

Supporting Information for: Understanding Flavin-Dependent Halogenase Reactivity via Substrate Activity Profiling

Mary C. Andorfer,¹ Jonathan E. Grob,² Christine E. Hajdin,² Julia R. Chael,¹ Piro Siuti,² Jeremiah Lilly,²
Kian L. Tan,^{2,*} Jared C. Lewis^{1,*}

¹Department of Chemistry, University of Chicago, Chicago, IL 60637. ²Global Discovery Chemistry, Novartis Institutes for Biomedical Research, 250 Massachusetts Ave, Cambridge, MA 02139.

Corresponding Authors

*Department of Chemistry, University of Chicago, Chicago, IL 60637

E-mail: jaredlewis@uchicago.edu

*Global Discovery Chemistry, Novartis Institutes for Biomedical Research, 250 Massachusetts Ave, Cambridge, MA 02139

E-mail: kian.tan@novartis.com

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Materials:

All reagents were obtained from commercial suppliers and used without further purification. Deuterated solvents were obtained from Cambridge Isotope labs. Silicycle silica gel plates (250 mm, 60 F254) were used for analytical TLC, and preparative chromatography was performed using SiliCycle SiliaFlash silica gel (230-400 mesh). Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, Iowa), and the sequences of the primers used in this study are reported below. All PCR was done using New England Biolabs (Ipswich, Massachusetts) Q5 high fidelity 2x mix catalog #M0494. Vector pET303-Chis was purchased from Thermo Fisher/Invitrogen catalog #K630203 (Cambridge, Massachusetts). NEBuilder HiFi DNA Assembly Master Mix catalog #E2621L was purchased from NEB. Dh5 alpha cells catalog #C2987on and BL21DE3 cells catalog #C2527 were purchased from NEB. LB Carbenicillin 100 plates were purchased from Teknova (Hollister, California). Plasmids pET-28a/RebF and pET-28a/RebH in BL-21 DE3 *E. coli* were provided by the Walsh group of Harvard Medical School, Boston, MA.¹ The pLIC-MBP plasmid was provided by the Bottomley group of Monash University, Clayton, Australia.² The pGro7 plasmid encoding the groES and groEL chaperone set was purchased from Takara (Otsu, Shiga, Japan). The Thal gene was provided by Prof. Karl-Heinz van Pée.³ BL21(DE3) *E. coli* cells were purchased from Invitrogen (Carlsbad, CA). Luria broth (LB) and Terrific broth (TB) media were purchased from Research Products International (Mt. Prospect, IL). All FDH genes were confirmed by sequencing at the University of Chicago Comprehensive Cancer Center DNA Sequencing & Genotyping Facility (900 E. 57th Street, Room 1230H, Chicago, IL 60637). Electroporation was carried out on a Bio-Rad MicroPulser using method Ec2. Ni-nitrilotriacetic acid (Ni-NTA) resin and Pierce[®] BCA Protein Assay Kits were purchased from Fisher Scientific International, Inc. (Hampton, NH), and the manufacturer's instructions were followed when using both products. Amicon[®] 30 kD spin filters for centrifugal concentration were purchased from EMD Millipore (Billerica, MA) and used at 4,000 g at 4 °C. The glucose dehydrogenase (GDH-105), FAD, and NAD were purchased from Codexis (Redwood City, CA). Biotage reverse phase columns (SNAP-KP-C18-HS) were purchased from Biotage.

General Procedures:

Reactions were analyzed using an Agilent Technologies 1200 BGM LCMS or an Agilent Technologies 6130 LC-MS. Reverse phase preparative chromatography was carried out using a Biotage Isolera One or a Waters Autopure 2545 BGM preparative LCMS. ^1H spectra were recorded at either 400 or 500 MHz and ^{13}C NMR spectra were recorded at 126 MHz on a Bruker DMX-500 at the University of Chicago Mass Spectrometry Service Center, or were recorded at either 400 or 600 MHz on a Bruker cryoprobe at the Global Discovery Chemistry at Novartis Institutes for Biomedical Research. High-resolution mass spectra were obtained on an Agilent Technologies 6224 TOF LC-MS or a [Water LCT Premier](#). FDH protein concentrations were determined by Pierce BCA Protein Assay Kit.

Expression and purification of MBP-RebF and FDHs: For all bioconversions in these studies, large-scale cultures (750 mL) of MBP-RebF and RebH were grown, expressed and purified as previously reported⁴. RebH variants, Thal, Rdc2, and Gsf1 were grown, expressed and purified according to the procedure described for RebH. This procedure produced more than 35 mg/L of purified enzyme for all FDHs. Concentrations of enzymes were measured using the Pierce BCA Protein Assay Kit. Following purification, MBP-RebF and FDHs were buffer exchanged into HEPES buffer (25 mM, pH 7.4), concentrated to 7 mg/mL, and lyophilized.

LCMS Methods:

LCMS method 1: Agilent Technologies 1200 BGM LCMS. Waters Sunfire C18 3 x 30mm column, 3.5 μM particle size; solvent A: $\text{H}_2\text{O}/0.1\%$ formic acid, solvent B: acetonitrile with 0.1% formic acid; 0-0.3 min, B = 95%; 0.3-3.7 min, B = 95-5%; 3.7-4.5 min, B = 5%; 4.5-4.75 min, B = 5-95%; 4.75-5 min, B = 95%. Flow rate 2.1 mL/min. Absorbance at 254 nm was measured.

LCMS method 2: Agilent Technologies 6130 LC-MS. Agilent Eclipse Plus C18 4.6 x 150 mm column, 3.5 μM particle size; solvent A = $\text{H}_2\text{O}/0.1\%$ TFA, solvent B = CH_3CN ; 0-10 min, B = 15%; 10-17 min, B = 15-80%; 17-20 min, B = 80-100%; 20-24 min, B = 100%. Flow rate 1 mL/min. Absorbance at 280 nm and 254 nm was measured.

LCMS method 3a-d: Waters Autopure 2545 BGM preparative LCMS. Waters Sunfire C18 10 x 100mm column, 5 μ M particle size; solvent A: H₂O/0.1% formic acid, solvent B: acetonitrile with 0.1% formic acid. Flow rate 15 mL/min. Absorbance at 254 nm was measured. Fraction collection was triggered by mass.

3a. 0-0.35 min, B = 2%; 0.35-4.3 min, B = 2-20%; 4.3-5.3 min, B = 20%; 5.3-5.5 min, B = 20-95%; 5.5-6.1 min, B = 95%; 6.1-6.2 min, B = 95-2%; 6.2-7.5 min, B = 2%.

3b. 0-0.35 min, B = 2%; 0.35-4.3 min, B = 2-5%; 4.3-5.3 min, B = 5%; 5.3-5.5 min, B = 5-95%; 5.5-6.1 min, B = 95%; 6.1-6.2 min, B = 95-2%; 6.2-7.5 min, B = 2%.

3c. 0-0.35 min, B = 12%; 0.35-4.3 min, B = 12-30%; 4.3-5.3 min, B = 30%; 5.3-5.5 min, B = 30-95%; 5.5-6.1 min, B = 95%; 6.1-6.2 min, B = 95-12%; 6.2-7.5 min, B = 12%.

3d. 0-0.35 min, B = 22%; 0.35-4.3 min, B = 22-40%; 4.3-5.3 min, B = 40%; 5.3-5.5 min, B = 40-95%; 5.5-6.1 min, B = 95%; 6.1-6.2 min, B = 95-22%; 6.2-7.5 min, B = 22%.

Preparative RebH bioconversions: Preparative bioconversions were conducted similarly for all substrates tested. A solution of 2, 4 or 10 mg substrate (1 equiv., 1.0 mM final concentration) in 5% v/v methanol was added to a reaction vessel (beaker, 25 or 50 mL). Solutions in HEPES buffer (25 mM, pH 7.4) of NAD (0.1 equiv., 100 μ M final concentration), FAD (0.1 equiv., 100 μ M final concentration), NaCl (10 equiv., 10 mM final concentration), and a glucose dehydrogenase (9 U/mL final concentration GDH) were added. Solutions in HEPES/glycerol buffer (25 mM, pH 7.5, 10% glycerol v/v) of RebH (0.025-0.05 equiv., 25-50 μ M final concentration) and MBP-RebF (0.0025 equiv., 2.5 μ M final concentration) were added. The bioconversion was diluted with HEPES buffer (25 mM, pH 7.4) to the appropriate reaction volume and an aqueous solution of glucose (20 equiv., 20 mM final concentration) was added to initiate the cofactor regeneration cycle. The beaker was agitated on a stir plate at 25 °C until quenched.

Reactions were monitored by LCMS method 1 as described in the General Procedures and were quenched with one volume of dichloromethane upon completion. The reaction mixture was transferred to a 50 mL falcon tube and centrifuged to separate the aqueous and organic layers. The organic layer was collected and the aqueous layer was extracted twice more with dichloromethane. Organic layers were combined and concentrated. The crude material was then

AAACAACATTCCGTGGGCACAAGGGACCCCGCGCGAAAATCAACCGTTCTTCGAAG
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GsfI Sequence:

ATGGCGATTCCCCAGTCTTGCACGGTCCTGGTTGCCGGCGGGGGACCGGGCGGAAG
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TTGTCATCCTCGTTACCATATCGGGGAAAGCTTACTCCCGAGTATGCGTTATCTTCTG
CGGTTTCATTGATCTGGAAGATACCTTCGAGCAGCATGGTTTTTCAGAAAAGCTTGGC
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GGTGAATTGGAAGACTTTGAAAATTCTAACATCGATGGGTTCAAAGCCCGCCTGGA
AAAGGGAAGTCTCGGTCTCCGCCGTGAACGTGCGCTGTGCCGCGATCACGCAGGTG
ACTTACAGATGTAA

Phylogenetic Tree (Figure 1 in manuscript): The amino acid sequences of all 8 FDHs were obtained through sequencing at the University of Chicago Comprehensive Cancer Center DNA Sequencing & Genotyping Facility. These sequences were exported into Geneious and a tree was built using a global alignment and the cost matrix Blosum62. The genetic distance model was Jukes-Cantor and the tree build method was neighbor-joining. The sequence identities of RebH vs. Thal, and RebH vs. Rdc2 was calculated using an alignment of the amino acid sequences in protein-protein Blast (Blastp).

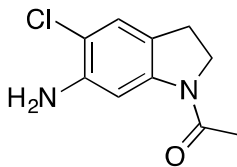
Bioconversions of Panel 1 and Panel 2: Bioconversions of substrates in Panel 1 and 2 were conducted in 96-well microtiter plates. Substrates were added to each well (3.75 μ L of a 10 mM stock in DMSO, 1 equiv., 0.5 mM final concentration). To each of these wells, a solution containing FAD (0.2 equiv., 100 μ M final concentration), NAD (0.2 equiv., 100 μ M final

concentration), NaCl (20 equiv., 10 mM final concentration), and glucose (40 equiv., 20 mM final concentration) was added. To initiate reaction, each FDH (0.05 equiv., 25 μ M final concentration), MBP-RebF (0.005 equiv., 2.5 μ M final concentration), and glucose dehydrogenase (9 U/mL final concentration) were added as solutions (25 mM HEPES, pH 7.4). The final reaction volume of all bioconversions was 75 μ L. Reaction plates were sealed using a plate sealer and were either agitated overnight at 650 rpm on a plate shaker (RebH, Thal, RebH variants) or left unshaken on the benchtop (Rdc2, Gsfl) at room temperature. These were quenched with 75 μ L of methanol, centrifuged to remove precipitated protein, filtered, and analyzed by LCMS Method 1, as described in the General Procedures.

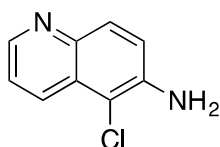
Analytical reactions with NCS and FDHs (Table 1 in manuscript): Analytical reactions with the chlorinating agent N-chlorosuccinimide (NCS) were conducted for several substrates in Panel 1 and 2. Substrate (5 mg, 1 equiv.) and NCS (1 equiv.) were added as solids to an eppendorf tube. Acetonitrile was added as solvent (500 μ L) and the reaction mixture was agitated at 650 rpm at 80°C. Bioconversions with these same substrates were conducted according to the procedure described in the section entitled *Bioconversions of Panel 1 and Panel 2*. Reactions with both NCS and FDHs were analyzed by LCMS Method 2 (substrates **23**, **38**, **40**, **41**, **43**, **44**, **45**, **46**) or GCMS (substrate **27**). Reported selectivity differences for all substrates in Table 1, with the exception of **37**, were determined by comparison of the areas of mono- or di-chlorinated product peaks at different retention times, either by LCMS or GCMS. The selectivity of **37** was determined through preparative reaction and isolation of the mono-chlorinated products from NCS and RebH.

Detailed isolation and characterizations - bioconversions:

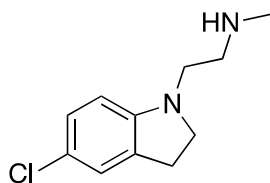
For each compound isolated, the ^1H NMR and HRMS of the chlorinated compound is shown. In some cases, impurity peaks were observed in the ^1H NMR of the chlorinated products, which is due to the small scale of these reactions and the use of formic acid during prep LC/MS purification. For this reason, the ^1H NMR of the starting material for each is included as well as a reference and yields are not reported (only % conversions based on LCMS UV traces).



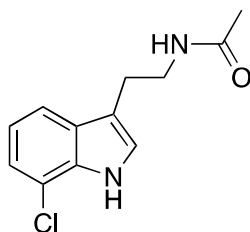
1-(6-Amino-5-chloro-2,3-dihydro-1H-indol-1-yl)ethanone (29a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude reaction mixture was concentrated onto Celite, packed into a Biotage samplet, and loaded into a reverse phase column (Biotage SNAP-KP-C18-HS). The crude material was purified by reverse phase chromatography to afford compound **29a** as the TFA salt. 1 H NMR (500 MHz; DMSO): δ 7.68 (s, 1H), 7.04 (s, 1H), 4.04 (t, J = 8.4, 2H), 2.98 (t, J = 8.3, 2H), 2.13 (s, 3H). HRMS (ESI-MS) calcd for $C_{10}H_{11}ClN_2O$ $[M + H]^+$: 211.0633 and 213.0604, found: 211.0604 and 213.0577.



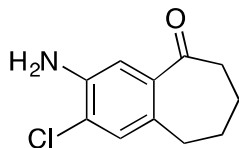
6-Amino-5-chloroquinoline (30a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3a) to afford compound **30a**. 1 H NMR (400 MHz; DMSO): δ 8.59 (dd, J = 4.2, 1.6, 1H), 8.22 (ddd, J = 8.5, 1.5, 0.8, 1H), 7.74 (d, J = 9.1, 1H), 7.48 (dd, J = 8.5, 4.2, 1H), 7.37 (d, J = 9.1, 1H), 5.99 (s, 2H). HRMS (ESI-MS) calcd for $C_9H_7ClN_2$ $[M + H]^+$: 179.0371 and 181.0341, found: 179.0343 and 181.0319.



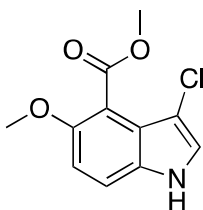
5-chloro-2,3-dihydro-N-methyl-1H-Indole-1-ethanamine (31a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3b) to afford compound **31a**. 1 H NMR (400 MHz; DMSO): δ 7.08-7.03 (m, 2H), 6.56 (dd, J = 8.2, 3.4, 1H), 3.36 (dd, J = 8.0, 4.9, 2H), 3.28 (s, 2H), 3.05 (s, 2H), 2.91 (t, J = 3.8, 2H), 2.54 (s, 3H). HRMS (ESI-MS) calcd for $C_{11}H_{15}ClN_2$ $[M + H]^+$: 211.0997 and 213.0967, found: 211.0974 and 213.0959.



N-[7-chloro-2-(1H-indol-3-yl)ethyl]-acetamide (32a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3d) to afford compound **32a**. 1 H NMR (400 MHz; DMSO): δ 11.21 (s, 1H), 7.95 (t, J = 5.5, 1H), 7.51 (d, J = 7.9, 1H), 7.23 (d, J = 2.4, 1H), 7.15 (d, J = 7.5, 1H), 7.00 (t, J = 7.7, 1H), 3.30 (t, J = 6.6, 2H), 2.80 (t, J = 7.3, 2H), 1.79 (s, 3H). HRMS (ESI-MS) calcd for $C_{12}H_{13}ClN_2O$ $[M + H]^+$: 237.0789 and 239.0670, found: 237.0799 and 239.0787.

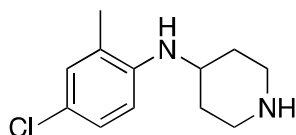


3-amino-2-chloro-6,7,8,9-tetrahydro-*H*-benzocyclohepten-5-one (33a): The bioconversion was conducted in a beaker according to the general procedure, using 0.05 equiv. of RebH (50 μ M final concentration) at 25 $^{\circ}$ C, with 4 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3d) to afford compound **33a**. 1 H NMR (400 MHz; DMSO): δ 7.16 (s, 1H), 7.07 (s, 1H), 5.42 (s, 2H), 2.76 (t, J = 6.1, 2H), 2.63-2.60 (m, 2H), 1.68 (td, J = 12.6, 6.4, 4H). HRMS (ESI-MS) calcd for $C_{11}H_{12}ClNO$ $[M + H]^+$: 210.0680 and 212.0651, found: 210.0737 and 212.0709. Note: Partial reduction of the ketone to an alcohol was observed. This side product, 3-amino-2-chloro-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol was isolated and characterized as well. 1 H NMR (400 MHz; DMSO): δ 6.94 (s, 1H), 6.87 (s, 1H), 5.15 (d, J = 4.0, 1H), 5.06 (s, 2H), 4.57 (dd, J = 9.5, 3.9, 1H), 2.62 (dd, J = 13.8, 7.2, 1H), 1.86 (t, J = 9.3, 2H), 1.65 (dd, J = 20.2, 9.7, 2H), 1.43 (q, J = 10.2, 1H), 1.17 (dt, J = 22.1, 10.9, 1H). HRMS (ESI-MS) calcd for $C_{11}H_{14}ClNO$ $[M + H]^+$: 212.0837 and 214.0808, found: 212.0827 and 214.0806.

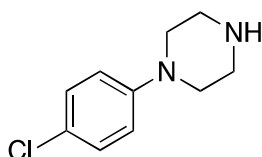


Methyl 3-chloro-5-methoxy-1H-indole-4-carboxylate (34a): The bioconversion was conducted in a beaker according to the general procedure, using 0.05 equiv. of RebH (50 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse

phase chromatography (LCMS method 3d) to afford compound **34a**. ^1H NMR (400 MHz; DMSO): δ 11.51 (s, 1H), 7.57 (d, $J = 2.7$, 1H), 7.47 (d, $J = 9.0$, 1H), 7.04 (d, $J = 9.0$, 1H), 3.83 (s, 3H), 3.79 (s, 3H). HRMS (ESI-MS) calcd for $\text{C}_{11}\text{H}_{10}\text{ClNO}_3$ $[\text{M} + \text{H}]^+$: 240.0422 and 242.0393, found: 240.0442 and 242.0224.

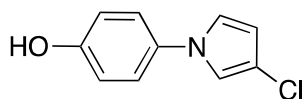


N-(4-chloro-2-methylphenyl)-4-piperidinamine (35a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μM final concentration) at 25 $^\circ\text{C}$, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3c) to afford compound **35a**. ^1H NMR (400 MHz; DMSO): δ 7.02-7.00 (m, 2H), 6.62 (d, $J = 9.3$, 1H), 3.51 (s, 1H), 3.26 (d, $J = 12.3$, 2H), 2.95-2.86 (m, 2H), 2.08 (s, 3H), 1.99 (d, $J = 12.1$, 2H), 1.60 (q, $J = 11.0$, 2H). HRMS (ESI-MS) calcd for $\text{C}_{12}\text{H}_{17}\text{ClN}_2$ $[\text{M} + \text{H}]^+$: 225.1153 and 227.1124, found: 225.1160 and 227.1131.

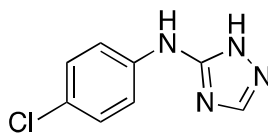


para-chlorophenylpiperazine (36a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μM final concentration) at 25 $^\circ\text{C}$, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude reaction mixture was concentrated onto Celite, packed into a Biotage samplet, and loaded into a reverse phase column (Biotage SNAP-KP-C18-HS). The crude material was purified by reverse phase chromatography to afford compound **36a** as the TFA salt. ^1H NMR (500 MHz; DMSO): δ 8.91 (s, 2H), 7.31-7.28 (m, 2H), 7.03-7.00 (m, 2H), 3.34 (m,

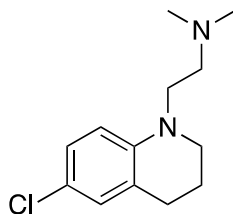
$J = 5.1, 4\text{H}$), 3.23 (s, 4H). HRMS (ESI-MS) calcd for $\text{C}_{10}\text{H}_{13}\text{ClN}_2$ $[\text{M} + \text{H}]^+$: 197.0840 and 199.0811, found: 197.1111 and 199.1042.



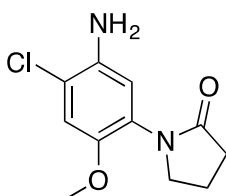
4-(3-chloro-1H-pyrrol-1-yl)phenol (37a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μM final concentration) at 25 $^\circ\text{C}$, with 1 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3c) to afford compound **37a**. ^1H NMR (500 MHz; DMSO): δ 7.36-7.33 (m, 3H), 7.21 (dd, $J = 3.0, 2.6$, 1H), 6.84-6.81 (m, 2H), 6.25 (dd, $J = 3.0, 1.8$, 1H). HRMS (ESI-MS) calcd for $\text{C}_{10}\text{H}_8\text{ClNO}$ $[\text{M} - \text{H}]^-$: 192.0211 and 194.0182, found: 192.0247 and 194.0278.



4-chloro-N-phenyl-1H-1,2,4-Triazol-5-amine (38a): The bioconversion was conducted in a beaker according to the general procedure, using 0.05 equiv. of RebH (50 μM final concentration) at 25 $^\circ\text{C}$, with 4 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3d) to afford compound **38a**. ^1H NMR (400 MHz; DMSO): δ 13.41 (s, 1H), 9.42 (s, 1H), 8.21 (s, 1H), 7.59-7.55 (m, 2H), 7.27-7.23 (m, 2H). HRMS (ESI-MS) calcd for $\text{C}_8\text{H}_7\text{ClN}_4$ $[\text{M} + \text{H}]^+$: 195.0432 and 197.0403, found: 195.0431 and 197.0409.

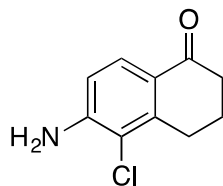


6-chloro-3,4-dihydro-N,N-dimethyl-1(2H)-quinolineethanamine (39a): The bioconversion was conducted in a beaker according to the general procedure, using 0.05 equiv. of RebH (50 μ M final concentration) at 25 $^{\circ}$ C, with 10 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3b) to afford compound **39a**. 1 H NMR (400 MHz; DMSO): δ 6.97 (dd, $J = 8.8, 2.7$, 1H), 6.91 (d, $J = 2.6$, 1H), 6.56 (d, $J = 8.8$, 1H), 3.38 (t, $J = 7.3$, 2H), 3.26 (t, $J = 5.6$, 2H), 2.65 (t, $J = 6.3$, 2H), 2.55 (t, $J = 7.3$, overlapping solvent peak), 2.32 (s, 6H), 1.84-1.78 (m, 2H). HRMS (ESI-MS) calcd for $C_{13}H_{19}ClN_2$ $[M + H]^+$: 239.1310 and 241.1281, found: 239.1317 and 241.1299. Note: A peak with a mass corresponding to 2 amu less than the mass of the starting material was consistently observed for this reaction. This “ $M_{SM} - 2$ ” side product could not be isolated.

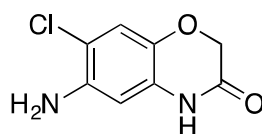


1-(5-amino-4-chloro-2-methoxyphenyl)-2-pyrrolidinone (40a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude reaction mixture was concentrated onto Celite, packed into a Biotage samplet, and loaded into a reverse phase column (Biotage SNAP-KP-C18-HS). The crude material was purified by reverse phase chromatography

to afford compound **40a** as the TFA salt. ^1H NMR (500 MHz; DMSO): δ 7.05 (s, 1H), 6.80 (s, 1H), 3.71 (s, 3H), 3.62 (t, $J = 7.0$, 2H), 2.36 (t, $J = 8.1$, 2H), 2.06 (quintet, $J = 7.6$, 2H). HRMS (ESI-MS) calcd for $\text{C}_{11}\text{H}_{13}\text{ClN}_2\text{O}_2$ $[\text{M} + \text{H}]^+$: 241.0738 and 243.0709, found: 241.0739 and 243.0727.

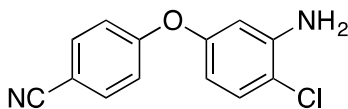


6-amino-5-chloro-3,4-dihydro-1(2H)-naphthalenone (41a): The bioconversion was conducted in a beaker according to the general procedure, using 0.05 equiv. of RebH (50 μM final concentration) at 25 $^\circ\text{C}$, with 4 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3d) to afford compound **41a**. ^1H NMR (400 MHz; DMSO): δ 7.62 (d, $J = 8.6$, 1H), 6.71 (d, $J = 8.6$, 1H), 6.32 (s, 2H), 2.87 (t, $J = 6.1$, 2H), 2.43 (t, $J = 6.5$, 2H), 1.99 (dt, $J = 12.7$, 6.3, 2H). HRMS (ESI-MS) calcd for $\text{C}_{10}\text{H}_{10}\text{ClNO}$ $[\text{M} + \text{H}]^+$: 196.0524 and 198.0495, found: 196.0710 and 198.1047.

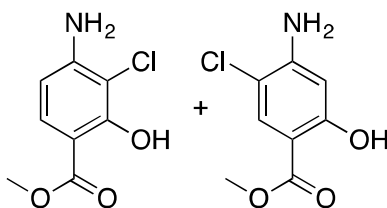


6-Amino-7-chloro-2H-1,4-benzoxazin-3(4H)-one (42a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μM final concentration) at 25 $^\circ\text{C}$, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3c) to afford compound **42a**. ^1H NMR (400 MHz; DMSO): δ 10.64 (s, 1H), 6.84 (s, 1H), 6.38 (s, 1H), 5.09 (s, 2H), 4.42 (s, 2H). HRMS (ESI-MS) calcd for

$C_8H_7ClN_2O_2$ $[M + H]^+$: 199.0269 and 201.0240, found: 199.0321 and 200.9913.

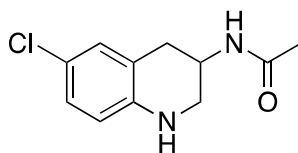


4-(3-amino-4-chlorophenoxy)-benzonitrile (43a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 °C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3d) to afford compound **43a**. 1H NMR (400 MHz; DMSO): δ 7.85-7.82 (m, 2H), 7.25 (d, J = 8.6, 1H), 7.13-7.10 (m, 2H), 6.50 (d, J = 2.8, 1H), 6.29 (dd, J = 8.6, 2.8, 1H), 5.61 (s, 2H). HRMS (ESI-MS) calcd for $C_{13}H_9ClN_2O$ $[M + H]^+$: 245.0476 and 247.0447, found: 245.0401 and 247.4741.

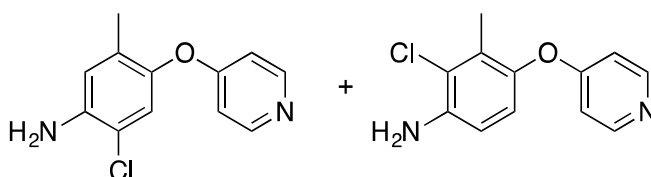


Methyl 4-amino-3-chlorosalicylate and Methyl 4-amino-5-chlorosalicylate (44a and 44b): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 °C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3d) to afford compound **44a** and **44b**. **44a:** 1H NMR (400 MHz; DMSO): δ 11.49 (s, 1H), 7.46 (d, J = 8.9, 1H), 6.46 (s, 1H), 6.34 (d, J = 8.9, 1H), 3.83 (s, 2H). HRMS (ESI-MS) calcd for $C_8H_8ClNO_3$ $[M + H]^+$: found: 202.0550 and 204.0287 **44b:** 1H NMR (400 MHz; DMSO): δ 10.57 (s, 1H), 7.56 (s, 1H), 6.36 (s, 2H), 6.24 (s, 1H), 3.81 (s, 3H). HRMS (ESI-MS). calcd for $C_8H_8ClNO_3$ $[M + H]^+$: found: 202.0483 and

204.0473.

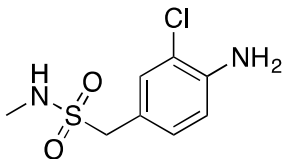


N-(6-chloro-1,2,3,4-tetrahydro-3-quinolinyl)-acetamide (45a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3c) to afford compound **45a**. **45a:** 1 H NMR (500 MHz; DMSO): δ 7.82 (d, J = 7.1, 1H), 6.91-6.88 (m, 2H), 6.47 (d, J = 8.3, 1H), 5.92 (s, 1H), 3.91-3.99 (m, 1H), 3.23 (d, J = 9.8, 1H), 2.92 (t, J = 9.0, 1H), 2.84 (dd, J = 16.0, 4.7, 1H), 2.59 (dd, J = 16.2, 8.5, 1H), 1.82 (s, 3H). HRMS (ESI-MS) calcd for $C_{11}H_{13}ClN_2O$ [$M + H$] $^+$: 225.0789 and 227.0760, found: 225.0764 and 227.0745.

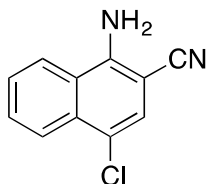


6-chloro-3-methyl-4-(4-pyridinyloxy)-benzenamine and **2-chloro-3-methyl-4-(4-pyridinyloxy)-benzenamine (46a and 46b):** The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3b) to afford compounds **46a** and **46b**. **46a:** 1 H NMR (500 MHz; DMSO): δ 8.72 (d, J = 7.1, 2H), 7.34 (d, J = 7.1, 2H), 7.20 (s, 1H), 6.79 (s, 1H), 1.97 (s, 3H). HRMS (ESI-MS) calcd for $C_{12}H_{11}ClN_2O$ [$M + H$] $^+$: 235.0633 and 237.0604, found: 235.0643 and 237.0607. **46b:** 1 H

NMR (500 MHz; DMSO): δ 8.70 (d, $J = 7.1$, 2H), 7.31 (d, $J = 7.1$, 2H), 6.96 (d, $J = 8.8$, 1H), 6.80 (d, $J = 8.8$, 1H), 2.07 (s, 3H). HRMS (ESI-MS) calcd for $C_{12}H_{11}ClN_2O$ $[M + H]^+$: 235.0633 and 237.0604, found: 235.0622 and 237.0600.

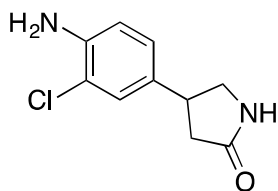


4-Amino-3-chlorophenyl-N-methyl methanesulfonamide (48a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3c) to afford compound **48a**. 1 H NMR (400 MHz; DMSO): δ 7.17 (d, $J = 1.9$, 1H), 6.99 (dd, $J = 8.3$, 2.0, 1H), 6.83 (q, $J = 4.9$, 1H), 6.75 (d, $J = 8.3$, 1H), 5.44 (s, 2H), 4.13 (s, 2H), 2.52 (m, overlapping solvent peak). HRMS (ESI-MS) calcd for $C_8H_{11}ClN_2O_2S$ $[M + H]^+$: 235.0302 and 237.0273, found: 235.0638 and 237.0078.

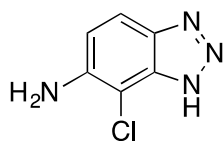


1-amino-4-chloro-2-naphthalenecarbonitrile (52a): The bioconversion was conducted in a beaker according to the general procedure, using 0.05 equiv. of RebH (50 μ M final concentration) at 25 $^{\circ}$ C, with 4 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3d) to afford compound **52a**. 1 H NMR (400 MHz; DMSO): δ 8.44 (d, $J = 8.4$, 1H), 8.08 (dd, $J = 8.4$, 0.9, 1H), 7.79 (ddd, $J = 8.3$, 7.0, 1.2, 1H), 7.64 (ddd, $J = 8.4$, 7.0, 1.3, 1H), 7.58 (s, 1H), 7.13 (s, 2H). HRMS (ESI-MS) calcd for $C_{11}H_{10}ClNO_3$ $[M + H]^+$:

240.0422 and 242.0393, found: 240.0217 and 241.9712.

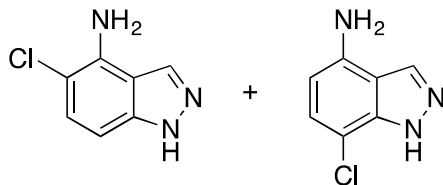


4-(4-amino-3-chlorophenyl)-2-pyrrolidinone (62a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude reaction mixture was concentrated onto Celite, packed into a Biotage samplet, and loaded into a reverse phase column (Biotage SNAP-KP-C18-HS). The crude material was purified by reverse phase chromatography. Product containing fractions were pooled, basified with K_2CO_3 , and extracted into dichloromethane to afford compound **62a**. 1H NMR (500 MHz; DMSO): δ 7.65 (s, 1H), 7.13 (d, J = 1.9, 1H), 6.97 (dd, J = 8.4, 1.9, 1H), 6.75 (d, J = 8.3, 1H), 3.55-3.51 (m, overlapping with solvent), 3.48-3.41 (m, overlapping with solvent), 3.10 (t, J = 8.5, 1H), 2.42 (dd, J = 16.2, 8.6, 1H), 2.22 (dd, J = 16.3, 9.4, 1H), 1.24 (s, 2H). HRMS (ESI-MS) calcd for $C_{10}H_{11}ClN_2O$ $[M + H]^+$: 211.0633 and 213.0604, found: 211.0712 and 213.0650.

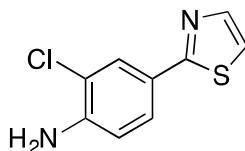


7-chloro-1H-benzotriazol-6-amine (64a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3a) to afford compound **64a**. Note: Upon concentration of LCMS-purified product with

formic acid, the formamide partially formed (can be observed in HNMR and HRMS). ^1H NMR (400 MHz; DMSO): δ 7.62 (d, $J = 8.8$, 1H), 6.92 (d, $J = 8.8$, 1H), 5.74 (s, 2H). HRMS (ESI-MS) calcd for $\text{C}_6\text{H}_5\text{ClN}_4$ $[\text{M} + \text{H}]^+$: 169.0276 and 171.0246, found: 169.1359 and 170.9829.

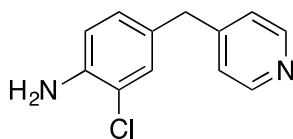


5-chloro-1H-indazol-4-amine and **7-chloro-1H-indazol-4-amine (67a and 67b)**: The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μM final concentration) at 25 $^\circ\text{C}$, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3b) to afford compounds **67a** and **67b**. **67a**: ^1H NMR (400 MHz; DMSO): δ 13.07 (s, 1H), 8.18 (s, 1H), 7.01 (d, $J = 8.0$, 1H), 6.11 (d, $J = 8.0$, 1H), 5.93 (s, 2H). HRMS (ESI-MS) calcd for $\text{C}_7\text{H}_6\text{ClN}_3$ $[\text{M} + \text{H}]^+$: 168.0323 and 170.0294, found: 168.2418 and 170.1853. **67b**: ^1H NMR (400 MHz; DMSO): δ 12.85 (s, 1H), 8.19 (s, 1H), 7.08 (d, $J = 8.7$, 1H), 6.66 (d, $J = 8.6$, 1H), 5.98 (s, 2H). HRMS (ESI-MS) calcd for $\text{C}_7\text{H}_6\text{ClN}_3$ $[\text{M} + \text{H}]^+$: 168.0323 and 170.0294, found: 168.0796 and 170.0637.

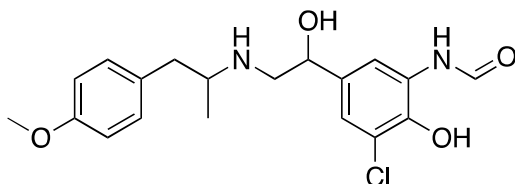


2-chloro-4-(2-thiazolyl)benzenamine (77a): The bioconversion was conducted in a beaker according to the general procedure, using 0.05 equiv. of RebH (50 μM final concentration) at 25 $^\circ\text{C}$, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude material was purified by reverse phase chromatography (LCMS method 3d) to afford compound **77a**. ^1H NMR (400 MHz; DMSO): δ 7.78 (d, $J = 3.3$, 1H), 7.76

(d, $J = 2.1$, 1H), 7.61-7.59 (m, 2H), 6.85 (d, $J = 8.5$, 1H), 5.93 (s, 2H). HRMS (ESI-MS) calcd for $C_9H_7ClN_2S$ $[M + H]^+$: 211.0091 and 213.0062, found: 211.0114 and 213.0093.



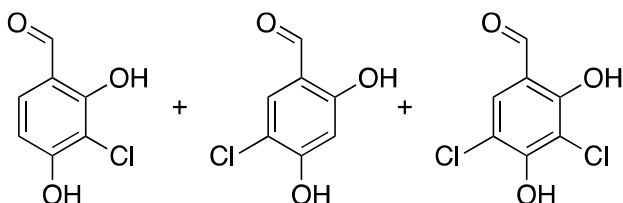
2-chloro-4-(4-pyridinylmethyl)-benzenamine (82a): The bioconversion was conducted in a beaker according to the general procedure, using 0.025 equiv. of RebH (25 μ M final concentration) at 25 $^{\circ}$ C, with 2 mg of substrate. After maximum conversion to monohalogenated product was observed by LCMS, one volume of dichloromethane was added to the reaction mixture and vortexed to quench. The reaction mixture was extracted into dichloromethane according to the general procedure. The crude reaction mixture was concentrated onto Celite, packed into a Biotage samplet, and loaded into a reverse phase column (Biotage SNAP-KP-C18-HS). The crude material was purified by reverse phase chromatography. Product containing fractions were pooled, basified with K_2CO_3 , and extracted into dichloromethane to afford compound **82a**. 1H NMR (500 MHz; DMSO): δ 8.44 (d, $J = 5.6$, 2H), 7.21 (d, $J = 5.9$, 2H), 7.09 (d, $J = 1.9$, 1H), 6.91 (dd, $J = 8.2$, 1.9, 1H), 6.73 (d, $J = 8.2$, 1H), 5.23 (s, 2H), 3.79 (s, 2H). HRMS (ESI-MS) calcd for $C_{12}H_{11}ClN_2$ $[M + H]^+$: 219.0684 and 221.0655, found: 219.0647 and 221.0627.



***N*-[3-chloro-2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]-formamide (25a):** A solution of 10 mg of **25** (1 equiv., 0.5 mM final concentration) in 5% v/v methanol was added to a crystallization dish (90 x 50 mm). Solutions in HEPES buffer (25 mM, pH 7.4) of NAD (0.2 equiv., 100 μ M final concentration), FAD (0.2 equiv., 100 μ M final concentration), NaCl (20 equiv., 10 mM final concentration), and a glucose dehydrogenase (9 U/mL final concentration GDH) were added. Solutions in HEPES/glycerol buffer (25 mM, pH 7.5, 10% glycerol v/v) of Rdc2 (0.05 equiv., 25 μ M final

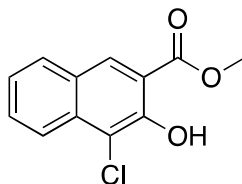
concentration) and MBP-RebF (0.0025 equiv., 2.5 μ M final concentration) were added. The bioconversion was diluted with HEPES buffer (25 mM, pH 7.4) to the appropriate reaction volume and an aqueous solution of glucose (40 equiv., 20 mM final concentration) was added to initiate the cofactor regeneration cycle. The dish was covered with aluminum foil and left on the benchtop. The reaction was monitored by LCMS Method 1 and was quenched with aqueous HCl (5.0 M, until pH \sim 1-2) upon completion (55% conv.). The quenched reaction mixture was transferred to 2 x 50 mL falcon tubes and was centrifuged at 15,000 rpm for 20 minutes. The aqueous supernatant was filtered through a 45 μ m filter and was concentrated by rotovap. The product was extracted from reaction salts through a solid-liquid extraction with MeOH (4 extractions with \sim 5 mL per extraction). The MeOH was filtered through filter paper and was concentrated onto Celite. The Celite was packed into a Biotage samplet, which was then loaded into a reverse phase column (Biotage SNAP-KP-C18-HS). The crude material was purified by reverse phase chromatography to afford **25a**. ^1H NMR (500 MHz; DMSO): δ 9.99 (s, 1H), 8.33 (d, J = 1.9, 1H), 8.05 (d, J = 2.0, 1H), 7.19 (d, J = 2.0, 1H), 7.15 (d, J = 8.7, 2H), 6.91 (d, J = 8.7, 2H), 4.85 (dd, J = 10.2, 2.5, 1H), 3.74 (s, 3H), 3.42 (m, 1H), 3.17-3.02 (m, 3H), 2.59 (dd, J = 13.1, 10.7, 1H), 1.09 (d, J = 6.5, 3H). ^{13}C NMR (126 MHz; DMSO): δ 161.06, 158.63, 143.02, 134.48, 130.71, 129.08, 128.87, 122.56, 121.59, 118.05, 114.53, 68.43, 55.52, 55.50, 51.04, 38.25, 15.21. HRMS (ESI-MS) calcd for $\text{C}_{19}\text{H}_{23}\text{ClN}_2\text{O}_4$ $[\text{M} + \text{H}]^+$: 379.1419 and 381.1391, found: 379.1449 and 381.1443.

Detailed isolation and characterizations – NCS reactions:

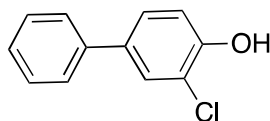


3-chloro-2,4-dihydroxy-benzaldehyde, 5-chloro-2,4-dihydroxy-benzaldehyde and 3,5-dichloro-2,4-dihydroxy-benzaldehyde (23a, 23b, and 23c): The reaction was conducted according to the general procedure. After maximum conversion to monohalogenated product was observed by LCMS Method 2, the crude reaction mixture was dry-loaded into Celite. The Celite was packed into a Biotage samplet, which was then loaded into a reverse phase column (Biotage SNAP-KP-C18-HS). The crude material was purified by reverse phase chromatography to afford

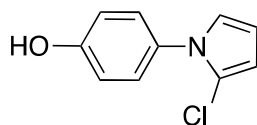
a purified mixture of **23a** and **23b** in 55% yield (34 mg, 0.198 mmol) and **23c** in 7% yield (5.5 mg, 0.027 mmol). **23a** and **23b**: $^1\text{H NMR}$ (500 MHz; CD_3CN): δ 9.73 (s, 1H), 9.69 (s, 1H), 7.65 (s, 1H), 7.51 (d, $J = 8.6$, 1H), 6.70 (d, $J = 8.6$, 1H), 6.52 (s, 1H). HRMS (ESI-MS) calcd for $\text{C}_7\text{H}_5\text{ClO}_3$ $[\text{M} - \text{H}]^-$: 170.9843 and 172.9814, found: 170.9874 and 172.9840. **23c**: $^1\text{H NMR}$ (500 MHz; CD_3CN): δ 9.73 (s, 1H), 7.71 (s, 1H). HRMS (ESI-MS) calcd for $\text{C}_7\text{H}_4\text{Cl}_2\text{O}_3$ $[\text{M} - \text{H}]^-$: 204.9454 and 206.9425, found: 204.9450 and 206.9470.



4-chloro-3-hydroxy-2-naphthalenecarboxylic acid, methyl ester (24a): The reaction was conducted according to the general procedure. After maximum conversion to monohalogenated product was observed by LCMS Method 2, the reaction mixture was concentrated. Because nearly complete conversion to one monohalogenated product was observed (94% by LCMS), it was not necessary to purify the crude reaction mixture to determine selectivity. **24a**: ^1H (500 MHz; CD_2Cl_2): δ 11.08 (s, 1H), 8.41 (s, 1H), 8.14 (dd, $J = 8.6, 0.7$, 1H), 7.83 (dd, $J = 8.2, 0.4$, 1H), 7.67 (ddd, $J = 8.5, 7.0, 1.3$, 1H), 7.43 (ddd, $J = 8.1, 6.9, 1.1$, 1H), 4.05 (s, 3H). HRMS (ESI-MS) calcd for $\text{C}_{12}\text{H}_9\text{ClO}_3$ $[\text{M} - \text{H}]^-$: 235.0156 and 237.0128, found: 235.0214 and 237.0140.



3-chloro-[1,1'-biphenyl]-4-ol (28a): The reaction was conducted according to the general procedure. After maximum conversion to monohalogenated product was observed by GCMS, the reaction mixture was concentrated onto silica gel. Purification by flash chromatography (SiO_2 , 5% ethyl acetate/hexanes) afforded **28a** in 69% yield (41.1 mg, 0.201 mmol). $^1\text{H NMR}$ (500 MHz; CD_2Cl_2): δ 7.64 (d, $J = 2.2$, 1H), 7.59-7.57 (m, 2H), 7.50-7.46 (m, 3H), 7.40-7.37 (m, 1H), 7.14 (d, $J = 8.4$, 1H). HRMS (ESI-MS) calcd for $\text{C}_{12}\text{H}_9\text{ClO}$ $[\text{M} - \text{H}]^-$: 203.0258 and 205.0229, found: 203.0266 and 205.0275.



4-(2-chloro-1H-pyrrol-1-yl)phenol (37b): The reaction was conducted according to the general procedure. After maximum conversion to monohalogenated product was observed by LCMS Method 2, the reaction mixture was concentrated onto silica gel. Purification by flash chromatography (SiO₂, 4% ethyl acetate/hexanes) afforded **37b**. ¹H NMR (500 MHz; DMSO): δ 7.19-7.17 (m, 2H), 6.93 (m, 1H), 6.87-6.85 (m, 2H), 6.20 (m, 2H). HRMS (ESI-MS) calcd for C₁₀H₈ClNO [M + H]⁺: 194.0367 and 196.0338, found: 194.0484 and 196.0932. Note: polymerization of this product occurred within hours of isolation. For this reason, spectra were collected immediately after purification.

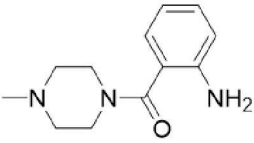
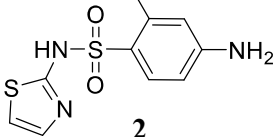
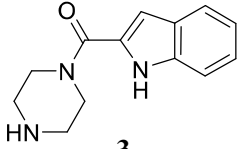
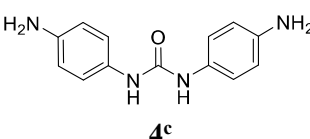
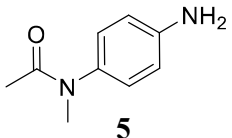
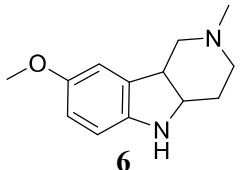
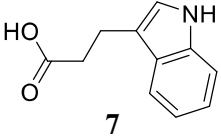
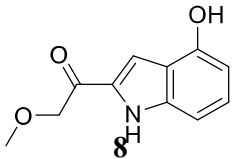
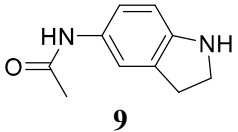
Note: 2-chlorophenol and 4-chlorophenol were purchased from commercial sources.

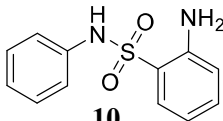
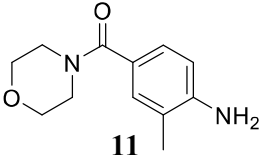
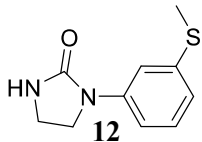
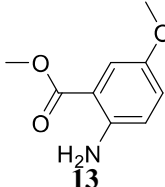
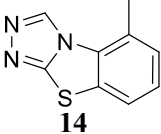
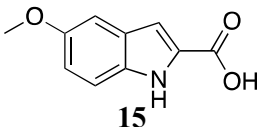
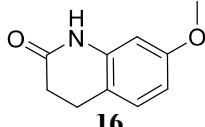
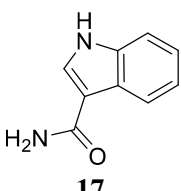
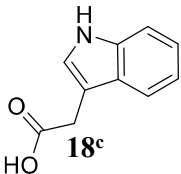
Halenium affinity calculations: Halenium affinity calculations were performed for all substrates as previously described.⁵

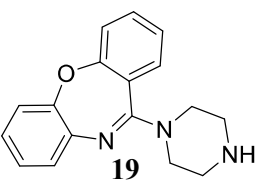
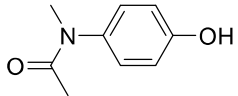
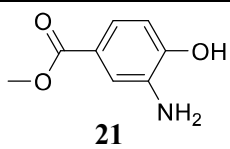
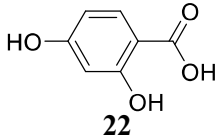
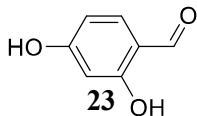
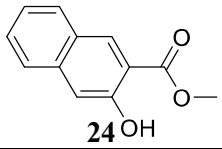
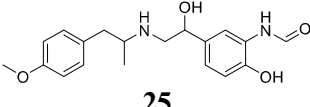
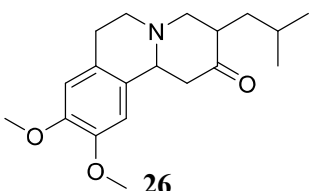
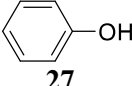
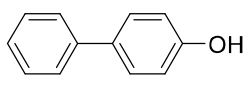
Docking studies: To identify the potential poses of the compounds in the halogenase enzyme, compounds were docked via two approaches (1) ICM Molsoft docking and (2) ROCS pharmacophoric overlay. Due to the availability of structural information available on RebH (PDB ID: 2OA1, 2O9Z, 2OAM, 2E4G, 2OAL), this enzyme was chosen as the sample system. For the first method, molecules were docked using MolSoft ICM version 3.8.4. A multi-conformer approach with a thoroughness score of 10 was utilized and the top 10 poses were scored. Scored poses with energetically favorable scores were considered as potential binding modes and were utilized in identifying sights of potential halogenation.

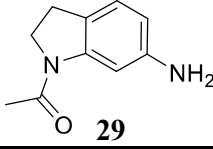
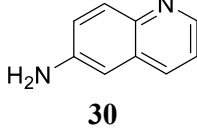
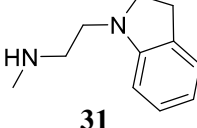
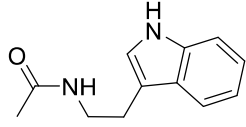
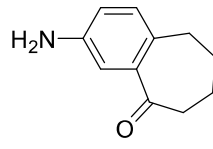
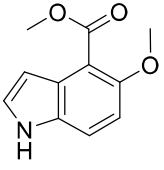
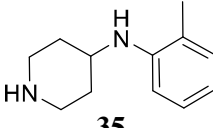
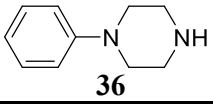
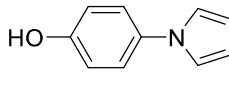
For the second method, the crystalized tryptophan was used as the template for pharmacophoric searching (ROCS OpenEye version 3.2). The molecule with the best Tanimoto similarity score was considered as a potential binding mode and was used for consideration in identifying sights of potential halogenation.

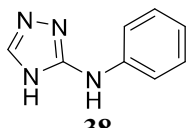
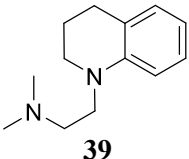
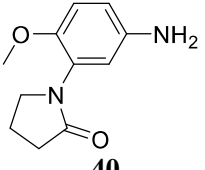
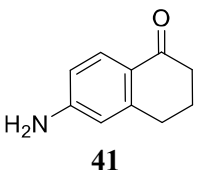
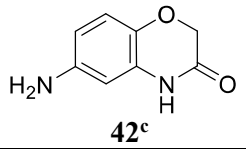
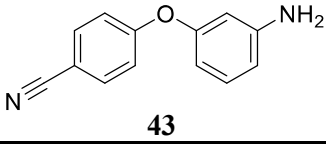
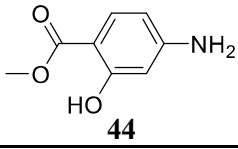
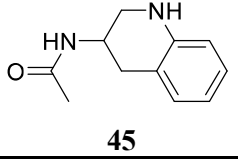
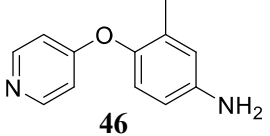
Table S1: Initial conversion data from panel 1 and 2.

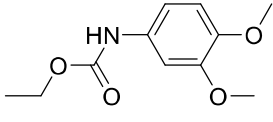
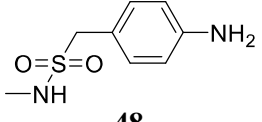
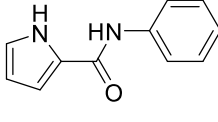
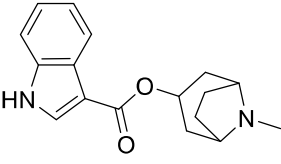
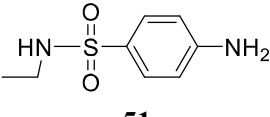
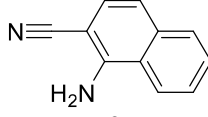
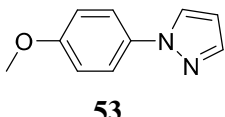
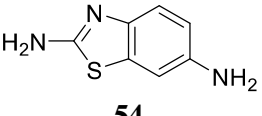
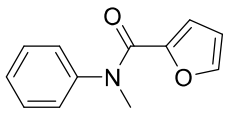
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% Conversion (LCMS)									
 1	1h	1	0	0	54	0	100	0	0
	ON	1	1	1	100	0	100	0	0
 2	1h	21	0	0	22	0	0	0	0
	ON	30	1	0	69	1	9	0	0
 3	1h	0	0	0	0	0	11	0	0
	ON	1	1	0	0	0	42	0	0
 4^c	1h	-	-	-	-	-	-	-	-
	ON	100	1	0	0	1	1	0	0
 5	1h	0	0	0	9	0	15	0	0
	ON	0	1	0	1	0	1	0	0
 6	1h	0 ^a	0	0 ^a	0 ^a	100	1	0	0
	ON	0	0	50 ^a	35 ^{ab}	50 ^a	65 ^{ab}	0	0
 7	1h	8	0	0	0	0	1	0	0
	ON	9	0	0	14	33	100	0	0
 8	1h	0	0	0	6	1	0	0	0
	ON	100	0	12	0	0	0	0	0
 9	1h	0	0	0	0	0	0	0	0
	ON	0	0	1 ^b	0	0	100 ^b	0	0

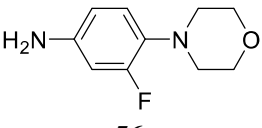
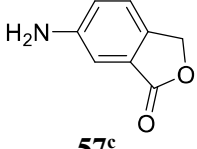
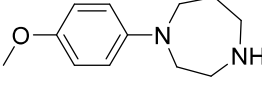
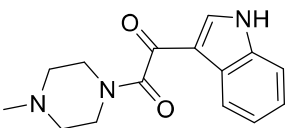
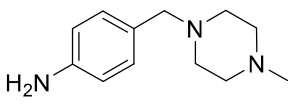
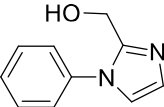
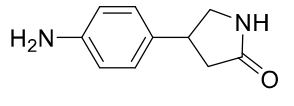
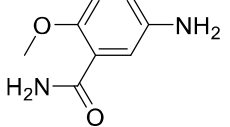
Compound	Time	Thal	RebH	10S	6TL	0K	4V	Rdc2	GsfI
 10	1h	0	0	0	1	1	1	0	0
	ON	1	0	0	11	1	1	0	0
 11	1h	4	0	0	0	0	0	0	0
	ON	4	0	0	5	0	1	0	0
 12	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	5	0	0
 13	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	1	0	1	0	0
 14	1h	0	0	0	0	0	1	0	0
	ON	0	0	0	0	1	0	0	0
 15	1h	0	0	1	1	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 16	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	1	0	0
 17	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	1	0	0
 18^c	1h	-	-	-	-	-	-	-	-
	ON	0	0	1	0	0	0	0	0

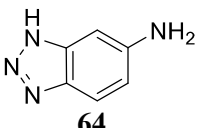
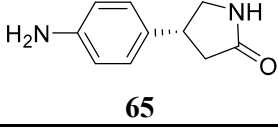
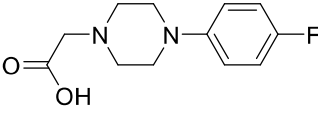
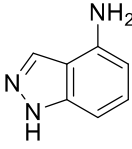
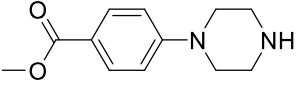
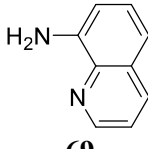
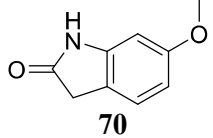
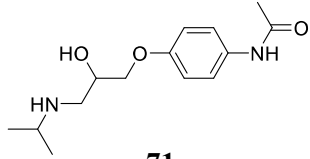
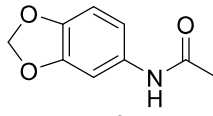
Compound	Time	Thal	RebH	10S	6TL	0K	4V	Rdc2	Gsfl
 19	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	1	0	0
 20	1h	0	0	0	0	0	1	0	0
	ON	0	0	0	0	0	0	0	0
 21	1h	0	0	0	0	1	0	0	0
	ON	0	0	0	0	0	0	0	0
 22	ON	0	0	0	0	0	0	2	2
 23	ON	0	0	0	0	18	4	88	15
 24	ON	1	0	0	32	0	0	24	7
 25	ON	0	0	0	0	0	0	22	2
 26	ON	0	0	2	5	0	0	35	6
 27	ON	1	1	0	0	0	10	4	0
 28	ON	0	0	0	0	0	0	11	1

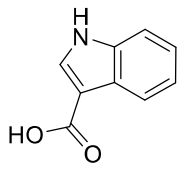
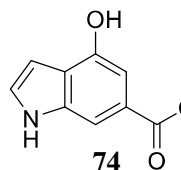
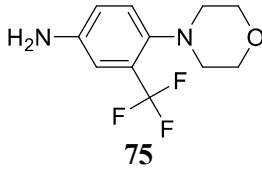
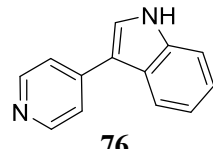
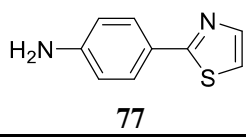
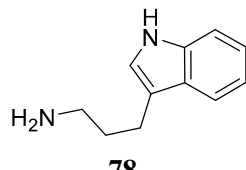
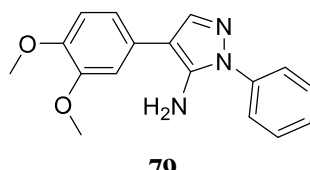
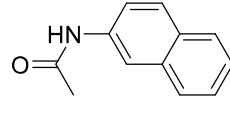
Compound	Time	Thal	RebH	10S	6TL	0K	4V	Rdc2	Gsfl
 29	1h	27	100	10	85	22	0	5	1
	ON	23	100	16	100	50	100	5	1
 30	1h	38	100	0	28	25	66	0	0
	ON	88	90	20	85	89	90	0	0
 31	1h	100	100	19	100	10	100	0	0
	ON	100	100	44	100	14	100	0	0
 32	1h	100	100	0	1	1	0	0	0
	ON	51	100	7	36	37	>100 ^d (36%)	0	0
 33	1h	0	1	0	1	0	1	0	0
	ON	32	13	0	44	0	67	0	0
 34	1h	26	19	1	24	0	0	4	0
	ON	23	20	1	100	1	80	4	0
 35	1h	100	100	31	100	12	100	0	0
	ON	100	100	44	100	19	100	0	0
 36	1h	100	100	1	72	1	100	0	0
	ON	100	100	50	98	0	100	0	0
 37	1h	100	100	14	100	18	100	0	0
	ON	100	0	50	100	100	100	0	0

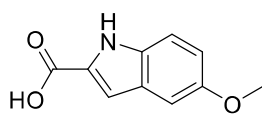
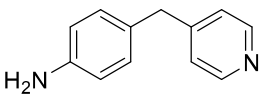
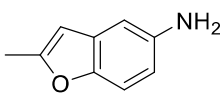
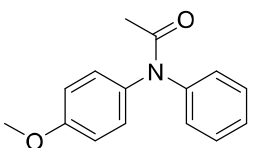
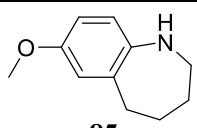
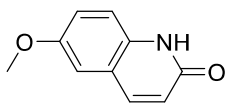
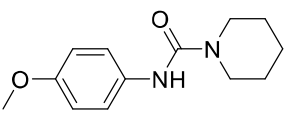
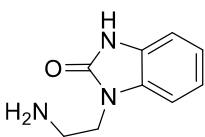
Compound	Time	Thal	RebH	10S	6TL	0K	4V	Rdc2	Gsfl
 38	1h	9	1	1	1	0	13	0	0
	ON	12	14	1	35	1	100	0	0
 39	1h	100	0 ^a	0	100	0	0	0	0
	ON	100 ^b	25 ^b	35 ^b	100 ^b	0	100 ^b	0	0
 40	1h	0	100	0	1	0	34	0	0
	ON	25	50	0	54	5	80	0	0
 41	1h	0	0	0	1	0	0	0	0
	ON	0	13	1	42	0	1	0	0
 42^c	1h	100	100	0	100	0	100	0	0
	ON	-	-	-	-	-	-	-	-
 43	1h	26	100	0	33	35	29	4	0
	ON	73	100	13	100	100	100	4	0
 44	1h	14	55	0	50	1	31	0	0
	ON	8	0	11	56	46	100	0	0
 45	1h	46	39	15	26	50	25	1	1
	ON	49	50	41	70	70	70	1	1
 46	1h	1	100	0	1	0	100	0	0
	ON	100	100	1	100	1	100	0	0

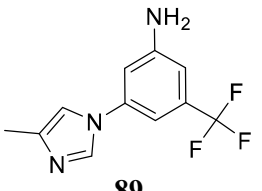
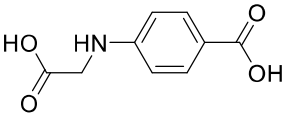
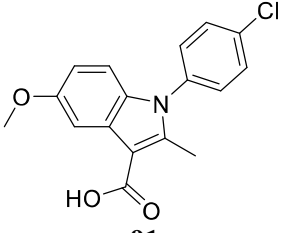
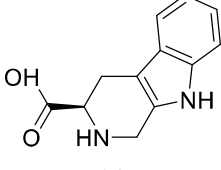
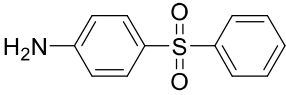
Compound	Time	Thal	RebH	10S	6TL	0K	4V	Rdc2	Gsfl
 47	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 48	1h	35	100	0	9	0	100	0	0
	ON	100	100	0	100	1	100	0	0
 49	1h	0	1	0	1	1	1	0	0
	ON	1	6	1	26	1	30	0	0
 50	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 51	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 52	1h	0	0	0	0	0	0	0	0
	ON	0	10	0	42	0	26	0	0
 53	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 54	1h	100	1	1	100	1	1	1	1
	ON	100	100	0	100	0	100	1	1
 55	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0

Compound	Time	Thal	RebH	10S	6TL	0K	4V	Rdc2	Gsfl
 56	1h	0	12	0	1	1	1	0	0
	ON	1	38	0	1	1	43	0	0
 57^c	1h	-	-	-	-	-	-	-	-
	ON	0	0	0	0	0	0	0	0
 58	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 59	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 60^c	1h	-	-	-	-	-	-	-	-
	ON	0	0	0	0	0	0	0	0
 61^c	1h	0	0	0	0	0	0	0	0
	ON	-	-	-	-	-	-	-	-
 62	1h	100	>100 ^d (54%)	100	100	100	>100 ^d (N/A)	0	0
	ON	>100 ^d (7%)	>100 ^d (59%)	100	100	>100 ^d (22%)	>100 ^d (62%)	0	0
 63^c	1h	-	-	-	-	-	-	-	-
	ON	0	1	0	1	0	1	0	0

Compound	Time	Thal	RebH	10S	6TL	0K	4V	Rdc2	Gsfl
 64	1h	100	100	0	1	0	100	0	0
	ON	100	100	0	100	100	30	0	0
 65	1h	100	>100 ^d (57%)	100	100	100	>100 ^d (N/A)	0	0
	ON	100	>100 ^d (99%)	100	100	>100 ^d (N/A)	>100 ^d (99%)	0	0
 66^c	1h	0	0	0	0	0	0	0	0
	ON	-	-	-	-	-	-	-	-
 67	1h	0	>100 ^d (99%)	1	0	100	>100 ^d (N/A)	10	5
	ON	>100 ^d (99%)	>100 ^d (100%)	69	>100 ^d (100%)	>100 ^d (99%)	>100 ^d (100%)	10	5
 68	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 69	1h	0	100	1	1	51	0	0	0
	ON	100	100	37	100	0 ^a	100	0	0
 70	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 71	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 72	1h	0	0	0	0	0	0	0	0
	ON	0	1	0	0	0	0	0	0

Compound	Time	Thal	RebH	10S	6TL	0K	4V	Rdc2	Gsfl
 73	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 74	1h	100	14	0	14	11	1	0	0
	ON	55	1	1	1	67	1	0	0
 75	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 76	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 77	1h	17	20	1	28	1	35	0	0
	ON	24	28	5	77	6	26	0	0
 78	1h	100	100	1	100	1	100	0	0
	ON	82	64	39	>100 ^d (99%)	1	>100 ^d (100%)	0	0
 79	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 80	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0

Compound	Time	Thal	RebH	10S	6TL	0K	4V	Rdc2	Gsfl
 81	1h	0	0	1	1	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 82	1h	100	100	100	100	1	100	0	0
	ON	100	100	1	100	1	100	0	0
 83	1h	100	84	1	100	25	100	10	1
	ON	37	100	1	1	86	100	10	1
 84	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 85	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 86	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 87	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 88	1h	0	0	0	0	0	0	0	0
	ON	0	100	0	0	0	0	0	0

Compound	Time	Thal	RebH	10S	6TL	0K	4V	Rdc2	Gsfl
 89	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 90^c	1h	0	0	0	0	0	0	0	0
	ON	-	-	-	-	-	-	-	-
 91	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0
 92	1h	0	0	0	0	0	8	0	0
	ON	10	11	10	6	0	24	0	0
 93	1h	0	0	0	0	0	0	0	0
	ON	0	0	0	0	0	0	0	0

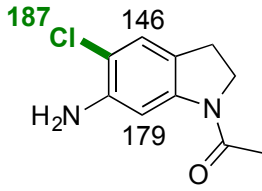
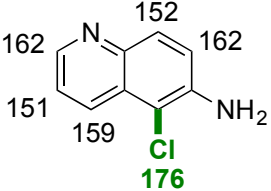
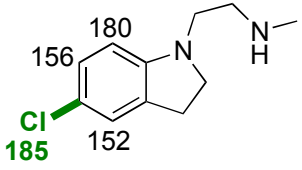
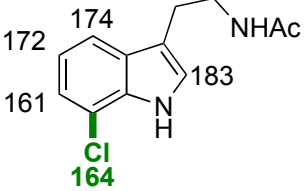
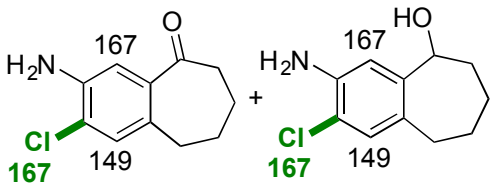
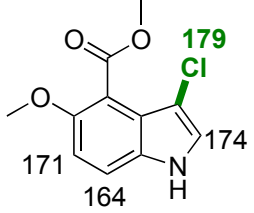
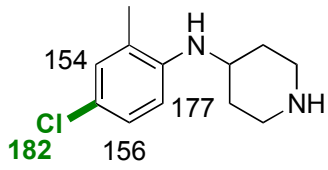
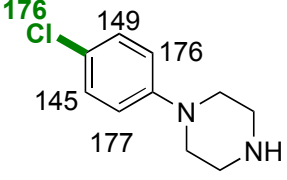
^a Species with a molecular weight "M-2" was observed.

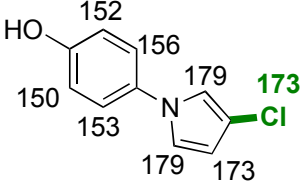
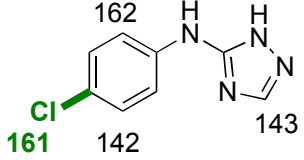
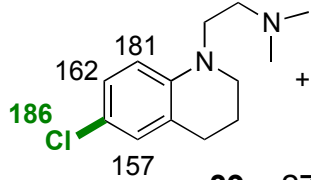
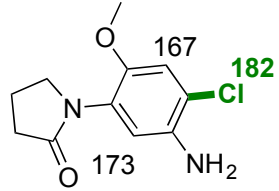
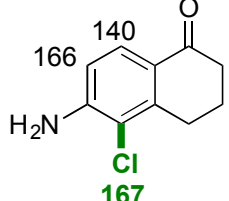
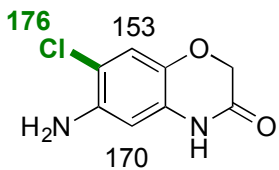
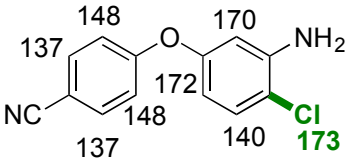
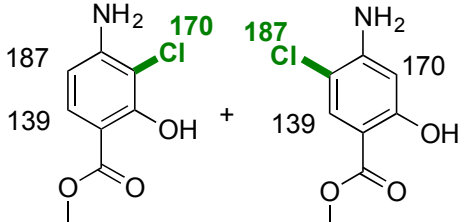
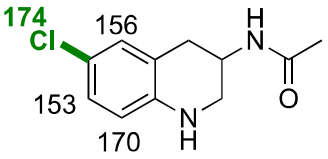
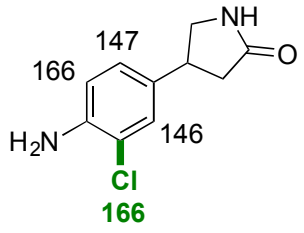
^b Species with a molecular weight "M-2+Cl" was observed. In this table, the listed conversion data includes both "M+Cl" as well as the "M-2+Cl" product.

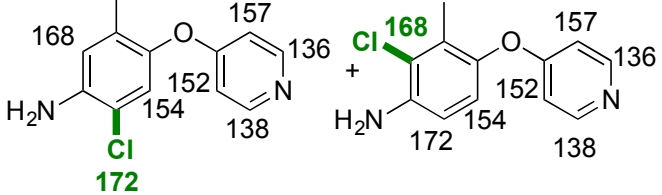
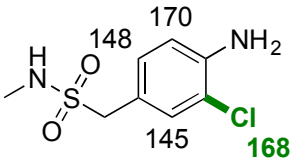
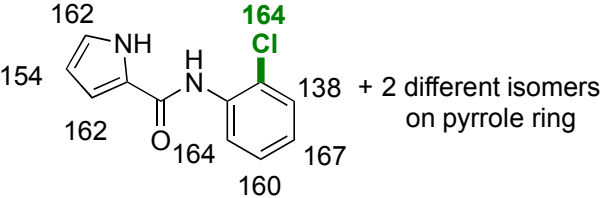
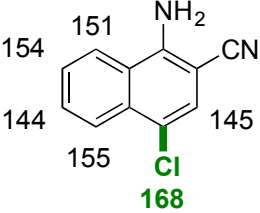
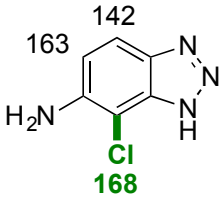
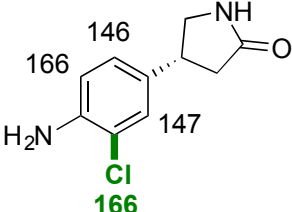
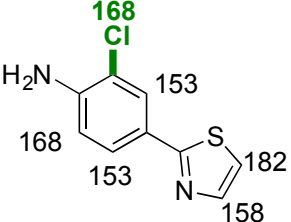
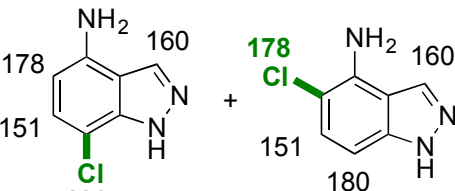
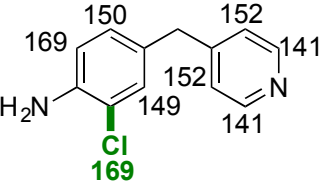
^c For these substrates, neither starting material nor product was observed.

^d When a substrate was completely halogenated at one site, and began dichlorination at another site, this is indicated by ">100%." Values in parentheses underneath this are % conversions to dichlorinated product.

Table S2: Isolated products from bioconversions. HalA values for all sp^2 hybridized C-H bonds are shown next to the respective C-H bond. The HalA value for the C-H bond that was chlorinated in each case is shown in green. Substrate numbers are bolded, followed by the % conversion to product (LCMS).

 <p>29a, 100%</p>	 <p>30a, 100%</p>
 <p>31a, 89%</p>	 <p>32a, 100%</p>
 <p>33a, 33b, 6%</p>	 <p>34a, 24%</p>
 <p>35a, 100%</p>	 <p>36a, 100%</p>

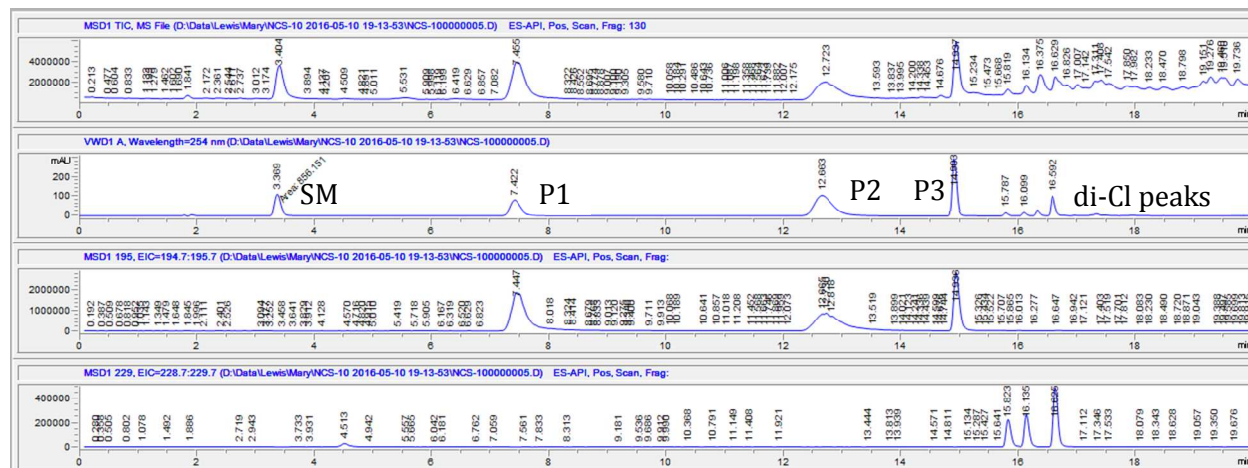
 <p>37a, 57%</p>	 <p>38a, 14%</p>
 <p>39a, 27%</p> <p>+ M-2 chlorinated product</p>	 <p>40a, 54%</p>
 <p>41a, 15%</p>	 <p>42a, 95%</p>
 <p>43a, 100%</p>	 <p>44a, 5% 44b, 81%</p>
 <p>45a, 41%</p> <p>+ dichlorinated product</p>	 <p>62a, 100%</p>

 <p>46a, 25% 46b, 60%</p>	 <p>48a, 33%</p>
 <p>49a, 6%</p>	 <p>52a, 10%</p>
 <p>64a, N/A</p>	 <p>65a, 100%</p>
 <p>77a, 33%</p>	 <p>67a, 4% 67b, 64%</p>
 <p>82a, 79%</p>	

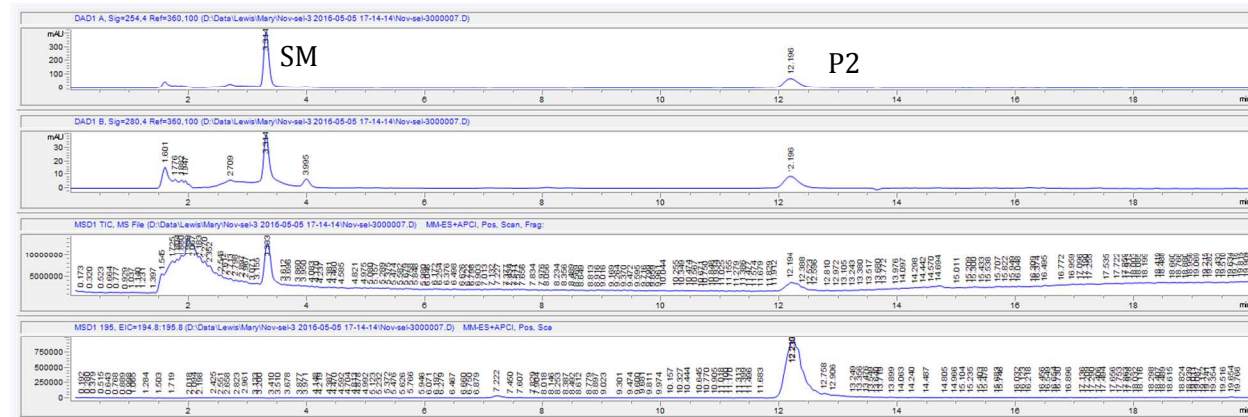
Selectivity comparison with NCS: Data for selectivities shown in Table 1 of the manuscript. Numbering refers to Table 1 entries.

1. HNMR characterization of both RebH and NCS products for comparison.
2. LCMS characterization. Shown below are UV traces at 254 nm, total ion count, and extracted ion for the mono-chlorinated product, as well as the di-chlorinated product when necessary.

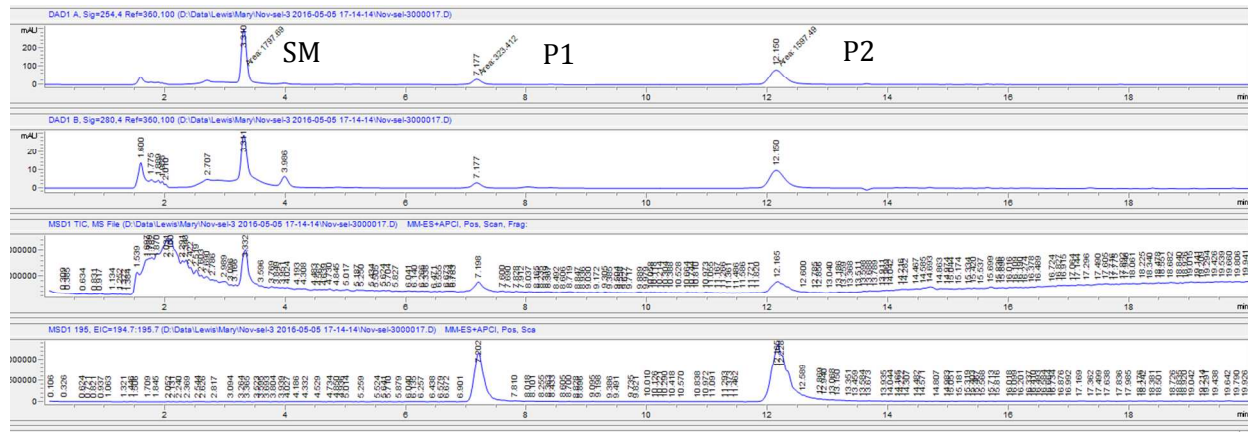
NCS



RebH

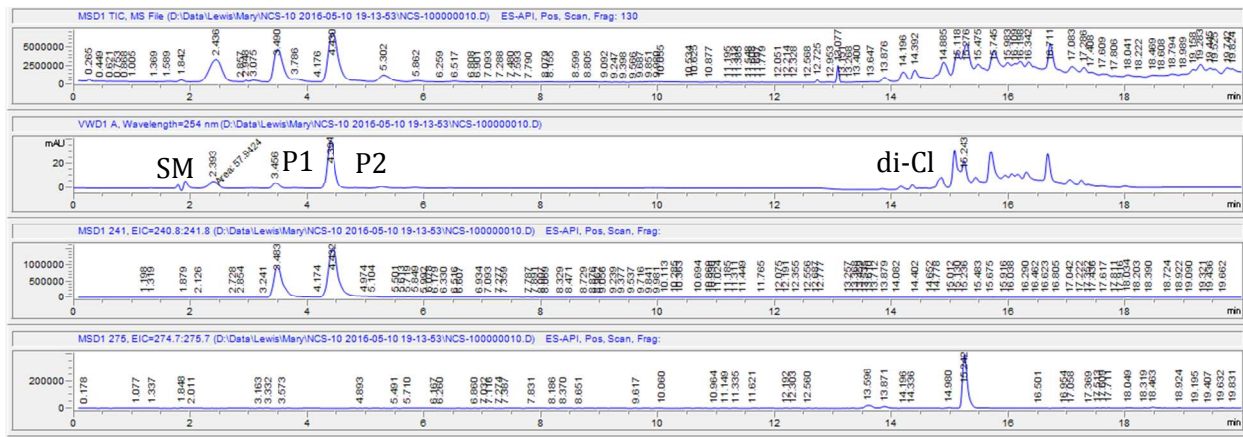


6TL



3. LCMS characterization. Shown below are UV traces at 254 nm, total ion count, and extracted ion for the mono-chlorinated product, as well as the di-chlorinated product when necessary.

NCS

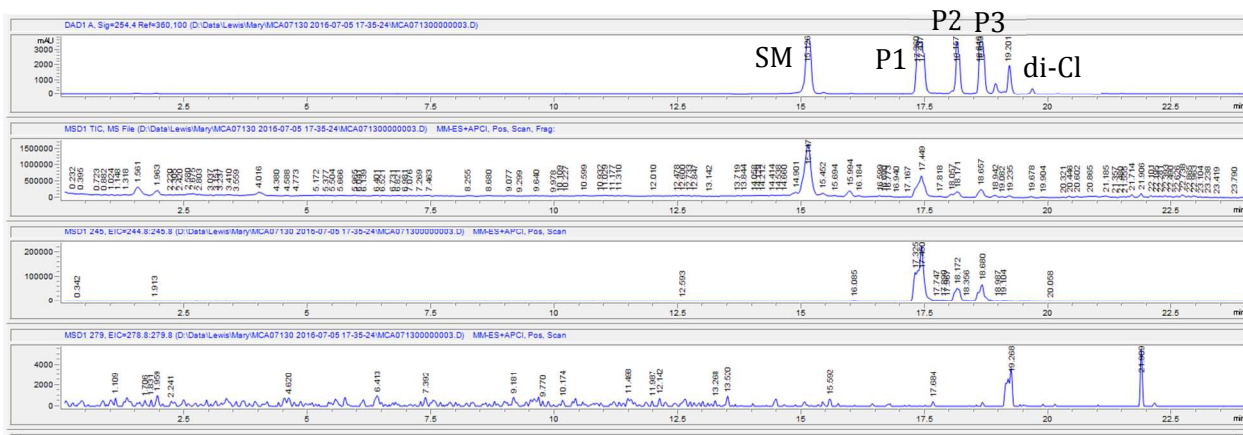


RebH

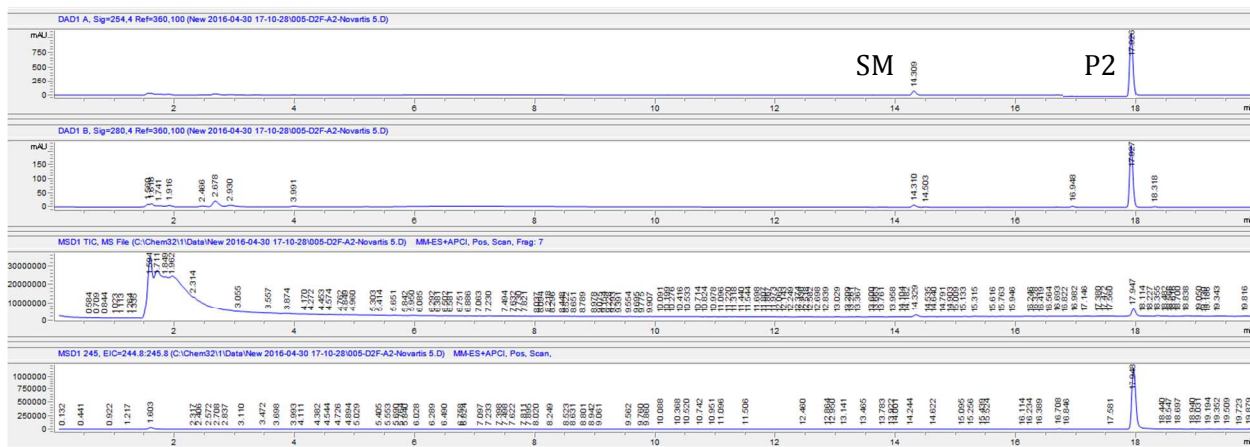


4. LCMS characterization. Shown below are UV traces at 254 nm, total ion count, and extracted ion for the mono-chlorinated product, as well as the di-chlorinated product when necessary.

NCS



RebH

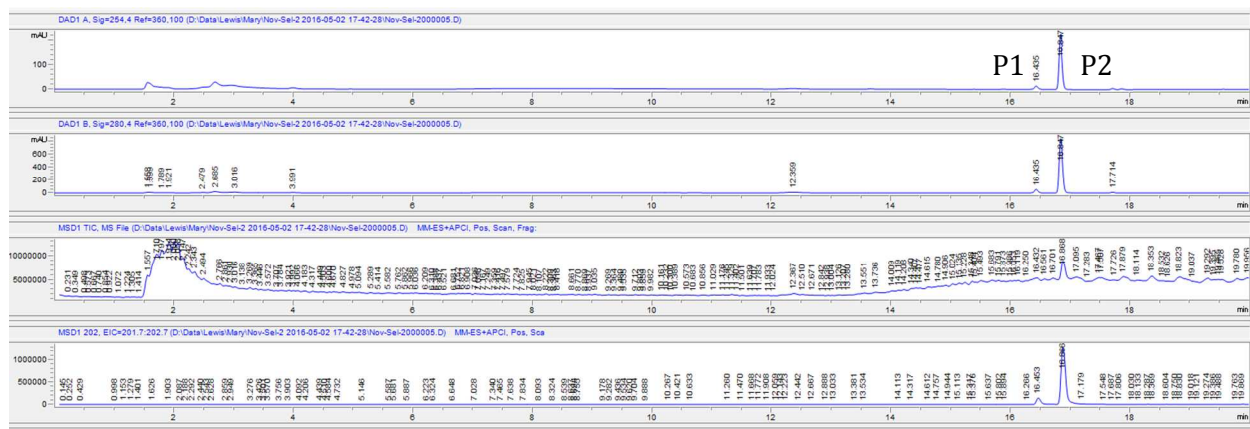


5. LCMS characterization. Shown below are UV traces at 254 nm, total ion count, and extracted ion for the mono-chlorinated product.

NCS

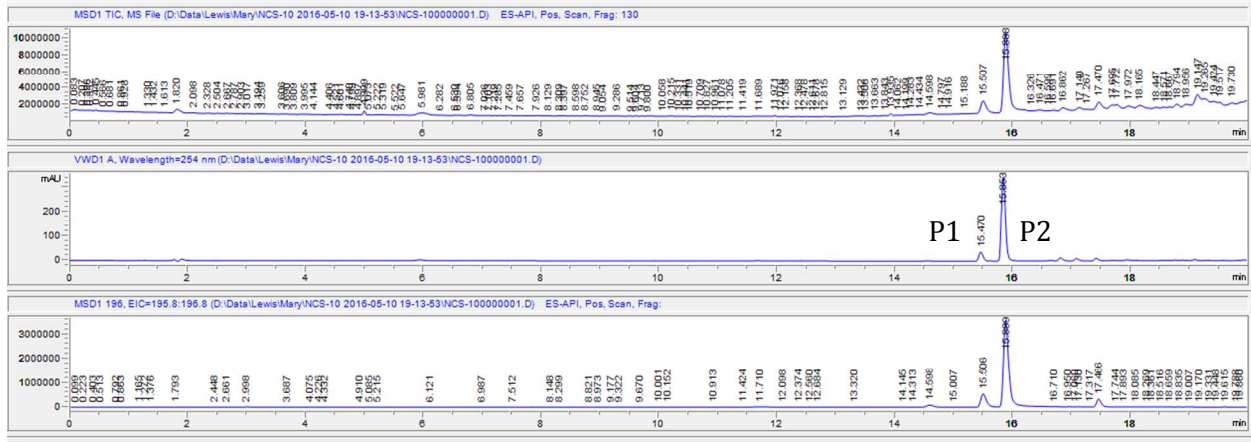


6TL



6. LCMS characterization. Shown below are UV traces at 254 nm, total ion count, and extracted ion for the mono-chlorinated product.

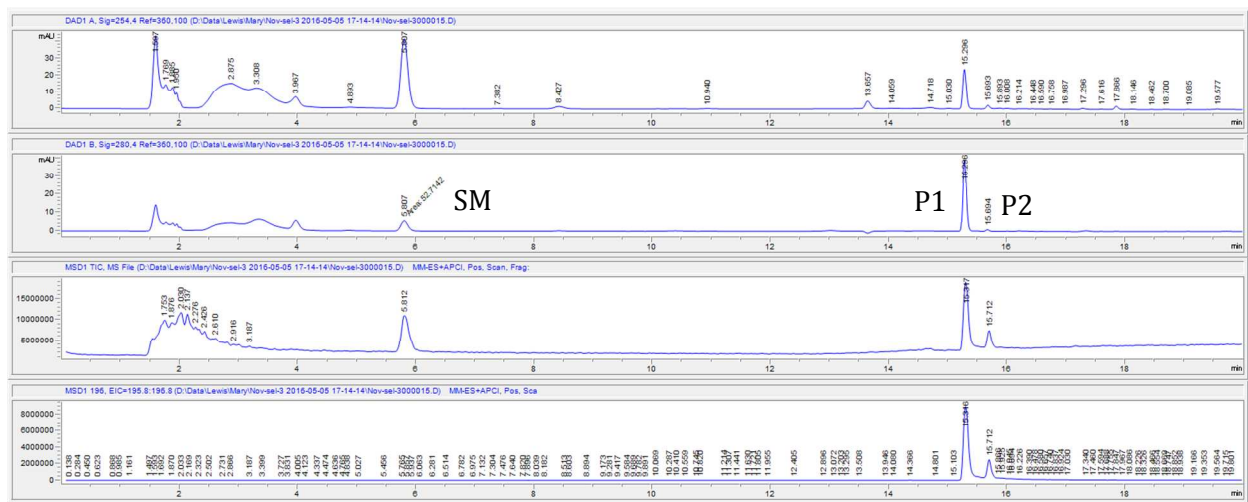
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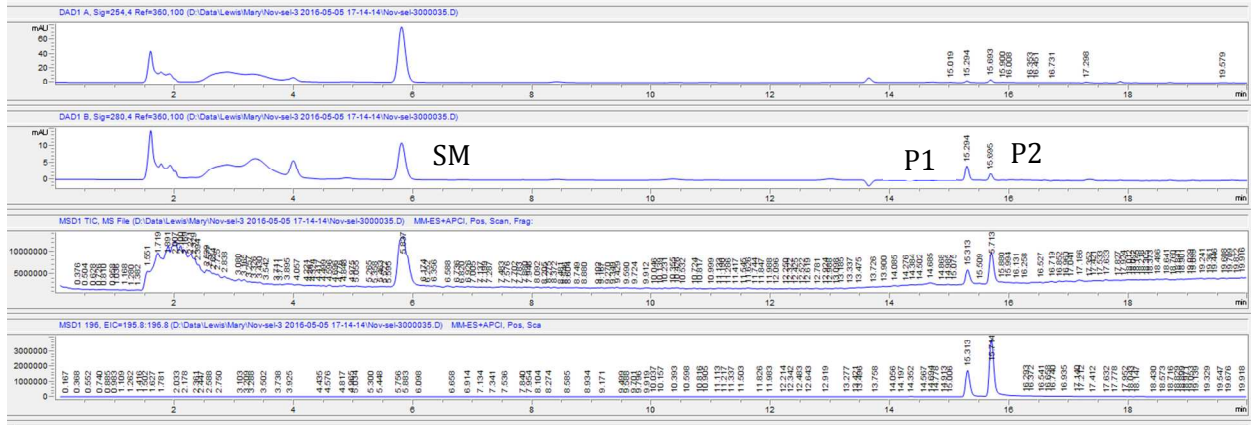
RebH



6TL

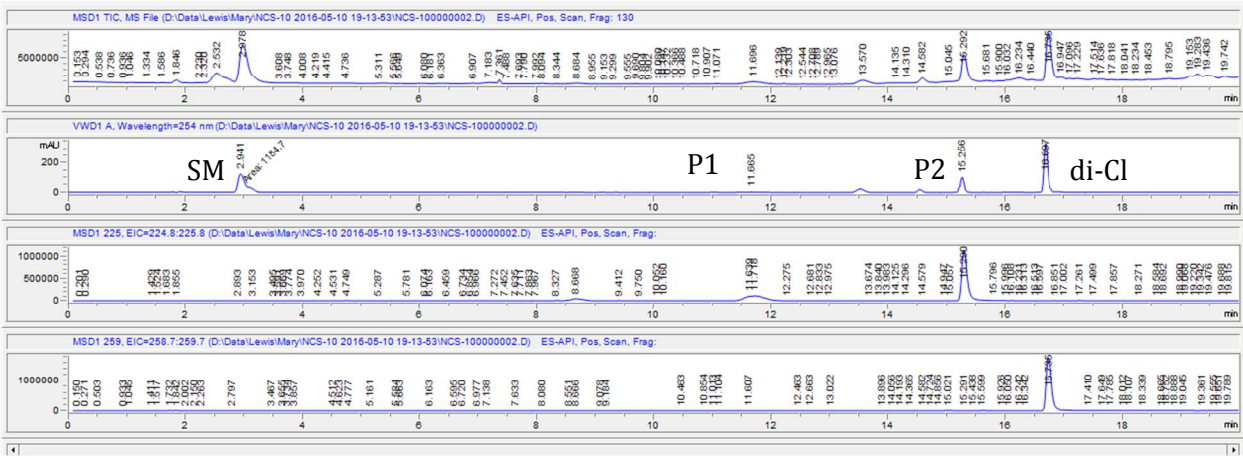


4V



7. LCMS characterization. Shown below are UV traces at 254 nm, total ion count, and extracted ion for the mono-chlorinated product, as well as the di-chlorinated product when necessary.

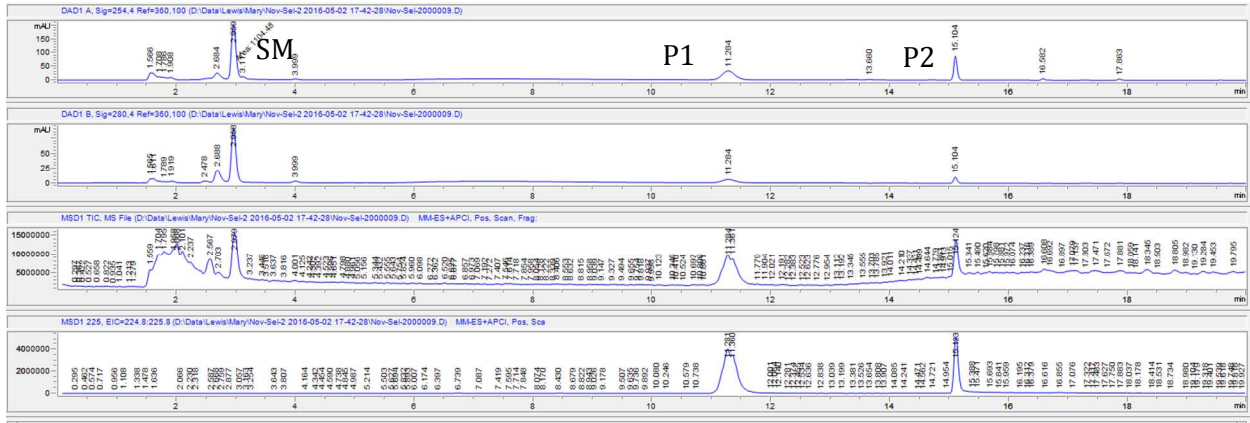
NCS



RebH

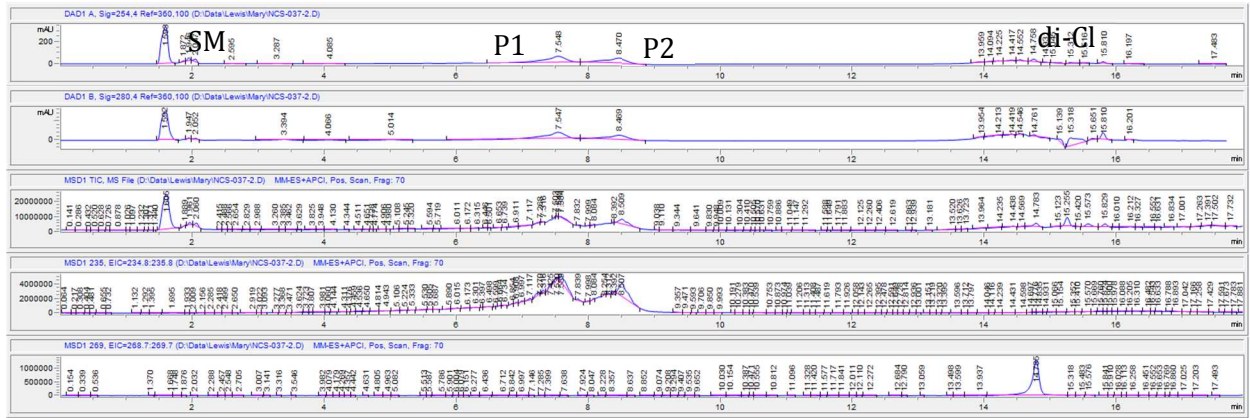


6TL

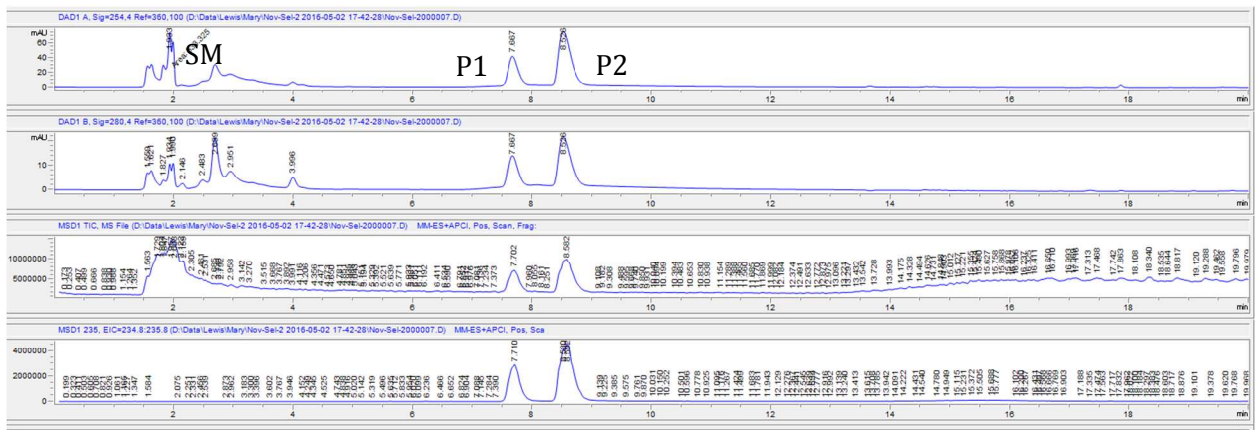


8. LCMS characterization. Shown below are UV traces at 254 nm, total ion count, and extracted ion for the mono-chlorinated product, as well as the di-chlorinated product when necessary.

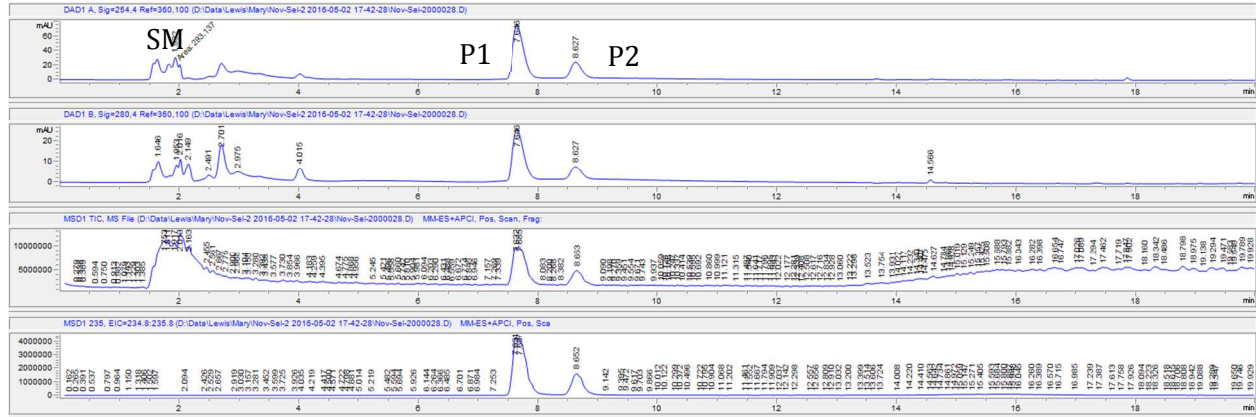
NCS



RebH

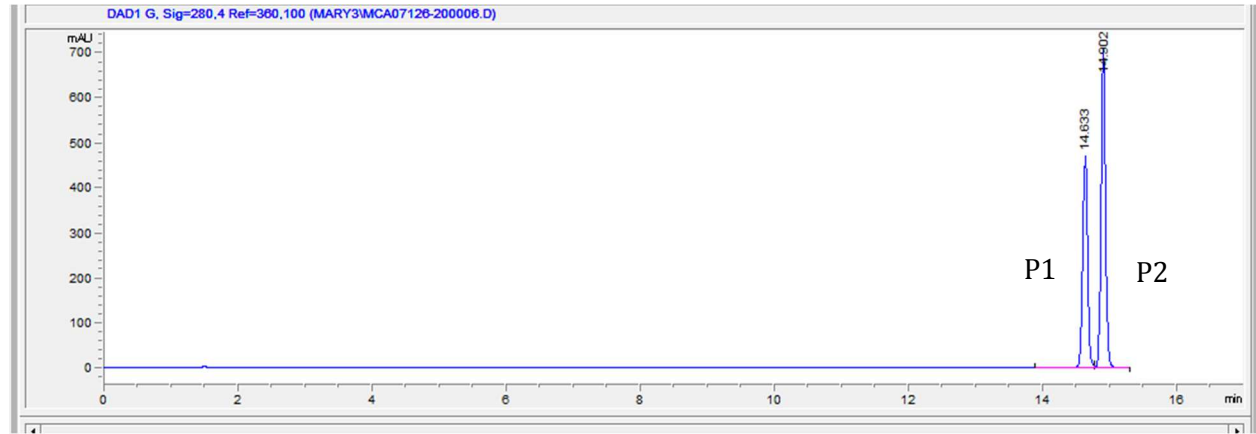


4V

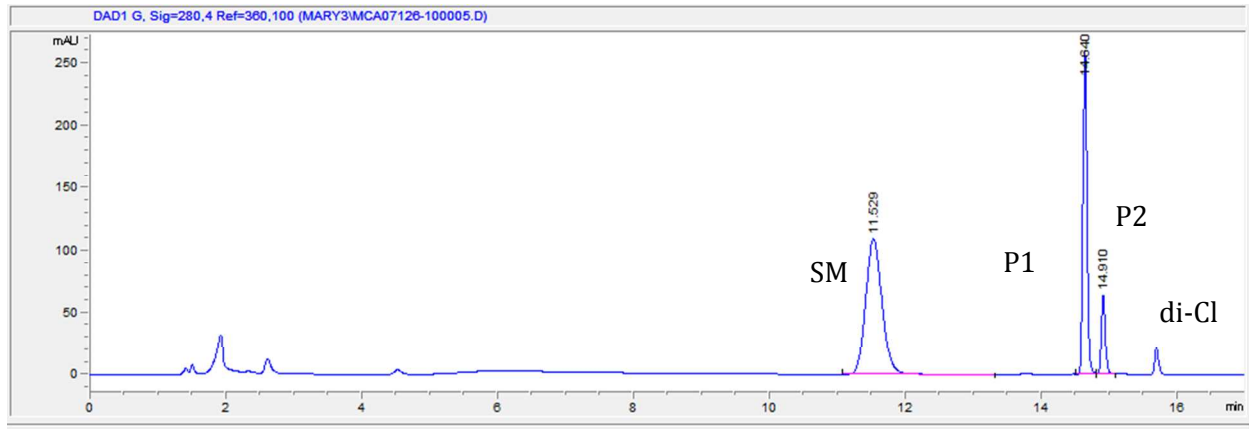


9. UHPLC characterization. The NCS product trace shown below was isolated and characterized by HNMR.

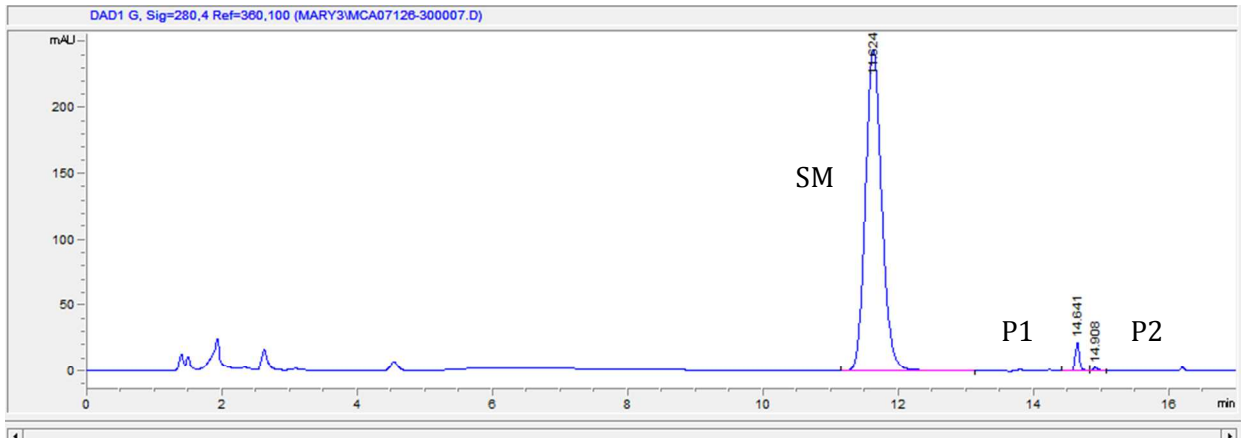
NCS



Rdc2

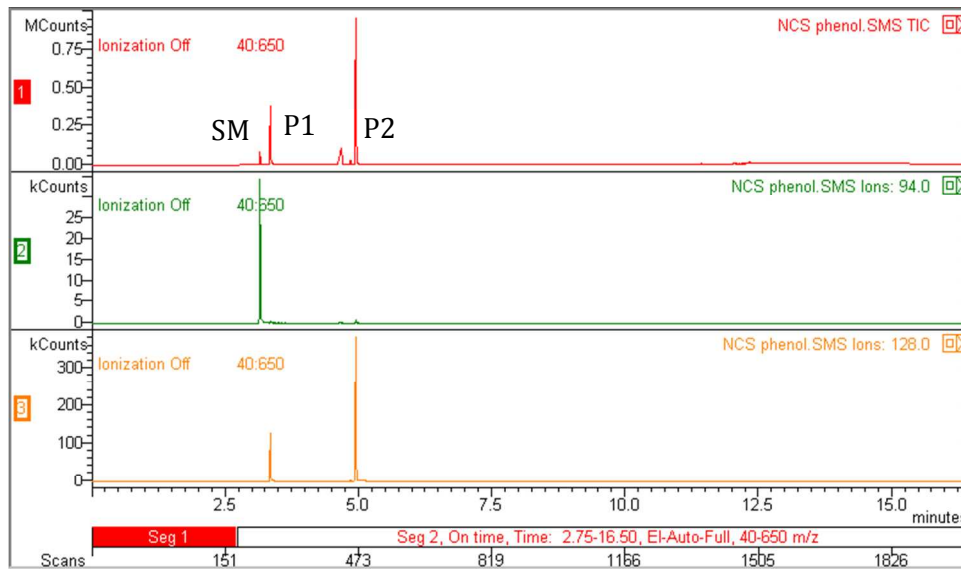


Gsfl

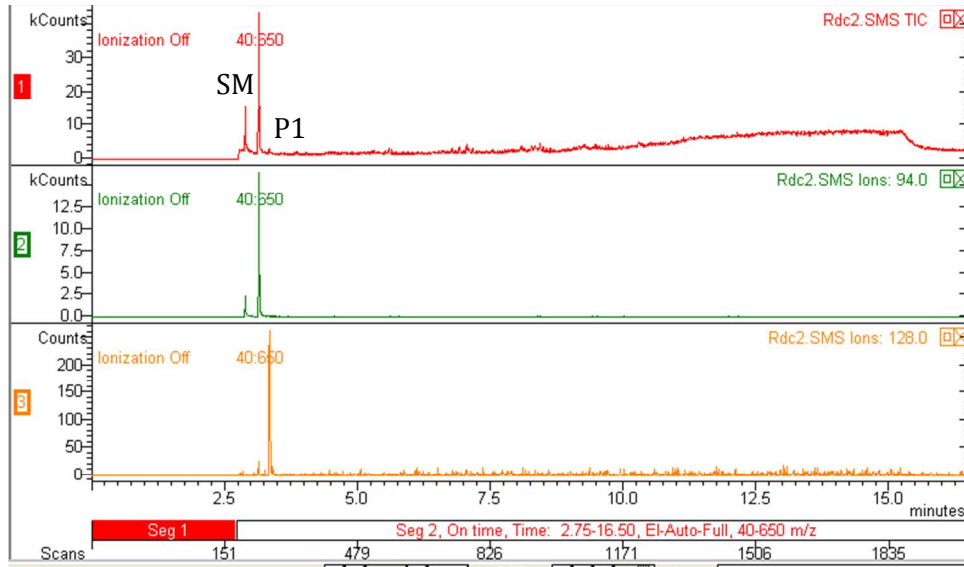


10. GCMS characterization. Shown below are the total ion count and extracted ion traces for the starting material and mono-chlorinated product.

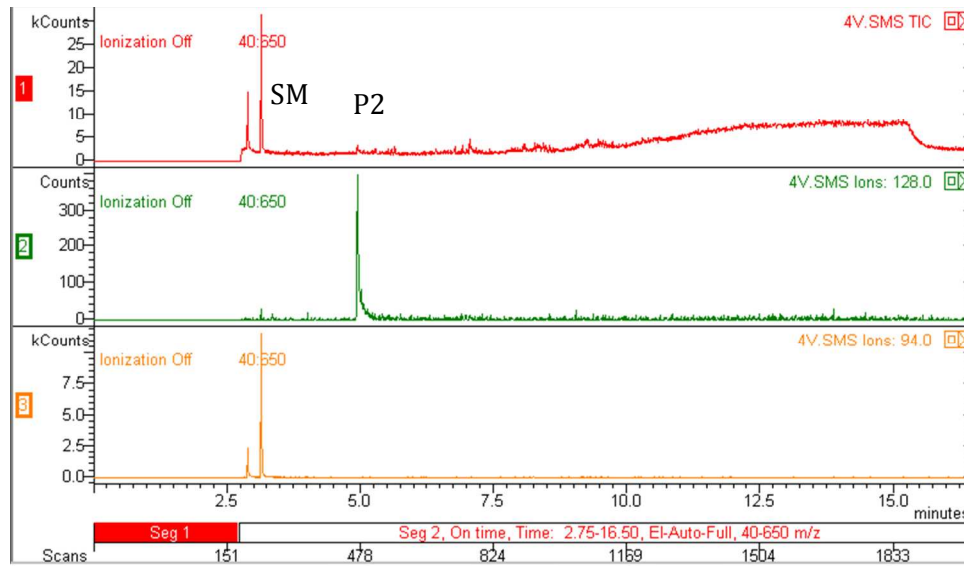
NCS



Rdc2



4V

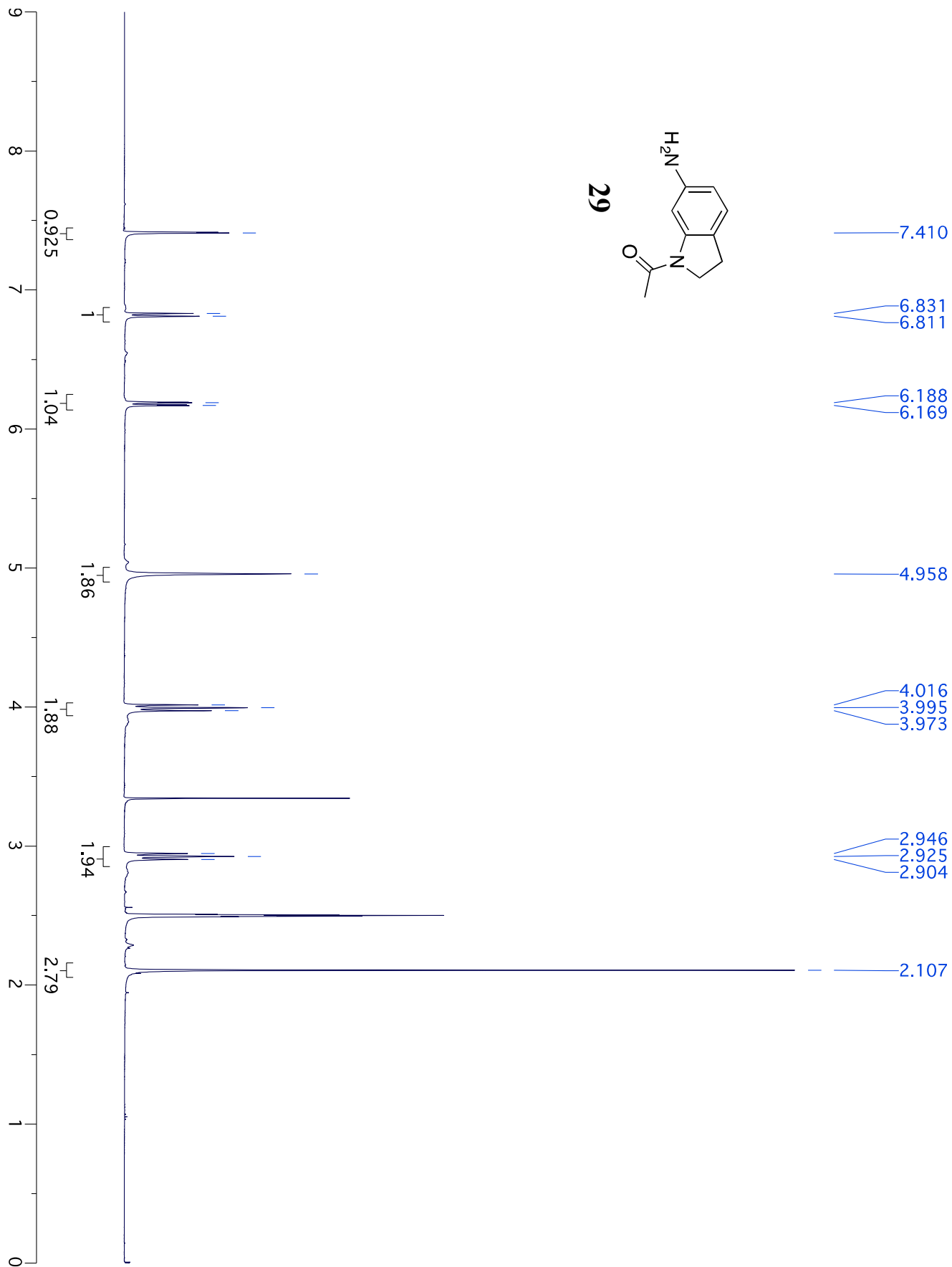
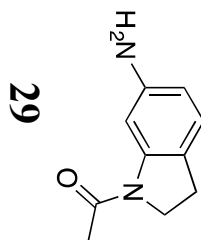


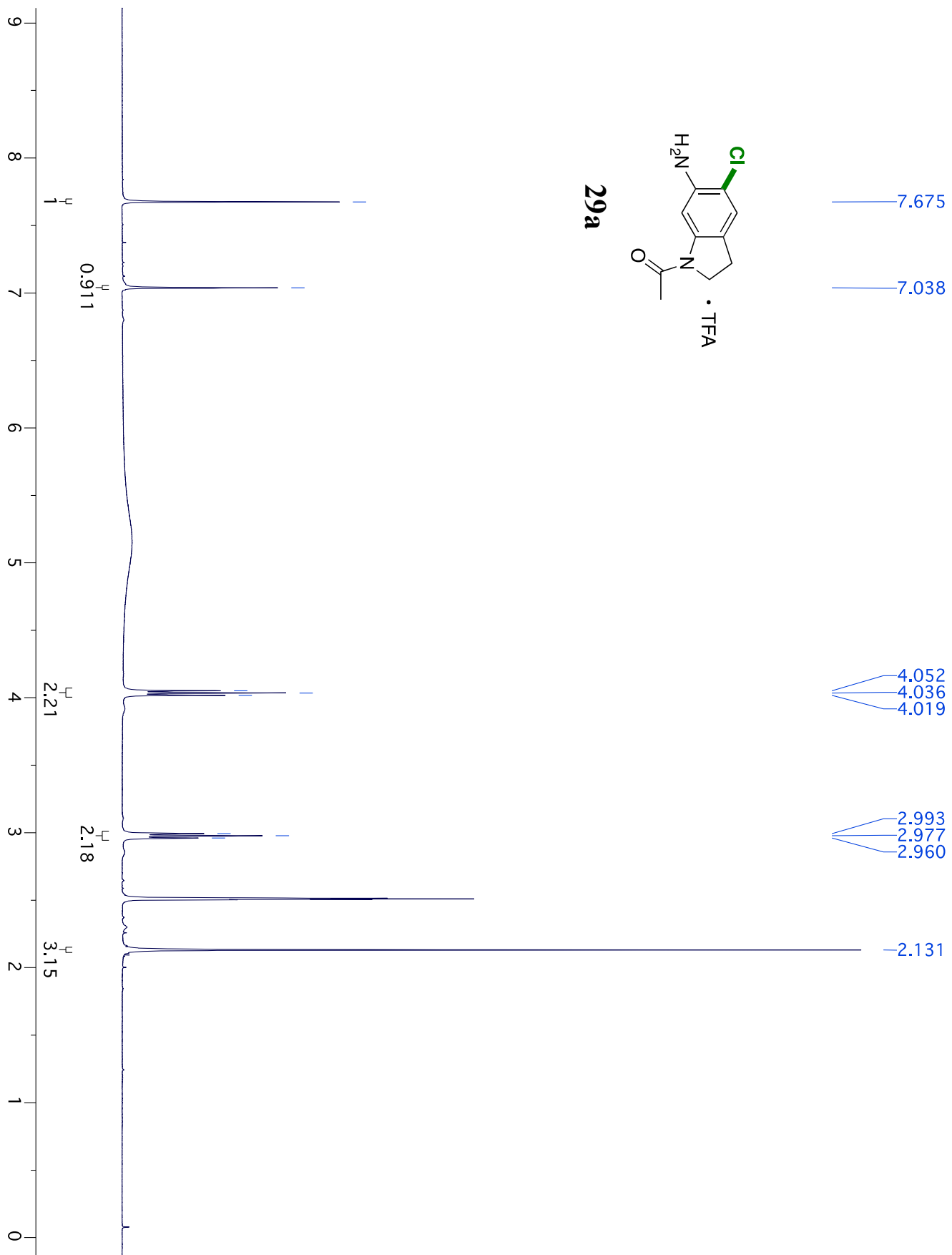
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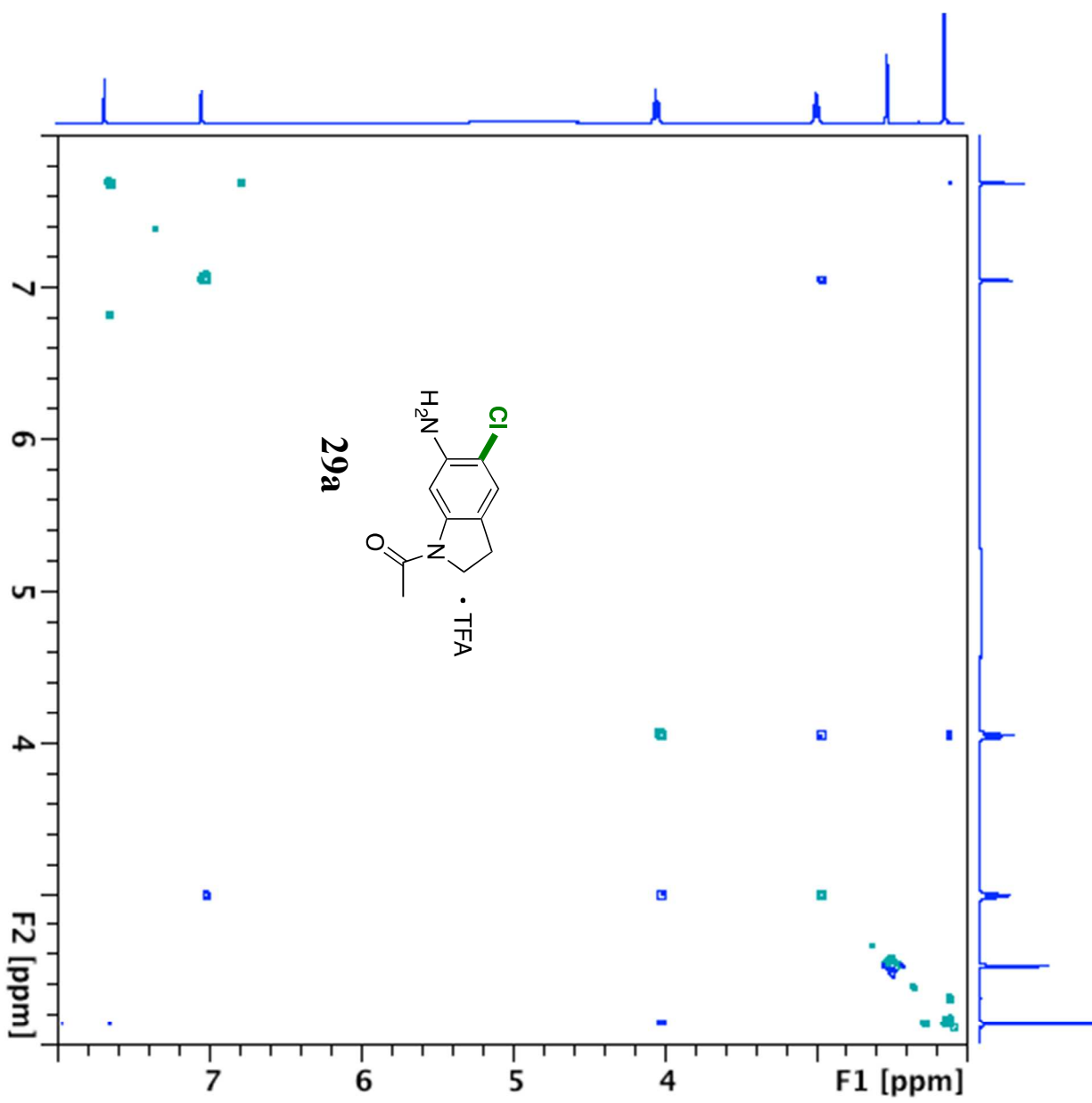
1. Yeh, E.; Garneau, S.; Walsh, C. T. *Proc. Nat. Acad. Sci.* **2005**, *102*, 3960-3965.
2. Cabrita, L. D.; Dai, W.; Bottomley, S. P. *BMC Biotech.* **2006**, *6*:12.
3. Seibold, C.; Schnerr, H.; Rumpf, J.; Kunzendorf, A.; Hatscher, C.; Wage, T.; Ernyei, A. J.; Dong, C.; Naismith, J. H.; van Pée, K.-H. *Biocatal Biotransfor* **2006**, *24*, 401–408.
4. Payne, J. T.; Andorfer, M.C.; Lewis, J.C. *Angew. Chem.* **2013**, *125*, 5379 –5382.
5. Andorfer, M. C.; Park, H.-J.; Vergara-Coll, J.; Lewis, J. C. *Chem. Sci.* **2016**, *7*, 3720-3729.

NMR Characterization

For each compound isolated, the ^1H NMR and HRMS of the chlorinated compound is shown. In some cases, impurity peaks were observed in the ^1H NMR of the chlorinated products, which is due to the small scale of these reactions and the use of formic acid during prep LC/MS purification. For this reason, the ^1H NMR of the starting material for each is included as well as a reference and yields are not reported (only % conversions based on LCMS UV traces).





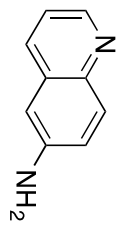


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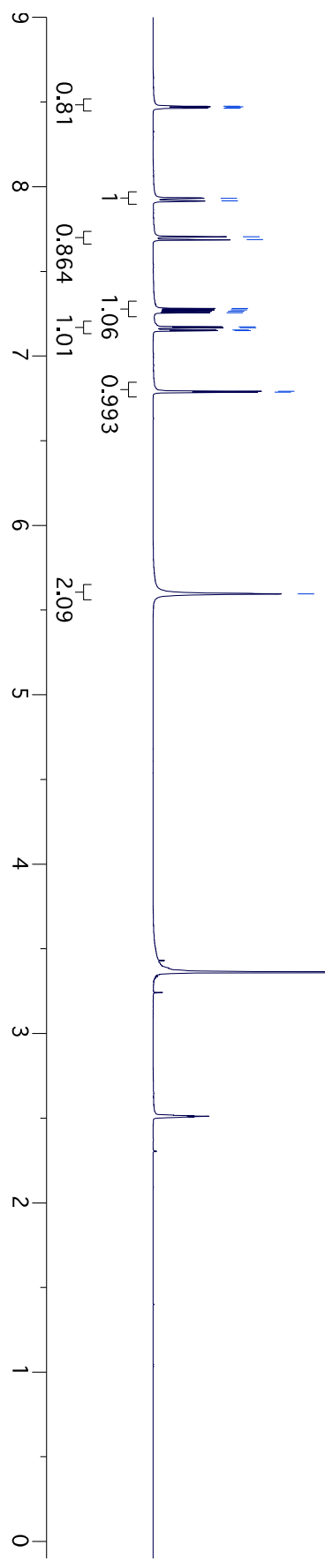
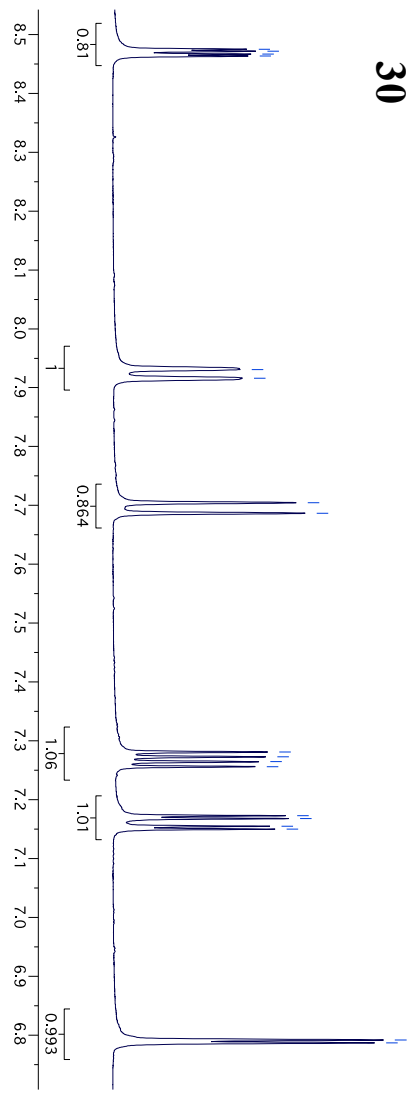
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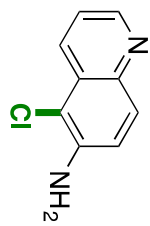


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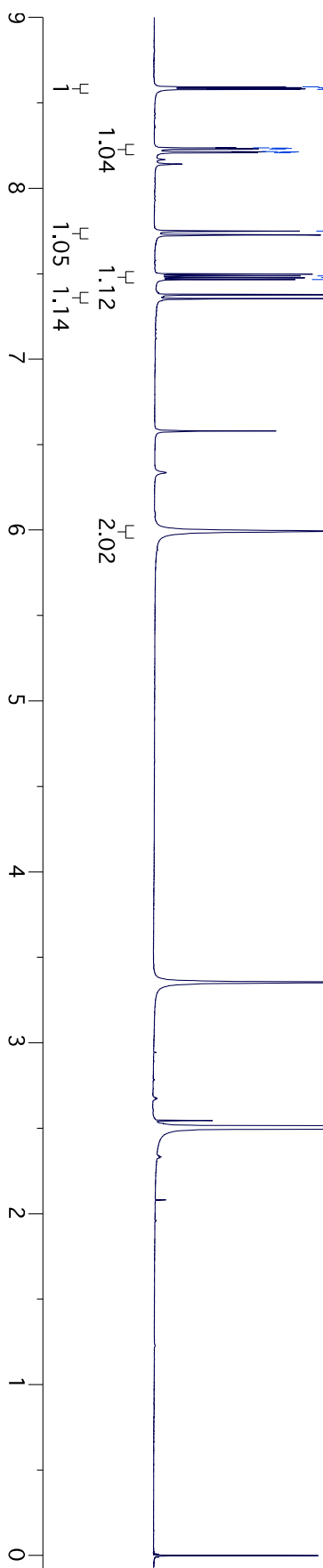
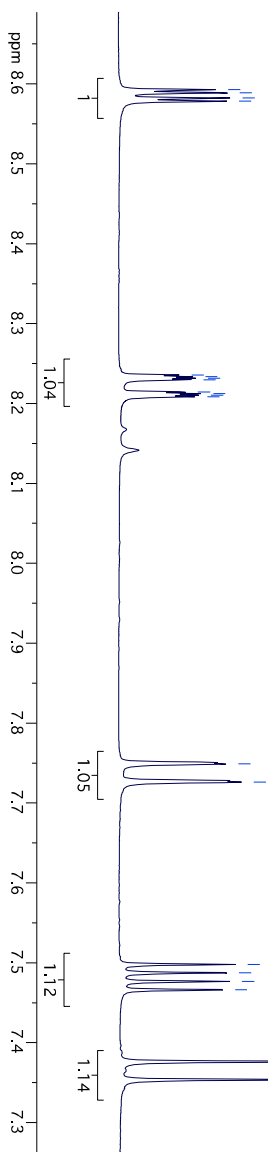


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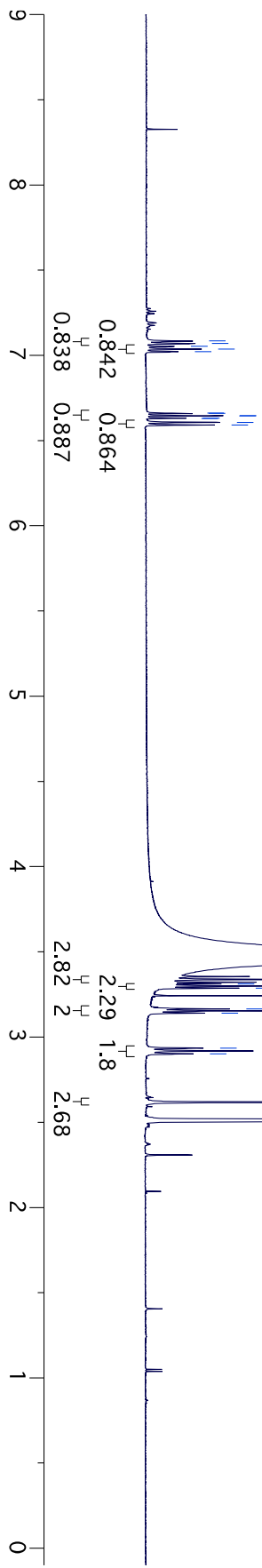
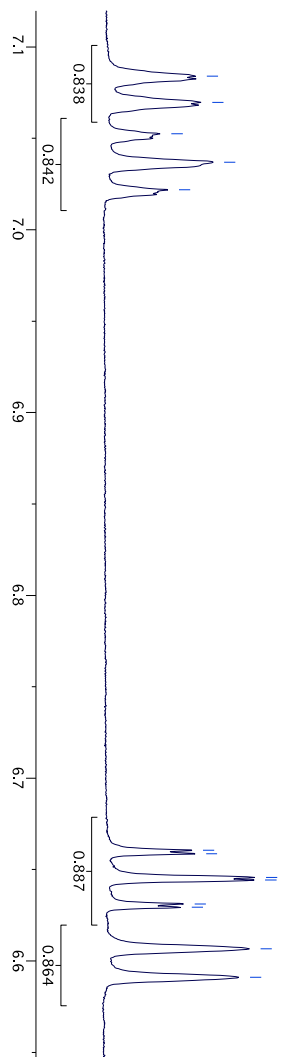
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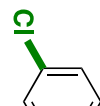




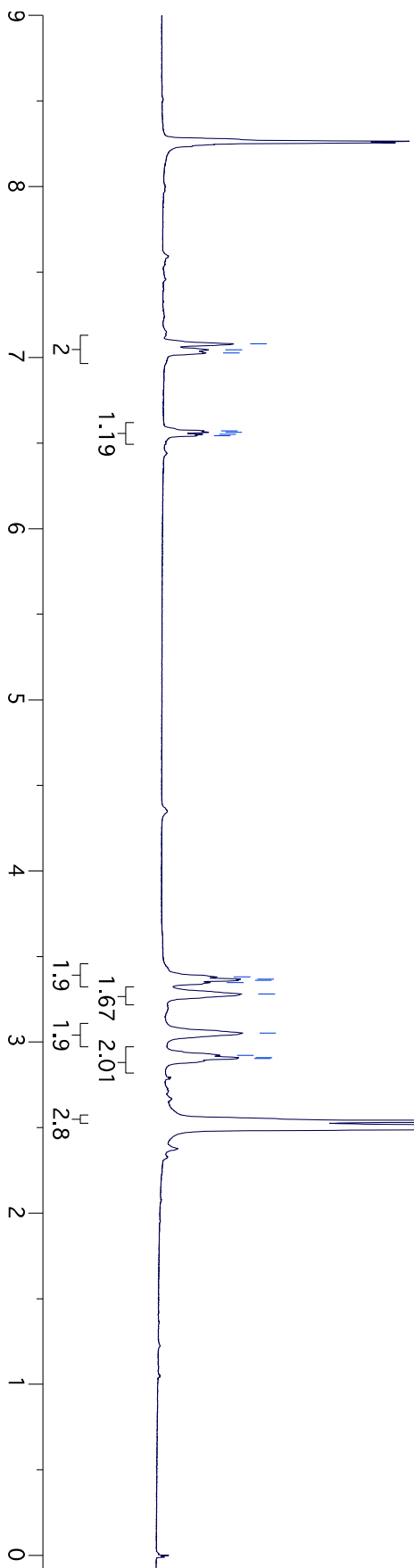
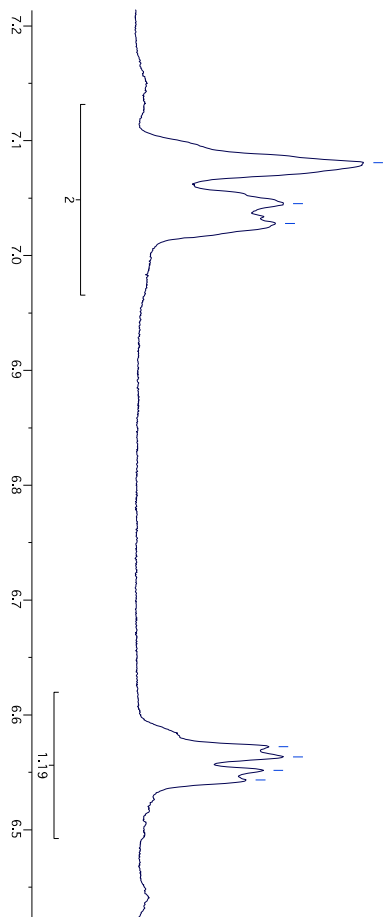
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- 2.902
- 2.617

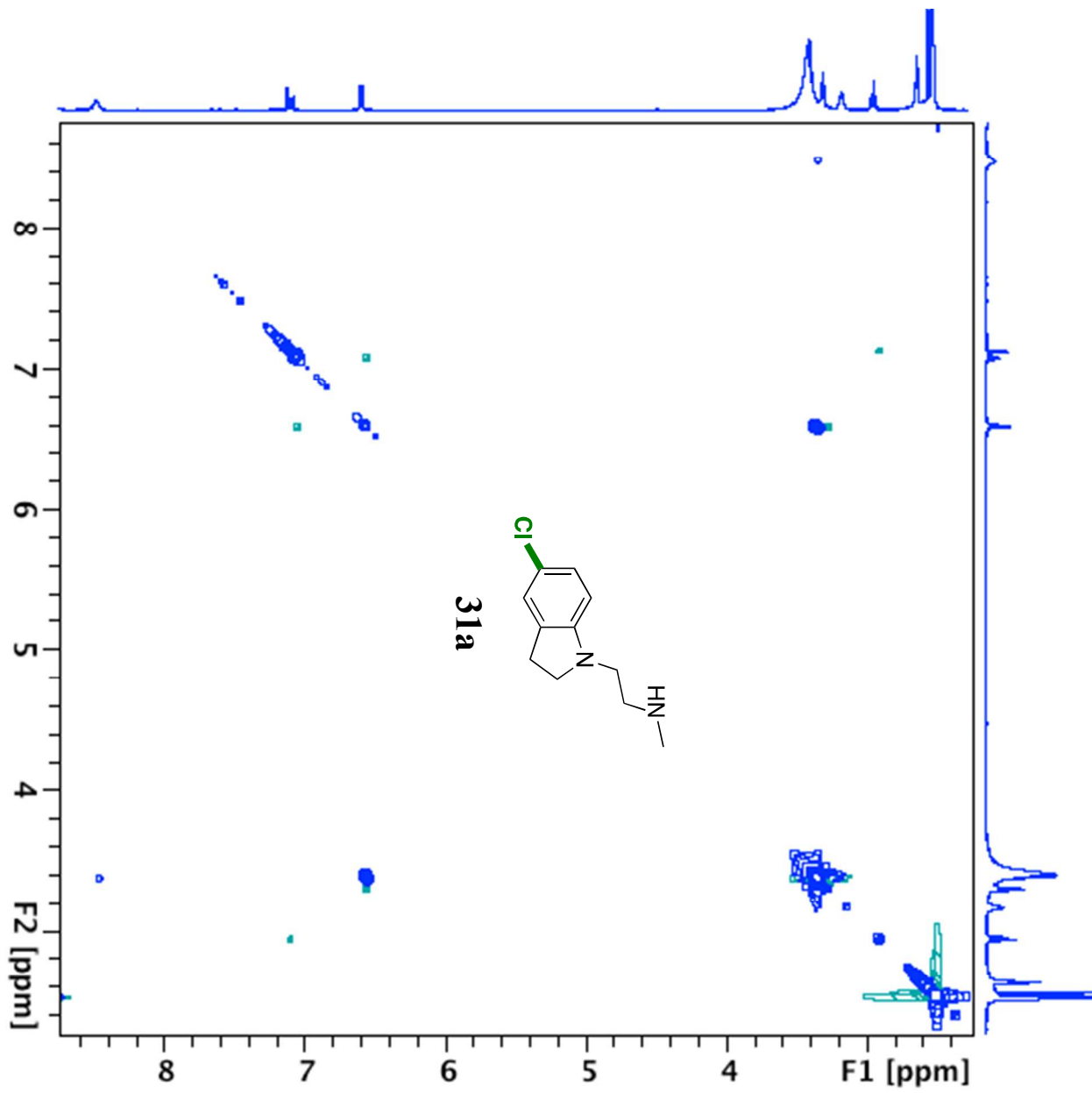
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31a

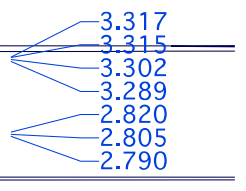
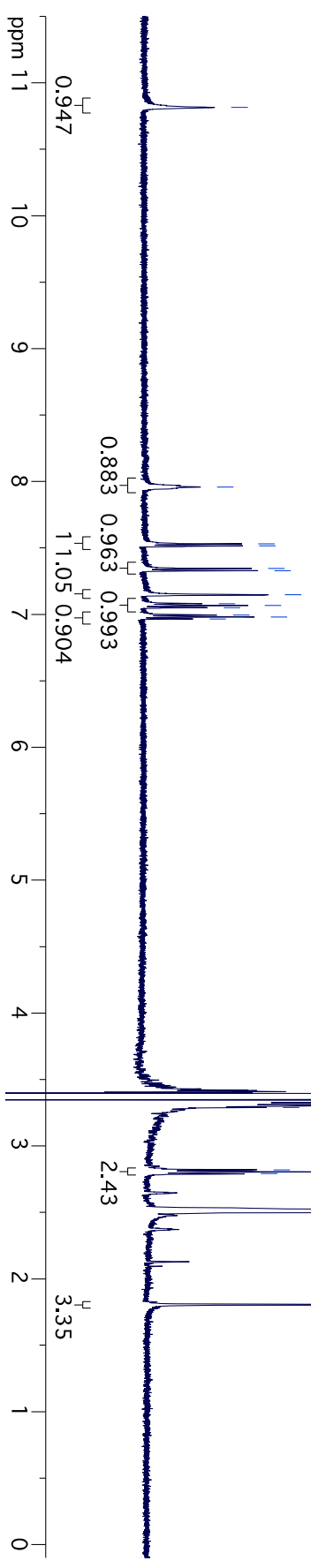
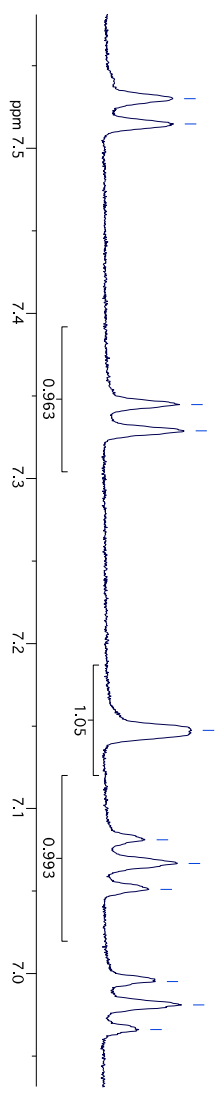




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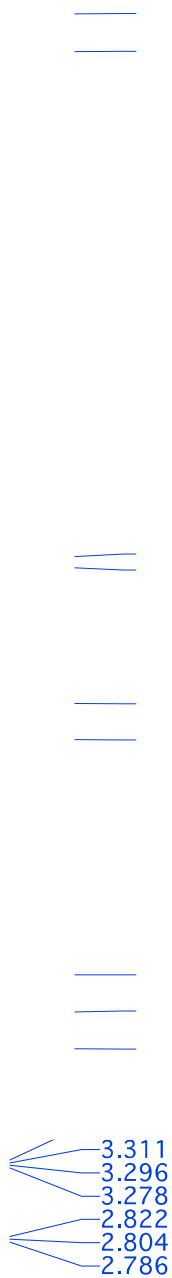
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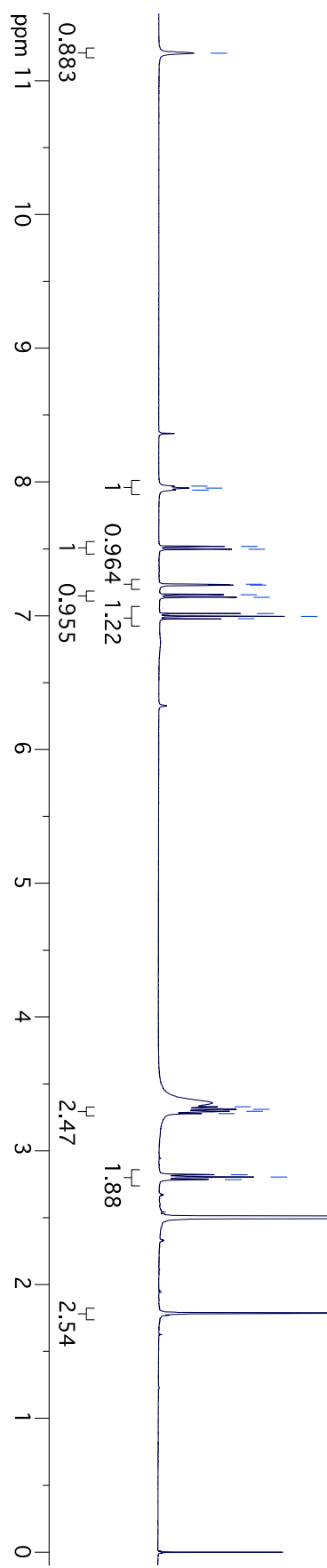
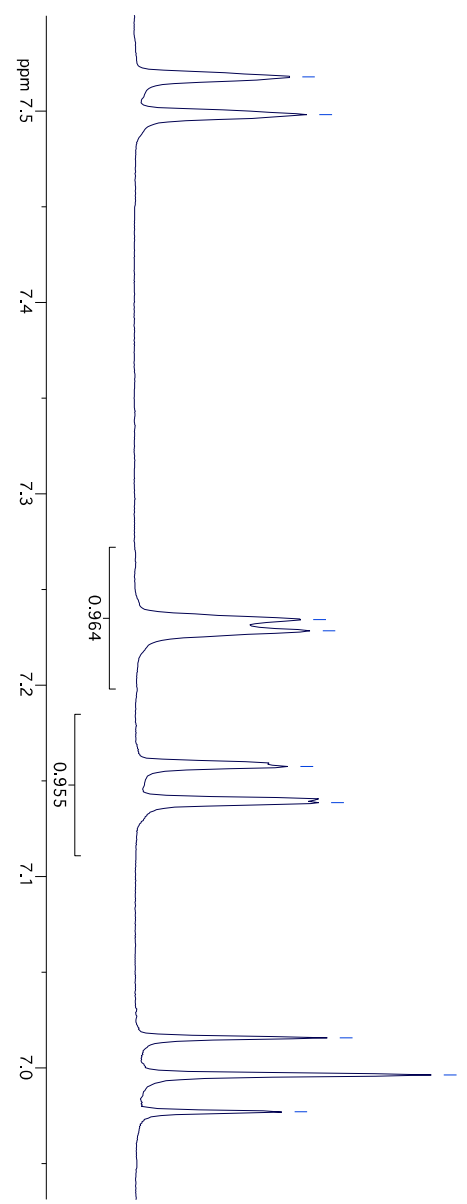
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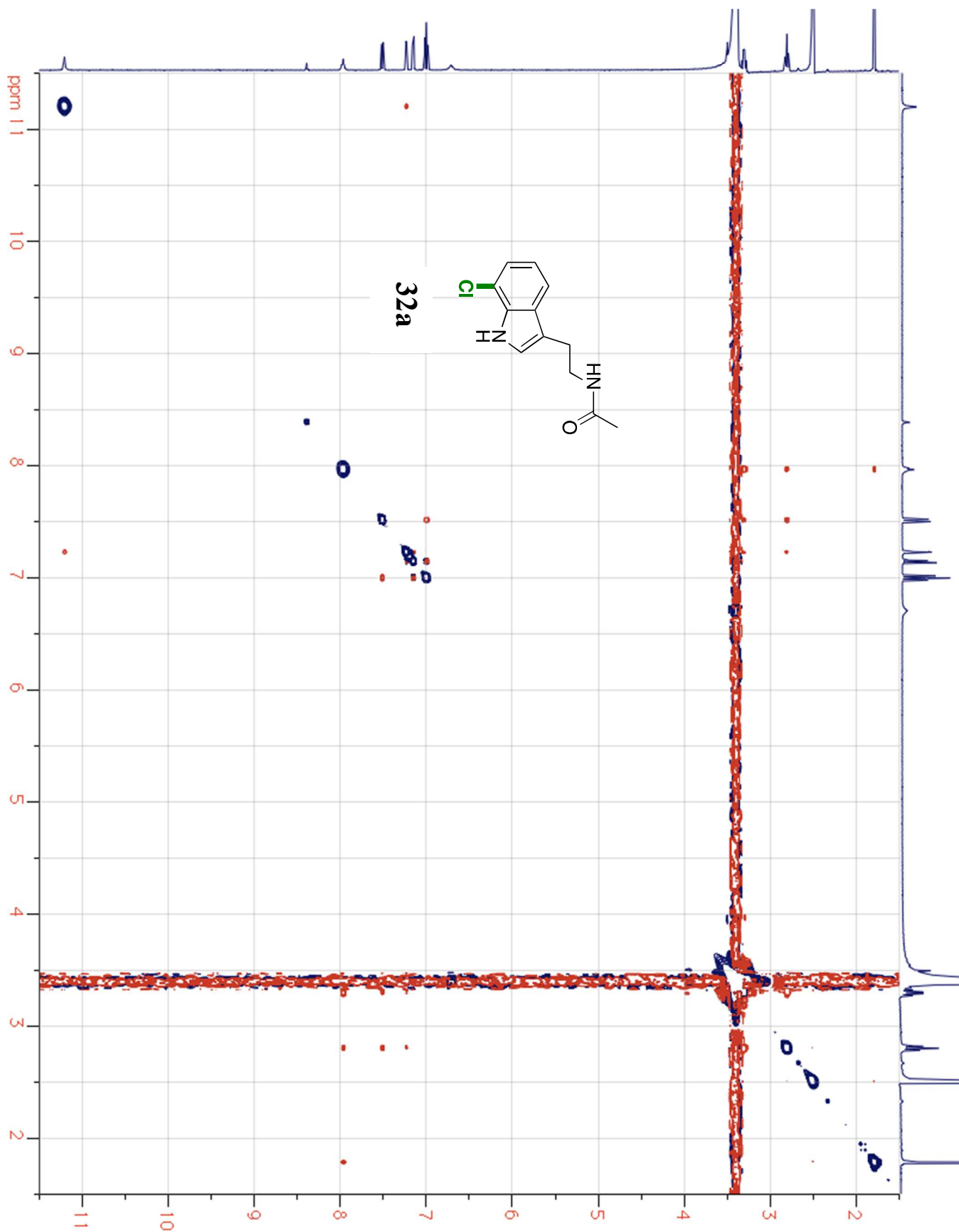
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1.787

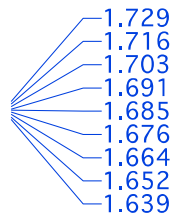
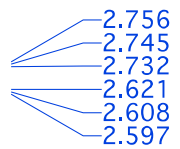
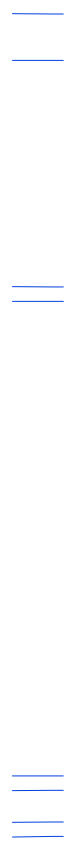
32a



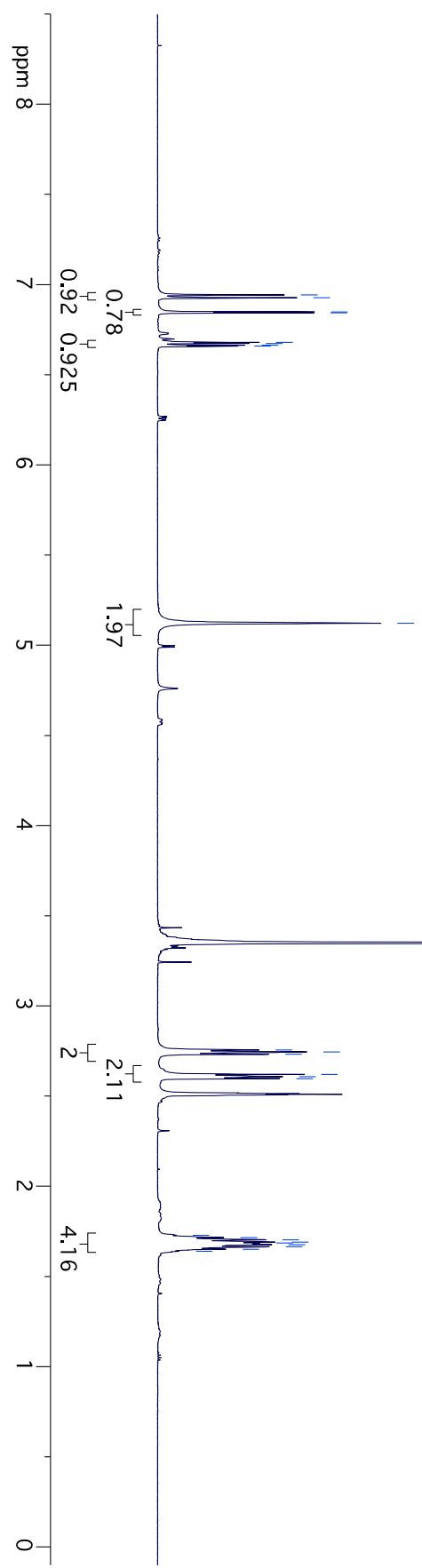
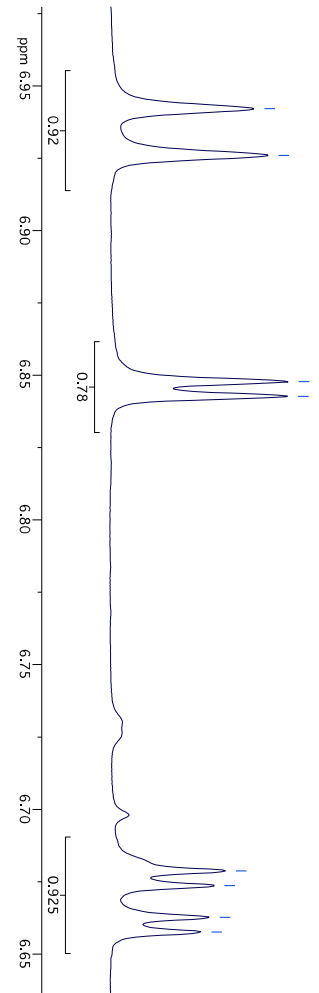


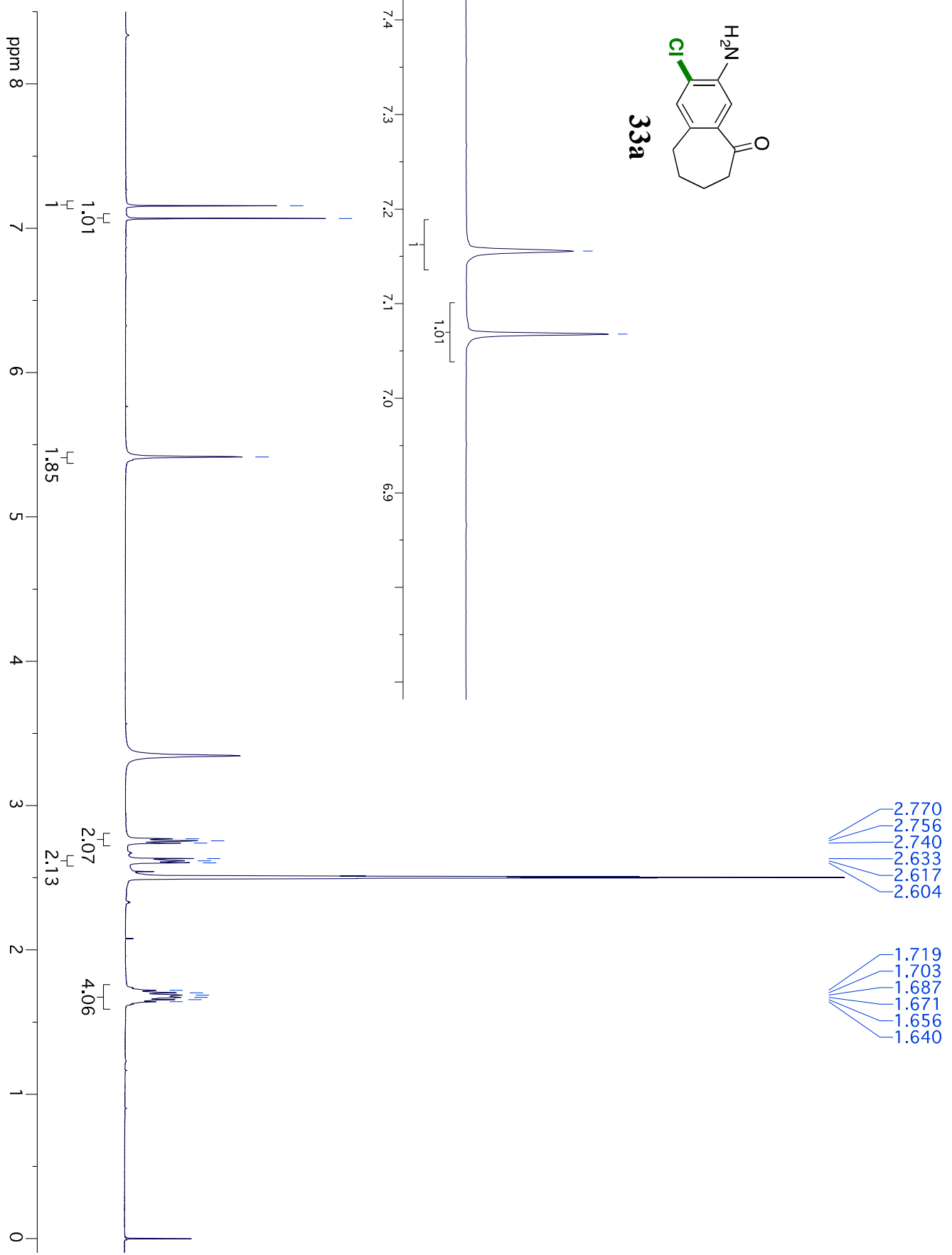
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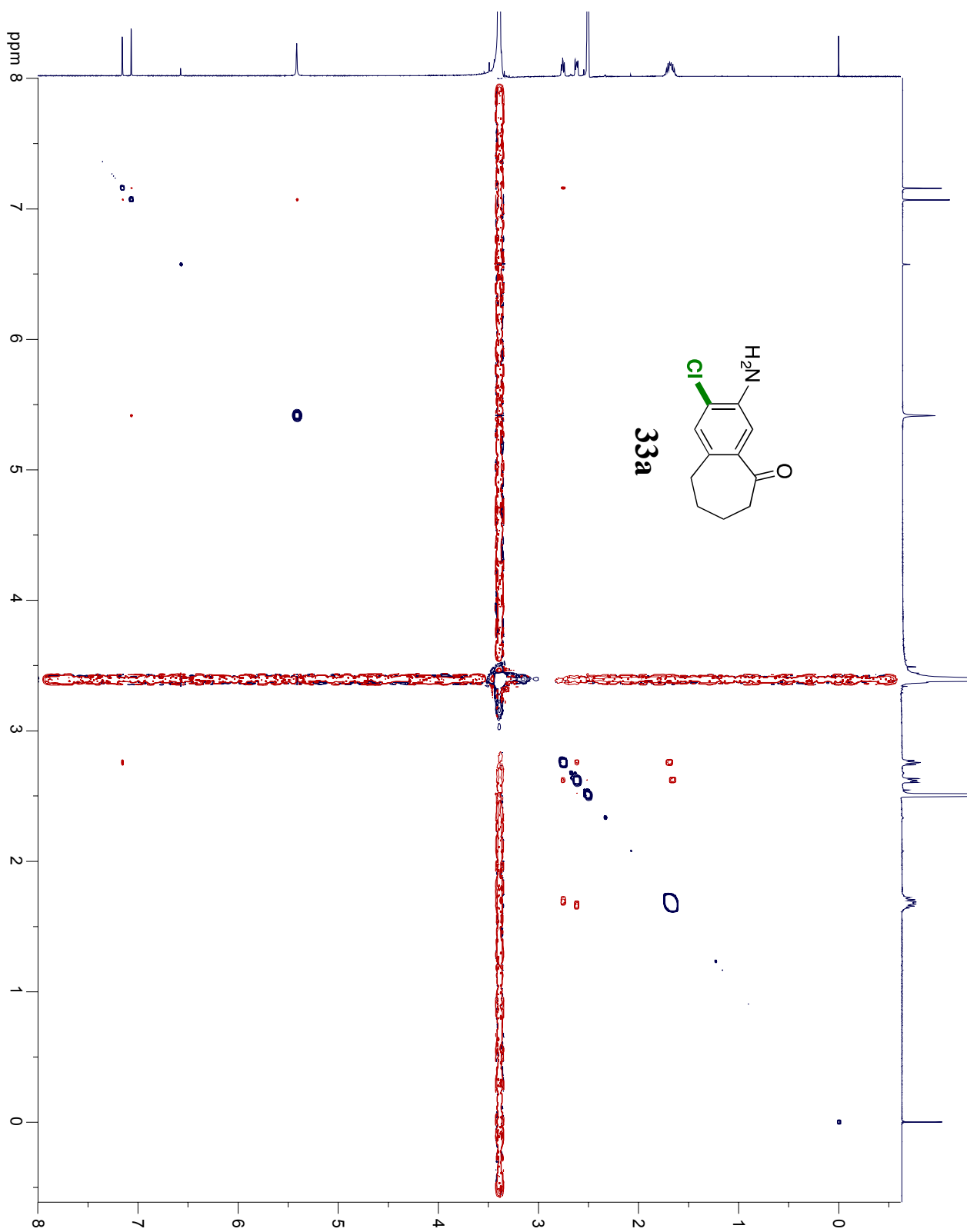
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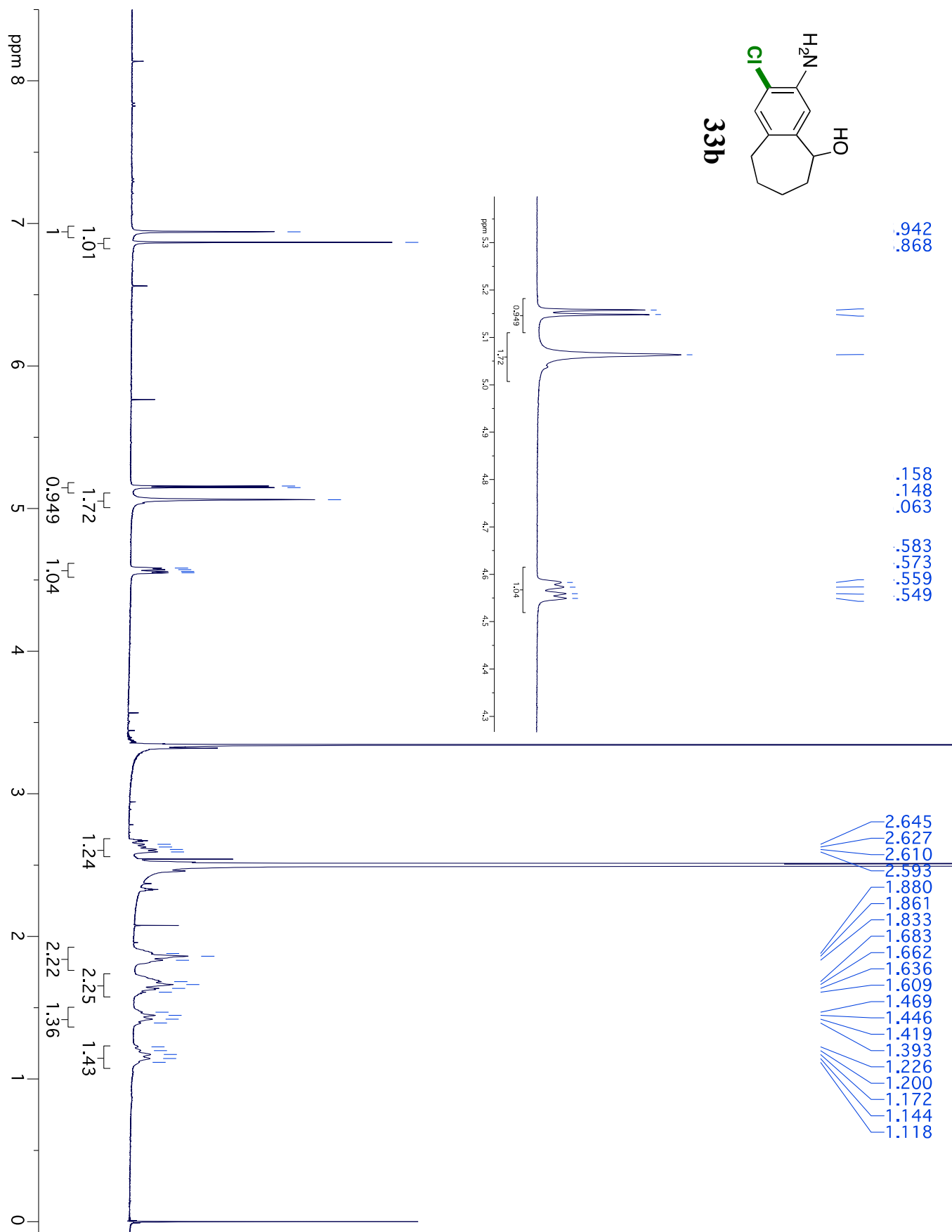
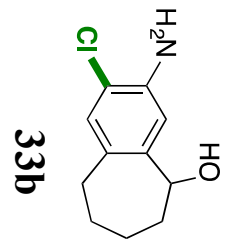


33







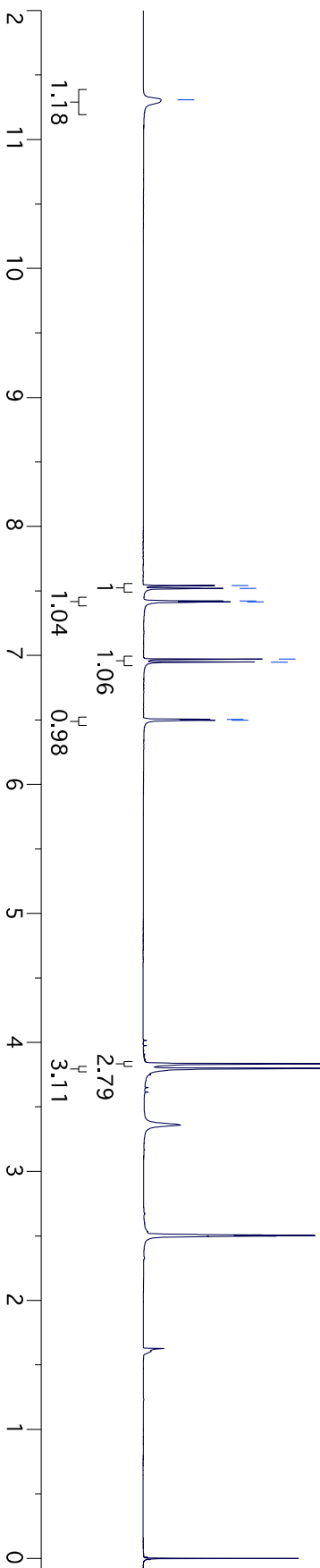
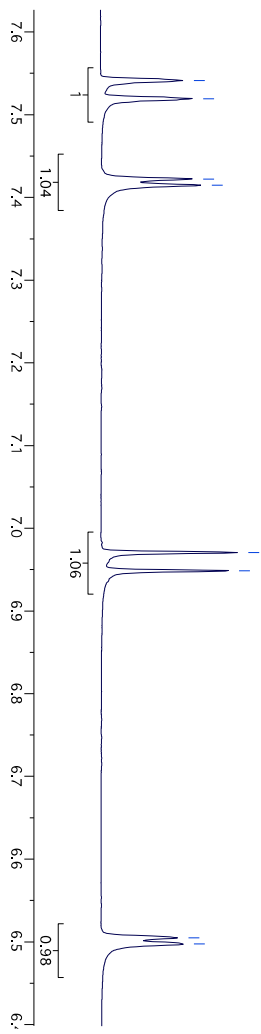


8

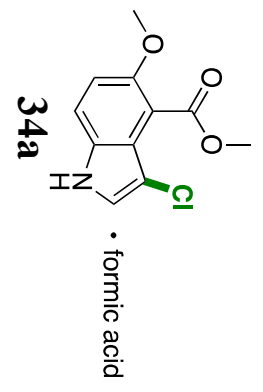


3.835
3.798

34



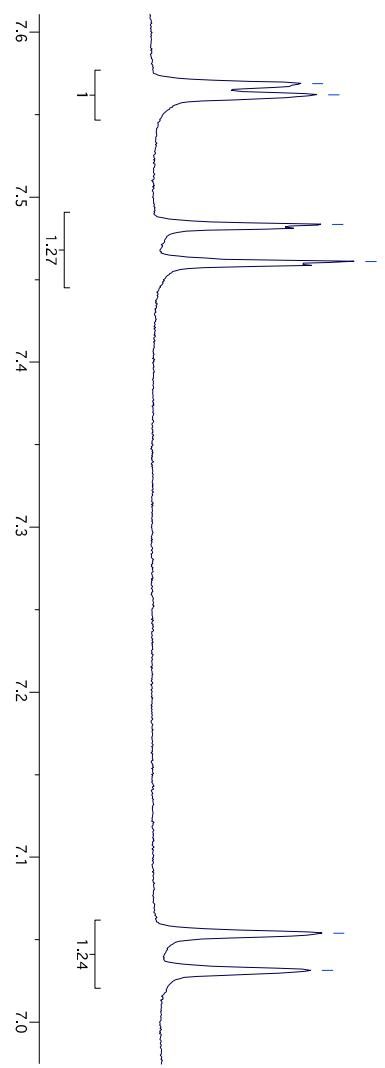
.512



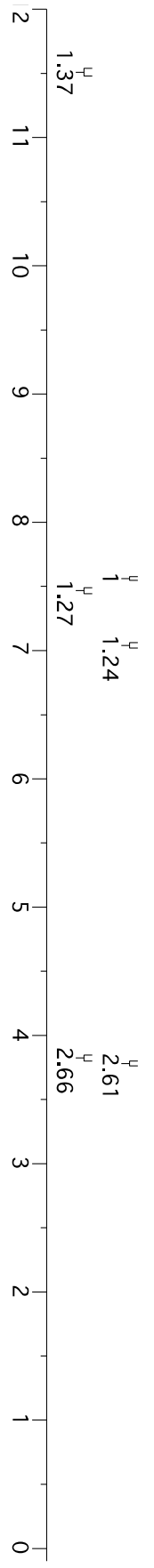
569
562
483
461
354
331

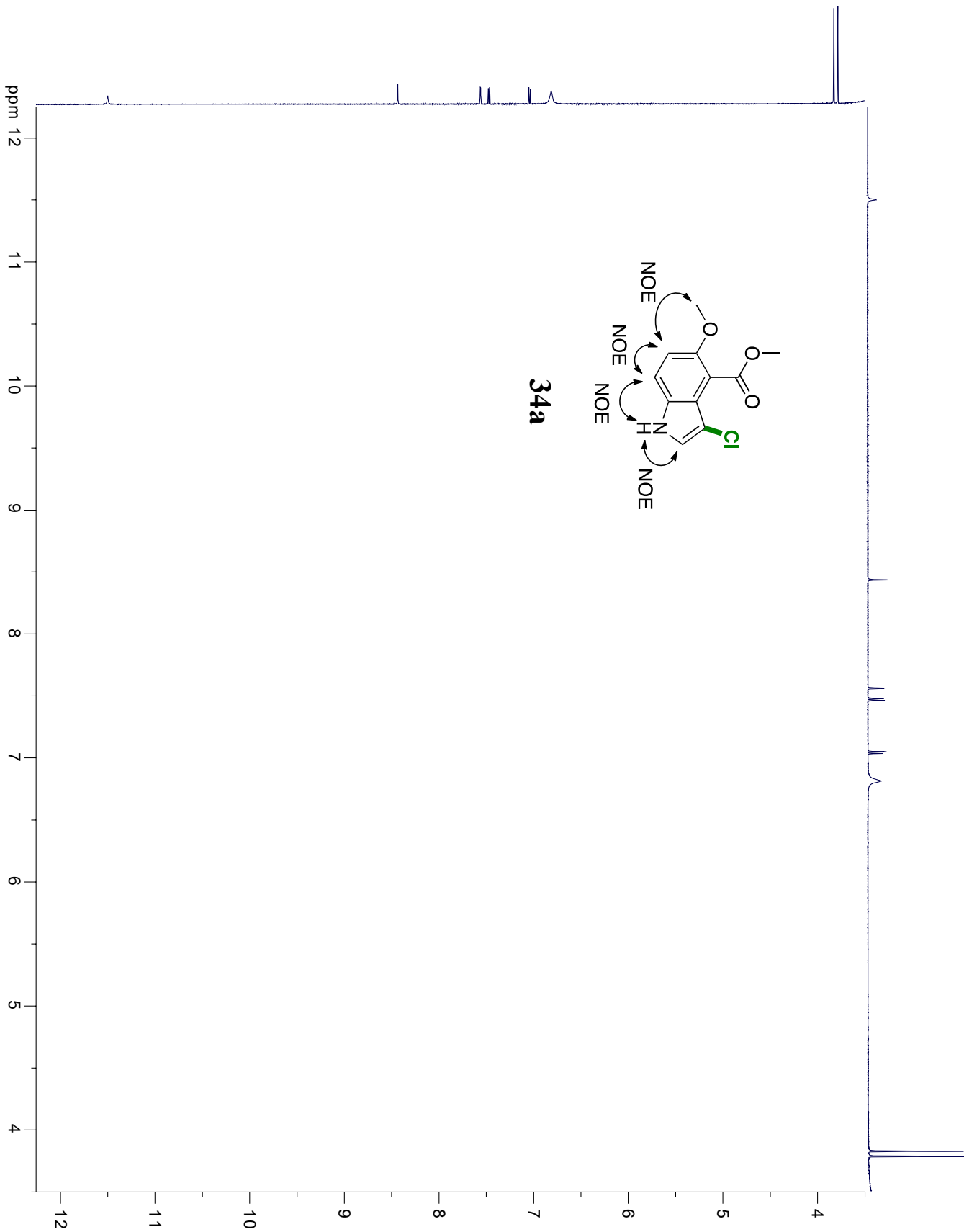


3.828
3.787



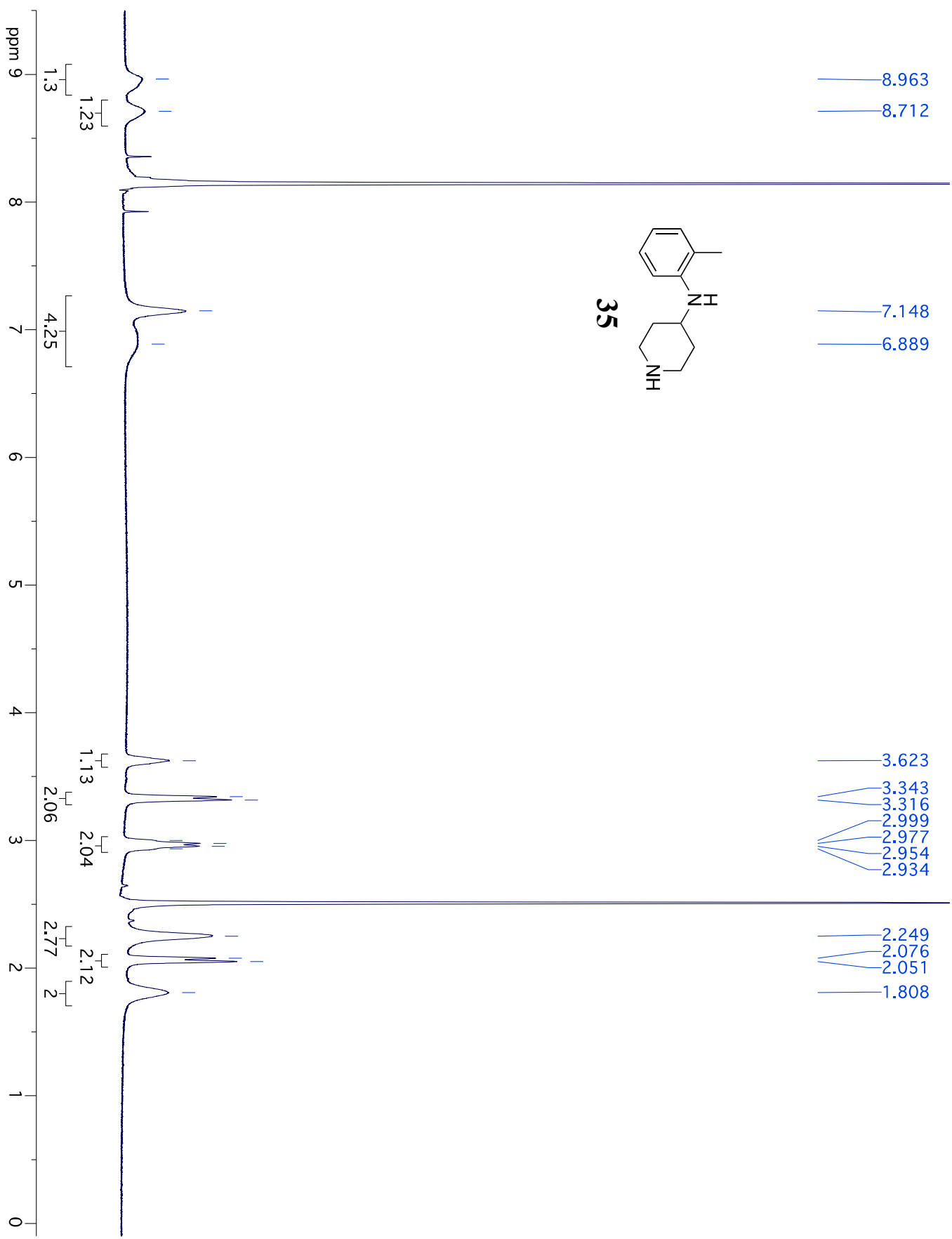
formic acid

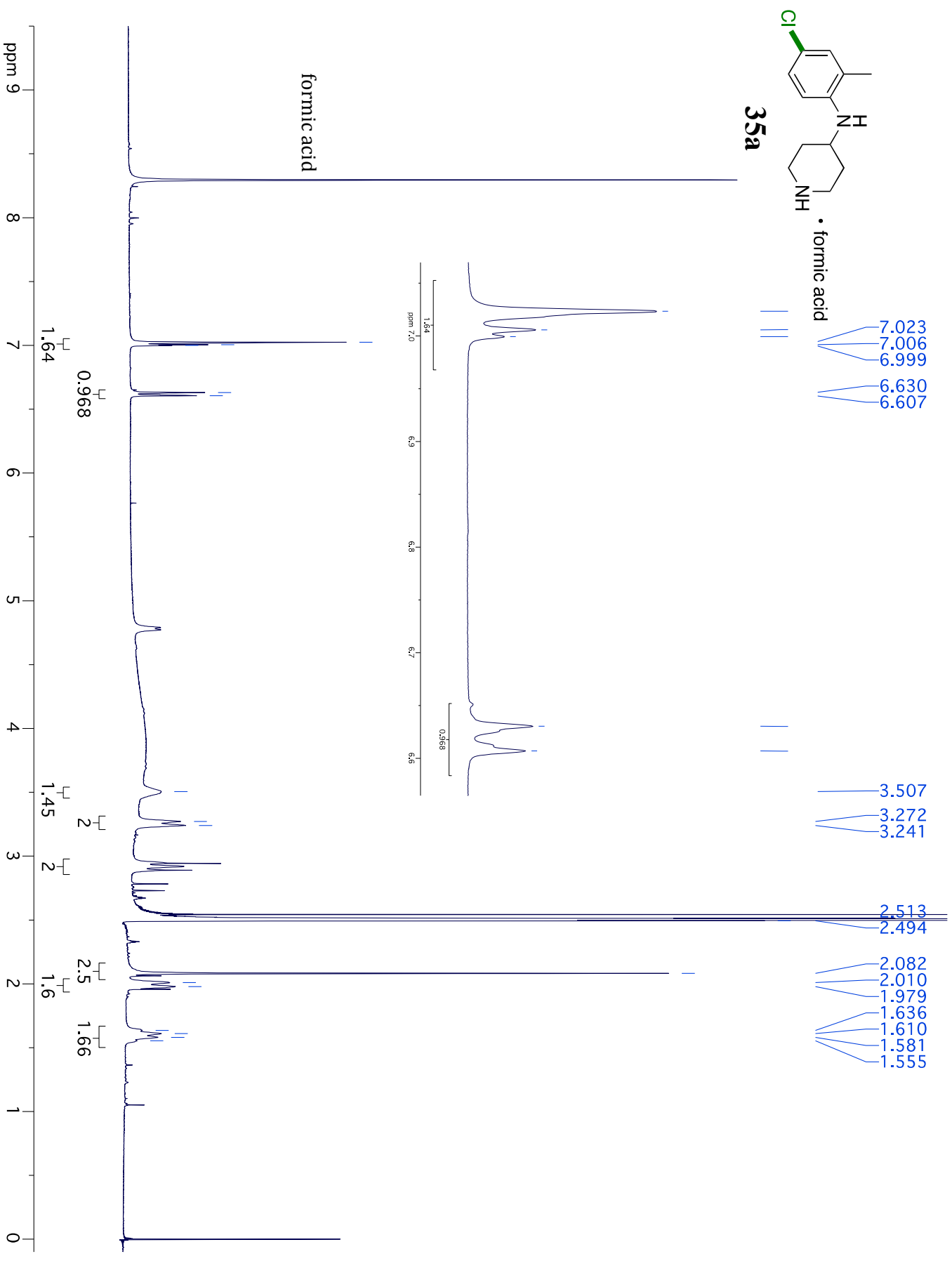
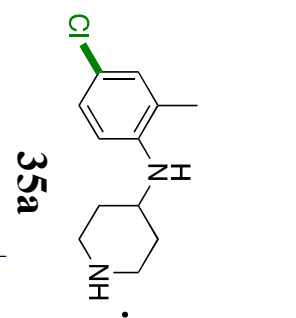


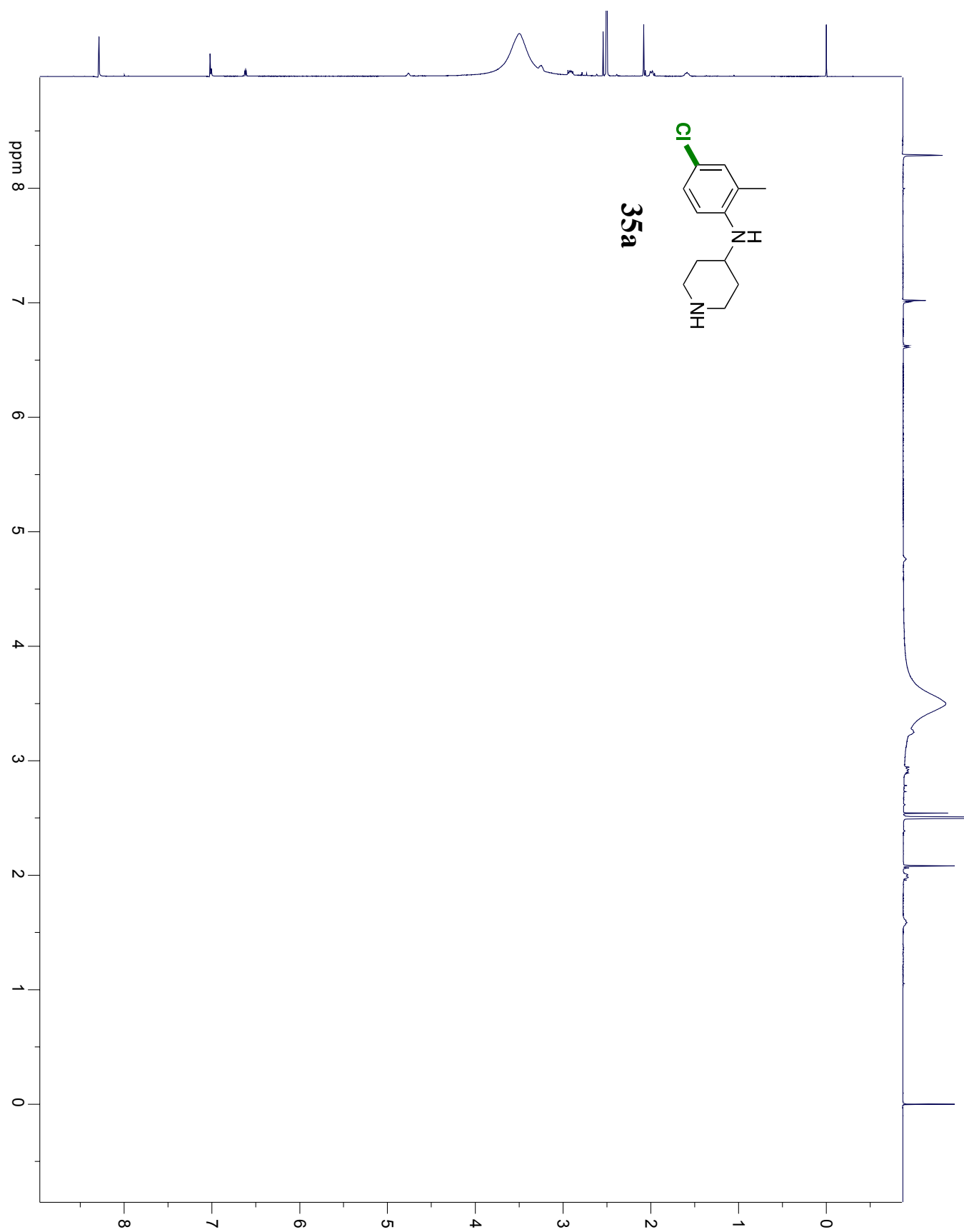


34a

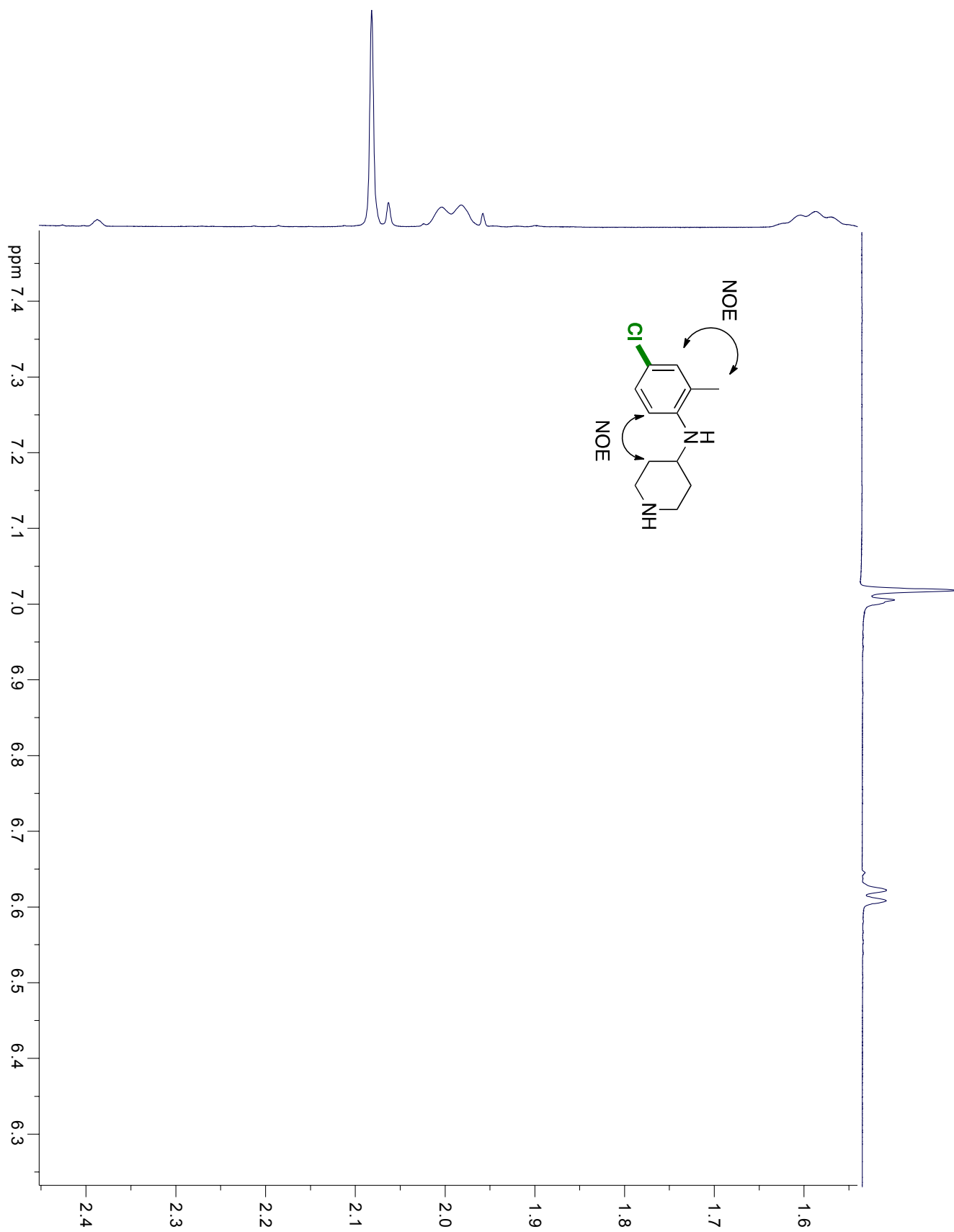
NOSEY of 34a







NOSEY of 35a.

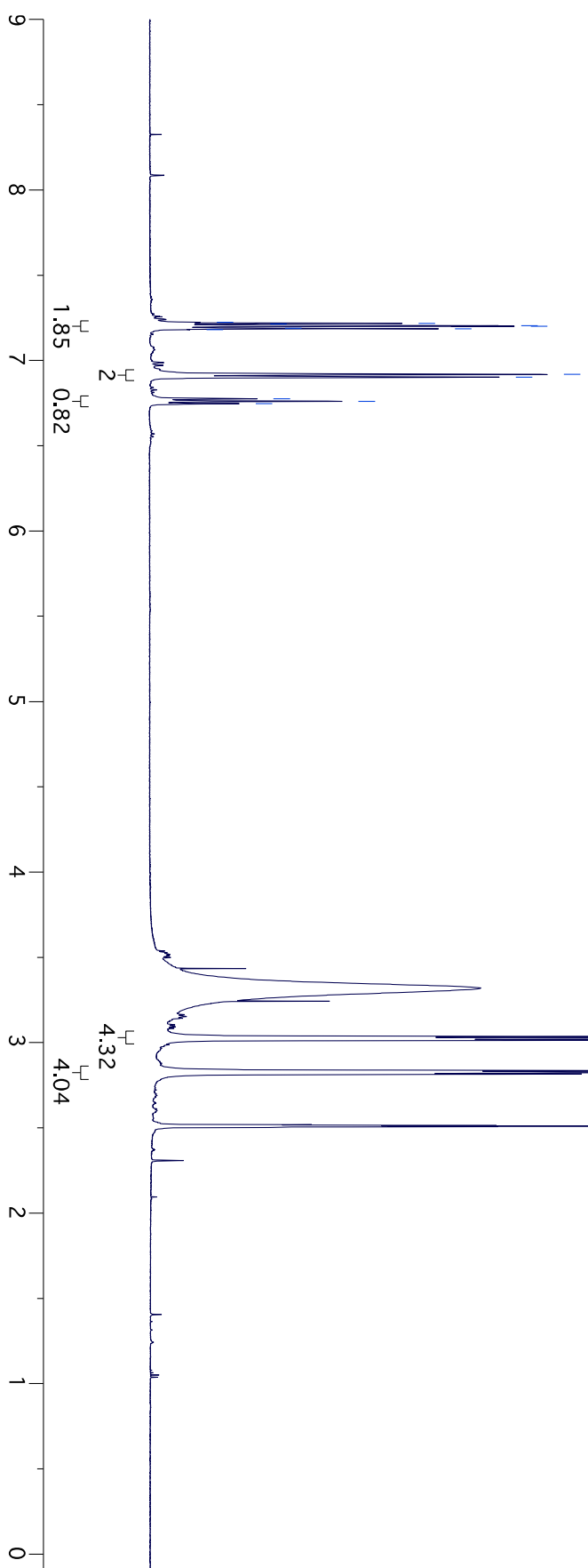
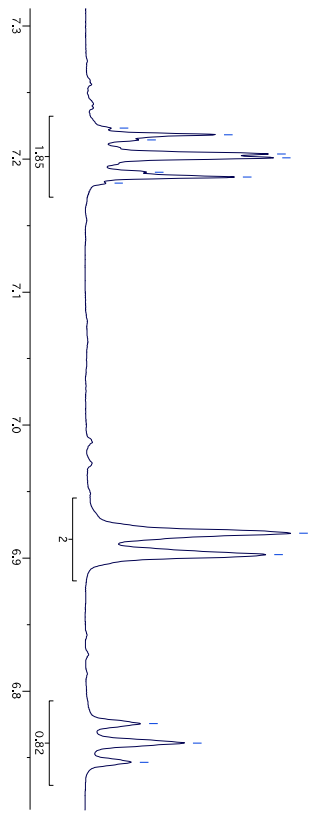


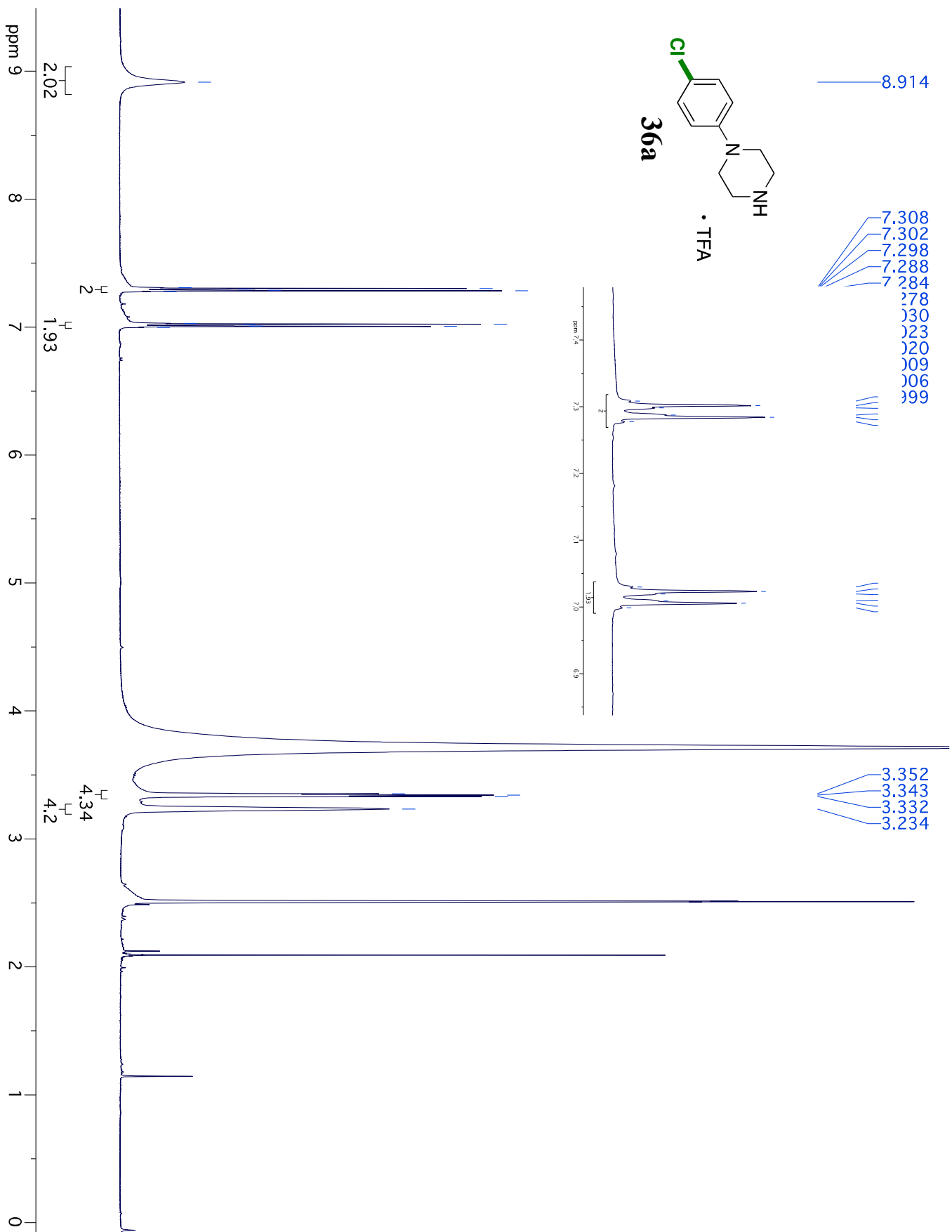
NOSEY of **35a**, zoomed in on particularly important NOEs for assigning selectivity.

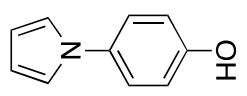


- 3.035
- 3.025
- 3.015
- 2.836
- 2.825
- 2.816

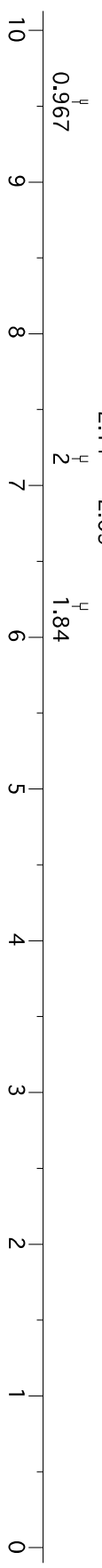
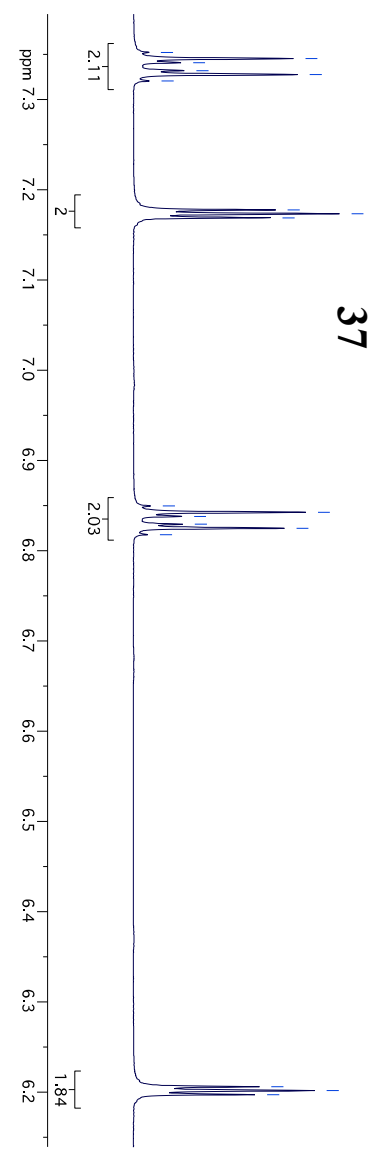
36

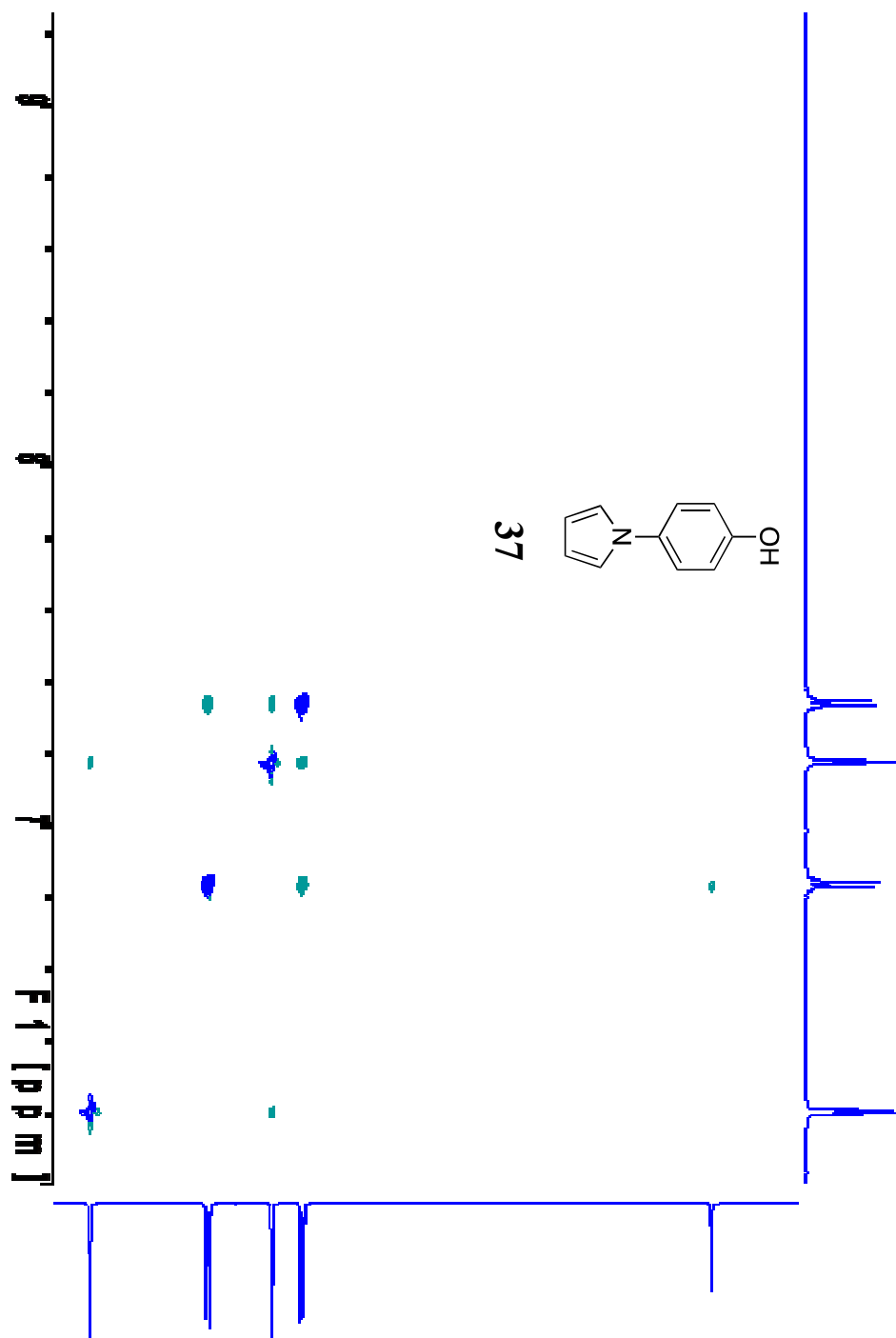






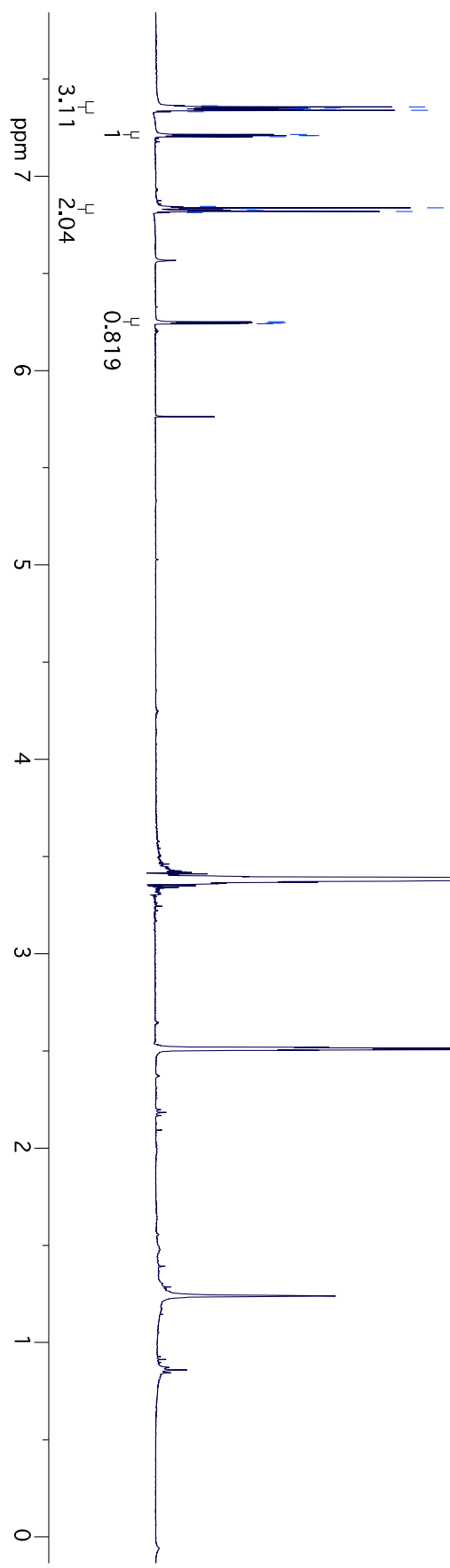
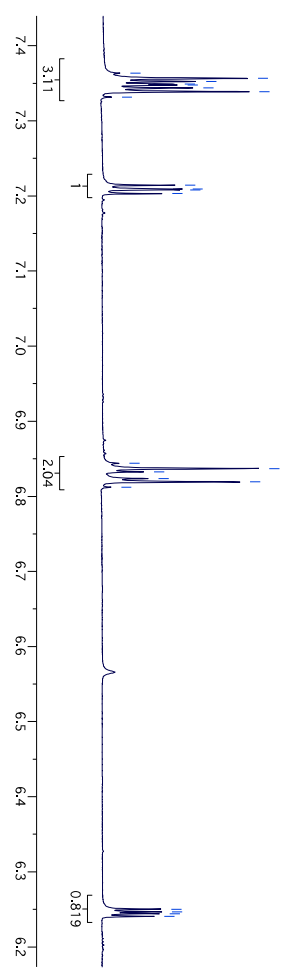
37

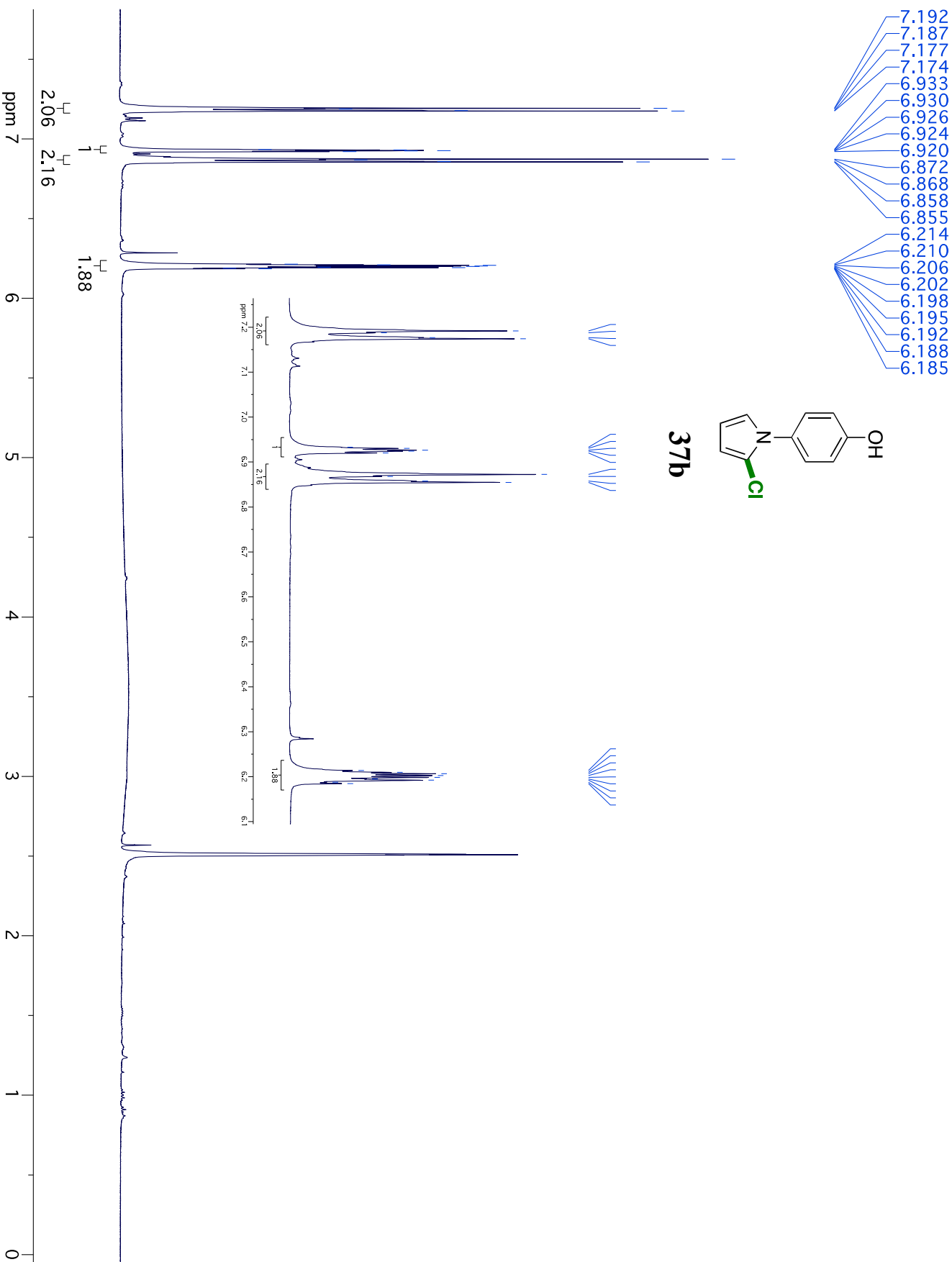




NOSEY of 37

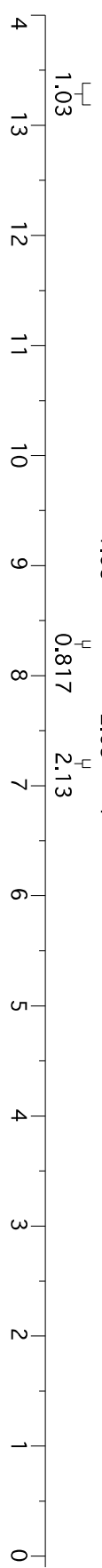
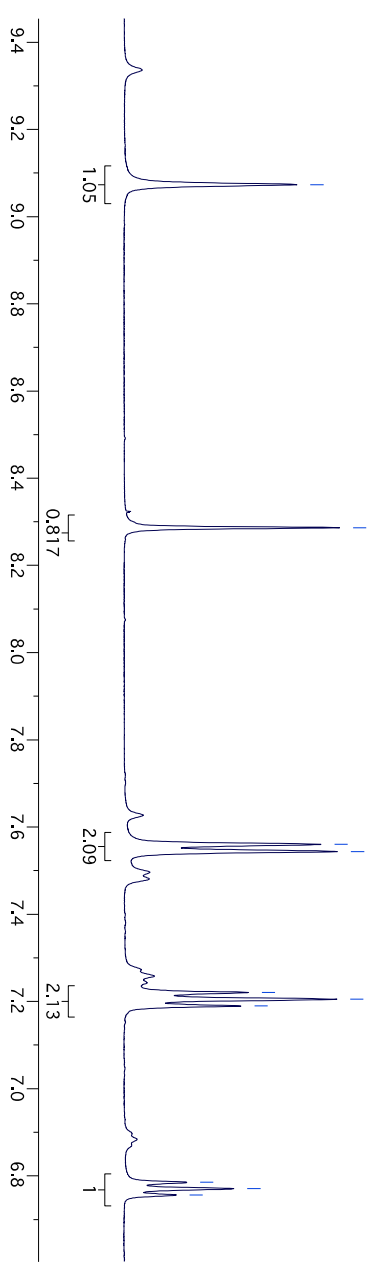
- 263
- 56
- 52
- 49
- 47
- 44
- 39
- 32
- 14
- 09
- 08
- 03
- 44
- 37
- 32
- 24
- 19
- 12
- 50
- 47
- 44
- 41

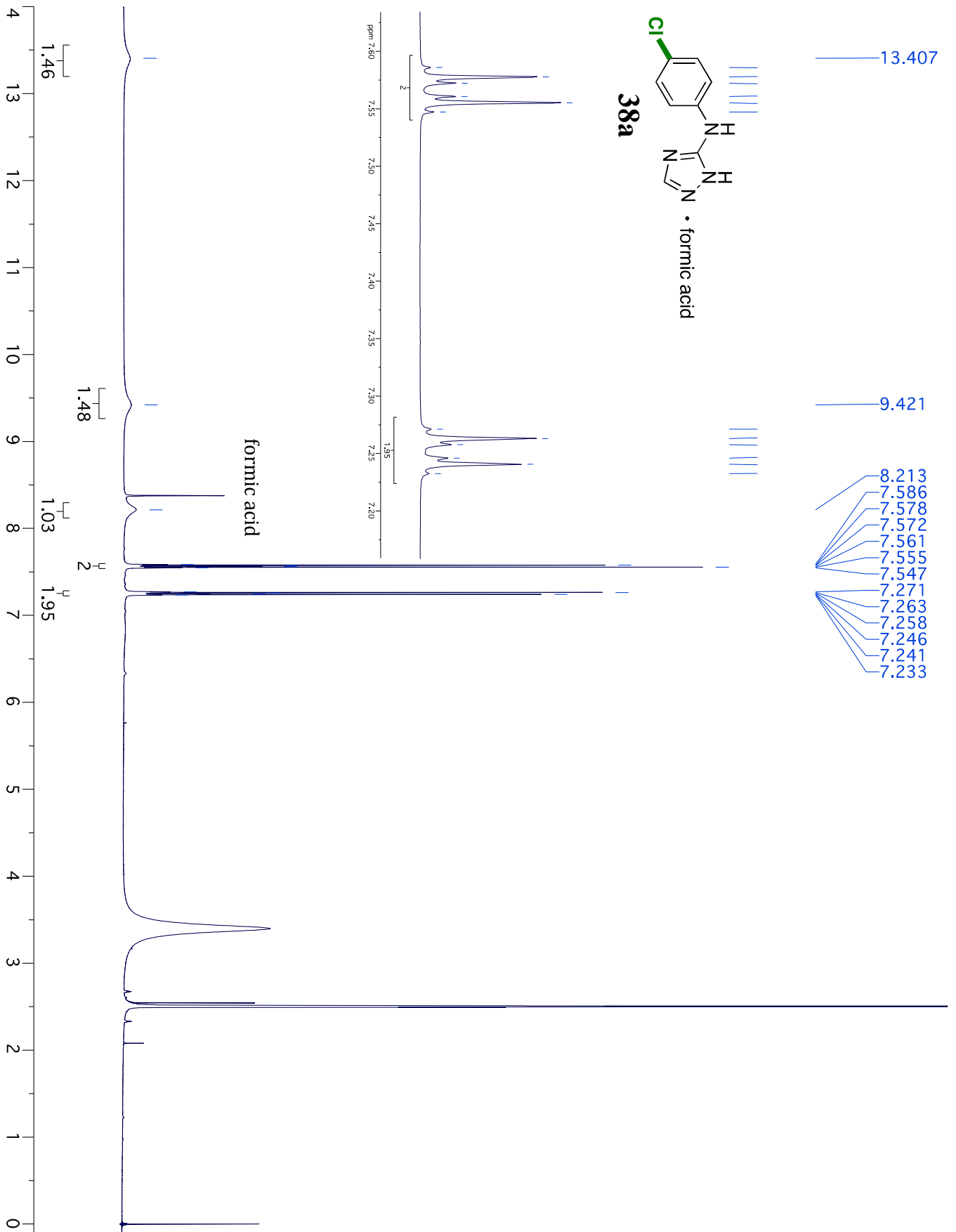




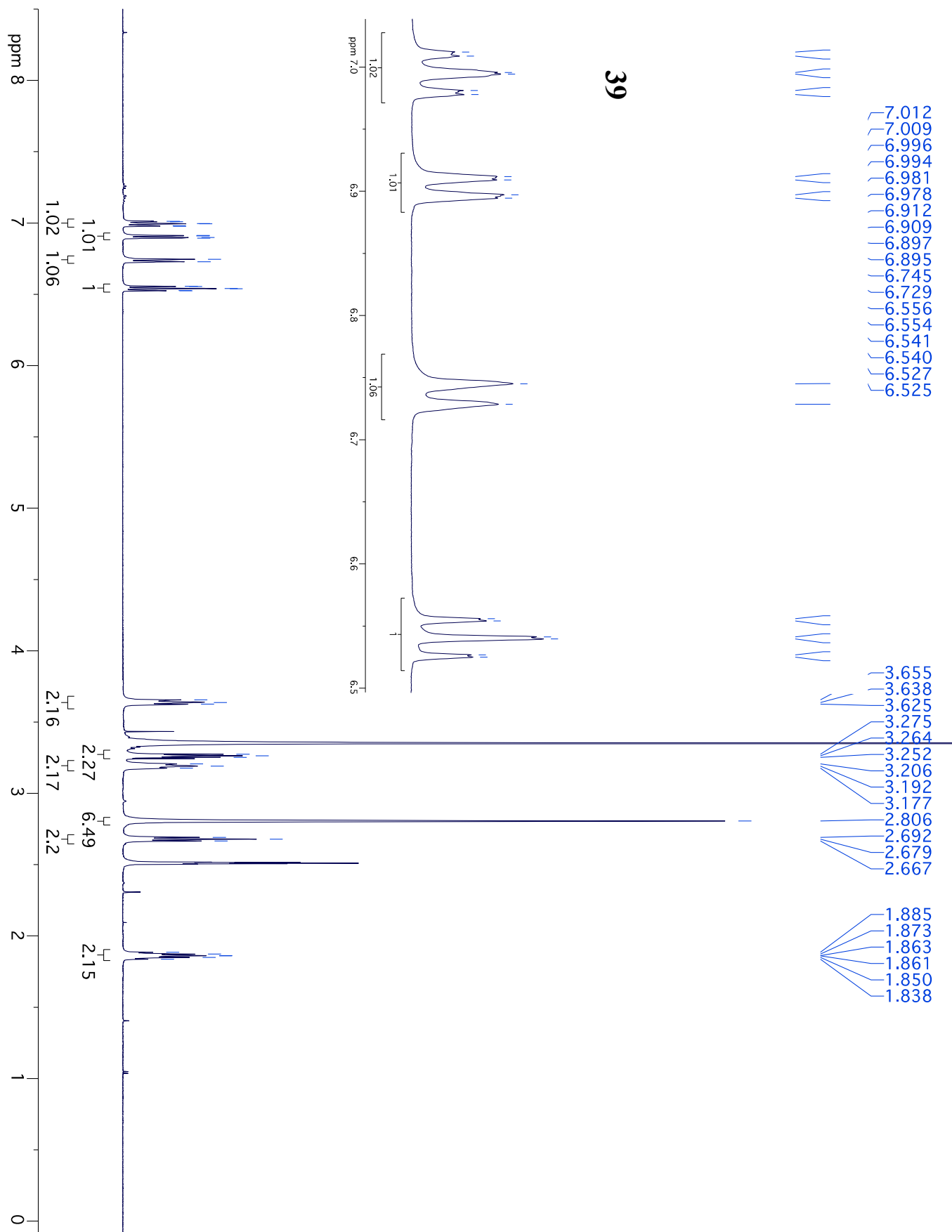


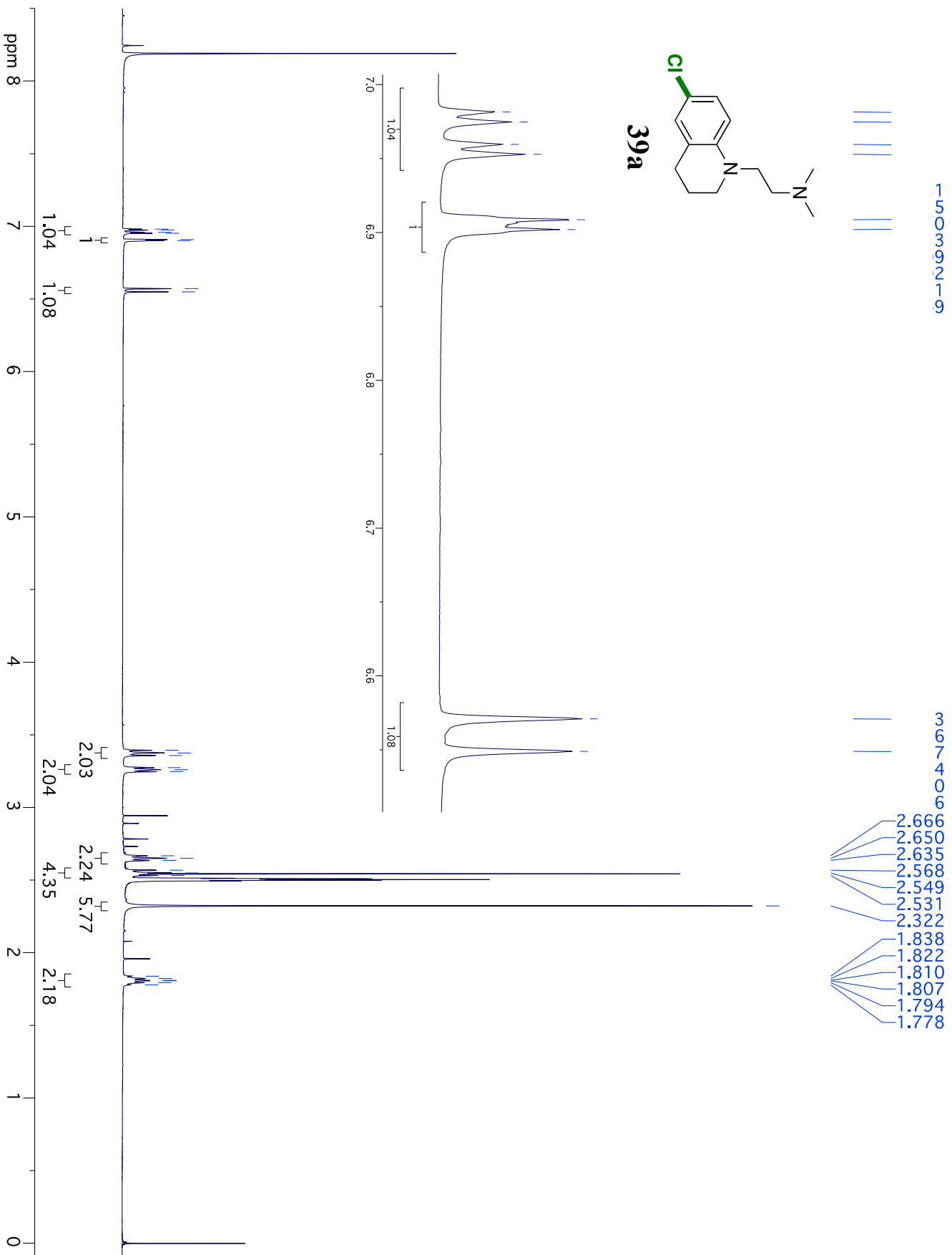
38





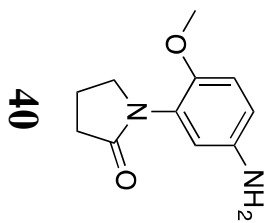
39



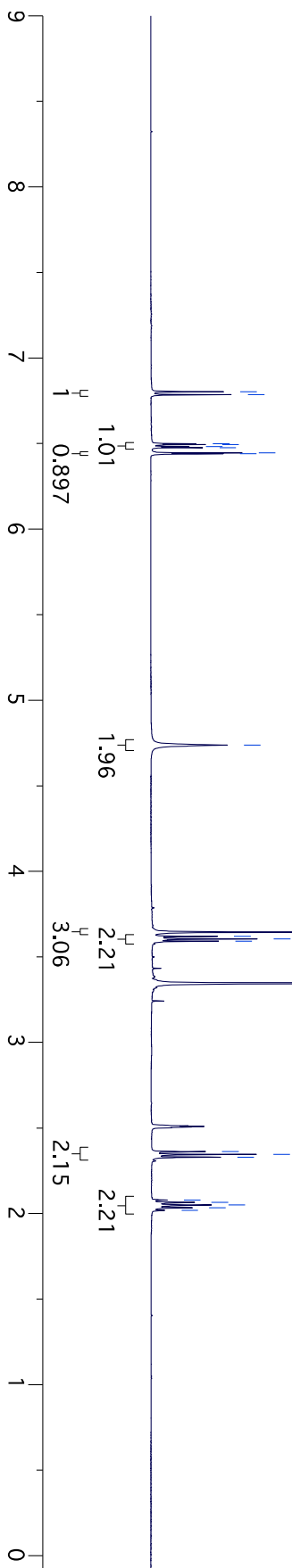
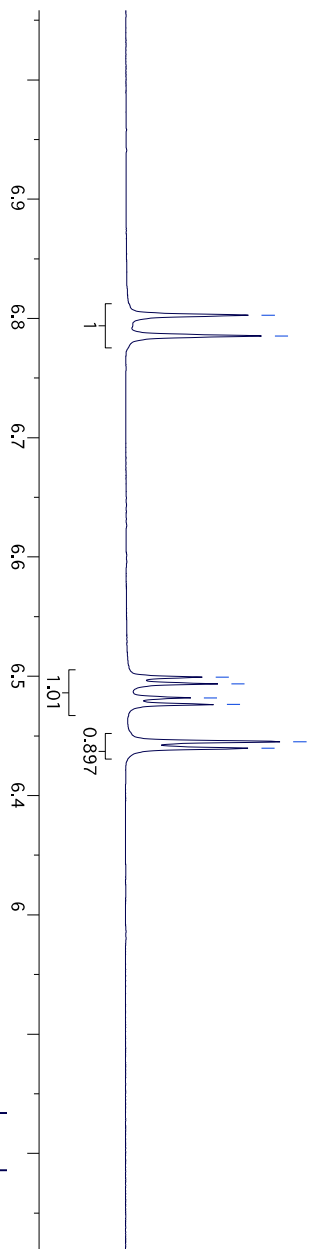


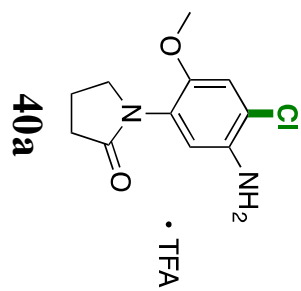


- 2.361
- 2.346
- 2.329
- 2.079
- 2.065
- 2.049
- 2.034
- 2.019



40

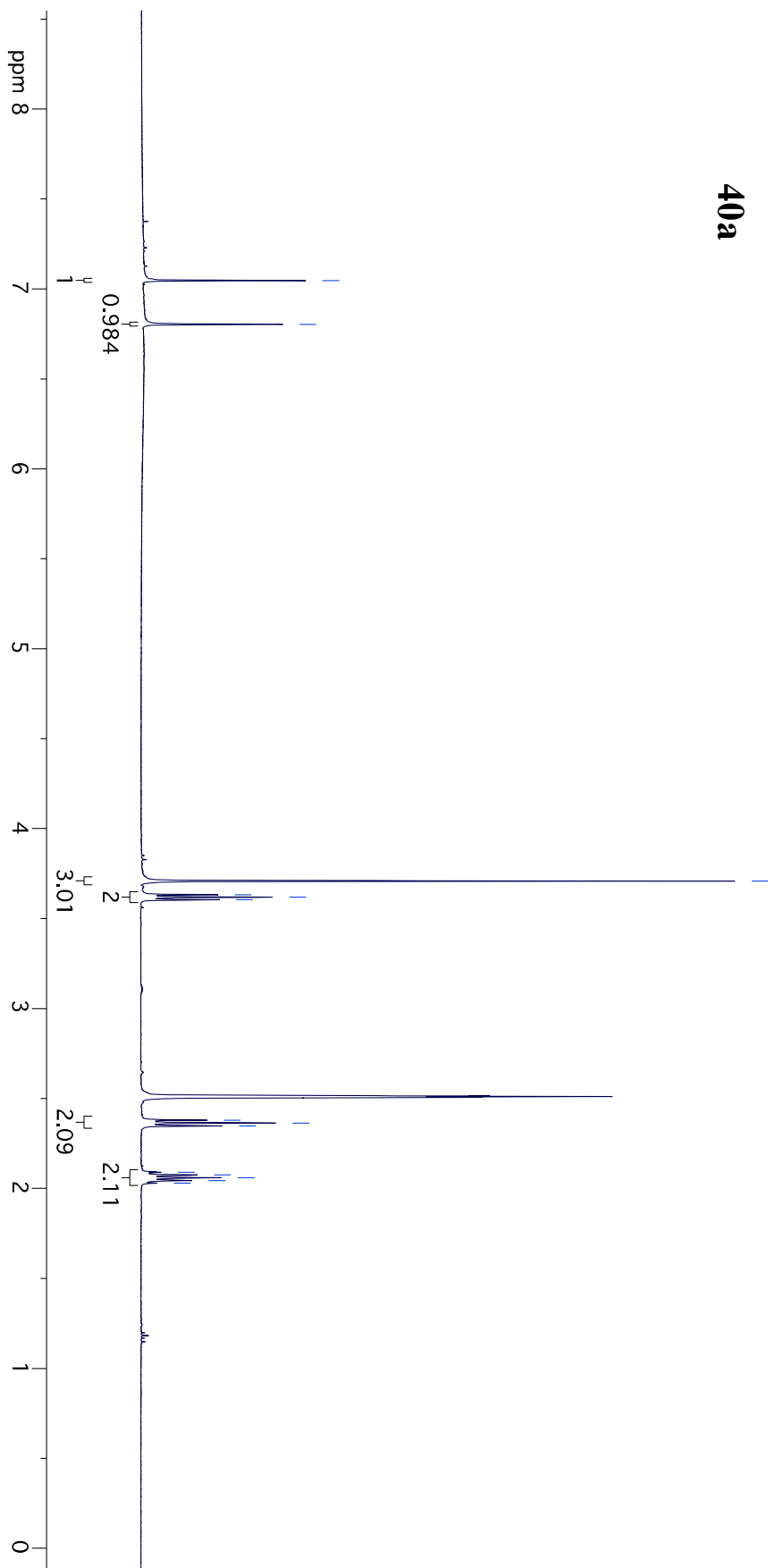


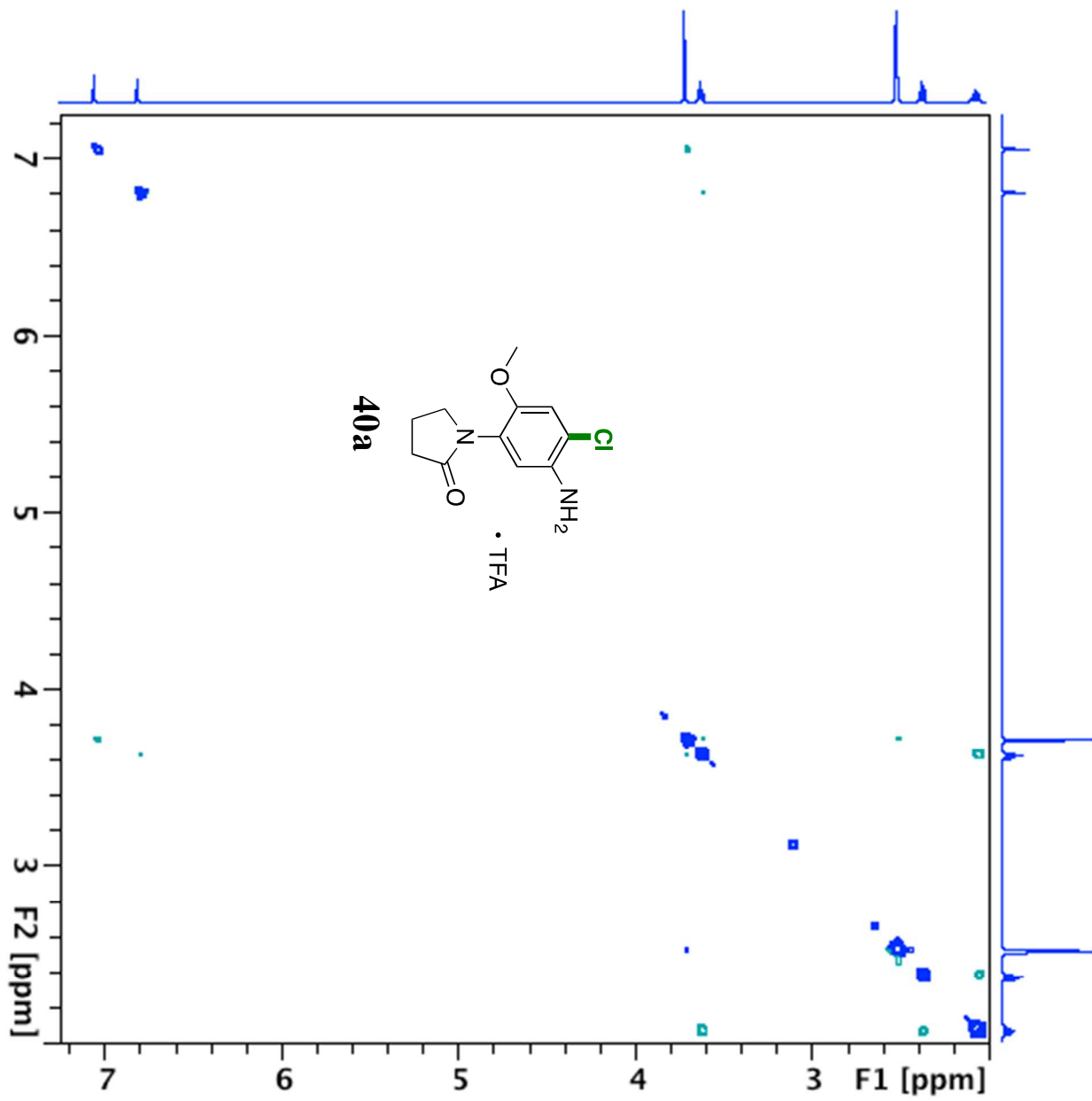


7.046
6.803

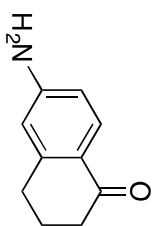
3.709
3.633
3.619
3.605

2.380
2.364
2.348
2.089
2.075
2.059
2.044
2.029

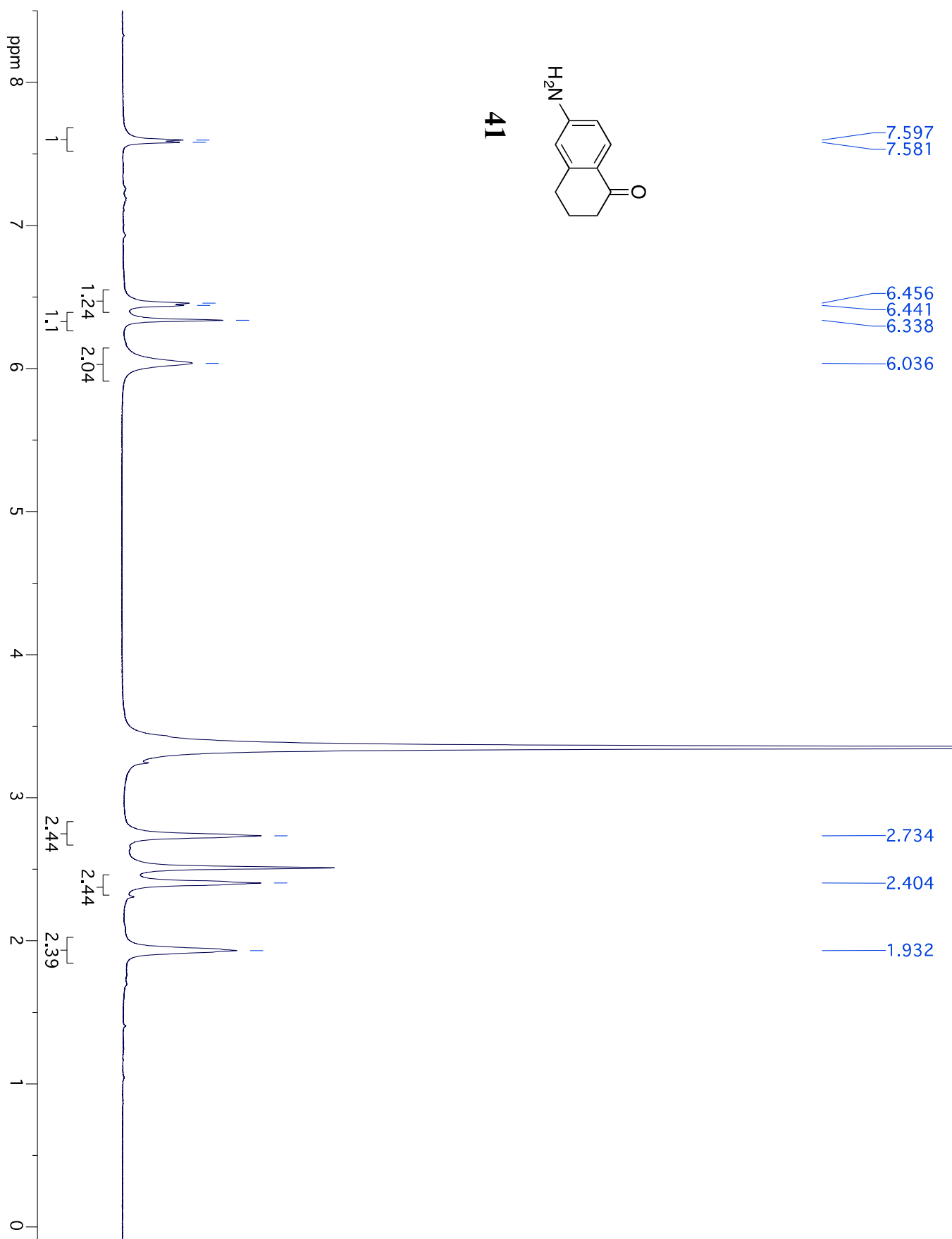


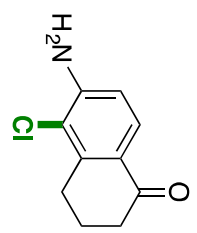


NOSEY of **40a**.

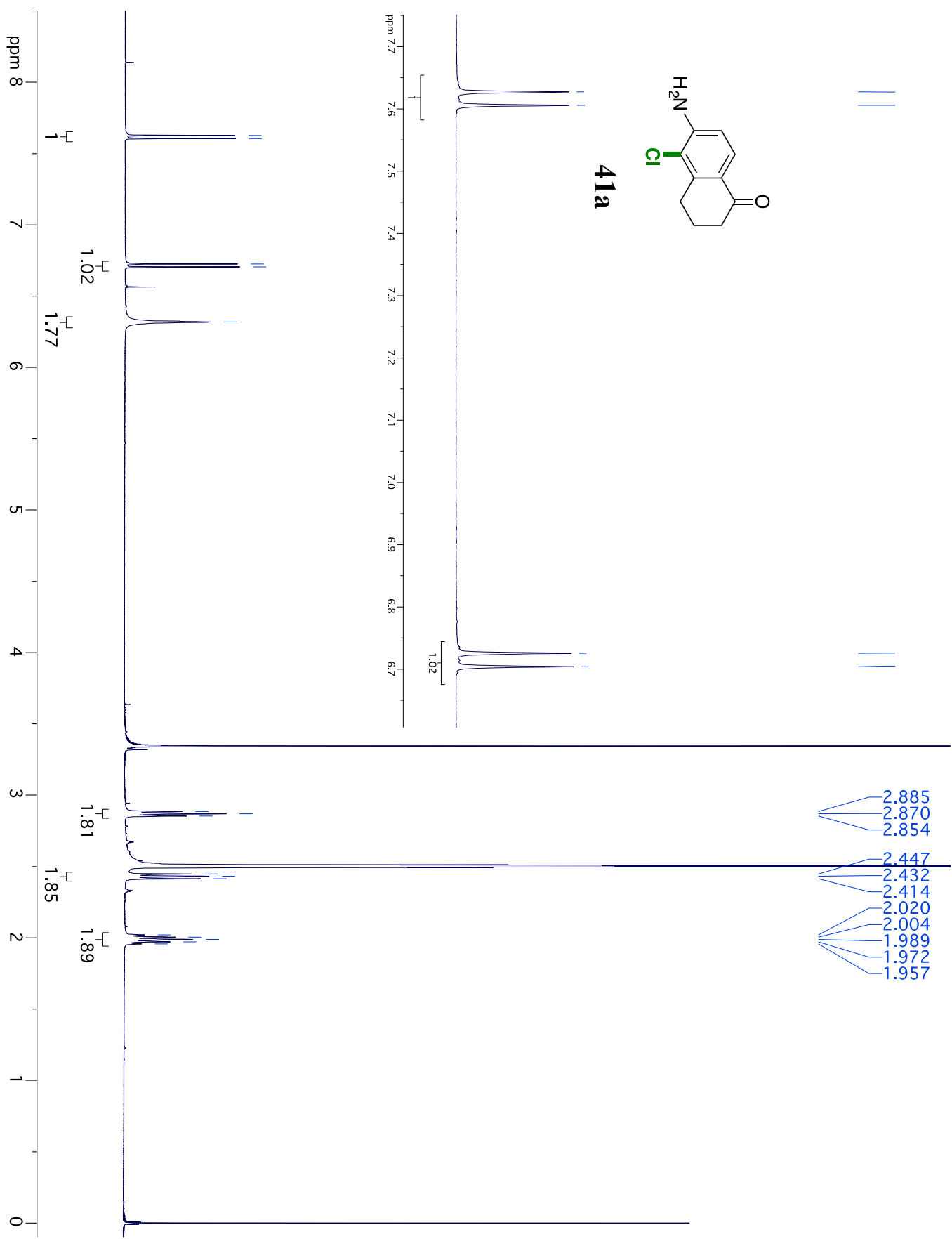


41





41a



471

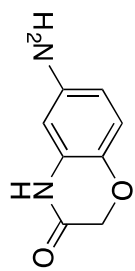


26
05
61
55
19
13
98
91

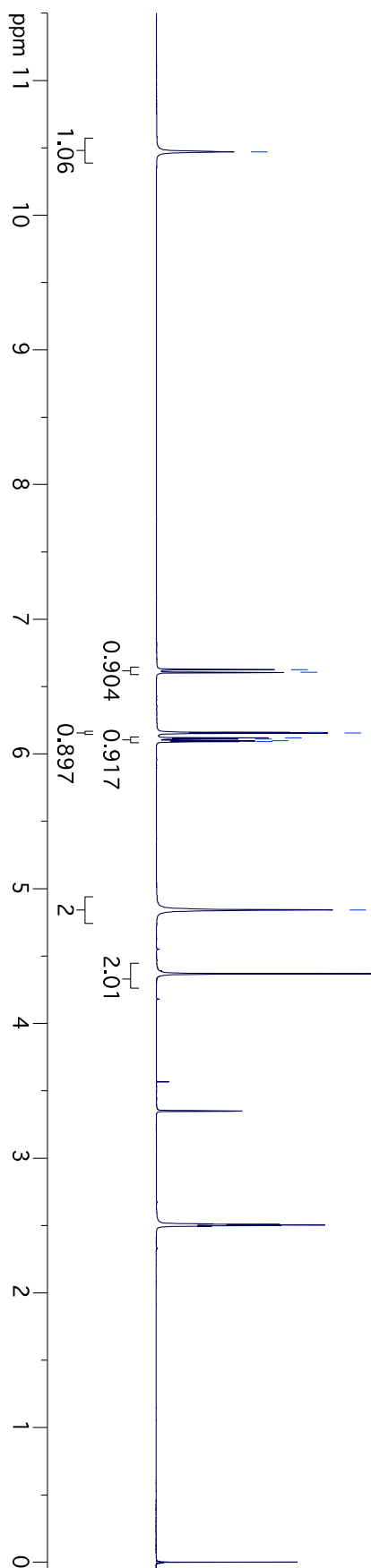
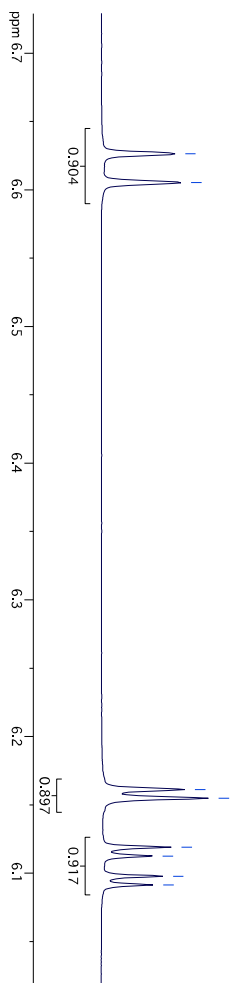


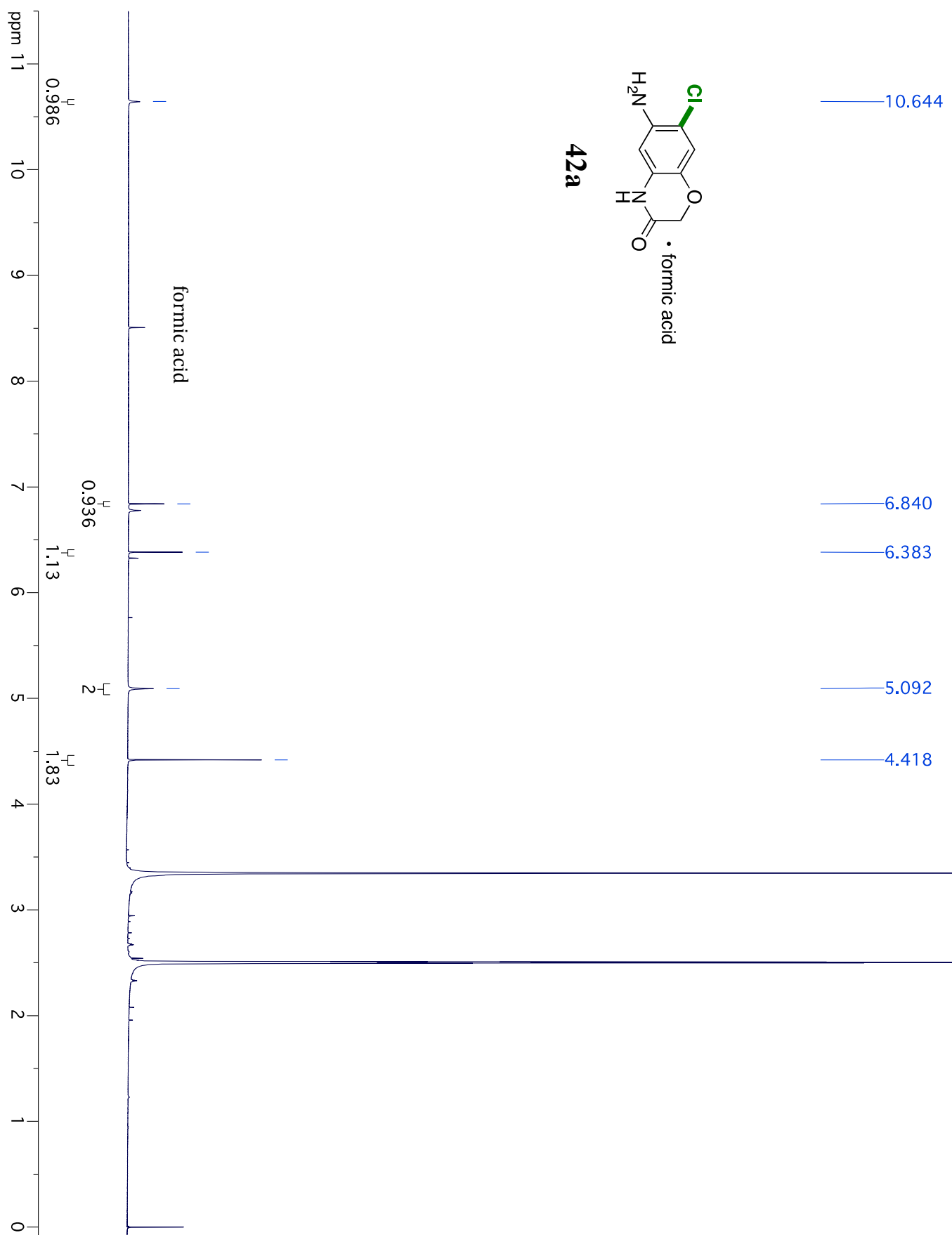
42

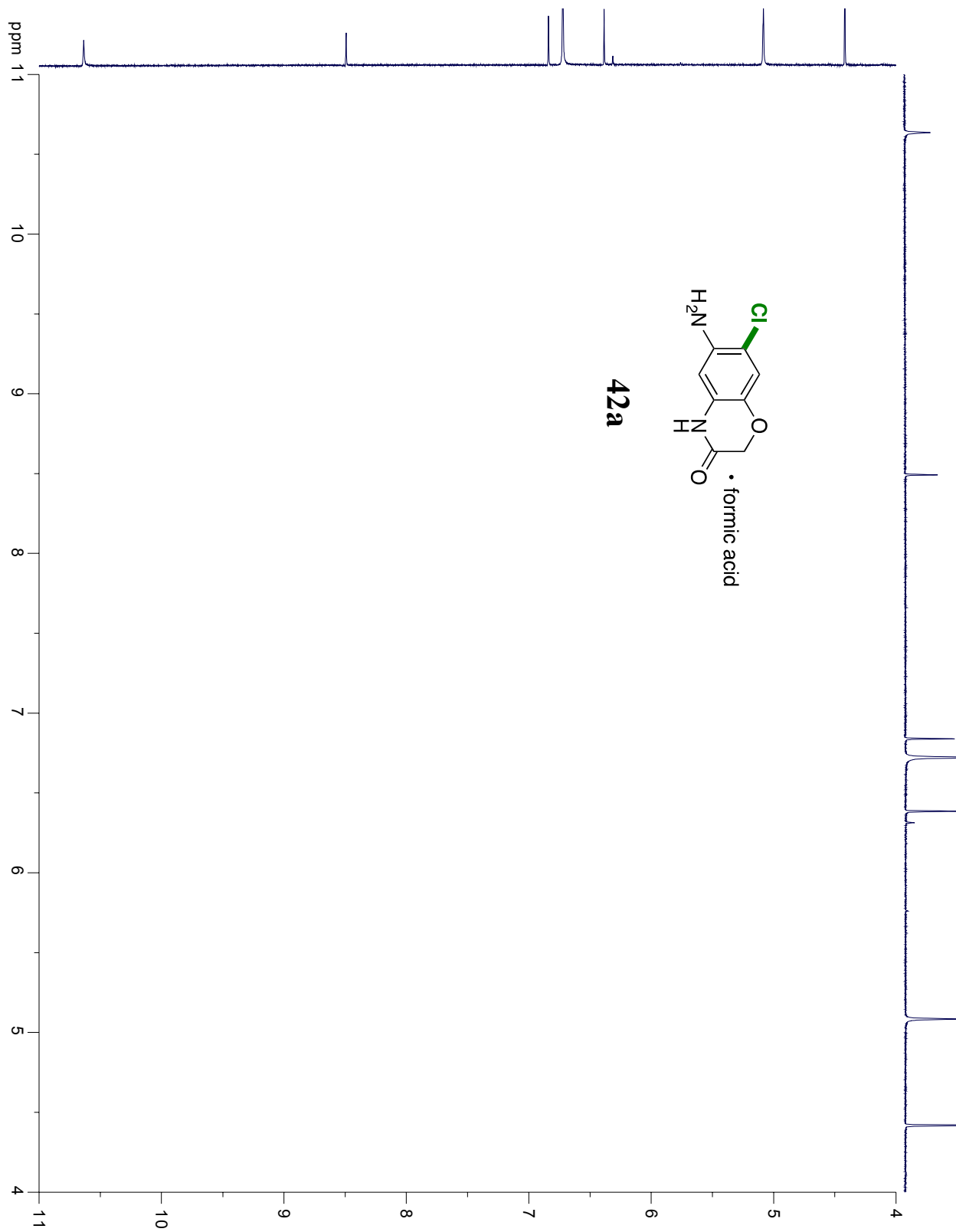
4.368



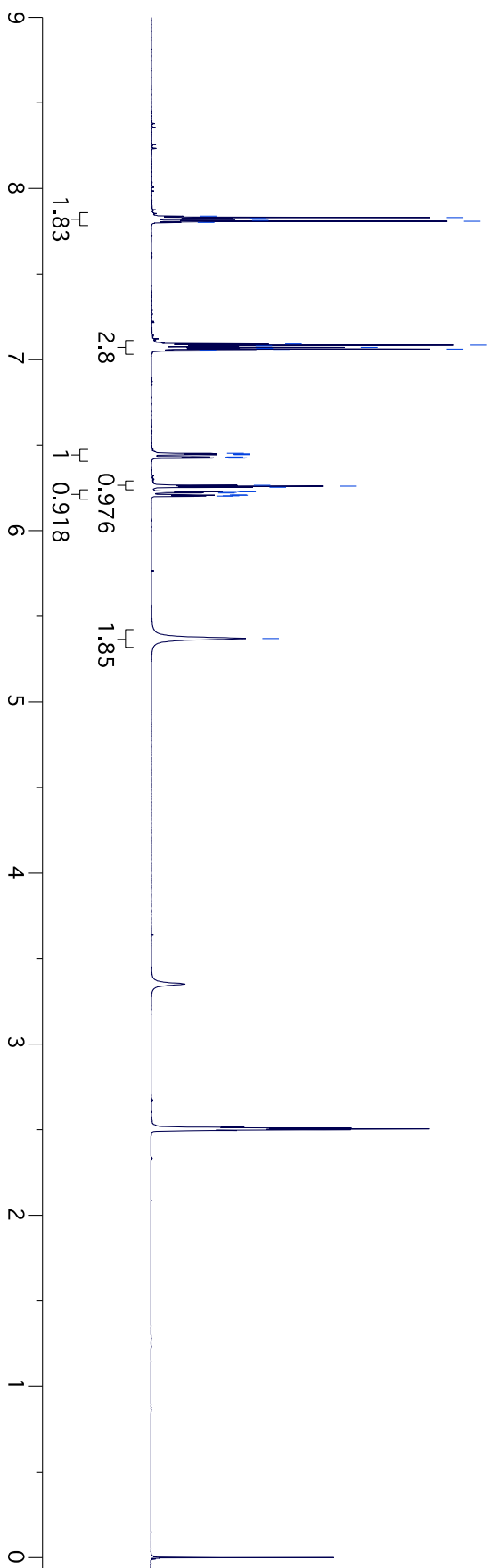
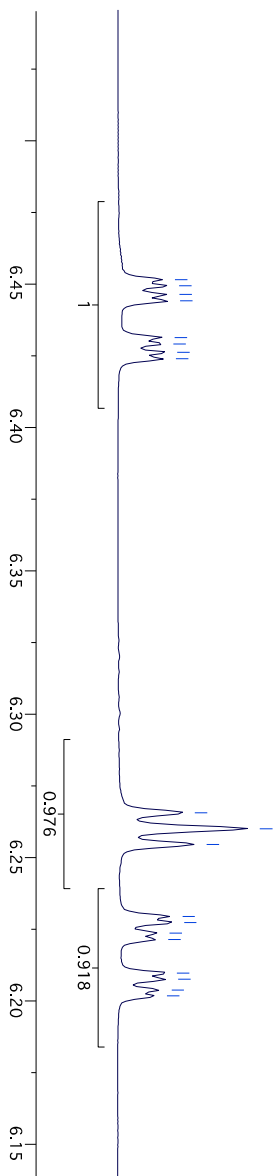
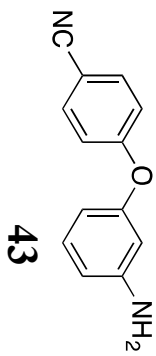
42



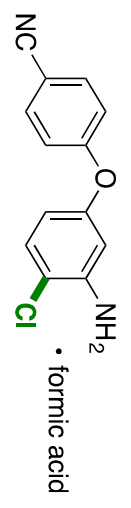




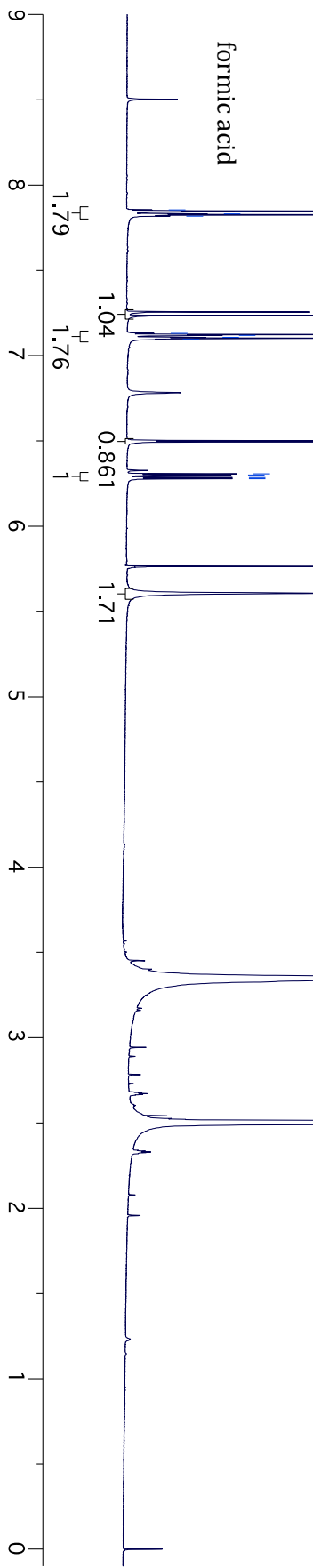
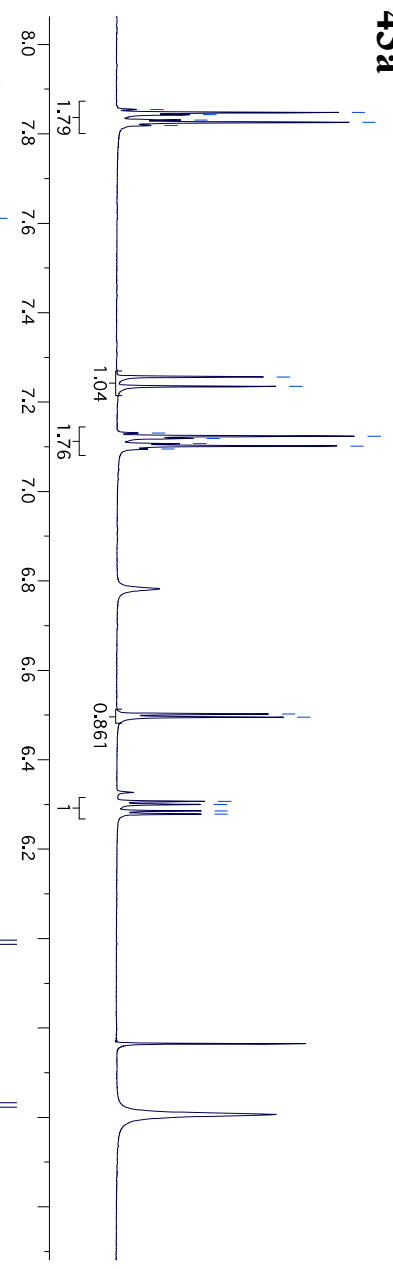
NOSEY of **42a**

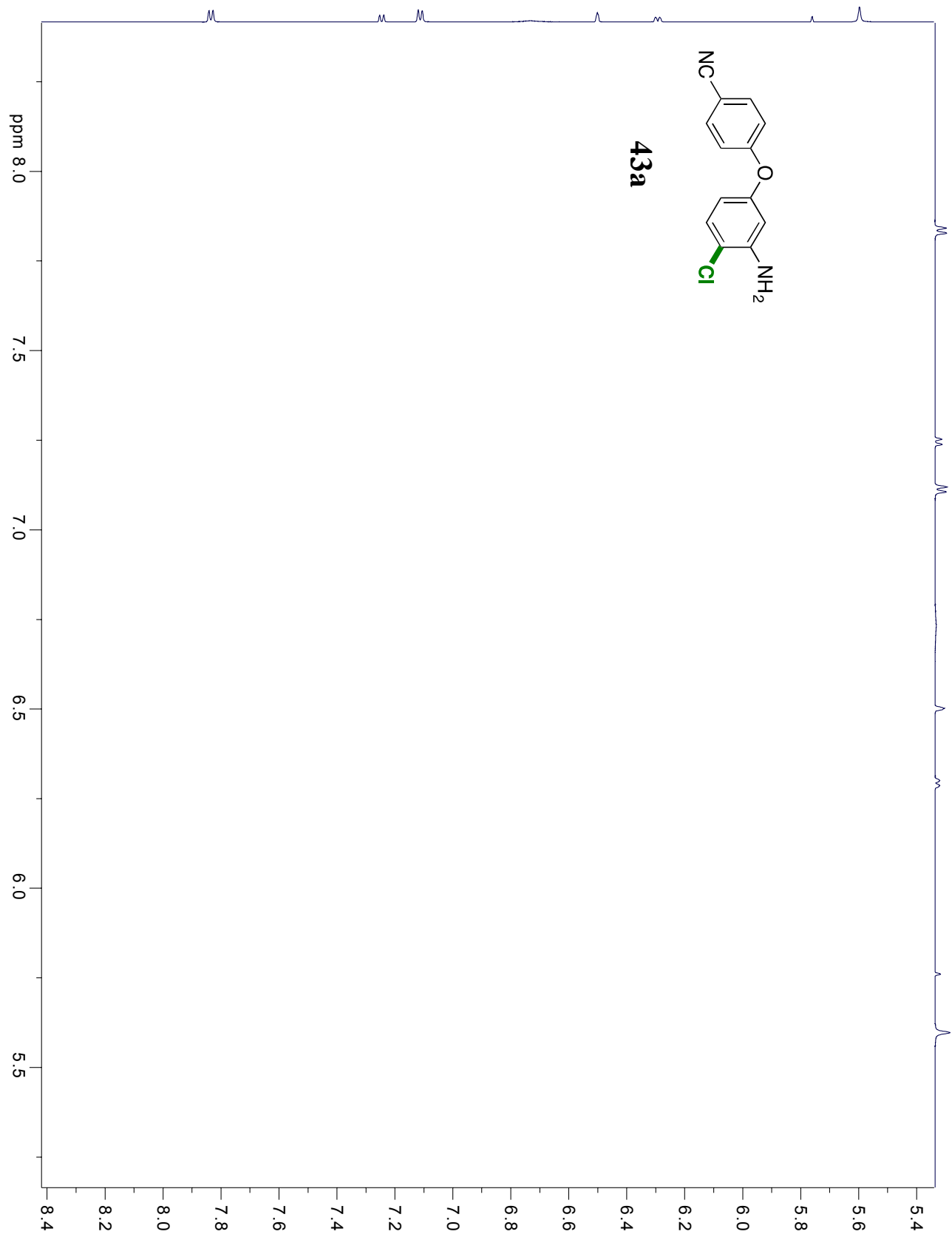


7.855

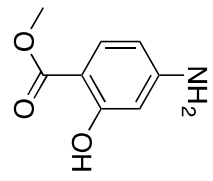


43a

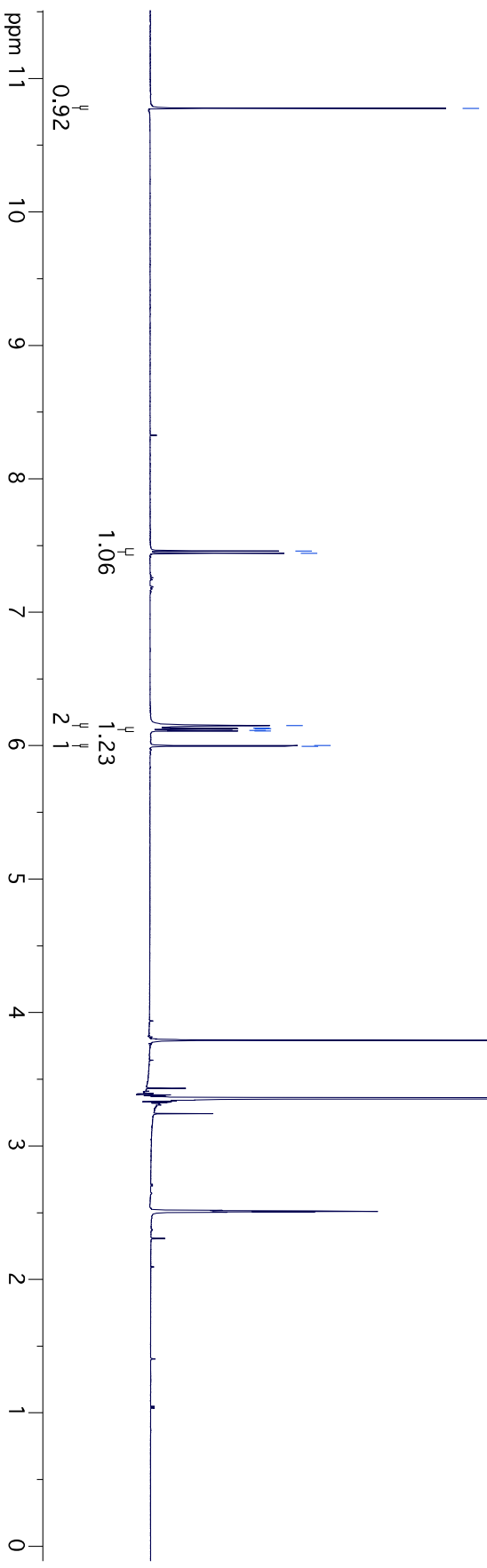
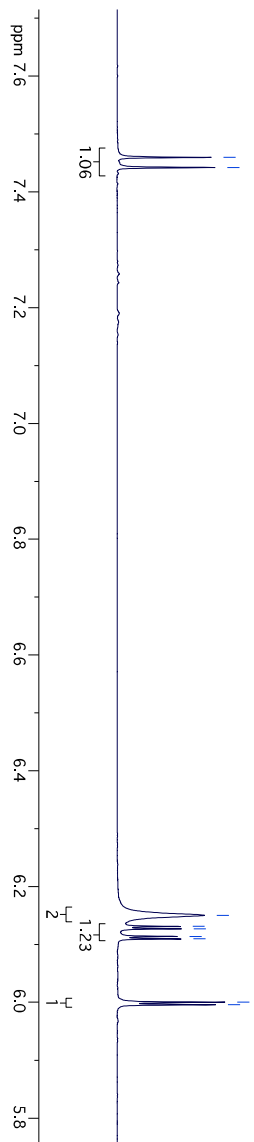


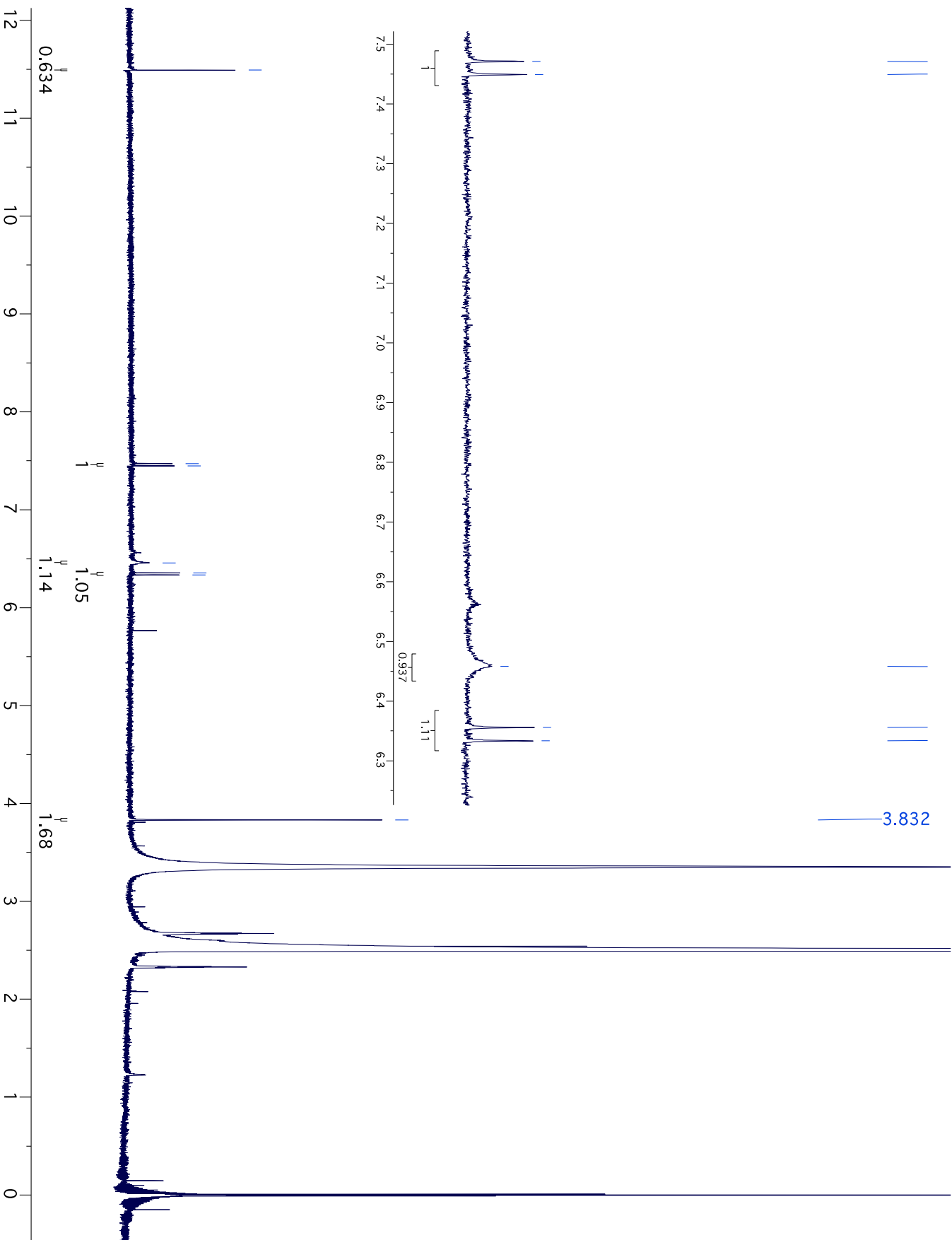


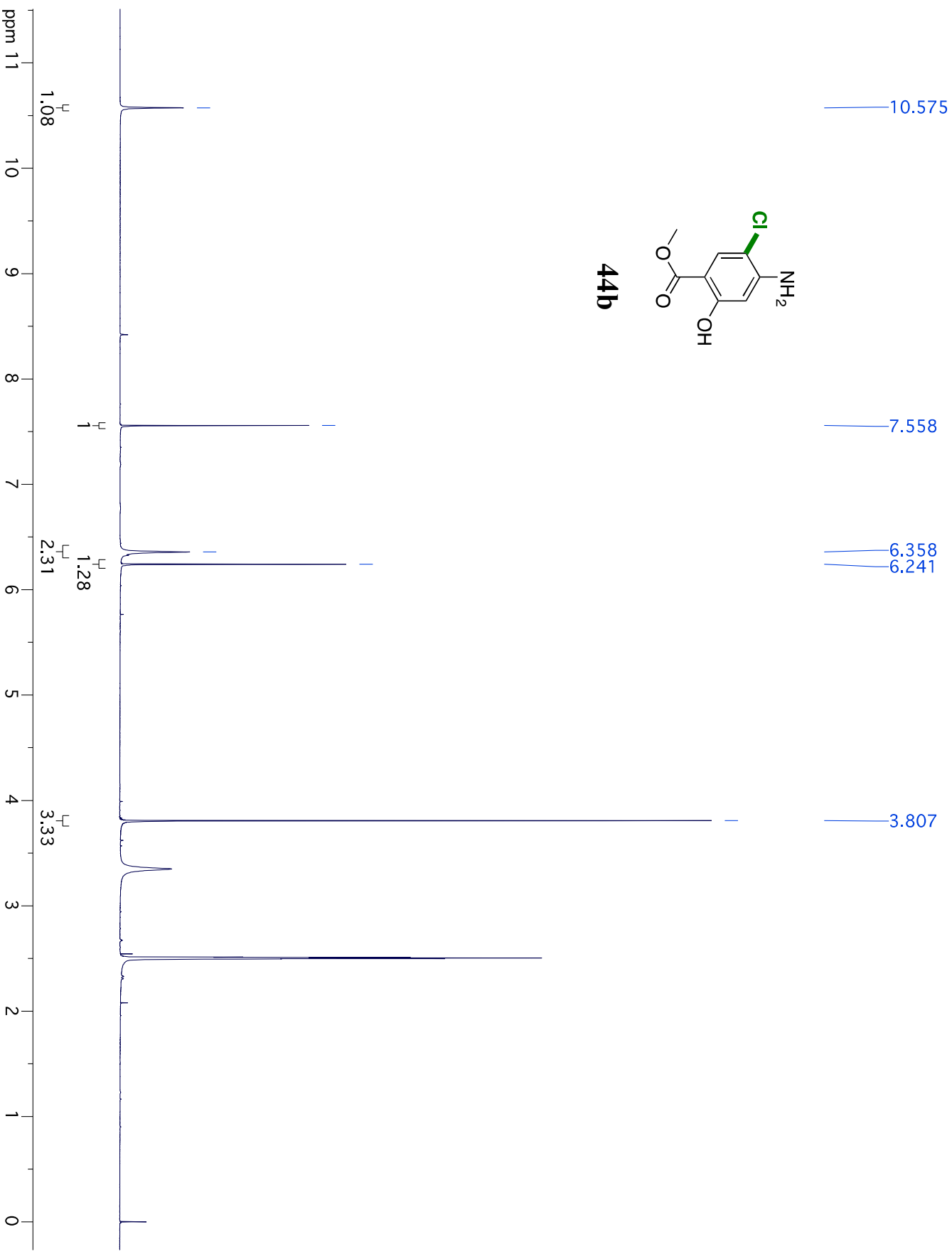
NOSEY of 43a

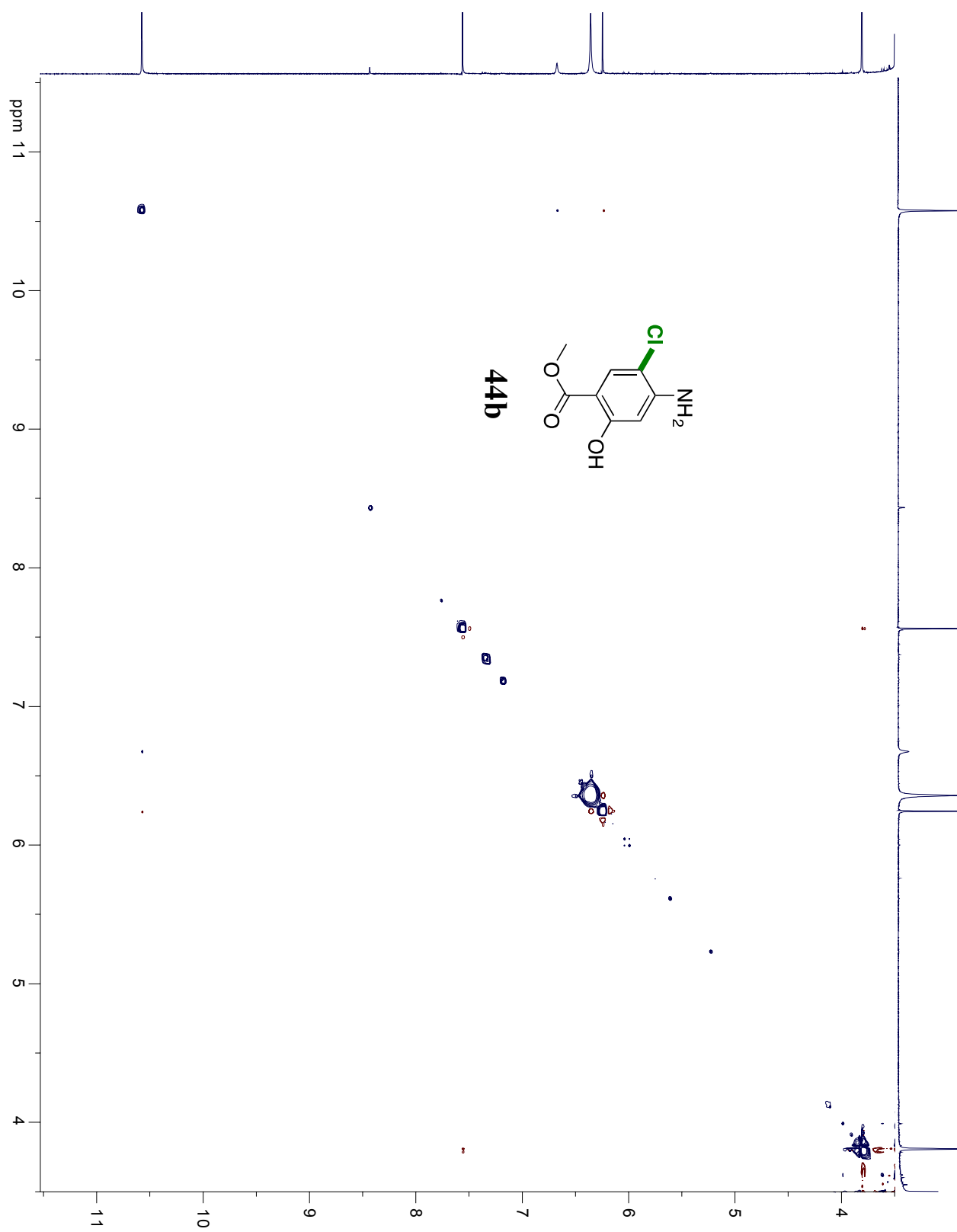


44







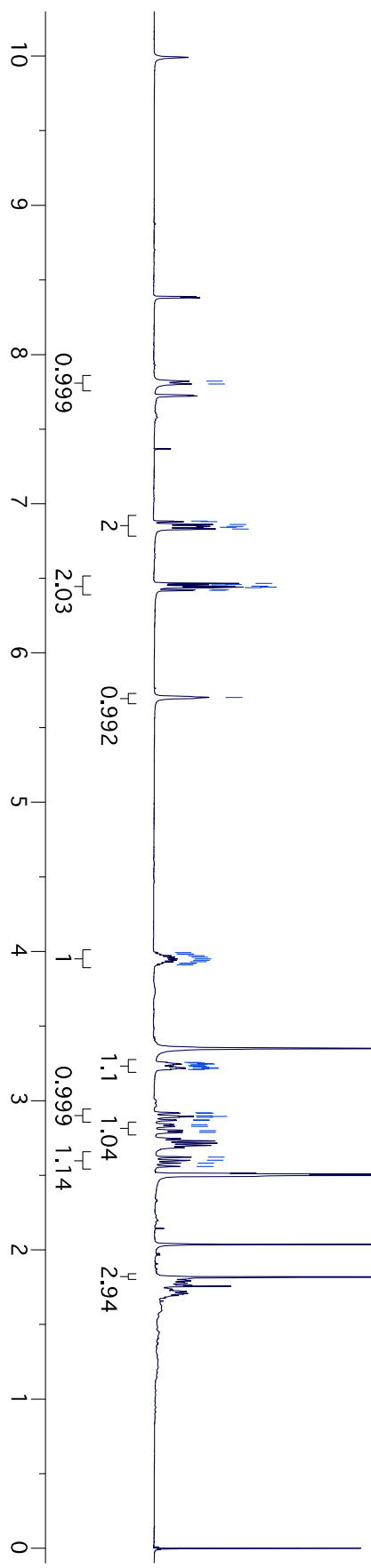
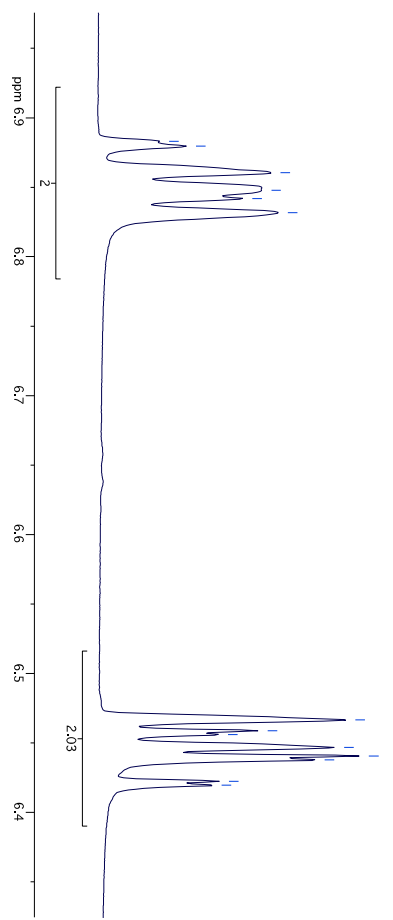


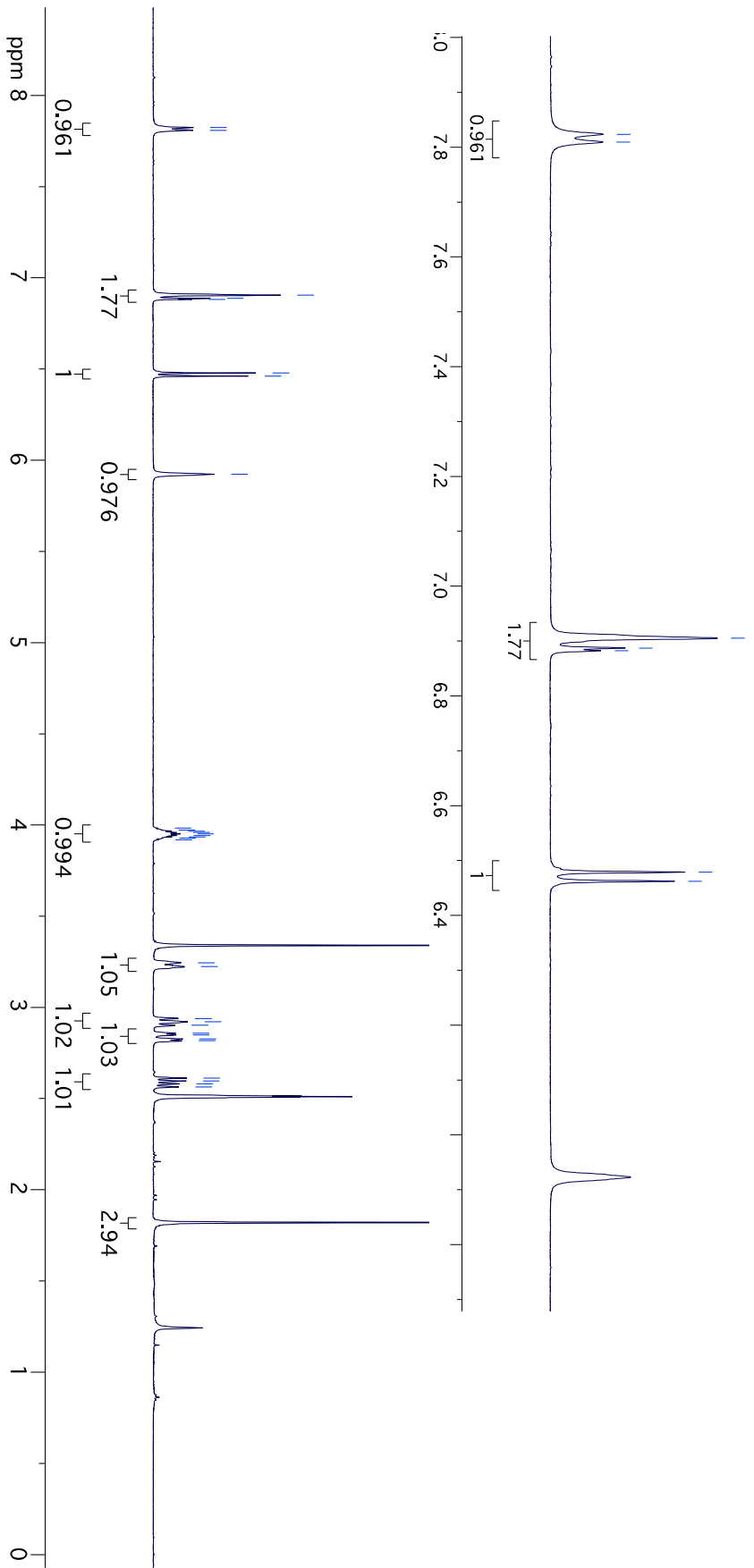
NOSEY of **44b**

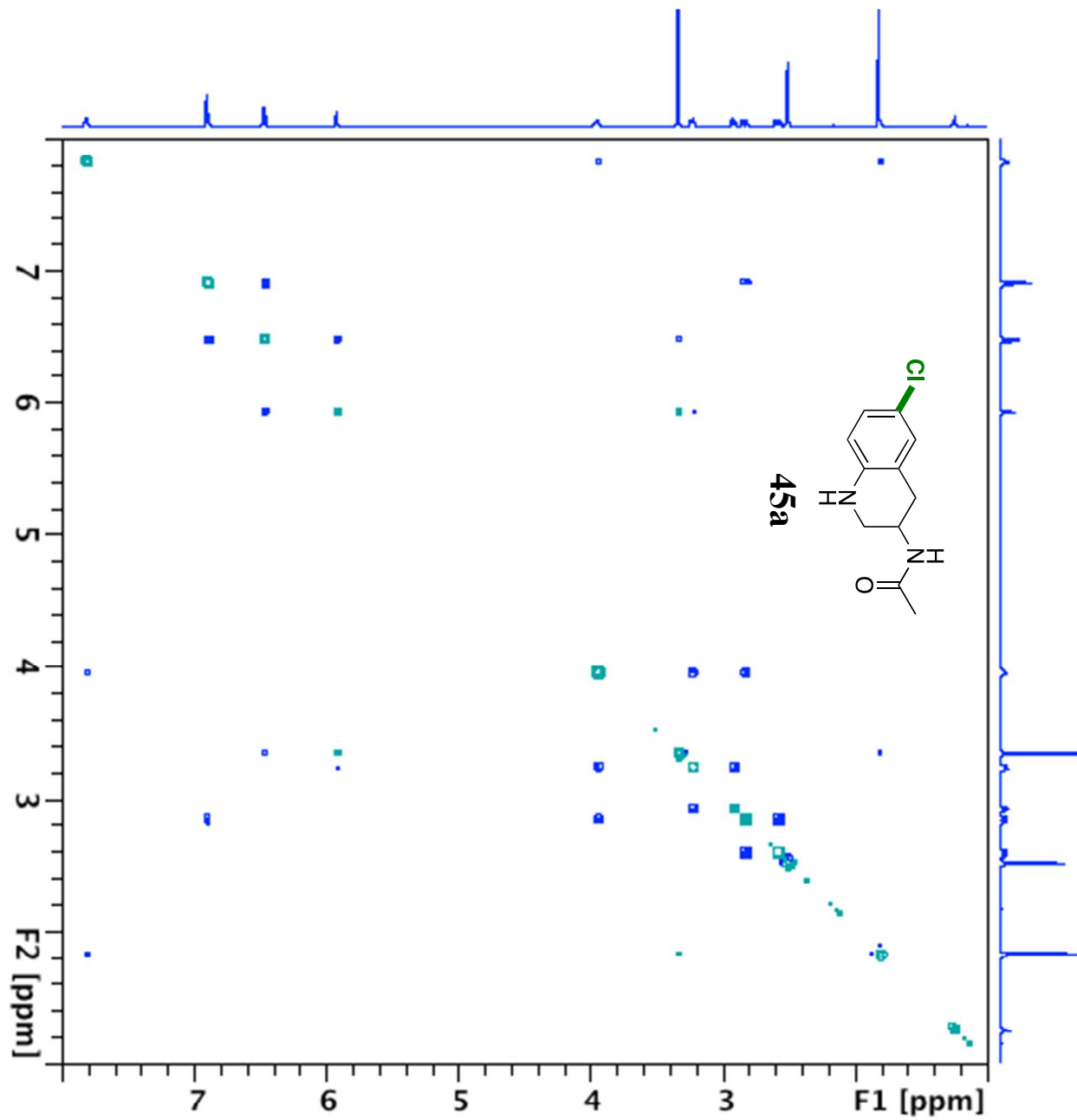
- 7.821
- 7.802
- 6.883
- 6.880
- 6.860
- 6.848
- 6.842
- 6.832
- 6.467
- 6.459
- 6.456
- 6.447
- 6.441
- 6.438
- 6.422
- 6.420
- 5.702
- 3.993
- 3.983
- 3.980
- 3.971
- 3.961
- 3.950
- 3.941
- 3.931
- 3.922
- 3.919
- 3.909
- 3.258
- 3.253
- 3.249
- 3.245
- 3.241
- 3.236
- 3.230
- 3.225
- 3.221
- 3.217
- 3.213
- 3.208
- 2.920
- 2.916
- 2.899
- 2.893
- 2.888
- 2.871
- 2.867
- 2.839
- 2.827
- 2.800
- 2.788
- 2.621
- 2.599
- 2.582
- 2.560
- 1.818



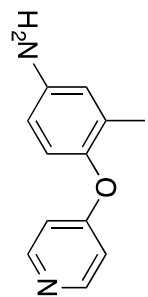
45



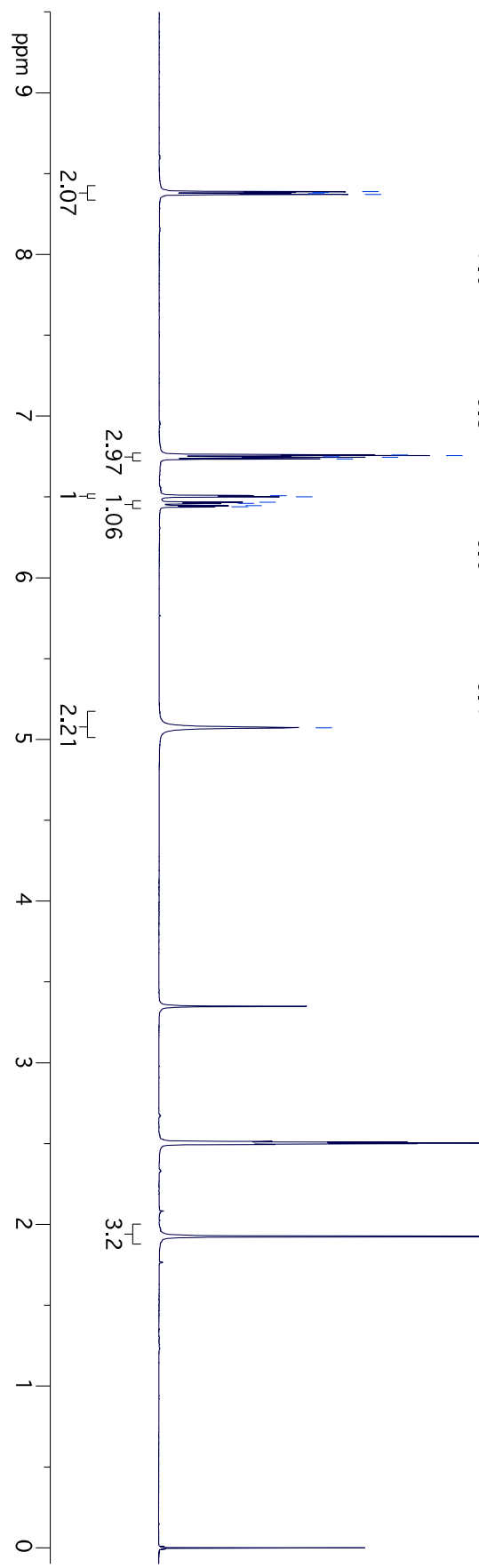
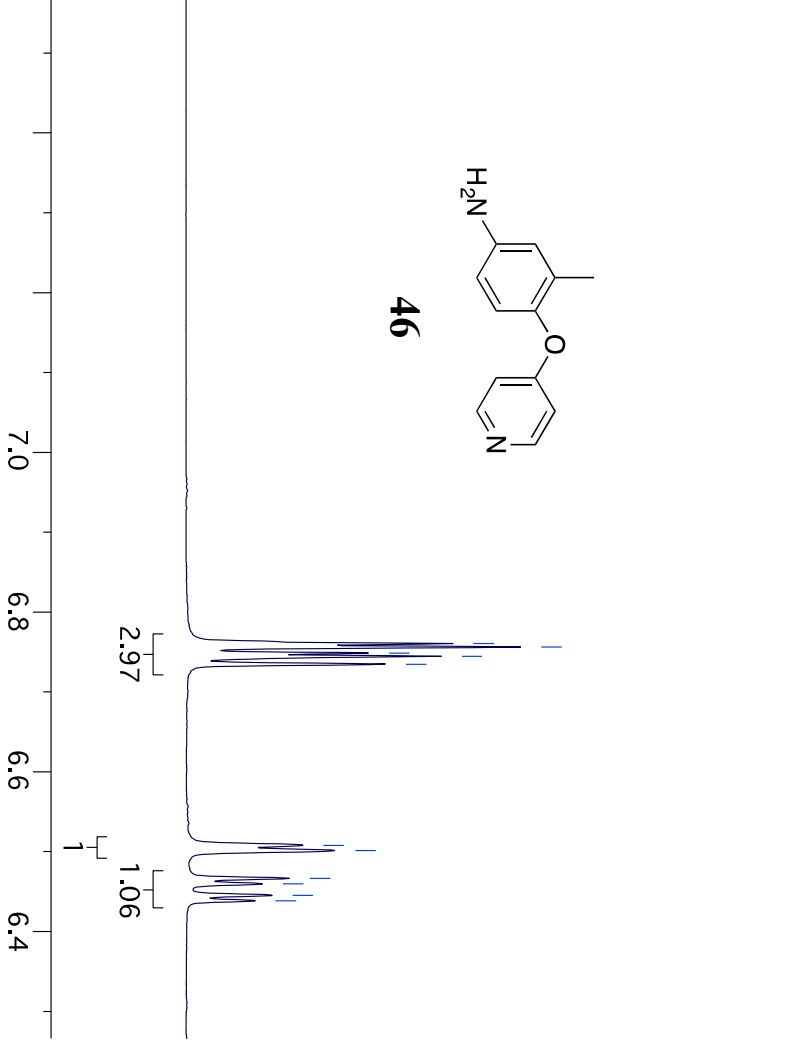




NOSEY of 45a



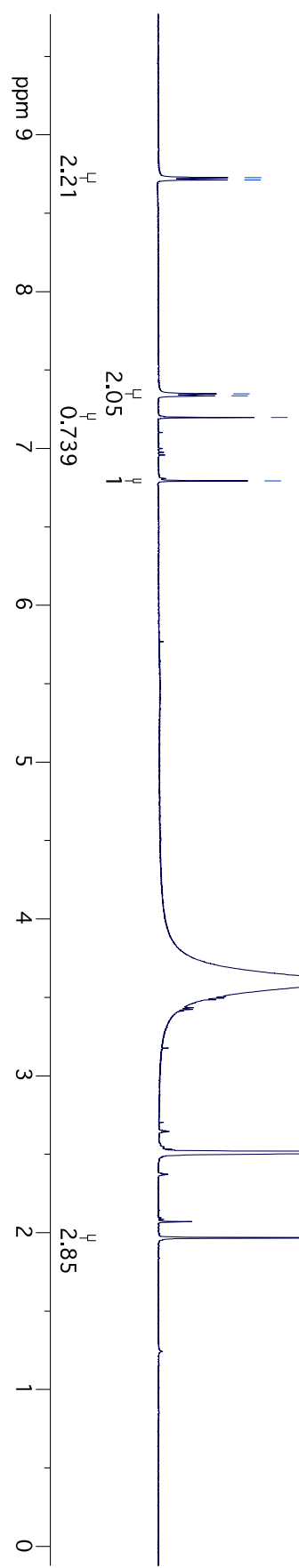
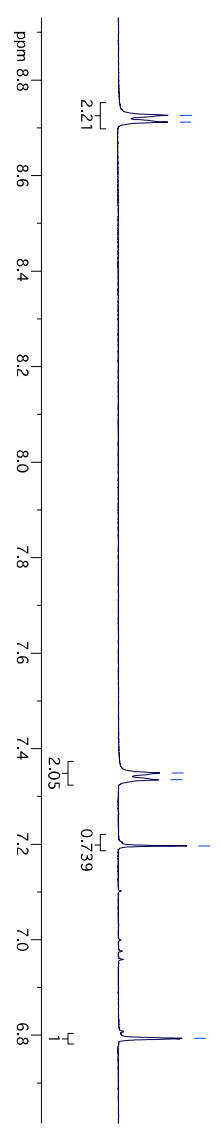
46

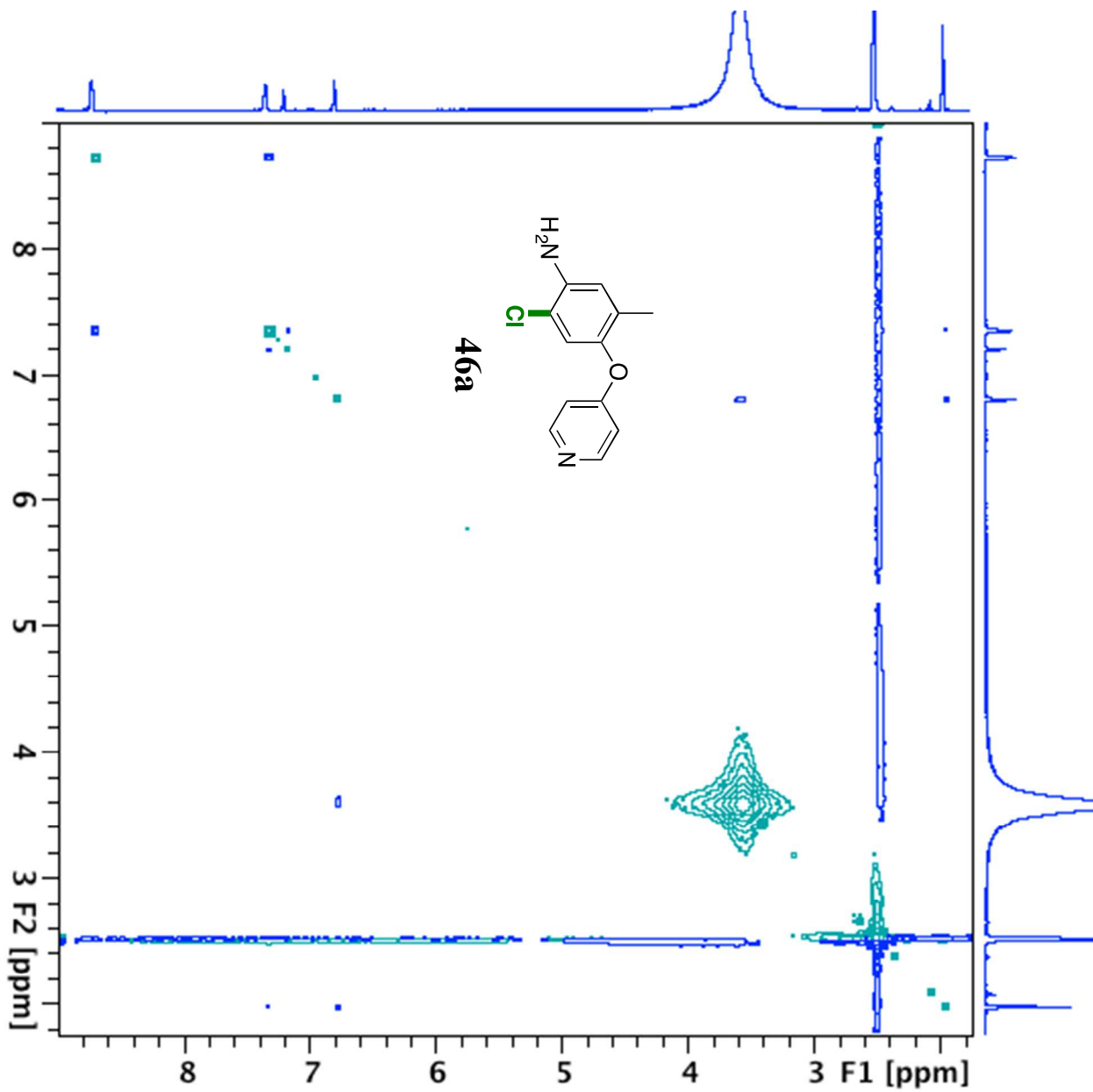


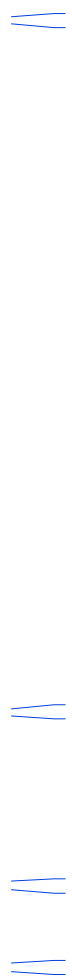
V

V

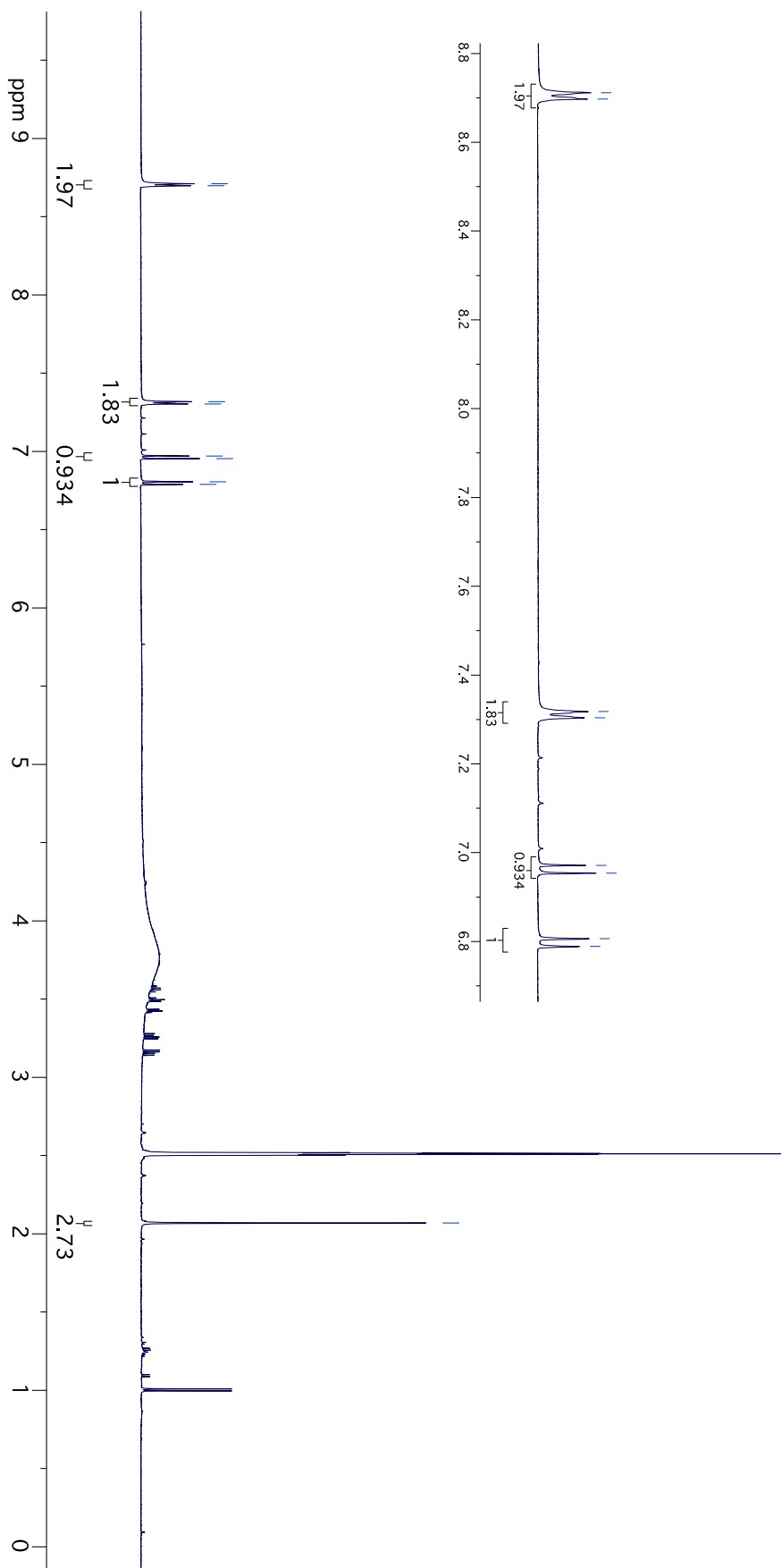
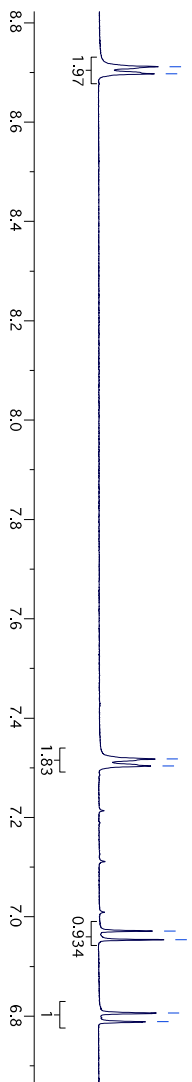
1.967

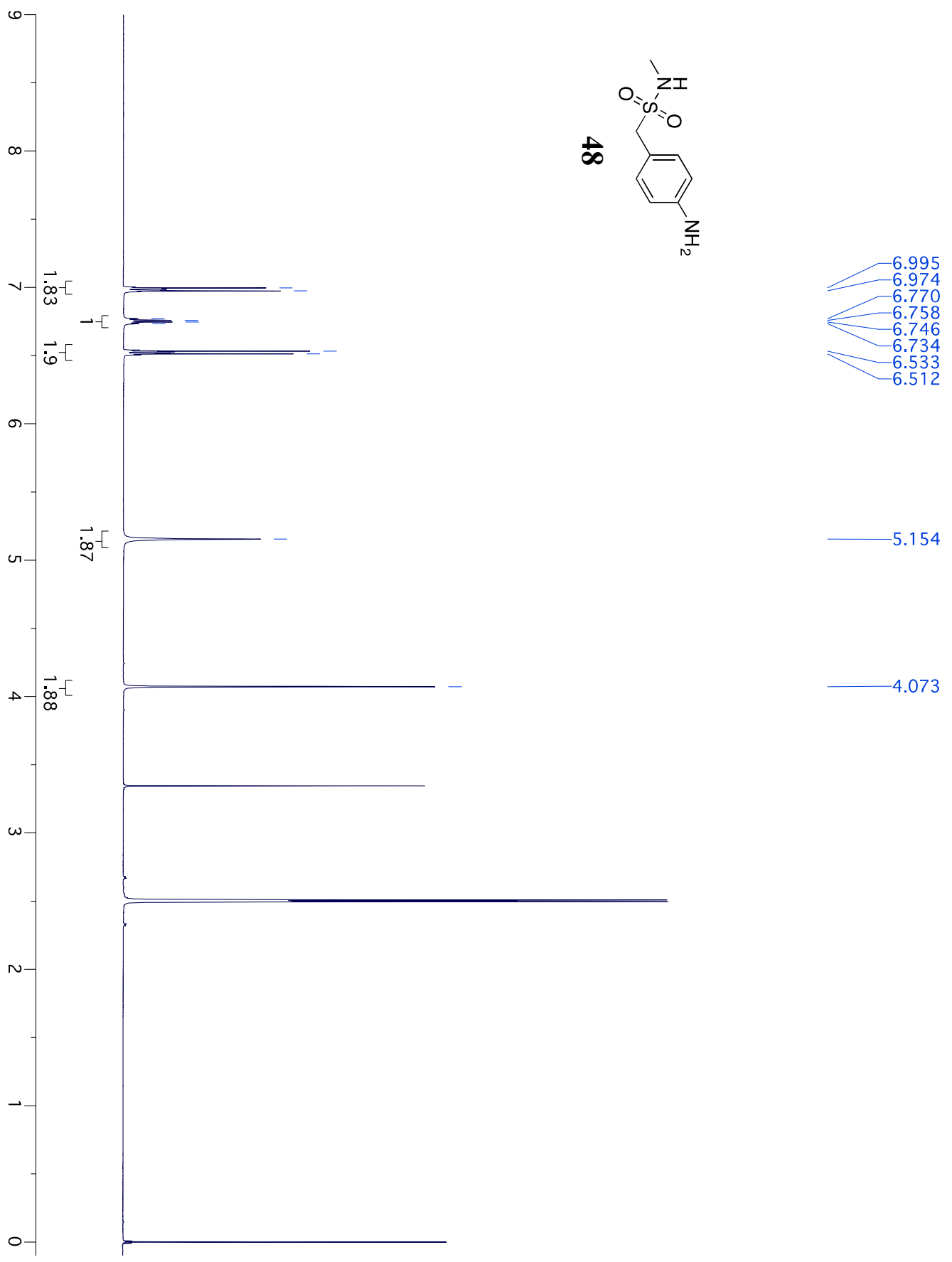
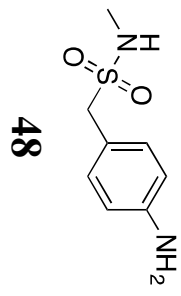


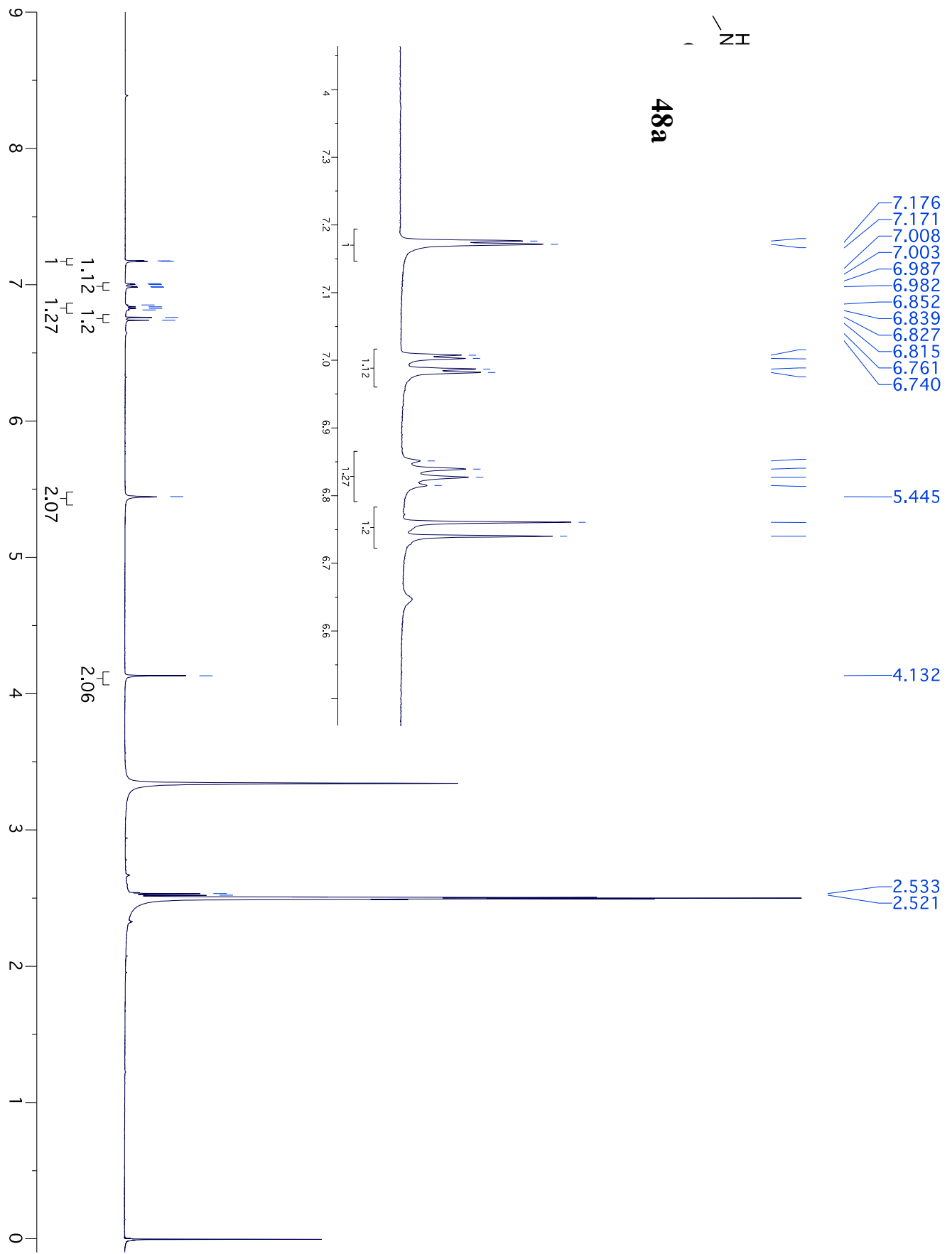


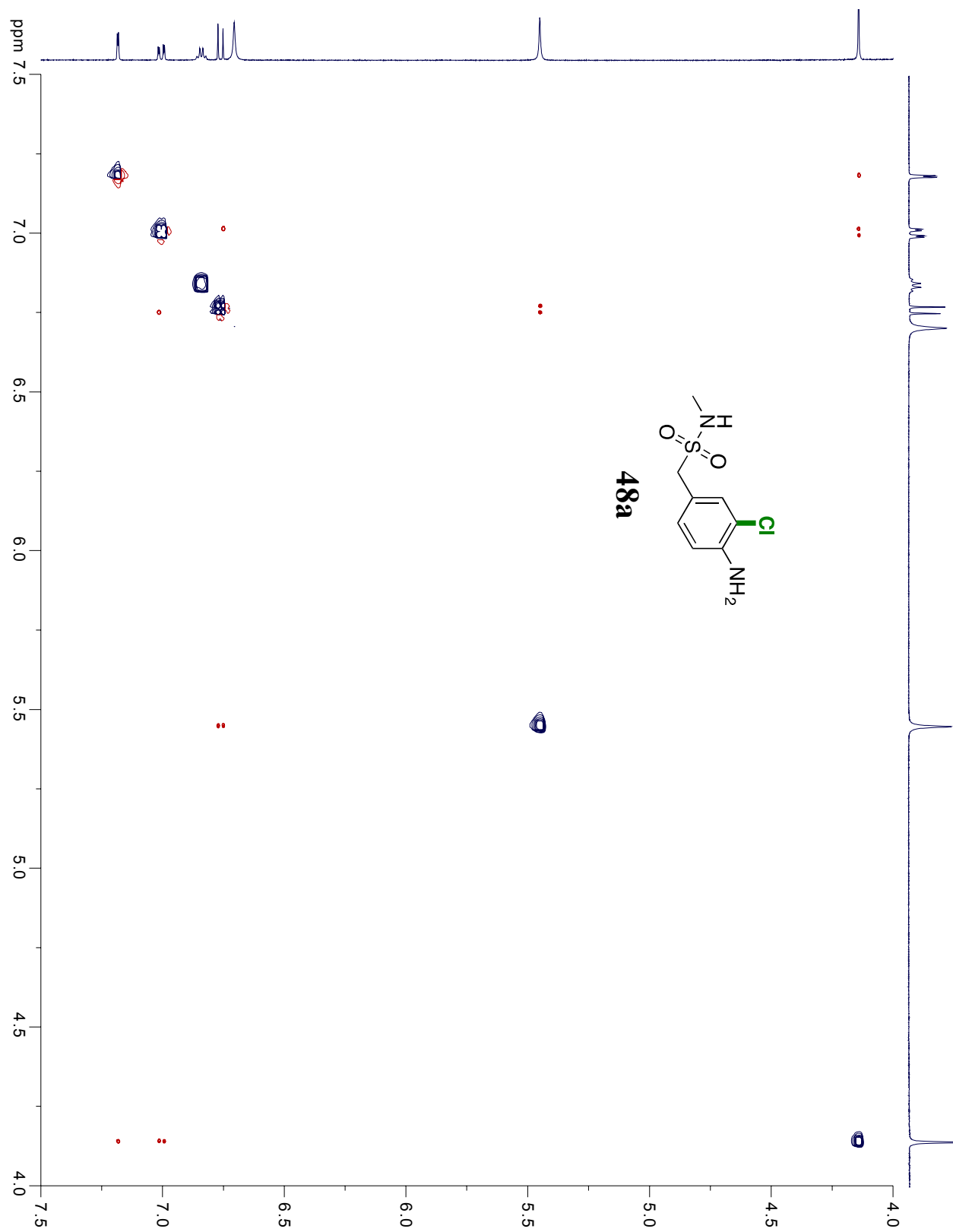


2.069





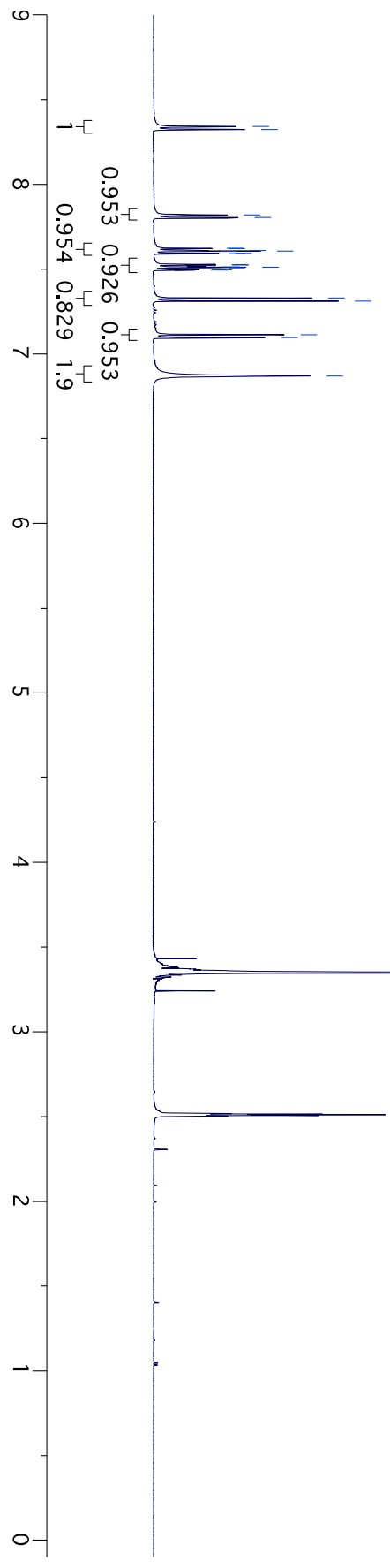
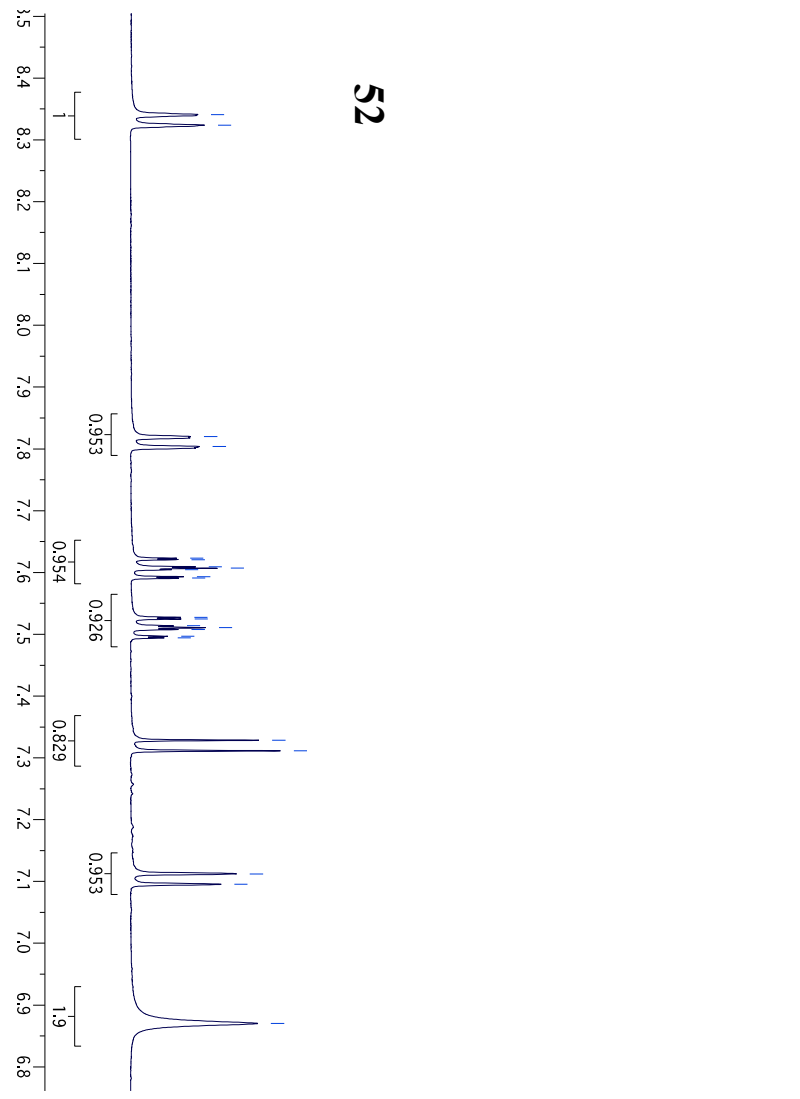


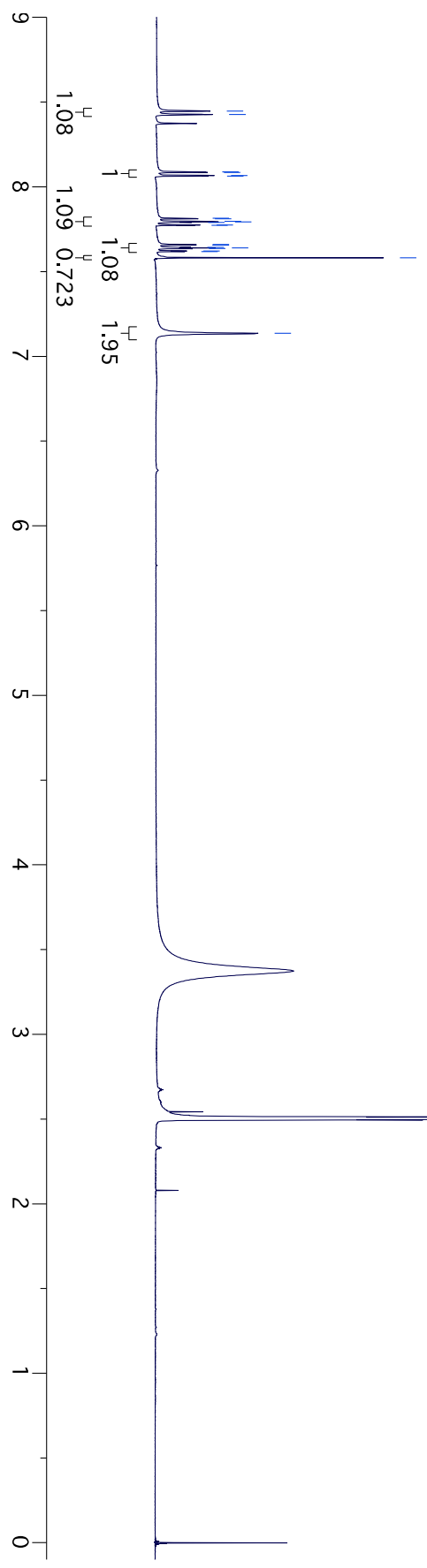
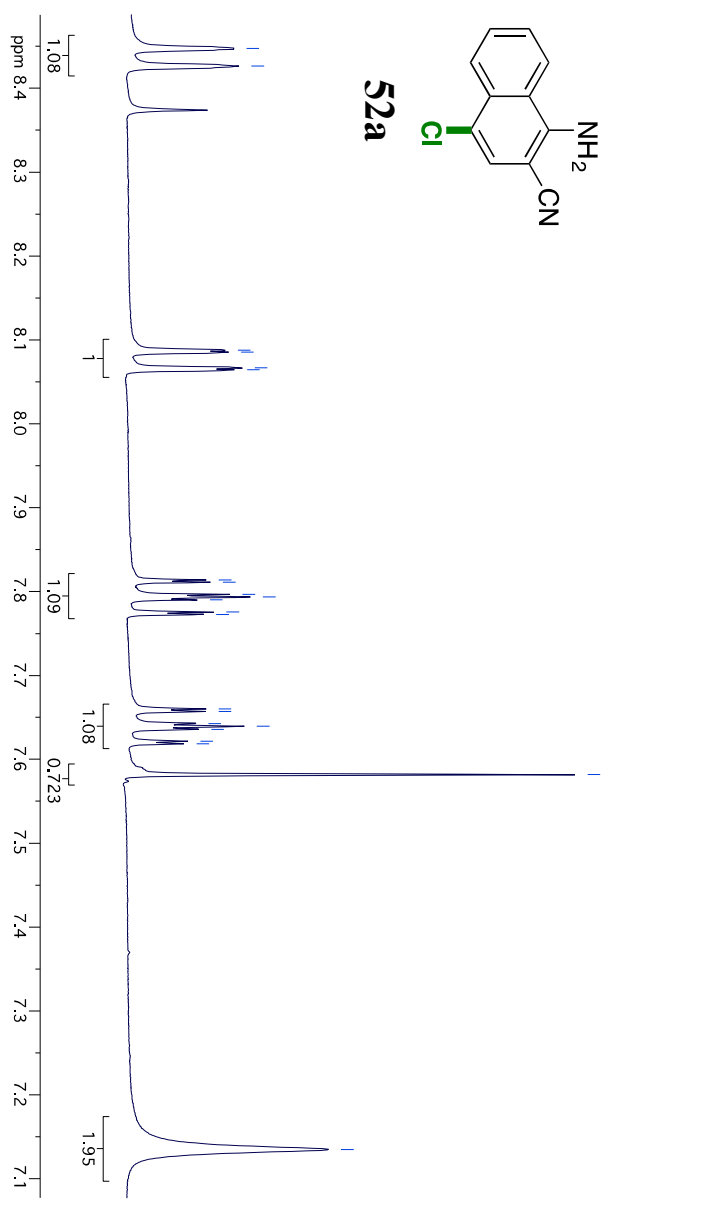
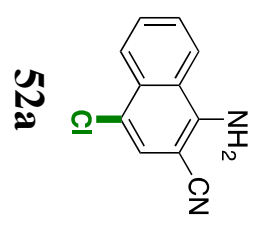
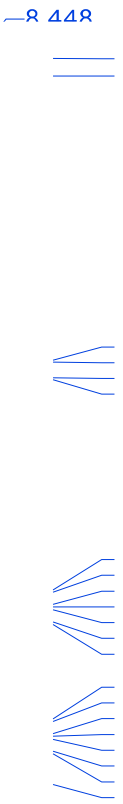


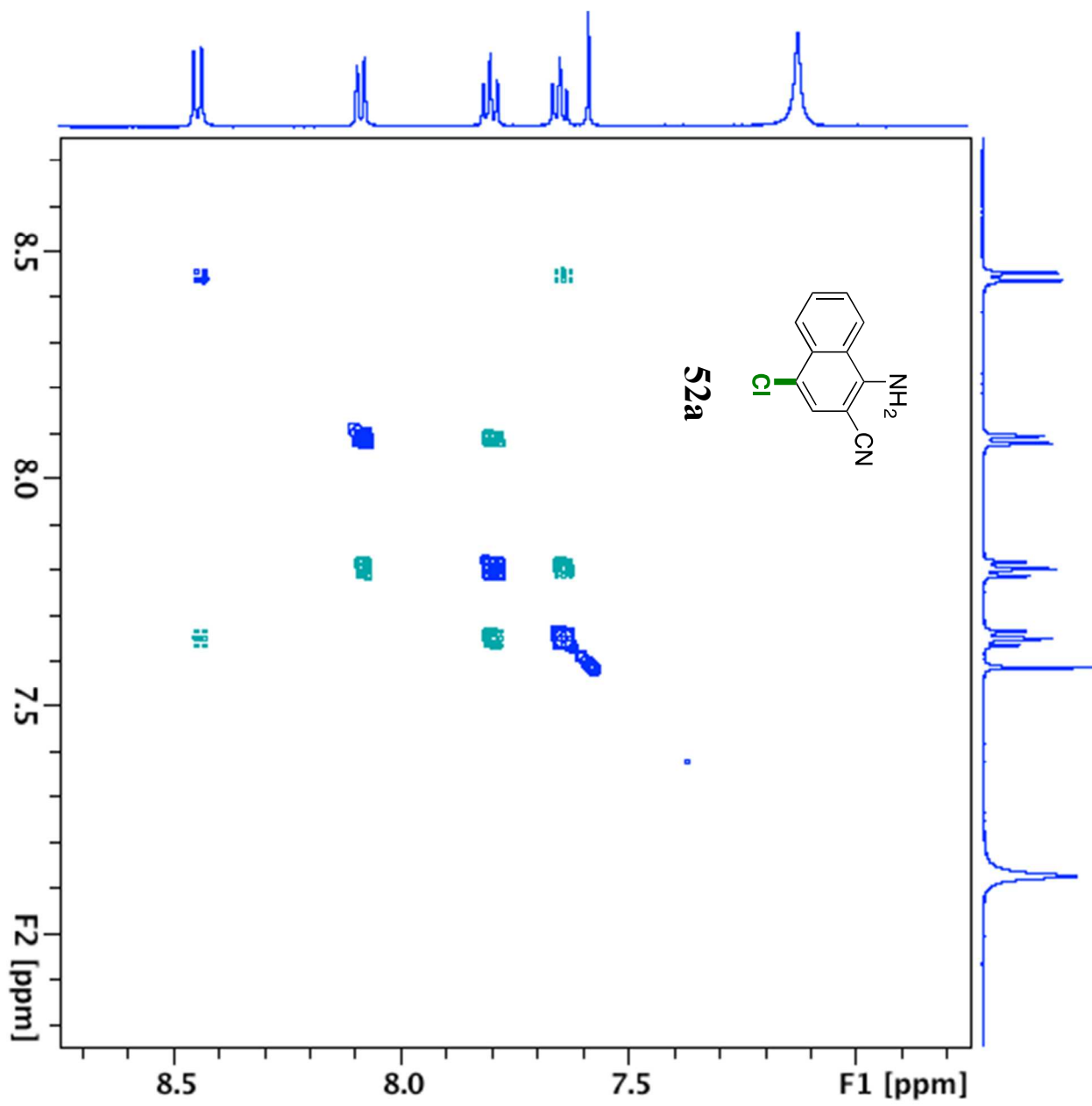
NOSEY of 48a



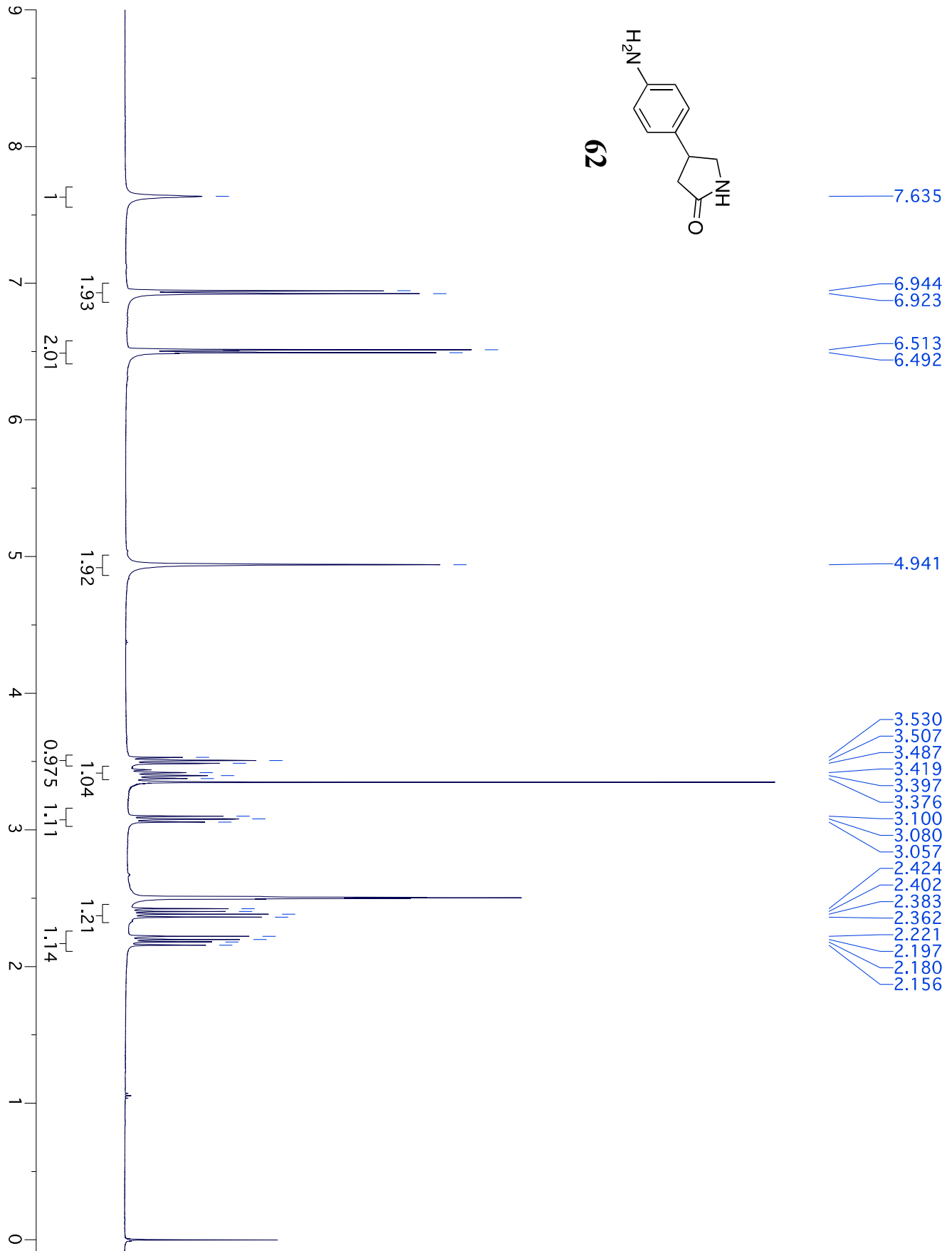
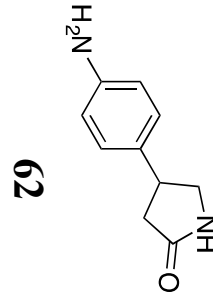
52





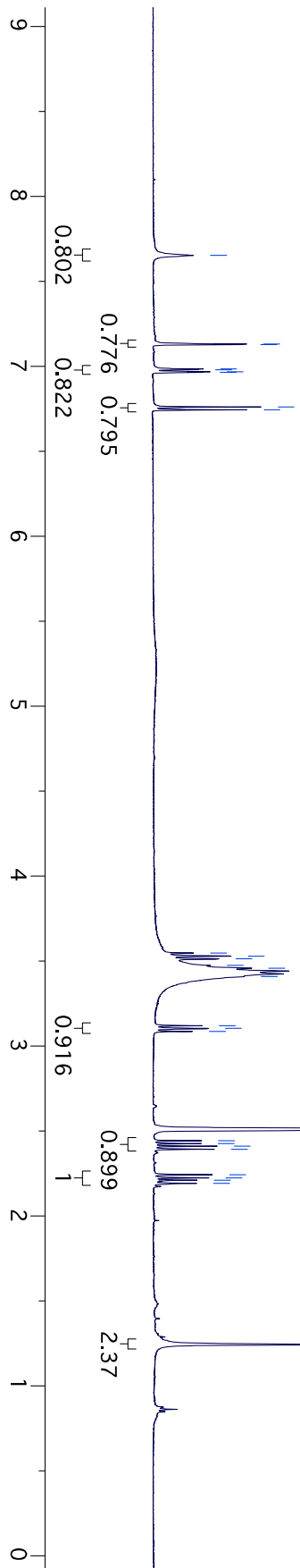
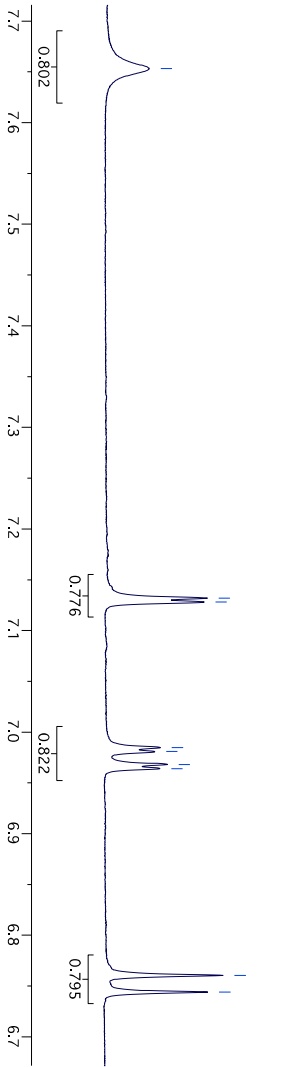
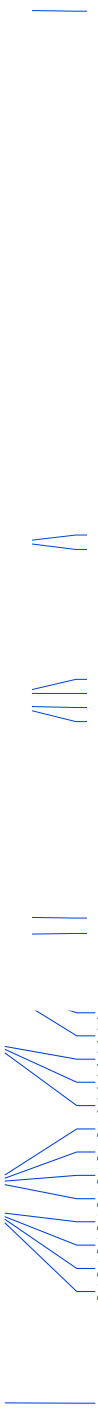


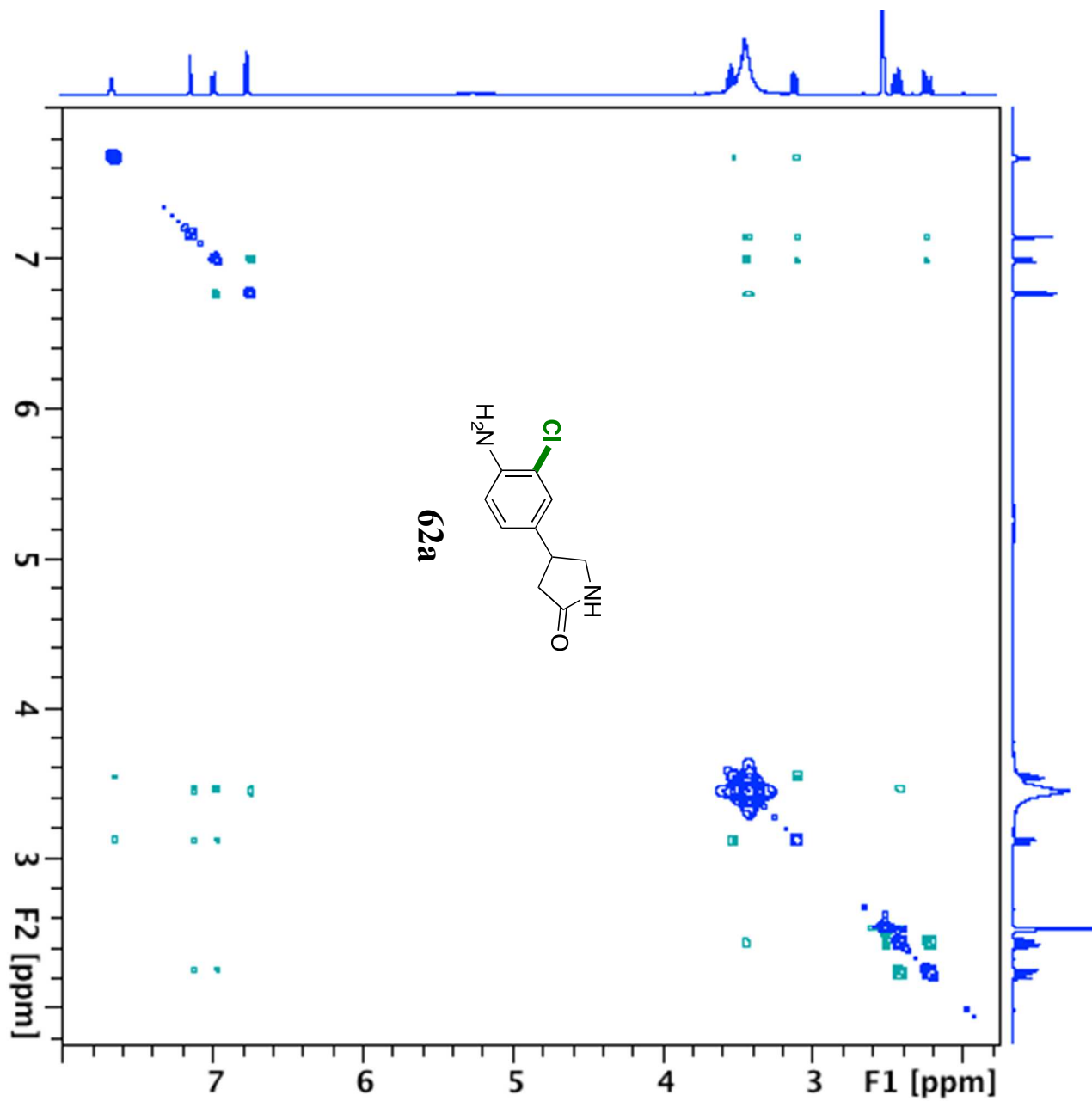
NOSEY of 52a



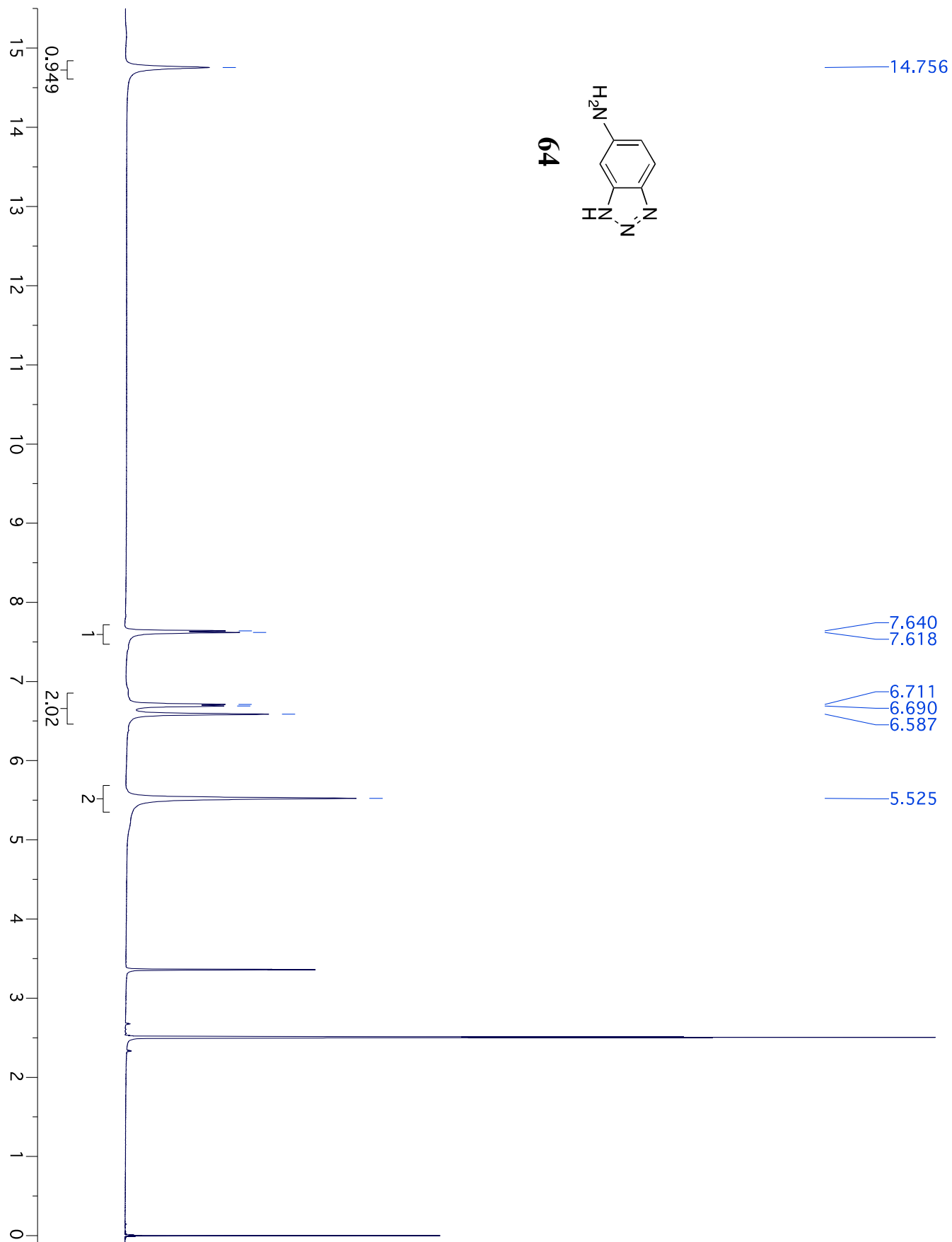
3
2
5
1
8
4
1
4

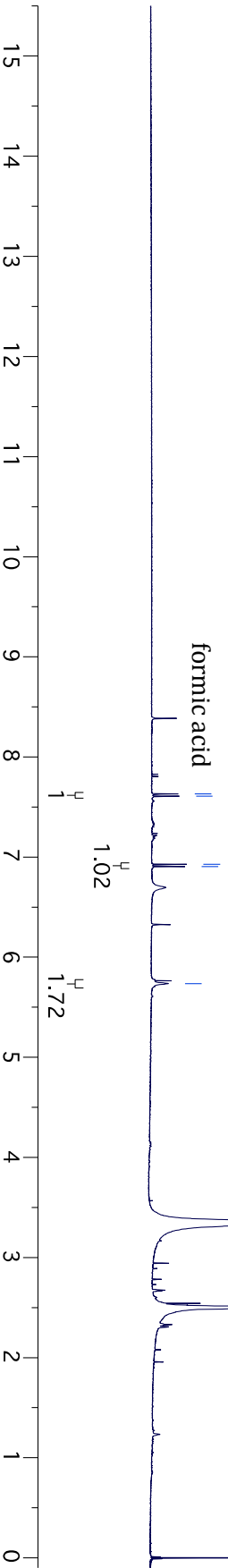
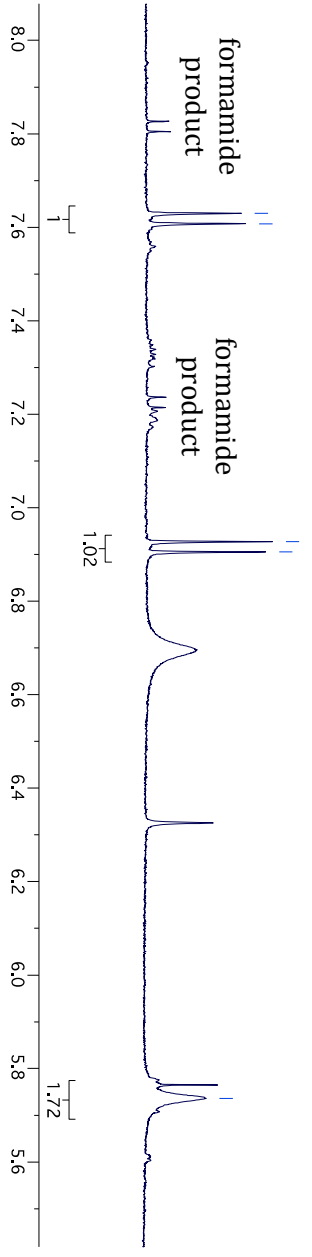
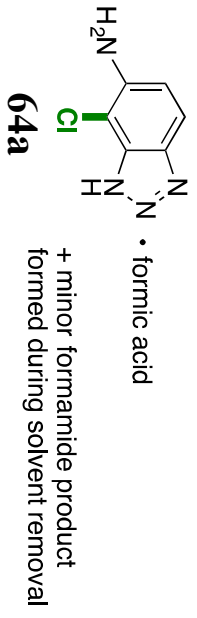
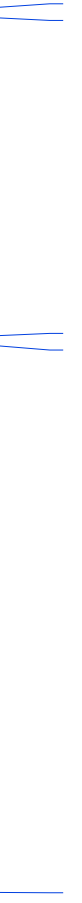
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9
2





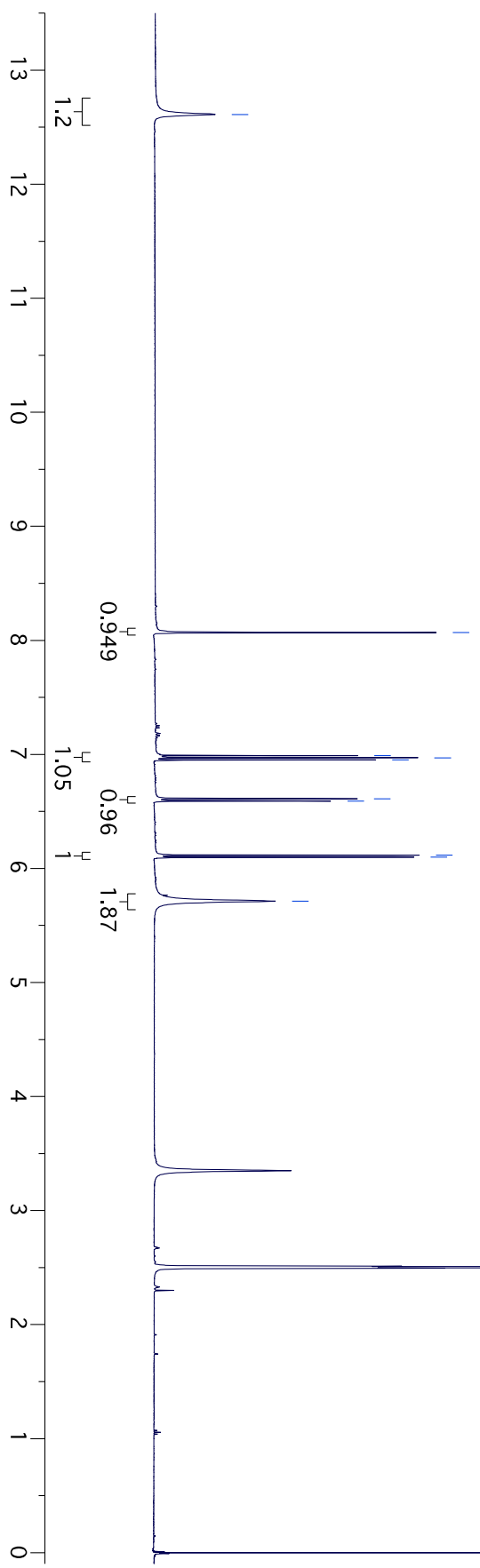
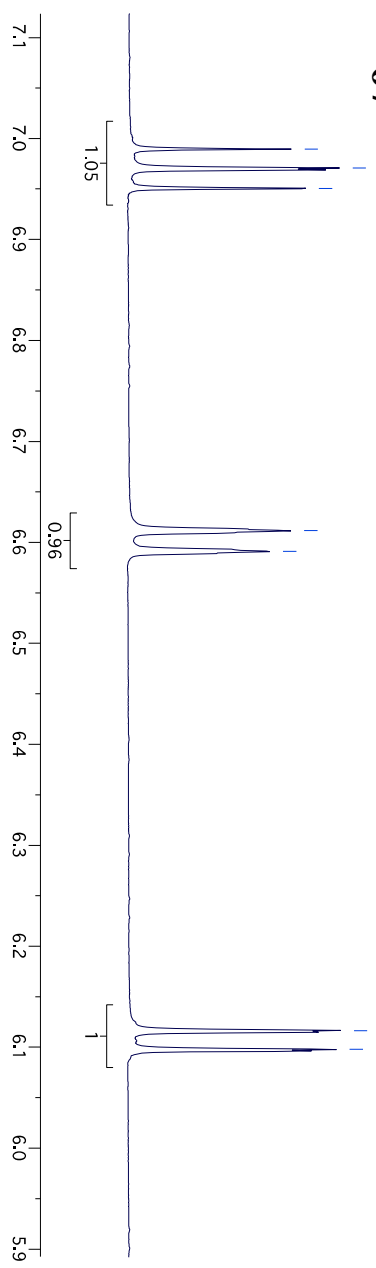
NOSEY of 62a

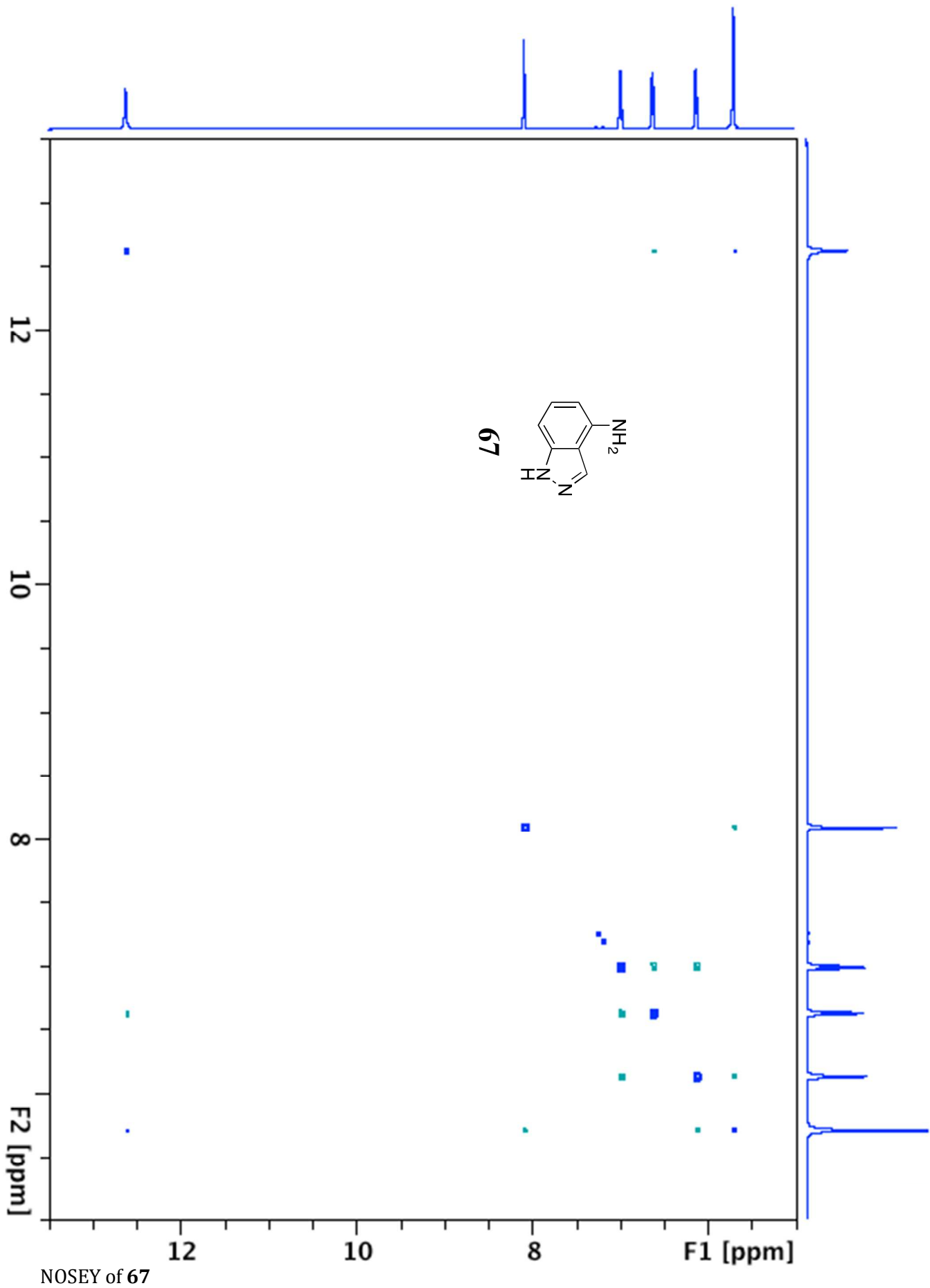


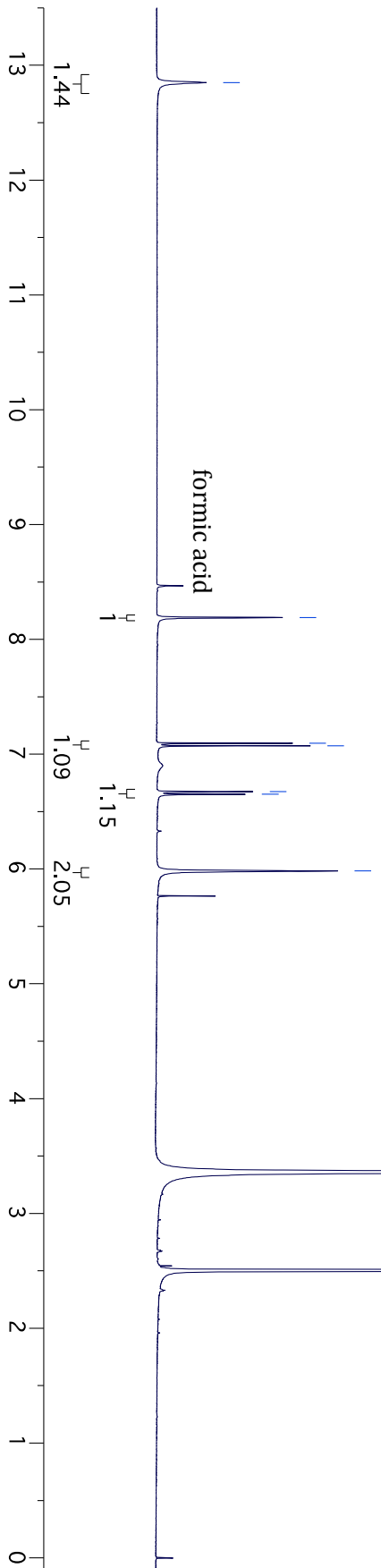
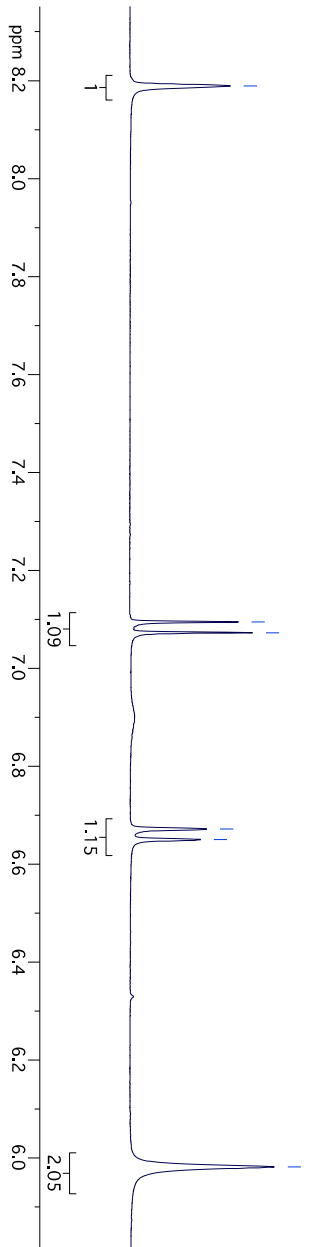
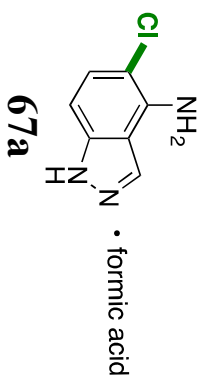


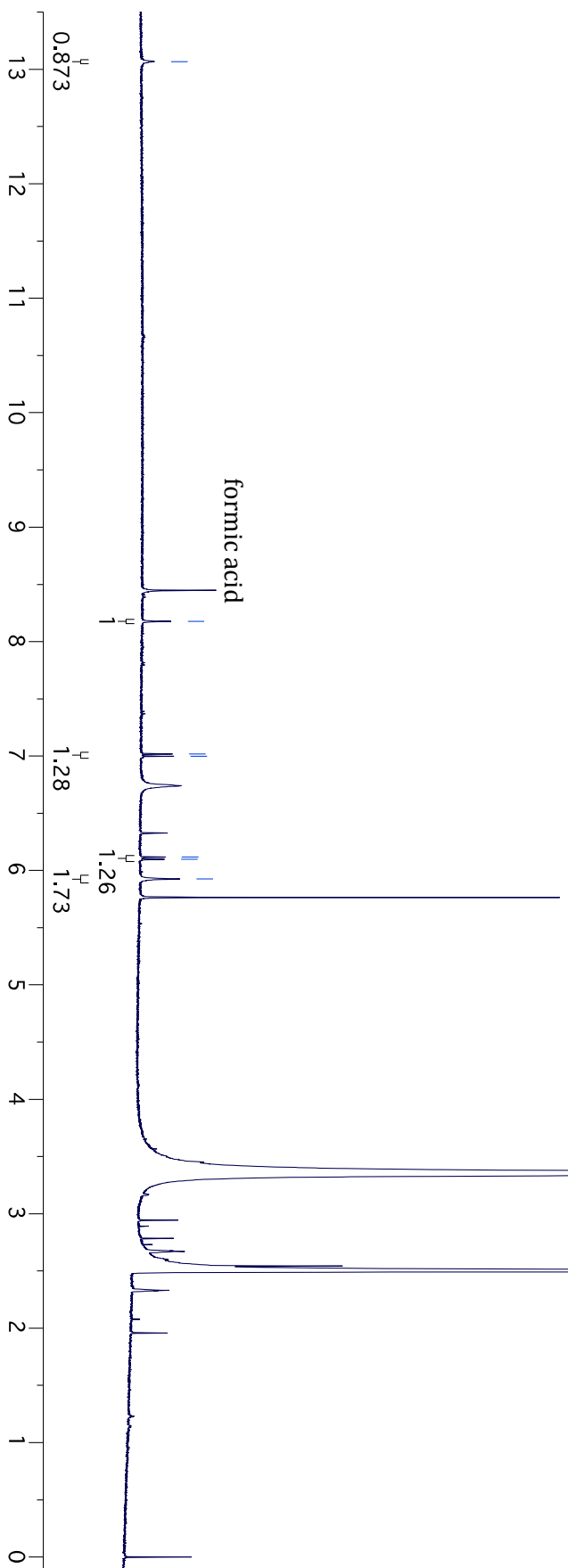
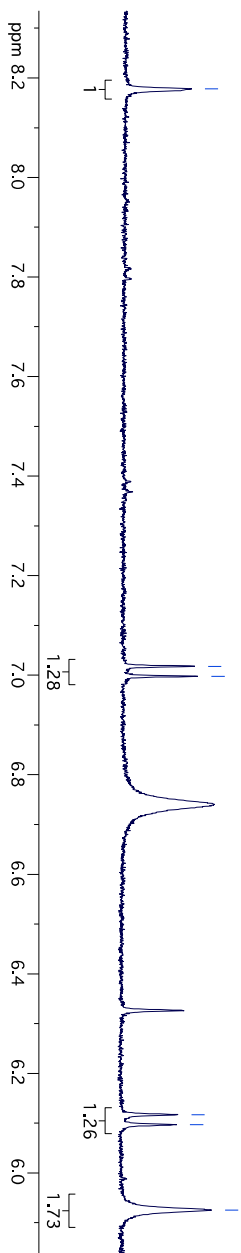
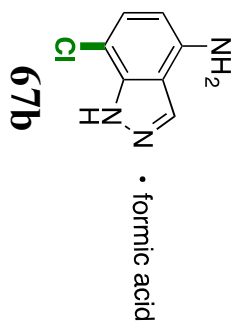


67



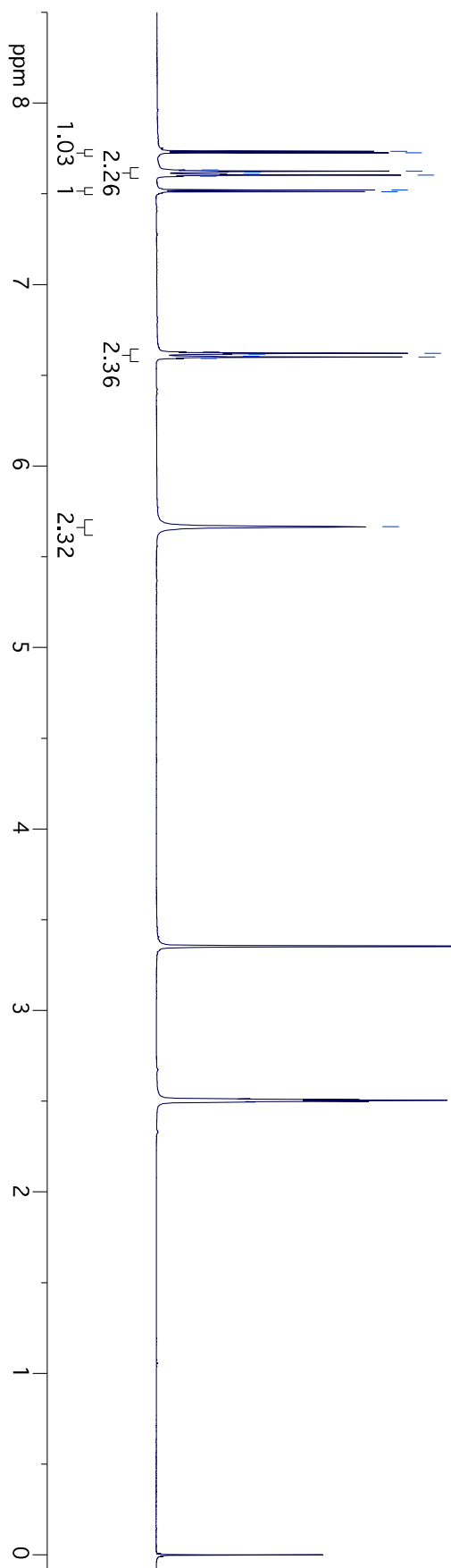
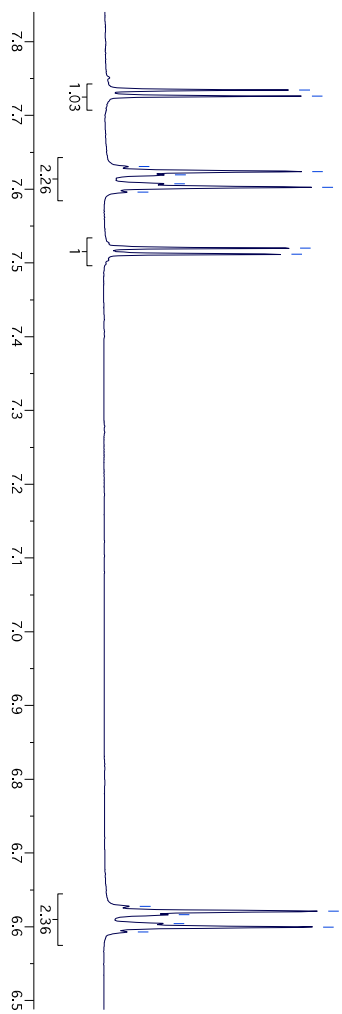






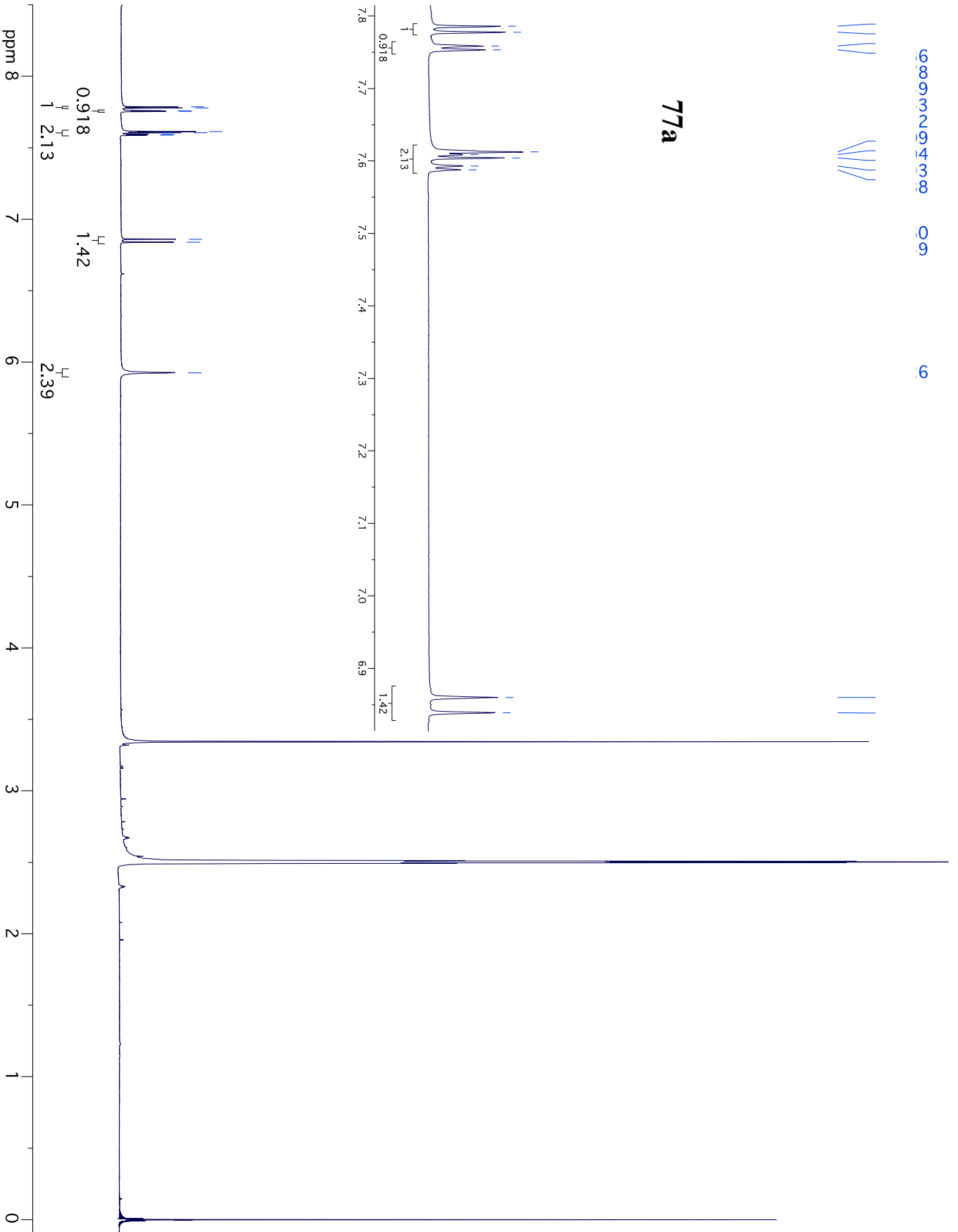


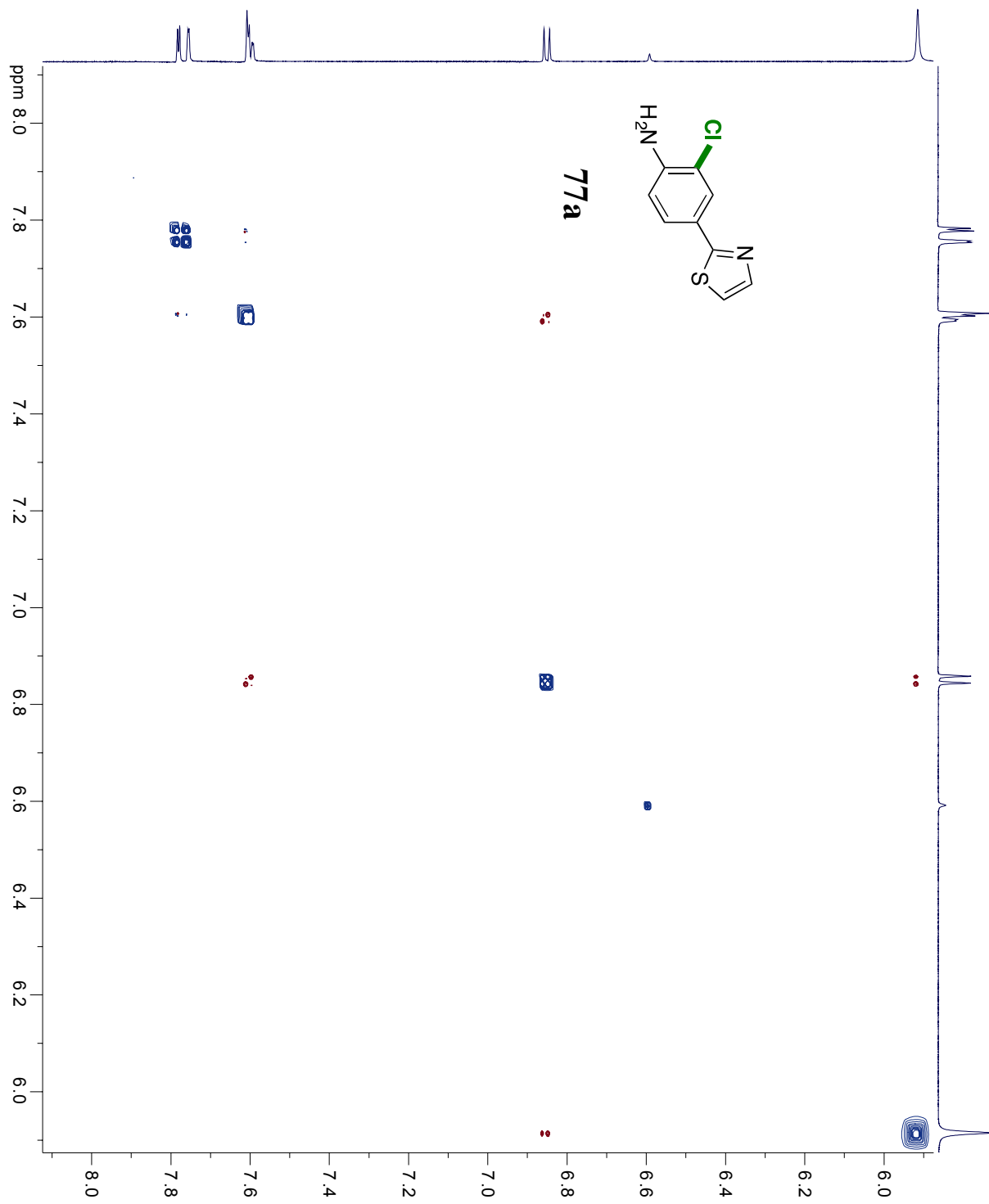
77



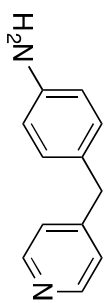
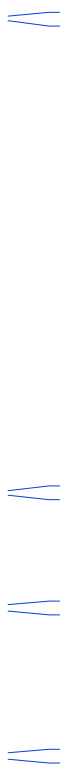
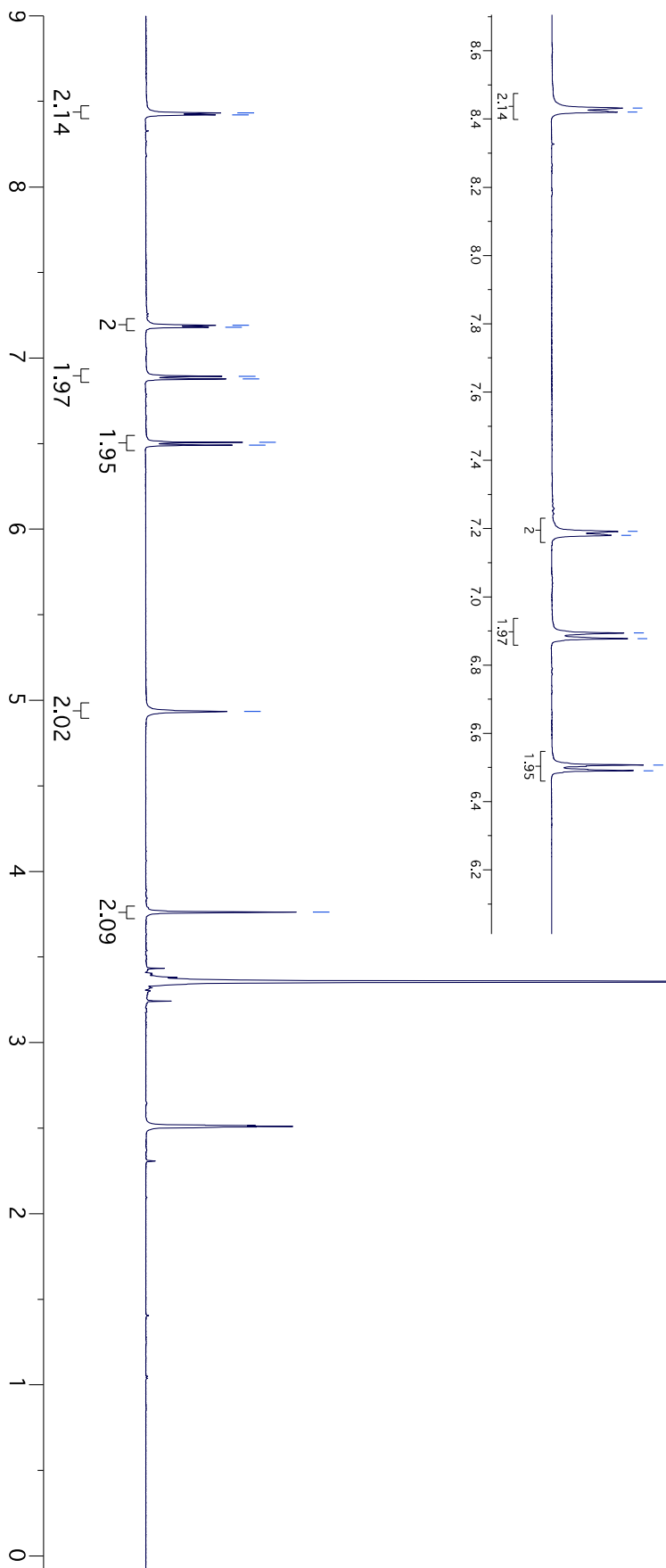


77a



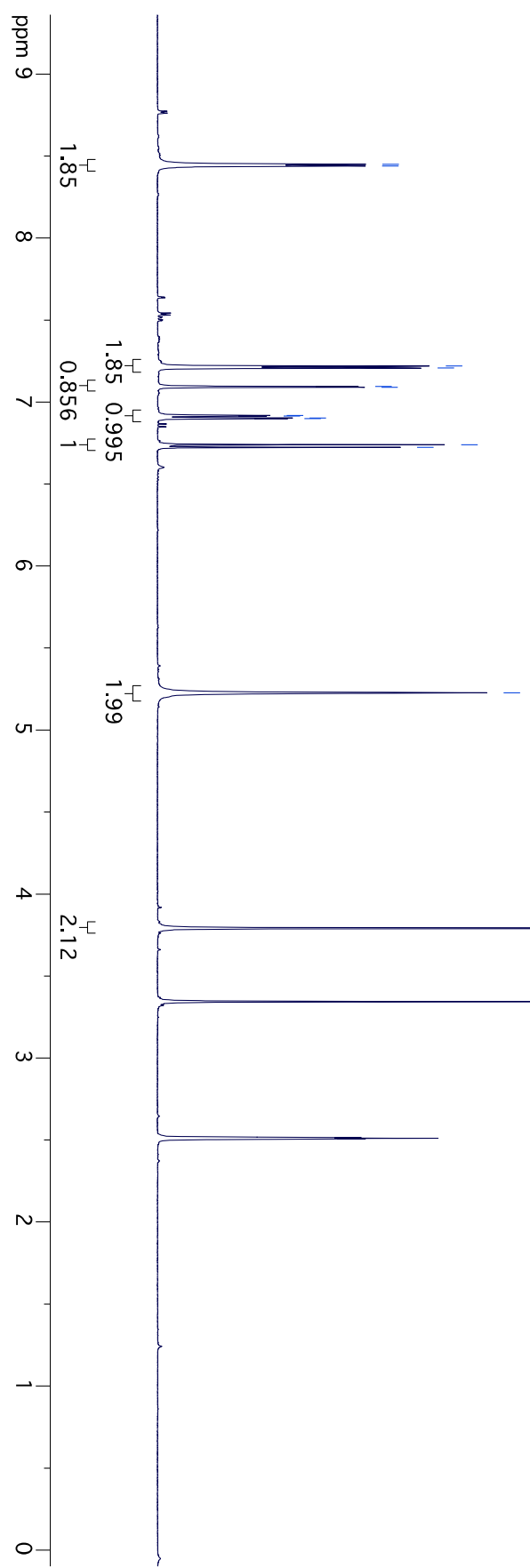
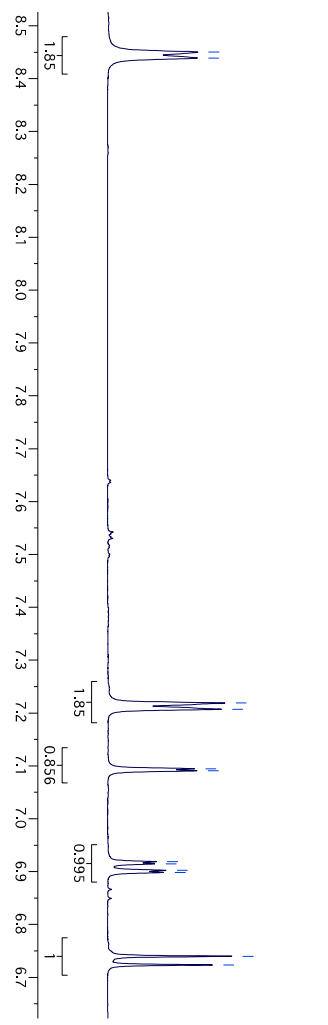


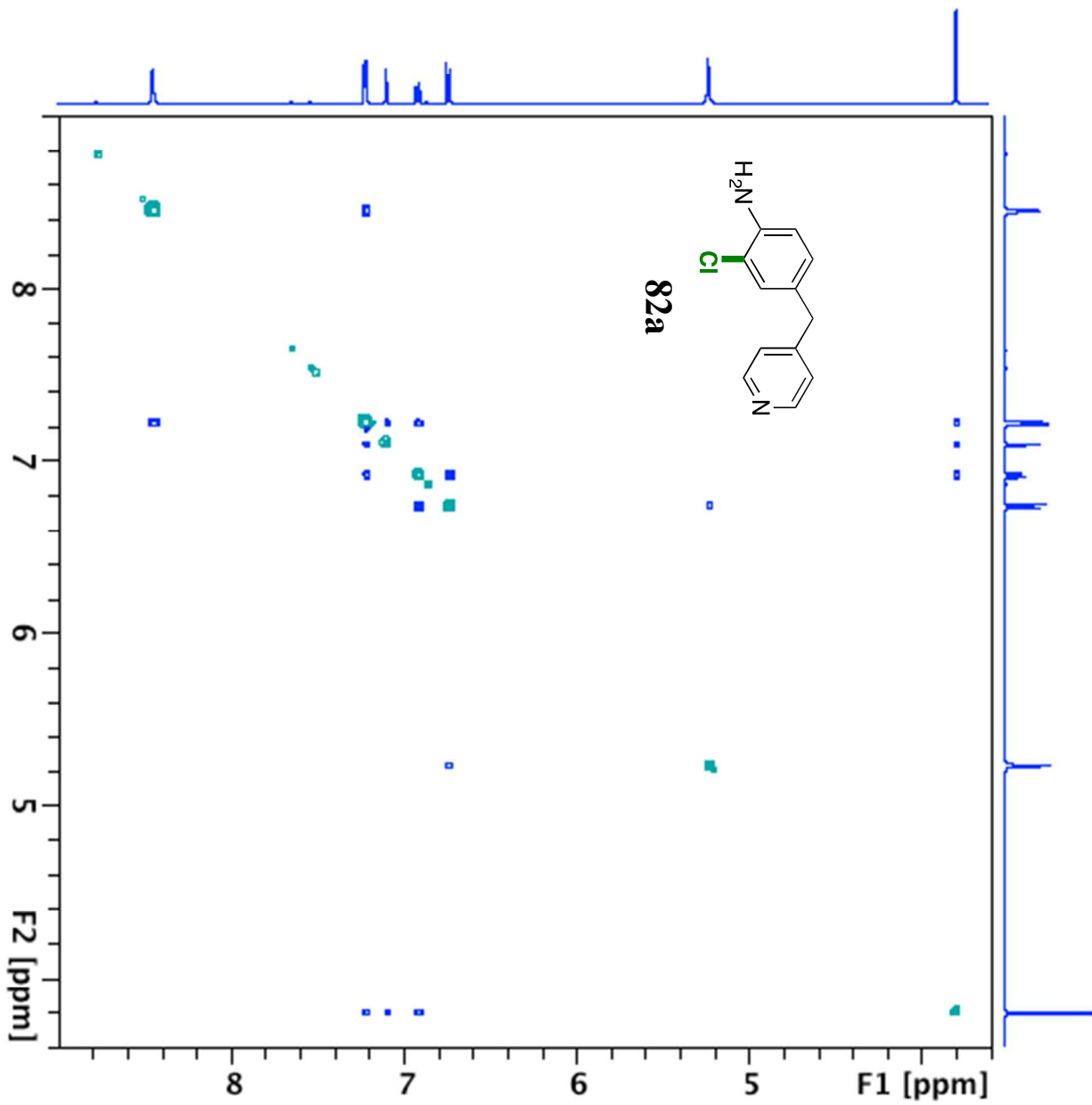
NOSEY of 77a

**82**

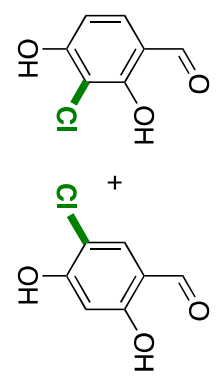
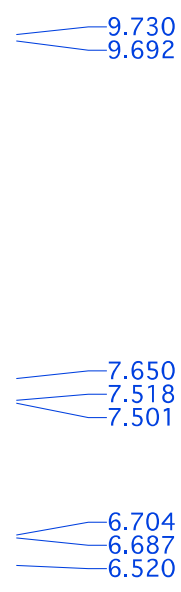


3.791

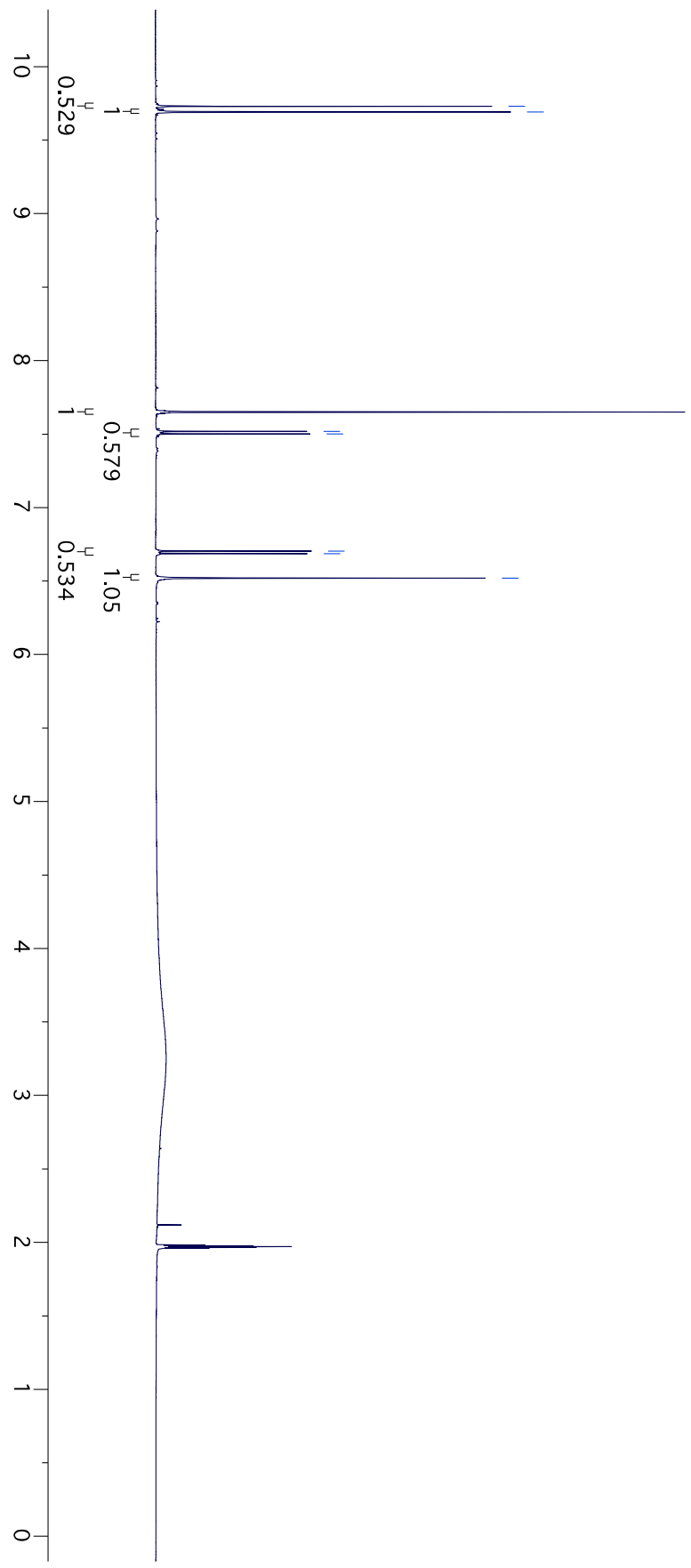


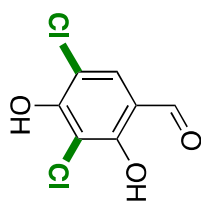


NOSEY of 82a

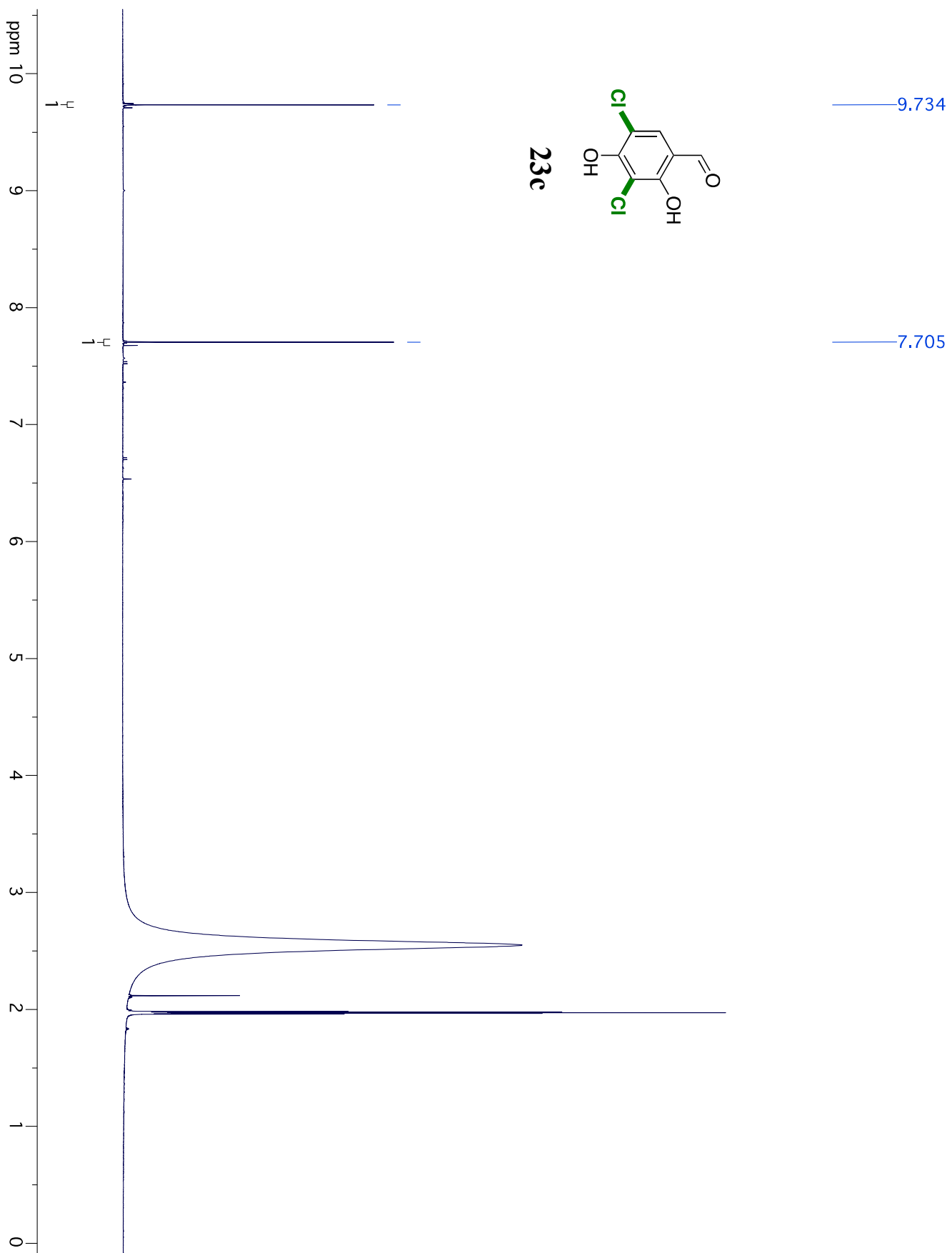


23a and 23b

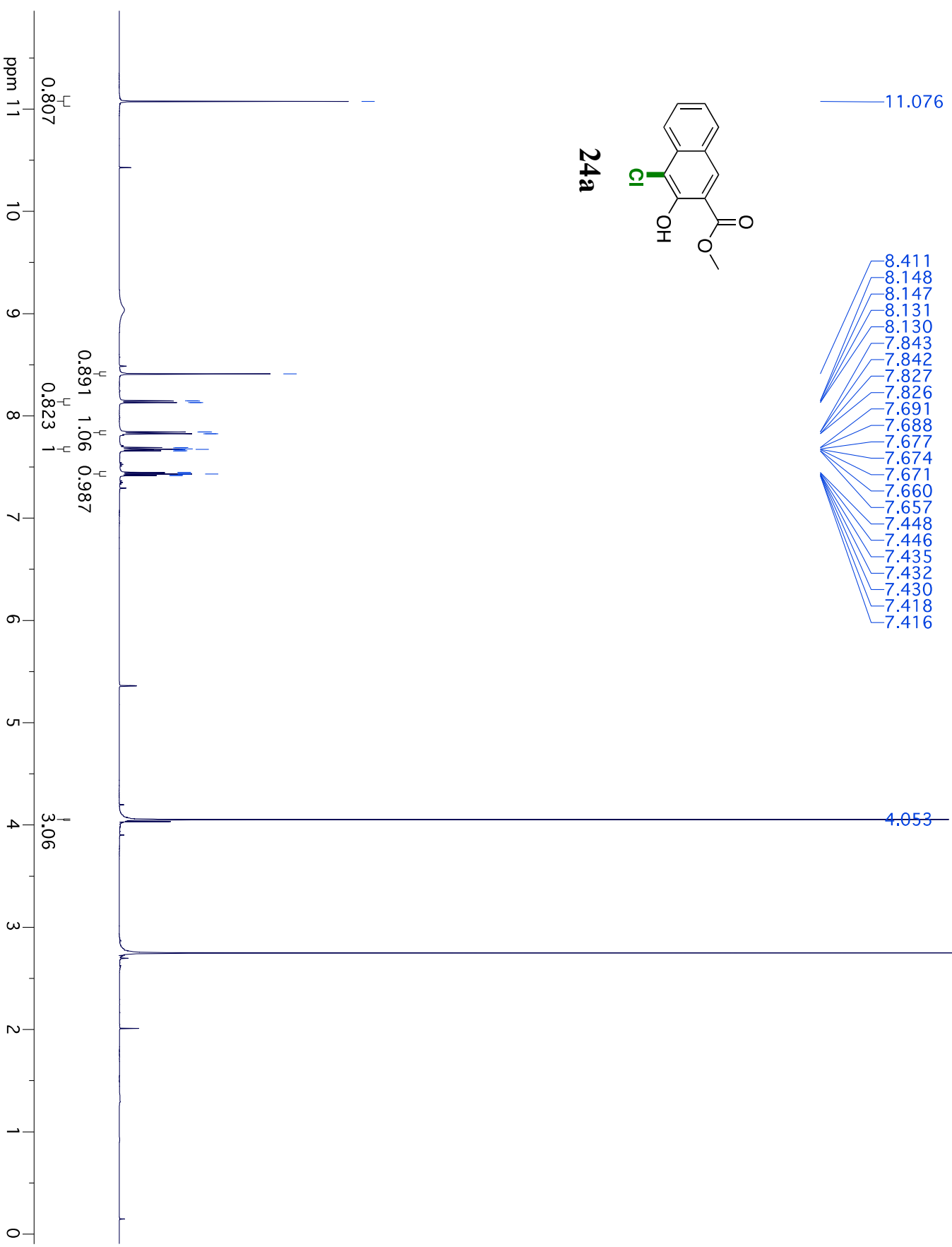
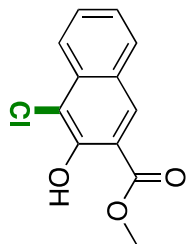


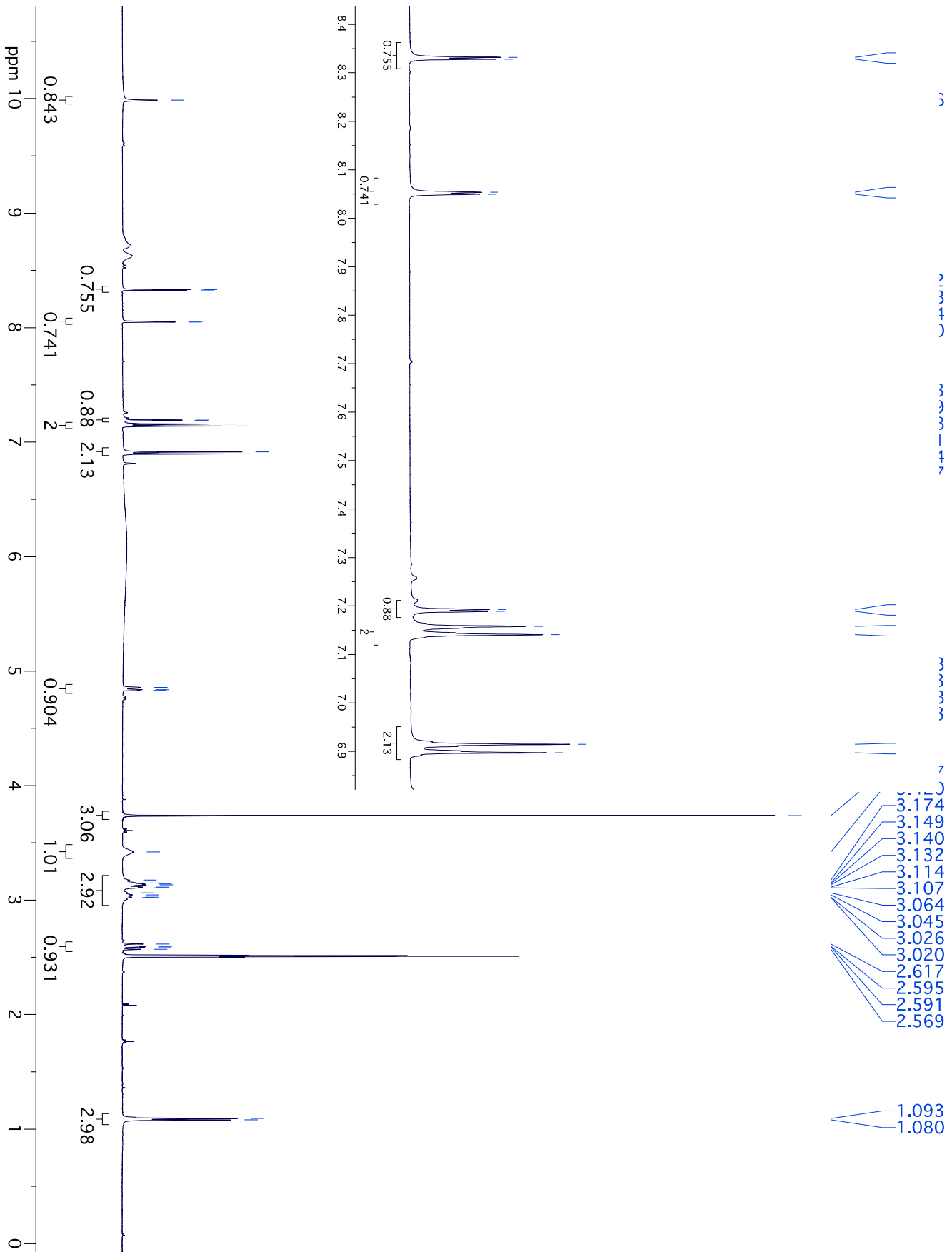


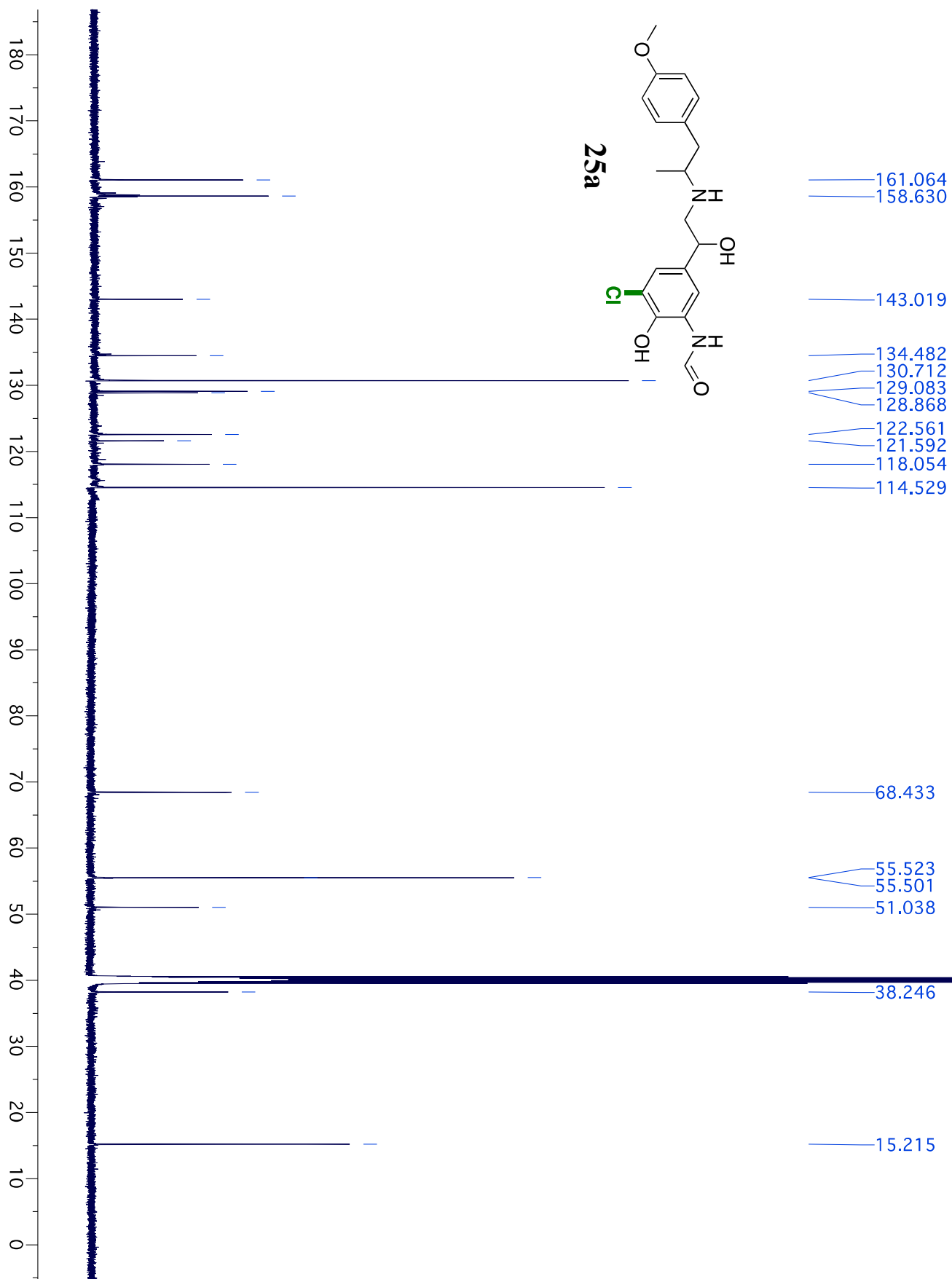
23c

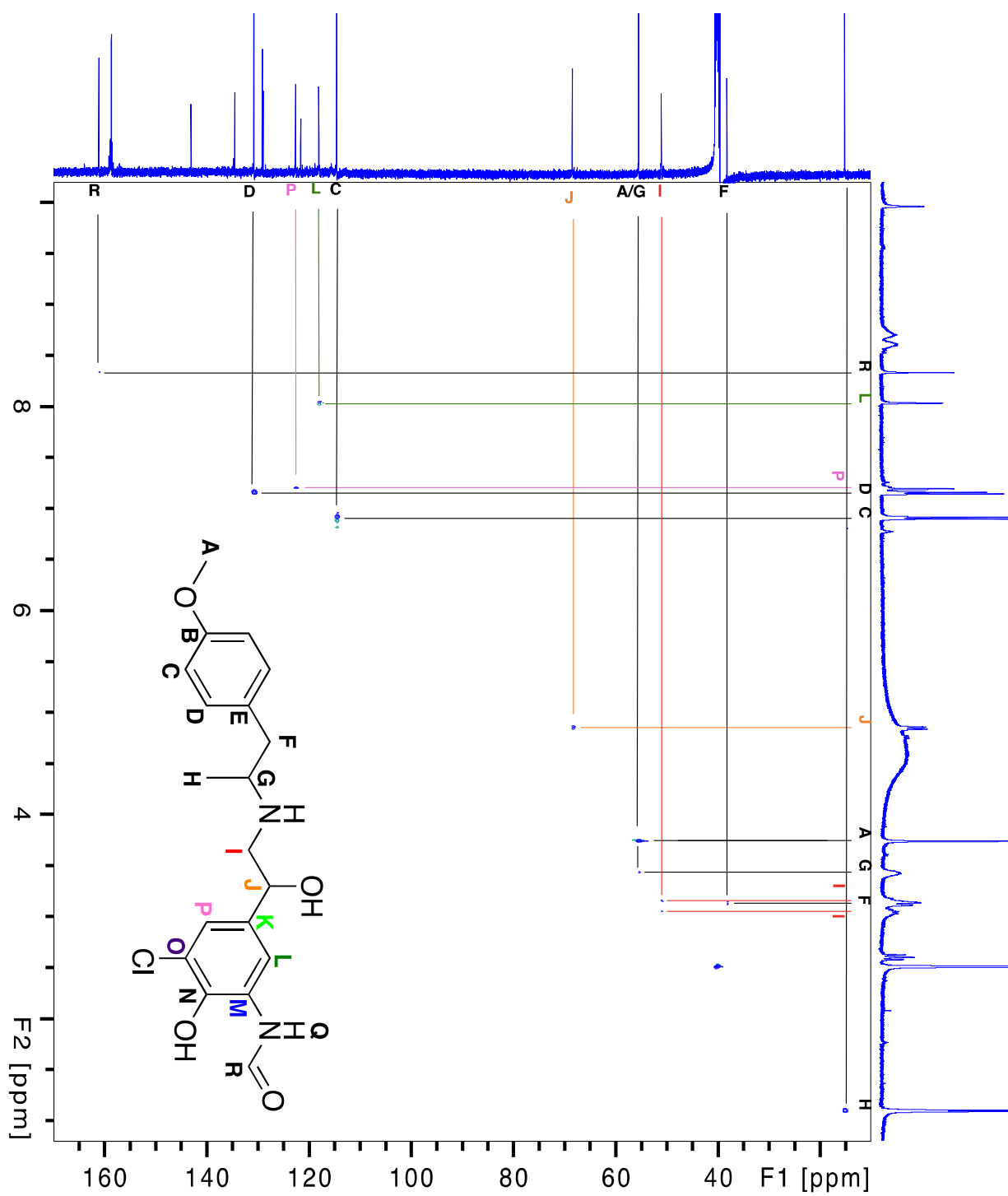


24a

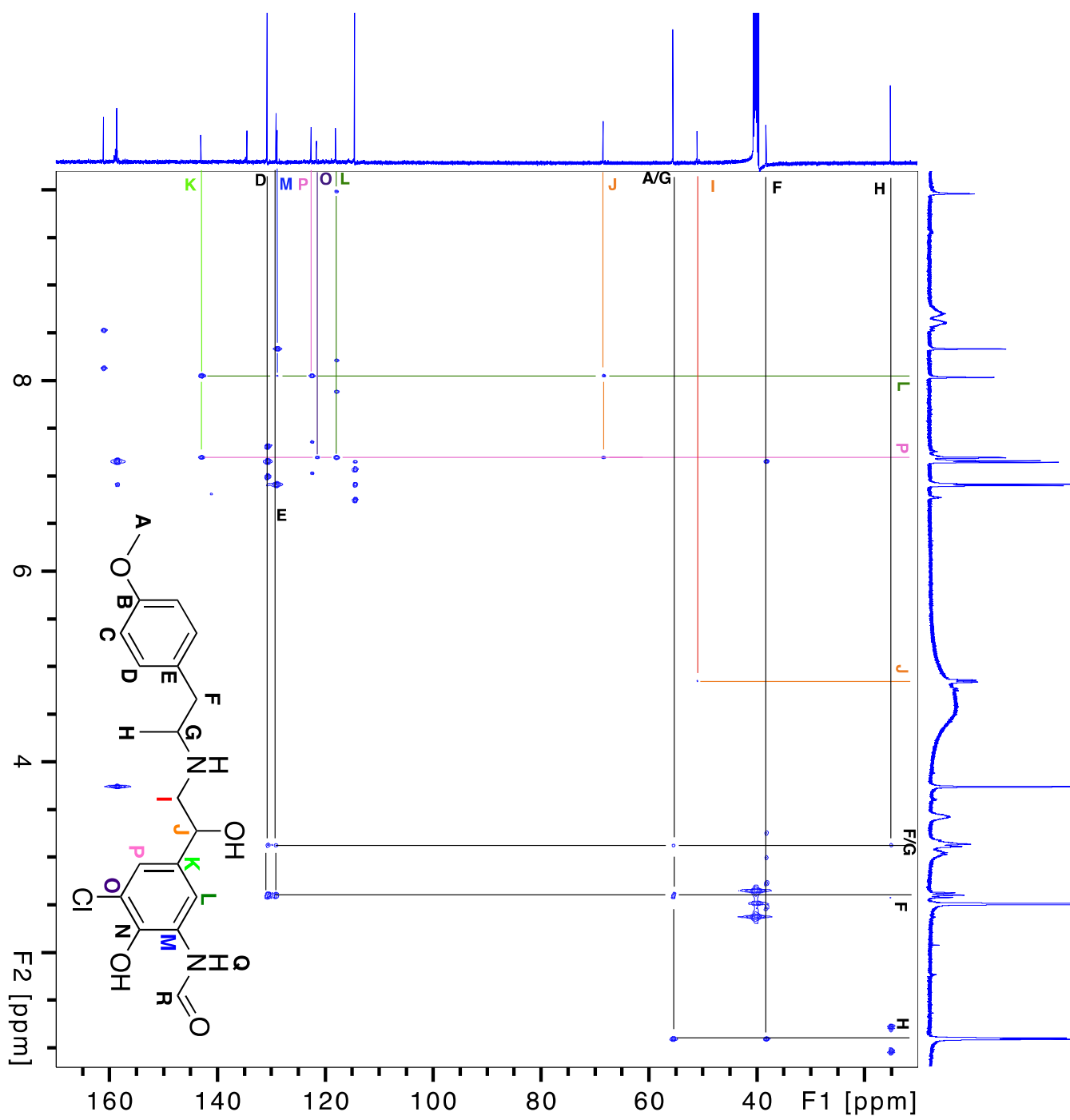








^1H - ^{13}C HSQC for 25a



^1H - ^{13}C HMBC for 25a

- 7.645
- 7.641
- 7.594
- 7.592
- 7.591
- 7.588
- 7.581
- 7.580
- 7.577
- 7.575
- 7.571
- 7.499
- 7.497
- 7.495
- 7.490
- 7.482
- 7.170

