

Supplementary Information:

Discovery of PqsE Thioesterase Inhibitors for *Pseudomonas aeruginosa* using DNA-Encoded

Small Molecule Library Screening

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Short Title: Small Molecule Screen for PqsE Inhibitors

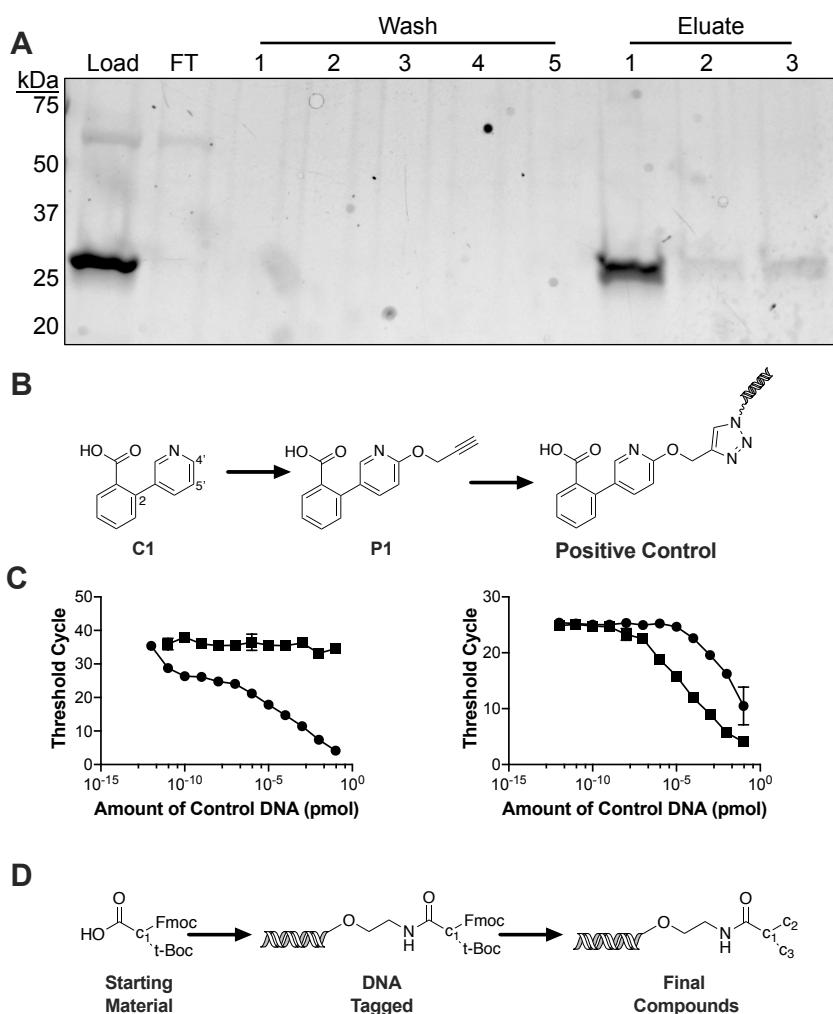


Figure S1. a) Coomassie stained SDS-PAGE gel monitoring 6xHis-PqsE protein during mock screening steps. FT denotes flow-through. Eluates 1 and 2 contained 500 mM and 1M imidazole, respectively. Eluate 3 refers to resin that had been placed at 60°C for 5 min and shows that significant 6xHis-PqsE protein remained on the cobalt resin after both imidazole elution steps, but it could be removed by heat denaturation. b) Chemical probe control molecules based on a known PqsE inhibitor called C1. c) Real time PCR analyses quantifying amplification of positive control (circles) and negative control (squares) DNA barcodes. The left panel shows the result when the primers used amplify the positive control. The right panel shows the results when the primers used amplify the negative control DNA barcode. Error bars represent SD of technical replicates, $n = 4$. d) DEL02 compound architecture.

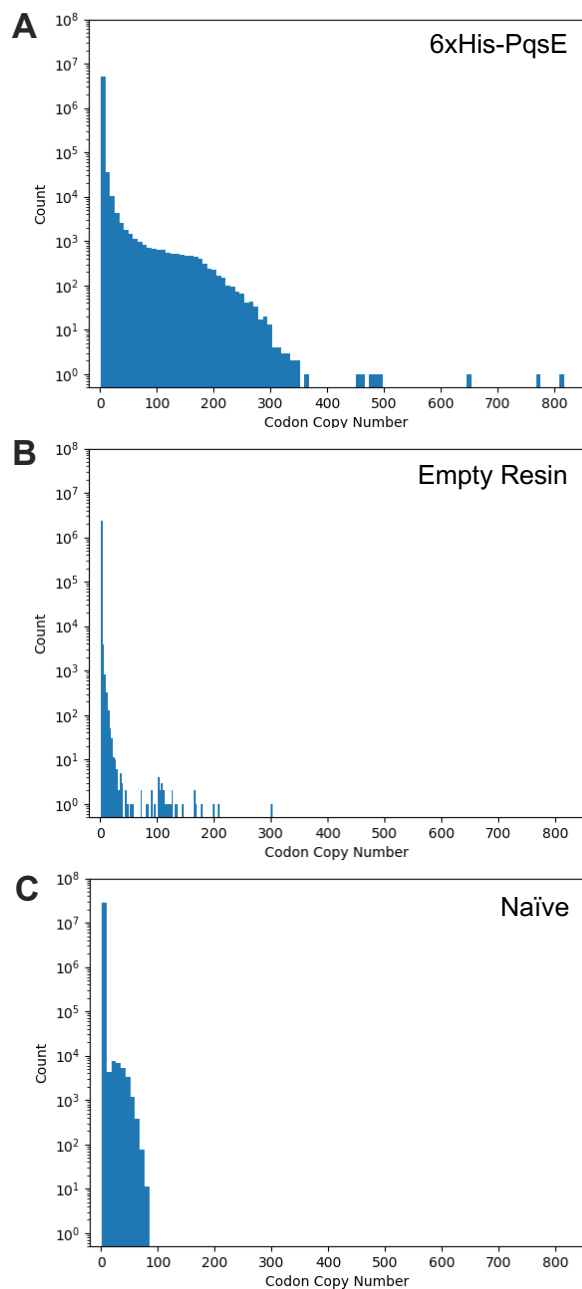


Figure S2. Distribution of sequence counts from DEL02 following selections. Histograms show the number of times a codon combination appears in recovered sequences from a) selection against PqsE; b) selection against naked resin; and c) sequencing of the naïve library. Sequences that were never present have been excluded. Plots were generated with Python/Matplotlib. The histograms had 100 bins for the PqsE and naked resin plots and 10 bins for the naïve plot.

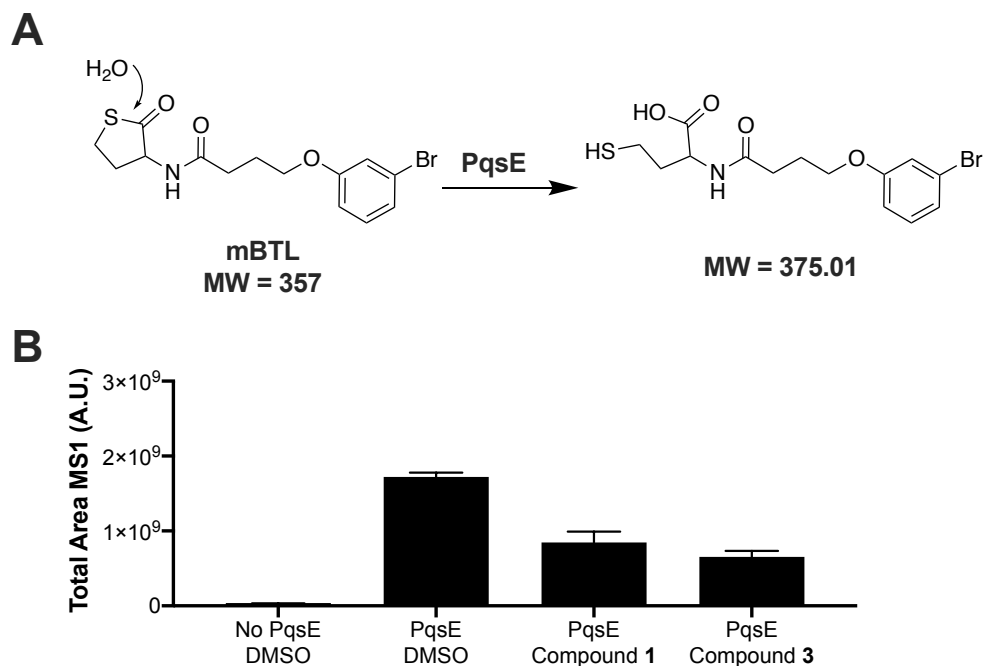


Figure S3. a) The proposed reaction carried out by the PqsE thioesterase with mBTL as substrate. b) Peak area under the curves for the 375.01 MW product from mass spectral analysis of 100 μ M mBTL combined with DMSO solvent, (first bar) 250 nM 6xHis-PqsE protein and DMSO solvent (second bar), or 250 nM 6xHis-PqsE protein and compounds **1** and **3** at 100 μ M (third and fourth bars, respectively). See Table 1 for structures of compounds **1** and **3**. Error bars represent SD of biological replicates, $n = 3$.

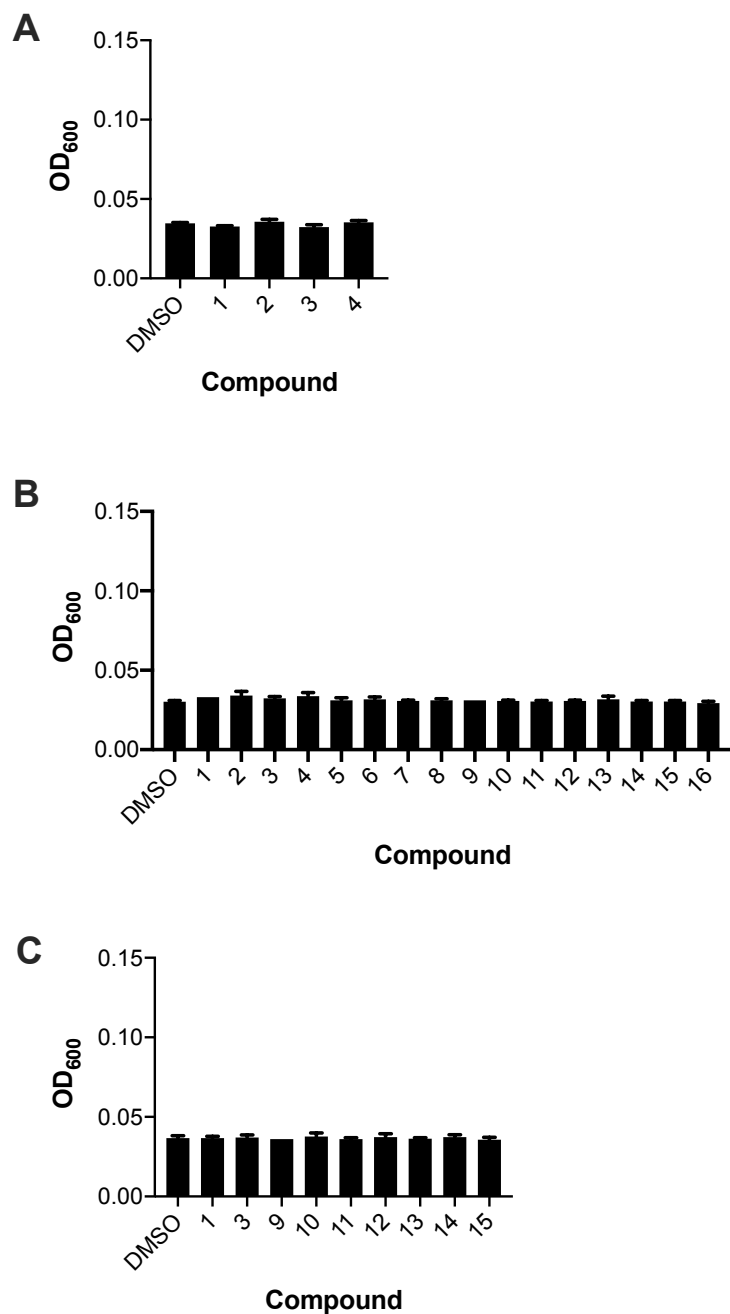


Figure S4. a) Solubility of resynthesized compounds at 2 mM in thermal shift assay buffer (50 mM Tris-HCl, pH 8.5, 150 mM NaCl, 10% (v/v) glycerol, and 20% (v/v) DMSO). b) At 2 mM in thioesterase buffer (50 mM Tricine, pH 8.5 with 0.01% (v/v) Triton X-100, and 20% (v/v) DMSO). c) At 100 μ M in LB medium. In all panels, solubility was assessed as OD₆₀₀. Error bars represent SD of biological replicates, $n = 3$.

Table S1 - Primers Used in this Work

Primer Name	Sequence
macroceutics-rt-f	TGACTCCCGACCGAAGG
macroceutics-rt-r-01	AGATGGCTAGTTCCATCACCAAT
macroceutics-rt-r-02	AGAAGCCTTTACGTCCCACAATA
DEL05-P-r	GAGAATGAATGCCACTATTACC
DEL05-Q-r	GATTAGTCCGCAAATAACCACA
Closing-tail-r	GGTTAGGCAGGGATGAGAG
Adapter primer 1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGACTCCCGACCGAAGGT
Adapter primer 2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTTAGGCAGGGA TGAGAG
Adapter primer 3	AATGATACGGCGACCACCGAACTGCGTGACTCCCGACCGAAGGT
Adapter primer 4	CAAGCAGAAGACGGCATACGAGATTGGTCAGGTTAGGCAGGGATGAG AG

All primers are listed in the 5' to 3' direction.

Table S2 – Summary of Sequencing Results from DEL02

	Total Processed Reads*	Total Unique Sequences	Average Hit Count per Compound**	Hit Count >75
Naïve Library	32244782	28382644	1.1	12
6xHis-PqsE	8609523	5096649	1.7	8797
Empty Resin	2570777	2392686	1.1	32

*Total processed reads includes all readable DNA sequences with PCR duplications removed

**Average hit count per compound is defined as the total processed reads divided by the total unique DNA sequences

METHODS

Strains and Growth Conditions

Escherichia coli BL21(DE3) containing pET28b-His6-PqsE (SM776)¹ was grown in LB medium (10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract) containing 50 µg/mL kanamycin. For *P. aeruginosa* assays, PA14 strain SM381¹ was grown in LB medium. All strains were grown at 37°C with aeration.

6xHis-PqsE purification

PqsE with a 6xHis tag fused to the amino terminus was purified as previously described². Briefly, 6xHis-PqsE production was induced in log phase *E. coli* BL21(DE3) cells by the addition of 1 mM IPTG for 4 h at 37°C. The cells were pelleted by centrifugation at 5000 rpm for 10 min and the pellet was frozen at -80°C. The thawed pellet was resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 20 mM imidazole), and the cells lysed by sonication with 15 1 sec pulses at the microtip limit with a 50% duty cycle (Sonifier 250, Branson). The lysate was cleared by centrifugation at 12000 rpm for 30 min and the protein purified by batch purification on Ni-NTA agarose (Qiagen) or by FPLC (Akta, GE). For batch purification, after adding 80 mL of lysate to 4 mL of resin, the mixture was washed with 10 column volumes of lysis buffer and the protein was eluted with 10 mL elution buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 500 mM imidazole). Ten 1 mL fractions were collected. Fractions containing protein were pooled and dialyzed into storage buffer [50 mM Tris-HCl, pH 8.5, 150 mM NaCl, 10% (v/v) glycerol]. For FPLC-based purification, lysate was loaded onto a HisTrap HP column and washed with 5 column volumes of lysis buffer, prior to elution with a gradient of imidazole from 20 mM to 500 mM. Eluted protein was monitored by UV at 280 nm and fractions containing protein were pooled. Purified PqsE protein was stored at -80°C prior to use. To judge purity, protein fractions were subjected

to SDS gel electrophoresis on a 4–20% Mini-PROTEAN®TGX™ Precast Gel (Bio-Rad) and stained with Coomassie (Biorad).

Quantification of positive and negative control DNA barcodes by RT PCR

To establish the screening protocol, positive and negative control DNA barcodes were synthesized. The molecule called C1 (2-(3'-pyridyl)benzoic acid), previously established to be an inhibitor of PqsE³, was modified with an alkyne attachment point to enable covalent attachment to a DNA barcode (see Supplementary Information). A subsequent copper-mediated click reaction to an azide-bearing DNA conjugate provided a suitable positive control. The negative control consisted only of a unique DNA barcode. Quantitation of the levels of the control DNAs was accomplished using RT-PCR. Reactions were carried out in a QuantStudio6 Real Time PCR system (Applied Biosystems) using PerfeCTa® SYBR® Green FastMix®, Low ROX (Quanta). The forward primer for these reactions, macroceutics-rt-f, hybridized to both the positive and negative control barcodes (Table S1). The reverse primer, macroceutics-rt-r-01, was specific for the positive control DNA barcode, while the macroceutics-rt-r-02 reverse primer was specific for the negative control DNA barcode (Table S1). To confirm the specificity of the primers, the positive and negative controls were diluted 12 times in a 10x dilution series and subjected to RT-PCR analysis with both primer sets.

Library Screening

The library (containing 5 sub-libraries named DEL01-05) was first cleared of molecules possessing intrinsic affinity for the cobalt resin. To accomplish this, 20 µL of the library (5500 pmol) was mixed with 50 µL of His-Tag Isolation & Pulldown Dynabeads® (ThermoFisher) in binding buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl) supplemented with 200 µg/mL BSA, 1 mg/mL ssDNA, 2 mM MnCl₂, and 0.05% (v/v) Tween20. The mixture was incubated for 30 min at

4°C with agitation. A magnet was used to remove the beads and the supernatant was used as the input library in subsequent steps.

The cleared library was combined with 150 µg purified PqsE protein, 200 µg/mL BSA, 1 mg/mL ssDNA, 2 mM MnCl₂, and 0.05% (v/v) Tween20 in binding buffer. This mixture was incubated for 30 min at 4°C with agitation. 75 µL of His-Tag Isolation & Pulldown Dynabeads® were washed twice with 1x PBS and added to the protein/library mixture. This mixture was incubated for 30 min at 4°C with agitation. The beads were removed using a magnet and washed 5 times with 100 µL wash buffer [50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% (v/v) Tween20, 2 mM MnCl₂]. The first three washes also contained 1 mg/mL ssDNA. The washed beads were added to 100 µL elution buffer [50 mM Tris-HCL, pH 8.0, 150 mM NaCl, 500 mM imidazole, 0.05% (v/v) Tween20] and incubated at room temperature for 30 min with agitation, and then heated at 60°C for 10 min without agitation. The beads were removed with a magnet. The eluent was subjected to centrifugation for 1 min at 13000 rpm to remove any denatured protein. The DNA-bound small molecules were purified with Axygen™ AxyPrep Mag™ PCR Clean-up Kits (Fisher) and eluted into 50 µL sterile water. 2 µL of this preparation was diluted 1:20 in sterile water for quantitation. We call the remainder “Round 1” of the selection and it was used as the input material for the subsequent round of selection as described in the next paragraph.

The small molecule selection outlined above was repeated two additional times, in succession, with the following changes. In the second round of selection, 50 µg of purified 6xHis-PqsE was combined with 48 µL of the library isolated in Round 1 and subjected to the selection described above. A 2 µL aliquot of the 6xHis-PqsE-selected small molecule binders was diluted 1:10 in sterile water for quantitation. The remainder of this preparation is called “Round 2” and it was used as the input material for the final round of selection. For the third round of selection, 50 µg of purified 6xHis-PqsE was combined with 43 µL of the Round 2 preparation and subjected to the above selection. A 1 µL aliquot of the 6xHis-PqsE-selected small molecule binders was diluted

1:50 in sterile water for quantitation. To assess yields, for the first two rounds of selection, primers macroceutics-rt-f and DEL05-P-r were employed in RT PCR analysis (Table S1). In the case of the third round of selection, primers macroceutics-rt-f and closing-tail-r were used (Table S1). We compared the PCR results to a standard curve generated with a dilution series of the cleared input library prior to screening. The assessment of binding to the naked resin was performed exactly as described above, except that 6xHis-PqsE protein was not included in any step. In the quantitation, the reverse primer used following selection Rounds 1 and 2 was DEL05-Q-r (Table S1).

Sequencing and hit analysis

Following library screening, adapters were attached to the collected DNA barcodes using Phusion High-Fidelity DNA polymerase (New England Biolabs). For the naïve library and the PqsE selection, adapter primers 1 and 2 were used for PCR amplification (Table S1). For the naked resin selection, adapter primers 3 and 4 were used (Table S1). The amplicon libraries were quantified by Qubit fluorometer (Invitrogen, CA), and examined on Agilent Bioanalyzer DNA High Sensitivity chips for size distribution. The DNA was sequenced using Illumina HiSeq 2500 Rapid flowcells as single-end 150 nt reads. Raw sequencing reads were filtered by Illumina HiSeq Control Software and only the Pass-Filter reads were used for further analysis. Sequences were analyzed with a suite of software including Patman⁴ for identifying codons, Barcode⁵ for sorting library tags, FASTX (http://hannonlab.cshl.edu/fastx_toolkit/), and in-house software for counting codon combinations.

Thioesterase inhibition assay

For testing the on-DNA specific PqsE binders for inhibitory activity, purified 6xHis-PqsE (at 500 nM) was combined with 500 μ M mBTL in 50 mM Tricine, pH 8.5, supplemented with 2 μ M MnCl₂ in 20 μ L total volume in a 384 well plate. The specific binders were added at 10 μ M.

Following 30 min incubation, 5 μ L 10 mM 5,5'-dithio-bis-[2-nitrobenzoic acid] (Ellman's reagent) were added to each well. The OD₄₀₅ from each well was measured on an Envision plate reader (Perkin Elmer).

For examining off-DNA specific binders, purified 6xHis-PqsE (at 250 nM) was combined with 100 μ M mBTL in 50 mM Tricine, pH 8.5, supplemented with 2 μ M MnCl₂ in 20 μ L total volume in a 384 well plate. The specific PqsE binders were tested for inhibitor activity in an 11-point, 4-fold dilution series starting at 100 μ M. Reactions proceeded for 20 min at room temperature prior to addition of the 5 μ L of 600 μ M CPM fluorescent reporter molecule. Fluorescence was monitored using the FITC filter set on an Envision plate reader (Perkin Elmer). When optimizing the assay in the absence of inhibitors, raw fluorescence values were plotted. When compounds were tested as inhibitors, thioesterase activity was plotted as the percent of the maximal response, which was calculated as the fluorescence output of the sample with putative inhibitor divided by the fluorescence output of the sample in the absence of the putative inhibitor. Each active molecule was tested in biological triplicate and the % inhibition data were subjected to nonlinear regression analysis to determine IC₅₀ values (Prism).

LC-MS detection of mBTL hydrolysis in the absence and presence of putative inhibitors

Solutions of PqsE (250 nM) with DMSO or test compounds at 100 μ M [final DMSO concentration of 1% (v/v)] and mBTL as the substrate (100 μ M) in a total volume of 500 μ L of assay buffer (50 mM Tricine, 2 μ M MnCl₂ pH 8.5) were incubated at room temperature for 20 min. A control sample containing no enzyme was included to determine the stability of mBTL under the assay conditions. Following incubation, the reactions were flash frozen and stored at -80 °C prior to LC-MS analysis. A standard of pure synthetic mBTL diluted to 10 μ M in assay buffer was used. Samples were injected on an LTQ Orbitrap XL instrument in line with a Shimadzu HPLC

system. Total ion counts were measured for the theoretical $[M+H]^+$ m/z values for hydrolyzed mBTL.

Thioesterase assay for Lineweaver-Burk analyses

Purified 6xHis-PqsE was incubated with DMSO or candidate inhibitor compounds in assay buffer [50 mM Tricine, 2 μ M $MnCl_2$, 0.01% (v/v) Triton X100, pH 8.5] for 10 min at room temperature. The mixtures were added to the wells of an opaque 384-well plate (Corning 3571). In a final reaction volume of 20 μ L per well, PqsE was held at a constant concentration of 250 nM, and compounds were tested at different concentrations, with a final DMSO concentration of 0.5% (v/v). 2 μ L of the thiol indicator, CPM, was added to each well, to give a final concentration of 120 mM [10% (v/v) DMSO] prior to the addition of the substrate, mBTL, which was also added at various concentrations. Immediately following the addition of mBTL, fluorescence was measured every minute for 15 min in an Envision 2104 plate-reader (Perkin Elmer) at excitation and emission wavelengths of 405 nm and 535 nm, respectively. The initial velocity was calculated from the first 5 min of the time-course experiments and reported as RFU/min. K_m and V_{max} were calculated from the average of three independent experiments, performed in triplicate using Prism8 (GraphPad).

Thermal shift assay

Thermal shift analyses were performed by incubating 5 μ M of purified PqsE with DMSO or test compounds in 50 mM Tris-HCl, pH 8.5, 150 mM NaCl, 10% (v/v) glycerol, supplemented with 200 μ M $MnCl_2$ in a total volume of 60 μ L. The positive control compound, C1, was tested at 100 μ M. Test compounds were added from a 5-point, 2-fold dilution series starting at 500 μ M. Following incubation, SYPRO Orange (ThermoFisher) was diluted to 200x in 50 mM Tris-HCl, pH 8.5, 150 mM NaCl, 10% (v/v) glycerol, supplemented with 200 μ M $MnCl_2$. 18 μ L of the protein-compound mixture was added to 2 μ L of the diluted SYPRO Orange in a 384 microtiter plate in

triplicate. Thermal shift was assessed using the QuantStudio6 Real Time PCR system (Applied Biosystems). Samples were incubated at 25°C for 2 min prior to being subjected to a linear heat gradient of 25°C to 99°C. The 99°C temperature was maintained for 2 min to terminate the assay. Fluorescence values were plotted in Prism8 (GraphPad). The first derivatives of the curves were plotted and the melting temperatures were determined as the peaks of the first derivative curves.

Pyocyanin Assay

Stationary phase *P. aeruginosa* overnight cultures were back-diluted 1:1000 in 2 mL fresh LB medium supplemented with 100 μ M test compound in 20 μ L DMSO or, when no test compound was included, 20 μ L DMSO solvent was added as a control. Following overnight incubation at 37°C with aeration, 1 mL of the cultures was subjected to centrifugation for 3 min at 13000 rpm. Clarified supernatants were transferred to plastic cuvettes and OD₆₉₅ was measured on a spectrophotometer (Beckman Coulter DV 730). Cell pellets were resuspended in 1 mL of PBS and 100 μ L of these preparations were transferred into wells of a 96-well plate. OD₆₀₀ was measured in an Envision plate reader (Perkin Elmer). Pyocyanin levels are reported as OD₆₉₅/OD₆₀₀.

Mass Spectrometry Analysis to Assess Compound Metabolism

Four independent overnight cultures of *P. aeruginosa* were subjected to centrifugation at 4000 rpm for 10 min. The cell pellets were resuspended in fresh LB medium. Aliquots of these cell suspensions were removed and saved. Subsequently, 100 μ M of compound **1**, compound **3**, or mBTL was added to the cell suspensions, followed by incubation with aeration at 37°C. At times 0, 0.5, 3, and 7 h, aliquots containing an amount of cells equal to OD₆₀₀=10.0 were removed and pelleted by centrifugation at 4000 rpm for 10 min. A 1 mL sample of each clarified cell-free culture fluid was removed, sterile filtered through a 0.22 μ m PES membrane (Millipore), and flash

frozen by plunging into a liquid nitrogen bath. The cell pellet was washed 4 times with 1x PBS and flash frozen. To prepare the overnight incubation samples, overnight cultures of *P. aeruginosa* were back-diluted 1:1000 into fresh LB medium containing 100 μ M compound **1**, compound **3**, or mBTL. These preparations were incubated for 16 h at 37°C with aeration followed by processing as described above. All of the cell pellets were thawed and resuspended in 1 mL of 1x Bugbuster (Millipore), supplemented with 50 μ g/mL Lysozyme (Sigma), and incubated for 30 min at 37°C with aeration. The samples were subjected to centrifugation at 4000 rpm for 10 min to remove debris. The lysates were flash frozen as above. The cell-free culture fluids and corresponding lysates were analyzed for mBTL, compound **1**, or compound **3** by mass spectrometry at WuXi App Tec. Solid phase extraction was performed using ISOLUTE PLD+ plates (Biotage) followed by injected onto a Triple Quad 6500+ system (SCIEX). Results were compared to standard curves of the compounds (2.5-2500 ng/mL for mBTL and 3-3000 ng/mL for compounds **1** and **3**). Mass spectral readings were interpolated using the standard curves to provide compound concentrations in the cell-free culture fluids and the cell lysates.

Compound Solubility assay

Compound solubility was assessed for each resynthesized molecule under investigation in each assay buffer used in these studies. All compounds were assessed for solubility at the highest concentration employed in the present work. Regarding the thioesterase assay buffer, compounds were diluted to 2 mM in 50 mM Tricine, pH 8.5 with 0.01% (v/v) Triton X100, and 20% (v/v) DMSO. For the thermal shift assay buffer, compounds were diluted to 2 mM in 50 mM Tris-HCl, pH 8.5, 150 mM NaCl, 10% (v/v) glycerol, and 20% (v/v) DMSO. For the pyocyanin assay, compounds were diluted to 100 μ M in LB medium. All dilutions were made in 50 μ L total volumes in a 96-well plate. The plate was shaken at 500 rpm for 30 sec and then incubated for 5 min at room temperature. OD₆₀₀ was measured on an Envision plate reader (Perkin Elmer).

Synthesis of Compounds 1-16 and Compound P1

Abbreviations:

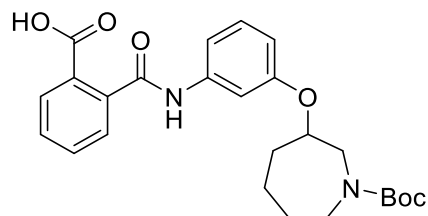
As used herein the symbols and conventions used in these processes, schemes and examples are consistent with those used in the contemporary scientific literature, for example, the Journal of the American Chemical Society or the Journal of Biological Chemistry. Specifically, the following abbreviations may be used in the examples:

g (grams);	mg (milligrams);
L (liters);	mL (milliliters);
μ L (microliters);	psi (pounds per square inch);
M (molar);	mM (millimolar);
μ mol (micromoles);	μ m (micron);
MHz (megahertz);	mm (millimeter);
mol (moles);	mmol (millimoles);
rt (room temperature);	h (hours);
min (minutes);	THF (tetrahydrofuran);
CDCl ₃ (deuterated chloroform);	DMSO-d ₆ (deuterated dimethylsulfoxide);
atm (atmosphere);	PE (petroleum ether);
EtOAc (ethyl acetate);	DCM (dichloromethane);
EtOH (ethanol);	<i>t</i> -Bu (<i>tert</i> -butyl);
MeOH (methanol);	ACN (acetonitrile);
NH ₄ CO ₃ (ammonium carbonate);	Na ₂ SO ₄ (sodium sulfate);
Boc (<i>tert</i> -butoxycarbonyl);	DIPEA (diisopropylethylamine);
NH ₄ Cl (ammonium chloride);	DIAD (diisopropyl azodicarboxylate);
Ph ₃ P (triphenylphosphine);	Fe (iron);

NaH (sodium hydride); LiHMDS (lithium hexamethyldisilazide);
K₃PO₄ (tripotassium phosphate); TBAF (tetrabutylammonium fluoride);
CuSO₄ (copper (II) sulfate); sec (seconds);
TBSOTf (*tert*-butyldimethylsilyl trifluoromethanesulfonate);
HATU (1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium3-oxide
hexafluorophosphate;
TBTA (tris((1-benzyl-4-triazolyl)methyl)amine);
EDTA (ethylenediaminetetraacetic acid)

Unless otherwise indicated, all temperatures are expressed in °C (degrees Centigrade). All reactions were conducted at room temperature unless otherwise noted. ¹H-NMR spectra were recorded on a Varian VXR-400, or a Varian Unity-400 at 400 MHz field strength. Chemical shifts are expressed in parts per million (ppm, δ units). Coupling constants (*J*) are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), quin (quintet) or br (broad). The mass spec was run on a Sciex API 100 using electrospray ionization (ESI). The LCMS was run using a C-18 reverse phase column (2.1 ID, 3.5 micron, 50 mm). The column conditions were 98% water with 0.05%TFA and 2% MeOH to 100% MeOH over 5.5 minutes. Analytical thin layer chromatography was used to verify the purity as well as to follow the progress of reaction(s). *Unless otherwise indicated, all final products were at least 95% pure as judged by HPLC / MS.*

2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)benzoic acid (1).



Step 1: *tert*-butyl 3-(3-nitrophenoxy)azepane-1-carboxylate.

To a solution of 3-nitrophenol (323 mg, 2.32 mmol, 461 μ L, 1.0 eq) in THF (15 mL) was added *tert*-butyl 3-hydroxyazepane-1-carboxylate (0.5 g, 2.32 mmol, 1.0 eq) and PPh_3 (914 mg, 3.48 mmol, 1.5 eq). The resulting reaction mixture was stirred at 20°C for 30 min, then the mixture was cooled to 0°C and DIAD (704 mg, 3.48 mmol, 677 μ L, 1.5 eq) in THF (5 mL) was added to the reaction mixture. The mixture was stirred at rt for 11 h. The reaction mixture was concentrated under reduced pressure to give a residue which was diluted with H_2O (30 mL) and extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with brine (1 x 50 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE/EtOAc = 50:1 to 1:1) to give *tert*-butyl 3-(3-nitrophenoxy)azepane-1-carboxylate (450 mg, 58% yield) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ = 7.85-7.71 (m, 2H), 7.43 (dd, J = 8.2, 16.4, 1H), 7.27 (m, 1H), 4.65 (m, 1H), 4.21-3.91 (ddd, J = 3.1, 18.4, 94.8, 1H), 3.83-3.72 (m, 1H), 3.31-3.01 (m, 2H), 2.01-1.64 (m, 6H), 1.47 (2 x s (hindered rotation), 9H).

Step 2: *tert*-butyl 3-(3-aminophenoxy)azepane-1-carboxylate.

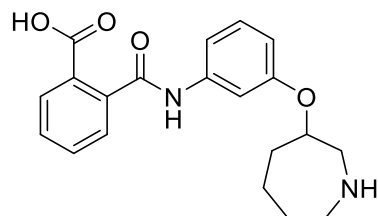
To a solution of *tert*-butyl 3-(3-nitrophenoxy)azepane-1-carboxylate (0.39 g, 1.16 mmol, 1.0 eq) in EtOH (43 mL) was added Fe (324 mg, 5.80 mmol, 5.0 eq) and NH_4Cl (434 mg, 8.12 mmol, 7.0 eq) at rt. The resulting reaction mixture was then heated to 80 °C and stirred for 12 h. The reaction mixture was then cooled to rt, filtered to remove particulates, and concentrated under reduced pressure. The residue was diluted with EtOAc (20 mL) and the pH adjusted to 9.0 by the addition of aqueous NaHCO_3 . The reaction mixture was transferred to a separatory funnel and extracted

with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (3 x 15 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude *tert*-butyl 3-(3-aminophenoxy)azepane-1-carboxylate (300 mg, 85% crude yield) which was used without further purification. ¹H NMR (400 MHz, CDCl₃) δ = 7.03 (dd, *J* = 7.6, 16.0, 1H), 6.40-6.24 (m, 3H), 4.61-4.39 (m, 1H), 4.32-3.96 (ddd, *J* = 3.3, 18.0, 92.9, 1H), 3.81 (m, 1H), 3.64 (m, 2H), 3.11-2.80 (m, 2H), 1.92-1.62 (m, 6H), 1.48 (s, 9H).

Step 3: 2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)benzoic acid.

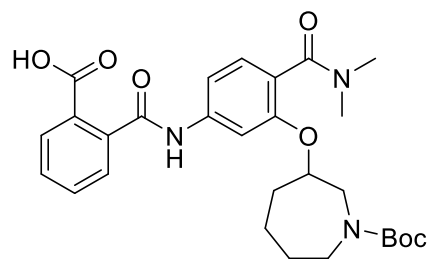
To a solution of *tert*-butyl 3-(3-aminophenoxy)azepane-1-carboxylate (0.25 g, 815 μmol, 1.0 eq) in DCM (5 mL) was added isobenzofuran-1,3-dione (120 mg, 815 μmol, 1.0 eq). The resulting reaction mixture was stirred at rt for 12 h under an N₂ atmosphere. The reaction mixture was combined and concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Luna C18 100x30mm 5μm column, mobile phase: [H₂O(0.05% HCl):ACN]; %B:50%-90%, 12 min) to give 60mg of impure 2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)benzoic acid as a white solid, which was further purified by reverse-phase HPLC (Waters Xbridge 150x25mm 5μm column; mobile phase: [H₂O(10mM NH₄HCO₃):ACN]; %B: 15%-55%, 10 min) to provide 2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)benzoic acid (39 mg, 11% yield, 99% purity by HPLC-UV) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 7.71 (m, 2H), 7.47 (m, 3H), 7.18 (m, 2H), 6.67 (m, 1H), 4.50 (m, 1H), 3.98-3.75 (ddd, *J* = 3.8, 16.6, 89.7, 1H), 3.54 - 3.08 (m, 4H), 1.93-1.58 (m, 5H), 1.39 (2 x s (hindered rotation), 9H); LCMS calculated for C₂₅H₃₀N₂O₆: *m/z* = 454; found: *m/z* = 455 (M+H).

2-((3-(azepan-3-yloxy)phenyl)carbamoyl)benzoic acid (2).



To a solution of 2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)benzoic acid (0.11 g, 242 μ mol, 1.0 eq) in EtOAc (1 mL) was added HCl/EtOAc (4M, 5 mL, 82.6 eq). The resulting reaction mixture was stirred at rt for 0.5 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was dissolved in THF (4 mL) and then NaOH (36 mg, 892 μ mol, 3.7 eq) in H₂O (0.9 mL) was added. The resulting mixture was stirred at rt for 1 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Xbridge 150x30mm 10 μ m column; mobile phase: [H₂O:ACN]; %B: 5%-25%, 10 min) to give 2-((3-(azepan-3-yloxy)phenyl)carbamoyl)benzoic acid (15 mg, 14% yield, 98% purity by HPLC-UV) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 12.75 (s, br, 1H), 7.66 (m, 2H), 7.43 (m, 3H), 7.18 (m, 2H), 6.63 (m, 1H), 4.54 (m, 1H), 3.98 (m, 1H), 3.17 (m, 1H), 2.97 (m, 2H), 1.91 - 1.41 (m, 6H); LCMS calculated for C₂₀H₂₂N₂O₄: *m/z* = 354; found: *m/z* = 355 (M+H).

2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-4-(dimethylcarbamoyl)phenyl)carbamoyl)benzoic acid (3).



Step 1: methyl 2-hydroxy-4-nitrobenzoate.

To a solution of 2-hydroxy-4-nitrobenzoic acid (5 g, 27.30 mmol, 1.0 eq) in MeOH (30 mL) was added H₂SO₄ (920 mg, 9.19 mmol, 0.5 mL, 98% purity, 3.37 eq). The resulting reaction mixture was heated to 65 °C and stirred for 12 h. The reaction mixture was cooled to rt and concentrated under reduced pressure to give a residue. The residue was diluted with H₂O (30 mL) and extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with brine (50 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE/EtOAc = 50:1 to 10:1) to give methyl 2-hydroxy-4-nitrobenzoate (1.5 g, 28% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ = 10.99 (s, 1H), 8.03 (d, *J* = 8.8, 1H), 7.83 (d, *J* = 2.2, 1H), 7.72 (dd, *J* = 2.2, 8.6, 1H), 4.03 (s, 3H).

Step 2: *tert*-butyl 3-(2-(methoxycarbonyl)-5-nitrophenoxy)azepane-1-carboxylate.

To a solution of methyl 2-hydroxy-4-nitrobenzoate (458 mg, 2.32 mmol, 1.0 eq) in THF (15 mL) was added *tert*-butyl 3-hydroxyazepane-1-carboxylate (0.5 g, 2.32 mmol, 1.0 eq) and PPh₃ (914 mg, 3.48 mmol, 1.5 eq). The resulting reaction mixture was stirred at 20°C for 30 min, then the mixture was cooled to 0°C and DIAD (704 mg, 3.48 mmol, 677 μL, 1.5 eq) in THF (5 mL) was added to the reaction mixture. The mixture was stirred at rt for 11 h. The reaction mixture was concentrated under reduced pressure to give a residue which was diluted with H₂O (30 mL) and extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with brine (1 x 50 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE/EtOAc = 20:1 to 1:1) to give *tert*-butyl 3-(2-(methoxycarbonyl)-5-nitrophenoxy)azepane-1-carboxylate (800 mg, 87% yield) as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ = 8.05-7.79 (m, 3H), 4.84-4.70 (m, 1H), 4.24-4.08 (m, 1H), 3.79 (s, 3H), 3.67 (m, 1H), 3.40-3.04 (m, 2H), 2.01-1.70 (m, 5H), 1.51-1.39 (s, 9H).

Step 3: 2-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-4-nitrobenzoic acid.

To a solution of *tert*-butyl 3-(2-methoxycarbonyl-5-nitro-phenoxy)azepane-1-carboxylate (0.7 g, 1.77 mmol, 1.0 eq) in THF (35 mL) was added LiOH·H₂O (372 mg, 8.87 mmol, 5.0 eq) in H₂O (9 mL). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between H₂O (20 mL) and EtOAc (15 mL) and extracted. The aqueous layer was acidified to pH=3 with 1N HCl and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (30 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give crude 2-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-4-nitrobenzoic acid (500 mg) which was used without further purification. ¹H NMR (400 MHz, MeOH-d₄) δ = 8.07 (d, *J* = 1.6, 1H), 7.88 (m, 2H), 4.84 (m, 1H), 4.02-3.81 (ddd, *J* = 4.0, 14.4, 56.7, 1H), 3.66 (m, 1H), 3.44 (m, 1H), 3.40-3.04 (m, 1H), 1.95 (m, 3H), 1.76 (m, 2H), 1.47 (m, 1H), 1.39 (s, 9H).

Step 4: *tert*-butyl 3-(2-(dimethylcarbamoyl)-5-nitrophenoxy)azepane-1-carboxylate.

A mixture of 2-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-4-nitrobenzoic acid (0.5 g, 1.31 mmol, 1.0 eq), *N,N*-dimethylamine hydrochloride (129 mg, 1.58 mmol, 1.2 eq), EDCI (378 mg, 1.97 mmol, 1.5 eq), HOBT (266 mg, 1.97 mmol, 1.5 eq) and DIPEA (340 mg, 2.63 mmol, 458 μL, 2.0 eq) in DMF (5 mL) was purged with N₂, and then the resulting mixture was stirred at rt for 12 h under an N₂ atmosphere. The reaction mixture was diluted with brine (50 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE:EtOAc = 1:1 to 100% EtOAc) to give *tert*-butyl 3-(2-(dimethylcarbamoyl)-5-nitrophenoxy)azepane-1-carboxylate (530 mg, 99% yield) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ = 7.87 (m, 2H), 7.40 (m, 1H), 4.69 (m, 1H), 4.09 (m, 1H), 3.74 (m, 1H), 3.30-2.97 (m, 5H), 2.85 (s, 3H), 1.99-1.58 (m, 6H), 1.58 (s, 9H); LCMS calculated for C₂₀H₂₉N₃O₆: *m/z* = 407; found: *m/z* = 408 (*M*+H).

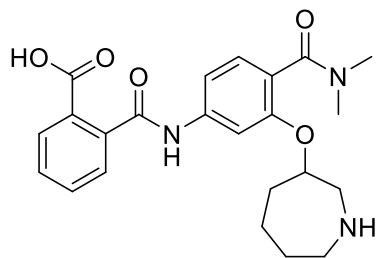
Step 5: *tert*-butyl 3-(5-amino-2-(dimethylcarbamoyl)phenoxy)azepane-1-carboxylate.

To a solution of *tert*-butyl 3-(2-(dimethylcarbamoyl)-5-nitrophenoxy)azepane-1-carboxylate (0.48 g, 1.18 mmol, 1.0 eq) in EtOAc (20 mL) was added 10% Pd/C (0.4 g) under N₂. The suspension was degassed under vacuum and purged with H₂(g) several times. The resulting reaction mixture was stirred under H₂(g) (15 psi) at rt for 3 h. The reaction mixture was filtered through a pad of Celite to remove particulates and concentrated under reduced pressure to give crude *tert*-butyl 3-(5-amino-2-(dimethylcarbamoyl)phenoxy)azepane-1-carboxylate (410 mg, 92% yield) as a yellow solid. The crude material was used without further purification. ¹H NMR (400 MHz, CDCl₃) δ = 7.05 (m, 1H), 6.29 (m, 2H), 4.60-4.40 (m, 1H), 4.25-4.07 (m, 1H), 3.96-3.68 (m, 2H), 3.07 (s, 3H), 2.94 (m, 1H), 2.87 (s, 3H), 1.89-1.56 (m, 5H), 1.47 (s, 9H); LCMS calculated for C₂₀H₃₁N₃O₄: m/z = 377; found: m/z = 378 (M+H).

Step 6: 2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-4-(dimethylcarbamoyl)phenyl)carbamoyl)benzoic acid.

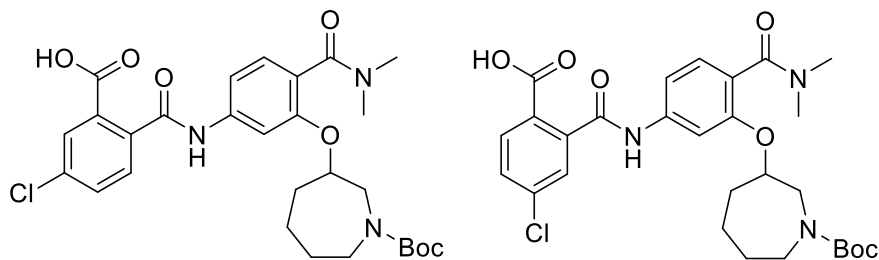
To a solution *tert*-butyl 3-(5-amino-2-(dimethylcarbamoyl)phenoxy)azepane-1-carboxylate (0.36 g, 954 μmol, 1.0 eq) in DCM (5 mL) was added isobenzofuran-1,3-dione (141 mg, 954 μmol, 1.0 eq). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Luna C18 100x30 mm 5μm column; mobile phase: [H₂O(0.225% formic acid):ACN]; %B: 30%-60%, 12 min) to give *tert*-butyl 3-(5-amino-2-(dimethylcarbamoyl)phenoxy)azepane-1-carboxylate (67 mg, 13% yield, 97% purity by HPLC-UV) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 13.09 (s, br, 1H), 10.42 (s, 1H), 7.89 (d, *J* = 7.5, 1H), 7.67 (m, 1H), 7.56 (m, 3H), 7.31 (m, 1H), 7.12 (d, *J* = 8.2, 1H), 4.51 (m, 1H), 3.91 (m, 1H), 3.54 (m, 1H), 3.33 (m, 1H), 3.13 (m, 1H), 2.96 (s, 3H), 2.79 (s, 3H), 1.81-1.59 (m, 5H), 1.40 & 1.33 (2 x s, 9H, *t*-butyl hindered rotation resonances); LCMS calculated for C₂₈H₃₅N₃O₇: m/z = 525; found: m/z = 526 (M+H).

2-((3-(azepan-3-yloxy)-4-(dimethylcarbamoyl)phenyl)carbamoyl)benzoic acid (4).



To a solution of *tert*-butyl 3-(5-amino-2-(dimethylcarbamoyl)phenoxy)azepane-1-carboxylate (150 mg, 285 μ mol, 1.0 eq) in EtOAc (1 mL) was added 4M HCl/EtOAc (3 mL, 42.05 eq). The resulting reaction mixture was stirred at rt for 2 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse phase HPLC (Waters Xbridge 150x25mm 5 μ m column; mobile phase: [H₂O(10mM NH₄HCO₃):ACN]; %B: 2%-25%, 7 min) to give 2-((3-(azepan-3-yloxy)-4-(dimethylcarbamoyl)phenyl)carbamoyl)benzoic acid (96 mg, 77% yield, 98% purity by HPLC-UV) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 11.80 (s, br, 1H), 7.75 (m, 1H), 7.56 (m, 2H), 7.47 (m, 2H), 7.29 (d, *J* = 7.9, 1H), 7.12 (d, *J* = 8.2, 1H), 4.57 (m, 1H), 3.23 (m, 1H), 2.89 (m, 6H), 2.78 (s, 3H), 1.89-1.48 (m, 6H); LCMS calculated for C₂₃H₂₇N₃O₅: *m/z* = 425; found: *m/z* = 426 (M+H).

2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)-5-chlorobenzoic acid (5)
and 2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)-4-chlorobenzoic acid (6).

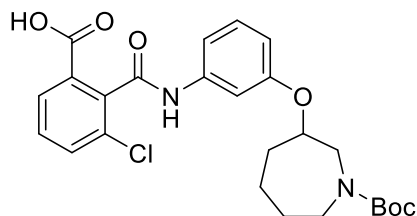


To a solution of *tert*-butyl 3-(3-aminophenoxy)azepan-1-carboxylate (0.3 g, 979 μ mol, 1.0 eq) in DCM (2 mL) was added 5-chloroisobenzofuran-1,3-dione (179 mg, 979 μ mol, 1.0 eq). The resulting reaction mixture was stirred at rt for 6 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse phase HPLC (Luna C18 100x30mm 5 μ m column; mobile phase: [H₂O(0.2% formic acid):ACN]; %B: 40%-80%, 10 min) to afford the two regioisomeric products 2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)-5-chlorobenzoic acid (67 mg, 13% yield, 95% purity by HPLC-UV) and 2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)-4-chlorobenzoic acid (75 mg, 15% yield, 93% purity by HPLC-UV) as white solids. The structures were confirmed by NOE and HSQC NMR experiments.

2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)-5-chlorobenzoic acid (5): ¹H NMR (400 MHz, DMSO-*d*₆) δ = 13.50 (s, br, 1H), 10.32 (s, 1H), 7.85 (d, *J* = 2.1, 1H), 7.74 (dd, *J* = 2.1, 8.2, 1H), 7.58 (dd, *J* = 7.6, 15.2, 1H), 7.35 (d, *J* = 18.6, 1H), 7.20 (m, 2H), 6.71 (dd, *J* = 7.5, 18.9, 1H), 4.49 (m, 1H), 3.99-3.71 (m, 1H), 3.55 (m, 1H), 3.36 (m, 2H), 3.25-3.07 (m, 1H), 1.97-1.51 (m, 5H), 1.64 & 1.41 (2 x s, 9H, *t*-butyl hindered rotation resonances); LCMS calculated for C₂₅H₂₉ClN₂O₆: *m/z* = 488; found: *m/z* = 511 (M+Na).

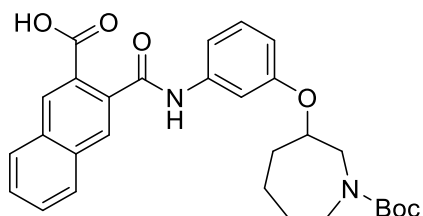
2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)-4-chlorobenzoic acid (6): ¹H NMR (400 MHz, DMSO-*d*₆) δ = 13.21 (s, br, 1H), 10.35 (s, 1H), 7.89 (d, *J* = 8.4, 1H), 7.64 (dd, *J* = 8.8, 16.8, 2H), 7.34 (d, *J* = 14.8, 1H), 7.20 (m, 2H), 6.72 (dd, *J* = 7.0, 18.5, 1H), 4.49 (m, 1H), 4.01-3.71 (m, 1H), 3.62-3.44 (m, 1H), 3.36 (m, 2H), 3.16 (m, 1H), 1.91-1.61 (m, 5H), 1.66 & 1.42 (2 x s, 9H, *t*-butyl hindered rotation resonances); LCMS calculated for C₂₅H₂₉ClN₂O₆: *m/z* = 488; found: *m/z* = 511 (M+Na).

2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)-3-chlorobenzoic acid (7).



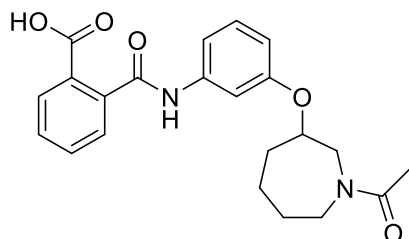
To a solution of *tert*-butyl 3-(3-aminophenoxy)azepane-1-carboxylate (0.45 g, 1.47 mmol, 1.0 eq) in DCM (8 mL) was added 4-chloroisobenzofuran-1,3-dione (402 mg, 2.20 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse phase HPLC (Xtimate C18 150x40mm 10 μ m column; mobile phase: [H₂O(10mM NH₄HCO₃):ACN]; %B: 20%-50%, 10 min) to give 2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)-3-chlorobenzoic acid (75 mg, 10% yield, 98% purity by HPLC-UV) as a white solid. The structure was confirmed by NOE and HSQC NMR experiments. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 13.21 (s, br, 1H), 10.23 (s, 1H), 7.87 (d, *J* = 7.7, 1H), 7.64 (d, *J* = 7.7, 1H), 7.48 (dd, *J* = 7.8, 15.8, 1H), 7.34 (d, *J* = 18.3, 1H), 7.20 (m, 2H), 6.72 (dd, *J* = 7.0, 18.9, 1H), 4.49 (m, 1H), 4.01-3.76 (m, 1H), 3.62-3.44 (m, 1H), 3.36 (m, 2H), 3.16 (m, 1H), 1.90-1.61 (m, 5H), 1.66 & 1.42 (2 x s, 9H, *t*-butyl hindered rotation resonances); LCMS calculated for C₂₅H₂₉ClN₂O₆: *m/z* = 488; found: *m/z* = 511 (M+Na).

3-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)-2-naphthoic acid (8).



To a solution of *tert*-butyl 3-(3-aminophenoxy)azepane-1-carboxylate (0.25 g, 816 μ mol, 1.0 eq) in DCM (4 mL) was added naphtho[2,3-*c*]furan-1,3-dione (242 mg, 1.22 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 12 h under an N₂ atmosphere. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Luna C18 100x30mm 5 μ m column; mobile phase: [H₂O(0.2% formic acid):ACN]; %B: 45%-75%, 12 min) to give 3-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)phenyl)carbamoyl)-2-naphthoic acid (171 mg, 42% yield, 95% purity by HPCL-UV) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 13.07 (s, br, 1H), 10.44 (s, 1H), 8.50 (s, 1H), 8.12 (m, 3H), 7.69 (m, 2H), 7.42 (d, *J* = 17.4, 1H), 7.25 (m, 2H), 6.72 (dd, *J* = 6.9, 18.0, 1H), 4.50 (m, 1H), 4.02-3.73 (m, 1H), 3.55 (m, 1H), 3.30 (m, 1H), 3.15 (m, 2H), 1.91-1.70 (m, 5H), 1.66 & 1.42 (2 x s, 9H, *t*-butyl hindered rotation resonances); LCMS calculated for C₂₉H₃₂N₂O₆: *m/z* = 504; found: *m/z* = 505 (M+H).

2-((3-((1-acetylazepan-3-yl)oxy)phenyl)carbamoyl)benzoic acid (9).



Step 1: 3-(3-nitrophenoxy)azepane.

To a solution of *tert*-butyl 3-(3-nitrophenoxy)azepane-1-carboxylate (0.98 g, 2.91 mmol, 1.0 eq) in EtOAc (5 mL) was added 4M HCl/EtOAc (5 mL, 6.86 eq). The resulting reaction mixture was stirred at rt for 6 h. The reaction mixture was concentrated under reduced pressure to give crude 3-(3-nitrophenoxy)azepane (0.83 g, 100% yield) as a yellow oil. This material was used without further purification. ¹H NMR (400 MHz, CDCl₃) δ = 10.21 (s, br, 1H), 7.82(m, 2H), 7.44 (m, 2H),

5.01 (m, 1H), 3.41 (m, 4H), 2.07 - 1.71 (m, 6H); LCMS calculated for $C_{12}H_{16}N_2O_3$: $m/z = 236$; found: $m/z = 237$ (M+H).

Step 2: 1-(3-(3-nitrophenoxy)azepan-1-yl)ethan-1-one.

To a solution of 3-(3-nitrophenoxy)azepane (0.8 g, 2.93 mmol, 1 eq, HCl salt) in pyridine (5 mL) was added Ac_2O (600 mg, 5.87 mmol, 550 μ L, 2.0 eq). The resulting reaction mixture was stirred at rt for 1 h under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to provide a residue. The residue was diluted with H_2O (15 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE: EtOAc = 10:1 to 0:1) to give 1-(3-(3-nitrophenoxy)azepan-1-yl)ethan-1-one (700 mg, 86% yield) as a yellow solid. 1H NMR (400 MHz, $CDCl_3$) δ = 7.87 (s, 1H), 7.79 (m, 1H), 7.44 (m, 2H), 4.65 (m, 1H), 4.46 (dd, $J = 4.7, 13.6$, 1H), 3.84-3.67 (m, 2H), 3.39 (m, 1H), 2.97 (dd, $J = 8.9, 13.6$, 1H), 2.15 (s, 3H), 2.07-1.74 (m, 4H), 1.50 (m, 1H); LCMS calculated for $C_{14}H_{18}N_2O_4$: $m/z = 278$; found: $m/z = 279$ (M+H).

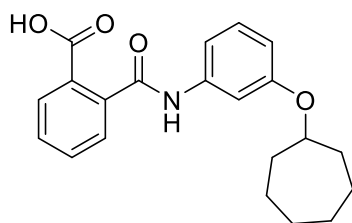
Step 3: 1-(3-(3-aminophenoxy)azepan-1-yl)ethan-1-one.

To a solution of 1-(3-(3-nitrophenoxy)azepan-1-yl)ethan-1-one (650 mg, 2.34 mmol, 1.0 eq) in EtOH (64 mL) was added Fe (652 mg, 11.68 mmol, 5.0 eq) and NH_4Cl (875 mg, 16.35 mmol, 7.0 eq) in H_2O (16 mL). The resulting reaction mixture was heated to 80 $^{\circ}C$ and stirred for 12 h. The reaction mixture was cooled to rt, filtered to remove solids and concentrated under reduced pressure. The resulting residue was diluted with H_2O (40 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give 1-(3-(3-aminophenoxy)azepan-1-yl)ethan-1-one (550 mg, 95% yield) as a yellow solid. The crude material was used without further purification. LCMS calculated for $C_{14}H_{20}N_2O_2$: $m/z = 248$; found: $m/z = 249$ (M+H).

Step 4: 2-(((3-(1-acetylazepan-3-yl)oxy)phenyl)carbamoyl)benzoic acid.

To a solution of 1-(3-(3-aminophenoxy)azepan-1-yl)ethan-1-one (550 mg, 2.21 mmol, 1.0 eq) in DCM (10 mL) was added isobenzofuran-1,3-dione (491 mg, 3.32 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Waters Xbridge Prep OBD C18 150x30mm 10 μ m column; mobile phase: [H₂O(10mM NH₄HCO₃):ACN];B%: 5%-35%, 11 min) followed by a second reverse-phase purification (Xtimate C18 150x25mm 5 μ m column; mobile phase: [H₂O:ACN];B%: 5%-55%, 10 min) to give 2-((3-((1-acetylazepan-3-yl)oxy)phenyl)carbamoyl)benzoic acid (61 mg, 7% yield, 91% pure by HPLC-UV) as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 13.03 (br s, 1H), 10.28 (d, *J* = 7.3, 1H), 7.87 (d, *J* = 7.2, 1H), 7.69-7.48 (m, 3H), 7.38 (d, *J* = 17.2, 1H), 7.21 (d, *J* = 5.5, 2H), 6.76 (m, 1H), 4.71-4.40 (m, 1H), 4.16 (dd, *J* = 4.2, 12.9, 1H), 3.82-3.50 (m, 2H), 3.37 (m, 1H), 3.08 (m, 1H), 2.02 (s, 3H), 1.93-1.56 (m, 5H), 1.42 (m, 1H); LCMS calculated for C₂₂H₂₄N₂O₅: *m/z* = 396; found: *m/z* = 397 (M+H).

2-((3-(cycloheptyloxy)phenyl)carbamoyl)benzoic acid (10).



Step 1: (3-nitrophenoxy)cycloheptane.

To a solution of 3-nitrophenol (1.95 g, 14.0 mmol, 2.78 mL, 1.0 eq) in THF (20 mL) was added cycloheptanol (1.6 g, 14.0 mmol, 1.69 mL, 1.0 eq) and PPh₃ (5.51 g, 21.0 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 30 min, then the mixture was cooled to 0°C and DIAD (4.25 g, 21.0 mmol, 4.09 mL, 1.5 eq) in THF (5 mL) was added to the reaction mixture. The

resulting reaction mixture was warmed to rt and stirred for 11 h. The reaction mixture was concentrated under reduced pressure to remove THF. The residue was diluted with H₂O (50 mL) and extracted with EtOAc (3 x 40 mL), and the combined organic layers were then dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE:EtOAc = 20:1 to 1:1) to give (3-nitrophenoxy)cycloheptane (2.9 g, 85% yield, 97% purity) as a yellow oil. ¹H NMR (400 MHz, Methanol-d₄) δ = 7.74 (dd, *J* = 1.6, 6.4, 1H), 7.66 (dd, *J* = 2.2, 4.4, 1H), 7.47 (dd, *J* = 8.3, 8.3, 1H), 7.27 (dd, *J* = 2.2, 6.4, 1H), 4.60 (m, 1H), 2.05 (m, 2H), 1.76 (m, 4H), 1.64 (m, 4H), 1.51 (m, 2H); LCMS calculated for C₁₃H₁₇NO₃: *m/z* = 235; found: *m/z* = 236 (M+H).

Step 2: 3-(cycloheptyloxy)aniline.

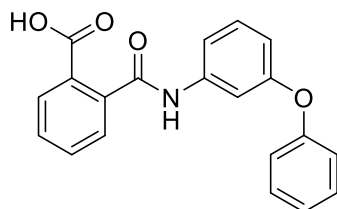
To a solution of (3-nitrophenoxy)cycloheptane (1.0 g, 4.12 mmol, 1.0 eq) in EtOH (120 mL) was added Fe (1.15 g, 20.61 mmol, 5.0 eq) and NH₄Cl (1.54 g, 28.86 mmol, 7.0 eq) in H₂O (30 mL). The resulting reaction mixture was heated to 80 °C and stirred for 12 h. The reaction mixture was cooled to rt and then filtered to remove solids, and then concentrated under reduced pressure to provide a residue. The residue was diluted with H₂O (60 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude 3-(cycloheptyloxy)aniline (860 mg, 100%) as a yellow oil, which was used without further purification. LCMS calculated for C₁₃H₁₉NO: *m/z* = 205; found: *m/z* = 206 (M+H).

Step 3: 2-((3-(cycloheptyloxy)phenyl)carbamoyl)benzoic acid.

To a solution of 3-(cycloheptoxy)aniline (0.5 g, 2.44 mmol, 1.0 eq) in DCM (10 mL) was added isobenzofuran-1,3-dione (541 mg, 3.65 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was then concentrated under reduced pressure to remove DCM. The residue was diluted with H₂O (40 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Waters Xbridge

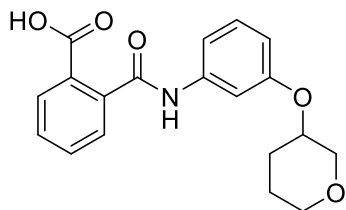
Prep OBD C18 150x30mm 10 μ m column; mobile phase: [H₂O(10mM NH₄HCO₃):ACN]; %B: 15%-45%, 11 min) to give 2-((3-(cycloheptyloxy)phenyl)carbamoyl) benzoic acid (122 mg, 14% yield, 99% purity by HPLC-UV) as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 12.21 (s, br, 1H), 7.70 (m, 2H), 7.47 (m, 2H), 7.37 (s, 1H), 7.17 (m, 2H), 6.60 (m, 1H), 4.43 (m, 1H), 1.97 (m, 2H), 1.77-1.60 (m, 4H), 1.55-1.30 (m, 6H); LCMS calculated for C₂₁H₂₃NO₄: m/z = 353; found: m/z = 354 (M+H).

2-((3-phenoxyphenyl)carbamoyl)benzoic acid (11).



To a solution of commercially available 3-phenoxyaniline (0.5 g, 2.70 mmol, 1.0 eq) in DCM (10 mL) was added isobenzofuran-1,3-dione (400 mg, 2.70 mmol, 1.0 eq). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Xtimate C18 150x25mm 5 μ m column; mobile phase: [H₂O(10mM NH₄HCO₃):ACN]; %B: 30%-60%, 8 min) to give 2-((3-phenoxyphenyl)carbamoyl)benzoic acid (129 mg, 14% yield, 98% purity by HPLC-UV) as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 7.8 -7.51 (m, 3H), 7.47-7.27 (m, 6H), 7.14 (m, 1H), 7.03 (d, *J* = 7.7, 2H), 6.69 (d, *J* = 7.7, 1H); LCMS calculated for C₂₀H₁₅NO₄: m/z = 333; found: m/z = 334 (M+H).

2-((3-((tetrahydro-2H-pyran-3-yl)oxy)phenyl)carbamoyl)benzoic acid (12).



Step 1: 3-(3-nitrophenoxy)tetrahydro-2*H*-pyran.

To a solution of DIAD (1.65 g, 8.16 mmol, 1.59 mL, 2.0 eq) and PPh₃ (2.14 g, 8.16 mmol, 2.0 eq) in THF (40 mL) was added tetrahydropyran-3-ol (0.5 g, 4.90 mmol, 1.2 eq). The resulting reaction mixture was stirred at rt for 10 min, then 3-nitrophenol (567 mg, 4.08 mmol, 810 μ L, 1.0 eq) was added to the reaction mixture. The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under reduced pressure to remove THF. The residue was diluted with H₂O (20 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (30 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE:EtOAc = 50:1 to 0:1) to give 3-(3-nitrophenoxy)tetrahydro-2*H*-pyran (0.6 g, 66% yield) as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ = 7.83 (m, 1H), 7.71 (dd, *J* = 2.3, 8.1, 1H), 7.41(m, 1H), 7.18 (dd, *J* = 2.3, 8.1, 1H), 4.44 (m, 1H), 3.97 (dd, *J* = 2.1, 11.7, 1H), 3.77 (m, 1H), 3.68 (m, 2H), 2.11 (m, 1H), 2.01 - 1.82 (m, 2H), 1.68 (m, 1H).

Step 2: 3-((tetrahydro-2*H*-pyran-3-yl)oxy)aniline.

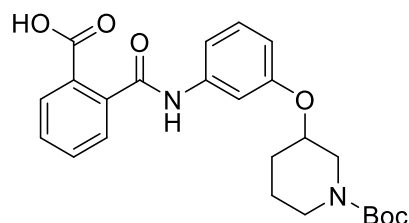
To a solution of 3-(3-nitrophenoxy)tetrahydro-2*H*-pyran (0.6 g, 2.69 mmol, 1.0 eq) in EtOH (75 mL) was added Fe (750 mg, 13.44 mmol, 5.0 eq) and NH₄Cl (1.01 g, 18.82 mmol, 7.0 eq) in H₂O (19 mL). The resulting reaction mixture was heated to 80 °C and stirred for 12 h. The reaction mixture was cooled to rt, filtered to remove solids and concentrated under reduced pressure to give a residue. The residue was diluted with H₂O (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (40 mL), dried over Na₂SO₄, filtered, and

concentrated under reduced pressure to give crude 3-((tetrahydro-2*H*-pyran-3-yl)oxy)aniline (440 mg) as a yellow oil, which was used without further purification.

Step 3: 2-((3-((3-((tetrahydro-2*H*-pyran-3-yl)oxy)phenyl)carbamoyl)benzoic acid.

To a solution of 3-((tetrahydro-2*H*-pyran-3-yl)oxy)aniline (0.35 g, 1.81 mmol, 1.0 eq) in DCM (10 mL) was added isobenzofuran-1,3-dione (402 mg, 2.72 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Waters Xbridge Prep OBD C18 150x30mm 10 μ m column; mobile phase: [H₂O (10mM NH₄HCO₃):ACN]; %B: 5%-30%, 10 min) to give 2-((3-((3-((tetrahydro-2*H*-pyran-3-yl)oxy)phenyl)carbamoyl)benzoic acid (230 mg, 37% yield, 97% purity by HPLC-UV) as a white solid. ¹H NMR (400 MHz, Methanol-*d*₄) δ = 7.69 (dd, *J* = 6.4, 20.4, 2H), 7.45 (m, 3H), 7.20(m, 2H), 6.70 (m, 1H), 4.37 (m, 1H), 3.90 (d, *J*=2.0, 11.4, 1H), 3.69 (m, 1H), 3.60 (m, 2H), 2.08 (m, 1H), 1.96-1.75 (m, 2H), 1.62 (m, 1H); LCMS calculated for C₂₀H₁₅NO₄: *m/z* = 341; found: *m/z* = 342 (M+H).

2-((3-((1-(*tert*-butoxycarbonyl)piperidin-3-yl)oxy)phenyl)carbamoyl)benzoic acid (13).



Step 1: *tert*-butyl 3-(3-nitrophenoxy)piperidine-1-carboxylate.

A solution of PPh₃ (2.61 g, 9.94 mmol, 1.0 eq) and DIAD (2.01 g, 9.94 mmol, 1.93 mL, 1.0 eq) in THF (40 mL) was cooled to 0°C and stirred for 30 min, then 3-nitrophenol (1.16 g, 8.35 mmol,

1.66 mL, 0.84 eq) and *tert*-butyl 3-hydroxypiperidine-1-carboxylate (2.0 g, 9.94 mmol, 1.0 eq) were added. The resulting reaction mixture was warmed to rt and stirred for 11.5 h. The reaction mixture was concentrated under reduced pressure to remove THF. The residue was diluted with H₂O (60 mL) and extracted with EtOAc (3 x 40 mL). The combined organic extracts were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The crude product was purified by reverse-phase HPLC (Luna C18 100x30mm 5µm column; mobile phase: [H₂O(0.2% formic acid):ACN]; %B: 45%-75%, 12 min) to give *tert*-butyl 3-(3-nitrophenoxy)piperidine-1-carboxylate (0.6 g, 15% yield) as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ = 7.83 (d, *J* = 8.2, 1H), 7.75 (dd, *J* = 2.3, 4.6, 1H), 7.44 (m, 1H), 7.25 (dd, *J* = 2.2, 8.6, 1H), 4.37 (m, 1H), 3.69-3.14 (m, 4H), 2.05 (m, 1H), 1.87 (m, 2H), 1.39 (m, 10H).

Step 2: *tert*-butyl 3-(3-aminophenoxy)piperidine-1-carboxylate.

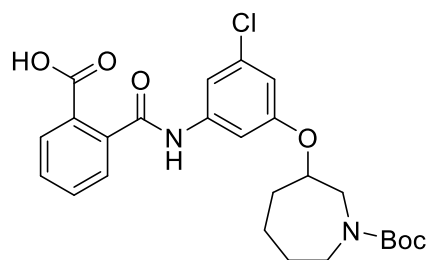
To a solution of *tert*-butyl 3-(3-nitrophenoxy)piperidine-1-carboxylate (0.55 g, 1.71 mmol, 1.0 eq) in EtOH (48 mL) was added Fe (476 mg, 8.53 mmol, 5.0 eq) and NH₄Cl (639 mg, 11.94 mmol, 7.0 eq) in H₂O (12 mL). The resulting reaction mixture was heated to 80 °C and stirred for 12 h. The reaction mixture was cooled to rt, filtered to remove solids, and concentrated under reduced pressure to give a residue. The residue was diluted with H₂O (40 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude *tert*-butyl 3-(3-aminophenoxy)piperidine-1-carboxylate (0.5 g, crude) as a yellow oil, which was used without further purification. ¹H NMR (400 MHz, CDCl₃) δ = 7.05 (dd, *J* = 8.0, 16.0, 1H), 6.39-6.23 (m, 3H), 4.25-4.14 (m, 1H), 3.83 (m, 2H), 3.40-2.88 (m, 2H), 2.11-1.77 (m, 3H), 1.44 (m, 10H).

Step 3: 2-(((1-(*tert*-butoxycarbonyl)piperidin-3-yl)oxy)phenyl)carbamoyl)benzoic acid.

To a solution of *tert*-butyl 3-(3-aminophenoxy)piperidine-1-carboxylate (0.45 g, 1.54 mmol, 1.0 eq) in DCM (10 mL) was added isobenzofuran-1,3-dione (342 mg, 2.31 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under

reduced pressure to remove DCM. The residue was diluted with H₂O (40 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Xtimate C18 150x25mm 5μm column; mobile phase: [H₂O(10mM NH₄HCO₃):ACN]; %B: 15%-45%, 8 min) to give 2-((3-((1-(*tert*-butoxycarbonyl)piperidin-3-yl)oxy)phenyl)carbamoyl)benzoic acid (213 mg, 31% yield, 98% purity by HPLC-UV) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ = 7.71 (m, 2H), 7.48 (m, 3H), 7.20 (m, 2H), 6.67 (m, 1H), 4.32(m, 1H), 3.81-3.39 (m, 3H), 3.23 (m, 1H), 2.01-1.65 (m, 3H), 1.52-1.16 (m, 10H); LCMS calculated for C₂₄H₂₈N₂O₆: m/z = 440; found: m/z = 463 (M+Na).

2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-5-chlorophenyl)carbamoyl)benzoic acid (14).



Step 1: *tert*-butyl 3-(3-chloro-5-nitrophenoxy)azepane-1-carboxylate.

To a solution of 3-chloro-5-nitrophenol (403 mg, 2.32 mmol, 1.0 eq) in THF (20 mL) was added *tert*-butyl 3-hydroxyazepane-1-carboxylate (0.5 g, 2.32 mmol, 1.0 eq) and PPh₃ (914 mg, 3.48 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 30 min, then the mixture was cooled to 0°C and DIAD (704 mg, 3.48 mmol, 677 μL, 1.5 eq) in THF (5 mL) was added to the reaction mixture. The resulting mixture was stirred at rt for 11 h. The reaction mixture was concentrated under reduced pressure to remove THF. The residue was diluted with H₂O (50 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over Na₂SO₄,

filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE: EtOAc = 20:1 to 1:1) to give *tert*-butyl 3-(3-chloro-5-nitrophenoxy)azepane-1-carboxylate (0.51 g, 53% yield) as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ = 7.79 (d, *J* = 8.8, 1H), 7.68 (d, *J* = 49.8, 1H), 7.26 (d, *J* = 51.2, 1H), 4.62 (m, 1H), 4.20-3.90 (m, 1H), 3.76 (m, 1H), 3.32-3.03 (m, 2H), 2.01-1.53 (m, 6H), 1.48 (2 x s, 9H, *t*-butyl hindered rotation resonances).

Step 2: *tert*-butyl 3-(3-amino-5-chlorophenoxy)azepane-1-carboxylate.

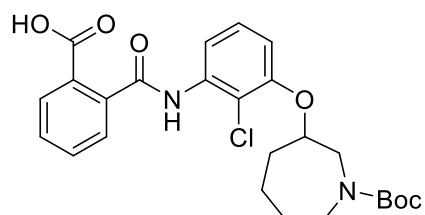
To a solution of *tert*-butyl 3-(3-chloro-5-nitrophenoxy)azepane-1-carboxylate (0.49 g, 1.19 mmol, 1.0 eq) in EtOH (37 mL) was added Fe (332 mg, 5.95 mmol, 5.0 eq) and NH₄Cl (445 mg, 8.32 mmol, 7.0 eq) in H₂O (10 mL). The resulting reaction mixture was heated to 80 °C and stirred for 12 h. The reaction mixture was cooled to rt and filtered to remove solids and the filtrate concentrated under reduced pressure. The residue was diluted with H₂O (40 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give the crude product *tert*-butyl 3-(3-amino-5-chlorophenoxy)azepane-1-carboxylate (0.45 g) as a yellow oil which was used without further purification.

Step 3: 2-(((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-5-chlorophenyl)carbamoyl)benzoic acid.

To a solution of *tert*-butyl 3-(3-amino-5-chlorophenoxy)azepane-1-carboxylate (0.25 g, 733.47 μmol, 1.0 eq) in DCM (4 mL) was added isobenzofuran-1,3-dione (163 mg, 1.10 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under reduced pressure to remove DCM. The residue was diluted with H₂O (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (Luna C18 100x30mm 5 μm column; mobile phase: [H₂O(0.225% formic acid):ACN]; %B: 40%-80%, 12 min) to give 2-(((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-5-

chlorophenyl)carbamoyl)benzoic acid (99 mg, 27% yield, 97% purity by HPLC-UV) as a yellow solid. ^1H NMR (400 MHz, Methanol- d_4) δ = 8.03 (d, J = 7.7, 1H), 7.65 (m, 1H), 7.59 (d, J = 7.7, 1H), 7.53 (m, 1H), 7.34 (d, J = 54.0, 1H), 7.23 (d, J = 54.2, 1H), 6.75 (d, J = 24.4, 1H), 4.57 (m, 1H), 4.08-3.74 (m, 1H), 3.72-3.32 (m, 2H), 3.27-3.12 (m, 1H), 1.94-1.73 (m, 5H), 1.53 (m, 1H), 1.48 and 1.41 (2 x s, 9H, *t*-butyl hindered rotation resonances); LCMS calculated for $\text{C}_{25}\text{H}_{29}\text{ClN}_2\text{O}_6$: m/z = 488; found: m/z = 489 ($\text{M}+\text{H}$).

2-((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-2-chlorophenyl)carbamoyl)benzoic acid (15).



Step 1: *tert*-butyl 3-(2-chloro-3-nitrophenoxy)azepane-1-carboxylate

To a solution of 2-chloro-3-nitrophenol (403 mg, 2.32 mmol, 1.0 eq) in THF (15 mL) was added *tert*-butyl 3-hydroxyazepane-1-carboxylate (0.5 g, 2.32 mmol, 1.0 eq) and PPh_3 (914 mg, 3.48 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 30 min, then the mixture was cooled to 0°C and DIAD (704 mg, 3.48 mmol, 677 μL , 1.5 eq) in THF (5 mL) was added to the reaction mixture. The resulting mixture was stirred at rt for 11 h. The reaction mixture was concentrated under reduced pressure to remove THF. The residue was diluted with H_2O (50 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE: EtOAc = 20:1 to 1:1) to give *tert*-butyl 3-(2-chloro-3-nitrophenoxy)azepane-1-carboxylate (0.61 g, 63% yield) as a yellow oil. ^1H NMR (400 MHz, CDCl_3) δ = 7.46-7.33 (m, 2H), 7.32-7.19 (m, 1H), 4.71-4.51 (m, 1H), 4.25-3.94 (m, 1H), 3.84 (m,

1H), 3.31-3.01 (m, 2H), 2.02-1.51 (m, 6H), 1.56 and 1.44 (2 x s, 9H, *t-butyl* hindered rotation resonances).

Step 2: *tert*-butyl 3-(3-amino-2-chlorophenoxy)azepane-1-carboxylate.

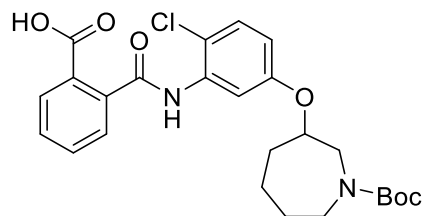
To a solution of *tert*-butyl 3-(2-chloro-3-nitrophenoxy)azepane-1-carboxylate (0.59 g, 1.59 mmol, 1.0 eq) in EtOH (45 mL) was added Fe (444 mg, 7.96 mmol, 5.0 eq) and NH₄Cl (596 mg, 11.14 mmol, 7.0 eq) in H₂O (11 mL). The resulting reaction mixture was heated to 80 °C and stirred for 12 h. The reaction mixture was cooled to rt and filtered to remove solids and the filtrate concentrated under reduced pressure. The residue was diluted with H₂O (40 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude *tert*-butyl 3-(3-amino-2-chlorophenoxy)azepane-1-carboxylate (0.49 g) as a yellow oil which was used without further purification.

Step 3: 2-(((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-2-chlorophenyl)carbamoyl)benzoic acid.

To a solution of *tert*-butyl 3-(3-amino-2-chlorophenoxy)azepane-1-carboxylate (0.20 g, 586.78 μmol, 1.0 eq) in DCM (4 mL) was added isobenzofuran-1,3-dione (130 mg, 887 μmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under reduced pressure to remove DCM. The residue was diluted with H₂O (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Luna C18 100x30mm 5μm column; mobile phase: [H₂O(0.225% formic acid):ACN]; %B: 40%-80%, 12 min) to give 2-(((3-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-2-chlorophenyl)carbamoyl)benzoic acid (35 mg, 11% yield, 96% purity by HPLC-UV) as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 13.06 (s, 1H), 9.89 (s, 1H), 7.87 (d, *J* = 7.9, 1H), 7.66 (m, 1H), 7.57 (m, 2H), 7.32 (m, 2H), 7.15 (d, *J* = 7.5, 1H), 4.61 (m, 1H), 3.99-3.75 (m, 1H), 3.52 (m,

1H), 3.21(m, 2H), 1.94-1.63 (m, 5H), 1.43 and 1.35 (2 x s, 9H, *t-butyl* hindered rotation resonances); LCMS calculated for C₂₅H₂₉ClN₂O₆: m/z = 488; found: m/z = 511 (M+Na).

2-((5-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-2-chlorophenyl)carbamoyl)benzoic acid (16).



Step 1: *tert*-butyl 3-(4-chloro-3-nitrophenoxy)azepane-1-carboxylate

To a solution of 4-chloro-3-nitrophenol (400 mg, 2.30 mmol, 1.0 eq) in THF (15 mL) was added *tert*-butyl 3-hydroxyazepane-1-carboxylate (496 mg, 2.30 mmol, 1.0 eq) and PPh₃ (97 mg, 3.46 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 30 min, then the mixture was cooled to 0°C and DIAD (700 mg, 3.46 mmol, 672 µL, 1.5 eq) in THF (5 mL) was added to the reaction mixture. The resulting mixture was stirred at rt for 11 h. The reaction mixture was concentrated under reduced pressure to remove THF. The residue was diluted with H₂O (50 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE: EtOAc = 20:1 to 1:1) to give *tert*-butyl 3-(4-chloro-3-nitrophenoxy)azepane-1-carboxylate (0.46 g, 51% yield) as a yellow oil. ¹H NMR (400 MHz, Methanol-d₄) δ = 7.58-7.45 (m, 2H), 7.25 (dd, *J* = 4.6, 29.2, 1H), 4.61 (m, 1H), 4.14-3.71 (m, 1H), 3.71-3.34 (m, 2H), 3.24(m, 1H), 2.05-1.71 (m, 6H), 1.48 and 1.38 (2 x s, 9H, *t-butyl* hindered rotation resonances); LCMS calculated for C₁₇H₂₃ClN₂O₅: m/z = 370; found: m/z = 393 (M+Na).

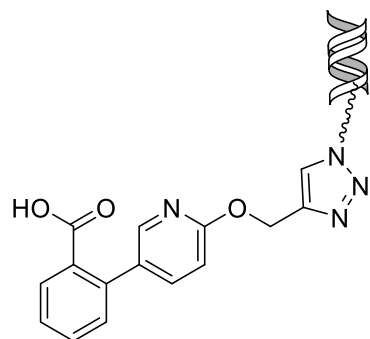
Step 2: *tert*-butyl 3-(3-amino-4-chlorophenoxy)azepane-1-carboxylate.

To a solution of *tert*-butyl 3-(4-chloro-3-nitrophenoxy)azepane-1-carboxylate (0.41 g, 1.05 mmol, 1.0 eq) in EtOH (32 mL) was added Fe (293 mg, 5.25 mmol, 5.0 eq) and NH₄Cl (393 mg, 7.35 mmol, 7.0 eq) in H₂O (8 mL). The resulting reaction mixture was heated to 80 °C and stirred for 12 h. The reaction mixture was cooled to rt and filtered to remove solids and the filtrate concentrated under reduced pressure. The residue was diluted with H₂O (40 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude *tert*-butyl 3-(3-amino-4-chlorophenoxy)azepane-1-carboxylate (0.34 g) as a yellow oil which was used without further purification.

Step 3: 2-((5-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-2-chlorophenyl)carbamoyl)benzoic acid.

To a solution of *tert*-butyl 3-(3-amino-4-chlorophenoxy)azepane-1-carboxylate (0.30 g, 880.17 μmol, 1.0 eq) in DCM (6 mL) was added isobenzofuran-1,3-dione (195 mg, 1.32 mmol, 1.5 eq). The resulting reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated under reduced pressure to remove DCM. The residue was diluted with H₂O (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by reverse-phase HPLC (Waters Xbridge 150x25mm 5μm column; mobile phase: [H₂O(10mM NH₄HCO₃):ACN]; %B: 15%-45%, 10 min) to give 2-((5-((1-(*tert*-butoxycarbonyl)azepan-3-yl)oxy)-2-chlorophenyl)carbamoyl)benzoic acid (65 mg, 15% yield, 96% purity by HPLC-UV) as a white solid. ¹H NMR (400 MHz, Methanol-d₄) δ = 7.89 (m, 1H), 7.79-7.49 (m, 4H), 7.33 (dd, *J* = 3.2, 8.7, 1H), 6.94-6.73 (dd, *J* = 6.8, 36.8, 1H), 4.59 (m, 1H), 4.09-3.76 (m, 1H), 3.68 (m, 1H), 3.52 (m, 1H), 3.36 (m, 1H), 3.24 (m, 1H), 2.02-1.85 (m, 3H), 1.80-1.69 (m, 2H), 1.49 and 1.41 (2 x s, 9H, *t*-butyl hindered rotation resonances); LCMS calculated for C₂₅H₂₉ClN₂O₆: *m/z* = 488; found: *m/z* = 489 (M+H).

Synthesis of Screening probe P1:



Step 1: 5-bromo-2-(prop-2-yn-1-yloxy)pyridine.

A slurry of NaH (3.57 g, 89.19 mmol, 60% purity, 1.0 eq) in THF (80 mL) was cooled to at 0°C, then prop-2-yn-1-ol (5 g, 89.19 mmol, 5.27 mL, 1.0 eq) was added dropwise over 3-5 minutes. The resulting reaction mixture was warmed to rt and stirred for 30 min, then the mixture was cooled to 0°C and 5-bromo-2-fluoro-pyridine (15.70 g, 89.19 mmol, 9.18 mL, 1.0 eq) in THF (20 mL) was added dropwise over 3-5 minutes. The reaction mixture was warmed to rt and stirred for 12 h. The reaction mixture was quenched by careful addition of H₂O (50 mL), and then concentrated under reduced pressure to remove THF. The remaining liquid was extracted with EtOAc (3 x 45 mL). The combined organic layers were washed with brine (80 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE: EtOAc = 1:0 to 30:1) to give 5-bromo-2-(prop-2-yn-1-yloxy)pyridine (13 g, 69% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ = 8.21 (d, *J* = 2.2, 1H), 7.67 (dd, *J* = 2.6, 8.8, 1H), 6.73 (d, *J* = 8.8, 1H), 4.95 (d, *J* = 2.4, 2H), 2.49 (t, *J* = 2.4, 1H).

Step 2: 5-bromo-2-((3-(*tert*-butyldimethylsilyl)prop-2-yn-1-yl)oxy)pyridine.

A solution of 5-bromo-2-(prop-2-yn-1-yloxy)pyridine (6.5 g, 30.65 mmol, 1.0 eq) in THF (100 mL) was cooled to -78 °C under N₂, and then LiHMDS (1 M, 46.0 mL, 1.5 eq) was added dropwise over 5 min. The resulting reaction mixture was stirred at -78 °C for 2 h, then TBSOTf (12.15 g,

45.98 mmol, 10.57 mL, 1.5 eq) in THF (10 mL) was added to the reaction mixture. The resulting reaction mixture was stirred at -78 °C for 2 h. The reaction mixture was quenched by adding aqueous NaHCO₃ (80 mL), and then was poured into a separatory funnel and extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (ISCO® 80 g SepaFlash® Silica Flash Column, PE:EtOAc 0-50% gradient at 100 mL/min) to give 5-bromo-2-((3-(*tert*-butyldimethylsilyl)prop-2-yn-1-yl)oxy)pyridine (13 g, 61% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ = 8.20 (d, *J* = 2.0, 1H), 7.67 (dd, *J* = 2.4, 8.7, 1H), 6.73 (d, *J* = 8.7, 1H), 4.94 (s, 2H), 0.92 (s, 9H), 0.12 (s, 6H); LCMS calculated for C₁₄H₂₀BrNOSi: *m/z* = 326; found: *m/z* = 327 (M+H).

Step 3: methyl 2-(6-((3-(*tert*-butyldimethylsilyl)prop-2-yn-1-yl)oxy)pyridin-3-yl)benzoate.

A mixture of 5-bromo-2-((3-(*tert*-butyldimethylsilyl)prop-2-yn-1-yl)oxy)pyridine (4 g, 12.26 mmol, 1.0 eq), (2-methoxycarbonylphenyl)boronic acid (5.52 g, 30.65 mmol, 2.5 eq), di-*tert* butyl(cyclopentyl)phosphane dichloropalladium iron (799 mg, 1.23 mmol, 0.1 eq), and K₃PO₄ (5.20 g, 24.52 mmol, 2.0 eq) in THF (60 mL) was degassed and purged with N₂ 3 times, and then the resulting reaction mixture was heated to 80 °C and stirred under an N₂ atmosphere for 12 h. The reaction mixture was cooled to rt and concentrated under reduced pressure to remove THF, then was diluted with H₂O (30 mL) and extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE:EtOAc = 1:0 to 10:1) to give methyl 2-(6-((3-(*tert*-butyldimethylsilyl)prop-2-yn-1-yl)oxy)pyridin-3-yl)benzoate (3 g, 64% yield) as a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ = 8.11 (d, *J* = 1.6, 1H), 7.91 (dd, *J* = 1.1, 7.7, 1H), 7.56 (m, 2H), 7.45 (m, 1H), 7.33 (dd, *J* = 0.9, 7.7, 1H), 6.84 (d, *J* = 7.7, 1H), 5.04 (s, 2H), 3.70 (s, 3H), 0.94 (s, 9H), 0.14 (s, 6H).

Step 4: methyl 2-(6-(prop-2-yn-1-yloxy)pyridin-3-yl)benzoate.

A solution of methyl 2-(6-((3-(*tert*-butyldimethylsilyl)prop-2-yn-1-yl)oxy)pyridin-3-yl)benzoate (2.61 g, 6.84 mmol, 1.0 eq) in THF (10 mL) was cooled to 0°C, then TBAF (1 M, 10.26 mL, 1.5 eq) was added dropwise over 2-3 min. The resulting reaction mixture was warmed to rt and stirred for 2 h. The reaction mixture was concentrated under reduced pressure to remove THF, then diluted with aq. NaHCO₃ (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (PE: EtOAc = 1:0 to 10:1) to give methyl 2-(6-(prop-2-yn-1-yloxy)pyridin-3-yl)benzoate (1.4 g, 77% yield) as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ = 8.12 (dd, *J* = 0.7, 2.4, 1H), 7.91 (dd, *J* = 1.3, 7.7, 1H), 7.57 (m, 2H), 7.45 (dd, *J* = 1.3, 7.6, 1H), 7.34 (dd, *J* = 1.0, 7.6, 1H), 6.85 (dd, *J* = 0.7, 8.6, 1H), 5.04 (d, *J* = 2.4, 2H), 3.71 (s, 3H), 2.51 (t, *J* = 2.5, 1H); LCMS calculated for C₁₆H₁₃NO₃: *m/z* = 267; found: *m/z* = 268 (M+H).

Step 5: 2-(6-(prop-2-yn-1-yloxy)pyridin-3-yl)benzoic acid.

To a solution of methyl 2-(6-(prop-2-yn-1-yloxy)pyridin-3-yl)benzoate (0.65 g, 2.43 mmol, 1.0 eq) in THF (3 mL) was added LiOH·H₂O (510 mg, 12.16 mmol, 5.0 eq) in H₂O (3 mL). The resulting reaction mixture was stirred at rt for 12 h. LCMS analysis showed approx. 35% of starting material remained, so an additional amount of LiOH·H₂O (306 mg, 7.30 mmol, 3.0 eq) in MeOH (2 mL) was added to the reaction mixture and the resulting reaction was heated to 50°C and stirred for 4 h. The reaction mixture was concentrated to remove THF and MeOH and the residue was partitioned between H₂O (15 mL) and EtOAc (15 mL) and extracted. The organic layer was discarded, and the aqueous was acidified to pH = 2 with 1N HCl and extracted with EtOAc (3 x 15 mL). The organic layers were combined, dried over Na₂SO₄ and concentrated to give a residue. The residue was purified by reverse phase HPLC (Nano-micro Kromasil C18 100x30mm 5μm column; mobile phase: [H₂O(0.2% formic acid):ACN]; %B: 10%-100%, 18 min) to give 2-(6-(prop-2-yn-1-yloxy)pyridin-3-yl)benzoic acid 352 mg, 29% yield, 97% purity by HPLC-UV) as a yellow

solid. ^1H NMR (400 MHz, Methanol- d_4) δ = 8.08 (d, J = 2.1, 1H), 7.89 (dd, J = 1.2, 7.8, 1H), 7.68 (dd, J = 2.5, 8.5, 1H), 7.59 (d, J = 1.3, 1H), 7.47 (s, 1H), 7.39 (d, J = 1.0, 1H), 6.86 (d, J = 8.8, 1H), 4.99 (d, J = 2.4, 2H), 2.88 (t, J = 2.4, 1H); LCMS calculated for $\text{C}_{15}\text{H}_{11}\text{NO}_3$: m/z = 253; found: m/z = 254 (M+H).

Step 6: 3-(2-azidoethoxy)propanoic acid (1.59 mg, 13.8 μmol) was dissolved in N,N-dimethylacetamide (50 μL), and then treated with HATU (3.8 mg, 13.8 μmol) and N-methylmorpholine (1 mg, 9.8 μmol), and the resulting reaction mixture allowed to shake 10 minutes at 4 $^\circ\text{C}$. Next, a solution of headpiece DNA with a PEG5 spacer (400 μL of 5 mM stock solution, 200 nmol) was dissolved in 250 mM phosphate buffer pH 9.4 (260 μL) and added to the reaction mixture. After shaking overnight at rt, the mixture was treated with 30 μL of 5 M NaCl and 990 μL of 95% ethanol. After cooling at -20 $^\circ\text{C}$ for 1 h, the DNA was recovered by centrifugation at 13000 rpm for 15 min, followed by decanting of the ethanol layer. The pellet was dissolved in water (200 μL), and sampled for MS. Sampling after reverse-phase UPLC-MS and deconvolution by ProMass, the azide-linked DNA conjugate was shown at 95% purity by MS (measured m/z of 5370, expected m/z of 5369).

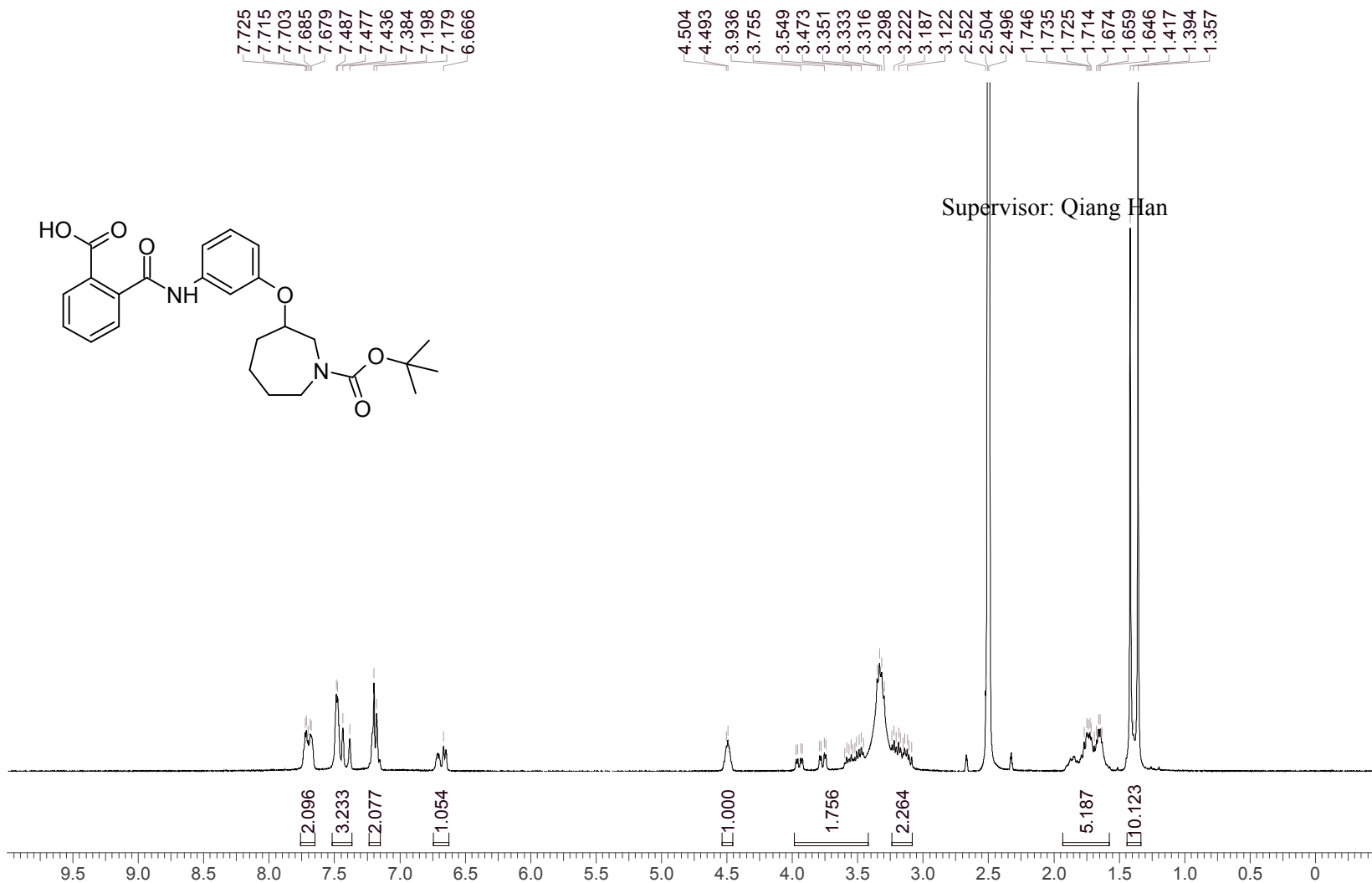
Step 7: Sodium ascorbate (2 μL of 50 mM stock in water, 100 nmol) was treated with CuSO_4 (2 μL of 50 mM stock in water, 100 nmol) and TBTA (4 μL of 25 mM stock in DMF, 100 nmol). The resulting catalyst mixture was shaken at rt for 10 min, then added to a solution of azide-coupled DNA Headpiece from step 6 (10 μL of 1 mM stock, 10 nmol) dissolved in 50 μL of pH 8 phosphate buffer (250 mM). Next, a solution of 2-(6-(prop-2-yn-1-yloxy)pyridin-3-yl)benzoic acid (10 μL of 1 mM stock, 10 nmol) was added, and the resulting reaction mixture was shaken at rt for 3 h, then treated with EDTA (8 μL , 500 mM), shaken for 10 min at rt, and spin-filtered (Amicon 3000 MW cutoff, 3 X washes, 12000 rpm, 20 min). Final compound was sampled by reverse-phase UPLC-MS to deliver the desired probe compound P1: 88% purity by MS (measured m/z of 5623, expected m/z of 5622).

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- (5) Frank, D. N. BARCRAWL and BARTAB: Software Tools for the Design and Implementation of Barcoded Primers for Highly Multiplexed DNA Sequencing. *BMC Bioinformatics* **2009**, *10* (1), 362. <https://doi.org/10.1186/1471-2105-10-362>.

Compound ID: BB0356

ET23679-31-P1D1 DMSO Bruker_C_400MHz



Supervisor: Qiang Han

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Points Count	65536
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Temperature (degree C)	24.213

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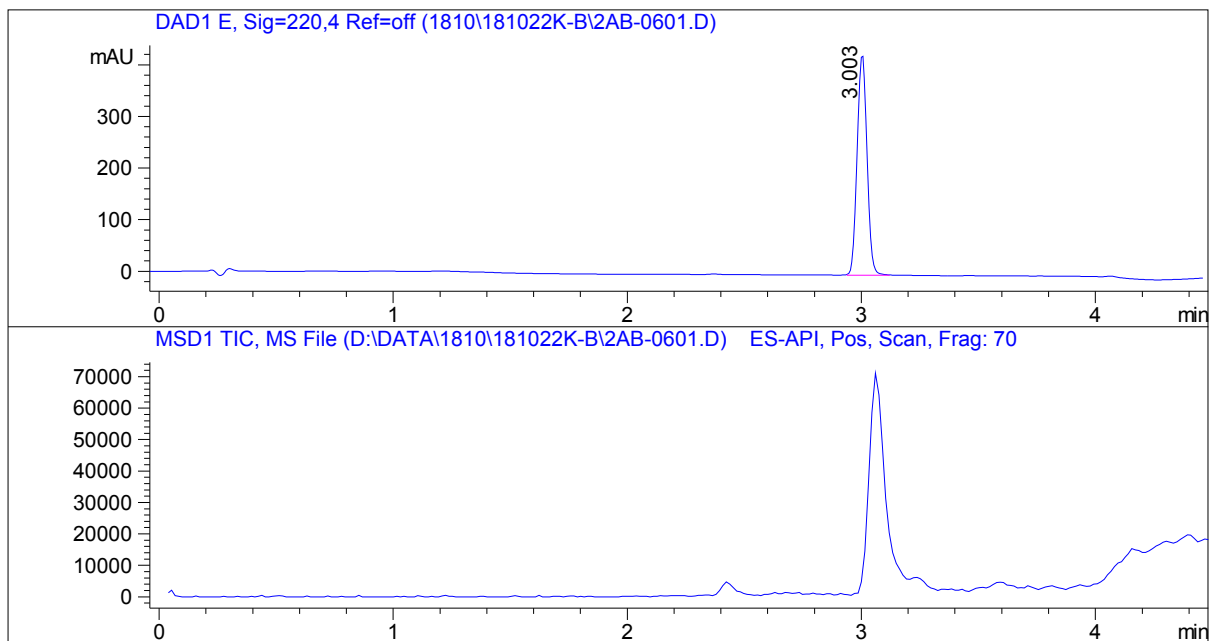
Operator:

Date:

LCMS REPORT

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Instrument : LCMS-K

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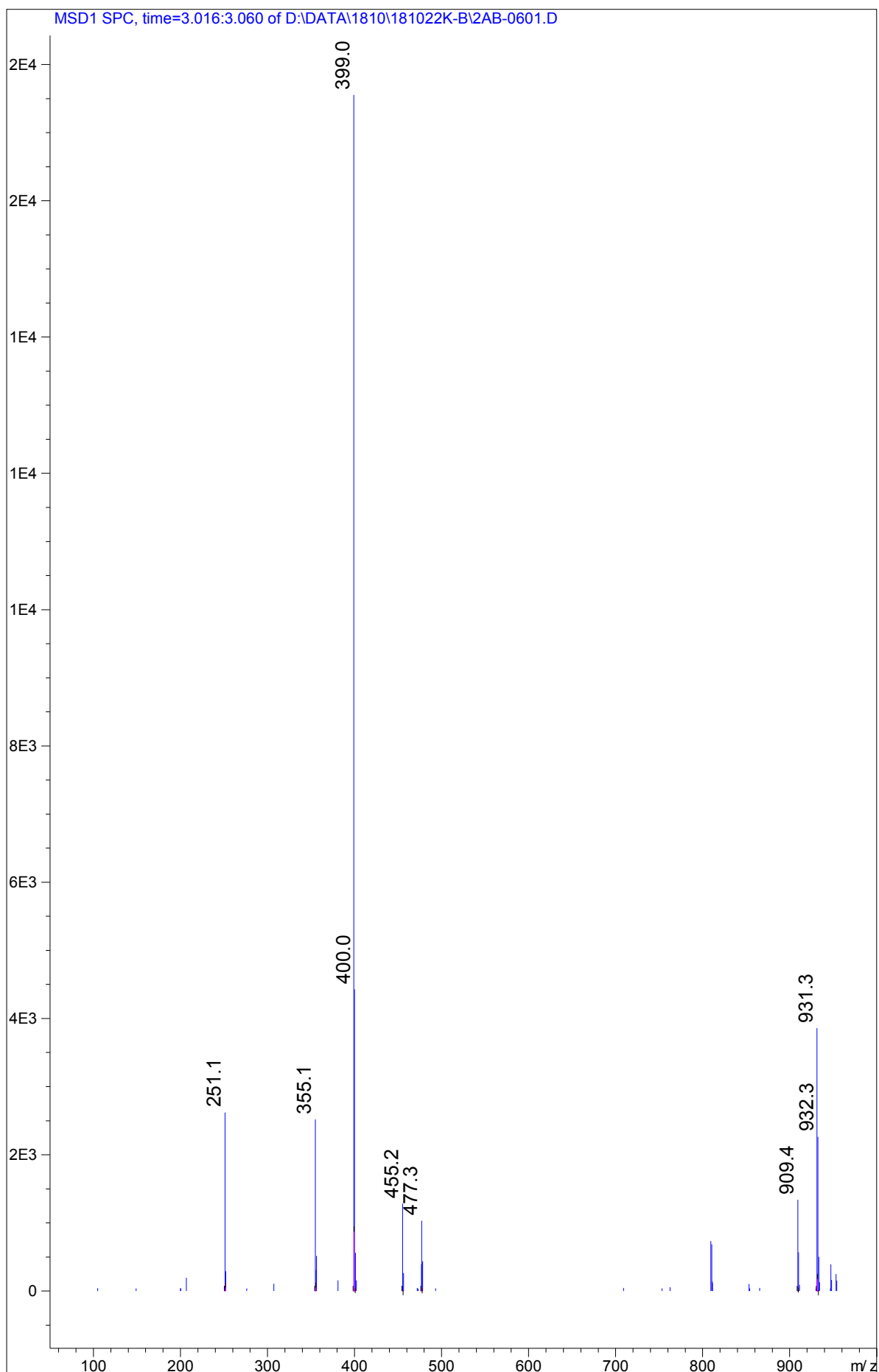
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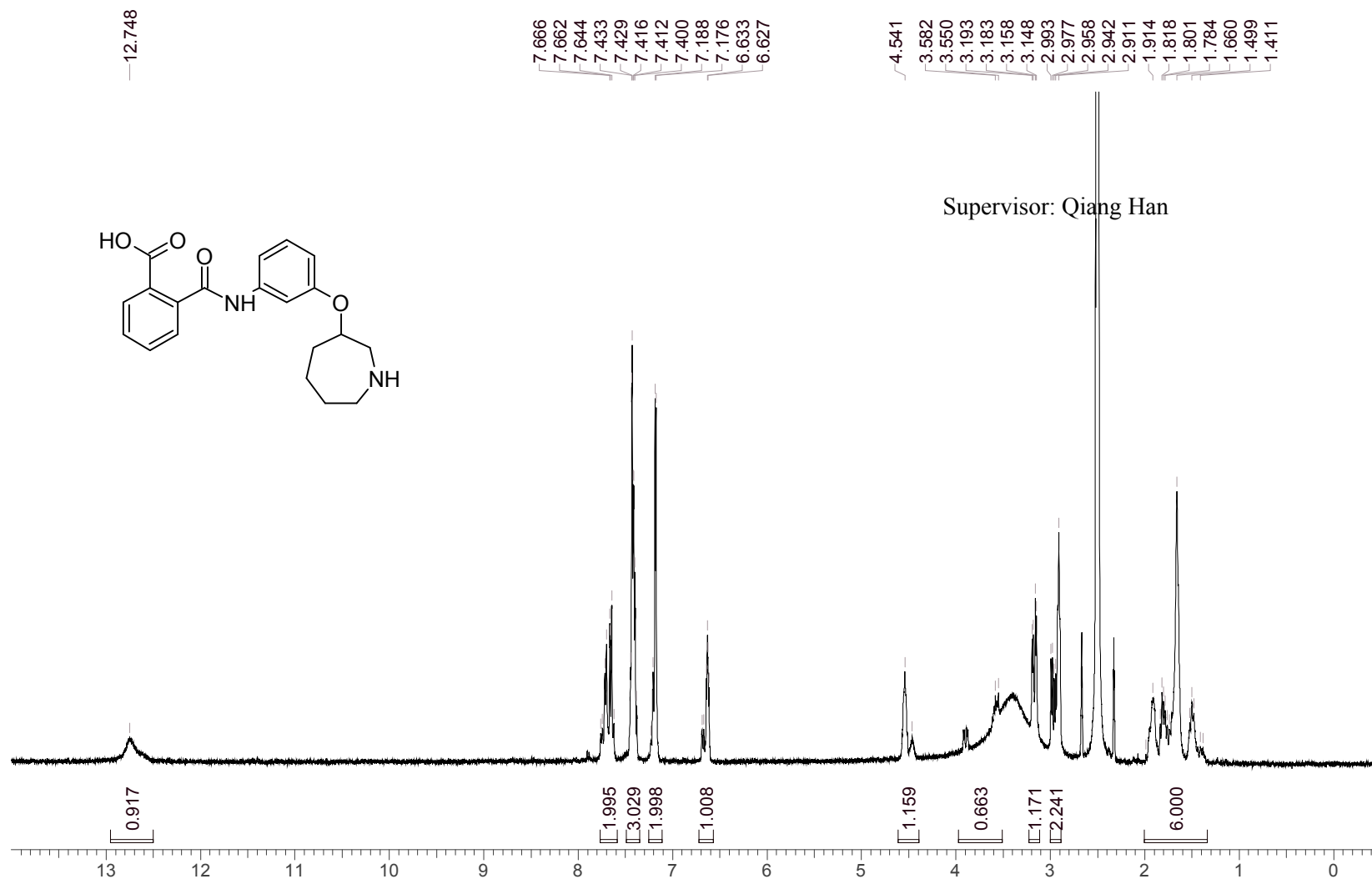
Operator: _____

Date: _____



Compound ID: BB0357

ET23679-60-P1BB DMSO Bruker_E_400MHz



Supervisor: Qiang Han

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Temperature (degree C)	-273.000

Confidential. For research only Not for regulatory filing

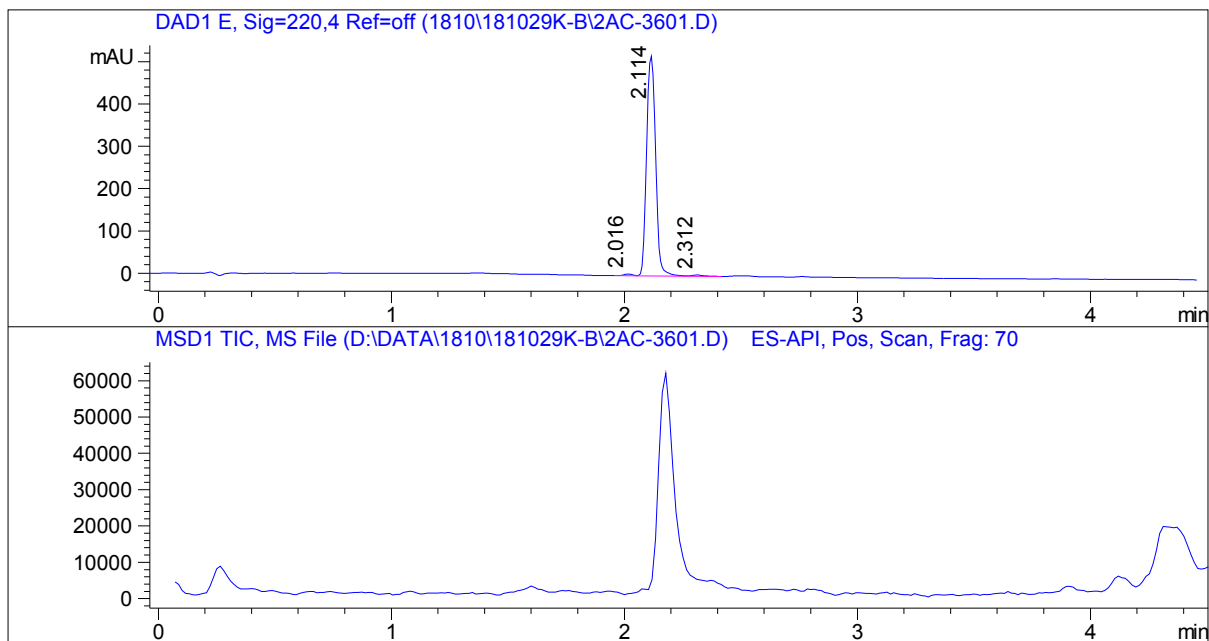
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Date:

LCMS REPORT

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Instrument : LCMS-K

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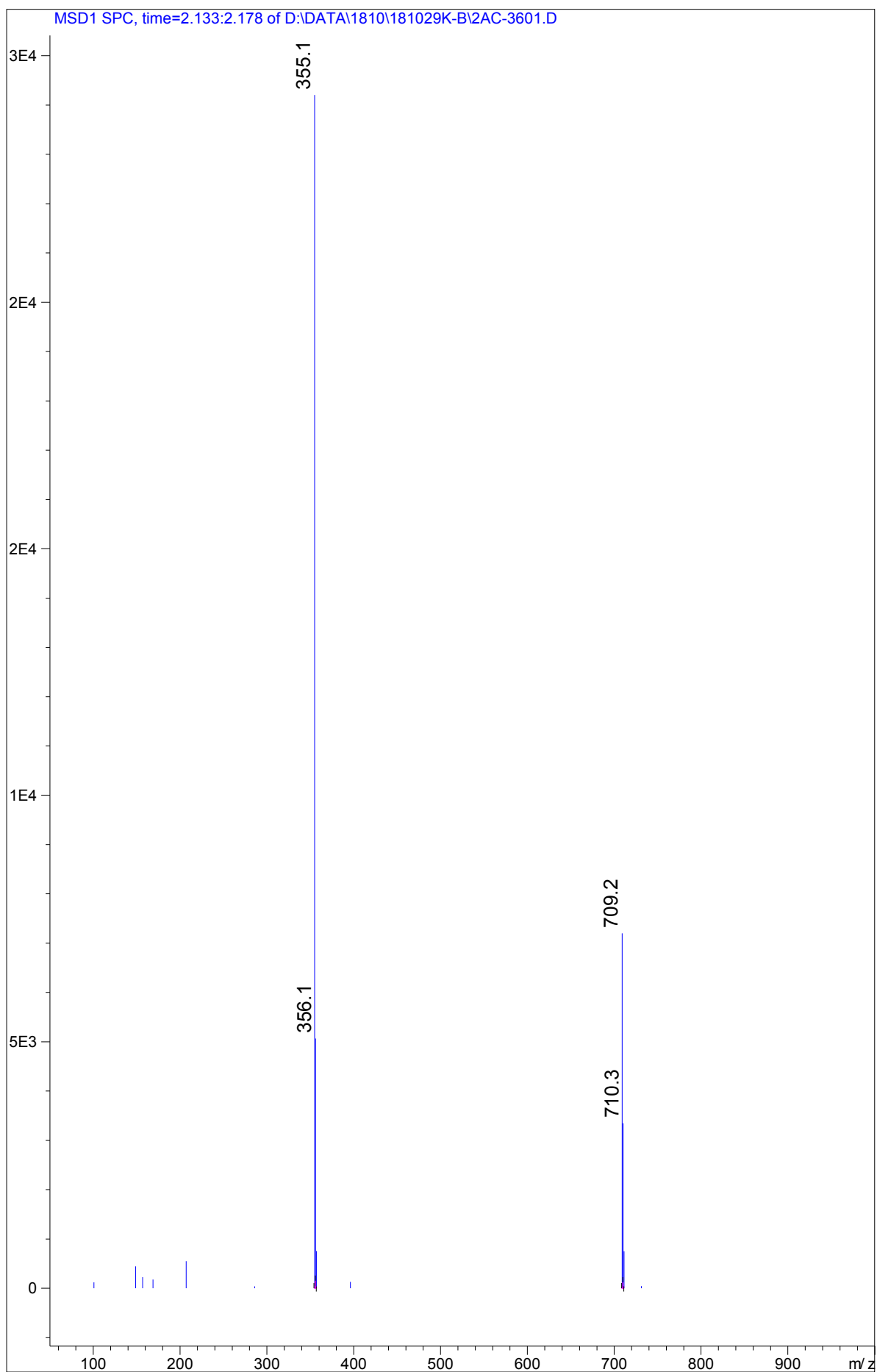
Integration Result

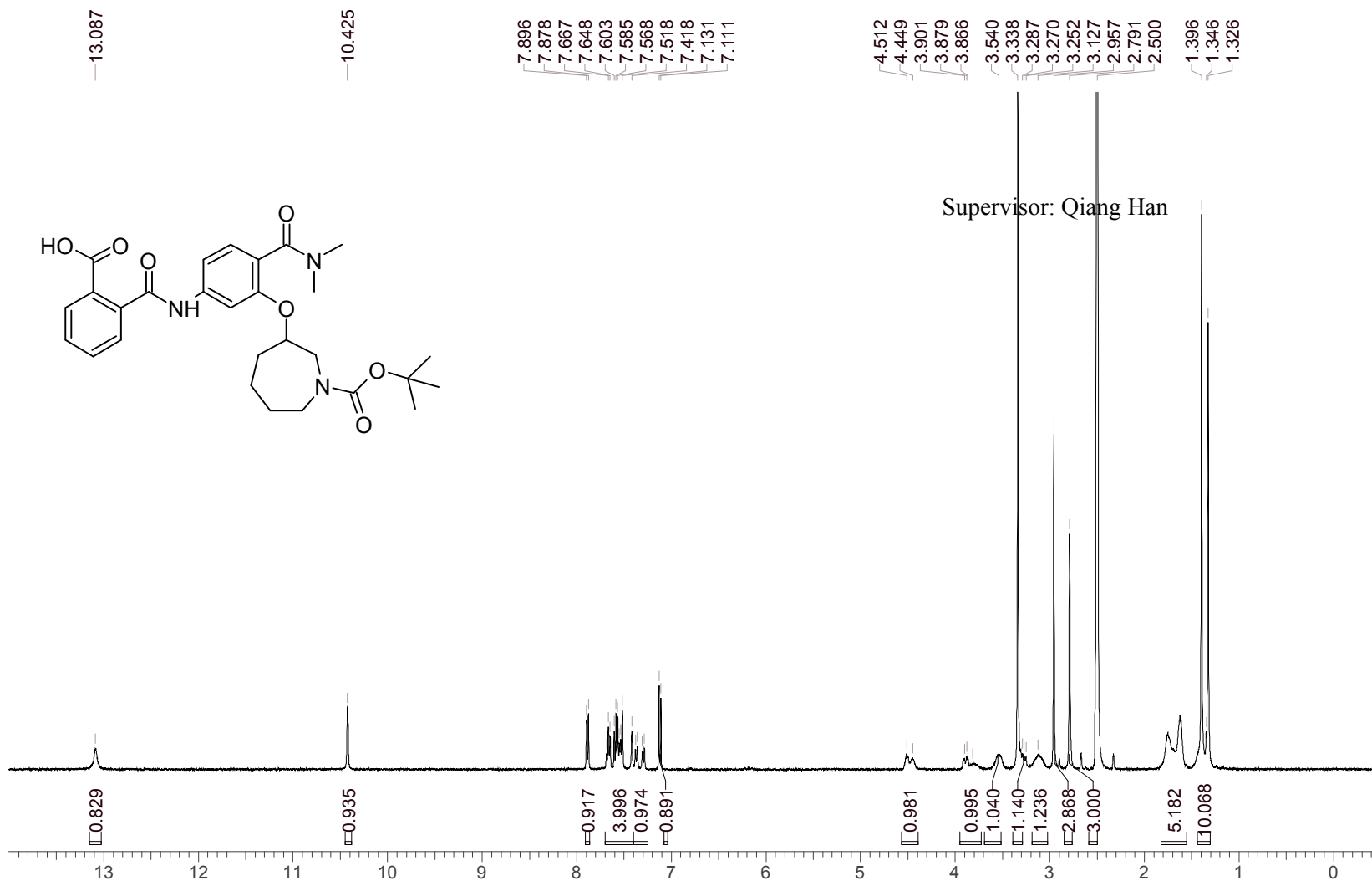
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Operator: _____

Date: _____



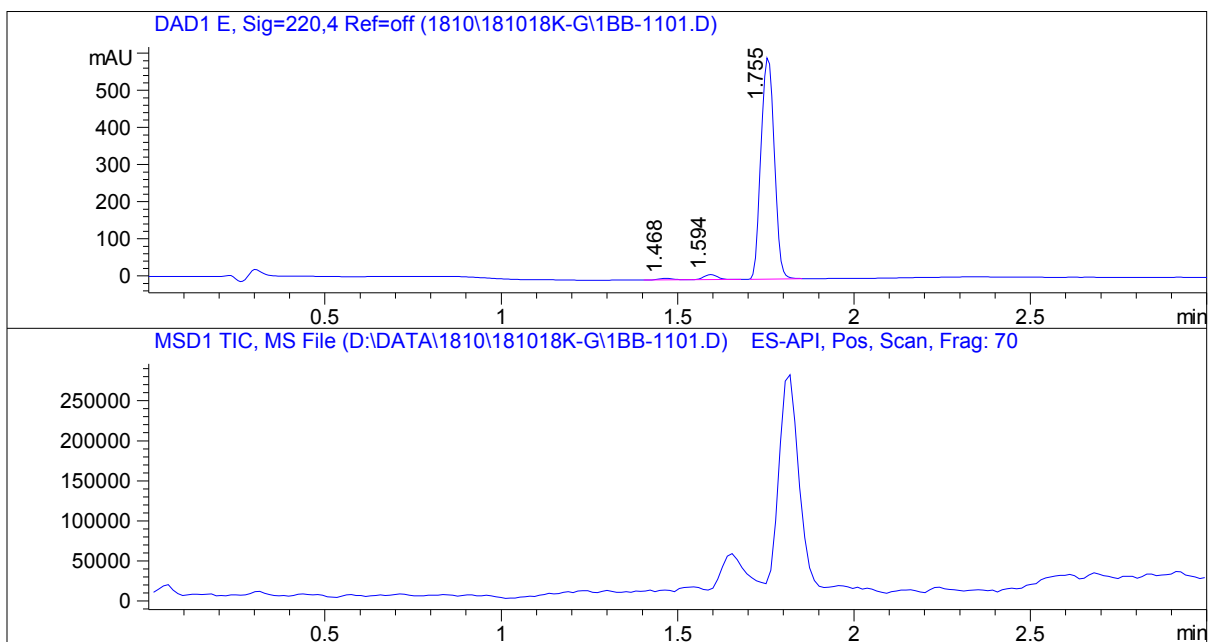


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LCMS REPORT

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Instrument : K

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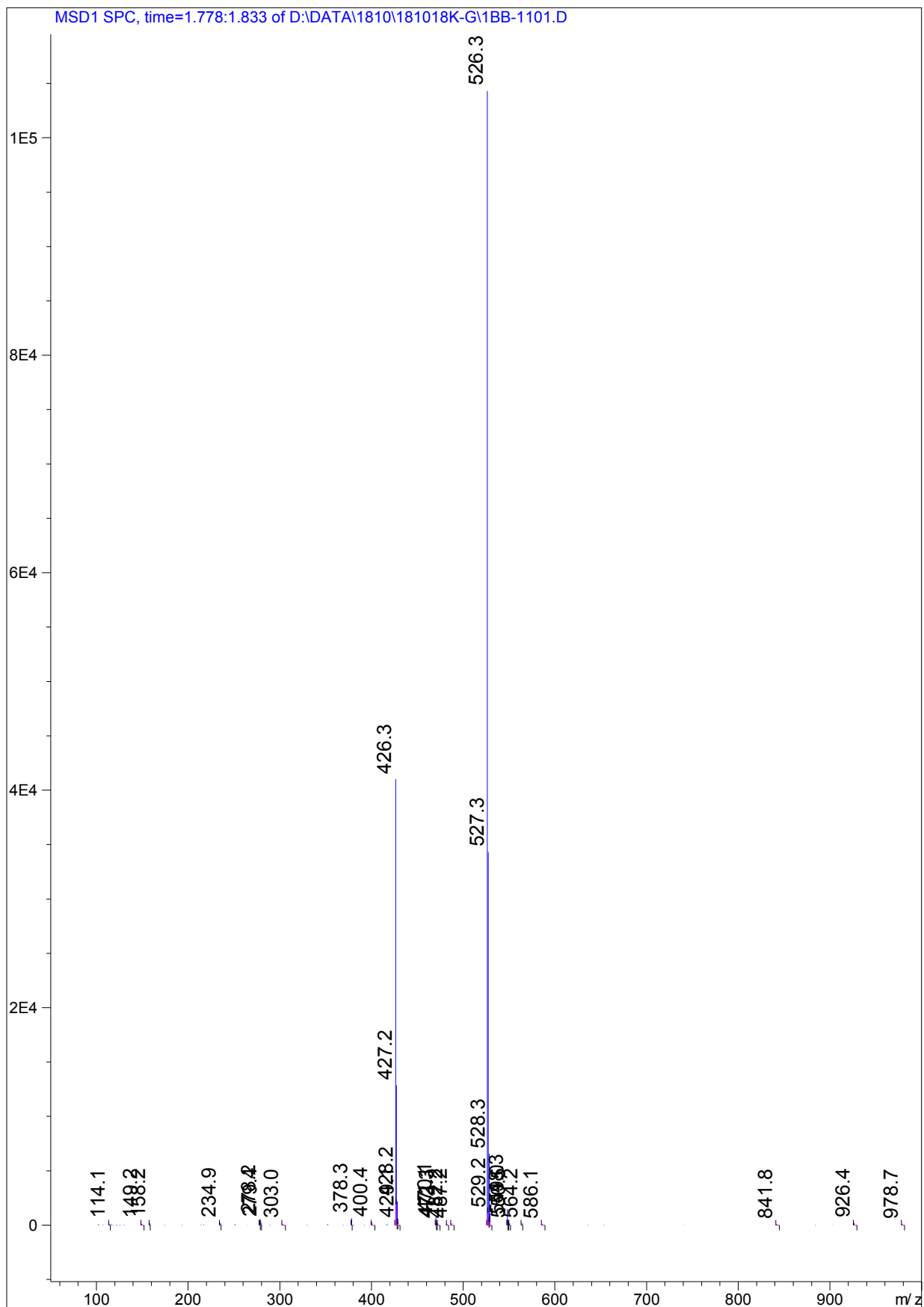
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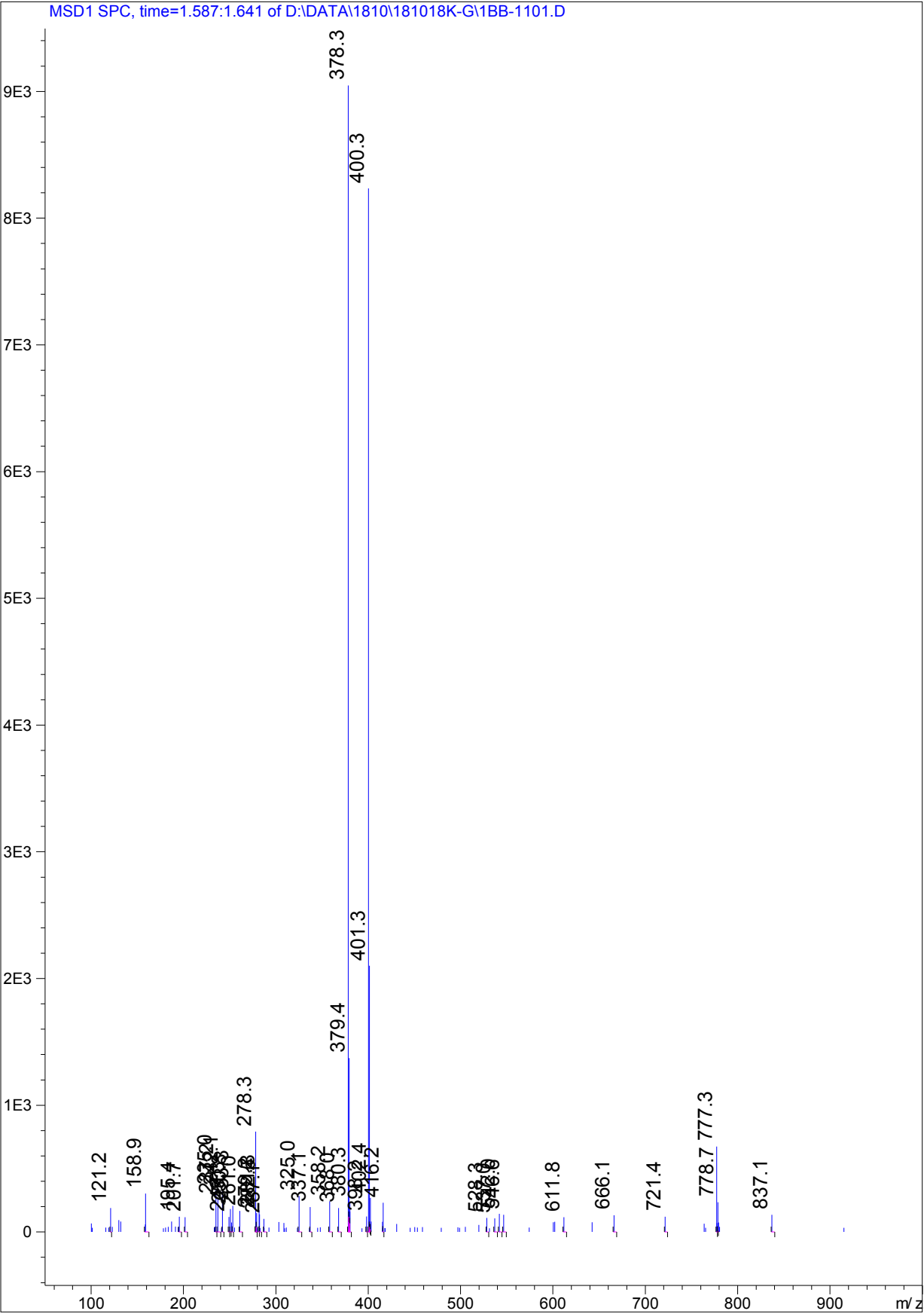
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Operator: _____

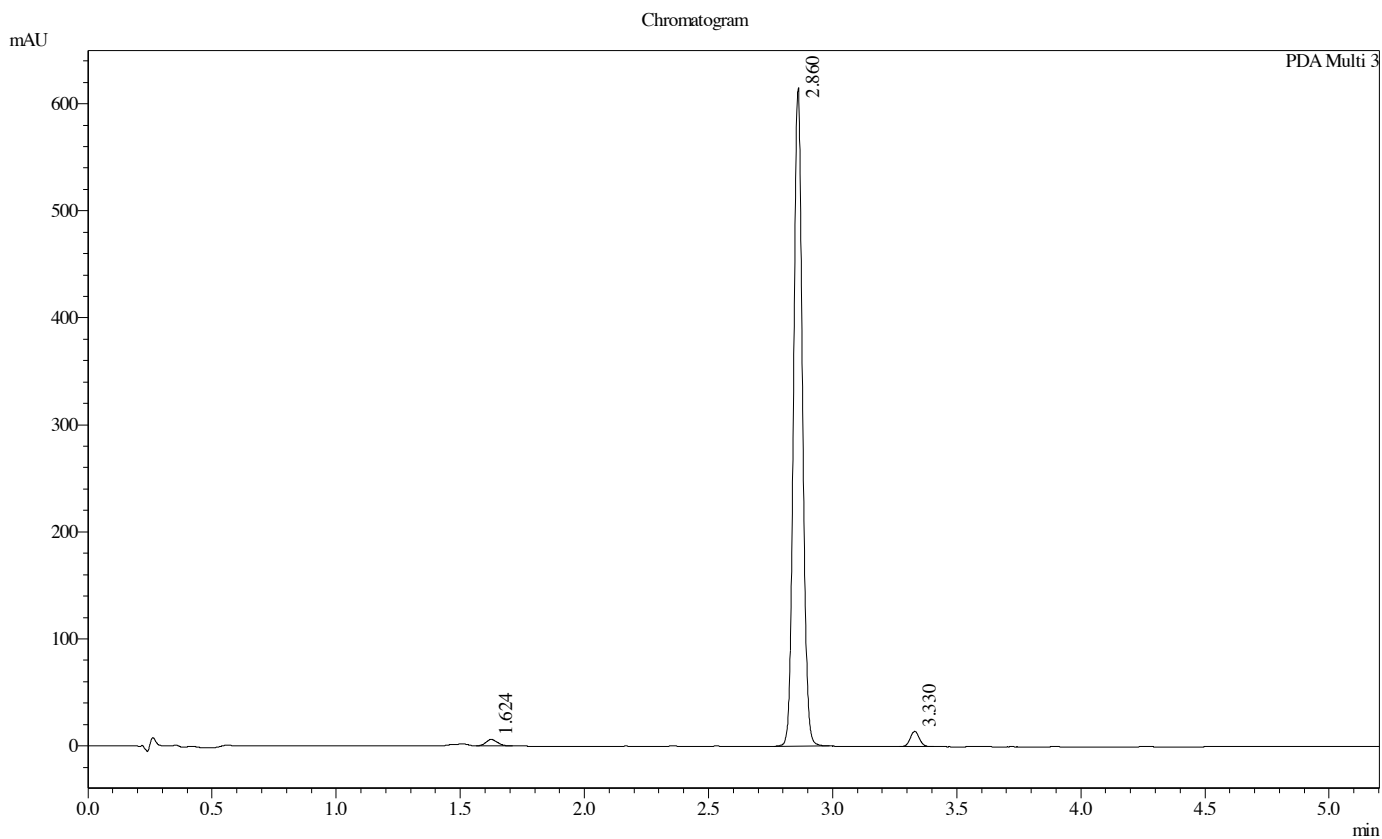
Date: _____





HPLC REPORT

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Injection Vol : 1ul
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Instrument : HPLC-011



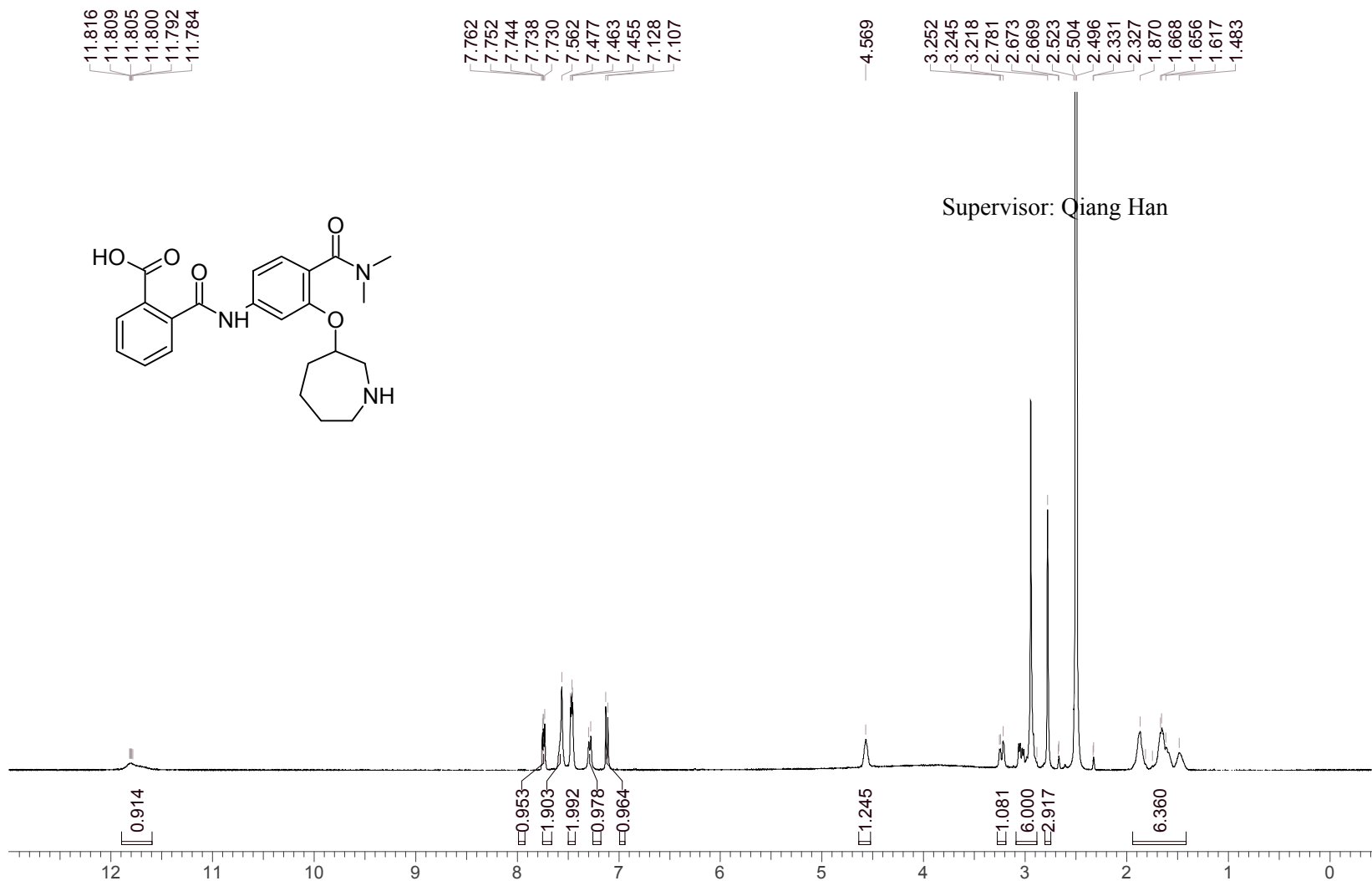
Integration Result

PDA Ch3 254nm 4nm

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Operator: _____

Date: _____



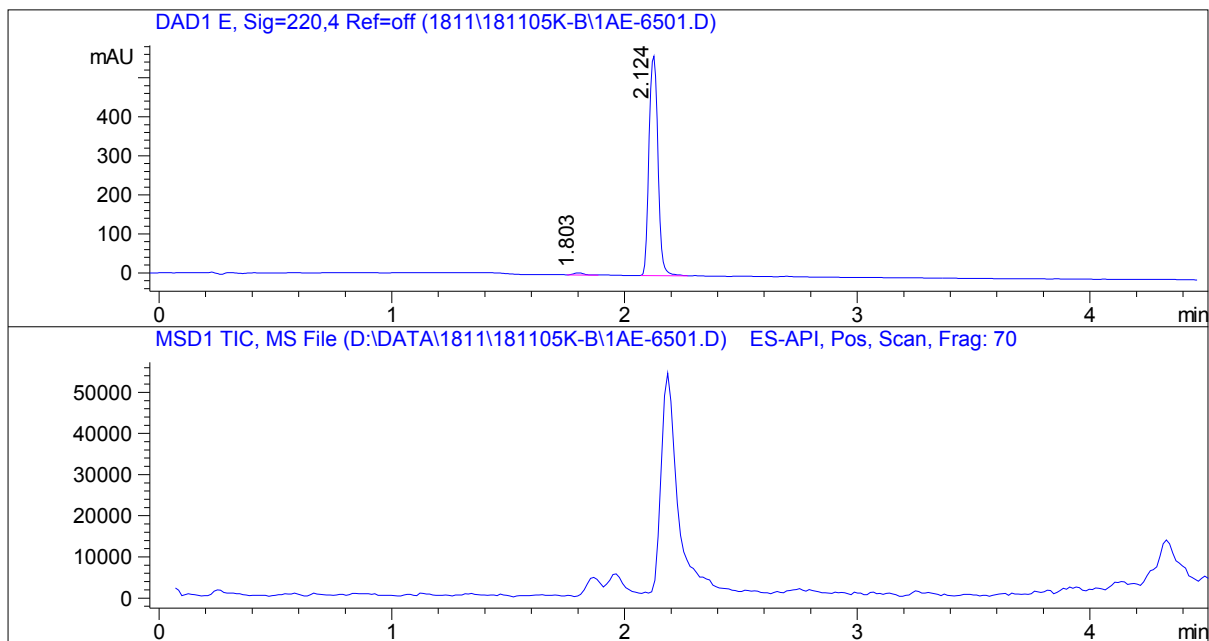
Supervisor: Qiang Han

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Date Nov 5
2018
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LCMS REPORT

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Instrument : LCMS-K

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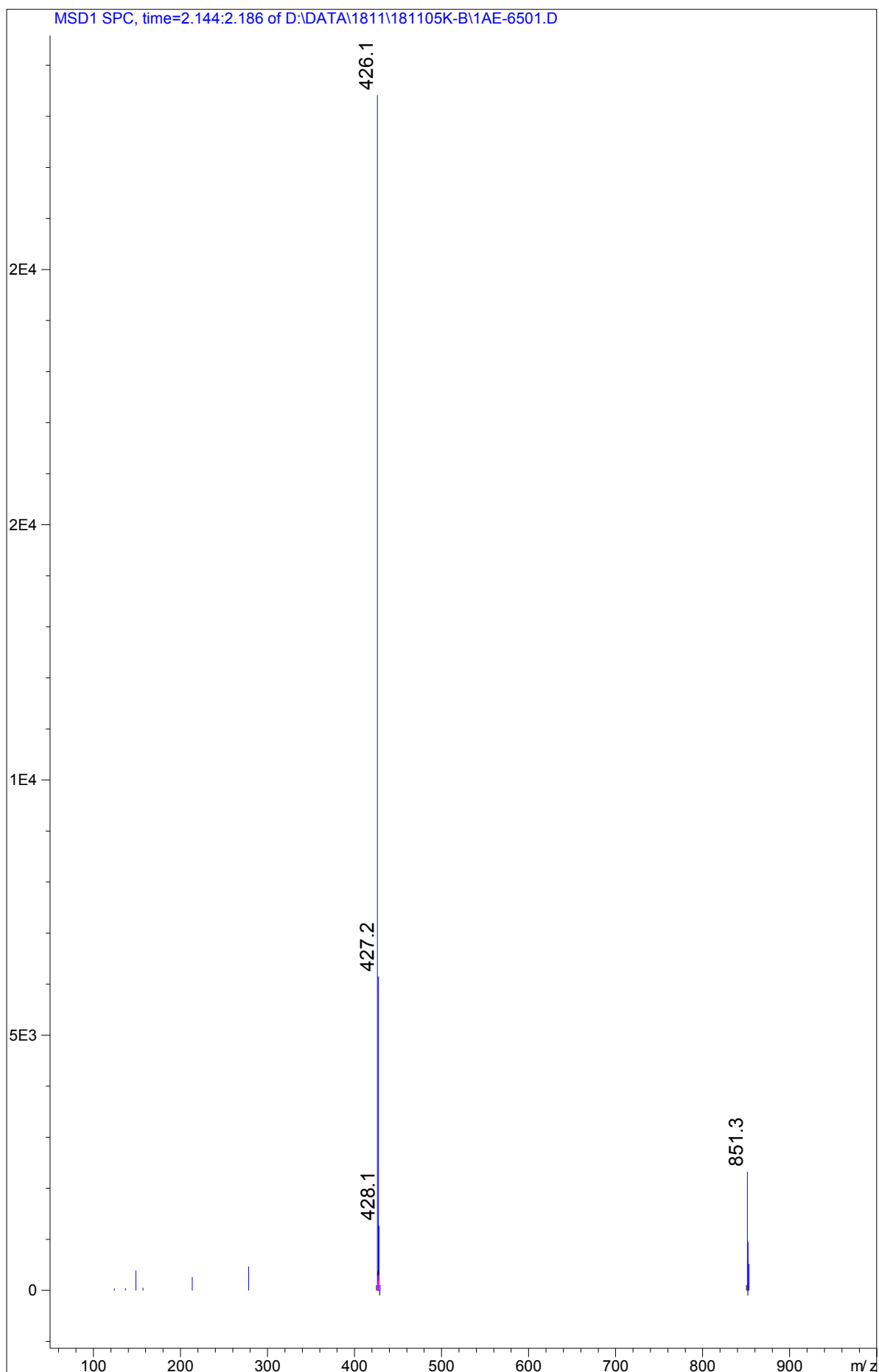
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	1.803	15.610	5.545	0.973	0.045	1.022
2	2.124	1512.383	564.364	99.027	0.043	98.978

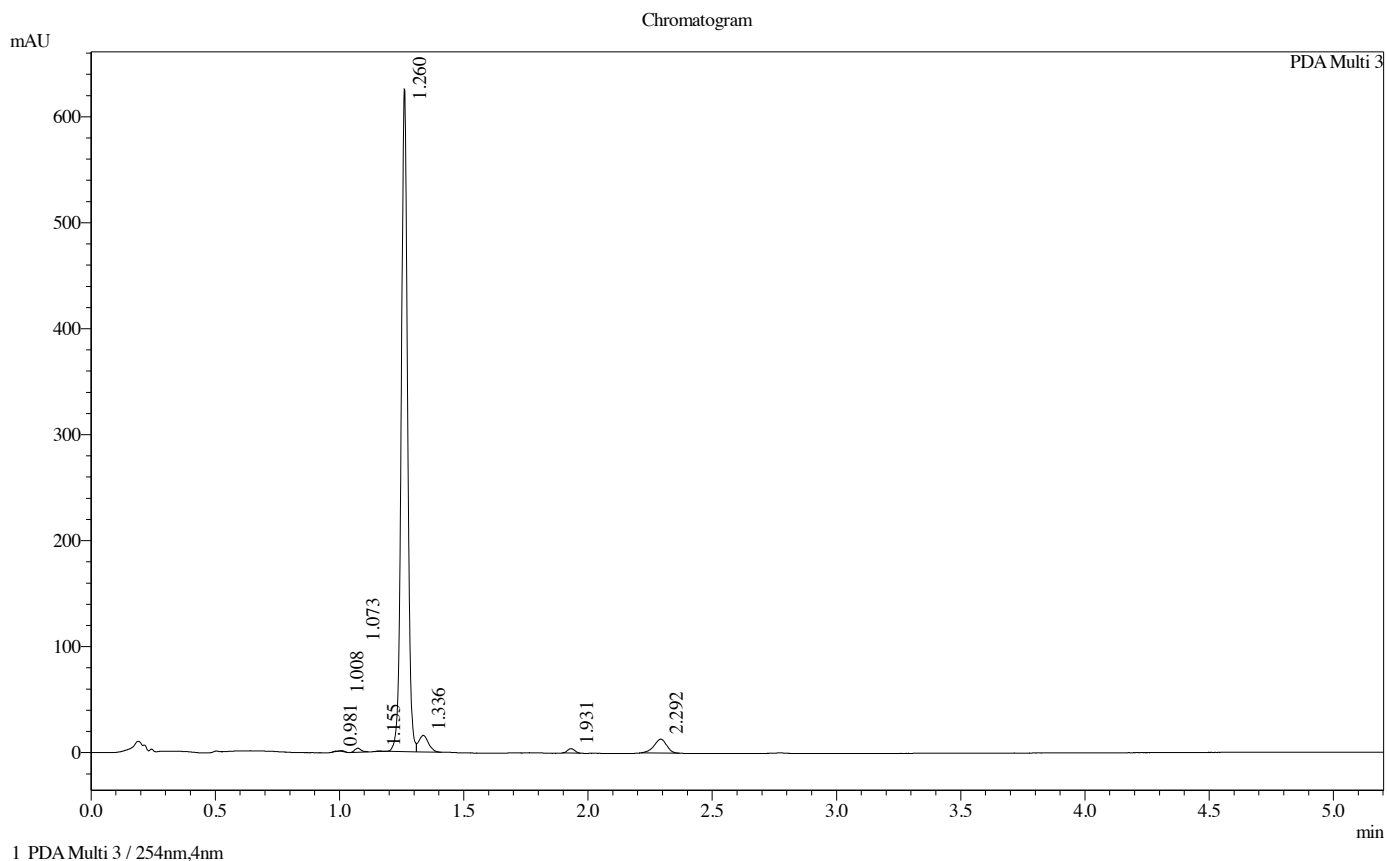
Operator: _____

Date: _____



HPLC REPORT

Compound ID :
Sample ID : ET23679-66-P1H
Injection Date : 2018/11/1 9:39:06
Injection Vol : 1ul
Location : tray1 vial90
Acq Method : D:\method\10-80HPLC-CD.lcm
Org DataFile : D:\data\2018\1811\181101\ET23679-66-P1H.lcd
Instrument : HPLC-031



Integration Result

PDA Ch3 254nm

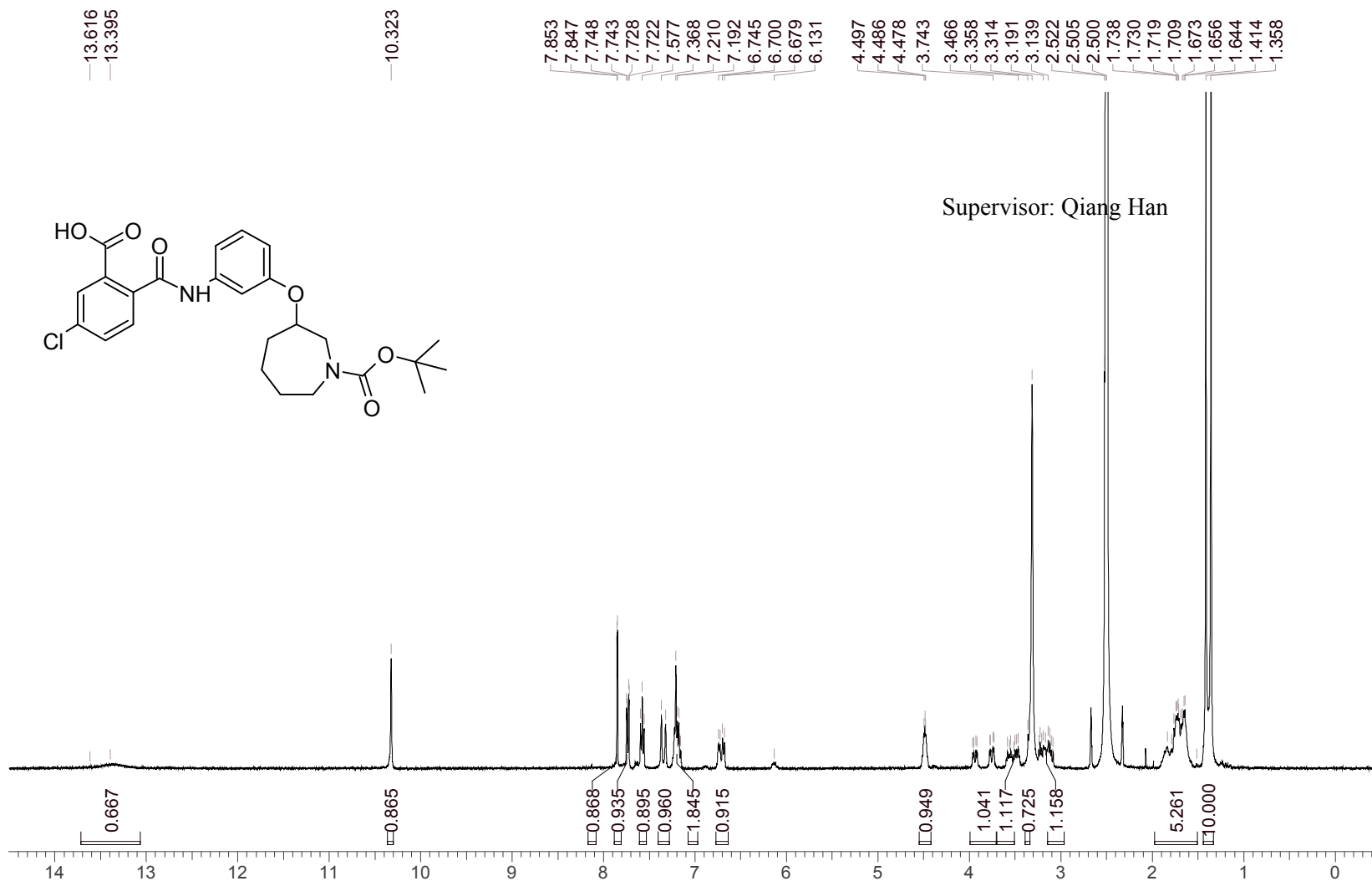
Peak#	Ret. Time	Height	Height %	USP Width	Area	Area %
1	0.981	586	0.088	0.025	554	0.046
2	1.008	907	0.137	0.030	995	0.082
3	1.073	3865	0.582	0.048	6741	0.559
4	1.155	628	0.095	0.038	887	0.073
5	1.260	625185	94.151	0.046	1100548	91.185
6	1.336	15560	2.343	0.081	42267	3.502
7	1.931	4175	0.629	0.056	8543	0.708
8	2.292	13120	1.976	0.093	46403	3.845

Operator: _____

Date: _____

Compound ID: BB0373

ET23679-146-P1AA DMSO Bruker_C_400MHz



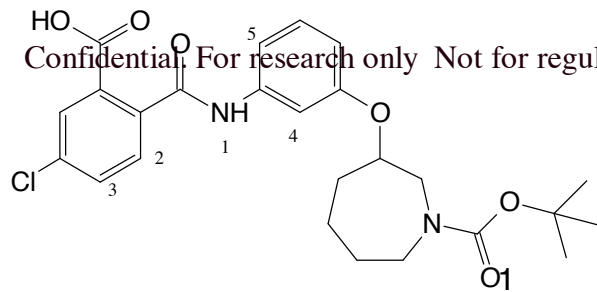
Supervisor: Qiang Han

Acquisition Time (sec)	2.0447
Comment	ET23679-1 46-P1AA DMSO Bruker_C_4 00MHz
Date	14 Dec 2018 12:01:51
Frequency (MHz)	400.1500
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	16384
Owner	nmr
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	106.30
SW(cyclical) (Hz)	8012.82
Solvent	DMSO-d6
Spectrum Offset (Hz)	2468.0044
Spectrum Type	standard
Sweep Width (Hz)	8012.70

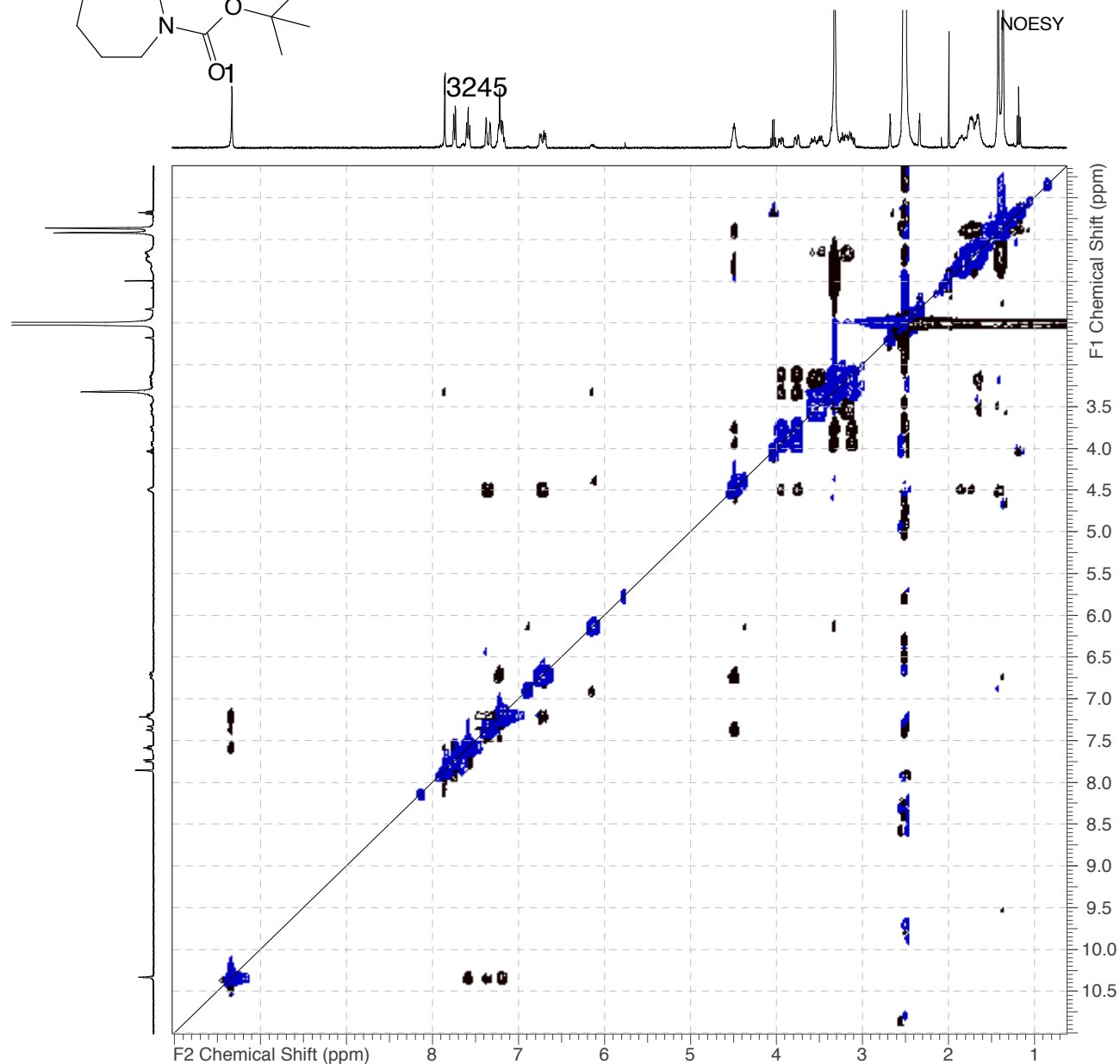
Confidential. For research only Not for regulatory filing

Operator:

Date:

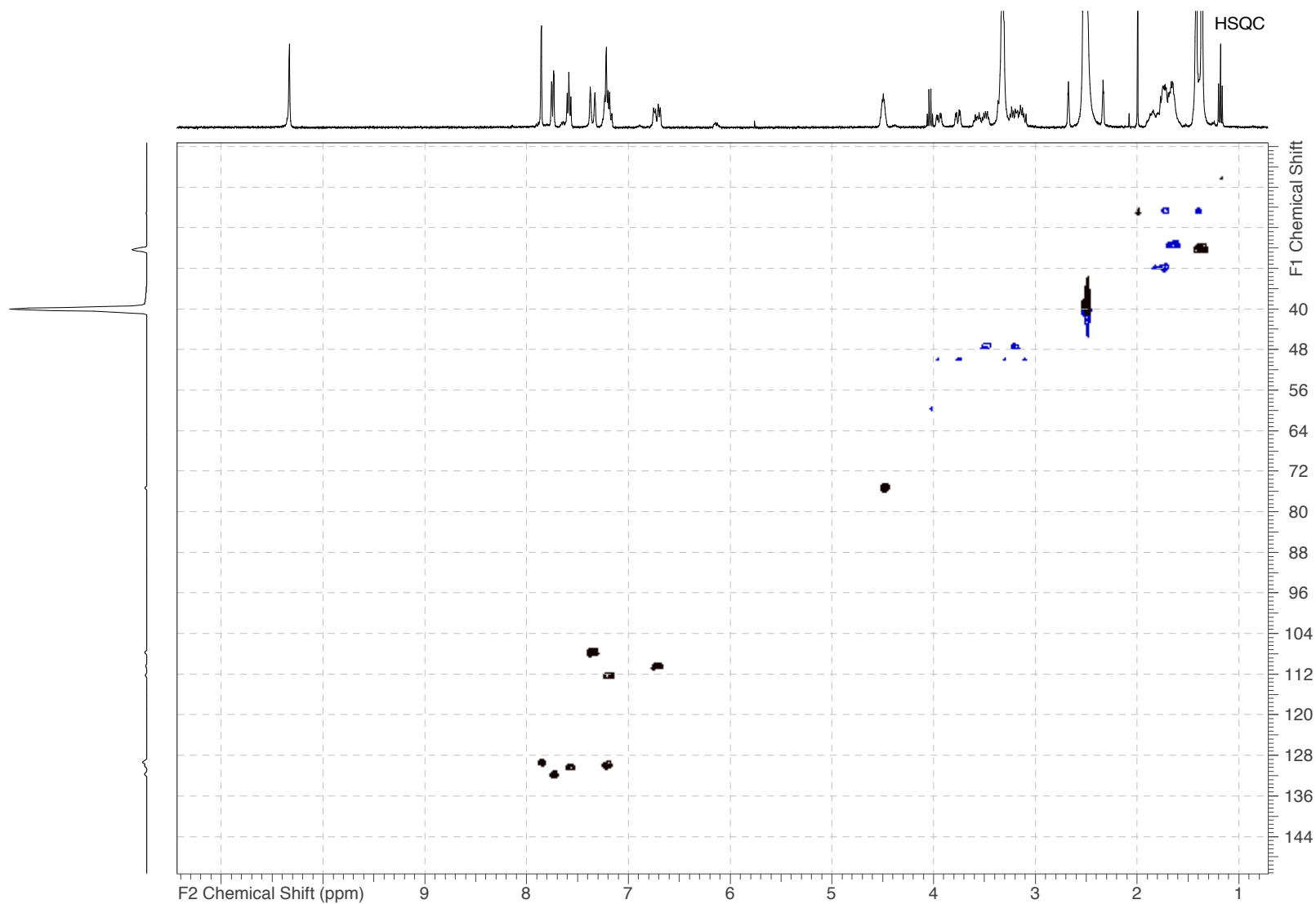


ET23679-146-P1A2 DMSO Bruker_F_400MHz



Acquisition Time (sec)	(0.2232, 0.0558)
Comment	ET23679-146-P1A2 DMSO Bruker_F_400MHz
Date	15 Dec 2018 15:39:00
File Name	\\10.25.3.239\data\ET23679-146-P1A2\2\PDAT A\1\2rr
Frequency (MHz)	(400.1700, 400.1700)
Nucleus	(1H, 1H)
Origin	spect
Original Points Count	(1024, 256)
Owner	nmr
Points Count	(1024, 1024)
Pulse Sequence	noesygp.php p
Solvent	DMSO-d6
Spectrum Type	NOESY
Sweep Width (Hz)	(4582.68, 4582.68)

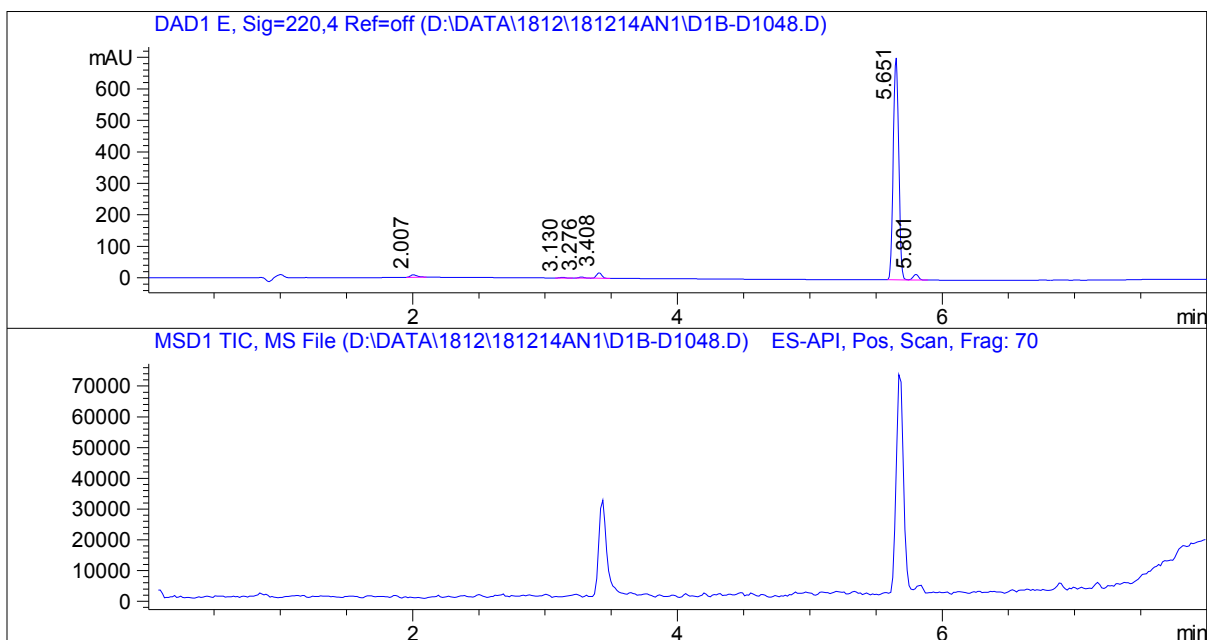
ET23679-146-P1A2 DMSO Bruker_C_400MHz



Acquisition Time (sec) (0.1597, 0.0154)
Comment ET23679-1
46-P1A2
DMSO
Bruker_C_400MHz
Date 17 Dec 2018 20:08:14
File Name \\10.25.3.194\data\ET23679-146-P1A2\2\PDAT A\1\2rr
Frequency (MHz) (400.1500, 100.6178)
Nucleus (1H, 13C)
Origin spect
Original Points Count (1024, 256)
Owner nmr
Points Count (1024, 1024)
Pulse Sequence hsqcedetgps isp2.3
Solvent DMSO-d6
Spectrum Type HSQC
Sweep Width (Hz) (6404.00, 16595.07)

LCMS REPORT

Compound ID : BB0373
Sample ID : ET23679-146-P1A1
Injection Date : 14. Dec. 2018
Inj. Vol. : 0.70 ul
Location : D1B-D1
Acq Method : D:\DATA\1812\181214AN1\30_90AB_8MIN_ASC.M
Data Filename : D:\DATA\1812\181214AN1\D1B-D1048.D
Instrument : AN



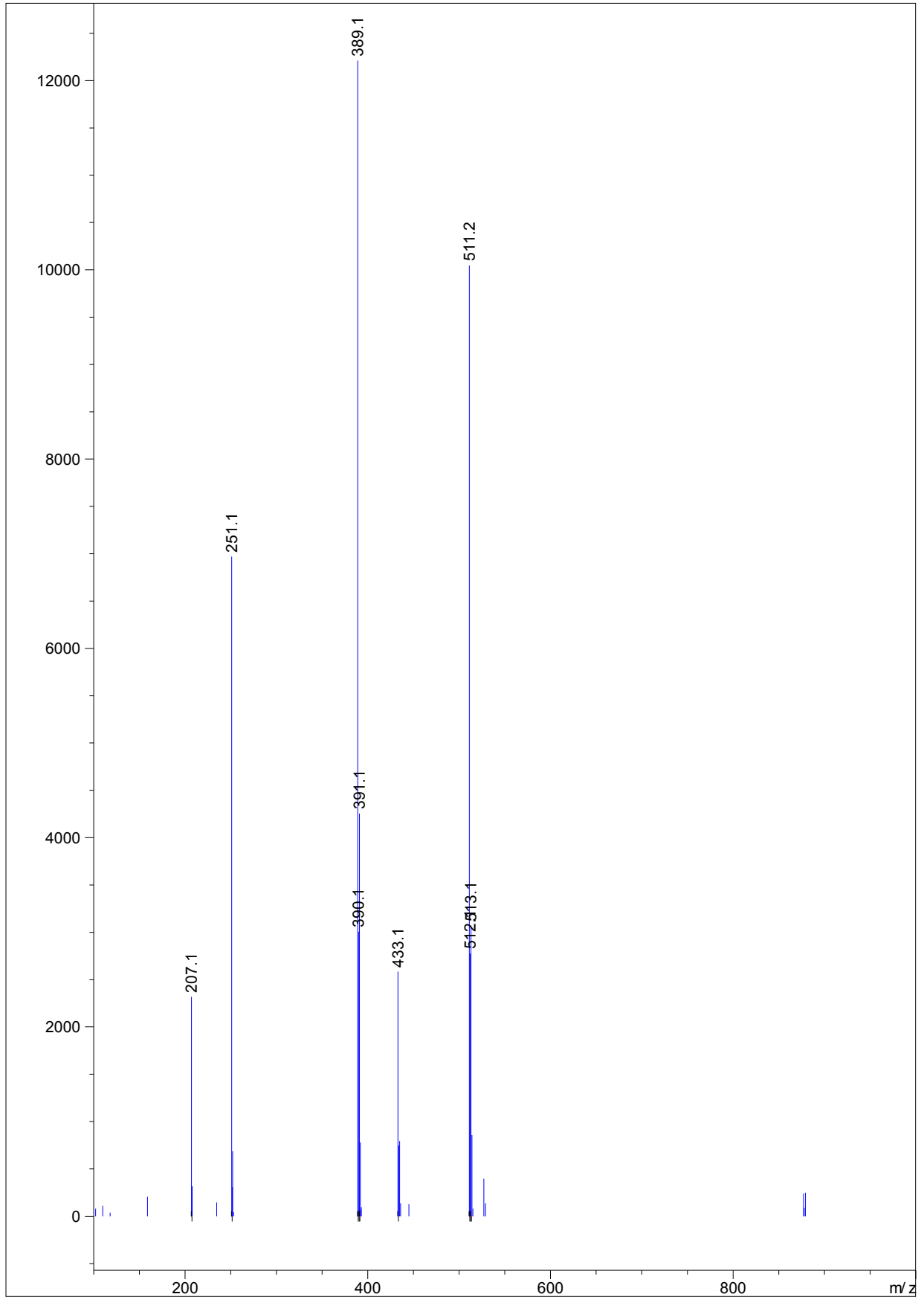
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	2.007	29.553	7.838	1.040	0.058	1.353
2	3.130	5.845	2.129	0.283	0.044	0.268
3	3.276	9.957	3.631	0.482	0.044	0.456
4	3.408	47.568	17.116	2.272	0.044	2.177
5	5.651	2040.570	705.237	93.607	0.046	93.401
6	5.801	51.257	17.453	2.317	0.046	2.346

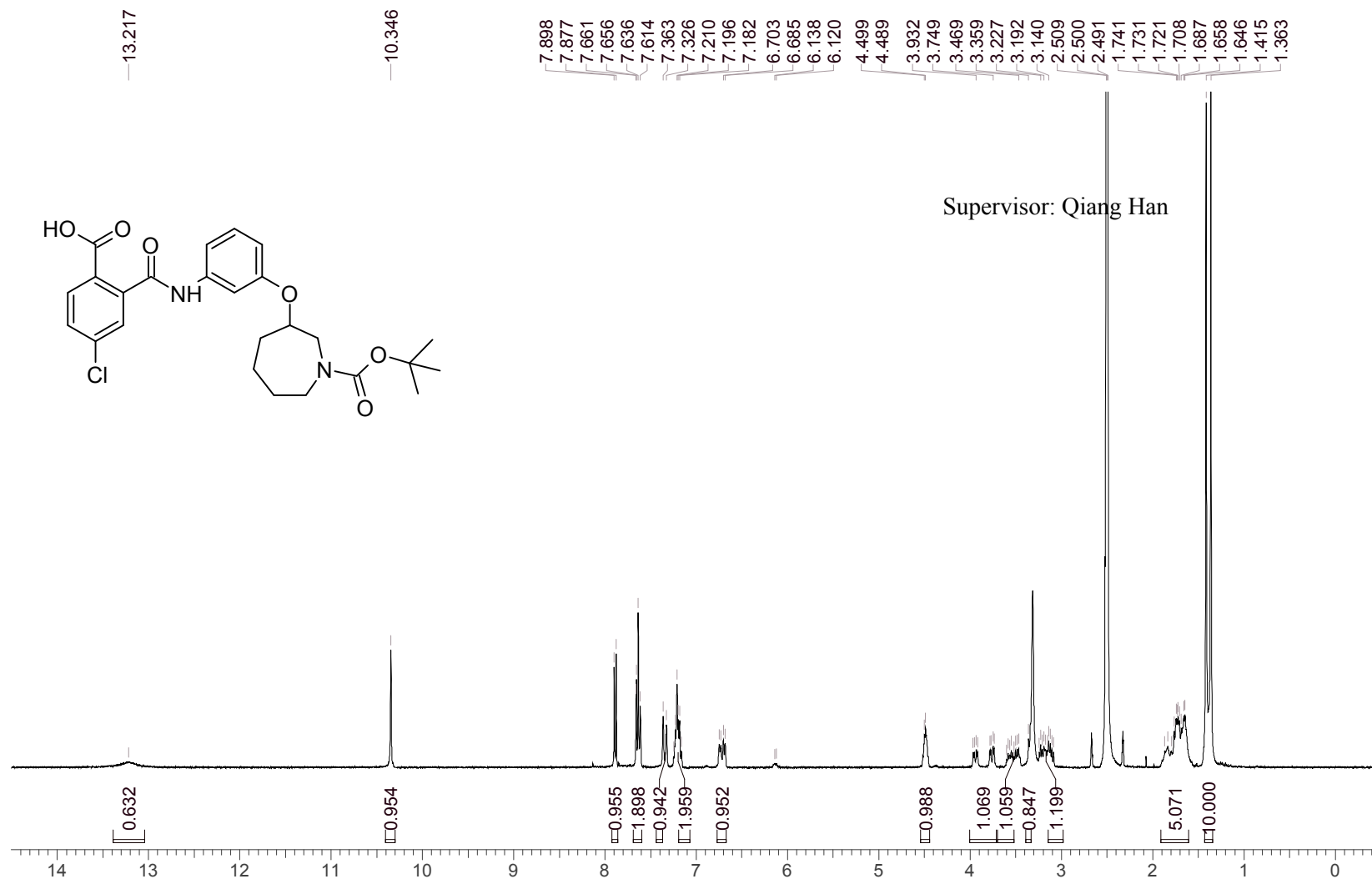
Operator: _____

Date: _____



Compound ID: BB0374

ET23679-146-P1BB DMSO Bruker_C_400MHz

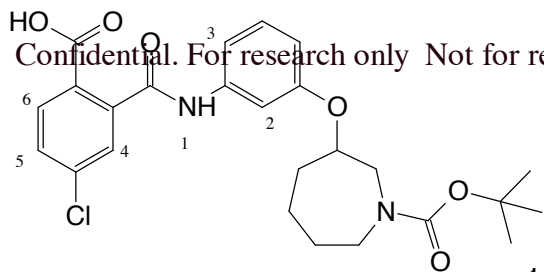


Acquisition Time (sec)	2.0447
Comment	ET23679-1 46-P1BB DMSO Bruker_C_4 00MHz
Date	14 Dec 2018 12:04:20
Frequency (MHz)	400.1500
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	16384
Owner	nmr
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	92.44
SW(cyclical) (Hz)	8012.82
Solvent	DMSO-d6
Spectrum Offset (Hz)	2468.0044
Spectrum Type	standard
Sweep Width (Hz)	8012.70

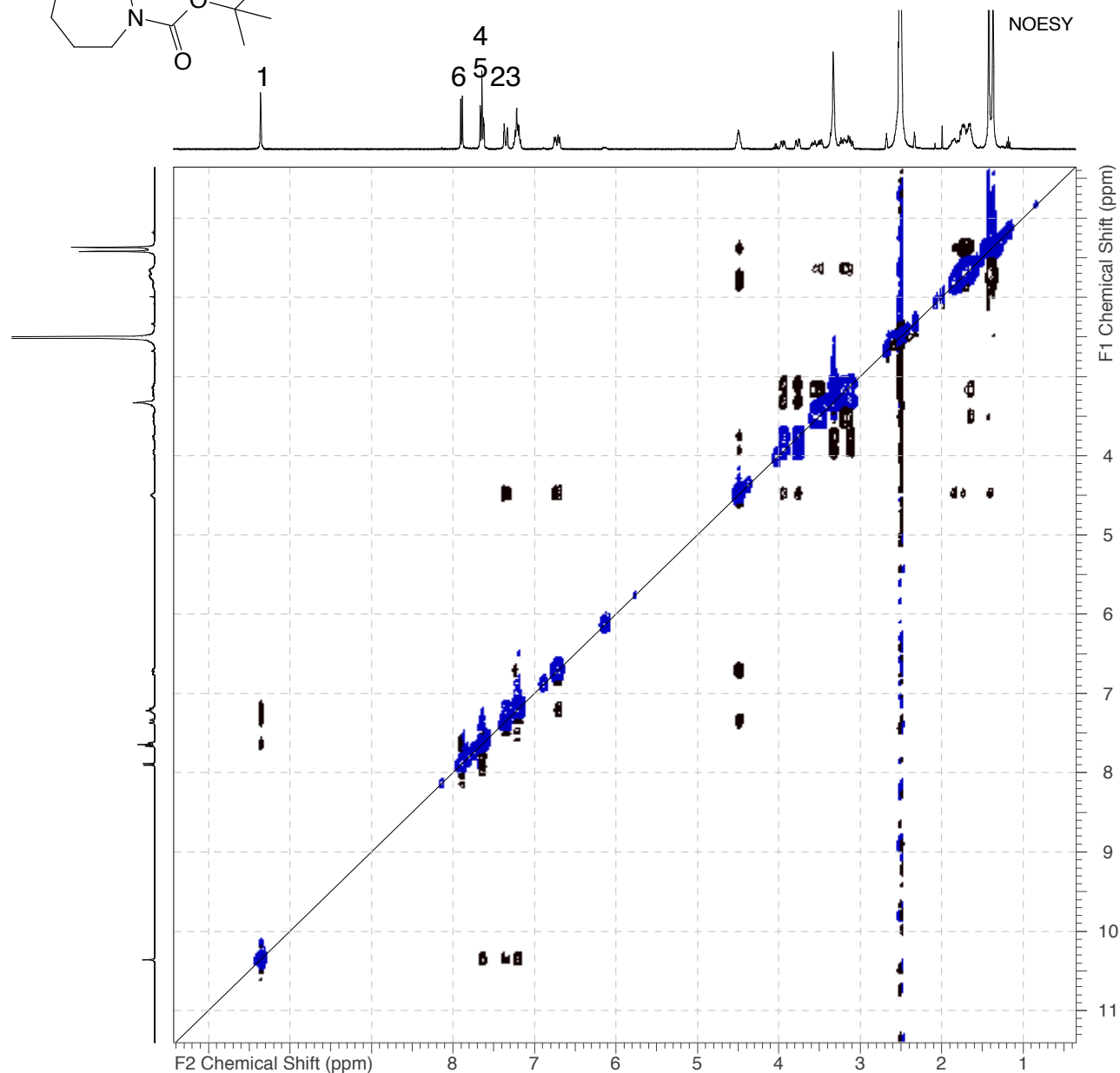
Confidential. For research only Not for regulatory filing

Operator:

Date:

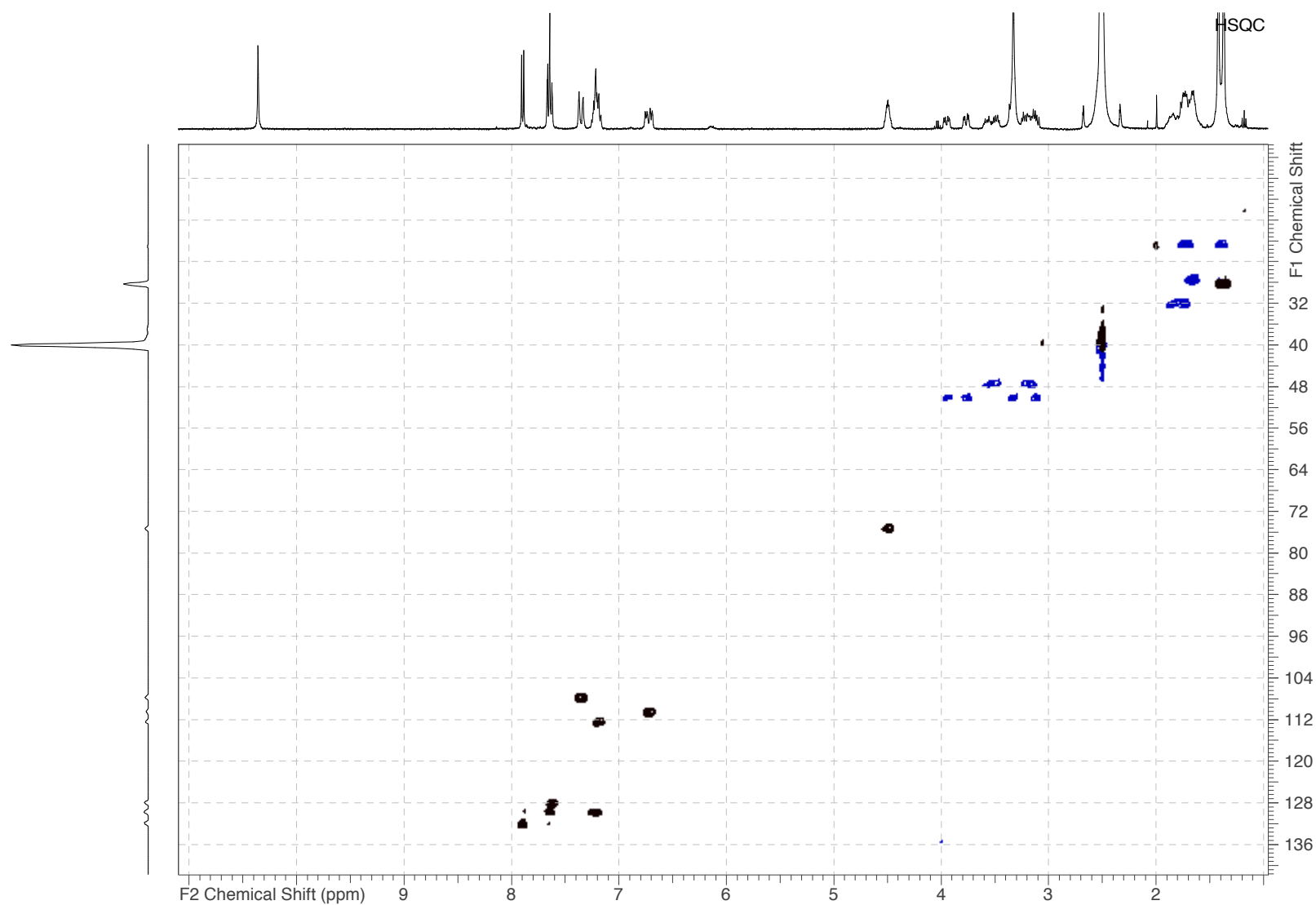


ET23679-146-P1B2 DMSO Bruker_F_400MHz



Acquisition Time (sec)	(0.1679, 0.0420)
Comment	ET23679-1 46-P1B2 DMSO Bruker_F_400MHz
Date	15 Dec 2018 17:26:38
File Name	\\10.25.3.23 9\data\ET23 679-146-P1 B2\2\PDAT A\1\2rr
Frequency (MHz)	(400.1700, 400.1700)
Nucleus	(1H, 1H)
Origin	spect
Original Points Count	(1024, 256)
Owner	nmr
Points Count	(1024, 1024)
Pulse Sequence	noesygp.php p
Solvent	DMSO-d6
Spectrum Type	NOESY
Sweep Width (Hz)	(6091.61, 6091.61)

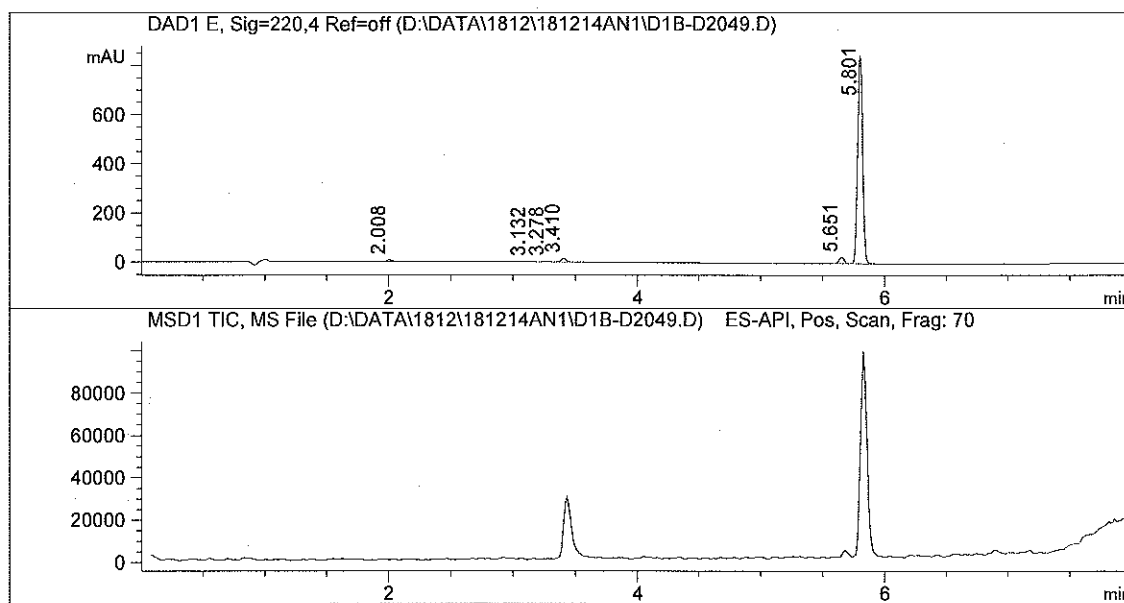
ET23679-146-P1B2 DMSO Bruker_C_400MHz



Acquisition Time (sec) (0.1597, 0.0154)
Comment ET23679-1
46-P1B2
DMSO
Bruker_C_400MHz
Date 17 Dec 2018 20:31:44
File Name \\10.25.3.194\data\ET23679-146-P1B2\2\PDAT A\1\2rr
Frequency (MHz) (400.1500, 100.6178)
Nucleus (1H, 13C)
Origin spect
Original Points Count (1024, 256)
Owner nmr
Points Count (1024, 1024)
Pulse Sequence hsqcedetgps isp2.3
Solvent DMSO-d6
Spectrum Type HSQC
Sweep Width (Hz) (6404.00, 16595.07)

LCMS REPORT

Compound ID : ~~BB0373~~ BB0374 Zhong JieLing
Sample ID : ET23679-146-P1B1
Injection Date : 14. Dec. 2018
Inj. Vol. : 0.70 ul 2018.12.19
Location : D1B-D2
Acq Method : D:\DATA\1812\181214AN1\30_90AB_8MIN_ASC.M
Data Filename : D:\DATA\1812\181214AN1\D1B-D2049.D
Instrument : AN



Integration Result

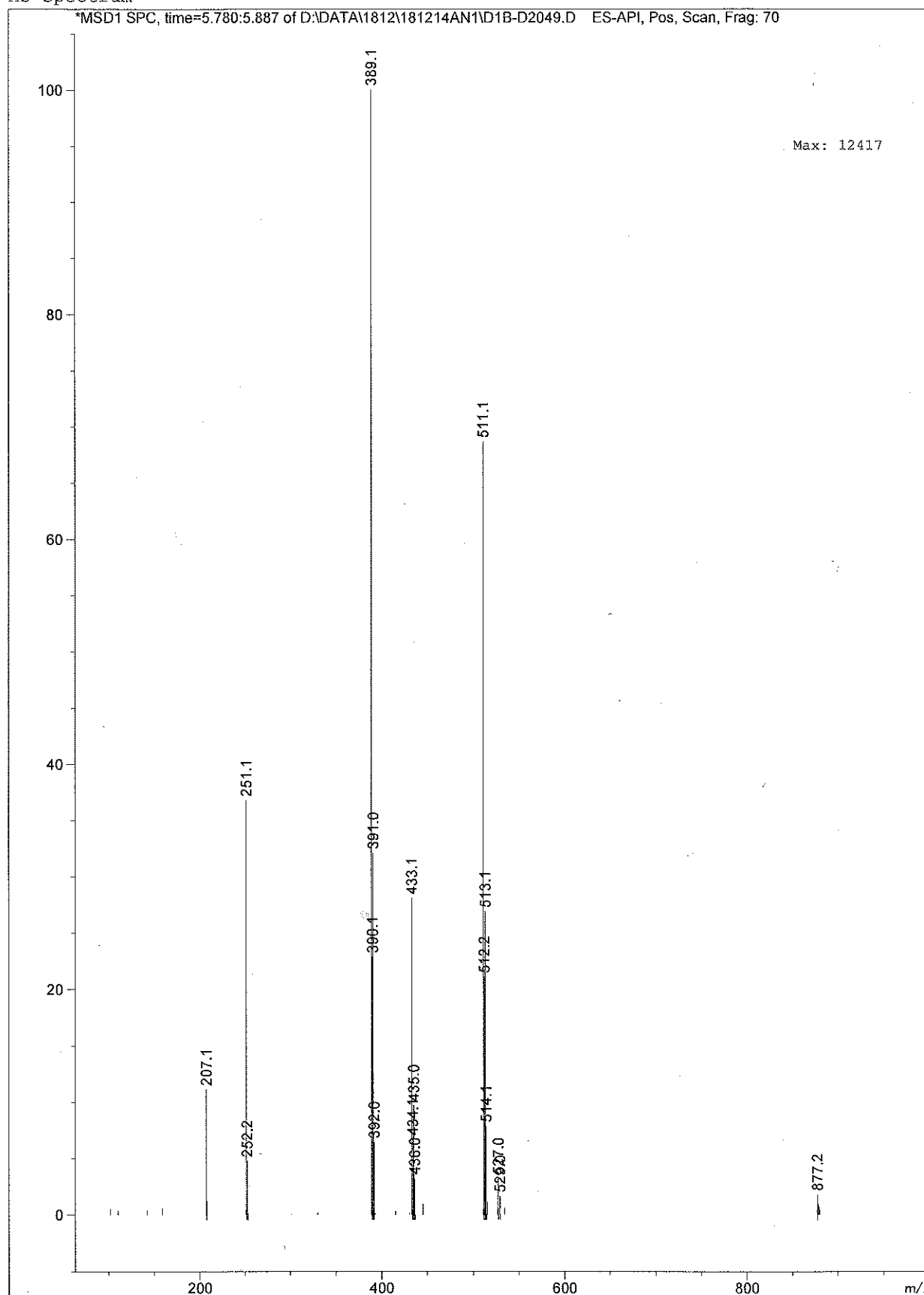
Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	2.008	25.770	6.706	0.747	0.057	0.991
2	3.132	5.672	2.053	0.229	0.044	0.218
3	3.278	9.621	3.504	0.390	0.044	0.370
4	3.410	42.697	15.363	1.712	0.044	1.642
5	5.651	74.061	25.429	2.834	0.046	2.847
6	5.801	2443.140	844.303	94.088	0.046	93.932

Operator: _____

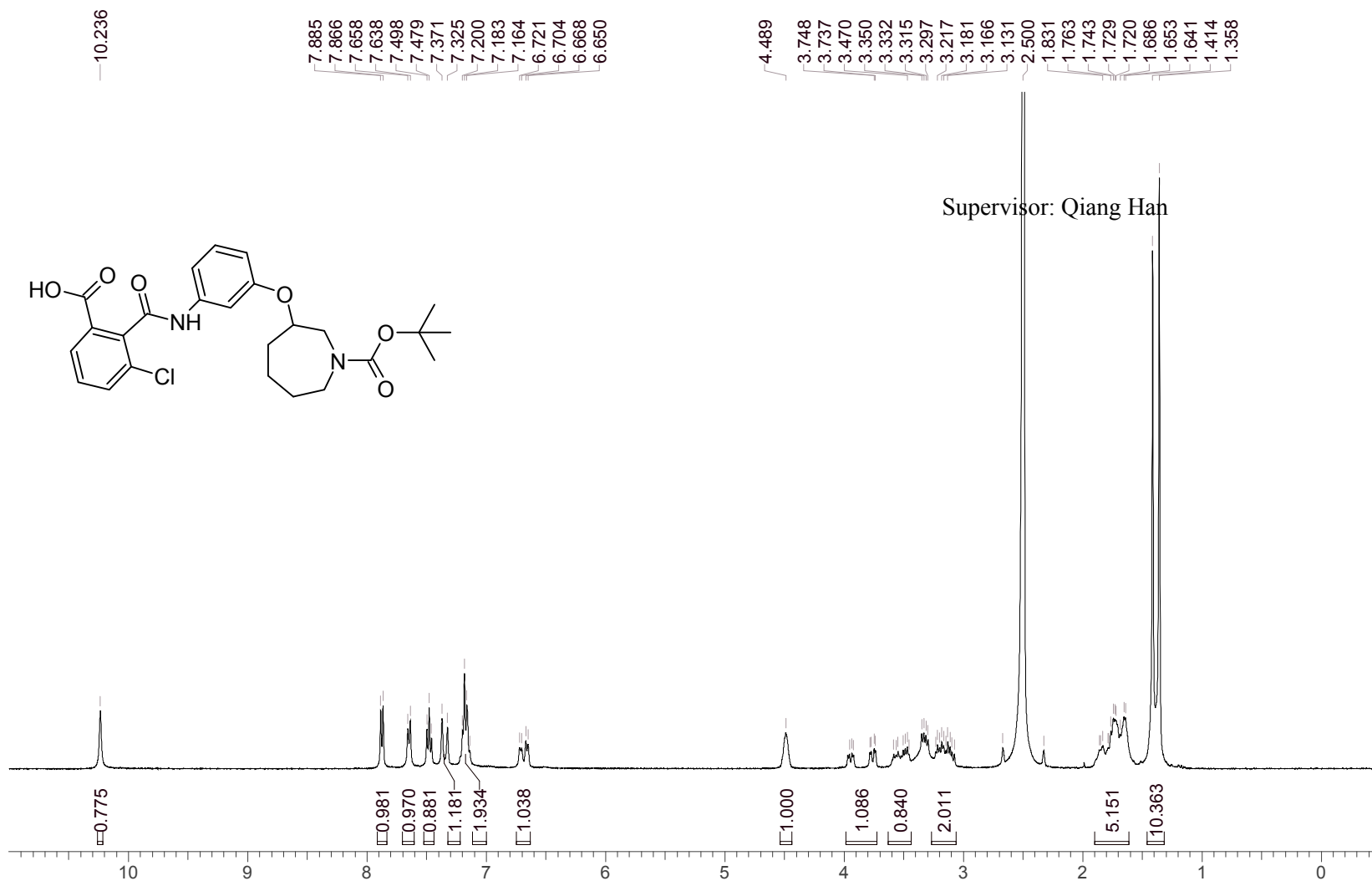
Date: _____

MS Spectrum



Compound ID: BB0376

ET23679-195-P1BB DMSO Varian_S_400MHz



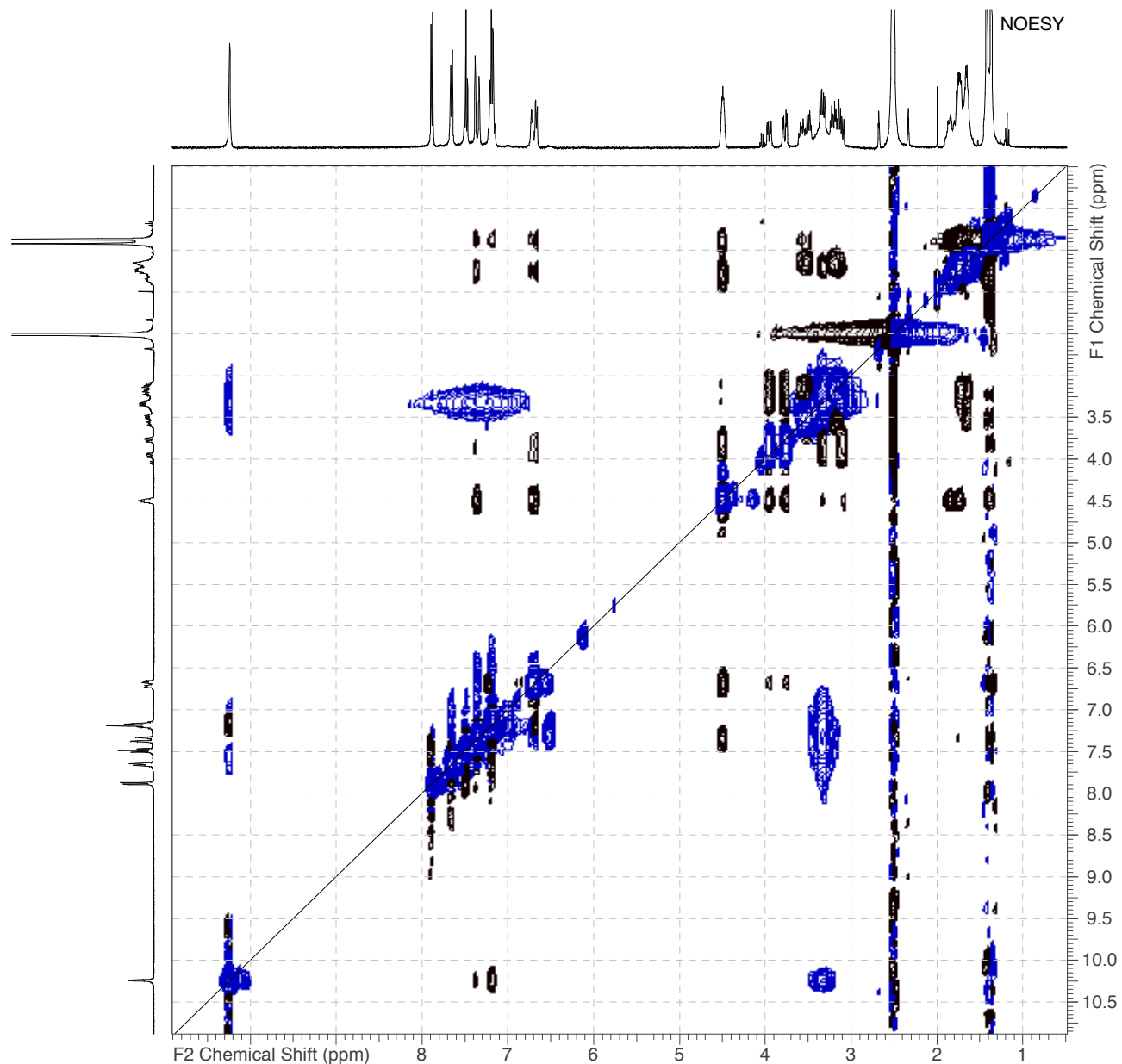
Acquisition Time (sec) 2.0486
 Comment ET23679-1
 95-P1BB
 DMSO
 Varian_S_
 400MHz
 Date Dec 27
 2018
 Frequency (MHz) 400.3377
 Nucleus 1H
 Number of Transients 8
 Original Points Count 14802
 Points Count 32768
 Pulse Sequence s2pul
 Receiver Gain 20.00
 SW(cyclical) (Hz) 7225.43
 Solvent DMSO-d6
 Spectrum Offset (Hz) 2812.8162
 Spectrum Type standard
 Sweep Width (Hz) 7225.21
 Temperature (degree C) 25.000

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Operator:

Date:

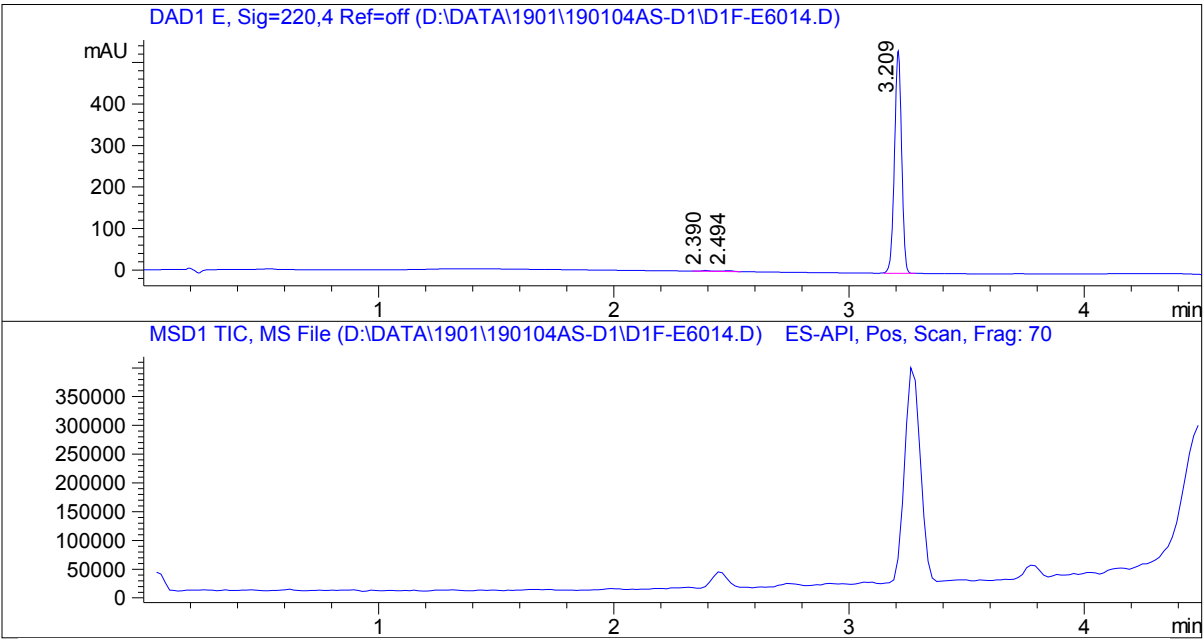
ET23679-195-P1B2 DMSO Bruker_F_400MHz



Acquisition Time (sec)	(0.2232, 0.0279)
Comment	ET23679-1 95-P1B2 DMSO Bruker_F_400MHz
Date	29 Dec 2018 08:42:20
File Name	E:\2D-temp\ ET23679-1 95-P1B2\2 PDATA\1\2 rr
Frequency (MHz)	(400.1700, 400.1700)
Nucleus	(1H, 1H)
Origin	spect
Original Points Count	(1024, 128)
Owner	nmr
Points Count	(1024, 1024)
Pulse Sequence	noesygp.php p
Solvent	DMSO-d6
Spectrum Type	NOESY
Sweep Width (Hz)	(4582.68, 4582.68)

LCMS REPORT

Compound ID : BB0376
Sample ID : ET23679-195-P1C1
Injection Date : 4. Jan. 2019
Inj. Vol. : 0.50 ul
Location : D1F-E6
Acq Method : D:\Data\1901\190104AS-D1\WUXIAB10.M
Data Filename : D:\DATA\1901\190104AS-D1\D1F-E6014.D
Instrument : AS



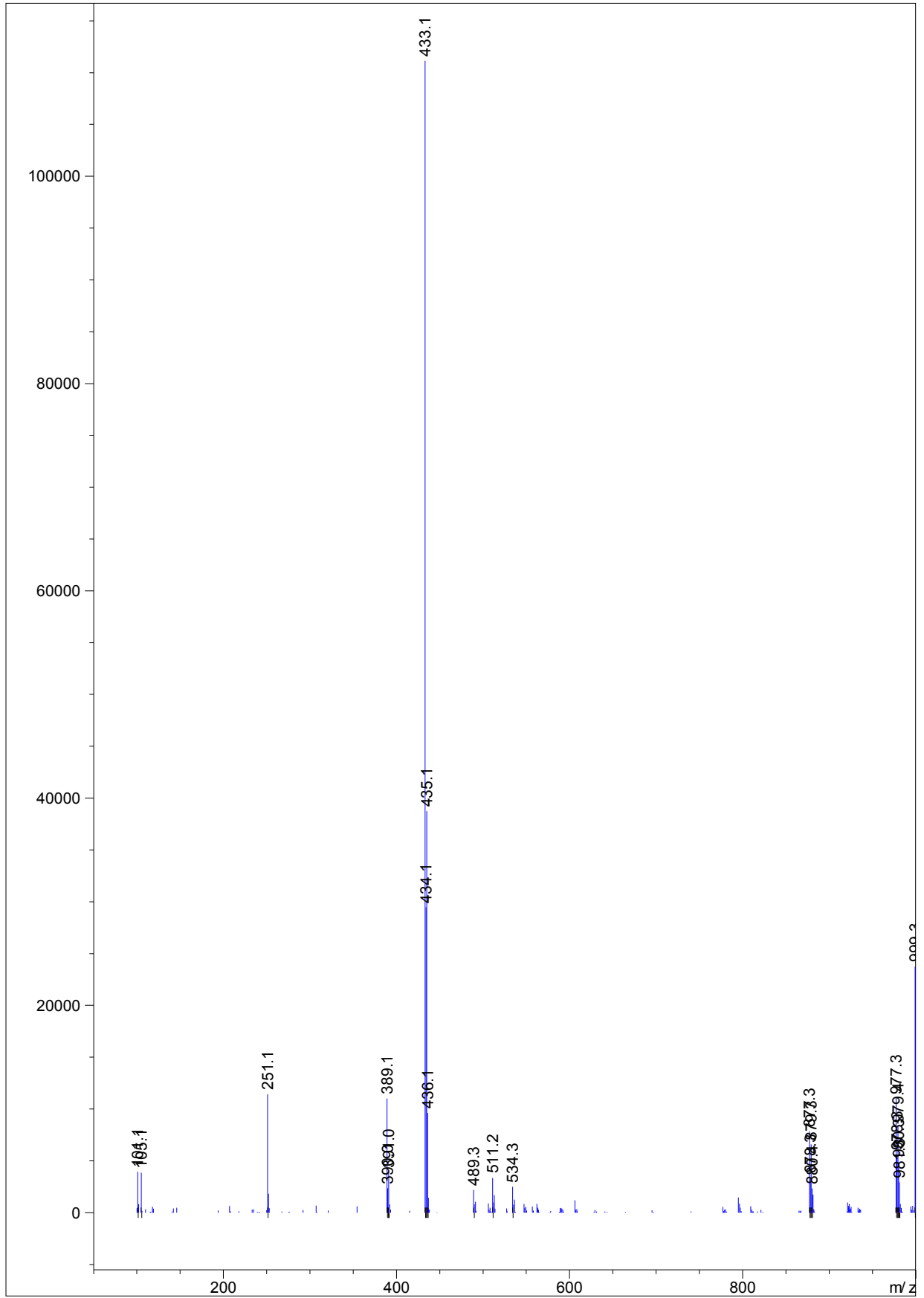
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	2.390	4.584	1.963	0.363	0.039	0.401
2	2.494	7.332	2.053	0.379	0.060	0.642
3	3.209	1130.135	537.177	99.258	0.034	98.957

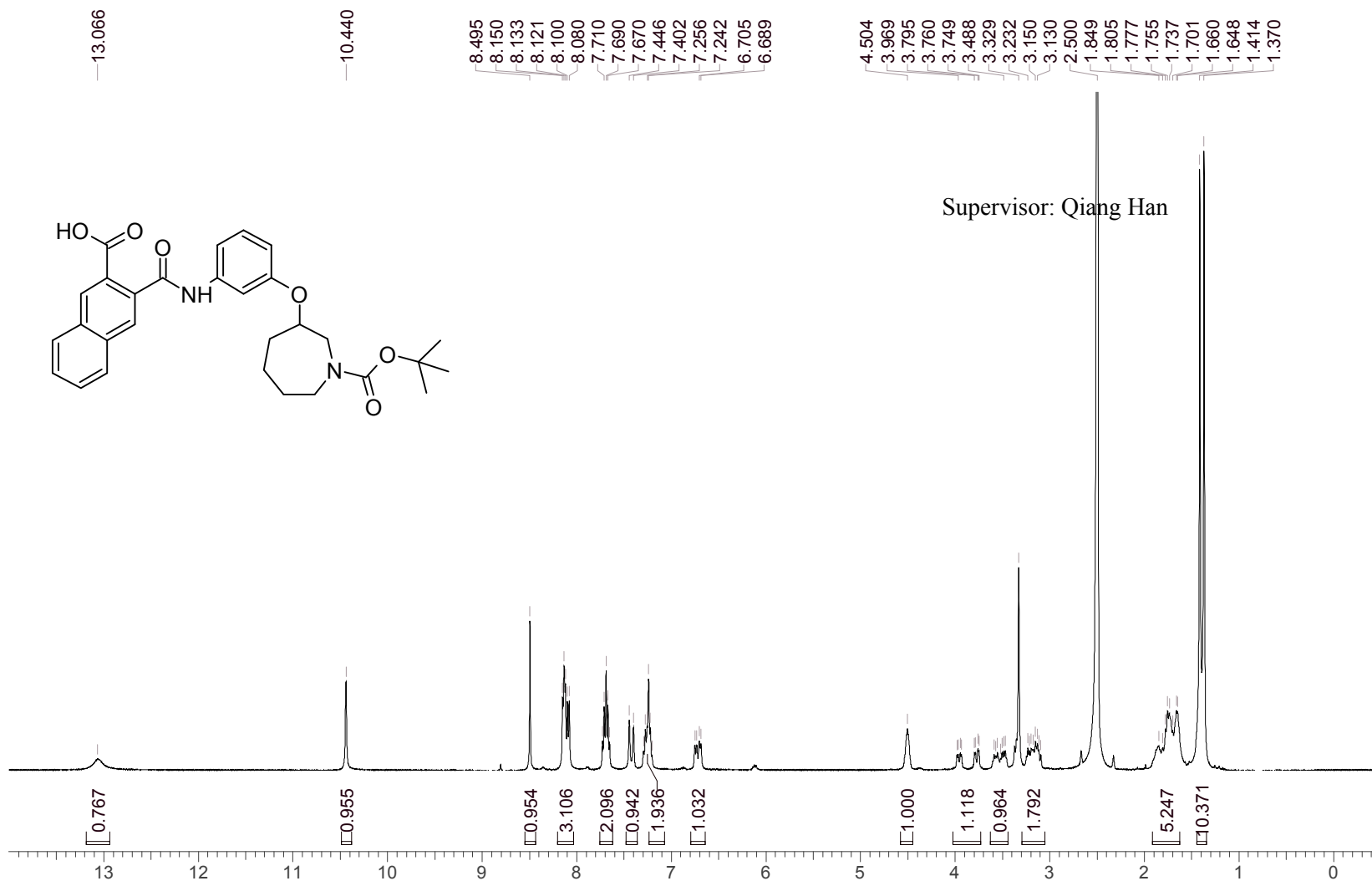
Operator:_____

Date:_____



Compound ID: BB0377

ET23679-196-P1AA DMSO Varian_S_400MHz



Acquisition Time (sec) 2.0486
Comment ET23679-1
96-P1AA
DMSO
Varian_S_
400MHz
Date Dec 27
2018
Frequency (MHz) 400.3377
Nucleus 1H
Number of Transients 8
Original Points Count 14802
Points Count 32768
Pulse Sequence s2pul
Receiver Gain 20.00
SW(cyclical) (Hz) 7225.43
Solvent DMSO-d6
Spectrum Offset (Hz) 2813.2566
Spectrum Type standard
Sweep Width (Hz) 7225.21
Temperature (degree C) 25.000

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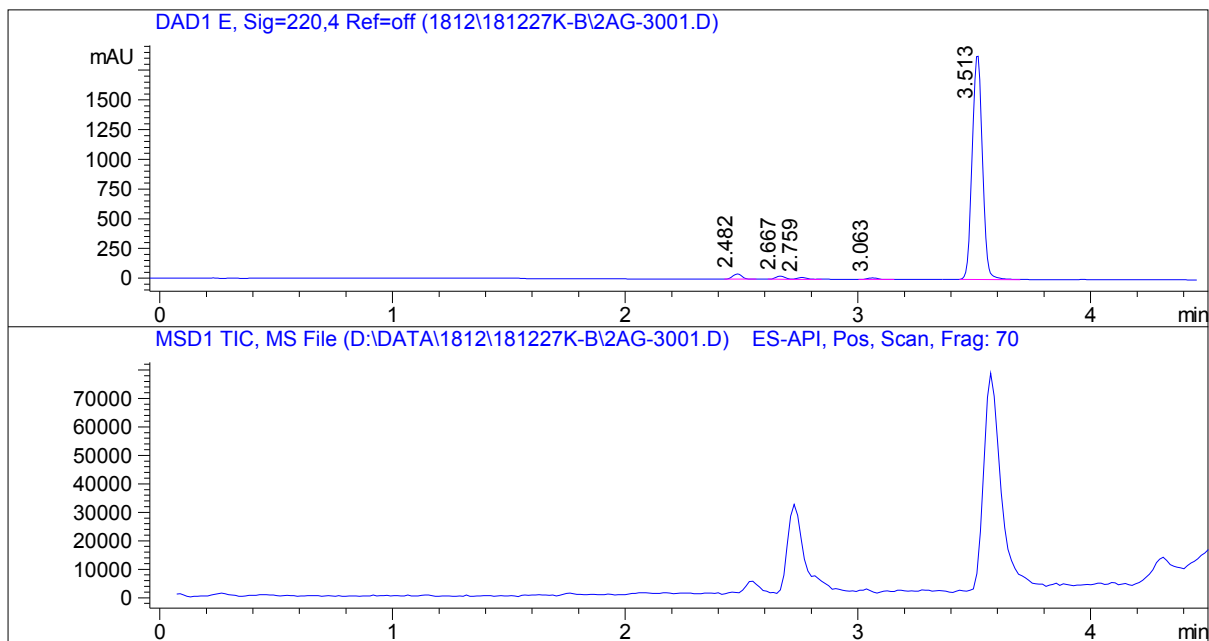
Operator:

Date:

LCMS REPORT

Compound ID : BB0377
Sample ID : ET23679-196-P1A1
Injection Date : 27. Dec. 2018
Inj. Vol. : 1.00 ul
Location : P2-A-07
Acq Method : D:\Data\1812\181227K-B\WUXIAB01_W.M
Data Filename : D:\DATA\1812\181227K-B\2AG-3001.D
Instrument : LCMS-K

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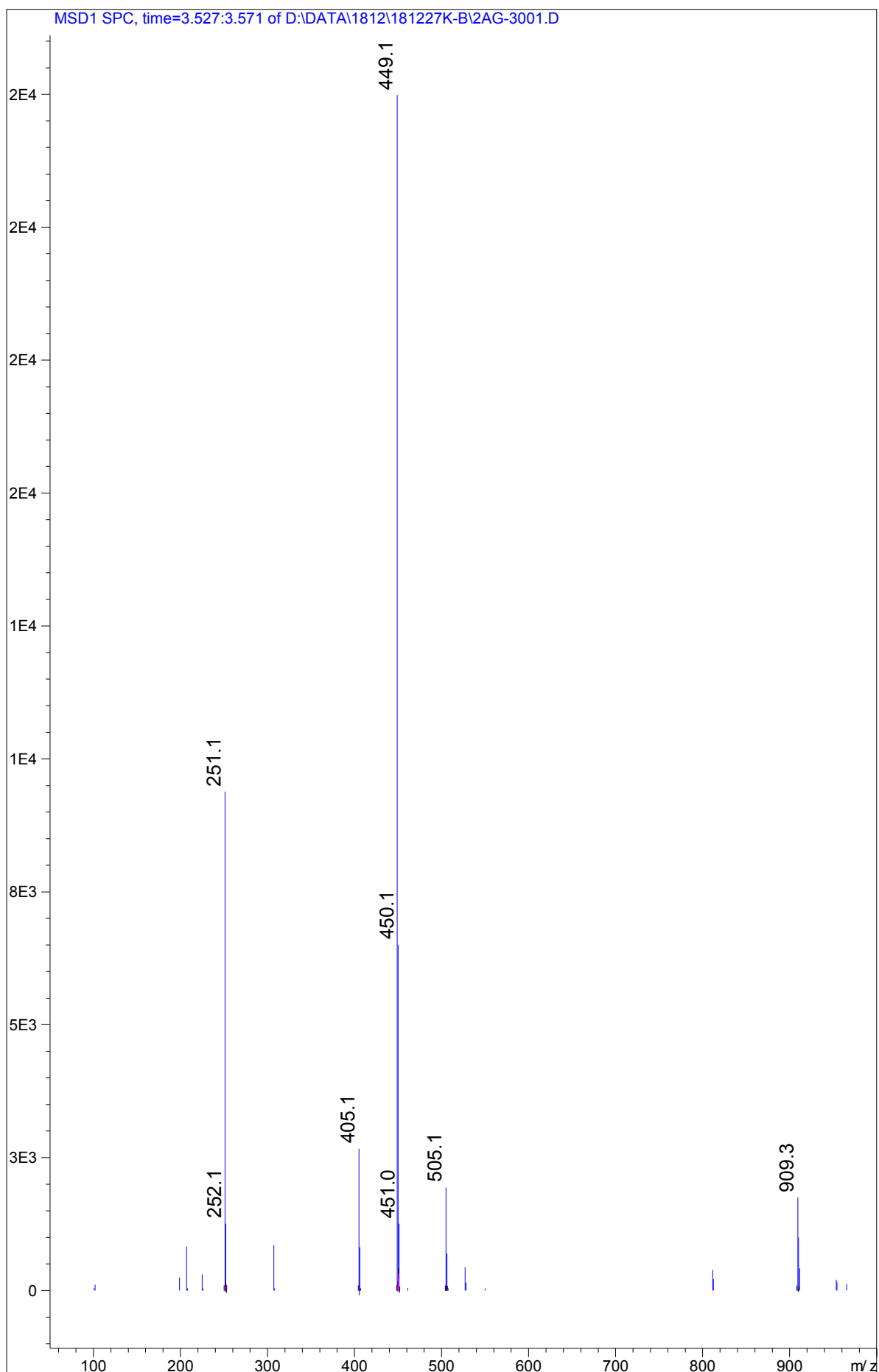
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	2.482	118.585	43.366	2.171	0.044	1.964
2	2.667	70.879	26.051	1.304	0.044	1.174
3	2.759	42.860	15.171	0.759	0.045	0.710
4	3.063	35.027	12.334	0.617	0.047	0.580
5	3.513	5772.023	1900.744	95.148	0.047	95.573

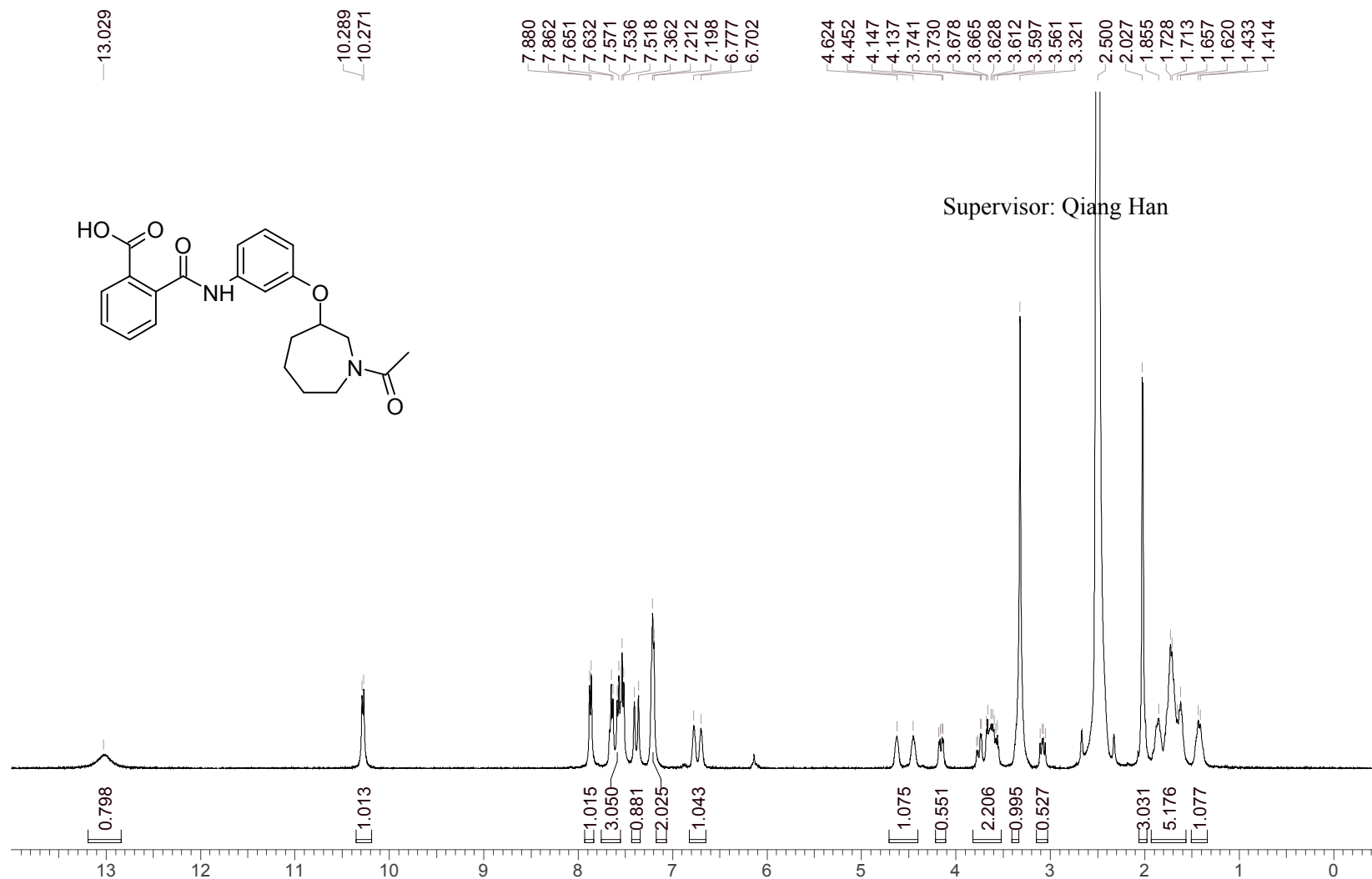
Operator: _____

Date: _____



Compound ID: BB0378

ET23679-206-P1B2 DMSO Bruker_F_400MHz



Supervisor: Qiang Han

Acquisition Time (sec)	2.0447
Comment	ET23679-2 06-P1B2 DMSO Bruker_F_ 400MHz
Date	09 Jan 2019 10:16:07
Frequency (MHz)	400.1700
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	16384
Owner	nmr
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	129.60
SW(cyclical) (Hz)	8012.82
Solvent	DMSO-d6
Spectrum Offset (Hz)	2467.6882
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	24.286

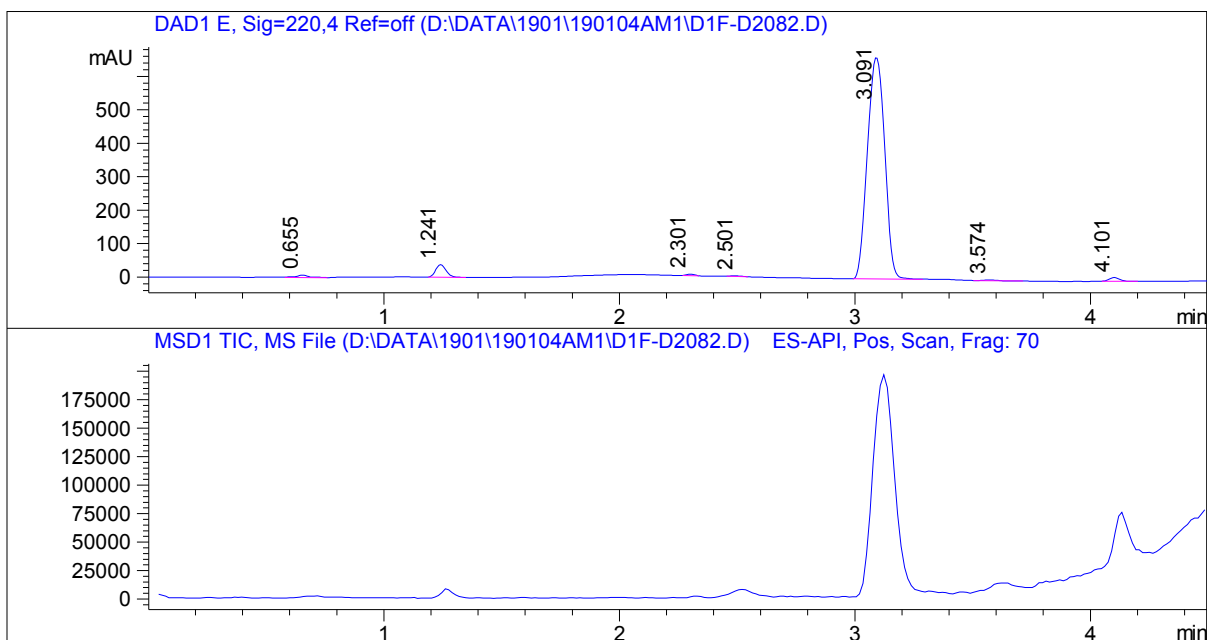
Confidential. For research only Not for regulatory filing

Operator:

Date:

LCMS REPORT

Compound ID : BB0378
Sample ID : ET23679-206-P1A1
Injection Date : 4. Jan. 2019
Inj. Vol. : 0.30 ul
Location : D1F-D2
Acq Method : D:\DATA\1901\190104AM1\WUXIAB10_XDB.M
Data Filename : D:\DATA\1901\190104AM1\D1F-D2082.D
Instrument : AM



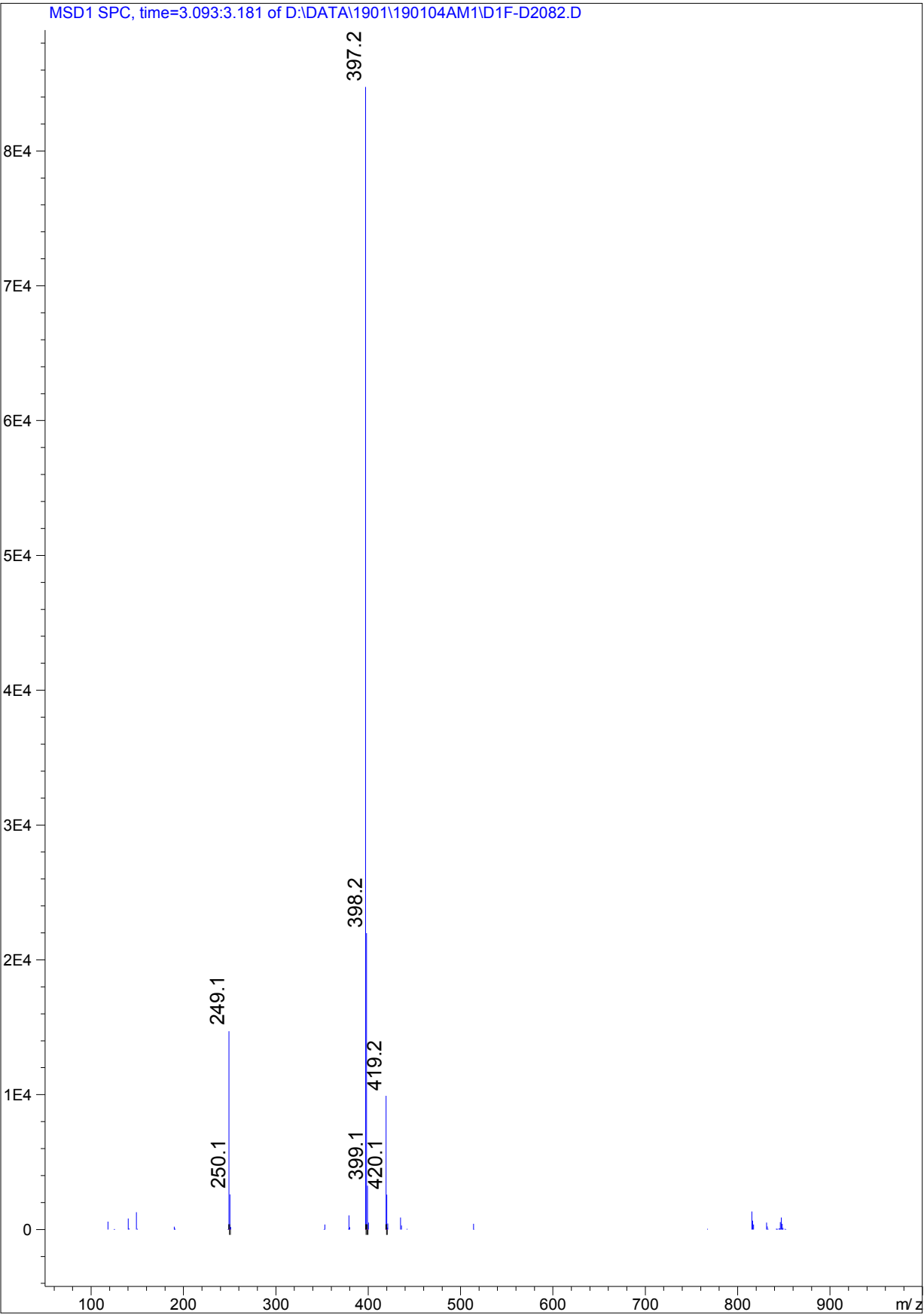
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	0.655	24.242	6.638	0.913	0.055	0.677
2	1.241	115.293	37.458	5.154	0.050	3.221
3	2.301	13.131	5.170	0.711	0.042	0.367
4	2.501	5.285	1.635	0.225	0.054	0.148
5	3.091	3380.202	662.543	91.155	0.084	94.443
6	3.574	7.541	2.268	0.312	0.055	0.211
7	4.101	33.411	11.116	1.529	0.049	0.933

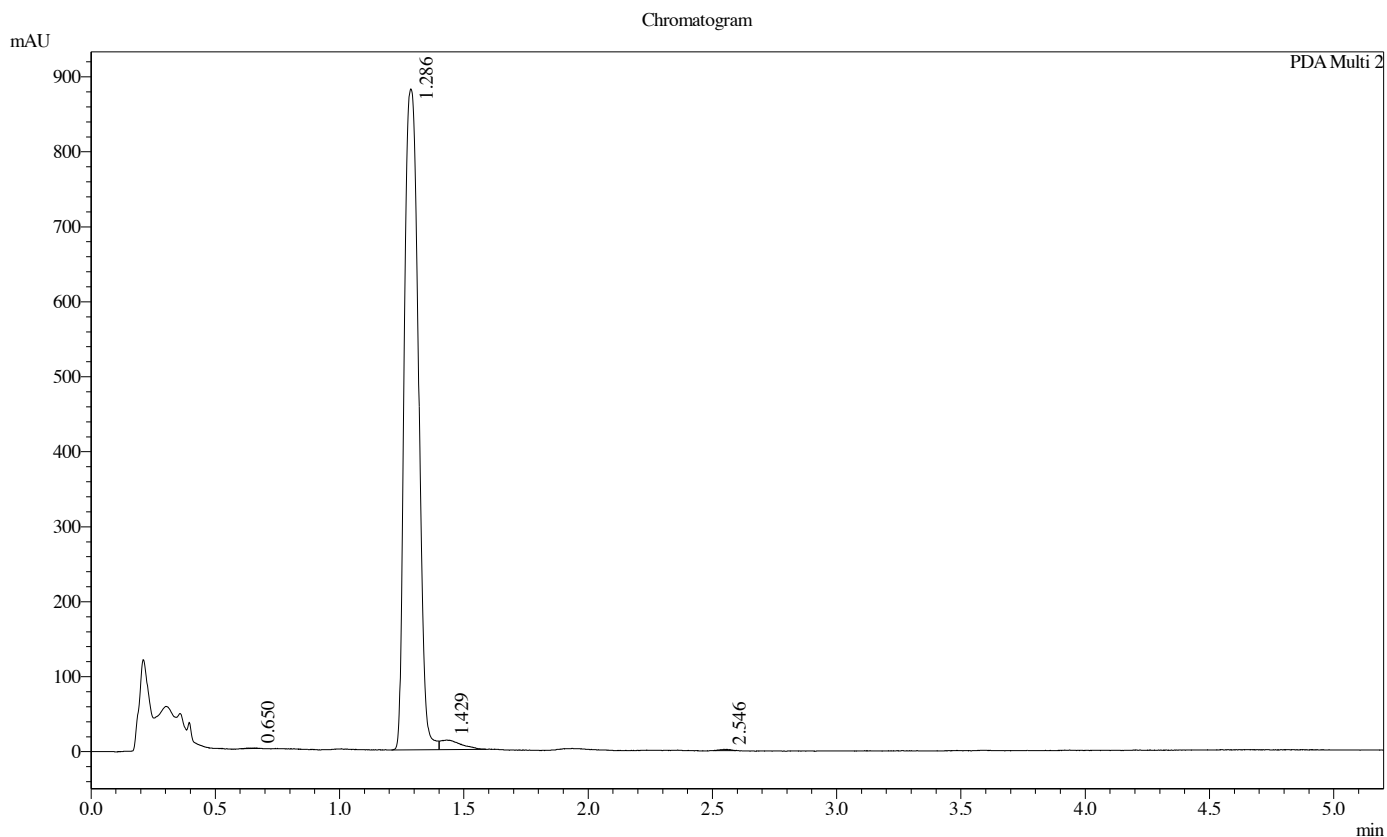
Operator: _____

Date: _____



HPLC REPORT

Compound ID :
Sample ID : ET23679-206-P1H
Injection Date : 12/27/2018 3:54:54 PM
Injection Vol : 0.1ul
Location : tray1 vial33
Acq Method : D:\method\10-80HPLC-CD.lcm
Org Data Name : D:\DATA\2018\1812\181227\ET23679-206-P1H.lcd
Instrument : HPLC-023



Integration Result

PDA Ch2 215nm

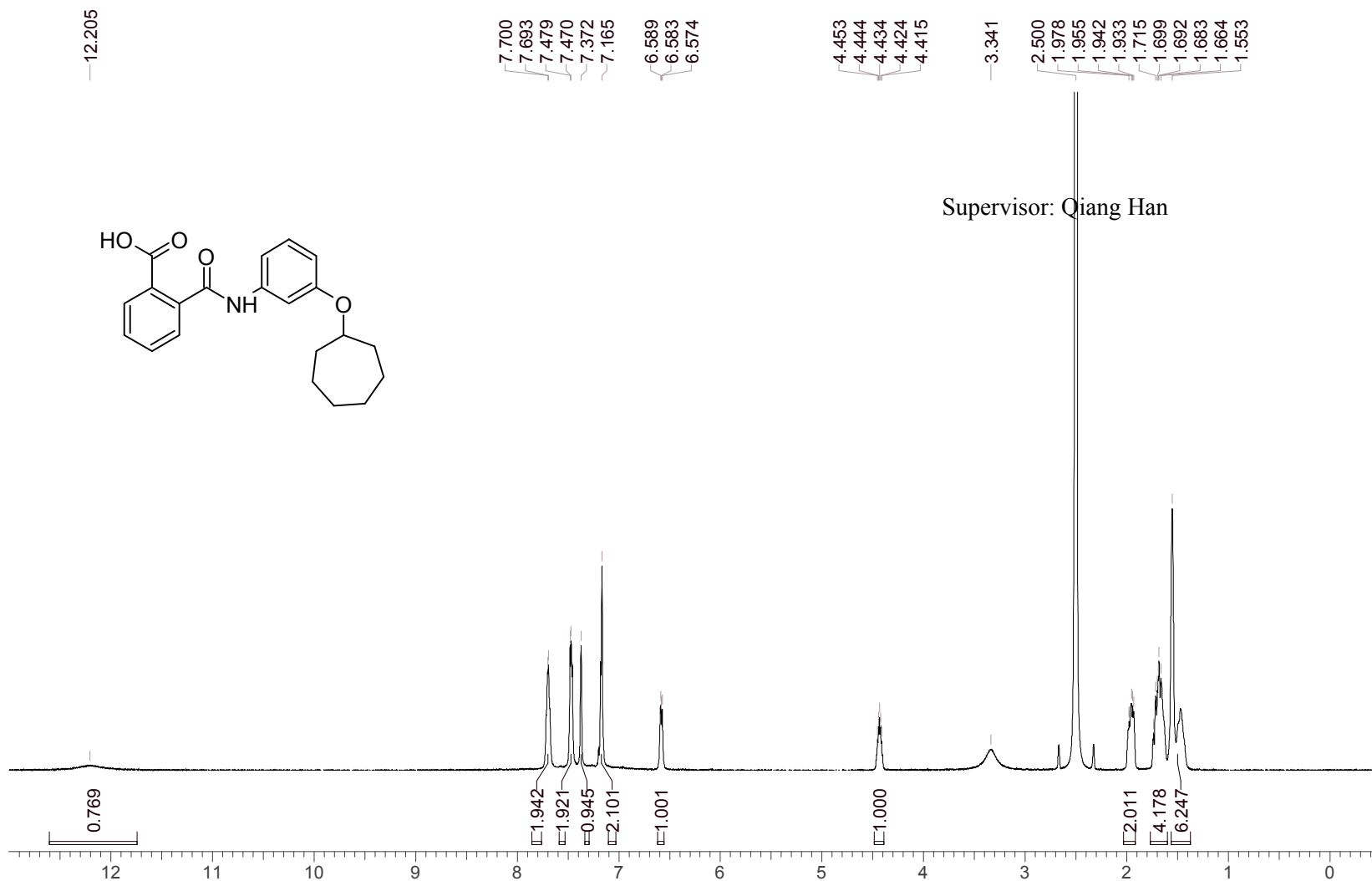
Peak#	Ret. Time	Height	Height %	USP Width	Area	Area %
1	0.650	840	0.094	0.067	2036	0.057
2	1.286	881283	98.297	0.098	3475898	97.715
3	1.429	12846	1.433	0.297	74570	2.096
4	2.546	1584	0.177	0.083	4658	0.131

Operator: _____

Date: _____

Compound ID: BB0379

ET23679-189-P1AA DMSO Bruker_C_400MHz



Acquisition Time (sec)	2.0447
Comment	ET23679-1 89-P1AA DMSO Bruker_C_ 400MHz
Date	24 Dec 2018 11:19:20
Frequency (MHz)	400.1500
Nucleus	1H
Number of Transients	8
Origin	spect
Original Points Count	16384
Owner	nmr
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	92.44
SW(cyclical) (Hz)	8012.82
Solvent	DMSO-d6
Spectrum Offset (Hz)	2468.6047
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	22.072

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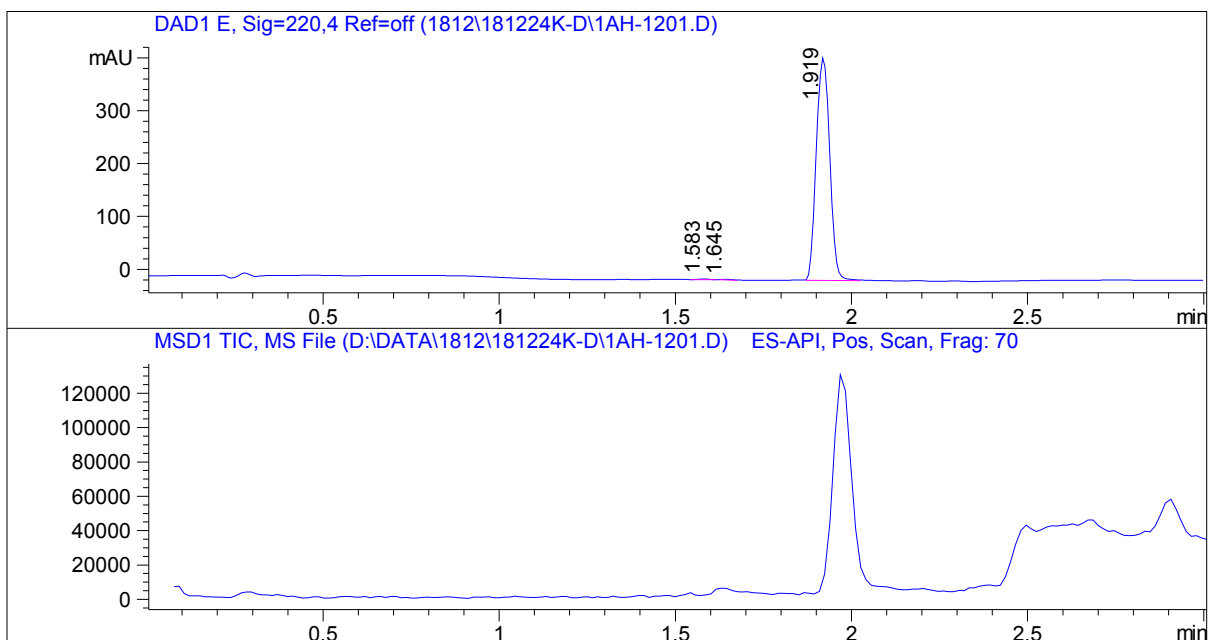
Operator:

Date:

LCMS REPORT

Compound ID : BB0379
Sample ID : ET23679-189-P1A1
Injection Date : 24. Dec. 2018
Inj. Vol. : 0.7 ul
Location : P1-A-08
Acq Method : D:\DATA\1812\181224K-D\DELIVER-K.M
Data Filename : D:\DATA\1812\181224K-D\1AH-1201.D
Instrument : K

->



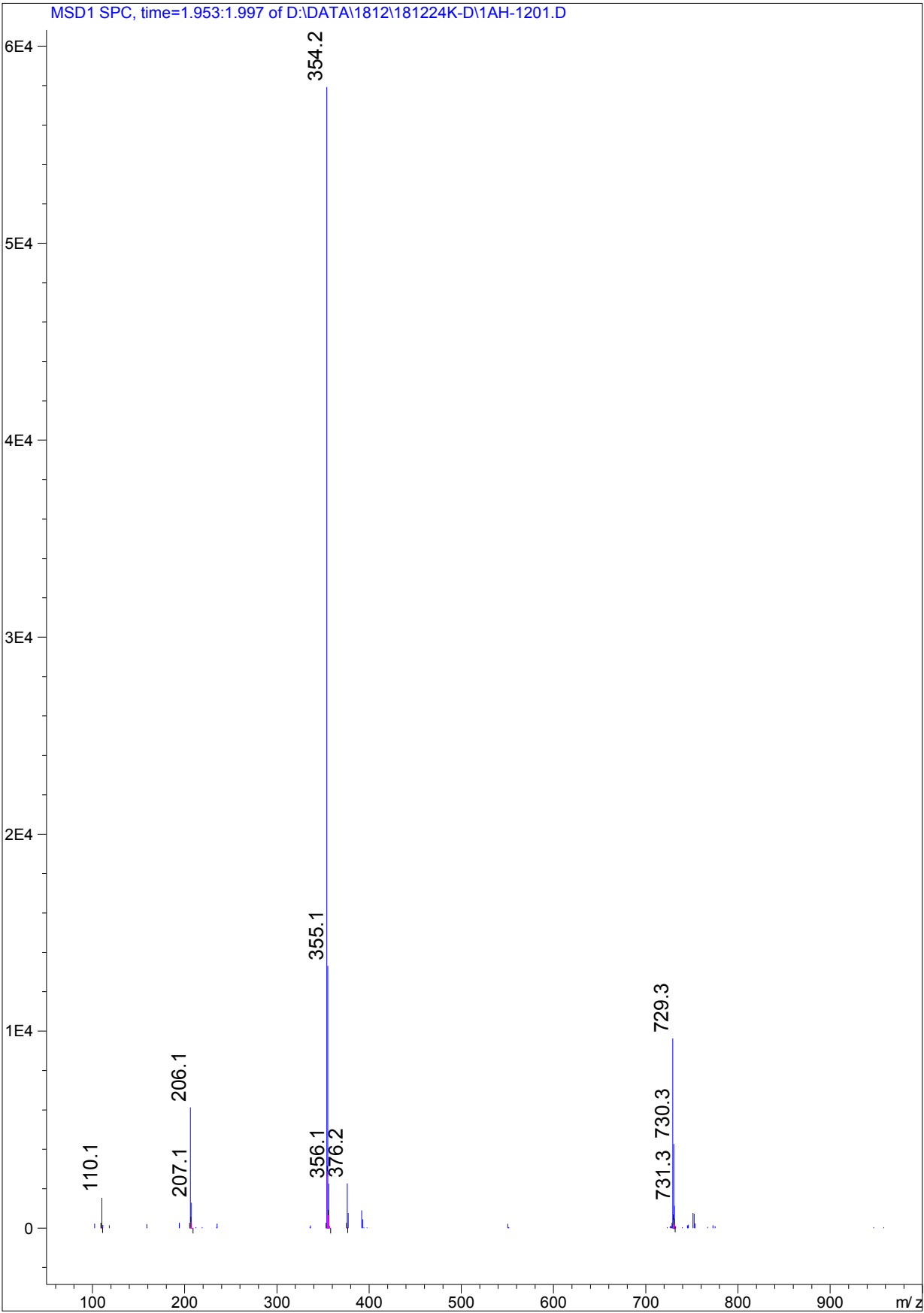
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	1.583	3.874	1.477	0.349	0.044	0.343
2	1.645	2.627	1.202	0.284	0.036	0.233
3	1.919	1121.484	421.157	99.368	0.045	99.424

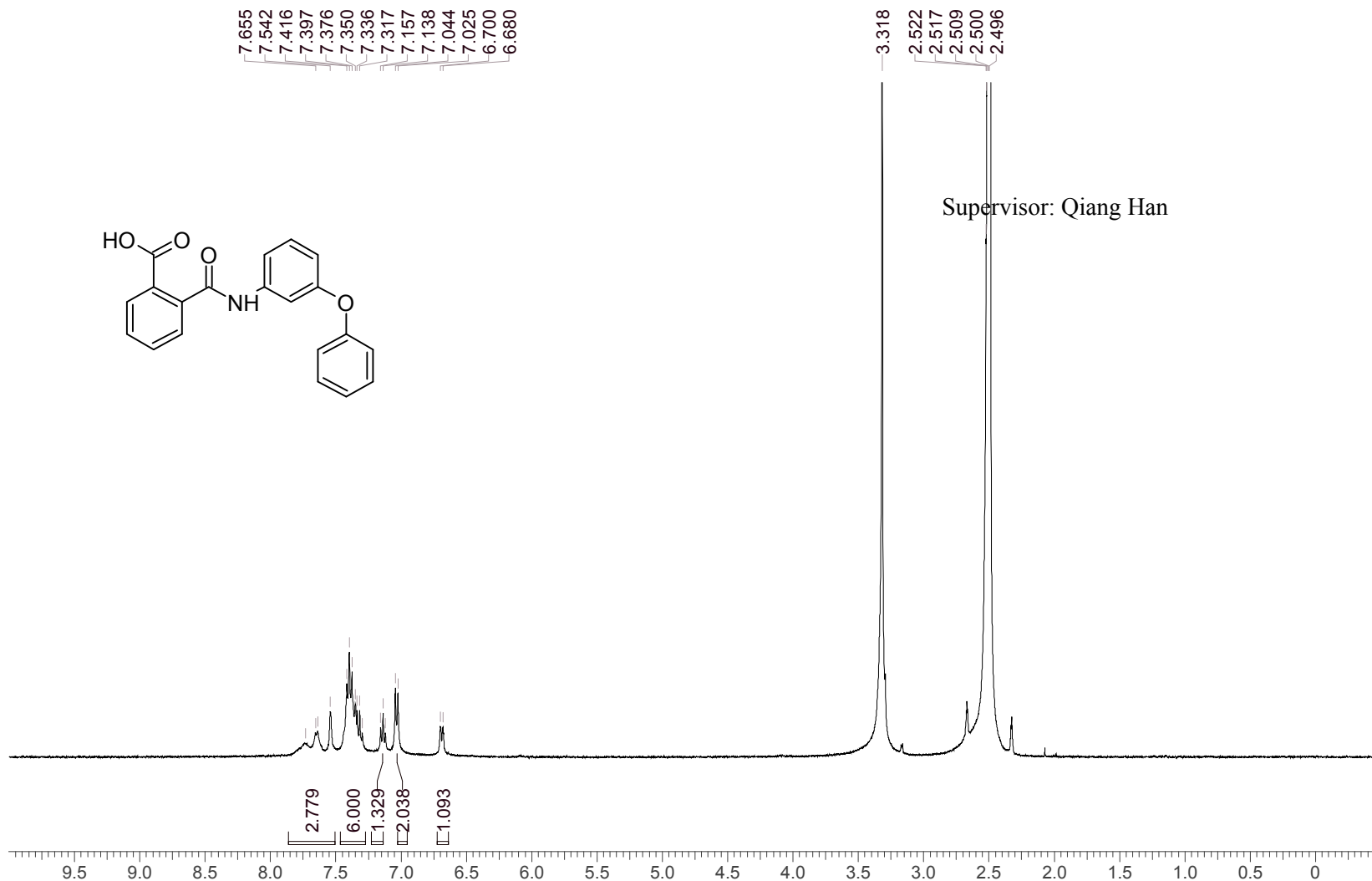
Operator: _____

Date: _____



Compound ID: BB0380

ET23679-182-P1AA DMSO Bruker_F_400MHz



Acquisition Time (sec)	2.0447
Comment	ET23679-1 82-P1AA DMSO Bruker_F_ 400MHz
Date	21 Dec 2018 10:42:55
Frequency (MHz)	400.1700
Nucleus	¹ H
Number of Transients	8
Origin	spect
Original Points Count	16384
Owner	nmr
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	145.41
SW(cyclical) (Hz)	8012.82
Solvent	DMSO-d6
Spectrum Offset (Hz)	2468.0483
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	25.148

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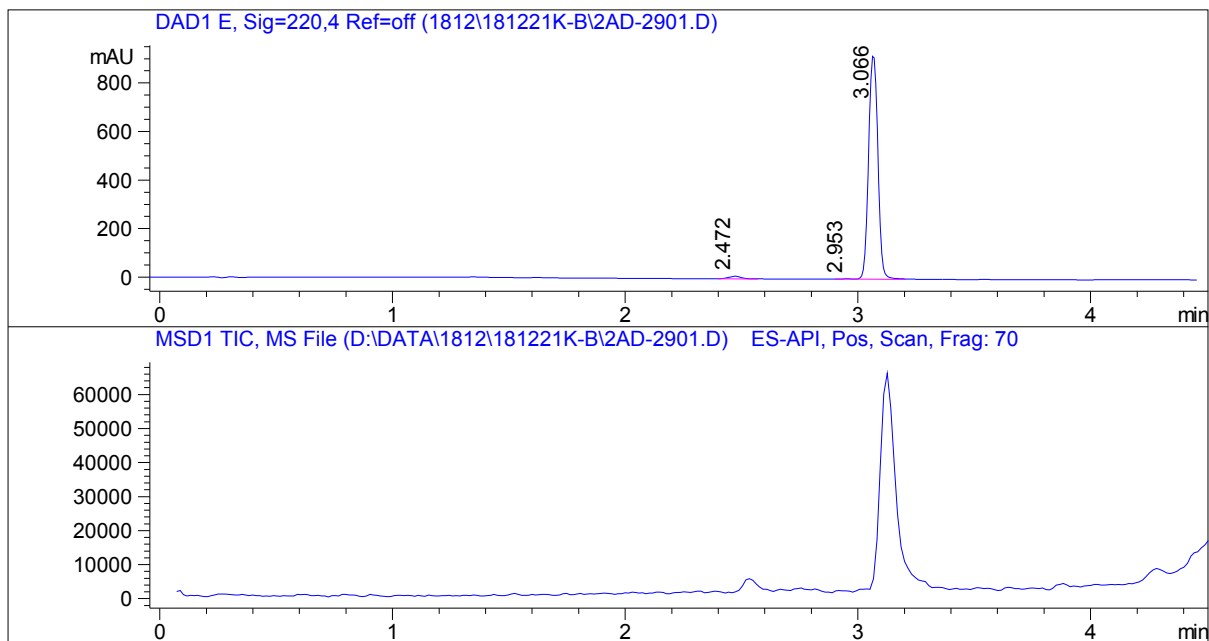
Operator:

Date:

LCMS REPORT

Compound ID : BB0380
Sample ID : ET23679-182-P1A1
Injection Date : 21. Dec. 2018
Inj. Vol. : 0.70 ul
Location : P2-A-04
Acq Method : D:\Data\1812\181221K-B\WUXIAB01_W.M
Data Filename : D:\DATA\1812\181221K-B\2AD-2901.D
Instrument : LCMS-K

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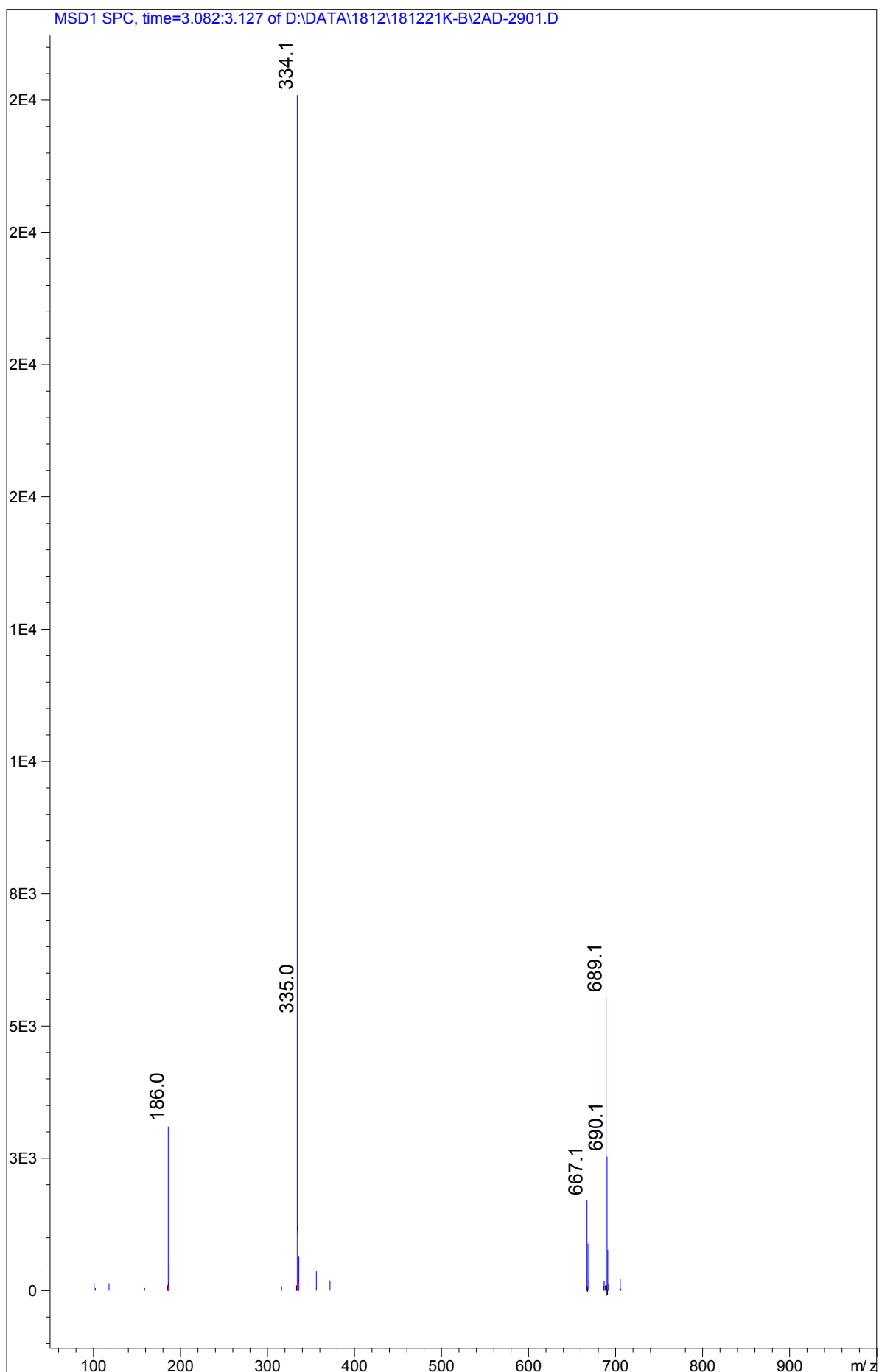
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	2.472	38.959	10.590	1.127	0.055	1.491
2	2.953	4.576	1.723	0.183	0.043	0.175
3	3.066	2569.784	927.758	98.690	0.044	98.334

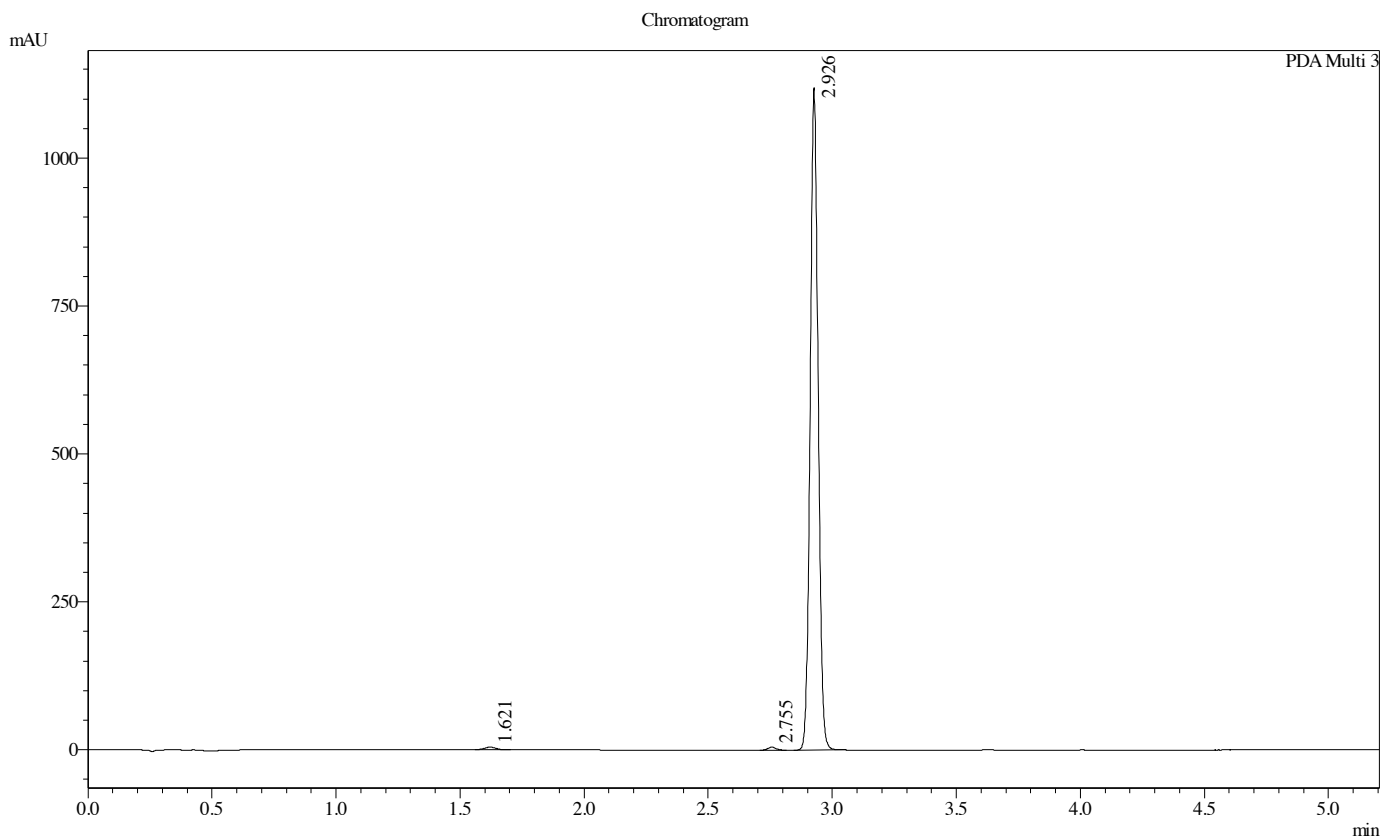
Operator: _____

Date: _____



HPLC REPORT

Compound ID :
Filename/Sample ID: ET23679-182-P1H
Injection Date : 12/18/2018 2:07:10 PM
Injection Vol : 0.5ul
Location : tray1 vial75
Acq Method : D:\method\10-80HPLC-AB.lcm
Org DataFile : D:\data\2018\1812\181218\ET23679-182-P1H.lcd
Instrument : HPLC-011



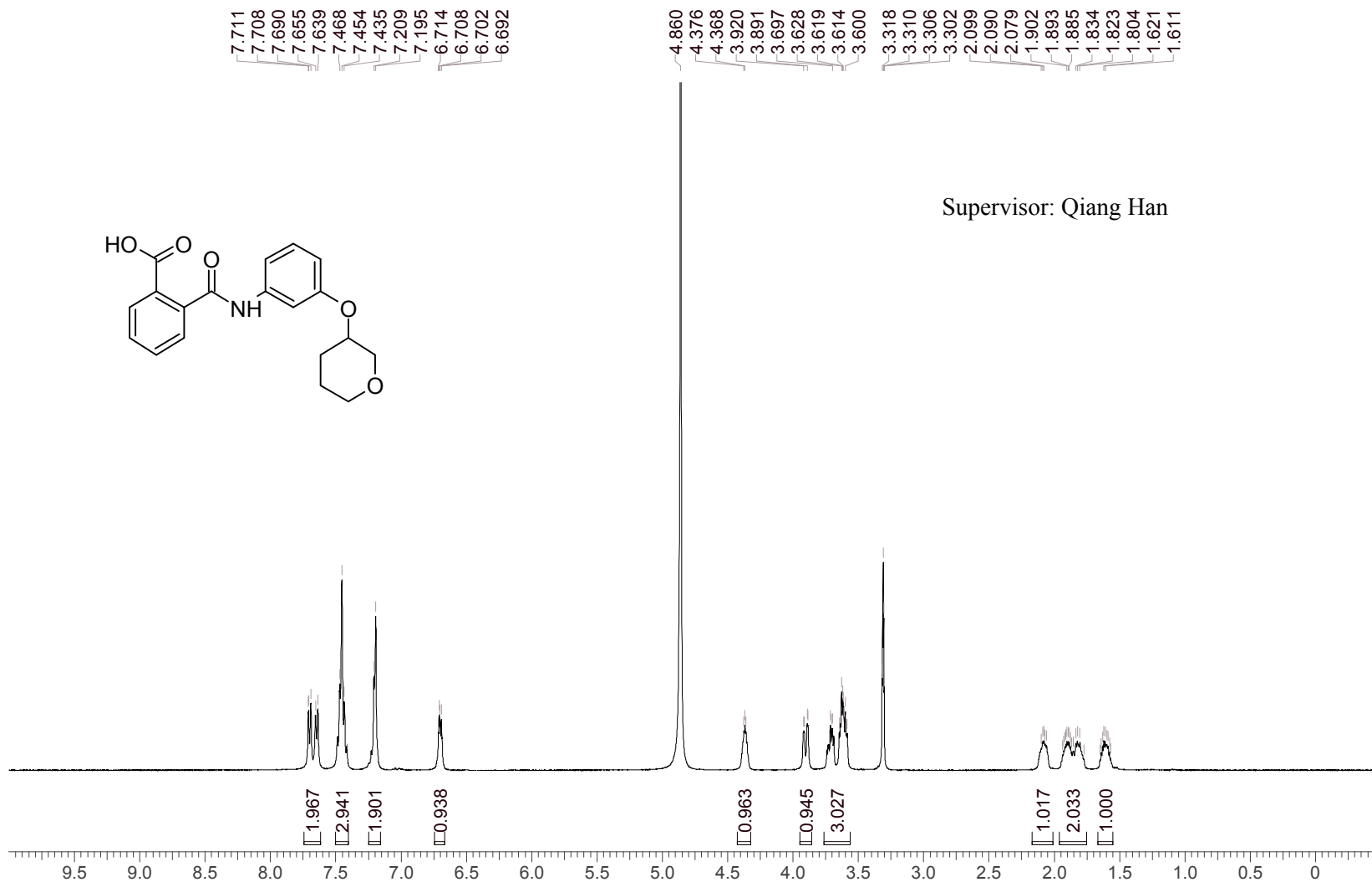
Integration Result

PDA Ch3 254nm 4nm

Peak#	Ret. Time	Height	Height %	USP Width	Area	Area %
1	1.621	4438	0.396	0.088	14680	0.579
2	2.755	5158	0.460	0.060	11621	0.459
3	2.926	1111903	99.144	0.060	2507622	98.962

Operator: _____

Date: _____

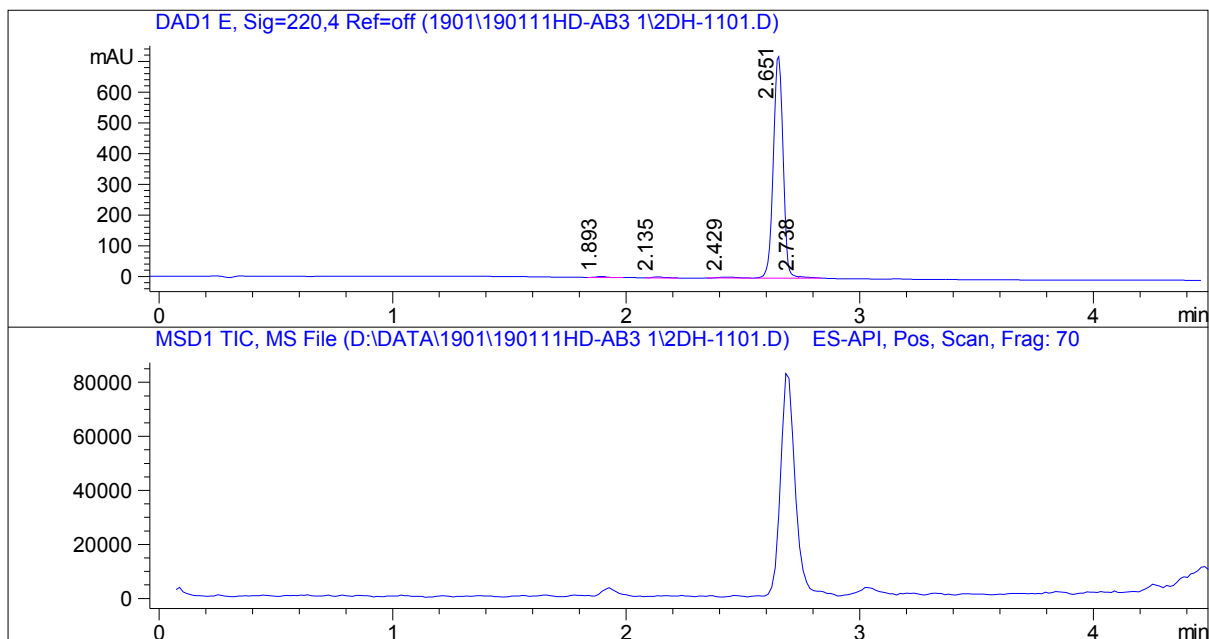


Acquisition Time (sec) 2.0447
Comment ET23679-2
10-P1BB
MeOD
Bruker_C_
400MHz
Date 11 Jan
2019
19:42:34
Frequency (MHz) 400.1500
Nucleus 1H
Number of Transients 8
Origin spect
Original Points Count 16384
Owner nmr
Points Count 65536
Pulse Sequence zg30
Receiver Gain 92.44
SW(cyclical) (Hz) 8012.82
Solvent METHAN
OL-d4
Spectrum Offset (Hz) 2463.3228
Spectrum Type standard
Sweep Width (Hz) 8012.70
Temperature (degree C) 26.863

LCMS REPORT

Compound ID : BB0381
Sample ID : ET23679-210-P1A1
Injection Date : 11. Jan. 2019
Inj. Vol. : 0.70 ul
Location : P2-D-08
Acq Method : D:\Data\1901\190111HD-AB3 1\WUXIAB01_W.M
Data Filename : D:\DATA\1901\190111HD-AB3 1\2DH-1101.D
Instrument : H

->



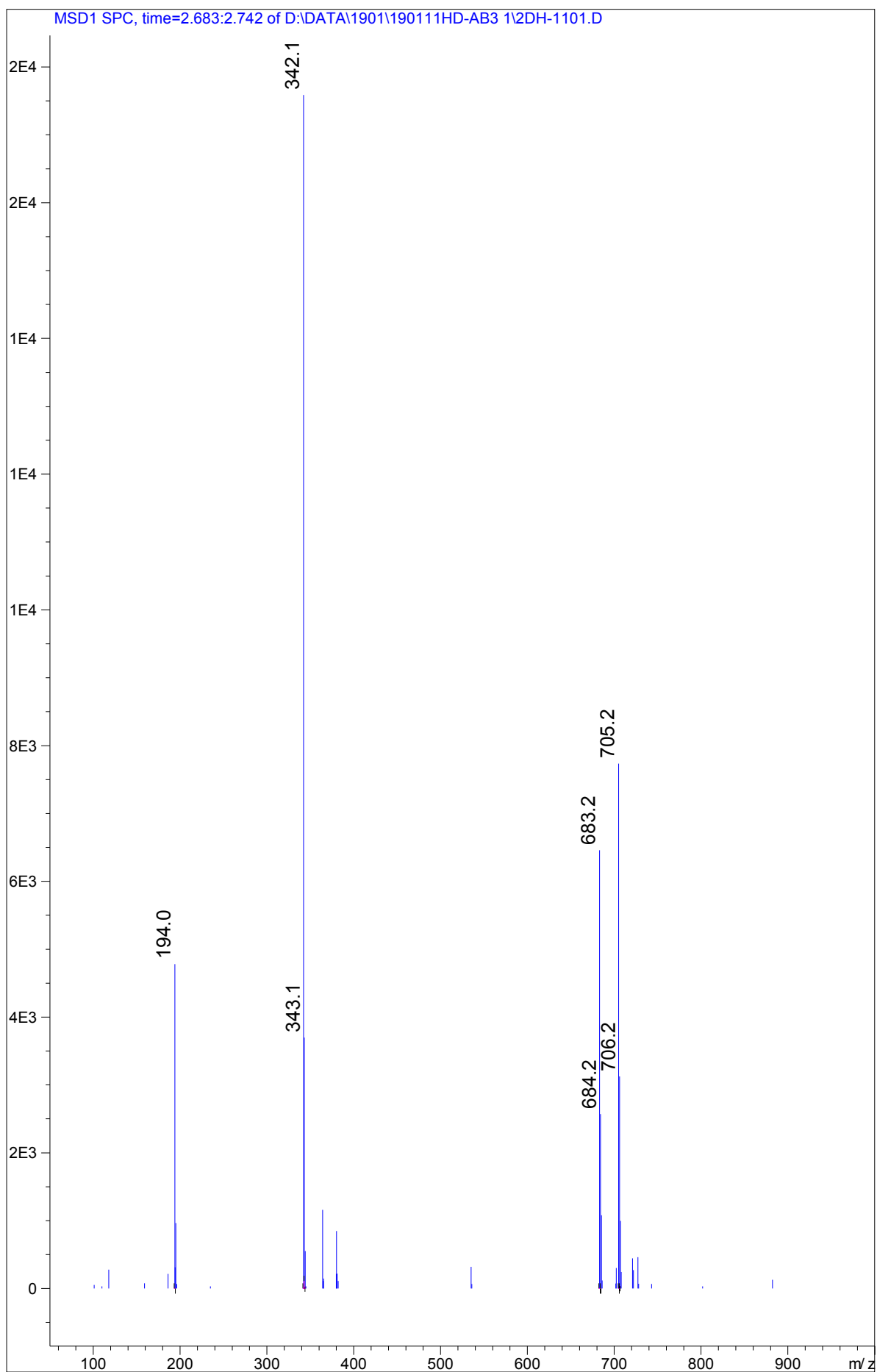
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	1.893	9.983	3.318	0.446	0.049	0.465
2	2.135	11.790	3.381	0.455	0.053	0.549
3	2.429	15.269	3.009	0.405	0.085	0.711
4	2.651	2094.813	728.689	97.999	0.048	97.609
5	2.738	14.271	5.168	0.695	0.046	0.665

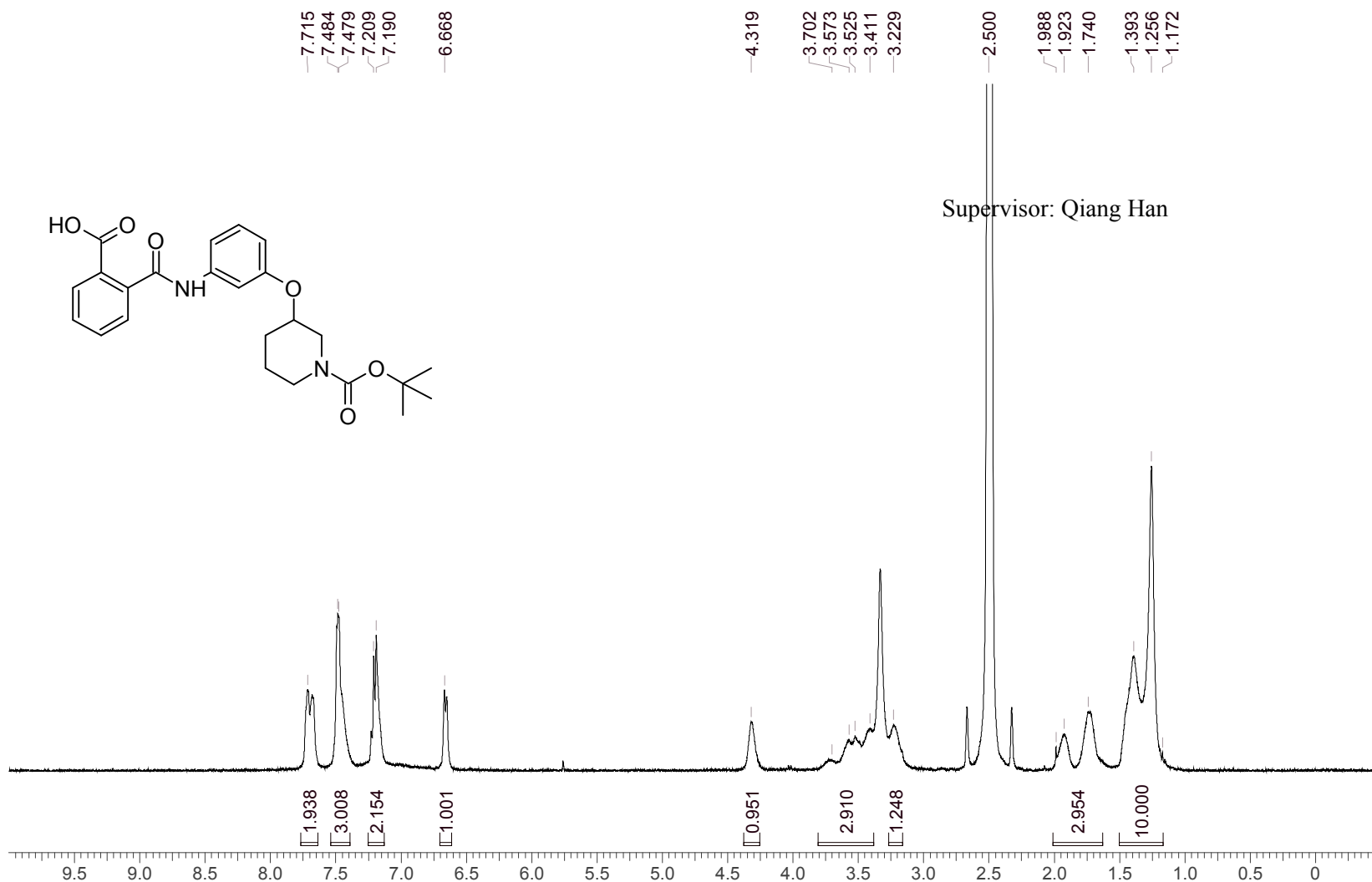
Operator: _____

Date: _____



Compound ID: BB0382

ET23679-178-P1AA DMSO Bruker_C_400MHz



Acquisition Time (sec) 2.0447
 Comment ET23679-1
 78-P1AA
 DMSO
 Bruker_C_
 400MHz
 Date 24 Dec
 2018
 11:16:47
 Frequency (MHz) 400.1500
 Nucleus 1H
 Number of Transients 8
 Origin spect
 Original Points Count 16384
 Owner nmr
 Points Count 65536
 Pulse Sequence zg30
 Receiver Gain 92.44
 SW(cyclical) (Hz) 8012.82
 Solvent DMSO-d6
 Spectrum Offset (Hz) 2468.0044
 Spectrum Type standard
 Sweep Width (Hz) 8012.70
 Temperature (degree C) 21.989

Supervisor: Qiang Han

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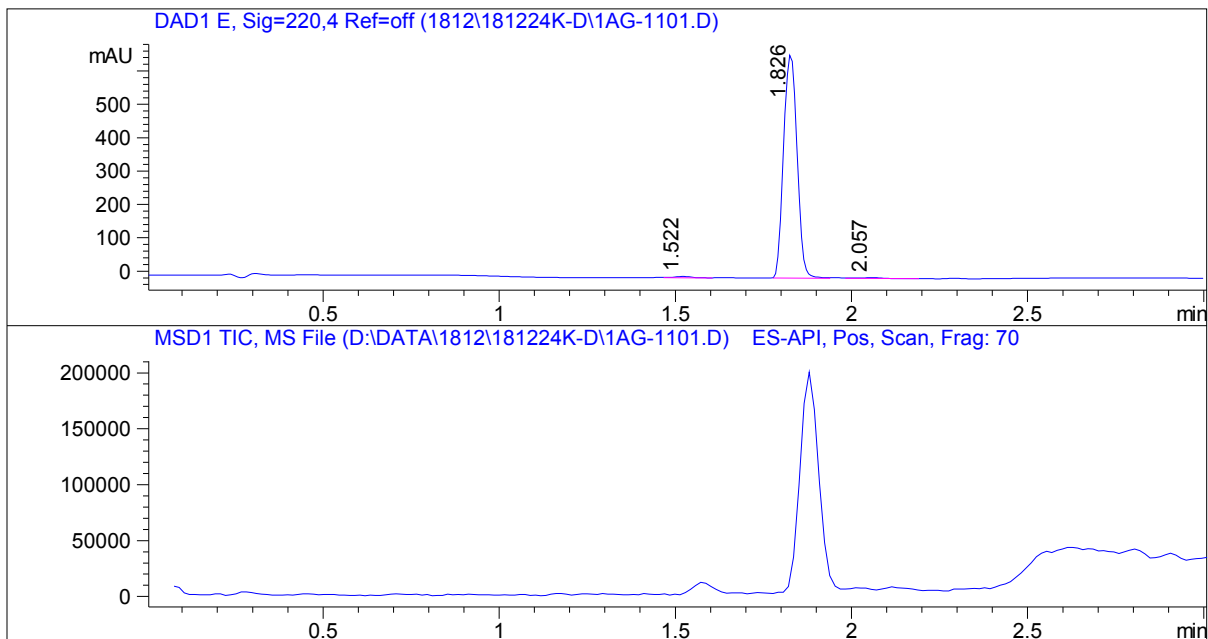
Operator:

Date:

LCMS REPORT

Compound ID : BB0382
Sample ID : ET23679-178-P1A1
Injection Date : 24. Dec. 2018
Inj. Vol. : 0.7 ul
Location : P1-A-07
Acq Method : D:\DATA\1812\181224K-D\DELIVER-K.M
Data Filename : D:\DATA\1812\181224K-D\1AG-1101.D
Instrument : K

->



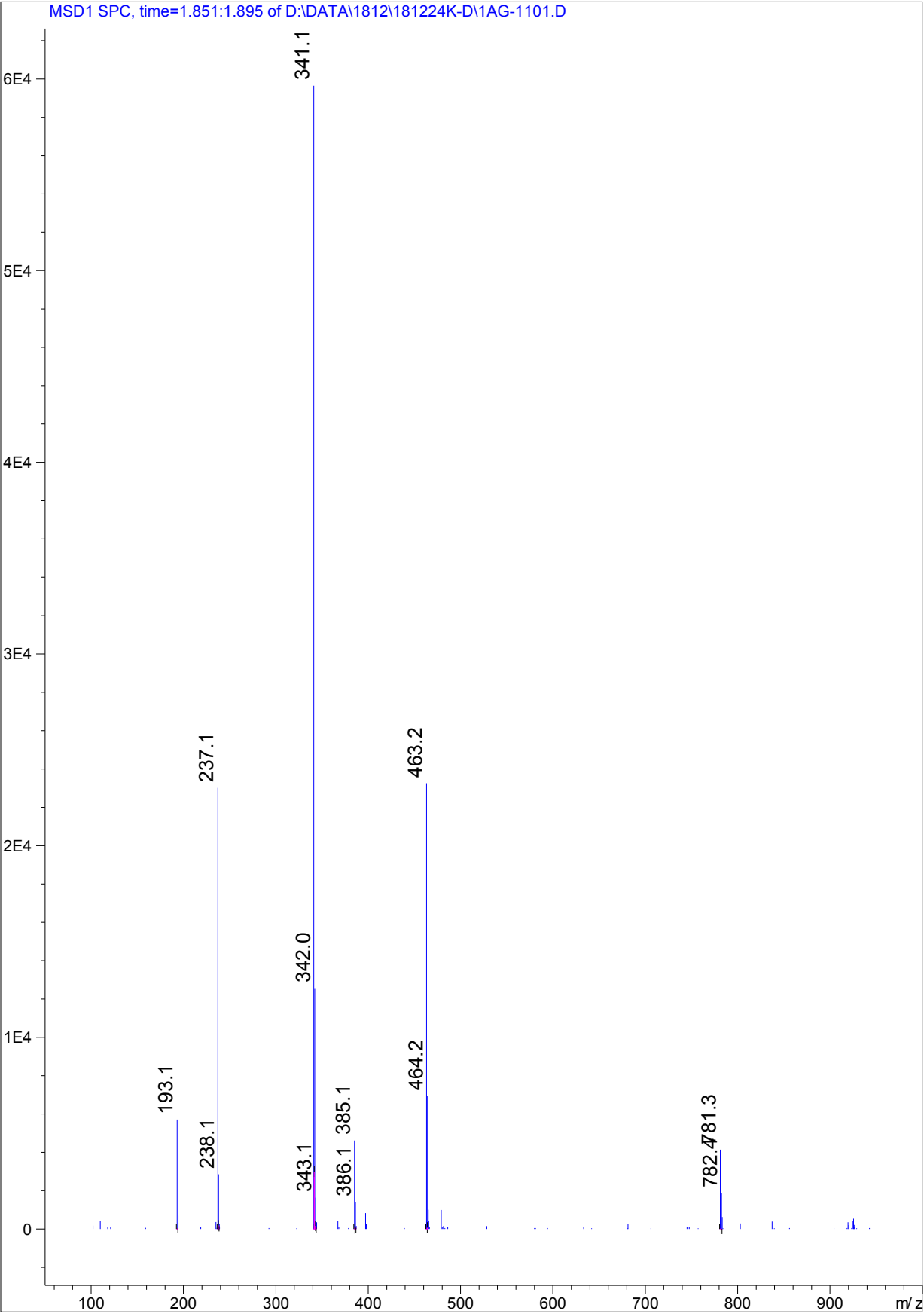
Integration Result

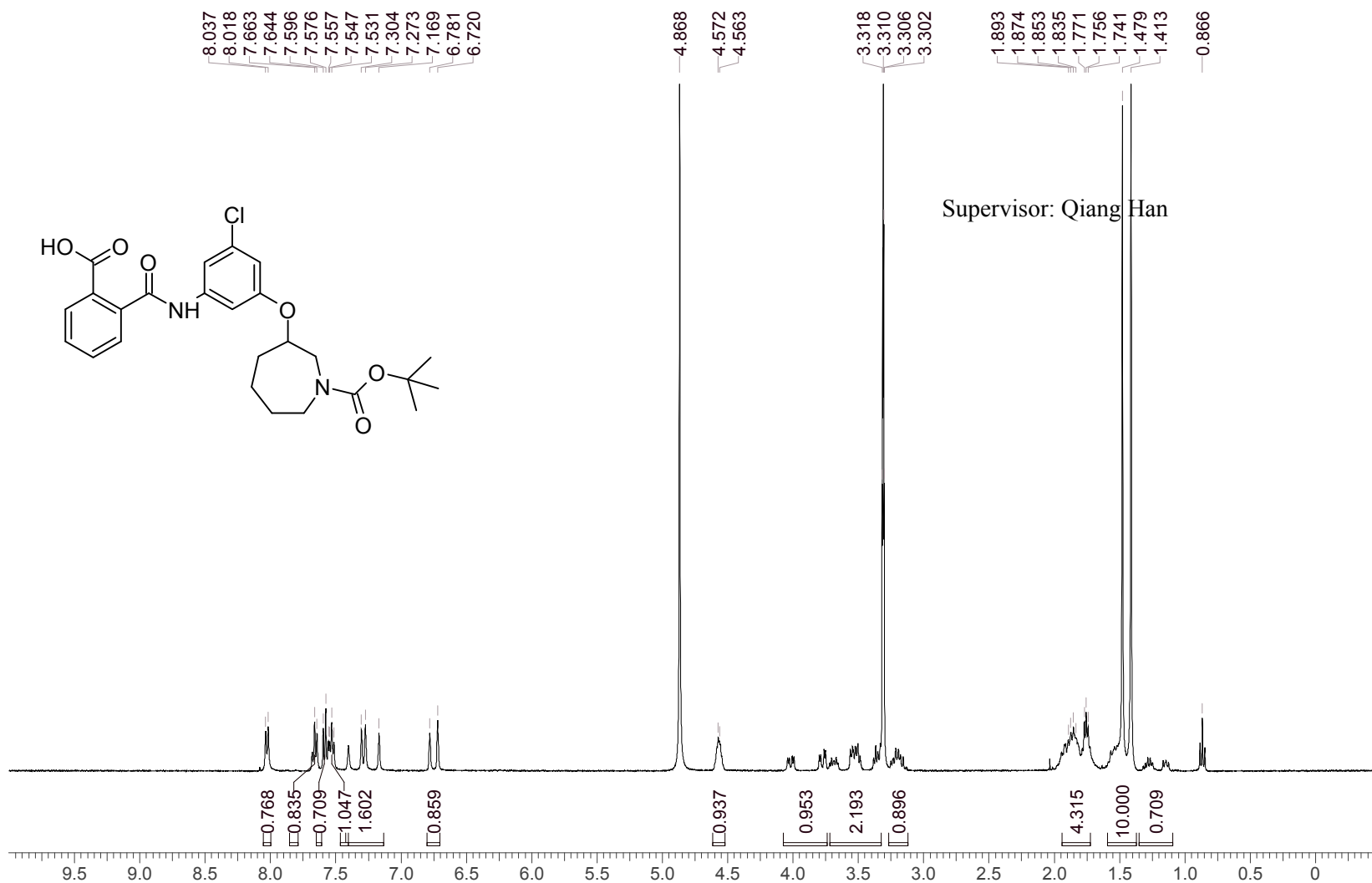
Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	1.522	12.753	3.881	0.574	0.050	0.708
2	1.826	1777.642	669.969	99.049	0.043	98.644
3	2.057	11.692	2.550	0.377	0.065	0.649

Operator: _____

Date: _____



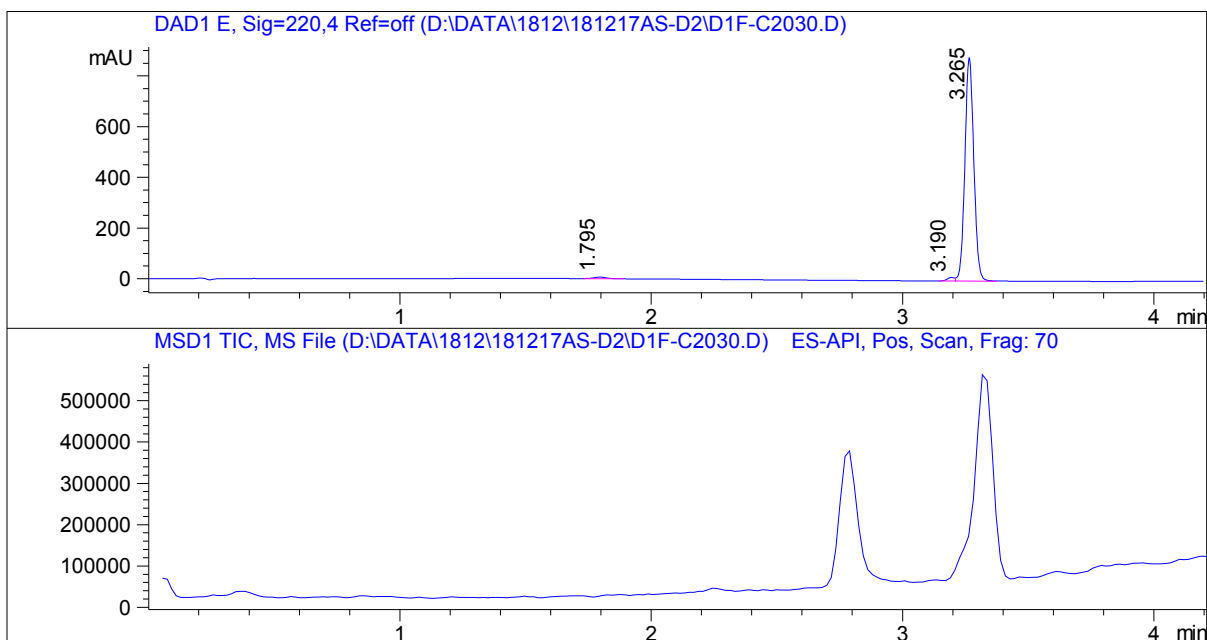


Acquisition Time (sec) 2.0486
Comment ET23679-1
65-P1N1
MeOD
Varian_S_
400MHz
Date Dec 18
2018
Frequency (MHz) 400.3374
Nucleus 1H
Number of Transients 8
Original Points Count 14802
Points Count 32768
Pulse Sequence s2pul
Receiver Gain 36.00
SW(cyclical) (Hz) 7225.43
Solvent METHAN
OL-d4
Spectrum Offset (Hz) 2805.0198
Spectrum Type standard
Sweep Width (Hz) 7225.21
Temperature (degree C) 25.000

Supervisor: Qiang Han

LCMS REPORT

Compound ID : BB0383
Sample ID : ET23679-165-P1K
Injection Date : 17. Dec. 2018
Inj. Vol. : 0.30 ul
Location : D1F-C2
Acq Method : D:\Data\1812\181217AS-D2\WUXIAB10.M
Data Filename : D:\DATA\1812\181217AS-D2\D1F-C2030.D
Instrument : AS



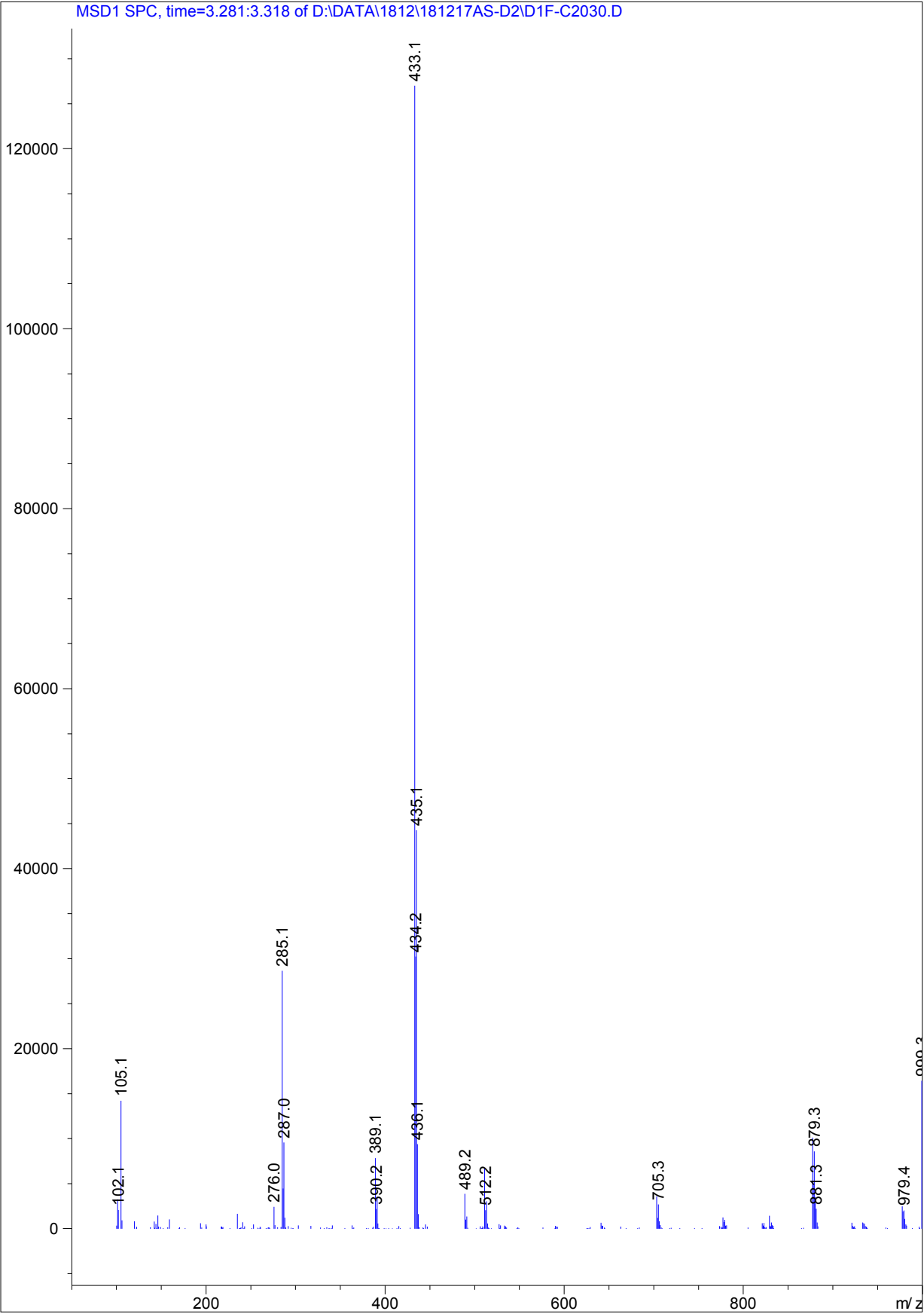
Integration Result

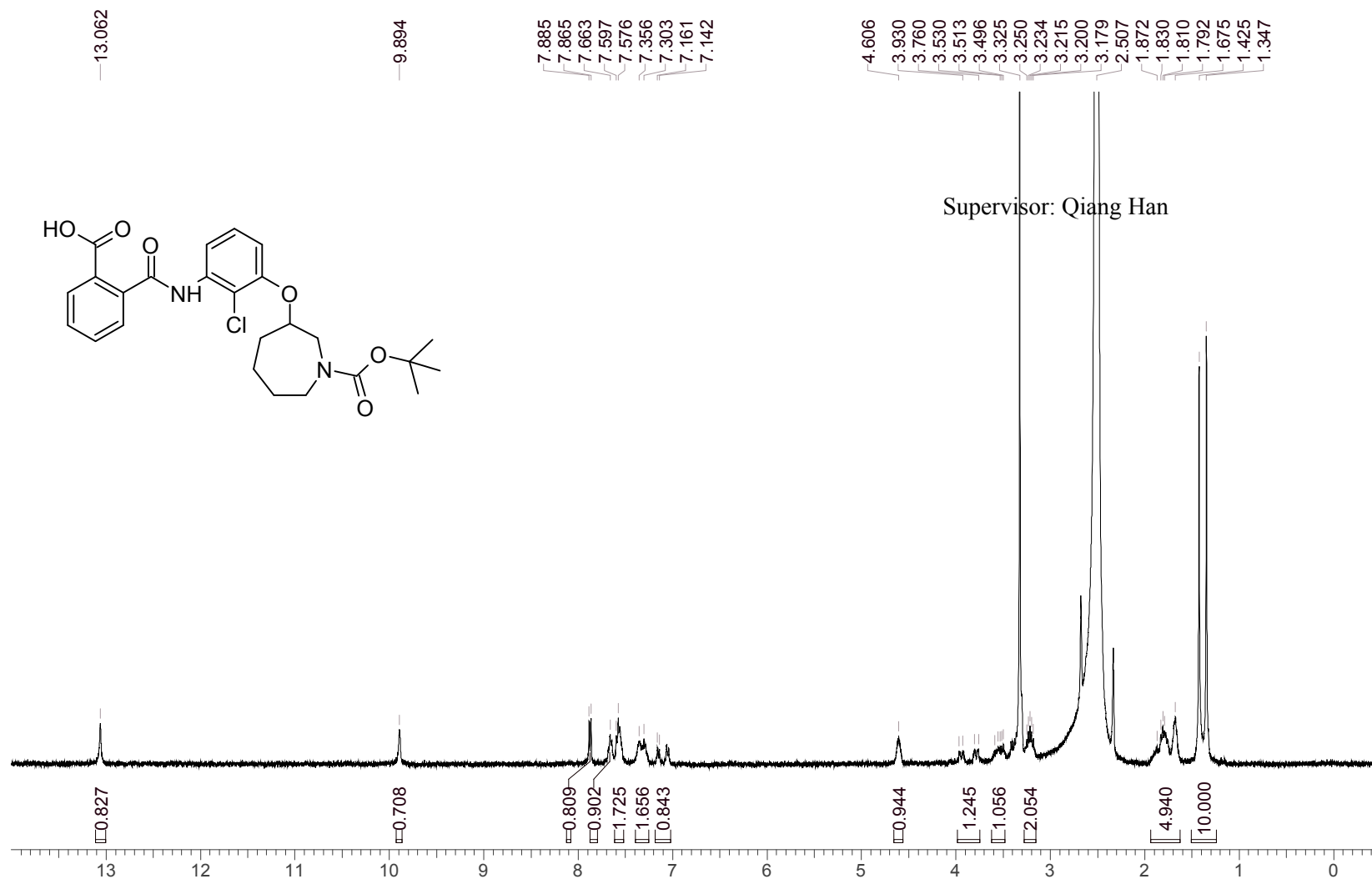
Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	1.795	22.699	6.250	0.693	0.056	1.019
2	3.190	27.746	13.169	1.460	0.032	1.246
3	3.265	2176.238	882.315	97.847	0.039	97.735

Operator: _____

Date: _____

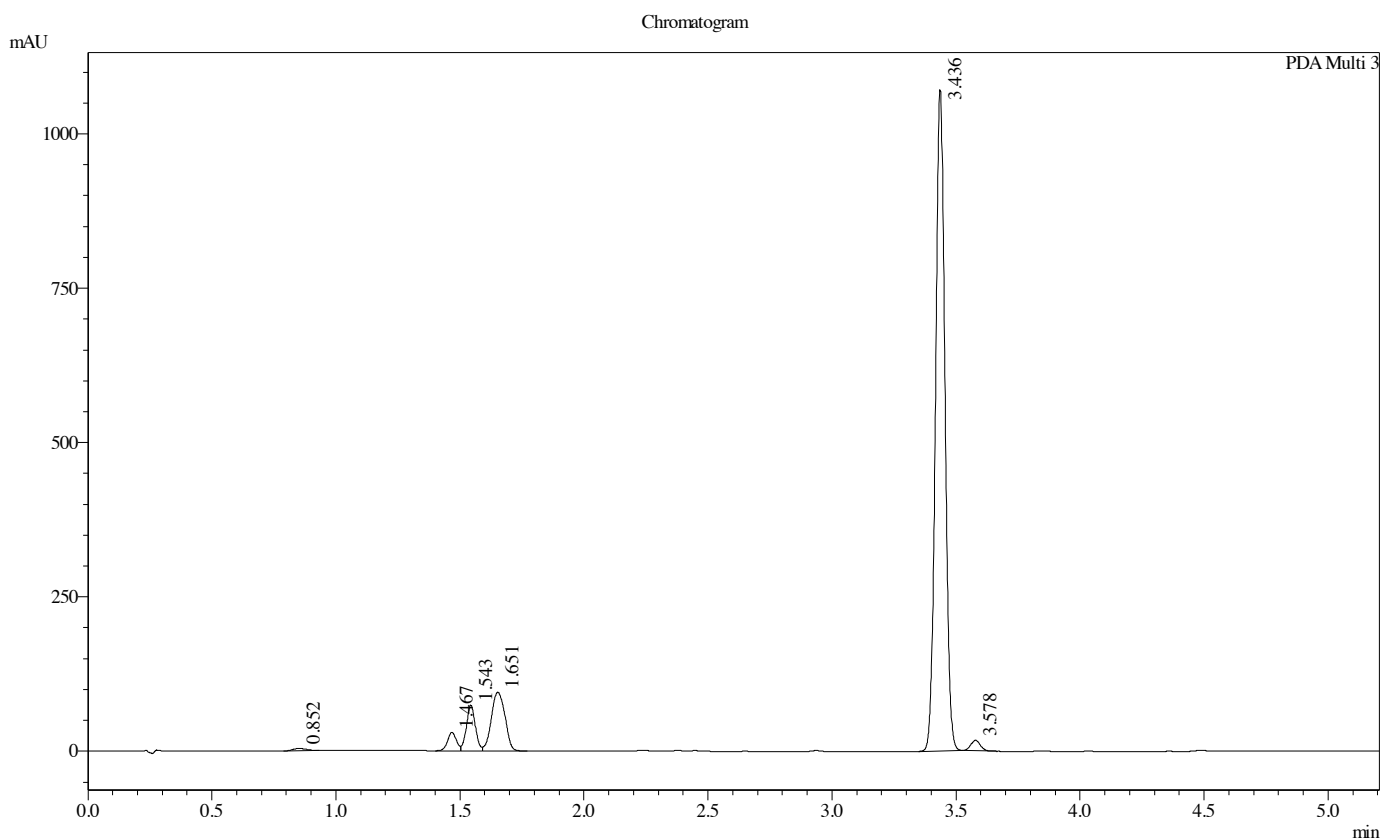




Acquisition Time (sec) 2.0447
Comment ET23679-1
66-P1D
DMSO
Bruker_F_
400MHz
Date 17 Dec
2018
10:57:10
Frequency (MHz) 400.1700
Nucleus 1H
Number of Transients 8
Origin spect
Original Points Count 16384
Owner nmr
Points Count 65536
Pulse Sequence zg30
Receiver Gain 145.41
SW(cyclical) (Hz) 8012.82
Solvent DMSO-d6
Spectrum Offset (Hz) 2471.0496
Spectrum Type standard
Sweep Width (Hz) 8012.70
Temperature (degree C) 24.256

HPLC REPORT

Compound ID :
Filename/Sample ID: ET23679-166-P1H
Injection Date : 12/14/2018 10:09:35 AM
Injection Vol : 1ul
Location : tray1 vial97
Acq Method : D:\method\10-80HPLC-AB.lcm
Org DataFile : D:\data\2018\1812\181214\ET23679-166-P1H.lcd
Instrument : HPLC-011



Integration Result

PDA Ch3 254nm 4nm

Peak#	Ret. Time	Height	Height %	USP Width	Area	Area %
1	0.852	3751	0.291	0.101	14090	0.400
2	1.467	29793	2.314	0.067	75508	2.144
3	1.543	73726	5.725	0.068	186240	5.289
4	1.651	95072	7.383	0.100	362046	10.281
5	3.436	1068552	82.982	0.071	2839084	80.620
6	3.578	16798	1.304	0.072	44582	1.266

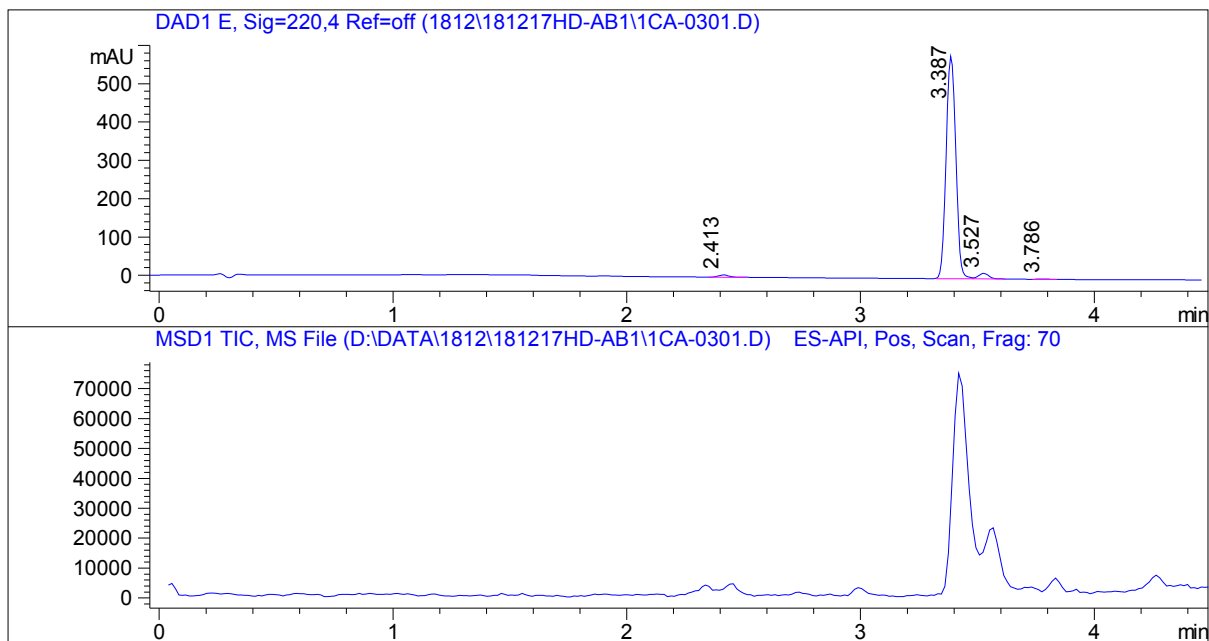
Operator: _____

Date: _____

LCMS REPORT

Compound ID : BB0384
Sample ID : ET23679-166-P1K
Injection Date : 17. Dec. 2018
Inj. Vol. : 0.70 ul
Location : P1-C-01
Acq Method : D:\Data\1812\181217HD-AB1\WUXIAB01_W.M
Data Filename : D:\DATA\1812\181217HD-AB1\1CA-0301.D
Instrument : H

->



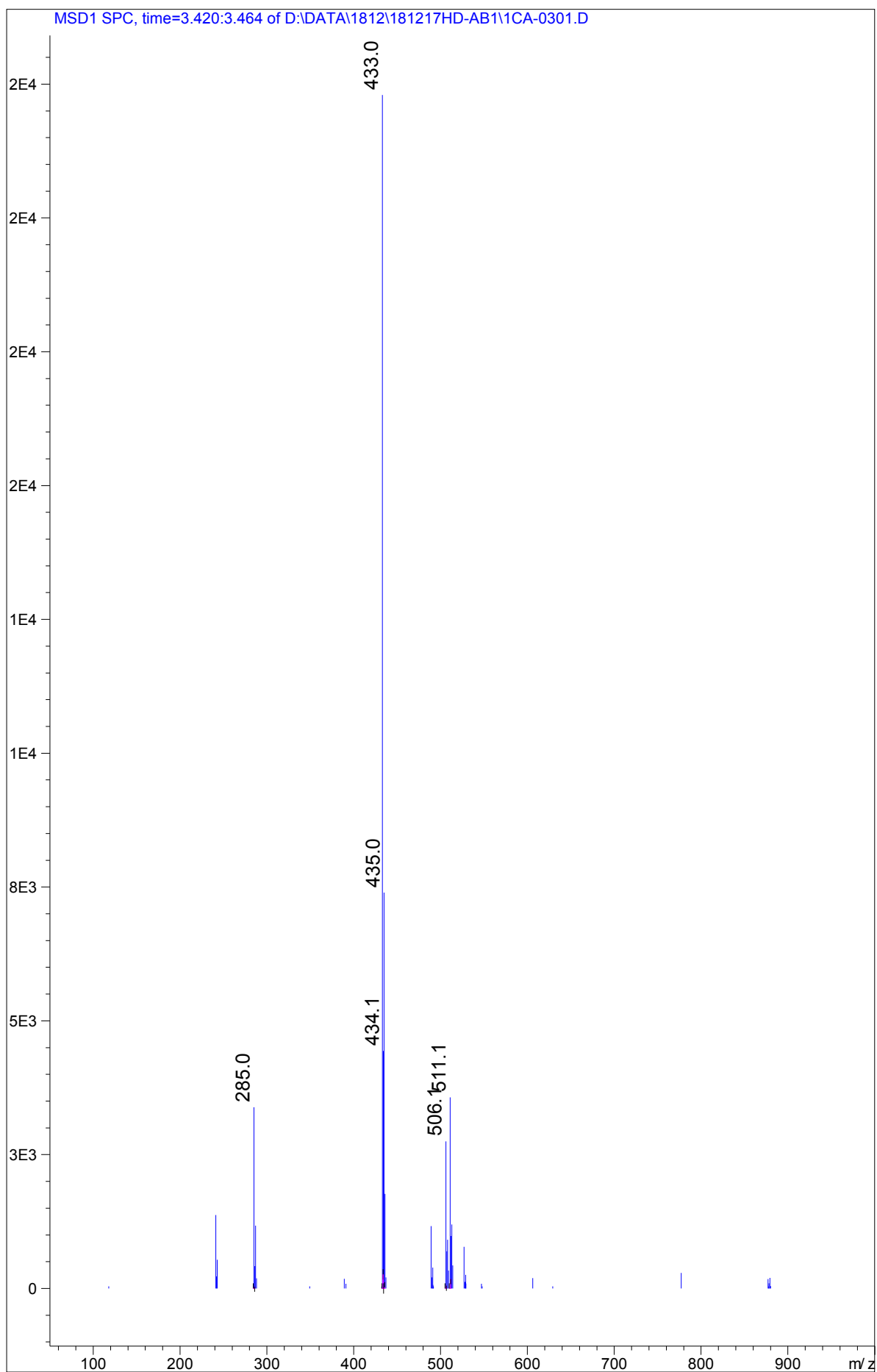
Integration Result

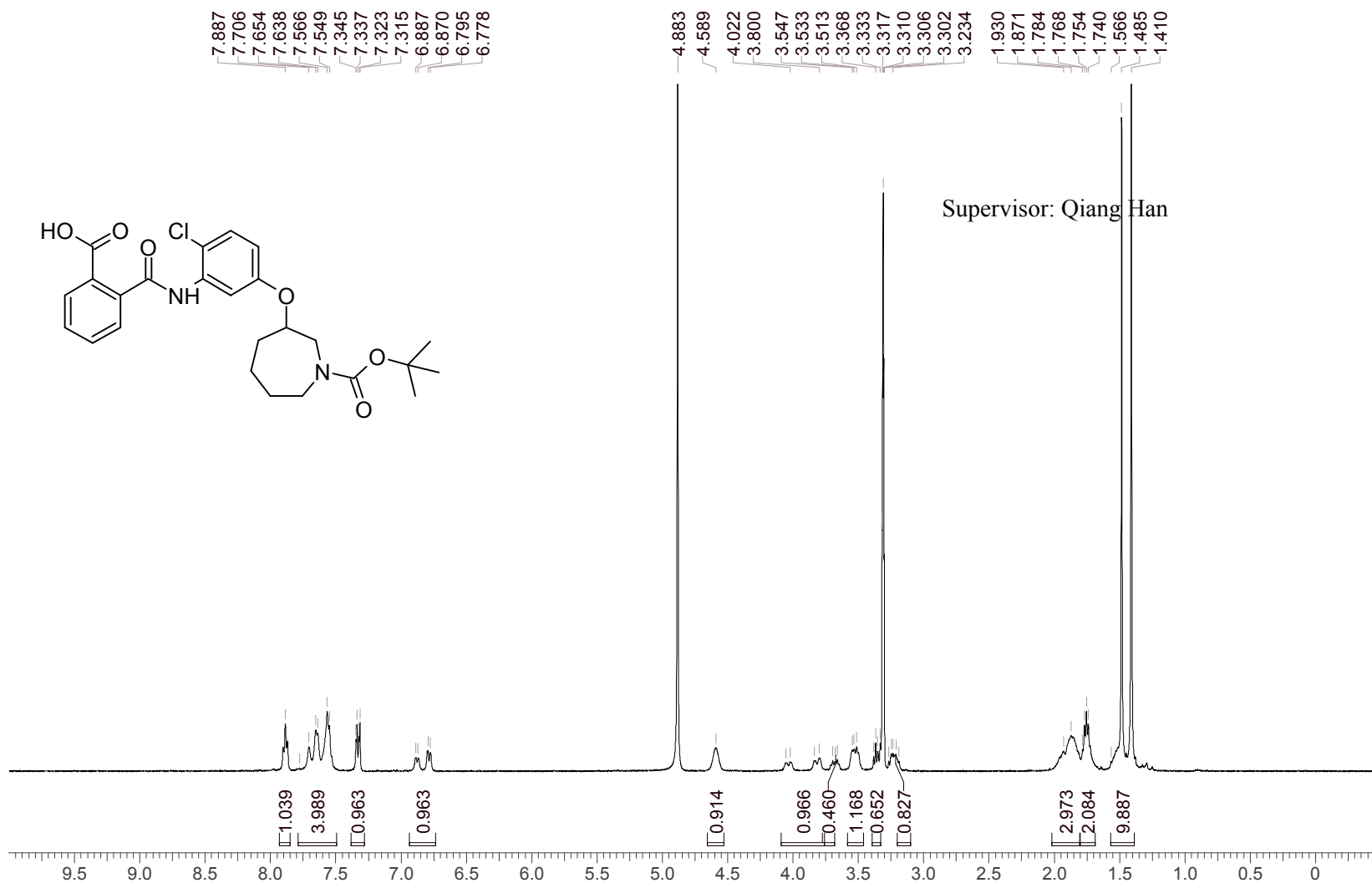
Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	2.413	18.843	5.888	0.978	0.051	1.083
2	3.387	1672.199	580.644	96.406	0.046	96.067
3	3.527	45.240	14.502	2.408	0.048	2.599
4	3.786	4.380	1.255	0.208	0.058	0.252

Operator: _____

Date: _____

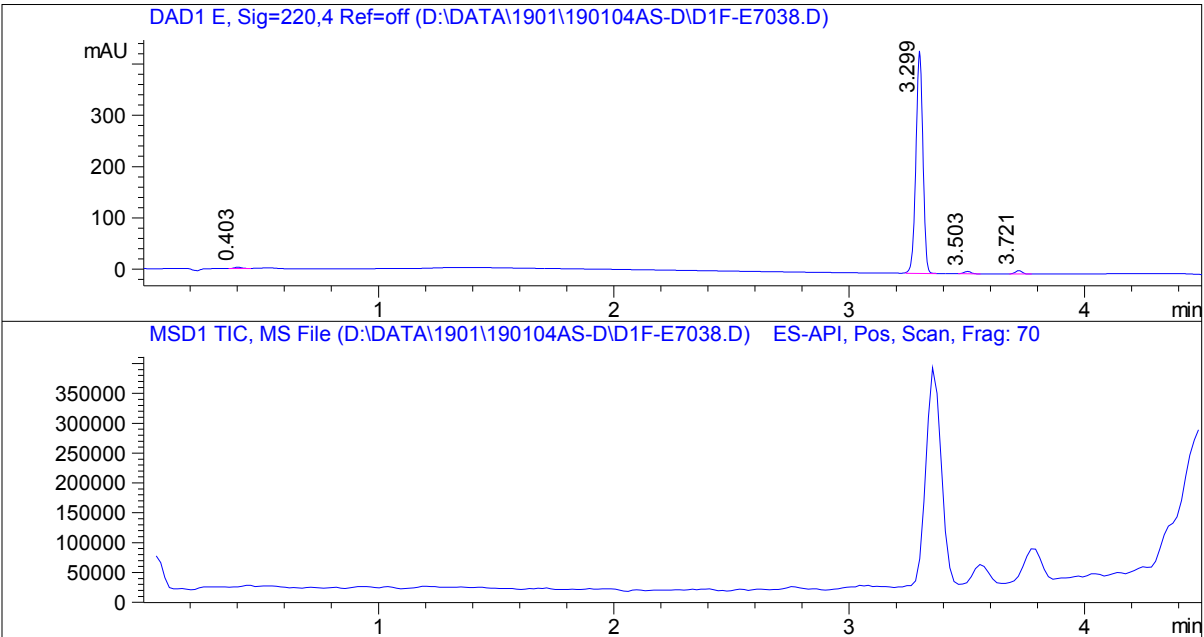




Acquisition Time (sec) 2.0447
Comment ET23679-1
99-P1CC
MeOD
Bruker_E_
400MHz
Date 08 Jan
2019
12:45:50
Frequency (MHz) 400.1300
Nucleus 1H
Number of Transients 8
Origin spect
Original Points Count 16384
Owner nmr
Points Count 65536
Pulse Sequence zg30
Receiver Gain 102.62
SW(cyclical) (Hz) 8012.82
Solvent METHAN
OL-d4
Spectrum Offset (Hz) 2463.6404
Spectrum Type standard
Sweep Width (Hz) 8012.70
Temperature (degree C) 22.542

LCMS REPORT

Compound ID : BB0385
Sample ID : ET23679-199-P1A1
Injection Date : 4. Jan. 2019
Inj. Vol. : 0.10 ul
Location : D1F-E7
Acq Method : D:\Data\1901\190104AS-D\WUXIAB10.M
Data Filename : D:\DATA\1901\190104AS-D\D1F-E7038.D
Instrument : AS



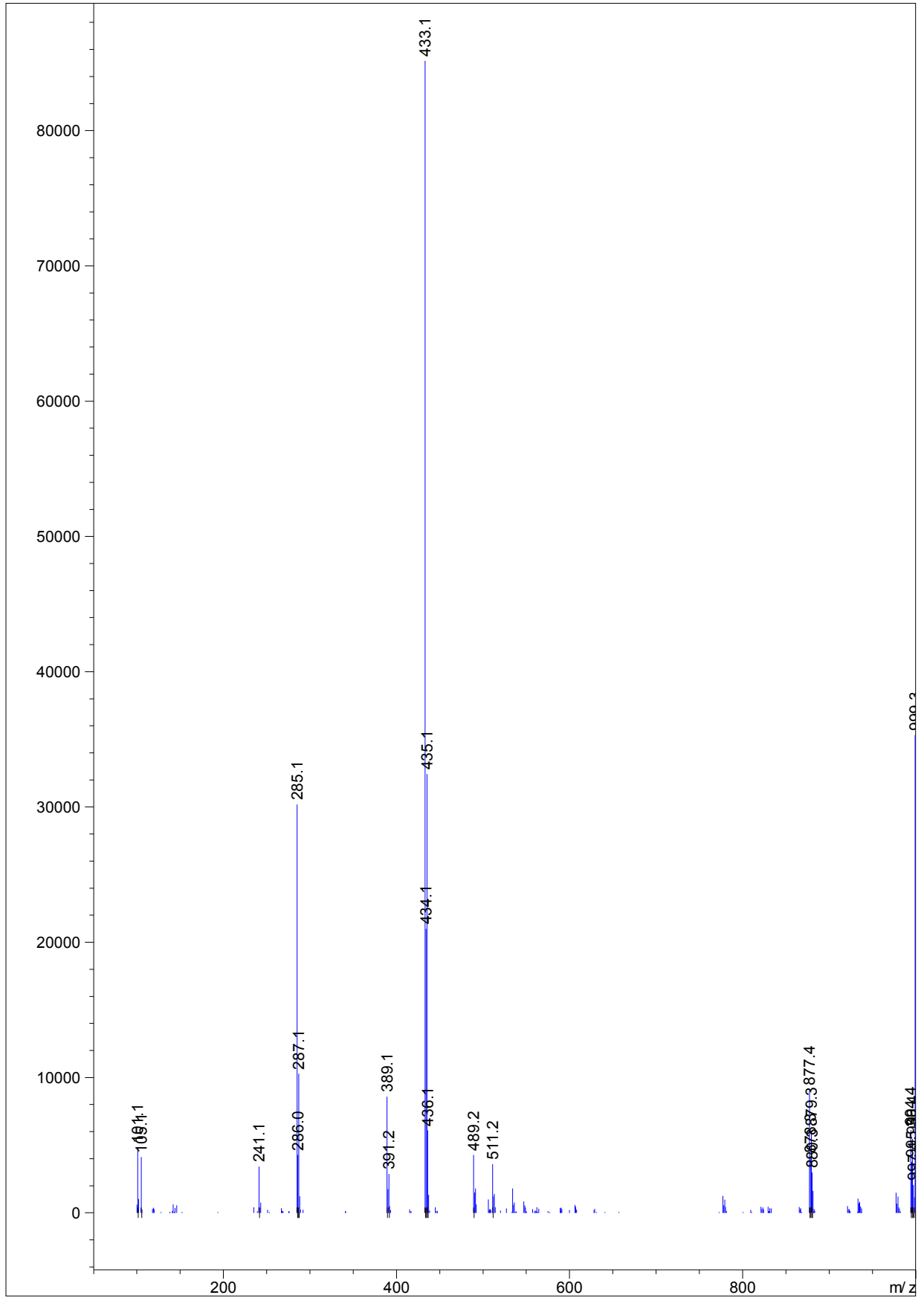
Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	0.403	5.323	2.640	0.590	0.032	0.578
2	3.299	893.315	433.489	96.896	0.033	96.953
3	3.503	9.257	4.556	1.018	0.032	1.005
4	3.721	13.495	6.690	1.495	0.032	1.465

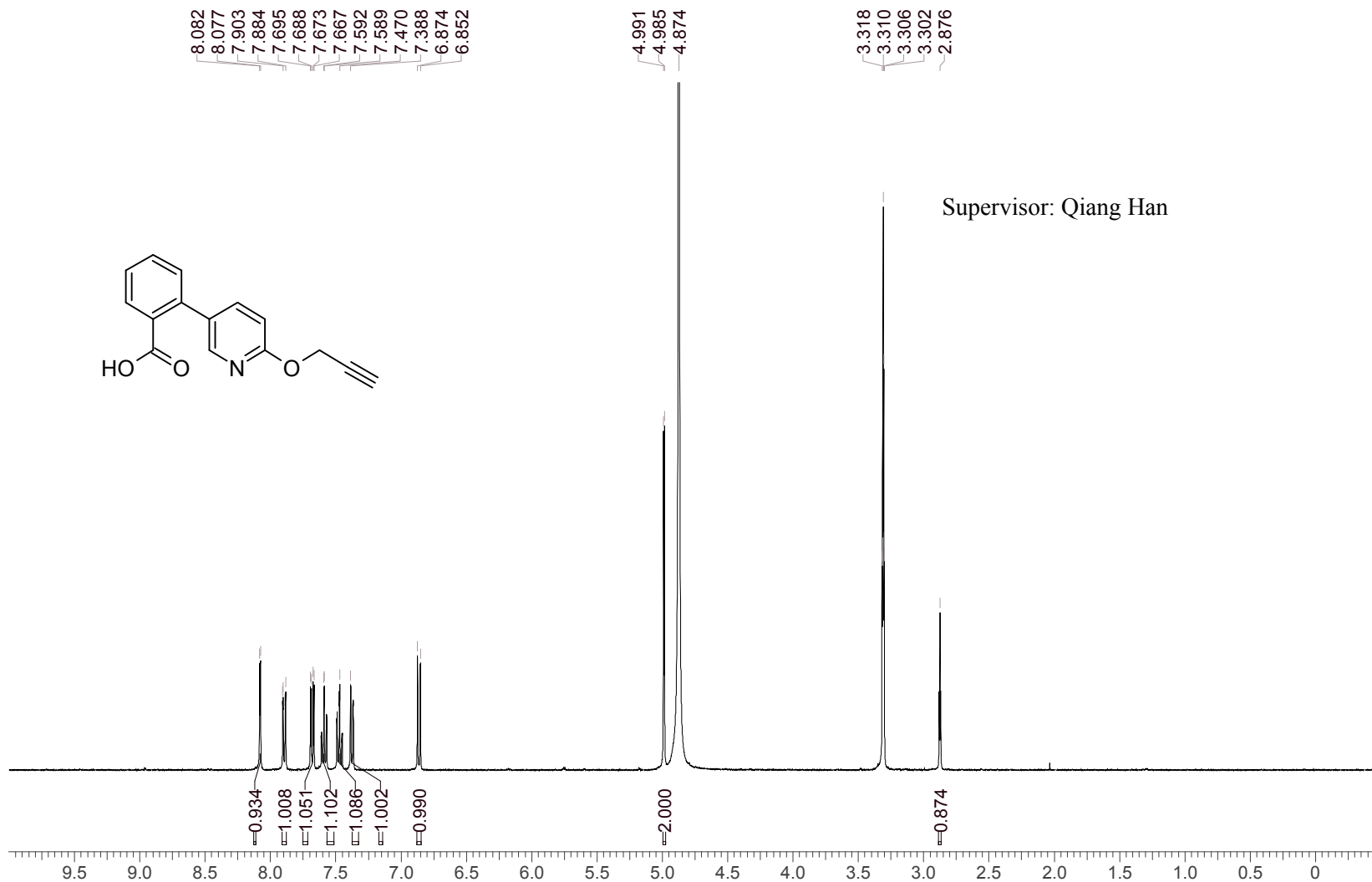
Operator:_____

Date:_____



Compound ID: BB0301

ET12347-572-P1AA MeOD Bruker_E_400MHz



Acquisition Time (sec)	2.0447
Comment	ET12347-5 72-P1AA MeOD Bruker_E_ 400MHz
Date	16 Oct 2017 11:10:58
Frequency (MHz)	400.1300
Nucleus	¹ H
Number of Transients	8
Origin	spect
Original Points Count	16384
Owner	nmr
Points Count	65536
Pulse Sequence	zg30
Receiver Gain	193.91
SW(cyclical) (Hz)	8012.82
Solvent	METHAN OL-d4
Spectrum Offset (Hz)	2463.2803
Spectrum Type	standard
Sweep Width (Hz)	8012.70
Temperature (degree C)	23.798

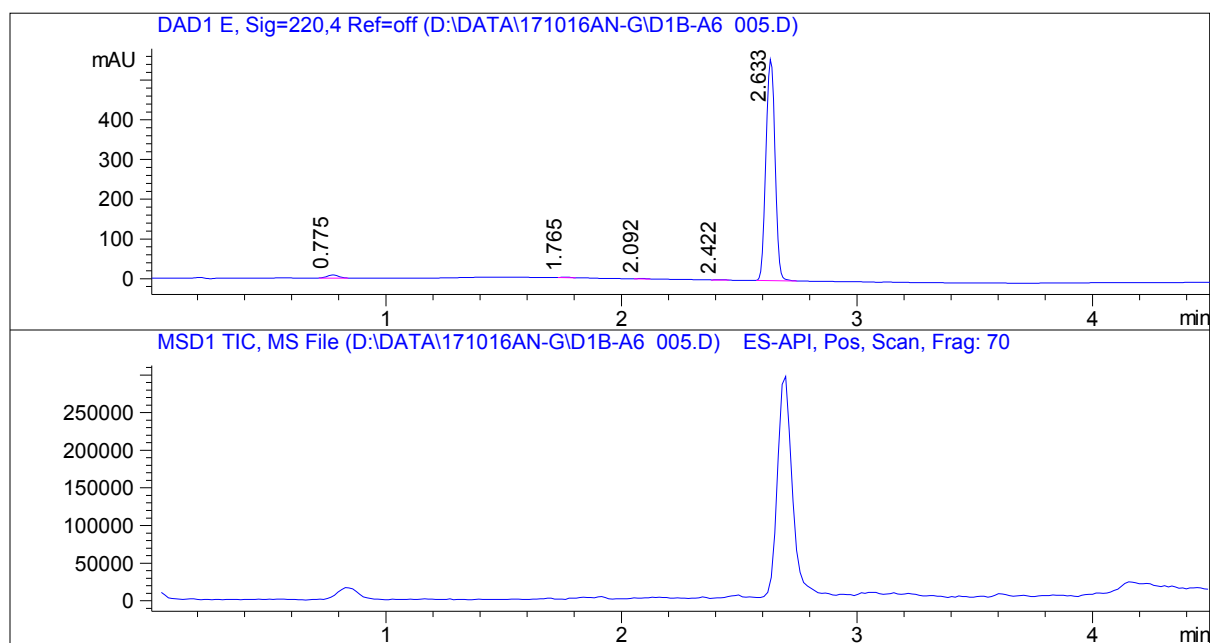
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Operator:

Date:

LCMS REPORT

Compound ID : BB0301
Sample ID : ET12347-572-P1A1
Injection Date : 16. Oct. 2017
Inj. Vol. : 0.20 ul
Location : D1B-A6
Acq Method : D:\Data\171016AN-G\WUXIAB10.M
Data Filename : D:\DATA\171016AN-G\D1B-A6 005.D
Instrument : AN



Integration Result

Signal 1 : DAD1 E, Sig=220,4 Ref=off

Peak #	RT [min]	Area	Height	Height %	Width [min]	Area %
1	0.775	26.252	7.766	1.363	0.056	1.700
2	1.765	4.368	1.736	0.305	0.042	0.283
3	2.092	3.719	1.294	0.227	0.048	0.241
4	2.422	3.332	1.195	0.210	0.046	0.216
5	2.633	1506.779	557.605	97.895	0.044	97.561

Operator: _____

Date: _____

