Synergistic Photoenzymatic Hydrogenation of Heteroaromatic Olefins by 'Ene'-Reductases

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Experimental Procedures

General. Unless otherwise noted, all chemicals and reagents for chemical reactions were obtained from commercial suppliers and used as received (Sigma-Aldrich, Oakwood Chemical, Combi-Blocks, Chem-Impex, and Acros Chemicals). GDH-105 was purchased from Codexis as cell free lysate and used as received. Silica gel chromatography purifications were carried out using AMD Silica Gel 60. ^1H- and ^{13}C- NMR spectra were recorded on a Bruker UltraShield Plus (500 and 125 MHz, respectively) instrument, and are internally referenced to residual proton signals in $CDCl_3$ (7.26 ppm). Data for ^1H-NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, ddd = doublet of doublet of doublet), coupling constant (Hz), and integration. Data for ^{13}C NMR are reported in terms of chemical shift relative to $CDCl_3$ (77 ppm). High- resolution mass spectra were obtained on an Agilent 6220 LC/MS with an electrospray ionization time-of-flight (ESI-TOF) detector. IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer and peaks are reported in terms of frequency of absorption (cm^{-1})

Chromatography. Analytical high-performance liquid chromatography (HPLC) was carried out using an Agilent 1260 Infinity LCMS System. Analytical chiral HPLC was conducted using an Agilent 1260 Infinity Chiral HPLC system with isopropanol and hexanes as the mobile phase. Chiral IA, IB, IC, ID, and OJ-H columns were used to separate enantiomers (4.6 x 250 mm, 5 µm). For samples requiring chiral SFC: chiral SFC screening and optimization was carried out using a Waters Acquity UPC2 system (Waters Corp, Milford, MA, USA). This system contained a photodiode array detector, column managers to allow six orthogonal columns to be run in series, a sample manager, and a fluid delivery module (liquid CO_2 pump as well as a modifier pump). The system was controlled by MassLynx software.

Cyclic Voltammetry. Electrochemical studies were carried out in a 25 mL three-necked flask at the given concentrations. All potentials were measured using a CH Instruments Electrochemical Workstation potentiostat, and were measured against a saturated calomel reference electrode (SCE). Before each measurement, the glassy-carbon working electrode was polished with 50 nm alumina powder and rinsed with deionized water. The platinum wire counter electrode was flame activated until red-hot three times prior to measurement.

CD Spectroscopy. CD spectra were acquired with an Applied Photophysics Chirascan instrument using a 200 μL cuvette with 200 μL of relevant protein solution at a concentration of 0.1 mg/mL.

Cloning. pET22b(+) was used as a cloning and expression vector for all enzymes described in this study. Codon optimized genes for 'ene' reductase enzymes were purchased as gBlocks from IDT and cloned using Gibson Cloning ([i]). All genes were cloned between the NdeI and XhoI restriction sites and contained an N-terminal (GluER) or C-terminal (OYE1, OYE2, OYE3, MorB, PETN, OPR1, NostocER, LacER) 6xHis tag. Cloning for each construct was carried out using DH5α E. coli.

Protein Expression and Purification. Enzymes used in purified protein experiments were expressed in BL21(DE3) E. coli cultures transformed with plasmid encoding ERED variants. Transformed glycerol stocks were used to initiate 25 mL overnight cultures (37 °C, 250 rpm). Expression cultures (500 mL of TB with ampicillin (100 μg/ml final concentration) in a 2L flask) were inoculated with 1-2 ml of the overnight culture and grown to OD_{600} = 0.6 (37 °C, 250 rpm). Once the cell cultures reached an OD of 0.6 they were chilled on ice for 15 minutes prior to the addition of IPTG. For GluER, expression was induced with 0.5 mM IPTG (20 h 25 °C 250 rpm). For OYE1, expression was induced with 0.1 mM IPTG (24 h 18 °C 250 rpm). For MorB Y72F, expression was induced with 0.5 mM IPTG (24 h 18 °C 250 rpm). For LacER, expression was induced with 0.1 mM (24 h 25 °C 250 rpm). For NostocER, expression was induced 0.1 mM IPTG (24 h 25 °C 250 rpm). Following expression, cells were pelleted and frozen at -80 °C for storage. For purification, frozen cells were thawed in ice-cold water and resuspended in buffer A (for GluER: 50 mM TEOA 25 mM imidazole pH 7.0, for all other proteins reported herein: 20 mM KPi, 300 mM NaCl, 30 mM imidazole, pH 7.0). Lysozyme (1 mg/mL), DNAseI (0.1 mg/mL), FMN (1 mg/mL), and PMSF (1 mg/mL, added as a 35 mg/mL solution in absolute ethanol) were added to the resuspended cells, followed by shaking at room temperature for 30 minutes. The resuspended cells were disrupted by sonication (2 x 4 min, output control 5, 35% duty cycle; Sonicator QSonica Q500 Ultra Sonicator). To pellet insoluble material, lysates were centrifuged at 14,000 x g for 1.5 h at 4 °C. Proteins were purified using a nickel NTA column (5 mL HisTrap HP, GE Healthcare, Piscataway, NJ) using an AKTAStart purifier FPLC system (GE healthcare). ERED enzymes were eluted with 100 % buffer B (for GluER: 50 mM TEOA 250 mM imidazole pH 7.0, for all other proteins reported herein: 20 mM KPi, 300 mM NaCl, 250 mM imidazole pH 7.0) over 5 column volumes. Fractions containing enzyme were pooled, concentrated, and subjected to three exchanges with no-imidazole buffer (for GluER: 50 mM triethanolamine (TEOA), 10% glycerol, pH=7.0, for all other EREDs reported: 20 mM KPi, 300 mM NaCl, pH 7.0) to remove excess salt and imidazole. Concentrated (1.0-1.5 mM) proteins were aliquoted, flash-frozen in liquid N_2, and stored at -80 °C until later use. Protein concentration was determined by A_{465} with calculated extinction coefficients. All proteins other than GluER were used as aliquots pre-expressed and purified according to the procedures detailed in previously published work from the Hyster lab (27).

Determination of Extinction Coefficients. Extinction Coefficients for ERED enzymes were calculated based on the extinction coefficient (12.2×10^{-3} $M^{-1}cm^{-1}$ at 464 nm) for free FMN released after protein denaturation [ii]. Extinction coefficient for GluER: $\varepsilon_{464} = 11.4 \times 10^{-3}$ $M^{-1}cm^{-1}$, OYE1: $\varepsilon_{465} = 10.8 \times 10^{-3}$ $M^{-1}cm^{-1}$ (465 nm), MorB Y72F: $\varepsilon_{463} = 10.7 \times 10^{-3}$ M^{-1} cm^{-1}, LacER: $\varepsilon_{463} = 11.6 \times 10^{-3}$ M^{-1} cm^{-1}, NostocER: $\varepsilon_{461} = 10.6 \times 10^{-3}$ M^{-1} cm^{-1}

Sequence Information

Ene-reductase 1 from Nostoc sp. PCC7120 (NostocER1)

NCBI Reference Sequence: WP_010996029.1

Protein sequence:

MSDEAERQRGNNLYKNSPLLPVSISQVSTSQLRETEIMSTNINLFSSYQLGELELPNRIVMAP
LTRQRAGEGNVPHQLNAIYYGQRASAGLIIAEATQVTPQGQGYPHTPGIHSPEQVAGWKL
VTDTVHQQGGRIFLQLWHVGRISHPDLQPDGGLPVAPSAIAPKGEVLTYEGKKPYVTPRAL
DTSEIPAIVEQYRQGAANALAAGFDGVEIHAANGYLIDQFLRDGTNQRTDEYGGAIENRAR
LLLEVTEAITSVWDSQRVGVRLSPSGTFNDIRDSHPLETFGYVAQALNRFNLSYLHIFEAIDAD
IRHGGTVVPTSHLRDRFTGTLIVNGGYTREKGDTVIANKAADLVAFGTLFISNPDLPERLEVN
APLNQADPTTFYGGGEKGYTDYPFLAVANKLEHHHHHH

DNA Sequence:

ATGAGTGATGAAGCGGAGCGCCAACGTGGAAATAATCTTTACAAAAATTCGCCCCT
TCTGCCTGTCTCTATAAGTCAGGTATCTACAAGCCAGTTGCGGGAGACCGAAATAAT
GAGTACCAATATAAACTTGTTCAGCAGTTACCAATTGGGTGAGTTAGAATTACCCAA
TCGCATAGTGATGGCACCTCTGACTCGCCAACGGGCTGGAGAGGGTAATGTGCCC
CACCAATTAAACGCGATATATTACGGCCAACGCGCCTCTGCCGGTCTGATCATAGC
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GAACCTTATTTATATCGAACCCGGACCTGCCTGAACGTTTGGAGGTAAATGCTCCGT
TAAATCAAGCAGACCCGACTACGTTTTATGGGGGCGGGGAGAAGGGCTACACAGA
TTATCCTTTTCTTGCTGTAGCAAATAAGCTCGAGCACCACCATCACCACCACTGA

Gluconobacter Oxydans Ene-Reductase (GluER)

GenBank Accession Code: WP_011252080.1

Protein Sequence:

HHHHHHMPTLFDPIDFGPIHAKNRIVMSPLTRGRADKEAVPTPIMAEYYAQRASAGLIITEAT
GISREGLGWPFAPGIWSDAQVEAWKPIVAGVHAKGGKIVCQLWHMGRMVHSSVTGTQPV
SSSATTAPGEVHTYEGKKPFEQARAIDAADISRILNDYENAARNAIRAGFDGVQIHAANGYL
IDEFLRNGTNHRTDEYGGVPENRIRFLKEVTERVIAAIGADRTGVRLSPNGDTQGCIDSAPET
VFVPAAKLLQDLGVAWLELREPGPNGTFGKTDQPKLSPQIRKVFLRPLVLNQDYTFEAAQT
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DNA Sequence:

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ACTATACTTTTGAGGCGGCACAGACGGCCCTGGCTGAGGGCAAGGCGGACGCTAT
TGCGTTTGGCCGTAAGTTCATTTCAAATCCAGACTTGCCTGAGCGCTTTGCCCGTGG
CATCGCACTGCAACCAGACGATATGAAAACATGGTACTCCCAAGGCCCAGAGGGTT
ACACAGACTATCCATCCGCAACTTCTGGGCCGAAC

Old Yellow Enzyme 1 (OYE1)

UniProtKB - Q02899 (OYE1_SACPS)

Protein Sequence:

MSFVKDFKPQALGDTNLFKPIKIGNNELLHRAVIPPLTRMRALHPGNIPNRDWAVEYYTQRA
QRPGTMIITEGAFISPQAGGYDNAPGVWSEEQMVEWTKIFNAIHEKKSFVWVQLWVLGW
AAFPDNLARDGLRYDSASDNVFMDAEQEAKAKKANNPQHSLTKDEIKQYIKEYVQAAKNS
IAAGADGVEIHSANGYLLNQFLDPHSNTRTDEYGGSIENRARFTLEVVDALVEAIGHEKVGL
RLSPYGVFNSMSGGAETGIVAQYAYVAGELEKRAKAGKRLAFVHLVEPRVTNPFLTEGEGEY
EGGSNDFVYSIWKGPVIRAGNFALHPEVVREEVKDKRTLIGYGRFFISNPDLVDRLEKGLPLN
KYDRDTFYQMSAHGYIDYPTYEEALKLGWDKK

DNA Sequence:

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CTTGGTGGACCGTTTAGAGAAAGGTCTTCCCTTGAACAAATATGATCGTGACACCTT
TTATCAGATGTCGGCGCACGGATACATTGATTACCCGACCTATGAAGAGGCTTTAAA
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Old yellow enzyme 2 (OYE2)

UniProtKB - Q03558 (OYE2_YEAST)

Protein Sequence:

MPFVKDFKPQALGDTNLFKPIKIGNNELLHRAVIPPLTRMRAQHPGNIPNRDWAVEYYAQR
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AFPDTLARDGLRYDSASDNVYMNAEQEEKAKKANNPQHSITKDEIKQYVKEYVQAAKNSI
AAGADGVEIHSANGYLLNQFLDPHSNNRTDEYGGSIENRARFTLEVVDAVVDAIGPEKVGL
RLSPYGVFNSMSGGAETGIVAQYAYVLGELERRAKAGKRLAFVHLVEPRVTNPFLTEGEGEY
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KYDRDTFYKMSAEGYIDYPTYEEALKLGWDKNHHHHHH

DNA Sequence:

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Old yellow enzyme 3 (OYE3)

P41816 (OYE3_YEAST)

Protein Sequence:

MPFVKGFEPISLRDTNLFEPIKIGNTQLAHRAVMPPLTRMRATHPGNIPNKEWAAVYYGQR
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ASFPDVLARDGLRYDCASDRVYMNATLQEKAKDANNLEHSLTKDDIKQYIKDYIHAAKNSI
AAGADGVEIHSANGYLLNQFLDPHSNKRTDEYGGTIENRARFTLEVVDALIETIGPERVGLRL
SPYGTFNSMSGGAEPGIIAQYSYVLGELEKRAKAGKRLAFVHLVEPRVTDPSLVEGEGEYSE
GTNDFAYSIWKGPIIRAGNYALHPEVVREQVKDPRTLIGYGRFFISNPDLVYRLEEGLPLNKYD
RSTFYTMSAEGYTDYPTYEEAVDLGWNKNHHHHHH

DNA Sequence:

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Thermophilic Old Yellow Enzyme (TOYE)

NCBI Reference Sequence: WP_012268805.1

Protein Sequence:

MSILHMPLKIKDITIKNRIMMSPMCMYSASTDGMPNDWHIVHYATRAIGGVGLIMQEATAV
ESRGRITDHDLGIWNDEQVKELKKIVDICKANGAVMGIQLAHAGRKCNISYEDVVGPSPIKA
GDRYKLPRELSVEEIKSIVKAFGEAAKRANLAGYDVVEIHAAHGYLIHEFLSPLSNKRKDEYGN
SIENRARFLIEVIDEVRKNWPENKPIFVRVSADDYMEGGINIDMMVEYINMIKDKVDLIDVSS
GGLLNVDINLYPGYQVKYAETIKKRCNIKTSAVGLITTQELAEEILSNERADLVALGRELLRNP
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DNA sequence

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Morphinone reductase (MorB)

UniProtKB - Q51990 (Q51990_PSEPU)

Protein Sequence:

MPDTSFSNPGLFTPLQLGSLSLPNRVIMAPLTRSRTPDSVPGRLQQIYYGQRASAGLIISEATN
ISPTARGYVYTPGIWTDAQEAGWKGVVEAVHAKGGRIALQLWHVGRVSHELVQPDGQQP
VAPSALKAEGAECFVEFEDGTAGLHPTSTPRALETDEIPGIVEDYRQAAQRAKRAGFDMVE
VHAANACLPNQFLATGTNRRTDQYGGSIENRARFPLEVVDAVAEVFGPERVGIRLTPFLELF
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DNA Sequence:

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Pentaerythritol tetranitrate reductase (PETNr)

UniProtKB - P71278 (P71278_ENTCL)

Protein Sequence:

MSAEKLFTPLKVGAVTAPNRVFMAPLTRLRSIEPGDIPTPLMGEYYRQRASAGLIISEATQISA
QAKGYAGAPGLHSPEQIAAWKKITAGVHAEDGRIAVQLWHTGRISHSSIQPGGQAPVSAS
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YLLHQFLSPSSNQRTDQYGGSVENRARLVLEVVDAVCNEWSADRIGIRVSPIGTFQNVDNG
PNEEADALYLIEELAKRGIAYLHMSETDLAGGKPYSEAFRQKVRERFHGVIIGAGAYTAEKAE
DLIGKGLIDAVAFGRDYIANPDLVARLQKKAELNPQRPESFYGGGAEGYTDYPSLHHHHHH

DNA Sequence:

ATGTCGGCCGAGAAGTTGTTCACGCCCTTAAAGGTCGGTGCGGTGACCGCTCCTAA
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12-Oxophytodienoate reductase 1 (OPR1)

UniProtKB - Q9XG54 (OPR1_SOLLC)

Protein Sequence:

MENKVVEEKQVDKIPLMSPCKMGKFELCHRVVLAPLTRQRSYGYIPQPHAILHYSQRSTNG
GLLIGEATVISETGIGYKDVPGIWTKEQVEAWKPIVDAVHAKGGIFFCQIWHVGRVSNKDFQ
PNGEDPISCTDRGLTPQIRSNGIDIAHFTRPRRLTTDEIPQIVNEFRVAARNAIEAGFDGVEIH
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GDTNPTALGLYMVESLNKYDLAYCHVVEPRMKTAWEKIECTESLVPMRKAYKGTFIVAGGY
DREDGNRALIEDRADLVAYGRLFISNPDLPKRFELNAPLNKYNRDTFYTSDPIVGYTDYPFLET
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DNA Sequence:

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LacER from Lactobacillus casei str. Zhang

NCBI Reference Sequence: WP_016363397.1

LacER Protein Sequence:

MSGYHFLKPFTFKHQTITLKNRIVIPPMTTRLSFEDGTVTRDEIRYYQQRAGGVGMFITGTAN
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LacER DNA Sequence:

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GGCTATCCGTTATCAAATTGCACCGGCGGACTTGGAAGAACTGGGCATCGCCCCTA
CGTTTTTAGATTTTATCGAGAGCATCTCTGGTGGAGCCAAGGGGGTGCCCTTGACG
ACGGCGCAGTCGGTCACTAGCAGTAACGTCACACAAGACCTCGAGCACCACCATC
ACCACCACTGA

Nicotinamide-dependent cyclohexanone reductase (NCR)

Genbank Accession Number: AAV90509

Protein Sequence:

MPSLFDPIRFGAFTAKNRIWMAPLTRGRATRDHVPTEIMAEYYAQRASAGLIISEATGISQEG
LGWPYAPGIWSDAQVEAWLPITQAVHDAGGLIFAQLWHMGRMVPSNVSGMQPVAPSAS
QAPGLGHTYDGKKPYDVARALRLDEIPRLLDDYEKAARHALKAGFDGVQIHAANGYLIDEFI
RDSTNHRHDEYGGAVENRIRLLKDVTERVIATIGKERTAVRLSPNGEIQGTVDSHPEQVFIPA
AKMLSDLDIAFLGMREGAVDGTFGKTDQPKLSPEIRKVFKPPLVLNQDYTFETAQAALDSG
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DNA Sequence:

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Bacillus subtilis NADPH dehydrogenase (YqjM)

Genbank Accession Number: BAA12619

Protein Sequence:

MHHHHHHMARKLFTPITIKDMTLKNRIVMSPMCMYSSHEKDGKLTPFHMAHYISRAIGQVG
LIIVEASAVNPQGRITDQDLGIWSDEHIEGFAKLTEQVKEQGSKIGIQLAHAGRKAELEGDIF
APSAIAFDEQSATPVEMSAEKVKETVQEFKQAAARAKEAGFDVIEIHAAHGYLIHEFLSPLSN
HRTDEYGGSPENRYRFLREIIDEVKQVWDGPLFVRVSASDYTDKGLDIADHIGFAKWMKEQ
GVDLIDCSSGALVHADINVFPGYQVSFAEKIREQADMATGAVGMITDGSMAEEILQNGRA
DLIFIGRELLRDPFFARTAAKQLNTEIPAPVQYERGW

DNA Sequence:

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CCAAGTTGGTCTTATTATCGTGGAGGCAAGTGCCGTAAATCCCCAGGGACGTATTA
CGGATCAAGATTTGGGTATCTGGAGCGATGAACACATCGAAGGCTTCGCGAAGCTG
ACAGAACAGGTTAAGGAACAAGGGTCTAAGATCGGCATTCAACTGGCCCACGCCG
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TGCAATACGAGCGTGGTTGGTGA

Ene-reductase from Deinococcus radiodurans (DrER)

NCBI Reference Sequence: WP_010888821.1

Protein sequence:

MTVSSAAAPQPASPAAPLLFTPLKLRSLELPNRVVVSPMCTYSATDGVANEFHLVHLGQYAL
GGAGLILAEATAVSPEGRITPEDLGLWDDRQIVPLGHITDFVHQHGGHIGVQLAHAGRKAS
TYAPWRGKGAVPAELGGWQVIGPDENSFHDLFPTPAMMGADELRGVVDAFSAAARRAQ
VAGFDAVEVHAAHGYLLHQFLSPLANTRTDDYGGSFENRTRLLLEVVRAVRHVWPAHLPLF
VRLSATDWAEGGWDLEQTVQLSKLLKYEGVDVLDISSGGLTAAQQIEVGPGYQVPFAAAV
SRAETEISVMAVGLIETGAQAEAILQAGDADLIALGRPFLRDPHWAQRAARELGLRPVSIDQ
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DNA sequence:

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Ene-reductase from Tersenia bercovieri (YersER)
NCBI Reference Sequence: WP_032896199.1

Protein sequence:

MKTAKLFSPLKVGALTLPNRVFMAPLTRLRSIEPGDIPTPLMAEYYRQRASAGLIITEATQISFQ
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DNA sequence:

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Ene-reductase from Cupriavidus metallidurans (RmER)

NCBI Reference Sequence: WP_011519282.1

Protein sequence:

MPHLFDPYRIGNLELANRIAIAPMCQYSAQEGNATDWHMIHLGQMALSGAGLLIIEATAVS
PEGRITPTDLGLYNDANEAALGRVLGAVRNHSPIAVTIQLAHAGRKASSEAPWDGGGQIRP
DQPRGWQTFAPSAVPHAAGEVPPAALDKAGMKKIRDDFVAAAKRAARLGIEGIEVHGAH
GYLLHQFLSPIANHRTDEYGGSLENRMRFPLEVFDAVREAFPAERPVWMRVSATDWVPNG
WDIEGTIALSHELKARGSAAVHVSTGGVSPQQAIKIGPGYQVPYAQRVKAEVGLPTMAVGLI
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DNA sequence:

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Flavin reductase family protein from Pyrococcus horikoshii (PhENR)
NCBI Reference Sequence: WP_010884948.1

Protein sequence:

MEGYRLLYPMRTYLIVSGHGEETNVMAADWVTVVSFDPFIVGVAVAPKRTTHKLIKKYGEFVI
SVPSLDVLRDVWIAGTKKGPSKLKEMSVTLIPSKKVKVPSIEEALANIECRVIDARSYGDHTFFV
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DNA sequence:

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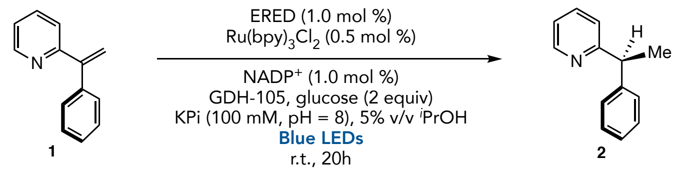
Supplementary Tables

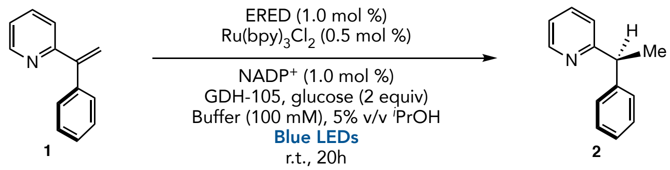
Table S1. Enzyme Screen for Photoenzymatic Reduction of Vinyl Pyridines



entry	ERED	yield (%)[a]	e.r.[b]
1	PhENR	Trace	–
2	YersER	71	55:45
3	RmER	>99	50:50
4	TsOYE	85	50:50
5	DrER	75	50:50
6	LacER	41	52:48
7	NostocER	57	70:30
8	GluER	32	51:49
9	MorB	84	75:25
10	NCR	0	–
11	OPR1	95	58:42
12	OYE1	85	58:42
13	OYE2	84	75:25
14	OYE3	64	65:35
15	PETNr	95	59:41
16	XenA	23	54:46
17	YqjM	72	50:50

[a]Yield calculated by ^{1}H-NMR analysis against internal standard. [b]Determined by HPLC over chiral stationary phase.

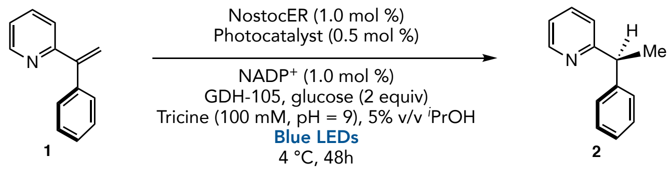
Table S2. Buffer and pH Screen for Photoenzymatic Reduction of Vinyl Pyridines



entry	ERED	Buffer (pH)	yield (%)[a]	e.r.[b]
1	OYE2	KPi (6.5)	43	58:42
2	OYE2	KPi (7.0)	64	57:43
3	OYE2	KPi (7.5)	89	59:41
4	OYE2	KPi (8.0)	84	75:25
5	OYE2	KPi (8.5)	59	70:30
6	OYE2	TEOA (8.0)	58	67:33
7	OYE2	Tricine (8.0)	28	78:22
8	OYE2	Tricine (8.5)	86	82:18
9	OYE2	Tricine (9.0)	60	89:11
10	OYE2	HEPES (8.0)	72	80:20
11	OYE2	Borate (8.0)	18	66:34
12	OYE2	Tris (8.0)	57	61:39
13	OYE2	MOPS (8.0)	88	66:34
14	NostocER	KPi (8.0)	57	70:30
15	NostocER	TEOA (8.0)	83	74:26
16	NostocER	Tricine (8.0)	81	75:25
17	NostocER	Tricine (8.5)	86	81:19
18	NostocER	Tricine (9.0)	70	86:14
19[c]	NostocER	Tricine (9.0)	96	92:8
20	NostocER	HEPES (8.0)	55	83:17

[a]Yield calculated by ^{1}H-NMR analysis against internal standard. [b]Determined by HPLC over chiral stationary phase. [c]Reaction temperature 4 °C, reaction time 48 h.

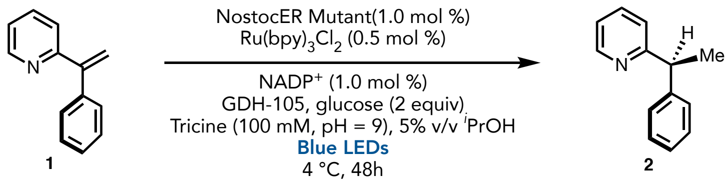
Table S3. Photocatalyst Screen for Photoenzymatic Reduction of Vinyl Pyridines



entry	Photocatalyst	yield (%)[a]	e.r.[b]
1	$Ru(bpy)_3Cl_2$ ($\lambda_{max} = 452$)	96	92:8
2	$Ru(phen)_3Cl_2$ ($\lambda_{max} = 422$)	82	92:8
3	$Ru(bpz)_3(PF_6)_2$ ($\lambda_{max} = 443$)	0	–
4	$Ir[dCF3ppy](bpy)PF_6$	3	–
5	$Ir[dF(CF_3)ppy]_2(dtbbpy)PF_6$ ($\lambda_{max} = 380$)	3	–
6	$Ir(ppy)_3$ ($\lambda_{max} = 375$)	8	–
7[c]	Rose Bengal ($\lambda_{max} = 520$)	Trace	–
8[c]	Eosin Y ($\lambda_{max} = 549$)	Trace	–
9	Fluorescein ($\lambda_{max} = 437$)	0	–

[a]Yield calculated by ^{1}H-NMR analysis against internal standard. [b]Determined by HPLC over chiral stationary phase. [c]Green LED used instead (maximum emission 530 nm).

Table S4. Point mutants of NostocER tested for redox activation of substrate.



entry	Mutation	yield (%)[a]	e.r.[b]
1	Y106F	60	79:21
2	W140F	91	84:16
3	Y219F	66	86:14
4	Y384F	65	82:18
5	H214A	47	75:25
6	N217A	44	85:15
7	H214A+N217A	18	55:45

[a]Yield calculated by ^{1}H-NMR analysis against internal standard. [b]Determined by HPLC over chiral stationary phase.

Supplementary Figures

Figure S1. View of NostocER (PDB code: 6UFF) active site with residues selected for mutation from Table S4. FMN is shown in yellow, targeted residues are highlighted.

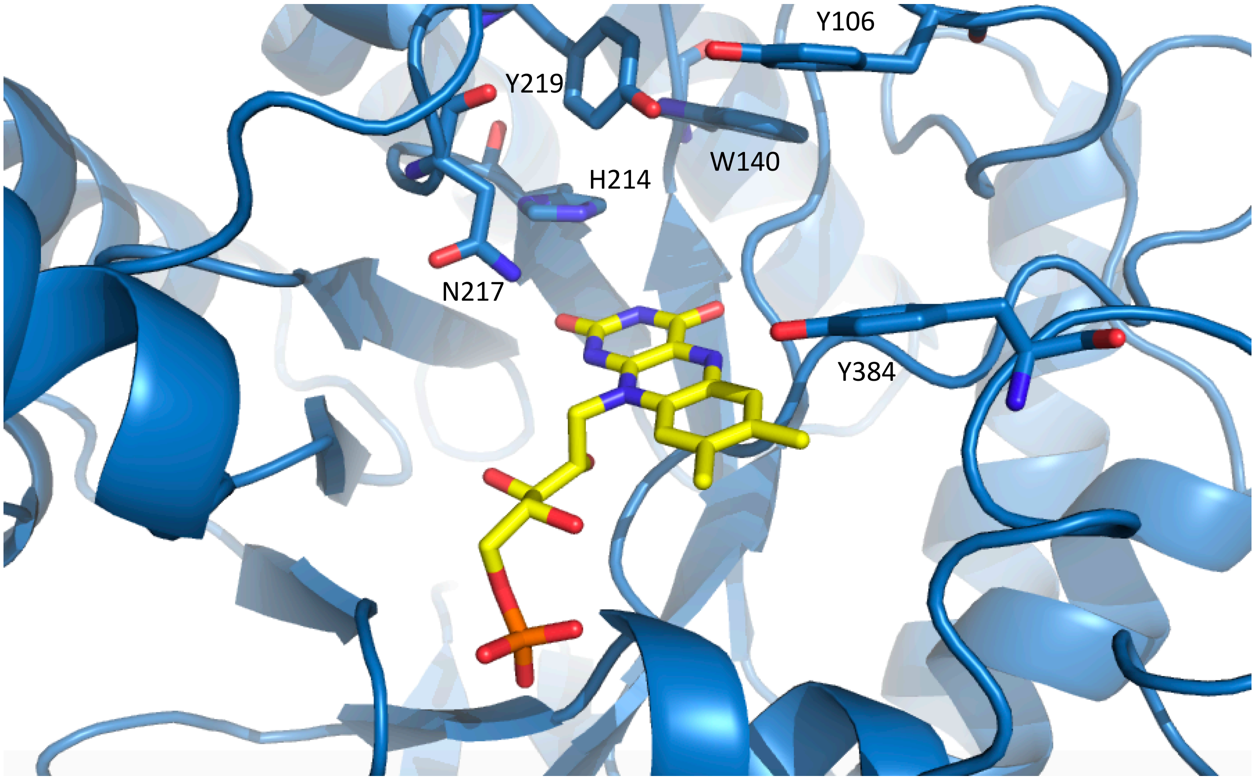


Figure S2. Resubjection of reduced product **2** to deuterated buffer reaction conditions. No deuterium exchange is noted, indicating deuterium labelling studies are not an artefact of washing out labels in buffer.

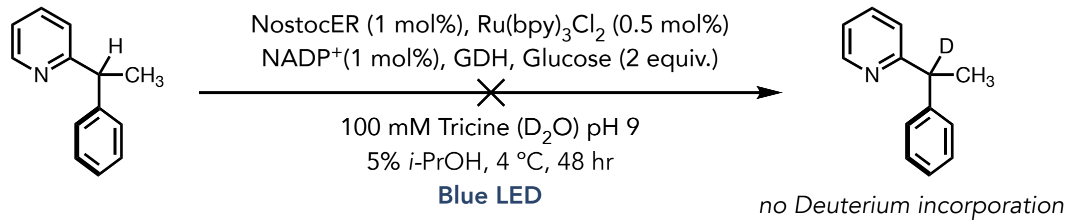


Figure S3. Free flavin and bovine serum albumin (BSA) control of model reaction. Racemic outcome indicates reduction of the pyridine is not occurring on the surface residues of a generic enzyme.

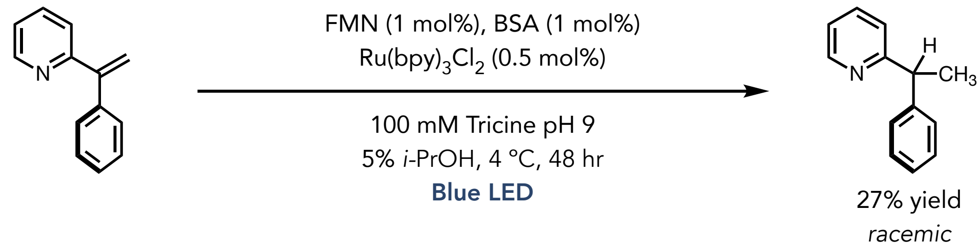
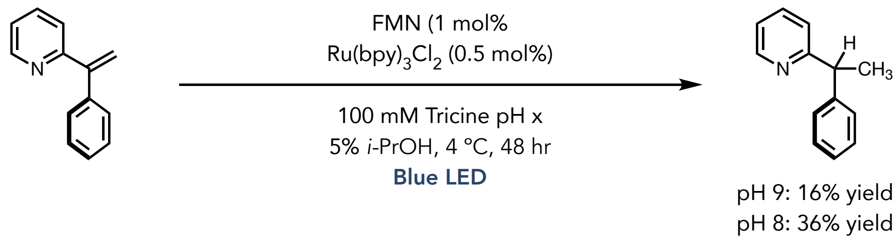


Figure S4. Free Flavin control reactions. Lower pH leads to higher background reactivity.



Preparation of Apo-Nostoc

In a falcon tube, a solution of 1500 nmol NostocER was diluted to a concentration of 300 nmol/mL in 20 mM KPi pH 7, 300 mM NaCl. The solution was then transferred to a dialysis bag and sealed with clips. Three independent 1 L buffers of 200 mM KPi pH 5.3, 2 M NaCl were prepared and cooled to 4 °C on ice. NostocER was then dialyzed against 1 L of 200 mM KPi pH 5.3, 2 M NaCl in a cold room at 4 °C with stirring. Buffer was exchanged every 8 hours for 24 hours, or until all of the yellow color of flavin was absent from dialysis bag. A white precipitate was observed forming. After all yellow color was removed from dialysis, the dialysis bag was removed. Apo-Nostoc was then dialyzed against 20 mM sodium pyrophosphate pH 8.5 with 3 buffer exchanges every 8 hours for 24 hours. After complete buffer exchange, Apo-Nostoc was removed from the dialysis bag and placed into a falcon tube. The falcon tube was centrifuged at 14,000 xg to pellet denatured Nostoc. The supernatant was carefully removed and concentrated. Protein concentration was measured using an extinction coefficient of 40,340 M^{-1} cm^{-1} at 280 nm.

Figure S5. Circular dichroism spectra of NostocER and Apo-NostocER after flavin removal, both solutions at 0.1 mg/mL. Close overlap of spectra indicate secondary structure of Apo-NostocER has not changed dramatically from that of the native, flavin-containing enzyme.

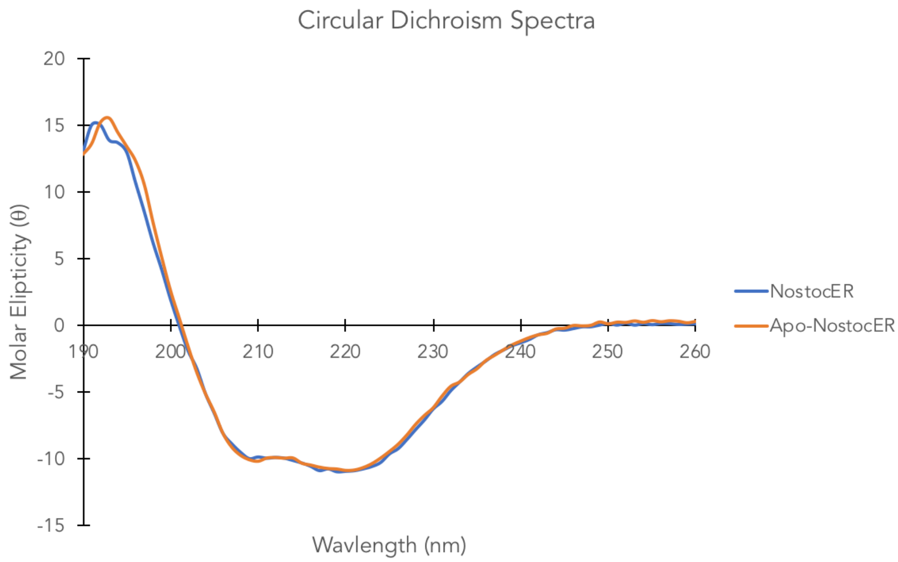


Figure S6. Attempted Apo-Nostoc riboflavin reconstitution. Blue Trace is UV-Vis spectra of Apo-Nostoc, distinctly lacking clear flavin signals in the visible region, indicating all flavin has been removed. Apo-Nostoc was incubated with 1 equiv. riboflavin with respect to enzyme at room temperature for 4 hours. Orange trace is the UV-Vis spectra of this incubation reaction before centrifuging. Post-centrifugation, enzyme loses any yellow color and flavin signals in the UV-Vis, shown in the grey trace. This indicates riboflavin without the negatively charged phosphate group does not bind to NostocER, and can serve as a model for freely floating flavin in solution with an apo-Nostoc protein in a reaction.

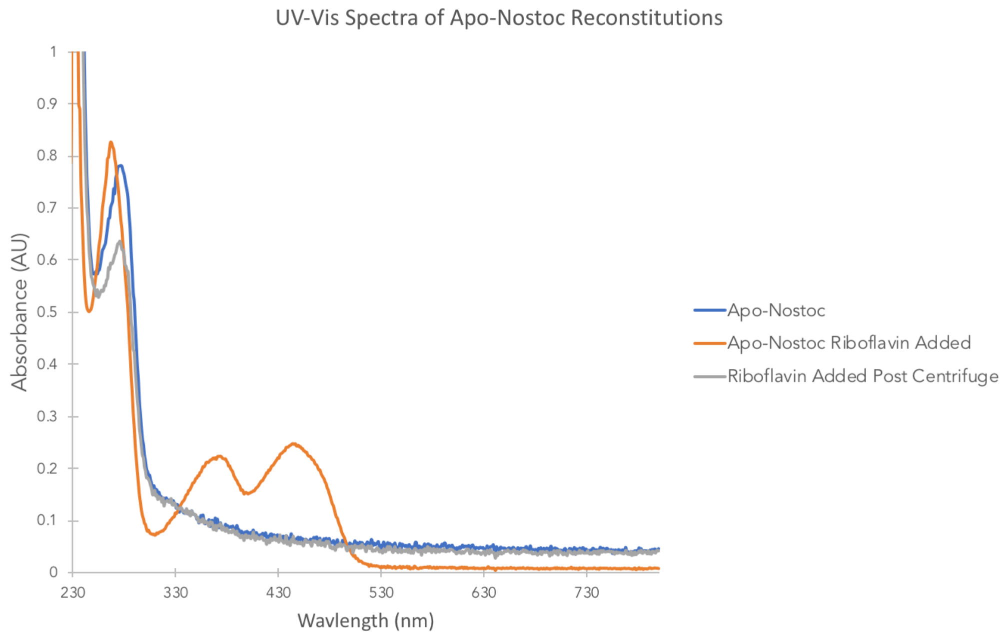


Figure S7. Model reaction using Apo-NostocER and riboflavin. Figure S6 demonstrates riboflavin does not bind to NostocER without the negatively charged phosphate in the ribityl side chain to form a salt bridge with the enzyme. Thus, riboflavin can be used as a model system for a dissociated, freely floating flavin in the model reaction with Apo-protein. Enantioseletivity of the product is racemic, indicating there is no pocket on the surface of NostocER imparting enantioselectivity to the product; instead, enantioselectivity is a direct result of radical quenching by flavin within the canonical NostocER active site.

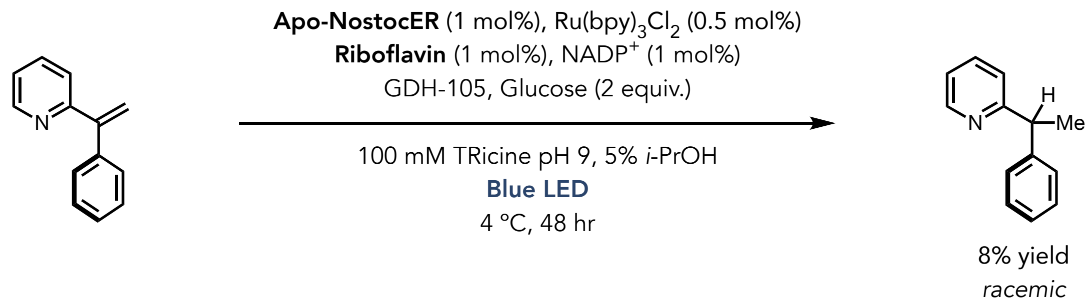
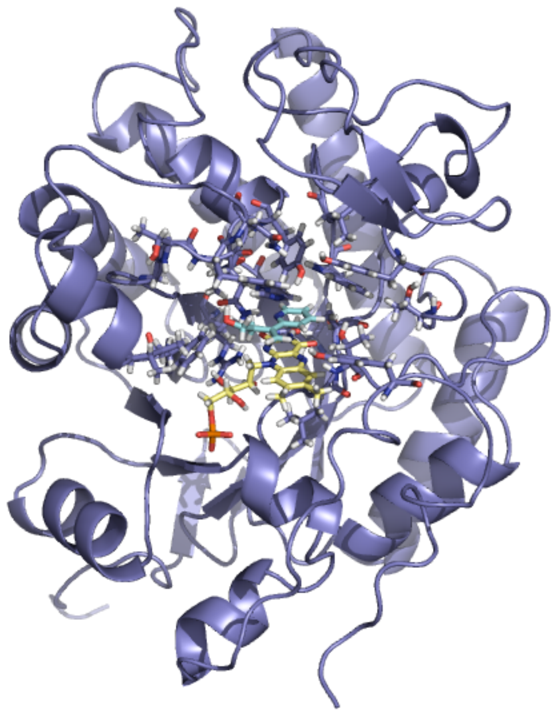
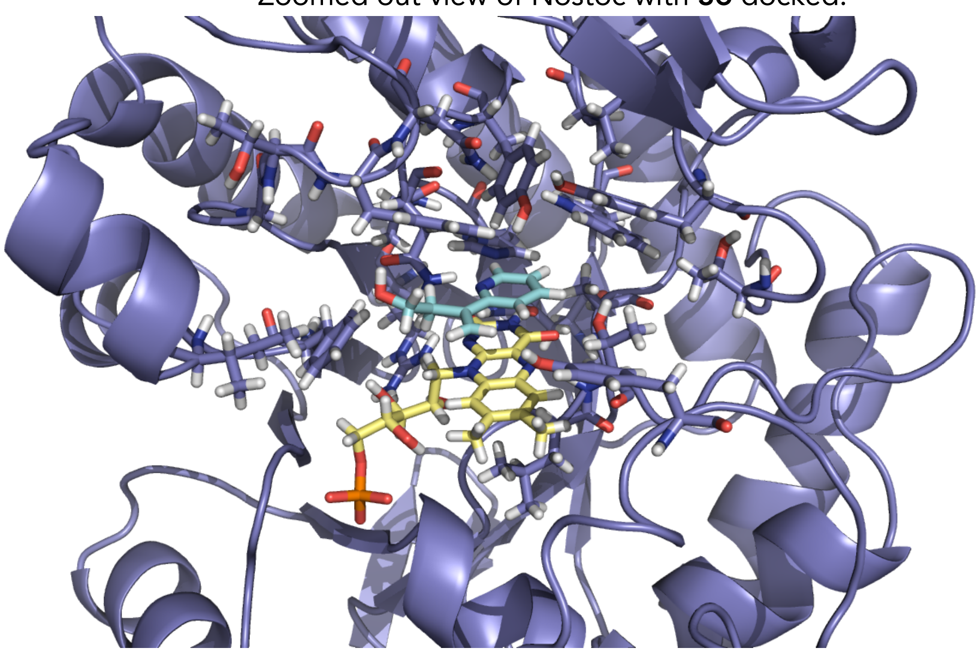


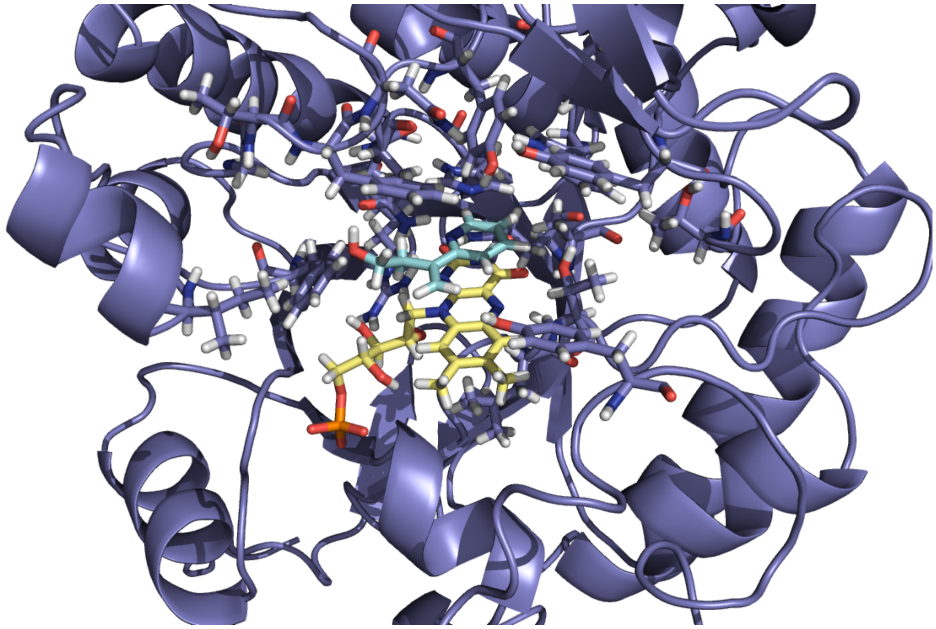
Figure S8. Docking model of substrate **3o** inside NostocER (PDB = 6UFF) showing lowest energy pose. After H-atom transfer from flavin, the shown pose corresponds to the observed stereochemistry of the product. Docking was conducted using YASARA docking program.



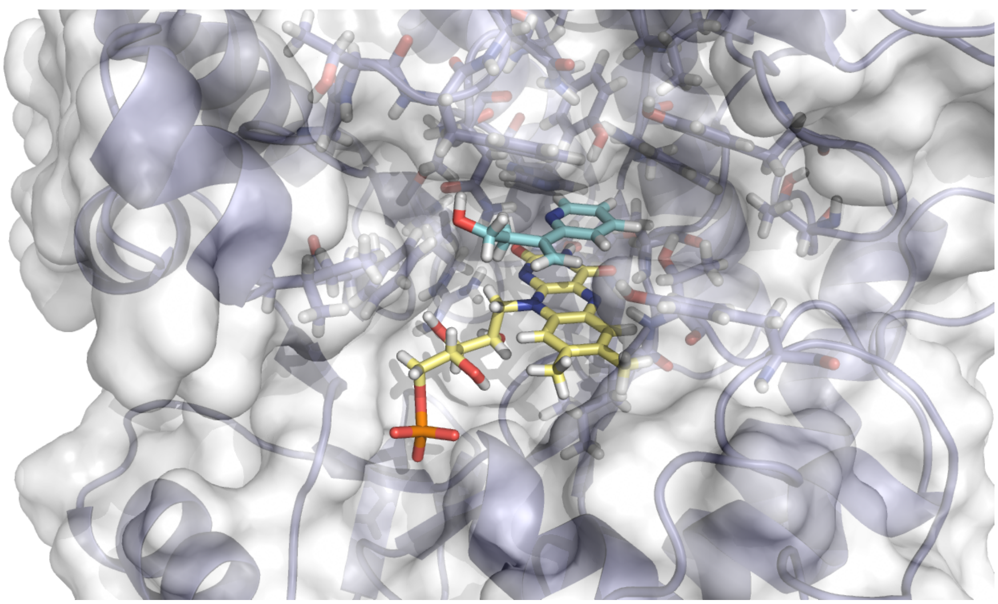
Zoomed out view of Nostoc with **3o** docked.



Zoomed in view of Nostoc with **3o** docked.



Alternate view of **3o** docking model.



Docking model of **3o** docked in NostocER with surface density shown.

Figure S9. SDS-PAGE gel of purified NostocER. Protein ladder in lane 1, BSA standards (5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.3125 mg/mL) in lanes 2, 3, 4, 5, 6, respectively, purified NostocER in lane 7.

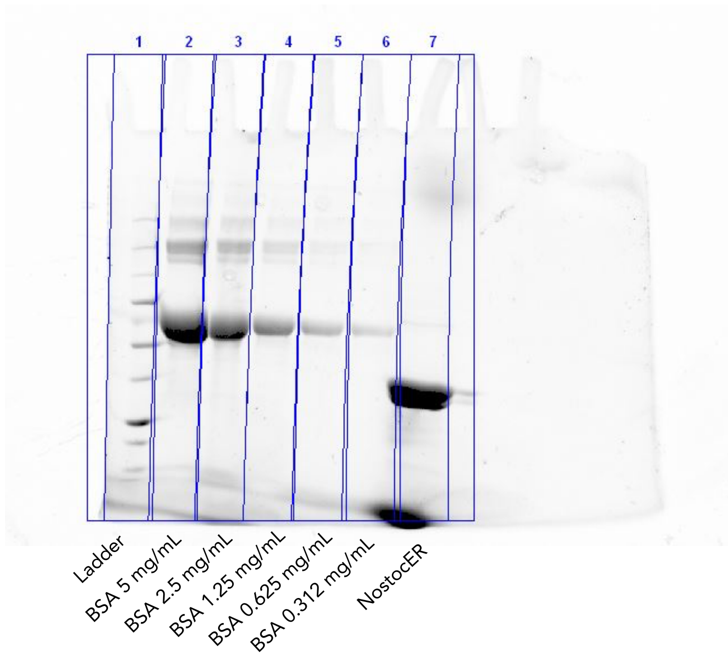
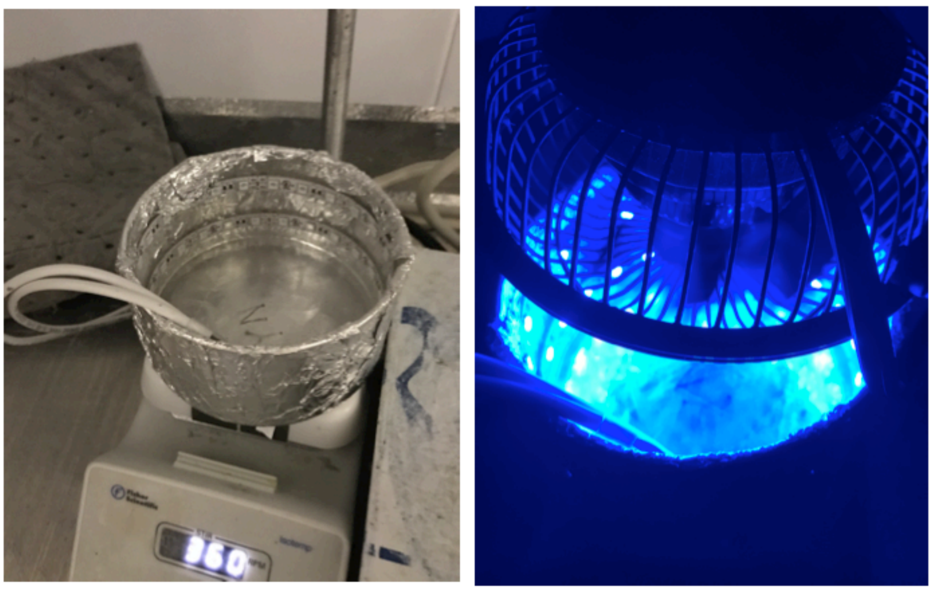
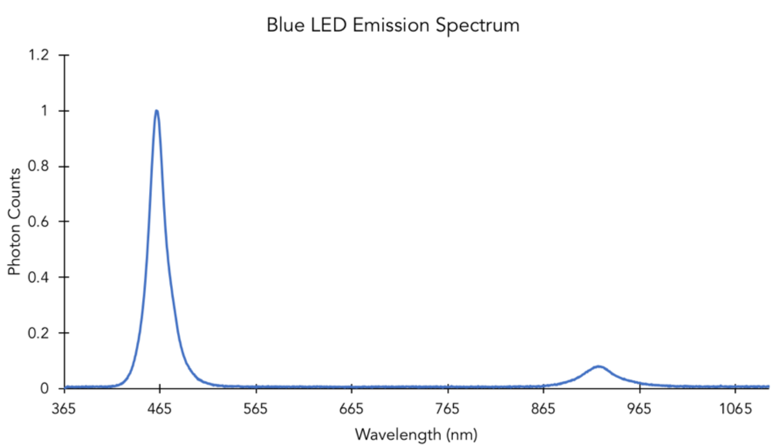
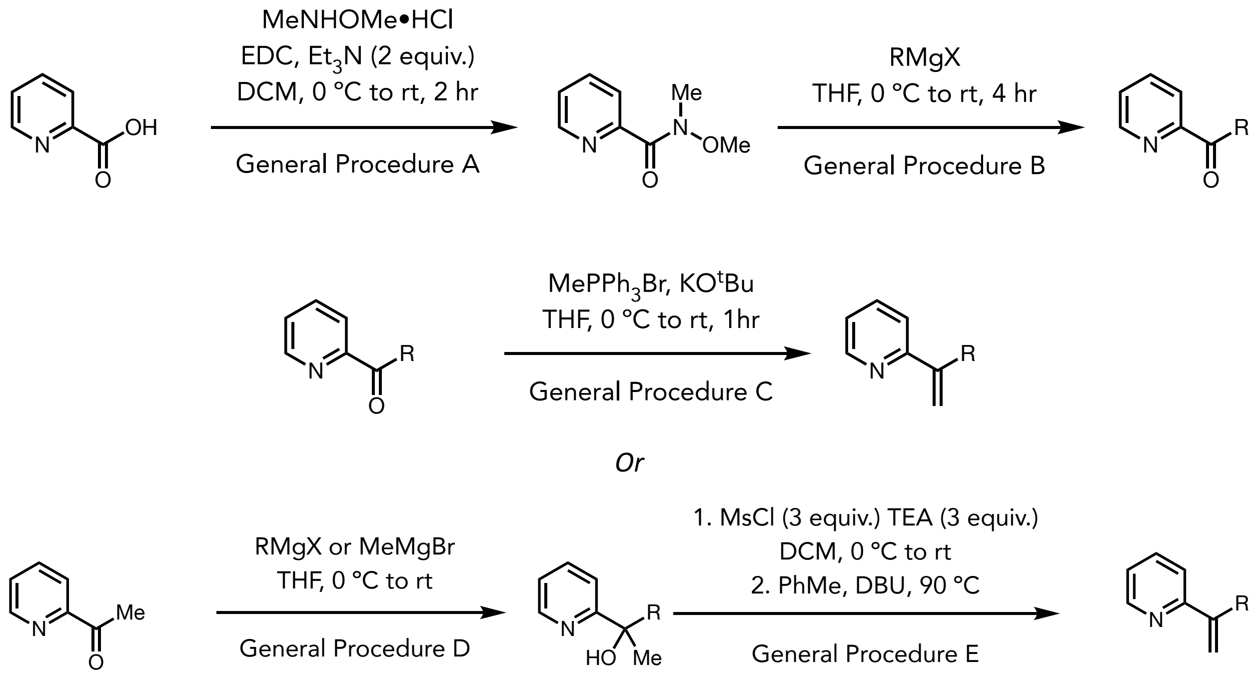


Figure S10. Photographic depiction of Blue LED light setup. LED strips were bought from www.superbrightleds.com (STN-BBLU-A3A-10B5M-12V). Emmission spectra for blue LED's are shown below. Light intensity is 3 W cm^{-2} s^{-1}. LED's are glued to the inside walls of a crystallization dish, and wrapped on the outside with aluminum foil. Left picture shows blue dish when no lights are on, right picture shows reaction setup with a cooling fan overtop. **NOTE**: During normal reaction setup, the blue dish is shielded with a cardboard box (not shown) to prevent eye damage. During reaction setup, orange glasses with blue cutoff are worn to mitigate possible eye damage from looking at the setup.





General Procedures for Substrate Synthesis



Procedure for Weinreb amide synthesis (General Procedure A):

To a stirred solution of 2-picolinic acid (1 equiv.), EDC (1.1 equiv.) and N,O-dimethylhydroxylamine hydrochloride (1.1 equiv.) in DCM (0.2 M) at 0 °C was added triethylamine (2 equiv.). Upon complete addition, the reaction mixture was warmed to ambient temperature. The reaction was monitored by TLC, and upon completion (approx. 2 hours) the mixture was diluted with DCM, treated with H_2O. The organic layer was separated and washed with saturated $NaHCO_3$ aqueous solution, brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography with 100% ethyl acetate.

Nucleophile addition to Weinreb amide (General Procedure B):

To a solution of aryl Grignard, as either the commercially available solution or freshly generated from magnesium turnings and 1,2-dibromoethane at reflux, in anhydrous THF (0.5 M, 1.5 equiv.) at 0 °C was added the Weinreb amide derived from picolinic acid (1 equiv.). The reaction mixture was warmed to ambient and monitored by TLC. Upon completion (usually <4 h), the reaction mixture was diluted with EtOAc and treated with H_2O. The aqueous layer was separated and extracted with EtOAc. Combined organics were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography.

Ketone olefination (General Procedure C):

To a flame-dried flask containing KOtBu (1.5 equiv.) and methyltriphenylphosphonium bromide (1.5 equiv.) at 0 °C was added anhydrous THF (20 mL/mmol substrate). The bright yellow suspension was warmed to ambient and stirred for 1 hour, before being cooled back to 0 °C. The 2-pyridyl ketone was added in one portion, and the reaction was monitored by TLC. Upon completion (typically <1 h), the reaction was diluted with hexanes, and filtered through a celite pad. The collected filtrate was concentrated under reduced pressure, and purified by flash column chromatography.

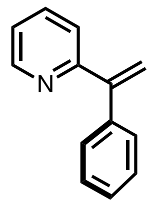
Nucleophile addition to 2-pyridyl ketones (General Procedure D):

A flame-dried flask under nitrogen atmosphere was charged with aryl bromide (1.25 equiv.), magnesium turnings (1.25 equiv.), anhydrous THF (0.5 M solution), and 3 drops of 1,2-dibromoethane. The solution was gently heated with a heat gun until bubbling was observed from the magnesium turnings. The solution was then heated at 70 °C until magnesium turnings were consumed, producing a light brown solution. The solution was subsequently cooled to 0 °C, and 2-acetylpyridine (1 equiv.) was added in one portion, and monitored by TLC. Upon completion, the reaction was quenched with water and extracted three times with ethyl acetate. The organic portions were pooled together, washed with brine, and dried with Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified via flash column chromatography.

Mesylation of tertiary alcohols and elimination (General Procedure E):

A flame-dried flask was charged with tertiary alcohol (1 equiv.) from General Procedure D/E and DCM (0.2 M). Triethylamine (3 equiv.) was added, and the solution cooled to 0 °C. Methanesulfonyl chloride (3 equiv.) was added dropwise over five minutes, upon which the reaction was warmed to room temperature and stirred for three hours. The reaction concentrated under pressure to obtain crude mesylated tertiary alcohol. This material was then redissolved in toluene (0.2 M), charged with DBU (6 equiv.), and heated at 90 °C overnight. The reaction was quenched with $NaHCO_3$, and extracted three times with ethyl acetate. Organic portions were pooled together, washed with brine, dried with Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified via flash column chromatography to yield desired 2-vinylpyridines.

Characterization of Vinyl-Pyridine Substrates



2-(1-phenylvinyl)pyridine (1)

Compound was prepared in 2 steps from phenylmagnesium bromide and Weinreb amide derived from picolinic acid to provide phenyl(pyridin-2-yl)methanone (1.208 g, 73% yield) via General Procedure B (1.500 g, 9.026 mmol). Reaction of phenyl(pyridin-2-yl)methanone (1.208 g, 6.549 mmol) via General Procedure C yielded title compound as a yellow oil (255.5 mg, 21% yield).

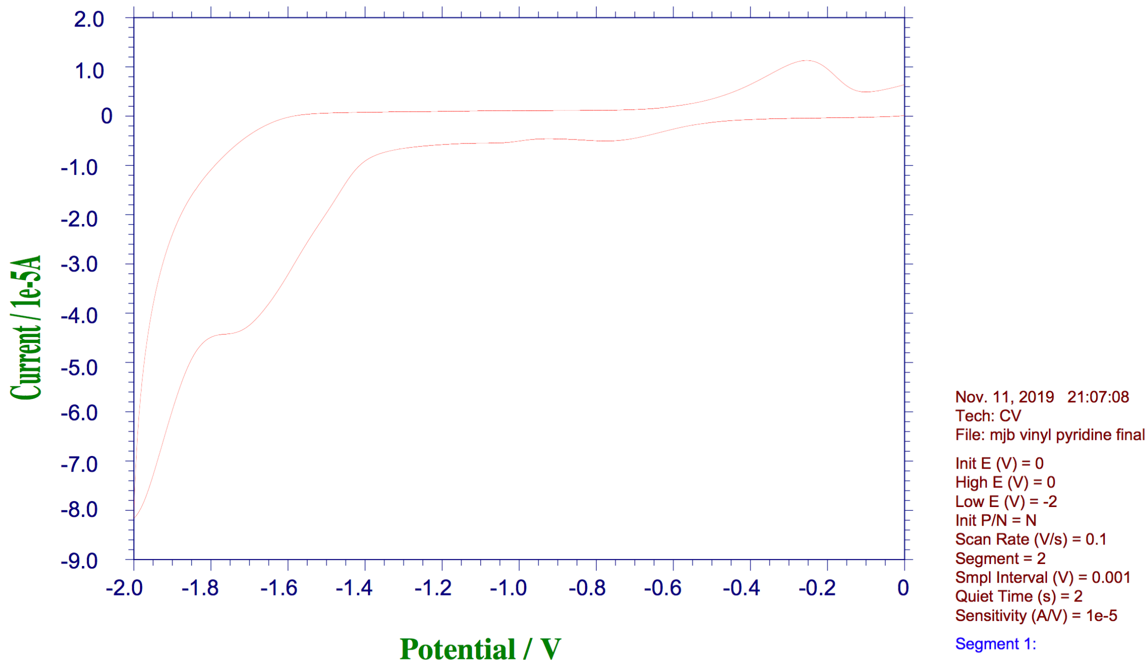
R$_f$ 0.54 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.65 (ddd, *J* = 5.0, 1.5, 1.0 Hz, 1H), 7.64 (td, *J* = 7.5, 1.5 Hz, 1H), 7.36 – 7.33 (m, 5H), 7.28 (dt, *J* = 7.5, 1.0 Hz, 1H), 7.22 (ddd, *J* = 7.5, 5.0, 1.0 Hz, 1H), 5.99 (d, *J* = 1.0 Hz, 1H), 5.61 (d, *J* = 1.0 Hz, 1H).

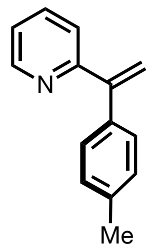
^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 158.67, 149.50, 149.26, 140.48, 136.47, 128.56, 128.43, 127.98, 123.04, 122.61, 117.91.

IR (neat, cm^{-1}): 1581, 1563, 1493, 1466, 1429, 913, 802, 775, 747, 702, 663.

HRMS calculated for $C_{13}H_{11}N$ $[M + H]^+$: 181.0891 Found: 181.0891



Cyclic voltammogram of substrate **1** 25 mM concentration in 500 mM KCl.



2-(1-phenylvinyl)pyridine (3a)

Compound was prepared in 2 steps from 4-iodotoluene and Weinreb amide **S1** to provide pyridin-2-yl(p-tolyl)methanone (591 mg, 75% yield) via General Procedure B (664 mg, 3.995 mmol). Reaction of pyridin-2-yl(p-tolyl)methanone (197 mg, 1.000 mmol) via General Procedure C yielded title compound as a yellow oil (95.0 mg, 49% yield).

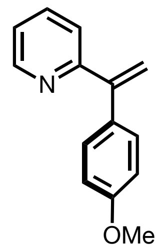
R_f 0.60 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.64 (ddd, *J* = 5.0, 2.0, 1.0 Hz, 1H), 7.63 (td, *J* = 7.5, 2.0 Hz, 1H), 7.28 (dt, *J* = 7.5, 1.0 Hz, 1H), 7.26 – 7.24 (m, 2H), 7.20 (ddd, *J* = 7.5, 5.0, 1.0 Hz, 1H), 7.18 – 7.16 (m, 2H), 5.93 (d, *J* = 1.5 Hz, 1H), 5.58 (d, *J* = 1.5 Hz, 1H), 2.38 (s, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 158.90, 149.51, 149.20, 137.77, 137.63, 136.36, 129.12, 128.45, 122.98, 122.51, 117.22, 21.36.

IR (neat, cm^{-1}): 1584, 1562, 1510, 1468, 1429, 1242, 913, 825, 802, 747, 678, 580

HRMS calculated for $C_{14}H_{13}N$ $[M + H]^+$: 195.1048 Found: 195.1043



2-(1-(4-methoxyphenyl)vinyl)pyridine (3b)

Compound was prepared in 2 steps from 4-bromoanisole and Weinreb amide **S1** to provide (4-methoxyphenyl)(pyridin-2-yl)methanone (503 mg, 59% yield) via General Procedure B (664 mg, 4.0 mmol). Reaction of (4-methoxyphenyl)(pyridin-2-yl)methanone (106 mg, 0.5 mmol) via General Procedure D and E with methylmagnesium bromide yielded title compound as a yellow oil (31.3 mg, 30% yield).

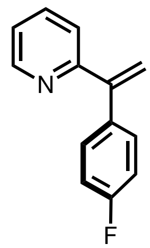
R_f 0.43 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.64 (ddd, J = 5.0, 2.0, 1.0 Hz, 1H), 7.64 (td, J = 7.5, 2.0 Hz, 1H), 7.31 – 7.27 (m, 3H), 7.21 (ddd, J = 7.5, 5.0, 1.0 Hz, 1H), 6.90 – 6.88 (m, 2H), 5.86 (d, J = 1.5 Hz, 1H), 5.56 (d, J = 1.5 Hz, 1H), 3.83 (s, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 159.51, 159.01, 149.50, 148.81, 136.39, 132.97, 129.71, 123.02, 122.52, 116.59, 113.82, 55.46.

IR (neat, cm^{-1}): 1584, 1510, 1247, 835, 802, 750, 677, 582, 558, 509, 482, 447, 456.

HRMS calculated for $C_{14}H_{13}NO$ $[M + H]^+$: 211.0997 Found: 211.0993



2-(1-(4-fluorophenyl)vinyl)pyridine (3c)

Compound was prepared in 2 steps from 4-bromofluorobenzene and Weinreb amide **S1** to provide (4-fluorophenyl)(pyridin-2-yl)methanone (484 mg, 32% yield) via General Procedure B (830.9 mg, 5.000 mmol). Reaction of (4-fluorophenyl)(pyridin-2-yl)methanone (201.2 mg, 1.000 mmol) via General Procedure C yielded title compound as a yellow oil (79.3 mg, 40% yield).

R_f 0.54 (1 : 4, EtOAc : hexanes)

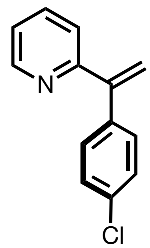
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.64 (d, *J* = 5.0 Hz, 1H), 7.66 (td, *J* = 7.5, 2.0 Hz, 1H), 7.34 – 7.31 (m, 2H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.23 (ddd, *J* = 7.5, 5.0, 0.5 Hz, 1H), 7.05 (t, *J* = 8.5 Hz, 2H), 5.94 (d, *J* = 1.0 Hz, 1H), 5.58 (d, *J* = 1.0 Hz, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 162.70 (d, *J* = 123 Hz), 158.58, 149.55, 148.36, 136.56, 136.49 (d, *J* = 3.3 Hz), 130.21 (d, *J* = 8.0 Hz), 122.89, 122.70, 117.81, 115.34 (d, *J* = 21 Hz)

^{19}F NMR (282 MHz, $CDCl_3$) **δ** -114.55.

IR (neat, cm^{-1}): 1584, 1507, 1468, 1222, 913, 840, 801, 748, 720, 677, 617, 578, 544, 518, 501, 472, 456, 442, 434, 423, 418, 406, 401.

HRMS calculated for $C_{14}H_{10}FN$ $[M + H]^+$: 199.0797 Found: 199.0791



<u>**2-(1-(4-chlorophenyl)vinyl)pyridine (3d)**</u>

Compound was prepared from 2-bromopyridine and 4'-chloroacetophenone. In a flame-dried flask, 2-bromopyridine (2.000 g, 12.658 mmol, 1 equiv.) was cooled to -78 °C in 25 mL anhydrous THF. To this solution, *n*-BuLi (12.658 mmol, 1 equiv.) was added and stirred for 30 min. After 30 min. elapsed, the solution was warmed to 0 °C, and 4'-chloroacetophenone (2.935 g, 18.987 mmol, 1.5 equiv.) was added in one portion. The workup for General Procedure D was followed, yielding 1-(4-chlorophenyl)-1-(pyridin-2-yl)ethan-1-ol (2.073 g, 70% yield). Reaction of 1-(4-chlorophenyl)-1-(pyridin-2-yl)ethan-1-ol (1.700 g, 7.274 mmol) via General Procedure E yielded title compound as a colorless oil (804.1 mg, 51% yield).

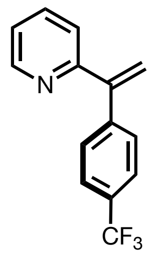
R_f 0.54 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.64 (ddd, *J* = 5.0, 1.5, 1.0 Hz, 1H), 7.66 (td, *J* = 7.5, 1.5 Hz, 1H), 7.35 – 7.32 (m, 2H), 7.30 – 7.28 (m, 3H), 7.23 (ddd, *J* = 7.5, 5.0, 1.0 Hz, 1H), 5.96 (d, *J* = 1.0 Hz, 1H), 5.61 (d, *J* = 1.0 Hz, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 158.32, 149.57, 148.31, 138.90, 136.59, 132.63, 129.88, 128.62, 122.88, 122.77, 118.21

IR (neat, cm^{-1}): 1584, 1489, 1091, 913, 834, 801, 747, 674, 617, 472, 457.

HRMS calculated for $C_{13}H_{11}ClN$ $[M + H]^+$: 215.0501 Found: 215.0500



2-(1-(4-(trifluoromethyl)phenyl)vinyl)pyridine (3e)

Compound was prepared from 2-bromopyridine and 4'-(trifluoromethyl)acetophenone. In a flame-dried flask, 2-bromopyridine (2.000 g, 12.658 mmol, 1 equiv.) was cooled to -78 °C in 25 mL anhydrous THF. To this solution, *n*-BuLi (12.658 mmol, 1 equiv.) was added and stirred for 30 min. After 30 min. elapsed, the solution was warmed to 0 °C, and 4'-(trifluoromethyl)acetophenone (3.572 g, 18.987 mmol, 1.5 equiv.) was added in one portion. The workup for General Procedure D was followed, yielding 1-(pyridin-2-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (1.757 g, 51% yield). Reaction of 1-(pyridin-2-yl)-1-(4-(trifluoromethyl)phenyl)ethan-1-ol (1.000 g, 3.741 mmol) via General Procedure E yielded title compound as a colorless oil (134 mg, 14% yield).

R_f 0.57 (1 : 4, EtOAc : hexanes)

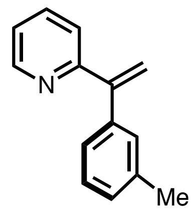
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.66 (dd, *J* = 5.0, 0.5 Hz, 1H), 7.71 (td, *J* = 8.0, 1.5 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.28 (dd, *J* = 7.0, 5.0 Hz, 1H), 6.10 (s, 1H), 5.70 (s, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 157.65, 149.28, 148.48, 123.88, 137.29, 137.17, 128.91, 128.20, 125.48 (q, *J* = 3.8 Hz), 123.03, 119.83.

^{19}F NMR (376 MHz, $CDCl_3$) **δ** -62.62.

IR (neat, cm^{-1}): 1325, 1165, 1123, 1065, 1017, 913, 748, 676

HRMS calculated for $C_{14}H_{11}F_3N$ $[M + H]^+$: 249.0765 Found: 249.0766



2-(1-(*m*-tolyl)vinyl)pyridine (3f)

Compound was prepared in 2 steps from *m*-tolylmagnesium bromide and Weinreb amide **S1** to provide pyridin-2-yl(*m*-tolyl)methanone (982.9 mg, 55% yield) via General Procedure B (1.000 g, 6.017 mmol). Reaction of pyridin-2-yl(*m*-tolyl)methanone (750 mg, 3.802 mmol) via General Procedure C yielded title compound as a colorless oil (266.1 mg, 35% yield).

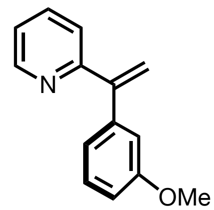
R_f 0.50 (1 : 4, EtOAc : hexanes)

^{1}H NMR (500 MHz, Chloroform-*d*) **δ** 8.68 – 8.62 (m, 1H), 7.64 (td, *J* = 7.7, 1.9 Hz, 1H), 7.31 – 7.12 (m, 7H), 5.98 (d, *J* = 1.5 Hz, 1H), 5.59 (d, *J* = 1.5 Hz, 1H), 2.36 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 158.75, 149.44, 149.34, 140.45, 138.00, 136.46, 129.25, 128.74, 128.30, 125.67, 123.03, 122.54, 117.76, 21.60.

IR (neat, cm^{-1}): 1769, 1758, 1582, 1467, 1429, 1377, 1241, 1048, 800, 749, 747, 683.

HRMS calculated for $C_{14}H_{13}N$ $[M+H]^+$: 195.1048 Found: 195.1049



<u>**2-(1-(3-methoxyphenyl)vinyl)pyridine (3g)**</u>

Compound was prepared in 2 steps from 3-bromoanisole (1.500 g, 8.020 mmol) via General Procedure D (776.6 mg, 56% yield). Reaction of 1-(3-methoxyphenyl)-1-(pyridin-2-yl)ethan-1-ol (700 mg, 3.053 mmol) via General Procedure E yielded title compound as a colorless oil (329 mg, 51% yield).

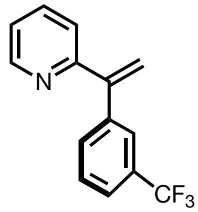
R_f 0.38 (1 : 4, EtOAc : hexanes)

^{1}H NMR (500 MHz, Chloroform-*d*) **δ** 8.65 (ddd, *J* = 4.9, 1.9, 0.9 Hz, 1H), 7.63 (td, *J* = 7.7, 1.9 Hz, 1H), 7.34 – 7.24 (m, 2H), 7.21 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 6.97 – 6.86 (m, 3H), 6.00 (d, *J* = 1.5 Hz, 1H), 5.61 (d, *J* = 1.5 Hz, 1H), 3.80 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) **δ** 159.60, 158.45, 149.46, 149.09, 141.91, 136.48, 129.41, 123.02, 122.60, 121.12, 118.03, 114.29, 113.42, 55.38.

IR (neat, cm^{-1}): 1662, 1581, 1485, 1465, 1457, 1448, 1429, 1306, 1285, 1230, 1148, 1042, 993, 912, 877, 784, 748, 719, 684, 617

HRMS (ESI-TOF) calculated for $C_{14}H_{13}NO$ $[M+H]^+$: 212.1069 Found: 212.1069



2-(1-(3-(trifluoromethyl)phenyl)vinyl)pyridine (3h)

Compound was prepared in 2 steps from 1-bromo-3-(trifluoromethyl)benzene (2.500 g, 11.110 mmol) via General Procedure D (658.7 mg, 22% yield). Reaction of 1-(pyridin-2-yl)-1-(3-(trifluoromethyl)phenyl)ethan-1-ol (914 mg, 3.420 mmol) via General Procedure E yielded title compound as a colorless oil (337.6 mg, 39% yield).

R_f 0.42 (1 : 4, EtOAc : hexanes)

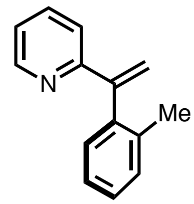
^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.68 – 8.62 (m, 1H), 7.72 – 7.57 (m, 4H), 7.56 – 7.45 (m, 2H), 7.31 (d, *J* = 7.9 Hz, 1H), 7.29 – 7.22 (m, 1H), 6.05 (s, 1H), 5.67 (d, *J* = 1.0 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 157.95, 149.63, 148.25, 141.22, 136.73, 131.91, 130.87 (q, *J* = 32.2 Hz), 128.88, 125.32 (q, *J* = 3.9 Hz), 124.74 (q, *J* = 3.8 Hz), 122.91, 122.80, 119.12.

^{19}F NMR (282 MHz, $CDCl_3$) δ -62.62.

IR (neat, cm^{-1}): 1583, 1468, 1431, 1329, 1308, 1280, 1163, 1122, 1070, 913, 802, 747, 699, 657

HRMS (ESI-TOF) calculated for $C_{14}H_{10}F_3N$ $[M+H]^+$: 249.0765 Found: 249.0759



2-(1-(*o*-tolyl)vinyl)pyridine (3i)

Compound was prepared in 2 steps from *o*-tolylmagnesium bromide and Weinreb amide **S1** to provide pyridin-2-yl(*o*-tolyl)methanone (809 mg, 45% yield) via General Procedure B (1.500 g, 9.026 mmol). Reaction of pyridin-2-yl(*o*-tolyl)methanone (500 mg, 2.535 mmol) via General Procedure C yielded title compound as a colorless oil (170.6 mg, 35% yield).

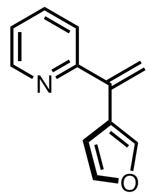
R_f 0.53 (1 : 4, EtOAc : hexanes)

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.65 (dt, *J* = 4.5, 1.5 Hz, 1H), 7.54 (td, *J* = 7.7, 1.9 Hz, 1H), 7.26 (tdd, *J* = 13.3, 7.1, 3.9 Hz, 4H), 7.15 (ddd, *J* = 7.4, 4.8, 1.2 Hz, 1H), 6.97 (dt, *J* = 7.9, 1.1 Hz, 1H), 6.46 (d, *J* = 2.0 Hz, 1H), 5.41 (d, *J* = 2.0 Hz, 1H), 2.09 (s, 3H)

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 157.36, 149.56, 148.61, 140.54, 136.60, 136.31, 130.19, 130.13, 127.89, 126.01, 122.37, 121.64, 118.75, 20.18.

IR (neat, cm^{-1}): 1580, 1562, 1487, 1466, 1456, 1428, 1040, 989, 920, 804, 766, 746, 730, 626, 608, 583, 404

HRMS (ESI-TOF) calculated for $C_{14}H_{13}N$ $[M+H]^+$: 195.1048 Found: 195.1049



2-(1-(furan-3-yl)vinyl)pyridine (3j)

Compound was prepared from 2-bromopyridine and Weinreb amide derived from furan-3-carboxylic acid. In a flame-dried flask, 2-bromopyridine (1.388 g, 8.790 mmol, 1 equiv.) was cooled to -78 °C in 18 mL anhydrous THF. To this solution, *n*-BuLi (8.790 mmol, 1 equiv.) was added and stirred for 30 min. After 30 min. elapsed, the solution was warmed to 0 °C, and *N*-methoxy-*N*-methylfuran-3-carboxamide (1.500 g, 9.669 mmol, 1.1 equiv.) was added in one portion. The workup for General Procedure B was followed, yielding furan-3-yl(pyridin-2-yl)methanone as a white solid (888.1 mg, 58% yield). Reaction of furan-3-yl(pyridin-2-yl)methanone (950 mg, 5.485 mmol) via General Procedure C yielded title compound as a brown oil (348 mg, 37% yield).

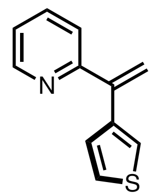
R_f 0.57 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, Chloroform-*d*) δ 8.64 (ddd, *J* = 4.9, 1.9, 0.9 Hz, 1H), 7.69 (td, *J* = 7.7, 1.8 Hz, 1H), 7.59 (t, *J* = 1.1 Hz, 1H), 7.50 – 7.40 (m, 2H), 7.24 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 6.57 (dd, *J* = 1.9, 0.9 Hz, 1H), 5.69 (d, *J* = 1.1 Hz, 1H), 5.63 (d, *J* = 1.1 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) **δ** 158.33, 149.16, 142.98, 141.35, 140.23, 136.62, 124.76, 122.70, 122.29, 115.82, 109.70.

IR (neat, cm^{-1}): 1584, 1563, 1467, 1457, 1430, 1159, 1065, 1020, 960, 898, 872, 796, 747, 650.

HRMS (ESI-TOF) calculated for $C_{11}H_9NO$ $[M+H]^+$: 171.0684 Found: 171.0679



2-(1-(thiophen-3-yl)vinyl)pyridine (3k)

Compound was prepared in 2 steps via an adaptation of General Procedure D. In a flame-dried flask, 3-bromothiophene (2.000 g, 12.266 mmol,1 equiv.) was added with 24 mL anhydrous THF and cooled to -78 °C. To this solution, 4.9mL *n*-BuLi (2.5 M in hexanes) (1 equiv.) and allowed to stir for 30 min. The reaction was warmed to 0 °C, upon which 2-acetylpyridine (1.634 g, 13.493 mmol, 1.1 equiv.) was added in one portion. The remainder of General Procedure D was followed to yield tertiary alcohol (610.4 mg, 24% yield). Reaction of 1-(pyridin-2-yl)-1-(thiophen-3-yl)ethan-1-ol (601.4 mg, 2.929 mmol) via General Procedure E yielded title compound as a yellow oil (442.1 mg, 80% yield).

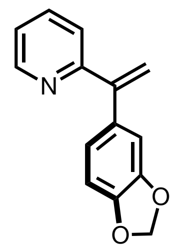
R_f 0.53 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, Chloroform-*d*) δ 8.65 (d, *J* = 4.7 Hz, 1H), 7.68 (td, *J* = 7.7, 1.8 Hz, 1H), 7.39 (d, *J* = 7.9 Hz, 1H), 7.35 – 7.22 (m, 3H), 7.21 (dd, *J* = 23.9, 4.9 Hz, 2H), 5.82 (s, 1H), 5.69 (s, 1H).

^{13}C-NMR (125 MHz, CDCl3) **δ** 158.56, 149.20, 143.67, 140.88, 136.52, 131.00, 128.18, 127.45, 125.44, 123.50, 122.67, 122.61, 116.76.

IR (neat, cm^{-1}): 1582, 1561, 1467, 1429, 908, 867, 836, 794, 747, 689, 668, 650, 623, 603.

HRMS (ESI-TOF) calculated for $C_{11}H_9NS$ $[M+H]^+$: 187.0456 Found: 187.0456



<u>**2-(1-(benzo[d][1,3]dioxol-5-yl)vinyl)pyridine (3l)**</u>

Compound was prepared in 2 steps from 5-bromobenzo[*d*][1,3]dioxole and Weinreb amide **S1** to provide benzo[d][1,3]dioxol-5-yl(pyridin-2-yl)methanone (407 g, 36% yield) via General Procedure B (831 mg, 5.000 mmol). Reaction of benzo[d][1,3]dioxol-5-yl(pyridin-2-yl)methanone (227 mg, 1.000 mmol) via General Procedure C yielded title compound as a pale yellow oil (176 mg, 78% yield).

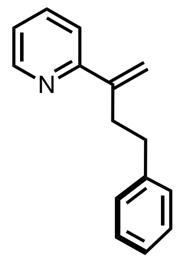
R_f 0.46 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.63 (ddd, *J* = 5.0, 2.0, 1.0 Hz, 1H), 7.65 (td, *J* = 7.5, 2.0 Hz, 1H), 7.32 (dt, *J* = 7.5, 1.0 Hz, 1H), 7.21 (ddd, *J* = 7.5, 5.0, 1.0 Hz, 1H), 6.85 – 6.79 (m, 3H), 5.97 (s, 2H), 5.85 (d, *J* = 1.0 Hz, 1H), 5.55 (d, *J* = 1.0 Hz, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 158.92, 149.50, 148.93, 147.66, 147.49, 136.45, 134.62, 123.04, 122.59, 122.28, 116.97, 109.03, 108.30, 101.24.

IR (neat, cm^{-1}): 1583, 1501, 1488, 1430, 1234, 1038, 913, 802, 747, 677, 457.

HRMS calculated for $C_{14}H_{11}NO_2$ $[M + H]^+$: 225.0789 Found: 225.0783



2-(4-phenylbut-1-en-2-yl)pyridine (3m)

Compound was prepared in 2 steps from phenethylmagnesium bromide and Weinreb amide **S1** to provide 3-phenyl-1-(pyridin-2-yl)propan-1-one (534.5 mg, 51% yield) via General Procedure B (830 mg, 5.000 mmol). Reaction of 3-phenyl-1-(pyridin-2-yl)propan-1-one (105 mg, 0.500 mmol) via General Procedure C yielded title compound as a yellow oil (43 mg, 41% yield).

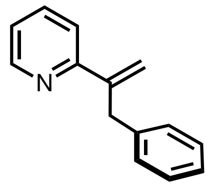
$\mathbf{R_f}$ 0.70 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.61 (ddd, *J* = 5.0, 2.0, 1.0 Hz, 1H0, 7.66 (td, *J* = 8.0, 2.0 Hz, 1H), 7.48 (dt, *J* = 8.0, 1.0 Hz, 1H), 7.29 – 7.26 (m, 2H), 7.22 – 7.16 (m, 4H), 5.74 (d, *J* = 0.5 Hz, 1H), 5.27 (dd, *J* = 2.5, 0.5 Hz, 1H), 2.97 – 2.94 (m, 2H), 2.85 – 2.82 (m, 2H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 158.45, 149.14, 147.71, 142.19, 136.44, 128.63, 128.39, 125.91, 122.27, 120.56, 115.47, 35.74, 34.89.

IR (neat, cm^{-1}): 1585, 1563, 1466, 1455, 1430, 912, 801, 744, 698, 677, 617, 557, 501, 472, 458.

HRMS calculated for $C_{15}H_{15}N$ $[M + H]^+$: 209.1204 Found: 209.1201



<u>2-(3-phenylprop-1-en-2-yl)pyridine (3n)</u>

Compound was prepared in 2 steps from benzylmagnesium bromide and Weinreb amide **S1** to provide 2-phenyl-1-(pyridin-2-yl)ethan-1-one (705 mg, 71% yield) via General Procedure B (830 mg, 5.000 mmol). Reaction of 2-phenyl-1-(pyridin-2-yl)ethan-1-one (107 mg, 1.000 mmol) via General Procedure C yielded title compound as a yellow oil (101.6 mg, 52% yield).

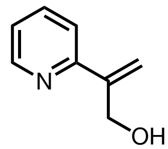
R_f 0.68 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.59 (ddd, *J* = 4.5, 2.0 1.0 Hz, 1H), 7.60 (td, *J* = 8.0, 2.0 Hz, 1H), 7.44 (dt, *J* = 8.0, 1.0 Hz, 1H), 7.29 – 7.25 (m, 4H), 7.20 – 7.16 (m, 1H), 7.14 (ddd, *J* = 8.0, 4.5, 1.0 Hz, 1H), 5.93 (dt, *J* = 1.0, 0.5 Hz, 1H), 5.19 (dt, *J* = 1.5, 1.0 Hz, 1H), 3.98 (brs, 2H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 157.99, 149.04, 147.22, 139.82, 136.44, 12925, 128.43, 126.16, 122.35, 120.70, 117.29, 39.92.

IR (neat, cm^{-1}): 1584, 1563, 1466, 1430, 913, 800, 743, 698.

HRMS calculated for $C_{15}H_{15}N$ $[M + H]^+$: 195.1048 Found: 195.1049



2-(pyridin-2-yl)prop-2-en-1-ol (3o)

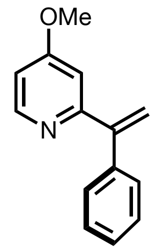
Compound prepared from 2-bromopyridine and allyl alcohol. In a flame-dried flask, 2-bromopyridine (3.0 mmol,1 equiv.), triethylamine (4.8 mmol, 1.6 equiv.), $Pd(OAc)_2$ (0.12 mmol, 0.04 equiv.) and 1,3-bis(diphenylphosphino)propane (0.24 mmol, 0.08 equiv.) were combined with 3 mL [bmim][BF_4] ionic liquid. The solution was degassed with three freeze-pump-thaw cycles. Allyl alcohol (15 mmol, 5 equiv.) was then added and the flask sealed. The reaction mixture was then heated to 125 °C for 24 hours. The reaction was cooled to room temperature, and 10 mL of 3M HCl were added and allowed to stir for 1 hour. Saturated Na_2CO_3 was added dropwise, and the solution was extracted three times with DCM. Organic extracts were combined, washed with water and brine, and dried with Na_2SO_4. The dried solution was filtered and concentrated under reduced pressure. The resulting brown residue was purified via column chromatography to give 22 mg of title compound (5% yield).

R_f 0.54 (100% EtOAc)

^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 8.55 (dd, *J* = 5.1, 1.7 Hz, 1H), 7.72 (td, *J* = 7.8, 1.8 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.27 – 7.20 (m, 1H), 5.81 (s, 1H), 5.52 (s, 1H), 4.60 (s, 2H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 157.49, 148.32, 145.54, 136.74, 122.59, 120.13, 116.22, 77.28, 77.03, 76.77, 66.20.

HRMS calculated for C_8H_9NO $[M + H]^+$: 135.0684 Found: 135.0680



4-methoxy-2-(1-phenylvinyl)pyridine (3p)

Compound prepared via an adapted procedure from Hilton *et al.*

In a flame-dried flask, 2-(1-phenylvinyl)pyridine (622.6 mg, 3.435 mmol) was dissolved in 34 mL anhydrous DCM. The solution was cooled to -78 °C, and triflic anhydride (969 mg, 3.435 mmol, 1 equiv.) was added dropwise. The resulting solution was stirred for 30 min., at which PPh_3 (990 mg, 3.778 mmol, 1.1 equiv.) was added in one portion. To this solution, DBU (522 mg, 3.435 mmol, 1 equiv.) was added dropwise and stirred for 30 min. at room temperature. The reaction was then quenched with water, diluted with DCM, and the resulting organic layer washed three times with water. The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure to approximately ~5 mL volume. Chilled Et_2O was then added to the concentrated solution. The resulting phosphonium salt precipitate was filtered and used crude in the next step.

A flame-dried flask was charged with 60 wt% NaH (205 mg, 5.152 mmol, 1.5 equiv.) and 6 mL anhydrous THF. The solution was cooled to 0 °C, and MeOH (165 mg, 5.152 mmol, 1.5 equiv.) was added dropwise. The solution was stirred for 30 min. at 0 °C, at which point the phosphonium salt was added in one portion. The reaction was then subjected to nitrogen backfill, and allowed to react for 12 hours. The reaction was then quenched with water and extracted three times with ethyl acetate. The organic solutions were combined, washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified via flash column chromatography, yielding title compound as a colorless oil (68.5 mg, 10% yield).

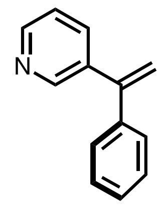
R_f 0.26 (1 : 4, EtOAc : hexanes)

^{1}H NMR (500 MHz, Chloroform-*d*) **δ** 8.49 (d, J = 5.6 Hz, 1H), 7.35 (d, J = 3.9 Hz, 5H), 6.82 – 6.74 (m, 2H), 6.05 (s, 1H), 5.63 (s, 1H), 3.82 (s, 3H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 166.01, 160.21, 150.62, 149.15, 140.27, 128.41, 128.29, 127.84, 117.71, 109.38, 108.28, 55.14.

IR (neat, cm^{-1}): 1585, 1562, 1470, 1442, 1300, 1261, 1231, 1142, 1037, 1028, 913, 779, 704.

HRMS (ESI-TOF) calculated for $C_{14}H_{13}NO$ $[M+H]^+$: 211.0997 Found: 211.1004



3-(1-phenylvinyl)pyridine (3q)

Compound was prepared by addition of phenylmagnesium bromide (10.565 mmol, 1.1 equiv.) to nicotinonitrile (1.000 g, 9.605 mmol) in 20 mL anhydrous THF at 0 °C. Water was added to the resulting solution, and extracted three times with ethyl acetate. Organic residues were combined, dried with Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified via flash column chromatography to yield phenyl(pyridin-3-yl)methanone (1.302 g, 74% yield). Reaction of phenyl(pyridin-3-yl)methanone (1.000 g, 5.458 mmol) via General Procedure C yielded title compound as a yellow oil (741 mg, 75% yield).

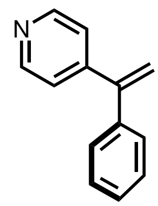
R_f 0.37 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 8.66 (d, *J* = 2.3 Hz, 1H), 8.58 (dd, *J* = 4.8, 1.7 Hz, 1H), 7.63 (dt, *J* = 7.9, 2.0 Hz, 1H), 7.42 – 7.30 (m, 5H), 7.31 – 7.25 (m, 1H), 5.59 (d, *J* = 0.9 Hz, 1H), 5.52 (d, *J* = 1.0 Hz, 1H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 149.37, 149.02, 147.00, 140.44, 137.18, 135.63, 128.54, 128.27, 128.12, 123.13, 115.91, 77.41, 77.16, 76.90.

IR (neat, cm^{-1}): 1609, 1564, 1493, 1473, 1444, 1410, 1070, 1022, 902, 815, 776, 719, 701, 624, 596, 573.

HRMS calculated for $C_{13}H_{11}N$ $[M + H]^+$: 181.0891 Found: 181.0889



4-(1-phenylvinyl)pyridine (3r)

Compound was prepared in 2 steps from phenylmagnesium bromide and Weinreb amide **derived from isonicotinic acid (via General Procedure A)** to provide phenyl(pyridin-4-yl)methanone (726 mg, 79% yield) via General Procedure B (831 mg, 5.000 mmol). Reaction of phenyl(pyridin-4-yl)methanone (183 mg, 1.000 mmol) via General Procedure C yielded title compound as a yellow oil (89.4 mg, 43% yield).

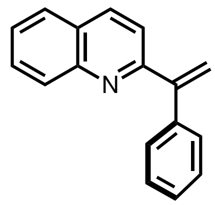
R_f 0.27 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.59 – 8.57 (m, 2H), 7.38 – 7.36 (m, 3H), 7.31 – 7.29 (m, 2H), 7.25 – 7.24 (m, 2H), 5.61 (d, *J* = 1.0 Hz, 1H), 5.60 (d, *J* = 1.0 Hz, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 150.03, 139.90, 128.80, 128.58, 128.38, 128.29, 128.06, 122.93, 117.10.

IR (neat, cm^{-1}): 1595, 1240, 913, 743, 720, 677, 617, 575, 525, 517, 502, 457.

HRMS calculated for $C_{13}H_{12}N$ $[M + H]^+$: 181.0891 Found: 181.0891



<u>**2-(1-phenylvinyl)quinoline (3s)**</u>

Compound was prepared by addition of phenylmagnesium bromide (8.400 mmol, 1.2 equiv.) to quinoline-2-carbaldehyde (1.096 g, 7.000 mmol) in 35 mL anhydrous THF at -78 °C. The brown solution was allowed to warm to ambient temperature overnight. The reaction was then quenched with 35 mL water, and the workup for General Procedure D was followed, yielding phenyl(quinolin-2-yl)methanol. The resulting alcohol was dissolved in 50 mL DCM at 0 °C, and Dess-Martin periodinane (2.97 g, 7.000 mmol) was added. The milky-cream color reaction was diluted with DCM, treated with saturated $NaHCO_3$ solution. The aqueous solution was separated and extracted three times with ethyl acetate. Organic solutions were combined, dried with Na_2SO_4 and concentrated under reduced pressure and purified via flash column chromatography yielding phenyl(quinolin-2-yl)methanone (812.5 mg, 50% yield).

Reaction of phenyl(quinolin-2-yl)methanone (233 mg, 1.000 mmol) via General Procedure C yielded title compound as a colorless oil (87.3 mg, 38% yield).

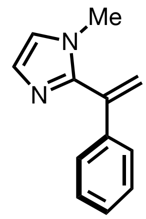
R_f 0.72 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.15 (d, *J* = 8.5 Hz, 1H), 8.10 (d, *J* = 8.5 Hz, 1H), 7.81 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.72 (ddd, *J* = 8.0, 7.0, 1.5 Hz, 1H), 7.54 (ddd, *J* = 8.0, 7.0, 1.5 Hz, 1H), 7.42 – 7.36 (m, 6H), 6.11 (d, *J* = 1.5 Hz, 1H), 5.77 (d, *J* = 1.5 Hz, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 158.97, 149.67, 148.11, 140.24, 136.21, 129.94, 129.73, 128.54, 128.46, 128.06, 127.55, 127.53, 126.59, 121.37, 118.94.

IR (neat, cm^{-1}): 1595, 1501, 913, 837, 744, 720, 669, 617, 545, 472, 419

HRMS calculated for $C_{17}H_{13}N$ $[M + H]^+$: 231.1048 Found: 231.1053



1-methyl-2-(1-phenylvinyl)-1H-imidazole (3t)

To a stirred solution of *N*-methylimidazole (0.821 g, 10 mmmol, 1 equiv.) and benzoyl chloride (2.108 g, 15 mmol, 1.5 equiv.) in acetonitrile at 0 °C was added triethylamine (1.517 g, 15 mmol, 1.5 equiv.) dropwise. After addition was finished, the solution was warmed to ambient temperature. The solution was then diluted with dichloromethane and treated with water. The organic phase was separated and washed with saturated $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting brown solid was purified via flash column chromatography to yield (1-methyl-1H-imidazol-2-yl)(phenyl)methanone (497 mg, 27% yield). Reaction of (1-methyl-1H-imidazol-2-yl)(phenyl)methanone (186 mg, 1.000 mmol) via General Procedure C yielded title compound as a yellow oil (128.6 mg, 70% yield).

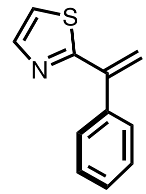
R_f 0.27 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 7.36 – 7.28 (m, 5H), 7.09 (d, *J* = 1.0 Hz, 1H), 6.91 (d, *J* = 1.0 Hz, 1H), 5.84 (d, *J* = 1.5 Hz, 1H), 5.66 (d, *J* = 1.0 Hz, 1H), 3.34 (s, 3H)

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 147.79, 139.45, 139.18, 132.20, 128.76, 128.39, 126.95, 121.90, 119.78, 34.15

IR (neat, cm^{-1}): 913, 744, 676.

HRMS calculated for $C_{12}H_{12}N_2$ $[M + H]^+$: 184.1000 Found: 184.0994



2-(1-phenylvinyl)thiazole (3u)

To a stirred solution of thiazole (425 mg, 5.000 mmol, 1 equiv.) in 10 mL acetonitrile was added 4-dimethylaminopyridine (183 mg, 1.5 mmol, 0.3 equiv.), and triethylamine (1.517 g, 15 mmol, 3 equiv.). At room temperature, benzoyl chloride (1.405 g, 10 mmol, 2 equiv.) was added, and a white precipitate was observed forming. A reflux condenser was attached to the flask, and the reaction heated to 80 °C overnight. The reaction was cooled to room temperature, poured into 100 mL water, and extracted two times with ethyl acetate. The organic solutions were combined, and subsequently washed with saturated NH_4Cl solution, saturated $NaHCO_3$ solution, and brine. The organic phase was then separated, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting brown oil was purified via flash column chromatography to yield a phenyl(thiazol-2-yl)methanone as a yellow solid (908 mg, 96% yield). Reaction of phenyl(thiazol-2-yl)methanone (189 mg, 1.000 mmol) via General Procedure C yielded title compound as a yellow oil (128.8 mg, 69% yield).

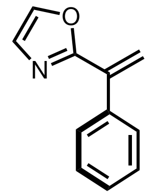
R_f 0.64 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 7.85 (d, *J* = 3.0 Hz, 1H), 7.50 – 7.48 (m, 2H), 7.41 – 7.39 (m, 3H), 7.31 (d, *J* = 3.5 Hz, 1), 6.11 (d, *J* = 0.5 Hz, 1H), 5.58 (d, *J* = 0.5 Hz, 1H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 168.97, 143.64, 143.09, 139.45, 128.67, 128.59, 128.48, 119.64, 118.47.

IR (neat, cm^{-1}): 1483, 1444, 1105, 1070, 1025, 913, 775, 743, 696, 582.

HRMS calculated for $C_{11}H_9NS$ [M + H]$^+$: 187.0455 Found: 187.0450



2-(1-phenylvinyl)oxazole (3v)

In a flame-dried flask, oxazole (200 mg, 2.89 mmol, 1 equiv.), benzoyl chloride (810 mg, 5.78 mmol, 2 equiv.) and 4-dimethylaminopyridine (106 mg, 0.87 mmol, 0.3 equiv.) were combined in 6 mL anhydrous acetonitrile. To this solution, triethylamine (870 mg, 8.67 mmol, 3 equiv.) were added. A reflux condenser was attached to the flask, and the reaction mixture heated at 80 °C for 24 hours. Upon completion, the reaction mixture was cooled to room temperature, and diluted with ethyl acetate and saturated $NaHCO_3$. Ethyl acetate was used to extract the aqueous layer three times. The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting residue was purified by column chromatography yielding oxazol-2-yl(phenyl)methanone (283 mg, 56% yield). Reaction of oxazol-2-yl(phenyl)methanone (280 mg, 1.620 mmol) via General Procedure C yielded title compound as a yellow oil (170.0 mg, 61% yield).

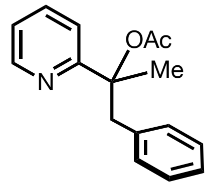
R_f 0.64 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 7.66 (s, 1H), 7.57 – 7.51 (m, 2H), 7.46 – 7.34 (m, 3H), 7.21 (s, 1H), 6.24 (s, 1H), 5.73 (s, 1H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 161.99, 141.67, 138.73, 137.37, 137.15, 134.15, 130.98, 128.65, 128.58, 128.46, 128.45, 128.40, 120.35.

IR (neat, cm^{-1}): 1665, 1482, 1448, 1370, 1286, 1173, 1139, 1071, 955, 913, 743, 720, 683, 617, 555, 456, 444.

HRMS calculated for $C_{11}H_9NO$ $[M + H]^+$: 171.0684 Found: 171.0680



1-phenyl-2-(pyridin-2-yl)propan-2-yl acetate (7)

To a stirred solution of 2-acetylpyridine (1.211 g, 10 mmol, 1 equiv.) in 50 mL anhydrous THF at 0 °C was added benzylmagnesium chloride (2.0 M in Et_2O) (7.5 mL, 15 mmol, 1.5 equiv.). The reaction was allowed to stir for 30 min., at which point acetic anhydride (3.062 g, 30 mmol, 3 equiv.), causing the solution to turn from brown, to greenish-yellow, to bright yellow over the course of 30 min. After consumption of starting material was observed via TLC, the reaction was diluted with Et_2O, then treated with water. The aqueous layer was extracted with Et_2O, and the organic layers were washed with saturated $NaHCO_3$, saturated NH_4Cl, and brine. The organic layer was separated and dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting red oil was purified via flash column chromatography to yield the title compound (1.561 g, 61% yield).

R_f 0.44 (1 : 4, EtOAc : hexanes)

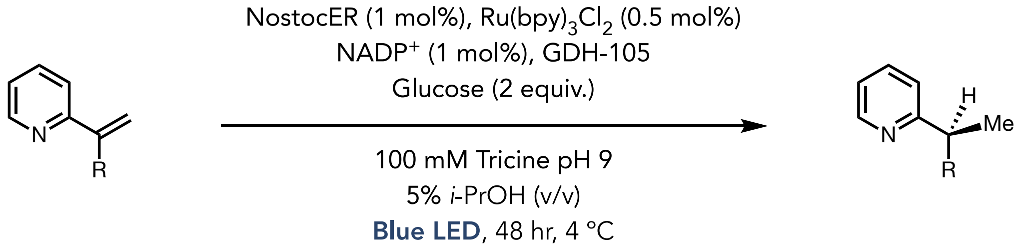
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.62 (ddd, J = 5.0, 1.5, 1.0 Hz, 1H), 7.57 (td, J = 8.0, 2.0 Hz, 1H), 7.18 – 7.15 (m, 4H), 7.12 (dt, J = 8.0, 1.0 Hz, 1H), 6.91 – 6.89 (m, 2H), 3.41 and 3.35 (ABq, J_{AB} = 13.5 Hz, 2H), 2.08 (s, 3H), 1.86 (s, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 170.06, 162.54, 148.83, 136.44, 136.20, 130.67, 127.91, 126.62, 122.09, 120.15, 84.69, 46.88, 23.90, 22.33.

IR (neat, cm^{-1}): 1737, 1730, 1590, 1432, 1367, 1251, 1234, 1167, 1105, 1079, 1016, 913, 781, 747, 700, 674.

HRMS calculated for $C_{14}H_{13}N$ $[M + H]^+$: 195.1048 Found -AcOH 195.1042

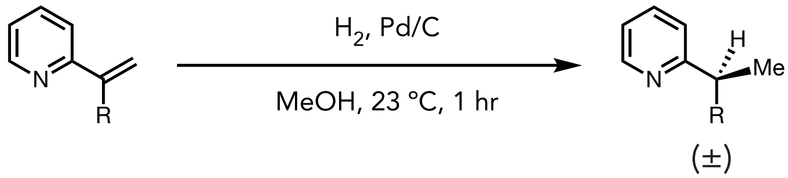
General Procedures for Vinyl Pyridine Reductions



Enzymatic Reduction (General Procedure F):

In the Coy chamber was introduced: protein aliquots (NostocER, in "OYE concentration buffer" [20 mM KPi pH 7.4, 300 mM NaCl], generally 2~4 mM concentration, 100 nmol total in each aliquot), shell vial with magnetic cross stir-bar, substrate in a one-dram vial, and a "master mix" vial containing 1.0 mg $Ru(bpy)_3Cl_2•6H_2O$ and 2.0 mg $NADP^+$ and 10.7 mg GDH-105 and 96.0 mg D-glucose. To the "master mix" was added 4.018 mL Tricine buffer (100 mM, pH 9,). ^{i}PrOH was added to substrate vial (such that concentration was 1 M). To each reaction shell vial was added 450 μL of "master mix", 30 μL of substrate in ^{i}PrOH, and 3 aliquots of protein. A rubber septum was affixed to the reaction vial, brought out of the Coy chamber and irradiated with blue LED's at 0 °C for 48 hours.

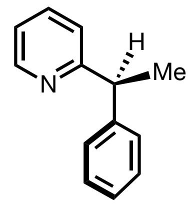
Upon completion, the reaction vials were treated with MeCN (0.9 mL) and an internal standard (1,3,5-tribromobenzene, 100 μL of a 10 mg/mL solution in MeCN [1 mg total]). The resultant mixture was centrifugated (10,000 xg, 5 min), and supernatant was partitioned between H_2O:DCM (3 mL : 3 mL). The aqueous layer was separated and extracted with DCM. Combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was dissolved in $CDCl_3$ and analyzed by ^{1}H-NMR for yield calculation. The $CDCl_3$ solution was reconcentrated and dissolved in HPLC grade hexanes for HPLC analysis.



Pd/C Hydrogenation (General Procedure G):

To a solution of olefin (typically ~ 0.2 mmol) in MeOH (10 mL/mmol substrate) was added 10 wt% Pd on activated carbon (100 mg/mmol substrate). A balloon of H_2 was bubbled through the solution and monitored by TLC until complete consumption of starting material was observed. The reaction was filtered through celite and concentrated under reduced pressure. The resulting residue was purified via flash column chromatography with hexanes/ethyl acetate as eluent.

Characterization of Vinyl-Pyridine Products



(*S*)-2-(1-phenylethyl)pyridine (2)

Title compound was obtained following General Procedure F from **1**. Yellow oil (96% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 96% (Run 1), 91% (Run 2), 96% (Run 3)

R_f 0.55 (1 : 4, EtOAc : hexanes)

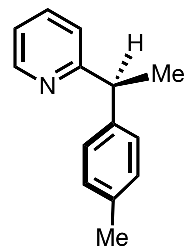
^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 8.59 – 8.54 (m, 1H), 7.56 (td, *J* = 7.7, 1.9 Hz, 1H), 7.30 (d, *J* = 1.4 Hz, 2H), 7.21 (qd, *J* = 5.3, 4.8, 2.2 Hz, 1H), 7.20 – 7.06 (m, 2H), 4.31 (q, *J* = 7.2 Hz, 1H), 1.71 (d, *J* = 7.3 Hz, 3H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 165.03, 149.12, 145.08, 136.45, 128.48, 127.70, 126.33, 122.15, 121.24, 77.31, 77.06, 76.81, 47.38, 20.76.

IR (neat, cm^{-1}): 1588, 1568, 1493, 1471, 1450, 1431, 1027, 993, 804, 746, 698, 639, 609, 584, 546.

HRMS calculated for $C_{13}H_{13}N$ $[M + H]^+$: 183.1048 Found: 183.1047

HPLC OJ-H column, 99.5 : 0.5 (hexane : iPrOH), 1.0 mL-min, t_R 31.4 min (major), 29.4 min (minor), 92 : 8 e.r.



<u>(*S*)-2-(1-(p-tolyl)ethyl)pyridine (4a)</u>

Title compound was obtained following General Procedure F from **3a**. Yellow oil (83% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 86% (Run 1), 82% (Run 2), 82% (Run 3)

R_f 0.56 (1 : 4, EtOAc : hexanes)

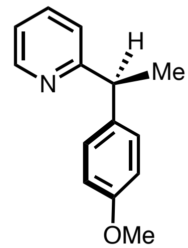
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.55 (ddd, *J* = 5.0, 1.0, 0.5 Hz, 1H), 7.55 (td, *J* = 7.5, 2.0 Hz, 1H), 7.20 – 7.18 (m, 2H), 7.12 – 7.07 (m, 4H), 4.26 (q, *J* = 7.0 Hz, 1H), 2.31 (s, 3H), 1.69 (d, *J* = 7.0 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 165.42, 149.24, 142.25, 136.52, 135.96, 129.30, 127.68, 122.18, 121.27, 47.14, 21.15, 20.93.

IR (neat, cm^{-1}): 1587, 1568, 1512, 1471, 1456, 1432, 1241, 1047, 1033, 913, 824, 788, 747, 720, 678, 549.

HRMS calculated for $C_{14}H_{15}NO$ $[M + H]^+$: 197.1204 Found: 197.1199

HPLC IA column, 98 : 2 (hexane : iPrOH), 1.0 mL-min, t_R 6.71 min (major), 6.21 min (minor), 90 : 10 e.r.



<u>(*S*)-2-(1-(4-methoxyphenyl)ethyl)pyridine (4b)</u>

Title compound was obtained following General Procedure F from **3b**. Yellow oil (76% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 78% (Run 1), 77% (Run 2), 72% (Run 3)

R$_f$ 0.41 (1 : 4, EtOAc : hexanes)

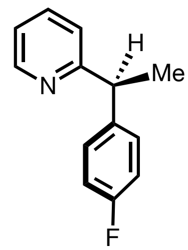
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.55 (ddd, *J* = 5.0, 1.5, 1.0 Hz, 1H), 7.55 (td, *J* = 8.0, 1.5 Hz, 1H), 7.23 – 7.20 (m, 2H), 7.10 (d, *J* = 8.0 Hz, 1H), 7.08 (ddd, *J* = 8.0, 5.0, 1.0 Hz, 1H), 6.85 – 6.82 (m, 2H), 4.24 (q, *J* = 7.0 Hz, 1H), 3.78 (s, 3H), 1.68 (d, *J* = 7.0 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 165.56, 158.17, 149.26, 137.40, 136.53, 128.73, 122.12, 121.26, 113.98, 53.38, 46.71, 21.05.

IR (neat, cm^{-1}): 1587, 1569, 1558, 1511, 1470, 1432, 1245, 1178, 1032, 913, 835, 788, 747, 720, 677, 617, 558.

HRMS calculated for $C_{14}H_{15}NO$ $[M + H]^+$: 213.1153 Found: 213.1147

HPLC IA column, 99.5 : 0.5 (hexane : iPrOH), 1.0 mL-min, t_R 8.05min (major), 7.42 min (minor), 85 : 15 e.r.



<u>(*S*)-2-(1-(4-fluorophenyl)ethyl)pyridine (4c)</u>

Title compound was obtained following General Procedure F from **3c**. Yellow oil (57% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 56% (Run 1), 53% (Run 2), 62% (Run 3)

$\mathbf{R_f}$ 0.53 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.56 (dd, *J* = 5.5, 2.0 Hz, 1H), 7.58 (td, *J* = 7.5, 2.0 Hz, 1H), 7.26 – 7.24 (m, 2H), 7.12 – 7.09 (m, 2H), 6.99 – 6.96 (m, 2H), 4.27 (q, *J* = 8.0 Hz, 1H), 1.68 (d, *J* = 8.0 Hz, 3H).

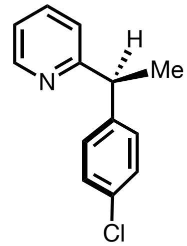
^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 164.91, 161.53 (d, *J* = 243 Hz), 149.38, 140.90, 136.65, 129.21 (d, *J* = 7.8 Hz), 122.13, 121.47, 115.22 (d, *J* = 21 Hz), 46.77, 21.08.

^{19}F NMR (282 MHz, $CDCl_3$) **δ** -117.08.

IR (neat, cm^{-1}): 1509, 1240, 1052, 1033, 913, 744, 720, 677, 617, 517, 502.

HRMS calculated for $C_{13}H_{12}FN$ [M + H]$^+$: 201.0953 Found: 201.0953

HPLC OJ-H column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 17.05 min (major), 13.64 min (minor), 90 : 10 e.r.



<u>(*S*)-2-(1-(4-chlorophenyl)ethyl)pyridine (4d)</u>

Title compound was obtained following General Procedure F from **3d**. Yellow oil (61% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 54% (Run 1), 69% (Run 2), 60% (Run 3)

R$_f$ 0.53 (1 : 4, EtOAc : hexanes)

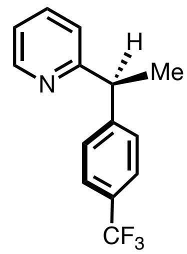
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.56 (dd, *J* = 5.5, 2.0 Hz, 1H), 7.58 (td, *J* = 7.5, 2.0 Hz, 1H), 7.27 – 7.22 (m, 4H), 7.12 – 7.10 (m, 2H), 4.26 (q, *J* = 7.0 Hz, 1H), 1.68 (d, *J* = 7.0 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 164.57, 149.43, 143.72, 136.67, 132.19, 129.18, 128.70, 122.16, 121.55, 46.91, 20.89.

IR (neat, cm^{-1}): 1588, 1492, 1471, 1432, 1245, 1091, 1051, 1014, 913, 836, 747, 720, 676, 617, 548, 501, 472, 457.

HRMS calculated for $C_{13}H_{12}ClN$ $[M + H]^{+}$: 217.0658 Found: 217.0658

HPLC OJ-H column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 13.20 min (major), 11.23 min (minor), 80 : 20 e.r.



<u>(*S*)-2-(1-(4-(trifluoromethyl)phenyl)ethyl)pyridine (4e)</u>

Title compound was obtained following General Procedure F from **3e**. Yellow oil (26% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 24% (Run 1), 31% (Run 2), 24% (Run 3)

R$_f$ 0.56 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.58 – 8.57 (m, 1H), 7.60 (td, *J* = 7.5, 1.5 Hz, 1H), 7.55 – 7.33 (m, 2H), 7.42 – 7.40 (m, 2H), 7.14 – 7.12 (m, 2H), 4.35 (q, *J* = 7.5 Hz, 1H), 1.72 (d, *J* = 7.5 Hz, 3H).

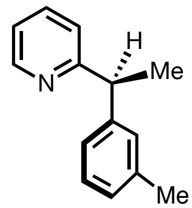
^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 164.00, 149.44, 149.23, 136.84, 128.74 (q, *J* = 32 Hz), 128.17, 126.31, 125.55 (q, *J* = 3.8 Hz), 122.29, 121.74, 47.32, 20.79.

^{19}F NMR (376 MHz, $CDCl_3$) **δ** -62.43.

IR (neat, cm^{-1}): 1325, 1164, 1118, 1069, 1033, 1016, 913, 744, 720, 676, 617, 502, 472, 458, 443.

HRMS calculated for $C_{14}H_{12}F_3N$ [M + H]$^+$: 251.0921 Found: 251.0923

HPLC IA column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 8.33 min (major), 7.13 min (minor), 72 : 28 e.r.



(*S*)-2-(1-(*m*-tolyl)ethyl)pyridine (4f)

Title compound was obtained following General Procedure F from **3f**. Yellow oil (73% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 86% (Run 1), 68% (Run 2), 65% (Run 3)

R$_f$ 0.59 (1 : 4, EtOAc : hexanes)

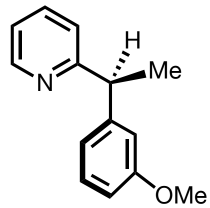
^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 8.56 (d, *J* = 6.0 Hz, 1H), 7.56 (td, *J* = 7.7, 1.9 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.11 (td, *J* = 12.3, 10.9, 5.6 Hz, 4H), 7.01 (d, *J* = 7.5 Hz, 1H), 4.26 (q, *J* = 7.2 Hz, 1H), 2.32 (s, 3H), 1.69 (d, *J* = 7.2 Hz, 3H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 165.13, 149.07, 145.00, 138.03, 136.45, 128.49, 128.37, 127.10, 124.67, 122.13, 121.20, 47.32, 21.51, 20.74.

IR (neat, cm^{-1}): 1588, 1567, 1470, 1456, 1431, 913, 775, 747, 720, 702, 672.

HRMS (ESI-TOF) calculated for $C_{14}H_{15}N$ $[M+H]^+$: 197.1204 Found: 197.1198

HPLC OJ-H column, 98 : 2 (hexane : iPrOH), 1.0 mL-min, t_R 15.17 min (major), 14.24 min (minor), 80:20 e.r.



<u>(*S*)-2-(1-(3-methoxyphenyl)ethyl)pyridine (4g)</u>

Title compound was obtained following General Procedure F from **3g**. Yellow oil (51% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 48% (Run 1), 52% (Run 2), 51% (Run 3)

$\mathbf{R_f}$ 0.56 (1 : 4, EtOAc : hexanes)

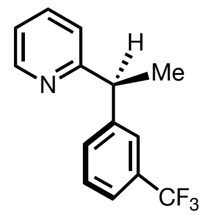
^{1}**H-NMR** (500 MHz, Chloroform-*d*) **δ** 8.59 – 8.53 (m, 1H), 7.56 (td, *J* = 7.7, 1.9 Hz, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 7.17 – 7.06 (m, 2H), 6.92 – 6.83 (m, 2H), 6.74 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 4.28 (q, *J* = 7.2 Hz, 1H), 3.77 (s, 3H), 1.70 (d, *J* = 7.2 Hz, 3H).

^{13}**C-NMR** (126 MHz, $CDCl_3$) **δ** 164.84, 159.66, 149.02, 146.69, 136.52, 129.43, 122.15, 121.28, 120.11, 113.69, 111.46, 55.16, 47.35, 20.69.

IR (neat, cm^{-1}): 1585, 1567, 1485, 1471, 1456, 1430, 1249, 1149, 1037, 993, 775, 747, 719, 697, 582.

HRMS (ESI-TOF) calculated for $C_{14}H_{15}NO$ $[M+H]^{+}$: 213.1153 Found: 213.1152

HPLC ID column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 13.07 min (major), 16.10 min (minor), 84 : 16 e.r.



<u>(*S*)-2-(1-(3-(trifluoromethyl)phenyl)ethyl)pyridine (4h)</u>

Title compound was obtained following General Procedure F from **3h**. Yellow oil (62% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 62% (Run 1), 64% (Run 2), 61% (Run 3)

R$_f$ 0.54 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 8.60 – 8.55 (m, 1H), 7.64 – 7.54 (m, 2H), 7.47 (dd, *J* = 16.4, 7.7 Hz, 2H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.13 (dd, *J* = 7.6, 3.0 Hz, 2H), 4.35 (q, *J* = 7.2 Hz, 1H), 1.72 (d, *J* = 7.1 Hz, 3H).

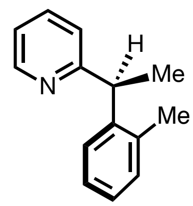
^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 164.01, 149.39, 146.07, 136.89, 130.83 (q, *J* = 32.0 Hz), 130.45, 129.02, 124.52 (q, *J* = 3.8 Hz), 123.39 (q, *J* = 3.8 Hz), 122.25, 121.74, 47.27, 20.89.

^{19}F NMR (282 MHz, $CDCl_3$) **δ** -62.50.

IR (neat, cm^{-1}): 1589,1472, 1432, 1326, 1162, 1121, 1074, 810, 780, 747, 701, 673, 657.

HRMS (ESI-TOF) calculated for $C_{14}H_{13}F_3N$ $[M+H]^+$: 251.0921 Found 251.0918

HPLC OJ-H column, 99.5 : 0.5 (hexane : iPrOH), 1.0 mL-min, t_R 6.73 min (major), 8.00 min (minor), 83 : 17 e.r.



<u>(*S*)-2-(1-(*o*-tolyl)ethyl)pyridine (4i)</u>

Title compound was obtained following General Procedure F from **3i**. Yellow oil (49% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 45% (Run 1), 51% (Run 2), 50% (Run 3)

R_f 0.56 (1 : 4, EtOAc : hexanes)

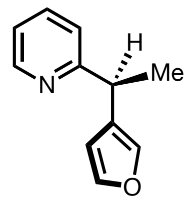
^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 8.56 (ddd, *J* = 4.9, 1.9, 0.9 Hz, 1H), 7.53 (td, *J* = 7.7, 1.9 Hz, 1H), 7.32 (d, *J* = 7.5 Hz, 1H), 7.21 (dt, *J* = 7.9, 4.3 Hz, 1H), 7.21 – 7.10 (m, 2H), 7.08 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 6.98 (dd, *J* = 7.9, 1.1 Hz, 1H), 4.49 (q, *J* = 7.2 Hz, 1H), 2.25 (s, 3H), 1.67 (d, *J* = 7.2 Hz, 3H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 165.39, 149.19, 142.97, 136.55, 136.39, 130.55, 126.93, 126.45, 126.29, 122.00, 121.16, 43.77, 20.86, 19.97.

IR (neat, cm^{-1}): 1586, 1567, 1471, 1457, 1430, 1419, 1048, 1033, 992, 786, 747, 675, 614, 596, 558.

HRMS (ESI-TOF) calculated for $C_{14}H_{15}N$ $[M+H]^+$: 197.1204 Found: 197.1208

HPLC OD-H column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 7.69 min (major), 6.88 min (minor), 56 : 44 e.r.



<u>(*S*)-2-(1-(furan-3-yl)ethyl)pyridine (4j)</u>

Title compound was obtained following General Procedure F from **3j**. Yellow oil (82% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 85% (Run 1), 83% (Run 2), 77% (Run 3)

R$_f$ 0.51 (1 : 4, EtOAc : hexanes)

Yield: 85% (Run 1), 83% (Run 2), 77% (Run 3)

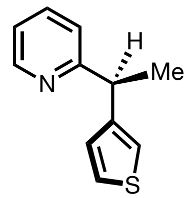
^{1}**H-NMR** (500 MHz, Chloroform-*d*) **δ** 8.58 – 8.53 (m, 1H), 7.61 (td, *J* = 7.7, 1.8 Hz, 1H), 7.35 (t, *J* = 1.7 Hz, 1H), 7.29 (q, *J* = 1.2 Hz, 1H), 7.19 – 7.10 (m, 2H), 6.27 (dd, *J* = 1.8, 0.9 Hz, 1H), 4.15 (p, *J* = 7.3 Hz, 1H), 1.62 (d, *J* = 7.2 Hz, 3H).

^{13}**C-NMR** (126 MHz, $CDCl_3$) **δ** 164.91, 149.29, 143.11, 139.01, 136.73, 129.01, 121.63, 121.55, 110.47, 38.96, 20.79.

IR (neat, cm^{-1}): 1589, 1568, 1457, 1433, 1158, 1054, 1021, 873, 778, 748, 729, 679, 663, 599.

HRMS (ESI-TOF) calculated for $C_{11}H_{11}NO$ $[M+H]^+$: 173.0840 Found 173.0840

HPLC OJ-H column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 15.77 min (major), 18.81 min (minor), 86: 14 e.r.



<u>(*S*)-2-(1-(thiophen-3-yl)ethyl)pyridine (4k)</u>

Title compound was obtained following General Procedure F from **3k**. Yellow oil (87% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 74% (Run 1), 82% (Run 2), 78% (Run 3)

R$_f$ 0.52 (1 : 4, EtOAc : hexanes)

Yield: 74% (Run 1), 82% (Run 2), 78% (Run 3)

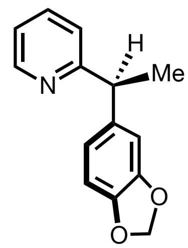
^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 8.58 (d, *J* = 4.8 Hz, 1H), 7.61 (td, *J* = 7.7, 1.8 Hz, 1H), 7.26 (dd, *J* = 5.0, 3.0 Hz, 1H), 7.17 – 7.07 (m, 3H), 6.99 (dd, *J* = 4.9, 1.3 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 1H), 1.72 (d, *J* = 7.2 Hz, 3H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 164.82, 148.96, 145.58, 136.77, 127.64, 125.56, 121.83, 121.42, 120.30, 43.14, 20.93.

IR (neat, cm^{-1}): 1590, 1568, 1472, 1457, 1432, 1419, 770, 748, 678, 658.

HRMS (ESI-TOF) calculated for $C_{11}H_{11}NS$ [M+H]$^{+}$: 189.0612 Found: 189.0612

HPLC OJ-H column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 21.80 min (major), 23.05 min (minor), 93 : 7 e.r.



<u>(*S*)-2-(1-(benzo[*d*][1,3]dioxol-5-yl)ethyl)pyridine (4l)</u>

Title compound was obtained following General Procedure F from **3l**. Yellow oil (87% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 92% (Run 1), 82% (Run 2), 87% (Run 3)

$\mathbf{R_f}$ 0.43(1 : 4, EtOAc : hexanes)

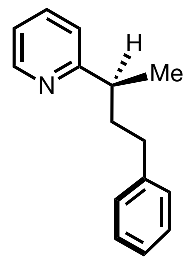
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.55 (ddd, *J* = 5.0, 2.0, 1.0 Hz, 1H), 7.57 (td, *J* = 7.5, 2.0 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 7.09 (ddd, *J* = 7.5, 5.0, 1.0 Hz, 1H), 6.78 – 6.75 (m, 2H), 6.74 (td, *J* = 7.5, 0.5 Hz, 1H), 5.91 and 5.90 (ABq, J_{AB} = 1.5 Hz, 2H), 4.21 (q, *J* = 7.5 Hz, 1H), 1.66 (d, *J* = 7.5 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 165.16, 149.31, 147.79, 146.06, 139.29, 136.57, 122.11, 121.37, 120.67, 108.35, 108.29, 100.99, 47.18, 21.09.

IR (neat, cm^{-1}): 1588, 1501, 1485, 1472, 1456, 1432, 1234, 1037, 935, 911, 814, 748, 669.

HRMS calculated for $C_{14}H_{13}NO_2$ [M + H]$^+$: 227.0946 Found: 227.0941

HPLC ID column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 18.09 min (major), 20.96 min (minor), 83 : 17 e.r.



(*S*)-2-(4-phenylbutan-2-yl)pyridine (4m)

Title compound was obtained following General Procedure F from **3m**. Yellow oil (73% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 73% (Run 1), 75% (Run 2), 69% (Run 3)

R$_f$ 0.54 (1 : 4, EtOAc : hexanes)

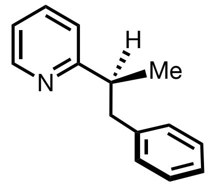
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.58 (dd, *J* = 5.0, 1.0 Hz, 1H), 7.61 (td, *J* = 7.5, 1.5 Hz, 1H), 7.28 – 7.25 (m, 2H), 7.18 – 7.10 (m, 5H), 2.93 (sextet, *J* = 7.0 Hz, 1H), 2.60 – 2.48 (m, 2H), 2.16 – 2.08 (m, 1H), 1.96 – 1.88 (m, 1H), 1.33 (d, *J* = 7.0 Hz, 2H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 166.24, 149.42, 142.56, 136.45, 128.50, 128.38, 125.77, 121.88, 121.28, 41.69, 38.85, 34.01, 21.08.

IR (neat, cm^{-1}): 1589, 1568, 1494, 1473, 1455, 1432, 1029, 990, 786, 746, 697, 624, 541, 517, 497.

HRMS calculated for $C_{15}H_{17}N$ [M + H]$^+$: 211.1361 Found: 211.1365

HPLC OJ-H column, 99.5 : 0.5 (hexane : iPrOH), 1.0 mL-min, t_R 20.17 min (major), 15.10 min (minor), 86 : 14 e.r.



<u>(*S*)-2-(1-phenylpropan-2-yl)pyridine (4n)</u>

Title compound was obtained following General Procedure F from **3n**. Yellow oil (57% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 57% (Run 1), 61% (Run 2), 68% (Run 3)

R$_f$ 0.51 (1 : 4, EtOAc : hexanes)

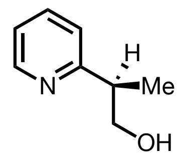
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.58 (ddd, *J* = 5.0, 2.0, 1.0 Hz, 1H), 7.54 (td, *J* = 7.5, 2.0 Hz, 1H), 7.23 – 7.20 (m, 2H), 7.16 – 7.13 (m, 1H), 7.11 – 7.08 (m, 3H), 7.03 (d, *J* = 7.5 Hz, 1H), 3.18 (sextet, *J* = 7.0 Hz, 1H), 3.11 and 2.84 (ABX, J_{AB} = 13.5 Hz, *J* = 7.0 Hz, 2H), 1.29 (d, *J* = 7.0 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 165.66, 149.37, 140.79, 136.37, 129.27, 128.24, 125.97, 122.10, 121.37, 43.98, 43.46, 20.14.

IR (neat, cm^{-1}): 1589, 1568, 1494, 1473, 1456, 1433, 1419, 990, 913, 786, 742, 698, 532.

HRMS calculated for $C_{14}H_{15}N$ $[M + H]^+$: 198.1277 Found: 197.1198

HPLC OJ-H column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 12.63 min (major), 8.81 min (minor), 96 : 4 e.r.



<u>(*R*)-2-(pyridin-2-yl)propan-1-ol (4o)</u>

Title compound was obtained following General Procedure F from **3o**. Yellow oil (72% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 72% (Run 1), 69% (Run 2), 70% (Run 3)

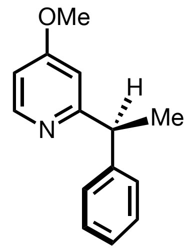
R_f 0.33 (100% EtOAc)

^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 8.46 – 8.38 (m, 1H), 7.58 (td, *J* = 7.7, 1.9 Hz, 1H), 7.18 – 7.04 (m, 2H), 4.37 – 4.01 (b, 1H), 3.87 (dd, *J* = 10.7, 3.8 Hz, 1H), 3.77 (dd, *J* = 10.8, 6.7 Hz, 1H), 3.01 (pd, *J* = 7.1, 3.8 Hz, 1H), 1.26 (d, *J* = 7.2 Hz, 3H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 163.94, 147.56, 135.80, 121.15, 120.50, 66.12, 40.77, 16.16.

HRMS calculated for $C_8H_{11}NO$ $[M + H]^+$: 137.0840 Found: 137.0843

HPLC AJ-H column, 97 : 3 (hexane : iPrOH), 1.0 mL-min, R_t 21.9 min (major), 18.3 min (minor), 96 : 4 e.r.



<u>(*S*)-4-methoxy-2-(1-phenylethyl)pyridine (4p)</u>

Title compound was obtained following General Procedure F from **3p**. Yellow oil (43% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 43% (Run 1), 43% (Run 2), 40% (Run 3)

R_f 0.14 (1 : 4, EtOAc : hexanes)

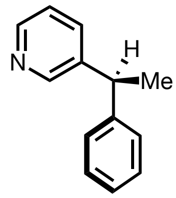
^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 8.42 – 8.36 (m, 1H), 7.32 – 7.25 (m, 4H), 7.19 (tt, *J* = 5.4, 3.2 Hz, 1H), 6.66 – 6.61 (m, 2H), 4.24 (q, *J* = 7.2 Hz, 1H), 3.78 (s, 3H), 1.69 (d, *J* = 7.3 Hz, 3H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 166.85, 166.20, 150.51, 145.06, 128.58, 127.77, 126.45, 108.37, 107.38, 55.12, 47.50, 20.83.

IR 1590, 1565, 1478, 1450, 1419, 1300, 1285, 1153, 1028, 815, 746, 698.

HRMS (ESI-TOF) calculated for $C_{14}H_{15}NO$ $[M+H]^+$: 213.1153 Found: 213.1156

HPLC IC column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 21.63 min (major), 24.28 min (minor), 93 : 7 e.r.



<u>(*S*)-3-(1-phenylethyl)pyridine (4q)</u>

Title compound was obtained following General Procedure F from **3q**. Yellow oil (95% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 95% (Run 1), 95% (Run 2), 92% (Run 3)

R$_f$ 0.51 (1 : 4, EtOAc : hexanes)

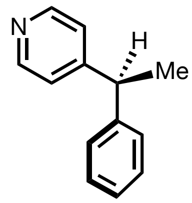
^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 8.53 (s, 1H), 8.44 (d, *J* = 4.8 Hz, 1H), 7.49 (dt, *J* = 8.0, 1.9 Hz, 1H), 7.30 (t, *J* = 7.6 Hz, 2H), 7.21 (dt, *J* = 8.6, 2.6 Hz, 4H), 4.18 (q, *J* = 7.3 Hz, 1H), 1.67 (d, *J* = 7.3 Hz, 3H).

^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 149.38, 147.58, 145.08, 141.74, 135.13, 128.71, 127.65, 126.58, 123.51, 42.55, 21.68.

IR (neat, cm^{-1}): 1573, 1496, 1477, 1450, 1420, 1375, 1022, 912, 813, 763, 743, 713, 698, 624, 581.

HRMS calculated for $C_{13}H_{13}N$ [M + H]$^{+}$: 183.1048 Found: 183.1049

HPLC IC column, 90 : 10 (hexane : iPrOH), 1.0 mL-min, t_R 11.91 min (major), 12.57 min (minor), 89 : 11 e.r.



<u>(*S*)-4-(1-phenylethyl)pyridine (4r)</u>

Title compound was obtained following General Procedure F from **3r**. Yellow oil (99% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 99% (Run 1), 99% (Run 2), 99% (Run 3)

R$_f$ 0.23 (1 : 4, EtOAc : hexanes)

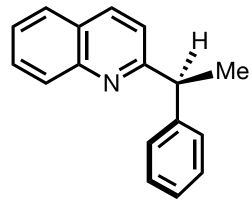
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.50 (brs, 2H), 7.33 – 7.30 (m, 2H), 7.24 – 7.21 (m, 1H), 7.20 – 7.19 (m, 2H), 7.14 – 7.13 (m, 2H), 4.12 (q, *J* = 7.0 Hz, 1H), 1.64 (d, *J* = 7.0 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 155.20, 149.91, 144.52, 128.76, 127.74, 126.77, 123.16, 44.36, 21.19.

IR (neat, cm^{-1}): 1595, 1462, 1058, 1033, 913, 744, 676, 617, 532, 471, 443.

HRMS calculated for $C_{13}H_{13}N$ [M + H]$^+$: 183.1048 Found: 183.1050

HPLC OJ-H column, 99 : 1 (hexane : iPrOH), 1.0 mL-min, t_R 32.44 min (major), 28.78 min (minor), 84 : 16 e.r.



<u>(*S*)-2-(1-phenylethyl)quinoline (4s)</u>

Title compound was obtained following General Procedure F from **3s**. Yellow oil (30% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yield 33% (Run 1), 29% (Run 2), 23% (Run 3)

R_f 0.69 (1 : 4, EtOAc : hexanes)

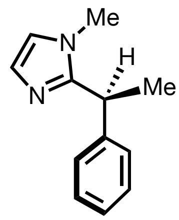
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 8.11 (d, *J* = 8.5 Hz, 1H), 8.00 (d, *J* = 8.0 Hz, 1), 7.75 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.70 (ddd, *J* = 8.5, 7.0, 1.5 Hz, 1H), 7.49 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 7.36 – 7.35 (m, 2H), 7.32 – 7.29 (m, 2H), 7.23 – 7.19 (m, 1H), 7.19 (d, *J* = 8.5 Hz, 1H), 4.51 (q, *J* = 7.0 Hz, 1H), 1.80 (d, *J* = 7.0 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 165.33, 147.78, 144.79, 136.44, 129.44, 129.35, 128.63, 127.96, 127.59, 126.99, 126.55, 126.05, 120.81, 48.22, 20.57.

IR (neat, cm^{-1}): 1598, 1501, 1450, 1425, 1027, 913, 832, 748, 720, 699, 675, 617, 551, 501, 476, 449.

HRMS calculated for $C_{17}H_{15}N$ $[M + H]^+$: 233.1204 Found: 233.1207

HPLC IA column, 99.5 : 0.5 (hexane : iPrOH), 1.0 mL-min, t_R 9.85 min (major), 7.36 min (minor), 92 : 8 e.r.



<u>**(*S*)-1-methyl-2-(1-phenylethyl)-1H-imidazole (4t)**</u>

Title compound was obtained following General Procedure F from **3t**. Yellow oil (56% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 55% (Run 1), 55% (Run 2), 58% (Run 3)

R_f 0.28 (1 : 4, EtOAc : hexanes)

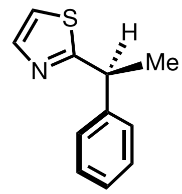
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 7.28 – 7.25 (m, 2H), 7.20 – 7.17 (m, 1H), 7.13 – 7.12 (m, 2H), 7.00 (d, *J* = 1.0 Hz, 1H), 6.77 (d, *J* = 1.0 Hz, 1H), 4.10 (q, *J* = 7.0 Hz, 1H), 1.72 (d, *J* = 7.0 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 150.18, 143.83, 128.84, 127.23, 126.94, 126.65, 121.09, 38.28, 32.71, 21.82.

IR (neat, cm^{-1}): 1492, 1451, 1280, 1055, 1032, 913, 743, 701, 540.

HRMS calculated for $C_{12}H_{14}N_2$ $[M + H]^+$: 186.1157 Found: 186.1154

HPLC IC column, 90 : 10 (hexane : iPrOH), 1.0 mL-min, t_R 16.01 min (major), 18.15 min (minor), 92 : 8 e.r.



<u>**(*S*)-2-(1-phenylethyl)thiazole (4u)**</u>

Title compound was obtained following General Procedure F from **3u**. Yellow oil (76% yield by ^{1}H-NMR analysis against internal standard, averaged over 3 runs).

Yields 76% (Run 1), 77% (Run 2), 74% (Run 3)

$\mathbf{R_f}$ 0.58 (1 : 4, EtOAc : hexanes)

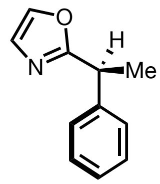
^{1}H-NMR (500 MHz, $CDCl_3$) **δ** 7.71 (d, *J* = 3.5 Hz, 1H), 7.36 – 7.32 (m, 4H), 7.27 – 7.25 (m, 1H), 7.19 (d, *J* = 3.5 Hz, 1H), 4.51 (q, *J* = 7.0 Hz, 1H), 1.80 (d, *J* = 7.0 Hz, 3H).

^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 175.76, 143.86, 142.32, 128.74, 127.54, 127.11, 118.52, 43.96, 21.77.

IR (neat, cm^{-1}): 1588, 1493, 1472, 1450, 1431, 1027, 912, 803, 747, 698, 582, 546, 502, 457, 441.

HRMS calculated for $C_{11}H_{11}NS$ $[M + H]^{+}$: 189.0612 Found: 189.0612

HPLC ID column, 99.5 : 0.5 (hexane : iPrOH), 1.0 mL-min, t_R 20.24 min (major), 23.41 min (minor), 88 : 12 e.r.



2-(1-phenylethyl)oxazole (4v)

Title compound was obtained following General Procedure F from **3v**. Yellow oil (40% yield by ^{1}H-NMR analysis against internal standard).

Yields 36% (Run 1), 41% (Run 2), 40% (Run 3)

R$_f$ 0.53 (1 : 4, EtOAc : hexanes)

^{1}H-NMR (500 MHz, Chloroform-*d*) **δ** 7.55 (s, 1H), 7.55 (s, 0H), 7.36 – 7.29 (m, 2H), 7.33 – 7.21 (m, 3H), 7.05 (d, *J* = 0.8 Hz, 1H), 4.28 (q, *J* = 7.2 Hz, 1H), 1.72 (d, *J* = 7.2 Hz, 3H).

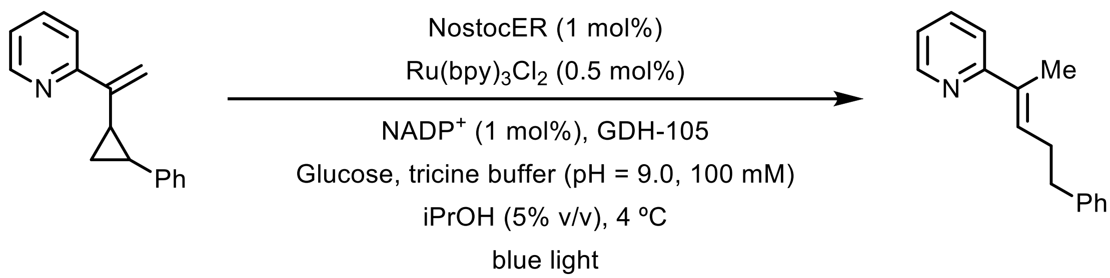
^{13}C-NMR (126 MHz, $CDCl_3$) **δ** 166.91, 141.99, 138.67, 128.84, 127.42, 127.19, 127.01, 39.73, 20.13.

IR (neat, cm^{-1}): 1647, 1566, 1492, 1451, 1376, 1136, 1086, 1049, 1027, 913, 744, 720, 697, 676, 617, 527, 492, 478, 472, 450.d

HRMS calculated for $C_{11}H_{11}NO$ [M + H]$^+$: 173.0840 Found: 173.0839

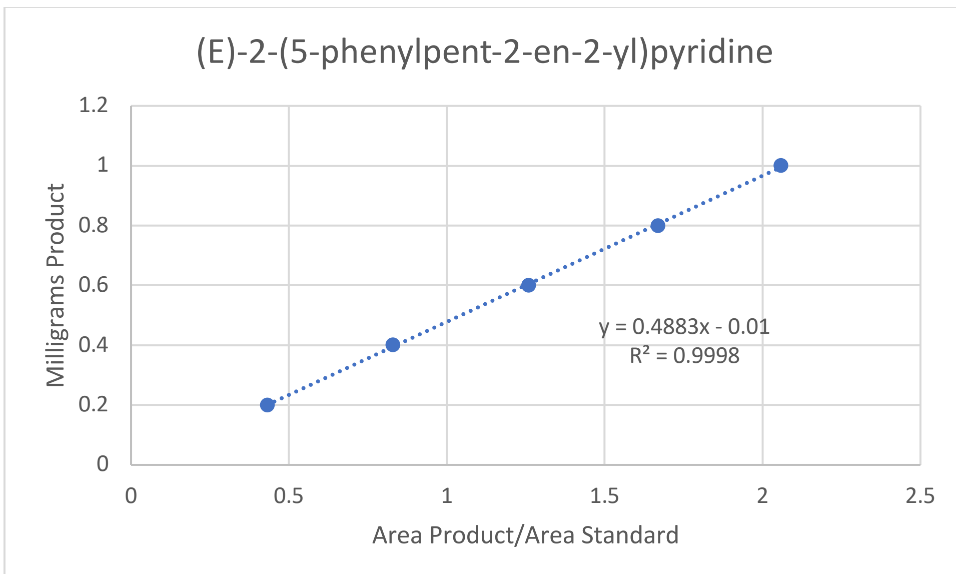
HPLC OJ-H column, 95 : 5 (hexane : iPrOH), 1.0 mL-min, t_R 15.28 min (major), 12.97 min (minor), 70 : 30 e.r.

Radical Clock Experiments:



Yield: 12%

Standard Curve:



Crystal Structure Determination

Structure determination and refinement

The gene for NostocER1 was expressed from pET22b in BL21(DE3) *E. coli* and purified as described above. The purified protein was brought to a final concentration of 1.00 mM, approximately 40 mg/mL in 20 mM KPi pH 7.4, 300 mM NaCl. Crystals used for data collection were grown using the sitting drop method (1:1 ratio of protein to crystallization solution with a 3 μL total drop size), 500 μL of buffer per well. The crystallization solution contained 0.1M Tricine HCl pH 9.0, 0.1 -0.2M $CaCl_2$, 28-30% (w/v) PEG3350. Up to 25 mM of ligand **30** was included in the crystallization medium, but no ordered ligand was evident in the active site of the protein. Crystals typically appeared after 3-5 days at 20^{o}C. Crystals were prepared for flash cryocooling by rapid equilibration in the crystallization solution supplemented with 20% (v/v) ethane-1,2-diol. Diffraction data for NostocER1 was obtained at NSLS-II beam line 17-ID-1 (AMX) at a wavelength of 0.9790 Å from crystals flash-cooled to 100 K. Data extended to a maximum resolution of 2.0 Å. All data was integrated with the program XDS (1) and scaled with the program AIMLESS (2). Crystals grew in space group *P1* with eight molecules in the asymmetric unit. The structure for NostocER1 was determined by the method of molecular replacement using the PDB entry 3GKA (3) as the model and the program PHASER (4). Electron density was clearly visible for residues 38-398. COOT (5) was used for model building and structure refinement was carried out with PHENIX.REFINE (6). Data collection, processing and refinement statistics are summarized in **Table S5**. The final model was deposited in the Protein Data Bank as entry 6UFF.

Ref 1: W. Kabsch, XDS. Acta Crystallogr. D Biol. Crystallogr. 66, 125–132 (2010). doi:10.1107/S0907444909047337 Medline

Ref2: P. R. Evans, G. N. Murshudov, How good are my data and what is the resolution? Acta Crystallogr. D Biol. Crystallogr. 69, 1204–1214 (2013). doi:10.1107/S0907444913000061 Medline

Ref 3: PDB ID: 3GKX, Crystal structure of N-ethylmaleimidine reductase from Burkholderia pseudomallei, Seattle Structural Genomics Center for Infectious Disease (SSGCID)

Ref4: A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni, R. J. Read, Phaser crystallographic software. J. Appl. Crystallogr. 40, 658–674 (2007). doi:10.1107/S0021889807021206 Medline

Ref 5: Paul Emsley and Bernhard Lohkamp and William G. Scott and Kevin Cowtan, Features and Development of Coot, Acta Crystallogr.D Biol. Crystallogr,2010,66,486-501.

Ref 6: Towards automated crystallographic structure refinement with phenix.refine. P.V. Afonine, R.W. Grosse-Kunstleve, N. Echols, J.J. Headd, N.W. Moriarty, M. Mustyakimov, T.C. Terwilliger, A. Urzhumtsev, P.H. Zwart, and P.D. Adams. Acta Crystallogr D Biol Crystallogr 68, 352-67 (2012).

Table S5. Data collection, processing and refinement.

Data Collection	
Space group	*P1*
Cell Dimensions	
a,b,c (Å)	81.56, 95.59, 99.90
α, β, γ (°)	66.79 , 89.91 , 82.58
Resolution (Å)	90.0 – 2.01 (2.13 – 2.01)
R_{sym}	0.102 (0.668)
R_{meas}	0.120 (0.796)
I/σI	8.7 (1.6)
Completeness (%)	97.3 (94.5)
Redundancy	3.6 (3.4)
Refinement	
Resolution (Å)	29.5 – 2.01
No. reflections	178926
R_{work}/R_{free}	0.173/0.222
No. atoms	
Protein	22186
Ligand	248
Water	1656
B-factors	
Overall ($Å^2$)	29.8
Protein ($Å^2$)	29.5
Ligand ($Å^2$)	21.4
Wilson B-factor ($Å^2$)	28.7
R.m.s. deviations	
Bond lengths (Å)	0.007

Bond Angles (°)	0.92
Ramachandran Plot	
Favored (%)	96.5
Outliers (%)	0.1

Density functional theory (DFT) calculations

All DFT computations were carried out using the PySCF quantum chemistry software package[1]. We performed spin-polarized gas-phase ground state geometry optimizations for radical anionic states of 2-, 3-, and 4-(1-phenylvinyl)pyridine using the B3LYP exchange-correlation functional[2] in the 6-31+G(d) basis set[3]. We then performed population analyses on optimized geometries using the intrinsic atomic orbital (IAO) approach[4] in PySCF. The IAO analysis has been shown to be a robust method for computing partial charges from DFT wave functions. The anionic partial charge results are shown in Fig. S1. As can be seen, the IAO analysis shows that approximately 50% of the anionic charge resides on the C2 position of the alkene in all cases. In contrast, almost no anionic charge appears on the C1 position of the alkene.

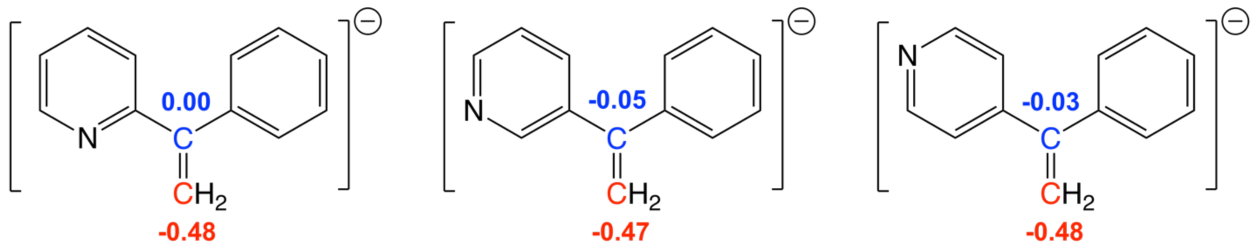
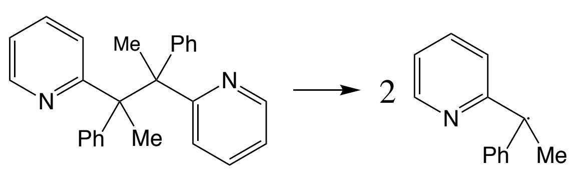
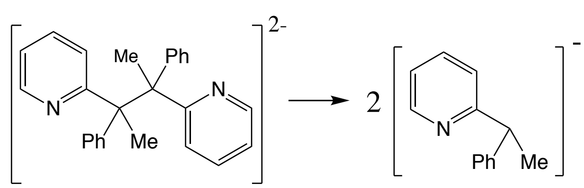


Fig. S1. Anionic partial charges on radical anionic states of 2-, 3-, and 4-(1-phenylvinyl)pyridine from DFT calculations and IAO population analysis.

We further performed DFT calculations to determine whether the radical anion or radical states of 2-(1-phenylvinyl)pyridine are persistent in water. The B3LYP exchange-correlation functional and COSMO solvent model[5] were used with the 6-31+G(d) basis set. The free energies of two C-C bond breaking reactions in Fig. S2 were computed. The free energy of Reaction 1 was computed to be 17.7 kcal/mol, while the free energy of Reaction 2 was -93.0 kcal/mol, indicating the C-C bond is highly unstable in the dianion.



Reaction 1



Reaction 2

Fig. S2. C-C bond breaking reactions to form radical (Reaction 1) and radical anion (Reaction 2) states of 2-(1-phenylvinyl)pyridine.

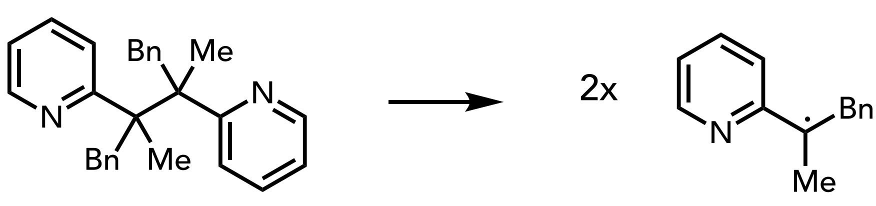


Figure S3. C–C bond breaking reaction to form radical (Reaction 3).

The optimized geometries of all compounds in the radical anionic state are shown below:

Cartesian coordinates of anionic 2-(1-phenylvinyl)pyridine:

H	0.9887100362	-0.1247365419	2.3062857295
C	0.5126291670	-0.0329133381	1.3349718524
C	-0.8045659944	0.3835711375	1.2607353334
H	-1.3618023439	0.5911144166	2.1740148271
C	-1.4067454510	0.5565749804	-0.0101789637
H	-2.4379711585	0.8820906831	-0.1263962184
C	-0.5919079923	0.3199080657	-1.1210285986
H	-1.0001537524	0.4725393445	-2.1251390511
N	0.6767694954	-0.0852234520	-1.0821850267
C	1.2664041601	-0.3050793116	0.1524544541
C	2.6673271729	-0.6918591649	0.1649151414
C	3.4412488899	-0.5825738793	-0.9928346399
H	3.0088628415	-0.1945116890	-1.9070240311
H	4.4568131140	-0.9677718629	-1.0270067529
C	3.3060990387	-1.1798185832	1.4025523086
C	2.6619542944	-2.0602797138	2.3108251244
C	3.3095113761	-2.5648543389	3.4369531486
C	4.6435696384	-2.2254121670	3.7137932288
C	5.3080632722	-1.3697423034	2.8237940284
C	4.6588507191	-0.8637992450	1.6984259581
H	5.1861431338	-0.1854180485	1.0326165610
H	5.1505771016	-2.6230853711	4.5908885722
H	6.3428022437	-1.0853480971	3.0155342226
H	1.6425161116	-2.3732929697	2.1026459507
H	2.7738848859	-3.2449585499	4.0989968407

Cartesian coordinates of anionic 3-(1-phenylvinyl)pyridine:

C	-4.5710803650	0.8800147004	1.3211415254
C	-5.6872903239	0.1340471972	0.9085569976
N	-5.5611858254	-0.9747028020	0.1505387547
C	-4.3340410517	-1.3546059395	-0.2069281750
C	-3.1169811902	-0.6833269768	0.1338261275
C	-3.2981276905	0.4750943349	0.9418524167
H	-2.4294814729	1.0300396503	1.2858044239
H	-4.7055232094	1.7619662111	1.9469455209
H	-6.6999230852	0.4232484356	1.1877175137
H	-4.2811394563	-2.2414497226	-0.8352827526
C	-1.8155113670	-1.2267762557	-0.2435870192
C	-0.6582541973	-0.3384611512	-0.3997810487
C	-1.6650242561	-2.6057105600	-0.4493168252
H	-2.4524355729	-3.3100168865	-0.1988597611
H	-0.7641085102	-3.0202720018	-0.8921923230
C	-0.7715903510	1.0177201246	-0.8178326336
C	0.3416843105	1.8234697684	-1.0340059295
C	1.6450551557	1.3234562700	-0.8523512646
C	1.7866208165	-0.0130292242	-0.4521080726
C	0.6731387543	-0.8220854913	-0.2341451241
H	0.8145123259	-1.8447524676	0.1047971408
H	-1.7601699125	1.4282119547	-1.0049221363
H	0.1965793204	2.8525750638	-1.3626020659
H	2.5156840805	1.9530176607	-1.0248947493
H	2.7826630738	-0.4286518924	-0.2965205404

Cartesian coordinates of anionic 4-(1-phenylvinyl)pyridine:

C	-3.3797616793	2.9235648377	-0.7095408744
N	-4.6423427904	2.6141801891	-0.3191657083
C	-4.7662318359	1.3759659802	0.2075073515

C	-3.7364127687	0.4598697101	0.3628650056
C	-2.3974434074	0.7824574250	-0.0309812244
C	-2.2783767913	2.0905707328	-0.5946537796
H	-3.2559744930	3.9128852137	-1.1542586789
H	-5.7725202215	1.1052619851	0.5355168328
H	-3.9493924238	-0.4992198383	0.8264706866
H	-1.3211760293	2.4379879401	-0.9732055812
C	-1.3146185888	-0.1799906496	0.0501814563
C	-1.5776585842	-1.5539997292	0.1377943029
H	-2.5784790261	-1.9560410761	0.0162341228
H	-0.7869580273	-2.2690976488	0.3453544125
C	0.0895430563	0.2703088845	0.0320614534
C	0.5174536288	1.4733958769	0.6525147451
C	1.8581892477	1.8489468151	0.6900646399
C	2.8504777838	1.0377019553	0.1154727449
C	2.4561459103	-0.1612736303	-0.4932350380
C	1.1135615275	-0.5363573379	-0.5325093971
H	-0.2226202553	2.1052601382	1.1353869704
H	2.1369361049	2.7787070525	1.1852893855
H	3.8982221071	1.3300804991	0.1476213639
H	3.2036946590	-0.8075947541	-0.9532488523
H	0.8283128971	-1.4582705709	-1.0323663398

Cartesian coordinates of neutral reactant in Reaction 1:

C	-1.737709	-0.060621	0.315990
C	-0.398280	0.123325	-0.648118
C	0.467180	1.356566	-0.239617
C	-0.852595	0.306313	-2.118711
H	-1.627811	1.069578	-2.210998
H	-1.254133	-0.620672	-2.527358

H	-0.015376	0.613807	-2.752800
C	0.513096	-1.134285	-0.567955
C	-1.399039	0.194517	1.810354
H	-2.304079	0.068569	2.415633
H	-1.036267	1.209053	1.972899
H	-0.628795	-0.488097	2.166394
C	-2.810162	1.017118	-0.039580
C	-2.307396	-1.499654	0.147291
C	0.409876	2.564185	-0.961623
C	1.195960	3.668782	-0.616293
C	2.073709	3.601144	0.466588
C	2.151391	2.411237	1.195004
C	1.364168	1.311250	0.845767
C	1.088738	-1.709381	-1.716174
C	1.930427	-2.815045	-1.591627
C	2.188343	-3.336784	-0.325085
C	1.584652	-2.704206	0.761554
N	0.781836	-1.641273	0.648241
C	-2.607888	2.376125	0.280060
C	-3.601139	3.311450	-0.003779
C	-4.792818	2.884679	-0.592361
C	-4.915474	1.524306	-0.870562
N	-3.959704	0.624237	-0.607873
C	-2.895759	-1.953377	-1.050725
C	-3.375083	-3.257427	-1.192361
C	-3.300481	-4.162221	-0.130798
C	-2.739688	-3.733542	1.071688
C	-2.253607	-2.428028	1.202308
H	1.452653	0.399954	1.424497
H	2.689491	4.456380	0.734672

H 2.831981 2.331007 2.039826

H -0.260609 2.667711 -1.806588

H 1.118147 4.581650 -1.202887

H 0.893074 -1.311227 -2.703461

H 2.374835 -3.261048 -2.478426

H 2.832417 -4.199619 -0.179874

H 1.751434 -3.066043 1.775925

H -1.816147 -2.152213 2.152918

H -3.028605 -1.276192 -1.881288

H -3.824674 -3.557701 -2.136583

H -3.678472 -5.176451 -0.237852

H -2.671919 -4.412852 1.918867

H -5.598287 3.576605 -0.823382

H -5.823875 1.129230 -1.324839

H -1.688507 2.710210 0.742467

H -3.444806 4.359725 0.240848

Cartesian coordinates of radical 2-(1-phenylvinyl)pyridine in Reaction 1:

C -3.157552 -0.263758 0.972171

C -2.157406 -0.138454 2.098256

H -2.526979 -0.640915 3.004493

H -1.972568 0.899017 2.380889

H -1.192995 -0.604157 1.851386

C -3.784068 0.950207 0.457713

C -3.461060 -1.594198 0.475655

C -3.132281 2.211262 0.525603

C -3.773471 3.351145 0.053743

C -5.061522 3.239091 -0.477649

C -5.631758 1.961374 -0.522547

N -5.030631 0.856823 -0.082272

C -4.108512 -1.829728 -0.769871

C -4.330330 -3.120194 -1.239655

C -3.925180 -4.235470 -0.494496

C -3.279746 -4.034456 0.730931

C -3.045129 -2.745229 1.202327

H -2.544782 -2.630644 2.157911

H -4.433384 -0.986795 -1.364650

H -4.822557 -3.258645 -2.199721

H -4.105445 -5.241823 -0.864394

H -2.956689 -4.887249 1.323835

H -5.604667 4.103089 -0.849874

H -6.632105 1.821646 -0.932503

H -2.122115 2.290392 0.912612

H -3.270978 4.314873 0.094394

Cartesian coordinates of dianion reactant in Reaction 2:

C -2.343681 -0.160476 0.481645

C -1.007734 -0.081985 -0.486651

C -0.285918 1.292683 -0.263494

C -1.431127 -0.170319 -1.974546

H -2.159176 0.591309 -2.241142

H -1.907833 -1.126235 -2.199967

H -0.551671 -0.061829 -2.621458

C -0.047394 -1.291840 -0.204204

C -1.917622 -0.023234 1.965469

H -2.796014 -0.108327 2.617007

H -1.439124 0.939005 2.157214

H -1.187612 -0.776015 2.252035

C -3.325996 1.024060 0.170853

C -3.041995 -1.552088 0.291247

C	-0.570408	2.393120	-1.098883
C	0.075391	3.624009	-0.952700
C	1.057078	3.797137	0.027195
C	1.339723	2.731237	0.882798
C	0.666671	1.511336	0.756183
C	0.213298	-2.278274	-1.172277
C	1.097158	-3.328570	-0.898372
C	1.782028	-3.333493	0.322617
C	1.464667	-2.334074	1.234028
N	0.556508	-1.370259	1.008762
C	-3.549130	2.067927	1.086383
C	-4.458189	3.091768	0.795553
C	-5.206248	3.012357	-0.388388
C	-4.915789	1.966016	-1.254339
N	-3.983390	1.027570	-1.020086
C	-3.932302	-1.834700	-0.769690
C	-4.589496	-3.064961	-0.869900
C	-4.354366	-4.081267	0.058067
C	-3.434795	-3.846063	1.084223
C	-2.803601	-2.604712	1.200582
H	0.900524	0.706626	1.442228
H	1.572841	4.751796	0.135872
H	2.084152	2.844301	1.672604
H	-1.322022	2.313986	-1.877133
H	-0.196200	4.449289	-1.612849
H	-0.276670	-2.267169	-2.138628
H	1.258946	-4.113462	-1.638949
H	2.512673	-4.103812	0.572507
H	1.944322	-2.309321	2.216452
H	-2.099286	-2.479481	2.015754

H -4.138391 -1.067483 -1.504896

H -5.284593 -3.226097 -1.695521

H -4.858204 -5.044417 -0.028914

H -3.200565 -4.631252 1.805022

H -5.959392 3.757125 -0.647442

H -5.440260 1.879436 -2.210241

H -3.009949 2.121277 2.024760

H -4.591628 3.919155 1.493600

Cartesian coordinates of neutral reactant in Reaction 3:

C	-0.564079	-0.17109	1.160469
C	-0.227274	-1.202554	2.047159
C	-0.497246	-1.063117	3.406115
C	-1.10106	0.105067	3.858436
C	-1.417056	1.074961	2.910713
N	-1.166692	0.944219	1.605802
H	-0.240635	-1.85946	4.097168
H	0.233764	-2.112724	1.690254
H	-1.331656	0.259895	4.906429
H	-1.903689	1.999326	3.213646
C	-1.62719	0.15091	-1.097292
C	0.041801	-1.70078	-0.77503
H	1.0156	-2.029067	-0.41801
H	-0.706923	-2.398959	-0.397947
H	0.04248	-1.797599	-1.861024
C	-0.30151	-0.256286	-0.355837
C	0.916899	0.737954	-0.782555
C	1.295604	0.505024	-2.263393
H	2.027951	1.250654	-2.583787
H	1.740309	-0.476346	-2.413151
H	0.44663	0.577416	-2.942338
C	0.512445	2.229136	-0.620496
C	-0.20666	2.912461	-1.61445
C	-0.531364	4.253279	-1.439361
H	-0.5136	2.414508	-2.523436
C	0.604316	4.162035	0.647423

C	-0.123601	4.902451	-0.277914
H	-1.089568	4.782079	-2.205166
H	0.960159	4.626664	1.564286
H	-0.348515	5.947767	-0.098297
N	0.915301	2.872653	0.485843
C	2.210863	0.544834	0.103135
H	1.924865	0.631048	1.150083
H	2.82321	1.428939	-0.091974
C	3.127186	-0.65908	-0.049912
C	3.210834	-1.625508	0.962689
C	4.014161	-0.780985	-1.131295
C	4.104841	-2.693553	0.882299
H	2.572625	-1.536577	1.835342
C	4.908072	-1.847767	-1.221068
H	4.023573	-0.023911	-1.907662
C	4.952748	-2.816088	-0.21767
H	4.142493	-3.424559	1.684083
H	5.579588	-1.914436	-2.07155
H	5.650489	-3.644616	-0.284607
C	-2.792287	-0.816101	-0.981888
C	-3.049228	-1.748225	-1.997338
C	-3.671001	-0.78217	0.110497
C	-4.129972	-2.626782	-1.920413
H	-2.397623	-1.784181	-2.865525
C	-4.753672	-1.658479	0.193546
H	-3.512379	-0.055799	0.899578
C	-4.986262	-2.58859	-0.819881
H	-4.305162	-3.336847	-2.722767
H	-5.420567	-1.608718	1.048903
H	-5.82983	-3.268625	-0.757417
H	-1.941947	1.124973	-0.727895
H	-1.403749	0.265306	-2.158844

Cartesian coordinates of radical product in Reaction 3:

C	-0.503251	1.069484	0.250425
C	-0.216401	2.300728	-0.558651
H	-0.893831	3.1261	-0.301118
H	0.80292	2.651595	-0.390927
H	-0.325588	2.130779	-1.635814
C	-1.735325	0.364953	0.107545
C	-2.760399	0.815028	-0.771932
C	-3.940708	0.10036	-0.874809
H	-2.621276	1.715631	-1.357159
C	-3.053616	-1.437242	0.728945
C	-4.105985	-1.05712	-0.111757
H	-4.727205	0.439164	-1.542157
H	-3.143208	-2.33409	1.339314
H	-5.013586	-1.647963	-0.161168
N	-1.914821	-0.771354	0.847301
C	0.548836	0.605949	1.222623
H	0.127495	-0.166892	1.866095
H	0.835643	1.449243	1.864575
C	1.799072	0.075118	0.533664
C	1.738392	-1.101064	-0.225358
C	3.025769	0.736599	0.638461
C	2.873996	-1.600842	-0.857134
H	0.793507	-1.628879	-0.311352
C	4.165898	0.239865	0.004809
H	3.095024	1.645871	1.229635
C	4.0932	-0.930435	-0.746358
H	2.808998	-2.517108	-1.43603
H	5.109748	0.767725	0.101515
H	4.978236	-1.319985	-1.239354

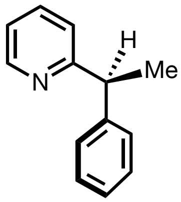
Competition Experiment Raw Data

FMN (equiv.)	Observed enantioselectivity	Predicted enantioselectivity	y-y	(y-y)^2	Rate Enhancement
0.01	92	92	0	0	1.18
0.5	76	79.5	3.5	12.25	
1	72	72.73394495	0.734	0.539	
2	66	65.58490566	-0.42	0.172	
5	61	58.01941748	-2.98	8.884	
10	58	54.43291592	-3.57	12.72	

References

1. Sun, Q.; Berkelbach, T. C.; Blunt, N. S.; Booth, G. H.; Guo, S.; Li, Z.; Liu, J.; McClain, J. D.; Sayfutyarova, E. R.; Sharma, S.; Wouters, S.; Chan, G. K.-L., *WIREs Comput. Mol. Sci.* **2018**, *8*, e1340.
2. Becke, A. D., *J. Chem. Phys.* **1993**, *98*, 1372.
3. Hariharan, P. C.; Pople, J. A., *Theoret. Chim. Acta* **1973**, *28*, 213.
4. Knizia, G., *J. Chem. Theory Comput.* **2013**, *9*, 4834-4843.

HPLC Traces



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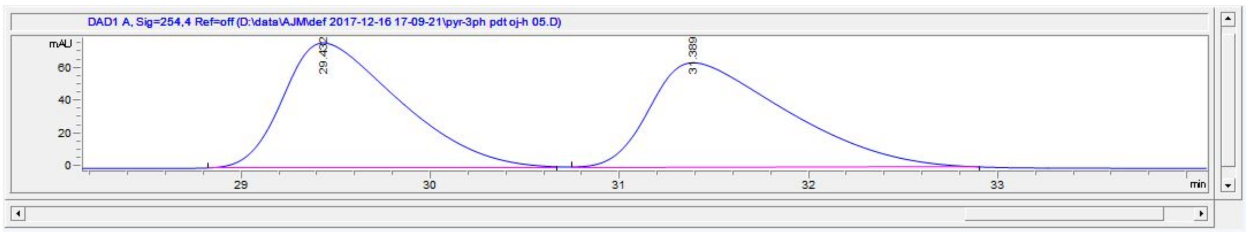

#	Time	Type	Area	Height	Width	Area%	Symmetry
1	29.432	BB	3246.6	75.7	0.6085	50.206	0.505
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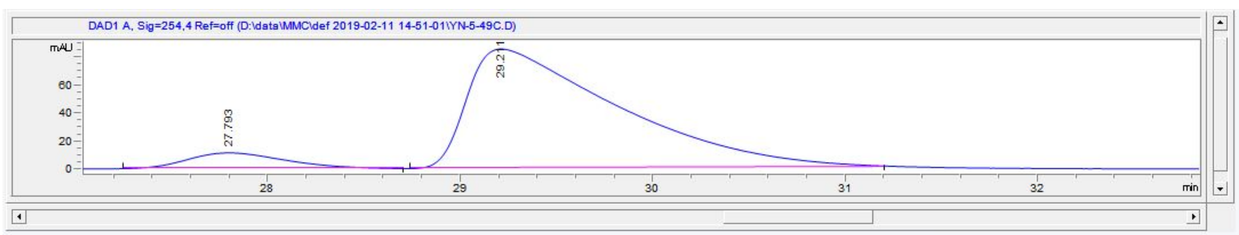
NostocER



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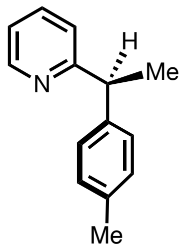

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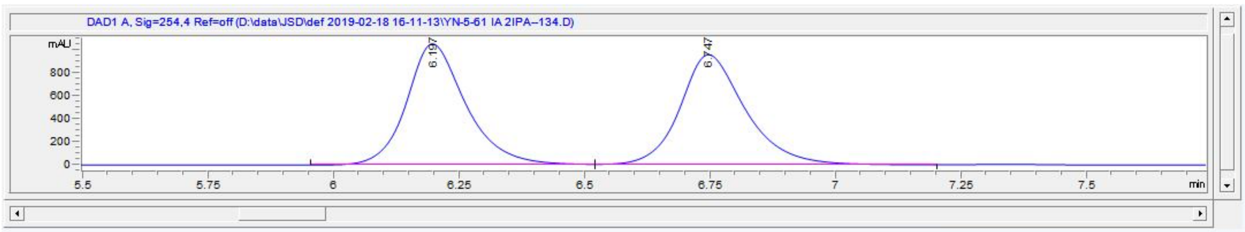
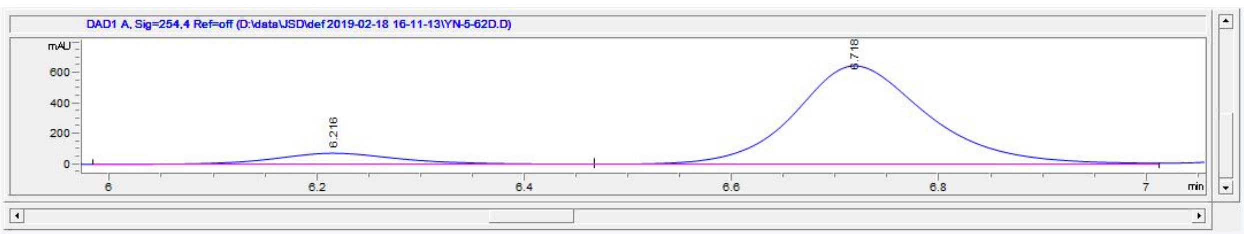


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	6.197	BV	8773.1	1056.3	0.1229	49.787	0.738
2	6.747	VV	8848	961.1	0.1364	50.213	0.741

NostocER



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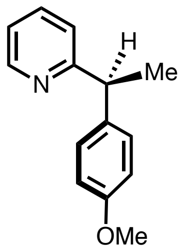

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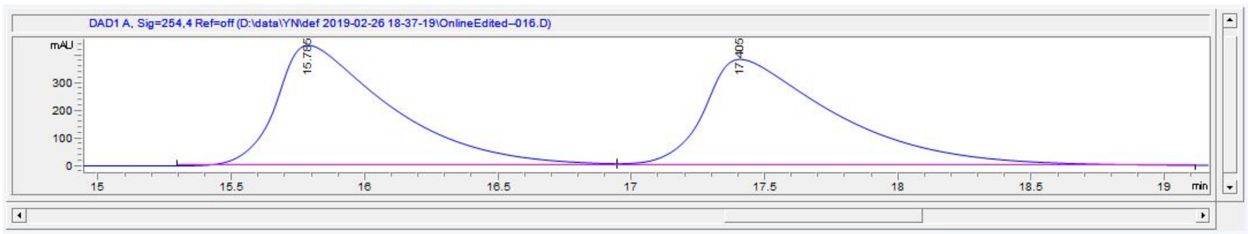
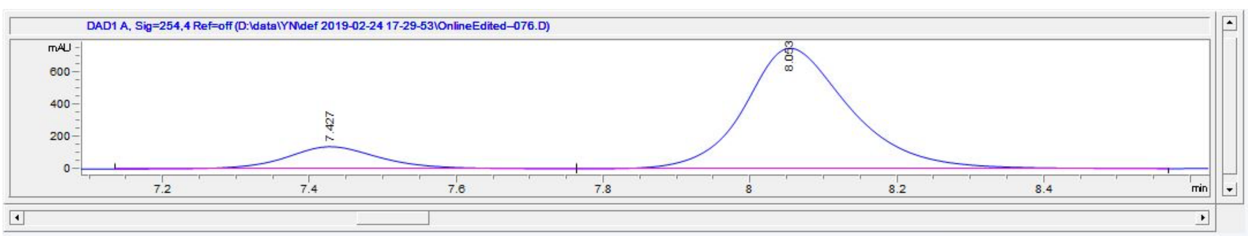


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	15.785	BV	13092.2	435.9	0.4264	49.724	0.366
2	17.405	VB	13237.5	384.5	0.4787	50.276	0.365

NostocER



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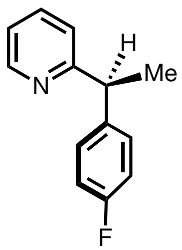
1	7.427	VV	1272	138.6	0.1371	14.354	0.844
2	8.053	VV	7589.6	750.3	0.15	85.646	0.731



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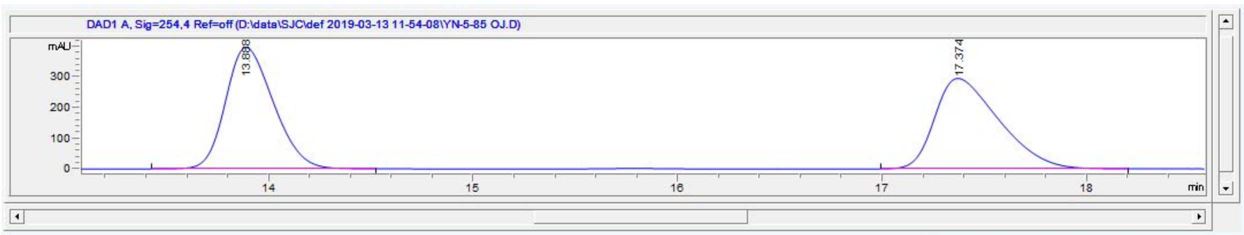

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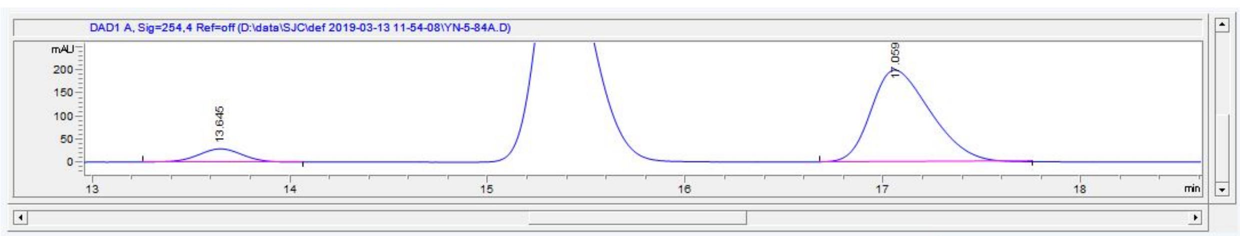

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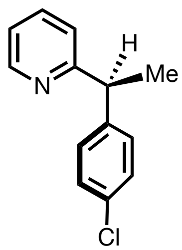

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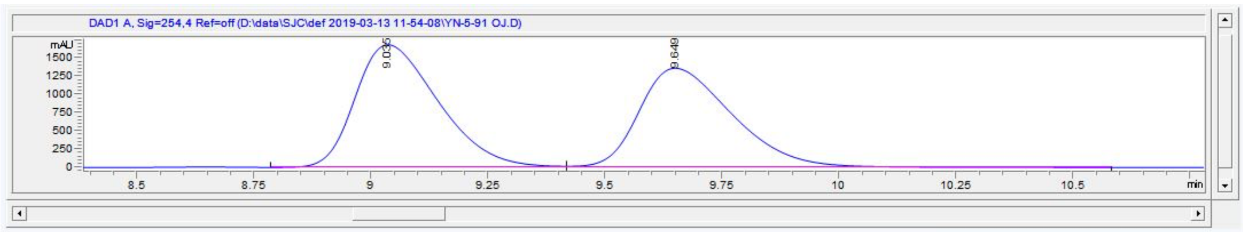

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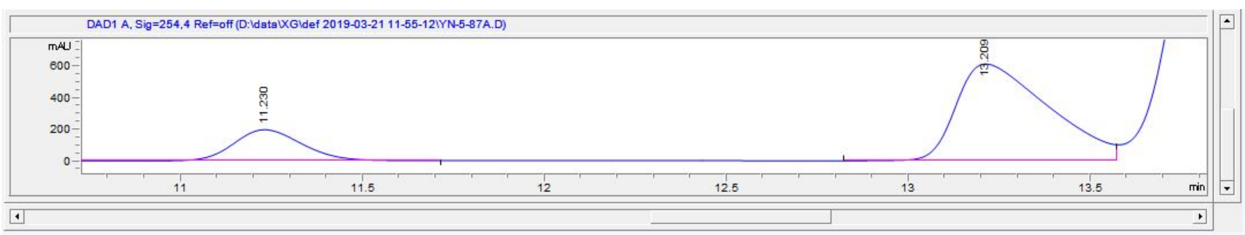
1	9.035	VV	20763	1681.1	0.1931	52.328	0.618
2	9.649	VB	18915.7	1358.3	0.2145	47.672	0.6

NostocER



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	11.23	BB	2695	199	0.207	19.750	0.845
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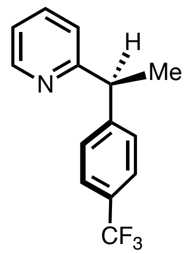

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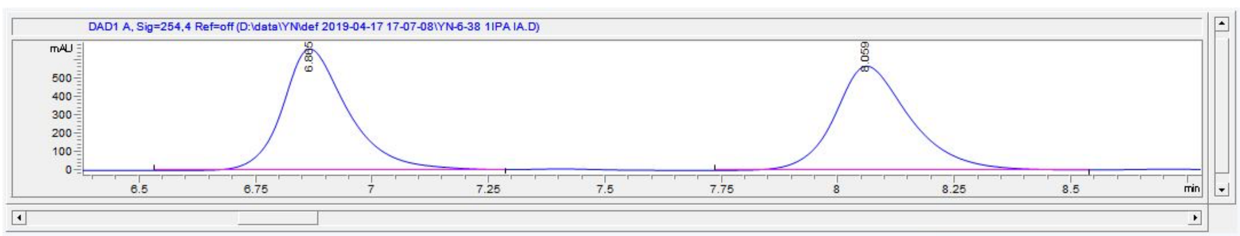
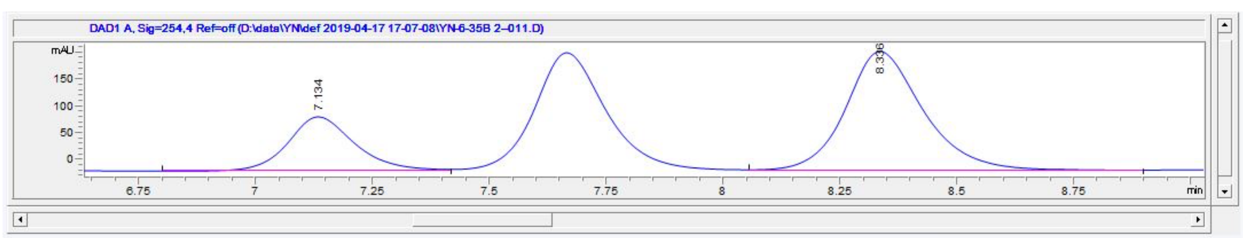


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	6.865	BV	6527.7	663.2	0.1458	50.189	0.677
2	8.059	VV	6478.5	569	0.1698	49.811	0.708

NostocER



#	Time	Type	Area	Height	Width	Area%	Symmetry
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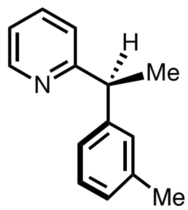

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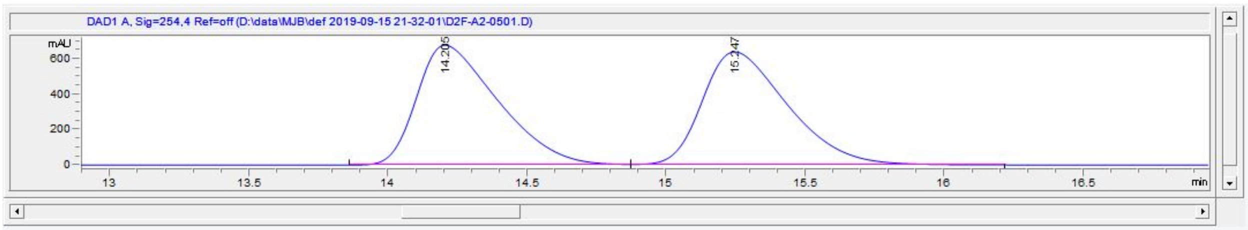
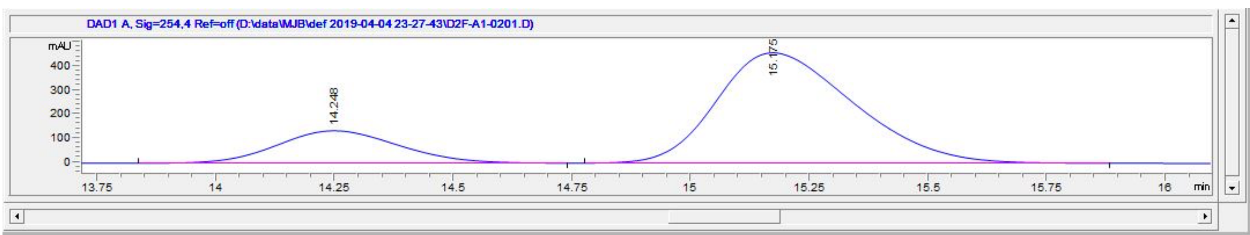


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	14.205	BV	13947.5	684.8	0.3056	50.195	0.512
2	15.247	VB	13839.1	646.1	0.3259	49.805	0.583

NostocER



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1	14.248	BB	2425.5	134.6	0.2782	20.752	0.85
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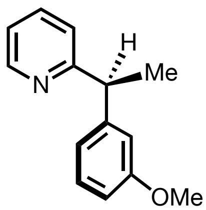

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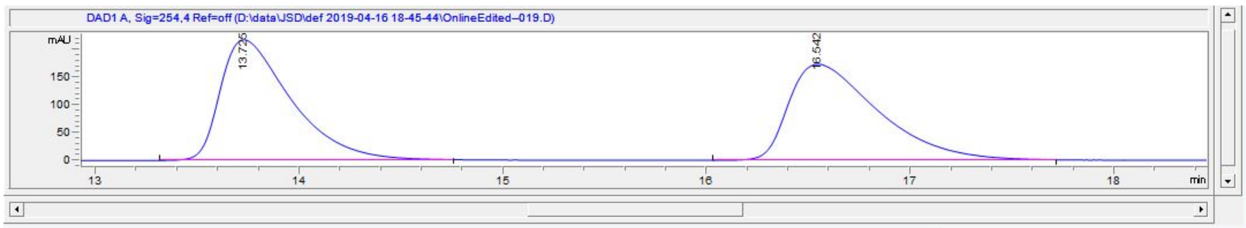
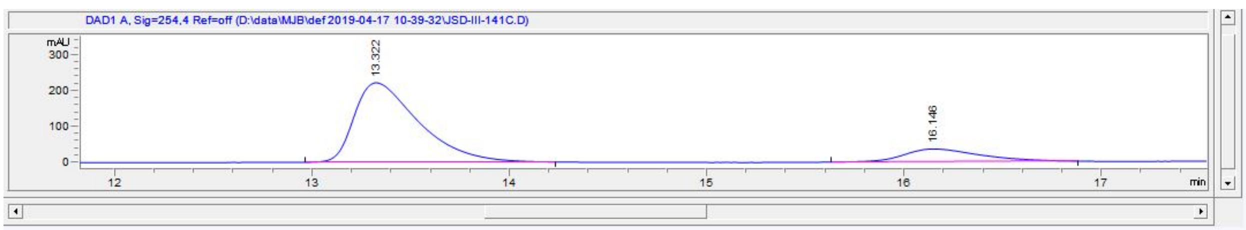


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	13.725	VV	5263.1	217.4	0.3525	49.839	0.469
2	16.542	VB	5297.1	173.3	0.4485	50.161	0.458

NostocER



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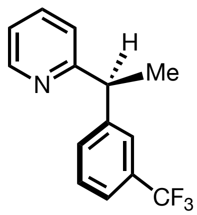

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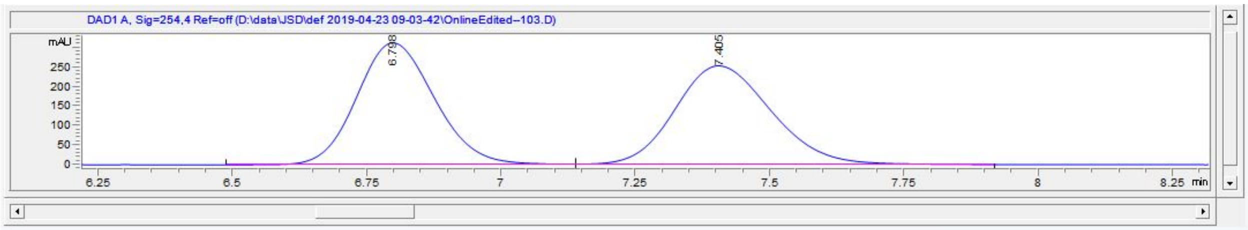
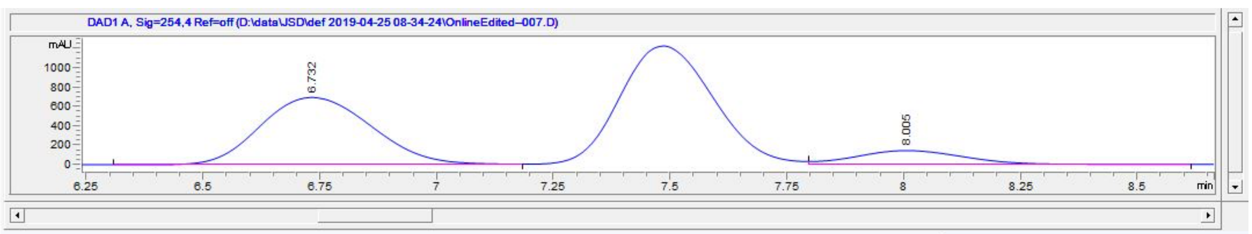


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	6.798	VV	3190.6	314.3	0.1574	49.866	0.839
2	7.405	VB	3207.7	255.2	0.1947	50.134	0.798

NostocER



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1	6.732	VV	11434.2	688.4	0.2648	82.275	0.832
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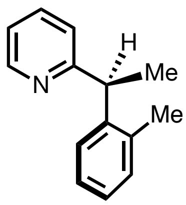

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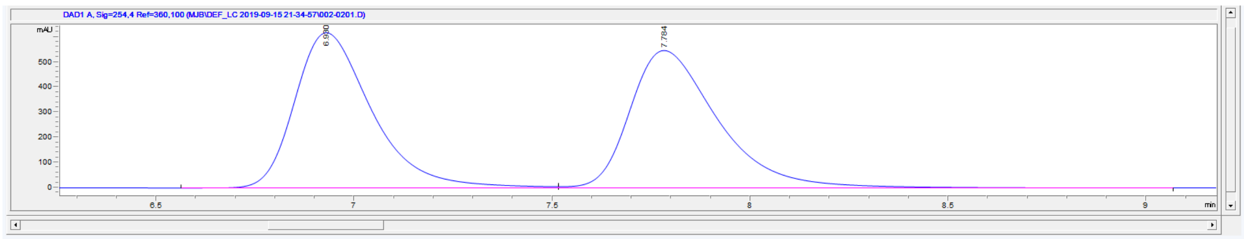

#	Time	Area	Height	Width	Area%	Symmetry
1	6.93	8371.6	615.9	0.2086	49.756	0.649
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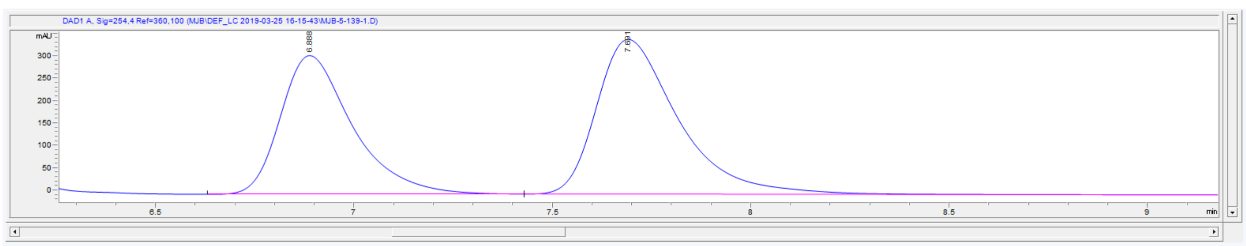
NostocER



#	Time	Area	Height	Width	Area%	Symmetry
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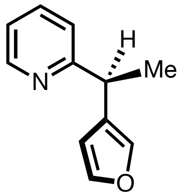

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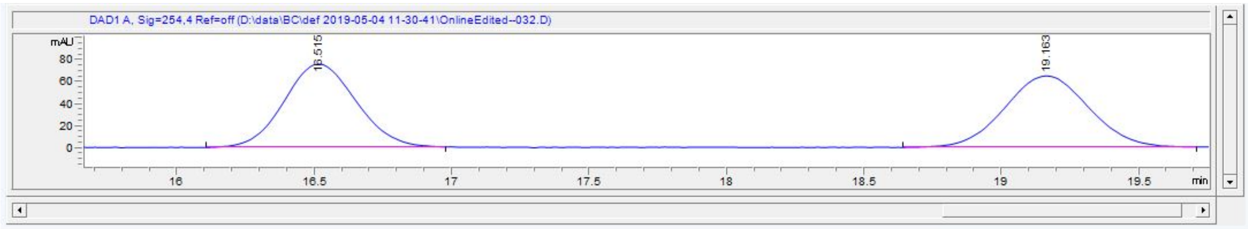

#	Time	Type	Area	Height	Width	Area%	Symmetry
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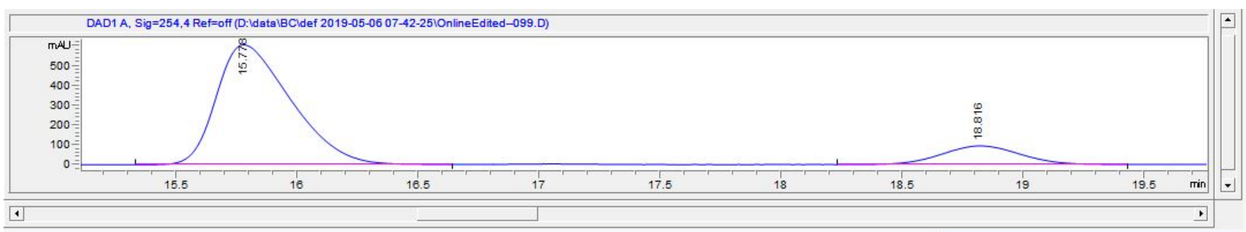
NostocER



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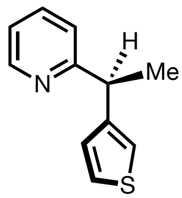
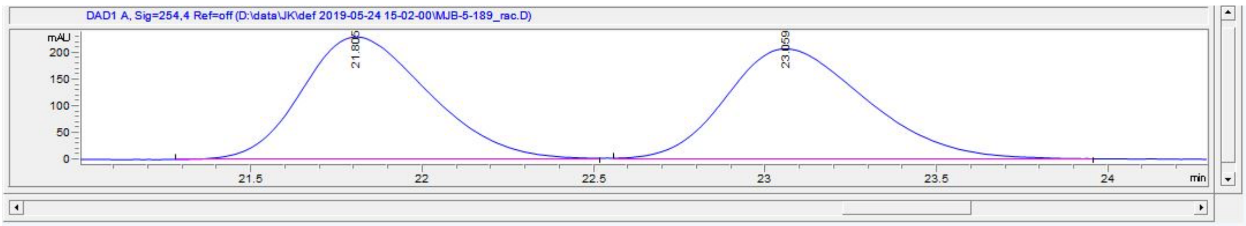
1	15.778	VB	12912.5	603.4	0.3247	85.843	0.551
2	18.816	BB	2129.5	93.6	0.3514	14.157	0.928

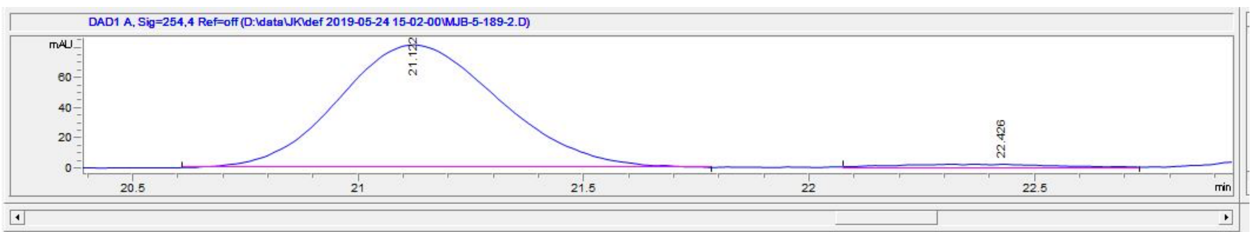


Racemate

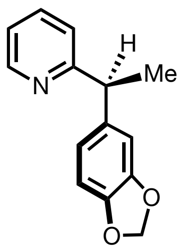


#	Time	Area	Height	Width	Symmetry
1	21.805	5955.1	228.9	0.3775	0.719
2	23.059	5984.1	206.7	0.4008	0.708

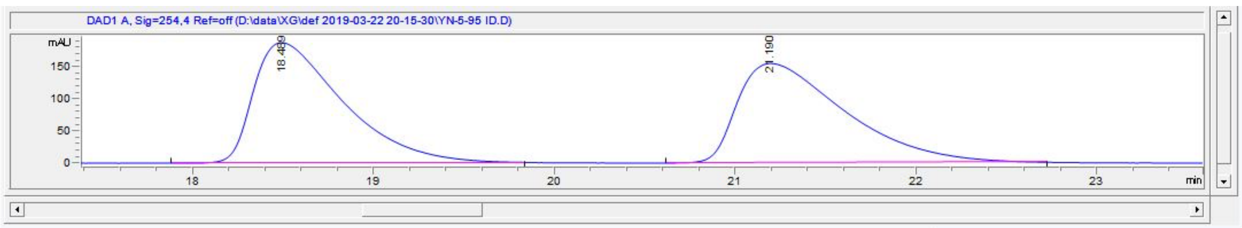
NostocER



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	21.122	BV	2032.2	81.6	0.3544	96.914	0.846
2	22.426	BV	64.7	2.5	0.3107	3.086	1.642

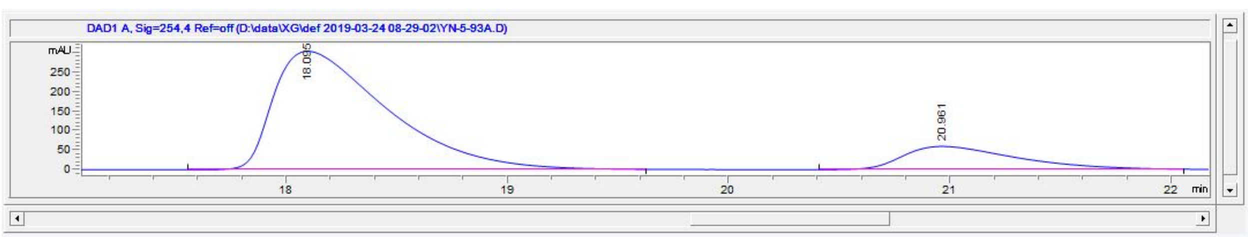


Racemate

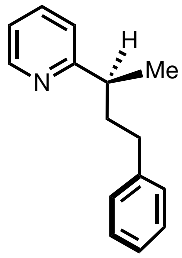


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	18.489	BB	6422.2	187.5	0.4826	50.040	0.43
2	21.19	BB	6411.9	155	0.5506	49.960	0.407

NostocER



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	18.095	BB	10885.8	305.4	0.5098	83.577	0.405
2	20.961	BB	2139.1	59.8	0.5057	16.423	0.491



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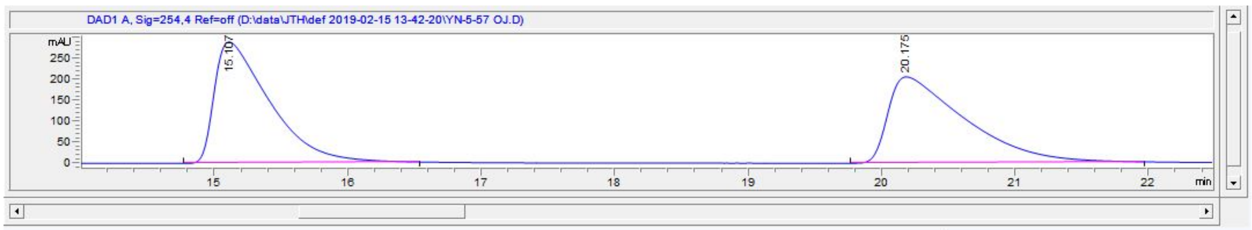
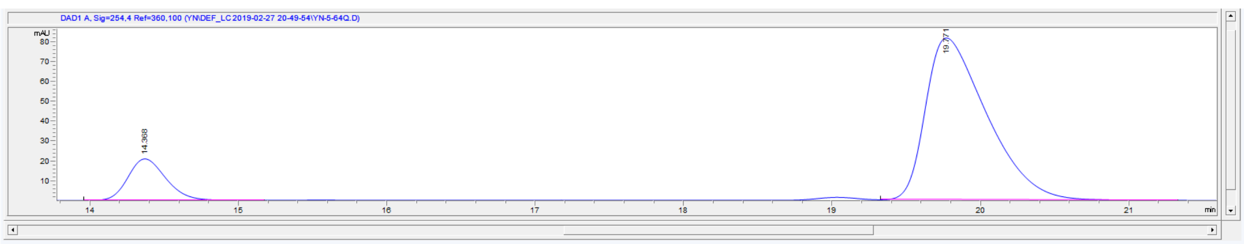
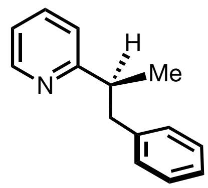


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	15.107	BB	8352.1	289.6	0.4165	50.277	0.334
2	20.175	BB	8260.1	206	0.5568	49.723	0.287

NostocER



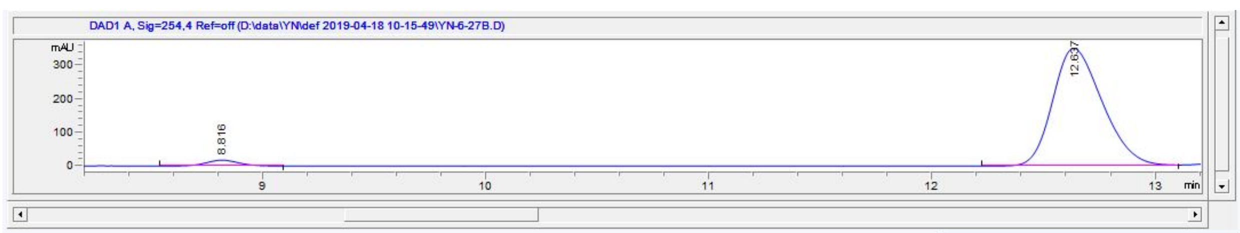
#	Time	Area	Height	Width	Area%	Symmetry
1	14.368	372.1	21	0.2746	13.659	0.772
2	19.771	2352.2	82	0.4346	86.341	0.485



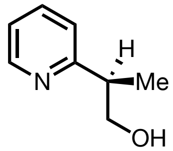
Racemate

#	Time	Type	Area	Height	Width	Area%	Symmetry
1	9.028	VB	12054	1098.5	0.169	49.988	0.608
2	13.117	VB	12059.6	688	0.2595	50.012	0.512

NostocER



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	8.816	BB	185.1	17.5	0.1614	3.319	0.886
2	12.637	VV	5392.4	347.5	0.2381	96.681	0.706



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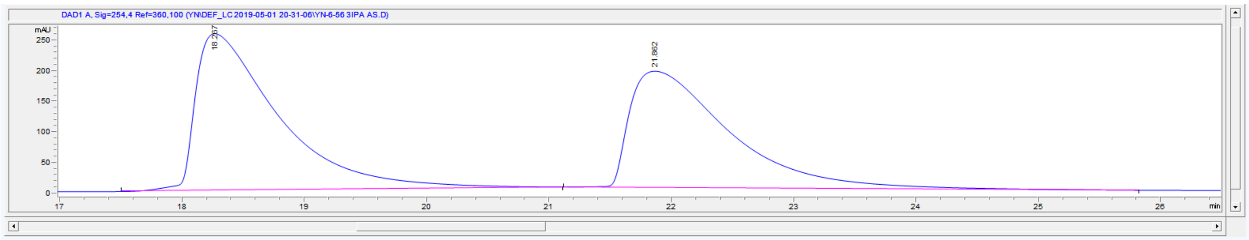
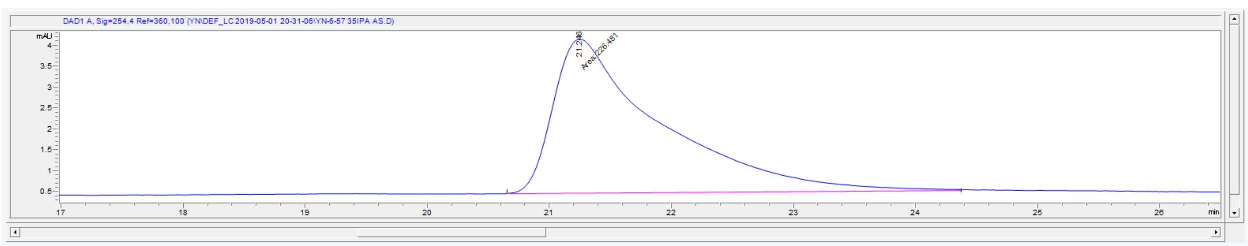
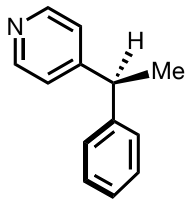


#	Time	Area	Height	Width	Area%	Symmetry
1	18.267	12078.1	256.3	0.6939	52.978	0.278
2	21.862	10720.2	189.8	0.8589	47.022	0.309

NostocER



#	Time	Area	Height	Width	Area%	Symmetry
1	21.246	226.5	3.7	1.0255	100.000	0.322



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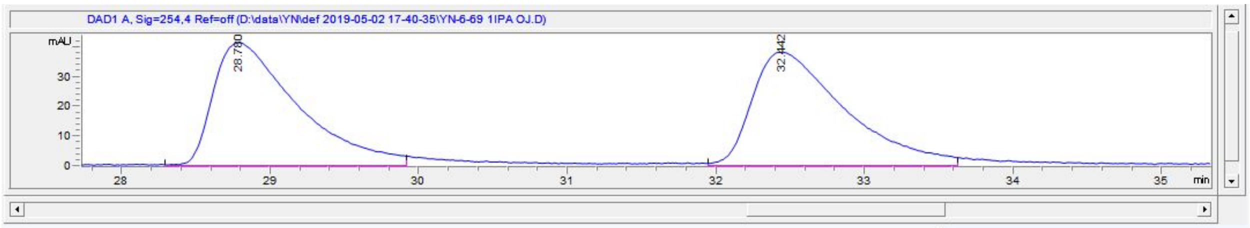
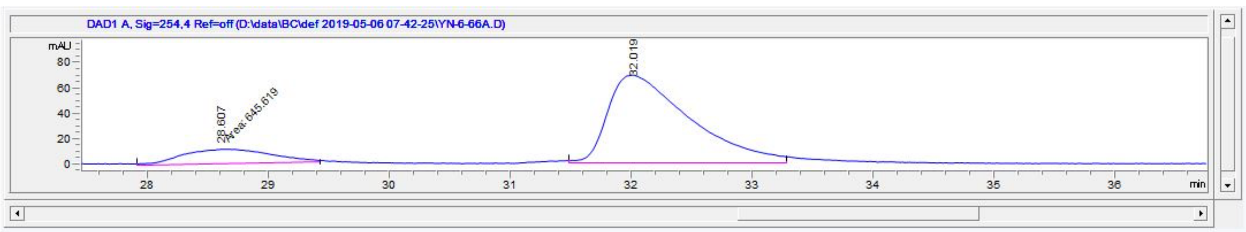
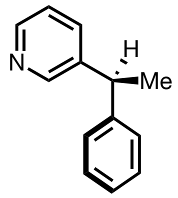


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	28.78	VB	1659.1	41.8	0.4701	49.648	0.409
2	32.442	BB	1682.6	38.6	0.5156	50.352	0.473

NostocER



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	28.607	MM	645.6	12.1	0.8902	16.029	0.756
2	32.019	VV	3382.2	71	0.562	83.971	0.44



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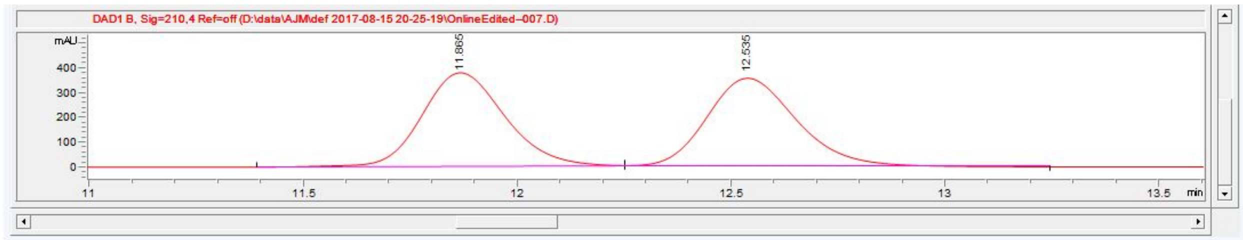
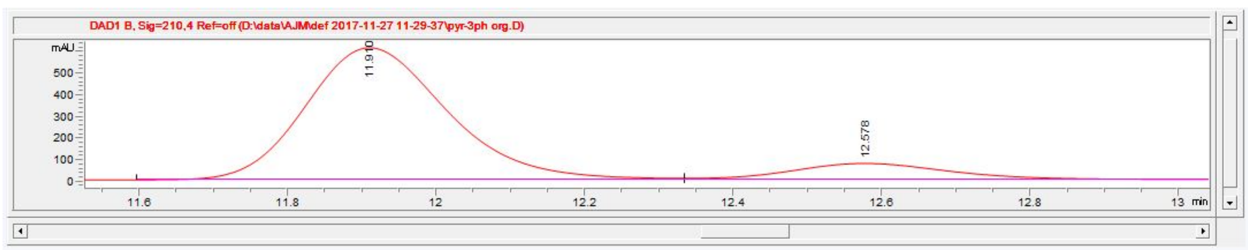
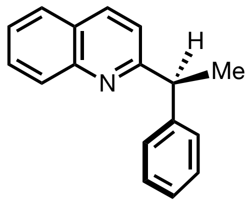


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	11.865	BV	5255.1	383.2	0.211	49.607	0.824
2	12.535	VB	5338.4	360.6	0.2277	50.393	0.79

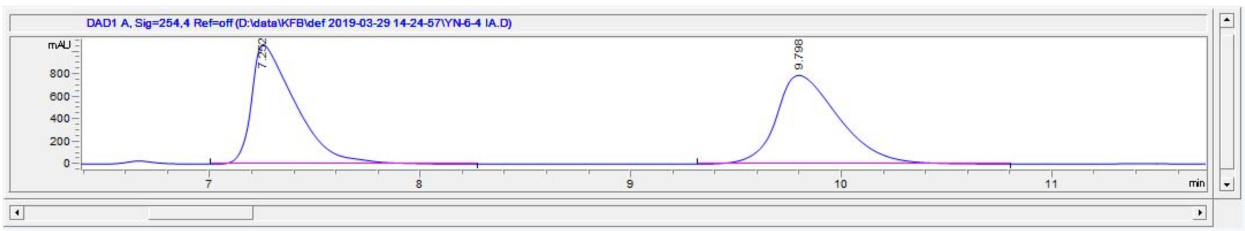
GluER



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	11.91	BV	8086.1	608	0.2042	87.805	0.788
2	12.578	VB	1123	74.7	0.2294	12.195	0.832

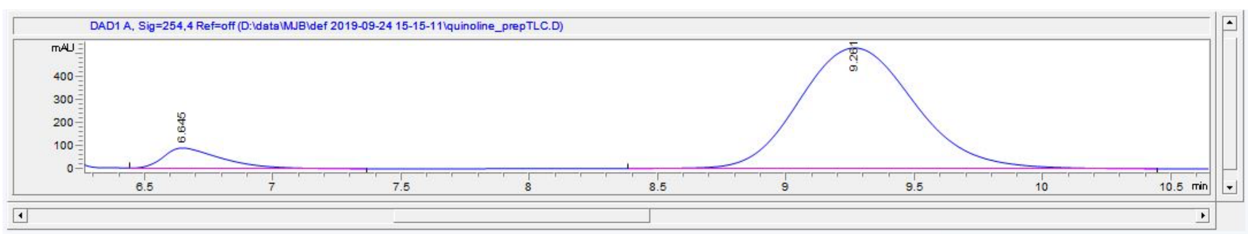


Racemate

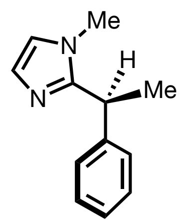


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	7.252	VB	15431	1060.3	0.2068	48.529	0.346
2	9.798	BB	16366.5	786.5	0.3128	51.471	0.562

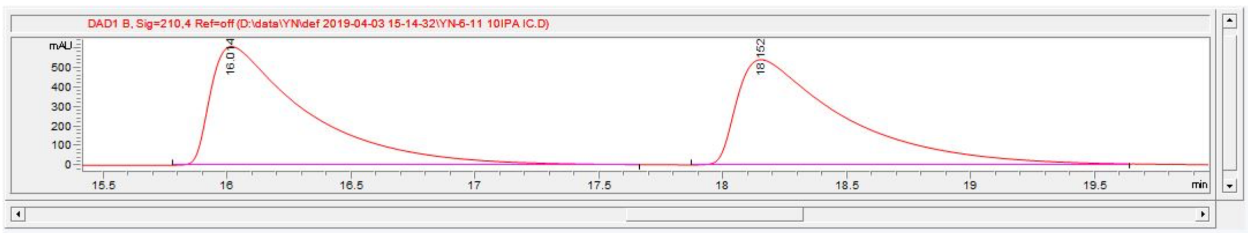
NostocER



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	6.645	VB	1475.3	90.7	0.2387	8.219	0.476
2	9.261	BB	16473.1	525.2	0.4949	91.781	0.878

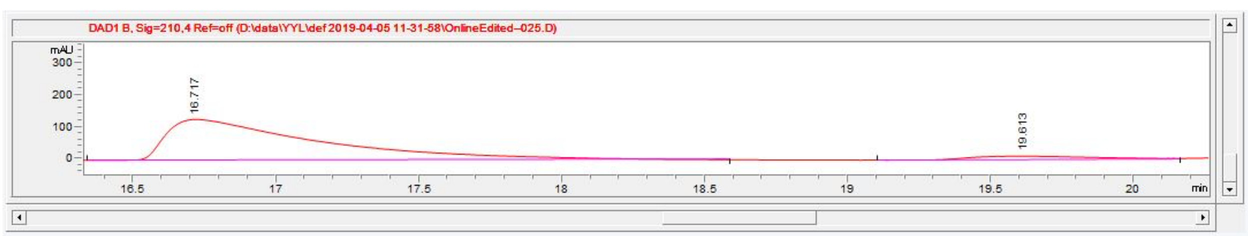


Racemate

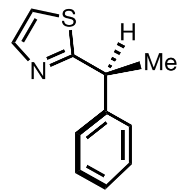


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	16.014	VV	16647.5	613.1	0.3718	50.117	0.248
2	18.152	BV	16570.1	545.3	0.4075	49.883	0.266

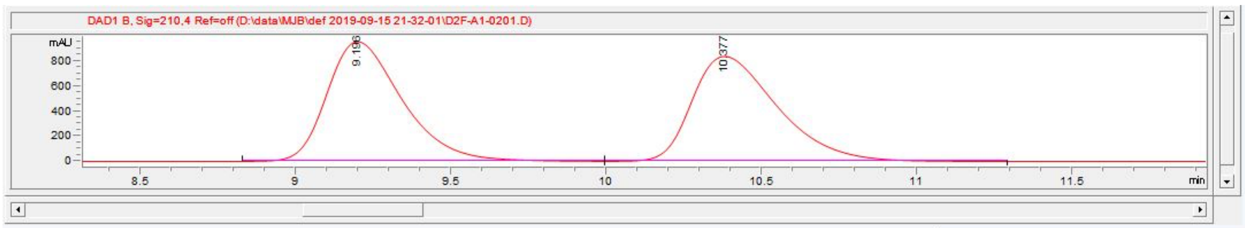
NostocER



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	16.717	VB	4749.1	127	0.5092	92.228	0.216
2	19.613	BV	400.2	11.8	0.4014	7.772	0.581

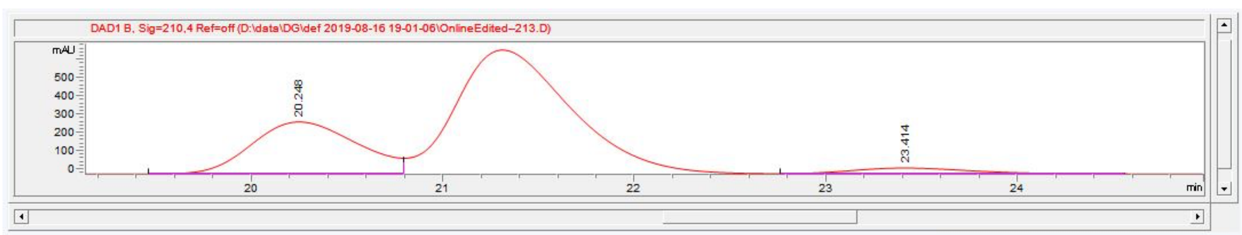


Racemate

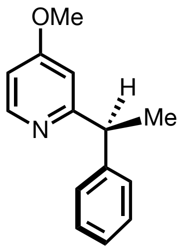


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	9.196	BV	16014.4	949.5	0.2588	50.135	0.651
2	10.377	VB	15928.5	833.3	0.295	49.865	0.584

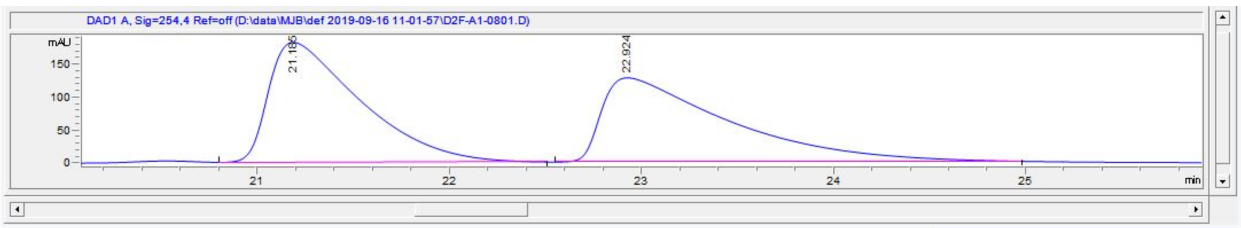
YersER



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	20.248	BV	10925.1	284.8	0.5935	88.345	0.798
2	23.414	BB	1441.3	32.4	0.6465	11.655	0.759

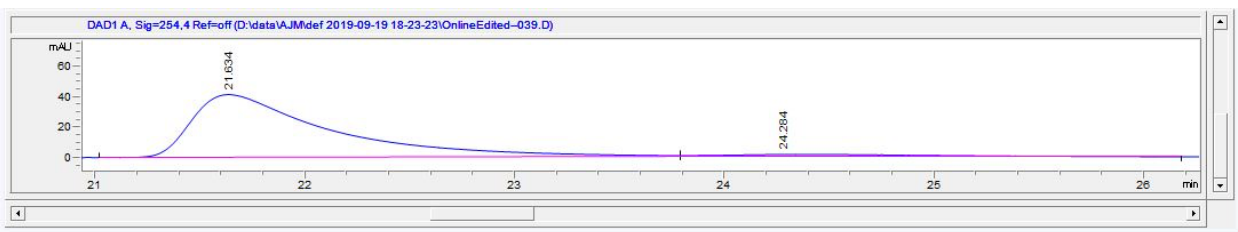


Racemate

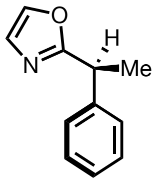


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	21.185	VB	6067.6	182.5	0.4668	50.831	0.344
2	22.924	BB	5869.3	127.9	0.6353	49.169	0.234

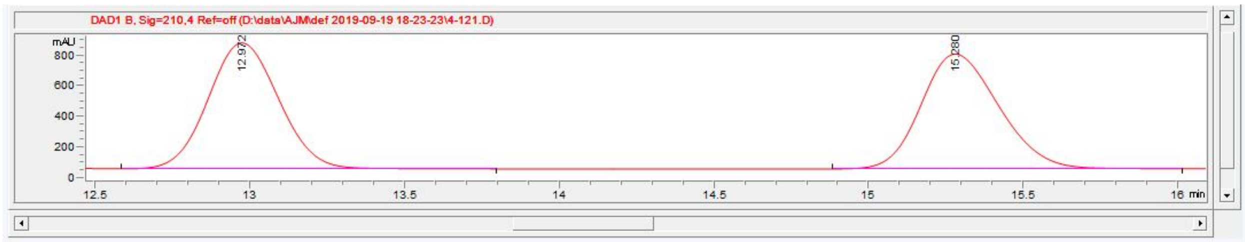
NostocER



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	21.634	BB	1883.4	40.9	0.6311	93.277	0.335
2	24.284	BB	135.8	1.7	0.9199	6.723	0.415



Racemate



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	12.972	VB	13143.4	823.3	0.2502	50.031	0.886
2	15.28	BB	13126.9	749.9	0.2722	49.969	0.76

NostocER

#	Time	Type	Area	Height	Width	Area%	Symmetry
1	12.921	VB	3977.1	239.6	0.2586	30.405	0.969
2	15.334	BB	9103.2	557.3	0.2546	69.595	0.812

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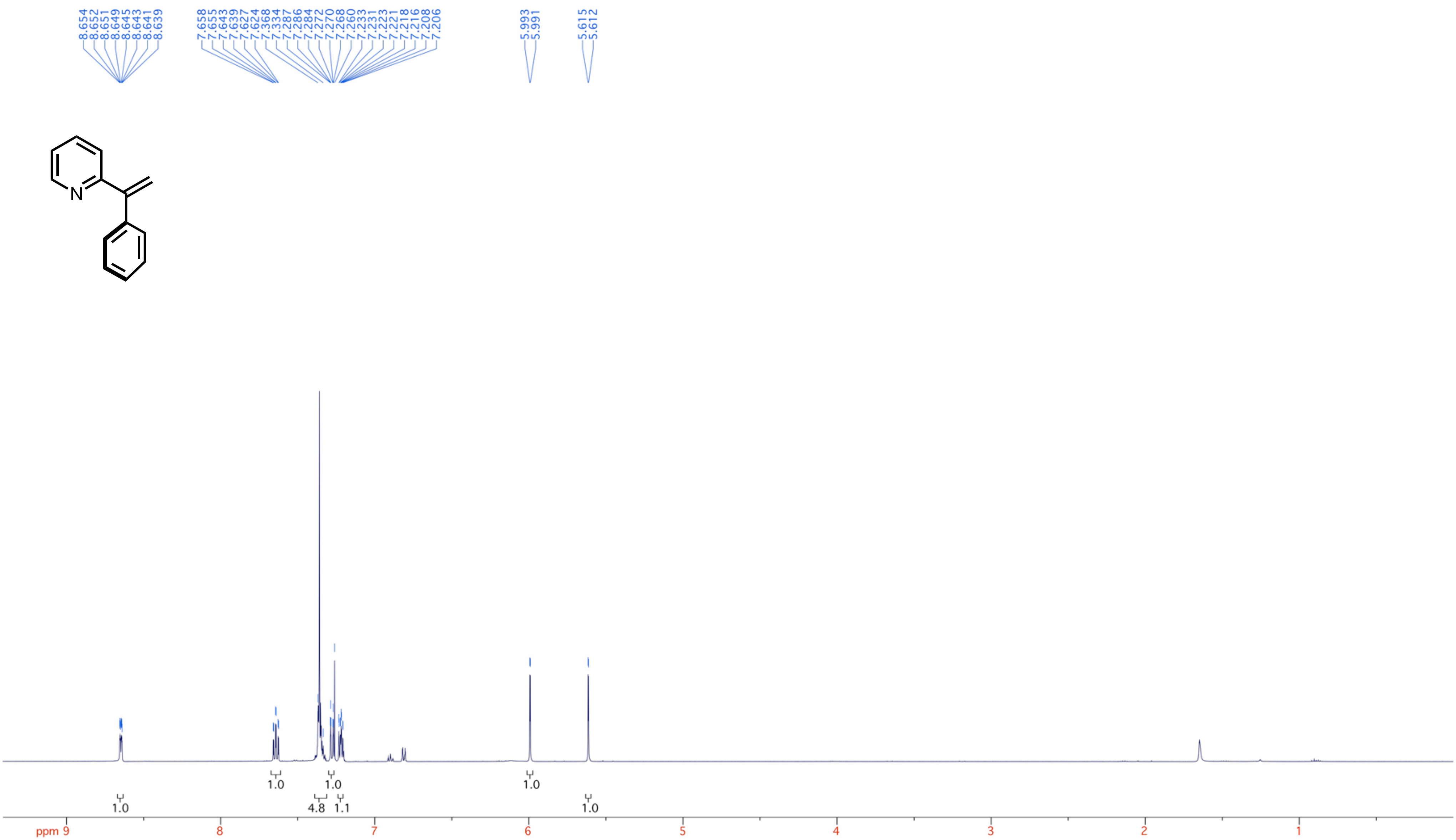
[i] D. G. Gibson, L. Young, R.-Y. Chuang, J. C. Venter, C. A. Hutchison, H. O. Smith *Nat. Methods* **6**, 343-345 (2009).

[ii] A. Aliverti, B. Curti, M. A. Vanoni *Indentifying and Quantitating FAD and FMN in Simple and in Iron-Sulfur-Containing Flavoproteins. In Flavoprotein Protocols*, Chapman, S.K.; Reid, G.A., Eds.; Humana Press: Totowa, New Jersey, **131**, 9 (1999).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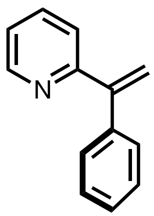

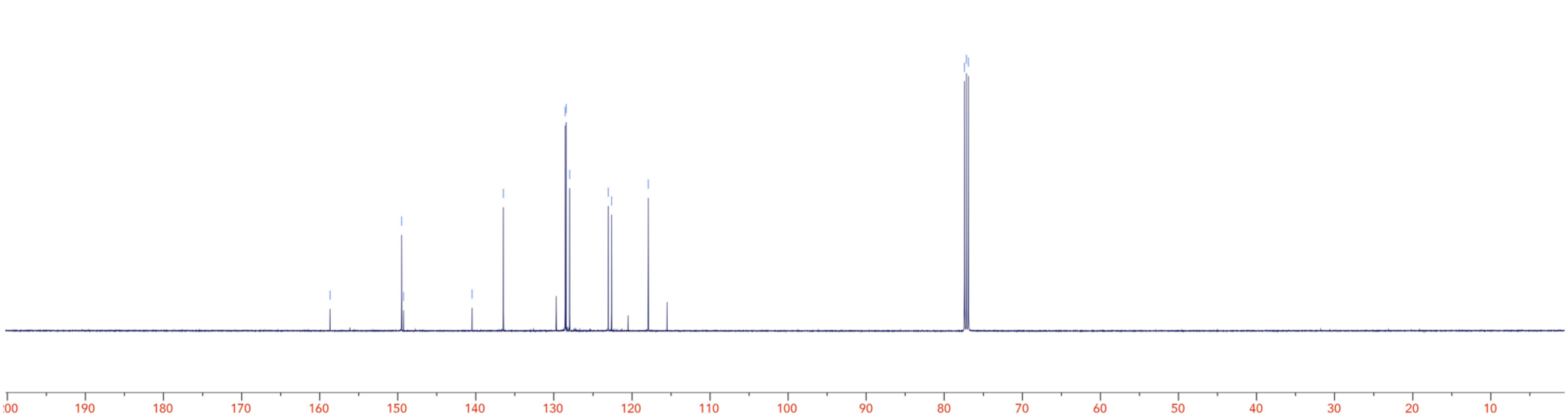
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76.906
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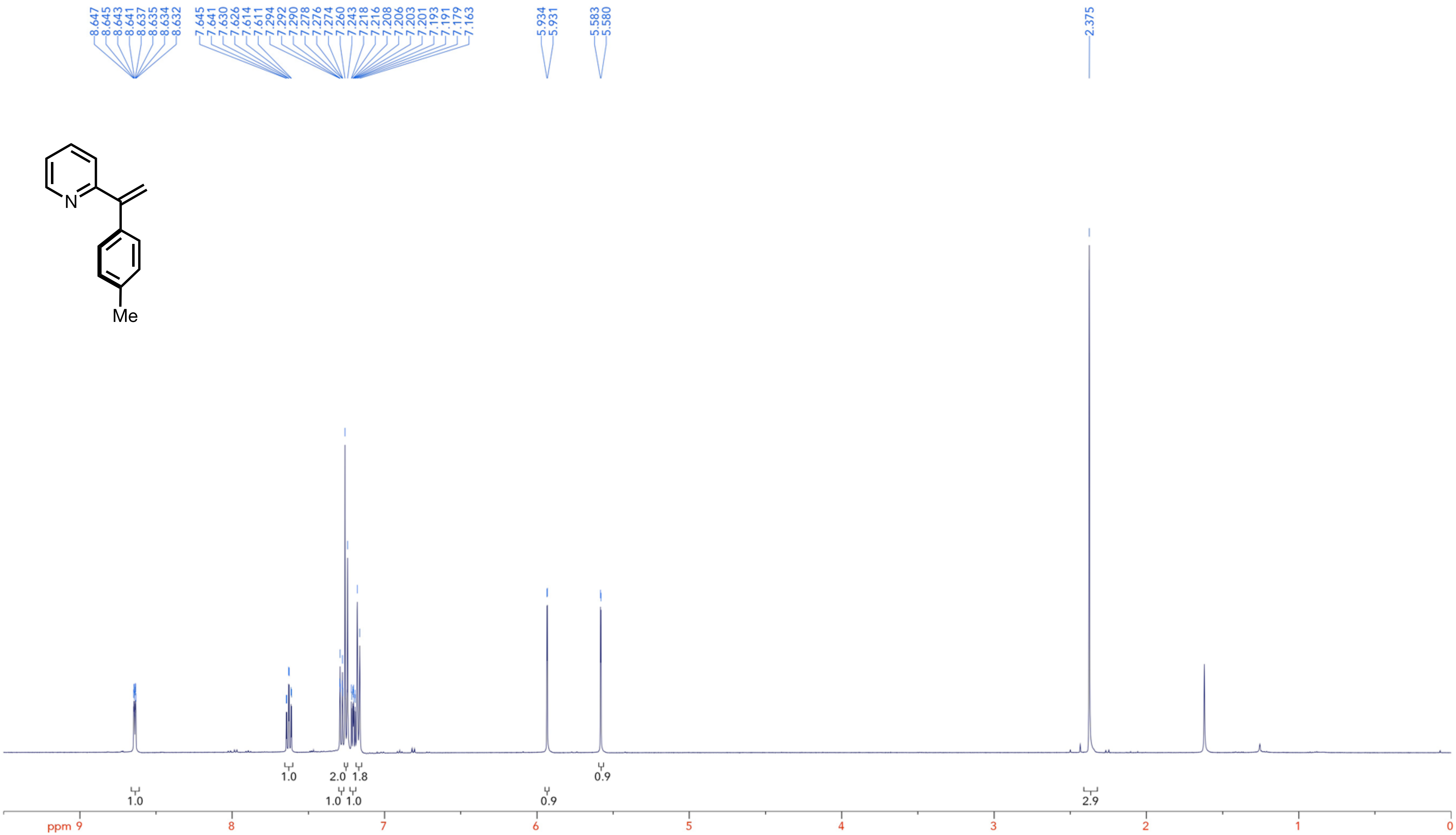
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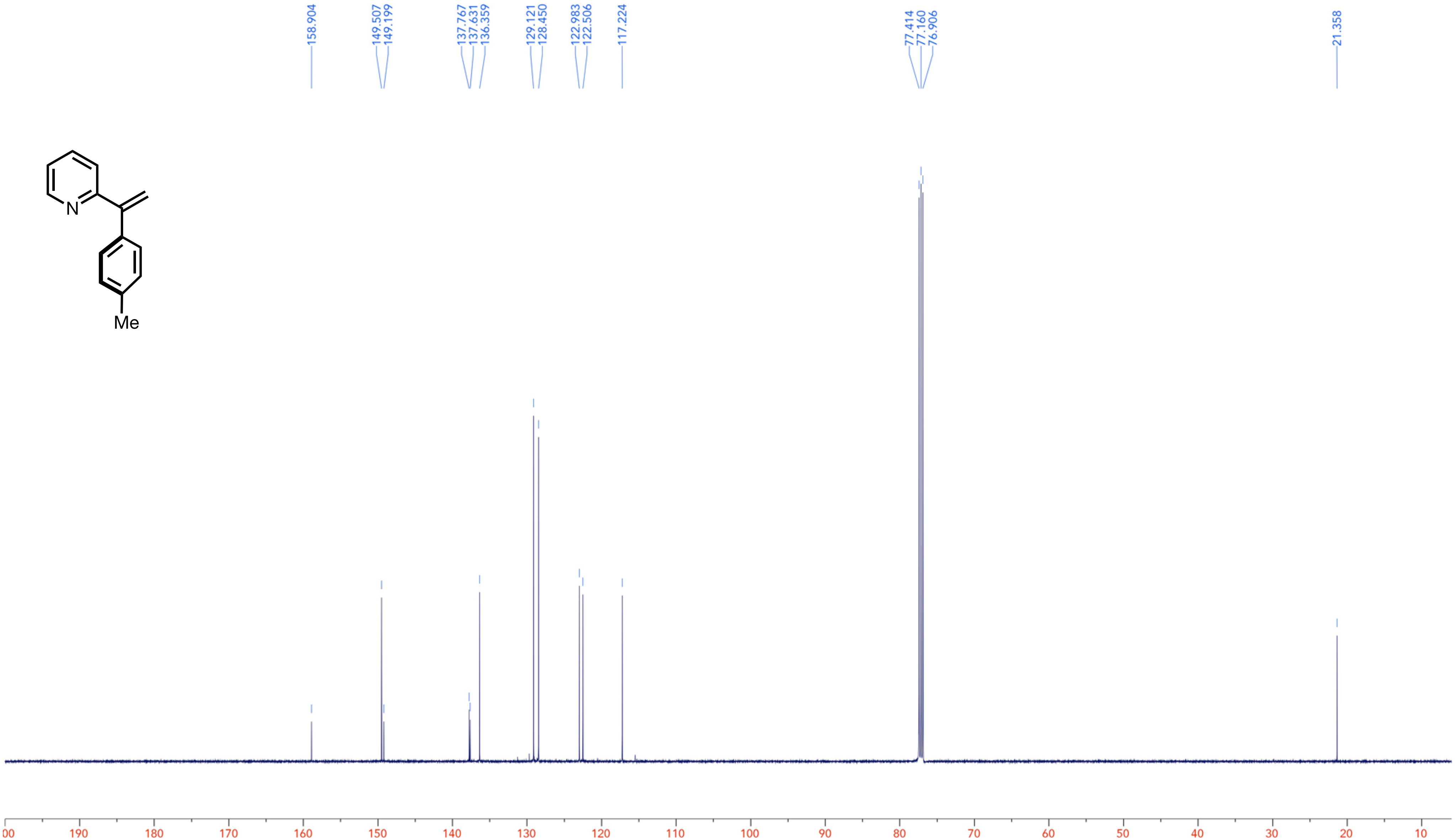
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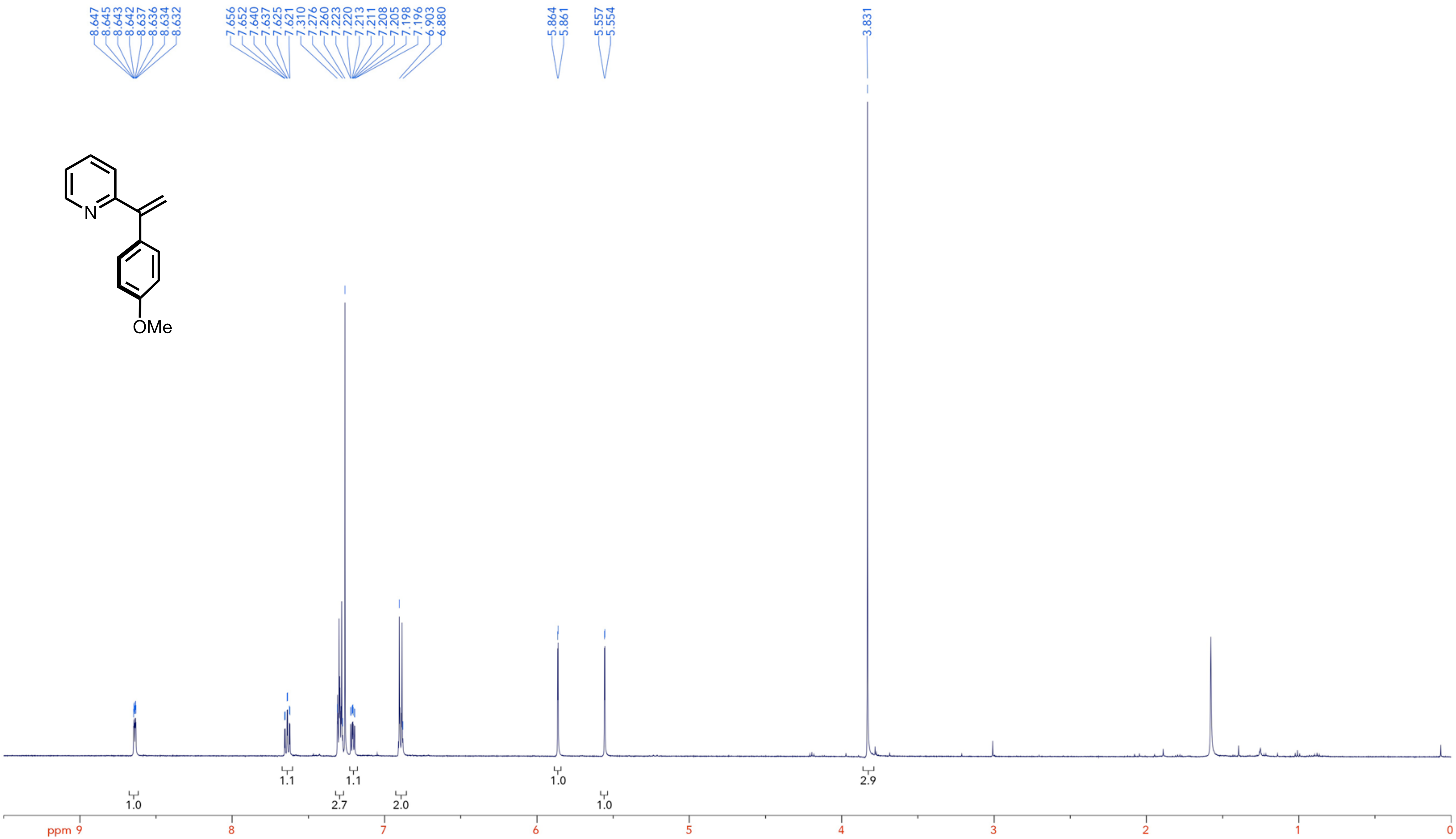
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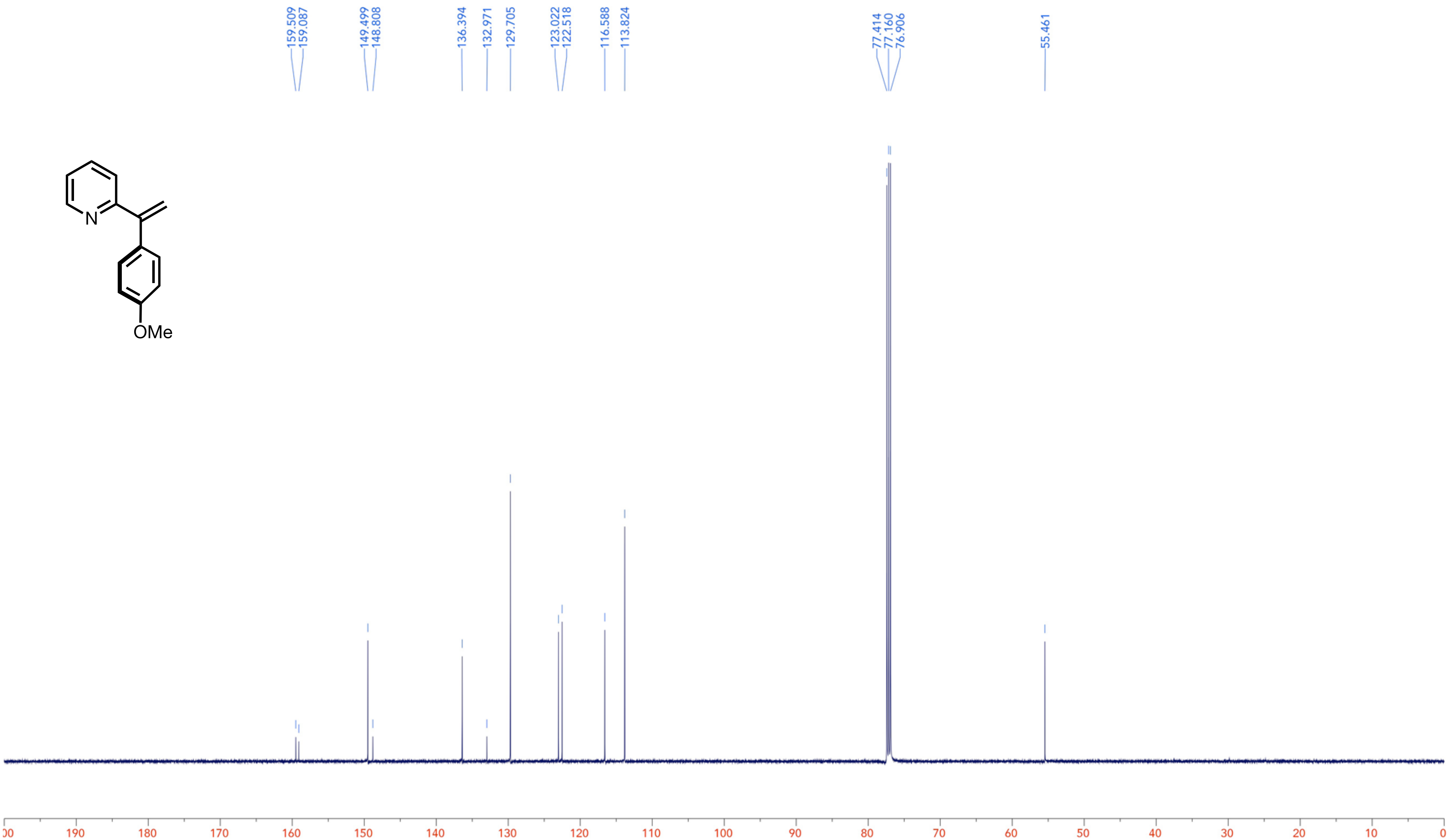
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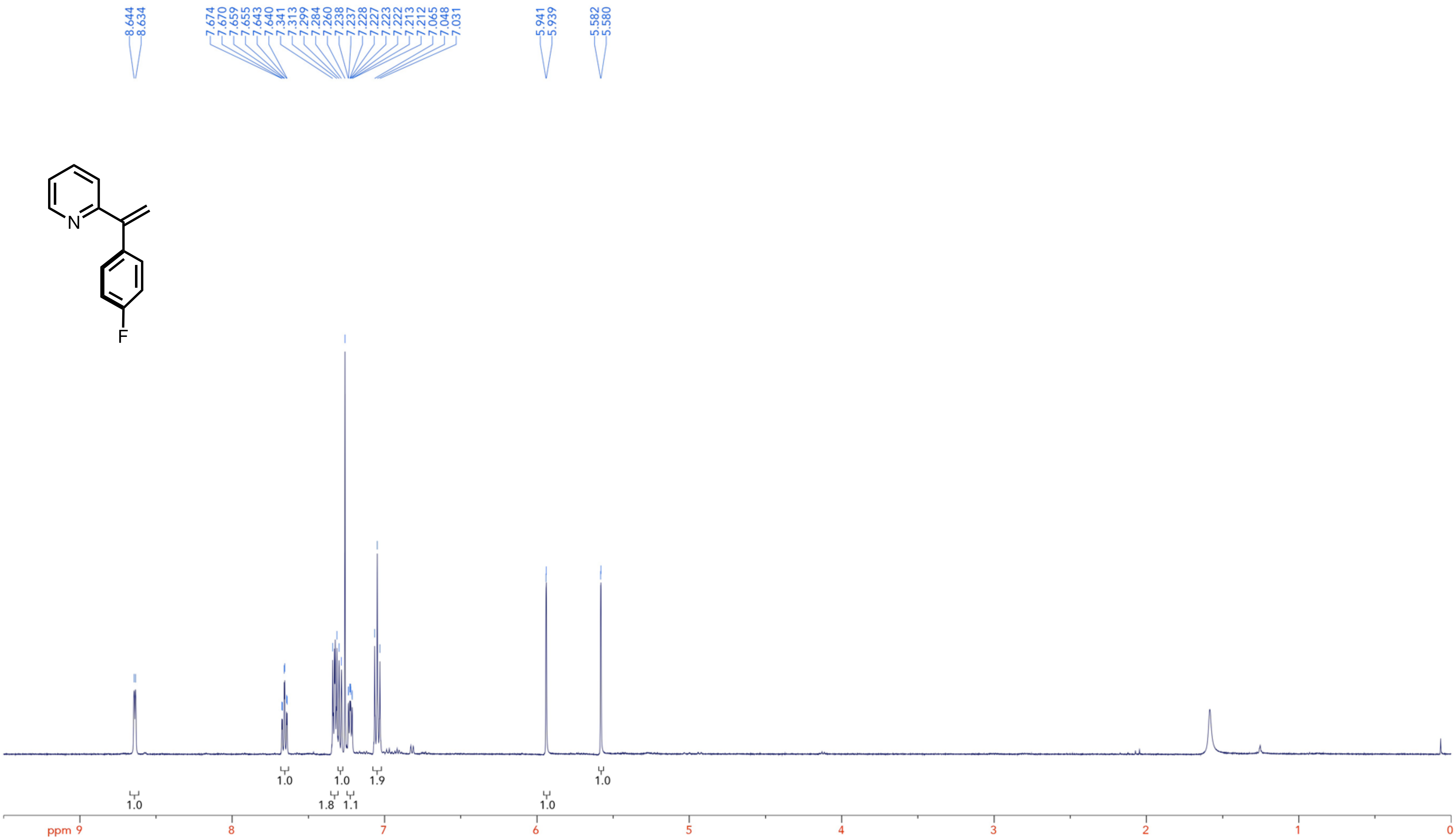

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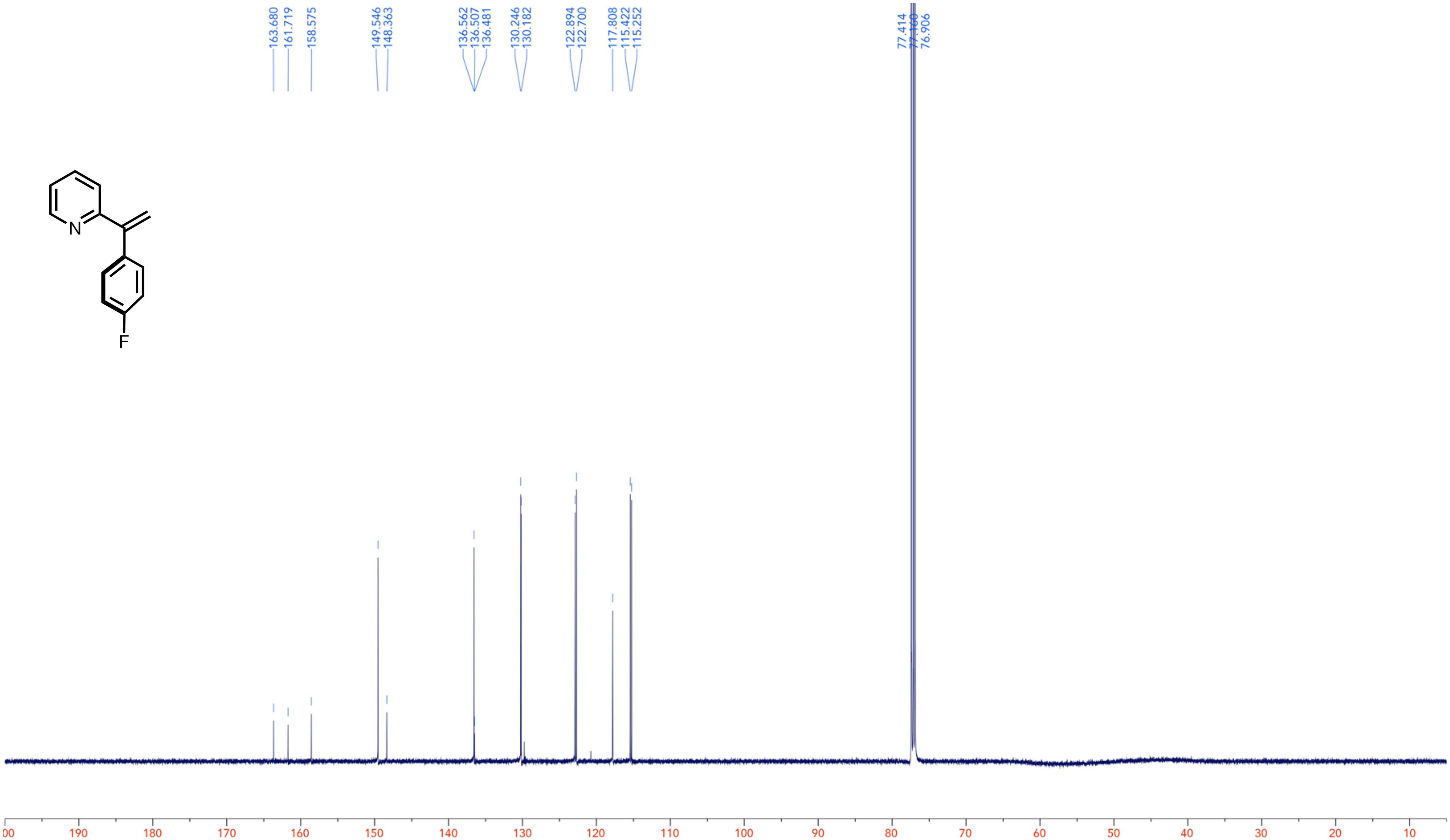
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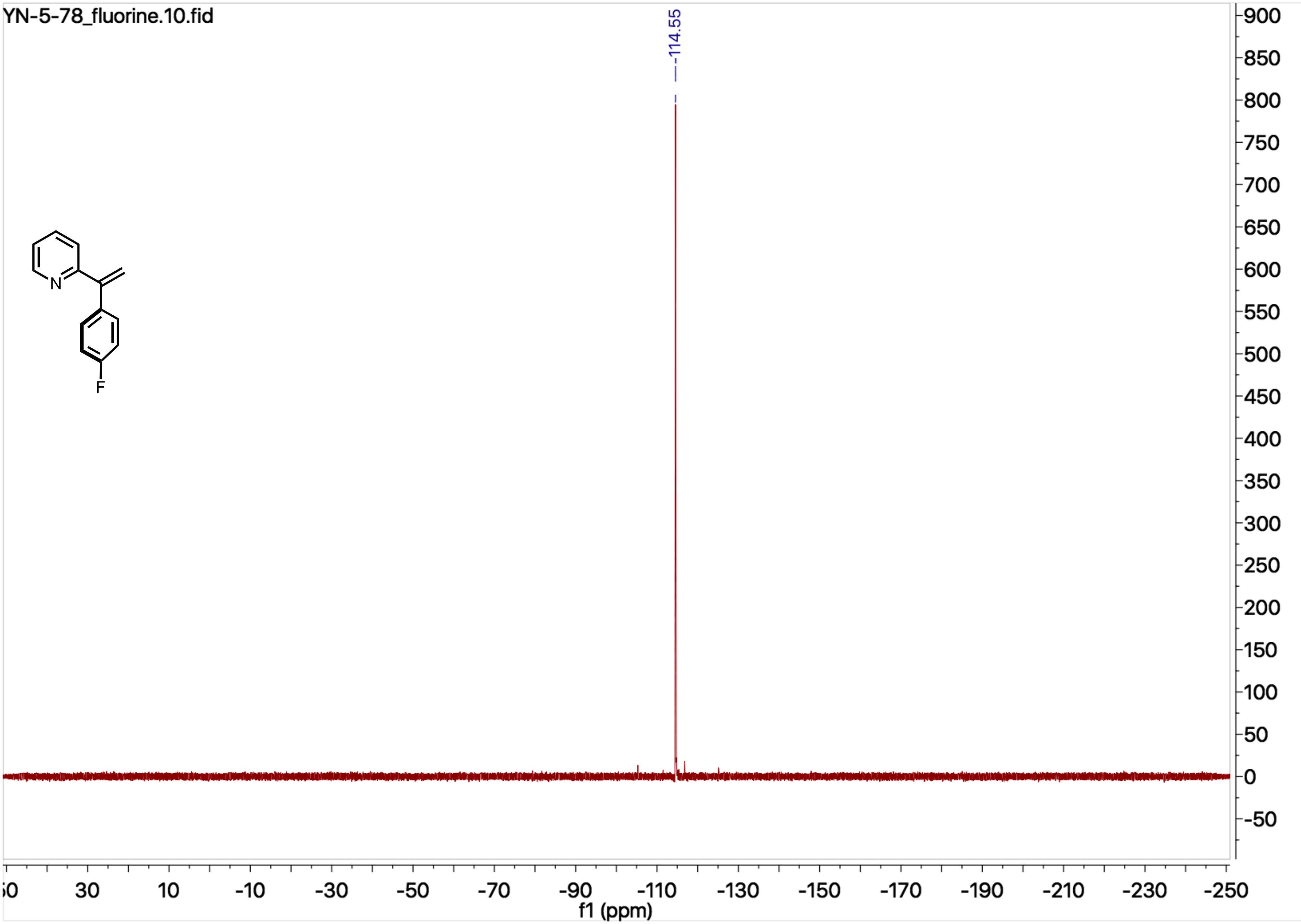
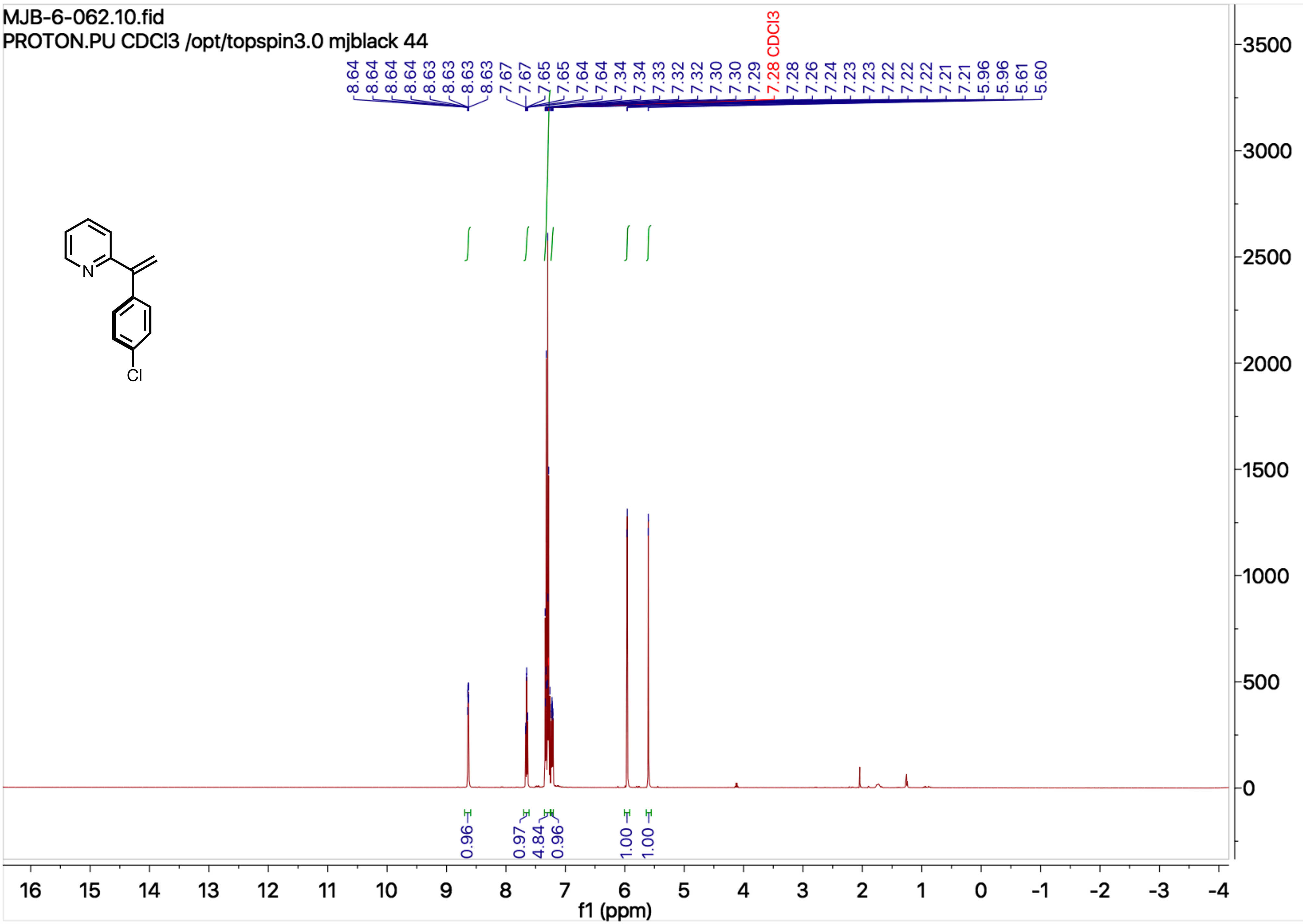
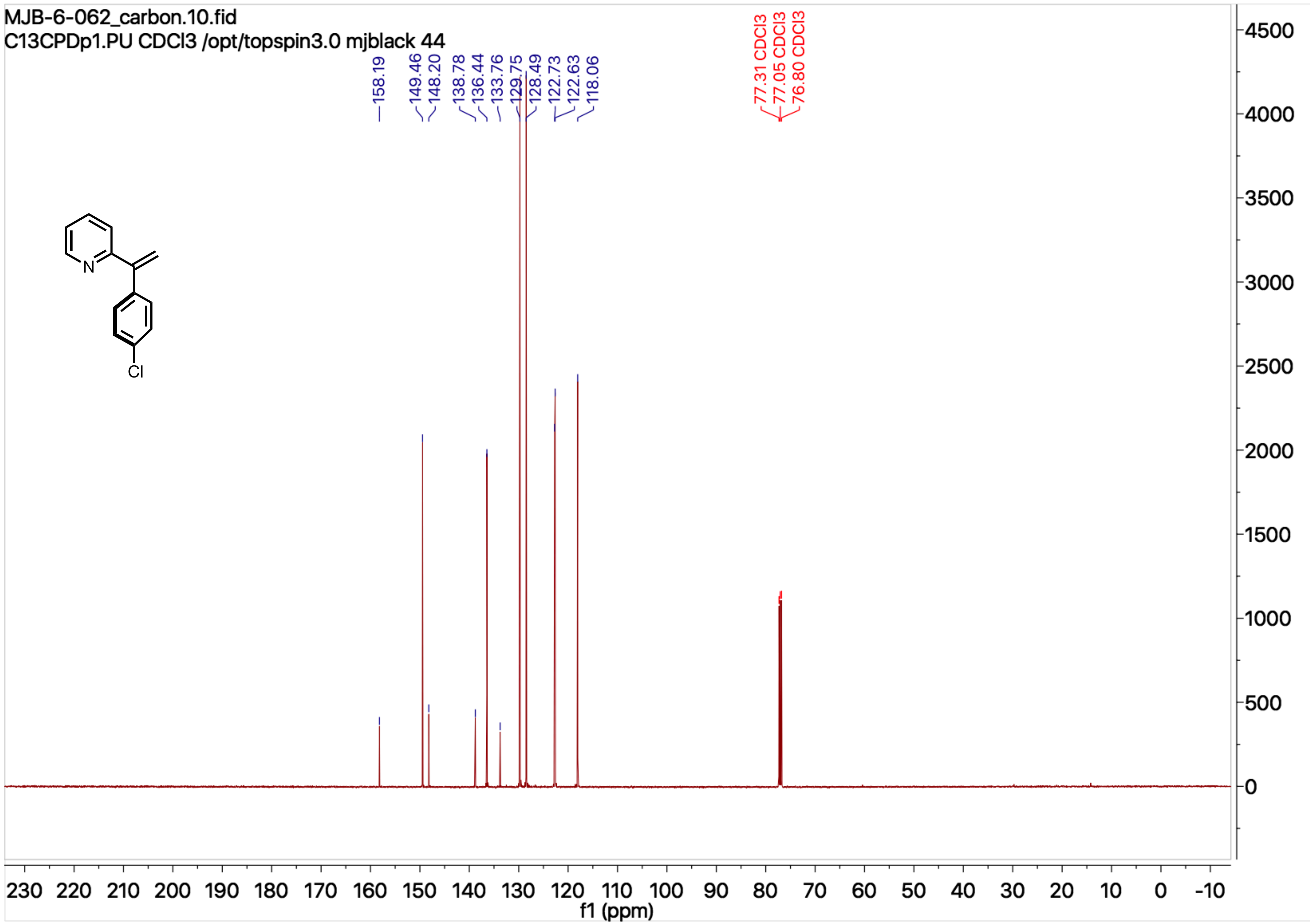
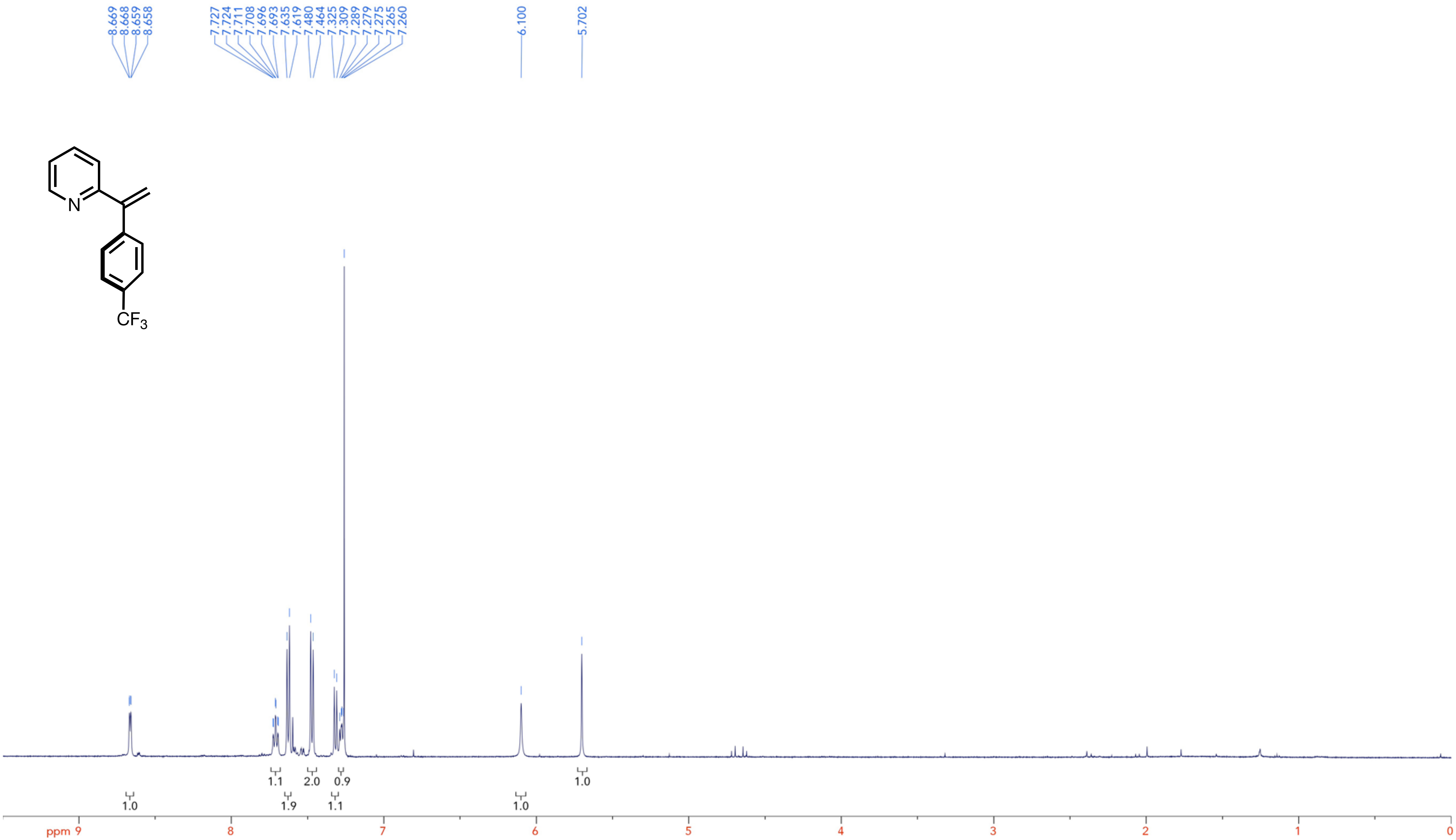
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10



YN-5-78_fluorine.10.fid
-114.55
N
F
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
30
10
-10
-30
-50
-70
-90
-110
-130
-150
-170
-190
-210
-230
-250
f1 (ppm)

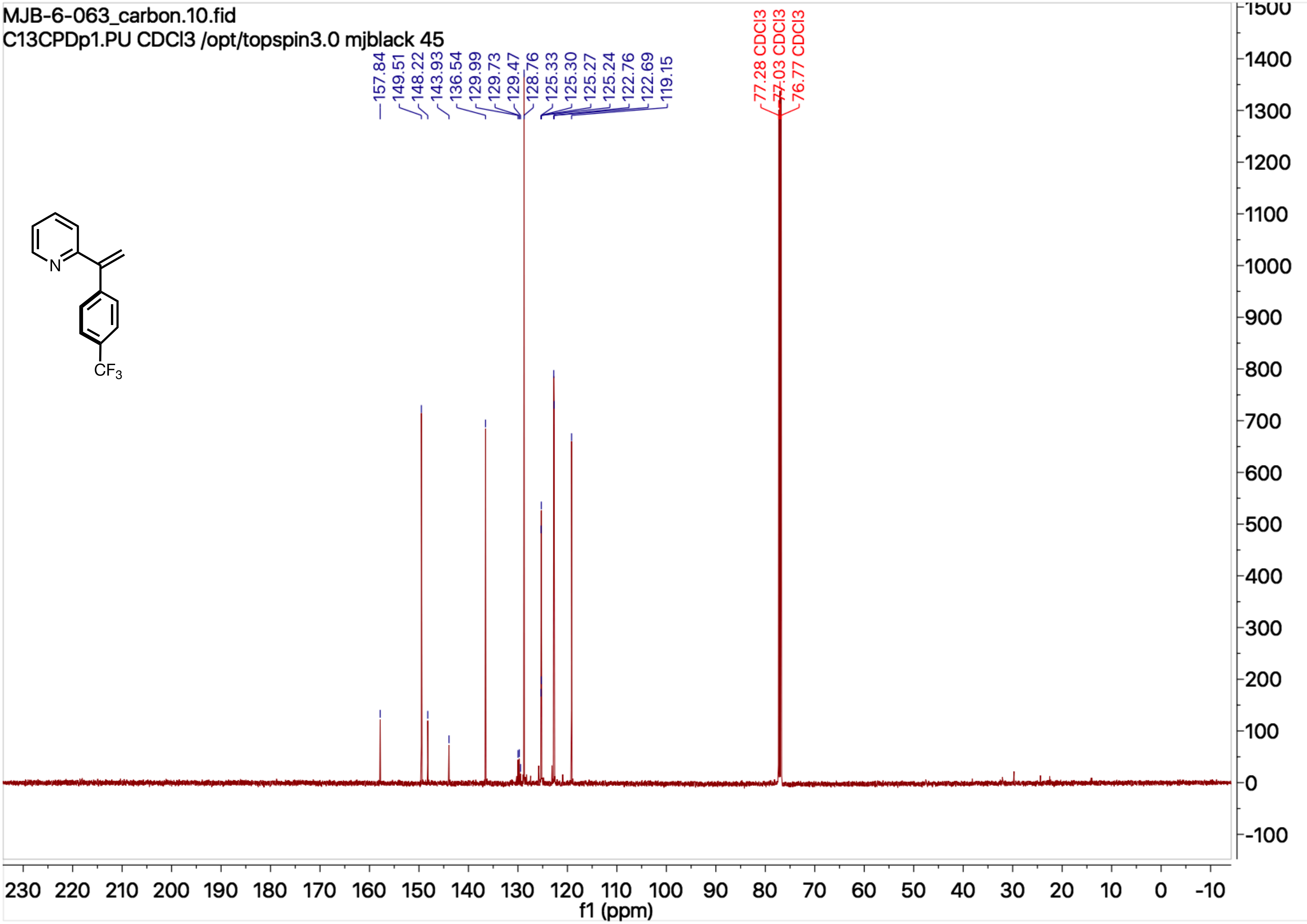

MJB-6-062.10.fid
PROTON.PU CDCl3 /opt/topspin3.0 mjblack 44
8.64 8.64 8.64 8.64 8.63 8.63 8.63 8.63 7.67 7.67 7.65 7.65 7.64 7.64 7.34 7.34 7.33 7.32 7.32 7.30 7.30 7.29 7.28 CDCl3 7.28 7.26 7.24 7.23 7.23 7.22 7.22 7.22 7.21 7.21 5.96 5.96 5.61 5.60
N
Cl
0.96
0.97
4.84
0.96
1.00
1.00
3500
3000
2500
2000
1500
1000
500
0
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)

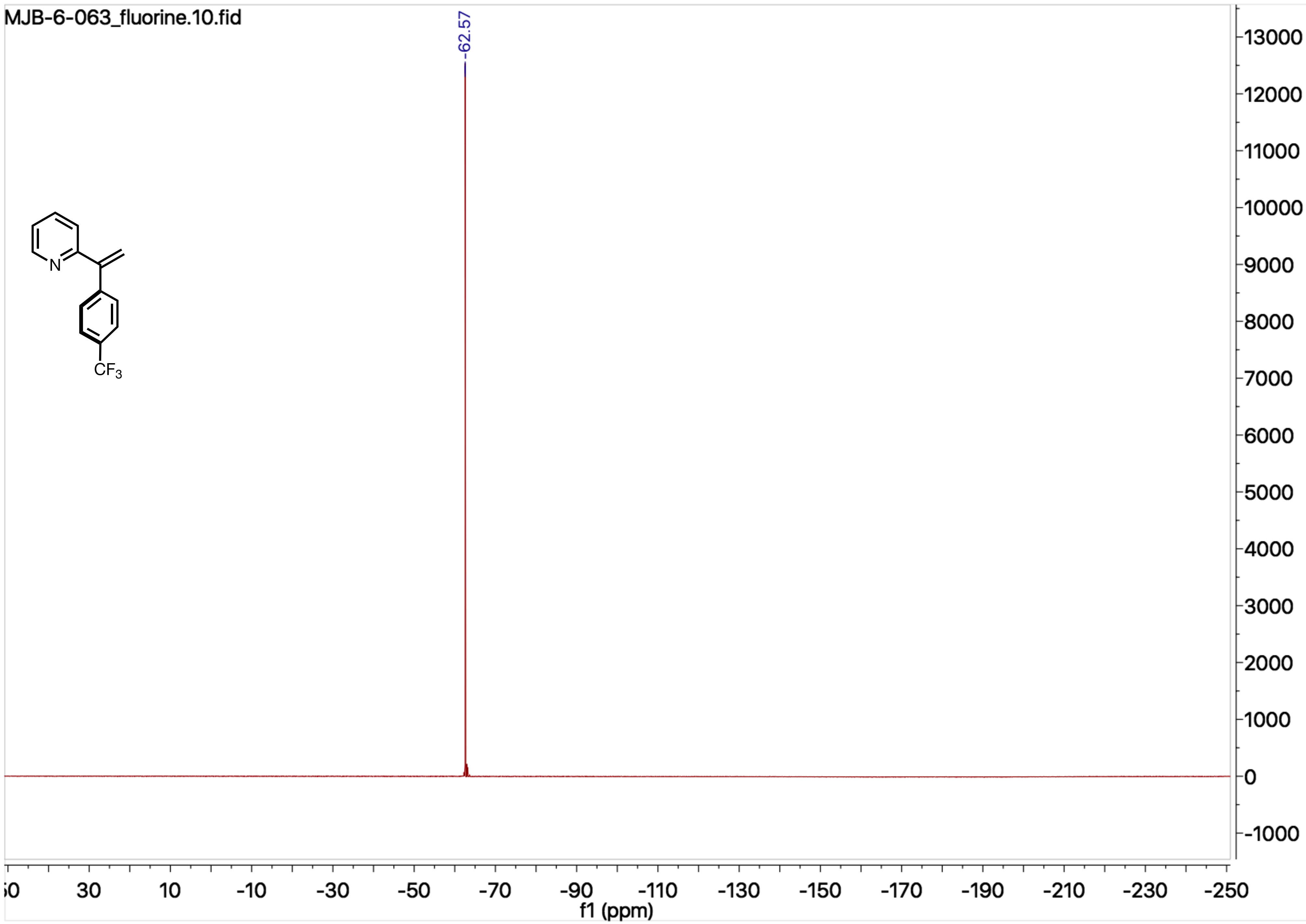

MJB-6-062_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 44
158.19
149.46
148.20
138.78
136.44
133.76
129.75
128.49
122.73
122.63
118.06
77.31 CDCl3
77.05 CDCl3
76.80 CDCl3
N
Cl
4500
4000
3500
3000
2500
2000
1500
1000
500
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

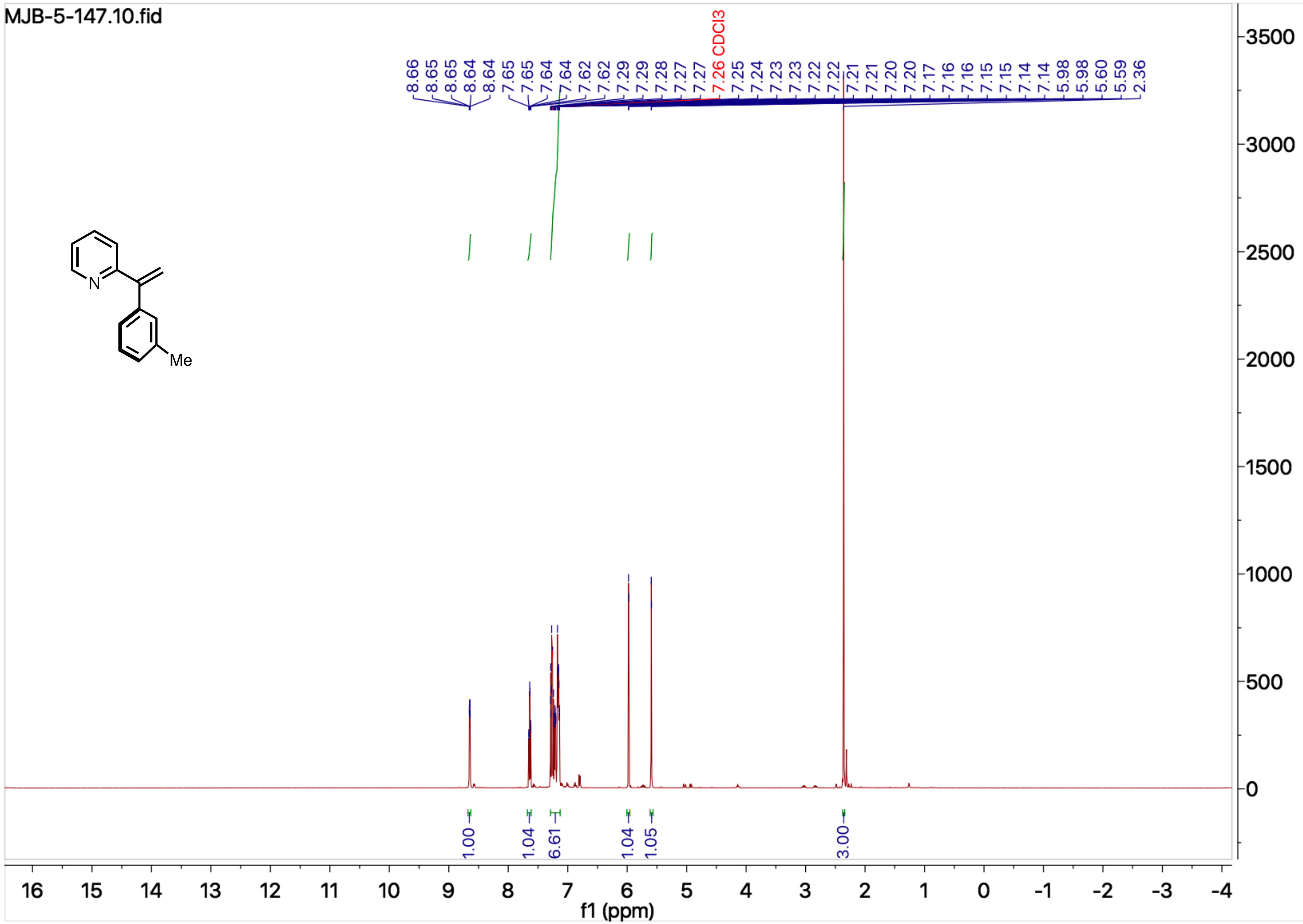


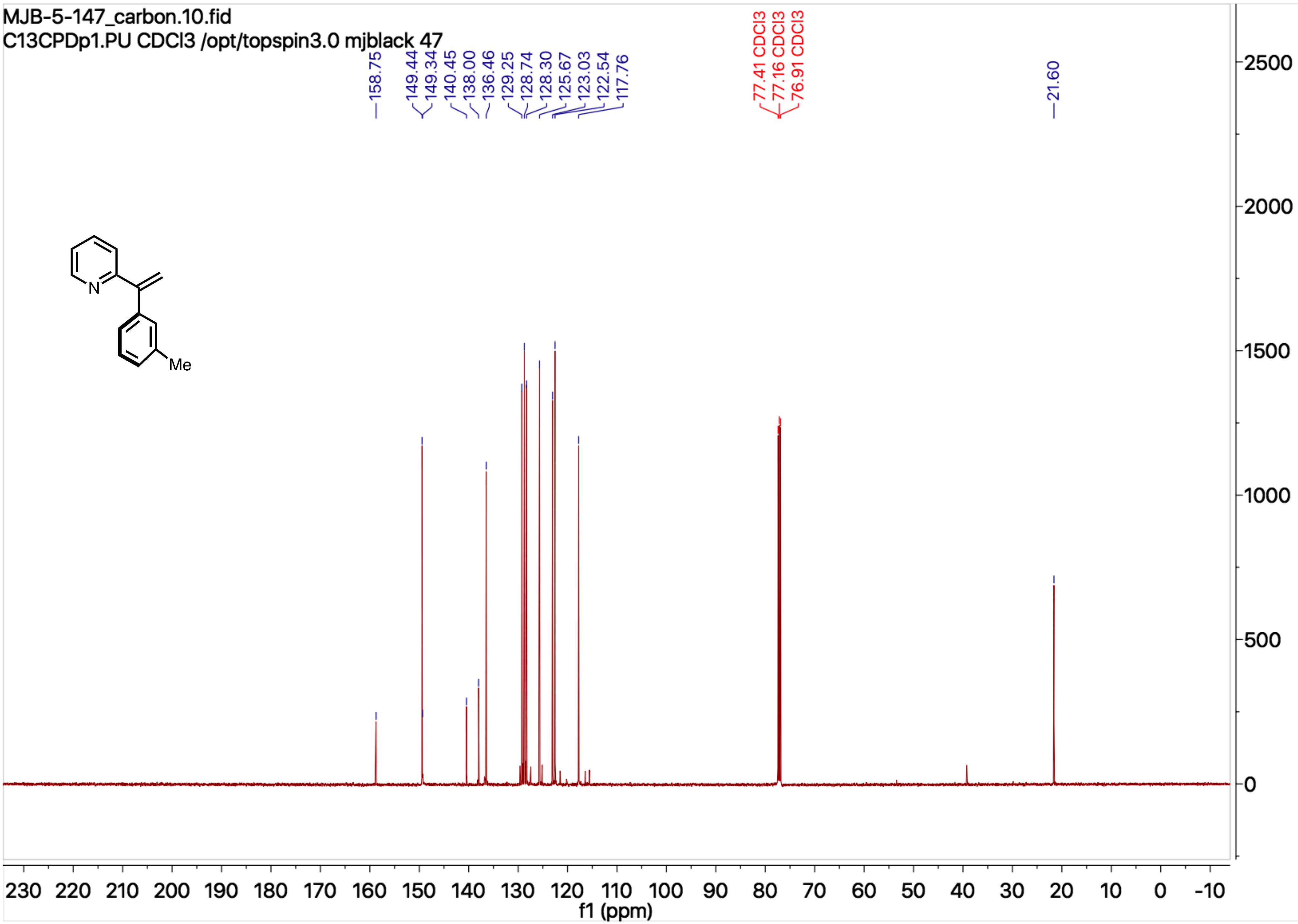
8.669
8.668
8.659
8.658
7.727
7.724
7.711
7.708
7.696
7.693
7.635
7.619
7.480
7.464
7.325
7.309
7.289
7.279
7.275
7.265
7.260
6.100
5.702
N
CF3
1.0
1.1
1.9
2.0
1.1
0.9
1.0
1.0
ppm 9
8
7
6
5
4
3
2
1
0

MJB-6-063_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 45

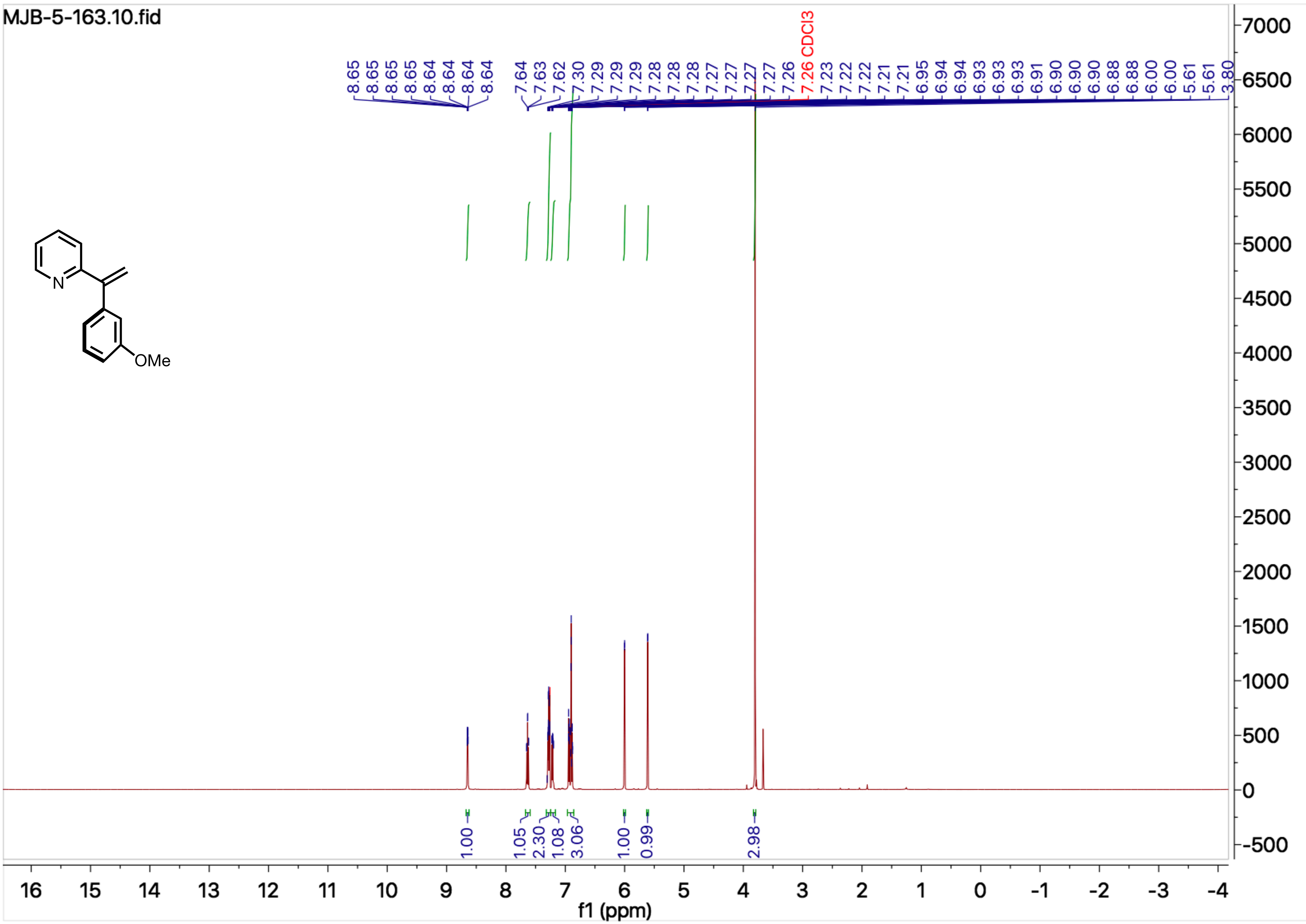



MJB-6-063_fluorine.10.fid
-62.57
N
CF3
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
30
10
-10
-30
-50
-70
-90
-110
-130
-150
-170
-190
-210
-230
-250
f1 (ppm)

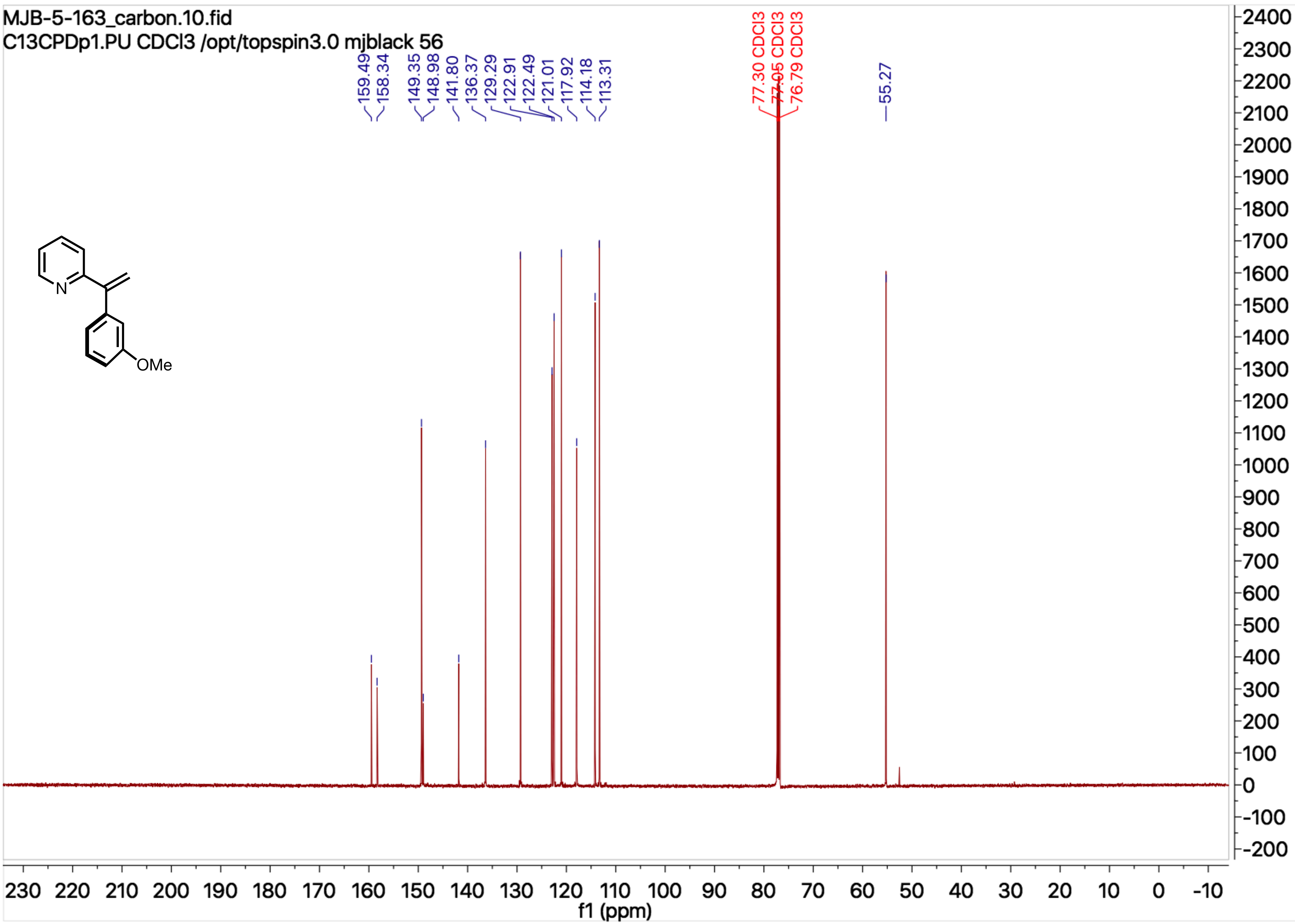

MJB-5-147.10.fid
8.66
8.65
8.65
8.64
8.64
7.65
7.65
7.64
7.64
7.62
7.62
7.29
7.29
7.28
7.27
7.27
7.26 CDCl3
7.25
7.24
7.23
7.23
7.22
7.22
7.21
7.21
7.20
7.20
7.17
7.16
7.16
7.15
7.15
7.14
7.14
5.98
5.98
5.60
5.59
2.36
N
Me
1.00
1.04
6.61
1.04
1.05
3.00
f1 (ppm)

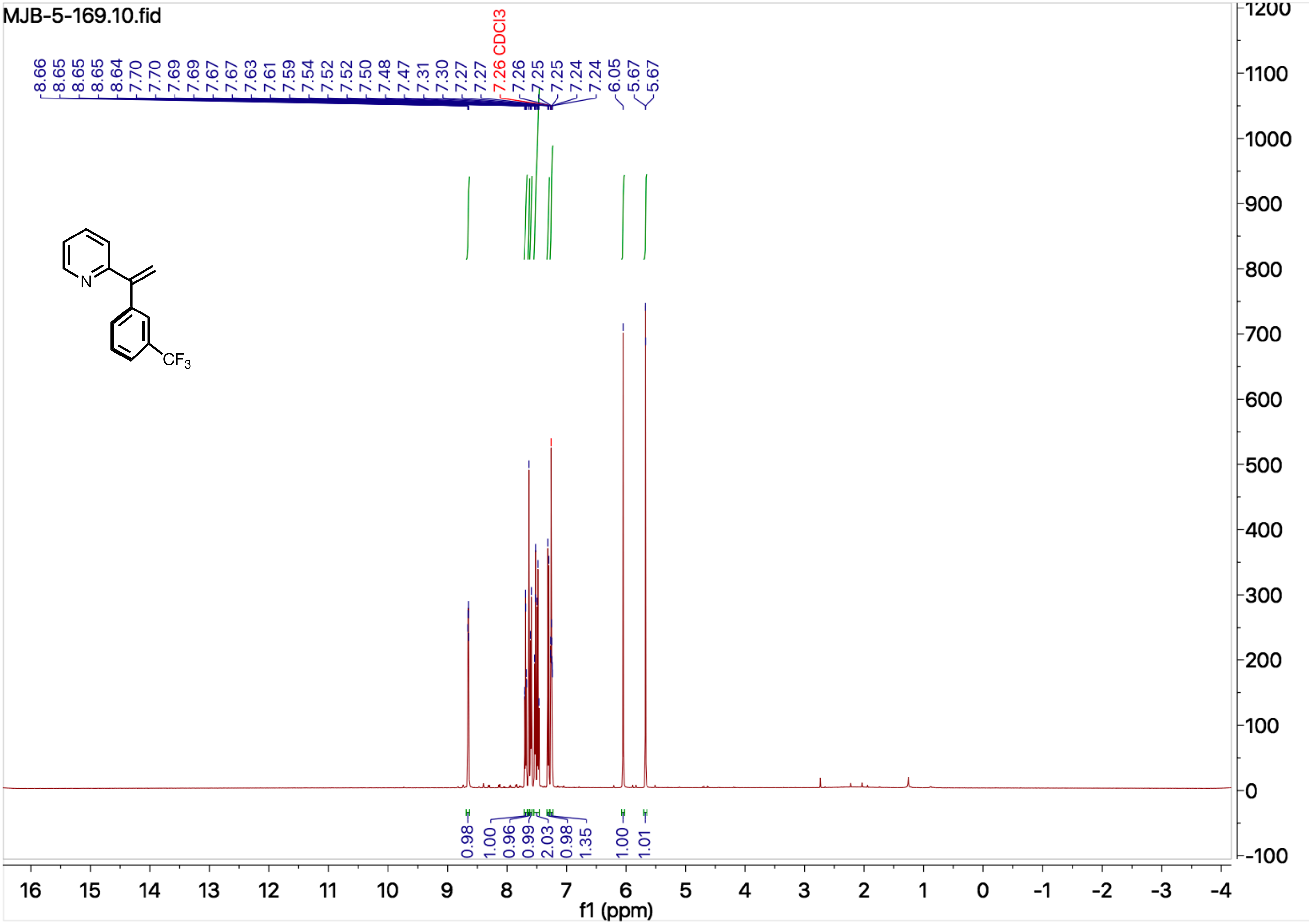


MJB-5-147_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 47
158.75
149.44
149.34
140.45
138.00
136.46
129.25
128.74
128.30
125.67
123.03
122.54
117.76
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
21.60
N
Me
2500
2000
1500
1000
500
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

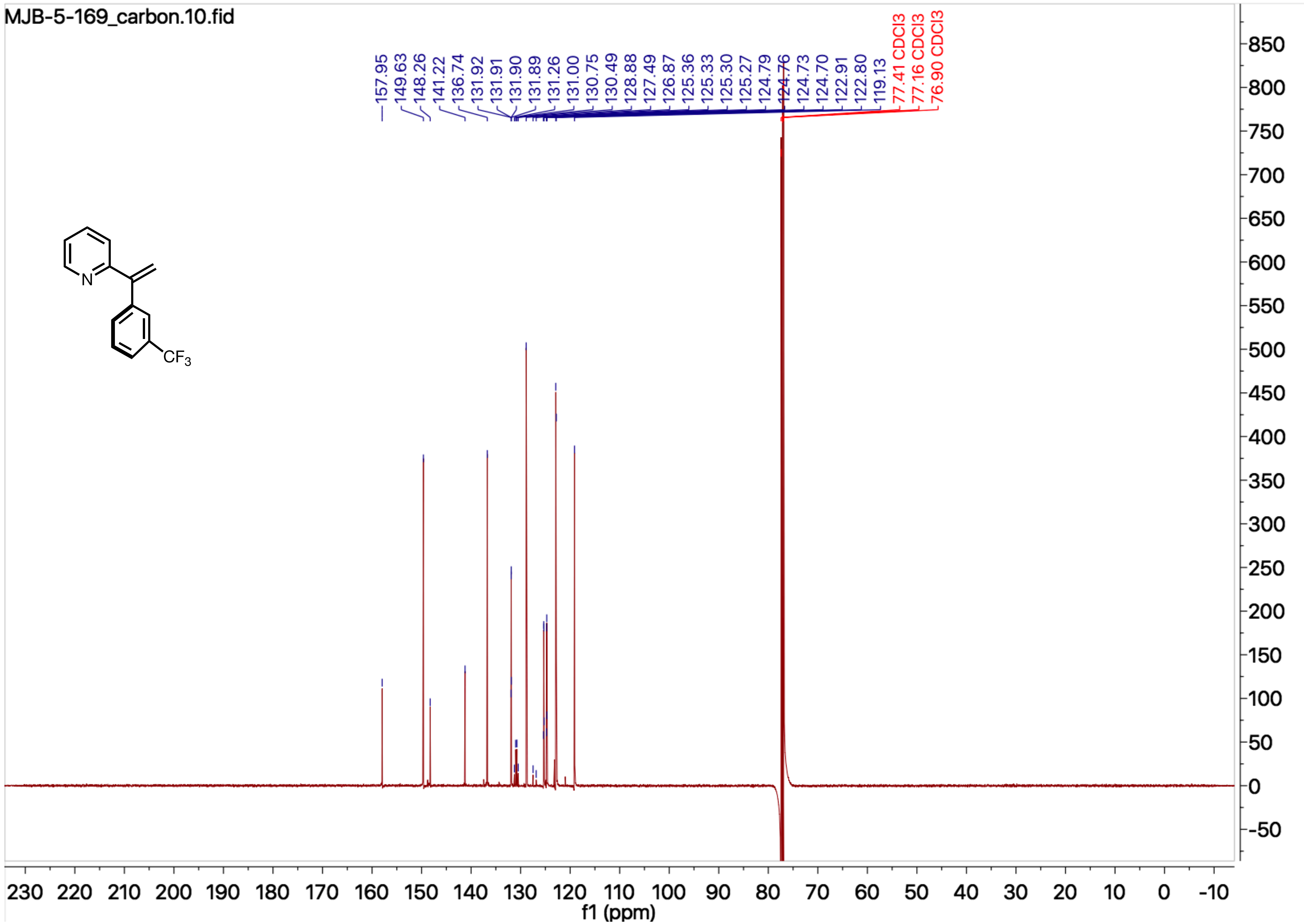


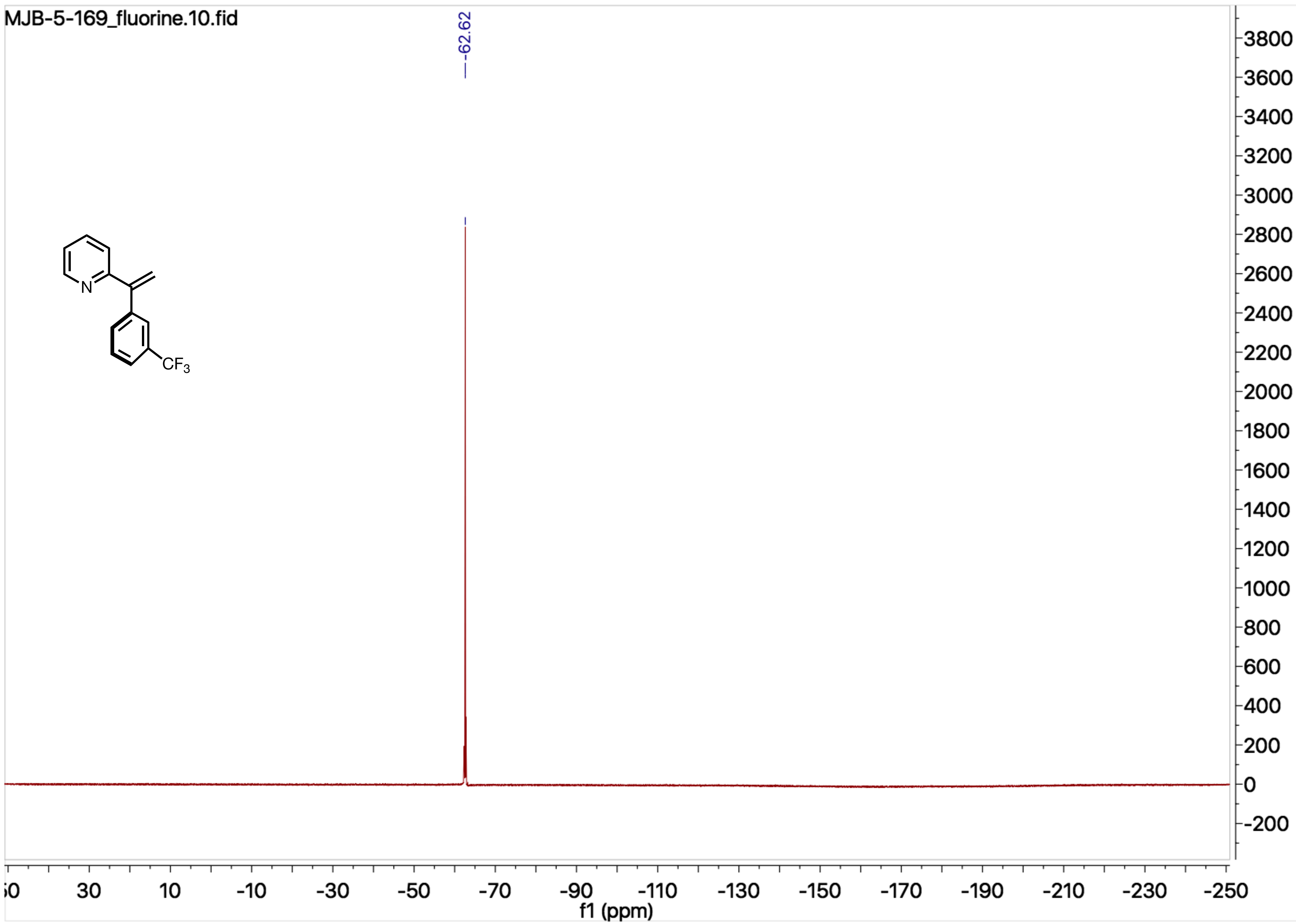
MJB-5-163.10.fid
OMe
N
f1 (ppm)

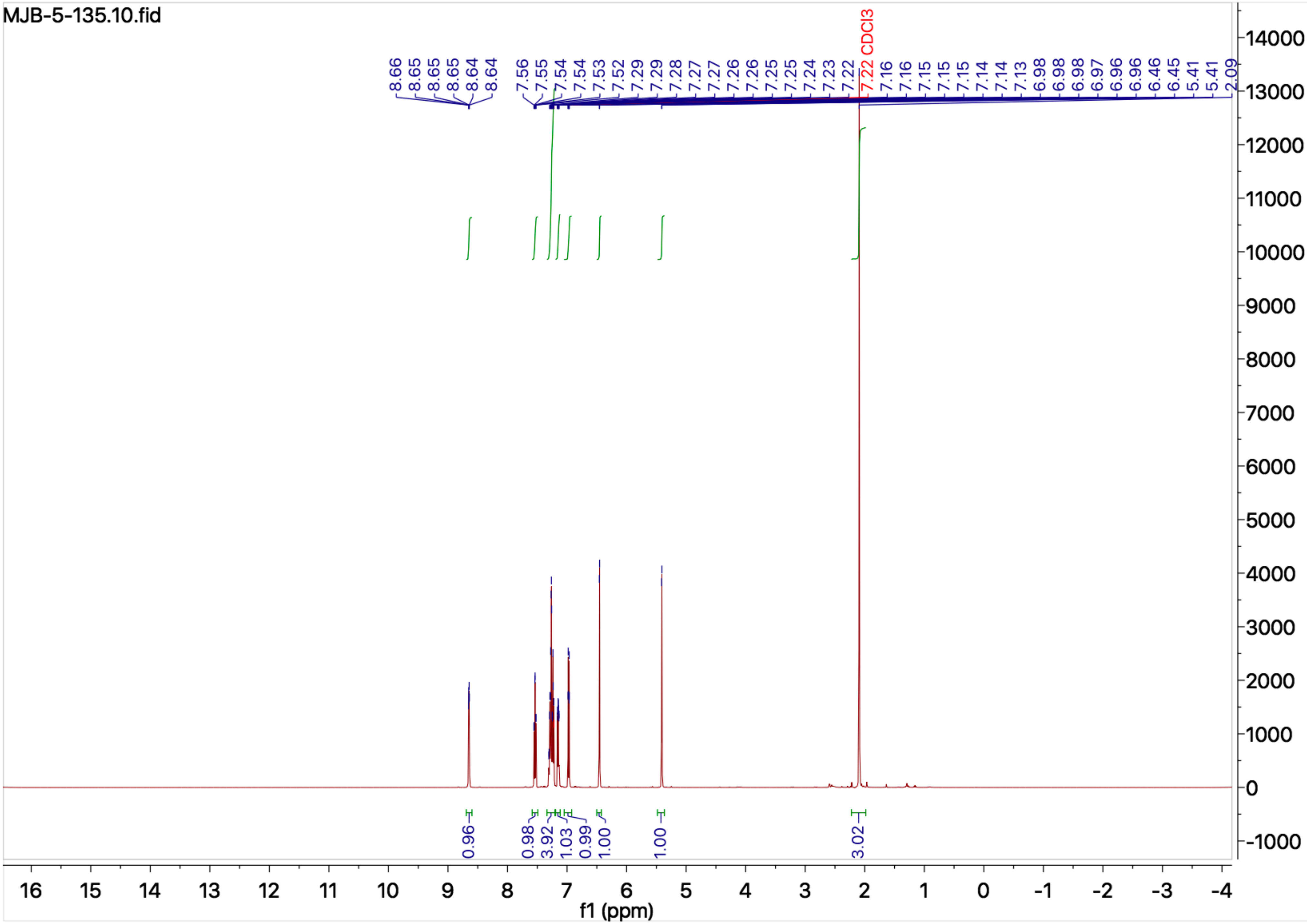

MJB-5-163_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 56
159.49
158.34
149.35
148.98
141.80
136.37
129.29
122.91
122.49
121.01
117.92
114.18
113.31
77.30 CDCl3
77.05 CDCl3
76.79 CDCl3
55.27
N
OMe
f1 (ppm)



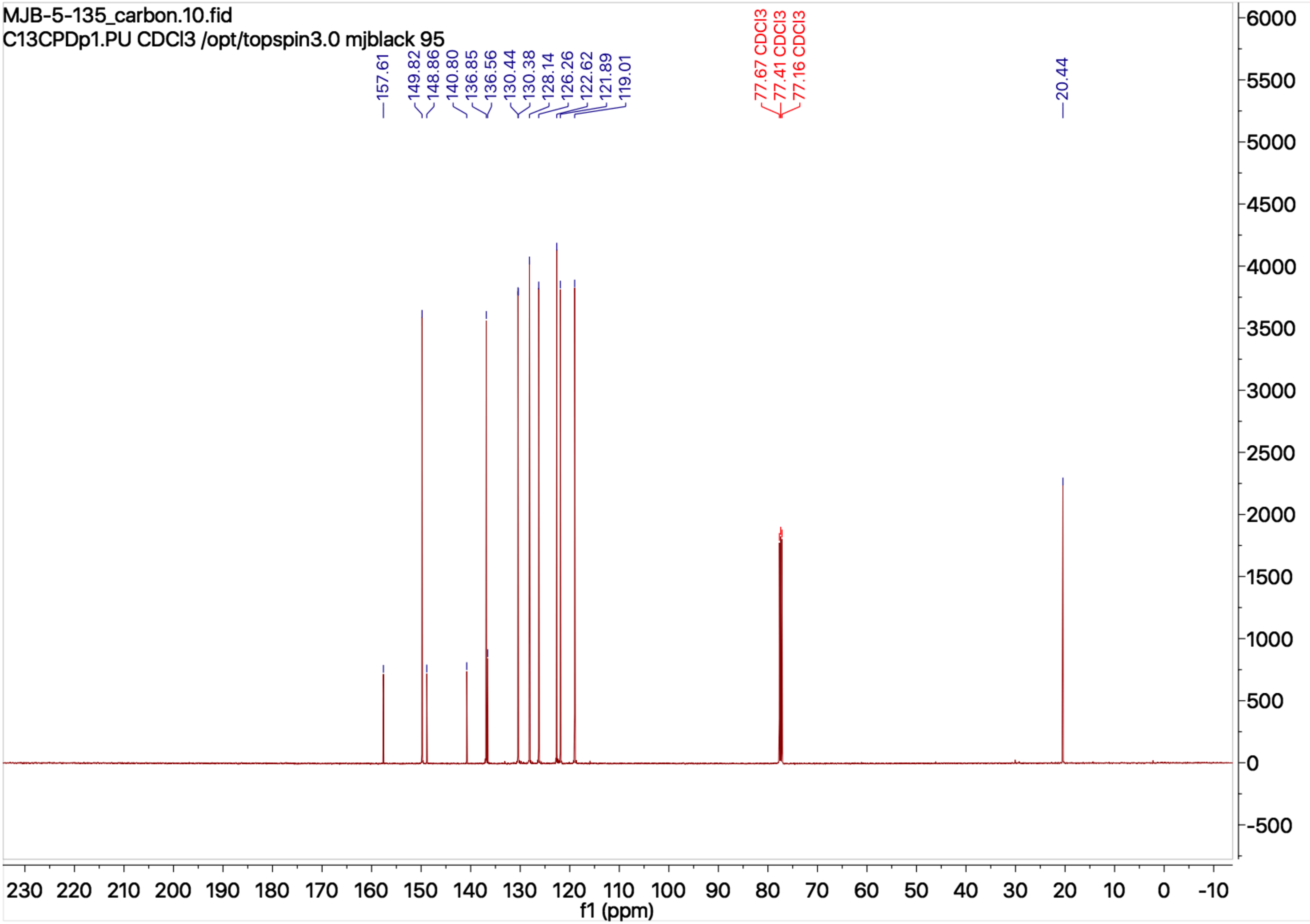
MJB-5-169.10.fid
8.66
8.65
8.65
8.65
8.64
7.70
7.70
7.69
7.69
7.67
7.67
7.63
7.61
7.59
7.54
7.52
7.52
7.50
7.48
7.47
7.31
7.30
7.27
7.27
7.26 CDCl3
7.26
7.25
7.25
7.24
7.24
6.05
5.67
5.67
N
CF3
0.98
1.00
0.96
0.99
2.03
0.98
1.35
1.00
1.01
f1 (ppm)

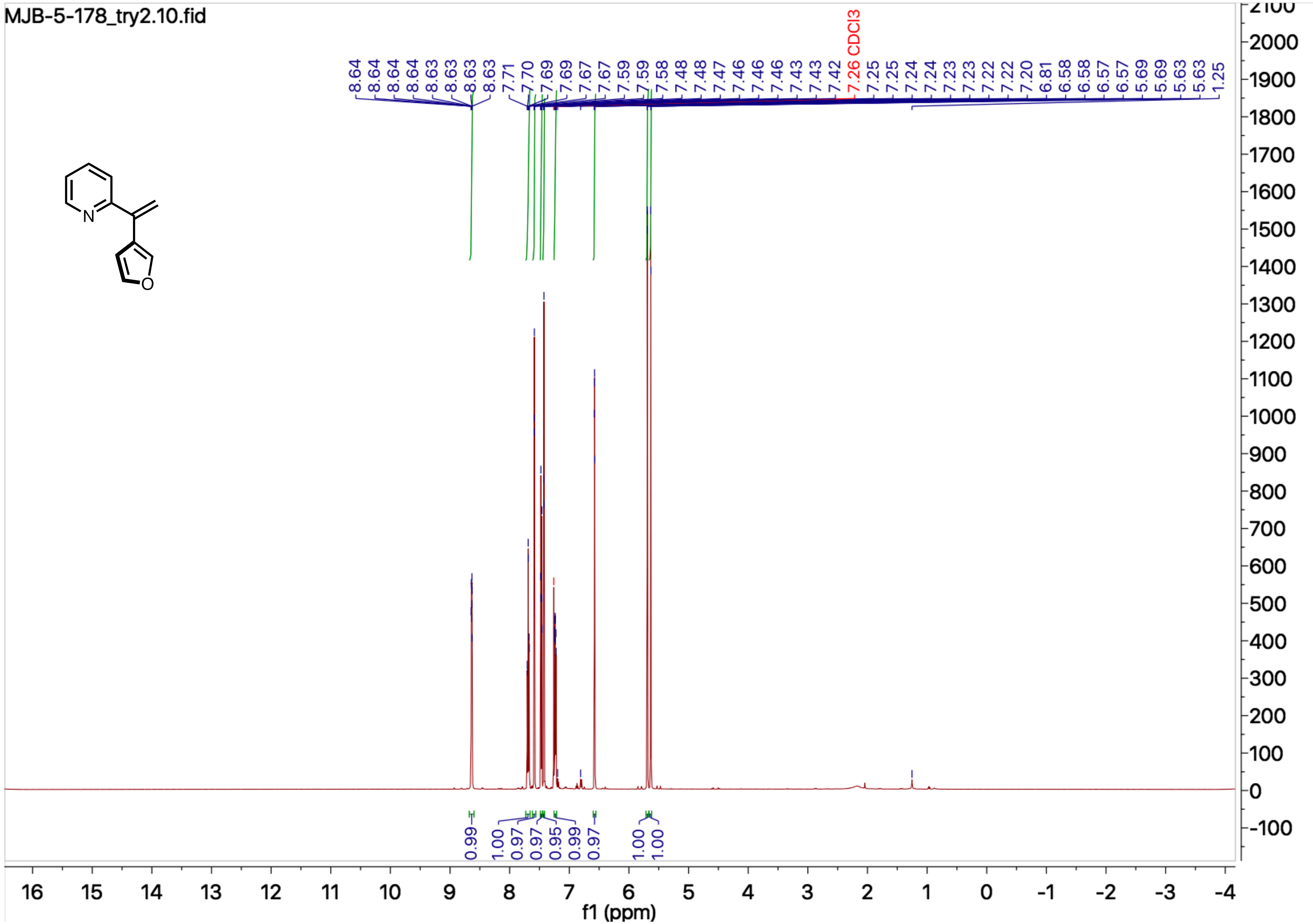

MJB-5-169_carbon.10.fid
157.95
149.63
148.26
141.22
136.74
131.92
131.91
131.90
131.89
131.26
131.00
130.75
130.49
128.88
127.49
126.87
125.36
125.33
125.30
125.27
124.79
124.76
124.73
124.70
122.91
122.80
119.13
77.41 CDCl3
77.16 CDCl3
76.90 CDCl3
N
CF3
f1 (ppm)

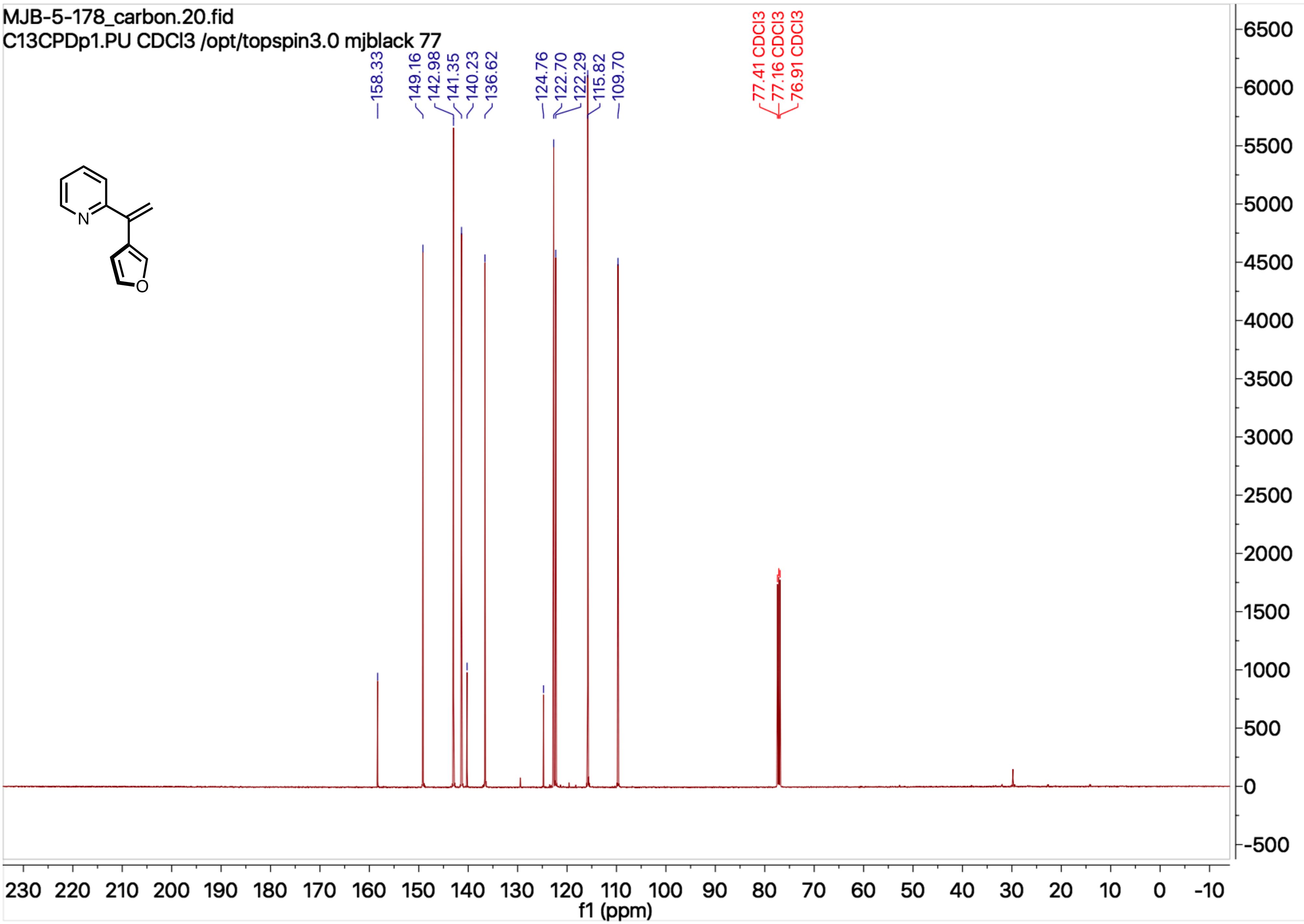

MJB-5-169_fluorine.10.fid
-62.62
N
CF3
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
30
10
-10
-30
-50
-70
-90
-110
-130
-150
-170
-190
-210
-230
-250
f1 (ppm)

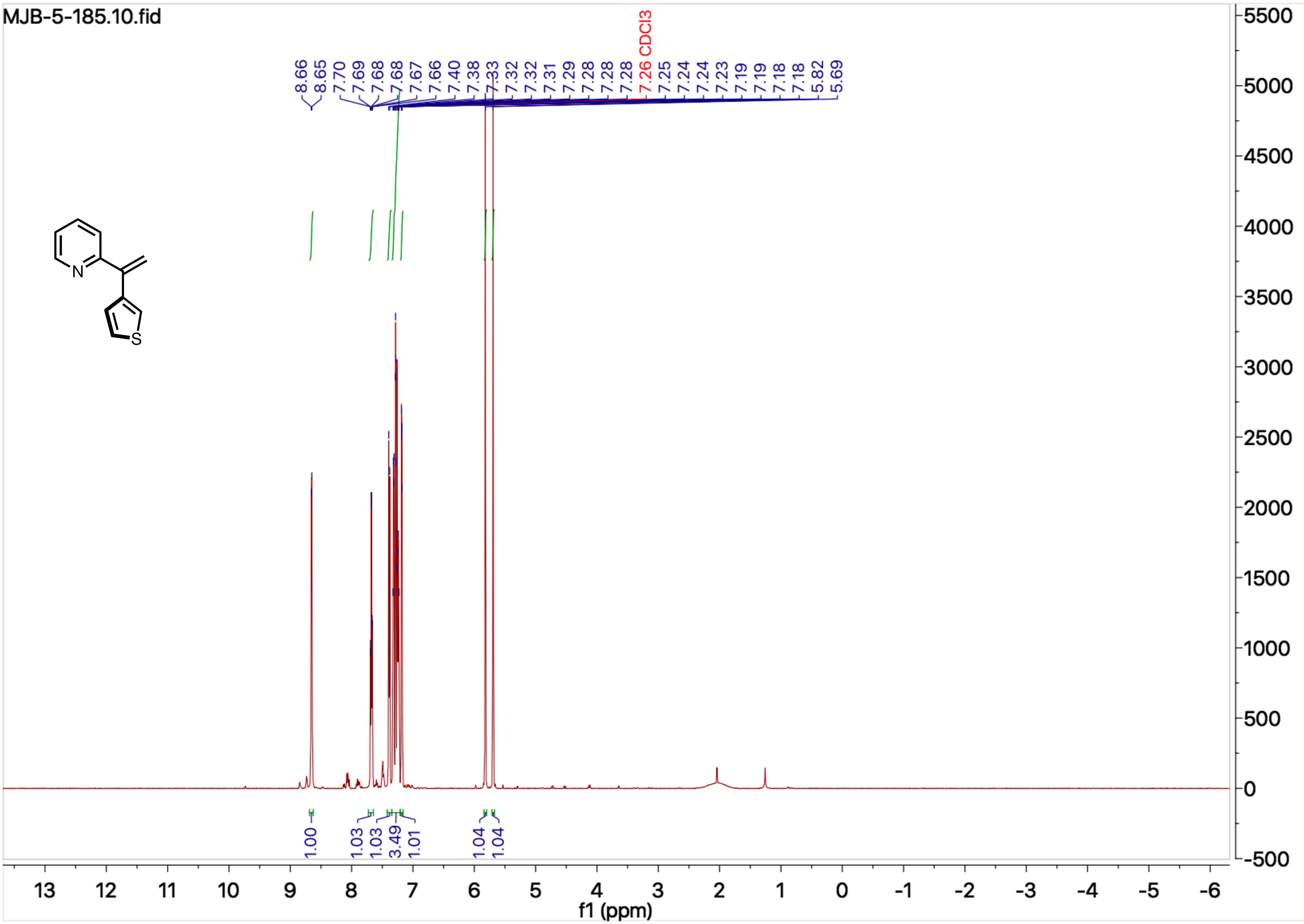


MJB-5-135.10.fid
8.66
8.65
8.65
8.65
8.64
8.64
7.56
7.55
7.54
7.54
7.53
7.52
7.29
7.29
7.28
7.27
7.27
7.26
7.26
7.25
7.25
7.24
7.23
7.22
7.22 CDCl3
7.16
7.16
7.15
7.15
7.15
7.14
7.14
7.13
6.98
6.98
6.98
6.97
6.96
6.96
6.46
6.45
5.41
5.41
2.09
0.96
0.98
3.92
1.03
0.99
1.00
1.00
3.02
f1 (ppm)

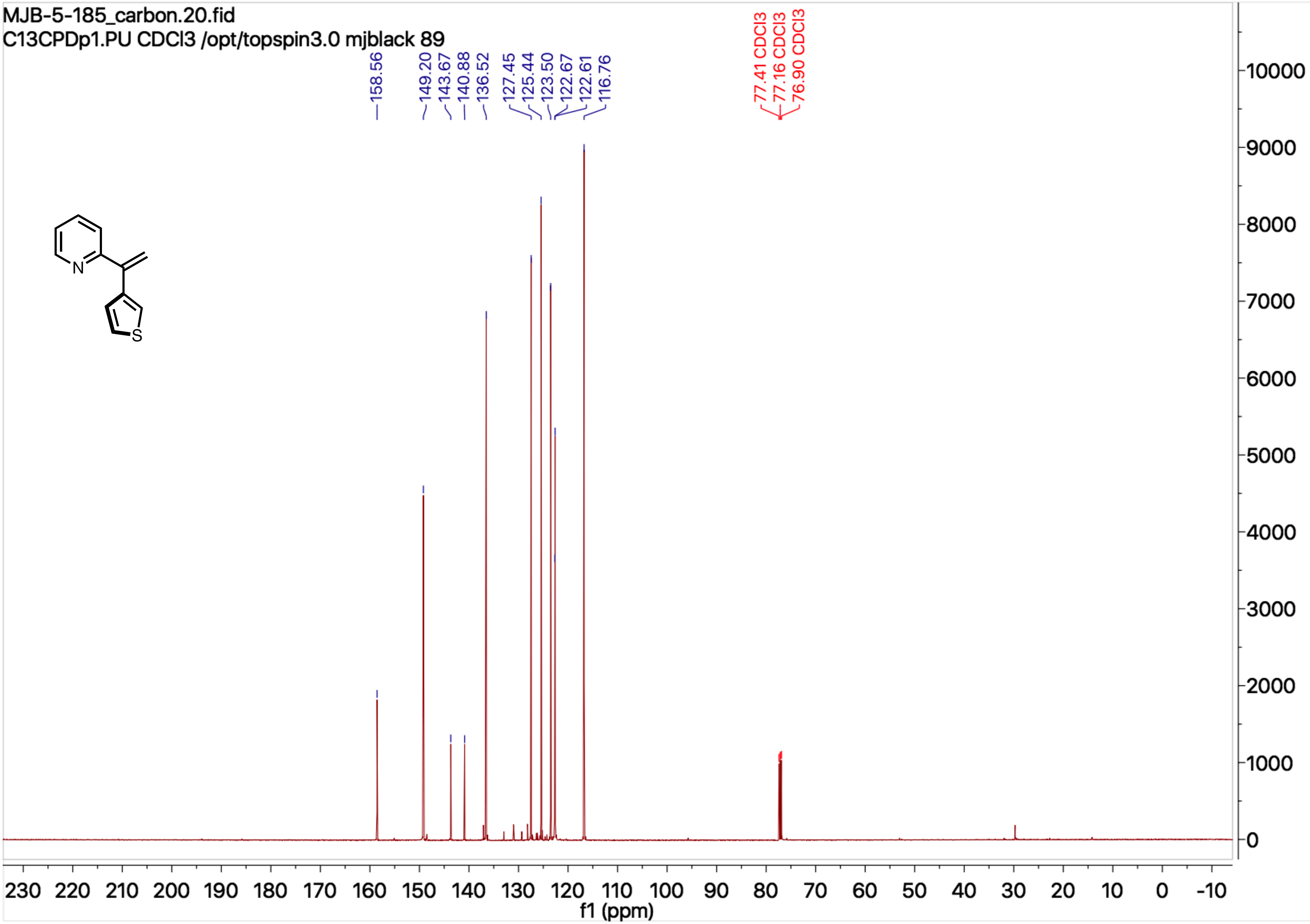

MJB-5-135_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 95
157.61
149.82
148.86
140.80
136.85
136.56
130.44
130.38
128.14
126.26
122.62
121.89
119.01
77.67 CDCl3
77.41 CDCl3
77.16 CDCl3
20.44
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

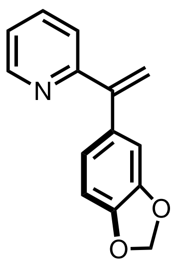

MJB-5-178_try2.10.fid
8.64 8.64 8.64 8.64 8.63 8.63 8.63 8.63 7.71 7.70 7.69 7.69 7.67 7.67 7.59 7.59 7.58 7.48 7.48 7.47 7.46 7.46 7.46 7.43 7.43 7.42 7.26 CDCl3 7.25 7.25 7.24 7.24 7.23 7.23 7.22 7.22 7.20 6.81 6.58 6.58 6.57 6.57 5.69 5.69 5.63 5.63 1.25
N
O
0.99 1.00 0.97 0.97 0.95 0.99 0.97 1.00 1.00
f1 (ppm)

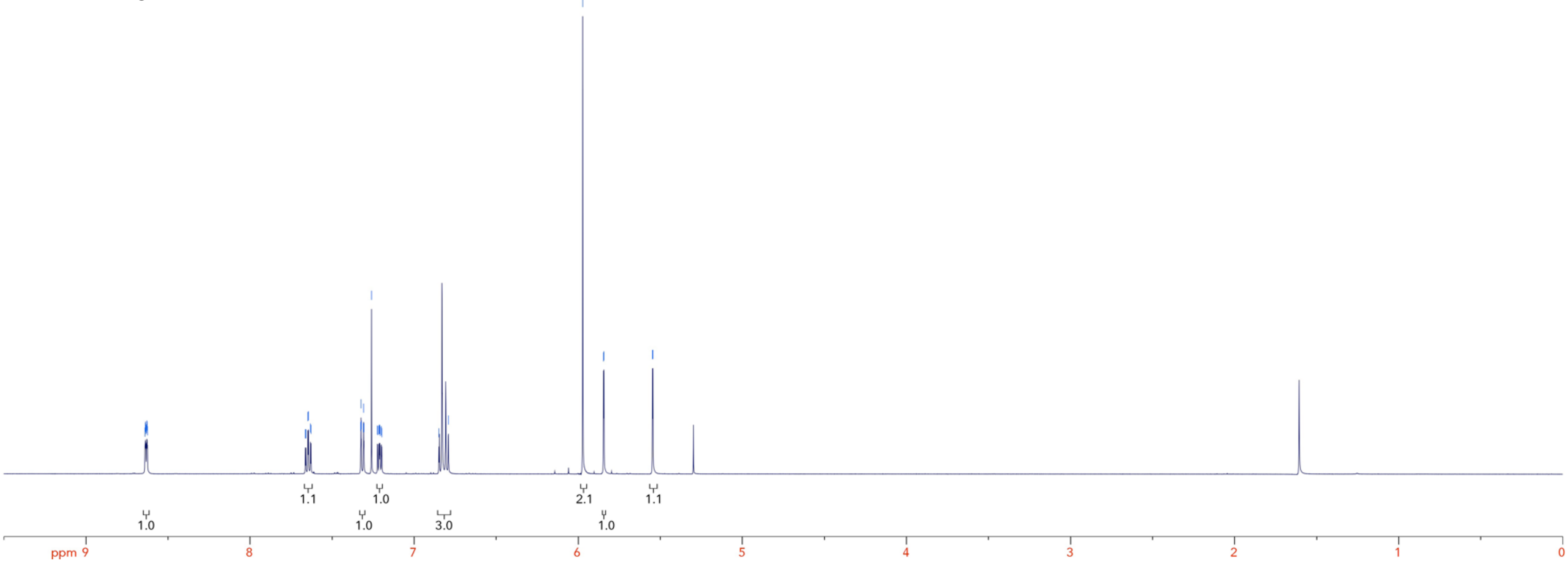

MJB-5-178_carbon.20.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 77
158.33
149.16
142.98
141.35
140.23
136.62
124.76
122.70
122.29
115.82
109.70
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
N
O
f1 (ppm)


MJB-5-185.10.fid
8.66
8.65
7.70
7.69
7.68
7.68
7.67
7.66
7.40
7.38
7.33
7.32
7.32
7.31
7.29
7.28
7.28
7.28
7.26 CDCl3
7.25
7.24
7.24
7.23
7.19
7.19
7.18
7.18
5.82
5.69
N
S
1.00
1.03
1.03
3.49
1.01
1.04
1.04
f1 (ppm)

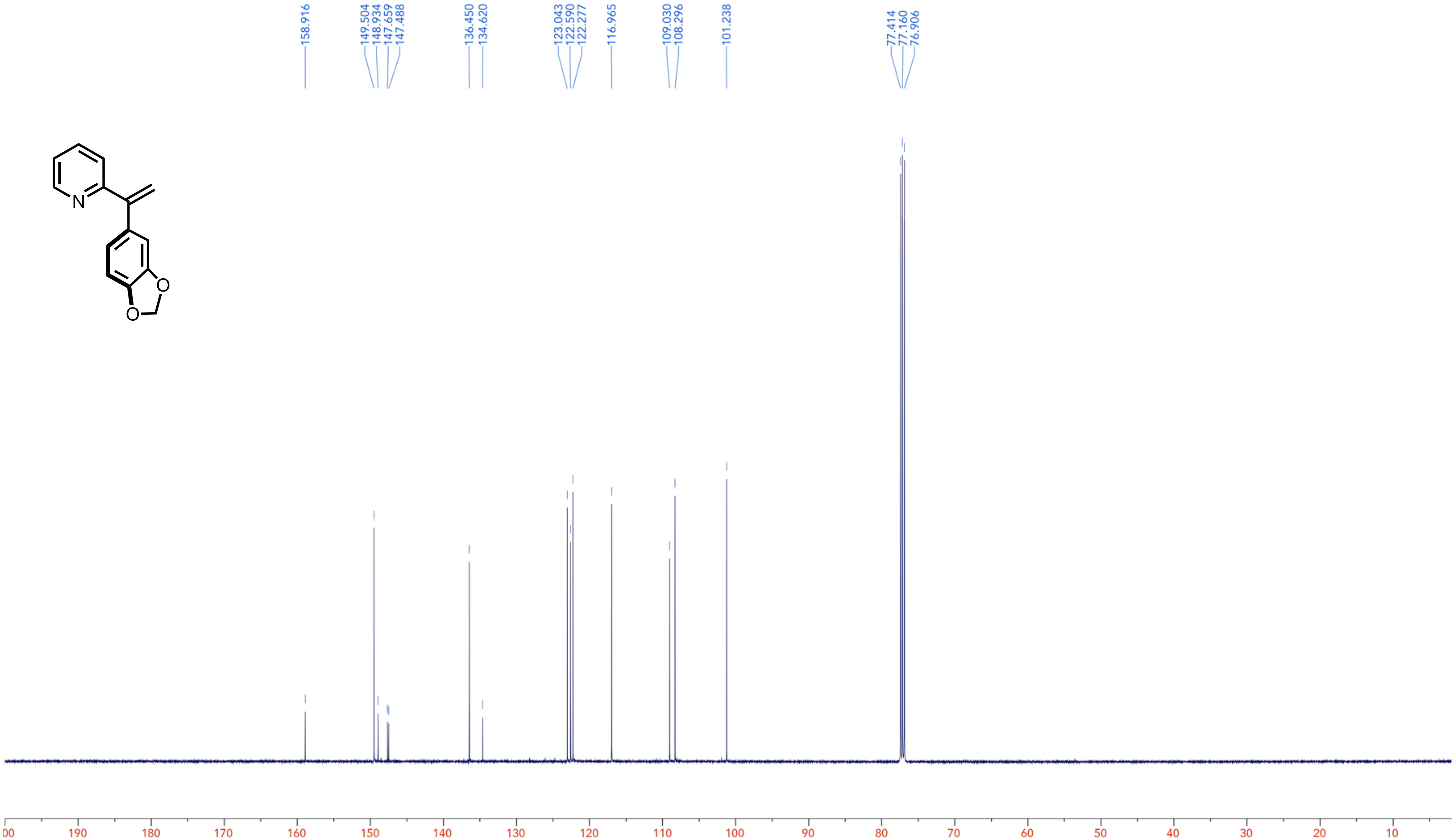
MJB-5-185_carbon.20.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 89



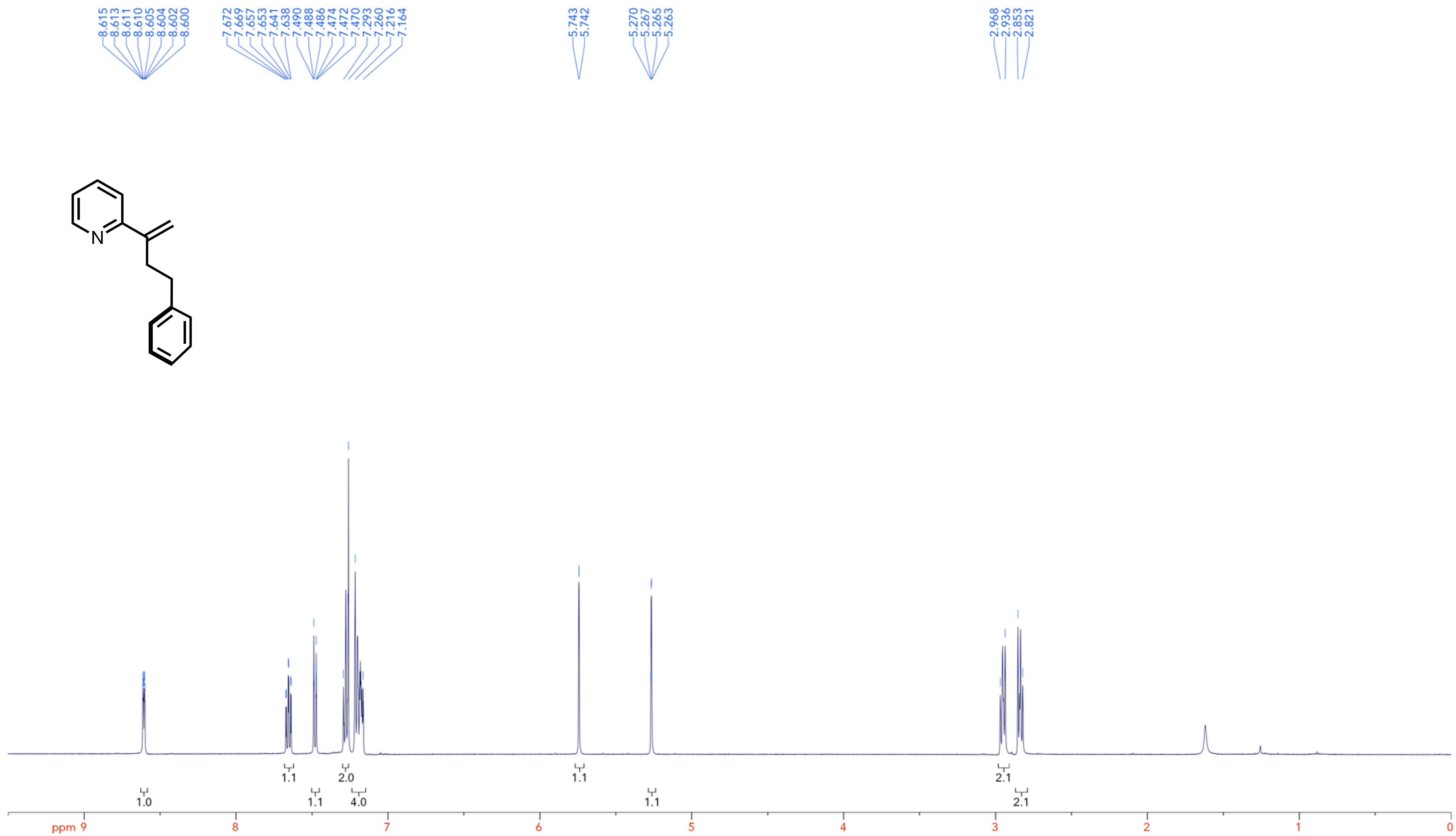


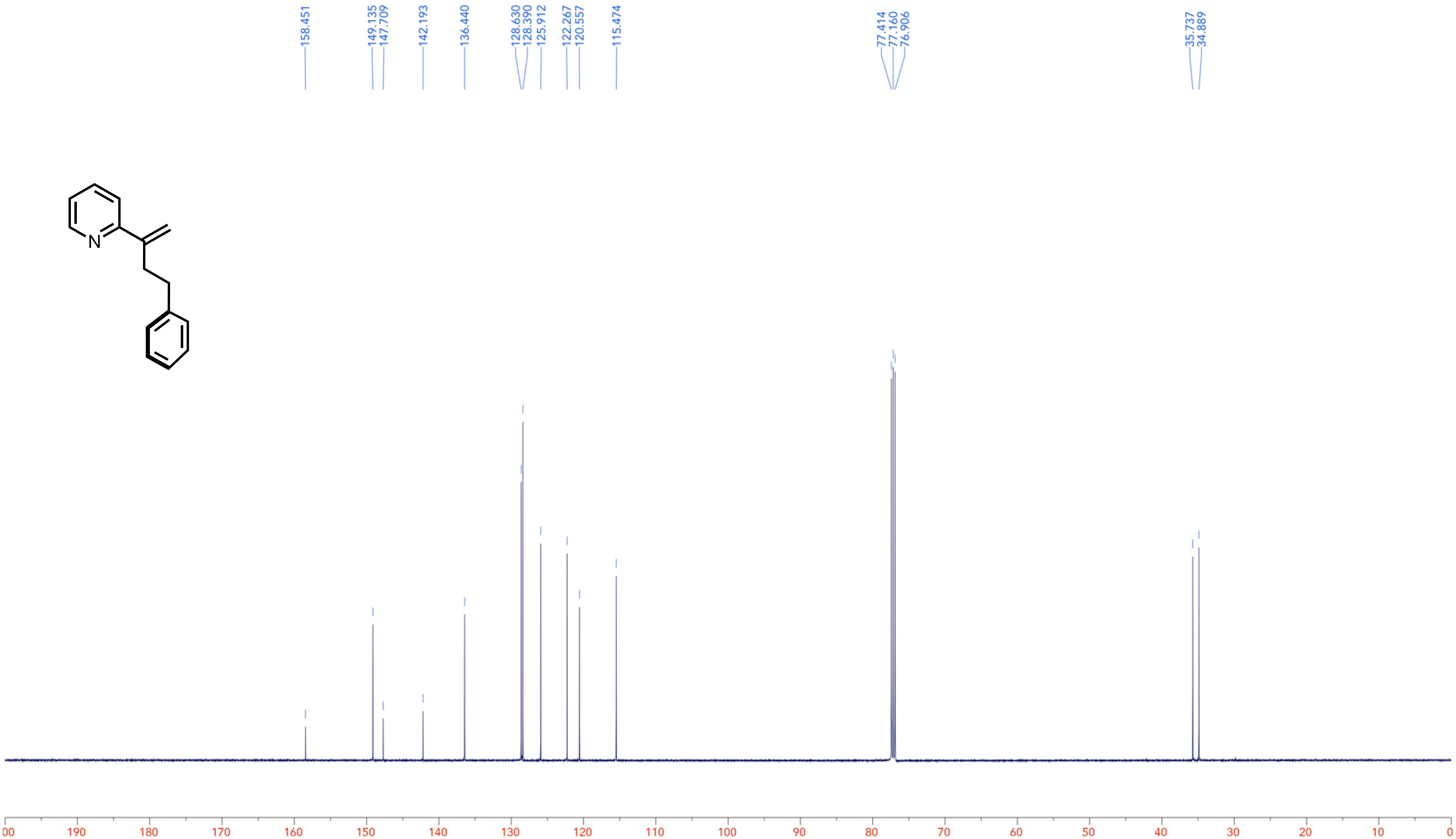


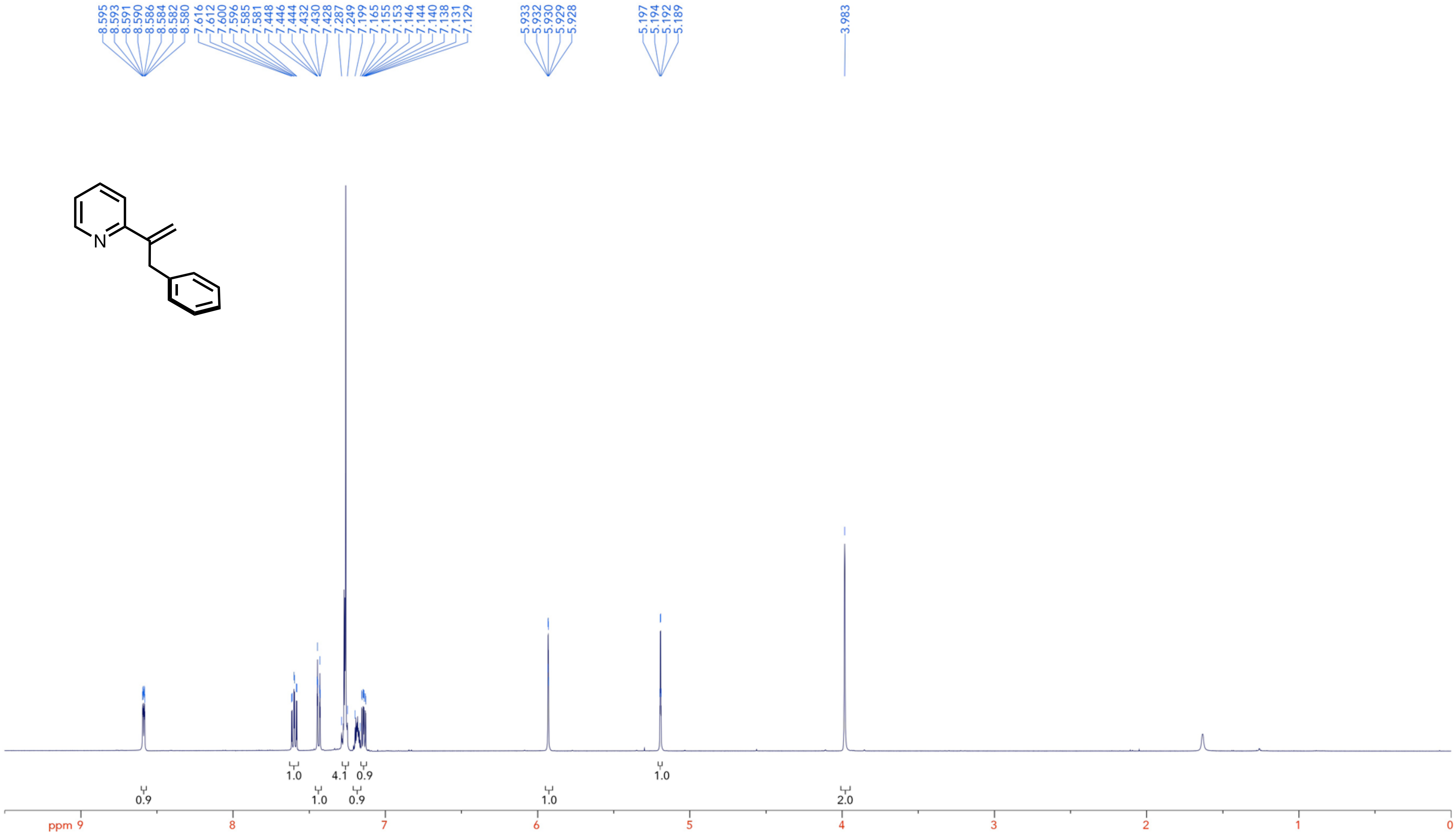
8.641
8.639
8.637
8.635
8.631
8.629
8.627
8.625
7.663
7.660
7.648
7.644
7.633
7.629
7.326
7.324
7.322
7.310
7.308
7.306
7.260
7.224
7.222
7.214
7.212
7.209
7.207
7.199
7.197
6.850
6.791
5.973
5.846
5.844
5.548
5.545
1.1
1.0
2.1
1.1
1.0
1.0
3.0
1.0
ppm 9
8
7
6
5
4
3
2
1
0



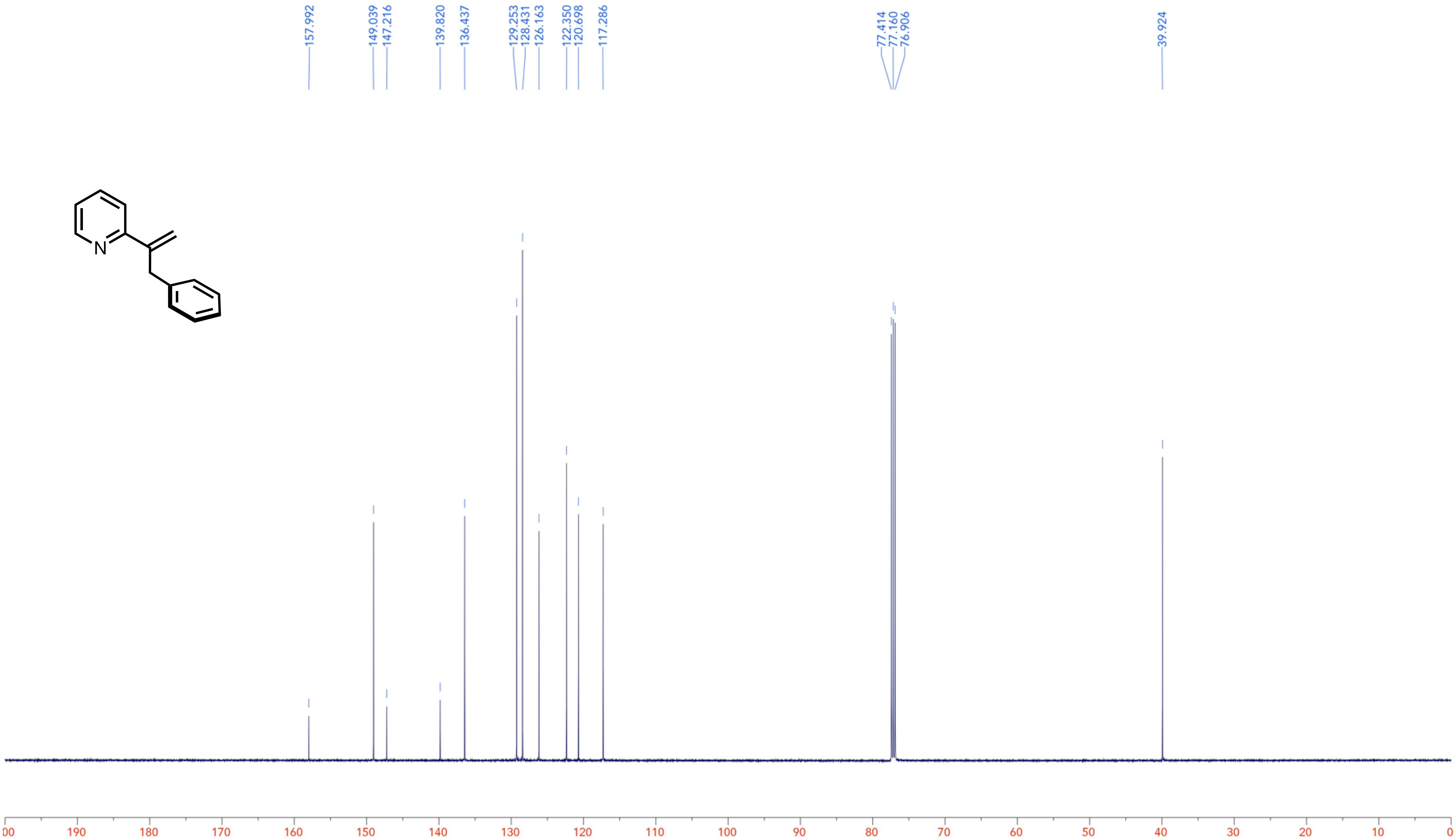
158.916
149.504
148.934
147.659
147.488
136.450
134.620
123.043
122.590
122.277
116.965
109.030
108.296
101.238
77.414
77.160
76.906
N
O
O
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

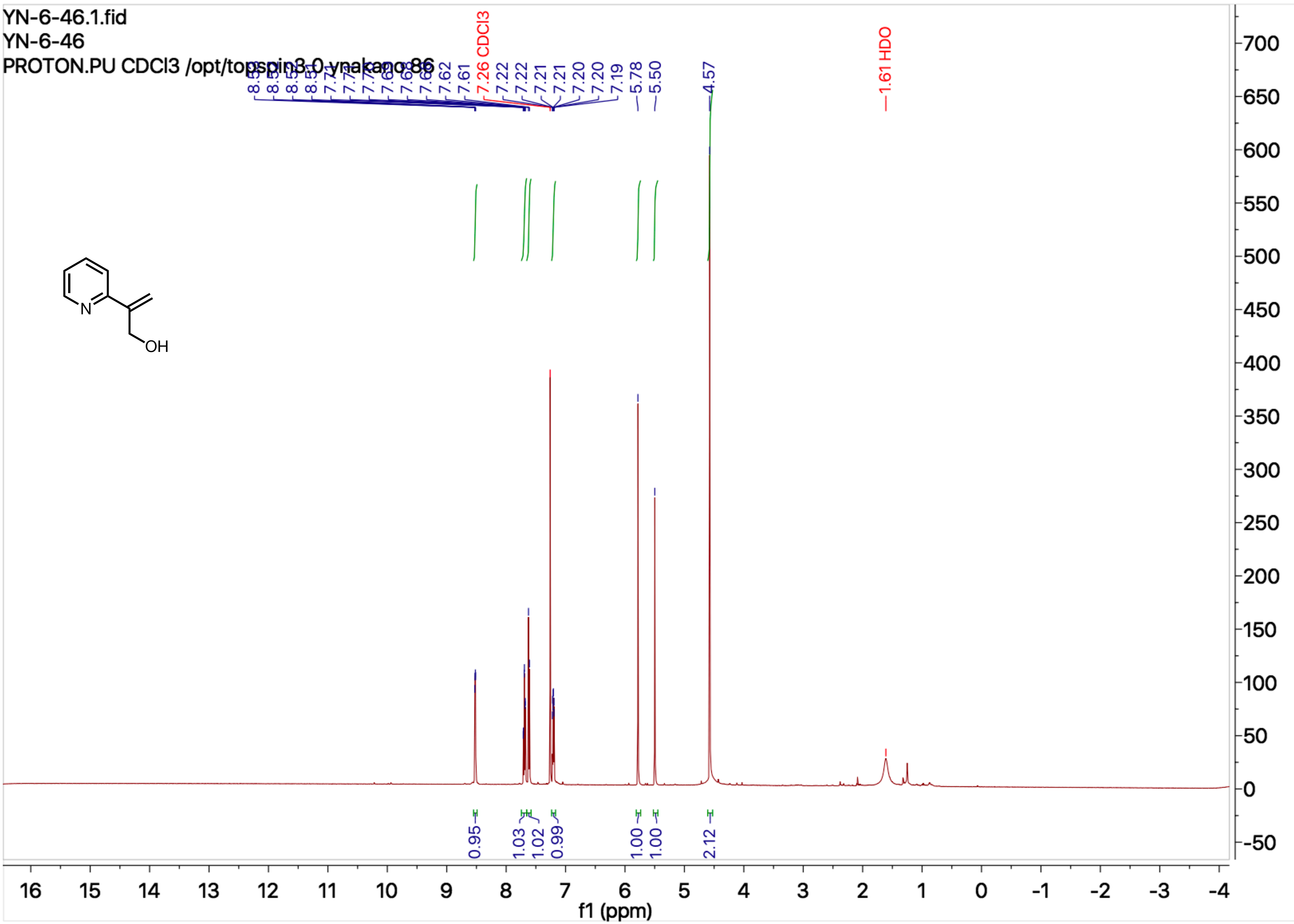

8.615
8.613
8.611
8.610
8.605
8.604
8.602
8.600
7.672
7.669
7.657
7.653
7.641
7.638
7.490
7.488
7.486
7.474
7.472
7.470
7.293
7.260
7.216
7.164
5.743
5.742
5.270
5.267
5.265
5.263
2.968
2.936
2.853
2.821
N
1.0
1.1
1.1
2.0
4.0
1.1
1.1
2.1
2.1
ppm 9
8
7
6
5
4
3
2
1
0

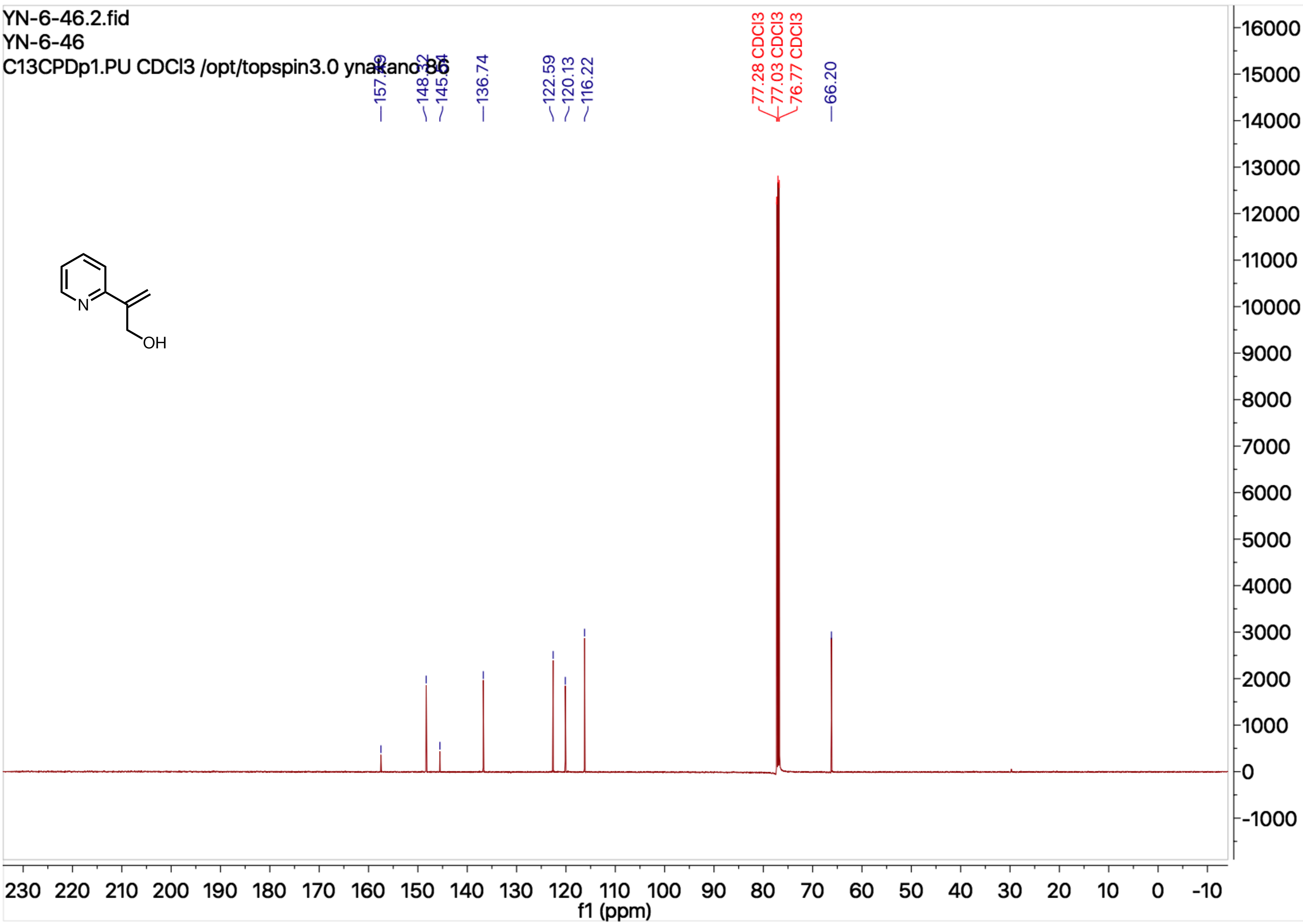

N
158.451
149.135
147.709
142.193
136.440
128.630
128.390
125.912
122.267
120.557
115.474
77.414
77.160
76.906
35.737
34.889
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0



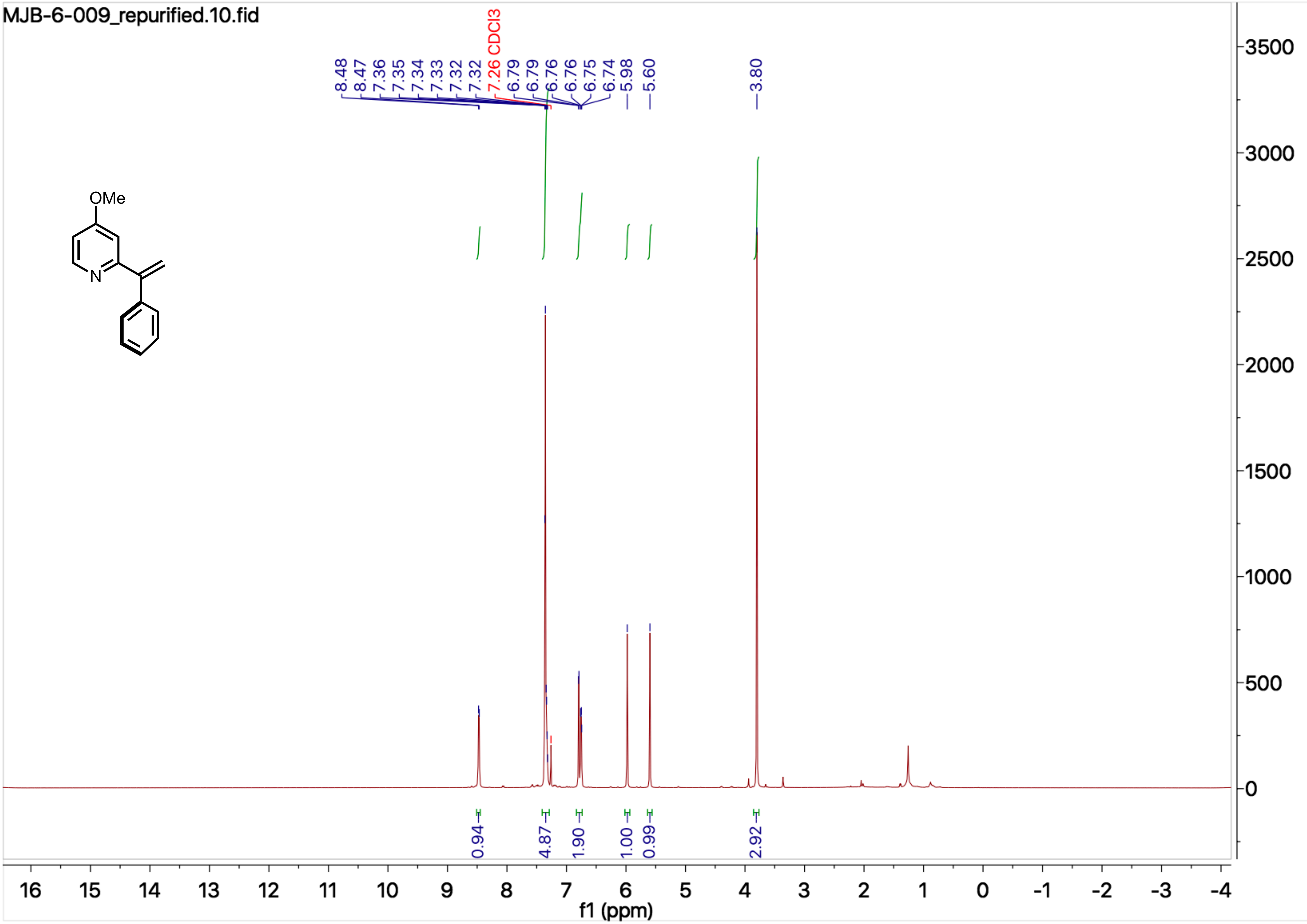
8.595
8.593
8.591
8.590
8.586
8.584
8.582
8.580
7.616
7.612
7.600
7.596
7.585
7.581
7.448
7.446
7.444
7.432
7.430
7.428
7.287
7.249
7.199
7.165
7.155
7.153
7.146
7.144
7.140
7.138
7.131
7.129
5.933
5.932
5.930
5.929
5.928
5.197
5.194
5.192
5.189
3.983
N
0.9
1.0
1.0
4.1
0.9
0.9
1.0
1.0
2.0
ppm 9
8
7
6
5
4
3
2
1
0

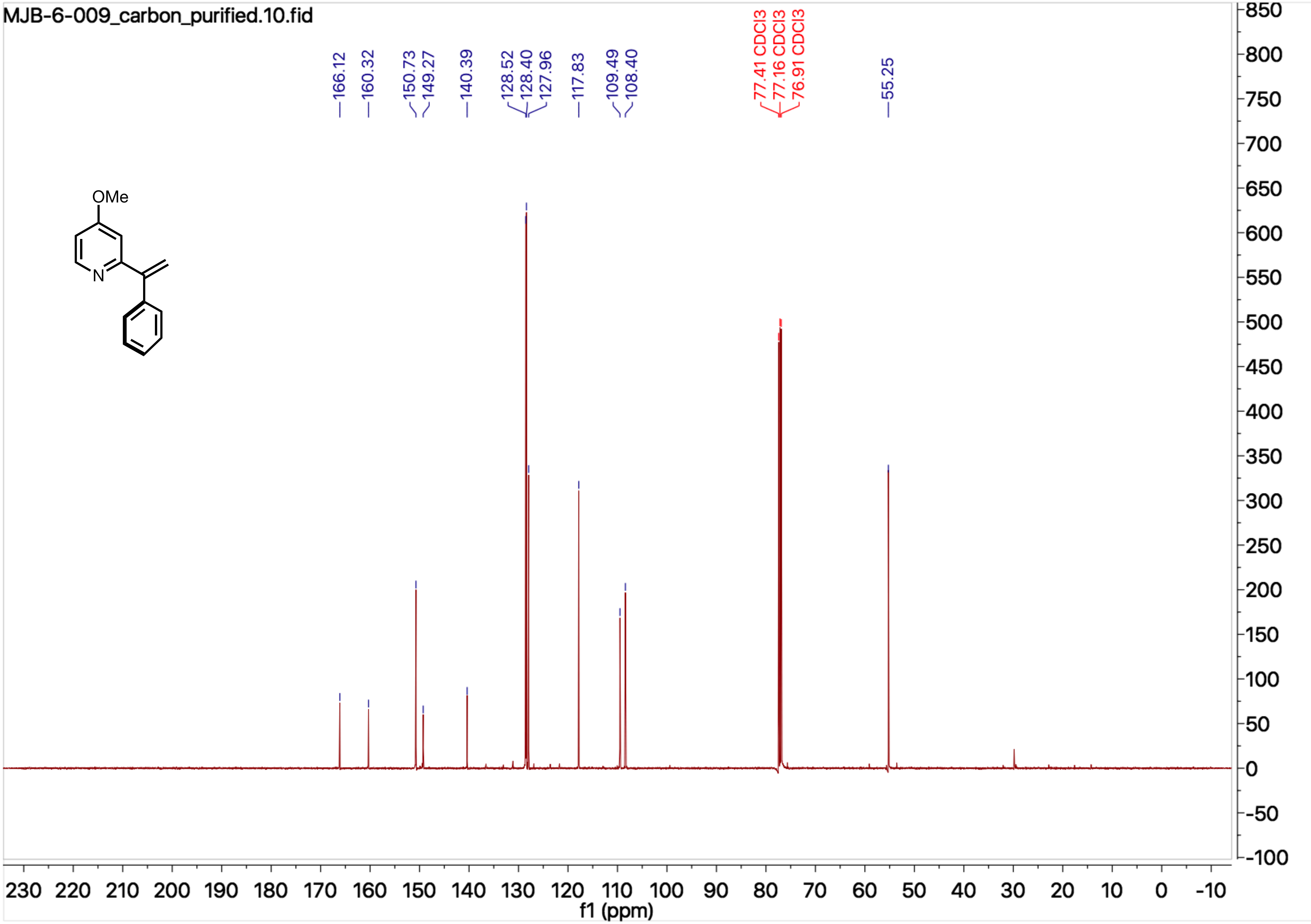

157.992
149.039
147.216
139.820
136.437
129.253
128.431
126.163
122.350
120.698
117.286
77.414
77.160
76.906
39.924
N
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

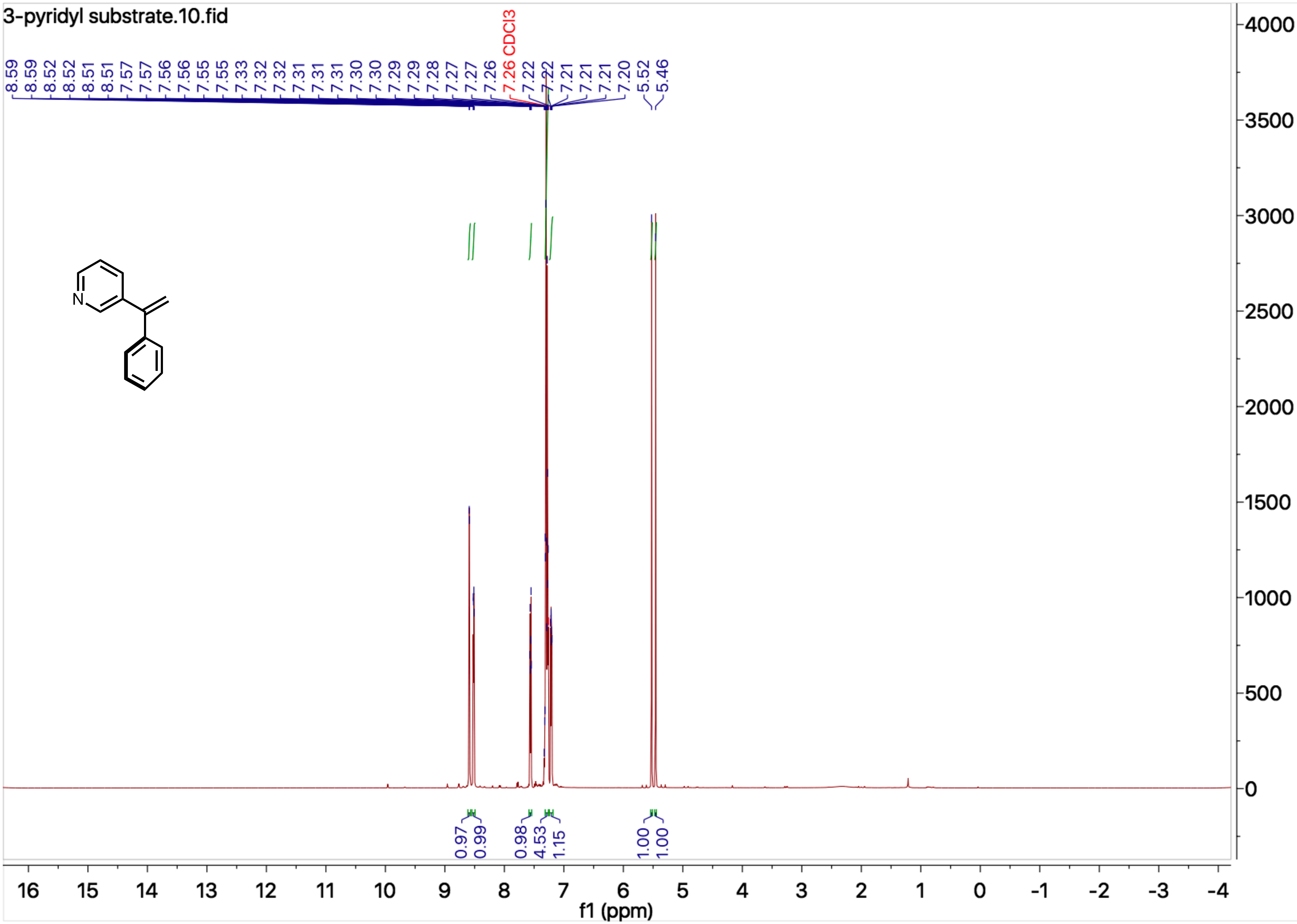

YN-6-46.1.fid
YN-6-46
PROTON.PU CDCl3 /opt/topspin3.0-ynakano 86
7.26 CDCl3
1.61 HDO
7.62
7.61
7.22
7.22
7.21
7.21
7.20
7.20
7.19
5.78
5.50
4.57
N
OH
0.95
1.03
1.02
0.99
1.00
1.00
2.12
f1 (ppm)



YN-6-46.2.fid
YN-6-46
C13CPDp1.PU CDCl3 /opt/topspin3.0 ynakano 86
157.49
148.32
145.54
136.74
122.59
120.13
116.22
77.28 CDCl3
77.03 CDCl3
76.77 CDCl3
66.20
N
OH
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


MJB-6-009_repurified.10.fid
8.48
8.47
7.36
7.35
7.34
7.33
7.32
7.32
7.26 CDCl3
6.79
6.79
6.76
6.76
6.75
6.74
5.98
5.60
3.80
OMe
N
0.94
4.87
1.90
1.00
0.99
2.92
f1 (ppm)


MJB-6-009_carbon_purified.10.fid
166.12
160.32
150.73
149.27
140.39
128.52
128.40
127.96
117.83
109.49
108.40
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
55.25
OMe
N
f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
850 800 750 700 650 600 550 500 450 400 350 300 250 200 150 100 50 0 -50 -100


3-pyridyl substrate.10.fid
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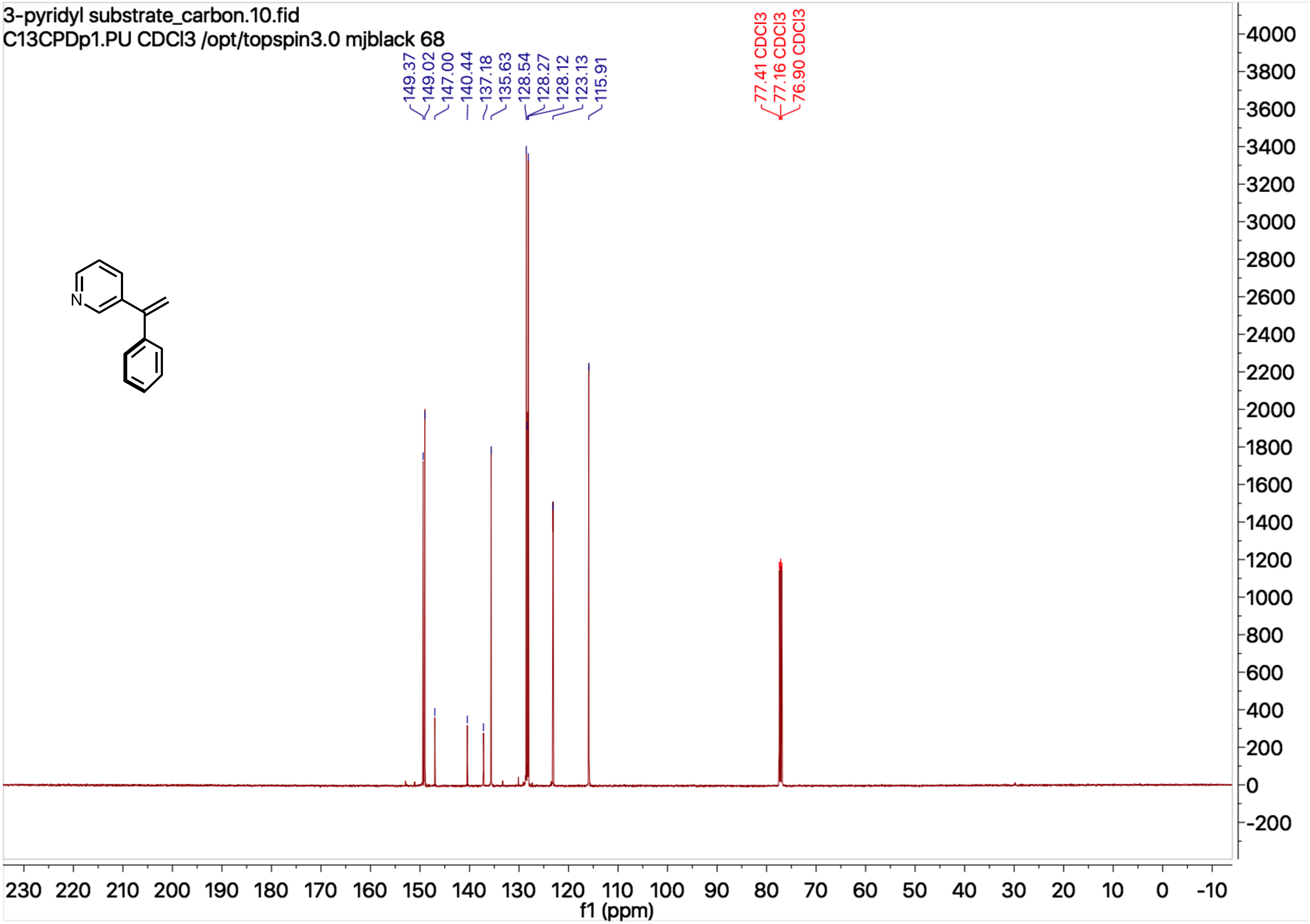
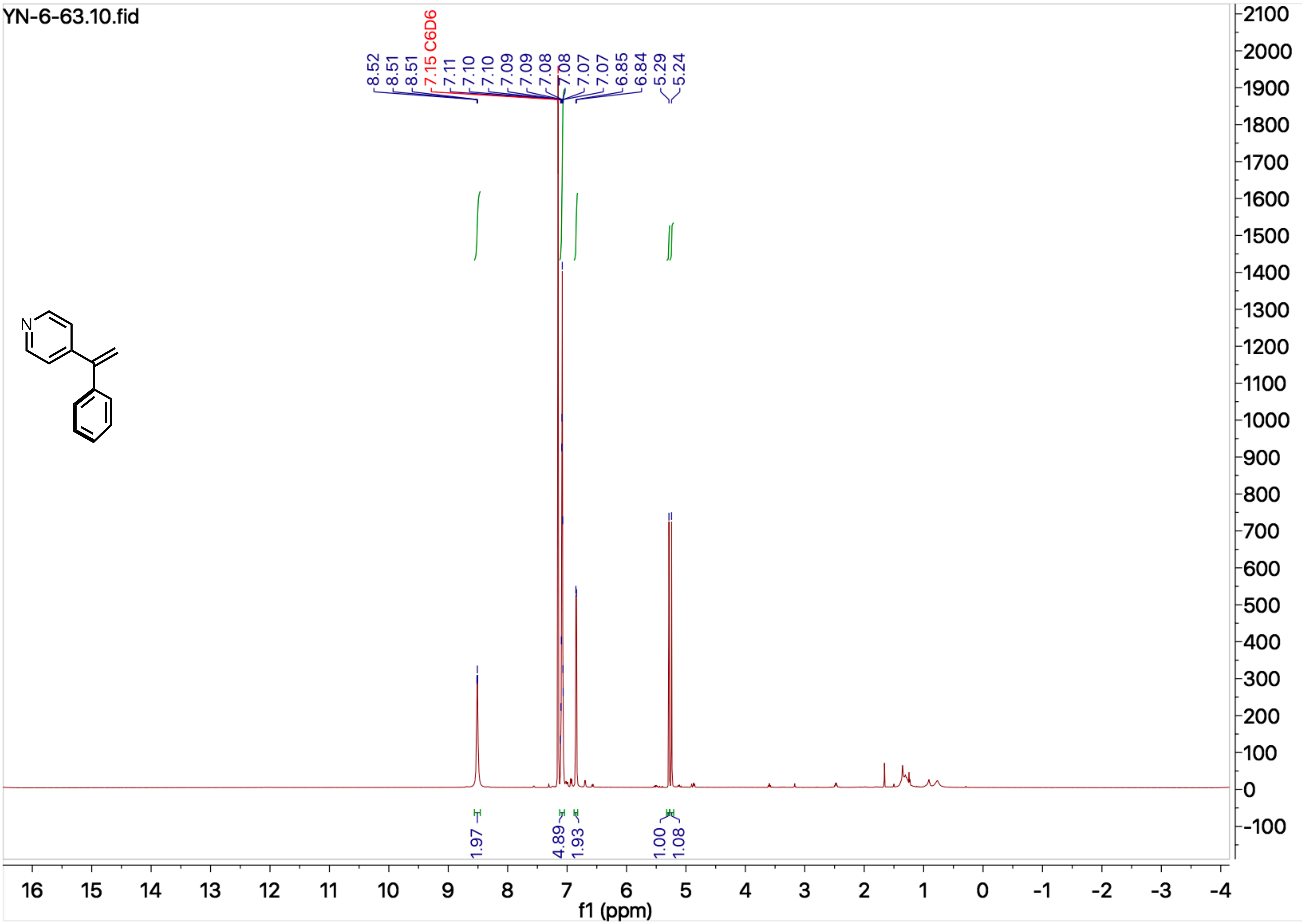
8.59
8.52
8.52
8.51
8.51
7.57
7.57
7.56
7.56
7.55
7.55
7.33
7.32
7.32
7.31
7.31
7.31
7.30
7.30
7.29
7.29
7.28
7.27
7.27
7.26
7.26 CDCl3
7.22
7.22
7.21
7.21
7.21
7.20
5.52
5.46
N
0.97
0.99
0.98
4.53
1.15
1.00
1.00
f1 (ppm)


3-pyridyl substrate_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 68
149.37
149.02
147.00
140.44
137.18
135.63
128.54
128.27
128.12
123.13
115.91
77.41 CDCl3
77.16 CDCl3
76.90 CDCl3
N
f1 (ppm)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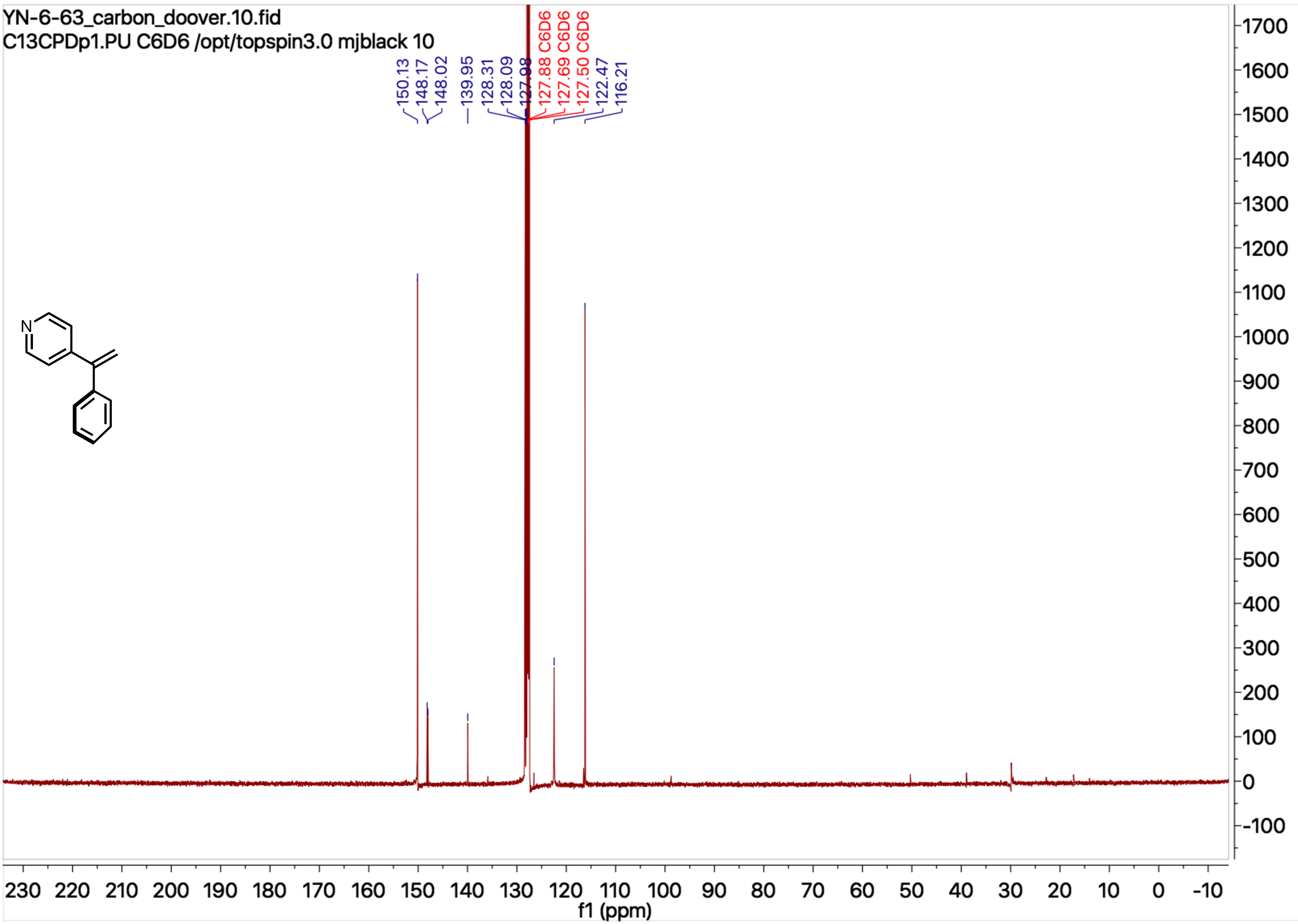
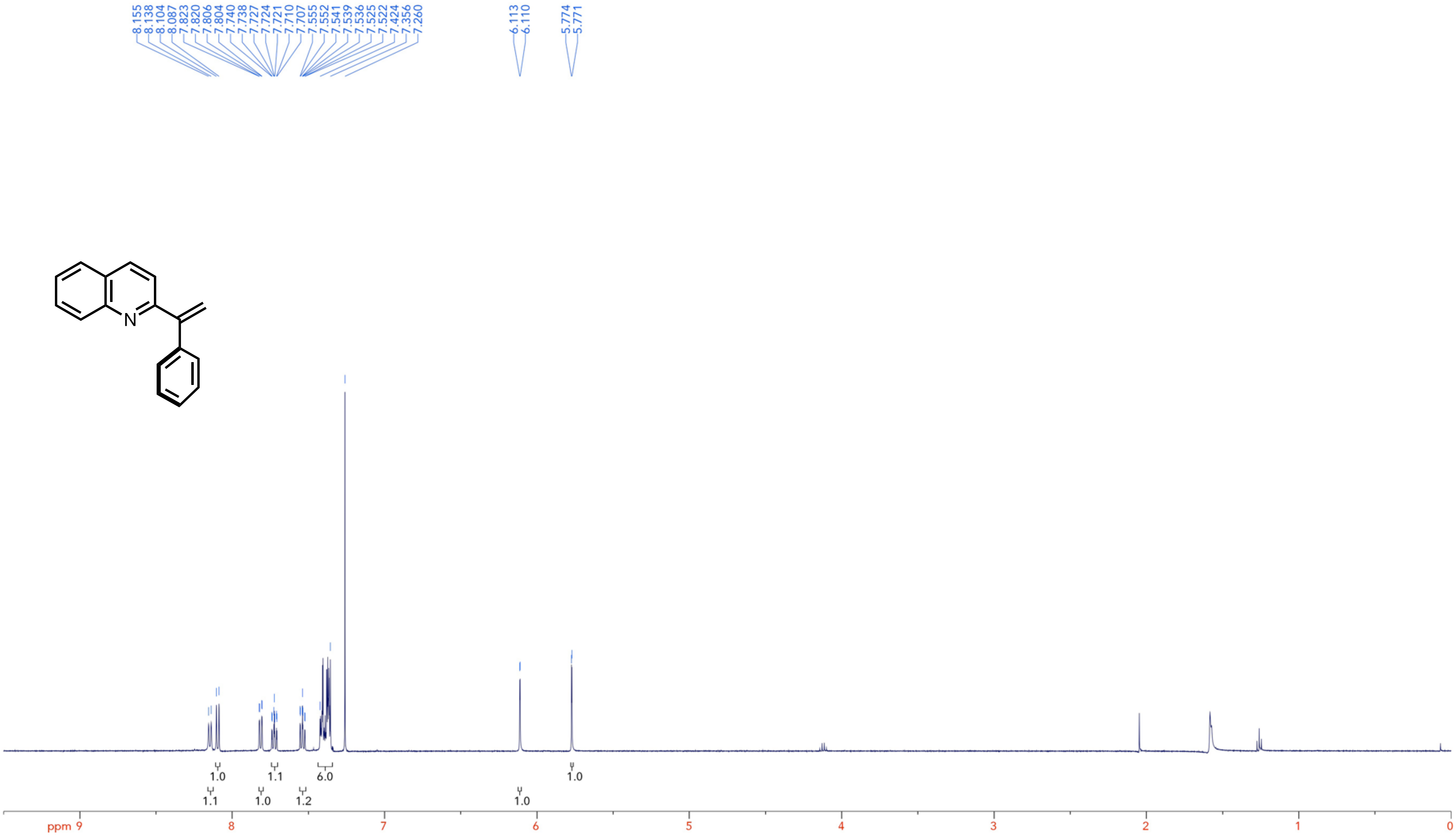
8.51
8.51
7.15 C6D6
7.11
7.10
7.10
7.09
7.09
7.08
7.08
7.07
7.07
6.85
6.84
5.29
5.24
N
1.97
4.89
1.93
1.00
1.08
f1 (ppm)


YN-6-63_carbon_doover.10.fid
C13CPDp1.PU C6D6 /opt/topspin3.0 mjblack 10
150.13
148.17
148.02
139.95
128.31
128.09
127.98
127.88 C6D6
127.69 C6D6
127.50 C6D6
122.47
116.21
N
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
1700 1600 1500 1400 1300 1200 1100 1000 900 800 700 600 500 400 300 200 100 0 -100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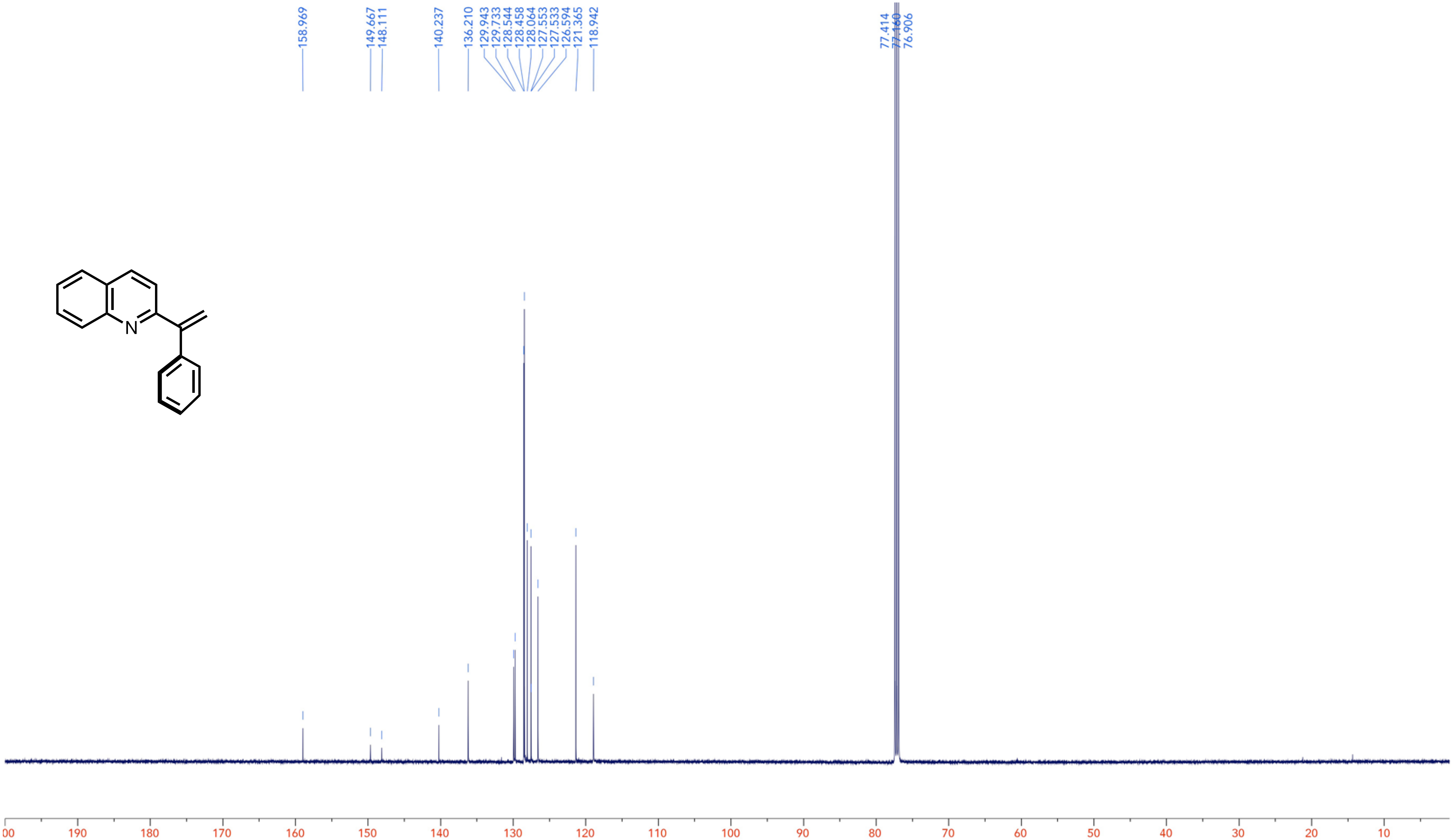
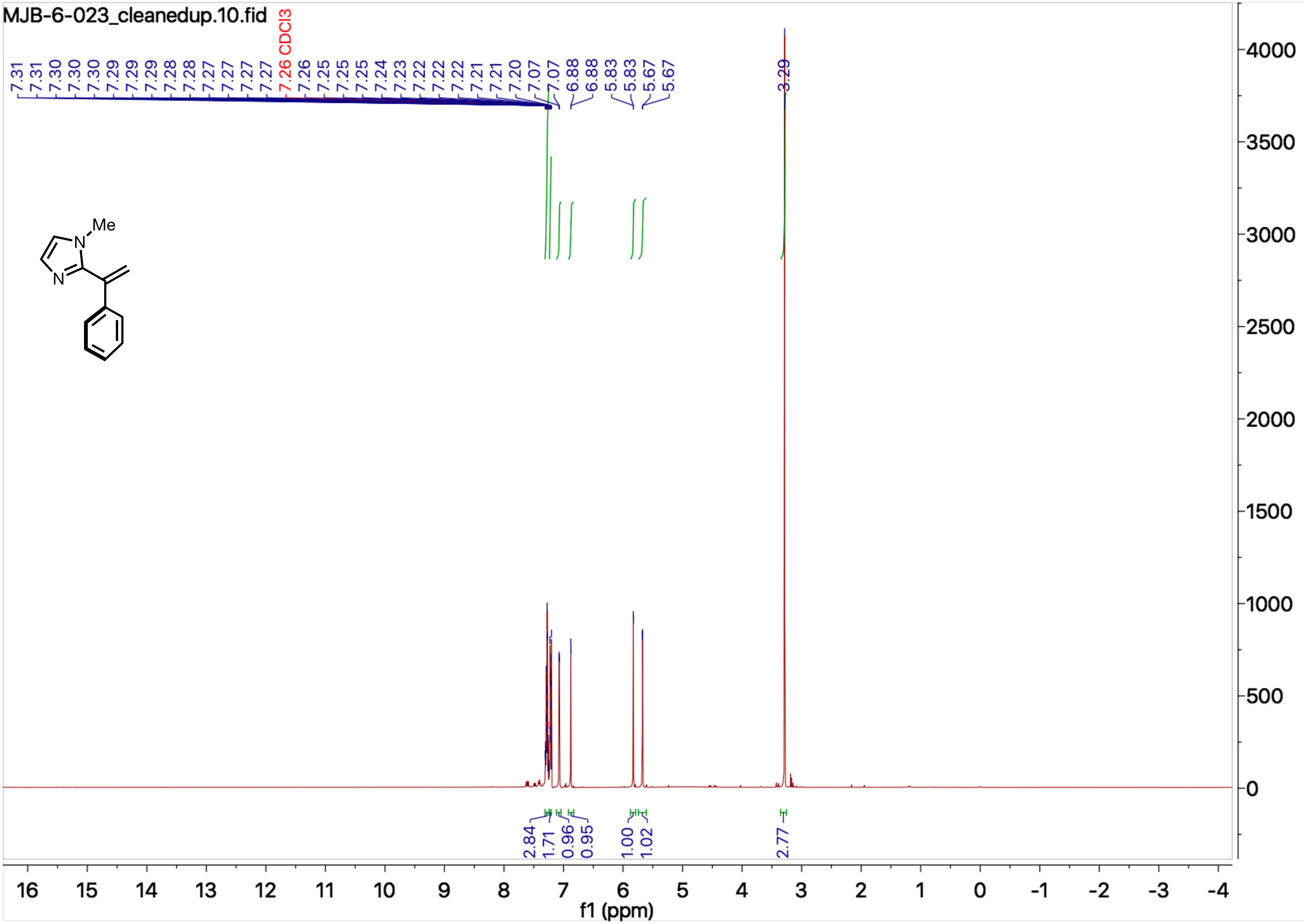
8.138
8.104
8.087
7.823
7.820
7.806
7.804
7.740
7.738
7.727
7.724
7.721
7.710
7.707
7.555
7.552
7.541
7.539
7.536
7.525
7.522
7.424
7.356
7.260
6.113
6.110
5.774
5.771
N
1.0
1.1
6.0
1.0
1.1
1.0
1.2
1.0
ppm 9
8
7
6
5
4
3
2
1
0


158.969
149.667
148.111
140.237
136.210
129.943
129.733
128.544
128.458
128.064
127.553
127.533
126.594
121.365
118.942
77.414
77.160
76.906
N
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


MJB-6-023_cleanedup.10.fid
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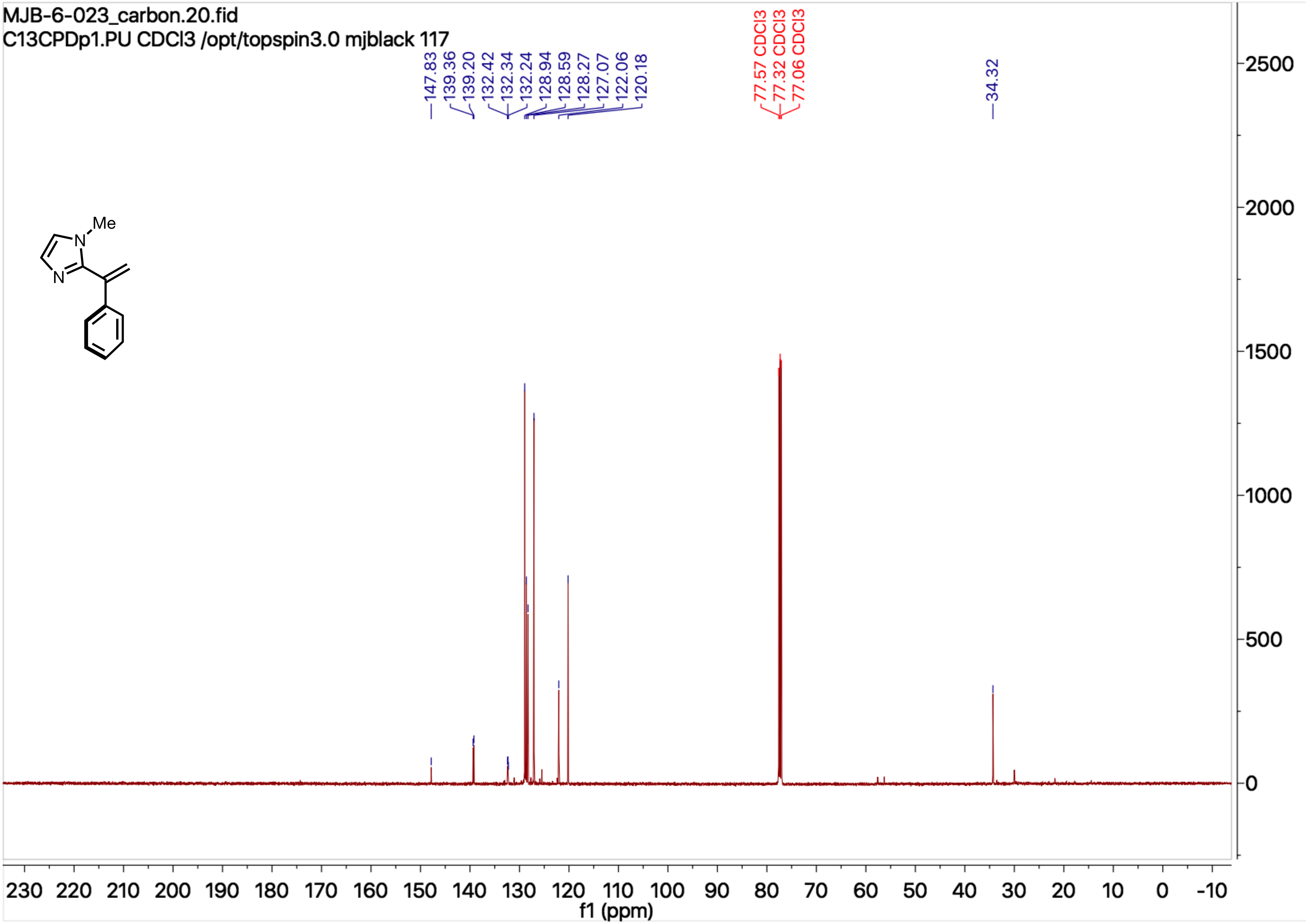
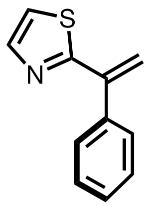
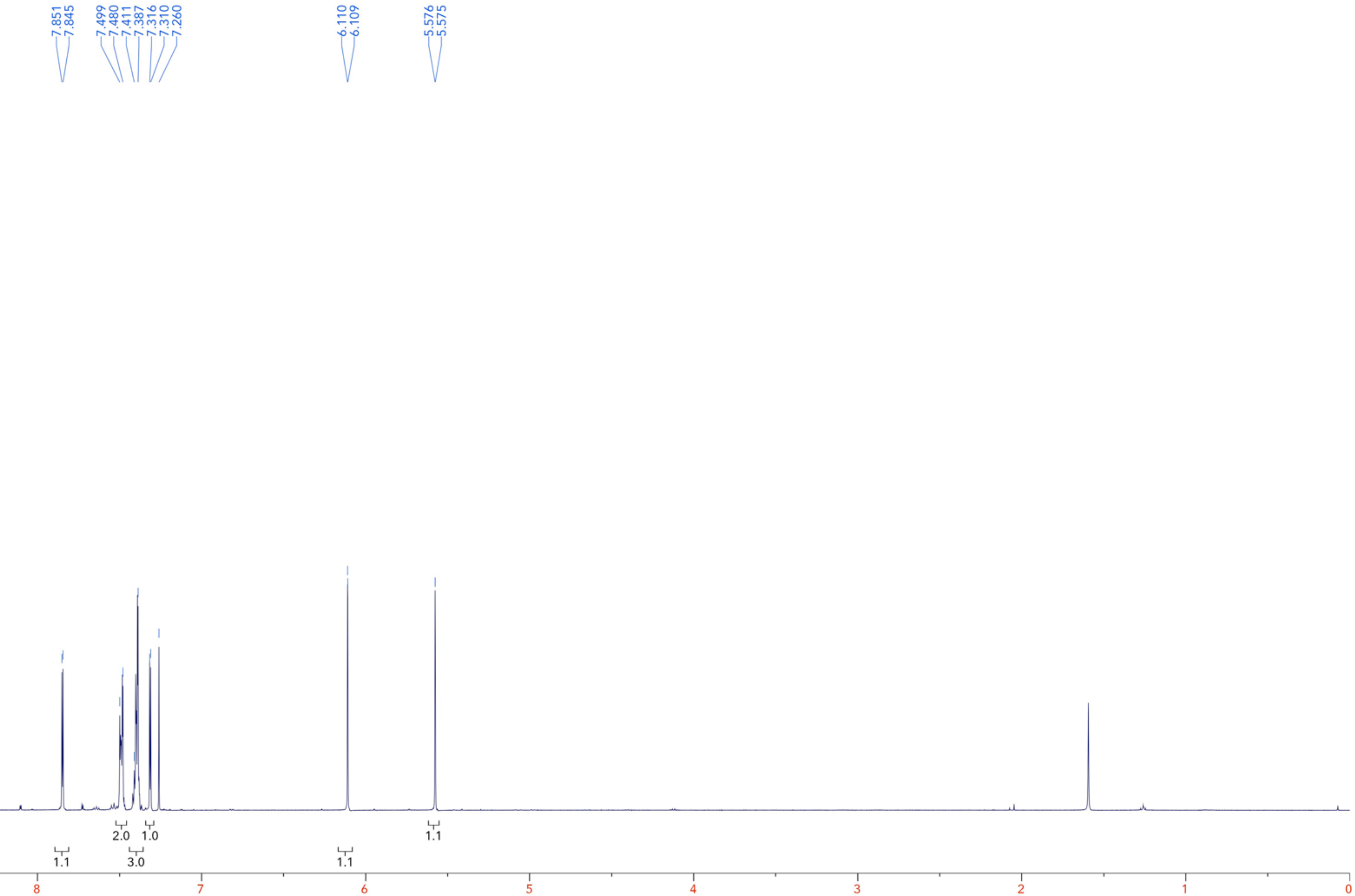
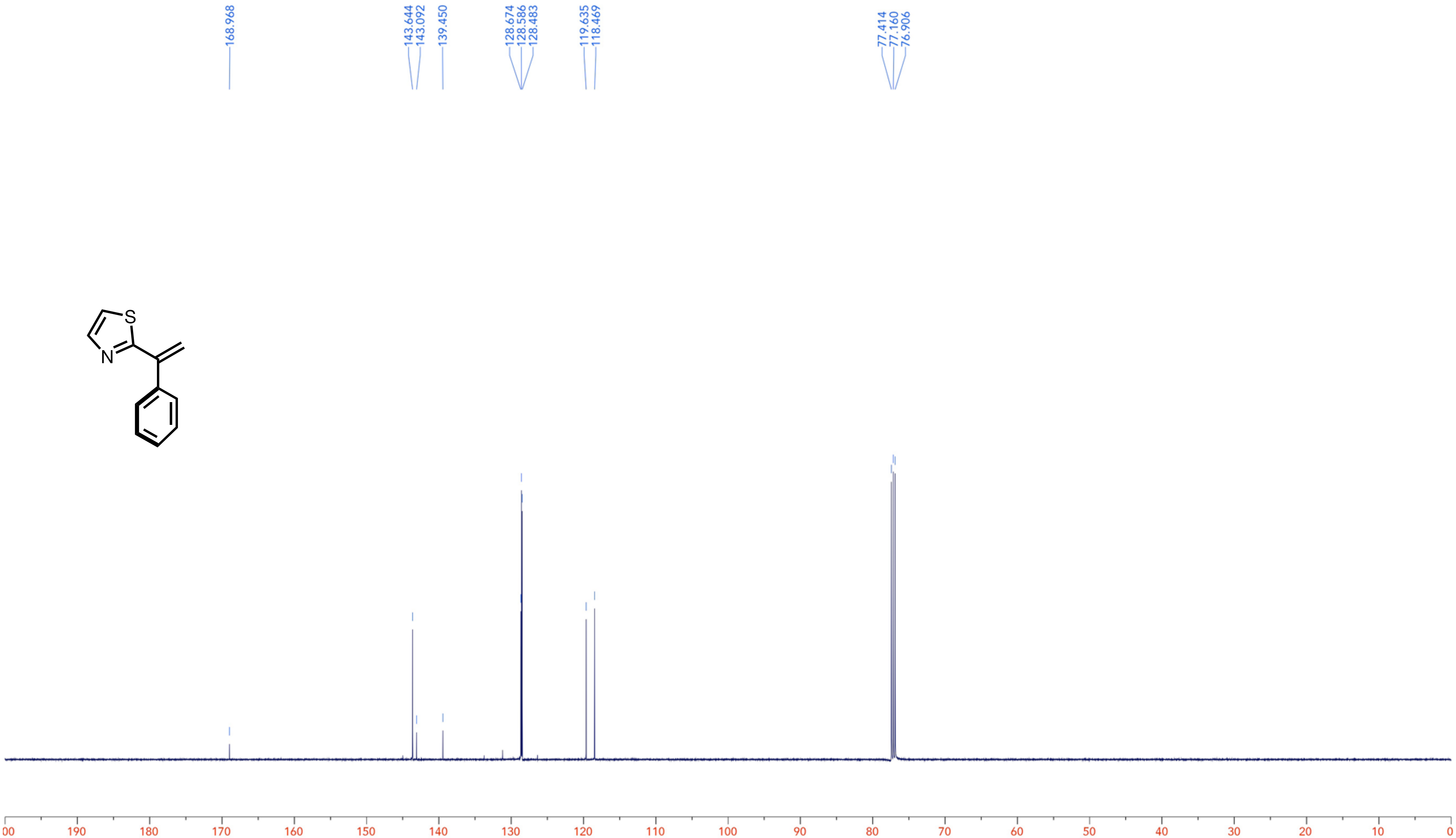
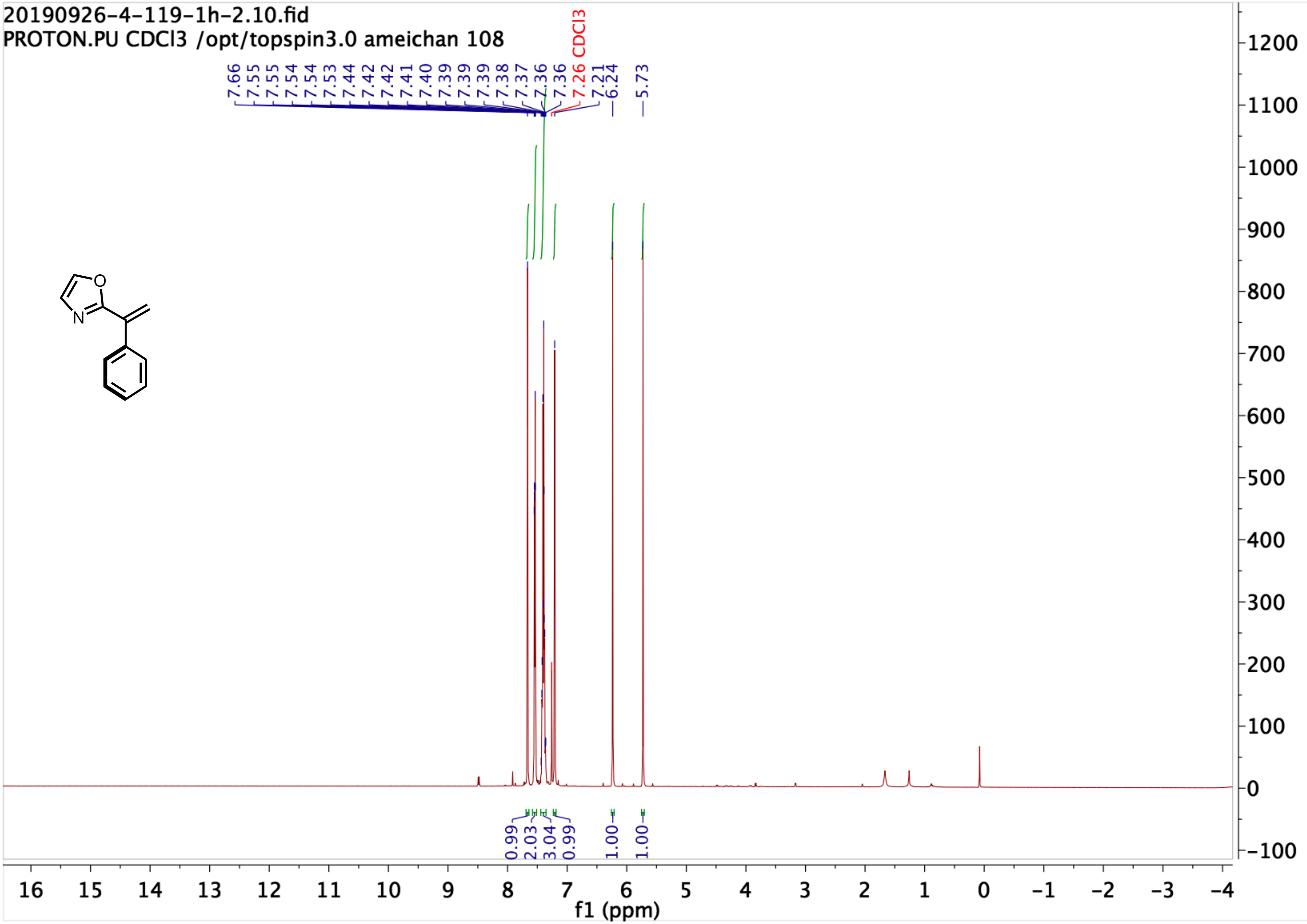
3.29
Me
N
N
2.84 1.71 0.96 0.95 1.00 1.02 2.77
4000
3500
3000
2500
2000
1500
1000
500
0
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)


MJB-6-023_carbon.20.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 117
147.83
139.36
139.20
132.42
132.34
132.24
128.94
128.59
128.27
127.07
122.06
120.18
77.57 CDCl3
77.32 CDCl3
77.06 CDCl3
34.32
Me
N
N
2500
2000
1500
1000
500
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

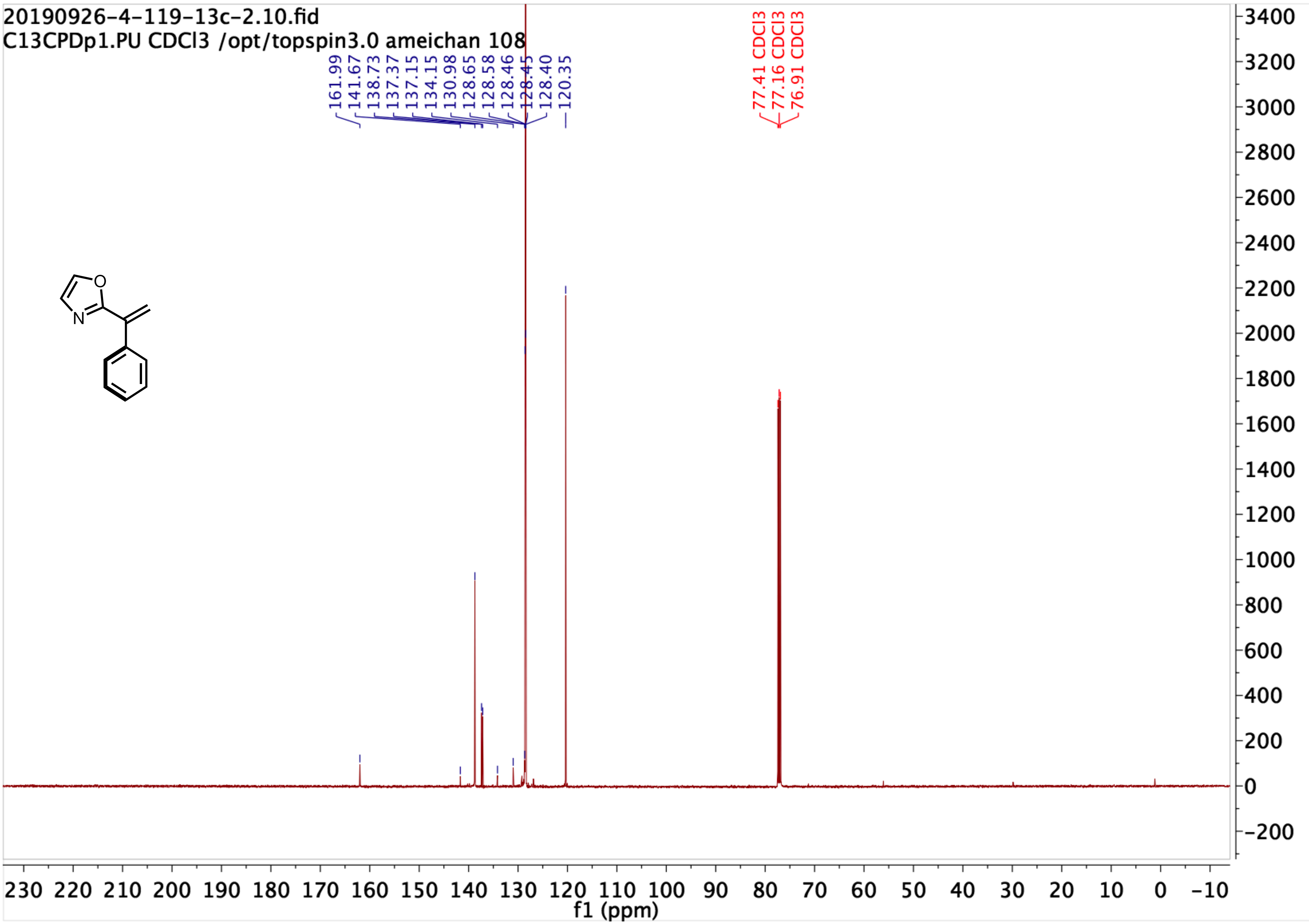

S
N

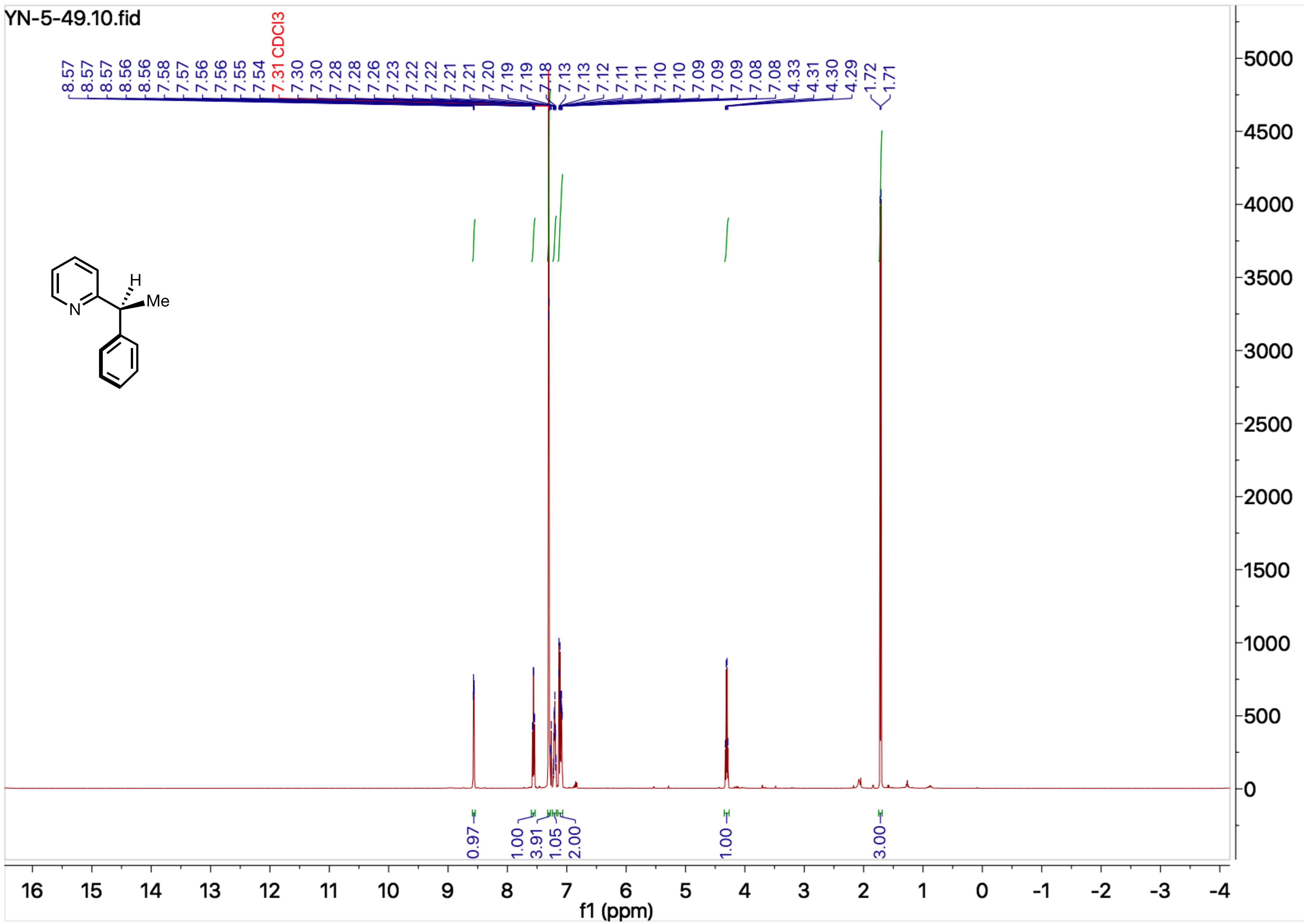

7.851
7.845
7.499
7.480
7.411
7.387
7.316
7.310
7.260
6.110
6.109
5.576
5.575
2.0
1.0
1.1
1.1
3.0
1.1
ppm 9
8
7
6
5
4
3
2
1
0


168.968
143.644
143.092
139.450
128.674
128.586
128.483
119.635
118.469
77.414
77.160
76.906
S
N
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0



20190926-4-119-1h-2.10.fid
PROTON.PU CDCl3 /opt/topspin3.0 ameichan 108
7.66 7.55 7.55 7.54 7.54 7.53 7.44 7.42 7.42 7.41 7.40 7.39 7.39 7.39 7.38 7.37 7.36 7.36 7.26 CDCl3 7.21 6.24 5.73
O
N
0.99 2.03 3.04 0.99 1.00 1.00
f1 (ppm)


20190926-4-119-13c-2.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 ameichan 108
161.99
141.67
138.73
137.37
137.15
134.15
130.98
128.65
128.58
128.46
128.45
128.40
120.35
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
O
N
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


YN-5-49.10.fid
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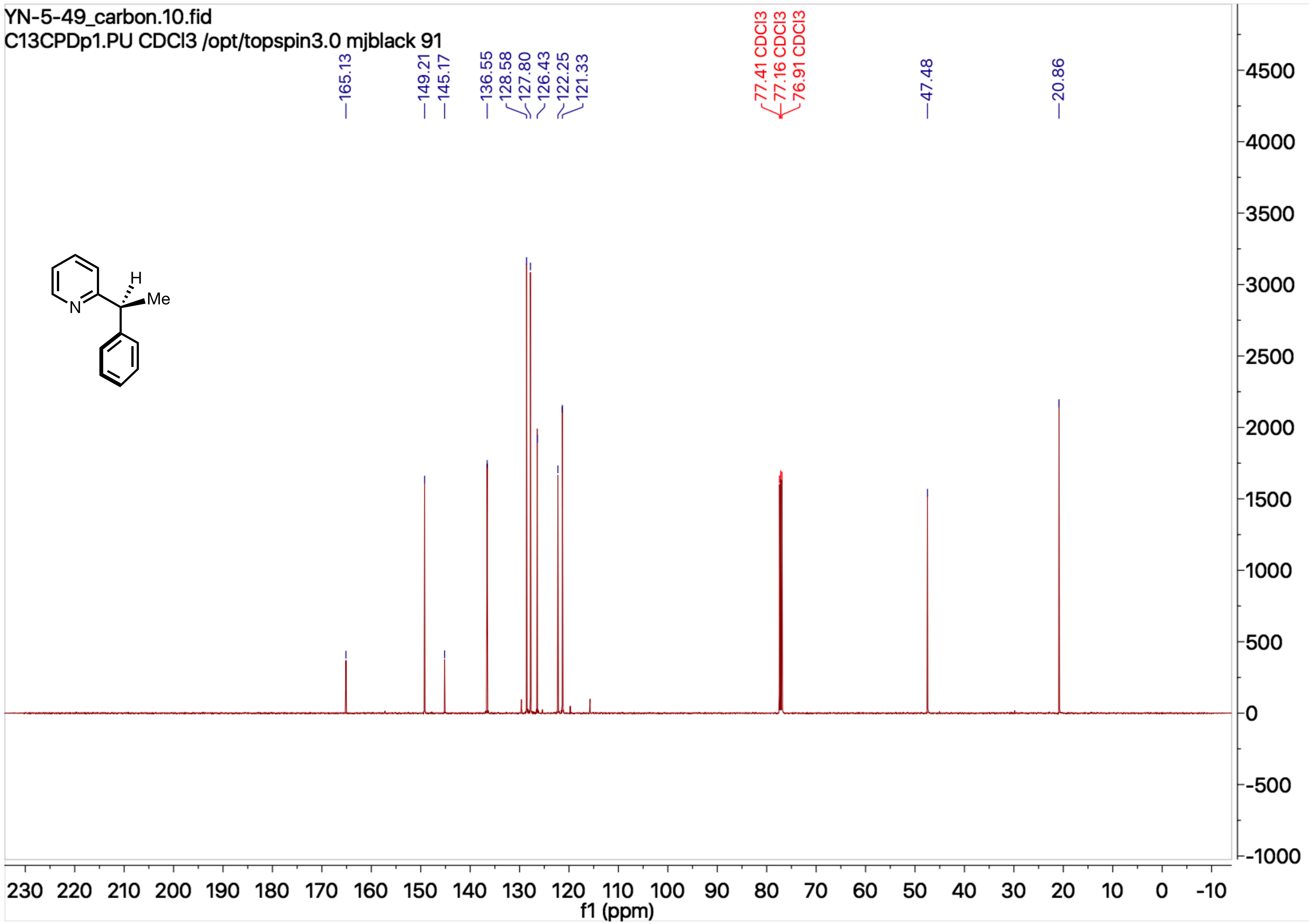
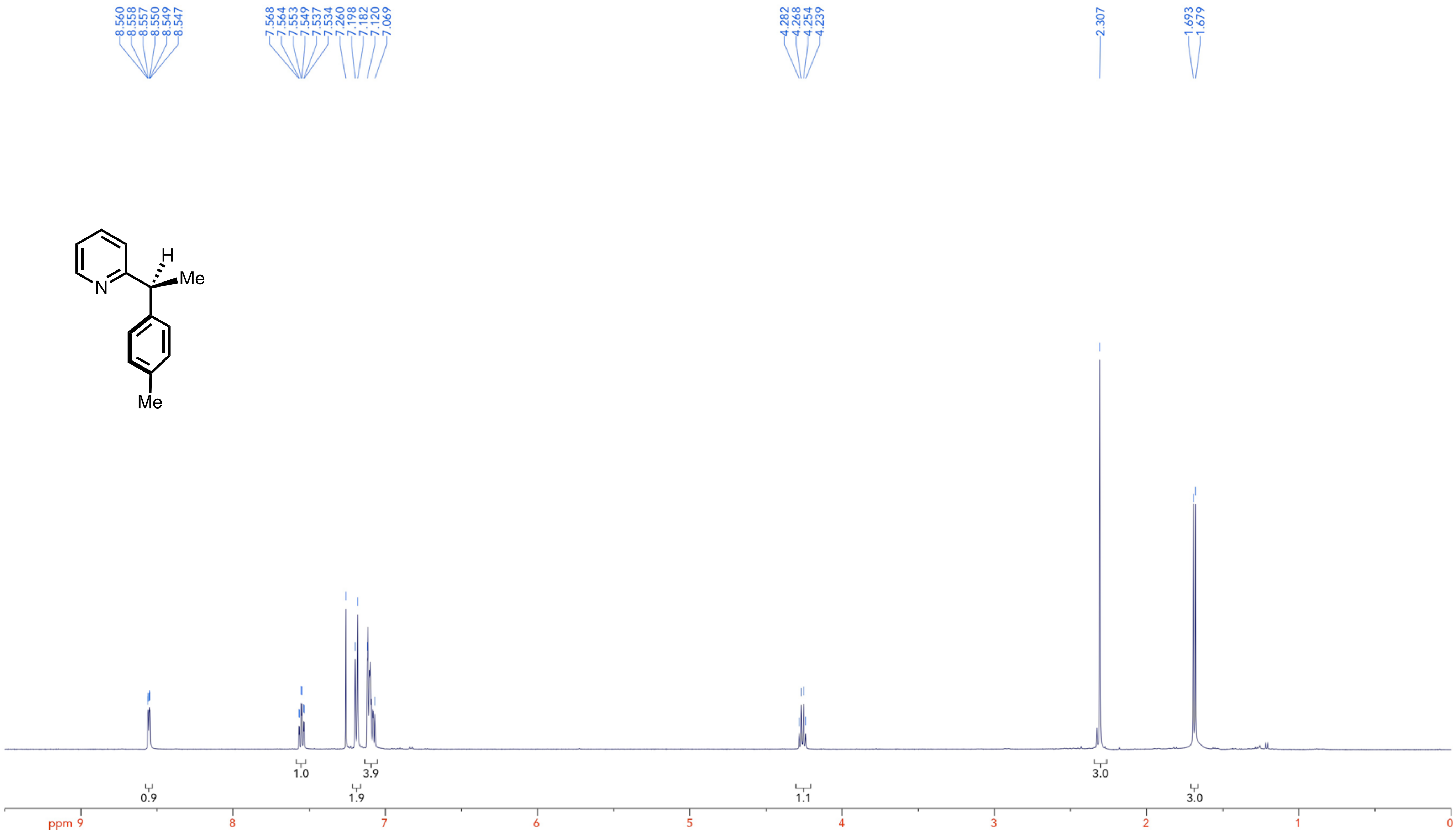
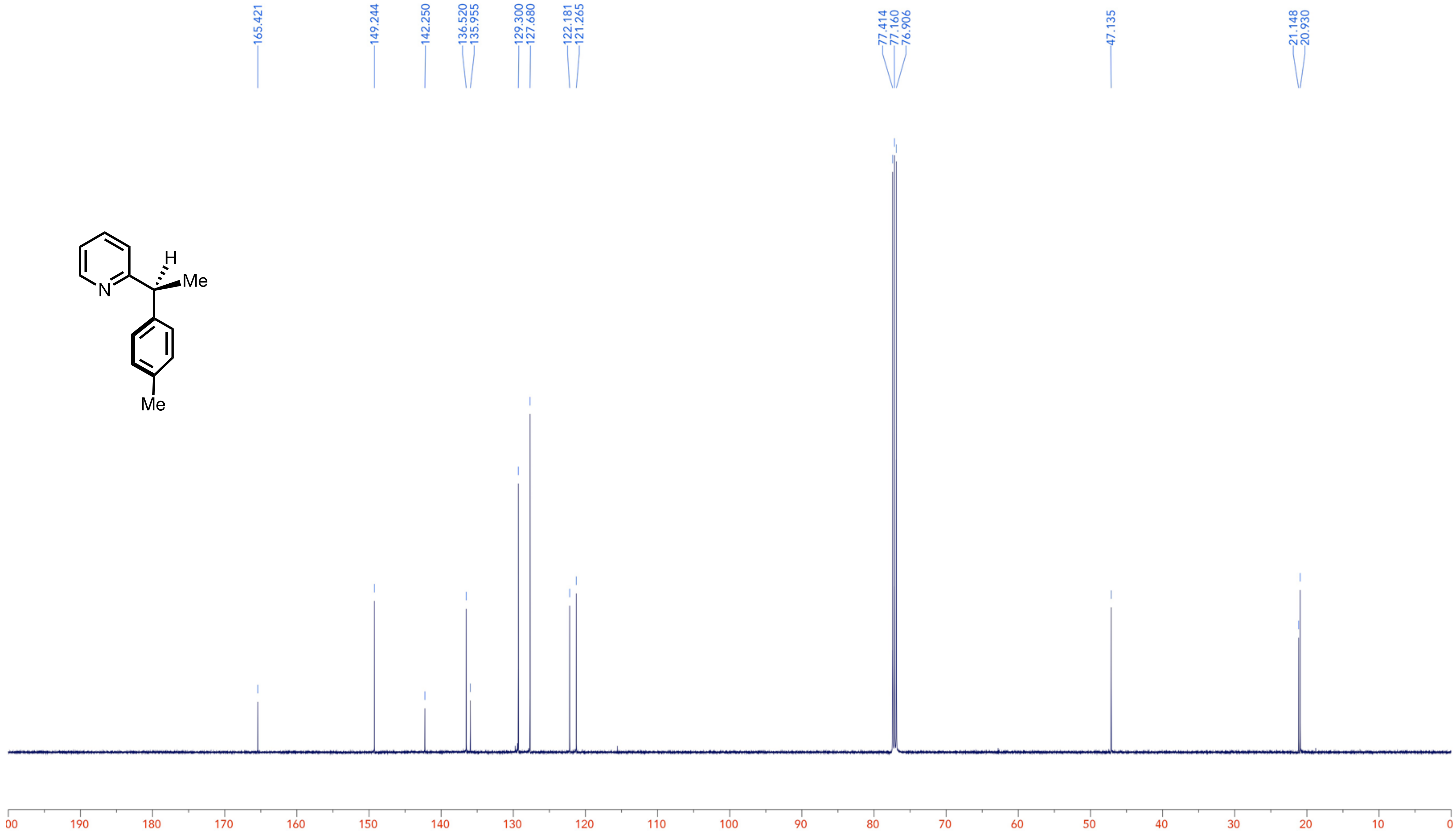
H
Me
N
0.97
1.00
3.91
1.05
2.00
1.00
3.00
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)

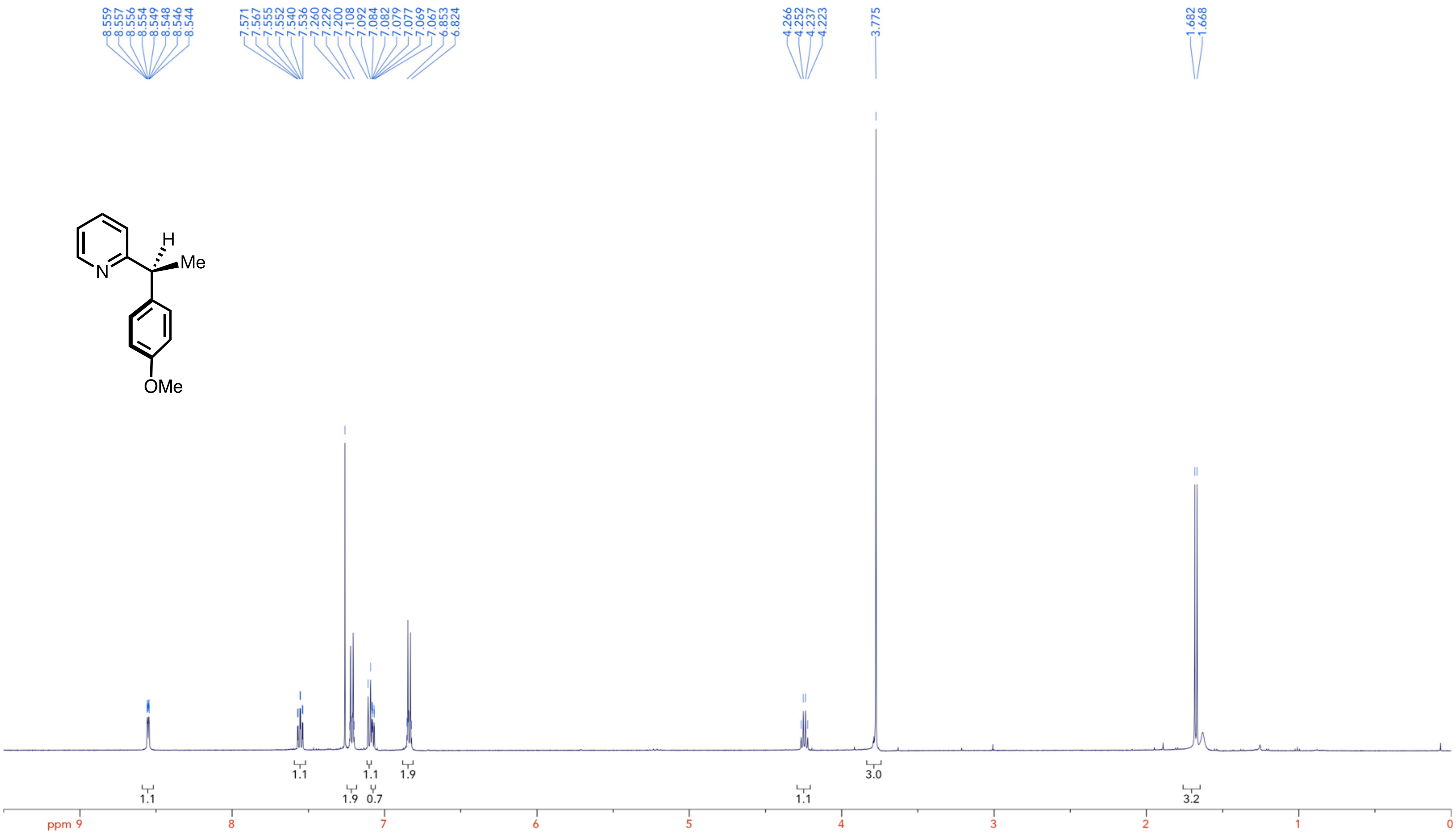


YN-5-49_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 91
165.13
149.21
145.17
136.55
128.58
127.80
126.43
122.25
121.33
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
47.48
20.86
H
Me
N
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
-1000
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



8.560
8.558
8.557
8.550
8.549
8.547
7.568
7.564
7.553
7.549
7.537
7.534
7.260
7.198
7.182
7.120
7.069
4.282
4.268
4.254
4.239
2.307
1.693
1.679
H
Me
N
Me
0.9
1.0
1.9
3.9
1.1
3.0
3.0
ppm 9
8
7
6
5
4
3
2
1
0


165.421
149.244
142.250
136.520
135.955
129.300
127.680
122.181
121.265
77.414
77.160
76.906
47.135
21.148
20.930
H
Me
N
Me
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0



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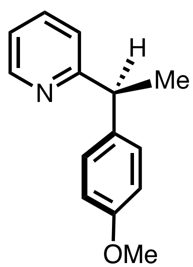
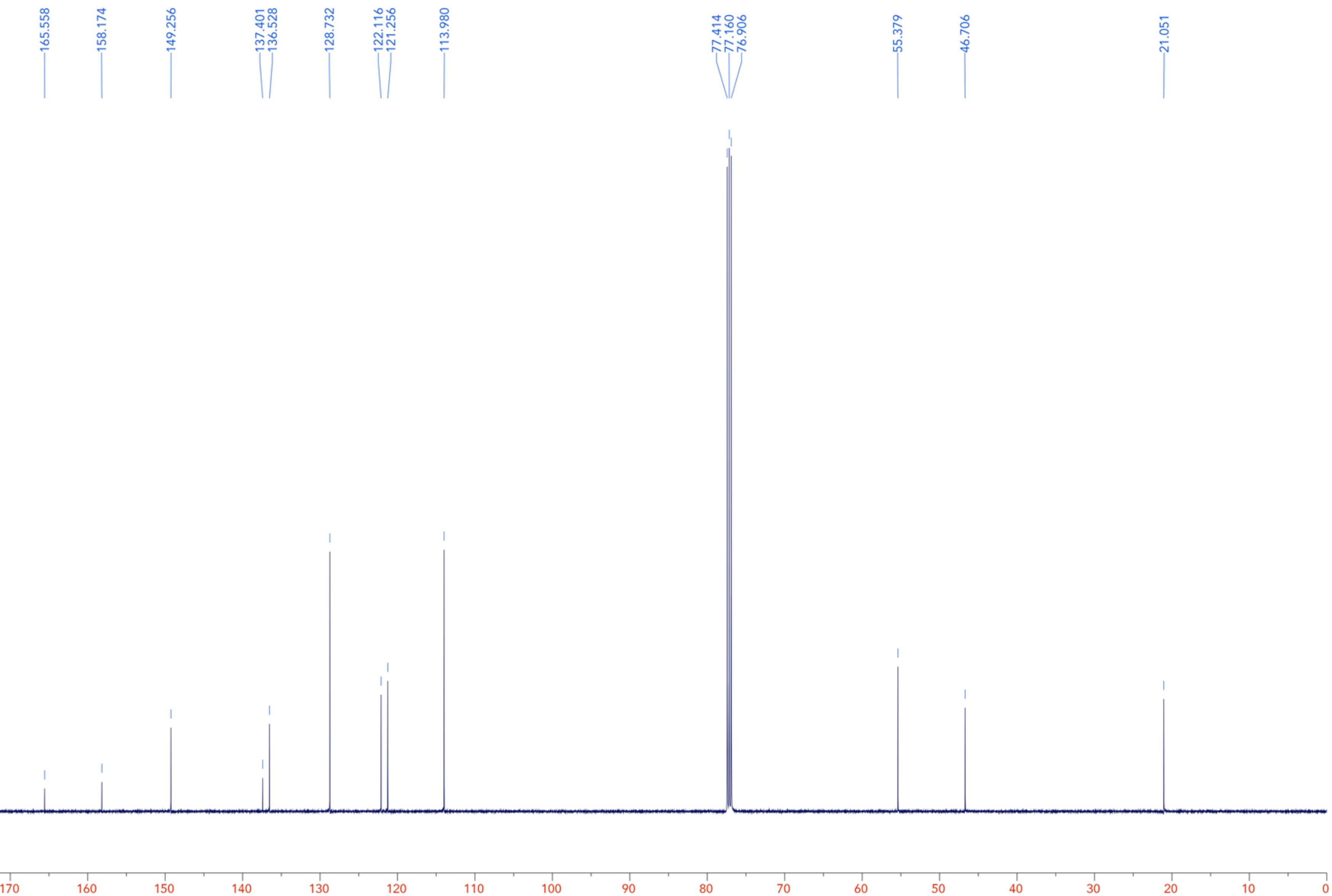
8.557
8.556
8.554
8.549
8.548
8.546
8.544
7.571
7.567
7.555
7.552
7.540
7.536
7.260
7.229
7.200
7.108
7.092
7.084
7.082
7.079
7.077
7.069
7.067
6.853
6.824
4.266
4.252
4.237
4.223
3.775
1.682
1.668
H
Me
N
OMe
1.1
1.1
1.9
1.1
0.7
1.9
1.1
3.0
3.2
ppm 9
8
7
6
5
4
3
2
1
0


H
Me
N
OMe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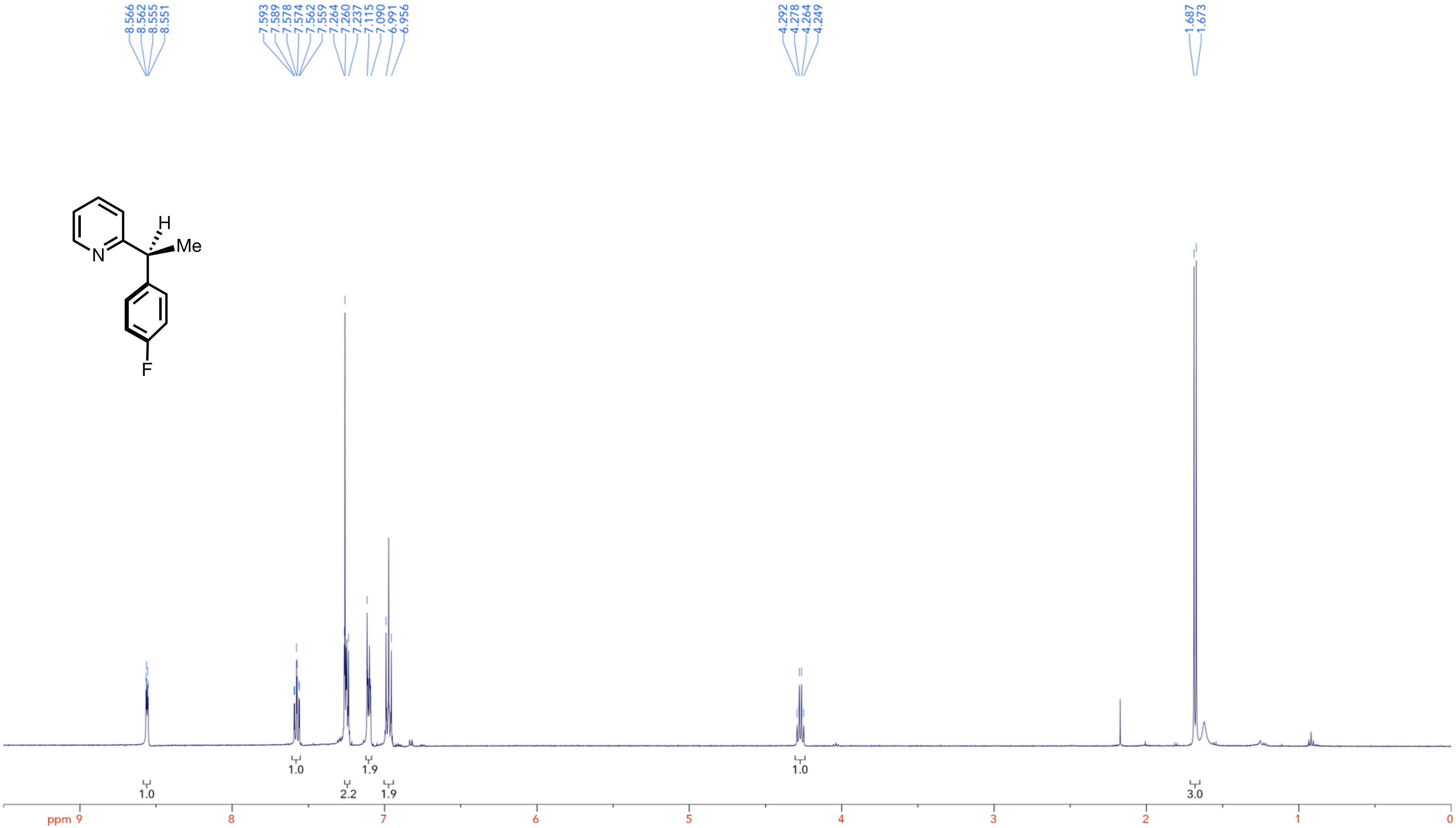
137.401
136.528
128.732
122.116
121.256
113.980
77.414
77.160
76.906
55.379
46.706
21.051
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0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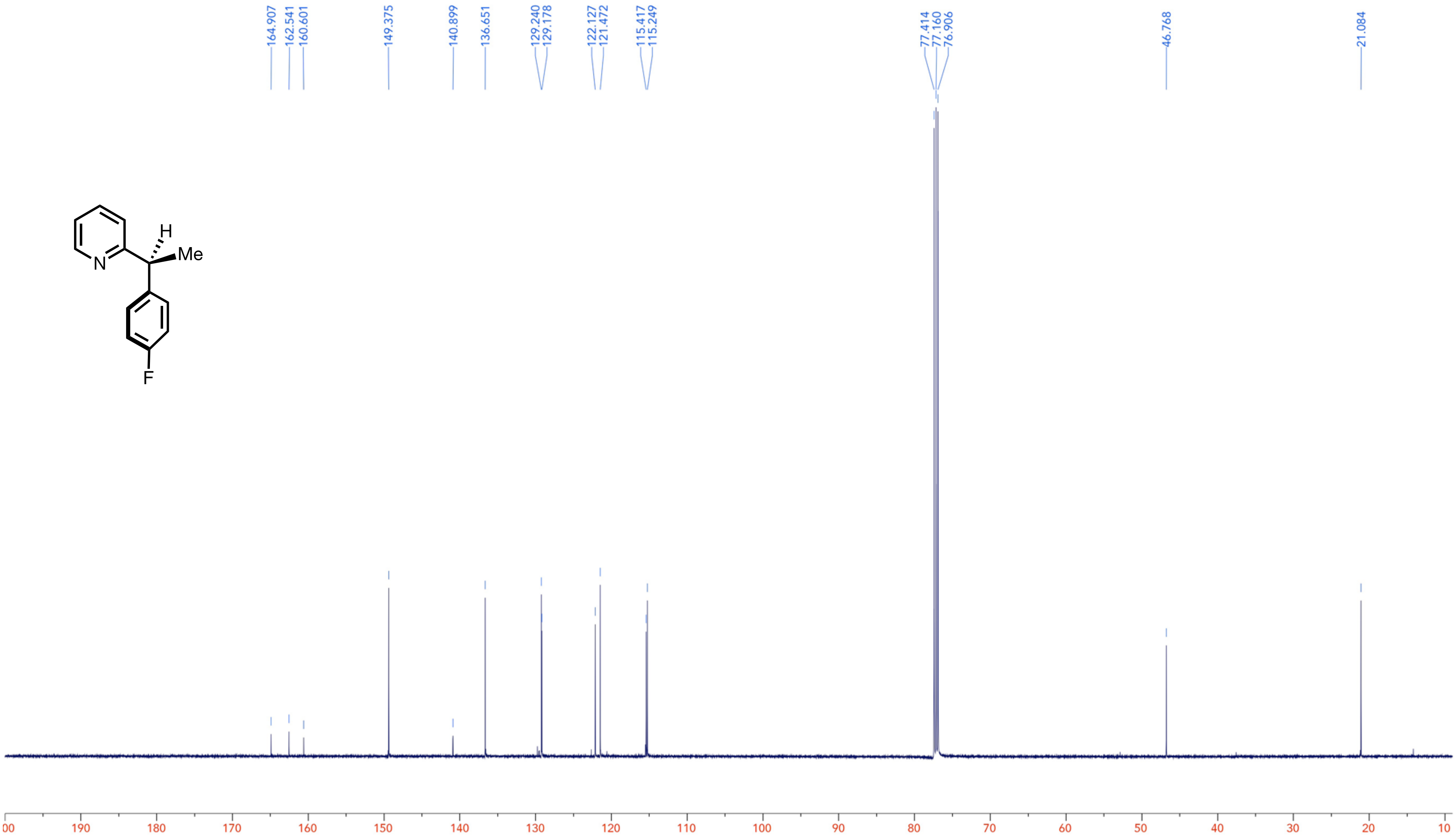
8.555
8.551
7.593
7.589
7.578
7.574
7.562
7.559
7.264
7.260
7.237
7.115
7.090
6.991
6.956
4.292
4.278
4.264
4.249
1.687
1.673
H
Me
N
F
1.0
1.0
2.2
1.9
1.9
1.0
3.0
ppm 9
8
7
6
5
4
3
2
1
0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129.178
122.127
121.472
115.417
115.249
77.414
77.160
76.906
46.768
21.084
H
Me
N
F
00
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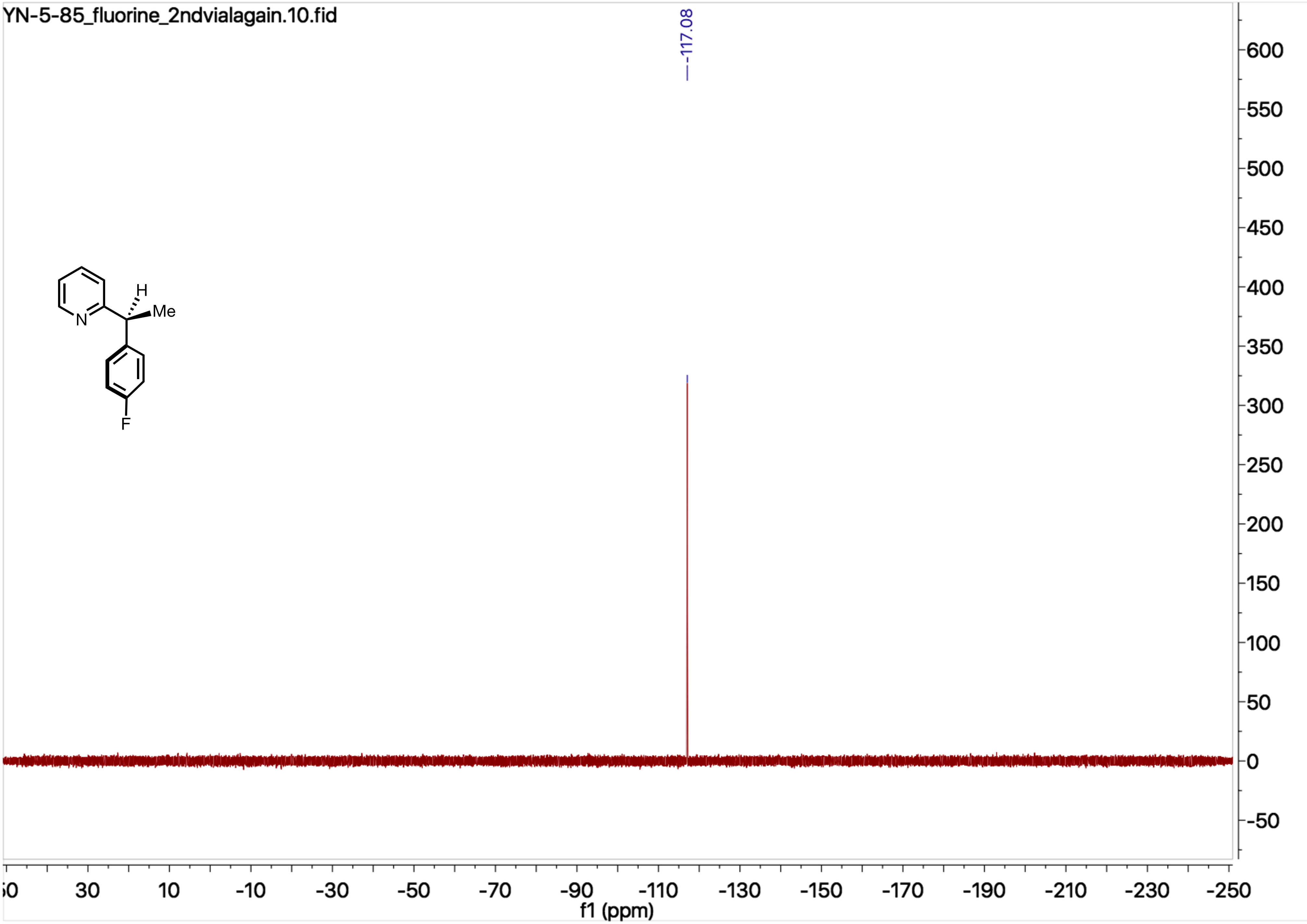
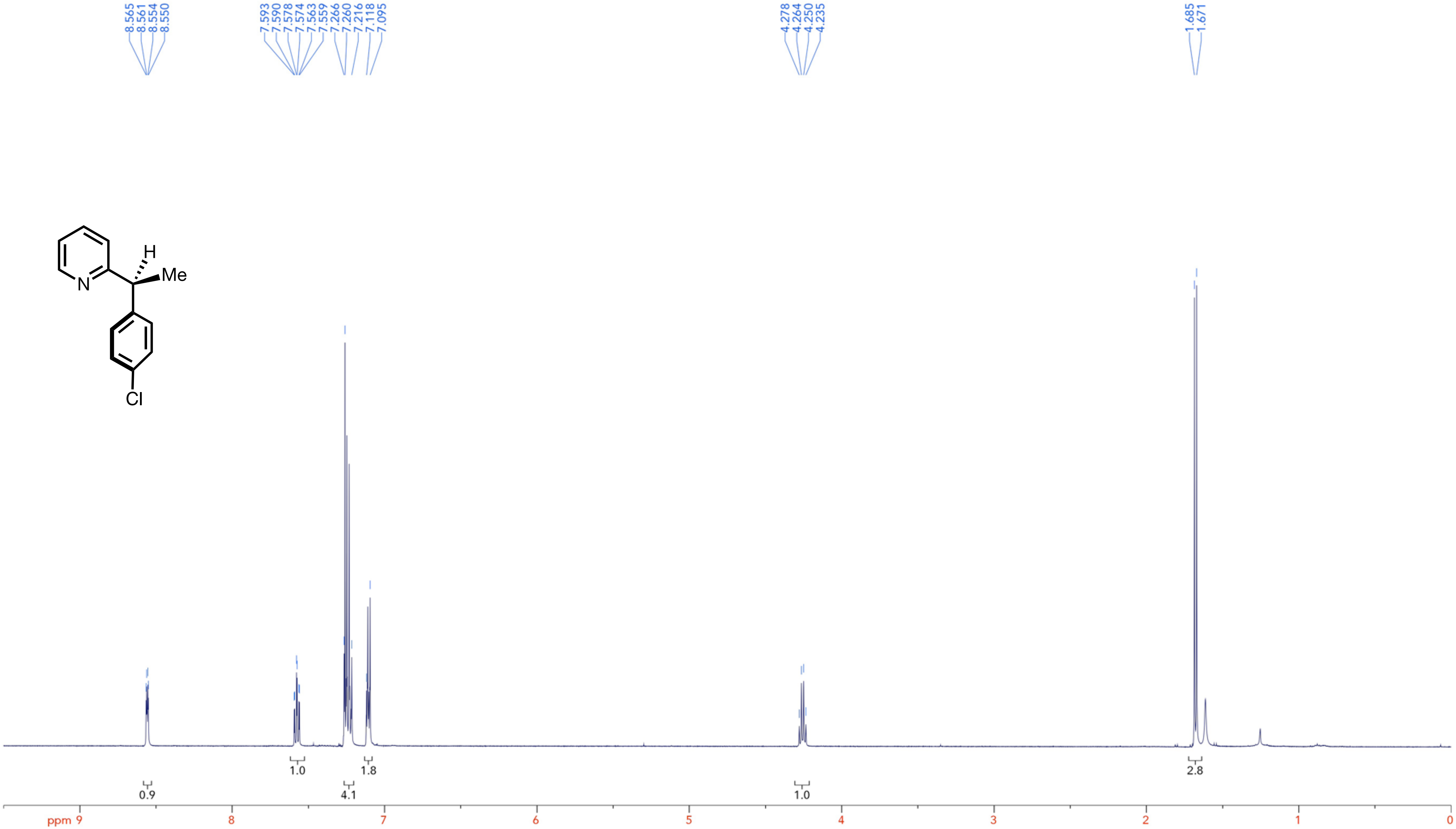
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


YN-5-85_fluorine_2ndvialagain.10.fid
-117.08
H
Me
N
F
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
30
10
-10
-30
-50
-70
-90
-110
-130
-150
-170
-190
-210
-230
-250
f1 (ppm)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8.554
8.550
7.593
7.590
7.578
7.574
7.563
7.559
7.266
7.260
7.216
7.118
7.095
4.278
4.264
4.250
4.235
1.685
1.671
H
Me
N
Cl
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4.1
1.8
1.0
2.8
ppm 9
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7
6
5
4
3
2
1
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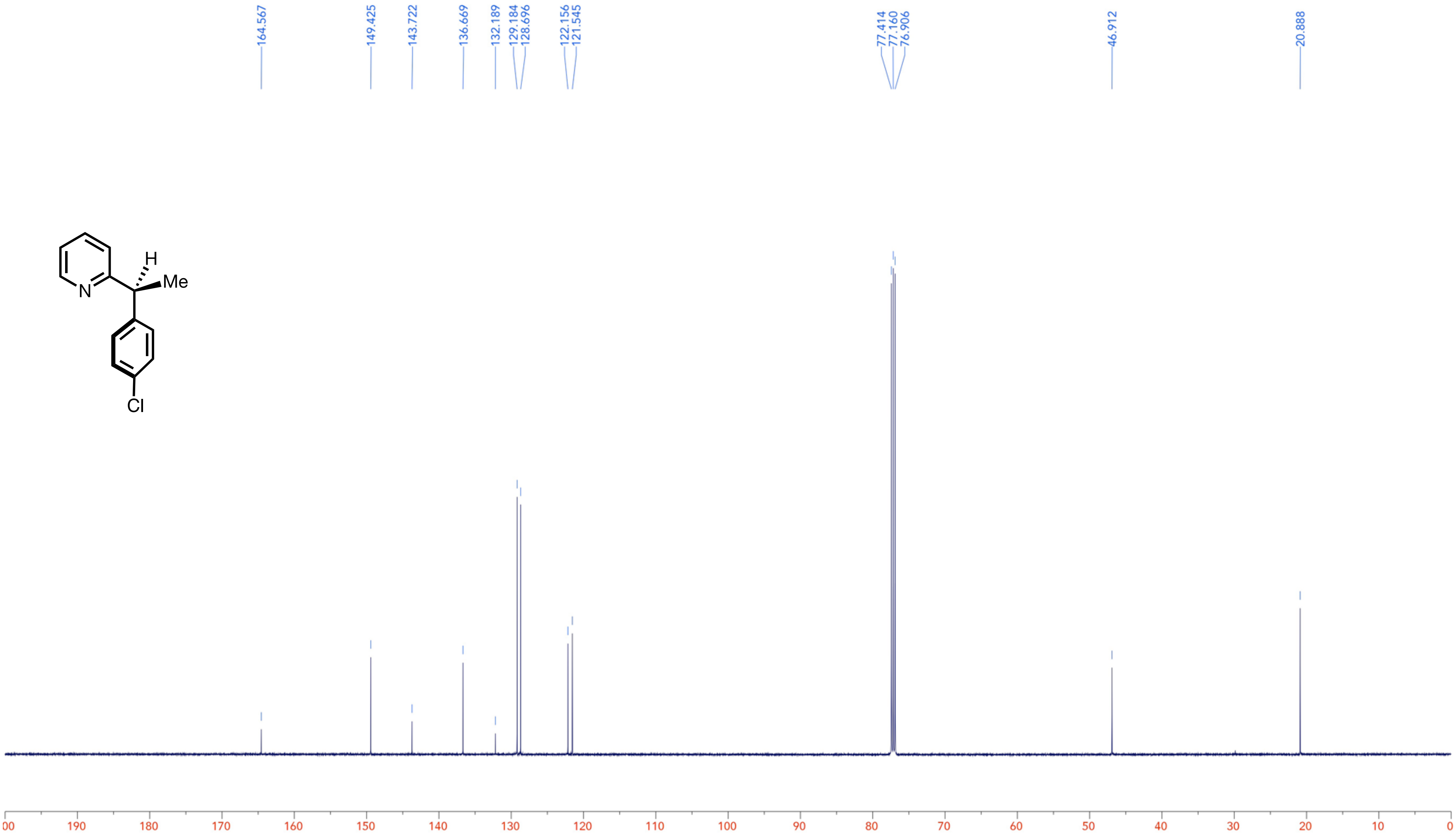
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143.722
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129.184
128.696
122.156
121.545
77.414
77.160
76.906
46.912
20.888
N
H
Me
Cl
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180
170
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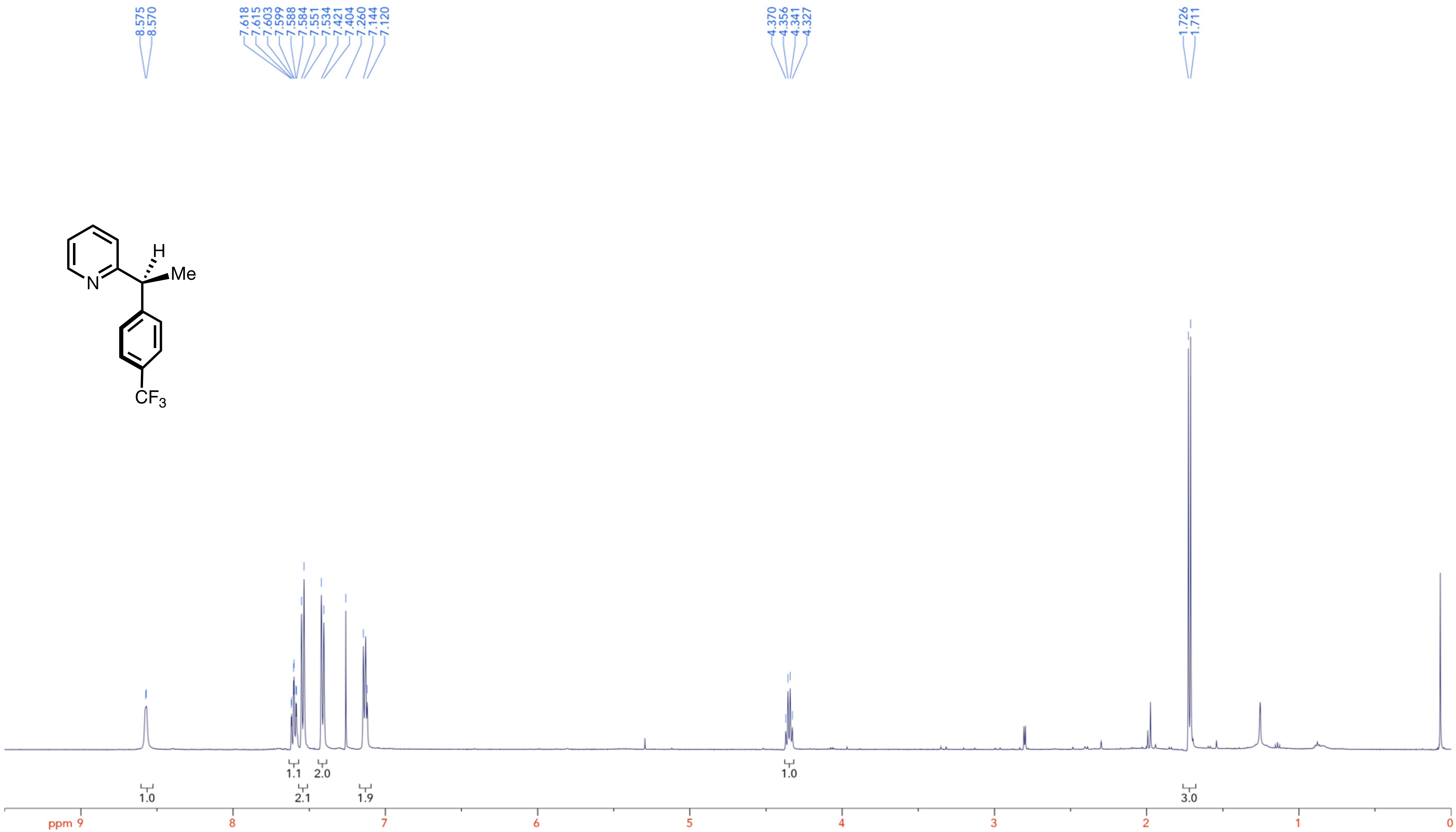
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0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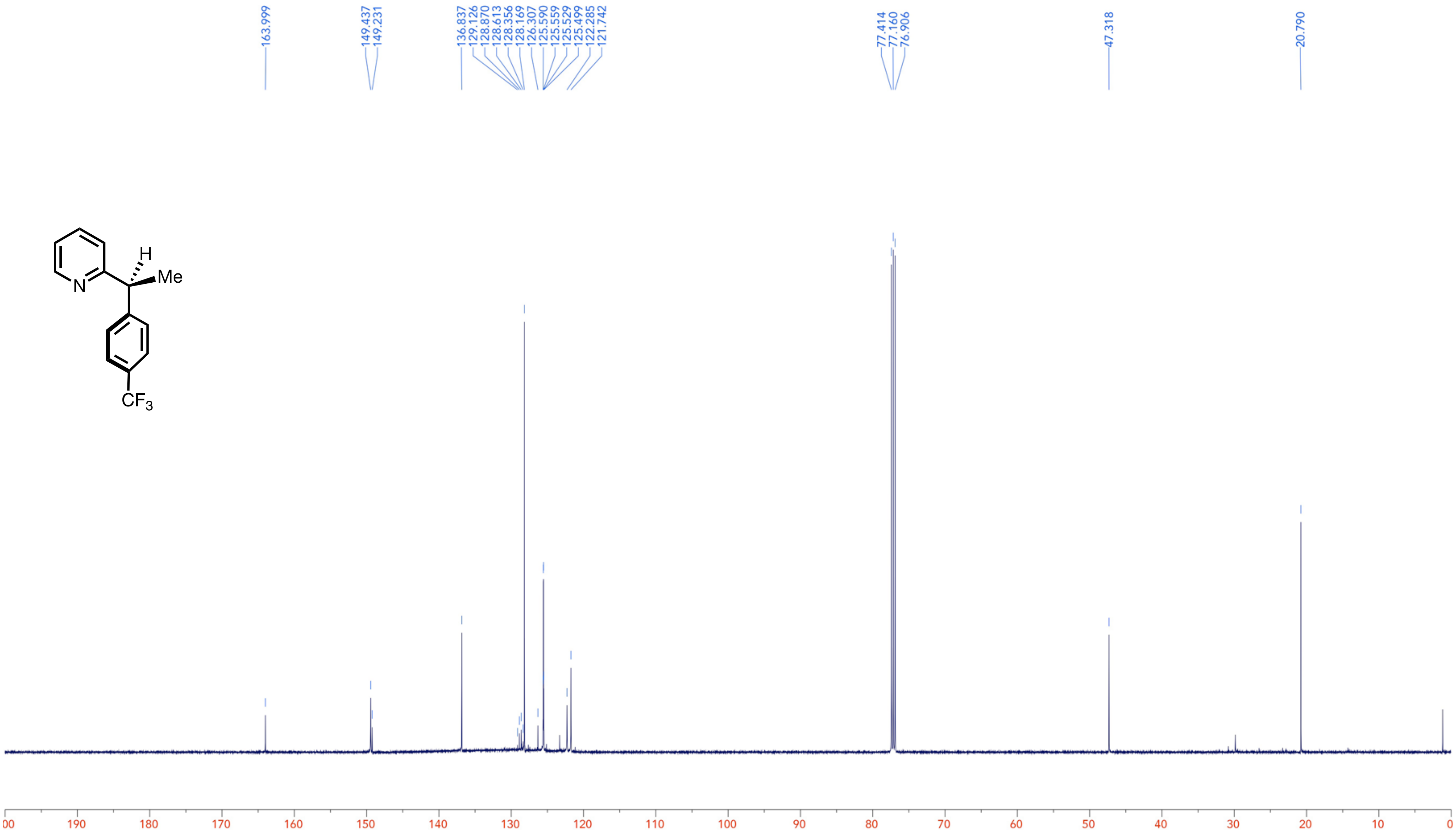
7.615
7.603
7.599
7.588
7.584
7.551
7.534
7.421
7.404
7.260
7.144
7.120
4.370
4.356
4.341
4.327
1.726
1.711
H
Me
N
CF3
1.1
2.0
1.0
1.0
2.1
1.9
3.0
ppm 9
8
7
6
5
4
3
2
1
0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149.231
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128.870
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128.356
128.169
126.307
125.590
125.559
125.529
125.499
122.285
121.742
77.414
77.160
76.906
47.318
20.790
H
Me
N
CF3
00
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180
170
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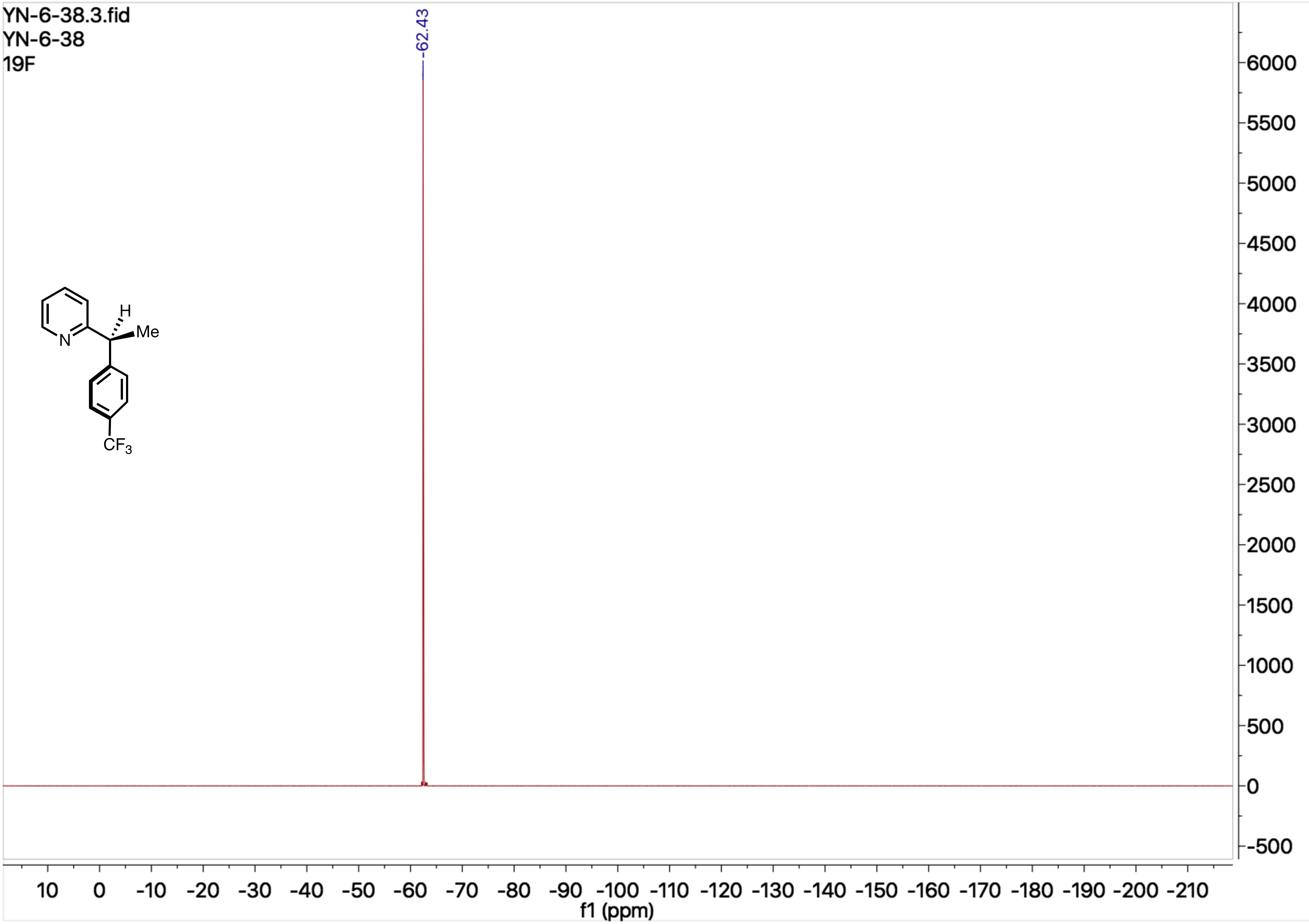
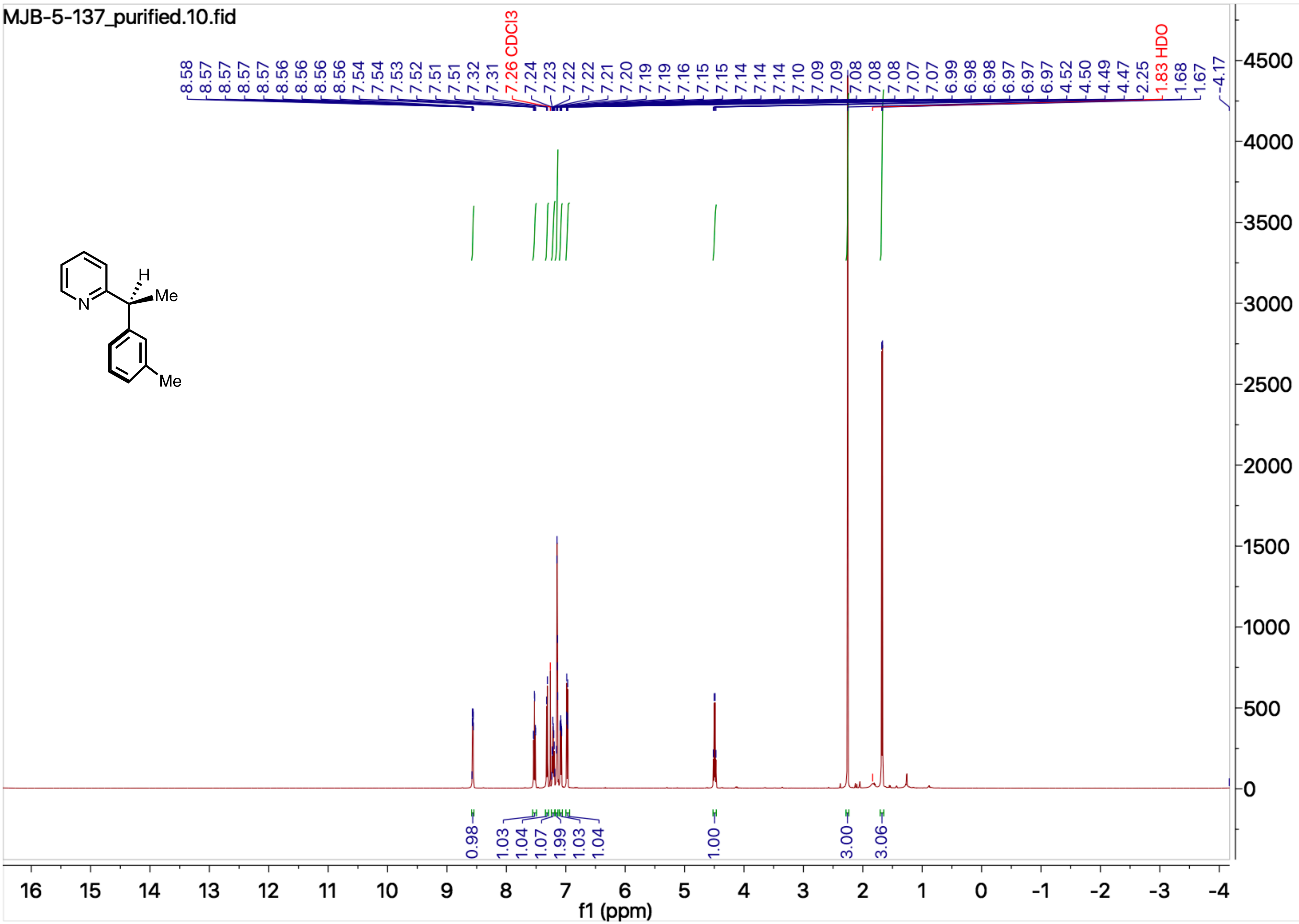
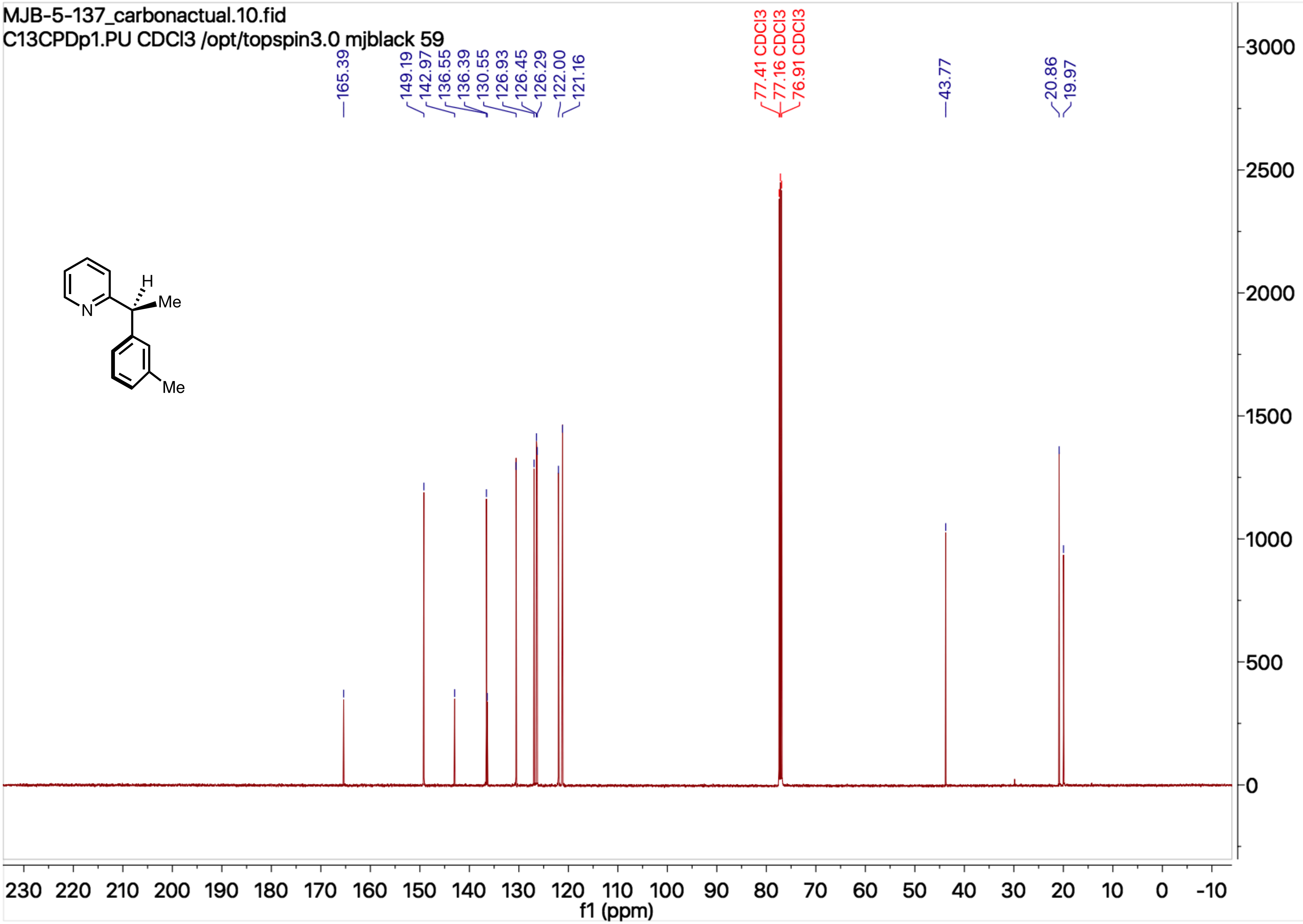
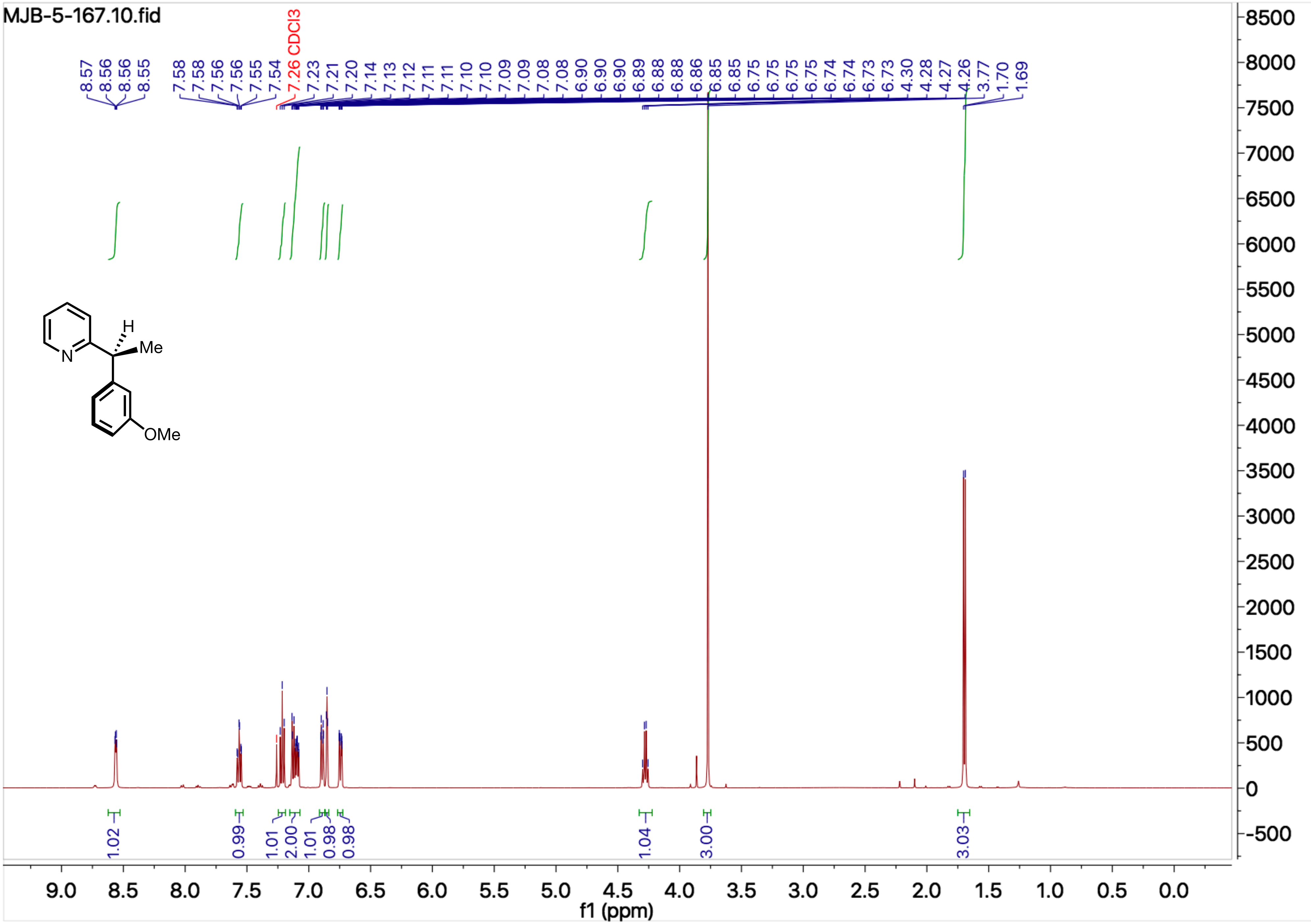
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


YN-6-38.3.fid
YN-6-38
19F
-62.43
H
Me
N
CF3
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)


MJB-5-137_purified.10.fid
8.58 8.57 8.57 8.57 8.57 8.56 8.56 8.56 8.56 7.54 7.54 7.53 7.52 7.51 7.51 7.32 7.31 7.26 CDCl3 7.24 7.23 7.22 7.22 7.21 7.20 7.19 7.19 7.16 7.15 7.15 7.14 7.14 7.14 7.10 7.09 7.09 7.08 7.08 7.08 7.07 7.07 6.99 6.98 6.98 6.97 6.97 6.97 4.52 4.50 4.49 4.47 2.25 1.83 HDO 1.68 1.67 -4.17
N
H
Me
Me
0.98 1.03 1.04 1.07 1.99 1.03 1.04 1.00 3.00 3.06
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)
4500 4000 3500 3000 2500 2000 1500 1000 500 0


MJB-5-137_carbonactual.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 59
165.39
149.19
142.97
136.55
136.39
130.55
126.93
126.45
126.29
122.00
121.16
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
43.77
20.86
19.97
H
Me
N
Me
3000
2500
2000
1500
1000
500
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


MJB-5-167.10.fid
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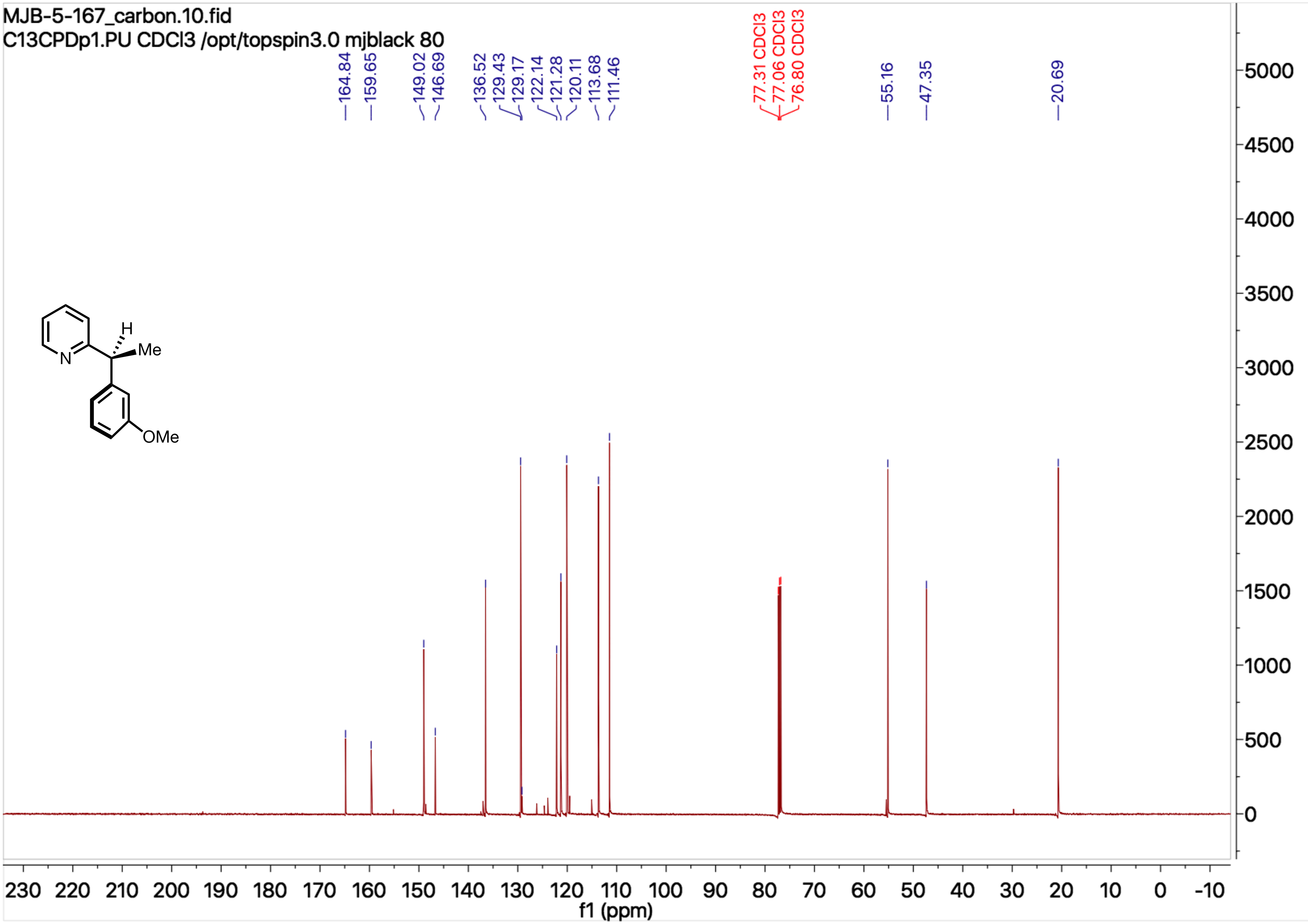
N
H
Me
OMe
1.02 0.99 1.01 2.00 1.01 0.98 0.98 1.04 3.00 3.03
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
8500 8000 7500 7000 6500 6000 5500 5000 4500 4000 3500 3000 2500 2000 1500 1000 500 0 -500


MJB-5-167_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 80
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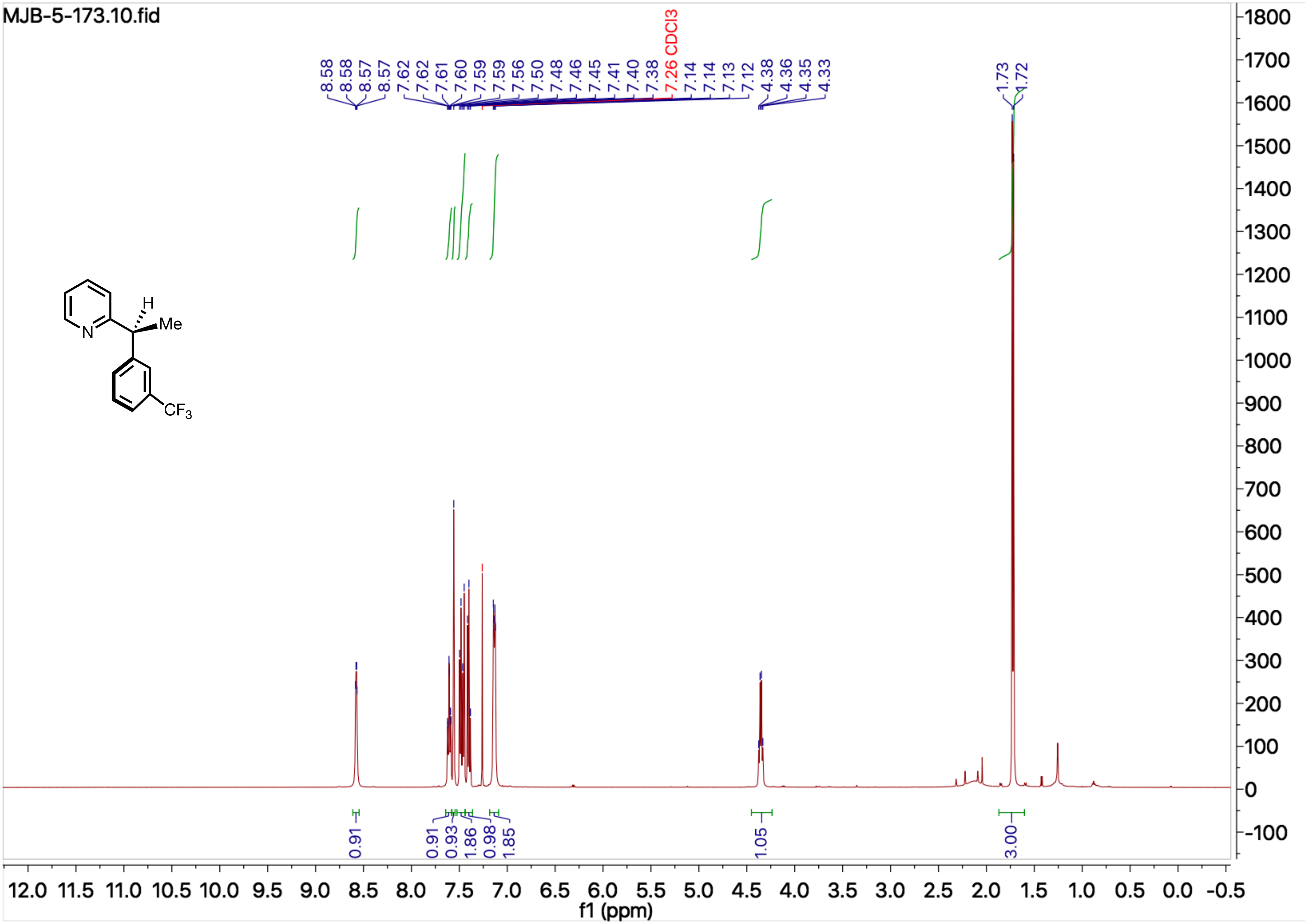
159.65
149.02
146.69
136.52
129.43
129.17
122.14
121.28
120.11
113.68
111.46
77.31 CDCl3
77.06 CDCl3
76.80 CDCl3
55.16
47.35
20.69
N
H
Me
OMe
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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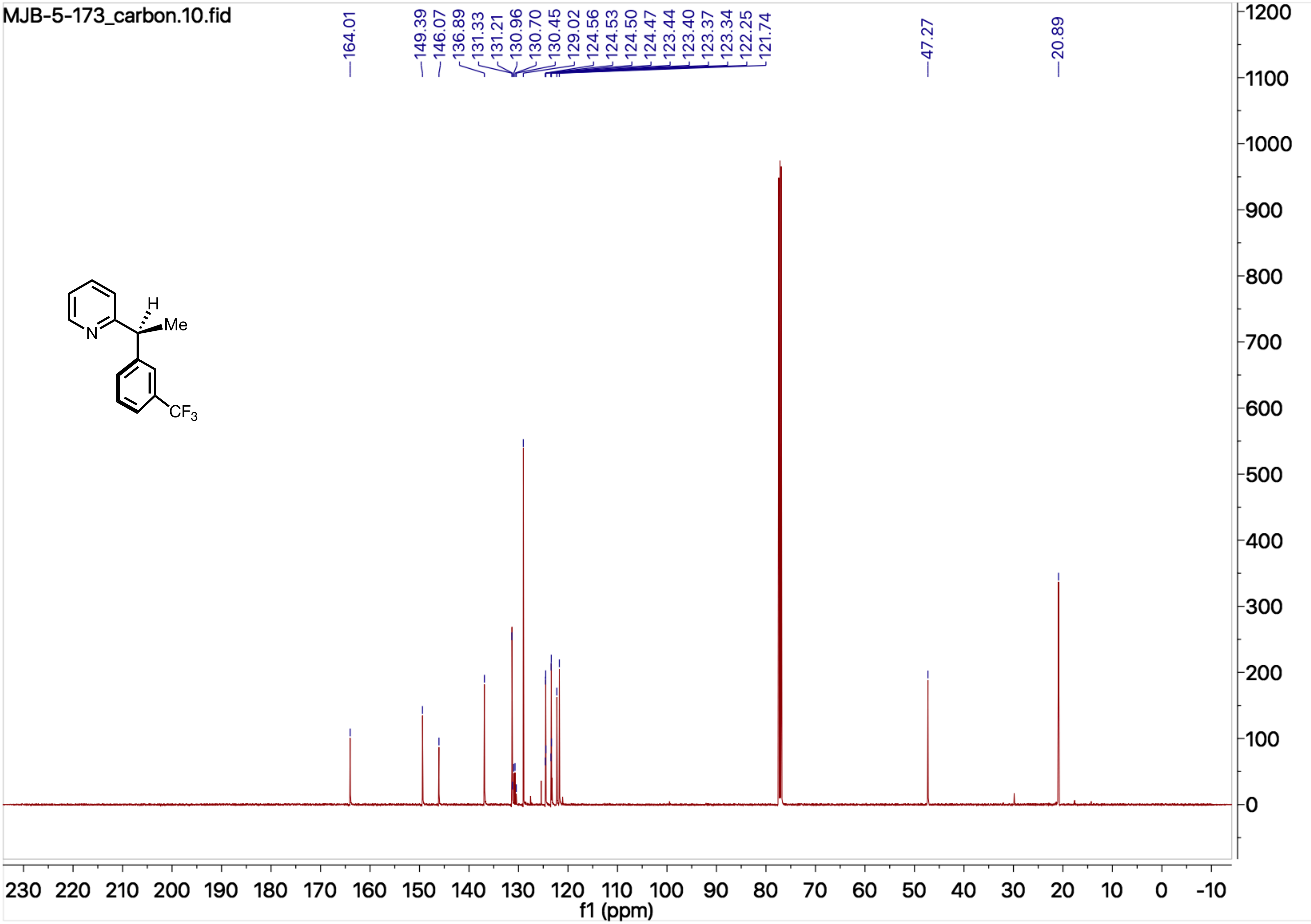
8.57
8.57
7.62
7.62
7.61
7.60
7.59
7.59
7.56
7.50
7.48
7.46
7.45
7.41
7.40
7.38
7.26 CDCl3
7.14
7.14
7.13
7.12
4.38
4.36
4.35
4.33
1.73
1.72
H
Me
N
CF3
0.91
0.91
0.93
1.86
0.98
1.85
1.05
3.00
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


MJB-5-173_carbon.10.fid
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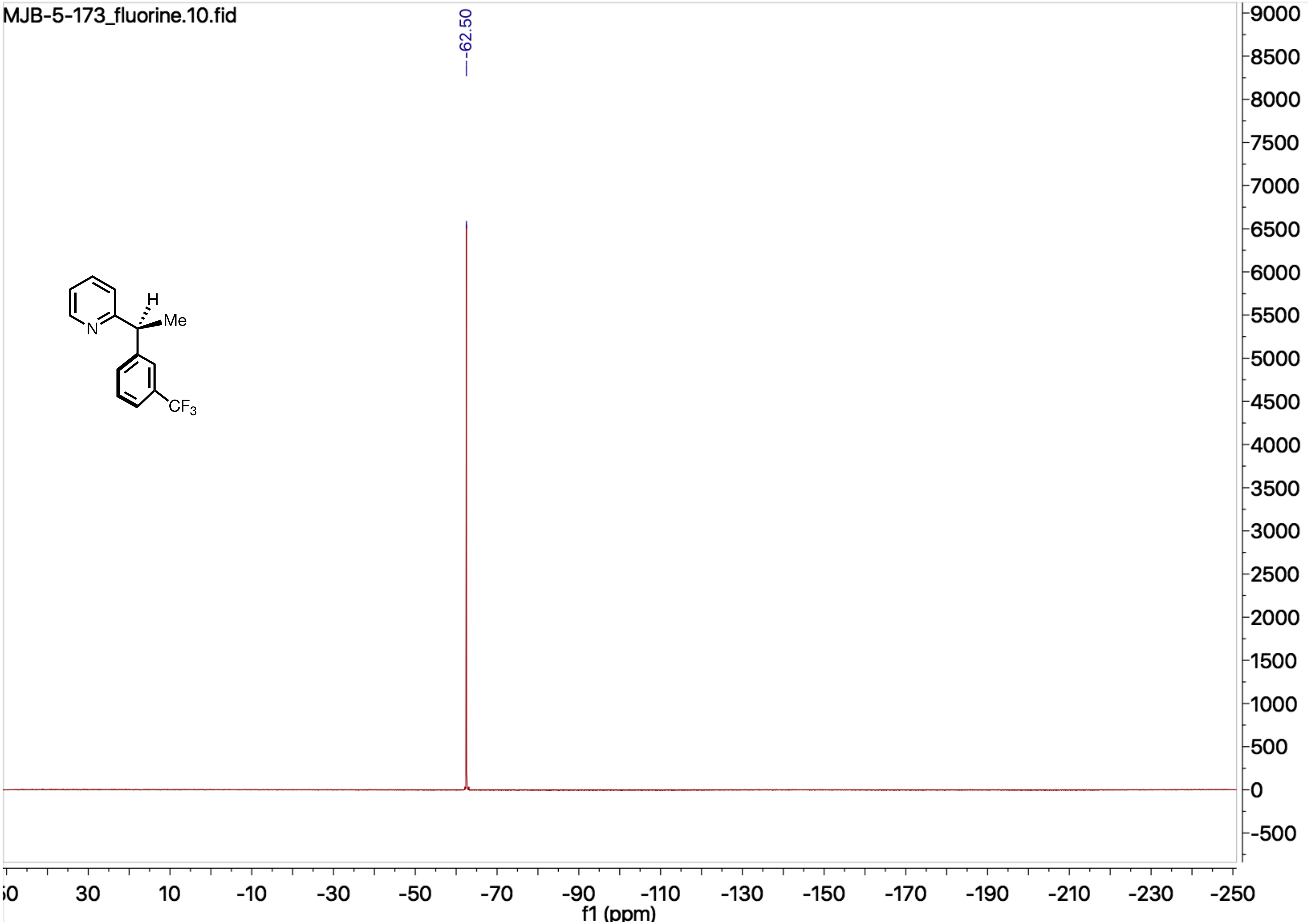
149.39
146.07
136.89
131.33
131.21
130.96
130.70
130.45
129.02
124.56
124.53
124.50
124.47
123.44
123.40
123.37
123.34
122.25
121.74
47.27
20.89
N
H
Me
CF3
f1 (ppm)


MJB-5-173_fluorine.10.fid
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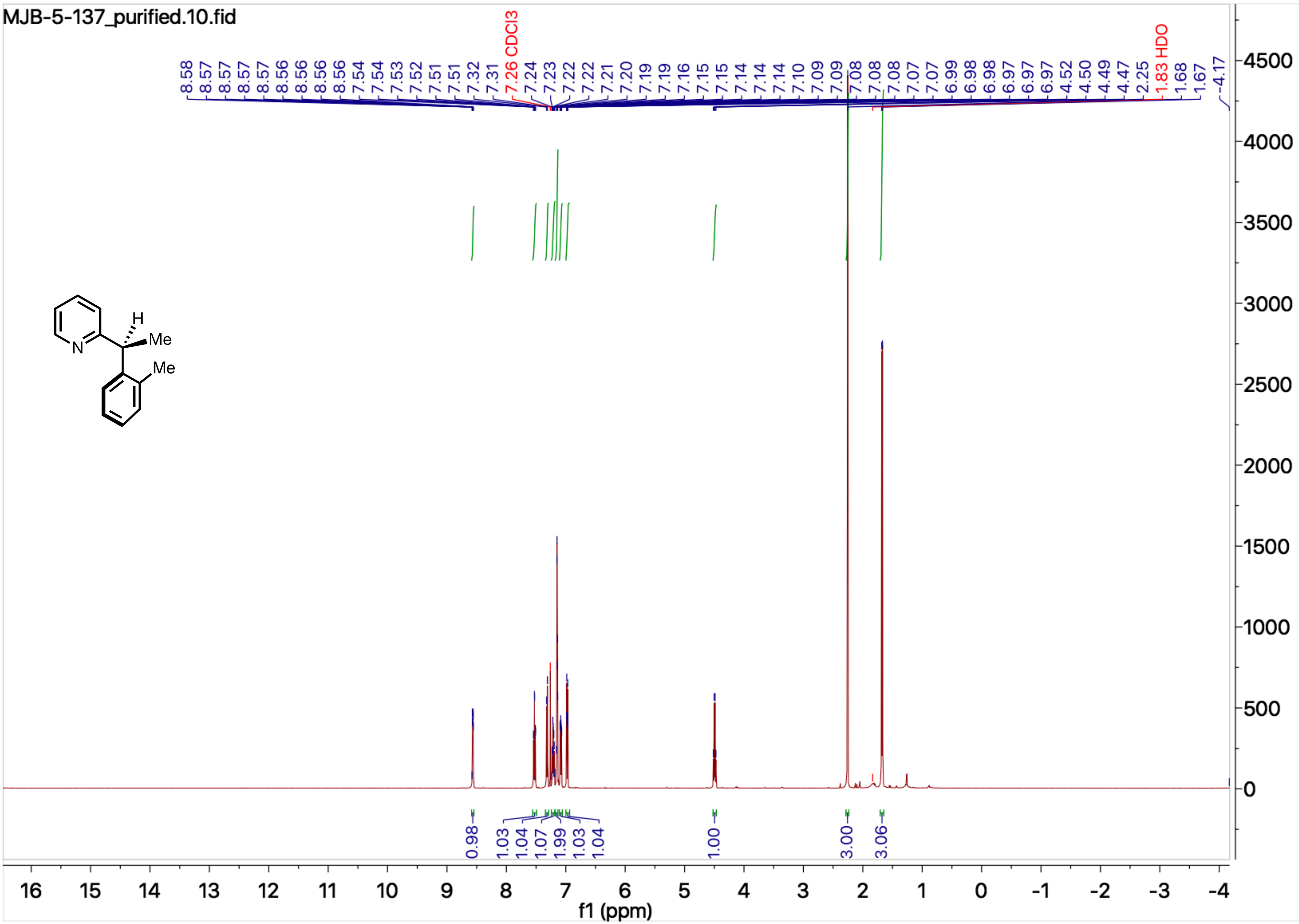
H
Me
N
CF3
9000
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
30
10
-10
-30
-50
-70
-90
-110
-130
-150
-170
-190
-210
-230
-250
f1 (ppm)


MJB-5-137_purified.10.fid
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8.57
8.57
8.57
8.57
8.56
8.56
8.56
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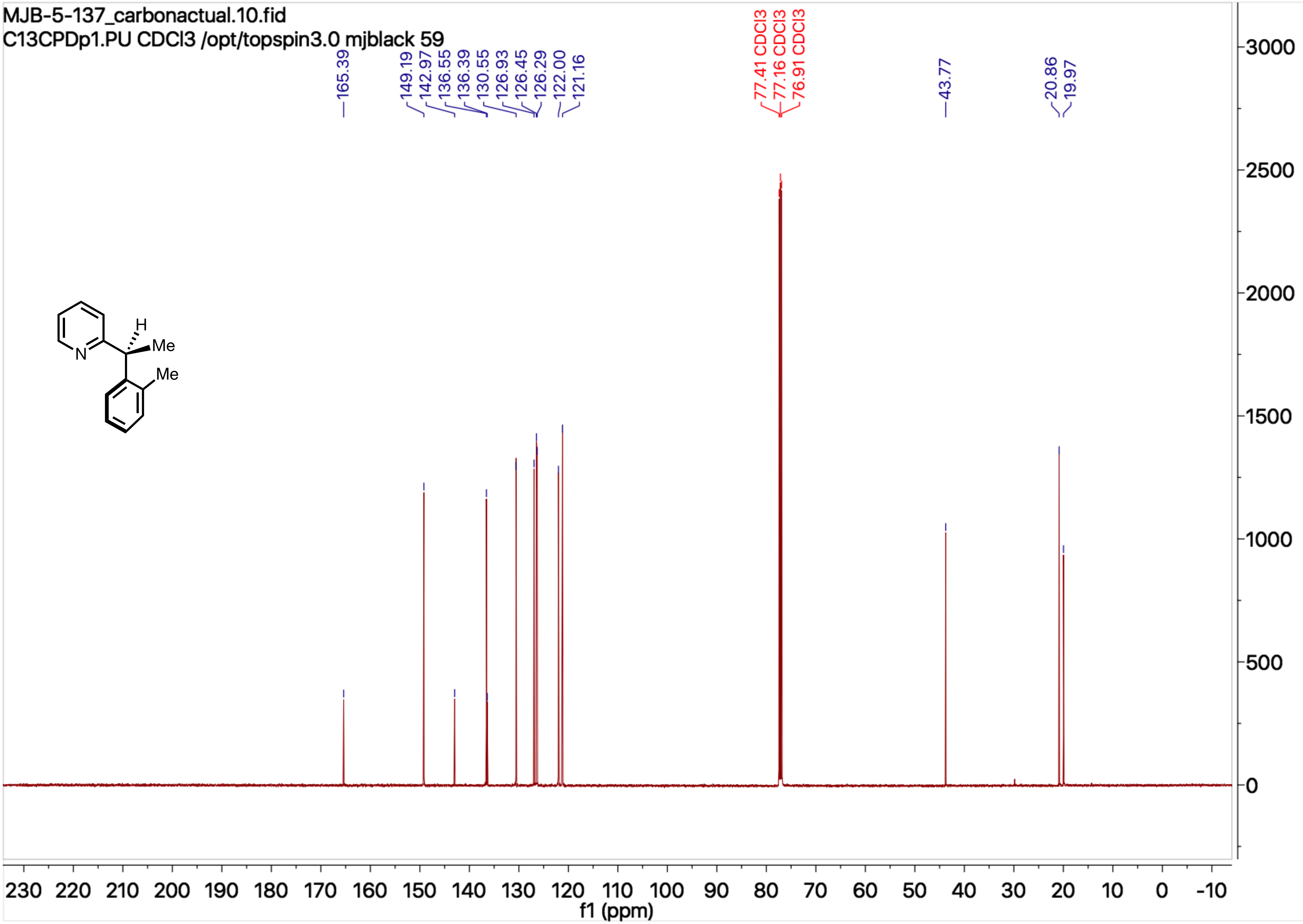
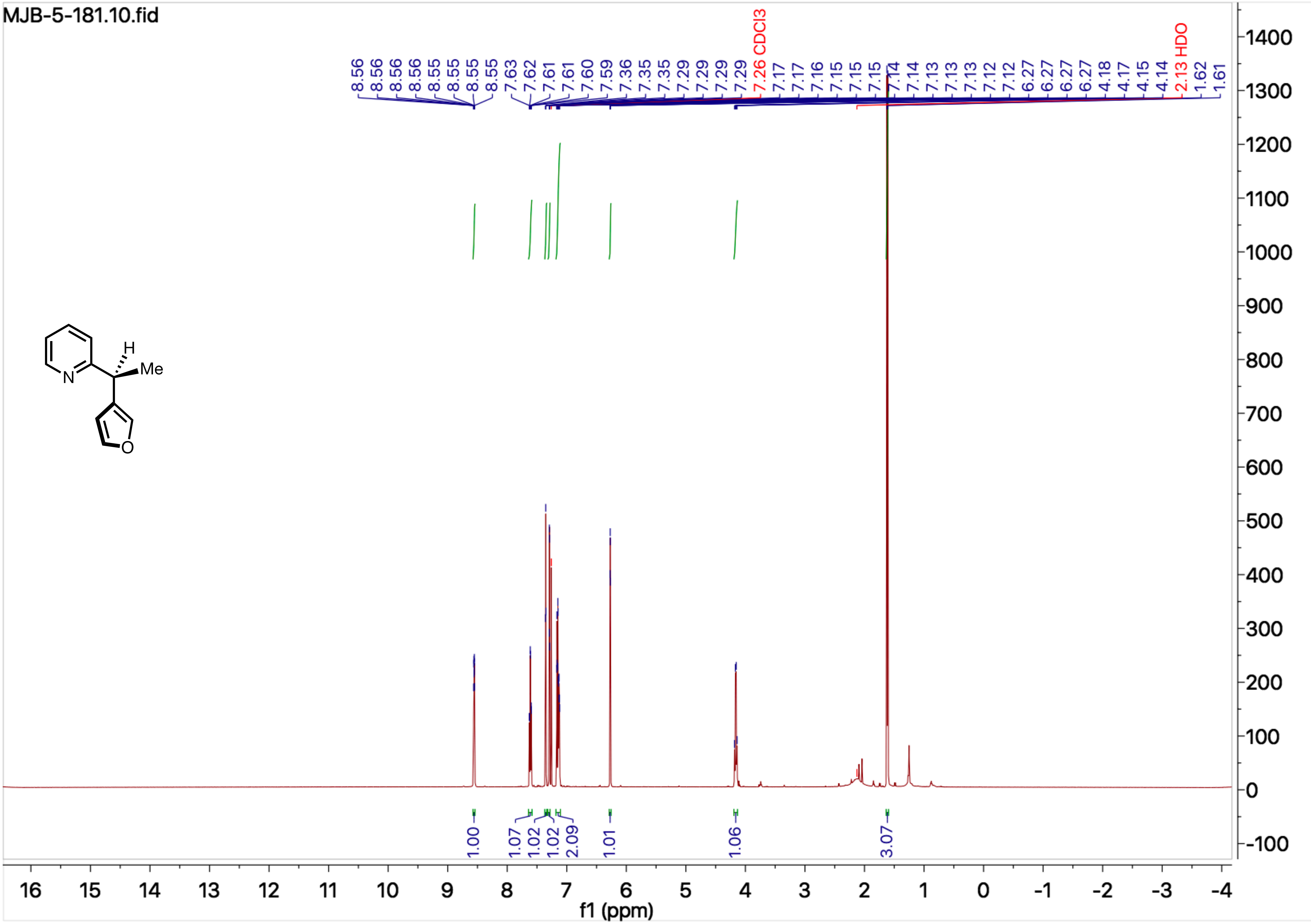
7.54
7.54
7.53
7.52
7.51
7.51
7.32
7.31
7.26 CDCl3
7.24
7.23
7.22
7.22
7.21
7.20
7.19
7.19
7.16
7.15
7.15
7.14
7.14
7.14
7.10
7.09
7.09
7.08
7.08
7.08
7.07
7.07
6.99
6.98
6.98
6.97
6.97
6.97
4.52
4.50
4.49
4.47
2.25
1.83 HDO
1.68
1.67
-4.17
H
Me
N
Me
0.98
1.03
1.04
1.07
1.99
1.03
1.04
1.00
3.00
3.06
f1 (ppm)


MJB-5-137_carbonactual.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 59
165.39
149.19
142.97
136.55
136.39
130.55
126.93
126.45
126.29
122.00
121.16
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
43.77
20.86
19.97
N
H
Me
Me
f1 (ppm)


MJB-5-181.10.fid
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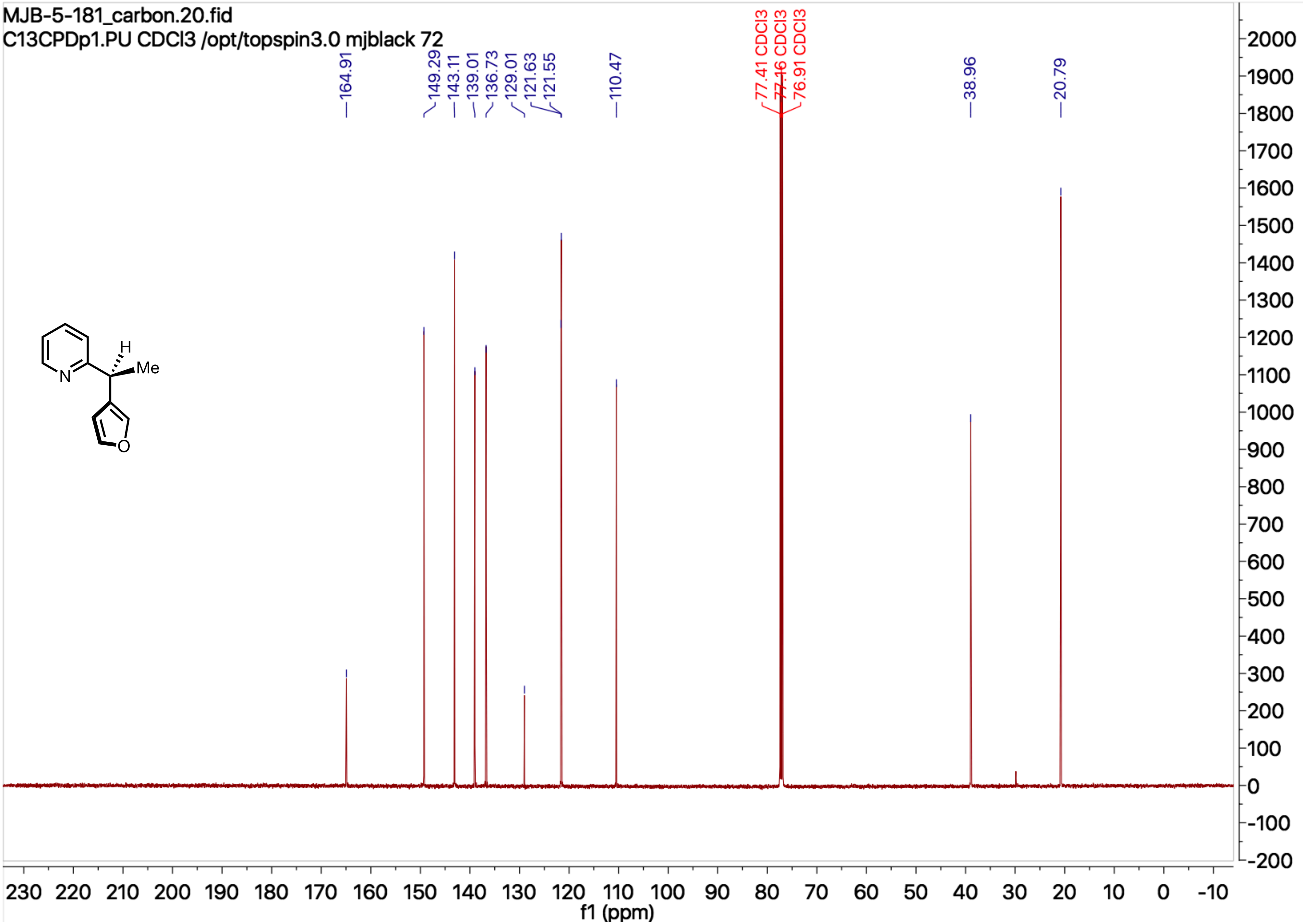
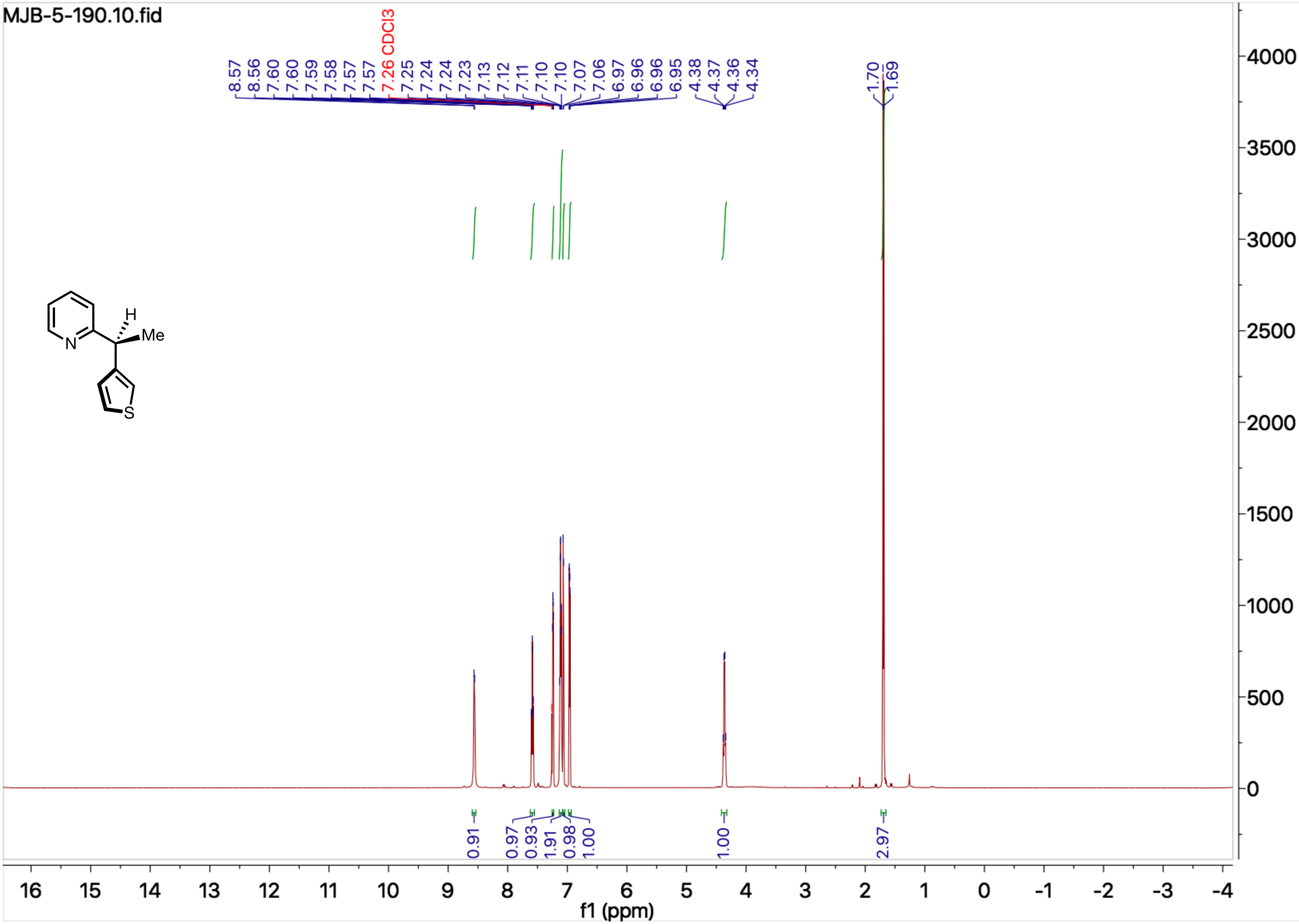
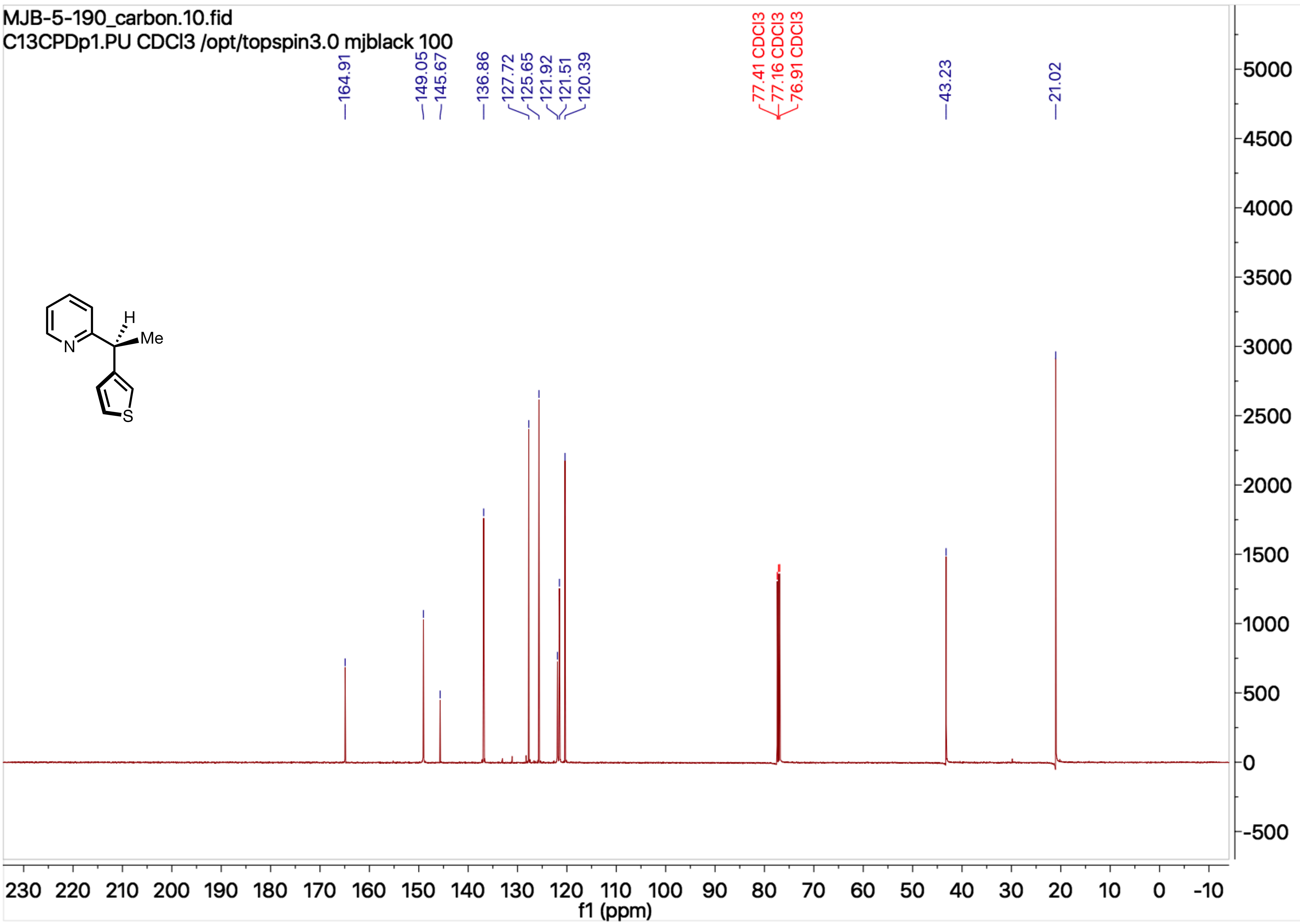
8.56
8.56
8.56
8.55
8.55
8.55
8.55
7.63
7.62
7.61
7.61
7.60
7.59
7.36
7.35
7.35
7.29
7.29
7.29
7.29
7.26 CDCl3
7.17
7.17
7.16
7.15
7.15
7.15
7.14
7.14
7.13
7.13
7.13
7.12
7.12
6.27
6.27
6.27
6.27
4.18
4.17
4.15
4.14
2.13 HDO
1.62
1.61
1.00
1.07
1.02
1.02
2.09
1.01
1.06
3.07
H
Me
N
O
f1 (ppm)


MJB-5-181_carbon.20.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 72
164.91
149.29
143.11
139.01
136.73
129.01
121.63
121.55
110.47
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
38.96
20.79
H
Me
N
O
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
-200
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


MJB-5-190.10.fid
8.57 8.56 7.60 7.60 7.59 7.58 7.57 7.57 7.26 CDCl3 7.25 7.24 7.24 7.23 7.13 7.12 7.11 7.10 7.10 7.07 7.06 6.97 6.96 6.96 6.95 4.38 4.37 4.36 4.34
1.70 1.69
H
Me
N
S
0.91 0.97 0.93 1.91 0.98 1.00 1.00 2.97
4000 3500 3000 2500 2000 1500 1000 500 0
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4
f1 (ppm)


MJB-5-190_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 100
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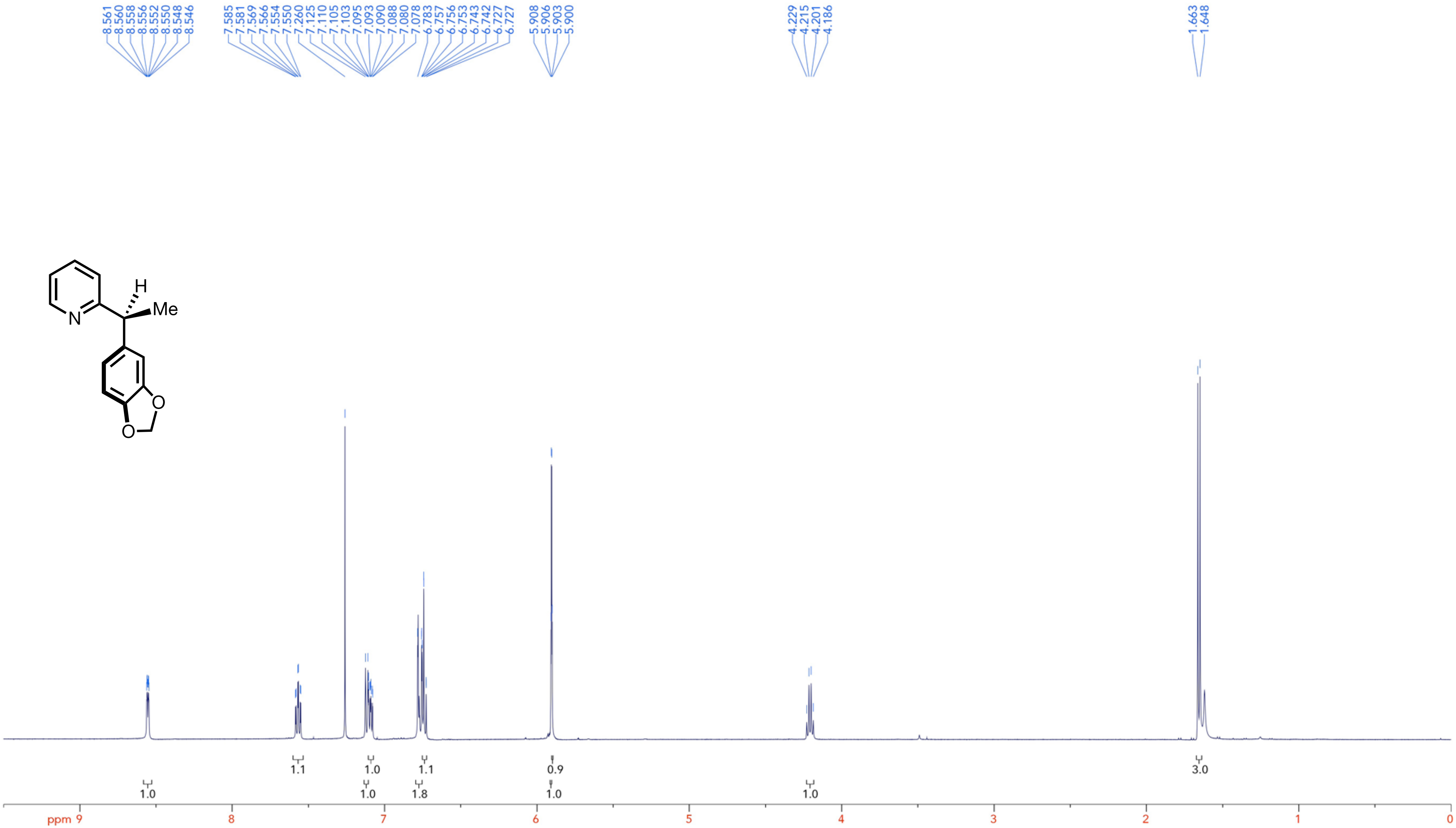
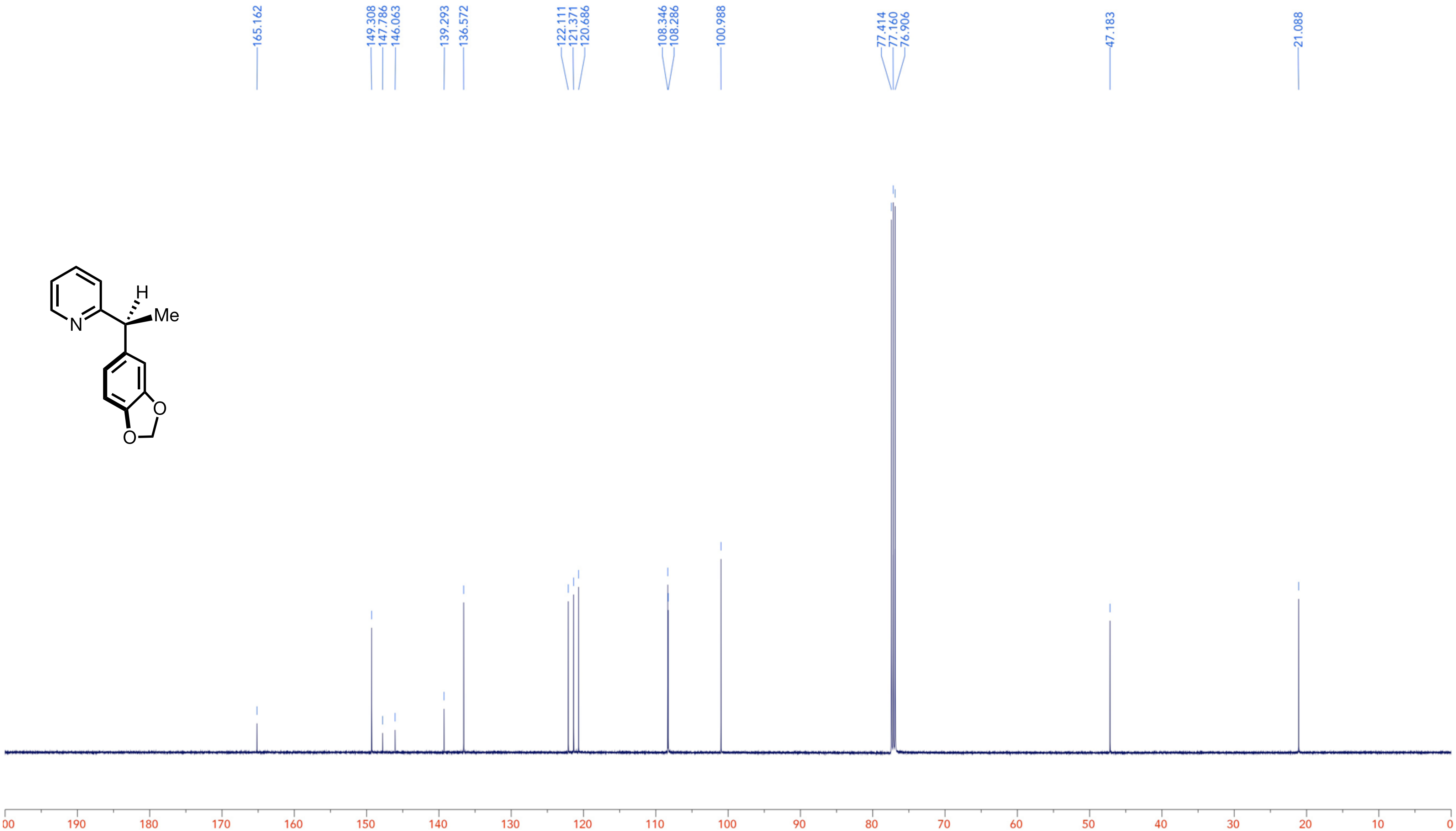
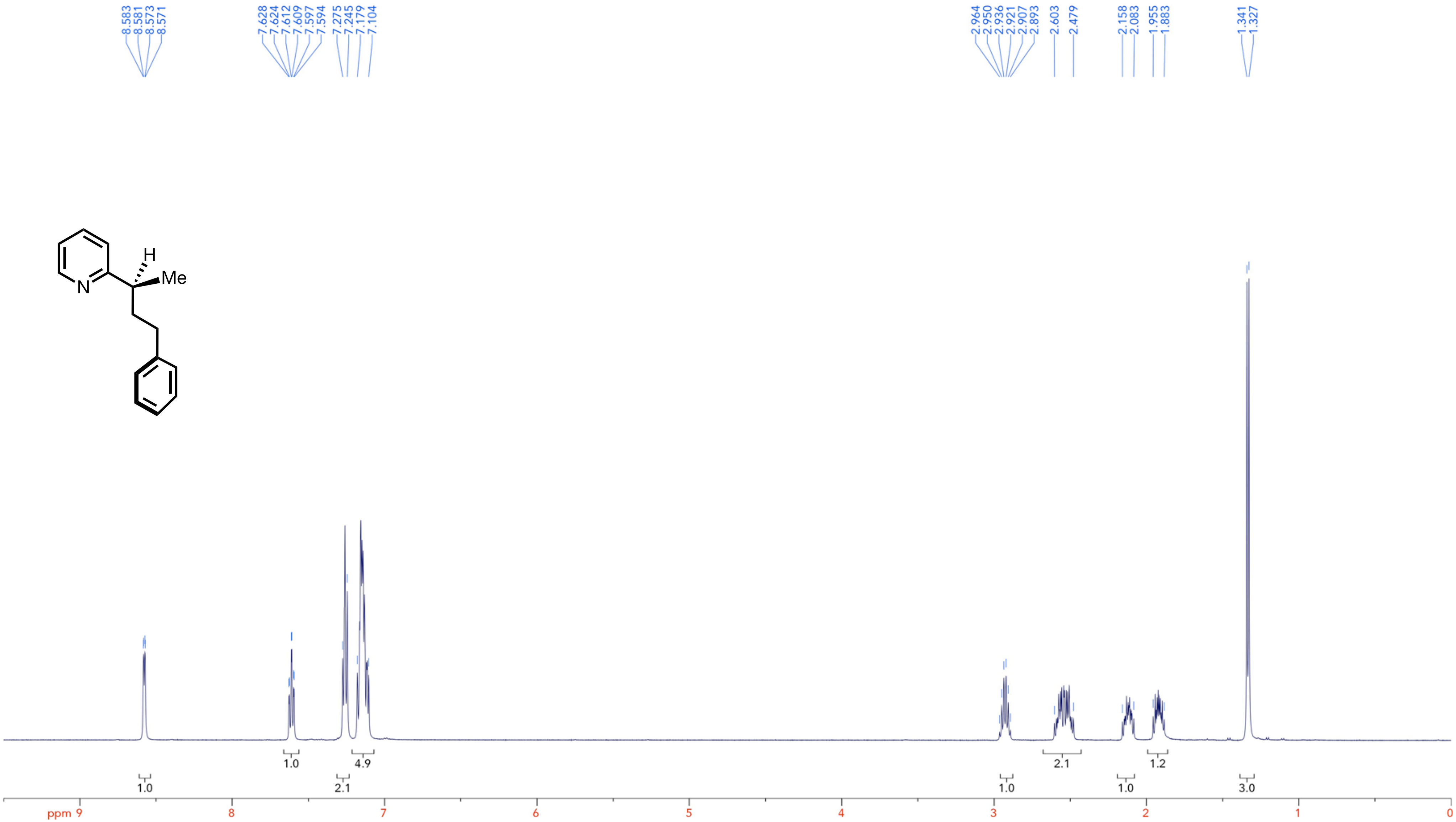
149.05
145.67
136.86
127.72
125.65
121.92
121.51
120.39
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
43.23
21.02
N
H
Me
S
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



8.561
8.560
8.558
8.556
8.552
8.550
8.548
8.546
7.585
7.581
7.569
7.566
7.554
7.550
7.260
7.125
7.110
7.105
7.103
7.095
7.093
7.090
7.088
7.080
7.078
6.783
6.757
6.756
6.753
6.743
6.742
6.727
6.727
5.908
5.906
5.903
5.900
4.229
4.215
4.201
4.186
1.663
1.648
H
Me
N
O
O
1.1
1.0
1.1
0.9
3.0
1.0
1.0
1.8
1.0
1.0
ppm 9
8
7
6
5
4
3
2
1
0



165.162
149.308
147.786
146.063
139.293
136.572
122.111
121.371
120.686
108.346
108.286
100.988
77.414
77.160
76.906
47.183
21.088
N
H
Me
O
O
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0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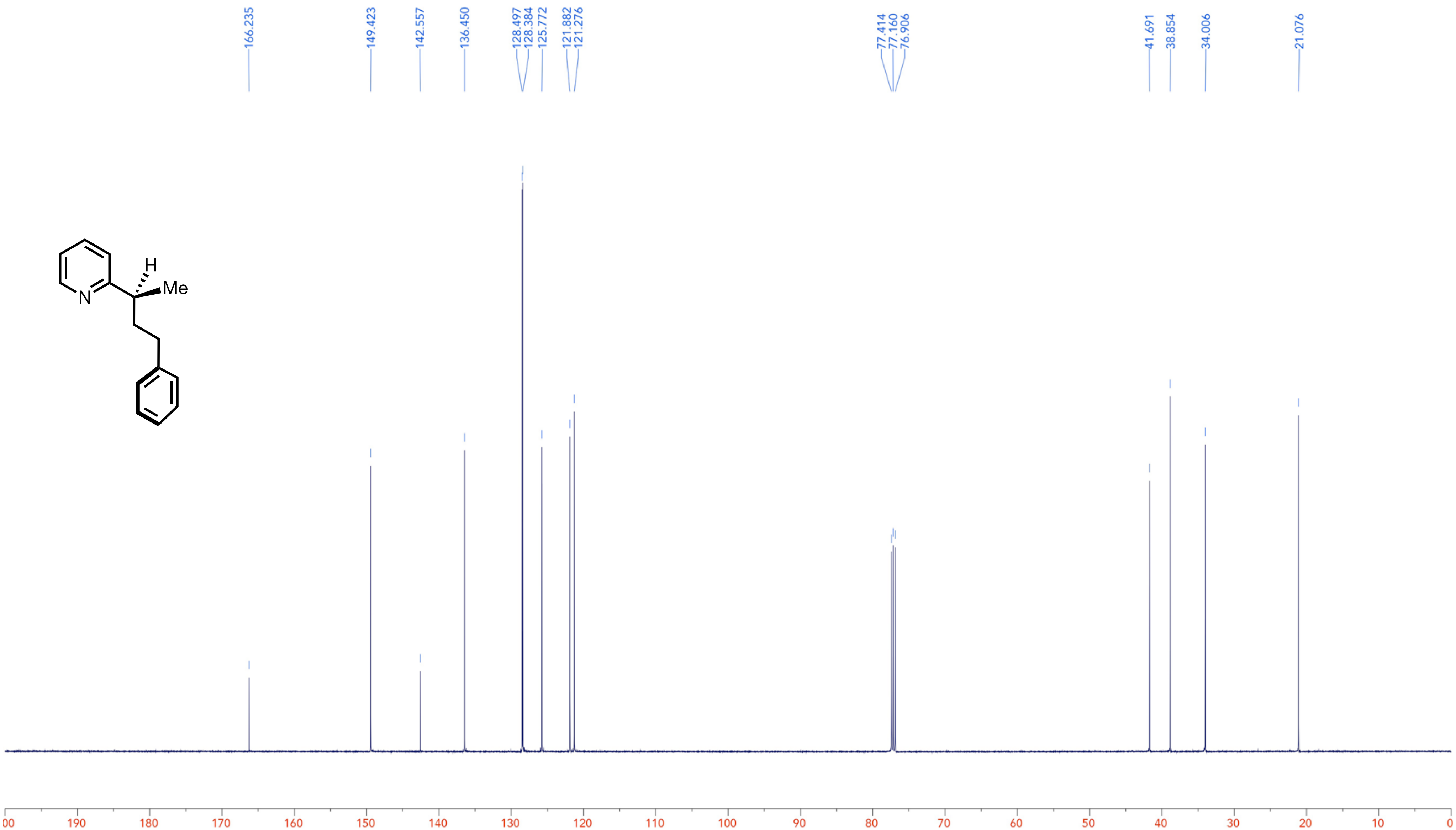
8.581
8.573
8.571
7.628
7.624
7.612
7.609
7.597
7.594
7.275
7.245
7.179
7.104
2.964
2.950
2.936
2.921
2.907
2.893
2.603
2.479
2.158
2.083
1.955
1.883
1.341
1.327
H
Me
N
1.0
1.0
2.1
4.9
1.0
2.1
1.0
1.2
3.0
ppm 9
8
7
6
5
4
3
2
1
0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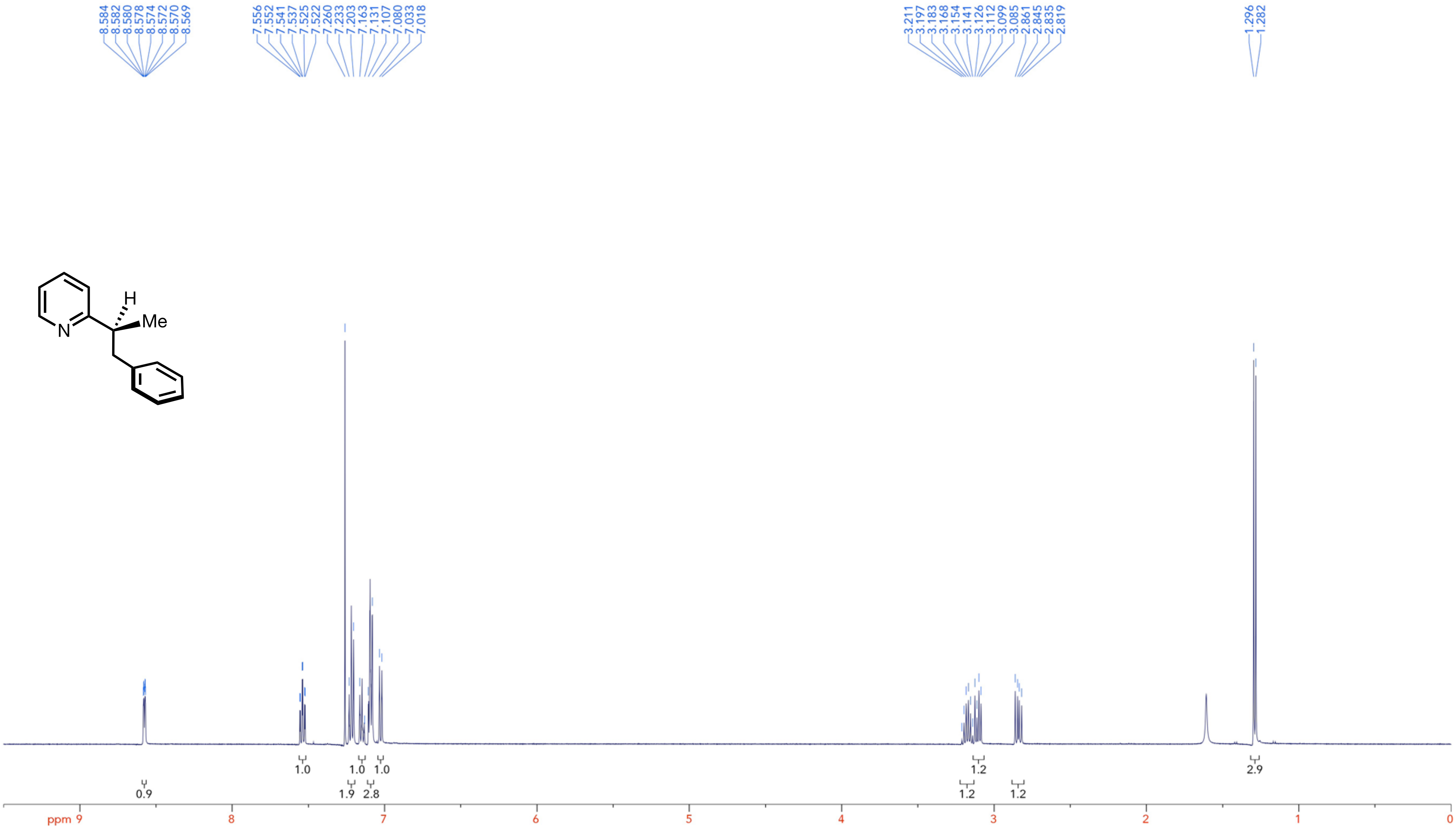
142.557
136.450
128.497
128.384
125.772
121.882
121.276
77.414
77.160
76.906
41.691
38.854
34.006
21.076
H
Me
N
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0



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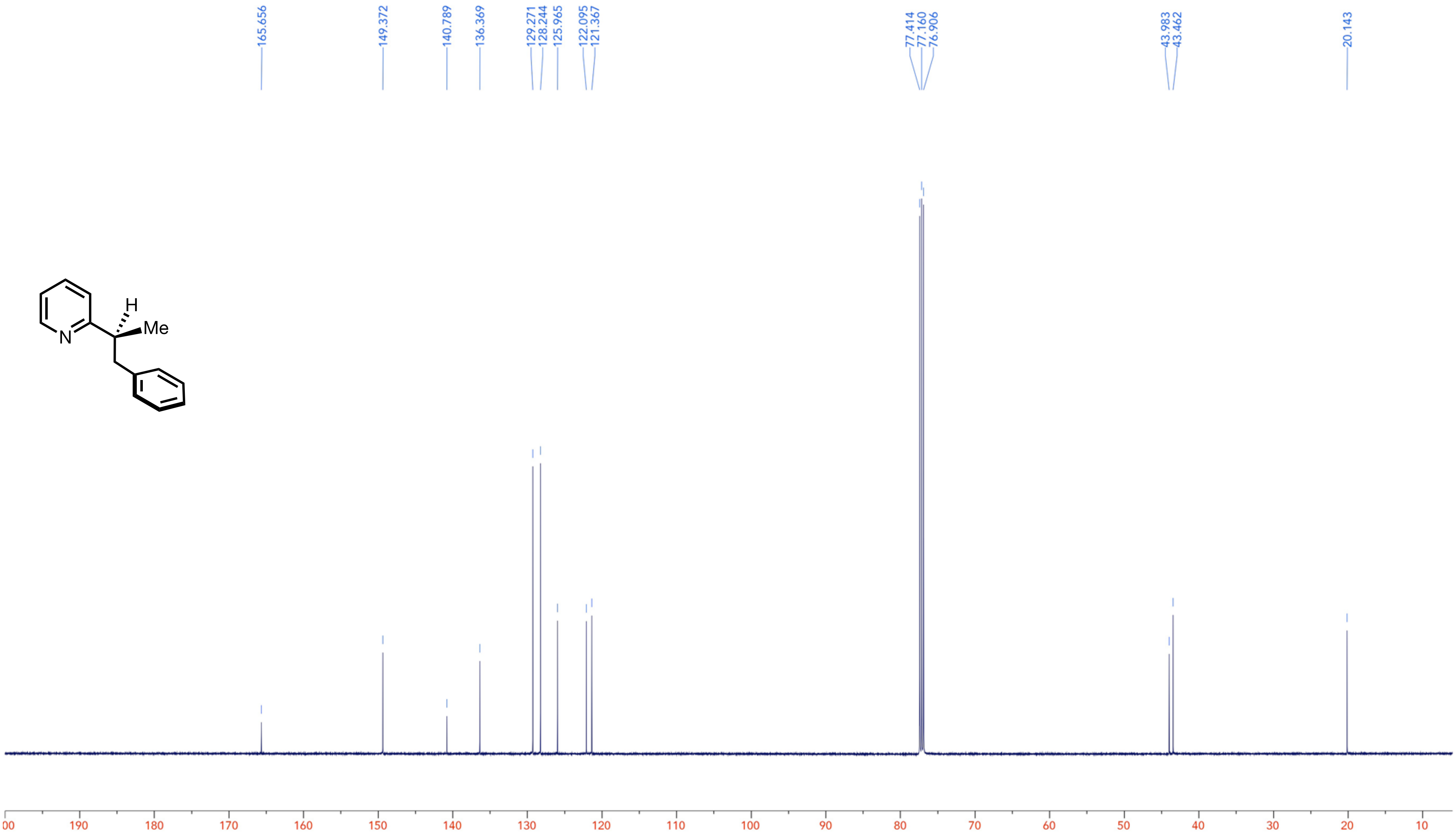
8.582
8.580
8.578
8.574
8.572
8.570
8.569
7.556
7.552
7.541
7.537
7.525
7.522
7.260
7.233
7.203
7.163
7.131
7.107
7.080
7.033
7.018
3.211
3.197
3.183
3.168
3.154
3.141
3.126
3.112
3.099
3.085
2.861
2.845
2.835
2.819
1.296
1.282
H
Me
N
0.9
1.0
1.9
1.0
2.8
1.0
1.2
1.2
1.2
2.9
ppm 9
8
7
6
5
4
3
2
1
0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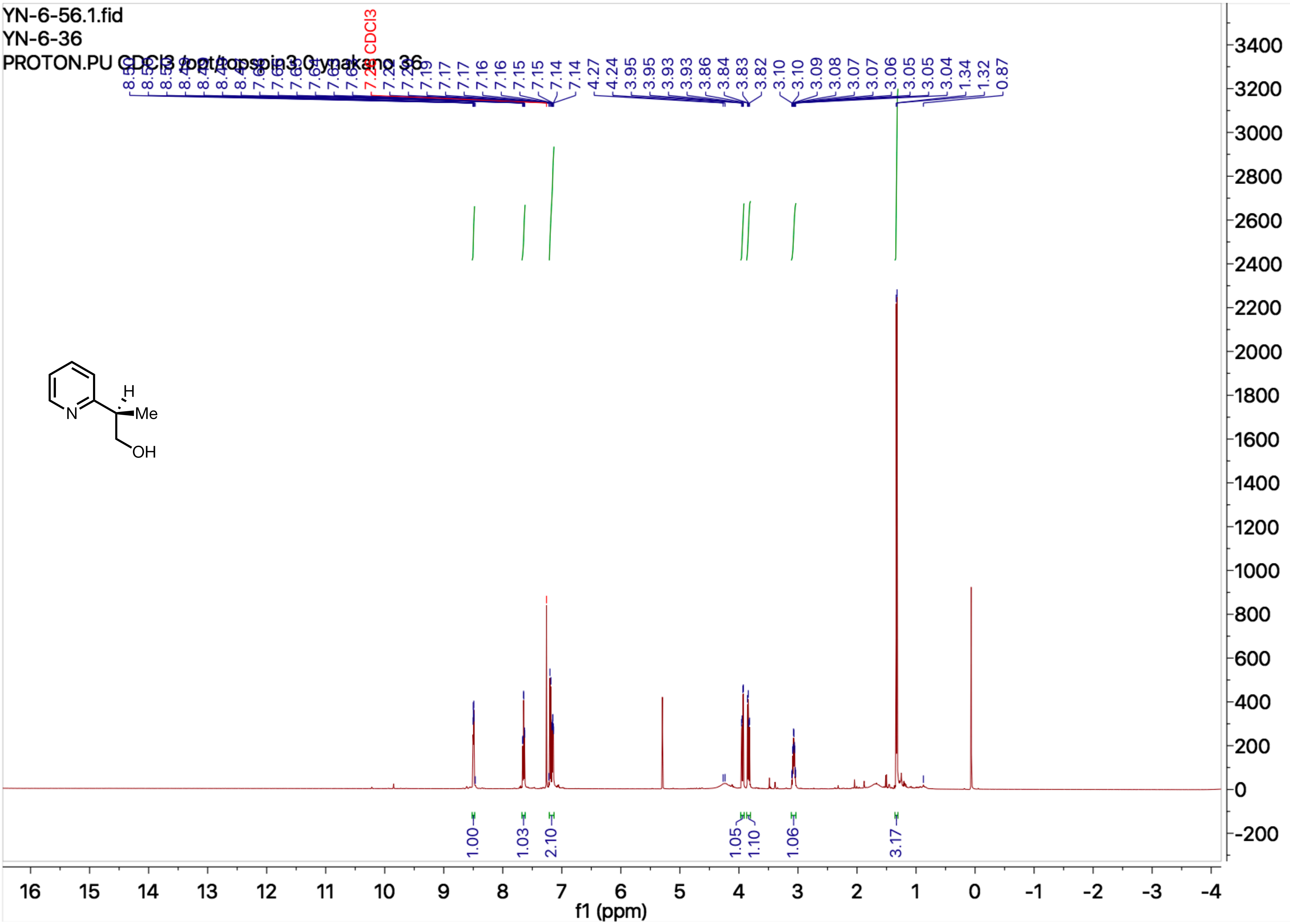
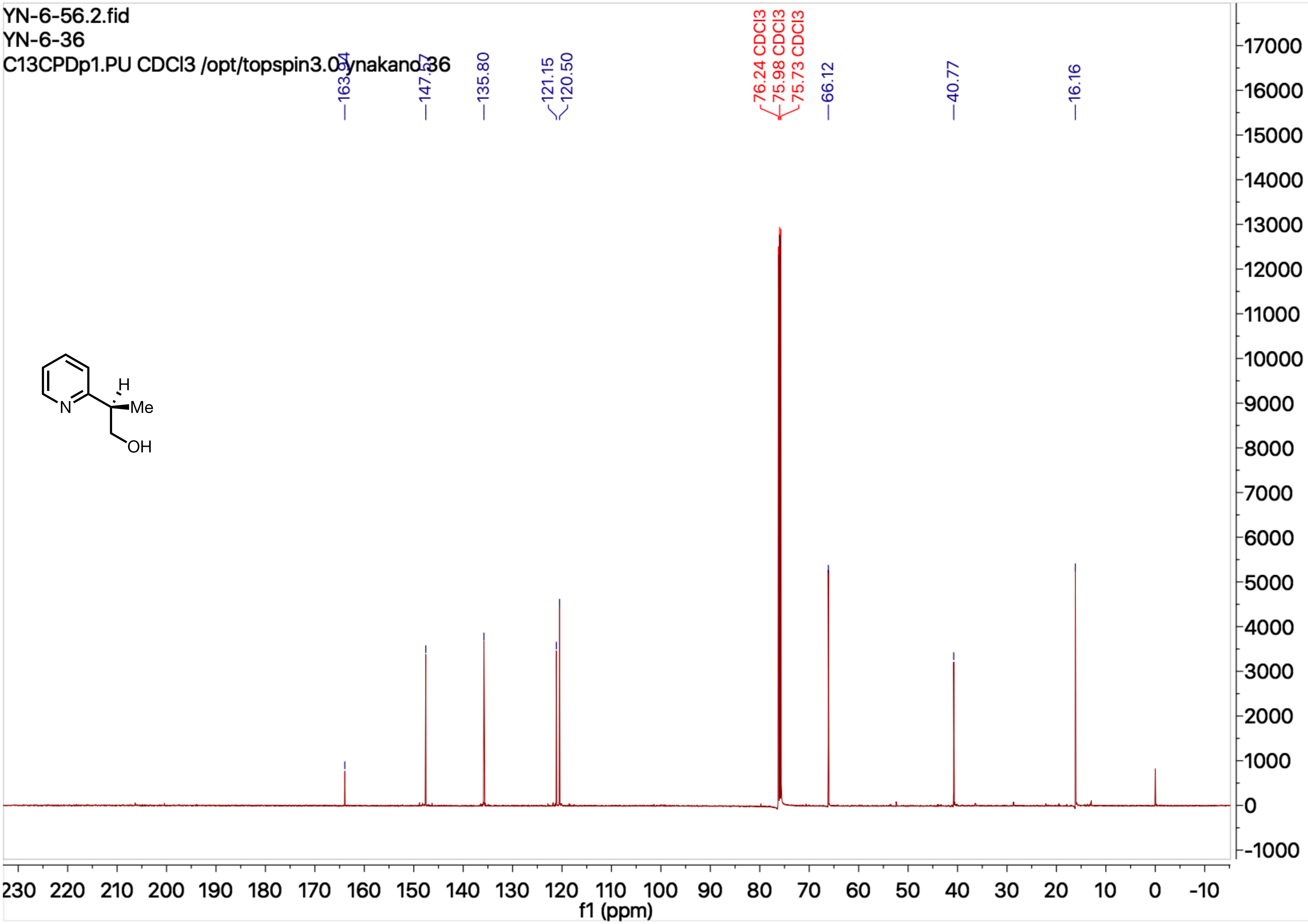
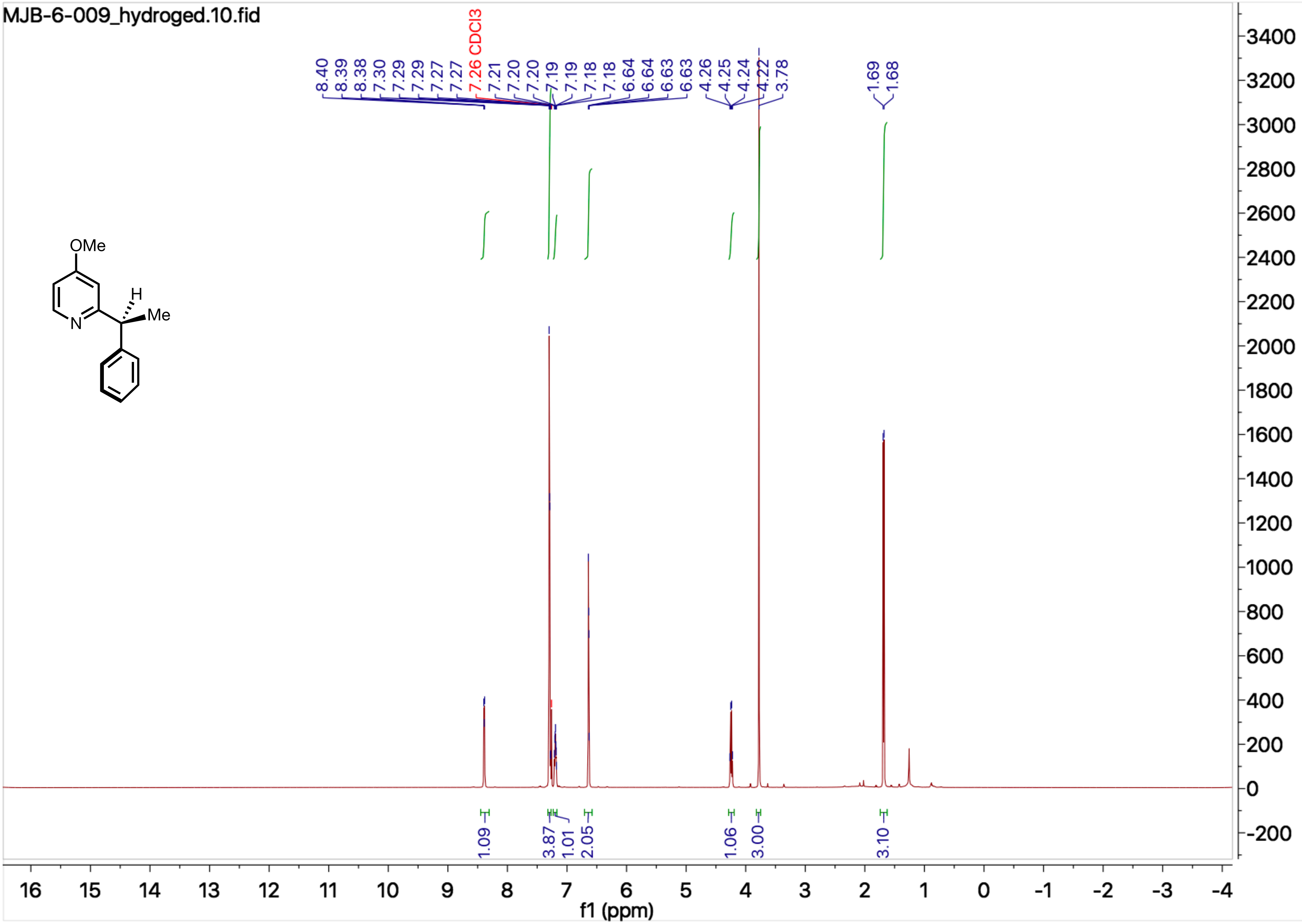
128.244
125.965
122.095
121.367
77.414
77.160
76.906
43.983
43.462
20.143
H
Me
N
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

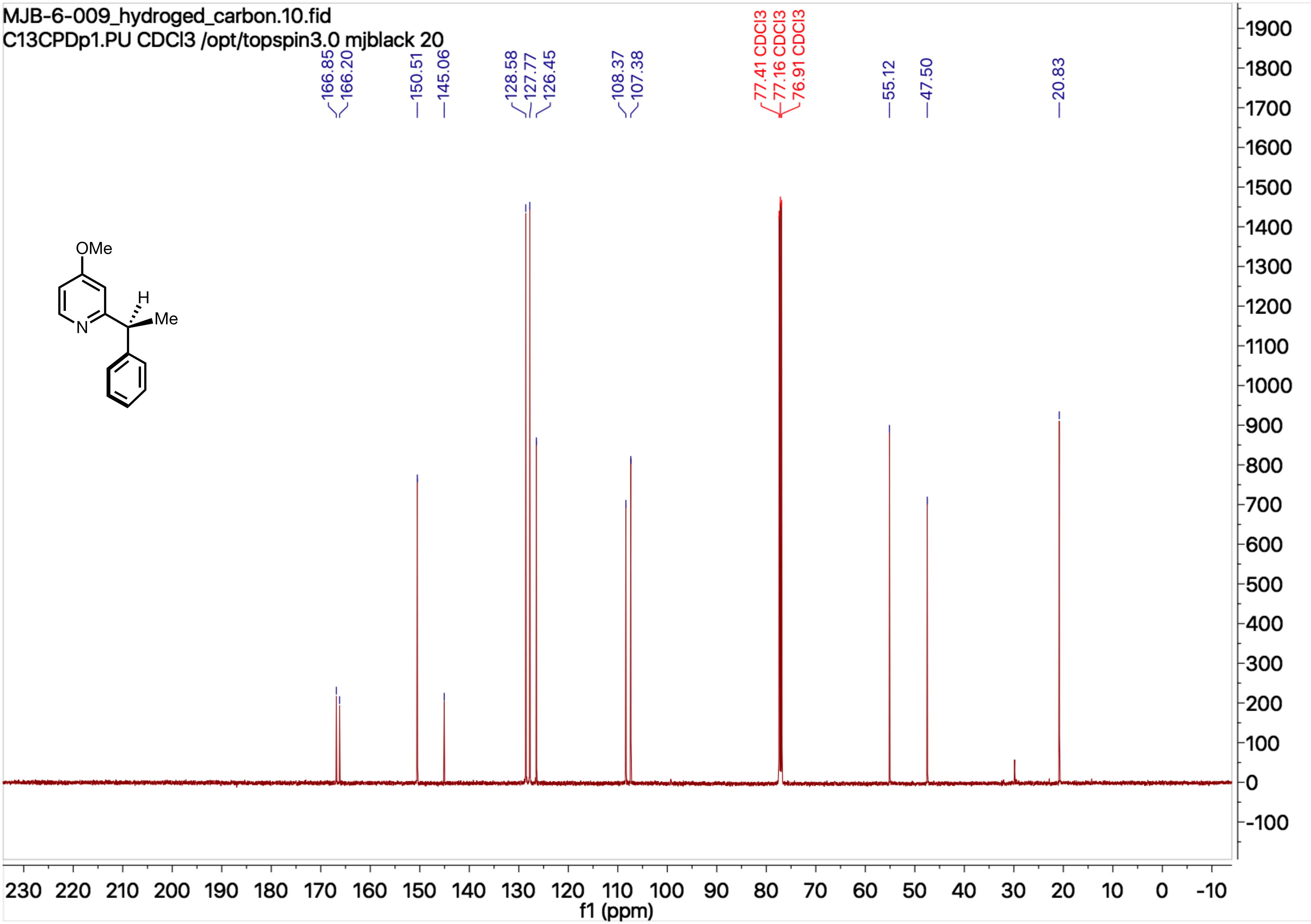


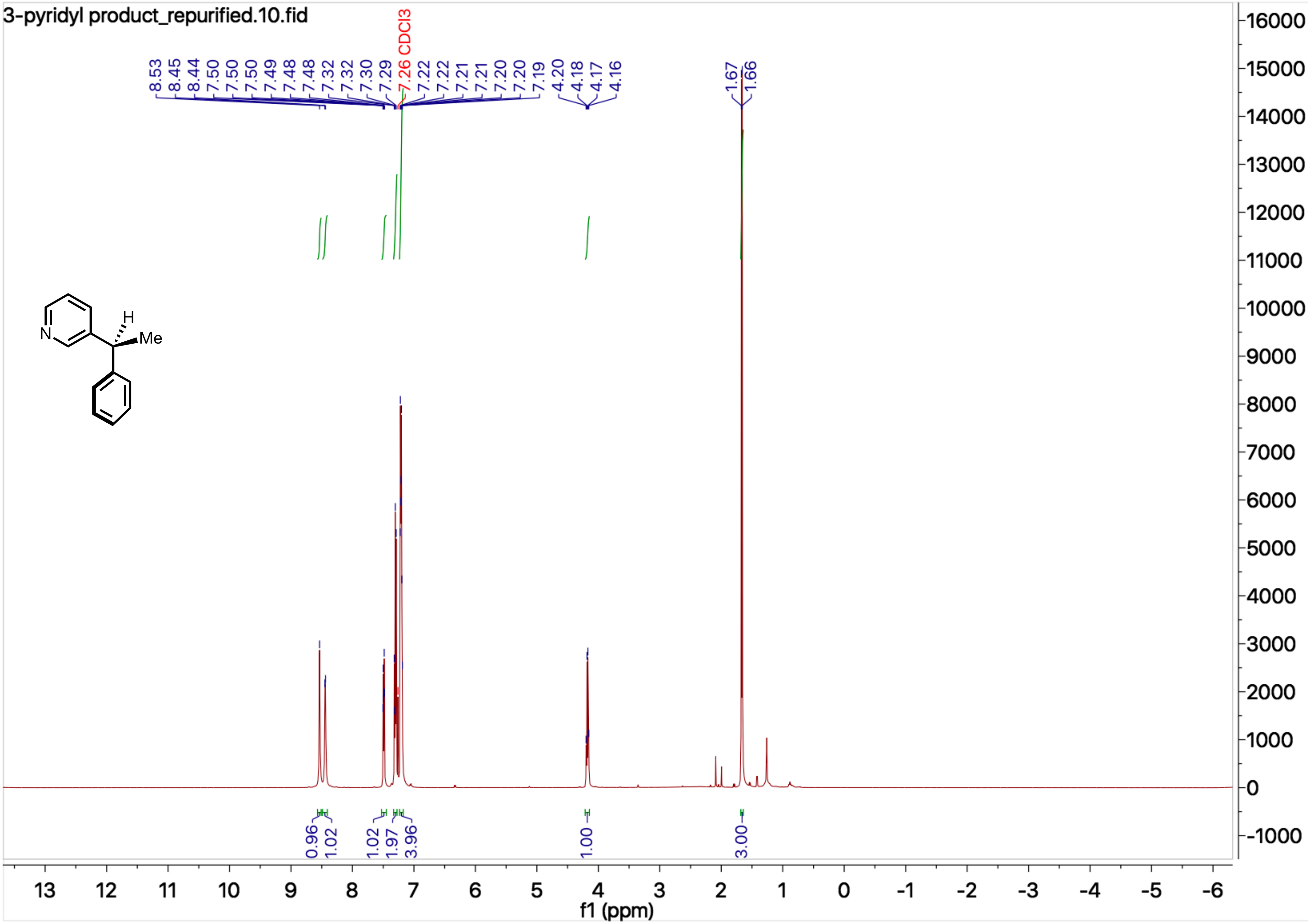
YN-6-56.1.fid
YN-6-36
PROTON.PU CDCl3 /opt/topspin3.0 ynakano 36
CDCl3
H
Me
N
OH
1.00
1.03
2.10
1.05
1.10
1.06
3.17
f1 (ppm)



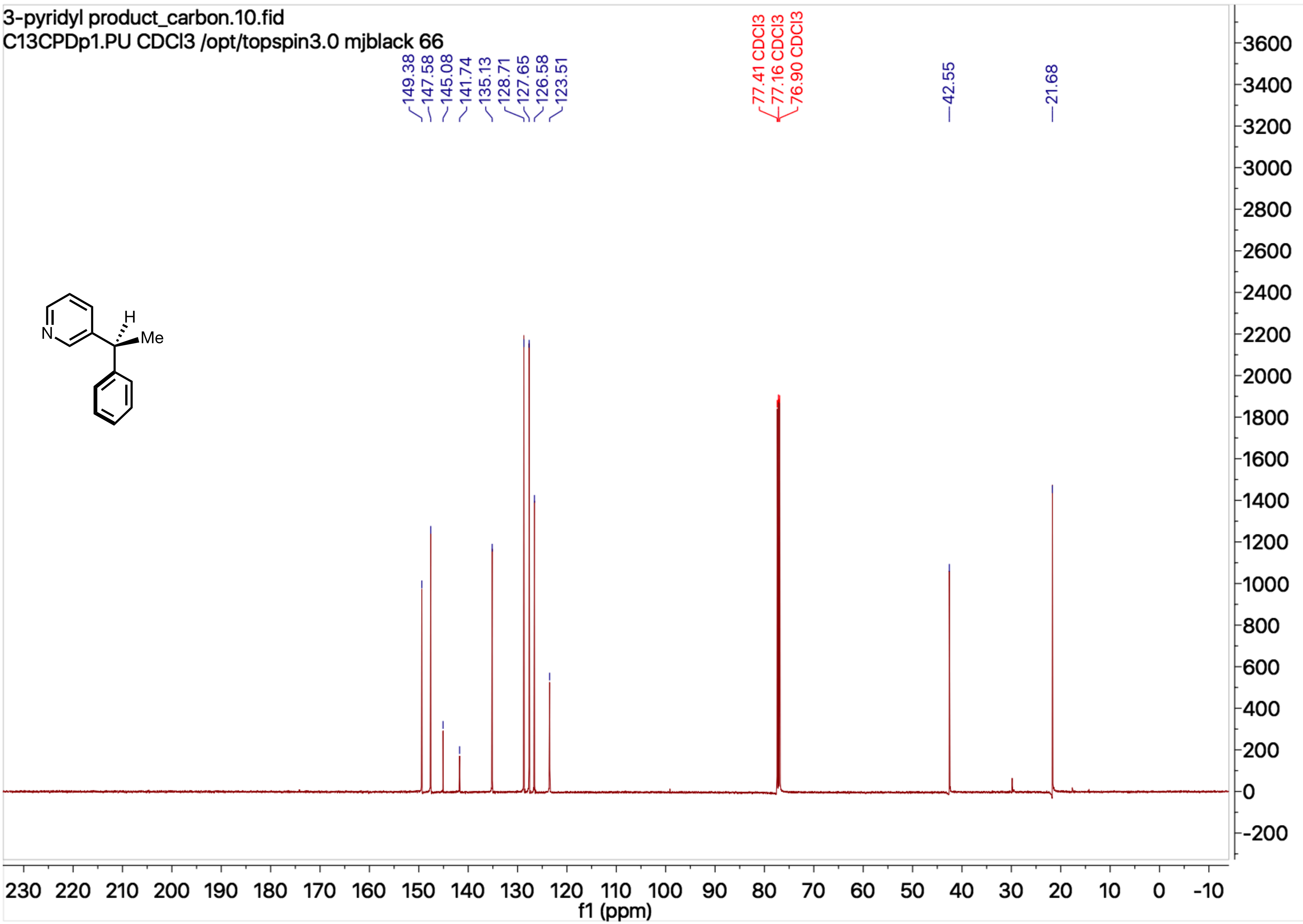
YN-6-56.2.fid
YN-6-36
C13CPDp1.PU CDCl3 /opt/topspin3.0/ynakano36
163.94
147.57
135.80
121.15
120.50
76.24 CDCl3
75.98 CDCl3
75.73 CDCl3
66.12
40.77
16.16
H
Me
N
OH
f1 (ppm)

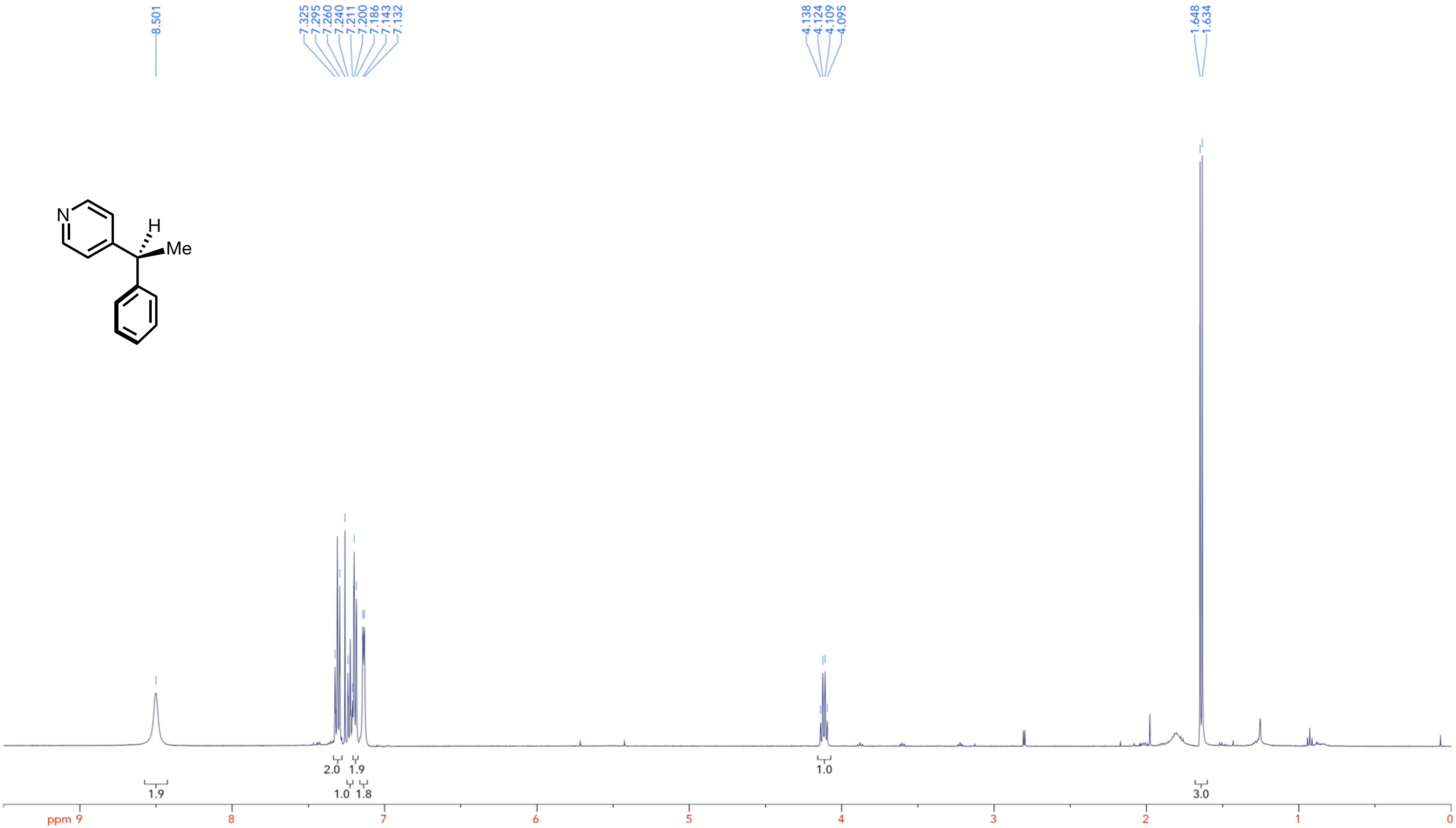

MJB-6-009_hydroged.10.fid
8.40
8.39
8.38
7.30
7.29
7.29
7.27
7.27
7.26 CDCl3
7.21
7.20
7.20
7.19
7.19
7.18
7.18
6.64
6.64
6.63
6.63
4.26
4.25
4.24
4.22
3.78
1.69
1.68
OMe
H
Me
N
1.09
3.87
1.01
2.05
1.06
3.00
3.10
f1 (ppm)


MJB-6-009_hydroged_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 20
166.85
166.20
150.51
145.06
128.58
127.77
126.45
108.37
107.38
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
55.12
47.50
20.83
OMe
H
Me
N
f1 (ppm)

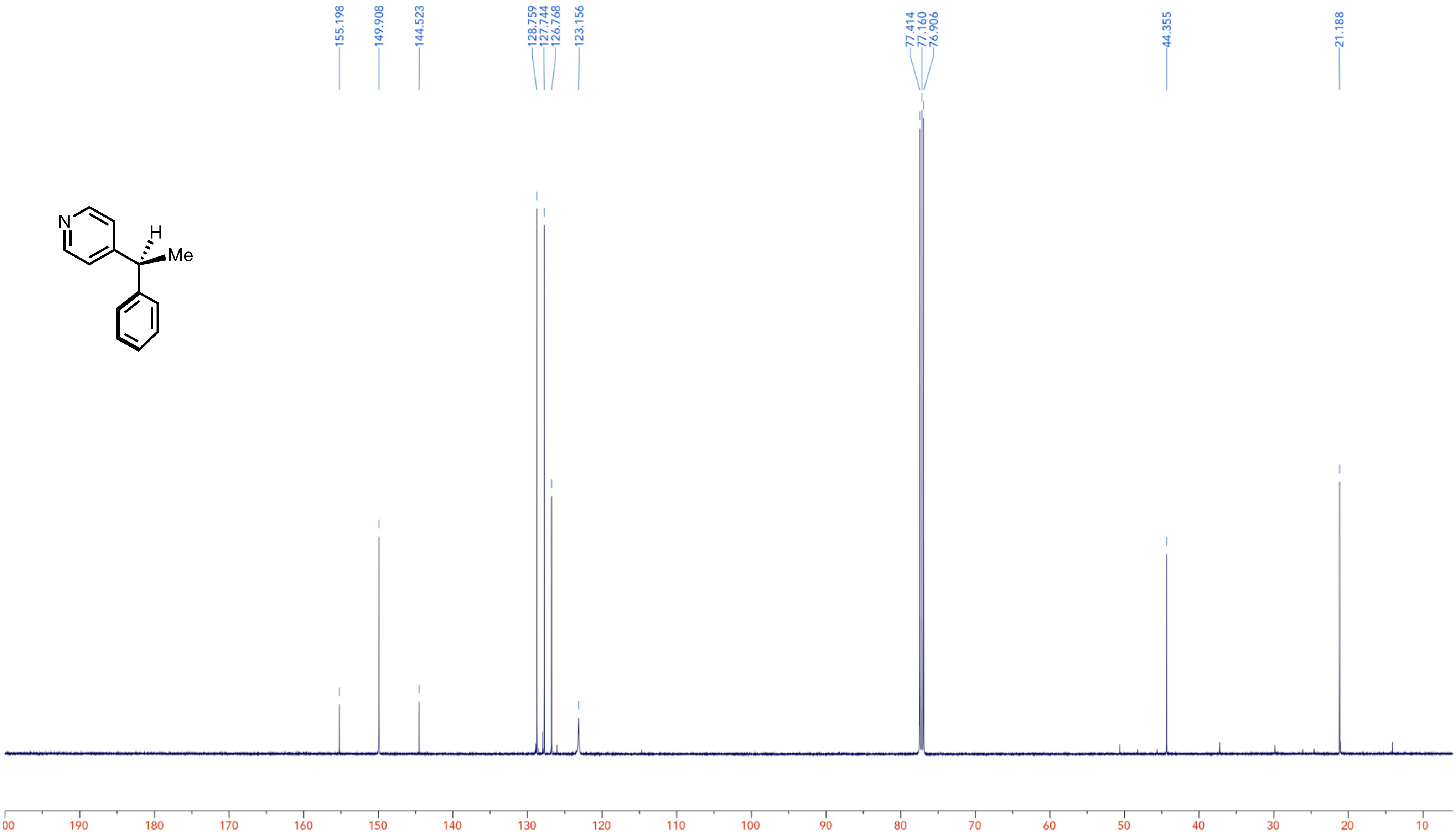


3-pyridyl product_repurified.10.fid
8.53 8.45 8.44 7.50 7.50 7.50 7.49 7.48 7.48 7.32 7.32 7.30 7.29 7.26 CDCl3 7.22 7.22 7.21 7.21 7.20 7.20 7.19 4.20 4.18 4.17 4.16 1.67 1.66
N
H
Me
0.96 1.02 1.02 1.97 3.96 1.00 3.00
f1 (ppm)


3-pyridyl product_carbon.10.fid
C13CPDp1.PU CDCl3 /opt/topspin3.0 mjblack 66
149.38
147.58
145.08
141.74
135.13
128.71
127.65
126.58
123.51
77.41 CDCl3
77.16 CDCl3
76.90 CDCl3
42.55
21.68
N
H
Me
f1 (ppm)



8.501
7.325
7.295
7.260
7.240
7.211
7.200
7.186
7.143
7.132
4.138
4.124
4.109
4.095
1.648
1.634
N
H
Me
1.9
2.0
1.0
1.9
1.8
1.0
3.0
ppm 9
8
7
6
5
4
3
2
1
0


N
H
Me
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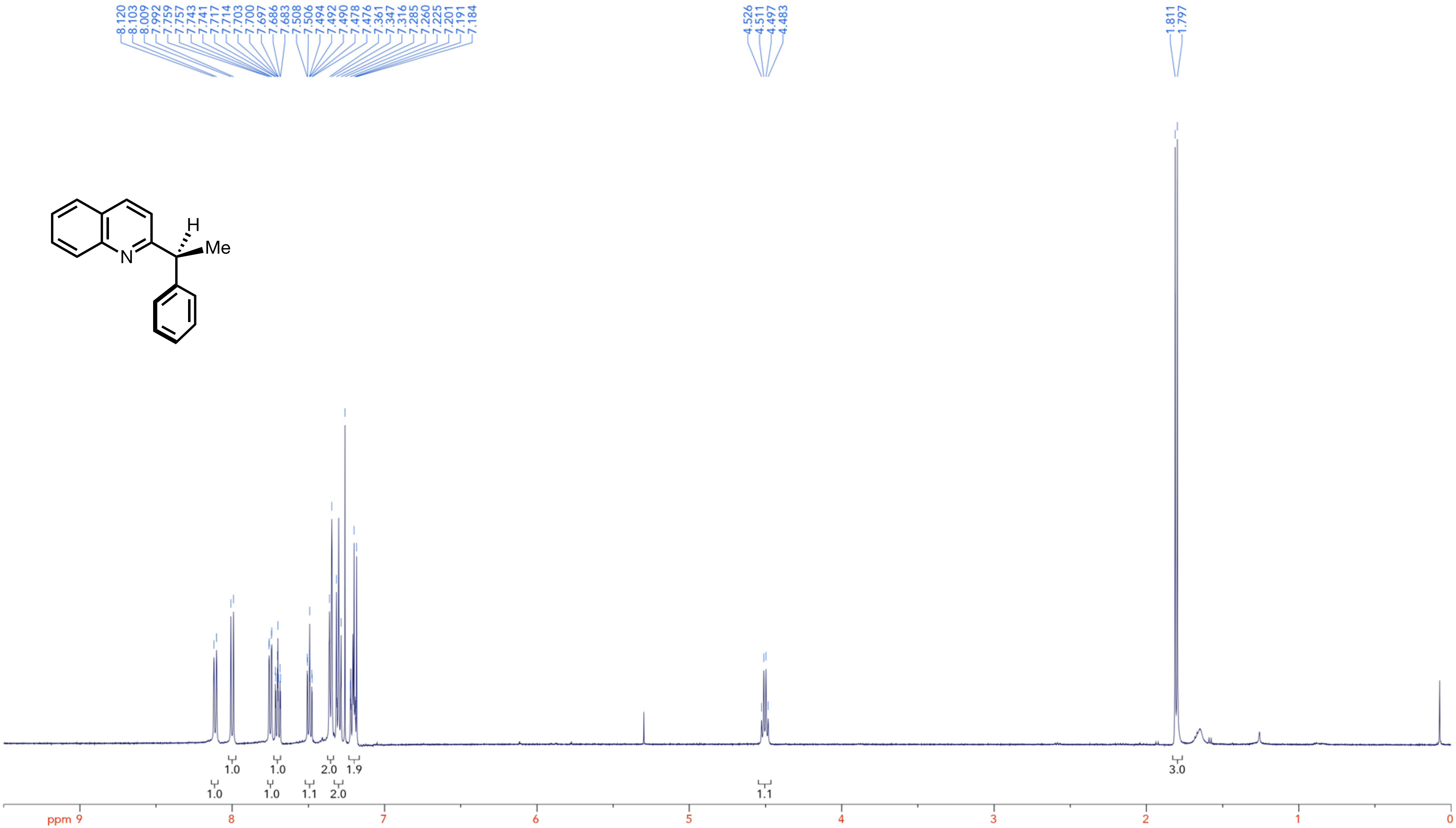
149.908
144.523
128.759
127.744
126.768
123.156
77.414
77.160
76.906
44.355
21.188
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10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7.757
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7.700
7.697
7.686
7.683
7.508
7.506
7.494
7.492
7.490
7.478
7.476
7.361
7.347
7.316
7.285
7.260
7.225
7.201
7.191
7.184
4.526
4.511
4.497
4.483
1.811
1.797
H
Me
N
1.0
1.0
1.0
1.0
1.1
2.0
2.0
1.9
1.1
3.0
ppm 9
8
7
6
5
4
3
2
1
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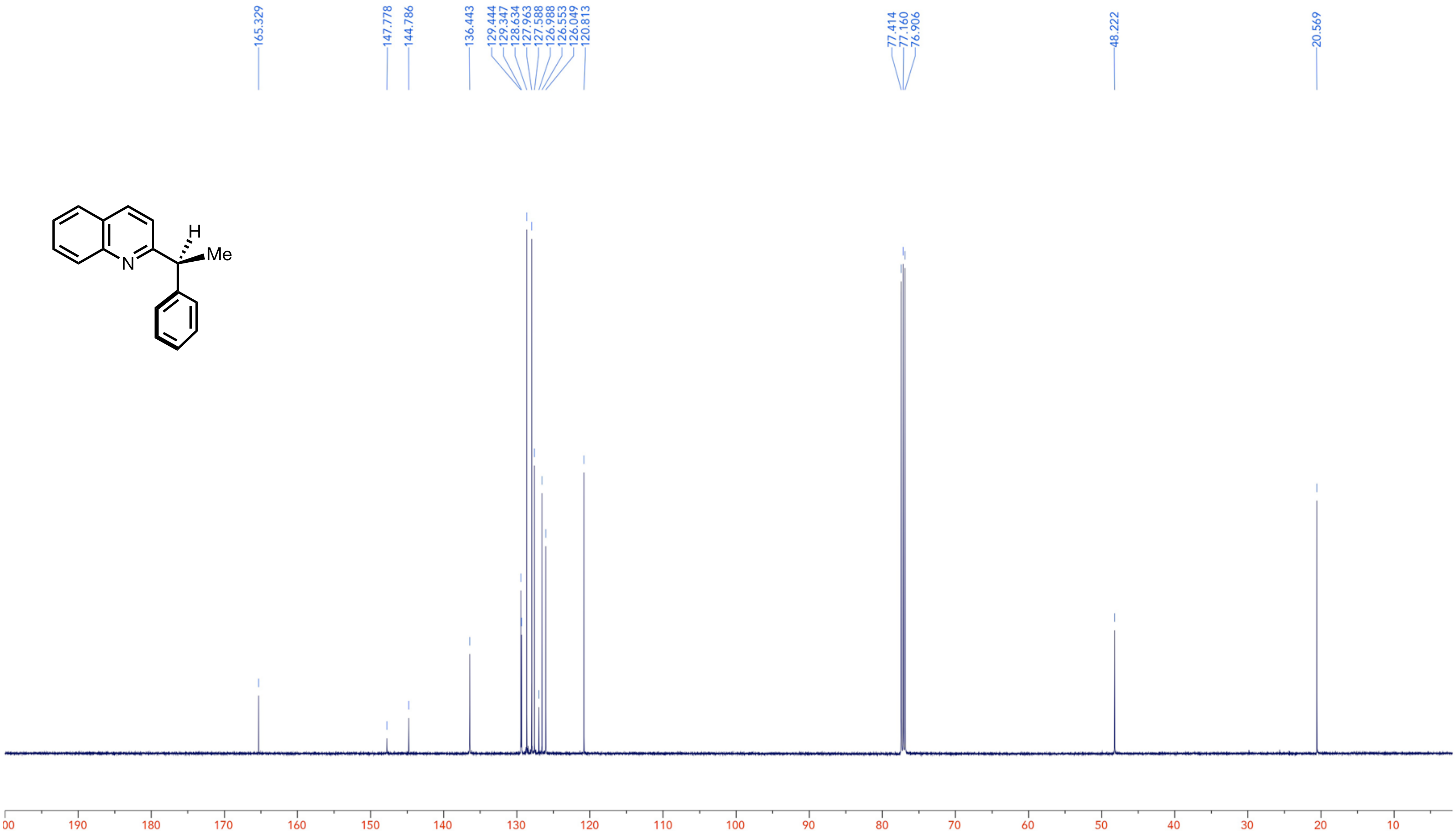

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144.786
136.443
129.444
129.347
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127.588
126.988
126.553
126.049
120.813
77.414
77.160
76.906
48.222
20.569
H
Me
N
00
190
180
170
160
150
140
130
120
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100
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80
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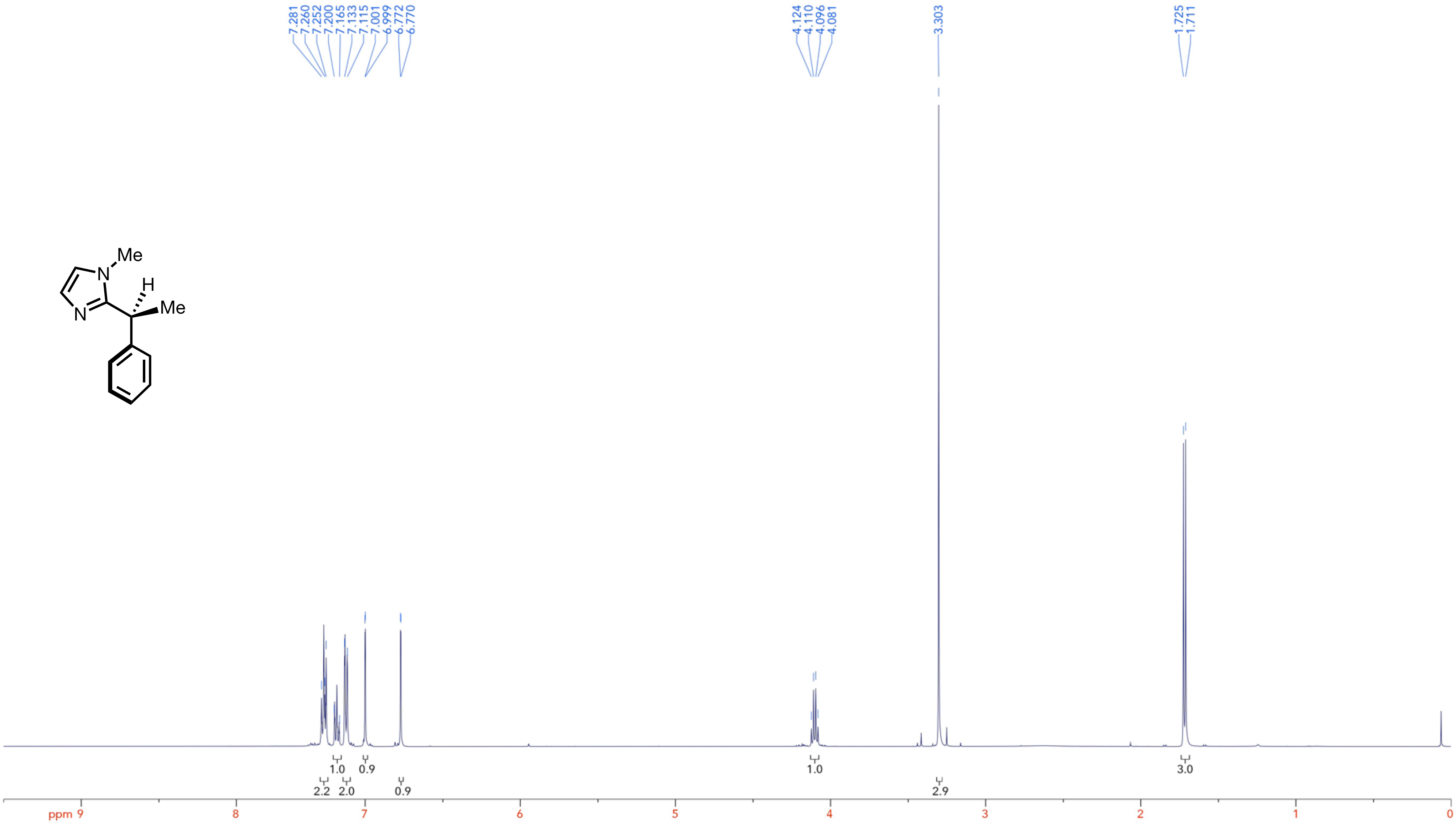
30
20
10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N
H
Me
N
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7.165
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7.115
7.001
6.999
6.772
6.770
4.124
4.110
4.096
4.081
3.303
1.725
1.711
1.0
0.9
2.2
2.0
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1.0
2.9
3.0
ppm 9
8
7
6
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1
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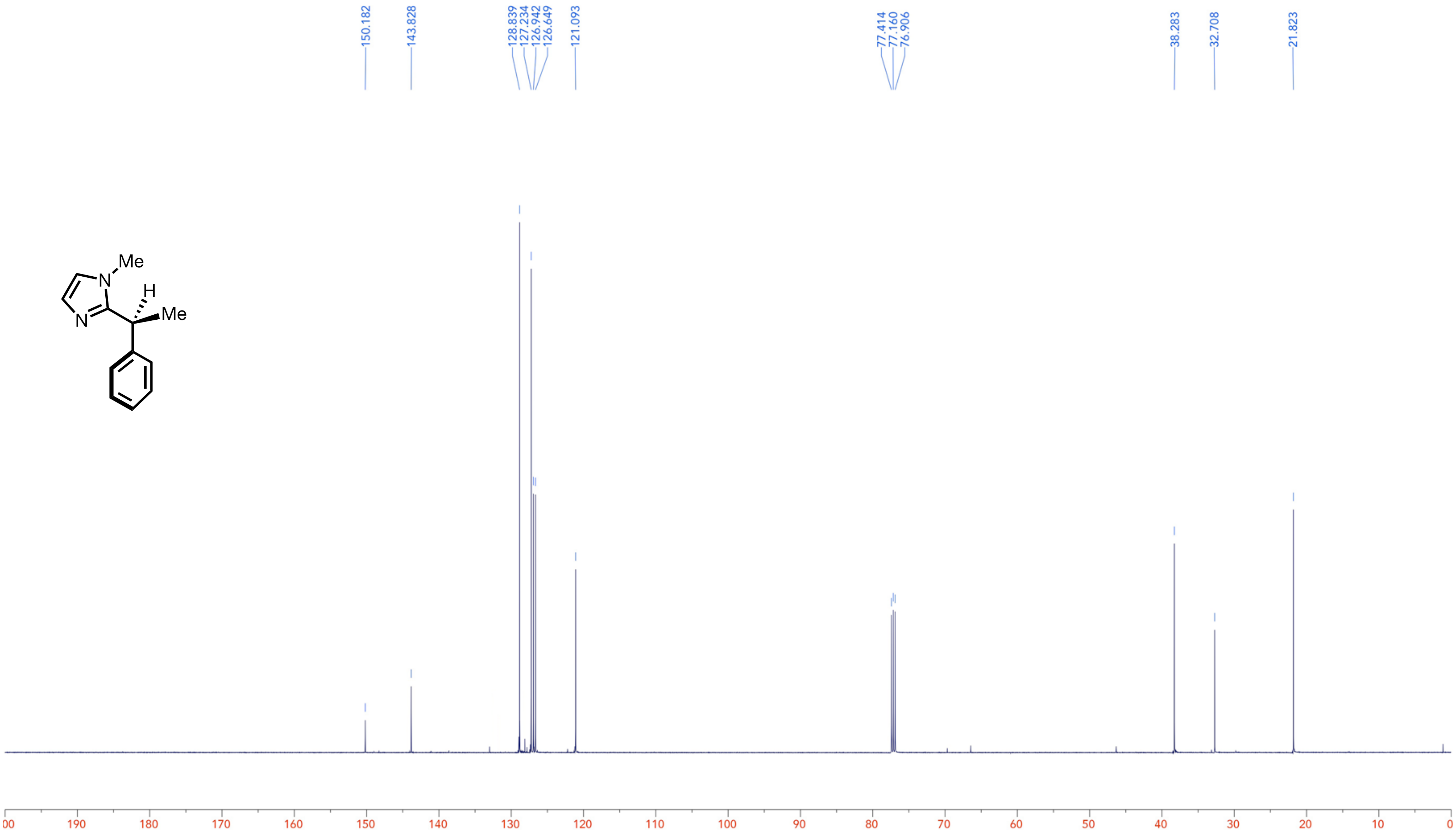
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121.093
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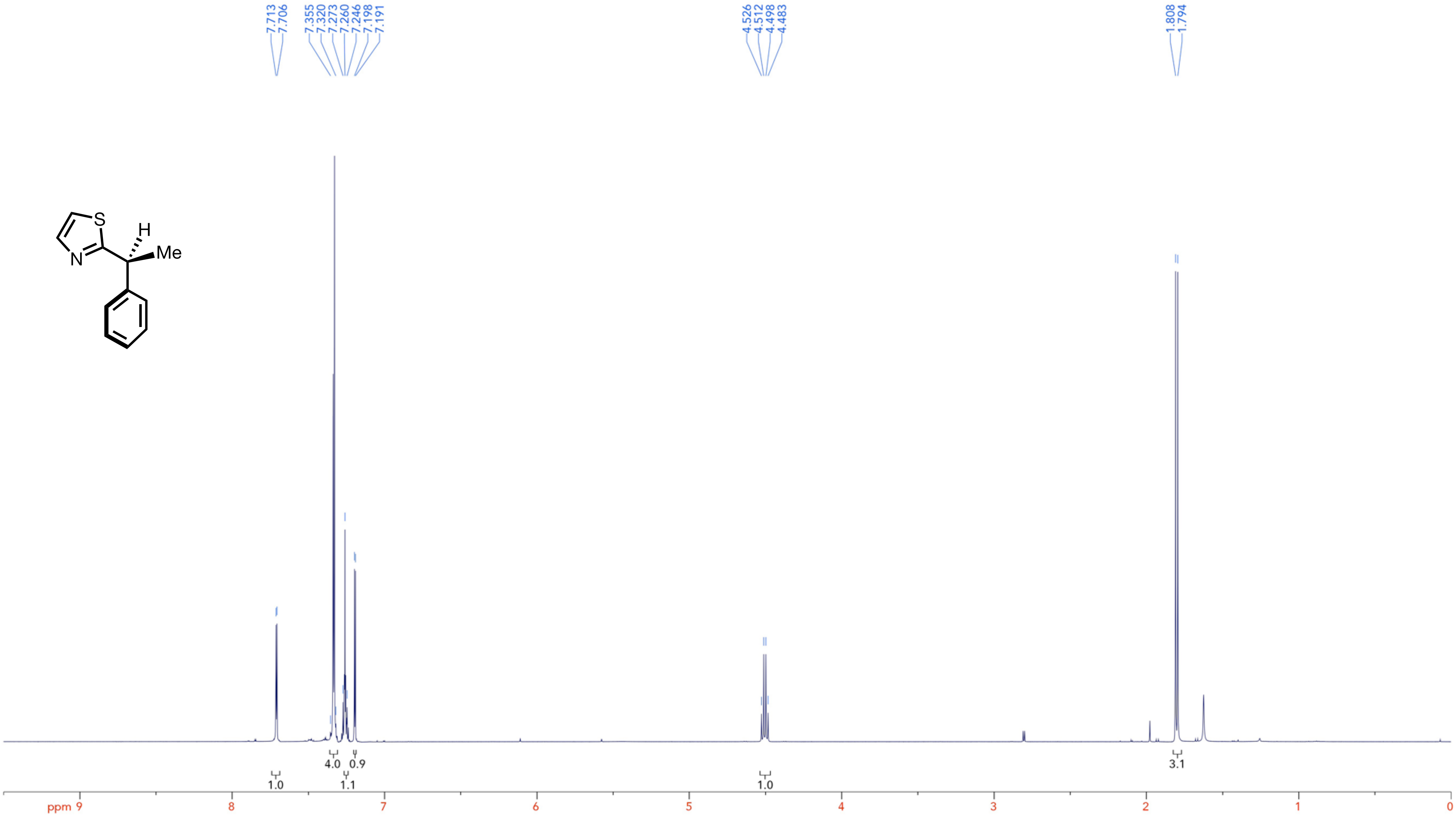
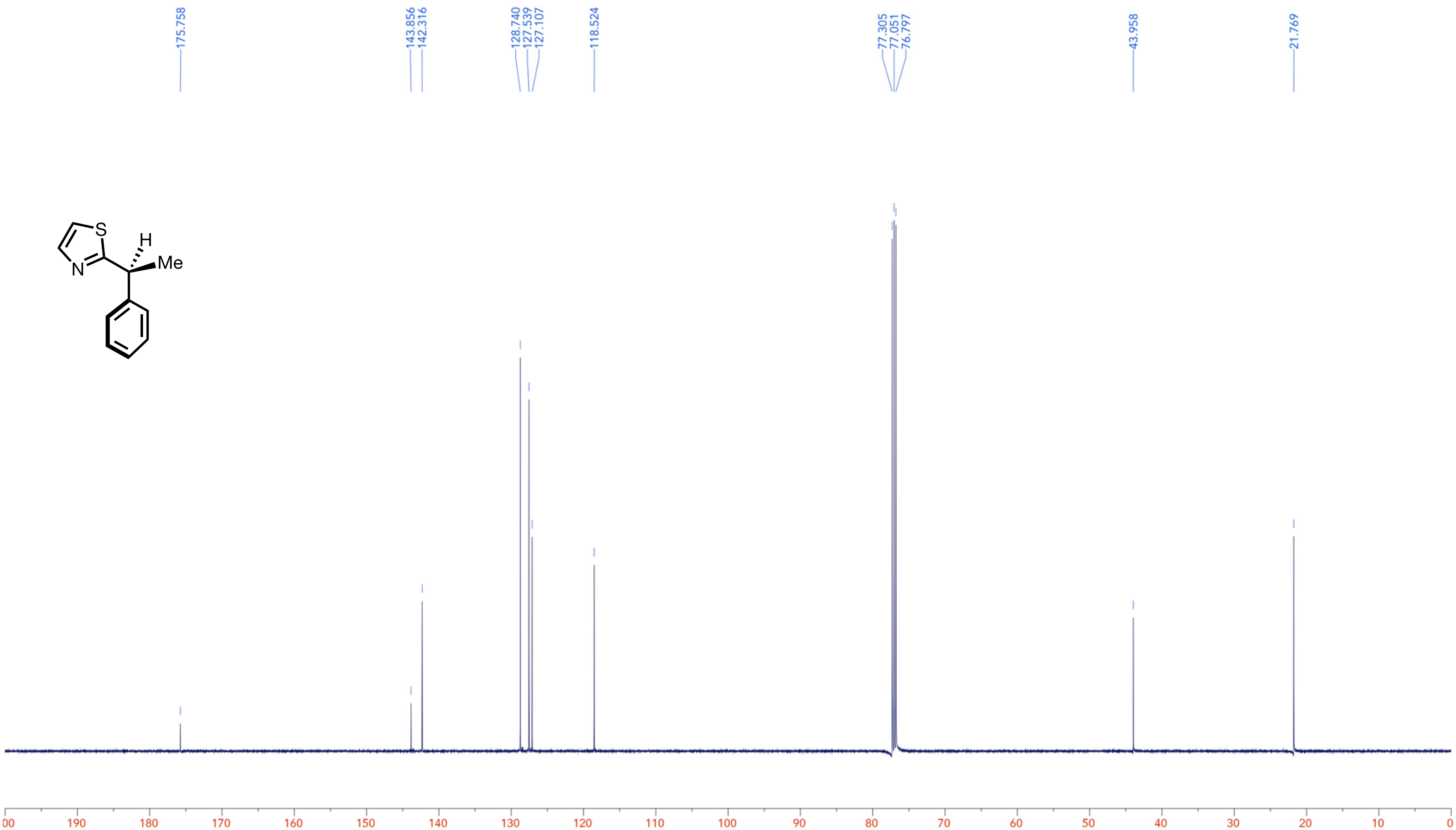
77.160
76.906
38.283
32.708
21.823
Me
N
H
N
Me
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0



7.713
7.706
7.355
7.320
7.273
7.260
7.246
7.198
7.191
4.526
4.512
4.498
4.483
1.808
1.794
S
N
H
Me
4.0
0.9
1.0
1.1
1.0
3.1
ppm 9
8
7
6
5
4
3
2
1
0



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143.856
142.316
128.740
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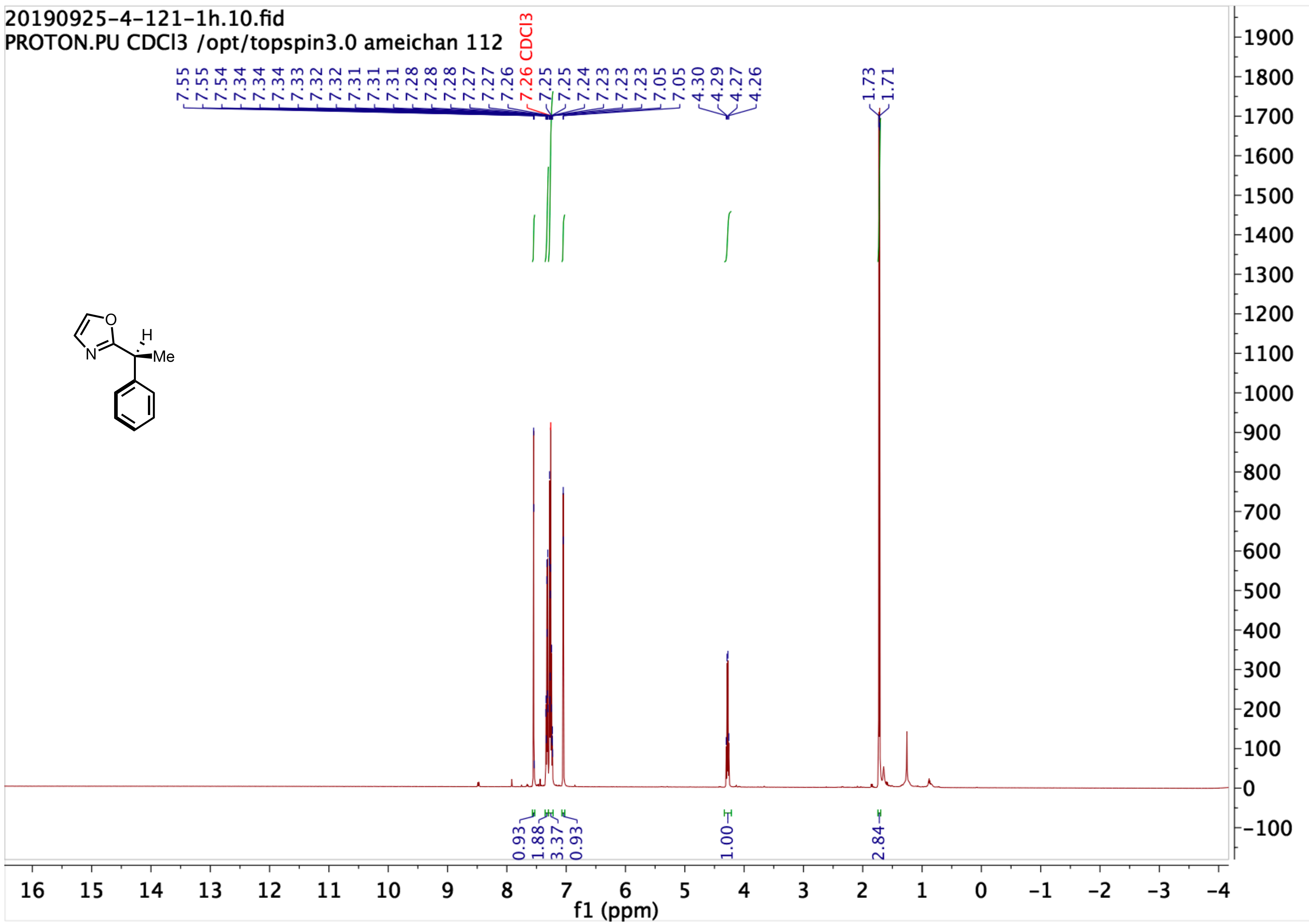
127.107
118.524
77.305
77.051
76.797
43.958
21.769
S
N
H
Me
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0



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PROTON.PU CDCl3 /opt/topspin3.0 ameichan 112
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7.55
7.54
7.34
7.34
7.34
7.33
7.32
7.32
7.31
7.31
7.31
7.28
7.28
7.28
7.27
7.27
7.26
7.26 CDCl3
7.25
7.25
7.24
7.23
7.23
7.23
7.05
7.05
4.30
4.29
4.27
4.26
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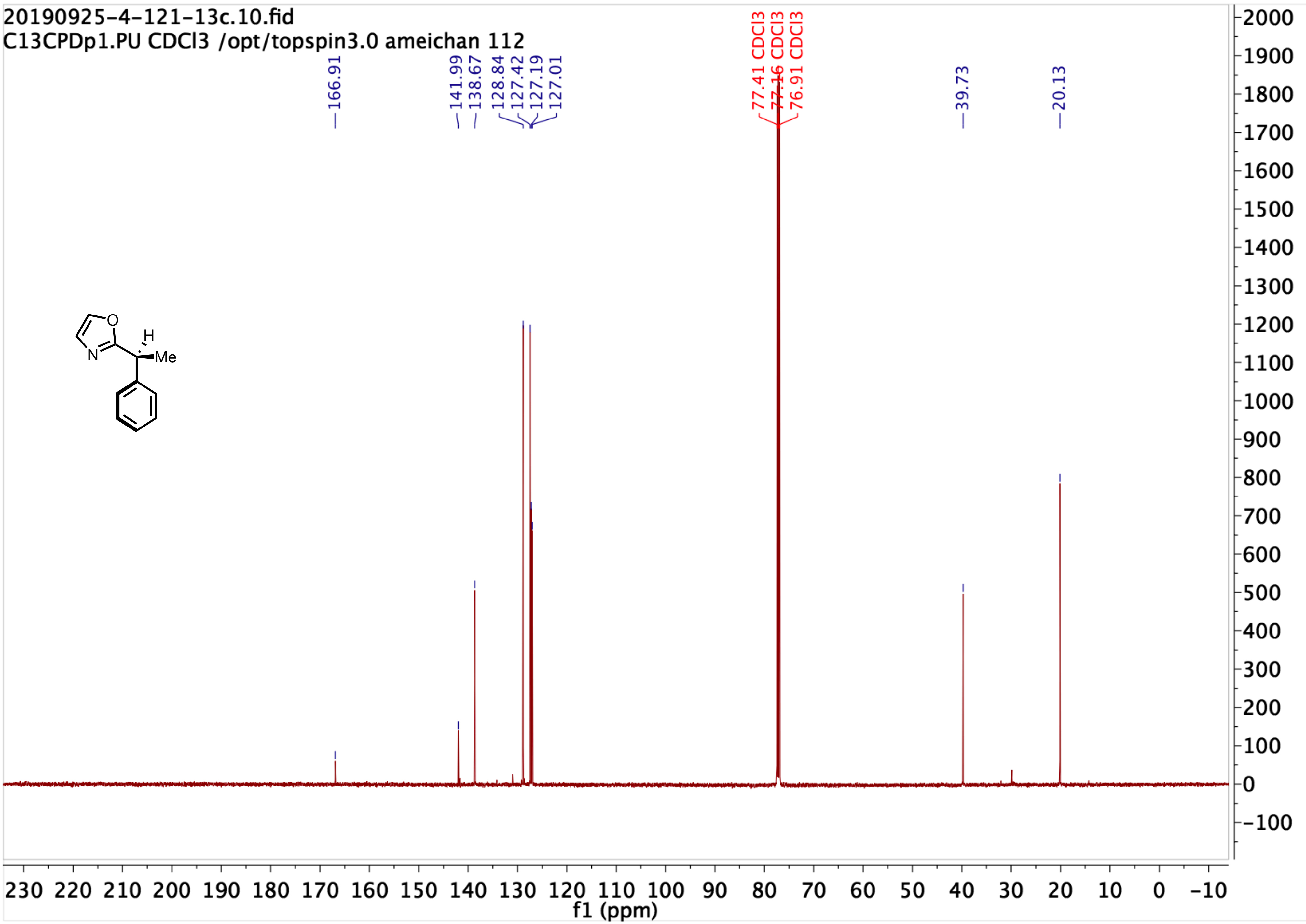
1.71
O
N
H
Me
0.93
1.88
3.37
0.93
1.00
2.84
f1 (ppm)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C13CPDp1.PU CDCl3 /opt/topspin3.0 ameichan 112
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141.99
138.67
128.84
127.42
127.19
127.01
77.41 CDCl3
77.16 CDCl3
76.91 CDCl3
39.73
20.13
O
N
H
Me
f1 (ppm)