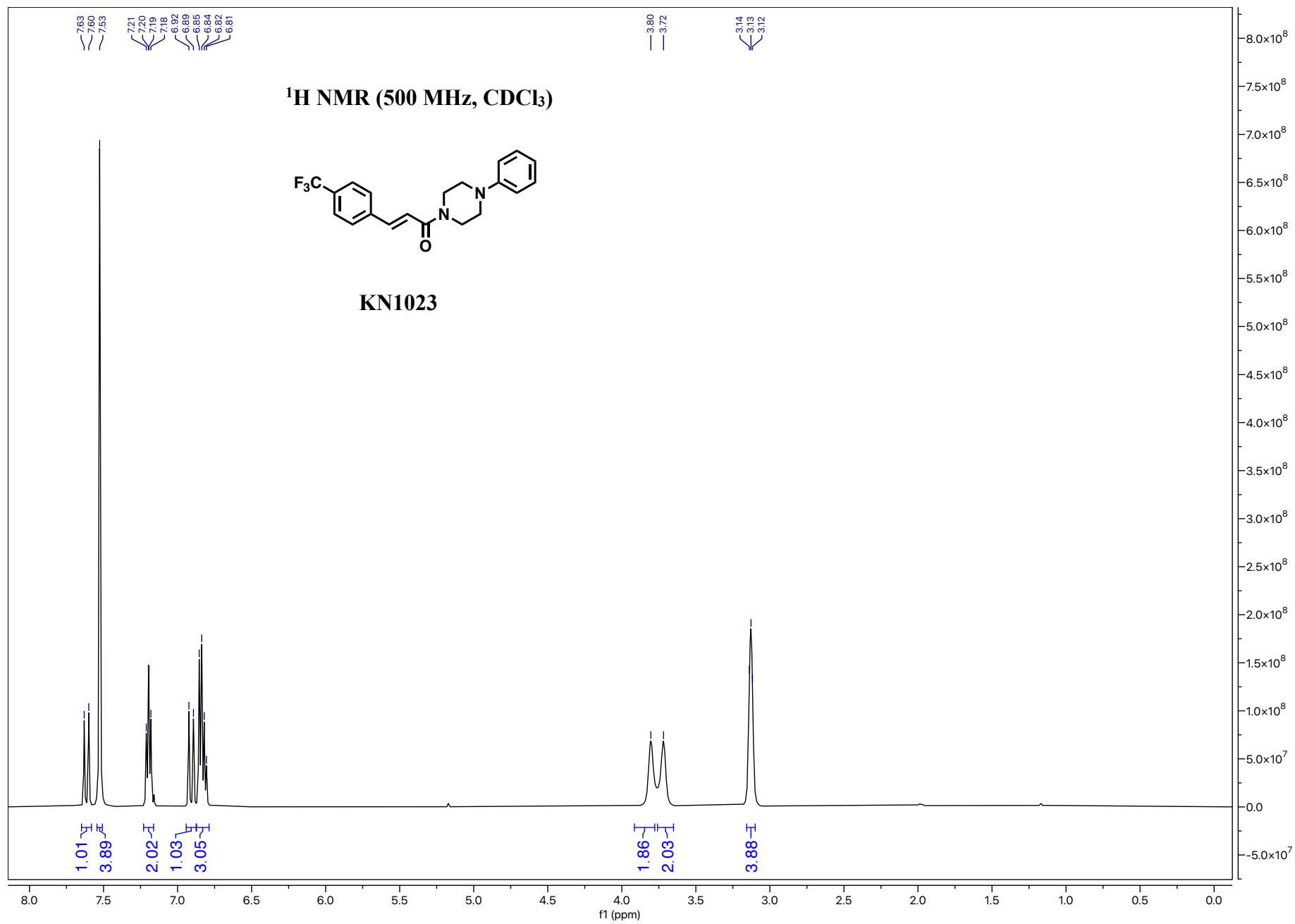


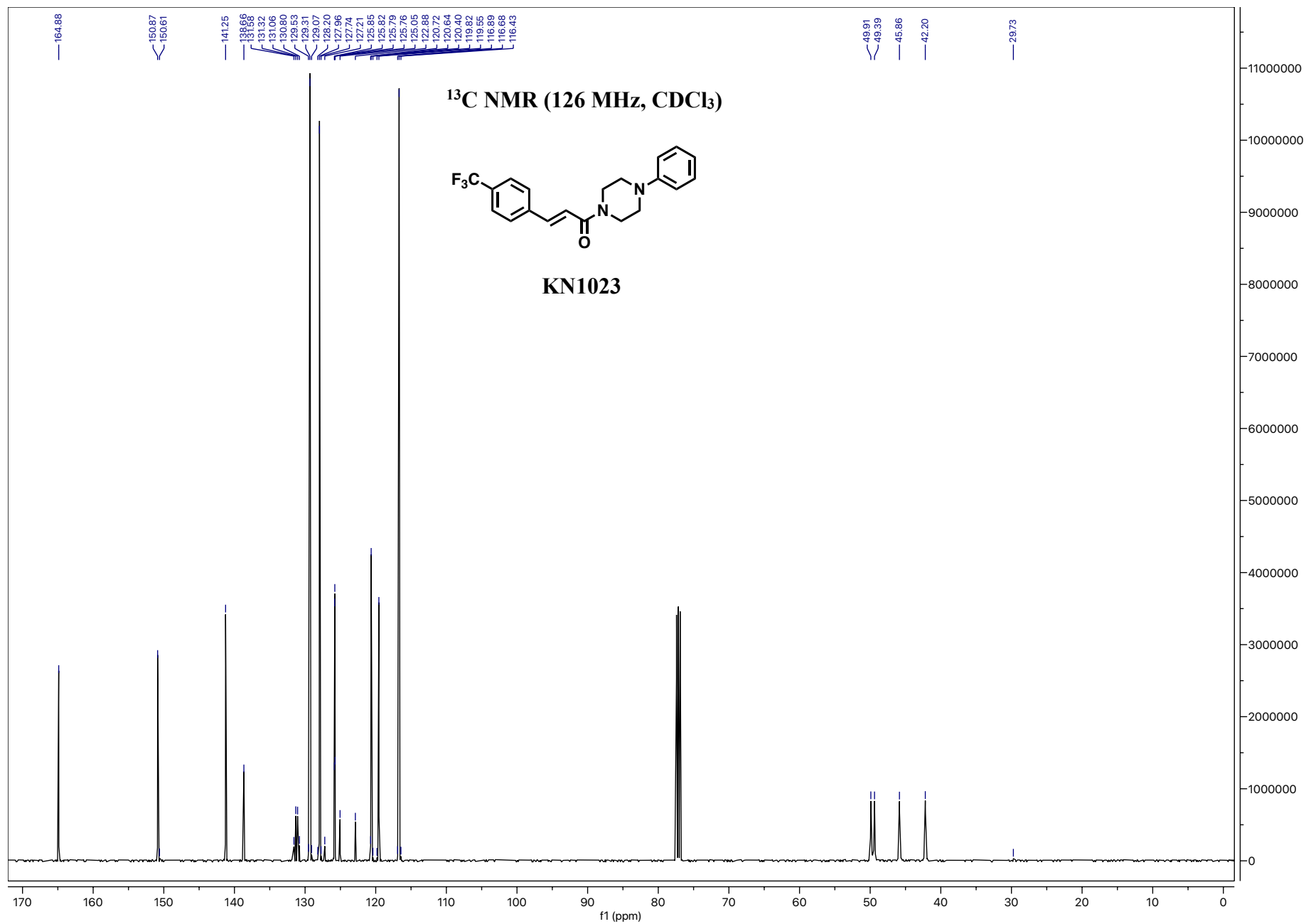


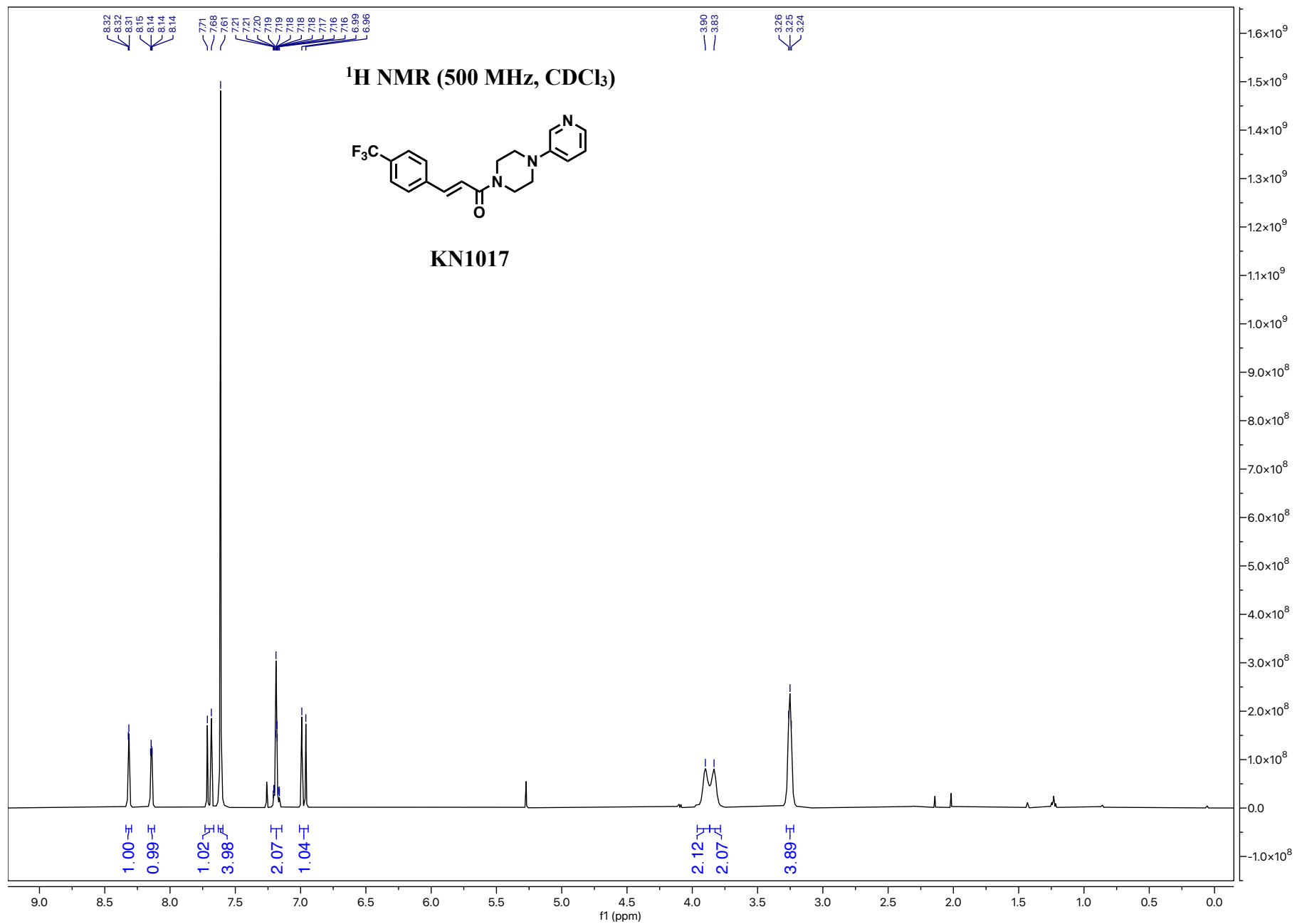




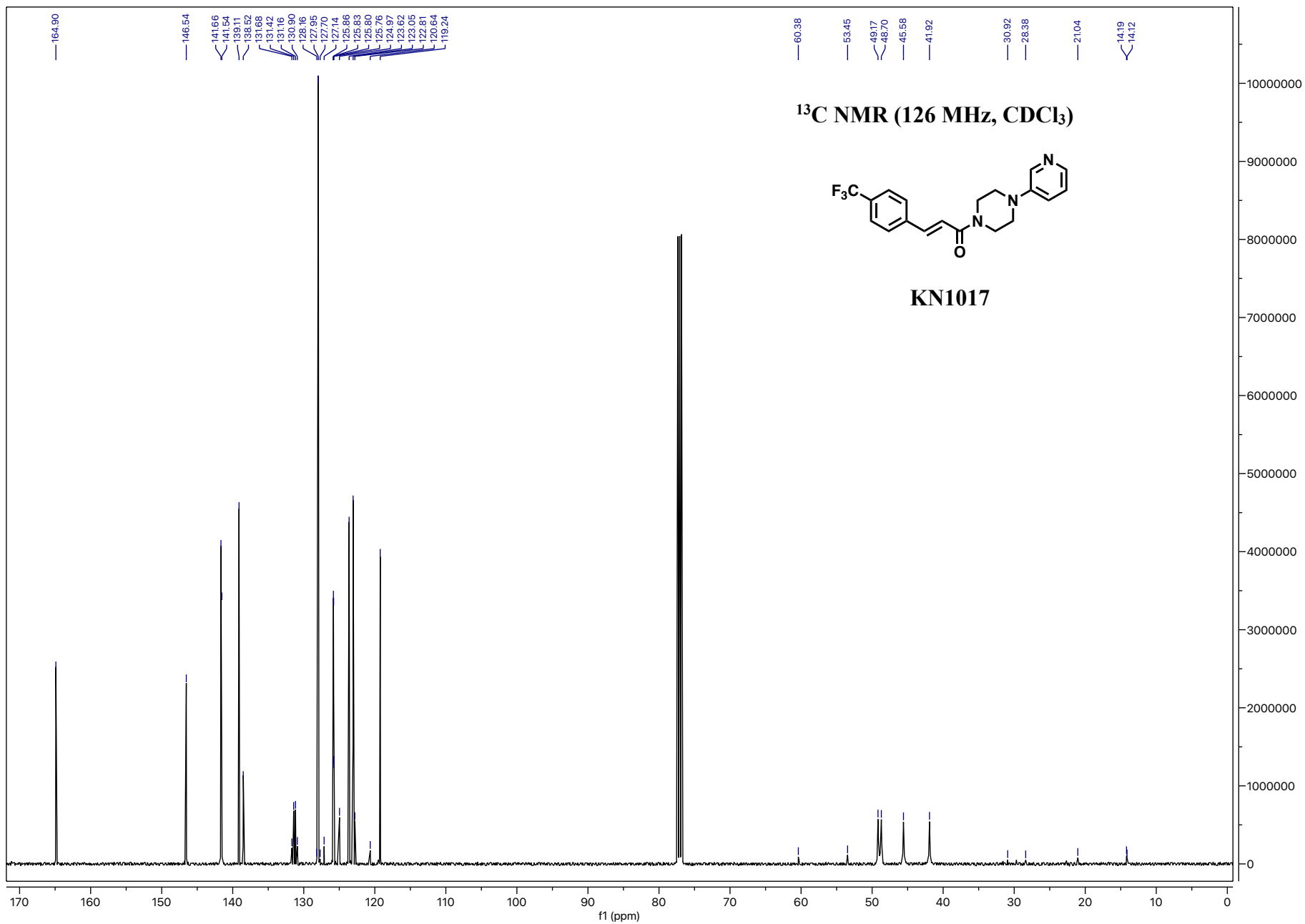


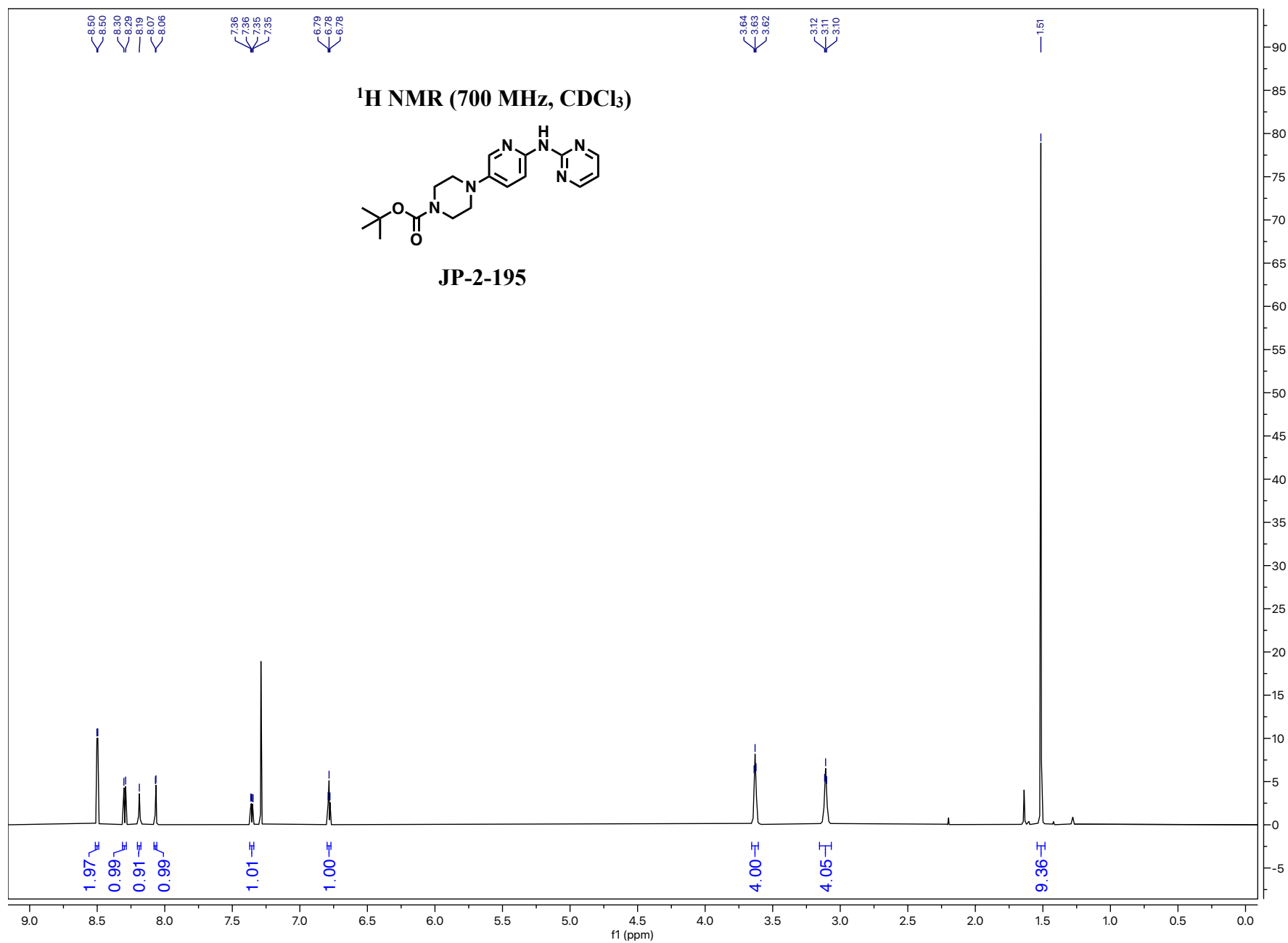


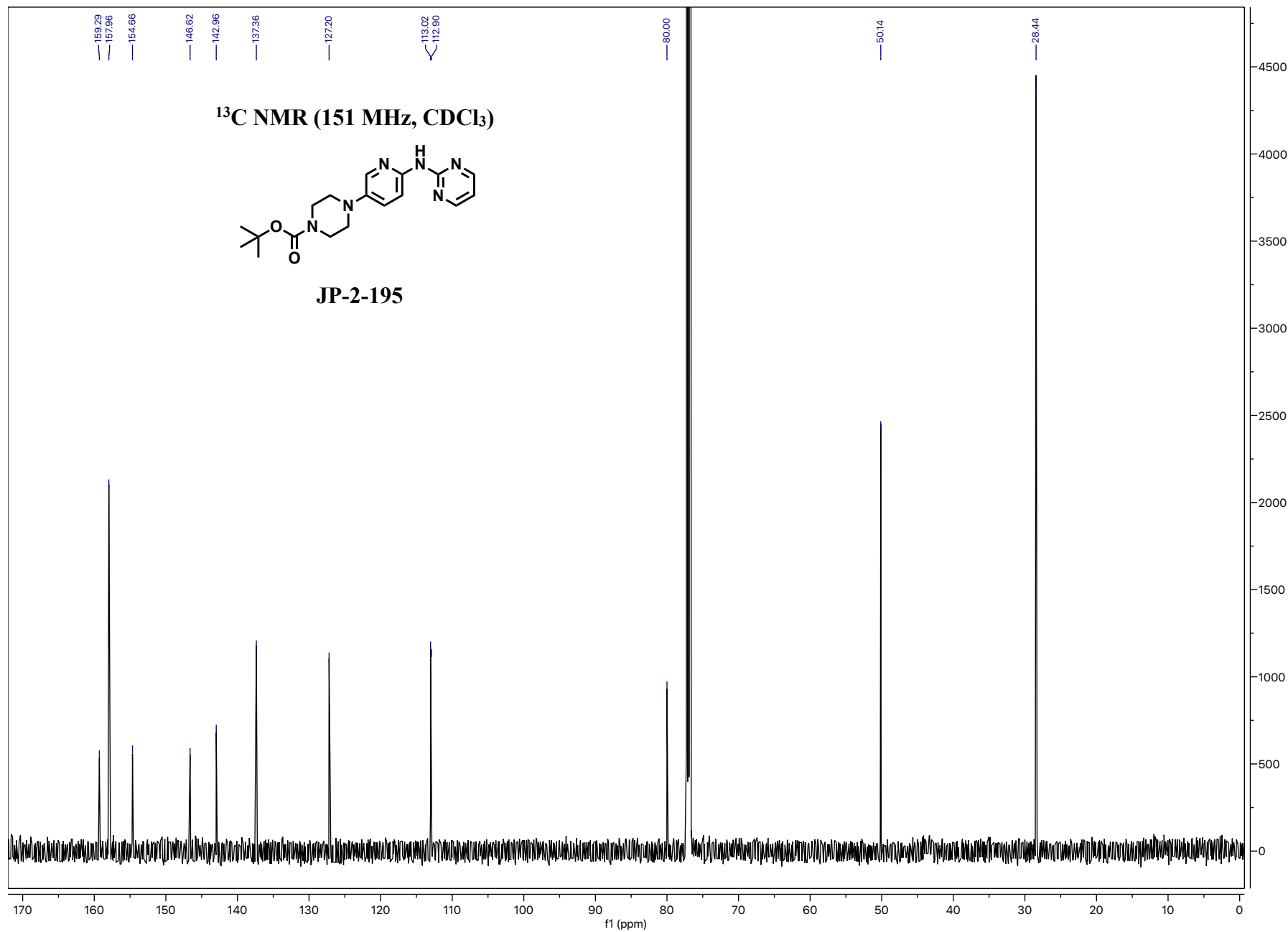


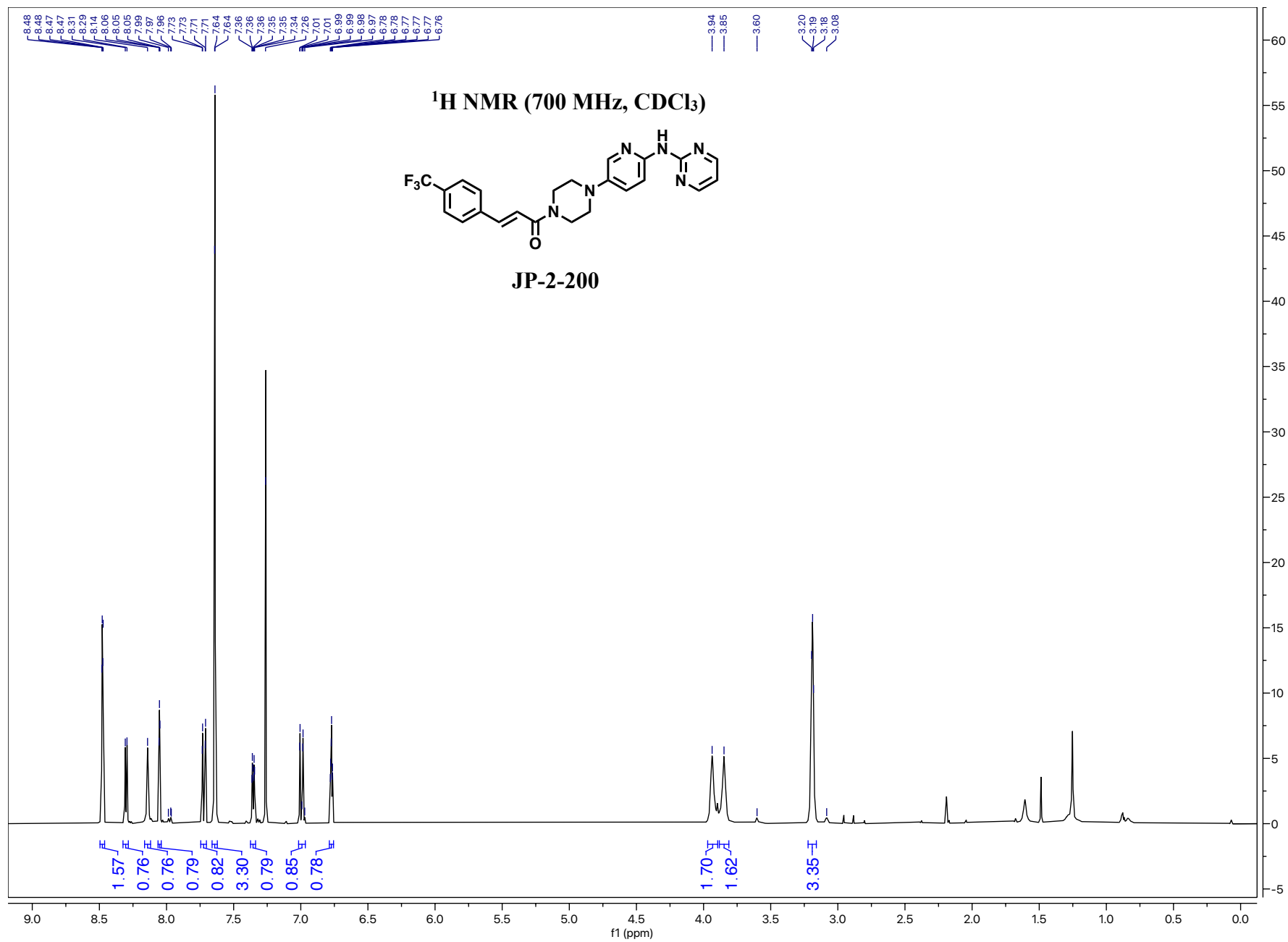


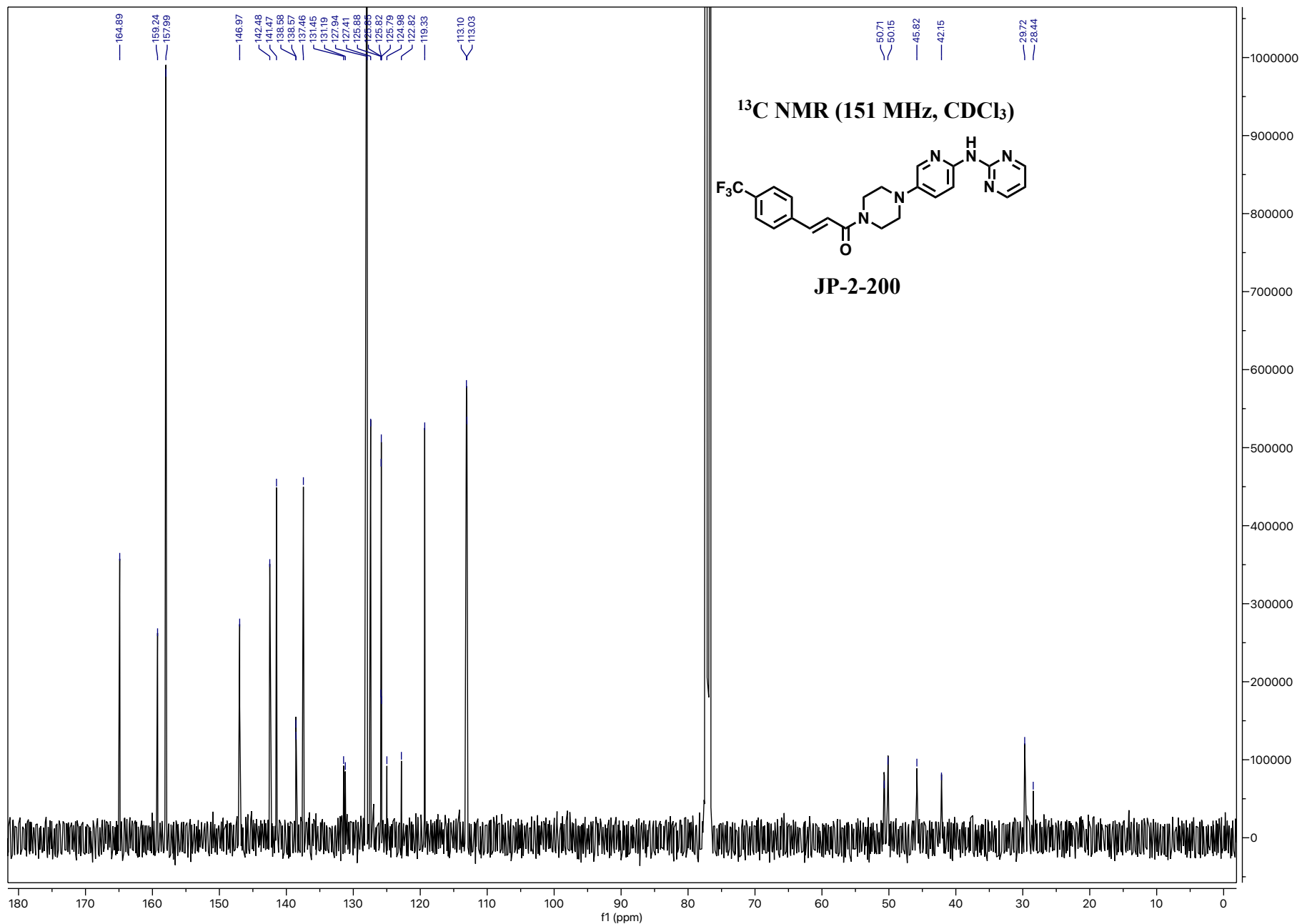


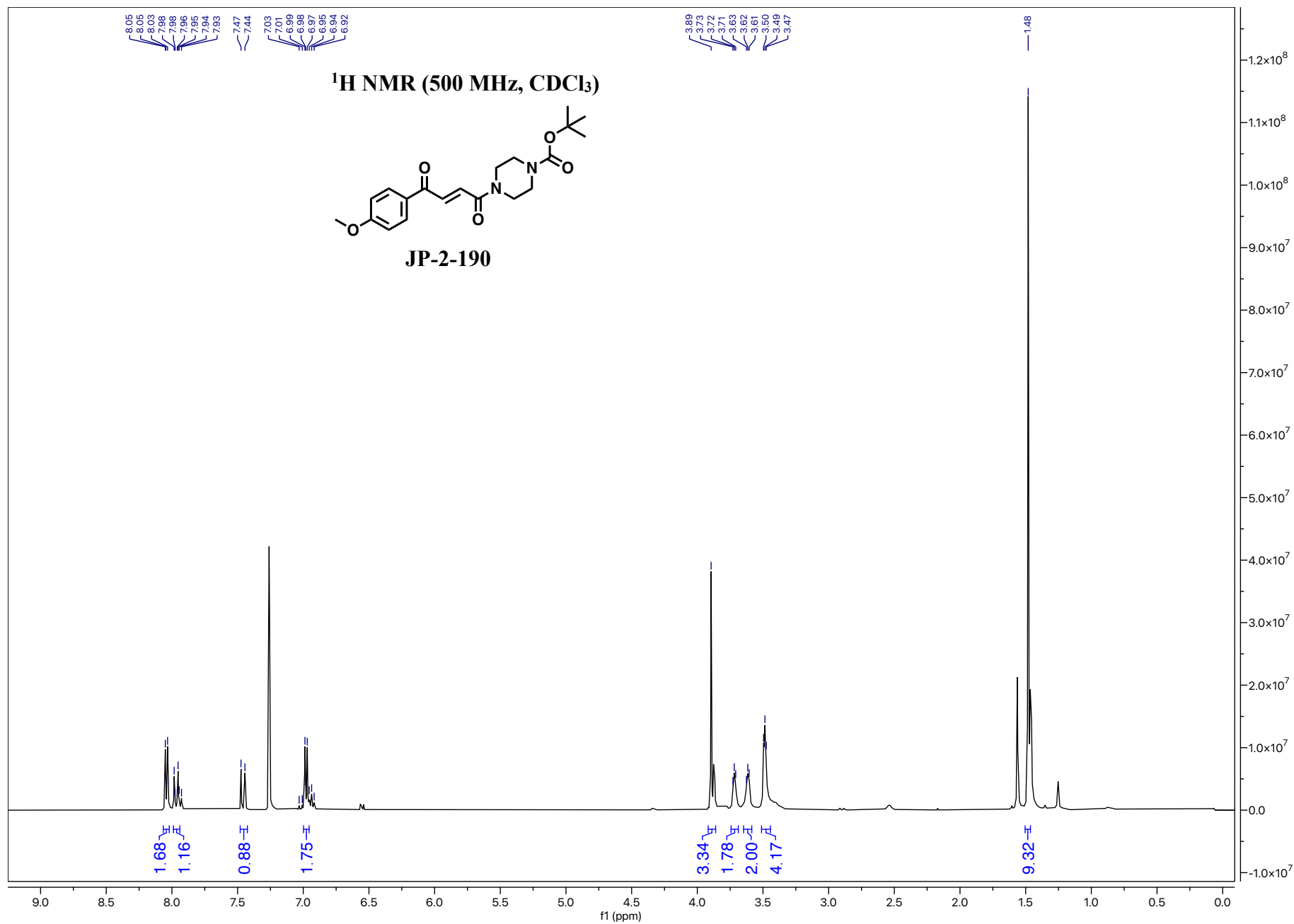


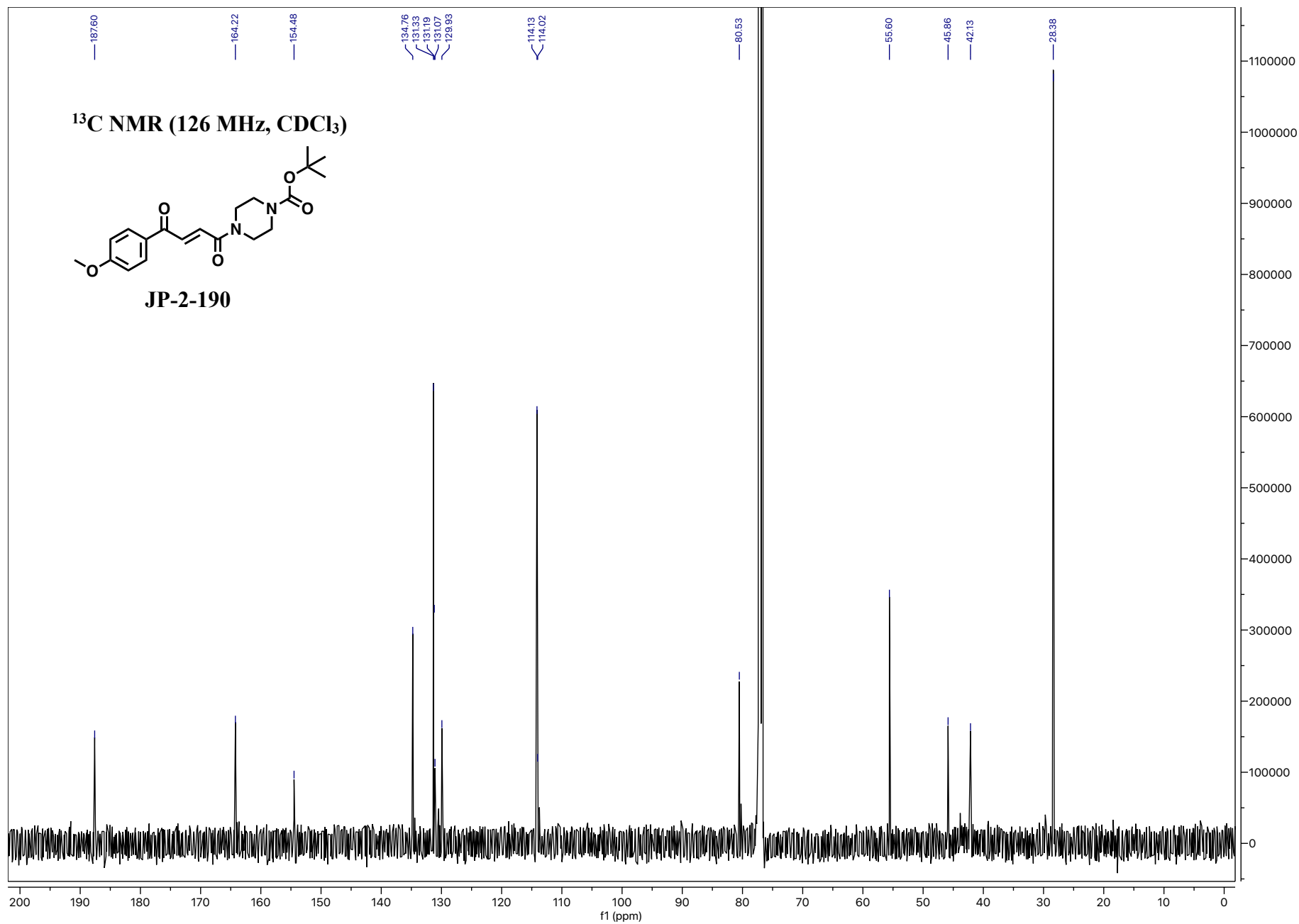


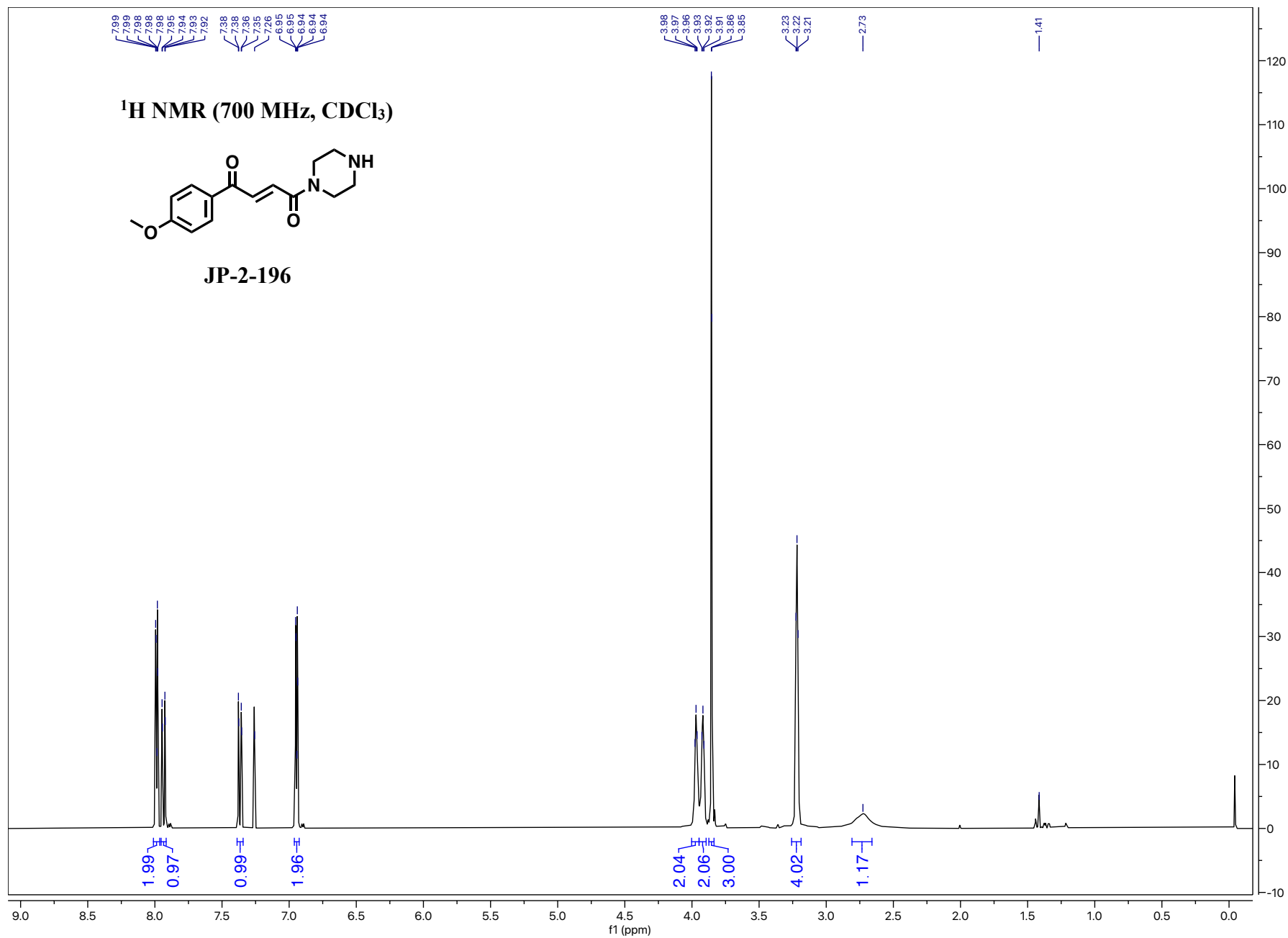




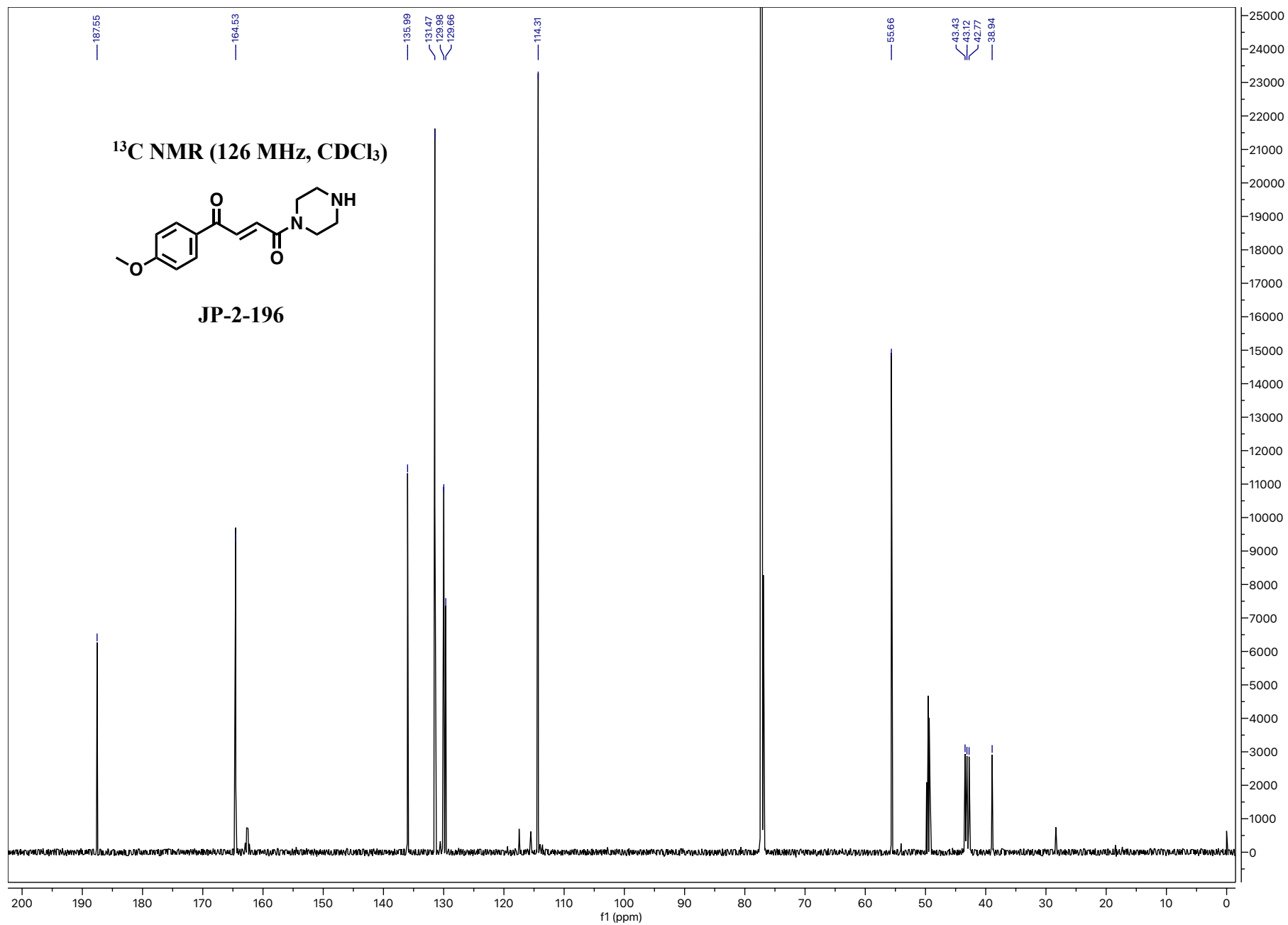












HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

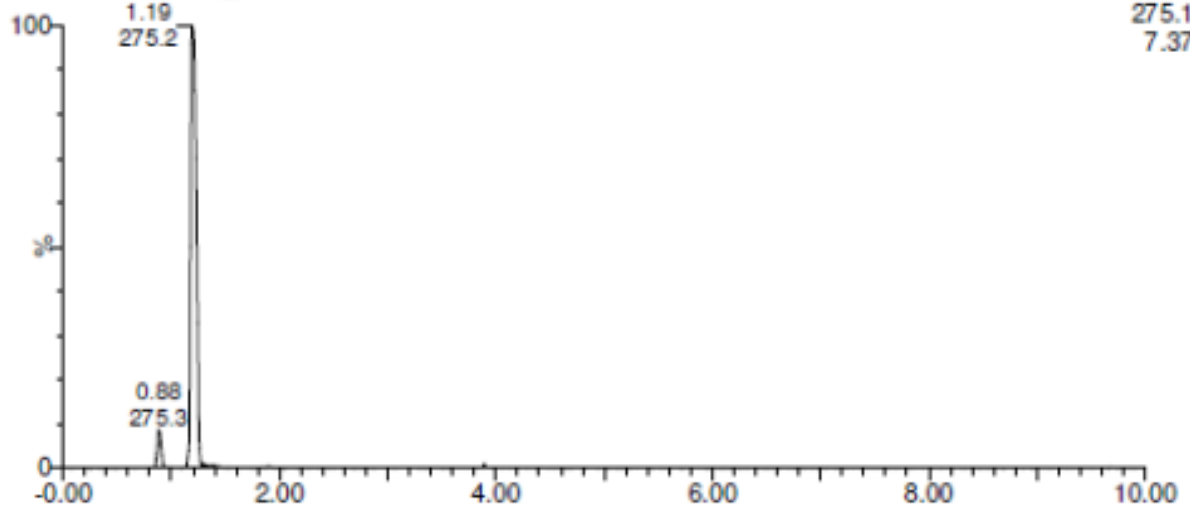
EM23001594446

08-Feb-2023  
17:28:17  
strubhe1-0208A-v21

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
275.132  
7.37e7

HESSEMA4-101-EXP236-S01\_OC

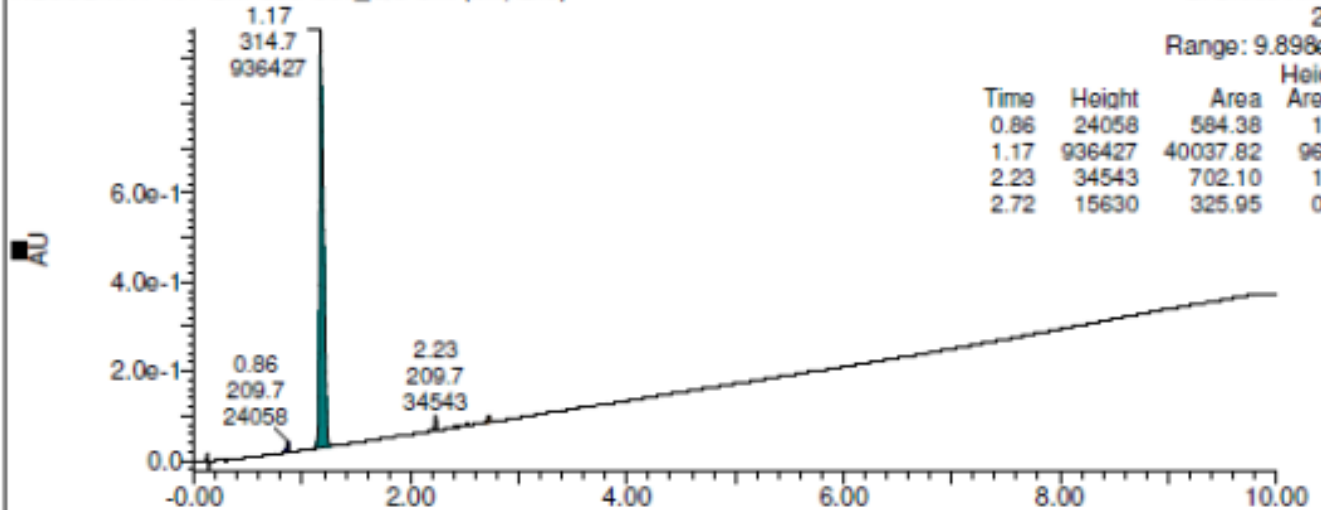


HESSEMA4-101-EXP236-S01\_OC Sm (Mn, 2x3)

2: Diode Array  
220

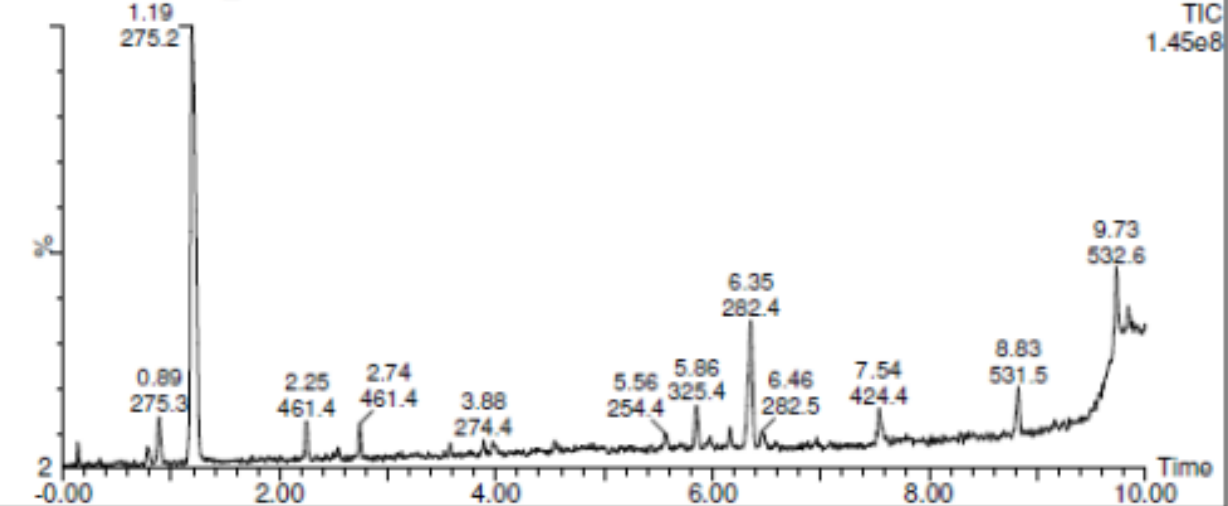
Range: 9.898e-1  
Height

Time	Height	Area	Area%
0.86	24058	584.38	1.40
1.17	936427	40037.82	96.13
2.23	34543	702.10	1.69
2.72	15630	325.95	0.78



HESSEMA4-101-EXP236-S01\_OC

1: Scan ES+  
TIC  
1.45e8



HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

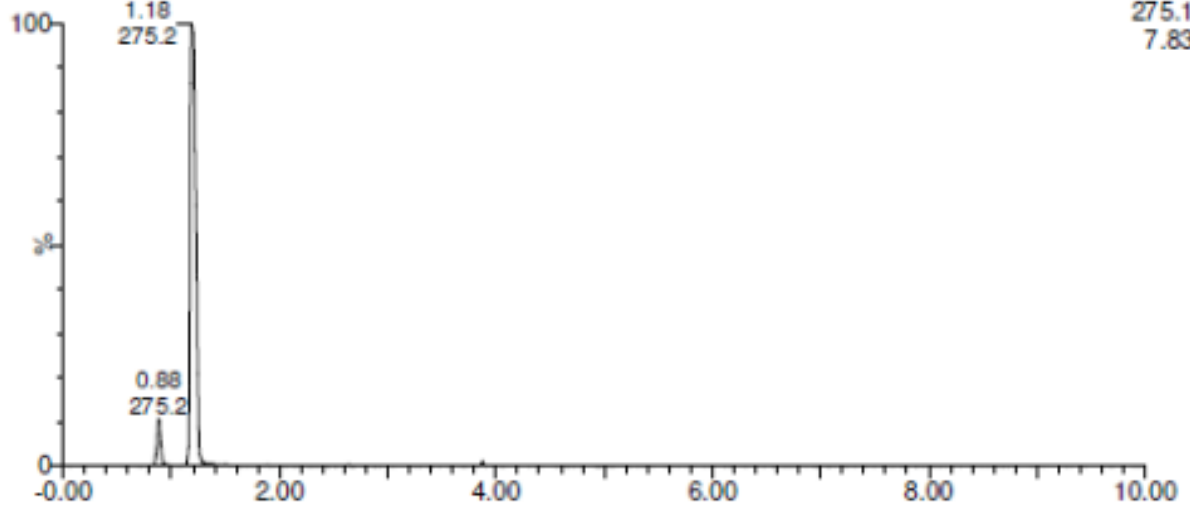
EM23001594446

14-Feb-2023  
13:28:46  
strubhe1-0208A-v21

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
275.132  
7.83e7

HESSEMA4-101-EXP236-S01\_6d\_QC

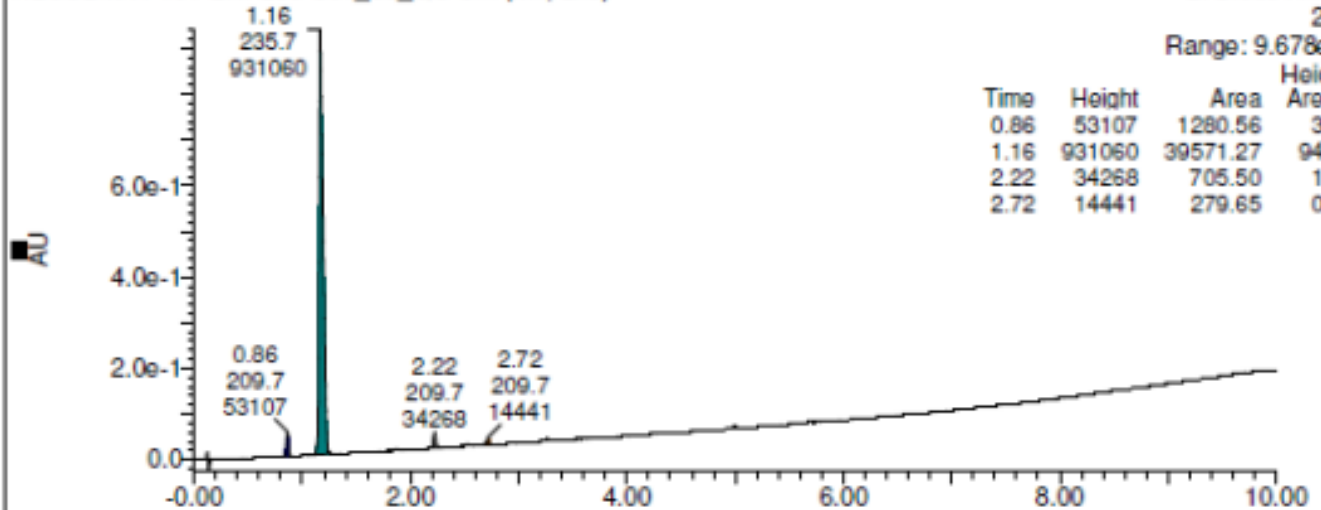


HESSEMA4-101-EXP236-S01\_6d\_QC Sm (Mn, 2x3)

2: Diode Array  
220

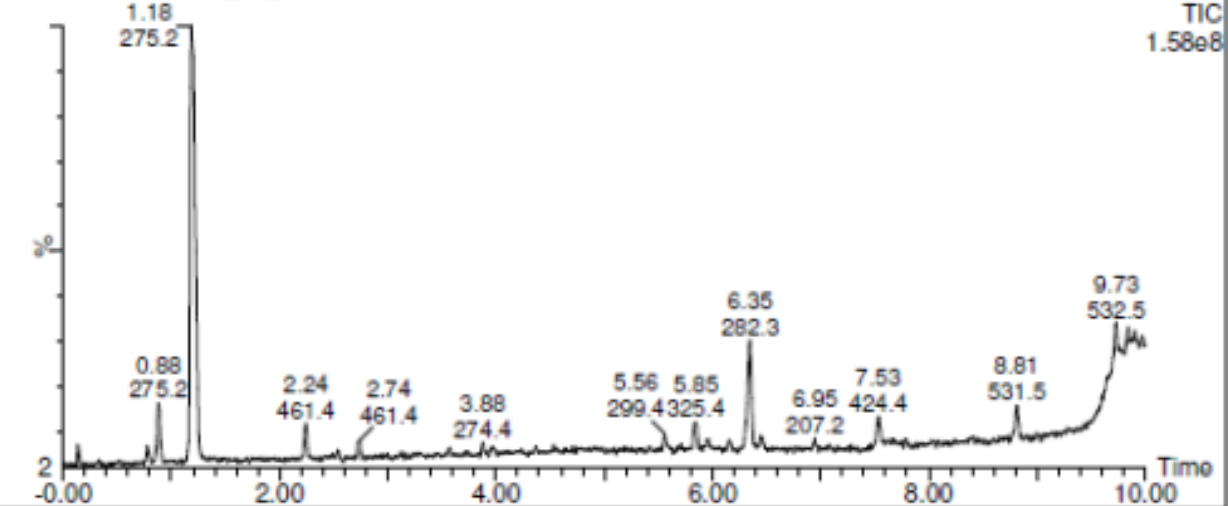
Range: 9.678e-1  
Height

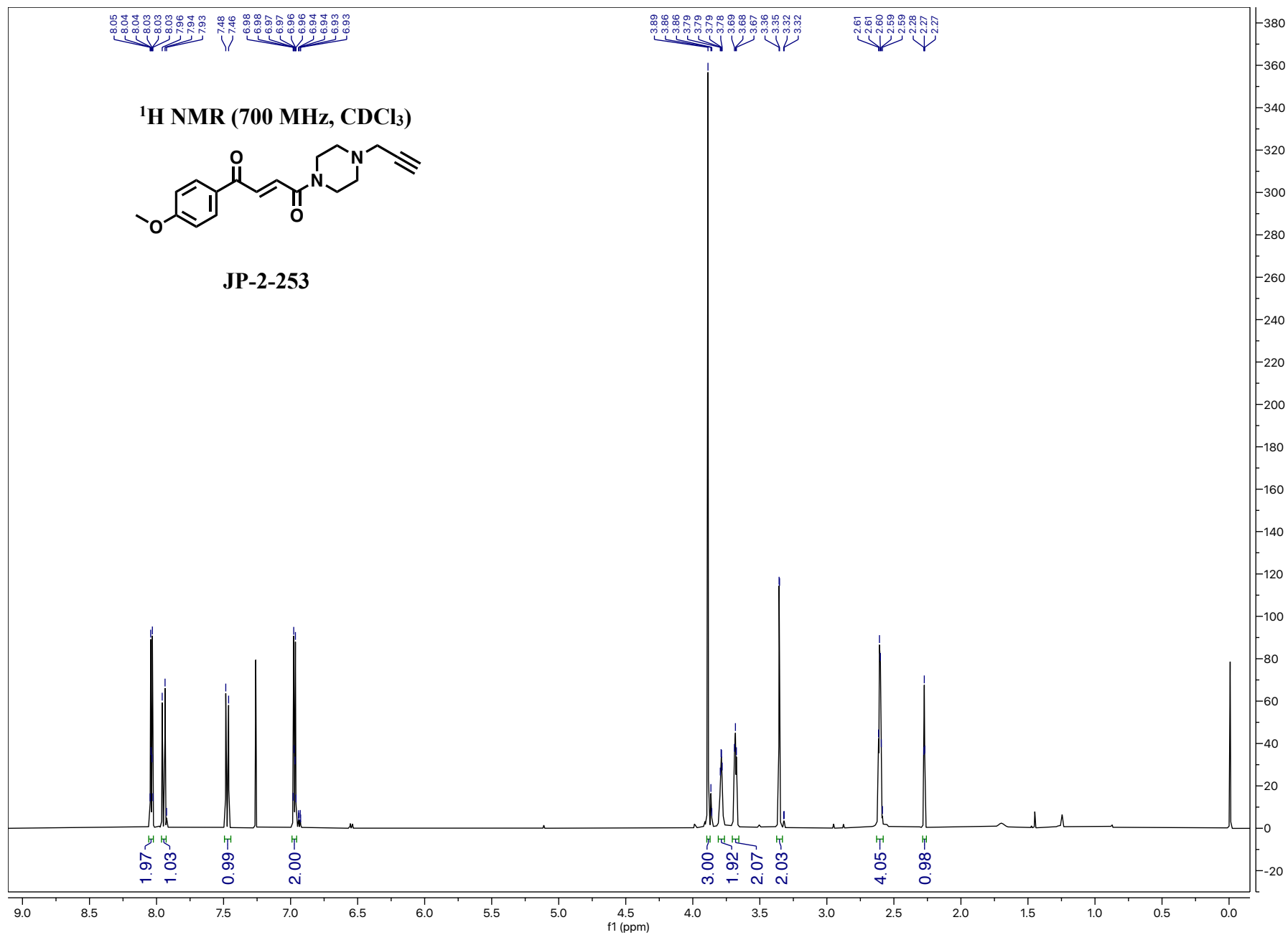
Time	Height	Area	Area%
0.86	53107	1280.56	3.06
1.16	931060	39571.27	94.58
2.22	34268	705.50	1.69
2.72	14441	279.65	0.67

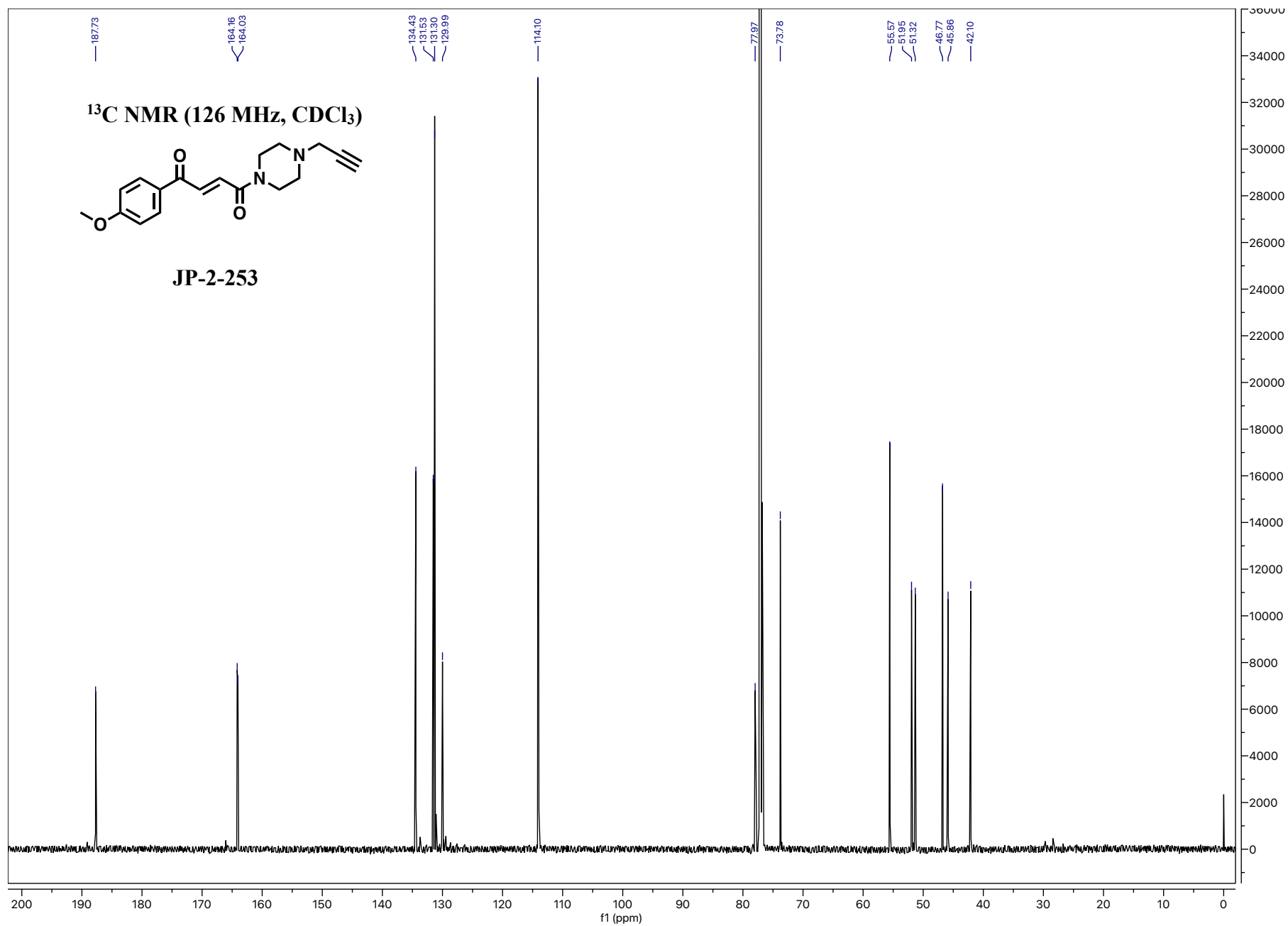


HESSEMA4-101-EXP236-S01\_6d\_QC

1: Scan ES+  
TIC  
1.58e8







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

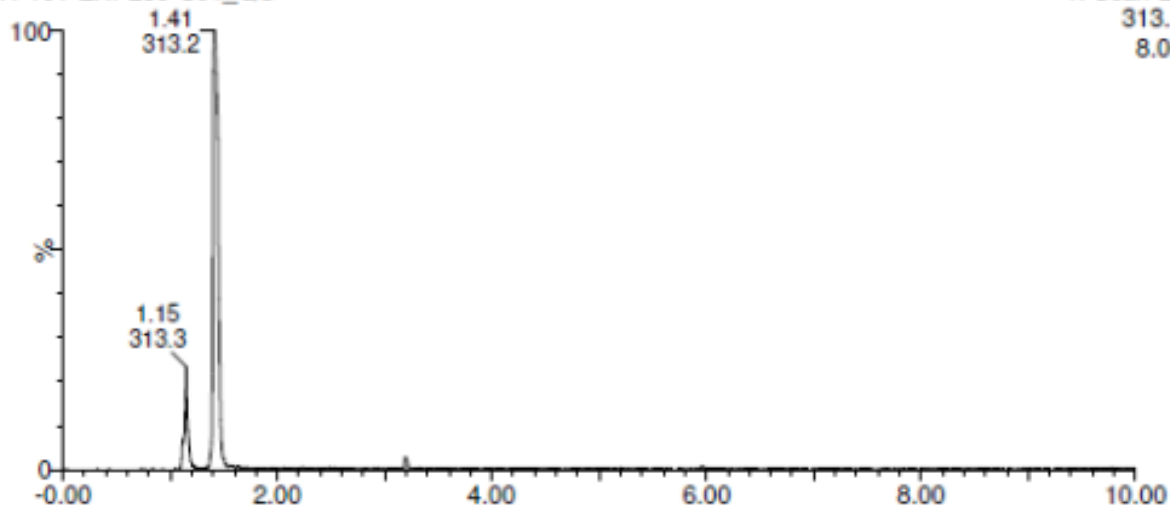
EM23001594453

08-Feb-2023  
18:01:28  
strubhe1-0208A-v24

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

HESSEMA4-101-EXP239-S01\_OC

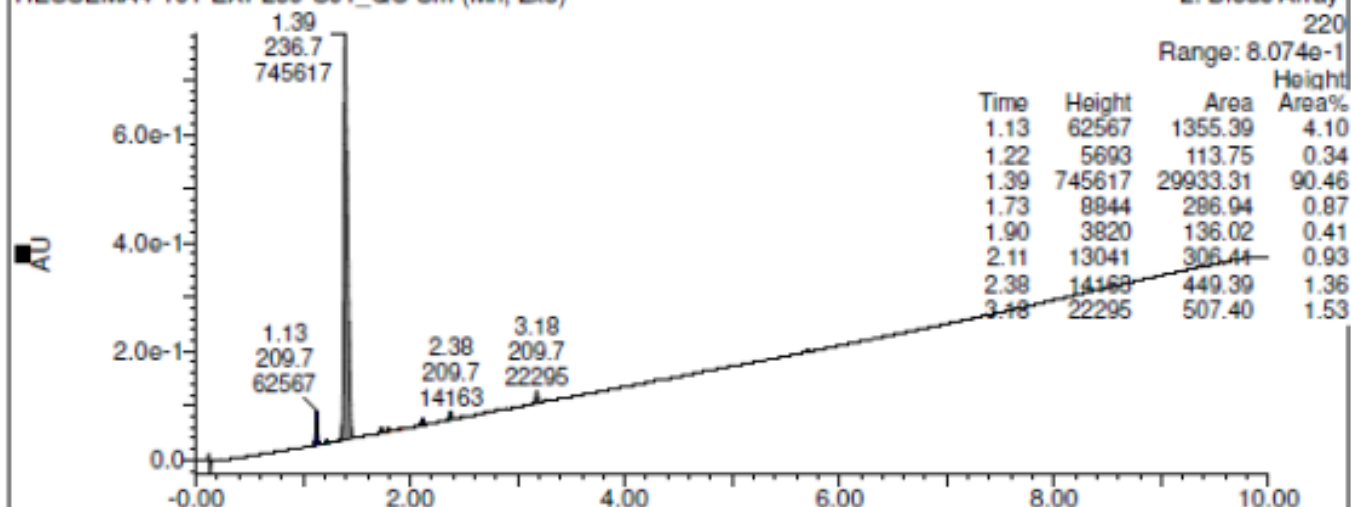
1: Scan ES+  
313.147  
8.06e7



HESSEMA4-101-EXP239-S01\_OC Sm (Mn, 2x3)

2: Diode Array  
220

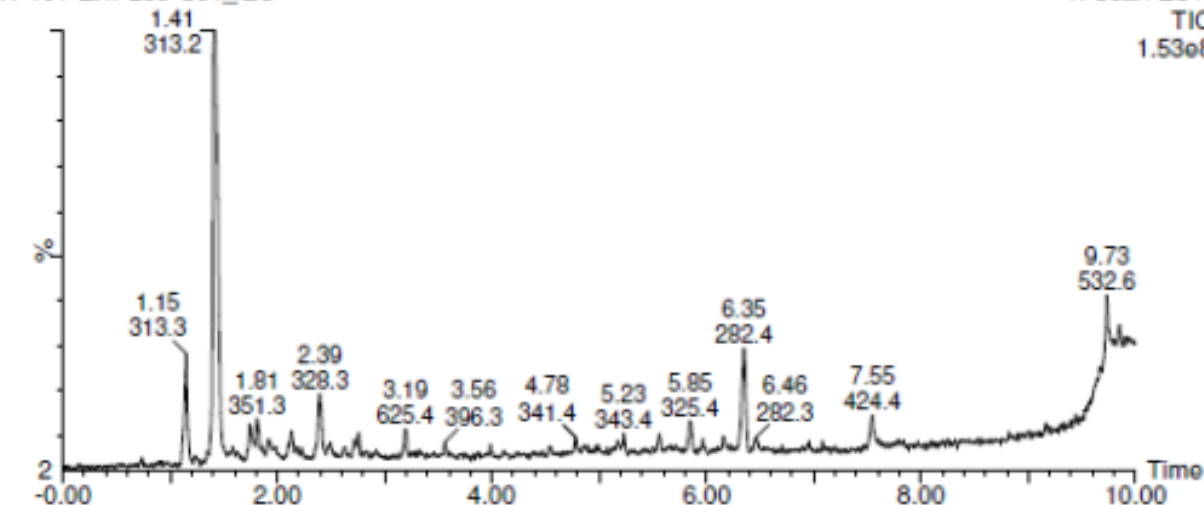
Range: 8.074e-1



HESSEMA4-101-EXP239-S01\_OC

1: Scan ES+

TIC  
1.53e8



HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

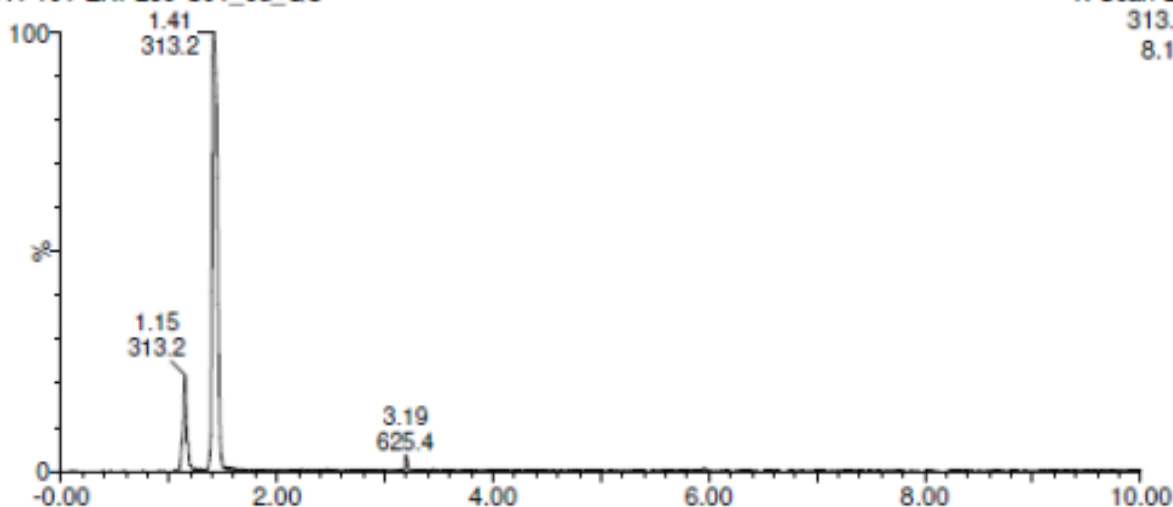
EM23001594453

14-Feb-2023  
13:51:33  
strubhe1-0208A-v24

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

HESSEMA4-101-EXP239-S01\_6d\_QC

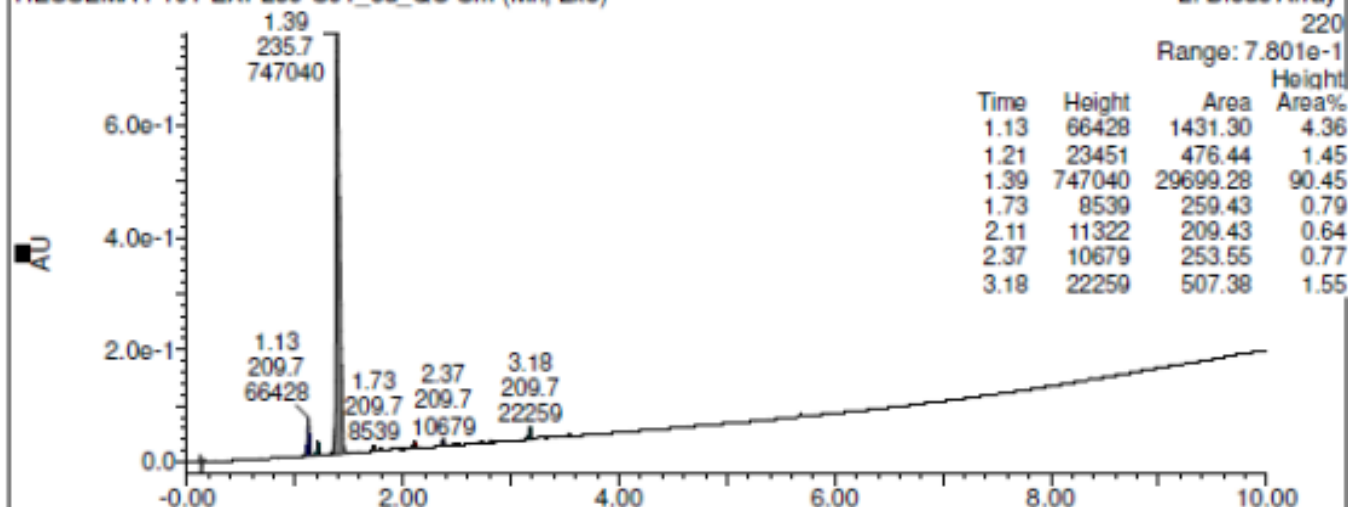
1: Scan ES+  
313.147  
8.13e7



HESSEMA4-101-EXP239-S01\_6d\_QC Sm (Mn, 2x3)

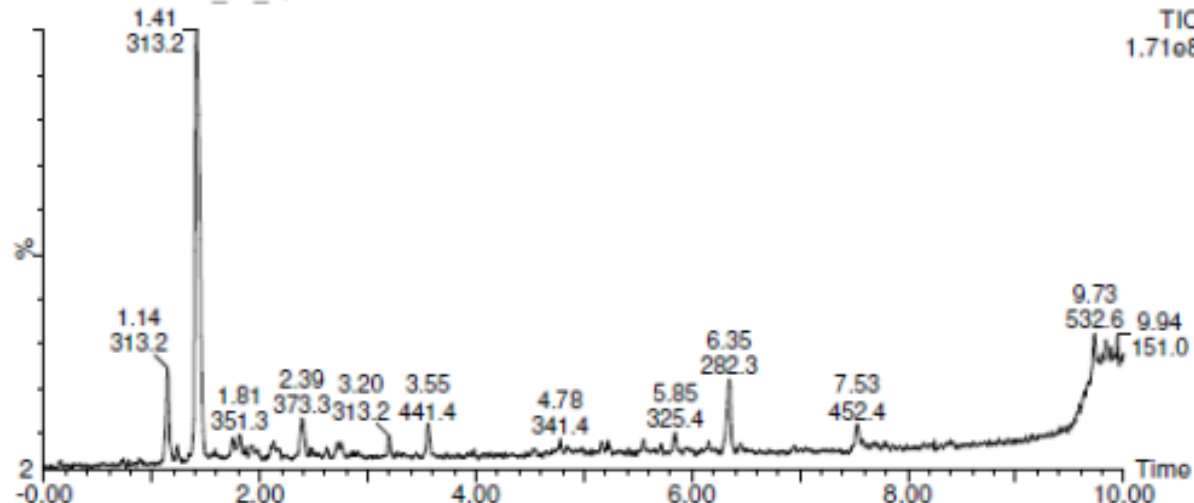
2: Diode Array  
220

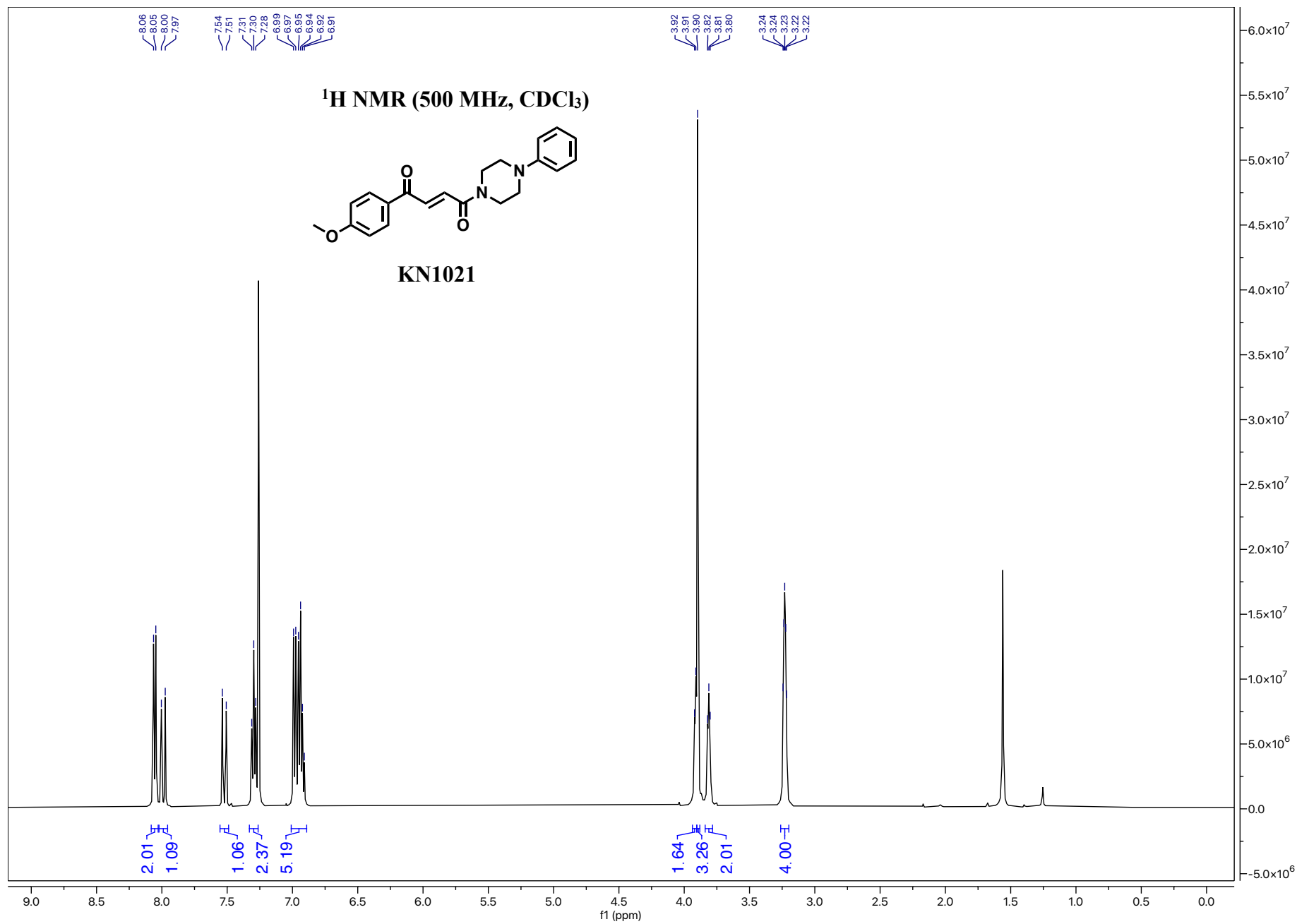
Range: 7.801e-1



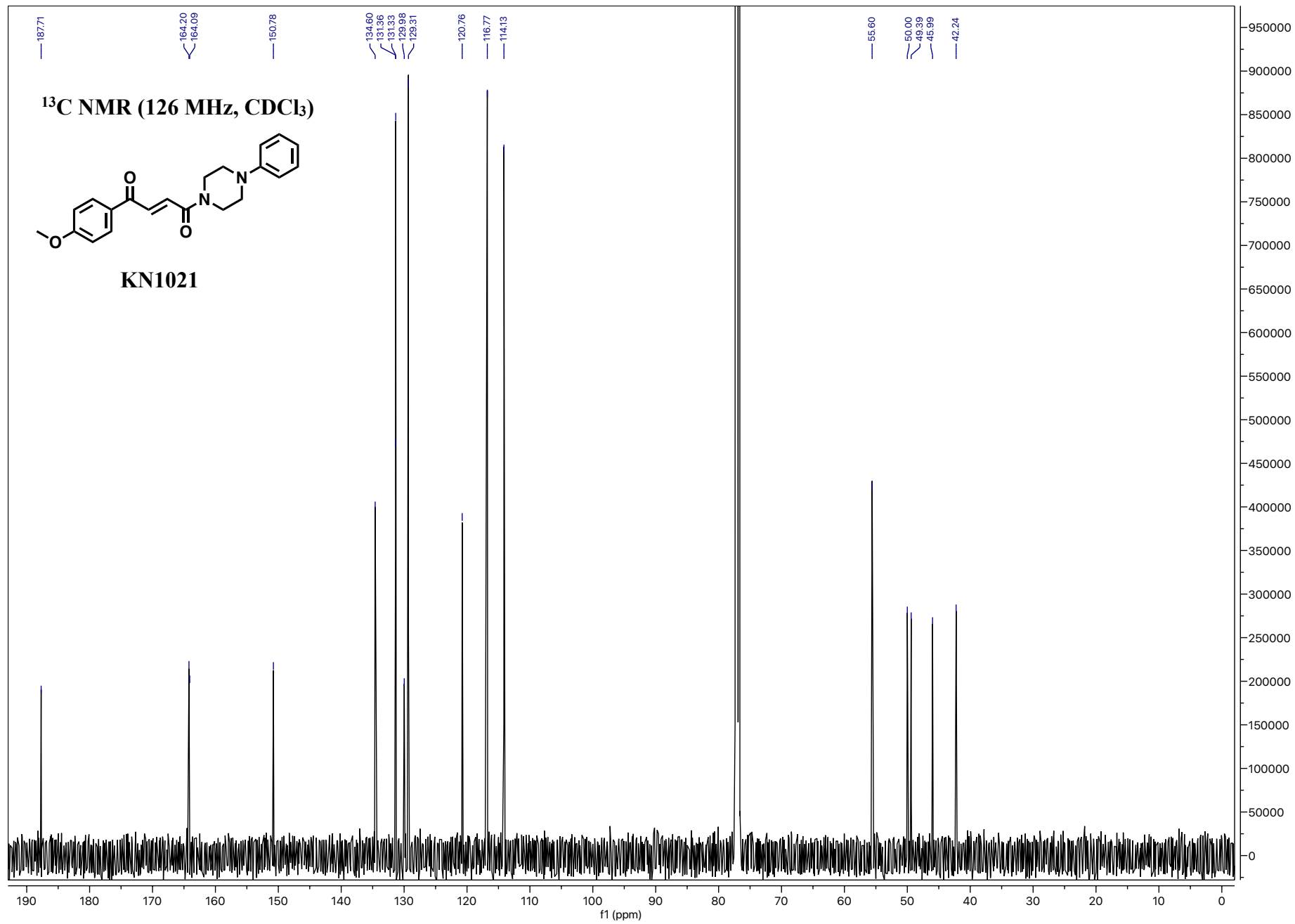
HESSEMA4-101-EXP239-S01\_6d\_QC

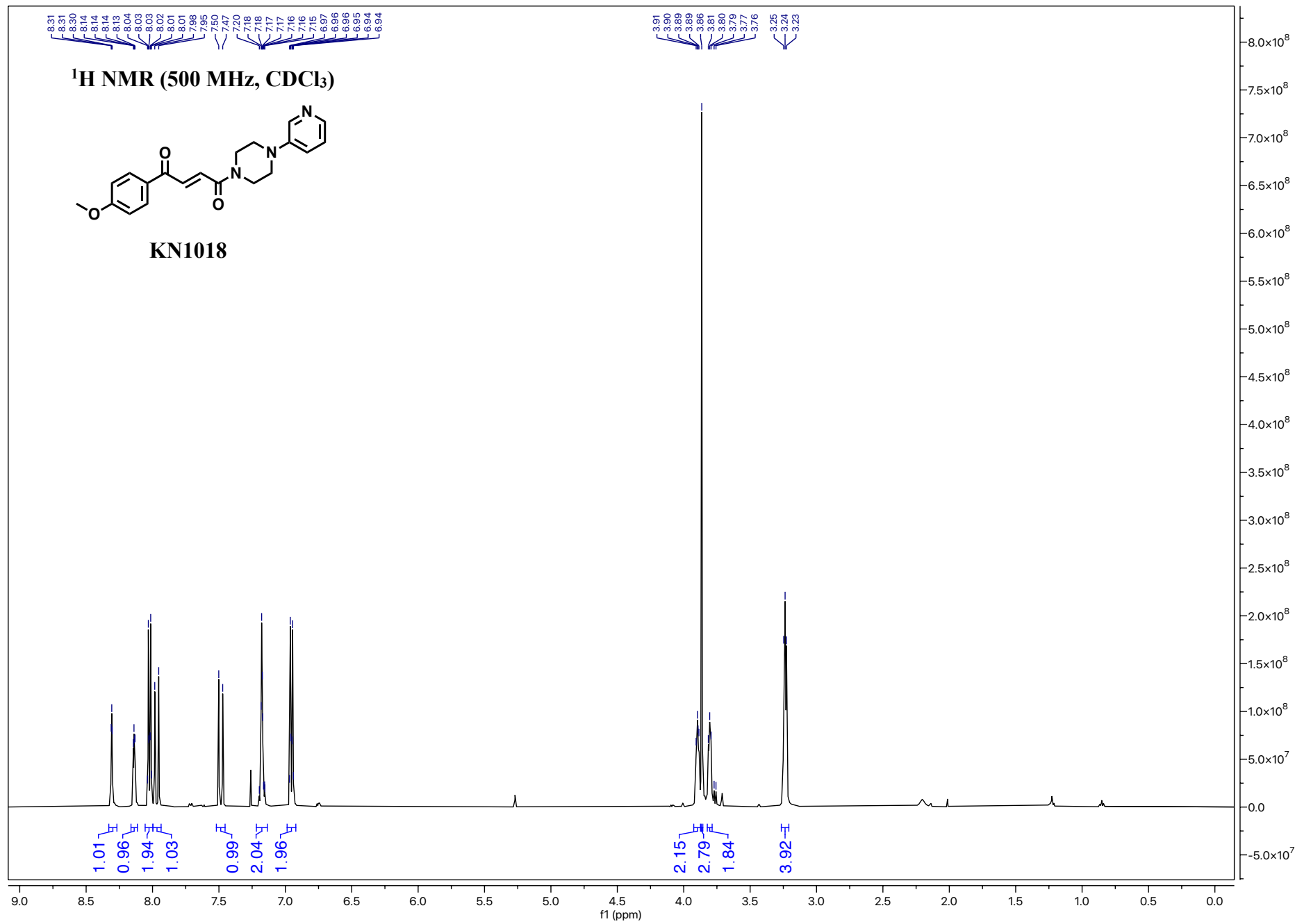
1: Scan ES+  
TIC  
1.71e8

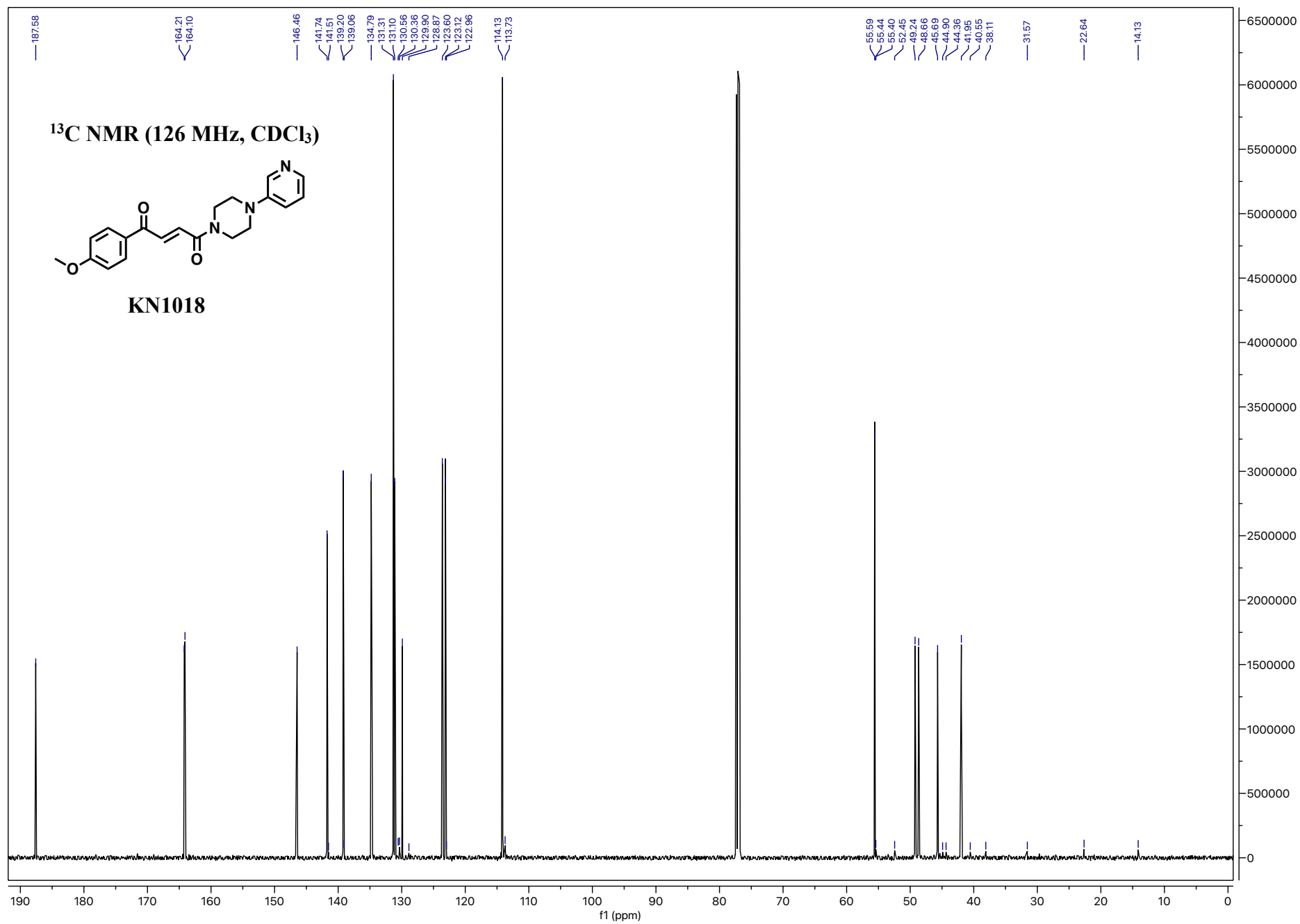


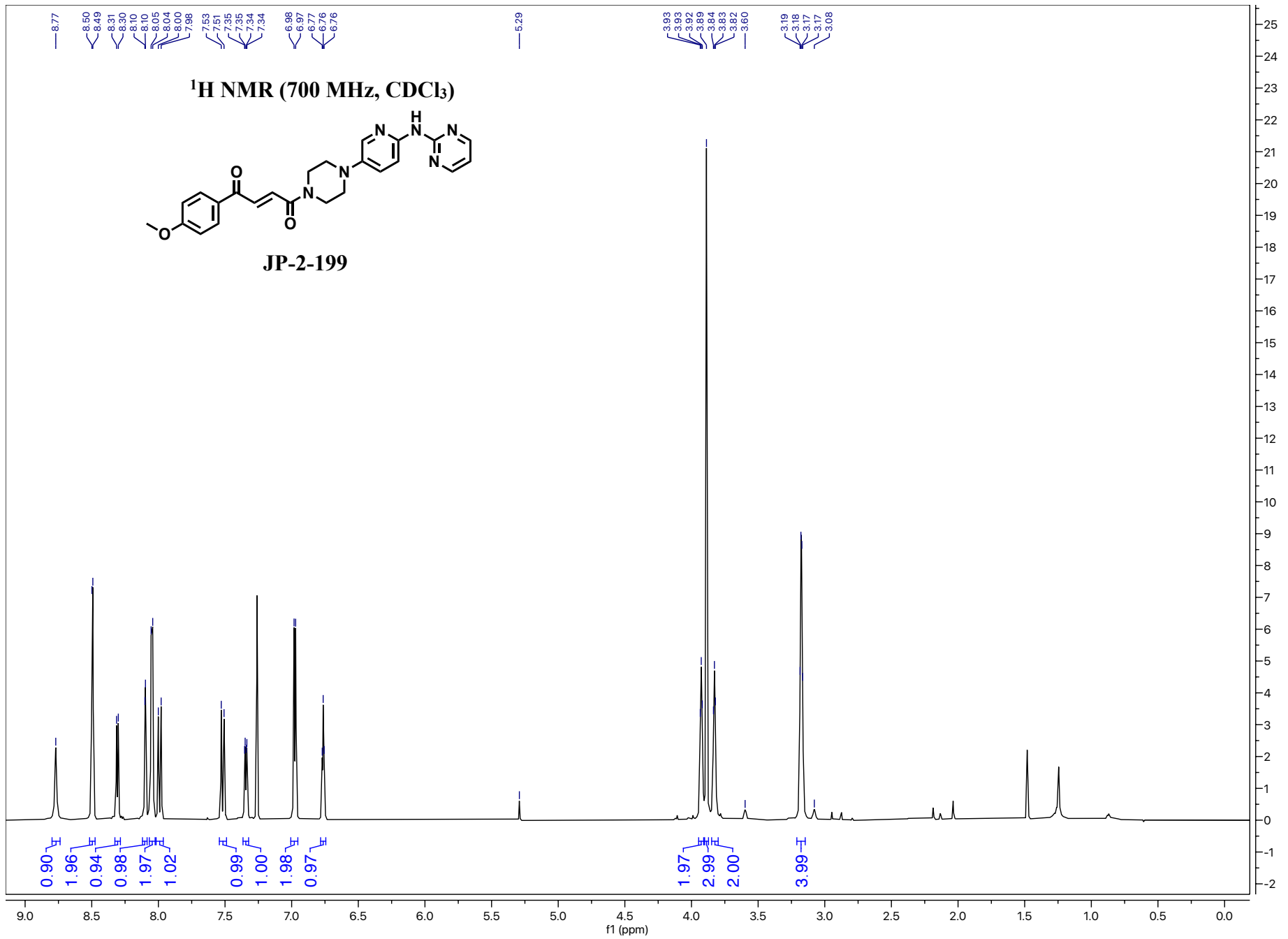


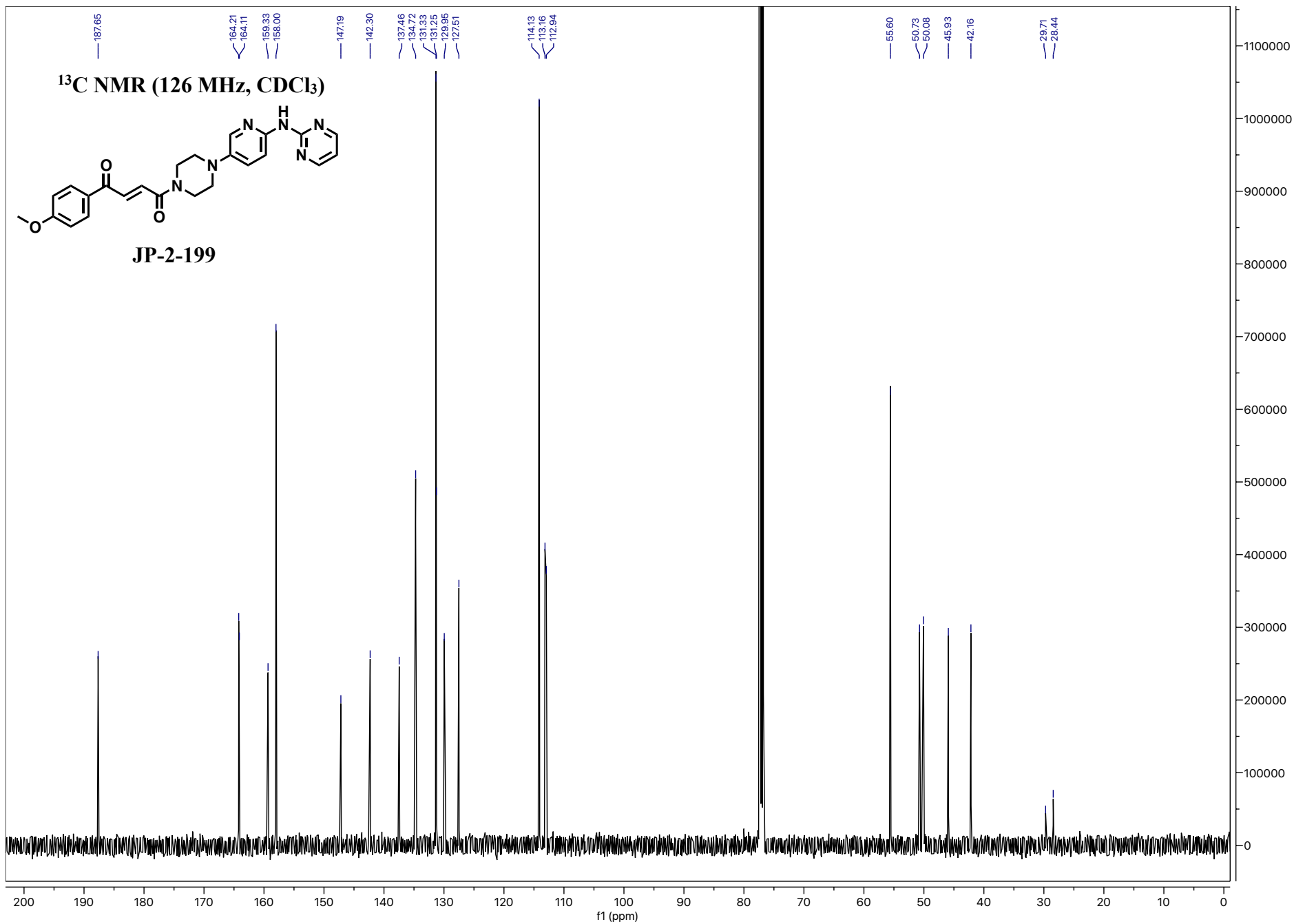


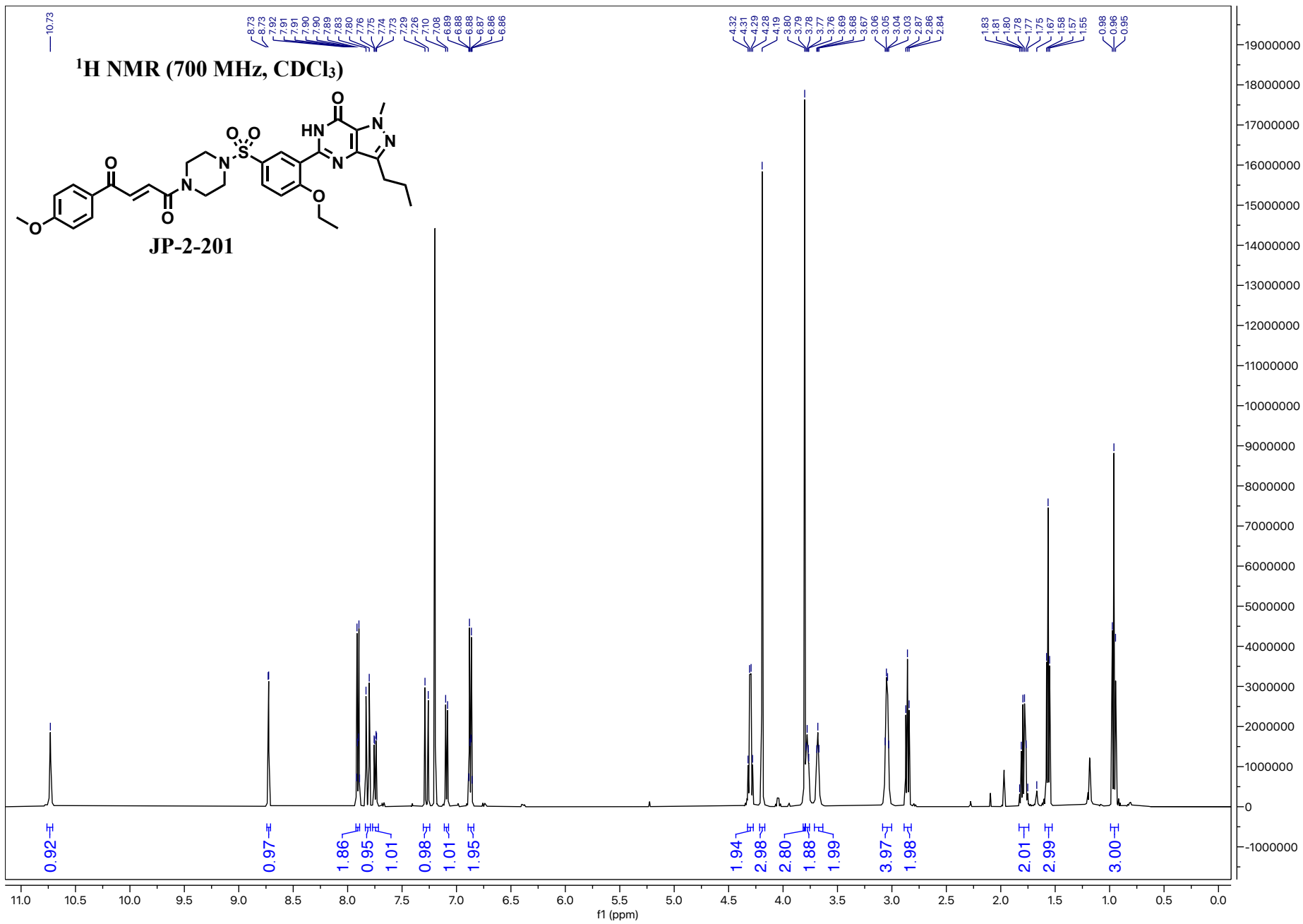


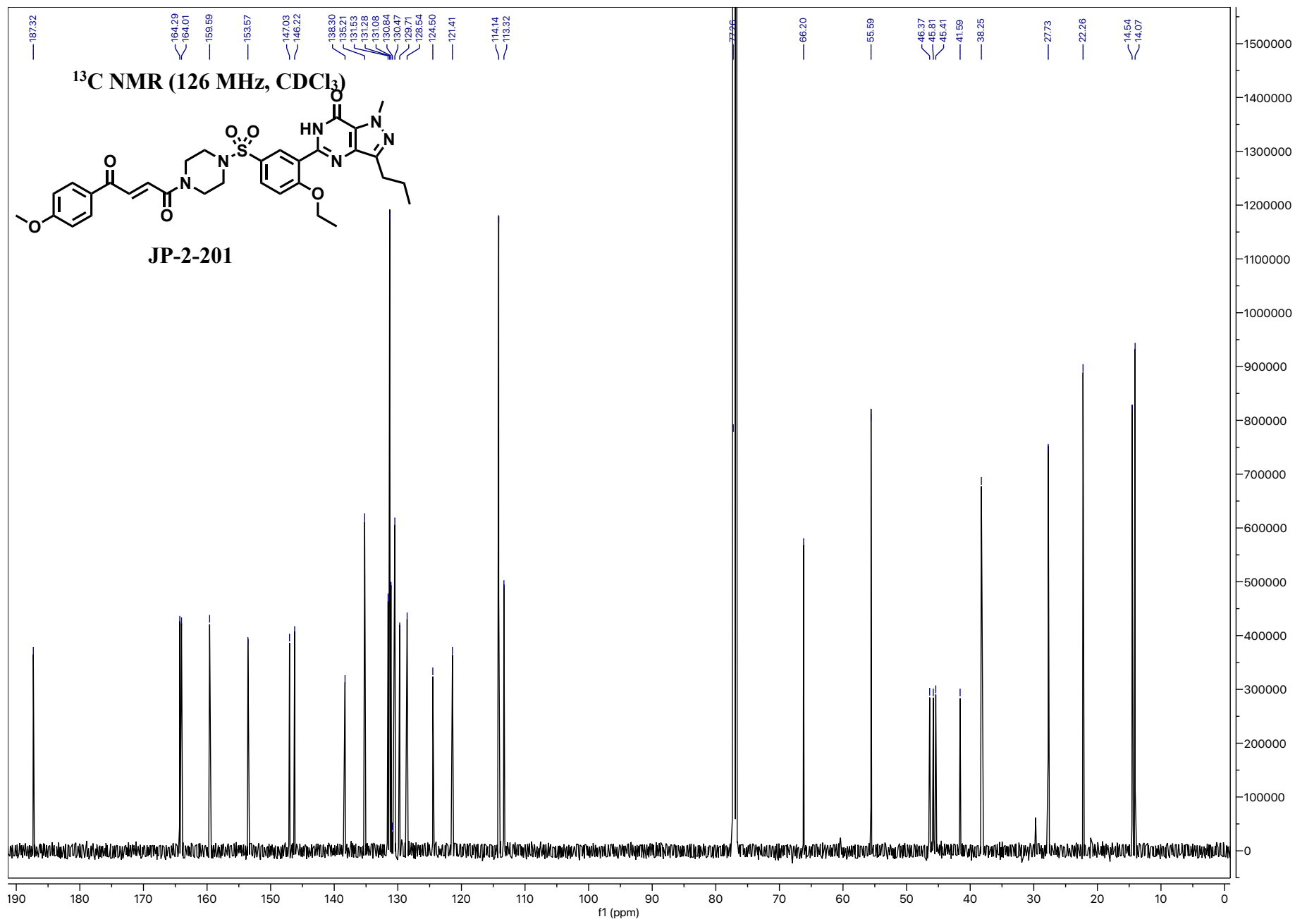












HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

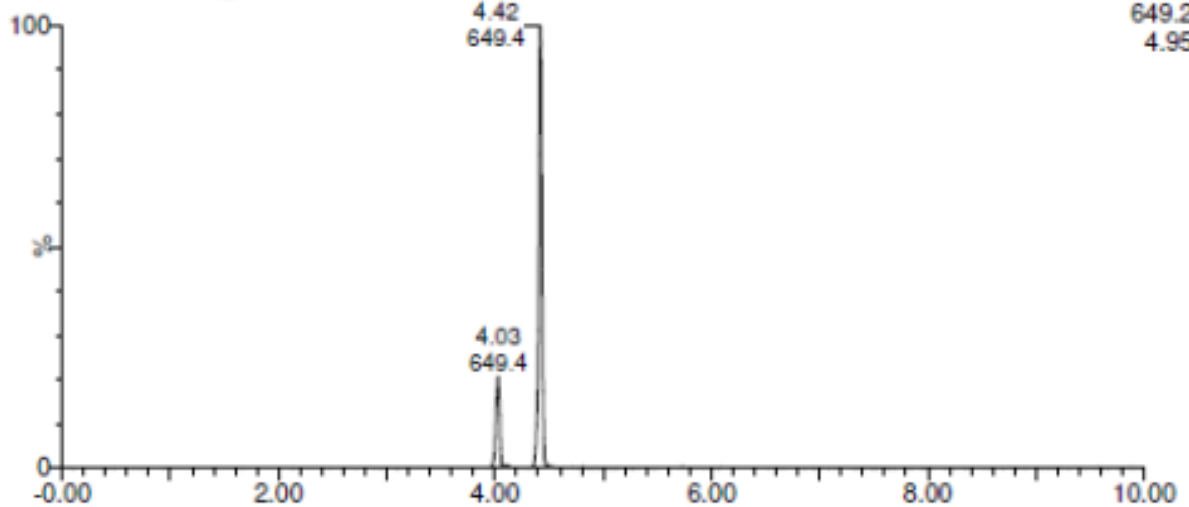
EM23001590294

13-Jan-2023  
16:27:51  
strubhe1-0112A-v08

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

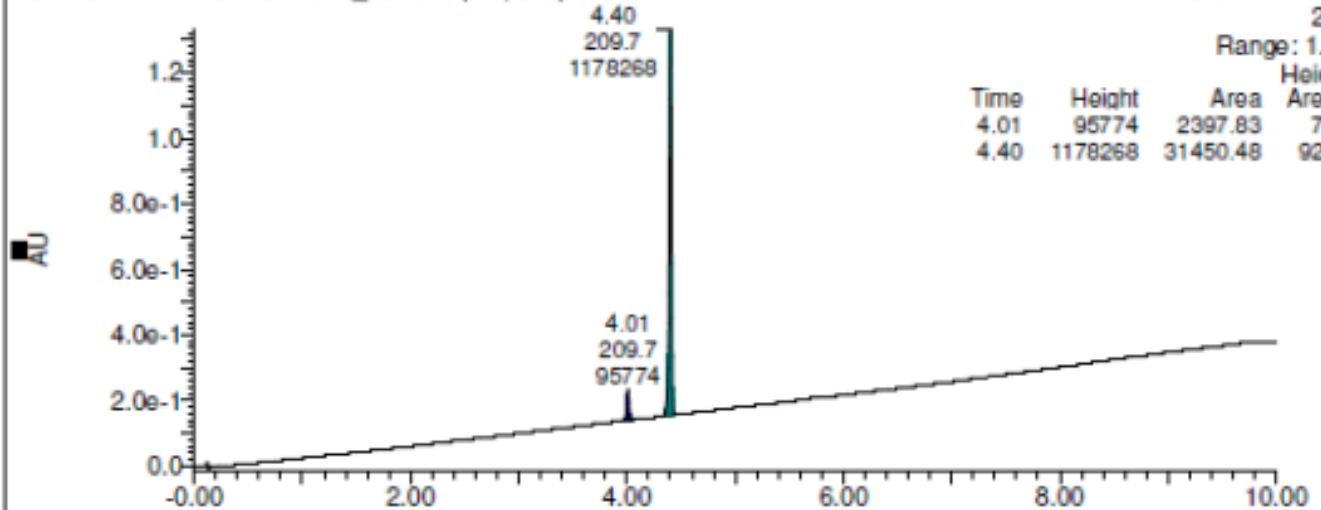
1: Scan ES+  
649.237  
4.95e7

HESSEMA4-101-EXP211-S01\_QC



HESSEMA4-101-EXP211-S01\_QC Sm (Mn, 2x3)

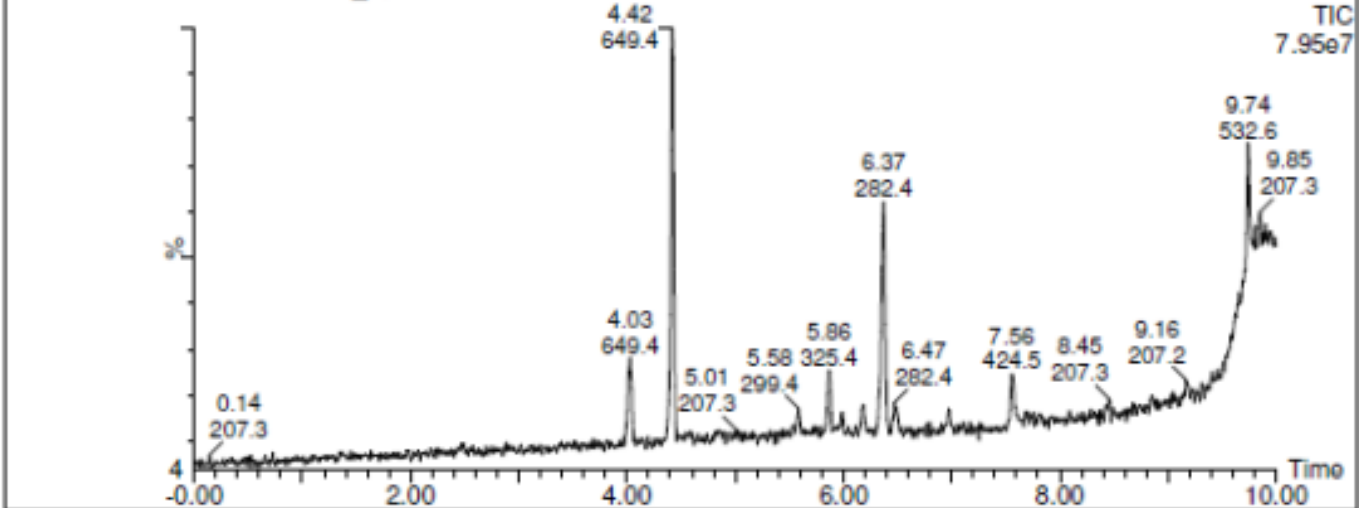
2: Diode Array  
220  
Range: 1.35  
Height



Time	Height	Area	Area%
4.01	95774	2397.83	7.08
4.40	1178268	31450.48	92.92

HESSEMA4-101-EXP211-S01\_QC

1: Scan ES+  
TIC  
7.95e7





HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

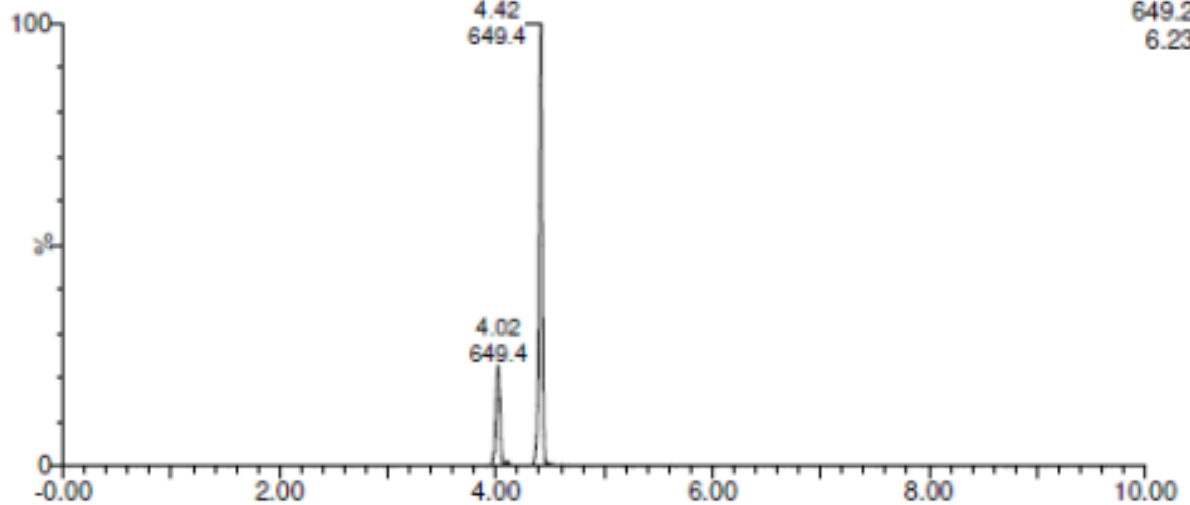
EM23001590294

19-Jan-2023  
16:53:50  
strubhe1-0118A-v08

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

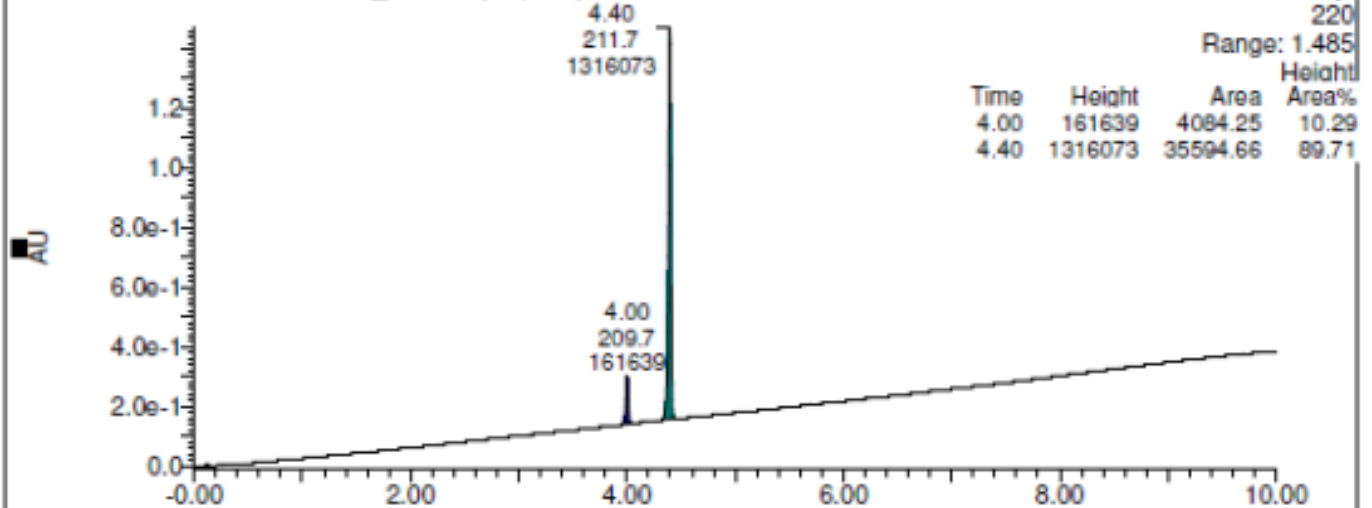
1: Scan ES+  
649.237  
6.23e7

HESSEMA4-101-EXP211-S01R\_OC



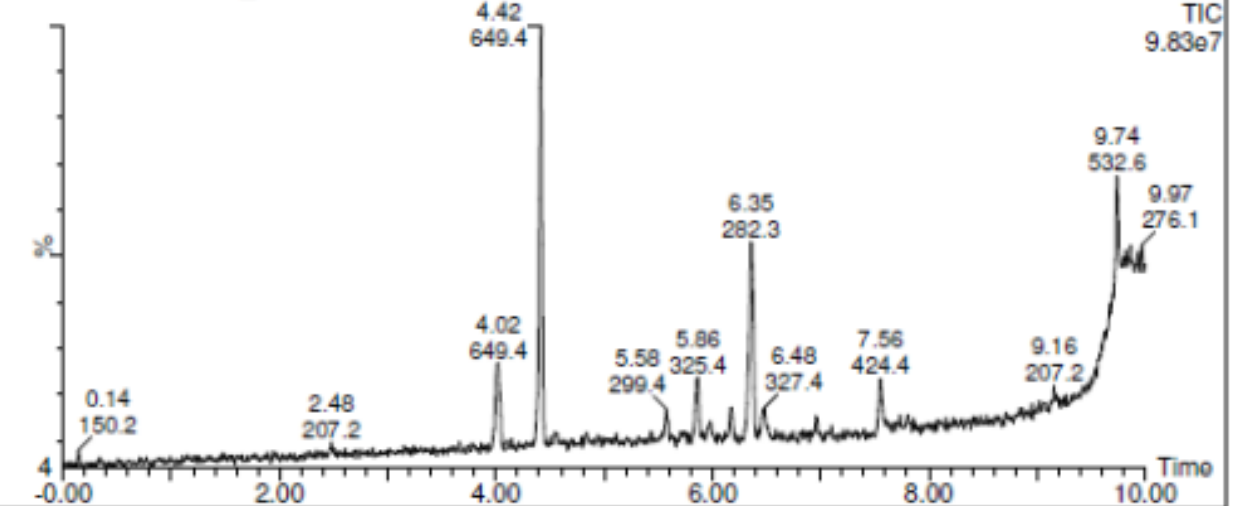
HESSEMA4-101-EXP211-S01R\_OC Sm (Mn, 2x3)

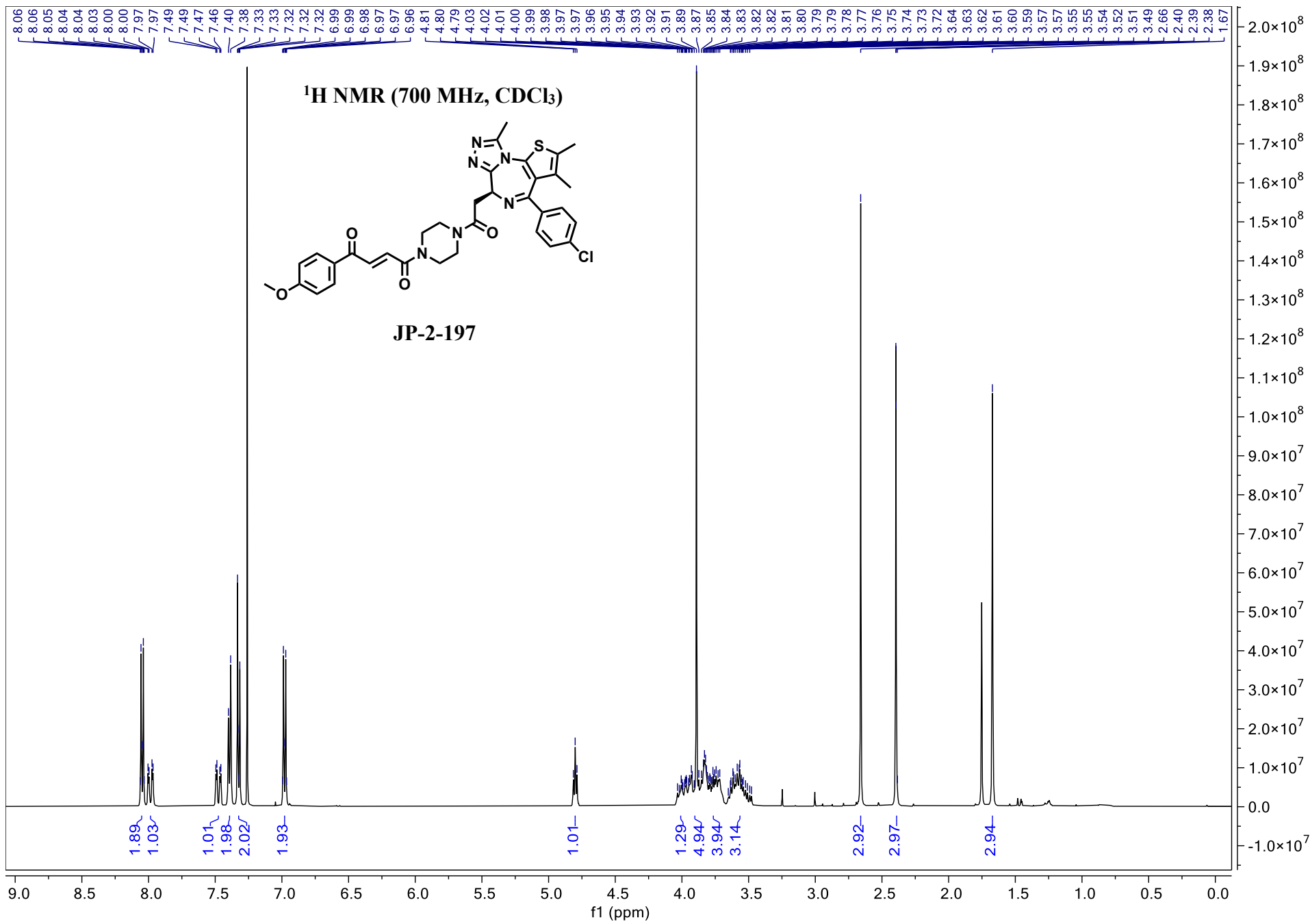
2: Diode Array  
220  
Range: 1.485  
Height

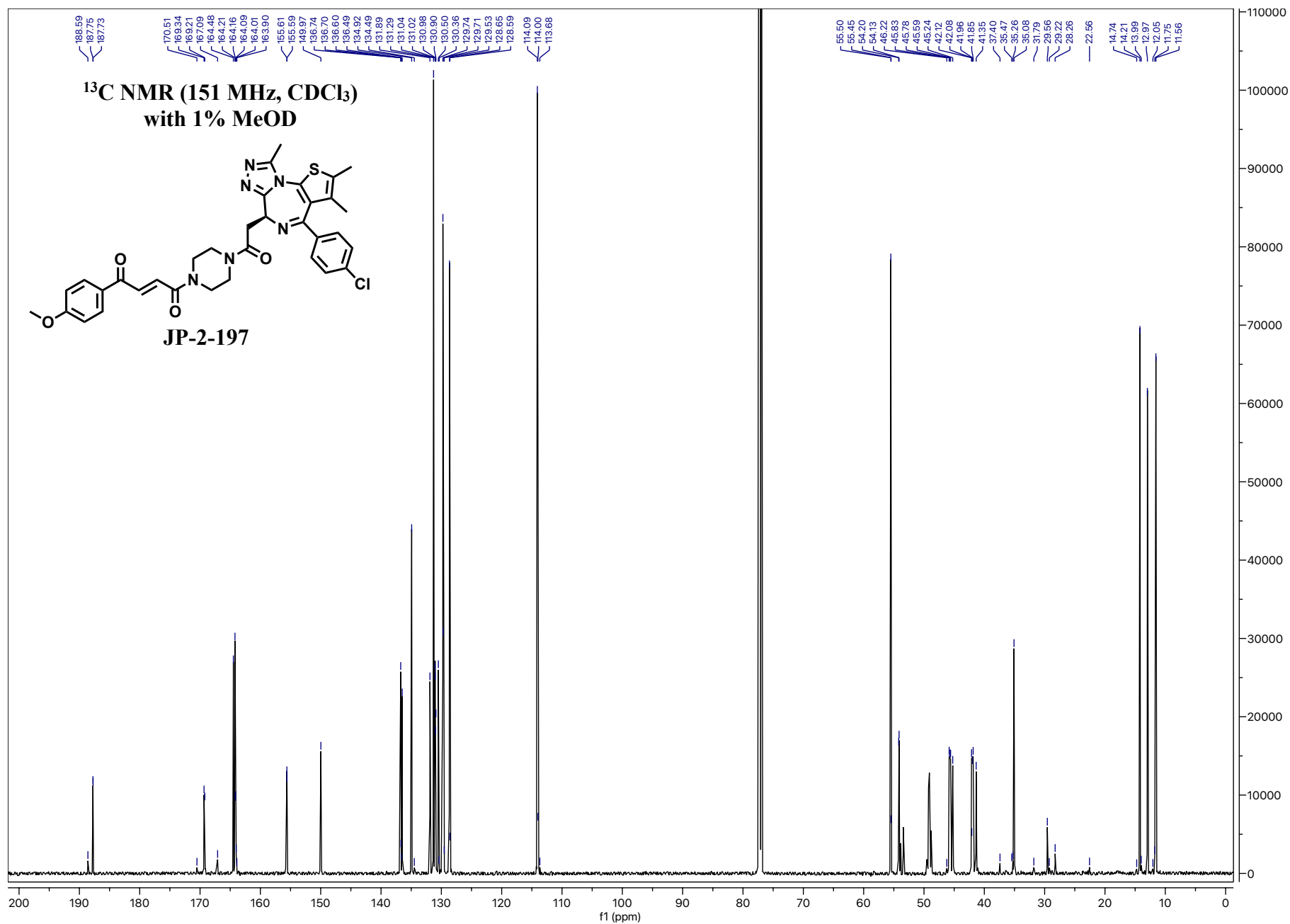


HESSEMA4-101-EXP211-S01R\_OC

1: Scan ES+  
TIC  
9.83e7







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

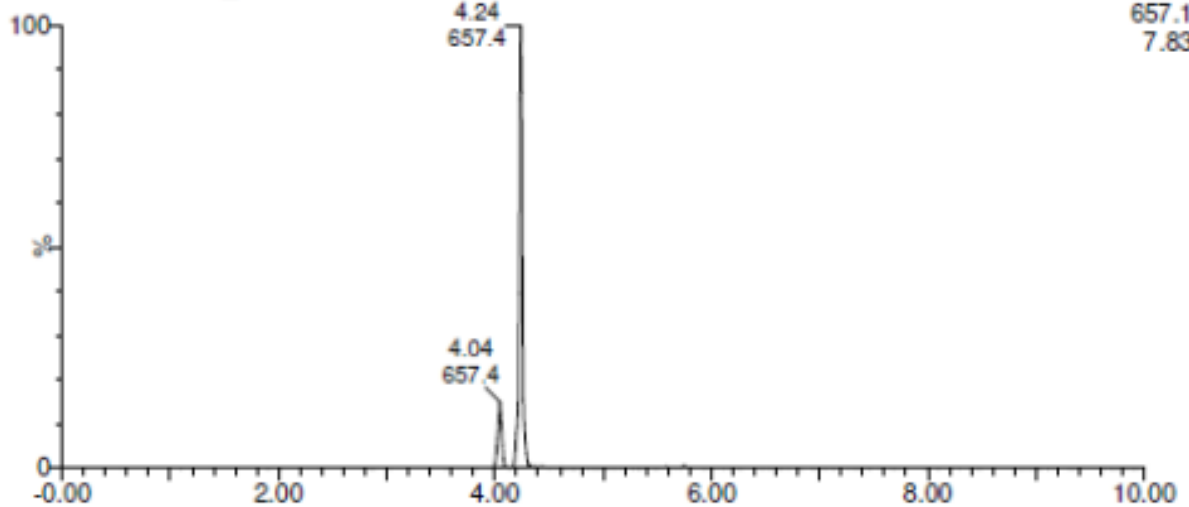
EM23001594422

08-Feb-2023  
14:09:20  
strubhe1-0208A-v03

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

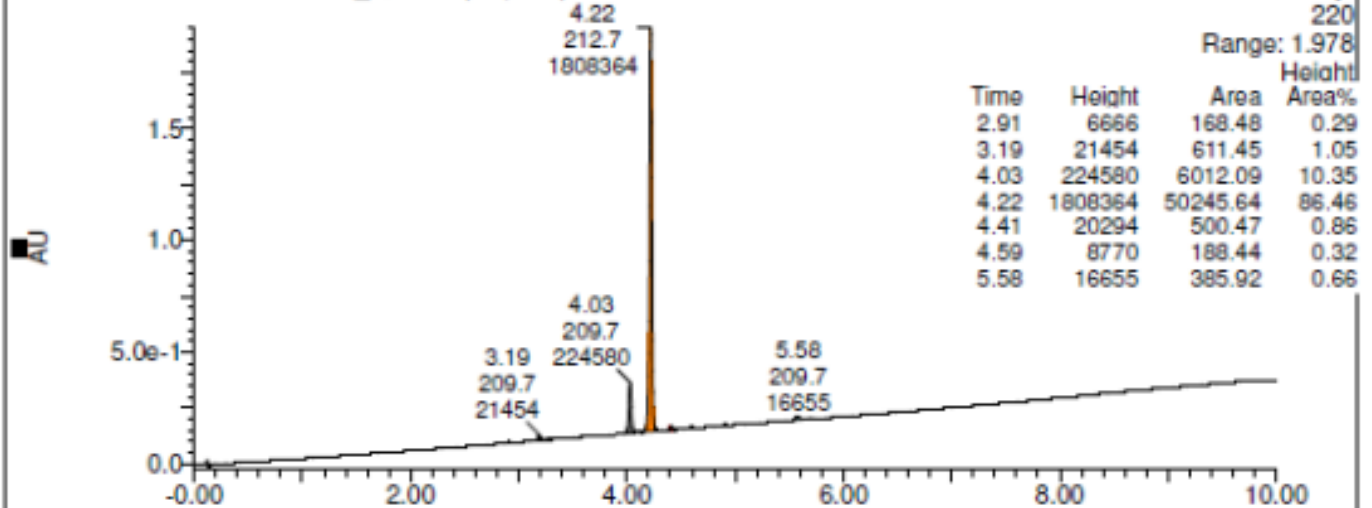
1: Scan ES+  
657.197  
7.83e7

HESSEMA4-101-EXP218-S01\_OC



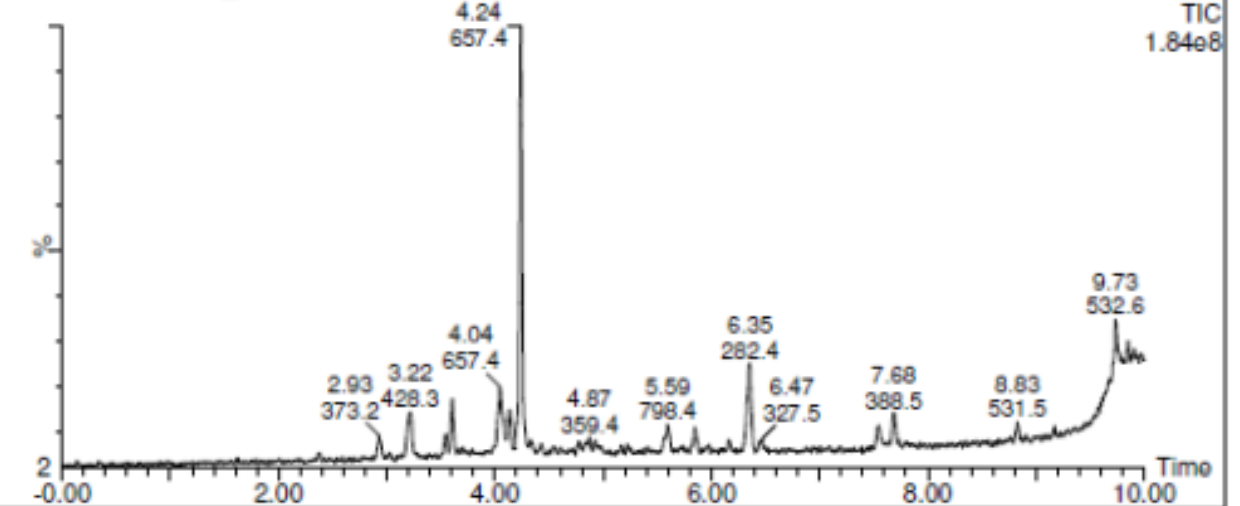
HESSEMA4-101-EXP218-S01\_OC Sm (Mn, 2x3)

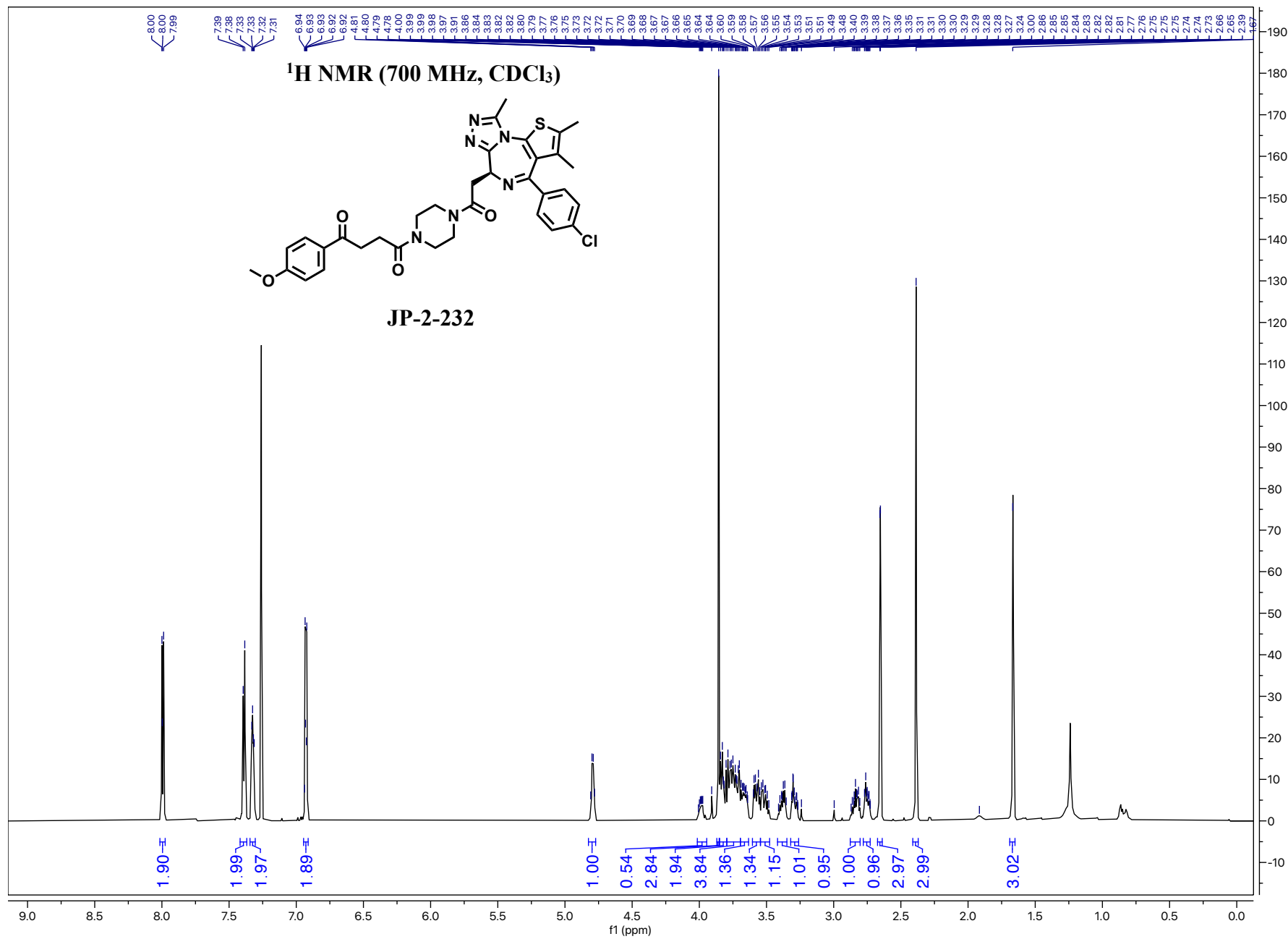
2: Diode Array  
220  
Range: 1.978  
Height

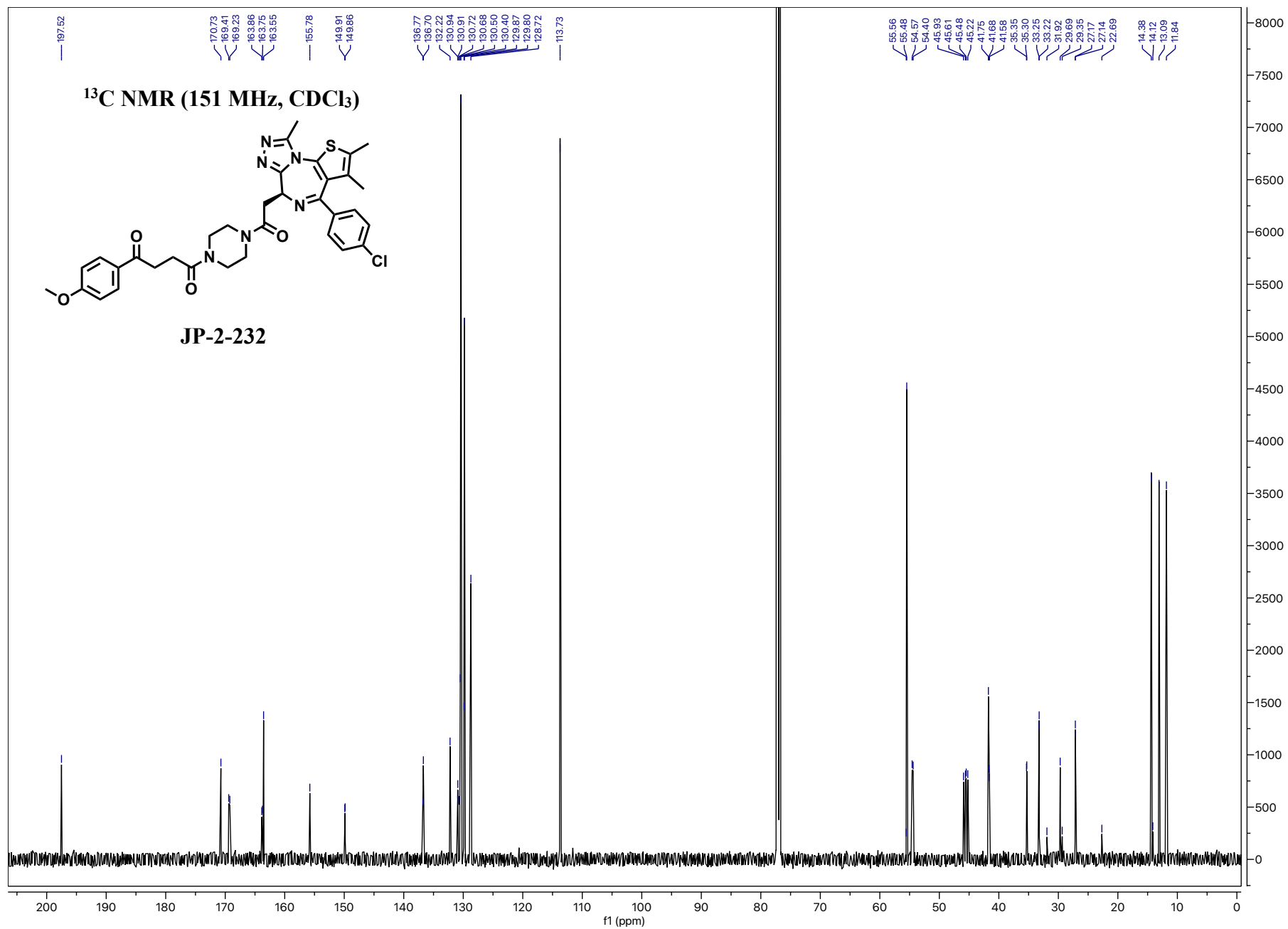


HESSEMA4-101-EXP218-S01\_OC

1: Scan ES+  
TIC  
1.84e8







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

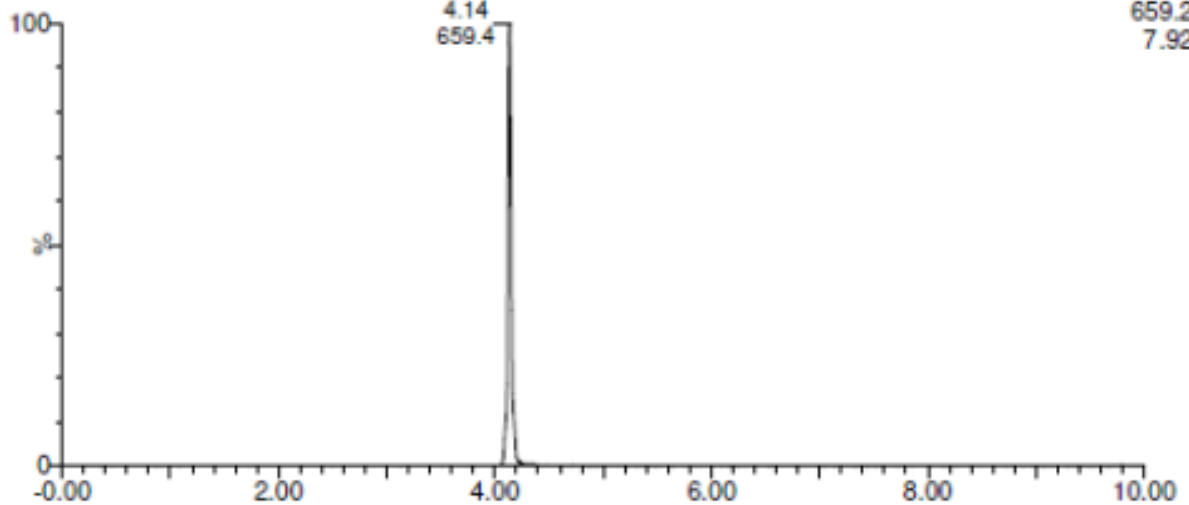
EM23001594447

08-Feb-2023  
17:39:20  
strubhe1-0208A-v22

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

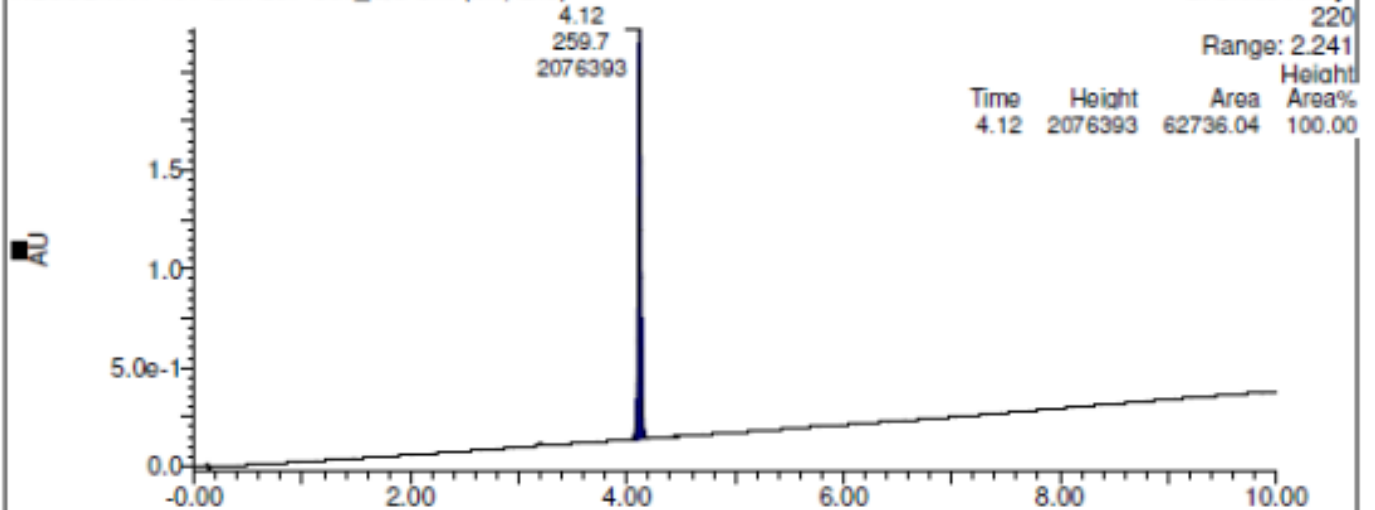
HESSEMA4-101-EXP237-S01\_OC

1: Scan ES+  
659.213  
7.92e7



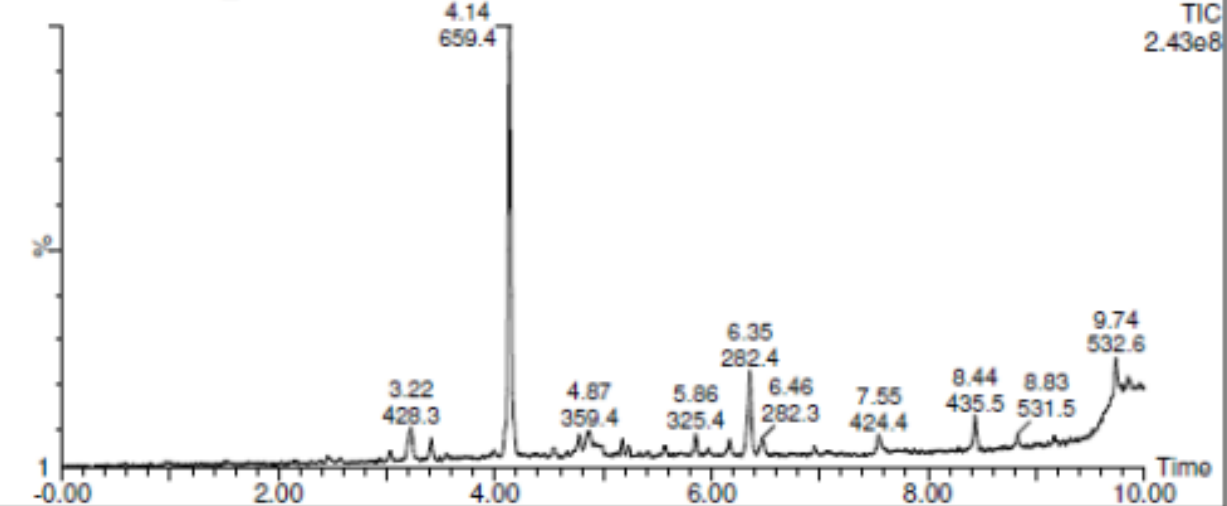
HESSEMA4-101-EXP237-S01\_OC Sm (Mn, 2x3)

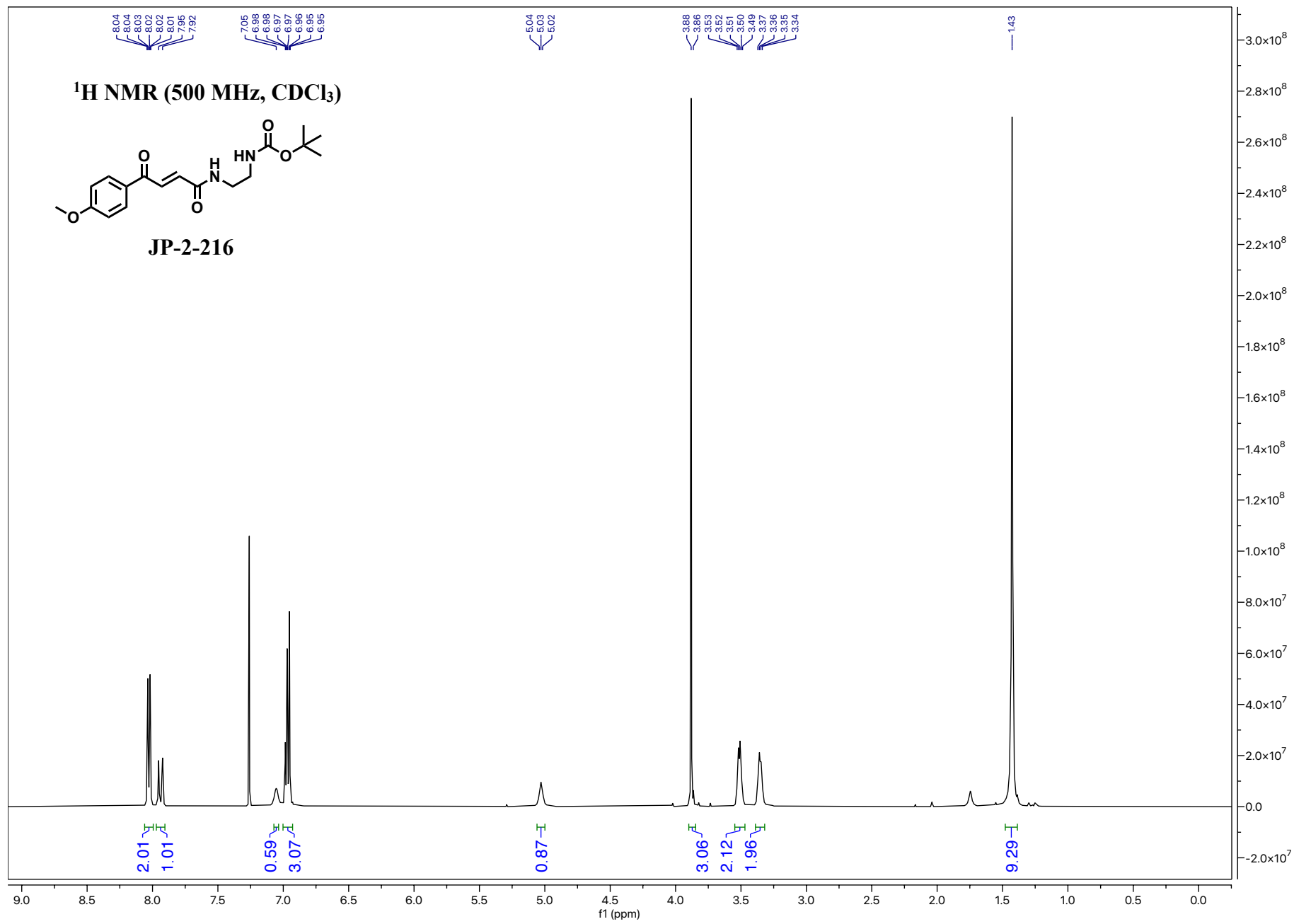
2: Diode Array



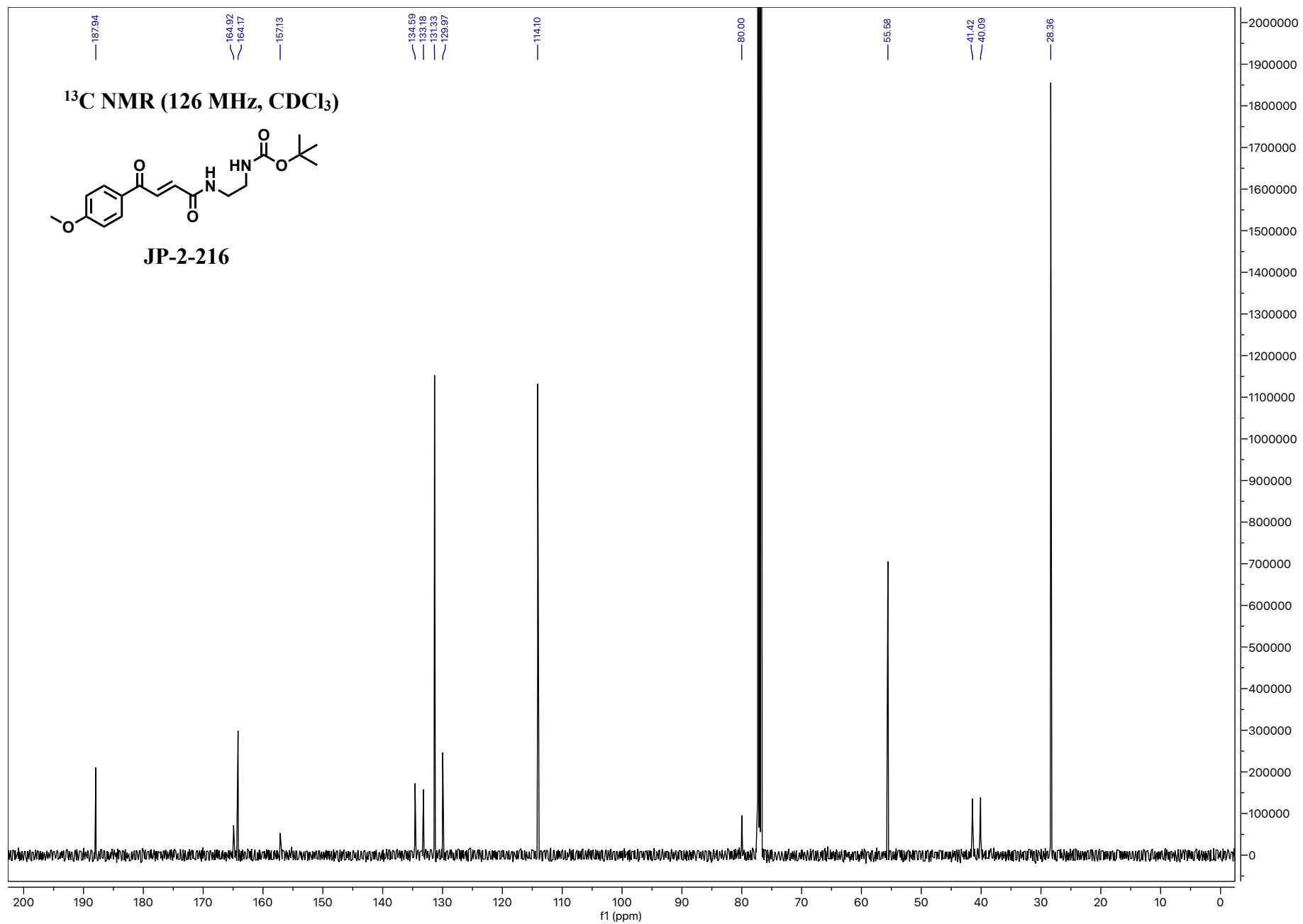
HESSEMA4-101-EXP237-S01\_OC

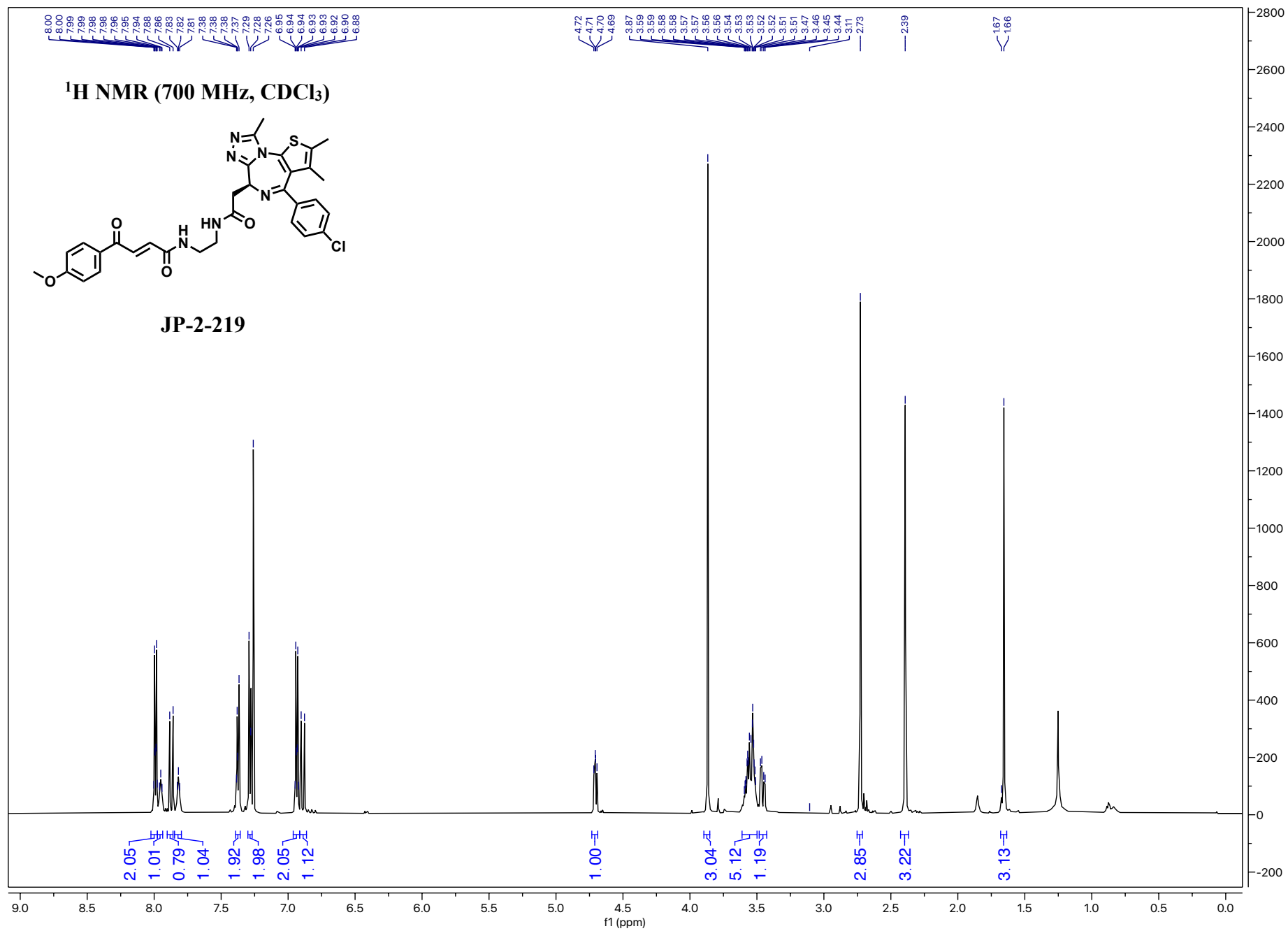
1: Scan ES+  
TIC  
2.43e8

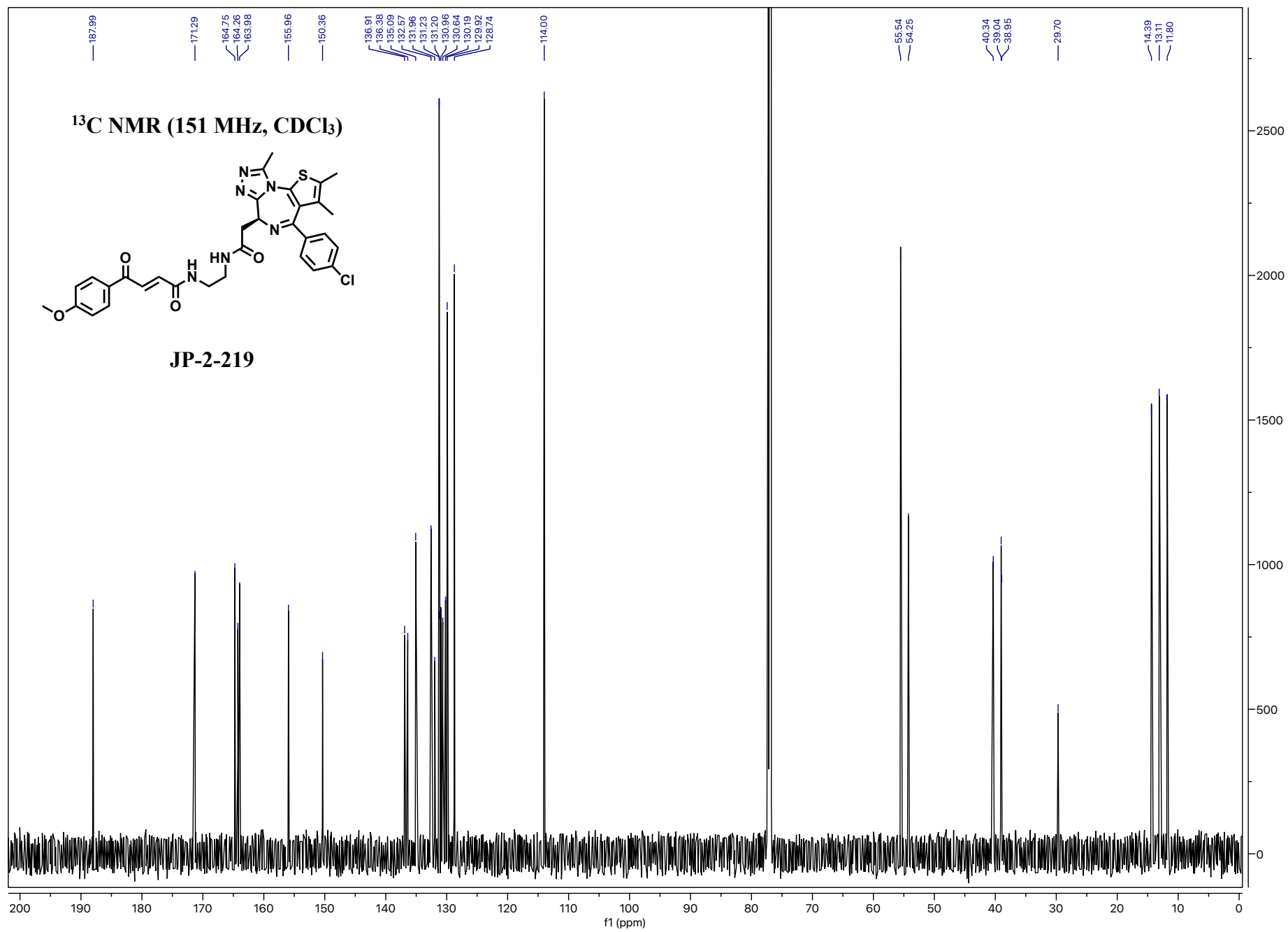












HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

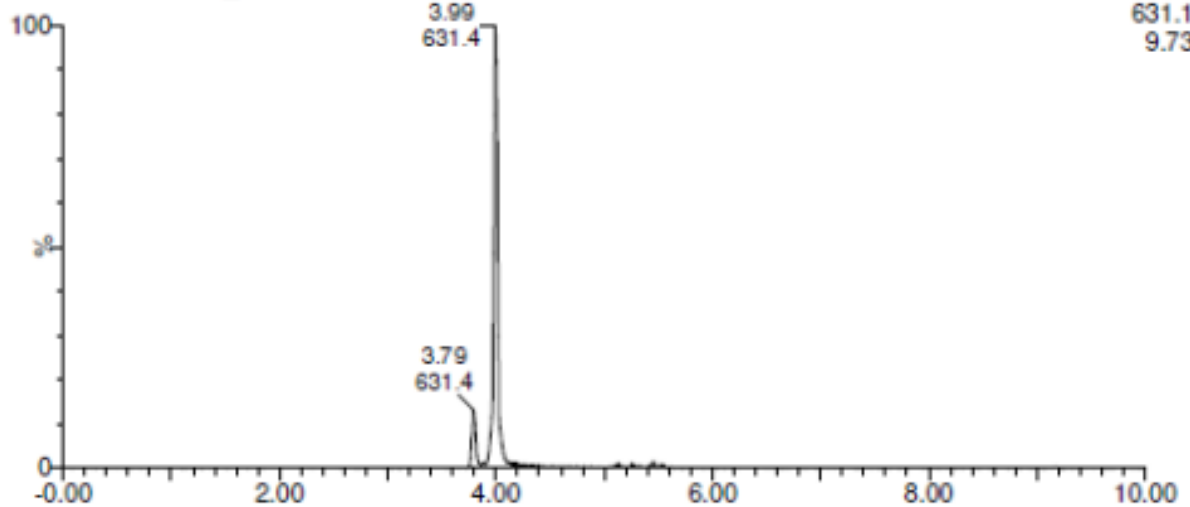
EM23001594448

08-Feb-2023  
17:50:24  
strubhe1-0208A-v23

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

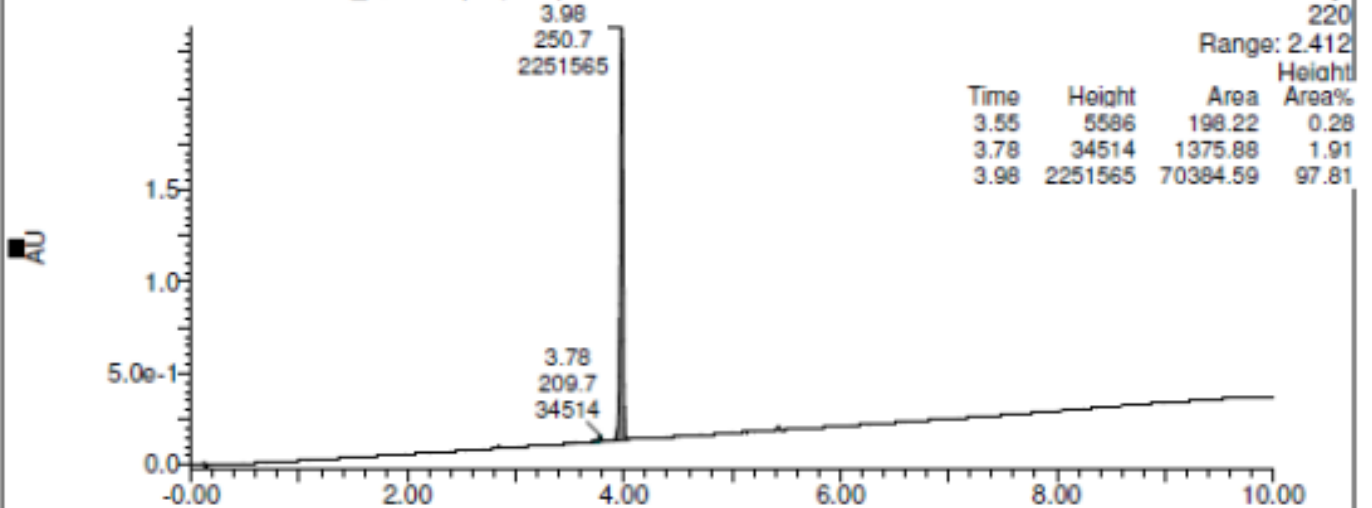
1: Scan ES+  
631.182  
9.73e7

HESSEMA4-101-EXP238-S01\_OC



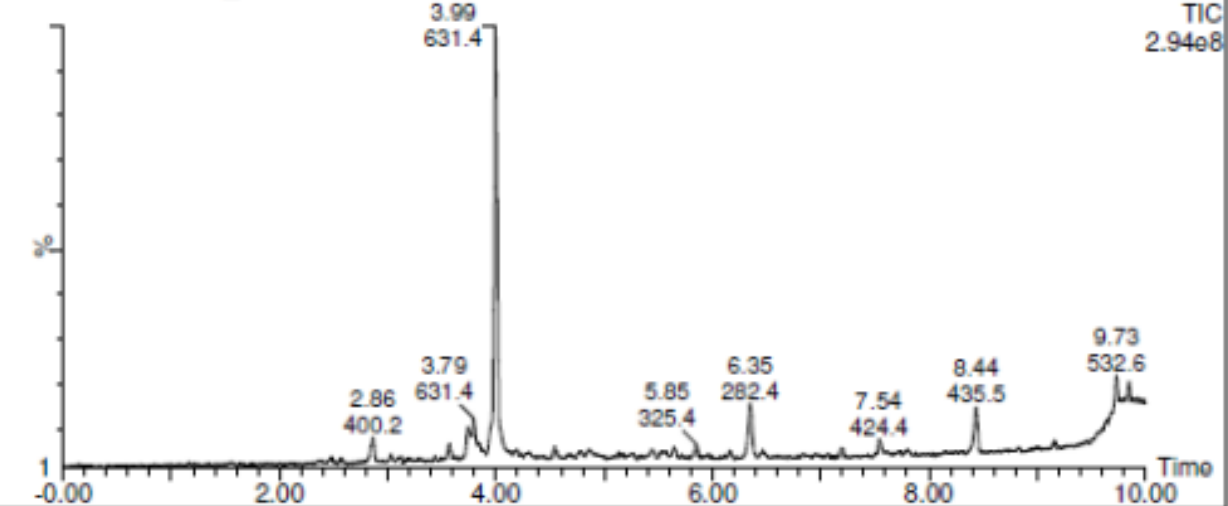
HESSEMA4-101-EXP238-S01\_OC Sm (Mn, 2x3)

2: Diode Array  
220  
Range: 2.412  
Height



HESSEMA4-101-EXP238-S01\_OC

1: Scan ES+  
TIC  
2.94e8



HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

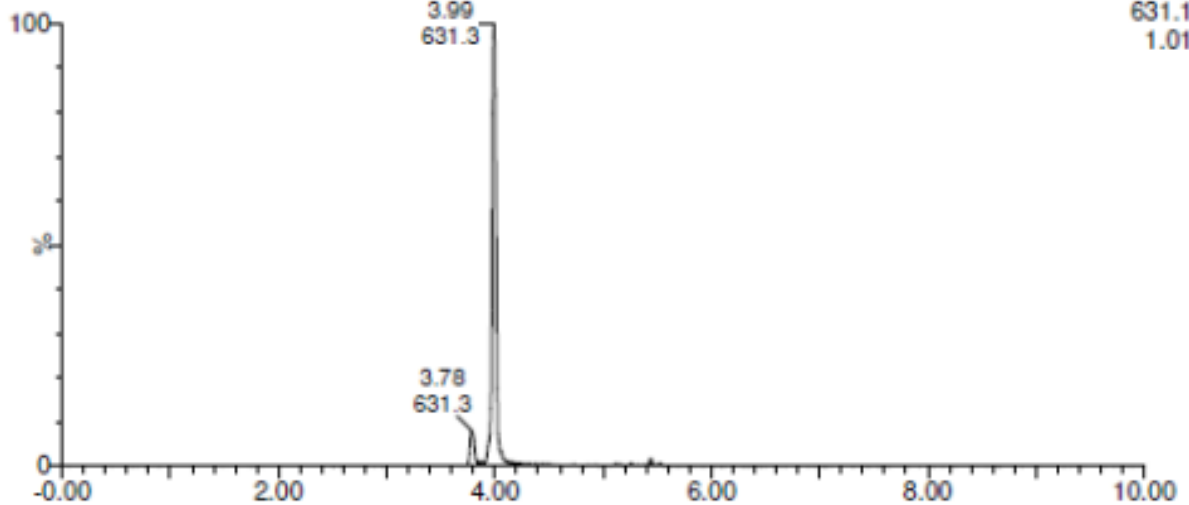
EM23001594448

14-Feb-2023  
13:40:30  
strubhe1-0208A-v23

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

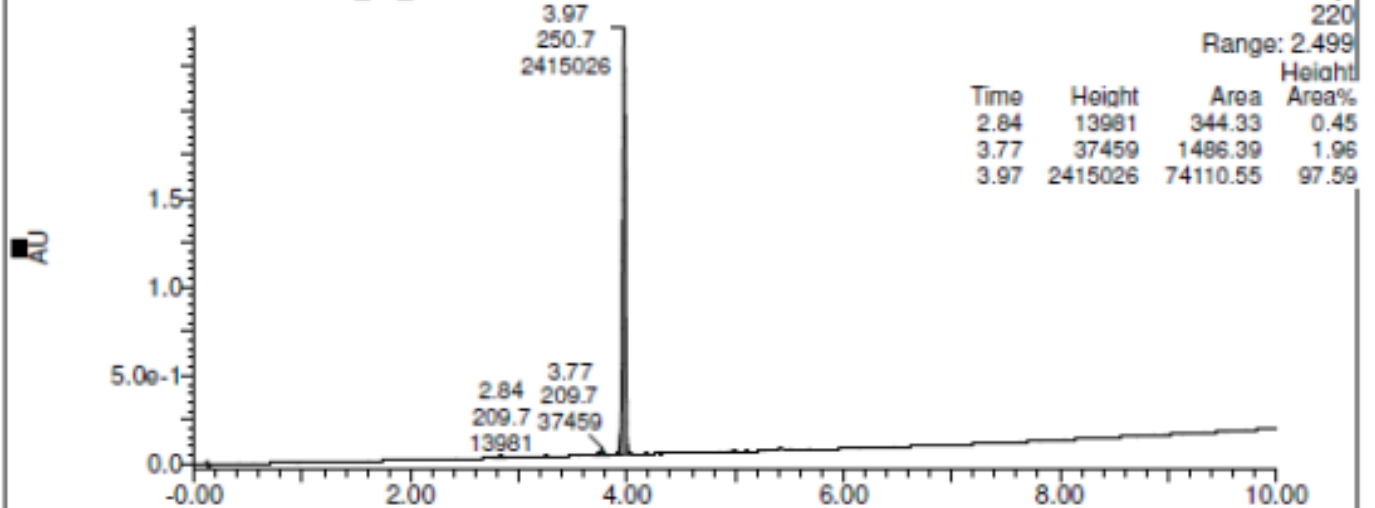
HESSEMA4-101-EXP238-S01\_6d\_QC

1: Scan ES+  
631.182  
1.01e8



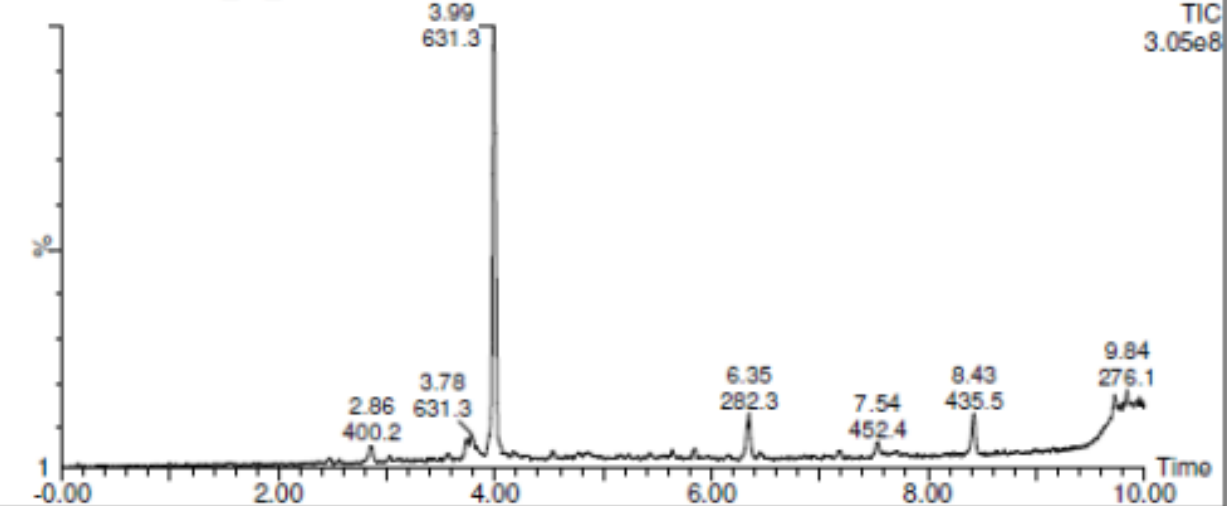
HESSEMA4-101-EXP238-S01\_6d\_QC

2: Diode Array  
220  
Range: 2.499  
Height

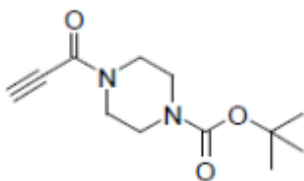


HESSEMA4-101-EXP238-S01\_6d\_QC

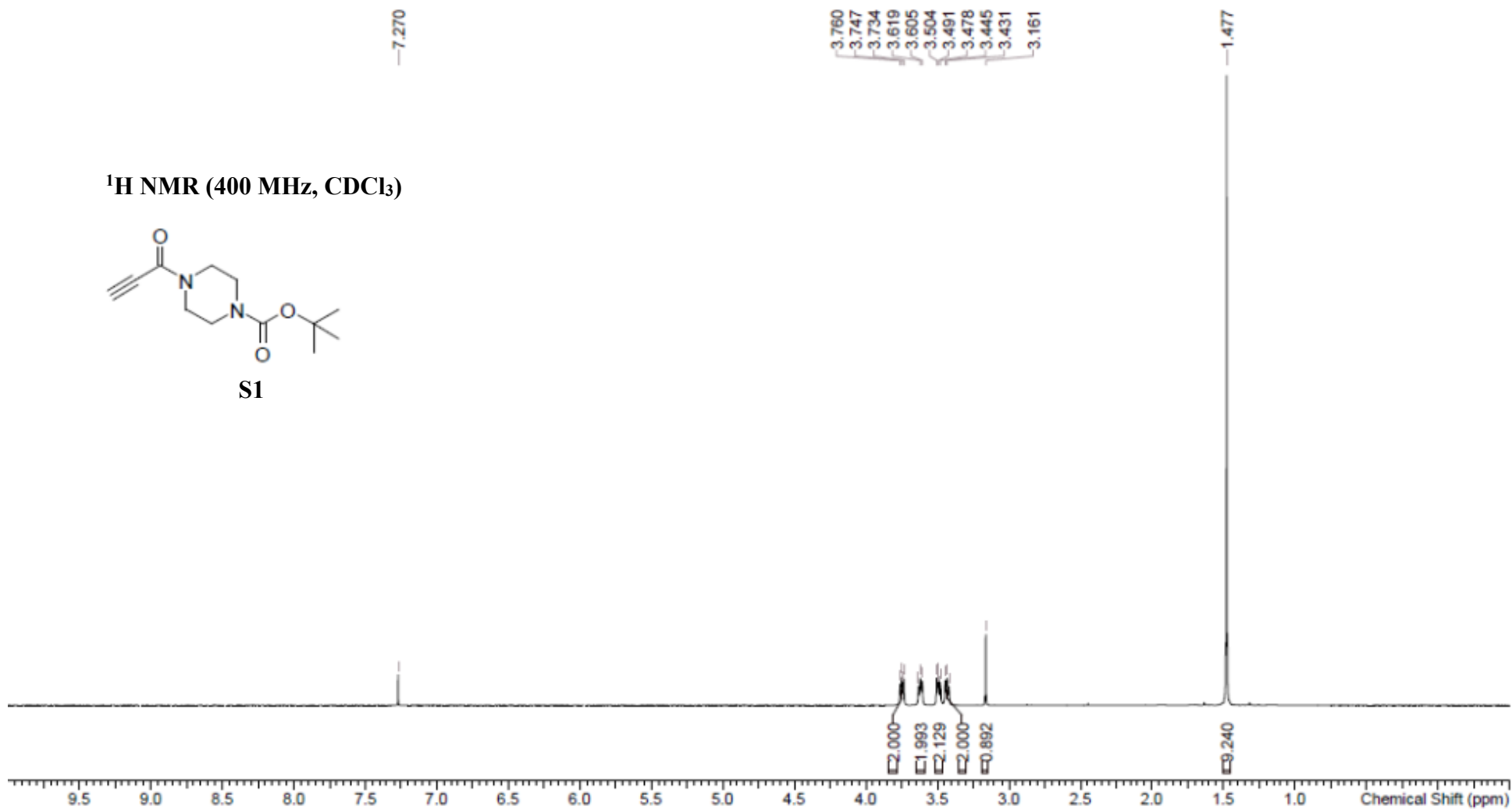
1: Scan ES+  
TIC  
3.05e8



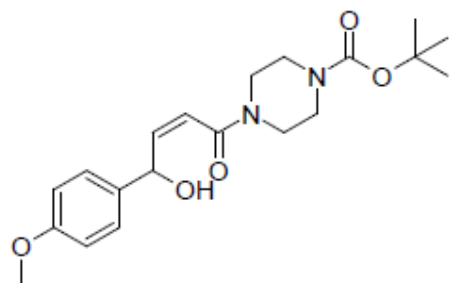
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)



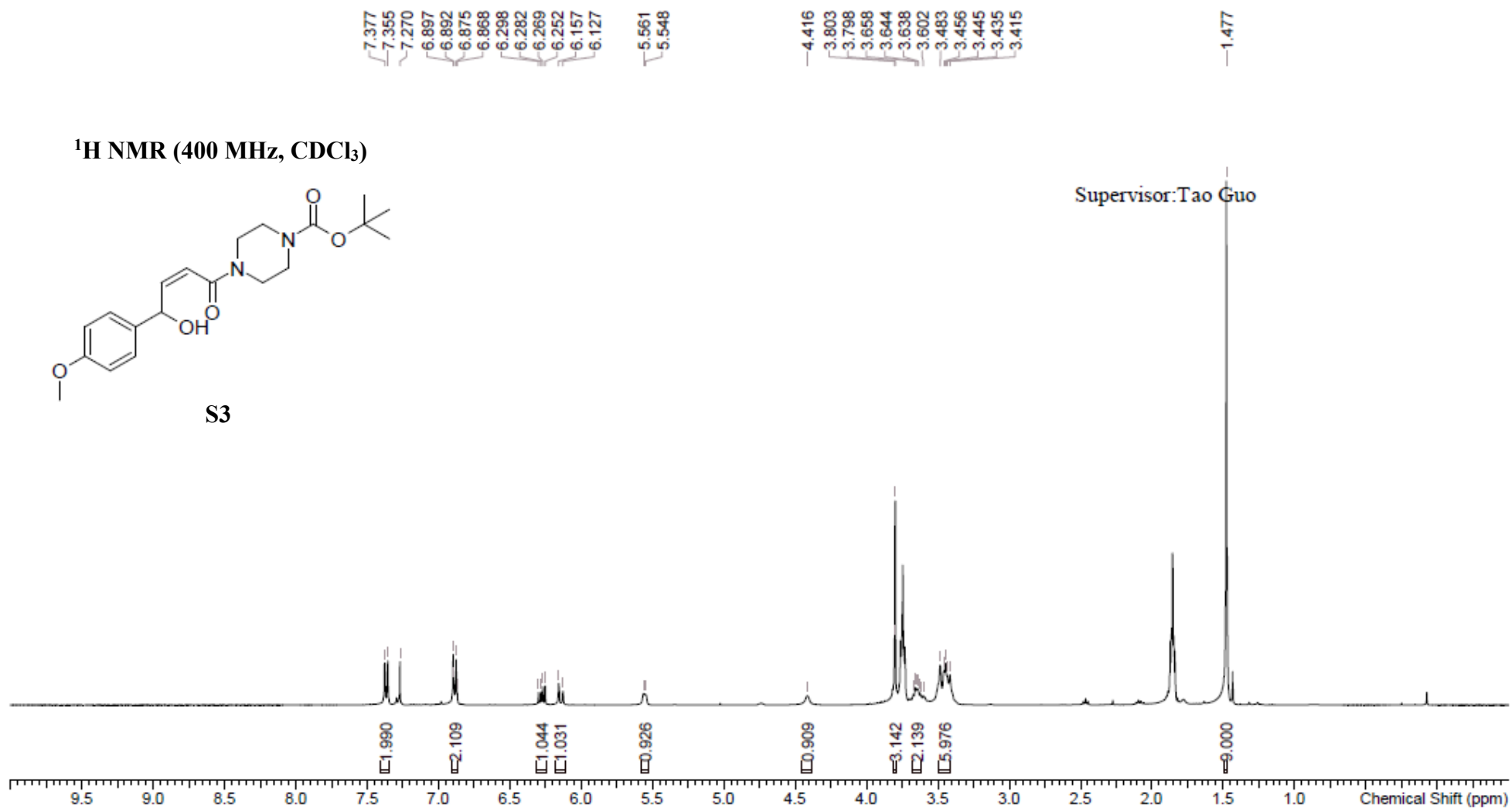
S1

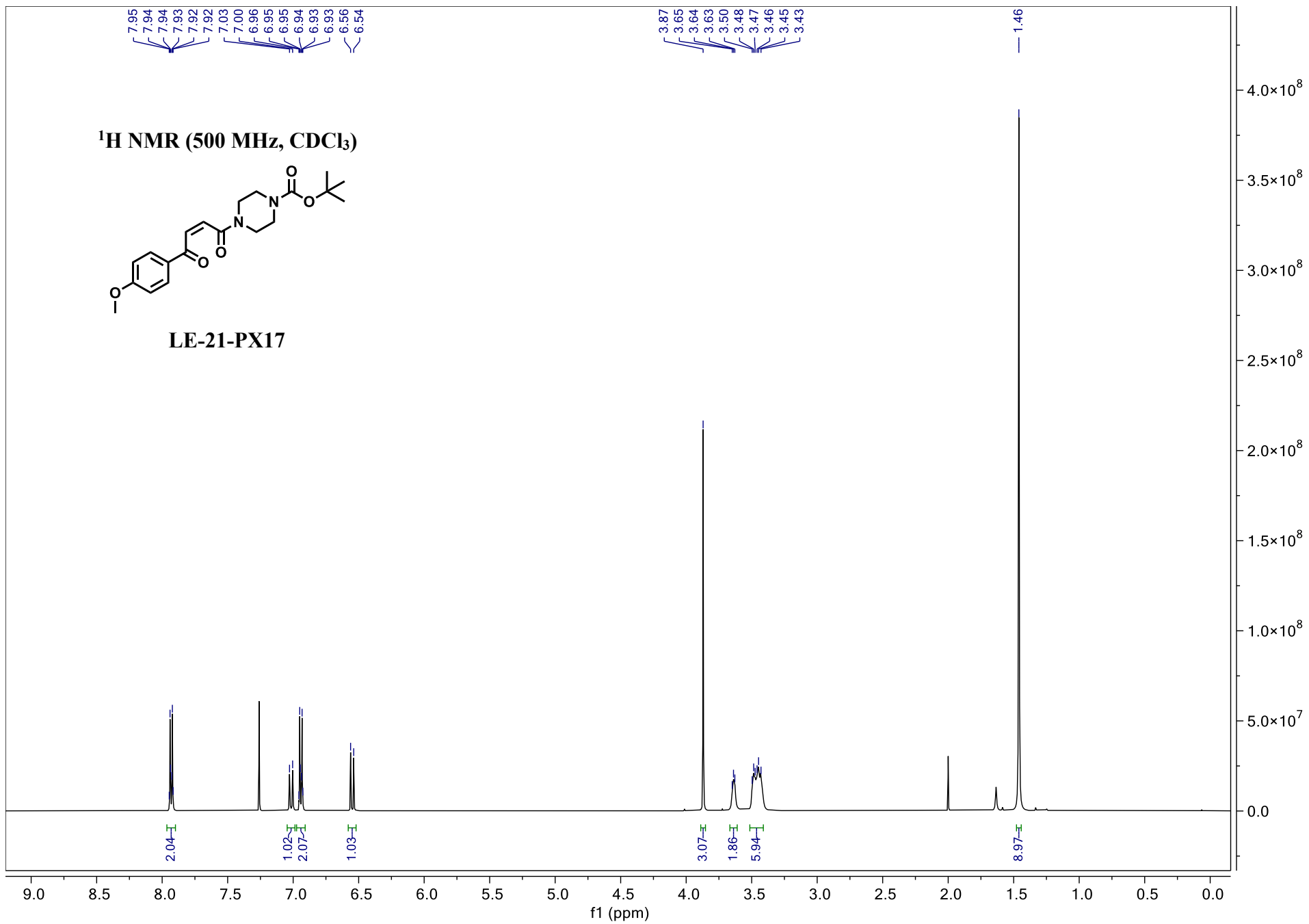


<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)

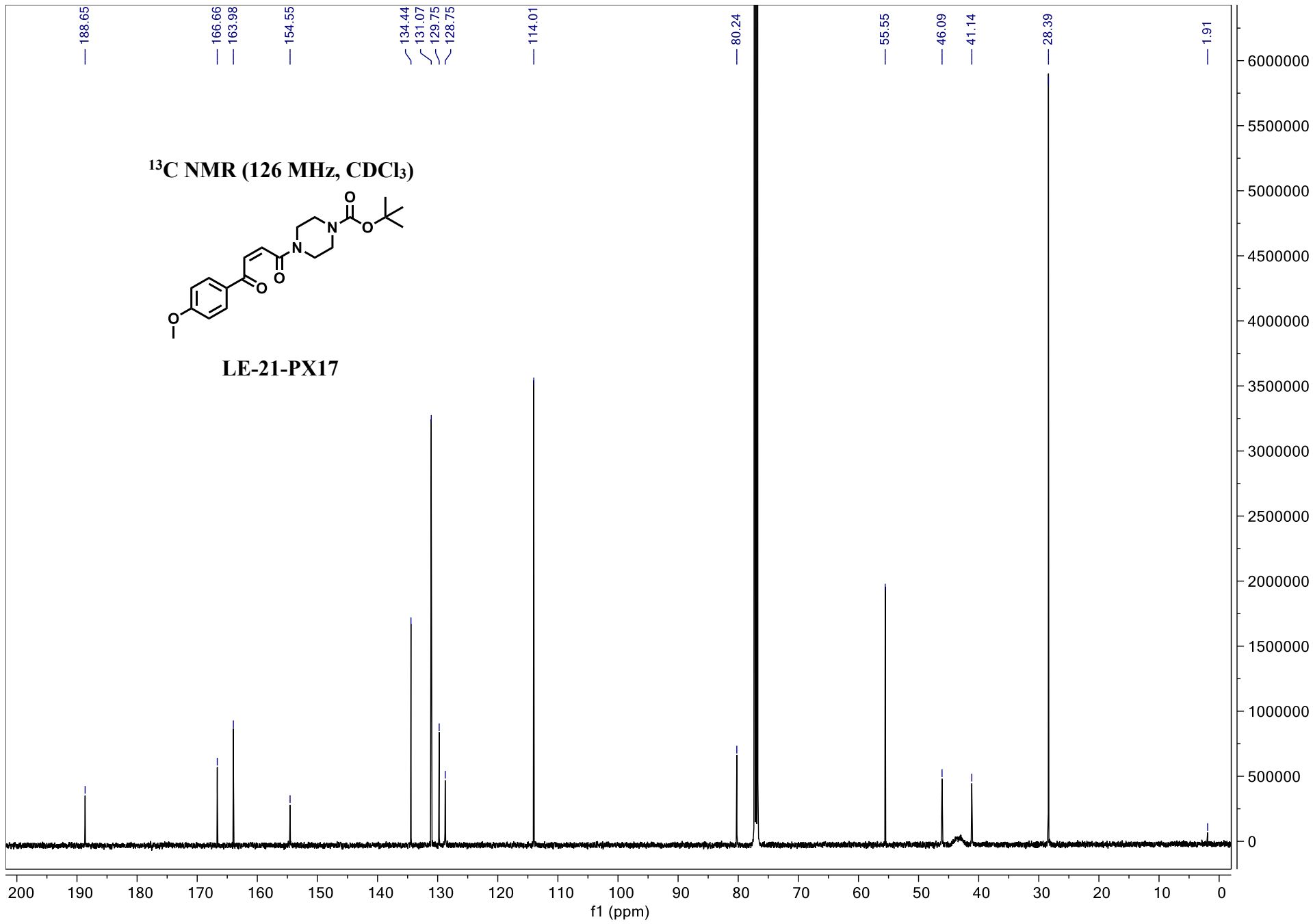


S3









HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

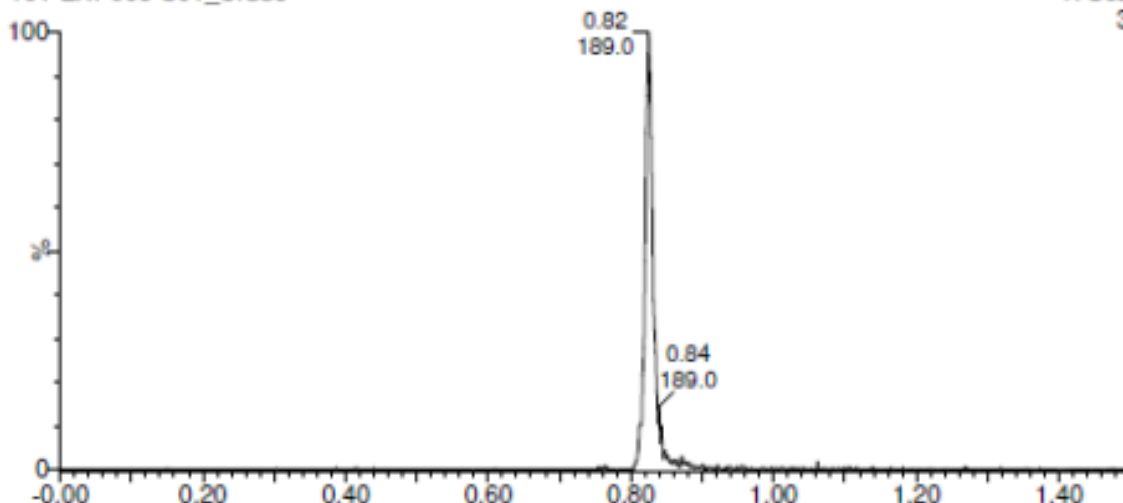
EM23001597792

02-Mar-2023  
14:16:04  
strubhe1-0302-v03

LCMS-QDa1  
Kinetex column,  
QDa1\_2m\_Acidic

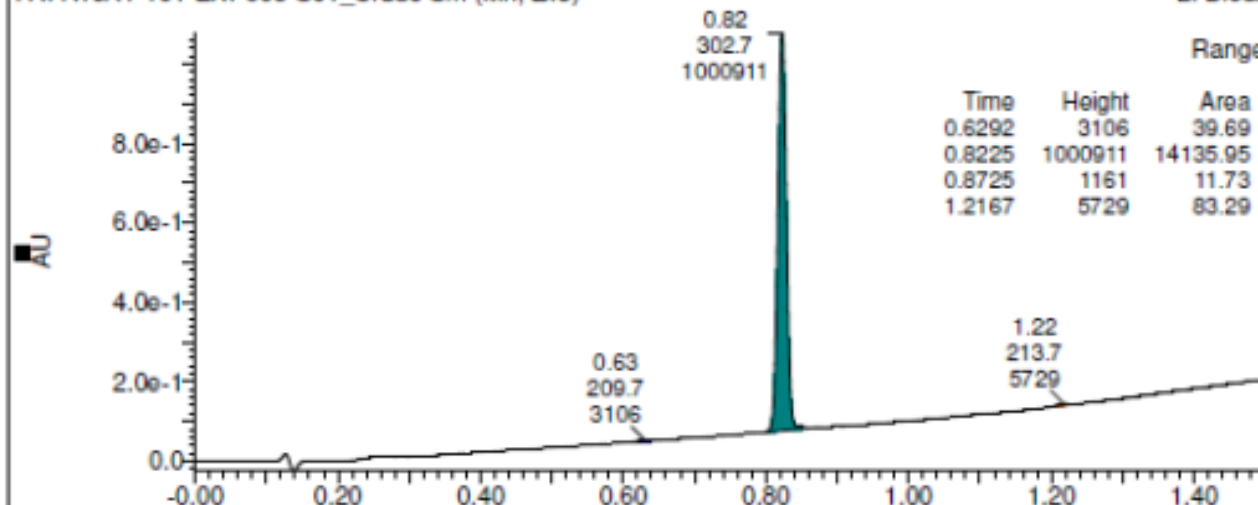
PAPATJA1-101-EXP005-S01\_Crude

1: Scan ES+  
375.184  
3.84e6



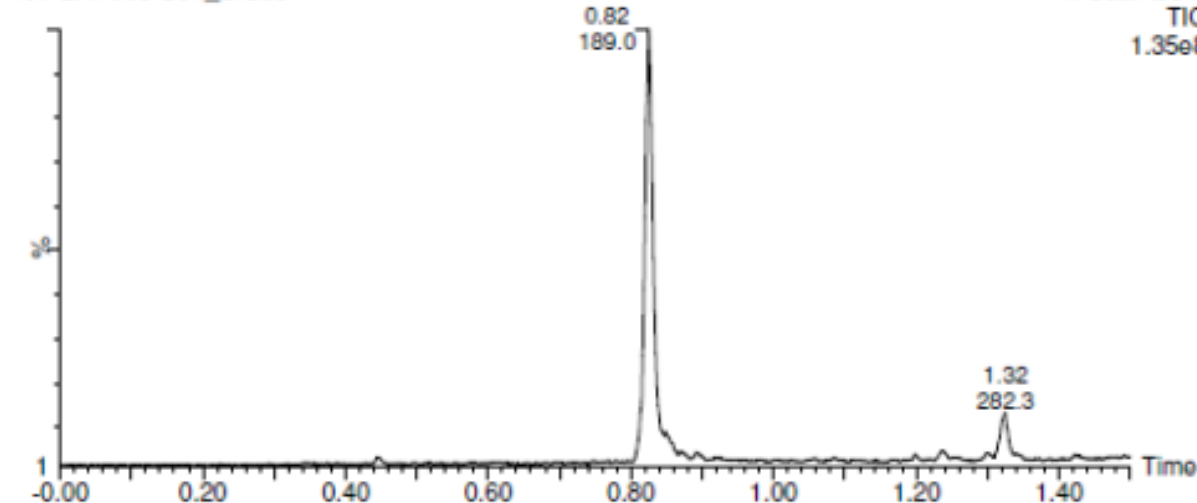
PAPATJA1-101-EXP005-S01\_Crude Sm (Mn, 2x3)

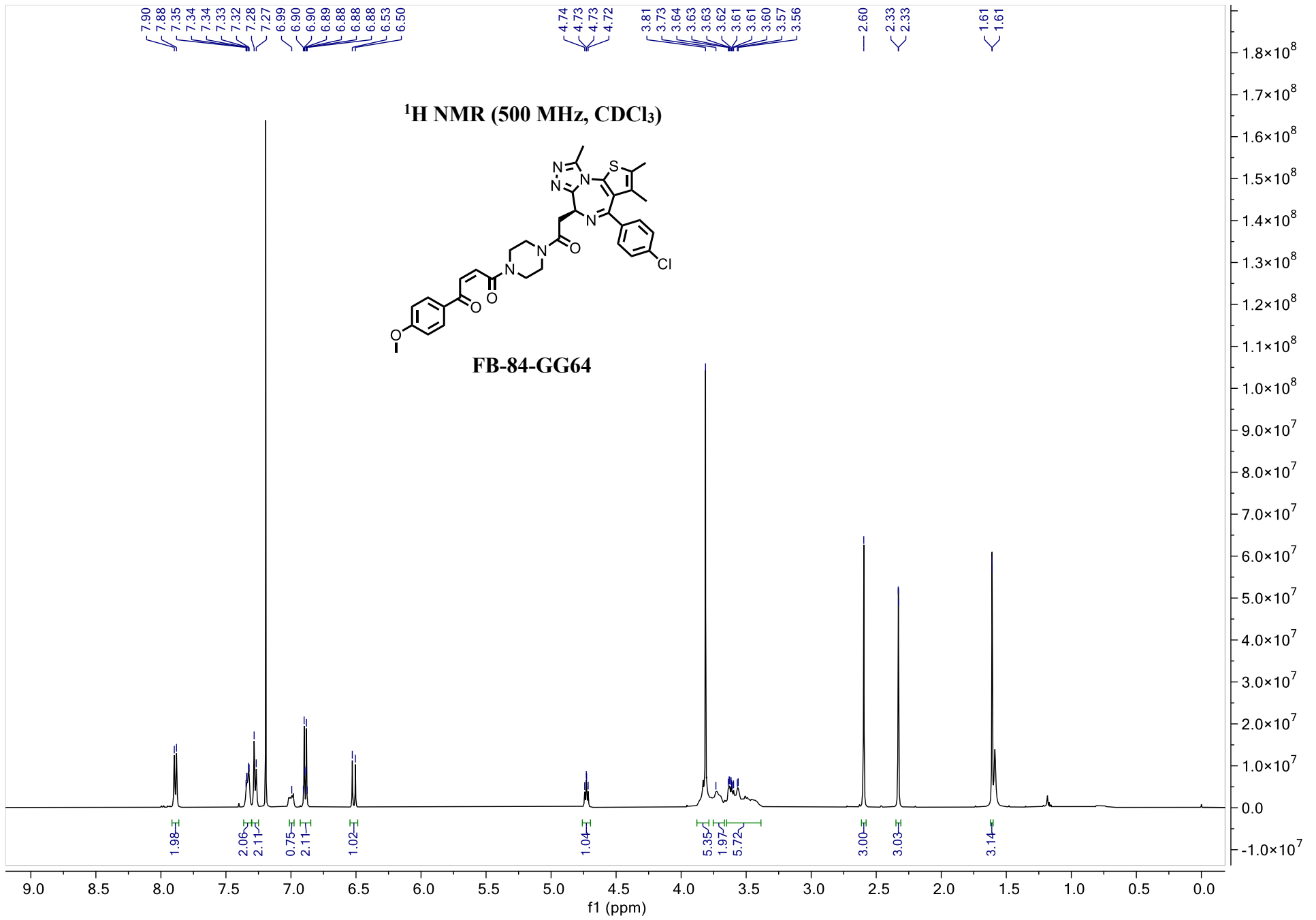
2: Diode Array  
220

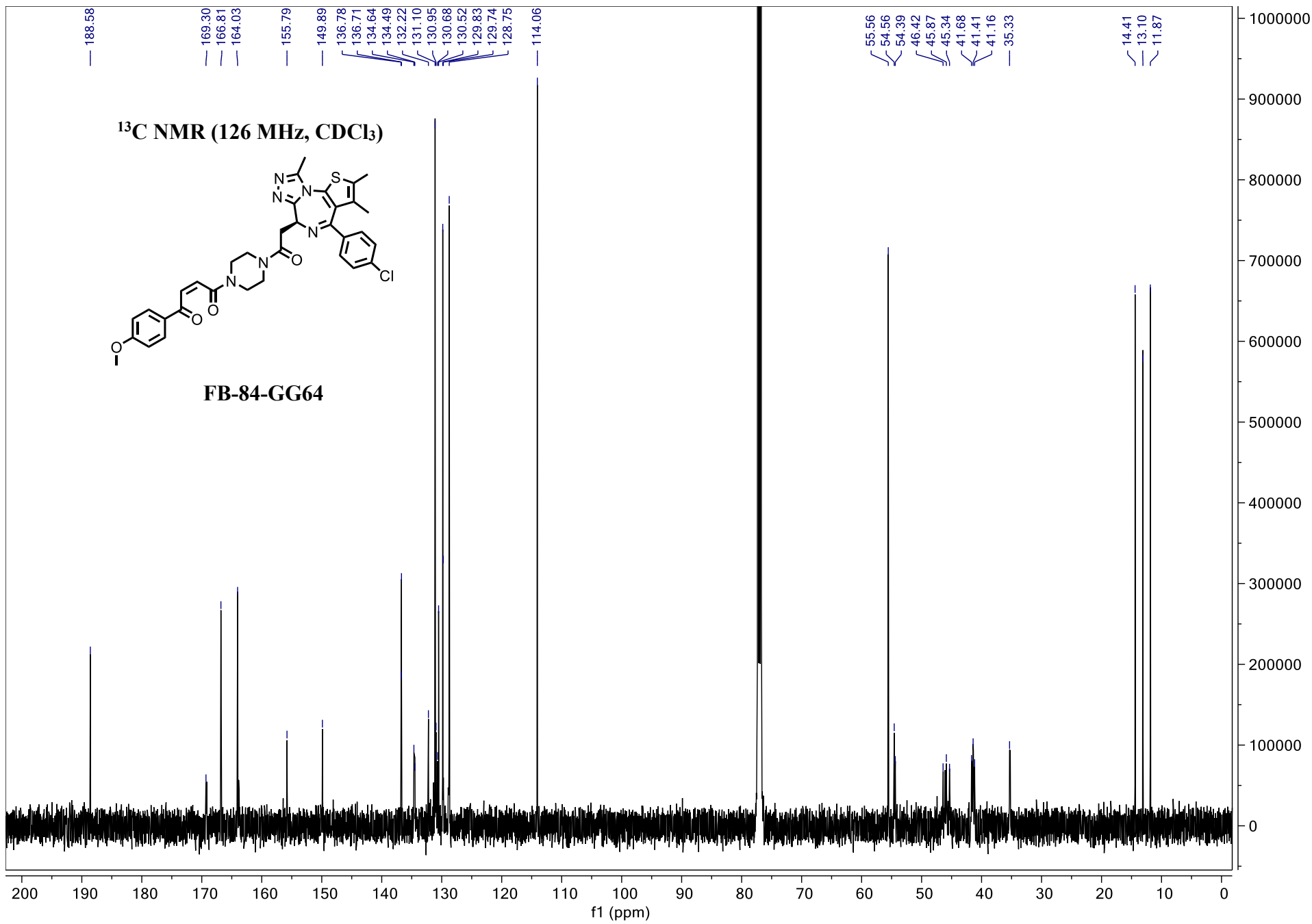


PAPATJA1-101-EXP005-S01\_Crude

1: Scan ES+  
TIC  
1.35e8







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

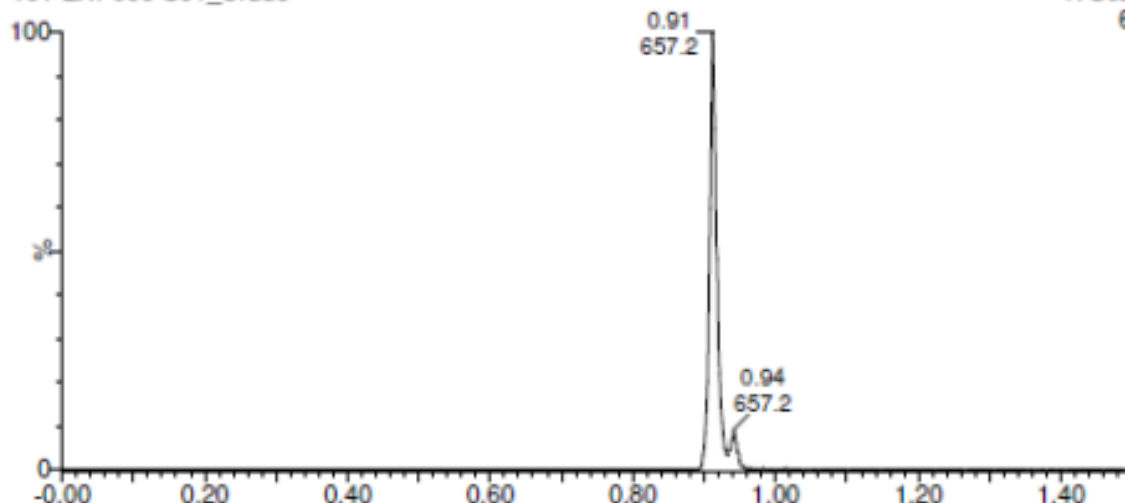
EM23001597796

02-Mar-2023  
14:18:33  
strubhe1-0302-v04

LCMS-QDa1  
Kinetex column,  
QDa1\_2m\_Acidic

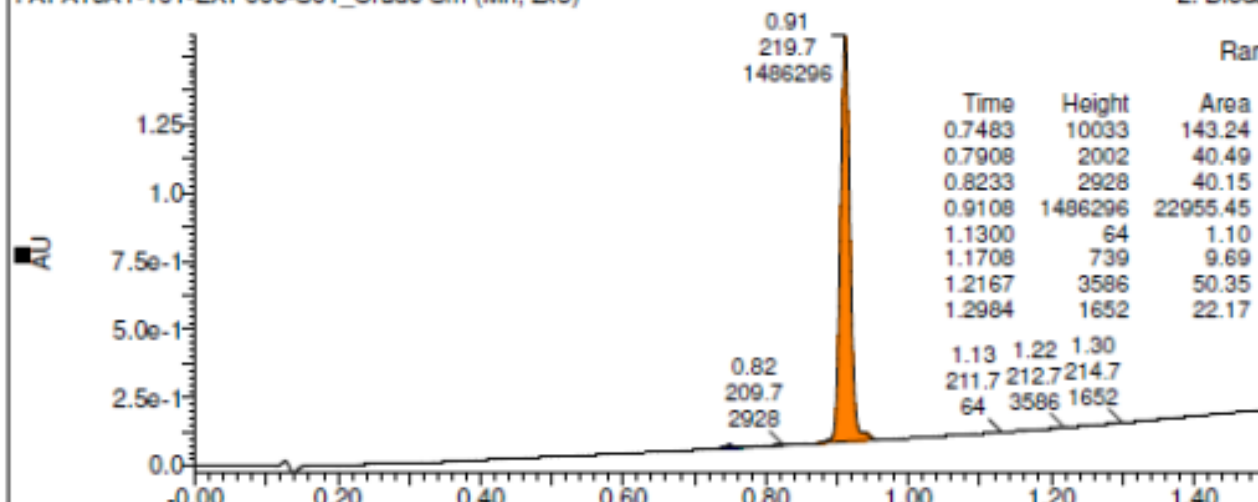
PAPATJA1-101-EXP006-S01\_Crude

1: Scan ES+  
657.197  
2.14e7



PAPATJA1-101-EXP006-S01\_Crude Sm (Mn, 2x3)

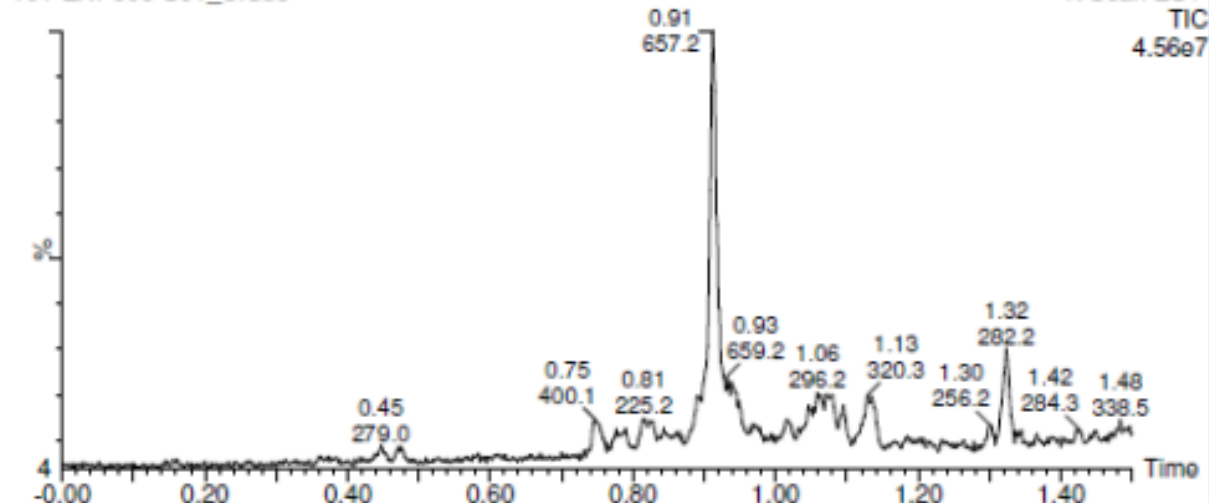
2: Diode Array  
220

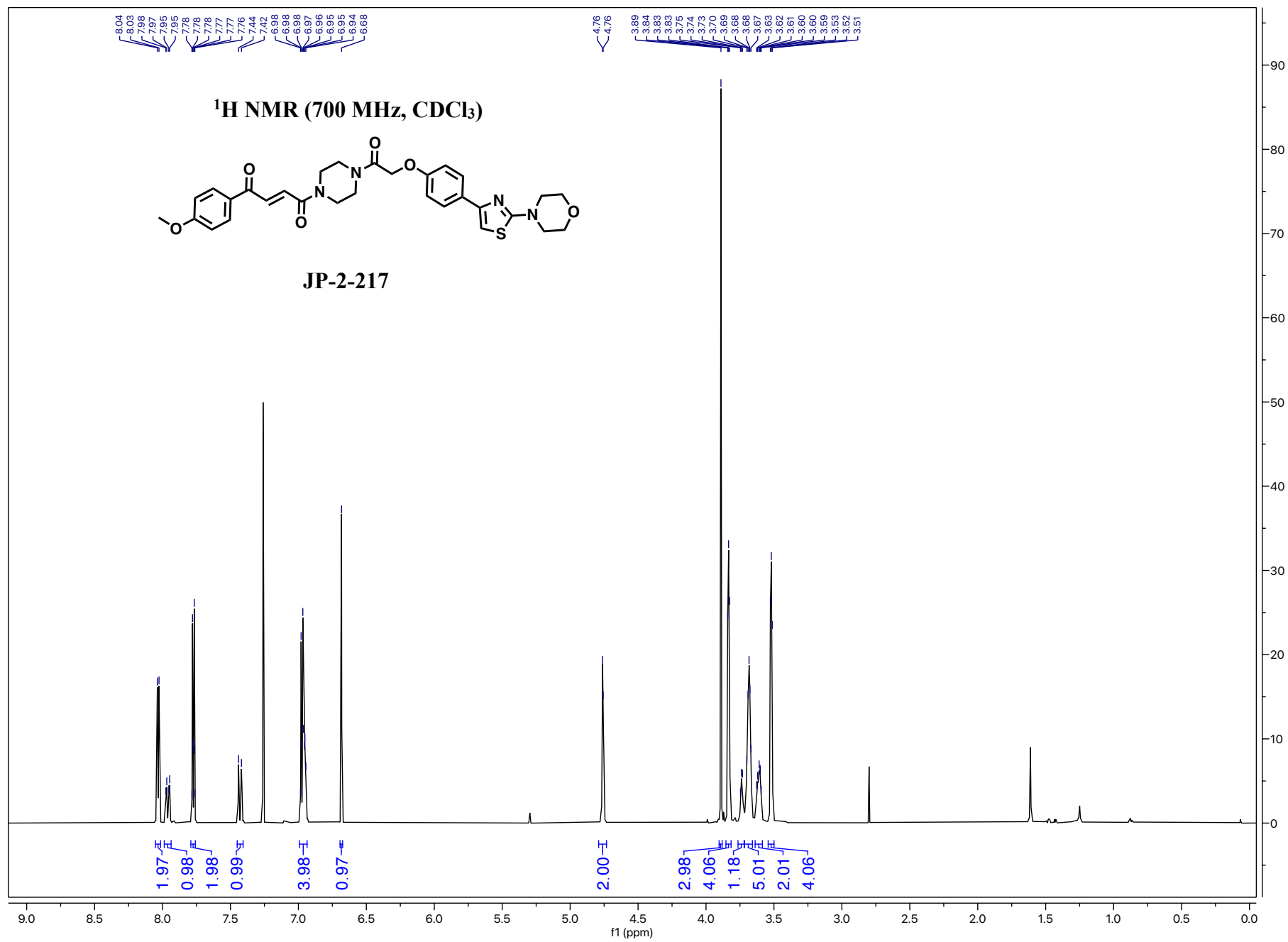


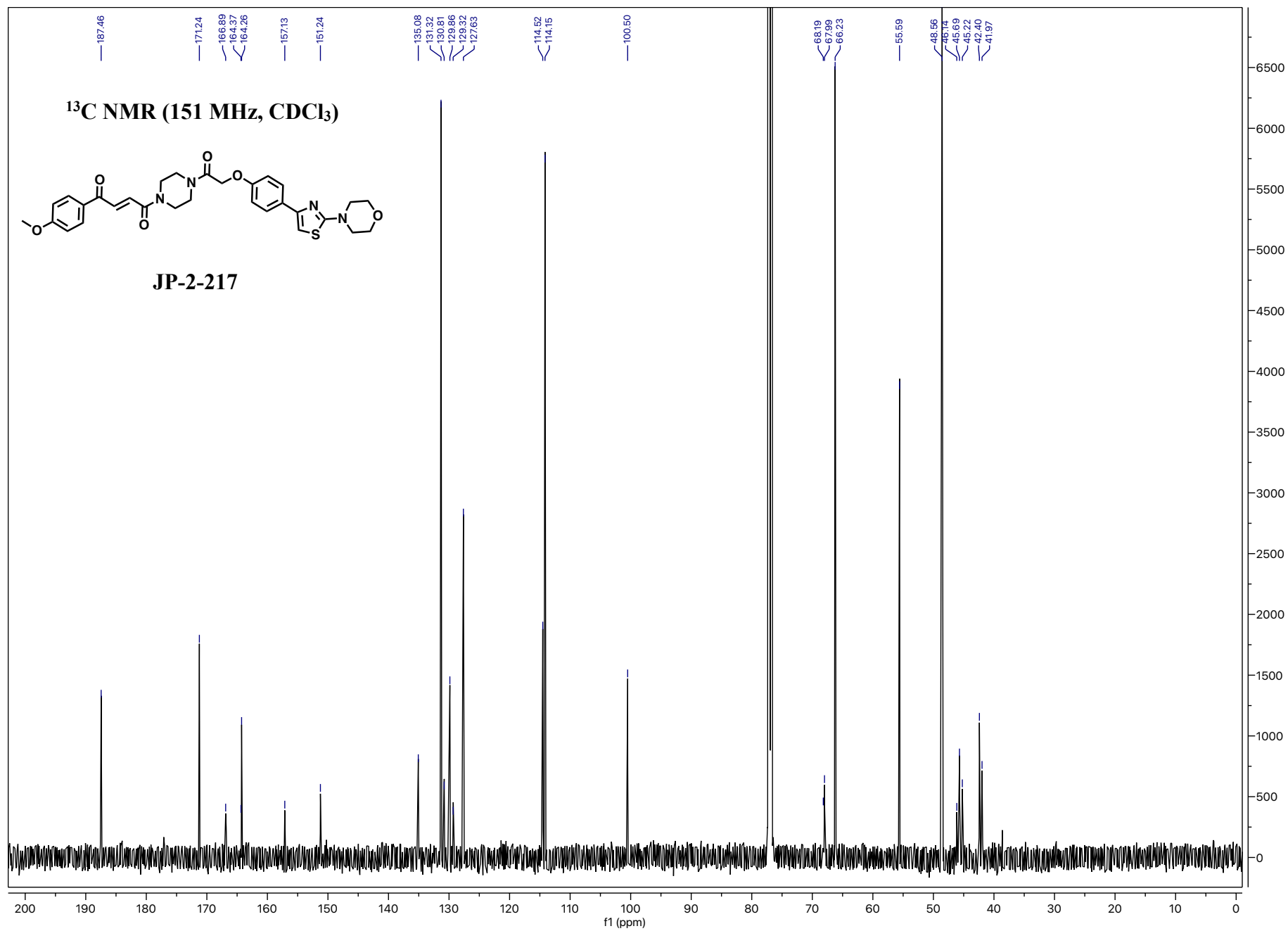
Time	Height	Area	Area%
0.7483	10033	143.24	0.62
0.7908	2002	40.49	0.17
0.8233	2928	40.15	0.17
0.9108	1486296	22955.45	98.68
1.1300	64	1.10	0.00
1.1708	739	9.69	0.04
1.2167	3586	50.35	0.22
1.2984	1652	22.17	0.10

PAPATJA1-101-EXP006-S01\_Crude

1: Scan ES+  
TIC  
4.56e7







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

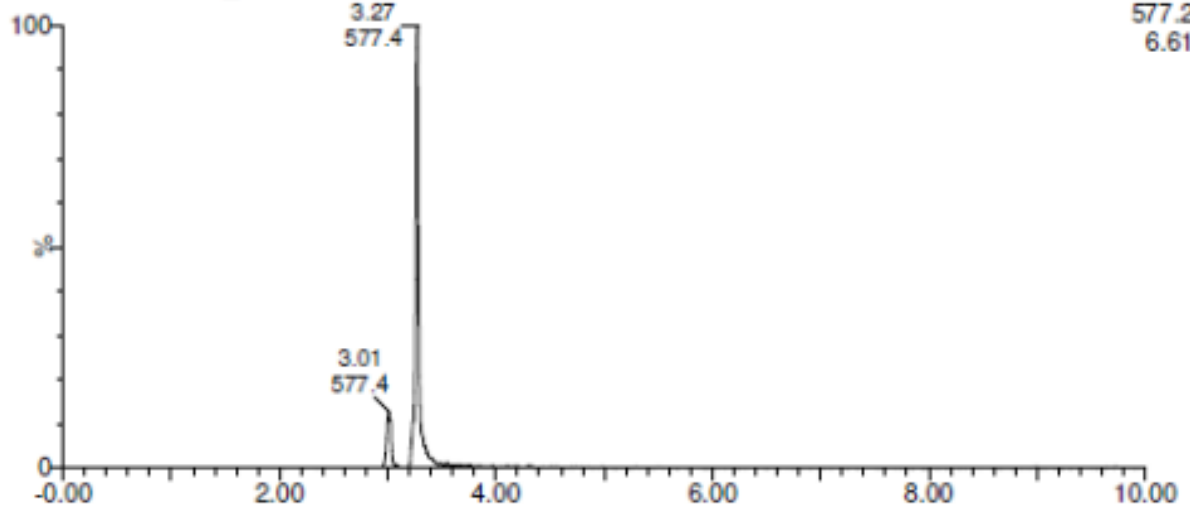
EM23001590293

13-Jan-2023  
16:16:48  
strubhe1-0112A-v07

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
577.204  
6.61e7

HESSEMA4-101-EXP210-S01\_OC



HESSEMA4-101-EXP210-S01\_OC Sm (Mn, 2x3)

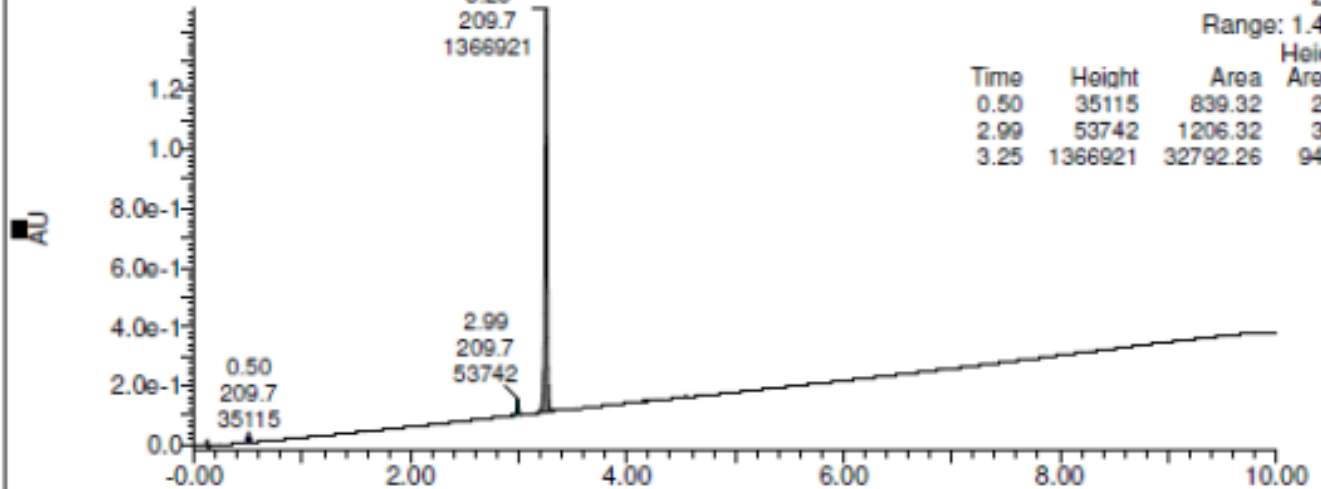
2: Diode Array

220

Range: 1.497

Height

Time	Height	Area	Area%
0.50	35115	839.32	2.41
2.99	53742	1206.32	3.46
3.25	1366921	32792.26	94.13

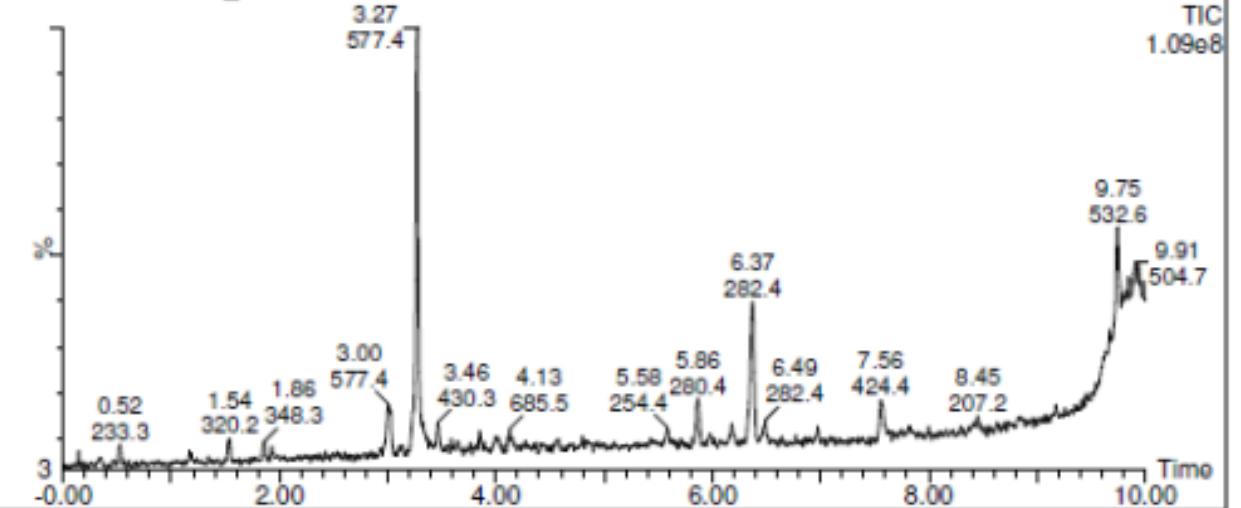


HESSEMA4-101-EXP210-S01\_OC

1: Scan ES+

TIC

1.09e8





HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

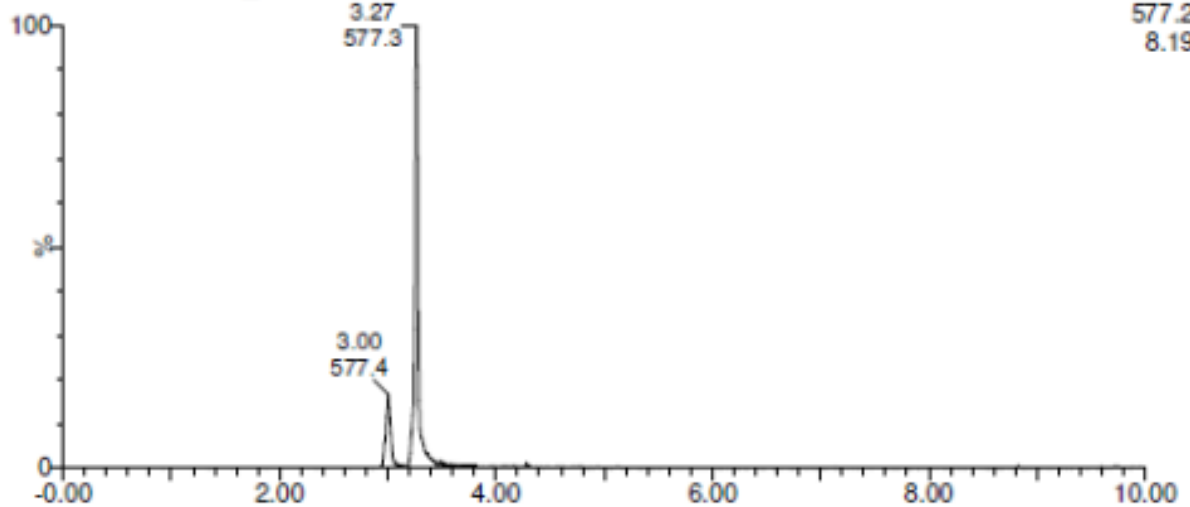
EM23001590293

19-Jan-2023  
16:42:45  
strubhe1-0118A-v07

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

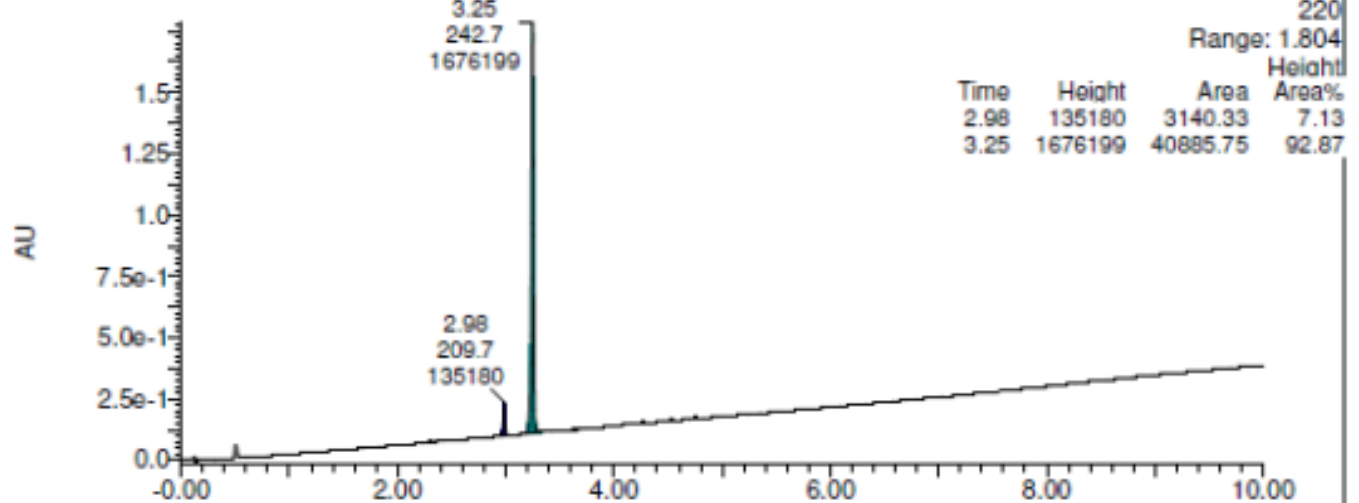
1: Scan ES+  
577.204  
8.19e7

HESSEMA4-101-EXP210-S01R\_QC



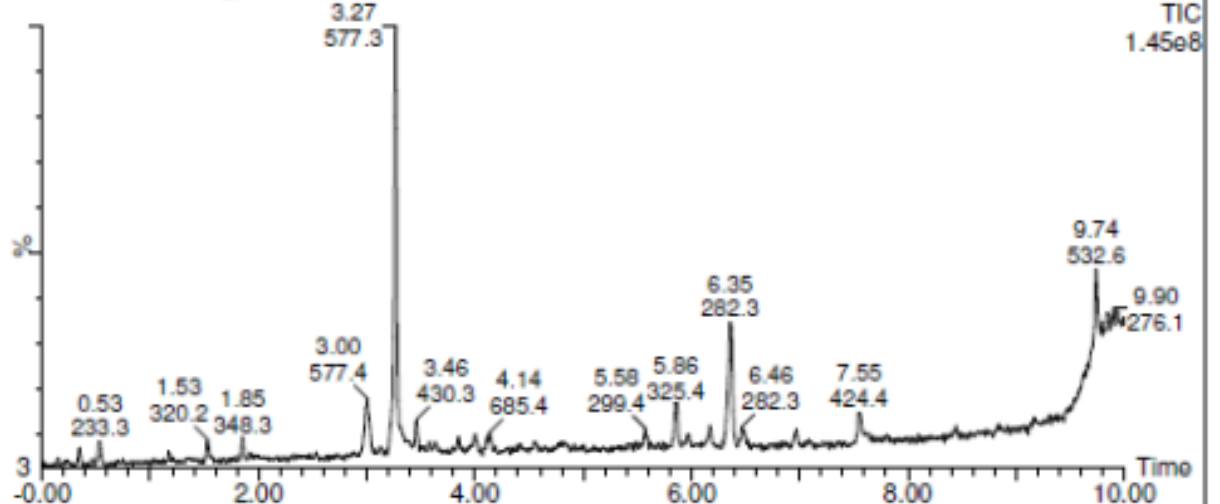
HESSEMA4-101-EXP210-S01R\_QC Sm (Mn, 2x3)

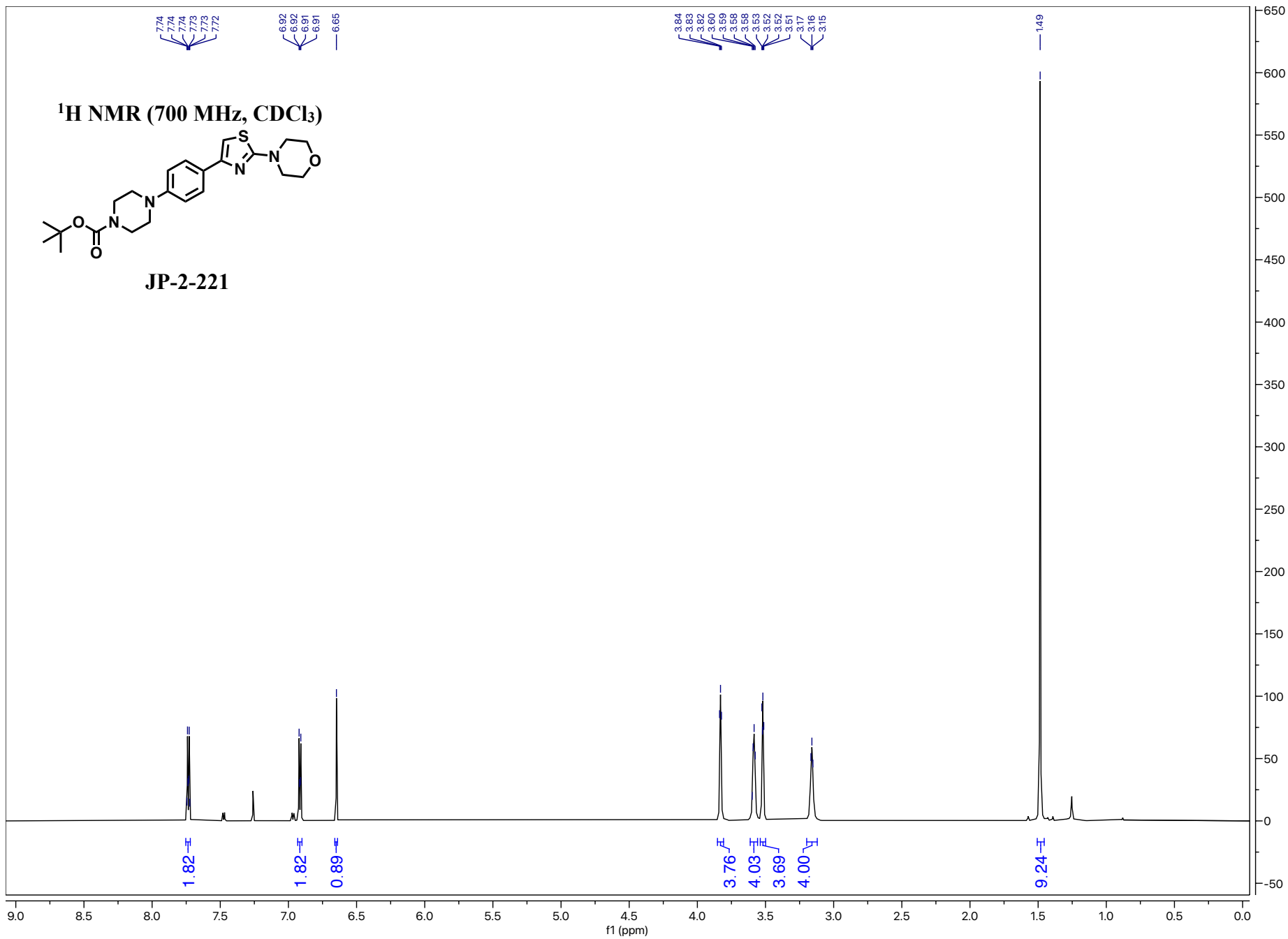
2: Diode Array

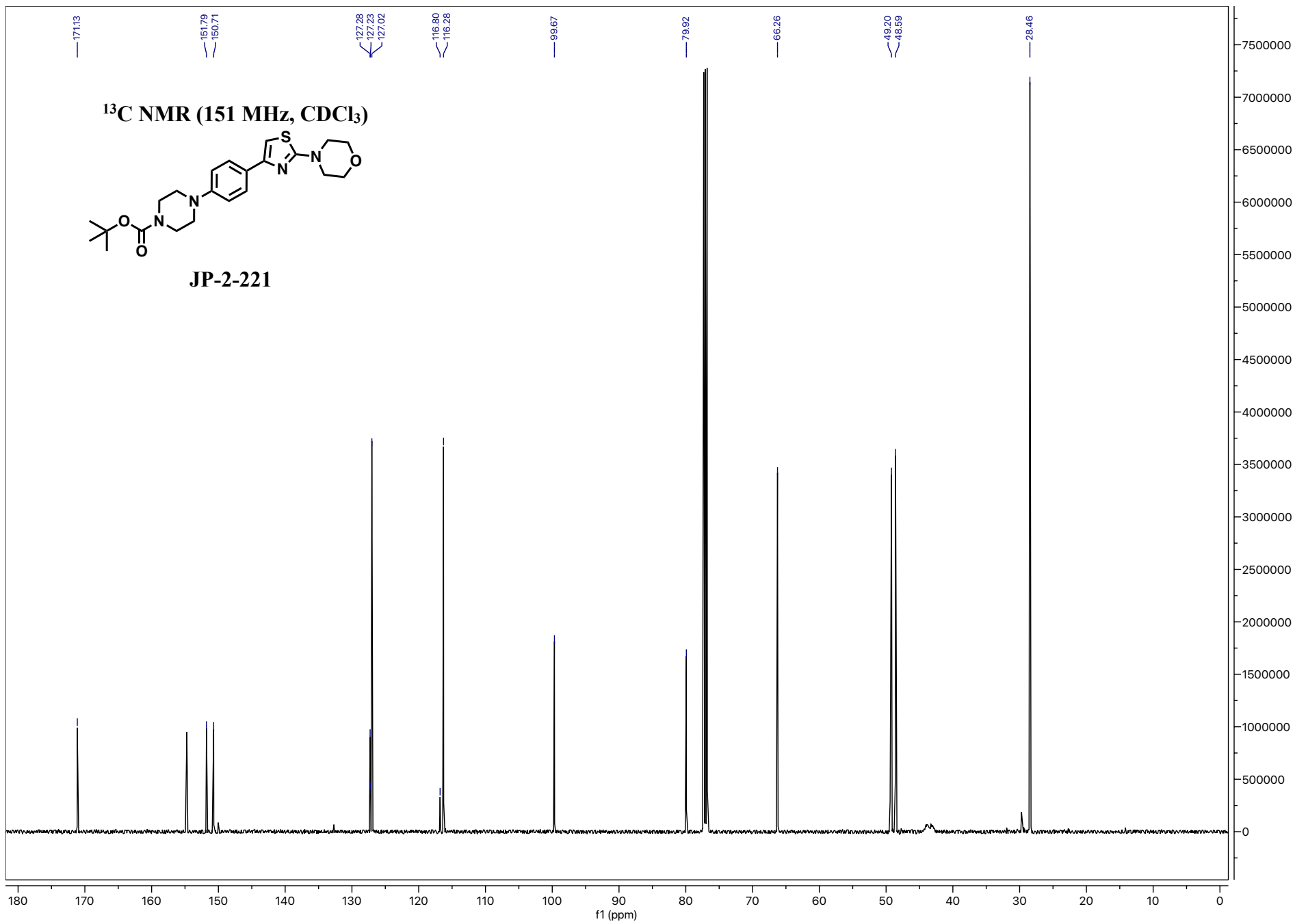


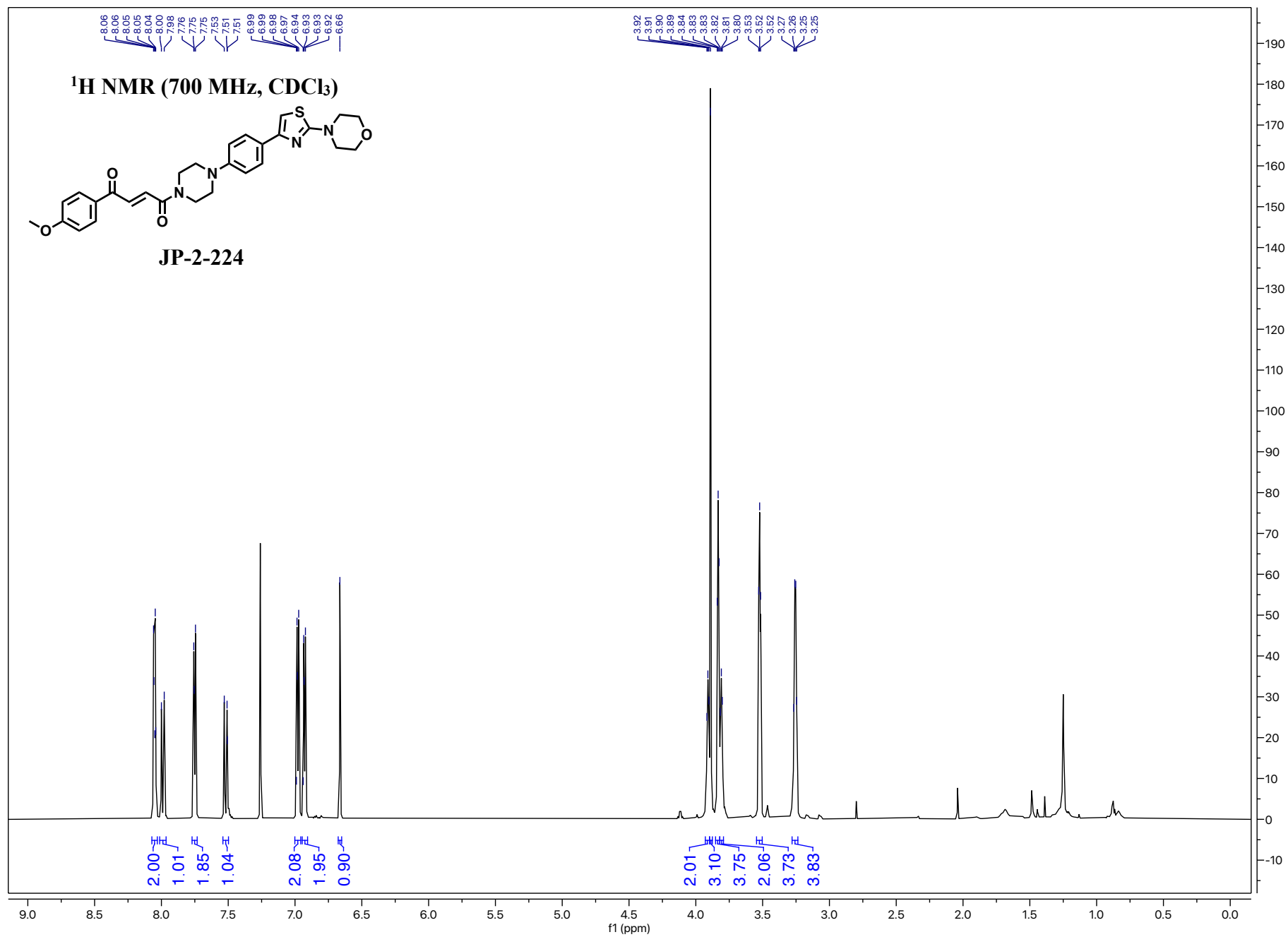
HESSEMA4-101-EXP210-S01R\_QC

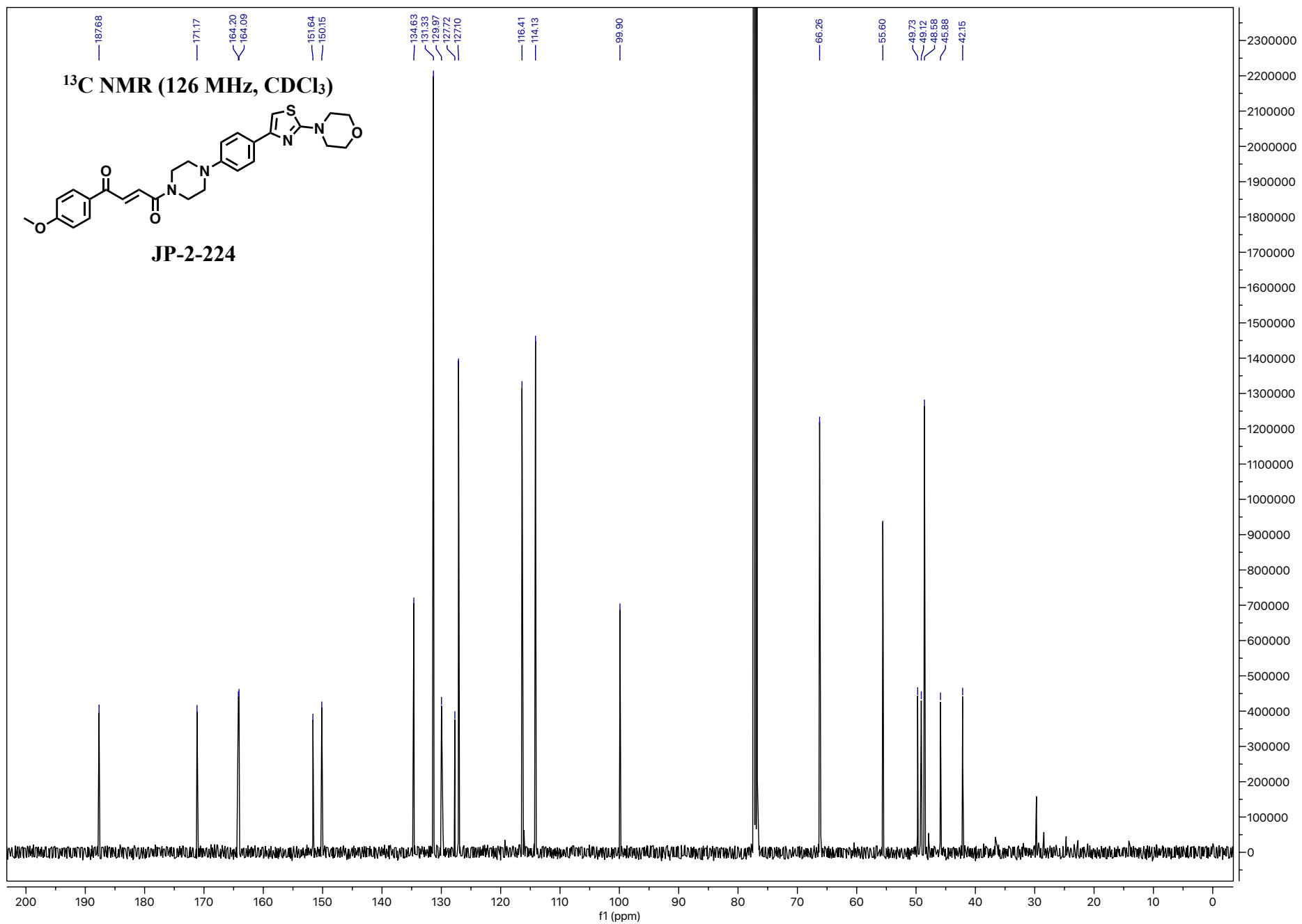
1: Scan ES+











HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

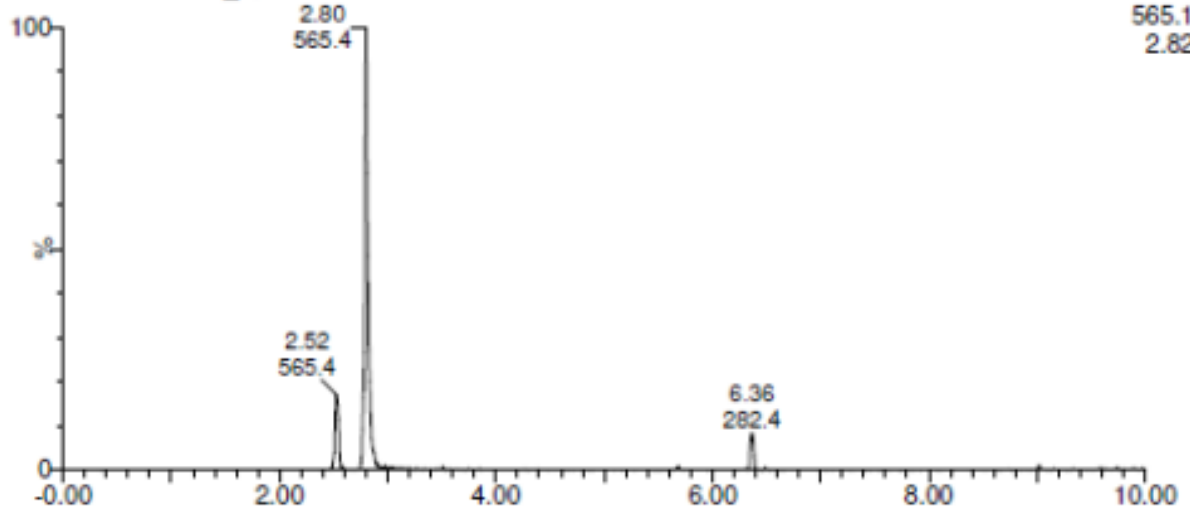
EM23001590285

13-Jan-2023  
15:54:05  
strubhe1-0112A-v03

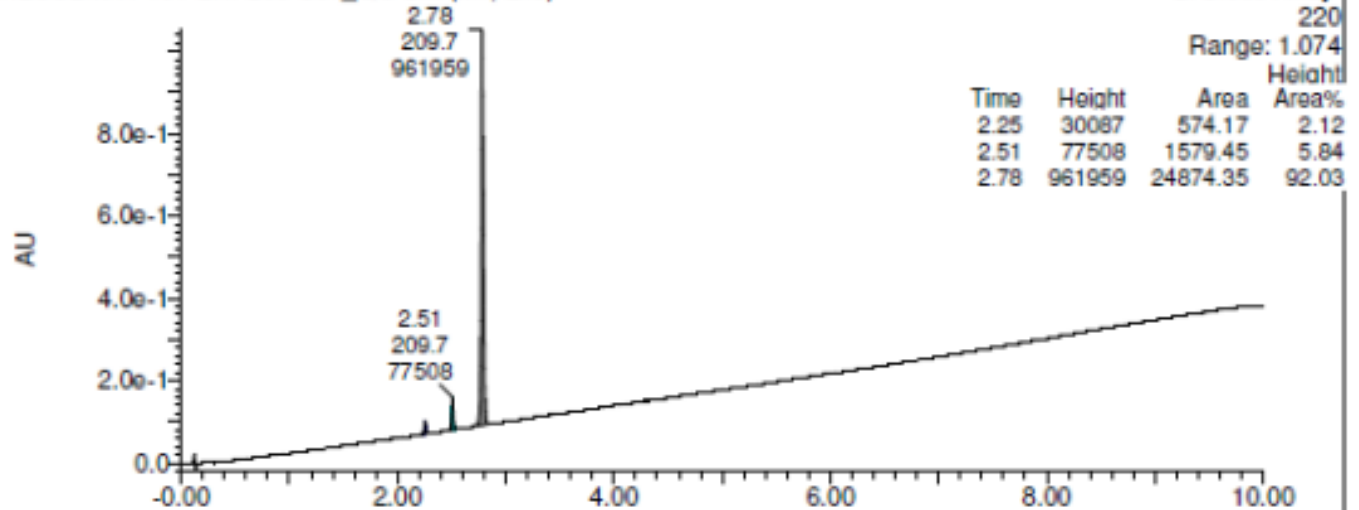
LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
565.189  
2.82e7

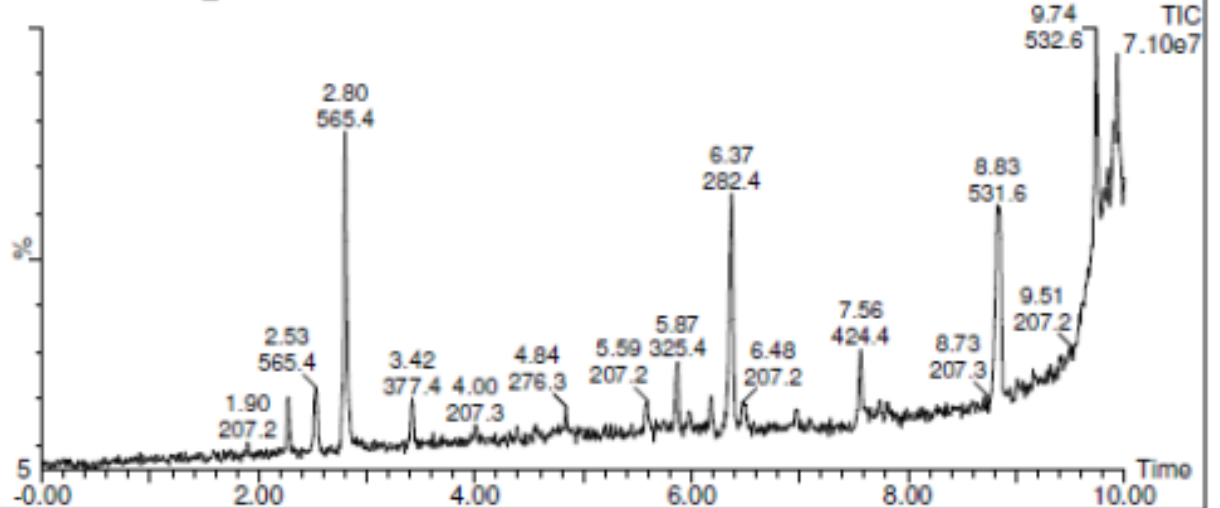
HESSEMA4-101-EXP206-S01\_OC



HESSEMA4-101-EXP206-S01\_OC Sm (Mn, 2x3)



HESSEMA4-101-EXP206-S01\_OC



HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

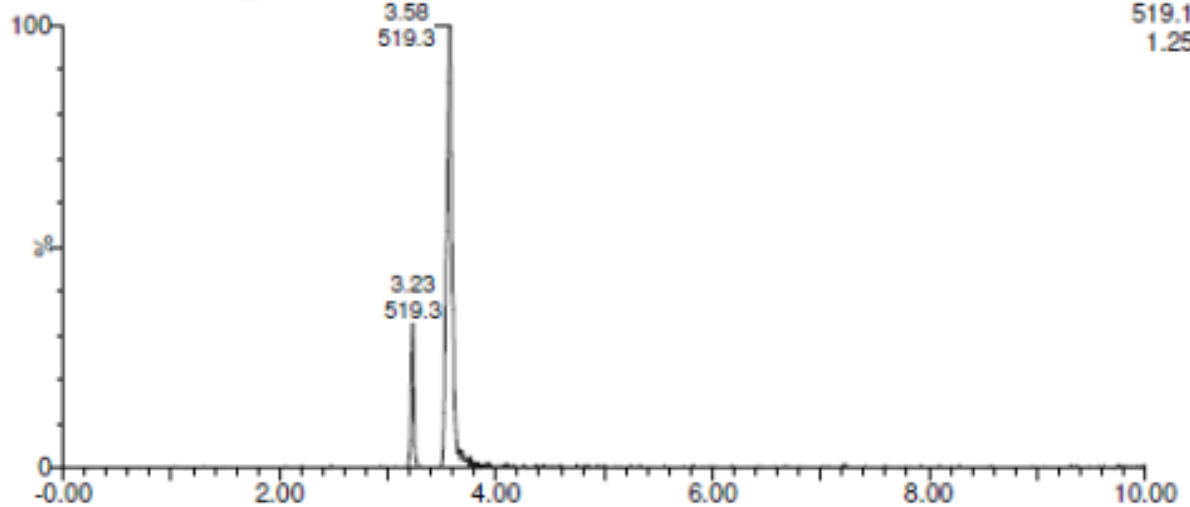
EM23001590291

19-Jan-2023  
16:20:41  
strubhe1-0118A-v05

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

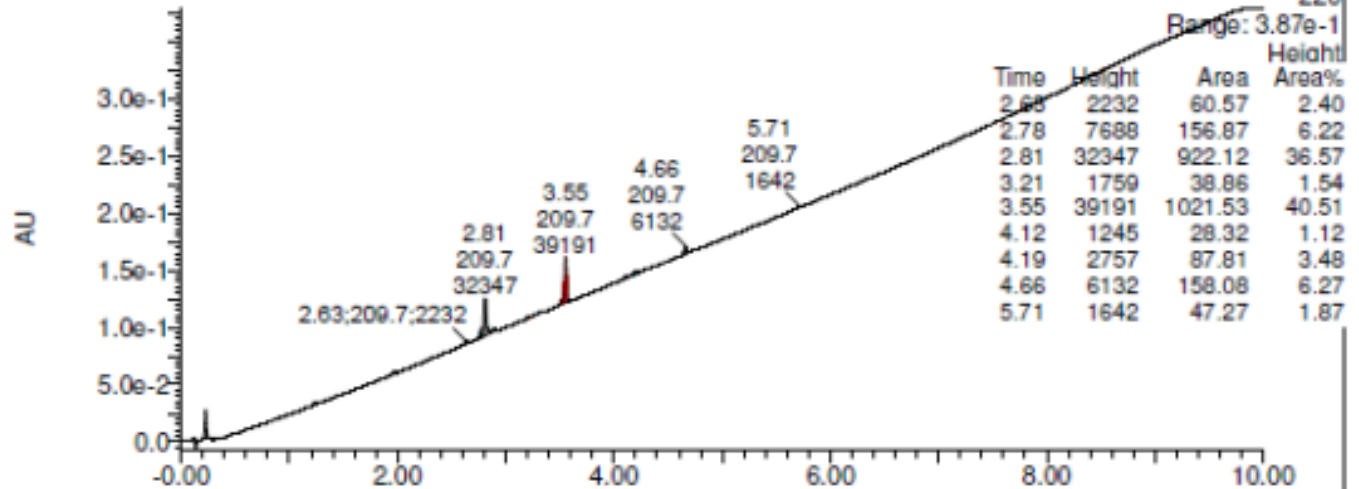
1: Scan ES+  
519.199  
1.25e7

HESSEMA4-101-EXP208-S01R\_OC



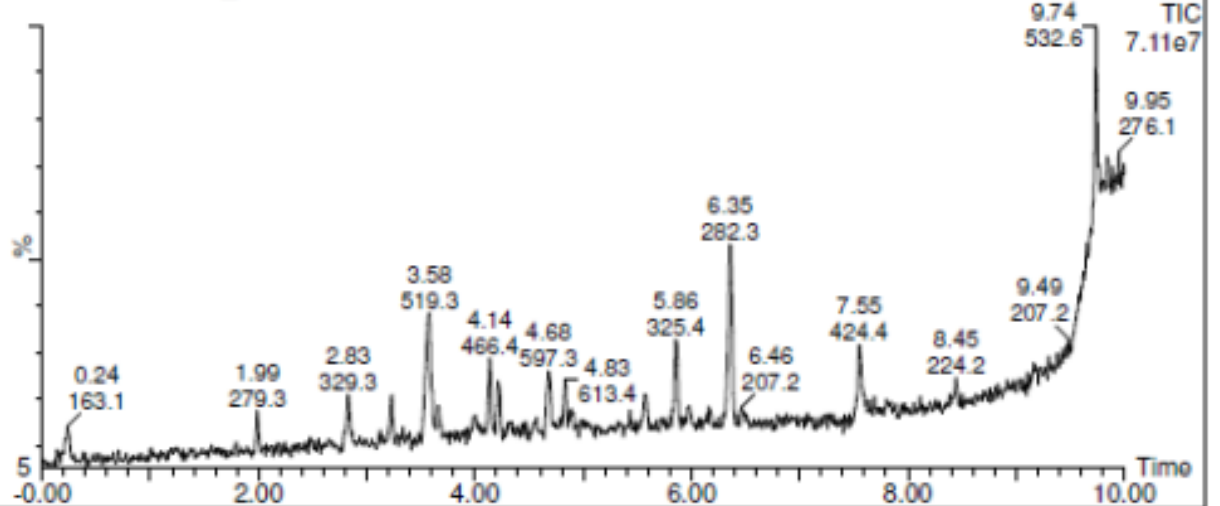
HESSEMA4-101-EXP208-S01R\_OC Sm (Mn, 2x3)

2: Diode Array  
220  
Range: 3.87e-1



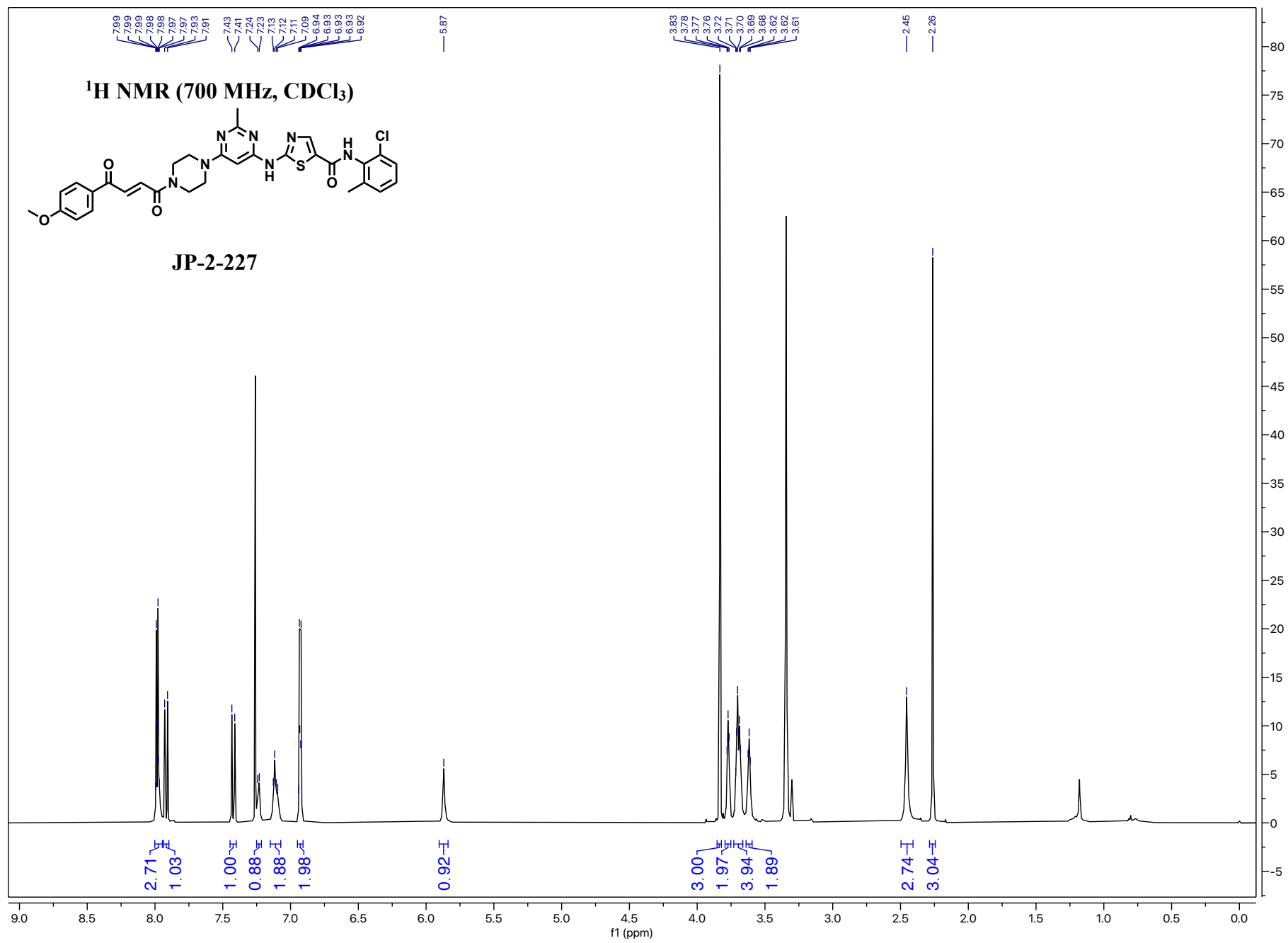
HESSEMA4-101-EXP208-S01R\_OC

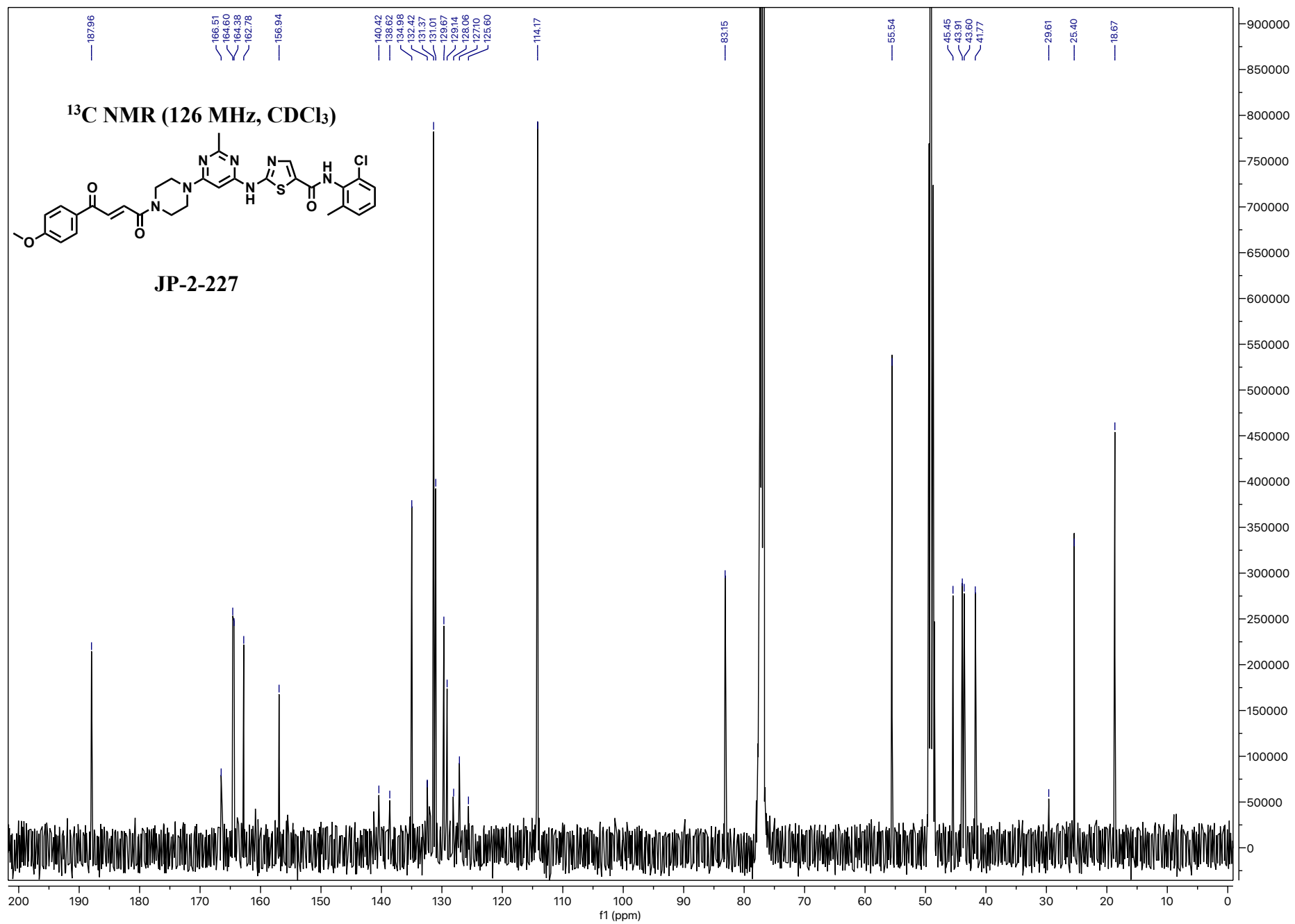
1: Scan ES+  
TIC  
9.74  
532.6  
7.11e7











HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

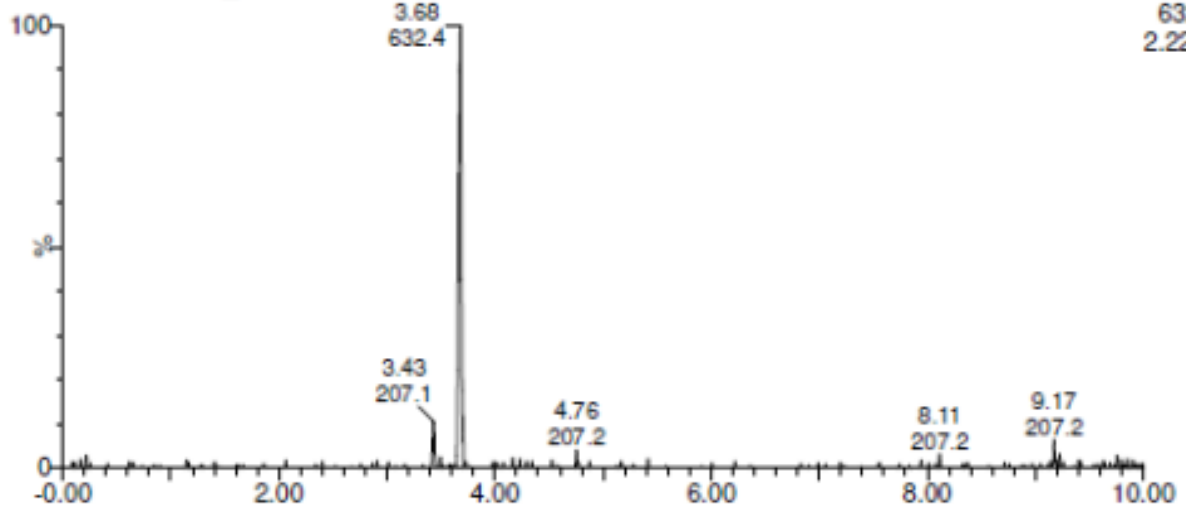
EM23001590289

13-Jan-2023  
15:30:39  
strubhe1-0112A-v04

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

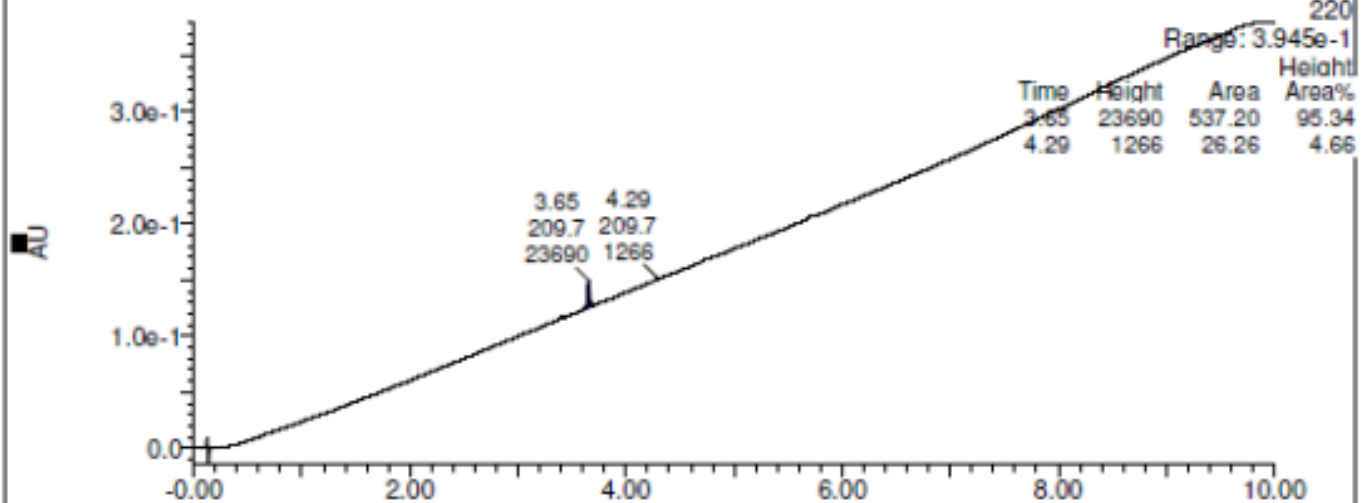
HESSEMA4-101-EXP207-S01\_OC-S

1: Scan ES+  
632.1  
2.22e6



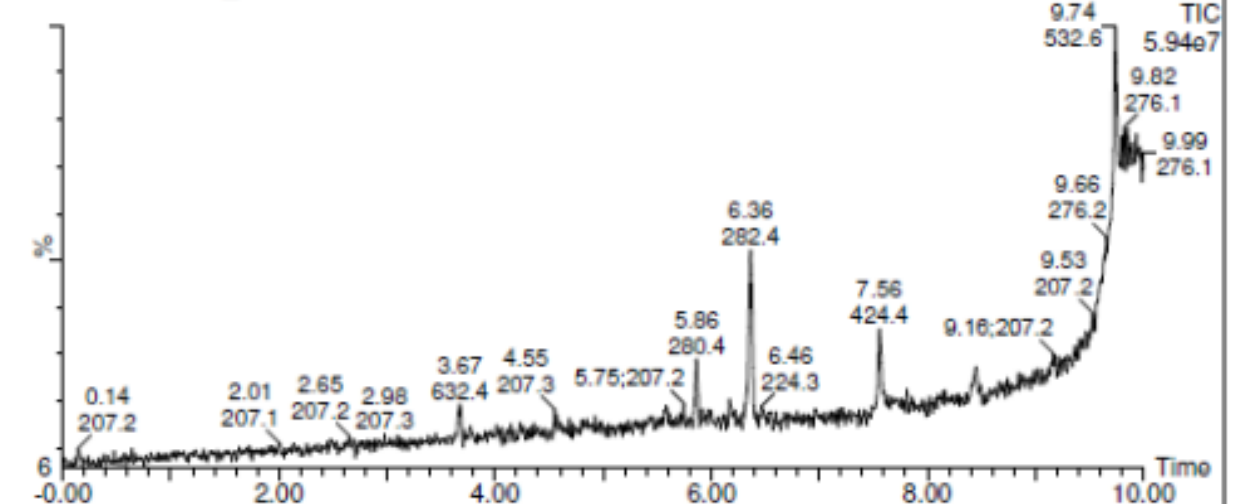
HESSEMA4-101-EXP207-S01\_OC-S Sm (Mn, 2x3)

2: Diode Array



HESSEMA4-101-EXP207-S01\_OC-S

1: Scan ES+ TIC



HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

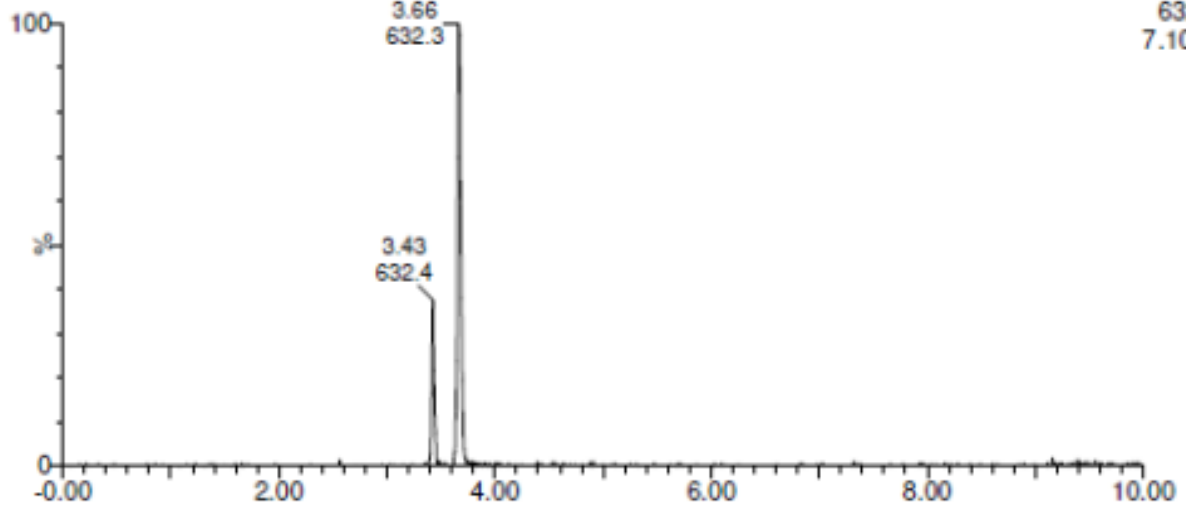
EM23001590289

19-Jan-2023  
16:09:36  
strubhe1-0118A-v04

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

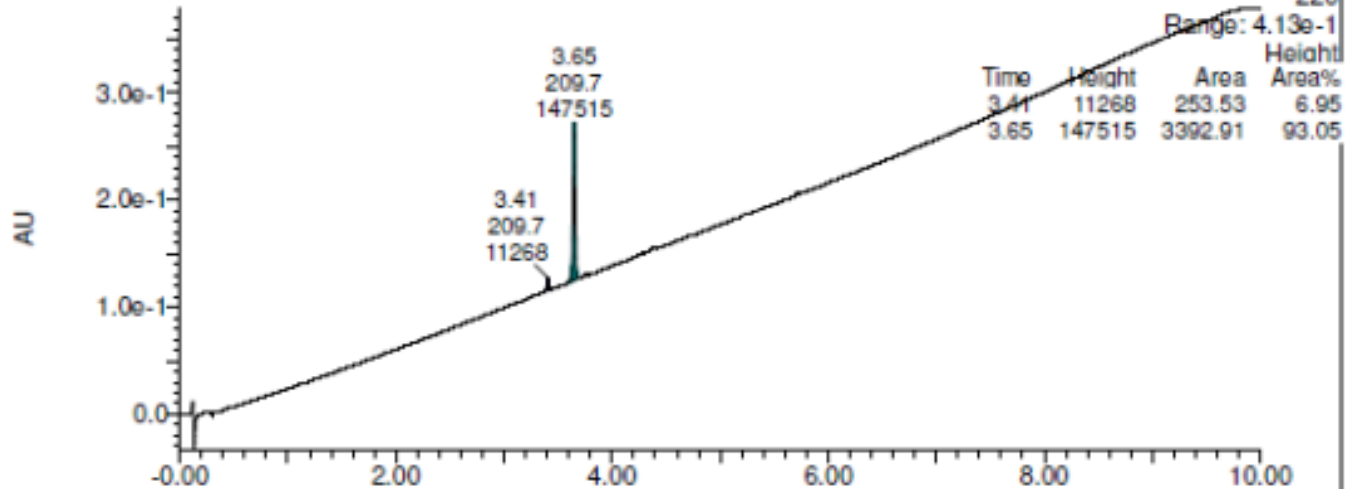
1: Scan ES+  
632.1  
7.10e6

HESSEMA4-101-EXP207-S01R\_OC



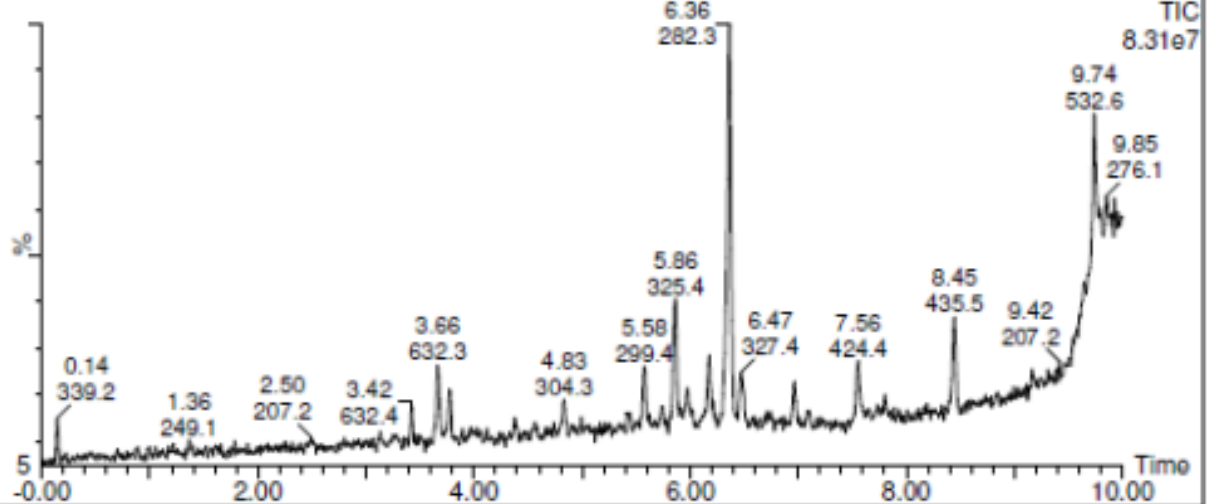
HESSEMA4-101-EXP207-S01R\_OC Sm (Mn, 2x3)

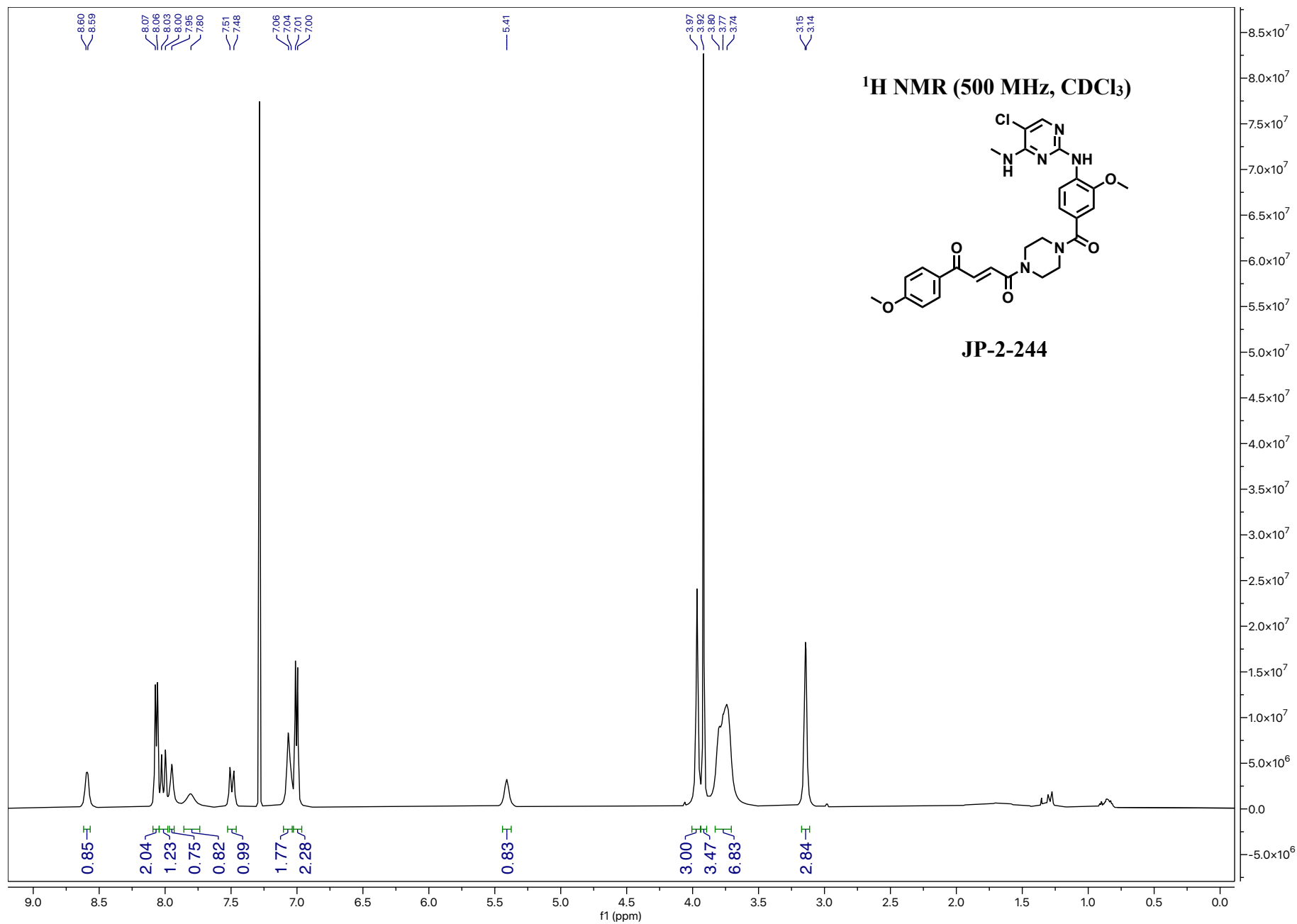
2: Diode Array

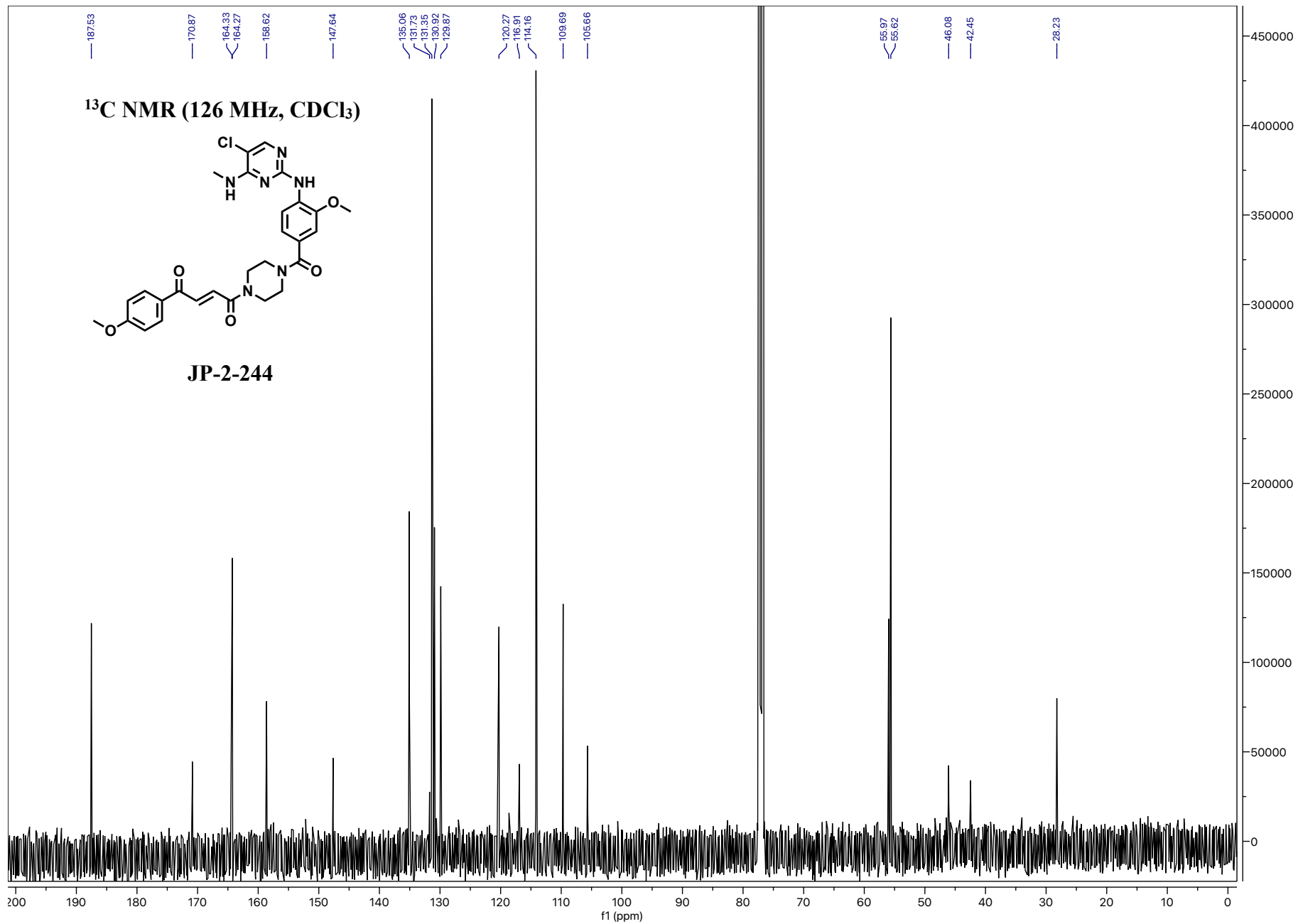


HESSEMA4-101-EXP207-S01R\_OC

1: Scan ES+







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

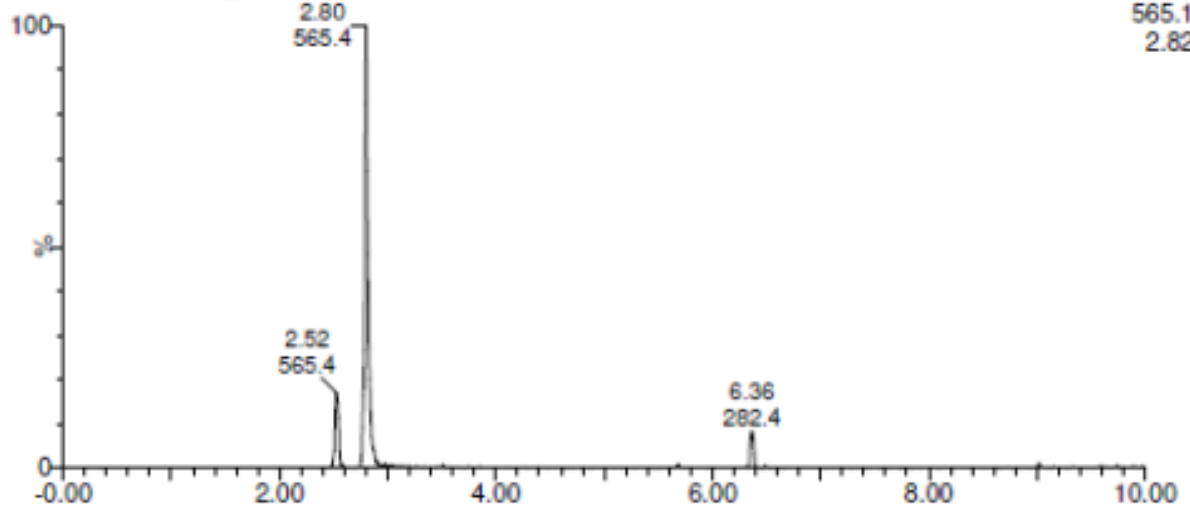
EM23001590285

13-Jan-2023  
15:54:05  
strubhe1-0112A-v03

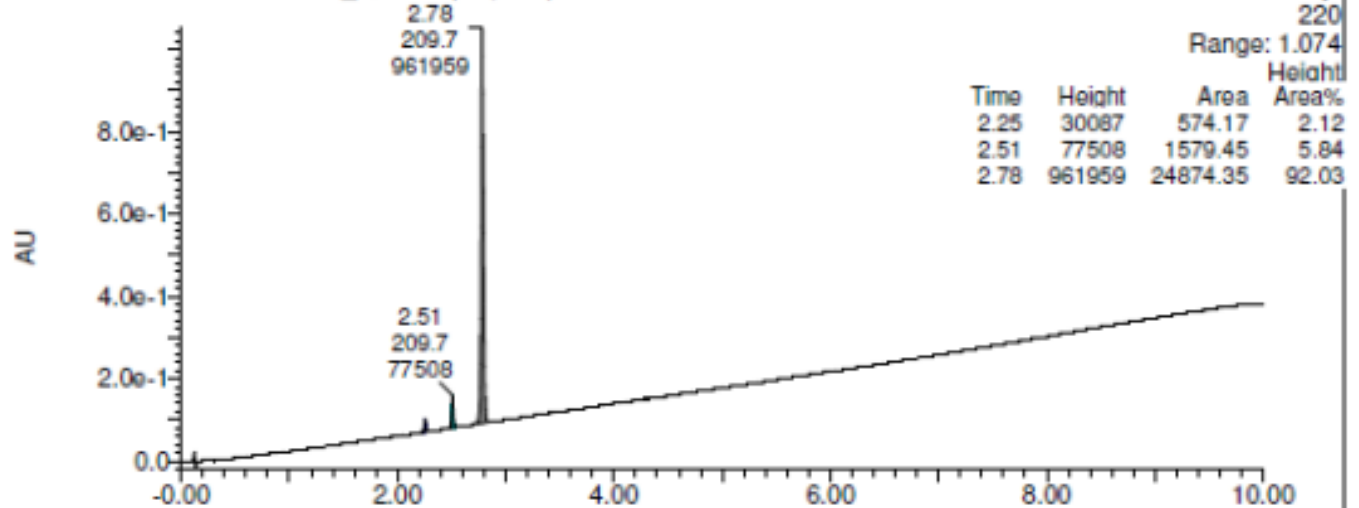
LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
565.189  
2.82e7

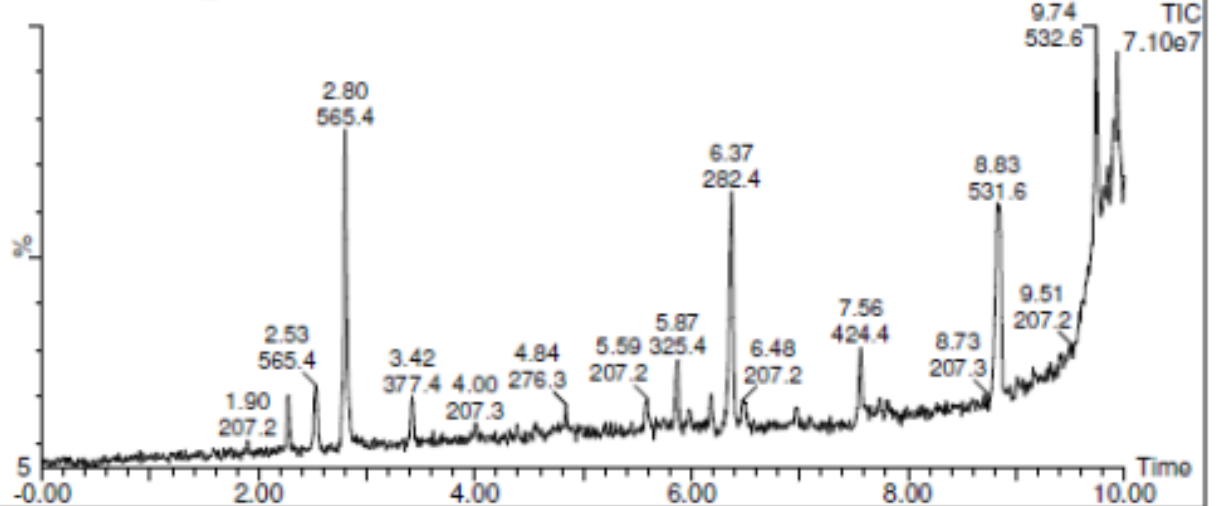
HESSEMA4-101-EXP206-S01\_OC



HESSEMA4-101-EXP206-S01\_OC Sm (Mn, 2x3)



HESSEMA4-101-EXP206-S01\_OC



HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

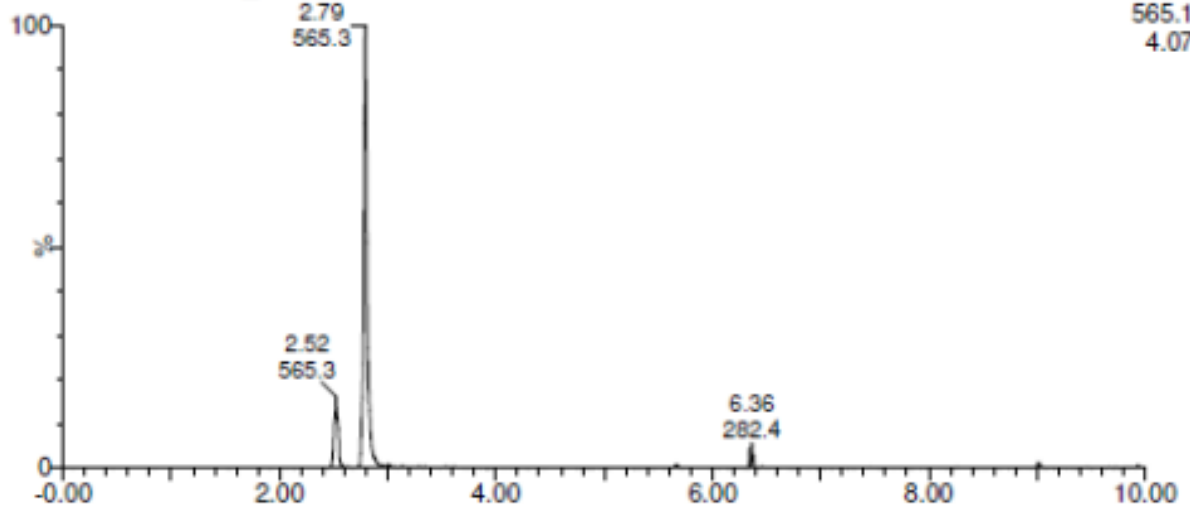
EM23001590285

19-Jan-2023  
15:58:35  
strubhe1-0118A-v03

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

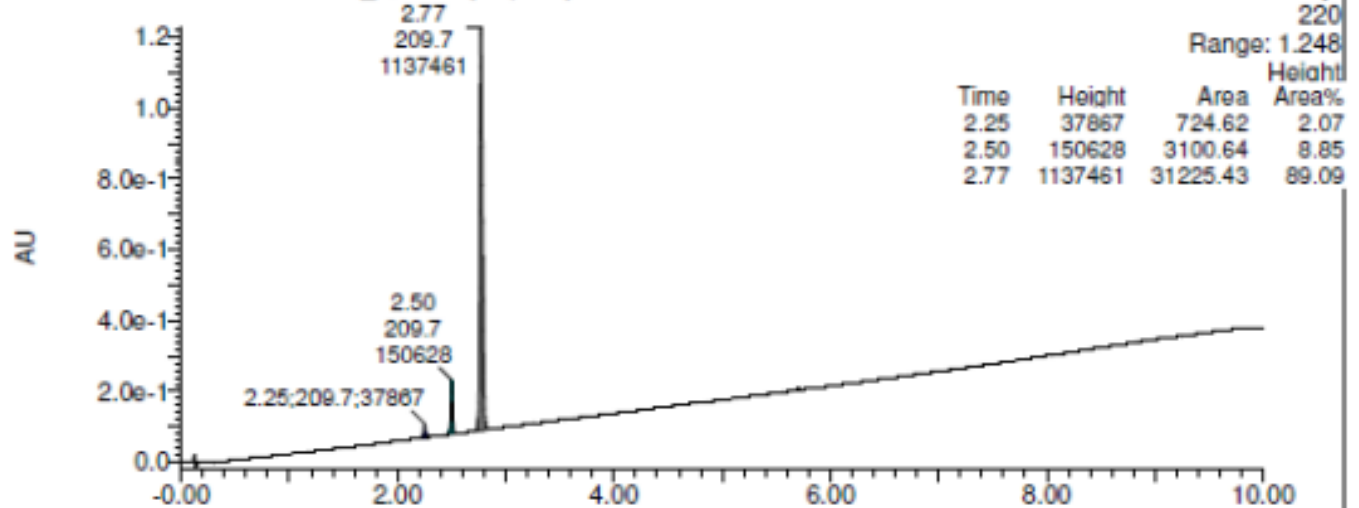
1: Scan ES+  
565.189  
4.07e7

HESSEMA4-101-EXP206-S01R\_QC



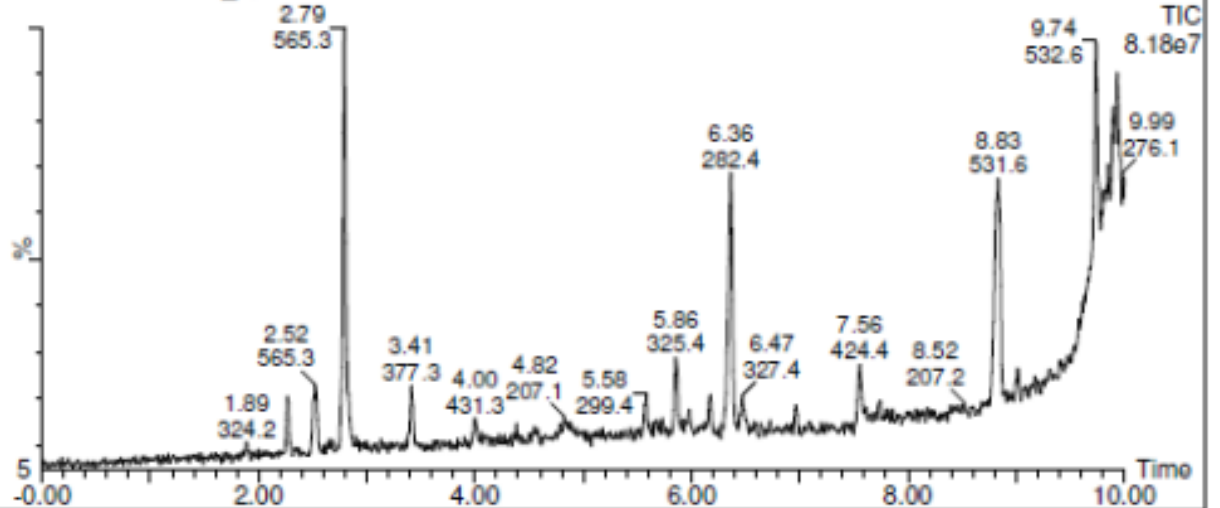
HESSEMA4-101-EXP206-S01R\_QC Sm (Mn, 2x3)

2: Diode Array  
220  
Range: 1.248  
Height



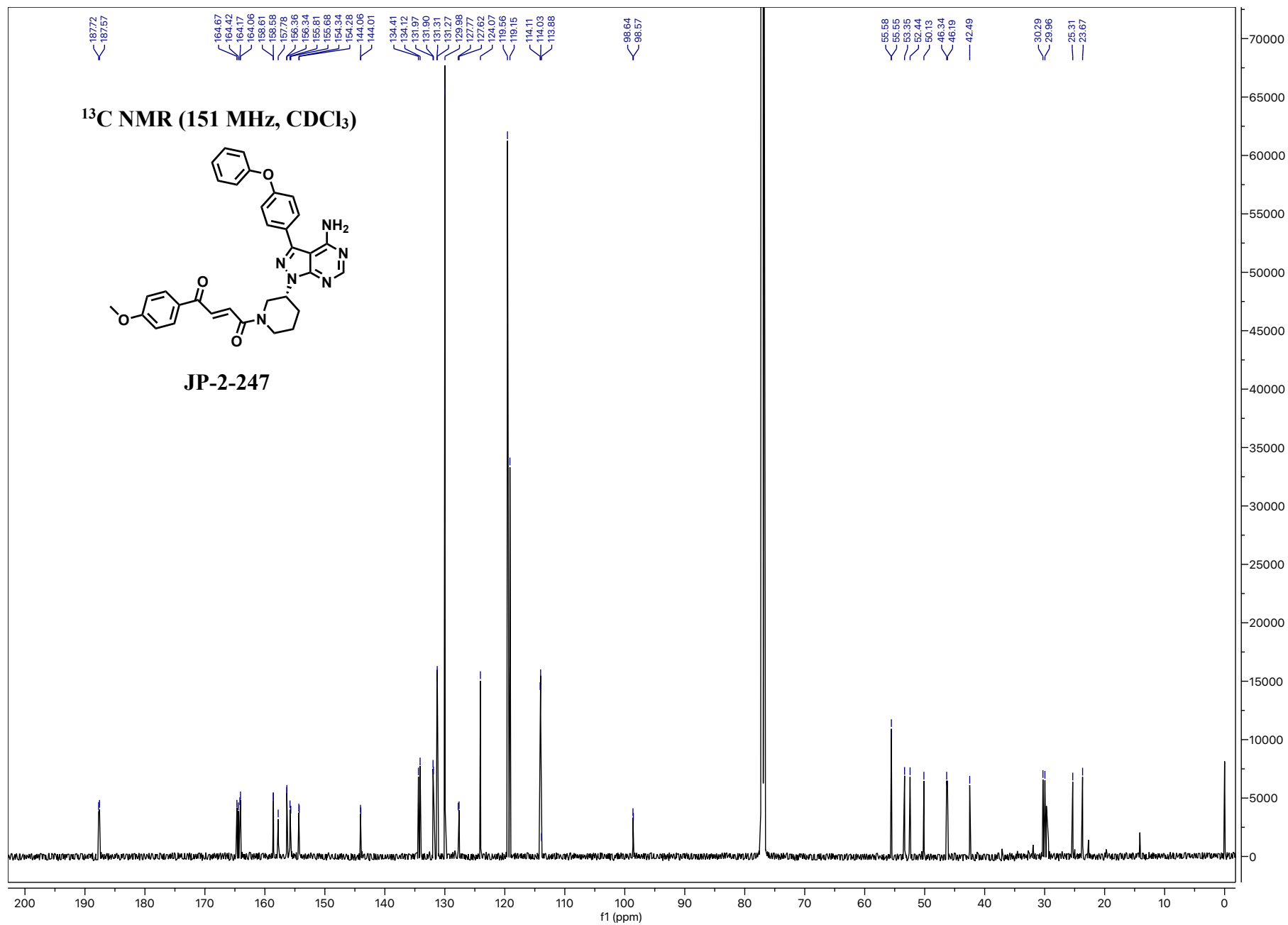
HESSEMA4-101-EXP206-S01R\_QC

1: Scan ES+  
TIC  
8.18e7









HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

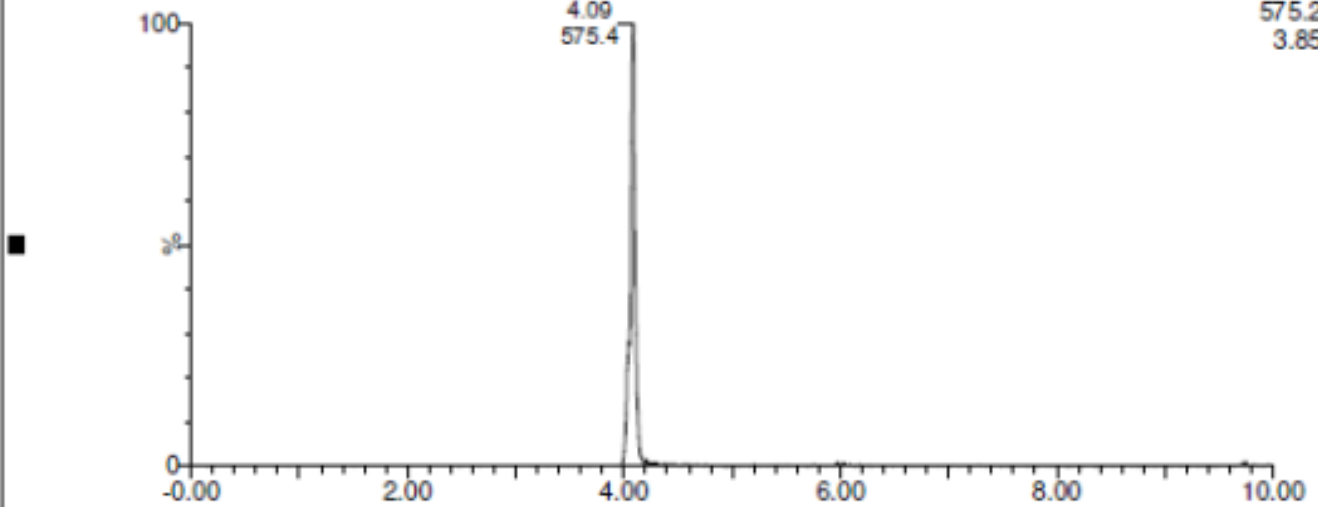
EM23001590284

13-Jan-2023  
14:56:11  
strubhe1-0112A-v02

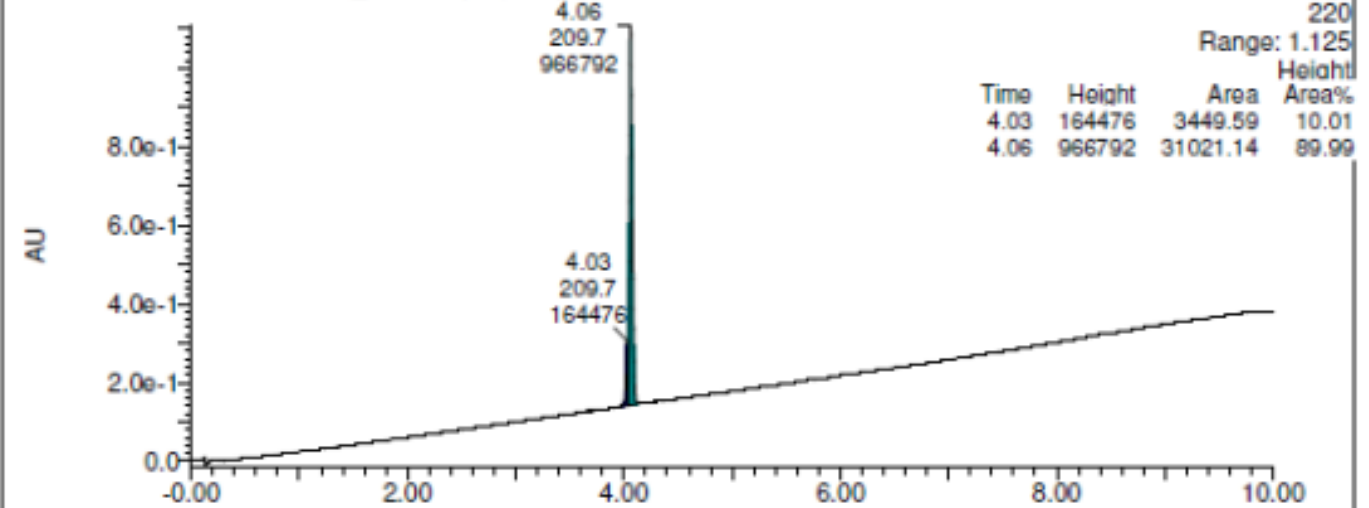
LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

1: Scan ES+  
575.233  
3.85e7

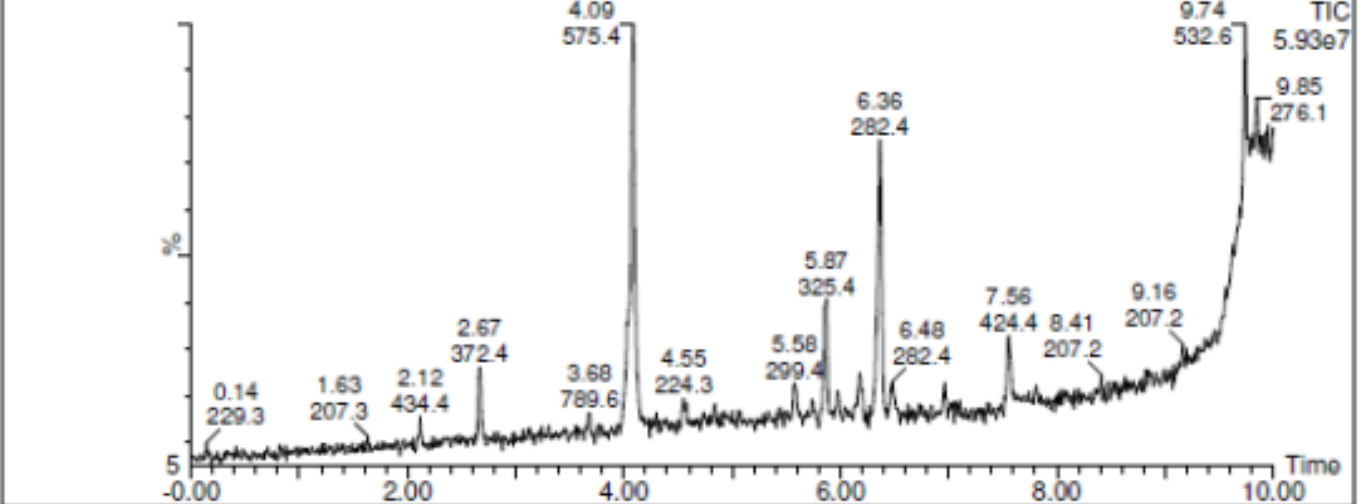
HESSEMA4-101-EXP205-S01\_OC



HESSEMA4-101-EXP205-S01\_OC Sm (Mn, 2x3)



HESSEMA4-101-EXP205-S01\_OC



HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

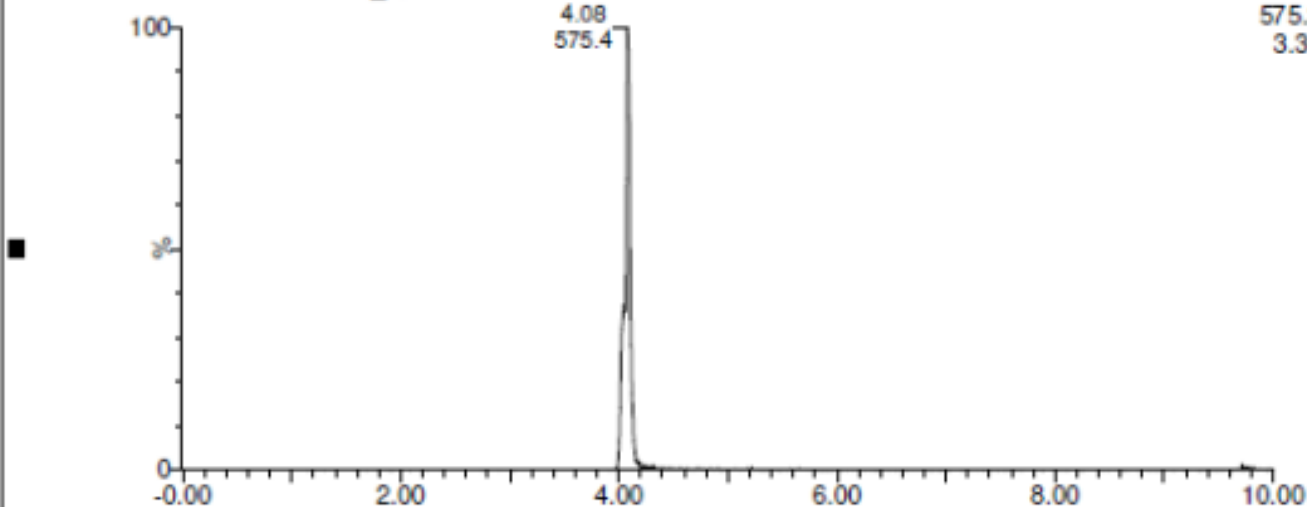
EM23001590284

19-Jan-2023  
15:46:53  
strubhe1-0118A-v02

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

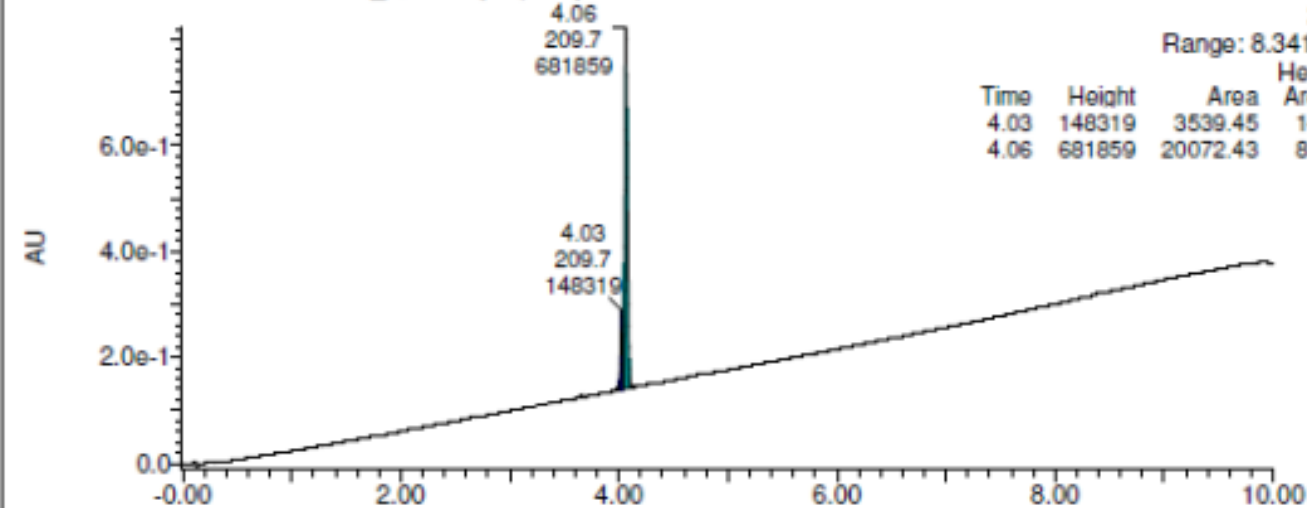
1: Scan ES+  
575.233  
3.36e7

HESSEMA4-101-EXP205-S01R\_OC



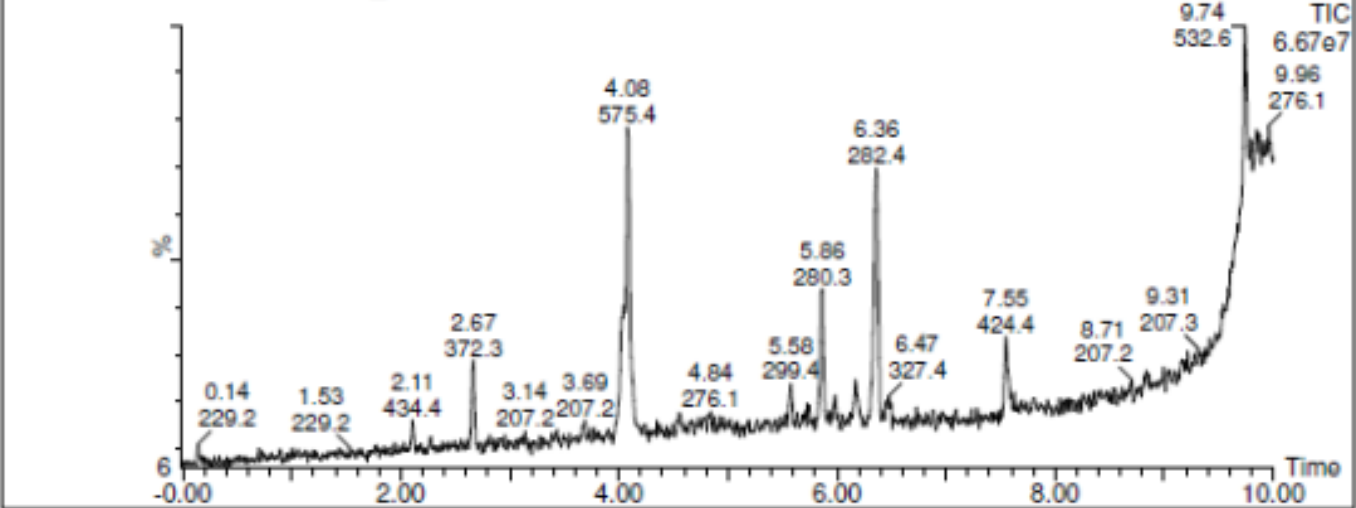
HESSEMA4-101-EXP205-S01R\_OC Sm (Mn, 2x3)

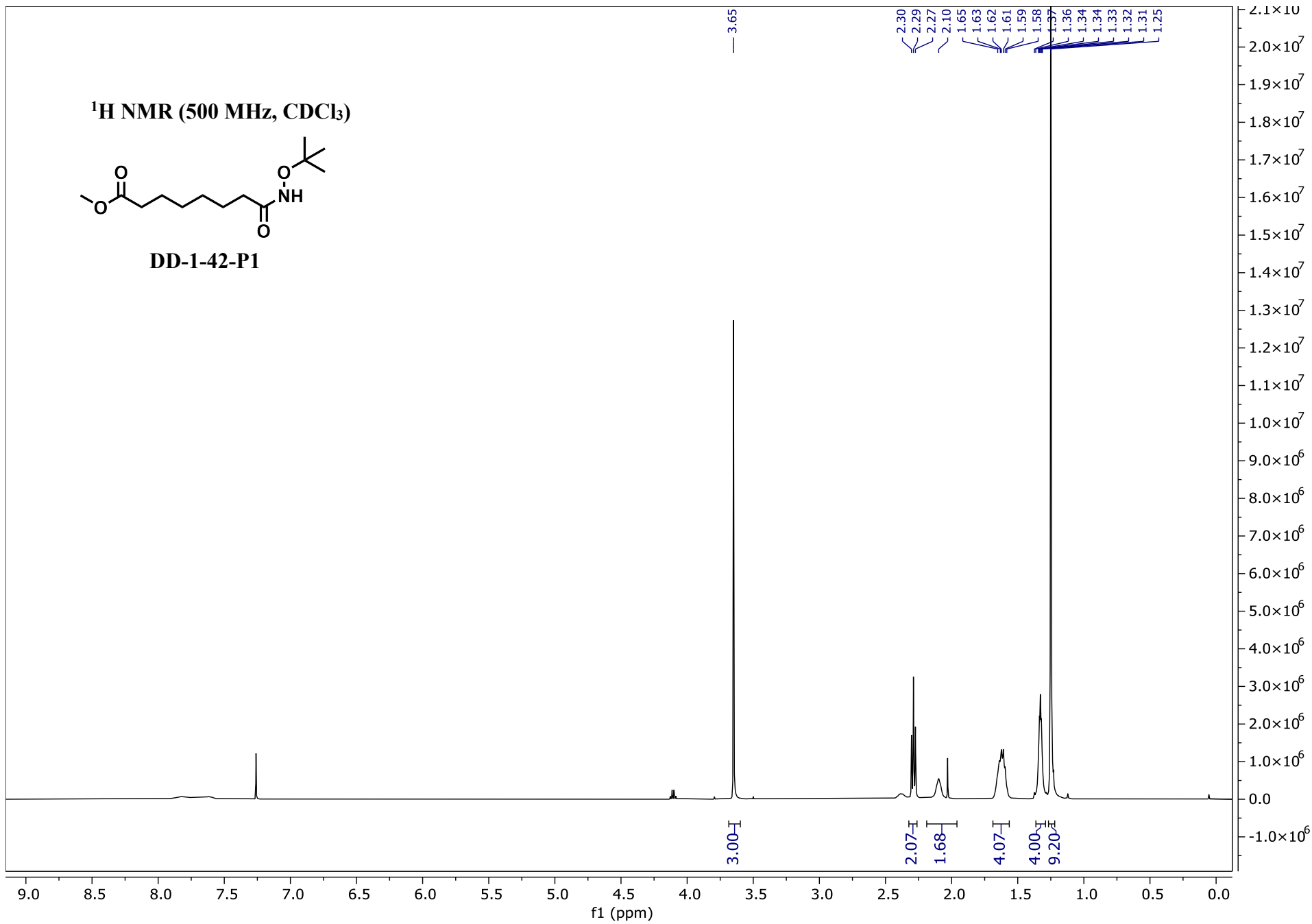
2: Diode Array  
220

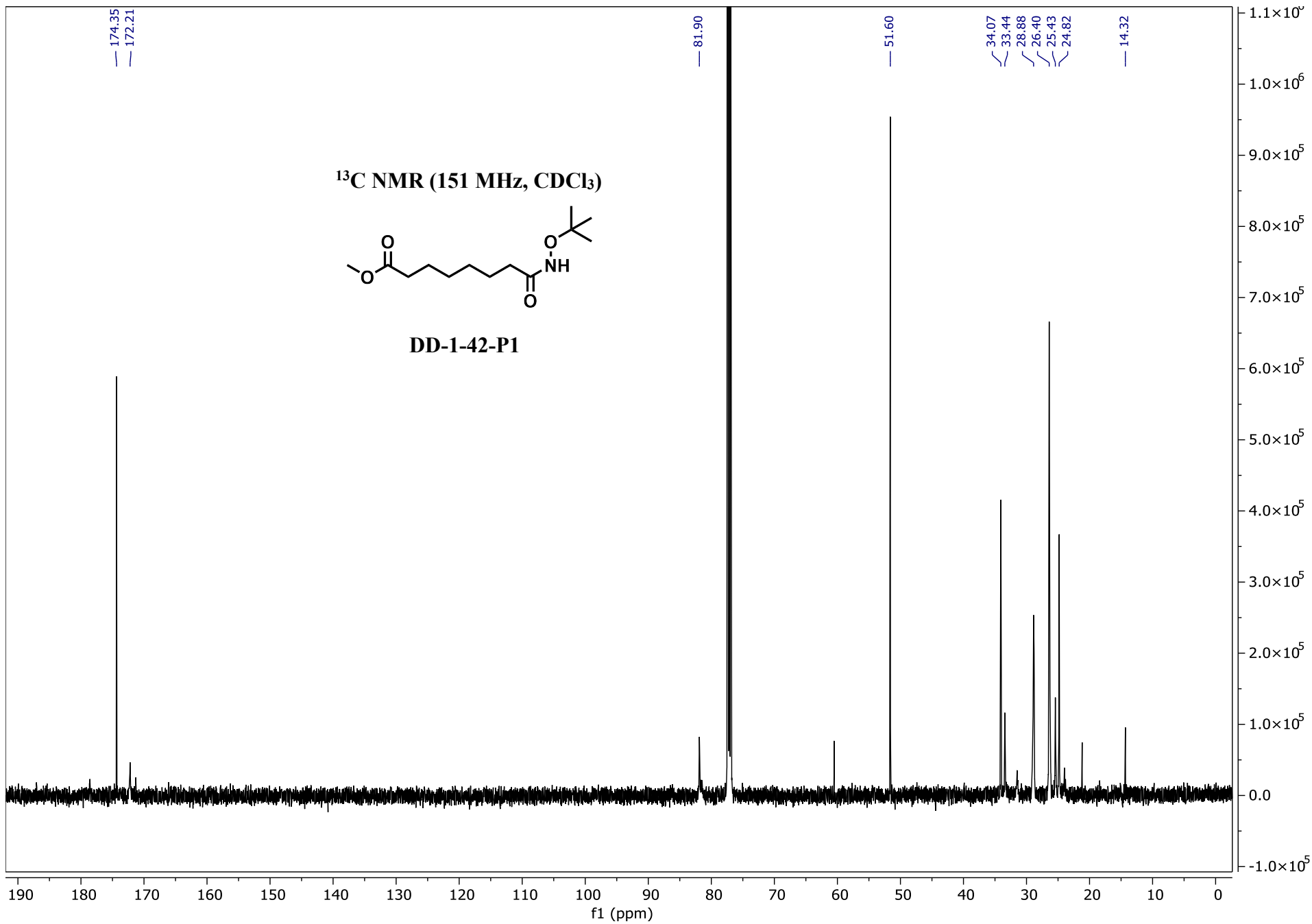


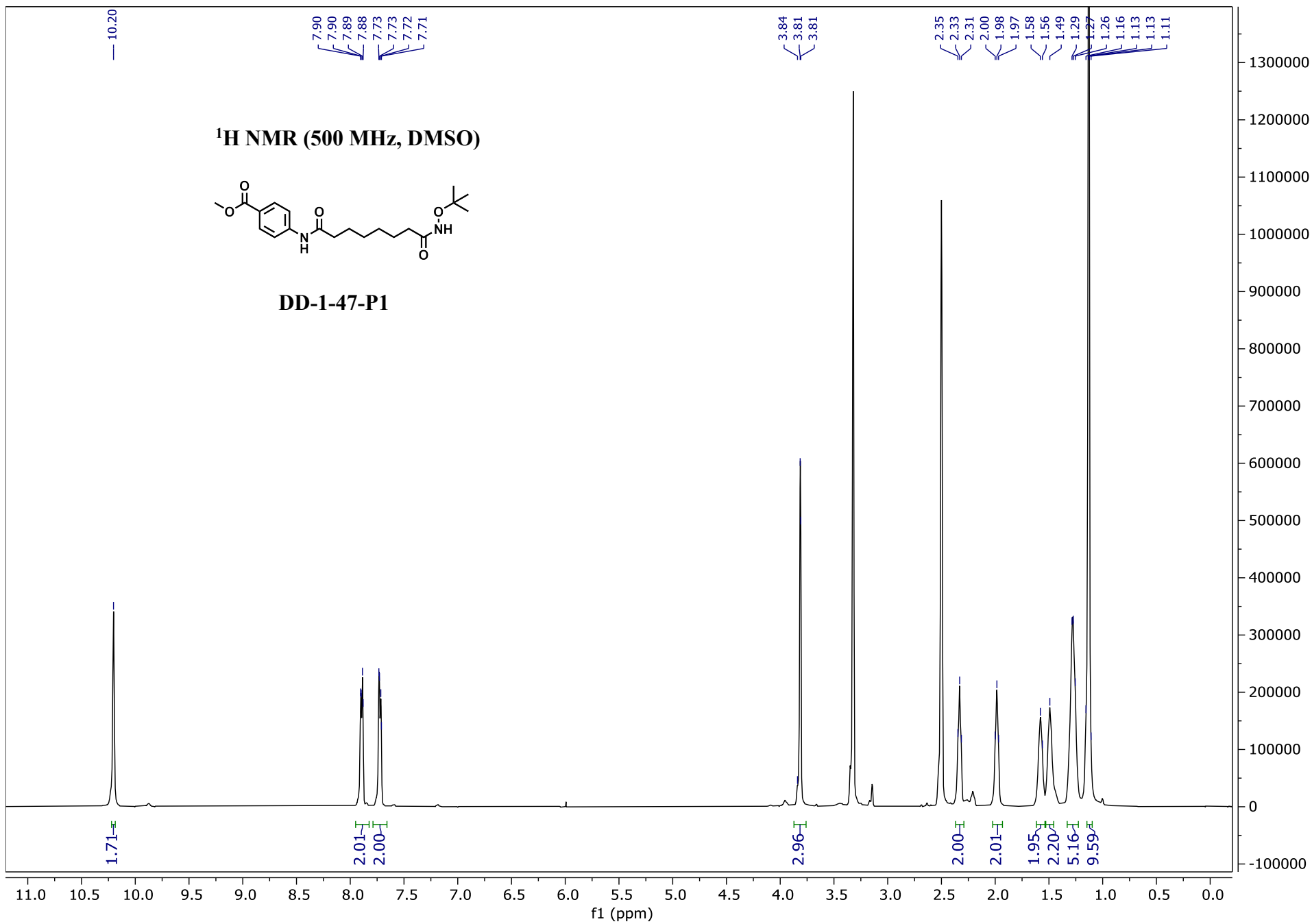
HESSEMA4-101-EXP205-S01R\_OC

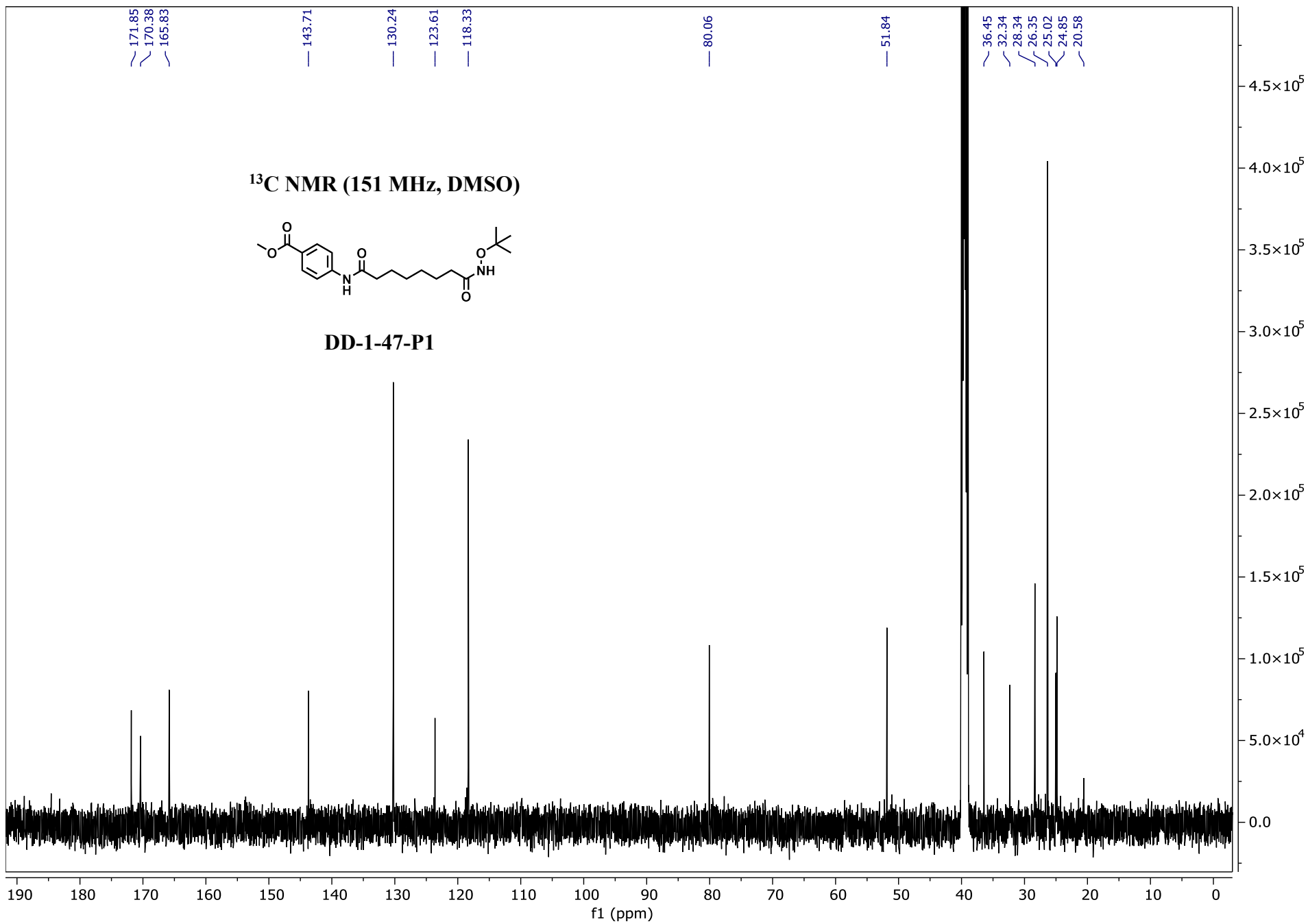
1: Scan ES+  
TIC



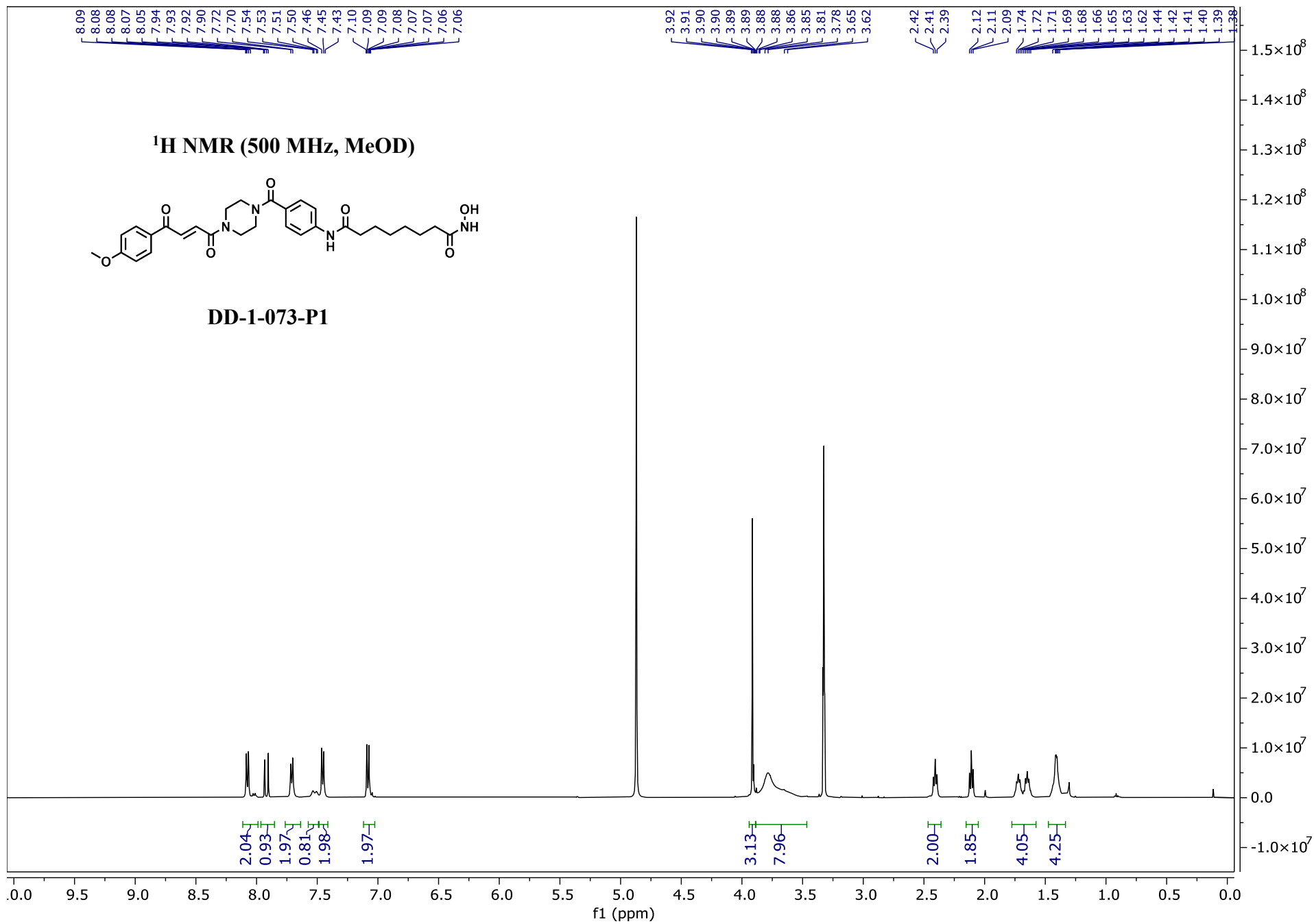


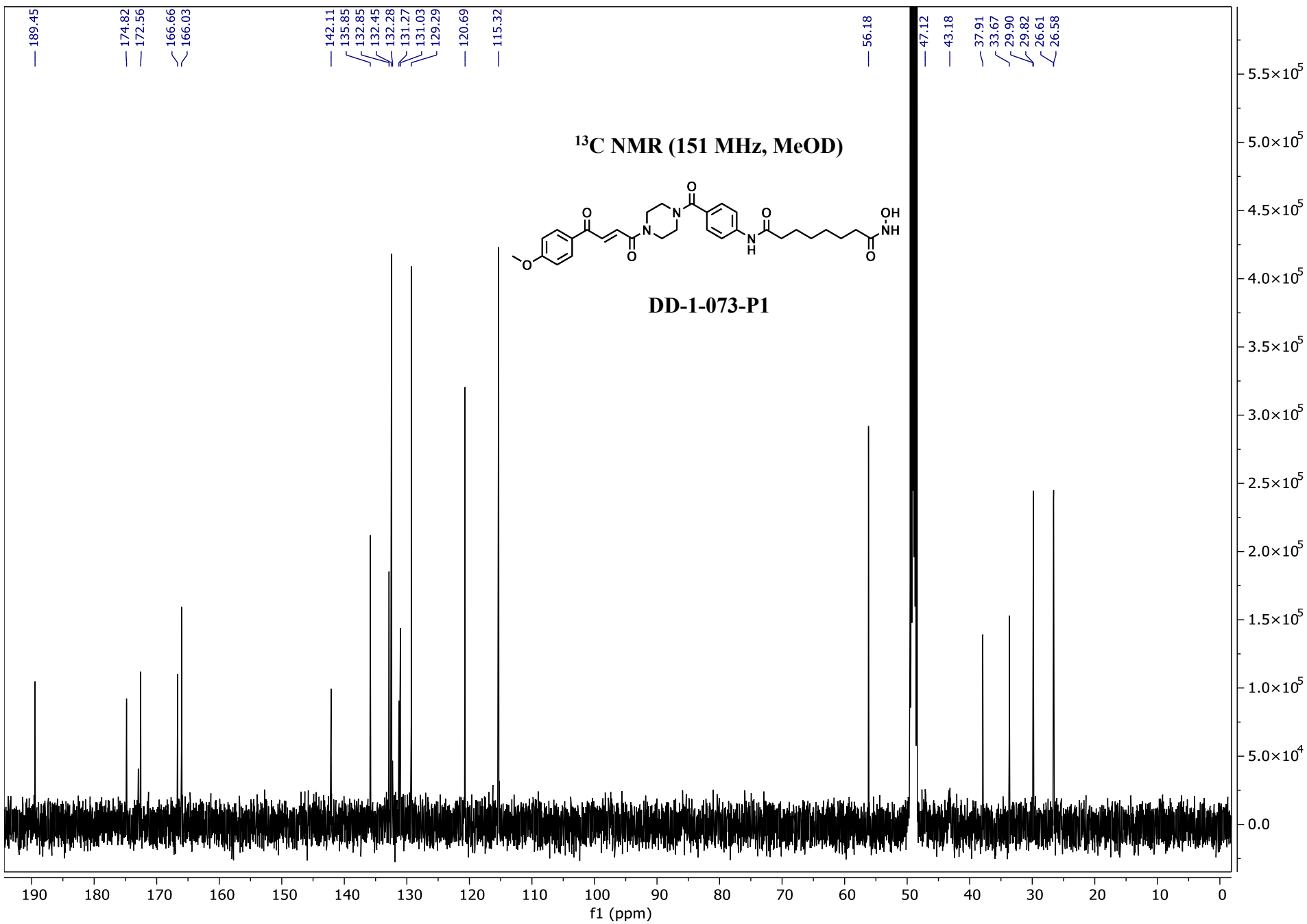














HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

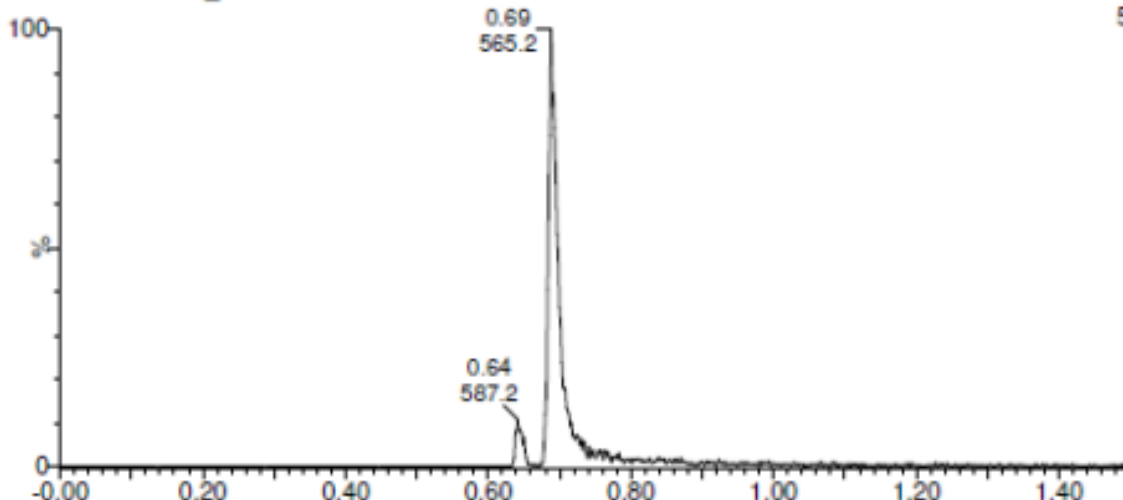
EM23001597802

02-Mar-2023  
14:21:04  
strubhe1-0302-v05

LCMS-QDa1  
Kinetex column,  
QDa1\_2m\_Acidic

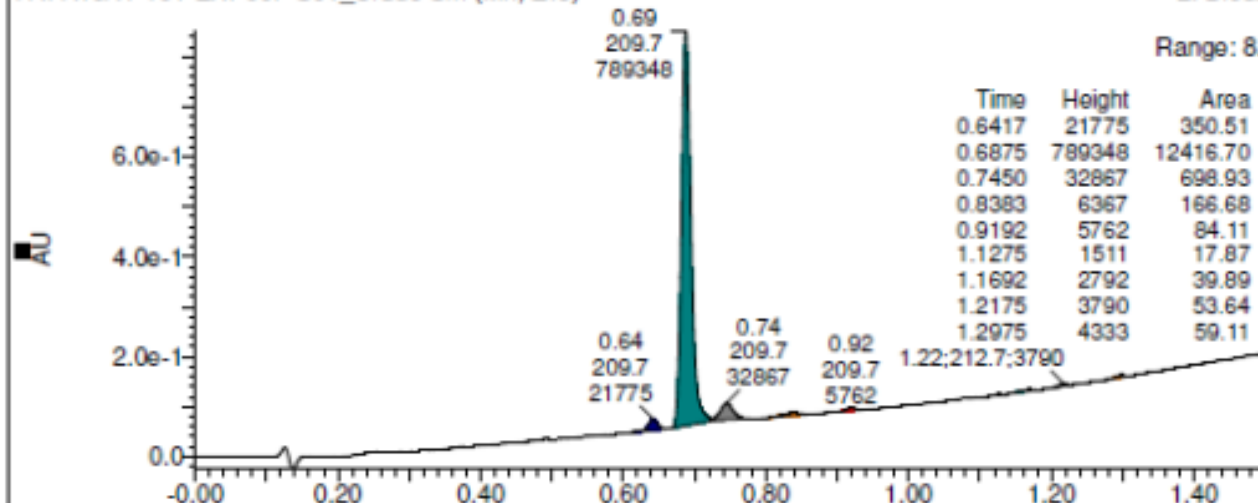
PAPATJA1-101-EXP007-S01\_Crude

1: Scan ES+  
565.258  
9.66e6



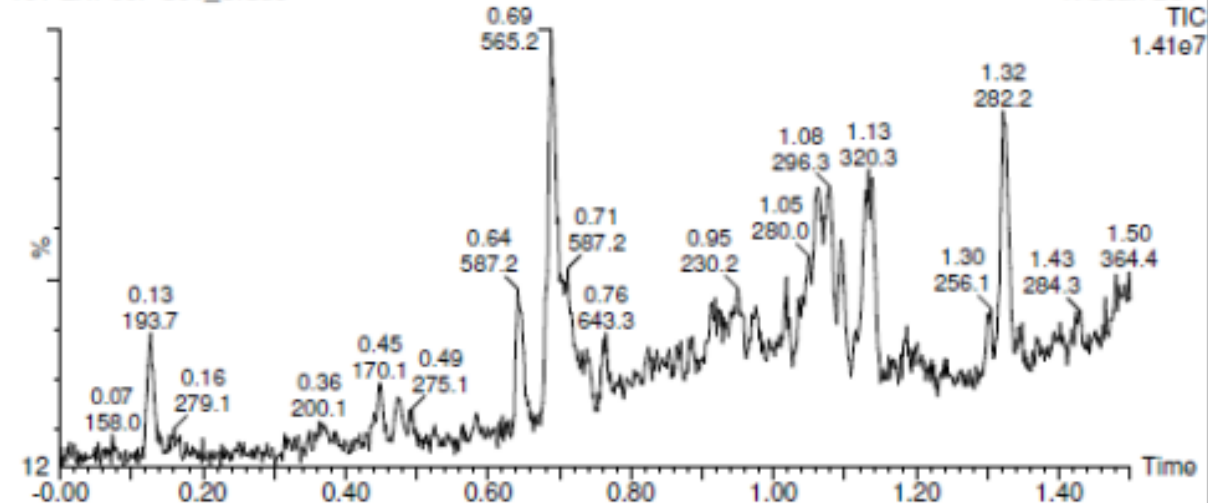
PAPATJA1-101-EXP007-S01\_Crude Sm (Mn, 2x3)

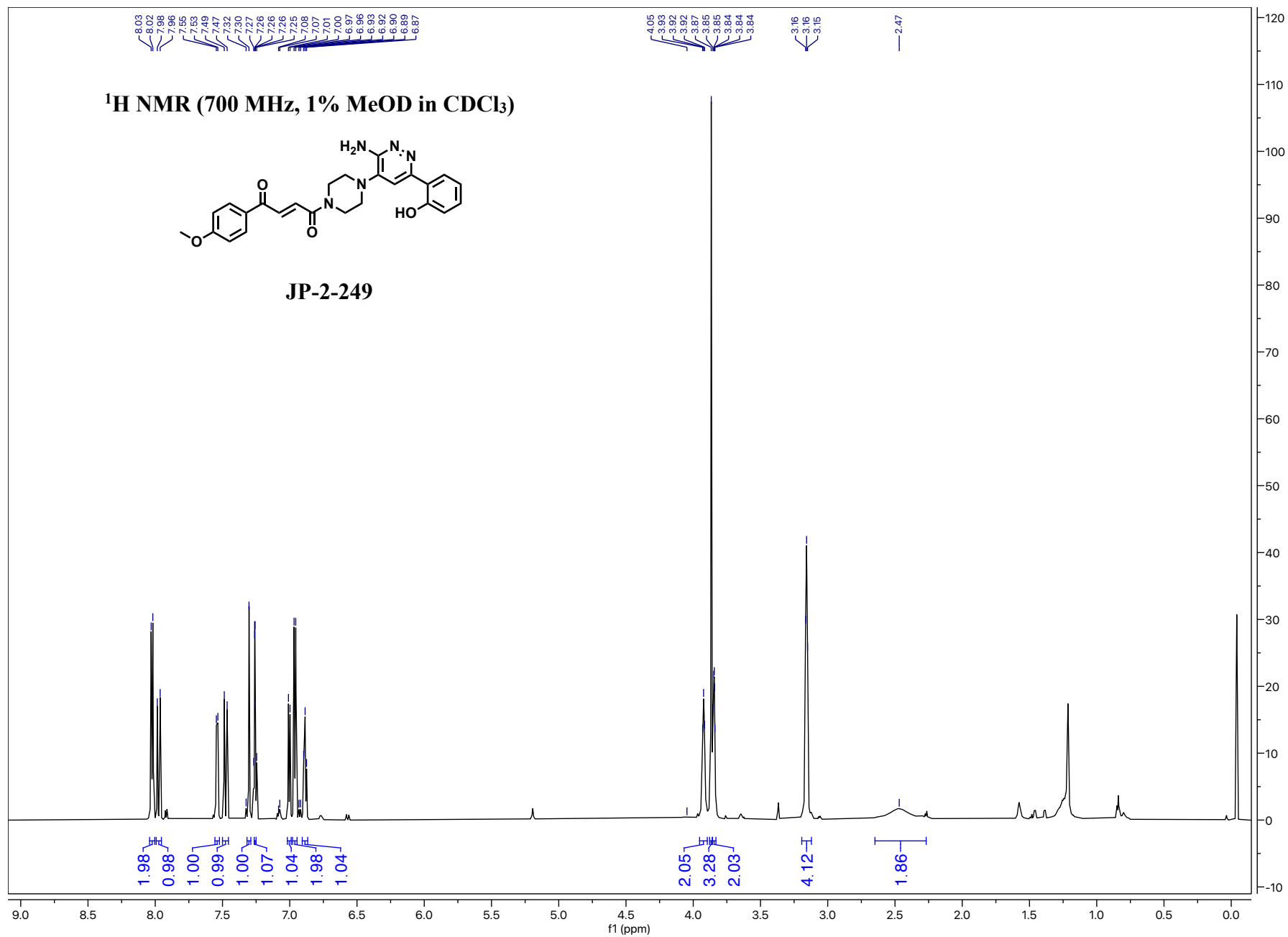
2: Diode Array  
220

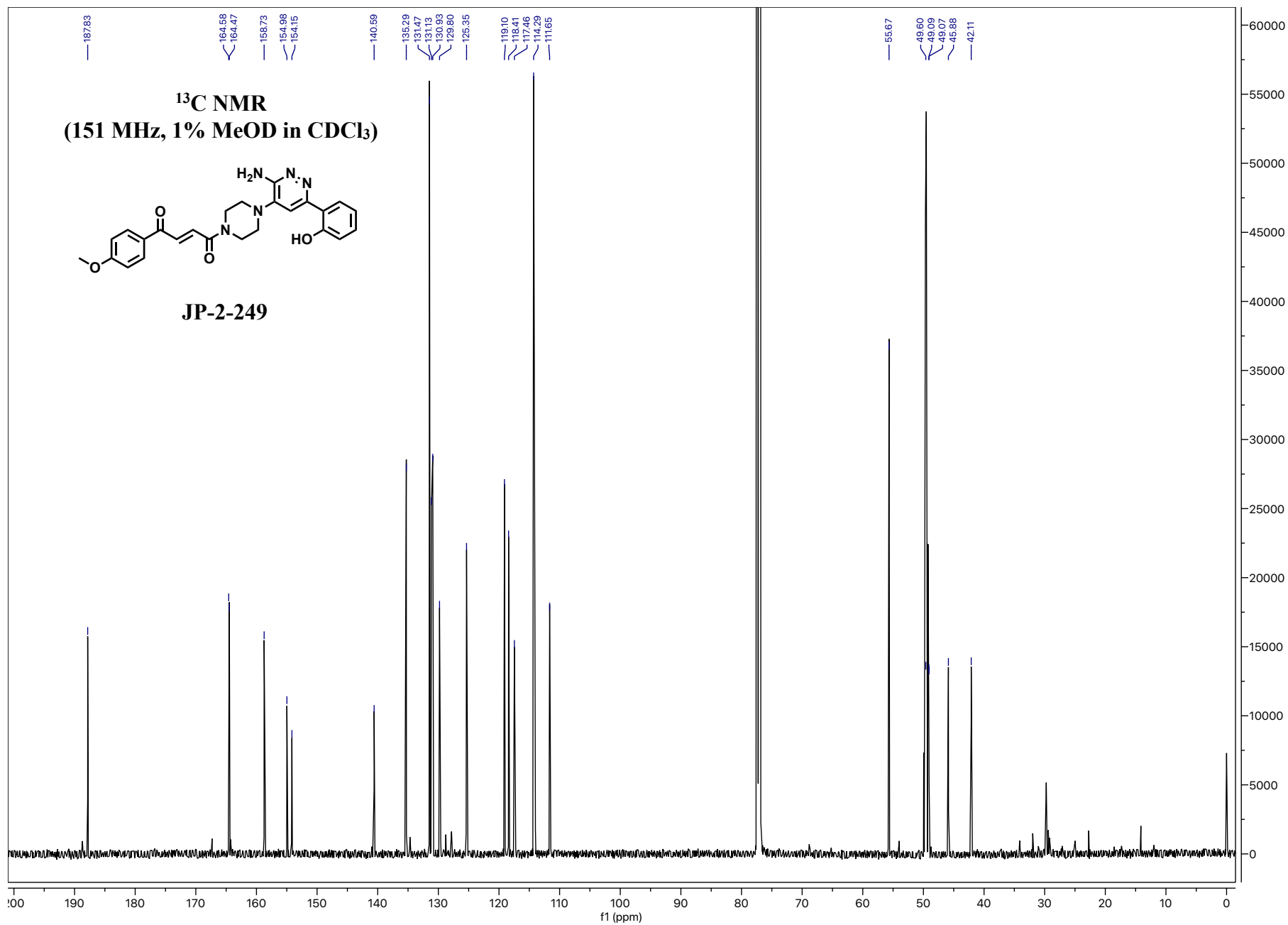


PAPATJA1-101-EXP007-S01\_Crude

1: Scan ES+  
TIC  
1.41e7







HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

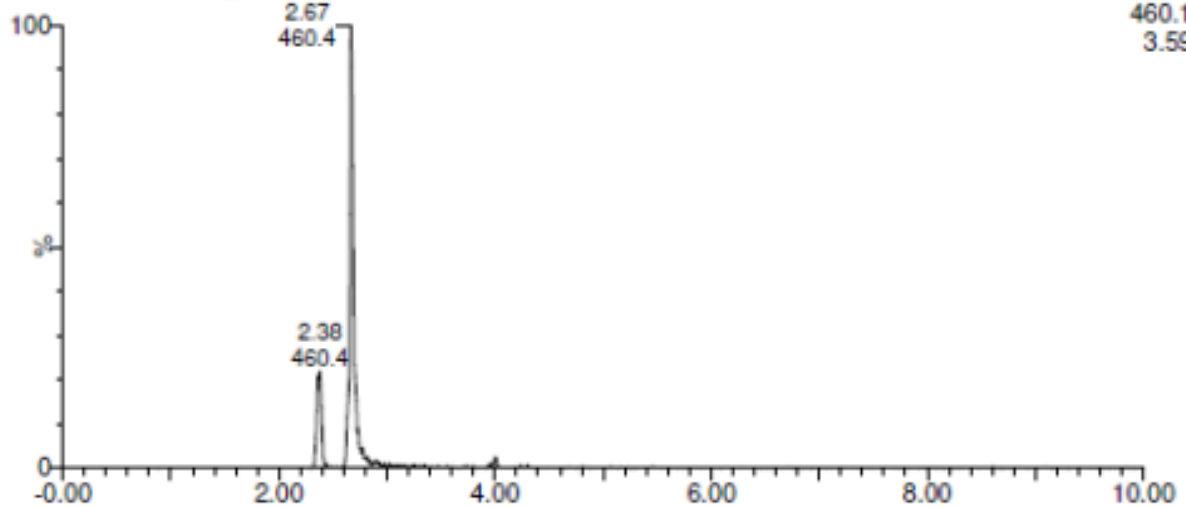
EM23001590282

13-Jan-2023  
14:43:28  
strubhe1-0112A-v01

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

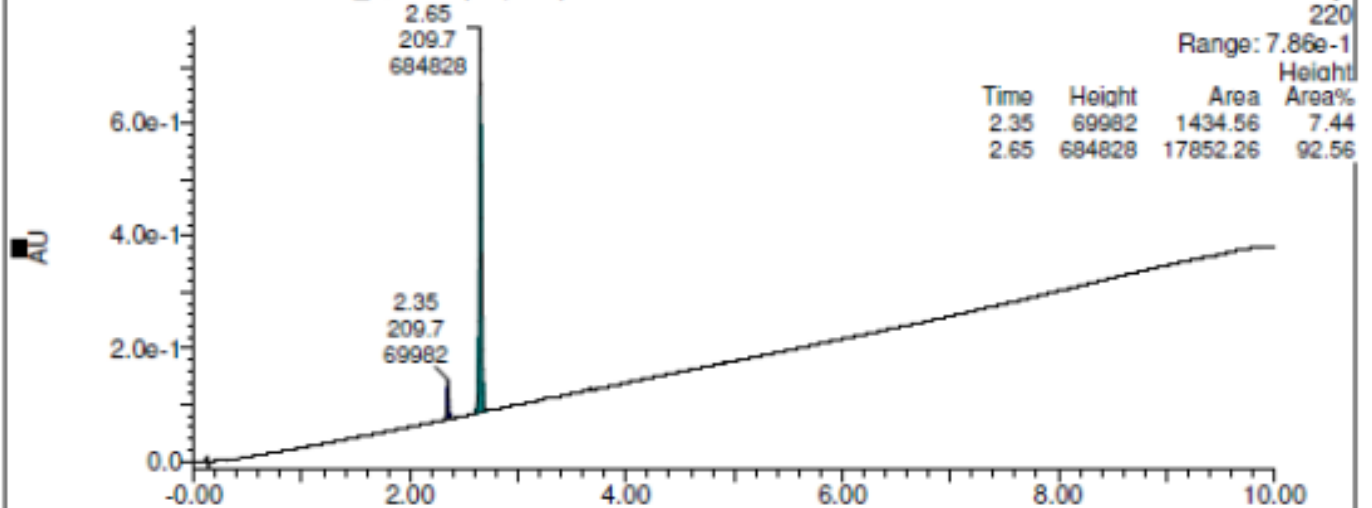
1: Scan ES+  
460.191  
3.59e7

HESSEMA4-101-EXP204-S01\_OC



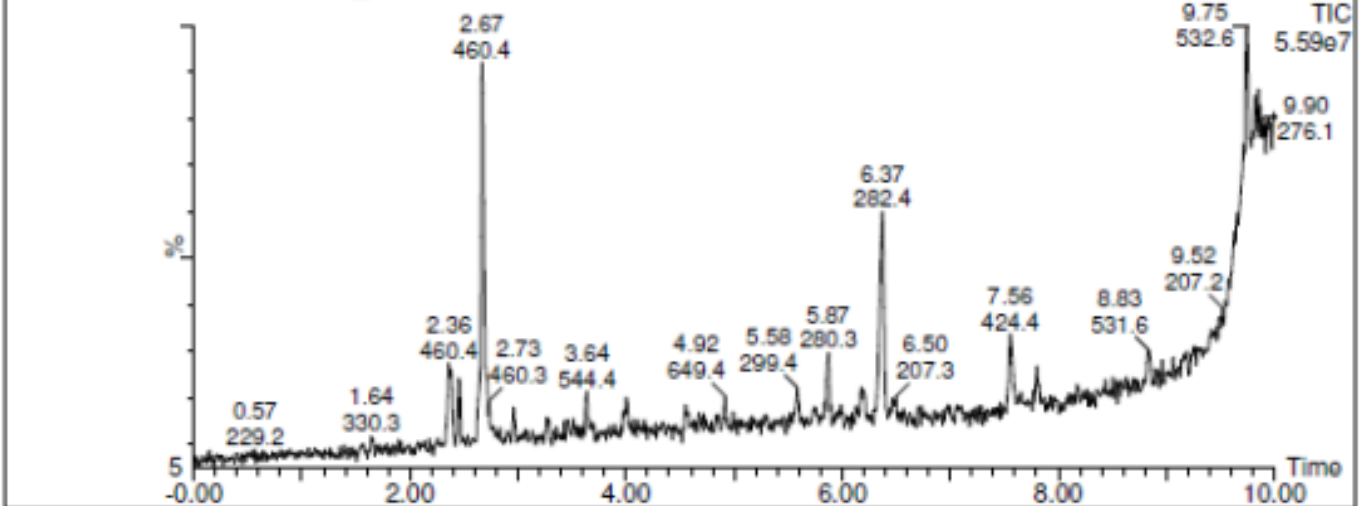
HESSEMA4-101-EXP204-S01\_OC Sm (Mn, 2x3)

2: Diode Array  
220  
Range: 7.86e-1  
Height



HESSEMA4-101-EXP204-S01\_OC

1: Scan ES+  
9.75  
532.6  
TIC  
5.59e7



HPLC=0.1%TFA in H2O/ACN with 1.2mL/min

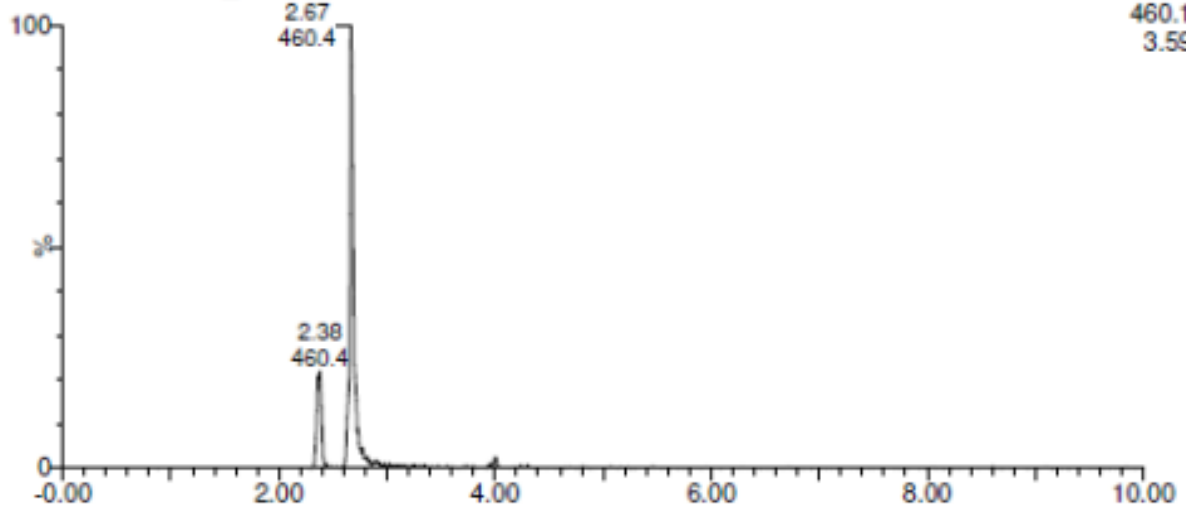
EM23001590282

13-Jan-2023  
14:43:28  
strubhe1-0112A-v01

LCMS-ZQ1  
Kinetex column,  
ZQ1\_10m\_Acidic

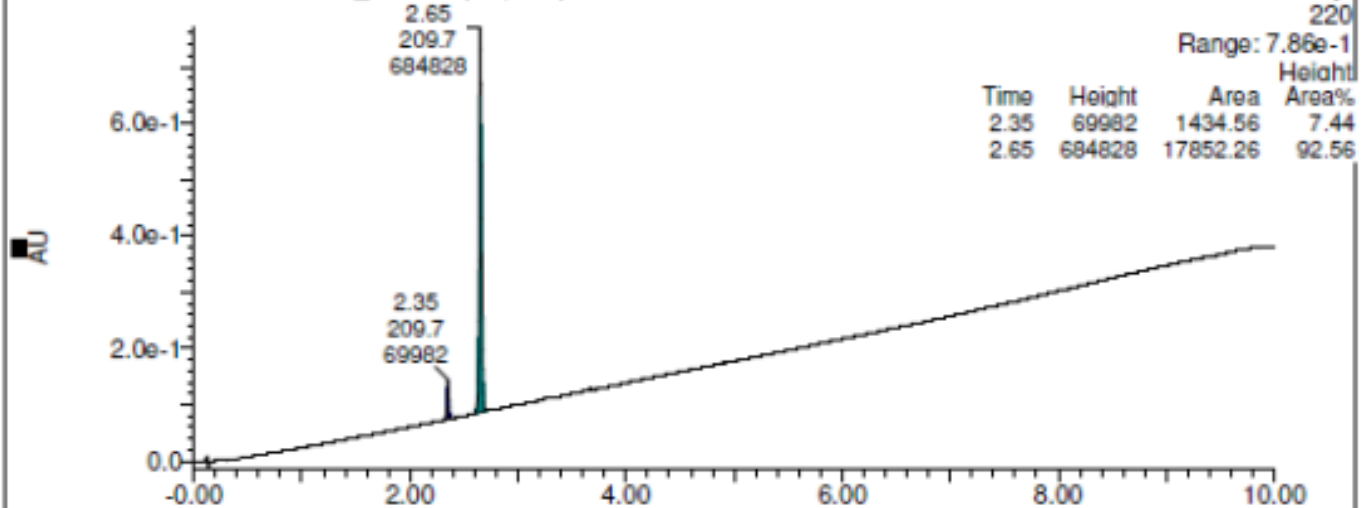
1: Scan ES+  
460.191  
3.59e7

HESSEMA4-101-EXP204-S01\_OC



HESSEMA4-101-EXP204-S01\_OC Sm (Mn, 2x3)

2: Diode Array  
220  
Range: 7.86e-1  
Height



HESSEMA4-101-EXP204-S01\_OC

1: Scan ES+  
9.75  
532.6  
TIC  
5.59e7

